Multi-Target Pharmacology of Drugs

What is an intended drug target?

What is the real pharmacology?

Implications for
  drug discovery
  drug repurposing
  beneficial effects
  adverse effects
  mutations & drug resistance
Die ZauberKugel

Magic Bullet, from a bullet that kills a specific invading microbe (eg Salvarsan vs syphilis) to a specific agent specific to a target
Protein-Ligand Binding

- Compound (ligand) binds to its target in 1:1 stoichiometry

- **Association Reaction:** \( P + L \rightleftharpoons PL \)

- \( K_a = [PL] / [P][L] \) (association constant, binding constant, affinity constant, binding affinity…, \( M^{-1} \))

- \( K_d = [P][L] / [PL] \) (dissociation constant, \( M \)) = \( 1/K_a \)

- \( \Delta G_{bind} = -RT \ln K_a \quad \text{AND} \quad \Delta G_{bind} = RT \ln K_d \)

- \( K_d \) unit is \( M \) (moles/Liter)

- Other \( K_d \) units:
  - Milli (10\(^{-3}\)): mM, or
  - Micro (10\(^{-6}\)): \( \mu M \) / \( \mu M \)
  - Nano (10\(^{-9}\)): nM
  - Pico (10\(^{-12}\)): pM
  - Femto (10\(^{-15}\)): fM
Fraction of drug-bound targets depends on \([D]\) and \(K_d\)

- **Bosutinib** targets
- Notations: \(T\) and \(D\), a.k.a. \(P\) and \(L\)
- \(pK_d = -\log_{10}(K_d)\)
- \(pD = -\log_{10}([D])\)

**Bound target fraction**

\([TD]/[T] = [D]/K_d\)

If we assume that \(D_0 \gg T_0\), therefore

\([D_0] - [TD] \approx [D_0]\), then

\([TD]/[T_0] \approx D_0/(K_d + D_0)\)

**Example:** **Bosutinib/Bosulif**

“BCR-ABL & SRC kinase inhibitor” for chronic myelogenous leukemia

**Actual Pharmacology is shown below**
Derivation of the bound fraction equation

• Convenient notations for the derivation:
  – $k$ is $K_d$, $c$ is [PL] complex concentration
  – $d$ is unbound drug or ligand, total drug is $d+c$
  – $t$ is unbound target; total target is $t+c$

• Derivation:
  – Definition of $K_d$: $k = td/c$, therefore $t/c = k/d$
  – Bound fraction is $f = c/(t+c)$, $1/f = (t+c)/c = (t/c) + 1$
  – Substituting $t/c$ for $k/d$: $1/f = k/d + 1 = (k+d)/d$
  – From $1/f$ to $f$: $f = d/(k+d)$
  – Drug is at high concentration and free $d$ is close to $[D_0]$
  – Back to the main notation: $f = [PL]/[P_0] = [D_0]/(K_d + [D_0])$
Target Map of **Imatinib**: Target Expression, Drug-resistant **Mutants**
Example of a protein-ligand binding problem, from $K$ to $\Delta G$

$$\Delta G^0 = -RT \ln K$$

- In the solution at equilibrium, the concentrations of unbound drug and protein are 13.5 nM and 0.5 nM, respectively, while the concentration of protein/drug complex is 4.5 nM. Find $\Delta G^0_b$ binding.

- **Solution:**
  - Reaction: $P + L \rightleftharpoons PL$
  - $K_d = [P][L] / [PL] = 13.5 \times 10^{-9} \times 0.5 \times 10^{-9} / (4.5 \times 10^{-9}) = 1.5 \text{ nM}$
  - $K_a = 1 / (1.5 \times 10^{-9}) \sim 0.67 \times 10^9 \text{ M}^{-1}$
  - $\Delta G^0 = -RT \ln K_a = RT \ln K_d$
  - $\Delta G^0 = 0.6 \times (-9 \ln 10 + \ln 1.5) \approx -12.19 \text{ kcal/mol}$

- **Answer:** $\Delta G^0_b \approx -12.19 \text{ kcal/mol}$
Shortcut: $K$ vs $\Delta G$, and $K_2/K_1$ to $\Delta\Delta G$

- $K_2 / K_1 = 10$

\[
\Delta G^0 = -RT \ln K
\]

- $\Delta\Delta G = \Delta G_2 - \Delta G_1 = -RT \ln K_2 + RT \ln K_1 = -RT \ln (K_2 / K_1)$

- $\Delta\Delta G = \Delta G_2 - \Delta G_1 = -0.6 \ln 10 \approx -0.6 \times 2.3 \approx -1.4 \text{ kcal/mol}$

- $K$ increases by a factor of 10 when $\Delta G$ decreases by 1.4 kcal/mol

- For example: correspondence between $\Delta G$ and $K_d$ for protein/ligand binding:

<table>
<thead>
<tr>
<th>$\Delta G \text{ bind}$ $[\text{kcal/mole}]$</th>
<th>$K_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-4.14</td>
<td>1 mM</td>
</tr>
<tr>
<td>-8.23</td>
<td>1 $\mu$M</td>
</tr>
<tr>
<td>-12.43</td>
<td>1 nM</td>
</tr>
<tr>
<td>-16.58</td>
<td>1 pM</td>
</tr>
<tr>
<td>-20.72</td>
<td>1 fM</td>
</tr>
</tbody>
</table>
Problem: Protein/drug binding, using K to $\Delta G$ shortcut

• A drug candidate was chemically optimized to reduce the therapeutic concentration 1000 times. Estimate the binding energy improvement required to reach that goal.

• **Solution:**
  • 10-fold Kd improvement $\equiv$ 1.4 kcal/mol decrease in $\Delta G$
  • 100-fold Kd improvement $\equiv$ 2.8 kcal/mol decrease in $\Delta G$
  • 1000-fold Kd improvement $\equiv$ 4.2 kcal/mol decrease in $\Delta G$

• **Answer:** The binding energy needs to be decreased by 4.2 kcal/mol.
Protein-ligand binding: concentrations vs drug-bound target fraction

**Problem:** In the solution at equilibrium, the concentrations of unbound drug and protein are 13.5 nM and 0.5 nM, respectively. Given the $K_d$ of 1.5 nM, estimate the fraction of total protein which is bound (the binding reaction has 1:1 stoichiometry).

**Solution:**

- Reaction: $P + L \rightleftharpoons PL$, Dissociation: $PL \rightleftharpoons P + L$
- $K_d = [P][L] / [PL] = 1.5 \times 10^{-9}$ M
- $[PL] = [P][L] / K_d = 0.5 \times 10^{-9} \times 13.5 \times 10^{-9} / (1.5 \times 10^{-9}) = 4.5 \times 10^{-9}$ M = 4.5 nM
- $[P_0]$: Unbound protein 0.5 nM, bound protein 4.5 nM, total $[P_0]$ = 5 nM
- Fraction bound = $[PL] / [P_0] = 4.5 / 5 = 90\%$

**Answer:** 90\% of the protein is bound.
Protein-Ligand Binding Equilibration

- Simplest case, 1:1 binding stoichiometry. \( P + L \rightleftharpoons PL \)
- Full equation: \( [PL] \) defined as \( x \)
  - At equilibrium, \( K_d = [P][L] / [PL] \) \( \Rightarrow \)
    - \( x \times K_d = (P_0 - x)(L_0 - x) \)
    - \( x \times K_d = x^2 - (P_0 + L_0)x + P_0L_0 \)
    - \( x^2 - (P_0 + L_0 + K_d) \times x + P_0L_0 = 0 \) - quadratic equation
  - \( a = 1; \ b = - (P_0 + L_0 + K_d); \ c = P_0L_0 \)
  - Solve \( ax^2 + bx + c = 0 \)

<table>
<thead>
<tr>
<th>Start (no equilibrium)</th>
<th>Protein ( [P] = P_0 )</th>
<th>Ligand ( [L] = L_0 )</th>
<th>Complex ( 0 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equilibration</td>
<td>Protein ( [P] = P_0 - x )</td>
<td>Ligand ( [L] = L_0 - x )</td>
<td>Complex ( [PL] = x )</td>
</tr>
</tbody>
</table>
Equilibrium [PL] as a function of total ligand, target and $K_d$

- Given a test tube with the initial protein concentration $P_0$, how much complex is formed upon addition of $L_0$ (concentration) of ligand with a given $K_d$?

$$x = L_{\text{bound}} = P_{\text{bound}} = \frac{P_0 + L_0 + K_d - \sqrt{(P_0 + L_0 + K_d)^2 - 4P_0L_0}}{2}$$
Example: bound fraction at equilibrium

- 0.30 µM of protein is mixed with 0.36 µM of drug. The dissociation constant is $K_d = 0.01$ µM. Evaluate the bound protein concentration after the system equilibrates.

- Solution:

  \[
  x^2 - (P_0 + L_0 + K_d) \times x + P_0L_0 = 0 - \text{quadratic}
  \]

  Assuming that $x$, $P_0$, $L_0$, and $K_d$ are all measured in the same units (e.g. µM), we can cancel out the prefix factor (e.g. $10^{-6}$)
Example continued

- \( a = 1 \)
- \( b = -(0.30+0.36+0.01) = -0.67 \)
- \( c = 0.30 \times 0.36 = 0.108 \)
- Solve \( ax^2 + bx + c = 0 \)
- \( x = (-b \pm \sqrt{b^2 - 4ac}) / 2a = 0.27 \text{ \(\mu\)M or 0.40 \text{ \(\mu\)M} \)
- \( x \) cannot exceed \( P_0 \) or \( L_0 \), so \( x = 0.27 \text{ \(\mu\)M} \) (use the solution with \(-\))

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Ligand</th>
<th>Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start (no equilibrium)</td>
<td>([P] = 0.3 \text{ (\mu)M})</td>
<td>([L] = 0.36 \text{ (\mu)M})</td>
<td>0</td>
</tr>
<tr>
<td>Equilibration</td>
<td>([P] - x = 0.03\text{(\mu)M})</td>
<td>([L] - x = 0.09\text{(\mu)M})</td>
<td>(x = 0.27\text{(\mu)M})</td>
</tr>
</tbody>
</table>

- And, BTW, \((0.03\text{\(\mu\)M} \times 0.09\text{\(\mu\)M}) / 0.27\text{\(\mu\)M} = 0.01 \text{ \(\mu\)M} = Kd \)

- **Answer:** 0.27\text{\(\mu\)M}
Total protein vs $K_d$:  

**K_d = [P][L] / [PL]**

Two common cases

1. $[P_{\text{total}}] << K_d$  
   $\implies [P] << K_d$  
   $\implies [P] / K_d << 1$  
   $[PL] << [L]$  
   - Ligand is **not depleted** by binding to the protein target  
   - True for **most biological targets** in vivo

2. $[P_{\text{total}}] > K_d$  
   $\implies [P] \sim K_d$  
   $\implies [P] / K_d \sim 1$  
   $[PL] \sim [L]$  
   - Ligand is **depleted** by binding to the protein target  
   - True for *albumin, antitrypsin* and other abundant plasma proteins  
   - Only unbound fraction exhibits pharmacological action.
[Target] $< \text{K}_d < \text{[Ligand]}$

- $P_{\text{total}} \ll \text{K}_d$ and $[\text{PL}] \ll [\text{L}]$
- $[\text{L}_{\text{total}}] \approx [\text{L}]$
- Target bound/unbound ratio (from definition of $\text{K}_d$):
  \[
  [\text{PL}] / [\text{P}] = [\text{L}] / \text{K}_d \approx [\text{L}_{\text{total}}] / \text{K}_d
  \]
- When $[\text{L}_{\text{total}}] \approx \text{K}_d$, $[\text{PL}] = [\text{P}]$, i.e.
  \[
  \text{K}_d \text{ is the ligand concentration at which 50% target is bound.}
  \]
- Similarly, $[\text{L}_{\text{total}}] \approx \text{K}_d \times [\text{PL}] / [\text{P}]$ for any bound/unbound ratio.
- Fraction of bound receptor:
  \[
  [\text{PL}] / [\text{P}_{\text{total}}] \approx [\text{L}_{\text{total}}] / (\text{K}_d + [\text{L}_{\text{total}}])
  \]
Example: \([\text{total Lig}] \approx ([\text{PL}] / [\text{P}]) \times K_d\)

- The concentration of the target protein in the patient’s body is 5 pM. Given a drug with \(K_d\) of 10 nM, what concentration of the drug is needed for 80% of the protein to be bound?

- **Solution:**

  \[ [\text{P}] \ll K_d \]

  Desired \([\text{PL}] / [\text{P}]\) ratio is 80/20 = 4/1

  Total ligand = \([\text{PL}] / [\text{P}] \times K_d \approx 40\text{nM} \]

  Another solution: **bound target fraction** is \(0.8 = x / (x + K_d)\), \(x=40\text{nM}\)

- **Answer:** \( \approx 40\text{nM} \)

- **Note:** if \(K_d\) is 10 nM, we need 90 nM drug for 90% bound protein, and 190 nM for 95% bound protein

- **Dose:** 90 nM for 500g/mole drug and 30L corresponds to 1.35mg dose
Case of high protein concentration

• $P_{\text{tot}} > K_d$; $[PL]$ is proportional to $[L]$ 

• If protein is in excess, i.e. $[PL] \ll [P]$ and $[P_{\text{tot}}] \approx [P]$

• Ligand bound / unbound ratio (from definition of $K_d$):

\[
\frac{[LP]}{[L]} = \frac{[P]}{K_d} \approx \frac{[P_{\text{tot}}]}{K_d}
\]

• $[P_{\text{tot}}] / K_d$ defines bound/unbound ratio for the ligand

• *Bound ligand fraction* $= \frac{[P_{\text{tot}}]}{([P_{\text{tot}}] + K_d)}$
High affinity drug-albumin binding

• Problem: $K_d$ (Albumin, warfarin) is $\sim5\ \mu$M, calculate drug fraction found to albumin in %, assuming albumin in physiological range or 35 to 50 g/L. MM = 66.5 kDa

• Solution:
  • $[P_{\text{tot}}] / ([P_{\text{tot}}] + K_d)$
  • with albumin concentration at 526 $\mu$M: $526 / 531 \sim 99.05\%$
  • with albumin concentration at 752 $\mu$M: $752 / 757 \sim 99.34\%$

• Note: unbound warfarin varies between 0.66% and $\sim0.95\%$, i.e. 43% increase for a skinny fasting person

• For drugs with high plasma protein binding, small changes in plasma protein can dramatically affect free drug concentration.
Low affinity drug-albumin binding

- **Example:** $K_d$ (Albumin, drug B) is ~5 mM, calculate albumin binding in %, assuming albumin concentration is in a physiological range of 450 to 750 $\mu$M.

- **Solution:**
  - $f = \frac{\text{[P}_{\text{tot}]}}{\left[\text{P}_{\text{tot}}\right] + K_d}$
  - with albumin concentration at 450 $\mu$M: $f = 450 / 5450 \sim 8.3$
  - with albumin concentration at 750 $\mu$M: $f = 750 / 5750 \sim 13$

- **Unbound** drug B (1-$f$, or 100-$f[\%]$) varies between 87% and 91.7%, only 5.5% increase for a skinny fasting person

- Variations of plasma concentrations of unbound drug B are not so dramatic.