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Your PI's Name: Maofu Liao

Project Name (3-5 words): Structural characterization of human ABCA1

Describe specimen, biological relevance, and brief background (maximum 500 words):

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 diseases. The membrane protein, ATP-Binding Cassette protein A1 (ABCA1) specifically recognizes cholesterol and phospholipids in the plasma membrane, and by harnessing the energy of ATP binding and hydrolysis, translocates these lipids to the extracellular acceptor protein, apolipoprotein AI (apo-AI) for high-density lipoprotein (HDL) formation and expulsion from the body. ABCA1 also flips lipids across the membrane bilayer to assist in the maintenance of the asymmetric bilayer composition that is characteristic of the plasma membrane. It is currently unknown how ABCA1 selects specific lipids and cholesterol for translocation and how this lipid-flipping function and ATP-hydrolysis are coupled to apo-AI binding and HDL formation. To better understand the complex interplay between the multiple functions of ABCA1, this project aims to utilize single-particle cryo-electron microscopy to characterize the specific interactions between ABCA1 and cholesterol/phospholipids by determining structures of ABCA1 reconstituted into a lipid membrane (i.e., nanodisc). The specimen supplied for screening will contain ABCA1 reconstituted in nanodiscs containing different lipids, in the absence or presence of various nucleotides such as ATP, ATP- γ -S, ADP-vanadate and ADP.

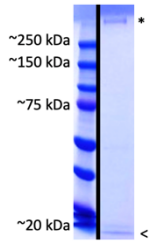
Specimen molecular weight: ~300 kDa

Specimen dimensions: Height ~ 200 Å, Diameter ~ 100 Å

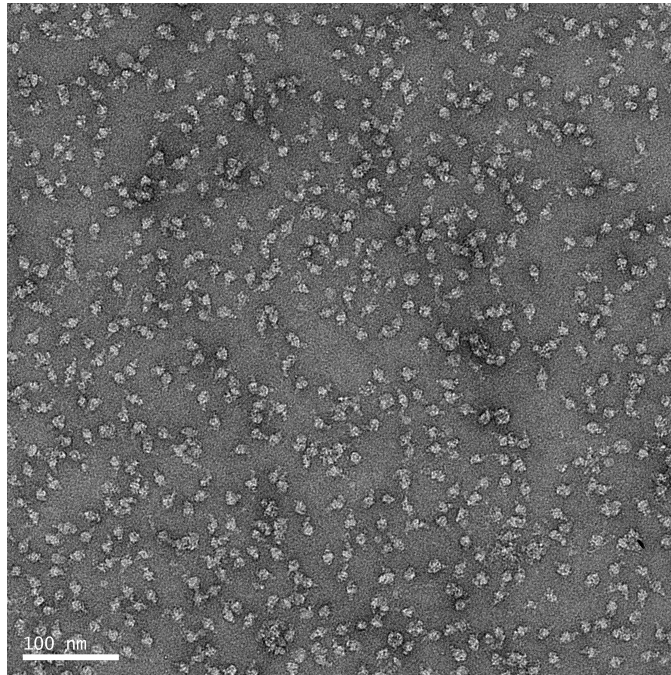
Specimen screening on the Talos Arctica requires biochemistry-related files (evidence of biochemical homogeneity such SDS page, SEC traces etc.) and negative stain images or 2D classes: See attached page.

Requested number of days (up to 4) on the Talos Arctica: 4 days

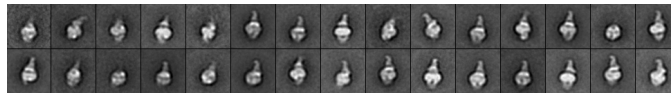
A)



B)



C)



A) SDS-PAGE gel illustrating the purity of nanodisc-reconstituted ABCA1, with ABCA1 and the nanodisc scaffold protein bands demarcated by an asterisk and arrow, respectively. **B)** Representative negative stain EM image of 0.02 mg/mL nanodisc-reconstituted ABCA1 collected on a Tecnai T12 at 67000x magnification. **C)** 2D class averages of nanodisc-reconstituted ABCA1 from a small negative stain EM dataset collected on a Tecnai T12.