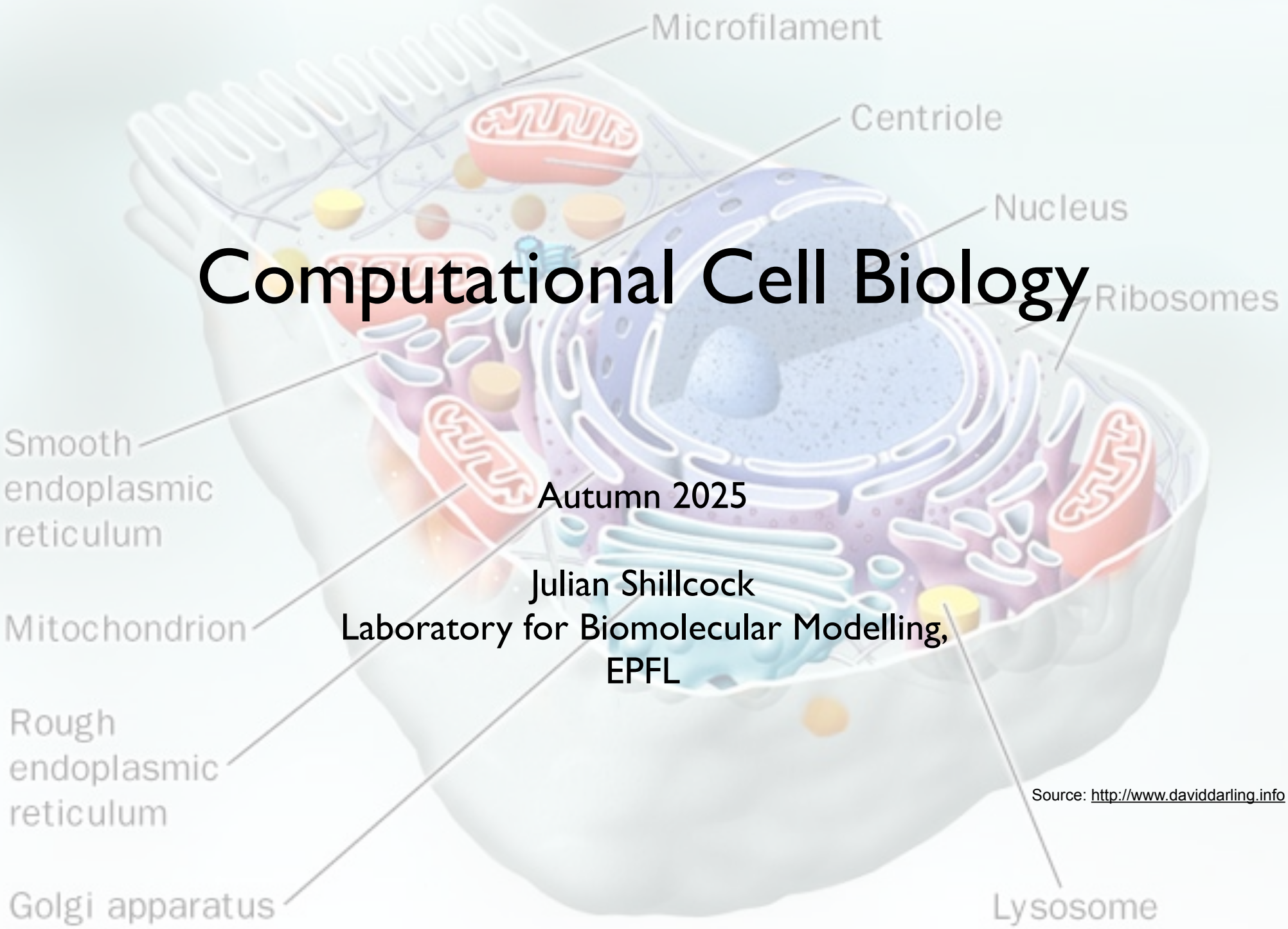


Computational Cell Biology

Autumn 2025

Julian Shillcock
Laboratory for Biomolecular Modelling,
EPFL

Source: http://www.daviddarling.info

Core Concepts

Building a computational model requires answering two questions:

What is important?

What is ignorable?

e..g, energy, entropy, phase, shape, flexibility, barrier, fluctuations, detailed chemistry, initial conditions, diffusion, …

Today: chemical binding, biochemistry are ignorable while symmetry, material properties (rigidity/size) of proteins are not.

Bacteria are sneaky in getting their way

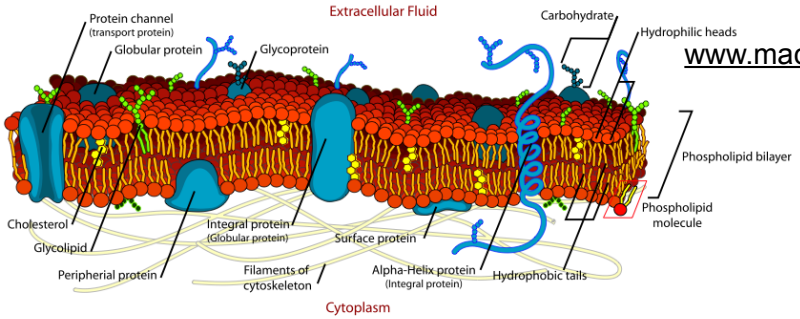
(If you're lucky - your model ignores what Nature ignores … that's what important means)

Who cares about symmetry at room temperature?

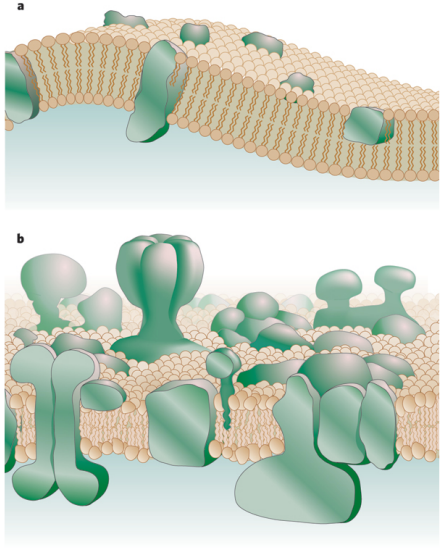
Wikipedia: Crystal Growth

Crystals have the following properties:

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



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



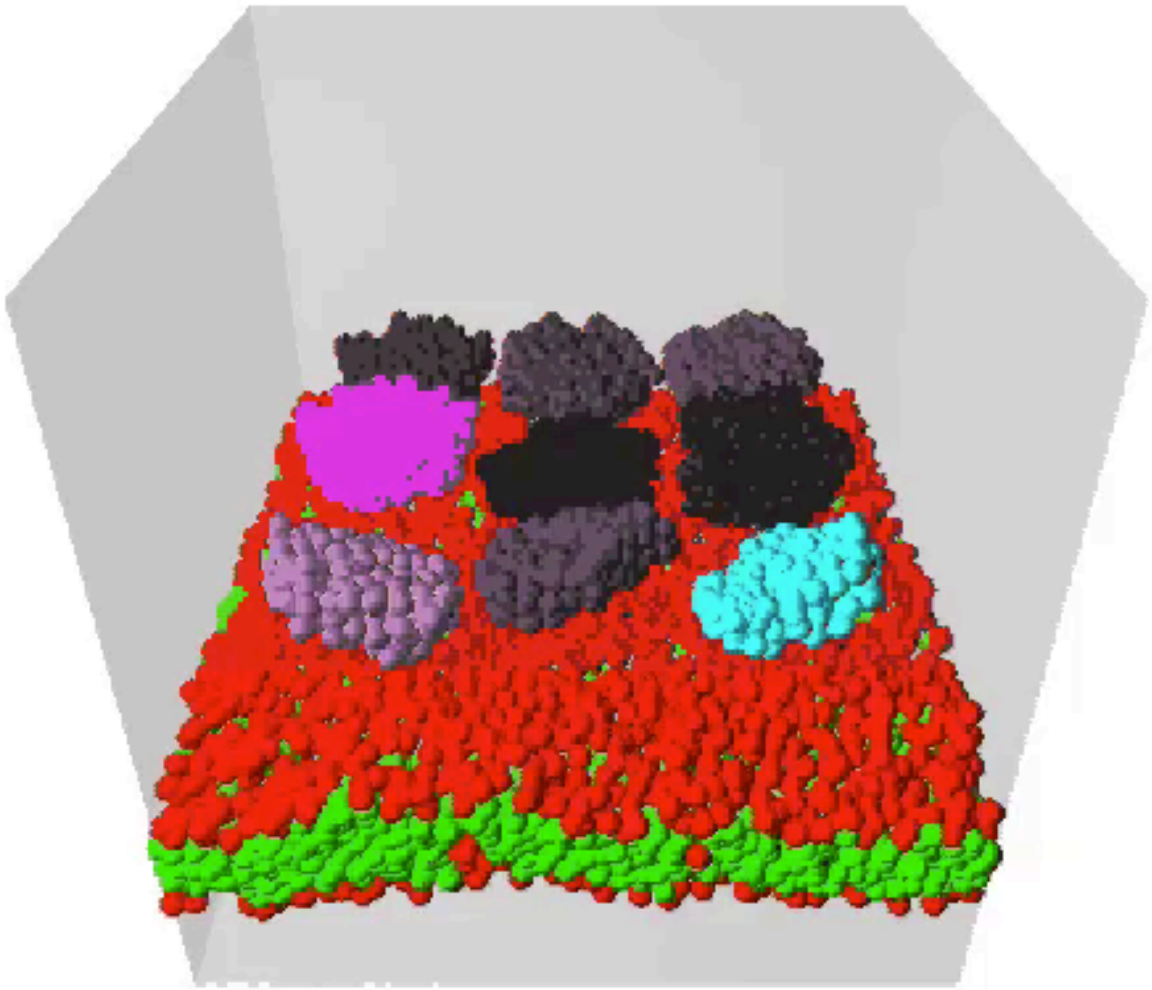
a, The Singer–Nicholson 'fluid mosaic model' (ref. 1). b, An amended and updated version.

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

It's important to remember who you meet



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

2008 - Curious effect in DPD simulations:
nanoparticles cluster in a line on a fluctuating membrane

2010 - Grant application combining expts. and sims. EU Innovative Training Network "Transpol"

2014 - Explanation and PhD
Weria Pezeshkian, Univ. Southern Denmark

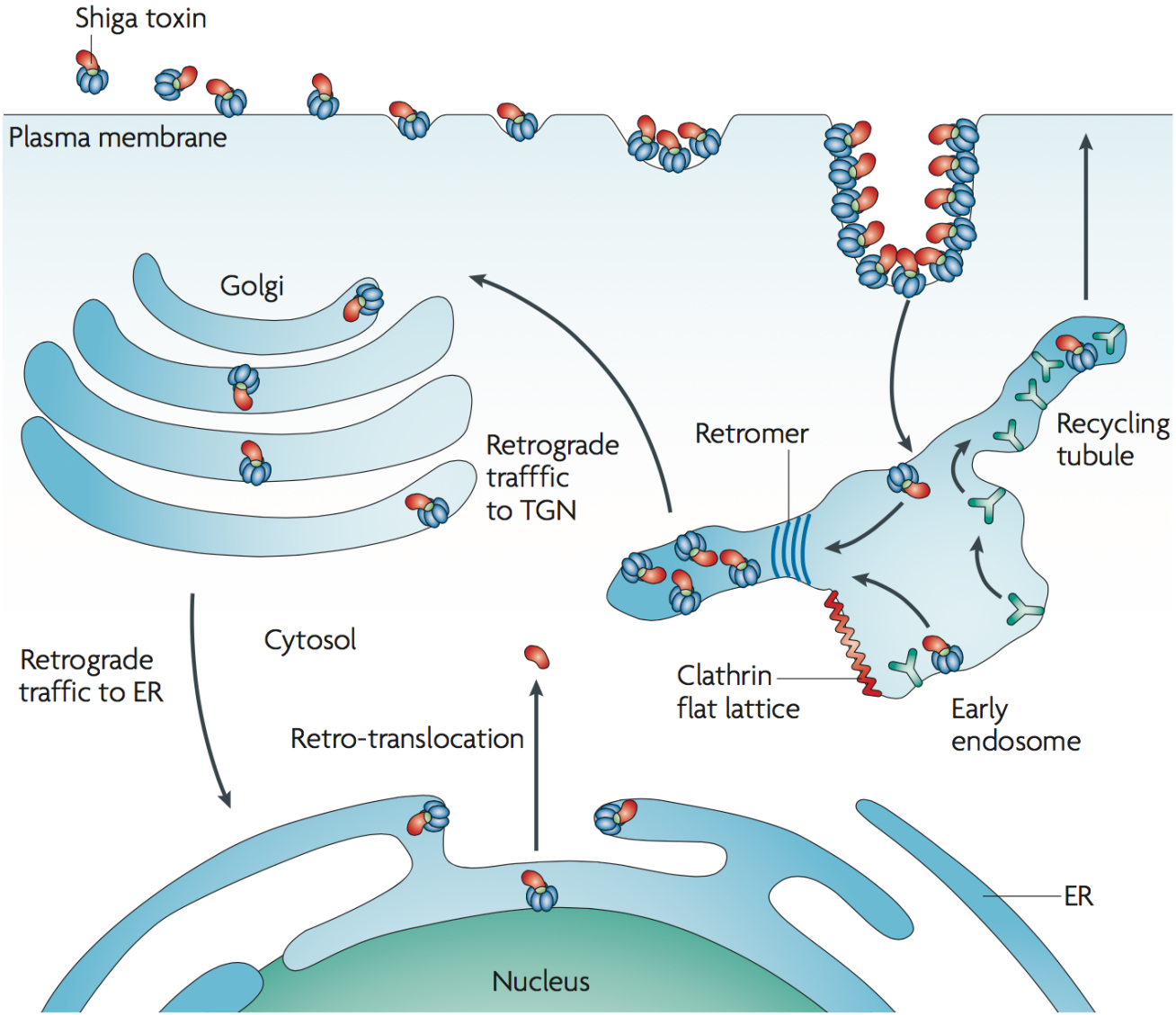
W. Pezeshkian et al. *ACS Nano* **11**:314 (2017)

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?



E. Coli and Shigella bacteria use a novel, non-CCP, endocytosis mechanism

L. Johannes Curie Inst.

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.

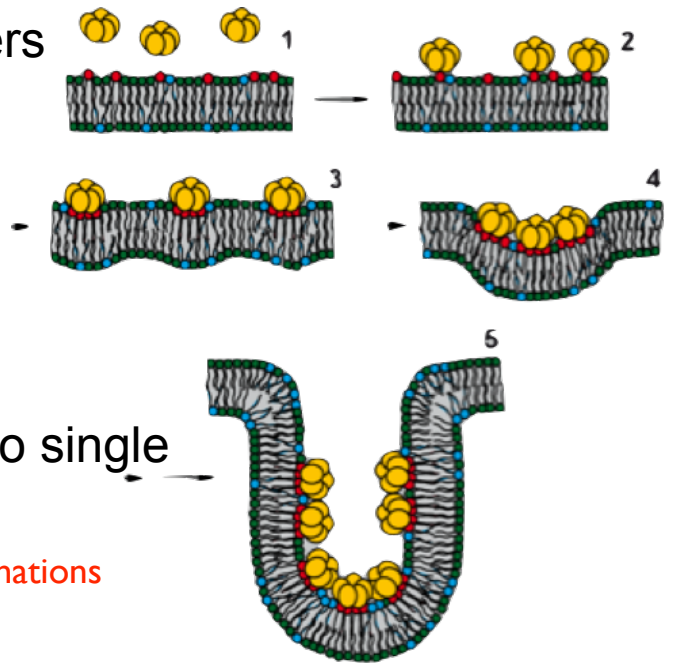
Shigella bacteria release small toxin particles (*shiga* toxin, ~ 7 nm) onto the plasma membrane that co-opt the cellular scission machinery to enter the cell and infect it. Each toxin particle can bind up to 15 Globotriaosylceramide (Gb3) lipids.

There are 3 stages to toxin entry into a cell:

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

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

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



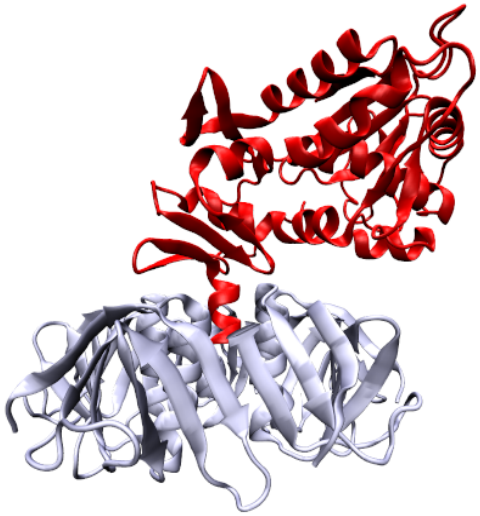
These stages occur on **different** length scales that mean no single simulation technique can follow the whole sequence.

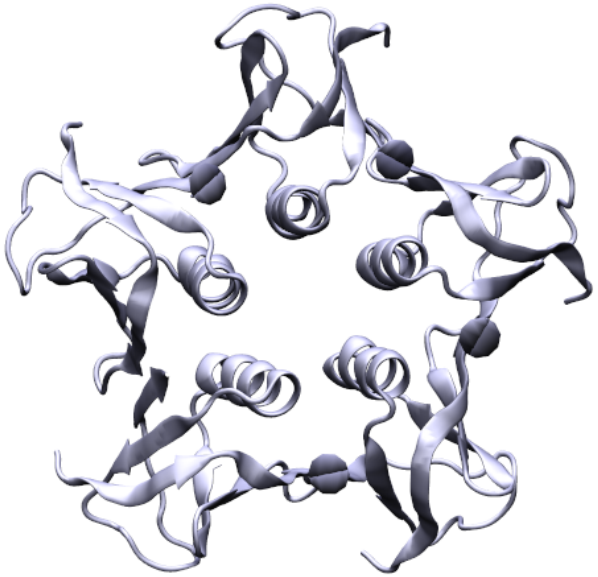
Römer et al. Shiga toxin induces tubular membrane invaginations for its uptake into cells, Nature 450:670 (2007)

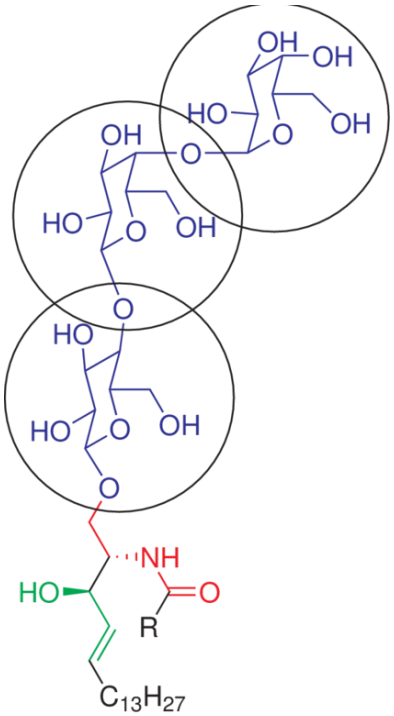
Shiga and shiga-like toxin

- 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 overexpressed by various human tumours.

A subunit







B subunit

B subunit

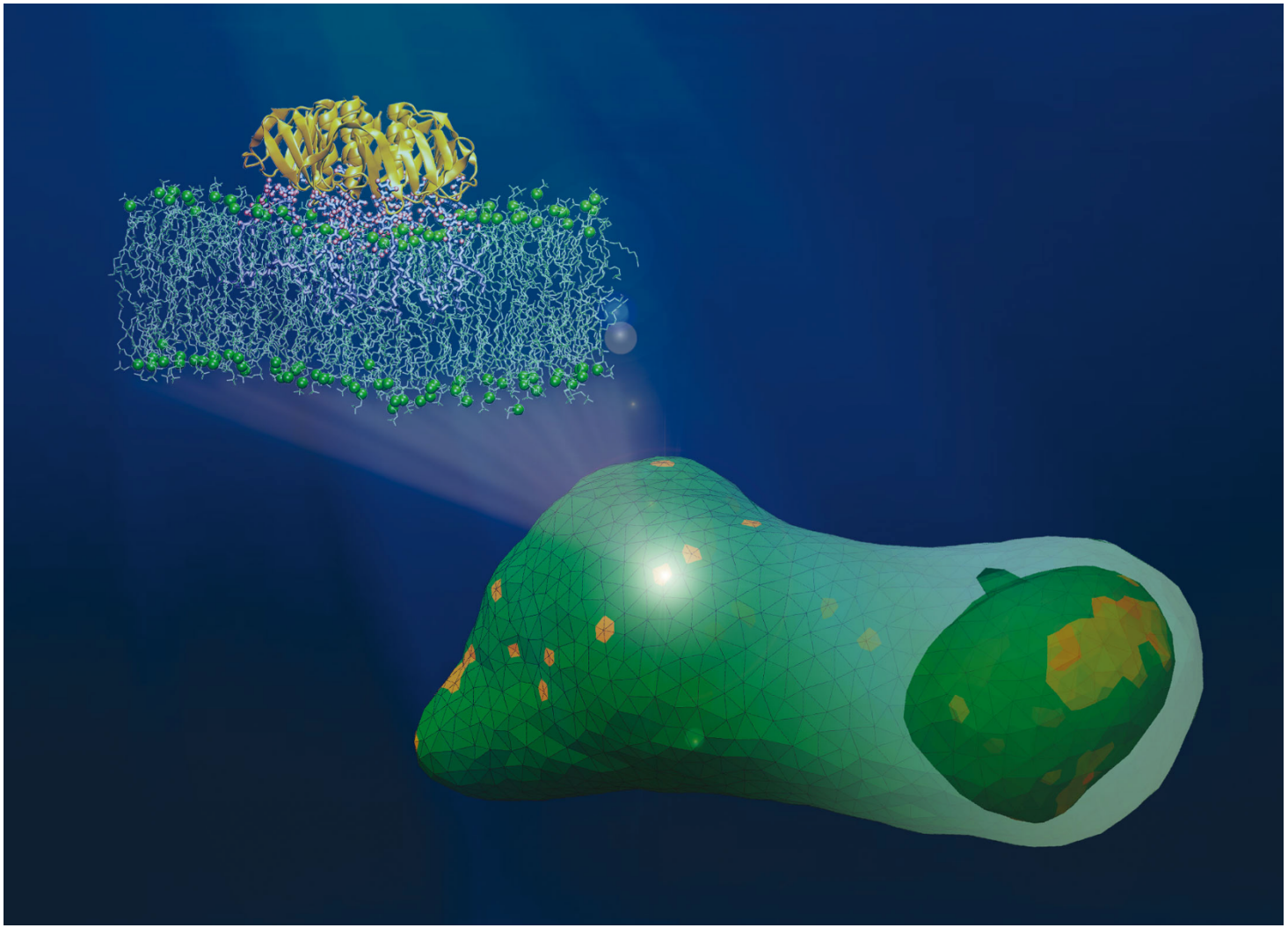
Gb3: STxB receptor

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)

Single simulation technique is not enough



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

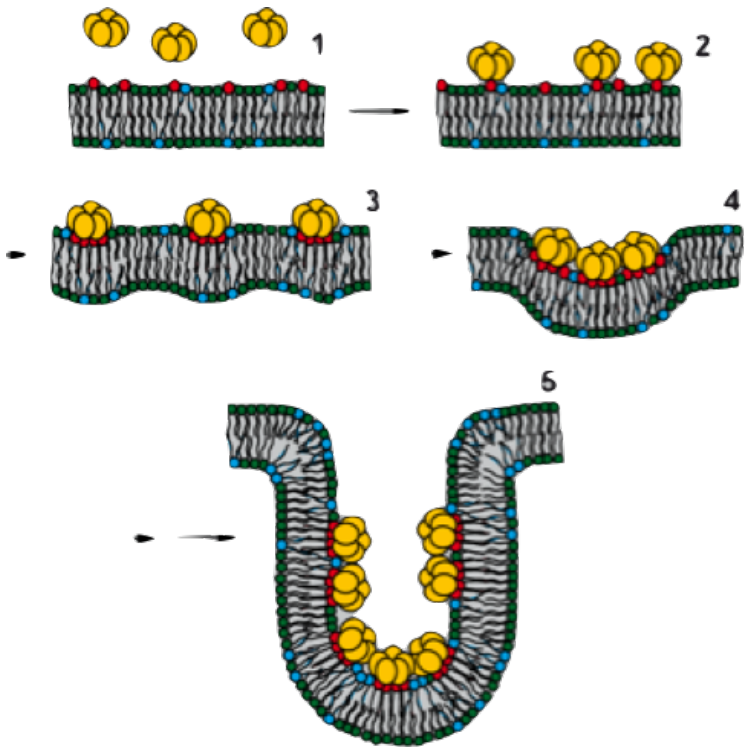
Modelling Shiga toxin entry to a cell

How these toxins enter the cell is a question that **cannot** be answered only by looking at dynamics on a single scale, we need a multi-scale technique.

1) Single toxin protein interacts with a membrane.
~ 7 nm, all atom molecular dynamics

2) Nano-domain formation and clustering.
~ 180 nm, dissipative particle dynamics

3) Membrane tubular invagination.
1 - 5 microns, Monte Carlo simulations/ triangulated network (only elastic/curvature properties of membrane are relevant once binding has occurred)

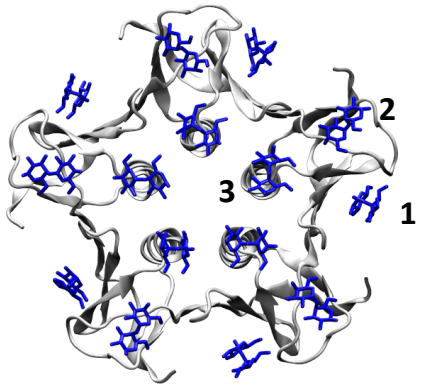


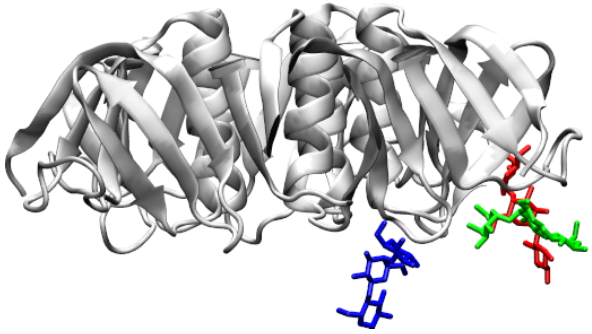
Weria Pezeshkian, PhD thesis, U. Southern Denmark 2015

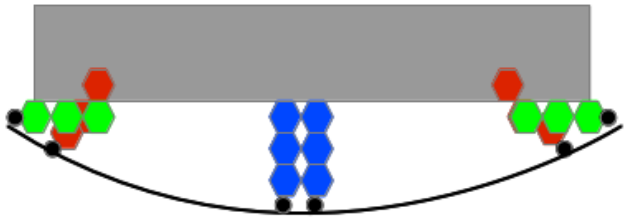
Step 1: STxB induces a small local membrane curvature

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

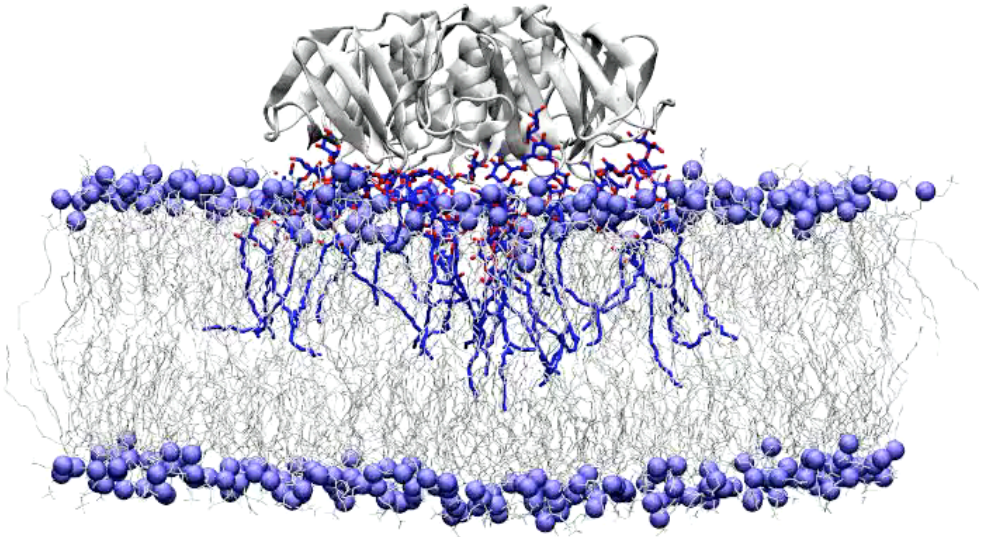
349 DOPC + 15 Gb3 + STxB
pre-bound to Gb3

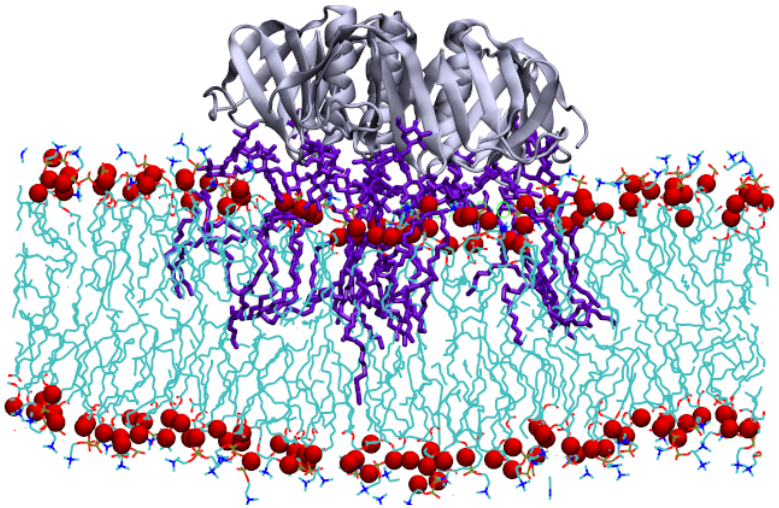






Angle of binding pockets creates curvature

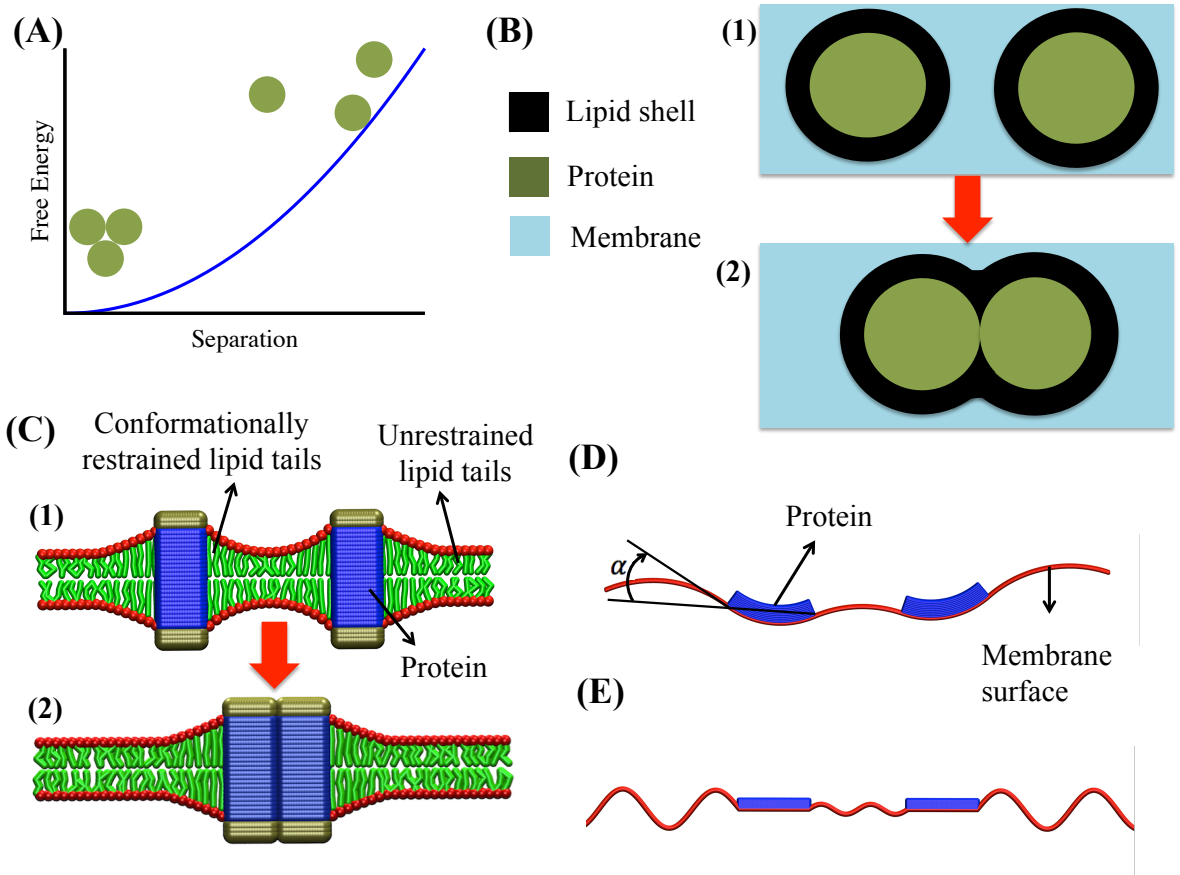




W. Pezeshkian et al. Soft Matter 11:1352-1361 (2015).

How might Shiga toxin perturb the membrane?

Unlike bare forces, membrane-mediated forces arise when two (or more) proteins/nanoparticles adsorb to/embed in a membrane and perturb its state.



Johannes et al. Trends Cell Biol. 2018

A) All operate by lowering the total free energy of membrane+proteins

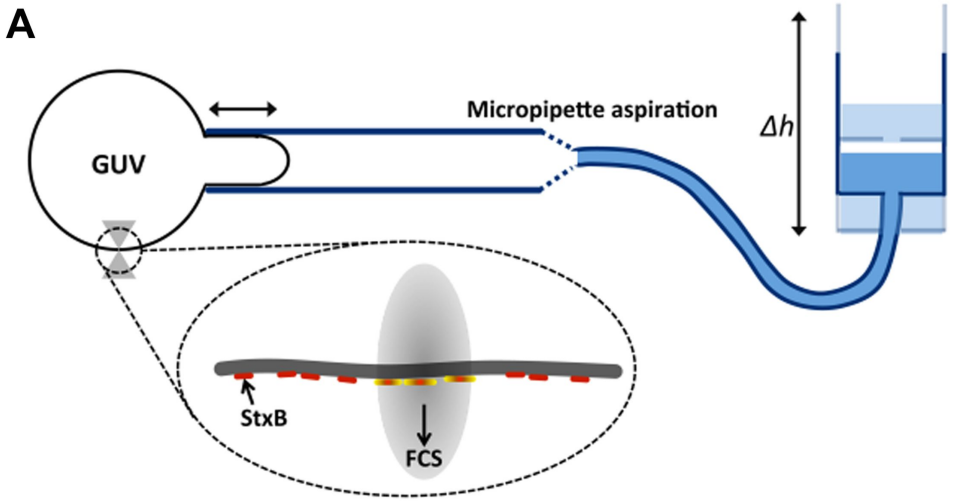
B) Capillary force/line tension

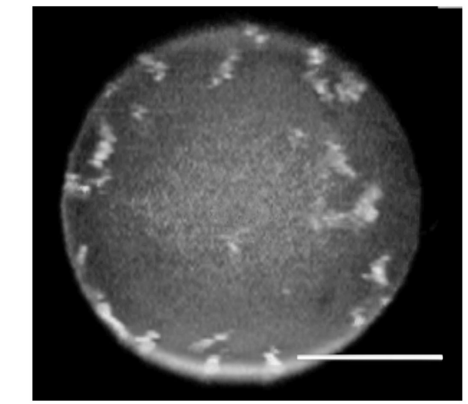
C) Depletion force

D) Curvature force

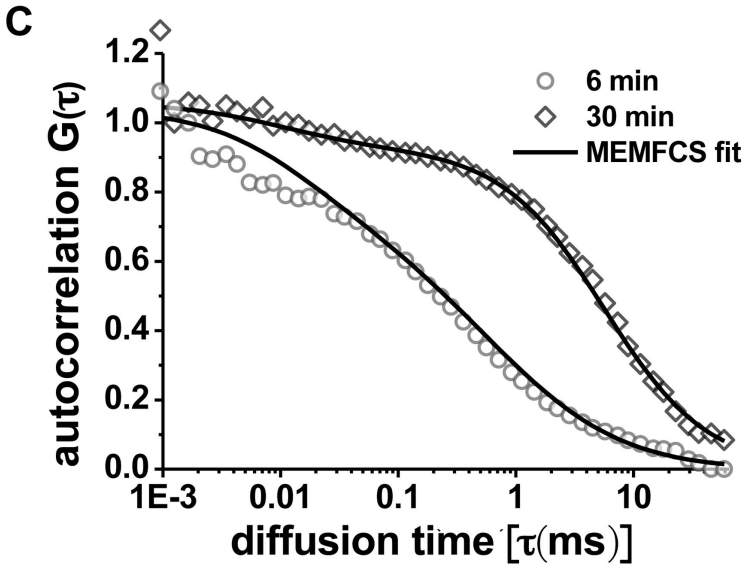
E) Fluctuation-induced force (Lecture 11)

FCS experiments confirm STxB cluster growth

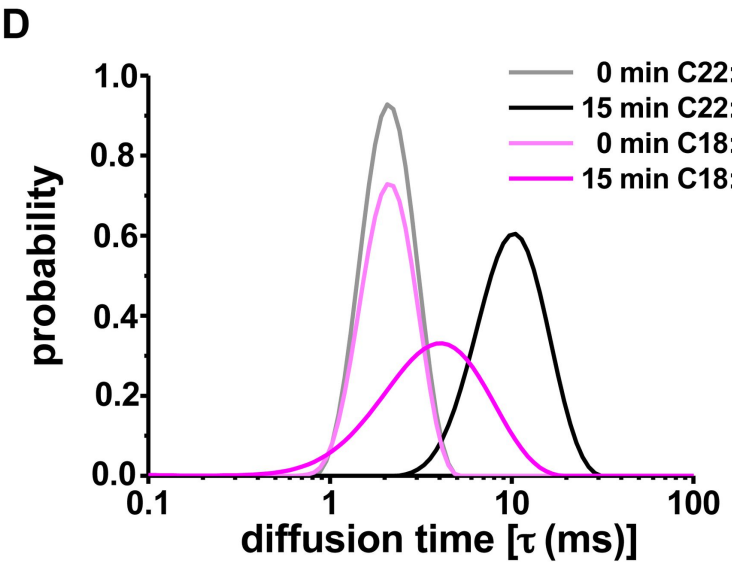




Composition: 70% DOPC + 30% Gb3



Diffusion time increases implying cluster size increases over time



Gb3 tail length matched to DOPC

Senthil Arumugan, Curie Inst.

Potential drivers of STxB clustering

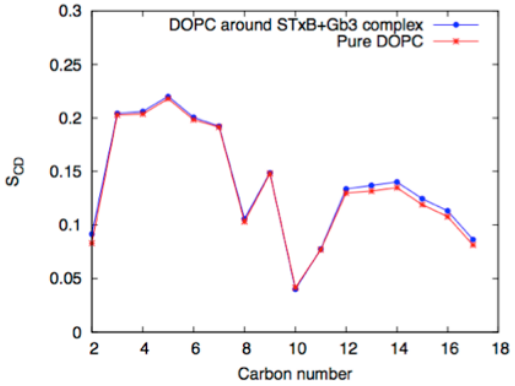
1) Line tension effects?

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

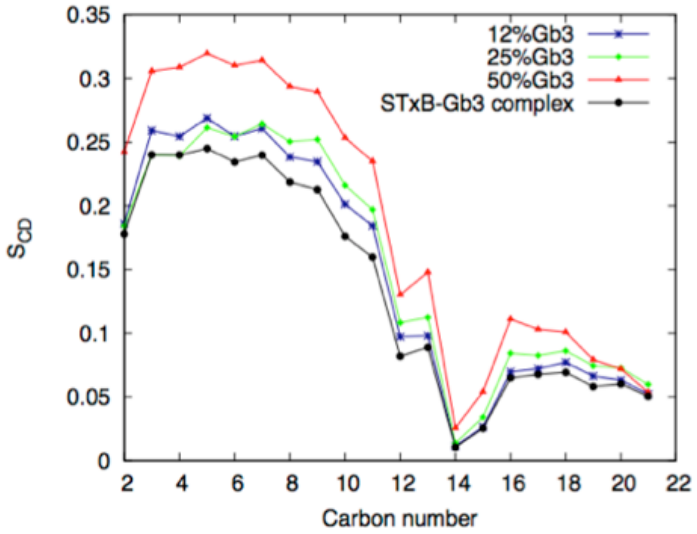
compositional mismatch - STxB clusters on 70% DOPC + 30% Gb3

(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:

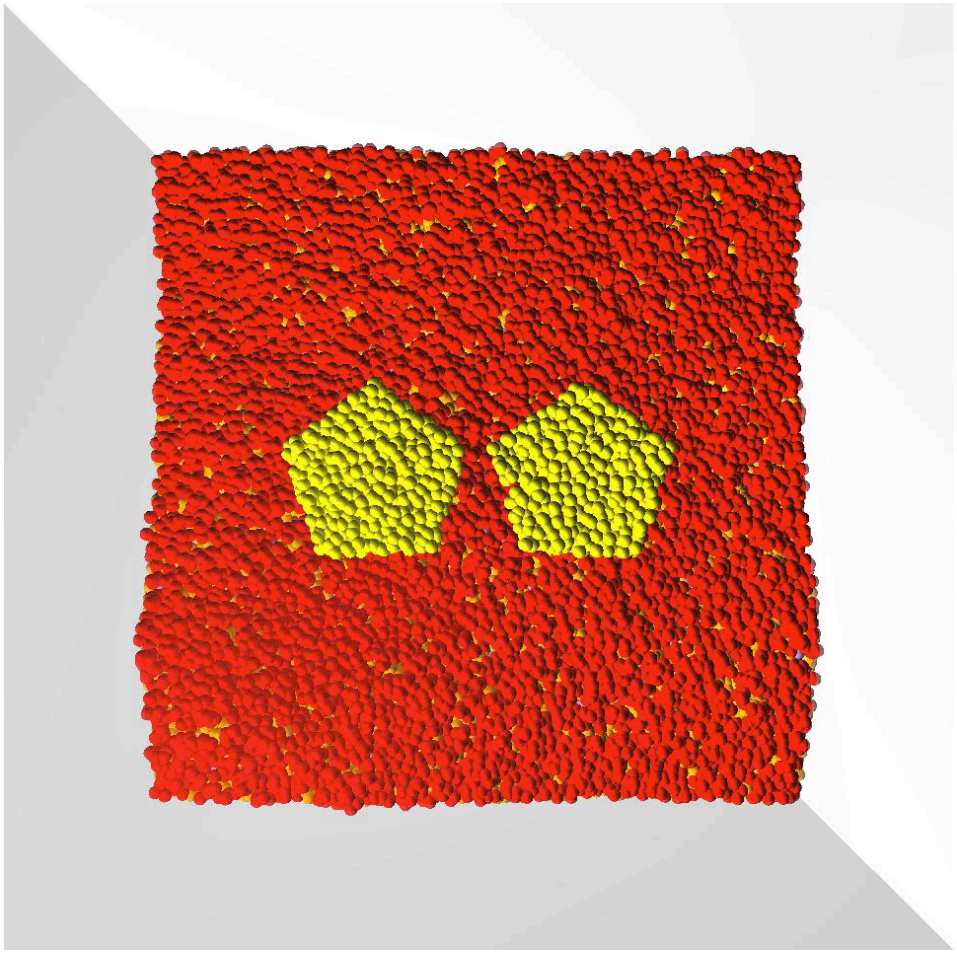
Reynwar and Deserno, Soft Matter 7:8567-8575 (2011)

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.

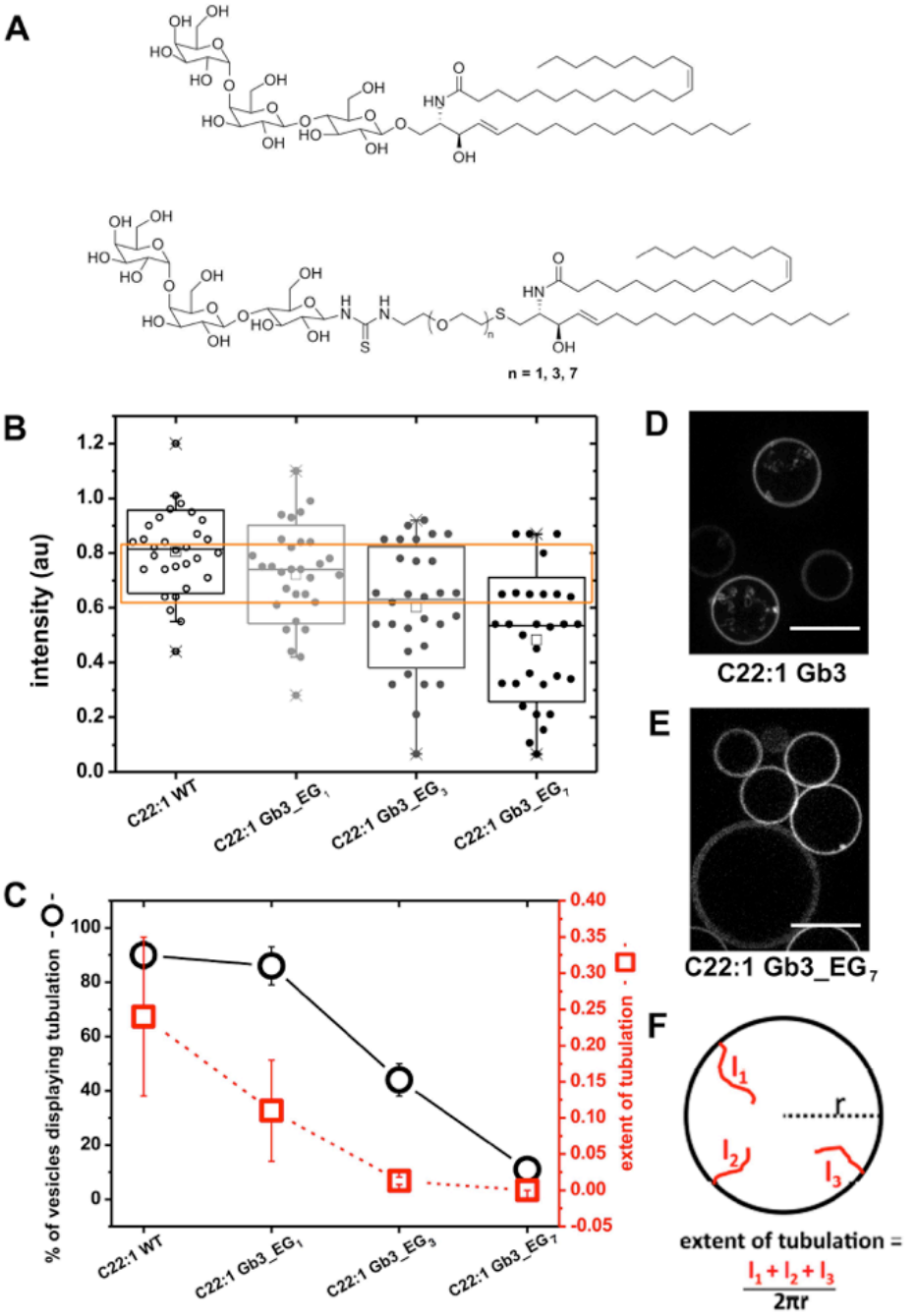
(Weria Pezeshkian, John Ipsen, SDU; Julian Shillcock, EPFL)

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.)

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.)

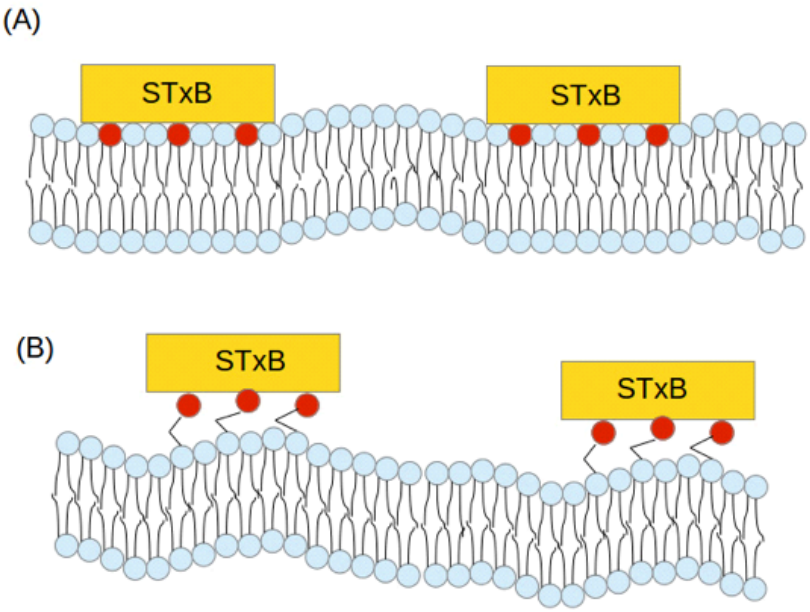


A thermal Casimir force may drive clustering

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

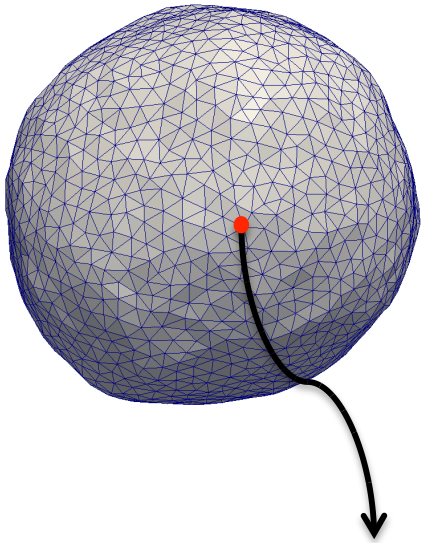


$V(H) \sim -k_B T (a/H)^{1/2}$ for disks of radius a (Lin. et al. PRL 107:228104 (2011))

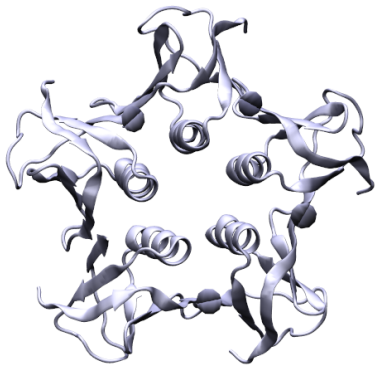
$V(H) \sim -k_B T (L/H)$ for pentagons of size L (Pezeshkian et al. ACS Nano 11:314-324 (2017))

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

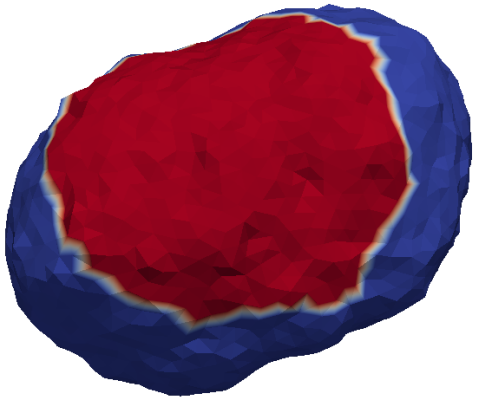


D≈150 nm
A≈0.3µm²

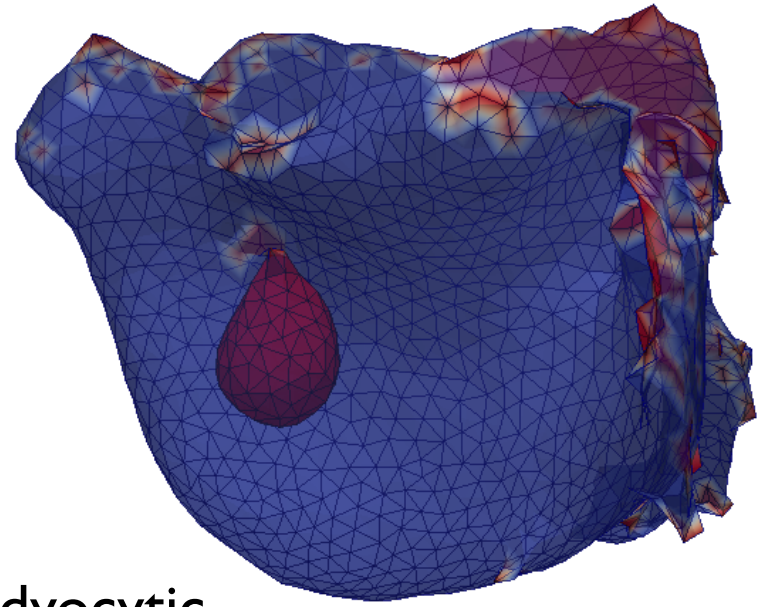


Monte Carlo simulations of a triangulated surface vesicle

Protein bound area
of membrane surface (red)

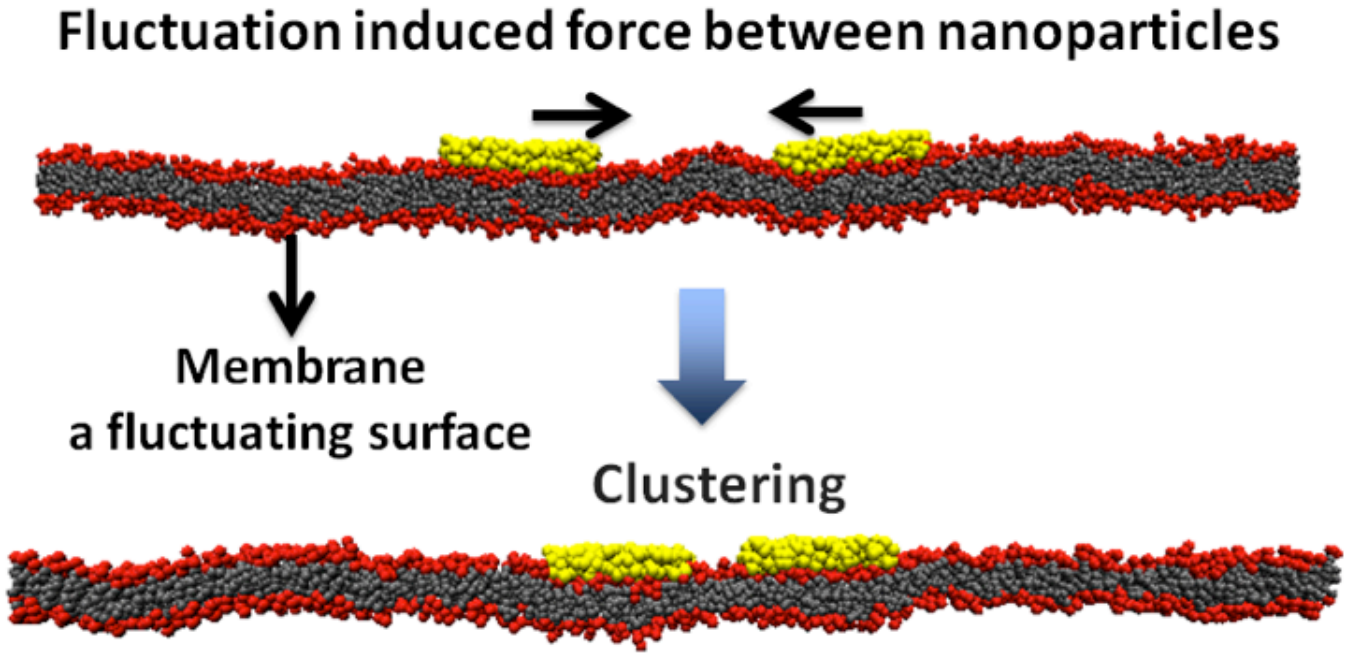


Tubular invagination inside cell



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

Once the clusters have curved inwards, cellular endyocytic machinery takes over and tubule is pinched off.



3 levels of *simulation*: each level focuses on different scales in the problem:

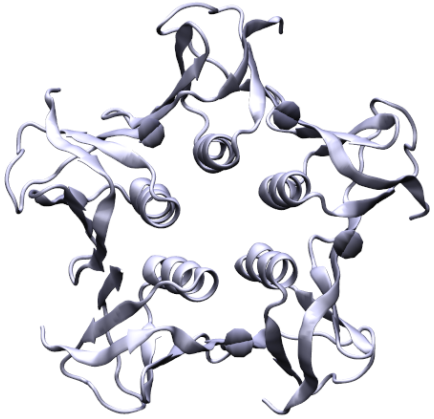
aaMD focuses on tight binding of Gb3 lipids, predicts the small induced curvature

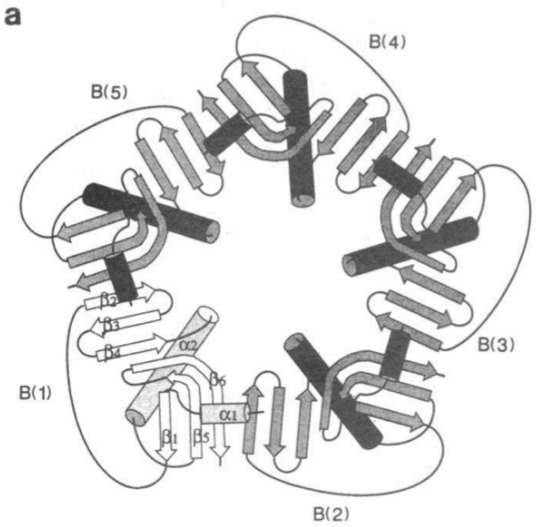
DPD treats toxin as a rigid pentagon, ignores atoms, emphasise membrane fluctuations

MC treats whole cell as an infinitely thin, flexible 2d surface

Q. Why is STxB a pentamer?

5 mins.





and Cholera toxin?

and enterotoxin, pertussis toxin, etc.?

The B-subunits of cholera and Shiga toxins are functionally and structurally related proteins with different chain lengths and no sequence similarity. They are responsible for toxin binding to specific glycosphingolipid receptors and

Pines and Johannes, Toxicon 45:389-393 (2005)

Acknowledgements

Weria Pezeshkian, John Ipsen
University of Southern Denmark - http://www.sdu.dk

Ludger Johannes and colleagues
Curie Institute, France - http://www.institut-curie.org

Funding and Support

European Community's Seventh Framework Programme (FP7/2007-2013) - TRANSPOL-264399
Agence Nationale pour la Recherche (ANR-09-BLAN-283, ANR-11 BSV2 014 03)
Human Frontier Science Program grant RGP0029-2014
ERC Advanced Grant 340485
Fondation ARC pour la Recherche sur le Cancer
Fondation Pierres-Giles de Gennes
DeiC National HPC Centre, University of Southern Denmark.

Summary

- Nature uses all *mechanisms* at her disposal: not just the methods we might expect: genes = protein machines, membrane = barrier

  e.g., Hydrophobic effect leads to membranes as barriers, but membranes then create new forces that act on nearby or transmembrane proteins

- Bacteria use mechanical properties - *Shiga toxin rigidity + non-covalent binding to Gb3 lipids + membrane curvature* - to infect a cell

- Shiga toxin clustering is not driven by ***positive*** attraction between the particles but by a ***suppression*** of membrane fluctuations leading to an effective attraction.

What is important? What is ignorable?

energy, entropy, phase, shape, flexibility, barrier, fluctuations, …

detailed chemistry, initial conditions, diffusion, …

Break

10 mins.

2 Questions

1) Are there other courses similar to this one in combining long simulations with theoretical concepts in Life Sciences?

2) Do your other Master's courses have many more students?

Free books


Bhushan Editor
Springer Handbook of Nanotechnology
Monticelli · Salonen Eds.
Biomolecular Simulations
BetaSys
Nanostructured Soft Matter
ICIAM 07
Butt · Graf · Kappl
Physics and Chemistry of Interfaces
PTASHNE
A GENETIC SWITCH
Phage Lambda Revisited
Soft Machines
Soft Condensed Matter
Spiking Neuron Models
SOFT CONDENSED MATTER PHYSICS IN MOLECULAR AND CELL BIOLOGY
UNDERSTANDING MOLECULAR SIMULATION
Frenkel
Smit
Rapaport
THE ART OF MOLECULAR DYNAMICS SIMULATION
Computer Simulation of Liquids
HAILE
MOLECULAR DYNAMICS SIMULATION
Elementary Methods
Knowledge is Beautiful
Understanding Non-equilibrium Thermodynamics
Flory · Statistical Mechanics of Chain Molecules
The Theory of Polymer Dynamics
Time Series
Gardiner
Handbook of Stochastic Methods
Risken
The Fokker-Planck Equation
GRIMMETT and STIRZAKER
Probability and Random Processes
Problems and Solutions
Modern C++ Programming with Test-Driven Development
Langr
The Joy of Patterns
STL Tutorial and Reference Guide, Second Edition
Musser Derge Saini
Design Patterns
Gamma · Helm Johnson · Vlissides
Effective C++ Second Edition
Meyers
Effective C++ Third Edition
More Effective C++
WRITE PORTABLE CODE
STROUSTRUP
The Design and Evolution of C++
Standard C++ IOStreams and Locales
Langer Kreft
C++ Templates
Vandevoorde · Josuttis
TCL/TK TOOLS
Mark Harrison
O'REILLY
Secure Programming Cookbook for C and C++
The VISUALIZATION TOOLKIT

Exercise

1) Homework 2 is a precis of your project goals, and any results so far. Also include any potential problems or constraints due to simulation time or numbers, so we can discuss it.

2) In writing your precis, take into account that part of the project is to become familiar with estimating what you are able to simulate, and what questions you can answer in the time available, e.g., # of parameters to vary, # of simulations, system size, time required to run the simulations, accuracy to get significant results.

3) In the final report, creativity in the project is more important than getting very precise results IF you can state the sources of error.

4) Include your thoughts and comments about what you have done, what you could improve, etc