Methods for measuring physical interactions:

***In vivo*/*ex vivo* methods.**

- Pull down. Use an antibody for some protein X and see what else comes out with it.
- Two hybrid (usually yeast or bacteria). Express a "bait" protein attached to the one half of a transcription factor and a "target" protein to the other half. If the "bait" and "target" interact, the transcription factor turns on. (Usually this expresses either a visible marker like *lacZ*, *GFP* or a nutritional maker that allows the creature to grow in some environment).
- Display (usually phage or bacteria): express a partner on the surface of a critter, stick the other partner to a plate, and enrich for critters that bind to the plate.
- High throughput variations on these themes.

These are not at equilbirium and are measures of k_{off} *not* binding constant! These should be validated *in vitro* if you want to argue they are actual binding.

***In vitro* methods**

- Gel shift: titrate one partner while holding the other fixed and then see how the fixed partner titrates on a native gel. If they interact, the fixed partner will migrate more slowly. (Often used for nucleic acid/protein interactions).
- Spectroscopy: titrate your favorite interaction partner into a protein solution and follow some spectroscopic signal of the protein (fluorescence, circular dichroism, NMR)
- Fluorescence anisotropy: attach a fluorescent tag to a partner of interest, titrate in your protein, and observe how the "tumble" of the fluorophore is altered by binding.
- Surface Plasmon Resonance: Fix either partner to a plate and flow the other partner over the plate. If they bind, you will see a difference in the plasmon resonance and have a signal to follow.
- Isothermal Titration Calorimetry: titrate your favorite interaction partner into a protein solution and follow the heat that is evolved. Hard to pull of, but gives you both enthalpy and free energy of binding.

All *in vitro* methods–and some *in vivo* ones–require fitting a model to extract information from your experiment.

Fitting exercise

1. Go to http://aclarke.uoregon.edu:8000 and open the "regression.ipynb" notebook
2. Follow the stuff I tell you to get started

Data file 1

1. Fit all four models to the observed binding data in "datafile_0.txt"
2. Which model would you choose and why?

Data file 2

1. Fit all four models to the observed binding data in "datafile_1.txt"
2. Which model would you choose and why?

Synthesis:

1. Can you think of away to formalize what you came up with?
2. Which models are most informative and why?
3. Can you think of a problem in your current lab where you might want to employ regression?

Avoid a crappy model by looking for random residuals

Avoid adding too many parameters with a likelihood ratio test.

(Basically, don't add parameters that don't improve your fit).

1. Measure your test-statistic D:

$$D = 2\Sigma(f_{complex} - y_{obs})^2 - 2\Sigma(f_{simpler} - y_{obs})^2$$

   where $f_{complex}$ are the calculated values for the complex model, $f_{simpler}$ are the calculated values for the simpler model, and y_{obs} is the real set of observations.

2. Calculate the difference in the complexity of the complex and simple models by:

$$n_{df} = n_{complex} - n_{simpler}$$

   where n_{df} is the difference in the number of degrees of freedom, $n_{complex}$ is the number of fitting parameters for the complex model, and $n_{simpler}$ is the number of fitting parameters for the simpler model.

3. Look up a p value from a χ^2 distribution using D as your test statistic and n_{df} degrees of freedom. This will give you a p value for accepting or rejecting the new model.