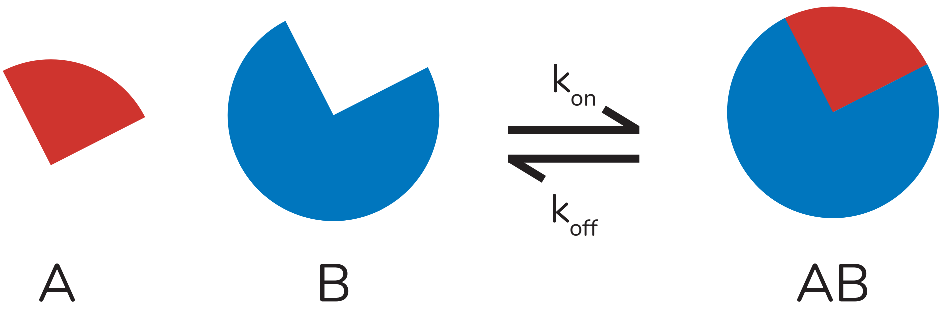
The K_D cheat sheet

Interactions between two species (e.g. Ligand A and Protein receptor B) can be characterized by their K_D - this value indicates how strong the interaction is.



$$K_D = \frac{k_{off}}{k_{on}}$$

$$K_D = \frac{[A][B]}{[A - B]}$$

Terms

Term	Units	Definition
K_D	Mols (M) (and pM, μM etc...)	Dissociation constant; the concentration at which half of the receptors present are bound to a binding partner.
k_{on}	Mols per second ($M\ s^{-1}$)	The second order rate constant of binding reaction. The rate at which binding occurs. This is concentration dependent.
k_{off}	Per second (s^{-1})	The first order rate constant for dissociation of the complex. The rate at which dissociation occurs. This is concentration indepdenent.

Key points to remember

- When K_D is low, binding is strong
- When K_D is high, binding is weak
- Lower case k = rate
- Upper case K = constant
- Don't forget **time** - as k_{on} is concentration dependent, the time for equilibrium to be reached varies with concentration.

Experimental Determination

- Keep the concentration of one species constant (A in the diagram below)
  - This should be the one which gives a signal in your detection method
  - This should preferably be the smaller species if measuring via size change
  - To avoid long reaction times ensure that [A] is close to K_D

- Change the concentration of the other species (B in the diagram below) logarithmic dilution is best to cover the full range.

- Plan each test in advance;
  1. Measure A on its own
  2. Measure the same concentration of A with a large excess of B, so that 90% or more of A is in bound form

     (you can tell that 90% is bound by measuring another similar concentration of B, and seeing that it gives a similar result)
  3. Measure the same concentration of A with a concentration of B you suspect will be at the K_D
  4. Measure 3 samples with [B] below the K_D
  5. Measure 3 samples with [B] above the K_D