Fluorescence correlation spectroscopy to measure the metabolism of high-density lipoprotein

RUSSELL DEITRICK, EMILY GIBSON, University of Colorado Denver, Dept of Physics, HAMID RAZZAGHI, University of Colorado Denver, Dept of Cardiology — High-density lipoprotein (HDL), referred to as the “good cholesterol”, carries free cholesterol to the liver to be filtered from the bloodstream and is important to our understanding of atherosclerosis. HDL is metabolized in part by the enzyme Endothelial Lipase (EL). With this project we will use fluorescence correlation spectroscopy (FCS) to study the metabolism of HDL by EL comparing wild type with different genetic mutations. FCS is an advanced microscopy technique in which we record fluctuations in the fluorescence of dye-labeled molecules (in this case, HDL labeled with Nile Red) as they freely diffuse through a small focal volume. This data can be analyzed mathematically using the cross-correlation function, from which we can ultimately ascertain much information. In our case, we are interested in the diffusion coefficient which, via the Stokes-Einstein relation for a sphere, we can determine the size of HDL as it undergoes the process of metabolism. Preliminary results seem to indicate that the metabolic process occurs very quickly, that the final size of HDL depends primarily on the concentration of EL, and that the wild and mutant variants of EL have a similar effectiveness. In following experiments, we hope to investigate these relationships further.

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