CBI Training Program Sabbatical Proposal

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For my sabbatical, I propose to learn how to image proteins by Atomic Force Microscopy (AFM) in Prof. Mark Banaszak Holl's lab at the University of Michigan. The Banaszak Holl group has used AFM in their study of materials for drug delivery and we have had previous collaborations with them for studying protein assemblages. Learning the technique of AFM from his group would greatly expand my research skills and help toward the advancement of my professional career. While there would be benefits to doing a sabbatical outside the University of Michigan, the close proximity will be beneficial for performing research after my sabbatical term has expired, since further experiments could be performed easily and at short notice.

The sabbatical portion of the CBI training grant presents a unique opportunity for the advancement of my research in the development of new protein biomaterials. Details of my research in designed and directed protein assembly have been presented previously. Briefly, I am exploring the assembly of protein building blocks (PBBs) into protein "cage" structures, similar in concept to artificial protein capsids. The strategy we have adopted for assembling protein cages is to utilize highly symmetrical proteins as PBBs which will mediate the assembly into geometrically favored structures, i.e. cages, and locked together using noncovalent linking agents. To demonstrate proof of concept, we are utilizing the KDPG Aldolase from *T. maritima* as a PBB, which consists of three identical subunits that form a quaternary structure that closely resembles a triangle. Geometry dictates that triangles can form various highly symmetrical 3-D structures; for example tetrahedrons, octahedrons, and/or icosahedrons depending on the number of units in the assembly. KDPG Aldolase was chosen for reasons including thermal stability, ease purification and obtaining useful amounts of protein, and catalytic activity for determining proper folding. To link the building blocks together we are using peptides designed to form heterodimeric coiled coils, which can be linked to any PBB by splicing genes encoding each together into a cloning vector and expressing in a host, such as *E. coli*.

To date genes have been made encoding the fusion proteins KDPG Aldolase and the coiled coil peptides and the proteins expressed and purified to yield good quantities of soluble proteins. Currently I am investigating the relative sizes of these fusion proteins using size exclusion chromatography (SEC) and analytical ultracentrifugation (AUC). While these techniques can give information about the size of protein assemblages, they do not directly indicate or show the structure(s) that are being formed. For my sabbatical I want to utilize the time in a lab in which I can learn a technique for visualizing such protein structures, which is vital to characterize the products of assembly.

Several techniques are available which can be used to image the proposed protein cages. These include transmission electron microscopy (TEM), atomic force microscopy

(AFM), and cryo-elecron microscopy (CEM). TEM is a relatively common technique and opportunities to learn the technique through EMAL is available in a one day session. However, AFM and CEM are more specialized techniques that would require more training time and would provide essential data that would not be obtained using TEM. AFM for instance can be used for obtaining heights of samples relative the surface the sample is appended to. Such information would be vital in the event that PBBs dimerize into a "sandwich" structure, a possibility previously not discussed. TEM would likely give images showing single PBBs that seemly did not assemble into a higher order structures because one PBB would be lying on top of the other PBB. Dimers of PBBs would give heights twice that of individual PBBs by AFM. Furthermore, AFM can be performed on proteins in solution, allowing one to investigate structures in their natural environment. Additionally, a broad range of AFM tips are available with different properties and functionalities appended, allowing a broad array of imaging to be performed. CEM would also be a useful technique for imaging and could be used to get more highly resolved images than can be obtained by TEM.