Biochemistry Lab

Hypothesis Design and Planning Handout

What is a good research project?

A good research project has a variety of essential elements: is original, explores an interesting research area, has a testable and falsifiable hypothesis, and has well-designed experiments that can provide believable data that are presented in a clear manner (research proposal), and culminates with a presentation of the rationale, results and conclusions, which often includes modifications of the original hypothesis and suggestions for future experiments.

What is a good hypothesis?

A scientific approach often depends on a good hypothesis, and in developing ideas for a research project it is well worth considering the attributes of a good hypothesis, and thinking about what it takes to both develop and present a good hypothesis driven research project. It is also worth remembering that it is very difficult to prove a hypothesis correct: science is usually based upon eliminating reasonable alternatives - you can prove a hypothesis wrong, or you can collect evidence that is consistent with the hypothesis.

A good hypothesis is:

Based upon prior observations : These can be your own preliminary results or they can be others work found in the literature, or often a combination of both- to develop a good hypothesis you need to find out what is already known. Is original If the answer to your question is known (ie is already in the scientific literature) it is not original research. You can make a hypothesis that further develops others ideas, but if the answer is known, it is not a hypothesis.

Is testable: Whatever hypothesis you make it must have predictions as to results you will get in experiments in support of the hypothesis

Is Falsifiable: The predictions you can make based upon your hypothesis must give rise to experiments where the outcome can "falsify" (disprove) your hypothesis

MDH-CS interaction Hypothesis Development.

1. Background: Record your observations based on the publications and other data you've studied.
   - Start general: What is the scientific overall understanding of protein-protein interactions, what are the driving forces for these interactions, what is the overall purpose of a protein interaction/channeling. What is a metabolon?
   - Then get specific: What do we know about MDH-CS interactions? What thermodynamic and metabolic reasons are there for MDH and CS to interact. That has been tested and learned in the literature (not just the last two or so papers, historically). What papers show that cytosolic MDH and CS do NOT interact? What data is there that hint there may be some level of interaction? Review the last four MDH-CS and identify the three different models of interface between MDH and CS.

2. Models and Bioinformatics: Compare the interface connections/regions/domains between MDH-CS for the pdb files shared with you on the website. Use your PyMOL skills here…
   - How are each model different (which were cross-linked, which PDB used what protein as the starting source, which PDB model was restricted and which not.
   - What do these models tell us that are in common and different?
   - Do these models, with or without lysine crosslinked restrictions tell us EVERY amino acid interacting at the MDH-CS interface?
   - Review the aligned MDH protein primary sequences. Identify similar and unique homology as they correlate to the possible interface between MDH and CS. Recognize that different models will have a slightly different answer. Create a graphic approach and a written description.
   - Review the mutants available and analyze if and how they may impact MDH-CS binding. Increase, decrease, no effect are all possible options. Use HawkDock to interpret key binding residues.
   - Make PyMOL models of the site of interaction. Compare wild-type MDH-CS interactions and mutate to match your selected mutation. Measure the strengths, distances and create a table of possible interactions before and after mutations.

3. What do we know and what is the gap?
   - A good hypothesis has to explain what we know, how we know it (published literature and modeling based on experimental/computational evidence, primary sequence alignments). This can also be based on experiments conducted but not yet published by others in a lab.
   - A good hypothesis will define a "gap in knowledge" We know this…. What is unclear or yet determine is this… you must explain what is unknown and WHY filling the gap of knowledge in is important. Reasons can include basic science and or applied needs including disease/clinical issues.

4. Draft your hypothesis.
   - Hypothesis start with … because of this (see #1-2) … we don't know (see #3)… therefore xxx is important/is key/will cause… some hypothesis are an "if – then" statement. If xxx is true then yyy will happen.
   - A hypothesis is a statement not a question. Review the key for a good hypothesis written above.
   - Is your hypothesis a testable thing? Consider how you will test your hypothesis? What controls will you need to include in your testing.

5. NEXT: planning the experiments to test your hypothesis.
   - Comparing wild-type models for possible interface surfaces and interactions between MDH and CS
   - Measuring the free energies of association between key residues in the various wild-type docking models will inform the role of the various mutants
   - Impact of the mutants on substrate binding and catalysis (i.e. are the mutants overlapping the substrate binding domain or the catalytic amino acid residues?) by both homology alignment/structural analysis and enzyme kinetic measurement – specific activity and Km/Vmax
   - Measuring the interactions – what conditions to support/push the interactions, how will the assay be conducted, how will you prepare the proteins, how will you remove the tag on either MDH or CS? How will you identify interacting proteins?