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2009 Rosalinde and Arthur Gilbert Foundation/AFAR New Investigator Award in Alzheimer Disease

Crystal structure of an intramembrane aspartyl protease

Summary of Progress

We have been focused on improving the quality of our crystals of signal peptide peptidase (SPP), which is a prerequisite for structure determination, and a major rate-limiting step of our project. Our ability to obtain initial crystals strongly indicates that this project ultimately will be successful. We are taking a multi-pronged approach to improve the crystals, including testing for stability and crystallization in different detergents, improving purification protocols, exploring 2D cryo-electron diffraction, and crystallization in complex with a chaperone. Our progress is summarized in further detail below.

Review of Specific Aims/Objectives

Aim #1: Express and purify SPP orthologs.

This aim was well underway last year, and we are now able to express and purify all variants of SPP for our studies, including catalytically impaired mutants. Variants include *H. marismortui* (mSPP), *A. fulgidus* (A. ful SPP), *H. salinarum* (H. sal SPP), *T. acidophilum* and *T. volcanium* SPPs and their respective double catalytic mutants (DM), discussed below, among others. Thus, our focus is now on achieving Aims 2 and 3, described in more detail below.

Aim #2: Crystallize SPP.

Crystallization of wild-type mSPP. Last year we reported crystallization of mSPP with diffraction limits of ~ 6 Å resolution. This continues to be the diffraction limit of our three-dimensional crystals (see Aim 3). To expand our crystallographic horizons, we have been working in collaboration with Prof. Ingeborg Schmidt Krey, an electron crystallographer at Georgia Tech who specializes in solving 2D membrane protein structures. Indeed, initial results look promising. Dialysis to remove detergent and reconstitute in liposomes (Lipid to Protein Ratio 15, T = 24°C) results in two-dimensional crystals with ordered mSPP as judged by Fourier transform analysis of the electron diffraction pattern (Figure 1). Optimization is underway.

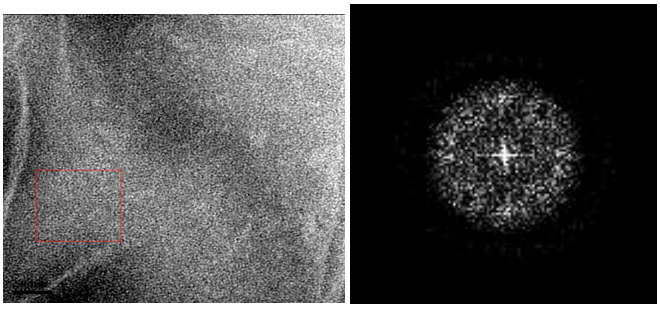

Figure 1. 2D crystallization of mSPP. mSPP reconstituted in proteoliposomes produces membranes (left). Fourier transform analysis of a small region of the membranes (right) demonstrates a lattice.

New complexes for membrane protein crystallization.

In parallel to efforts to extend the diffraction limit of wild-type mSPP crystals, we are investigating new chaperones for complexation with mSPP. My lab has teamed up with Jennifer Maynard at UT Austin, a protein engineer, to generate tailored antibody fragments with high affinity for epitopes on SPP. The first antibody fragment generated has rather high affinity for an EE-tag installed in SPP and was readily crystallized (Figure 2). We are now pursuing the cocrystallization of mSPP and this new chaperone, as well as developing this method more broadly, including different types of antibody fragments and different epitopes for recognition.

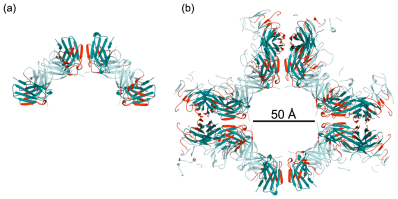

Figure 2. Crystal lattice of antibody fragment 3D5/EE_48. (a) Asymmetric unit. (b) Lattice structure with size of central cavity indicated. Lighter hue indicates the light variable chain while the darker hue indicates heavy variable chain. Complementary determining regions are depicted in red.

Aim #3: Solve the crystal structure of SPP by itself and with inhibitors.

Our best apo SPP crystals to date (~5x5x20 μm) still diffract to ~6 Å resolution, with several reflections observed at ~4 Å resolution, as reported in the original application**.** We have not yet improved the resolution of the crystals.

Presentations/Publications

Pai, J. C.; Culver, J. A.; Drury, J. E.; Lieberman, R. L., Maynard, J. A. Peptide-binding single chain antibody fragment (scFv) chaperones for protein cocrystallization. *Prot. Eng. Des. Sel.* 24 (2011) 419-428. (DOI: 10.1093/protein/gzq120).

Lieberman, R. L., Peek, M. E.; Watkins, J. D. Macromolecular X-ray crystallography. In Methods in Molecular Biology: Electron Microscopy (I. Schmidt-Krey and Y. Cheng, eds.), Humana Press: Springer, Totowa, NJ, in press, 2010.

Overall impressions/comments

I am incredibly appreciative for the AFAR funds. Since the start of the AFAR grant, I hired a new postdoctoral fellow, Dr. Jason Drury, and attracted several graduate and undergraduate students to the "SPP subgroup." These funds have been indispensible in giving my lab more freedom in pursuing new avenues for the structure of an intramembrane aspartyl protease. For example, the tailored antibody fragment work was awarded an NIH R21 (PI: Lieberman), and subsequently expanded to a 4-year NIH R01 (PI: Maynard) grant.