Supporting Information

Chen et al. 10.1073/pnas.1106420108

SI Materials and Methods

High-Level Expression of Human apoE3. A monomeric, biologically active, full-length human apoE3 is generated using an engineered pET30(+) vector (Novagen) in which the long his-tag in the N terminus was replaced by a short his-tag (eight histidines) as described (1). The expressions were carried out using *Escherichia coli* BL21(DE3) cells. The purification procedures essentially followed the manuals. Using high cell density method (2), we can routinely produce 17–30 mg isotope-labeled apoE3 in 100-mL minimum medium.

Preparation of Segmental Labeled apoE3 Proteins. The preparation of segmental labeled apoE using on-column native chemical ligation was following the protocol described previously (3).

NMR Spectroscopy Assignment. All NMR samples contained ~1 mM uniform/segmental triple-labeled human apoE3, in a buffer containing 100 mM sodium phosphate, at pH 6.8, 0.01 mM NaN_3, 10 mM EDTA, 10 mM d_{10}-DTT, 0.03 mM 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS), and 10% D_2O. For 3D/4D Nuclear Overhauser Effect Spectroscopy (NOESY) spectral collection, we used 50% deuterated triple-labeled apoE3 in 100 mM sodium phosphate, 500 mM urea at pH 6.8, 0.01 mM NaN_3, 10 mM EDTA, 10 mM d_{10}-DTT, 0.03 mM DSS, and 10% D_2O. All spectra were acquired at 30 °C on 600 MHz Varian INOVA spectrometer equipped with a cryogenic probe. Backbone assignment of full-length human apoE3 was achieved as described (4). HCC-TOCSY-NNH, CCC-TOCSY-NNH, 15N-edited NOESY and 4D ^{13}C, ^{15}N-edited NOESY were collected using a 50% deuterated triple-labeled apoE3 for the side chain atom assignment. NMR data were processed on a SGI workstation using nmrPipe (5) and the assignment was achieved using the PIPP and nmrview software (6).

NMR Structure Determination. NOE distance restraints were generated using 3D/4D NOESY experiments, including 3D ^{15}N-edited NOESY and 4D-^{13}C, ^{15}N-edited NOESY using both uniformly and segmentally labeled apoE3 samples. Unique segmental labeling strategy was developed using a ^{2}H, ^{15}N-apoE-NT-^{13}C, ^{15}N-apoE-CT sample. The 3D ^{15}N-edited NOESY spectrum of this unique NMR sample allowed us to unambiguously identify 78 interdomain NOEs between apoE3-NT amide protons and apoE3-CT aliphatic protons (7). CSI program was used to predict secondary structure. TALOS program was used to obtain the backbone dihedral angles (ϕ and ψ based on chemical shift information (8).

A total of 3,459 NOE derived distance restraints, 408 dihedral angle restraints, and 318 hydrogen bond restraints were used for performing the structure calculation using the software package CYANA (9). Structure calculations were carried out in an iterative manner and each iteration generated and energy minimized 200 NMR structures in CYANA, including 10,000 steps of simulated annealing. The generated structures were analyzed for distance and dihedral angle restraint violations. A new restraint set was generated after these analyses for the next calculation. Final twenty best-fit NMR structures were used for further analysis.

Structural Analysis. The NMR structures of apoE3 were analyzed using Insight II (*MSI, San Diego*) and VADAR (10) programs. The VADAR provides the detailed information of buried hydrophilic residues, buried H-bonds, including the fractional solvent-accessible surface area of the side chain of each residue as well as H-bonds present in each structure. Insight II was used for superimposition and manual generating the apoE opening models based on structural and functional data of apoE. The structures are displayed using Pymol (*DeLano Scientific, Palo Alto*).

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Fig. S1. Selected cross-sections of the 4D ^{15}N, ^{13}C-edited NOESY spectrum of apoE3. The ^{15}N and HN (amide proton) chemical shifts are labeled on each plane respectively. The spectral assignments are based on chemical shift assignment of apoE3 (BMRB database accession number: 15744). The crosspeaks labeled in red are long-range interdomain NOEs. The crosspeaks labeled in black are intradomain NOEs.

Fig. S2. A comparison of the NMR structures of the N-terminal domain of full-length apoE3 and apoE3(1–183). (*A*) NMR structure of the N-terminal domain of the full-length apoE3. (*B*) NMR structure of apoE3(1–183) (PDB accession code: 2kc3). (*C*) Superposition of the four helix bundle of the apoE-NT. The full-length apoE3 is colored in blue and apoE3(1–183) is in green.

Black Mutations: No functional difference
Red Mutations: Type III hyperlipoproteinemia
Brown Mutations: Lipoprotein Glomerulopathy
Green Mutations: IV/V hyperlipoproteinemia
Purple Mutations: SEA-BLUE HISTIOCYTE DISEASE

Fig. S3. Possible lipoprotein-associated apoE3 structures on HDL and VLDL particles. To simplify situation, we do not discuss apoAI and apoAII structures on spherical HDL particles. The diameters of the HDL (*A*) and VLDL (*B*) shown are 12 and 45 nm respectively. The apoE structures are shown in ribbons, with the apoE-CT in pink, the hinge domain in green, and the apoE-NT in blue, respectively. The surface-located cholesterols are shown with yellow balls in HDL and with purple balls in VLDL. The phospholipids are shown with gray balls in both HDL and VLDL. Triacylglycerols (TGs) are shown schematically in yellow whereas cholesterol-esters are shown schematically in purple inside the lipid-core of VLDL. Two states: a binding intermediate and a completely opened apoE3 conformation are shown to bind to both HDL and VLDL surfaces. In VLDL, we also showed apoB schematically.

Fig. S4. Human Natural Occurring Mutants of apoE based on OMIM database.

Table S1. Structural statistics of the 20 best-fit NMR structures of human apoE3

Number of NMR restraints	
Total NOE restraints	3,459
Intraresidue	427
Sequential ($\|i-j\|=1$)	887
Medium range ($1<\|i-j\|<5$)	1,573
Long-range ($\|i-j\|\geq 5$)	572
INTER-DOMAIN **NOEs**	224
Hydrogen bonds restraints	318
Total dihedral angle restraints	408
ϕ	204
ψ	204
Structural statistics	
Distance violations (>0.1 Å, %)	0.61
Average distance violations ±rmsd	0.012 ± 0.001
Dihedral angle violations (>2.5°, %)	0.31
Average dihedral angle violations ±rmsd	0.311 ± 0.059
Deviation from idealized geometry	
Bond lengths (Å)	0.00385 ± 0.00018
Bond angles (°)	0.28308 ± 0.01157
Impropers (°)	0.06395 ± 0.00242
Ramachandran plot (%)	
Residues in most favored regions	76.8
Residues in additional allowed regions	17.7
Residues in generally allowed regions	4.4
Residues in disallowed regions	1.1
Average pairwise rmsd (Å)	
All helix regions (13–22, 25–40, 45–52, 55–80, 89–125, 131–164, 168–180, 188–199, 209–223, 238–266, 271–276)	
Backbone heavy atoms	0.58 ± 0.05
All heavy atoms	1.13 ± 0.08
Long helix regions (25–40, 55–80, 89–125, 131–164, 238–266)	
Backbone heavy atoms	0.48 ± 0.05
All heavy atoms	1.02 ± 0.10
Superposition of the four helix-bundle between apoE and apoE N-terminal domain: (28–39, 56–79, 90–123, 132–162)	
Backbone heavy atoms	1.70

Table S2. Secondary structure locations of human apoE3

ApoE N-terminal domain:						
Helix N1	Helix N2	Helix1	Helix1′	Helix 2	Helix 3	Helix 4
6–9	12–22	26–40	45–52	55–79	89–125	131–164
ApoE Hinge domain:						
Hinge helix1	Hinge helix2					
168–180	190–199					
ApoE C-terminal domain:						
Helix C1	Helix C2	Helix C3				
210–223	236–266	271–276				

Table S3. Buried hydrophilic residues within apoE3 *

	LoopN (1–5)	HelixN1 (6–9)	HelixN2 (12–22)	Helix 1 (26–40)	Loop (41–44)	Helix 1′ (45–52)	Helix 2 (55–79)	Loop (80–88)	Helix 3 (89–125)	Helix 4 (131–164)
ApoE-NT										
LoopN										R142, K146
Helix N1										
Helix N2				E27						D153, Q156, K157
Helix 1							T67, E70		Y118 [†]	
Helix 1′										
Helix 2				Y36						Y162
Helix 3							Q55, T57, K75			
Helix 4	E3, Q4	T8	Q17, T18 S22						D107, R114 Y118	
The Hinge Domain										
HingeH1							E79		R90, S94, Q98	H140, D151
Hingeloop									Q101	
HingeH2							Q55, T57, R61, D65, S197		C112, R119	
ApoE-CT										
Helix C1					Q41, T42 S44	Q46, E49 E50, S53, S54, R213				R134,
Helix C2	R142, R146						R217	T83	T89, R92, E96, Q98, R103,D107 D110,E245 S263,E266	K143,R147 R150,D154 Q246 Q249,R264
Loop	T225								E238,K242 Q235	D227,E234,
Helix C3			Q275							D154,K157 R158, D271
LoopC	E281									S139 R150 Q279,E281 E287

*This table lists all the buried hydrophilic residues among apoE3 within the apoE-NT and between apoE domains. Based on orientations of these buried hydrophilic residues, we show them between the two helices. For example, S197 is between helices 2 and HingeH2

[†]The bold buried hydrophilic residues are between the two lobes of the allowed helix bundle opening of the apoE-NT (see Table S6, *11*). The blue buried hydrophilic residues are between LoopN, Helices N1, N2, and Helix 4 that maintain the Loop N, Helices N1 and N2 in the defined position to interaction with Helix 4

Table S4. Hydrophobic residue distribution of the apoE-CT

Buried hydrophobic residues of the apoE-CT between apoE domains.
M221, T225, L229, A237, V239, L243, L252, A256, A259, V280, A291, V294.

ApoE-CT exposed hydrophobic residues
W210, L214, A216, M218, V232, V236, A241, A247, I250, A254, A257, L261, F265, L268, A269, M272, W276, A277, V283, A285, A286, T289, A292, P295, H299.

Table S5. H-bonds and salt-bridges within apoE N-terminal domain

	LoopN (1–5)	HelixN1 (6–9)	HelixN2 (12–22)	Helix 1 (26–40)	Loop (41–44)	Helix 1′ (45–52)	Helix 2 (55–79)	Loop (80–88)	Helix 3 (89–125)	Helix 4 (131–164)
Helix 1							W26-E70 L30b-T67 Y36-E59			
Helix 1′						E50-Q55				
Helix 2										
Helix 3						R119-L52b				
Loop (126–130)					T130-L43b T130-S44b					
Helix 4	R142-E3 K146-Q4	D153-L14b Q156-Q21 Q156-S22 K157-Q17			R145-Q41				L148b-D107 D151-A100b D151-L104b R158-E96	
HingeH1									R172-S94 A176b-S94 E179-Q98	

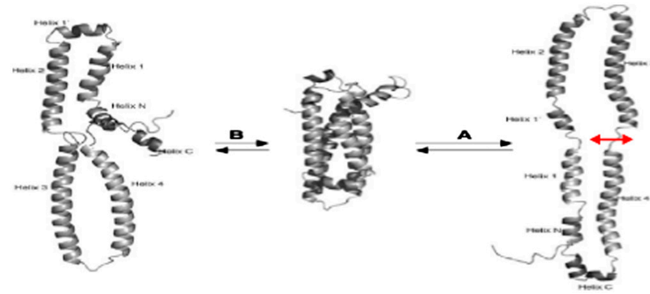

Table S6. A summary of published kinetic and thermodynamic data of lipid-free apoE3 and apoE3 interactions with lipid surface, lipoproteins and heparin and comparisons with our proposed two-step conformational adaptations of apoE3 upon binding to lipid surface, lipoproteins and heparin

	Lipid-free *	Lipid emulsion †					DMPC ‡			Lipoproteins §		Heparin¶							ApoE3
	$\Delta G_D^{H_2O}$ (kcal/mol)	$k^{35/120}$ min^{-1}	$\Delta G^{35/120}$ kcal/mol	$\Delta H^{35/120}$ kcal/mol	$\Delta S^{35/120}$ cal/molK	$K_d^{35/120}$ μM	K_m μM	k_1 min^{-1}	k_2 min^{-1}	K_d^{VLDL} μM	K_d^{HDL3} μM	k_{a1}	k_{d1}	k_{a2}	k_{d2}	K_1	K_2	K_d	conformational adaptations‖
ApoE3	11–12	F/F	−12/ − 11	−1.7/ − 69	34/ − 192	0.12/0.24	2.2	0.12	1.10	0.6 ± 0.1	4 ± 3	9.8	9.2	7.2	2.2	107	3.3	0.02	two-step
E3-CT	2–4	F/F	−11/ − 12	−3.2/ − 34	27/ − 76	0.28/0.19	1.1	0.20	2.50	0.3 ± 0.1	0.5 ± 0.1	1.6	30	1.1	6.7	5.2	0.2	1.6	step one (CT-opening, fast)
E3-NT	8–9	S/S	−10/ − 10	−2.4/ − 43	26/ − 109	1.80/1.30	6.3	0.09	0.44	2.0 ± 1.8	1.7 ± 1.3	0.1	8.4	12	3.2	0.8	3.9	2.6	step two (NT-opening, slow)
Reference	1–3			4				5		6					7				

*The stability data of lipid-free apoE3 and two apoE3 domains are summarized from three publications listed in the column.
†Two different particles sizes of emulsion are reported in this publication, including 35 nm and 120 nm emulsion particles. The $k^{35/120}$ is the result of Fig. 1 of both particles in the cited publication.
‡In the DMPC clearance time course of this publication, two phases of DMPC clearance were noted: Rapid phase and Slow phase. The k_1 and k_2 are the rate constants of rapid phase and slow phase respectively.
§K_d^{VLDL} and K_d^{HDL3} are the dissociation constants of apoE binding to VLDL and HDL3.
¶$k_{a1}(10^5\ M^{-1}\ s^{-1})$, $k_{d1}(10^{-2}\ s^{-1})$, $k_{a2}(10^{-3}\ s^{-1})$ and $k_{d2}(10^{-3}\ s^{-1})$ are the rate constants of association (*a*) and dissociation (*d*) of first step and second step binding of apoE to heparin using a two-step mechanism proposed in the cited publication. $K_1(10^5\ M^{-1})$ and K_2 are the binding constants of apoE to heparin of first step and second step. K_d *(μM)* is the overall dissociation constant.
‖The apoE3 conformational adaptation is also two steps which is described in the text. This two-step conformational adaptation of apoE3 upon lipoprotein-binding and heparin-binding is based on specific structural topology of apoE3, providing a unique and specific mechanism of apoE3 interacting with lipoproteins including HDL and VLDL and with heparin/HPSG. This two-step conformational adaptation of apoE3 provides details of the structural rationale of apoE3 in response to lipoprotein-binding and heparin-binding.

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