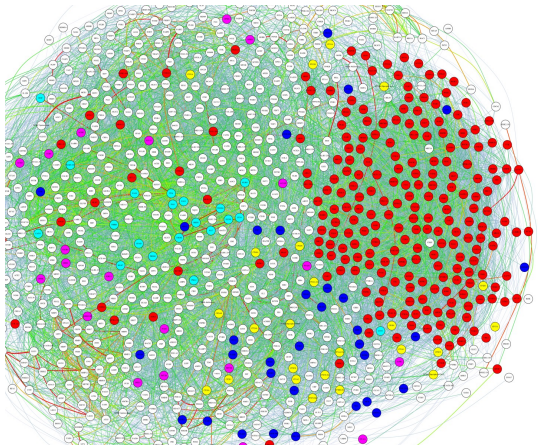
Target Pharmacology of Drugs

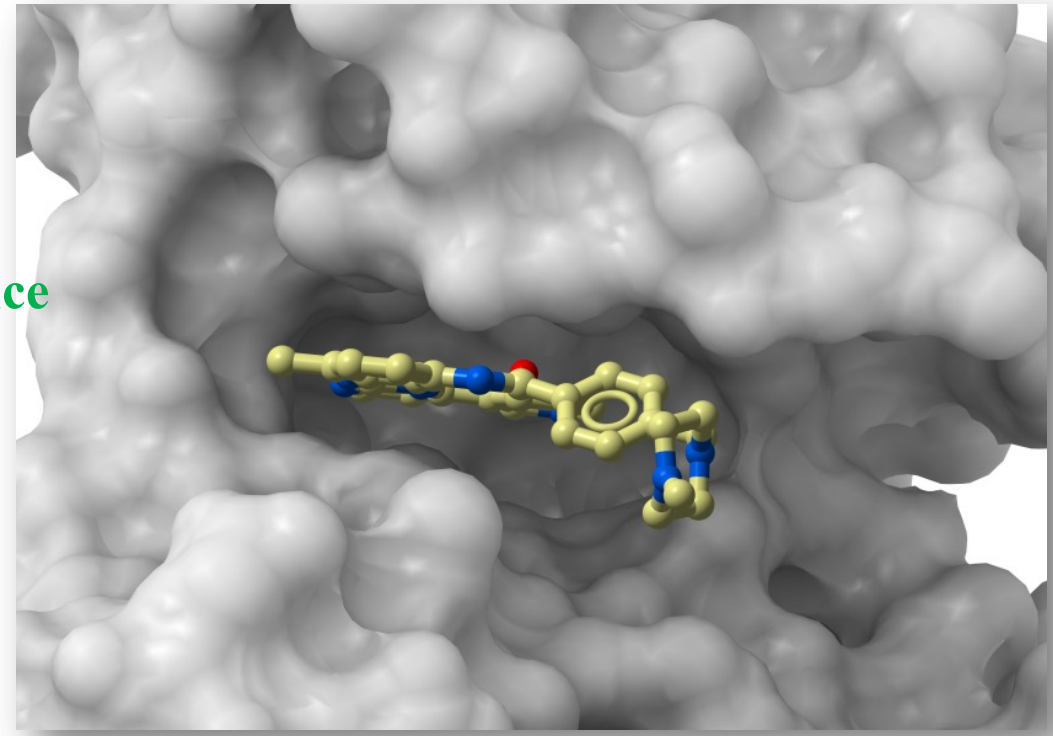
What is an intended drug target?

What is the real pharmacology?

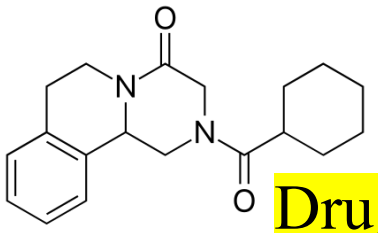
Implications for

- **drug discovery**
- **drug repurposing**
- **beneficial effects**
- **adverse effects**
- **mutations & drug resistance**



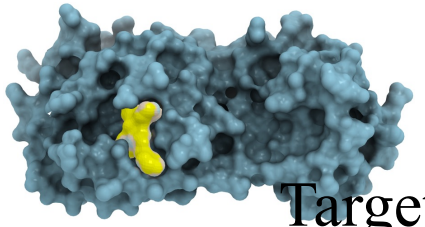


Die ZauberKugel

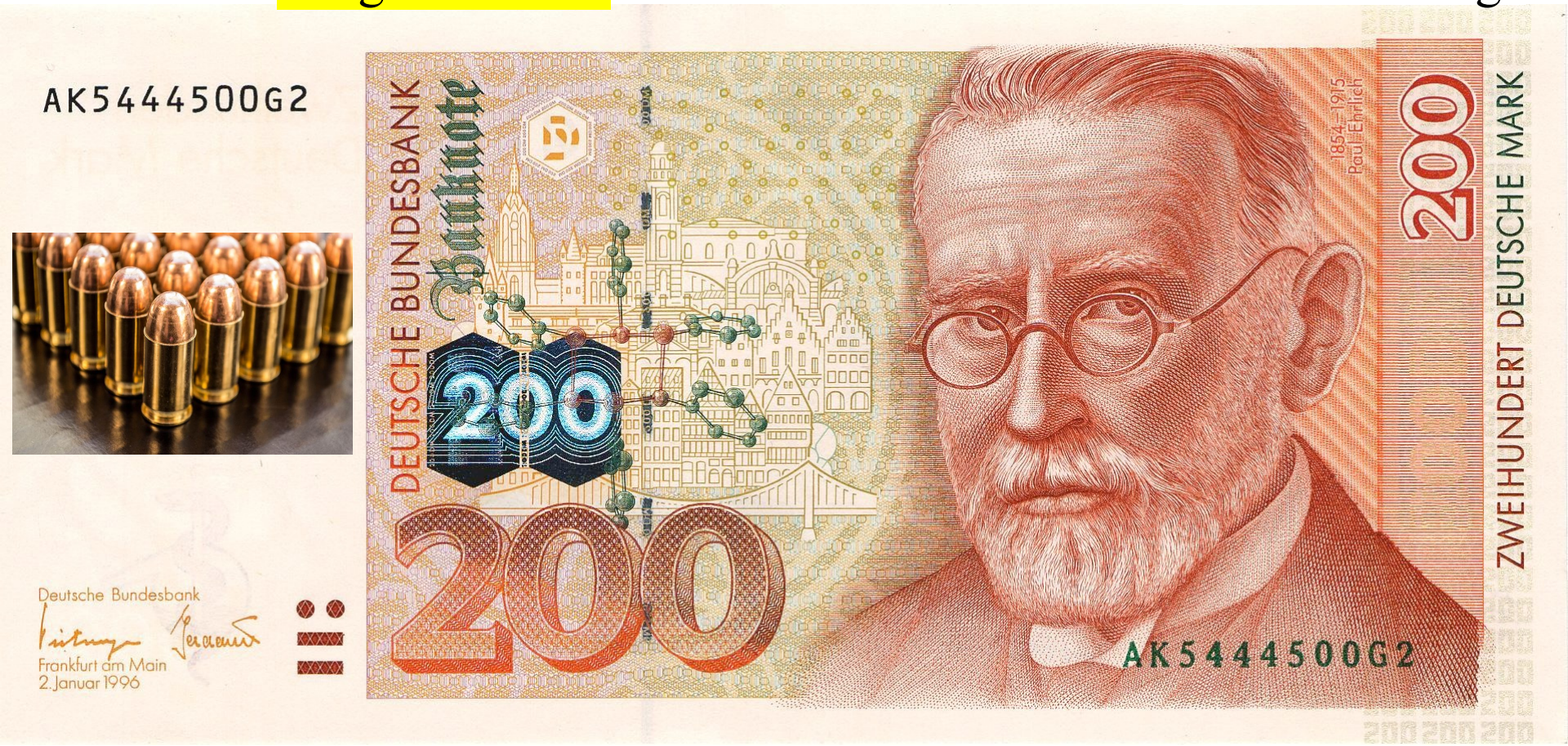


Drug/Candidate

In Silico Model



Target



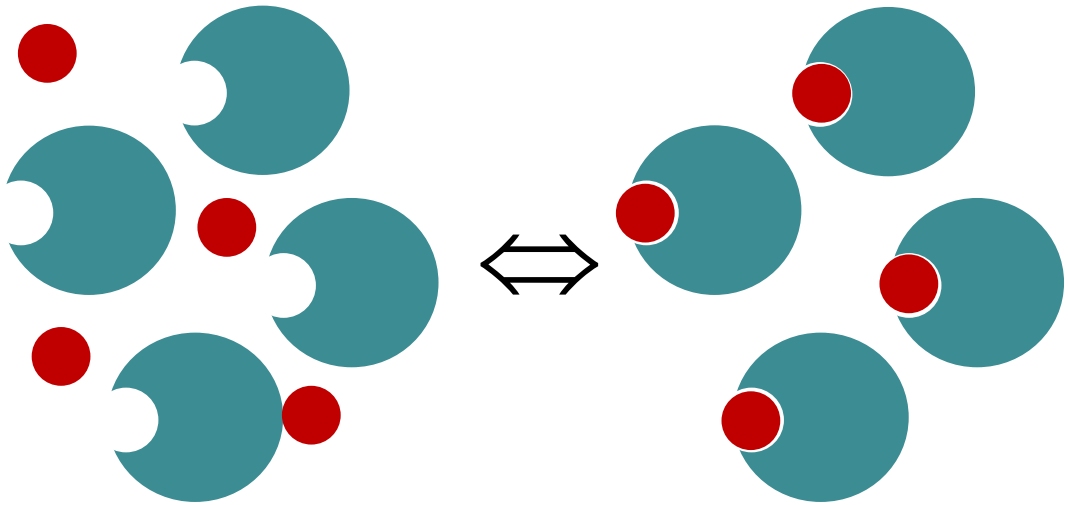
- Magic Bullet, from a bullet that kills a specific invading microbe (eg **Salvarsan vs syphilis**) to a specific agent specific to a target

Protein-Ligand Binding

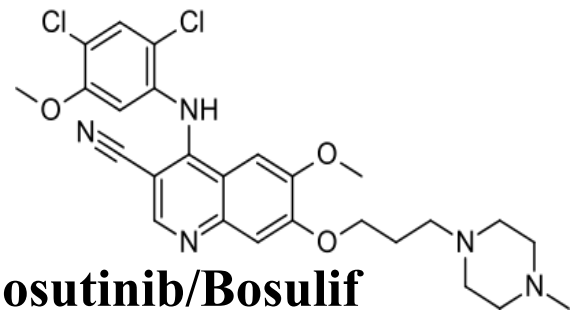
- Compound (ligand) binds to its target in 1:1 stoichiometry
- **Association Reaction**: $P + L \Leftrightarrow PL$
- K_a = [PL] / [P][L] (***a****ssociation constant*, *binding constant*, *affinity constant*, *binding affinity…*, M^{-1})
- K_d = [P][L] / [PL] (***di****ssociation* constant, M) = $1/K_a$
- $\Delta G_{bind} = -RT \ln K_a$ AND $\Delta G_{bind} = RT \ln K_d$

- K_d unit is M (moles/Liter)
- Other K_d units:

Milli (10^{-3}): mM, or
Micro(10^{-6}): µM / uM
Nano (10^{-9}): nM
Pico (10^{-12}): pM
Femto(10^{-15}): fM



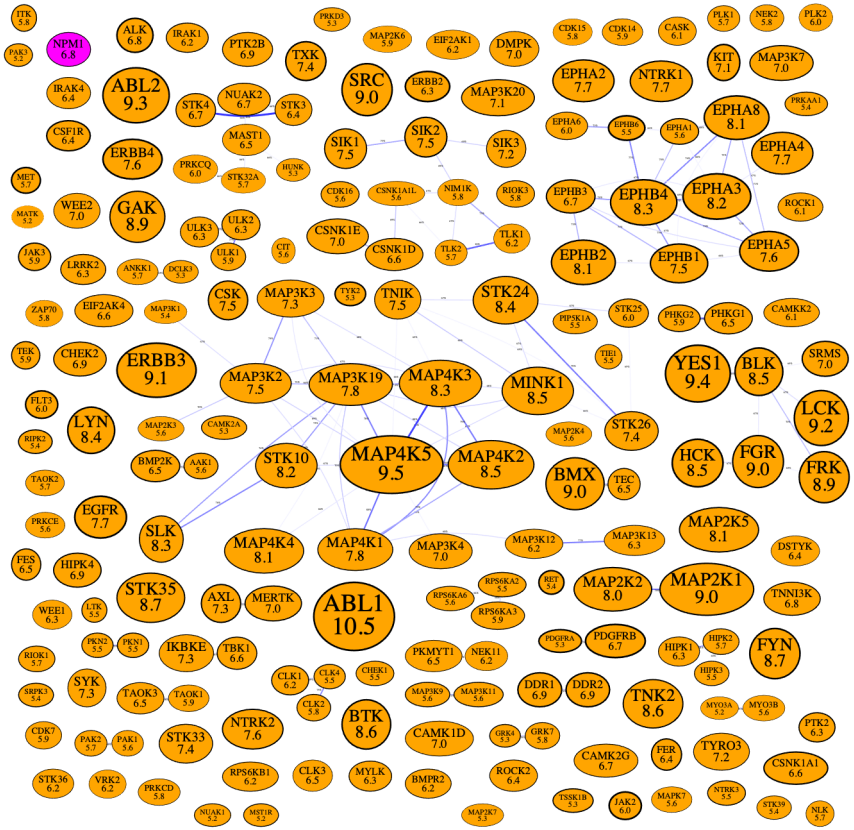
Fraction of drug-bound targets depends on [D] and K_d



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

Example: **Bosutinib/Bosulif**
"BCR-ABL & SRC kinase inhibitor"
for chronic myelogenous leukemia

Actual Pharmacology (pKd values)



$\mathbf{K_d = [T][D] / [TD]}$ (definition)

Bound/unbound target ratio

$\mathbf{[TD]/[T]=[D]/K_d}$

If we assume that $D_0 >> T_0$, therefore $[D_0]-[TD] \approx [D_0]$, then we get

Bound Target Fraction

Bound target/Total target

$\mathbf{[TD]/[T_0] = D / (K_d+D)}$

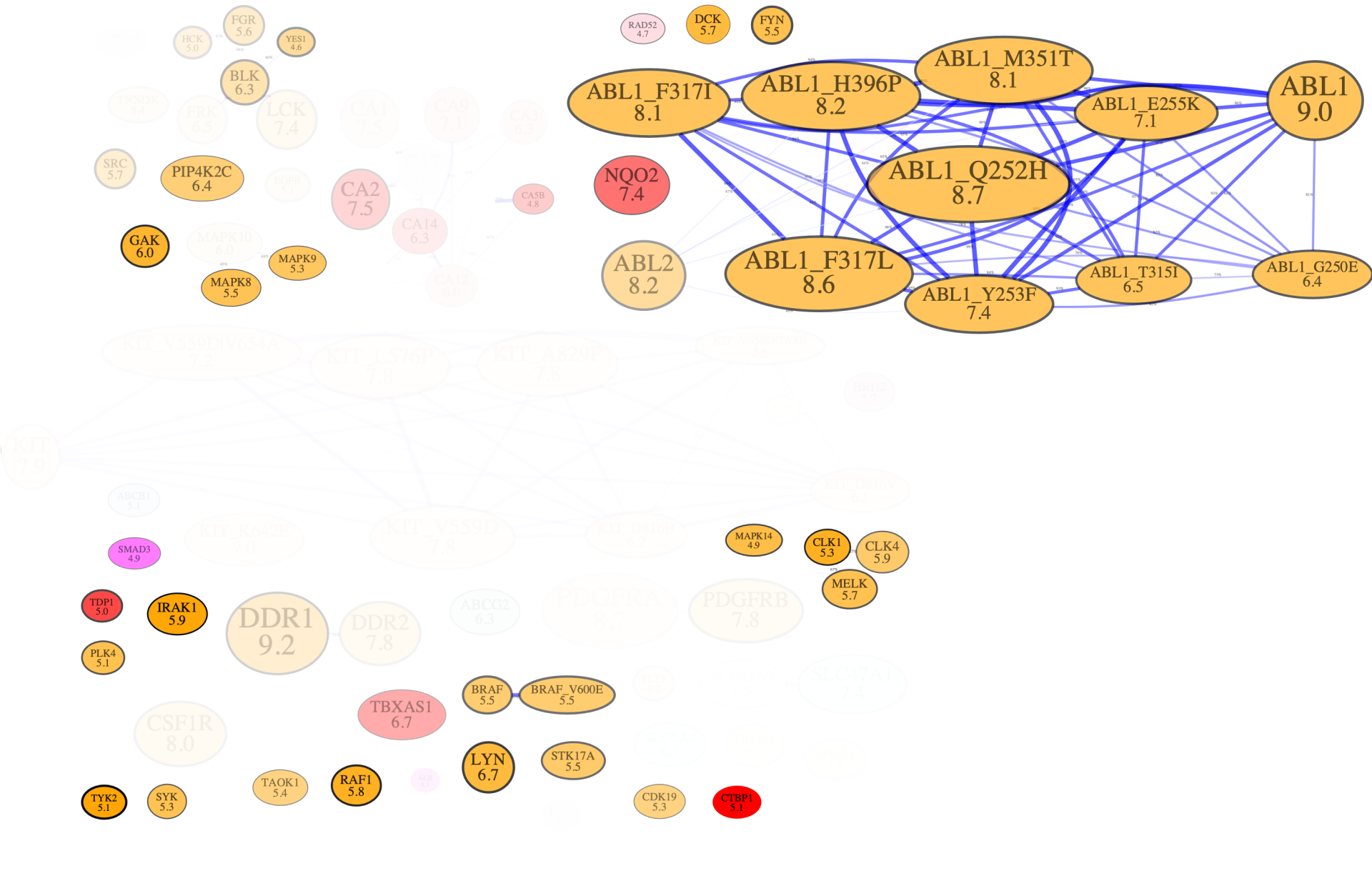
$\mathbf{[TD]/[T_0] \approx D_0/(K_d+D_0)}$

(* optional)

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]/$[P_0]$ = $\mathbf{[D_0] /(K_d + [D_0])}$

Multiple targets of **Imatinib**. Target **Expression**, Drug-resistant **Mutants**



Example of a **protein-ligand binding** problem, from **K** to ΔG

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

- 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 ΔG^0_b binding.
- **Solution:**

  Reaction: P + L $\Leftrightarrow$ PL

  $\mathbf{K_d}$ = [P][L] / [PL] = 13.5 nM $\times$ 0.5 nM/ (4.5 nM) = 1.5 nM

  $K_a = 1 / (1.5 \times 10^{-9}\ \text{M}) \sim 0.67 \times 10^{9}\ \text{M}^{-1}$

  $\Delta G^0 = -RT \ln K_a = RT \ln K_d$

  $\Delta G^0 = (0.002\ \text{kcal/(K mol)} * 300\text{K}) \ln (1.5 \times 10^{-9}) \approx -12.19\ \text{kcal/mol}$
- **Answer:** $\Delta G^0_b \approx -12.19$ kcal/mol

Shortcut: *K* vs ΔG, and K2/K1 to $\Delta\Delta G$

- $K_2 / K_1 = 10$

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

- $\Delta\Delta G = \Delta G_2 - \Delta G_1 = -RT \ln K_2 + RT \ln K_1 = -RT \ln (K_2 / K_1)$
- $\Delta\Delta G = \Delta G_2 - \Delta G_1 = -0.6 \ln 10 \approx -0.6 \times 2.3 \approx -1.4$ kcal/mol

- K increases **10-fold**, if ΔG decreases by **1.4** kcal/mol

- For example: correspondence between ΔG and K_d for protein/ligand binding:

1.4 kcal/mol ➔10 fold K drop

ΔG bind [kcal/mole]	**K_d**
-4.14	1 mM
-8.23	1 μM
-12.43	1 nM
-16.58	1 pM
-20.72	1 fM

Problem: Protein/drug binding, using K to ΔG 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 K_d improvement $\equiv$ 1.4 kcal/mol decrease in ΔG
  - 100-fold K_d improvement $\equiv$ 2.8 kcal/mol decrease in ΔG
  - 1000-fold K_d improvement $\equiv$ 4.2 kcal/mol decrease in ΔG
- **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 Reaction: P + L $\Leftrightarrow$ PL, Dissociation: PL$\Leftrightarrow$ P + L).
- **Solution:**
  - K_d = [P][L] / [PL] = 1.5×10^{-9} M = 1.5 nM
  - [PL] = [P][L] /K_d = 0.5×13.5 / (1.5) = 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%

  - Also, directly from: [PL]/ [P_0] = D/(K_d+D) = 13.5/(13.5+1.5) = 0.9
- **Answer:** 90% of the protein is bound.

Protein-Ligand Binding Equilibration

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

	Protein	**Ligand**	**Complex**
Start (no equilibrium)	$[P] = P_0$	$[L] = L_0$	0
Equilibration	$[P] = P_0 - x$	$[L] = L_0 - x$	$[PL] = x$

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?

$$x = L_{bound} = P_{bound} = \frac{P_0 + L_0 + K_d - \sqrt{\left(P_0 + L_0 + K_d\right)^2 - 4P_0L_0}}{2}$$

Example: bound fraction at equilibrium *from total concentrations*

- 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:**

	Protein	**Ligand**	**Complex**
Start (no equilibrium)	[P] = P_0	[L] = L_0	0
Equilibrium	**[P] = P_0 – *x***	**[L] = L_0 – *x***	**[PL] = *x***

$x^2 - (P_0+L_0+K_d) \times x + P_0L_0 = 0$ – 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 $ax^2 + bx + c = 0$
- $x = (-b \pm \sqrt{(b^2 - 4ac)}) / 2a = 0.27\ \mu M$ or $0.40\ \mu M$
- x cannot exceed P_0 or L_0, so $x = 0.27\ \mu M$ (use the solution with –)

	Protein	**Ligand**	**Complex**
Start (no equilibrium)	[P] = 0.3 μM	[L] = 0.36 μM	0
Equilibration	[P] – x = 0.03μM	[L] – x = 0.09μM	x = 0.27μM

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

- **Answer:** 0.27μM

Total protein vs K_d: Two common cases

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

1. True for **most biological targets** in vivo

- $\mathbf{[P_{total}] << K_d}$ $\Rightarrow$ $[P] << K_d$ $\Rightarrow$ $[P] / K_d << 1$

  $\mathbf{[PL] << [L]}$

- *Ligand **is not depleted*** by binding to the protein target

2. True for *albumin, antitrypsin,* **abundant** plasma proteins

- $\mathbf{[P_{total}] > K_d}$ $\Rightarrow$ $[P] \sim K_d$ $\Rightarrow$ $[P] / K_d \sim 1$

  $\mathbf{[PL] \sim [L]}$

- Ligand ***is depleted*** by binding to the protein target
- Only unbound ligand fraction acts on therapeutic targets.

[Target] <K_d< [Ligand]

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

- $P_{total} << K_d$ and $[PL] << [L]$
- $[L_{total}] \approx [L]$
- Target bound/unbound ratio (from definition of K_d):

$$[PL] / [P] = [L] / K_d \approx [L_{total}] / K_d$$

- When $[L_{total}] \approx K_d$, $[PL] = [P]$, i.e.

K_d is the ligand concentration at which 50% target is bound.

- Similarly, $[L_{total}] \approx Kd \times [PL]/[P]$ for any bound/unbound ratio.
- Fraction of bound receptor: $[PL] / [P_{total}] \approx [L_{total}] / (K_d + [L_{total}])$

Example: [*total Lig*] ≈ ([PL]/[P]) 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:**

  $[P] << K_d$

  Desired [PL]/[P] ratio is 80/20 = 4/1

  Total ligand = $[PL]/[P] \times K_d \approx 40nM$

  Another solution: *bound target fraction* is $0.8 = x/(x+K_d)$, x=40nM
- **Answer:** ≈ 40 nM
- **Note:** if K_d is 10nM, we need 90 nM drug for 90% bound protein, and 190 nM for 95% bound protein
- **Dose**: 90nM for 500g/mole drug and 30L corresponds to 1.35mg dose

Case of **high protein concentration**

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

- $P_{tot} > K_d$; [PL] is proportional to [L]
- If protein is in excess, i.e. [PL] << [P] and $[P_{tot}] \approx [P]$
- Ligand bound / unbound ratio (from definition of K_d):

$$\mathbf{[LP] / [L] = [P] / K_d \sim [P_{tot}] / K_d}$$

- $\mathbf{[P_{tot}] / K_d}$ defines bound/unbound ratio for the ligand
- *Bound ligand fraction* = $\mathbf{[P_{tot}] / ([P_{tot}] + K_d)}$

High affinity drug-albumin binding

- **Problem:** K_d (Albumin, warfarin) is ~5 μM, calculate drug fraction found to albumin in %, assuming albumin in physiological range or 35 to 50 g/L. MM = 66.5 kDa
- **Solution:**
  - $[P_{tot}] / ([P_{tot}] + K_d)$
  - with albumin concentration at 526 μM: 526 / 531 ~ 99.05%
  - with albumin concentration at 752 μM: 752 / 757 ~ 99.34%

- **Note:** unbound warfarin varies between 0.66% and ~0.95%, 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 μM.
- **Solution:**
  - Bound drug fraction: $f=D_{albumin\_bound} / D_0 \approx$ **$[P_{tot}] / ([P_{tot}] + K_d)$**
  - with albumin concentration at 450 μM: f = 450 / 5450 ~ 8.3%
  - with albumin concentration at 750 μM: f = 750 / 5750 ~ 13%

- **Unbound** drug B (1-f, or 100(1-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.