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

Electronic Supplementary Information article "Conformational fluctuations in molten globule state of α-lactalbumin"

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Table S1: Predicted pKa values of titrable residues during CpHMD simulations at pH=2. The offset value is defined as the difference between predicted pKa and system pH. Fraction of time titrable residues remain protonated during simulations.

Residue Name	Residue	Predicted pKa Value	Offset Value	Fraction of protonation
7	GLU	3.675	1.675	0.956
11	GLU	3.334	1.334	0.987
14	GLU	3.869	1.869	0.794
25	GLU	2.586	0.586	0.998
37	ASP	3.239	1.239	0.945
46	ASP	3.652	1.652	0.978
49	GLU	3.933	1.933	0.988
63	ASP	2.472	0.472	0.748
64	ASP	3.270	1.270	0.949
78	ASP	2.714	0.714	0.838
82	ASP	2.594	0.594	0.797
83	ASP	3.077	1.077	0.923
84	ASP	2.784	0.784	0.859
87	ASP	2.052	0.052	0.530
88	ASP	3.499	1.499	0.969
97	ASP	3.392	1.392	0.961
113	GLU	4.661	2.661	0.998
116	ASP	3.282	1.282	0.950
121	GLU	4.091	2.091	0.992

Table S2: Putative binding sites of Oleic acid (OA) with nature and secondary element in crystal structure.

Residue Number	Residue	Initial Structure	Nature
8	VAL	Helix	Hydrophobic
9	PHE	Helix	Hydrophobic
10	ARG	Helix	Basic
12	LEU	Helix	Hydrophobic
13	LYS	Helix	Basic
16	LYS	Helix	Basic
17	GLY	Helix	Hydrophobic
19	GLY	Helix	Hydrophobic
20	GLY	Helix	Hydrophobic
21	VAL	Helix	Hydrophobic
23	LEU	Helix	Hydrophobic
26	TRP	Helix	Hydrophobic
27	VAL	Helix	Hydrophobic
31	PHE	Helix	Hydrophobic
32	HIS	Helix	Basic
51	GLY	Loop	Hydrophobic
52	LEU	Loop	Hydrophobic
53	PHE	Loop	Hydrophobic
55	ILE	Sheet	Hydrophobic
58	LYS	Loop	Basic
59	ILE	Loop	Hydrophobic
60	TRP	Loop	Hydrophobic
89	ILE	Helix	Hydrophobic
90	MET	Helix	Hydrophobic
92	VAL	Helix	Hydrophobic
93	LYS	Helix	Basic
94	LYS	Helix	Basic
95	ILE	Helix	Hydrophobic
96	LEU	Helix	Hydrophobic
98	LYS	Helix	Basic
99	VAL	Helix	Hydrophobic
100	GLY	Helix	Hydrophobic
101	ILE	Helix	Hydrophobic
104	TRP	Helix	Hydrophobic

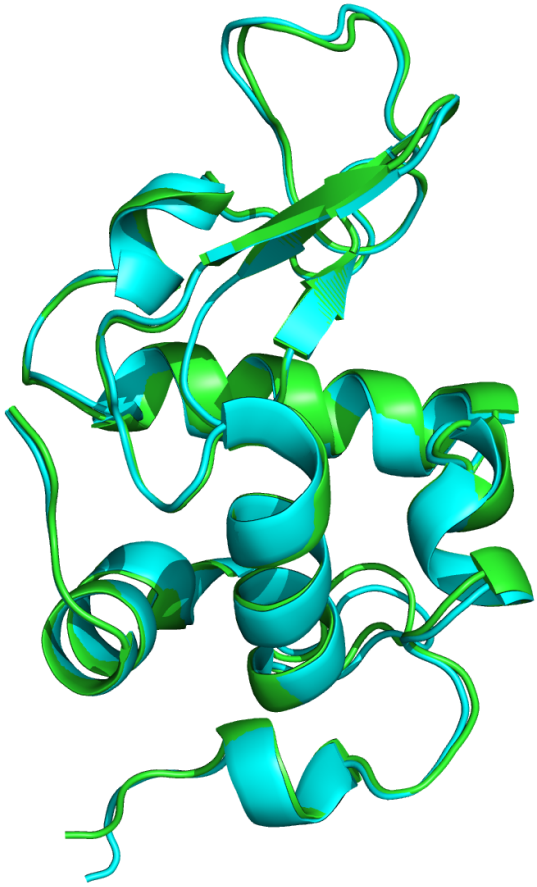

Figure S1: Overlapped average structure obtained from normal MD simulation (green) and constant pH MD simulations (cyan) at neutral pH. Root mean square distance between two structure is 0.479 A^o.

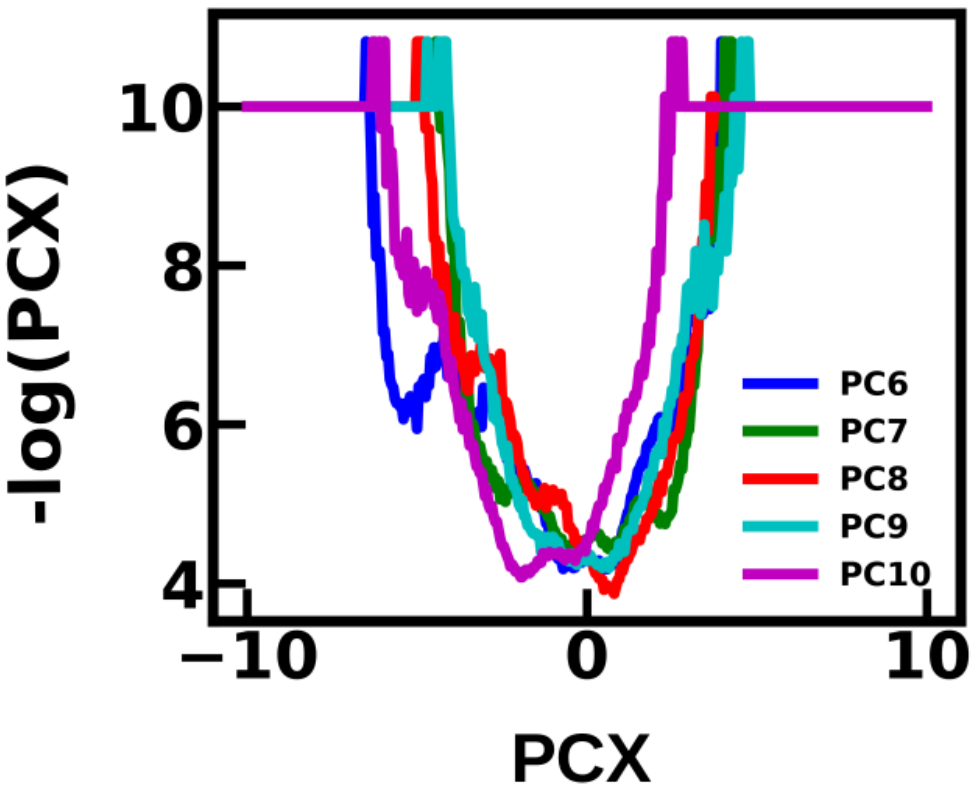

Figure S2: Free energy landscape obtained from dPCA+ for PC6-PC10. Metastability decrease gradually for higher PCs.

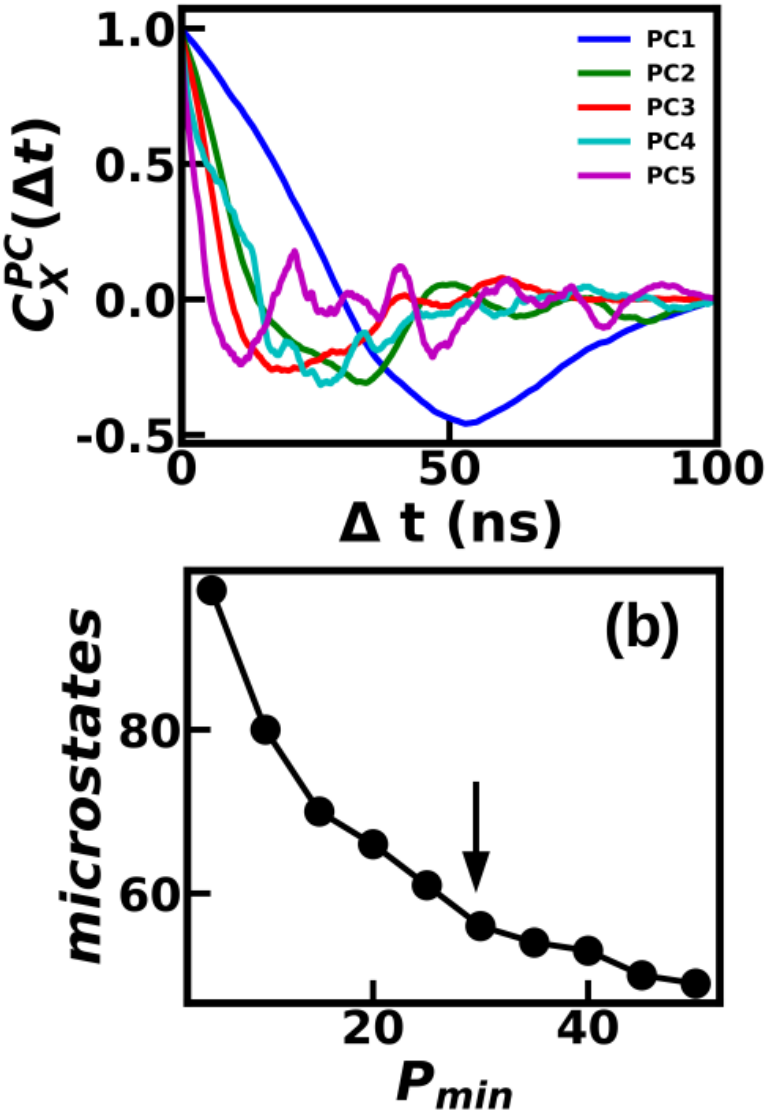

Figure S3: (a) Autocorrelation function of principal component 1-5, (b) Number of microstate, plotted as function of the minimal population P_{min}. P_{min}=30 (shown by arrow) value used in the analysis to avoid initial drop .

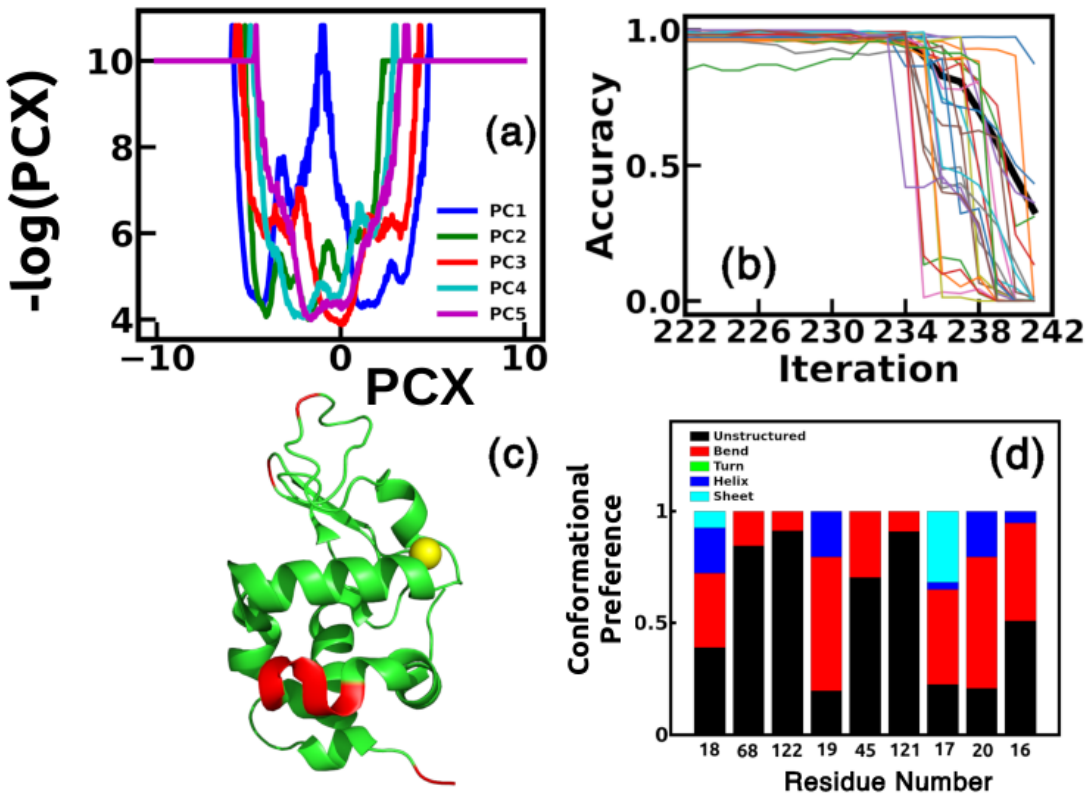

Figure S4: Identification of essential coordinate for apo (Ca^{2+} ion is shown for better understanding) protein applying constant pH simulation at pH7.(a) Principal component obtained from dPCA+ analysis. PCs 1-5 are shown in figure. (b) Accuracy loss plot of XGBoost classifier. The figure is shown as a function of number of discarded coordinate. Accuracy of all metastable states drops drastically upon removing of mostly last 10 coordinates., (c) Residues having essential coordinates are marked in initial crystal structure. They are colored in red. For better understanding,k we keep the calcium ion. (d) Conformational preference of those residues having essential coordinates.

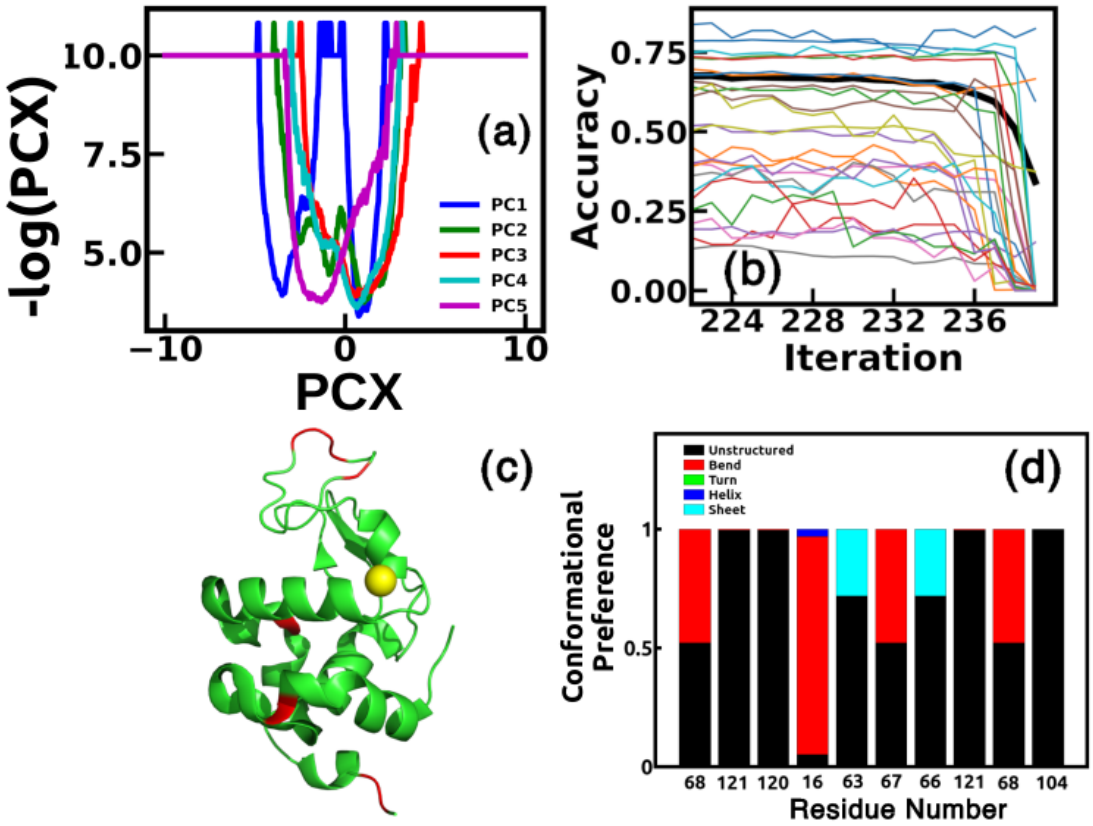

Figure S5: Identification of essential coordinate for holo protein using unbiased molecular dynamics simulation at neutral pH. (a) Principal component obtained from dPCA+ analysis. PCs 1-5 are shown in figure. (b) Accuracy loss plot of XGBoost classifier. The figure is shown as a function of number of discarded coordinate. Accuracy of all metastable states drops drastically upon removing of mostly last 10 coordinates., (c) Residues having essential coordinates are marked in initial crystal structure. They are colored in red. All non-essential residues belong to loop region. For better understanding, we keep the calcium ion. (d) Conformational preference of those residues having essential coordinates.

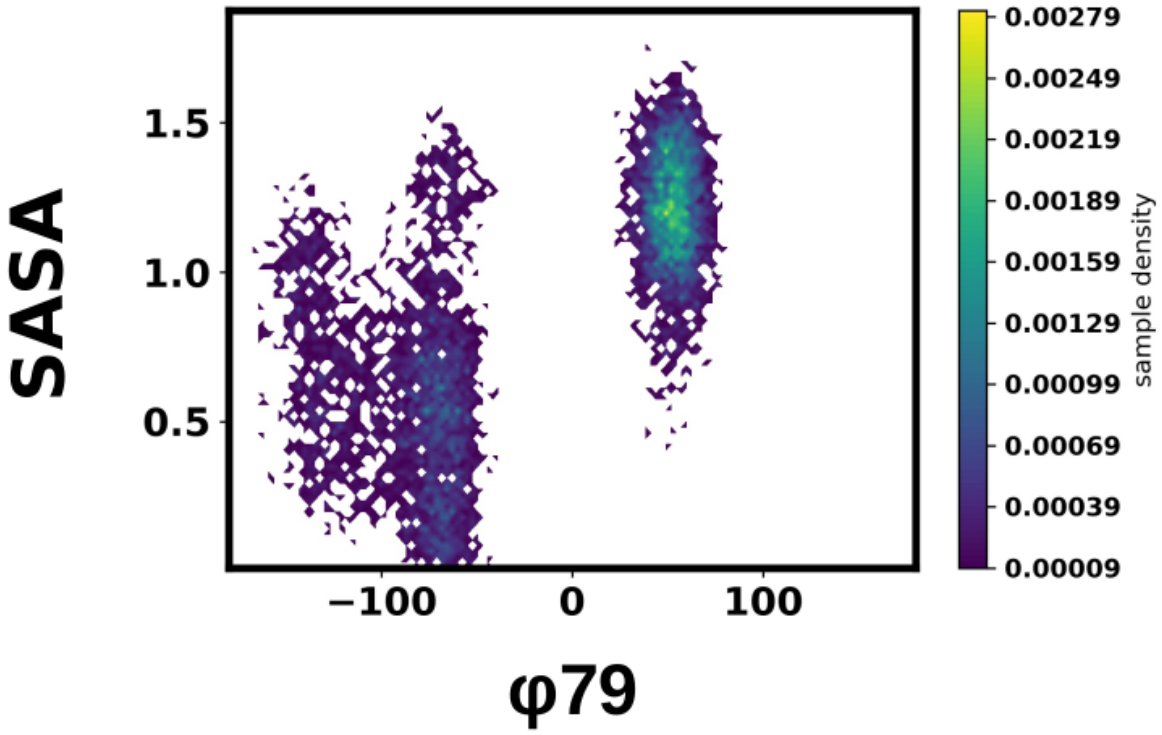

Figure S6: Correlation plot between SASA value of ILE89 with dihedral ϕ fluctuations of res LYS79.