Perspective on biophysics of the cell

EPFL

Biophysics is:

mapping biological processes onto (simpler) physical ones for study
(random walks, membrane surfaces, rigid particles)

hiding the complexity of biology within simple models including simulations
(opposing forces in lipids, chemical potential, Langevin equation)

predicting the consequences of physical principles in biological structures
(escape of a particle from potential, rigidity of SNARE anchors)

an approach that uses a toolbox of ideas and mathematical procedures that can be assembled into a quantitative description of biology:

what is important? energy, entropy, shape, flexibility, barrier, fluctuations, …

what is ignorable? detailed chemistry, initial conditions, diffusion, …

Symmetry in cellular processes

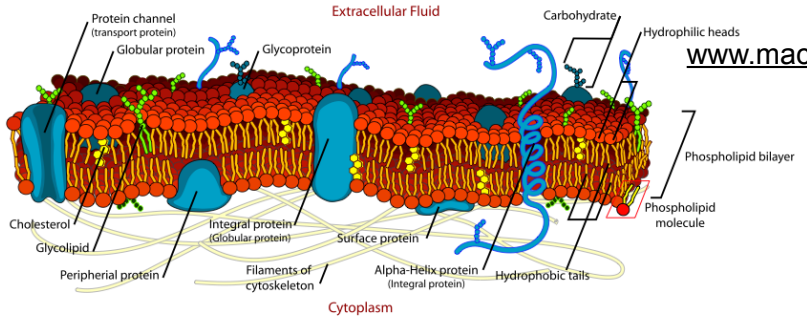
- Who cares about symmetry for soft matter at room temperature?
- Endocytosis — modelling shiga toxin infection biology
- Identifying toxin clustering mechanisms — line tension, curvature, composition, depletion
- Casimir force on membranes, theory and simulations

Who cares about symmetry at room temperature?

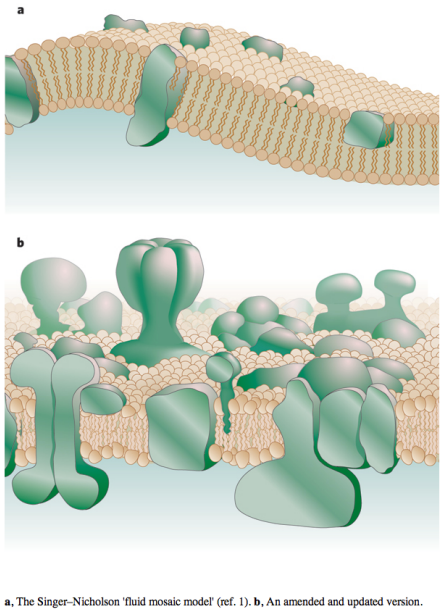
Wikipedia: Crystal Growth

Crystals have the following properties:

- regular unit cell
- translational symmetry in 3 dimensions
- rate of growth is very asymmetric (imperfections)
- transport properties that can be highly asymmetric, e.g., electrical conduction



www.macroevolution.net/fluid-mosaic-model.html



a, The Singer–Nicholson 'fluid mosaic model' (ref. 1). b, An amended and updated version.

Engelman DM, Membranes are more mosaic than fluid, Nature 438:578-580 (2005)

Biological cells have none of these properties, but symmetry is still important:

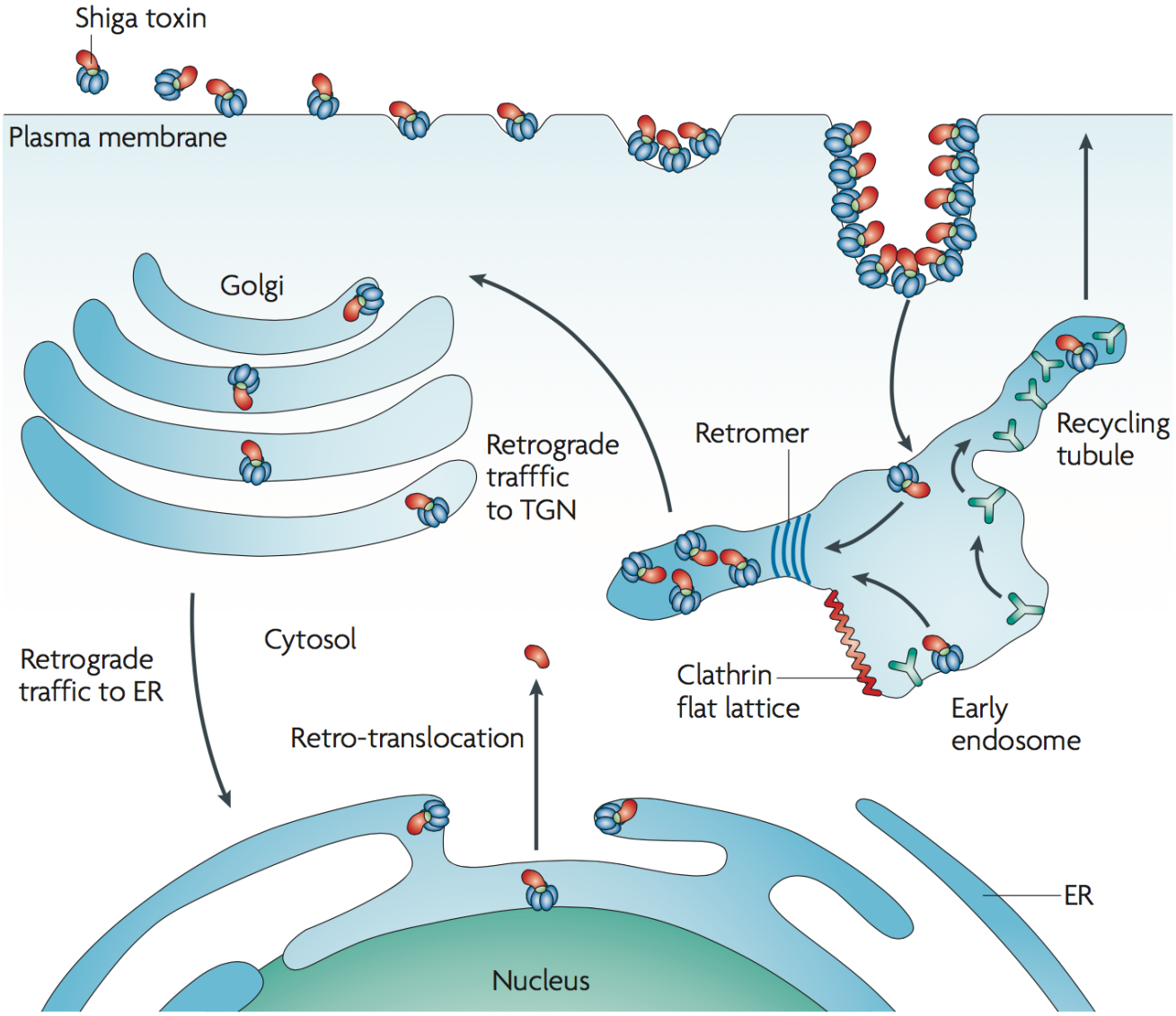
- actin/microtubule filaments are polar
- membranes are intentionally asymmetric (composition and charge)
- proteins have internal symmetry for binding sites, channel transport
- assembly of protein structures requires symmetry, e.g., clathrin
- some proteins have strange symmetry, e.g., actin monomers, shiga toxin proteins

How do bacteria infect a cell?

Clathrin-coated pits
- bind to surface receptor
- concentrate
- CCP forms internally
- endocytosis

Non-clathrin entry
- bind to surface receptor

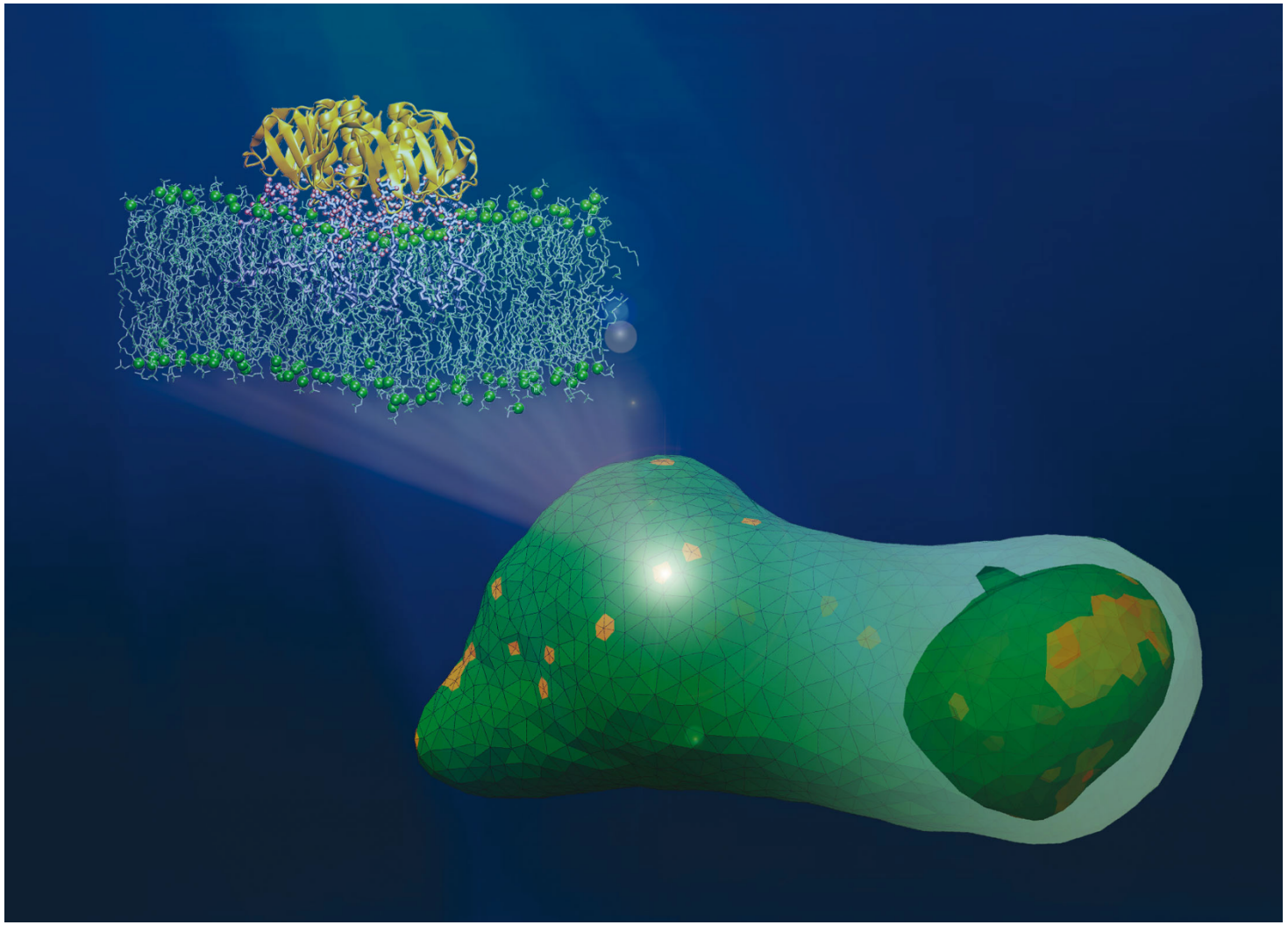
Shiga toxin entry
- bind to membrane lipid
- aggregate?
- invaginate?



Shigella bacterium seems to use a novel, non-CCP, endocytosis mechanism

L. Johannes Curie Inst.

Single simulation technique is not enough



Pezeshkian et al. Soft Matter 12:5164 (2016)

Biology of Shigella infection of a cell

Shigella is a genus of Gram-negative, facultative anaerobic, nonspore-forming, nonmotile, rod-shaped bacteria closely related to *Salmonella*. The genus is named after Kiyoshi Shiga, who first discovered it in 1897.[1]

Source: Wikipedia.

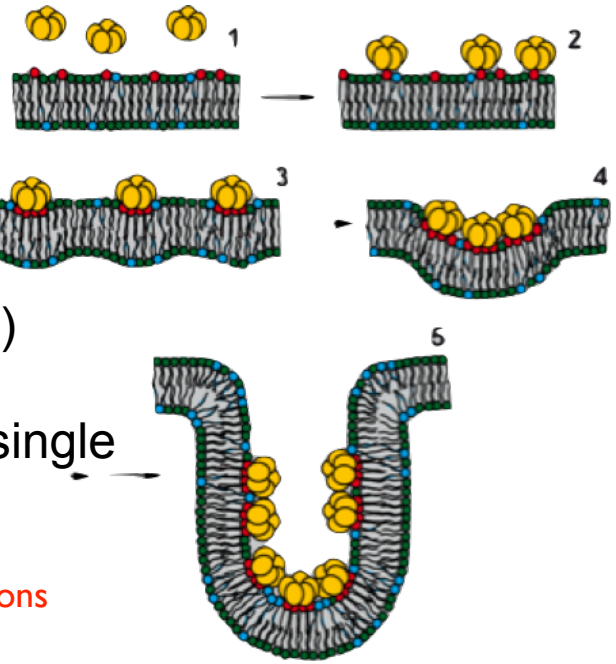
Shigella bacteria release small AB^5 toxin particles (*shiga* toxin, ~ 7 nm) onto the plasma membrane that co-opt the cellular scission machinery to enter the cell and infect it. Each toxin particle can bind up to 15 Globotriaosylceramide (Gb3) lipids.

There are 3 stages:

A) Single STxB particles bind to a specific membrane lipid, Gb3, and diffuse around
(length scale ~ 7 nm, all atom molecular dynamics)

B) Above a certain concentration, bound STxB forms clusters
(length scale ~ 180 nm, dissipative particle dynamics)

C) Large clusters bend the membrane and create a tube that is endocytosed by cellular machinery
(length scale ~ 1 - 5 µm, Monte Carlo,triangulated network)



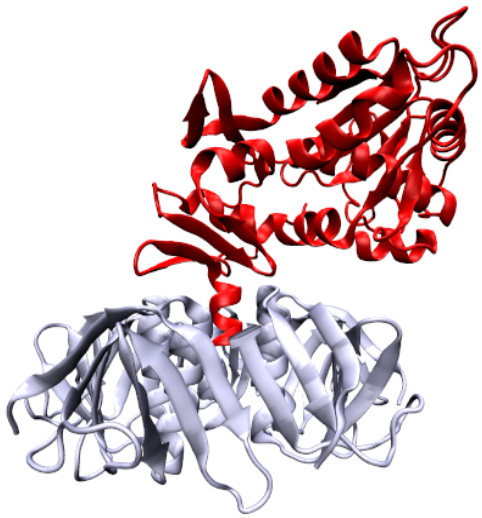
These stages occur on **different** length scales that mean no single simulation technique can follow the whole sequence.

Römer et al. Shiga toxin induces tubular membrane invaginations for its uptake into cells, Nature 450:670 (2007)

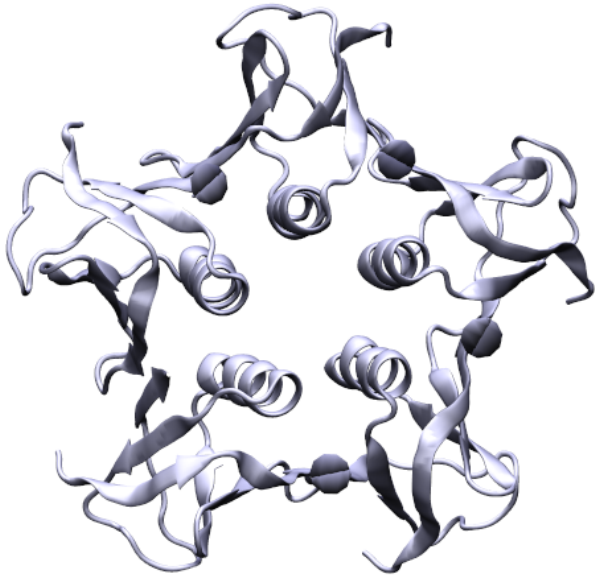
Shiga and shiga-like toxin

- Shiga Toxin and Shiga like toxin-1 are a part of an AB^5 protein family.
- A subunit modifies rRNA in the host cell and results in cell death
- B subunit (STxB) is a pentamer of identical units.
- STxB binds up to 15 Globotriaosylceramide (Gb3) lipids on the cell membrane surface (e.g. Gb3:22:0 and Gb3:22:1). It has no direct protein-protein interactions
- Gb3 is over-expressed by various human tumours.

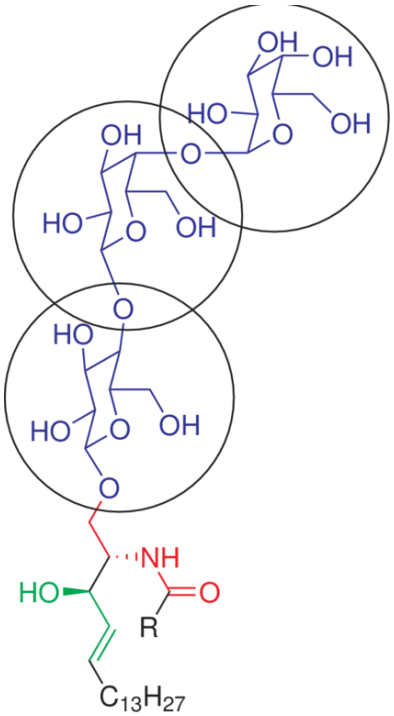
A subunit



B subunit



B subunit



Gb3: STxB receptor

A generic mechanism for entry

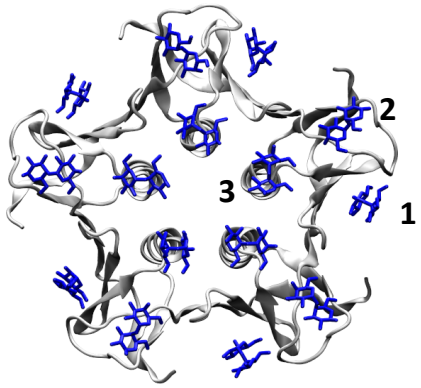
This binding site geometry is preserved for the receptor-binding parts of cholera toxin and simian virus 40 (SV40) (Figure 3c), for which it was shown previously that they also have curvature-active properties, endowing them with the capacity to drive tubular membrane invaginations through interaction with their GSL receptor molecules [25], as observed for Shiga toxin [9]. Strikingly, these GSL-binding pathogenic lectins do not have any sequence similarity, which suggests that this binding site geometry might be the result of convergent evolution towards a common function: membrane mechanical work in relation to inward-oriented curvature generation for the construction of endocytic pits. Of note, cholera toxin and SV40 have indeed both been described to be efficiently internalized into cells in which the clathrin pathway is inhibited [18,26]. Further pathogens and pathogenic factors exist that also interact with GSLs in one way or another to get into cells (reviewed for gangliosides in Reference [27]), suggesting that this mechanism is used more widely.

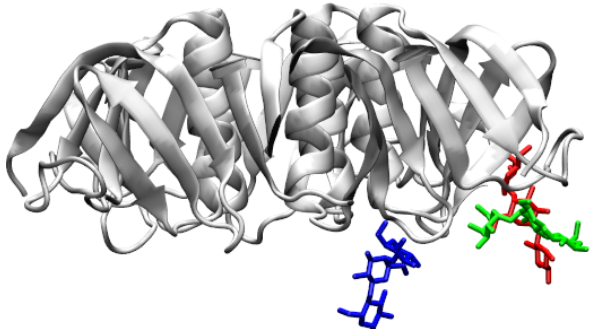
L. Johannes, Shiga Toxin — A model for glycolipid-dependent and lectin-driven endocytosis, Toxins 9:340 (2017)

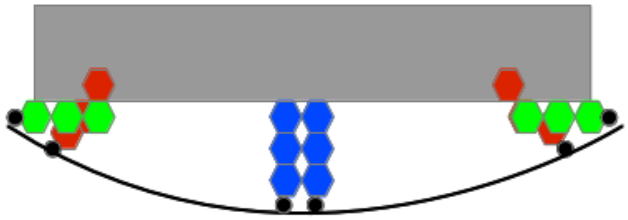
Step 1: STxB induces a small local membrane curvature

All atom MD ~200 + 400 ns, ~ 10 nm
Gromacs, CHARMM36 FF, TIP3P water,
Gb3 params from Pezeshkian 2015.

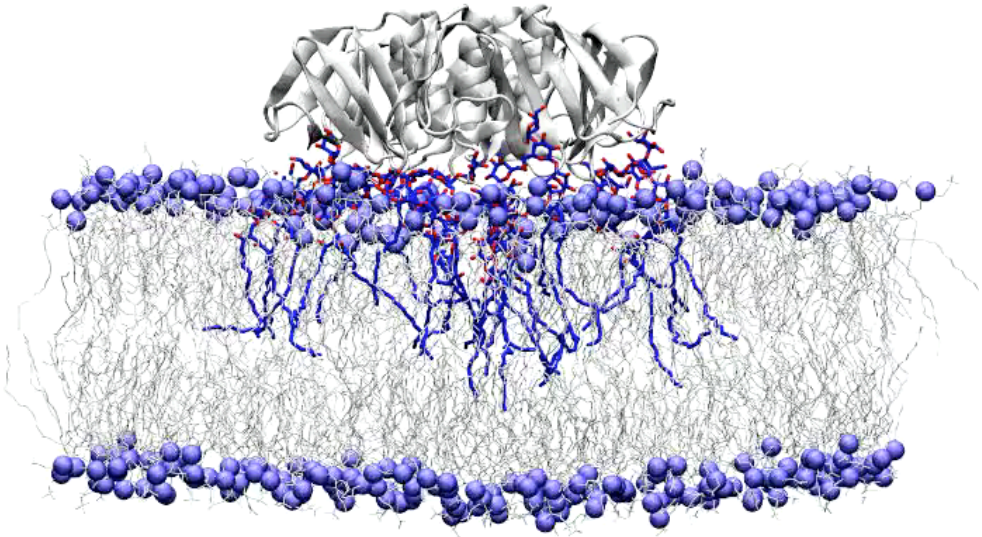
349 DOPC + 15 Gb3 + STxB
pre-bound to Gb3

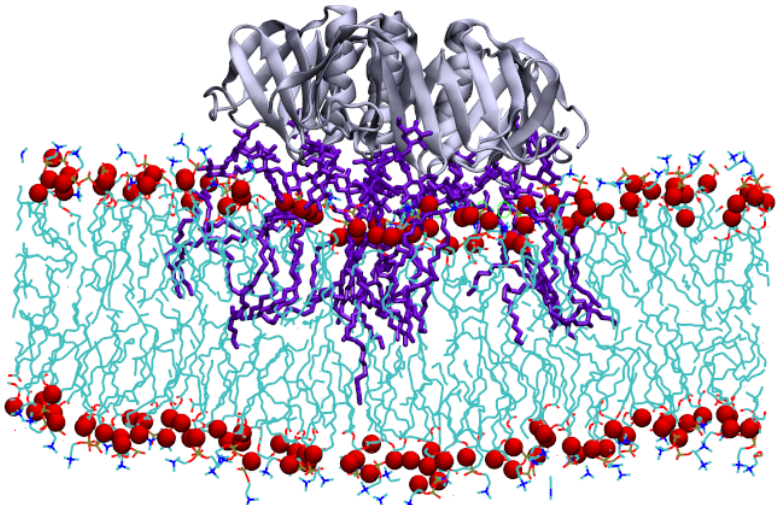






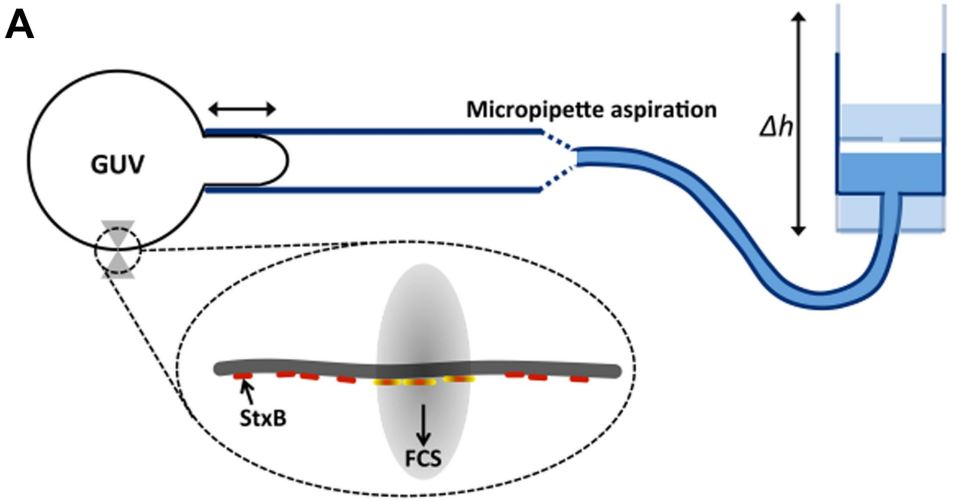
Angle of binding pockets creates curvature

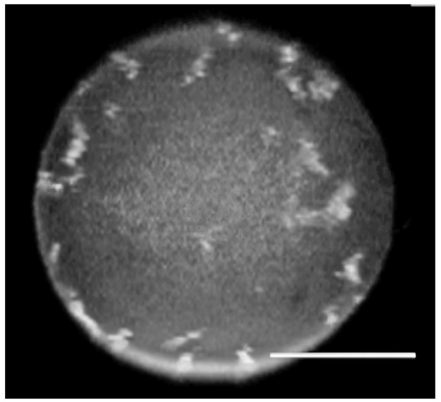




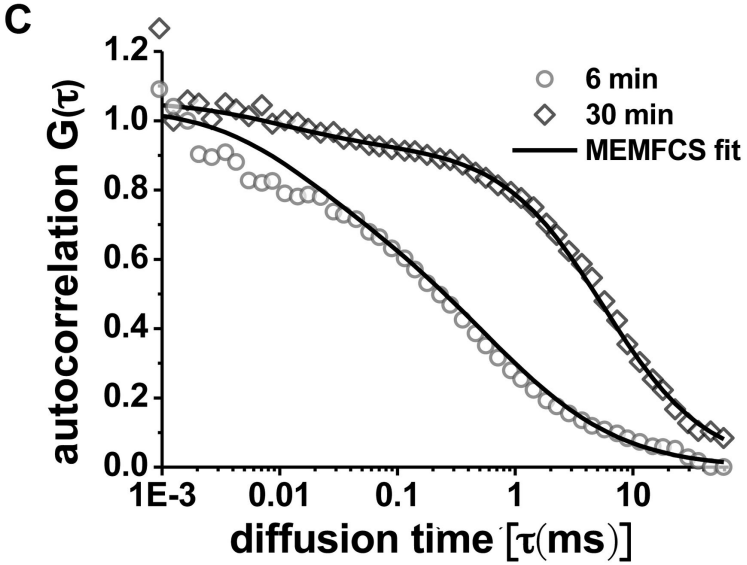
W. Pezeshkian et al. Soft Matter 11:1352-1361 (2015).

FCS experiments confirm STxB cluster growth

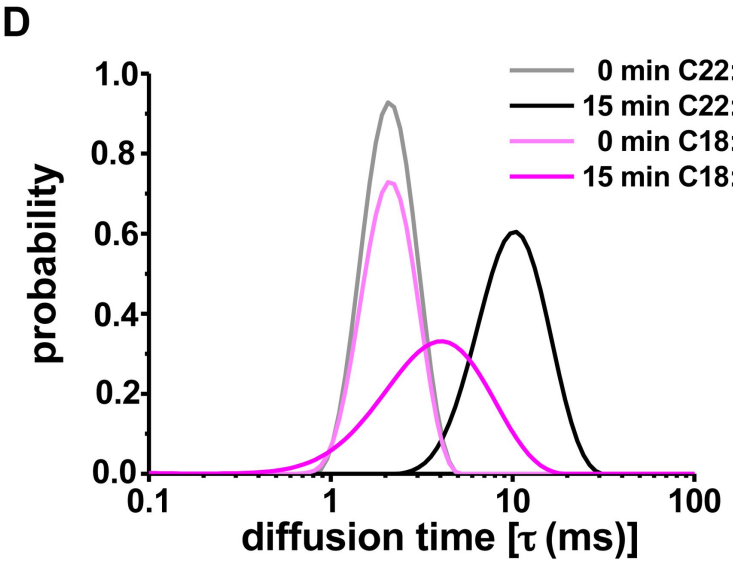




Composition: 70% DOPC + 30% Gb3



Diffusion time increases implying cluster size increases over time



Gb3 tail length matched to DOPC

Senthil Arumugan, Curie Inst.

Potential drivers of STxB clustering

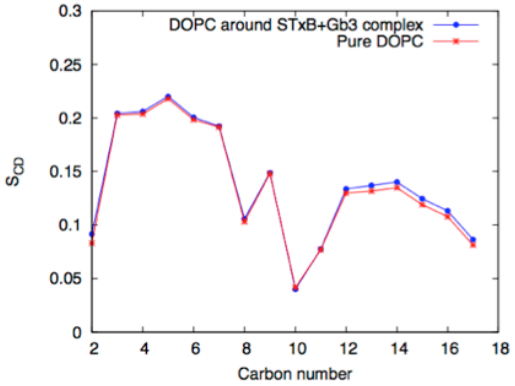
1) Line tension effects?

lipid chain length mismatch - STxB clusters on both C22:1 and C18:1 Gb3

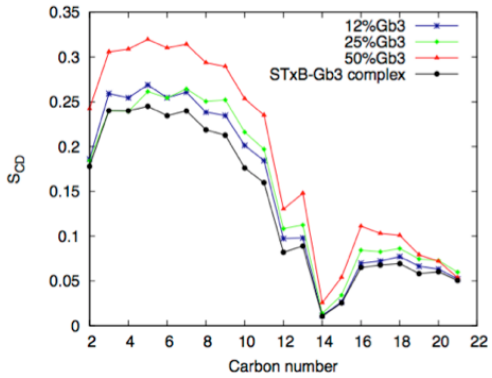
compositional mismatch - STxB clusters on 70% DOPC + 30% Gb3

(above phase transition temperature with same Gb3 conc. under toxin and in bulk vesicle)

2) Entropic perturbation of hydrophobic core?



Deuterium order parameter for pure DOPC bilayer and DOPC + Gb3-STxB complex (from MD)



Deuterium order parameter for Gb3 chains at various % concentrations, and STxB-Gb3 complex (from MD)

3) Curvature-mediated force?

STxB has low contact angle of 7^0 that is predicted analytically to give rise to a *repulsive* force:

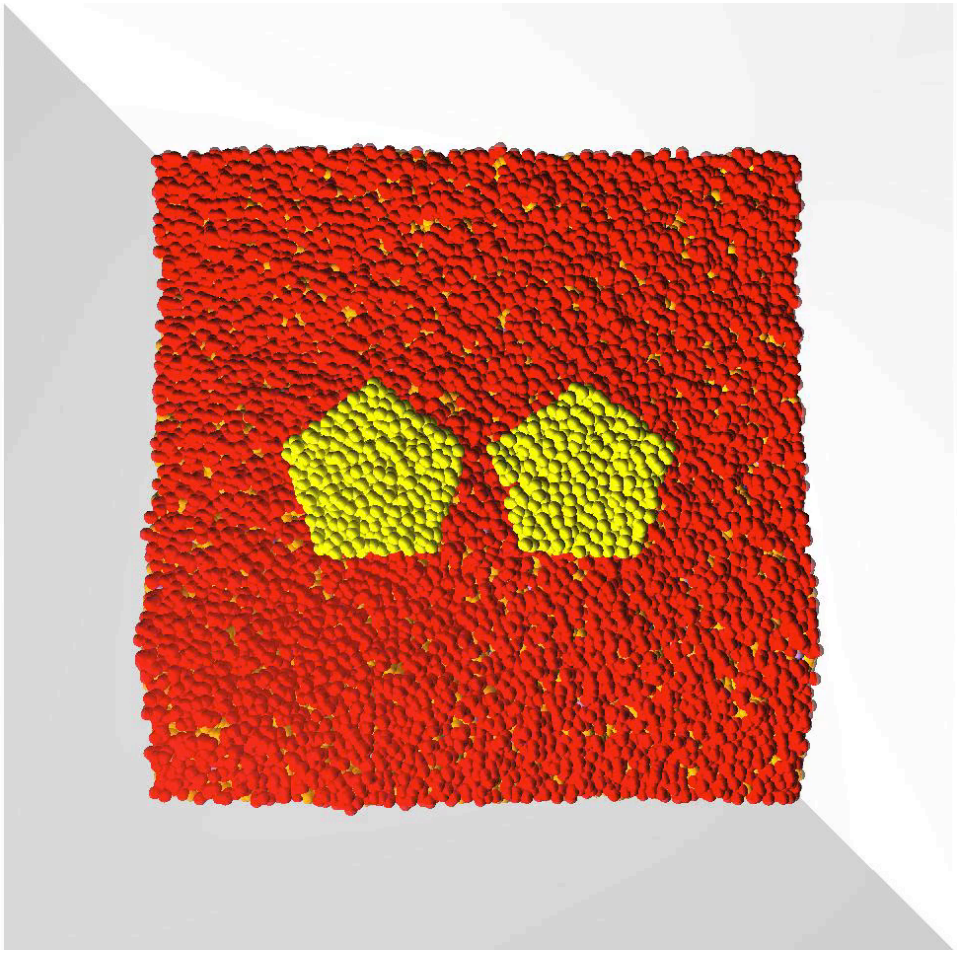
Reynwar and Deserno, Soft Matter 7:8567-8575 (2011)

Goulian et al., Europhys. Lett. 22:145-150 (1993)

Step 2: Membrane-bound STxB forms clusters

DPD simulations

NP size: 6 nm
Simulation time: 12 μs
Box size: $(40\ nm)^3$
Solvent is invisible for clarity



Aggregation only occurs for **rigid** NPs with linear size > ~5 nm that are tightly bound to the membrane

NB. There is a small repulsive a_{ij} force between the pentamers.

Tight binding to membrane is necessary for clustering

DPD simulations predicted that small (< 5 nm) or floppy nanoparticles do not cluster, nor do particles that are displaced from the membrane by linkers.

Size and shape are hard to change for STxB, but we can displace them.

(Weria Pezeshkian, John Ipsen, SDU; Julian Shillcock, EPFL)

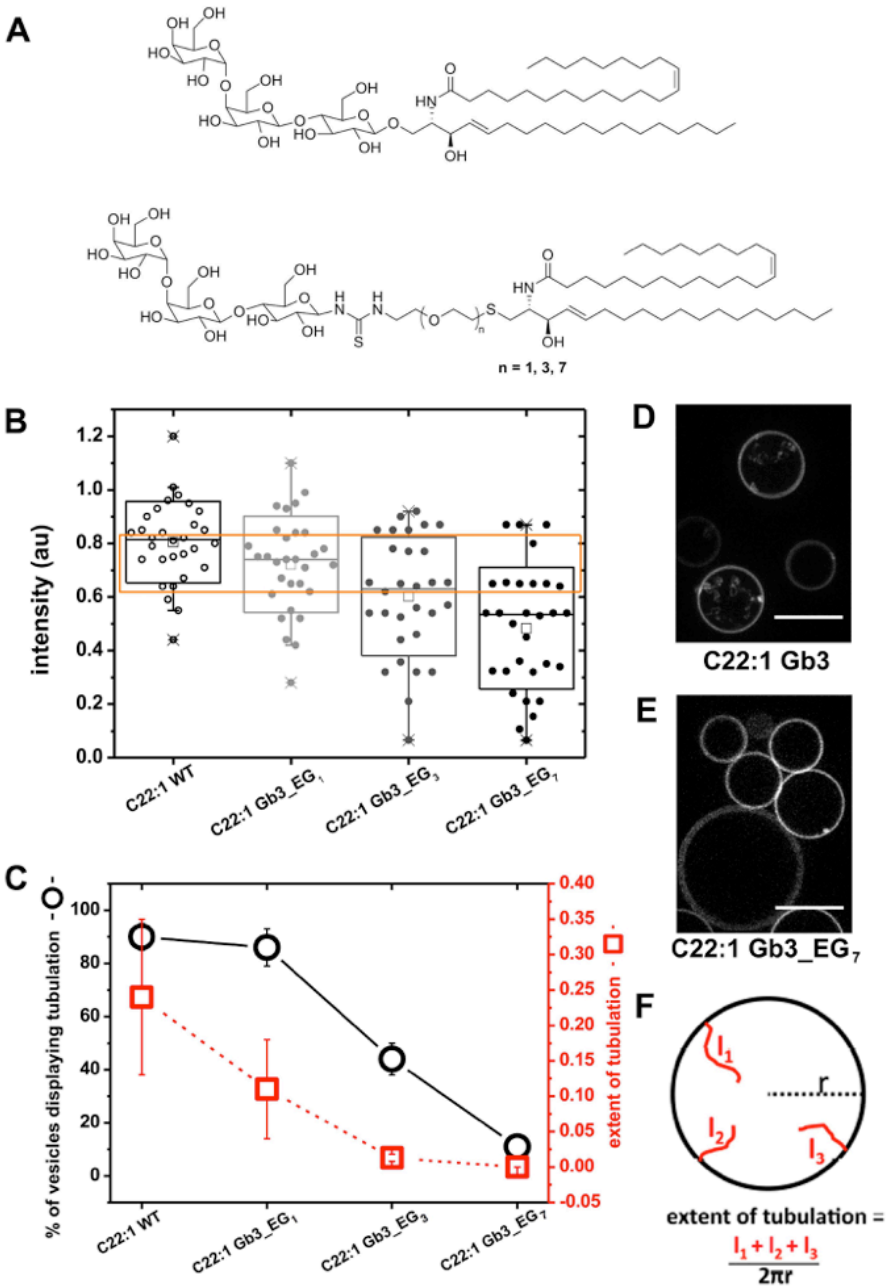
We synthesized novel lipid species (A) with the carbohydrate head separated from the ceramide backbone by EG linker

(Haifei Gao, Jean-Claude Florent, Curie Inst.)

NB 12/40 pages in Supp Mat describes the synthetic chemistry.

STxB still binds to membrane (B) but tubulation is reduced (C) for linkers > 3 EG,
vesicles of Gb3 exhibit tubules (D) while those with Gb3:EG7 do not (E)

(Ludger Johannes, Senthil Arumugan, Curie Inst.)

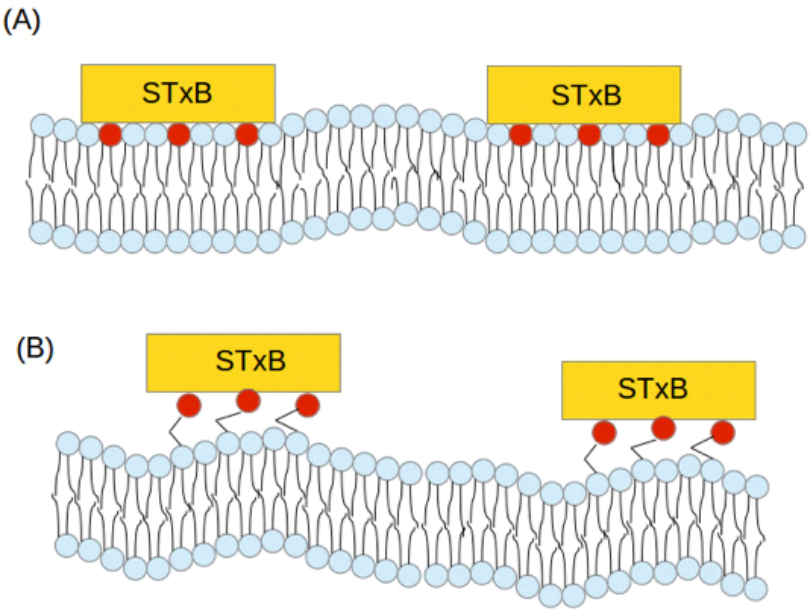


A thermal Casimir force may drive clustering

Whereas line tension effects, if present, are *insufficient* to drive clustering, tight binding of the STxB to the membrane is *essential*, which makes a fluctuation induced force the most likely explanation for the clustering process.

Phase separation of flat, rigid inclusions on a membrane due to a thermal Casimir force is also predicted from MC simulations (T. Weikl. PRE 66:061915 (2002))

Theoretical calculations also predict a strong attraction at separations (H) small compared to particle size:

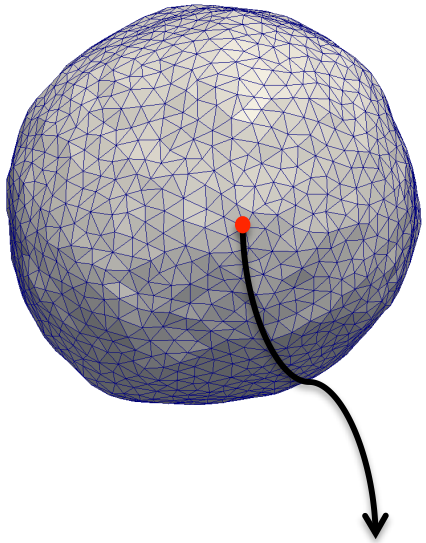


$V(H) \sim -k_BT\,(a/H)^{1/2}$ for disks of radius a (Lin. et al. PRL 107:228104 (2011))

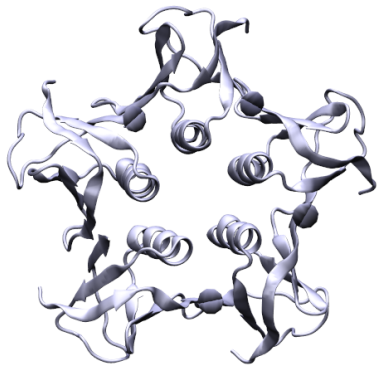
$V(H) \sim -k_BT\,(L/H)$ for pentagons of size L (Pezeshkian et al. ACS Nano 11:314-324 (2017))

Recent review of Casimir forces:
L. Woods et al. Materials perspective on Casimir and van der Waals interactions, Rev. Mod. Phys. 88:045003 (2016)

Step 3: STxB clusters create tubular invaginations

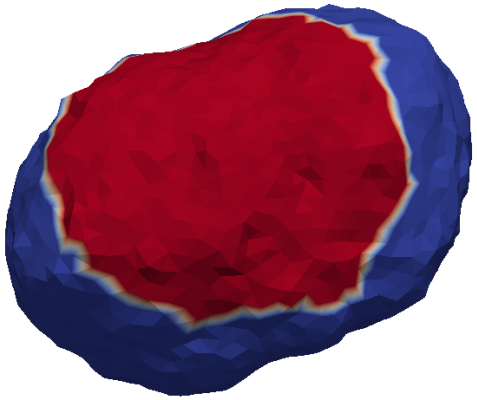


D≈150 nm
A≈0.3μm²

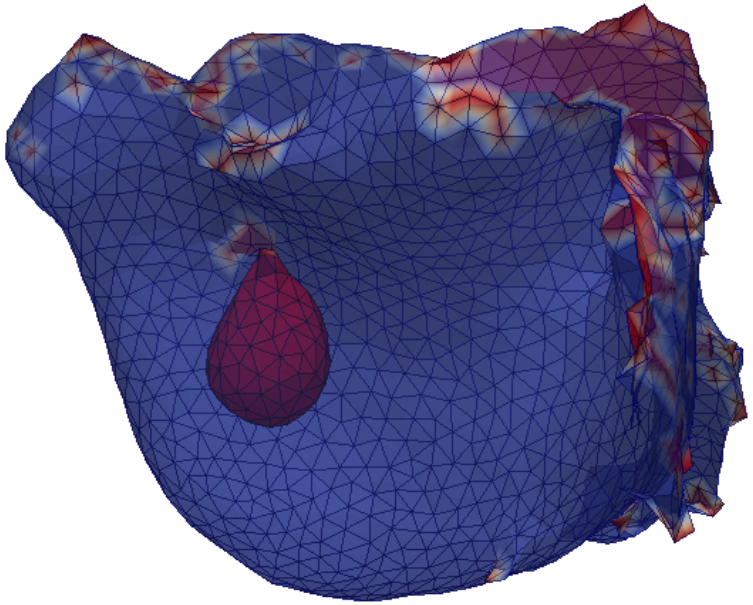


Monte Carlo simulations of a triangulated surface vesicle

Protein bound area
of membrane surface (red)



Tubular invagination inside cell

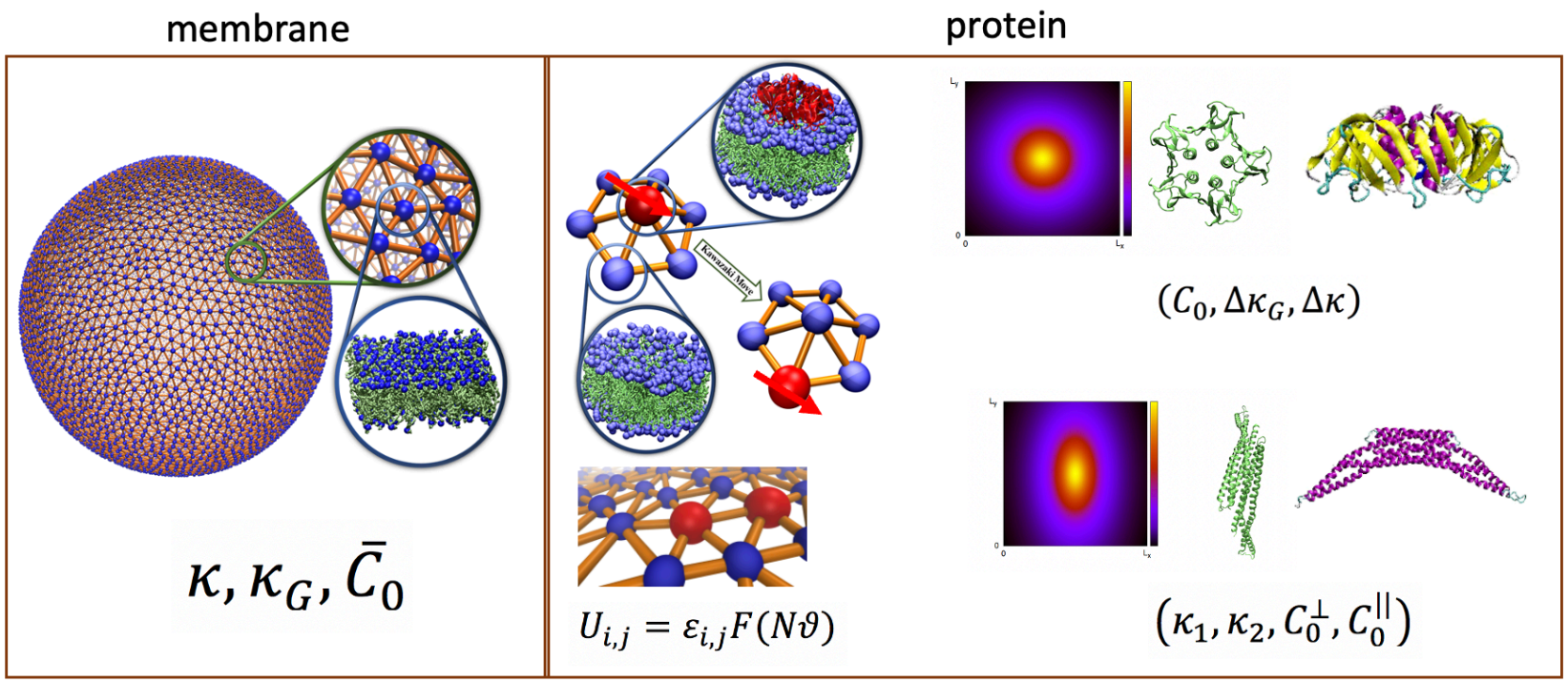


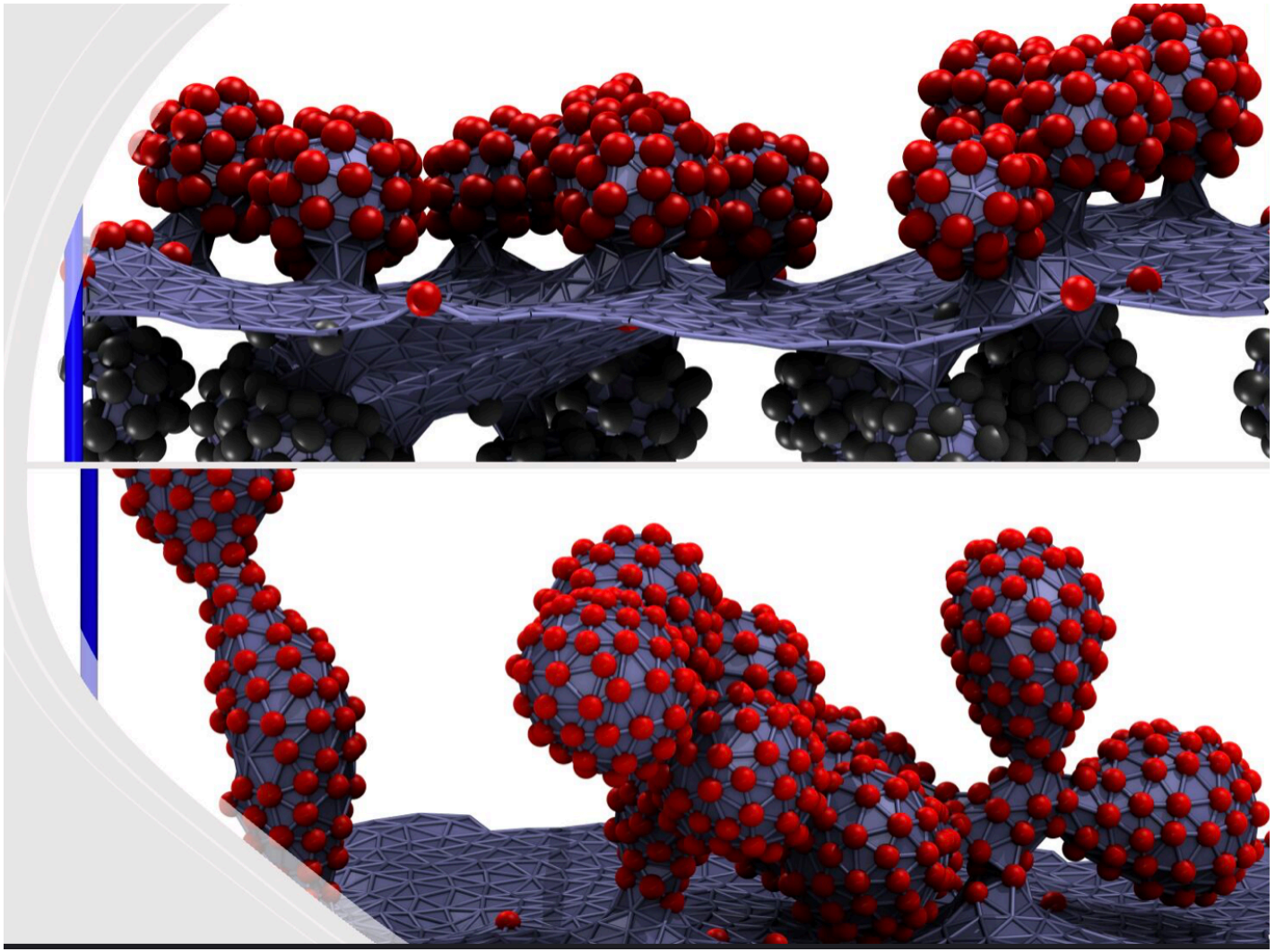
W. Pezeshkian et al. Soft Matter 12:5164-5171 (2016)

About FreeDTS

Weria Pezeshkian edited this page 2 weeks ago · 2 revisions

FreeDTS is software to perform computational research on biomembranes at messocpic length-scale. In FreeDTS, a membrane is represented by a dynamically triangulated surface equipped with vertex-based inclusions to integrate the effects of integral and peripheral membrane proteins. Several algorithms are included into the software that allow for simulation of framed membrane with constant tension, vesicles with various fixed volume or constant pressure difference, confined membranes into the fixed region of the space, constant fixed global curvature and application for external forces on regions of the membrane. In addition, the software allows one to turn off the shape evolution of the membrane and only explore inclusions organization. This allows to take realistic membrane shapes obtained from Cryo-ET and obtain heterogeneous organization of biomolecules which can be backmapped to finer simulations models.



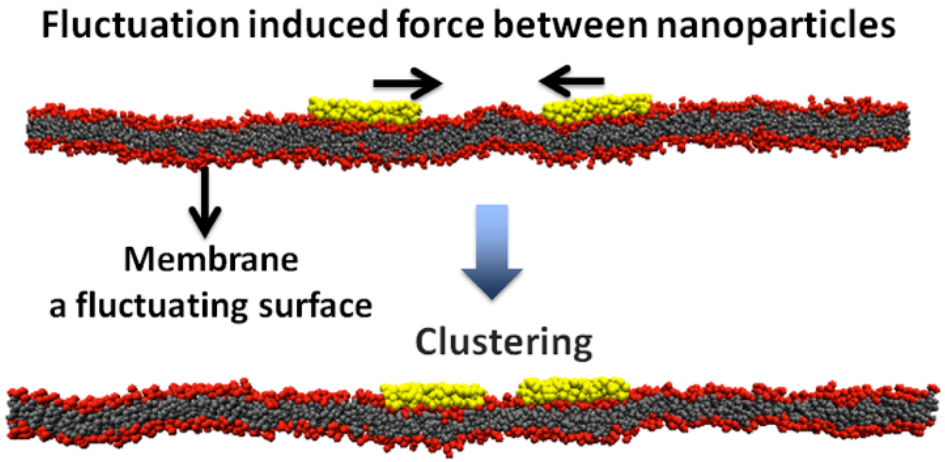


Paper https://www.biorxiv.org/content/10.1101/2023.05.05.539540v1

Source code https://github.com/weria-pezeshkian/FreeDTS

Summary

- STxB binds to signalling lipids Gb3
- Suppression of membrane's thermal fluctuations gives rise to an attractive Casimir-like interaction that drives STxB to form domains
- STxB domains induce membrane curvature that creates invaginations by which toxin enters the cell



Cell cannot prevent this pathway without mutating its signalling lipids.

Manufactured nanoparticles could also bind to target lipids in the plasma membrane and so enter the cell for therapeutic purposes.

W. Pezeshkian et al. ACS Nano 11:314-324 (2017)

Acknowledgements

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