Supplementary Information

A novel folding pathway of the villin headpiece subdomain HP35

Ercheng Wang[2,#], Peng Tao[1,#], Jun Wang[1], Yi Xiao[1*]

[1] *Institute of Biophysics, School of Physics, Huazhong University of Science and Technology, Wuhan 430074, China.*
[2] *College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, Zhejiang 310058, China*

Table S1 The fraction of each cluster and the RMSDs of the important components of the representative structures of each cluster, the RMSD of each component lower than the corresponding native state cutoff are in boldface, the native state cutoff for Global, N-seg, C-seg , PLWK, Helix1, Helix2 and Helix3 are 2Å, 2Å, 2Å, 2Å, 1Å, 1Å, 1Å and 1Å, respectively.

Cluster	Frames	Fraction	Global RMSD(Å)	N-seg RMSD(Å)	Hycore RMSD(Å)	C-seg RMSD(Å)	PLWK RMSD(Å)	Helix1 RMSD(Å)	Helix2 RMSD(Å)	Helix3 RMSD(Å)
1	21230	0.380	6.056	5.886	4.909	4.613	1.970	3.336	2.510	2.534
2	8070	0.144	7.651	4.438	3.816	**1.504**	**0.176**	**0.294**	2.002	**0.320**
3	7278	0.130	3.848	**1.170**	**0.870**	3.492	**0.146**	1.049	**0.321**	3.468
4	6527	0.117	7.354	3.902	4.739	5.524	2.143	**0.510**	2.599	3.846
5	3713	0.110	5.347	3.670	2.209	3.400	1.102	1.921	**0.183**	3.675
6	3713	0.066	**1.524**	**0.826**	**0.248**	**0.761**	**0.181**	**0.561**	**0.244**	**0.733**
7	1227	0.022	16.338	5.560	4.663	7.775	2.298	**0.369**	3.030	5.371
8	1032	0.018	9.817	7.535	7.269	6.413	**0.427**	3.472	2.878	4.136
9	664	0.012	7.482	8.037	3.800	4.643	1.992	4.079	3.338	2.357
10	9	< 0.001	26.639	12.175	10.598	13.049	2.458	4.083	3.306	7.165
11	6	< 0.001	22.270	9.860	9.149	10.147	2.593	3.943	2.578	5.469

[#] They made equal contributions to this work.

[*] Corresponding author.

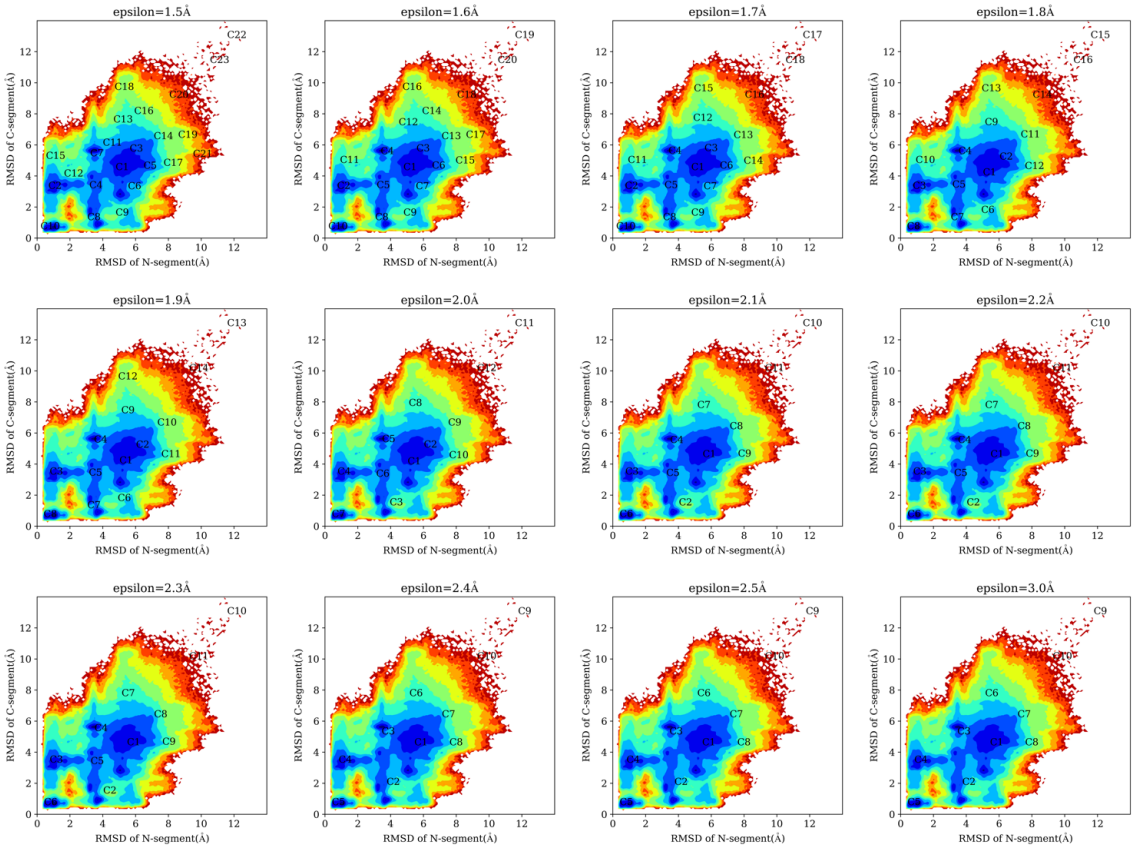

Fig. S1 Cluster results with different epsilon ranged from 1.5 to 3.0Å. The definition of epsilon is the distance cutoff between points for forming a cluster. Generally, bigger epsilon will produce lesser clusters.

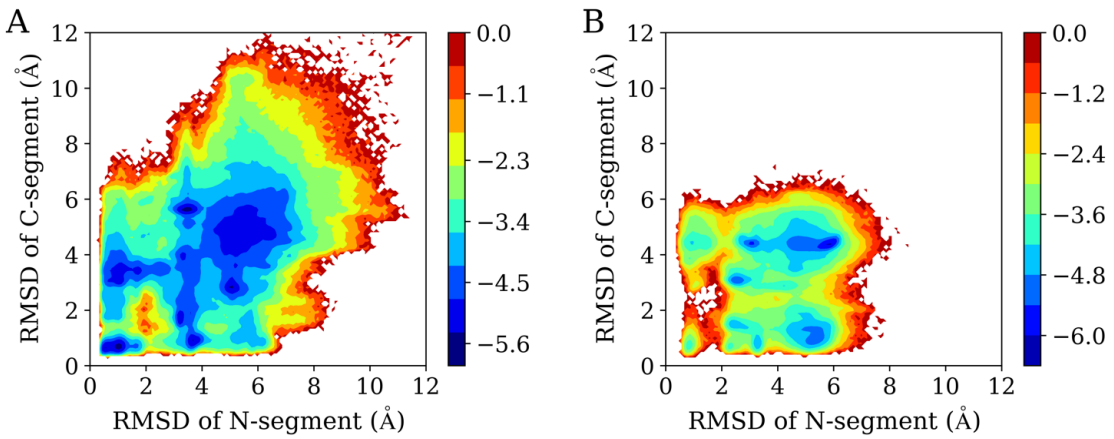

Fig. S2 Free energy landscapes that are built from CMD simulations in this study (A) and from 9μs REMD simulations (B, unpublished data). We performed REMDs with the AMBER ff14SB force field. To represent the solvation effect, the Generalized Born (GB) model (*igb* = 5) was selected with 0.2 M salt concentration and default surface tension of 0.005 kcal/mol/Å^2. There were 16 replicas in the REMD, and the targeting temperatures were 276, 286, 298, 309, 322, 334, 348, 362, 376, 390, 405, 421, 437, 453, 471, and 489 K, created by a python library *remd_temperature* with an exchange probability of 0.45. Due to that GPU accelerated implicit solvent GB simulations do not support a cutoff, the cutoff was set to 999.0 Å.

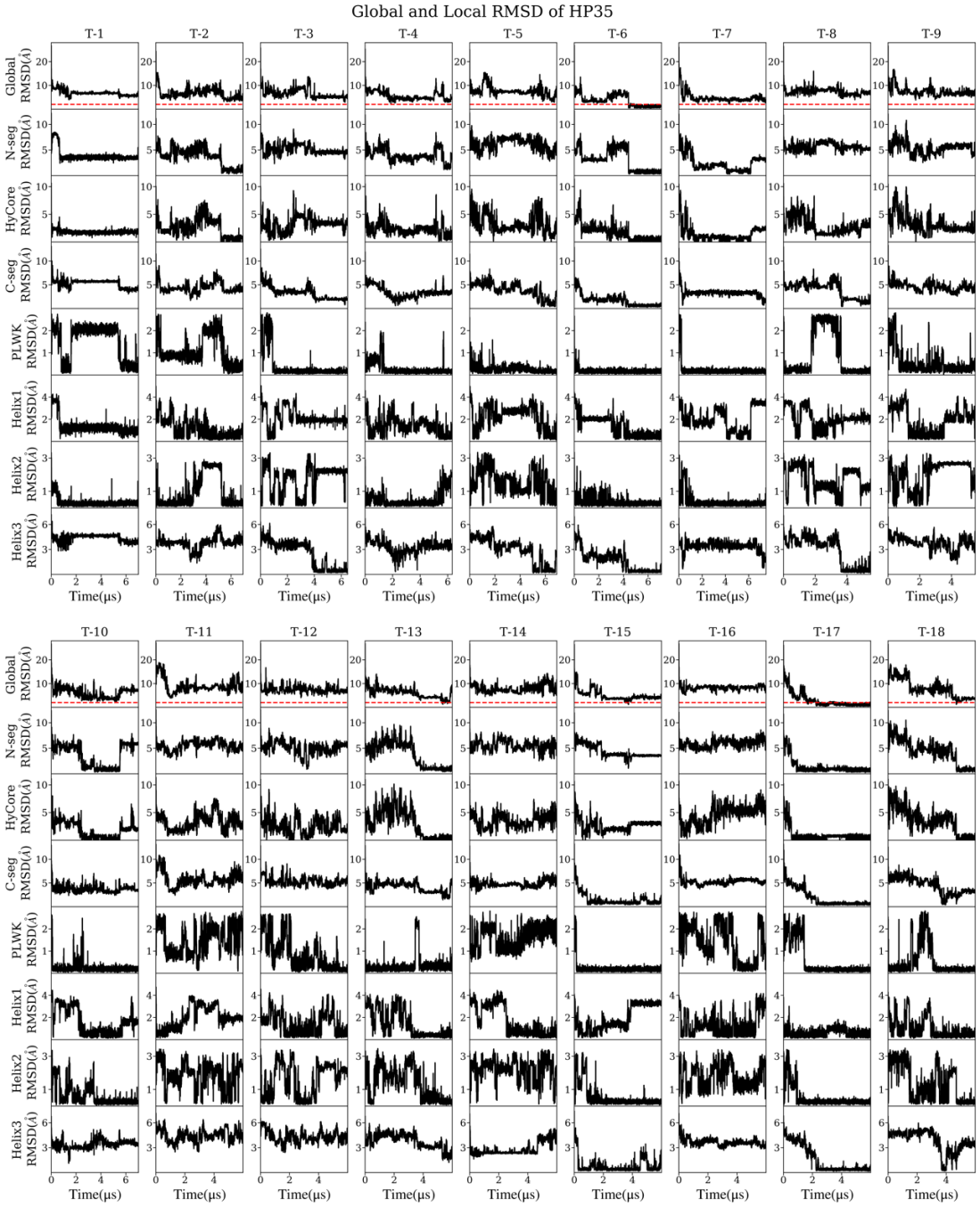

Fig. S3 Time courses of Cα-RMSDs of the global and local structures of HP35 simulated by CMD using AMBER ff14SB force field. “Global” denotes the entire chain without the two ending residues, “N-seg” the N-segment, “C-seg” the C-segment, “HyCore” the hydrophobic core and “PLWK” the PLWK motif. The red dashed represents Cα-RMSD = 2.0 Å.

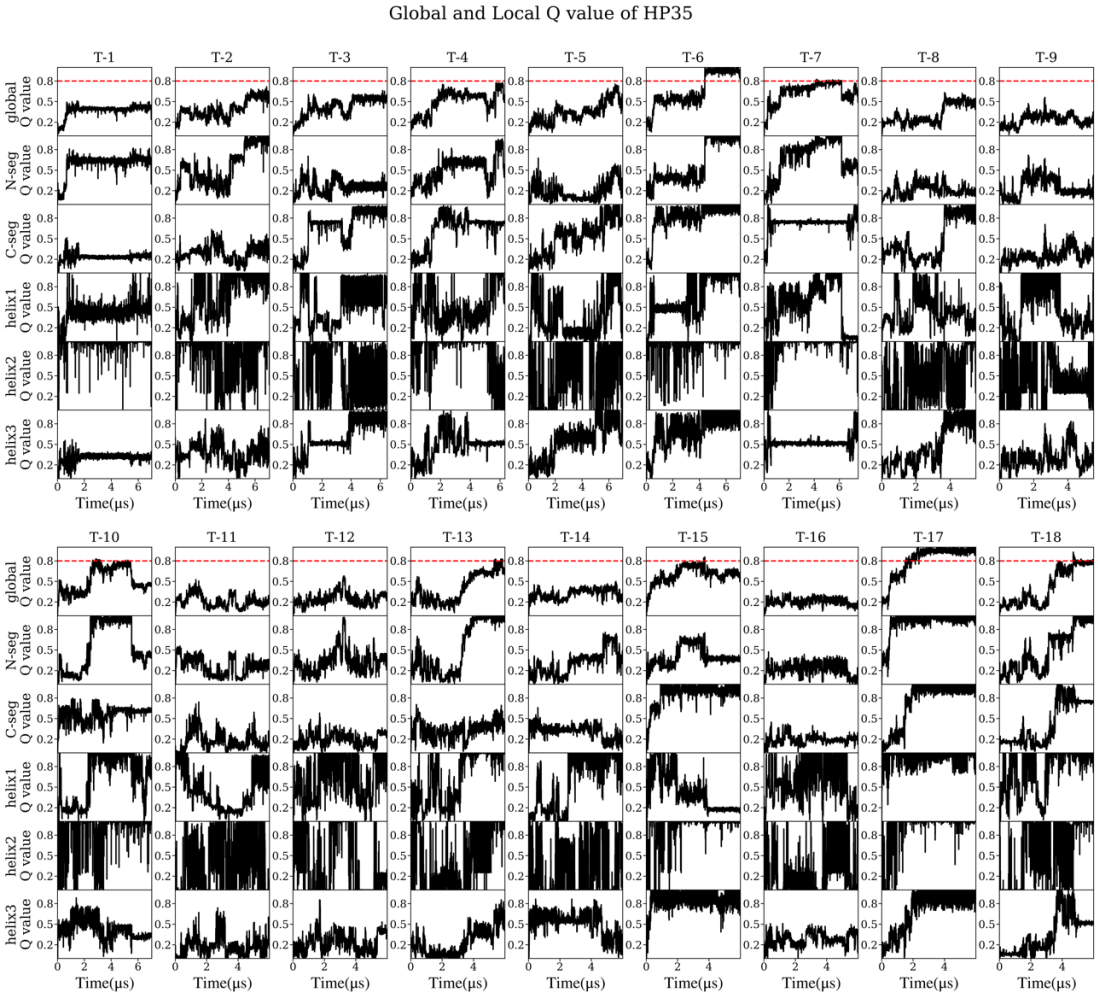

Fig. S4 Time courses of Q values of the global and local structures of HP35 simulated by CMD using AMBER ff14SB force field. “Global” denotes the entire chain without the two ending residues, “N-seg” the N-segment, “C-seg” the C-segment. The red dashed represents Q = 0.8.

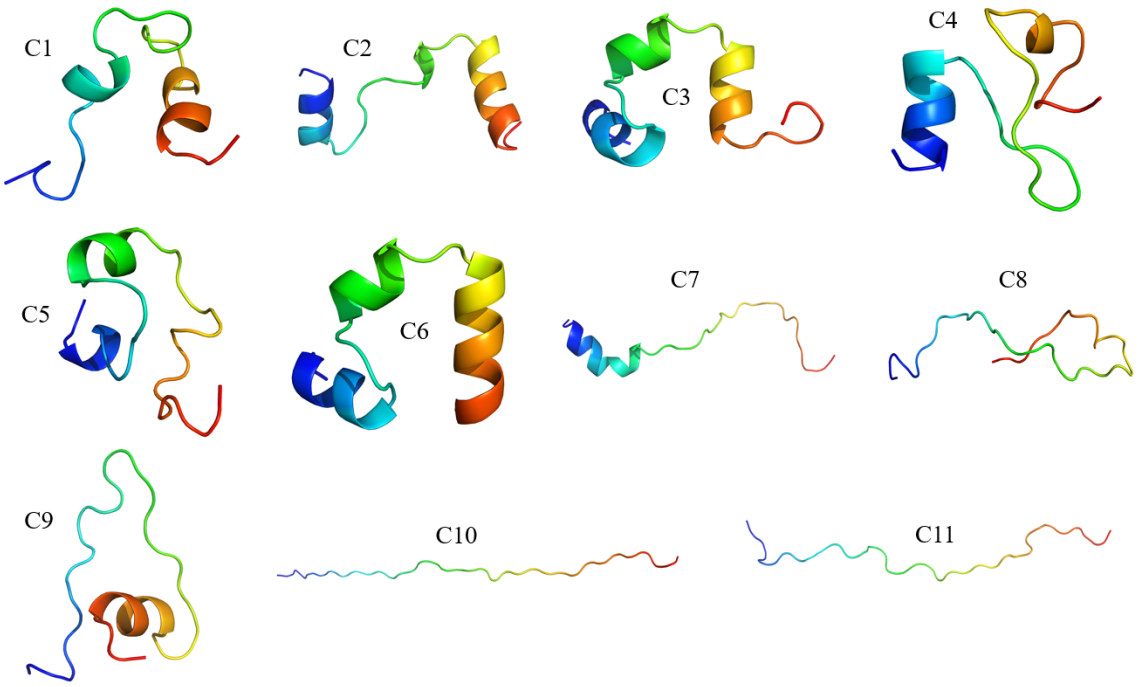

Fig. S5 Representative structures for all clusters discussed in the main text.

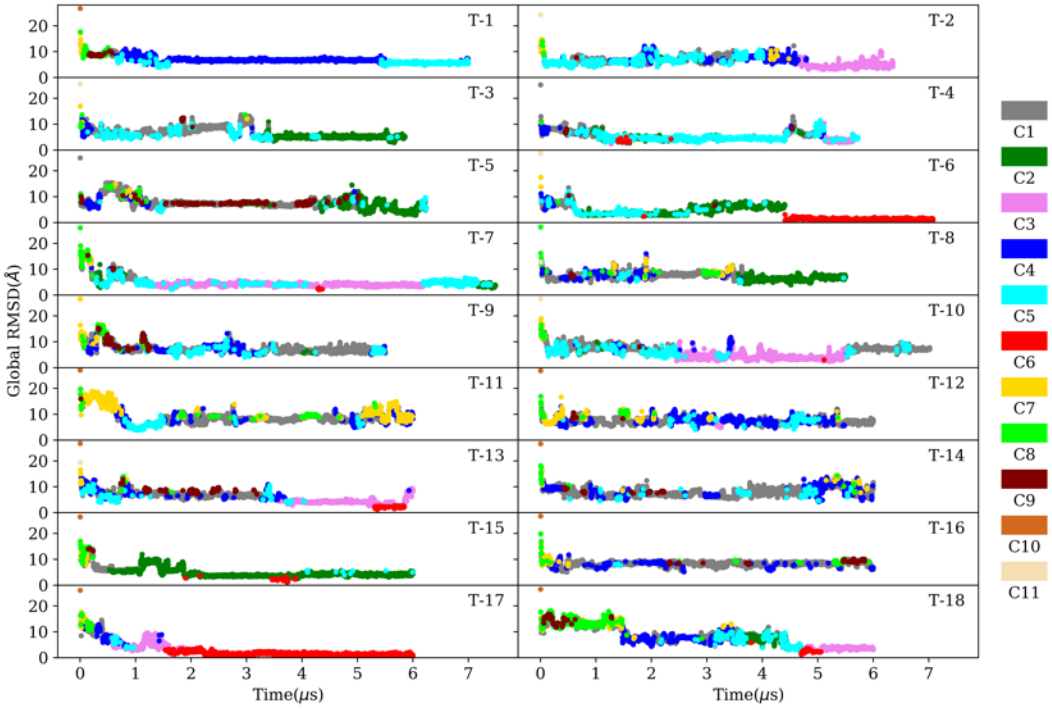

Fig. S6 Time courses of RMSDs of the global structures of HP35, and the colors of the RMSDs are determined from the cluster results of global structures.

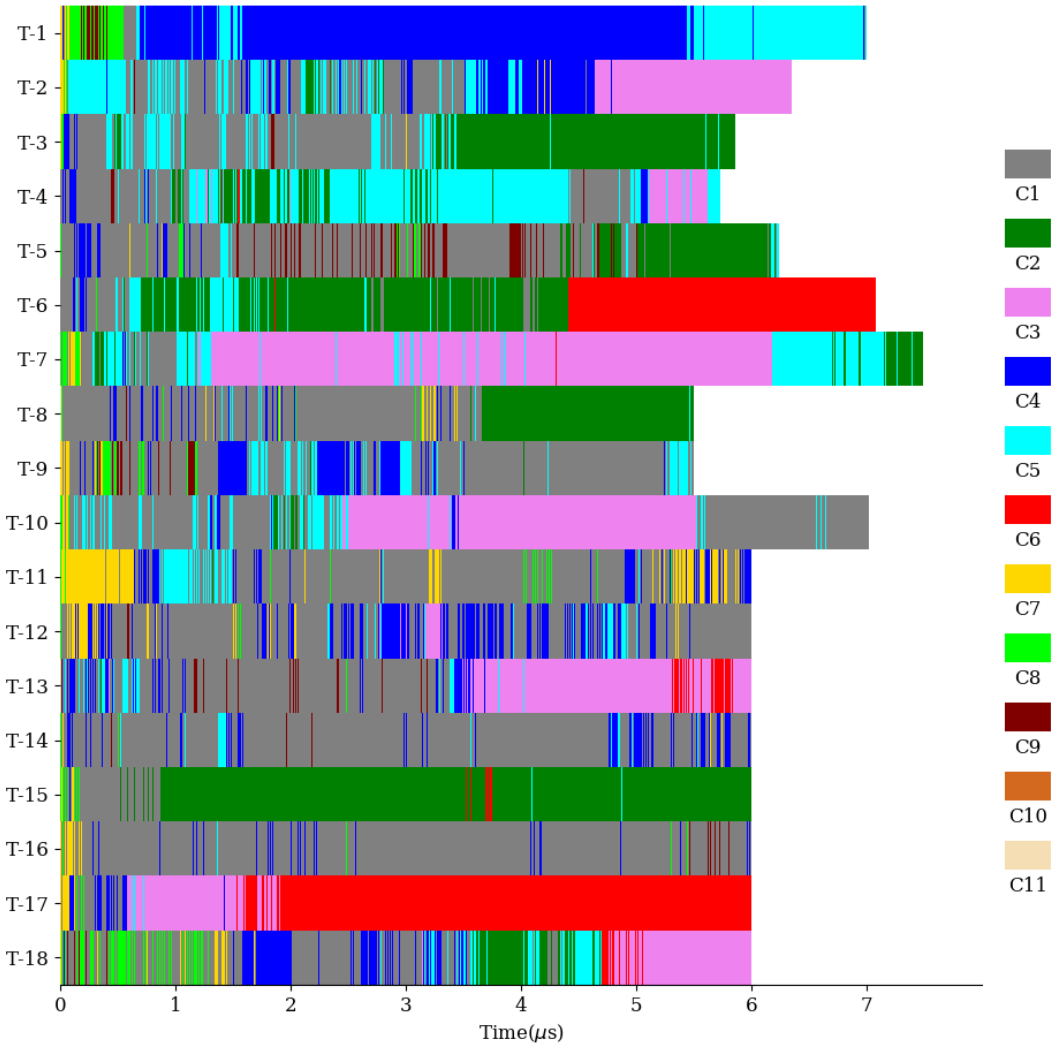

Fig. S7 Time courses of clusters for all 18 trajectories.

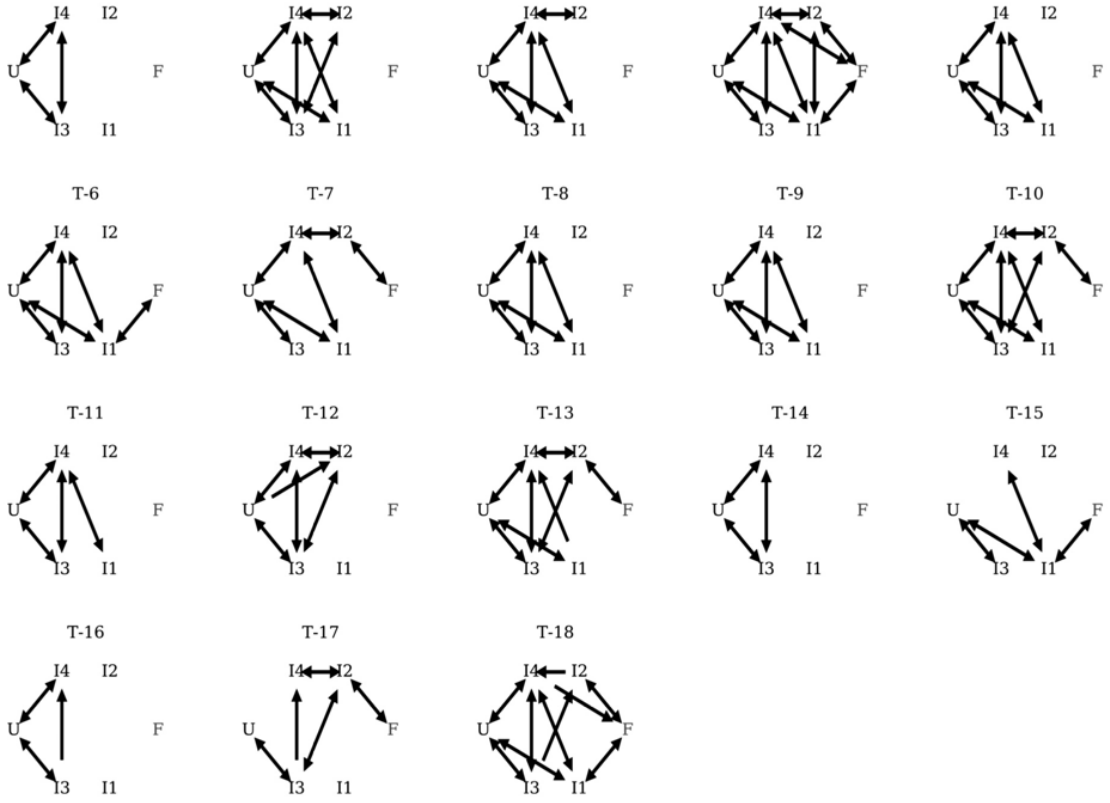

Fig. S8 Folding pathways for all 18 independent trajectories.

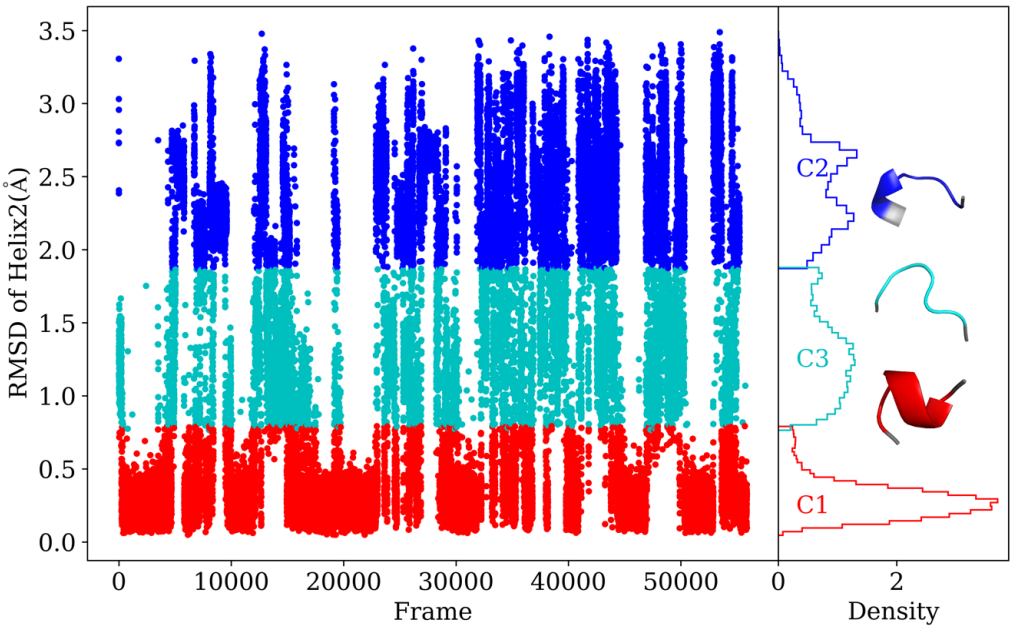

Fig. S9 Cluster results of Helix2 based on its RMSD. The distributions and representative structures for Cluster 1-3 (C1-C3) are shown in right panel (colored in red, blue and cyan respectively).

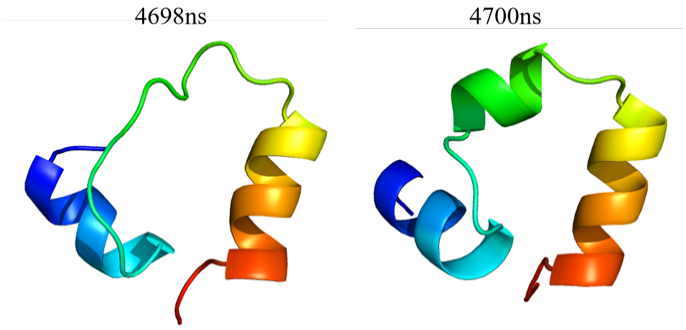

Fig. S10 In T-18, at 4698ns, Helix1 and Helix3 have formed and arranged to form a near-native structure (left). After 2ns, Helix2 formed to carry out the simultaneous folding of N-segment and C-segment (right).

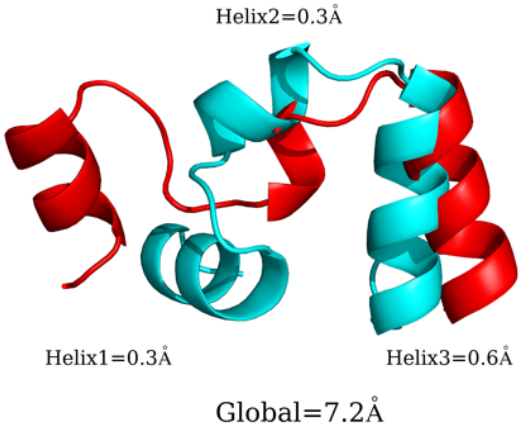

Fig. S11 An example of structure alignment of HP35. Native and non-native structures are colored in cyan and red, respectively.