Supplementary Note

Due to space restrictions of the Journal, we were asked to abbreviate our discussion of the model in the main paper. What follows is the original, more complete discussion.

Consistency with previously reported data. Analytical ultracentrifugation (AUC) can provide low-resolution molecular shape information. **Supplementary Table 7** summarizes three analytical ultracentrifugation studies[31-33] on lipid-free apoA-I, which conclude that monomeric apoA-I is asymmetrical in shape with an axial ratio of ~6.5 (161 x 24 Å). This contrasts with our SAXS envelopes which had an axial ratio of 2.1-2.8. The discrepancy between AUC and SAXS measurements may reflect the dynamics of apoA-I in solution vs the 'locked' state after cross-linking. Using Forster resonance energy transfer, Brouillette and colleagues later concluded apoA-I is more compact[35] and that the AUC results[31] were probably reflective of an unfolded protein due to high external centrifugal forces. The molecular length measured by AUC is about double what we measured by SAXS while the width is about half. This is consistent with the notion that monomeric apoA-I, like apoA-IV[36], can open up like a pocket knife to oligomerize or bind lipids. This may be further supported by EPR studies identifying residues 26, 44, 64, 167, 217, and 226 to be in the same plane[37]. In the time-averaged structure all but residues 217 and 226 fall within the same plane. Given that EPR studies were executed on multimeric apoA-I, it's plausible that when H6 opens and apoA-I self-associates, residues 217 and 226 fall in-plane with the remaining residues. Several laboratories[38-40] have suggested that helix 5 may be the center of such a hinge. Additionally, the positioning of H6 on the new model appears poised to unfold for interaction with lipid or another molecule. The exact nature of this unfolding must await further studies focused specifically on apoA-I oligomerization.

Limitations of the model. A limitation of the current model stems from our attempt to represent a highly dynamic protein with a single, time-averaged model. Numerous studies have documented the "molten globule" nature of apoA-I[16,41]. H-DX quantifies the rate of proton transfer to amide groups in the protein and allows one to determine locations and stabilities of elements of secondary structure[26]. Given that the observed rate constant for hydrogen exchange is lower than the rate constant for helical closing and H-bond formation in apoA-I, the observed hydrogen exchange rate is related to the α-helix open-closed equilibrium constant (K_{op}), a measure of the free energy (ΔG) of helix stabilization. This concept is illustrated in **Supplementary Figure 4** which compares hydrogen-exchange rates at three temperatures for a peptide segment in an unprotected random coil state (dashed line) and in a protected helical state (solid line). Given the degree of protection, expressed as the protection factor P_f ($=1/K_{op}$), one can derive K_{op} and the ΔG of helix stabilization. ApoA-I has ΔG of helix stabilization ranging from ~3-5 kcal/mol[26]. At neutral pH and room temperature, P_f for these helices is ~$10e^4$, which corresponds with complete hydrogen exchange into the α-helical segments occurring in ~10 minutes (**Supplementary Figure 4B**); i.e. all the helical segments of native apoA-I have opened and closed at least once in this timeframe. To put this in context, a more stable globular protein such as cytochrome C has a ΔG of helix stabilization of 10 kcal/mol corresponding to a P_f of ~$10e^8$, indicating that complete hydrogen exchange would require ~10 weeks. Given this degree of secondary structure dynamics, the overall high content of random coil, and the number of solvent exposed hydrophobic residues in apoA-I, it may not be possible to fully capture apoA-I structure in a single model. At physiological temperatures, apoA-I may adopt many of the structures shown in **Fig. 1**. However, our model is a time-averaged structure derived from experimental data obtained on a time scale that is much longer than typical secondary structure oscillations. For this reason, we think of it as a base model upon which hypothesized dynamics and conformational alterations can be further tested or modeled.

Another issue relates to the notion of solvent accessibility of the cross-linking reagents. While most cross-links fit the model in terms of Euclidian distance ('as the crow flies'), nearly half are impeded by some obstruction. For example, the cross-link path may be sterically hindered by a side chain rotamer from a non-participating residue or it may pass through the backbone of an adjacent helical domain. The answer to how this can happen most likely lies in protein dynamics. Cross-linking

experiments are completed on the time scale of minutes to hours, substantially longer than the timescale described above for helix opening and closing. Thus, α-helical domains in apoA-I have unfolded and refolded multiple times allowing cross-linker access to amines that are otherwise inaccessible. Furthermore, it is possible that cross-links may stabilize low probability structures, which would facilitate these observations. However, previous reports have shown excellent consistency between observed cross-links on solution structures with crystal structures of apoA-I$^{1\text{-}184\ (42)}$ and apoA-IV[36] and non-apolipoproteins[43-46] validating the approach. It's important to recognize that *in vitro* studies presented here are performed on an ensemble of structures that vary at any given time point during the experiment. Additionally, it's likely that most of the dynamics are localized; i.e. lipid-free apoA-I likely exists as a discrete structure that exhibits characteristics of a molten globular protein. Thus, the model represents a time-average of those ensembles and cross-links that appear sterically hindered or solvent inaccessible likely occur on an alternate conformation within the boundaries of the experimental system. Additional studies are needed to better define these boundaries and the extent of rearrangement apoA-I can achieve *in vivo* and *in vitro*.

Finally, despite its consistency with much of the known structural data, we note that the model is still limited in resolution compared to NMR or X-ray crystallography. The general backbone configuration is likely correct, but more refined molecular interactions such as salt-bridging and hydrogen bonding are still unclear. While the protein clearly has a somewhat defined structure and shape as captured by SAXS, the exact lengths of helical domains, the precise positions of N- and C-termini and even the integrity of the helical bundle itself are likely to be in flux on the timescale of seconds. Highly unstable helices (P_f<10) that have ΔG stabilization of < 1.3 kcal/mol and are open > 10% of the time are not detected in the timescale of the H-DX kinetic experiments but are detected by CD measurements. However, truncation of the C-terminus from residue 243 to residues 221 and 231 reduces CD-detectable helix content by 14 and 7 amino acid residues, respectively[16,40]. On the basis of such observations, Mei and Atkinson[40] suggested that the segment spanning residues 231-241 contains α-helical structure. This C-terminal segment is located near the N-terminus (**Fig. 5c**) and it may contribute to the stabilization of the NT helix bundle induced by the C-terminal domain[47]. The existence of such a C-terminal helical domain is further supported by preliminary molecular dynamics simulations of the new model (Segrest et al, unpublished observation).

References

1. Silva, R.A., Hilliard, G.M., Fang, J., Macha, S. & Davidson, W.S. A three-dimensional molecular model of lipid-free apolipoprotein A-I determined by cross-linking/mass spectrometry and sequence threading. *Biochemistry* **44**, 2759-69 (2005).
2. Pollard, R.D., Fulp, B., Samuel, M.P., Sorci-Thomas, M.G. & Thomas, M.J. The conformation of lipid-free human apolipoprotein A-I in solution. *Biochemistry* **52**, 9470-81 (2013).
3. Segrest, J.P., Jones, M.K., Shao, B. & Heinecke, J.W. An experimentally robust model of monomeric apolipoprotein A-I created from a chimera of two X-ray structures and molecular dynamics simulations. *Biochemistry* **53**, 7625-40 (2014).
4. Davidson, W.S., Hazlett, T., Mantulin, W.W. & Jonas, A. The role of apolipoprotein AI domains in lipid binding. *Proc Natl Acad Sci U S A* **93**, 13605-10 (1996).
5. Bergeron, J. et al. Characterization of human apolipoprotein A-I expressed in Escherichia coli. *Biochim Biophys Acta* **1344**, 139-52 (1997).
6. Rogers, D.P. et al. Truncation of the amino terminus of human apolipoprotein A-I substantially alters only the lipid-free conformation. *Biochemistry* **36**, 288-300 (1997).
7. Sparks, D.L., Frank, P.G., Braschi, S., Neville, T.A. & Marcel, Y.L. Effect of apolipoprotein A-I lipidation on the formation and function of pre-beta and alpha-migrating LpA-I particles. *Biochemistry* **38**, 1727-35 (1999).
8. Suurkuusk, M. & Hallen, D. Denaturation of apolipoprotein A-I and the monomer form of apolipoprotein A-I(Milano). *Eur J Biochem* **265**, 346-52 (1999).
9. Davidson, W.S. et al. Structural organization of the N-terminal domain of apolipoprotein A-I: studies of tryptophan mutants. *Biochemistry* **38**, 14387-95 (1999).
10. Huang, W. et al. A single amino acid deletion in the carboxy terminal of apolipoprotein A-I impairs lipid binding and cellular interaction. *Arterioscler Thromb Vasc Biol* **20**, 210-6 (2000).
11. Panzenbock, U., Kritharides, L., Raftery, M., Rye, K.A. & Stocker, R. Oxidation of methionine residues to methionine sulfoxides does not decrease potential antiatherogenic properties of apolipoprotein A-I. *J Biol Chem* **275**, 19536-44 (2000).
12. Weinberg, R.B. et al. Structure and interfacial properties of chicken apolipoprotein A-IV. *J Lipid Res* **41**, 1410-8 (2000).
13. Sigalov, A.B. & Stern, L.J. Oxidation of methionine residues affects the structure and stability of apolipoprotein A-I in reconstituted high density lipoprotein particles. *Chem Phys Lipids* **113**, 133-46 (2001).
14. Gorshkova, I.N., Liu, T., Zannis, V.I. & Atkinson, D. Lipid-free structure and stability of apolipoprotein A-I: probing the central region by mutation. *Biochemistry* **41**, 10529-39 (2002).
15. Reschly, E.J. et al. Apolipoprotein A-I alpha -helices 7 and 8 modulate high density lipoprotein subclass distribution. *J Biol Chem* **277**, 9645-54 (2002).
16. Saito, H. et al. Domain structure and lipid interaction in human apolipoproteins A-I and E, a general model. *J Biol Chem* **278**, 23227-32 (2003).
17. Fang, Y., Gursky, O. & Atkinson, D. Structural studies of N- and C-terminally truncated human apolipoprotein A-I. *Biochemistry* **42**, 6881-90 (2003).
18. Saito, H. et al. alpha-Helix formation is required for high affinity binding of human apolipoprotein A-I to lipids. *Journal of Biological Chemistry* **279**, 20974-20981 (2004).
19. Han, J.M., Jeong, T.S., Lee, W.S., Choi, I. & Cho, K.H. Structural and functional properties of V156K and A158E mutants of apolipoprotein A-I in the lipid-free and lipid-bound states. *J Lipid Res* **46**, 589-96 (2005).
20. Zhu, X., Wu, G., Zeng, W., Xue, H. & Chen, B. Cysteine mutants of human apolipoprotein A-I: a study of secondary structural and functional properties. *J Lipid Res* **46**, 1303-11 (2005).

21. Arnulphi, C., Sanchez, S.A., Tricerri, M.A., Gratton, E. & Jonas, A. Interaction of human apolipoprotein A-I with model membranes exhibiting lipid domains. *Biophys J* **89**, 285-95 (2005).
22. Gorshkova, I.N. et al. Structure and stability of apolipoprotein a-I in solution and in discoidal high-density lipoprotein probed by double charge ablation and deletion mutation. *Biochemistry* **45**, 1242-54 (2006).
23. Tanaka, M. et al. Influence of tertiary structure domain properties on the functionality of apolipoprotein A-I. *Biochemistry* **47**, 2172-80 (2008).
24. Fukuda, M. et al. Conformational change of apolipoprotein A-I and HDL formation from model membranes under intracellular acidic conditions. *J Lipid Res* **49**, 2419-26 (2008).
25. Kono, M. et al. Disruption of the C-terminal helix by single amino acid deletion is directly responsible for impaired cholesterol efflux ability of apolipoprotein A-I Nichinan. *J Lipid Res* **51**, 809-18 (2010).
26. Chetty, P.S. et al. Helical structure and stability in human apolipoprotein A-I by hydrogen exchange and mass spectrometry. *Proc.Natl.Acad.Sci.U.S.A* **106**, 19005-19010 (2009).
27. Jayaraman, S., Cavigiolio, G. & Gursky, O. Folded functional lipid-poor apolipoprotein A-I obtained by heating of high-density lipoproteins: relevance to high-density lipoprotein biogenesis. *Biochemical Journal* **442**, 703-712 (2012).
28. Zehender, F., Ziegler, A., Schonfeld, H.J. & Seelig, J. Thermodynamics of protein self-association and unfolding. The case of apolipoprotein A-I. *Biochemistry* **51**, 1269-80 (2012).
29. Ryan, R.O., Forte, T.M. & Oda, M.N. Optimized bacterial expression of human apolipoprotein A-I. *Protein Expr Purif* **27**, 98-103 (2003).
30. Roberts, L.M. et al. Structural analysis of apolipoprotein A-I: limited proteolysis of methionine-reduced and -oxidized lipid-free and lipid-bound human apo A-I. *Biochemistry* **36**, 7615-24 (1997).
31. Rogers, D.P., Roberts, L.M., Lebowitz, J., Engler, J.A. & Brouillette, C.G. Structural analysis of apolipoprotein A-I: effects of amino- and carboxy-terminal deletions on the lipid-free structure. *Biochemistry* **37**, 945-55 (1998).
32. Barbeau, D.L., Jonas, A., Teng, T. & Scanu, A.M. Asymmetry of apolipoprotein A-I in solution as assessed from ultracentrifugal, viscometric, and fluorescence polarization studies. *Biochemistry* **18**, 362-9 (1979).
33. Edelstein, C. & Scanu, A.M. Effect of guanidine hydrochloride on the hydrodynamic and thermodynamic properties of human apolipoprotein A-I in solution. *J Biol Chem* **255**, 5747-54 (1980).
34. Mei, X. & Atkinson, D. Crystal structure of C-terminal truncated apolipoprotein A-I reveals the assembly of high density lipoprotein (HDL) by dimerization. *J Biol Chem* **286**, 38570-82 (2011).
35. Brouillette, C.G. et al. Forster resonance energy transfer measurements are consistent with a helical bundle model for lipid-free apolipoprotein A-I. *Biochemistry* **44**, 16413-25 (2005).
36. Walker, R.G. et al. The Structure of Human Apolipoprotein A-IV as Revealed by Stable Isotope-assisted Cross-linking, Molecular Dynamics, and Small Angle X-ray Scattering. *Journal of Biological Chemistry* **289**, 5596-5608 (2014).
37. Lagerstedt, J.O. et al. The "beta-clasp" model of apolipoprotein A-I--a lipid-free solution structure determined by electron paramagnetic resonance spectroscopy. *Biochim Biophys Acta* **1821**, 448-55 (2012).
38. Rogers, D.P. et al. The lipid-free structure of apolipoprotein A-I: effects of amino-terminal deletions. *Biochemistry* **37**, 11714-25 (1998).
39. Pollard, R.D., Fulp, B., Sorci-Thomas, M.G. & Thomas, M.J. High-Density Lipoprotein Biogenesis: Defining the Domains Involved in Human Apolipoprotein A-I Lipidation. *Biochemistry* **55**, 4971-81 (2016).
40. Mei, X., Liu, M., Herscovitz, H. & Atkinson, D. Probing the C-terminal domain of lipid-free apoA-I demonstrates the vital role of the H10B sequence repeat in HDL formation. *J Lipid Res* **57**, 1507-17 (2016).

41. Gursky, O. & Atkinson, D. Thermal unfolding of human high-density apolipoprotein A-1: implications for a lipid-free molten globular state. *Proc Natl Acad Sci U S A* **93**, 2991-5 (1996).
42. Melchior, J.T. et al. An Evaluation of the Crystal Structure of C-terminal Truncated Apolipoprotein A-I in Solution Reveals Structural Dynamics Related to Lipid Binding. *J Biol Chem* **291**, 5439-51 (2016).
43. Huang, B.X., Kim, H.Y. & Dass, C. Probing three-dimensional structure of bovine serum albumin by chemical cross-linking and mass spectrometry. *J Am Soc Mass Spectrom* **15**, 1237-47 (2004).
44. Jacobsen, R.B. et al. Structure and dynamics of dark-state bovine rhodopsin revealed by chemical cross-linking and high-resolution mass spectrometry. *Protein Sci* **15**, 1303-17 (2006).
45. Young, M.M. et al. High throughput protein fold identification by using experimental constraints derived from intramolecular cross-links and mass spectrometry. *Proc Natl Acad Sci U S A* **97**, 5802-6 (2000).
46. Peng, L., Rasmussen, M.I., Chailyan, A., Houen, G. & Hojrup, P. Probing the structure of human protein disulfide isomerase by chemical cross-linking combined with mass spectrometry. *J Proteomics* **108**, 1-16 (2014).
47. Koyama, M. et al. Interaction between the N- and C-terminal domains modulates the stability and lipid binding of apolipoprotein A-I. *Biochemistry* **48**, 2529-2537 (2009).