4) **Propose experiments or computations that allow further characterization of how the lead compound interacts with your molecular target. Justify and carry out at least one computation to compare some property of your optimized lead to your original lead compound.**

Due date: Fri, Nov 30, 2005

As a fourth part of your project, you are expected to come up with a plan how to optimize your lead compound's interaction with the target. One of the first steps is to characterize the interaction of the lead with the target. Quantitative characterization typically involves determination the mode of action of the lead (agonist, antagonist, competitive inhibitor, mechanism-based inactivator, …). There are a variety of ways to do this, and some common examples are discussed below.

a) If you developed a biochemical assay in the previous part, you may be able to modify this assay to measure the binding of your lead to the target over a range of lead concentrations. If your target is an enzyme, rate measurements at different inhibitor concentration allow characterizing the kinetic mechanism and binding strength of the inhibitor.

b) If you developed a cell-based assay for a known target in the previous part, you may have to design a different assay or use the same assay for quantitative characterization of lead-target interactions. This depends of weather the response signal from your assay is directly related to the binding affinity of your lead.

c) If you developed a cell-based assay for an unknown target, you need to describe here how to identify what this target is.

d) Determination of the structure of the target in complex with your lead is a useful step. Research if your target has been ever crystallized, and if yes, identify appropriate crystallization conditions. It is very reasonable to propose a crystallization experiment based on known conditions. If it has not been crystallized, you could outline a strategy how you could attempt crystallization; however the chance of success is small and you should not build the rest of your case on the assumption that you somehow will get the crystal structure of the lead-target complex.

e) Free-energy calculations and thermodynamic integrations are applicable in cases where your screening strategy involved virtual screening, e.g. docking. Explain how you plan to identify better binders using the TI, FEP, or LIE approaches.

Some of you already have an idea by now what molecule(s) are your leads. If you do not, you could consider any known ligand (natural substrate, previously failed drug) as a lead for the following part. You may also have some idea how to modify your lead to make it a better binder, more soluble, more stable, or less toxic.

Carry out at least one set pf computations to compare your optimized lead with the original. In the simplest case, you could perform a conformational analysis or calculate the electrostatic potential surface of the original and modified lead. However, you are encouraged to try more challenging approaches. Docking is pretty straightforward and allows you to create a nice picture of your optimized lead in the binding pocket. Free energy calculations are more challenging but allow you to compare solubilities or binding affinities to assess the success of modifications that you made.