Structure of Serum Amyloid A Suggests a Mechanism for Lipoprotein Binding and Functions: SAA as a Hub in Macromolecular Interaction Networks

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SUPPLEMENTAL DATA

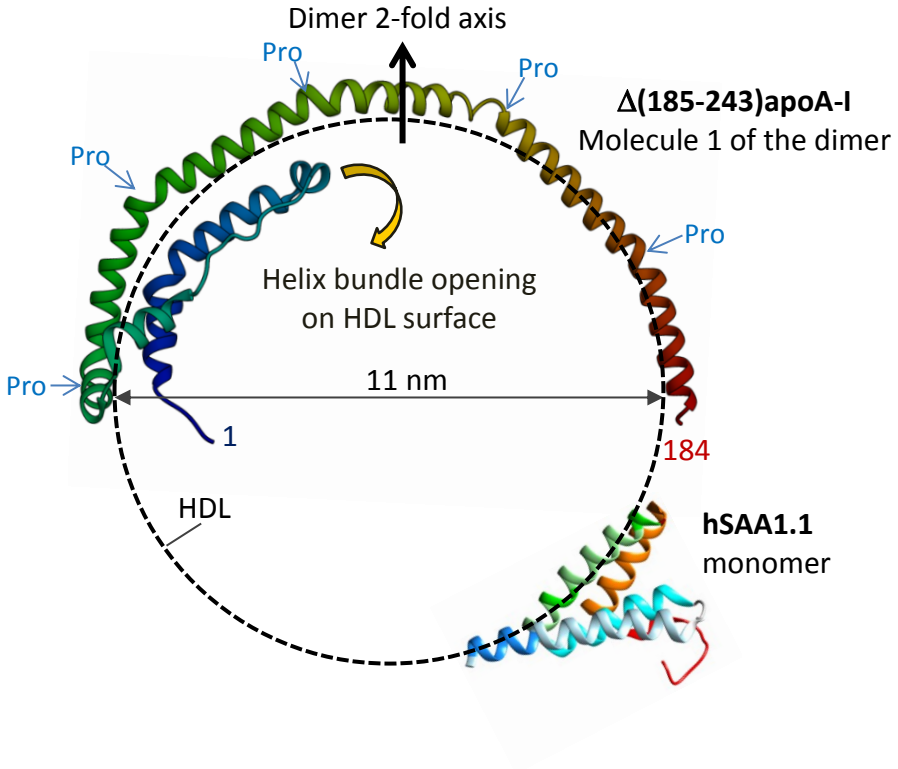

Figure S1 Structural basis for apoA-I binding to HDL surface: comparison with SAA. ApoA-I, which is the major protein on normal HDL, is partially replaced with SAA on acute-phase HDL. The apoA-I sequence is comprised of 11/22-mer tandem helical repeats punctuated by Pro. Pro-induced helical kinks (shown by arrows) confer the overall molecular curvature that is commensurate with the HDL diameter. X-ray crystal structure of the C-terminally truncated human lipid-free apoA-I, Δ(185-243)apoA-I (PDB ID 3R2P) [1] is shown for one molecule from the crystallographic dimer. The highly dynamic hydrophobic C-terminal tail 185-243 (not shown) was truncated to augment crystallization. In the crystal, 1-184 fragment of lipid-free apoA-I dimerizes to form a semi-circular four-helix bundle; three helices come from molecule 1 of the dimer and the fourth from molecule 2 (not shown). The dimer two-fold axis is indicated. Upon HDL binding, two N-terminal helical segments (in dark blue and teal) rotate around the flexible hinge away from the other apoA-I helices (circular arrow), thereby opening the four-helix bundle to expose the apolar helical faces to the lipid surface. The resulting pairs of helices wrap around the HDL perimeter in an antiparallel "double-belt" conformation [2]. A similar mode of protein-lipid surface binding was inferred for other apolipoproteins. It is distinctly different from the proposed binding mode of SAA monomer to HDL, in which the relative orientation of helices 1 and 3 is similar to that observed in the crystal structures of lipid-free SAA (illustrated at the bottom, PDB ID 4IP8).

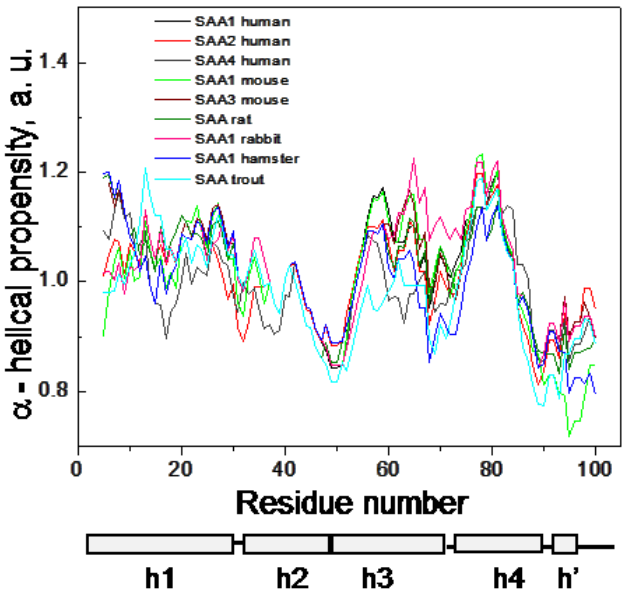

Figure S2 Alpha-helical propensity of selected SAA proteins obtained by using the ExPASy server with Levitt's algorithm [3]. Other methods (e. g. Deleage and Roux, [4]) yielded similar profiles. Secondary structural elements observed by x-ray crystallography in lipid-free oligomeric hSAA1.1 and mSAA3 are shown at the bottom: rectangles – α-helices; lines – non-helical structure.

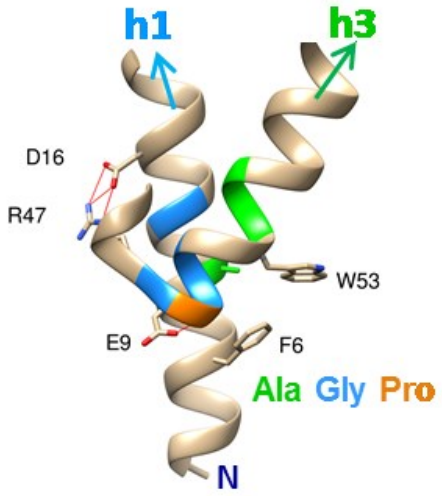

Figure S3 Specific interactions between conserved residues near the h1-h3 helical junction which contribute to the relative packing of these helices at an ~45° angle. A part of hSAA1.1 structure (PDP ID 4IP8) that encompasses h1, h2-h3 linker and h3 is shown. Main chains of the strictly conserved Gly, Ala and Pro groups that facilitate close helical spacing are color-coded to show A10, G13, G48, P49, G50, G51, A54, and A55. Main chains of A10 and G50 are packed unusually close to each other, with only a 3.6Å distance between A10 C_α and G50 C, a 3.6Å distance between A10 N and G50 C, and a 4.0 Å distance between A10 C_α and G50 C_α.
Selected side chains involved in h1-h3 packing in this region are shown. Their interactions include aromatic stacking (F6 – W53), a salt bridge (D16 – R47), and an H-bond (COO^- of E9 to the main chain N of G50). All these residues are highly conserved in the SAA family (Fig. 2).

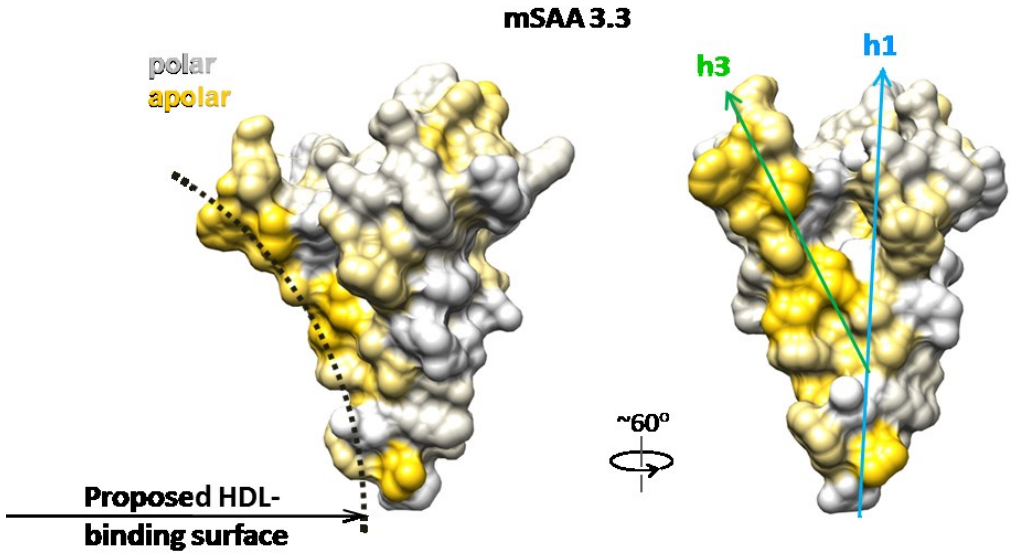

Figure S4 Space-filling model of murine SAA3.3 (PDB ID 4Q5G) showing surface hydrophobicity.

References

1. Borhani, D. W., Rogers, D. P., Engler, J. A., Brouillette, C. G. (1997) Crystal structure of truncated human apolipoprotein A-I suggests a lipid-bound conformation. *Proc. Natl. Acad. Sci. USA 94(23)*, 12291-12296.
2. Mei, X., Atkinson, D. (2011) Crystal structure of C-terminal truncated apolipoprotein A-I reveals the assembly of high density lipoprotein (HDL) by dimerization. *J. Biol. Chem. 286(44)*, 38570-38582.
3. Levitt, M. (1978) Conformational preferences of amino acids in globular proteins. Biochemistry. 17(20), 4277-4285.
4. Deleage, G., Roux, B. (1987) An algorithm for protein secondary structure prediction based on class prediction. *Protein Engineering 1*, 289-294.