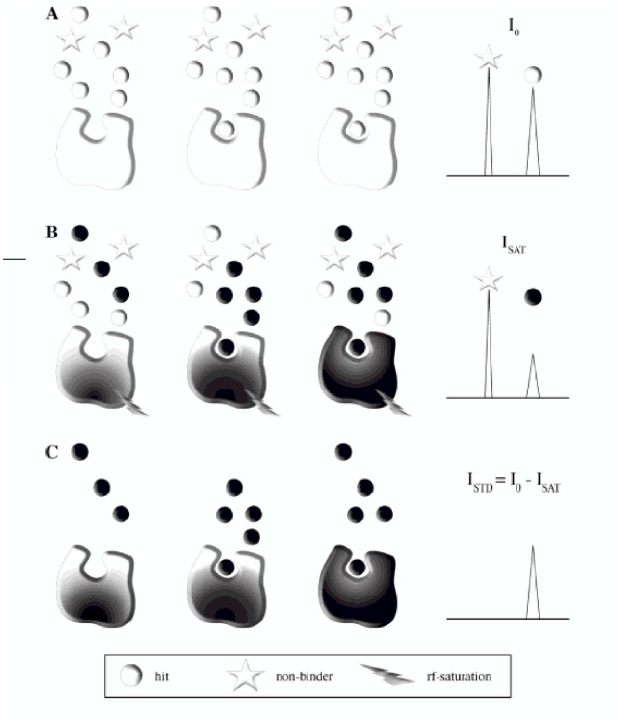
18– Special Topics: Saturation Transfer Difference (STD) Spectroscopy

This is a solution state NMR method, which is used to identify which ligands bound best to a target receptor. In other words, this technique is used to probe one-site ligand binding of the type:

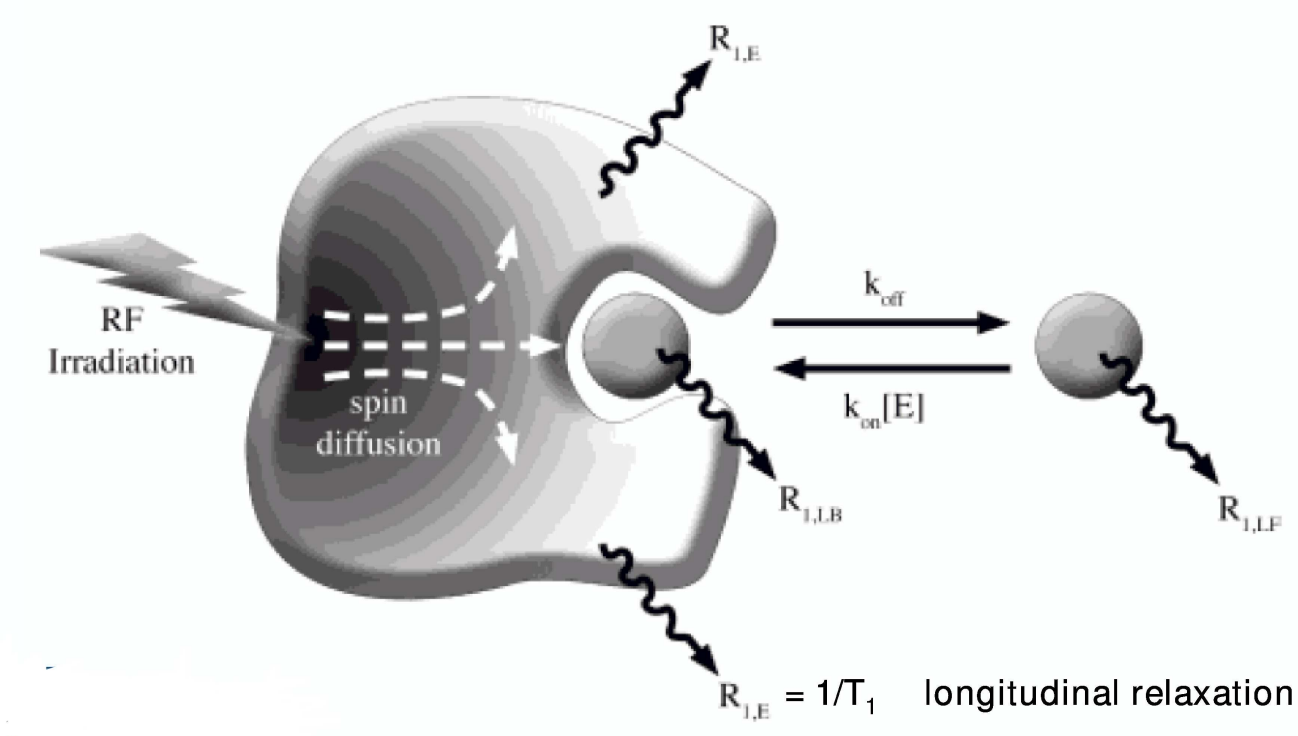
$$[E] + [L] \longleftrightarrow [EL] \tag{18.1}$$

by seeing which ligand NMR signal is most affected.

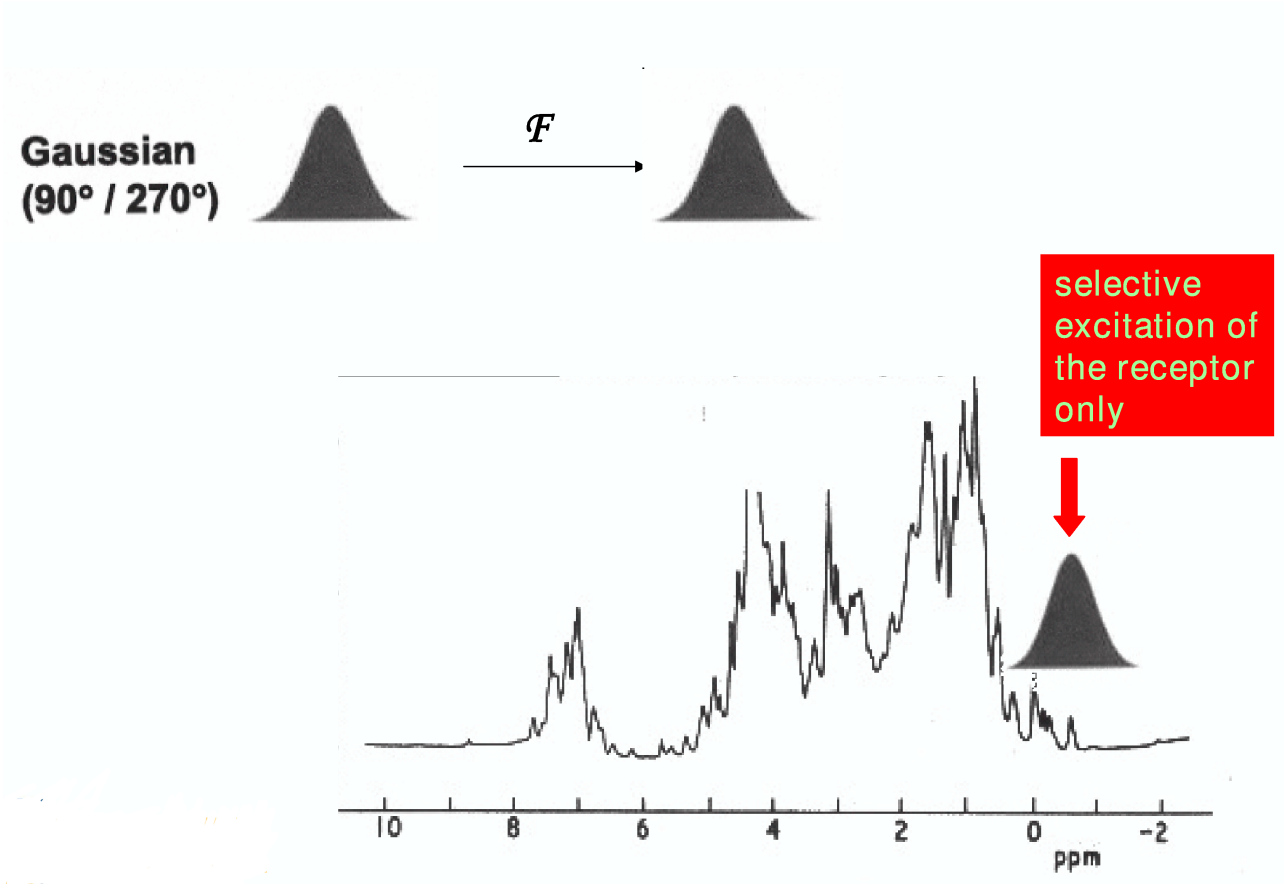


ref: Lepre et al., *Chemical Rev.*, 104, 3641 (2004)

The basic idea of the method is shown in the picture below:



The receptor (protein) molecule is selectively (saturated) irradiated using a selective pulse, e.g. Gaussian or any of the ones we saw in Chapter 3.



Since only the protein resonates in the range -1 ppm - 0 ppm, it is easy to achieve this selective irradiation. The phenomenon which leads to a "labelling" of the ligand and of the rest of the receptor molecule is spin diffusion, so let's take a closer look at this.

18.1 Spin diffusion in Solution

Spin diffusion is a process by which spin polarization is transported between spatially separated equivalent spins. There are two types: spatial spin diffusion and spectral spin diffusion. Both arise because of the

dipole-dipole interaction between the spins. They can be distinguished by how they are detected:

1. Spatial: measured by deviations from exponentiality during relaxation processes

2. Spectral: exchange of magnetization between resolved resonance lines

So for our purposes here, we are dealing with the effects on relaxation, i.e. spatial spin diffusion. We can think of this process in terms of NOEs.

As we saw in Chapter 14, we can define the steady state NOE enhancement as

$$\eta = \frac{\Gamma_{12} I_{2o}}{\Gamma_{11} I_{1o}} \tag{18.2}$$

or

$$\eta = \frac{\sigma}{\rho} \tag{18.3}$$

where the numerator is the cross-relaxation rate and the denominator is the overall relaxation rate.

For a spin pair, this enhancement can be written in terms of the Larmor frequency and the correlation

time as:

and for spin pair (denoted with subscript 2):

$$\eta_2 = \frac{\sigma_2}{\rho_2}\frac{1}{1+f_{ex}} = \frac{5+\omega_0^2\tau_c^2-4\omega_0^4\tau_c^4}{10+23\omega_0^2\tau_c^2+4\omega_0^2\tau_c^2}\frac{1}{1+f_{ex}} \qquad (2)$$

Parameter f_{ex} represents the relative contribution of external relaxation sources, τ_c is the correlation time of the process that modulates dipole interaction, and ω_0 is the resonance frequency of the observed nuclei. In diamagnetic systems, external relaxation is negligible, $f_{ex} \ll 1$.

In NOE Methods, Binding Is Defined by the Lifetime of the Interaction. When protein protons are irradiated, their fast cross-relaxation with other protein protons (spin diffusion) (Macura and Ernst, 1980) rapidly distributes spin saturation throughout the network of protein protons. Saturation further transfers to any bound molecule (anesthetics) that satisfies the condition that the effective correlation time (τ_{eff}), which is the combined complex lifetime (τ_B) and the protein tumbling correlation time (τ_c), is longer than the period of proton resonance frequency (ω_0):

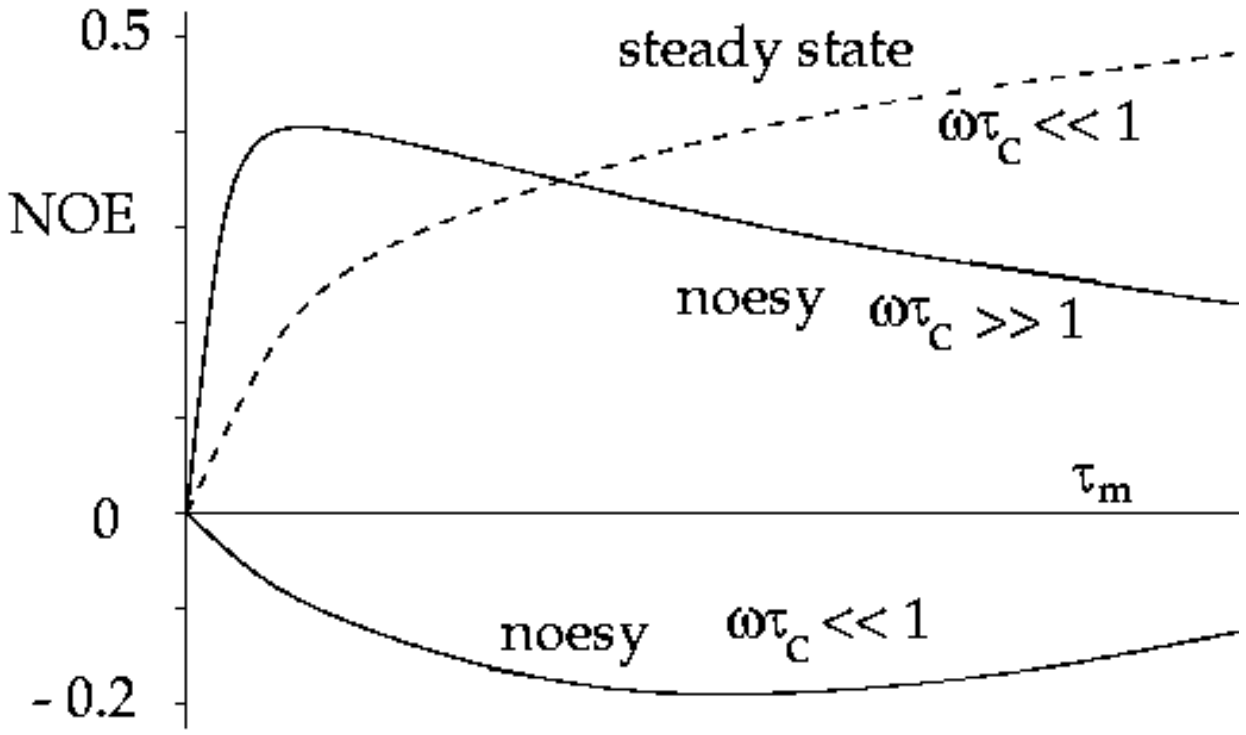
$$\frac{1}{\tau_{eff}} = \frac{1}{\tau_B} + \frac{1}{\tau_c} < \omega_0 \qquad (3)$$

This means that at resonance frequency of 500 MHz, the lifetime limit of the anesthetic-protein complex is 360 ps; shorter living complexes will not saturate by the double resonance method. Thus, the only criterion on anesthetic binding in the NOE-type experiments is an effective correlation time, i.e., average lifetime of the complex.

Streiff et al., Mol. Pharm., 66, 2004

The sign and the magnitude of η will depend on the overall mobility and structure of the complex. If the complex tumbles isotropically then τ_{eff} can simply replace τ_c in the equation for η_2 above.

Spin diffusion is a process which involves multispins systems, therefore we need to take more than two-spins into account. This requires specific models to determine the enhancement quantitatively. Generally speaking, the relaxation rates σ and ρ are proportional to the number of spins involved. Typically, though, ρ will increase faster as a function of buildup time than σ, therefore η will be lower for multispin systems (vs two-spin systems).



For the saturation transfer difference experiment the network of spins can be approximated as three groups of equivalent spins: the irradiated spins A (receptor), a group which is not irradiated but participates in the magnetization transfer B (receptor), and finally, group C at which saturation transfer is measured (ligand). By dividing the spin system in this way, saturation transfer can be approximated by two steps:

1. Step 1: Intramolecular NOE from A to B,

2. Step 2: Intermolecular NOE from B to C.

18.2 Pulse sequence

The basic pulse sequence for STD is:

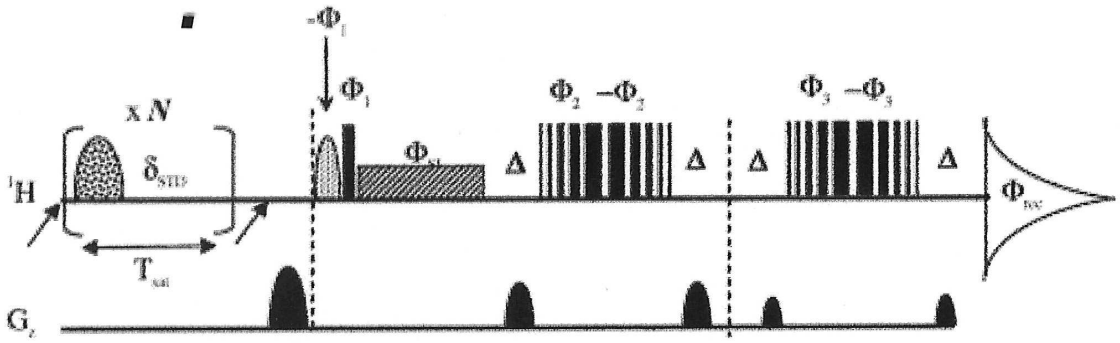


Figure 6. Example of an STD pulse sequence.[48] The upper and lower staffs show proton rf and field gradient pulses, respectively. Selective rf saturation occurs for a time $T_{SAT} = 1-3$ s via a train of N frequency selective rf pulses (e.g. 50 ms Gaussian or Seduce-1[49] 90° pulses separated by an interpulse delay of ~1 ms). Two experiments are performed, which apply saturation on and off resonance with receptor protons in an interleaved fashion. The 1D signals are stored in separate locations, and their spectral differences are taken via inverting the receiver phase. A WATERGATE-5[28] readout sequence polls the residual z-magnetization. Suppression of receptor signals is achieved by relaxation filtering during the spin–echo delay Δ and the optional $R_{1\rho} = 1/T_{1\rho}$ spin-lock. An optional water flip-back 90° pulse precedes the first hard 90° pulse (e.g. 2 ms Seduce-1 90° pulse). Phase cycling is as follows: $\Phi_1 = (16x, 16\ -x)$; $\Phi_2 = (x, y, -x, -y)$; $\Phi_3 = (4x, 4y, 4-x, 4-y)$; $\Phi_{SL} = y$; and $\Phi_{rec} = 2(x, -x, x, -x, -x, x, -x, x)$, $2(-x, x, -x, x, x, -x, x, -x)$. Φ_{rec} flips 180° between the on and off resonance spectra.

A key element to the sequence is the application of on- and off-resonance (with respect to the protein signals) irradiation (what does this accomplish and how?). For ligands which do not interact with the receptor, the difference between on- and off-resonance irradiation

$$\Delta I = 0 \tag{18.4}$$

whereas, for ligands which do interact with the receptor

$$\Delta I > 0 \tag{18.5}$$

The relative amount of saturation (see the first figure) can be determined by plotting $\frac{\Delta I}{I_{off}}$ as a function of the saturation time. For long saturation times, this ratio will give η_2.

18.3 Application of STD

Turning low affinity ligands to higher affinity ligands

For a reaction of the type:

$$[\mathrm{E}] + [\mathrm{L}] \underset{k_{\mathrm{off}}}{\overset{k_{\mathrm{on}}}{\leftrightarrow}} [\mathrm{EL}]$$

we can define a dissociation constant $K_{\mathrm{D}} = [\mathrm{E}][\mathrm{L}]/[\mathrm{EL}]$

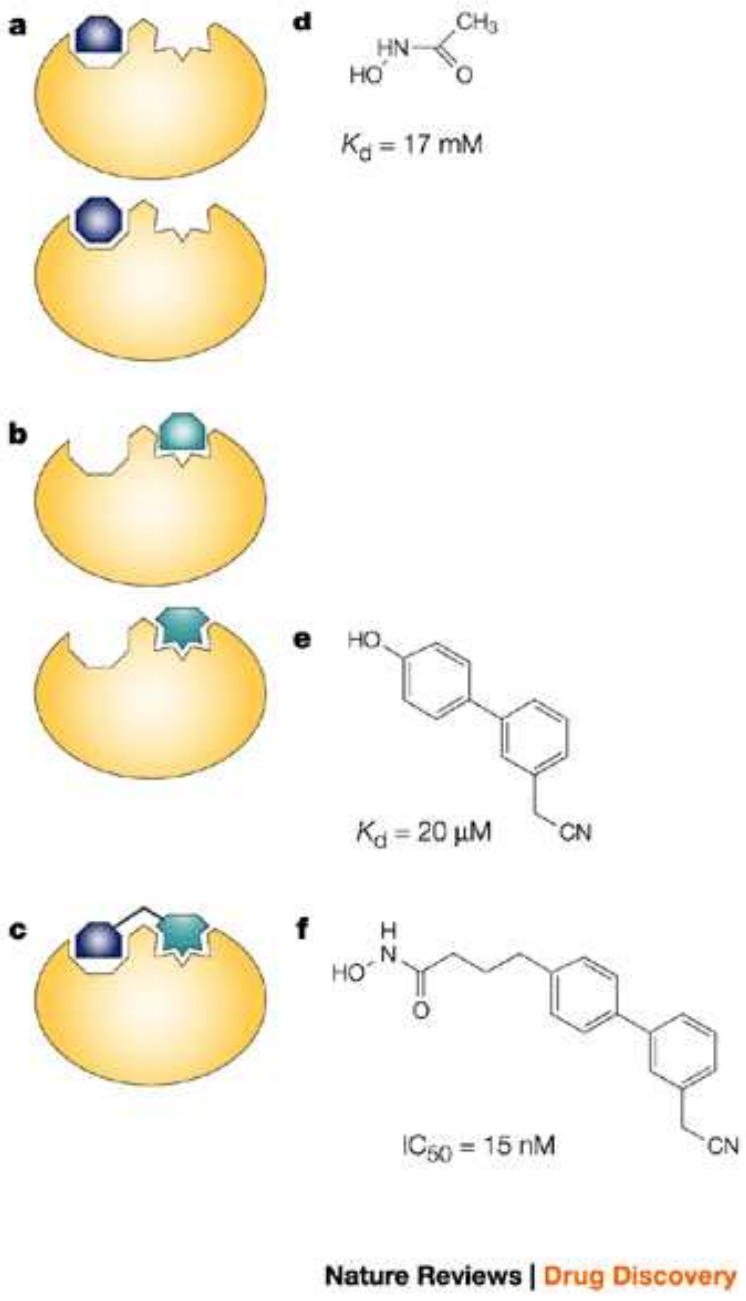
The objective in drug design is to make a ligand where K_D is in the nM range.

Three strategies are used to achieve this:

1) Combination strategies
2) Elaboration strategies
3) Variation strategies

Example 1

Combination strategies: SAR by NMR



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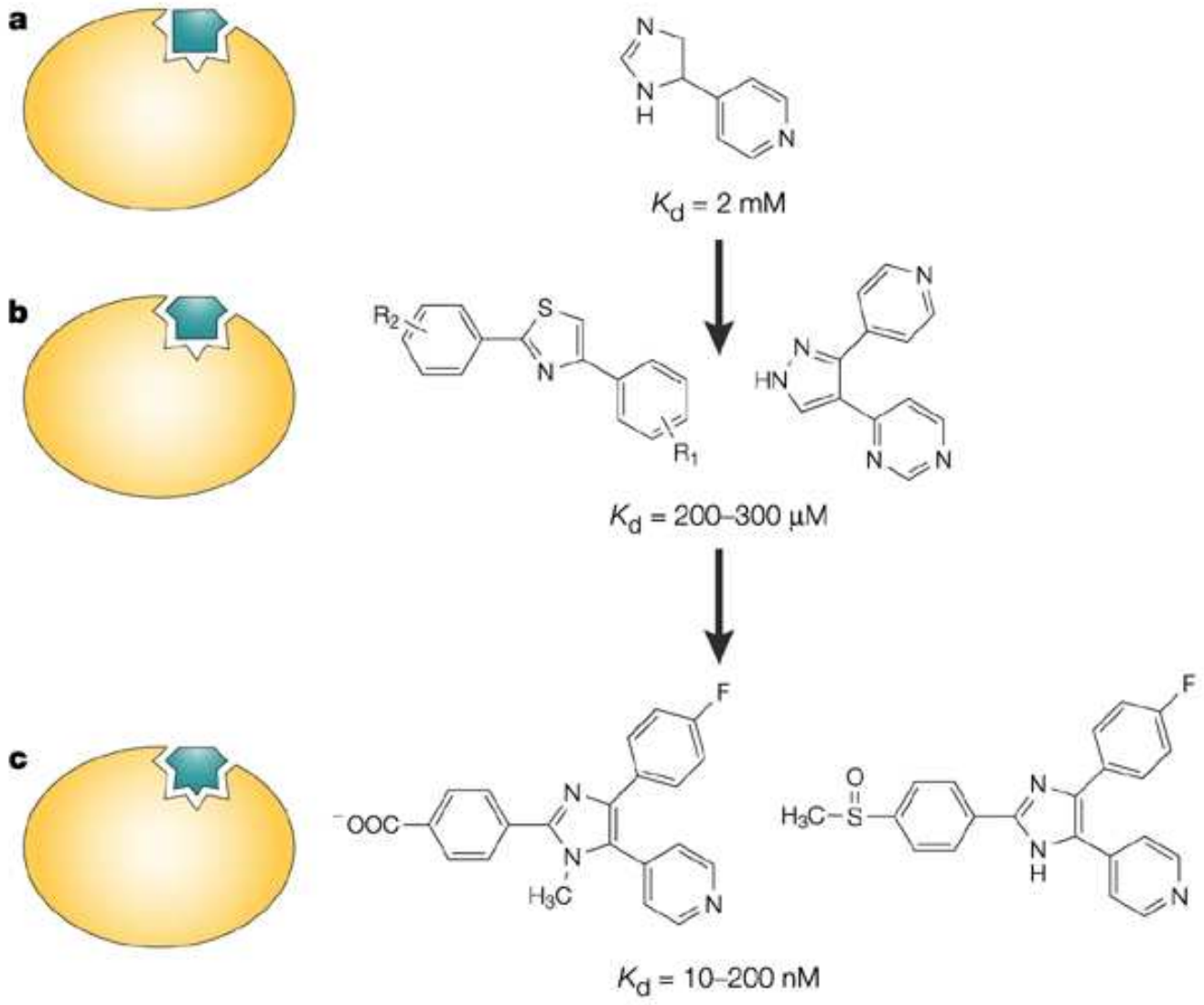
Example 2

Elaboration strategies: Shapes


a
b
c
K_d = 2 mM
K_d = 200–300 μM
K_d = 10–200 nM