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

C-Terminal Tail Insertion of Bcl-x_L in Membrane Occurs via Partial Unfolding and Refolding Cycle Associating Micro-solvation

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Supporting information

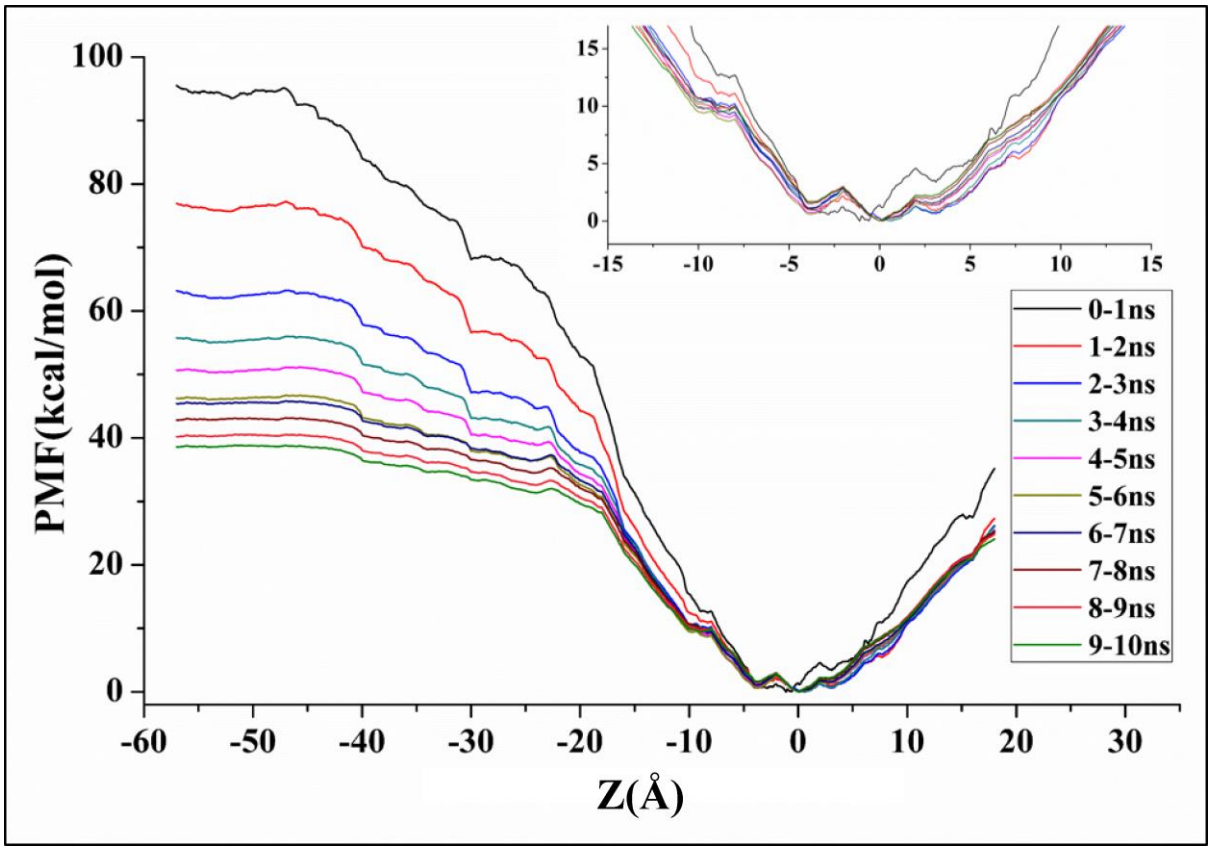

Figure S1: PMF profile computed over different windows of simulation, showing a gradual change in the Energy profile leading to convergence. The difference between two consecutive interval decreases gradually. The region near the minima is shown in inset. The horizontal axis Z is same as defined in figure 1.

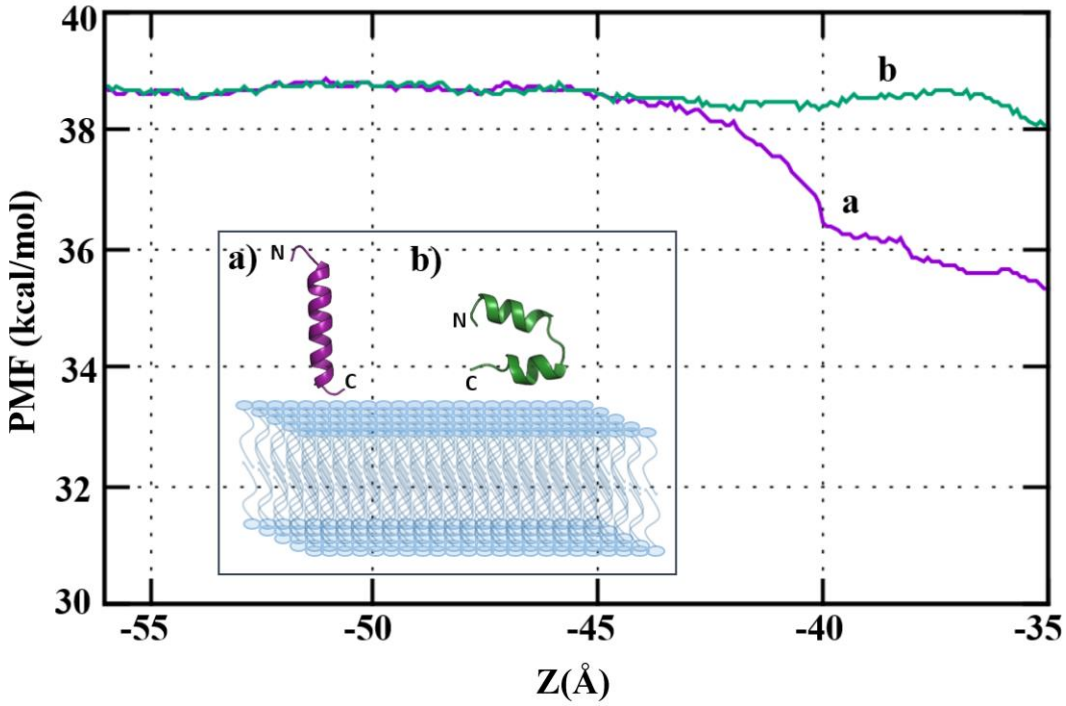

Figure S2: Overlay of PMF near the membrane surface, obtained using two different starting conformation a) completely helical and b) partially unfolded.

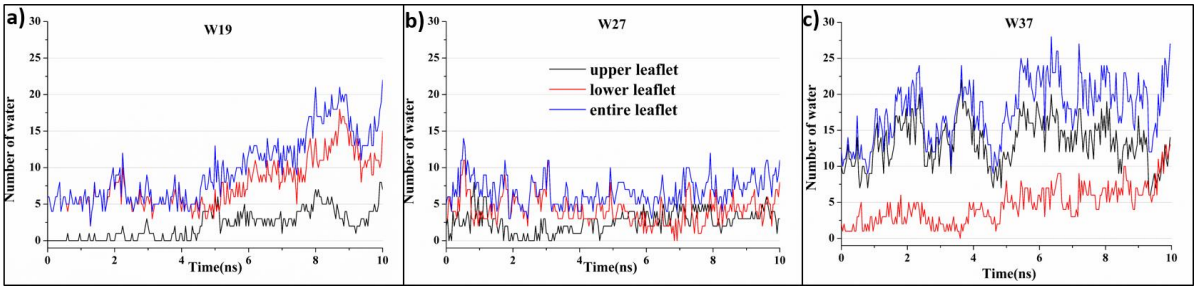

Figure S3: Distribution of water within the hydrophobic core of the membrane. Number of water within membrane along the simulation for window a) w19, b) w27 and c) w37 respectively.

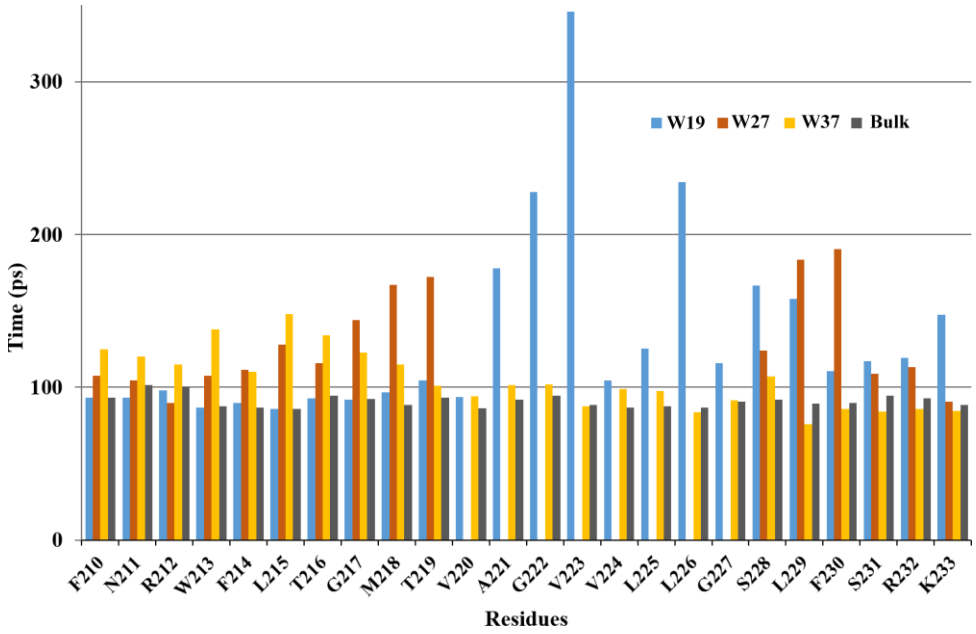

Figure S4: Average residence time of water present within a cutoff of 8Å of various amino acid residues inside the membrane. The data has been averaged over the last 5ns of selected windows separately: w19, w27, w37. The 'bulk' refers to the water around the peptide in bulk water, i.e. not inside the membrane.

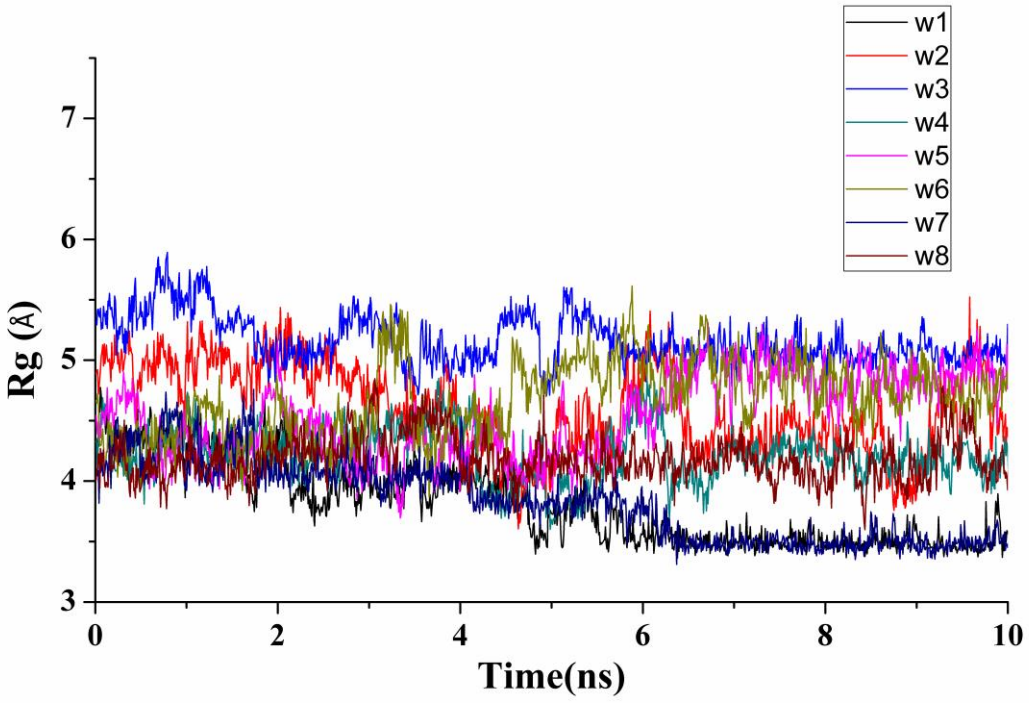

Figure S5: Radius of gyration of alpha carbons of C-terminal residues (S228 – K233) of CTPEP along the trajectory in the windows w1 to w8 (z = -40 to -56Å).

Table S1: Detail of simulation of the stratified windows. Each of them was set for 5ns of equilibration and 10ns of production.

	System identifier	**Distance of COM of protein from COM of membrane (Å)**	
		Upper boundary	***Lower boundary***
1	w1	-56	-54
2	w2	-54	-52
3	w3	-52	-50
4	w4	-50	-48
5	w5	-48	-46
6	w6	-46	-44
7	w7	-44	-42
8	w8	-42	-40
9	w9	-40	-38
10	w10	-38	-36
11	w11	-36	-34
12	w12	-34	-32
13	w13	-32	-30
14	w14	-30	-28
15	w15	-28	-26
16	w16	-26	-24
17	w17	-24	-22
18	w18	-22	-20
19	w19	-20	-18
20	w20	-18	-16
21	w21	-16	-14
22	w22	-14	-12
23	w23	-12	-10
24	w24	-10	-8
25	w25	-8	-6
26	w26	-6	-4
27	w27	-4	-2
28	w28	-2	0
29	w29	0	2
30	w30	2	4
31	w31	4	6
32	w32	6	8
33	w33	8	10
34	w34	10	12
35	w35	12	14
36	w36	14	16
37	w37	16	18

Table S2: Detail of simulation of the stratified windows for 2D PMF. The production run was 10 ns for each system

	System identifier	Distance of COM of protein from COM of membrane (Å)		Inclination angle (θ)	
		Upper boundary	***Lower boundary***	***Upper boundary***	***Lower boundary***
1	w2d_1	-56	-54	180	150
2	w2d_2	-56	-54	150	120
3	w2d_3	-56	-54	120	90
4	w2d_4	-54	-52	180	150
5	w2d_5	-54	-52	150	120
6	w2d_6	-54	-52	120	90
7	w2d_7	-52	-50	180	150
8	w2d_8	-52	-50	150	120
9	w2d_9	-52	-50	120	90
10	w2d_10	-50	-48	180	150
11	w2d_11	-50	-48	150	120
12	w2d_12	-50	-48	120	90
13	w2d_13	-48	-46	180	150
14	w2d_14	-48	-46	150	120
15	w2d_15	-48	-46	120	90
16	w2d_16	-46	-44	180	150
17	w2d_17	-46	-44	150	120
18	w2d_18	-46	-44	120	90
19	w2d_19	-44	-42	180	150
20	w2d_20	-44	-42	150	120
21	w2d_21	-44	-42	120	90
22	w2d_22	-42	-40	180	150
23	w2d_23	-42	-40	150	120
24	w2d_24	-42	-40	120	90
25	w2d_25	-40	-38	180	150
26	w2d_26	-40	-38	150	120
27	w2d_27	-40	-38	120	90

Supporting information text:

Method to compute PMF starting from a partially folded conformation of peptide, as shown in Figure S2: To consider the possibility of the partially folded conformation as initial structure the completely helical peptide (used in ABF simulations) was solvated in a 64 Å cubic TIP3P waterbox and simulated for 100 ns in NPT condition (Temperature = 300 K, Pressure = 1 atm.) after adequate minimization (ABNR and SD) and equilibration (NVT). From simulation the peptide was found to sample two major conformation, the completely helical (figure S2a) and a helical hairpin (figure S2b). The helical hairpin is found structurally almost similar to that obtained from window w6 after ~8ns of simulation (figure 4b). So as a possibility that partially unfolded structure was used as starting conformation and a PMF was built in the association and absorption regions (Z = -34 to -56 Å). All the conditions for ABF simulation were kept similar except the atoms on which the collective variable was applied. The distance between center of mass of membrane and center of mass of alpha carbons of residues 221 to 225 which is connecting between two helical parts in the hairpin like structure is chosen as collective variable.

Notes of capping the peptides: Terminal charges often take part in polar interactions in both the ways: intermolecular and intra-molecular. Thus it can lock the molecule in specific conformations due to over-stabilization of electrostatic interaction. This can slow down the conformational sampling and can introduce artefacts. Even, near the membrane surface, uncapped C-terminal can drastically influence the dynamics of a trans-membrane protein insertion. It is likely that near the negatively charged membrane surface the equilibrium would shift to make the C-terminus neutral to absorb the repulsion. Since no such polarizable force field is available till date to replace the static point charges, the strategic way out is to cap the terminal ends to make them neutral and uniform throughout, which also takes care of the sampling efficiency.