Lecture 9: Theory of Non-Covalent Binding Equilibria

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General chemical reaction: \[ \nu_A A + \nu_B B \rightleftharpoons \nu_C C + \nu_D D \]

Very important type of “reaction”: **bimolecular non-covalent binding**

\[ R_{(sol)} + L_{(sol)} \rightleftharpoons RL_{(sol)} \]

• Small molecule dimerization/association
• Supramolecular complexes
• Protein-ligand binding
• Protein-protein binding/dimerization
• Protein-nucleic acids interactions
• ...

**Note**: We are implicitly assuming above that we can describe the system as being composed of 3 distinct chemical “species”, R, L, and RL (**quasi-chemical description**).

If interactions between R and L are weak/non-specific then it would be more appropriate to treat the system as a **non-ideal solution** of R and L.
Based on the earlier results for general reactions:

\[ \nu_A = \nu_B = 1; \nu_C = 1; \nu_D = 0 \]

\[ K_b(T) = \frac{\text{Binding constant}}{e^{-\Delta A_b(T)/kT}} = \frac{\left( \frac{C_{\text{RL}}}{C^\circ} \right)_{\text{eq.}}}{\left( \frac{C_R}{C^\circ} \right)_{\text{eq.}} \left( \frac{C_L}{C^\circ} \right)_{\text{eq.}}} \]

\[ K_b(T) = (V^\circ)^{\nu_C + \nu_B - \nu_A - \nu_B} \frac{\varphi_C(T)^{\nu_C} \varphi_D(T)^{\nu_D}}{\varphi_A(T)^{\nu_A} \varphi_B(T)^{\nu_B}} = C^\circ \frac{\varphi_{\text{RL}}(T)}{\varphi_R(T) \varphi_L(T)} \]

\[ \varphi_i(T) = \frac{1}{n_u \Lambda u_j} \int d x_u^{3n_u-3} e^{-\beta \Psi_i(x_u)} \]

\[ \Psi_i(x_u) \quad \text{Effective potential energy of solute } i \text{ in solution.} \]
If the solution is isotropic (\(\Psi_i(x_u)\) invariant upon rotation of solute), integrate analytically over rotational degrees of freedom (ignoring roto-vibarational couplings, OK at physiological temperatures.

\[
\varphi_i(T) = 8\pi^2 \varphi_{\theta i} = \frac{8\pi^2}{n_u} \prod_j \Lambda_{uj}^{3n_u} e^{-\beta \Psi_i(x_u)} = \frac{8\pi^2 Z'_i}{n_u} \prod_j \Lambda_{uj}^{3n_u}
\]

Internal coordinates

When inserting into the expression for \(K_b(T)\), \(\Lambda\)'s cancel because \(n_{RL} = n_R + n_L\)

We get:

\[
K_b(T) = \frac{C^\circ}{8\pi^2} \frac{Z'_{RL}}{Z'_R Z'_L}
\]

\[
Z'_R = \int d x_R^{3n_R-6} e^{-\beta \Psi_R(x_R)} \quad \quad Z'_L = \int d x_L^{3n_L-6} e^{-\beta \Psi_L(x_L)}
\]

\[
Z'_{RL} = ???
\]

In the complex, RL, the “external” coordinates (translations, rotations) of the ligand become internal coordinates of the complex.

\[ Z'_{RL} = \int_{"BOUND"} d\mathbf{x}_R^{3n_R-6} d\mathbf{x}_L^{3n_L-6} d\zeta_L^6 e^{-\beta \psi_{RL}(\mathbf{x}_R, \mathbf{x}_L, \zeta_L)} \]

- **External coordinates of the ligand relative to receptor**
  \[ \zeta_L^6 = \left\{ (r, \theta, \varphi), (\omega_1, \omega_2, \omega_3) \right\} \]

- **Position of the ligand relative to receptor frame**
- **Orientation of the ligand relative to receptor frame**

**It is up to us to come up with a reasonable definition of “BOUND”.** That is we need to define the RL species before we can compute its partition function. The binding constant will necessarily depend on this definition. **Must match experimental reporting.**

If the binding is strong and specific the exact definition of the complexed state is often not significant.
It is convenient to introduce an “indicator” function for the complex:

\[
I(\zeta_L) = \begin{cases} 
1 & \text{ligand is in binding site} \\
0 & \text{ligand is outside binding site} 
\end{cases}
\]

then:

\[
Z'_{RL} = \int_{\text{BOUND}} \ldots = \int d\mathbf{x}_R^{3n_R-6} d\mathbf{x}_L^{3n_L-6} d\zeta_L^6 I(\zeta_L) e^{-\beta \Psi_{RL}(\mathbf{x}_R, \mathbf{x}_L, \zeta_L)}
\]

Next, define “binding energy” of a conformation of the complex:

\[
u = u(\mathbf{x}_R, \mathbf{x}_L, \zeta_L) = \Psi_{RL}(\mathbf{x}_R, \mathbf{x}_L, \zeta_L) - \Psi_R(\mathbf{x}_R) - \Psi_R(\mathbf{x}_L)
\]

basically, change in effective energy for bringing ligand and receptor together at fixed internal conformation:
In terms of binding energy:

\[ \Psi_{RL}(x_R, x_L, \xi_L) = \Psi_R(x_R) + \Psi_R(x_L) + u(x_R, x_L, \xi_L) \]

then:

\[ K_b(T) = \frac{C^\circ}{8\pi^2} \frac{Z'_{RL}}{Z'_R Z'_L} = \]

\[ \frac{C^\circ}{8\pi^2} \int d x_R^{3n_R-6} d x_L^{3n_L-6} d \xi_L^6 I(\xi_L) e^{-\beta \Psi_R(x_R)} e^{-\beta \Psi_L(x_L)} e^{-\beta u(x_R, x_L, \xi_L)} \]

\[ \int d x_R^{3n_R-6} d x_L^{3n_L-6} e^{-\beta \Psi_R(x_R)} e^{-\beta \Psi_L(x_L)} \]

Now: we are not very good at computing partition functions. We are much better at computing ensemble averages:

\[ \langle O \rangle = \frac{\int dx O(x) e^{-\beta U(x)}}{\int dx e^{-\beta U(x)}} = \int dx O(x) \rho(x) \]
To transform the expression for $K_b$ so that it looks like an average:

multiply and divide by: \[
\int d\zeta_L I(\zeta_L) = V_{\text{site}} \Omega_{\text{site}}
\]

then:

\[
K_b(T) = \frac{C^\circ Z'_{RL}}{8\pi^2 Z'_R Z'_L} = \\
\frac{C^\circ}{8\pi^2} \frac{\int d\mathbf{x}_R^{3n_R - 6} d\mathbf{x}_L^{3n_L - 6} d\zeta_L^6 I(\zeta_L) e^{-\beta\Psi_R(x_R)} e^{-\beta\Psi_L(x_L)} e^{-\beta u(x_R, x_L, \zeta_L)}}{\int d\mathbf{x}_R^{3n_R - 6} d\mathbf{x}_L^{3n_L - 6} e^{-\beta\Psi_R(x_R)} e^{-\beta\Psi_L(x_L)}} = \\
\frac{V_{\text{site}} \Omega_{\text{site}}}{V^\circ 8\pi^2} \frac{\int d\mathbf{x}_R^{3n_R - 6} d\mathbf{x}_L^{3n_L - 6} d\zeta_L^6 I(\zeta_L) e^{-\beta\Psi_R(x_R)} e^{-\beta\Psi_L(x_L)} e^{-\beta u(x_R, x_L, \zeta_L)}}{\int d\mathbf{x}_R^{3n_R - 6} d\mathbf{x}_L^{3n_L - 6} e^{-\beta\Psi_R(x_R)} e^{-\beta\Psi_L(x_L)}}
\]

or:

\[
K_b(T) = \frac{V_{\text{site}} \Omega_{\text{site}}}{V^\circ 8\pi^2} \left< e^{-\beta u(x_R, x_L, \zeta_L)} \right>_{R+L+I()}
\]
We can see that binding constant can be expressed in terms of an **average** of the exponential of the binding energy over the **ensemble** of conformations of the complex in which the ligand and the receptor are **not interacting** while the ligand is placed **in the binding site**.

Standard free energy of binding: \( \Delta A_b^\circ(T) = -kT \ln K_b(T) \)

\[
\Delta A_b^\circ(T) = -kT \ln \frac{V_{\text{site}}}{V^\circ} - kT \ln \frac{\Omega_{\text{site}}}{8 \pi^2} - kT \ln \left\langle e^{-\beta u(x_R, x_L, \zeta_L)} \right\rangle_{R+L+I} \\
\]

\( \Delta A_b^\circ(\text{translational}) \quad \Delta A_b^\circ(\text{rotational}) \quad \Delta A_b^\circ(\text{excess}) \)

(analytic formulas) \quad (numerical computation)
Interpretation in terms of binding thermodynamic cycle:

\[ \text{R} + \text{L} \xrightarrow{\Delta A_b^\circ} \text{RL} \]

Ligand in solution at concentration \( C^\circ \)

\[ \Delta A_b^\circ (t + r) \]

Loss of translational, rotational freedom (to fit binding site definition)

\[ \Delta A_b^\circ (\text{exc.}) \]

Binding while in receptor site (independent of concentration)

\[ \text{R}(\text{L}) \]

“Virtual” state in which ligand is in binding site without interacting with receptor

\[ \Delta A_b^\circ = \Delta A_b^\circ (t + r) + \Delta A_b^\circ (\text{exc.}) \]

A future lecture (BEDAM) and computer exercise will focus on the computation of \( \Delta A_b^\circ (\text{exc.}) \) by computer simulations.
From the definition of entropy:

$$\Delta S_b^\circ = -\frac{\partial \Delta A_b^\circ (T)}{\partial T}$$

with

$$\Delta A_b^\circ (T) = -kT \ln \frac{V_{\text{site}}}{V^o} - kT \ln \frac{\Omega_{\text{site}}}{8\pi^2} + \Delta A_b(\text{exc.})$$

get

$$\Delta S_b^\circ (T) = k \ln \frac{V_{\text{site}}}{V^o} \frac{\Omega_{\text{site}}}{8\pi^2} - \frac{\partial \Delta A_b(\text{exc.})}{\partial T}$$

the first term is the entropy loss due to the loss of translational and rotational freedom of the ligand:

$$\Delta S_{b, t+r}^\circ = k \ln \frac{V_{\text{site}}}{V^o} \frac{\Omega_{\text{site}}}{8\pi^2}$$

Note that it's temperature-independent. It depends only on the standard concentration and the definition of the binding site.
Now let's turn to the “interesting” piece:

$$\Delta S_b(\text{exc.}) = -\frac{\partial \Delta A_b(\text{exc.})}{\partial T}$$

recall that:

$$\Delta A_b(\text{exc.}) = -kT \ln \langle e^{-\beta u} \rangle_{R+L+I()} = -kT \ln \frac{Z'_{RL}}{Z'_{R+L+I()}}$$

where:

$$u = u(x_R, x_L, \zeta_L; T) = \Psi_{RL}(x_R, x_L, \zeta_L; T) - \Psi_R(x_R; T) - \Psi_R(x_L; T)$$

with:

$$\Psi(x; T) = U(x) + W(x; T):$$

is the binding energy (think of it as the ligand-receptor interaction energy) of a given conformation of the complex ($U$ is the potential energy and $W$ is the solvent potential of mean force ~solvation free energy).

and:

$$\langle \cdots \rangle_{R+L+I()}$$

denotes an average over the ensemble of conformations of the complex in which the receptor and ligand are not interacting, but the ligand is restrained to within the binding site.
\[\Delta A_b^{(\text{exc.})} = -kT \ln \left( e^{-\beta u} \right)_{R+L+I} = -kT \ln \frac{Z_{RL}'}{Z_{R+L+I}'}\]

so:

\[-\frac{\partial \Delta A_b^{(\text{exc.})}}{\partial T} = k \ln \frac{Z_{RL}'}{Z_{R+L+I}'} + kT \frac{\partial}{\partial T} \ln \frac{Z_{RL}'}{Z_{R+L+I}'} =\]

\[\Delta A_b^{(\text{exc.})} \quad \frac{1}{T} \left[ \frac{1}{Z_{RL}'} \frac{\partial Z_{RL}'}{\partial \beta} - \frac{1}{Z_{R+L+I}'} \frac{\partial Z_{R+L+I}'}{\partial \beta} \right] =\]

\[\left( kT \frac{\partial}{\partial T} = -\frac{kT}{KT^2} \frac{\partial}{\partial \beta} = -\frac{1}{T} \frac{\partial}{\partial \beta} \right)\]

generally:

\[\frac{1}{Z} \frac{\partial Z}{\partial \beta} = \frac{1}{Z} \frac{\partial}{\partial \beta} \int dx \ e^{-\beta U(x)} = -\int dx \ U(x) e^{-\beta U(x)} = -\left\langle U \right\rangle\]

so:

\[-\frac{\partial \Delta A_b^{(\text{exc.})}}{\partial T} = -\frac{\Delta A_b^{(\text{exc.})}}{T} + \frac{1}{T} \left[ \left\langle \Psi \right\rangle_{RL} - \left\langle \Psi \right\rangle_{R+L+I} \right] = -\frac{\Delta A_b^{(\text{exc.})}}{T} + \frac{\Delta \left\langle \Psi \right\rangle}{T}\]

Nice, but … WRONG!
… because $\Psi$ is temperature-dependent!

The derivative has an additional piece:

$$\frac{1}{Z} \frac{\partial Z}{\partial \beta} = \frac{1}{Z} \frac{\partial}{\partial \beta} \int dx e^{-\beta \Psi(x; T)} = - \frac{1}{Z} \int dx \left[ \Psi + \beta \frac{\partial \Psi}{\partial \beta} \right] e^{-\beta \Psi(x; T)}$$

$$= - \langle \Psi \rangle - \beta \langle \frac{\partial \Psi}{\partial \beta} \rangle = - \langle \Psi \rangle + T \langle \frac{\partial \Psi}{\partial T} \rangle$$

Only the solvent potential of mean force part, $W$, of $\Psi$ is temperature-dependent:

$$\frac{1}{Z} \frac{\partial Z}{\partial \beta} = - \langle U + W \rangle + T \langle \frac{\partial W}{\partial T} \rangle$$

So we get:

$$\Delta S_b^{\text{(exc.)}} = - \frac{\partial \Delta A_b^{\text{(exc.)}}}{\partial T} = - \frac{\Delta A_b^{\text{(exc.)}}}{T} + \frac{\Delta \langle U + W \rangle}{T} - \Delta \left\langle \frac{\partial W}{\partial T} \right\rangle$$

where:

$$\Delta \left\langle \frac{\partial W}{\partial T} \right\rangle = \left\langle \frac{\partial W}{\partial T} \right\rangle_{\text{RL}} - \left\langle \frac{\partial W}{\partial T} \right\rangle_{\text{R+L+I}}$$
Including the ideal term: \[ \Delta S_{b,t+r}^\circ = k \ln \frac{V_{\text{site}}}{V^\circ} \frac{\Omega_{\text{site}}}{8\pi^2} \]

we finally obtain the following formula for the standard entropy of binding:

\[
\Delta S_b^\circ = -\frac{\Delta A_b^\circ}{T} + \frac{\Delta \langle U + W \rangle}{T} - \Delta \left\langle \frac{\partial W}{\partial T} \right\rangle
\]

The entropy of binding measures the reduction of conformational freedom of receptor/ligand upon binding + the change of entropy of the solvent (hydrophobicity, etc.).

\[
\Delta S_b^\circ = \Delta S_{\text{conf.}}^\circ + \Delta S_{\text{solv.}}^\circ
\]

The total binding entropy can be measured from, for example, the temperature variation of the binding free energy. However, the conformational and solvation contributions can not be measured individually (although people have designed NMR experiments to try to probe \( \Delta S_{\text{conf.}}^\circ \) ).
Energy of binding (=enthalpy of binding apart from $p\Delta V$, which is often negligible):

From the definition of free energy ...

\[ \Delta E_b = \Delta A^\circ_b + T \Delta S^\circ_b \]

substituting ...

\[ \Delta S^\circ_b = - \frac{\Delta A^\circ_b}{T} + \frac{\Delta \langle \Psi \rangle}{T} - \Delta \left( \frac{\partial W}{\partial T} \right) \]

we get:

\[ \Delta E_b = \Delta \langle \Psi \rangle - T \Delta \left( \frac{\partial W}{\partial T} \right) \]

Change in internal energy due to receptor-ligand interactions.

Change in internal energy of the solvent.

Note that $\Delta E_b$ does not depend on standard state concentration.
Combining the formulas for $\Delta E_b$ and $\Delta S_b^\circ$ we see that the solvation term cancels in the expression for the free energy of binding:

$$\Delta A_b^\circ = \Delta E_b - T \Delta S_b^\circ = \Delta \langle \Psi \rangle - T \Delta S_{conf}^\circ.$$ 

It follows that the effective energy and configurational entropy pair is an equally valid decomposition of the free energy as the energy/entropy pair.

One important distinction is that $(\Delta E_b, \Delta S_b^\circ)$ are directly measurable whereas $(\Delta \langle \Psi \rangle, \Delta S_{conf}^\circ)$ are not.

Given that they are easier to compute $(\Delta \langle \Psi \rangle, \Delta S_{conf}^\circ)$ are encountered more often in theoretical models than $(\Delta E_b, \Delta S_b^\circ)$.

Historical note: the fact that the solvation term cancels in the expression of the binding free energy has caused endless confusion in the literature. Some have even claimed that the solvent does not affect the enthalpy/entropy of binding, which is of course not true. The effect of the solvent is included implicitly in the values of $(\Delta \langle \Psi \rangle, \Delta S_{conf}^\circ)$.
A side note: why is \( \Delta S_{\text{conf}} \) called the “configurational” entropy change?

Consider the configurational entropy (of the solute) \( S_{\text{conf}} \):

\[
-\frac{A}{T} + \frac{\langle \Psi \rangle}{T} = k \ln Z + \frac{1}{T} \int dx \Psi(x) \frac{\exp(-\beta \Psi(x))}{Z} = k \ln Z + \frac{1}{T} \int dx \Psi(x) \rho(x)
\]

We can write:

\[
\Psi(x) = -kT \ln e^{-\beta \Psi(x)} = -kT \ln \frac{e^{-\beta \Psi(x)}}{Z} = -kT \ln \rho(x) - kT \ln Z
\]

So:

\[
S_{\text{conf}} = k \ln Z - k (\ln Z) \int dx \rho(x) - k \int dx \rho(x) \ln \rho(x)
\]

\[
S_{\text{conf}} = -k \int dx \rho(x) \ln \rho(x)
\]

Shannon's information theory definition of the entropy of a probability distribution.
Notice that to compute $S_{\text{conf}}$ all we need is the distribution of conformations of the solute in solution (no energies, etc.). In that sense it is a “configurational” entropy.

The entropy expression:

$$S = -k \int dx \rho(x) \ln \rho(x)$$

Is one of the most fundamental relations of statistical physics.
Reorganization free energy of binding

Consider the following thermodynamic cycle:

\[ \Delta S_b^\circ = -\frac{\partial \Delta A_b^\circ(T)}{\partial T} \]

Standard entropy of binding

with

\[ \Delta A_b^\circ(T) = -kT \ln \frac{V_{\text{site}}}{V^\circ} - kT \ln \frac{\Omega_{\text{site}}}{8\pi^2} + \Delta A_b(\text{exc.}) \]

get

\[ \Delta S_b^\circ(T) = k \ln \frac{V_{\text{site}}}{V^\circ} \frac{\Omega_{\text{site}}}{8\pi^2} - \frac{\partial \Delta A_b(\text{exc.})}{\partial T} \]

the first term is the entropy loss due to the loss of translational and rotational freedom of the ligand:

\[ \Delta S_{b,t+r}^\circ = k \ln \frac{V_{\text{site}}}{V^\circ} \frac{\Omega_{\text{site}}}{8\pi^2} \]

Note that it's temperature-independent. It depends only on the standard concentration and the definition of the binding site.
Reorganization free energy of binding

Consider the following thermodynamic cycle:

\[
\Delta G_{\text{conf}} = \Delta G_{\text{reorg}} + \Delta E_b
\]

- \( \Delta G_{\text{conf}} \) represents the loss of conformational freedom and energetic strain.
- \( \Delta G_{\text{reorg}} \) is the reorganization free energy of binding.
- \( \Delta E_b \) is the energy contribution from interatomic interactions.

The equation for translational/rotational entropy loss is:

\[
\Delta S^\circ_{b,t+r} = k \ln \frac{V_{\text{site}} \Omega_{\text{site}}}{V^\circ 8\pi^2}
\]
$$
\Delta E_b \equiv \langle u \rangle_{RL} = \langle \Psi_{RL}(x_R, x_L, \zeta_L; T) - \Psi(R; T) - \Psi_R(x_L; T) \rangle_{RL}
$$

This is usually straightforward to compute from, say, a MD trajectory of the complex

If we know $\Delta G^\circ_b$ then $\Delta G^\circ_{\text{reorg}}$ can be obtained by difference:

$$
\Delta G^\circ_{\text{reorg}} = \Delta G^\circ_b - \Delta E_b
$$

Key facts about reorganization:

- Reorganization always opposes binding $\Delta G^\circ_{\text{reorg}} > 0$ (because it involves shifting the unbound conformational ensembles of binding partners away from equilibrium).
- The only favorable thermodynamic driving force towards binding is the formation of receptor-ligand interactions.
- The relative binding affinity of two complexes can be understood