Enabling Structure Determination of Challenging Samples with new cryo Electron Microscopy Methods

Jose A. Rodriguez [1], David Eisenberg,[1] Logan Richards,[1] Ambarneil Saha,[1] Matthew Agdanowski,[1] Roger Castells-Graells (rcastellsg@ucla.edu),[1] and **Todd O. Yeates**[1]

[1]UCLA-DOE Institute for Genomics and Proteomics; University of California, Los Angeles. Los Angeles, CA 90095

https://www.doe-mbi.ucla.edu/ucla-doe-institute-missions/

Project Goals:

Research in the UCLA-DOE Institute for Genomics and Proteomics (IGP) includes major efforts in the area of imaging science, with key applications in microbial biosystems, from genomics to function. Our team is pioneering new enabling capabilities that facilitate the discovery of molecular structural features affecting protein function and specificity. These capabilities span the broad areas of X-ray diffraction, electron microscopy, and micro-electron diffraction, along with protein engineering and selection methods designed to advance those techniques. Emerging MicroED techniques present challenges in processing, refinement and phasing of diffraction data that our team is tackling in part by enabling rapid access to robust public-facing tools (webservers) that facilitate ED structure determination. Our team is also making critical advances to resolve protein structures smaller than about 50 kDa in size by cryo-EM. Success in that goal will be transformative for applying cryo-EM to broad problems in bioenergy science.

Abstract:

Breakthroughs in cryo-EM –
Cryo-electron microscopy has become a major tool for atomic structure determination. While its power has been widely demonstrated for analyzing large macromolecular complexes, signal-to-noise challenges make it difficult or impossible to resolve the structure of smaller proteins by cryo-EM. With a practical lower limit around 50 kDa, a large fraction of cellular proteins of interest remain outside the scope of this leading structural method. In recent work, our DOE-UCLA IGP team has broken through this barrier by engineering novel scaffolds with sufficient rigidity and modularity to achieve resolution useful for interpreting atomic structure. These unique scaffolds are based on designed protein cages fused to Darpins as modular protein adaptors. We will discuss soon-to-be published work, where we have created a second generation of scaffolds that are rigid enough to reach the critical resolution of 3 Å (Fig. 1). Our scaffolding approaches should open up EM-based structural studies on wide-ranging proteins and enzymes with bioenergy importance. Applications to select enzymes involved in cellulosic breakdown are planned, along with future applications of cage-based scaffolds as markers in cryo-EM tomography.

Enabling micro-ED methods -
Electron diffraction is reemerging as a frontier method for atomic structure determination from three-dimensional microcrystalline proteins and peptides. With micro-electron diffraction

(MicroED), the ability to work with very small crystals circumvents the most common obstacle to successful x-ray crystallographic work: obtaining large well-ordered crystals. However, other distinctions of the MicroED method bring up new challenges that need to be overcome in order for the method to reach its potential. Our UCLA-DOE IGP team is making important inroads on multiple key challenges in both crystal imaging and diffraction.

(i) We are developing new atomic scattering parameterizations, which are distinctly different from X-ray scattering profile for charged atoms, in order to clarify the important issues of charge states using MicroED. We have developed a public web server to make those parameters fully accessible at: https://srv.mbi.ucla.edu/faes/. This resource enables Gaussian parameterization of elastic electron scattering factors in a form amenable to refinement in the program Phenix. Current applications will be discussed.

(ii) For biomolecules that are within the size range of total synthesis (which includes many small proteins), we are applying methods of racemic crystallography, where phasing challenges are greatly mitigated, and for larger molecules with potentially novel folds, we are taking advantage of fragment-based phasing approaches (Fig. 2) and, in collaboration with PNNL, direct crystal imaging.

We are also developing and updating tools for validating and assessing the quality of three-dimensional protein structure models. That important topic is reemerging as a major challenge and opportunity in wake of the coming flood of predicted models from recent advances in machine-learning-based structure prediction.

Collectively, the enabling capabilities we are developing will broadly facilitate the determination and refinement of unknown macromolecular structures with importance for bioenergy.

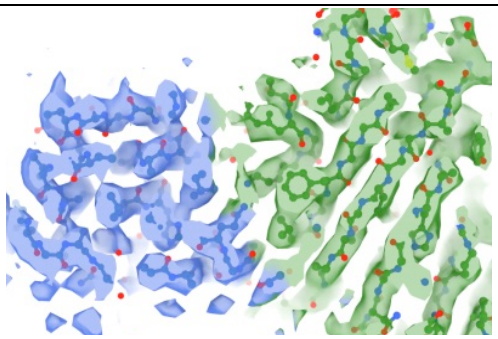

Figure 1. A cryo-EM map of a small protein (26 kDa GFP) bound to its modular adaptor (Darpin). The small cargo protein (green) has a resolution of 3.0 Å. The large designed cage that serves as the scaffold is not shown.

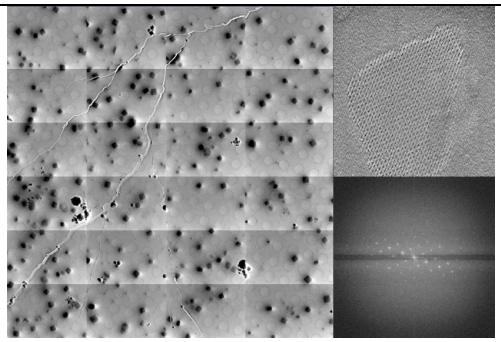

Figure 2. A field of cell-grown frozen-hydrated nanocrystals (left) is surveyed for tomographic reconstruction of a single crystal (right, top), whose Fourier Transform shows a lattice (right, bottom).

References

1. M.C. Thompson, T.O. Yeates, J.A. Rodriguez (2020). Advances in methods for atomic resolution macromolecular structure determination. *F1000Res* **9**: doi: 10.12688/f1000research.25097.1.

This work is supported by DOE Grant DE-FC02-02ER63421.