Electron diffraction and atomic force microscopy for protein structure determination

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Cryo-electron crystallography is an emerging structural biology technique for determining protein structures. Up to now, atomic structures of model proteins as well as unknown peptide structures, have been solved using this technique. Besides being more easily accessible than synchrotron facilities, electron crystallography requires only small crystals in the nanometer size range. Due to the strong interaction of electrons with matters, electron crystallography is especially suitable for studying weakly diffracting crystals, such as many membrane protein crystals. Atomic force microscopy (AFM) is a near-field microscopy technique that allows topography (surface) imaging of single molecules at the nanometer scale. This project combines atomic force microscopy with electron crystallography to study nanometer sized protein crystals to extract maximum structural information on the proteins.

Protein crystals that are resistant to radiation damage (e.g. insulin, feruloyl esterase A) will be used as initial test systems, then, ultra-thin three-dimensional crystals will be tested. The student will learn how to produce these crystals, obtain electron diffraction data, and analyze the diffraction data. The student in this project will use atomic force microscopy to screen crystal sizes and establish the best conditions for crystal preparation for electron diffraction. Atomic force microscopy will also be used to determine the cell parameters of the crystals to guide the indexing of electron diffraction data. The student will be trained in scientific computing allowing the development of computational tools to optimize the data collection strategy for minimum exposure to the electron beam.

In the first year, the student will be trained in electron microscopy and atomic force microscopy. Model protein crystals will be prepared and techniques for preparing protein crystals for cryo-electron microscopy will be mastered. Data collection strategies for X-ray diffraction (e.g. BEST) will be adapted for electron diffraction in the second year. Electron diffraction data will be collected on model protein crystals using the developed programs and analyzed using existing macromolecular crystallography packages. The third year will be dedicated to determining a complete protein structure using molecular replacement and drafting manuscripts with the obtained results.