Team 21 – Protein Crystallization

Project Brief

Ross Kieser, Braxton Bliss, Ben Feikema, Zack Ardavanis, Abraham Mena

Project Description

Protein crystallization is the process of formation of a protein crystal by prompting protein molecules to form crystals by supersaturating the solution they are dissolved in. Protein crystallization is an important industrial and scientific procedure since it allows to determine the protein structure via X-ray crystallography. Once crystallized, proteins structures are used for biological and medical applications for example.

Crystallization can be executed with different techniques; among them, and the most popular, is the hanging-drop vapor diffusion technique. Team 21 aims to optimize this process for lysozyme and bovine serum albumin (BSA) by constructing a device that allows for air at different relative humidity to flow through the wells.

Requirements Summary

Team 21 aims to develop new, albeit limited, knowledge in the field of protein crystallization that could trust the field forward. The following are goals the team has set for itself and the requirements of each step:

- Crystallize basic protein (lysozyme)
    - The team should familiarize itself with the hanging drop technique and laboratory procedures.
    - Proteins crystals should be produced easily.
- Crystallize advanced protein (BSA)
    - The advanced protein should be relative cheap (~ 150$).
    - The advanced protein should be of interest and not so well understood.
- Design a novel device and method for protein crystallization
    - The team has decided to develop a method that controls relative humidity in an air flow into the wells.
- Show that device and method work with basic protein
    - After producing lysozyme crystals via hanging-drop vapor diffusion the team should apply its novel relative humidity method to lysozyme.
- Show that device and method work with advanced protein
    - Apply the novel relative humidity method to BSA. Expected results should be the same observed in the lysozyme experiments.

Major Design Decisions

The major design decisions came mostly from the first protein we selected, lysozyme. Calvin Chemistry and Biochemistry faculty helped us decide on this protein due to its readily available crystallization methodology. Another major design decision was to determine which variable will be changed as a function of concentration to construct a solubility curve. Relative humidity was chosen as a design variable since it represents the core of the current science of crystallization and is an area of crossover between chemical engineering and biochemistry. BSA was selected as our second protein because success in crystallizing a more difficult protein will prove the usefulness of our humidity control device and process.

Significant Issues

The biggest issue the team has faced has been related to finding its own path. As with any research project, goals and objectives change as we learn and during the first few months of the project, as the team learned more, the research scope changed. Another big issue the team has faced was learning the chemistry and biochemistry of protein crystallization via research papers and interactions with professionals, such as Calvin professors and Professor Mo Jiang. A detailed list of all major issues encountered can be found below.

- Learning enough biochemistry to understand the function of proteins and the body of research around their crystallization.
- Gaining access to lab space, materials, and equipment.
- Understanding team strengths and weaknesses.
- Finding a direction and focus for research and development and setting realistic goals.

Status

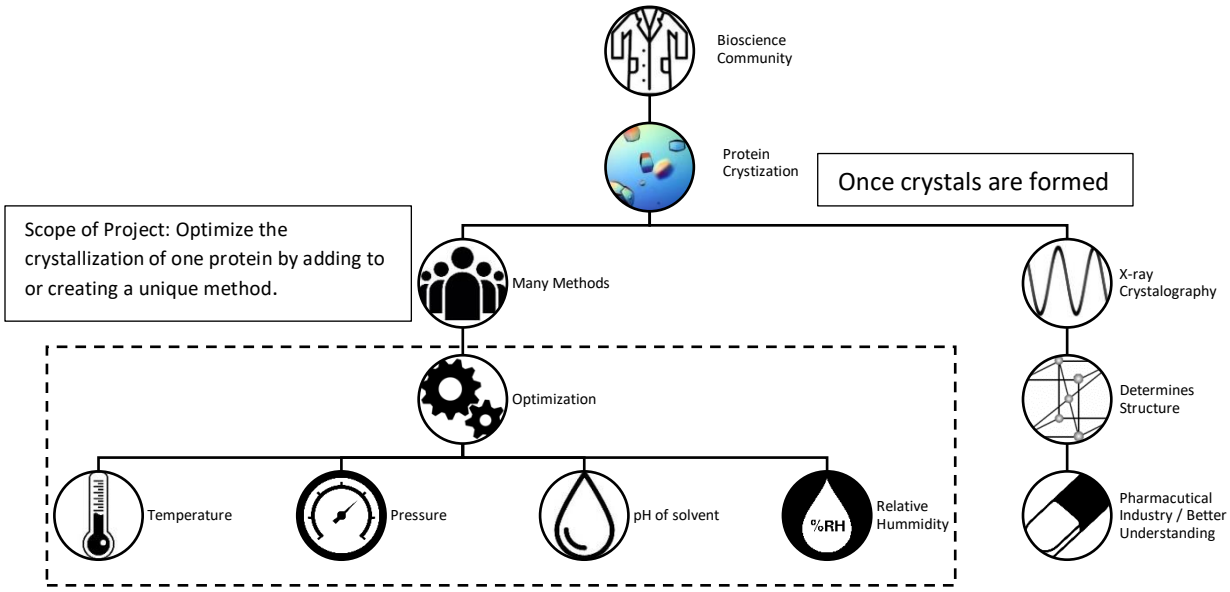
As of February 23, 2018, Team 21 has successfully crystallized Hen Egg-White Lysozyme (HEWL) following the procedure written by the team during fall semester and applying techniques learned through research. The team used information put together from different papers where HEWL was specified and used a hanging-drop vapor diffusion technique. The crystals took approximately 36 hours to become visible to the naked eye.

The team is currently working on an apparatus that saturates air following the descriptions of a similar device used by Baba, Hoshino, Ho and Kumasaka in their 2013 paper, *Humidity control and hydrophilic glue coating applied to mounted protein crystals improves X-ray diffraction experiments*. Ben Feikema is currently leading the design and testing of this device. The team next intends to test that the air is in fact saturated and then devise a system to deliver that air to the wells in a crystallization plate or to a single drop of dissolved protein.

The team will carry out HEWL crystallization experiments again under these conditions to determine the effect of relative humidity the rate of crystallization. After success with lysozyme, the team intends to prove the value of their device by crystallizing BSA. The procedure to be used will be the hanging-drop vapor diffusion using optimized conditions published by Krauss, Sica, Mattia and Merlino[1]. Materials for this experiment will include the protein itself, methoxy poly(ethylene glycol) 5K, $MgCl_2$, and Tris HCl.

[1] Krauss, Sica, et al, *Increasing the X-ray diffraction Power of Protein Crystals by Dehydration: The case of Bovine Serum Albumin and a Suvery of Literature Data,* International Journal of Molecular Science (2012).

A Brief Overview



Protein Crystallization

- Hanging Drop Vapor Diffusion
    - Lysozyme
    - Bovine Serum Albumin

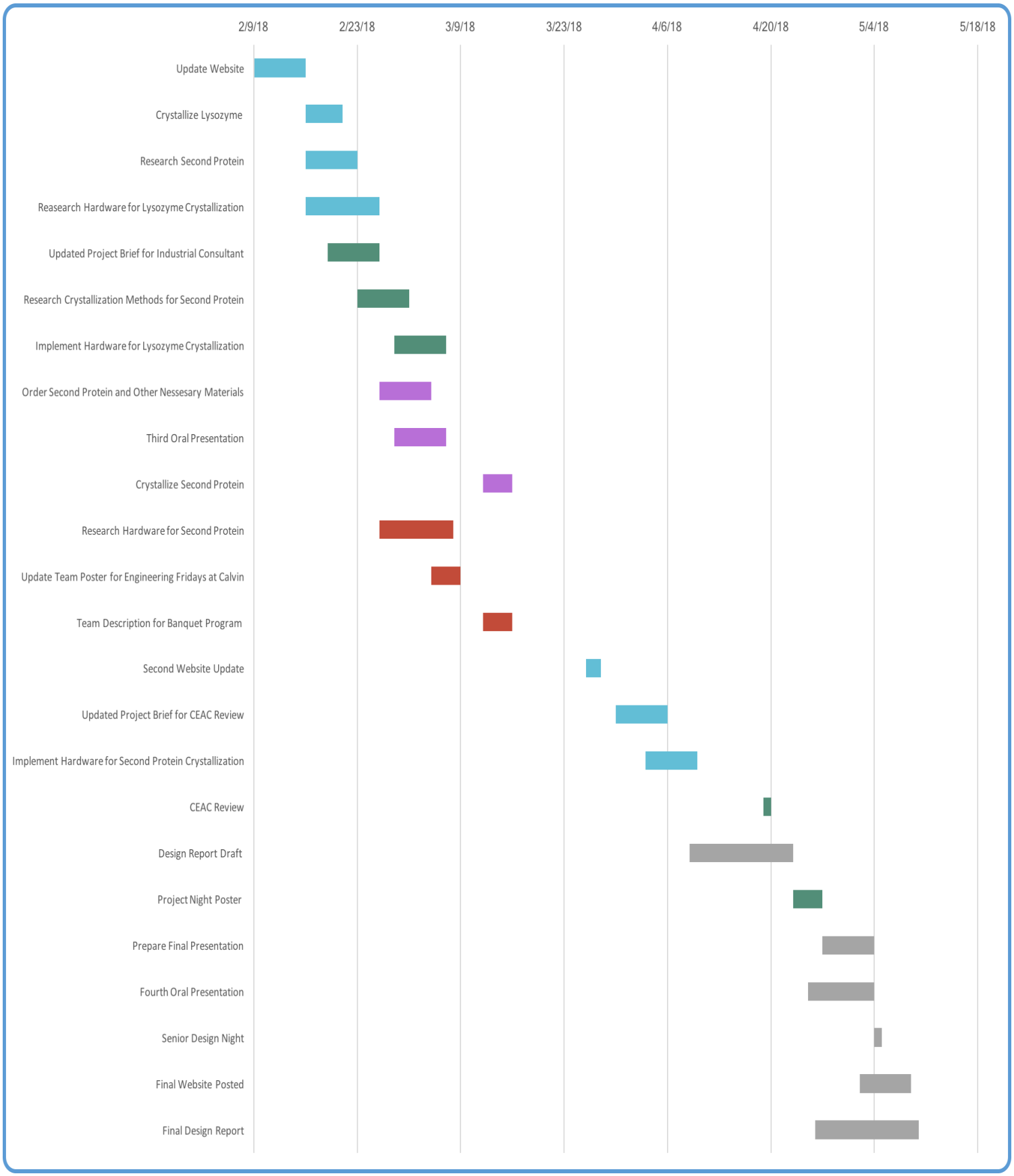
Optimization

- Relative humidity control device

Goal

- Observe if humidity control improves the crystallization process as judged by crystal size, speed of crystallization, microscope-observable quality of crystal, or other relevant factors.

Schedule


2/9/18
2/23/18
3/9/18
3/23/18
4/6/18
4/20/18
5/4/18
5/18/18
Update Website
Crystallize Lysozyme
Research Second Protein
Reasearch Hardware for Lysozyme Crystallization
Updated Project Brief for Industrial Consultant
Research Crystallization Methods for Second Protein
Implement Hardware for Lysozyme Crystallization
Order Second Protein and Other Nessesary Materials
Third Oral Presentation
Crystallize Second Protein
Research Hardware for Second Protein
Update Team Poster for Engineering Fridays at Calvin
Team Description for Banquet Program
Second Website Update
Updated Project Brief for CEAC Review
Implement Hardware for Second Protein Crystallization
CEAC Review
Design Report Draft
Project Night Poster
Prepare Final Presentation
Fourth Oral Presentation
Senior Design Night
Final Website Posted
Final Design Report