Your Name: Ashlee Plummer

Your PI's Name: Maofu Liao

Project Name: Structural characterization of the human cholesterol exporter, ABCA1

Describe specimen, biological relevance, and brief background:

Cholesterol homeostasis requires the efficient removal of this sterol from the plasma membrane that encapsulates human cells. Disruption of this expulsion pathway leads to cholesterol accumulation in the plaques that are associated with cardiovascular disease. Cholesterol and phospholipids are selected from the membrane by the membrane protein, ATP-Binding Cassette Protein A1 (ABCA1), and transferred to the extracellular acceptor protein, Apo-lipoprotein AI (apo-AI) for High-Density Lipoprotein (HDL) formation and expulsion from the body. ABCA1 also flips lipids across the membrane bilayer by hydrolyzing ATP to assist in the maintenance of the asymmetric bilayer composition that is characteristic of the plasma membrane. It is currently unknown how ABCA1 selects certain lipids and cholesterol for translocation and how this lipid-flipping function and ATP-hydrolysis are related to apo-AI binding and HDL formation. To better understand the complex interplay between the multiple functions of ABCA1, this project aims to utilize cryo-electron microscopy to resolve the specific interactions between ABCA1 and cholesterol/phospholipids by determining the structure of ABCA1 reconstituted into a lipid membrane. The specimen supplied for analysis will contain ABCA1 reconstituted into a nanodisc, which contains a patch of lipid bilayer surrounded by a membrane-scaffold protein.

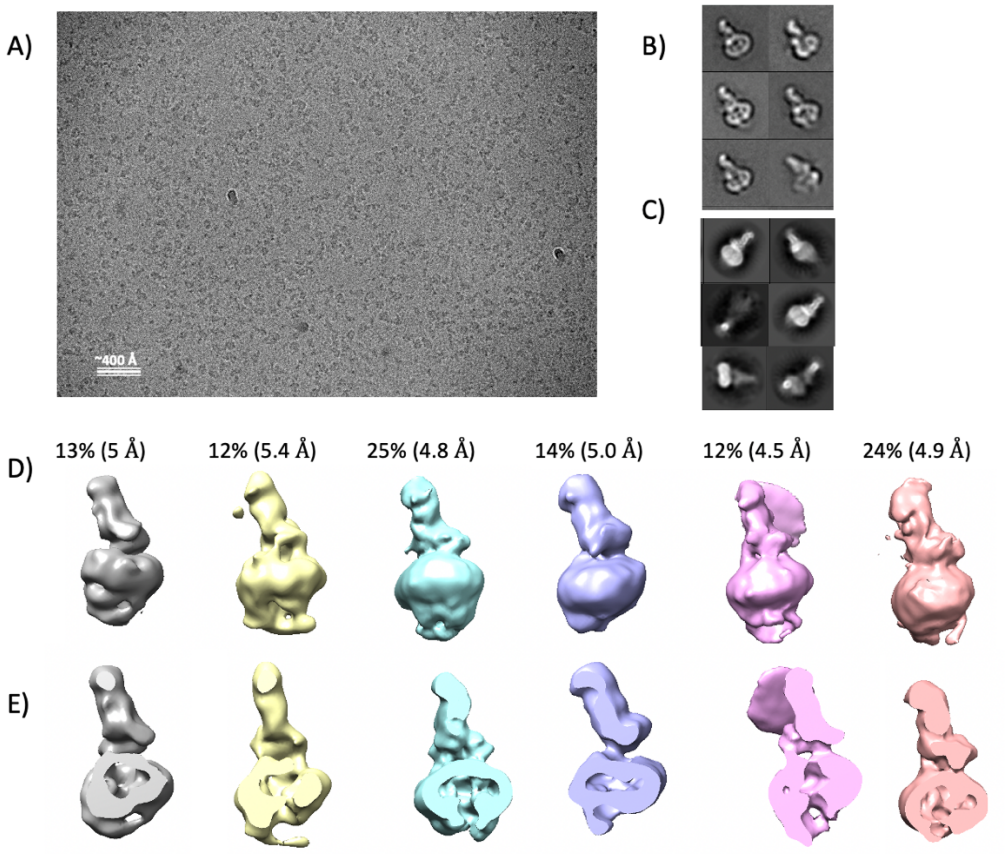
Prior results for a data collection of ABCA1 reconstituted into nanodiscs (Talos Project ID CEMC0010) are shown in Figure 1 and show promising particle density and homogeneity. Samples including ATP analogs such as ATP-γ–S will be provided to elucidate conformations of various states of ABCA1 in its ATP hydrolysis cycle.

Specimen molecular weight: ~350 kDa (~250 kDa protein with ~100 kDa nanodisc)

Specimen dimensions: ~250 Å height, ~120 Å nanodisc diameter

Data collection on either the Talos Arctica or Titan Krios requires upload of cryo images (with scale bars) and 2D classes with a description of your previous TEM usage and processing scheme:

Figure 1



A) Representative image of 1 mg/mL ABCA1 reconstituted into nanodiscs, collected on the Talos Arctica at 36,000x magnification. This dataset included ~ 1200 micrographs collected in counting mode with a pixel size of 1.13 Å and dose rate of 1.09 $e^-/Å^2$. **B)** Representative 2D class averages generated with 'samclasscas.py' of nanodisc-reconstituted ABCA1 (~45,000 particles). **C)** Representative Relion3 2D class averages of nanodisc-reconstituted ABCA1 (~260,000 particles). **D)** Relion3 3D classification results for a selection of ABCA1 particles (~65,000 particles). Each of the six classes is labelled with its percentage population of particles and reported resolution from Relion3. **E)** Slices of the 3D classes from **D)** to indicate transmembrane densities.

Requested microscope (Talos Arctica or Titan Krios): Titan Krios

Phase plate (yes or no): No

Requested number of days (up to 4): 4