You are studying a member of the Guanylate Kinase family. As part of its enzymatic cycle, two "lobes" open and close. (It is active when closed, but must be open to bind substrate and release product). You design an inhibitor and want to understand which structure it binds to.

You use a technique called FRET (Förster resonance energy transfer), which lets you measure an apparent distance between two fluorophores (the stars in the picture). You excite one flourophore (say, green star) but not the other (say purple star). If you choose your fluorophores wisely, the emission from the green fluorophore will excite the purple fluorophore. This means you can excite green, but observe emssion on purple. One can measure the FRET efficiency (E), which is basically how well energy is transferred from the first to the second fluorophore. This can be related to distance by the following:

$$E = \frac{1}{1 + (r/R_0)^6}$$

where r is the distance between the fluorophores and R_0 depends on the fluorophores chosen. Some fluorphores transfer efficiently only at short range, others have very long range. Thus, y choosing specific dye pairs, one can build an assay that is sensitive to specific distance changes.

1. You measure the total fluorescence change for a cuvette containing billions of molecules. You measure an apparent distance of 7.5 nm in the absence of the drug, and 5.0 nm in the presence of the drug. Come up with a mechanistic model for what is happening when the drug binds.

2. You repeat this experiment using TIRF microscopy, which lets you measure the apparent distance between lobes for individual molecules. You make 2,000 single-molecule measurements in the abscence of the drug, and 2,000 more measurements in the presence of the drug. Download the Excel spreadsheet containing these data. (Each group will be assigned a different spreadsheet).

   (a) Are these observations consistent with your "bulk" observations?

   (b) Are these observations consistent with your model? If not, is there a model you can come up with that is consistent with both the bulk and individual observations?

3. On the board, draw the following. Be prepared to present your work to the rest of the class.

(a) Your initial mechanism

(b) Your single-molecule data

(c) Your mechanism consistent with the data.

4. What do your results tell you about *average* versus *individual* molecular measurements?