BIOPHYSICAL CHEMISTRY

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This text was compiled on 07/17/2025

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Licensing

*A detailed breakdown of this resource's licensing can be found in **Back Matter/Detailed Licensing**.*

Preface

Dear Reader,

Thank you for your interest in this book. First a couple of words about what this book is and what it is not. We wrote this text to serve as the primary source for a series of Biophysical Chemistry courses that we regularly teach as faculty members of the Chemistry Department at Western Washington University (WWU; Bellingham, WA, USA). These courses are currently listed as CHEM 466 and CHEM 467 and serve the needs of WWU undergraduate Biochemistry program. The present text is based on the assumption of what WWU Biochemistry majors know and do not know at the time they enter their final year of study toward Bachelor of Science (B.S.) degree in Biochemistry. The structure and style of the text reflects our combined experience in teaching this course and our views on the content and pedagogical practices. Although there are many highly valuable books on various topics of Biophysical Chemistry available commercially today, we decided to design our own text because not a single text we know covers all the topics needed for our particular program at WWU with the scope and depth required and with the needed pedagogical elements present. Because of its focus and design, the book is not meant to be a general purpose textbook in Biophysical Chemistry.

As of Fall 2021, the text consist of five Parts, each in turn composed of several Chapters. Every Chapter starts with a one-paragraph introduction describing the Chapter's focus. The principal Learning Objectives are listed next to guide the students in their work with the Chapter. Within the main body of the Chapter, figures, formulas, tables and side notes are utilized in a coherent way to facilitate the comprehension. At the end of each Chapter or within it, we present some relevant problem solving examples, which are followed by a list of practice problems for the students to self-assess their level of comprehension and in some cases help them to advance to the next Chapters.

The book is currently under active development. So, we are thankful for feedback from the Readers who might choose to provide suggestions on how to improve this text.

With best regards,
The Authors
September 2022

CHAPTER OVERVIEW

1: Biochemical Thermodynamics

1.1: Thermodynamic Variables and Equations of State
1.2: The First Law of Thermodynamics
1.3: Thermochemistry
1.4: The Second Law of Thermodynamics
1.5: The Boltzmann Distribution and the Statistical Definition of Entropy
1.6: The Gibbs and Helmholtz Energy
1.7: Equilibria in Biochemical Systems

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1.1: Thermodynamic Variables and Equations of State

Classical thermodynamics provides a conceptual framework from which we can understand the behavior of molecular systems in the biological sciences at a quantitative level. This chapter introduces some of the concepts relating to properties of a system and its surroundings that we will need to study classical thermodynamics. Applications to biological systems will be presented in later chapters. In this chapter, we will focus on how the macroscopic properties of a system are related to and depend on the properties of the constituent atoms and molecules. As an example we will discuss the ideal-gas equation, its range of validity, and how it can be extended to real gases or fluids of interacting molecules.

Learning Objectives

- Build a precise vocabulary of thermodynamic definitions before applying them to biochemical systems.
- Understand state variables and how they are mathematically related in an equation of state.
- Be able to manipulate the ideal gas equation of state.
- Understand how real gases deviate from ideality and how real gases and fluids can be modeled by the virial equation of state, which is an expression for the pressure of a gas as a polynomial in the density.

Basic Definitions

We begin our discussion of biochemical thermodynamics with some definitions that will allow us to make general statements about how energy is exchanged and converted into various forms.

A **system** is any part of the universe that is of interest to us. This might be the Sun-Earth-Moon system, a human lung, fruit fly, a single bacteria cell, or a beaker on a bench top. Some example systems are shown in **Figure I.1.A:**

Figure I.1.A: Some systems of varying complexity include the sun-earth-moon system, a human lung, a fruit fly, a single bacteria cell, or a beaker

Everything else in the universe that is not part of the system is called the **surroundings.** The system + the surroundings constitutes the **universe.**

We can classify systems into 3 types: open systems, closed systems, or isolated systems. An **open system** is able to exchange both matter and heat with the surroundings. A **closed system** cannot exchange matter with the surroundings but *can* exchange heat with the surroundings. An **isolated system** cannot exchange any heat or any matter with surroundings. These three types of systems are depicted in **Figure I.1.B**:

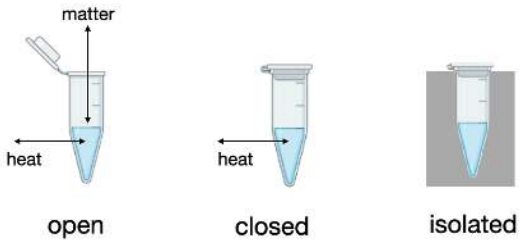

Figure I.1.B: An open system can exchange both matter and heat with the surroundings. A closed system cannot exchange matter with the surroundings but can exchange heat. An isolated system cannot exchange heat or matter with the surroundings.

The branch of science called **thermodynamics** is interested in the relationships between properties of a system and how properties change as the system changes state. A **property** is any mathematically quantifiable parameter of the system. Some properties could include the pressure, the temperature, the density, the index of refraction, etc ….

We can distinguish between two types of properties: intensive and extensive. **Intensive** properties are *independent* of the quantity (amount of matter) being measured. Some intensive properties include the density, pressure, and temperature. On the other hand, **Extensive** properties *depend* on the quantity (amount) being measured. Some extensive properties are the mass, the volume, and the number of moles.

Intensive properties can be constructed as the ratio between two extensive properties. For example the **density** is

$$\text{density} = \frac{\text{mass}}{\text{volume}} \tag{1.1.1}$$

Notice that both **mass** and **volume** are extensive (depend on the amount), but the density (the ratio of the mass over volume) is intensive.

Similarly, the **pressure** is defined as

$$\text{pressure} = \frac{\text{force}}{\text{area}} \tag{1.1.2}$$

The SI units of pressure is the Pascal (Pa) and 1 Pa = 1 N· m^{-2} = 1 kg·m^{-1}· s^{-2} = 1 J· m^{-3}. **Table I.1.i** relates some common units of pressure.

Table I.1.i: Some common units of pressure

1 Pa = 1 N m^{-2}
1 bar = 100 kPa (10^5 Pa)
1 atm = 101.32 kPa
1 torr (mm Hg) = 1/760 atm

Note: **Energy** by itself (measured in Joules or calories) is an *extensive* property. Often, we report energies as a molar ratio in units of J/mol or cal/mol which is an *intensive* property.

A common way to define an intensive property is to define the **molar** quantity by dividing some extensive variable by the number of moles. For example, the **molar volume** is defined as:

$$\bar{V} = \frac{V}{n} \tag{1.1.3}$$

Here **n** is the number of moles and $\bar{\mathbf{V}}$ is called the molar volume and is an intensive quantity.

Temperature is another important thermodynamic parameter that will be defined in serval ways throughout this text. For now, we will define the **temperature** as the measure of the motion of the atoms within the system. This definition of temperature implies that the "thermodynamic" temperature is measured in Kelvin, because the Kelvin scale is the absolute temperature scale. In the limit that T=0 K (absolute zero), the motion of the atoms approaches zero. We can convert between temperature in Kelvin and Celsius scales using the relation:

$$T(\text{in Kelvins}) = T(\text{in }^\circ C) + 273.15 \tag{1.1.4}$$

Caution

Note: Absolute temperatures (in K) must be used in thermodynamic calculations.

See Practice Problems 1 and 2.

Thermodynamic Equations of State

An **equation of state** is a mathematical expression that fully describes the thermodynamic state of the system in terms of a set of physical properties. The most familiar example is the ideal gas law:

$$PV = nRT \tag{1.1.5}$$

or, introducing the molar volume (Equation 1.1.3):

$$P\bar{V} = RT \tag{1.1.6}$$

where $\mathbf{P}$ is the pressure, $\mathbf{V}$ is the volume, $\mathbf{n}$ is the number of moles, $\mathbf{T}$ is the temperature. $\mathbf{R}$ is the gas constant given in **Table I.1.ii**. Equation 1.1.5 is known as the **ideal gas equation of state**. The ideal gas equation of state (PV=nRT) allows us to see how the properties of an ideal gas are related.

Table I.1.ii: Common units for the ideal gas constant R.

R = 8.314 J K^{-1} mol^{-1} = 0.08206 L atm K^{-1} mol^{-1} L atm = 101.34 J

The ideal gas equation of state will be a useful model for us to work with as we derive thermodynamic relationships because it is intuitive and algebraically easy to manipulate. At this point, it is worthwhile to make some comments concerning the ideal gas equation of state:

1. The ideal gas equation of state can be derived from first principles (kinetic theory of gases).
2. At sufficiently *high* temperature and *low* pressure, all gases fit the ideal gas law.
3. Assumptions made in the ideal gas law:
   - The gas molecules themselves occupy no volume.
   - There are no attractive or repulsive forces between gas molecules.
   - All collisions are perfectly elastic.

See Practice Problems 3 and 4.

Because of these assumptions, we expect all **real gases** to deviate from ideal behavior. To quantify this we define the **compressibility factor, Z,** as

$$Z = \frac{P\bar{V}}{RT} \tag{1.1.7}$$

Notice from Equation 1.1.5 that for an ideal gas, $Z = 1$. All real gases will deviate from this ideal behavior. **Figure I.1.C** shows the compressibility factor Z as a function of pressure for N_2 gas at different temperatures. A perfect ideal gas would have $Z = 1$ for all pressures and temperatures. For N_2 gas we see that at sufficiently low pressure $Z \to 1$ and that at higher temperature (purple curve), the gas behalves more like an ideal gas (dotted line).

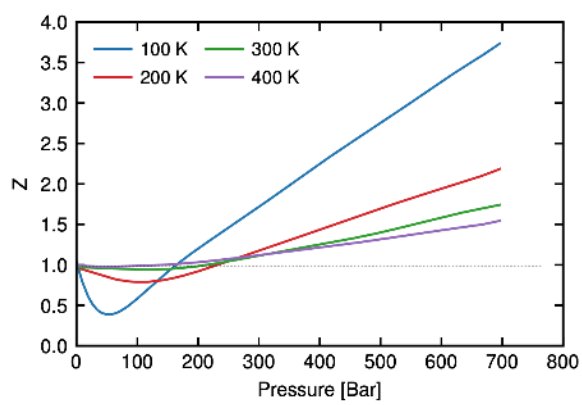

Figure I.1.C: Compressibility factor of nitrogen gas as a function of pressure for different temperatures. The ideal gas limit is shown by the dotted line at Z=1.

In order to derive an equation of state for a non-ideal gas, we can consider a series expansion of the compressibility factor, $\mathbf{Z}$, in powers of the inverse molar volume, $\mathbf{1/\bar{V}}$:

$$Z = 1 + \frac{B_2}{\bar{V}} + \frac{B_3}{\bar{V}^2} + \frac{B_4}{\bar{V}^3} + \ldots \tag{1.1.8}$$

Equation 1.1.8 is called the **virial equation of state**, and $\mathbf{B_2}$ is called the **second virial coefficient**, $\mathbf{B_3}$ is called the third virial coefficient, etc…. The virial coefficients (B_2, B_3, …) are typically fit to experimental data and are temperature dependent. Notice that for a perfect ideal gas, the second and higher virial coefficients are all zero. The virial equation of state works well to describe any gas, but has the drawback of needing the virial coefficients from fitting to experimental data.

For gases that exhibit small deviations from ideal gas behavior, we can truncate Equation 1.1.8 to include just the second virial coefficient:

$$Z \approx 1 + \frac{B_2}{\bar{V}} \tag{1.1.9}$$

The second viral coefficient, $\mathbf{B_2}$ is related to the interactions between atoms described by a potential energy function $U(r)$, where r is the distance between atom pairs. For a dilute system of non-polar molecules, the relationship between the second virial coefficient and the potential energy is

$$B_2 = N_A \frac{1}{2} \int_0^\infty \left[1 - e^{-U(r)/k_B T}\right] 4\pi r^2 dr \tag{1.1.10}$$

where $\mathbf{N_A}$ is Avogadro's number, $\mathbf{k_B}$ is Boltzmann's constant, and $\mathbf{T}$ is the temperature. In most cases, we cannot analytically solve the integral in Equation 1.1.10. Note that in the absence of interactions, $U(r) = 0$, then from Equation 1.1.10, $B_2 = 0$ and the gas behaves like an ideal gas as we would expect for non-interacting gas molecules.

See Practice Problems 5-7.

Osmotic Pressure and Osmotic Virial Coefficients

In dealing with solutions (either solutions of small molecule solutes or macromolecules in solution), an important colligative property is the **osmotic pressure**. The osmotic pressure is given by the symbol Π and is different from the pressure of a gas (P) because it does not arise from collisions of molecules against the wall of a container. The osmotic pressure is a *hydrostatic* pressure that arises when solvent molecules pass through a semipermeable membrane to the more concentrated side of the membrane as shown in **Figure I.1.D.** The flow of solvent across the semipermeable membrane is called **osmosis**. Consider the osmotic pressure (Π) that develops on the more concentrated side of the membrane due to solvent molecules moving from the dilute to the concentrated side of the membrane. This situation illustrated in **Figure I.1.D.**

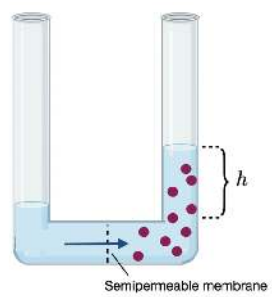

Figure I.1.D: Definition of osmotic pressure. The difference in concentration across the membrane causes a chemical potential. Solvent flows from the dilute to the concentrated side in an attempt to equalize the chemical potential. As water flows from the dilute to concentrated side, the water level will rise on the concentrated side. At equilibrium, the pressure differential is equal to the osmotic pressure.

As the pressure builds up on the high concentration side, the solvent level will rise by a height, h. The hydrostatic pressure is the difference between the pressure on the two sides of the semipermeable membrane and is given as

$$\Pi = \rho g h \tag{1.1.11}$$

where ρ is the solvent density, and g is the acceleration due to gravity. For an **ideal solution**, the osmotic pressure, Π, resembles the form of the ideal gas law:

$$\Pi V = nRT \tag{1.1.12}$$

Using the fact that n/V is the **concentration** (i.e. number of moles per unit volume of solution), we can rewrite Equation 1.1.12 in terms of the **molarity** (M):

$$\Pi = \underline{M}RT \tag{1.1.13}$$

If we define the **mass concentration** (C) of the solute (in units of g • L^{-1}), then we can rewrite 1.1.13 as:

$$\frac{\Pi}{RTC} = \frac{1}{M_w} \tag{1.1.14}$$

where M_w is the molar mass of the solute molecule (in units of g • mol^{-1}), and C is the mass concentration of the solute ($C = nM_w/V$).

Equation 1.1.14 assumes we are dealing with an ideal solution in which the solute particles are non-interacting. Instead, if we want to consider a *non-ideal* solution of weakly interacting molecules, we can expand Equation 1.1.14 in powers of C to obtain a virial equation of state for the osmotic pressure of a real solution:

$$\frac{\Pi}{RTC} = \frac{1}{M_w}\left[1 + B'_2C + B'_3C^2 + B'_4C^3 + \ldots\right] \tag{1.1.15}$$

where $B'_2, B'_3, B'_4 \ldots$ are the second, third, and fourth osmotic virial coefficients.

In the dilute limit (C << 1), we can truncate the expansion at the second virial coeffient:

$$\frac{\Pi}{RTC} = \frac{1}{M_w}\left[1 + B'_2C\right] \tag{1.1.16}$$

The second virial coefficient B' is related to the interactions between atoms describe by the potential energy function $U(r)$ through the relation given above in Equation 1.1.10. The second virial coefficent may be positive or negative depending on the nature of the particle-particle interactions. A negative coefficient corresponds to net attractive interactions, and a positive coefficient correspond to repulsive interactions.

Note: The second virial coeffient, B'_2, as written in Equation 1.1.16 is related to the second virial coefficients, B_2, in Equation 1.1.9 by the relation:

$$B'_2 = \frac{B_2}{M_w} \tag{1.1.17}$$

Osmotic pressure plays an important role in biology. For example, trees use osmotic pressure to transport water from the roots to the upper branches. The effect of osmotic pressure on the cell is illustrated in **Figure I.1.E**. When red blood cells are placed in a salt solution having a lower concentration than the intracellular fluid, the solution is **hypotonic**, and the cell will gain water through osmosis in an attempt to equalize the osmotic pressure. This situation is illustrated in Figure I.1.E (a). The cells will swell and potentially burst. When red blood cells are placed in a salt solution with the same osmotic pressure as the intracellular fluid, the solution is **isotonic** with respect to the cytoplasm. This situation is illustrated in See Figure I.1.E (b). Finally, when red blood cells are placed in a solution with a higher salt concentration than the intracellular fluid, the solution is **hypertonic** and water inside the cell flows outside the cell in an attempt to equalize the osmotic pressure, causing the cell to shrink. This is the situation illustrated in Figure I.1.E (c).

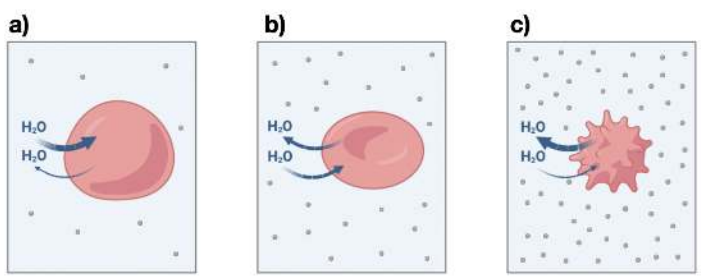

Figure I.1.E: a) When red blood cells are placed in a hypotonic solution whose osmotic pressure is less than that of the intracellular fluid, water flows into the cells and the cell swells and eventually bursts. b) When red blood cells are placed in an isotonic salt solution having the same osmotic pressure as the intracellular fluid, the rate of flow of water into and out of the cells is the same and the cell does not change shape. c) When red blood cells are placed in a hypertonic solution, the osmotic pressure is greater outside than that of the intracellular fluid, and water flows out of the cells. The cells shrink.

Examples

Example 1.1.1

Classify each of the following systems as either open, closed, or isolated. (a) A red blood cell, (b) a gas in a piston without valves, (c) boiling water in a kettle on the stove, (d) A closed Thermos flask of hot coffee (approximately).

Solution

(a) open; (b) closed; (c) open; (d) isolated

Example 1.1.2

Classify each of the following properties as intensive or extensive: (a) molar mass, (b) pressure, (c) temperature, (d) mass

Solution

(a) intensive; (b) intensive; (c) intensive; (d) extensive

Example 1.1.3

A Bellingham homebrewer collects the amount of gas evolved during the fermentation process. Later, the brewer measures the volume of gas to be 0.64 L at a cold temperature of 12.3 °C and 1 atm. Assuming ideal gas behavior, what was the volume of the gas at the fermentation temperature of 37.0 °C and 1 atm.

Solution

We use the ideal gas equation: PV=nRT to set up a ratio between the low temperature and high temperature system:

$$\frac{V_1}{V_2} = \frac{T_1}{T_2} \tag{1.1.18}$$

$$\frac{0.64\ \mathrm{L}}{V_2} = \frac{285.45\ \mathrm{K}}{310.15\ \mathrm{K}} \tag{1.1.19}$$

$$V_2 = 0.695\ \mathrm{L} \tag{1.1.20}$$

Practice problems

Problem 1. Classify each of the following systems as either open, closed, or isolated. (a) perfectly insulated water heater, (b) a glass thermometer (c) the universe (d) soup cooking on a stove (e) the earth (f) automobile (g) a sealed reaction flask

Problem 2. Classify each of the following properties as intensive or extensive: (a) density, (b) force, (c) molar volume, (d) heat.

Problem 3. Under which of the following sets of conditions would you expect a real gas to be adequately described by the ideal gas model (a) low pressure and low temperature; (b) low pressure and high temperature, (c) high pressure and high temperature, and (d) high pressure and low temperature.

Problem 4. An ideal gas in a piston is originally at a pressure of 118.0 atm and 85 °C. When the piston expands, its final volume, pressure, and temperature were 3.5 L, 1.0 atm, and 45 °C, respectively. What was the initial volume of the gas?

Problem 5. At 300 K, the second virial coefficient (B_2) of CO_2 gas is -120.5 $cm^3\ mol^{-1}$, for methane gas, CH_4, the second virial coefficient is -41.9 $cm^3\ mol^{-1}$, and for N_2 gas the second virial coefficient is -4.2 $cm^3\ mol^{-1}$. Rank these gases from most ideal gas to least idea gas at this temperature? Explain your reasoning.

Problem 6. Calculate the pressure of methane at 398.15 K if the molar volume is 0.2 L mol^{-1}, given that the second virial coefficient (B_2) of methane is -0.0163 L mol^{-1}. Compare your results with that obtained using the ideal gas equation. Is methane more or less compressible than an ideal gas at this temperature? (Assume that all other higher order virial coefficients can be neglected).

Problem 7. The **Boyle temperature** is the temperature at which the coefficient B_2 is zero. Therefore, a real gas behaves like an ideal gas at this temperature. **(a)** give a physical interpretation of this behavior. **(b)** Calculate the Boyle temperature for a gas whose second virial coefficient has the following form: $B_2 = a - \frac{b}{T}$ with the experimentally determined second virial coefficient measured at the following temperatures:

Second virial coefficient (B_2) (L mol^{-1})	Temperature (K)
-0.0237	292.95
-0.0231	296.15
-0.0228	298.15
-0.0218	303.15
-0.0201	313.15
-0.0185	323.15
-0.0117	373.15
-0.0065	423.15

Problem 8. The osmotic pressure of a protein in solution at 298 K (under crystallization conditions) was measured at the following concentrations:

Concentration (g L^{-1})	osmotic pressure (10^{-2} kPa)
0.50	1.85
1.00	3.68
1.50	5.48
2.00	7.25
2.50	9.00

Assuming that the osmotic virial equation of state can be truncated after the second virial coefficient, **a)** What is the molar mass of the protein?

b) What is the value of the second osmotic virial coefficient in units of L/g?

c) Based on the sign of the second virial coefficient, what might you speculate about the average contribution of intermolecular interactions under crystallization conditions? (For reference see: A. George and W.W. Wilson, "Predicting Protein Crystallization from Dilute Solution Property," *Acta Cryst.* (1994). D50, 361-365).

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1.2: The First Law of Thermodynamics

Systems can undergo a change of state from some initial state to a final state accompanied by a change in the system's energy. In this chapter, we analyze two types of energy: heat and work. This leads to a presentation of the first law of thermodynamics that deals with the conservation of energy, stating that any changes in the total internal energy of the system must be due to exchanges of either heat or work with the surroundings. Emphasis is placed on ideal gases because their equation of state is known. We also introduce an important property of a material, the heat capacity, that allows us to calculate the change in temperature as a function of heat. Finally, the concept of enthalpy is introduced that plays an important role in biochemical reactions.

Learning Objectives

- Be able to explain and give some examples of state properties.
- Be able to define heat and work and understand heat and work are types of energy.
- Understand that heat and work are path-dependent (not state properties).
- Understand that energy is conserved: changes in the total internal energy must be due to either heat flow into/out of the system in relation to the surroundings or work being done on/ being performed by the system in relation to the surroundings.
- Be able to calculate the change in energy, enthalpy, work and heat exchange for the compression/expansion of an ideal gas and generalize these observations to other systems.

State properties and the internal Energy (U)

In Chapter I.1, we introduced the concept of an equation of state that relates **state** properties (also called **state** variables or **state** functions). **State properties** describe the thermodynamic state of the system in terms of quantifiable observables such as temperature, pressure, volume, and number of moles for an ideal gas.

An important feature of state properties is that they are independent of the system's history. In other words, if the system is changed from some *initial* state to a *final* state, the corresponding change in any state function does not depend on the path taken from the *initial* to *final* state or on any intermediate states. An example of a state property is the **total internal energy** of the system, which we will give the symbol **U**. The total internal energy includes the translational, rotational, vibrational, electronic, and nuclear energies of all the particles, as well as the energy due to intermolecular interactions.

Figure I.2.A shows a schematic energy diagram of changing the system from some initial state *A* into some final state *B*.

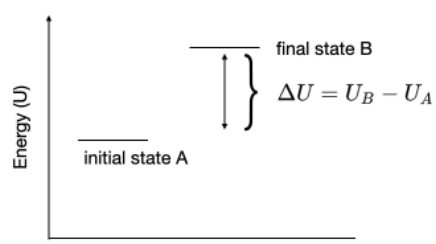

Figure I.2.A The change in internal energy (U) in going from some initial state A to some final state B does not depend on the process of going from A to B but only on the difference in energy between A and B.

Notice that the overall change in energy, ΔU does not depend on how the system was change from $A \rightarrow B$. For state variables we write the change if energy

$$\Delta U = U_f - U_i \tag{1.2.1}$$

As an example, consider a piston containing an ideal gas as shown in **Figure I.2.B**:

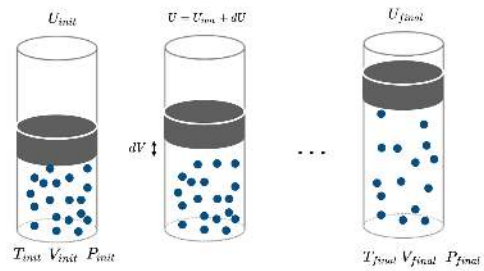

Figure I.2.B: A gas at an initial temperature, pressure, and volume has an initial energy given by U_{init}. Expanding the piston an infinitesimal amount dV, the infinitesimal change in energy is dU. After expansion of the gas the system is in a new state with a different temperature, volume, pressure. The final energy is U_{final}.

Since the molecules do not interact, the total internal energy depends only on the kinetic energy of all the gas molecules, which is related to the temperature. We now expand the piston through a series of infinitesimal steps such that the temperature of the gas changes as the volume increases. At each infinitesimal step, the infinitesimal change in the internal energy is dU. Summing over all the infinitesimal steps gives the total change in internal energy as

$$\int_{U_i}^{U_f} dU = U_f - U_i = \Delta U \tag{1.2.2}$$

Mathematically, $\mathbf{dU}$ is an *exact differential* (see Appendix), meaning that integration from an initial state to a final state is independent of the path. For any state property dz, the differential is exact, and we can write:

$$\int_{z_i}^{z_f} dz = \Delta z \tag{1.2.3}$$

Equation 1.2.3 will serve as a definition of a state property since all state properties are exact differentials because they are independent of the path.

Work

We have seen how the total internal energy is a state property of system. At this point, we might ask ourselves what would be an example of a quantity that is *not* a state property? One such example would be the work done by the system in going from an initial to a final state. **Work** is the physical activity directed towards the production of accomplishing something. Work can be *performed on* the system by the surroundings, or work can be *done by* the system on the surroundings; however, work is not an intrinsic property of the system itself and is not a state property. The amount of work performed will depend on the path taken to change the state from some initial state to some final state. As an example, we can consider a hiker walking in the Sehome Arboretum. **Figure I.2.C** shows the situation of a hiker starting at some initial position *A* and reaching the final position B by two different paths. Because these paths are different, the hikers will have performed a different amount of work in traveling from point A to point B, depending on the path taken.

Figure I.2.C: Two hikers in the Sehome Arboretum travel from the same initial point **A** to the same final point **B**, but they travel by different paths. Because the paths differ, the hikers will have performed a different amount of work in traversing from A to B.

In classical mechanics the work is defined as the force times the distance. For an infinitesimal displacement $\mathbf{dx}$ the work is

$$\delta w = F \cdot dx \tag{1.2.4}$$

where $\mathbf{F}$ is the applied force.

Note

Note: The quantity δw denotes an infinitesimal increment of work. It should be noted that work is not a state function, meaning that the differential is inexact and Equation 1.2.3 cannot be applied. Instead, we write the total work over a path taken from some initial state to some final state as:

$$w = \int_{path} \delta w \tag{1.2.5}$$

Because the differential δw is *inexact*, the integral over different paths with the same end points will be different.

In thermodynamics we generalize this concept of work to consider different types of work summarized in **Table I.2.i:**

Table I.2.i: Various types of work and their mathematical expressions

Types of work	δw	Generic force, displacement
mechanical work	F · dx	F is the force, dx is the distance
Surface work	γ · dA	γ is the surface tension, dA is the change in area
Electrical work	φ · dQ	φ is the electric potential difference, dQ is the electric charge
Gravitational work	mg · dh	mg is the gravitational acceleration, dh is the change in height
Stretching work	τ · dL	τ is the tension, dL is the change in length
Expansion (compression)	P · dV	P is the pressure, dV is the change in volume

We will mainly focus our attention on the expansion (compression) type work of work (sometimes called PV-work) of a gas in a cylinder. **Figure I.2.D** shows the situation of a gas in a cylinder with a piston with some opposing, external pressure, $\mathbf{P_{ex}}$, acting on the gas.

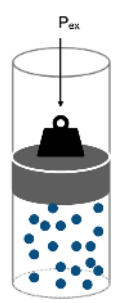

Figure I.2.D: A gas in a cylinder with a piston. An external pressure pushing down on the piston acts to oppose the expansion of the gas.

Because the external pressure is pushing against the gas (the direction of the force is pointing down on the piston), we define the work of expansion as:

$$\delta w \equiv -P_{ex} \cdot dV \tag{1.2.6}$$

where $\boldsymbol{\delta}\mathbf{w}$ is some infinitesimal amount of work and $\mathbf{dV}$ is an infinitesimal change in the volume due to expansion or compression of the piston. Equation 1.2.6 is the definition of the work of expansion/compression of a gas. Let's look at some examples for how to calculate the work for a gas in a cylinder.

Example 1: Expansion of a gas against a constant pressure

As our first example, we consider the expansion of a gas against a constant pressure. The situation is shown in **Figure I.2.E**. Initially, two masses oppose the piston. Removal of one of the masses, causes the gas in the cylinder to expand against the constant pressure due to the remaining mass from an initial volume $\mathbf{V_i}$ to a final volume $\mathbf{V_f}$.

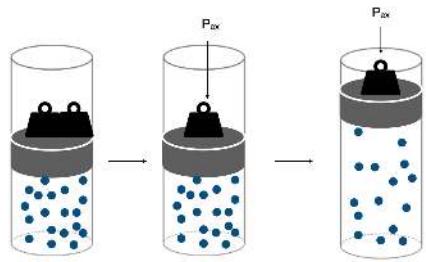

Figure I.2.E: **Expansion of a gas against a constant pressure.** A gas is held at an initial volume $\mathbf{V_i}$ by two masses on a piston. Upon removal of one of the masses, the gas expands to a new volume $\mathbf{V_f}$ against a constant pressure $\mathbf{P_{ext}}$.

The total work performed by the gas can be calculated by integrating Equation 1.2.6

$$w = -\int_{V_i}^{V_f} P_{ext} \cdot dV \tag{1.2.7}$$

Because the external applied pressure in this case is constant, it can be taken out of the integral to give:

$$\begin{aligned} w &= -P_{ext} \int_{V_i}^{V_f} dV \\ &= -P_{ext} \Delta V \end{aligned} \tag{1.2.8}$$

where $\Delta V = (V_f - V_i)$. Notice that in the last line we have used the fact that the volume is a state variable so that $\Delta V = \int dV$.

> Key Result: The work performed during the expansion of a gas against a constant external pressure is $w = -P_{ext} \cdot \Delta V$

See Practice Problem 1.

Example 2: Free expansion of a gas against a vacuum.

We now consider a slightly different situation when a gas is allowed to freely expand into a vacuum. The situation is illustrated in **Figure I.2.F.** A gas is compressed to a volume $\mathbf{V_i}$ by a piston that is held in placed by a mass, and a vacuum is created by removal of air from the space above the piston. When the mass is removed, the gas will freely expand against the vacuum to fill the cylinder to a final volume of $\mathbf{V_f}$.

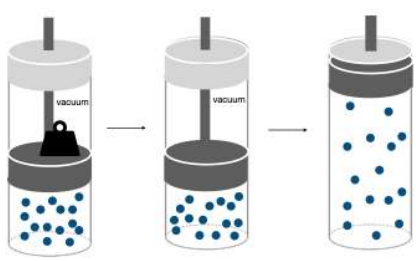

Figure I.2.F: **Expansion of a gas into a vacuum.** In the cylinder on the left, an applied pressure is used to compress a gas, and a vacuum is created in the space above the piston. When the weight is removed (middle cylinder), the compressed gas will expand from an initial volume $\mathbf{V_i}$ against the vacuum until it occupies the available space (right cylinder) with final volume $\mathbf{V_f}$.

We can again use Equation 1.2.6 to obtain:

$$w = -P_{ext} \cdot \Delta V \tag{1.2.9}$$

However, here we note that the external pressure $P_{ext} = 0$ because the gas is expanding into a vacuum. Thus, the work performed by the expansion is $w = 0$.

> Key Result: There is no work performed in the free expansion of a gas against a vacuum. $w = 0$.

See Practice Problems 2 and 3.

Work is not a state property

Notice that in the first example the work performed in expanding the gas was $w = -P_{ext} \cdot \Delta V$, but in the second example, expanding the gas to the same final volume against a vacuum resulted in no work being performed. Thus, we see that the amount of work being performed *depends on the path taken* and hence work is not a state property.

Note: In this book we adopt the convention that work being performed **on the system** by the surroundings is positive. This means that work being performed **by the system** on the surroundings will be negative by convention. Work being done on the system by the surrounding is shown on the left panel of **Figure I.2.G** with an arrow showing the direction of work pointing from the surroundings to the system. The right panel of **Figure I.2.G** shows the situation of work being performed by the system with an arrow pointing form the system to the surroundings. For the situation on the right, the work will have a negative sign.

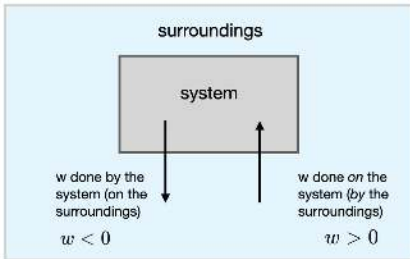

Figure I.2.G: Sign convention for work. Work performed by the system on the surrounding is depicted by an arrow pointing form the system to the surroundings and has a negative sign by convention. Work performed on the system (by the surroundings) is depicted by an arrow pointing from the surroundings into the system and is positive by convention.

Heat

Heat is another type of energy that can flow across the system boundary causing the system to change its state. As an example we can consider the situation shown in **Figure 1.2.H** of two liquids: one at 25 °C and one at 75 °C. If the two containers are brought into thermal contact, heat will flow out of the hotter (75 °C) liquid into the cooler (25 °C) liquid until thermal equilibrium is reached and both liquids reach the same final temperature.

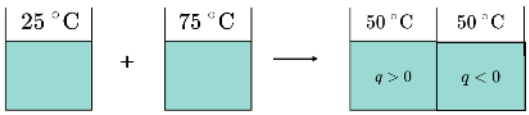

Figure I.2.H: When two systems at different temperature are brought into thermal contact, heat will flow from the hotter system into the colder system until the two systems are at thermal equilibrium.

Heat is not a state property

Just like work, heat is not a state property, and the amount of heat flow into or out of the system depends on the path taken from the initial state to the final state. We will use the symbol q to represent heat.

Note: In this book we adopt the same sign convention that we used for work. Heat being **absorbed by the system** from the surroundings will be defined as positive and represented with an arrow pointing from the surroundings to the system to indicate the direction of heat flow. On the other hand, heat **given off by the system** into the surroundings will have a negative sign and will be depicted with an arrow pointing from the system into the surroundings as shown in **Figure I.2.I**

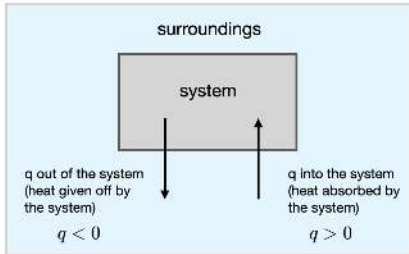

Figure I.2.I **Sign convention for heat.** Heat flowing out of the system into the surroundings (exothermic) is depicted by an arrow pointing form the system to the surroundings and has a negative sign by convention. Heat flowing into the system from the surroundings (endothermic) is depicted by an arrow pointing from the surroundings into the system and is positive by convention.

The Zeroth Law of Thermodynamics

The concept that heat flows from hot to cold establishes what is known as the **zeroth law of thermodynamics**. The zeroth law states that if system A is in thermal equilibrium with system B, and system B is in thermal equilibrium with system C, then system A must also be in thermal equilibrium with systems C. **Figure I.2.J** summarizes the zeroth law of thermodynamics.

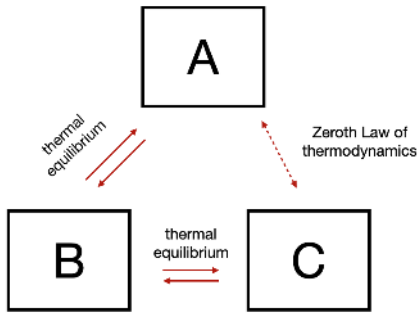

Figure I.2.J: The Zeroth Law of Thermodynamics establishes that if two systems (A and B) are in thermal equilibrium with each other and another system (C) is brought into thermal equilibrium with one of the systems (B), then C is also in thermal equilibrium with system A.

Although conceptually simple, the zeroth law is important because it formally establishes temperature as a well-defined quantity.

First Law of Thermodynamics

Having defined **heat** and **work** as types of energy, we are now in a position to state the **first law of thermodynamics**. The first law states that the change in energy (ΔU) of a system is equal to the sum of the work done on the system (w) and heat (q) put into the system. Mathematically, we can express the first law as:

$$\Delta U = q + w \tag{1.2.10}$$

We see from Equation 1.2.10 that the first law of thermodynamics is a statement about the **conservation of energy**. In order for the energy of the system to change, energy must be transferred to/from the surroundings in the form of either heat or work.

For dealing with infinitesimal changes of energy (dU) the first law can be written in differential form:

$$dU = \delta q + \delta w \tag{1.2.11}$$

Reversible vs. Irreversible Processes

Both **Example 1** and **Example 2** above are examples of an *irreversible* process, meaning that the expansion against a constant pressure or against a vacuum occurs spontaneously in one direction, but the reverse process does not occur spontaneously. This directionality of spontaneous change results from the fact that the system is not maintained at equilibrium during the expansion. When the gas is expanding, the pressure inside is not equal to the external applied pressure causing a net force to push the gas molecules into the larger volume.

Imagine the situation shown in **Figure I.2.K** where the internal pressure of the gas is equal to the external pressure applied by the piston. **Figure I.2.K** represents the system at equilibrium.

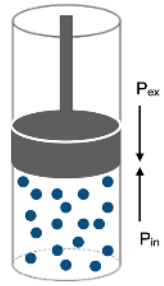

Figure I.2.K: A gas in a cylinder at equilibrium with the surroundings. At equilibrium the pressure exerted by the gas on the piston ($\mathbf{P_{in}}$) is equal and opposite to the pressure exerted by the surroundings on the piston ($\mathbf{P_{ex}}$).

We could now consider expanding the gas through a series of infinitesimal steps where equilibrium is maintained throughout the expansion by adjusting the external pressure so that it is always equal to the internal pressure of the gas. Note that in this case the external applied pressure will not be constant since it is adjusted to maintain equilibrium at each step. The situation is illustrated in **Figure I.2.L**. At each infinitesimal step, there is no net force pushing the gas to change volume in any one direction so the process is said to be **reversible**.

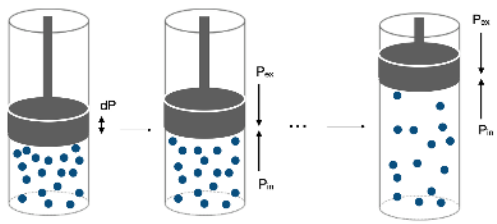

Figure I.2.L: Reversible expansion of a gas. A gas in a cylinder initially at volume $\mathbf{V_i}$ is slowly expanded to a final volume $\mathbf{V_f}$ such that the internal pressure $\mathbf{P_{in}}$ remains equal and opposite to the external pressure $\mathbf{P_{ex}}$ at each infinitesimal step. During a reversible process, quasi-equilibrium is maintained throughout.

A **reversible process** is a process which is at equilibrium at each infinitesimal step. Because work depends on the path (not a state property), the amount of work performed (and thus also the amount of heat flow) will depend on if the process occurs reversibly or irreversibly. As an example we will consider the *reversible* expansion of a gas.

Example 3: Isothermal Reversible Expansion of a Gas

Consider the isothermal reversible expansion of a gas. The situation is similar to the situation illustrated in **Figure I.2.L.** The gas is initially at a volume $\mathbf{V_i}$ and is expanded reversibly to a final volume of $\mathbf{V_f}$ while maintaining a constant temperature. Because the processes is reversible, the external pressure ($\mathbf{P_{ext}}$) is always equal to the internal pressure of the gas ($\mathbf{P_{in}}$) which can both be replaced by the variable $P = P_{in} = P_{ext}$. The work is again calculated by integrating Equation 1.2.6:

$$w = -\int_{V_i}^{V_f} P \cdot dV \tag{1.2.12}$$

Note that in this case, we cannot take the variable $\mathbf{P}$ outside of the integral because the pressure is not constant but depends on the volume during the expansion. In order to proceed, we need to know how the pressure $\mathbf{P}$ depends on the volume $\mathbf{V}$. For an ideal gas, we know the relation from the ideal gas equation of state:

$$P = \frac{nRT}{V} \tag{1.2.13}$$

Substituting this into Equation 1.2.12 gives:

$$w = -\int_{V_i}^{V_f} \frac{nRT}{V} \cdot dV \tag{1.2.14}$$

We can now use the fact that $\mathbf{n}$ (constant number of moles), $\mathbf{R}$, and $\mathbf{T}$ (isothermal = constant temperature) are constants and can be pulled out of the integral so that the integrand only depends on the volume:

$$w = -nRT \int_{V_i}^{V_f} \frac{dV}{V}$$
$$= -nRT \ln\left(\frac{V_f}{V_i}\right) \tag{1.2.15}$$

Note

We have used the integral: $\int \frac{1}{x} dx = \ln x + C$

Key Result: For the *reversible* isothermal expansion of a gas, the work performed is $w = -nRT \ln\left(\frac{V_f}{V_i}\right)$

See Practice Problems 4 and 5

Maximum Work Theorem

A comparison of Equation 1.2.15 with the result above from Equation 1.2.8 shows that the amount of work performed is different if the process is performed reversibly than if the process is performed irreversibly. **Figure I.2.M** shows a plot of the Pressure vs. the Volume for the two different expansions. A plot of P vs. V is called a PV-diagram and the work performed is the area under the curve. Notice that the work performed (area under the curve) is *greater* for the reversible expansion than the work performed for the irreversible expansion. It turns out that for any reversible process, the amount of work performed is maximized as compared to the equivalent irreversible processes. This principle is called the **maximum work theorem.**

Key result: The **maximum work theorem** states that for all processes leading from a specified initial state to some specified final state, the work performed is a maximum for a reversible process.

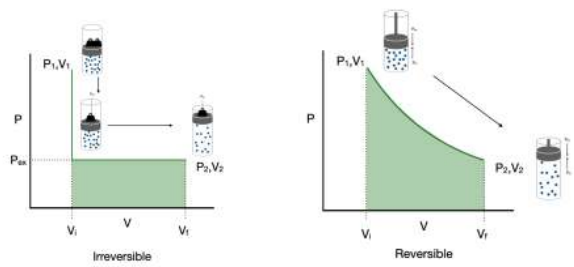

Figure I.2.M Left: The situation described in Example 1 of the irreversible expansion of a gas against a constant pressure. **Right**: The situation described in Example 3 of the reversible expansion of a gas. The area under the curve between the initial state and final state is the magnitude of the work performed by the piston. Note that that area under the curve is always greater in the reversible process.

The Heat Capacity

A useful property of a system is the **heat capacity**. The **heat capacity** is the amount of energy (heat) required to raise the temperature of a material by some infinitesimal amount dT.

$$\delta q = C \cdot dT \tag{1.2.16}$$

Equation 1.2.16 says that the amount of heat transferred to the systems (δq) is proportional to the change in temperature (dT) and the proportionality factor is the **heat capacity** of the material. How much the temperature rises in a material as it is heated depends on:

- the amount of heat delivered (q)
- the amount of substance present
- the chemical nature and physical state of the material
- the conditions under which the energy is added

Integrating both sides of Equation 1.2.16 from an initial temperature $\mathbf{T_i}$ to a final temperature $\mathbf{T_f}$ gives:

$$q = \int_{T_i}^{T_f} C \cdot dT \tag{1.2.17}$$

In general, the heat capacities $\mathbf{C}$ also depend on the temperature. However, for an ideal gas, the heat capacity is independent of temperature and can be taken out of the integral to give:

$$q = C \int_{T_i}^{T_f} dT$$

$$q = C\Delta T \tag{1.2.18}$$

where $\Delta T = T_f - T_i$ and we have used the fact that temperature is a state property.

For a gas, the heat capacity depends on whether the gas undergoes a change of state at constant volume conditions (isochoric) or at constant pressure conditions (isobaric).

We denote the heat capacity at constant volume as C_v and the heat capacity at constant pressure as C_p. At constant volume we have:

$$\delta q_v = C_v \cdot dT \tag{1.2.19}$$

and at constant pressure we have:

$$\delta q_p = C_p \cdot dT \tag{1.2.20}$$

where the subscript indicates whether we are under conditions of constant volume or pressure. Rearranging Equation 1.2.19 and Equation 1.2.20 we see that the heat capacity is given by:

$$C_v = \left(\frac{\partial q}{\partial T}\right)_V \tag{1.2.21}$$

and at constant pressure we have:

$$C_p = \left(\frac{\partial q}{\partial T}\right)_P \tag{1.2.22}$$

The heat capacities C_p and C_v are the slope of the curve of heat vs. temperature.

Enthalpy

We saw above that work and heat depend on the path taken from an initial state to a final state. Let's consider in more detail the *reversible* expansion/compression of a gas in a cylinder under the conditions of constant pressure (isobaric). From the first law of the thermodynamics we can write the change of energy as:

$$dU = \delta q_p + \delta w$$

$$dU = \delta q_p - P \cdot dV \tag{1.2.23}$$

where the subscript on δq_p indicates we are under conditions of constant pressure (isobaric) and we have used the fact that the reversible work is $\delta w = -P \cdot dV$. Rearranging Equation 1.2.23 for δq_p we have:

$$\delta q_p = dU + PdV$$

$$\delta q_p = d(U + PV) \tag{1.2.24}$$

where the second line is true because the pressure $\mathbf{P}$ is constant. We now introduce a new quantity called the **enthalpy** that gets the symbol $\mathbf{H}$ that is defined as the energy + PV:

$$H = U + PV \tag{1.2.25}$$

Equation 1.2.25 is the definition of the enthalpy. Substituting Equation 1.2.25 into Equation 1.2.24 gives:

$$\delta q_p = dH \tag{1.2.26}$$

or, upon integrating both sides:

$$q_p = \Delta H \tag{1.2.27}$$

From Equation 1.2.26 and Equation 1.2.27, we see that the change in enthalpy of the system is equal to the amount of heat gained by the system under the conditions of constant pressure.

Key result: Enthalpy is defined as: $H = U + PV$

Key result: The change in enthalpy of the system is equal to the heat gained by the system **at constant pressure**: $q_p = \Delta H$

Note

Note: Although heat is not a state property and thus depends on the path, the enthalpy H *is* a state property and so the change of enthalpy ΔH will be *independent* of the path taken.

Heat Capacity Revisited

Constant Pressure

Recall that the heat capacity for a gas depends on if the conditions are isobaric (constant P) or isochoric (constant V). At constant pressure, we copy the expression from Equation 1.2.20 above:

$$\delta q_p = C_p \cdot dT$$

We have also shown that the heat gained at constant pressure is equivalent to the change in enthalpy $\mathbf{dH}$ (Equation 1.2.26). Substituting Equation 1.2.26 into Equation 1.2.20 gives:

$$dH = C_p \cdot dT \tag{1.2.28}$$

or, rearranging in terms of C_p:

$$C_p = \left(\frac{\partial H}{\partial T}\right)_P \tag{1.2.29}$$

Equation 1.2.29 shows that the slope of the enthalpy $\mathbf{H}$ with respect to the temperature $\mathbf{T}$ at constant pressure is the heat capacity $\mathbf{C_p}$.

Constant Volume

At constant volume, we copy the expression from Equation 1.2.19 above:

$$\delta q_v = C_v \cdot dT \tag{1.2.30}$$

From the first law of thermodynamics we have the change in energy $\mathbf{dU}$ is given by: $dU = \delta q_v - PdV$ where the subscript $\delta \mathbf{q_v}$ indicates we are at constant volume conditions. Since the volume is constant, there is *no work being performed* since $\mathbf{dV = 0}$. *At constant volume conditions* we can write:

$$dU = \delta q_v \tag{1.2.31}$$

since all the change in energy at constant volume is due to heat flow (no work). Substituting Equation 1.2.31 into Equation 1.2.30 gives:

$$dU = C_v \cdot dT \tag{1.2.32}$$

or, rearranging in terms of $\mathbf{C_v}$:

$$C_v = \left(\frac{\partial U}{\partial T}\right)_V \tag{1.2.33}$$

Equation 1.2.33 shows that the slope of the energy $\mathbf{U}$ with respect to the temperature $\mathbf{T}$ at constant volume is the heat capacity $\mathbf{C_v}$.

Relation between C_v and C_p for an ideal gas

To relate $\mathbf{C_v}$ and $\mathbf{C_p}$ for an ideal gas, we begin with the definition of the enthalpy: $H = U + PV$. Recall that for an ideal gas, $PV = nRT$. Substituting the ideal gas law into the definition of the enthalpy gives:

$$H = U + nRT \tag{1.2.34}$$

Note that because we have use the ideal gas law, Equation 1.2.34 only applies to an ideal gas. Taking $\mathbf{n}$ and $\mathbf{R}$ to be constants, we can write the differential form of Equation 1.2.34 as:

$$dH = dU + nRdT \tag{1.2.35}$$

Substituting Equation 1.2.32 and Equation 1.2.28 into Equation 1.2.35 and dividing out the dT gives:

$$C_p = C_v + nR \tag{1.2.36}$$

Equation 1.2.36 relates the heat capacity at constant pressure $\mathbf{C_p}$ and the heat capacity at constant volume $\mathbf{C_v}$ for an ideal gas.

> Key result: For an ideal gas the heat capacity at constant pressure C_p and the heat capacity at constant volume C_v are related by $C_p = C_v + nR$

See Practice Problem 6

Examples

Example 1.2.1

One mole of an ideal gas in a cylinder is initially held at 12 atm of pressure and 298 K. Calculate the work done by a piston if the gas is expanded against a constant external pressure of 1.0 atm while the temperature is kept constant at 298 K.

Solution

For the expansion of an ideal gas at constant external pressure the work is given by Equation 1.2.8

$w = -P_{ext} \cdot \Delta V$

First, we use PV=nRT to find the initial and final volumes of the gas:

$V_i = \frac{1 \text{ mole} \times 0.08206 \text{ L atm K}^{-1} \text{ mol}^{-1} \times 298 \text{ K}}{12 \text{ atm}}$

$V_i = 2.038 \text{ L}$

Note that the final pressure inside the cylinder is equal to the external pressure of 1.0 atm:

$V_f = \frac{1 \text{ mole} \times 0.08206 \text{ L atm K}^{-1} \text{ mol}^{-1} \times 298 \text{ K}}{1 \text{ atm}}$

$V_f = 24.45 \text{ L}$

This gives the total work as:

$w = -1.0 \text{ atm}(24.45 \text{ L} - 2.038 \text{ L})$

$w = -22.4 \text{ L atm}$

$w = -22.4 \text{ L atm} \times \frac{101.325 \text{ J}}{1 \text{ L atm}} = -2270 \text{ Joules}$

Notice in the final step we convert L atm to Joules, which is the more conventional energy units. Also notice that the sign of the work is *negative* by our convention because the system is doing work on the surroundings (expansion).

Example 1.2.2

During the reversible isothermal compression of 3.0 moles of an ideal gas at T=291 K, the pressure changes from 3.0 atm to 5.0 atm. Calculate the work done.

Solution

We use the fact that for the *reversible* isothermal expansion of a gas, the work performed is $w = -nRT\ln\left(\frac{V_f}{V_i}\right)$ (See Equation 1.2.15).

For an ideal gas at constant number of moles and constant temperature:

$\frac{V_f}{V_i} = \frac{P_i}{P_f}$

So the work (for an isothermal process) can be expressed in terms of the pressure as:

$w = -nRT\ln\left(\frac{P_i}{P_f}\right)$

$w = -3.0 \text{ moles } \times \ 8.314 \text{ J K}^{-1} \text{ mol}^{-1} \ \times \ 291 \text{ K } \ln\left(\frac{3.0}{5.0}\right)$

$w = 3707.6 \text{ Joules}$

Note that the work is *positive* by our convention since the surroundings is doing work on the system (compression).

Example 1.2.3

The molar heat capacity at constant volume of a monatomic idea gas is 3/2 R. How much heat must be added to raise the temperature of 2.0 moles of an ideal gas by 10 °C?

Solution

We use the fact that the heat at constant volume is given by Equation 1.2.19:

$\delta q_v = C_v \cdot dT$

Integrating both sides gives:

$q_v = C_v \Delta T$

Inserting the definition of the molar heat capacity $\bar{C}_v = C_v/n$ into our expression for the heat gives:

$q_v = n\bar{C}_v \Delta T$

where n is the number of moles. Now we solve for the heat:

$q_v = 2.0 \text{ moles } \times \ \frac{3}{2} \ \times 8.314 \text{ J K}^{-1} \text{ mol}^{-1} \ \times \ 10 \text{ K}$

$q_v = 249.42 \text{ Joules}$

Practice Problems

Problem 1. Consider an experiment where a gas is compressed isothermally (constant temperature) from an initial pressure of 1.0 atm and a volume of 3.0 L to a final pressure of 4.0 atm and a volume of 1.0 L. The temperature remains constant at 20.0 °C. Calculate the work done if the compression is performed in a single step against a constant external pressure of 4.0 atm. (Report your answer in Joules).

Problem 2. You are traveling in a space ship whose cabin volume is 10.0 m^3. The air inside the cabin is maintained at 1 atm and 298 K, suitable for human space travel. Suddenly, the cabin springs a leak, allowing 1.8 moles of gas to escape into the vacuum before you are able to repair the damage. Calculate **a)** q, **b)** w, and **c)** ΔU for this process, assuming that the walls of the cabin are perfect insulators (adiabatic).

Problem 3. Calculate the work done when 50.0 g of iron reacts with hydrochloric acid:

Fe (s) + 2 HCl (aq) → $FeCl_2$ (aq) + H_2 (g)

assuming the reaction takes place in **a)** a **closed** vessel of fixed volume, and **b)** an **open** beaker at 25 °C. (The atomic mass of iron is 55.85 g/mol)

Problem 4. In *Example 3* of the main text an expression was derived for the reversible isothermal expansion of an ideal gas. Now consider the reversible expansion of a gas under **isobaric** (constant P) conditions. Show that for the reversible isobaric expansion of an ideal gas the work is

$$w = -nR\Delta T \tag{1.2.37}$$

(Hint: start with the definition of the work of expansion as $dw = -P_{ex} \cdot dV$.)

Problem 5. Derive an expression for ΔH, ΔU, w, and q for the reversible expansion of an ideal gas under the following conditions: **a)** isobaric (Hint: see Problem 4), **b)** isochoric (constant V), **c)** isothermal (Hint: see Example 3), **d)** adiabatic (no heat flow, Hint: q=0).

Problem 6. The molar heat capacity for **liquid** methanol is $\bar{C}_v = 68.624$ J K^{-1} mol^{-1}. The density of methanol is 0.792 g/mL. How much heat is released when 50 mL of liquid methanol is cooled from 60 °C to 10 °C? If the process is performed in an open container, what is the change in enthalpy ΔH? (Hint: $\bar{C}_v \approx \bar{C}_p$ for a liquid).

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1.3: Thermochemistry

In this chapter we apply the first law of thermodynamics and the concept of enthalpy introduced in Chapter I.2 to chemical reactions. At standard state conditions we can use tabulated heats of formation to calculate the change in enthalpy for any reaction. At temperatures other than standard conditions we use the temperature dependence of the enthalpy to derive an expression for the change in enthalpy of a reaction at any temperature in relation to a reference temperature.

Learning Objectives

- Understand how the first law of thermodynamics describes energy changes in chemical reactions.
- Be able to calculate the change in enthalpy for a reaction using the standard enthalpy of formation.
- Be able to calculate the change in enthalpy for a reaction at non-standard temperature from a reference value and reference temperature.

Thermochemistry: Heat of reaction

Chemical reactions almost always involve heat transfer. In an **exothermic reaction**, heat is given off during the reaction, meaning that heat is transferred from the system to the surroundings. In an **endothermic reaction**, heat is absorbed during the reaction, meaning that heat is transferred from the surroundings into the system. Recall from Chapter I.2 that under the conditions of constant pressure, the amount of heat, $\mathbf{q_p}$, is equal to the change in enthalpy. Most biochemical reactions occur under condition of constant pressure and so we can equate the change in enthalpy of a reaction $\mathbf{\Delta_r H}$ with the **heat of the reaction**. The subscript in $\mathbf{\Delta_r H}$ indicates that we are here concerned with a chemical reaction and the change of enthalpy associated with that reaction.

Since enthalpy is a state function (does not depend on the path) we can write:

$$\Delta_r H = H_{\text{products}} - H_{\text{reactants}} \tag{1.3.1}$$

Equation 1.3.1 says that the change in enthalpy of a reaction is the difference in enthalpies of the products and reactants. Consider the following reaction of the dimerization of nitrogen dioxide to form dinitrogen tetroxide:

$$2\,\mathrm{NO_2(g)} \rightarrow \mathrm{N_2O_4(g)} \qquad \text{(Reaction I)}$$

For this reaction, 2 moles of NO_2*(g)* react to form 1 mole of N_2O_4*(g)*. The molar change in enthalpy will be:

$$\Delta_r H = H(\mathrm{N_2O_4}) - 2H(\mathrm{NO_2}) \tag{1.3.2}$$

Notice that because we have two moles of reactant NO_2*(g)* for every one mole of product N_2O_4*(g)* we multiply the molar enthalpy of NO_2*(g)* by the stoichiometric coefficient 2. The absolute molar enthalpies of the individual reactants and products are not easily measured. Fortunately, because enthalpy is a state function, the change in enthalpy for the reaction will be the same no matter what path is taken from reactant to product. To calculate $\mathbf{\Delta_r H}$ for the reaction, we break the reaction into two intermediate steps, shown schematically in **Figure I.3.A**. We first break the reactants into their constituent elements at standard state and then form the products from their constituent elements in the standard state. The reason for breaking the reaction into these intermediate steps is that we can look-up tabulated values for the standard heats of formation for these reactions. We can then sum together the change in enthalpy for the individual steps to obtain the overall change in enthalpy.

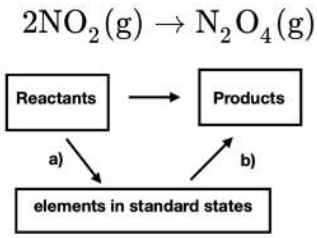


Figure I.3.A Schematic description of Hess's law. The total reaction is broken into two elementary steps. In step a) the reactants are broken into their constituent elements, and in step b) the products are formed from the constituent elements.

In our example, we first write the reaction for breaking reactant NO_2*(g)* into its elements at standard state:

$$2\ \mathrm{NO_2}\ (g) \rightarrow \mathrm{N_2}\ (g) + 2\ \mathrm{O_2}\ (g) \quad \textit{Reaction I.a}$$

We can look up the change in molar enthalpy of formation, $\Delta_f \bar{H}^\circ$, for NO_2 *(g)*. Notice that the superscript $^\circ$ indicates standard state conditions. The change in enthalpy of formation, $\Delta_f \bar{H}^\circ$ for NO_2*(g)* is the formation of one mole of NO_2 *(g)* from its constituent elements:

$$0.5\ N_2\ (g) + O_2\ (g) \rightarrow NO_2\ (g)\ \Delta_f \bar{H}^\circ\ (NO_2)$$

Notice that the $\Delta_r H^\circ$ for *Reaction I.a* above is -2 $\Delta_f \bar{H}^\circ$ for NO_2 *(g)*.

Similarly, for the products we write the reaction for the formation of products from their constituent elements:

$$N_2\ (g) + 2\ O_2\ (g) \rightarrow N_2O_4(g)\ \textit{Reaction I.b}$$

We can look up in a table the value of $\Delta_f \bar{H}^\circ$ for N_2O_4 *(g)*. Notice that the $\Delta_r H^\circ$ for *Reaction I.b* is equal to $\Delta_f \bar{H}^\circ$ for N_2O_4 *(g)*.

$$N_2\ (g) + 2\ O_2\ (g) \rightarrow N_2O_4(g)\ \Delta_f \bar{H}^\circ\ (N_2O_4)$$

Summing *Reaction I.a* and *Reaction I.b* gives the overall reaction:

$$\begin{array}{lll}
\text{a)} & 2NO_2(g) \rightarrow \cancel{N_2}(g) + \cancel{2O_2} & -2\Delta_f H(NO_2) \\
\text{b)} & \cancel{N_2}(g) + \cancel{2O_2}(g) \rightarrow N_2O_4(g) & \Delta_f H(N_2O_4) \\
\hline
\textit{Reaction I} & 2NO_2(g) \rightarrow N_2O_4(g) & \Delta_r H^\circ
\end{array}$$

and we can write the $\Delta_r H^\circ$ for the overall reaction as the sum of the two intermediate steps:

$$\Delta_r H^\circ = \Delta_f \bar{H}^\circ(N_2O_4) - 2\Delta_f \bar{H}^\circ(NO_2) \tag{1.3.3}$$

In general, we can write the change of enthalpy of a reaction at standard conditions as

$$\Delta_r H^\circ = \sum \nu \Delta_f \bar{H}^\circ(\text{products}) - \sum \nu \Delta_f \bar{H}^\circ(\text{reactants}) \tag{1.3.4}$$

where $\Delta_f \bar{H}^\circ$ are the standard molar heats of formation, ν are the stoichiometric coefficients, and the sum is over the products minus the sum over the reactants. Equation 1.3.4 is known as **Hess's law** which states that in a chemical reaction $\Delta_r H^\circ$ does not depend on the intermediate steps.

See Practice Problems 1 and 2.

Temperature dependence of heat of reaction

Hess's Law and Equation 1.3.4 allows us to calculate $\mathbf{\Delta_r H^\circ}$ at standard conditions using tabulated data for standard heats of formation. In order to calculate $\Delta_r H$ at non-standard state conditions, we need to know how $\Delta_r H$ varies with temperature. Recall that the slope of the enthalpy vs. temperature at constant pressure gives the heat capacity:

$$C_p = \left(\frac{\partial H}{\partial T}\right)_P \tag{1.3.5}$$

or, rearranging we get:

$$dH = C_p \cdot dT \tag{1.3.6}$$

For considering changes in $\Delta_r H$ with temperature, we replace $\mathbf{H}$ in Equation 1.3.6 with $\mathbf{\Delta_r H}$ to get:

$$d\Delta_r H = \Delta_r C_p \cdot dT \tag{1.3.7}$$

where $\mathbf{\Delta_r C_p}$ is the difference in heat capacities of products and reactants and is given by a Hess's Law-type analog:

$$\Delta_r C_p = \sum \nu C_p^{(\text{products})} - \sum \nu C_p^{(\text{reactants})} \tag{1.3.8}$$

where the sum is over the individual heat capacities of the products minus the reactants weighted by their stoichiometric coefficients. We integrate both sides of Equation 1.3.7 from an initial reference state to a final state:

$$\int_{T^\circ}^{T_2} d\Delta_r H = \int_{T^\circ}^{T_2} \Delta_r C_p \cdot dT$$

$$\Delta_r H_{T_2} - \Delta_r H_{T^\circ} = \int_{T^\circ}^{T_2} \Delta_r C_p \cdot dT$$

$$\Delta_r H_{T_2} = \Delta_r H_{T^\circ} + \int_{T^\circ}^{T_2} \Delta_r C_p \cdot dT \tag{1.3.9}$$

Equation 1.3.9 allows us to compute the $\mathbf{\Delta H}$ of a reaction at any non-standard temperature if we know how $\mathbf{\Delta_r C_p}$ depends on temperature. Usually this is determined experimentally and $\mathbf{\Delta_r C_p}$ is fit to a polynomial function that interpolates the data. However, if the heat capacity is independent of temperature (as it is for an ideal gas), we can take $\mathbf{\Delta_r C_p}$ out of the integral to obtain:

$$\Delta_r H_{T_2} = \Delta_r H_{T^\circ} + \Delta_r C_p \int_{T^\circ}^{T_2} dT$$

$$\Delta_r H_{T_2} = \Delta_r H_{T^\circ} + \Delta_r C_p (T_2 - T^\circ) \tag{1.3.10}$$

Equation 1.3.10 is known as **Kirchhoff's law** and is valid if $\mathbf{\Delta_r C_p}$ is independent of temperature. We can see schematically that Kirchhoff's law results from taking the reaction at T_2 and breaking it into three steps:

1. Heat/cool the reactant from T_2 to T° using $\Delta H = C_p^{reactants} \Delta T$.
2. Find $\Delta_r H$ of the reaction at T° using the tabulated heats of formation and Hess's law.
3. Cool/heat the products from T° back to T_2 using $\Delta H = C_p^{products} \Delta T$

Summing these three steps results in Kirchhoff's law Equation 1.3.10 where $\mathbf{\Delta_r C_p}$ is the difference in heat capacities between the products and reactants. **Figure I.3.B** shows a schematic of this process.

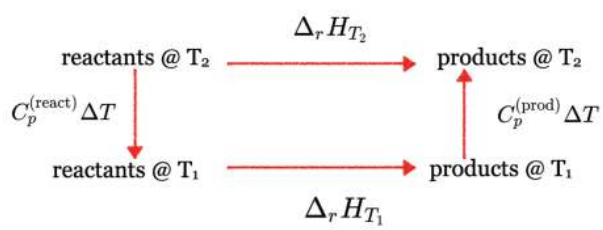


Figure I.3.B: Schematic depiction of Kirchhoff's law: The $\Delta_r H$ at some non-standard temperature T_2 is calculated in a three-step process where in step 1) the reactants are heated/cooled from T_2 to T_1. In step 2) the reactants are converted into products at a reference temperature for which $\Delta_r H$ is known. In step 3) the products are cooled/heated from the reference temperature T_1 back to the temperature of interest T_2.

See Practice Problem 3

Examples

Example 1.3.1

Methane gas is burned in a Bunsen burner. Use Hess's law to calculate $\Delta_r H^\circ$ of the combustion of methane at 298 K and 1 bar. The standard molar enthalpies of formation at 298 K and 1 bar are:

CH_4 (g)	$\Delta_f \bar{H}^\circ$ = -74.85 kJ/mol
CO_2 (g)	$\Delta_f \bar{H}^\circ$ = -393.5 kJ/mol
H_2O (g)	$\Delta_f \bar{H}^\circ$ = -241.8 kJ/mol

Solution

For the combustion of methane, the balanced chemical reaction is:

$CH_4\,(g) + 2\,O_2\,(g) \rightarrow CO_2(g) + 2\,H_2O\,(g)$

From Equation 1.3.4:

$\Delta_r H^\circ$ = [$\Delta_f H^\circ$ (CO_2)+ 2 $\Delta_f H^\circ$ (H_2O)] - [$\Delta_f H^\circ$ (CH_4) + 2 $\Delta_f H^\circ$ (O_2)]

By convention, we assigne a value of zero to $\Delta_f \bar{H}^\circ$ for elements in their most stable allotropic state, so $\Delta_f \bar{H}^\circ$ (O2) = 0 at 298 K. Using the tabulated values of $\Delta_f H^\circ$:

$$\Delta_r H^\circ = [-393.5 \text{ kJ/mol} + 2(-241.8 \text{ kJ/mol})] - (-74.85 \text{ kJ/mol})$$

$$\Delta_r H^\circ = -802.3 \text{ kJ/mol}$$

Example 1.3.2

Calculate the value of $\Delta_r \bar{H}$ for the dimerization of nitrogen dioxide to form dinitrogen tetroxide at 600 K.

$$2\,\mathrm{NO_2(g)} \rightarrow \mathrm{N_2O_4(g)} \tag{1.3.11}$$

The standard molar enthalpies of formation and molar heat capacities are:

$\mathrm{NO_2(g)}$	$\Delta_f \bar{H}^\circ$ = 33.9 kJ/mol	$\bar{C}_p$ = 37.9 J K^{-1} mol^{-1}
$\mathrm{N_2O_4(g)}$	$\Delta_f \bar{H}^\circ$ = 9.7 kJ/mol	$\bar{C}_p$ = 79.1 J K^{-1} mol^{-1}

Solution

First, we use Hess's law to find $\Delta_r \bar{H}^\circ$ of the reaction at standard conditions. From Equation 1.3.3 we have:

$$\Delta_r H^\circ = \Delta_f \bar{H}^\circ(\mathrm{N_2O_4}) - 2\Delta_f \bar{H}^\circ(\mathrm{NO_2})$$

$$\Delta_r H^\circ = 9.7 \text{ kJ/mol} - 2(33.9 \text{ kJ/mol}$$

$$\Delta_r H^\circ = -58.1 \text{ kJ/mol}$$

To find $\Delta_r \bar{H}$ at 600 K we use Equation 1.3.10. Assuming the molar heat capacity is independent of temperature we use:

$$\Delta_r \bar{H} = \Delta_r H_{T^\circ} + \Delta_r C_p (T_2 - T^\circ)$$

We find $\Delta_r C_p$ from Equation 1.3.8:

$$\Delta_r \bar{C}_p = \bar{C}_p(\mathrm{N_2O_4}) - 2\bar{C}_p(\mathrm{NO_2})$$

$$\Delta_r \bar{C}_p = 79.1 \text{ J K}^{-1} \text{ mol}^{-1} - 2(37.9 \text{ J K}^{-1} \text{ mol}^{-1})$$

$$\Delta_r \bar{C}_p = 3.3 \text{J K}^{-1} \text{ mol}^{-1}$$

$$\Delta_r \bar{C}_p = 0.0033 \text{kJ K}^{-1} \text{ mol}^{-1}$$

Finally, we compute $\Delta_r \bar{H}$ at 600 K:

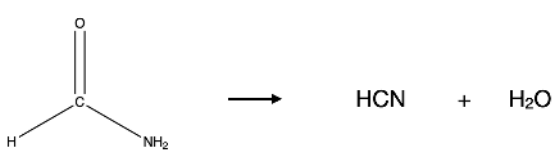
$$\Delta_r \bar{H} = -58.1 \text{ kJ/mol} + 0.0033 \text{kJ K}^{-1} \text{ mol}^{-1}(600 - 298)$$

$$\Delta_r \bar{H} = -57.1 \text{ kJ/mol}$$

Notice that $\Delta_r \bar{H}$ at 600 K is not very different from $\Delta_r H^\circ$ at 298 K. For gas-phase reactions, the change in enthalpy due to the molar heat capacities tends to cancel between the reactants and products.

Practice Problems

Problem 1. Formamide ($HCONH_2$) is used in the industrial production of hydrogen cyanide. Calculate the change in enthalpy for the dehydration of formamide to form water and hydrogen cyanide.



using the tabulated heat of formation data:

Water (g)	$\Delta_f \bar{H}^\circ$ = -241.8 kJ/mol
Formamide	$\Delta_f \bar{H}^\circ$ = -188.79 kJ/mol
Hydrogen cyanide	$\Delta_f \bar{H}^\circ$ = 129.286 kJ/mol

Problem 2. Alcohol fermentation is the process in which carbohydrates are broken down into ethanol and carbon dioxide. The overall reaction is

$C_6H_{12}O_6$ (s) → 2 C_2H_5OH (l) + 2 CO_2 (g)

Given that $\Delta_f \bar{H}^\circ$ for C_2H_5OH (l) is -277.0 kJ/mol, $\Delta_f \bar{H}^\circ$ for CO_2 (g) is -393.5 kJ/mol, and $\Delta_f \bar{H}^\circ$ for $C_6H_{12}O_6$ (s) is -1274 kJ/mol, calculate $\Delta_r H^\circ$ for the fermentation reaction shown.

Problem 3. The thermal denaturation of a globular protein with $T_m = 40$ °C has $\Delta \bar{H} = 300$ kJ/mol for unfolding at the melting temperature (T_m). The difference in the constant-pressure molar heat capacity between the denatured state (unfolded) and the folded state (N) is 9.0 kJ mol^{-1} K^{-1}. Find the molar enthalpy of denaturation at $T = 25$ °C. (Assume the heat capacity does not depend on temperature).

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1.4: The Second Law of Thermodynamics

The first law of thermodynamics (Chapter I.2) describes the conservation of energy but does not tell us anything about the direction or spontaneity of a reaction. In this chapter we introduce the concept of entropy as derived by Rudolf Clausius and formulate the second law of thermodynamics. The second law of thermodynamics is of central importance in science and tells us the direction of spontaneous change for any process. We then calculate the change of entropy for a number of exemplary cases.

Learning Objectives

- Be able to provide a thermodynamic definition of entropy as the reversible heat divided by the temperature.
- Be able to articulate the second law of thermodynamics and understand its meaning and significance: the entropy of the universe increases for all spontaneous processes and the entropy of the universe remains unchanged for an equilibrium (reversible) process.
- Be able to calculate the change in entropy for the compression/expansion of an ideal gas.
- Be able to calculate the change in entropy for the various processes: heating/cooling, phase transition, ideal mixing.

Relevance of the first law of thermodynamics

So far we have been concerning ourselves with the interconversion of heat and work as two form of energy. The first law sets a limit on the *magnitude* of energy transfer by stating that energy cannot be created or destroyed, thus any change in energy (ΔU) must be due to work (w) or heat (q) transferred to/from the system with the surroundings.

The mathematical statement of the first law, $\Delta U = q + w$ is a consequence of the fact that energy is neither created or destroyed but flows from one part of the universe (the system) to another (the surroundings) or is converted from one form to another. Although the first law limits the magnitude of energy change, it says nothing about the *directionality* of energy transfer or whether or not a process will be *spontaneous*.

Consider an example sketched in **Figure I.4.A**. A rubber ball is held some height h above a table. The ball has some potential energy due to gravity. When the ball is released, its potential energy is converted to kinetic energy, and the ball bounces off the table. Each time the ball bounces, it does not reach the same height as the previous bounce because some of the kinetic energy is being dissipated to the molecules in the table. Eventually, the ball comes to rest, and all its kinetic energy has been transferred to the molecules in the table. This increase in kinetic energy results in the temperature of the table rising, and we can say that the kinetic energy of the ball was converted into heat. This process occurs spontaneously once the ball is dropped and is indicated by the forward arrow labeled **a)** in **Figure I.4.A.**

Let's now consider the reverse process in which the ball at rest on the table absorbs the kinetic energy of the molecules in the table and converts this energy into work by spontaneously levitating against the force of gravity. During this process, the molecules in the table lose some kinetic energy, causing the temperature of the table to decrease. Thus, heat has been converted to work needed to raise the ball to some height h. This process is indicated by the reverse arrow labeled **b)** in **Figure I.4.A.**

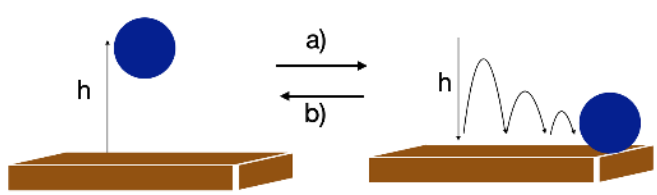

Figure I.4.A: **a)** A ball suspended at a height h will spontaneous bounce off the table, converting its potential energy into kinetic energy and heat. **b)** In the reverse process, the ball absorbs heat from the table, converts this heat to kinetic energy to elevate a distance h above the table.

In both cases, the total energy is conserved in compliance with the first law of thermodynamics, but the first process occurs spontaneously, while the second process does not occur spontaneously. For the second process to occur, all the molecules in the table would need to spontaneously synchronize their random motion and transfer their kinetic energy to the ball. If there are sufficiently many molecules in the table, the probability for these molecules to synchronize their random motion is extremely unlikely. The second law of thermodynamics will set a limit on the *direction* of energy transfer, such that case 1 (potential energy → kinetic energy → heat) is spontaneous, but that the reverse process, case 2 (heat → kinetic energy → potential energy), will not happen.

Thermodynamic Definition of Entropy

In this chapter we consider the **thermodynamic definition of entropy** as formulated by Rudolf Clausius (1822-1888). Let's consider again the *reversible* heat transfer of a system going from some initial state (P_1, T_1, V_1) to some final state (P_2, T_2, V_2). From the first law of thermodynamics we have $dU = \delta w + \delta q_{rev}$ where the subscript on δq_{rev} indicates that the heat transfer we are considering is for a reversible process. Recall that both work and heat are not state properties and thus depend on the path taken. For reversible work we know that $\delta w = -P \cdot dV$. Also, we have that $dU = C_v \cdot dT$ from Chapter I.2 (See **Equation I.2.31**). Inserting these into the equation for the first law (**Equation I.2.9**) and solving for the unknown δq_{rev}, we have:

$$\delta q_{rev} = C_v \cdot dT + P \cdot dV \tag{1.4.1}$$

Equation 1.4.1 is an expression for the reversible heat. Integrating both sides of Equation 1.4.1 from an initial state i to a final state f:

$$q_{rev} = C_v \Delta T + \int_i^f P \cdot dV \tag{1.4.2}$$

We see that the reversible heat depends on the path due to the work term $P \cdot dV$. This is not surprising because the heat is not a state property.

Following Clausius, let's now consider a slightly different quality $\frac{\delta q_{rev}}{T}$. From Equation 1.4.1, dividing both side by T, we have:

$$\frac{\delta q_{rev}}{T} = C_v \cdot \frac{dT}{T} + \frac{P}{T} \cdot dV \tag{1.4.3}$$

Using the fact that $\frac{P}{T} = \frac{nR}{V}$ for an ideal gas, we can integrate both sides of Equation 1.4.3 *independently of the path!*

$$\begin{aligned} \int_i^f \frac{\delta q_{rev}}{T} &= C_v \int_i^f \frac{dT}{T} + nR \int_i^f \frac{dV}{V} \\ &= C_v \ln\left(\frac{T_f}{T_i}\right) + nR \ln\left(\frac{V_f}{V_i}\right) \end{aligned} \tag{1.4.4}$$

Therefore, we conclude that the quantity $\frac{\delta q_{rev}}{T}$ is a state property! We define this new state property **S**, so that:

$$dS = \frac{\delta q_{rev}}{T} \tag{1.4.5}$$

and, integrating both sides from an initial state i to a final state f:

$$\int_i^f dS = \int_i^f \frac{\delta q_{rev}}{T} = \Delta S \tag{1.4.6}$$

where $\Delta S = S_f - S_i$. The state property **S** is called the **entropy**.

> Key Result: The infinitesimal change in entropy is defined as $dS = \frac{\delta q_{rev}}{T}$. Notice the entropy is defined in terms of the *reversible* heat.

From Equation 1.4.4 and **Equation I.4.**1.4.6 **we have that for the expansion of an ideal gas, the change in entropy is:**

$$\Delta S = C_v \ln\left(\frac{T_2}{T_1}\right) + nR \ln\left(\frac{V_2}{V_1}\right) \tag{1.4.7}$$

Notice that Equation 1.4.7 is valid for any expansion/compression of an ideal gas regardless of whether the process was carried our reversibly or irreversibly because entropy is a state property.

> Key Result: The change in entropy for the reversible or irreversible expansion/compression of an ideal gas is: $\Delta S = C_v \ln\left(\frac{T_2}{T_1}\right) + nR \ln\left(\frac{V_2}{V_1}\right)$

Note

Note: Clausius was able to find a new state property by dividing the reversible heat $\delta \mathbf{q_{rev}}$ (not a state property) by the temperature. The temperature in this case is called an *integrating factor* that makes the integral $\int \frac{\delta q_{rev}}{T}$ exact.

Entropy for an irreversible vs. reversible process

So far, we have defined $\mathbf{dS}$ from Equation 1.4.5 in terms of the *reversible* heat $\delta \mathbf{q_{rev}}$. Note that the subscript *rev* indicates the process is reversible. While the entropy is a state property (independent of the path), the heat is not and will depend on the path. Therefore, the equivalence in Equation 1.4.5 is valid only for a reversible path. Even though the entropy is the same, the magnitude of the heat transfer is greater for the *reversible* process, so we have:

$$\delta q_{rev} > \delta q_{irrev} \tag{1.4.8}$$

Dividing both sides of Equation 1.4.8 by $\mathbf{T}$ and inserting the definition of entropy from **Equation I.4.**1.4.5 **gives:**

$$dS > \frac{\delta q_{irrev}}{T} \tag{1.4.9}$$

While the entropy change would be the same for an irreversible or reversible process, $\Delta S = \Delta S_{rev} = \Delta S_{irrev}$, the heat flow is not the same, so for any irreversible process $\Delta S > q_{irrev}/T$.

Key Result: For any process the change in entropy is $dS \geq \frac{\delta q}{T}$. The equality holds only for a *reversible* process, whereas the inequality holds if the process is *irreversible*.

Entropy of the Surroundings

So far we have only focused on the entropy of the system. In this section we will consider the change in entropy of the *surroundings*. First, we notice that any heat gained (or lost) by the system must have come from (or gone to) the surroundings:

$$\delta q_{sys} = - \delta q_{surr} \tag{1.4.10}$$

We consider the surroundings as an infinitely large reservoir. *Any* amount of heat transferred to the surroundings (δq_{surr}) will only lead to an infinitesimally small change in the reservoir, given that the reservoir is sufficiently large. Infinitesimally small changes are characteristic of a *reversible* process, so any heat transfer from the perspective of the surroundings can be treated as reversible, since it will have the same effect on the surroundings as a reversible process. Thus, from **Equation I.4.**1.4.5**,** we can always write for the surroundings:

$$dS_{surr} = \frac{\delta q_{surr}}{T} \tag{1.4.11}$$

or

$$\Delta S_{surr} = \frac{q_{surr}}{T} \tag{1.4.12}$$

The Second Law of Thermodynamics

The Second Law of Thermodynamics deals with the change in entropy of the universe. The change of entropy of the universe is:

$$\Delta S_{universe} = \Delta S_{sys} + \Delta S_{surr} \tag{1.4.13}$$

Substituting Equation 1.4.12 into Equation 1.4.13 gives:

$$\Delta S_{universe} = \Delta S_{sys} + \frac{q_{surr}}{T} \tag{1.4.14}$$

For a reversible process we can substitute the equality of Equation 1.4.5 to give:

$$\Delta S_{universe} = \frac{q_{rev}}{T} + \frac{q_{surr}}{T}$$

$$\Delta S_{universe} = 0 \tag{1.4.15}$$

where the last line follows from the fact that the heat gained by the surroundings is equal and opposite to the heat lost by the system ($q_{sys} = -q_{surr}$). We see that for a *reversible process* the change of entropy of the universe is zero.

Now, if the processes is *irreversible* we have to use the inequality of Equation 1.4.9. Substituting this into **Equation I.4.**1.4.14 gives for an *irreversible processes:*

$$\Delta S_{universe} > \frac{q_{rev}}{T} + \frac{q_{surr}}{T}$$

$$\Delta S_{universe} > 0 \tag{1.4.16}$$

where again the last line follows from the fact that the heat gained by the surroundings is equal and opposite to the heat lost by the system ($q_{sys} = -q_{surr}$). Thus, for an *irreversible* process, the change in entropy of the universe must be greater than zero.

This result is known as the **Second Law of Thermodynamics** which can be expressed in mathematical form as:

$$\Delta S_{universe} \geq 0 \tag{1.4.17}$$

Where the equality holds if the process is reversible, and the inequality applies if the process is irreversible.

Key Result: The entropy of an *isolated* system always increases in an irreversible process and remains unchanged in a reversible process. It can never decrease. This statement is known as the **second law of thermodynamics** and is expressed mathematically as: $\Delta S_{universe} \geq 0$

See Practice Problem 1

Some applications of calculating the entropy

Having defined the second law of thermodynamics, we will now consider some specific examples of calculating ΔS.

Example 1: Cyclic process

A cyclic process is any series of steps that returns the system to its original state. Because entropy is a state property:

$$\Delta S = \sum_i \Delta S_i = 0 \tag{1.4.18}$$

where ΔS_i is the entropy change for the ith step and the change in entropy for the cycle is zero because S is a state property.

Example 2: Reversible adiabatic process

An **adiabatic process** is a process in which no heat is exchanged between the system and surroundings. For a reversible adiabatic processes, $\delta q_{rev} = 0$ since no heat is exchanged. Integrating Equation 1.4.5 gives:

$$\Delta S = \int \frac{\delta q_{rev}}{T}$$

$$\Delta S = 0 \tag{1.4.19}$$

where the second line follows for an adiabatic process ($\delta q_{rev} = 0$).

Example 3: Reversible phase change at constant T and P

At the phase transition temperature, both the forward and reverse reactions are in equilibrium. Therefore, at precisely the phase transition temperature, the phase transition is reversible. For example, the freezing of liquid water is reversible (at equilibrium) at the phase transition temperature of 0 °C.

$$H_2O\ (l) \leftrightarrow H_2O\ (s)\ T = 0\ °C$$

From Equation 1.4.5 at constant **T** we can write:

$$\Delta S = \frac{q_{rev}}{T} \tag{1.4.20}$$

Recalling, that under constant pressure conditions, $q_p = \Delta H$, we can write, for a reversible phase transition:

$$\Delta S = \frac{\Delta H}{T} \tag{1.4.21}$$

where ΔH is the change in enthalpy of the associated phase transition.

Example 4: Constant pressure (reversible) heating (no phase change)

Consider heating a substance from some initial temperature $\mathbf{T_i}$ to some final temperature $\mathbf{T_f}$ reversibly. We assume that there are no phase transitions between $\mathbf{T_i}$ and $\mathbf{T_f}$. At constant pressure, we begin with the definition of the heat capacity:

$$\delta q = C_p \cdot dT \tag{1.4.22}$$

Substituting Equation 1.4.22 into the definition of the entropy, Equation 1.4.5 gives:

$$\begin{aligned} \Delta S &= \int_{T_i}^{T_f} C_p \frac{dT}{T} \\ \Delta S &= C_p \int_{T_i}^{T_f} \frac{dT}{T} \\ \Delta S &= C_p \ln\left(\frac{T_f}{T_i}\right) \end{aligned} \tag{1.4.23}$$

where we have assumed the heat capacity $\mathbf{C_p}$ is independent of temperature.

See Practice Problem 2

Example 5: Ideal mixing of two inert gases at constant T and P

In this example, we consider the mixing of two ideal gases. Consider the situation shown in **Figure I.4.B.** Two gases of different chemical identities A and B are contained in two flasks of volume V_A and V_B. Let n_A be the number of moles of gas A, and n_B be the number of moles of gas B. When the stopcock separating the two flasks is open, the gases will spontaneously mix so that both gases fill the final volume of $V = V_A + V_B$. Similarly, the total number of moles of the combined system is $n = n_A + n_B$.

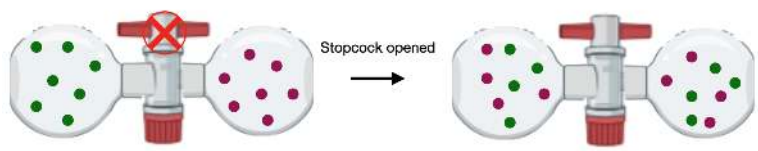

Figure I.4.B. Ideal mixing of two inert gases. When the stopcock is opened, gas A initially contained in a volume VA with nA number of moles spontaneously mixes with nB moles of gas B initially contained in volume VB. The final volume is V=VA + VB and the total number of moles of gas is n = nA + nB.

The overall change in entropy for the mixing of two gases is the sum of the change in entropy for each gas:

$$\Delta S = \Delta S_A + \Delta S_B \tag{1.4.24}$$

At constant $\mathbf{T}$, the entropy change for gas A is given by the second term in Equation 1.4.7:

$$\Delta S_A = n_A R \ln\left(\frac{V}{V_A}\right) \tag{1.4.25}$$

and similarly for gas B:

$$\Delta S_B = n_B R \ln\left(\frac{V}{V_B}\right) \tag{1.4.26}$$

Substituting Equation 1.4.25 and Equation 1.4.26 into **Equation I.4.**1.4.24**gives:**

$$\Delta S = n_A R \ln\left(\frac{V}{V_A}\right) + n_B R \ln\left(\frac{V}{V_B}\right) \tag{1.4.27}$$

Using the fact that the total volume is $V = nRT/P$ for an ideal gas, Equation 1.4.27 becomes:

$$\begin{aligned} \Delta S &= -n_A R \ln\left(\frac{n_A}{n}\right) - n_B R \ln\left(\frac{n_B}{n}\right) \\ \Delta S &= -n_A R \ln(x_A) - n_B R \ln(x_B) \end{aligned} \tag{1.4.28}$$

where x_A is the mole fraction of gas A defined as $x_A = n_A/n$ and x_B is the mole fraction of gas B. Notice that because the mole fraction is less than one, ΔS for mixing of two ideal gases is be greater than zero.

See Practice Problem 3

Examples

Example 1.4.1

What is the change in entropy when one mole of liquid water is heated from its freezing point to its boiling point in an open container. The molar heat capacity of liquid water is 75.38 J mol^{-1} K^{-1}.

Solution

Since the water is being heated at constant pressure, we use Equation 1.4.23:

$$\Delta S = C_p \ln\left(\frac{T_f}{T_i}\right)$$

or, after introducing the definition of the molar heat capacity:

$$\Delta S = n\bar{C}_p \ln\left(\frac{T_f}{T_i}\right)$$

Substituting in the values of the initial and final temperatures gives:

$$\Delta S = 1 \text{ mole } \times 75.38 \text{ J mol}^{-1} \text{ K}^{-1} \ \ln\left(\frac{373.15 \text{ K}}{273.15 \text{ K}}\right)$$

$$\Delta S = 23.52 \text{ J K}^{-1}$$

Notice that the entropy increases upon heating.

Example 1.4.2

Show that for a *reversible* adiabatic expansion of an ideal gas $\Delta S = 0$, but for an *irreversible* adiabatic expansion of an (isolated) ideal gas $\Delta S > 0$

Solution

For a *reversible* process, (see Example 2), we have from the definition of the entropy:

$$\Delta S = \int \frac{\delta q_{rev}}{T}$$

Since the process is adiabatic, there is no heat exchange with the surroundings, meaning that $q_{rev} = 0$ and

$$\Delta S = 0$$

For an *irreversible* process, we have from Equation 1.4.9:

$$\Delta S > \int \frac{\delta q_{irrev}}{T}$$

Again, the process is adiabatic so there is no heat exchange with the surroundings, meaning that $q_{irrev} = 0$, giving:

$$\Delta S > 0$$

Note that this result applies only to an isolated system. It is possible to reduce the entropy of the system with the aid of the external surroundings. The entropy change of both system + surroundings taken together, however, cannot decrease.

Practice Problems

Problem 1. One mole of an ideal gas is isothermally expanded from 5.0 L to 10 L at 300 K. Compare the entropy changes for the system, surroundings, and the universe if the process is carried out **a)** reversibly, and **b)** irreversibly against a constant external pressure of 2.0 atm.

Problem 2. The molar enthalpy of vaporization of water is $\Delta H_{vap} = 40.6$ kJ/mol at the boiling temperature of 100 °C. **a)** What is the value of ΔS when one mole of liquid water is converted to a gas at 100 °C. **b)** What is ΔS for the conversion of one mole of liquid water to a gas at a temperature of 120 °C. The molar heat capacity of liquid water is 75.38 J mol^{-1} K^{-1} and the molar heat

capacity of water vapor is 36.57 J mol^{-1} K^{-1}.

Hint consider ΔS of heating/cooling from *Example 4 (see Example 1.4.1)* in addition to ΔS for a phase transition in *Example 3*.

Problem 3. Suppose you have a compartment that contains 1 mole of NO and a second compartment that contains 0.3 moles of O_2. Calculate the change in entropy ΔS of mixing the two gasses together. Assume the gasses do not react and are ideal gasses.

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1.5: The Boltzmann Distribution and the Statistical Definition of Entropy

In this chapter we introduce the statistical definition of entropy as formulated by Boltzmann. This allows us to consider entropy from the perspective of the probabilities of different configurations of the constituent interacting particles in an ensemble. This conception of entropy led to the development of modern statistical thermodynamics. For systems that can exchange thermal energy with the surroundings, the equilibrium probability distribution will be the Boltzmann distribution. Knowing this equilibrium probability distribution allows us to calculate various thermodynamics observables.

Learning Objectives

- Understand the statistical definition of entropy and be able to use the number of available microstates of the system to calculate the entropy.
- Understand how all thermodynamic properties ultimately can be traced back to the number of occupied microstates and their probability.
- Be able to use the Boltzmann distribution to calculate the ratio of occupied energy states at a given temperature and the probability of a particle being in any particular energy level.

Microstates and Boltzmann Entropy

In our discussion of thermodynamics, thus far we have neglected the molecular nature of reality: that molecular systems are composed of many interacting atoms. In this chapter we will explore how the large number of atoms in a macromolecular system gives rise to thermodynamic observables. We have seen that the thermodynamic state of the system can be designed by a set of state properties (P, V, n, T, U, H, S). The macroscopic state of the system is called a **macrostate.** In contrast, a **microstate** is a specific configuration of all the constituent atoms that make up the system.

As an illustrative example of a microstate is to consider the number of ways any given state may be constructed. Consider the question of how many unique ways there are to arrange four particles as shown in **Figure I.5.A** into two bins.

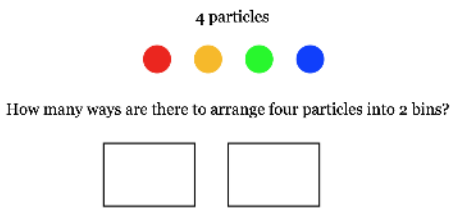

Figure I.5.A Consider the number of possible ways to arrange four particles into two bins. The are a total of 16 unique configurations as shown in **Figure 1.5.B**

It turns out that there are 16 different ways we could arrange four particles into two bins as shown in **Figure I.5.B.**

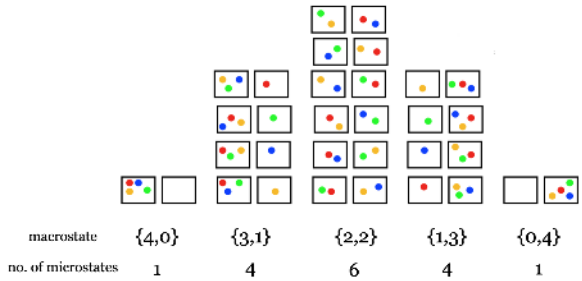

Figure I.5.B The total number of ways to arrange four particles into two bins. There are five possible macrostates defined by the number of particles in each bin ({4,0}, {3,1}, {2,2}, {1,3},{0,4}). Whereas the macrostate {4,0} can only be achieved in one way, the macrostate {2,2} can be achieved in six different ways.

If we define each unique arrangement of the four particles as a microstate, then there are 16 possible microstates. The overall number of particles in each bin defines the macrostate, so from **Figure I.5.B** we see that there are four macrostates, and that the number of microstates making up the different macrostates is not equal. The macrostate that corresponds to a configuration with two particles in each bin ({2,2}) has the highest number of microstates (6), while the macrostates that correspond to configurations with all the particles in one of the bins ({4,0} and {0,4}) have the fewest number of microstates (1).

In general, we can calculate the number of microstates in a given distribution according to the formula:

$$W = \frac{N!}{n_1!n_2!,\ldots} \tag{1.5.1}$$

where W is the number of ways of distributing N total particles into bins, and n_1, n_2, … is the number of particles in each bin.

Example 1.5.1

Example 1: Use Equation 1.5.1 to calculate the number of ways to arrange four particles into two bins such that there are two particles in each bin ({2,2}).

Solution

We have four total particles, so $N = 4$. To calculate the number of ways to arrange four particles into a configuration with $n_1 = 2$ and $n_2 = 2$ we have:

$$W = \frac{4!}{2!2!}$$
$$W = \frac{4 \times 3 \times 2 \times 1}{2 \times 1 \times 2 \times 1}$$
$$W = 6 \tag{1.5.2}$$

We calculate that there are six different microstates corresponding to the macrostate with two particles in each bin ({2,2}), which agrees with the drawing in **Figure I.5.B.**

See Practice Problems 1 and 2

The **Boltzmann definition of entropy** relates the entropy as the natural logarithm of the number of microstates, **W**:

$$S = k_B \ln W \tag{1.5.3}$$

where $\mathbf{k_B}$ is a constant of proportionality known as Boltzmann's constant:

$$k_B = 1.380658 \times 10^{-23}\ \mathrm{J\ K^{-1}} \tag{1.5.4}$$

Since W is unitless, the units on $\mathbf{k_B}$ give entropy the correct thermodynamic units, and the value of Boltzmann's constant ensures that the statistical definition of entropy from Equation 1.5.3 is in agreement with the thermodynamic definition of entropy from Chapter I.4.

The Boltzmann Distribution

Equation 1.5.3 allows us to compute the entropy **S** for any given configuration of particles. Looking again at **FigureI.5.B**, we see that the configuration with two particles in each bin ({2,2}) has more accessible microstates (W=6) than a configuration with all four particles in one bin ({4,0} with W=1). Therefore, the macrostate with configuration {2,2} has a greater entropy than the macrostate with configuration {4,0}.

As a second example consider a system of four particles with two discrete energy levels as shown in **Figure I.5.C.**

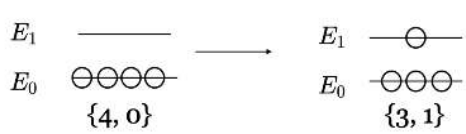

Figure I.5.C: Two level system. Consider a system of four particles each with two accessible energy levels: a ground state energy with energy E_0 and a single excited state with energy E_1. If all particles are in the ground state, the total energy is $E = 4E_0$ as shown on the left. There is only one way to have all the particles in the ground state so W=1. If one of the particles is in the excited state, the total energy is $E = 3E_0 + E_1$ and W=4 since there are four possible ways to have a configuration with one particle in the excited state.

The particles can either be at the ground state energy E_0 or the excited state energy E_1. For example, the left side of **Figure I.5.C** shows the microstate with all four particles in the ground state. In this state, the total energy is $E = 4E_0$, and there is only one possible microstate (W=1). The configuration on the left corresponds to the macrostate with the lowest entropy. The right side of

Figure I.5.C shows three particles in the ground state and one particle in the excited state. In the configuration on the right, the total energy is $E = 3E_0 + E_1$ and there are four possible microstates since any one our of the four particles could be in the excited state (W=4). The entropy of each of these configurations can be calculated from Equation 1.5.3 using **Equation I.5.**1.5.1**.**

We now ask ourselves, is there a dominant configuration of particles? The dominant configuration will be the configuration that maximizes $\mathbf{W}$, or equivalently the configuration that maximizes the entropy $\mathbf{S}$. Since W is a function of all the n_i, the dominant configuration (that maximizes S) will be given by:

$$\left(\frac{\partial \ln W}{\partial n_i}\right) = 0 \tag{1.5.5}$$

where $\mathbf{n_i}$ is the number of particles in energy level i.

In solving for the maximum entropy condition of Equation 1.5.5, we must also enforce the constraint that the total energy of the system is:

$$E_{total} = \sum_i n_i E_i \tag{1.5.6}$$

and that the total number of particles $\mathbf{N}$ is:

$$N = \sum_i n_i \tag{1.5.7}$$

Maximizing a function subject to constraints can be done using a mathematical technique called *Lagrange's method of undetermined multipliers*. The result is that at a given temperature $\mathbf{T}$, the fraction of particles with energy E_i is given by:

$$\frac{n_i}{N} = \frac{e^{-E_i/k_BT}}{q}. \tag{1.5.8}$$

Equation 1.5.8 is known as the **Boltzmann distribution** and gives the probability of a particle occupying a given energy level $\mathbf{E_i}$ at temperature $\mathbf{T}$. The term in the denominator $\mathbf{q}$ is called the **partition function** and is given by:

$$q = \sum_i e^{-E_i/k_BT} = e^{-E_0/k_BT} + e^{-E_1/k_BT} + \ldots \tag{1.5.9}$$

The ratio of the number of particles (populations) between any two energy levels $\mathbf{n_1}$ and $\mathbf{n_2}$ is given by:

$$\frac{n_2}{n_1} = e^{-\Delta E/k_BT} \tag{1.5.10}$$

where $\mathbf{\Delta E}$ is the difference in energy between the two energy levels $\Delta E = E_2 - E_1$

> Key Result: The Boltzmann distribution gives the distribution of particles that corresponds to the most probable populations and is given by the formula:
>
> $$\frac{n_i}{N} = \frac{e^{-E_i/k_BT}}{\sum_i e^{-E_i/k_BT}}.$$
>
> The ratio of the number of particles between any two energy levels is
>
> $$\frac{n_2}{n_1} = e^{-\Delta E/k_BT}.$$

See Practice Problem 3

Degenerate Energy Levels

Equation 1.5.8 and Equation 1.5.10 were derived without considering the case of **degeneracy**. In practice, there may be two or more distinct states with the same energy. Two distinct states with the same energy are called **degenerate** energy levels. We specify the degeneracy of a particular energy level i with the symbol $\mathbf{g_i}$**. Figure I.5.D** shows a situation where the ground state is not

degenerate ($\mathbf{g_0 = 1}$), and the excited state has a degeneracy of $\mathbf{g_1 = 2}$ because there are two possible states with the same energy E_1.

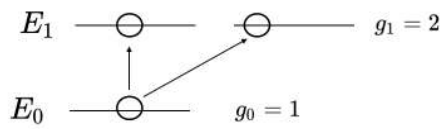

Figure I.5.D. Degenerate energy levels: In this example there is only one ground state level with energy E_0, but there are two possible excited state energy levels with the same energy E_1. A particle in the ground state can be excited into either of the two excited states. Because these two states have the same energy they are called **degenerate** energy levels. In this example, the excited state E_1 has a degeneracy $g_1 = 2$ while the ground state has a degeneracy $g_0 = 1$.

For the case where there are degenerate energy levels, we generalize the probabilities given by Equation 1.5.8 and **Equation I.5.** 1.5.10. The general Boltzmann distribution will be:

$$\frac{n_i}{N} = \frac{g_i e^{-E_i/k_BT}}{q}. \tag{1.5.11}$$

with g_i the degeneracy of state i and the partition function:

$$q = \sum_i g_i e^{-E_i/k_BT} = g_0 e^{-E_0/k_BT} + g_1 e^{-E_1/k_BT} + \ldots \tag{1.5.12}$$

The ratio of the number of particles (populations) between any two energy levels $\mathbf{n_1}$ and $\mathbf{n_2}$ is given by:

$$\frac{n_2}{n_1} = \frac{g_2}{g_1} e^{-\Delta E/k_BT} \tag{1.5.13}$$

Examples

Example 1.5.2

Calculate the number of microstates for 8 particles to be arranged into 4 bins such that the configuration of particles is {6,0,0,2}

Solution

The number of microstates is given by Equation 1.5.1:

$W = \frac{N!}{n_1!n_2!,\ldots}$

$W = \frac{8!}{6!0!0!2!}$

$W = 28$

Example 1.5.2

a) Write an expression for the partition function for the two-level system shown in Figure I.5.D with energy in the ground state E_0 and degeneracy $g_0 = 1$ and energy in the excited state E_1 and degeneracy $g_1 = 2$. **b)** Let the energy of the ground state be $E_0 = 0$ and the energy in the excited state be $E_1 = 2.41$ kJ mol^{-1}. Evaluate the value of the partition function at T=298 K. **c)** What is the probability to find the system in the ground state at T=298 K?

Solution

a) Using Equation 1.5.12, for a two-level system we have:

$q = g_0 e^{-E_0/k_BT} + g_1 e^{-E_1/k_BT}$

and substituting the values of the degeneracy gives:

$q = e^{-E_0/k_BT} + 2e^{-E_1/k_BT}$

b) When energies are given in molar units (kJ mol^{-1}), it is useful to use the gas constant $R = 8.314$ J mol^{-1} K^{-1} $= N_A k_B$. The the partition function becomes:

$q = 1 + 2e^{-2410/(8.314 \cdot 298)}$

$q = 1.756$

c) The probability for the system to be in the ground state is given by Equation 1.5.11:

$p_0 = \frac{g_0 e^{-E_0/k_B T}}{q}$

$p_0 = \frac{1}{q}$

Inserting the value of q from part b):

$p_0 = 0.569$

Practice Problems

Problem 1. Calculate the total number of ways to arrange 16 particles into four bins such that there are four particles in each bin ({4,4,4,4}). What can you say about the entropy of this macrostate as compared to any other macrostate? Explain your answer.

Problem 2. Suppose we have 8 particles that are arranged into 4 bins. Calculate the number of microstates (W) for each of the following distributions of particles: **a)** {5,1,1,1}, **b)** {4,2,2,0}, **c)** {2,6,0,0}

Problem 3. Suppose a protein can exist in two conformations that have an energy difference of 2.0 kJ/mol. What is the estimated ratio of the two conformations at 300 K?

Problem 4.

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1.6: The Gibbs and Helmholtz Energy

In this chapter we introduce two additional state properties: the Gibbs energy and the Helmholtz energy. These additional variables are useful for allowing us to determine the direction of spontaneous change without having to directly calculate the change in entropy of the universe from the second law. The Gibbs energy has particular importance in biochemistry. We next introduce the (intensive) molar Gibbs and the chemical potential. Emphasis is placed on the thermodynamics of mixtures and phase separations.

Learning Objectives

- Know the thermodynamic definitions of the Gibbs energy and Helmholtz energy and why these two properties are important.
- Understand that the change in Gibbs energy has both an enthalpic and entropic contribution.
- Know that at constant pressure and temperature the Gibbs energy decreases for a spontaneous process, and at constant volume and temperature the Helmholtz energy decreases for a spontaneous process.
- Be able to identify the fundamental differentials for dU, dH, dG, and dA and how these can be used to arrive at thermodynamic relationships.

The Gibbs and Helmholtz energy

The first law of thermodynamics (Chapter I.2) accounts for the conservation of energy and the second law of thermodynamics (Chapter I.4) determines the spontaneity. Together, these laws should allow us to deal with any biophysical problem, but their direct application is not always convenient. In addition to our current set of state properties it is useful to define two additional state properties: the **Gibbs energy** and the **Helmholtz energy**.

Recall that we have already define the **total internal energy** $\mathbf{U}$ and the **enthalpy** $\mathbf{H = U + PV}$. Similarly, we now define the **Gibbs energy:**

$$G = H - TS \tag{1.6.1}$$

The Gibbs energy ($\mathbf{G}$) is the enthalpy minus the product of the temperature and the entropy.

We also define the **Helmholtz energy:**

$$A = U - TS \tag{1.6.2}$$

The Helmholtz energy ($\mathbf{A}$) is the total internal energy minus the product of the temperature and the entropy.

If we are interested in infinitesimal changes in the Gibbs or Helmholtz energies we can consider the differential form of Equation 1.6.1 and Equation 1.6.2

$$dG = dH - TdS - SdT \tag{1.6.3}$$

$$dA = dU - TdS - SdT \tag{1.6.4}$$

Note

Note: In the differential form of Equation 1.6.4 we have made use of the product rule for derivatives: $d(AB) = BdA + AdB$

The Significance of the Gibbs and Helmholtz energy

In order to understand why the Gibbs and Helmholtz energies are important, we need to recall the second law of thermodynamics which states:

$$dS_{sys} + dS_{surr} \geq 0 \tag{1.6.5}$$

Recalling that for the surroundings:

$$\begin{aligned} dS_{surr} &= \frac{\delta q_{surr}}{T} \\ &= -\frac{\delta q_{sys}}{T} \end{aligned} \tag{1.6.6}$$

We can substitute Equation 1.6.6 into Equation 1.6.5 to obtain an alternative expression for the second law in terms of only the system variables:

$$dS - \frac{\delta q_{sys}}{T} \geq 0$$

$$TdS - \delta q_{sys} \geq 0 \tag{1.6.7}$$

Equation 1.6.7 follows from the second law of thermodynamics. We will now consider two particular cases.

Case 1: Constant T and P Conditions

At constant P, the heat transfer is equivalent to the enthalpy (see Chapter I.2):

$$\delta q_p = dH \tag{1.6.8}$$

Substitution of Equation 1.6.8 into Equation 1.6.7 gives:

$$TdS - dH \geq 0 \tag{1.6.9}$$

Substituting the differential form of the Gibbs energy (Equation 1.6.4) into Equation 1.6.9 gives:

$$dG + SdT \leq 0 \tag{1.6.10}$$

At constant T, the second term $SdT = 0$ because T is not changing, giving the final result:

$$dG \leq 0 \tag{1.6.11}$$

or upon integrating both sides from an initial to final state:

$$\Delta G \leq 0 \tag{1.6.12}$$

The equality holds for a reversible (equilibrium) process, and the inequality holds for any spontaneous process at constant T and P.

> Key Result: At constant T and P conditions $\Delta G < 0$ for a spontaneous process and $\Delta G = 0$ for a reversible process.

Since we are at constant T, the differential form of dG from Equation 1.6.4 simplifies to:

$$dG = dH - TdS \tag{1.6.13}$$

Integrating both sides at constant T and P from an initial state to a final state gives:

$$\Delta G = \Delta H - T\Delta S \tag{1.6.14}$$

from which we see that the Gibbs energy has an enthalpic term and an entropic term.

> Key Result: $\Delta G = \Delta H - T\Delta S$
>
> The expression is valid at constant T and P.

Case 2: Constant T and V Conditions

For the case of constant volume, the heat transfer is equivalent to the total internal energy (see Chapter I.2):

$$\delta q_v = dU \tag{1.6.15}$$

Substitution of Equation 1.6.15 into Equation 1.6.7 gives:

$$TdS - dU \geq 0 \tag{1.6.16}$$

Substituting the differential form of the Helmholtz energy (Equation 1.6.4) into Equation 1.6.16 gives:

$$dA + SdT \leq 0 \tag{1.6.17}$$

Again at constant T, the second term $SdT = 0$ because T is not changing, giving the final result:

$$dA \leq 0 \tag{1.6.18}$$

or upon integrating both sides from an initial to final state:

$$\Delta A \leq 0 \tag{1.6.19}$$

The equality holds for a reversible (equilibrium) process, and the inequality holds for any spontaneous process at constant T and V.

> Key Result: At constant T and V conditions $\Delta A < 0$ for a spontaneous process and $\Delta A = 0$ for a reversible process.

Since we are at constant T, the differential form of dA from Equation 1.6.4 simplifies to:

$$dA = dU - TdS \tag{1.6.20}$$

Integrating both sides at constant T and V from an initial state to a final state gives:

$$\Delta A = \Delta U - T\Delta S \tag{1.6.21}$$

> Key Result: $\Delta A = \Delta U - T\Delta S$
>
> The expression is valid at constant T and V.

Four Fundamental differentials of thermodynamics

The first law of thermodynamics in differential form is:

$$dU = \delta q + \delta w \tag{1.6.22}$$

For a reversible process we have defined the entropy as $dS = \delta q_{rev}/T$ and the reversible work as $\delta w = -P \cdot dV$. Substituting these identities into Equation 1.6.22 gives the following differential form of the first law:

$$dU = TdS - PdV \tag{1.6.23}$$

Note that Equation 1.6.23 is valid for a reversible process in which the only work is due to compression/expansion.

The enthalpy was defined in Chapter I.2 as:

$$H = U + PV \tag{1.6.24}$$

From Equation 1.6.24 we can write a differential form of the enthalpy as:

$$dH = dU + PdV + VdP \tag{1.6.25}$$

where we have again used the product rule from calculus on the PV term. Substituting Equation 1.6.23 into Equation 1.6.25 for the dU term gives another differential relation for dH:

$$\begin{aligned} dH &= TdS - PdV + PdV + VdP \\ dH &= TdS + VdP \end{aligned} \tag{1.6.26}$$

Similarly, substitution of Equation 1.6.23 into the dU term in the differential form for dA in Equation 1.6.4 gives another differential for dA:

$$\begin{aligned} dA &= TdS - PdV - TdS - SdT \\ dA &= -PdV - SdT \end{aligned} \tag{1.6.27}$$

Finally, substitution of Equation 1.6.26 into the dH term differential form for dG in Equation 1.6.4 gives another differential for dG:

$$\begin{aligned} dG &= TdS + VdP - TdS - SdT \\ dG &= VdP - SdT \end{aligned} \tag{1.6.28}$$

Table I.6.i summarizes the four fundamental differential relations for dU, dH, dG, and dA:

Table I.6.i

$dU = TdS - PdV$	(Equation 1.6.23)
$dH = TdS - VdP$	(Equation 1.6.26)
$dA = -PdV - SdT$	(Equation 1.6.27)
$dG = VdP - SdT$	(Equation 1.6.28)

Pressure dependence of ΔG

The fundamental differentials from **Table I.6.i** are useful for deriving various thermodynamic relationships. As an example, we can use Equation 1.6.28 to derive the pressure dependence of ΔG. Starting from Equation 1.6.28, at **constant T** the second term $SdT = 0$, giving:

$$dG = VdP \tag{1.6.29}$$

or

$$\left(\frac{\partial G}{\partial P}\right)_T = V \tag{1.6.30}$$

Integrating both sides of Equation 1.6.29 from an initial pressure $\mathbf{P_i}$ to a final pressure $\mathbf{P_f}$ gives:

$$\Delta G = \int_{P_i}^{P_f} VdP \tag{1.6.31}$$

Solids and liquids are nearly incompressible, so the volume does not change significantly with changes in the pressure. Therefore, for **solids and liquids** the volume can be treated as constant in Equation 1.6.31, and upon integration gives:

$$\Delta G = V\Delta P \tag{1.6.32}$$

Note that Equation 1.6.32 is valid for solids and liquids. For an ideal gas we can substitute $V = nRT/P$ for the volume in Equation 1.6.31:

$$\Delta G = \int_{P_i}^{P_f} \frac{nRT}{P} dP$$

$$\Delta G = nRT \int_{P_i}^{P_f} \frac{dP}{P}$$

$$\Delta G = nRT \ln\left(\frac{P_f}{P_i}\right) \tag{1.6.33}$$

If we set the initial pressure to 1 bar (standard pressure), and replace the initial Gibbs energy $\mathbf{G_i}$ with the symbol for G at the standard state $\mathbf{G^\circ}$, then Equation 1.6.33 becomes:

$$G = G^\circ + nRT \ln\left(\frac{P}{1\text{ bar}}\right)$$

$$\bar{G} = \bar{G}^\circ + RT \ln\left(\frac{P}{1\text{ bar}}\right) \tag{1.6.34}$$

where in the last line we have use the expression for the **molar Gibbs, $\bar{\mathbf{G}}$**.

See Practice Problem 1

Examples

Example 1.6.1

Show that at constant pressure, the entropy is given by:

$$S = -\left(\frac{\partial G}{\partial T}\right)_P$$

Solution

Starting with Equation 1.6.28:

$$dG = VdP - SdT$$

At constant pressure ($dP = 0$), we have:

$$dG = -SdT$$

or, solving for S:

$$S = -\left(\frac{\partial G}{\partial T}\right)_P$$

Practice Problems

Problem 1. At high pressure, graphite (density $\rho = 2.25$ g/cm^3) can be spontaneously converted into diamond (density $\rho = 3.51$ g/cm^3) through a solid-to-solid phase transition:

C (graphite) → C (diamond)

At 1 atm of pressure the standard molar Gibbs energy of this reaction is $\Delta\bar{G}^\circ = 2.84$ kJ/mol. At what pressure does the reaction become spontaneous (i.e. at what pressure does $\Delta\bar{G} = 0$)?

Problem 2. Consider the *reversible* freezing of liquid water into ice at a constant temperature of 0 °C and constant pressure of 1 atm.

H_2O (l) ↔ H_2O (g) 0 °C

Show that ΔG for this process is 0. (Hint: use the relation $\Delta G = \Delta H - T\Delta S$).

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1.7: Equilibria in Biochemical Systems

In this chapter we extend the concept of the Gibbs energy to mixtures. In the case of mixtures, the number of moles of the different components can change as a result of a chemical reaction or a phase transition. The partial molar Gibbs energy or chemical potential can be used to determine the spontaneity of a chemical reaction or of a phase transition. We first derive an expression for the chemical potential of gases, volatile liquids, and ideal solutions. We then introduce the concept of the activity to write a general expression for the chemical potential. For a reaction, the reaction quotient can be expressed in terms of the activities of the constituent species. Finally, we express the equilibrium constant in terms of the change in Gibbs free energy.

Learning Objectives

- Know the definition of the chemical potential as the partial molar Gibbs energy, and be able to analyze the spontaneity of a phase transition based on the change in chemical potential.
- Be able to use the chemical potential to calculate the change in Gibbs energy for a process involving changing number of moles.
- Understand the definition of the activity and how it can be used to describe both ideal and real solutions.
- Be able to write the reaction quotient in terms of the activities and modalities of the components of a reaction.
- Be able to use relation between the standard change in Gibbs energy and the equilibrium constant.

Gibbs energy and phase equilibria

For a phase transition in equilibrium at the phase transition temperature, such as the freezing of liquid water at 0 °C, the process is reversible. At equilibrium, the change in the *molar* Gibbs energy $\Delta\bar{G} = 0$, meaning that if two phases are at equilibrium,

$$\bar{G}_{solid} = \bar{G}_{liquid} \tag{1.7.1}$$

Notice here that the molar Gibbs, $\bar{\mathbf{G}}$ is the same between the two phases. The molar Gibbs energy is an *intensive* variable (Gibbs energy per mole). We must use the molar Gibbs because the phase equilibrium is independent on the amount of substance. For example, we could have a small ice cube in equilibrium with a large volume of water at 0 °C.

If we have multiple species in our system, the intensive variable of interest is the **partial molar Gibbs energy** that is defined for the ith component of the system as:

$$\bar{G}_i = \left(\frac{\partial G}{\partial n_i}\right)_{T,P,n_j} \tag{1.7.2}$$

where n_i is the number of moles of the ith component, and n_j is the number of moles of all the other components in the system. The total Gibbs energy is a function of the number of moles of each species:

$$G = \sum_i \bar{G}_i n_i \tag{1.7.3}$$

The partial molar Gibbs, $\bar{G}_i$ shows how infinitesimal changes in the Gibbs energy, $\mathbf{dG}$, depend on infinitesimal changes in the number of moles of a component (n_1, n_2, …)

$$dG = \bar{G}_1 dn_1 + \bar{G}_2 dn_2 + \ldots \tag{1.7.4}$$

The chemical potential

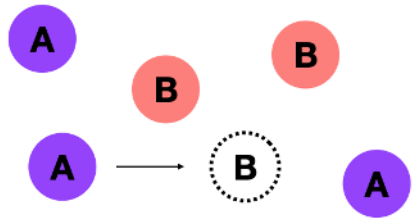
The partial molar Gibbs is an important property for determining phase equilibria. We give this quantity a special name called the **chemical potential** which gets the Greek symbol μ, and we write the chemical potential of the ith component as:

$$\mu_i = \bar{G}_i = \left(\frac{\partial G}{\partial n_i}\right)_{T,P,n_j} \tag{1.7.5}$$

Substituting Equation 1.7.5 into Equation 1.7.4 gives:

$$dG = \mu_1 dn_1 + \mu_2 dn_2 + \ldots$$
$$dG = \sum_i \mu_i dn_i \tag{1.7.6}$$

Consider the spontaneous transfer of some moles of a molecule from state A to state B as shown in **Figure I.7.A**.



$$dn_B = -dn_A$$

Figure I.7.A. The conversion of a molecule from state A into state B. The number of moles converted from state A into state B is given by dn_B, and is equal and opposite to dn_A.

The change in the number of moles in state A, dn_A will be equal and opposite to the change in the number of moles in state B, dn_B, so we can write the total change in Gibbs energy from Equation 1.7.6 as

$$dG = \mu_A dn_A + \mu_B dn_B$$
$$dG = (\mu_B - \mu_A) dn_B \tag{1.7.7}$$

We now ask ourselves, when will the transition from state A into state B become spontaneous? For a spontaneous process $dG < 0$. Thus, the transition of dn_B moles from state A to state B, will be *spontaneous* if

$$(\mu_B - \mu_A) dn_B < 0$$
$$\mu_B < \mu_A \tag{1.7.8}$$

We see from Equation 1.7.8 that matter flows in the direction of lower chemical potential.

> Key Result: Matter flows spontaneously from high chemical potential to low chemical potential. The flow of matter will continue until the chemical potentials are equal, which is the equilibrium condition.

See Practice Problem 1

Recall that in Chapter I.6 we calculated the pressure dependence of the Gibbs energy for an ideal gas (See **Equation I.6.33**):

$$\bar{G} = \bar{G}^\circ + RT \ln\left(\frac{P}{P_0}\right) \tag{1.7.9}$$

where P_0 is the pressure at standard conditions (1 bar). For a multi-component system of ideal gases, the partial molar Gibbs energy for each component is related to the partial pressure P_i of each species from Equation 1.7.9:

$$\bar{G}_i = \bar{G}_i^\circ + RT \ln\left(\frac{P_i}{P_0}\right)$$
$$\mu_i = \mu_i^\circ + RT \ln\left(\frac{P_i}{P_0}\right) \tag{1.7.10}$$

Notice that Equation 1.7.10 gives an expression for the chemical potential of each species in the mixture, μ_i, given the chemical potential at the standard state μ_i° and the partial pressures P_i.

> Key Result: For a mixture of ideal gases, the chemical potential of the ith species is $\mu_i = \mu_i^\circ + RT \ln\left(\frac{P_i}{P_0}\right)$ where P_i is the partial pressure of the gas and μ_i° is the standard chemical potential of component i when its partial pressure is 1 bar.

Thermodynamics of mixing volatile liquids

Figure I.7.B shows a pure liquid at equilibrium with its vapor in a closed container.

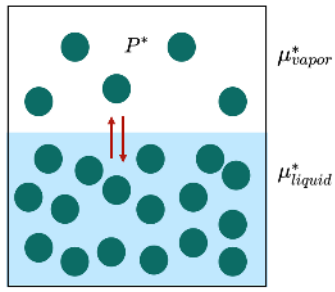


Figure I.7.B.

Since the system is at equilibrium the chemical potentials (partial molar Gibbs energy) are equal:

$$\mu^*_{vapor} = \mu^*_{liquid} \tag{1.7.11}$$

where the asterisk (*) indicates a pure substance. From Equation 1.7.9 for the gas phase we can write:

$$\mu^*_{vapor} = \mu^*_{liquid} = \mu^\circ_{vapor} + RT\ln\left(\frac{P^*}{1\text{ bar}}\right) \tag{1.7.12}$$

where P^* is the vapor pressure and μ°_{vapor} is the chemical potential at P^* = 1 bar.

Now consider a mixture of volatile liquids as shown in **Figure I.7.C**.

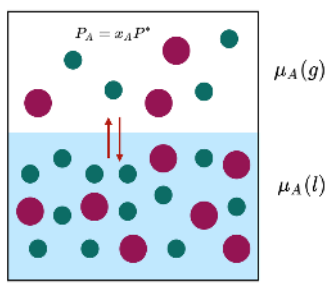


Figure I.7.C.

Since both components are in equilibrium with their vapors, the chemical potential for each component is still equal in the two phases. For example, for component A, we have:

$$\mu_A(l) = \mu_A(g) = \mu^\circ_A(g) + RT\ln\left(\frac{P_A}{1\text{ bar}}\right) \tag{1.7.13}$$

where P_A is the partial pressure of vapor A. Because, $\mu^\circ_{vapor} = \mu^\circ_A(g)$, we subtract Equation 1.7.12 from Equation 1.7.13 to obtain:

$$\mu_A(l) - \mu^*_A(l) = RT\ln\left(\frac{P_A}{1\text{ bar}}\right) - RT\ln\left(\frac{P^*}{1\text{ bar}}\right)$$

$$\mu_A(l) = \mu^*_A(l) + RT\ln\left(\frac{P_A}{P^*}\right) \tag{1.7.14}$$

Thus, from Equation 1.7.14, the chemical potential of a liquid in a mixture, $\mu_A(l)$, is given in terms of the chemical potential of the pure liquid ($\mu^*_A(l)$) and the ratio of the vapor pressure in the pure state over the vapor pressure in the mixture.

The relationship between the two vapor pressures in Equation 1.7.14 is given by **Raoult's law** which states that the vapor pressure of a substance in a mixture is the product of its vapor pressure as a pure liquid and its mole fraction:

$$P_A = x_A P^* \tag{1.7.15}$$

where x_A is the mole fraction of component A in the mixture. Inserting Equation 1.7.15 into Equation 1.7.14 gives the final expression for the chemical potential of a liquid in a mixture:

$$\mu_A(l) = \mu_A^*(l) + RT \ln x_A \tag{1.7.16}$$

> Key Result: For a volatile liquid in a mixture, the chemical potential of component A is given by $\mu_A(l) = \mu_A^*(l) + RT \ln x_A$ where $\mu_A^*(l)$ is the chemical potential of the pure liquid and x_A is the mole fraction.

See Practice Problems 2 and 3

Thermodynamics of ideal solutions

For the case of a solute dissolved in a solvent (liquid), the chemical potential of the solvent is the same as for a mixture of volatile liquids:

$$\mu_{solvent}(l) = \mu_{solvent}^*(l) + RT \ln x_{solvent} \tag{1.7.17}$$

For the case of the solute, it is often more convenient to express the chemical potential in terms of the *molality* m defined as

$$\text{molality} = \frac{\text{moles of solute}}{\text{mass of solvent in kg}}$$

For the solute, the chemical potential is:

$$\mu_{solute}(l) = \mu_{solute}^\circ(l) + RT \ln\left(\frac{m_{solute}}{m^\circ}\right) \tag{1.7.18}$$

Note here the careful choice of the reference state for the solute. The references state is defined as a state of unit molality where $m^\circ = 1 \text{ mol kg}^{-1}$.

Thermodynamics of real solutions

Typically, the conditions inside the cell are far from the conditions of an ideal solution. In general, we can write an expression for the chemical potential as:

$$\mu_i = \mu_i^\circ + RT \ln a_i \tag{1.7.19}$$

where a_i is called the **activity** and μ_i° is a reference state. For real solutions, the activity is given as

$$a_i = \gamma_i (m_i / m^\circ) \tag{1.7.20}$$

where γ_i is called the **activity coefficient** that is a measure of the deviation from ideality. For an ideal solution, $\gamma = 1$ and $a_i \to m_i/m^\circ$. **Table I.7.i** summarizes the expression of the activity and standard state for various substances. Note that for a pure sold and a pure liquid the activity is one.

Table I.7.i. Activity and standard state for various substances.

Substance	Standard State (μ°)	activity (a)
solid	pure solid, 1 bar	1
liquid	pure liquid, 1 bar	1
gas	pure gas, 1 bar	P^*/(1 bar)
solvent	pure solvent	mole fraction x_i
ideal solute	molality of 1 mol kg^{-1}	m_i/m°
real solution	molality of 1 mol kg^{-1}	$\gamma_i\ (m_i/m^\circ)$

The reaction quotient and chemical equilibrium

Consider a reaction in which the forward and reverse reaction can occur:

$aA \rightleftharpoons bB$ where a and b are the stoichiometric coefficients. The total change in the Gibbs energy from Equation 1.7.6 is

$$\Delta G = b\mu_B - a\mu_A \tag{1.7.21}$$

Substituting Equation 1.7.20 into Equation 1.7.21 gives:

$$\begin{aligned} \Delta G = & \ b\mu_B^\circ - a\mu_A^\circ + bRT\ln a_B - aRT\ln a_A \\ \Delta G = & \ \Delta G^\circ + RT\ln\left(\frac{a_B^b}{a_A^a}\right) \\ \Delta G = & \ \Delta G^\circ + RT\ln Q \end{aligned} \tag{1.7.22}$$

where

$$Q = \left(\frac{a_B^b}{a_A^a}\right) \tag{1.7.23}$$

is the **reaction quotient** and

$$\Delta G^\circ = b\mu_B^\circ - a\mu_A^\circ \tag{1.7.24}$$

is the change in Gibbs energy at standard conditions. The **reaction quotient** tells us which direction of the reaction will be favored. Let K_{eq} be the equilibrium constant. If $Q < K_{eq}$, then the forward reaction will be favored. If $Q > K_{eq}$, then the reverse reaction will be favored, and at equilibrium $Q = K_{eq}$.

Recall that at equilibrium $\Delta G = 0$. Inserting this result into Equation 1.7.22 gives the result:

$$\Delta G^\circ = -RT\ln K_{eq} \tag{1.7.25}$$

where we have use the fact that at equilibrium $Q = K_{eq}$.

See Practice Problems 4 and 5

The equilibrium constant

At equilibrium, $K_{eq} = Q$, so the equilibrium constant can be written in terms of the activities of each species as:

$$K_{eq} = \frac{a_B^b}{a_A^a} \tag{1.7.26}$$

For a real solution, because $a = \gamma(m/m^\circ)$ we can write the equilibrium constant as:

$$\begin{aligned} K_{eq} &= \frac{\gamma_B^b}{\gamma_A^a}\frac{(m_B/m^\circ)^b}{(m_A/m^\circ)^a} \\ &= K_\gamma K_{obs} \end{aligned} \tag{1.7.27}$$

where K_{obs} is the apparent or observed equilibrium constant given by

$$K_{obs} = \frac{(m_B/m^\circ)^b}{(m_A/m^\circ)^a} \tag{1.7.28}$$

and K_γ is a correction for non-ideal solutions given by (γ_B^b/γ_A^a). For an ideal solution $\gamma = 1$ and the thermodynamic equilibrium constant $K_{eq} = K_{obs}$. Often, we express the solute concentrations in molarities (moles/L) instead of molalities. If we redefine the reference state to be 1 molar, we obtain the more familiar form of the equilibrium constant:

$$K_{eq} = \frac{[B]^b}{[A]^a} \tag{1.7.29}$$

where the square bracket indicates a concentration in units of mol L^{-1}.

See Practice Problem 6

Temperature dependence of K_{eq}

At standard state conditions the equilibrium constant is given by Equation 1.7.25

$$\ln K_{eq} = -\frac{\Delta G^\circ}{RT} \tag{1.7.30}$$

Inserting the relation $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$ at two different temperatures T_1 and T_2 gives the **van 't Hoff equation**:

$$\ln \frac{K_2}{K_1} = \frac{\Delta H^\circ}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \tag{1.7.31}$$

It follows from Equation 1.7.31

$$\ln K_{eq} = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \tag{1.7.32}$$

From Equation 1.7.32 we see that a plot of $\ln K$ vs. $1/T$ gives a straight line with a slope of $-\Delta H^\circ/R$ and an intercept of $\Delta S^\circ/R$.

See Practice Problem 7

Practice Problems

Problem 1. Which of the following has a higher chemical potential? (If neither, answer "same")

(a) H_2O (l) or H_2O (s) at water's normal melting point (0 ºC).

(b) H_2O (l) or H_2O (s) at -5 ºC and 1 bar.

Problem 2. Which would have the higher chemical potential? Benzene at 25 ºC and 1 bar or benzene in a 0.1 M toluene solution at 25 ºC and 1 bar.

Problem 3. Calculate the chemical potential of ethanol in solution relative to that of pure ethanol when its mole fraction is 0.40 at its boiling point (78.3 ºC.)

Problem 4. The first step in the metabolic breakdown of glucose is its phosphorylation to G6P:

$$\text{glucose (aq)} + P_i \rightarrow \text{G6P (aq)}$$

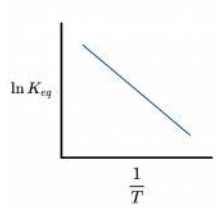
The standard Gibbs energy for the reaction is +14.0 kJ/mol at 37 ºC. What is the equilibrium constant? Hint: $\Delta G^\circ = -RT \ln K_{eq}$.

Problem 5. Consider a motor protein (such as the F_0F_1 ATP synthase) that converts the free energy of ATP hydrolysis into mechanical work. The reaction is ATP → ADP + P_i which has a ΔG° = −30 kJ/mol. Under experimental conditions at T = 298 K, [ATP] = [P_i] = 10^{-3} M and [ADP] = 10^{-4} M. What is ΔG of the reaction under these conditions?

Problem 6. For the following reaction, **(a)** write an expression for the (thermodynamic) equilibrium constant in terms of the activities of each species, **(b)** write an expression for the apparent (observed) equilibrium constant (K_{obs}).

$$CH_3COOH\ (aq) + H_2O\ (l) \leftrightarrow CH_3COO^-\ (aq) + H_3O^+\ (aq)$$

Problem 7. Consider the plot below that shows the temperature dependence of the equilibrium constant for a particular reaction. Is the reaction endothermic, exothermic, or neither?



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CHAPTER OVERVIEW

2: Chemical Kinetics

2.1: Kinetic Rate Laws
2.2: Reaction Mechanisms
2.3: Transition State Theory

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2.1: Kinetic Rate Laws

Kinetics deals with the rates of chemical processes *i.e.* how rapidly is a reactant consumed and a product formed? In this chapter, we will first define the reaction rate as the instantaneous change in concentration with respect to the time. Often we are interested in how the reaction rate depends on the concentrations of the species involved. This depends on the order of the reaction and leads to the various differential rate laws. On the other hand, if we are interested in how the concentration of species changes with time, we integrate the differential rate laws to obtain the integrated rate laws. Given an initial concentration and a kinetic rate constant, the integrated rate laws allow us to calculate the concentration at any future time.

Learning Objectives

- Understand the definition of the reaction rate and be able to write down a differential equation for any proposed mechanism.
- Understand the difference between the differential vs. integrated rate laws and be able to recognize the order of a reaction from a plot of either rate vs. concentration or concentration vs. time.
- Be able to use the integrated rate laws to solve for the half life.
- Be able to solve the integrated rate laws algebraically, given the initial concentration, to find the concentration at a later time.

The Reaction Rate

The **rate of a reaction** is the change in concentration of either the reactant or product with respect to time. During the course of a reaction, the concentration of the reactants will decrease and the concentration of the products will increase as shown in **Figure II.1.A**.

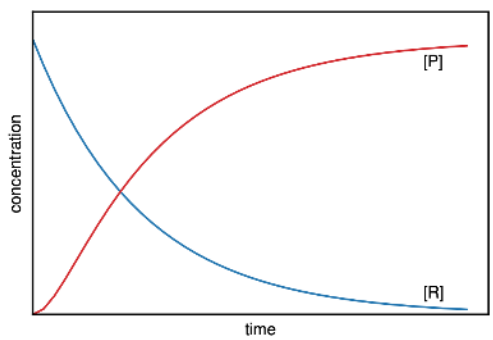


Figure II.I.A. Schematic depiction of the concentration of the reactants [R] and the concentration of products [P] as a function of time for a hypothetical reaction. The reactant [R] is consumed while the production [P] is formed. The rate at any given time is related to the slope of the concentration vs. time curve.

The rate of change of reactant and product is related to the slope of the concentration curves shown in **Figure II.1.A.** In general, for the reaction:

$$aA + bB \rightarrow cC + dD \quad \textbf{(Reaction 1)}$$

where the coefficients (a, b, c, and d) are the stoichiometric coefficients, the **rate** can be expressed in terms of any of the reactants or products as:

$$\text{rate} = -\frac{1}{a}\frac{d[A]}{dt} = -\frac{1}{b}\frac{d[B]}{dt} = \frac{1}{c}\frac{d[C]}{dt} = \frac{1}{d}\frac{d[D]}{dt} \tag{2.1.1}$$

where the expression in brackets refers to the concentration at a given time t. Notice that the rate expression in terms of the reactants contains a negative sign because the reactant is being used up, so its concentration is decreasing, whereas the concentration of the product is increasing.

Reaction Order

The rate of a reaction usually (but not always) depends on the concentration. Conceptually, it makes sense that the reaction rate increases with concentration because the frequency of collisions between reactive species will be greater at higher concentrations. However, the precise relationship between concentration and the reaction order must be determined experimentally. In most cases, for the general reaction of the form of **Reaction 1**, the reaction rate can be expressed in terms of the concentrations as:

$$\text{rate} = k[A]^n[B]^m \tag{2.1.2}$$

where k is called the **rate constant** and n and m are the **order** of the reaction with respect to A or B and must be determined experimentally.

> **Note**
>
> The reaction orders m and n in Equation 2.1.2 are *not* the same as the stoichiometric coefficients and must be determined from experiment.

Equation 2.1.2 is known as the *rate law* and the overall ***reaction order*** is determined by the sum of the orders n and m for each reactant. We will now consider a few cases.

Zero-Order Reaction

Consider the reaction of the form:

A → products **(Reaction 2)**

If the reaction is **zero order**, the rate law (Equation 2.1.2) is given by:

$$\text{rate} = k[A]^0 \tag{2.1.3}$$

Substituting the definition of the rate from Equation 2.1.1 we have:

$$-\frac{d[A]}{dt} = k[A]^0$$

$$-\frac{d[A]}{dt} = k \tag{2.1.4}$$

where the negative sign is because the concentration of reactant A is decreasing. Equation 2.1.4 gives us a first-order differential equation for the concentration [A]. This is known as the *differential rate law* since it is in differential form. For a zero-order reaction, the rate constant k is in units of $M\ s^{-1}$.

Notice from Equation 2.1.4 for a zero-order reaction, the rate is simply a constant k and does not depend on the concentration. A plot of the rate vs. concentration for a zero-order reaction gives a horizontal line as shown in **Figure II.1.B (a)**

Integrating both sides of Equation 2.1.4 from concentration $[A]_0$ at $t = 0$ to concentration $[A]$ at $t = t$ gives

$$\int_{[A_0]}^{[A]} d[A] = -k\int_0^t dt$$

$$[A] - [A]_0 = -kt$$

$$[A] = [A]_0 - kt \tag{2.1.5}$$

Equation 2.1.5 is the *integrated rate law* for a zero-order reaction and gives an expression for the time-dependence of the concentration [A]. From Equation 2.1.5 we see that for a zero-order reaction, a plot of the concentration [A] vs. time yields a straight line with slope $-k$. A plot of Equation 2.1.5 is show in **Figure II.1.B (b)**

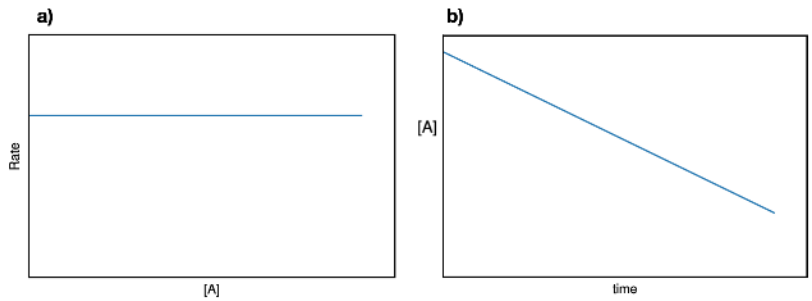


Figure II.1.B. Zero-order reaction. **(a)** For a zero-order reaction, the rate is independent of time and a plot of the rate vs. concentration is a constant. **(b)** The integrated rate law for a zero-order reaction exhibits a linear dependence of the concentration with respect to time.

First-Order Reaction

In a first-order reaction, the rate is proportional to the concentration.

$$\text{rate} = k[A]$$

Again, for a reaction of the form of **Reaction 2:**

$$\text{A} \rightarrow \text{products}$$

we have:

$$-\frac{d[A]}{dt} = k[A] \tag{2.1.6}$$

For a first-order reaction, the rate constant k has units of s^{-1}. A plot of Equation 2.1.6 is shown in **Figure II.1.C (a)** showing a linear dependence of the rate vs. time with a slope of $-k$. The first order differential equation of Equation 2.1.6 can be solved by *separation of variables* to obtain the *integrated rate law:*

$$\frac{d[A]}{[A]} = -kdt$$

$$\int_{[A_0]}^{[A]} \frac{d[A]}{[A]} = -k\int_0^t dt$$

$$\ln\frac{[A]}{[A]_0} = -kt$$

$$[A] = [A]_0 e^{-kt} \tag{2.1.7}$$

Notice that in a first-order reaction, the initial concentration decays *exponentially* with time. **Figure II.1.C (b)** shows the concentration vs. time for a first-order reaction.

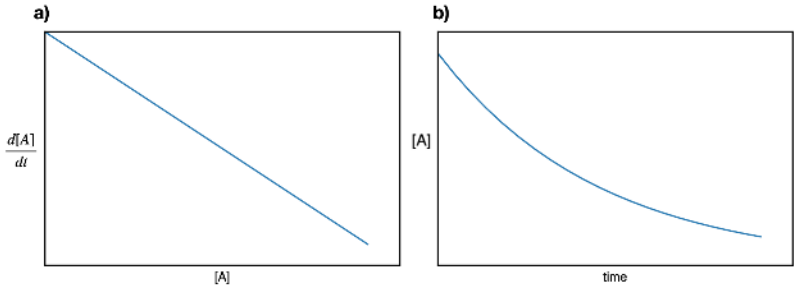


Figure II.I.C First-order reaction. (a) In a first-order reaction, the rate depends linearly on the time with the magnitude of the slope equal to the rate constant. **(b)** A plot of the concentration with respect to time for a first-order reaction is an exponential function.

Second-Order Reaction

There are two types of reactions we will consider that are second-order.

Case 1: Reaction of the type A → products

The first type of second-order reaction that we will consider is for a reaction of the form of **Reaction 2:**

A → products

For a second-order reaction of this type, the rate is:

$$\text{rate} = -\frac{d[A]}{dt} = k[A]^2 \tag{2.1.8}$$

For a second-order reaction of this type, the rate constant k has units of $M^{-1}\ s^{-1}$ and the rate is proportional to the concentration raised to the power of two. A plot of Equation 2.1.8 is shown in **Figure II.1.D (a)** showing a quadratic dependence of the rate vs. time. Equation 2.1.8 is a *second* order differential equation that can be solved by *separation of variables* to obtain the *integrated rate law:*

$$\int_{[A_0]}^{[A]} \frac{d[A]}{[A]^2} = -k\int_0^t dt$$

$$\frac{1}{[A]} - \frac{1}{[A]_0} = kt$$

$$\frac{1}{[A]} = kt + \frac{1}{[A]_0} \tag{2.1.9}$$

Notice that for a second-order reaction of this kind, the *inverse* of the concentration is linear with time with a slope of k. **Figure II.1.D (b)** shows the concentration vs. time for a second-order reaction.

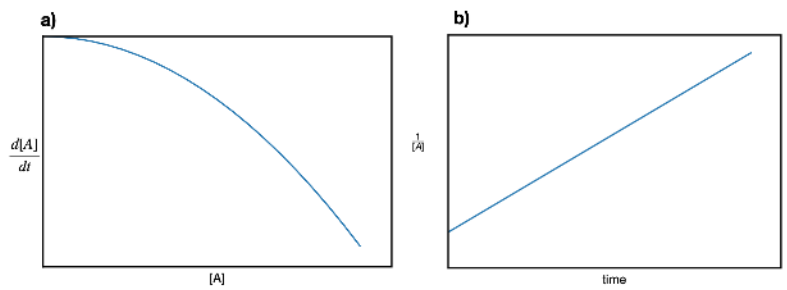


Figure II.1.D. Second order reaction. (a) For a second-order reaction describing **Reaction 2**, the rate has a quadratic dependence on the time. **(b)** A plot of the *inverse* concentration with respect to time gives a straight line.

Case 2: Reaction of the type A + B → products

We can also consider a second type of second-order reaction that is represented by two different molecular species (A and B) coming together to form products:

A + B → products **(Reaction 3)**

The rate is given by:

$$\text{rate} = -\frac{d[A]}{dt} = -\frac{d[B]}{dt} = k[A][B] \tag{2.1.10}$$

In this case, the rate is first-order with respect to A and first-order with respect to B, but the overall reaction order is second-order. An example of this type of second-order reaction is the renaturation of DNA from two complementary single-stranded DNA chains to form double-stranded DNA.

The integrated rate law from Equation 2.1.10 is

$$\frac{1}{[B]_0 - [A]_0}\ln\left(\frac{[B][A]_0}{[A][B]_0}\right) = kt \tag{2.1.11}$$

where $[A]_0$ is the initial concentration of species A at time $t = 0$ and $[B]_0$ is the initial concentration of species B at time $t = 0$. A plot of Equation 2.1.11 is shown in **Figure II.1.E**

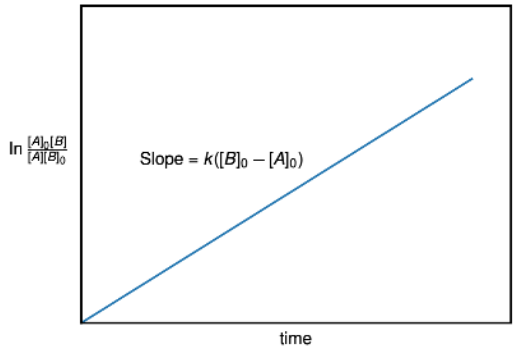


Figure II.1.E. Integrated rate law for a second-order reaction of the type according to Reaction 3. In this type of reaction, two different species A and B react to form a product. The reaction is first order with respect to both A and B, so the overall reaction order is two.

Key Result: **Table II.2.i** summarizes the various *rate laws* for a zero, first, and second order reaction

Table II.2.i

order	differential form	integrated form	units of k
0	$\frac{d[A]}{dt} = -k$	$[A] = [A]_0 - kt$	M s^{-1}
1	$\frac{d[A]}{dt} = -k[A]$	$[A] = [A]_0 e^{-kt}$	s^{-1}
2	$\frac{d[A]}{dt} = -k[A]^2$	$\frac{1}{[A]} = \frac{1}{[A]_0} + kt$	M^{-1} s^{-1}
2[a]	$\frac{d[A]}{dt} = -k[A][B]$	$\frac{1}{[B]_0 - [A]_0} \ln \frac{[B][A]_0}{[A][B]_0} = kt$	M^{-1} s^{-1}

a) A + B → products

The half-Life

The **half-life** of a reaction is the time required for the concentration of a reactant to decrease to half of its initial concentration. To find the half-life of any reaction, we can substitute the quantity:

$$\frac{1}{2}[A]_0 = [A] \tag{2.1.12}$$

into any of the integrate rate laws in **Table II.2.i** and solve for the time t. For example, for a first order rate law given by Equation 2.1.7:

$$[A] = [A]_0 e^{-kt} \tag{2.1.13}$$

Substitution of $\frac{1}{2}[A]_0$ for $[A]$ gives:

$$\begin{aligned} \frac{1}{2}[A]_0 &= [A]_0 e^{-kt_{1/2}} \\ \frac{1}{2} &= e^{-kt_{1/2}} \\ -kt_{1/2} &= \ln 2 \\ t_{1/2} &= \frac{\ln 2}{k} \end{aligned} \tag{2.1.14}$$

Notice that for a *first-order* reaction, the half-life does not depend on the initial concentration since $[A]_0$ does not appear in the expression for $t_{1/2}$.

The half-lives for the the other reaction orders can be solved in a similar way for each of the integrated rate laws in **Table II.1.i**. The expression for the half-lives for reaction orders 0, 1, and 2 are summaries in **Table II.1.ii.**

Table II.1.ii. Half life for some common reactions

Table II.2.ii

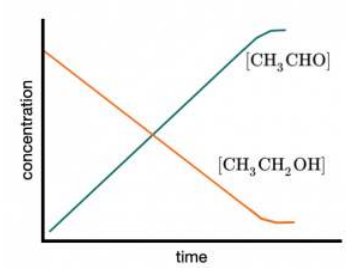
order	half life
0	$t_{1/2} = \frac{[A]_0}{2k}$
1	$t_{1/2} = \frac{\ln 2}{k}$
2	$t_{1/2} = \frac{1}{k[A]_0}$

Practice problems

Problem 1. The first step in the metabolism ethanol is the conversion of ethanol into acetaldehyde by the enzyme ethanol dehydrogenase according to the reaction:

$$\text{ethanol} + NAD^+ \rightarrow \text{acetaldehyde} + NADH + H^+$$

The figure below shows the concentration of the reactant ethanol and the product acetaldehyde. What is the order of the reaction? Sketch a plot of the rate of alcohol metabolism vs. concentration of ethanol.



Problem 2. For a certain first order reaction, the reaction is 40% complete after 120 min at 298 K. What is the value of the rate constant?

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2.2: Reaction Mechanisms

In Chapter II.1 Kinetic Rate Laws we calculated the integrated rate law for zero, first, and second order reactions. The rate law must be determined from experiment and is consistent with an underlying mechanism. In this chapter we will consider schemes for different reaction mechanisms involving one or more elementary steps. We will consider reaction schemes for reversible reactions, parallel reactions, and sequential reactions. These type of reaction schemes are the building blocks for building more complex biochemical reaction pathways and networks.

Learning Objectives

- Understand what is meant by the molecularity of a reaction.
- Be able to analyze three types of reactions: reversible reactions, parallel reactions, and consecutive reactions.
- Understand the plot of concentration vs. time for each of these types of reactions.
- Be able to write down a differential equation (without needing to solve it) for the rate of any species in any complex mechanism.

Reaction Mechanism and Molecularity

We saw in Chapter II.1 Kinetic Rate Laws that the rate law for a given reaction must be determined from experiment. The **order** of the reaction refers to the overall reaction in terms of reactants being converted into products. However, the overall reaction often involves the sum of several, single, definitive, elementary kinetic steps that make up the reaction mechanism. .

An elementary reaction cannot be further subdivided into a smaller sequence. Consider the *elementary reaction:*

$$\mathrm{aA} + \mathrm{bB} \longrightarrow \mathrm{cC}$$

If the reaction is an elementary reaction, we can write the rate law as

$$\text{rate} = \frac{1}{c}\frac{d[C]}{dt} = -\frac{1}{a}\frac{d[A]}{dt} = -\frac{1}{b}\frac{d[B]}{dt} = k[A]^a[B]^b \tag{2.2.1}$$

where a, b, and c are now the stoichiometric coefficients in the elementary reaction.

The number of reacting molecules in an elementary step determines the **molecularity** of the reaction. The **molecularity** of the reaction refers to a single elementary step in the mechanism that reflects what is actually happening in the reaction at a molecular level. An elementary step that involves only one reactant molecule are called **unimolecular**, and typically occurs with *cis-trans* isomerization, thermal decomposition, or ring-opening reactions. An example would be the decomposition of N_2O_4 (g) into NO_2 (g) according to the reaction:

$$\mathrm{N_2O_4(g)} \rightarrow 2\,\mathrm{NO_2(g)}$$

For this example the rate would be

$$\text{rate} = \frac{1}{2}\frac{d[\mathrm{NO_2}]}{dt} = k[\mathrm{N_2O_4}] \tag{2.2.2}$$

which is a first order reaction since the rate depends linearly on the concentration of the reactant N_2O_4 (g).

A second example is the conversion of *trans* proline to *cis* proline shown in **Figure II.2.A**

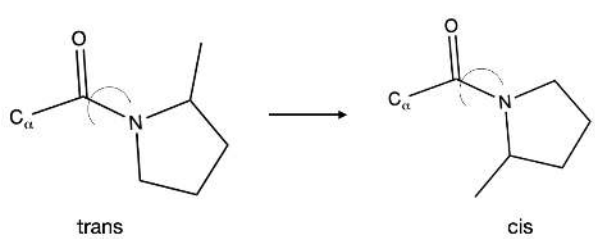


Figure II.2.A. The cis-trans isomerization of proline is an example of a unimolecular reaction.

An elementary step that involves two reactant molecules is called a **bimolecular reaction**. A simple example of a bimolecular reaction is reaction of H_2 (g) with I_2 (g) to form HI (g) according to the reaction:

$$\mathrm{H_2(g) + I_2(g) \rightarrow 2\,HI(g)}\cdot$$

For this example, the rate is

$$\text{rate} = \frac{1}{2}\frac{d[\mathrm{HI}]}{dt} = k[\mathrm{H_2}][\mathrm{I_2}] \tag{2.2.3}$$

An elementary step that involves the simultaneous encounter of three reactant molecules is called **termolecular**. Termolecular reactions are very rare because the probability of three reactant molecules colliding at the same time with the correct orientation and sufficient energy is very small. There are only a few known termolecular interactions, mostly involving nitric oxide. An example is the reaction of NO (g) with Cl_2 (g):

$$\mathrm{2\,NO(g) + Cl_2(g) \rightarrow 2\,NOCl}$$

For this example the reaction rate is

$$\text{rate} = \frac{1}{2}\frac{d[\mathrm{NOCl}]}{dt} = k[\mathrm{NO}]^2[\mathrm{Cl_2}] \tag{2.2.4}$$

which is overall a third-order reaction.

So far we have considered reaction mechanisms of individual, isolated reactions. In biochemistry, various elementary reaction steps are coupled or combined into complex reaction pathways. We will now look at a few of types of reaction pathways involving two or more reactions that can serve as building blocks for developing more complex reaction schemes.

Reversible Reaction

In a ***reversible reaction***, the forward reaction (A → B) occurs with rate constant k_1 while the reverse reaction (B → A) simultaneously occurs with rate constant k_{-1}. The reversible reaction scheme is:

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$$

Reaction Scheme 1: Reversible Reaction

Examples of reactions of the type shown in **Reaction Scheme 1** include protein folding and unfolding for a two-state model, a conformational change between an open and closed configuration of a protein, and a reversible isomerization reaction, such as the interconversion between dihydroxyacetone phosphate and glyceraldehyde-3-phosphate in glycolysis.

For a reaction scheme according to **Reaction Scheme 1** the rate of change of the reactant is given by:

$$\frac{d[A]}{dt} = -k_1[A] + k_{-1}[B] \tag{2.2.5}$$

At equilibrium, the concentration of reactant and product become constant, and so there is no change in the concentration of A with time ($d[A]/dt = 0$), so at equilibrium:

$$k_1[A] = k_{-1}[B] \tag{2.2.6}$$

The ratio of [B]/[A] is the equilibrium constant K_{eq}, leading to the expression:

$$\frac{[B]}{[A]} = K_{eq} = \frac{k_1}{k_{-1}} \tag{2.2.7}$$

The relationship between the equilibrium constant and the forward and reverse reaction rates stems from the **principle of microscopic reversibility** that states that at equilibrium the rates of the forward and reverse process are equal for every elementary step of the reaction.

Figure II.2.B shows a plot of the concentration vs. time for a reaction according to **Reaction Scheme 1**. Before equilibrium, the concentrations of both A and B will change with time until the equilibrium state is reached, at which point the concentrations of A and B remain constant with a ratio given by Equation 2.2.7.

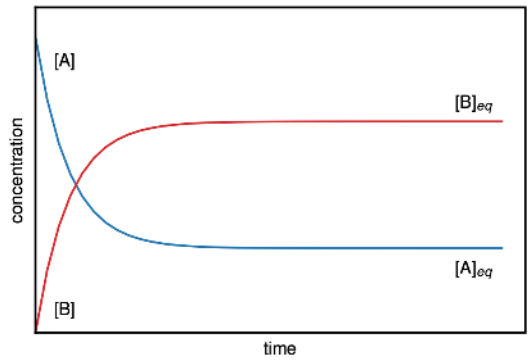


Figure II.2.B. Concentration of A and B vs. time for the reversible reaction according to **Reaction Scheme 1**. At equilibrium the rates of the forward and reverse reaction are equal, and the concentration of A and B are not changing. The ratio of $[B]_{eq}$ and $[A]_{eq}$ at equilibrium gives the equilibrium constant.

Another example is the bimolecular formation of a complex such as a ligand/protein complex:

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C$$

Reaction Scheme 2: reversible bimolecular reaction

In this case the forward rate constant k_1 is for a second order reaction with units of M^{-1} s^{-1} and the reverse rate constant k_{-1} is for a first order reaction with units of s^{-1}. The rate constants for the complex formation is:

$$K_A = \frac{[AB]}{[A][B]} = \frac{k_1}{k_{-1}} \tag{2.2.8}$$

where K_A is the association constant (in units of M^{-1}), and for the dissociation:

$$K_D = \frac{[A][B]}{[AB]} = \frac{k_{-1}}{k_1} \tag{2.2.9}$$

where K_D is the dissociation constant (in units of M).

Parallel Reactions

In metabolism, a metabolic precursor can be shunted into different metabolic pathways at a metabolic branching point. Consider for example that glyceraldehyde-3-phosphate (GAP) can be converted to 1,3-bisphosphoglycerate in the glycolysis pathway or can be used to convert sugars via the pentose phosphate pathway as shown in **Figure II.2.C**

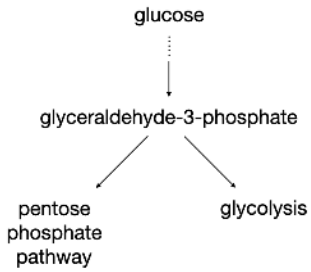
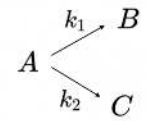


Figure II.2.C. Example of a branching point in metabolism that leads to parallel reactions. Glucose is first converted to glyceraldehyde-3-phosphate which can enter the pentose phosphate pathway for the conversion of sugars or can be metabolized via glycolysis.

This kind of reaction scheme is a parallel reaction and there are numerous examples in metabolism. The overall reaction scheme is shown in **Reaction Scheme 3:**



Reaction Scheme 3: Parallel reaction

For the parallel reaction scheme of **Reaction Scheme 3** the rate of consumption of A depends on both rate constants:

$$\frac{d[A]}{dt} = -k_1[A] - k_2[A]$$
$$\frac{d[A]}{dt} = -(k_1 + k_2)[A] \tag{2.2.10}$$

Equation 2.2.10 is a first order reaction that can be integrated to give:

$$[A] = [A]_0 e^{-(k_1+k_2)t} \tag{2.2.11}$$

The rate of formation of the products B and C are given by:

$$\frac{d[B]}{dt} = k_1[A]$$
$$\frac{d[C]}{dt} = k_2[A] \tag{2.2.12}$$

Substitution of Equation 2.2.11 into Equation 2.2.12 gives after integration:

$$[B] = [A]_0 \frac{k_1}{k_1 + k_2}\left(1 - e^{-(k_1+k_2)t}\right) \tag{2.2.13}$$

and

$$[C] = [A]_0 \frac{k_2}{k_1 + k_2}\left(1 - e^{-(k_1+k_2)t}\right) \tag{2.2.14}$$

Figure II.2.D shows the concentrations as a function of time for the reactant A and the products B and C for a parallel reaction. Notice that [A] decrease to zero and gets used up, at which point the [B] and [C] remain constant, since there is no more reactant.

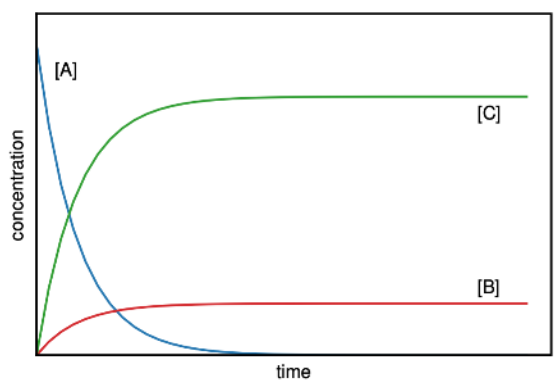


Figure II.2.D Concentration of A, B, and C vs. time for the parallel reaction according to **Reaction Scheme 3**. [A] decreases exponentially (first order kinetics) unit A is used up. When A is used up, [B] and [C] remain constant since there is no more reactant. The ratio of [B] or [C] formed can be determined from the ratio of rate constants k_1 and k_2.

If we are interested in the final concentration of [B] and [C] after A is used up, (as shown in **Figure II.2.D**), we can take the limit of Equation 2.2.13 and Equation 2.2.14 as the time goes to infinity:

$$[B]_\infty = [A]_0 \frac{k_1}{k_1 + k_2}$$
$$[C]_\infty = [A]_0 \frac{k_2}{k_1 + k_2} \tag{2.2.15}$$

From Equation 2.2.15 we see that the final concentration of [B] and [C] depends on the rate constants of the two parallel reactions (k_1 and k_2). The ratio of rate constants is equal to the ratio of the final products:

$$\frac{[B]_\infty}{[C]_\infty} = \frac{[A]_0 \frac{k_1}{k_1+k_2}}{[A]_0 \frac{k_2}{k_1+k_2}}$$

$$\frac{[B]_\infty}{[C]_\infty} = \frac{k_1}{k_2} \tag{2.2.16}$$

See practice problem ???

Consecutive Reactions

Another common feature of metabolic pathways are consecutive reactions in which the product from the first step become the reactant for a second step, etc…. The metabolism of ethanol is an example. Ethanol is first oxidized to acetaldehyde by the enzyme alcohol dehydrogenase. In a second step, acetaldehyde is oxidized to acetic acid by acetaldehyde dehydrogenase:

$$\text{ethanol} \xrightarrow{k_1} \text{acetaldehyde} \xrightarrow{k_2} \text{acetic acid}$$

The accumulation of acetaldehyde causes nausea and headaches.

For a two-step consecutive reaction of the form of **Reaction Scheme 4**:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

Reaction Scheme 4: Consecutive reactions

the rate law equations are:

$$\frac{d[A]}{dt} = -k_1[A]$$

$$\frac{d[B]}{dt} = k_1[A] - k_2[B]$$

$$\frac{d[C]}{dt} = k_2[B] \tag{2.2.17}$$

Plots of the concentrations of A, B, and C as a function of time are shown in **Figure II.2.E**

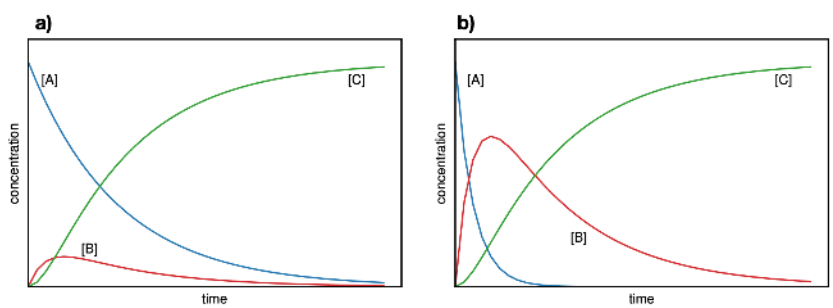


Figure II.2.E. Concentration of A, B, and C vs. time for the consecutive reaction of Reaction Scheme 4. **a)** Case where $k_1 < k_2$. The conversion of B → C is much faster than the formation of B from A, therefore [B] does not build up. **b)** Case where $k_1 > k_2$. The reaction B → C is much slower than the formation of B from A, leading to accumulation of B.

From **Figure II.2.E** and from Equation 2.2.17 we see that A decreases according the first-order rate law:

$$[A] = [A]_0 e^{-k_1 t} \tag{2.2.18}$$

Equation 2.2.18 can be substituted into the middle Equation 2.2.17, which can be integrated to give:

$$[B] = [A]_0 \frac{k_1}{k_2 - k_1}\left(e^{-k_1 t} - e^{-k_2 t}\right) \tag{2.2.19}$$

Finally, [C] can be determined from the conservation of mass requirement that $[C] = [A]_0 - [A] - [B]$ to obtain:

$$[C] = [A]_0 \left(1 + \frac{k_1 e^{-k_2 t} - k_2 e^{-k_1 t}}{k_2 - k_1}\right) \tag{2.2.20}$$

Figure II.2.D shows the time dependence of A, B, and C for **Reaction Scheme 4**. Notice that B is an intermediate and is only produced transiently. Eventually, the intermediate B will be used up and converted to C. The amount of accumulation of B depends on the relative rate constants k_1 and k_2. **Figure II.2.E (a)** shows the case where $k_1 << k_2$. In this case, the conversion of B → C is much faster than the formation of B from A. In this case we see that the concentration of B does not appreciate because as soon as B is formed, it is converted into C. **Figure II.2.E (b)** shows the case where $k_1 >> k_2$. In this case, the reaction B → C is much slower than the formation of B from A. In this case, B will initially accumulate until A is used up. Once A is used up, B begins to decrease as it is slowly converted into C.

Practice Problems

Problem 1. Initially a system has only species A at a concentration of 0.1 M. If a process proceeds from A to both B and C in parallel first-order reactions, and the forward rate to produce B is ten times larger than the rate to produce C, what are the final amounts of species B and C?

Problem 2. Consider the consecutive reaction in which ATP binds to an enzyme with a rate constant of $k_1 = 0.3$ s^{-1} in the first step and then is hydrolyzed with a rate constant of $k_2 = 1 \times 10^{-3}$ s^{-1}. **a)** Do you expect to be able to detect the intermediate ATP-bound enzyme complex? Why? **b)** Do How long is the lifetime of the intermediate ATP-bound enzyme complex?

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2.3: Transition State Theory

Thus far we have not considered the temperature dependence of the rate. Temperature affects a reaction rate because the rate "constant" k that enters into the rate equation is temperature dependent. Typically, the rate of a reaction will increase with temperature because a higher kinetic energy leads to more molecular collisions with sufficient orientation and energy. However, for enzyme-catalyzed reactions, a higher temperature can lead to a decrease in the rate as the enzyme denatures at higher temperatures. In this chapter we discuss how the rate of a reaction depends on the temperature and on the activation energy. This will lead to a discussion on transition state theory. In transition state theory a reaction proceeds through a high-energy intermediate whose formation is a kinetic bottleneck, making the reaction a rare event.

Learning Objectives

- Understand how the rate constant depends on temperature and be able to calculate the change in the rate at two different temperatures, given the activation energy.
- Understand the meaning of the potential energy surface and how it can be used as a reaction coordinate for simple reactions.
- Be able to use transition state theory to provide a meaning to the terms in the Arrhenius equation and to relate the rate constant to thermodynamic properties.

Temperature dependence of Rate

For a typical reaction, A + B → products, (shown in Figure II.3.A), the reaction requires the reactants A and B to collide with sufficient energy at the correct orientation in space. The rate law for this second order reaction is:

$$\text{rate} = k\,[A]\,[B] \tag{2.3.1}$$

One can image that as the temperature increase the kinetic energy of the molecules increases and so does the number of collisions with sufficient energy. Thus, the rate typically increases as the temperature is increased. The **Arrhenius equation** describes the usual observed dependence of the rate constant k with the temperature:

$$k = Ae^{-E_a/RT} \tag{2.3.2}$$

where T is the temperature, E_a is the activation energy, R is the gas constant (in J mol^{-1} K^{-1}), and A is a pre-exponential factor. Taking the natural log of both sides of Equation 2.3.2 gives a linearized form:

$$\ln k = \ln A - \frac{E_a}{RT} \tag{2.3.3}$$

A plot is shown in **Figure II.3.A**, where the slope gives the activation energy E_a/R and the intercept is the $\ln A$.

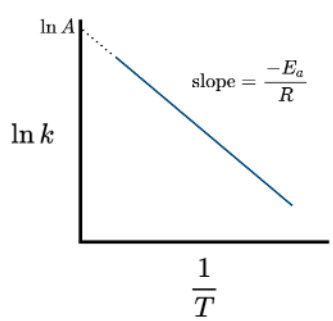

Figure II.3.A. Plot of $\ln k$ vs. 1/T. For Arrhenius behavior, the plot is linear with a slope equal to $-E_a/R$.

If the rate constant k can be measured at two temperatures, T_1 and T_2, then the pre-exponential factor drops out of Equation 2.3.3, giving:

$$\ln \frac{k_2}{k_1} = -\frac{E_a}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \tag{2.3.4}$$

See practice problem 1

Potential energy surface

To better understand the concept of the activation energy, we turn to a discussion of the **potential energy surface** and the energetics of a reaction. A simple reaction diagram is shown in **Figure II.3.B** which shows the energy of the reactants, transition state, and products along a hypothetical reaction coordinate. The reaction coordinate is a representation of the degree to which a reaction has progressed.

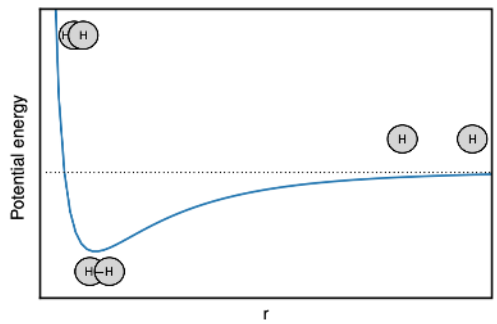

Figure II.3.B. The potential energy for the H_2 molecule as a function of the internuclear distance **r**. The minimum in energy corresponds to the equilibrium bond length.

A simple way to think about the reaction coordinate is to consider the combination of two atoms to form a diatomic molecule, such as H + H → H_2. The potential energy curve describing the interaction energy between the H atoms is shown in **Figure II.3.B**. At large distances, the individual atoms do not feel each other. As the distance between them decreases, there is a net attraction leading to a stable bond formation in the H_2 molecule. For a slightly more complex reaction, such as the substitution of a halogen via the SN_2 mechanism:

$$Cl^- + Br{-}CH_3 \rightarrow Br^- + Cl{-}CH_3$$

a three-dimensional potential energy surface plot is required. **Figure II.3.C** shows a hypothetical potential energy surface for this reaction.

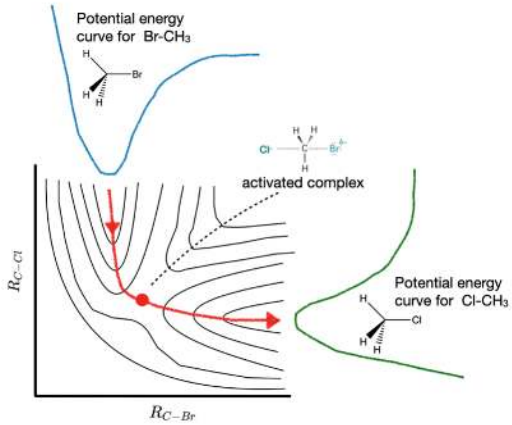

Figure II.3.C. A contour plot of the two dimensional potential energy surface for the SN_2 reaction Cl^- + Br-CH_3 → Br^- + Cl-CH_3. The red line traces the path along the minimum energy between reactant and product. The reaction proceeds via an activated complex located at the red dot between the reactant and product.

The potential energy curve for the formation of the Cl-CH_3 bond is plotted along the y-axis, and the potential energy curve for the formation of the Br-CH_3 bond is plotted along the x-axis. The contour plot shows the energies corresponding to different combinations of the atomic distances R_{C-Br} and R_{C-Cl}. The red curve traces out the minimum amount of energy along a reaction path from reactants to products. The minimum energy path has a saddle point that represents the location of the **activated complex** [Cl ··· CH_3 ··· Br]. The minimum potential energy path can be represented as the reaction coordinate shown in **Figure II.3.D**.

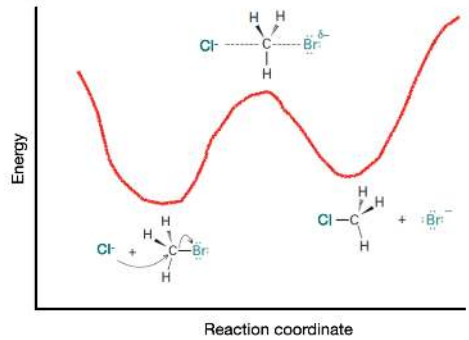

Figure II.3.D. A one dimensional reaction coordinate along the minimum in energy path given by the red curve in **Figure II.3.C**. The formation of the high-energy activated complex at the energy barrier is the rate limiting step.

Transition state theory

In Equation 2.3.2 we introduced two parameters: the pre-exponential factor A and the activation energy E_a. These value of these parameters determine the temperature dependence of the rate constant. In this section, we will consider one theory of reaction dynamics (transition state theory) to account for these parameters in the Arrhenius equation. **Transition state theory** can be used to explain the rates of biochemical reactions in fluid environments and provides insight into the details of a reaction at the molecular scale.

Transition state theory assumes that as two reactants come together, the potential energy increases and reaches a maximum. The maximum corresponds to the formation of an **activated complex** that can either continue on to form the products or can collapse back to the reactants. Consider a reaction of the form of **Reaction Scheme 1**:

$$\mathrm{A}+\mathrm{B} \rightleftharpoons [\mathrm{AB}^{\ddagger}] \longrightarrow \mathrm{P}$$

where A and B are reactants that form an activated complex $[\mathrm{AB}^{\ddagger}]$ that can collapse back into A and B or continue to products, P. If the reactants $\mathrm{A}+\mathrm{B}$ are at equilibrium with the activated complex, an equilibrium constant can be defined as

$$K^{\ddagger}=\frac{[AB^{\ddagger}]}{[A][B]} \tag{2.3.5}$$

The rate of the reaction is proportional to the concentration of the activated complex $[\mathrm{AB}^{\ddagger}]$ at the top of the energy barrier. It follows that the rate constant can be written as:

$$k=\kappa \nu K^{\ddagger} \tag{2.3.6}$$

where ν is the frequency of vibration of the activated complex along the degree of freedom that leads to the formation of the product. (From statistical thermodynamics it can be shown that the vibrational frequency is $\nu=k_B T/h$ where k_B is Boltzmann's constant and h is Planck's constant). The parameter κ is called the **transmission coefficient** and is introduced to account for the fact that the activated complex does not always form product.

The rate constant in Equation 2.3.6 can be related to the thermodynamic properties through the relation:

$$\Delta G^{\circ\ddagger}=-RT\ln K^{\ddagger} \tag{2.3.7}$$

where $\Delta G^{\circ\ddagger}$ is the change in standard molar Gibbs energy of activation as shown in Figure X. Combining Equation 2.3.7 and Equation 2.3.6 gives an expression for the rate constant:

$$\begin{aligned} k &= \kappa \nu e^{-\Delta G^{\circ\ddagger}/RT} \\ &= \kappa \nu e^{\Delta S^{\circ\ddagger}/R} e^{-\Delta H^{\circ\ddagger}/RT} \end{aligned} \tag{2.3.8}$$

where in the second line we have used the fact that $\Delta G^{\circ\ddagger}=\Delta H^{\circ\ddagger}-T\Delta S^{\circ\ddagger}$. Comparing Equation 2.3.8 with the **Arrhenius equation** Equation 2.3.2 we see that the pre-exponential factor **A** is given by transition state theory to be

$$A=\kappa \nu e^{\Delta S^{\circ\ddagger}/R} \tag{2.3.9}$$

and the activation energy $\mathbf{E_a}$ is identified as the **enthalpy of activation** $\boldsymbol{\Delta H^{\circ\ddagger}}$ as shown in **Figure II.3.E**.

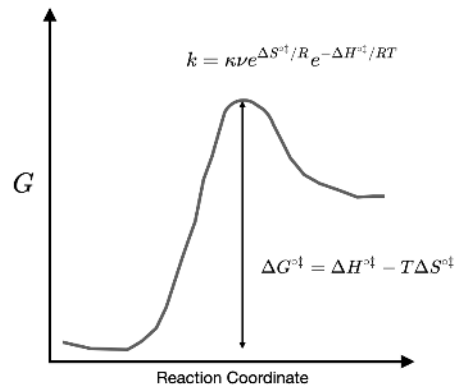

Figure II.3.E. Gibbs energy change for a reaction. The standard molar Gibbs energy of activation $\Delta G^{\circ\ddagger}$ is the difference in Gibbs energy between the activated complex and the reactant.

Practice Problems

Problem 1. What is the rate constant at 300 K for a reaction with activation energy $E_a = 50$ J mol^{-1} and a pre-exponential factor of $A = 10^{11}$ s^{-1}.

Problem 2.

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CHAPTER OVERVIEW

3: Molecular Mechanics and Statistical Thermodynamics

3.1: Potential Energy Surface and Bonding Interactions
3.2: Intermolecular Forces
3.3: Newtonian Mechanics
3.4: Molecular Dynamics Simulations
3.5: Analysis of Molecular Dynamics Trajectories
3.6: Advanced topics in Molecular Dynamics

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3.1: Potential Energy Surface and Bonding Interactions

In this chapter, we return to the idea of a potential energy surface and consider models for a covalent bond. The potential energy changes as a function of the relative distance between bonded atoms. As atoms come together to form molecules, the potential energy will have a minimum at the equilibrium bond distance. For diatomic molecules, the potential energy surface is one-dimensional and depends only on the distance between the two atoms in the diatomic molecule. For polyatomic molecules with more complex structure, the potential energy surface will be a high-dimensional function of the positions all the atoms. To model the secondary structure of proteins and other macromolecules, we need potential energy functions to describe the equilibrium bond stretching of covalent bonds, the equilibrium angle bending (involving an angle between three atoms), and dihedral torsional rotations (involving four atoms). In this chapter we will look at empirical potential energy functions for these three type of interactions that govern the conformational energy of a polypeptide chain.

Learning Objectives

- Understand the difference between a harmonic potential energy and the Morse potential, and what is meant by the terms *harmonic* or *anharmonic* vibrations.
- Be able to explain and justify the different parameters used to describe single, double, and triple bonds.
- Be able to sketch the dihedral potential energy for the butane molecule.
- Understand the physical justification for the different types of empirical potential energy functions that describe bonding interactions.

The chemical bond

The starting place for understanding chemical bonding and molecular structure is the **Born Oppenheimer approximation**. Since atomic nuclei are much heavier than the electrons, the electrons move much faster than the nuclei. Thus, to a good approximation, the motion of the atomic nuclei and electrons can be treated separately. The energy due to the electrons in the ground state can be calculated assuming a fixed position of the nuclei. This calculation can be repeated at different values of the internuclear distance between the atoms. This gives the potential energy for the nuclear positions as a function of the internuclear distance. As a consequence of the Born Oppenheimer approximation, we can think of the atomic nuclei as moving along a **potential energy surface** that is a function only of the nuclei position.

We begin by considering the simplest case of the potential energy curve for a diatomic molecule, such as H_2 that was introduced in **Chapter II.3** in the context of transition state theory. **Figure III.1.A** again shows the potential energy curve for the H_2 molecule. Notice that the **equilibrium bond length**, labeled r_e, is located at the minimum of the bond potential energy curve.

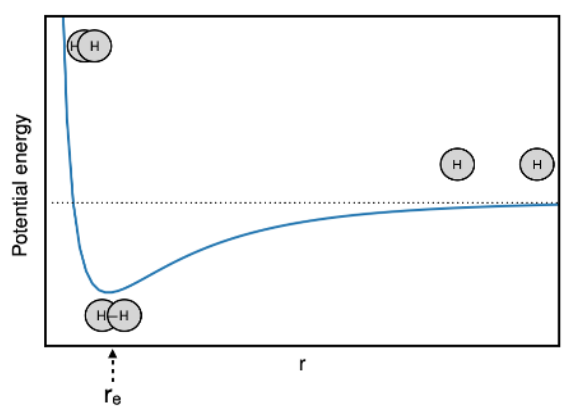

Figure III.1.A. Potential energy surface for molecular hydrogen as a function of the internuclear distance. The equilibrium bond distance corresponds to the minimum on the potential energy surface.

If the displacement away from the **equilibrium** bond length is small, the potential energy surface in the vicinity of the equilibrium bond distance can be described by a harmonic oscillator (see Appendix for the derivation)

$$V = \frac{1}{2}k(r - r_e)^2 \tag{3.1.1}$$

In Equation 3.1.1, r is the distance between the two atoms and r_e is the equilibrium bond distance. **Figure III.1.B** compares the full potential energy curve for H_2 with Equation 3.1.1. Notice that at sufficiently small displacements from r_e, vibrations in the bond are accurately described by Equation 3.1.1. Oscillations that are described by Equation 3.1.1 are called **harmonic**. In a harmonic bond, the force that results from stretching or compressing the bond distance is proportional to the displacement from the equilibrium position (Hook's law).

$$\text{Force} = -\frac{dV}{dr} \tag{3.1.2}$$

$$= -k\left(r - r_e\right) \tag{3.1.3}$$

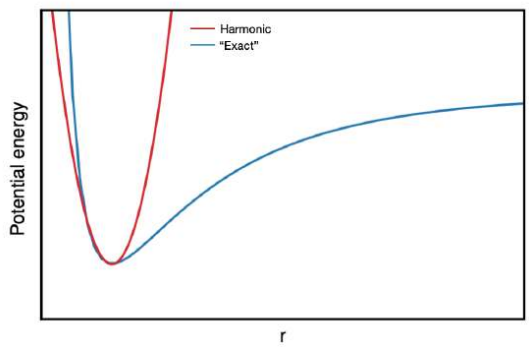

Figure III.1.B. Comparison between Harmonic bond approximation (Equation 3.1.1) with the exact potential energy surface. The harmonic approximation is valid for small displacements from the equilibrium bond distance.

Equations III.1.3.1.1 and III.1.3.1.3 describe small perturbative fluctuations about the equilibrium bond distance. In this case, the bonds are said to be harmonic bonds. Large displacements of the bond away from r_e lead to *anharmonic* vibrations. Anharmonic vibrations describe bond stretching that is far from the equilibrium bond distance. To model anharmonic vibrations, we could consider including higher-order terms in the Taylor expansion in Appendix. Alternatively, a common potential that can model bond breaking is the **Morse potential:**

$$V = D_e\left(1 - e^{-a(r-r_e)}\right)^2 \tag{3.1.4}$$

where D_e is the bond dissociation energy, and a controls the potential width. **Figure III.1.C** shows a plot of the Morse potential.

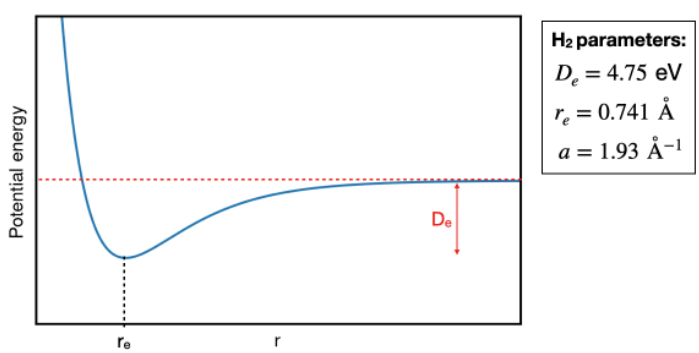

Figure III.1.C. To a good approximation anharmonic bond vibrations can be modeled using the Morse potential given by Equation 3.1.4. Figure III.1.C show the Morse potential using parameters for the H_2 molecule.

The potential energy surface for macromolecules is high-dimensional

In the previous section we described the potential energy for a diatomic molecule, such as H_2, O_2, N_2, NO, etc …, in terms of the equilibrium bond distance $\mathbf{r_e}$ and the internuclear distance $\mathbf{r}$. As we saw in **Chapter II.3** (see **Figure II.3.C**), for polyatomic molecules involving more than two bonded atoms, the potential energy surface is a function of all the internuclear distances and quickly becomes high-dimensional. For a proteins, nucleic acids, lipids, polysaccharides, or other macromolecules, if we let $\mathbf{R}$ be the set of positions of all the atoms ($\mathbf{R} = \{\mathbf{R}_1, \mathbf{R}_2, \ldots, \mathbf{R}_N\}$), then the potential energy surface $U(\mathbf{R})$ will be a function of all atomic coordinates. Although we cannot draw in more than three dimensions, we can consider an abstract high-dimensional space

where a point is given by the set of all the atomic positions: $(\mathbf{R}_1, \mathbf{R}_2, \ldots, \mathbf{R}_N)$. Local minima in this high-dimensional space will represent metastable conformations and local maxima represent kinetic barriers.

At constant number of particles, temperature, and volume, the probability of a given conformation is given by the normalize Boltzmann weight of the energy:

$$P(\mathbf{R}) = \frac{e^{-U(\mathbf{R})/k_BT}}{\int \ldots \int e^{-U(\mathbf{R})/k_BT}\, d\mathbf{R}_1, \ldots, d\mathbf{R}_N} \tag{3.1.5}$$

where the integral in the denominator is a multi-dimensional integral over all the atomic coordinates. It is common practice in computational biophysics to reduce the high-dimensional potential energy landscape to a limited number of variable that can describe the conformational state of the system, called **order parameters** or **collective variables**. An example of this was already present in **Chapter II.3** where we reduce the two-dimensional potential energy surface for the S_N2 substitution to a one-dimensional picture by constructing a path along the lowest energy contour from the reactant to product state as shown in **Figure III.1.D**

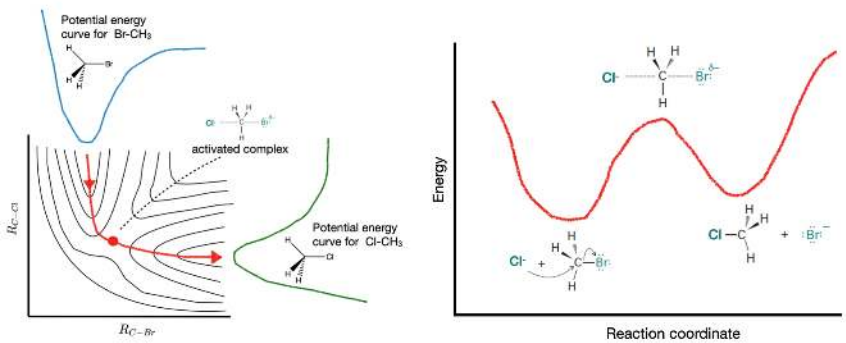

Figure III.1.D. The "high dimensional" representation of the potential energy surface for the S_N2 reaction as a function of the C-Cl bond distance and C-Br bond distance. A "low dimensional" representation along the hypothetical reaction coordinate is shown on the right along the red curve corresponding to the lowest energy path between reactant and product states. The transition state is the highest energy intermediate state between the reactant and product along the reaction coordinate.

As another example, we can consider the two dihedral angels, ϕ and ψ for the Nme-Ala-Ace peptide shown here:

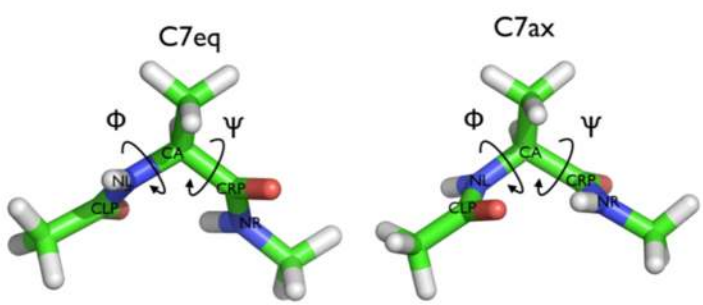

Figure III.1.E. Two metastable states of the Nme-Ala-Ace peptide are characterized by the two Ramachandran dihedral angles, ϕ and ψ.

Imagine we could sample randomly the equilibrium configurations drawn from a Boltzmann distribution of states given by Equation 3.1.5. In this case, we could construct a histogram of all ϕ, ψ angle combinations to obtain the probability $P(\phi, \psi)$. From statistical biophysics, we can define a **free energy surface** from the probabilities as

$$F(\phi, \psi) = -k_BT \ln P(\phi, \psi) \tag{3.1.6}$$

The free energy surface is a low-dimensional representation of system that provides information about metastable states, their relative stability, and the free energy barriers separating them. An example two-dimensional free energy surface for the Nme-Ala-Ace peptide is shown in **Figure III.1.F**. From this surface, we can define the **transition state** as the separatrix where there is equal probability for the system to flow into either metastable basin.

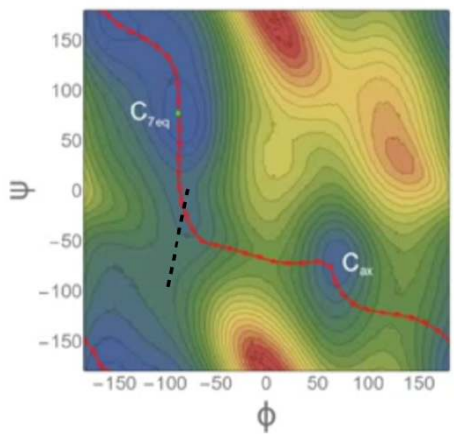

Figure III.1.F. The two-dimensional free energy surface for the Nme-Ala-Ace peptide projected on the space of the two Ramachandran dihedral angles, ϕ and ψ. The transition state is defined as the separatrix where there is equal probability for the system to flow into either metastable basin.

The covalent bond revisited

In molecular mechanics, we are often interested in equilibrium fluctuations of chemical bonds. For small fluctuations, it is customary to use a harmonic potential for the form of 3.1.7:

$$V = \frac{1}{2}k(r - r_e)^2 \tag{3.1.7}$$

The spring constant k determines the stiffness of the bond and the value of r_e is the equilibrium bond distance. These values are **parameters** of the model and are usually empirically determined to fit to experimentally observed values (for example from IR spectroscopy). Each type of chemical bond will need to be parameterized to agree with experiment. For example, a carbon-carbon single bond, between two sp^3 carbon atoms, such as in the ethane molecule shown in **Figure III.1.G (a)** will have a slightly *larger* value of r_e and a slightly smaller value of k as compared to a carbon-carbon double bond, between two sp^2 carbon atoms, such as in the ethene molecule shown in **Figure III.1.G (b)**.

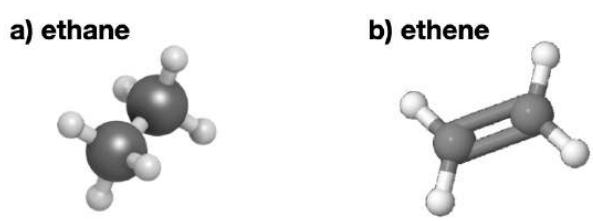

Figure III.1.G. The single bond between the sp^3 hybridized carbons in the ethane molecule **(a)** has a longer equilibrium bond length and is weaker than the double bond between the sp^2 hybridized carbons in the ethene molecule **(b)**. As a result the parameters in the harmonic bond potential energy function will be different for different hybridizations of carbon.

Table III.1.a shows the values for the spring constant and equilibrium bond distance for the different bond orders of carbon bonds.

hybridization	bond order	spring constant, k	equilibrium bond distance, r_e
sp^3	1	100 kcal/mol/Å^2	1.5 Å
sp^2	2	200 kcal/mol/Å^2	1.3 Å
sp	3	400 kcal/mol/Å^2	1.2 Å

Figure III.1.H shows a plot of the harmonic bond for each type of bond (single, double, and triple). We can see that as the bond order increases, the equilibrium bond distance, r_e, decreases, and the stiffness of the bond, k increases.

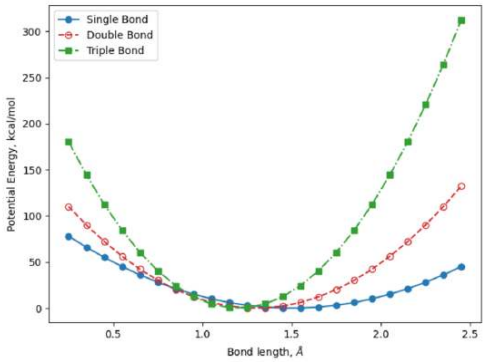

Figure III.1.H. The potential energy function of a carbon-carbon single bond (sp^3 hybridization) vs. a carbon-carbon double bond (sp^2 hybridization) vs. a carbon-carbon triple bond (sp hybridization). The parameters for each bond type are given in **Table III.1.a**

Angular and dihedral potentials for polyatomic molecules

In molecules with more than two bonded atoms it is possible to define a bond angle shown in **Figure III.1.I**. A bond angle is formed by three consecutive atoms, here labeled i, j, and k. The corresponding bond angle is θ. If we let $\mathbf{r}_{ij}$ be the bond distance between atoms i and j and $\mathbf{r}_{jk}$ the bond distance between atoms j and k, the bond angle can be expressed in terms of the atomic positions as

$$\theta = \arccos\left(\frac{\mathbf{r}_{ij} \cdot \mathbf{r}_{jk}}{|r_{ij}||r_{jk}|}\right) \tag{3.1.8}$$

where the dot indicates the dot product between bond vectors $\mathbf{r}_{ij}$ and $\mathbf{r}_{jk}$ and $|r_{ij}|$ and $|r_{jk}|$ are the lengths of the bond vectors.

It is common to describe fluctuations about the equilibrium bond angle with a harmonic potential of the form:

$$V_{angle} = \frac{1}{2}k(\theta - \theta_e)^2 \tag{3.1.9}$$

where θ_e is the equilibrium bond angle and k is a force constant that parameterizes the stiffness of the bond angle.

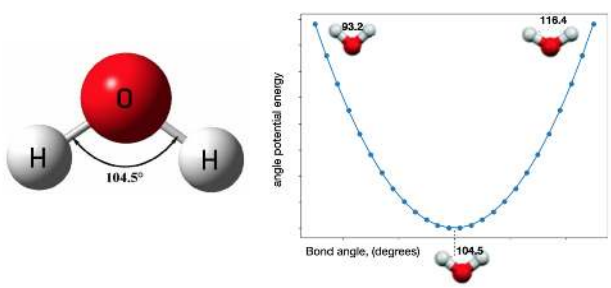

Figure III.1.I. Fluctuations about the equilibrium bond angle, θ, can be modeled using a harmonic potential energy function about the equilibrium bond angle θ_e.

If we are interested in the force acting on atom i, as we would need for solving Newton's equation of motion, we can use the chain rule:

$$\frac{dV}{dr_i} = \frac{dV}{d\theta} \cdot \frac{d\theta}{dr_i} \tag{3.1.10}$$

where the derivate $\frac{dV}{d\theta}$ can be computed by taking the derivative of Equation 3.1.9 with respect to θ, and the derivative $\frac{d\theta}{dr_i}$ can be computed by taking the derivative of Equation 3.1.8 with respect to r_i.

For atoms with four or more bonded atoms, there is a potential energy barrier to internal rotation about the dihedral angles. As a simple example we will consider the rotation about the methyl dihedral in butane as shown below:

The lowest energy configuration is the staggered, anti-, geometry at a bond angle of 180º. There are two equivalent staggered configurations with bond angles of 300º and 60º which are 3.8 kJ mol^{-1} higher in energy. The large energy barrier of 19 kJ mol^{-1} for the eclipsed, sys-, geometry means that rotations from on gauche configuration (300º) into another gauche configuration (60º) will be a rare event. To model a periodic potential energy function as seen in **Figure III.1.J**, the torsional energy is given by:

$$V_{torsion} = A[1 + \cos(n\phi - \phi_0)] \tag{3.1.11}$$

where ϕ is the dihedral angle of interest and the parameter A is a constant with units of energy, n is an integer, and ϕ_0 is a reference dihedral angle.

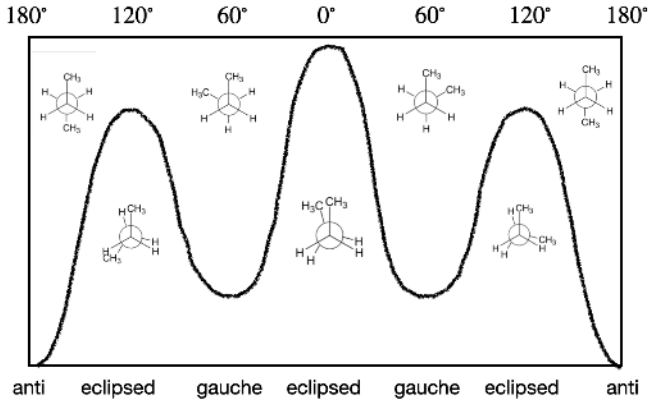

Figure III.1.J. The dihderal potential energy about the methyl bond in the butane molecule that can be modeled by Equation 3.1.11. The potential energy function has a period of 360º with the lowest energy configuration in the staggered, anti, configuration with a bond angle of 180º. The maximum in energy occurs at for the eclipsed configuration with a bond angle of 0º.

As an example, consider the the structure of the retinal chromophore shown in **Figure III.1.K** which has a conjugated π bond system. Absorption of a photon induces an excited state of the chromophore which is followed by the isomerization of the retinal protonated Schiff base around the C_{13}=C_{14} bond (adjacent to the Schiff base group). Rotation of the dihedral bonds can be modeled using Equation 3.1.11. **Table III.1.b** reports the parameter set used in molecular dynamics simulations of the retinal Schiff base.

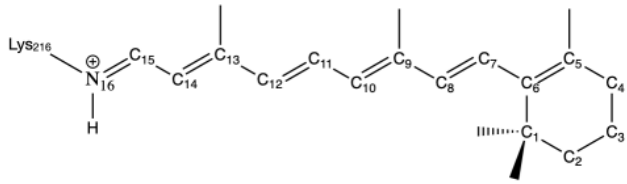

Figure III.1.K. Structure of retinal chromophore covalently bound to Lys216. Photoisomerization leads to rotation of the dihedral angle about the C_{13}=C_{14} bond

Table III.1.b. The parameter set for the torsion potentials of the main polyene chain of the retinal Schiff base. Values taken from Tajkhorshid et al. 1999. The form of the torsion potential is given by Equation 3.1.11.

ϕ	A kcal/mol	n	ϕ_0 (degrees)
C_5-C_6-C_7-C_8	5.62	2.0	180.0
C_6-C_7-C_8-C_9	19.99	2.0	180.0
C_7-C_8-C_9-C_{10}	8.515	2.0	180.0
C_8-C_9-C_{10}-C_{11}	18.64	2.0	180.0
C_9-C_{10}-C_{11}-C_{12}	11.25	2.0	180.0
C_{10}-C_{11}-C_{12}-C_{13}	17.54	2.0	180.0
C_{11}-C_{12}-C_{13}-C_{14}	14.15	2.0	180.0
C_{12}-C_{13}-C_{14}-C_{15}	14.73	2.0	180.0
C_{13}-C_{14}-C_{15}-N_{16}	15.215	2.0	180.0
C_{14}-C_{15}-N_{16}-C_ϵ	14.38	2.0	180.0

Practice Problems

Coming soon ...

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3.2: Intermolecular Forces

In Chapter III.1 we investigated interactions between bonded molecules, and to model the configurational energy of a macromolecule we introduced an empirical potential energy function for the covalent bonding potential energy, the angular potential energy, and the torsional potential energy. We now discuss interactions between atoms that are not directly bonded together, called non-bonding interactions. These give rise to two common types of intermolecular interactions: electrostatic interactions between charged molecules, and van der Waals (dispersion) interactions. The Coulomb interaction between charges plays an important role in the assembly of biological structures. Other important types of intermolecular forces are the dipole-dipole interaction between polar molecules and the dipole-induced-dipole between a polar molecule and a non-polar molecule with a given polarizability. The formation of biological complexes, secondary structure, and protein/ligand complexes is governed by the energetic contributions of each of these types of interactions.

Learning Objectives

- Be able to sketch and label the attractive and repulsive Coulomb potential between ions.
- Be able to sketch and label the Lennard Jones potential for dispersion interactions.
- Know how the different potential energy terms for the Coulomb, van der Waals, dipole, and induced dipole scale with the interatomic distance. Know which of these potentials is considered long-ranged.
- Be able to compute the potential energy between two ions given the interatomic distance or vice versa.
- Be able to predict how the addition of counter ions or salts in aqueous solvent screens the Coulombic interaction

The Coulomb potential between ions

The potential energy acting between two charged species in a vacuum, as shown in **Figure III.2.A**, depends on the separation distance between the ions, r, and the charge on the ions z_A and z_B:

$$V = \frac{z_A z_B}{4\pi\epsilon_0 r} \tag{3.2.1}$$

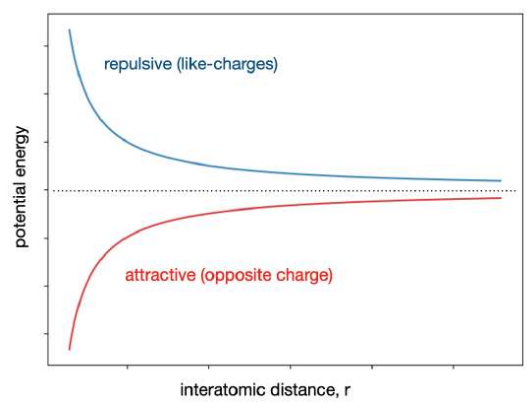

Figure III.2.A. The Coulomb potential energy given by Equation 3.2.1 describes the electrostatic interactions between ions. The potential energy is attractive between ions of opposite charge and repulsive between ions of the same charge.

Notice that the potential energy from Equation 3.2.1 is positive (repulsive) for like-charges and negative (attractive) for opposite-charges. The constant ϵ_0 is the **vacuum permittivity** as is $\epsilon_0 = 8.854187816 \times 10^{-12}\ J\,C^2\ m^{-1}$. Equation 3.2.1 is know as the **Coulomb interaction** because the force acting between two ions attract or repel each other according to **Coulomb's law**:

$$F = -\frac{z_A z_B}{4\pi\epsilon_0 r^2} \tag{3.2.2}$$

Equations 3.2.1 and 3.2.2 describe the interactions between charged species in a vacuum. For atoms that are not in a vacuum, we need to take account of the fact that the solvent or surrounding molecules lie between the two interacting ions and mediate the strength of this interaction. For example, in aqueous solvent, the bare ions are surrounded by water molecules which weakens the interaction between ions as shown in **Figure III.2.B**.

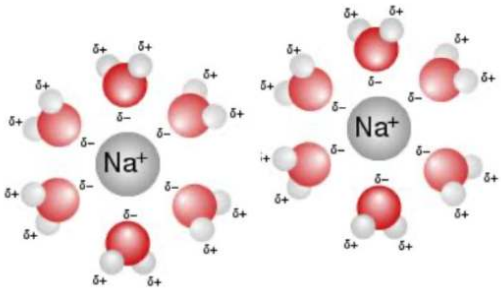

Figure III.2.B. The Coulomb potential energy between bare charges is screened in solution as the water molecules form a solvation shell around the ions.

To account for the solvent, the permittivity of the medium is expressed as the vacuum permittivity times the **relative permittivity** ϵ_r. For ions in a medium the Coulomb interaction becomes

$$V = \frac{z_A z_B}{4\pi\epsilon_0\epsilon_r r} \tag{3.2.3}$$

Table III.2.a reports the relative permittivity ϵ_r for different solvents. Note that highly polar solvents like water ($\epsilon_r = 78$) can have a large effect on the strength of the Coulomb interaction.

Table III.2.a. Relative dielectric strength of some common solvents

Solvent	relative dielectric constant, ϵ_r
Acetic acid	6
Methanol	33
Formic acid	58
Water	78

Ion-dipole and dipole-dipole interactions

Polar molecules have a net dipole moment μ. In the presence of an ion, there will be an ion-dipole interaction resulting from an attractive interaction between the ion and one end of the dipole moment, and a repulsive interaction between the ion and the other end of the dipole moment. In general, the strength of the interaction will depend on the orientation between the dipole moment and the ion as shown in Figure X. The interaction potential in a vacuum is given by

$$V = -\frac{\mu_A z_B \cos\theta}{4\pi\epsilon_0 r^2} \tag{3.2.4}$$

where θ is the angle that measures the alignment between the dipole moment and the ion (shown in Figure X). Notice that if z_B is positive, the lowest energy occurs when $\theta = 0$ (and $\cos\theta = 1$) because the positive ion will be closest to the negative end of the dipole moment.

For the interaction between two polar molecules, **dipole-dipole** interaction will depend on the orientation of the two polar molecules and will be *attractive* when the two dipole moments are aligned tail to tip because oppositely charged ends of the molecule attract (see Figure X). The dipole dipole interaction between two molecules in a vacuum is

$$V = \frac{\mu_A \mu_B (1 - 3\cos^2\theta)}{4\pi\epsilon_0 r^3} \tag{3.2.5}$$

Note that this potential energy decrease more rapidly as function of distance ($1/r^3$) than Equation 3.2.4.

From Equation 3.2.5, we see that the *attractive* dipole-dipole is strongest (most negative) when $\theta = 0$ or θ = 180º (and $1 - 3\cos^2\theta = -2$). This corresponds to the case when the dipole moments of the two polar molecules are perfectly aligned as in Figure X. In this case, the dipole-dipole interaction energy is

$$V = -\frac{2\mu_A \mu_B}{4\pi\epsilon_0 r^3} \tag{3.2.6}$$

It should be emphasized that Equation 3.2.6 is the dipole-dipole attractive potential energy if the molecules have a fixed orientation in which the dipole moments are perfectly aligned as shown in Figure X.

In macroscopic systems, where molecules are free to rotate, the average dipole-dipole interaction energy represents the average orientation of the dipole moments. When molecules are free to rotate, the dipole-dipole interaction energy is

$$V = -\frac{2}{3}\frac{\mu_A^2\mu_B^2}{(4\pi\epsilon_0)^2 r^6}\frac{1}{k_B T} \tag{3.2.7}$$

The exact form of Equation 3.2.7 is not important to us here, but it is important to notice that the distance dependence on the average interaction energy between polar molecules scales as the inverse *sixth* power of the interatomic distance. (which turns out to be the same as the van der Waals interaction)

Key Result

The average interaction energy between polar molecules scales as $V\ \frac{1}{r^6}$ where r is the distance between atoms.

Induced-dipole interactions and dispersion interactions

dipole-induced-dipole interaction energy

$$V = -\frac{\mu_A^2\alpha_B}{\pi\epsilon_0 r^6} \tag{3.2.8}$$

scales as $1/r^6$ (same scaling as average dipole-dipole interaction).

dispersion interaction energy

$$V = -\frac{3}{2}\frac{\alpha_A\alpha_B}{r^6}\frac{I_A I_B}{I_A + I_B} \tag{3.2.9}$$

where I_A and I_B are the ionization energies of the two molecules and α_A and α_B are the polarizabilities. scales as $1/r^6$ (same scaling as average dipole-dipole interaction and dipole-induced-dipole interaction).

In general, we can summarize all the non-Coulomb interactions (average dipole-dipole interaction, dipole-induced-dipole, and dispersion interaction) as scaling as $1/6$. These potentials are often lumped together in a single attractive "van der Waals" term

$$V = -\frac{A}{r^6} \tag{3.2.10}$$

where A is a parameter that depends on the identity of the atoms and molecules and the types of interactions present between them. Equation 3.2.10 will be used in the remainder of this book to describe attractive van der Waals type interactions.

Key Result

The van der Waals attractive energy is represent as a contribution from three different types of interactions: average dipole-dipole, dipole-induced-dipole, and dispersion interactions. The potential energy is written as $V = -\frac{A}{r^6}$ such that the van der Waals attractive energy scales as the inverse separation distance to the sixth power and A is a coefficient that depends on the identity of the atoms and molecules.

The Lennard-Jones (12,6) potential

$$V = \frac{B}{r^n} \tag{3.2.11}$$

where B is another parameter, and n is an integer (typically set to 12). For the case where $n = 12$, the potential energy is a balance between short-ranged repulsion ($1/r^{12}$) and van der Waals attractions at larger distances ($1/r^6$). The sum of the repulsive and van der Waals attractive interaction can be expressed by the **Lennard-Jones potential**

$$V = 4\epsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right] \tag{3.2.12}$$

where ϵ is a parameter with units of energy that characterizes the depth of the attractive minima and σ is a parameter with units of distance that parameterizes the range of the potential. Figure X shows a comparison of the Lennard-Jones potential with the Born-Oppenheimer potential energy surface between two argon atoms.

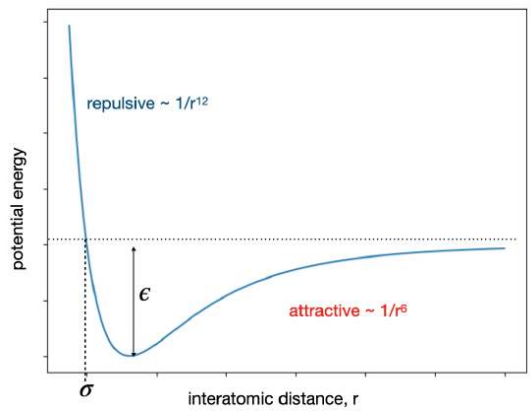

Figure III.2.?. The form of the Lennard-Jones potential given by Equation 3.2.12 is a common potential to describe both the attractive van der Waals interaction that scales as ~$1/r^6$, along with a short-ranged repulsive interaction that scales as ~$1/r^{12}$. The Lennard-Jones potential is parameterized by the value of ϵ, which defines the well depth, and the value of σ which is the interparticle distance at which the potential energy is zero.

The Lennard-Jones potential is a good approximation for the interaction energy between two non-charged species. Table X presents the values of the parameters ϵ and σ for some atoms and molecules.

Hydrogen Bonds

$$V = \frac{E}{r^{12}} - \frac{E}{r^{10}} \tag{3.2.13}$$

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3.3: Newtonian Mechanics

Introduction goes here

Learning Objectives

- Objective 1
- Objective 2

Newton's equation of motion

While the electronic structure of atoms is described by quantum mechanics, the motion of atomic nuclei and larger molecules can be described by Newton's laws of motion. Consider two interacting particles as shown in Figure 3.3.A. According to Newton's second law of motion the force acting on a particle is equal to the product of the mass of the particle and its acceleration:

$$\vec{F} = m\vec{a} \tag{3.3.1}$$

where F is the net force acting on a particle with mass m. Equation 3.3.1 describes how a force acting on a body can produce motion of a body. Force and acceleration are both vector quantities. The acceleration is the first derivative of the velocity with respect to time:

$$\vec{a} = \frac{d\vec{v}}{dt} \tag{3.3.2}$$

and the second derivative of the position with respect to time:

$$\vec{a} = \frac{d^2\vec{x}}{dt^2} \tag{3.3.3}$$

As shown in Figure 3.3.A, if two particles interact, they exert forces on one another that are equal in magnitude and opposite in direction, according to Newton's third law.

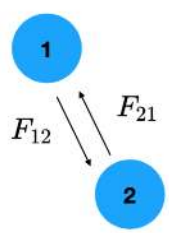

Figure III.3.A. Schematic depiction of two interacting particles. F_{12} is the force acting on particle 2 due to the interaction with particle 1. F_{21} is the equal in magnitude and opposite in direction force on particle 1 due to particle 2.

Given the initial positions and velocities of a system of particles and a suitable expression for the forces acting on each particle, the goal of classical mechanics is to calculate the positions and velocities of the particles at all future times.

Assuming that the potential energy is a function of the atomic positions, $U(\mathbf{R}) = U(R_1, R_2, \ldots, R_N)$, the force is:

$$F(\mathbf{R}) = -\nabla U(\mathbf{R}) \tag{3.3.4}$$

where ∇ is the differential operator:

$$\nabla = \hat{x}\frac{\partial}{\partial x} + \hat{y}\frac{\partial}{\partial y} + \hat{z}\frac{\partial}{\partial z} \tag{3.3.5}$$

.

Using Equation 3.3.4 and Equation 3.3.3, Newton's second law can be rewritten as a second order differential equation in terms of the position:

$$-\frac{\partial U(\mathbf{R})}{\partial R_i} = m_i \frac{d^2 \vec{R}_i}{dt^2} \tag{3.3.6}$$

Harmonic motion

As an illustrative example of Newtonian mechanics, consider a body of mass m, attached to a spring, and able to move along the x-direction only as shown in Figure 3.3.B. The potential energy is that of a harmonic oscillator:

$$U(x) = \frac{1}{2}k(x - x_0)^2 \tag{3.3.7}$$

where k is the spring constant that parameterizes the stiffness of the spring. The equilibrium position is when the spring is relaxed and the position of the mass is at x_0. This position corresponds to the minimum in potential energy. The net force acting on the mass at x_0 is zero, meaning that the acceleration is zero at this point, consistent Newton's equation of motion. If the spring is compressed or stretched along the x-axis, the mass will feel a restoring force given by Hook's law:

$$F = -\frac{dU}{dt}$$

$$F = -k(x - x_0) \tag{3.3.8}$$

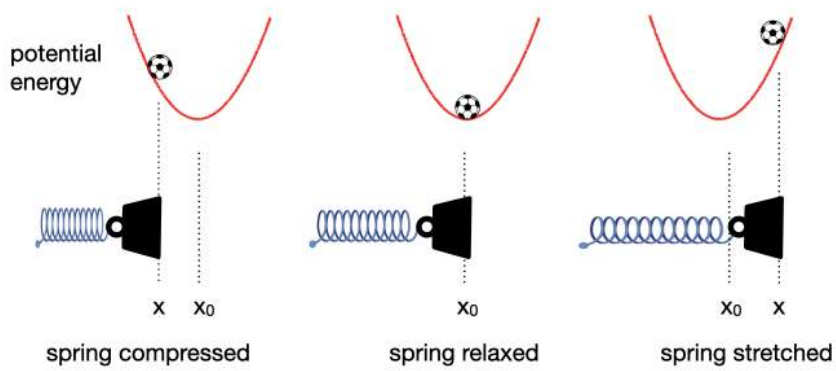

Figure III.3.B. A mass on a spring along the x-direction. The equilibrium position is represented by the position of the mass at x_0 where the spring is relaxed. If the spring is compressed or stretched the mass will feel a restoring force towards the equilibrium position. The potential energy curve is a harmonic oscillator, sketched above showing a minimum at the equilibrium position.

Newton's second law for the harmonic oscillator is:

$$-k(x - x_0) = m\frac{d^2x}{dt^2} \tag{3.3.9}$$

Equation 3.3.9 is a second order differential equation. The solution for the position as a function of time is

$$x(t) = A\cos(\omega_0 t + \phi) \tag{3.3.10}$$

where A is the amplitude of the oscillation, $\omega_0 = \sqrt{k/m}$, and ϕ is a phase factor that depends on the initial conditions.

Phase space

Equation 3.3.9 is a second order differential equation because the largest derivative order is a second derivative. In general, any n-th order differential equation can be expressed as a n first order differential equations:

$$\frac{dx_1}{dt} = f_1(x_1, \ldots, x_n)$$

$$\vdots$$

$$\frac{dx_n}{dt} = f_n(x_1, \ldots, x_n)$$

For the harmonic oscillator, the second order differential Equation 3.3.9 can be equivalently written as two first order differential equations:

$$\frac{dx}{dt} = v(t)$$

$$\frac{dv}{dt} = -\frac{k}{m}(x - x_0) \tag{3.3.11}$$

where $v(t)$ is the velocity. We can completely describe the dynamical state of the system with 2 variables: the position x(t) and the velocity v(t).

Figure 3.3.C shows a plot of Equation 3.3.10 (left) and the phase space plot (right) representing the time evolution of the dynamical system.

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3.5: Analysis of Molecular Dynamics Trajectories

Coming soon ...

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3.6: Advanced topics in Molecular Dynamics

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CHAPTER OVERVIEW

4: Spectroscopy - Types, Key Features, Examples

4.1: Spectroscopy- Basic Elements and Principles

4.2: “Two Masses on a Spring” Model and Infrared (IR) Spectroscopy

4.3: Quantum Mechanics and Quantum Oscillator Model

4.4: Fluorescence and Phosphorescence

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4.1: Spectroscopy- Basic Elements and Principles

In this Chapter, we will introduce a general notion of spectroscopy as a method and of its most basic type of data, a spectrum. We will also introduce the most basic features of each spectroscopic signal (spectral line or resonance): position, intensity and width/lineshape. Lastly, we will take a look at a relationship between quantum chemistry features of a sample (energy levels of electrons and other quantized particles) and spectral properties a such a sample.

Learning Objectives

- Grasp the definition of spectroscopy and a spectrum as the most basic item reported by spectroscopic measurements.
- Distinguish between the three key features of a spectral line (aka signal or peak): position, intensity, line-width.
- Master the quantitative relationships between frequency, energy and wavelength.
- Get familiar with the concept of spectral width: frequency, energy or wavelength range covered by the spectrum.

Spectroscopy: definition and examples

In general, the term "spectroscopy" can be applied to an experimental method, which reports a set of values ("intensities") as function of certain "frequency" or related variables. In this sense, spectroscopy is very different from other types of measurements. For example, reporting mass of an object takes only one value, same for an object's speed or concentration of solution, etc. Contrary to that, a spectrum inherently represents a function relating intensity (Y axis) to frequency (X axis). The X-axis may be represented by other variables related to frequency, e.g. energy, wavelength or some derivative of those. Figure IV.1.A shows an NMR spectrum of a protein sample with the X-axis labeled in ppm, a type of units representing frequency.

The spectrum has many tens if not hundreds of resonance lines (or peaks, signals). Some of these signals stand alone (are well-resolved) and thus are easy to be distinguished from others, e.g. peaks on the left at around 10 ppm. Most other peaks however strongly overlap, which may complicate their analysis.

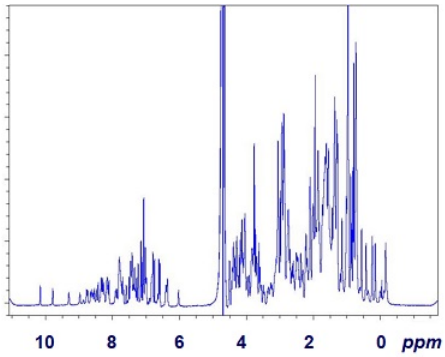

Figure IV.1.A A ^{1}H NMR spectrum of a protein. The X-axis is labeled in units of ppm, which effectively is a measure of frequency (can be converted to units of Hz). The data was collected by Serge L. Smirnov (WWU, Bellingham, WA).

Spectroscopy: basic parameters

For each spectral line (aka signal or peak), three foundational parameters provide its complete description:

- Position: the X-axis value of the maximum of the peak;
- Intensity: the Y-axis value of the maximum of the signal or the area under the curve of the peak
- Linewidth: the X-axis measure of how broad or narrow the spectral line is (for example reported as line width at half-height, LWHH).

These three parameters determined for every peak in the spectrum provide the complete description of the spectral data. In addition, many types of spectroscopy need to take into account the level of noise of the recording: the value of uncertainty along the Y-axis for every measured point of the spectrum. Usually, noise level is uniform throughout the sample but there can be some exceptions.

Two integrated parameters, sensitivity and resolution are often used to describe the quality or usefulness of a particular spectrum.

- **Sensitivity** Qualitative definition: the ability of a user to distinguish real signals from noise "blips" or background. Quantitative estimate: signal-to-noise ratio, value much greater than 1 represents a high-sensitivity spectrum). Higher ("stronger") peaks and

lower noise help to increase the sensitivity.

- **Resolution** Qualitative definition: the ability of a user to distinguish adjacent spectral signals from each other. Quantitative estimate: two peaks can be reliably "resolved" if the difference along the X-axis between their positions is much greater than the average of their respective line width values. Spectra with narrower ("sharper") peaks have greater resolution.

Quantitatively, sensitivity can be estimated as the signal/noise ratio using the weakest peak's signal intensity. A resolution value can be quantified for any two signals (peaks) as a ratio of the inter-peak distance over the average peak linewidth, e.g. LWHH. Calculated in this way, sensitivity and resolution are both unitless quantities.

Solid resolution and sensitivity afford reliable determination of all the three fundamental signal parameters: signal position, intensity and line shape. Insufficient sensitivity may prevent correct quantification of the peak intensity and is some cases signal position. Likewise, poor spectral resolution could result in obscured peaks positions, distort ed line shape and misrepresented signal intensity (see **Figure IV.1.B**).

> Big picture: Common types of spectroscopic techniques widely utilized in biochemistry and life sciences include the following varieties: fluorescent/phosphorescent, Nuclear Magnetic Resonance spectroscopy, ultraviolet (UV) and visual range (VIS) methods, circular dichroism, infrared (IR) spectroscopy and others.

Relationship between quantum particle energy, frequency and wavelength

In many spectroscopic applications, physics describes the fundamental properties of the target system, including the energy differences between the relevant states of the system. Depending on the process under study, the laws of either classical physics or quantum physics may be the most applicable. Applications of both of these types of physics to specific variants of spectroscopic methods will be discussed in the next sections of this Chapter as well as in the next Chapters.

In general quantum physics provides the following quantitative relationships between energy E, frequency ν and wavelength λ values of a wave-particle quantum:

Equation 4.1.2

$$E = h \cdot \nu \tag{4.1.1}$$

$$c = \lambda \cdot \nu \tag{4.1.2}$$

These relationships are parametrized via two universal constants, speed of light c and Plank's constant h. From these formulas, we see that quantum's frequency and energy values are inversely proportional to its wavelength. Thus, in **Table IV.1.I** the frequency and energy values grow from left to right, whereas the wavelength values grow from right to left (and hence each wavelength range is bracketed from its largest to smallest values).

Table IV.1.I	Radio	Microwave	Infrared	Visible	Ultraviolet	x-Ray	γ-Ray
Frequency, Hz	$< 3.0 \times 10^{9}$	$3.0 \times 10^{9} - 3.0 \times 10^{11}$	$3.0 \times 10^{11} - 4.0 \times 10^{14}$	$4.0 \times 10^{14} - 7.5 \times 10^{14}$	$7.5 \times 10^{14} - 3.0 \times 10^{16}$	$3.0 \times 10^{16} - 3.0 \times 10^{19}$	$> 3.0 \times 10^{19}$
Energy, J	$< 2.0 \times 10^{-24}$	$2.0 \times 10^{-24} - 2.0 \times 10^{-26}$	$2.0 \times 10^{-26} - 2.7 \times 10^{-19}$	$2.7 \times 10^{-19} - 5.0 \times 10^{-19}$	$5.0 \times 10^{-19} - 2.0 \times 10^{-17}$	$2.0 \times 10^{-17} - 2.0 \times 10^{-14}$	$> 2.0 \times 10^{-14}$
Wavelength	> 10 cm	10 - 0.10 cm	1000 - 0.7 μm	700 - 400 nm	400 - 10 nm	10 - 0.01 nm	< 0.01 nm

Table IV.1.I. Most common types of irradiation and corresponding ranges of frequency, wavelength and energy. From this table we can see that for example infrared spectroscopy deals with radiation frequency values of 3.0×10^{2} – 3.0×10^{5} GHz (1 Hz = 1 oscillation per second; 1 GHz = 10^{9} Hz), energy values of 2.0×10^{-29} – 2.7×10^{-22} kJ and wavelength values of 700 nm – 1 mm.

Examples (Quiz MC questions with a new Spectrum will be presented from the Examples here)

Let's take a quick look at the spectrum in **Figure IV.1.A** above. Here are some comments on sensitivity and resolution with some peaks as study cases.

1. General assessment: the spectrum allows reliable observation of some individual signals and shows some groups of signals which appear as collectives. Thus, the spectrum has some value but its sensitivity and resolution need to be analyzed critically.
2. The sensitivity of this recording is overall strong as the noise level (wiggles of the baseline) is obviously lower than the intensity of most peaks and the signal-to-noise level is way greater than the value of 1.
3. An example of well resolved peaks: two signals, one right below 10 ppm and one right above it are well-resolved. Another example of a well-resolved peak is the line at 6 ppm. For these peaks, the recording provides an excellent resolution.

4. An example of poor/insufficient resolution: most peaks within 1-2 ppm and 8-9 ppm are not resolved from the adjacent spectral lines.
5. The frequency (X-axis) of the spectrum is labeled in units of parts-per-million (ppm). The X-axis covers a range of values form -1.5 ppm (on the right) to 11.0 ppm (on the left). Thus the *spectral width* of the spectrum is 12.5 ppm (11.0 - (-1.5)).

Practice problems

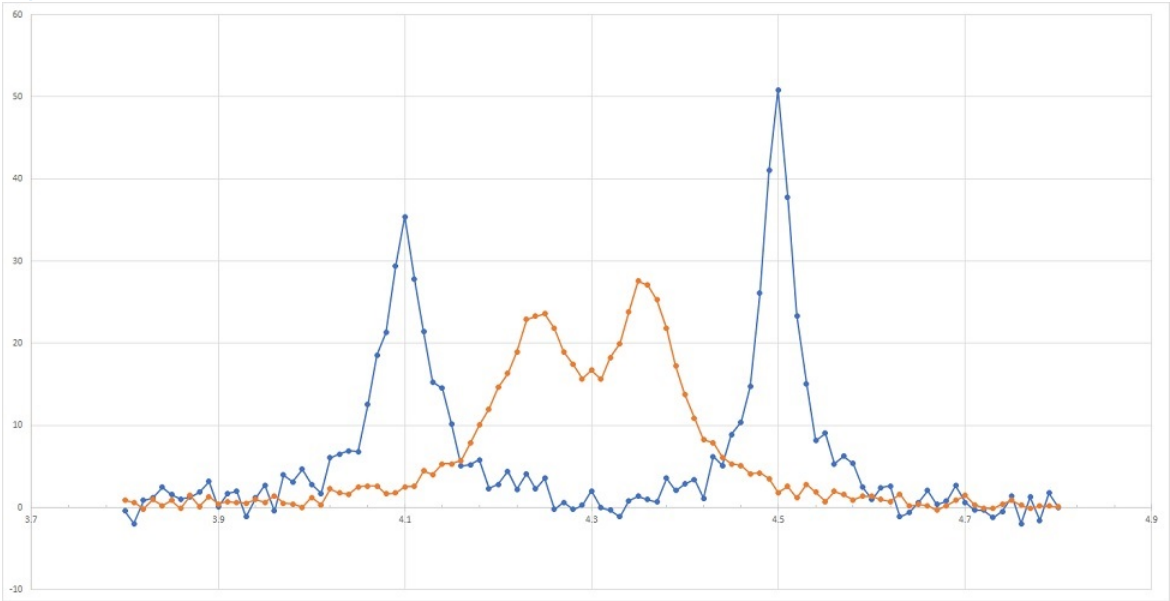

Figure IV.1.B Two spectra (two peaks in each) are shown.

Problems 1 – 3 below request your analysis of these spectra's sensitivity and resolution. Problems 4 and 5 target the relationship between the wavelength, frequency and energy of UV light often used in spectroscopic measurements.

Practice problem 1. Which spectrum in Figure IV.1.B (orange or blue) has greater sensitivity? Provide both qualitative and quantitative justification.

Practice problem 2. What are the peak positions of the two peaks of the blues spectrum? What are the peak positions of the two peaks of the orange spectrum?

Practice problem 3. Which spectrum (orange or blue) has greater resolution? Provide quantitative justification.

Practice problem 4. The absorbance of nucleic acids in UV range peaks at about 260 nm. What is the energy value (in Joules) of a quantum of UV light at this wavelength? Is this energy higher or lower than the energy of a covalent carbon-carbon bond ?

Practice problem 5. The absorbance of nucleic acids in UV range peaks at about 260 nm. What is the energy value (in Joules) of a quantum of UV light at this wavelength? Is this energy higher or lower than the energy of a hydrogen bond ?

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4.2: “Two Masses on a Spring” Model and Infrared (IR) Spectroscopy

This Chapter presents a basic view of a classical physics model of “two masses on a spring” and shows how this model can help understand and interpret infrared (IR) spectroscopy data. This will be one of our examples of some theoretical foundation for a certain type of spectroscopy. This theory-experiment connection is important for every type of spectroscopy because spectroscopic data always need to be interpreted and it is not possible without a sufficiently accurate theory.

Learning Objectives

- Understand the “mass on a spring” and “two masses on a spring” models and their capacity to predict the frequency of vibrations based on the mass and spring stiffness values
- Develop an appreciation of the relationship between a model (“two masses on a spring”) and a physical process (“covalent bond atomic vibrations”)
- Develop a sense how atomic masses and bond energy values define the resonance frequency (wavenumbers) in IR spectra

“One Mass on a Spring” Model

Figure IV.2.A shows an object of mass ***m*** attached to a massive wall via a classical spring of stiffness (spring force constant) ***k***. In classical physics, the motion of the object is guided by the Newton’s 2nd law stating that an object of mass m moving under the influence of force ***F*** will be moving with acceleration such that: ***F***= ***m•a***. Based on **Figure IV.2.A**, we can apply tools of Calculus to calculate the trajectory, ***x***(***t***), of the object.

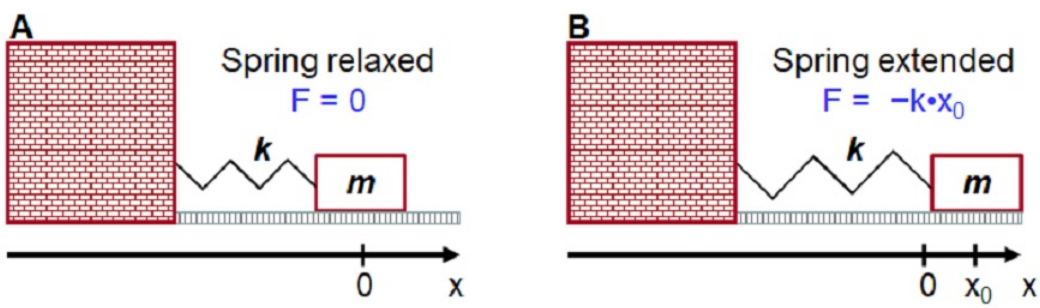

Figure IV.2.A. “A mass on a spring” model. (**A**) a weightless spring of stiffness ***k*** connects an object of mass ***m*** to a wall of an infinitely large mass. The spring is initially relaxed (neither extended nor compressed) and thus it exerts zero force on the object of mass ***m***. (**B**) The spring is extended by the initial value of x_0. The extended spring exerts initial force $F=-k * x_0$ on the object. Once the extended spring and mass are left alone, the system starts oscillatory motions driven by the force whose magnitude is directly proportional to the extension of the spring and direction is opposite to the spring compression or expansion. Equation IV.2.1 below describes the trajectory of the object of mass ***m***.

The oscillation frequency ν can be calculated from the Hooke’s law as a function of the object mass m and spring force constant k as follows:

$$F = -k \cdot x \tag{4.2.1}$$

$$m \cdot a = -k \cdot x \tag{4.2.2}$$

$$m \cdot \frac{d^2x}{dt^2} = -k \cdot x \tag{4.2.3}$$

$$x(t) = x_0 \cdot cos(\sqrt{\frac{k}{m}} \cdot t) \tag{4.2.4}$$

The obtained solution for the trajectory $x(t)$ corresponds to a type of motion called harmonic oscillator (position depends on time as a cosine function). The mass on a spring model and its calculus-based treatment can be extended to any system, in which the restoring force is pulling in the direction opposite to the displacement from equilibrium and its absolute value is proportional to the displacement.

Big picture: The trajectory, $x(t)$, of any system in which the absolute value of the restoring force is directly proportional to the value of the perturbation (displacement from equilibrium) and the direction of the restoring force is the opposite to the direction of the perturbation can be described by differential equations similar to **Equation IV.2.3** above. For any such a system, its dynamics over time will be based on the harmonic oscillator model: $x(t) = x_0 \cdot \cos(\omega \cdot t)$. Moreover, for any system in which the second derivative of its coordinate is directly proportional to —coordinate, $d^2x/dt^2 = -\omega^2 \cdot x(t)$, the dynamics of its trajectory will be harmonic: $x(t) = x_0 \cdot \cos(\omega \cdot t + \theta)$. In this equation, parameter ω defines the frequency of harmonic oscillations whereas parameters x_0 (initial amplitude or displacement) and θ (phase) are defined by the state of the system, $x(t)$, at time t=0.

"Two Masses on a Spring" Model

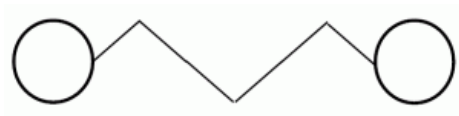

Figure IV.2.B. Two masses connected by a compressible spring can undergo harmonic/oscillatory back-and-forth motions. Click on the image to see the action.

Two masses, m_1 and m_2, are connected by a spring with the force constant k (**Figure IV.2.B**). If the spring is compressed or extended and then let go, the two masses will be performing back-and-forth oscillatory motions.

The oscillation frequency ν can be calculated from the Hooke's law as a function of reduced mass μ and spring force constant k *as follows:*

$$\nu = \frac{1}{2\pi} \cdot \sqrt{\frac{k}{\mu}} \tag{4.2.5}$$

$$\mu = \frac{m_1 \cdot m_2}{m_1 + m_2}$$

The units of frequency is 1/s (the number of oscillations per second) or s^{-1}. This unit has a special name: Hertz or **Hz**.

Infrared (IR) Spectroscopy

Oscillations of two atoms connected by a covalent bond can be approximated by the model described with the "two masses on a spring" model shown above. The atoms have small but very specific mass values defined by the chemical type of the atoms corrected for their specific isotopic makeup (the number of neutrons). The bond energy values can be related to the stiffness of the spring (k value in **equation IV.2.5** above).

Although this model lacks the capacity to describe some complicated cases, it is adequate to predict some trends in the IR signatures of a number of simple samples.

Infrared (IR) spectroscopy probes oscillations with wavelength values of 0.8 – 250 μm. The respective frequency values fall into the range of 1.20×10^{12} – 3.75×10^{14} Hz. Note that the smallest frequency value corresponds to the longest wavelength and vice versa. This is because the irradiation wavelength λ (in meters) and oscillation frequency ν (in Hz or 1/sec) are inversely proportional via the speed of light constant c (300,000,000 m/s):

$$\nu = c/\lambda \tag{4.2.6}$$

The characteristic oscillation frequency values measured by infrared spectroscopy are reported as so-called wavenumber, W, in units of cm^{-1}:

$$W = 1/\lambda \tag{4.2.7}$$

Thus, the wavenumber values for the IR wavelengths (0.8 – 250 μm) cover the range 40-12,500 cm^{-1}.

Big picture: Infrared spectroscopy of small molecules allows calculation of the bond stiffness (k constant) for a great range of covalent bonds from the experimentally observed wavenumbers and atomic mass values known from the periodic table. The stiffness constant values obtained via such IR measurements then are used as bond stiffness parameters for the molecular dynamics force fields outlined in Chapter III.1 Potential Energy Surface and Bonding Interactions. Likewise, crystallographic

structure of small molecules allow determination of the equilibrium covalent bond length and bond angle values, which are also used as corresponding force field parameters in molecular dynamics simulations.

IR Isotope Effect

Many types of atoms have several isotopic variants : ^{1}H, ^{2}H and ^{3}H for hydrogen, ^{12}C and ^{13}C for carbon etc. Isotopes of the same chemical type of an atom differ in the numbers of neutrons inside the nucleus: no (zero) neutrons in ^{1}H, one neutron in ^{2}H and two neutrons in ^{3}H. Therefore, the atomic mass values of isotopes of the same chemical type differ. This mass difference leads to different wavenumber values for IR bands when multiple isotopes of relevant atoms are present (the so-called IR "isotope effect").

Examples

Example 4.2.1

1. Two objects of 1.0 kg each are connected by a spring with stiffness value $\boldsymbol{k}$ = 5,000 N/m. What is the reduced mass $\boldsymbol{\mu}$ of this system? What will be the frequency $\boldsymbol{\nu}$ of free oscillations of this system if it gets excited and then left alone?

Answer: $\boldsymbol{\mu}$ = 0.50 kg; $\boldsymbol{\nu}$ = 15.9 Hz (oscillations per second)

Example 4.2.2

2. Two objects of 4.0 kg each are connected by a spring with stiffness value $\boldsymbol{k}$ = 5,000 N/m. What is the reduced mass $\boldsymbol{\mu}$ of this system? What will be the frequency $\boldsymbol{\nu}$ of free oscillations of this system if it gets excited and then left alone?

Answer: $\boldsymbol{\mu}$ = 2.0 kg; $\boldsymbol{\nu}$ = 7.96 Hz

Note that quadrupling of both mass values (in comparison with problem 1 above) resulted in quadrupling of the reduced mass, which in turn resulted in decreasing by one-half of the oscillation frequency.

Practice Problems

Practice problem 1. Two objects of 1.0 kg each are connected by a spring with stiffness value $\boldsymbol{k}$ = 20,000 N/m. What is the reduced mass $\boldsymbol{\mu}$ of this system? What will be the frequency $\boldsymbol{\nu}$ of free oscillations of this system if it is excited and then left alone?

Practice problem 2. What is the approximate value of reduced mass $\boldsymbol{\mu}$ on a system with one object having mass $\boldsymbol{M}$ much larger than the mass value $\boldsymbol{m}$ of the other object ?

Practice problem 3. An IR measurement of chemically pure carbon monoxide gas sample reported a stretch band (signal) with wavenumber of 2045 cm^{-1}. What is the vibration frequency of the carbon-oxygen bond in this sample?

Practice problem 4. Predict the IR wavenumber for carbon monoxide gas if all carbon atoms are of ^{13}C isotope type.

Practice problem 5. Your colleague suggested that multiple stretch bands at various frequency (wavenumber) values and of possibly lower intensity could be detected for the sample described in Practice Problem 3 above. State how many stretch bands you would expect to register for this sample, explain what the reason for the multiple bands can be and predict their wavenumber values in units of cm^{-1}.

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4.3: Quantum Mechanics and Quantum Oscillator Model

This Chapter describes basic models which allow to explain the phenomenon of quantization of energy levels for a system of electrons in atoms (aka quantum mechanical models of electron energy levels). The models presented here are much more basic than the ones used by quantum chemists today, yet the models below allow understand the basics of quantum mechanics as a branch of theory vital for modern experimental physics and chemistry.

Learning Objectives

- Grasp the scope and limits of applicability of quantum mechanics and the Schrödinger equation
- Develop understanding of the central variables of the Schrödinger equation: the potential energy and wave function
- Solve the Schrödinger equation for a “particle in box” model (quantum oscillator)
- Obtain quantitative description of the “orbitals”, their shapes and quantized energy levels for the “particle in box” model
- Make connections between the selection of the potential energy model and its validation via spectroscopic experiments

Quantum Mechanics (QM)

When the size of the system under investigation is too small (much smaller than the Bohr's radius, ~0.5 Angstrom, which describes sub-atomic scales), the laws of Newtonian mechanics are not describing the system correctly. Specifically, energy values available to sub-atomic systems cannot be correctly predicted by classical physics as we can learn from experimental observations. It was determined that laws of quantum mechanics need to be used for systems of sub-atomic scales. One of the key features of quantum mechanics is that every element of the system (e.g., an electron) is considered a wave rather than a particle. This leads to probabilistic descriptions of the system's states, which is fundamentally different from a deterministic description afforded for bulky systems by classical physics.

The quantum mechanical state of a sub-atomic system is described not by exact positions of all its elements but by a specific measure of probability called a **wave function**, $\boldsymbol{\Psi(x)}$, of the items to be located at any point ***x*** of the coordinate space. Two quantities, mass ***m*** and energy ***E***, have more or less the same meaning in quantum physics and in classical physics. Thus, let's consider the relationship between the wave function, mass and measurable energy forming the foundation of quantum mechanics in the form of the **Schrödinger equation**:

Equation 4.3.1

$$-\frac{h^2}{8\pi^2 m}\cdot\frac{d^2\Psi(x)}{dx^2}+U(x)\cdot\Psi(x) = E\cdot\Psi(x) \tag{4.3.1}$$

In this equation, ***h*** represents the **Plank's constant** (6.626 × 10^{-34} J×s or J/Hz), which describes a relationship between energy (measured in Joules) and frequency (measured in Hz) for our entire Universe. The quantity ***U(x)*** represents the potential energy of the system at any given point ***x*** of the coordinate space. The exact mathematical formula for ***U(x)*** depends on the system under investigation and on the model of the system researchers propose. The low values of ***U(x)*** in some range of ***x*** values correspond to a “low penalty” and thus high probability for the system to be located in this particular set of points. Numerically, this probability can be expressed as the square of the absolute value of the wave function over this range of ***x*** vales. Higher ***U(x)*** values correspond to “higher penalty” for the system to be at particular values of ***x*** and thus low probability of its to be found at these locations. For any subatomic particle under consideration (e.g. an electron), the ***U(x)*** is expected to be low within the atomic boundaries and high – outside. See a specific example below.

Quantum “Particle in a Box” Model and Electron Orbitals

The “particle in a box” model is one of the simplest ones for quantum particles (e.g. for an electron within an atom). Under this model, ***U(x)*** has the value of zero (no penalty) within a certain range of ***x*** (from 0 to *L*, *where L represents the linear size of the system*) around a “center of the atom” at ***x***=*L/2*. Outside of this range (***x***<*0* and ***x***>*L*), ***U(x)*** = +∞, which is equivalent of $\boldsymbol{\Psi(x)}$ = 0 outside the atom (the penalty for the electron to leave the atom is infinitely high, which means that the electron stays within the atom). Within the atom defined as a one-dimensional segment of space ($0 \le x \le L$) the Schrödinger equation adopts a simple form:

Equation IV.3.4.3.2

$$-\frac{h^2}{8\pi^2 m}\cdot\frac{d^2\Psi(x)}{dx^2} = E\cdot\Psi(x) \tag{4.3.2}$$

It can be noted that in the formula above the second derivative of the target function ($\Psi(x)$ in this case) is directly proportional to the function itself with the negative proportionality factor. As we saw in the previous Chapter (see equations IV.2.3, IV.2.4 and the Big picture comment which follows them), a harmonic function of the sine or cosine type works as an answer to this differential equation. Specifically, the following linked forms for $\boldsymbol{\Psi(x)}$ and $\boldsymbol{E}$ provide solutions for the differential equation above and satisfies the condition of the wave function adopting the value of zero at the walls of the well:

Equation IV.3.4.3.4

$$\Psi_n(x) = \sqrt{\frac{2}{L}} sin(\frac{n\pi}{L}x) \tag{4.3.3}$$

$$E_n = \frac{h^2 n^2}{8mL^2} \tag{4.3.4}$$

Analysis of Eq. IV.3.3 shows that at ***n***=1 the "orbital" described by the wave function is centered or has highest probability around the center of the well (***x***=*L*/2). The energy of the particle occupying this "first" orbital (***n***=1) has the energy of $\boldsymbol{E_1} = \{\boldsymbol{h}^2\}/\{8\boldsymbol{mL}^2\}$, a value specific only to this "orbital". As per equation IV.3.3 above, the energy values will grow proportionately to $\boldsymbol{n^2}$, thus $\boldsymbol{E_2} = 4\boldsymbol{E_1}$, $\boldsymbol{E_3} = 9\boldsymbol{E_1}$, etc. Actual probability of a quantum particle to be located within a range of dx around a certain value of x is proportional to $[\boldsymbol{\Psi(x)}]^2\boldsymbol{dx}$ Thus, the actual "shape" of the orbital is defined by $[\boldsymbol{\Psi(x)}]^2$ and not by the wave function itself.

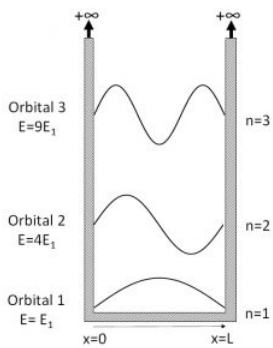

Figure IV.3.A Quantum "particle in a box" model. Wave function shapes and the energy values for the orbitals with the principal quantum number ***n*** of 1, 2 and 3 are shown.

> Big picture: Developing mathematical descriptions for ***U(x)*** for various atoms and molecules belongs to the domain of **quantum physics** and constitutes a focus of **computational chemistry**. The models developed by computational chemists need to be verified by experimental **spectroscopic measurements** (see next chapter) in order to be confirmed and accepted or to be found incompatible with the experimental data and modified or discarded. This process is **iterative**: currently, many models of ***U(x)*** are accepted for various relevant chemical systems while others have been rejected.

QM Example: electrons in a hydrogen atom vs. in a diatomic hydrogen molecule

As the environment for the quantum particle changes, its potential energy ***U***(***x***) changes as well (that is our models need to take these changes into account). Let's consider a case of formation of a diatomic H_2 molecule from two isolated hydrogen atoms already discussed in this textbook, see **Figure II.3.B**. As the di-atomic H_2 molecule forms, the potential energy ***U***(***x***) for each out of the two electrons changes: from being a part of an atom attracted to just a single nucleus (a single proton), each of these electrons now exist in a di-nuclear molecule being shared (that is influenced) by two nuclei. This is a major change for the potential energy: ***U***(***x***) is now different and two Molecular Orbitals (MO) now are available for each of the two electrons as opposed to two Atomic Orbitals, AO, available initially for each electron separately. According to the Aufbau principle (recall the General Chemistry material), the two electrons occupy the lowest energy Molecular Orbital, leaving the other one – higher energy MO – unoccupied.

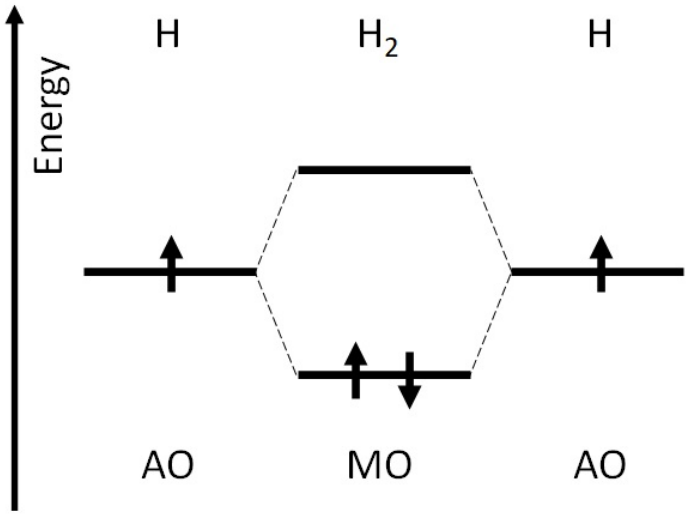

Figure IV.3.B Atomic Orbitals (AO) for isolated hydrogen atoms, Molecular Orbitals (occupied and unoccupied) for an H_2 molecule and their relative energy values. Dashed lines indicate that the MOs are "derived" from specific AOs.

Key result: An exact mathematical description of $\boldsymbol{U}(\boldsymbol{x})$ for an electron in the field of two nuclei is too complex for the scope of this textbook, so we just see the outcome of the respective modeling and experiments. One key result here is that the energy value for the electrons in the di-atomic H_2 system is lower than the respective value in the isolated H atoms. This explains why **bond formation is an exothermic process**.

Practice Problems

Practice problem 1. Under the "particle in a box" model, the first orbital ($\boldsymbol{n}$=1) has its shape similar to that of an *S-orbitals* in atoms. What can you say about the shape of the second orbital ($\boldsymbol{n}$=2) predicted by this model? What an actual electron orbital is the second orbital predicted here is similar to in terms of its "shape"?
Hint: take into account that the actual shape of the orbital in the 3D space is described by the *square* of the wave function.

Practice problem 2. Working within the "particle in a box" model, prove that the wave function represented by equation 3 in this chapter actually works as a solution for differential equation 2 (Schrödinger equation) "within the well" or within an atom.

Practice problem 3. According to the "quantum particle in a box" model, the potential energy outside of the box or well is infinitely high: $\boldsymbol{U}(\boldsymbol{x}) = +\infty$. What mathematical function describes the wave function outside of the well (or box, or an atom) according to this model? Does this function describe correctly the fundamental fact that an electron stays within the atom and does not venture outside of it?

Practice problem 4*. If one applies the "particle in a box" model to the electron within a hydrogen atom (H), is the distribution of electron energy levels ($\boldsymbol{E_n} \propto \boldsymbol{n^2}$) consistent with the light emission bands of this atom?

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4.4: Fluorescence and Phosphorescence

This Chapter describes fluorescent spectroscopy based on the QM theory outlined in the previous Chapter. Electron energy levels within the sample define the spectra of absorption as well as of emission (fluorescence and phosphorescence). Relationship between fluorescent spectroscopy and UV range of light is described.

Learning Objectives

- Understand how irradiation absorption leads to the excitation of electrons in the system
- Distinguish emission via fluorescence from emission via phosphorescence
- Grasp the kinetics effect: why phosphorescence often occurs much slower than fluorescence within the same system
- Become familiar with the processes described by the Jablonski diagram
- Develop a sense of how the average wavelength values for related absorption, fluorescence and phosphorescence are related

Absorption

In a system with quantized energy levels, its particles (e.g. electrons) in general occupy the lowest energy levels/states. An incoming quantum of radiation with the energy $\boldsymbol{E}$ and frequency $\boldsymbol{v}$ ($\boldsymbol{E} = \boldsymbol{hv}$) can excite an electron from a lower energy level (ground state) to an unoccupied higher energy level (excited state) if the energy of the incoming quantum (e.g. photon) matches or resonates with the difference between the energy levels of the excited and ground states ($\boldsymbol{E} \geq \boldsymbol{E_{excited}} - \boldsymbol{E_{ground}}$). **Figure IV.4.A** shows the processes of irradiation absorption and subsequent emission via two types of pathways, fluorescence and phosphorescence.

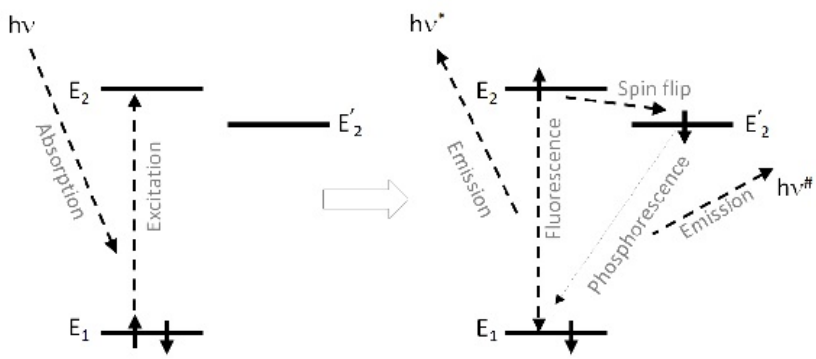


Figure IV.4.A The processes of radiation absorption (left) and emission (right).

If the energy of an incoming quantum is lower than the $\boldsymbol{E_{excited}} - \boldsymbol{E_{ground}}$ difference ($\boldsymbol{E} < \boldsymbol{E_{excited}} - \boldsymbol{E_{ground}}$), the absorption of the incoming irradiation will not happen. For example, two incoming quanta of the same energy will not excite a "ground ⇒ excited" transition with the energy double of the individual quantum of radiation.

If the energy of an incoming quantum is equal to the $\boldsymbol{E_{excited}} - \boldsymbol{E_{ground}}$ difference ($\boldsymbol{E} = \boldsymbol{E_{excited}} - \boldsymbol{E_{ground}}$), the upward transition will happen and the excited particle (e.g. electron) will have its energy increased. In terms of the quantum description (Schrödinger equation, equation IV.3.1), the potential energy function defining the states of the excited electron will stay the same, but the wave function will change to the one with a higher energy level and different orbital shape.

If the energy of an incoming quantum is greater than the $\boldsymbol{E_{excited}} - \boldsymbol{E_{ground}}$ difference ($\boldsymbol{E} > \boldsymbol{E_{excited}} - \boldsymbol{E_{ground}}$), the absorption of the incoming irradiation will take place and the system can go to a higher energy state (excited). If the energy of the incoming quantum is high enough, its absorption might cause major alterations of the system, e.g. bond breakage (excited electrons leave shared molecular orbitals thus depopulating them), or ionization (excited electrons leaving the atom/molecule).

Fluorescence

An electron excited to a higher unoccupied level with energy value $\boldsymbol{E_2}$ can "relax" back to the original ground level $\boldsymbol{E_1}$ (**Figure IV.4.A**) via a process called fluorescence. Fluorescence takes place rather quickly, in 10^{-8}-10^{-5} seconds, due to the fact that the excited electron simply comes back to its original ground state without any quantum number alterations apart from the orbital number. During fluorescence, most of the energy of the initial incoming irradiation is emitted back because the excitation and

relaxation occur between the same two levels with energies of E_2 and E_1. Note that the fluorescence emission frequency v^* is somewhat lower than the absorption frequency ($v^* < v$) due to inevitable energy losses during absorption and fluorescence.

Phosphorescence

Another pathway through which the energy of excitation can be recovered is called phosphorescence. During phosphorescence, the excited particle (e.g., an electron), first relaxes to another "excited state" with slightly lower energy level of E'_2 ($E'_2<E_2$). With high likelihood, a spin flip occurs during such a step ($E_2 \Rightarrow E'_2$), which prevents this still excited electron from relaxing back to its original ground state (E_1) right away if there is another electron there with the same spin (**Figure IV.4.A**). Therefore, it will take a substantial time to reverse the spin flip and allow the excited electron to relax back to the ground state. As a result, phosphorescence may last a lot longer than fluorescence: from milliseconds to days and even years, depending on the specific chemical system. Because $E'_2<E_2$, the main phosphorescence frequency $v^{\#}$ is smaller than fluorescence frequency v^* ($v^{\#} < v^*$).

Jablonski Diagram

In real systems, most molecules consist of multiple atoms, which complicates the set of absorption, fluorescence and phosphorescence transitions. In addition, most electronic quantum energy levels (E_1, E_2, E'_2 etc) undergo internal split due to vibrational motion of the relevant nuclei (vibrational relaxation). Thus, the overall picture includes many transitions of each type and many levels and sub-levels. A complete set of relevant transitions for a specific molecule is referred to as the Jablonksi diagram (**Figure IV.4.B**).

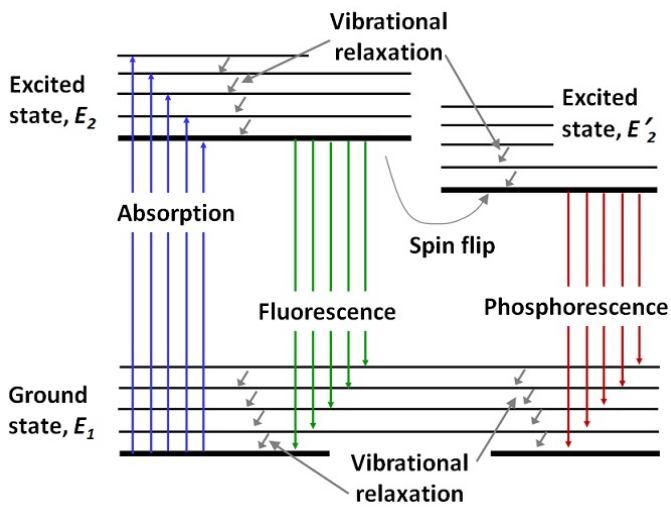


Figure IV.4.B The Jablonski diagram. Note, that each electronic energy level (E_1, E_2, E'_2 etc) is in turn split into vibrational sublevels. The energy difference between these vibrational sublevels within an electronic level are small enough, so vibrational relaxation between the sublevels occurs actively all the time, which complicates the overall absorption/excitation and relaxation/emission spectra and their interpretation.

Because of the complexity of the Jablonski diagram for real molecules, the respective absorption, fluorescence and phosphorescence spectra can be messy in terms of the line shapes (**Figure IV.4.C**). But in general and on average, the relationship between the energy (and thus frequency v as $E=hv$) of the absorption, fluorescence and phosphorescence is clear : $E_{absorption} \geq E_{fluorescence} \geq E_{phosphorescence}$. Due to the inverse relationship between the frequency v and wavelength λ ($c=\lambda v$), the trends in wavelength values are the opposite: $\lambda_{absorption} \leq \lambda_{fluorescence} \leq \lambda_{phosphorescence}$ (**Figure IV.4.C**).

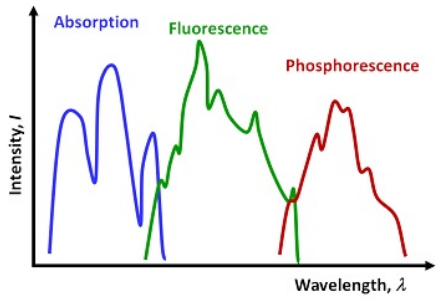
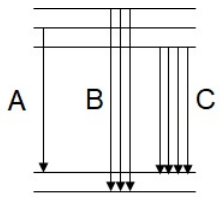


Figure IV.4.C General spectra for absorption, fluorescence and phosphorescence. Note, that the relative wavelengths of the three respective groups are based on the energy of the respective transition presented on the Jablonski diagram (Figure IV.4.B).

Practice problems



In a certain system, three pathways, A, B and C (see figure above), are available for relaxation of excited electrons via **fluorescence**. The energy change of the downward transitions are as follows:
$\Delta E_A = 7.9\times10^{-22}$ kJ, $\Delta E_B = 9.9\times10^{-22}$ kJ, and $\Delta E_C = 4.9\times10^{-22}$ kJ

The number of arrows for each transition is proportional to the probability of each relaxation pathway: the more arrows for the pathway, the more exited electrons follow the pathway to relax.

Practice problem 1. Which pathway(s) generate photons which are capable of disrupting a double –C=C – bond? Justify quantitatively.

Practice problem 2. Provide a spectrum representing the three transitions with the X axis in nm. The spectrum should accurately represent the relative probability of the transitions. Each spectral line should be drawn as a thin vertical bar: assume uniform line widths for all the peaks.

Practice problem 3. Consider the same system at thermal equilibrium. If the system has at its ground state energy levels 1,000,000 moles of electrons, how many electrons will spontaneously occupy all of the "excited" states combined? Justify quantitatively.

Practice problem 4. You are analyzing the Jablonski diagram for two molecules **X** and **Y**. Molecule **X** is double the size of molecule **Y** (has twice as many atoms). In what ways do you expect the Jablonski diagram for molecule **X** could be different from that of molecule **Y**?

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CHAPTER OVERVIEW

5: Nuclear Magnetic Resonance (NMR) Spectroscopy - Introduction

5.1: Nuclear Spin and Magnetic Field

5.2: Basic NMR Excite-Record Experiment and Related Spectrum

5.3: Chemical shift in units of Hz and ppm

5.4: Fourier Transformation (FT)- from an FID to a Spectrum

5.5: Effects of the Sample, Equipment and Recording Regimes on the NMR Spectral Sensitivity and Resolution.

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5.1: Nuclear Spin and Magnetic Field

In this Chapter, we will discuss why certain types of atomic nuclei are sensitive to the external magnetic field due to their quantum property known as the "spin". We further will cover the dependence between the magnetic sensitivity of atomic nuclei and quantization of their energy levels once these atoms are exposed to the magnetic fields. After completing this Chapter, you will be able to quantitatively determine the frequency of magnetic resonance of a specific type of atomic nucleus exposed to a magnetic filed of certain strength.

Learning Objectives

- Understand the connection between nuclear quantum spin and nuclear magnetic moment
- Grasp the effect of external magnetic field on the quantization of nuclear spin energy levels (Zeeman split)
- Develop appreciation of the dependence of the resonance nuclear frequency on the strength of the magnetic field.

Nuclear "Spin"

Atomic nucleus is a particle of subatomic scale. Thus, it can be described by a set of "quantum properties". One of them is called "nuclear spin", which fundamentally is related to the sensitivity of the nucleus to the effects of external magnetic fields (**Figure V.I.A**). In some sense (which is an oversimplification), an atomic nucleus with a non-zero spin value can be viewed as a little magnet, sensitive to the presence of an external magnetic field.

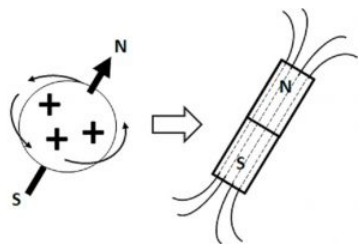

Figure V.I.A Non-zero nuclear "spin" provides a "quantum magnetic moment" to the atomic nucleus.

The nuclear spin value depends on the proton/neutron composition of the nucleus the atom or isotope. **Table V.I.I** lists some examples of nuclei with spin values of 0, ½, and 1 (the table does not include examples of spin values above 1):

Table V.1.I

Spin	Relevant Isotopes	Common features of the nuclei/isotopes
0	^{12}C, ^{16}O	Nuclei composed of even numbers of protons and even numbers of neutrons
½	^{1}H, ^{13}C, ^{15}N, ^{31}P, ^{19}F, ^{129}Xe	Nuclei composed of odd number of nucleons (protons and neutrons)
1	^{2}H, ^{14}N	Nuclei composed of odd numbers of protons and odd numbers of neutrons

Zeeman Splitting of Spin-½ Nuclei

In this textbook, we will almost exclusively consider the spin-½ nuclei and their interactions with external magnetic fields. Such interactions are at the heart of Nuclear Magnetic Resonance (NMR) spectroscopy, a central experimental method according to the classification of the American Chemical Society. As we will see in this and next chapters, modern NMR spectroscopy is widely utilized to address many problems in biochemistry, biophysics and other areas of life sciences in general. **Figure V.I.B** shows a modern NMR spectroscopy system and its central components: the superconducting magnet with the probe (where the sample in an NMR tube is subjected to external magnetic fields) and consoleor spectrometer (an elaborate assembly of advanced electronics circuits collectively controlling the process of magnetic field application and data recording).

Figure V.I.B A modern NMR spectrometer: Bruker Avance III HD (a cabinet on the background) equipped with a 16.4 Tesla (700 MHz, ^{1}H frequency) superconducting magnet (a large cylinder on three vibration-canceling suspension legs).

Under an external magnetic field B_o, the energy ***E(m)*** of a quantum particle with a spin projection m on the axis of B_o (magnetic field is a vector!) is given by the following formula:

Equation V.1.5.1.1

$$E(m) = -m \cdot h \cdot \gamma \cdot B_0 \tag{5.1.1}$$

In the formula above, m denotes quantized spin projection value on the axis of B_o: only m values of +½ and -½ are possible for spin-½ nuclei. Next, ***h*** denotes Plank's constant, which we first introduced in Chapter IV.3. The value of **γ** is specific to each isotope type, with γ values for some spin-½ isotopes listed in **Table V.I.II**.

Table V.1.II

Spin-½ Nucleus	^{1}H	^{19}F	^{31}P	^{129}Xe	^{13}C	^{15}N
γ, MHz/Tesla	42.58	40.05	17.24	-11.78	10.71	-4.32
|**γ**| relative to ^{1}H	1.0	0.94	0.40	0.28	0.25	0.10

Because only two spin projection values (½ and -½) are possible for spin-½ nuclei, formula (**1**) above tells that only two states (projections on B_o) with their respective two energy levels ***E(½)*** , ***E(-½)*** and energy difference (split) ***ΔE = E(-½) – E(½)*** are possible for such spins placed in an external magnetic field B_o:

Equation 5.1.4

$$E(1/2) = -\frac{1}{2} \cdot h \cdot \gamma \cdot B_0 \tag{5.1.2}$$

$$E(-1/2) = \frac{1}{2} \cdot h \cdot \gamma \cdot B_0 \tag{5.1.3}$$

$$\Delta E = h \cdot \gamma \cdot B_0 \tag{5.1.4}$$

A Split Spin-½ System and Nuclear Magnetic Resonance effect

Thus, a population of spin-½ particles will split into two sub-populations ***N(½)*** and ***N(-½)*** with their respective energy levels: lower value ***E(½)*** and higher value ***E(-½)***. At thermal equilibrium, the respective values of ***N(½)*** and ***N(-½)*** will be related via the Boltzmann distribution (covered in Chapter I.5):

Equation V.1.5.1.5

$$\frac{N(-1/2)}{N(1/2)} = e^{-\frac{\Delta E}{k_B \cdot T}} \tag{5.1.5}$$

Equation V.1.5.1.6

$$\frac{N(-1/2)}{N(1/2)} = e^{-\frac{h \cdot \gamma \cdot B_0}{k_B \cdot T}} \tag{5.1.6}$$

Such a separation into two sub-population of a nuclear spin-½ system under an external magnetic field B_o is called the **Zeeman splitting**. This split, equilibrated system of spin-½ nuclei can be perturbed out of equilibrium by an energy quanta whose frequency corresponds to the ***ΔE*** value (resonance excitation). After the system is perturbed in this way, it can be allowed to relax back to the thermal equilibrium (Boltzmann distribution) and the energy emitted during relaxation can be detected. Energy required to excite a nuclear spin-½ system and energy detected during relaxation of the excited system constitute the essence of **Nuclear Magnetic Resonance (NMR)** effect.

Example 5.1.1

Let's calculate the value of the Zeeman energy splitting **Δ*E*** for protons (^{1}H) under an external magnetic field B_0 = 11.7 Tesla (as in eq. (2) above):

Solution

Δ*E* = 6.626 × 10^{-34} {J/Hz} × 42.58 {MHz/Tesla} × 10^6 {Hz} / 1 {MHz} × 11.7 {Tesla} = 3.30 × 10^{-25} J

Practice Problems

Problem 1. List at least four types of atoms/isotopes whose nuclei have spin-½ properties.

Problem 2. Calculate **Δ*E*** value (Zeeman split) in units of Joules for each of the spin-½ nuclei listed in **Table V.1.II** above in an external magnetic field B_o of 11.7 Tesla. What is the effect of the gyromagnetic ratio γ on the value of **Δ*E*** ?

Problem 3. Explore the effect of the strength of the external magnetic field value B_o on the value of the Zeeman energy split **Δ*E*** by doing problem 2 above at B_o = 23.4 Tesla.

Problem 4. The term **γ**·B_o appears in equations (1), (2) and (3) above and will appear in several key formulas later. What are the units of this product **γ**·B_o? What does this observation tell you about the physical meaning of **γ**·B_o (what processes it might represent)? Take a note of it as it will become relevant in the next chapter.

Problem 5. Compare the nuclear spin-½ Zeeman energy splitting (**Δ*E***) value calculated in the Examples above with the electron energy level gap corresponding to UV irradiation of **λ**=280 nm. Which energy gap is greater: the one for a ^{1}H system at B_o=11.7 Tesla or the one corresponding to UV light of **λ**=280 nm?

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5.2: Basic NMR Excite-Record Experiment and Related Spectrum

In this Chapter, we will consider the most basic type of NMR experiment: acquiring a 1D data for a spin-½ sample pre-equilibrated in an external, static magnetic field. We will see how the properties of the NMR setup (e.g., static magnet power B_o) and of the target system (e.g. the gyromagnetic ratio value γ) are affecting the obtained spectra. Finally, we will briefly explain why we are seeing spectra at all, that is why nuclei of the same chemical type (and thus γ value) report different NMR frequencies.

Learning Objectives

- Distinguish the three basic stages of a 1D NMR experiment (equilibration, excitation pulse, recording FID) and understand their purpose
- Get the basic idea of an excitation pulse of alternating B_1 field and of its roles in NMR experiments (system excitation)
- Develop understanding between the NMR resonance frequency of a spin-½ nuclei of a certain chemical type and magnetic field strength $\boldsymbol{B_0}$
- Understand why we are getting a spectrum with multiple lines instead of just one single peak from all the nuclei of the same type (the concept of shielding/de-shielding and B_{eff})

The Basic One-Dimensional NMR experimental scheme for a spin-½ system

Figure V.2.1 shows the three main stages of a basic NMR experiment used to record a one-dimensional or 1D spectrum for a spin-½ sample (e.g. ^{1}H, ^{13}C, ^{31}P, ^{19}F etc). The three main stages of the experiment are (1) equilibration, (2) excitation and (3) recording. Let's consider each one in some detail.

During the first stage, the sample (spin-½) system is operating solely under the influence of a constant magnetic field B_o, oriented along the long axis of the main magnet (Z axis). As described in the previous section (Chapter V.1), the spin population is split into two subgroups (the Zeeman splitting) with their distinct energy values. According to the Boltzmann distribution formula (Chapter I.5 , Chapter V.1), the population of the lower-energy state is greater than the population of the higher energy state, which creates an overall excess of the lower-energy state spins and population imbalance.

Stage (2) is designed to perturb the spin system out of the Boltzmann equilibrium established during the initial stage of the experiment. This is typically achieved by applying the second magnetic field (called B_1) oriented in the direction perpendicular to B_o (magnetic fields are vector quantities, they have a direction!). The parameters of B_1 field (e.g. its orientation, power frequency and duration of acting) are chosen in such a way as to "excite" a substantial fraction of the lower-energy spins to the higher energy level. **Figure V.2.1** demonstrates this by showing more spin arrows located on the higher energy level at the expense of the lower-energy state. Once "excited", the system has a new, non-Boltzmann distribution of populations at the two energy levels.

The last, third stage of the experiment starts at the moment when B_1 field is turned off. At this stage, the spin system is operating again under the influence of B_o only, just like during stage (1), but the system is out of equilibrium at the start. Thus the spins start "flipping" (changing energy levels stochastically) until the system restores its Boltzmann populations at the two energy levels. During this process of equilibration and spin "flipping" the magnetic field of the sample is changing ("flipping" spins are little "flipping" magnets!). According to the laws of electricity and magnetism, changing magnetic field will create alternating electric current in the nearby electric circuits. This very current is being detected as the raw NMR "signal" or Free Induction Decay (FID).

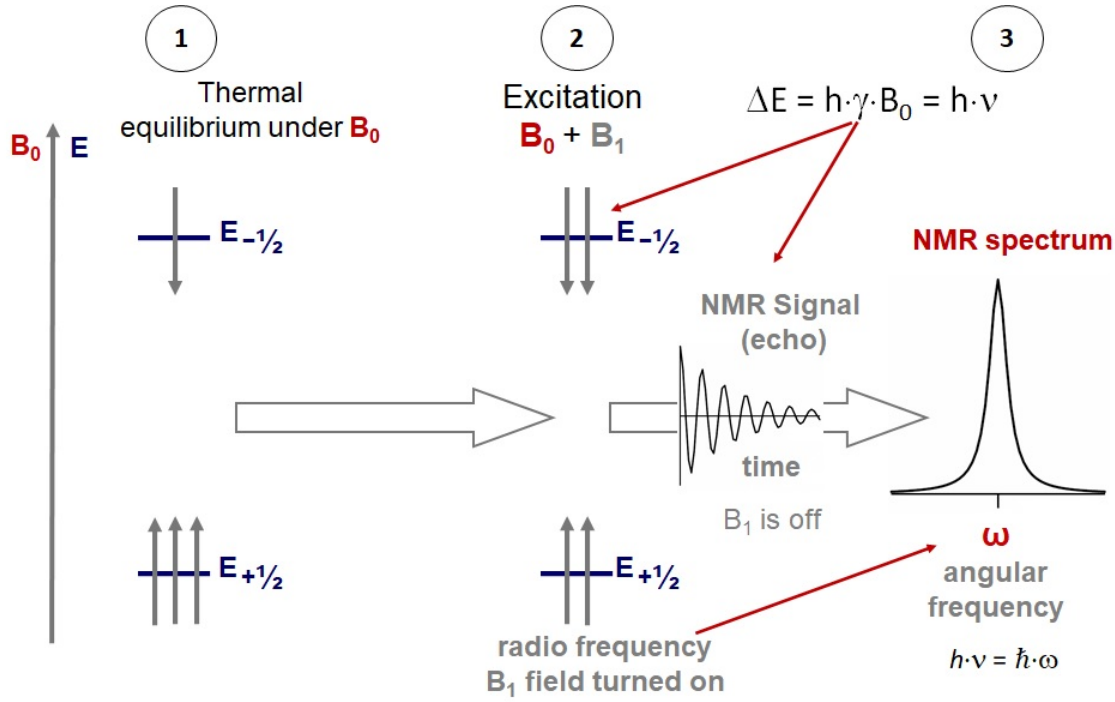

Figure V.2.1 Basic 1D NMR experiment. The three basic stages are labeled with numbers in circles as follows: (1) Thermal equilibration of the sample under constant magnetic field B_o; (2) perturbing the sample out of equilibrium (excitation) with an alternating magnetic field B_1 ; (3) Recording the "echo" (FID) from the perturbed system as it relaxes back to the equilibrium while field B1 is turned off (field B0 is always on). Constant magnetic field strength value B_o (stages 1 and 2) and angular frequency $\omega = 2\cdot\pi\cdot\nu$ (stage 3) are shown red text color to highlight the fact that these values are strongly interconnected: B_o value defines the properties of the spectrum including the angular frequency or the position of the peak along the X-axis.

Proton NMR: a Spectrum with Multiple Peaks or a Single Peak for All the Protons ?

From the description above, it appears that for all the spin-½ nuclei with the same gyromagnetic ratio value γ (e.g., for all ^{1}H in the sample) the energy gap **Δ*E*** between the spin-up (spin projection +½) and spin-down (spin projection -½) states should be the same since this value is defined by the product $\gamma\cdot B_o$, [Chapter V.1, equation (2)]. It is correct that the γ value is the same for all the nuclei of the same type in the sample (and in the Universe for that matter) but the intensity of the magnetic field is not equal to exactly B_o for every nucleus.

Here is why. A biological molecule (e.g. a protein or DNA) contains protons (^{1}H) existing in a variety of local electronic environments, e.g. a methyl group protons (CH_3) are sharing electrons with a single carbon atom, whereas aromatic protons in a phenylalanine side chain "share" multiple electrons of the aromatic ring. Under the influence of a combination of external magnetic fields B_o (constant) and B_1 (alternating), these electron groups react differently and thus produce different local "current effects", which in turn generate different local (small but still relevant!) magnetic fields of their own. Thus, the methyl group protons and aromatic protons experience different effective magnetic fields $\mathbf{B_{eff}}$, which in most cases will be *not* exactly equal to B_o.

Equation V.2.???

$$\Delta E_{eff} = h\cdot\gamma\cdot B_{eff} \tag{5.2.1}$$

$$\Delta E_{eff} = h\cdot\nu_{eff} \tag{5.2.2}$$

Equation V.2.1 establishes that the magnitude of Zeeman energy splitting is defined by the gyromagnetic ratio γ of the specific nucleus type and the effective value of the magnetic field B_{eff}. To see how this logic extends even further, let us consider backbone amide protons (^1H_N) in a protein sample. Depending on the chemical type of each amino acid residue, its amide proton will have slightly different chemical and thus electronic environment. Moreover, for the residues positioned inside a folded protein structure local non-covalent neighbors also differ and would influence the local magnetic field through their local electric currents. Thus, for all the amide protons (which are chemically the same type of nuclei but structurally are different), the local magnetic fields B_{eff} may be different for each individual amide proton ^1H_N. The same logic applies to al other types of protons or other spin-½ nuclei in a protein molecule. Combined, the local electron effects or currents may lead to B_{eff} be lower than the main

magnet field B_o (so-called "shielding effect") or greater than B_o ("de-shielding"). The quantitative shielding effect is often described explicitly as a unitless quantity σ of relatively small absolute value and the overall algebraic relationships between B_o, B_{eff} and σ are as follows:

Equation V.2.5.2.7

$$\Delta E_{eff} = h \cdot \nu_{eff} \tag{5.2.3}$$

$$\Delta E_{eff} = \hbar \cdot \omega_{eff} \tag{5.2.4}$$

$$\nu_{eff} = \gamma \cdot B_{eff} \tag{5.2.5}$$

$$\omega_{ref} = 2 \cdot \pi \cdot \nu_{ref} \tag{5.2.6}$$

$$B_{eff} = B_0 \cdot (1 - \sigma) \tag{5.2.7}$$

In Equation V.2.2, we introduced another flavor of frequency, angular frequency ω, which is reported in units radians/sec (instead of oscillations/second as for the oscillation frequency ν). With the angular frequency, we also had to introduce the so-called reduced Plank constant $\hbar$, which is the Plank's constant divided by 2π. This seems a bit redundant (it is!) but the radial frequency can be simply more convenient to use for us later on, so stay tuned.

The frequency ν_{eff} value defined in this way is called *Larmor frequency*. So, why does a protein sample report a spectrum of ^{1}H signals with different frequencies instead of a single "proton peak"? This is because each type of proton experiences a slightly different effective magnetic field B_{eff} due to local field shielding and thus operates under a slightly different energy gap $\Delta E_{eff} = h\gamma \cdot B_{eff}$ as in equation (1) above. In turn, $\Delta E_{eff} = h \cdot \nu_{eff} = \hbar \cdot \omega_{eff}$ (the frequency of the transition across an energy gap is directly proportional to the energy gap value) and thus $\nu_{eff} = \gamma \cdot B_{eff}$. So, the registered frequency of transition (resonance frequency!) would be different for different protons because each one of them operates under a different effective magnetic field. **Figure V.2.1** shows an ^{1}H NMR spectrum of a modest size proteins sample.

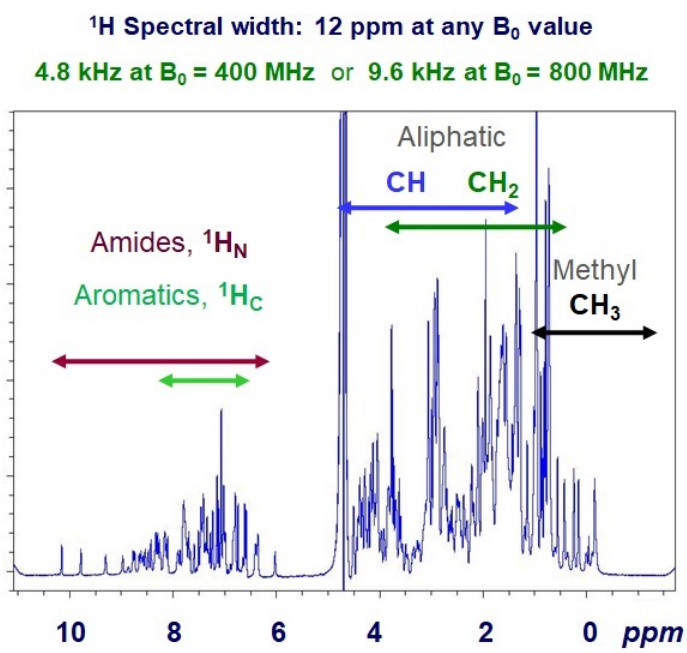

Figure V.2.2 A 1D ^{1}H spectrum of a protein sample (S.L. Smirnov, WWU, Bellingham, WA). Note that the spectrum width of the spectrum expressed in units of Hz does depend on the power of the magnet B_o (expressed in units of Hz).

In **Figure V2.2**, the central, strongest resonance (at 4.8 ppm; the one which goes way above the range of the Y-axis shown) comes from protons in water ([^1H_2O] ≈ 55 M !) and this position approximately corresponds to the B_o magnetic field. With respect to the water spectral line, ^{1}H nuclei from aliphatic groups (CH, CH_2 and CH_3) are di-shielded and thus experience B_{eff} fields exceeding B_o ($B_{eff} > B_o$, "up-field shift"). On the other side of the spectrum, amide and aromatic protons are experiencing B_{eff} lower than B_o ($B_{eff} < B_o$, such nuclei are shifted "down-field"). Fundamentally, the difference between the resonance frequency $\Delta\nu$ between any two nuclei a and b (of the same chemical type, e.g. ^{1}H) reports a respective difference between their respective B_{eff} values:

Equation V.2.5.2.8

$$\nu_{eff,a} - \nu_{eff,b} = \gamma \cdot (B_{eff,a} - B_{eff,b}) \tag{5.2.8}$$

Example 5.2.1

Determine the approximate value of resonance frequency ν of protons in an NMR spectrometer equipped with a magnet with a field strength of B_o=11.7 Tesla.

Solution

According to formula (1) above $\boldsymbol{\nu = \gamma \cdot B_0}$= 42.58 {MHz/Tesla} · 11.7 {Tesla} = 498.2 MHz.

Such a unit would be referred to (and labeled as) a 500 MHz magnet.

Example 5.2.2

It is stated in **Figure V.2.2** that a proton spectrum of a certain protein sample covers frequencies within the range of 4.8 kHz (4800 Hz) if the data recorded on a 400 MHz magnet. Calculate the field strength B_o of such a magnet and the difference in local fields B_{eff} between the two extreme frequency points (not peaks!) within the spectrum ($\boldsymbol{B_{ff,\ rightmost} - B_{eff,\ leftmost}}$). Calculate both requested values in units of Tesla. Compare the two numerical values.

Solution

B_o= $\boldsymbol{\nu / \gamma}$ = 400 {MHz} / 42.58 {MHz/Tesla} = 9.39 Tesla

$\boldsymbol{B_{ff,\ rightmost} - B_{eff,\ leftmost}}$ = ($\boldsymbol{\nu_{rightmost} - \nu_{leftmost}}$)/ $\boldsymbol{\gamma}$ = (***spectral width***) / $\boldsymbol{\gamma}$ = 4800 {Hz}· (1 {MHz}/10^6 {Hz}) / 42.58 {MHz/Tesla} = 1.13×10^{-4} Tesla

We see here that the biggest difference in B_{eff} is much, much smaller than the overall magnetic field B_o generated by the main magnet: $\boldsymbol{\Delta B_{eff}} << B_o$

This is a very general phenomenon in biomolecular NMR today.

Practice Problems

Problem 1. Why is the signal from water proton in Figure V.2.2 the strongest among all the resonances present? In other words, why is the water resonance intensity exceeds any signal originating from other ^{1}H nuclei in the protein sample?

Problem 2. For the magnet operating at $\mathbf{B_0}$ of 11.7 Tesla, calculate resonance base frequency for ^{1}H, ^{13}C, ^{15}N, and ^{31}P nuclei. Do these numbers tell you why the NMR magnet strength is represented as the ^{1}H frequency?

Problem 3. Looking at the spectrum in **Figure V.2.2**, compare the intensity values of various peaks: are the peak intensities uniform throughout the spectrum or not? What can be the reason(s) for this intensity (non?)-uniformity?

Problem 4. Example 2 above shows that the difference in resonance frequency between any two nuclei of the same type (e.g. protons) depends on the magnet strength B_o. This is not very convenient because such a measure is equipment-specific. Can you propose a resonance frequency unit, which would show such a difference as numerically the same quantity for any value of B_o? Hint: proportionating (normalizing) for B_o might be helpful.

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5.3: Chemical shift in units of Hz and ppm

This Chapter introduces the other most common unit to measure and report the NMR resonance frequency: *ppm*, parts-per-million. We will consider examples when the frequency units of *Hz* (1/*second*) are the most justified choice and the opposite cases- when ppm's should be used. We will also start describing quantitatively how raw NMR signal, ***S*(*t*)**, depend on time ***t***, initial current $\boldsymbol{S_0}$, and two properties of the target nucleus: resonance frequency $\boldsymbol{\Omega}$ (or $\boldsymbol{\nu}$) and relaxation rate ***R***.

Learning Objectives

- Understand the dependence of frequency expressed in units of *Hz* from the instrumentation used (magnet strength)
- Define the magnet strength- independent units of *ppm*, parts-per-million
- Grasp the benefits and limitations of both types of frequency units, *Hz* and *ppm*, and learn when each one is the most appropriate
- Get familiar with a mathematical description for an NMR signal ***S*** as a function of time ***t*** and three parameters: initial signal $\boldsymbol{S_0}$, angular frequency $\boldsymbol{\Omega}$ and relaxation rate ***R***.
- Appreciate why the angular resonance frequency $\boldsymbol{\Omega}$ (or oscillatory frequency $\boldsymbol{\nu}$) can be expressed in units of both *Hz* and *ppm* while rates ***R*** mostly use *Hz* as units.

NMR Chemical Shift

Formula V.2.1, $\boldsymbol{\nu_{eff}} = \boldsymbol{\gamma}\cdot\boldsymbol{B_{eff}} = \boldsymbol{\gamma}\cdot(1-\sigma)\cdot B_o$, indicates that the resonance frequency of a target spin-½ nucleus is defined by its intrinsic magnetic shielding property σ and the externally generated magnetic field B_o. Whereas the value for σ for the same nucleus in the same sample will remain the same for any recording on any NMR instrument, the numerical value of B_o changes from magnet to magnet. Therefore, the numerical value of the resonance frequency $\boldsymbol{\nu_{eff}}$ will change if we perform an NMR recording of the same sample on a different instrument/magnet. This is inconvenient as the same quantity $\boldsymbol{\nu_{eff}}$ may have different values depending on the instrument used.

In order to avoid such a complication, resonance frequency is often expressed in NMR in relative term as a so-called chemical shift value $\boldsymbol{\delta}$ with respect the to the resonance frequency of some standard chemical. Such a "standard" can be either added to the NMR sample as a separate chemical (e.g. DSS) or an existing chemical can be used (e.g. a solvent like H_2O, common in biochemistry). The numerical value for the chemical shift of a specific spin-½ nucleus (sample) $\boldsymbol{\delta_{sample}}$ is expressed via $\boldsymbol{\nu_{ref}}$ and $\boldsymbol{\nu_{sample}}$ in units of parts-per-million (*ppm*) as follows:

Equation V.3.5.3.1

$$\delta_{sample} = \frac{\nu_{ref} - \nu_{samle}}{\nu_{ref}} \cdot 10^6 (ppm) \tag{5.3.1}$$

Key Result: Because both values, $\boldsymbol{\nu_{ref}}$ and $\boldsymbol{\nu_{sample}}$, are directly proportional to the magnet strength B_o, the chemical shift value $\boldsymbol{\delta_{sample}}$ expressed as the ratio above in units of *ppm* is independent of B_o. In other words, the chemical shift expressed in units of *ppm* will have the *same numerical value* whether the sample is measured on a weaker or stronger magnet!

Please see how the chemical shift value can be calculate in **Example 1** below.

NMR Raw Signal: Free Induction Decay (FID), its Key Parameters and their Units

As figure **Figure V.2.1** shows in Chapter V.2, the NMR experiment produces its signal as an echo from the relaxation of the excited spin-½ system back to thermal equilibrium. This signal is registered as electric current generated in detection coils by the magnetic field oscillations caused by the spin-½ transitions between the two Zeeman split levels during relaxation.

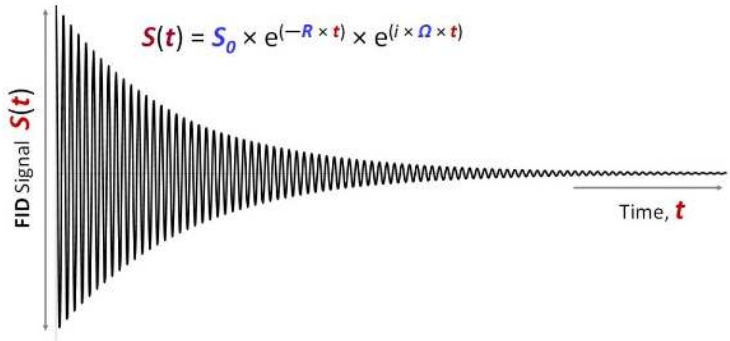

Figure V.3.1 Free induction decay (FID): the raw NMR signal detected as electric current in detection coils positioned near the sample. The electric current signal, ***S***(***t***), depends on time t and three parameters written in blue: $\boldsymbol{S_0}$ (initial current, ***S***(***t***=0)), relaxation rate ***R*** and angular resonance frequency ***Ω***.

Figure V.3.A above shows a typical shape of the FID signal (current) vs. time: it is a harmonically oscillating, exponentially decaying curve. The signal intensity depends on time approximately as follows:

Equation V.3.5.3.2

$$S(t) = S_0 \cdot e^{-R \cdot t} \cdot e^{i \cdot \Omega \cdot t} \tag{5.3.2}$$

Such a signal would be produced by a single type of a spin-½ nucleus, that is by a specific proton in our sample biomolecule (e.g. by $^1H_\alpha$ of Pro108, or $^{13}C_\beta$ in Ala35 etc.). Parameter ***Ω*** is the angular resonance frequency of this nucleus ***Ω*** = 2π***ν***, where ν is the effective oscillatory frequency of this nucleus ($\boldsymbol{\nu_{eff}}$) described in Chapter V.2. Angular frequency ***Ω*** is often used in NMR theory for rather technical reasons, but essentially it is linear resonance frequency ***ν*** scaled by factor 2π. Parameter ***R*** in the equation above specifies the rate of relaxation from the excited state to thermal equilibrium forthis particular spin. Just like the resonance frequency ***Ω***, relaxation rate ***R*** is specific to each nucleus as it depends on the local covalent and non-covalent environment of the nucleus (and not so much on the external magnetic field B_o). Lastly, factor $\boldsymbol{S_0}$ corresponds to the initial signal (detected electric current) when time ***t***=0. The value of $\boldsymbol{S_0}$ depends on the amount of the sample and the spin-up vs spin-down population polarization, which in turn depends on the magnitude of the main magnetic field B_o and gyromagnetic ratio ***γ*** (mathematical details will come a bit later). The units of ***S*** and $\boldsymbol{S_0}$ (they are the same as can be deduced from **Equation V.3.1**) usually do not play much role in practical use of NMR spectroscopy as we will see in later chapters. For clarity and simplicity, we can postulate here that ***S*** and $\boldsymbol{S_0}$ have units of electric current: Ampere (Amp).

It is important to appreciate that relaxation rate ***R*** is measured in units of *Hz*. Because R does not depend on the external magnetic field strength B_o (it is *almost* correct), expressing it in any units other than *Hz* (e.g. in *ppm*) really gives no benefit. Thus, we have an example of two types of quantities, R and Ω, which can both be expressed in units of *Hz* or *ppm*. Importantly, for only one quantity – resonance frequency Ω (or ν) – it is meaningful to express it in relative units (ppm) to normalize the quantity with respect to the magnet strength B_o. This is why relaxation rates ***R*** almost always are listed in units of *Hz*.

Example 5.3.1

Let us consider an NMR spectrometer with an 11.7 Tesla magnet (B_o = 11.7 T). If for two protons, H_{ref} (reference) and H_a (target sample), their resonance frequency values are measured as 500,010,000 *Hz* and 500,000,000 *Hz* respectively, what are the chemical shift values in ppm ($\boldsymbol{\delta_{ref}}$ and $\boldsymbol{\delta_a}$) ?

Solution

According to Equation V.3.1 above:

$\boldsymbol{\delta_{ref}}$ = (500,010,000 {*Hz*} – 500,010,000 {*Hz*}) / (500,010,000 {*Hz*}) × 10^6 ({*ppm*}) = 0 *ppm (*this makes sense: *chemical shift of a reference is zero)*

$\boldsymbol{\delta_a}$ = (500,010,000 {*Hz*} – 500,000,000 {*Hz*}) / (500,010,000 {*Hz*}) × 10^6 ({*ppm*}) = 20 *ppm*

The difference between the resonance frequencies of the reference and proton H_a are 20 *ppm* (this value is independent of magnet strength B_o) and 10,000 Hz (this value is proportional to B_o and thus will change proportionately to B_o if a different magnet is used for recording).

Example 5.3.2

If we have two non-interacting (e.g., remotely placed) spin-½ nuclei **a** and **b** within the sample molecule, each can generate an NMR signal described by formula 5.3.2 above, $\boldsymbol{S_a(t)}$ and $\boldsymbol{S_b(t)}$ respectively. How would a combined signal generated by the entire molecule can be mathematically described?

Solution

$\boldsymbol{S(t) = S_a(t) + S_b(t)}$

Practice Problems

Problem 1. Consider Example 1 above. Calculate the chemical shift of proton H_A and reference proton H_{ref} as well as the chemical shift difference if the same recording is done on a magnet with: **a**) B_o= 9.4 Tesla, **b**) B_o = 23.4 Tesla. Calculate all the values in units of both *Hz* and *ppm* and compare the obtained numbers with the results of Example 1 above.

Problem 2. Example 1 above shows how to calculate chemical shift $\boldsymbol{\delta}$ in units of ppm using oscillatory resonance frequency $\boldsymbol{v}$. Will the value $\boldsymbol{\delta}$ change if instead oscillatory frequency one uses angular frequency ω and if yes, what will the difference be?.

Problem 3. Let's consider two spin-½ nuclei: nucleus *a* having relaxation rate $\boldsymbol{R_a}$=10 Hz, the other, nucleus *b*, having relaxation rate $\boldsymbol{R_b}$ = 100 Hz. Which signal, the one from nucleus *a* or the one from nucleus *b*, will be reduced faster below 10% of their initial values, $\boldsymbol{S_{0a}}$ and $\boldsymbol{S_{0b}}$ respectively? Justify quantitatively.

Problem 4. Let's consider two spin-½ nuclei described in Example 1 above. What nucleus, H_{ref} or H_a, will give more oscillations per unit of time when their NMR signals, $\boldsymbol{S_{ref}(t)}$ and $\boldsymbol{S_a(t)}$ respectively, are recorded and analyzed? Justify quantitatively.

Problem 5*. Imagine that both axes on Figure V.3.1 are labeled with actual numbers. Can you determine the numerical value of the resonance frequency $\boldsymbol{\Omega}$ from this graph? If so, describe the algorithm for such a determination.

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5.4: Fourier Transformation (FT)- from an FID to a Spectrum

This Chapter introduces the most generally applied method for conversion of raw NMR signal (FID) to NMR spectra- Fourier transformation (FT). Specifically, we will see how the key parameters of an FID introduced in the previous chapter (***S₀***, ***R*** and ***Ω***) affect the key properties of an NMR spectral resonance: position, intensity and linewidth. We will also demonstrate how Fourier transformation applied to a combined FID generated by multiple spin-½ nuclei leads to a spectrum with multiple respective resonance lines, that is we will demonstrate mathematical linearity of FT.

Learning Objectives

- Introduce the basic mathematical description of Fourier transformation (FT)
- Grasp how FT converts time-domain function into a frequency-domain function
- Learn how FT operates: it samples frequency values for the best matches to the oscillating FID
- Appreciate how FT deconvolutes an FID from multiple nuclei into respective NMR spectral lines or from FIDs of differing intensities.
- Determine how key FID parameters (***S₀***, ***R***, ***Ω***) affect the position, intensity and linewidth of the corresponding NMR spectral line via FT.

Fourier transformation as method to produce an NMR spectral line from FID

Historically, the very first NMR spectra for spin-½ nuclei were collected very inefficiently: one frequency was sampled at a time by subjecting a target system at thermal equilibrium to an excitation (B1 field) alternating at a specific frequency value. This was very slow: after each excitation the system needed time to relax back to thermal equilibrium. Worse yet, such a slow pace of recording did not allow for repetition of the data collection thus the sensitivity of the recordings was low and the resolution – very limited (more on sensitivity and resolution of NMR later). In order to bypass this bottleneck, Swiss scientist Richard Ernst applied a well-known method – Fourier transformation for processing of raw NMR data – FID or ***S***(***t***) signal vs. time ***t***. This allowed a much faster approach: the system is excited by a broad range of frequencies at once and the response is deconvoluted via digital Fourier transformation (FT). The resulting acceleration of recordings and gains in sensitivity/resolution were so dramatic that it immediately made NMR a central method: practical, wide-spread and rapidly developing into various applications. For this contribution, Richard Ernst was awarded a Nobel prize in Chemistry in 1991 for the "development of the method of high-resolution nuclear magnetic resonance (NMR) spectroscopy". Figure V.4.A and Equation V.4.1 introduce Fourier transformation and its application for converting an FID into a spectral line.

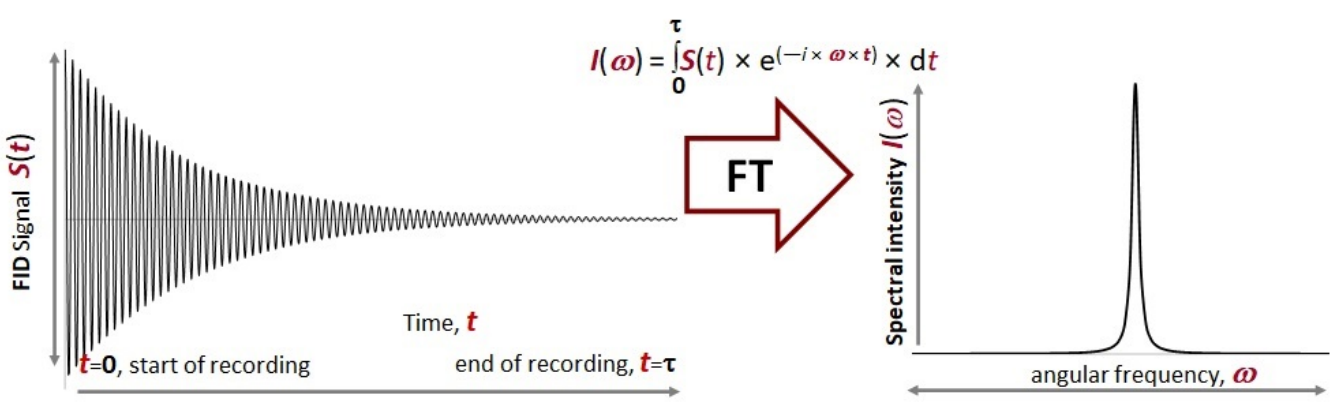

Figure V.4.A The most general way to produce an NMR spectral line from the raw FID recorded signal via Fourier transformation (FT). See Examples and Practice problems below for the actual application of FT for the FID approximation introduced in this text. For ***S***(***t***) shown here, a single angular frequency oscillation was chosen.

Mathematically, Fourier transformation is equivalent to trying to find the "best harmonic sin/cos match ***I***(ω) to the target oscillating function" by applying the following treatment to the target function of time ***S***(***t***):

Equation 5.4.1

$$I(\omega) = \int_0^{\tau} S(t) \cdot e^{-i \cdot \omega \cdot t}\, dt \tag{5.4.1}$$

Note

Mathematically, $e^{i \cdot x}$ or "complex exponent represents the following expression: $e^{i \cdot x} = \cos(x) + \mathbf{i} \cdot \sin(x)$ where **i** is called the "complex iota", which is defined as $\mathbf{i}^2 = -1$. In this equation, cos(*x*) is called the "real part" and sin(*x*) is called the "imaginary part" of the complex exponent. Complex exponent is a way to treat a trigonometric sin/cos combination (needed for the correct phase determination) via a much cleaner and simpler "exponential math".

From **Equation V.4.1** above, we can appreciate that FT operates by trying multiplication of the target function by a cos(ωt) for every angular frequency ω with the goal of detecting the specific ω value giving the maximum value for the integration. The maximum value I(ω) will be thus obtained at ω = Ω, the resonance angular frequency of the target spin-½ nucleus. Note that because FT is based on an integration (a linear mathematical operation), an FID composed of signals from multiple sources oscillating at multiple resonance frequencies, the produced spectrum I(ω) will have multiple "peaks", corresponding to those multiple frequencies of oscillations (see Examples below).

Example 5.4.1

On mathematical linearity of FT. Starting from Equation V.4.1 and Figure V.4.A above, show that the FID signal originating from a s system of two spin-½ protons will be converted via FT into a spectrum containing two peaks such that the resulting spectrum is a sum of the two individual spectra as if each of them were recorded and processed separately.

Solution

To prove the requested, first, let's describe mathematically the combined FID from two sources, S(t) = S1(t) + S2(t). Therefore:

Equation 5.4.4

$$I(\omega) = \int_0^{\tau} (S_1(t) + S_2(t)) \cdot e^{-i \cdot \omega \cdot t}\, dt \tag{5.4.2}$$

$$I(\omega) = \int_0^{\tau} S_1(t) \cdot e^{-i \cdot \omega \cdot t}\, dt + \int_0^{\tau} S_2(t) \cdot e^{-i \cdot \omega \cdot t}\, dt \tag{5.4.3}$$

$$I(\omega) = I_1(\omega) + I_2(\omega) \tag{5.4.4}$$

Thus we demonstrated that because of the mathematical linearity of the integration (and FT), the spectra resulting from FT-processed FIDs are additive as well.

Example 5.4.2

Parameter S_0 in **Equation V.3.2** describing how the FID signal depends on time (Chapter V.3) corresponds to the initial signal (electric current detected at time ***t***=0). Demonstrate that the spectrum resulting from FT processing of the FID grows linearly with S_0 : that is if S_0 doubles, the spectral intensity will double as well.

Solution

To prove the requested, we will plug S(t) as described in **Equation V.3.2** into Fourier transformation in **Equation V.4.1** and check how the result of the integration depends on S_0:

Equation 5.4.7

$$I(\omega) = \int_0^{\tau} S(t) \cdot e^{-i \cdot \omega \cdot t}\, dt \tag{5.4.5}$$

$$I(\omega) = \int_0^{\tau} S_0 \cdot e^{-R \cdot t} \cdot e^{i \cdot \Omega \cdot t} \cdot e^{-i \cdot \omega \cdot t}\, dt \tag{5.4.6}$$

$$I(\omega) = S_0 \cdot \int_0^{\tau} e^{-R \cdot t} \cdot e^{i \cdot \Omega \cdot t} \cdot e^{-i \cdot \omega \cdot t}\, dt \tag{5.4.7}$$

The last formula above shows that I(ω) depends linearly on $\mathbf{S_0}$ since S_0 is simply a multiplication factor for the rest of the expression.

Key Result: Examples 1 and 2 above show that Fourier transformation is a mathematically linear treatment. Specifically, FID composed of a sum of signals from multiple nuclei will be FT-processed into a spectrum, which is a sum of individual spectra originating from the respective nuclei. Likewise, scaling the initial signal intensity (and thus the entire FID) up or down will result in proportionately scaled up or down of the spectrum produced from this FID via FT.

This key property of Fourier transformation as well as that fact that it is very amenable for digital treatment (this is with computer software) explains why this method of producing NMR spectra from raw NMR data (FIDs) is the most widely used today although a number of respectable alternatives to FT do exist.

Practice Problems

Problem 1. Using Excel, Origin or any other suitable spreadsheet software, build a simple FID or $\boldsymbol{S(t)}$ function according to the following formula:
$\boldsymbol{S(t)} = \boldsymbol{S_0} \cdot e^{-\boldsymbol{R \cdot t}} \cdot \cos(\boldsymbol{\Omega} \cdot \boldsymbol{t})$ where time t is the independent variable, **S(t)** is the function of **t** , whereas $\boldsymbol{S_0}$, $\boldsymbol{R}$ and $\boldsymbol{\Omega}$ are parameters (constants). Specify $\boldsymbol{S_0}$, $\boldsymbol{R}$ and $\boldsymbol{\Omega}$ values in in their respective data cells. Using this function, build a plot $\boldsymbol{S}$ vs. $\boldsymbol{t}$ and explore how its shape changes in response to your alteration of each of the three parameters (vary them one-by-one).

Problem 2. Starting from **Equation 5.4.7** above, transform the FT for S(t) in such a way that under the exponent there is a single exponential function only (not a product of three exponents). Is this single-exponent integral *easy* to "take" to determine I(ω)?

Problem 3*. Starting from the result for Problem 2 above, determine the I(ω) under the assumption of the sufficiently long acquisition time $\boldsymbol{\tau} >> \mathbf{1}/\boldsymbol{R}$. This condition effectively states that NMR data recording will start at certain time $\boldsymbol{t}$=0 and continue until $\boldsymbol{t}=\boldsymbol{\tau}$, where $\boldsymbol{\tau}$ is much larger than the characteristic relaxation time $1/\boldsymbol{R}$. To determine I(ω), only the "real part" of the complex integration result is needed (the "complex part" can be ignored). Also, the following complex math hint can be useful:

$$\frac{1}{a+i\cdot b}=\frac{(a-i\cdot b)}{(a-i\cdot b)\cdot(a+i\cdot b)}=\frac{a}{a^2+b^2}-i\cdot\frac{b}{a^2+b^2}$$

Problem 4*. Each NMR spectrum is reporting signals only within a certain range of frequencies (SW: spectral width). With the idea of a "digital FID", a finite set of of current vs. time S(t) data points, processed with Fourier transformation and reporting frequencies ω within a certain range ($\omega_{min} < \omega < \omega_{max}$; ω_{max}-ω_{min} = SW, spectral width), predict how a resonance with a frequency outside of this range ($\omega < \omega_{min}$ or $\omega > \omega_{max}$) would be represented on the digital NMR spectrum?

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5.5: Effects of the Sample, Equipment and Recording Regimes on the NMR Spectral Sensitivity and Resolution.

This Chapter describes how key elements of NMR data collection (properties of sample molecules, their concentration, magnet strength B_o, recording regimes) affect the two fundamental qualities of the recorded data: spectral resolution and spectral sensitivity.

Learning Objectives

- Appreciate the effect of the magnetic field strength B_o on FID S_0 and spectral line intensity
- Develop a sense of difference between the signal and noise parts of NMR spectra
- Understand the link between the number of times the spectrum is recorded and spectral sensitivity
- Explore the connection between the magnetic field strength B_o and spectral resolution: linewidth in ppm and Hz vs peak separation in ppm and Hz
- Grasp the effects of the biomolecule size and concentration on the spectral line width and intensity

In the last practice problem of the previous Chapter, we applied Fourier transformation to a model FID originating from a single spin-½ system (nucleus). The resulting mathematical expression describing the NMR signal line-shape as a function of resonance angular frequency **ω** (=2·π·**ν**, where **ν** is the linear resonance frequency) is as follows:

Equation V.5.5.5.1

$$I(\omega) = S_0 \frac{R}{R^2 + (\omega - \Omega)^2} \tag{5.5.1}$$

In this expression, the three parameters S_0, R, and Ω have exactly the same meaning as in **Equation V.3.2** for FID (Chapter V.3) : initial current, FID relaxation rate and resonance frequency respectively. **Equation V.5.1** above allows us to appreciate the effects of the sample properties, concentration and magnet power B_o on the key properties of the respective NMR resonance line: its position, intensity and line-width.

Magnet Strength B_0 and Signal Intensity

The intensity of the current detected in response to an NMR excitation by a particular sample (spin-½ system) is defined by the fraction of spins, S_{up}, whose spin projection on the axis of B_o is parallel to the field and which have no compensatory "anti-parallel" spins under the initial condition of thermal equilibrium (for positive gyromagnetic ratio values):

Equation V.5.5.5.2

$$S_{up} = \frac{N(+1/2) - N(-1/2)}{N(+1/2) + N(-1/2)} \tag{5.5.2}$$

Utilizing the Boltzmann distribution formula for the ratio of spin-up over spin-down populations (Equation 5.1.3 in Chapter V.1), it can be shown that

Equation V.5.5.5.4

$$S_{up} = \frac{1}{2} \cdot \frac{\Delta E}{k \cdot T} \tag{5.5.3}$$

$$S_{up} = \frac{1}{2} \cdot \frac{h \cdot \gamma \cdot B_0}{k \cdot T} \tag{5.5.4}$$

Thus, we can see that the excess of the spin-up over spin-down populations is directly proportional to the value of gyromagnetic ratio γ and magnetic filed strength B_o. (NB! this is a serious oversimplification) The initial signal intensity S_0 and thus NMR peak intensity can be viewed as directly proportional to S_{up} ratio. Under these assumptions, we can state that doubling the magnetic field B_o leads to doubling the signal intensity.

Signal-to-Noise Ratio as a Measure of Spectral Sensitivity

The Free Induction Decay (FID) recorded as electric current in every NMR experiment consists of two major components: the currents induced by alternating magnetic moment of the sample (that is "signal" itself) and random, stochastic electric currents existing in every electronic circuit also known as "noise". The level of noise is defined by the properties of conductor and semiconductor elements of the circuits and temperature at which the electronics operates: the higher the temperature, the stronger the random noise currents. Unlike the noise, the signal from the sample is repeatable and if we rerun the NMR experiment, we will get exactly the same currents from the sample FID.

Spectral sensitivity is a measure of spectrum quality in the sense of the capacity of the scientist to distinguish the real peaks (signals) from noise. Figure V.5.A demonstrates how two spectra having very similar level (intensity) of its signals can differ in their capacity to distinguish real signals from elements of noise (see the features marked by red arrows).

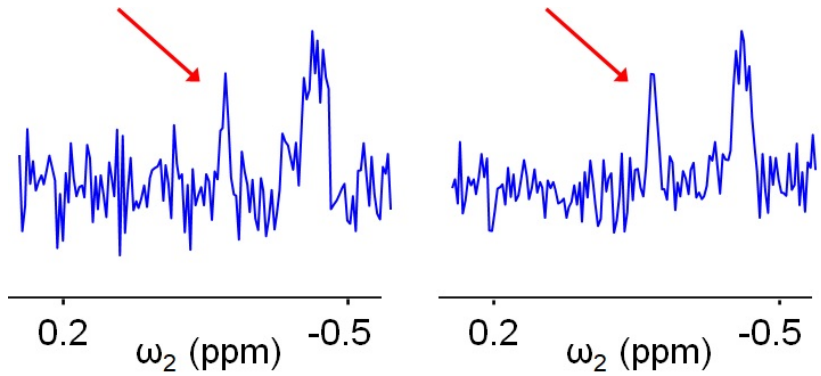

Figure V.5.A The two spectra are similar in many respects except for their clarity about the features marked by the arrows. Peak on the left leaves plenty of doubts whether this feature is a real peak or it is a noise component. The spectrum on the right has generally a lower level of noise with respect to the signals and is unambiguous regarding the nature of the feature marked by the arrow: it is a real peak. The recordings were made by S.L. Smirnov.

Figure V.5.A helps to appreciate why signal-to-noise (***S***/***N***) ratio is commonly used as a quantitative measure of spectral sensitivity. The higher the ***S***/***N*** ratio, the less the features of the noise can mask "real" peaks. Alternatively, the greater the ***S***/***N*** ratio, the smaller "real" signals can be identified.

Key Result: The **S/N** ratio offers a very practical approach to increase the sensitivity of NMR spectra: *repeat recordings multiple (**n**) times and sum up the obtained spectra* (the spectra can be summed up since Fourier transformation is a linear mathematical operation). Such a treatment will help to increase ***S/N*** ratio because the intensity of the real signals will be increased by factor ***n*** ("real" peaks will reproduce faithfully with every repetition of the experiment) , whereas the noise features being random or stochastic will have their intensities increased on average by a factor $\boldsymbol{n}^{0.5}$ (square root or **n**). Thus after the recording is repeated ***n*** times, signal-to-noise ratio will grow as $n/n^{0.5} = n^{0.5}$. For example, to increase spectra sensitivity by factor 2, one needs to repeat the recording 4 times ($2 = 4^{0.5}$).

Repeating NMR recordings multiple times offers a way to increase spectral sensitivity while working with the same instrumentation (that is without resorting to more powerful and thus less accessible spectrometers). Another obvious way to increase spectra sensitivity is to use a *more powerful magnet* (*with greater* B_o) as **Equation V.5.3** above suggests. This way, the signal intensity will go up proportionately to B0 or even faster. If the noise level stays the same, ***S***/***N*** will be greater. The same **Equation V.5.3** offers another avenue: *alter the temperature* (up? down?) to get stronger signal and greater ***S***/***N*** ratio. *Increasing the amount of the sample used for the recording* is another general and widely used way to increase the intensity of the signals while keeping the noise level the same (the noise level depends on the instrumentation and not on the amount of the sample in the NMR tube).

Broader picture: In addition to the generally used ways to increase sensitivity of NMR spectra listed above, more sensitive NMR probes have appeared as the most recent method to increase **S/N** of NMR recordings. Such probes are often referred to as *cryoprobes* since their electronics is cooled by liquid cryogens (e.g. helium or nitrogen) to drive the circuitry temperature and noise currents down. The temperature of the sample in an NMR cryoprobe is still controlled by the operator: in other words, in cryoprobes our biomolecular samples are *not* cryogen-cold.

Sample Relaxation Rates: Effects on Spectral Sensitivity and Resolution

Sensitivity: **Equation V.5.1** above shows that the NMR peak intensity depends on the value of the FID relaxation rate ***R***. This means that if for whatever reason the overall relaxation rate of a particular spin-½ changes, the signal intensity and this spectral sensitivity will change. There are multiple reasons, which can lead to alteration in rates of FID relaxation (to be discussed later in this text). Some of those reasons include: larger or smaller size of the biological molecule, a polypeptide adopting more folded or more disordered conformation, certain conformational or chemical exchanges phenomena etc. Algebraically, $\boldsymbol{I}(\omega)$ goes down as the relaxation rate ***R*** goes up. This spectral sensitivity goeas up when the FID relaxes slower and goes down when the relaxation rates increase. **Example 1** below provides a quick introduction into this relationship.

Resolution: Spectral resolution described the capacity of the operator tell two adjacent peaks apart. In other words, the user of the spectrum needs to know whether a particular spectral pattern shows a single peak or two peaks or three or more. Likewise, it is important to be able to determine the peak position (**Ω** values) and spectra of sufficient resolution allow this.

Examples

Example 1. What is the effect of doubling the relaxation rate R on spectral sensitivity?

Sensitivity can be quantified as the **S**/**N** ratio. Since we are discussing in this example changes in the properties of the sample, the noise level (property of the instrument) remains constant. According to **Equation V.5.1** above, the peak intensity at $\omega = \boldsymbol{\Omega}$, $\boldsymbol{I}(\omega) = \boldsymbol{S_0}/\boldsymbol{R}$. This increasing the relaxation rates by factor 2 (e.g. if the sample dimerize) will lead to reduction in signal intensities by factor 2. Thus with the noise level staying constant, ***S***/***N*** ratio and sensitivity will drop by factor 2.

Example 2. What is the effect of doubling the relaxation rate R on spectral resolution? Consider the case of two NMR signals of the same $\boldsymbol{S_0}$ value and separated by 100 Hz. For both of them, let their relaxation rates be 20 Hz initially and twice that value after a certain change (the separation between the peak position and $\boldsymbol{S_0}$ values remain the same).

Will the two peaks be better resolved or worse resolved after their respective relaxation rates double?

Equation V.5.1 above shows the at greater R (while $\boldsymbol{S_0}$ and the ***Ω*** stay the same), the peak will be generally broader: do Practice Problem 4 as a hint to appreciate the effect of ***R***. Thus as the R values double, the peaks' shoulders get closer to and peaks become less resolved.

Practice Problems

Problem 1. Calculate the $\boldsymbol{S_{up}}$ value for ^{1}H spins for NMR recordings performed at 25 ^{o}C and magnetic field value a) B_o = 11.7 tesla and b) B_o = 23.4 Tesla

Problem 2. Build in Excel a graph showing an NMR spectral lineshape as a function of angular frequency ω for spin-½ system with the following parameters: $\boldsymbol{S_0}$ = 1, ***Ω*** = 1000 rad/sec, ***R*** = 10 Hz

Problem 3. Predict how the changes in the line-shape in Problem 2 if the amount of the sample is doubled. Specifically, how will the following properties change ? a) peak position? b) peak height ? c) peak width?

Problem 4*. Spectral resolution is greater for spectra with narrower (sharper) peaks. NMR peak width is often reported as linewidth at half-height (LWHH). Utilize Equation V.5.1 above to determine how each of the three key parameters describing an NMR peak ($\boldsymbol{S_0}$, ***R*** and ***Ω***) affect LWHH of a peak?

Problem 5. The NMR frequency axis can be labeled in ppm or Hz (the two most common types of units). Consider a sample with just two NMR resonances (peaks). If two NMR recordings are done at $\boldsymbol{B_0}$ values of 300 MHz and 600 MHz (^{1}H frequency), what is the change in peak separation and LWHH values at the two field strength if expressed in units of ppm? Same question if the x-axis units are Hz?

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CHAPTER OVERVIEW

6: Solution NMR in Structural Biology of Proteins

6.1: 2D NMR Spectroscopy - Enhanced Spectral Resolution and Protein Backbone Conformation Reporters
6.2: Heteronuclear 3D NMR- Resonance Assignment in Proteins
6.3: Analyzing Protein Dynamics, Conformational States and Function with NMR

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6.1: 2D NMR Spectroscopy - Enhanced Spectral Resolution and Protein Backbone Conformation Reporters

This Chapter introduces 2D NMR spectroscopy, which greatly enhances the resolving power of the spectra in comparison with generic 1D data. Specifically, 2D ^{15}N-HSQC NMR spectra will be discussed in the context of analysis of protein samples in terms of assessing chemical and conformation states of the samples. In these applications, we will be relying on the NMR basics described above: 1D NMR spectroscopy, Fourier transformation, concepts of sensitivity and resolution.

Learning Objectives

- Learn how a heteronuclear 2D NMR spectrum can greatly increase spectral resolution for large biological samples: case of ^{15}N-HSQC
- Develop a basic sense for how ^{15}N-labeled protein samples can be generated in the lab
- Grasp the basic information inferred for protein samples from of ^{15}N-HSQC: peak count vs. the protein primary structure and protein states
- Learn to relate ^{15}N-HSQC resonance positioning patterns with protein backbone conformation (folded vs. disordered) proteins
- Appreciate the role of backbone $^{1}H_N$ chemical shift values as sensitive reporters of protein backbone conformation and the role of the 8.0-8.5 ppm range

In previous sections, we introduced a very basic type of NMR data: 1D spectra, in which signal intensity depends on resonance frequency of only one type of spin-½ nuclei (e.g., ^{1}H). These spectra can be very informative for small molecules (MW of hundreds of Daltons) studied in organic and inorganic chemistry. In these cases, each spectrum typically has a set of well-resolved peaks whose identity in many cases can be obtained from the chemical shift values alone. The utility of such 1D NMR spectra become much more limited if they are recorded for significantly larger samples, e.g. proteins whose molecular weights often are at the level of 10 kDa and higher.

Two-dimensional (2D) NMR spectra: the case of ^{15}N-HSQC for proteins

Severe spectral overlap and thus limited spectral resolution is one of the biggest limitations of 1D NMR spectra of complex biological molecules, e.g. proteins and nucleic acids. Modern NMR offers a fundamental solution to this limitation: introducing additional dimensions in the form of other types of spin-½ nuclei affecting the presence and intensity of the NMR signals. For example, let's consider a spectrum shown in **Figure VI.1.A**: ^{15}N-HSQC (Heteronuclear Single Quantum Coherence) also known as {^{1}H-^{15}N}-HSQC or ^{1}H-^{15}N HSQC. This is an example of a two-dimensional (2D) spectrum: the intensity of each resonance depends on two types of chemical shift values, ^{1}H (x-axis) and ^{15}N (y-axis) in this case. In this type of NMR data, each spectral line corresponds to a covalent ^{15}N-^{1}H group, that is a proton-nitrogen nuclear system connected by a shared electron system of a single covalent bond. This type of experiment displays significant sensitivity (high signal intensity) due to a relatively high value of proton-nitrogen scalar coupling (J-coupling) of 90-92 Hz. This value means that the the ^{1}H and ^{15}N covalent partners communicate to each other changes in their magnetization states 90-92 times a second, often enough to develop a strong proton-nitrogen cross-peaks (resonance lines). No signals from protons bound to other types of atoms (carbon, oxygen, etc.) are reported. As in any type of 2D NMR data, here the signals may display significant spectral overlap in the proton (^{1}H) domain but since they have different ^{15}N chemical shift values, the peaks are well-resolved. For example, for the four peaks marked with arrows their ^{1}H chemical shift values are very close around 8.0 ppm but they are well-resolved since their ^{15}N shift values are all different. Thus 2D experiments offer a significant enhancement in spectral resolution in comparison with basic 1D NMR data even for large biological samples.

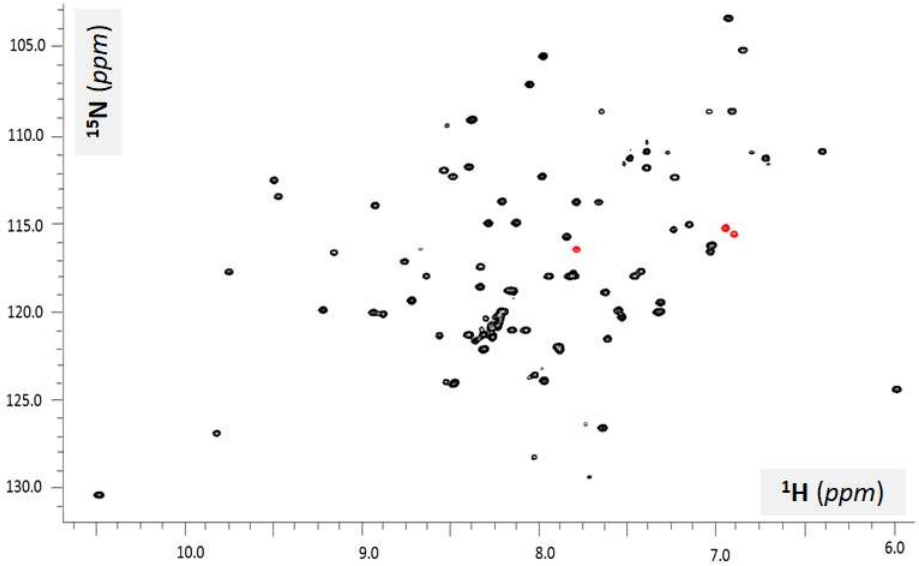


Figure VI.1.A ^{15}N-HSQC of a 76-residue protein fragment. The data is courtesy of the Smirnov lab (WWU/Chemistry, Bellingham, WA).

^{15}N-protein sample production and NH/NH2 groups contributing signals to ^{15}N-HSQC spectra of protein samples

In order to record 15N-HSQC data, one needs to have a sufficient fraction of nitrogen atoms in the sample to be present in the from of spin-½ ^{15}N nuclei. At their natural abundance level, 15N nuclei comprise 0.4% of all the nitrogen atoms. for protein samples, this is typically insufficient for NMR recordings since the concentration of a protein sample itself is no greater than 1 mM (typically less for most proteins). Thus methods of production of protein samples with an enhanced presence of 15N isotopes are needed. Currently, such an enrichment can be routinely achieved by expressing the target protein samples in bacterial cultures supplied with $^{15}NH_4Cl$ (^{15}N-labeled ammonium chloride) as the sole source of nitrogen for the cells. This approach allows to bring the level of uniform ^{15}N labeling in proteins to >95%: that is >95% of all the nitrogen atoms in the sample are represented by ^{15}N isotopes. This level is very sufficient for recording high-sensitivity ^{15}N-HSQC data for >0.1 mM protein with the most common NMR instrumentation, e.g. at B_0 of 11.7 tesla (500 MHz of ^{1}H frequency) and higher. Recently, more sophisticated methods were developed, which allow for segmental labeling of large protein samples: only a portion of the protein can be labeled with NMR active isotopes to simplify the relevant NMR spectra while keeping the entire target sample present.

Almost all amino acid residues have at least one NH group present : backbone amide group. Some residues have NH and NH_2 groups within their side chains, e.g. tryptophan, asparagine, glutamine etc. One type of residues, prolines, have no NH group at all. Thus statistically for protein samples, the majority of their ^{15}N-HSQC resonances originate from their backbone NH groups while some peaks originate from the side chains. Therefore, a simple peak count can inform the researcher about the state of the protein sample by answering a simple question: does the ^{15}N-HSQC peak count correspond (or not) to the primary structure of the sample? [Let's recall that a protein primary structure is defined as its amino acid sequence plus all the relevant post-translational covalent modifications like phosphorylation, disulfide bonds, ubiquitination, methylation, etc.]. If the number of the resonances ("cross-peaks") is equal to the number of signals expected from the protein primary structure than one can propose that the sample has a single conformation (and chemical) state. If the number of the observed ^{15}N-HQC peaks is greater than what is predicted from the protein's primary structure then one can conclude that the protein exists in multiple conformational and/or chemical states. If the number of observed peaks is too small for the sample of a certain primary structure, some more complex scenarios need to be expected: aggregation, complex dynamics behavior, chemical degradation etc. These scenarios will be discussed later in this and other chapters of this part of the textbook. **Table IV.1.I** sums up these scenarios.

Table IV.1.I

The number of ^{15}N-HSQC resonances: observed vs. expected	Most likely protein sample state
Observed ≈ Expected	Single average conformation and single chemical state (no degradation)

The number of ^{15}N-HSQC resonances: observed vs. expected	Most likely protein sample state
Observed >> Expected	Multiple conformational states or chemical degradation possible
Observed << Expected	Possible aggregation and/or complex dynamics regimes

^{15}N-HSQC and conformation states of protein backbone: folded/ordered vs. unfolded/disordered samples

In addition to the analysis of the number of ^{15}N-HSQC resonances, the way the signals are positioned within the spectrum (peak pattern) is very informative as well. Since most of the ^{15}N-HSQC peaks originate from the backbone amides, ^{1}H chemical shift values can be used to assess the chemical/electronic environment for the residues and thus can work as reliable reporters of the backbone state: folded vs. unfolded. The ^{15}N-HSQC spectrum in **Figure VI.1.A** corresponds to a mostly folded polypeptide as can be judged by the fact that most of the resonances have $^{1}H_N$ chemical shift values are found outside of 8.0-8.5 ppm window. This significant dispersion of the peaks in the amide proton dimension indicates that for most of residues, their electronic environment of their backbone amide groups is unique for each residues, which can only be the case for a folded protein (each backbone HN group has different set of non-covalent neighbors). Contrary to that, a disordered polypeptide exposes its backbone HN groups to nearly identical environments, which is mostly water. Therefore, the ^{15}N-HSQC resonances of an intrinsically disordered protein (IDP) or an intrinsically disordered region (IDR) will be clustered within a narrow range of 1HN chemical shift values, typically within 8.0-8.5 ppm as shown on **Figure VI.1.B**.

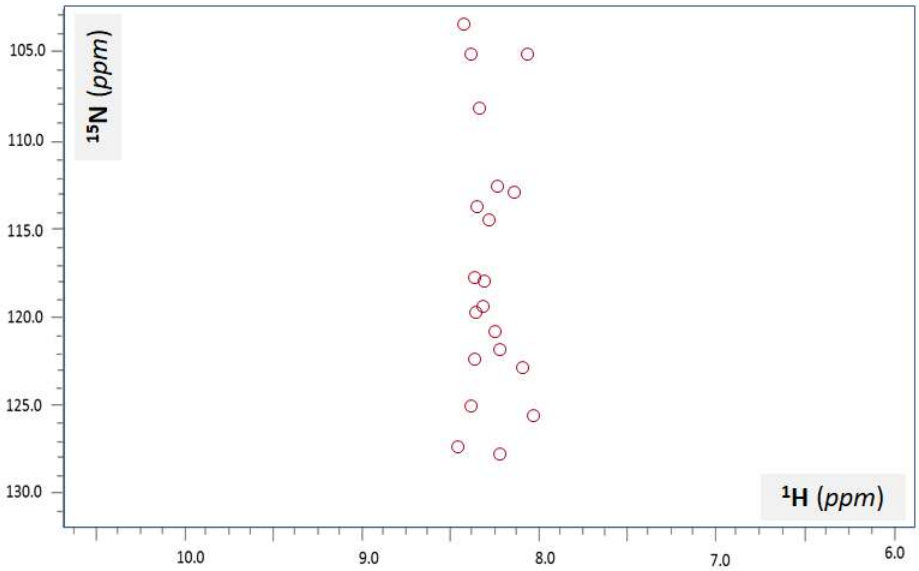


Figure VI.1.B A schematic ^{15}N-HSQC spectrum of an intrinsically disordered protein (IDP). Note that most of the signals are clustered within 8.0-8.5 ppm in ^{1}H dimension.

Examples

Example 6.1.1

A high number of chemical shift data is currently available for all the 20 amino acids in isolation or for their respective ^{1}H and ^{15}N resonances in the context of protein samples. Biological Magnetic Resonance Data Bank (BMRB) provides public access to this information with very useful statistics:

Using the BMRB chemical shift statistics, rank the average backbone ^{15}NH chemical shift values for the following types of amino acids: Ala, Gly, Trp.

Answer

BMRB chemical shift table gives the following average ^{15}NH values and rank for the three residue types:

Gly (109.6 ppm) < Trp (121.6 ppm) < Ala (123.3 ppm)

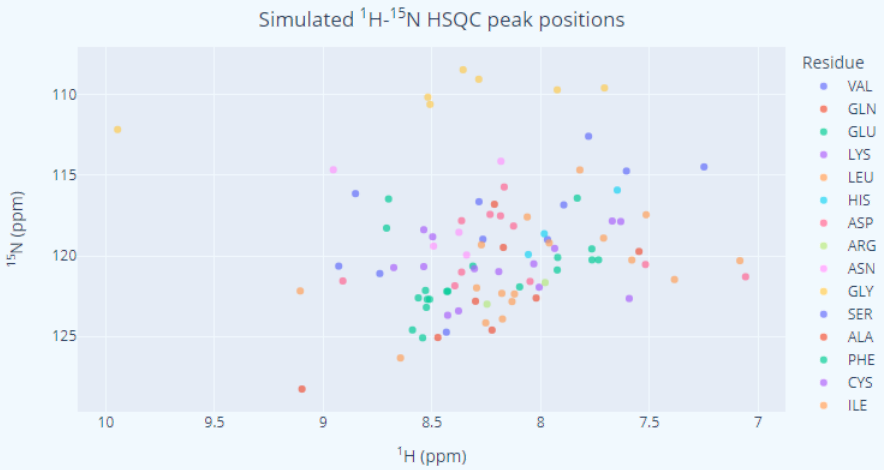
As we can see, Gly backbone nitrogen chemical shifts are noticeably lower than for the other two residue types. In fact, Gly is similarly different from all other residue types as well.

Example 6.1.2

How many residues can be estimated to belong to ordered (folded) segments of protein sample for BMRB Entry 50205?

Answer

Examination of the ^{15}N-HSQC of BMRB entry 50205 reveals about 75-85 signals outside of 8.0-8.5 ppm $^{1}H_N$ chemical shift range.



Some peaks corresponding to Asn and Gln side chains need to be excluded from the cumulative statistics (see Practice Problem 2 below).

Practice Problems

Problem 1. List all the standard amino acids whose side chains have NH or NH_2 groups. List all the standard amino acids which have no NH, NH_2 and NH_3^+ groups when place internally within a polypeptide chain.

Problem 2. Predict an ^{15}N-HSQC peak pattern and their approximate location on the spectrum (use **Figure VI.1.B** as template) for side chains of Asn and Gln residues.

Problem 3. Predict an ^{15}N-HSQC peak pattern and their approximate location on the spectrum (use **Figure VI.1.B** as template) for side chains of Trp residues.

Problem 4. Why are backbone $^{1}H_N$ chemical shift values are more reliable indicators or folded vs. disordered backbone states of a protein than backbone $^{15}N_H$ values? Thinking simplistically, both nuclei form covalent backbone amide groups and thus can be equally sensitive reporters of the protein backbone conformations. However, we see that the $^{15}N_H$ chemical shift values do not change much upon unfolding or folding of a polypeptide whereas $^{1}H_N$ ones undergo major and easily detectible changes as Figures VI.1A and VI.1.B above show. Explain.

Problem 5. Estimate (with a justification) how many residues may belong to disordered segments of the backbone for protein sample delivering the ^{15}N-HSQC spectrum shown in **Figure VI.1.A**.

Problem 6*. Estimate (with a justification) how many residues may belong to ordered (folded) segments of the backbone for protein sample delivering the ^{15}N-HSQC spectrum shown in **Figure VI.1.B**.

Problem 7*. Example 2 above indicates that protein sample for BMRB entry 50205 likely has 75-85 “folded” residues. How many residues can be estimated to belong to disordered (unfolded) segments in this 183-redsidue protein sample?

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6.2: Heteronuclear 3D NMR- Resonance Assignment in Proteins

In the previous Chapter we described 2D NMR spectroscopy, which offers significantly greater spectral resolution than basic 1D spectra. In this Chapter we will show how the well-resolved 2D ^{15}N-HSQC resonances can be assigned to specific residues and chemical groups within protein samples. As an example, we will consider a couple of complementary types of 3D NMR data: HNCACB and CBCA(CO)NH and their joint application for making heteronuclear NMR resonance assignment in proteins. Such an assignment opens a number of ways to probe structure and function (e.g. ligand binding) for the target protein samples.

Learning Objectives

- Grasp why the resonance assignment of 2D ^{15}N-HSQC can be beneficial : the case of ligand (drug) binding by a protein (therapeutic target)
- Familiarize with 3D heteronuclear through-bond (J-coupling) NMR : introduction and case of HNCACB and CBCA(CO)NH pair of 3D experiments
- Follow an example of assignment of heteronuclear NMR resonances ($^{1}H_N$, $^{15}N_H$, $^{13}C\alpha$, $^{13}C\beta$) from a combination of 2D ^{15}N-HSQC and 3D HNCACB/CBCA(CO)NH

^{15}N-HSQC as an assay for probing protein – ligand interactions: the need for the NMR resonance assignment

During the process of rational drug design, it is often necessary to characterize the interactions between the therapeutic target (protein) and candidate drug (ligand) beyond determination of the **binding affinity** (***K_d***). Heteronuclear solution NMR experiments ^{15}N-HSQC can provide significant insight for such interactions. Let's recall that most of the signals in this 2D NMR spectra originate from backbone H-N amide groups and some (minority) from the side chain NH and NH_2 groups. The position of ^{15}N-HSQC resonances are defined by the $^{1}H_N$ and $^{15}N_H$ chemical shift values, which in tern depend on the local electronic environment. Ligand binding changes such an environment for the residues forming the binding site even if the tertiary structure of the rest of the protein does not get perturbed. In such a case, the 15N-HSQC resonance pattern undergoes local changes: only the resonances representing NH groups involved in the binding site change their position significantly (>0.05 ppm in ^{1}H and/or >0.2 ppm in ^{15}N dimension) or signal intensity (including peak disappearance). **Figure VI.2.A** illustrates such a change.

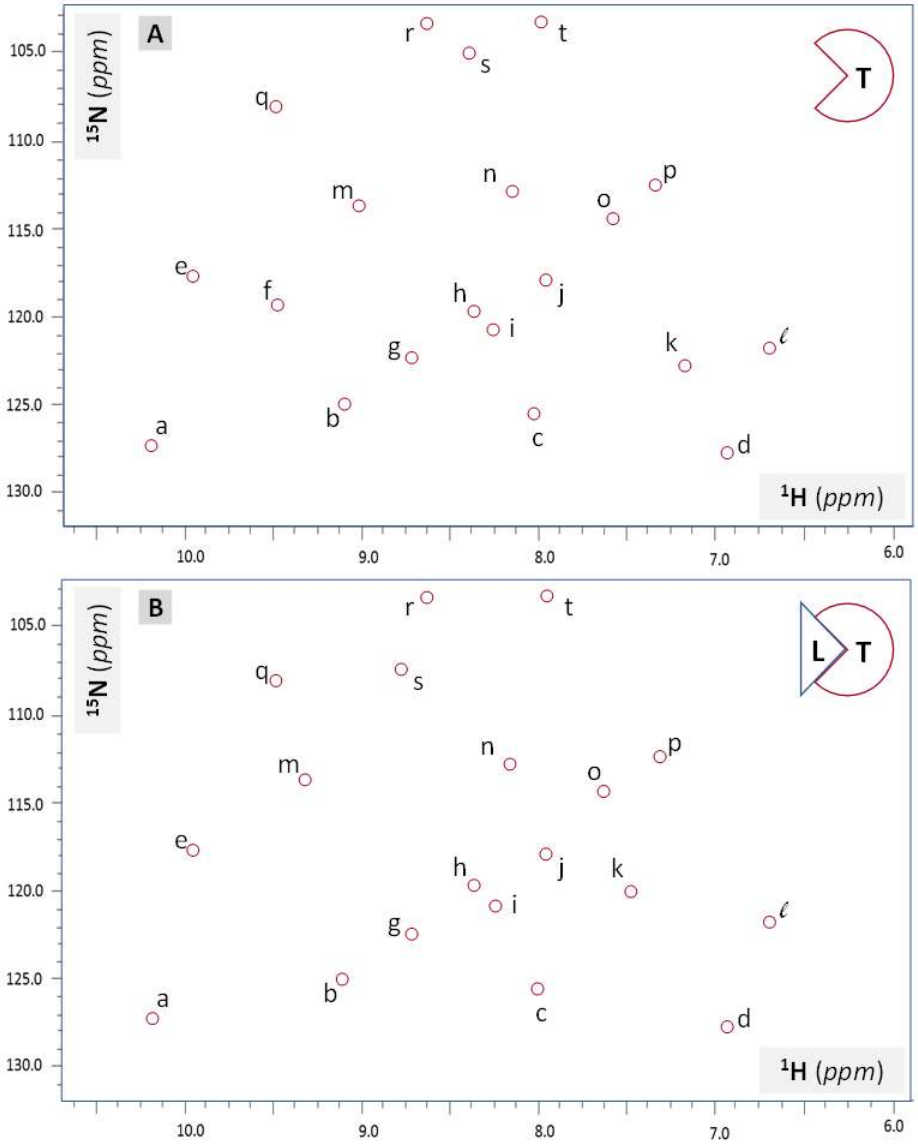

Figure VI.2.A ^{15}N-HSQC as a method to characterize ligand (L) – target (T) interactions. In this case, the target is an ^{15}N-labeled protein, the ligand has no ^{15}N labeling. The target resonance labels do not convey any information about the resonance assignment (yet). Panel A shows the ^{15}N-HSQC of the ^{15}N-labeled target protein (T) in isolation. Panel B depicts ^{15}N-HSQC of the ^{15}N-labeled target protein (T) in presence of a substantial amount of the unlabeled ligand (L).

Importantly, every ^{15}N-HSQC resonance in **Figure VI.2.A** is labeled with a single letter to help identify specific peaks which undergo spectral changes upon ligand binding. This data could have much greater impact if the peaks which underwent the most pronounced changes in position and/or intensity were assigned to specific amino acid residues within the polypeptide and chemical groups within those residues (backbone vs. side chain). The rest of this Chapter demonstrates some of the fundamentals of the heteronuclear NMR resonance assignment methodology.

Heteronuclear 3D NMR introduction: CBCA(CO)NH spectrum as an example

Just like every 2D 15N-HSQC resonance reports a J-coupling via a covalent bond between an 15N and 1H spin-½ nuclei, there are 3D NMR experiments which report resonances originating from J-coupling (through-bond) of three types of spin-½ nuclei (^{1}H, ^{13}C, ^{15}N). In this section we will introduce two such types of 3D NMR data: HNCAB and CBCA(CO)NH. In order to produce a protein sample with nearly complete uniform labeling with ^{13}C and ^{15}N isotopes, bacterial recombinant protein expression can be performed in a minimal media supplemented with ^{13}C-labeled glucose and ^{15}N-labeled ammonium chloride as the sole sources of carbon and nitrogen respectively. **Figure VI.2.B** introduces a general concept of a 3D NMR data and shows an element of 3D CBCA(CO)NH spectrum.

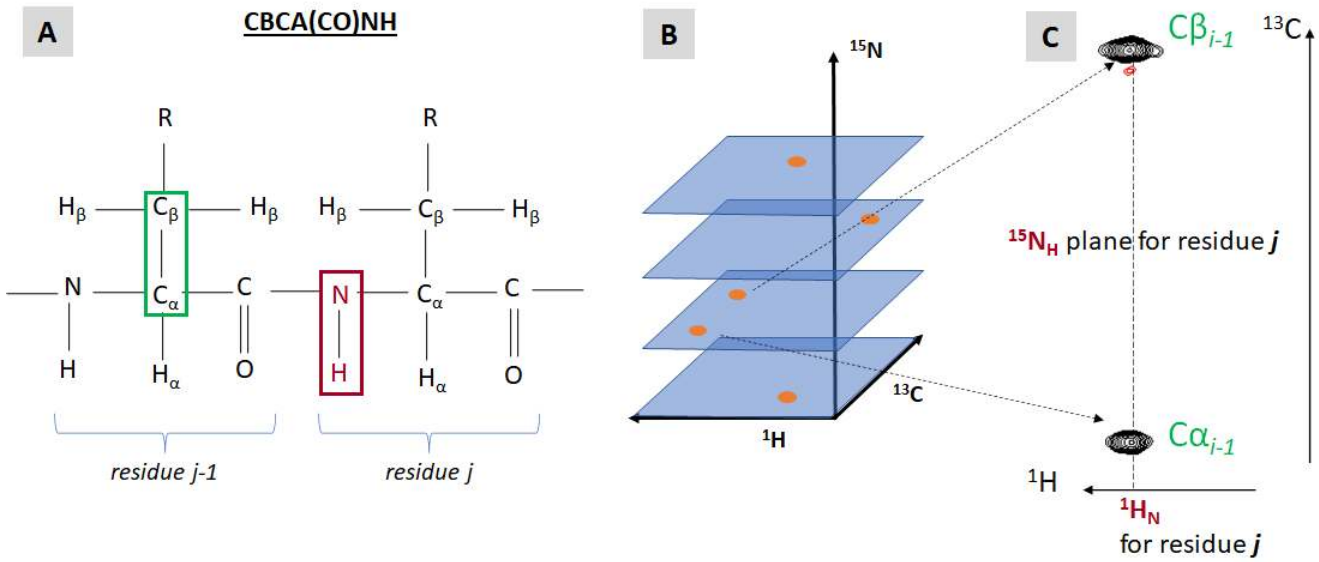

Figure VI.2.B An introduction into heteronuclear 3D NMR spectra in general and CBCA(CO)NH in particular. **A**: J-coupled spin systems involved into 3D CBCA(CO)NH through-bond resonance generation- backbone HN group of a residue and Cα and Cβ of a preceding residue. **B**: a schematic depiction of a heteronuclear (^{1}H, ^{13}C, ^{15}N) 3D NMR spectrum. **C**: a fragment of a single ^{15}N plane showing two 3D resonances originating from J-coupling of the backbone amide group of residue ***j*** and Cα and Cβ nuclei of preceding residue ***j***-1. The Figure design is courtesy of Heather Miears (the Smirnov lab, WWU, 2017).

Each resonance ("cross-peak") of a 3D CBCA(CO)NH spectrum indicates a through-bond (J-coupling scalar) interaction between two atoms of the backbone amide group ($^{1}H_N$ and $^{15}N_H$) or residue ***j*** and Cα and Cβ nuclei (^{13}C) of preceding residue ***j***-1. The name of the experiment, CBCA(CO)NH refers to the specific spin-½ nuclei involved (and not involved) in relevant J-coupling interactions: Cβ and Cα are J-coupled to NH while the connecting carbonyl carbon is not reporting any NMR signal (although its magnetization state is affected during the experiment). Two types of residues generate special CBCA(CO)NH peak pattern: prolines have no amide proton, so they do not have CBCA(CO)NH peaks linked with their amide groups. Glycine residues have no Cβ, therefore for any residue following a glycine only a single CBCA(CO)NH resonance will be observed (from glycine NH to previous Cα).

The NMR resonance assignment: combined use of two complementary datasets HNCACB and CBCA(CO)NH

By itself, CBCA(CO)NH does not convey much of sequential information. Another heteronuclear 3D NMR dataset, HNCACB, affords a powerful complement here. Just like CBCA(CO)NH, HNCACB reports resonances originating from J-coupling between backbone amide group and Cα / Cβ nuclei. The difference is that HNCACN reports two additional peaks, all intra-residual: between HN and Cα a Cβ spins (**Figure VI.2.C**).

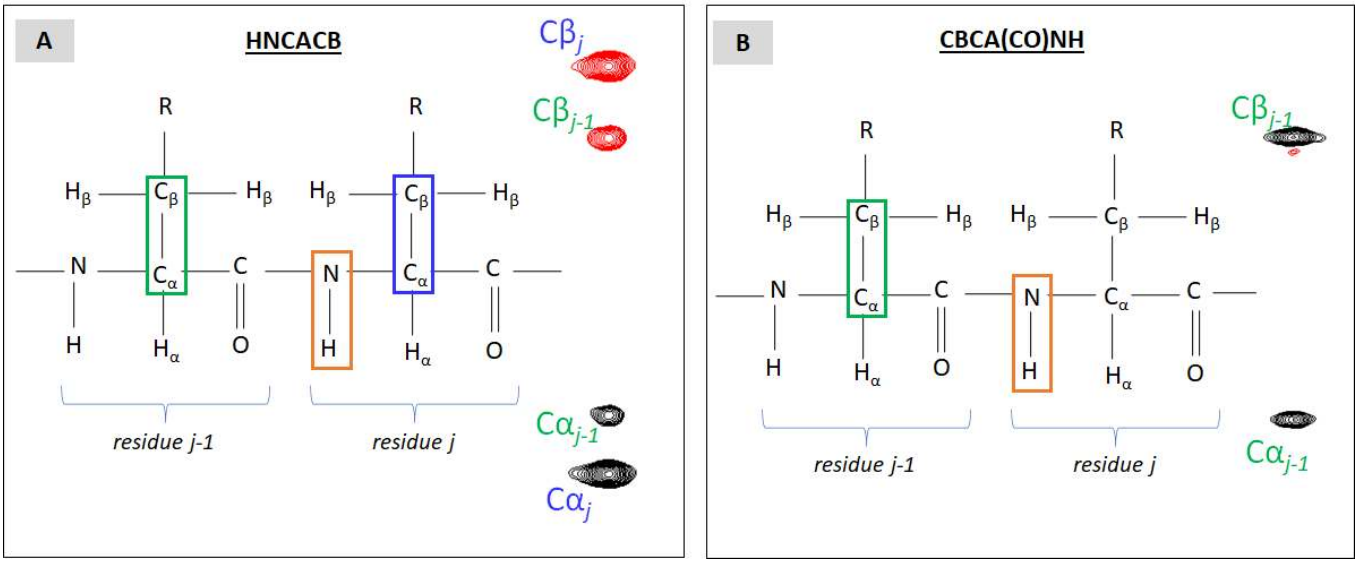

Figure VI.2.C Identical fragments of 1%N-planes from two complementary 3D NMR datasets. **A**: HNCACB and **B**: CBCA(CO)NH. The two CBCA(CO)NH resonances have exact HNCACB counterparts. In addition to those two peaks, HNCACB also has two more resonances originating from backbone HN grouped J-coupled with Cα and Cβ within the same residue. Importantly, two pairs of HNCACB resonances have the opposite signs: those involved $C\alpha_j$ and $C\alpha_{j-1}$ are positive (black contours) and those involved $C\beta_j$ and $C\beta_{j-1}$ are negative (red contours). All the four HNCACB and two CBCA(CO)NH peaks share the same ^{1}HN and ^{15}NH chemical shift values since they originate from the same backbone amide of residue ***j***. This combination of two related spectra and positive/negative HNCACB peak intensities allow unambiguous determination of the four carbon chemical shifts and their assignment to the current and previous residues. The Figure design is courtesy of Heather Miears (the Smirnov lab, WWU, 2017).

Typically, HNCACB and CBCA(CO)NH are acquired with identical parameters including spectral width in all three dimensions and the same number of data points in the ^{15}N dimension (or ^{15}N planes as on panel **B** of **Figure VI.2.B**) Now, let's imagine that we go through every ^{15}N plane and build the pairs of "residue ***j*** / residue ***j***-1″ HNCAB/CBCA(CO)NH peaks. This does not give us the sequence-specific NMR resonance assignments yet but already creates such pairs of 3D cross-peaks linked to di-peptides within the sequence. Now, let's take into account that for some types of residues their 13Cα and 13Cβ chemical shift values differs remarkably from those from other residue types. For details, take a look at BMRB chemical shift statistics for amino acid residues with emphasis on Gly, Ala, Ser, Thr. Knowing where such residues are positioned within the polypeptide sequence, we can start "connecting the dots" by mapping HNCACB/CBCA(CO)NH planes and di-peptides on actual amino acid sequence.

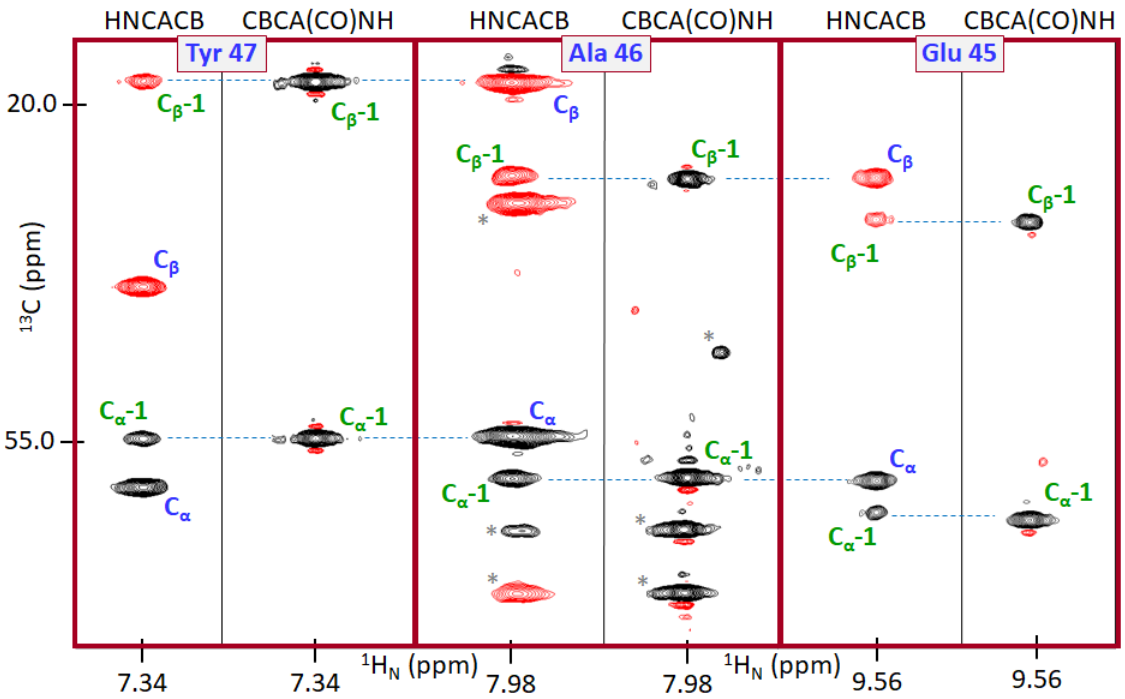

Figure VI.2.D An example of combined use of HNCACB and CBCA(CO)NH experiments for the backbone NMR resonance assignment in proteins. Cα and Cα labels are color coded: blue for intra-residual signals and green for preceding carbons (Cα-1, Cβ-1). HNCACB contours are color-coded: black for positive signals (Cα) and red for negative ones (Cβ). Asterisks (*) indicate resonances which originate from residues other than Alal46 (note that the $^{1}H_{N}$ chemical shift values for those peaks are different than for Alal46). The Figure design is courtesy of Heather Miears (the Smirnov lab, WWU, 2017).

Figure VI.2.D provides a general idea of how the two 3D NMR experiments HNCACB and CBCA(CO)NH can be utilized together to map the signals on the amino acid sequence of a protein sample. The C of Ala residues typically has chemical shift values below 20.0 ppm, which is unique. This allows identification of Ala patterns HNCACB/CBCA(CO)NH spectral patters. Starting from this starting points (as well from other distinct values, e.g. Cα for Gly and Cβ for Ser/Thr), one can continue "connecting the dots" process outlined in Figure VI.2.D to cover the entire sequence. If these two 3D NMR datasets encounter resonance overlaps, which are impossible to resolve, more 3D NMR dataset pairs are utilized in a similar way, e.g. HNCO/HN(CA)CO and others. This process allows assignment to specific residues and chemical groups of nearly all backbone and some side-chain resonances ($^{1}H_{N}$, $^{15}N_{H}$, $^{13}C\alpha$, $^{13}C\beta$). Methods for assigning side-chain chemical shift values are not discussed in this chapter but conceptually they are similar to the ones described here.

With the general process of the protein NMR resonance assignment described, let's assume that this method was successfully applied to the protein target (T) sample presented in Figure VI.2.A. The resonance assignment completion allows one to replace letter labels with residue-number labels (similar to the ones used in Figure VI.2.D). This in turn allows one to determine the specific residues affected directly or allosterically by binding of the ligand (L) to the target. In many cases, such information together with other data leads to the determination of the ligand binding residues within the target. If the ligand is a candidate therapeutic agent, identification of the ligand binding residues greatly advances ensuing efforts to optimize the drug.

Example 6.2.1

Analyze **Figure VI.2.A** and list at least two resonances which undergo major spectral changes upon binding of the unlabeled ligand (L) to the ^{15}N-labeled target protein (T). Major spectral changes for this model spectrum include resonances moving by >0.05 ppm in ^{1}H or >0.2 ppm in ^{15}N dimensions as well as peak disappearance (peak intensity going down to zero).

Solution

Upon ligand L binding target protein (T), resonance **f** disappears and resonance **s** moves by >0.05 ppm in ^{1}H dimension.

Example 6.2.2

Inspect BMRB entry 50205 and list all the heteronuclear NMR datasets utilized for the NMR resonance assignment.

Solution

BMRB entry 50205 contains the chemical shift assignment data for the target sample and offers several ways to look at its underlying NMR data including the list of experiments used to perform the NMR resonance assignment and the chemical shift values. E.g., the NMR-STAR v3 text file has a section titled _Experiment_list, which sums up the heteronuclear NMR data types used for making the assignments: 2D ^{1}H-^{15}N HSQC and 3D HNCACB, CBCA(CO)NH, HNCO and HN(CA)CO.

Example 6.2.3

How many 3D HNCACB resonances would you expect to originate from a Lys residue which is preceded by a Met?

Solution

four as both Lys and Met have backbone amide (HN) groups and both have Cα and Cβ atoms.

Practice Problems

Problem 1. Analyze **Figure VI.2.A** and list all the resonances which undergo major spectral changes upon binding of the unlabeled ligand (L) to the ^{15}N-labeled target protein (T). Example 1 above will help you start the analysis.

Problem 2. From BMRB entry linked to PDB 5VNT, list all the heteronuclear NMR datasets utilized for the NMR resonance assignment for the target sample.

Problem 3. Let's consider panel B of **Figure VI.2.B**. Imagine that the ^{13}C dimension is taken out of the spectrum (all ^{13}C planes are collapsed together). What type of 2D spectrum will remain after such a dimension reduction?

Problem 4. How many 3D HNCACB resonances would you expect to originate from a Gly residue which is preceded by a Pro?

Problem 5. How many 3D HNCACB resonances would you expect to originate from a Pro residue which is preceded by a Gly?

Problem 6*. Look up the amino acid NMR chemical shift values statistics table presented with BMRB repository and list the average values for the following resonances: ^{15}N, ^{13}Cα and ^{13}Cβ for Gly, Ala, Tyr, Glu, Arg, Ser, Thr, Pro. From this analysis, suggest what types of residues tend to report unusually low or high chemical shift values in comparison with the rest of the amino acids?

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6.3: Analyzing Protein Dynamics, Conformational States and Function with NMR

Learning Objectives

- Connect various types of molecular motion (e.g., side chain and backbone local motion, full-domain rotational motion) and transformations (e.g., chemical exchange) with specific times scale values, from sub-picosecond to hour-scale and beyond.
- Grasp the concept of the correlation time τ_C and how its values differ in small molecules vs. proteins and in folded/ordered vs. unfolded/disordered protein conformations.
- ps-ns dynamics: Consider how some basic types of NMR data (e.g., $^{15}\mathrm{N}$ T_1 and T_2 relaxation times/rates) can inform us of specific polypeptide conformational states, e.g. help distinguish **intrinsically disordered regions** (IDRs) from folded/ordered domains.

NMR spectroscopy allows site-specific probing of biomolecular structure and dynamics, which in turn offers powerful insight into the mechanisms of biomolecular function. In the previous Chapter, we described the basics of the heteronuclear NMR resonance assignment process for proteins, which allows site-specific mapping of the individual NMR resonance lines to the specific residues and atoms within the biomolecule. Advancing further, in this Chapter we will discuss how certain types of solution NMR data (T1, T_2) can inform us about protein structural dynamics and conformational states over ps-ns timescale. We will also briefly discuss how other types of NMR dynamics data can uncover the mechanisms of slower processes.

Conformational and Chemical Changes: Types of Motion and their Timescales

Biological molecules operate at non-zero temperatures (in units of Kelvin). Thus, motions of various types occur within each individual biomolecule (e.g., protein, DNA, etc.) and between molecules interacting in complexes (e.g., protein-protein, protein-DNA, protein-ligand interactions). From Newtonian physics, we know that mechanical objects move at certain rates and accelerations as defined by the mass values of the participating particles and forces exerted on one particle by all others. Atoms and small molecules, from smallest (e.g., $\mathrm{H_2O}$, $\mathrm{CH_4}$) to the larger ones (e.g. proteins) are still large enough to qualify as Newtonian objects. On the other hand, sub-atomic objects (electrons, protons, neutrons or smaller) follow non-Newtonian laws of Quantum Physics. For small Newtonian objects (e.g. atoms) or their groups (e.g. molecules), the frequency of their motions ν (Greek letter, pronounced "nu"), inversely related to timescale, is given by the **Eyring Equation**:

$$v = \frac{k_B \cdot T}{h} \cdot e^{-\frac{\Delta G^{\ddagger}}{R \cdot T}} \tag{6.3.1}$$

In this equation, k_B is the Boltzmann constant (1.38×10^{-23} J/K), T is the temperature in units of Kelvin (e.g., 310 K for normal adult human body temperature), h is Planck's constant (6.63×10^{-34} J $\cdot$ s), $\Delta G^{\ddagger}$ is the Gibbs free energy value (here in J/mol) of the kinetic barriers the system needs to "cross" or "jump over" to switch from one state to another and R is the gas constant, $R = k_B \times N_A = 8.31$ J/(K $\cdot$ mol). Thus, the lower the free energy barrier between the states, the higher the frequency of the transitions between separate states. In the absence of a barrier ($\Delta G^{\ddagger} = 0$ kJ/mol), this frequency is the highest attainable for biochemical systems and has a value of ~10^{13} Hz $(1/\mathrm{s})$ at temperature values typical for biological settings (room temperature, body temperature, etc.). In general, Equation 6.3.1 allows estimation of the frequency of transition between two states if one knows the value of the free energy barrier (see worked Examples and Practice Problems below). This equation also explains why the frequencies of transition between states separated by high activation energy barriers are lower (the transition times are higher) than the transition frequencies for low-barrier cases (Figure 6.3.1).

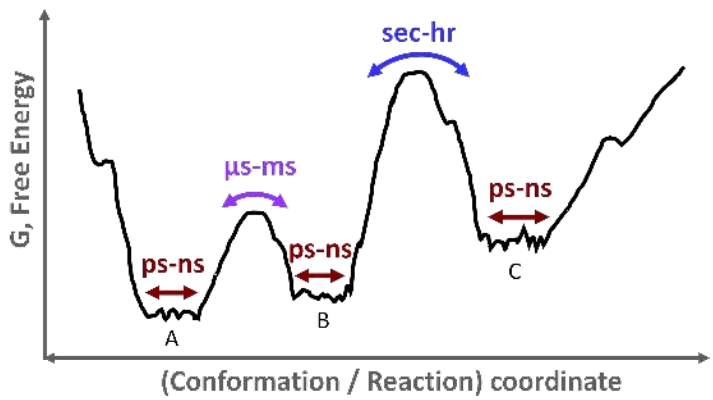


Figure 6.3.1: A generalized free energy space landscape of a molecular system of interest (e.g. a protein) showing three major hypothetical states (e.g., conformations): A, B and C. States A and B are separated by a medium energy barrier, which corresponds to μs-ms times of exchange between A and B. States B and C are separated by a significantly greater energy barrier, which results in a much slower exchange (than between A and B) of seconds-hours times. Within each of the three major states, there are sub-states separated by the smallest energy barriers (little wiggles of the Figure), which correspond to the fastest (occurring most readily) transitions within each major state, with a ps-ns time scale. Note that it is the peak height (i.e. the activation energy to be overcome) rather than absolute value of free energy that determines a transition's frequency.

From general Chemistry we know that temperature represents the average kinetic energy of moving atoms and their groups. Recall the basic equation for the average kinetic energy $\langle E_{kin} \rangle$ of monoatomic gas particles:

$$\begin{aligned} \langle E_{kin} \rangle &= \frac{1}{2} \langle m v^2 \rangle \\ &= \frac{3}{2} RT \end{aligned}$$

This formula indicates that for a given temperature, it is the mass m of the molecular group which would define the average speed v (distinct from the frequency, ν, or "nu"). Thus, the smaller the atomic or molecular entity, the faster its most rapid oscillatory motions will be at a given temperature. The speeds of atomic motions can be linked with the frequencies in case of oscillatory motion, e.g., in a bond vibration or protein backbone side chain rotation, etc. Therefore, we see that the smaller/lighter an oscillating molecular group is, the higher the frequency of those oscillations.

Figure 6.3.2 informs us about the characteristic times and frequency values of a number of typical biochemical processes, such as ligand binding/unbinding or enzyme catalysis acts, which are typically orders of magnitude slower than bond and side chain oscillations. The actual frequency value of a process tells a lot about is nature or mechanism. Thus, measuring these frequencies can be informative for biochemists. Solution NMR spectroscopy can be particularly helpful here because it can excite the target system with alternating magnetic fields of almost any desirable frequency.

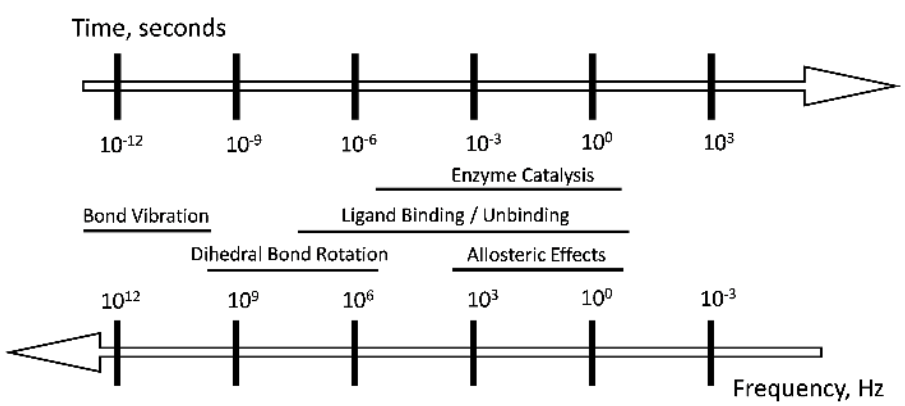


Figure 6.3.2: The time and frequency scales of the most common types of intra- and inter- molecular processes typical for biological molecules, e.g., proteins and nucleic acids. Note the numerical value of the fastest (highest frequency and lowest time-scale) processes: 10^{12} Hz or 10^{-12} s (1 ps).

Backbone bond and side chain dynamics (ps-ns time scale) is sensitive to the conformational state of the polypeptide chain. Specifically, folded and unfolded segments of a protein differ noticeably in terms of the types of motions their atomic groups (e.g., amino acid residues) undergo. All the groups in a folded protein or domain move together as one single object (a helpful oversimplification). On the other hand, individual amino acid residues in an intrinsically disordered region (IDR) move almost independently from one another. Thus, the Brownian motion of every amino acid in a folded domain is happening slower (since the domain is large), whereas residues in an IDR move at higher frequencies. Specifically designed NMR experiments can detect this difference in motion dynamics and thus help distinguish folded domains from IDRs. Figure $6.3.2$ also shows that many types of intermolecular interactions (ligand binding, enzymatic catalysis) are happening at significantly slower rates (larger time scales) than bond vibrations and dihedral rotations.

Correlation Times for Different Conformational States (folded vs. unfolded polypeptides)

Quantitatively, diffusional ("random") molecular motions can be characterized by the correlation time τ_C, a period after which the system loses any connection to ("forgets") its state at time zero. For example, a simplest type of random molecular motion - rotation of a spherical protein domain around its center of gravity - can be described by the **correlation function** $C(t)$ which is related to the probability that the randomly moving molecule adopts its original orientation (recorded at time t=0) or conformation after a certain time t. In the simplest case of an isolated folded protein, the correlation function can be expressed as

$$C(t) = C(0)e^{-t/\tau_c}$$

where parameter τ_C is the correlation time in this model and $C(0)$ is the normalization factor (a number). In practical terms, the correlation time of random rotational motion of a relatively spherical folded protein (e. g., ubiquitin) can be described by the average time it takes this object to rotate by 1 radian around its center of mass (Figure $6.3.2A$). The probability of any subset of the target molecules returning to the original state at $t = \tau_C$ is lower than 1 by a factor of $1/e$, a value substantially lower than 1 (only $1/e$ fraction of all the molecules have any chance of getting back to the original state at $t = \tau_C$). Thus, one can safely assume most of the target molecules "forget" their initial position or state after times equal or longer than τ_C. For folded proteins or domains, the correlation time values are relatively high ($\tau_C > 4$ ns) since these molecules pack tens of amino acid residues (a high-mass system) and their random rotational motion is thus quite slow. Since such molecules move and rotate roughly as a single unit, their τ_C value is the same for all the residues in the polypeptide. For motions of amino acids in an **intrinsically disordered protein** (IDP) or region (IDR), each residue would move to a large degree independently from the others (a useful simplification), as if each residue behaved as a small organic molecule (Figure $6.3.3B$). For the residues in an IDP/IDR, their individual correlation times are small ($\tau_C \approx 1$ ns), just like the values for small organic molecules. Unlike in the folded domain, an IDP would commonly need multiple correlation time values to model its internal conformational dynamics, since amino acids of various sizes can move at different rates at the same temperature (Figure $6.3.3B$).

Types of NMR data to probe ps-ns dynamics of biomolecules

Several types of NMR experiments produce data showing polypeptide motions in the ps-ns time range. In this chapter we will focus on T_1 and T_2 types of NMR data, both probing the rates (and times) of relaxation of NMR signals typically registered from the $\{^1\mathrm{H}-^{15}\mathrm{N}\}$ backbone groups in proteins. The T_1 relaxation mechanism, also called **spin-lattice relaxation**, refers to the loss of the spin-½ excitation (and thus signal) through protein interactions with the solvent (e.g., water). Figure $6.3.3$ (Left vs. Right) shows that the T_1 relaxation times are shorter (the relaxation rates higher) for the amino acids within disordered regions (because each such residue is in direct contact with water molecules) than for the residues within folded domains where many of such residues are hidden inside the domain. The T_2 relaxation mechanism, also called **spin-spin relaxation**, refers to the loss of the spin-½ signal coherence through internal interactions between the spins within the biomolecule itself (e.g., protein). The T_2 relaxation times are typically longer (the relaxation rates lower) for the residues in IDRs, where only two neighbors (on average) are covalently bonded to each internal residue, than for residues within folded domains where each residue has many covalently and non-covalently bonded neighbors.

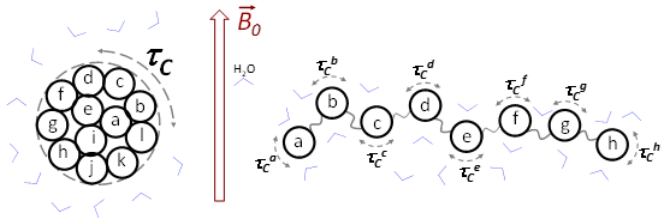


Figure 6.3.3: Left: Solvated folded protein model; Right: solvated intrinsically disordered protein (IDP); Middle: external magnetic field B0 and a graphic representing a water molecule. Note that in the IDP every residue forms a direct contact with the molecules of the solvent. Contrary to that, amino acid residues buried inside a folded protein domain are protected from interactions with water but have plenty of contact with other residues of the same protein. Finally, each "disordered" residue may have a unique correlation time value due to different sizes (masses) of the side chains. The τ_C value for the residues in the folded domain (Left) is depicted in a larger font than for those values in the IDR to highlight the relative value difference.

The generalized free energy profile of a biomolecule (Figure 6.3.1) informs us that various processes occur at drastically different time/frequency scales (Figure 6.3.2). In this chapter, we focused on once class of such events - dynamics of motion of amino acids. Figures 6.3.4 and 6.3.4 combined inform us that the correlation times τ_C for amino acid residues in folded/ordered and unfolded/disordered proteins are noticeably different (>4 ns vs. 1 ns) and that T_1 and T_2 rates depend on τ_C values. Thus, T_1 and T_2 NMR relaxation times combined with the residue-specific NMR resonance assignment (see Chapter 6.2) can help reliably distinguish folded/ordered and unfolded/disordered elements of the protein sample. In this regard, solution NMR is one of today's most powerful biophysical techniques. It is worth noting that in addition to T_1 and T_2 notation for the spin-lattice and spin-spin relaxation mechanisms, many authors use the complementary $R1$ ($R_1 = 1/T_1$) and $R2$ ($R_2 = 1/R_2$) notation which refers to the rates of relaxation (in units of Hz) as opposed to the relaxation times (in seconds). For a typical folded protein domain, the ^{15}N relaxation rates R1 fall into the single-digit range (in Hz) whereas the $R2$ rates are 20-40 Hz.

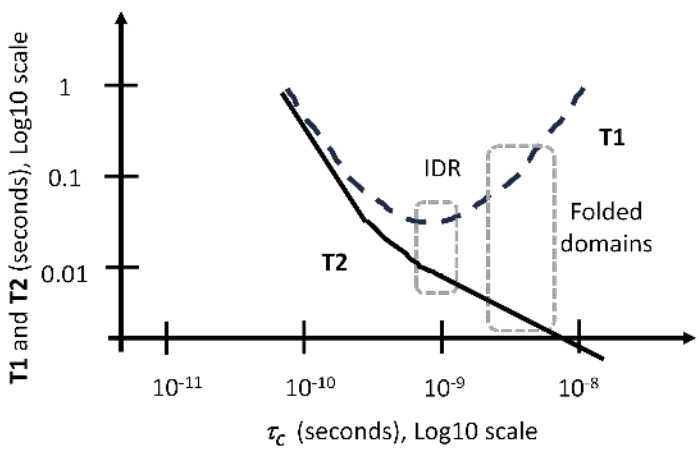


Figure 6.3.4: Modeled T_1 and T_2 values (Log scale) vs correlation time τ_C for individual residues in polymers (e.g., proteins), folded and not. Note that for small molecules or amino acids in a disordered polypeptide ($\tau_C \approx 1$ ns) the T_1 and T_2 values are relatively close to each other (dashed rectangle marked IDR). Contrary to that, for the residues within a typical folded domain ($\tau_C \geq 4$ ns, dashed rectangle marked "Folded domains"), the T_2 values are significantly shorter than T_1 (>10X difference).

Big Picture

A rich repertoire of solution NMR experiments have been developed and applied in recent decades to probe biochemical and biophysical processes over time scales ranging from below picoseconds to hours and above. In addition to T_1 and T_2, { ^{15}N$-^1$H}-NOE (Nuclear Overhauser Effect) data provides an independent experimental probing of the ps-ns backbone motions and can distinguish between folded/ordered and unfolded/disordered domains. Another group of methods ($R1\rho$, CPMG, CEST, etc.) produce data sensitive to motions or changes in the μs-ms time scale. More traditional techniques, e.g. reporting chemical shift values, can be utilized to distinguish states in slow exchange (seconds-hours). Although even a brief introduction of most of these methods is outside the scope of this text, a simple list of these approaches is enough to demonstrate that modern solution NMR is a uniquely powerful technique, capable of providing site-specific information about biomolecular structure, dynamics and function.

Further theory (optional)

A common mathematical approach relating the description of how spins involved in molecular motions and intermolecular communications at various frequencies interact with constant and alternating magnetic fields (e.g., NMR pulses) is based on the concept of the **spectral density function**, $J(\omega)$. The spectral density function essentially reports a probability value J indicative of the system's likelihood to exhibit motions or interactions happening at a given frequency ω. Mathematically, the spectral density function can be calculated as the Fourier transform of the correlation function $C(t)$. Thus, the more complex the motions of the system, the more sophisticated its model of the correlation function needs to be and thus the more laborious it will be to propose a realistic and practically useful spectral density function. Although, $C(t)$ and $J(\omega)$ can be complex and even very non-trivial in many scenarios, working them out is well worth the effort as it helps to design NMR experimental schemes targeting the frequency ranges relevant for the process or sample studied. The derivation of practically used forms of $J(\omega)$ is outside the scope of this Chapter, however at least one of the more advanced problems below provides an informative example to a curious reader.

Worked Problems

Problem 6.3.1

What is the highest frequency of periodic oscillations of the smallest Newtonian objects (e.g. atoms) at room temperature and at regular body (human) temperature?

Solution

Room temperature corresponds to 25 °C (298 K), normal human body temperature is 37 °C (310 K). The oscillation frequency will be highest at $\Delta G^{\ddagger} = 0$ (i.e., no barrier between the two states).

As per Equation 6.3.1, the highest frequency then is:

- at 298 K:

$$\frac{(1.38 \times 10^{-23}\ \mathrm{J/K})(298\ \mathrm{K})}{6.63 \times 10^{-34}\ \mathrm{J \cdot s}} = 0.620 \times 10^{13}\ (1/\mathrm{s})$$

- at 310 K:

$$\frac{(1.38 \times 10^{-23}\ \mathrm{J/K})(310\ \mathrm{K})}{6.63 \times 10^{-34}\ \mathrm{J \cdot s}} = 0.645 \times 10^{13}\ \mathrm{Hz}$$

Problem 6.3.2

What is the magnitude of the energy barrier $\Delta G^{\ddagger}$ between two states (A and B) of a macromolecule that corresponds to transition time of 1 ps and 1 μs at normal human body temperature?

Solution

Transition time of 1 μs matches to frequency $\nu = 1.00 \times 10^{6}\ \mathrm{Hz} = 1\ \mathrm{MHz}$; for 1 ps, $\nu = 1.00 \times 10^{12}\ \mathrm{Hz} = 1\ \mathrm{THz}$.

As per Equation 6.3.1, for the transition frequency of

- $1.00 \times 10^{6}\ \mathrm{Hz}$:

$$\begin{aligned}\Delta G^{\ddagger} &= RT\,\frac{\ln\left(\dfrac{k_B\,T}{h}\right)}{\nu} \\ &= 40.3\ \mathrm{kJ/mol}\end{aligned}$$

- $1.00 \times 10^{12}\ \mathrm{Hz}$:

$$\Delta G^{\ddagger} = RT\,\frac{\ln\left(\frac{k_B\,T}{h}\right)}{\nu}$$
$$= 4.80\ \mathrm{kJ/mol}$$

Practice Problem 6.3.1

Can the highest frequency of periodic oscillations of the smallest Newtonian objects (e.g., atoms) exceed 10^{13} Hz in a biological scenario on our planet

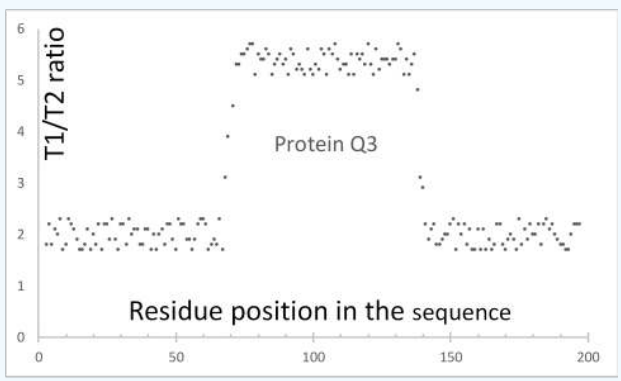
Practice Problem 6.3.2

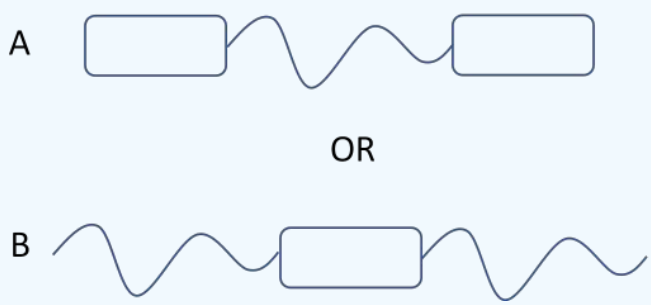
Chapter 3 of this text discusses molecular dynamics simulations of biological molecules. Commonly, the time step of 1 femtosecond (fs) or 2 fs are used today in these simulations to reevaluate the positions of al the atoms in the molecules and of all the forces acting upon them. The fact that these time steps are finite (non-zero) makes the simulations appear less than ideal. Given that the computational resources available today are quite extensive, would you (or would you not) suggest reducing these timesteps below 1 fs? Justify quantitatively.

Practice Problem 6.3.3

As a common practice, the "T_1/T_2 vs. residue number" graph for protein Q3 is provided.

(This way, less space in a journal article is used as opposed to showing two separate graphs, one for T_1 and the other – for T_2 as functions of the residue position in the amino acid sequence)





T_1/T_2 vs. residue number" graph: The round-edge rectangles correspond to folded domains, wiggly lines to IDRs

a. How many residues does protein Q3 have?
b. Describe the 2° / 3° structure of Q3 in terms of residues belonging to folded domains vs. residues belonging to intrinsically disordered regions (IDRs).
c. What structural model, A or B, (below) corresponds to T_1 and T_2 NMR data for protein Q3?

Practice Problem 6.3.4*

The correlation function for a spherical molecule (folded protein): $C(t) = C(0)\, e^{-t/\tau}$. What is the algebraic expression for the spectral density function $J(\omega)$ for this molecule? Use the obtained algebraic expression for $J(\omega)$ to estimate the "highest rates" of rotational motion such a sample. You can define the "highest rates" as the frequency ω value which corresponds to the $J(\omega)$ value at 50% of its maximum.

Practice Problem 6.3.5

What environmental factors and solvent properties affect the correlation time value τ in Practice Problem $6.3.4$ and how specifically?

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CHAPTER OVERVIEW

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7.1: Appendix I.

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7.1: Appendix I.

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Glossary

2D NMR Spectroscopy | A technique that provides information on atomic interactions in a protein, allowing higher spectral resolution and better peak assignment.

Advanced Topics in Molecular Dynamics | In-depth concepts like enhanced sampling techniques, free energy calculations, and multiscale modeling.

Analysis of Molecular Dynamics Trajectories | The process of interpreting the results from molecular dynamics simulations to understand molecular behavior.

Basic Elements of Spectroscopy | Components such as light sources, detectors, and sample cells used in spectroscopic analysis.

Boltzmann Distribution | Describes the distribution of particles among available energy states in a system.

Bonding Interactions | Forces that hold atoms together within molecules (e.g., covalent, ionic, or hydrogen bonds).

Chemical Shift | The displacement of a resonance frequency relative to a standard, measured in parts per million (ppm).

Conformational States | Different shapes that a protein can adopt, which can influence its biological activity.

Equations of State | Mathematical relationships that describe the state of a system, such as the ideal gas law.

Equilibria in Biochemical Systems | Describes the balance between forward and reverse reactions in biochemical processes.

Excite-Record Experiment | The process of exciting nuclei with a magnetic pulse and recording their response to generate an NMR spectrum.

First Law of Thermodynamics | Law of energy conservation, stating that energy cannot be created or destroyed, only transformed.

Fluorescence | The emission of light by a molecule that has absorbed light or other electromagnetic radiation.

Fourier Transformation | A mathematical method to convert data from the time domain (FID) into the frequency domain (NMR spectrum).

Gibbs Free Energy | A thermodynamic potential used to predict the spontaneity of a process at constant temperature and pressure.

Helmholtz Free Energy | A thermodynamic potential used to predict spontaneity at constant temperature and volume.

Heteronuclear 3D NMR | A three-dimensional NMR technique that involves multiple types of nuclei, enabling resonance assignments and structural analysis of proteins.

Infrared (IR) Spectroscopy | A technique that measures the absorption of infrared light by molecules, helping identify functional groups.

Intermolecular Forces | Forces that act between molecules, including van der Waals forces, dipole-dipole interactions, and hydrogen bonds.

Kinetic Rate Laws | Mathematical expressions that describe the rate of a chemical reaction as a function of reactant concentrations.

Magnetic Field | A field that affects the behavior of magnetic dipoles like nuclear spins, fundamental to NMR spectroscopy.

Molecular Dynamics Simulations | Computational simulations that model the behavior of atoms and molecules over time.

Newtonian Mechanics | A branch of physics that describes the motion of objects based on forces acting on them.

NMR for Protein Function | Using NMR spectroscopy to understand how protein structure relates to its biological function and dynamics.

Nuclear Spin | The intrinsic angular momentum of nuclei that can interact with magnetic fields.

Phosphorescence | Emission of light from a molecule following absorption, where the emission persists longer than fluorescence.

Potential Energy Surface | A multidimensional surface that describes the energy of a system in relation to the positions of the atoms.

Protein Dynamics | The study of how proteins change shape and function in response to their environment, often analyzed through NMR.

Quantum Mechanics and Quantum Oscillator Model | Describes molecular vibrations and energy levels as quantized.

Reaction Mechanisms | A step-by-step sequence of elementary reactions that leads to the overall chemical transformation.

Second Law of Thermodynamics | States that the total entropy of an isolated system always increases over time.

Spectral Sensitivity and Resolution | Factors that determine the clarity and detail of an NMR spectrum, influenced by the sample, equipment, and experimental conditions.

Spectroscopy | The study of the interaction between matter and electromagnetic radiation.

Statistical Definition of Entropy | Entropy is related to the number of possible microstates in a system, quantified by the Boltzmann constant.

Thermochemistry | Study of heat changes that occur during chemical reactions.

Thermodynamic Variables | Quantities that define the state of a system (e.g., pressure, volume, temperature, and chemical composition).

Transition State Theory | Theory that explains how chemical reactions occur by describing the transition between reactants and products via a high-energy state.

Two Masses on a Spring Model | A simplified model used to describe molecular vibrations, particularly relevant in infrared (IR) spectroscopy.

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    - 7.1: Appendix I. - *CC BY-NC-SA 4.0*
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