Thermal Transitions in Serum Amyloid A 1.1 in Solution and on the Lipid: Implications for Structure and Stability of Acute-Phase High-Density Lipoproteins

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SUPPLEMENTAL MATERIAL

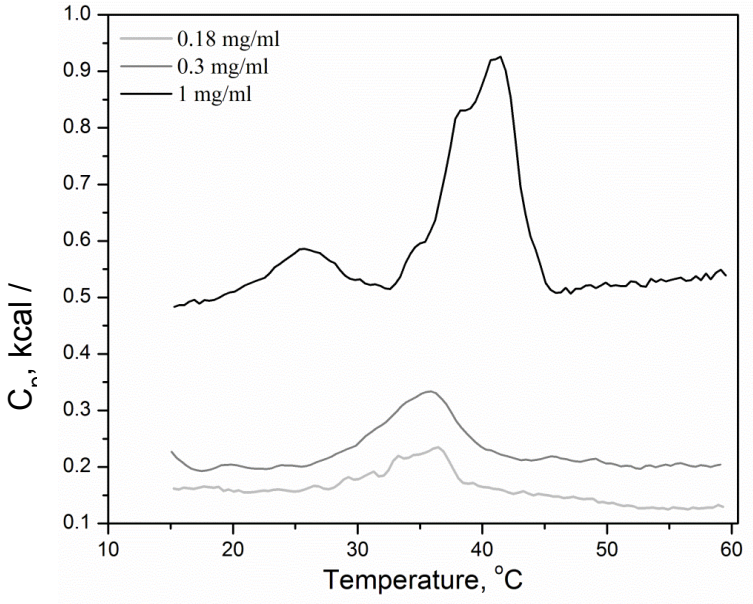

Figure S1. Concentration dependence of the calorimetric transition of lipid-free SAA.

DSC data were recorded from three samples containing 0.18, 0.3 or 1.0 mg/ml SAA in standard buffer.

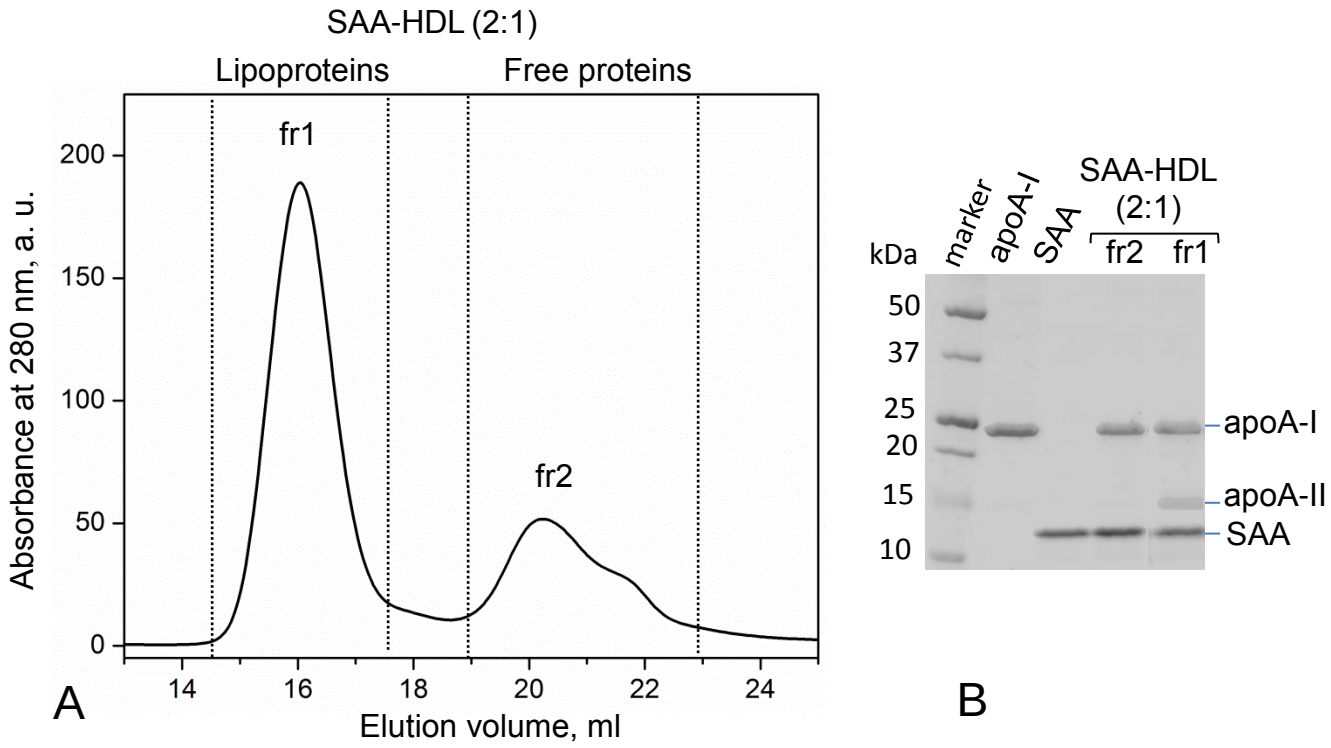

Figure S2. Analysis of SAA-HDL by analytical-grade size exclusion chromatography (A) and SDS PAGE (B). HDL were incubated with SAA at 2:1 SAA:apoA-I molar ratio. SEC was performed with a Superose 6 10/300 GL column controlled by an AKTA UPC 10 FPLC system (GE Healthcare). Elution by PBS (10 mM Na Phosphate, 150 mM NaCl, pH 7.5) was carried out at a flow rate of 0.5 ml/min. Two major peaks corresponding to lipoprotein-only fraction (fr1) and free proteins (fr2) were isolated and analyzed by SDS PAGE (B). Free apoA-I and SAA are shown for comparison. The major HDL proteins including apoA-I, apoA-II, and SAA are indicated.

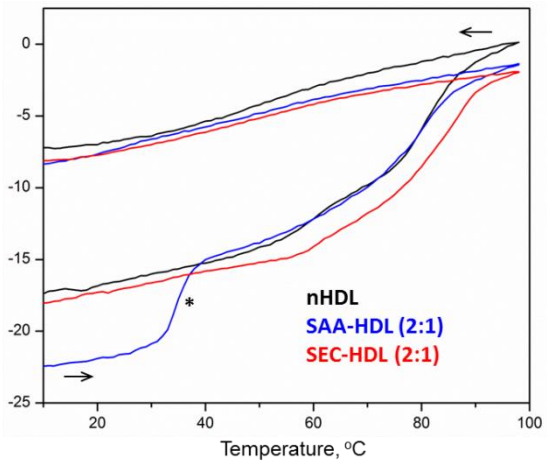

Figure S3. Thermal denaturation of SEC-HDL(2:1) analyzed by circular dichroism spectroscopy. Normal HDL contained 0.1 mg/ml apoA-I; SAA-HDL(2:1) contained 0.1 mg/ml apoA-I and 2:1 SAA:apoA-I molar ratio, which corresponds approximately to 0.085 mg/ml SAA. SEC-HDL was lipoprotein-only fraction isolated by SEC from SAA-HDL(2:1) as described in Figure S2; the total protein concentration in this fraction was 0.1 mg/ml. Heating and cooling data, $\Theta_{222}(T)$, were recorded at 222 nm at a scan rate of 6 °C/h. Arrows show direction of temperature changes. The unfolding transition at near-physiologic temperatures (*) is detected in the total SAA-HDL but not in its lipoprotein-only fraction, SEC-HDL.

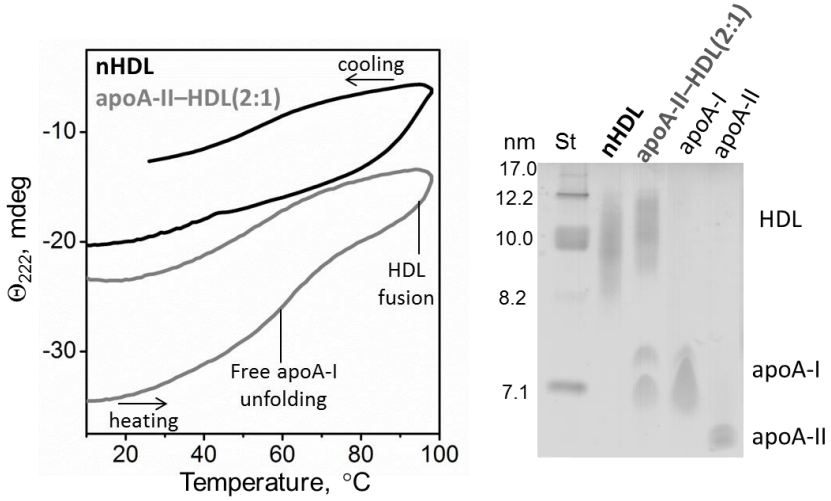

Figure S4. CD melting data of apoA-II-enriched HDL lack the helical folding / unfolding transition at near-physiologic temperatures. To test whether the structural transition observed in the entire sample of SAA-HDL(2:1) and SAA-HDL(4:1) at near-physiologic temperatures requires the presence of SAA, we carried out control experiments using recombinant human apoA-II. Similar to SAA, apoA-II can displace apoA-I from HDL. HDL were incubated with apoA-II using 2:1 mol/mol apoA-II:apoA-I at 37 °C. As a result, a substantial fraction of apoA-I was displaced from HDL, as indicated by the native gel (lane "apoA-II-HDL(2:1)"). Despite the presence of this dissociated apoA-I, CD melting data recorded of apoA-II–HDL(2:1) clearly showed the absence of the helical folding / unfolding transition at near-physiologic temperatures. Instead, free apoA-I has much more stable helical structure that undergoes folding / unfolding at much higher temperatures approaching 60°C (indicated). These results unambiguously show that the 38 °C transition in SAA-HDL is due to SAA.