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

Supplementary Figures:

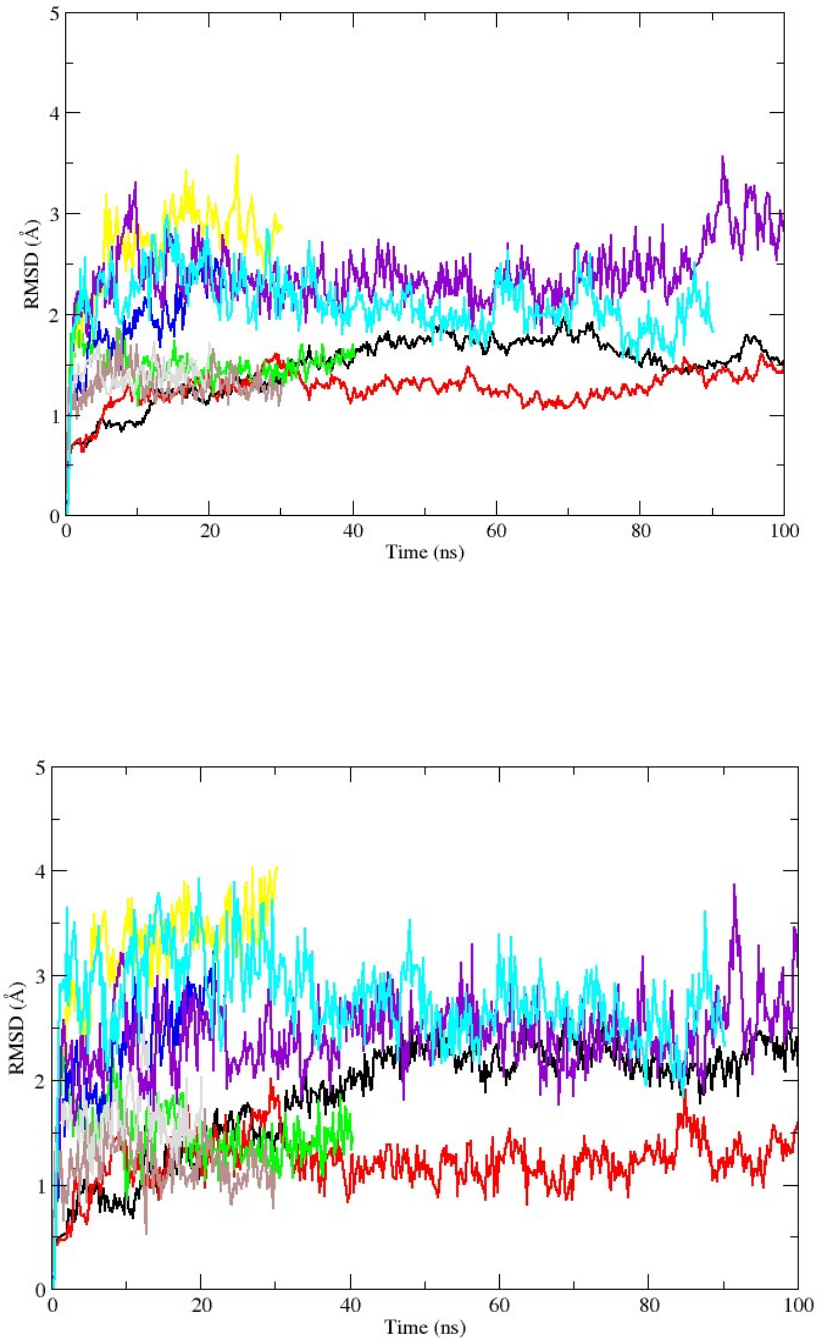
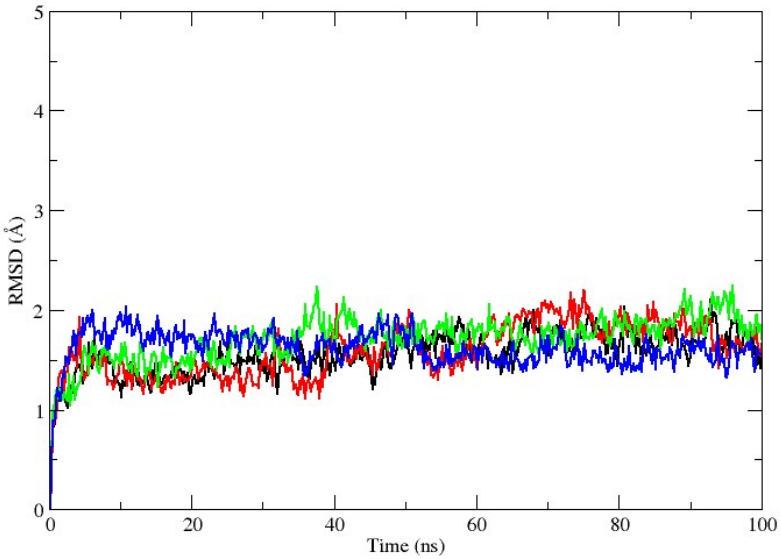


Figure S1: RMSD plots of WT system. Each color corresponds to RMSD of a different replica of the system. RMSD of the backbone atoms of the protein is shown on the upper panel, whereas RMSD of the backbone of the binding site residues, which are composed of S139, N175, N193, Y195 and Y196, is shown on the lower panel. Black, yellow, cyan, purple and blue correspond to PO-I, red corresponds to closed conformation. Brown, gray and green correspond to PO-II conformation.



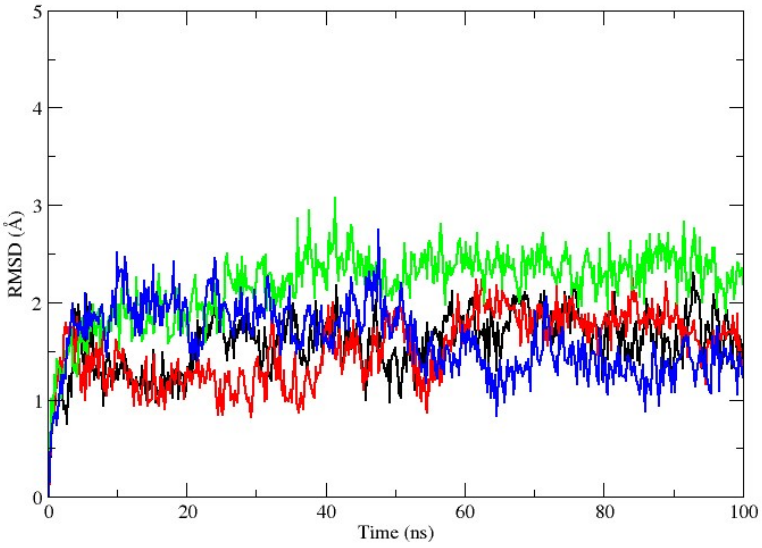


Figure S2: RMSD plots of WT_{150mM} system. Each color corresponds to RMSD of a different replica of the system. RMSD of the backbone atoms of the protein is shown on the upper panel, whereas RMSD of the backbone of the binding site residues, which are composed of S139, N175, N193, Y195 and Y196, is shown on the lower panel.

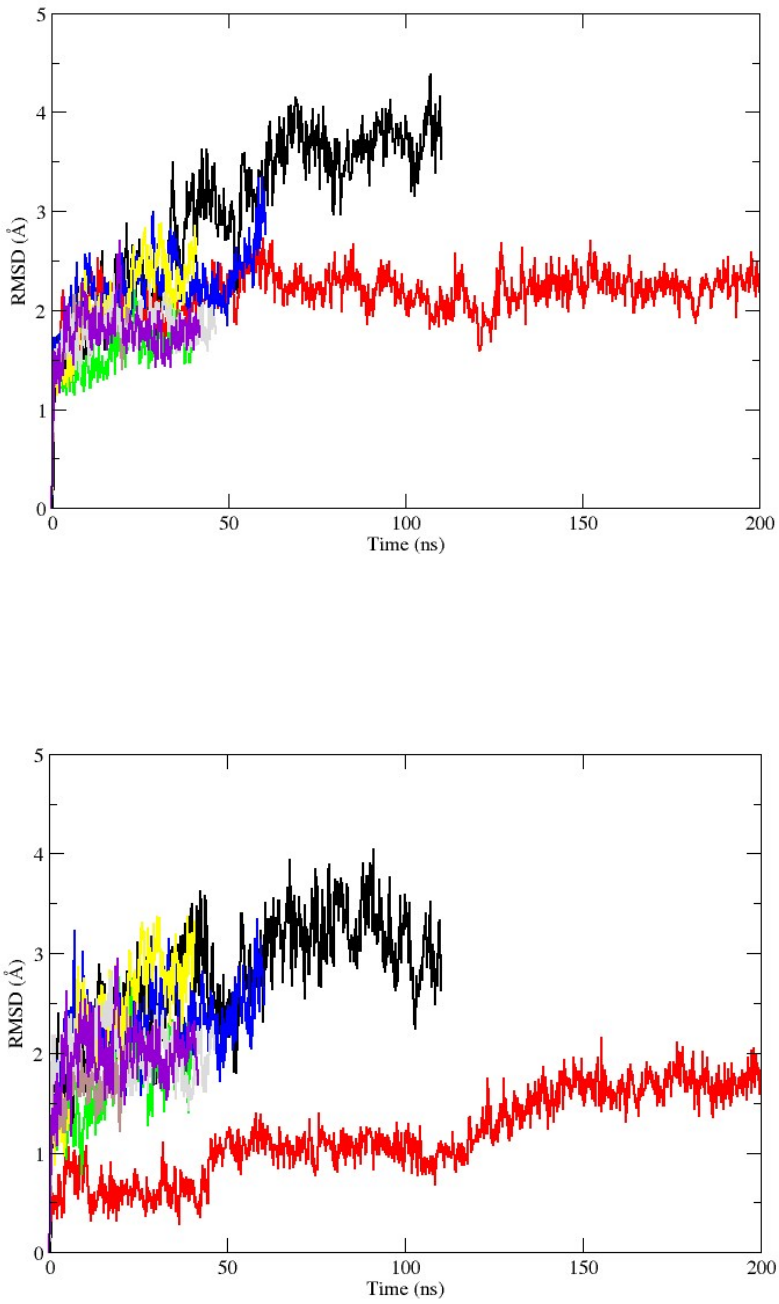


Figure S3: RMSD plots of D52A system. Each color corresponds to RMSD of a different replica of the system. RMSD of the backbone atoms of the protein is shown on the upper panel, whereas RMSD of the backbone of the binding site residues, which are composed of S139, N175, N193, Y195 and Y196, is shown on the lower panel.

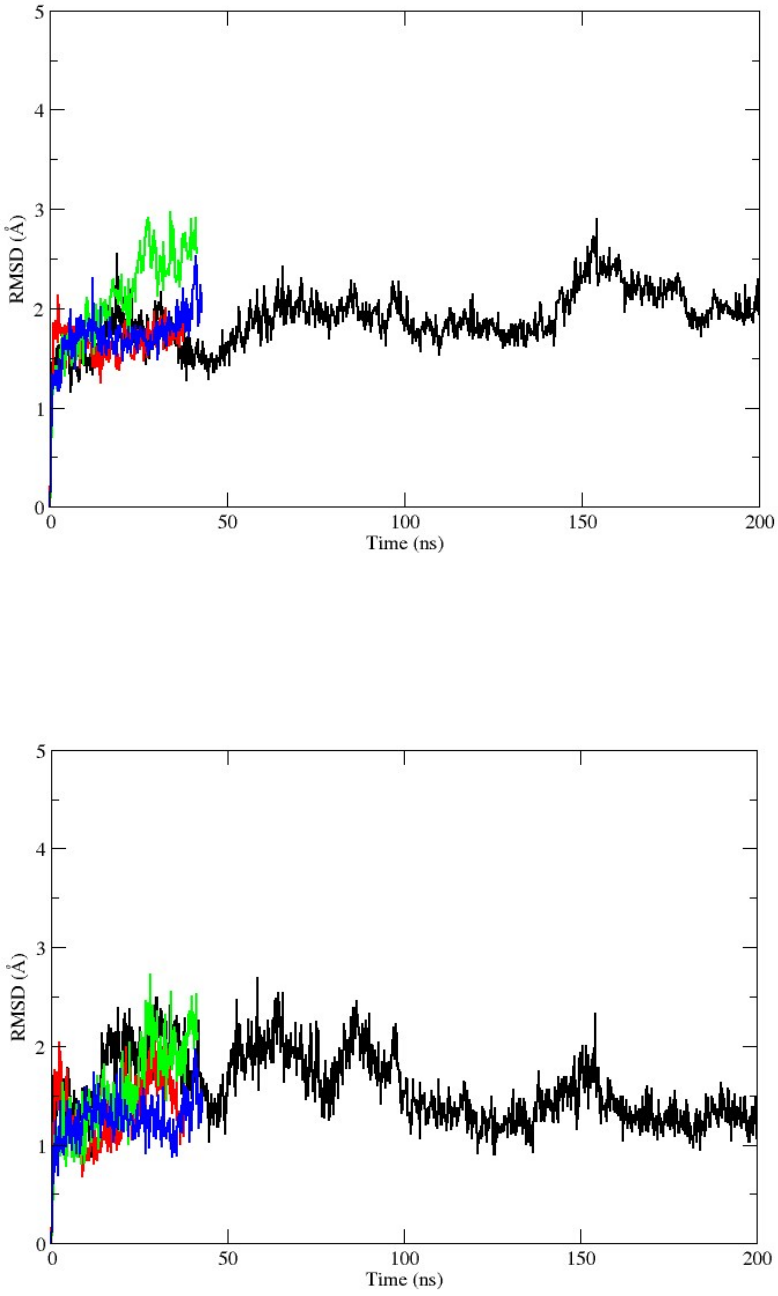


Figure S4: RMSD plots of D52A$_{150mM}$ system. Each color corresponds to RMSD of a different replica of the system. RMSD of the backbone atoms of the protein is shown on the upper panel, whereas RMSD of the backbone of the binding site residues, which are composed of S139, N175, N193, Y195 and Y196, is shown on the lower panel.

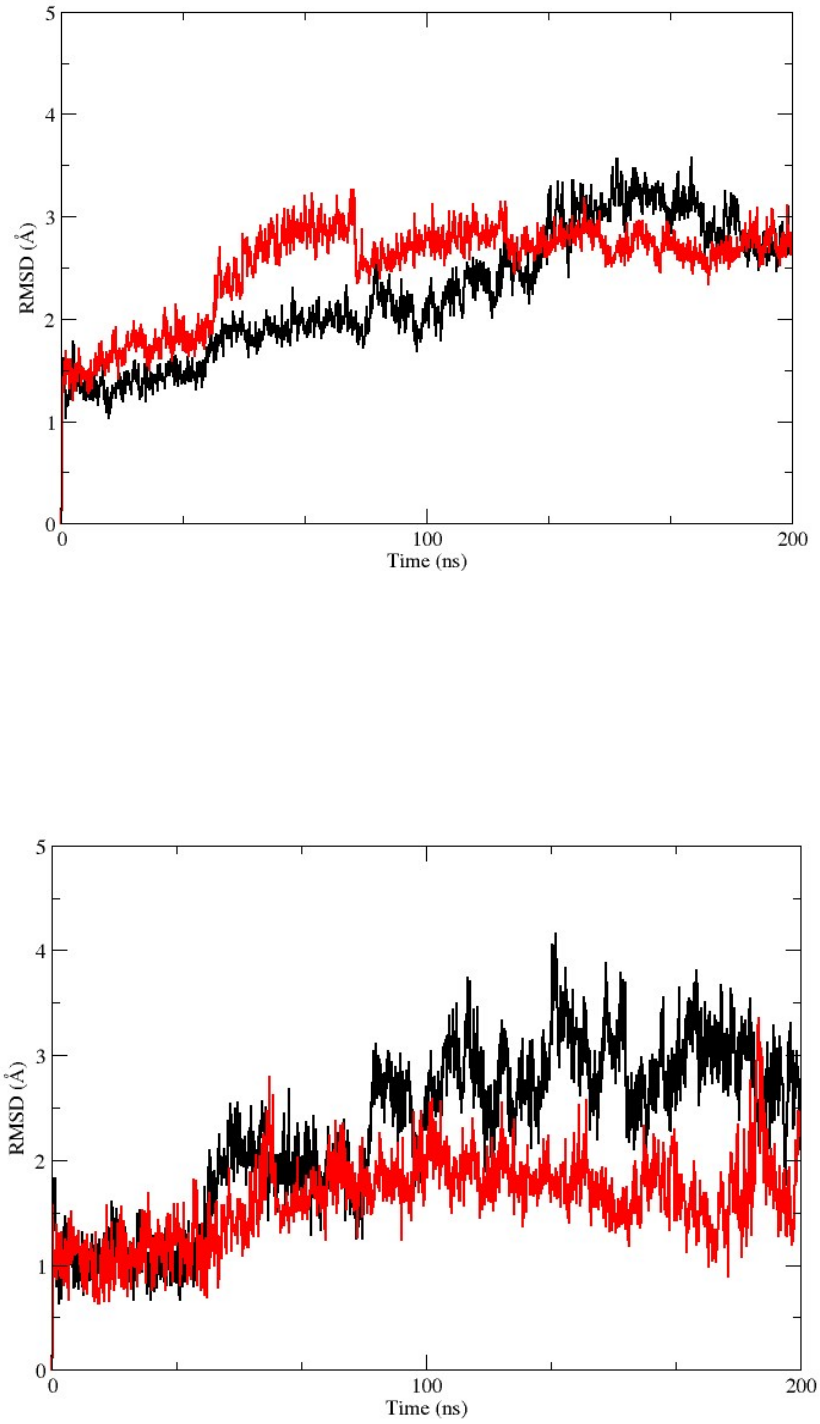


Figure S5: RMSD plots of $H^{+}D52^{0}$ system. Each color corresponds to RMSD of a different replica of the system. RMSD of the backbone atoms of the protein is shown on the upper panel, whereas RMSD of the backbone of the binding site residues, which are composed of S139, N175, N193, Y195 and Y196, is shown on the lower panel.

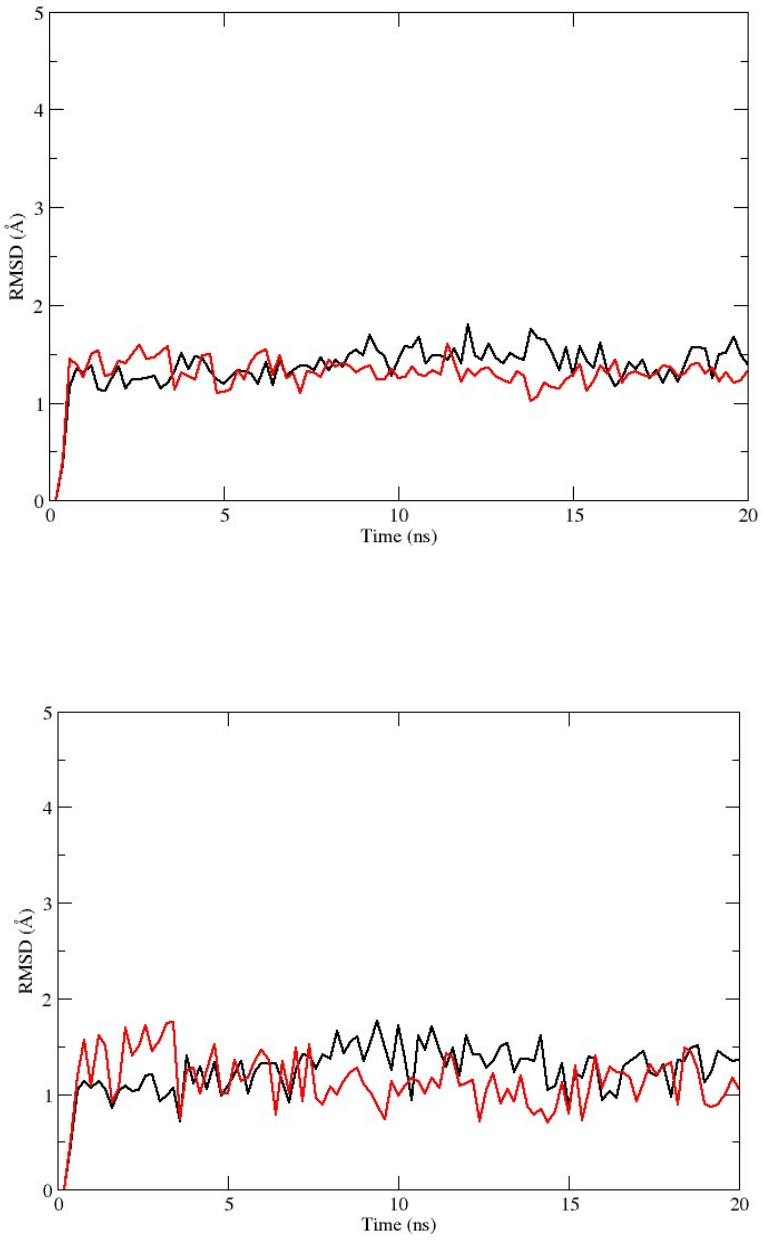


Figure S6: RMSD plots of WT* system. Black color corresponds to RMSD of WT* and red color corresponds to RMSD of WT^*_{150mM} system. RMSD of the backbone atoms of the protein is shown on the upper panel, whereas RMSD of the backbone of the binding site residues, which are composed of S139, N175, N193, Y195 and Y196, is shown on the lower panel.

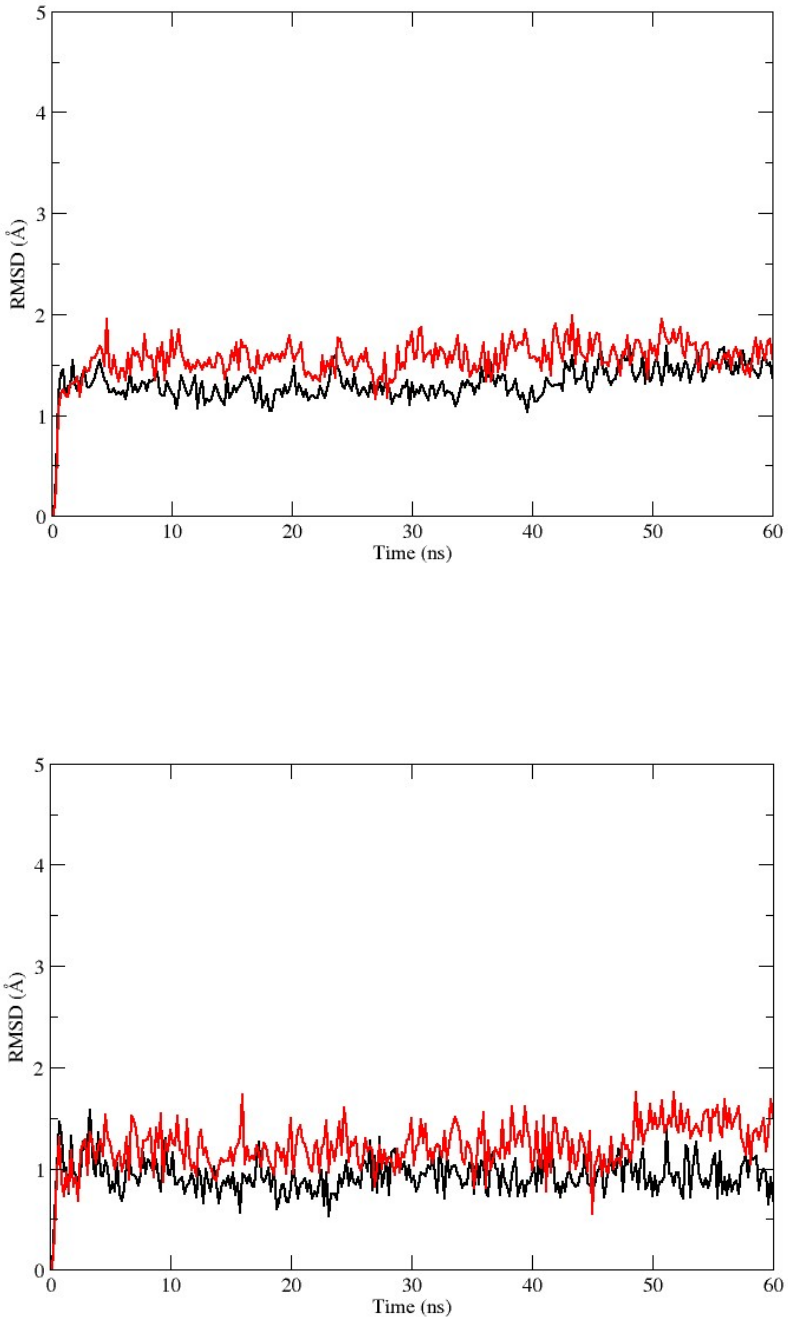


Figure S7: RMSD plots of D52A* system. Black color corresponds to RMSD of D52A* and red color corresponds to RMSD of D52A*$_{150mM}$ system. RMSD of the backbone atoms of the protein is shown on the upper panel, whereas RMSD of the backbone of the binding site residues, which are composed of S139, N175, N193, Y195 and Y196, is shown on the lower panel.

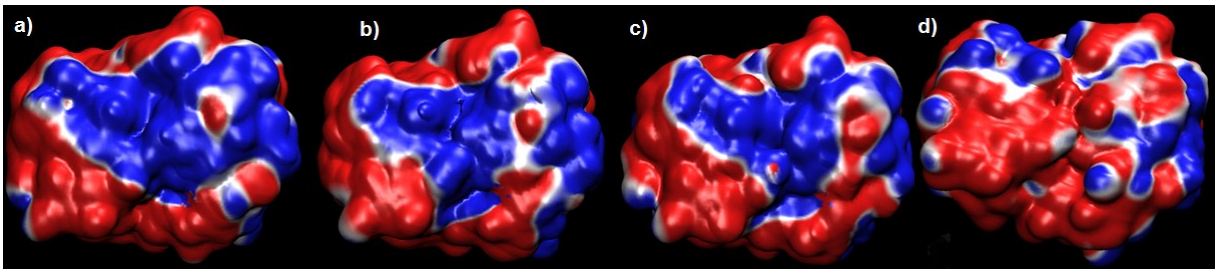


Figure S8: (a-c) Electrostatic potential distributions of 0th, 50th and the 100th frame of a 100-ns simulation belonging to low IS WT system. In the last panel **(d)**, the electrostatic potential distribution of the last frame of another 100 ns replica is shown.

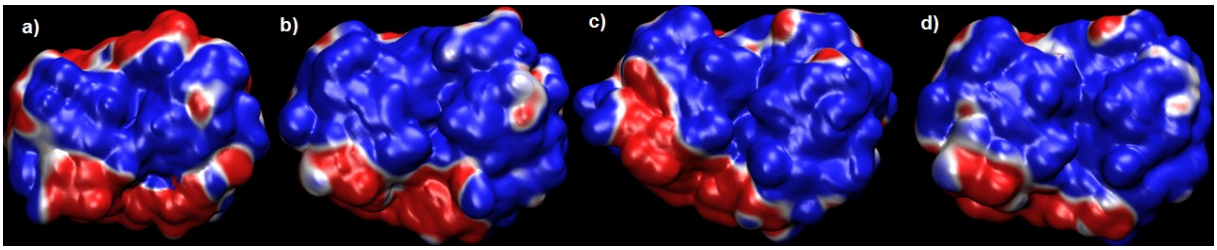


Figure S9: (a-c) Electrostatic potential distributions of 0th, 50th and the 100th frame of a 100-ns simulation belonging to low IS, D52A system. In the last panel **(d)**, the electrostatic potential distribution of the last frame of another 100 ns replica is shown.

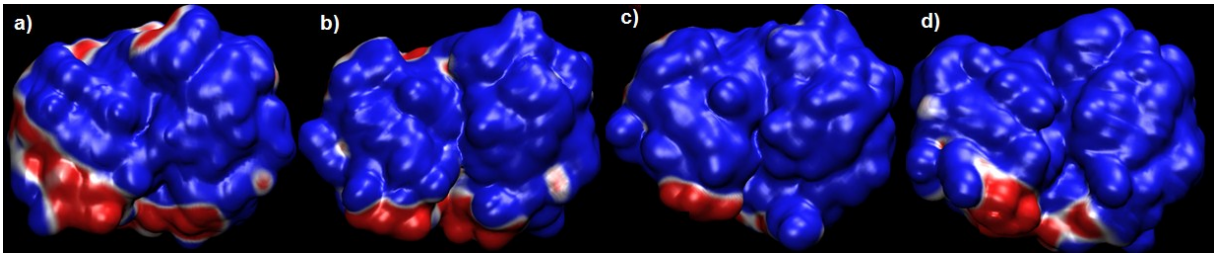


Figure S10: (a-c) Electrostatic potential distributions of 0th, 50th and the 100th frame of a 100-ns simulation belonging to low IS, $H^{+}D52^{0}$ system. In the last panel **(d)**, the electrostatic potential distribution of the last frame of another 100 ns replica is shown.

Table S1. Estimated mean and bias estimates for free energy differences for various values of *C* in equation 4.

System	ΔG_{bind} (kcal/mol) ; *C*=10	ΔG_{bind} (kcal/mol) ; *C*=15	ΔG_{bind} (kcal/mol) ; *C*=20
$WT^*_{,150mM}$	-22.0 ±0.5	-22.0 ±0.6	-22.1± 0.6
WT*	-16.6± 0.5	-16.7± 0.4	-16.6± 0.3
$WT_{,150mM}$	-10.5 ±0.6	-10.5 ±0.5	-10.6 ±0.5
WT	-8.4± 0.4	-8.4± 0.4	-8.4± 0.4
$D52A^*_{,\ 150mM}$	-22.8 ±0.8	-22.8 ±0.8	-22.8± 0.7
D52A*	-18.6± 0.6	-18.7± 0.6	-18.7 ±0.6
$D52A_{,150mM}$	-9.2± 0.7	-9.2± 0.6	-9.2 ±0.6
D52A	-5.8 ±0.8	-5.9 ±0.7	-5.9± 0.7
H^+D52^0	-5.4 ±0.2	-5.4 ±0.2	-5.4± 0.2