Molecular mechanism of membrane recognition and subsequent insertion of lipidated LC3 protein

Lipi Thukral, Durba Sengupta, Amrita Ramkumar, Divya Murthy, Nikhil Agrawal and Rajesh S. Gokhale

Supplementary Information

Table S1: Summary of Simulations: We have performed extensive coarse-grain molecular dynamics simulations of LC3-PE to understand the membrane binding and permeation of lipidated protein. Simulation set-up were different with respect to membrane lipids (charged and uncharged) and protein structures (Ala- and Ile-mutant). In addition, two sets of control simulations were performed, namely, non-lipidated and pure bilayer without protein. The details of all the simulations are listed below.

System	LC3	Membrane lipids	Water	No. of Simulations	Length of each simulation (μs)
LC3-PE in zwitterionic membrane	1	1136 POPC	52290	15	10
LC3-PE in charged membrane	1 1	568 POPC, 284 POPE, 284 CL	57455	15	10
Ala-mutant-LC3-PE	1	1136 POPC	52290	15	10
Ile-mutant-LC3-PE	1	1136 POPC	52290	15	10
LC3 (no lipid anchor)	1	1136	52290	5	5
POPC	-	1136	52290	5	5

Table S2: Details of primers for cloning and mutations.

L65A,R68A-FWD	GAGTGAGCTCATCGCGATAATTGCAAGGCGCCTGCAGCTCAATGC
L65A,R68A-REV	GCATTGAGCTGCAGGCGCCTTGCAATTATCGCGATGAGCTCACTC
R69A-FWD	GAGTGAGCTCATCGCGATAATTGCAGCGCGCTTACAGCTCAATGC
R69A-REV	GCATTGAGCTGTAAGCGCGCTGCAATTATCGCGATGAGCTCACTC
R69I-FWD	GAGTGAGCTCATCGCGATAATTGCAATCCGCTTACAGCTCAATGC
R69I-REV	GCATTGAGCTGTAAGCGGATTGCAATTATCGCGATGAGCTCACTC
R69E-FWD	GAGTGAGCTCATCGCGATAATTGCAGAGCGCTTACAGCTCAATGC
R69E-REV	GCATTGAGCTGTAAGCGCTCTGCAATTATCGCGATGAGCTCACTC
LC3B REV-XhoI	GGGGATCCTTACACTGACAATTTCATCCC
LC3B FWD-BamHI	GCCTCGAGCTATGCCGTCGGAGAAGACCTTC

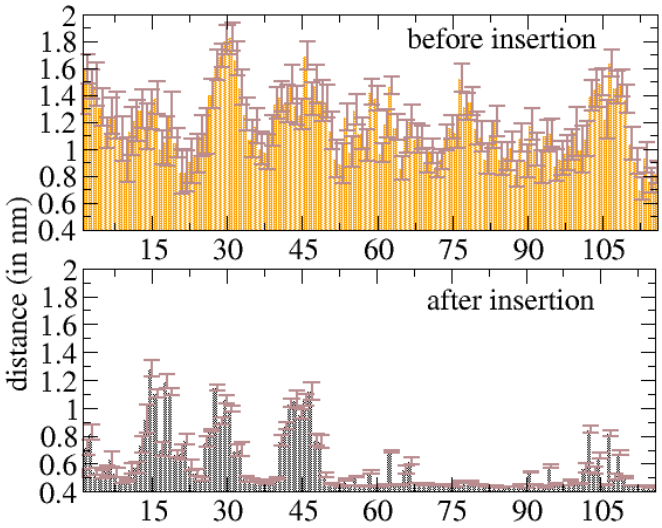

Figure S1: Residue-wise distribution of distance of the protein with the center of mass of the membrane. The trajectories were divided into before- and after insertion segments and is related to Figure 2. Error bars show the standard error calculated from fourteen productive trajectories.

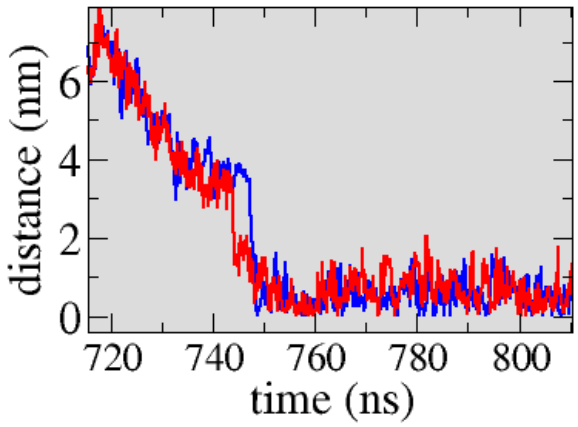

Figure S2: Time evolution of partitioning of individual acyl chain as a function of distance to the center of the membrane. The distance of last atom of each acyl chain was monitored and thus two chains are represented in blue and red, respectively. This is related to molecular mechanism shown in detail in Figure 2.

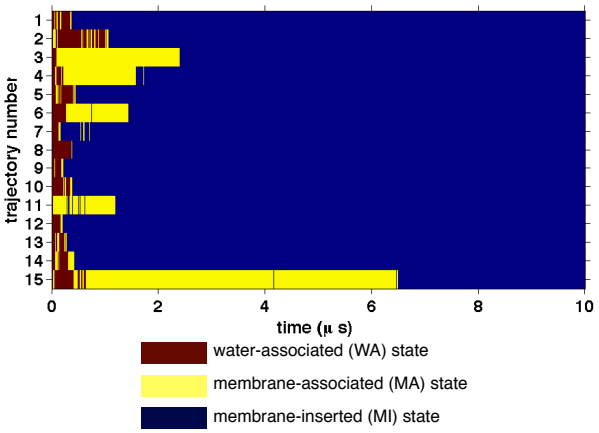

Figure S3: Time evolution of the distance between the PE chain of the LC3 and negatively charged bilayer along the fifteen trajectories. The lipid anchor of protein inserts in all trajectories. The rows represent the existence of the following: (1) Water-associated (maroon), (2) Membrane-associated (yellow), (3) Membrane-inserted (blue).

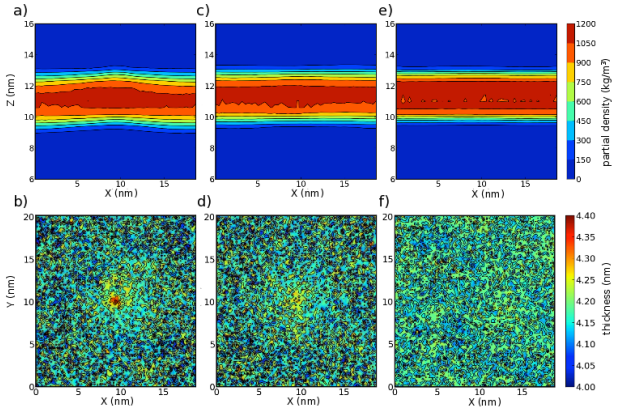

Figure S4: **Membrane Properties.** Upper and lower panel show the partial densities and membrane thickness, respectively, calculated for one of the representative trajectory. **A-B**: the point of insertion of the lipid anchor; **C-D**: 3 μs after insertion of the lipid anchor and **E- F**: for a pure bilayer. The partial densities are calculated along the membrane normal and one of the membrane lateral axis and averaging over the other lateral axis. Membrane thickness is calculated as the difference between the position of the headgroup phosphate beads. For further details see Methods section.

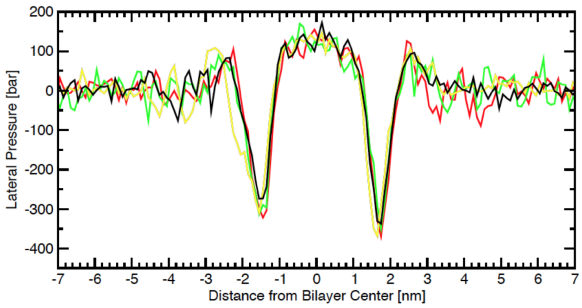

Figure S5: **Lateral Pressure Profile for all fourteen trajectories.** In particular, these profiles were calculated within 1 nm of the protein to assess the local changes.