Syllabus for MBTP e^{2\pi i(hx + ky + lz)}: Crystallographic Workshop

Note: One or two target proteins (depending on the number of students) will be selected for their likely ability to crystallize and the presence of a molecular replacement search model. Students may choose to bring a protein from their lab to work on side-by-side, however the TAs and workshop leaders will not perform any preparation on those outside proteins. Students may coordinate with the TAs if they wish to move their personal project forward in parallel (for example, putting their cultures in the same incubator, being present for harvesting bacteria). Each session is anticipated to take ~2 hrs. Ideally, the sessions should be weekly.

SESSION 1: Optimizing protein expression.
In the first session, students perform test expression of two target proteins. The protein will be in a CSB vector (N-terminal His-tag and T7 promoter) and will be IPTG inducible. Expression will be at three temperatures (37° 4 hr induction; 30° 5 hr induction; 22° overnight) and with two types of medium (TB or LB). Students will evaluate whether the protein is soluble or aggregated (in the pellet) and identify whether there is robust expression by SDS-PAGE.

In preparation for SESSION 1, workshop leaders and TAs will make medium and start cultures.

SESSION 2: Protein purification.
In the second session, students will start with cell lysate for one protein and perform Ni^{2+}-affinity and size exclusion chromatography on an Akta system. Different teams of students will purify different target proteins, and each team will purify in one of three different buffers (HEPES, Tris, MOPS).

In preparation for SESSION 2, workshop leaders and TAs will grow large cultures of each of the target proteins (2-4 L), harvest, spin, and lyse to provide clarified lysate. Teams may need to split up to find enough Akta systems.

SESSION 3: Protein crystallization.
In the third session, students will use the Vanderbilt high throughput crystallization facility to perform broad screening. Each team will screen their protein against ~500 conditions using two protein concentrations. In this initial screen, proteins with known ligands (metalloactive sites, Ca^{2+}, SAM, substrates, inhibitors, etc.) will be crystallized in the presence and absence of carefully selected ligands in parallel.

In preparation for SESSION 3, workshop leaders will ensure that the facility is properly stocked with crystallization screens.

Homework. Students are to monitor the digital images of their crystallization reactions and identify any crystals using light and UV microscopy. They can direct questions about whether they have a crystal hit to the workshop leader. Hit conditions should be gathered so that reagents for optimization are on hand.

SESSION 4/5: Crystal optimization (2 sessions)
In the fourth and fifth sessions, students will identify 3-4 promising crystallization conditions, make stock solutions for optimization, and set grid screens. Students will learn grid screens in the first session, and seeding and additive screening in the second session.

In preparation for SESSION 4/5, workshop leaders will ensure that reagents are available for optimization.

SESSION 6: Cryoprotection.
In the sixth session, we will learn to mount and cryoprotect crystals. Students will learn how to select cryoprotectants. Crystals will be placed into a synchrotron puck for data collection.
In preparation for SESSION 6, workshop leaders will ensure that reagents are available.

Homework. Students must register for a structbio computer account.

SESSION 7: Diffraction data.
In the seventh session, we will correlate the workshop with an available synchrotron shift. Each team of students will collect data from crystals that they have optimized. If no target diffracts, the remainder of the workshop is performed with lysozyme (data collected on campus and not at the synchrotron). Students will learn how to align and orient crystals in the beam and how to collect and process diffraction data. Students will learn what data collection statistics tell you and how to perform a data cut.

In preparation for SESSION 7, workshop leaders and TAs will cryo preserve at duplicate crystals to provide samples that have only experienced expert handling.

Homework. Students should have Phenix, CCP4, and Coot installed on their laptops and determined to be functional.

SESSION 8: Structure determination and refinement.
In the eighth session, students will learn Phenix and Coot. They will determine the structure by molecular replacement in the Phaser module of Phenix and display the electron density in Coot. Students will learn the basics of model building. Students will learn what refinement statistics tell you and how to balance R factors with geometry and real space fitting. Students will learn what resolution limits are appropriate for adding water molecules and protons to a molecule, what B-factors are, and when TLS refinement is appropriate.

In preparation for SESSION 8, workshop leaders and TAs don’t have to do anything, but may wish to run through the data processing independently to help guide the session.

Homework. Students refine the structure and install Pymol on their laptops.

SESSION 9: Making figures in Pymol.
In the ninth session, students will use Pymol to make figures for a manuscript.

In preparation for SESSION 9, workshop leaders and TAs don’t have to do anything.

At the end of the Workshop, workshop leaders and TAs will ensure that at least one set of coordinates is sufficiently refined for deposition into the PDB. Workshop leaders are responsible for a manuscript draft. Workshop leaders are requested to have a first version of the manuscript to all students within 6 months and the manuscript will be developed with input from all authors. TAs are anticipated to be co-first authors, workshop students are anticipated to be interior authors, and workshop leaders are anticipated to be corresponding authors. Workshop leaders are expected to make the final call in author order based upon their perception of the contributions of the different students. Workshop leaders are welcome to discuss some of the more difficult aspects of corresponding authorship (such as assigning final author order and communicating decisions to all authors) with faculty. Workshop leaders are anticipated to submit the manuscript within 6 months after starting an independent faculty position.