Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplement information

Fullerenol $C_{60}(OH)_{16}$ prevents amyloid fibrillization of $A\beta_{40}$:

in vitro and *in silico* approach

‡[a,b]Zuzana Bednarikova, ‡[c]Pham Dinh Quoc Huy, [d]Maria-Magdalena Mocanu, [a]Diana Fedunova, [c]Mai Suan Li*, [a,e]Gazova Zuzana*

[a]Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia

[b]Department of Biochemistry, Institute of Chemistry, Faculty of Science, Safarik University, Srobarova 2, 041 54 Kosice, Slovakia

[c]Institute of Physics, Polish Academy of Sciences, Al. Lotnikow 32/46, 02-668 Warsaw, Poland

[d]Department of Biophysics, "Carol Davila" University of Medicine and Pharmacy, 050474 Bucharest, Romania

[e]Department of Medical and Clinical Biochemistry, Institute of Chemistry, Faculty of Medicine, Safarik University, Trieda SNP 1, 040 11 Kosice, Slovakia

‡ These authors contributed equally

Table S1: Energy obtained by gaussian 09 (Hartree Fock method) for 7 isomers. The difference from the lowest energy of isomer L1 is also shown.

Isomers	Energy (kcal/mol)	Energy (Hatree)	Energy difference E-E(L1) (kcal/mol)
L1	-2183012.64	-3478.89	0
L2	-2182988.16	-3478.85	24.48
L3	-2182928.96	-3478.75	83.68
L4	-2182928.53	-3478.75	84.11
L5	-2182917.39	-3478.74	95.25
L6	-2182911.82	-3478.73	100.82
L7	-2182896.60	-3478.70	116.04

Table S2: Parameters of isomer L1 which have been used for MD simulations. Here, ε is the depth of the Lennard-Jones potential well and *R* is the van der Waals radius.

Atom name	Atom type	Charge	R(Angstrom)	e(kcal/mol)
C1	ca	-0.098838	1.9080	0.0860
C2	ca	-0.038046	1.9080	0.0860
C3	ca	0.115008	1.9080	0.0860
C4	ca	0.241845	1.9080	0.0860
C5	ca	-0.125665	1.9080	0.0860
C6	ca	-0.148336	1.9080	0.0860
C7	ca	0.150585	1.9080	0.0860
C8	ca	0.105281	1.9080	0.0860
C9	ca	-0.316049	1.9080	0.0860
C10	ca	-0.007806	1.9080	0.0860
C11	c3	0.397228	1.9080	0.1094
C12	ca	-0.201241	1.9080	0.0860
C13	ca	-0.039249	1.9080	0.0860
C14	ca	0.234673	1.9080	0.0860
C15	ca	0.231847	1.9080	0.0860
C16	ca	0.275651	1.9080	0.0860
C17	ca	-0.143573	1.9080	0.0860
C18	ca	-0.266987	1.9080	0.0860
C19	ca	0.017040	1.9080	0.0860
C20	ca	-0.154213	1.9080	0.0860
C21	c3	0.443897	1.9080	0.1094
C22	ca	0.027613	1.9080	0.0860
C23	ca	-0.099320	1.9080	0.0860
O1	oh	-0.737394	1.7210	0.2104
C24	c3	0.382274	1.9080	0.1094
O2	oh	-0.609322	1.7210	0.2104
C25	c3	0.069706	1.9080	0.1094
O3	oh	-0.599121	1.7210	0.2104
C26	ca	-0.189523	1.9080	0.0860
C27	c3	0.145490	1.9080	0.1094
C28	ca	-0.286384	1.9080	0.0860
C29	ca	-0.082145	1.9080	0.0860
C30	ca	-0.232211	1.9080	0.0860
C31	c3	-0.028825	1.9080	0.1094
C32	c3	0.457301	1.9080	0.1094
C33	c3	0.219942	1.9080	0.1094
O4	oh	-0.593563	1.7210	0.2104
C34	c3	0.603461	1.9080	0.1094
O5	oh	-0.527354	1.7210	0.2104
C35	ca	-0.345842	1.9080	0.0860
O6	oh	-0.647077	1.7210	0.2104
C36	ca	-0.212117	1.9080	0.0860
O7	oh	-0.546570	1.7210	0.2104
C37	ca	0.299873	1.9080	0.0860
C38	ca	-0.064014	1.9080	0.0860
C39	ca	-0.124707	1.9080	0.0860
C40	ca	0.048255	1.9080	0.0860
C41	ca	0.015016	1.9080	0.0860
C42	ca	-0.030701	1.9080	0.0860
C43	ca	-0.104143	1.9080	0.0860
C44	ca	-0.152960	1.9080	0.0860
C45	ca	0.225667	1.9080	0.0860
C46	c3	0.433485	1.9080	0.1094
O8	oh	-0.552526	1.7210	0.2104

C47	ca	-0.235044	1.9080	0.0860
O9	oh	-0.598552	1.7210	0.2104
C48	c3	0.345881	1.9080	0.1094
O10	oh	-0.474333	1.7210	0.2104
C49	c3	0.755559	1.9080	0.1094
O11	oh	-0.591031	1.7210	0.2104
C50	c3	0.229346	1.9080	0.1094
C51	c3	0.574169	1.9080	0.1094
C52	c3	0.266961	1.9080	0.1094
H1	ho	0.388878	0	0
H2	ho	0.351547	0	0
H3	ho	0.407926	0	0
H4	ho	0.354853	0	0
H5	ho	0.392802	0	0
H6	ho	0.352540	0	0
H7	ho	0.420668	0	0
H8	ho	0.367224	0	0
H9	ho	0.398003	0	0
H10	ho	0.418967	0	0
H11	ho	0.483522	0	0
C53	ca	0.026112	1.9080	0.0860
C54	ca	-0.139177	1.9080	0.0860
C55	ca	-0.141328	1.9080	0.0860
C56	ca	0.353539	1.9080	0.0860
C57	c3	-0.099611	1.9080	0.1094
C58	ca	-0.315448	1.9080	0.0860
C59	ca	-0.129596	1.9080	0.0860
C60	ca	0.003351	1.9080	0.0860
O12	oh	-0.687294	1.7210	0.2104
O13	oh	-0.559361	1.7210	0.2104
O14	oh	-0.527926	1.7210	0.2104
O15	oh	-0.529634	1.7210	0.2104
O16	oh	-0.655368	1.7210	0.2104
H12	ho	0.368695	0	0
H13	ho	0.379670	0	0
H14	ho	0.442100	0	0
H15	ho	0.391904	0	0
H16	ho	0.374174	0	0

Table S3: Parameters of isomer L7 which have been used for MD simulations. Here, ε is the depth of the Lennard-Jones potential well and *R* is the van der Waals radius.

Atom name	Atom type	Charge	*R* (Å)	ε (kcal/mol)
C1	c3	0.269200	1.9080	0.1094
C2	ce	-0.081200	1.9080	0.0860
C3	c2	-0.124400	1.9080	0.0860
C4	c3	0.236300	1.9080	0.1094
C5	c3	0.237200	1.9080	0.1094
C6	ce	0.030800	1.9080	0.0860
C7	ca	-0.013800	1.9080	0.0860
C8	ce	-0.103200	1.9080	0.0860
C9	ca	-0.079300	1.9080	0.0860
C10	ca	-0.086300	1.9080	0.0860
C11	ca	-0.116300	1.9080	0.0860
C12	c2	-0.188400	1.9080	0.0860
C13	ce	-0.054200	1.9080	0.0860
C14	cf	-0.026200	1.9080	0.0860
C15	cf	-0.085200	1.9080	0.0860
C16	c3	0.265200	1.9080	0.1094
C17	c2	-0.168400	1.9080	0.0860
C18	cf	-0.012200	1.9080	0.0860
C19	c3	0.235300	1.9080	0.1094
C20	ca	0.012200	1.9080	0.0860
C21	ca	-0.001800	1.9080	0.0860
C22	ce	-0.018400	1.9080	0.0860
C23	ce	-0.073400	1.9080	0.0860
C24	c3	0.235300	1.9080	0.1094
C25	c3	0.277400	1.9080	0.1094
C26	cf	0.058800	1.9080	0.0860
C27	ca	-0.010800	1.9080	0.0860
C28	c3	0.277300	1.9080	0.1094
C29	c3	0.227200	1.9080	0.1094
C30	cf	0.012000	1.9080	0.0860
C31	cf	-0.083200	1.9080	0.0860
C32	ca	-0.082300	1.9080	0.0860
C33	ca	-0.010000	1.9080	0.0860
C34	ca	-0.025300	1.9080	0.0860
C35	ca	0.001000	1.9080	0.0860
C36	cf	-0.069300	1.9080	0.0860
C37	ca	0.010000	1.9080	0.0860
C38	c3	0.228200	1.9080	0.1094
C39	c3	0.271200	1.9080	0.1094
C40	c3	0.236400	1.9080	0.1094

C41	cf	-0.006400	1.9080	0.0860
C42	ca	-0.000800	1.9080	0.0860
C43	ca	-0.043300	1.9080	0.0860
C44	ca	-0.009000	1.9080	0.0860
C45	ca	-0.099300	1.9080	0.0860
C46	ce	-0.072300	1.9080	0.0860
C47	c3	0.280400	1.9080	0.1094
C48	cf	-0.003000	1.9080	0.0860
C49	c2	-0.110400	1.9080	0.0860
C50	c3	0.241200	1.9080	0.1094
C51	ce	-0.047400	1.9080	0.0860
C52	cf	-0.095200	1.9080	0.0860
C53	ca	-0.094300	1.9080	0.0860
C54	ca	-0.018800	1.9080	0.0860
C55	ca	-0.083300	1.9080	0.0860
C56	ca	-0.084300	1.9080	0.0860
C57	c3	0.271300	1.9080	0.1094
C58	c3	0.283300	1.9080	0.1094
C59	ca	0.020000	1.9080	0.0860
C60	ca	0.025000	1.9080	0.0860
O1	oh	-0.563800	1.7210	0.2104
H11	ho	0.439000	0	0
O2	oh	-0.566800	1.7210	0.2104
H10	ho	0.423000	0	0
O3	oh	-0.577800	1.7210	0.2104
H6	ho	0.450000	0	0
O7	oh	-0.540800	1.7210	0.2104
H4	ho	0.435000	0	0
O5	oh	-0.570800	1.7210	0.2104
H1	ho	0.420000	0	0
O4	oh	-0.563800	1.7210	0.2104
H7	ho	0.442000	0	0
O6	oh	-0.565800	1.7210	0.2104
H5	ho	0.433000	0	0
O8	oh	-0.538800	1.7210	0.2104
H2	ho	0.431000	0	0
O9	oh	-0.563800	1.7210	0.2104
H8	ho	0.433000	0	0
O11	oh	-0.537800	1.7210	0.2104
H9	ho	0.435000	0	0
O13	oh	-0.575800	1.7210	0.2104
H16	ho	0.444000	0	0
O10	oh	-0.557800	1.7210	0.2104
H3	ho	0.432000	0	0

O14	oh	-0.561800	1.7210	0.2104
H15	ho	0.441000	0	0
O16	oh	-0.520800	1.7210	0.2104
H13	ho	0.415000	0	0
O15	oh	-0.529800	1.7210	0.2104
H14	ho	0.430000	0	0
O12	oh	-0.558800	1.7210	0.2104
H12	ho	0.434000	0	0

Table S4. Binding energy ΔE_{bind} (kcal/mol) obtained by the docking method for 7 isomers in five $A\beta_{40}$ models. Error bars come from averaging over five models.

	L1	L2	L3	L4	L5	L6	L7
M1	-5.9	-5.5	-6.5	-6.3	-6.6	-5.9	-5.9
M2	-6.7	-6.6	-6.1	-6.1	-6.7	-6.4	-6.3
M3	-7.4	-7.5	-6.4	-6.6	-6.9	-7.3	-6.2
M4	-7.8	-7.3	-6.5	-6.4	-6.7	-7.2	-7.0
M5	-6.6	-5.8	-6.1	-5.7	-6.0	-6.3	-5.3
Average	-6.9 ± 0.7	-6.5 ± 0.9	-6.3 ± 0.2	-6.2 ± 0.3	-6.6 ± 0.3	-6.6 ± 0.6	6.1 ± 0.6

Table S5. Binding energy ΔE_{bind} (kcal/mol) obtained by the docking method for the most unstable isomer L7. N_{hb} and N_{sc} refer to the number of hydrogen bonds and side-chain contacts, respectively. See Figure S3 and Figure S4 for more information about HBs and SC contact networks.

Model	ΔE_{bind}	N_{hb}	N_{sc}	Amino acids in side-chain contact with ligand
1	-5.90	3	11	Asp1 , Glu3 , Arg5 , Val18 , Ala21 , Asp23 , Ser26 , Lys28 , Ile32 , Gly33 , Leu34
2	-6.30	4	10	His6 , Asp7 , Ser8 , Gly9 , His13 , His14 , Lys16 , Asp23 , Val24 , Gly25
3	-6.20	0	6	Val12 , Gln15 , Lys16 , Leu17 , Phe19 , Glu22
4	-7.00	3	9	Phe4 , Ser8 , Tyr10 , His13 , Val36 , Gly37 , Gly38 , Val39 , Val40
5	-5.30	4	9	Ala2 , Gly9 , Tyr10 , Ile31 , Val36 , Gly37 , Gly38 , Val39 , Val40

Table S6. Binding free energy (kcal/mol) of isomer L7 to $A\beta_{40}$ obtained by the MM-PBSA method. The van der Waals (ΔE_{vdW}) and electrostatic interactions for OH groups and carbon atoms (ΔE_{elec}) are in red and blue, respectively.

Model	Traj	ΔE_{vdW}		ΔE_{elec}		ΔE_{PB}	ΔE_{sur}	$-T\Delta S$	ΔG_{bind}	ΔG_{bind}
1	1	-49.5		-85.5		100.9	-2.9	19.4	-17.3	-21.3
		-6.8	-42.7	73.9	-159.3					
	2	-55.5		-103.6		121.1	-3.6	16.0	-25.4	
		-6.6	-48.9	66.2	-169.8					
2	1	-51.6		-92.6		111.8	-2.9	20.4	-14.7	-23.7
		-6.3	-45.3	128.7	-221.2					
	2	-71.6		-107.6		134.4	-4.1	15.9	-32.8	
		-8.5	-63.1	83.1	-190.7					
3	1	-62.0		-128.8		144.8	-4.1	18.3	-31.6	-33.0
		-6.6	-55.4	84.6	-213.4					
	2	-75.8		-110.9		142.2	-4.6	14.3	-34.5	
		-12.1	-63.6	19.1	-129.9					
4	1	-46.0		-115.7		136.3	-3.3	19.6	-8.9	-7.7
		-1.2	-44.8	94.5	-210.2					
	2	-33.6		-98.9		110.2	-2.3	17.8	-6.6	
		0.4	-34.0	126.5	-225.3					
5	1	-49.6		-81.0		103.4	-3.1	19.4	-10.6	-10.3
		-8.8	-40.8	136.2	-217.2					
	2	-40.3		-60.8		78.9	-2.7	17.3	-9.9	
		-8.0	-34.9	110.2	-171.0					
Average		-53.8		-98.5		118.4	-3.4	17.8	**-19.2**	

Table S7. List of residues locating near isomer L7 of $C_{60}(OH)_{16}$. Results were obtained by the MD simulations. The adjacent amino acids are defined as those forming contact with fullerenol more than 50% of time in equilibrium (Figure 6).

Model	Residues
1	Glu3, Arg5, His14, Ala21, Glu22, Asp23, Val24, Gly25, Ser26, Asn27, Lys28, Gly29, Ile32, Gly33, Leu34
2	Phe4, His6, Asp7, Ser8, Gly9, Tyr10, His14, Val24, Gly25, Ser26, Asn27, Lys28, Ala30, Gly33, Gly37, Gly38
3	Ala2, Glu11, Val12, His13, His14, Gln15, Lys16, Glu22, Asp23, Val24, Gly25, Ser26, Asn27, Lys28, Ile31, Met35, Gly37, Gly38, Val39
4	Ala2, Glu3, Phe4, Arg5, Asp7, Tyr10, Ser26, Asn27, Lys28, Leu34, Val36, Gly37, Gly38, Val39, Val40
5	Ala2, Arg5, Gly9, Tyr10, Glu11, Val12, Phe19, Phe20, Met35, Val36, Gly37, Gly38, Val39, Val40

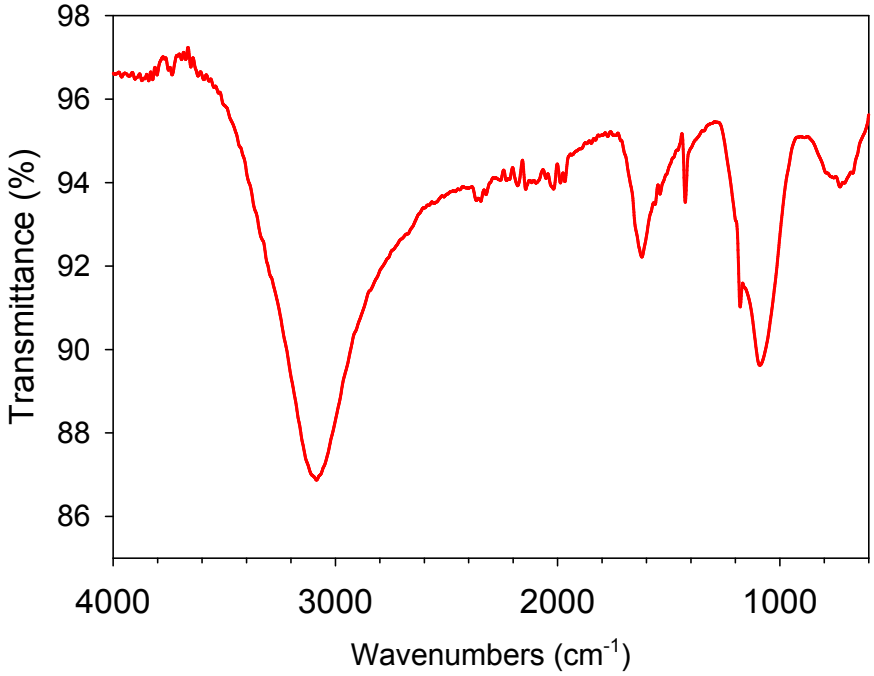

Figure S1. FTIR spectrum of fullerenol $C_{60}(OH)_{16}$ prepared according procedure published by Wang *et al.*.[30] The spectrum corresponds to spectrum of the fullerene C_{60} which carries 16 ± 2 OH groups as determined by Wang *et al.*.[30] Concentration of $C_{60}(OH)_{16}$ fullerenol was 15 mg/ml.

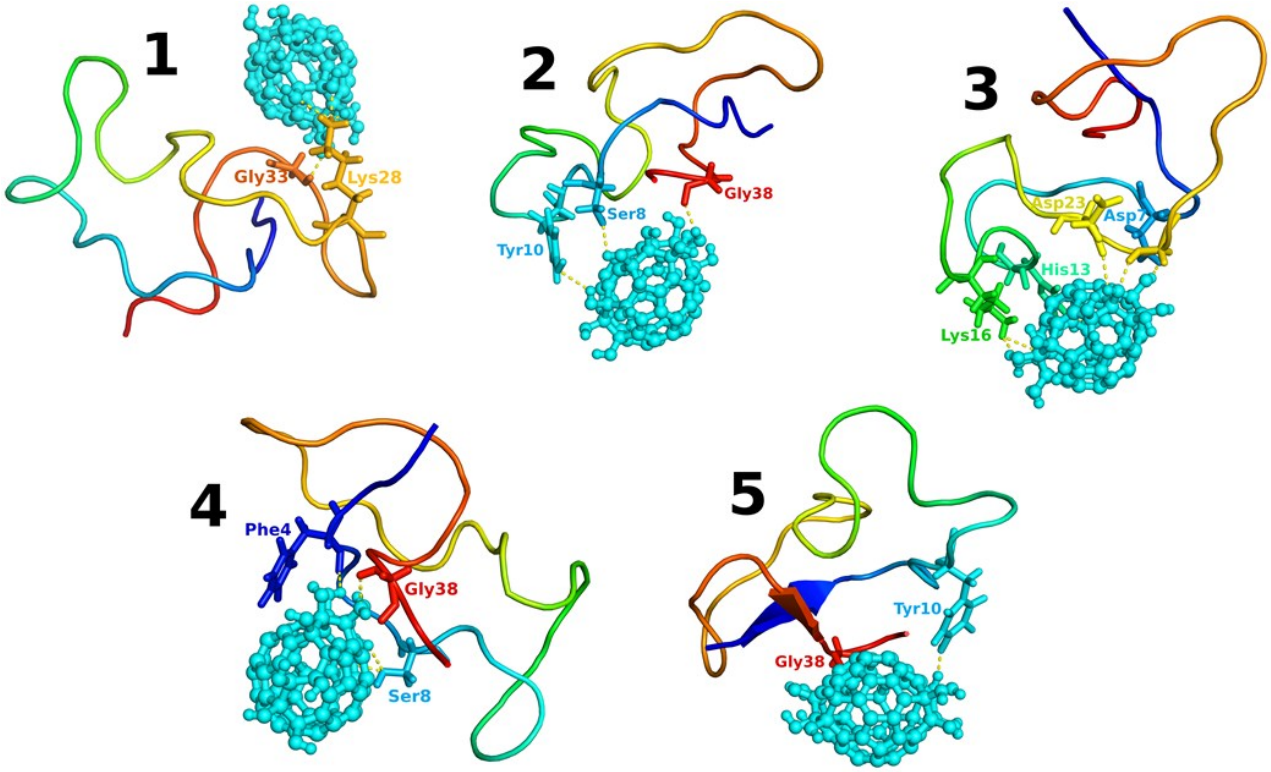

Figure S2: Docking poses of isomer L1 in the five models. Yellow dashed lines represent hydrogen bonds between L1 and Aβ40.

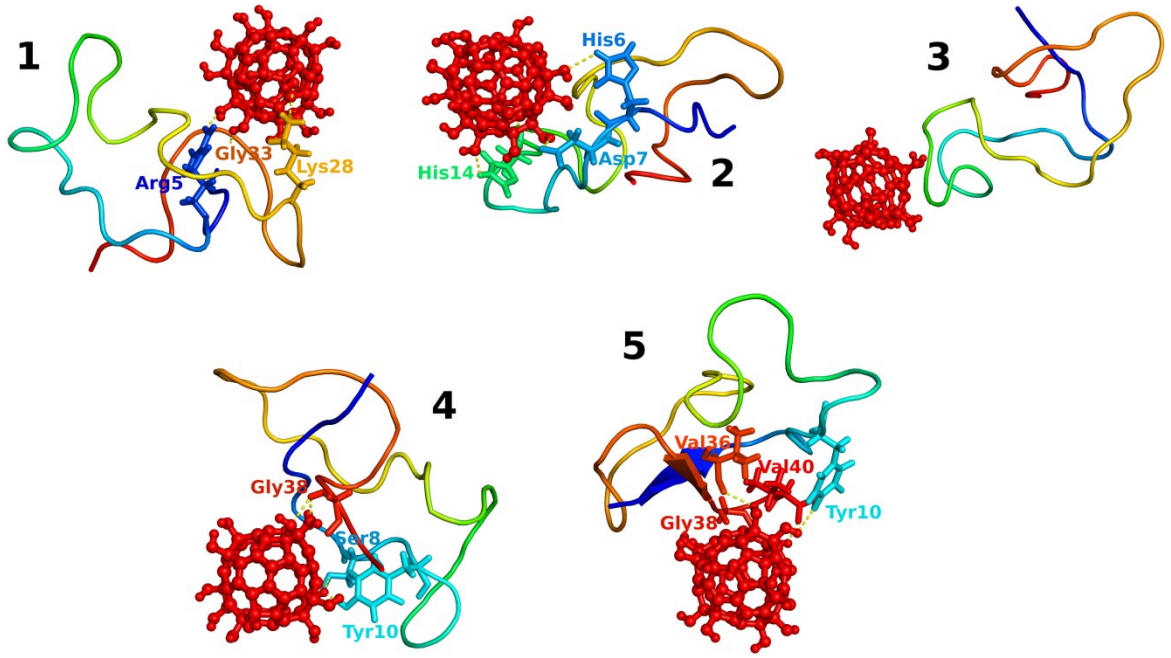

Figure S3. Hydrogen bond network formed by the most unstable isomer L7 with Aβ40 in five structure models. Yellow dashed line represents the hydrogen bond. The names of residues forming hydrogen bonds with fullerenol are explicitly shown. Results were obtained by the docking method.

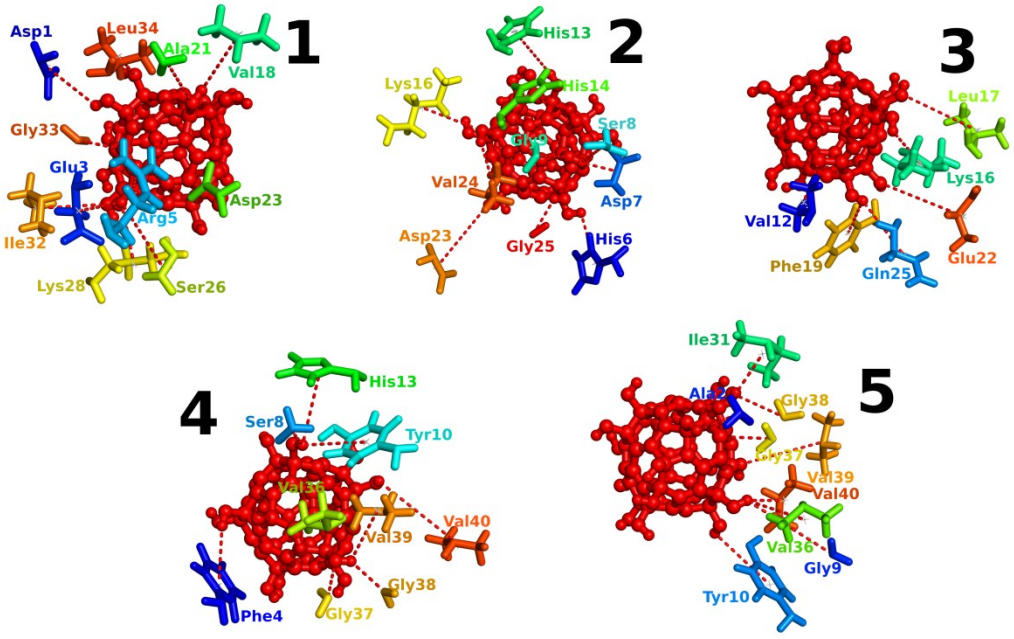

Figure S4. Side-chain contacts between Aβ40 and isomer L7 in the best docking poses of 5 structure models (red dashed lines). The names of residues forming SC with fullerenol are explicitly shown.

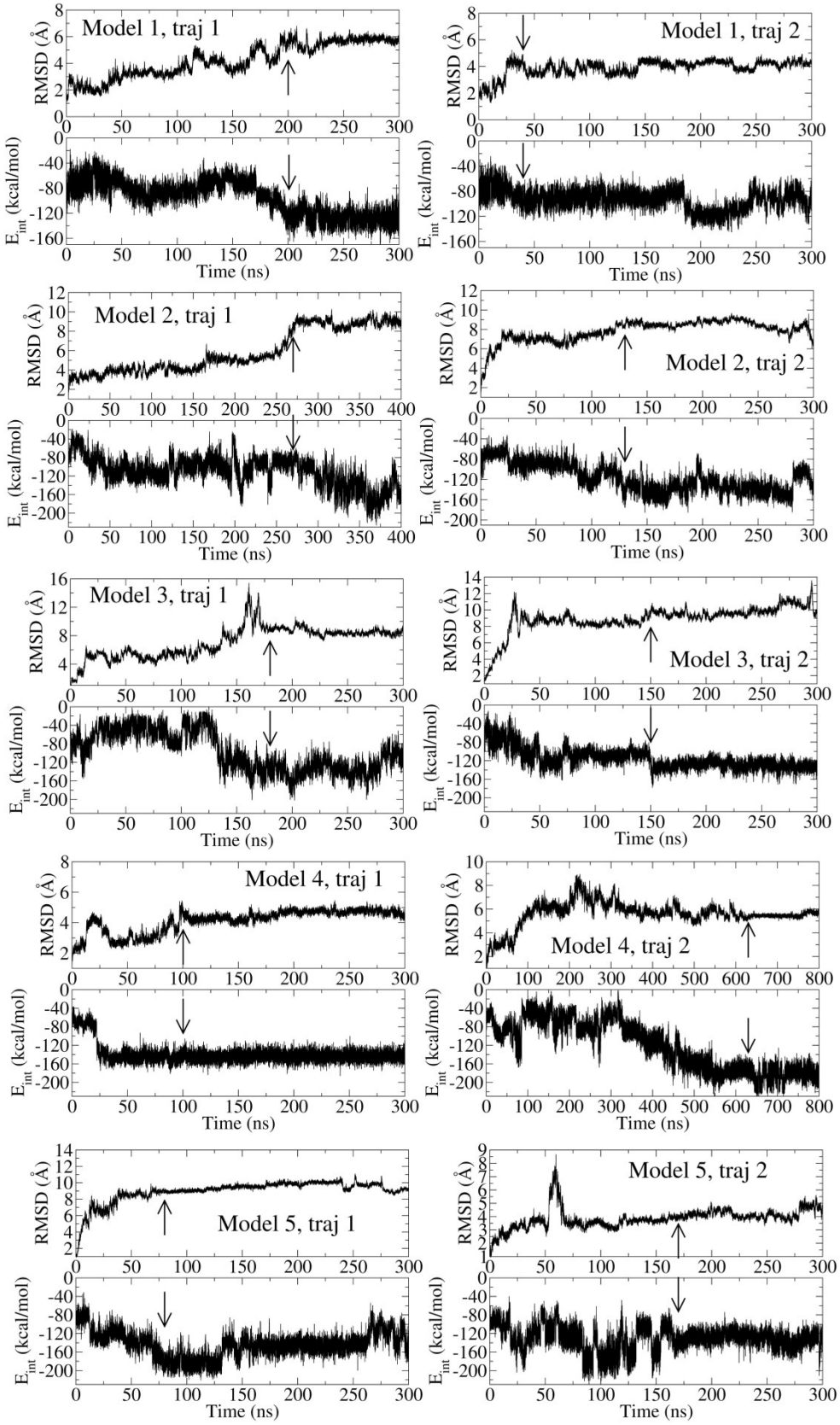

Figure S5. The time evolution of the RMSD and interaction energy for Aβ40-L1 complexes. The arrow refers to the time when the system reaches equilibrium. Snapshots collected after arrow was used for MM/PBSA calculation.

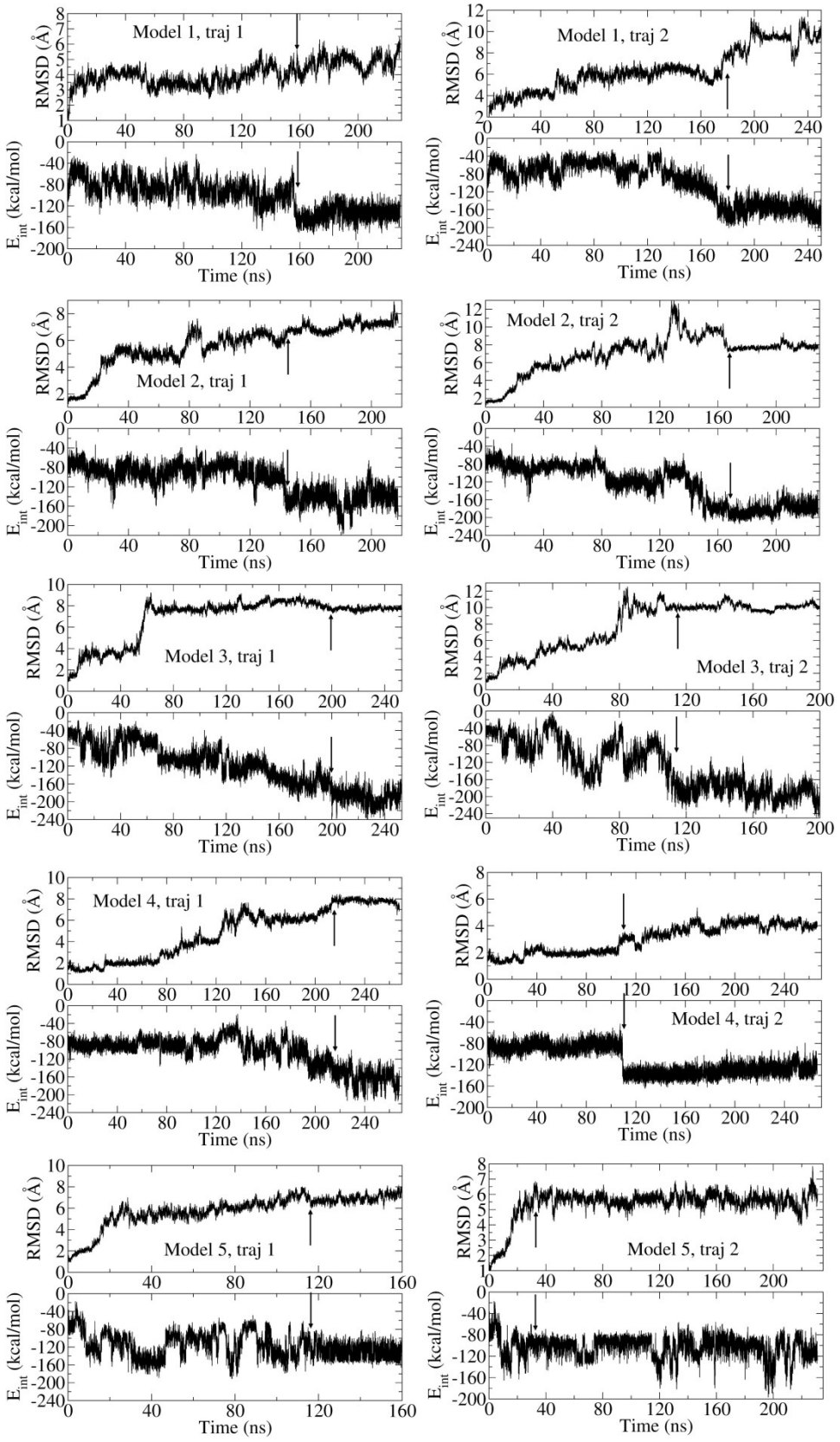

Figure S6. The time evolution of the RMSD and interaction energy for Aβ40-L7 complexes. The arrow refers to the time when the system reaches equilibrium. Snapshots collected after arrow was used for MM/PBSA calculation.

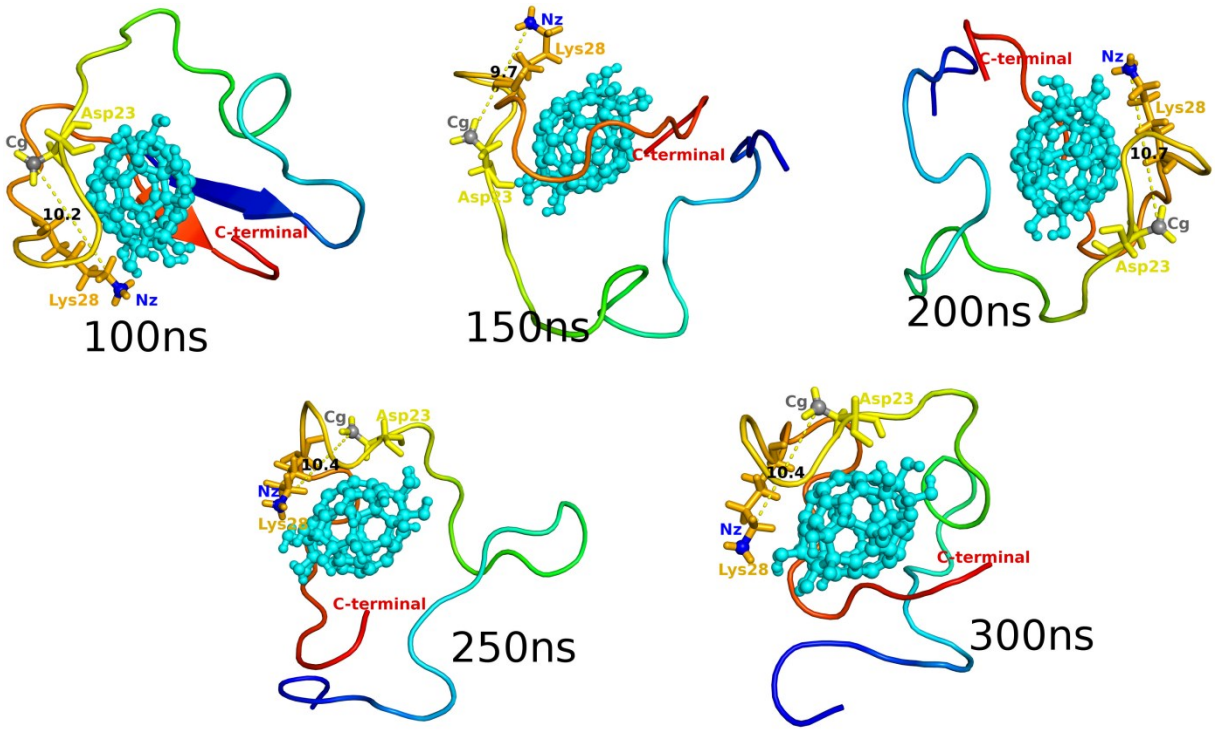

Figure S7. Representative snapshots where isomer L1 forms SC contacts with the salt bridge as well as with the C-terminus.

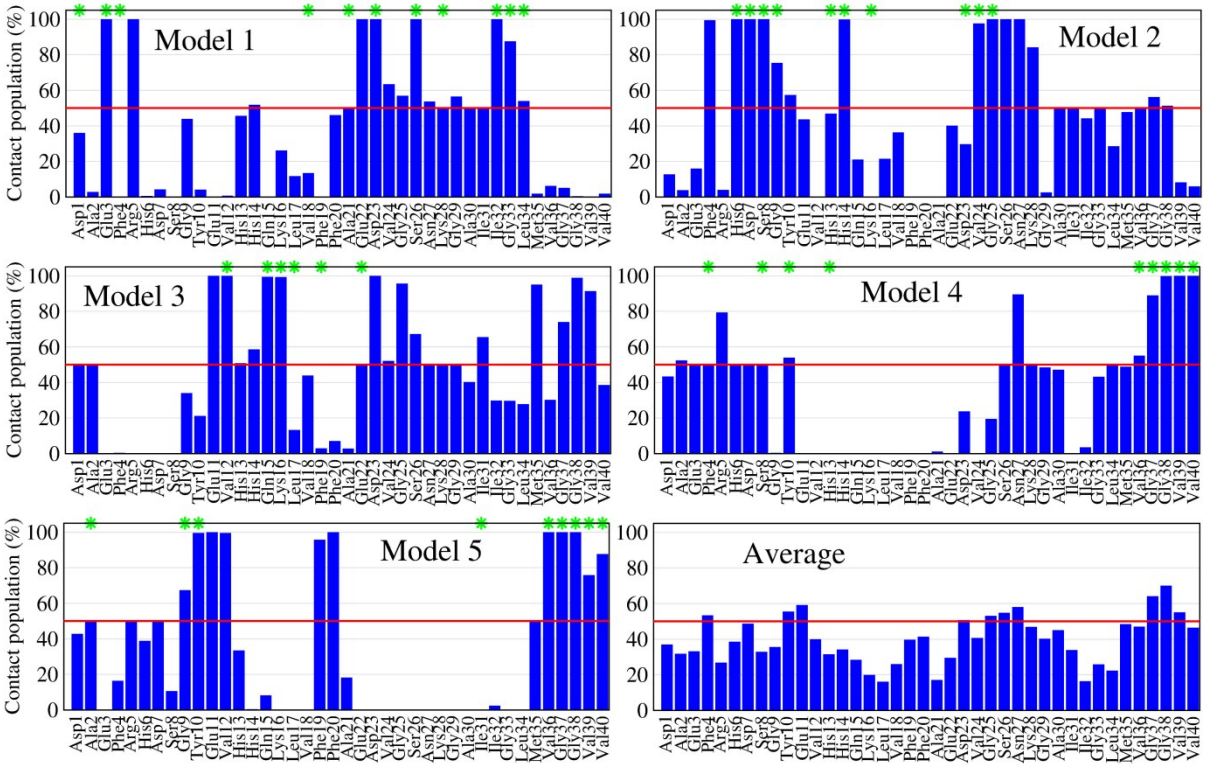

Figure S8. Probability that amino acid (AA) stays in contact with isomer L7 of fullerenol C60(OH)16 in equilibrium. AAs with green stars have contact with ligand by docking method. Red line refers to probability of 50% above which the binding is strong.

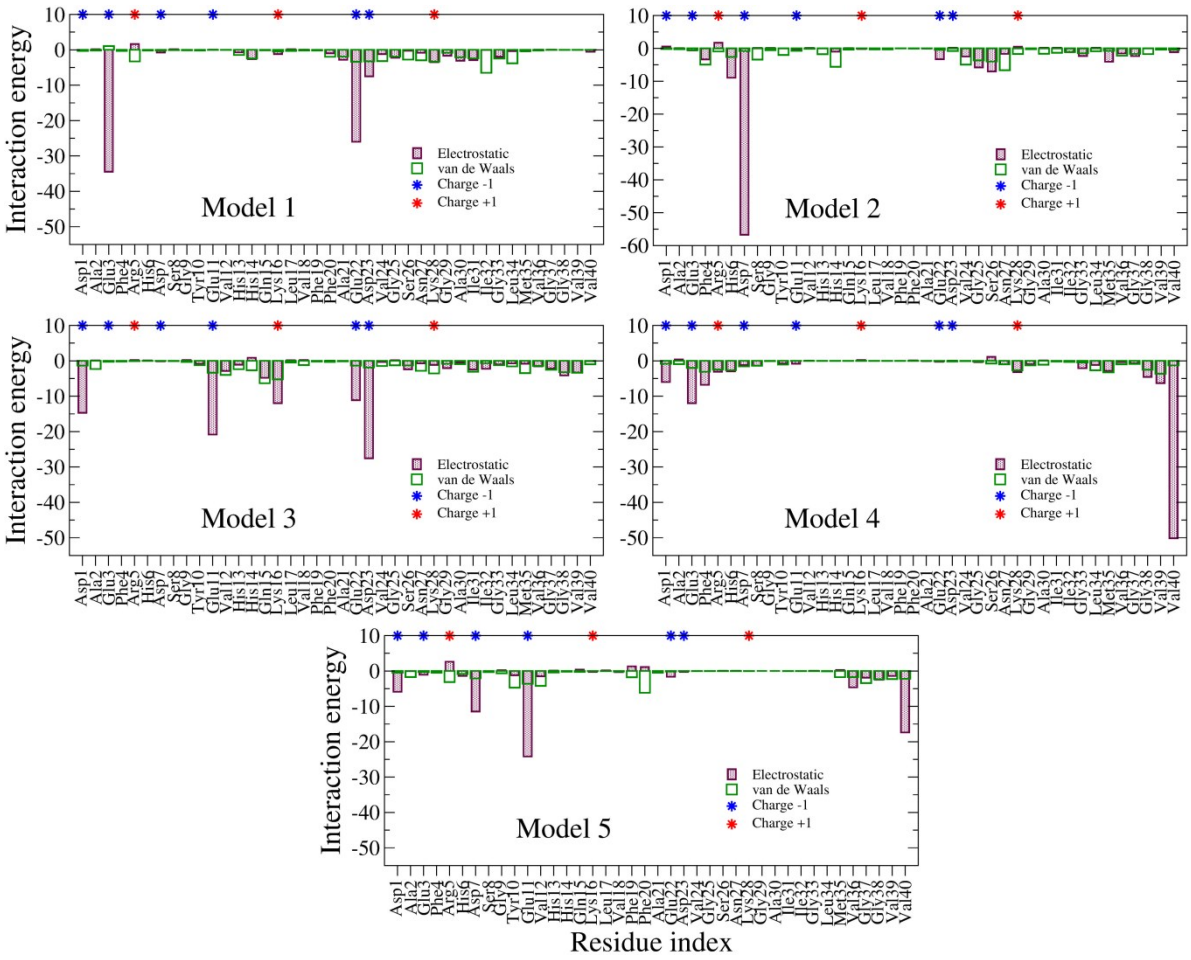

Figure S9. Per-residue distributions for vdW and Coulomb interactions with isomer L7 of fullerenol C60(OH)16. Results were obtained by MD simulations. The interaction energy is in kcal/mol.