Structural variability of high-density lipoproteins

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ABSTRACT

The in vivo function of high-density lipoprotein (HDL) is reverse cholesterol transport (RCT, Fig. 1), which is understood to inhibit cardiovascular diseases. Models of the detailed molecular structures of HDL remain controversial, and it is likely that individual HDL particles exhibit structural variations. To better understand the morphology of HDL and learn how particles grow and accommodate variable amounts of lipid, our approach is to image reconstituted and nascent HDL (r/nHDL) using electron cryomicroscopy (cryoEM) and measure individual particles to quantify their underlying size and shape distributions. Here we show the coarse 2D and 3D morphology of r/nHDL and describe visually and statistically the extent of their structural variability.

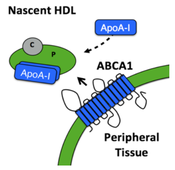


FIGURE 1: nHDL formation. nHDL is formed via the interaction of plasma apolipoprotein A-I (apoA-I) and ATP-Binding Cassette Transporter A-I (ABCA1).

HDL STRUCTURE

Despite its simple composition, the structure of HDL has been debated since the 1970s, and numerous others have been proposed on the basis of spectroscopic and imaging data (Fig. 2). nHDL particles and their synthetic analogues consist of a lipid bilayer believed to be circumscribed by antiparallel dimers of apolipoprotein A-I (apoA-I). Curiously, the purely discoidal models suggest no mechanism for the incorporation of additional lipid/protein. On the other hand, the "twisted-belt" (4) has received relatively few citations despite its ability to describe growth via changes in particle geometry (i.e. ellipse to disc).

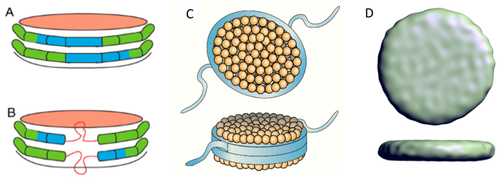


FIGURE 2: HDL structural models. **A.** "Double-belt" model (1,2). **B.** "Solar-flares" model (2,3). **C.** "Twisted-belt" model (4). Note that the tails on this particle represent HIS tags used in the experiments used derive this model. **D.** Top and side view of rHDL single particle cryoEM structure (5).

It is likely that individual HDL particles exhibit dynamic structural differences, but prior experiments have not rigorously verified this hypothesis. Here, we explore the underlying size and shape distributions of n/rHDL to identify structural motifs to better understand mechanisms responsible for the observed size and shape distributions. Since HDL size and shape are directly driven by the fundamental biophysics of protein-lipid interactions, our data is directly related to the energetic barriers governing this system. Unlike most biophysical methods, which provide information only about ensembles, our analyses permit true studies of individual HDL particles. Moreover, through statistical aggregation of our particle measurements, we can link relate our findings back to prior spectroscopic and chromatographic data, thereby relating instance properties to ensemble properties.

SAMPLE PREPARATION

Nascent HDL was obtained following a published protocol (6). BHK cells were loaded with free cholesterol (FC) via methyl-beta-cyclodextrin (mbCD) and incubated with human ApoA-I to produce 2.5mM mbCD-FC nHDL particles, which were purified using size-exclusion chromatography (SEC, Fig. 3). Reconstituted HDL nanodiscs were formed via a well-established protocol (7) involving the incubation of DMPC multilamelar vesicles (MLV) containing 10mol% free cholesterol (FC) with ApoA-I derived from human plasma.

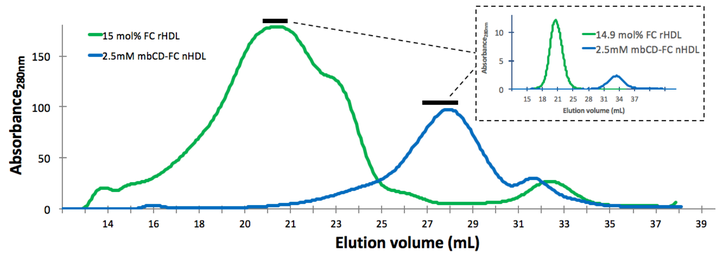
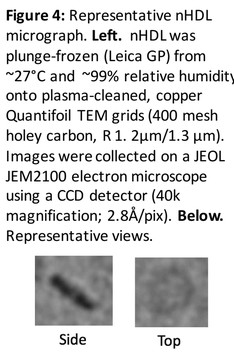


FIGURE 3: SEC of n/rHDL on Superose 6 column

2D ELECTRON CRYOMICROSCOPY

Figure 4: Representative nHDL micrograph. **Left.** nHDL was plunge-frozen (Leica GP) from ~27°C and ~99% relative humidity onto plasma-cleaned, copper Quantifoil TEM grids (400 mesh holey carbon, R 1. 2µm/1.3 µm). Images were collected on a JEOL JEM2100 electron microscope using a CCD detector (40k magnification; 2.8Å/pix). **Below.** Representative views.



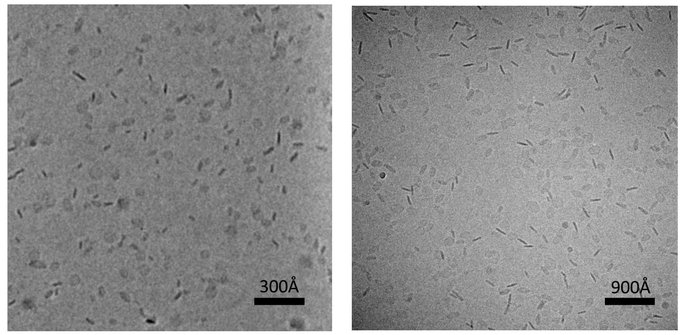
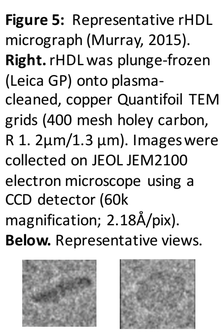


Figure 5: Representative rHDL micrograph (Murray, 2015). **Right.** rHDL was plunge-frozen (Leica GP) onto plasma-cleaned, copper Quantifoil TEM grids (400 mesh holey carbon, R 1. 2µm/1.3 µm). Images were collected on JEOL JEM2100 electron microscope using a CCD detector (60k magnification; 2.18Å/pix). **Below.** Representative views.



2D INDIVIDUAL PARTICLE ANALYSIS

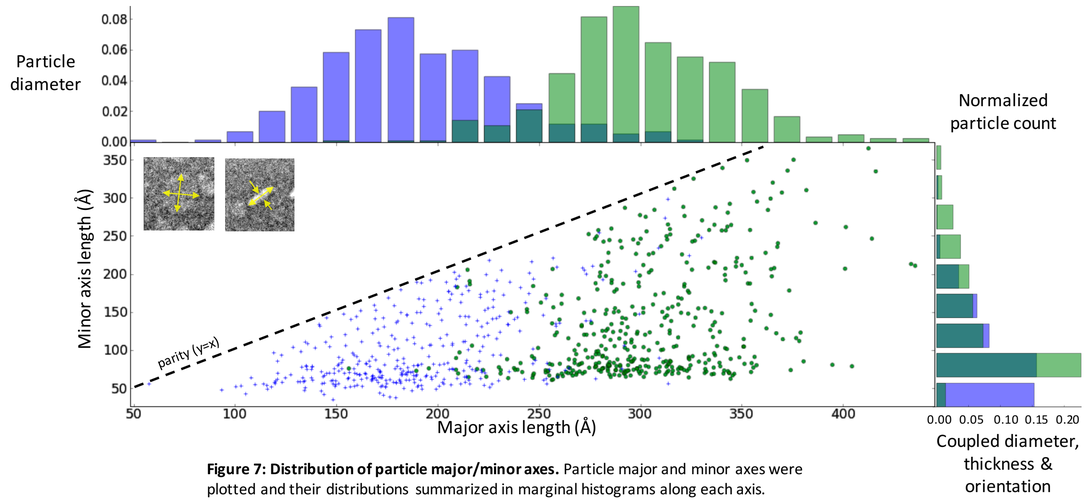


Figure 7: Distribution of particle major/minor axes. Particle major and minor axes were plotted and their distributions summarized in marginal histograms along each axis.

We measured the major and minor axis of a random population of 2D particle images. Measurements were plotted for nascent and reconstituted HDL to compare and contrast. The mean major and minor axis lengths of nascent HDL were 190.3±43.6Å and 100.7±49.2Å, whereas rHDL measured 303.0±41.1Å and 136.1±71.6Å. While particle diameter can be obtained from the major axis distributions and bilayer thickness can be approximately inferred from the peaks of the minor axis distributions, the minor axes represent a combination of particle orientation and thickness/diameter, which will require 3D imaging and reconstruction methods to decouple.

CONCLUSIONS

- rHDL is a good structural analogue for studying nHDL in-vitro
- Despite SEC, population remains heterogeneous
- Particle thickness cannot be decoupled from orientation in 2D
  - Tomographic (3D) imaging required
- Site-specific labels would help reduce orientational ambiguity
- Statistical aggregation of individual particle measurements can link instances to ensemble properties

FUTURE DIRECTIONS

We are now extending these studies by focusing on an even larger population of nascent and reconstituted HDL with nanogold labels to localize specific residues of apoA-I (Fig. 6). By imaging a larger distribution of particles in 3D, we hope to ascertain the existence of geometric constraints governing particle growth and lipid uptake. Our labeling will aid in subsequent alignment and averaging of homogeneous subgroups we observe. Additionally, to better understand the process of spontaneous and protein-mediated HDL formation, we will also image lipid vesicles and whole cells incubated with labeled apoA-I.

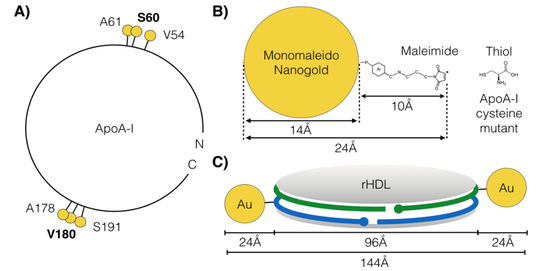


FIGURE 6: Nanogold labeling of apoA-I. **A.** We have computationally chosen specific apoA-I$_{CYS}$ mutants with the constraint that the hydropathy of apoA-I must change as little as possible. **B.** Our chosen label is 1.4nm nanogold, which can be bound to our CYS mutants via a functional maleimide group. **C.** Once labeled, we expect our particles to appear similar to this model.

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