Effect of tip-link protein(Cadherin23) on membrane Bending Rigidity

Hemraj Meena[1], Harshit[2], Rajni[2], Sabyasachi Rakshit[3], Tripta Bhatia[2]

Soft Matter Biophysics Lab

[1]Department of Biological Sciences, [2]Department of Physical Sciences,[3]Department of Chemical Sciences

Abstract: We used minimalistic membrane compartment systems namely Giant Unilamellar Vesicles (GUVs) for the study and we used a semi-experimental technique called vesicle fluctuation analysis(VFA) to get values of membrane bending rigidity an important elasto-mechanical parameter. We have found that attachment of full length protein{Cdh23 EC(1-27)} to the membrane increases the bending rigidity by $10k_BT$ whereas shorter Cdh23 EC(1-2) don't have any effect on membrane bending rigidity. The increase in bending rigidity is also observed when cholesterol is incorporated in the membrane.

- During biologically important processes, such as cell migration, endocytosis, exocytosis, cell fusion and fission, the cell membrane experiences mechanical stress at various length scales. To understand its response to the stress, knowledge of the elastic properties of the lipid bilayer is crucial and one of the main elastic parameters of membrane is bending rigidity.
- Many theoretical studies report that peripheral membrane proteins don't affect membrane bending rigidity, we have tried to test this idea.
- For the study we used Extracellular part of Cdh23 as model peripheral protein , further to compare effect of protein length and size we have used different constructs of the protein.

Giant Unilamellar Vesicles (GUVs)

- Simplified cell sized model membrane system which is composed of lipids.
- Lipids are amphiphilic molecules. When in contact with polar solutions the molecules self-assemble to form closed vesicles.
- We can increase the complexity of the system by changing membrane composition and attaching proteins.

Tip link protein: Cadherin23 EC(1-27)

- Cdh23 is a single pass transmembrane protein that forms tip links(a rope like structure) to connect stereocillia of hair cells found in inner ear of all mammals with hearing capacity.

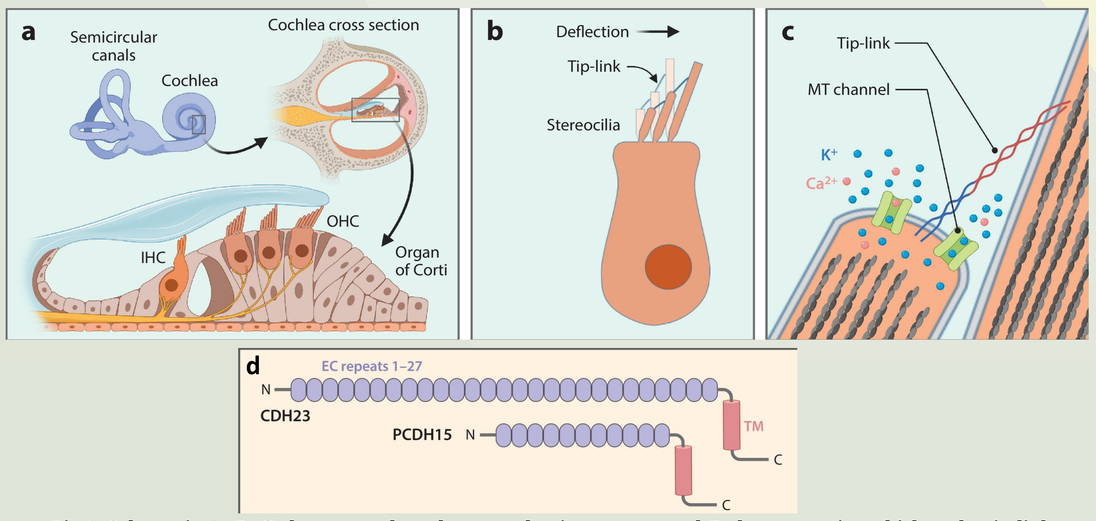


Fig.1 Schematic A, B, C shows sound mechantransduction process and D shows proteins which make tip link structure in hair cells.

Vesicle Fluctuation Analysis (VFA)

- VFA is a simple experimental technique for extracting information about membrane physical properties by analyzing the conformational fluctuations of giant vesicles visible by optical microscopy.
- Membrane deformation due to collisions with exchange of thermal (or active) energy with its environment in the membrane results in membrane flickering (or fluctuations) shown schematically in Fig.

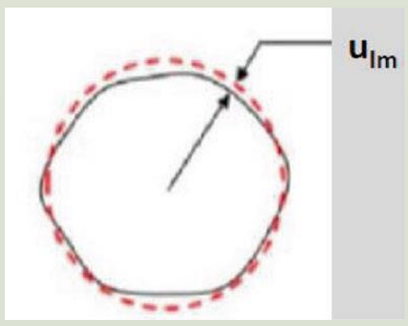


Fig.2 Schematic of membrane fluctuation (taken from ref.1)

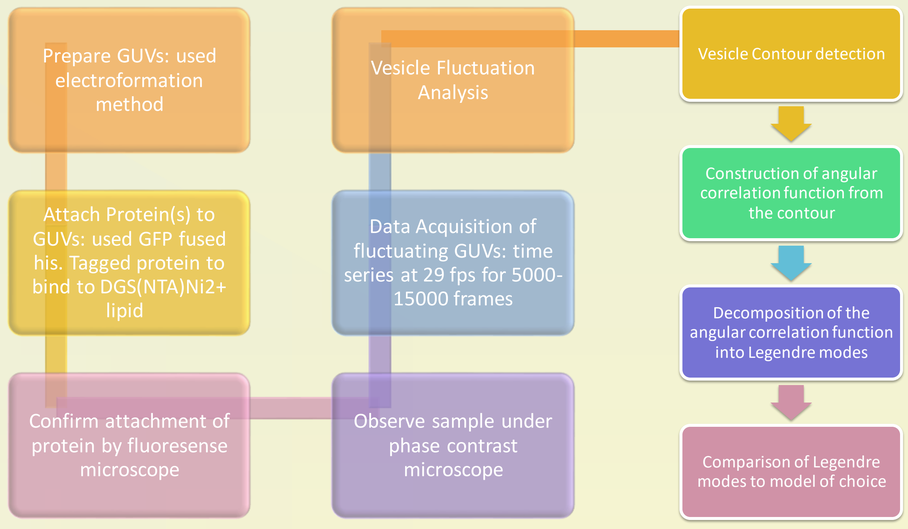
In the above figure the solid black is the vesicle boundary and red dotted line shown the fluctuations is happening and u_{lm} is the contour deviation function.

- Detailed analysis of membrane conformational fluctuations reveal information about material properties of the membrane, in particular the bending rigidity (κ)

References

- Zheng W, Holt JR. The Mechanosensory Transduction Machinery in Inner Ear Hair Cells. Annu Rev Biophys. 2021
- Ipsen, John Hjort, Allan Grønhøj Hansen, and Tripta Bhatia. " Vesicle fluctuation analysis." In The Giant Vesicle Book, pp. 333-345. CRC Press, 2019.

Methodology



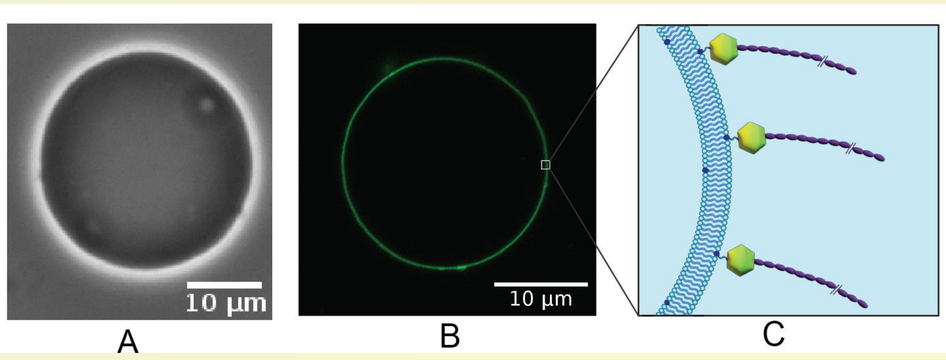


Fig.3 This image shows a GUV in phase contrast(A) and fluorescense microscope(B). Figure(C) shows attached protein to the membrane in which the protein binds to membrane by 6x his. tag to DGS-NTA(Ni)$^{2+}$ lipid via cooridnation bond.

Results

Table 3 Summary of all experiments, Standard Error(SE)

Experiment	Bending Rigidity(k_B T)	Mean $\pm SE$	Selected GUVs(n)	Analyzed GUVs
Control Expt. 1	73.09	±2.72	9	32
Cdh23 E.C.(1-27)	84.25	±2.99	7	26
Cdh23 E.C.(1-2) monomer	75.34	±3.01	11	46
Cdh23 E.C.(1-2) Fc dimer	74.42	±2.89	11	56

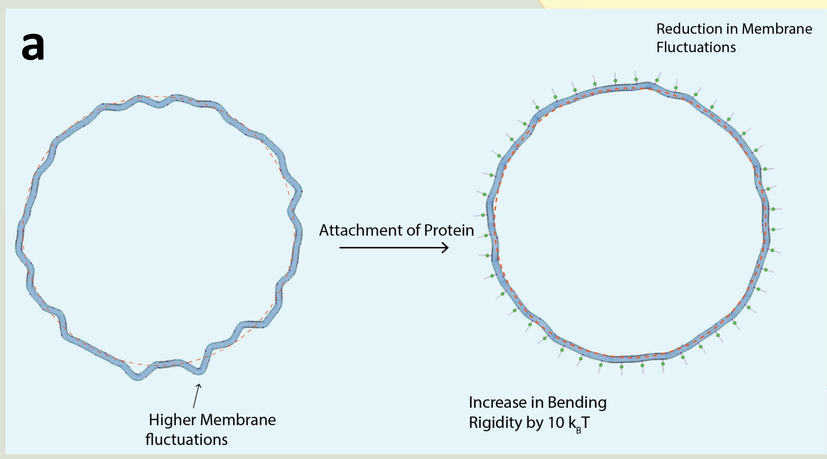


Fig.4 a) Protein attachment to the membrane leads to increase in bending rigidity, addition of Ca^{2+}(1.5mM) to the cadherin attached GUVs leads to GUV clusters ranging from 30-250µm.

Conclusion

- We have observed that Cdh23 EC(1-27) attachment to the membrane makes the membrane more stiff, by increasing its bending rigidity .
- When Ca^{2+} is added to the cadherin attached GUVs, GUVs start to form clusters ranging from 30-250µm supporting the role of cadherins as cell adhesion molecules.