Topic #3, lecture 3b

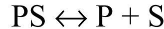
DNA binding proteins and DNA binding affinity

DNA binding affinity and the equilibrium dissociation constant:

The interaction of a **P**rotein with its **S**ite on DNA is:

$$P + S \leftrightarrow PS$$

or you can think of it as dissociation of a protein from its site:



$$PS \leftrightarrow P + S$$

The dissociation of this complex is denoted by a constant $\mathbf{K_d}$, which is a measure of DNA binding affinity.

Total protein concentration: $P_T = [P] + [PS]$

Total DNA sites: $S_T = [S] + [PS]$

***P** is the concentration of free protein, **S** is the concentration of free DNA sites, and **PS** is the concentration of the complex.*

The affinity, or $\mathbf{K_d}$, for the binding reaction is:

$$\mathbf{K_d} = \frac{[P][S]}{[PS]}$$

The ratio of [S] to [PS] (or [PS] to [S]) is an experimental variable that is most commonly determined by quantitating DNA binding by gel shift analysis. In this situation, [S] is the free probe (unbound sites), and [PS] is the bound probe. To use a gel shift, you must quantitate the amount of probe in the bound and unbound bands on the gel shift.

Operational Definition of K_d:
the concentration of DNA binding protein ([P]) when half the sites are occupied at equilibrium

Equilibrium:
New complexes are forming at the same rate that complexes are dissociating

For high affinity binding K_d values will be **low** (ie. it takes less protein to occupy half the sites)
For low affinity binding, K_d values will be **high** (ie. it takes more protein to occupy half the sites)

Typical K_d's for specific DNA binding proteins: $K_d = 1 \times 10^{-9}$M (1nM)
Typical K_d's for non-specific DNA binding: $K_d = 1 \times 10^{-7}$M (100nM)

What does the Kd equation mean in practical terms?

When the concentration of DNA binding sites is higher than the K_d ([S]>>K_d):
eg. [S] = 1 X 10^{-7}M

$$10^{-9}\text{M} = \frac{[P] \bullet 10^{-7}\text{M}}{[PS]} \quad \text{then,} \quad 0.01 = \frac{[P]}{[PS]}$$

this means that most of the protein is bound to DNA ([PS]>>[P])

However, when the DNA concentration is lower than the K_d ($[S] << K_d$): eg. $[S] = 1 \times 10^{-11}M$

$$10^{-9}M = \frac{[P] \cdot 10^{-11}M}{[PS]} \quad \text{then,} \quad 100 = \frac{[P]}{[PS]}$$

most of the protein is free, in solution

and when the DNA concentration is equal to the K_d ($[S]=10^{-9}M$):

$$10^{-9}M = \frac{[P] \cdot 10^{-9}M}{[PS]} \quad \text{then,} \quad 1 = \frac{[P]}{[PS]} \quad \text{or } [P]=[PS]$$

and free protein and bound protein are **equal**. We say that there is **50% fractional occupancy**

How do we determine K_d?

1. Set up a situation where the concentration of DNA binding sites is much lower than the K_d (**$[S]<<K_d$**) (there are ways to make an educated guess) in a **EMSA assay**.

2. Add increasing amounts of protein until 50% of the oligo is shifted - this means that 50% of the sites are occupied (we're assuming here that there is one site per oligonucleotide)

$$\textbf{fractional occupancy} \quad \nu = \frac{PS}{S_{total}} = \frac{PS}{S_{free} + PS}$$

remember this equation - it will be used below

In the gel shift assay, we are looking for the situation when $\nu = 0.5$. In other words, we will take note of the protein concentration that yields 50% fractional occupancy of the DNA probe.

It's important to point out that if $[S] <<<< K_d$, then the amount of bound P will never be significant and most of the protein will be free. This means it is fair to write:

$$[P_{total}] = [P_{free}]$$

Also, it is difficult to get an accurate measure of [PS] - we would rather not have to do this, and fortunately, we don't have to. Remember that:

$$K_d = \frac{[P][S]}{[PS]}$$

solve this equation for [PS] to get:

$$[PS] = \frac{[P][S]}{K_d}$$

Using the ***fractional occupancy equation*** *shown earlier (shown again here):*

$$\nu = \frac{PS}{S_{free} + PS}$$

we can substitute [PS] in the fractional occupancy equation with our new equation to get:

$$\nu = \frac{[P_{free}]}{K_d + [P_{free}]}$$

Remember!??! That $[P_{total}] = [P_{free}]$?? remember why??
Use this to our advantage:

$$\nu = \frac{[P_{total}]}{K_d + [P_{total}]}$$

we are looking for the point on our gel where fractional occupancy = 50%, $\nu = 0.5$

so,

$$0.5 = \frac{[P_{total}]}{K_d + [P_{total}]}$$

$$0.5\ K_d + 0.5[P_{total}] = [P_{total}]$$

$$0.5\ K_d = 0.5[P_{total}]$$

$$\boxed{K_d = [P_{total}]}$$

What happens when the DNA concentration is very high, >>>Kd?

$$10^{-9}M = \frac{[P][10^{-7}M]}{[PS]}$$

$$0.01 = \frac{[P]}{[PS]}$$

all the protein (99%) should be bound

Is there ever a physiological situation that approaches this calculation?

Theoretically yes….in the nucleus.

What are typical concentrations of DNA binding proteins?

What will occupancy be like for proteins with low Kd's?

What will occupancy be for proteins with high Kd's (eg. non-specific DNA binding proteins)?