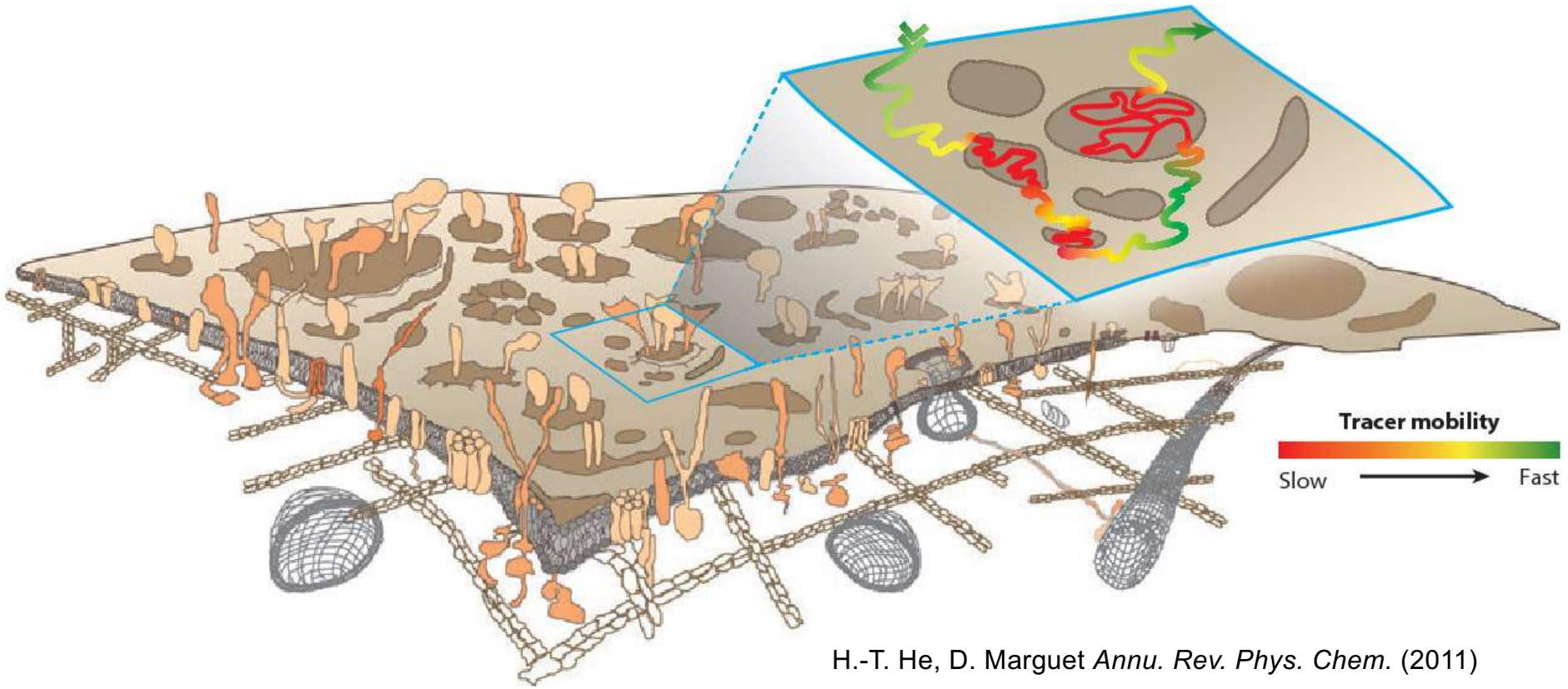
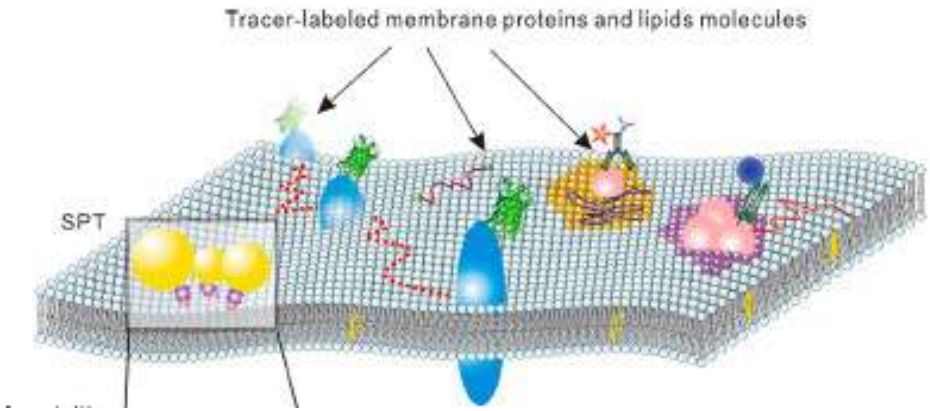


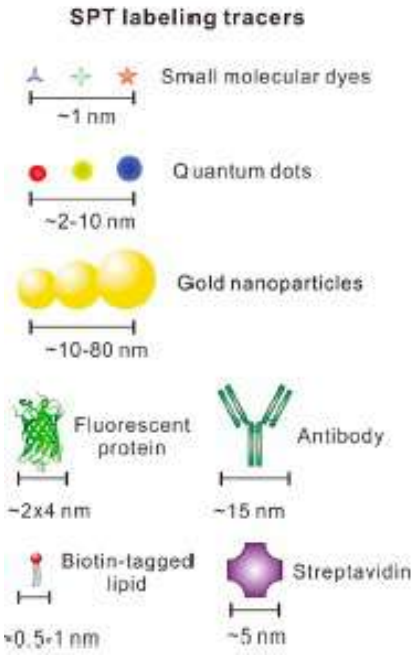
H.-T. He, D. Marguet *Annu. Rev. Phys. Chem.* (2011)

3. Protein Clustering on Membranes

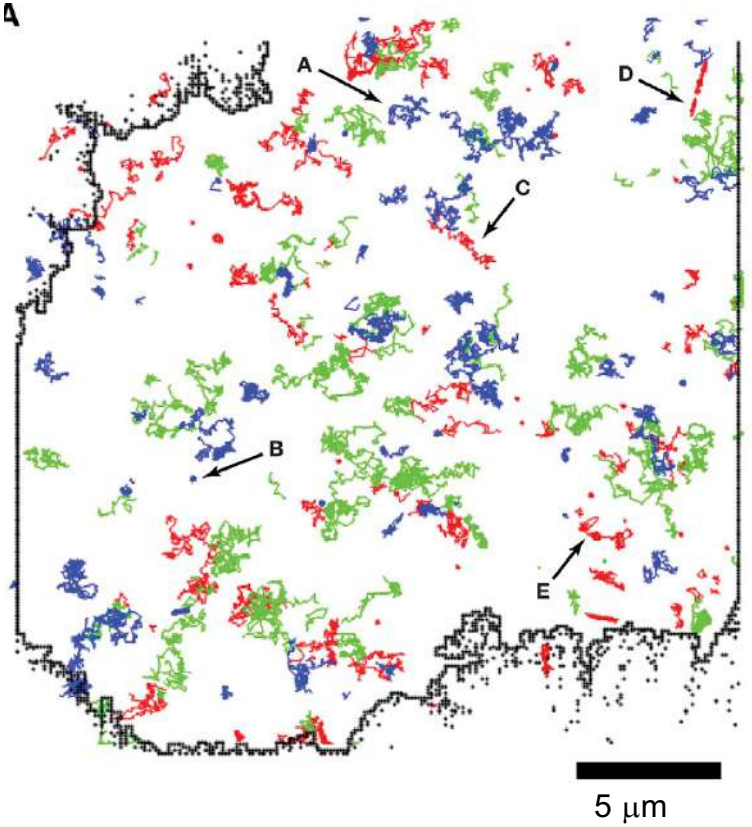
Heterogeneous Diffusion in the Plasma Membrane

Single Particle Tracking at the PM





Y. Yu, M. Li, Y. Yu *ACS Nano* (2019)



K. Jacobson et al *Cell* (2019)

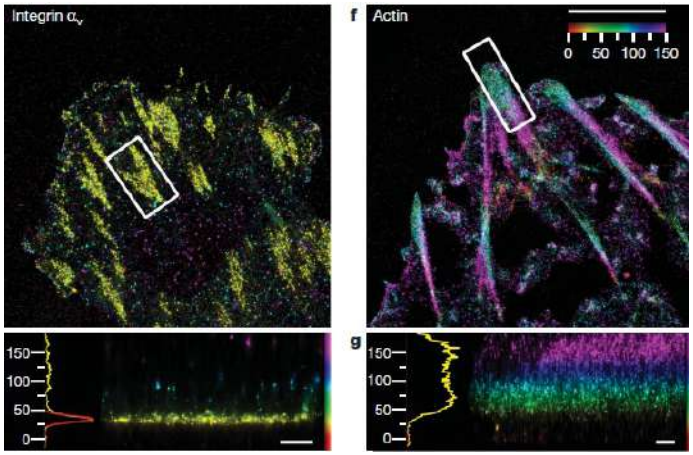
Heterogeneous mobility

But, difficult to determine if lipid or protein nano-domains

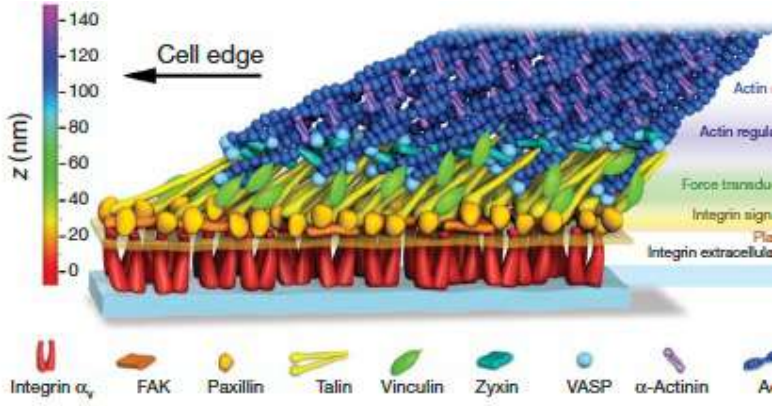
In Living Cells, *no Macroscopic* Lipid or Protein Domains in General

Except special cases, nanodomains *below optical resolution (<300 nm)*

- Focal adhesion (cell adhering)

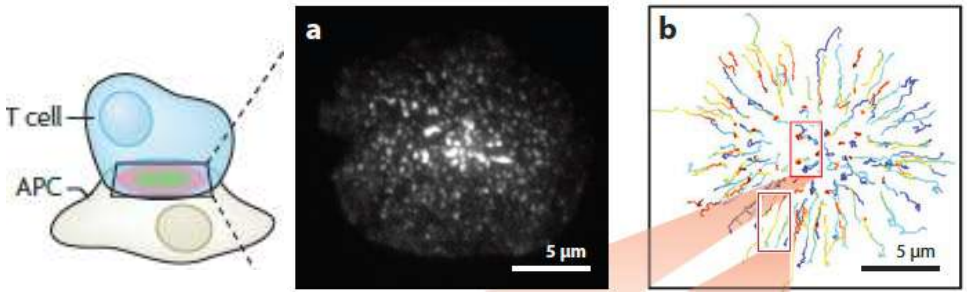


500 nm



P. Kanchanawong…. C. Waterman *Nature* (2010)

- Immune synapse



T-cell interacting with a bilayer containing T-cell receptors (fluo)

M.L. Dustin, J.T. Groves *Annu. Rev. Biophys.* (2012)

With super resolution microscopy (optics, CryoEM)

or sophisticated technics (high-speed AFM, FRET etc..)

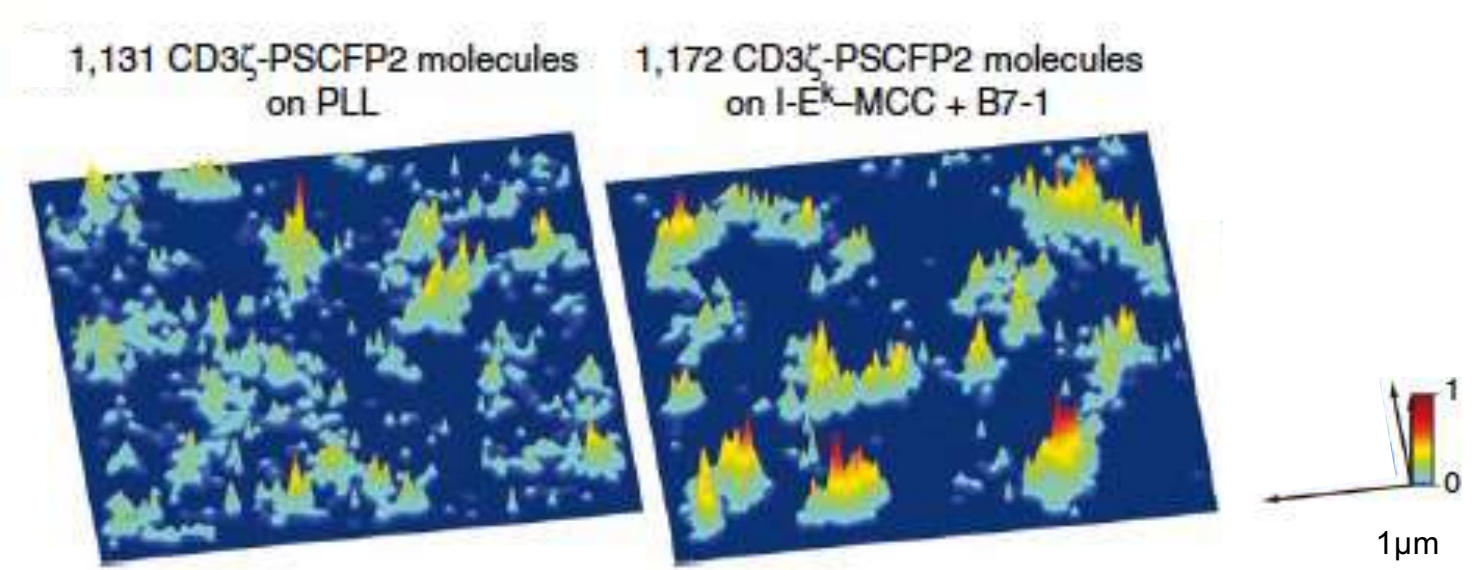
Small protein clusters in cell membranes

(<100 nm, some time also dimers, trimers…)

Membrane Protein Clustering

- Membrane Receptors

In native T-cells membranes



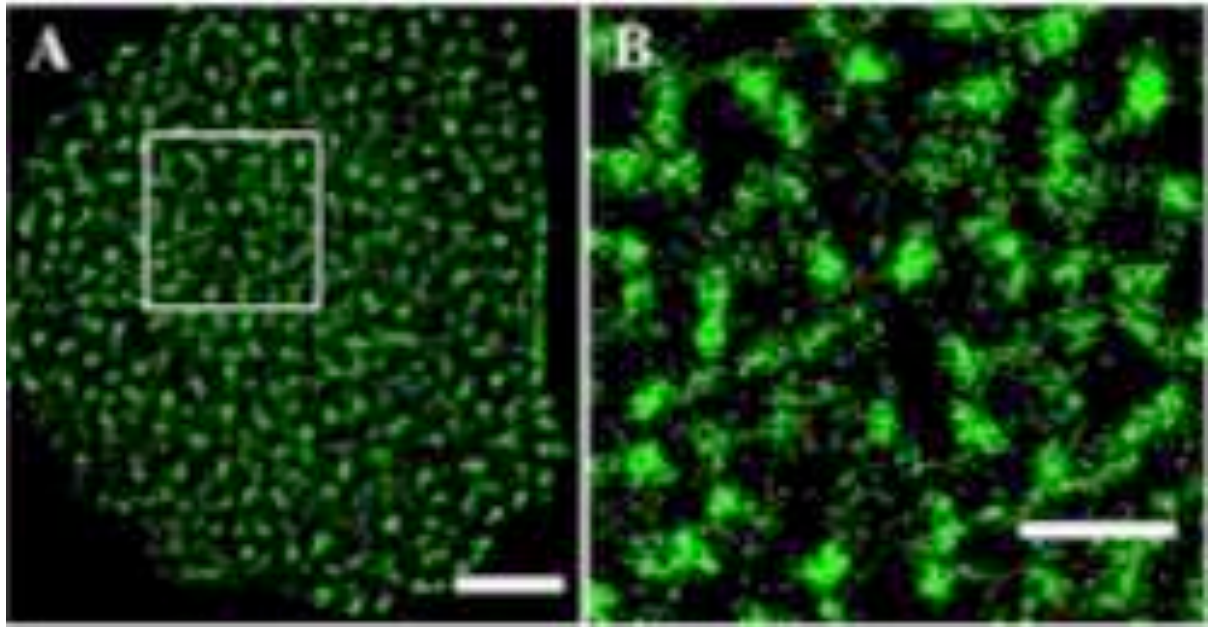
Non activated Activated

B.F. Lillemeier et al. *Nature Immun.* (2010)

Membrane Protein Clustering

- Glucose Transporter
  GLUT1

dSTORM

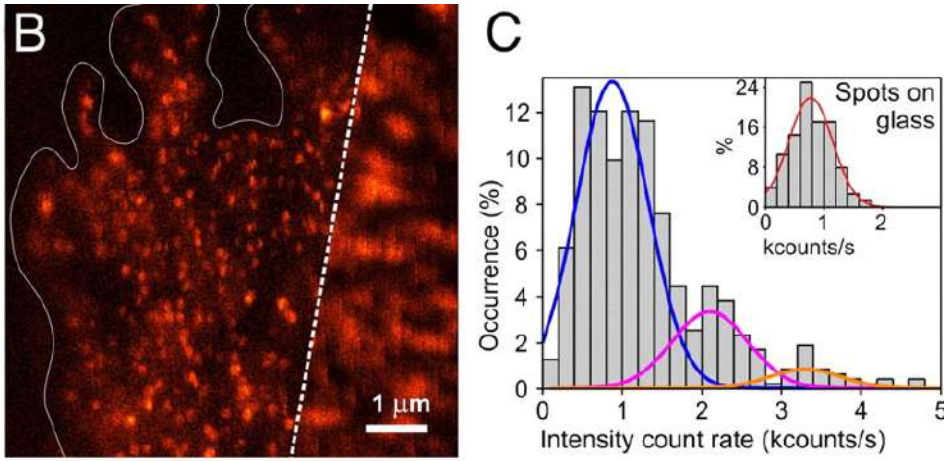


5 μm 2 μm

Yan Q…**Wang H.** *PNAS* (2018)

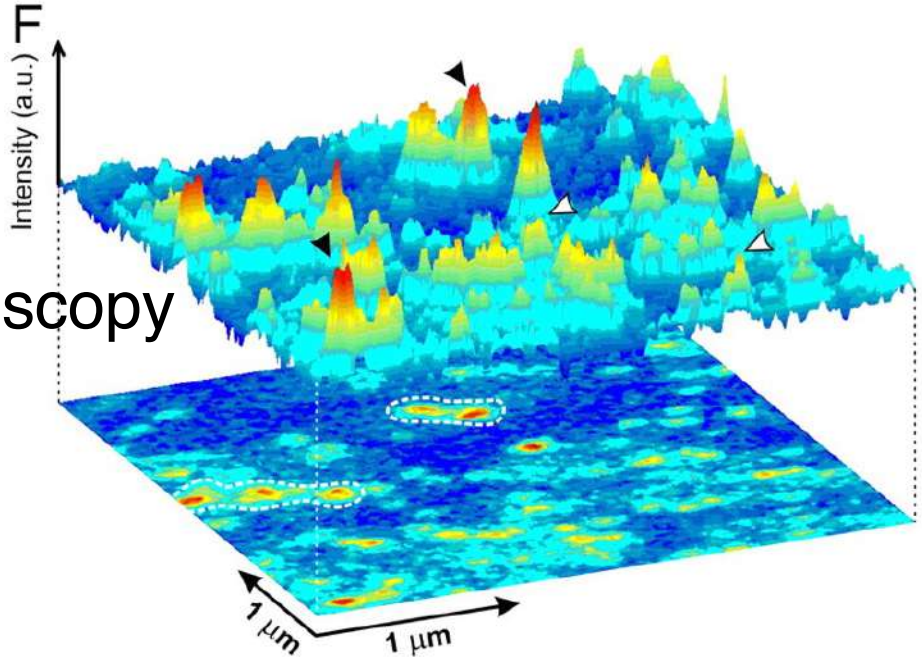
Membrane Protein Clustering

- GPI-anchored proteins



Single-molecule

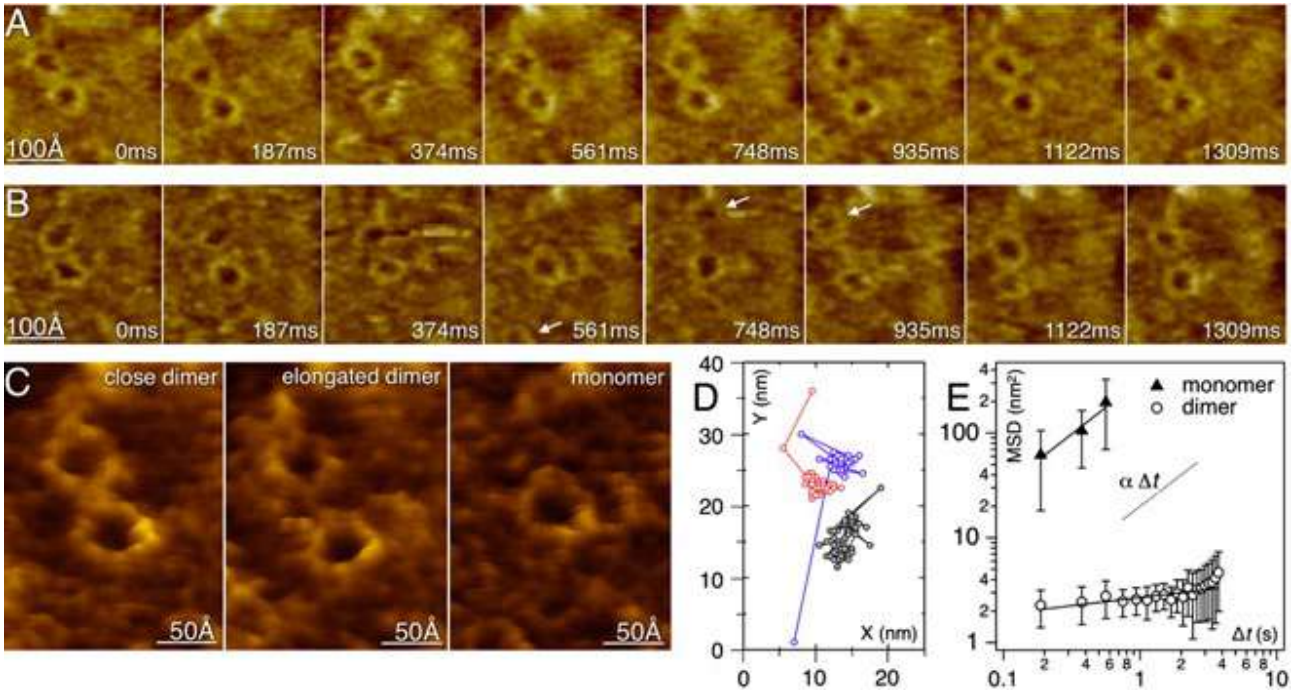
Near-field Optical Microscopy

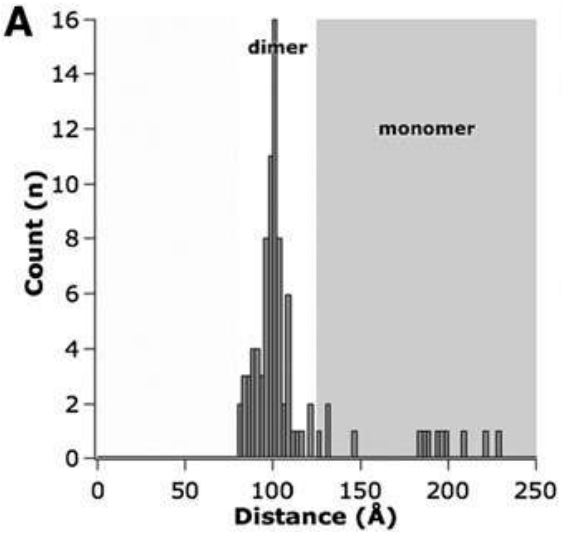


Van Zanten T. S., …..**Garcia-Parajo M. F**. *PNAS* (2009)

Membrane Protein Clustering

Direct visualisation of dimer formation





High-speed AFM

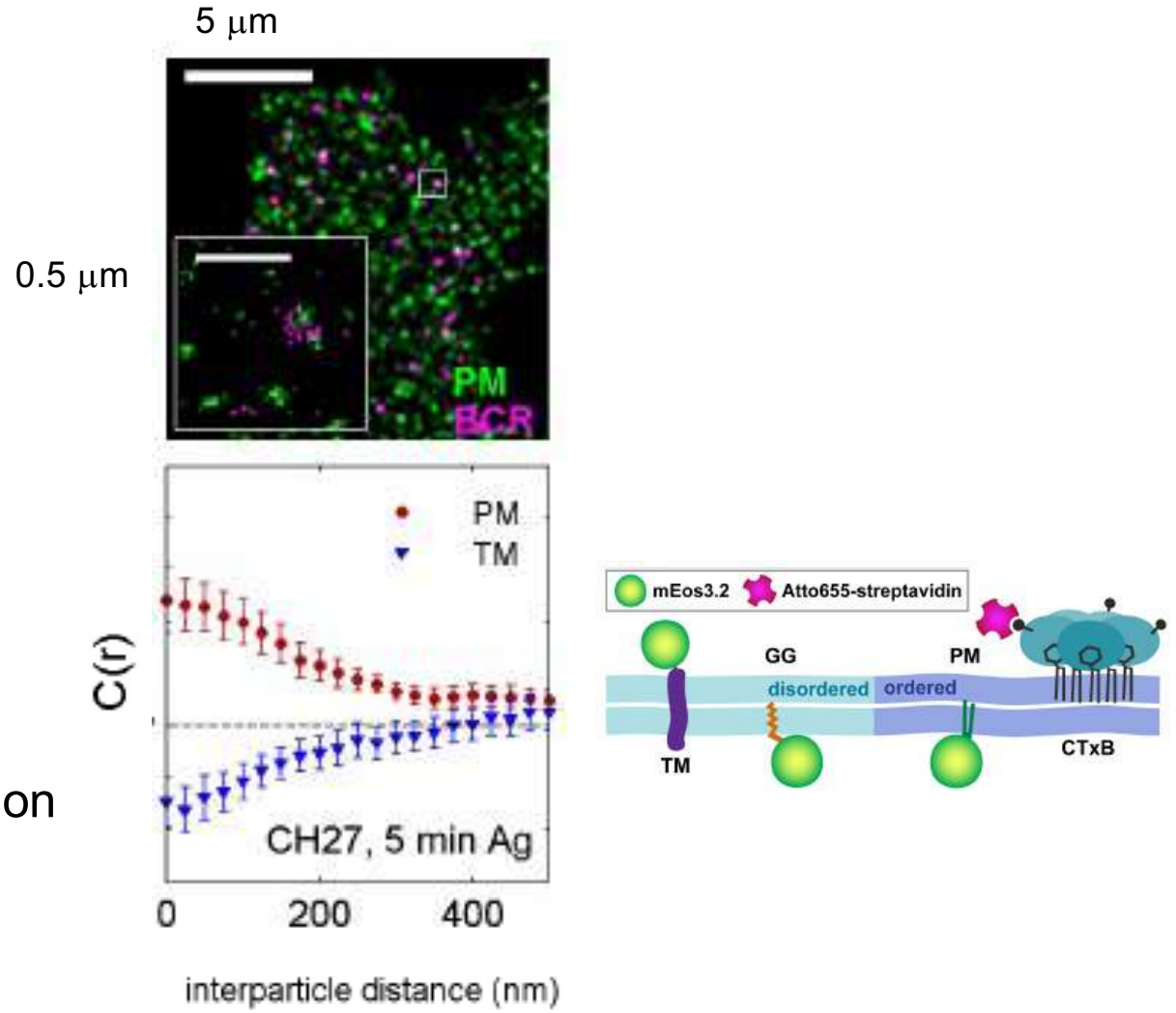
C-rings of the ATP-synthase moving in a supported bilayer

I. Casuso… S. Scheuring *Biophys. J.* (2010)

Role of Rafts in Receptor Clustering

B Cell Receptor
BCR

Super Resolution
Microscopy



Stone M. B… **Veatch S. L**. *Elife* (2017)

Which interactions can lead to protein clustering?

- Many theoretical work on protein on or embedded in membranes (inclusions)

  Much less in vitro work, or evidence in cells (recent)

- "Colloidal interactions" : electrostatic, van der Waals (attractive)

  More specific: Hydrophobic, hydrogen bonds

  Entropic repulsions between lipid bilayers (Cf. Helfrich)

- But, with fluid 2D membrane:

  membrane-mediated interactions between proteins

  Due to membrane elastic properties

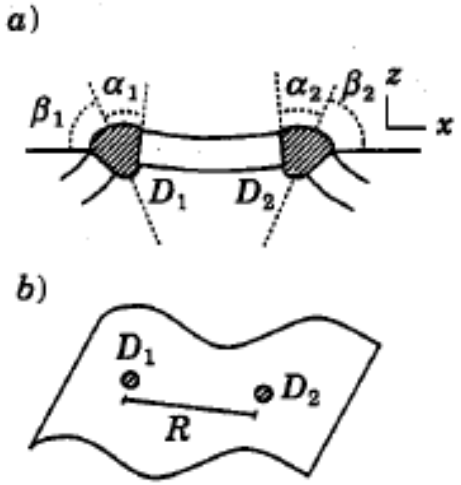
Long-Range Interactions

EUROPHYSICS LETTERS 10 April 1993

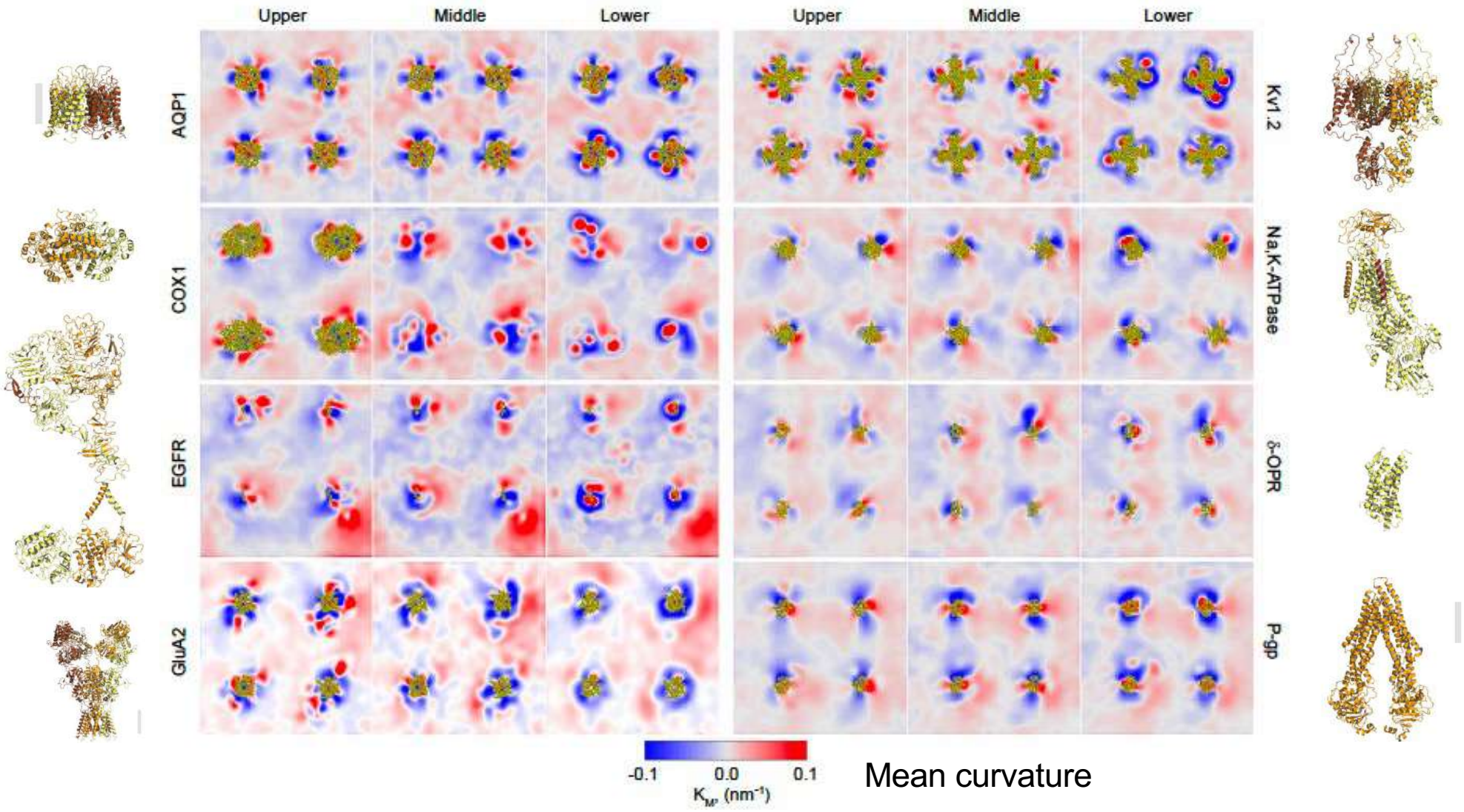
Europhys. Lett., **22** (2), pp. 145-150 (1993)

Long-Range Forces in Heterogeneous Fluid Membranes.

M. GOULIAN (*), R. BRUINSMA (**) (§) and P. PINCUS (*) (**)



Local Membrane Bending: "Fingerprint" of Membrane Proteins



V. Corradi….**D. P. Tieleman** *ACS Cent. Sci.* (2018)

Long-Range Interactions

EUROPHYSICS LETTERS 10 April 1993

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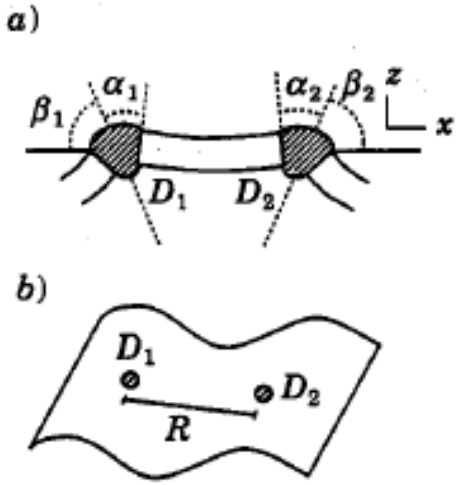


$$V^T(R) = \frac{\kappa_B T}{4\pi^2 \kappa_0^2 R^4} \int_{D_1} d^2r\, \delta\kappa(r) \int_{D_2} d^2r\, \delta\bar{\kappa}(r) +$$

Long range interaction

If R>>A $\quad V(R) = \frac{2}{\pi}(2\kappa + \bar{\kappa})(\alpha_1^2 + \alpha_2^2)\frac{A^2}{R^4}$, $(2\kappa + \bar{\kappa}) > 0$

Always repulsive

M. Goulian *Curr. Opin. Colloid Interf.* (1996)

Many follow up theoretical papers and simulations….

- A new type of interaction, for very stiff proteins that *perturb membrane fluctuations (Casimir-like)*

M. Goulian et al. *Europhys. Lett.* (1993)

$$V(R) = -6k_BTA^2\frac{1}{R^4}$$

Always attractive

Independent of membrane elastic constants

Significant at R ~ a few protein diameter

Fluctuations: peristaltic thickness fluctuations, local protrusion modes, lipid density fluctuations, and fluctuations of lipid tilt

See L. Johannes… J. C. Shillcock. *Trends Cell Biol.* (2018)

A.-F. Bitbol…J.B. Fournier in
Physics of Biological Membranes (2018)

- Curvature-mediated force

$$V_c(R) = \frac{2}{\pi}(2\kappa + \bar{\kappa})(\alpha_1^2 + \alpha_2^2)\frac{A^2}{R^4}$$

For vesicles that do not change topology, the $\bar{\kappa}$ term can be omitted

For identical inclusions:

$$V_c(R) = \frac{8}{\pi}\kappa\alpha^2 \frac{A^2}{R^4}$$

- Fluctuation-mediated force

$$V_f(R) = -6k_BTA^2\frac{1}{R^4}$$

$$V_c(R) = |V_f(R)| \longrightarrow \alpha = \sqrt{\frac{3}{4\pi}\frac{k_BT}{\kappa}}$$

$$\kappa \approx 25\, k_B\mathrm{T} \qquad \alpha \approx 6°$$

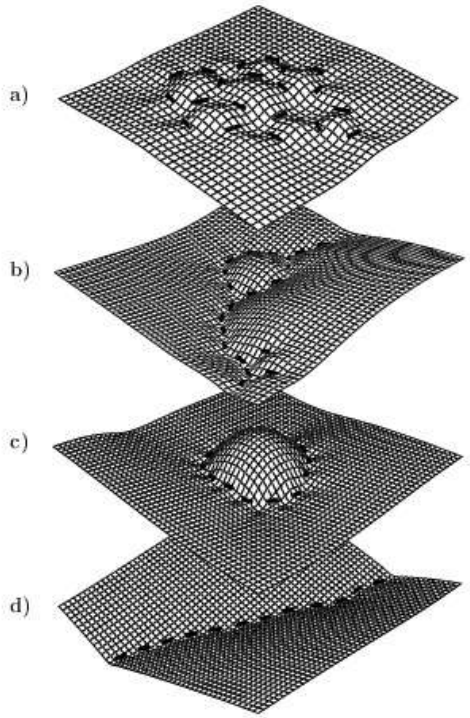
If *large* contact angles α, *curvature* forces dominate
For *quasi-flat* inclusions, *fluctuation* forces dominate

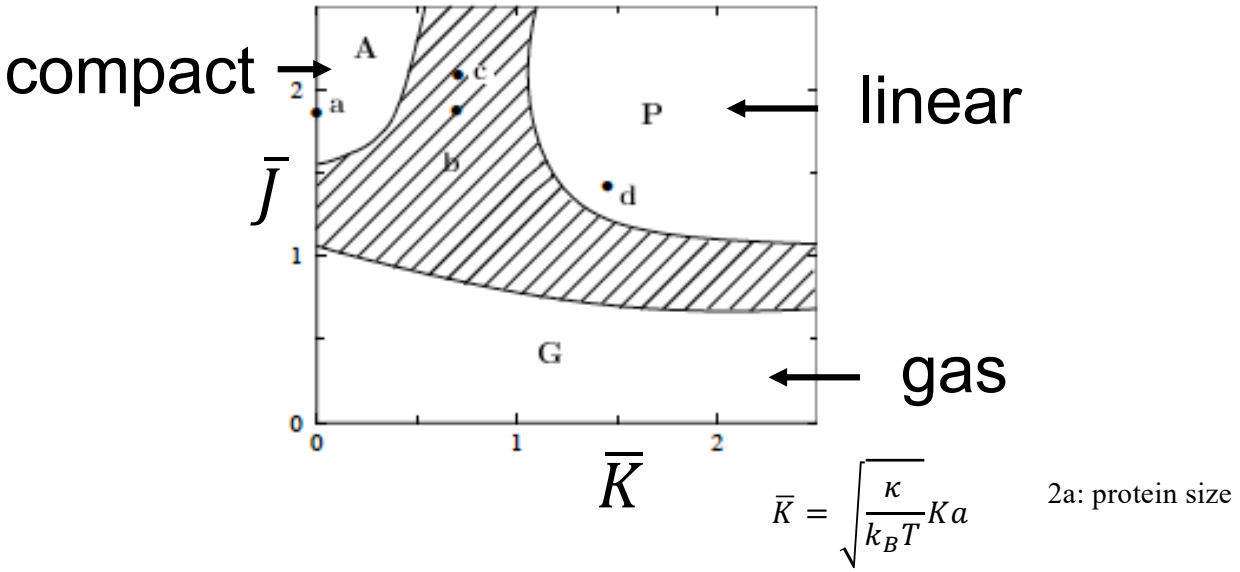
- *Multi-body effects*

Inclusions:

$\bar{K}$ mean curvature)

$\bar{J}$ mean Gaussian (anisotropic) curvature)

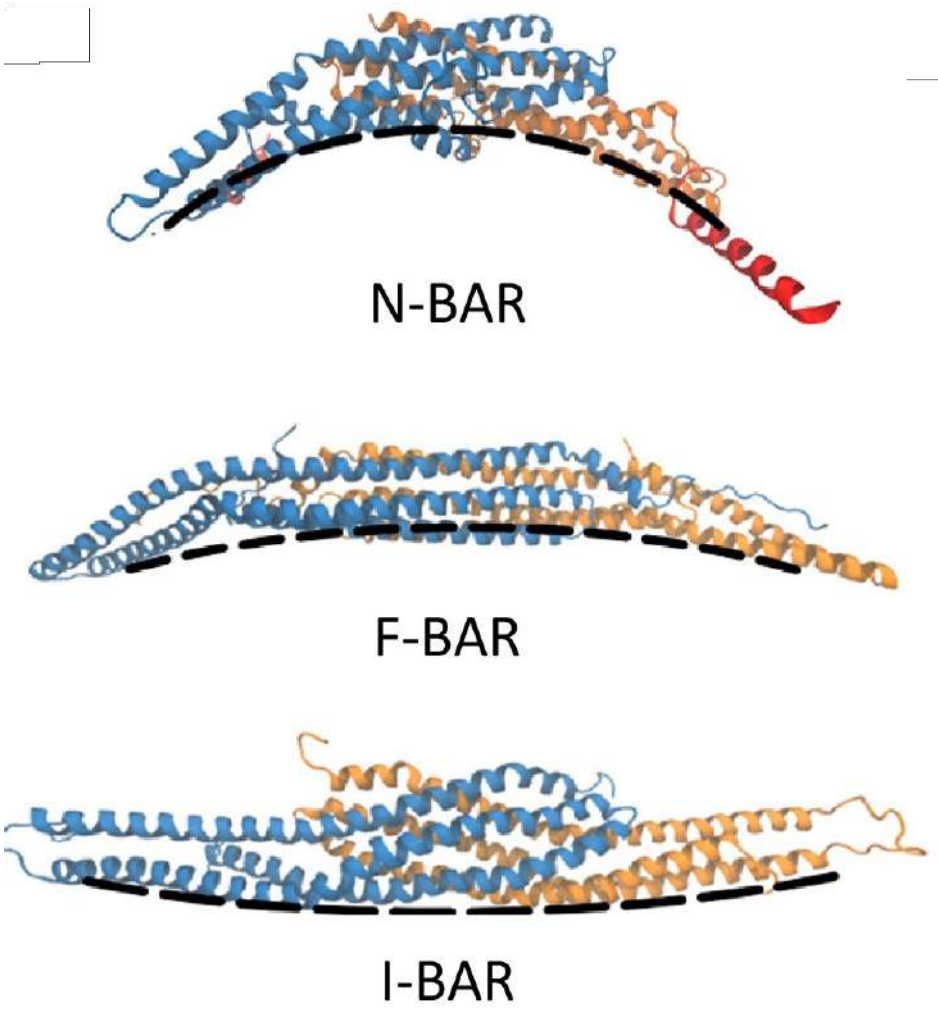




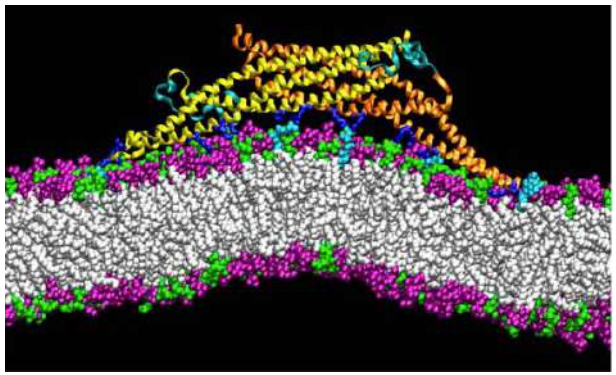
Monte Carlo simulations

P.G. Dommersnes, J.B. Fournier *Eur. Phys. J. B* (1999)

BAR-Domain Proteins: Dimers with Various Intrinsic Curvatures



Bind to
negatively-charged membranes
(PS, PI(4,5)P_2 etc…)

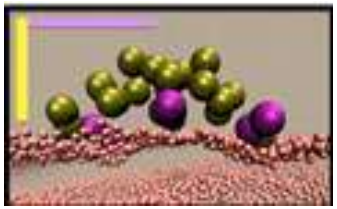


Blood P *et al*
Biophys. J. (2008)

M. Simunovic et al, *Trends Cell Biol.* (2015)

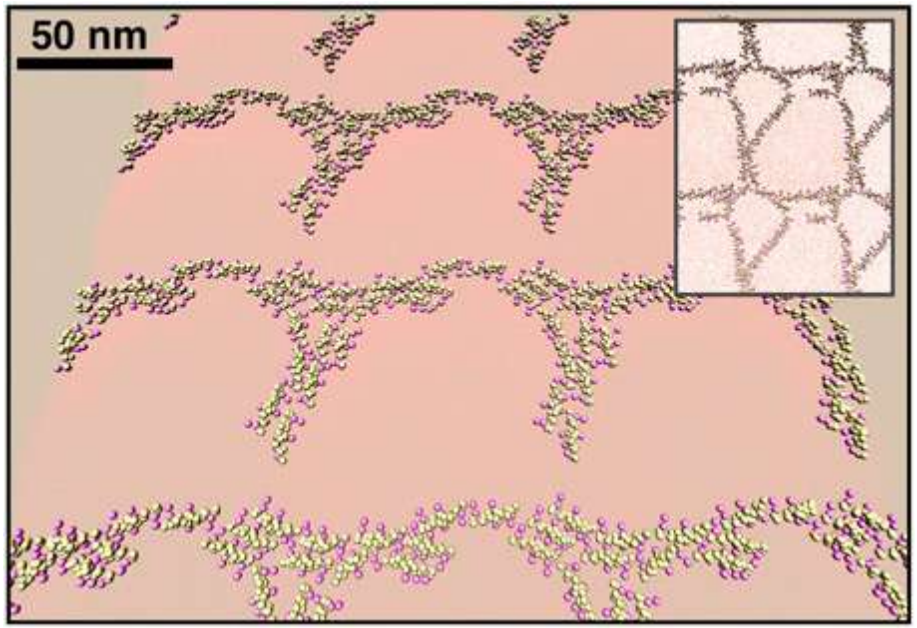
Local Membrane Deformation Induces Long Distance Protein-Protein Interaction

M. Simunovic, G. Voth (U. Chicago)



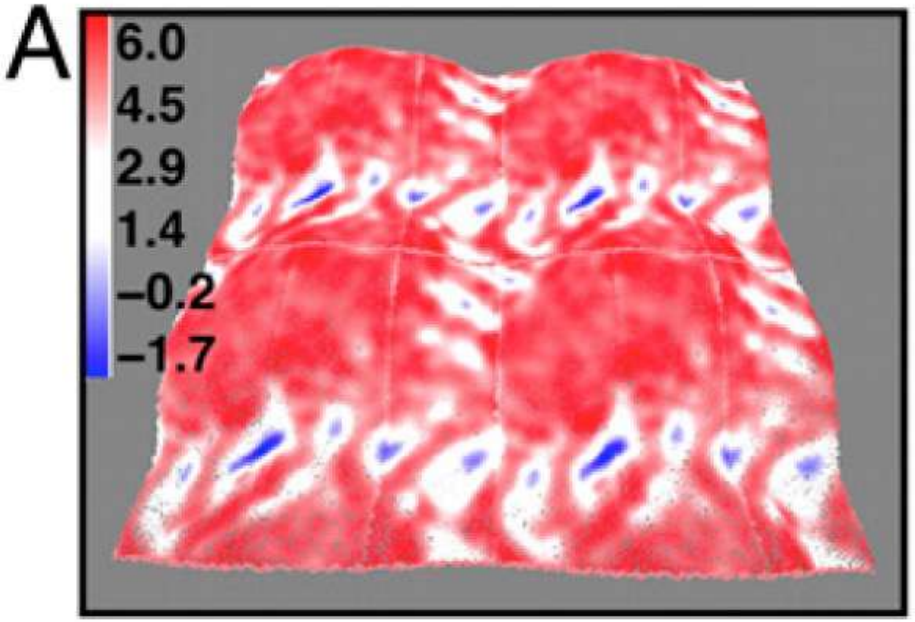
Coarse Grained model of N-BAR (endophilin)

Proteins: Linear organization



$\phi = 18\%$

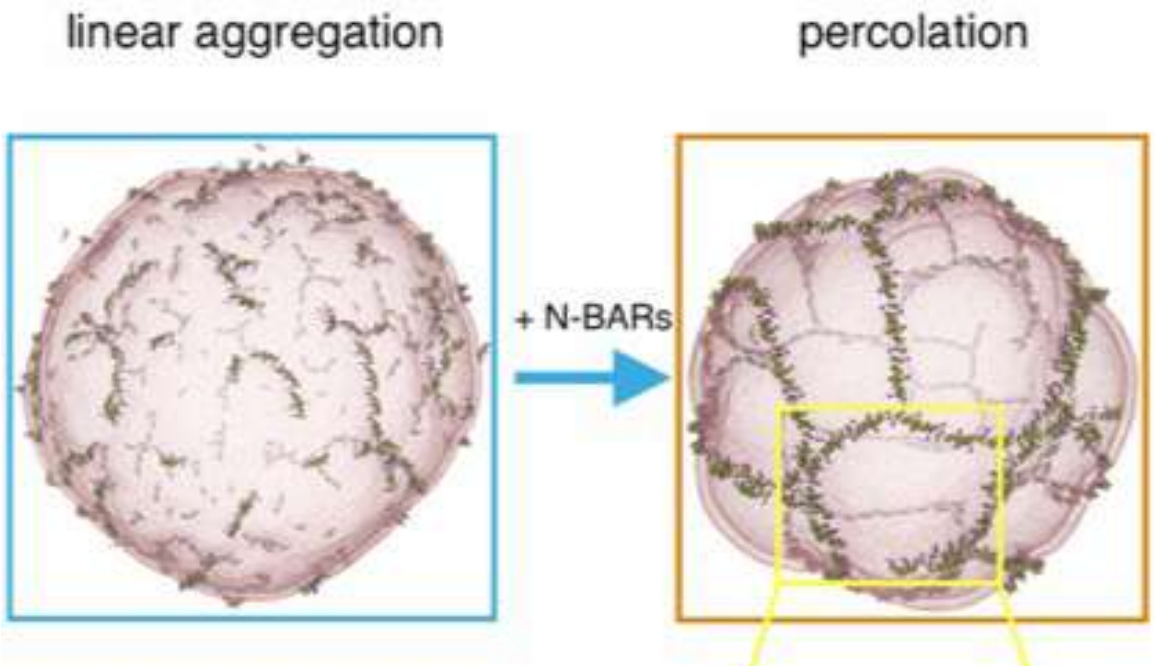
Membrane local mean-curvature

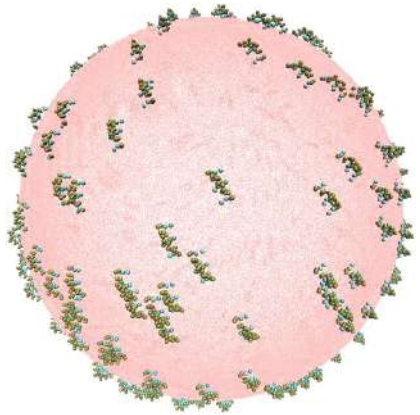


Simunovic, Srivastava, Voth, *PNAS* (2013)

Membrane Mediated N-BAR Assembly

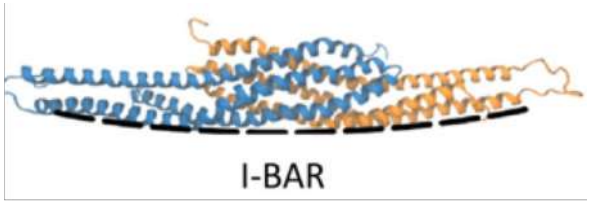
M. Simunovic,
G. Voth (U. Chicago)

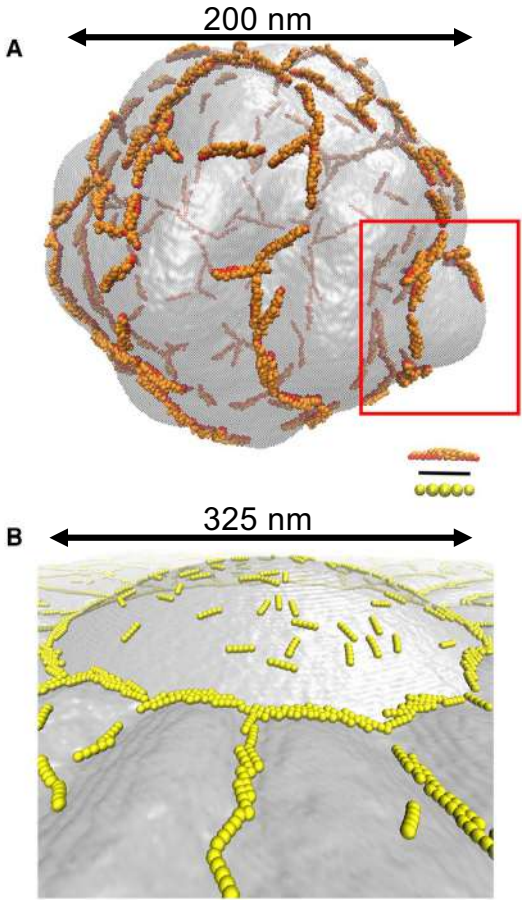




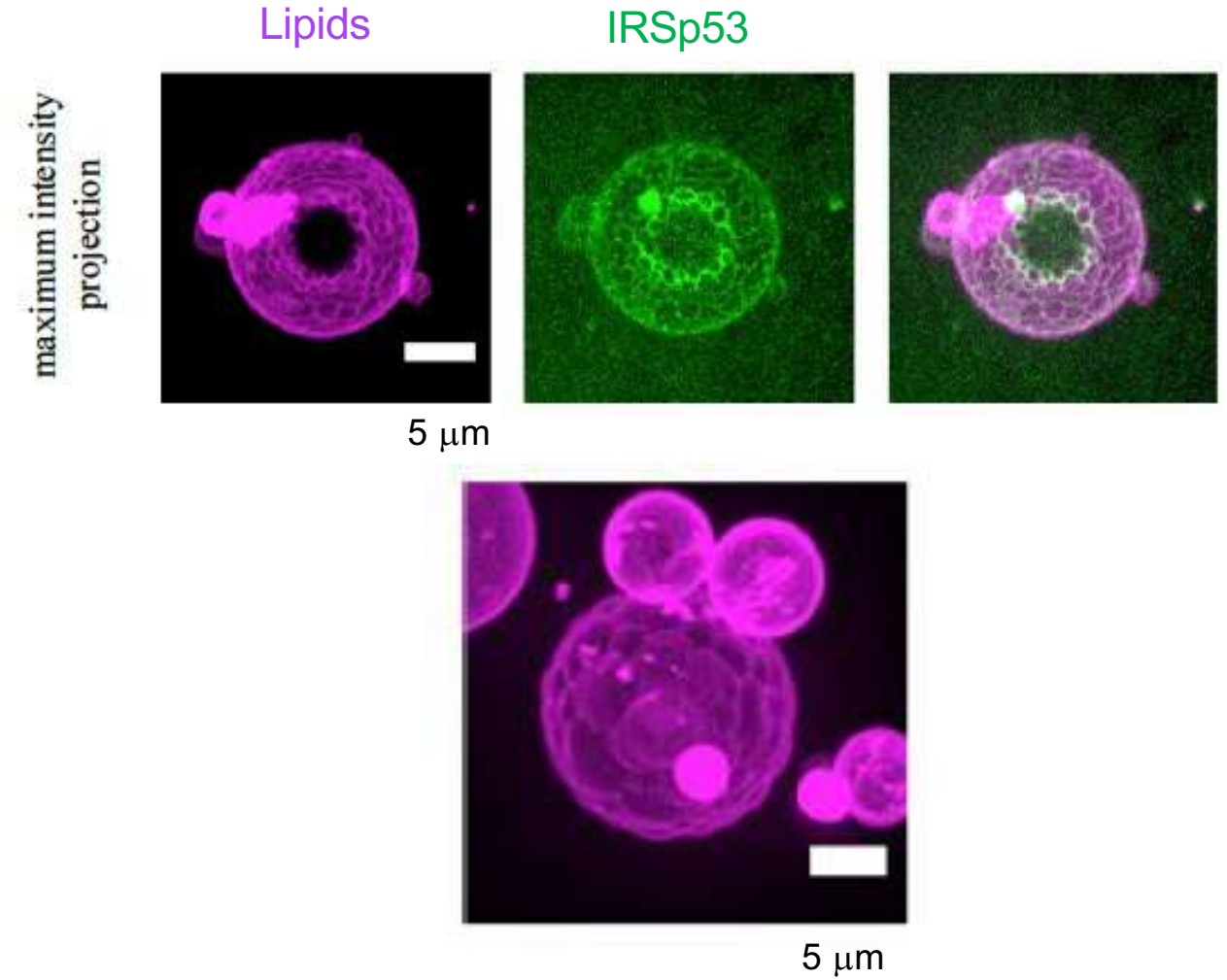
Simunovic,Srivastava,Voth, *PNAS* (2013)
Simunovic,…,Voth, *Biophys J.*((2013)
Simunovic, Voth, *Nat. Commun.* (2015)

Membrane Mediated I-BAR Assembly





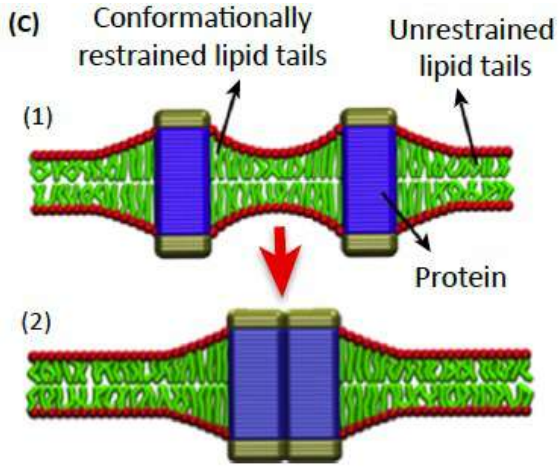
IRSp53



Jarin Z., Tsai F.-C…..Bassereau P., Voth G. A. *Biophys. J.* (2019)

Short Range Interactions

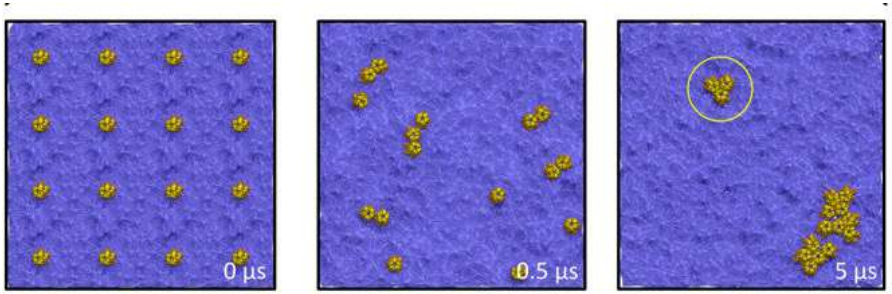
- Local membrane thickness deformations (hydrophobic mismatch)

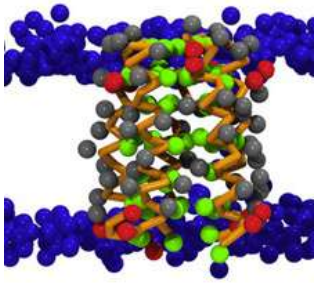


Many models

See A.-F. Bitbol…J.B. Fournier in *Physics of Biological Membranes* (2018)

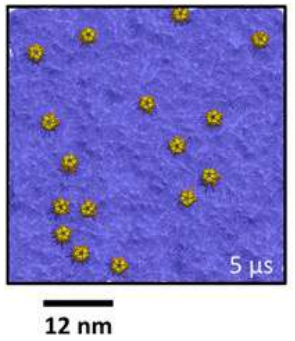
Positive mismatch





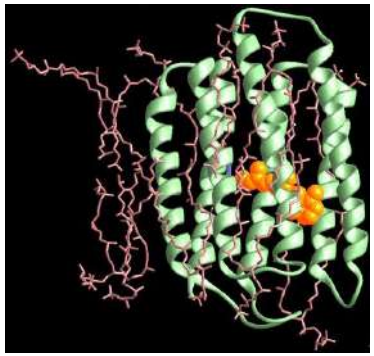
Martini force field

No mismatch



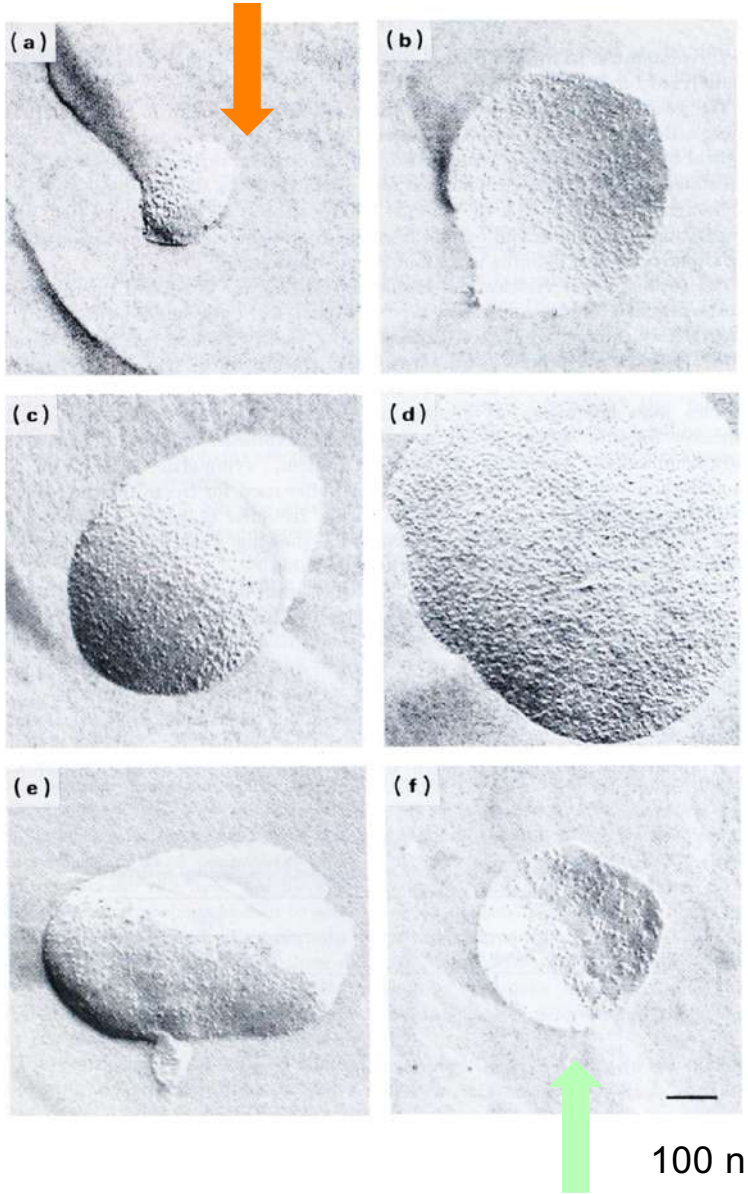
D.L. Parton…M. S. P. Sansom. *Biophys. J.* (2011)

Bacteriorhodopsin Aggregation due to Mismatch



Hydrophobic part

~30 Å



Phosphatidilcholine vesicles

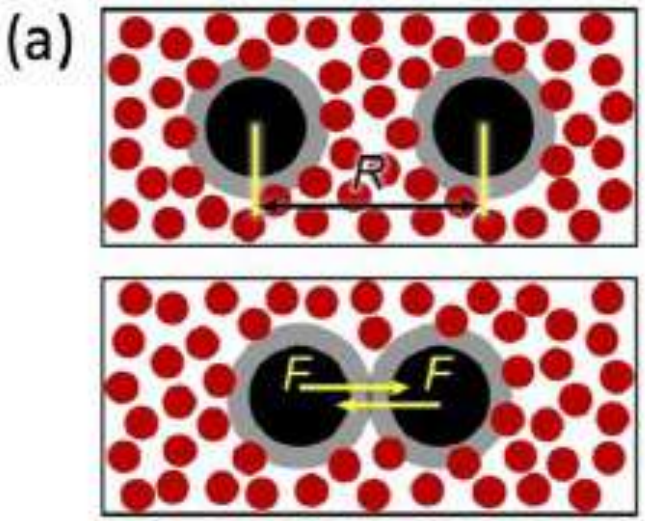
Acyl chain	Lipid t_m (°C)	Temperature (°C)	HC thicknes- (Å)†	Distribution
10 : 0	0	15	15·5	Aggregated
12 : 0	−1·8‡	22	19·5	Dispersed
14 : 0	23‡	34	23	Dispersed
16 : 0	41·4§	50	26	Dispersed
18 : 0	54·9§	60	29·5	Dispersed
22 : 1	11‖	35	34	Dispersed
24 : 1	24‖	45	37·5	Dispersed, aggregated and ordered arrays

Large mismatch (+/-) required

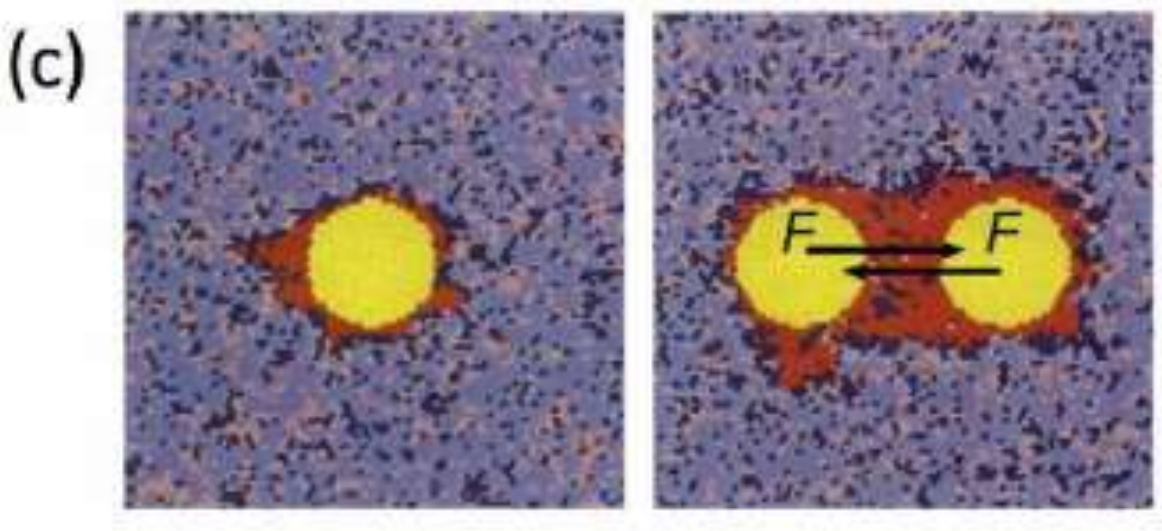
B.A. Lewis, D.M. Engelman *J. Mol. Biol.* (1983)

Short Range Interactions

Depletion/Capillary Force



"Wetting" Force



Multicomponent membrane

Destainville N et al *Current topics in membranes* (2016)

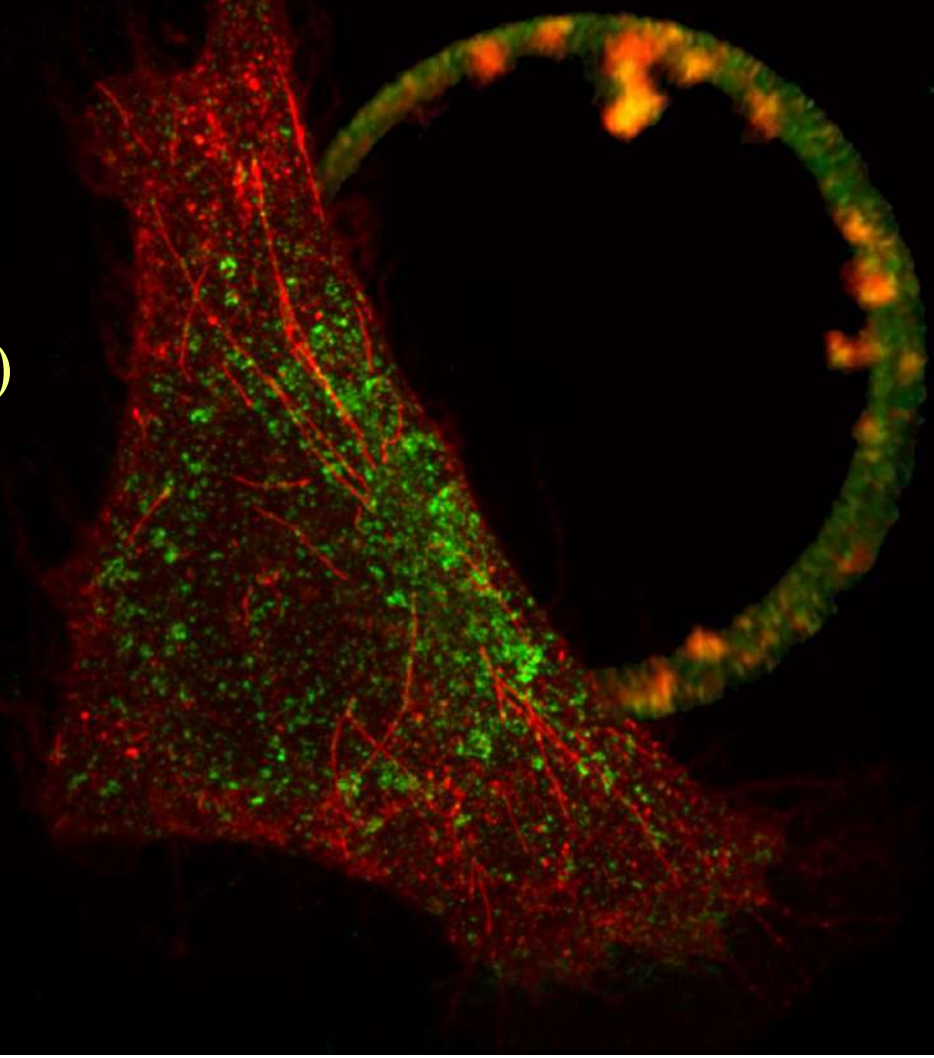
Attraction

ShigaToxin

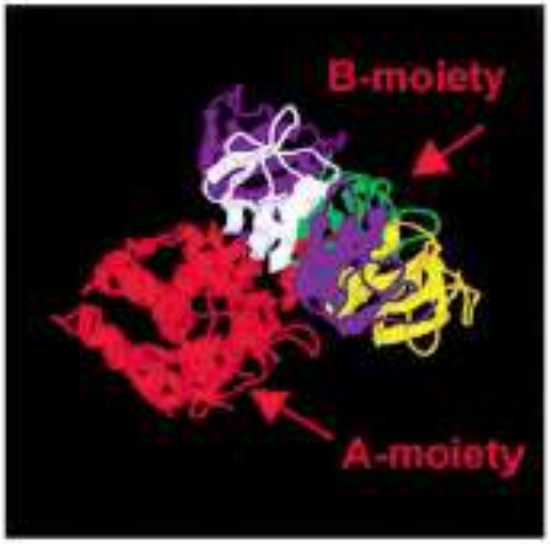
Collaborators:

L. Johannes (I. Curie)

W. Pezeshkian, J. Ipsen (Copenhagen)



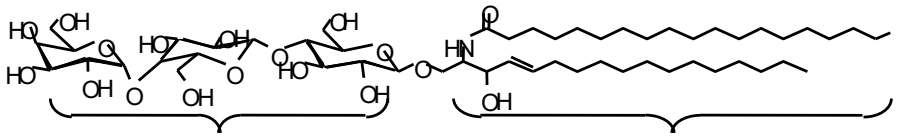
Shiga Toxin (STxB)



- B moiety: pentamer

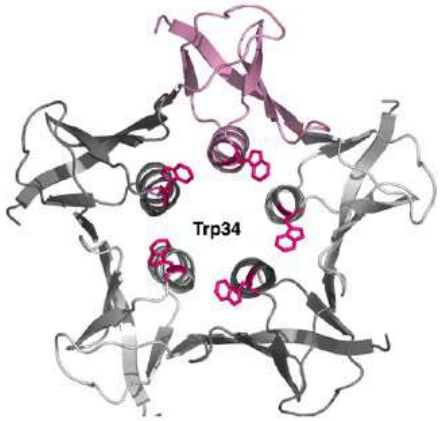
"Toxin transporter"

- Receptor = Gb3 LIPID



Globotriose Ceramide

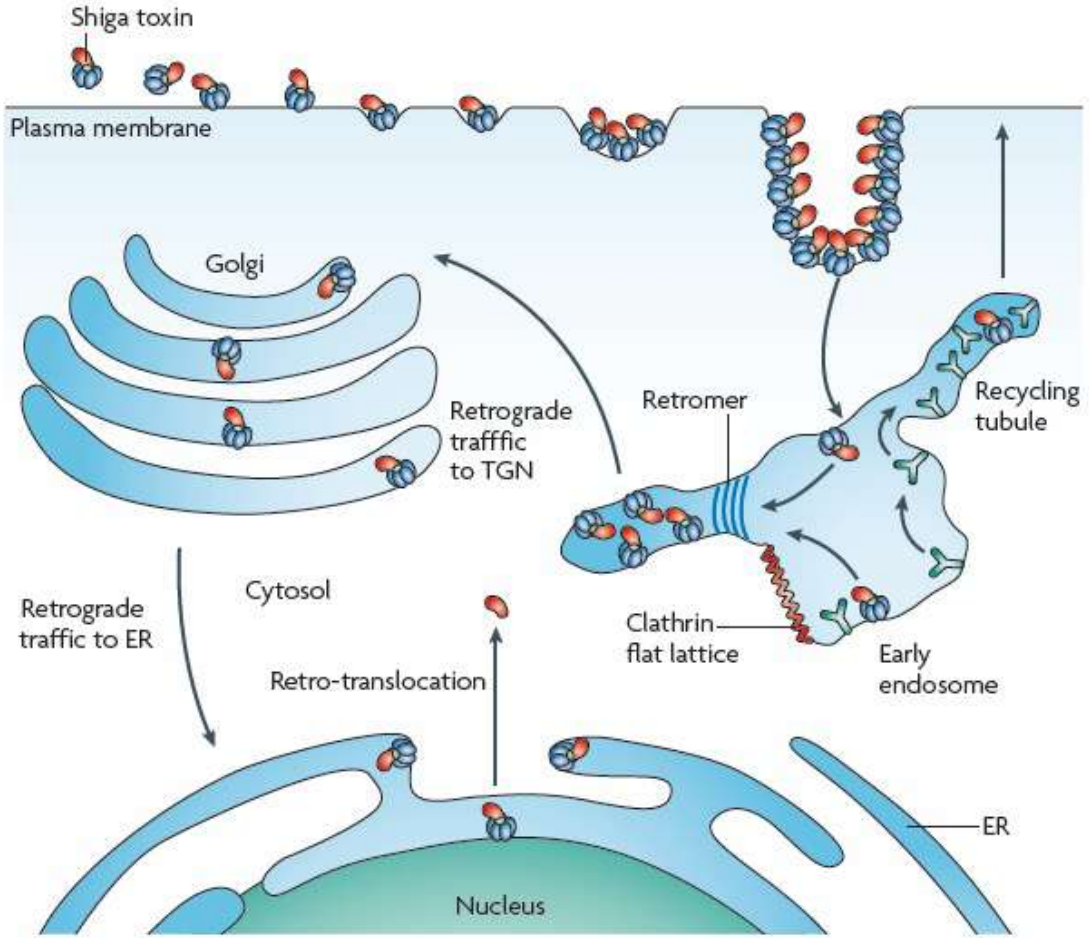
- 1 STxB pentamer ⟷ 10-15 Gb3



Top view of B pentamer

Shiga Toxin Endocytosis

Shiga-toxin: Clathrin–independent endocytosis
mediated by binding to its lipid ligand Gb3



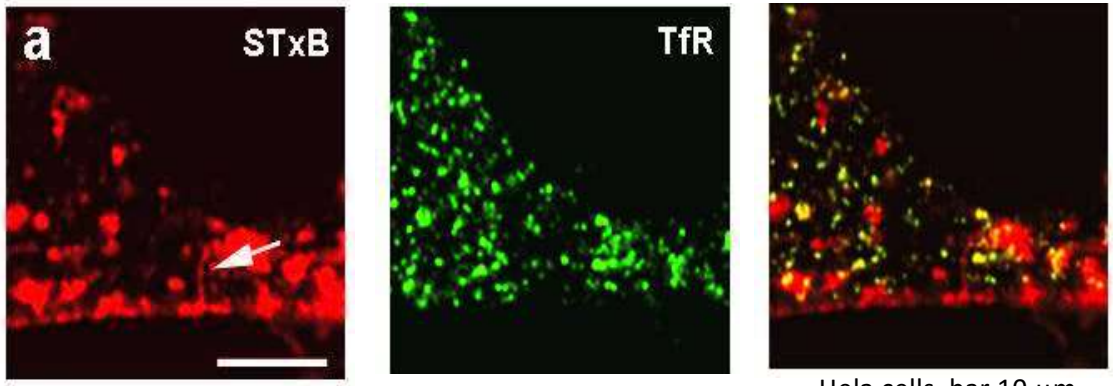
Similar for **Cholera-toxin** (binding to GM1)

L. Johannes, W. Romer, *Nat. Rev. Micro.* **8**, 105 (2010).

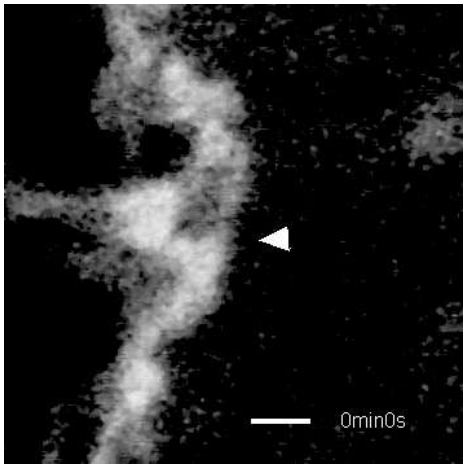
Tubular Structures for Toxin Endocytosis

W. Römer, L. Johannes

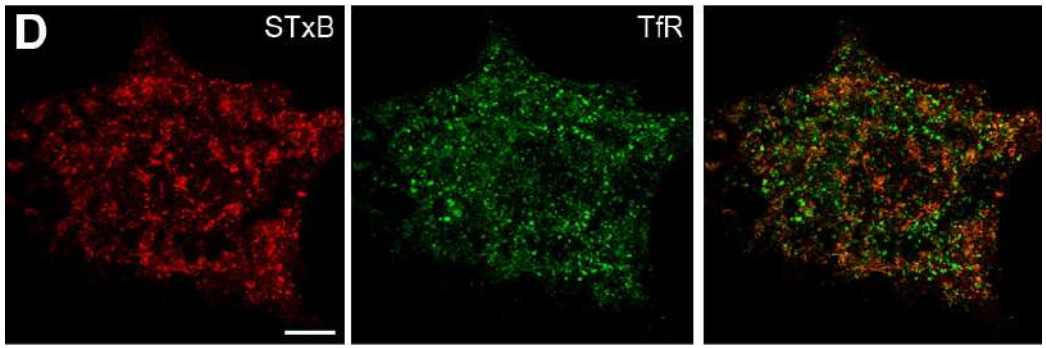
Toxin entry:

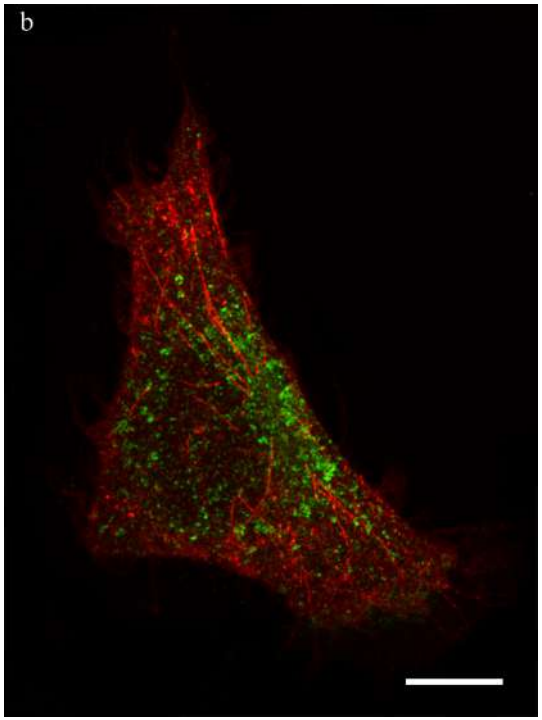


Hela cells, bar 10 µm



ATP-depleted HeLa cells : More tubes



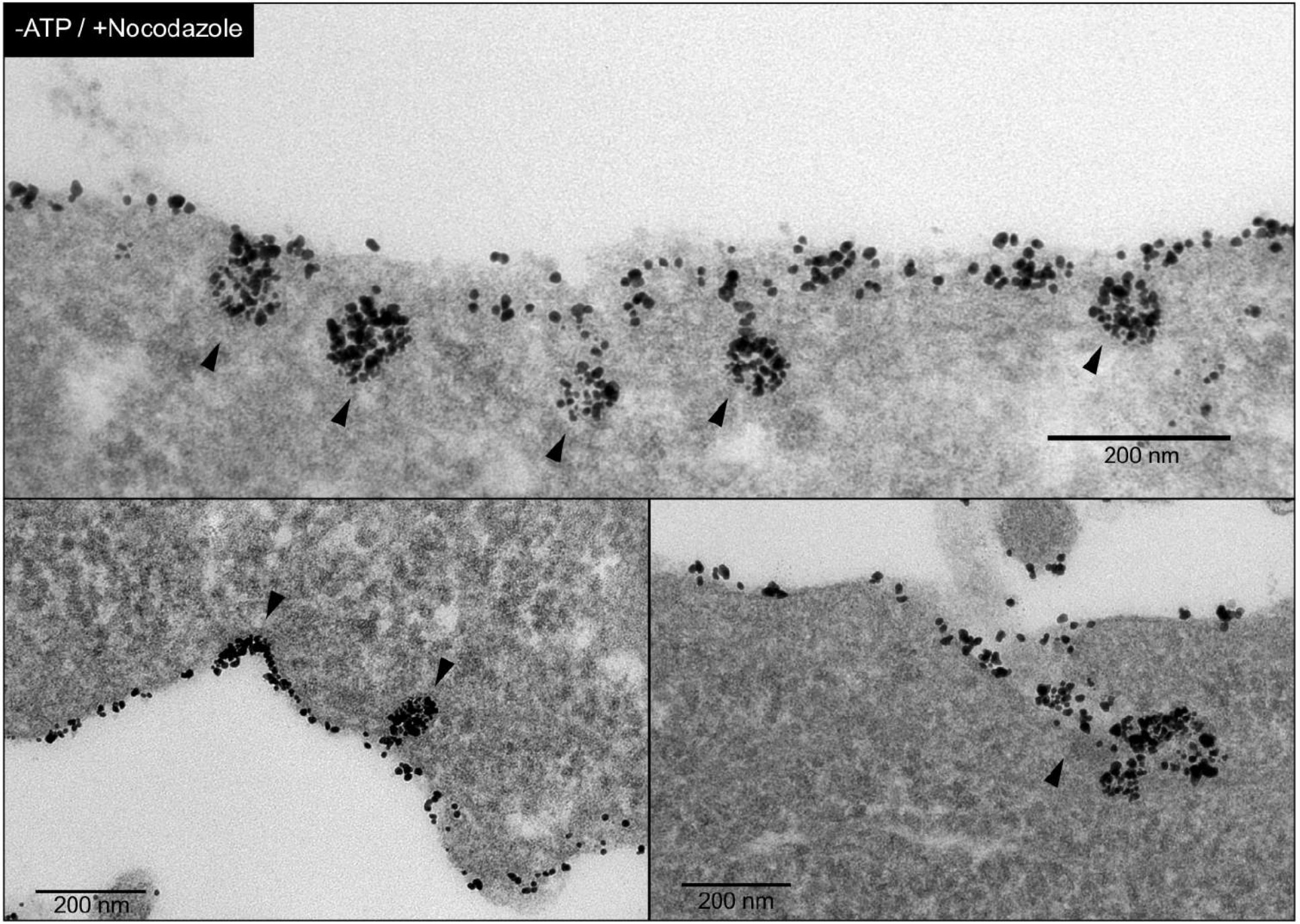


5 µm

Similar tubes if interfering with fission (dynamin)

STxB ⟺ Membrane tubulation?
Mechanism ?

W. Römer et al, Nature (2007)

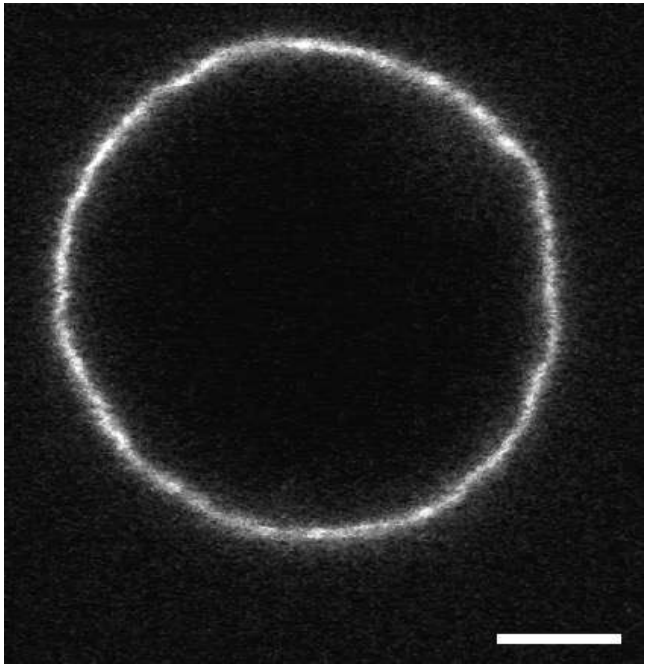

-ATP / +Nocodazole
200 nm
200 nm
200 nm

Similar Tubular Structures with Model Membranes

GUVs: DOPC + 30% Chol + 5 % Gb3

+ STxB (200 nM)

STxB



X3 accelerated

W. Römer, L. Berland et al, *Nature* (2007)

STxB alone Tubulates Membranes

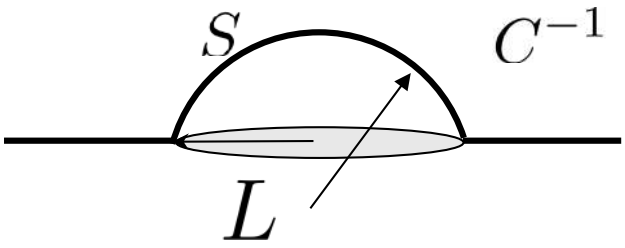
1 : Fast **uniform binding** of STxB

2 : Small **domains/cluster** of STxB

3 : **If initially low** membrane **tension**:
tubular deformations

W. Römer, L. Berland et al, *Nature* (2007)

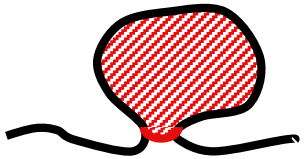
Effect of Membrane Tension



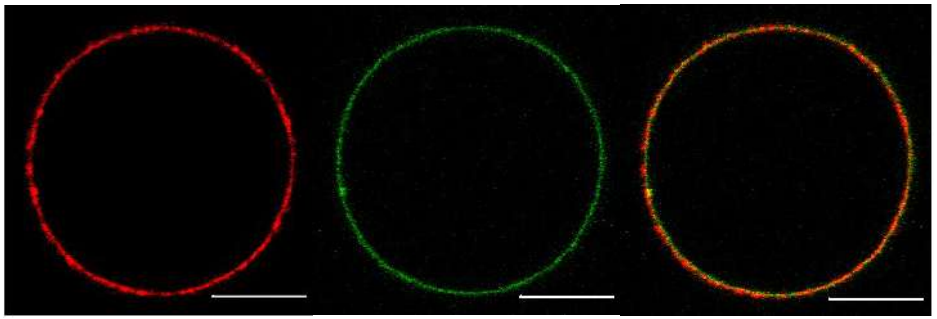
$$F = \gamma_L 2\pi L + \frac{1}{2}\kappa S(C - C_0)^2 + \sigma(S - \pi L^2)$$

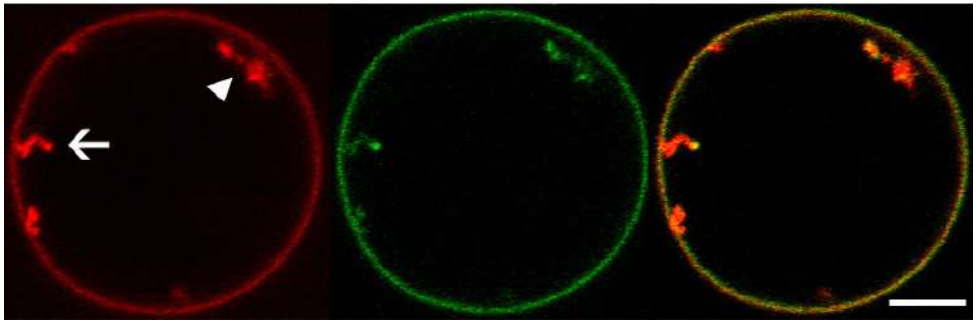
P. Sens, M.S. Turner *Phys. Rev. E* (2006)

High tension



Low tension





STxB Membrane

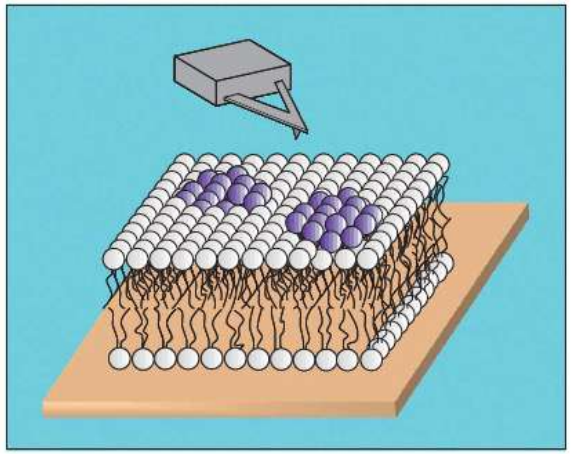
10 μm

No tubule
STxB clusters

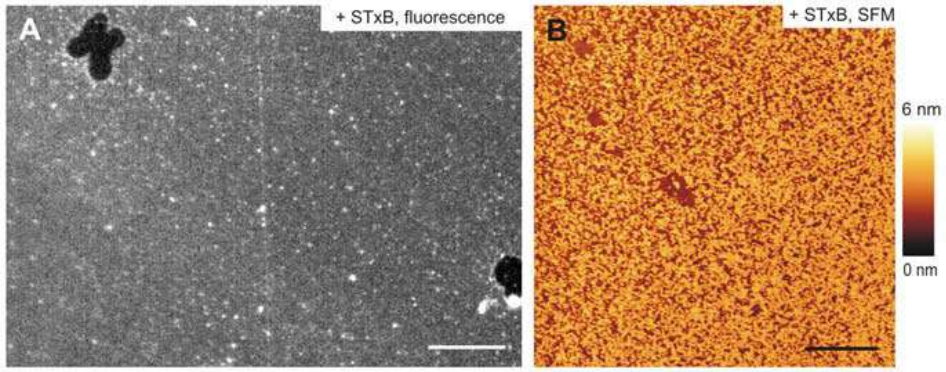
Tubules

STxB Clusters on Supported Membranes

AFM on SLB



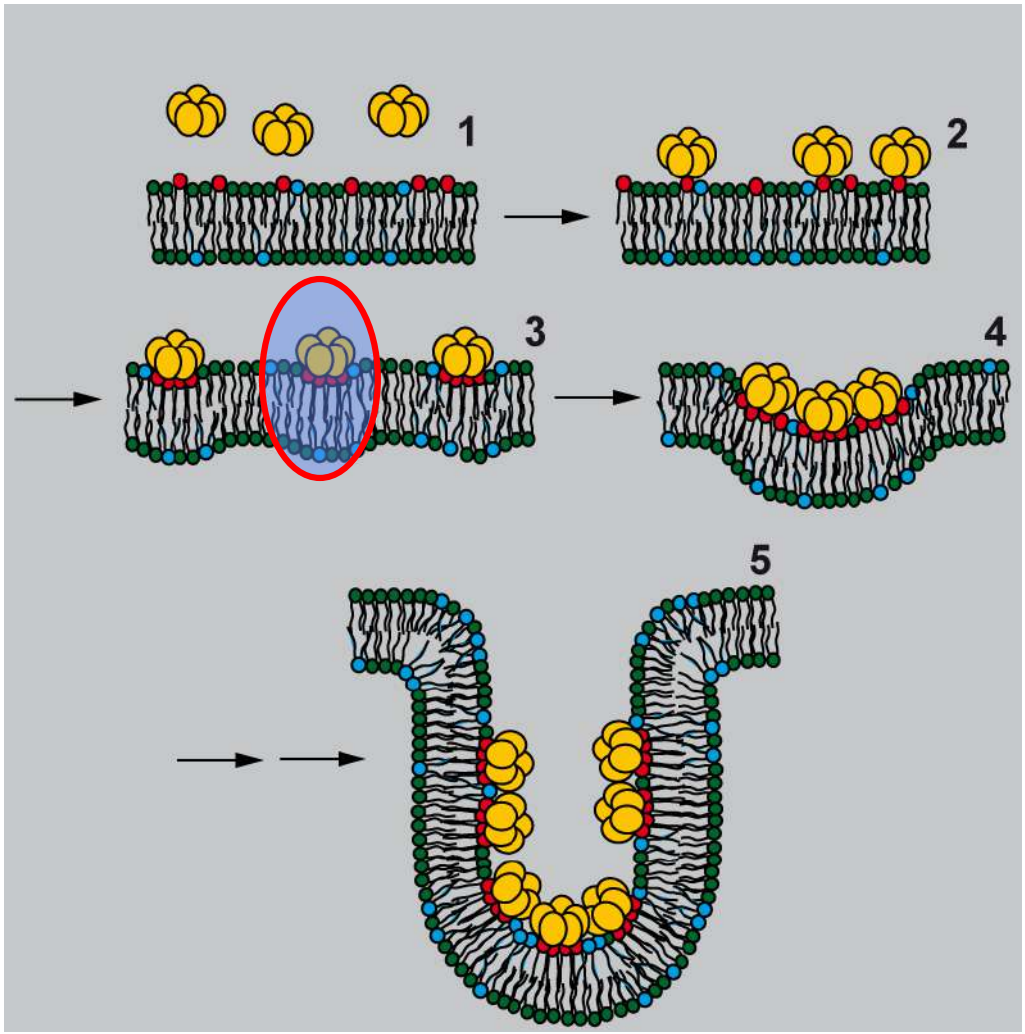
From M.-P. Mingeot-Leclercq et al. *Nat. Protoc.* (2008)



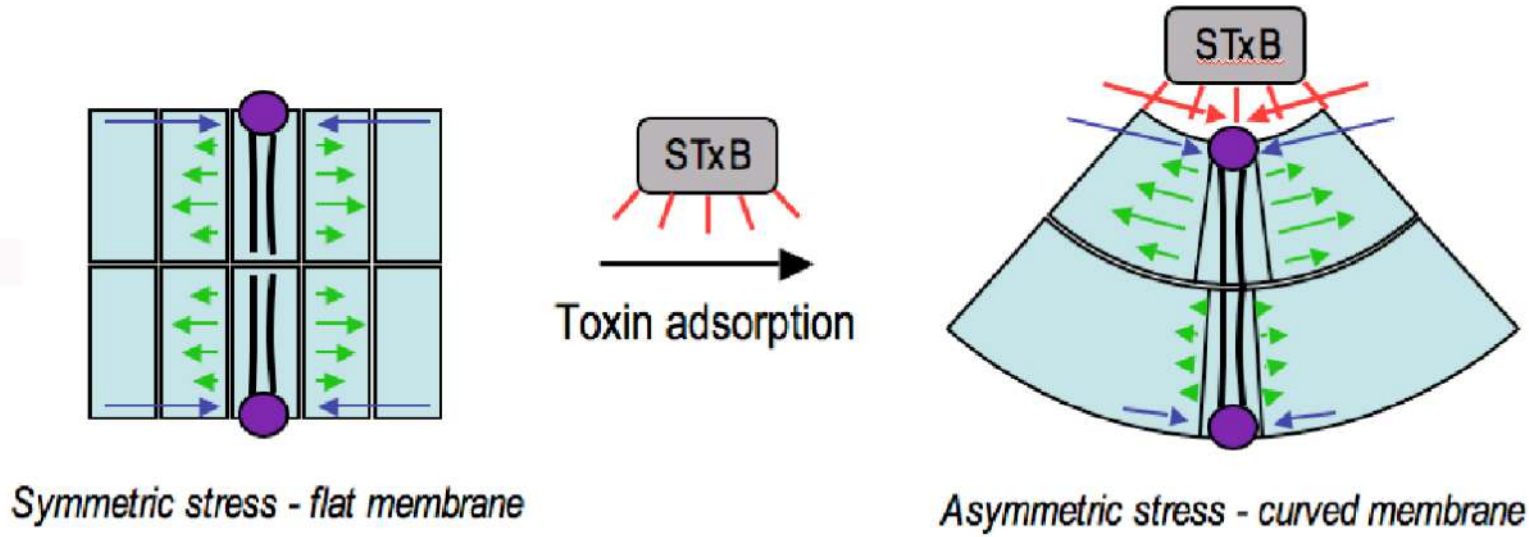
Clusters

B. Windschiegl….C. Steinem *PLoS ONE* (2009)

Shiga Toxin-induced Endocytic Pit Construction



Membrane Bending



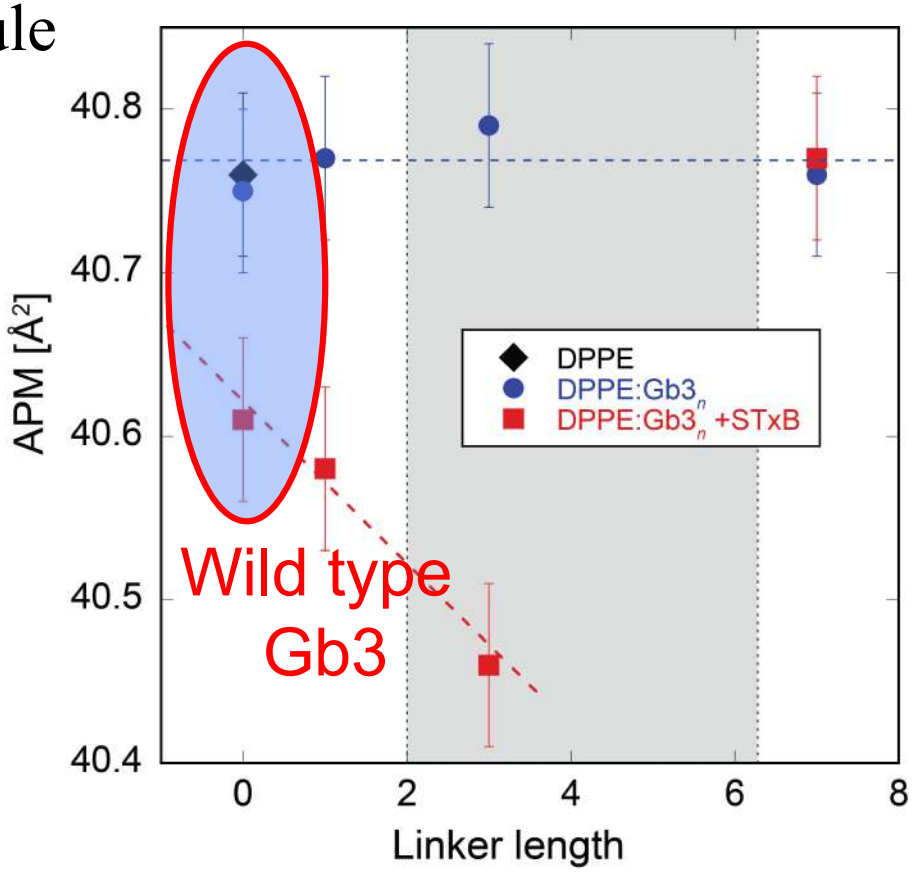
STxB induces Gb3 compaction on the external leaflet

W. Römer, L. Berland et al, *Nature* (2007)

STxB Induces Lipid Compression on One Leaflet

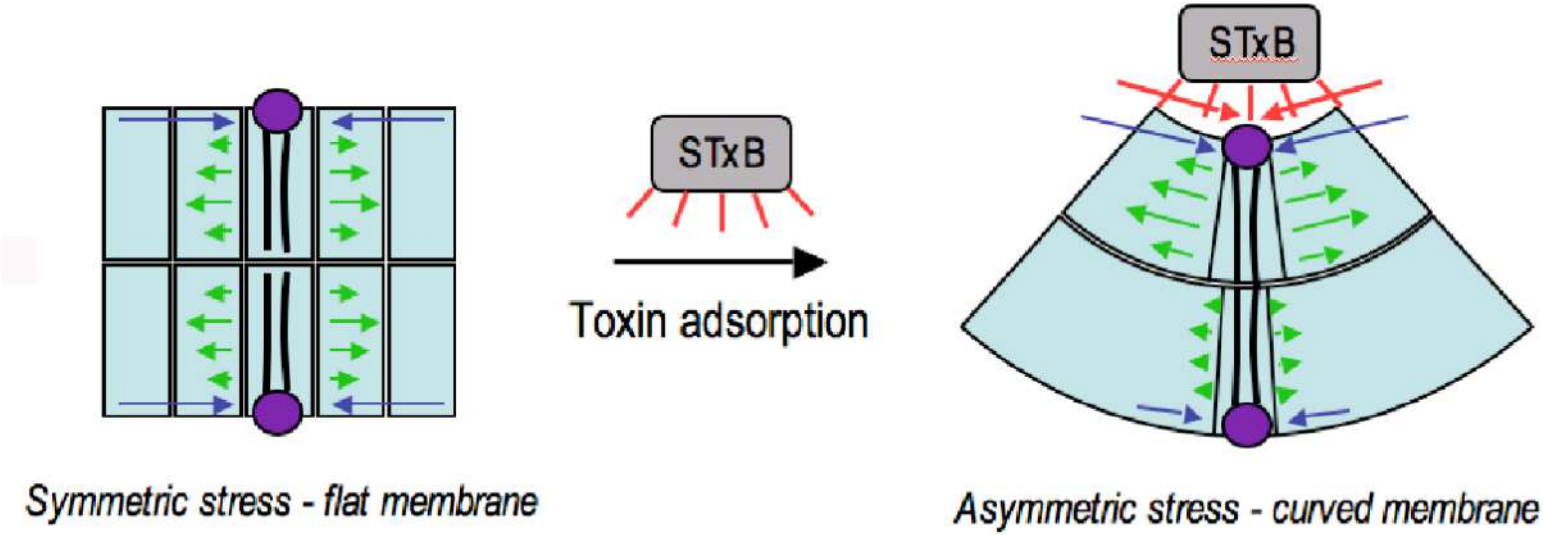
X-ray surface scattering – Monolayer on water

Area per Molecule

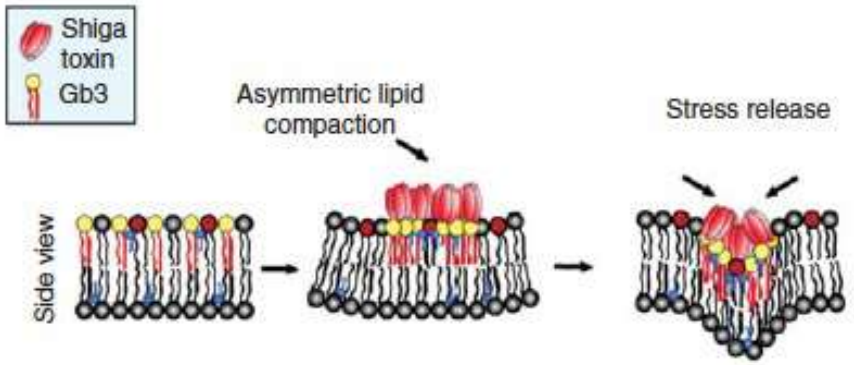


E.B. Watkins … L. Johannes *Nano Lett.* (2019)

Membrane Bending



STxB induces Gb3 compaction
on the external leaflet

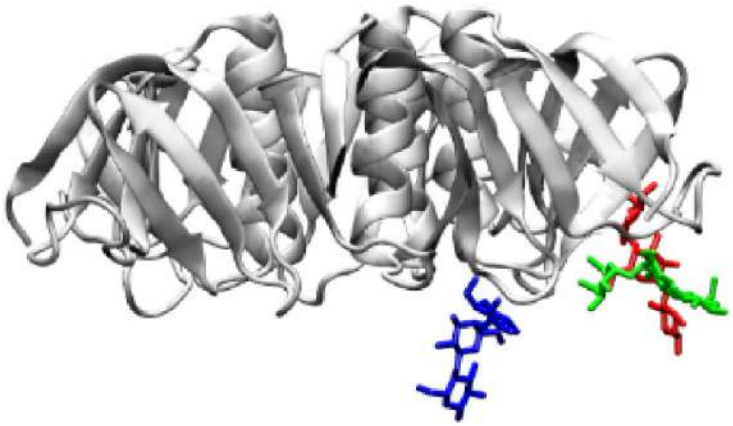


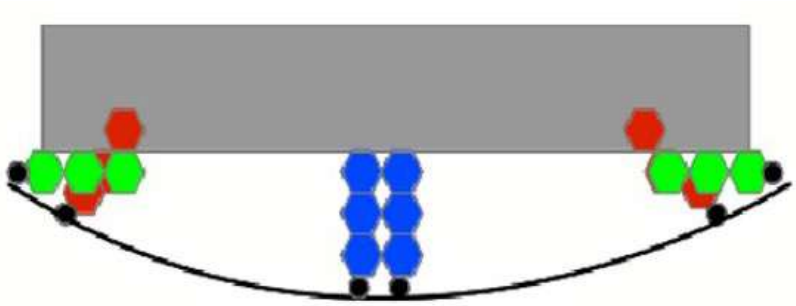
➡ Negative spontaneous curvature

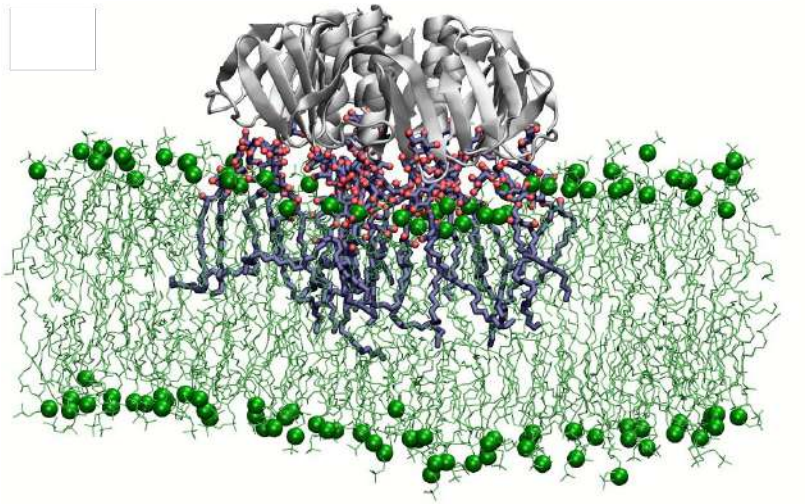
Membrane Bending: Molecular Scale

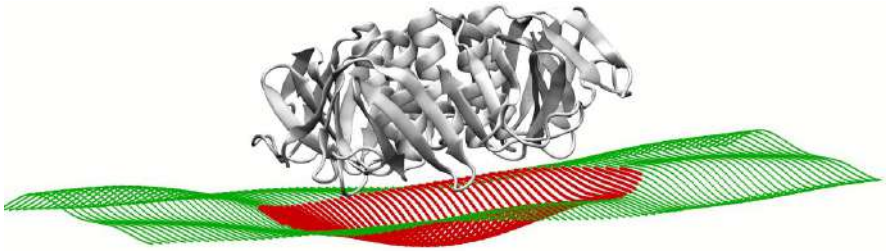
STxB binding → Gb3 lipid tilt

→ Negative membrane curvature





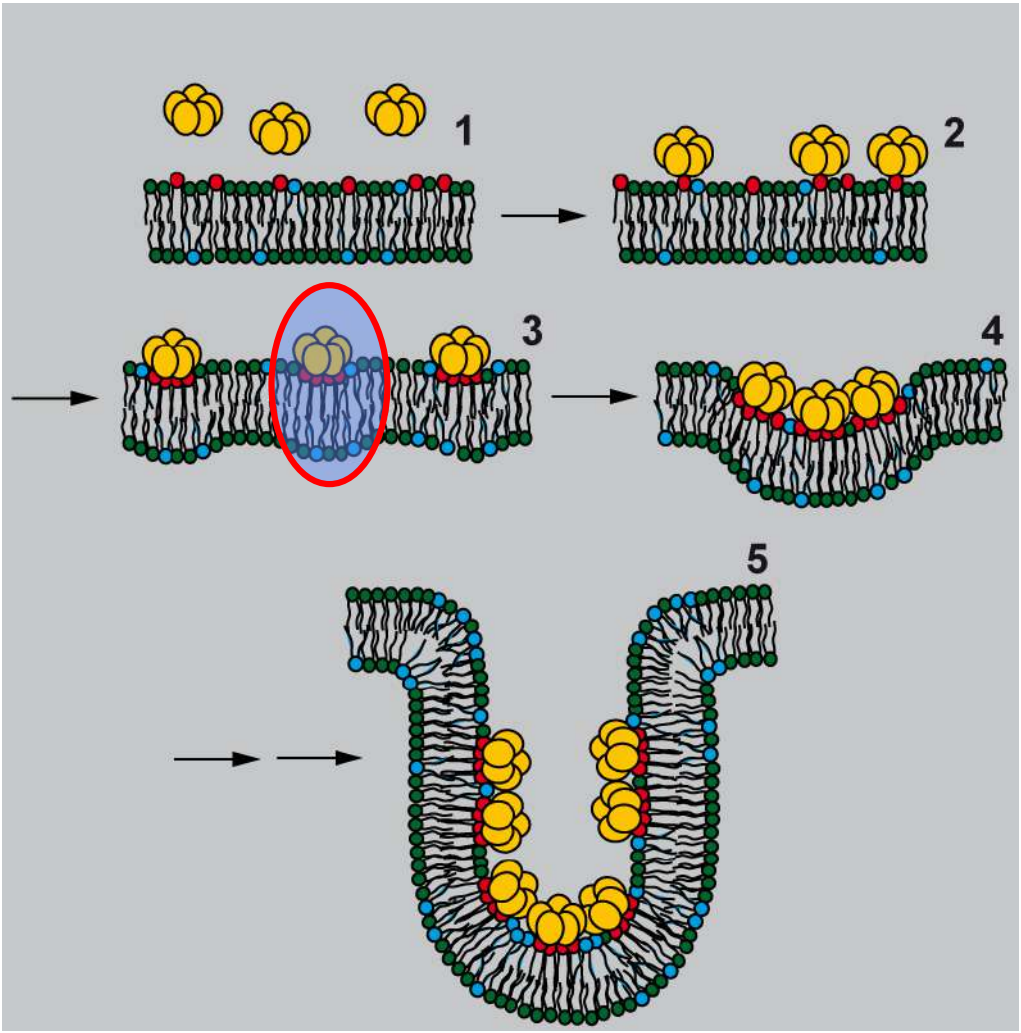




W. Pezeshkian…. J. Ipsen., *Soft Matter* (2016)

Membrane Bending

- Toxin-lipid geometry
- Asymmetric membrane compression

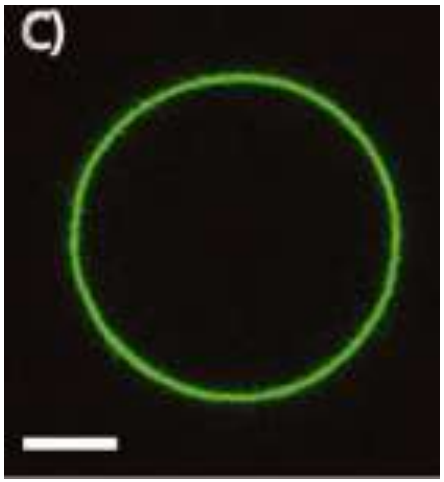


New mechanism for clathrin-independent endocytosis:
Cargo-Mediated budding

Lipid Composition in Tubules ?

DOPC:Chol:SM + 5% Gb3 :
(41:32:21)

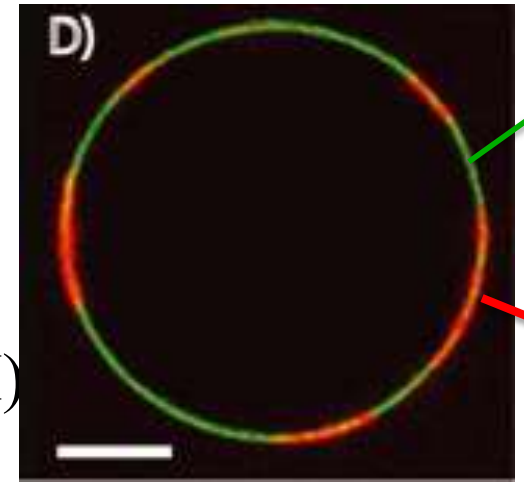
Homogeneous



+ STxB

(200 nM)

Phase separation



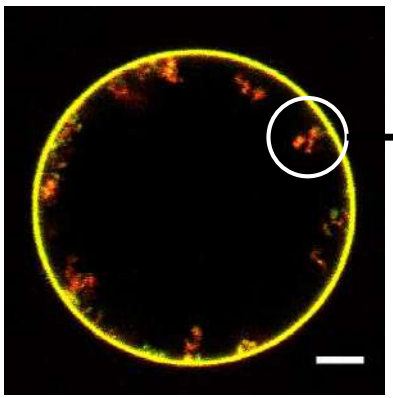
L_d phase:
PC- rich

STxB
in L_o phase:
SM- rich

Ld phase marker

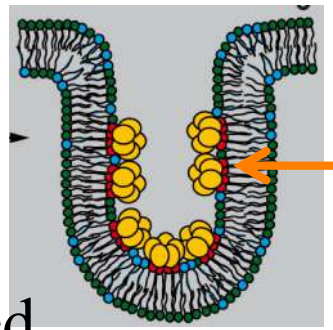
STxB : induction of *SM-rich* environment

In tubules?



STxB

enriched



Enriched
in SM ?

M. Safouane, L. Berland et al *Traffic* (2010)

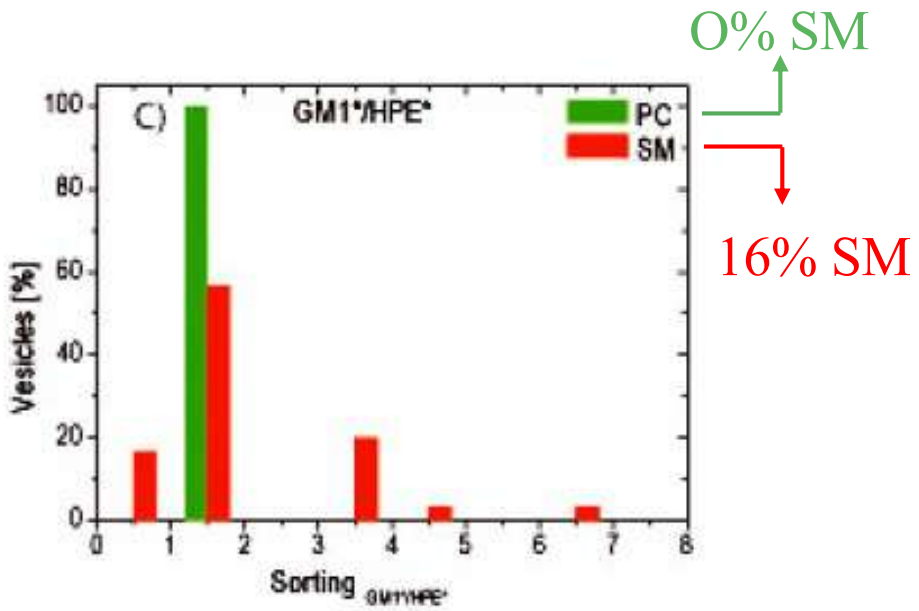
SM Enriched in STxB-induced Tubules

2 fluorescent lipid analogs:

GM1*: No phase preference

HPE*: Ld phase

$$Sorting_{GM1^*/DHPE^*} = \frac{I_t^{GM1^*} / I_t^{DHPE^*}}{I_v^{GM1^*} / I_v^{DHPE^*}}$$

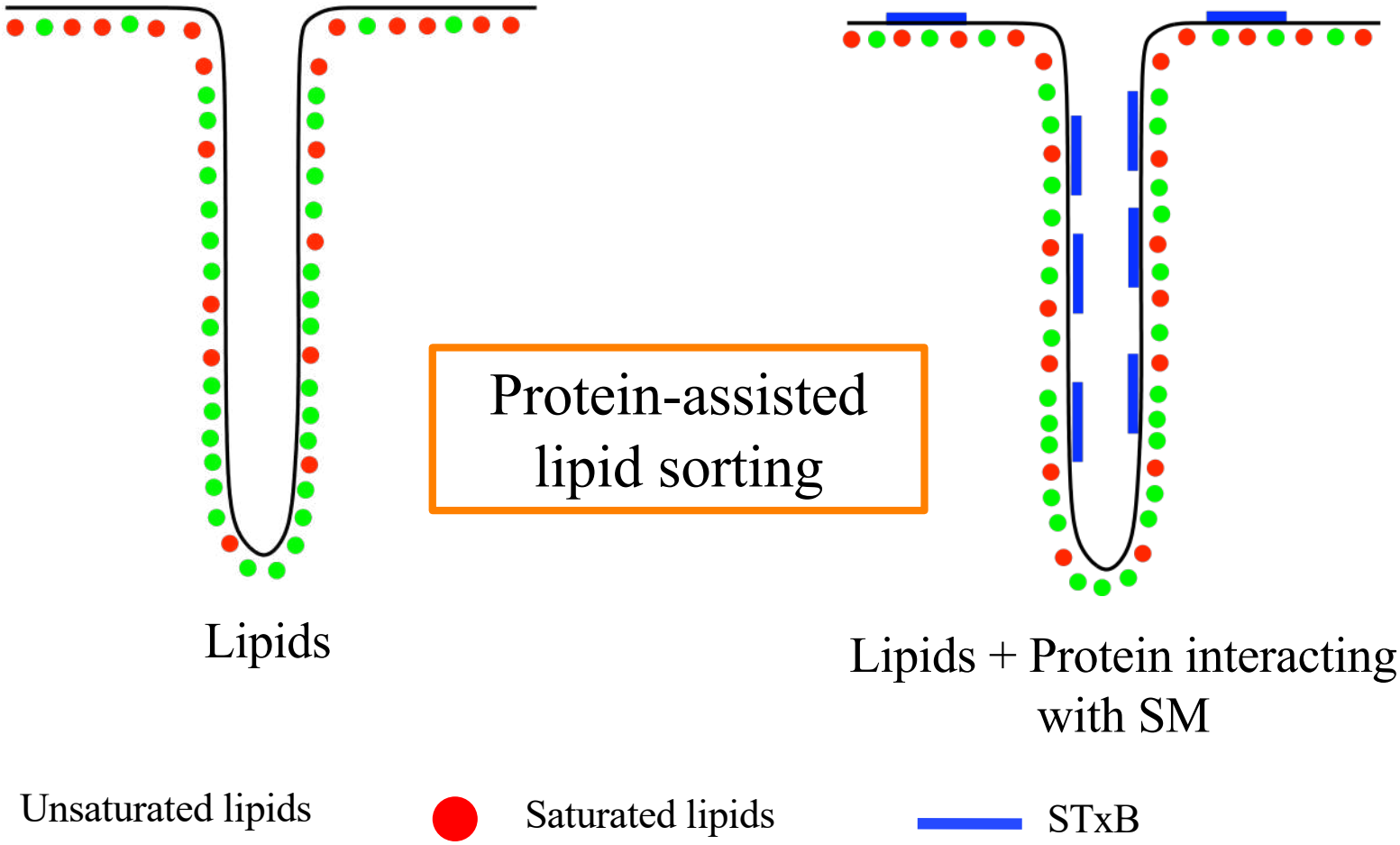


→ *SM enriched in the tubes*

Confirmed in vivo (Laurdan experiments)

M. Safouane, L. Berland et al *Traffic* (2010)

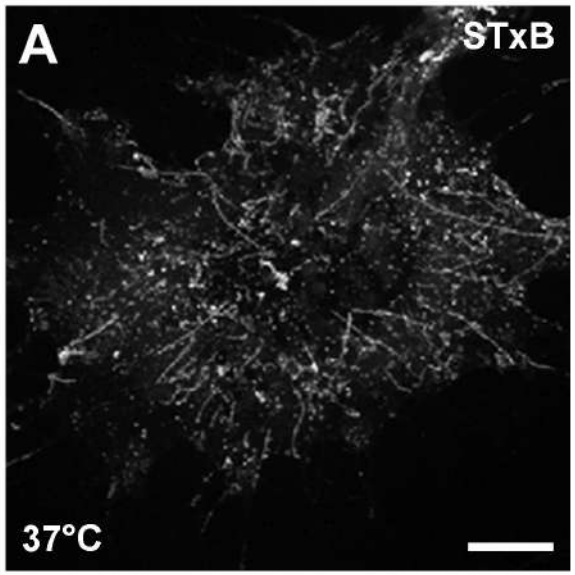
Enrichment of Gb3 by STxB in the Tubes drives SM Enrichment in a Curved Membrane

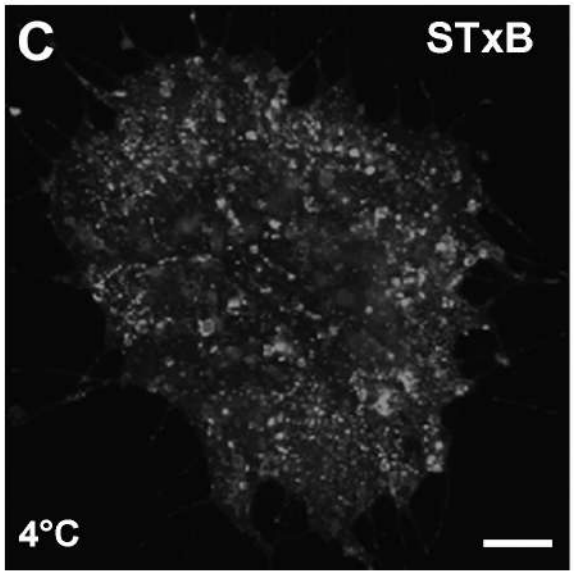


Interaction (direct or indirect) with proteins can influence lipid sorting

Temperature-induced Scission of STxB Tubules

37°C
Control





4°C
Control

37°C
- Cholesterol

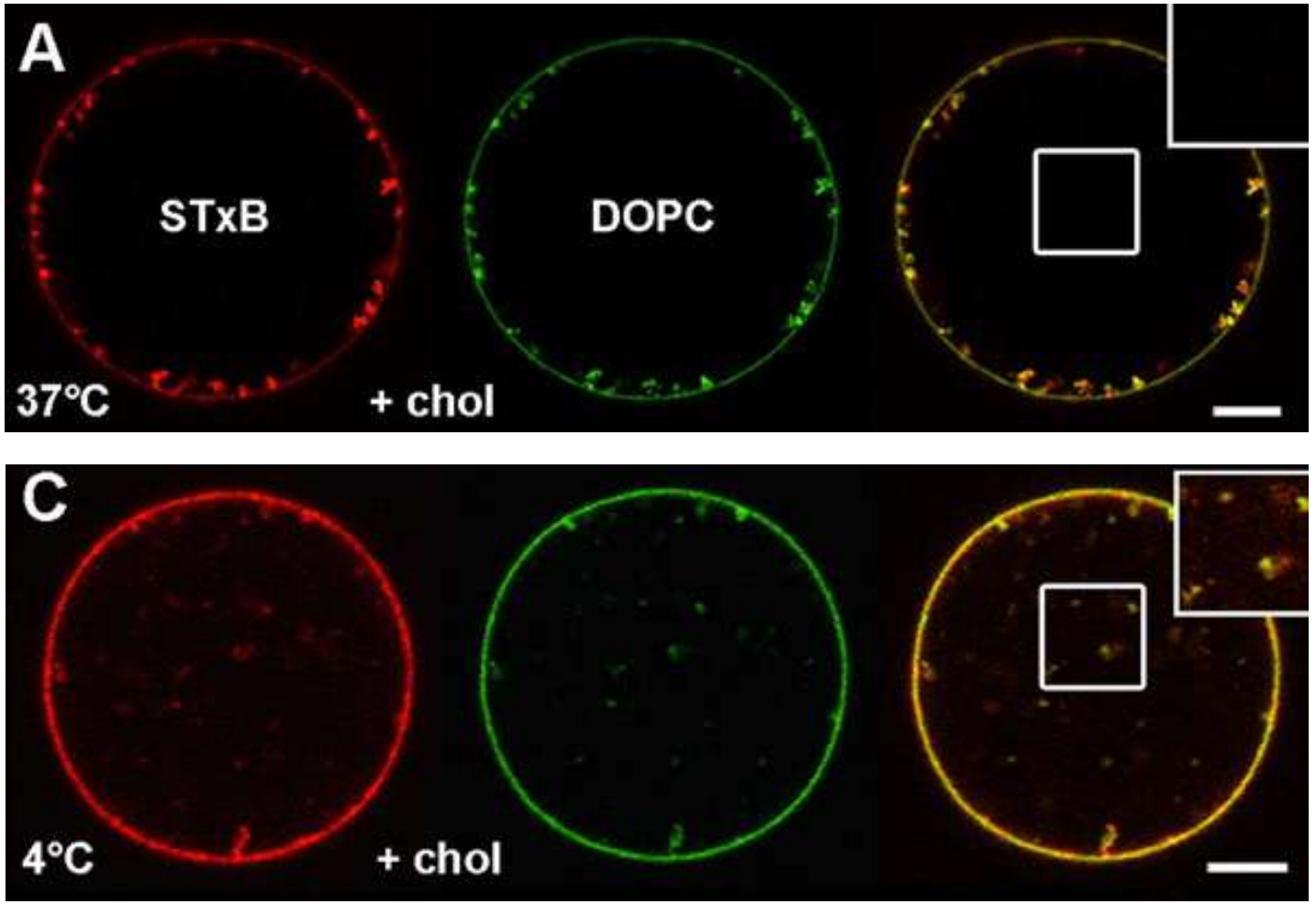
4°C
- Cholesterol

But not if cholesterol is decreased

W. Römer *et al., Cell* (2010)

Shiga Toxin Induces Fission of its own Tubules

DOPC + 5% HeLa Gb3(α-hydroxylated) + 30% Cholesterol

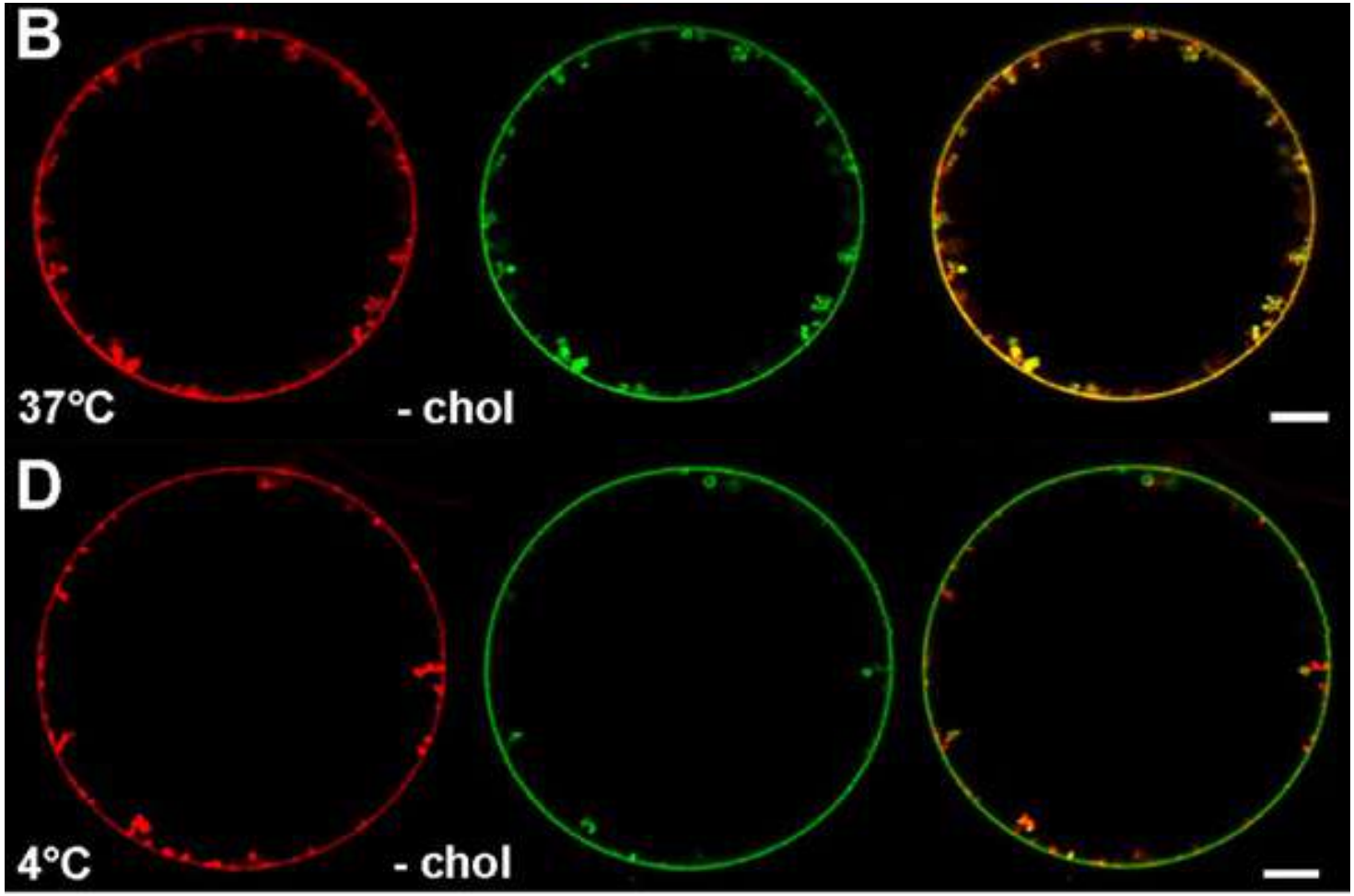


5 µm

W. Römer *et al., Cell* (2010)

No Fission in the Absence of Cholesterol

DOPC + 5% HeLa Gb3(α-hydroxylated) - *NO Cholesterol*

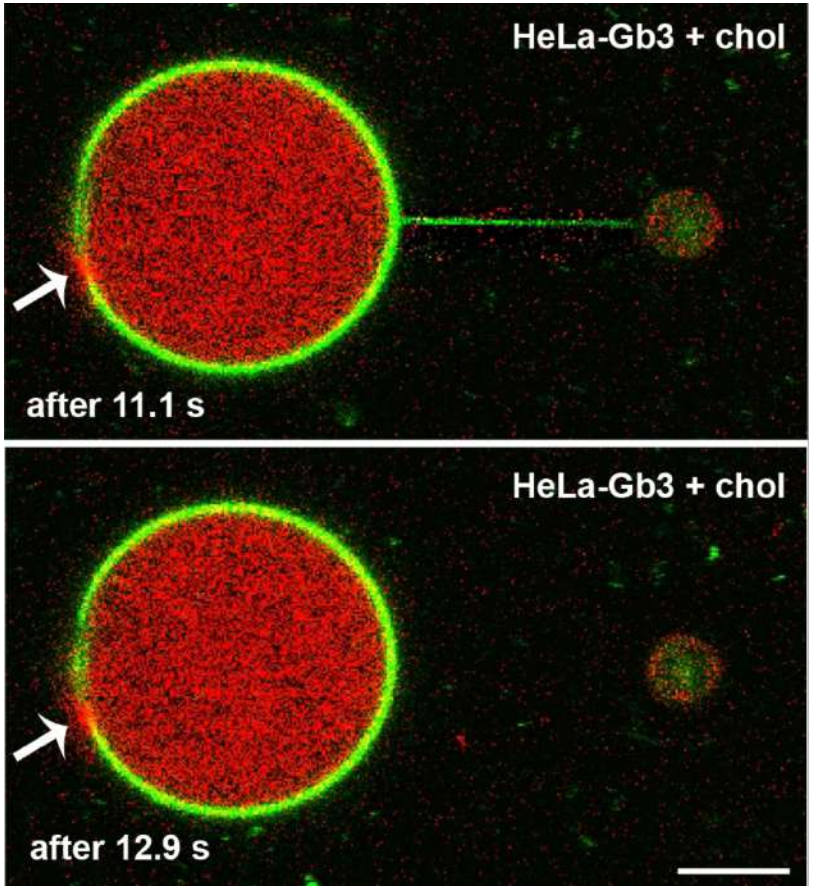


5 μm

W. Römer *et al., Cell* (2010)

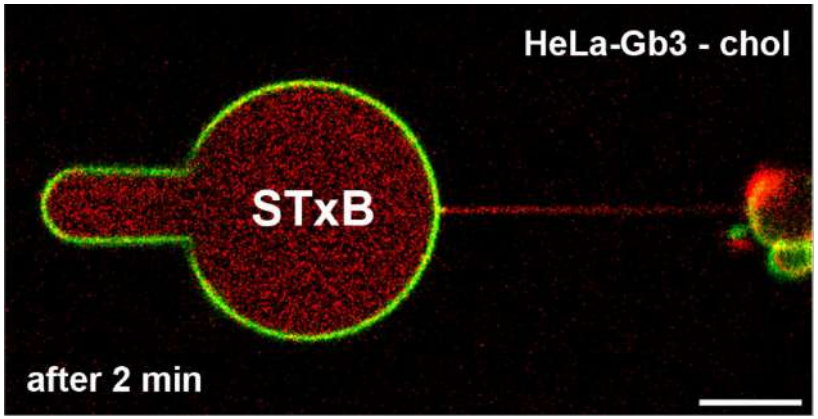
Line Tension-Induced Fission

+ cholesterol



Fission

- cholesterol



No domain ➡ no fission

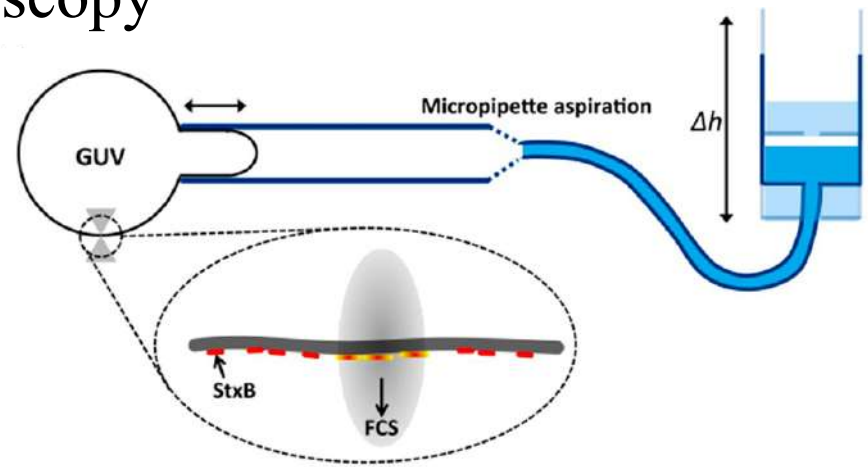
Encapsulated STxB

Fluorescent lipid

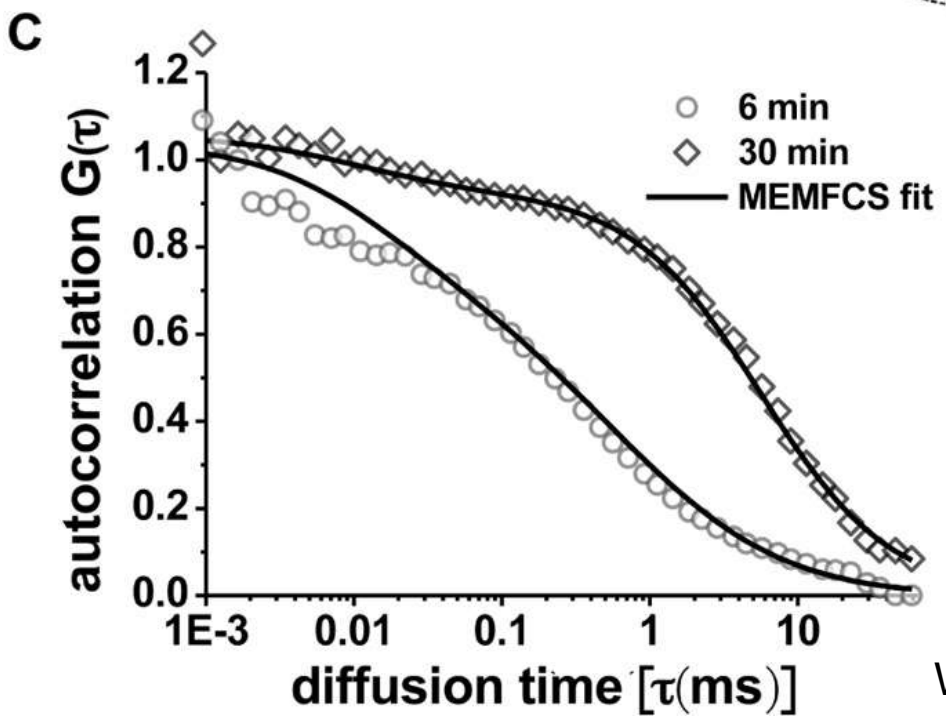
W. Römer *et al., Cell (2010)*

STxB Clustering

Fluorescence Correlation Spectroscopy



Autocorrelation function



Characteristic diffusion time τ_D

W. Pezeshkian et al. *ACS Nano* (2017)

$$\tau_D = \frac{r^2}{4D}$$ ⟵ Spot size

Diffusion coefficient D ⟷ Cluster size R

Small cluster size $$D = \frac{k_B T}{4\pi\mu_m h} \ln\left(\frac{R_{mem}}{R}\right)$$

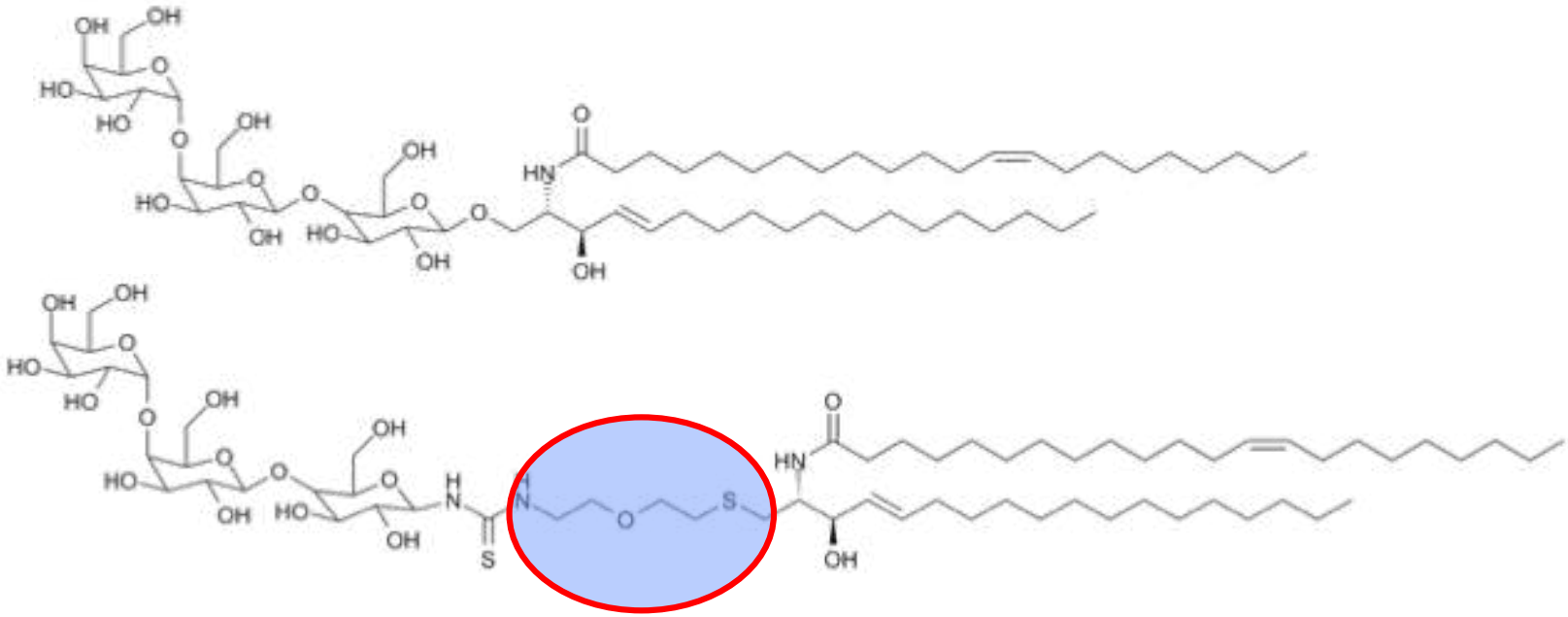
(for finite size membrane R_{mem})

Saffman and Delbrück, *PNAS* (1975)

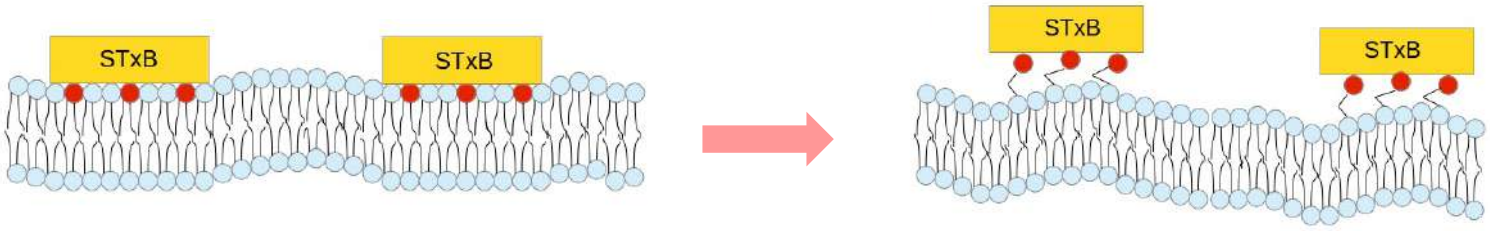
Large cluster size $$D = \frac{k_B T \lambda}{4\pi\mu_m h} \frac{1}{R}$$

Here R_{max}=180 nm (∼ 50 monomers)

Reduce Mechanical Coupling between Sugar and Ceramide



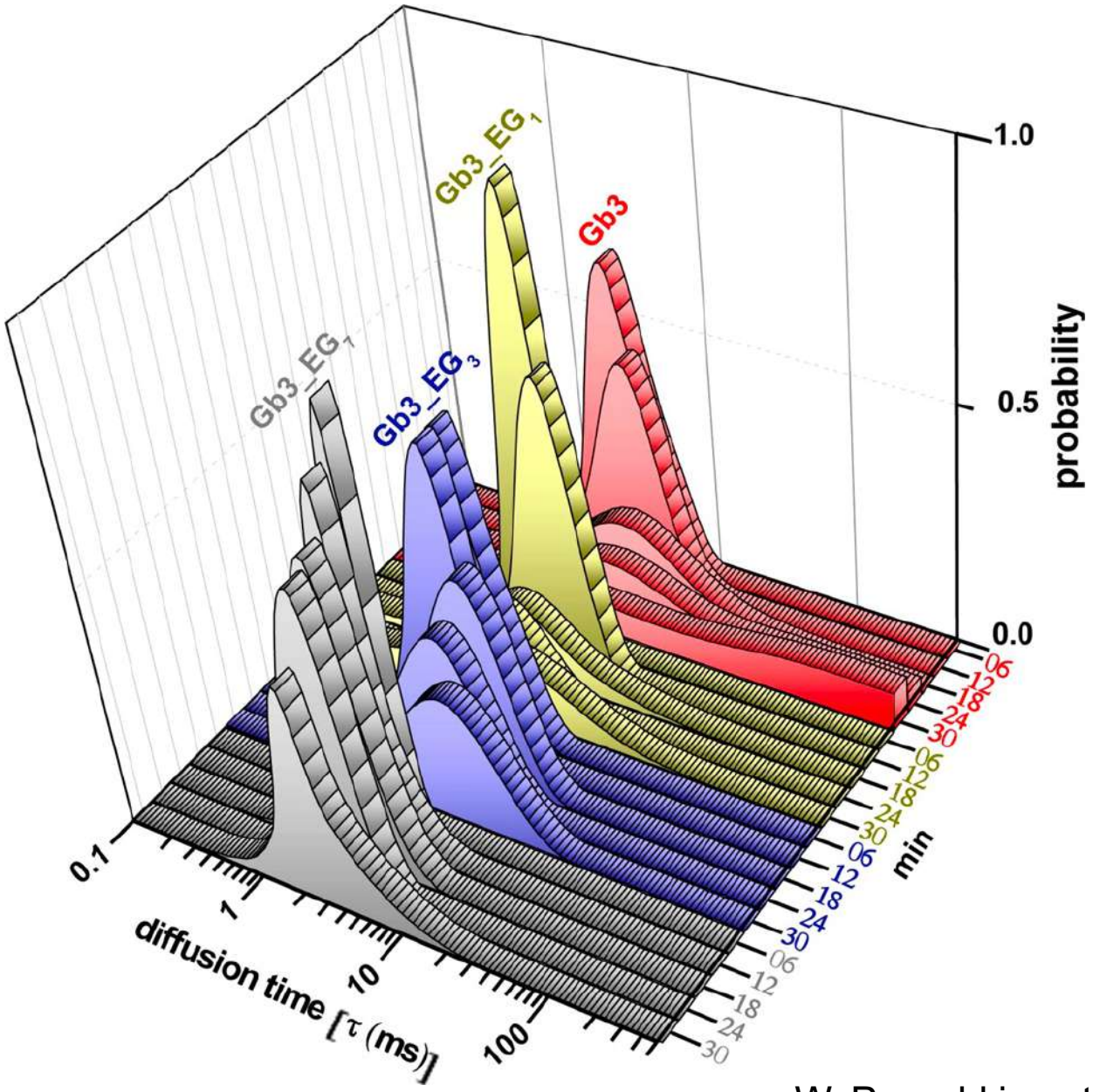
PEG linker: 1x, 3x, 7x



→ Reduce tight binding of STxB on the membrane

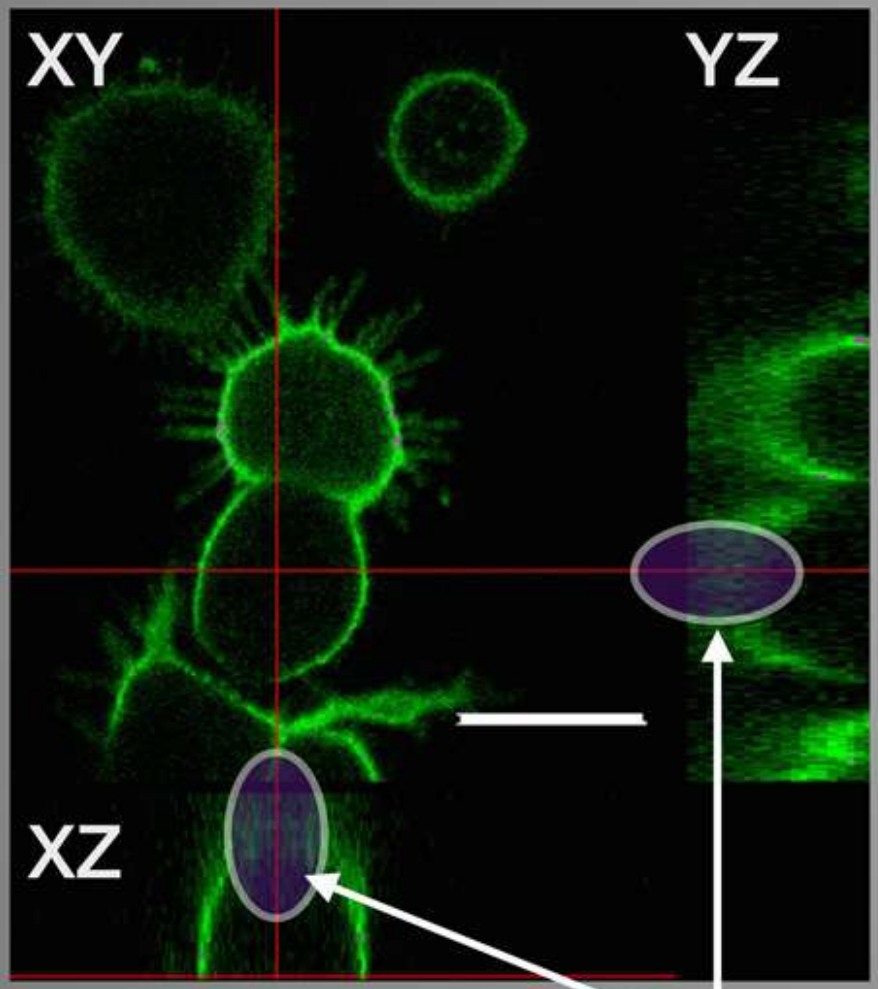
W. Pezeshkian et al. *ACS Nano* (2017)

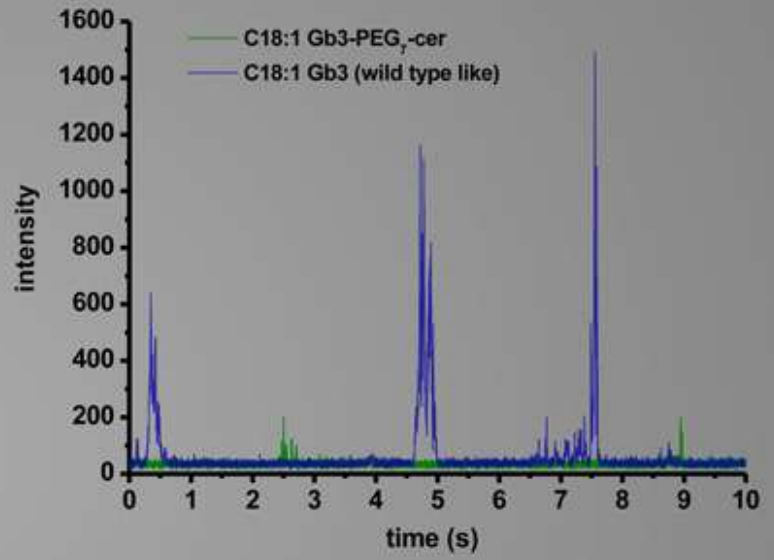
No Mechanical Coupling – No Clustering

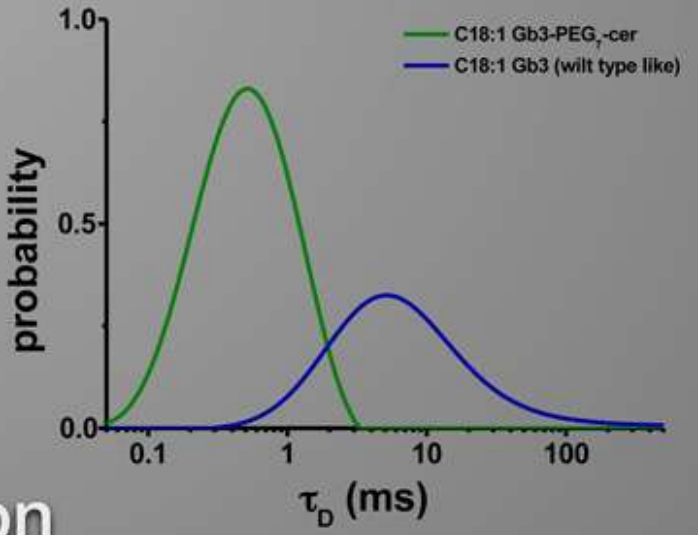


W. Pezeshkian et al. *ACS Nano* (2017)

Mechanical coupling is required for efficient clustering of STxB as measured by FCS

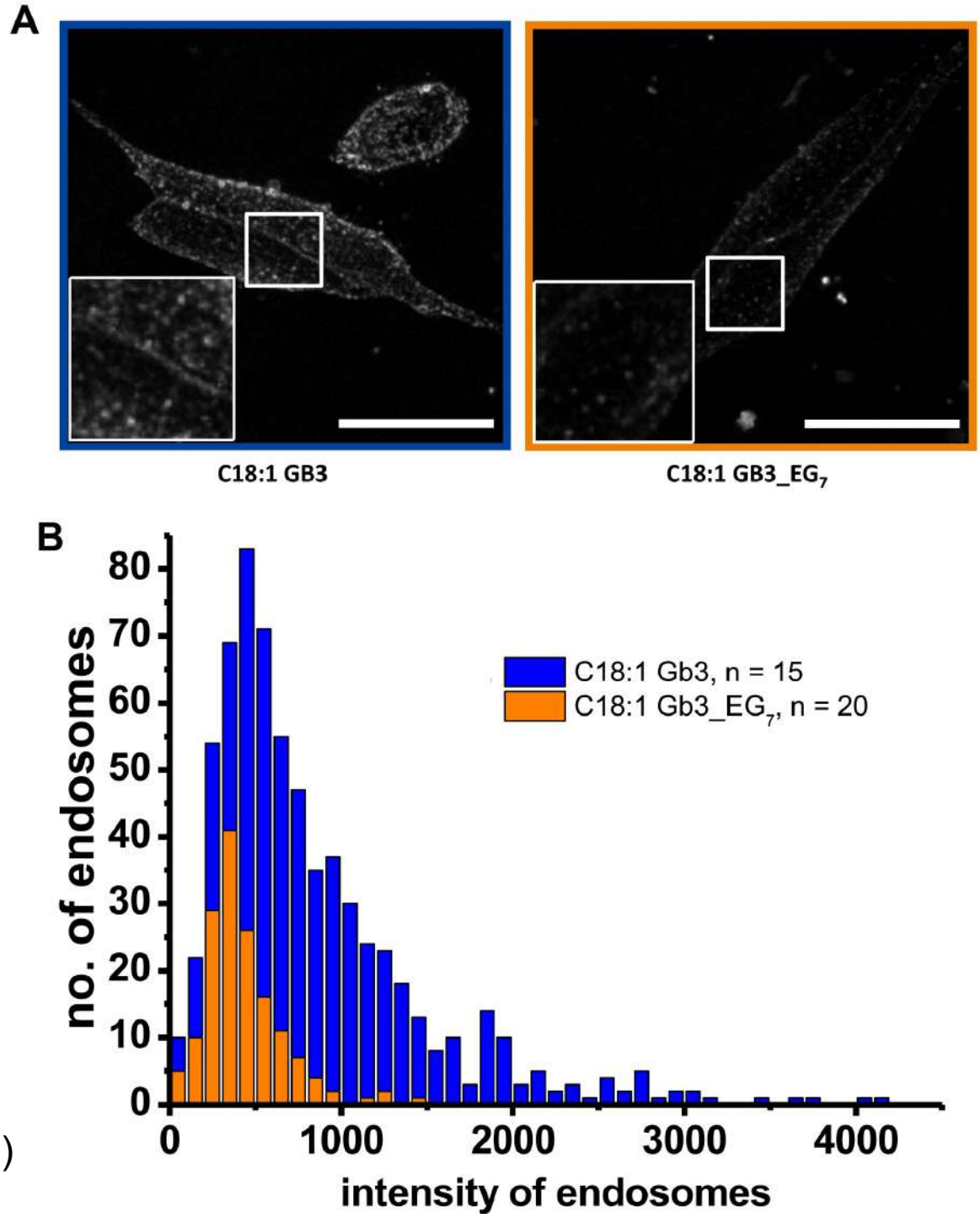






measurement region

Mechanical coupling is required for efficient endocytosis of STxB

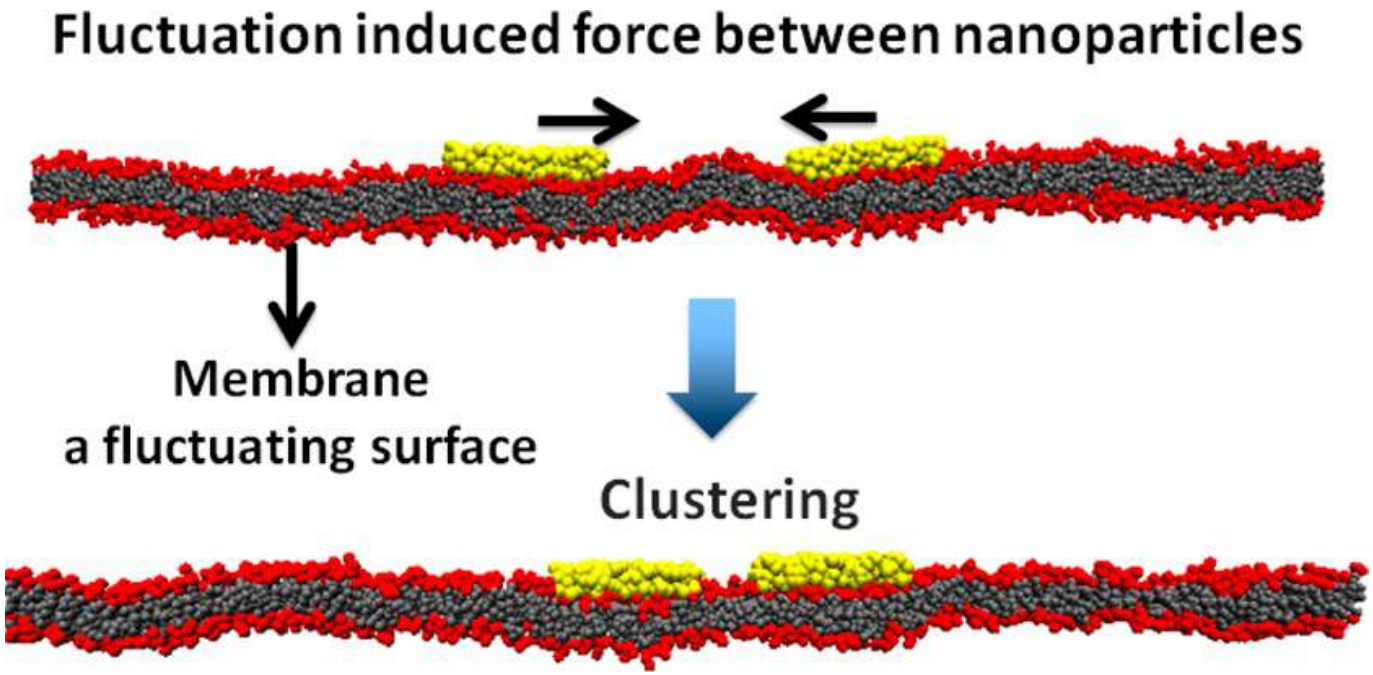


W. Pezeshkian et al. *ACS Nano* (2017)

Membrane Fluctuation Force

No hydrophobic mismatch (no line tension)

Membrane bending: repulsive force

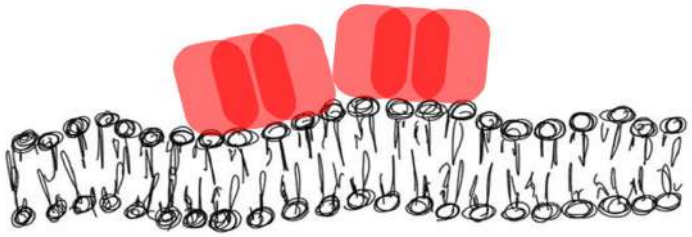


Force ~ a few pN

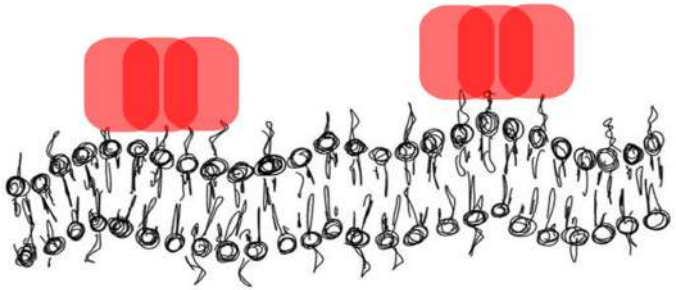
Range ~ protein size (5-10 nm)

W. Pezeshkian et al. *ACS Nano* (2017)

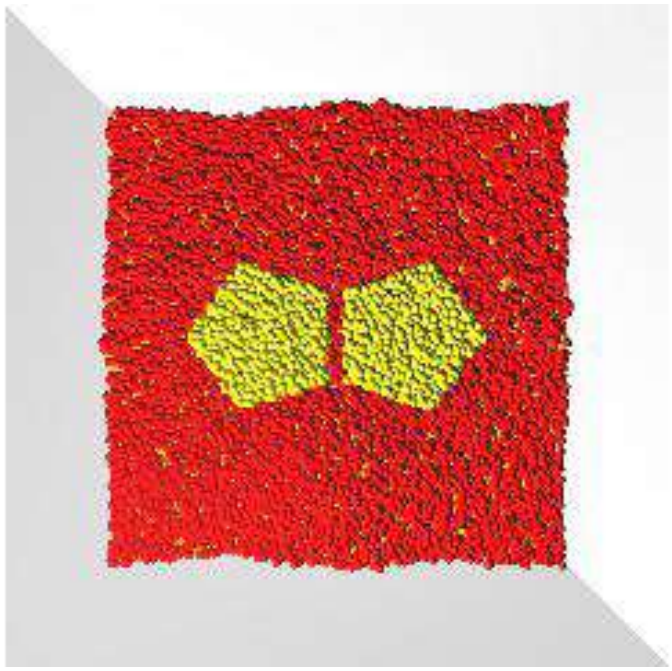
Membrane Fluctuation Force

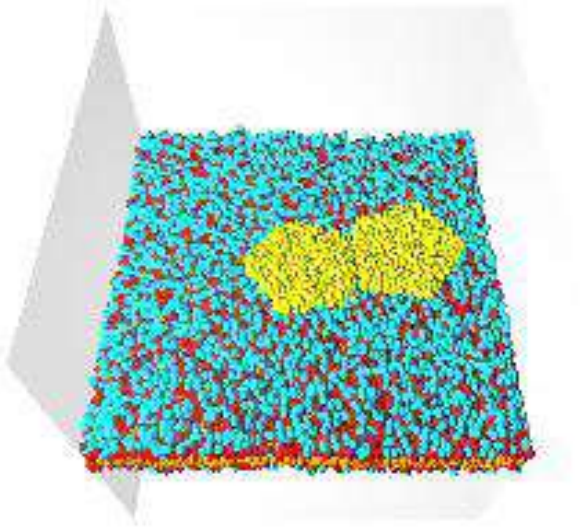


No linker



Linker

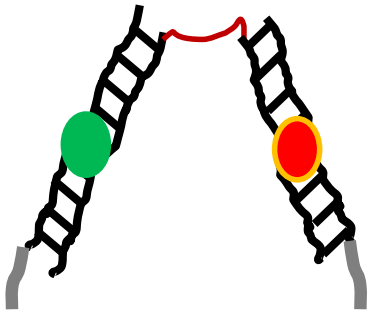




Coarse-grained simulation: *Dissipative Particle Dynamics (DPD)*

W. Pezeshkian et al. *ACS Nano* (2017)

DNA Nanosensor : STxB Clustering Energy



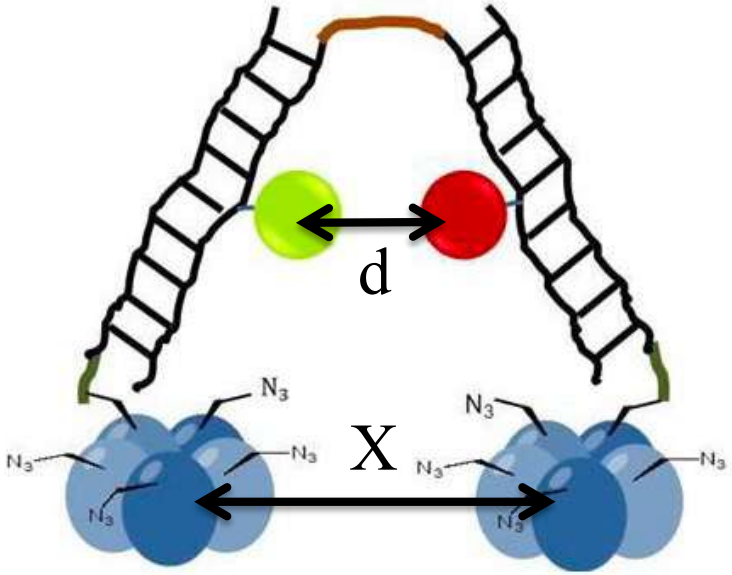
DNA nano-sensor

+

STxB

Conjugation

FRET
to measure distance



Free: d > 10 nm
NO FRET

STxB clustering:
d ≈ 4-8 nm FRET

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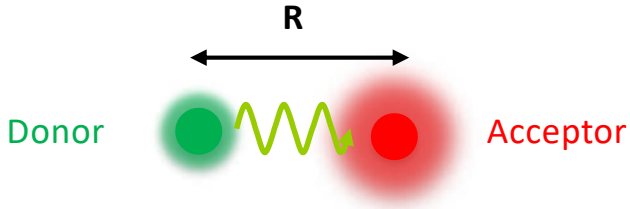
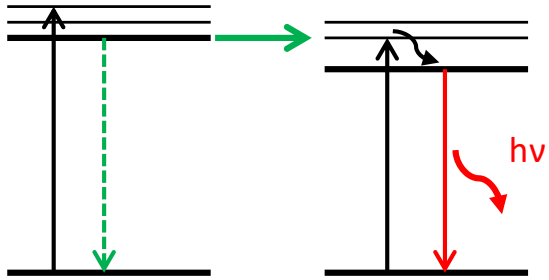
Principle of Förster Resonance Energy Transfer (FRET)

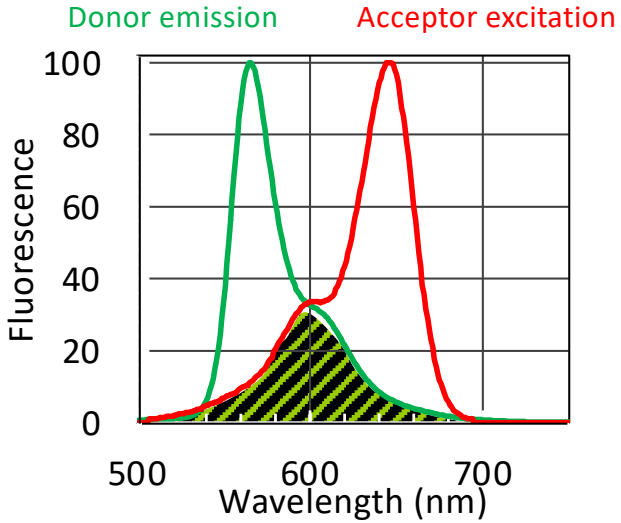




Transfer

Couple D/A
Förster radius: $\mathbf{R_0}$

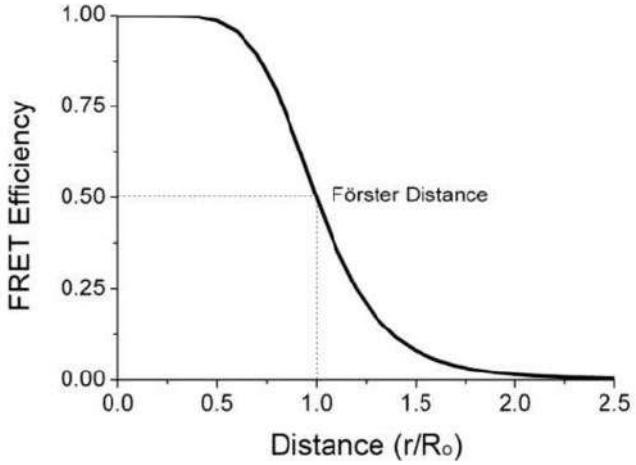
Distance between
D and A: **R**

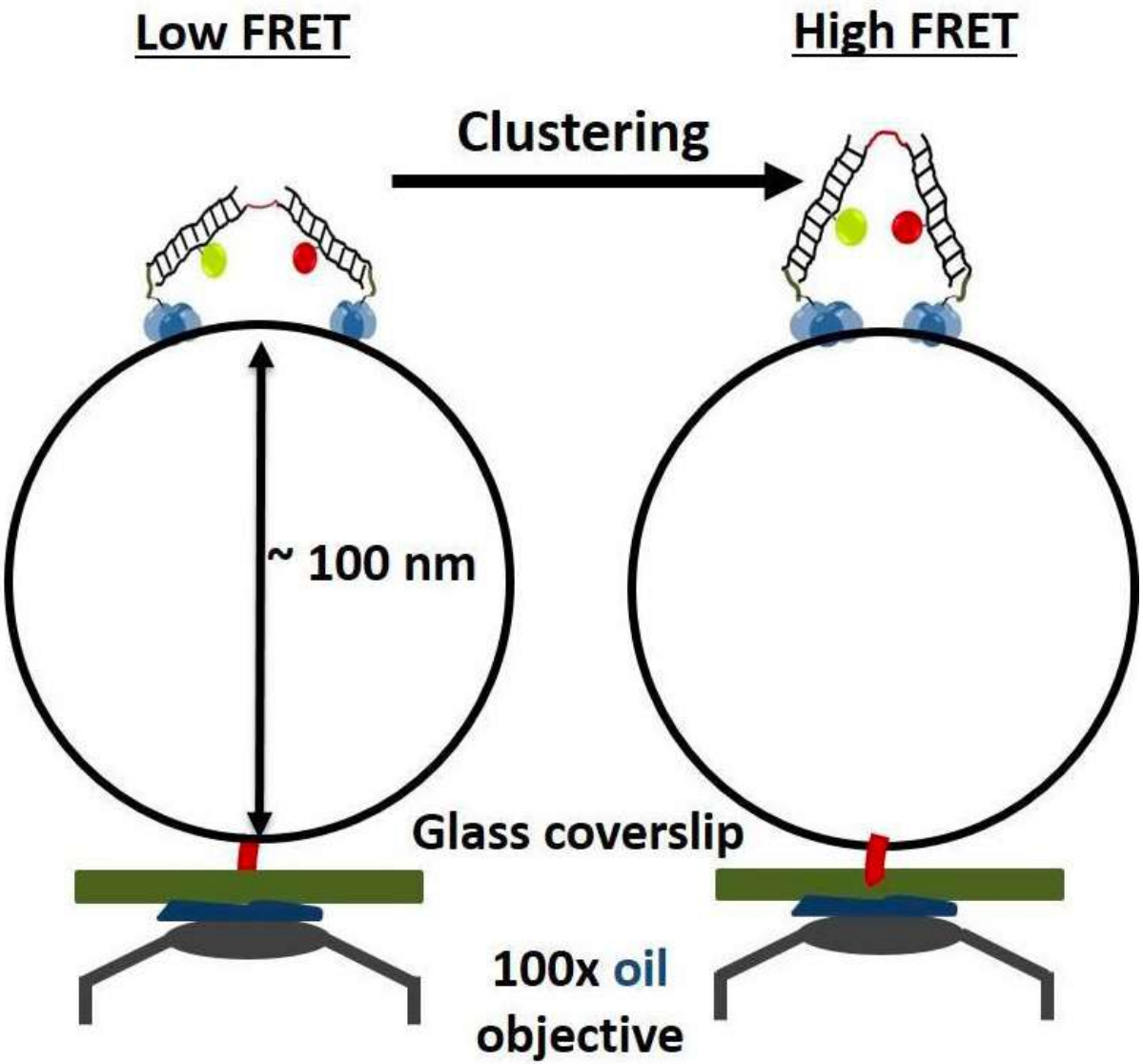


Spectral overlap
between D and A

$$E_{th} = \frac{R_0^6}{R_0^6 + R^6}$$

DNA Nanosensor : STxB Clustering Energy





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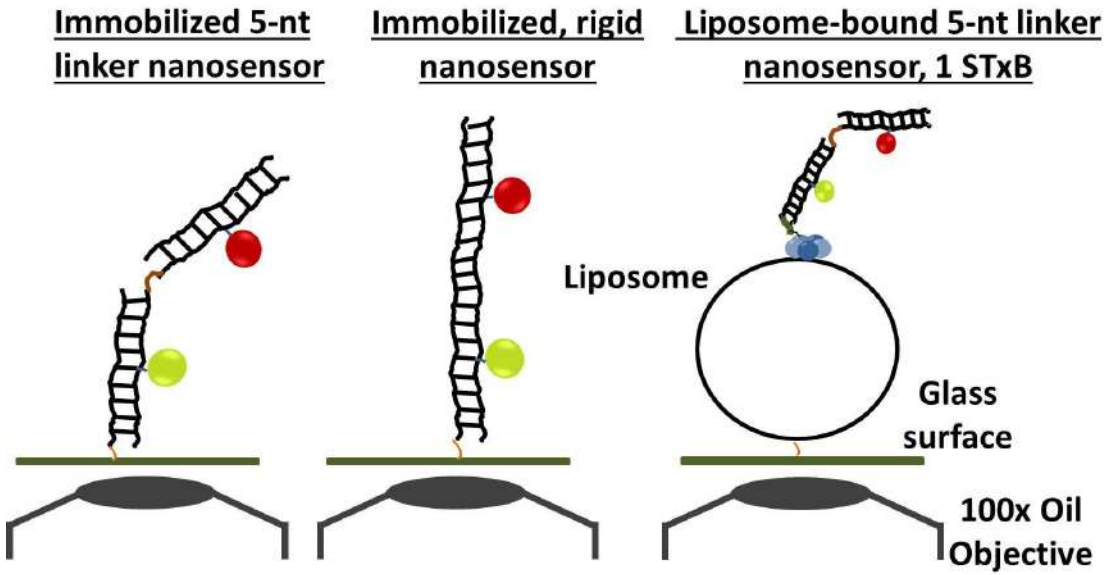
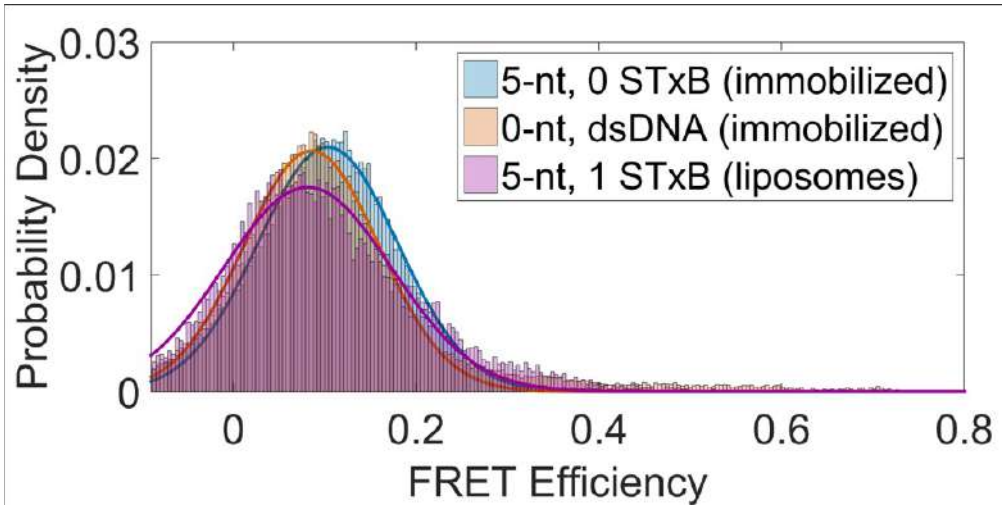
No Attraction
if the 2 STxB are not bound to a Membrane





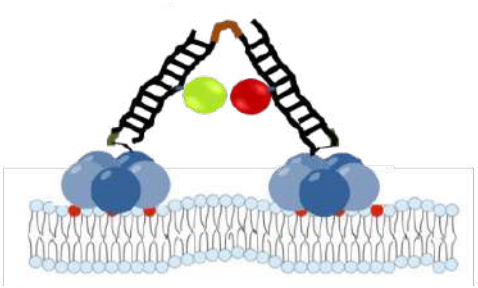
In solution

E= 0.132 (no STxB)

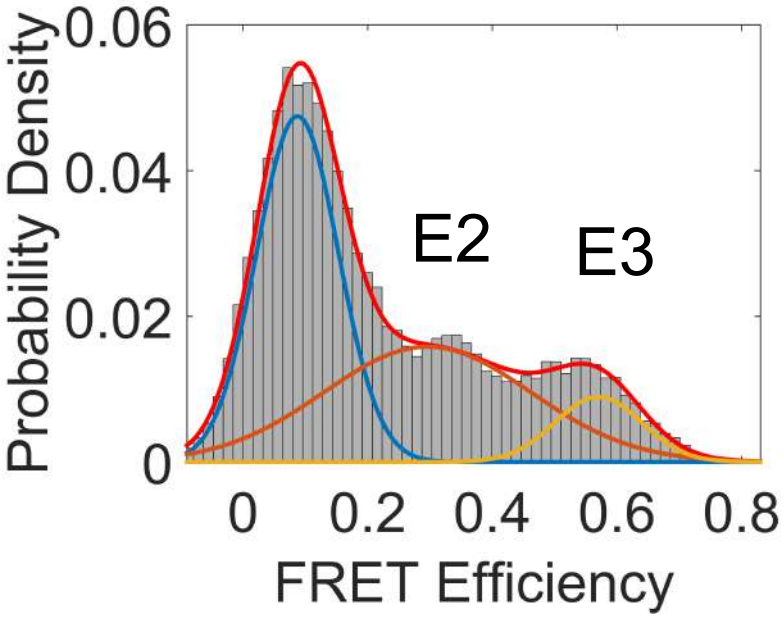
E=0.138 (with STxB)

Attraction when both STxB are bound to Membrane

Natural Gb3



E1 (single arm attached)



E3 = 0.571

E2 = 0.294

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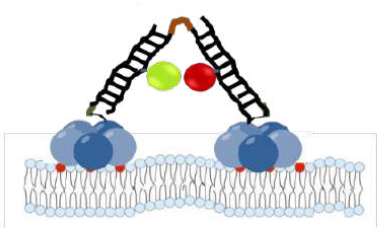
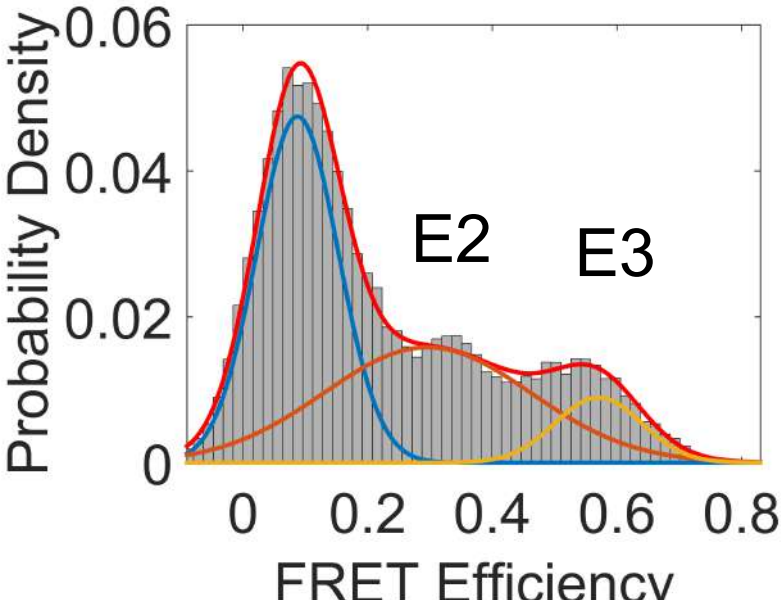
Reducing Mechanical Coupling Reduces Attraction

Natural Gb3



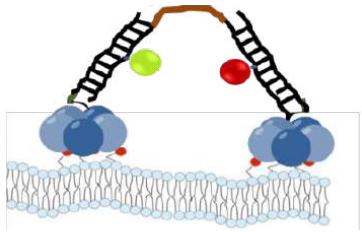
E1 (single arm attached)

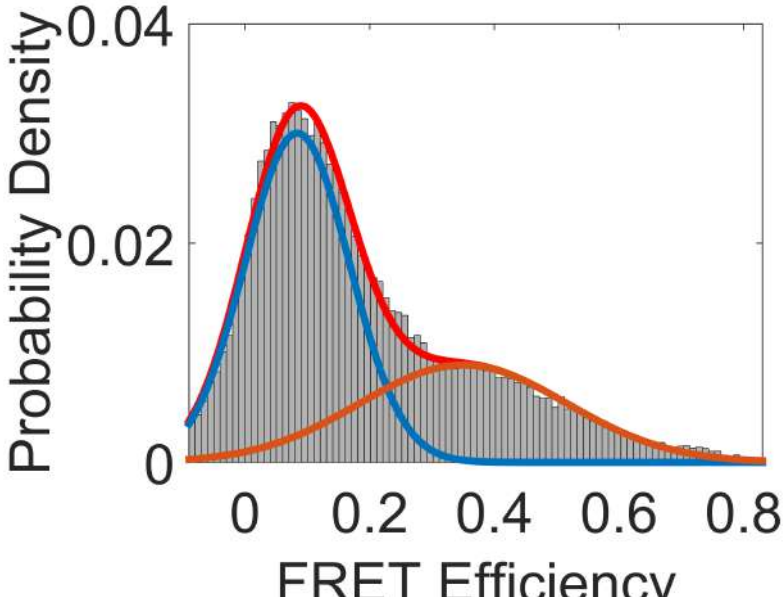


E3 = 0.571

E2 = 0.294

Gb3-EG$_7$





E3 = 0.351

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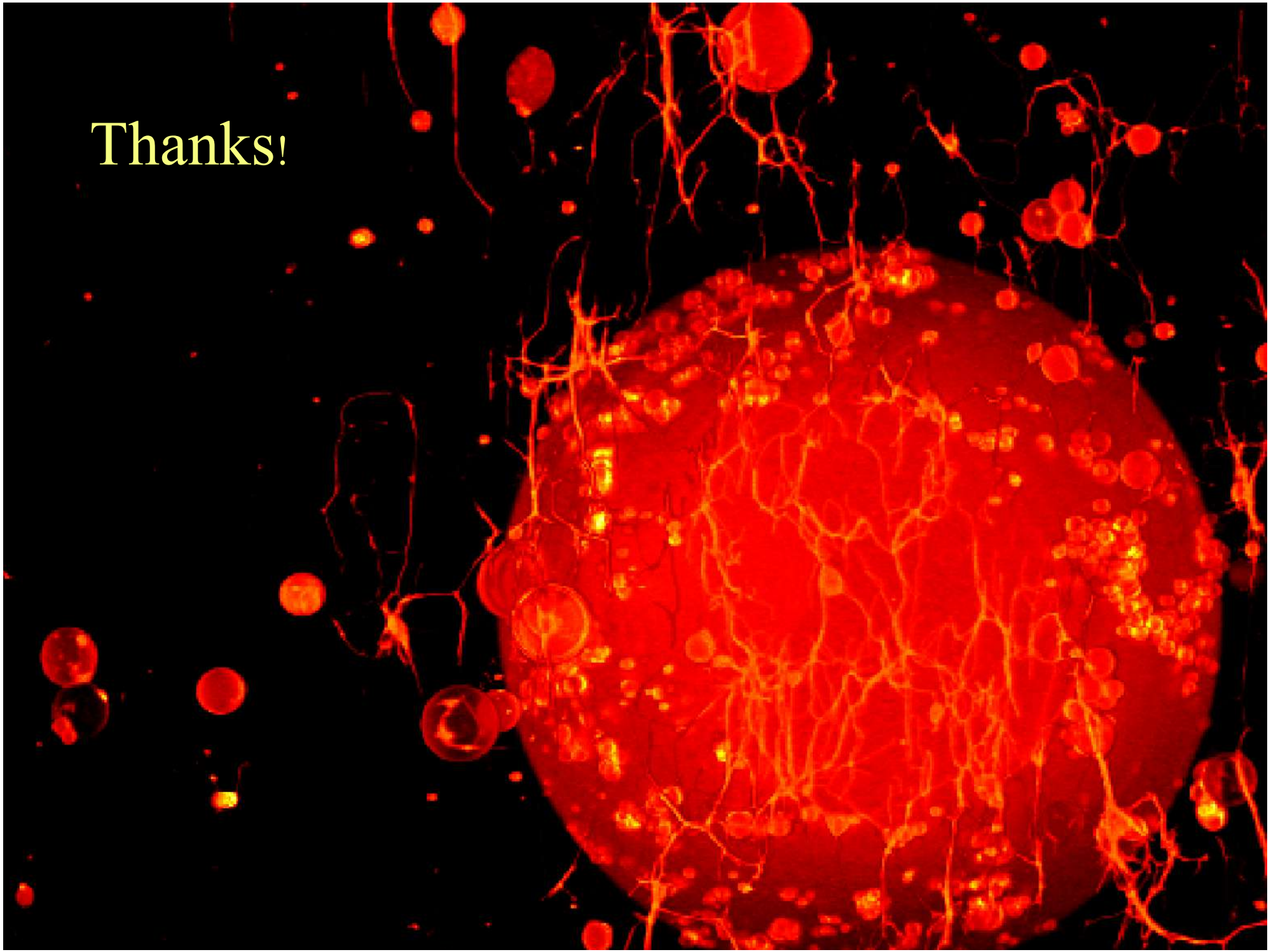
In progress:

Simulations and theory (Neda Rahmani, W. Pezeshkian and J. Ipsen)

Calculation of the attraction energy

Prediction of fluctuation force

Comparison with our FRET measurements


Thanks!