Supplementary information

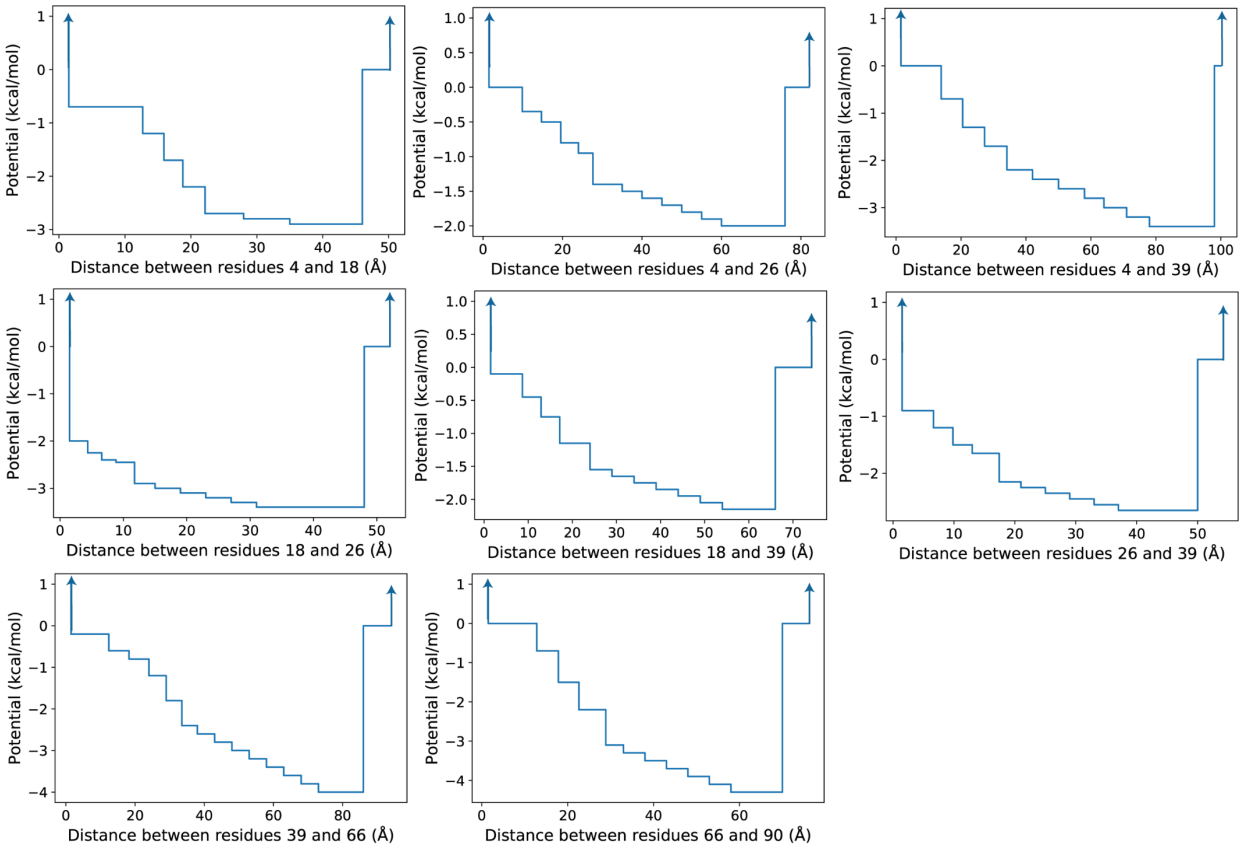

Figure S1. The discrete step function potential used as constraints for DMD simulations, Related to Figure 1.

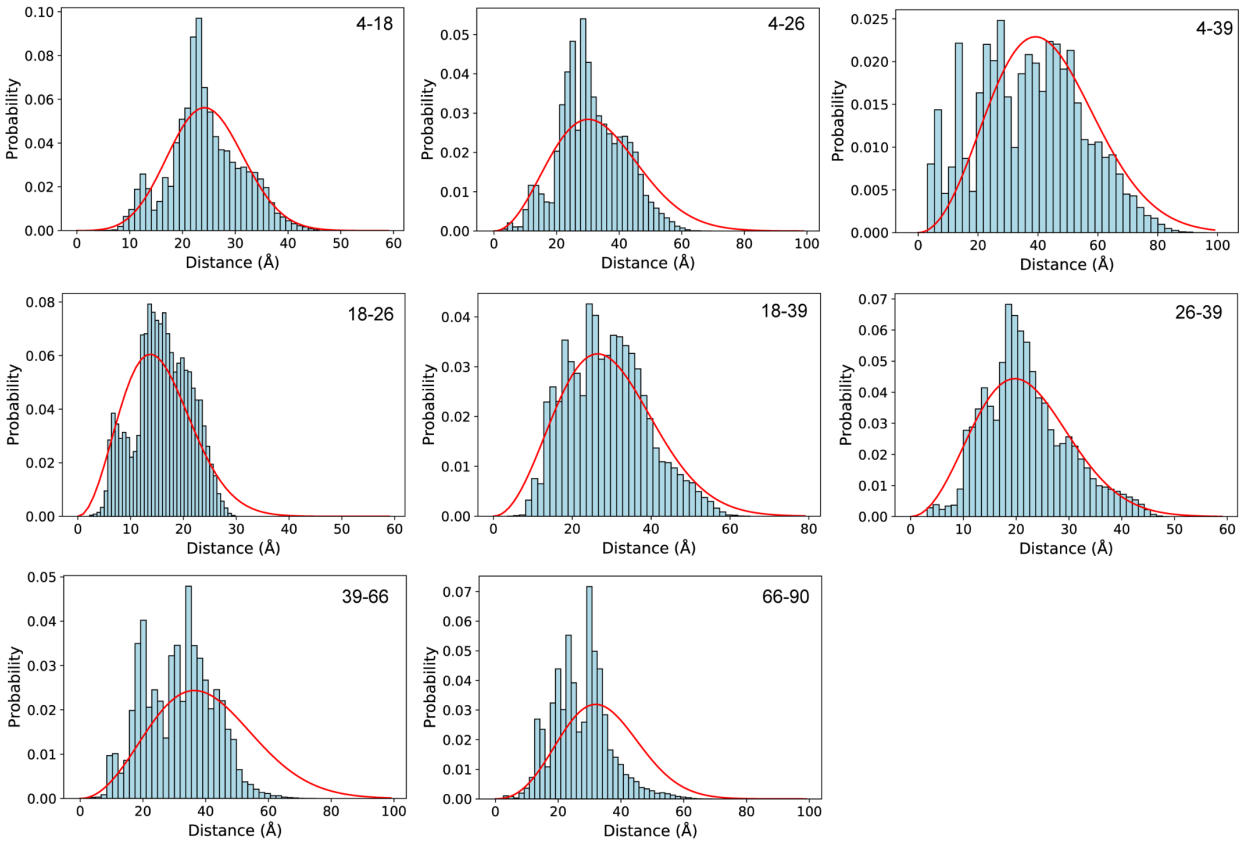

Figure S2. Comparison of computational (blue histogram) and experimental (red line) distance distributions, Related to Figure 1. The pair of residues are labeled in each panel.

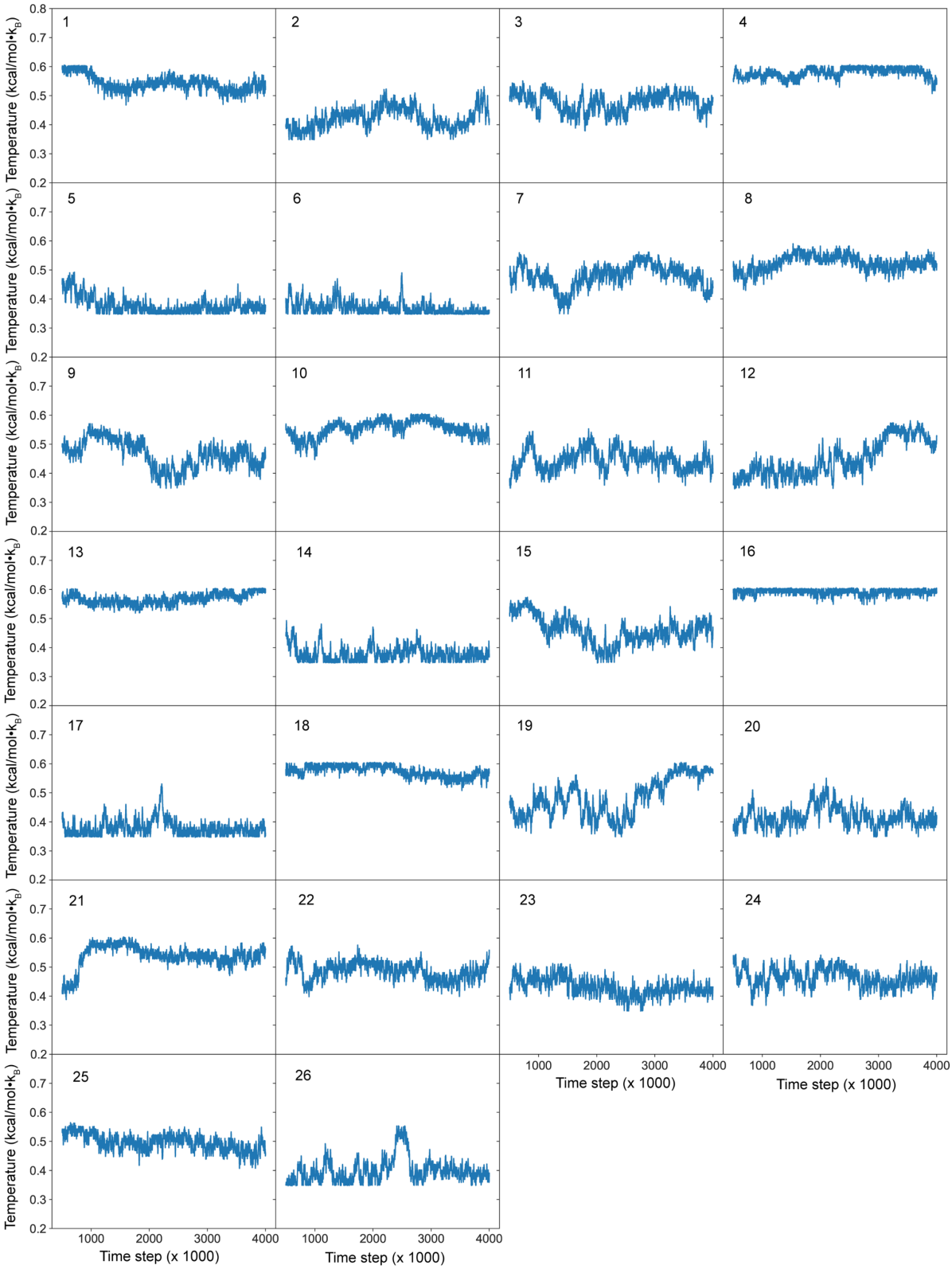

Figure S3. The time evolution of each replica in the temperature space, Related to STAR Methods. The number in each subplot indicates the replica number.

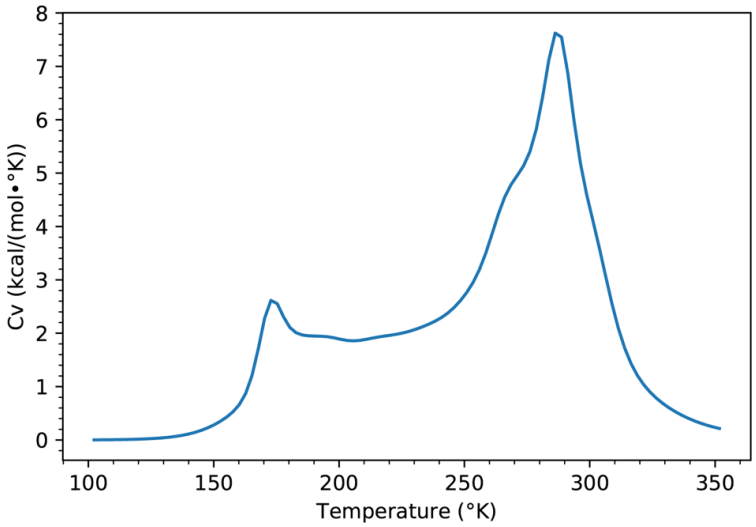

Figure S4. The heat capacity curve of α-syn simulations within time steps of $0.5x10^6$ ~ $4x10^6$, Related to STAR Methods.

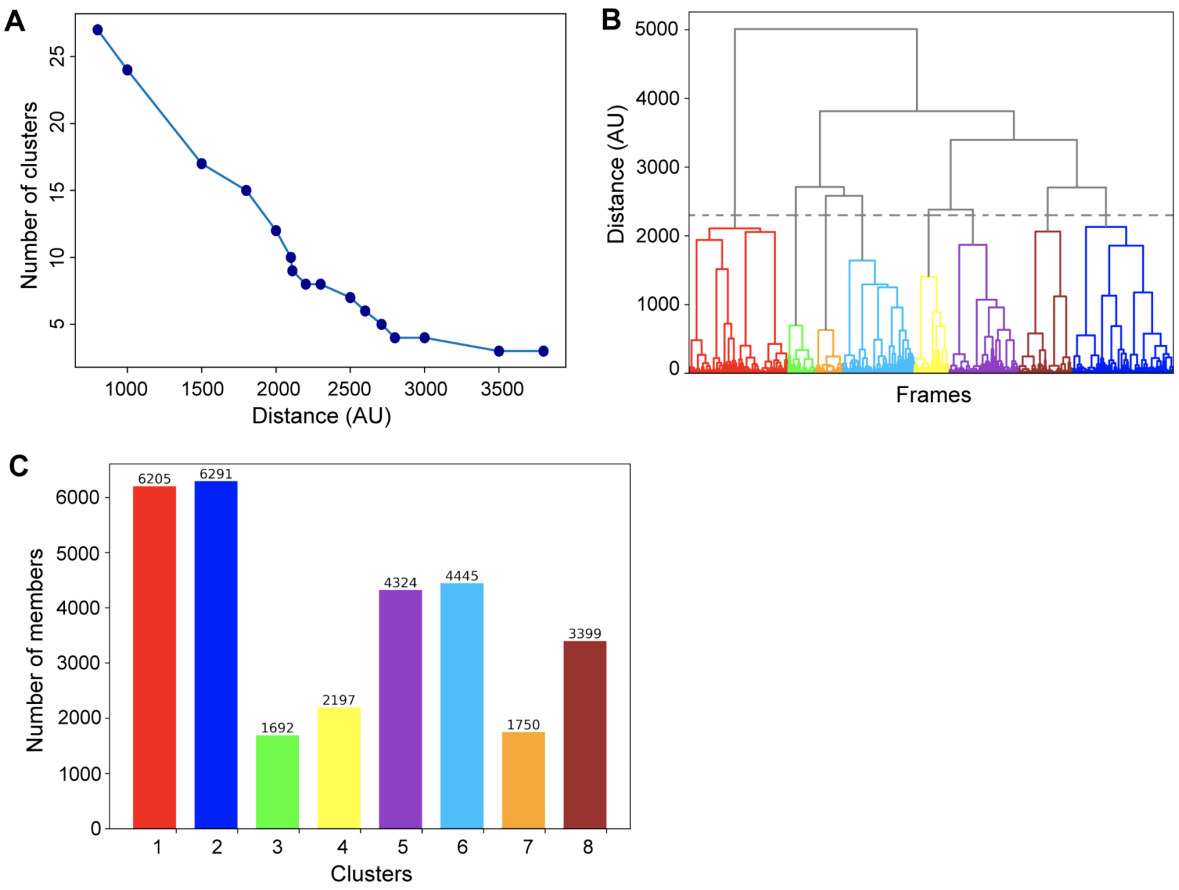

Figure S5. Hierarchical clustering of the conformational ensemble of α-syn monomer, Related to STAR Methods.

(A) The distance cutoffs are optimized for the hierarchical clustering.

(B) The clustering dendrogram is shown and the distance cutoff is selected at the dashed line.

(C) The number of structures within each cluster is shown for the selected distance cutoff.

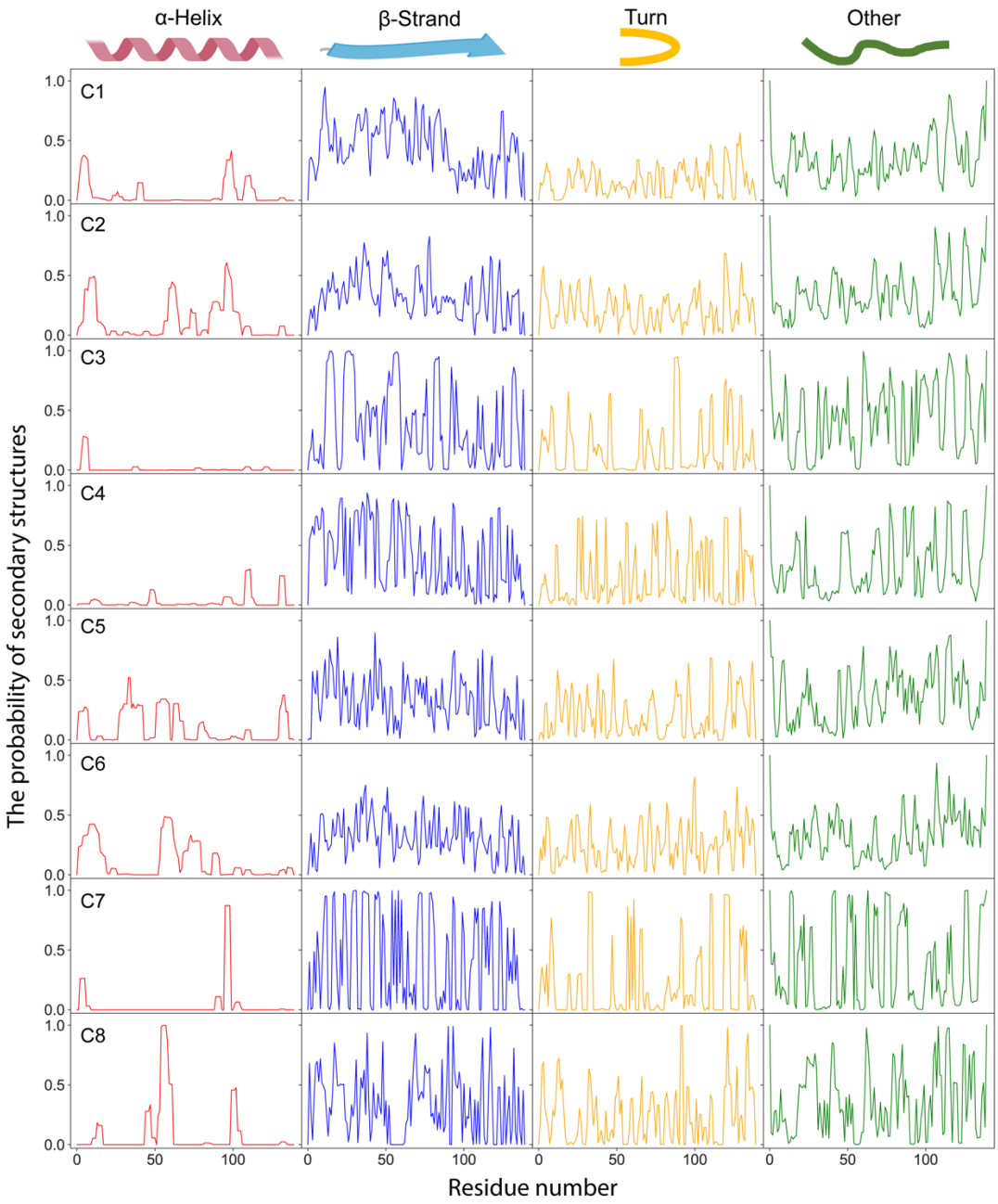

Figure S6. The probability of secondary structures for each residue of α-syn within clusters 1~8 (C1~8), Related to Figure 3.

The secondary structures of the residues in each structure are computed using GROMACS (*Methods*).

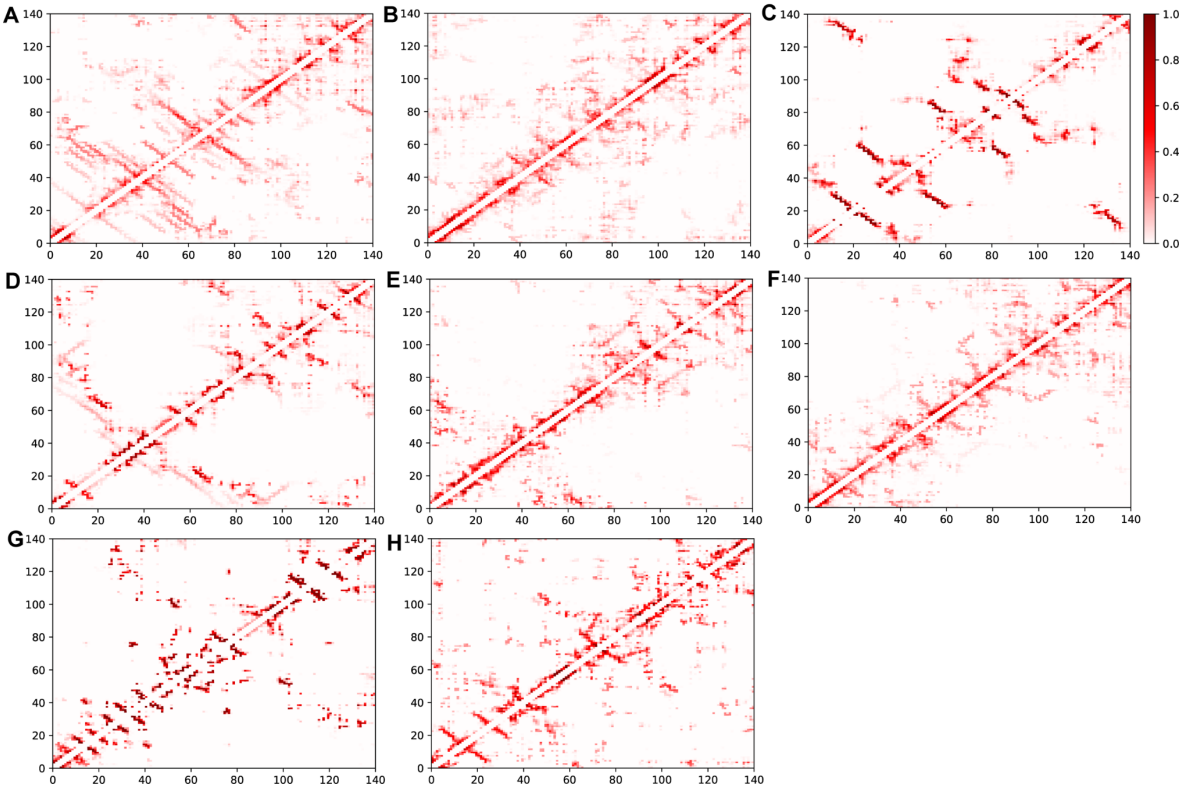

Figure S7. The contact frequency maps of clusters 1~8 (A~H), Related to Figure 3. The x- and y-axis indicate residue numbers.

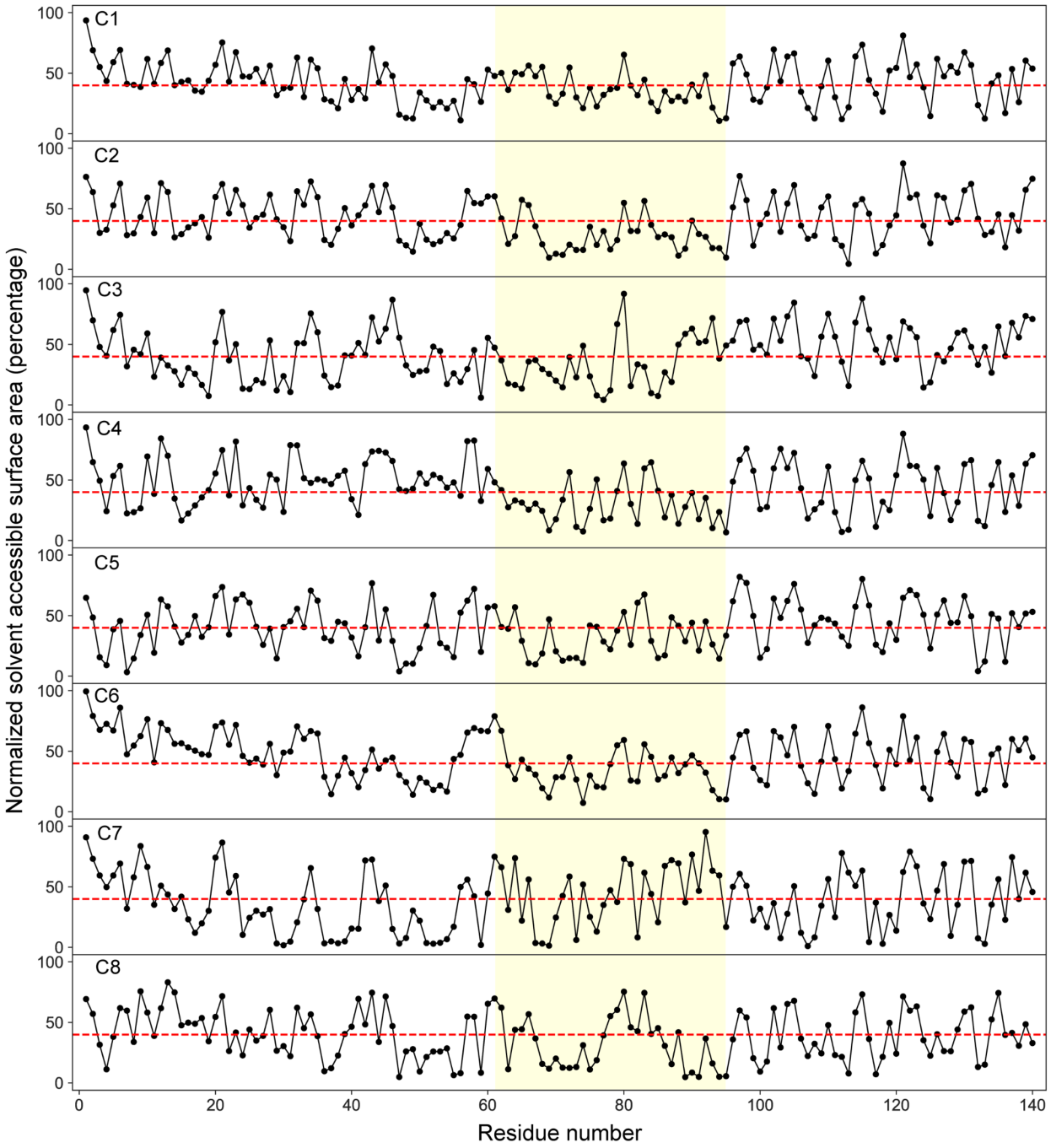

Figure S8. The normalized solvent accessible surface area (SASA) of each residue for all the structures that have N- and C- terminal long-range contacts in clusters 1~8 (C1~8), Related to Figure 4. Residues with SASA < 0.4 (red dashed line) are considered buried, while those > 0.4 are considered surface exposed. The NAC segment is highlighted in yellow.

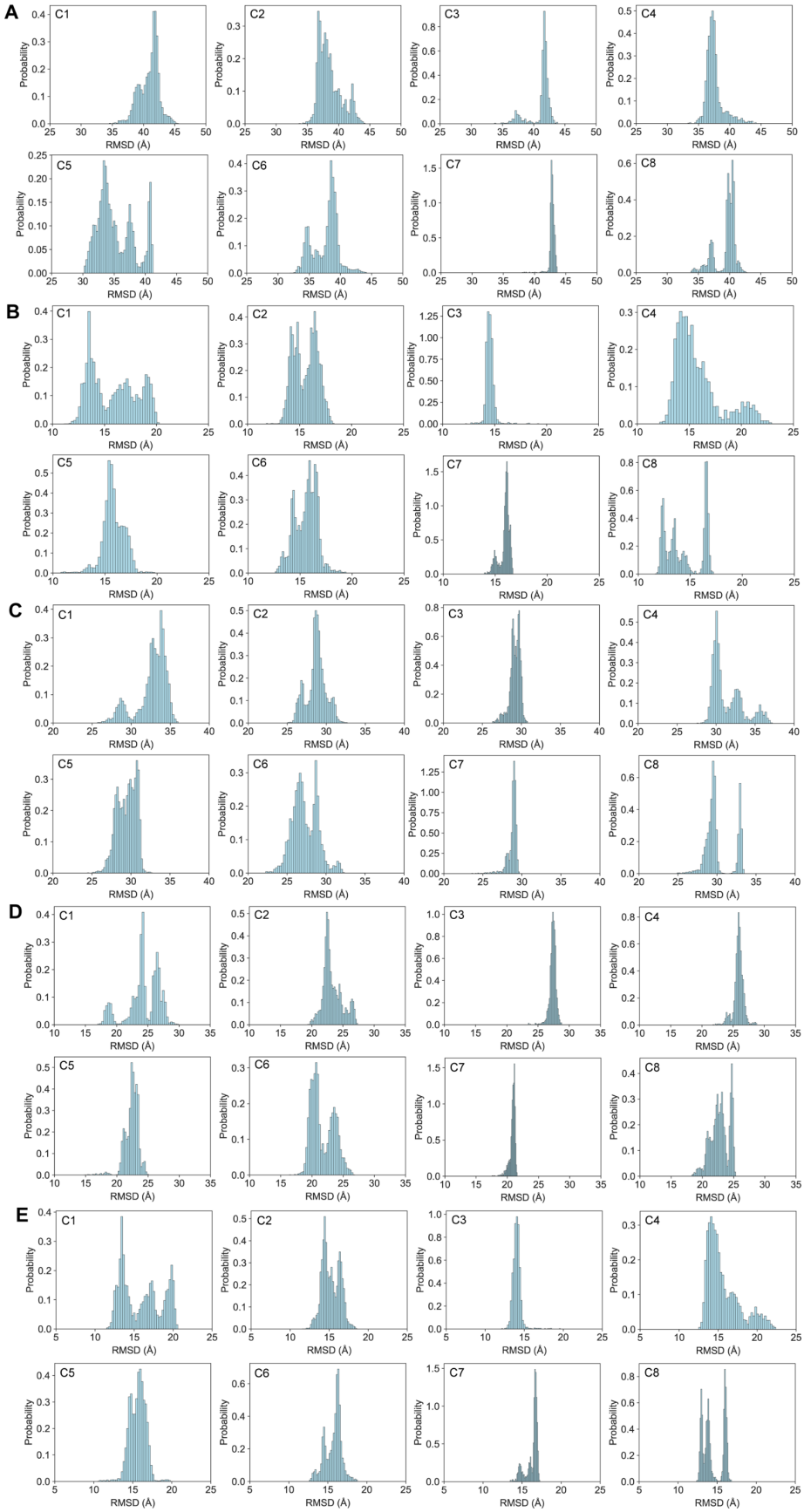
A
B
C
D
E
C1
C2
C3
C4
C5
C6
C7
C8
Probability
RMSD (Å)

Figure S9. Comparison of structures in PDB with the conformational ensemble of α-syn, Related to Figure 7.

(A) The RMSD between the structure of human micelle-bound α-syn (PDB 1XQ8) and all the structures in clusters 1~8 (C1~8). The RMSD values are calculated by comparing structures of the whole protein.

(B) The RMSD between the cryo-EM structure of α-syn fiber (PDB 6A6B, residues 37~99) and all the structures in clusters 1~8 (C1~8). The RMSD values are calculated by comparing structures of the truncated protein from residues 37 to 99.

(C) The RMSD between the NMR structure of α-syn fiber (PDB 2N0A, 30~98) and all the structures in clusters 1~8 (C1~8). The RMSD values are calculated by comparing structures of the truncated protein from residues 30 to 98.

(D) The RMSD between the cryo-EM structure of multiple system atrophy Type II-1 α-syn filament (PDB 6XYP, chain A, residues 14~94) and all the structures in clusters 1~8 (C1~8). The RMSD values are calculated by comparing structures of the truncated protein from residues 14 to 94.

(E) The RMSD between the cryo-EM structure of multiple system atrophy Type II-1 α-syn filament (PDB 6XYP, chain B, residues 36~99) and all the structures in clusters 1~8 (C1~8). The RMSD values are calculated by comparing structures of the truncated protein from residues 36 to 99.

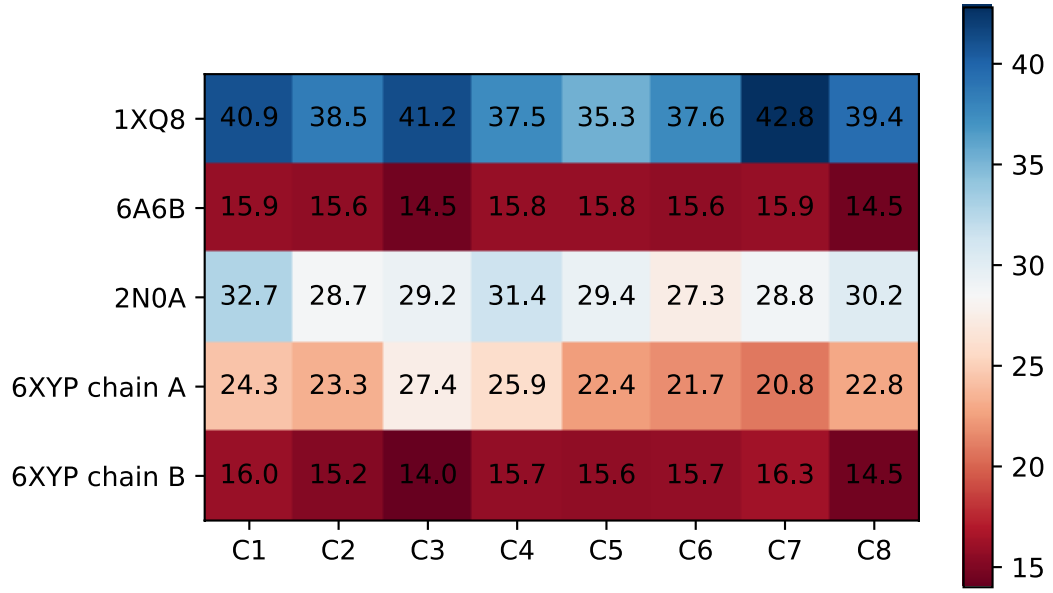

Figure S10. The average RMSD values between the eight clusters (C1~C8) and the experimental structures from PDB, Related to Figure 7. The average RMSD values are shown in each square.

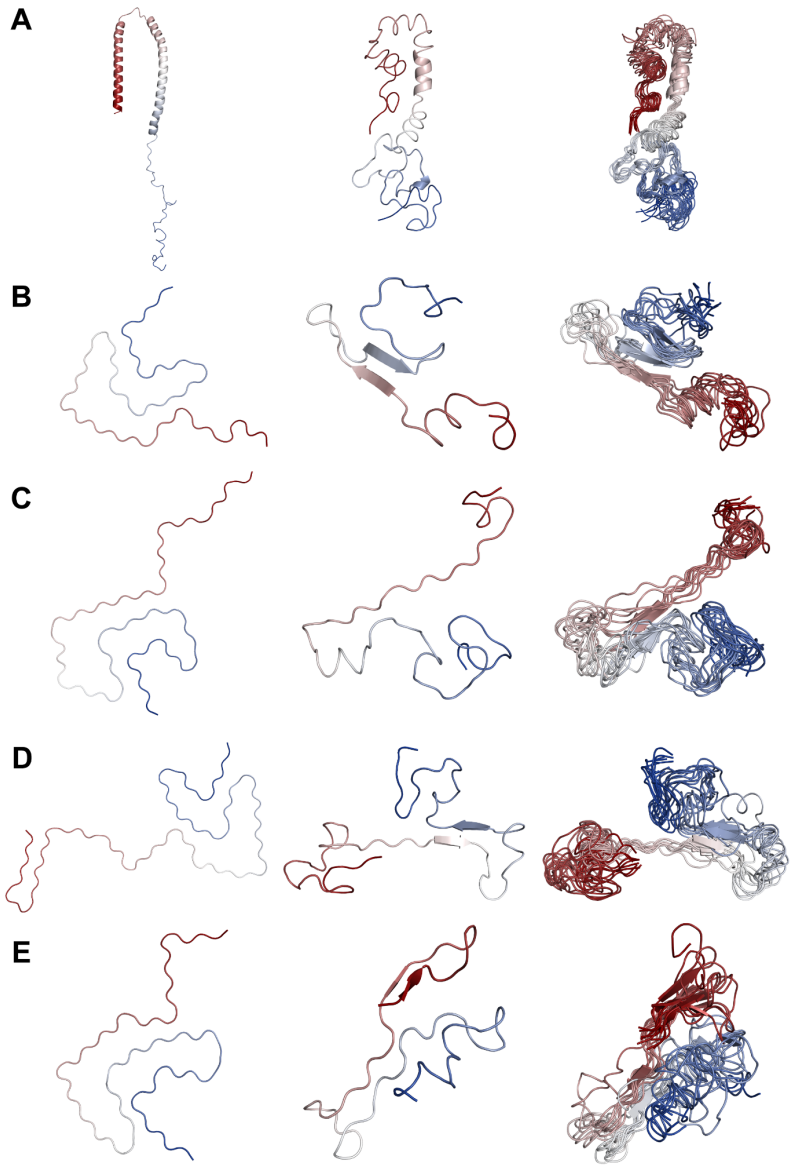

Figure S11. Comparison of α-syn structures in PDB with simulated structures, Related to Figure 7.

(A) The NMR structure of micelle-bound α-syn (PDB 1XQ8, residues 1~140) on the left panel is compared to the structure in cluster 5 with the lowest RMSD (in the middle). Right panel shows the top 10 structures in cluster 5 with the lowest RMSD.

(B) The cryo-EM structure of α-syn fiber (PDB 6A6B, residues 37~99) on the left panel is compared to the structure in cluster 1 with the lowest RMSD (in the middle). Right panel shows the top 10 structures in cluster 1 with the lowest RMSD.

(C) The NMR structure of α-syn fiber (PDB 2N0A, residues 30~98) on the left panel is compared to the structure in cluster 1 with the lowest RMSD (in the middle). Right panel shows the top 10 structures in cluster 1 with the lowest RMSD.

(D) The cryo-EM structure of multiple system atrophy Type II-1 α-syn filament (PDB 6XYP, chain A, residues 14~94) is compared to the structure in cluster 1 with the lowest RMSD (in the middle). Right panel shows the top 10 structures in cluster 1 with the lowest RMSD.

(E) The cryo-EM structure of multiple system atrophy Type II-1 α-syn filament (PDB 6XYP, chain B, residues 36~99) is compared to the structure in cluster 1 with the lowest RMSD (in the middle). Right panel shows the top 10 structures in cluster 1 with the lowest RMSD.

Table S1. The replica exchange rate of DMD simulations, Related to STAR Methods.

Temperature 1 (kcal/mol•k_B)	Temperature 2 (kcal/mol•k_B)	Exchange rate
0.35	0.36	0.551
0.36	0.37	0.549
0.37	0.38	0.577
0.38	0.39	0.577
0.39	0.40	0.555
0.40	0.41	0.591
0.41	0.42	0.605
0.42	0.43	0.591
0.43	0.44	0.589
0.44	0.45	0.599
0.45	0.46	0.636
0.46	0.47	0.605
0.47	0.48	0.605
0.48	0.49	0.598
0.49	0.50	0.594
0.50	0.51	0.594
0.51	0.52	0.577
0.52	0.53	0.535
0.53	0.54	0.540
0.54	0.55	0.554
0.55	0.56	0.494

0.56	0.57	0.469
0.57	0.58	0.493
0.58	0.59	0.449
0.59	0.60	0.550

Table S2. Pairwise RMSD (Å) between the eight clusters, Related to Figure 2.

Clusters	C1	C2	C3	C4	C5	C6	C7	C8
C1	0.0	16.3	20.9	19.1	19.7	21.7	17.3	15.6
C2	16.3	0.0	15.7	13.0	14.7	16.6	15.2	13.8
C3	20.9	15.7	0.0	16.7	18.3	17.4	15.7	16.1
C4	19.1	13.0	16.7	0.0	13.6	18.3	18.1	15.9
C5	19.7	14.7	18.3	13.6	0.0	17.0	18.9	15.3
C6	21.7	16.6	17.4	18.3	17.0	0.0	15.1	17.5
C7	17.3	15.2	15.7	18.1	18.9	15.1	0.0	14.2
C8	15.6	13.8	16.1	15.9	15.3	17.5	14.2	0.0

Table S3. The average pairwise RMSD within each cluster, Related to Figure 2.

Clusters	RMSD (Å)
C1	13.5
C2	12.2
C3	8.0
C4	11.2
C5	11.7
C6	13.6
C7	5.3
C8	10.0

Table S5. The average secondary structure content (in percentage) of each cluster and the conformational ensemble, Related to Figure 3.

Cluster	α-Helix	β-Strand	Turn	Other
C1	5.1	44.2	16.5	34.2
C2	11.4	32.4	21.4	34.8
C3	1.2	38.0	18.3	42.5
C4	2.8	41.6	23.1	32.5
C5	10.5	32.7	20.6	36.2
C6	11.1	31.7	24.6	32.6
C7	3.8	40.2	19.7	36.3
C8	8.2	33.1	23.4	35.3
Ensemble average	7.9	36.3	20.8	34.9