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

Supplementary Information for PEGylation within a Confined Hydrophobic Cavity of a Protein

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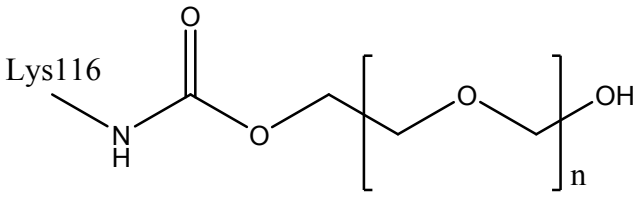

Figure S1. Schematic of carbamate linker attaching PEG chain to BSA's K116 residue. For 2, 5, 10, and 20 kDa K116 PEGylated BSA systems, corresponding repeating unit numbers for the PEG polymer were n = 45, 113, 227, and 454.

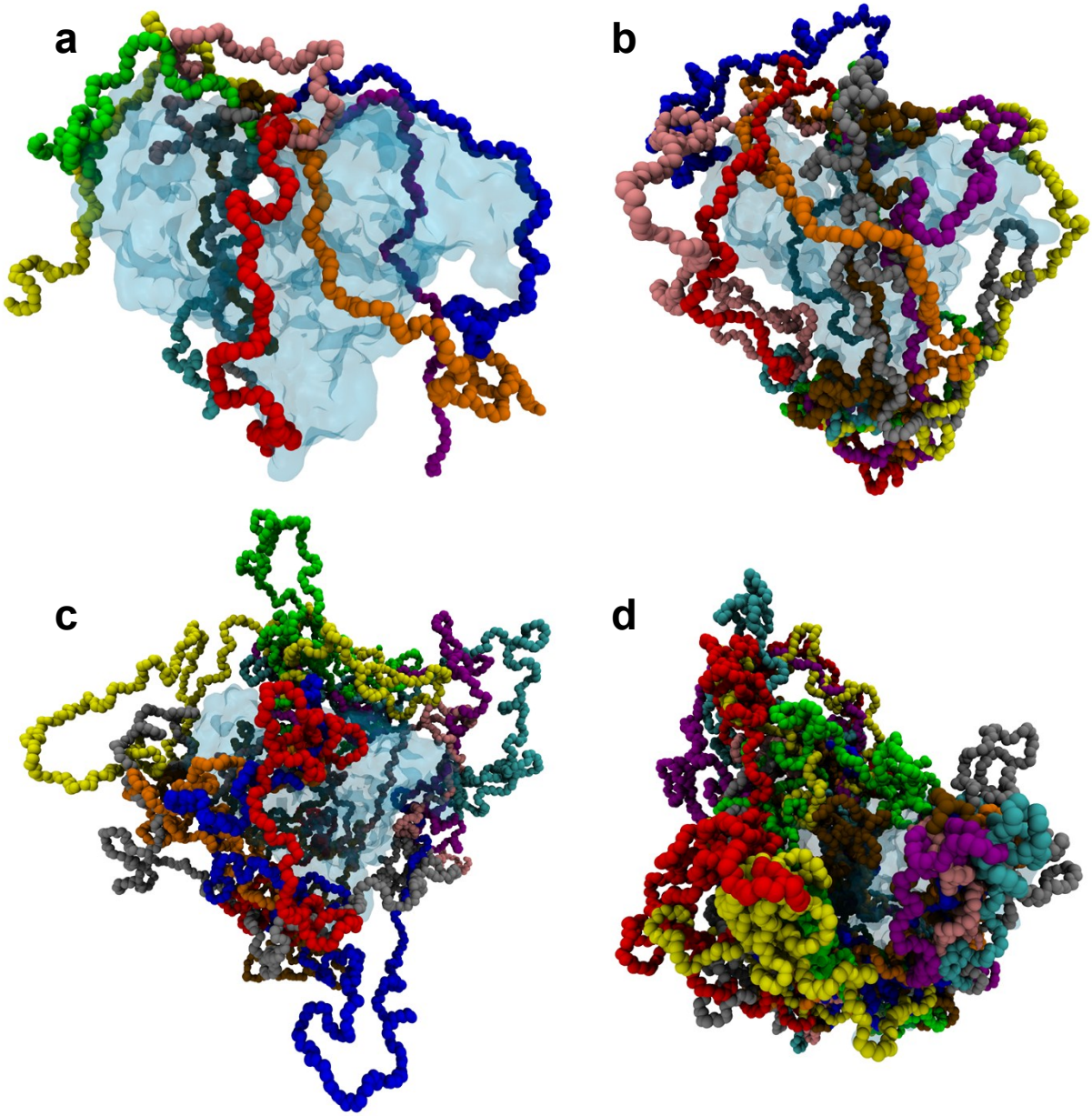

Figure S2. Superimposed initial equilibrated PEG conformations for 2 (a), 5 (b), 10 (c), and 20 (d) kDa K116 PEGylated BSA conjugates. PEG chains are colored according to sim IDs 1-10 as follows: blue, red, orange, yellow, green, cyan, purple, pink, brown, and gray.

PEG shape can be estimated using geometrical analysis by comparing the sum of the radius of gyration, R_g, of free protein and of free PEG to the distance between centers of mass of protein and PEG in PEGylated conjugates ($D_{PEG,BSA}^{COM}$). However, the conjugation site in K116 PEGylated conjugates is located within the volume defined by the R_g of the free BSA, which may bias this analysis toward indicating shroud-like conformations. To reduce this bias, a correction factor was calculated and added to measured $D_{PEG,BSA}^{COM}$ values. The R_g of the free BSA was calculated and shown to be a consistent value of 27.5 ± 0.3 Å. The distance between the center of mass of the BSA and the Cα atom of K116 was measured and shown to be 24.0 ± 1.6 Å. The correction factor was defined as the difference between these two values (3.5 Å) and added to measured $D_{PEG,BSA}^{COM}$ averages for K116 PEGylated systems, effectively "moving" the conjugation site to the edge of the spherical volume generated by the free BSA's R_g and reducing initial bias toward results indicating shroud-like conformations.

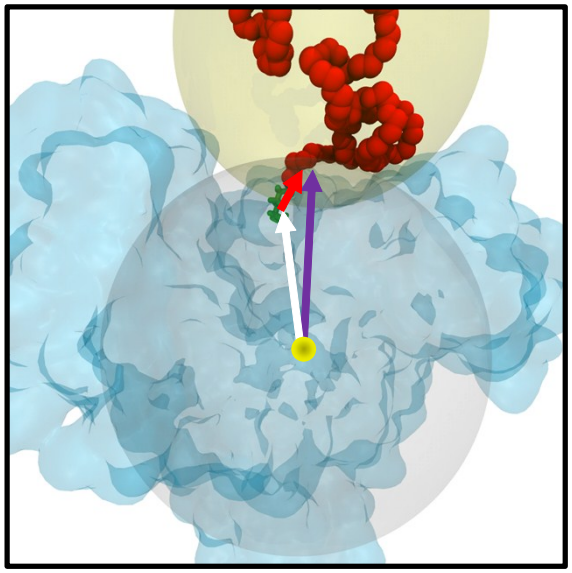

Figure S3. Generation of correction factor for K116 PEGylated BSA conjugate PEG chain shape analysis. Volumes generated by radii of gyration (R_g) of the free BSA and free PEG are represented as gray and yellow spheres, respectively. PEG atoms are shown as red van der Waals spheres and K116 is presented as a green licorice model. The purple arrow denotes the free BSA R_g, the white arrow denotes distance from BSA's center of mass to Cα of K116, and the red arrow denotes the correction factor.

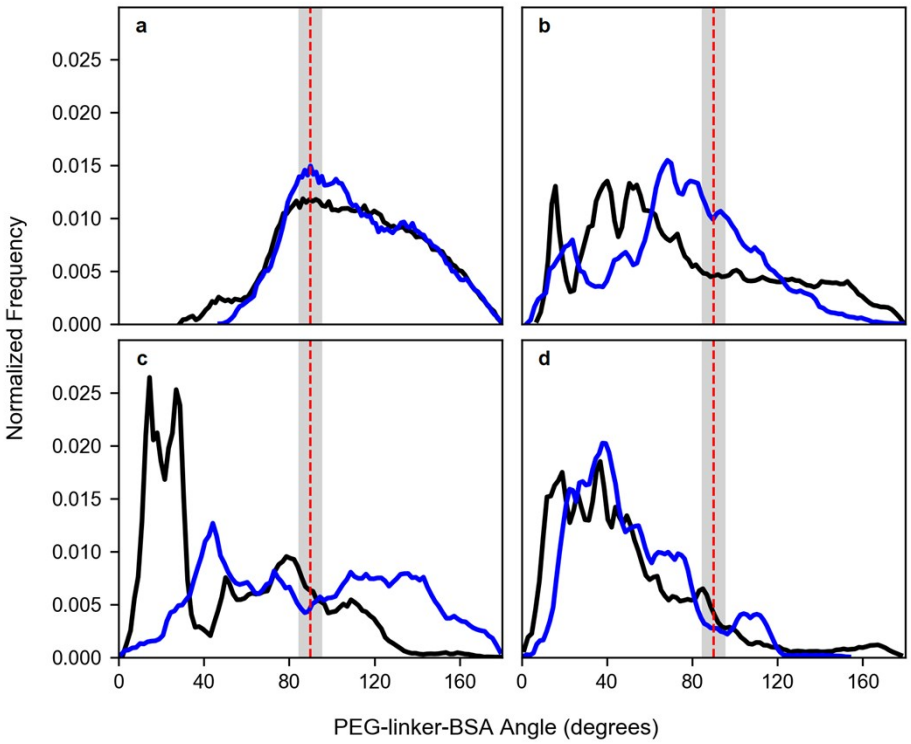

Figure S4. Frequency distribution of the angle between the centers of mass of the PEG chain, linker, and BSA, denoted θ, for ten simulations of 2 (a), 5 (b), 10 (c), and 20 (d) kDa PEGylated BSA conjugates for both N-terminal (black line) and K116 (blue line) grafting sites. Transition regime is demarcated by 90° (red line), with gray boundaries indicating ±5°.

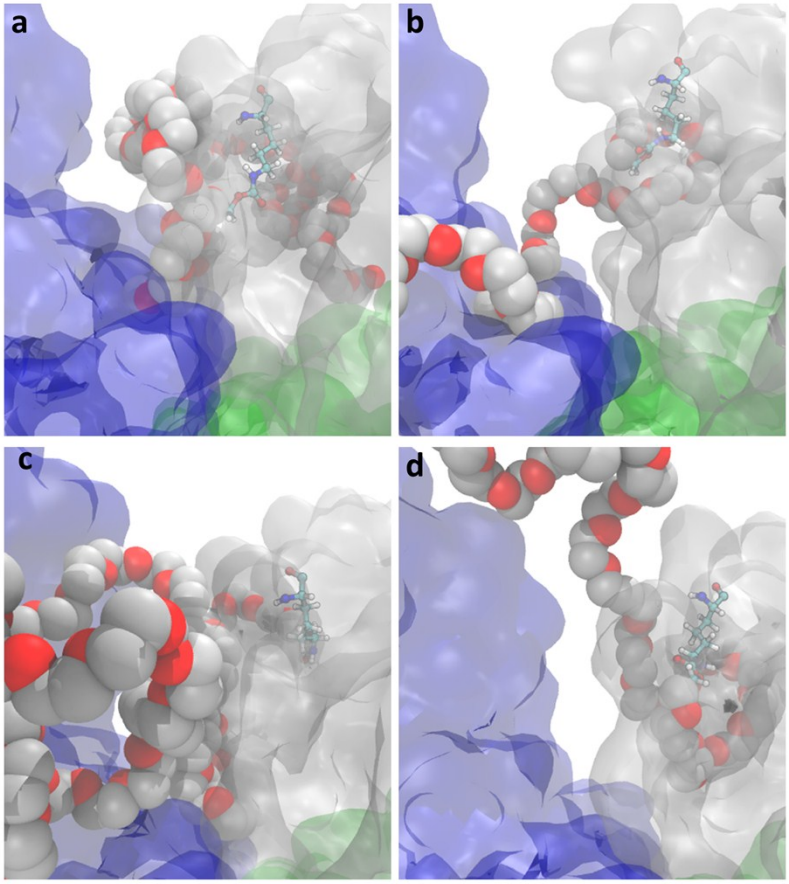

Figure S5. A snapshot showing the inward orientation of K116 and the carbamate linker (highlighted CPK representation) for (a) 2, (b) 5, (c) 10, and (d) 20 kDa PEGylated systems. PEG oxygen and carbon atoms are shown as red and gray van der Waals spheres, respectively. The BSA is partitioned into domain I (gray), domain II (green), and domain III (blue).

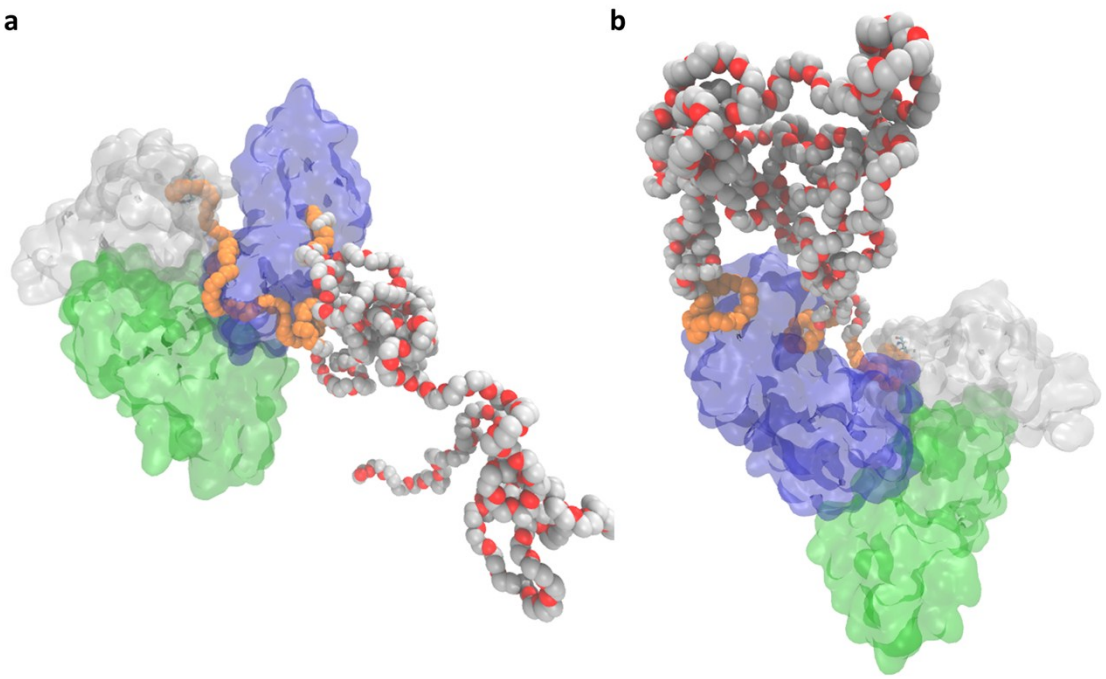

Figure S6. Snapshots of the two cases of (a) only the grafted end interacting with the BSA and (b) both the grafted end and the free end of the PEG chain interacting with the BSA. The BSA is partitioned into domain I (gray), domain II (green), and domain III (blue). PEG oxygen and carbon atoms are shown as red and gray van der Waals spheres, respectively, while PEG heavy atoms within 5 Å of BSA are colored orange.

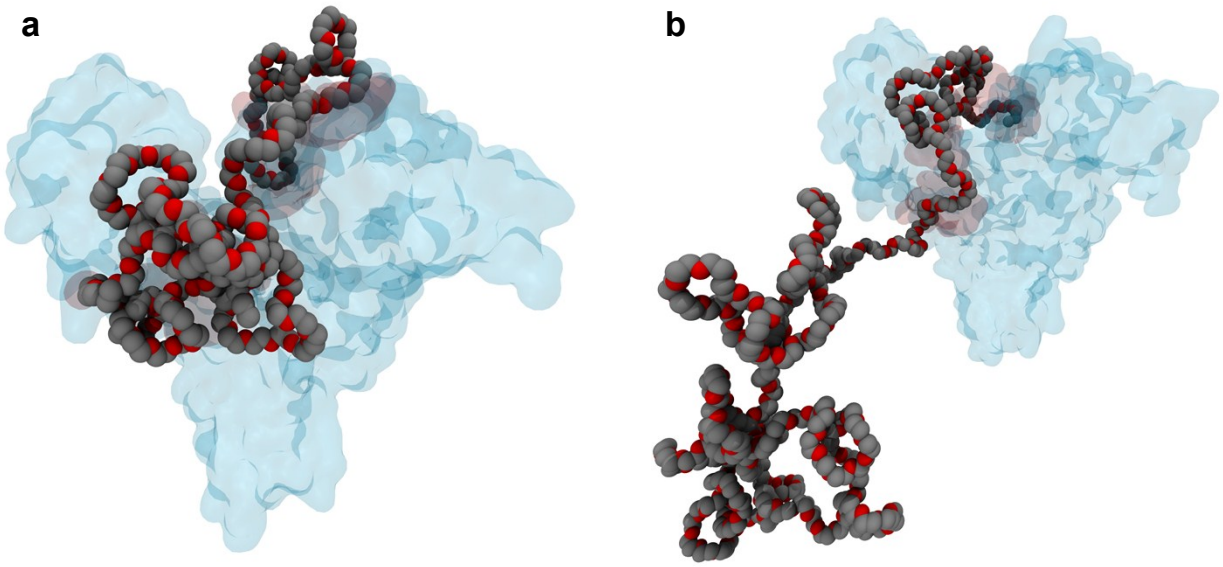

Figure S7. Snapshots of "wrapped" conformation of PEG chain in 5 kDa K116 PEGylated BSA conjugate (a) and partial interaction of PEG chain in 10 kDa K116 PEGylated BSA conjugate (b), with the rest of the chain protruding away from the protein. Visualizations are extracted from trajectories from sim ID 1. PEG oxygen and carbon atoms are shown as red and gray van der Waals spheres, respectively, and K116 is presented in a licorice model representation. Patches of the BSA's surface in contact (within 5 Å) with PEG atoms are colored red.

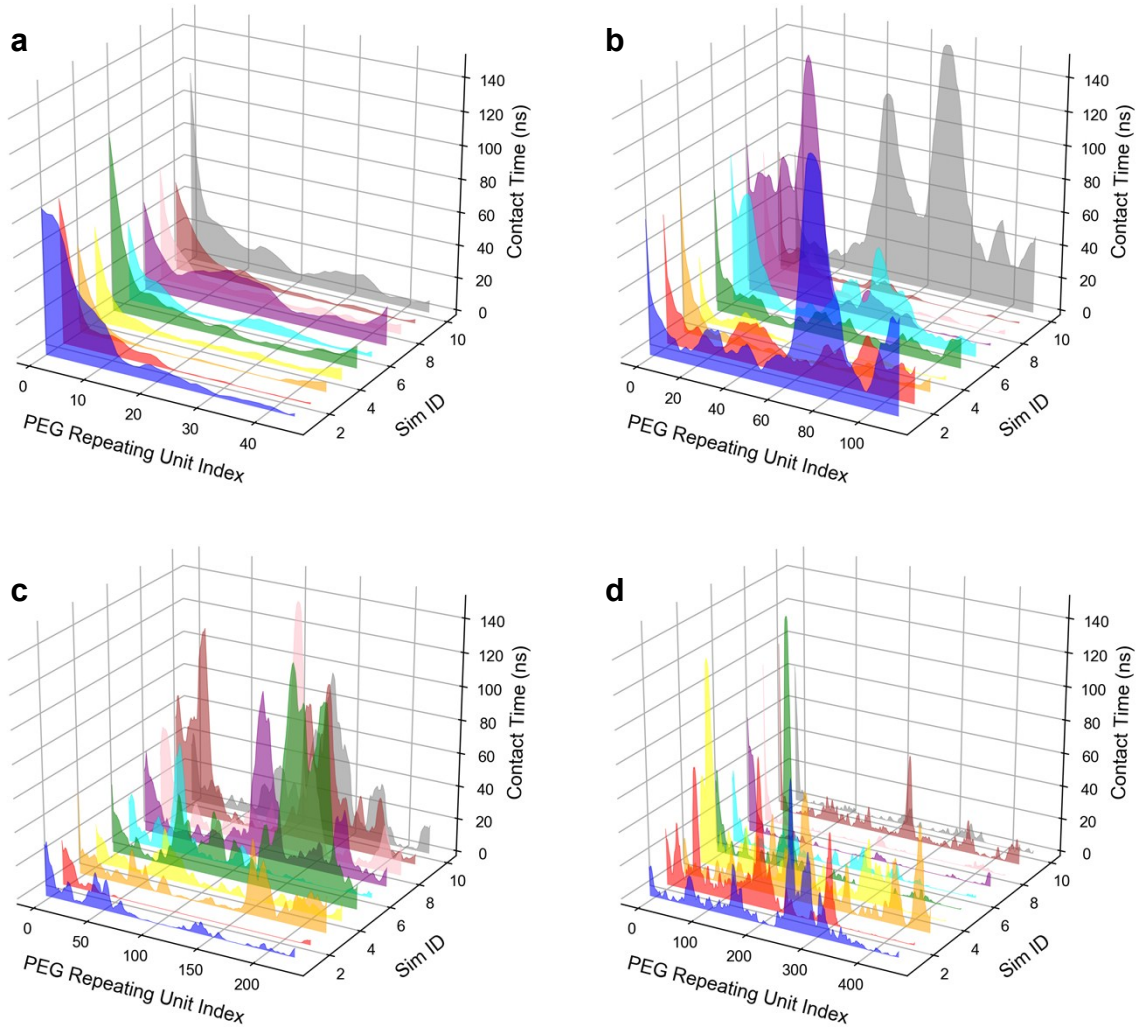

Figure S8. Contact time across repeating units of the grafted PEG polymer in 2 (a), 5 (b), 10 (c), and 20 (d) kDa N-terminal PEGylated BSA trajectories, where index 1 designates the repeating unit bonded to the linker. Contact time was measured for each of the ten simulations per PEG MM, referred to as "Sim ID" and colored as follows: blue, red, orange, yellow, green, cyan, purple, pink, brown, and gray.

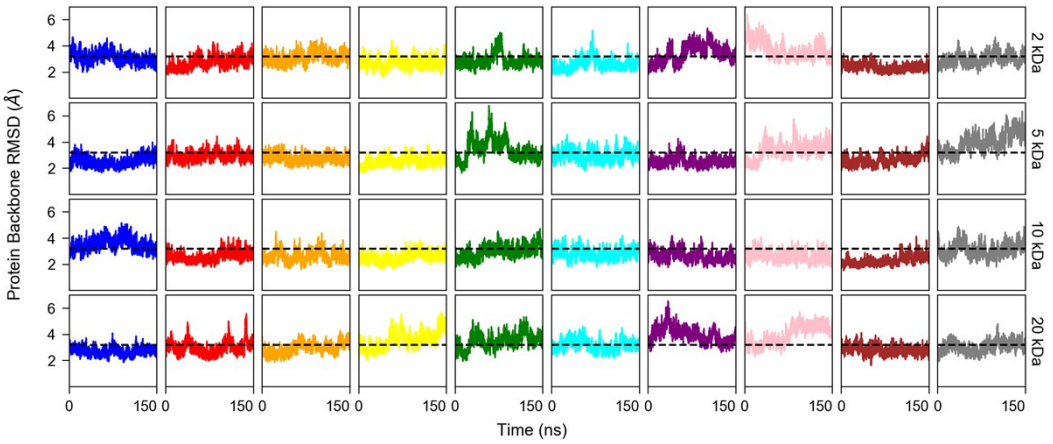

Figure S9. Evolution of root-mean-square-deviations (RMSDs) over time for ten simulations of K116 PEGylated BSA systems of each MM (2, 5, 10, and 20 kDa). Simulations were designated by "Sim ID" and are colored as follows: blue, red, orange, yellow, green, cyan, purple, pink, brown, and gray. The black dashed line represents the average RMSD value for the free BSA, calculated using the final 150 ns of the total 500 ns simulation time.

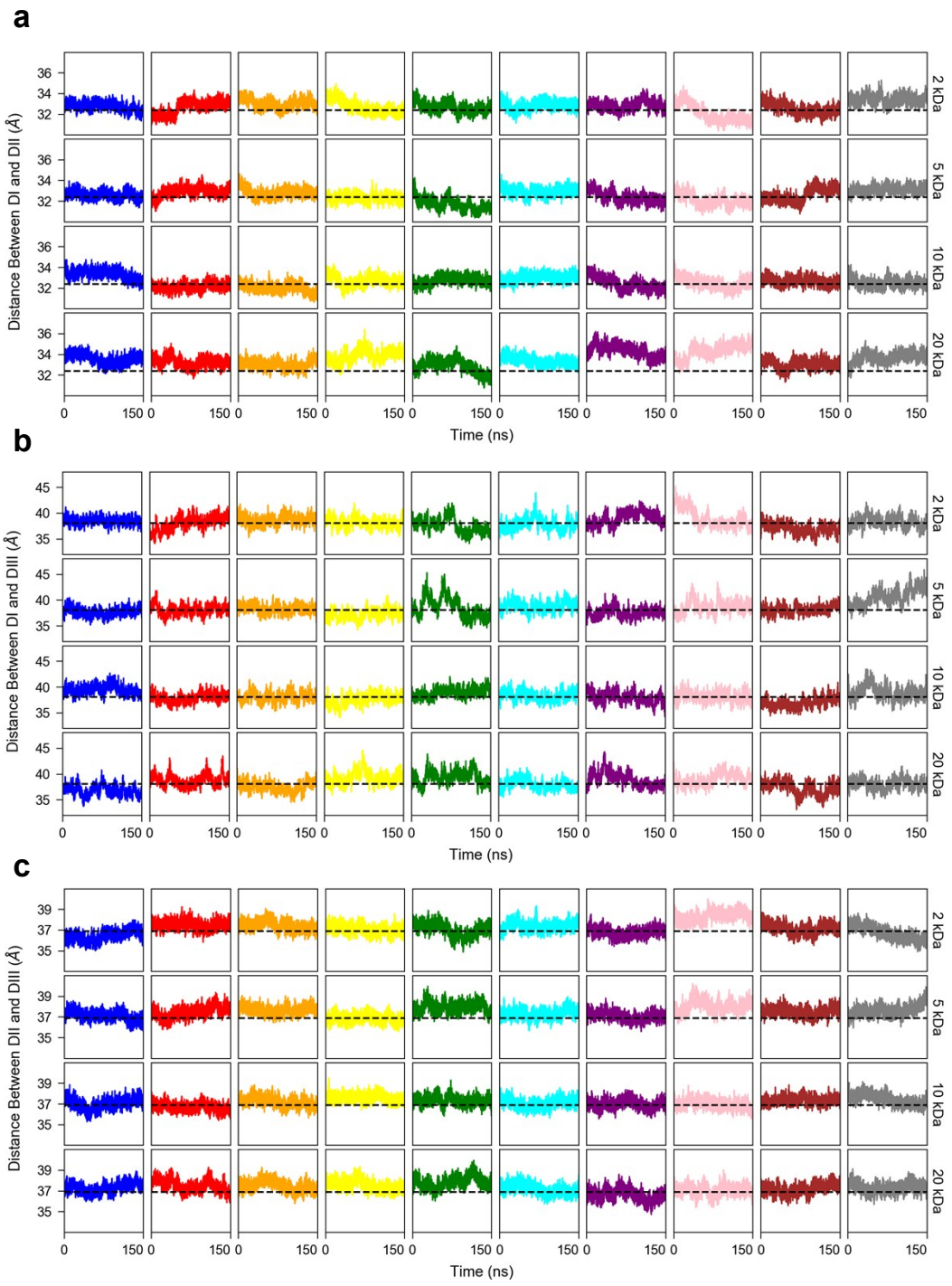

Figure S10. Evolution of distances between domains I & II (a), domains I & III (b), and domains II & III (c) over time for K116 PEGylated BSA systems. Simulations were designated by "Sim ID" and are colored as follows: blue, red, orange, yellow, green, cyan, purple, pink, brown, and gray. The black dashed line represents the average distance value for the free BSA, calculated using the final 150 ns of the total 500 ns simulation time.

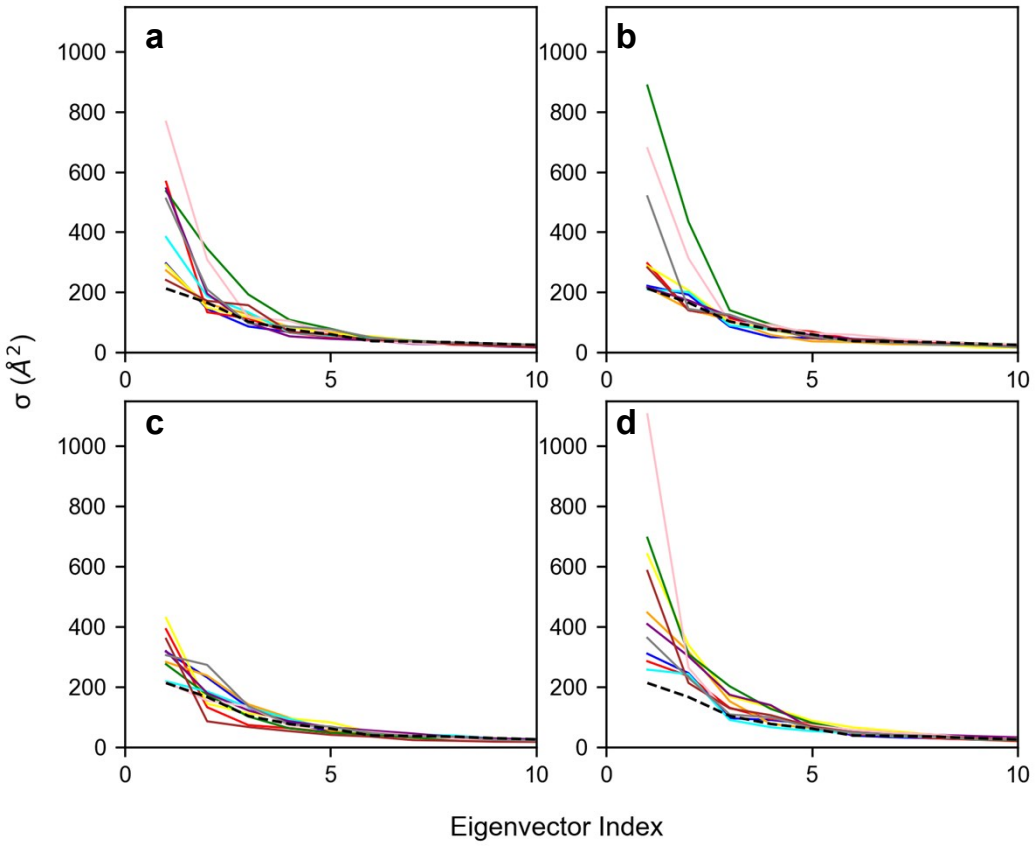

Figure S11. Scree plot for PCA analysis of Cα atoms in 2 (a), 5 (b), 10 (c), and 20 (d) kDa K116 PEGylated BSA systems. Simulations were designated by "Sim ID" and are colored as follows: blue, red, orange, yellow, green, cyan, purple, pink, brown, and gray. The black dashed line represents variance values for principal components of the free BSA, calculated using the final 150 ns of the total 500 ns simulation time.

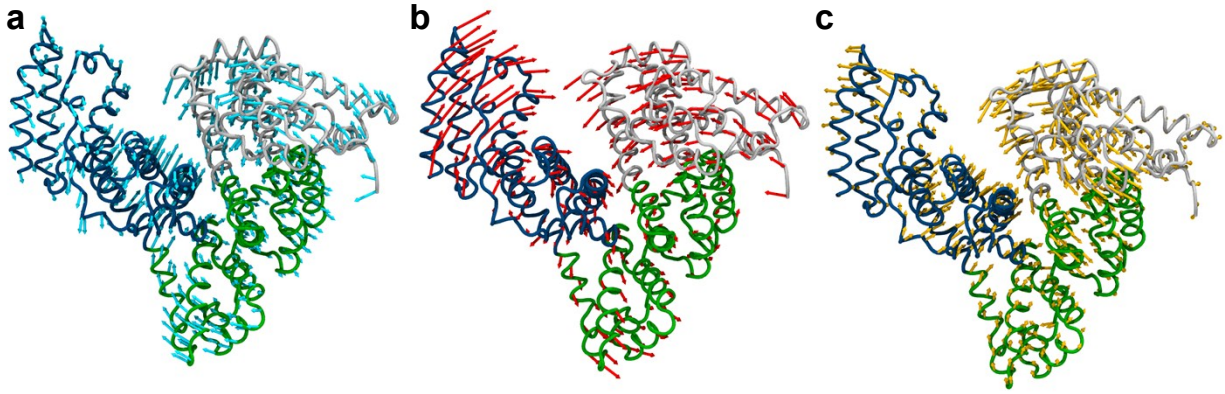

Figure S12. Cartoon representation of the BSA, which is partitioned into domain I (gray), domain II (green), and domain III (blue). The first three low mode vibrations are projected onto the protein, where principal components are colored as follows: correlated motions (a) as cyan arrows, anti-correlated motions (b) as red arrows, and tilt-like motions (c) as orange arrows.

Table S1. Simulation system sizes (number of atoms) for ten simulations, designated by "sim ID", for 2, 5, 10, and 20 kDa K116 PEGylated BSA conjugates.

sim ID	simulation system size (number of atoms)			
	2 kDa	5 kDa	10 kDa	20 kDa
1	230295	262631	322417	356549
2	187585	262439	233607	356387
3	178273	262490	323403	356537
4	221849	262685	323352	356513
5	230508	262436	293041	356939
6	189875	394678	224735	355971
7	230337	262658	323148	356192
8	170935	262520	323187	356522
9	230478	394855	322548	356648
10	230475	262604	257312	356729

Table S2. Radius of gyration for free PEG, grafted PEG, sum of free PEG and free protein (R_s), and distance between centers of mass of PEG and BSA ($D_{PEG,BSA}^{COM}$) in N-terminal PEGylated BSA conjugates. Fraction of time during which PEG assumed a shroud-like conformation ($R_s > D_{PEG,BSA}^{COM}$) is also reported.

PEG MM (kDa)	average radius of gyration (Å)			$D_{PEG,BSA}^{COM}$ (Å)	shroud-like conformation (%)
	free PEG	grafted PEG	R_s		
2	15.4 (3.1)	15.4 (3.1)	42.9 (3.1)	53.0 (3.4)	12.2
5	21.6 (4.7)	21.6 (4.7)	49.1 (6.0)	44.1 (15.0)	59.3
10	28.6 (5.0)	28.6 (5.0)	56.1 (6.3)	40.0 (13.5)	81.8
20	49.7 (7.4)	49.7 (7.4)	77.2 (8.8)	36.4 (12.3)	95.7

Table S3. Unique residues with top twenty maximum residence times for K116 PEGylated systems of each MM. Residence time is defined as any continuous time period of contact for within any given simulation.

2 kDa PEG		5 kDa PEG		10 kDa PEG		20 kDa PEG	
residue	time (ns)	residue	time (ns)	residue	time (ns)	residue	time (ns)
Lys180	127.6	Leu115	150.0	Arg435	150.0	Lys556	119.4 88.6
Arg185	106.4 57.4	Lys116	150.0 112.2	Cys436	150.0	Asp236	106.9
Lys114	95.2 36.3	Lys136	150.0	Lys439	150.0	Lys544	72.2
Arg144	94.9	Arg185	147.3 103.2	Arg444	150.0	Phe553	68.9
Lys116	54.1 29.4	Phe133	139.4	Arg185	99.2	Leu189	66.4
Ile181	42.9 40.0	Ile181	136.9 79.2	Lys544	99.1	Lys204	65.0
Tyr137	42.6 39.0	Leu122	124.1	Lys116	97.3	Glu570	61.8
Glu140	39.9 31.87	Tyr137	84.8 58.9	Asn404	95.1	Ala193	60.2
Ile141	38.6	Lys20	79.4	Pro440	88.5	Leu259	60.1
Leu115	34.1	Lys523	74.9	Ser442	81.4	Arg185	58.6
Leu189	32.2	Val40	69.1	Lys338	57.9	Leu178	58.3
Pro113	30.7 26.2	Lys114	67.3	Leu189	57.4	Lys106	56.3
Leu178	28.4 24.9	Leu505	61.8	Lys537	56.7	Asn549	53.7
Thr183	27.9	Lys350	61.4	Glu540	53.3	Lys474	52.5
Pro179	27.1	Lys474	60.7	Lys114	52.3	Lys239	52.1
Cys176	26.6	Tyr160	55.9	Glu443	50.4	Lys114	48.6
Val188	25.7	Thr526	51.7	Ser428	46.4	Tyr147	46.9
Pro572	24.7	Asn482	49.9	Ala405	45.1	Lys375	46.7
Tyr160	22.8	Phe508	38.9	Lys474	42.2	Lys180	46.6
Met184	21.2	Cys486	38.5	Met547	40.6	Leu397	44.3

Table S4. Average values for root-mean-square-deviations (RMSDs) in free BSA and K116 PEGylated BSA systems. For free BSA, the final 150 ns of the total 500 ns simulation time was used for analysis.

system	RMSD (Å)			
	backbone	domain I	domain II	domain III
Free BSA	3.2 (0.3)	2.3 (0.2)	1.9 (0.2)	2.0 (0.2)
2 kDa	3.0 (0.6)	1.9 (0.2)	1.3 (0.2)	1.9 (0.3)
5 kDa	3.0 (0.7)	1.8 (0.2)	1.3 (0.2)	1.8 (0.2)
10 kDa	2.8 (0.5)	1.8 (0.2)	1.2 (0.2)	1.8 (0.3)
20 kDa	3.3 (0.7)	1.9 (0.3)	1.9 (0.4)	1.8 (0.2)