Lab Sequence for cCURE with Collaborations

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| Week | Focus of Lab | Associated Assignments |
| 1 | Learning the Assay- specific activity of native gMDH |  |
| 2 | Km and Vmax determination of Native protein | Basics of Data Analysis |
| 3 | Effects of pH and Temperature on Native protein | Background literature |
| 4 | Bioinformatics and Molecular Visualization: Developing a Proposal | Hypothesis outline |
| 5 | Proposal Ideas, interactions with Collaborators | Proposal Draft/Collaboration experiments |
| 6 | Plasmid Purification and Primer Design |  |
|  | Spring Break |  |
| 7 | Expression and Purification of Mutants | Final Proposal: what you will do, what your collaborator will do |
| 8 | Basic Kinetics of Mutant protein (compare with weeks 1,2 & 3 as appropriate) | Is the Mutant different from Native  Statistics etc |
| 9 | Km and Vmax of mutants | Is the Mutant different from Native  Statistics etc |
| 10 | Inhibition studies | Figures preparation |
| 11 | Conformation & Stability (A) or Repeat key experiments | Poster Draft etc |
| 12 | Conformation & Stability (B) or repeat key experiments | Figures preparation  Data Exchange with Collaborators |
| 13 | Repeat key experiments to confirm data/Collaborator Interactions | Present & Discuss data/Figures with collaborators |
| 14 | Research Project Presentations |  |

**Key Techniques include**

**Basic pipetting and solutions**

**Rate measurements: uv spectroscopy**

**Standard Curves: Bradford Assay**

**Michaelis-Menten Kinetics and Inhibitors**

**Bioinformatics: Clustal omega analysis**

**DNA Isolation**

**PCR, DNA Gels**

**Protein Purification, NiNTA affinity chromatography, SDS PAGE**

**Protein CD and Thermal Melts**

**Typical Collaborations may include**

**Western Blotting**

**Size Exclusion Chromatography**

**Fluorescence Based Thermal Shift Assays**

**MALDI-tof and es-ms/peptide mapping**

**Ligand Binding studies**

**Fluorescence Spectroscopy**