SUPPLEMENTAL INFORMATION:

Aromatic Residues in the C-terminus of Apolipoprotein C-III Mediate Lipid Binding and LPL Inhibition

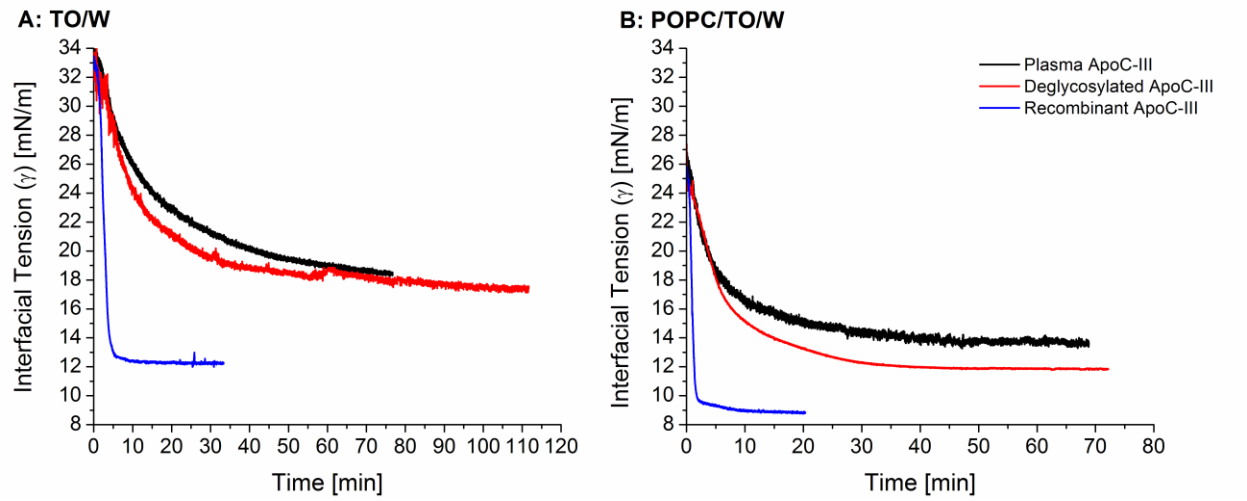
Nathan L. Meyers[1,4], Mikael Larsson[2], Evelina Vorrsjö[3], Gunilla Olivecrona[3], and Donald M. Small[1]

[1]Department of Physiology and Biophysics, Boston University School of Medicine, 700 Albany Street W302, Boston, MA 02118

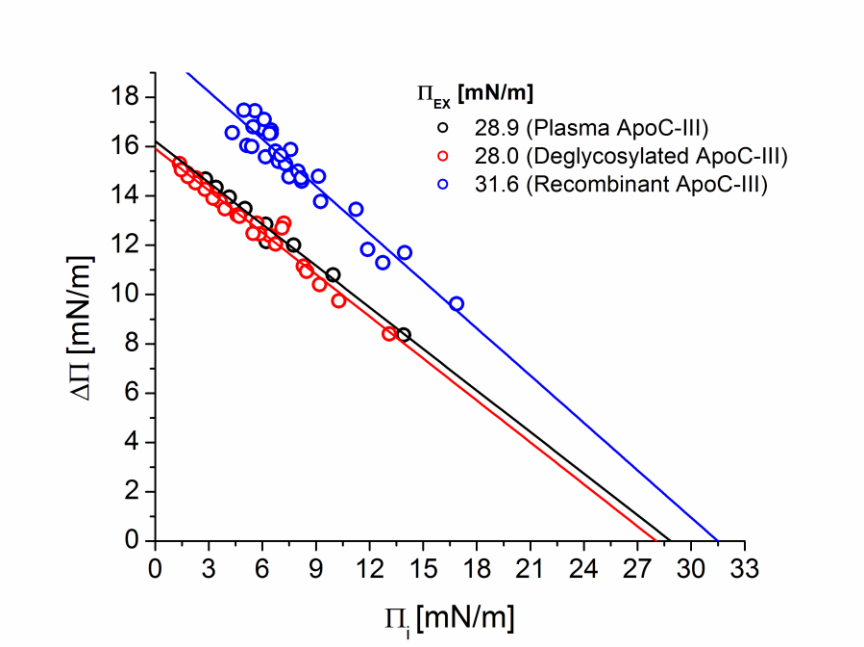
[2]Department of Medicine, University of California Los Angeles, 650 Charles E. Young Drive South, Los Angeles, CA 90095

[3]Department of Medical Biosciences/Physiological Chemistry, Umeå University, SE-901 87 Umeå, Sweden

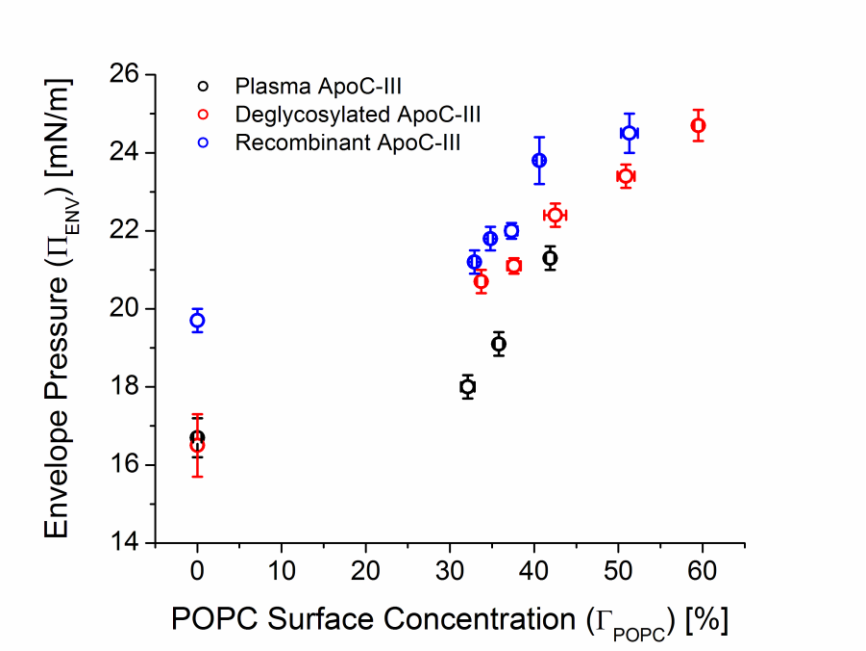
[4]Department of Virology and Immunology, Gladstone Institutes, 1650 Owens St 5th floor, San Francisco, CA 94158



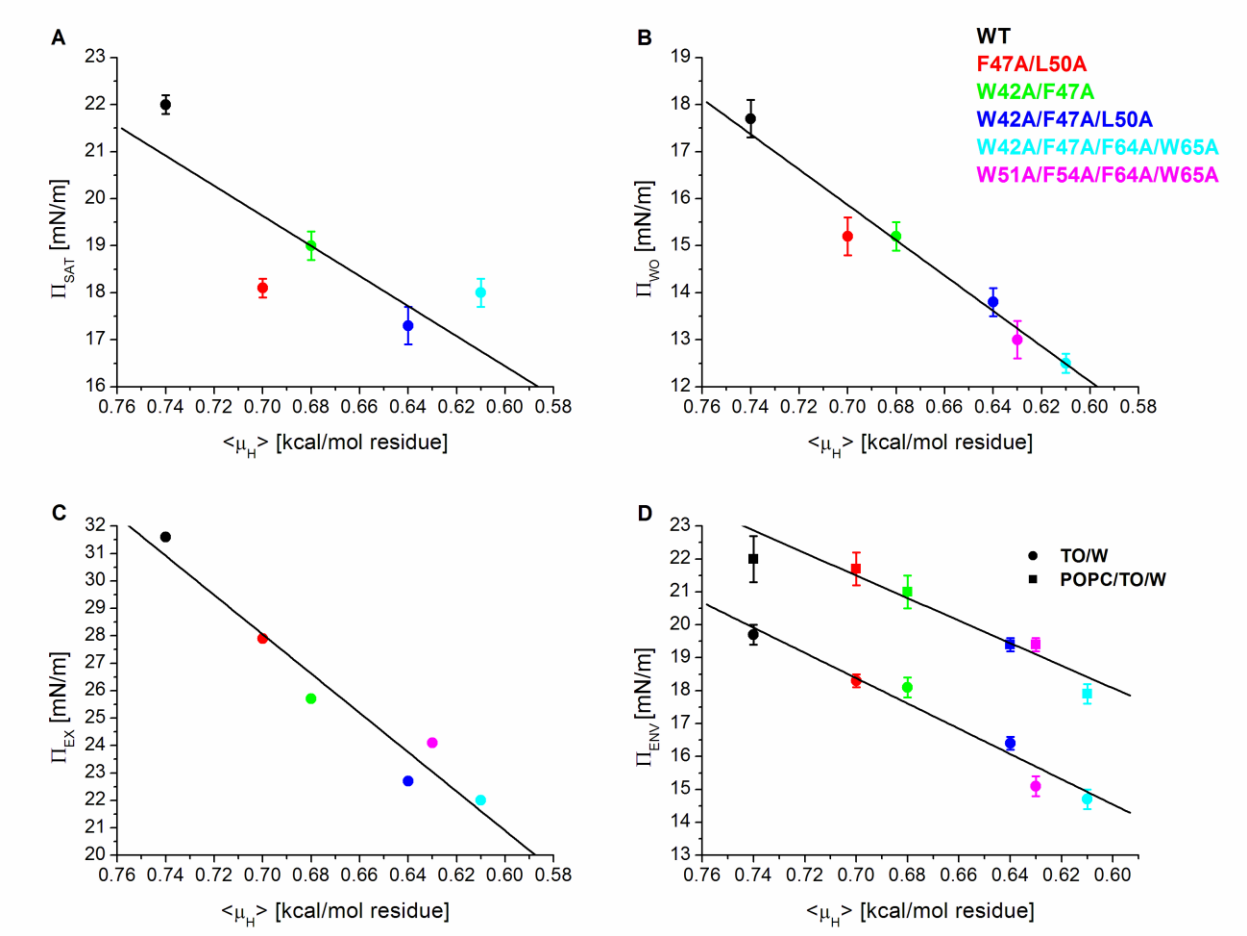
Supplemental Fig. S1: The C-terminal 6-His tag in recombinant apoC-III enhances the ability of the protein to bind to and modify lipid surfaces. (A) Representative interfacial tension (γ) versus time curves for the adsorption to a TO/W interface of plasma, de-glycosylated plasma, and recombinant apoC-III peptides. Plasma apoC-III sample was de-glycosylated by the Protein Deglycosylation Mix II (NEB) and isolated by HPLC. Successful de-glycosylation was shown by SDS Gel Electrophoresis (not shown). In individual experiments, recombinant, plasma, and de-glycosylated were added to the bulk phase at a concentration of 2.5 μg/mL and absorbed to a TO/W interface. Peptide adsorption decreased γ from γ_{TO} to an equilibrium value (γ_{eq}). This corresponded to an increase in surface pressure (Π) of ΔΠ. Plasma and de-glycosylated apoC-III exhibited similar adsorption kinetics, and both increased Π by 14 mN/m. By comparison, recombinant apoC-III exhibited faster adsorption kinetics and increased Π by 20 mN/m. (B) After generation of POPC/TO/W interfaces of Γ_{POPC} = 37.0±1.0% (Π_i = 6.5±0.2 mN/m), peptide was injected into the bulk phase at a concentration of 2.5 μg/mL in individual experiments with all three apoC-III variants. Similar to a TO/W interface, recombinant apoC-III exhibited fast adsorption kinetics and a large ΔΠ of 16.5 mN/m at a POPC/TO/W interface. By comparison, plasma and de-glycosylated apoC-III exhibited slower adsorption kinetics and smaller ΔΠ of 11.5 and 13.5 mN/m, respectively.



Supplemental Fig. S2: The C-terminal 6-His tag in recombinant apoC-III increases protein exclusion pressure (Π_{EX}). A series of experiments similar to Fig. S1B was conducted for plasma, de-glycosylated plasma, and recombinant apoC-III over a range of Π_i. $\Delta\Pi$ was plotted against Π_i for all three peptides. Each data set was fit linearly, and these linear regressions were significant ($-0.99 < R < -0.96$, $p < 0.0001$). X-intercepts represent Π_{EX}, the pressure at which a peptide cannot bind POPC/TO/W interfaces. Recombinant apoC-III modified these interfaces to a greater extent than native and de-glycosylated plasma forms of apoC-III, as indicated by $\Delta\Pi$ values that were 2 to 3 mN/m higher at all Π_i. Recombinant apoC-III also showed the ability to insert into POPC/TO/W interfaces of higher pressures, as indicated by higher Π_{EX} than the glycosylated and de-glycosylated plasma forms of apoC-III.



Supplemental Fig. S3: The C-terminal 6-His tag in recombinant apoC-III increases its retention pressure at lipid/water interfaces of varying POPC concentrations. In separate experiments, all three apoC-III variants were added to the bulk phase at 2. 5 µg/mL and adsorbed to a TO/W interface or POPC/TO/W interfaces of various initial pressures (Π_i). After a 150-mL washout, each peptide/lipid/water interface was expanded and compressed at a rate of 1.2 µL/min, similar to the protocol shown in Fig. 4A. Π_i values were converted to Γ_{POPC}, as described previously (49). γ values during the compression phase were converted to Π and the retention pressures (Π_{ENV}) of apoC-III peptides at lipid surfaces were calculated as described at a TO/W interface in Fig. 5. Π_{ENV} values were plotted as a function of Γ_{POPC} for each apoC-III variant. X- and y-error bars are the standard deviation from n = 2-3 experiments.



Supplemental Fig. S4: All biophysical parameters measured in this study correlate with the hydrophobicity of the apoC-III variants. The mean hydrophobic moment (<μ_H>) was calculated for each apoC-III variant, as described in Table 1. The biophysical parameters Π_{SAT} (A), Π_{WO} (B), Π_{EX} (C), and Π_{ENV} (D) were plotted against <μ_H> of the peptides. Π_{ENV} values were plotted for a TO/W interface and POPC/TO/W interface of Γ_{POPC} = 37.0±1.0%. Y-error bars are the standard deviation from n = 2-3 experiments. Linear regressions were applied to all plots. These fits were significant, with $0.95 < R < 0.99$, $p < 0.004$ for plots in panels (B-D). $R = 0.77$, $p = 0.12$ for the linear regression in (A).