Chemical Potential

Chemical reactions
Dissociation of salts
Self-assembly of amphiphiles
NB

Queste diapositive sono state preparate per il corso di Biofisica tenuto dal Dr. Attilio V. Vargiu presso il Dipartimento di Fisica nell’A.A. 2014/2015

Non sostituiscono il materiale didattico consigliato a piè del programma.
References

• Books and other sources
  • Biological Physics (updated 1st ed.), Philip Nelson, Chap. 8
  • Biochemistry (5th ed.), Berg et al., Chap. 1

• Movies

• Exercise
Chemical energy is a source of life

**Most important energy conversion mechanism in living organisms of all dimensions involves chemical energy**

- Food is source of energy, and most useful work done in cells involves metabolizing nutrients (chemical synthesis).
  - Energy is trapped in the *chemical bonds* of nutrient molecules.
- Multi-step metabolic pathways catalyzed by enzymes realize the conversion of food (substrates) into useful molecules (products), i.e. conversion of chemical energy into mechanical, electrical, or chemical (again, to store energy) work.
Chemical energy is a source of life

- Cells keep chemical reactions in balance essentially by recycling: they disassemble large molecules into building blocks used to create new components they require.
- Breaking down of complex organic molecules (e.g. proteins, nucleic acids, and polysaccharides into amino acids, nucleotides, and sugars, respectively) occurs via catabolic pathways and usually involves release of energy (exothermic process).
- Synthesis of new macromolecules occurs via anabolic pathways requiring energy.
Chemical energy is a source of life

- Cells must balance catabolic and anabolic pathways as to control levels of critical metabolites and ensure that sufficient energy is available.
  - For example, if supplies of glucose wane, cells synthesize glucose from other materials or send fatty acids into the citric acid cycle to generate ATP.
  - Conversely, excess glucose converted into storage forms (glycogen, starches, fats).
Chemical energy is a source of life

• As for eating food, also burning wood, coal, or gasoline combines carbon in energy source with oxygen from atmosphere to make carbon dioxide and energy.
Chemical potential

All of these processes involves creation (assembly by synthesis) and destruction (disassembly by digestion) of several “particles”.

• The availability of molecules of a species, and thus the amount of chemical energy stored therein, is measured by the chemical potential of the species.

• In open system of $N_1$ “particles” of type 1, ... , $N_\alpha$ of type $\alpha$, ..., where $N_\alpha$ and total energy $E$ can vary, chemical potential of species $\alpha$ is:

$$
\mu_\alpha = -T \frac{\partial S}{\partial N_\alpha} \bigg|_{E, N_\beta, \beta \neq \alpha}
$$

Derivative at fixed value of $E$ needed because internal (chemical energy can vary)!

$E \to$ total energy of the system.

Includes all kinds of contributions: kinetic, mechanical, electromagnetic, chemical, ... 

$$
E = K + U(R) + \sum_{\alpha} N_\alpha \varepsilon_\alpha
$$

$\varepsilon_\alpha \to$ internal energy of species $\alpha$, can change during any kind of reaction
Chemical potential

When two systems $A$ and $B$ can exchange energy and particles, both thermal and chemical flows must be balanced at equilibrium:

\[ T_A = T_B \quad \text{Thermal equilibrium} \]

\[ \Delta T \rightarrow \text{availability of energy: entropic force driving energy transfer between } A \text{ and } B \]

\[ \Delta \mu_{A,B} \rightarrow \text{availability of particles: entropic force driving net transfer of } \alpha \text{ particles between } A \text{ and } B \]

- For dilute solutions $\mu$ can be written as sum of a term dependent on concentration and a concentration-independent one involving internal energy of molecules (plus eventually a term depending on a conservative force – gravitational, electric, etc):

\[ \mu = k_B T \ln \left( \frac{c}{c_0} \right) + \mu^0(T) + U \left( \{R\} \right) \]

\[ c = \frac{N}{V} \rightarrow \text{number density} \]

\[ c_0 \rightarrow \text{reference concentration, usually 1M} \]

- $\mu_0$ standard chemical potential at temperature $T$ (defined with respect to $c_0$), for an ideal gas given by:

\[ \mu^0(T) = \varepsilon - \frac{3}{2} k_B T \ln \frac{m k_B T}{2 \pi \hbar^2 c_0^{2/3}} \]
Chemical potential

The activity of a species is defined as:

\[ \gamma = e^{(\mu - \mu_0)/k_BT} \]

- For dilute solutions \( \gamma \) approximately equals the ratio \( c/c_0 \).

- Cells are crowded in their interior, so equation for \( \mu \) and \( \mu_0 \) will not hold in cellular environments.

- However, general definition and condition for chemical equilibrium are universal laws.

A molecular species will be highly available for chemical reactions if its concentration \( c \) or its internal energy \( \varepsilon \) are big, and \( \mu \) describes the overall availability.
Chemical potential drives reactions

• Statistical state function are inter-convertible by means of Legendre transforms → \( \mu \) also expressed as generalized force of \( G(p,T), F(T,V), H(S,p) \).

• Particularly useful defining \( \mu \) as Gibbs free energy change per change in density (or number of particles of each species):

\[
\mu_\alpha = \frac{\partial G}{\partial N_\alpha} \bigg|_{T,p,N_\beta,\beta \neq \alpha}
\]

• For general reaction involving \( m \) species (\( k \) reactants and \( m-k \) products):

\[
\nu_1 X_1 + \ldots + \nu_k X_k \Leftrightarrow \nu_{k+1} X_{k+1} + \ldots + \nu_m X_m
\]

\( \Delta G \) of reaction given by algebraic sum of \( m \) chemical potentials each multiplied by its stoichiometric coefficient \( \nu_k \):

\[
\Delta G \equiv -\nu_1 \mu_1 - \ldots - \nu_k \mu_k + \nu_{k+1} \mu_{k+1} + \ldots + \nu_m \mu_m
\]
Chemical potential drives reactions

\[ \Delta G \equiv -\nu_1 \mu_1 - \ldots - \nu_k \mu_k + \nu_{k+1} \mu_{k+1} + \ldots + \nu_m \mu_m \]

• \( \Delta G \rightarrow \) net chemical force driving reaction: 
  this will run forward if \( \Delta G \) is negative, backward if it is positive.

• At equilibrium, \( \Delta G \) and flows in both directions compensate.

Since \( \mu = k_B T \ln \left( \frac{c}{c_0} \right) + \mu^0 \left( T \right) \)

also \( \Delta G \) expressed as sum of concentration-dependent part and concentration-independent standard free energy change:

\[ \Delta G^0 \equiv -\nu_1 \mu^0_1 - \ldots - \nu_k \mu^0_k + \nu_{k+1} \mu^0_{k+1} + \ldots + \nu_m \mu^0_m \]

Rearrangement of general formula gives **Mass Action rule:**

\[
\begin{bmatrix} X_{k+1} \\ X_1 \end{bmatrix}^{\nu_{k+1}} \ldots \begin{bmatrix} X_m \end{bmatrix}^{\nu_m} = K_{eq} = e^{-\Delta G^0 / k_B T} \]

\[
\begin{bmatrix} X \end{bmatrix} = \frac{c}{c_0}, \quad c_0 = 1M
\]


NB: Queste dispense non sostituiscono il materiale didattico suggerito a piè del programma!
Often useful to define logarithmic measure of equilibrium constant $K_{eq}$:

$$pK \equiv -\log_{10} K_{eq}$$

Particularly true when studying equilibrium and kinetics of reactions in solvent, where solution’s $pH$ can drive reactions:

- All hydrogenous molecules can dissociate in water, giving off one or more hydrogen atoms to solution.

- Also water can dissociate, the reaction being:

$$H_2O \rightleftharpoons H^+ + OH^-$$

with dissociation constant given by (in standard conditions):

$$K = \frac{[H^+][OH^-]}{[H_2O]} = 1.8 \cdot 10^{-16}$$
Remarks:

- Considering that in dilute solutions water concentration is always $\sim 55.5 \text{M}$, define new constant $K_W$ (ion product of water – at room T) independent from $[H_2O]$:

\[
[H_2O] = \frac{C_{H_2O}}{C_0} \approx 55.5 \rightarrow K_W \equiv K[H_2O] = [H^+][OH^-] \approx 1.8 \cdot 10^{-16} \cdot 55.5 \approx 10^{-14}
\]

\[K_W \equiv [H^+][OH^-] = 10^{-14} \rightarrow pK_W = 14\]

- Actually not $H^+$ but hydronium $H_3O^+$ and bigger ions are found in solution.
  - Ion product allows easily calculating $[H^+]$ from $[OH^-]$ and viceversa:
    \[
    [H^+] = 10^{-14}/[OH^-] \rightarrow [H^+] = 10^{-7} \iff [OH^-] = 10^{-7}
    \]
  - Concentration of hydronium and hydroxide ions in pure water $10^{-7} \text{M}$. 

Remarks:

- Considering that in dilute solutions water concentration is always ~55.5M, new constant $K_W$ (ion product of water – at room T) defined independent from $[H_2O]$:

$$[H_2O] = \frac{C_{H_2O}}{C_0} \approx 55.5 \quad \rightarrow \quad K_W \equiv K[H_2O] = [H^+][OH^-] \approx 1.8 \cdot 10^{-16} \cdot 55.5 \approx 10^{-14}$$

$$K_W \equiv [H^+][OH^-] = 10^{-14} \quad \rightarrow \quad pK_W = 14$$

- If concentration of $H_3O^+$ ($OH^-$) increases, e.g. by adding strong acid, $HCl$ (or base, $NaOH$), concentration of $OH^-$ ($H_3O^+$) must decrease (through reaction $2H_2O \rightleftharpoons H_3O^+ + OH^-$) in order for the Mass Action law still be valid

\[ \downarrow \]

Concentration of hydroxyl and hydroxide ions vary in opposite directions in order to maintain constant the value of water ion product
Summarize these facts by defining *pH of solution* as:

\[ pH = -\log_{10}[H^+] \]

- *pH* of pure water (neutral *pH*) is 7.
- Adding *H*⁺ to solution increases [H⁺], lowers *pH*: solution with *pH* < 7 called *acidic*.

  Acid: neutral substance releasing *H*⁺ in water.
- Adding *OH*⁻ to solution decreases [H⁺], rising *pH*: solution with *pH* > 7 called *basic*.

  Base: neutral substance releasing *OH*⁻ in water.

**Degree of dissociation of substances related to *pK* of reaction:**

- Strong acids and bases dissociate almost totally: e.g. *H*₃*PO*₄ and *NaOH*.
- Carboxyl (-*COOH* ⇌ -*COO⁻ + *H*⁺) and amine (-*NH*₃⁺ ⇌ -*NH*₂ + *H*⁺) groups most present in proteins dissociate only partially (weak acids and bases).
Acid-base reactions and pH

**pH influence reaction equilibria**

- $pK_a$ of generic acid-base reaction can be written as function of $pH$:

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

$$\downarrow$$

$$-\log_{10}(K_a) = -\log_{10}\left(\frac{[H^+][A^-]}{[HA]}\right) = -\log_{10}\left([H^+]\right) - \log_{10}\left([A^-]/[HA]\right)$$

$$\downarrow$$

$$pH = pK_a + \log_{10}\left([A^-]/[HA]\right)$$

**Henderson–Hasselbalch equation**

- When solution $pH$ equals reaction $pK_a$, deprotonation is halfway to completion:

$$pH = pK_a \rightarrow \log_{10}\left([A^-]/[HA]\right) = 0 \rightarrow [A^-] = [HA]$$
Acid-base reactions and pH

**pH influence protonation probability of acids and bases**

- In general, probability of an acid to be protonated given as:

\[
P_a^H = \frac{[HA]}{([A^-]+[HA])}
\]

\[
\downarrow
\]

\[
[HA] = \frac{[H^+][A^-]}{K_a}
\]

\[
P_a^H = \frac{1}{1 + K_a/\left[H^+\right]} = \frac{1}{1 + K_a 10^{\text{pH} - \text{pK}_a}} = \frac{1}{1 + 10^{\text{pH} - \text{pK}_a}}
\]

- Average charges on acidic and basic residues given by:

\[
[HA] \Leftrightarrow [H^+][A^-] \rightarrow \langle q_a \rangle = -e \left(1 - P_a^H\right)
\]

\[
[HB^+] \Leftrightarrow [H^+][B] \rightarrow \langle q_b \rangle = eP_b^H
\]

\(<q>\) decreases with increase in pH
pH and titration curves

• Acid and basic groups in macromolecules do not behave independently but affect each other.

• pH appearing in equations should be local value around each residue: variable and difficult to know.

• However, one can titrate a solution containing acids and/or bases, i.e. change global pH of solution and track changes in protonation states (and thus total charge).

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Titration curve of ribonuclease
**pH and titration curves**

- Titration curve depends on $pK_a$ of groups present in solution:
  - $P^H_a$ rapidly varying from 0 to 1 in ±2 $pH$ units around $pK_a$, otherwise constant 0 or 1 value.
  - Starting from high $[H^+]$ (low $pH$) all groups will be protonated → most acidic neutral, bases positively charged, macromolecule positively charged.
  - Increasing $pH$ first affect only acidic groups, which give off their $H$s, becoming negative and lowering total charge of macromolecule.
  - As $pH$ rises, weak and then strong bases will become neutral, still lowering total charge.

![Titration curve graph]
$pK_a$ values in proteins

<table>
<thead>
<tr>
<th>Group</th>
<th>Acid</th>
<th>Base</th>
<th>Typical $pK_a$*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal $\alpha$-carboxyl group</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
<td>3.1</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
<td>4.1</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
<td>6.0</td>
</tr>
<tr>
<td>Terminal $\alpha$-amino group</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
<td>8.0</td>
</tr>
<tr>
<td>Cysteine</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
<td>8.3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
<td>10.9</td>
</tr>
<tr>
<td>Lysine</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
<td>10.8</td>
</tr>
<tr>
<td>Arginine</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
<td>12.5</td>
</tr>
</tbody>
</table>

* $pK_a$ values depend on temperature, ionic strength, and the microenvironment of the ionizable group.

Each aminoacid has its characteristics $pK_a$ values

Range from $\sim$4 for most acidic aminoacids (GLU and ASP) to $\sim$12.5 for most basic ARG.

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**$pK_a$ values in proteins**

Each aminoacid has its characteristics $pK_a$ values of titration curve

- **Glycine**
  - $pK_1 = 2.34$
  - $pK_2 = 9.60$
  - $pI = 5.97$

- **Histidine**
  - $pK_1 = 1.82$
  - $pK_2 = 6.0$
  - $pK_R = 9.17$

- **Glutamate**
  - $pK_1 = 2.19$
  - $pK_2 = 9.67$
  - $pK_R = 4.25$
**pKₐ values in nucleic acids**

<table>
<thead>
<tr>
<th>Base</th>
<th>Group</th>
<th>Nucleoside</th>
<th>3’-Nucleotide</th>
<th>5’-Nucleotide</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>N1</td>
<td>3.52</td>
<td>3.70</td>
<td>3.88</td>
</tr>
<tr>
<td>C</td>
<td>N3</td>
<td>4.17</td>
<td>4.43</td>
<td>4.56</td>
</tr>
<tr>
<td>G</td>
<td>N7</td>
<td>3.30</td>
<td>3.50</td>
<td>3.60</td>
</tr>
<tr>
<td>G</td>
<td>N1</td>
<td>9.42</td>
<td>9.84</td>
<td>10.00</td>
</tr>
<tr>
<td>T</td>
<td>N3</td>
<td>9.93</td>
<td>10.47</td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>N3</td>
<td>9.38</td>
<td>9.96</td>
<td>10.06</td>
</tr>
</tbody>
</table>

*pKₐ values of nucleobases at 20°C without salt.*

Nucleobases are uncharged under physiological conditions (5 < pH < 9): functional to realization of intra-strand H-bonds.

- As pH approaches 9, proton on N1@G \((pK_{\alpha} \sim 9.7)\) participating in H-bond with C gets lost → DNA double helix destabilized.

- Below pH 5, some of H-bond acceptors participating in base-pairing become protonated → double helix separates.

\[\downarrow\]

**Acid–base reactions that remove or donate protons at specific positions on DNA bases can disrupt double helix.**

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Buffers can regulate pH in organism and in lab experiments

• Given drastic effect of pH changes on structure of macromolecules, organisms have engineered mechanisms to mitigate these changes.

• Mechanism involve a solution, buffer, that can absorb pH changes due to addition of an acid, a base, and to change in concentration (e.g. dilution).

Comparing titration curves obtained by adding 1M of HCl in pure water and in a 0.1M solution of sodium acetate (Na\(^+\)CH\(_3\)COO\(^-\)) reveals efficacy of buffer in control of pH changes.

Why?

• Sodium acetate dissociates completely in water, thus H\(^+\) ions react with acetate ions forming acetic acid.

• This happens until all acetate ions get protonated, thus limiting increase of hydronium ions concentration in solution and drop in pH.

• When no more ions are left to sequester protons, pH start again to drop with same pendency as in case of very dilute solutions.
Buffers can regulate pH in organism and in lab experiments

- Given drastic effect of pH changes on structure of macromolecules, organisms have engineered mechanisms to mitigate these changes.
- Mechanism involve a solution, buffer, that can absorb pH changes due to addition of an acid, a base, and to change in concentration (e.g. dilution).

Henderson–Hasselbalch equation gives ratio of acetate ion vs. acetic acid concentration:

\[
\frac{[A^-]}{[HA]} = 10^{pH - pK_a}
\]

Since \(pK_a\) of acetic acid is 4.75:

- At high pH very little acetic acid formed: 
  \(pH=9 \rightarrow \frac{[A^-]}{[HA]}=10^{9-4.75} \approx 17.800\).
- At \(pH=4.75\) half acetic acid formed: 
  \(\frac{[A^-]}{[HA]}=10^{4.75-4.75}=1\).
- At low pH most acetate ions get protonated: 
  \(pH=3 \rightarrow \frac{[A^-]}{[HA]}=10^{3-4.75} \approx 0.02\).
**pH buffers**

*A buffer solution works better in region around $pK_a$ of acid component*

- Key buffer solution found in biological system should have $pK_a$ close to 7.4.

- One buffer based on phosphoric acid ($H_3PO_4$), which can be deprotonated in three steps to form a phosphate ion:

  
  \[
  \begin{align*}
  H_3PO_4 &\rightarrow H_2PO_4^- \\
  &\quad \text{pK}_a = 2.12 \\
  H_2PO_4^- &\rightarrow HPO_4^{2-} \\
  &\quad \text{pK}_a = 7.21 \\
  HPO_4^{2-} &\rightarrow PO_4^{3-} \\
  &\quad \text{pK}_a = 12.67
  \end{align*}
  \]

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- Since $pK_a$ of 2nd reaction $\sim 7.21$, inorganic phosphate exists primarily as a nearly equal mixture of $H_2PO_4^-$ and $HPO_4^{2-}$ at physiological pH.

  - In blood *inorganic phosphate* $\sim 1\text{mM}$

  \[
  \downarrow
  \]

  Useful buffer against acid-base reactions: increases amount of acid required to produce drop in $pH$ from 7.4 to 7.3 by a factor of $\sim 6000$ compared with pure water.
Calculate amount of acid to add in order to change pH from 7.4 to 7.3:
(a) in water and (b) in 1 mM phosphate buffer.

**Water**

\[
\Delta pH = 7.3 - 7.4 \rightarrow \Delta[H^+] = (10^{-7.3} - 10^{-7.4}) M \approx (5 \cdot 10^{-8} - 4 \cdot 10^{-8}) M = 10^{-8} M
\]

**Phosphate buffer**

Total concentration of buffer: \(1mM = [H_2PO_4^-] + [HPO_4^{2-}]\)

At pH=7.4

\[
\text{ion/acid ratio: } \frac{[HPO_4^{2-}]}{[H_2PO_4^-]} = 10^{pH-pK_a} = 10^{0.19} \approx 1.55
\]

\[
[HPO_4^{2-}] \approx 1.55[H_2PO_4^-] = 1.55 \left(1 \text{ mM} - [HPO_4^{2-}]\right) \rightarrow [HPO_4^{2-}] = 1.55/2.55 \text{ mM} \approx 0.61 \text{ mM}
\]

\[
[H_2PO_4^-] = 1 \text{ mM} - [HPO_4^{2-}] \approx 0.39 \text{ mM}
\]
**pH buffers**

Calculate amount of acid to add in order to change pH from 7.4 to 7.3:
(a) in water and (b) in 1 mM phosphate buffer.

**Water**

\[ \Delta pH = 7.3 - 7.4 \rightarrow \Delta \left[ H^+ \right] = \left( 10^{-7.3} - 10^{-7.4} \right) M \approx \left( 5 \cdot 10^{-8} - 4 \cdot 10^{-8} \right) M = 10^{-8} M \]

**Phosphate buffer**

Total concentration of buffer: \( 1 \text{mM} = \left[ H_2PO_4^- \right] + \left[ HPO_4^{2-} \right] \)

At \( \text{pH}=7.3 \)

ion/acid ratio: \( \left[ HPO_4^{2-} \right]/\left[ H_2PO_4^- \right] = 10^{\text{pH}-pK_a} = 10^{0.09} \approx 1.23 \)

\[ \left[ HPO_4^{2-} \right] \approx 1.23 \left[ H_2PO_4^- \right] = 1.23 \left( 1 \text{mM} - \left[ HPO_4^{2-} \right] \right) \rightarrow \left[ HPO_4^{2-} \right] = 1.23/2.23 \text{ mM} \approx 0.55 \text{ mM} \]

\[ \left[ H_2PO_4^- \right] = 1 \text{ mM} - \left[ HPO_4^{2-} \right] \approx 0.45 \text{ mM} \]
**pH buffers**

Calculate amount of acid to add in order to change $pH$ from $7.4$ to $7.3$: (a) in water and (b) in $1 \text{ mM}$ phosphate buffer.

### Water

$$\Delta pH = 7.3 - 7.4 \rightarrow \Delta [H^+] = \left(10^{-7.3} - 10^{-7.4}\right) M \approx \left(5 \cdot 10^{-8} - 4 \cdot 10^{-8}\right) M = 10^{-8} M$$

### Phosphate buffer

Amount of $H^+$ consumed by buffer is equal to amount of $HPO_4^{2-}$ converted into $H_2PO_4^-$.  

$$\Delta \left[HPO_4^{2-}\right] = \Delta [H^+] \approx (0.61 - 0.55) \text{ mM} = 0.06 \text{ mM} = 6 \cdot 10^{-5} M$$

Useful buffer against acid-base reactions: increases amount of acid required to produce drop in $pH$ from $7.4$ to $7.3$ by a factor of $\sim 6000$ compared with pure water.
Kinetic interpretation of equilibria

Although thermodynamic of reactions well defined by Keq, kinetic interpretation can be tricky and needs additional source of information about every step of (apparently simple) complicated reactions

• Consider reaction between two diatomic molecules:

\[ X_2 + Y_2 \Leftrightarrow 2XY \]

• Simplistic reasoning would lead to conclude that rate at which a molecule \( X_2 \) find and bumps a molecule \( Y_2 \) is proportional to \([Y_2]\) and total number of bumps per time proportional to \([X_2]\). The same reasoning for backward reaction gives:

\[ r_+ = k_+ [X_2][Y_2] \quad \text{and} \quad r_- = k_- (c_{XY})^2 \]

• Reaction constants \( k_+ \) and \( k_- \) are related to equilibrium constant \( K_{eq} \) as \( r_+ \) and \( r_- \) are equal at equilibrium:

\[ \frac{r_+}{r_-} = 1 = \frac{k_+ [X_2][Y_2]}{k_- (c_{XY})^2} = \frac{k_+}{k_-} \frac{1}{K_{eq}} \quad \rightarrow \quad \frac{k_+}{k_-} = K_{eq} \]
Kinetic interpretation of equilibria

• Thus, reaction should be of first order both in $X_2$ and $Y_2$.

• Can happen that reaction is of zeroth order in $Y_2$ (increasing concentration does not affect $r_+$) and second order in $X_2$ (doubling concentration quadruplexes the rate).

• Often this is not the case since only initial and final steps of reaction are seen, thus only reagents and products but not intermediates (due to intermediate reaction in multi-step processes) accessible.

\( \downarrow \)

True reaction mechanism unknown!

• Reaction might well occur in three steps with different reaction rates (e.g. first step much slower than others)

• Slower step called bottleneck or rate-limiting process.
Kinetic interpretation of equilibria

Rate-limiting process controls overall rate of reaction and gives true concentration dependence.

• Mass Action rule imposes each step to be in equilibrium when overall reaction is in equilibrium:

\[
\frac{(c_X)^2 c_{X_2}}{(c_{X_2})^2} = K_{eq,1}c_0, \quad \frac{c_{XY_2}}{c_Xc_{Y_2}} = K_{eq,2}, \quad \frac{(c_{XY})^2}{c_{XY_2}c_X} = K_{eq,3}
\]

• Overall reaction rate found by multiplying three step-wise \( K_{eq,i} \):

\[
K_{eq} = K_{eq,1}K_{eq,2}K_{eq,3}
\]

\[\downarrow\]

Details of intermediate steps in a reaction are immaterial for its overall equilibrium
Chemical potential drives reactions

By definition, the reaction quotient

\[ \frac{[X_{k+1}]^{v_{k+1}} \ldots [X_m]^{v_m}}{[X_1]^{v_1} \ldots [X_k]^{v_k}} = K_{eq} = e^{-\Delta G^0/k_BT} \]

is always positive. The Mass Action Rule is a restatement of the Second Law of Thermodynamics.

When one or more chemical reactions occur fast enough to equilibrate on experimental time scale, equilibrium also implies relations between the various \( \mu_i \), namely one Mass Action rule for each relevant reaction.

**Le Châtelier’s Principle:** if equilibrium is removed system will react in direction opposite to induced change as to establish a new equilibrium (world’s entropy will increase):

- If \( X_1 \) is increased, reaction will run forward as to partly undoes equilibrium shift.
- If \( T \) is increased by heating system, equilibrium shifts towards higher-energy side of reaction: system absorb thermal energy, thus actual change in \( T \) lower than if no reaction had occurred. Again system reacts as to partly undoes original disturbance.
Chemical potential drives reactions

**Reaction quotient**

\[
\frac{[X_{k+1}]^{v_{k+1}} \cdots [X_m]^{v_m}}{[X_1]^{v_1} \cdots [X_k]^{v_k}} = K_{eq} = e^{-\Delta G^0/k_BT}
\]

_**Mass action rule is a restatement of Second Law of thermodynamics**_

When one or more chemical reactions occur fast enough to equilibrate on experimental time scale, equilibrium also implies relations between the various \( \mu_\alpha \), namely one Mass Action rule for each relevant reaction.

_Le Châtelier’s Principle:_ if equilibrium is removed system will react in direction opposite to induced change as to establish a new equilibrium (world’s entropy will increase):

• Le Châtelier’s Principle reminds us that when free energy barriers to cross are huge, systems can stay out of equilibrium (and thus apparently in equilibrium) for very long time (essentially indefinite).

• Deviation from full equilibrium represents stored free energy.
Self-assembly of amphiphiles

- Cells are defined by (thus can exists thanks to) plasma membranes protecting them from external environment.

- Biological membranes are mainly made up by amphiphiles, namely by elongated molecules containing one polar/charged end and a nonpolar one.

- Amphiphiles are also called surfactants or emulsifiers, and include detergents such as sodium dodecyl sulfate (SDS) and phospholipids such as phosphatidylcholine.

\[ \text{SDS} \quad \text{H}_3\text{C} \quad \begin{array}{c} \text{O} \\ \text{O} \\ \text{S} \\ \text{O} \\ \text{O} \\ \text{Na}^+ \end{array} \]

\[ \quad \text{PC} \quad \begin{array}{c} \text{O} \\ \text{O} \\ \text{P} \\ \text{O} \\ \text{O} \\ \text{N}^+ \end{array} \]
Self-assembly of amphiphiles

• Membranes and other structures such as micelles and vesicles (liposomes) form by self-assembling.

• Gradient in chemical potential, i.e. a (mainly entropic) chemical force drives aggregation of constitutive molecules.

• Amphiphiles inserted in a oil-water mixture migrate to the interface reducing its surface tension and removing direct contacts between the two substances.
Self-assembly of amphiphiles

- Membranes and other structures such as micelles and vesicles (liposomes) form by self-assembling.

- Gradient in chemical potential, i.e. a (mainly entropic) chemical force drives aggregation of constitutive molecules.

- Eventually oil droplets forms which are stabilized in water, creating an emulsion (such as mayonnaise).
Self-assembly of amphiphiles

• In water, surfactant molecules such as SDS assemble into micelles, thus minimizing the hydrophobic area exposed to solvent (hydrophobic effect).

• A given amphiphile forms micelles of typical size: too many will lead to some completely in the interior, too few will not shield hydrophobic tails.
Self-assembly of amphiphiles

- Formation of micelles occurs suddenly at values of \( c \) higher than a critical concentration, called \textit{critical micelle concentration (CMC)}.  

- In solution SDS (partly) dissociates into Na\(^+\) and SDS anions: number of anions can be measured from the osmotic pressure using the van’t Hoff relation:

\[
\Delta p = c_s k_B T
\]

- Comparing the behavior of this salt to KCl, the pressure due to free floating SDS anions dropped suddenly at solution concentration \( c=\text{CMC} \).
Self-assembly of amphiphiles

- At $c > \text{CMC}$ ratio of independently moving SDS anions to all ions dropped sharply.
  - No phase separation observed.

\[ \downarrow \]

Intermediate-size objects formed $\rightarrow$ micelles

- Same trend found for other properties such as electrical conductivity, polarization,…
Self-assembly of amphiphiles

- Results can be interpreted with simplified model:
  - All SDS molecules dissociate into Na\(^+\) and amphiphile SDS\(^-\).
  - The latter are in equilibrium between single molecules and N-units aggregates.

- Applying the Mass Action rule gives the monomer/micelle concentration ratio:
  \[
  c_N / (c_1)^N = K_{eq} / (c_0)^{N-1}
  \]

- Total concentration of monomers is then:
  \[
  c_{tot} = c_1 + Nc_N = c_1 \left( 1 + NK_{eq} \left( \frac{c_1}{c_0} \right)^{N-1} \right)
  \]

- CMC by definition concentration \(c_{tot} = c_*\) at which \(c_*\) = \(Nc_N = \frac{1}{2}c_*\), giving:
  \[
  \left( \frac{1}{2N} c_{N}^{*} \right) / \left( \frac{1}{2} c_1 \right)^N = K_{eq} / (c_0)^{N-1} \rightarrow c_{tot} = c_1 \left( 1 + \left( \frac{2c_1}{c_*} \right)^{N-1} \right)
  \]
Self-assembly of amphiphiles

\[ c_{tot} = c_1 \left( 1 + \left( 2 \frac{c_1}{c_*} \right)^{N-1} \right) \]

- Data can be fitted with varying parameters \( N \) and \( c_* \) and \( c_1 \) can be obtained as a function of the total amount \( c_{tot} \) of surfactant poured in solution.

- The limiting values obtained from this equation are:
  - Low concentration relative to CMC: \( c_{tot} \ll c_* \rightarrow c_1 \ll c_* \rightarrow c_{tot} \approx c_1 \)
  - High concentration relative to CMC: \( c_{tot} \gg c_* \rightarrow c_1 \gg c_* \rightarrow c_{tot} \approx Nc_N \)

- The osmotic pressure is given by van’t Hoff relation, taking into account that each micelle counts as one object. Thus, \( \Delta p \) relatively to a strong dissociating salt is:

\[
\frac{\Delta p_{SDS}}{\Delta p_{KCl}} = \frac{\Delta p_{SDS}}{2c_{tot}k_B T} = \frac{1}{2} \left( 1 + \frac{1 + N^{-1} \left( 2c_1/c_* \right)^{N-1}}{1 + \left( 2c_1/c_* \right)^{N-1}} \right)
\]
Self-assembly of amphiphiles

• Putting $c_*=1.4M$, poor fit obtained with $N=5$ (dashed line), while good fit with $N=30$ (solid line), typical dimension of SDS micelles.

• Sharpness of transition indicates that aggregates of 2, 3, etc SDS do not form as intermediates during micelle formation.

• Monomers cooperate to create a micelle, and cooperativity sharpens the transition as it mitigates the effects of random thermal motion.
Surfactant Action Mechanism

• To remove dirty material (usually containing hydrophobic molecules) from skin or clothes, surfactants (surface active agents, or detergents or tensioactives) sequester and enclose small droplets by the same mechanisms.

• Agitation and/or high temperature facilitate removal and wash out.

- Fat or oil stain
- Add surfactant
- Adsorbed surfactant lowers the interfacial tension between the fabric and the stain
- Stain does not desorb spontaneously
- Mechanical agitation
- Clean fabric + adsorbed surfactant: prevents re-adsorption of fat globule
- Removal of stain: Clean fabric
Surfactant Action Mechanism

Surface Active Agents
More phases...

Several more arrangements can be found varying the amphiphile/solvent ratio.
Self-assembly in cells: bilayers

Indeed, amphiphile molecules present in cells (phospholipids) do not have a cone shape compatible with formation of micelles, and they do form bilayers and thus vesicles of different dimensions.

- Self-assembly of two-chains phospholipids into bilayers driven by stronger entropic force than that causing micellization of single-chain amphiphiles.
- Exposing two hydrophobic chains to hydrophilic solvent has a free energy cost \( \Delta G \) about twice as great than exposing one.
- \( \Delta G \) enters equilibrium constant exponentially \( \rightarrow \) understood why membranes form and resist dissolving at concentration much lower than the CMC of micelles.
- Vesicles are suddenly formed to remove edges exposing hydrocarbon chains.
- Membrane are fluids assembled via hydrophobic effect and readily accepting embedded objects having a hydrophobic path roughly spanning twice the length of the phospholipid chain.
Self-assembly in cells: bilayers

- Membrane proteins can be solubilized using detergents above CMC concentration, which then form micelles incorporating both phospholipids and the protein.
- Detergents can also stabilize membrane fragments hindering vesicle formation.
Protein folding and surfactants

Anfinsen showed that for many proteins, at fixed external conditions (solvent, pH, ionic strength, pressure, temperature, etc.):

• Sequence completely determines folded structure.
• Native conformation corresponds to minimum of free energy.

• Native structures only marginally stable: $\Delta G$ rarely $> 20 k_B T$ at room T.
• Using organic solvent or adding small amount of surfactant to proteins suspended in polar solvent leads to protein denaturation $\rightarrow$ surfactant shield hydrophobic regions of polypeptide chain, reducing entropic force leading to their association.

$\downarrow$

Demonstrates that hydrophobic interactions are main forces driving protein folding
Hydrophobic effect and intermolecular forces

Hydrophobic effect also regulates aggregation of macromolecules:

- Fibrinogen proteins normally float in blood.
- Injury in blood vessels triggers an enzyme that clips off a part of fibrinogen, leaving an hydrophobic patch exposed to solvent in truncated protein (fibrin).
- Fibrins cluster together forming scaffold on which blood clot occurs.