IPC Report: 4 pieces of feedback

- General comments to all students from Alan
- In-line comments from Neal (writing)
- Individual comments from Agi (methods)
- Individual comments from Alan (rest of report), and score for each section

Comments from Alan, I

1. In general, strive for precision and conciseness. Try to use the minimum number of words and sentences you need to convey your points with clarity. Use consistent voice (generally passive) and tense (generally past). Make sure terminology is used correctly, and avoid colloquialisms or "unscientific-sounding" wording.

2. Abstract should be a precise summary of your rationale, approach, results, and significance. Make sure you tell the reader which mutations you made and at least qualitatively what changes in calcium affinity and cooperativity you observed.

3. Introduction should define and justify goals of your study, not merely state what you did (*e.g.* don't just say "In this study we used inverse pericam to..."). In the Discussion, you should revisit your goals and evaluate how well they were achieved.

Methods vs. Results

- **Methods**: “DNA was extracted from XL1-Blue cells by a miniprep procedure. Cells from 1.5 mL of each liquid culture were spun down and resuspended…”
- **Results**: “Amplified DNA was isolated in order to evaluate the success of the mutagenesis reaction, and ultimately produce mutant protein. Two individual colonies carrying X#Z DNA were grown in liquid culture, then lysed to obtain DNA. Both candidates, along with S101L DNA from a colleague, were tested by sequencing and restriction digest…”

Comments from Alan, II

4. In the Results section, make sure the reader knows what you are doing and can follow the logical progression from each step to the next. It might help to think of this section as a story. Although there is a separate section for detailed description of the methods, make sure the procedures you used (with essential details only) are clearly stated here also.

5. Discussion should begin with a brief summary of your approach and results (do not include this in the Results section). It is not necessary to discuss details of every procedure you implemented—discussion of procedural details (*e.g.* incomplete digestion by restriction enzymes) can often be placed in the Results section. Instead, integrate results from related experiments to discuss the major issues related to your study: (1) success of creating the mutant proteins you set out to make, (2) quality of titration data and explanation of titration results, (3) success and significance of overall goals of your study, and directions for future work.

Create a context at the start of your results

The purpose of the results section is **to present your data in a relatively unbiased way**, **but with some guiding framework**. Begin with a short overview of the entire experiment, and then delve into specific sub-sections that describe each piece of the work. Note that the sub-sections should be organized by functional content, not by what you did each day in lab. One potential division might be the following: construction of the mutant plasmid, verification of mutant DNA and protein production, and characterization of the mutant protein. However, other schemes could work as well or better.

Each sub-section should begin with an overview sentence that introduces the present experiment and end with a sentence stating the primary conclusion reached from that experiment. The overview and/or concluding sentences should also provide a transition to the previous/next piece of data. You may present your reader with the broad strokes of what your data indicate, particularly in the sub-section headings and concluding sentences, and in the figure caption titles. However, **you should reserve detailed interpretation of your data for the discussion section**.

From http://openwetware.org/wiki/20.109(S09):Protein_engineering_research_article

Create a context at the start of your results: Example

Inverse pericam was modified with two different mutations in order to modify binding affinity of the Ca2+ binding region CaM, and to alter cooperativity among the four binding sites CaM possesses. Mutations were chosen to minimize binding affinity. Mutant plasmids were selectively chosen and engineered out of pRSET plasmids, presence of abnormal DNA was tested, and this DNA was bacterially amplified, protein production was induced from bacteria, and characteristics of pure mutant proteins were assessed.

Create a context and establish a “story” at the start of your discussion

The purpose of the discussion section is to **interpret and contextualize your data**. You should **begin by reiterating your major findings**. Then you might do any or all of the following: connect your findings to other research (published or that of your peers); describe any ambiguities and sources of error in the data, and suggest future experiments to resolve uncertainties; explain where you expect your work may lead, and suggest specific experiments for extending your findings; describe any conceptual or technical limitations of the research. Finally, you should **explain the significance of your findings to basic science and to engineering applications**. Like the previous sections, the discussion should have a clear organization and narrative flow.

From http://openwetware.org/wiki/20.109(S09):Protein_engineering_research_article

Create a context and establish a “story” at the start of your discussion: Example 1 summarizing motive, approach, results and conclusions

We set out to modify Ca^{2+} sensitivity in inverse pericam derivatives and were successful at increasing cooperativity at the slight expense of calcium affinity in one mutation, and at entirely deleting the ability to sense calcium in the other mutation. Gene-level insertion of restriction sites was achieved through site-directed mutagenesis, resultant protein was readily transformed and expressed in a bacterial host, and mutant protein was purified and assayed for calcium sensitivity via fluorescence. We obtained qualitative data in the earlier phases of the project through gel electrophoresis and SDS-PAGE, and quantitative data in the later phases of the project through examination of calcium concentration titration curves and calculation of disassociation constant and Hill coefficient. However, our methods were not flawless, and there exist appropriate conclusions to be drawn and connections to be made between our experimental findings and future research.

Create a context and establish a "story" at the start of your discussion: Example 2 focusing on structure/function relationship

In this experiment, the D22W mutation was created to diminish IPC's affinity for calcium. To do so, the nature of the amino acids in the original protein were examined and analyzed in order to create an antithesis. Aspartic acid (D) is a medium-sized, hydrophilic, polar amino acid with a negative charge. By altering the DNA to encode for tryptophan (W) instead of aspartic acid, we aimed to vastly change IPC's affinity towards calcium. Tryptophan is the biggest amino acid, and is nonpolar, hydrophobic, and neutral in charge. The charge and the polarity (and thus the affinity for water) probably play the largest roles in determining the binding fractions for Ca^{2+}, which is a positively charged molecule. The size most likely also plays a role in blocking the ligand from binding effectively with other molecules in the EF-hand.

End your discussion with implications for further research and usefulness: Example

Clearly, these results warrant further analysis and experimentation. There are considerable limitations on site-direct mutagenesis at a single amino acid residue because mechanisms of cooperativity, for example, cannot be confirmed. In such a case, X-ray crystallography of CaM complexed with a target protein or calcium would be extremely revealing in terms of a more complete understanding of CaM binding. Additionally, *in vitro* kinetics experiments would be useful for understanding the cooperativity mechanism of the EF-hand motif.

While the results we obtained in this present were not necessarily exactly what we predicted, the fact that altering a single amino acid residue can cause such a dramatic change in the behavior of IPC is promising in terms of future research. By better understanding the 3-dimensional structure of Inverse Pericam, we can potentially engineer a more specific and more sensitive calcium sensor. Similarly, we can extend this thought process to engineer biomolecules and proteins that recognize not only calcium, but other target molecules as well.

Revision logistics

- Highlight major revisions! (e.g., red font)
- Due Thur/Fri after spring break
- Don't hesitate to contact any of us for help implementing the feedback