Oligomerization and Structural Reconstruction Characterization of an LPL Filament

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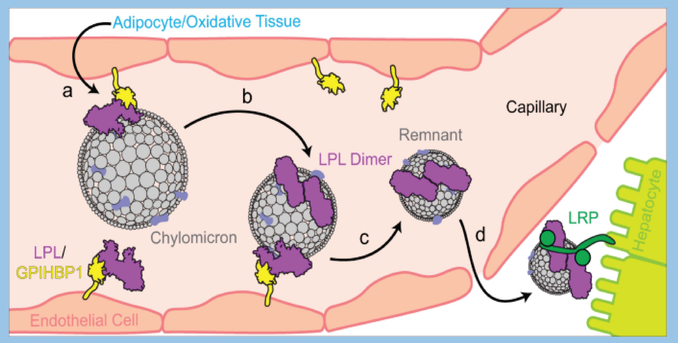


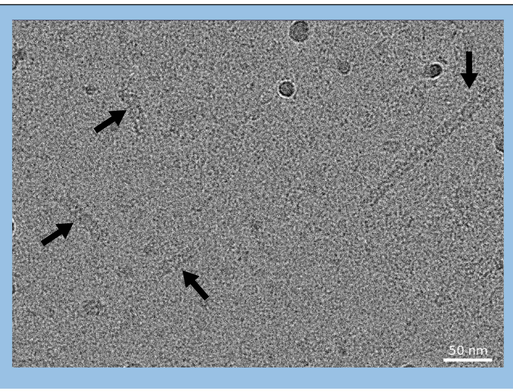
Gunn, Kathryn H., and Saskia B. Neher. "Structure of Dimeric Lipoprotein Lipase Reveals a Pore Adjacent to the Active Site." Nature News, May 4, 2023. https://www.nature.com/articles/s41467-023-38243-9#citeas.

Background

Lipoprotein lipase (LPL) is an enzyme that hydrolyzes triglycerides from lipoproteins like chylomicrons. The metabolization of triglycerides is vital for maintaining proper function in blood vasculature of the human body. When triglyceride rich lipoproteins build up in capillaries, they begin to clog, putting stress on the heart that can result in heart disease. Currently, cardiovascular disease ranks as the number one cause of death in the United States, with approximately one-half of American adults afflicted by it[1].

Previously, Dr. Gunn, an assistant professor at Stony Brook University, solved the structure of ~25 nm diameter helical LPL oligomer[2]. However, Dr. Gunn observed that the LPL helices inside of secretory vesicles were roughly 12 nm in diameter, prompting further investigation into the physiological form of the LPL helical oligomer.

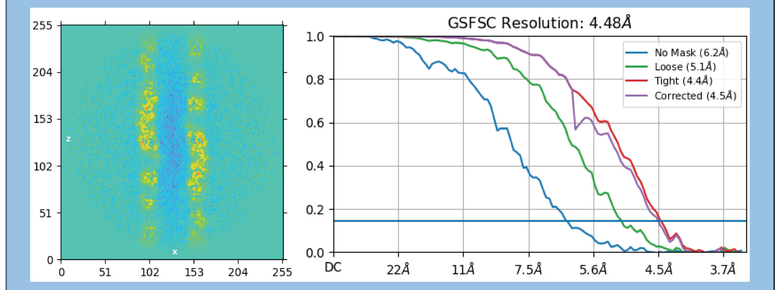
References
1. American Heart Association News (2019, January 31). Cardiovascular diseases affect nearly half of American adults, statistics show https://www.heart.org/en/news/2019/01/31/cardiovascular-diseases-affect-nearly-half-of-american-adults-statistics-show
2. Gunn, K. H., Roberts, B. S., Wang, F., Strauss J. D., Borgnia M. J., Egelman, E. H., & Neher, S. B. (2020, April 24). The structure of helical lipoprotein lipase reveals an unexpected twist in lipase storage. PNAS. Retrieved January 25, 2023, from https://www.pnas.org/doi/abs/10.1073/pnas.1916555117



Methods

Using negative stain microscopy, the concentration of 12 nm diameter LPL filaments was qualitatively compared between conditions varying in buffers, salt concentration, and pH level. After identifying the most promising condition for enrichment of 12 nm LPL filaments (consisting 20 mM HEPES buffer, 500 mM NaCl at a physiological pH of 7.4) samples were applied to 300-mesh holey carbon copper grids, coated with 2 nm continuous carbon. We used cryogenic electron microscopy (cryoEM) to collect micrographs of LPL in these conditions, as seen in the figure above.

These micrographs were analyzed using data processing software like CryoSPARC and Relion. Particles were picked through a variety of methods including by hand, template pickers, and Topaz, which utilizes a neural network picker. The picked particles were classified two-dimensionally before these classifications were used to create three-dimensional reconstructions. Through this workflow, over 3 million particles were sorted through, with over 200 thousand classified two-dimensionally, and almost 50 thousand used in the final helical reconstruction. Helical reconstruction was done using parameters determined from an average power spectrum of the individual particles, as seen in the figure to the right. The positioning of the layer lines are significant for elucidating the repeats inside the helix and determining the final structure. The parameters of the final structure were found to be 129 Å, 5.6 subunits per turn, a rise of 23.6 Å, and a twist of -66°.



Conclusion and Future Directions

From this processing, a model of the 12 nm LPL filament at 4.48 Å resolution was produced. With the findings during this research period, a crucial step in further work in understanding of the structure of LPL has been accomplished. In the future, pharmaceuticals targeting the mechanisms and pathways of this LPL oligomer may assist in lipoprotein lipase digestion of lipoproteins.