Supporting Information for

Simulation Study of Membrane Bending by Protein Crowding: A Case Study with the Epsin N-terminal Homology Domain

Taraknath Mandal*, Shivam Gupta , and Jatin Soni

Department of Physics, Indian Institute of Technology Kanpur, Kanpur-208016, India.
*taraknath@iitk.ac.in

Table 1: Summary of the simulations performed in this study. Bold numerical numbers indicates the number of replicas performed.

System	Number of lipids	Number of proteins	Total number of atoms/beads	Simulation time(μs)
Membrane-protein interface: **1** ENTH with H_0 (HMMM-AT)	162	1	81066	0.1
Membrane-protein interface: **1** ENTH with H_0 (AT)	162	1	74407	0.5
Membrane-protein interface **1** ENTH without H_0 (HMMM-AT)	162	1	79007	0.1
Membrane-protein interface: **1** ENTH without H_0 (AT)	162	1	75575	0.3
Membrane-protein interface: **3** ENTH with H_0 (CG, no elastic network)	2000	1	69376	10
Membrane-protein interface: **3** ENTH with H_0 (CG, with elastic network)	2000	1	69376	10
Curvature generation by two ENTH domain with H_0 (CG): **1**	2000	2	70033	10
Curvature generation by two ENTH domain without H_0 (CG): **1**	2000	2	71360	10
Curvature sensing by ENTH with H_0 (CG): **3**	3200	1	150617	5
Curvature sensing by ENTH without H_0 (CG): **3**	3200	1	150582	6
Curvature generation by four ENTH domain with H_0 (CG): **1**	8000	4	382038	10
Curvature generation by sixteen ENTH domain with H_0 (CG): **1**	8000	16	389834	10
Curvature generation by sixteen ENTH domain without H_0 (CG): **1**	8000	16	392676	10
Partial pressure in the presence of ENTH domain with H_0 (CG): **1**	1240	48	91941	2
Partial pressure in the presence of ENTH domain without H_0 (CG: **1**)	1240	48	91761	2
Aggregation of proteins ENTH domain with H_0 (CG): **1**	18000	16	940302	20
Aggregation of proteins ENTH domain with H_0 (CG): **2**	18000	16	962152	20
Membrane curvature with no protein (**CG**): **1**	18000	0	956968	3
Membrane curvature generation (**AT**): **1**	1440	4	572832	0.12

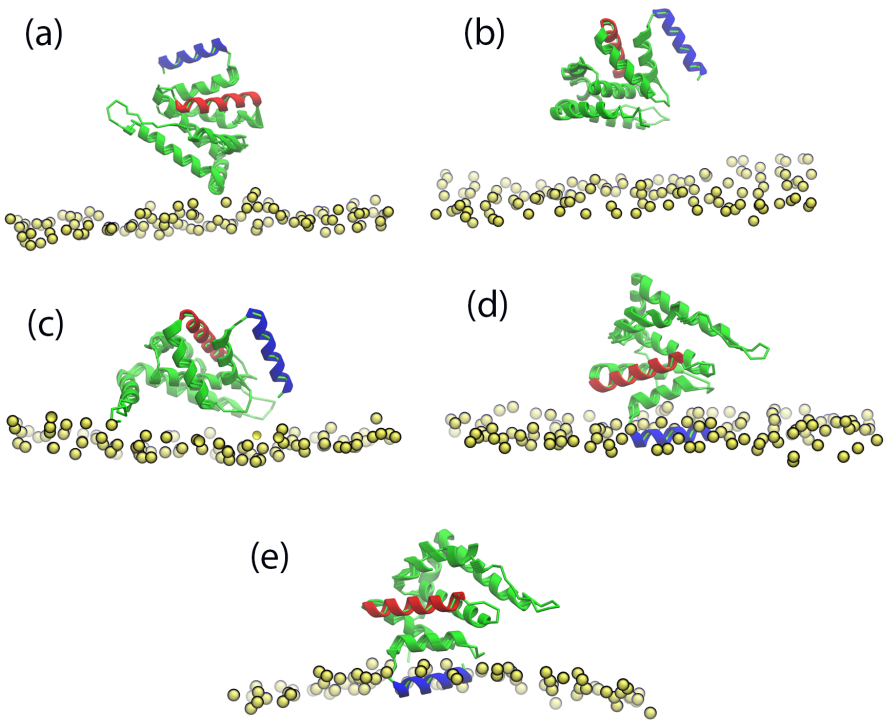

Figure S1: Simulation of the ENTH protein and membrane interaction with a different initial configuration where the H_0 helix (blue) is away from the membrane surface. With simulation time the protein spontaneously rotates and binds to the membrane through the H_0 helix. Snapshots are shown at (a) 0, (b) 0.4, (c) 0.5, (d) 0.6 and (e) 5.0 μs.

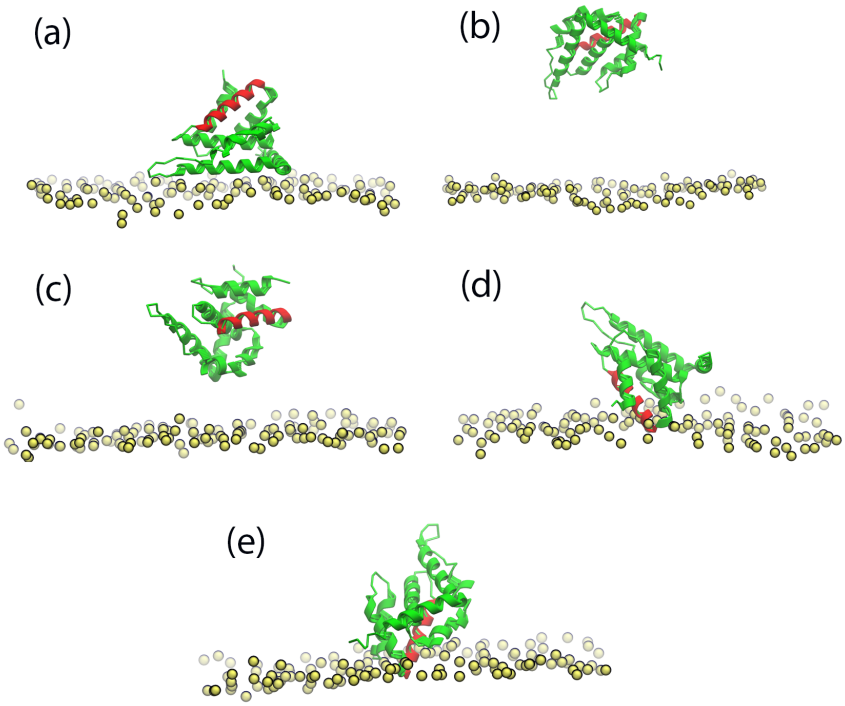

Figure S2: Simulation of the ENTH-H_0 protein and membrane interaction with a different initial configuration where the H_3 helix (red) is away from the membrane surface. With simulation time the protein spontaneously rotates and bind to the membrane through the H_3 helix. Snapshots are shown at (a) 0, (b) 1.0, (c) 1.15, (d) 1.5 and (e) 5.0 μs.

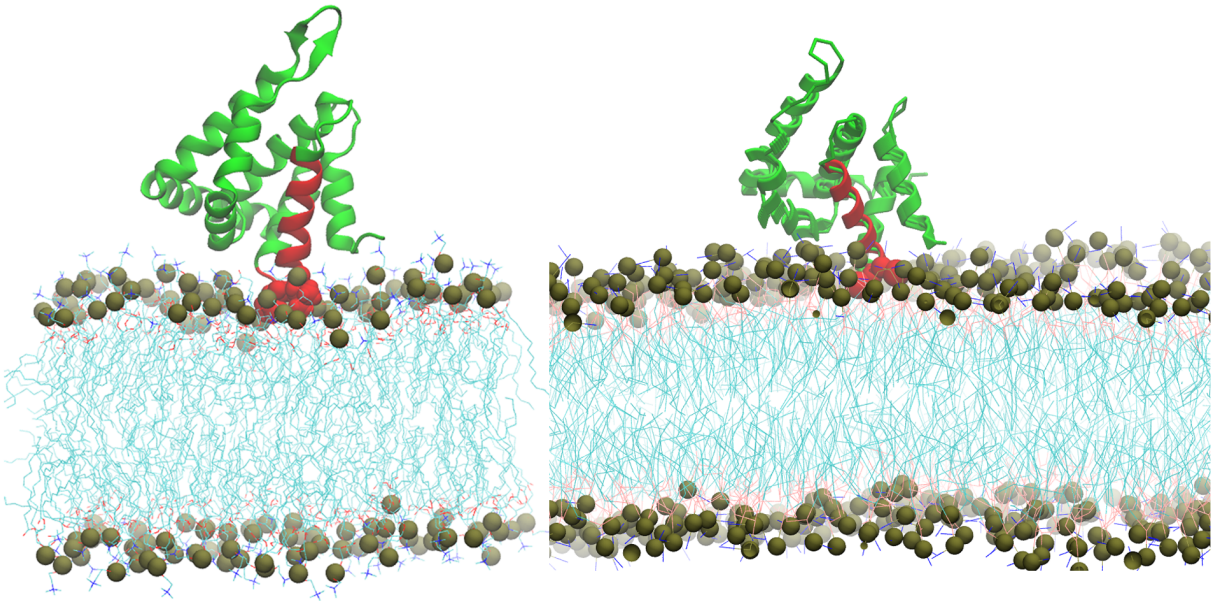

Figure S3: Equilibrated snapshots of the protein-membrane interface in the absence of H_0 helix (ENTH-H_0 domain), obtained from the (left) atomistic and (right) coarse-grained simulations. Red color indicates the H_3 helix. Residue 50VAL of the H_3 is highlighted by the red spheres. Water and ions are removed for clarity.

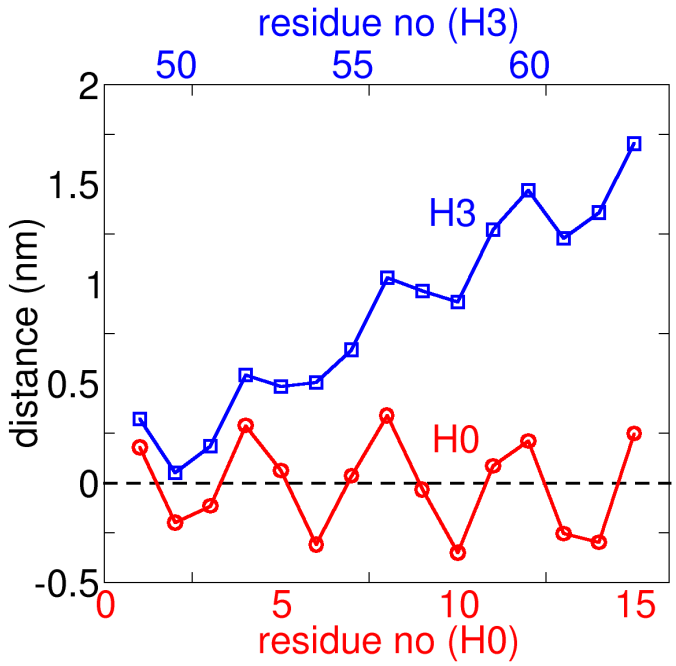

Figure S4: (Red) Distances of different residues of the H_0 helix from the top PO4 layer when the ENTH domain binds with the membrane through H_0 helix. (Blue) Distances of different residues of the H_3 helix from the top PO4 layer when the ENTH domain binds with the membrane through H_3 helix, in the absence of H_0 helix. In the later case, fewer number of residues are engaged in membrane binding, thus binding strength is likely to be less compared to the former case, where ENTH binds with the membrane through H_0.

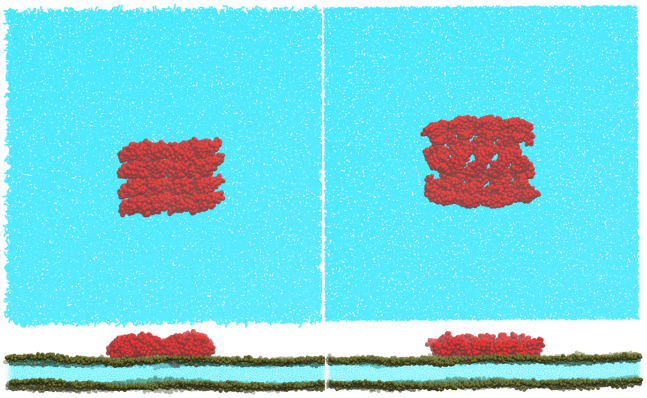

Figure S5: Initial structures of the protein-membrane complex used for calculating membrane curvature induced by sixteen (left) ENTH and (right) ENTH-H_0 domains. Red and cyan color represent the protein and membrane, respectively. Water and ions are not shown for clarity. Bottom panel shows the side view of the protein-membrane complex.

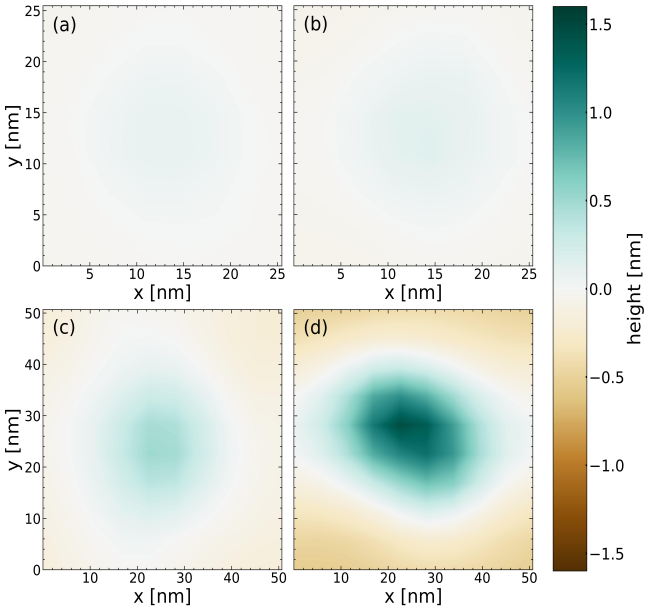

Figure S6: Membrane height profile in the presence of (a) one (b) two (c) four and (d) sixteen ENTH domains with the H_0 helix.

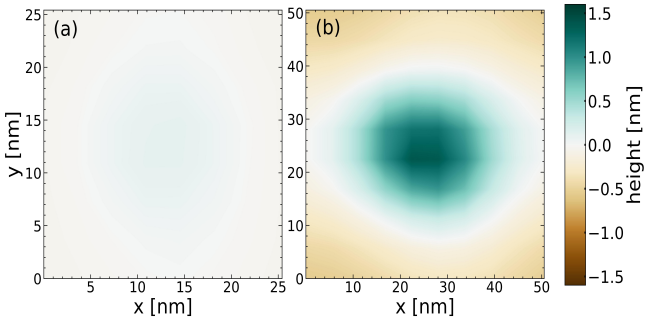

Figure S7: Membrane height profile in the presence of (a) one and (b) sixteen ENTH domains without the H_0 helix (ENTH-H_0 domains).

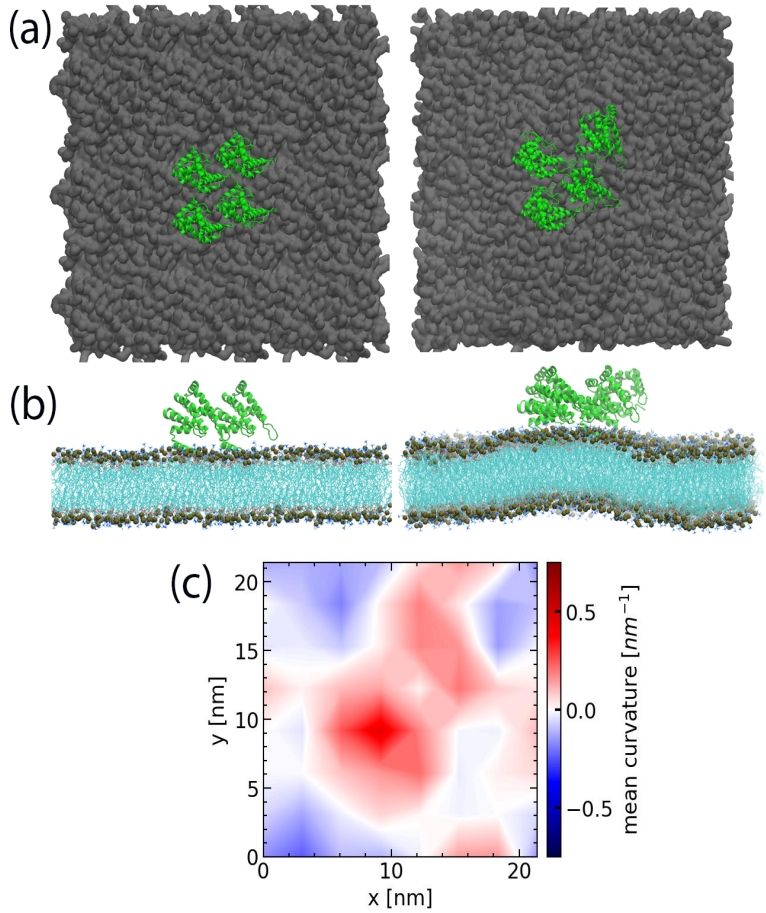

Figure S8: Atomistic simulation of the membrane curvature induced by a protein cluster. (a) Top view of the (left) initial and (right) equilibrated structure of the protein-membrane complex. The protein cluster maintains its compactness throughout the simulation. (b) Side view of the (left) initial and (right) equilibrated structure of the protein-membrane complex. (c) 2D plot of the membrane curvature. A positive curvature is generated by the protein at the membrane center.

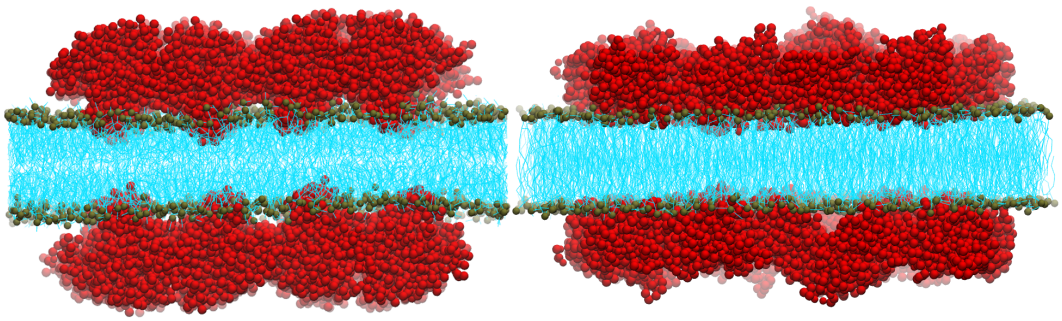

Figure S9: Initial structures of the protein-membrane complex used for the calculations of lateral pressure profile of the membrane in the presence of (left) ENTH and (right) ENTH-H_0 domains. Red and cyan color represent the protein and membrane, respectively. Water and ions are not shown for clarity. In both the cases there are a total of 48 proteins, 24 on each of the leaflets.

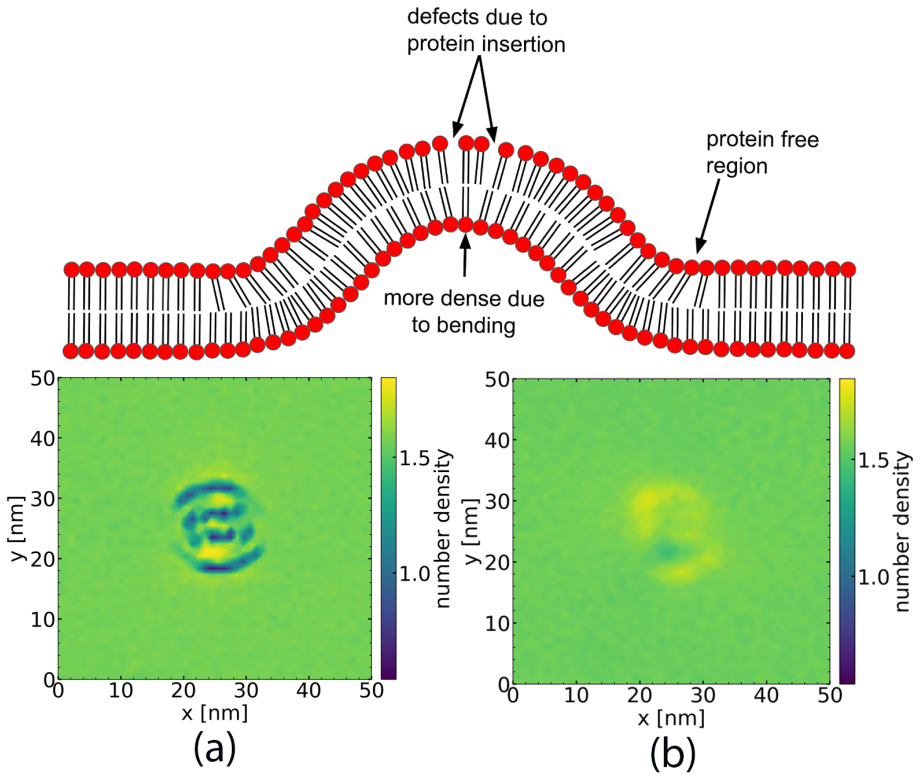

Figure S10: (top) schematic showing the membrane packing defects due to protein insertion. Lipids are relatively less dense in the protein binding region, and lipids are relatively more dense beneath the protein bound region. (Bottom) Number density of the PO4 beads for the (a) upper (protein bound) leaflet and (b) lower leaflet.

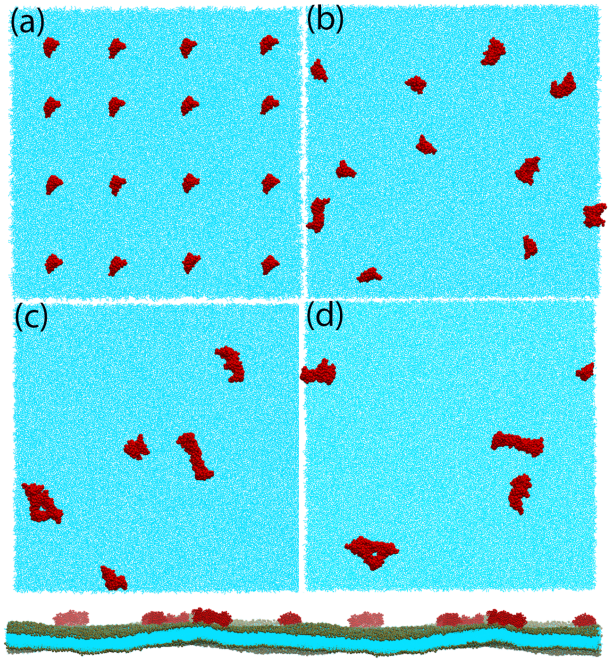

Figure S11: Aggregation of the proteins on the membrane surface. Sixteen ENTH domains are initially placed far away from each other on top of a 75 nm × 75 nm bilayer. Equilibrated structure of the protein-membrane complex at (a) 0 μs (b) 3 μs (c) 10 μs and (d) 20 μs. Cyan and red represent the membrane and proteins, respectively. Water and ions are removed for clarity. Proteins are not completely aggregated yet within 20 μs long simulation, but are very likely to aggregate completely in a longer simulation.

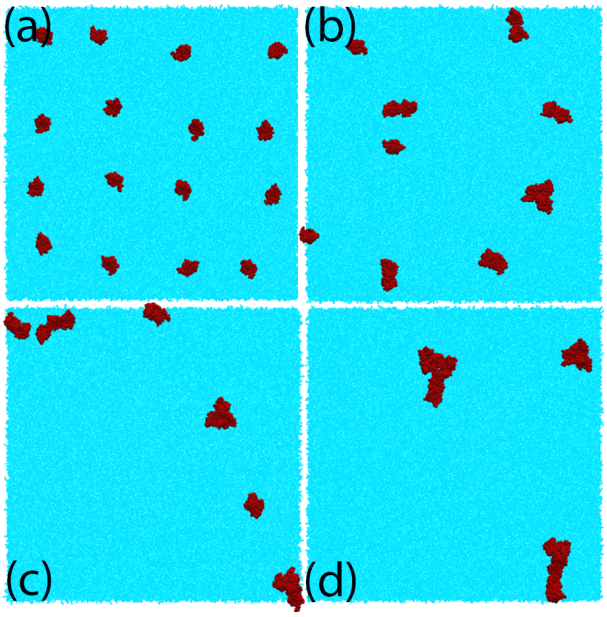

Figure S12: An independent simulation result showing the aggregation of the ENTH-H_0 on the membrane surface. Sixteen ENTH-H_0 domains are initially placed far away from each other on top of a 75 nm $\times$ 75 nm bilayer. Equilibrated structure of the protein-membrane complex at (a) 0 μs (b) 3 μs (c) 10 μs and (d) 20 μs. Cyan and red represent the membrane and proteins, respectively. Water and ions are removed for clarity. Proteins are not completely aggregated yet within 20 μs long simulation, but are very likely to aggregate completely in a longer simulation.