

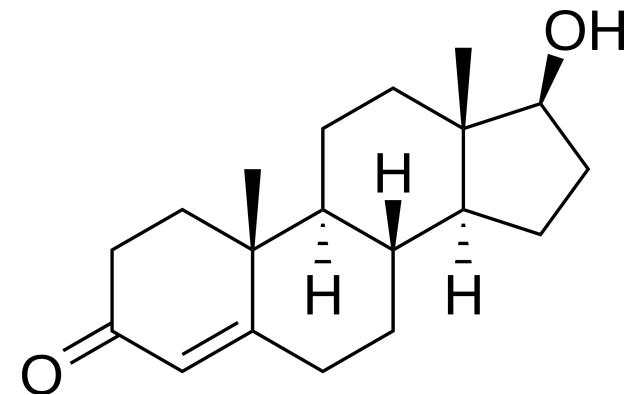
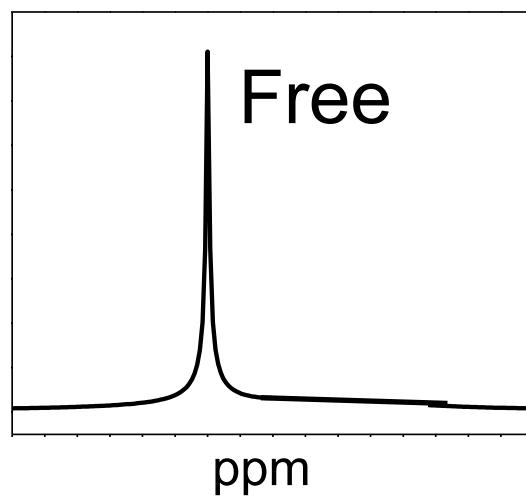
Drug Discovery I

Arthur G. Roberts

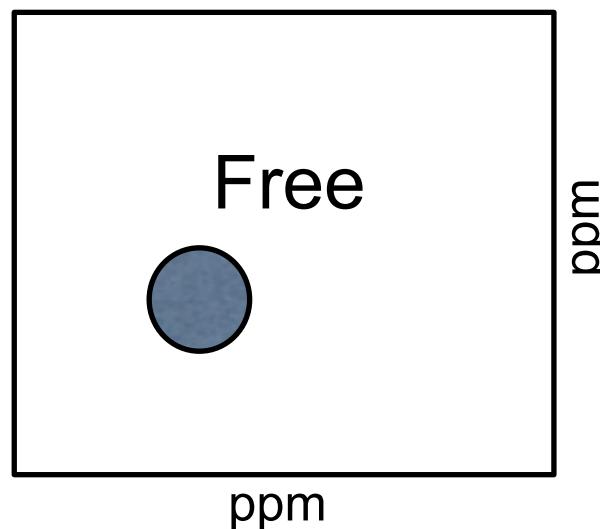
Review: Chemical Exchange

NMR

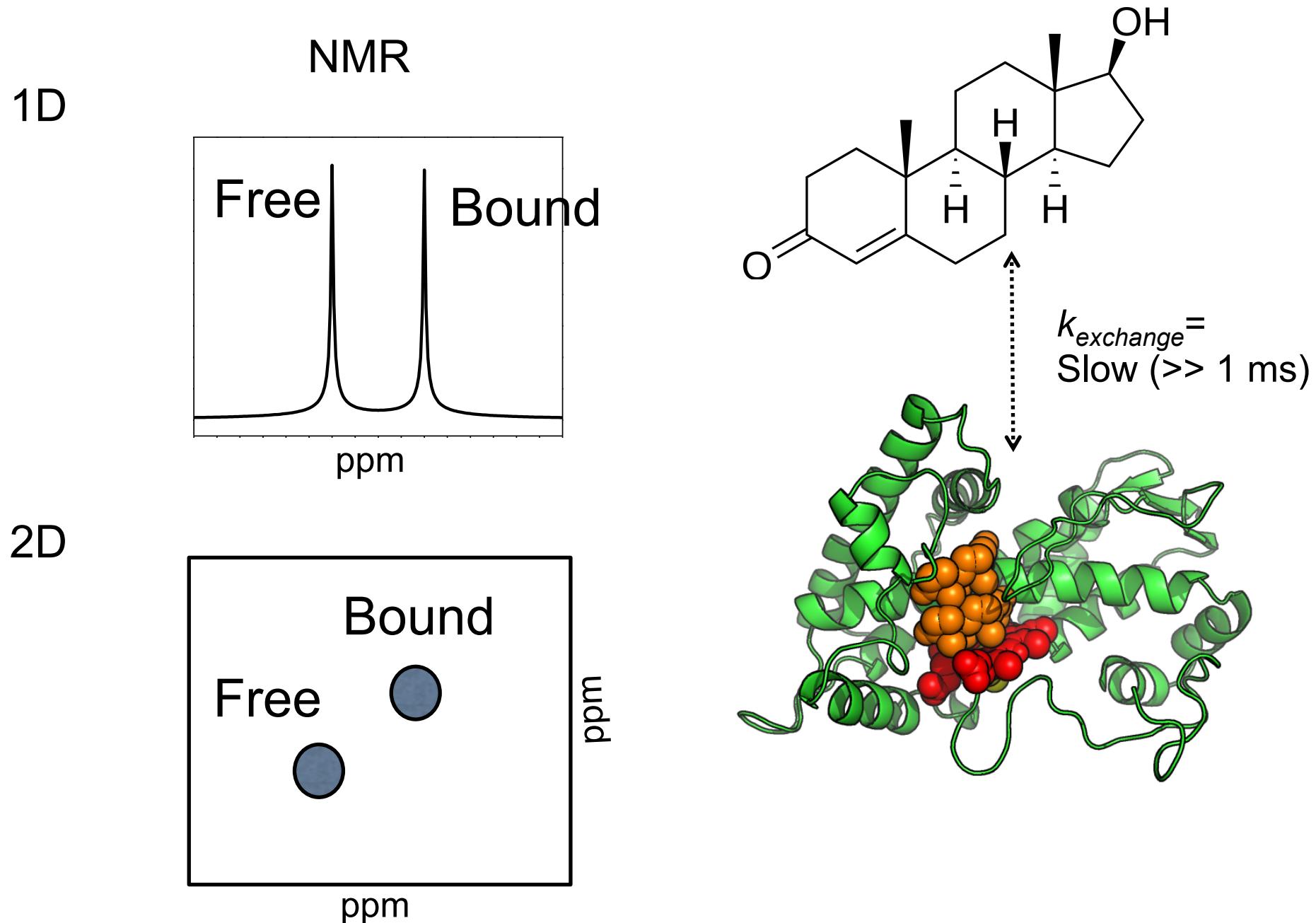
1D



2D



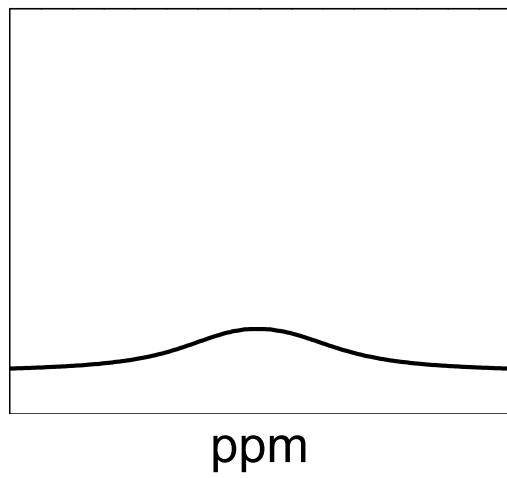
Review: Chemical Exchange



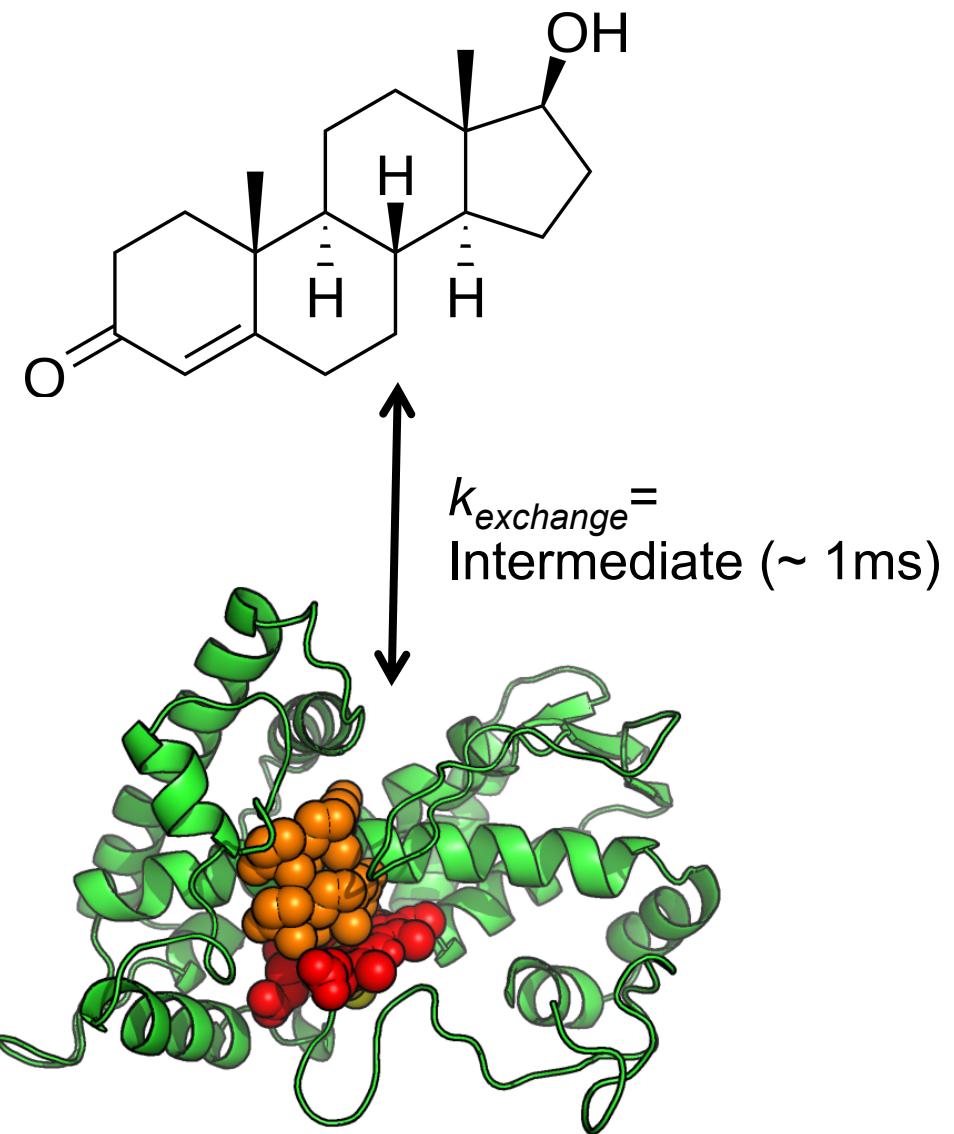
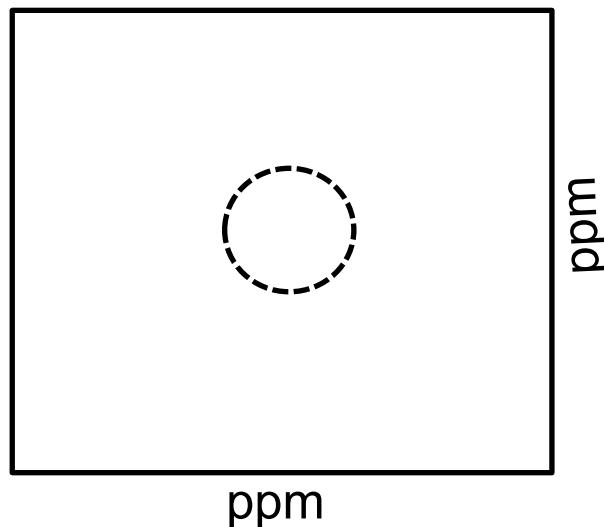
Review: Chemical Exchange

NMR

1D



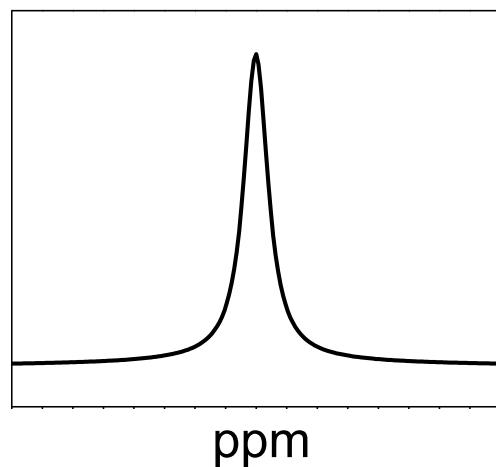
2D



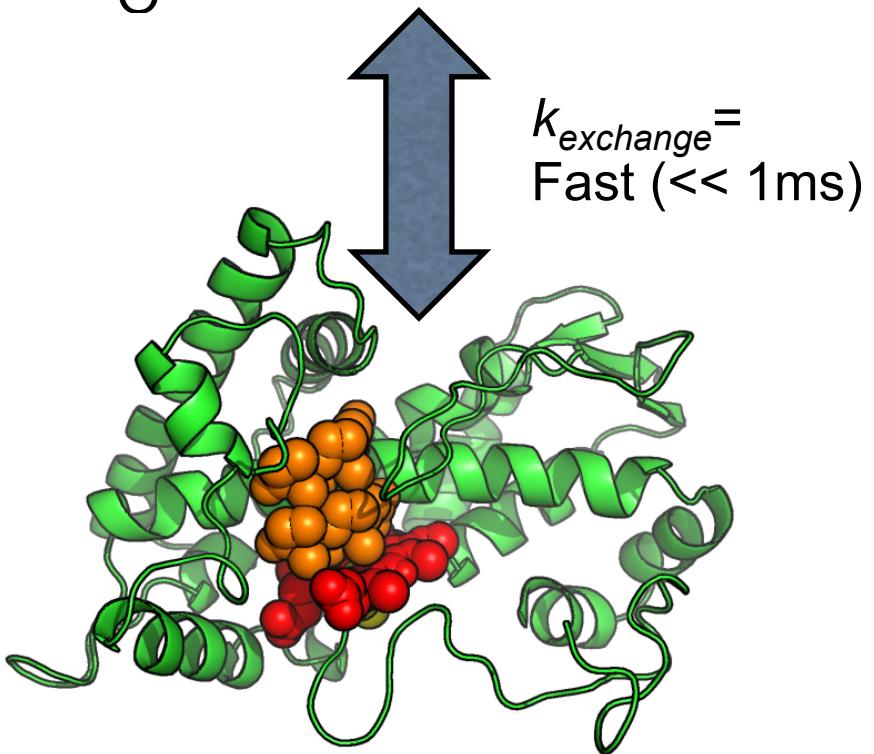
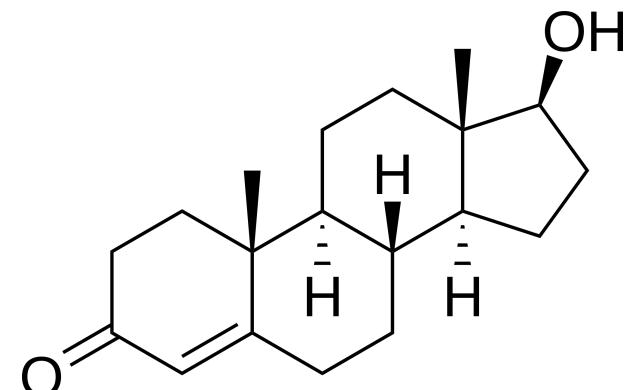
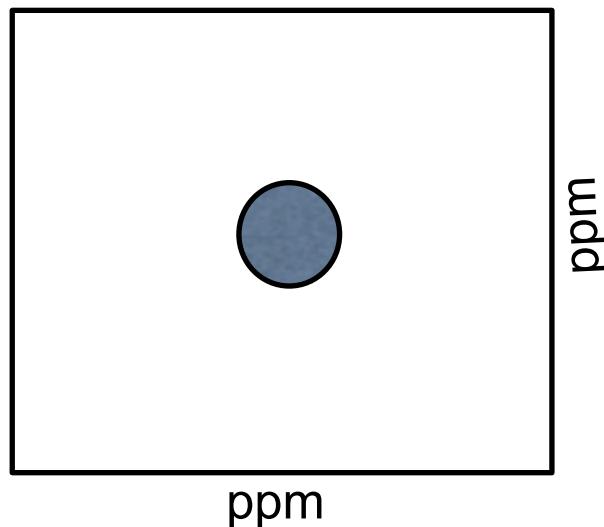
Review: Chemical Exchange

NMR

1D



2D



$$k_{\text{exchange}} = \text{Fast} (<< 1\text{ms})$$

Review: Chemical Exchange

- Slow Exchange ($k_{exchange} \gg 1 \text{ ms}$)
 - Peaks Split
- Intermediate Exchange ($k_{exchange} = \sim 1 \text{ ms}$)
 - Peaks Broaden (1D)
 - Peaks Disappear (2D)
- Fast Exchange ($k_{exchange} \ll 1 \text{ ms}$)
 - Peaks Shift

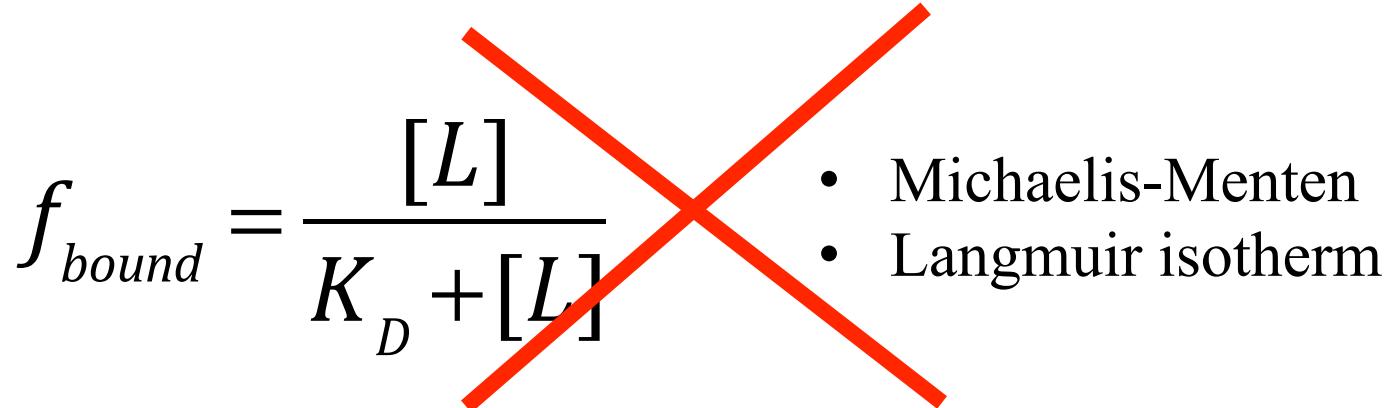
Binding Equations

$$f_{bound} = \frac{[L]}{K_D + [L]}$$

- Michaelis-Menten
- Langmuir isotherm

Binding Equations

$$f_{bound} = \frac{[L]}{K_D + [L]}$$

- 
- Michaelis-Menten
 - Langmuir isotherm

Binding Equations

$$f_{\text{bound}} = \frac{[L]}{K_D + [L]}$$

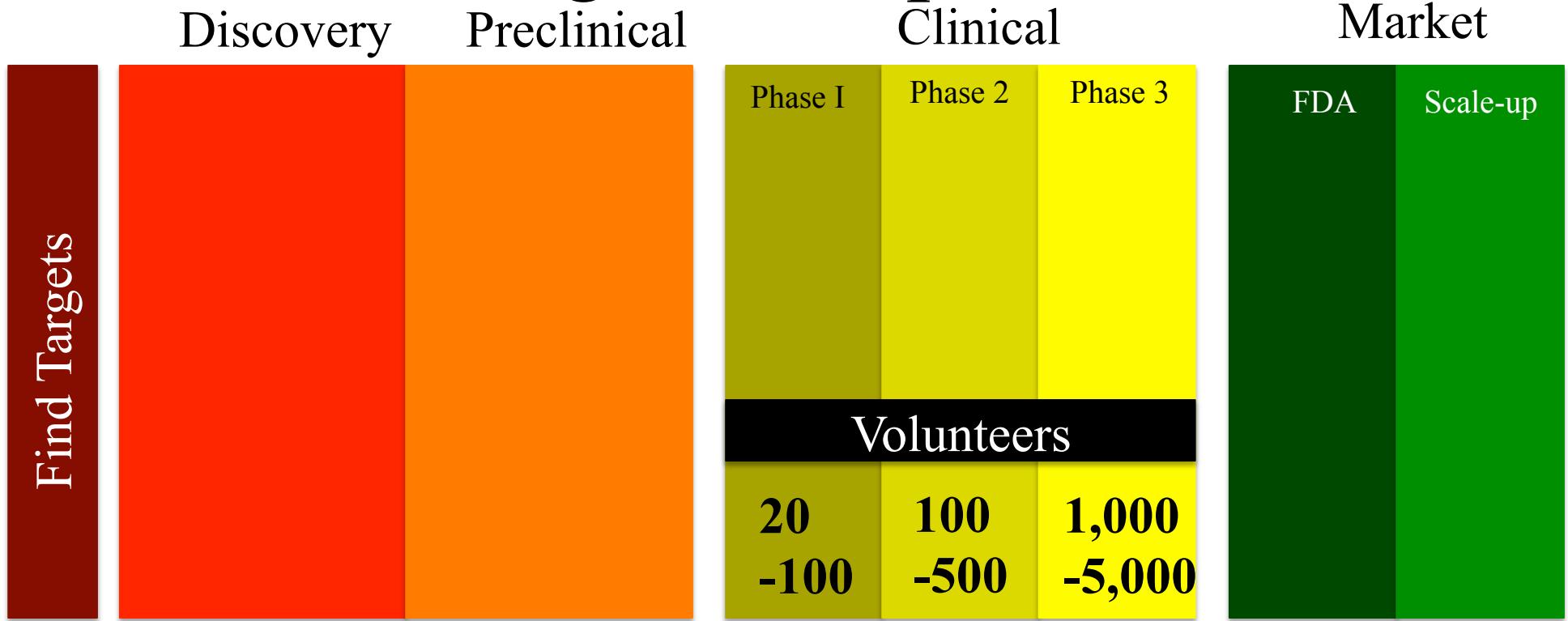
- Michaelis-Menten
- Langmuir isotherm

$$f_{\text{bound}} = \frac{[P] + [L] + K_D - \sqrt{([P] + [L] + K_D)^2 - 4[P][L]}}{2[P]}$$

Factors in Drug Discovery

- Program Selection (Disease)
- Target Selection
- Assay Development
- Lead Generation
- Lead Optimization
- Drug Candidate?
- Clinical Trials

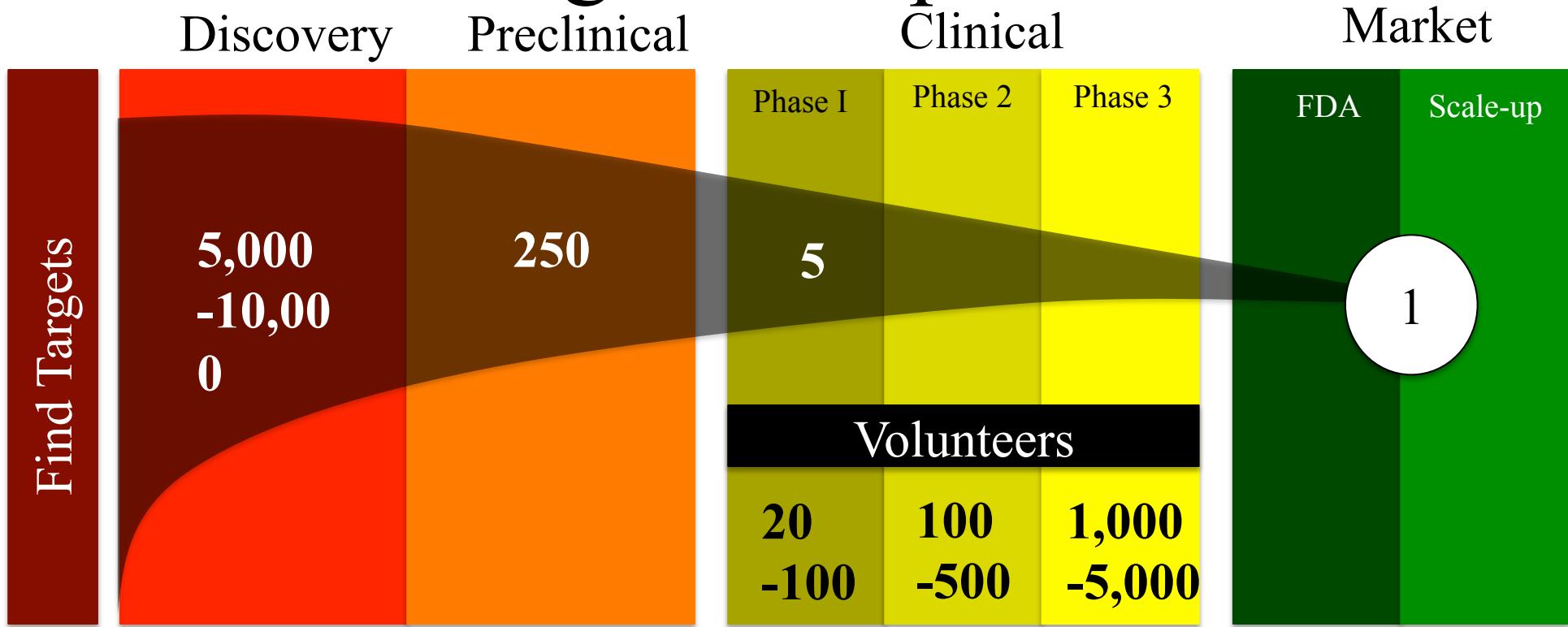
Drug Development



*Inflation Adjusted

Innovation.org and DiMasi, et al. 2003

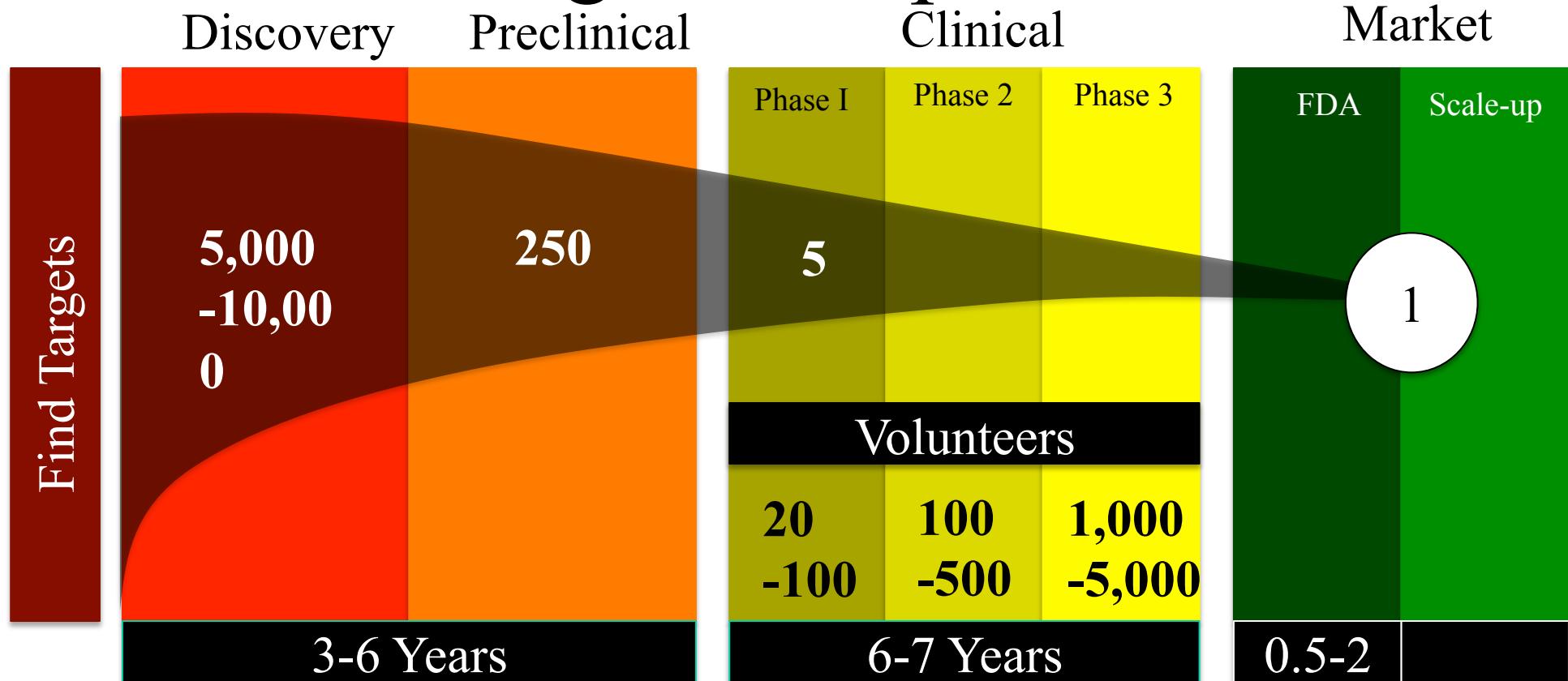
Drug Development



*Inflation Adjusted

Innovation.org and DiMasi, et al. 2003

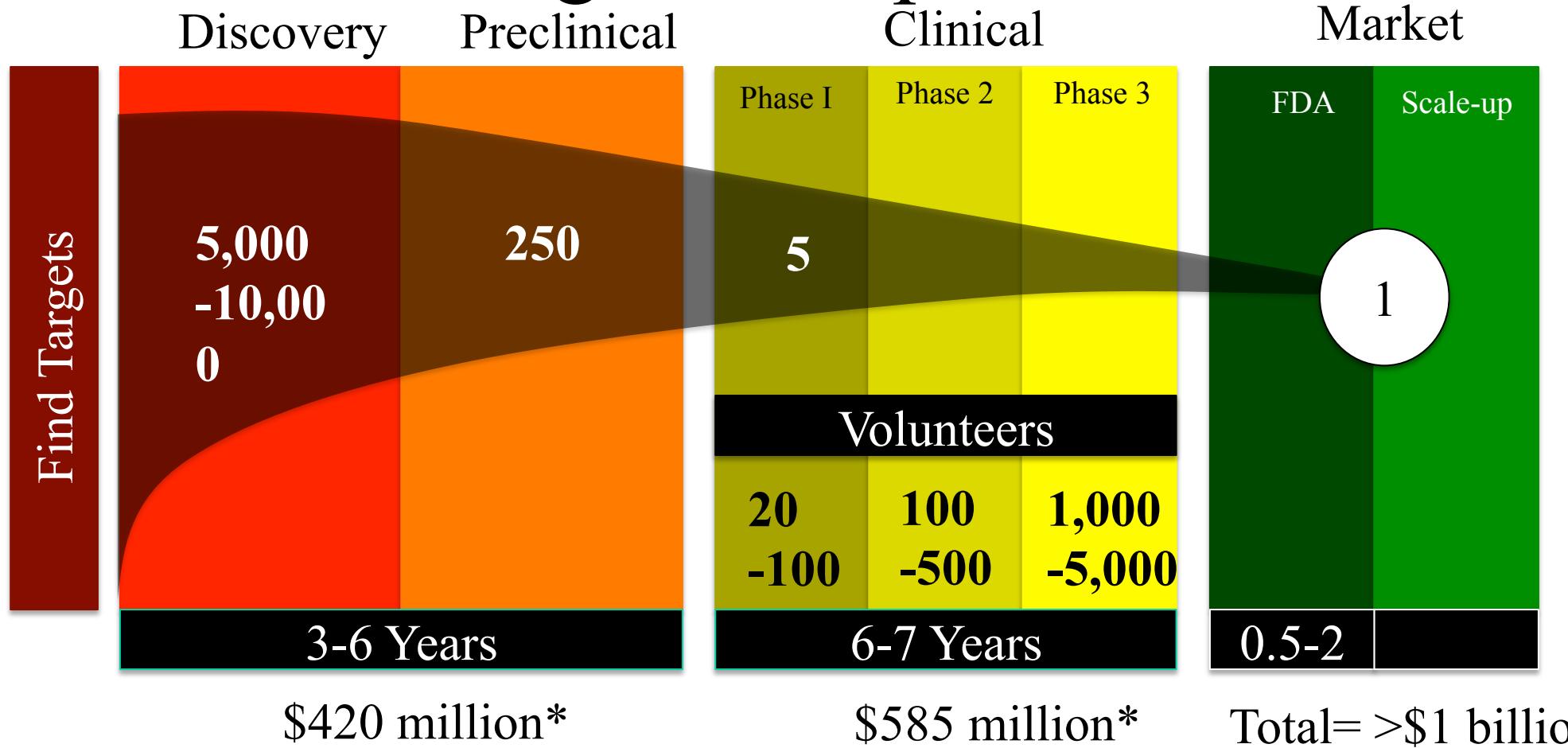
Drug Development



*Inflation Adjusted

Innovation.org and DiMasi, et al. 2003

Drug Development

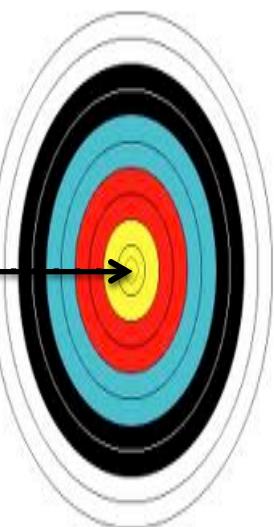


*Inflation Adjusted



Drug

Target



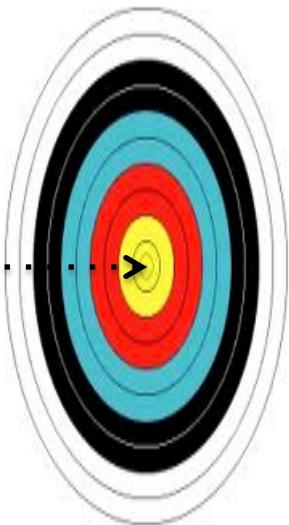


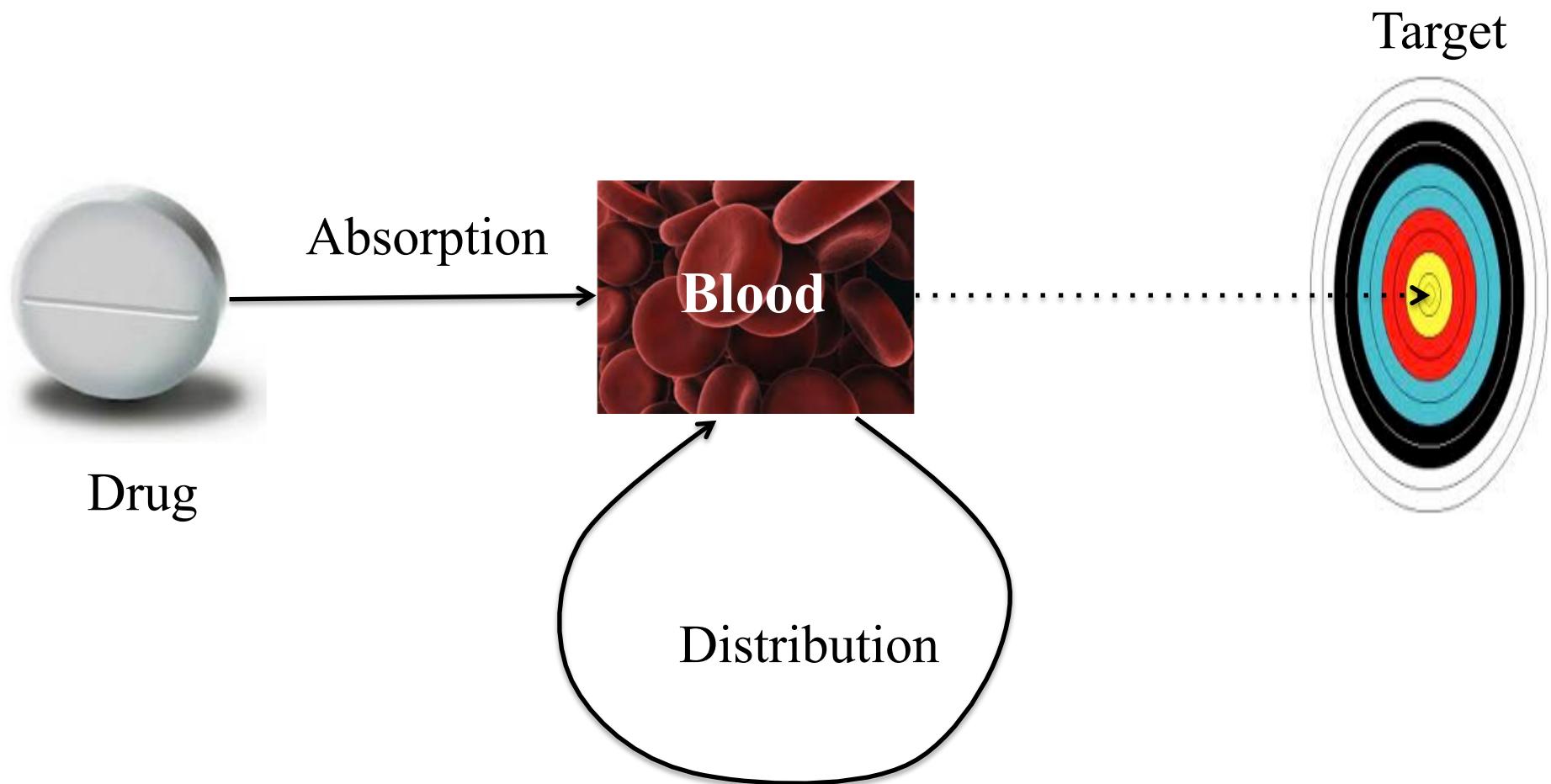
Absorption

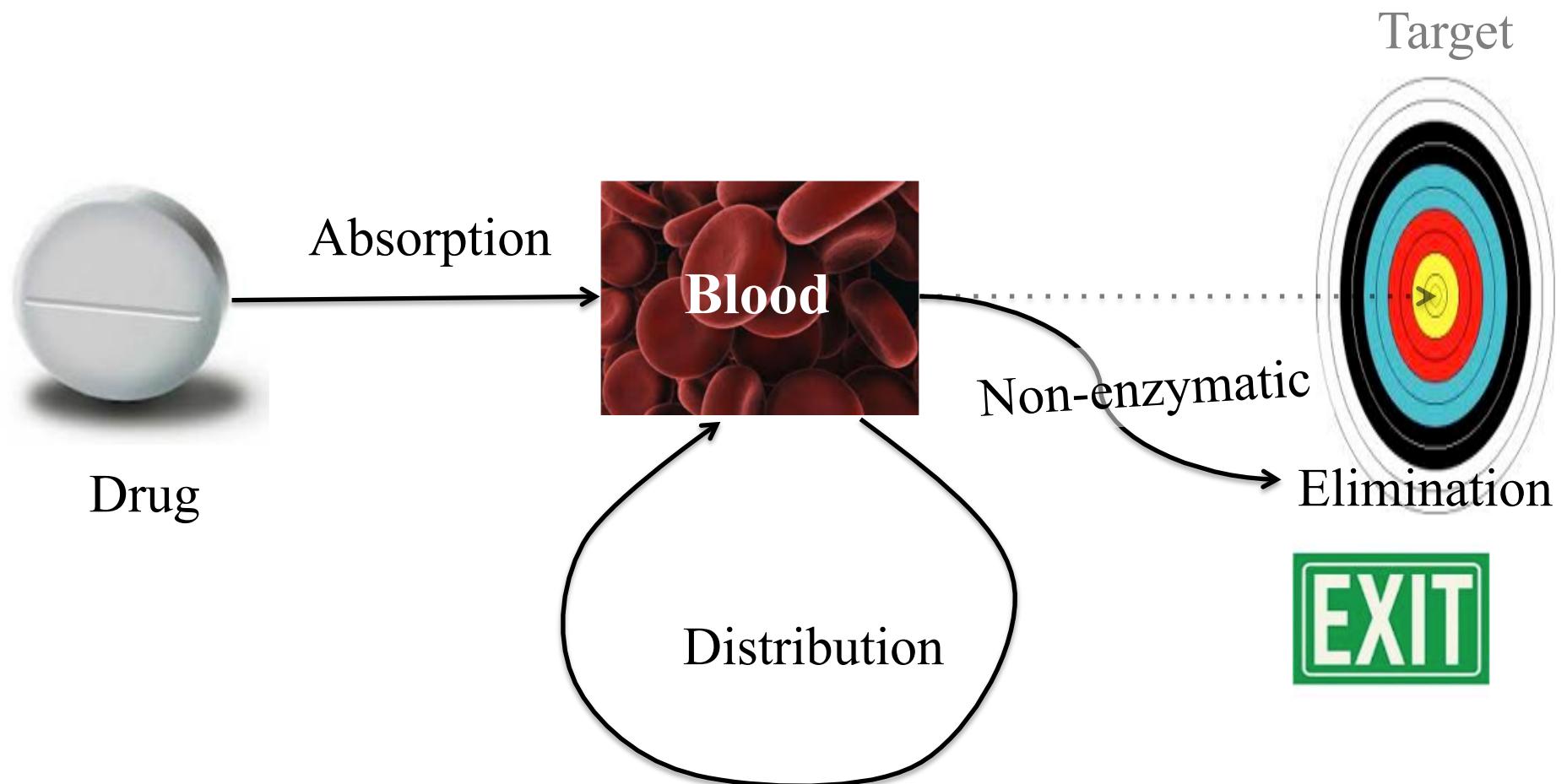


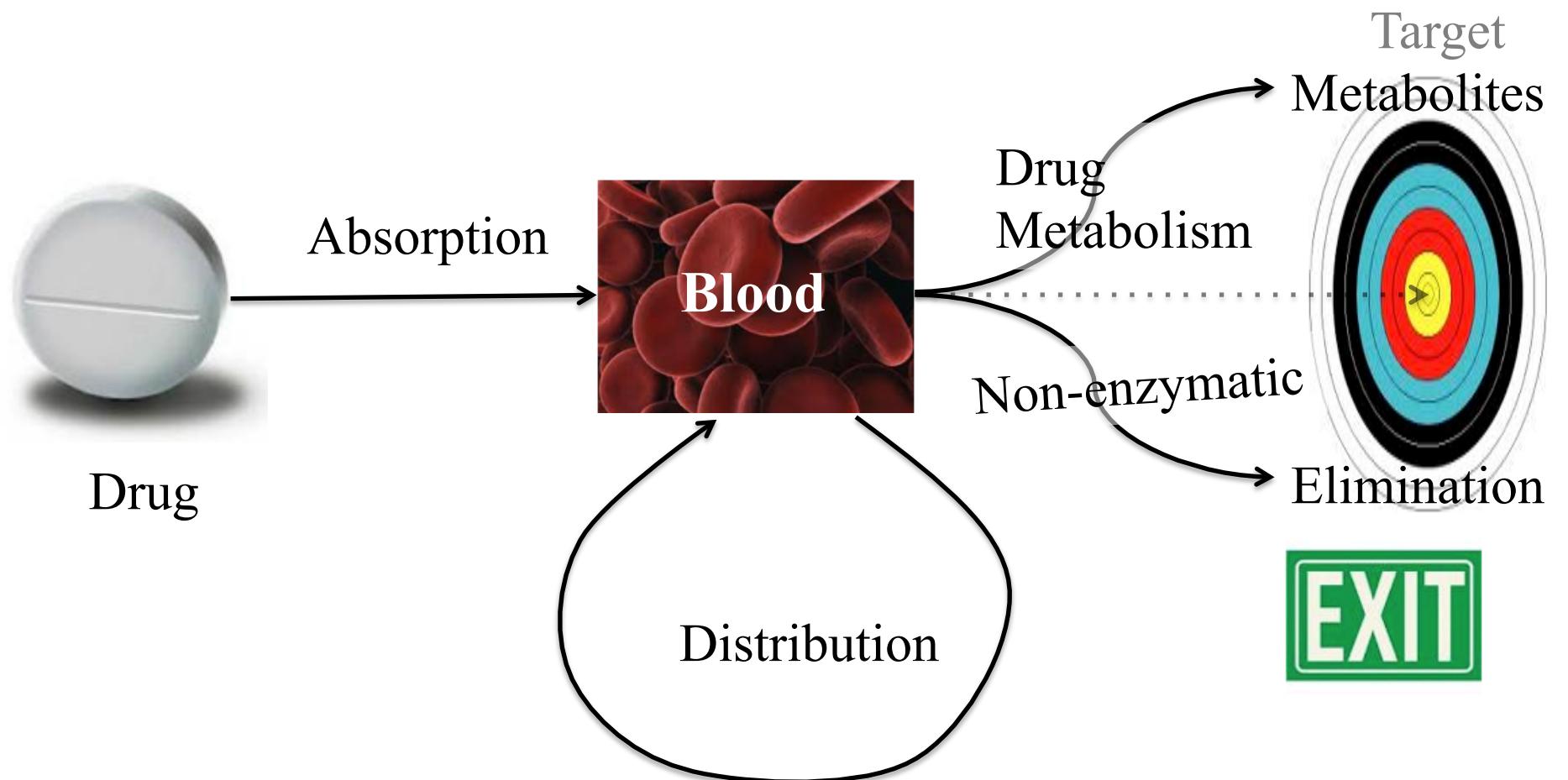
Drug

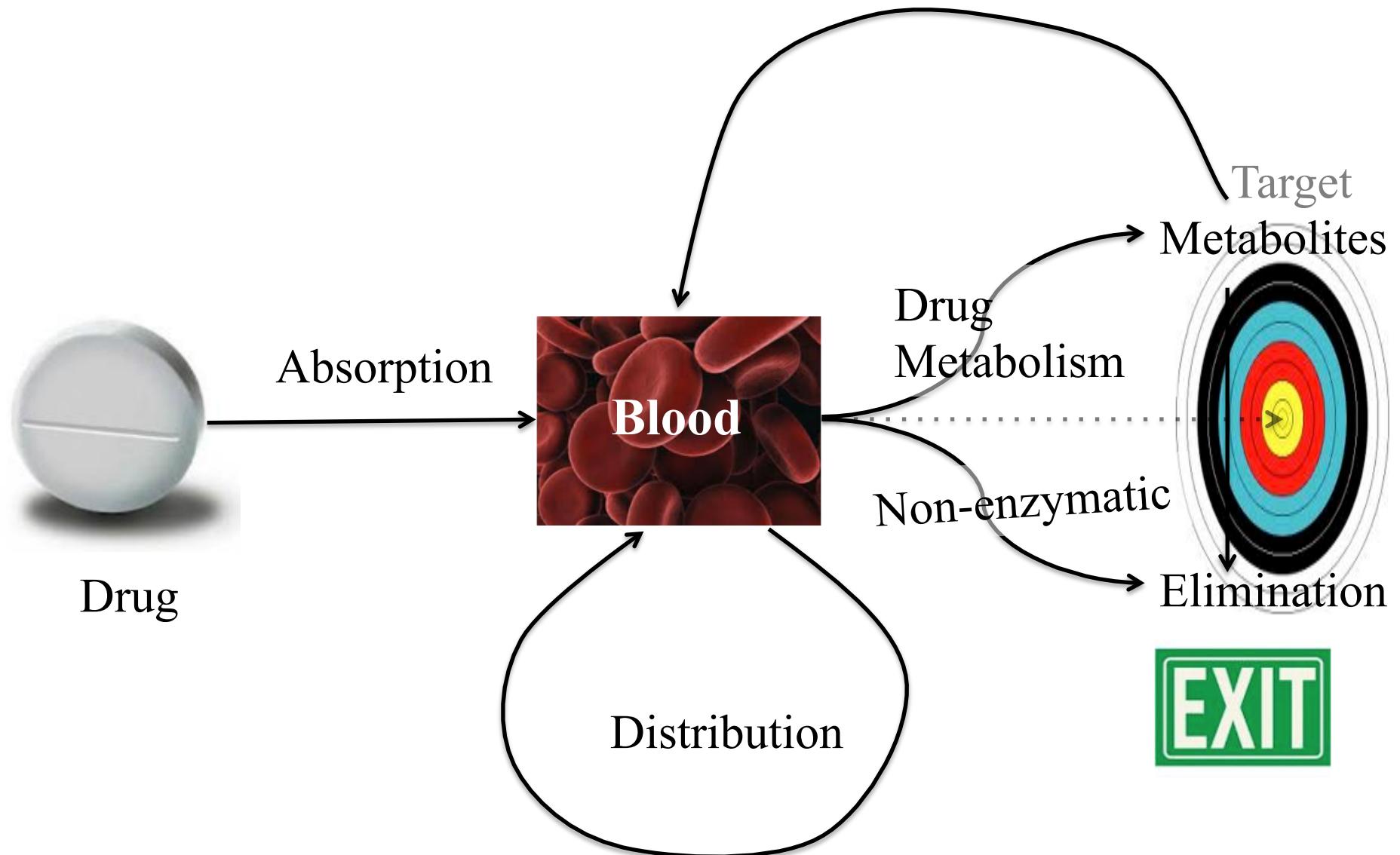
Target









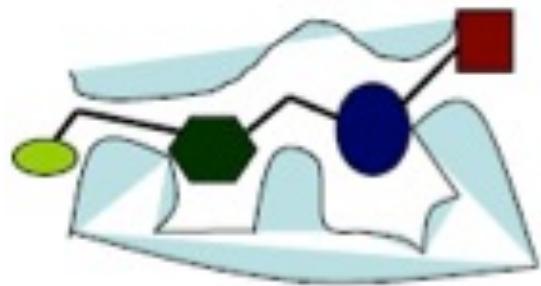


Lead Generation

- “leads”
 - activity against target
- lead optimization
 - potency
 - selectivity
 - pharmacokinetic parameters
- High Throughput Screening (HTS)
- Fragment Based Drug Design (FBDD)

HTS involves screening a large chemical library for compounds that bind to the target in the hopes of finding relatively potent drug leads ($K_d < 1 \mu M$). Although a large number (millions) of compounds can be screened, it is expensive and limited in terms of sampling the real “chemical space” ($10^{40} - 10^{100}$ possibilities!)

HTS



Low quality HTS hit with many non-optimised interactions with target site

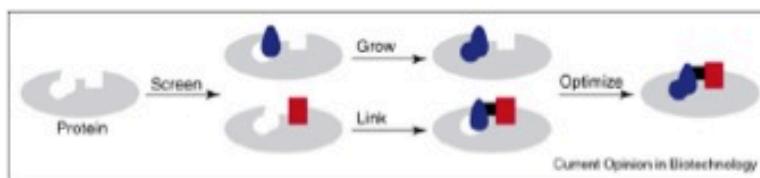
- Modest affinity
- Low ligand efficiency

Assumption: 1 ligand bound.

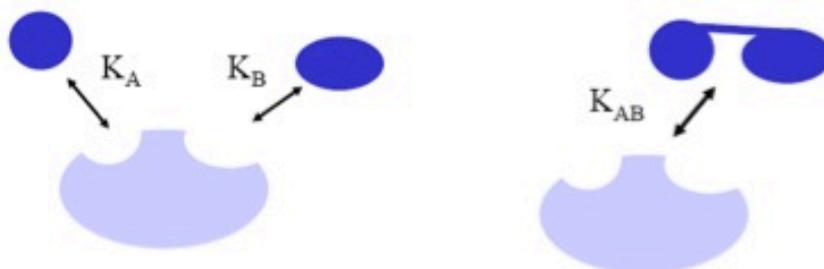
FBDD is based on screening smaller numbers of compounds (several thousand) in the hopes of finding low affinity (millimolar to micromolar) binders that can expanded to generate a high affinity lead which has “chemical diversity”

Fragment Based Drug Design

- screen few compounds (<10,000) using generic binding assay
 - Less resource intensive than HTS
 - Small compounds ("fragments"), typically 9 - 20 non-H atoms
(note: The more complex the screened molecules are, the less probable it is that they bind to a given site – IOW keep it simple!)
- find weak but efficient (high binding energy per atom) binders
- develop into more potent compounds using fragment expansion, merging or linking while maintaining a high ligand efficiency.



The basic concept of fragment-based lead discovery. The blue ovoid and red rectangle represent fragments that bind to the target protein. These can be linked or expanded to produce high-affinity ligands.

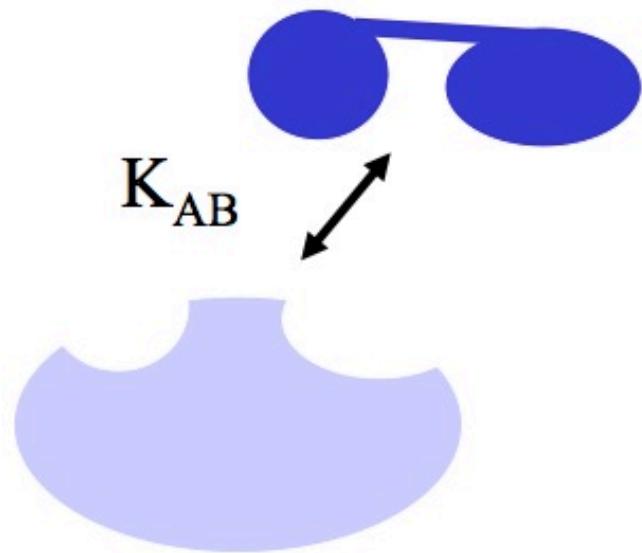
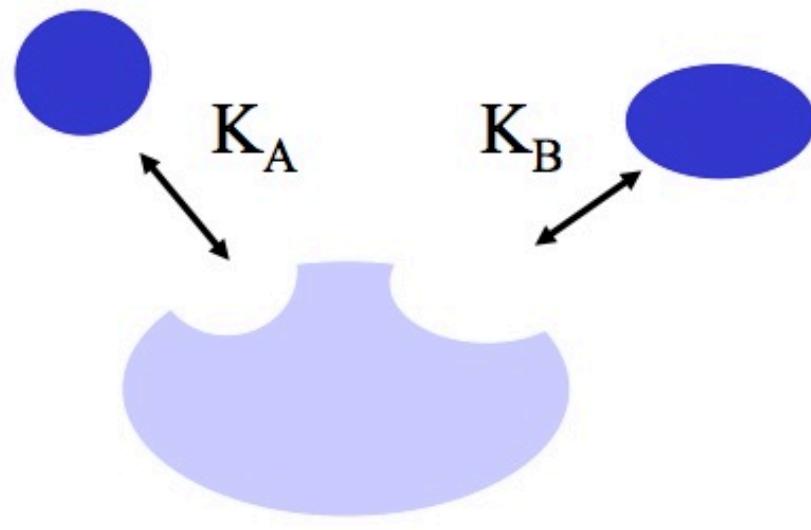


$$\Delta G_{AB} = \Delta G_A + \Delta G_B, \quad RT\ln(K) = -\Delta G_{AB}, \quad K_{AB} = K_A \times K_B$$

$$K_A = 2 \times 10^3, K_B = 5 \times 10^3, K_{AB} = 1 \times 10^7$$

SAR by NMR

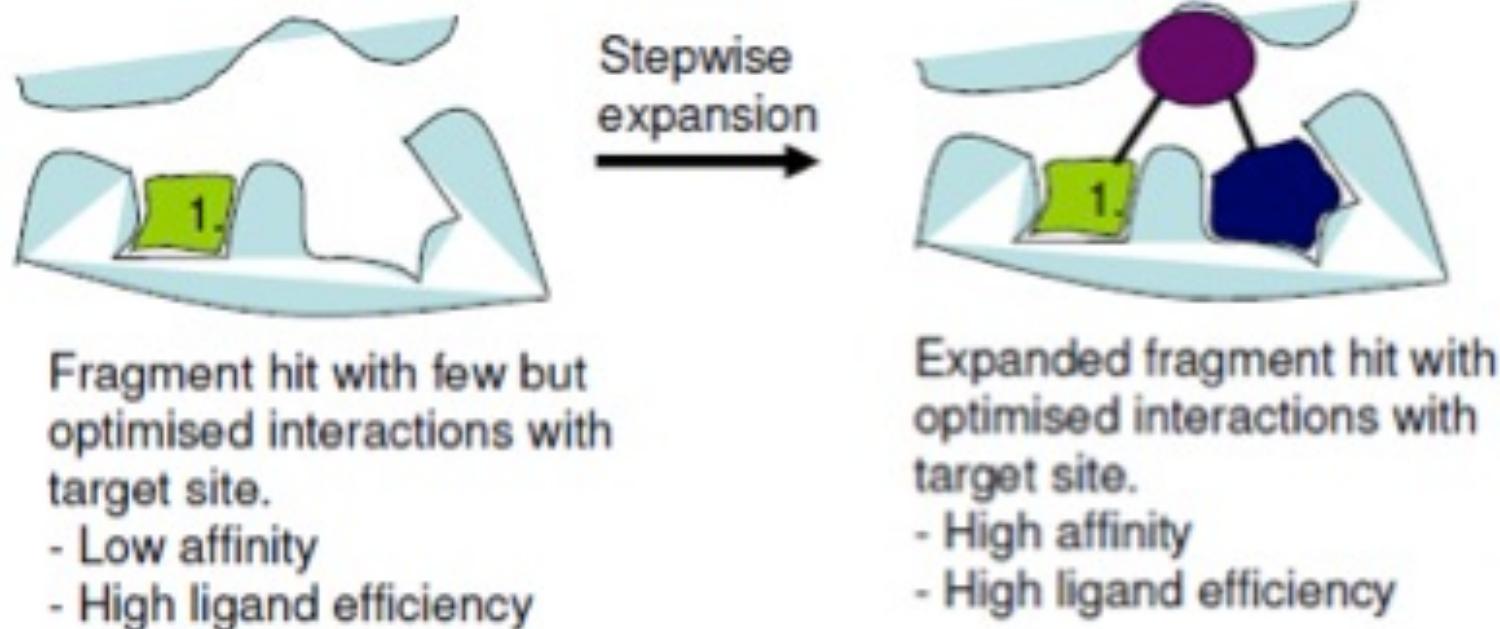
Shuker, Hajduk, Meadows, Fesik, Science, 274, 1531 (1996)



$$\Delta G_{AB} = \Delta G_A + \Delta G_B, \quad RT\ln(K) = -\Delta G_{AB}, \quad K_{AB} = K_A \times K_B$$

$$K_A = 2 \times 10^3, K_B = 5 \times 10^3, K_{AB} = 1 \times 10^7$$

FBDD



Assumption: Fragment only binds in one spot.

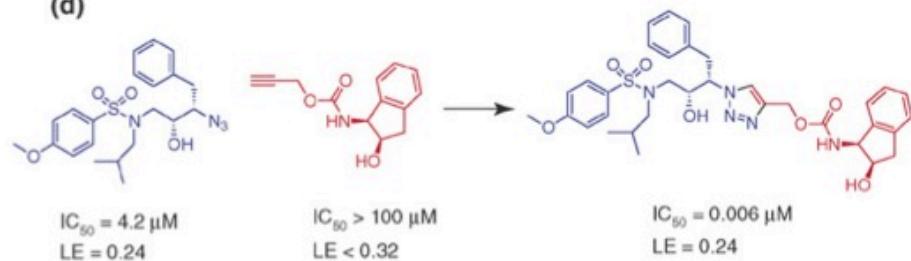
FBDD: Examples

(a)



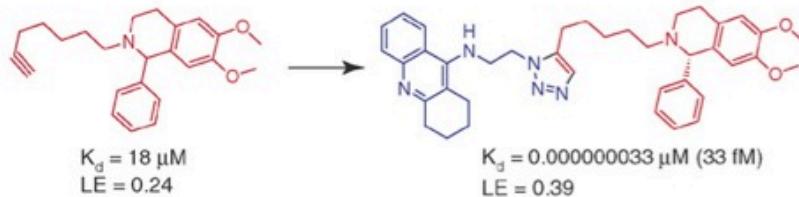
Thrombin

(d)



HIV-1 protease

(i)



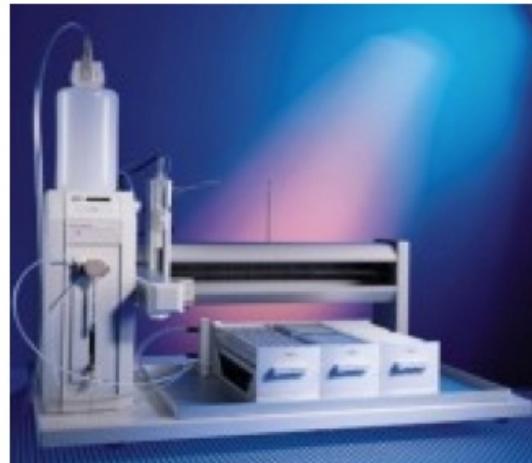
acetylcholinesterase

Erlanson, D. A. (2006). "Fragment-based lead discovery: a chemical update." *Current Opinion In Biotechnology* 17(6): 643-652.

NMR Drug Discovery Hardware



Cryoprobe



Flow Probe

Autosampler



NMR: FBDD

- Target/Receptor-Based NMR screening
 - chemical shift perturbation (CSP) mapping
- Ligand-Based NMR screening

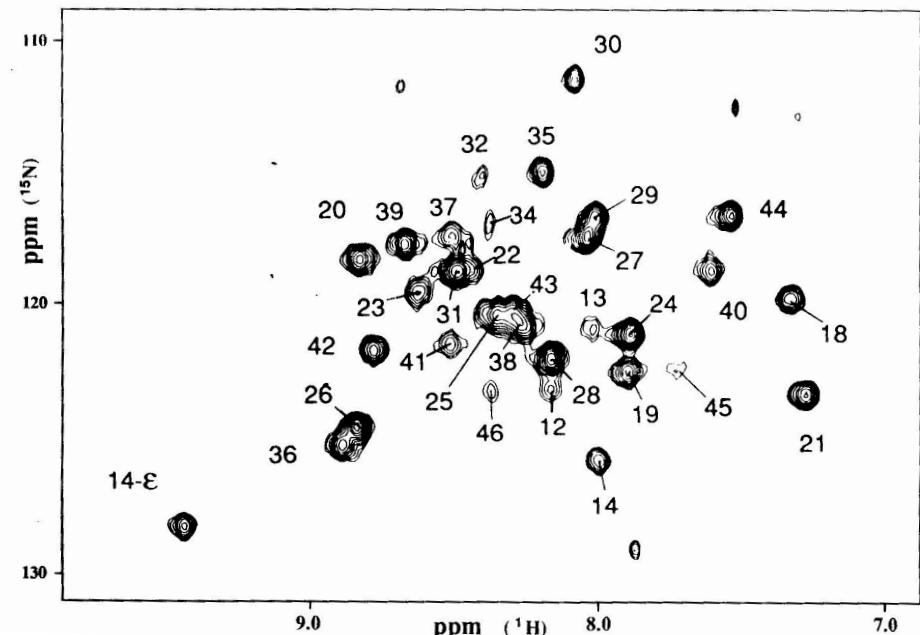
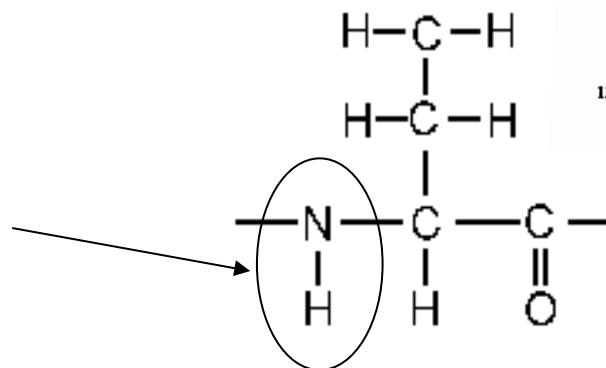
Target/Receptor-Based NMR screening

- Heteronuclear Single Quantum Correlation spectroscopy

^{15}N

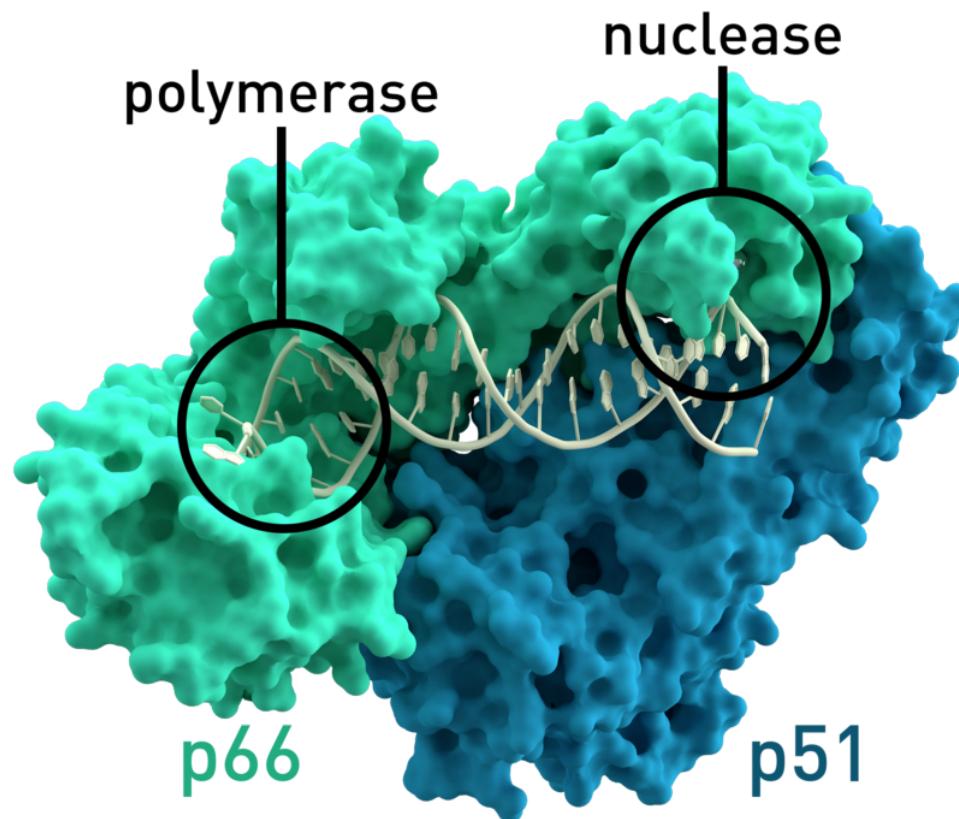
1 signal for each amide.

Only see
this



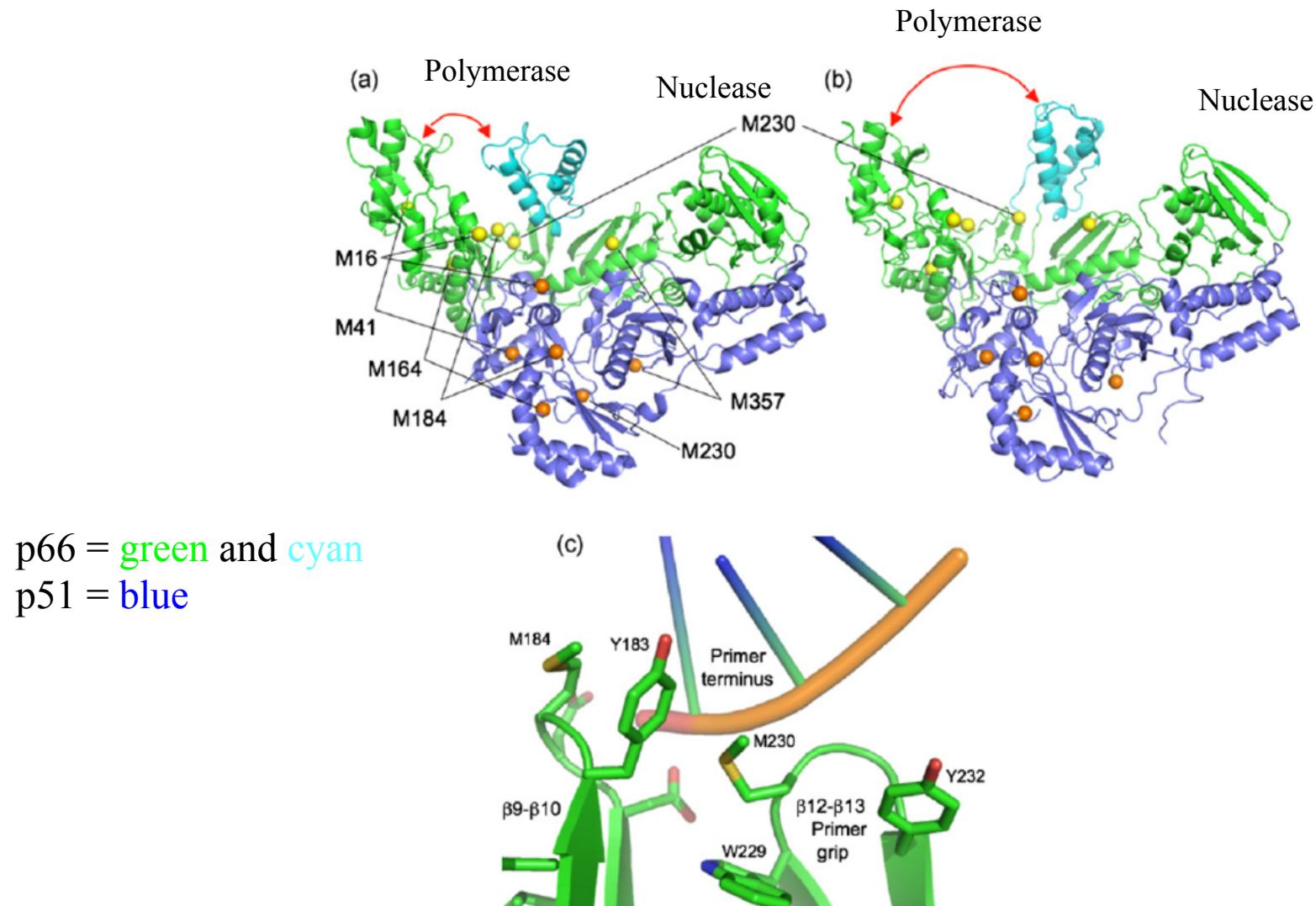
^1H

Example: HIV Reverse Transcriptase (RT)



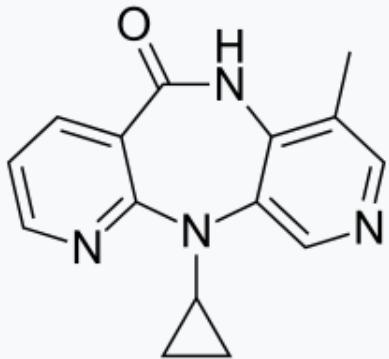
Single Stranded RNA → DNA

Example: HIV Reverse Transcriptase

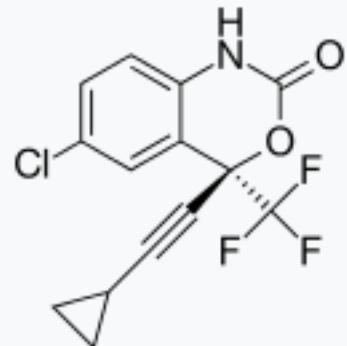


Non-nucleoside RT Inhibitors

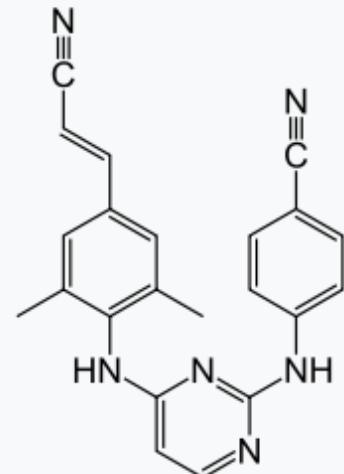
Nevirapine



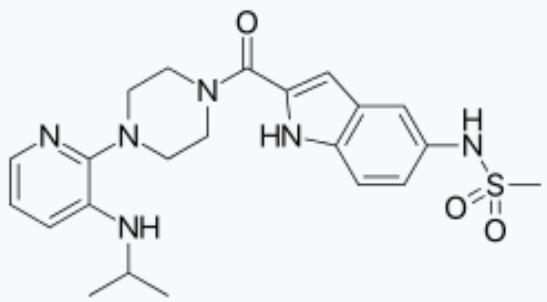
Efavirenz



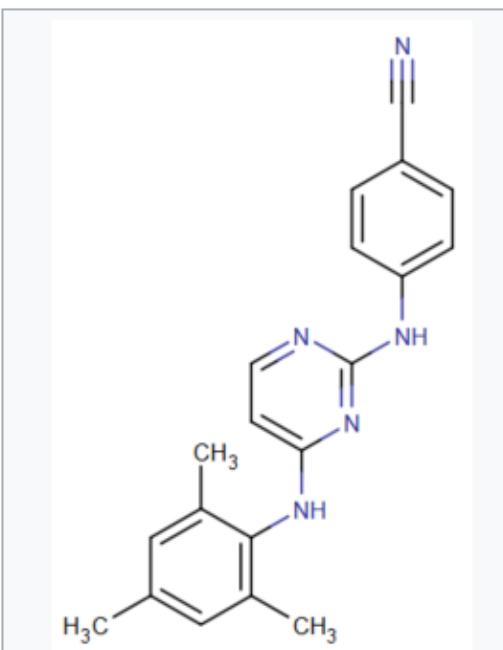
Rilpivirine



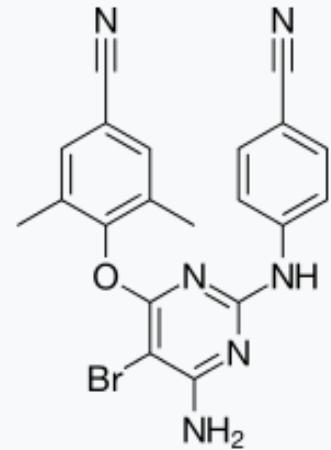
Delavirdine



Dapivirine

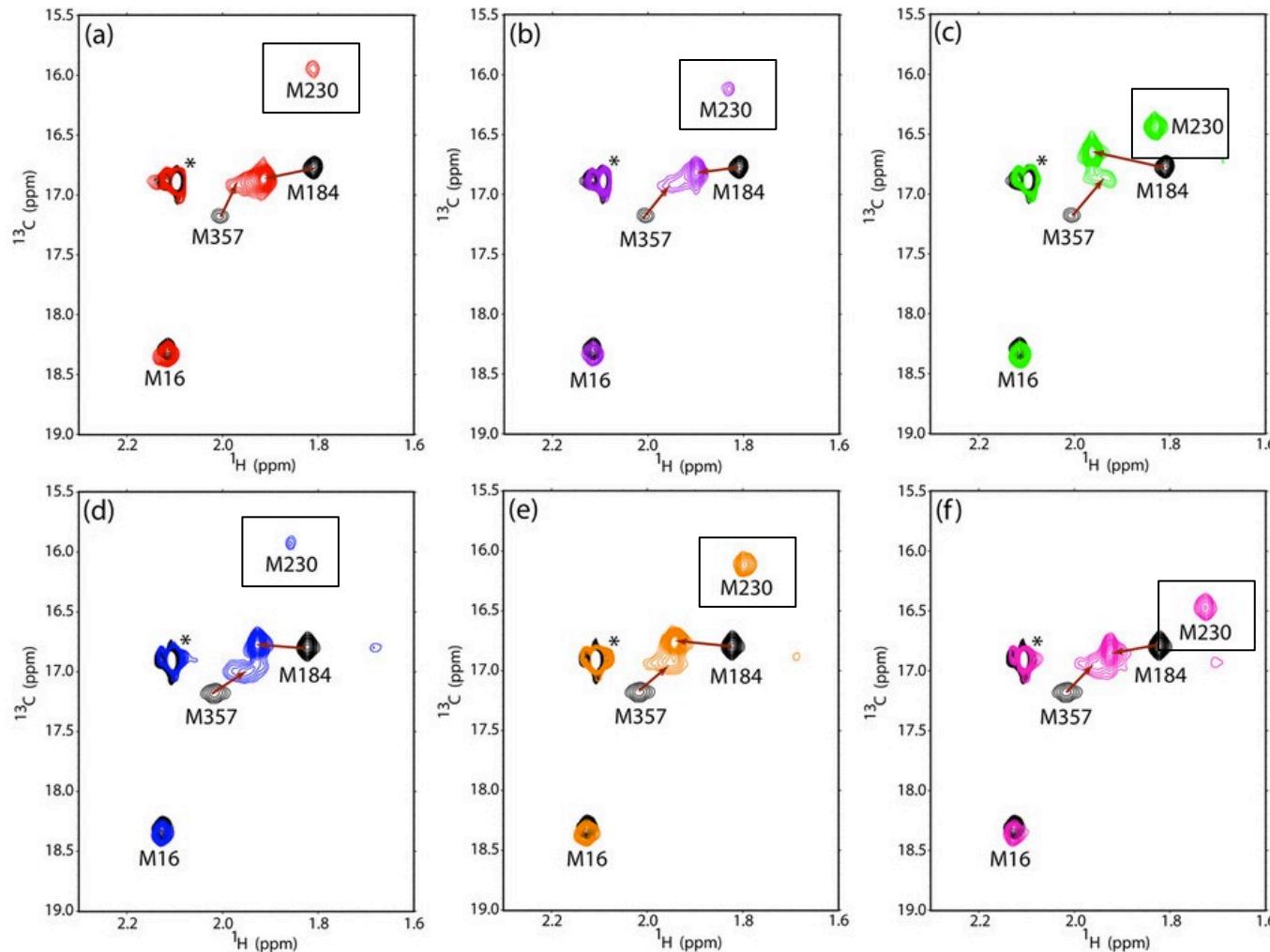


Etravirine



Drugs Induce Peak Shifts

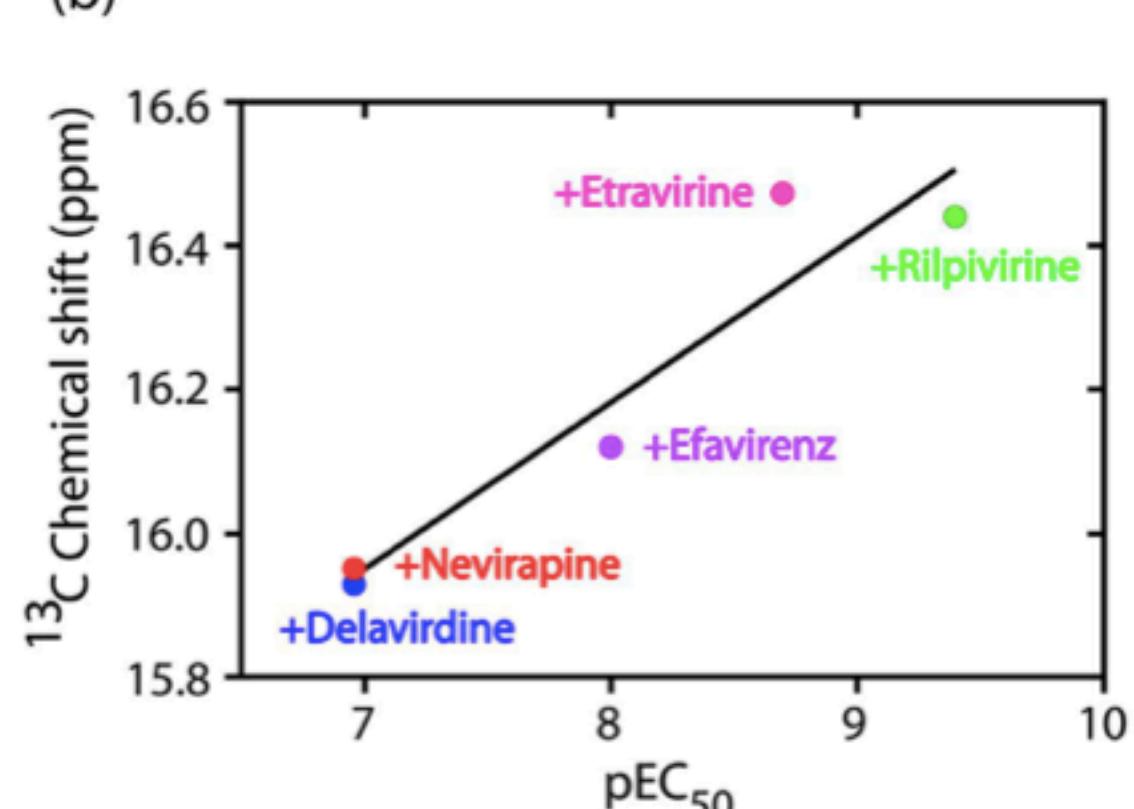
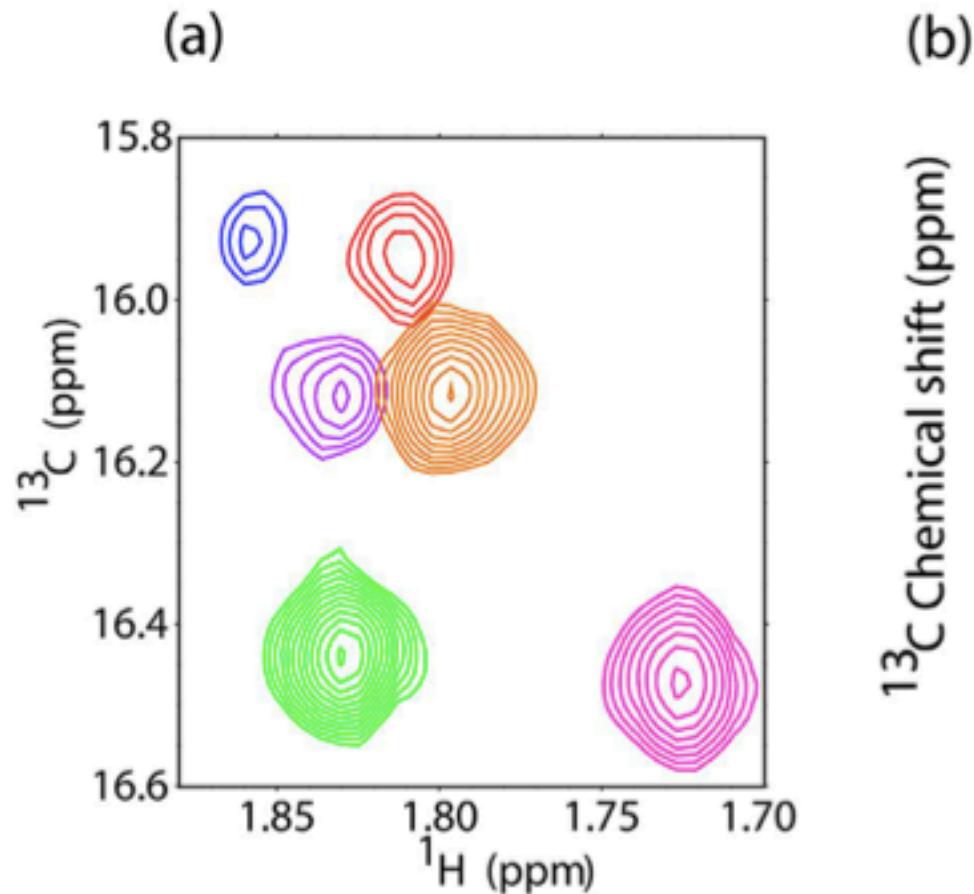
HIV-1 RT



(a) nevirapine, (b) efavirenz, (c) rilpivirine, (d) delavirdine, (e) dapivirine, and (f) etravirine

Thammaporn et al. NMR characterization of HIV-1 reverse transcriptase binding to various non-nucleoside reverse transcriptase inhibitors with different activities. Scientific Reports 5, Article number: 15806 (2015). p. 1-6.

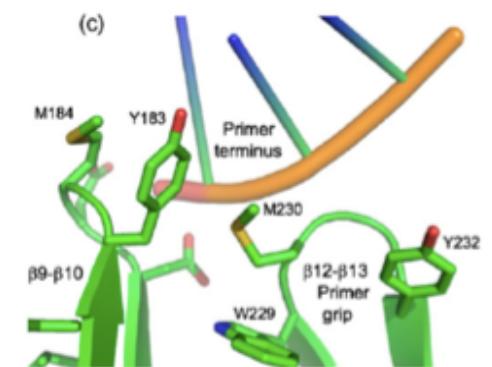
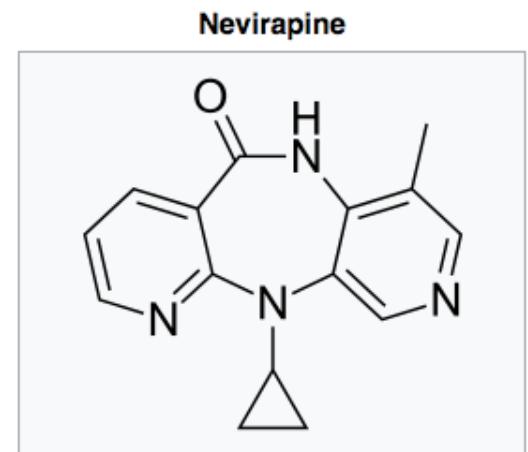
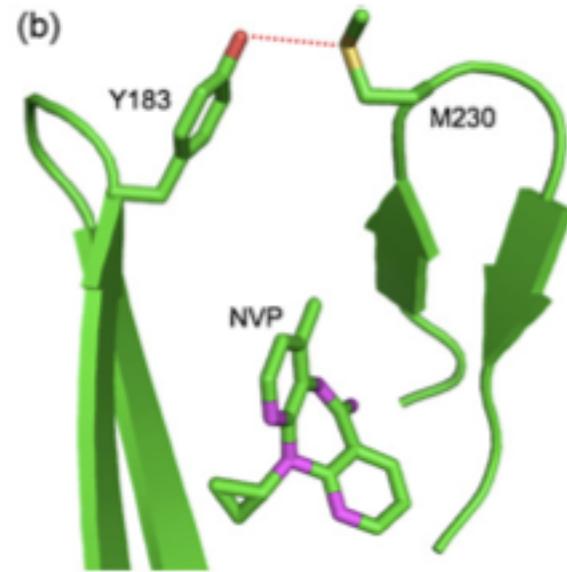
Correlation between chemical shift and effectiveness



(a) nevirapine, (b) efavirenz, (c) rilpivirine, (d) delavirdine, (e) dapivirine, and (f) etravirine

Thammaporn et al. NMR characterization of HIV-1 reverse transcriptase binding to various non-nucleoside reverse transcriptase inhibitors with different activities. Scientific Reports 5, Article number: 15806 (2015). p. 1-6.

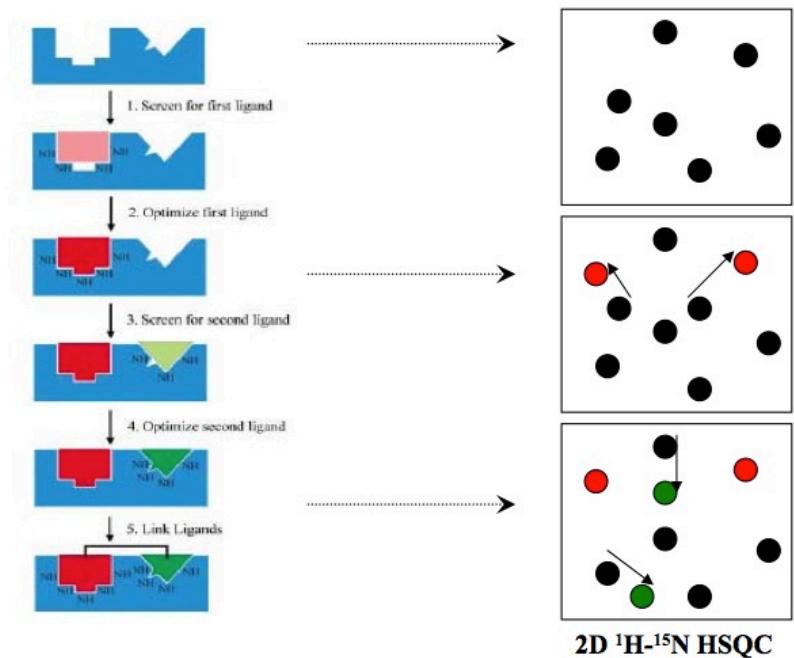
nevirapine effects



Ren et al. High resolution structures of HIV-1 RT from four RT-inhibitor complexes. Nat. Struct. Biol. p. 293-302.

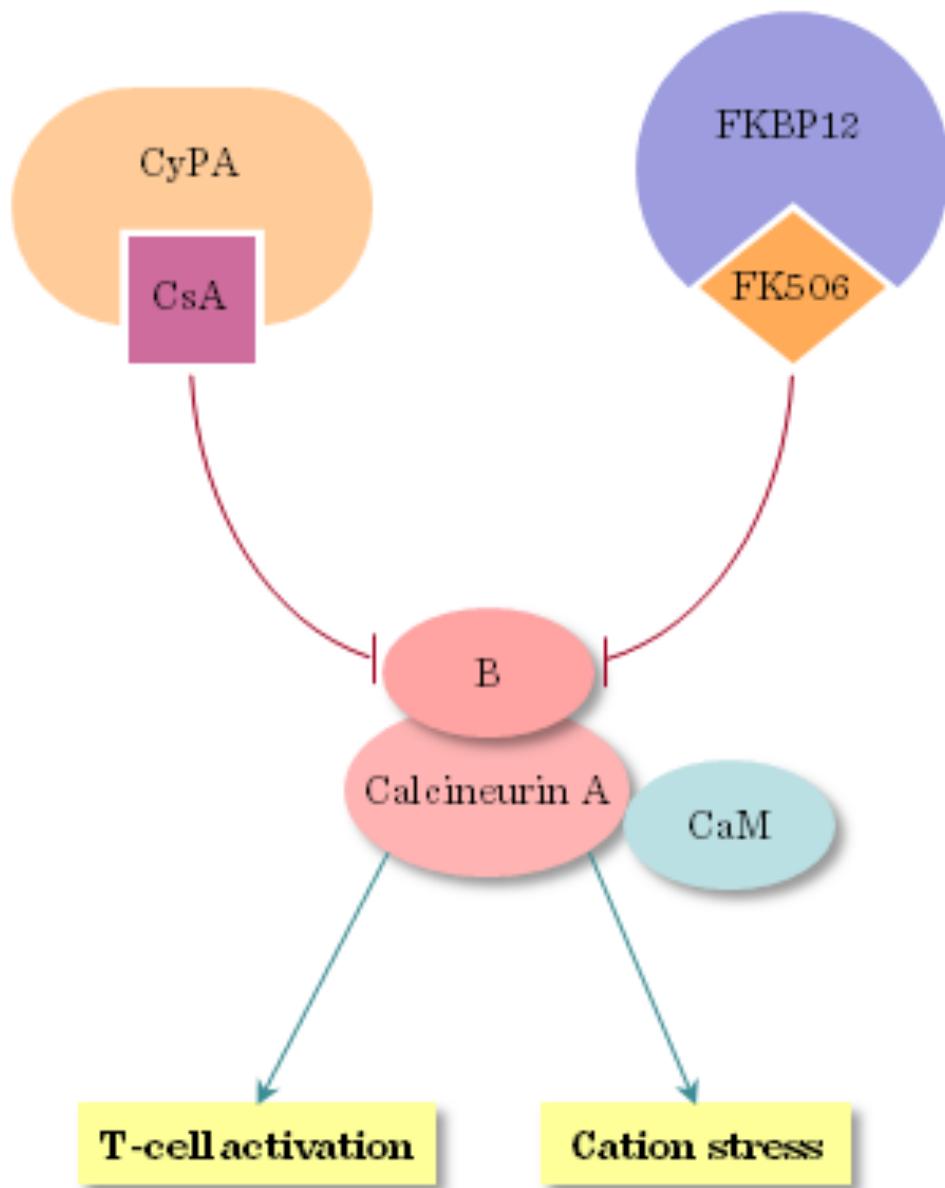
Strategy

1. Relatively High affinity compounds
(big CSP HSQC)
2. Optimize Compound
3. Add 2nd Compound with Optimized Compound (big shifts HSQC)
4. Optimize 2nd Compound
5. Tether Optimized Compounds Together



Shuker, S. B., P. J. Hajduk, R. P. Meadows and S. W. Fesik (1996). "Discovering high-affinity ligands for proteins: SAR by NMR." *Science* 274(5292): 1531-4.

Drug Target: FKBP



Inhibitors

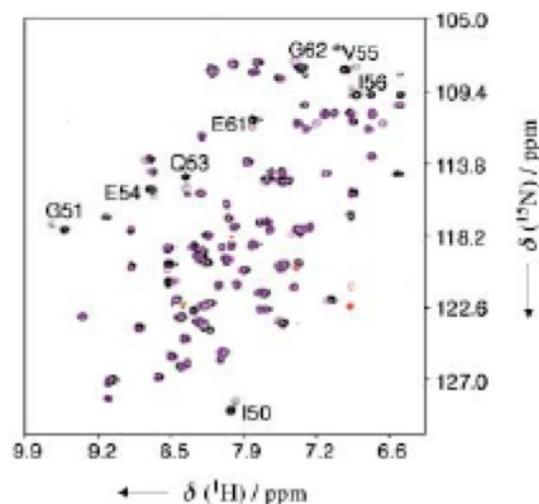
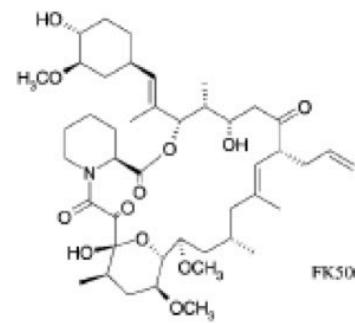
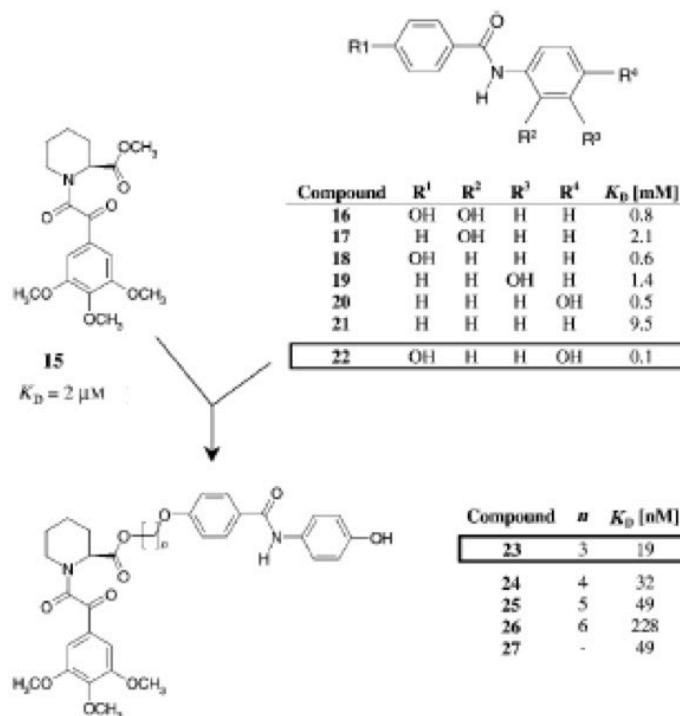
- CsA=Cyclosporin
- FK506=Tacrolimus

Proteins

- CyPA=cyclophilin A
- FKBP=FK506 Binding Protein
- B=Calcineurin B
- CaM=Calmodulin

Shuker et al. used SAR by NMR to screen 1000 substances for FK506-like binding activity. FK506 is an important drug that binds FKBP and suppresses rejection reactions after organ transplants but it has high toxicity. SAR by NMR yielded a tethered ligand with high affinity for FKBP ($K_D=19$ nM).

Example



Shuker, S. B., P. J. Hajduk, R. P. Meadows and S. W. Fesik (1996). "Discovering high-affinity ligands for proteins: SAR by NMR." *Science* **274**(5292): 1531-4.

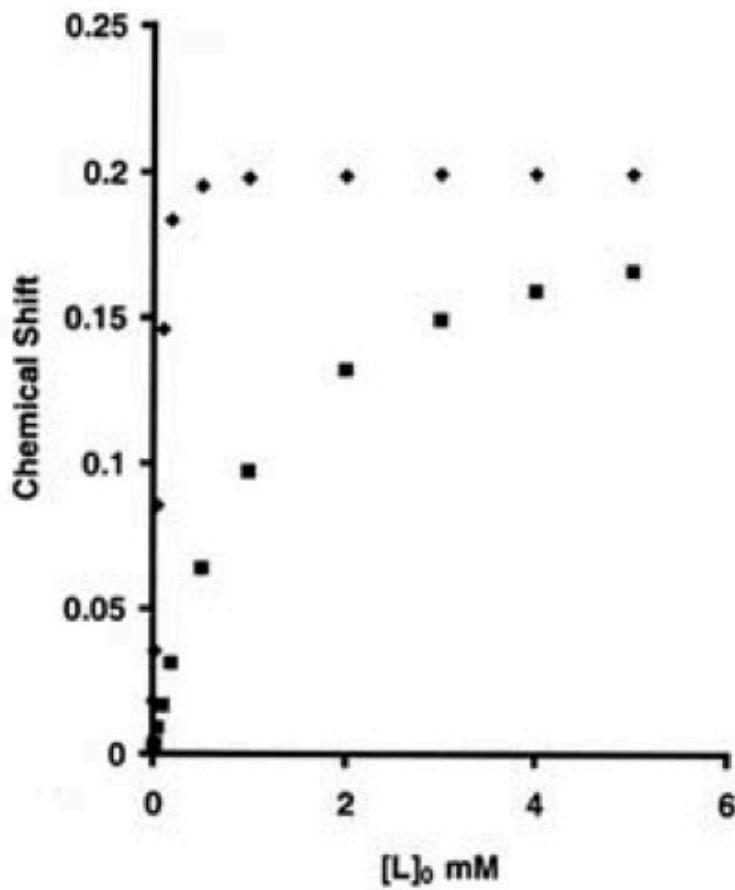
FKBP = FK506 Binding Protein (Protein Folding Chaperone)

Ligand-based NMR Screening

- **Chemical Shift**
- Saturation Transfer Difference
- Relaxation Methods
- Diffusion Editing
- NOE-based Methods
- Residual Dipolar Couplings
- Other

Fig. (1). Simple representation of a typical NMR binding curve. This plot was constructed according to a model of 1:1 binding for a virtual solution containing 0.1 mM protein and a range of ligand concentration from 10 μ M to 5 mM as given on the x axis. Curves for medium strength ($\blacksquare K_D = 1$ mM) and strong binding ($\blacklozenge K_D = 1$ μ M) are shown. In the model system shown here the observed NMR nucleus resides on the protein, and the chemical shift difference between the bound and free states is 0.20 ppm. The curve illustrates the non-linear relationship between $\Delta\delta$ and $[L]_0$.

Chemical Shift



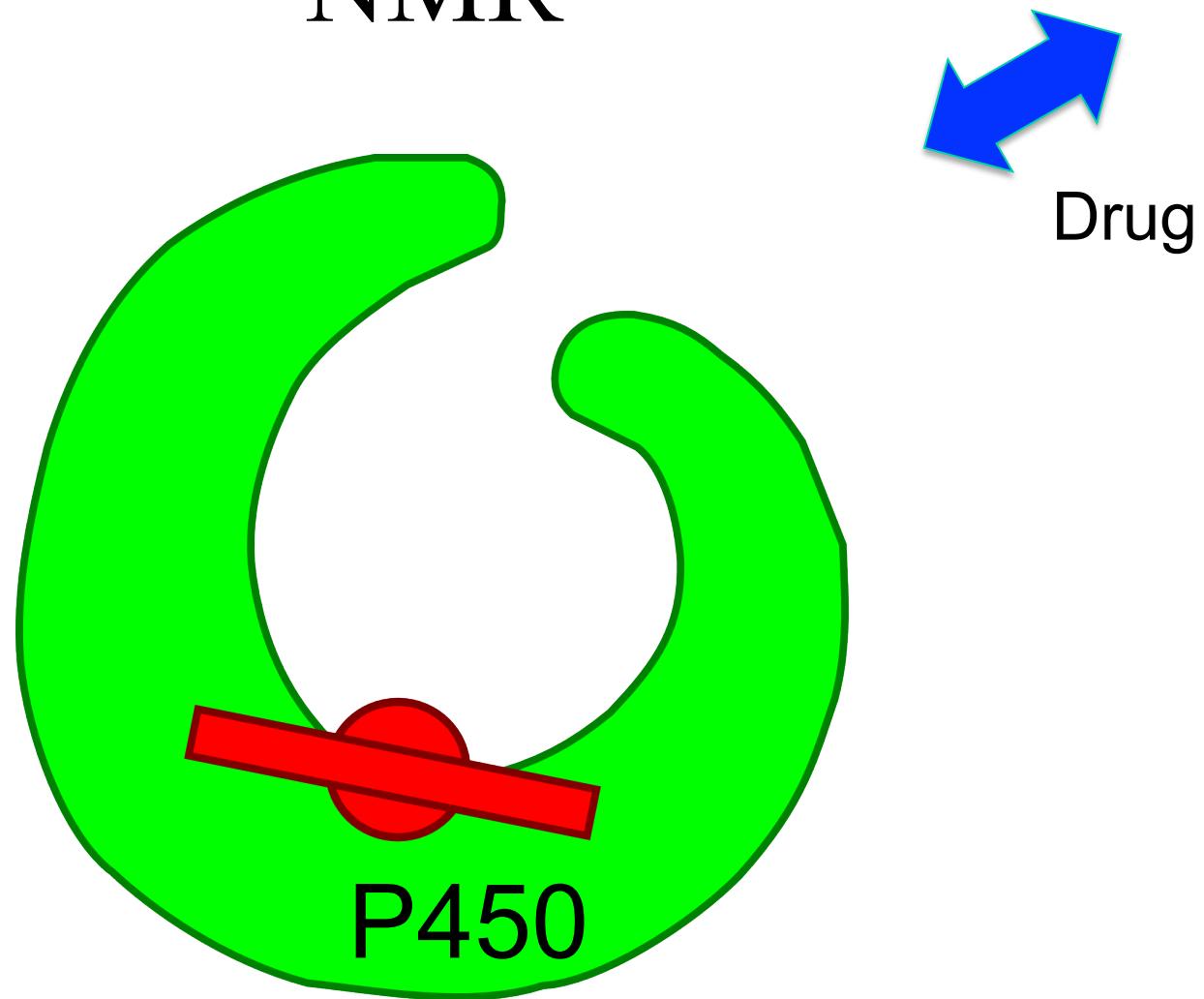
Current Topics in Medicinal Chemistry 2003, 3, 39-53

Ligand-based NMR Screening

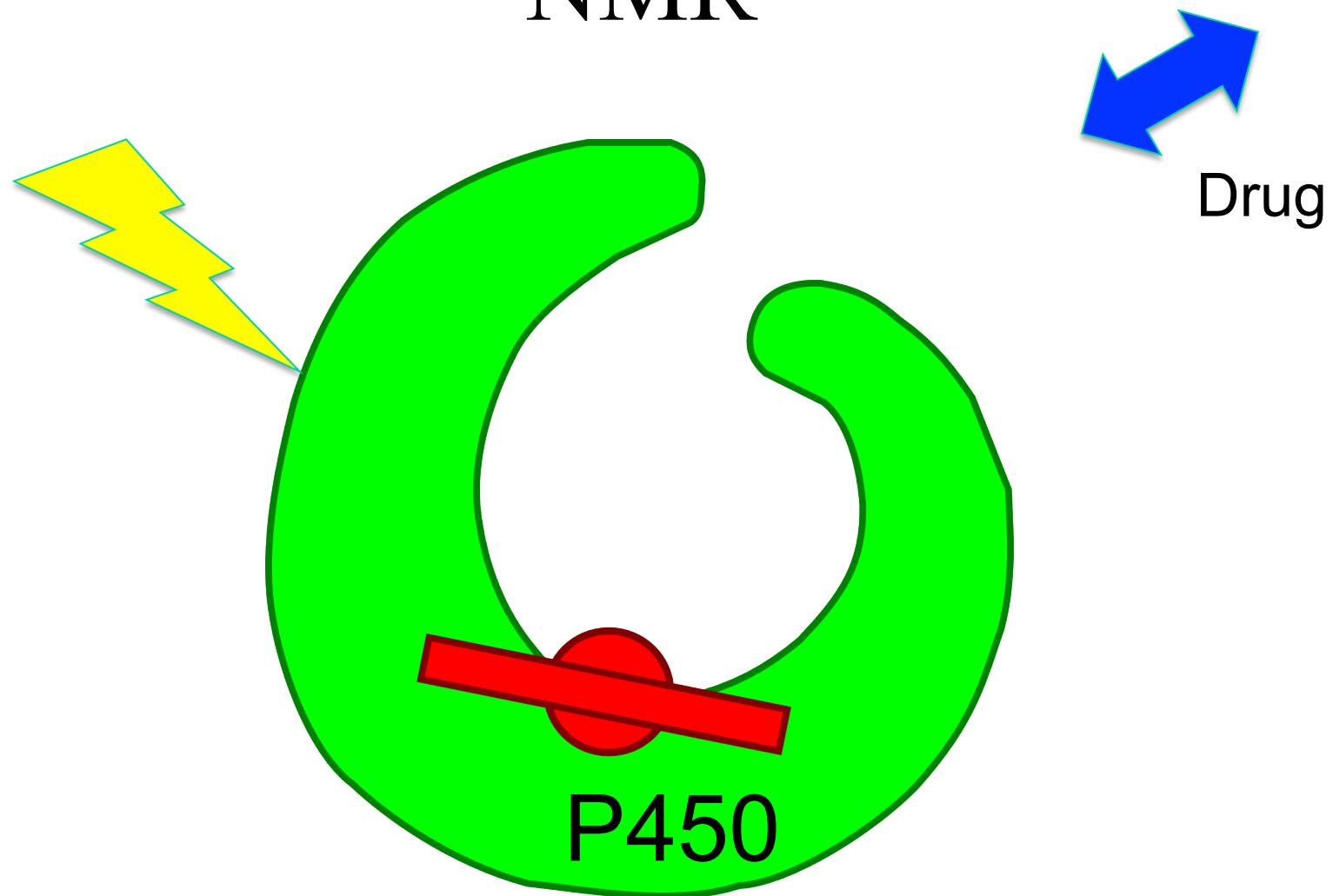
- Chemical Shift
- **Saturation Transfer Difference**
- Relaxation Methods
- Diffusion Editing
- NOE-based Methods
- Residual Dipolar Couplings
- Other

STD involves selectively saturating a resonance that belongs to the receptor (must find a region of the spectrum that contains only resonances from receptor such as 0 ppm to -1 ppm). If ligand binds, saturation will propagate from the selected receptor protons to other protons of receptor via spin diffusion and then the saturation is transferred to binding compounds by cross relaxation at the ligand-receptor interface. A control experiment is done by saturating a region of the spectrum that does not contain signal. The resulting difference spectrum yields only those resonances that have experienced saturation, namely those of the receptor and those of the compound that binds to the receptor. The receptor is typically present at very small concentrations so its resonances will not be visible.

Saturation Transfer Difference (STD) NMR

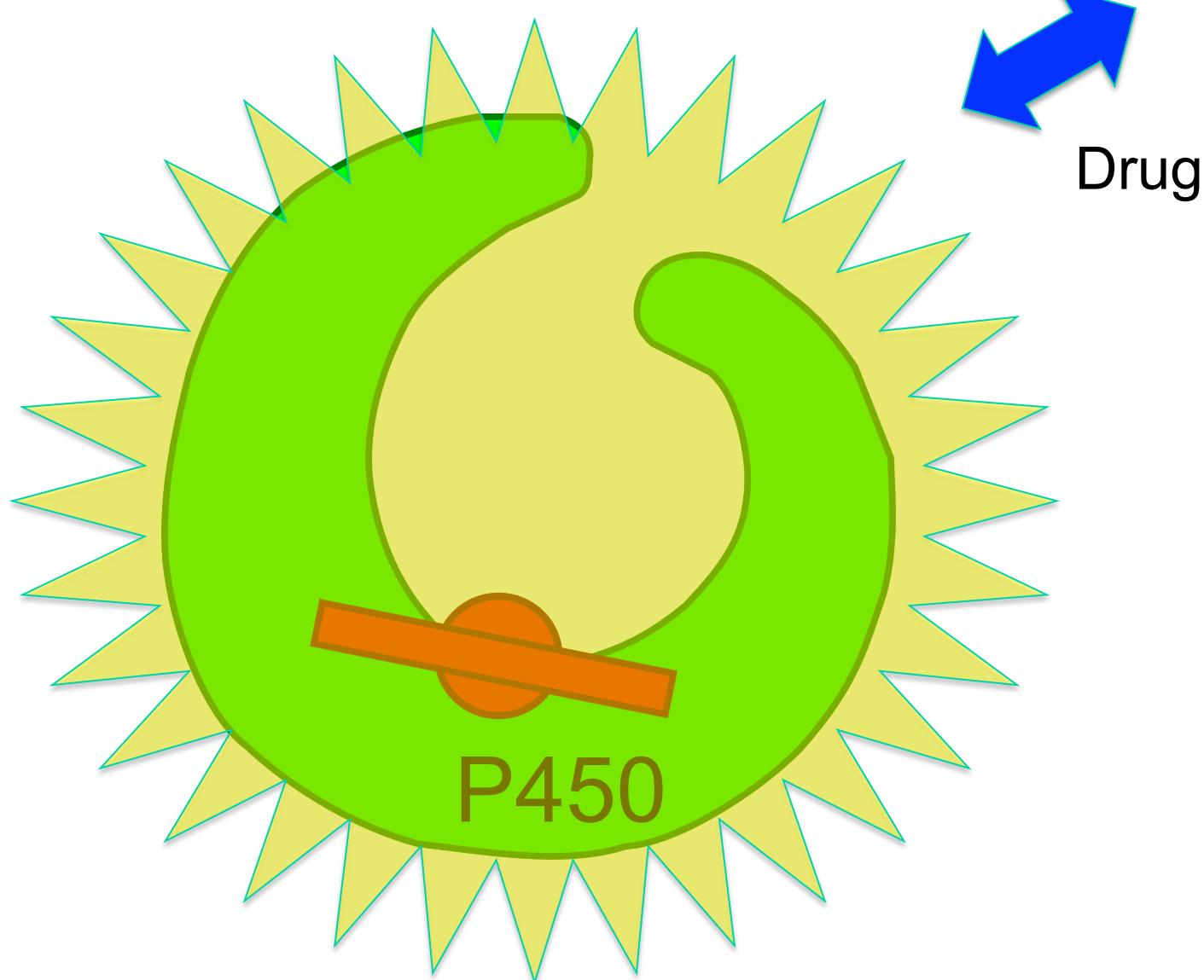


Saturation Transfer Difference (STD) NMR



Saturation Transfer Difference (STD)

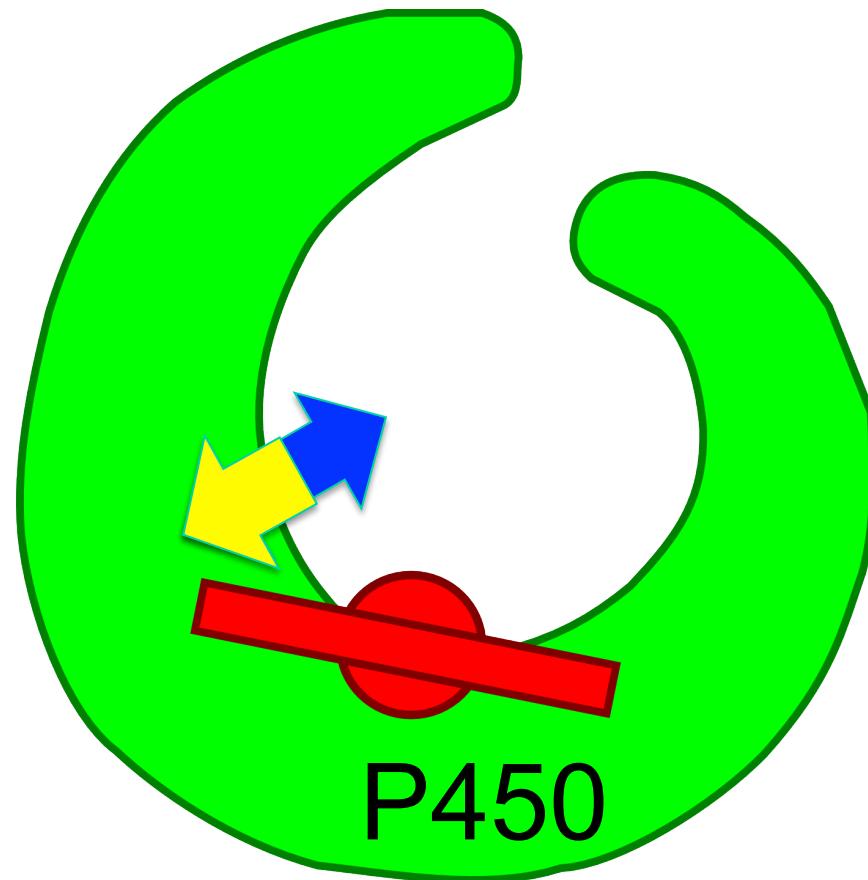
NMR



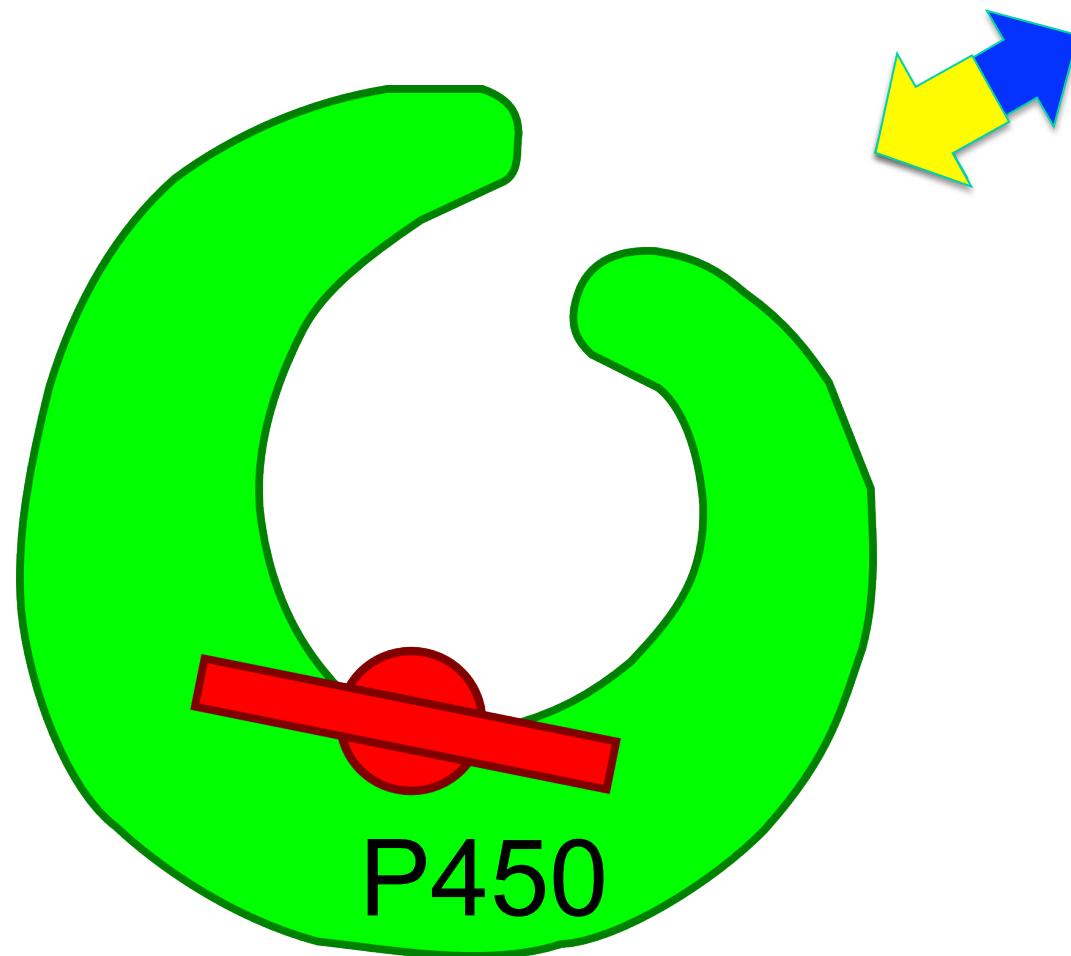
Saturation Transfer Difference (STD) NMR



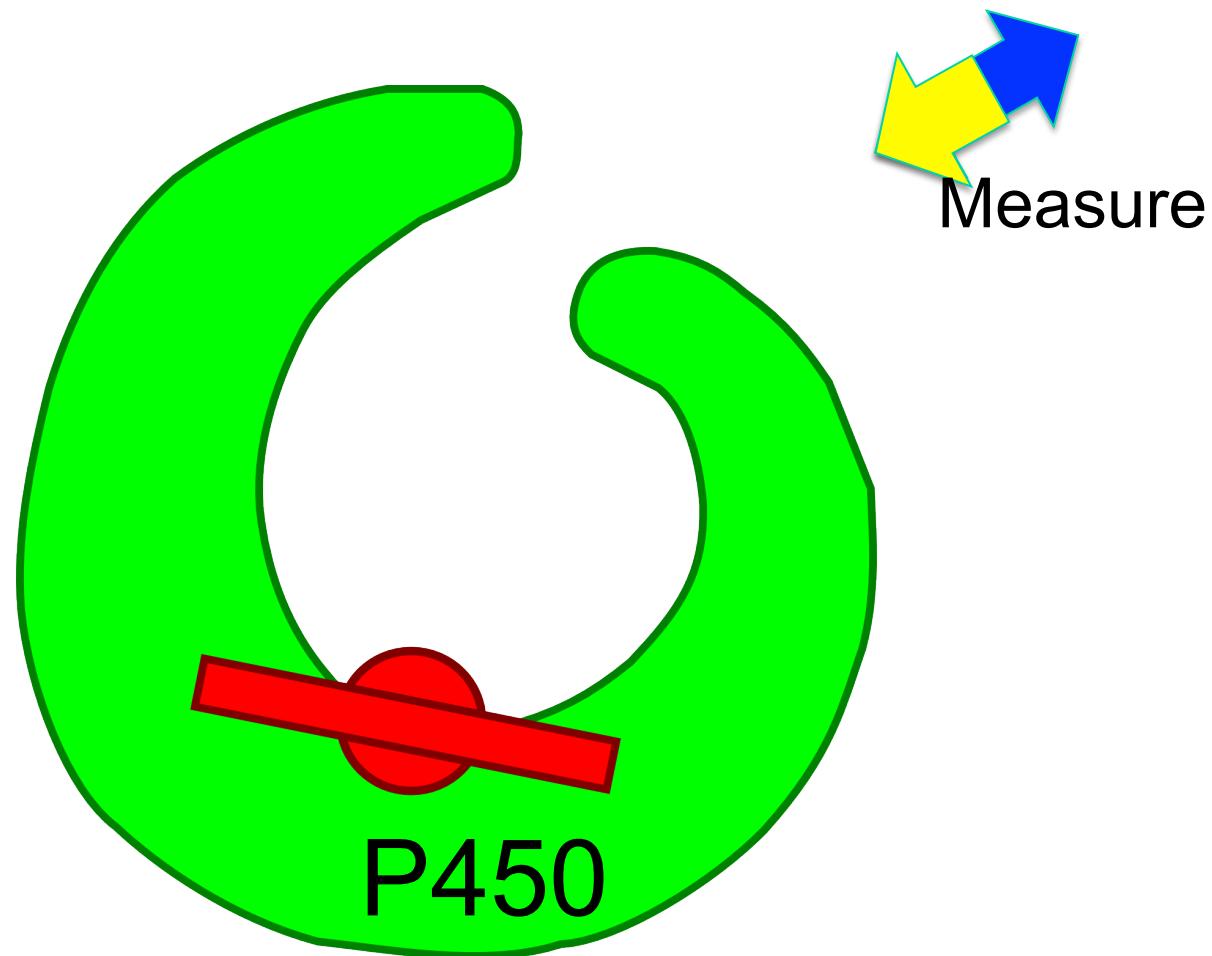
Saturation Transfer Difference (STD) NMR



Saturation Transfer Difference (STD) NMR



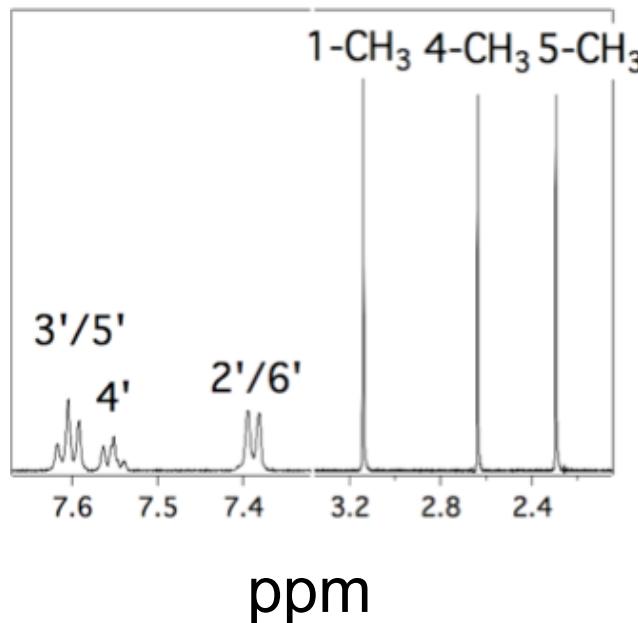
Saturation Transfer Difference (STD) NMR



Protein-Ligand Interactions: Saturation Transfer Difference (STD) NMR

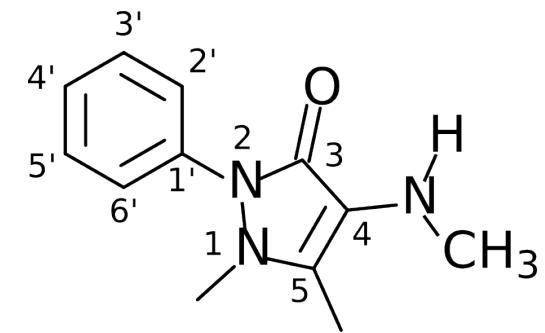
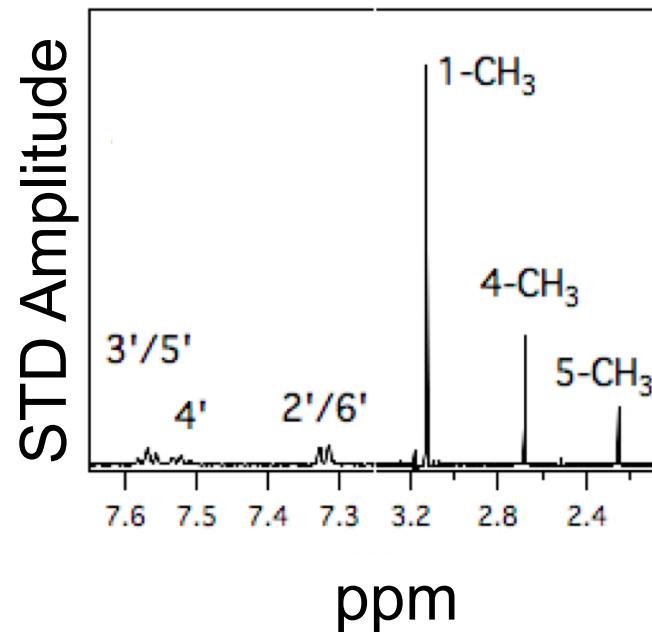
Proton NMR

NMR Amplitude



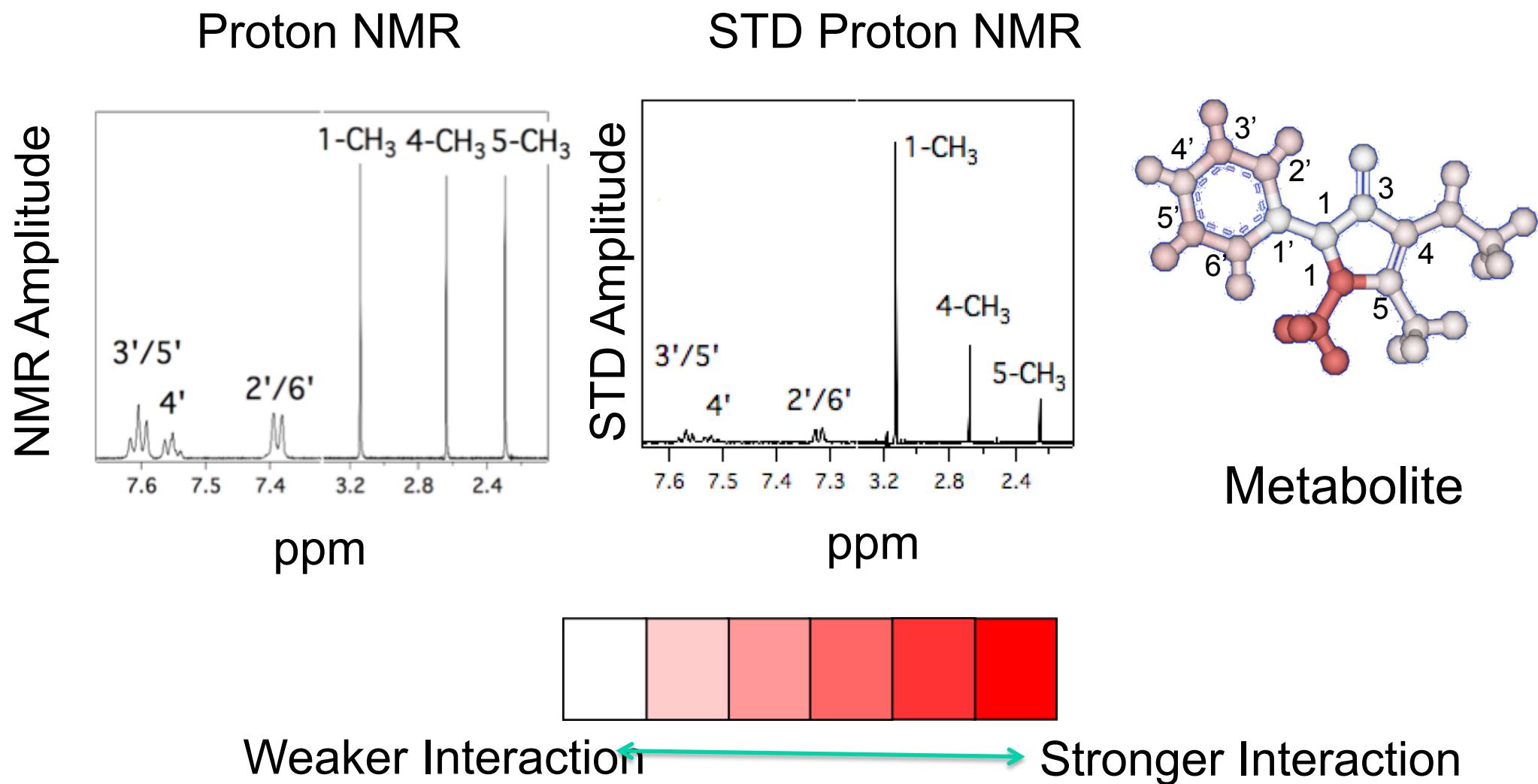
STD Proton NMR

STD Amplitude

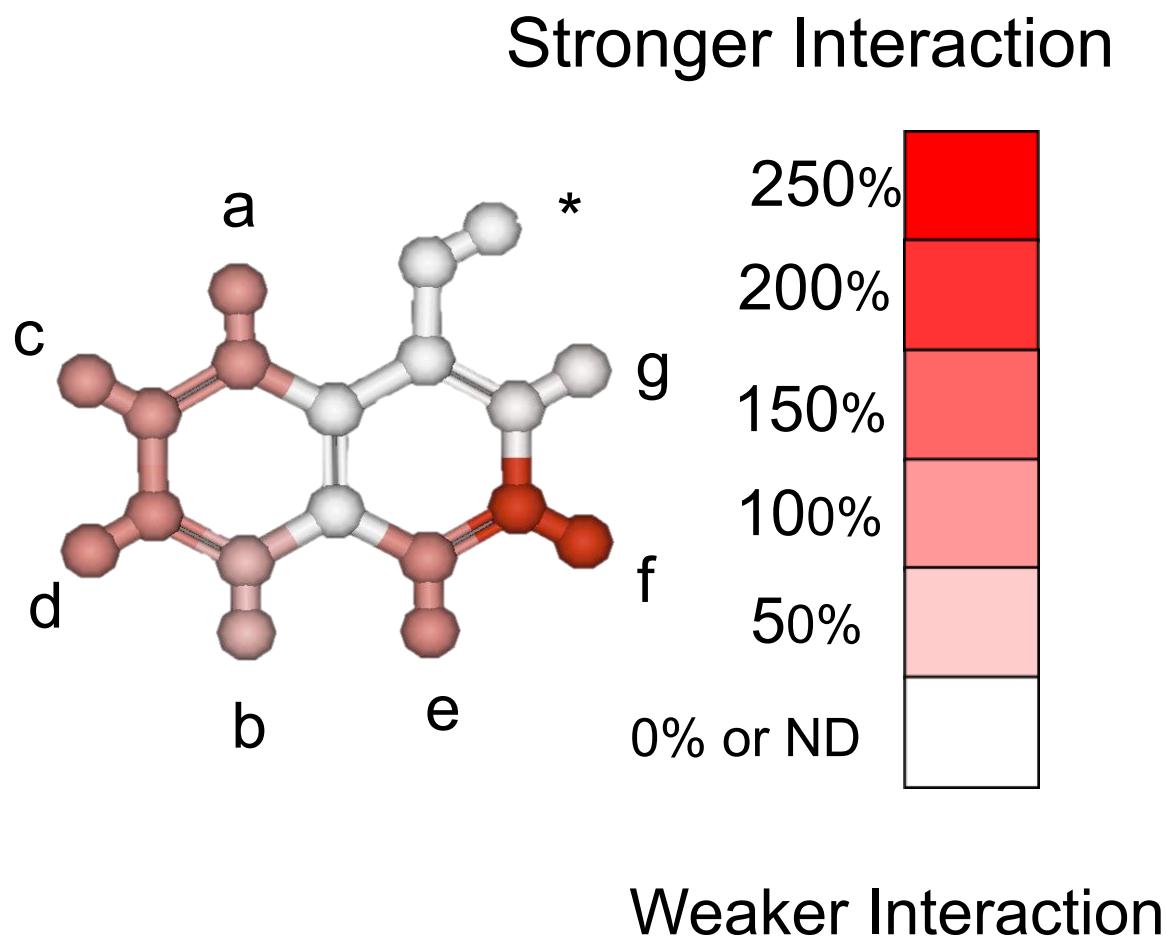
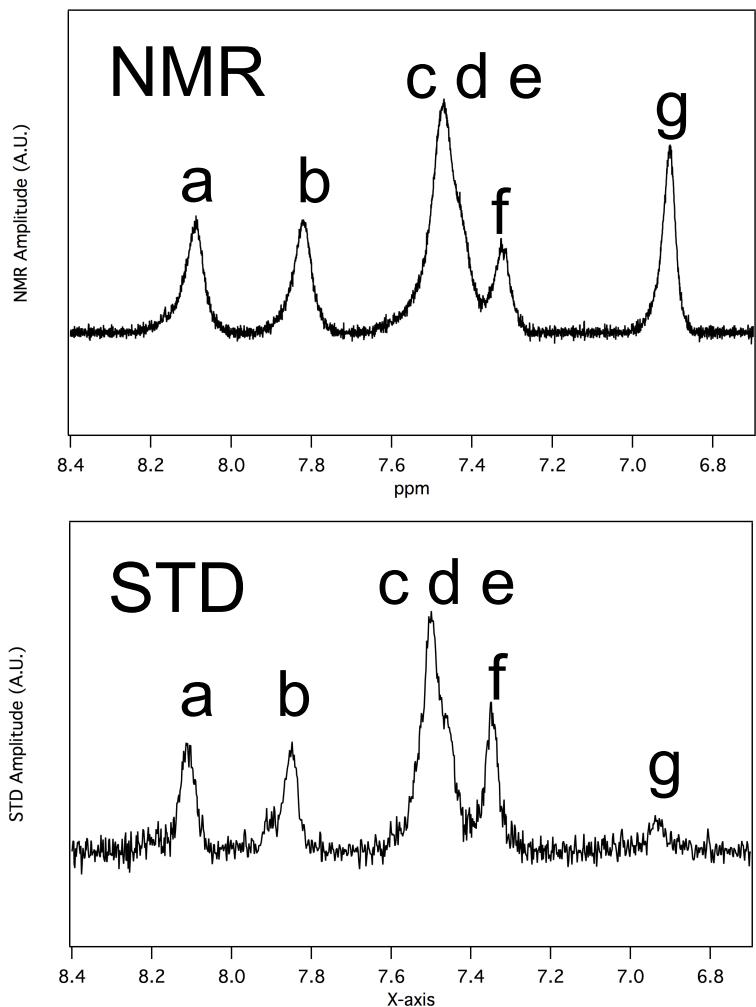


Metabolite

Protein-Ligand Interactions: Saturation Transfer Difference (STD) NMR



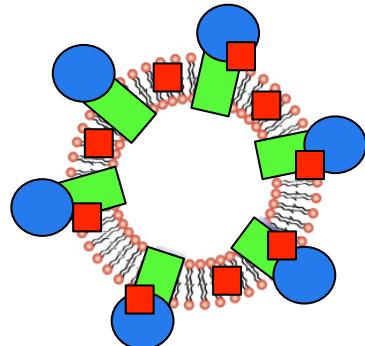
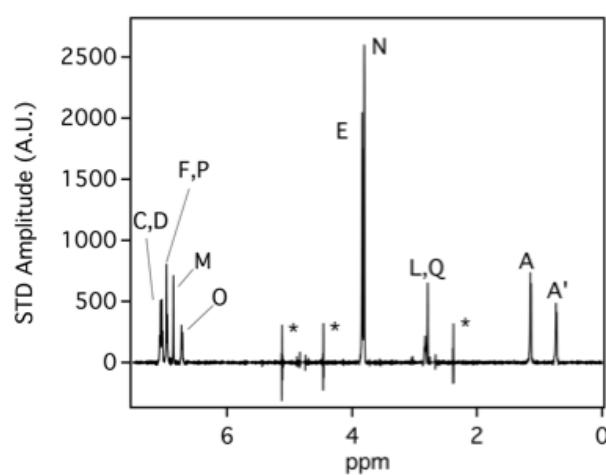
STD NMR: Whole Cell NMR



Sf9 cells with UGT1A1 and Substrate (1-naphthol)

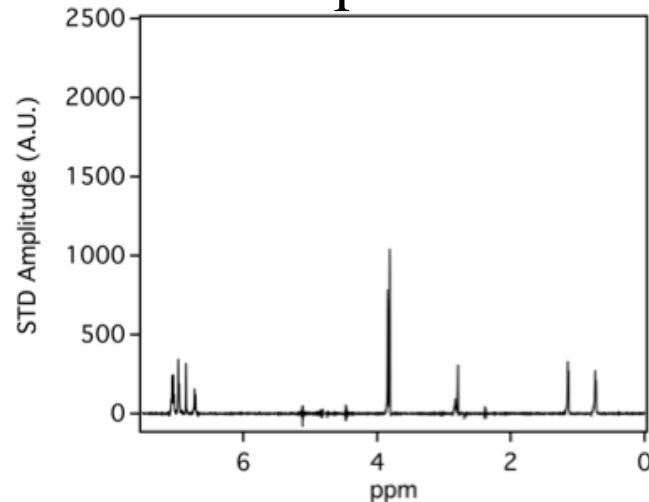
Saturation Transfer Double Difference (STDD) NMR

drug (verapamil)
and proteoliposome

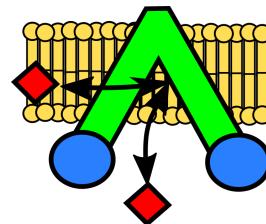
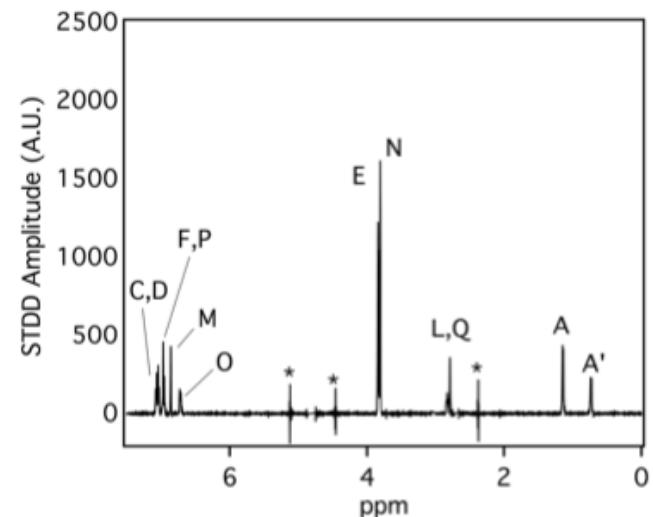


- Transporter
- Drug
- Liposome

drug (verapamil)
and liposome



drug (verapamil)-transporter
interactions



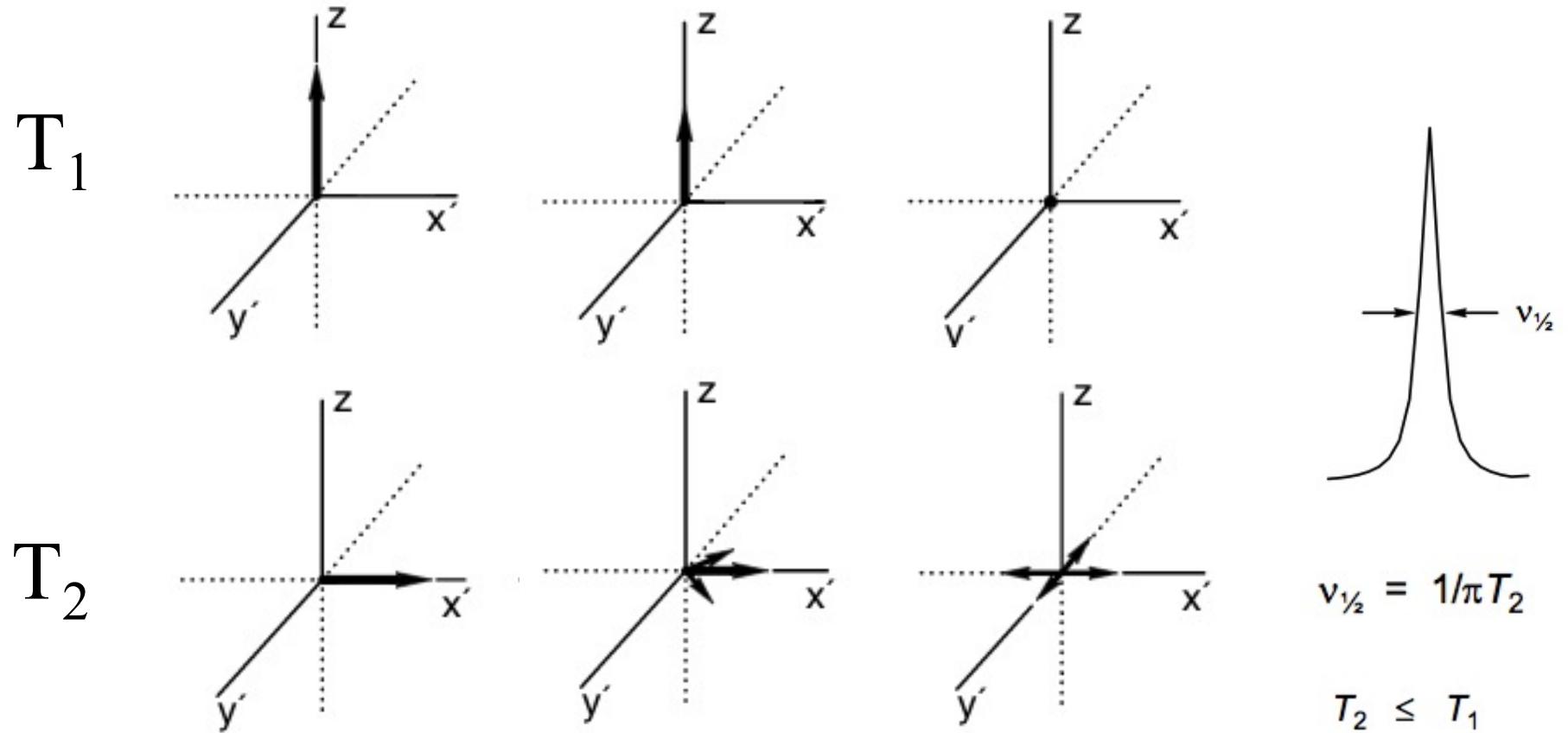
L

N

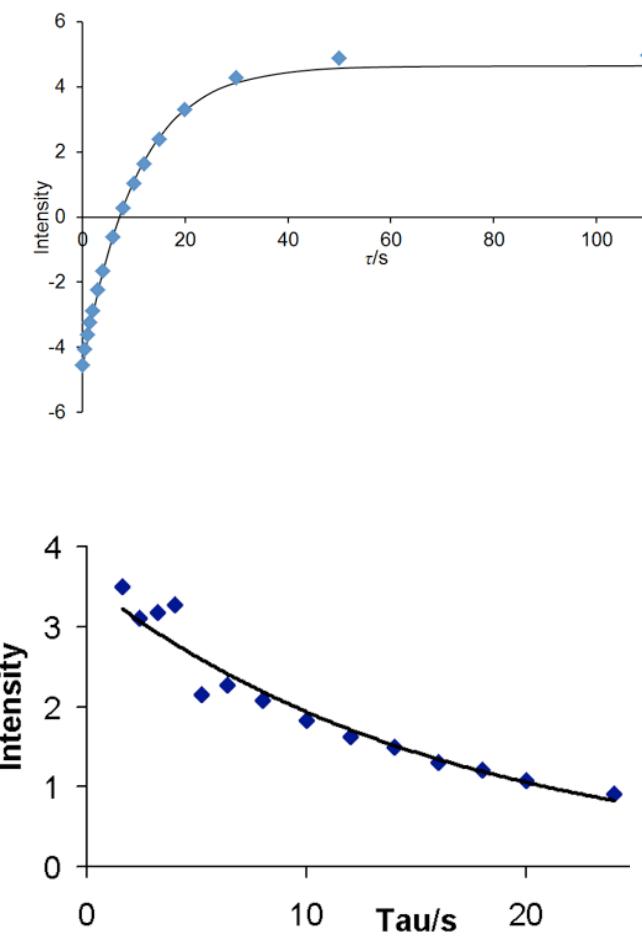
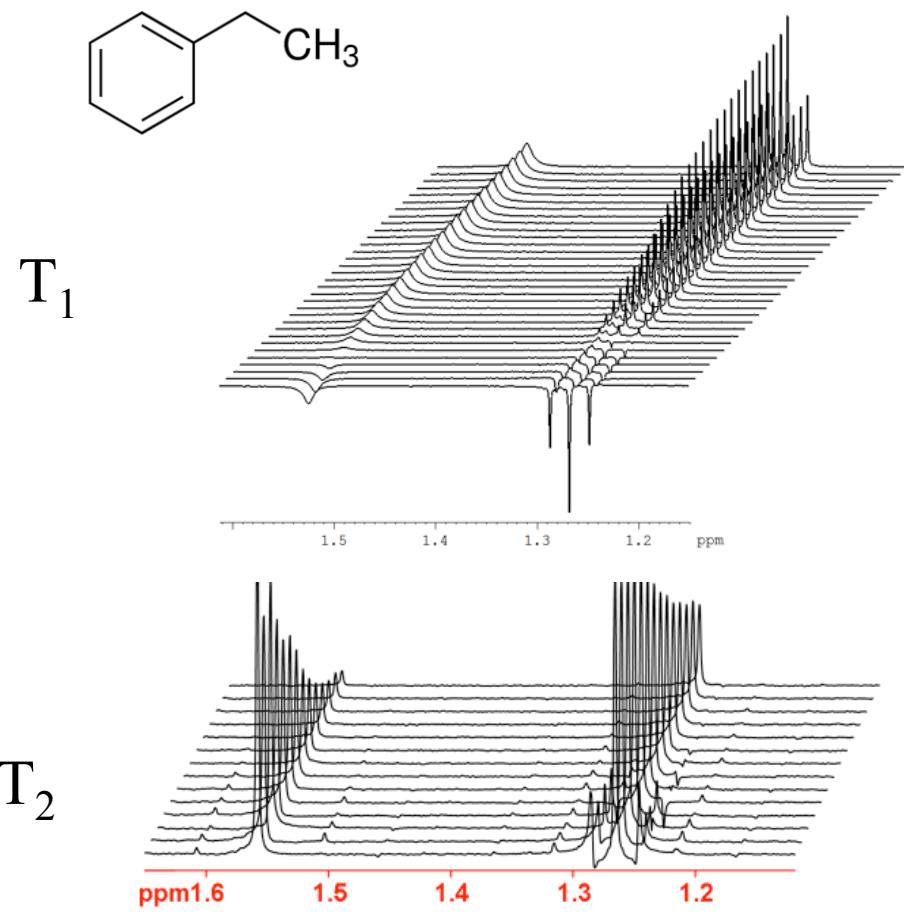
Ligand-based NMR Screening

- Chemical Shift
- Saturation Transfer Difference
- **Relaxation Methods**
- Diffusion Editing
- NOE-based Methods
- Residual Dipolar Couplings
- Other

Relaxation Methods: Types of Relaxation

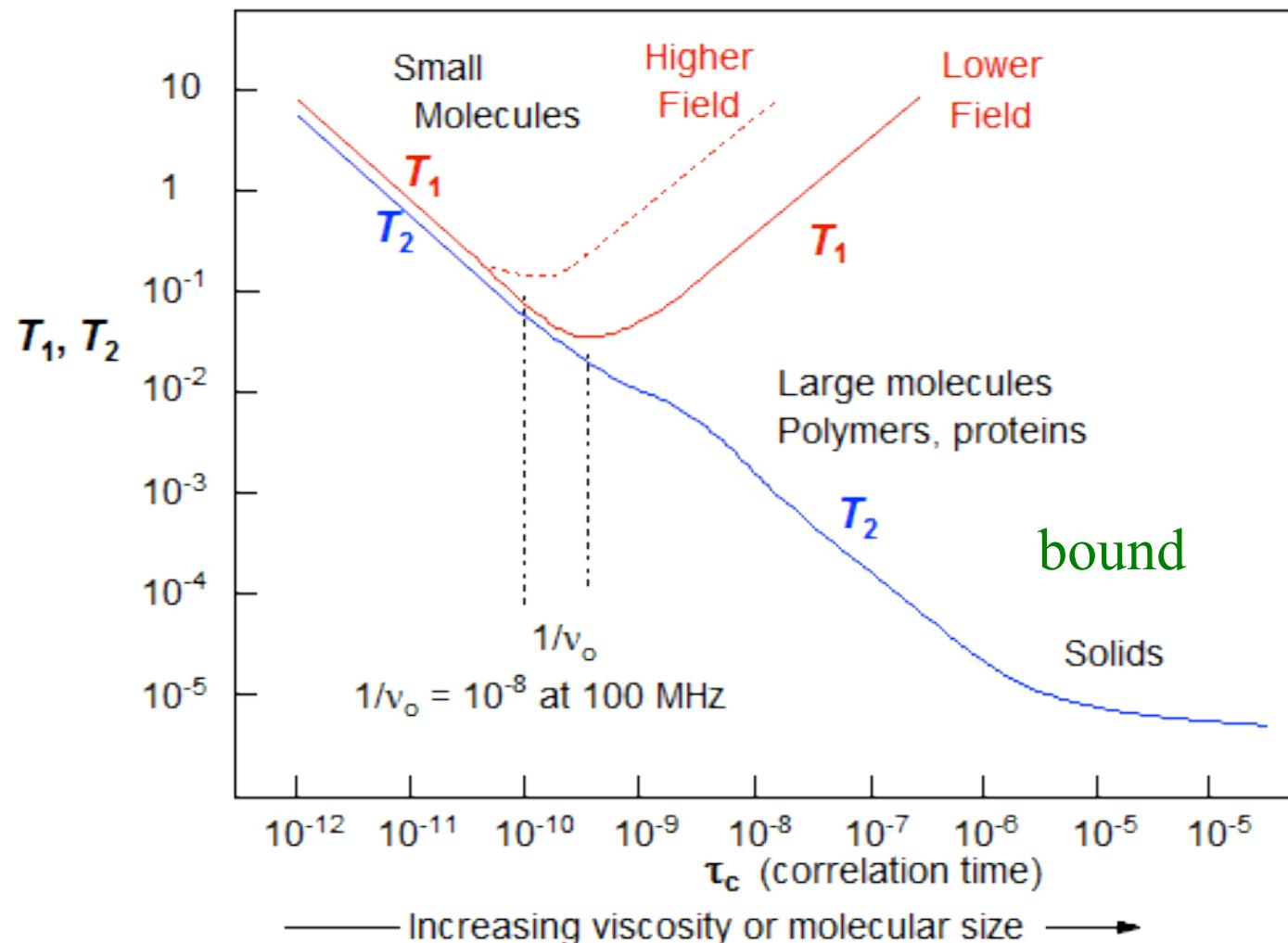


Relaxation Methods: Measurement

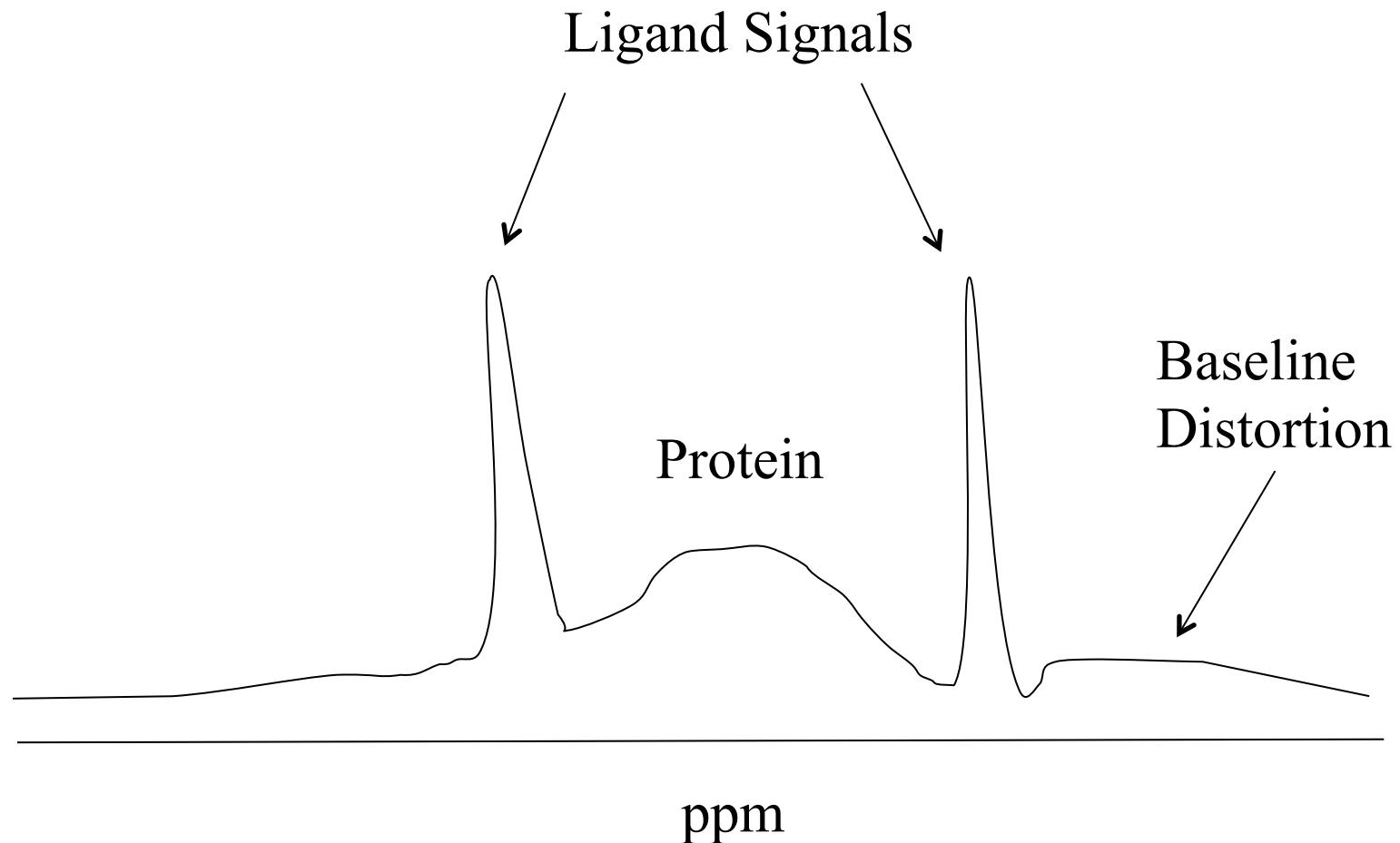


Relaxation Methods

unbound

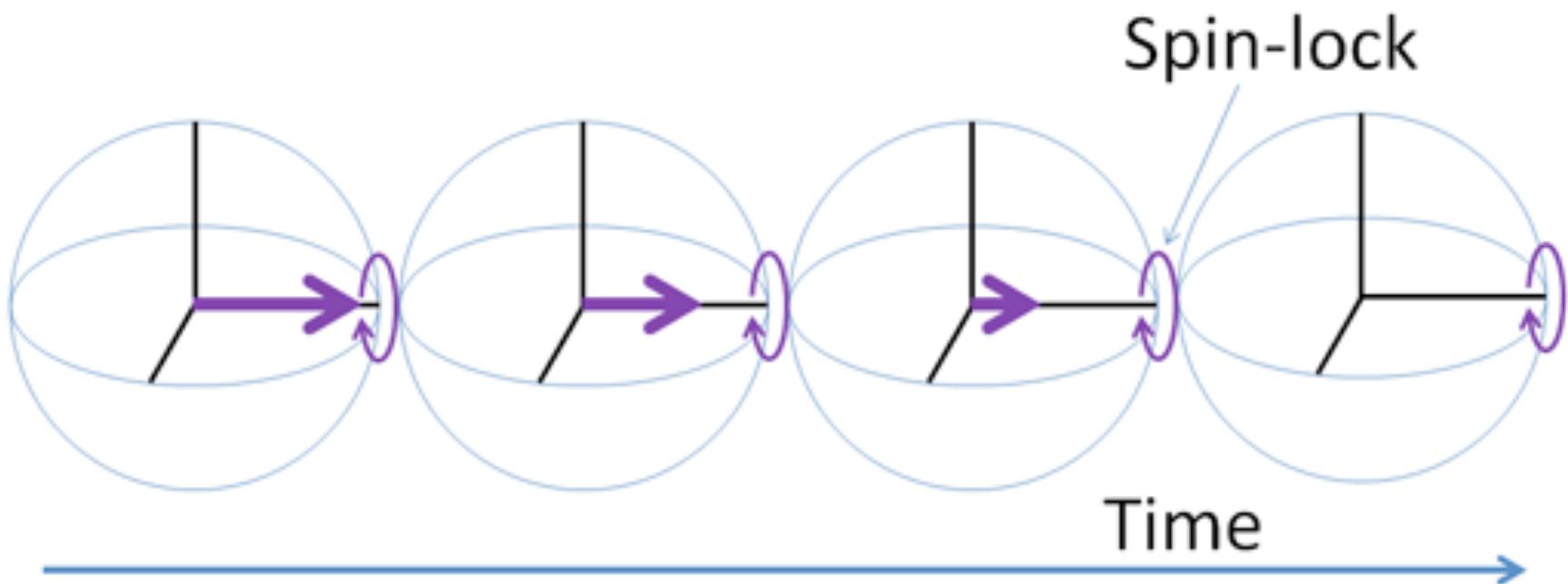


Ligands with Protein



When a ligand is bound to a protein, it behaves as if it is the same size as the protein.

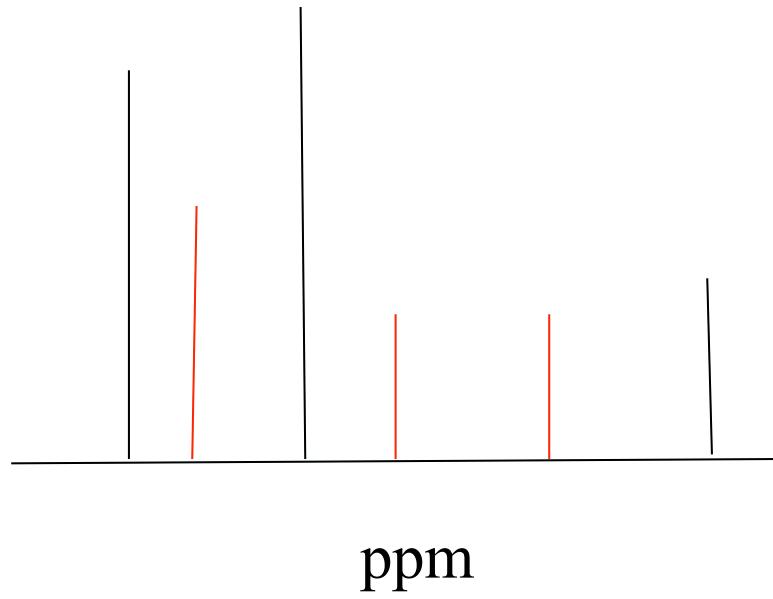
Spin Lock Pulse: Remove Protein Signal



<http://chem.ch.huji.ac.il/nmr/techniques/other/t1t2/t1t2.html>

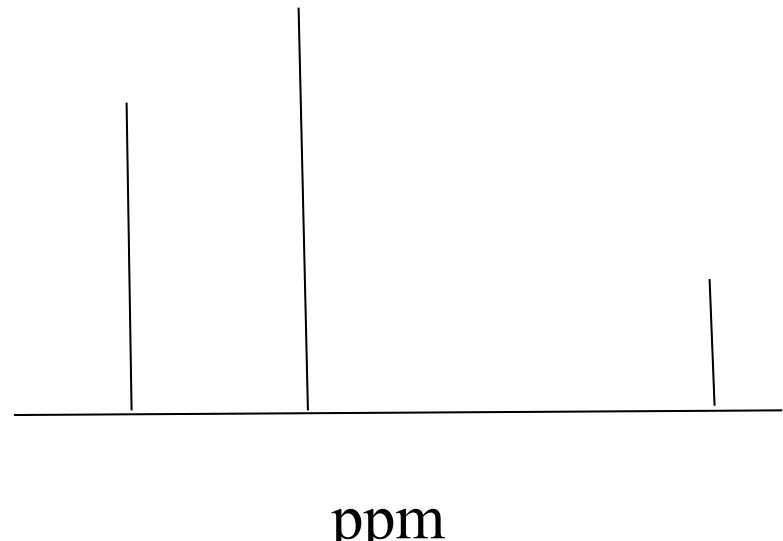
Relaxation Methods: $T_{1\rho}$ filter

Drugs with Protein



without spin lock pulse ($T_{1\rho}$ filter)

Drugs with Protein



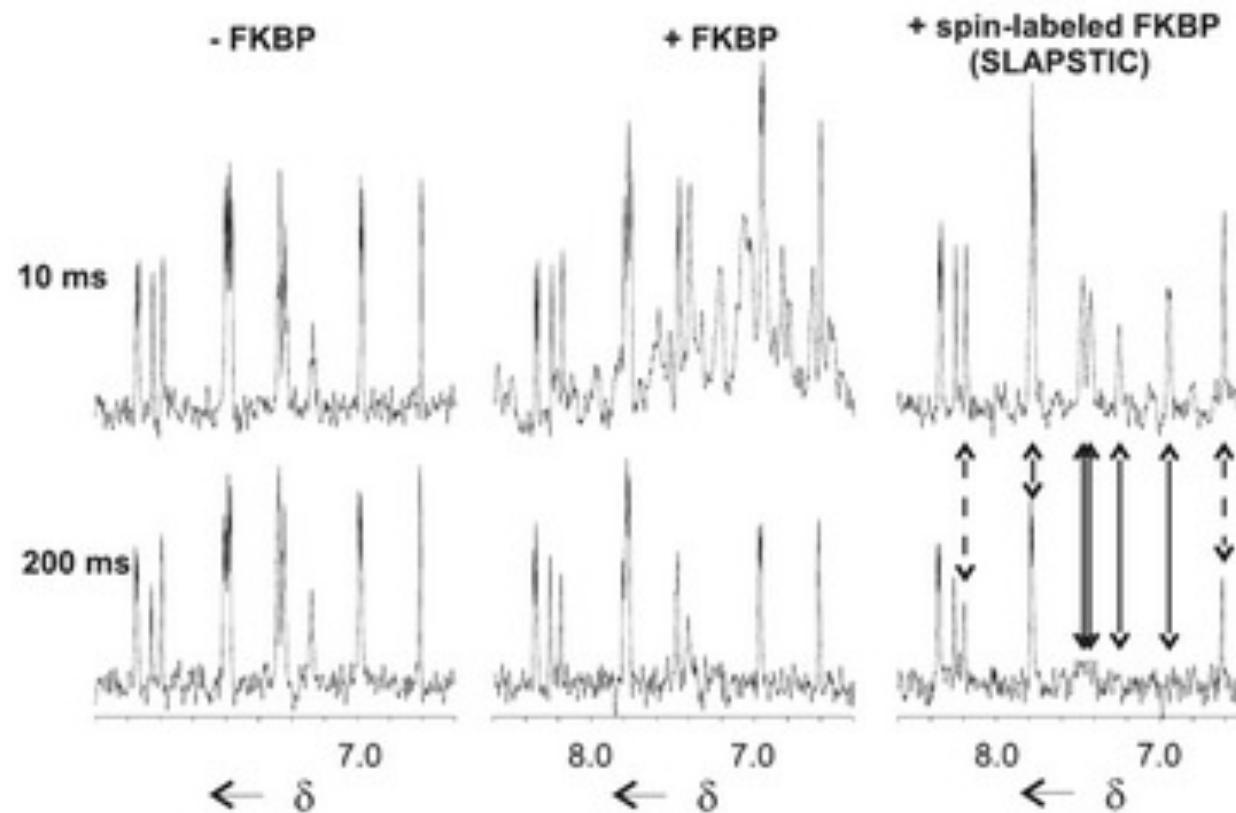
with spin lock pulse ($T_{1\rho}$ filter)

Unbound Ligand
Bound Ligand

Fig. 15.2 $T_{1\rho}$ experiments performed on a mixture of *para*-hydroxy-benzanilide (**1**) and five other compounds with FKBP. Spectra in the upper and lower row correspond to spin-lock periods of 10 ms and 200 ms, respectively. Higher attenuation at 200 ms, as visible in the SLAPSTIC spectra (right) means faster relaxation in the bound state

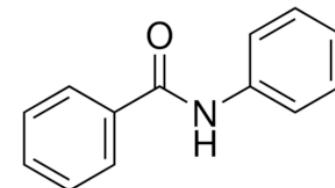
and easier detection of binding. Resonances of **1** are marked with black arrows, resonances of methyl-4-methoxythiophene-3-carboxylate, a compound that binds to FKBP very weakly, are marked with dashed arrows. The remaining signal at 7.8 ppm stems from another compound.

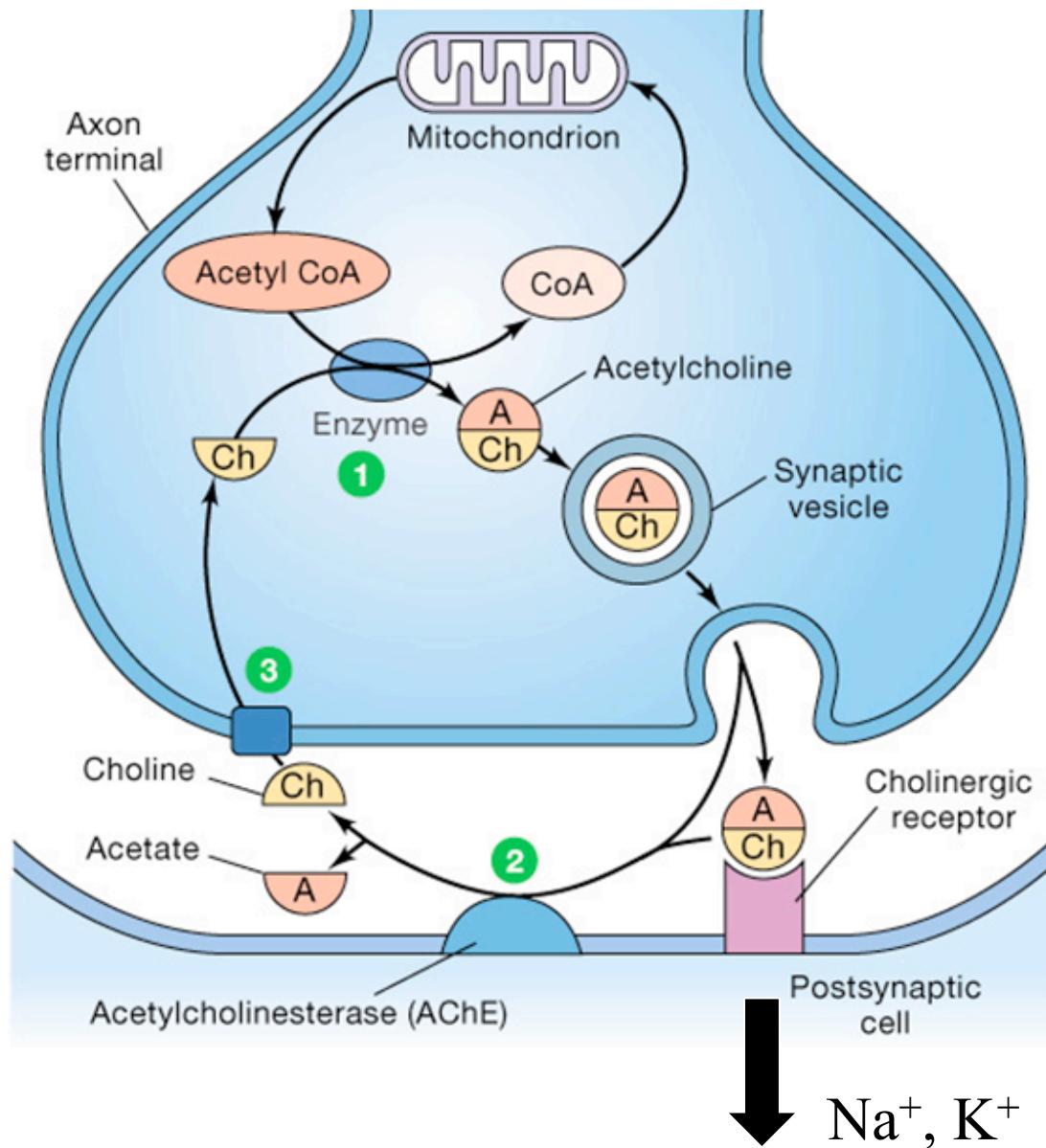
Relaxation Methods: SLAPSTIC (spin labels attached to protein side chains as a tool to identify interacting compounds)



FKBP= FK506 Binding Protein

W. Jahnke, S. Ru'dissor, M. Zurini, J. Am. Chem. Soc. 123
(2001) 3149.





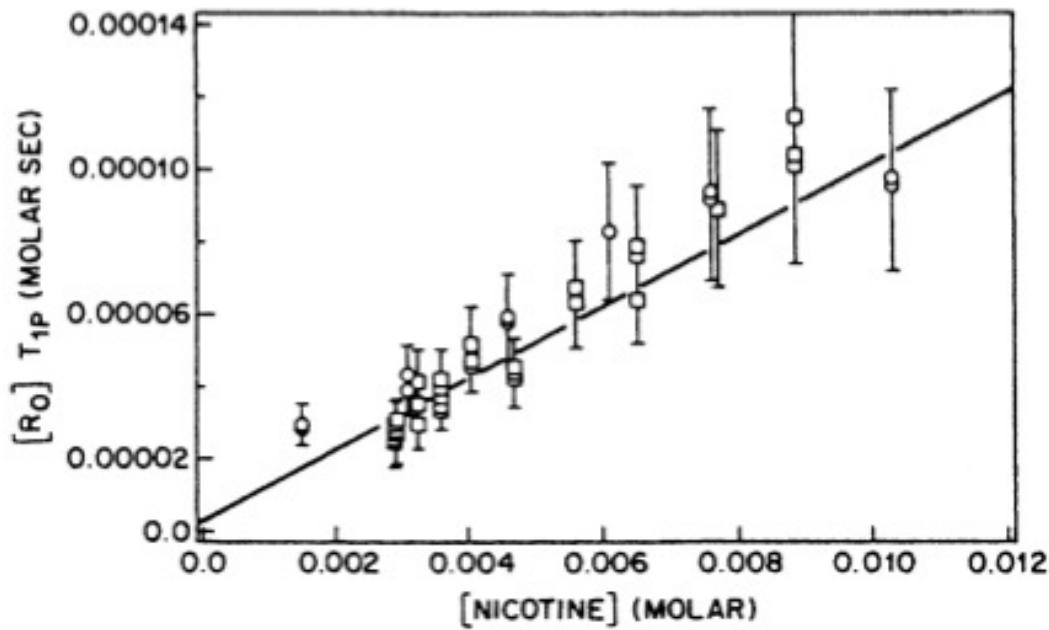
1 Acetylcholine (ACh) is made from choline and acetyl CoA.

2 In the synaptic cleft ACh is rapidly broken down by the enzyme acetylcholinesterase.

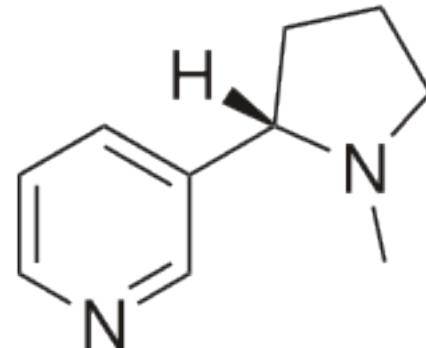
3 Choline is transported back into the axon terminal and is used to make more ACh.

Fig. 6. Plot of nicotine concentration versus $[P]_0 T_{1(\text{sel})}$ for multiple measurements on two different acetylcholine receptor preparations (circles and squares). The pyridinyl H-4 proton of nicotine was used for the relaxation measurements. Similar results were obtained from the other aromatic protons. The data show the typical scatter in the selective T_1 measurements, and estimated error bars [61]. Reproduced with permission. © 1988 Biophysical Society.

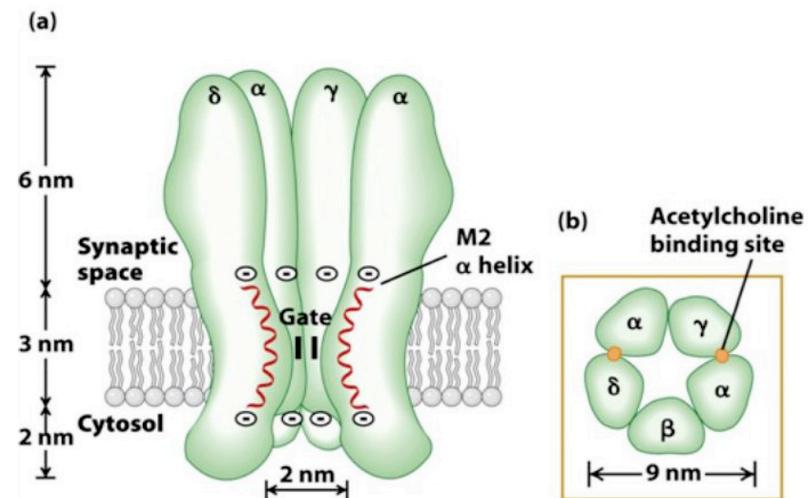
Relaxation Methods: T_1



$$T_{1P} = [(1/T_{1obs}) - (1/T_{1free})]^{-1}$$



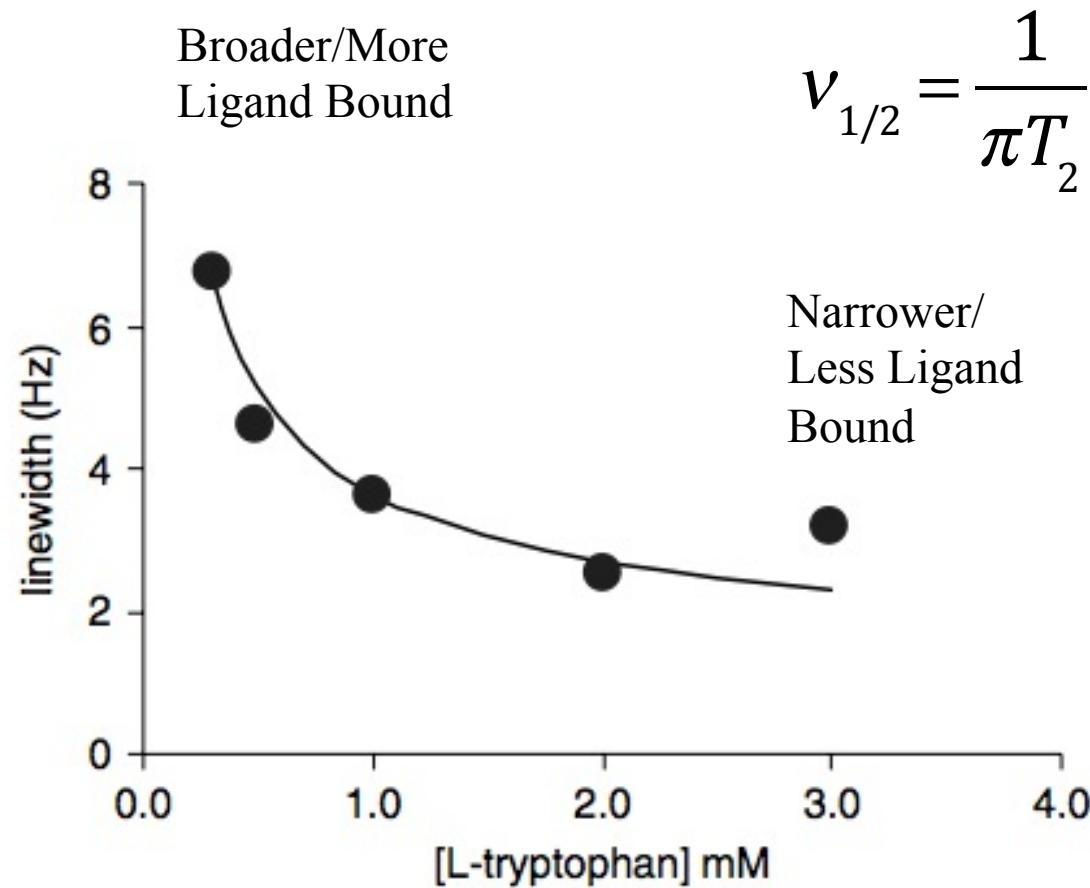
nicotine



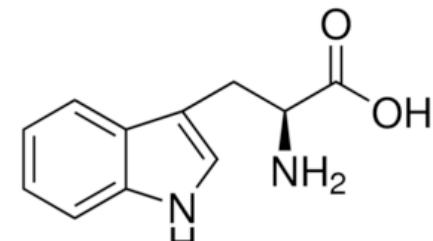
Behling et al., Measuring Relative Acetylcholine Receptor Agonist Binding by Selective Proton Nuclear Magnetic Resonance Relaxation Experiments. Biophys. J. 53 (1988) 947-954.

Figure 2. The L-tryptophan–BSA system as an example of a binding isotherm constructed from NMR linewidth data. The linewidth of H-4 is shown as a function of [L-tryptophan] at constant $[BSA] = 100 \mu\text{M}$. The ligand is in fast exchange between the free and bound states, so the observed linewidth is the mole fraction-weighted average of the free and bound forms. At high [L-tryptophan] the contribution of ν_{bound} becomes insignificant and the linewidth approaches values typical of small molecules. At low [L-tryptophan] the contribution of ν_{bound} is increasingly influential and the linewidth rises rapidly. The solid line is the calculated binding curve for a single BSA binding site ($n = 1$), $\nu_{\text{free}} = 1.6 \text{ Hz}$, $\nu_{\text{bound}} = 23 \text{ Hz}$ and $K_D = 107 \mu\text{M}$.

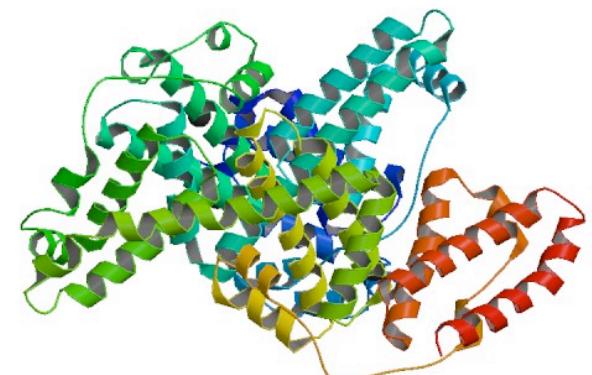
Relaxation Methods: T_2



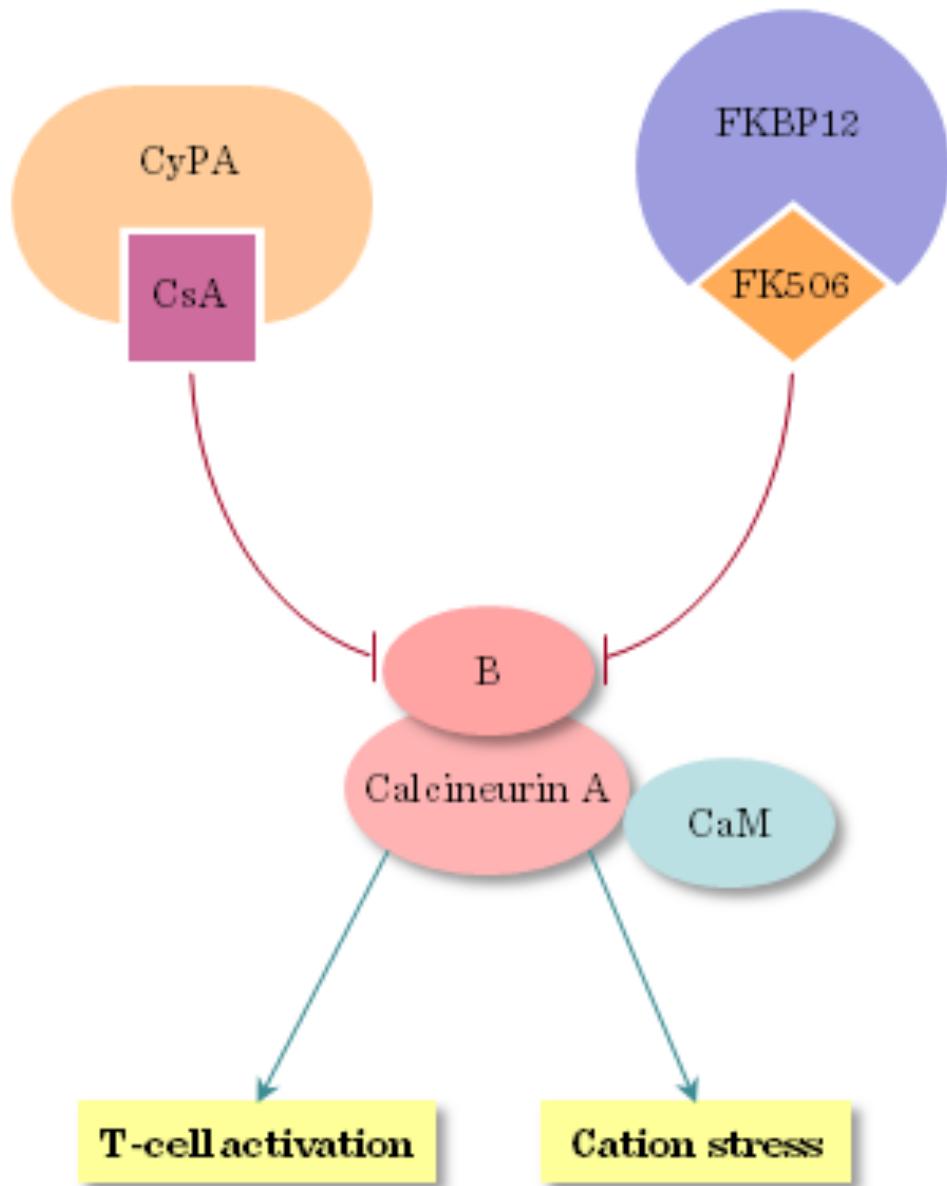
$$\nu_{1/2} = \frac{1}{\pi T_2}$$



L-tryptophan



bovine serum albumin



Inhibitors

- CsA=Cyclosporin
- FK506=Tacrolimus

Proteins

- CyPA=cyclophilin A
- FKBP=FK506 Binding Protein
- B=Calcineurin B
- CaM=Calmodulin

Ligand-based NMR Screening

- **Chemical Shift**
- **Saturation Transfer Difference**
- **Relaxation Methods**
- Diffusion Editing
- NOE-based Methods
- Residual Dipolar Couplings
- Other