Protein/protein interactions

• Protein/protein interactions are fundamental to biology.
• In the past much of the focus has been on qualitative information.
  – What proteins interact?
  – What is the function of the interaction?
  – What is the structure of the protein/protein complex?
• Now quantitative information is considered increasingly important
  – Helps understand molecular mechanisms
  – Essential for modelling complex processes
  – Important for drug discover
• This talk which focus on the quantitative properties of protein/protein interactions.
Binding models

• The vast majority of protein:protein interactions are simple 1-to-1 associations of the form
  \[ A + B \rightleftharpoons AB \]

• The remainder of this presentation will focus on this scheme.

• The most common variation of this scheme is where one protein (e.g. B) has additional binding sites.
  \[ AB + A \rightleftharpoons A_2B \text{ or} \]
  \[ AB + C \rightleftharpoons A\bar{B}C \]

• If these binding sites are independent then one simply treats each interaction as a 1-to-1 association and adds them together.

• If the binding sites are not independent then one has positive or negative cooperativity (i.e. allosteric effects)
Which binding properties are important?

- **Affinity**
  - $K_A$ (affinity constant) or $K_D$ (dissociation constant)
  - $K_D=1/K_A$

- **Kinetics**
  - $k_{ass}$ or $k_{on}$ association rate constant or on rate
  - $K_{diss}$ or $k_{off}$ dissociation rate constant or off rate

- **Thermodynamic properties**
  - $\Delta H$ enthalpy change
  - $\Delta S$ entropy change
  - $\Delta C$ heat capacity change

- **Mechanical properties** (e.g. unbinding force, elasticity)
Affinity

• This is a measure of how favourable an interaction is
• Best expressed as affinity constant or $K_A$
• For $A + B \leftrightarrow AB$

\[
K_A = \frac{[AB]}{[A][B]} = \frac{1}{K_D}
\]

– Best thought of as the ratio of [products] versus [reactants] at equilibrium
– Note the units (M$^{-1}$)
– Higher affinity = higher $K_A$

• Also expressed as dissociation constant or $K_D$
– Usually thought of as concentration of A at which half of B is bound ([B]=[AB])

\[
K_D = \frac{[A][B]}{[AB]}
\]

– Units are M
– Higher affinity = lower $K_D$
Measuring the affinity constant

- One could simply measure \([A]\), \([B]\) and \([AB]\) at equilibrium and calculate \(K_D\).
- In practice this is difficult and the following approach is used.
- Increasing fixed concentrations of one molecules A ([A]) are added to a fixed amount of its ligand B and you measure amount of bound A (Bound).
- Plot the results and fit the following equation to the data to determine \(K_D\) and \(\text{Bound}_{max}\):

\[
\text{Bound} = \frac{[A]\text{Bound}_{max}}{[A] + K_D}
\]

**DERIVATION**

\[
K_D = \frac{[A][B]}{[AB]} \quad \text{(1)}
\]

and

\[
[B] = [B_{total}] - [AB] = [AB]_{max} - [AB] \quad \text{(2)}
\]

By substitution of (2) into (1) and rearranging, we get

\[
[AB] = \frac{[A][B_{max}]}{[A] + K_D}
\]

or

\[
\text{Bound} = \frac{[A]\text{Bound}_{max}}{[A] + K_D}
\]
Measuring affinity constant

- Data are circles
- Line is non-linear fit of the equation performed by computer
- Gives the indicated values for $K_D$ and $Bound_{max}$
- If the fit is good it indicates that binding follows the simple 1:1 model
- Difficult to see if fit is poor in this plot

\[
Bound = \frac{[A]Bound_{max}}{[A] + K_D}
\]
Scatchard plot

- A plot of \( \frac{\text{Bound}}{[A]} \) versus \([A]\)
- Plot should be linear for a standard 1:1 interaction
- If curved it indicates wrong model and possible problem with the experiment
- Most commonly concave up
  Usually caused by experimental error (commonly heterogeneity of proteins)
  Sometimes due to negative cooperativity
- Far less common is to see concave down
  Usually caused by positive cooperativity

**DERIVATION**

\[
\text{Bound} = \frac{[A]\text{Bound}_{\text{max}}}{[A]+K_D}
\]

\[
\text{Bound}[A]+\text{Bound} \times K_D = [A]\text{Bound}_{\text{max}}
\]

Divide both sides by \([A]K_D\) and rearrange, giving

\[
\frac{\text{Bound}}{[A]} = -\frac{1}{K_D}[A]+\frac{\text{Bound}_{\text{max}}}{K_D}
\]

Plot of \( \frac{\text{Bound}}{[A]} \) versus \([A]\) gives

- slope = \(-\frac{1}{K_D}\)
- Y intercept = \(\frac{\text{Bound}_{\text{max}}}{K_D}\)
- Therefore
- X intercept = \(\text{Y intercept} - \text{slope} = \text{Bound}_{\text{max}}\)
Thermodynamics of binding

• Binding is favoured if it leads to a net increase in disorder or entropy.
• This includes entropy of:....
  – the system (interacting molecules and solvent)
    • represented as change in entropy or $\Delta S$
  – the environment (everything else)
    • as the system releases or absorbs heat it changes the entropy of the surroundings
    • heat release is measure as change in enthalpy or $\Delta H$
Gibbs free energy change

- The change in Gibbs free energy ($\Delta G$) is a measure of the net change in entropy - i.e. the extent to which binding is favoured.

$$\Delta G = \Delta H - T \Delta S$$

$\Delta G < 0$ then binding is favoured.

- The $\Delta G$ depends on the concentration. At equilibrium $\Delta G = 0$

- $\Delta G^o$ is the standard state $\Delta G$ which assumes all components are at the standard state concentration of 1 M (mol.L$^{-1}$)

- It can be calculate from the affinity constant

$$\Delta G^o = RT\ln K_D$$

where

- $R$ is the Gas Constant (2 cal.mol$^{-1}$.K$^{-1}$)
- $T$ is absolute temperature in Kelvin ($^\circ C+273.18$)
- $K_D$ is expressed in units M
Origins of enthalpy and entropy changes

\[ \Delta G^o = \Delta H - T\Delta S^o \]

- Change in enthalpy (\(\Delta H\))
  - Release of heat (\(\Delta H < 0\)) favours binding
  - This happens when bonds are formed
    - e.g. hydrogen bonds, salt bridges, van der Waals contacts
  - However bonds are also broken upon binding
    - displacement of water and ions (always)
    - conformational change (sometimes)

- Change in entropy (T\(\Delta S\))
  - Increase in entropy favours binding
  - The protein/protein interaction leads to decrease in entropy
    - Stabilise conformation at the binding interface
    - Decreased rotation/translation of proteins
  - However displacement of water from the binding interface leads to an increase in entropy (this is the hydrophobic effect)
The key role of water

• Water is present at very high concentrations (55 M) and interacts with protein surfaces
• Thus many water bonds need to be broken, which has unfavourable enthalpic effect
• Water can also act as glue filling in gaps between surfaces that lack surface shape complementarity

• Water is believed to form an organised shell over hydrophobic surfaces. Ejection of water from these surfaces into free solution has favourable entropic effect. This is the ‘hyrophobic effect’.
• Note that there is an unfavourable enthalpic effect as well since the water molecules in the shell interact weakly.
TCR and antibody binding have distinct thermodynamic properties
(Data from Willcox et al 1999 and Stites 1997)
Changes in conformation at a T cell receptor/peptide-MHC interface
Heat capacity change ($\Delta C$)

- $\Delta H$ and $T\Delta S$ usually vary with temperature
- The extent of this variation is given by $\Delta C$
- This is a consequence of changes in water with temperature

Low temp – binding disrupts water ‘shell’ with unfavourable effects on $\Delta H$ and favourable effects on $\Delta S$

High temp – water shell already ‘melted’ so both effects are lost
Why measure heat capacity change?

- The entropy change includes contributions from changes in solvent entropy (hydrophobic effect) and protein entropy.
- The heat capacity change can be used to estimate solvent entropy change, enabling estimation of the protein entropy change.

**DETAILED EXPLANATION**
*(see Spolar and Record (1994) Science 263:777)*

It has been shown that $\Delta C$ is correlated with the non-polar surface area that is buried by binding ($A_{np}$).

Thus $\Delta C$ can be used to estimate the contribution of the hydrophobic effect ($\Delta S_{he}$) to total entropy change ($\Delta S_{Total}$).

The change in rotational and translational entropy ($\Delta S_{rt}$) can be calculated, and is same for all protein/protein interactions.

This enables $\Delta S_{other}$ to be calculated since

$$\Delta S_{Total} = \Delta S_{he} + \Delta S_{rot/trans} + \Delta S_{other}$$

The main contribution to $\Delta S_{other}$ is thought to be reductions in conformational flexibility accompanying binding.
Measuring thermodynamic parameters

- $\Delta S$ cannot be measured directly
- $\Delta G$ and $\Delta H$ are measured and $\Delta S$ calculated from relationship $\Delta G = \Delta H - T \Delta S$
- $\Delta H$ can be measured in two ways
  - calorimetry (see later) or
  - van’t Hoff analysis

**Van’t Hoff analysis**

- $\Delta G$ is measured over a range of temperature and plotted
- The non-linear van’t Hoff equation* is fitted to the data to determine $\Delta H$, $\Delta S$ and $\Delta C$
- The slope of the plot represents $\Delta H$
- This plot is curved for macromolecular interactions since $\Delta H$ varies with temperature
- The curvature represents the $\Delta C$

*Non-linear van’t Hoff equation

$$\Delta G = \Delta H_{To} - T \Delta S_{To} + \Delta C(T - T_o) - T \Delta C \ln \left( \frac{T}{T_o} \right)$$

where $T_o$ is an arbitrary reference temperature.
Kinetics

• Since biological systems are not at equilibrium the rate of binding and dissociation is critical

• For a simple 1:1 interaction \((A + B \leftrightarrow AB)\)

• Rate of dissociation
  – \(\frac{dAB}{dt} = k_{\text{diss}}[AB]\)
  – where \(k_{\text{diss}}\) is the dissociation rate constant \((k_{\text{off}})\)

• Rate of association
  – \(\frac{dAB}{dt} = k_{\text{ass}}[A][B]\)
  – where \(k_{\text{ass}}\) is the association rate constant \((k_{\text{on}})\)

• At equilibrium the rate of association must equal the rate of dissociation
  – \(k_{\text{diss}}[AB] = k_{\text{ass}}[A][B]\)
  – \(k_{\text{diss}}/k_{\text{ass}} = [A][B]/[AB]\)
  – Since \(K_D = [A][B]/[AB]\) it follows that
  – \(k_{\text{diss}}/k_{\text{ass}} = K_D\)
Dissociation

- Any reaction of the form \( \frac{d[AB]}{dt} \propto [AB] \) will be exponential so
  - i.e. \([AB]_t = [AB]_0 e^{-k_{\text{diss}}t}\)
  - \(k_{\text{diss}}\) determined directly by curve fitting
- The half life \( (t_{1/2}) \) can be calculated as follows
- Since at the \( t = t_{1/2} \)
  \([AB]_t/[AB]_0 = 0.5 = e^{-k_{\text{diss}}t_{1/2}}\)
- It follows that
  \(-k_{\text{diss}}t_{1/2} = \ln 0.5 = 0.693\)
- Thus
  \(t_{1/2} = 0.693/k_{\text{off}}\)
Association

- In most experimental system it is impossible to follow association alone in the absence of simultaneous dissociation

- For the simple interaction \( A + B \leftrightarrow AB \)

- \( \frac{d[AB]}{dt} = k_{ass}[A][B] - k_{diss}[AB] \)

- It can be shown that \([AB]_t = [AF]_{final} (1 - e^{-k_{obs}t})\) where \( k_{obs} = k_{ass}[A] + k_{off} \)

- Thus one needs to know the \( k_{off} \) and the \([A]\) to calculate the \( k_{on} \)
Determination of binding kinetics

\[ k_{\text{obs}} = [A]k_{\text{ass}} + k_{\text{diss}} \]

- \( k_{\text{ass}} = 42000 \text{ M}^{-1}\cdot\text{s}^{-1} \)
- \( k_{\text{diss}} = 0.5 \text{ s}^{-1} \)
- \( K_D = 1.2 \times 10^{-5} \text{ M} \)

Residuals plot (difference between data and fitted curve)
## Summary of affinity and kinetic constants biological interactions

<table>
<thead>
<tr>
<th>Interaction</th>
<th>$k_{on}$ (M$^{-1}$s$^{-1}$)</th>
<th>$k_{off}$ (s$^{-1}$)</th>
<th>$K_D$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-cell recognition molecules</td>
<td>$10^5$</td>
<td>1-10</td>
<td>$10^{-5}$-$10^{-4}$</td>
</tr>
<tr>
<td>Antibody/antigen</td>
<td>$10^5$</td>
<td>$10^{-3}$</td>
<td>$10^{-8}$</td>
</tr>
<tr>
<td>Cytokine/receptor</td>
<td>$10^5$</td>
<td>$10^{-4}$</td>
<td>$10^{-9}$</td>
</tr>
<tr>
<td>Enzyme/inhibitor (eg barnase/barnstar)</td>
<td>$10^8$</td>
<td>$10^{-3}$</td>
<td>$10^{-11}$</td>
</tr>
</tbody>
</table>
Factors affecting kinetics

• The association rate constant does not vary that much
  – Association requires two proteins to collide in the correct orientation and in the correct conformation
  – This will be similar for most proteins
  – The basic rate is about $10^5 \text{ M}^{-1} \text{s}^{-1}$
  – The rate can be accelerated by long range electrostatic forces
    • Increased rate of collision
    • Steer binding sites into correct orientation
    • E.g. barnase/barnstar interaction

• The dissociation rate constant varies considerably and is responsible for most of the change in affinity constants
  – It is determined by the number and strength of bonds in the contact interface
  – Depends on size of interface and the degree of surface-shape and electrostatic complementarity
How the BIAcore works

- The BIAcore uses an optical method (surface plasmon resonance) to measure changes in refractive index.
- Macromolecules binding to a sensor surface leads to an increase in refractive index near the surface.
A BIACore sensorogram

- Resonance signal (kRU)
- Association
- Concentration
- Kinetics
- Dissociation
- Regeneration
The BIAcore has important advantages for measuring protein:ligand interactions

• No labelling is necessary
• Real-time analysis allows equilibrium binding levels to be measured even with extremely rapid off-rate.
• Small volumes allow efficient use of protein. Important when very high concentrations are required.
• All types of binding data can be obtained
Microcalorimetry

- Two proteins are mixed and the heat release upon binding is measured.
- Provides a direct measure of the $\Delta H$ (whereas van’t Hoff analysis is indirect).
- Allows more accurate measurement of $\Delta C$.
- Can also determine $\Delta G$ and $T \Delta S$.
- Its disadvantage compared with the BIAcore is that very large amounts of protein are required and no kinetic data provided.
Measuring key properties of protein-protein interactions

<table>
<thead>
<tr>
<th>Property</th>
<th>BIAcore</th>
<th>Calorimetry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affinity</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Enthalpy</td>
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<tr>
<td>Entropy</td>
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<tr>
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<tr>
<td>Stochiometry</td>
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