Supplementary Information (SI) for Physical Chemistry Chemical Physics.


# Supporting information for "Beyond the Crystal: Molecular Dynamics Investigations of CETP with Varied Lipid Substrates Reveal Asymmetric Dominant Motions"

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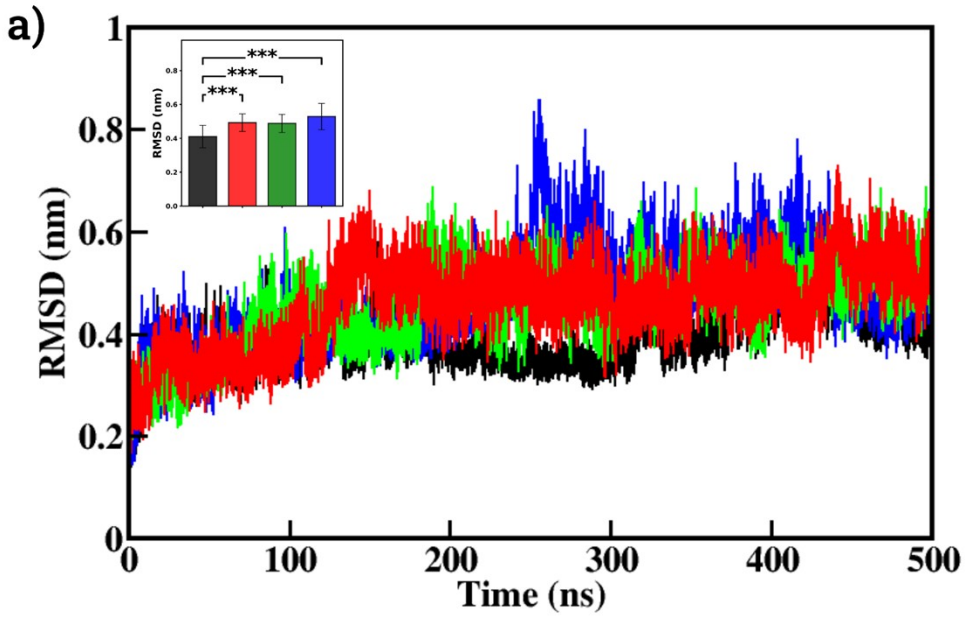
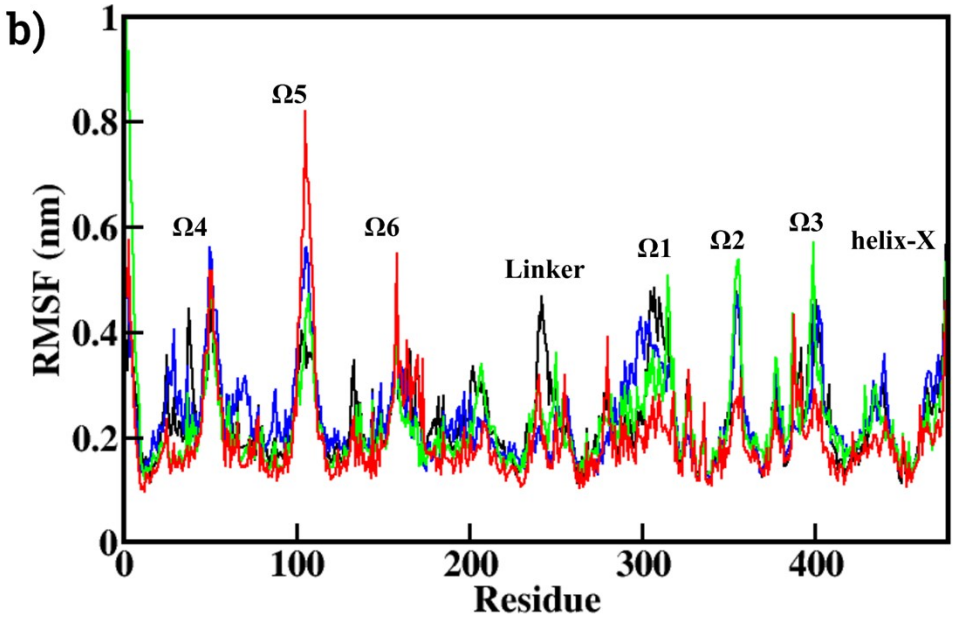
**Table S1:** List of Systems studied

| SI.NO | Systems | Simulation time | Box volume (Å$^3$) | Total number of atoms |
|---|---|---|---|---|
| 1 | CE_CETP_CE (M1) | 500 ns | 147.9 x 147.9 x 147.9 | 312806 |
| 2 | CE_CETP_TG (M2) | 500 ns | 148 x 148 x 148 | 312862 |
| 3 | TG_CETP_CE (M3) | 500 ns | 147.9 x 147.9 x 147.9 | 330127 |
| 4 | TG_CETP_TG (M4) | 500 ns | 148.1 x 148.1 x 148.1 | 312849 |
| 5 | Replica of M1 | 500 ns | 147.9 x 147.9 x 147.9 | 312806 |
| 6 | Replica of M2 | 500 ns | 148 x 148 x 148 | 312862 |
| 7 | Replica of M3 | 500 ns | 147.9 x 147.9 x 147.9 | 330127 |
| 8 | Replica of M4 | 500 ns | 148.1 x 148.1 x 148.1 | 312849 |
| | Total time | 4 μs | | |


a)
RMSD (nm)
Time (ns)
0
0.2
0.4
0.6
0.8
1
0
100
200
300
400
500
***
***
***
RMSD (nm)


b)
RMSF (nm)
Residue
Ω4
Ω5
Ω6
Linker
Ω1
Ω2
Ω3
helix-X
0
0.2
0.4
0.6
0.8
1
0
100
200
300
400

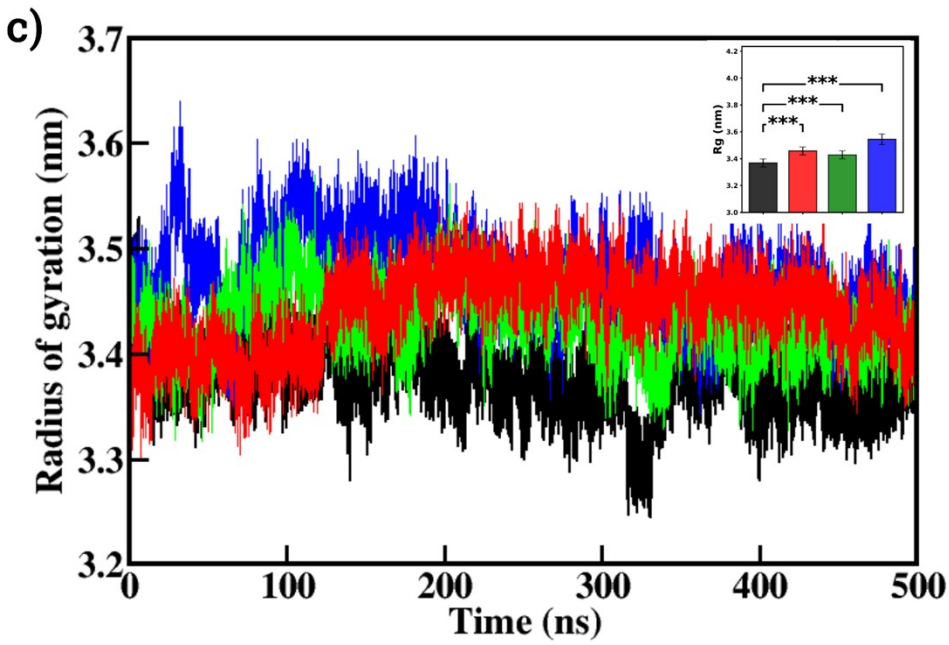



**Figure S1: Conformational Stability and Flexibility of CETP in Different Simulated Systems - Replica (a)** Time evolution of the root-mean-square deviation (RMSD) of CETP. The graph shows the deviation of the protein's Cα atoms from their initial conformation over the 500 ns simulation period; **(b)** Residue-level root-mean-square fluctuations (RMSF). The plot highlights key domains that exhibit significant conformational changes across the different systems, including the N- and C-terminal distal loops (Ω4–Ω6, Ω1–Ω3), the central linker region, and Helix-X; **(c)** Time evolution of the radius of gyration (Rg). The plot shows the compactness of the CETP protein over the simulation. All the plots are colored according to the simulated systems: CE_CETP_CE (black), CE_CETP_TG (red), TG_CETP_CE (green), and TG_CETP_TG (blue); **Insets:** Statistical significance was calculated using Welch's unpaired t-test (***$p < 0.001$) *for the* RMSD and Rg values (over the final 300 ns). Error bars represent the standard deviation, and asterisks indicate statistical significance compared to the control CE-CETP-CE system.

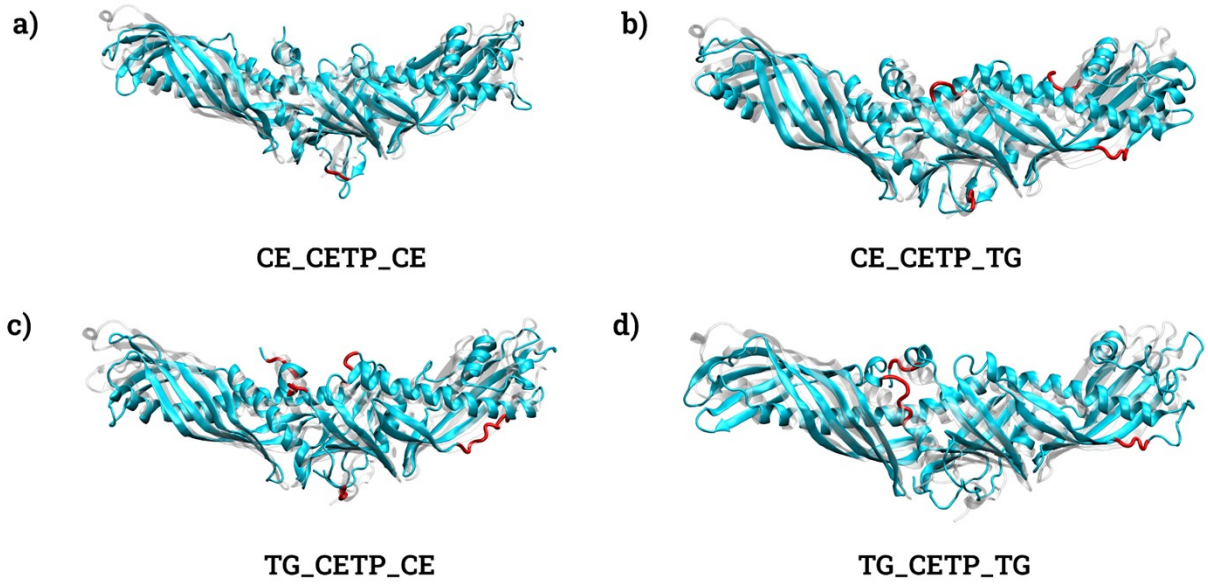



**Figure S2: Secondary structure distortions in the Cholesteryl Ester Transfer Protein (CETP) of different simulated systems - Replica.** The transparent grey structure represents the crystal structure (PDB ID: 2OBD) used as a reference, while the cyan cartoon structure depicts the final conformation of CETP after 500 ns of molecular dynamics simulation for each system. Regions where stable secondary structure elements (like helices or sheets) have distorted or unravelled into disordered coils/loops are highlighted in red. Results are compared across four distinct neutral lipid binding systems: a) CE-CETP-CE, b) CE-CETP-TG, c) TG-CETP-CE, and d) TG-CETP-TG.

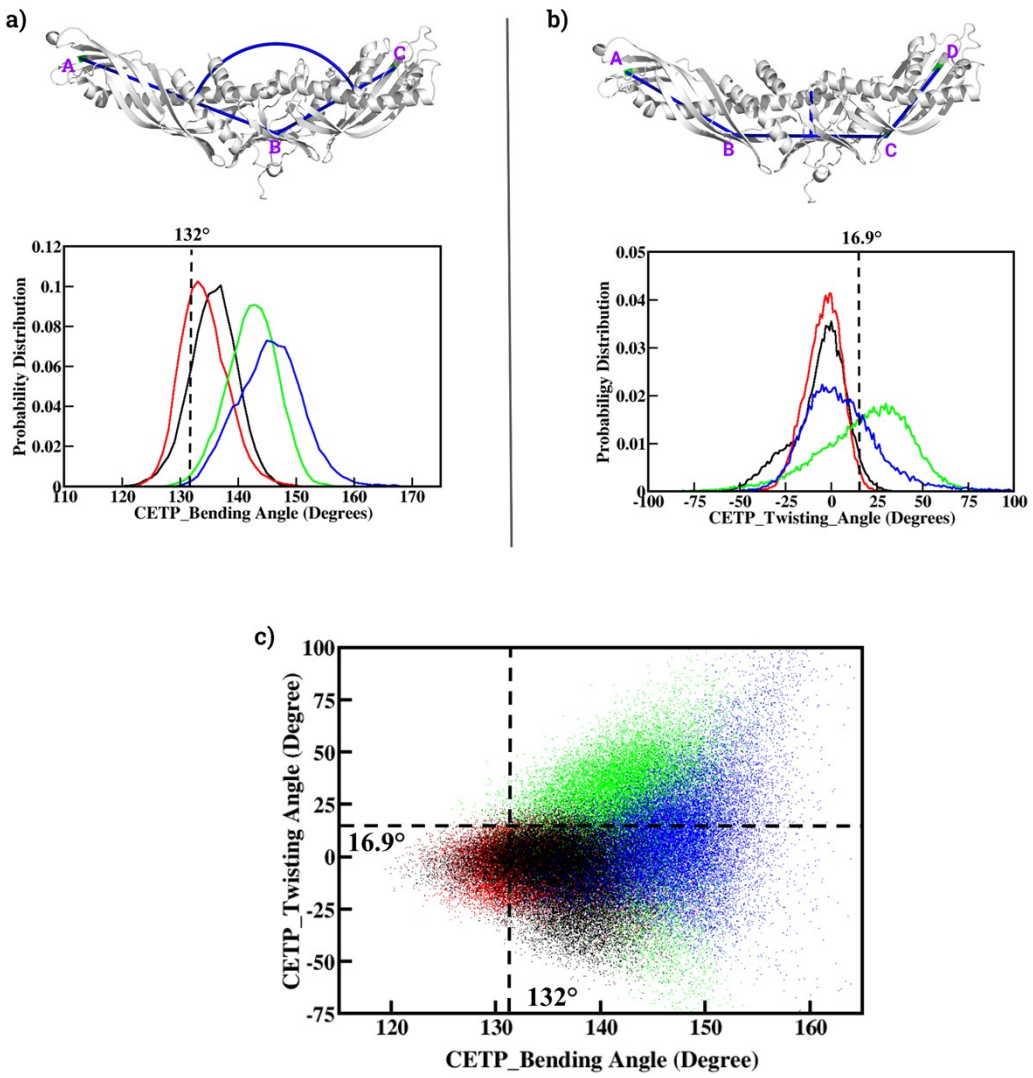



**Figure S3: Quantification of bending and twisting motions in the CETP protein - Replica.** **(a)** Probability distribution of the bending motion of CETP bound to different neutral lipids (CE and TG) during the simulations. The bending angle was calculated based on three reference points that define the angle, as illustrated in the schematic above. **(b)** Probability distribution of the twisting/untwisting motion of CETP under the same conditions. The twisting angle was determined by measuring the dihedral angle between four reference points, as shown in the schematic. (c) Two-dimensional joint distribution landscape correlating the Bending Angle (X-axis) and Twisting Angle (Y-axis). The dashed lines mark the crystal structure's conformations in all the figures. Results are shown for four simulated systems: CE-CETP-CE (black), CE-CETP-TG (red), TG-CETP-CE (green), and TG-CETP-TG (blue).

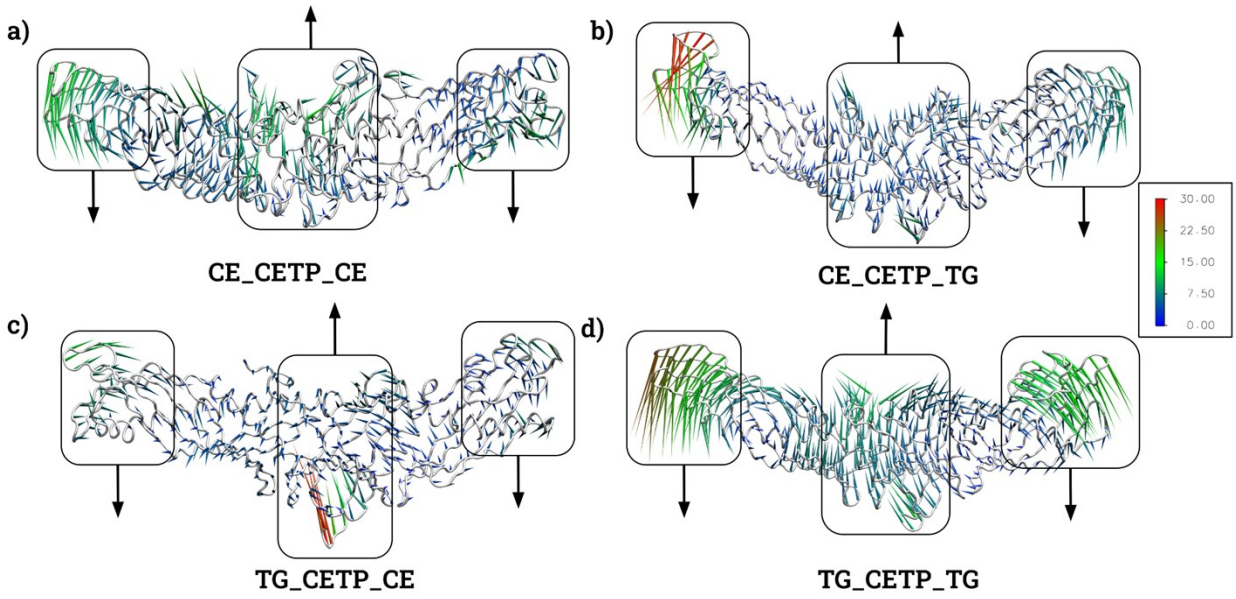



**Figure S4: Porcupine plot depicting the altered flexibility of the CETP residue's dominant motions along the first principal component - Replica.** These Porcupine plots illustrate the principal direction of dominant collective motion for CETP residues along the first principal component (PC1) trajectory. The length and direction of the arrows emanating from each C-alpha atom represent the magnitude and direction of the residue's flexibility during the simulation. Results are compared across four distinct neutral lipid binding systems: a) CE-CETP-CE, b) CE-CETP-TG, c) TG-CETP-CE, and d) TG-CETP-TG systems

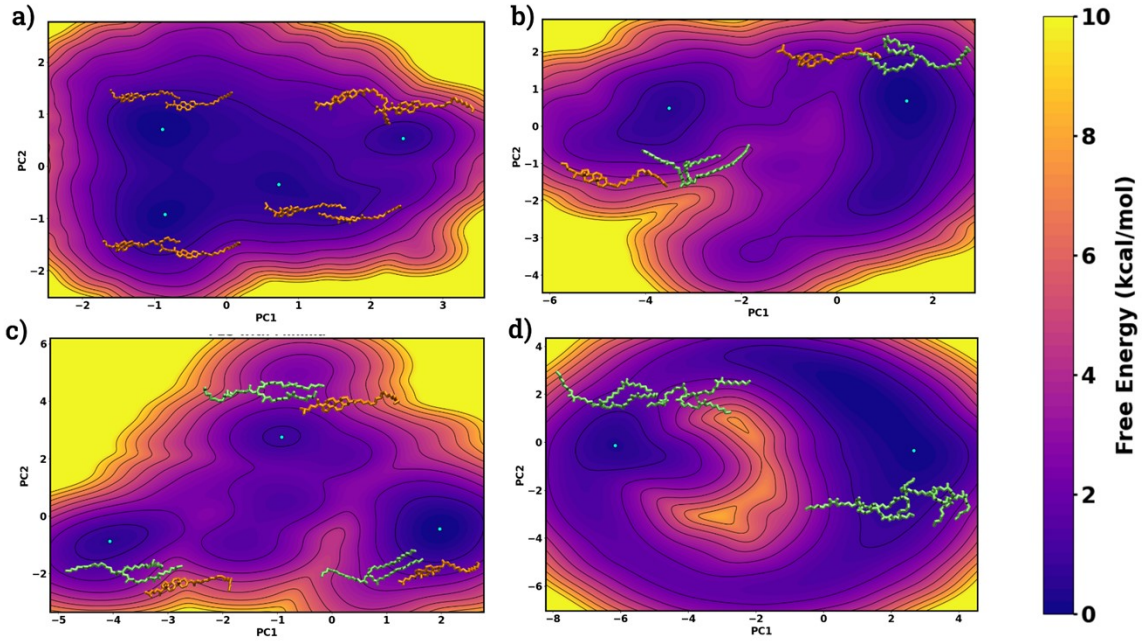



**Figure S5: Free energy landscapes (FELs) with contour mapping for the simulated CETP systems - Replica.** The landscapes are represented as two-dimensional projections of the simulated neutral lipid structures along the first two principal components (PC1 and PC2), overlaid with the corresponding free energy profiles. FELs are shown for CETP bound to different neutral lipid systems: **(a)** CE_CETP_CE, **(b)** CE_CETP_TG, **(c)** TG_CETP_CE, and **(d)** TG_CETP_TG. The identified energy minima indicate the most stable conformational states sampled during the simulations, reflecting the most probable orientations of the neutral lipids within the CETP cavity.