Protein Self-Organization

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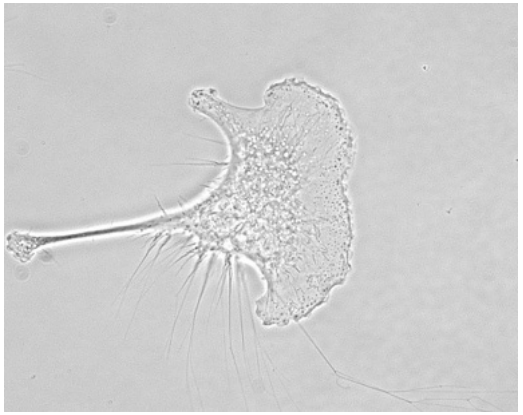
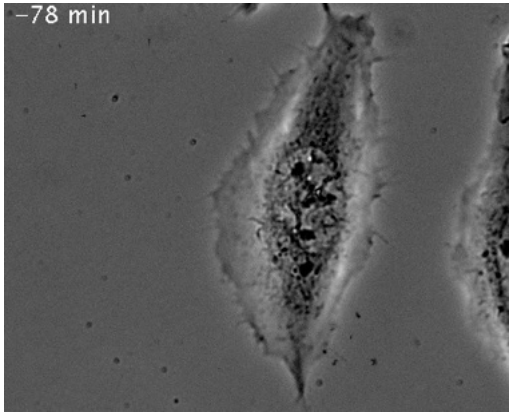
Cells can Change their External Shape and Interior

Macrophage motion



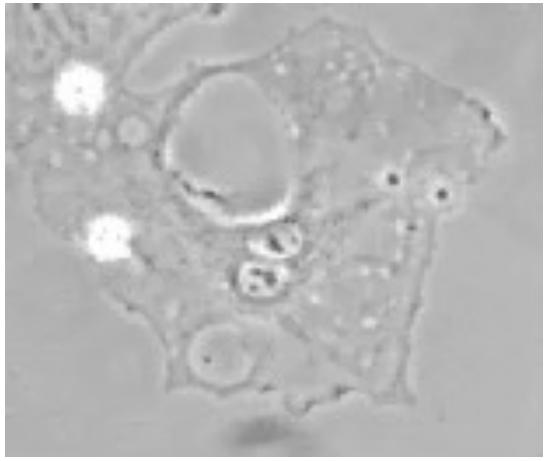
From P. Matsudaira, MIT

Cell division



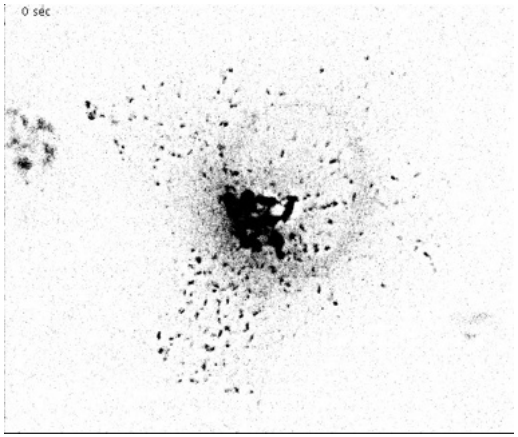
From A. Echard, Inst. Pasteur

Tunnel opening and healing



Boyer *et al.*, *J. Cell Biol.* **173**, 809 (2006)
E. Lemichez (Inst. Curie)

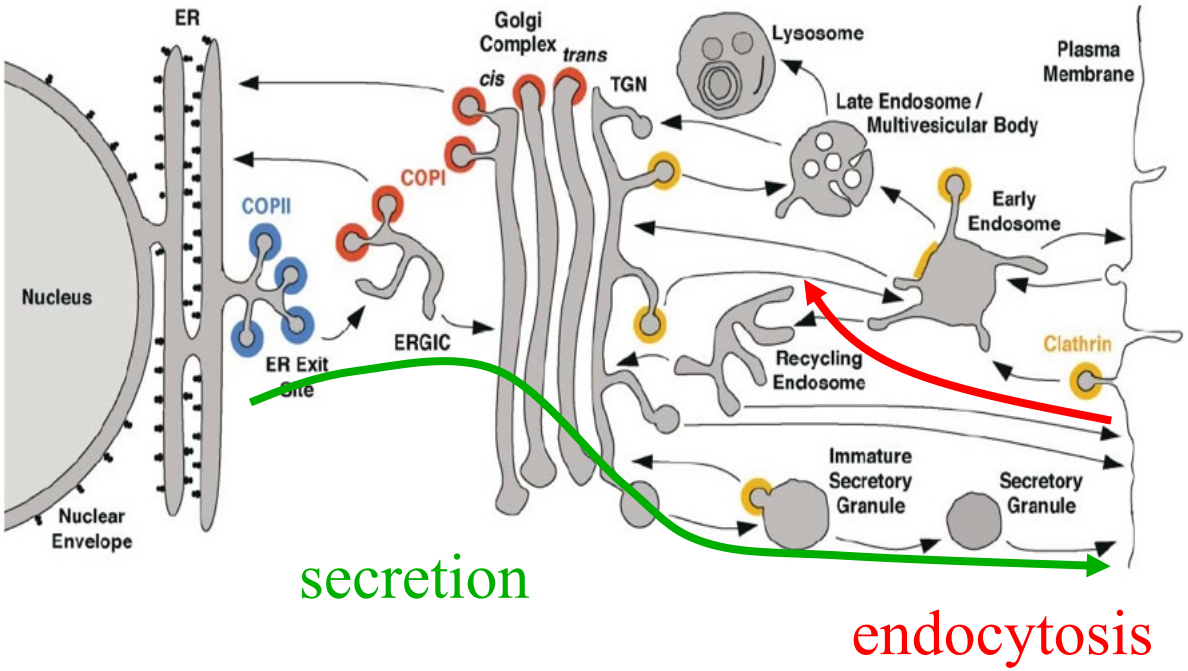
Traffic around Golgi



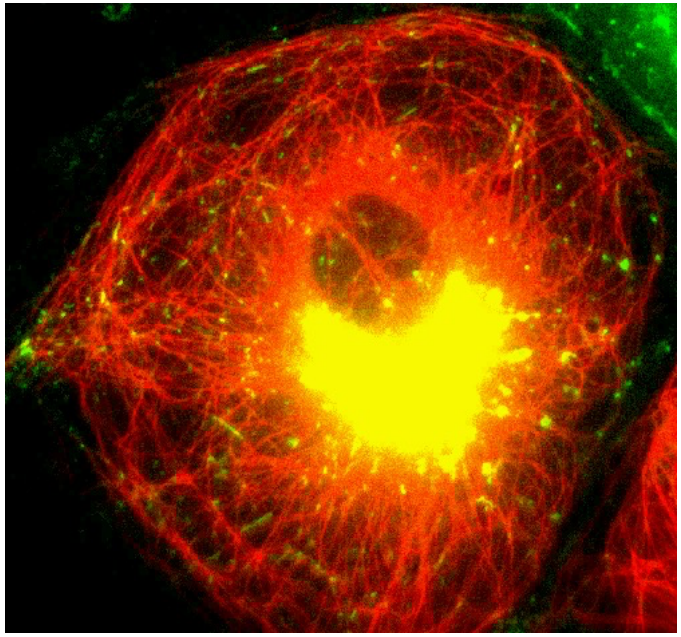
S. Miserey-Lenkei *et al.*, *Nat. Cell Biol.* **12**, 645 (2010)
B. Goud (Inst. Curie)

Shape results from interplay between membrane and cytoskeleton

Constant Traffic in Cells



Bonifaccio and Glick
Cell (2004)

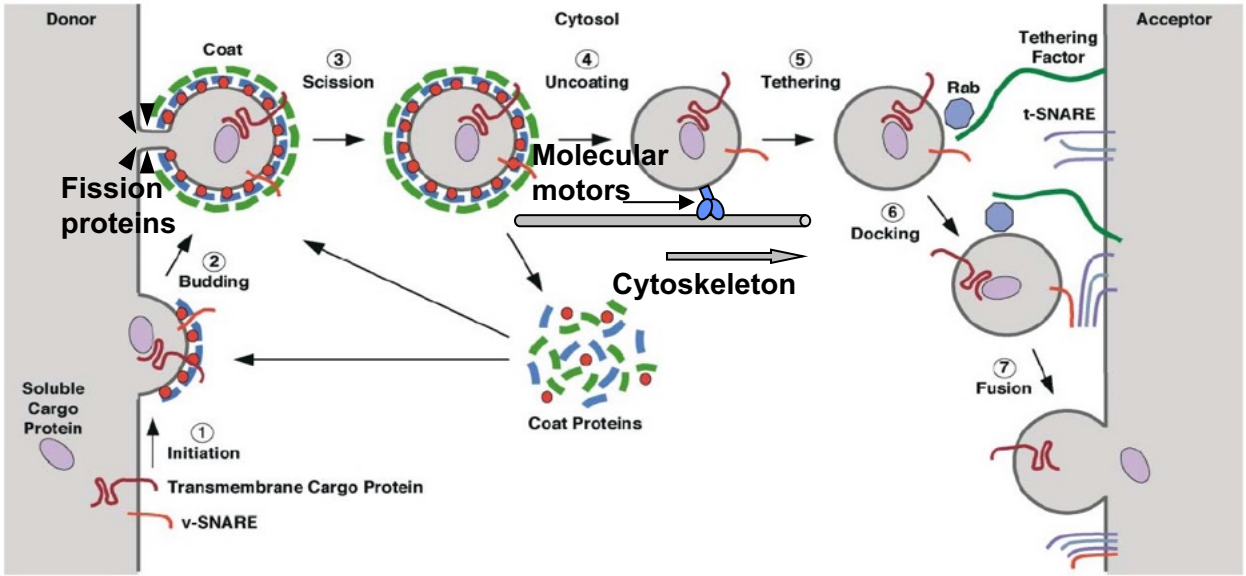


Toomre et al
J. Cell Sci. (1999)

Red: Microtubules
Green: Golgi (VSV-G)
Overlap: yellow

Formation of small vesicles, tubes:
requires to *strongly deform* membranes

Different Steps of "Vesicular" Transport



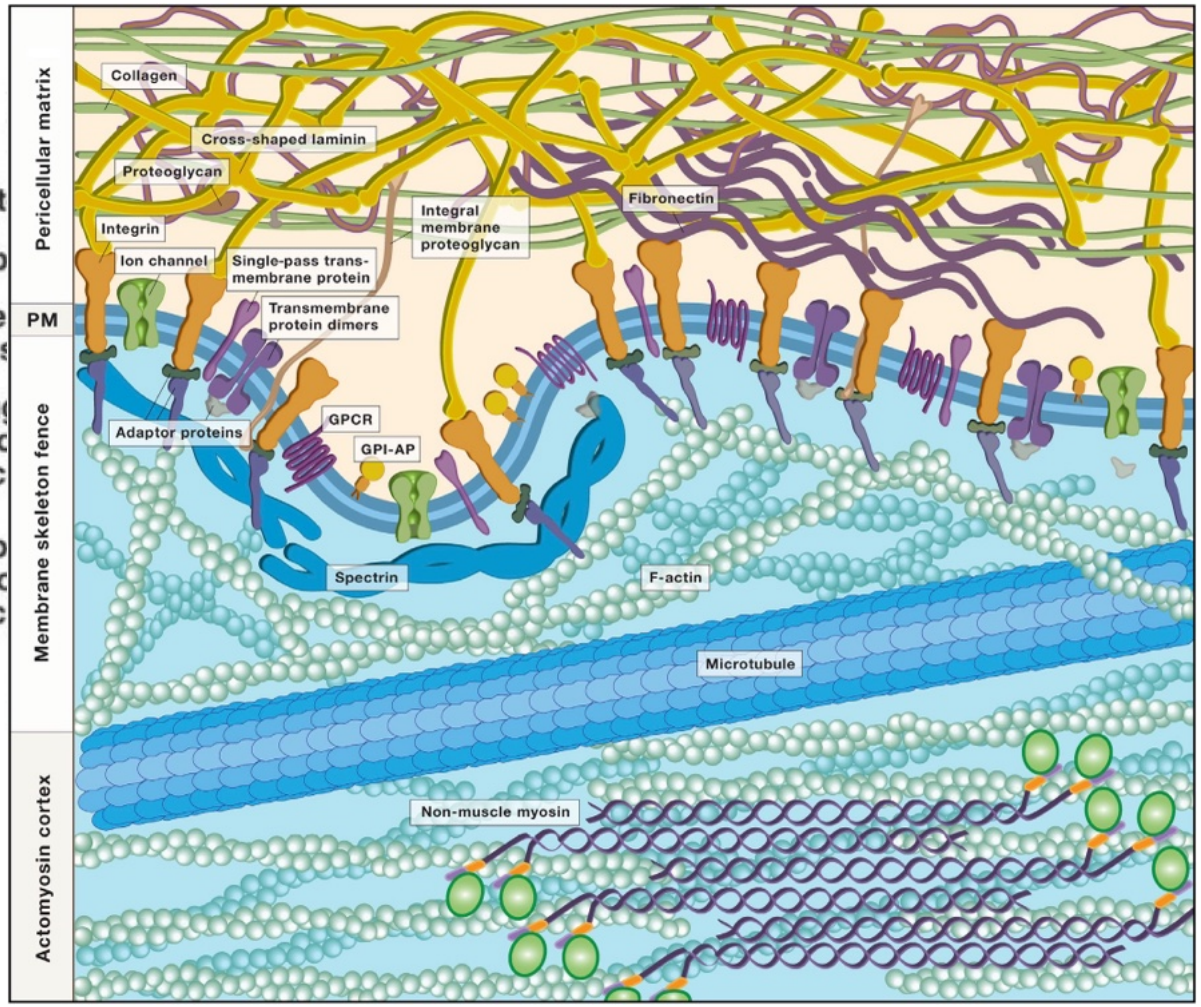
Bonifaccio and Glick
Cell (2004)

1) Sorting (Proteins and lipids) + Budding
2) Fission
3) Transport (Microtubules)
4) Targeting-Docking
5) Fusion

Cell Envelop: the Plasma Membrane

Outside

Inside



Lipid Membrane

~5 nm

K. Jacobson, ….B. Lagerholm, *Cell* (2019)

Lipid membrane reinforced by the actin cortex

Membrane Basis: Lipid Bilayer

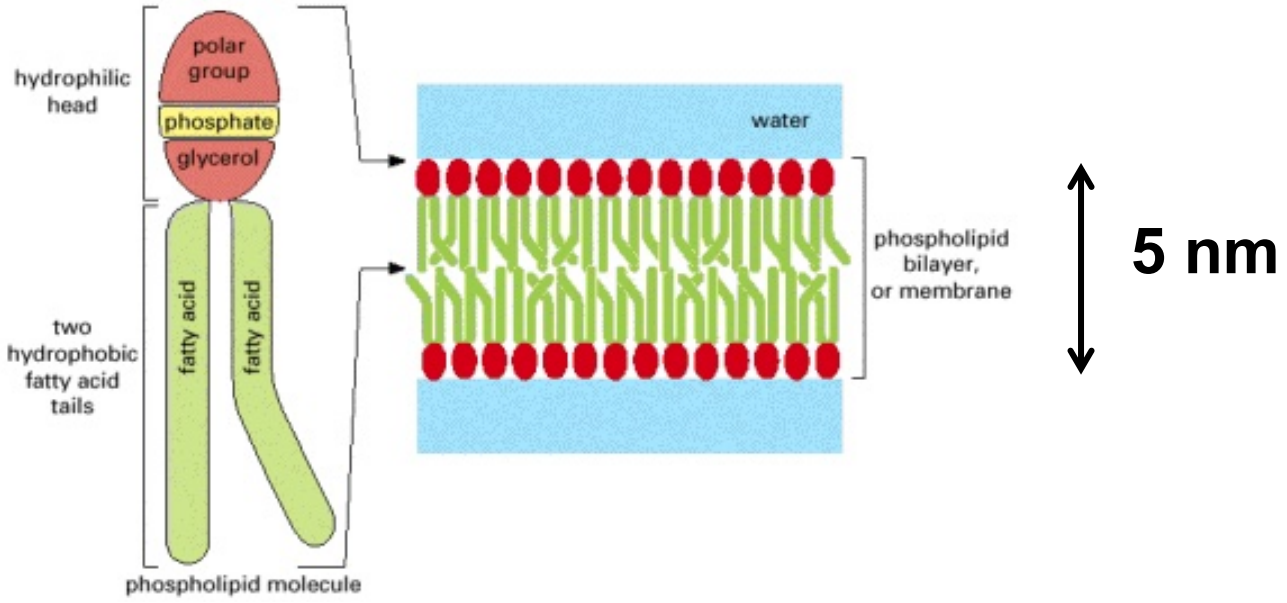
Self-assembly of *amphiphilic* molecules

Hydrophilic head group

Hydrophobic chains

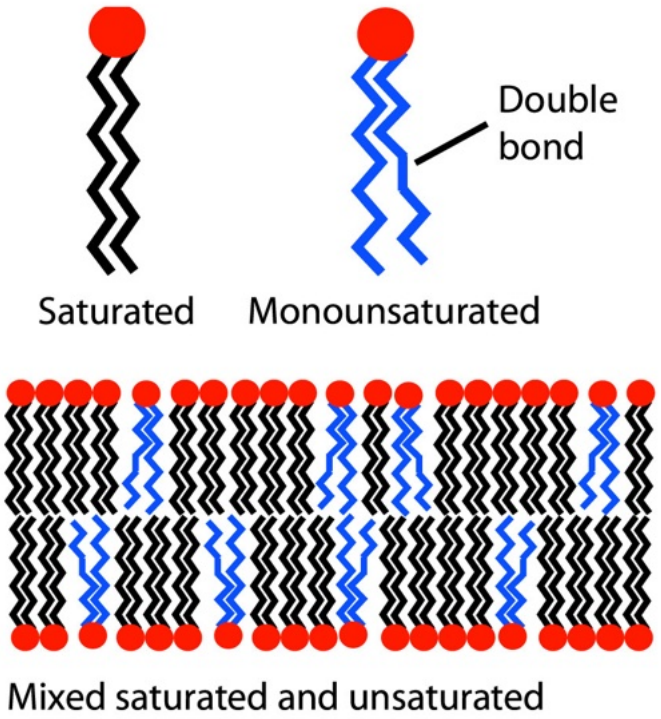
.....

Lipid bilayer



Membrane – Lipid Bilayer

Chains: fully saturated
or with unsaturations

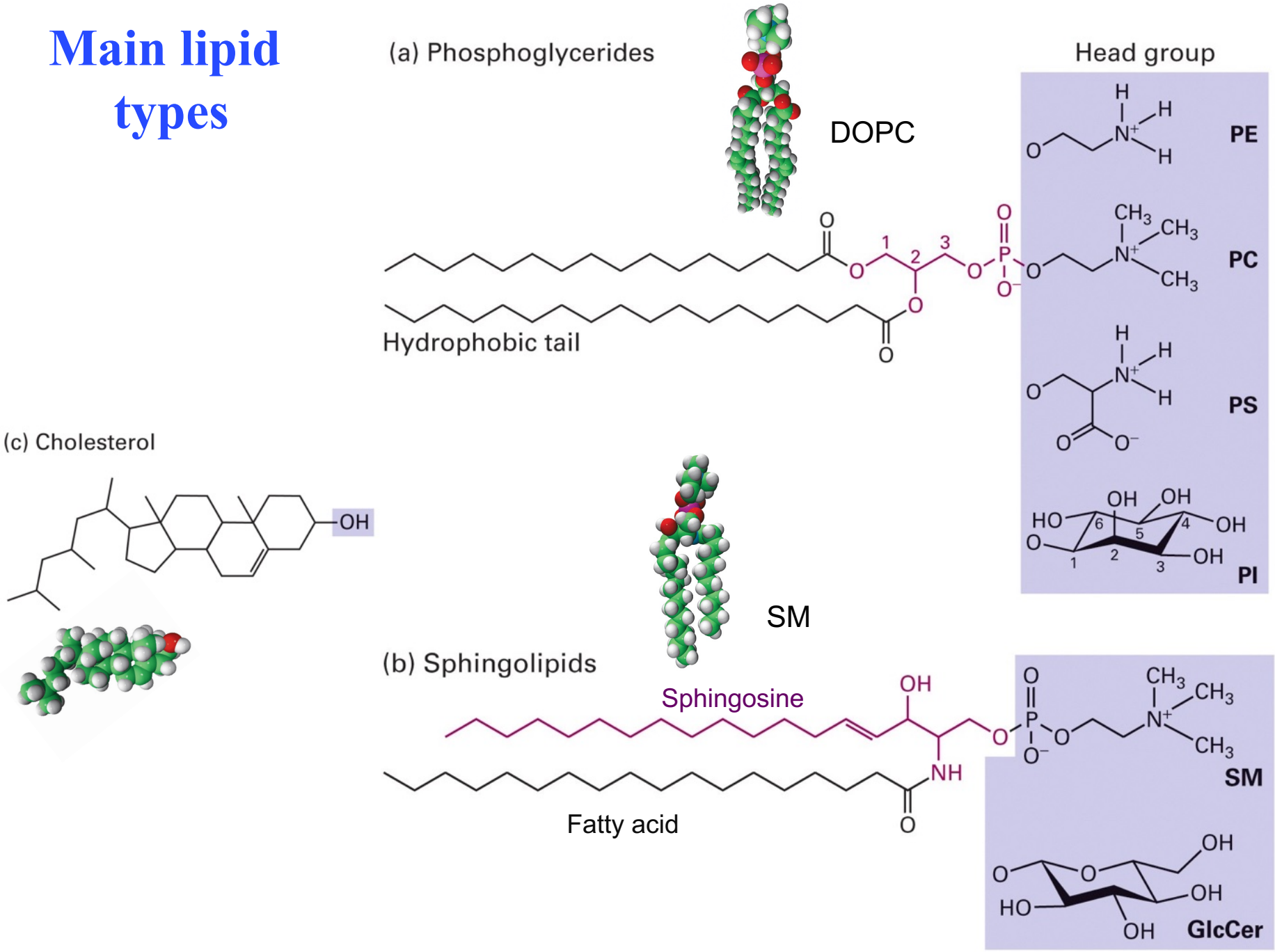


Commonly found in lipid membranes:

Saturated chains: C12 to C20

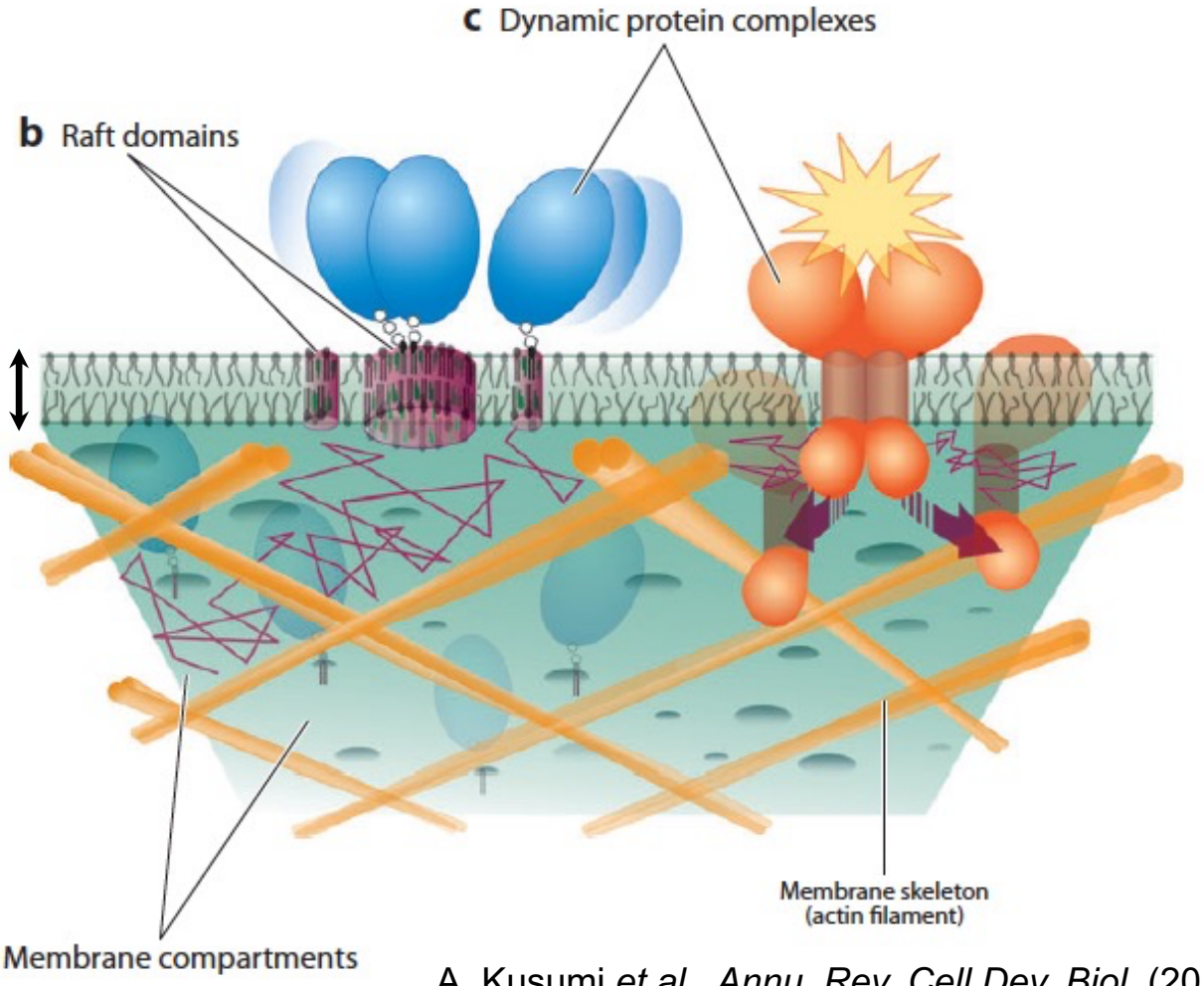
Unsaturated chains: C16:1, C18:1, C18:2, C18:3, C20:2, polyunsat. C22, C24 etc…

Main lipid types



Plasma Membrane: Heterogenous 2D-Fluid

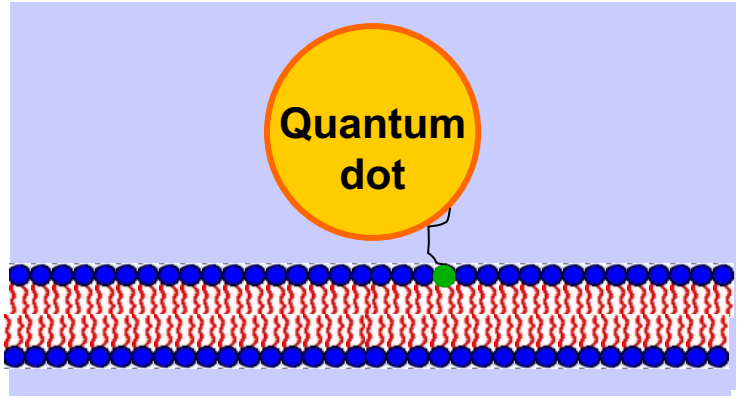
Lipid bilayer

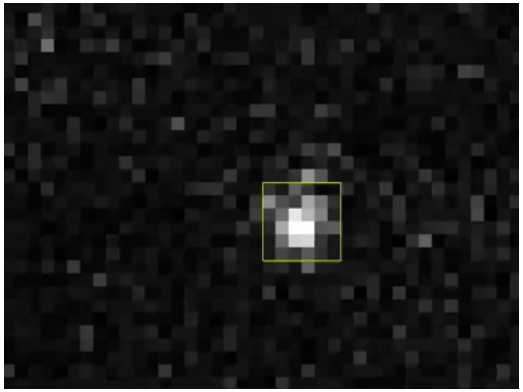


A. Kusumi *et al.*, *Annu. Rev. Cell Dev. Biol.* (2012)

Membrane: *Fluid= 2D liquid* (viscosity ~ 100 times water)

Membranes = 2-Dimensional Fluids

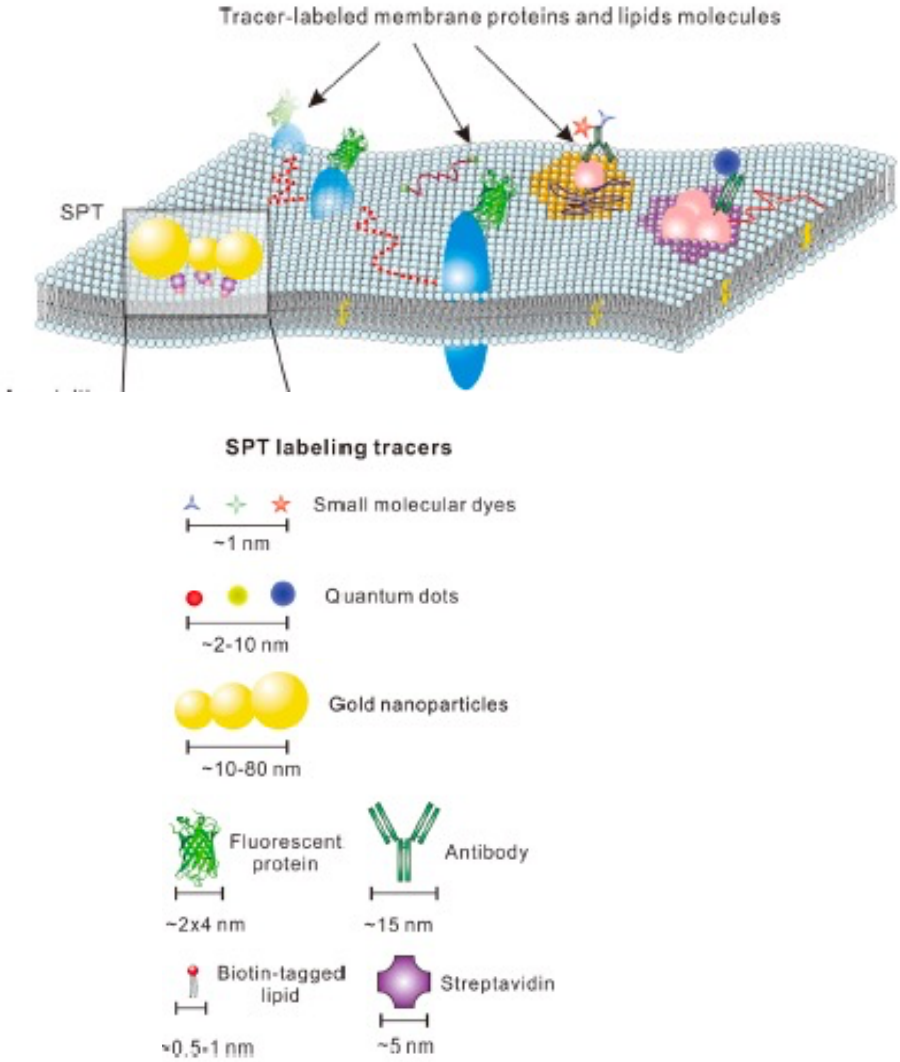




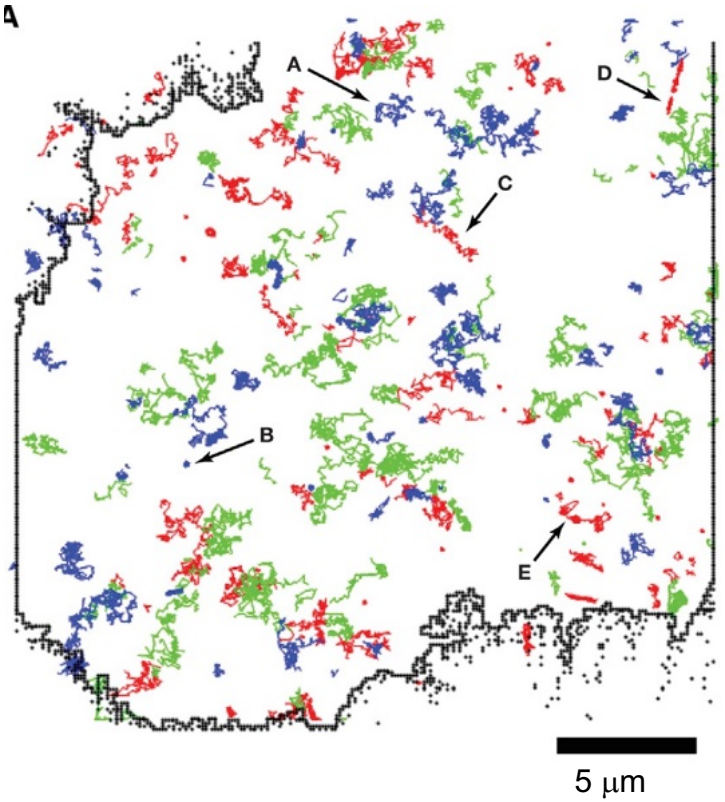
Single Particle Tracking

Heterogeneous Diffusion in the Plasma Membrane

Single Particle Tracking at the PM



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

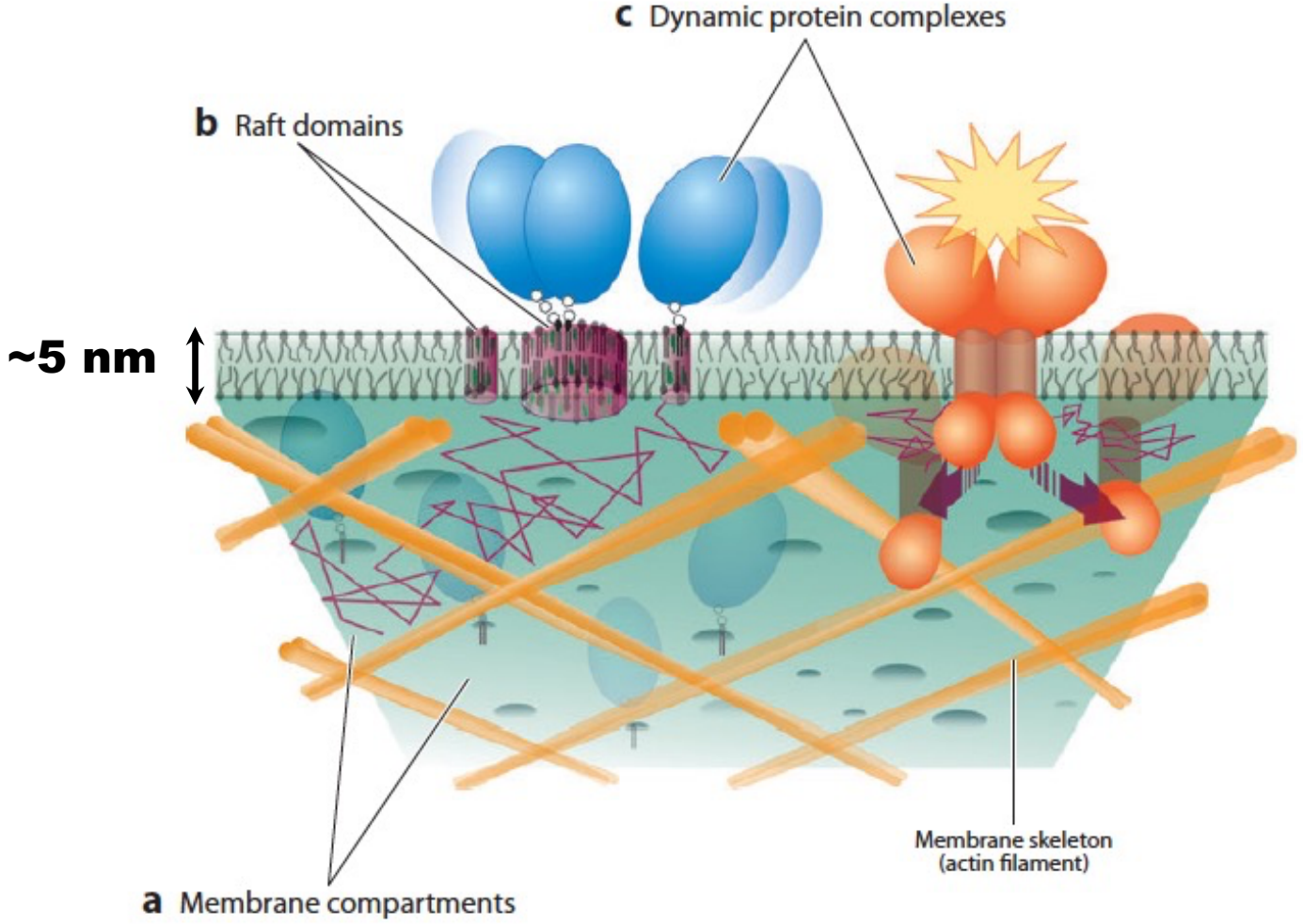


K. Jacobson et al *Cell* (2019)

Markers are mobile

But ***heterogeneous*** mobility

Plasma Membrane: Heterogenous 2D-Fluid



A. Kusumi *et al.*, *Annu. Rev. Cell Dev. Biol.* (2012)

Lipids, high density of membrane proteins (inclusions, bound)

Inhomogeneities (lipid rafts, protein clusters …)

Confinement due to cortical actin filaments

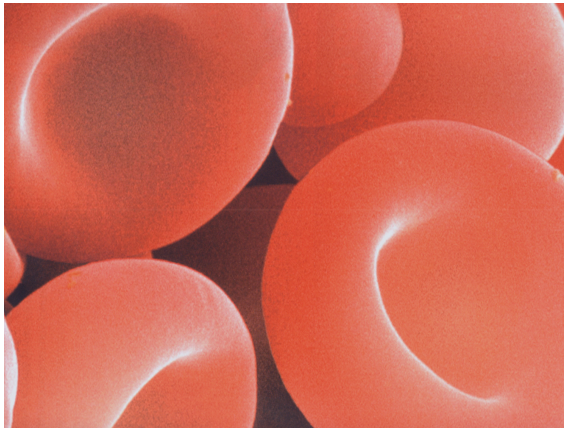
Lipid Membrane Mechanics

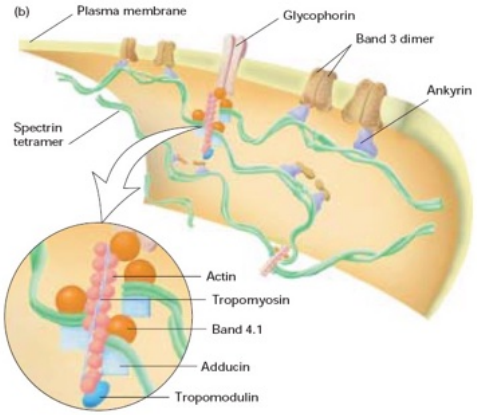
Objective: *modeling cell membranes*

W. Helfrich, E. Sackmann, E. Evans (>1973) etc…

Model membrane systems required to test theoretical models

1st model system available =Red Blood Cells





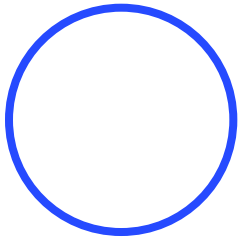
Still too complex…..

In the 80', first developments of giant liposomes with a single lipid type

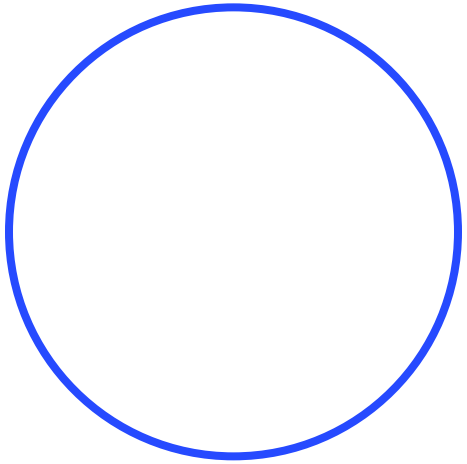
Model Membranes

Liposomes

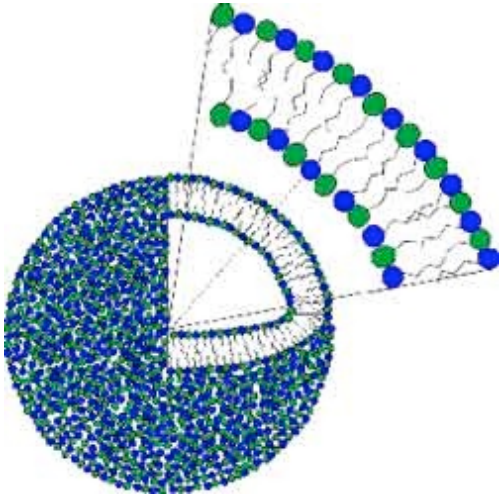
Small vesicles
(< 100 nm)



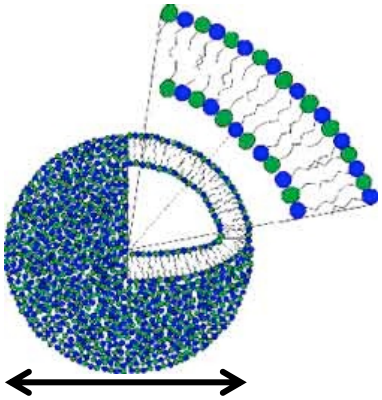
Large vesicles
(100 nm – 1 μm)



Giant vesicles
> a few μm - 100 μm)



Some Technics to Study GUVs



≈ 10 µm and above

Measurements on a Single GUVs

Same methods as in cell biology

Perfect with *optical microscopy*

But a bilayer:

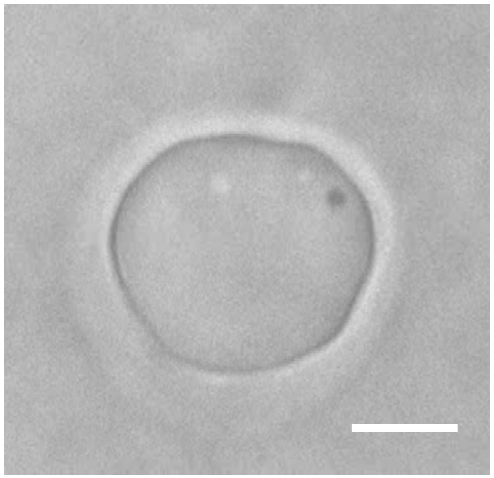
n=1.38-1.42 , e= 5nm, in water (n=1.33)….

➡ Increase the contrast

- Different media inside and outside the GUV (sugar, buffers + balance osmotic pressure)

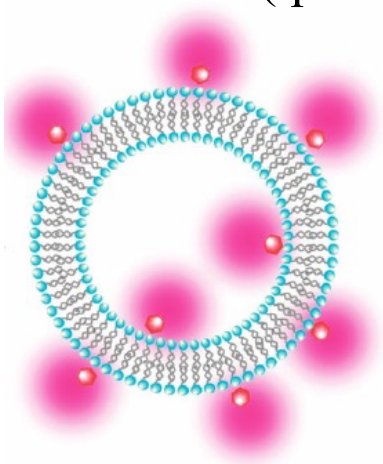
- Add a small fraction of fluorescent molecules (a few 0.1 % fluorescent lipids)

Use CONFOCAL microscopy (quantitative measurements)

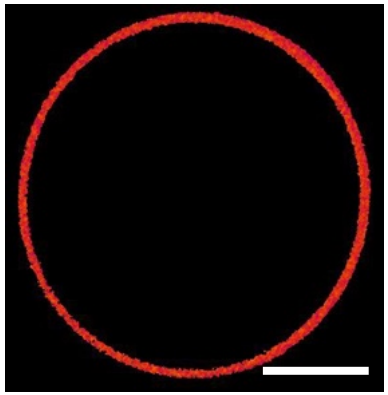


10 µm

Phase contrast microscopy



From: H. Robson Marsden et al, *Chem. Soc. Rev.*, (2011)

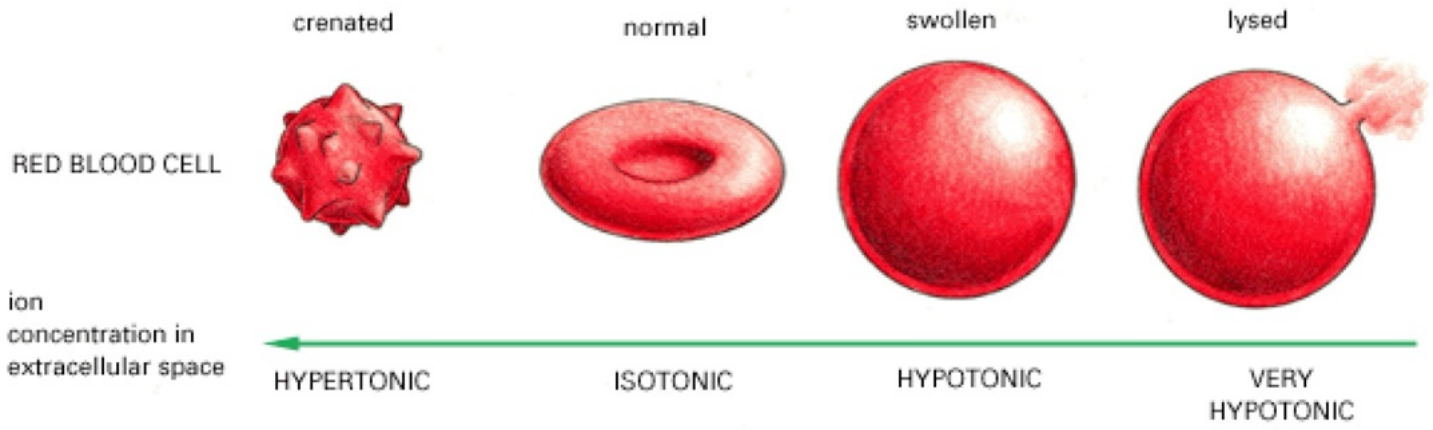


10 µm

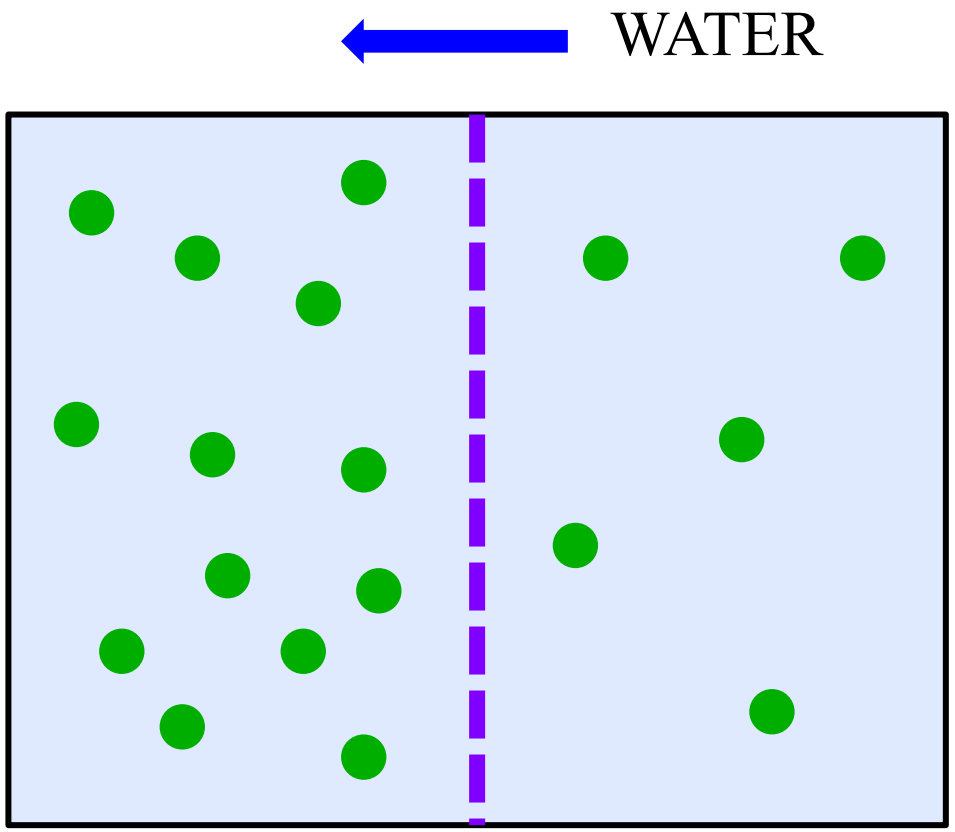
Membrane Tension

Cell Volume Changes
Due to Osmotic Pressure Differences

Red Blood Cells



Membrane Semi-Permeability and Water Flow



Permeability to water
But not to solute

Δc: molar concentration difference between compartments

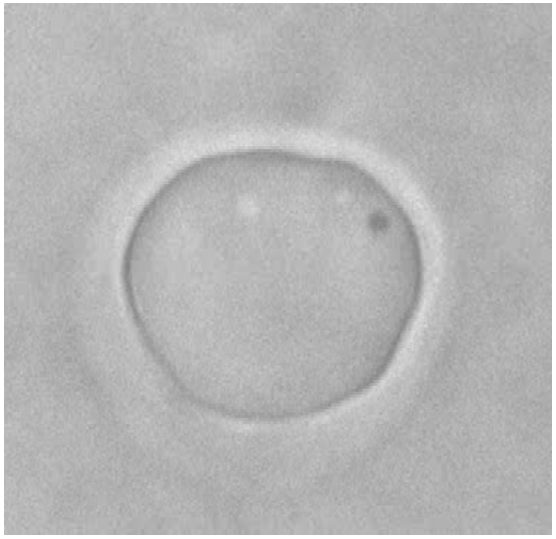
● Glucose or sucrose
(do not cross the membrane)

Osmotic pressure difference

$$\Delta\Pi = \Delta cRT \qquad (R = 8.31\ J/K.mol)$$

Water flows to equilibrate the differences

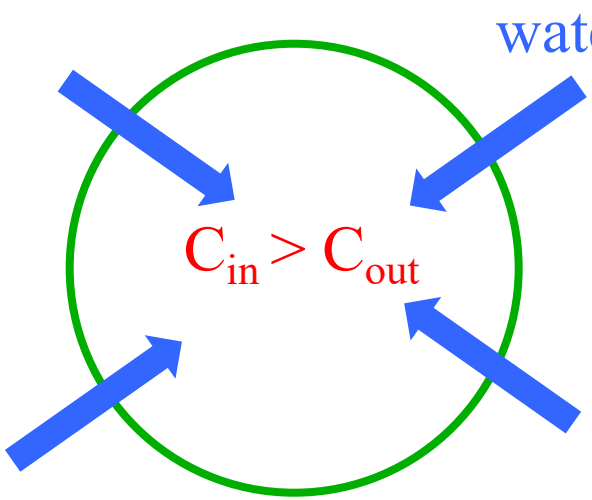
GUV Membrane can be Stretched



Changing tension by changing the osmotic pressure

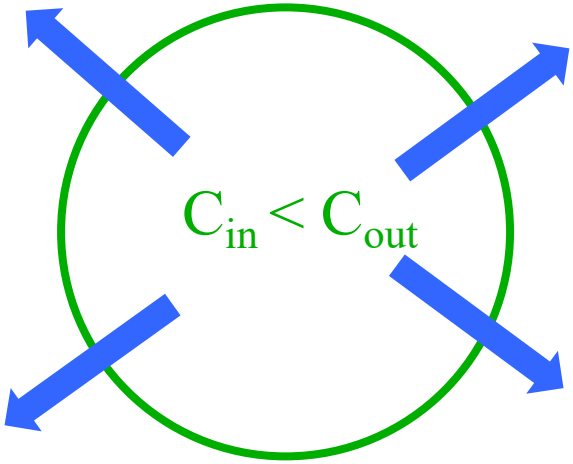
GUV Volume Changes Due to Osmotic Pressure Differences

Hypotonic

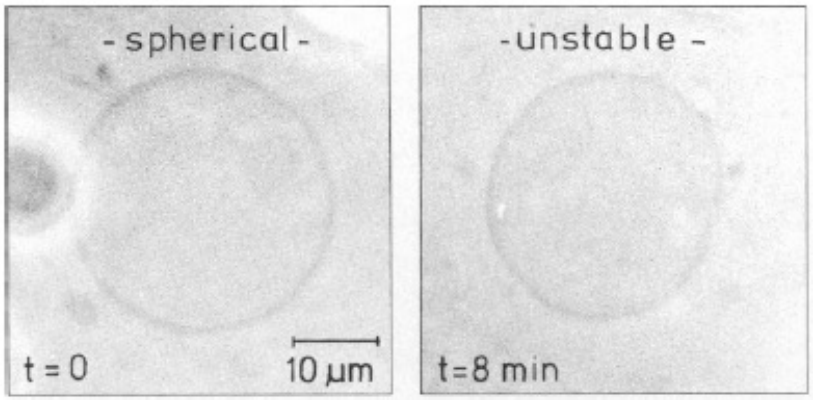


Swelling
Bursting…

Hypertonic

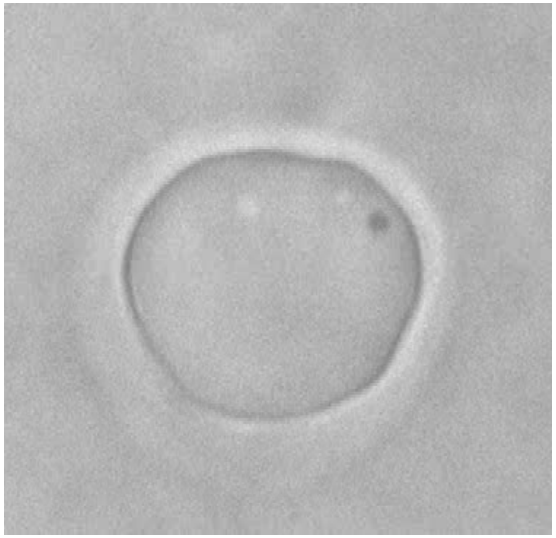


Deflating

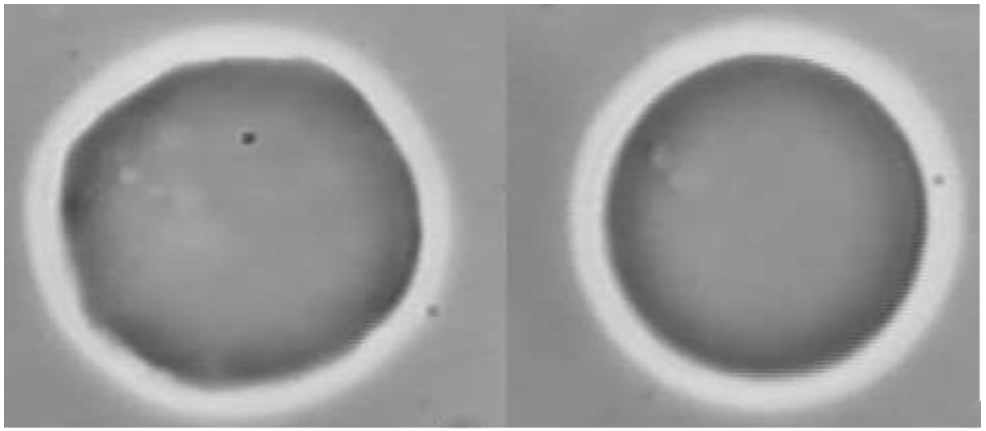


E. Boroske et al, *Biophys. J.* **34**, 95 (1981)

GUV Membrane can be Stretched



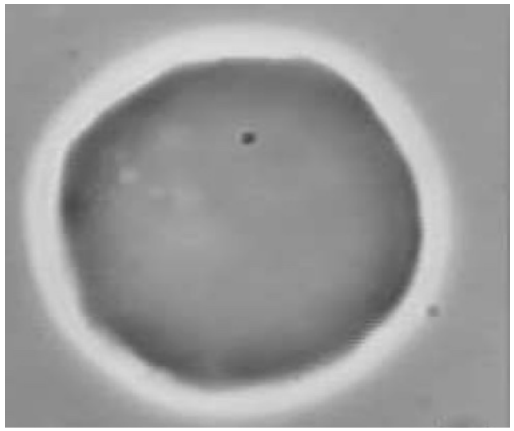
Hypertonic Hypotonic

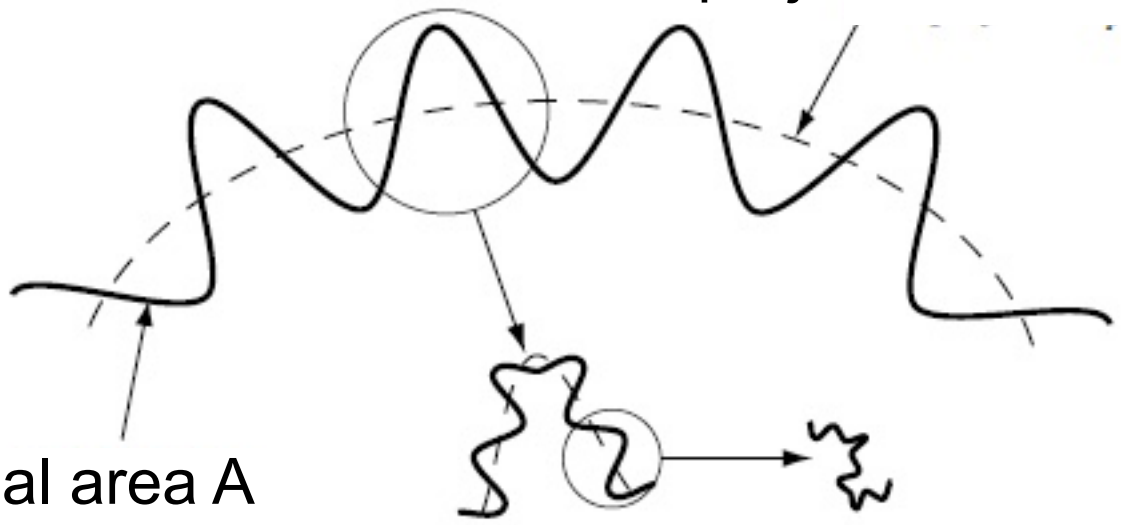


Low σ (10^{-8} - 10^{-6} N/m)

High σ (10^{-6} - 10^{-3} N/m)

Changing tension by changing the osmotic pressure



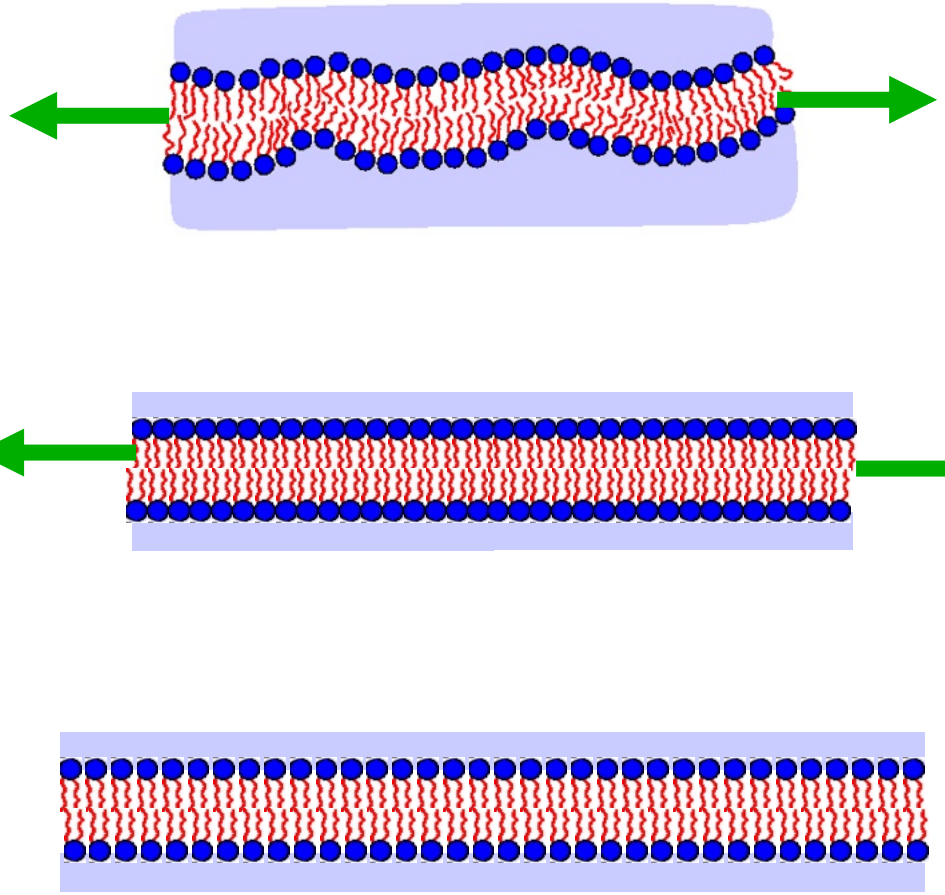


Excess area: $\Delta A = A - A_p$ Surface stored in fluctuations

Relative excess area: $\alpha = \frac{\Delta A}{A_p}$

Lipid Membrane Elasticity

Free-energy for membrane stretching H_s:



$$\sigma = \frac{\partial H_s}{\partial A}$$

A: membrane area

σ: membrane tension

(in Joules/m^2 or N/m)

Elastic regime

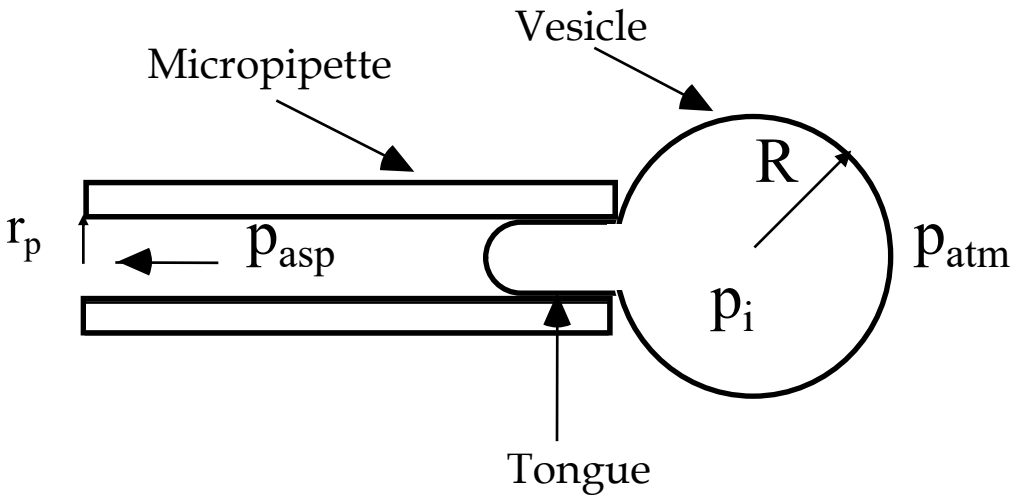
$$\sigma = K_a \frac{\Delta A}{A}$$

Cf. Hook's law

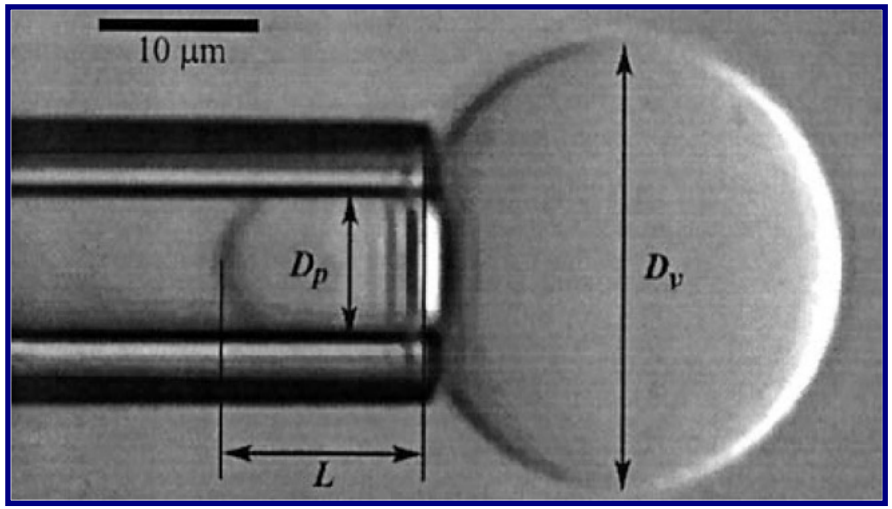
K_a: stretching modulus

Controlled Membrane Tension: Micropipette Aspiration

Evan Evans
UBC, Vancouver

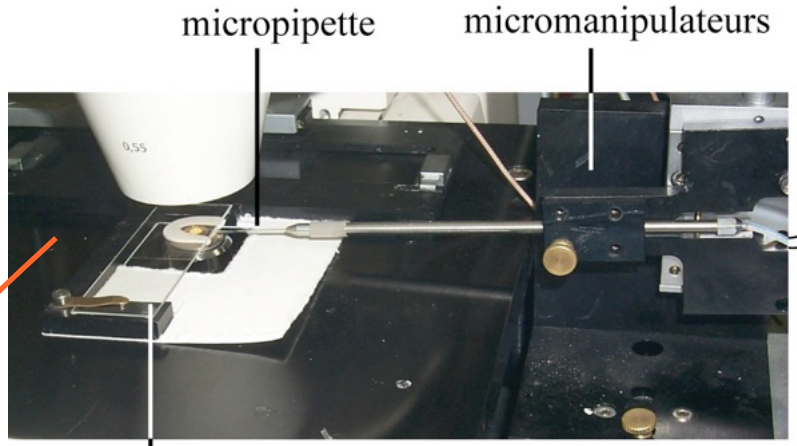


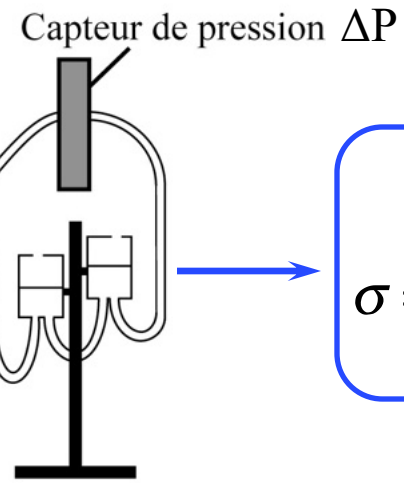
(Kwok et Evans, 1981)



Giant Unilamellar Vesicle (GUV)

Micropipette Aspiration (Kwok et Evans, 1981)





DIC

Tension:

$$\sigma = \frac{D_p}{4(1 - D_p / D_v)} \Delta P$$

ΔP

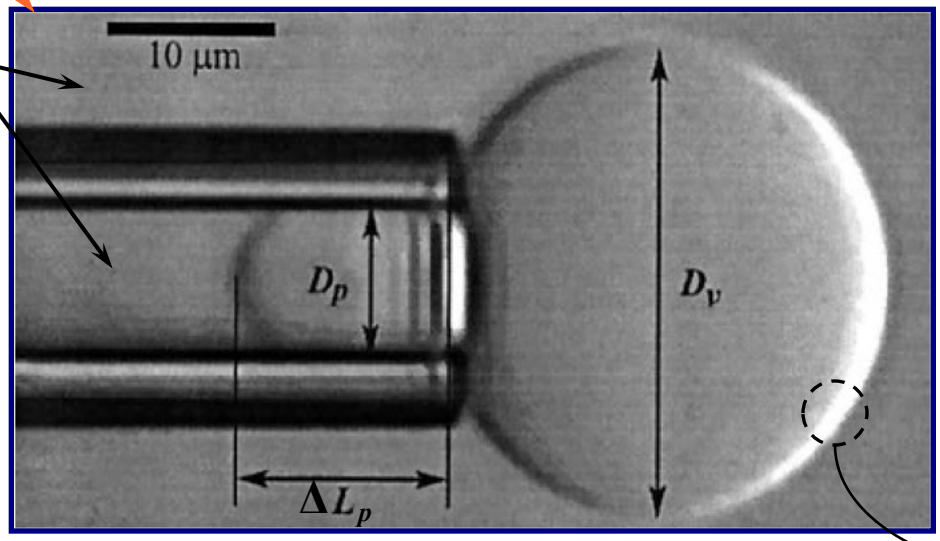
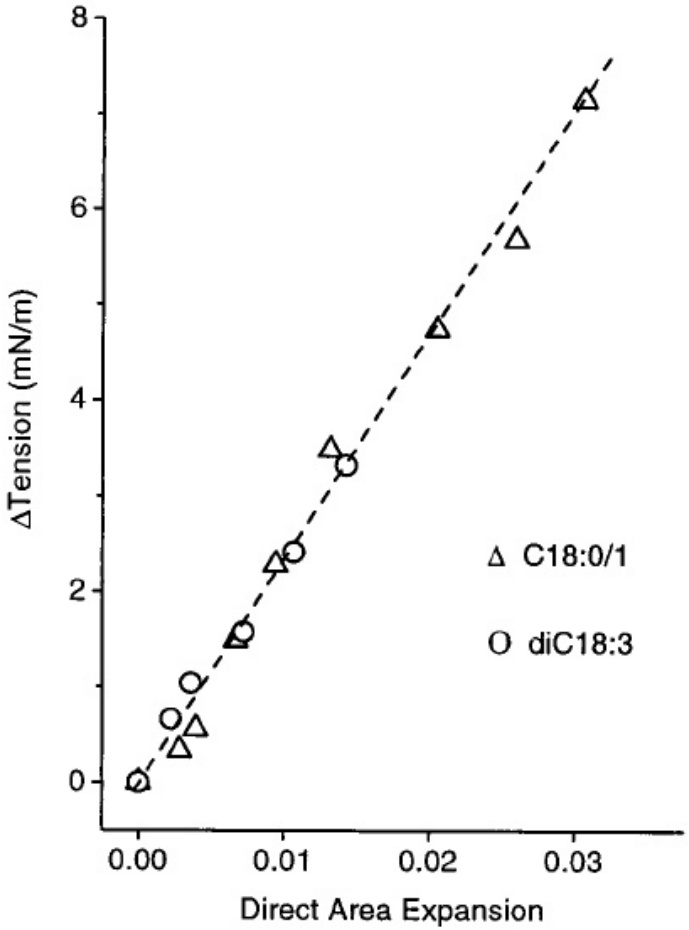


Image analysis

Excess area:

$$\Delta\alpha = \frac{(D_p/D_v)^2 - (D_p/D_v)^3}{D_p} L_p$$

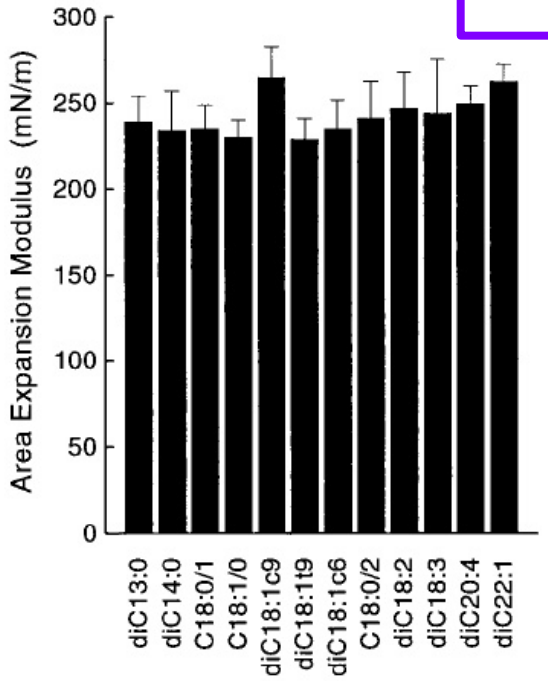


$$\sigma - \sigma_0 = K_a \Delta\alpha$$

W. Rawicz …E. Evans *Biophys. J.* **79**, 328-339 (2000)

Stretching moduli

$$\sigma = K_a \frac{\Delta A}{A}$$



W. Rawicz …E. Evans *Biophys. J.* **79**, 328-339 (2000)

System	$K_A \pm$ SD (mN/m) 32–35°C
One component	
DOPC	—
SOPC	290 ± 17
1:1 Binary	
DOPC/CHOL	870 ± 141
SOPC/CHOL	1130 ± 110
SM/CHOL	2193 ± 209
1:1:1 Ternary	
DOPC/SM/CHOL	610 ± 61
SOPC/SM/CHOL	880 ± 130
1:1:2 Ternary	
SOPC/SM/CHOL	1377 ± 172

W. Rawicz .. E. Evans *BJ* **94**, 4725-4736 (2008)

K_a:

PC lipids: *insensitive* to chain length, unsaturations

Larger with Sphingolipids

Increases with cholesterol

Lipid membranes:

Hard to stretch

(≈ 5 times harder/polyethylene)

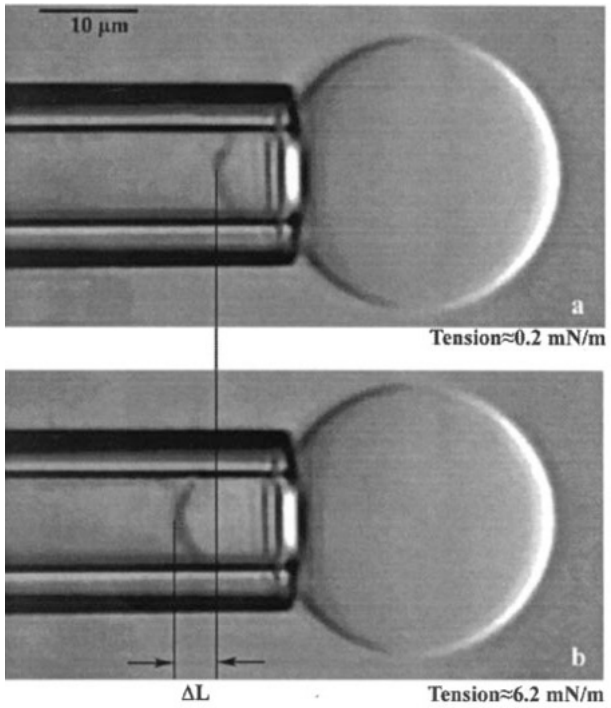
Lipid Membranes: Fragile upon Stretching

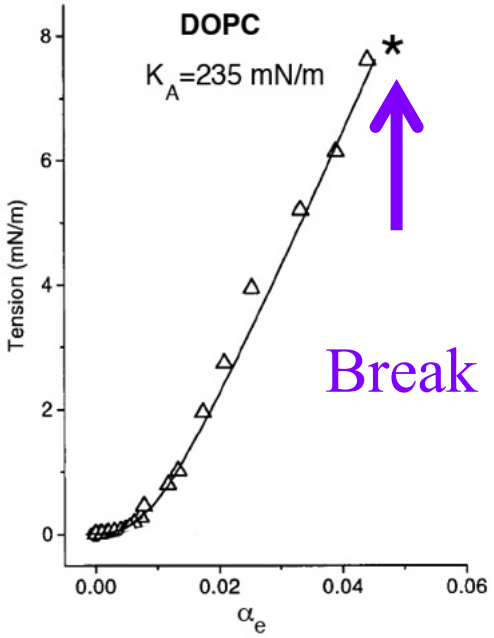
Strain limited to ≈ 5%

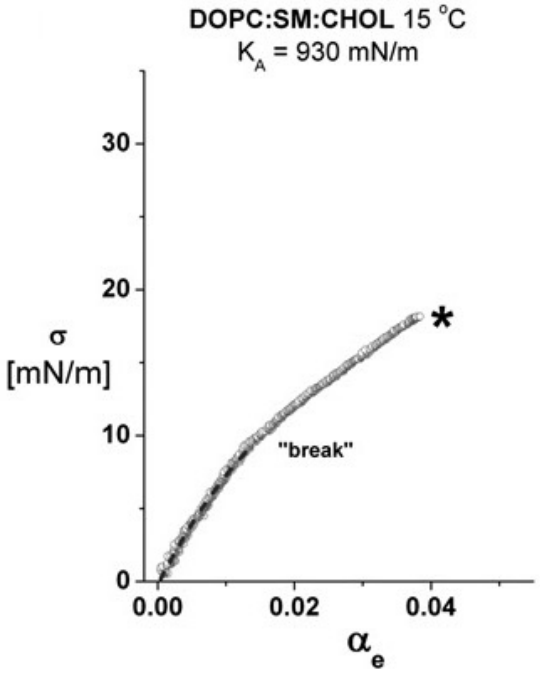
E. Evans

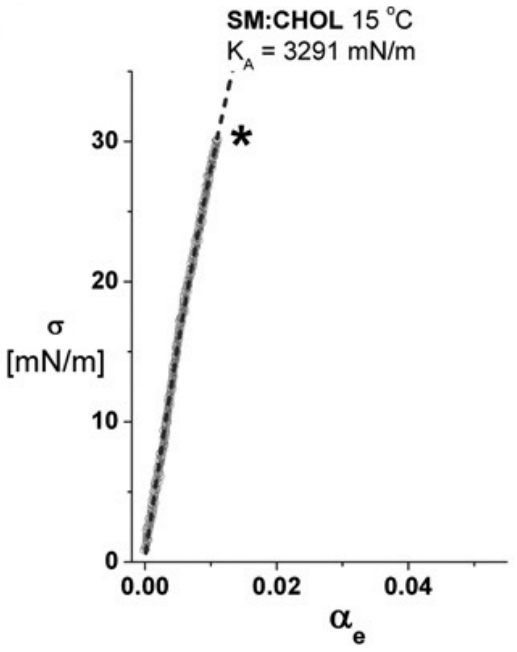
W. Rawicz, et al *Biophys. J.* **79**, 328 (2000)

W. Rawicz et al *Biophys. J.* **94**, 4725 (2008)

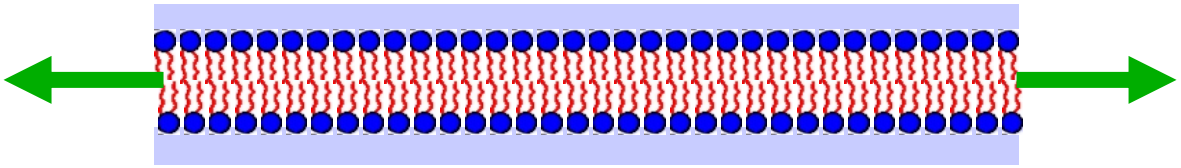




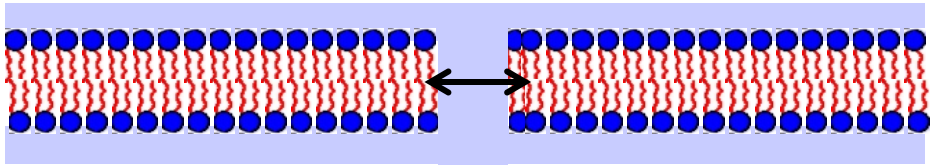




High Tension: Membrane Lysis

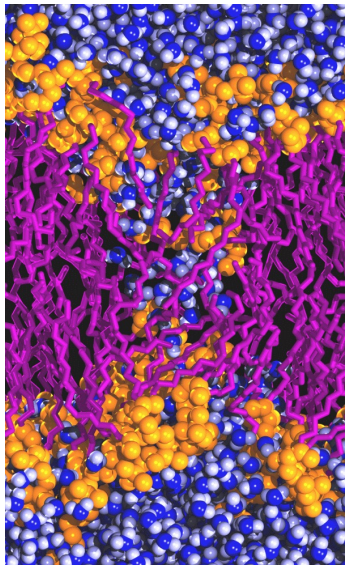


HOLE

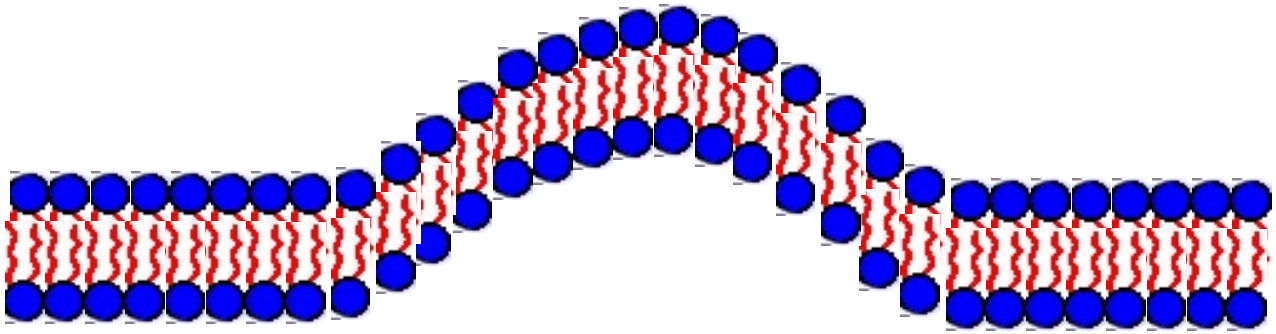


Max Strain $\Delta\alpha \approx 5\%$

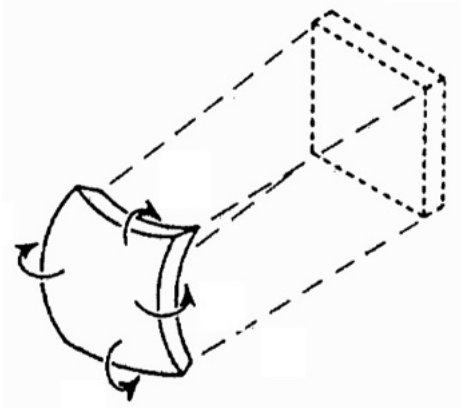
Numerical simulations



Lipid Membranes: Easily Bent



Allows for intracellular trafficking, endocytosis, exocytosis



Bending Energy of Fluid Membranes

W. Helfrich
P. Canham
E. Evans (≈1970)

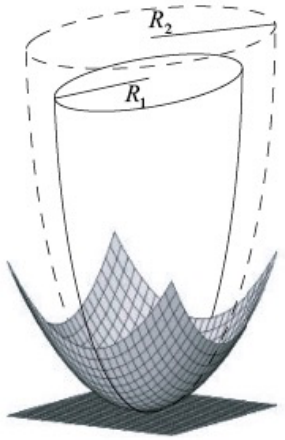
Energy/unit area:

$$f_{bending} = \frac{1}{2}\kappa C^2$$

W. Helfrich, *Zur Naturforschung* **28c**, 693 (1973)
"Elastic properties of lipid bilayers :
theory and possible experiments"

C: Mean curvature

$$C = C_1 + C_2 = \frac{1}{R_1} + \frac{1}{R_2}$$



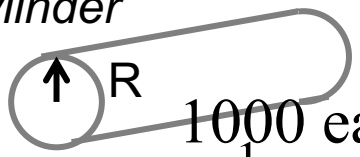
κ : **bending modulus**

from a few k_BT to ≈ 100 k_BT $(k_BT = 4.10^{-21} J)$

depends on composition

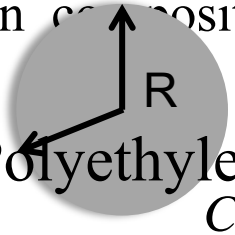
1000 easier than Polyethylene

cylinder



$$C = \frac{1}{R}$$

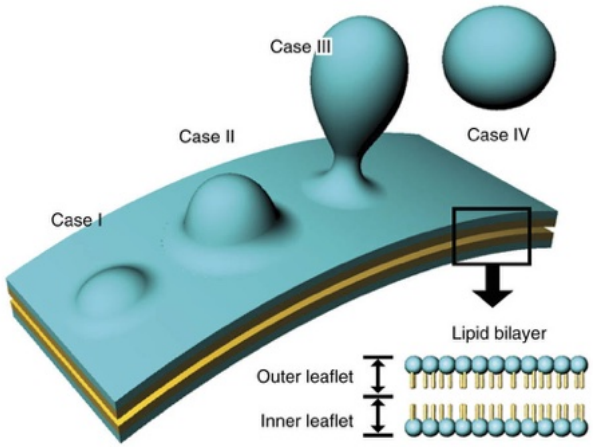
sphere

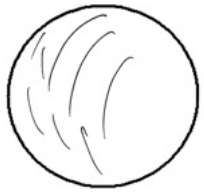


$$C = \frac{1}{R} + \frac{1}{R} = \frac{2}{R}$$

$$f_{bending} = \frac{1}{2}\kappa C^2$$

Energy to form a spherical vesicle from a flat membrane:



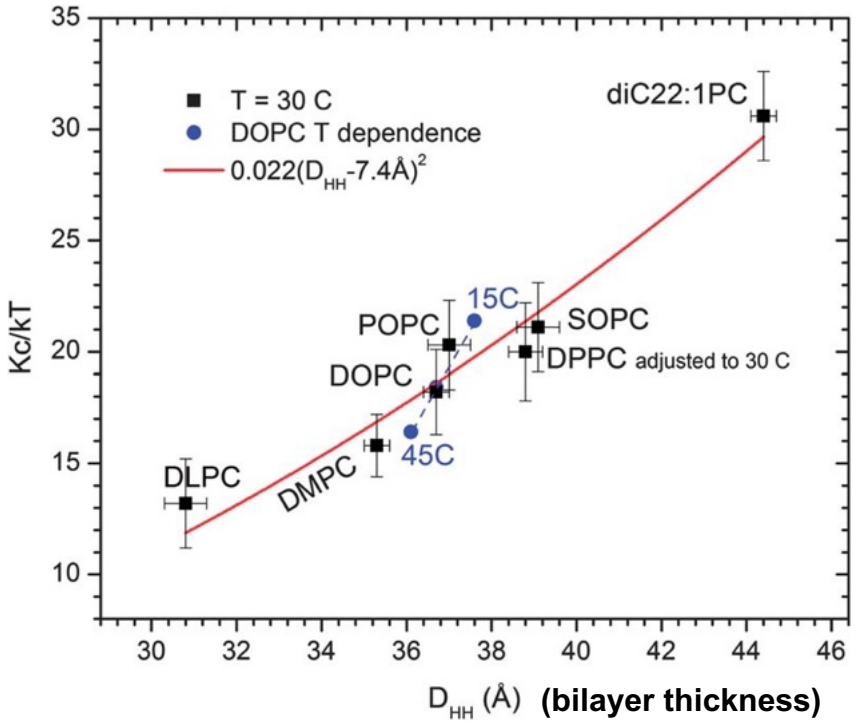
SPHERE  $C = \frac{2}{R}$

$$F = \frac{1}{2}\oiint \kappa \left(\frac{2}{R}\right)^2 dS = \frac{1}{2}\kappa \frac{4}{R^2} 4\pi R^2 = 8\pi\kappa$$

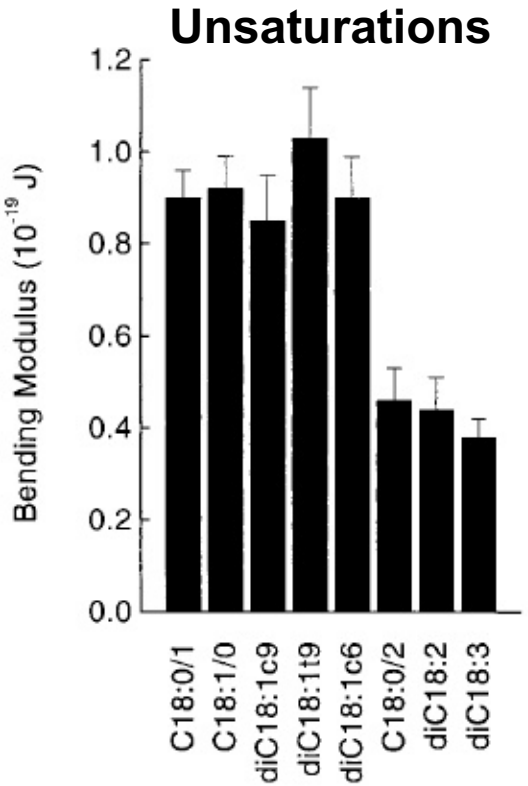
Independent of vesicle size!!!!

For κ=10 $k_B T$ $\quad$ $F \approx 250\ k_B T$

Effect of Chain Length and Unsaturation Numbers on κ



J. F. Nagle *Faraday Discuss.* **161**, 11 (2013)

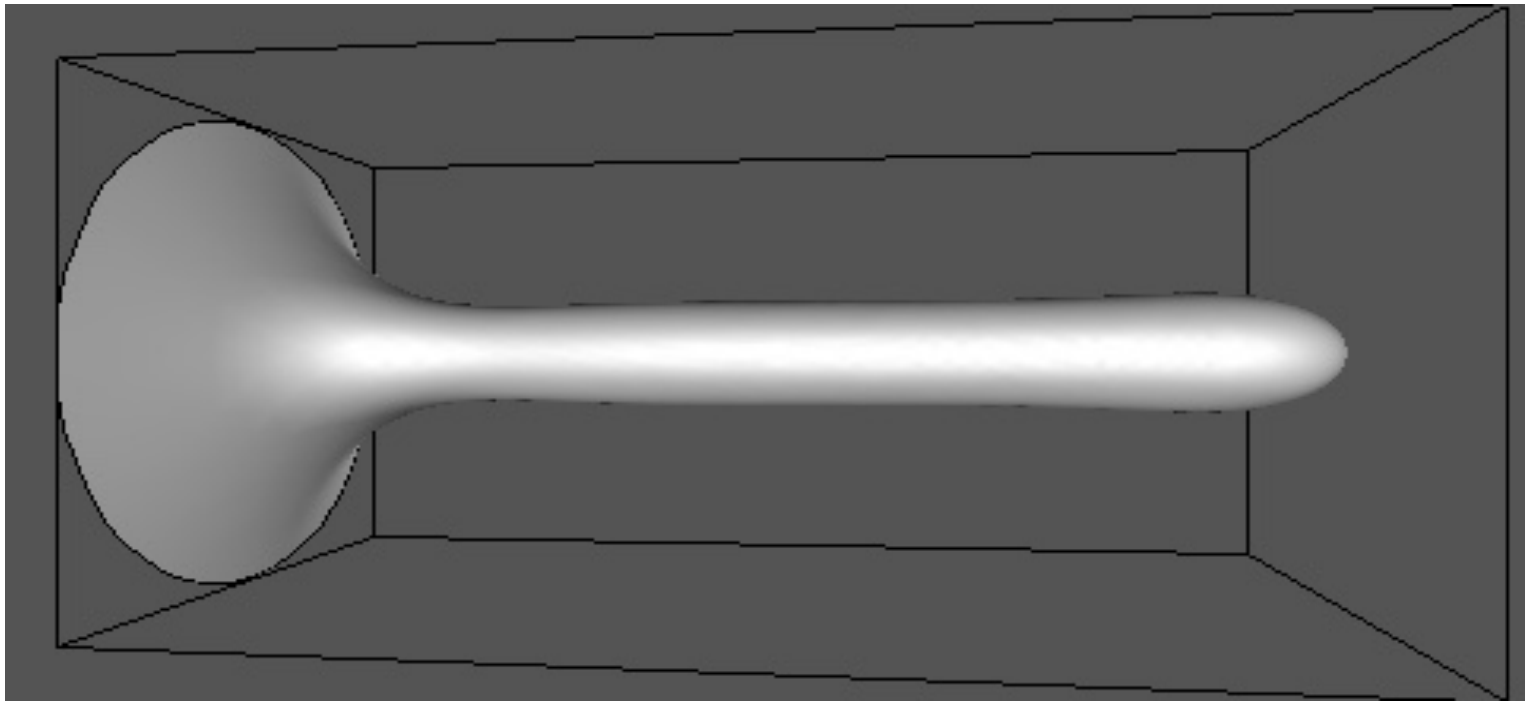


W. Rawicz …E. Evans *Biophys. J.* **79**, 328-339 (2000)

κ

Increases with chain length
Decreases with unsaturation #
Increases with cholesterol
Sphingolipids

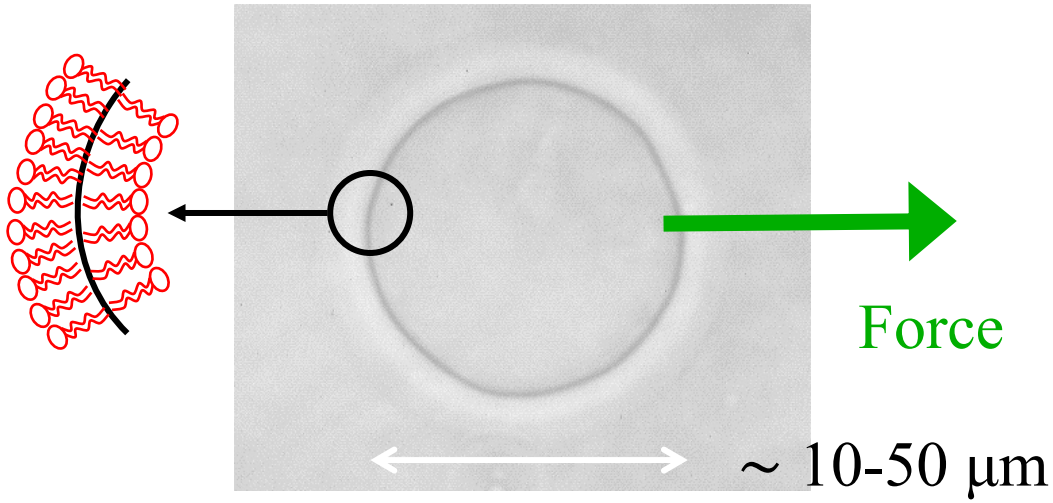
Membrane Nanotubes

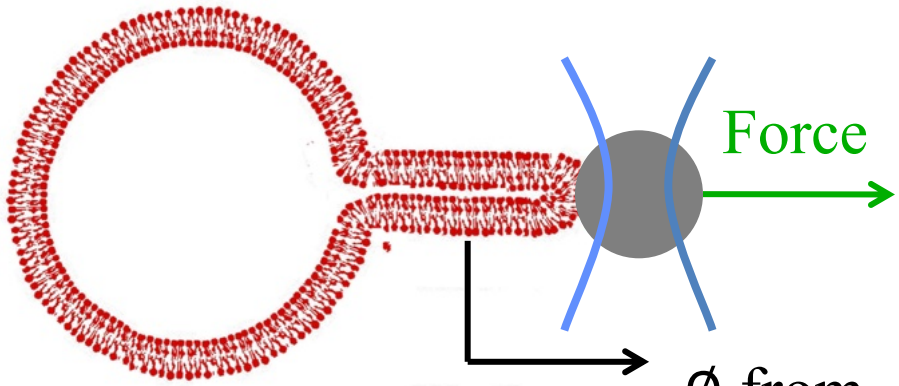


From Derényi et al, *PRL (2002)*

Membrane Nanotubes

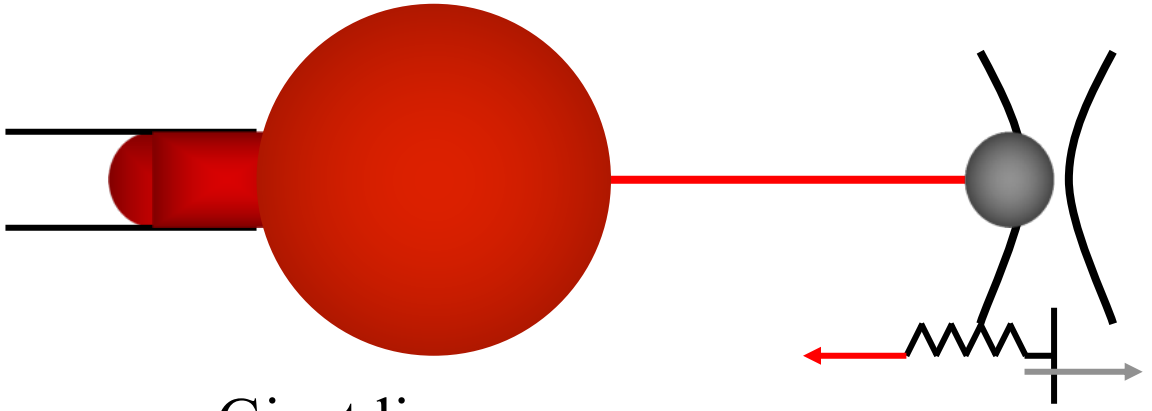
Giant Unilamellar Vesicle (GUV)

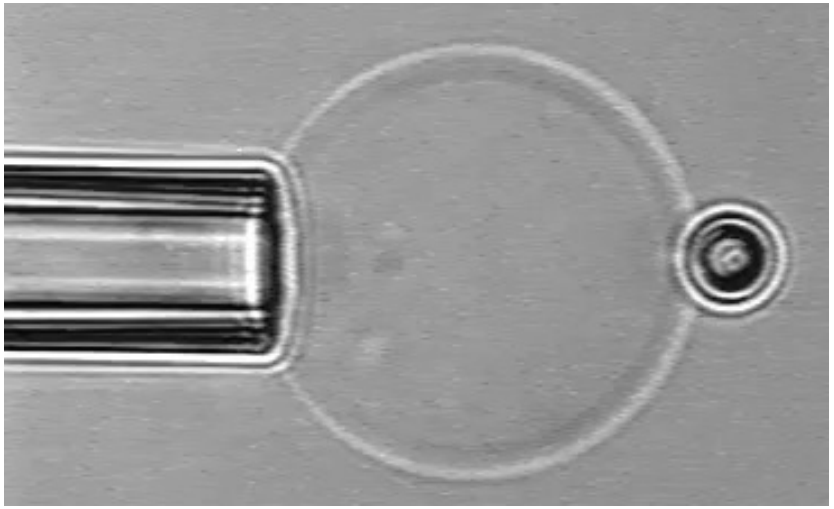


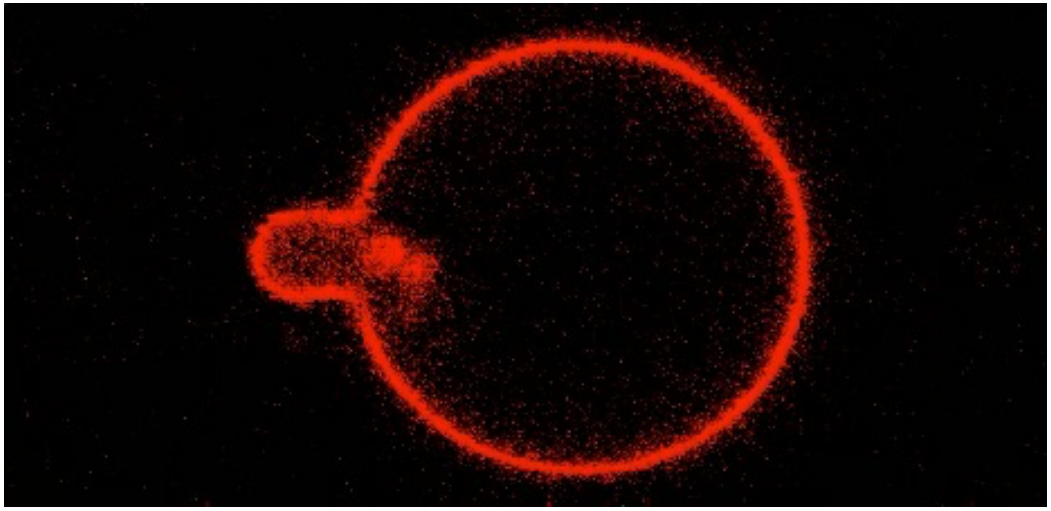


Ø from ~15 nm to a few 100 nm

Membranes can be Highly Bent : Nanotube



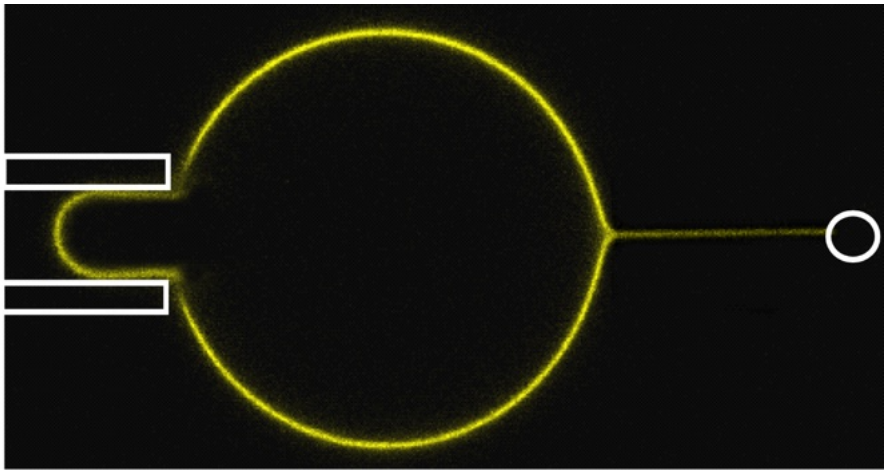




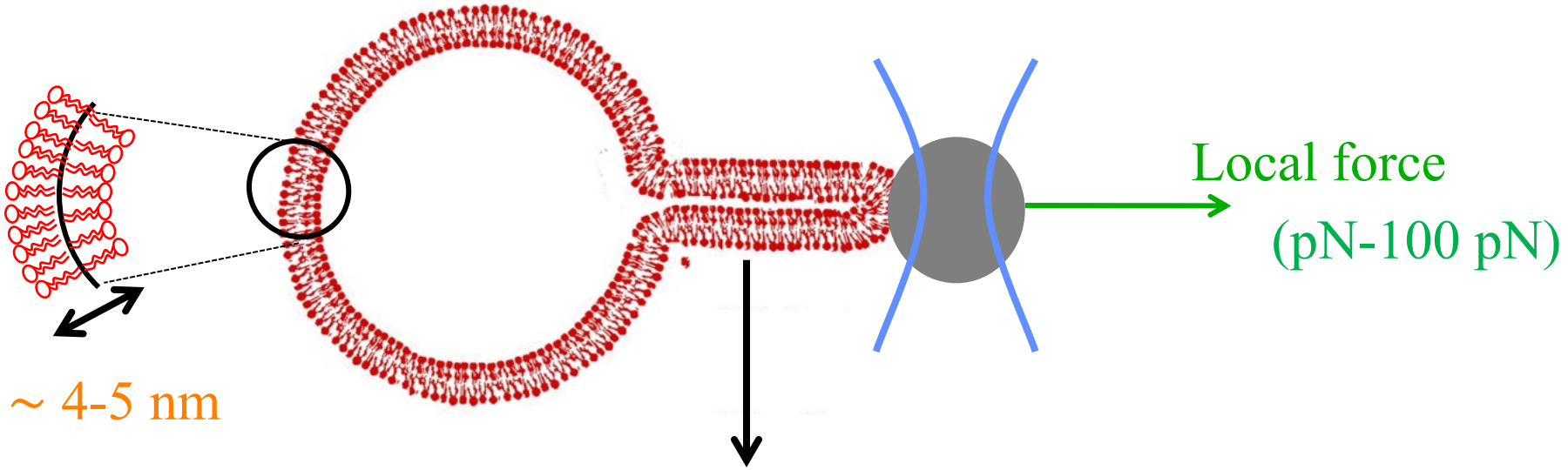
D. Cuvelier … P. Nassoy *Biophys. J* (2005)

B. Sorre et al, *PNAS* (2009)

Membrane Nanotubes : Highly Curved



~ 10-50 μm



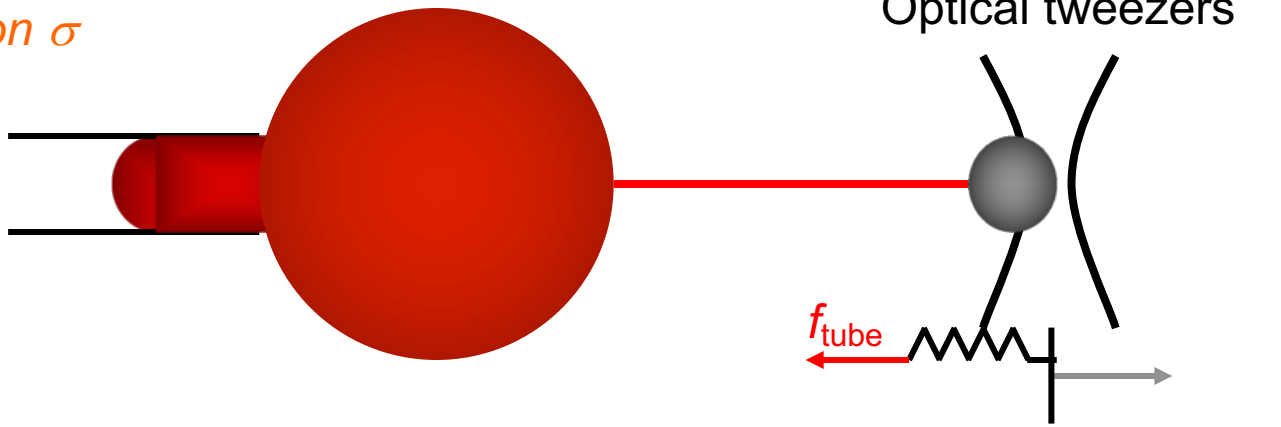
Nanotube Mechanics

Force

Optical tweezers

Control of membrane tension σ

Micropipette aspiration



$$R_0 = \sqrt{\frac{\kappa}{2\sigma}}$$

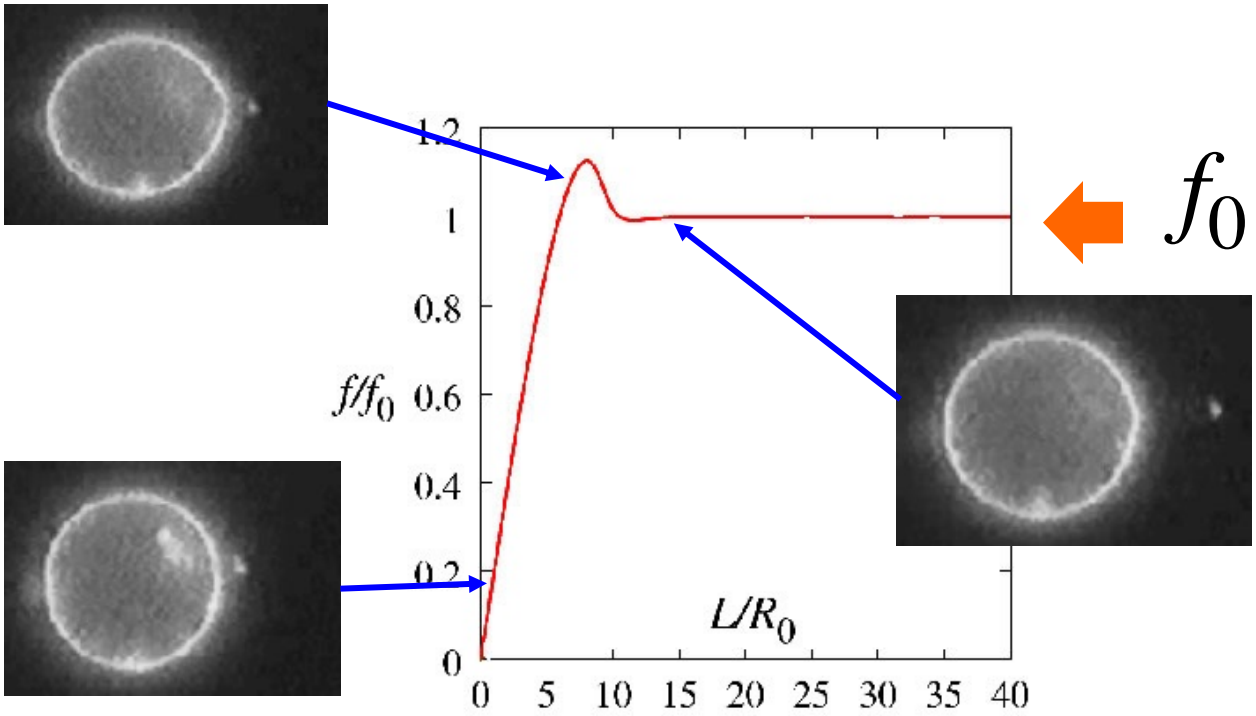
Confocal microscopy

$$f_0 = 2\pi\sqrt{2\kappa\sigma}$$

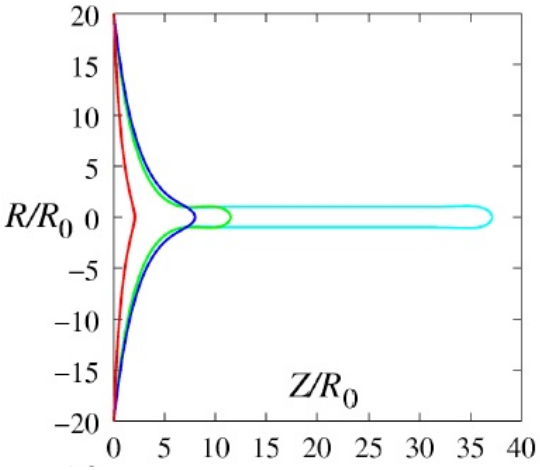
Some references

- Evans, Yeung C*hem. Phys. Lipids* (1994*)*
- Bozic, Svetina, Zeks *PRE* (1997)
- Svetina, Zeks, Waugh, Raphael *Eur. B. J.* (1998)
- Derényi, Jülicher*,* Prost *PRL (*2002)
- Powers, Huber, Goldstein *PRE* (2002)

Theory: Force/Extension at Constant Tension



$$f_0 = 2\pi\sqrt{2\kappa\sigma}$$

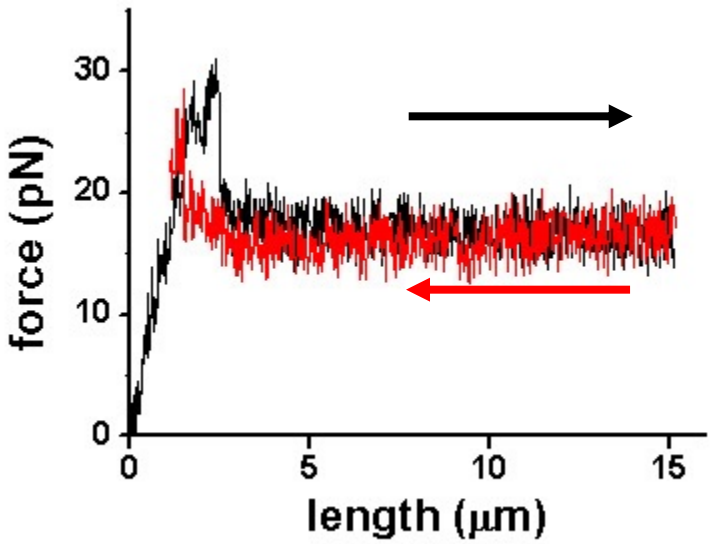


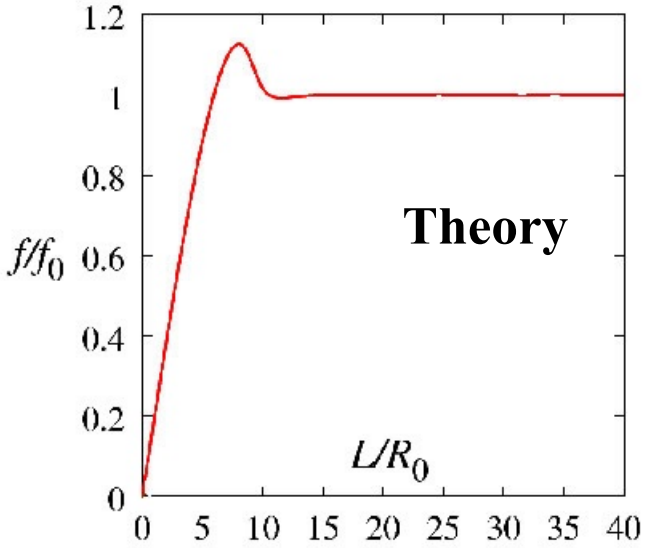
Dérényi et al, PRL ***88*** *(2002) 238101*

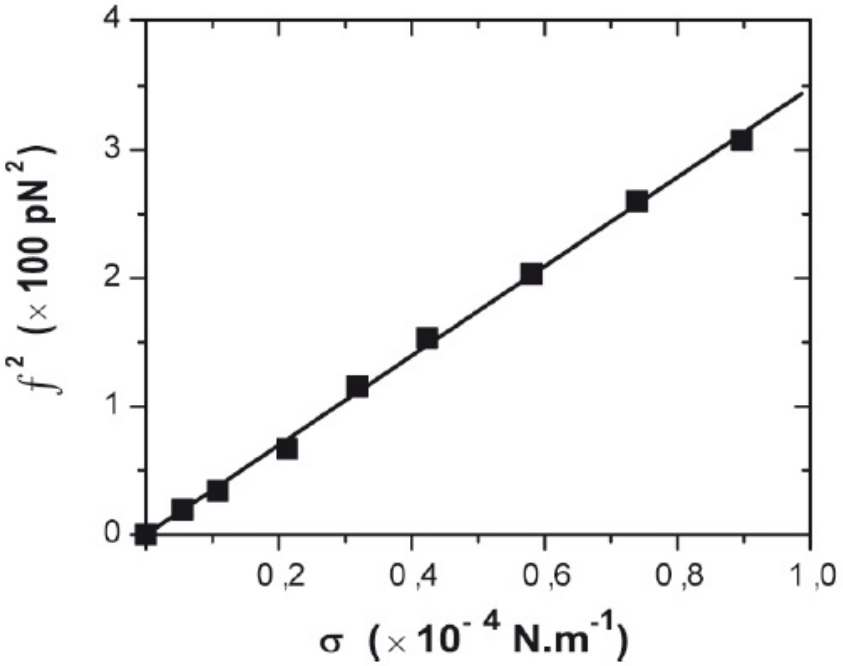
Powers et al PRE ***65*** *(2002) 041901.*

Experiments

EPC





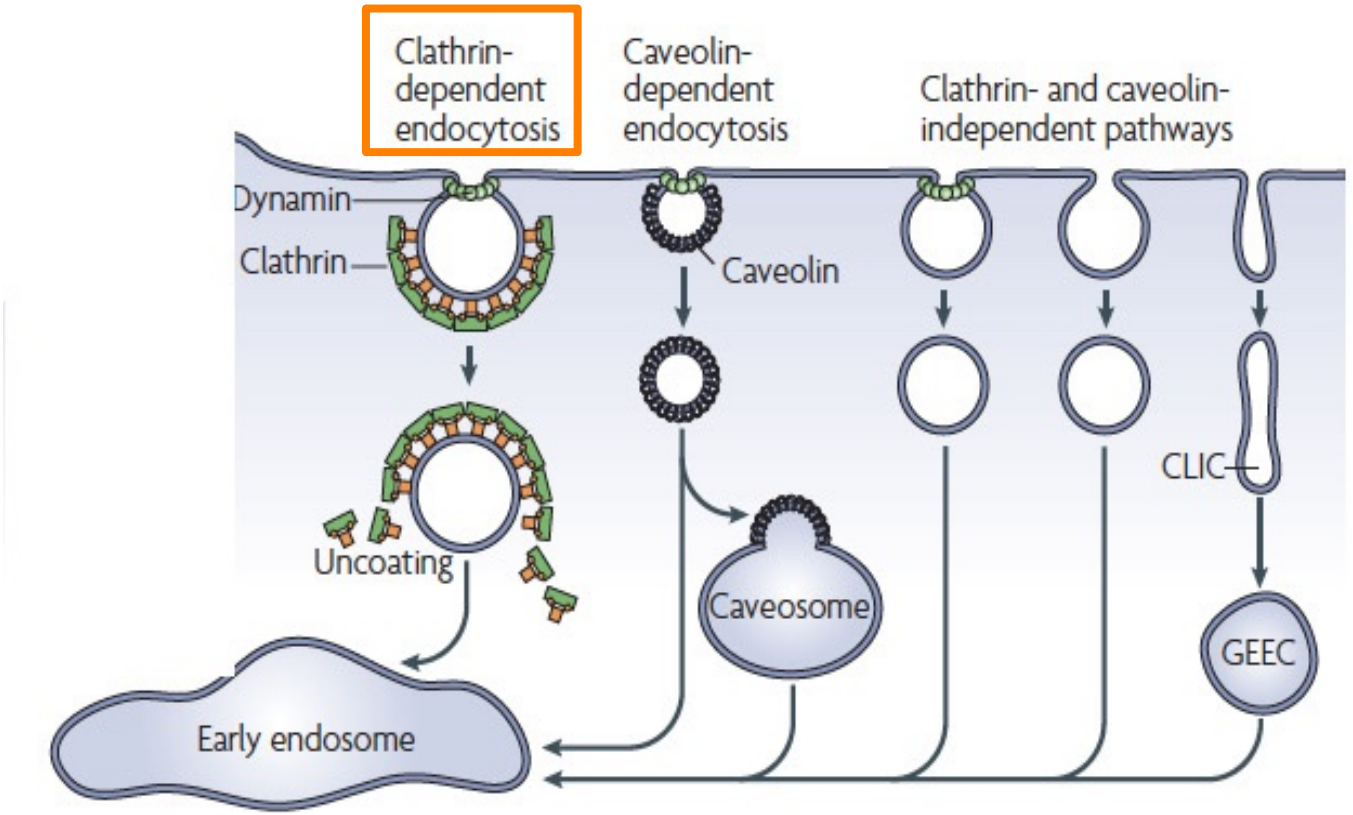


$$f_0 = 2\pi\sqrt{2\kappa}\sqrt{\sigma}$$

$$\kappa_{EPC} = 10 \pm 1\ k_B T$$

Bending Membranes with Proteins

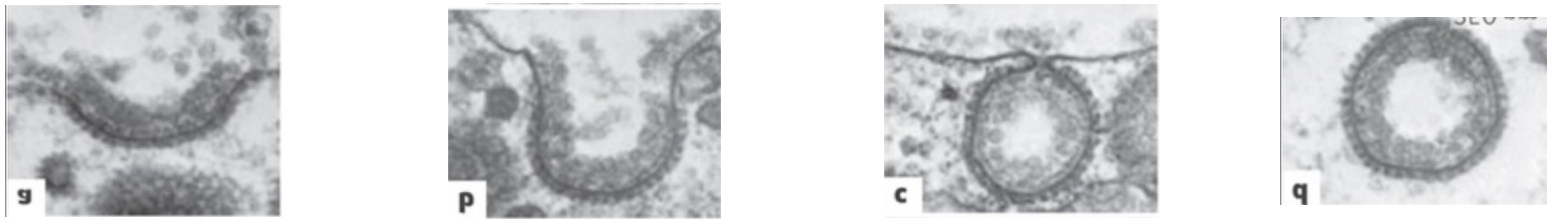
Clathrin-Mediated and Clathrin-Independent Endocytosis

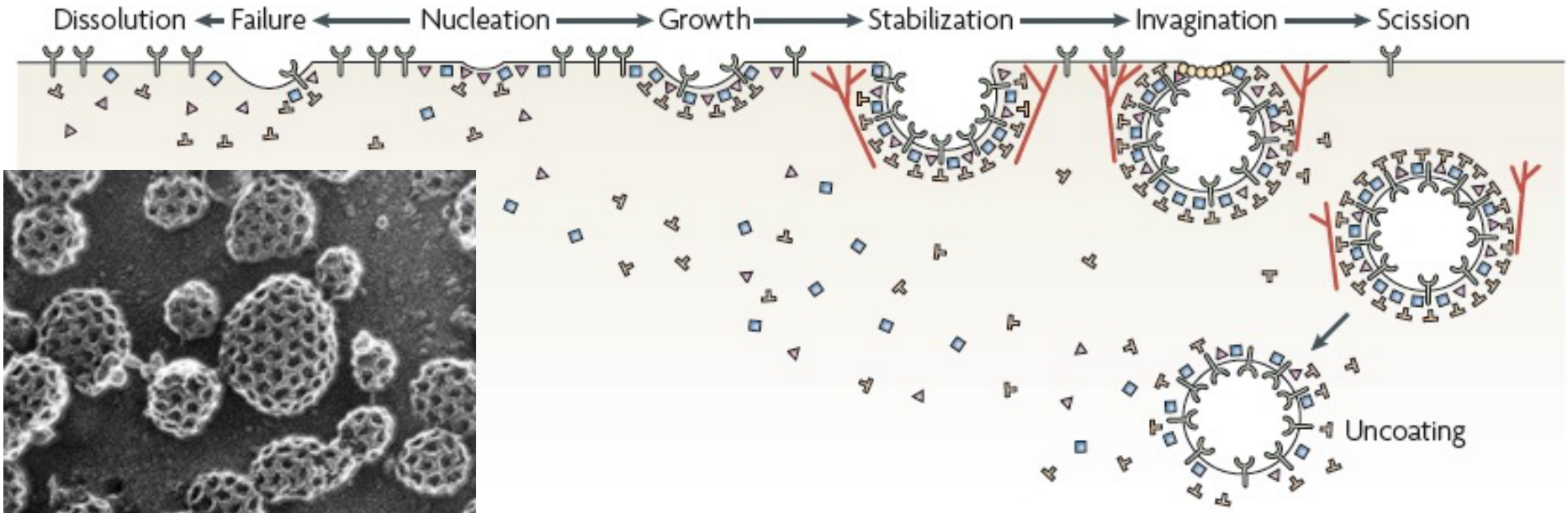


From S. Mayor, R. Pagano, *Nat. Rev. Mol. Cell Biol.* (2007)

Formation of a Clathrin-Coated Vesicle

From McMahon H. T., Gallop J. L.
Nature (2005)

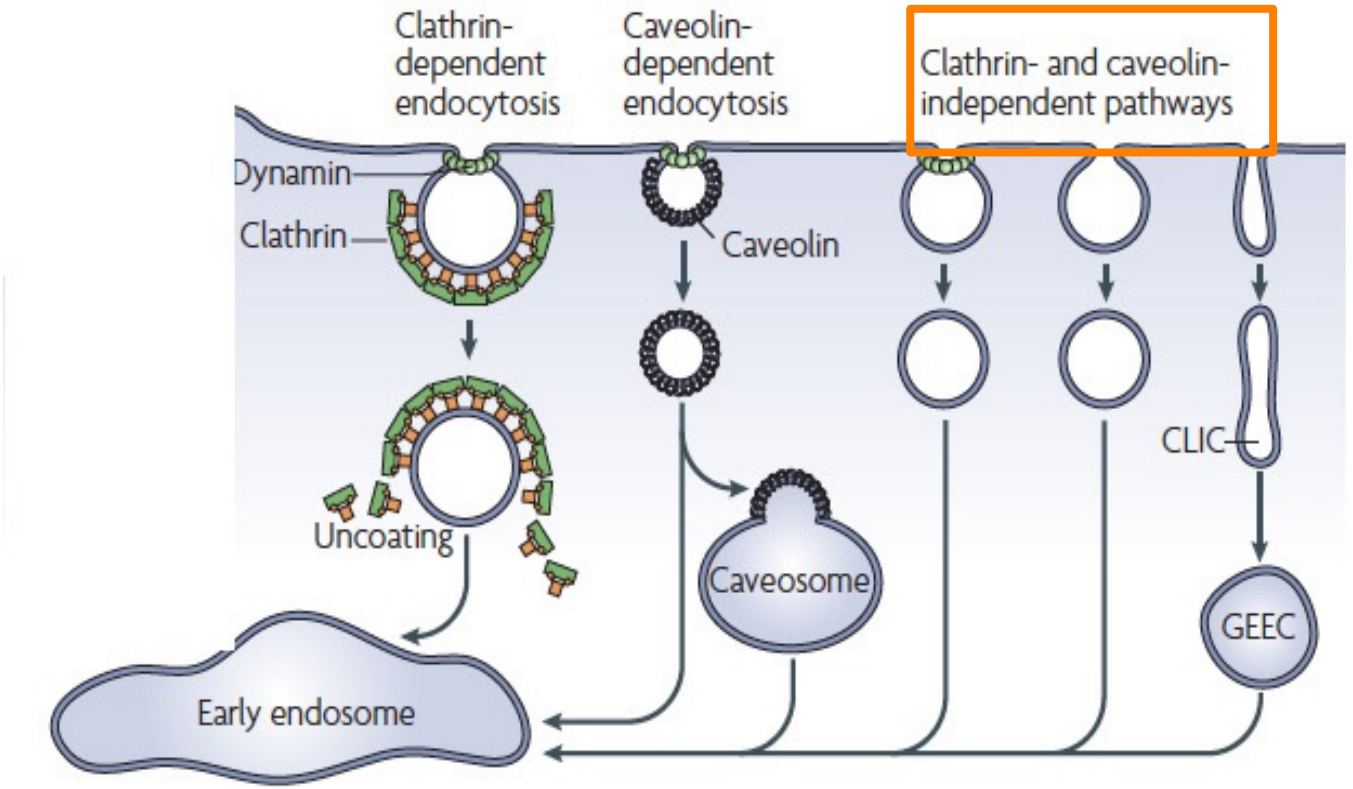




Fujimoto, L.M. et al. Traffic (2000)

Traub L. *Nat. Rev. Mol. Cell Biol* (2009)

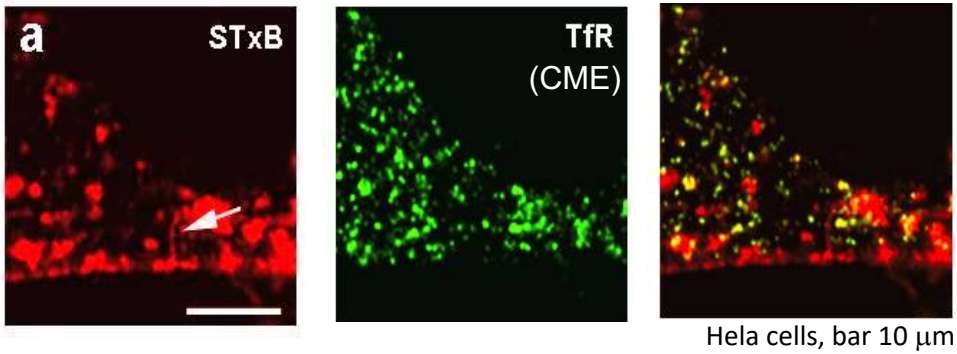
Clathrin-Mediated and Clathrin-Independent Endocytosis



From S. Mayor, R. Pagano, *Nat. Rev. Mol. Cell Biol.* (2007)

Clathrin-Independent Endocytosis

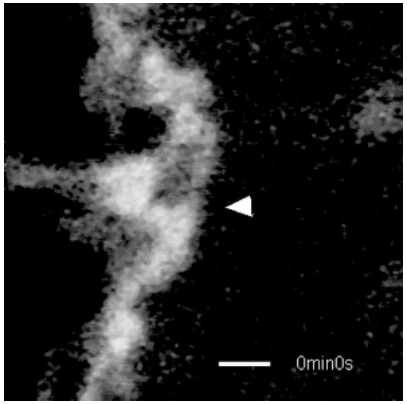
- Shiga and Cholera toxin

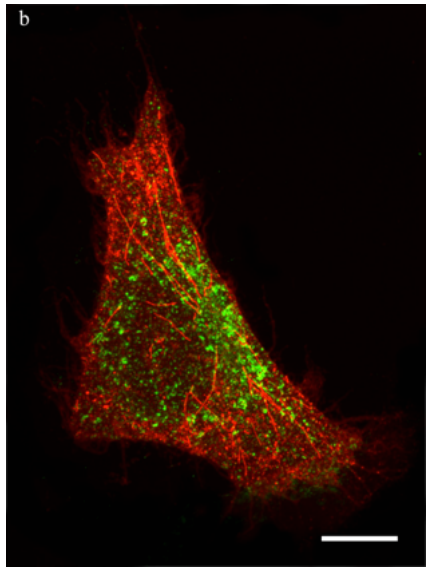


Hela cells, bar 10 μm

W. Römer … L. Johannes, *Nature* (2007)

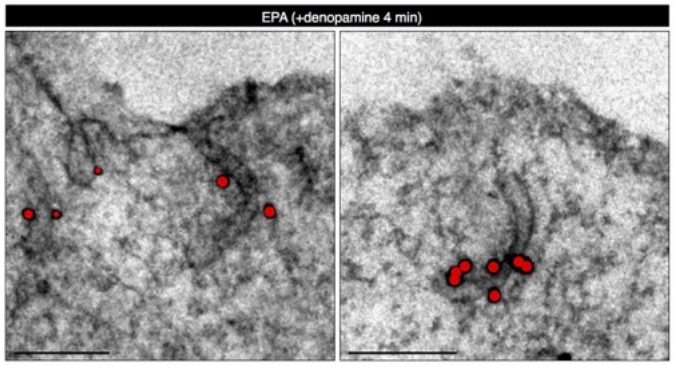
ATP-depleted cells





5 μm

- Activated receptors (GPCR, EGFR…) **FEME**



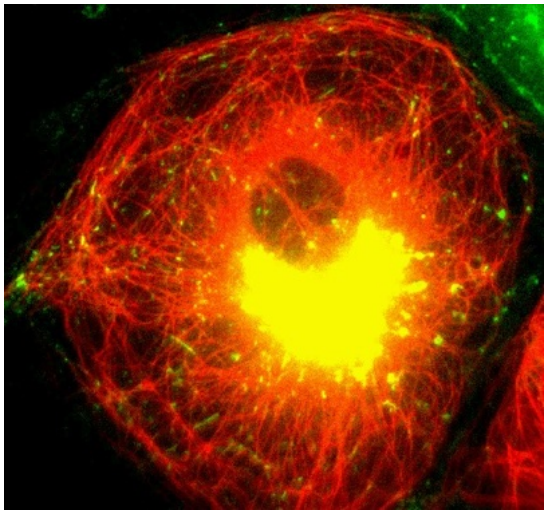
250 nm

E. Boucrot… H. McMahon, *Nature* (2015)

Tubules in Intracellular Traffic

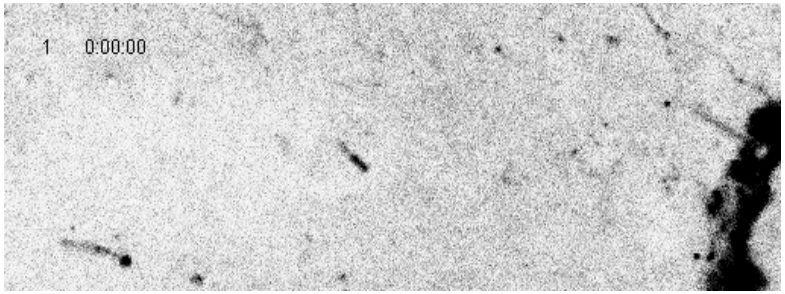
Narrow tubes (usually $\Phi \approx 50$ nm)

Golgi



Toomre et al
J. Cell Sci. (1999)

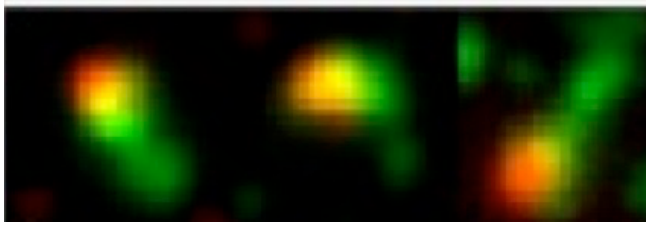
Microtubules
Golgi (VSV-G)

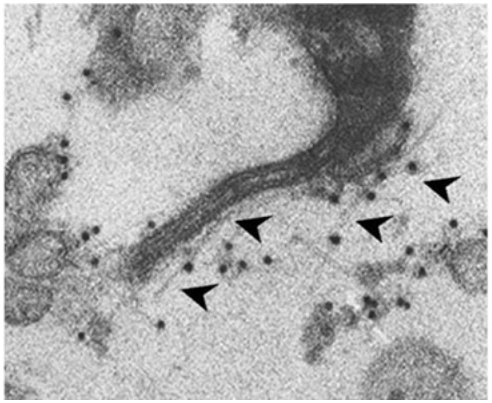


VSV-GFP

J. Lippincott Schwartz (CBMB-NIH)

Endosomes

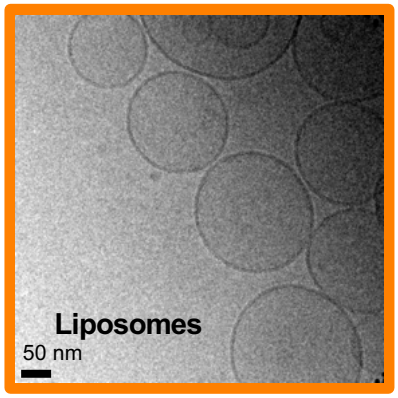




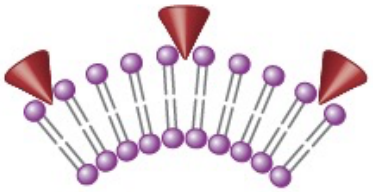
Puthenveedu *et al.*,
Cell (2010)

Gold NP=9 nm

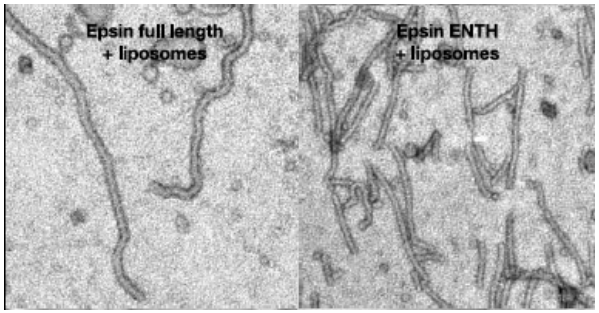
Spontaneous Membrane Bending due to Proteins



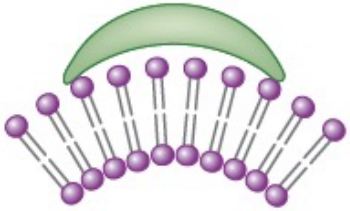
Cryo-EM (A. Bertin)

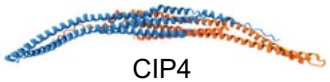


Insertion
(Amphipatic helix)



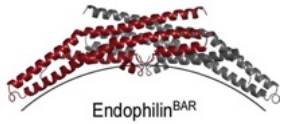
M. G. J. Ford *et al.*, *Nature* (2002)

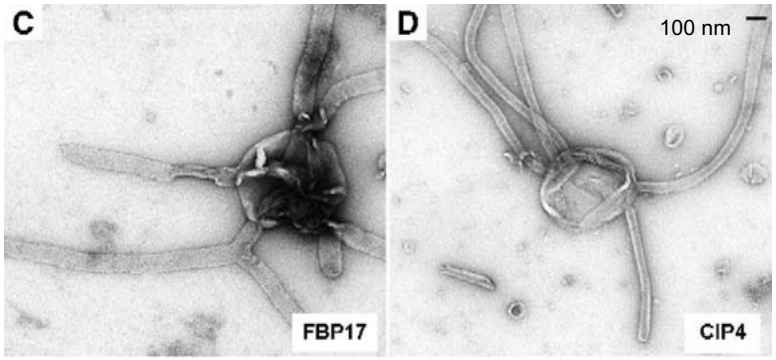




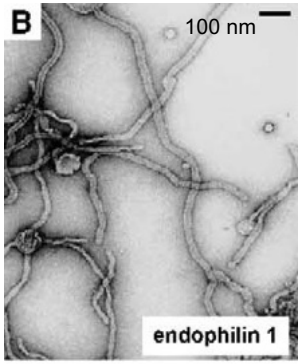
CIP4

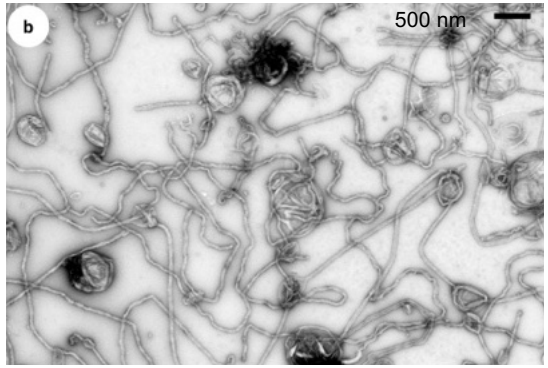
Molding (BAR-domain proteins)





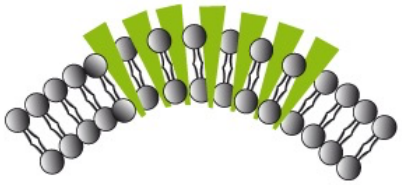
T. Itoh *et al.*, *Dev. Cell* (2005)

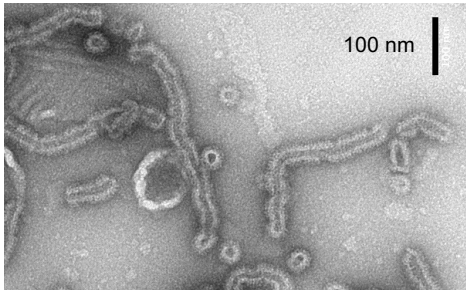




K. Takei et al *Nat. Cell Biol.* (1999)

Conical inclusions
(Trans-membrane proteins)

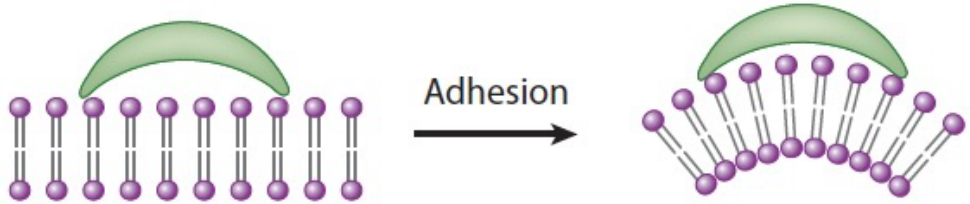




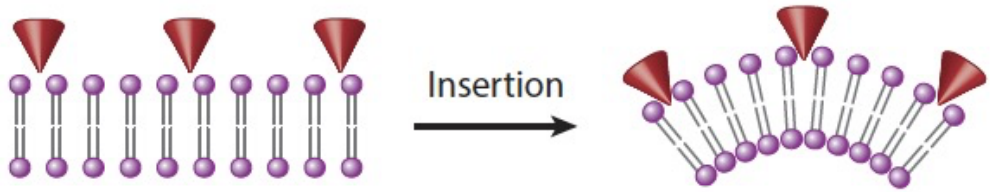
BmrA:
ABC transporter

P. Fribourg *et al.*,
J. Mol. Biol. (2014)

Asymmetric Protein Binding: Membrane Spontaneous Curvature

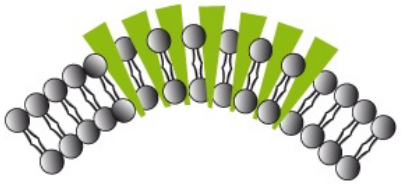


BAR-proteins



Amphipatic helices

Also:
Shape asymmetry



Conical membrane proteins

$$f_{bending} = \frac{\kappa}{2} \times \left(C - C_0\right)^2$$

C_0: *spontaneous curvature* of the *membrane*

W. Helfrich, *Zur Naturforschung* (1973)

Membrane Spontaneous Curvature

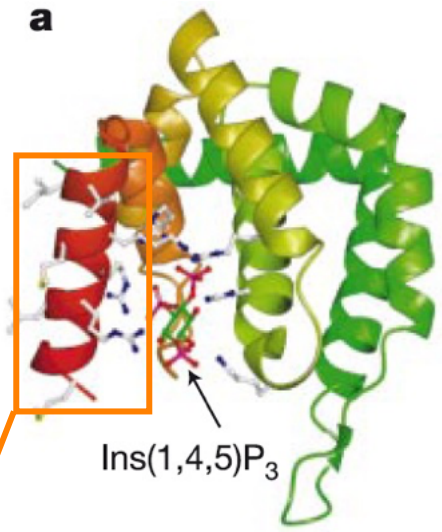
C_0 depends on *protein surface fraction* on the membrane: ϕ

$$f_{bending} = \frac{\kappa}{2} \times \left(C - C_0(\phi) \right)^2$$

Epsin

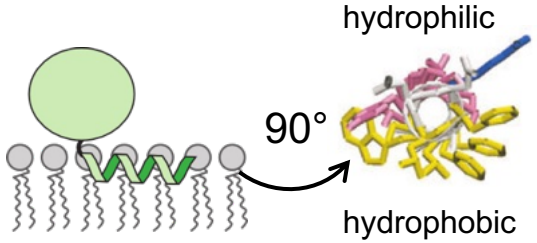
ENTH domain

with PiP_2



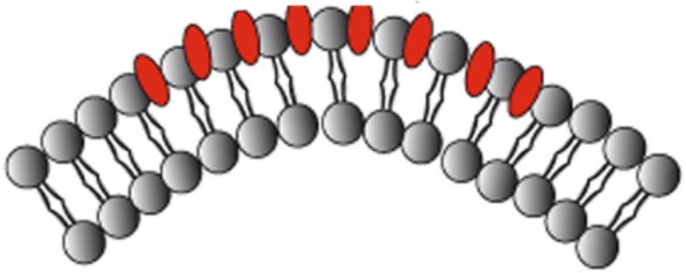
M. G. J. Ford *et al.*, *Nature* **419**, 361 (2002)

Amphipatic helix



Antonny, *Annu. Rev. Biochem.*, 2011

Inserts into the bilayer



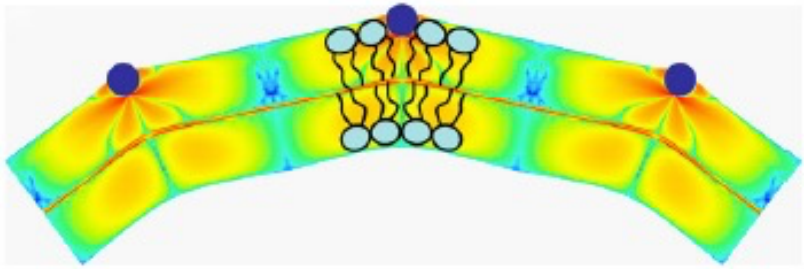
C_0 depends *on the depth of the insertion in the membrane*

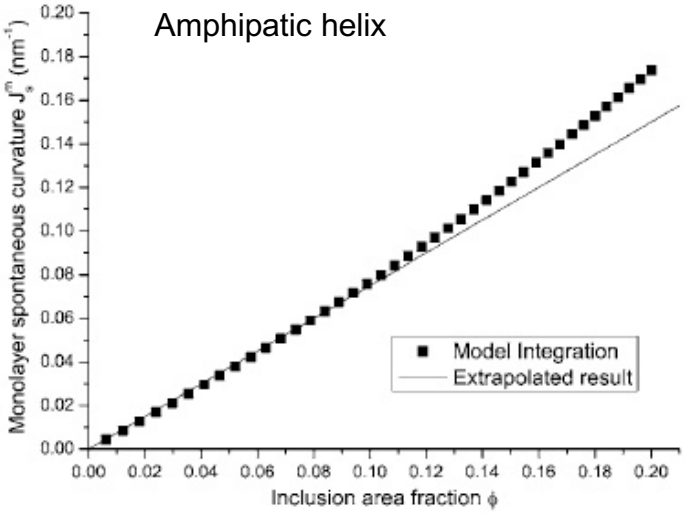
F. Campelo, H. Mc Mahon, M. Kozlov, *Biophys. J.*

Membrane Spontaneous Curvature

C_0 depends on *protein surface fraction* on the membrane: ϕ

$$f_{bending} = \frac{\kappa}{2} \times \left(C - C_0(\phi) \right)^2$$





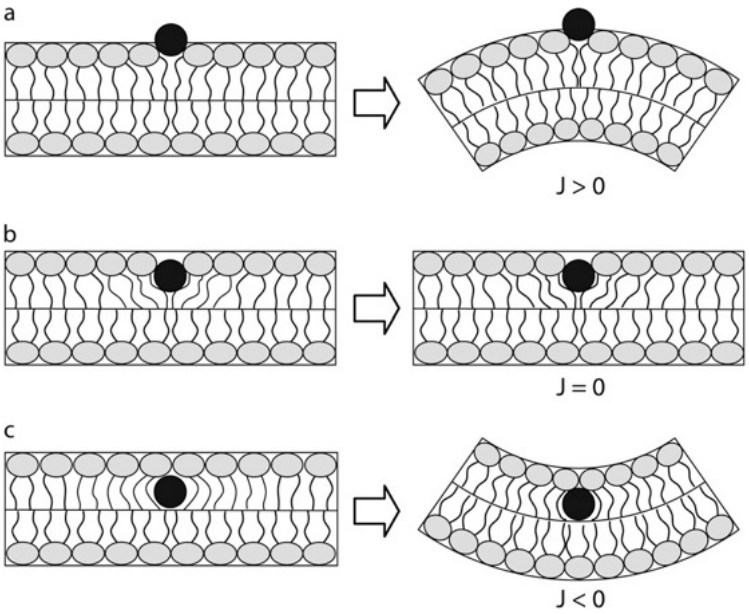
F. Campelo, H. Mc Mahon, M. Kozlov, *Biophys. J.* (2008)

At low ϕ (<10%):

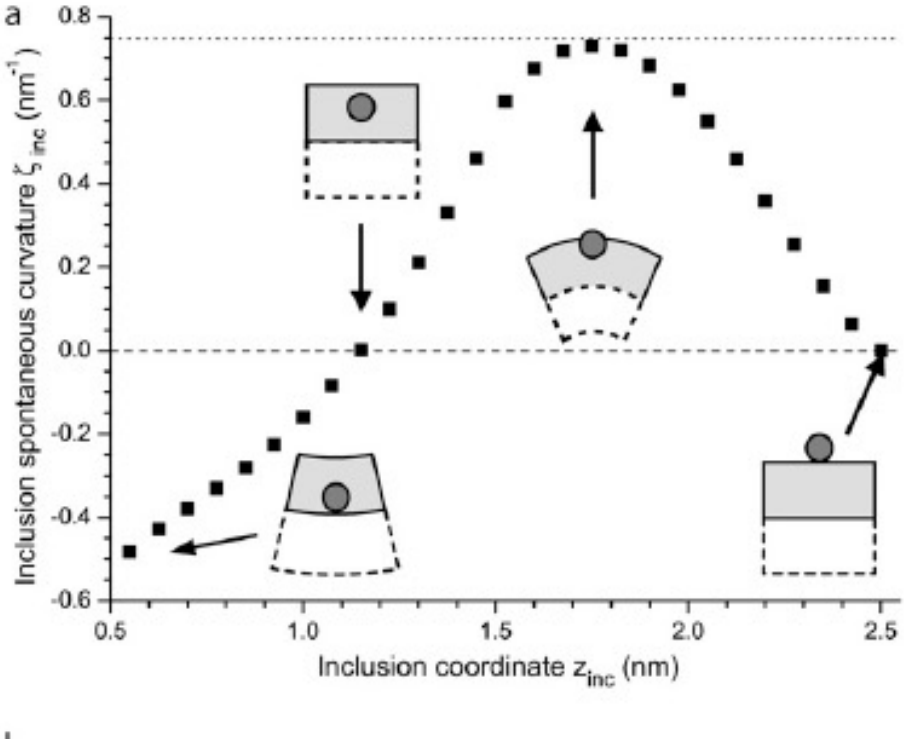
$$C_0(\phi) = \bar{C}_p \phi$$

$\bar{C}_p$: intrinsic spontaneous curvature of the *protein* in the membrane

- Membrane curvature depends on insertion depth

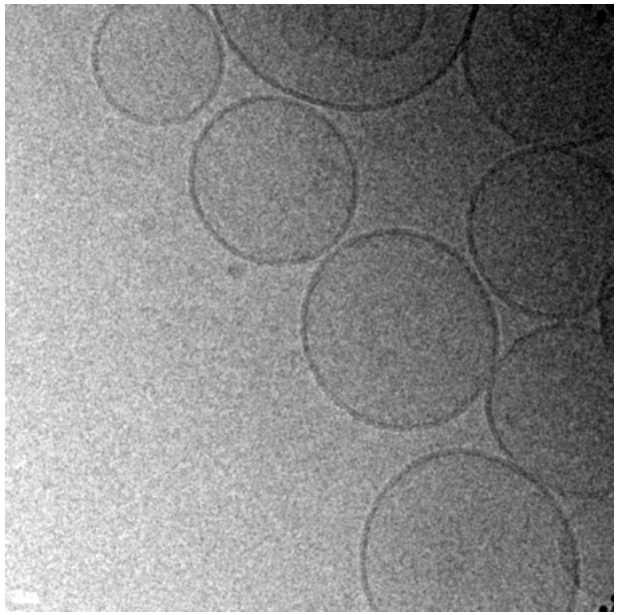


uncoupled bilayers



F. Campelo et al, *Biophys. J.* (2008)

At High Concentration: Tubulation



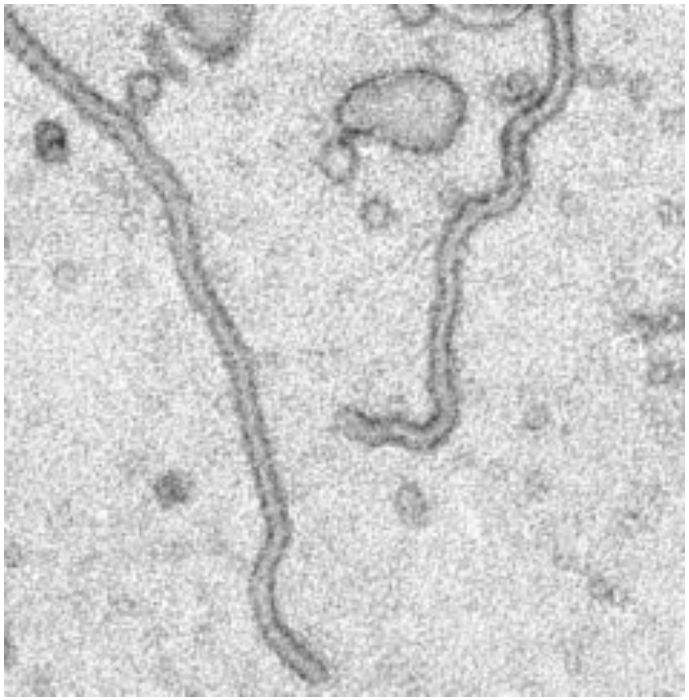
50 nm

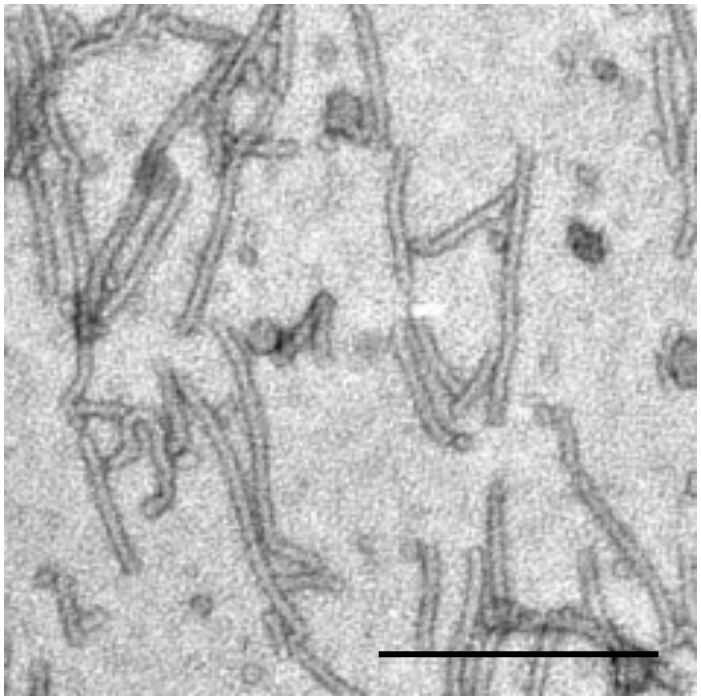
Liposomes (with PiP2)

full length

+ Epsin

ENTH domain





300 nm

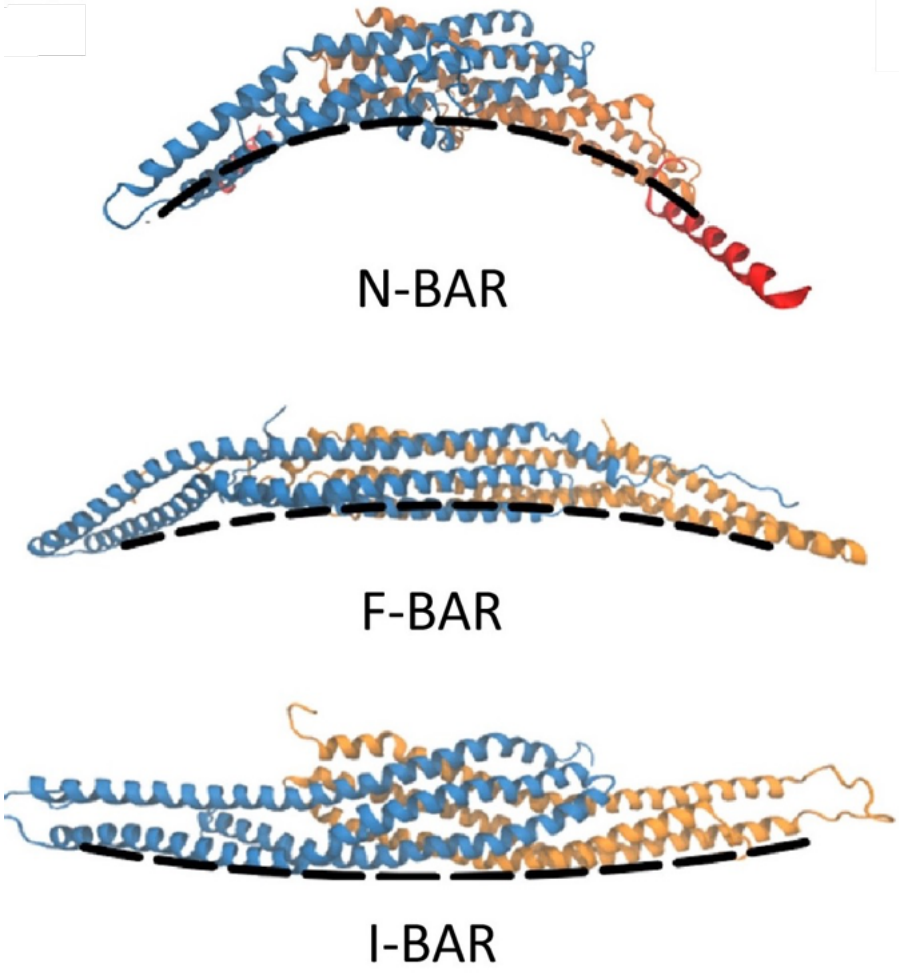
M. G. J. Ford *et al.*, *Nature* **419**, 361 (2002)

Membrane Curvature:

A Physical Cue

for Recruiting "Curved" Proteins

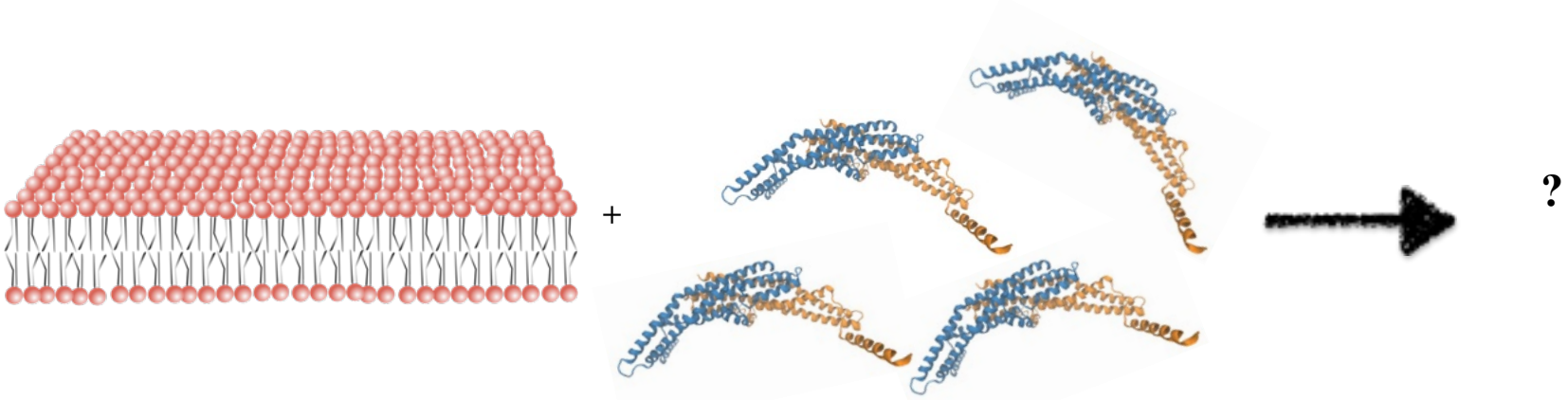
BAR-Domain Proteins: Dimers with Various Intrinsic Curvatures



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

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

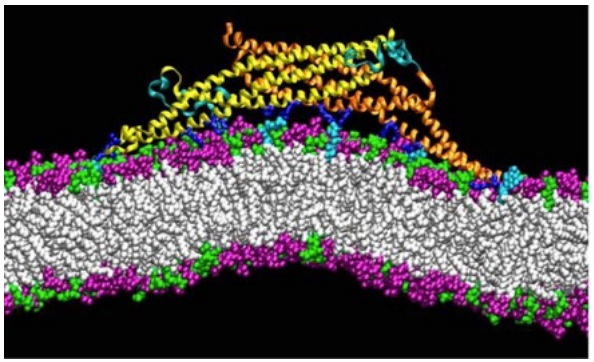
BAR-Domain Proteins on Membranes



Bind to *negatively-charged* membranes (PiP2, PS)

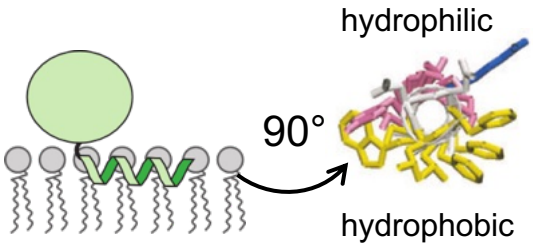
Locally: membrane bending

~ *"mold"*

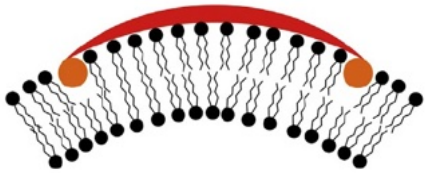


Blood P *et al*
Biophys. J. (2008)

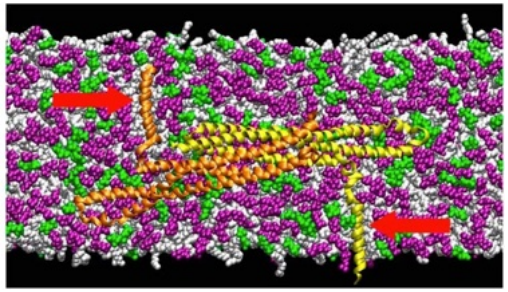
± amphipatic helices N-BAR



Antonny, *Annu. Rev. Biochem.*, 2011

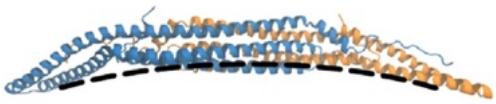


Top view

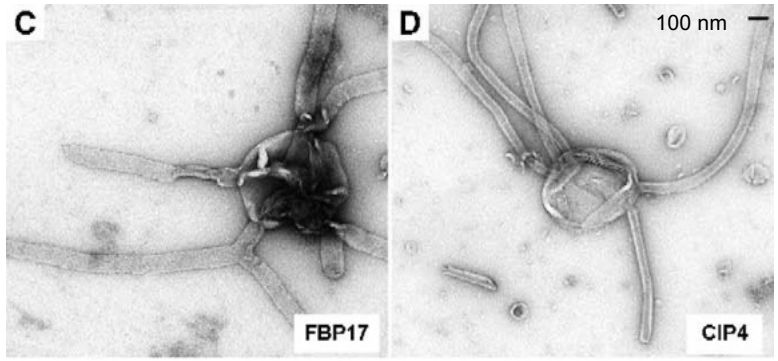


Liposome Tubulation with HIGH Bulk Concentration

$$R_{Tubule} \approx 1/\overline{C_p}$$



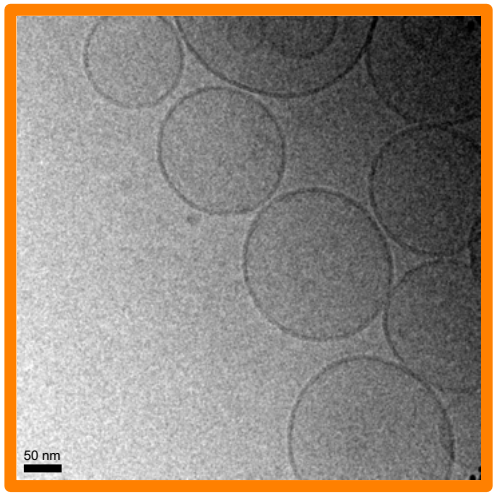
F-BAR



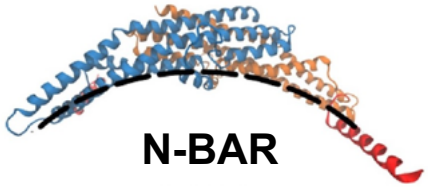
R ≃60 nm

T. Itoh *et al.*, *Dev. Cell* **9**, 791 (2005)

liposomes



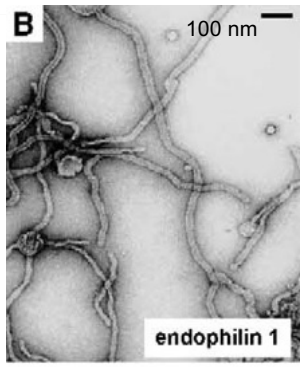
Cryo-EM (A. Bertin)

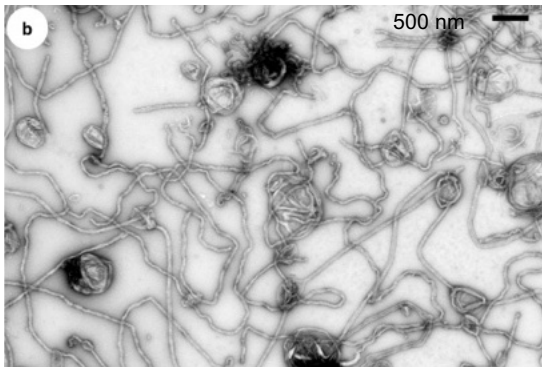


N-BAR

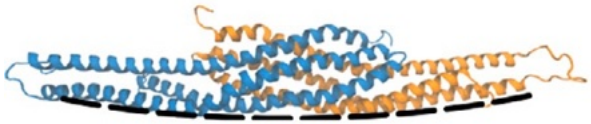
Mid- plane/membrane

R ≃10 nm



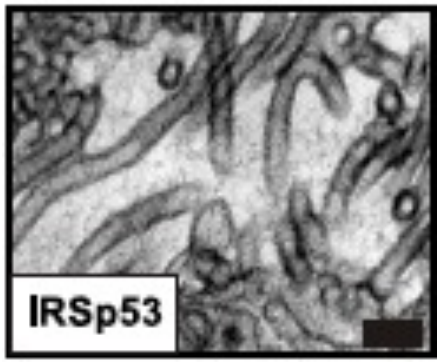


Farsad K, et al. *J. Cell Biol.* (2001)

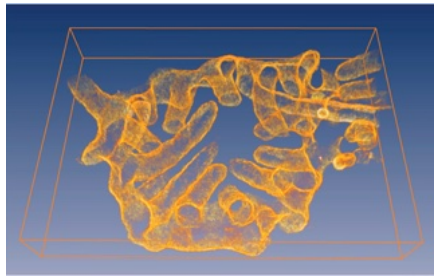


I-BAR

R=19 nm

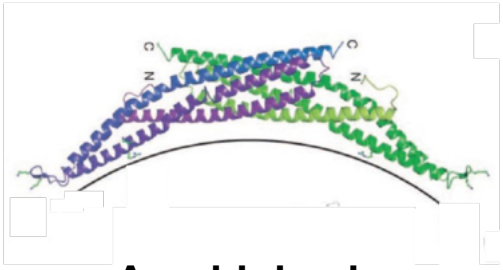


100 nm

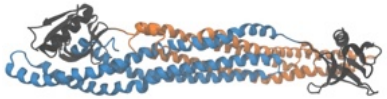


P. Mattila *et al.*, *J. Cell Biol.* (2007).

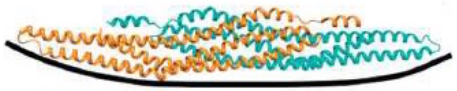
GUV Tubulation at HIGH Bulk Concentration (>μM)



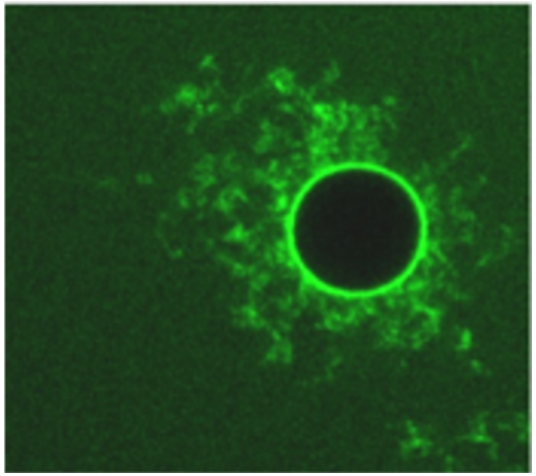
Amphiphysin

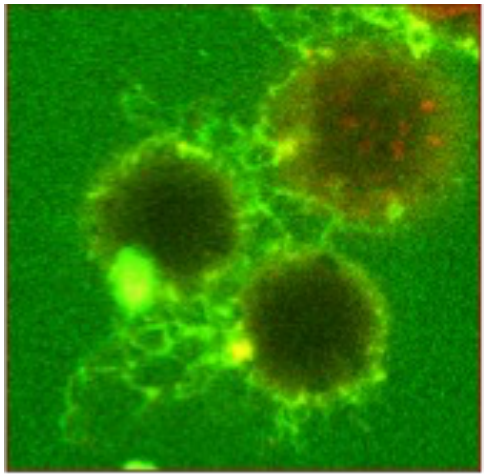


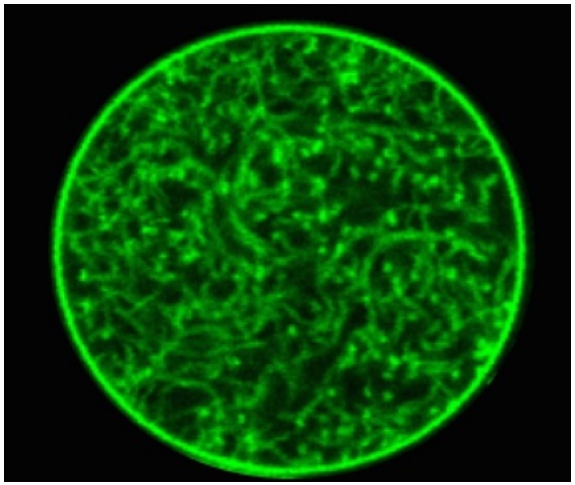
BAR: β-Centaurin



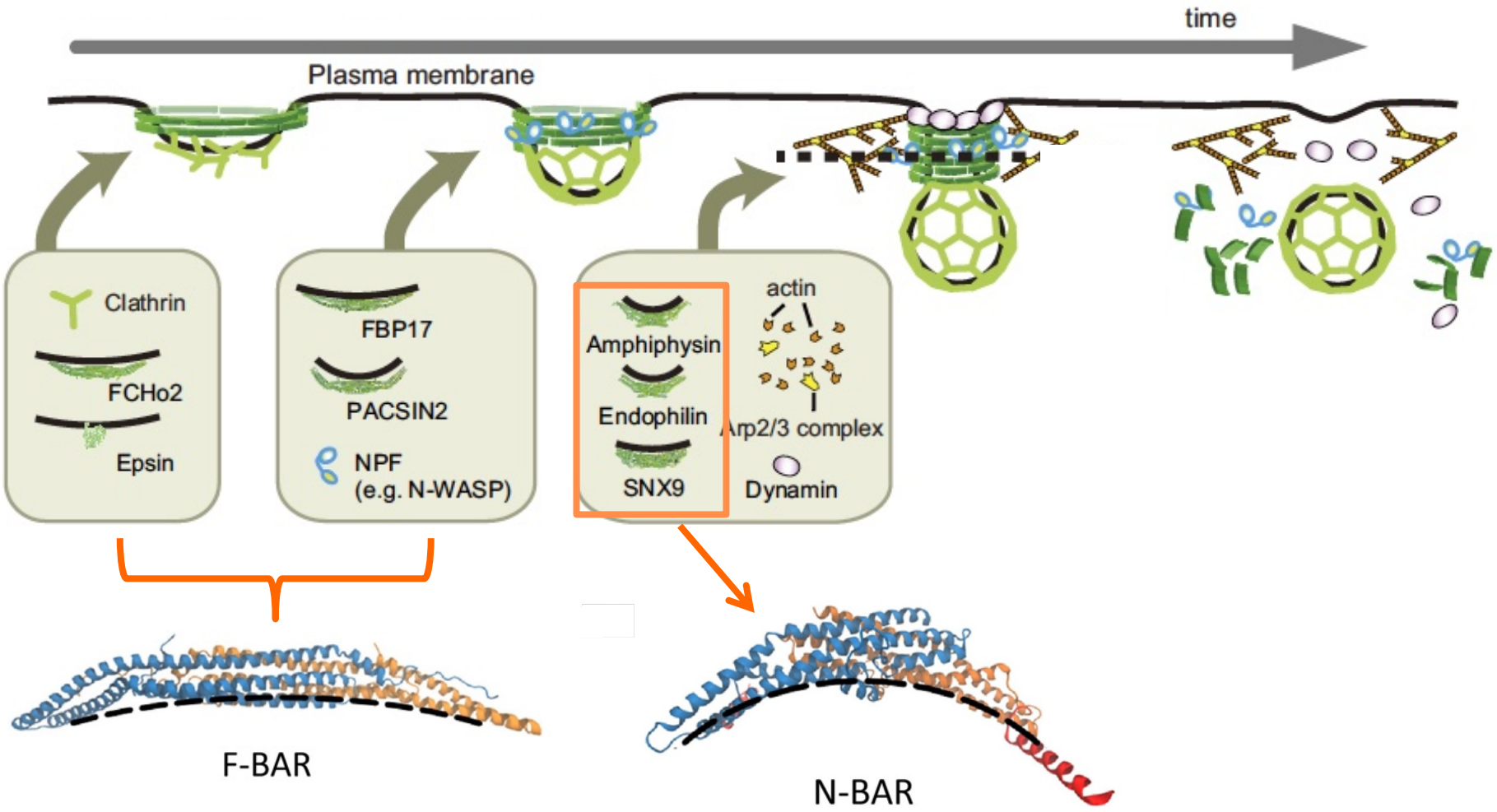
I-BAR: IRSp53







BAR-Domain Proteins and Endocytosis



S. Suetsugu, S. Kurisu, T. Takenawa, *Physiol. Rev.* (2014)

Membrane Spontaneous Curvature

$$f_{bending} = \frac{\kappa}{2} \times \left(C - C_0(\phi) \right)^2$$

(per unit area)

C_0 depends on *protein surface fraction* on the membrane: ϕ

At low ϕ (<10%):

$$C_0(\phi) = \bar{C}_p \phi$$

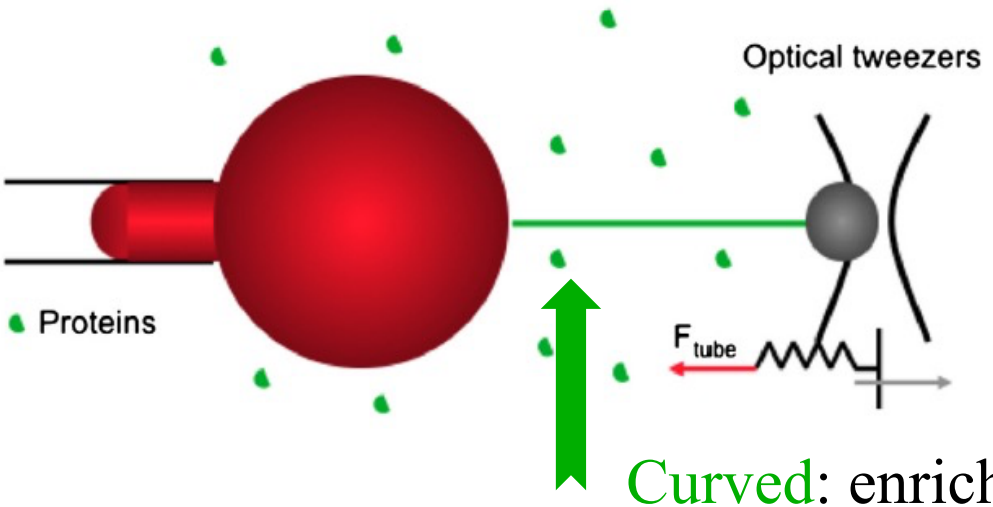
$\bar{C}_p$: effective spontaneous curvature of the *protein* (molecular)

F. Campelo …M. Kozlov *Biophys. J.* (2008)

Membrane curvature ⟺ Protein enrichment "curvature sensing"

Using Nanotubes to Investigate Curvature-Induced Protein Sorting

- Curvature-induced sorting of BAR domain proteins?
- Enrichment in tubes?



At low protein density

Curved: enrichment of proteins $\overline{C_p} \neq 0$

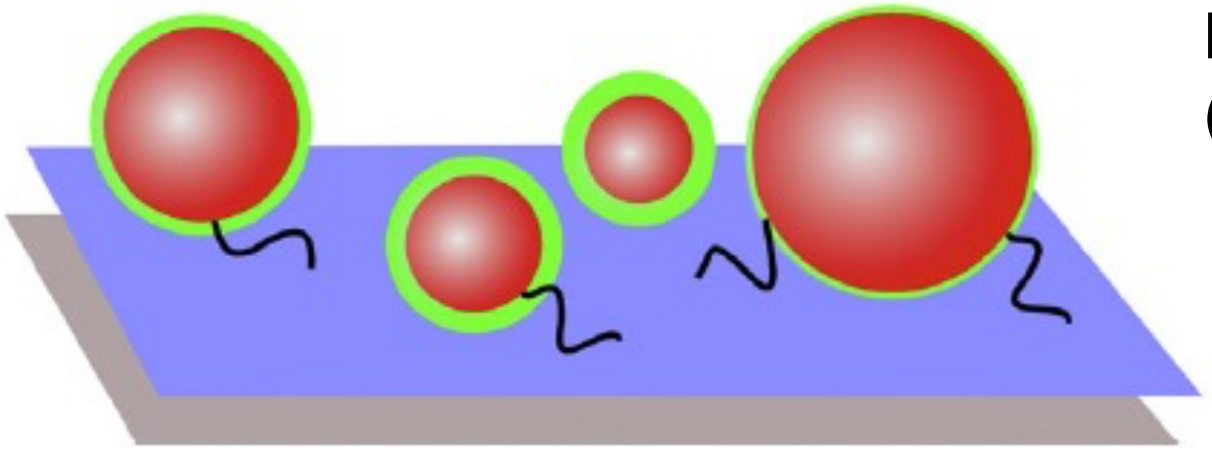
With confocal microscopy:

Sorting coefft: versus *Mean curvature*

$$S = \frac{\phi_t}{\phi_{ves}} = \frac{I_t^{prot}}{I_{ves}^{prot}} \Big/ \frac{I_t^{lip^*}}{I_{ves}^{lip^*}}$$

$$C = 1/R_{tube}$$ *at steady state*

Small Liposomes of Various Curvatures

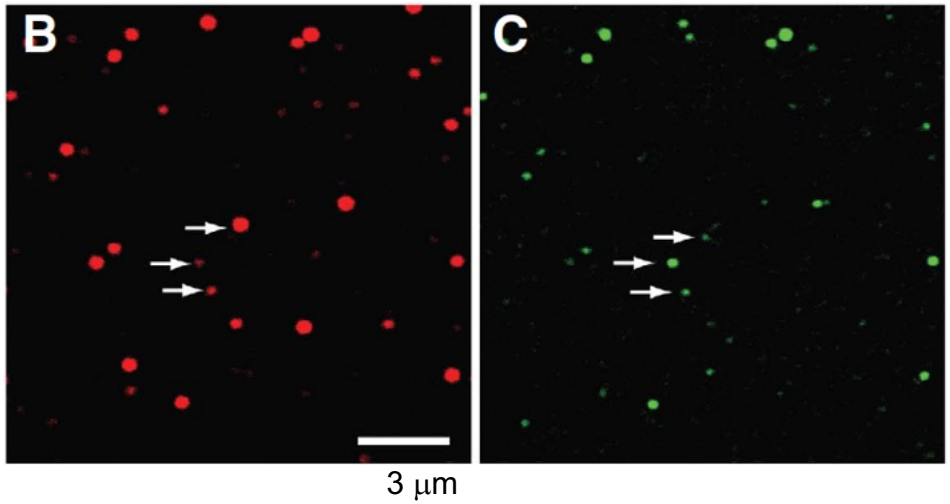


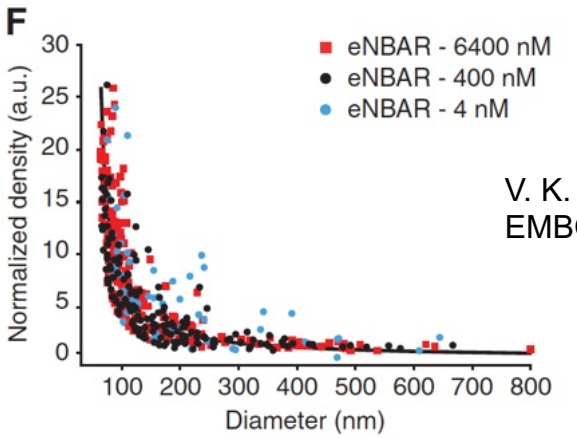
Dimitris Stamou
(Copenhagen)

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

TIRF detection of the fluorescence (proteins)
versus fluorescence of the liposomes (radius)

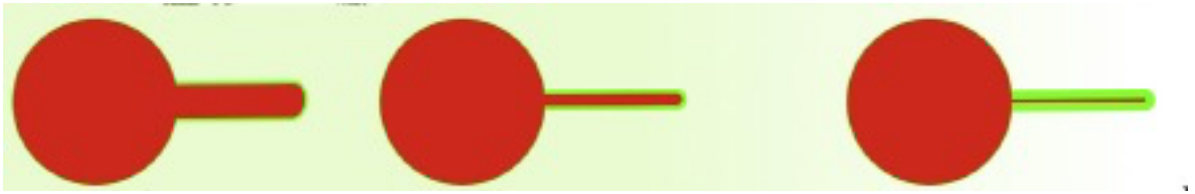
Lipids Endo-NBAR





V. K. Bhatia... D. Stamou
EMBO J. (2009)

Sorting of Molecules Induced by Membrane Curvature



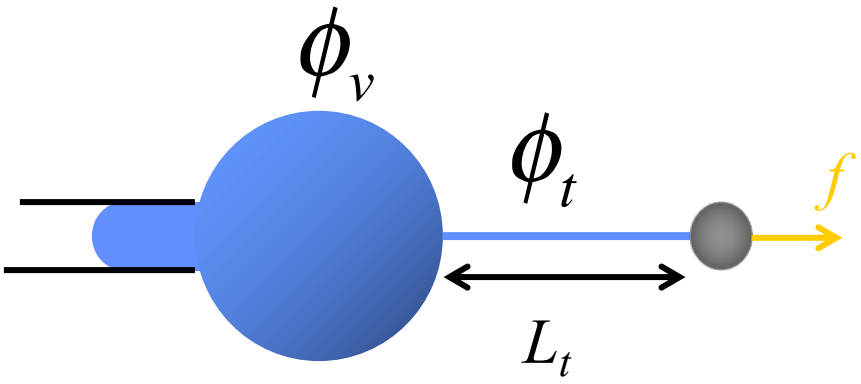
- Gain :
  Reduction of the bending energy
  due to spontaneous curvature (C_0)

- Penalty :
  Mixing Entropy
  Membrane stiffening (inclusions)

Curvature Sensing - Modeling

A. Callan-Jones

Tubes



$$\Delta\phi = \phi_t - \phi_v$$

In the dilute limit:

"Mixing entropy"

$$F_t = 2\pi R_t L_t \left[\frac{\kappa}{2}\left(\frac{1}{R_t^2} - \frac{2\overline{C}_p \phi_t}{R_t} \right) + \frac{1}{2}\chi\Delta\phi^2 + \sigma \right] - fL_t$$

bending

tension

with

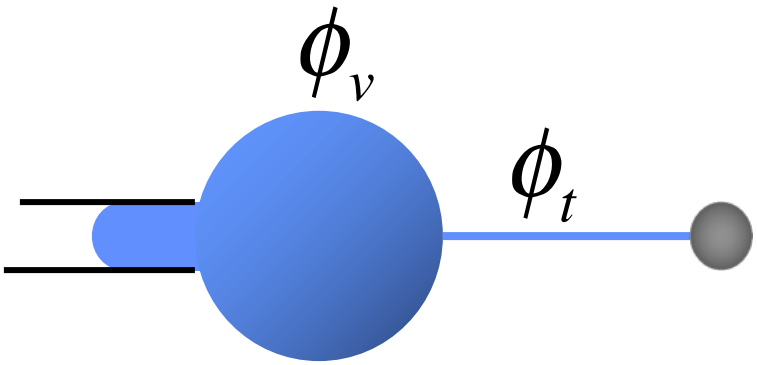
$$\chi = f_m''(\phi_v) + \kappa\overline{C_p^2} \approx \frac{k_B T}{A_p \phi_v} + \kappa\overline{C_p^2}$$

Sorre B., Callan-Jones A. et al, *PNAS* (2012)

Curvature Sensing - Modeling

A. Callan-Jones

Tubes



Protein sorting

$$S = \frac{\phi_t}{\phi_v} = 1 + \text{Cte}\ \frac{1}{R_t}$$

Linearly proportional to curvature

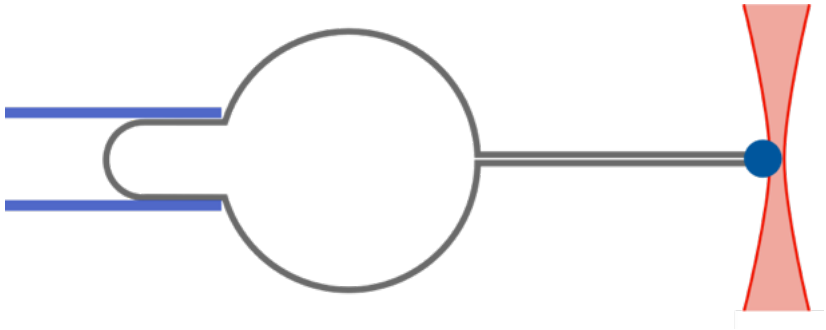
$$S = 1 + \frac{1}{\overline{C_p}\phi_v}\frac{1}{R_t}$$

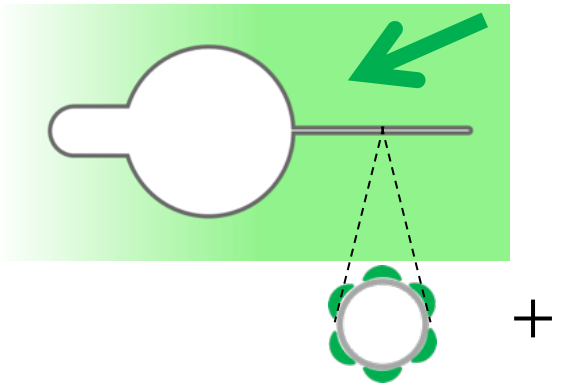
$\phi_v \longrightarrow 0$

$$S = 1 + \frac{A_p \kappa \overline{C_p}}{k_B T}\frac{1}{R_t}$$

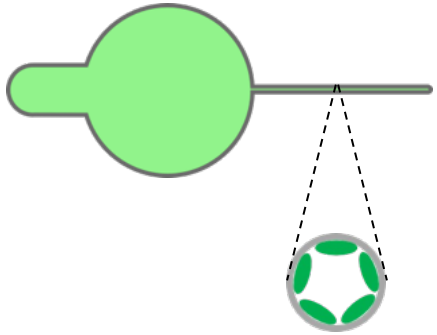
Sorre B., Callan-Jones A. et al, *PNAS* (*2012*)

Two Possible Geometries





+



−

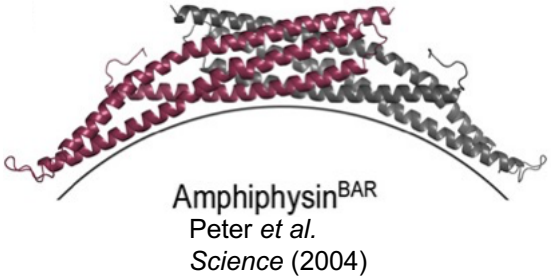
Interaction/*positive* curvature

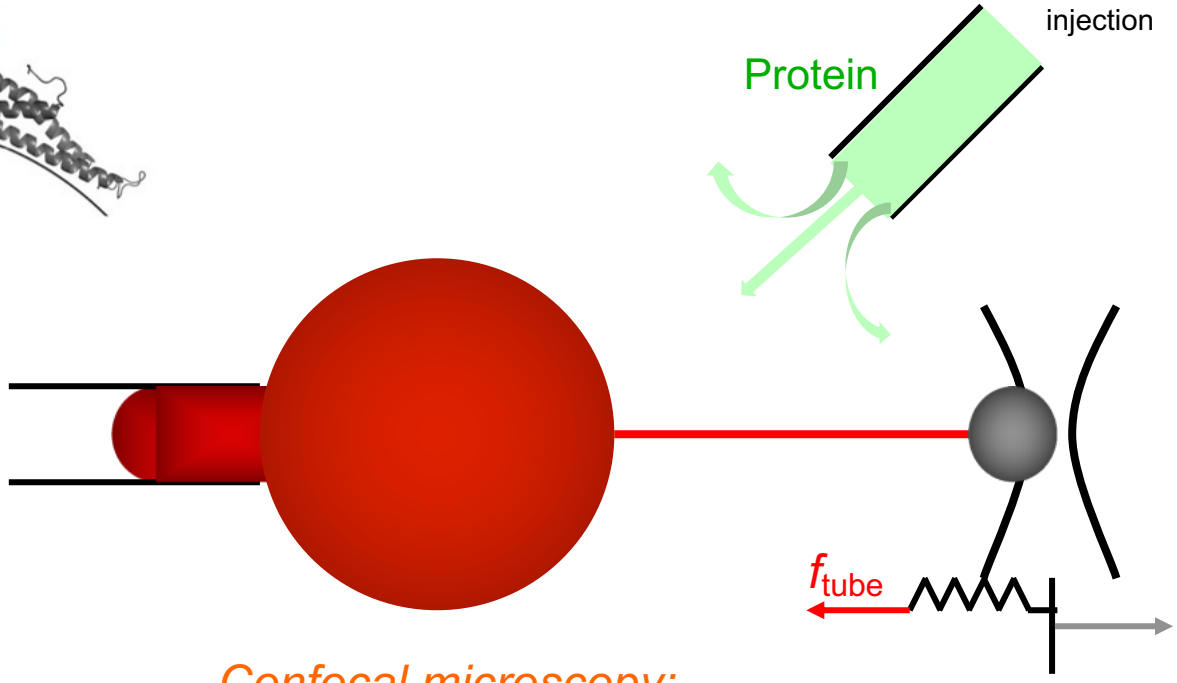
→ *inject* protein

Interaction/*negative* curvature

→ *encapsulate* protein

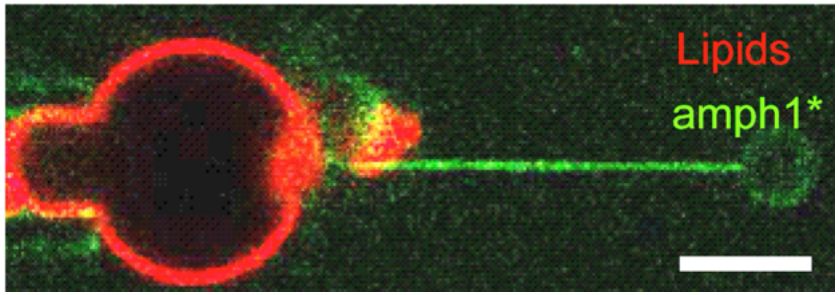
Measuring Curvature-Induced Enrichment





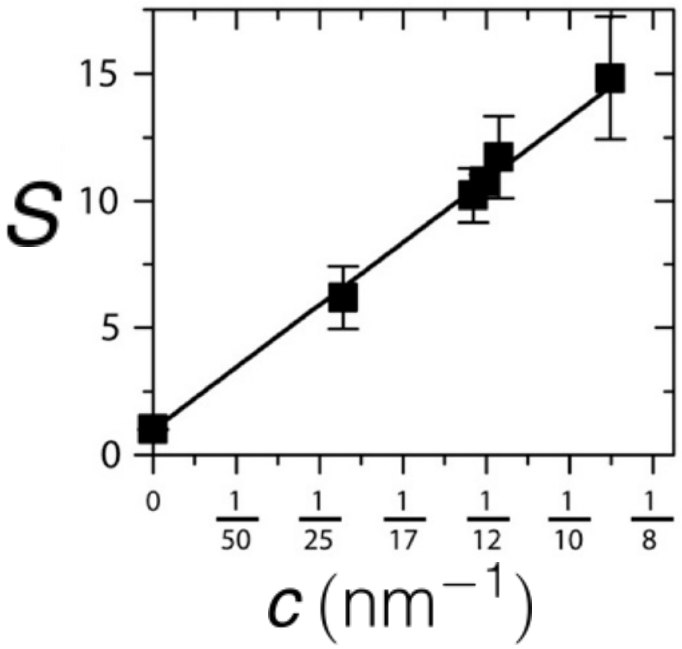
Confocal microscopy:
Protein binding

Low surface fraction on the GUV (1-5%)



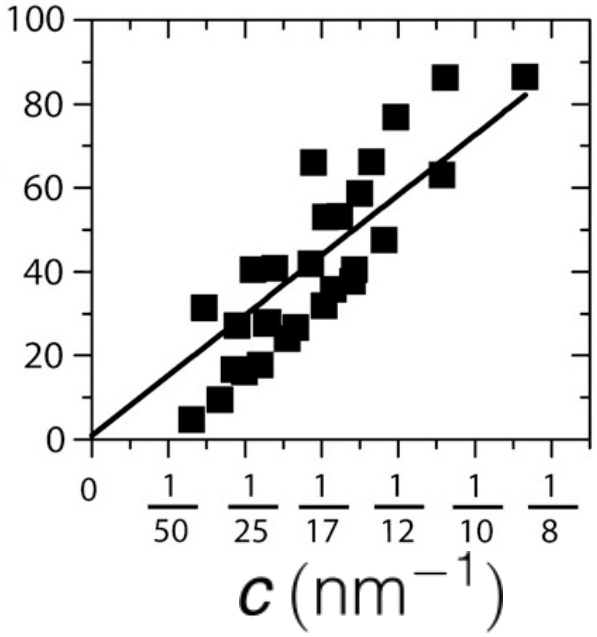
Amphiphysin 1 – Enrichment in Nanotubes

$\phi_v = 1.4\%$



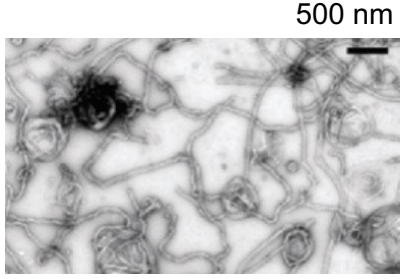
$\overline{C_p} = 1.9 \pm 0.4\ nm^{-1}$

$\phi_v < 0.25\%$

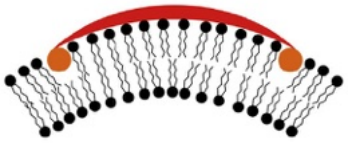


$\overline{C_p} = 0.8 \pm 0.4\ nm^{-1}$

R=11 nm….



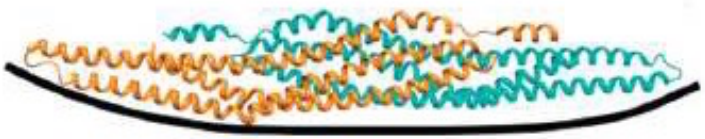
K. Takei …P. De Camilli *NCB* (1999)



Amphipatic helices dominant?

Sorre B., Callan-Jones A. et al*PNAS* (2012)

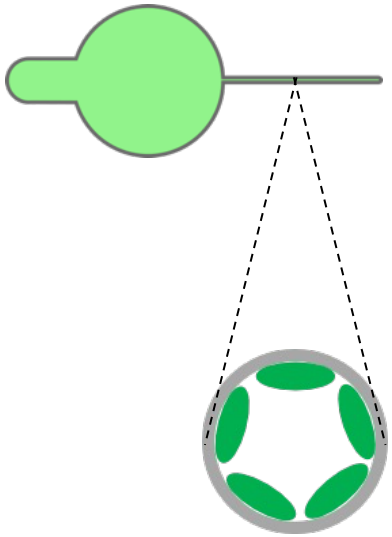
IRSp53 I-BAR Senses Negative Membrane Curvature

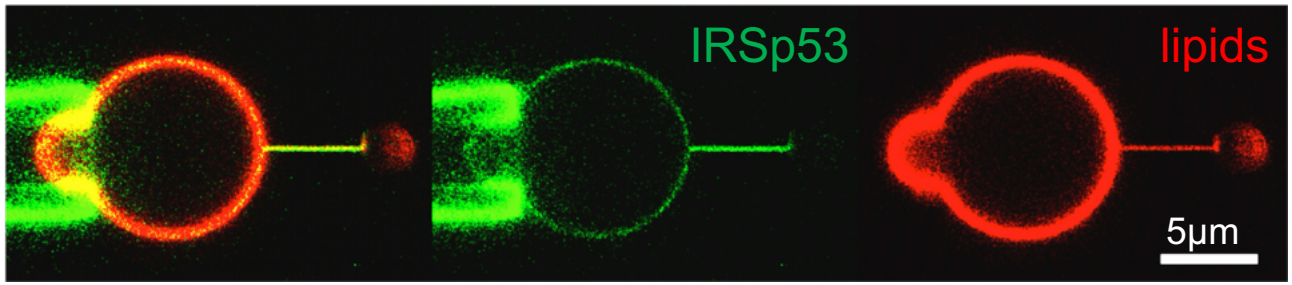


- Proteins *encapsulated* in the GUVs

  EPC (57%), cholesterol (15%), DOPE (10%)
  PI(4,5)P_2 (8%), DOPS (10%)

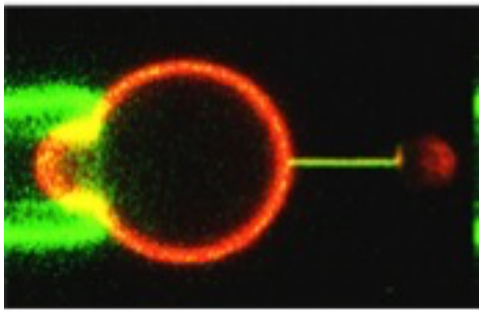
- Pulling tube – **Negative curvature** induces IRSp53 **enrichment**



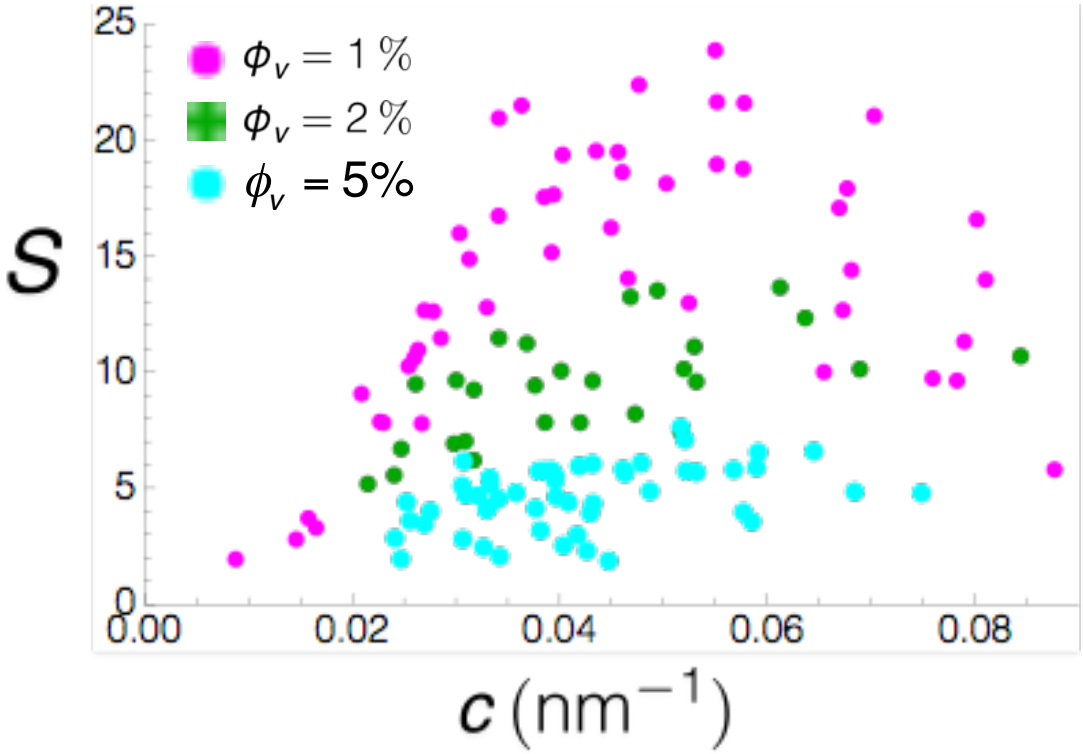


C. Prévost et al, *Nat. Comm.* (2015)

IRSp53 I-BAR Senses Negative Membrane Curvature



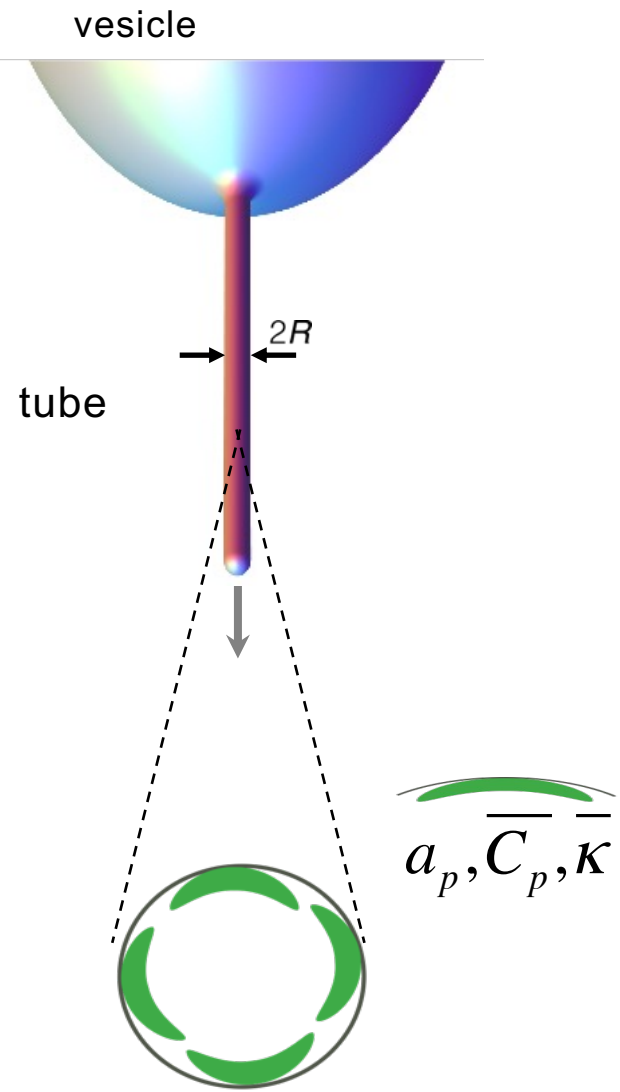
Not linear……



C. Prévost et al, *Nat. Comm.* (2015)

Another Sorting Model

A. Callan-Jones



- Tube energy:

$$F_t = 2\pi RL\left[\begin{array}{l} \dfrac{\kappa}{2}\dfrac{1}{R^2} \\ +\dfrac{\overline{\kappa}}{2}\phi_t\left(\dfrac{1}{R}-\left|\overline{C_p}\right|\right)^2 \\ +\ \textit{stretching}\ (\sigma) + \textit{mixing entropy} \end{array}\right]$$

Bare membrane

Coated membrane

Energy cost if $\dfrac{1}{R} \neq \overline{C}_p$

- No direct protein-protein interaction
- If $\phi_v \to 0$

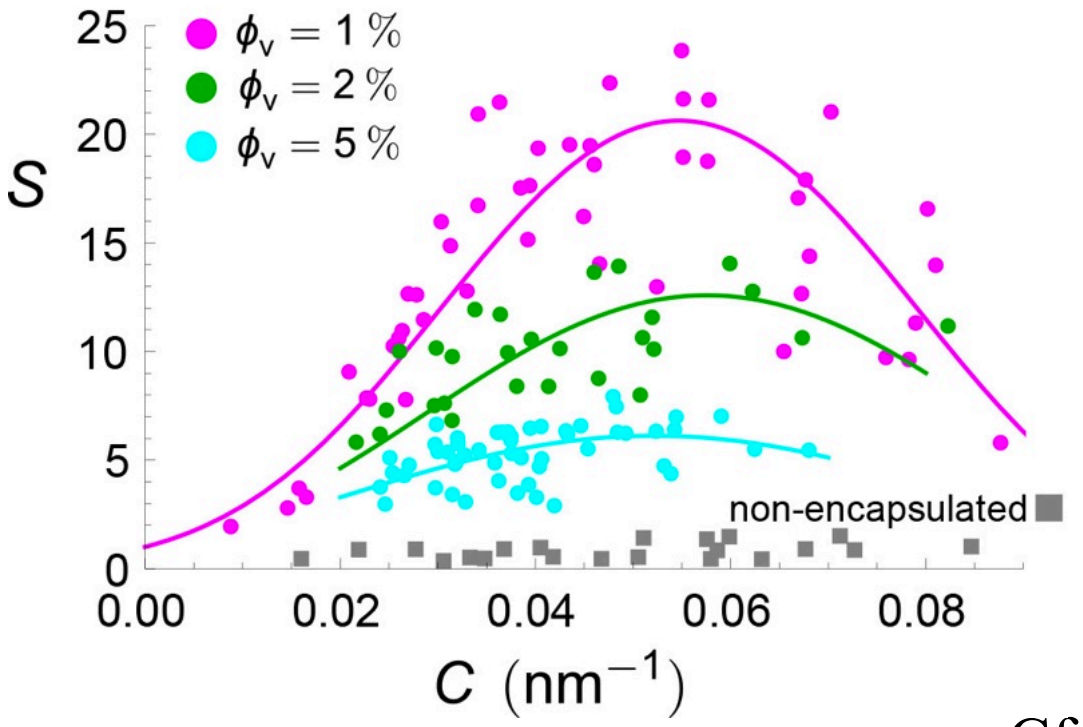
$$S \approx \exp\left[\frac{\bar{\kappa}\, A_p}{k_B T}\left(\frac{\left|\overline{C_p}\right|}{R}-\frac{1}{2R^2}\right)\right]$$

Otherwise, numerical solutions

C. Prévost et al, *Nat. Comm.* (2015)

IRSp53 Sorting

$$S = \frac{\phi_t}{\phi_v} = \frac{I^t_{prot}}{I^v_{prot}} \Big/ \frac{I^t_{lip^*}}{I^v_{lip^*}}$$



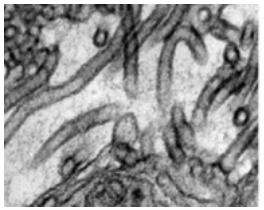
Fit ➡ $\overline{C_p}^{-1} = 18$ nm

$\bar{\kappa} \approx 35 \pm 7 k_B T$

$(\kappa \approx 20 k_B T)$

Radius ~ 19 nm

Cf liposomes/EM

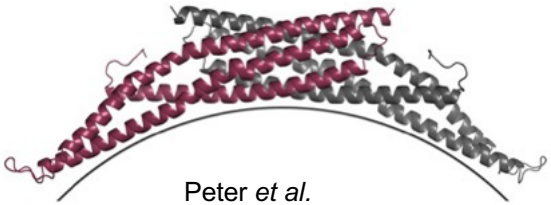


Saarikangas et al., *Current Biol.*, 2009

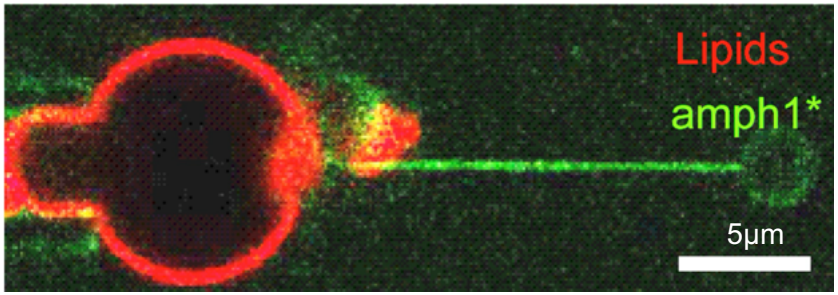
Optimal enrichment
in tubes of radius 18 nm

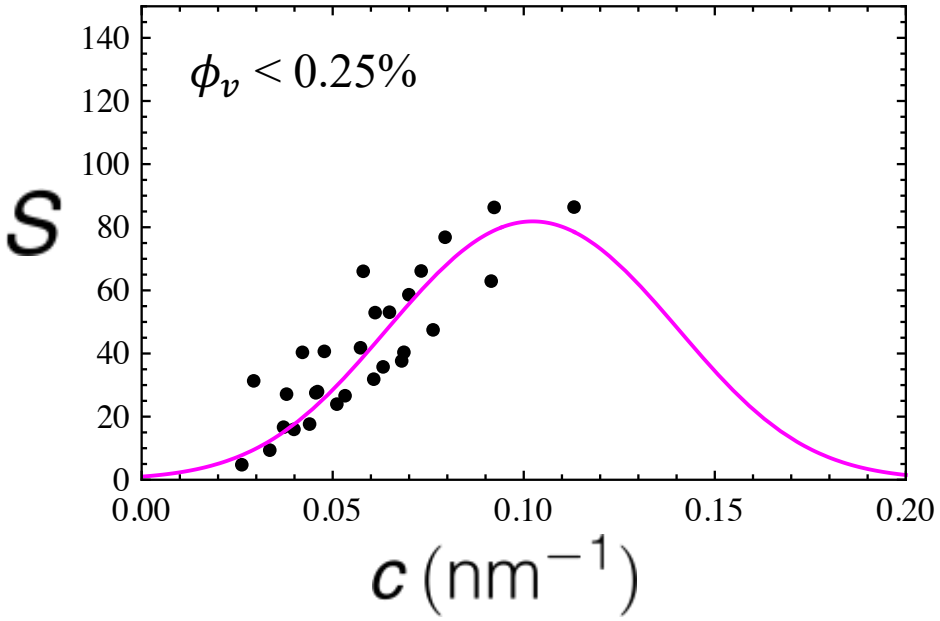
C. Prévost et al, *Nat. Comm.* (2015)

Amphiphysin 1 – New analysis



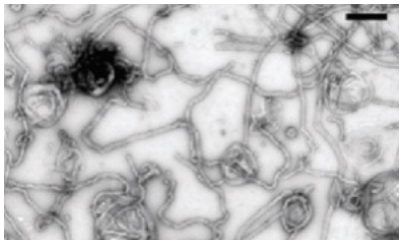
Peter *et al.*
Science (2004)





$\overline{C_p} = 1/10nm$

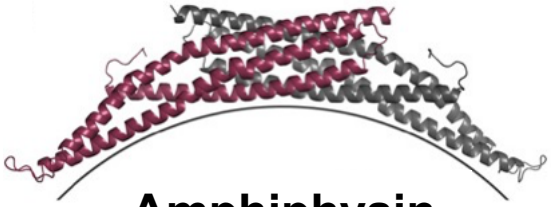
R=11 nm



K. Takei …P. De Camilli *Nat. Cell Biol.* (1999)

F.C. Tsai et al *Soft Matter* (2021)

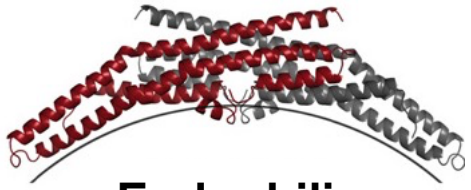
Sorting of BAR-Domain Proteins



Amphiphysin

$\overline{C_p} = 1/10nm$

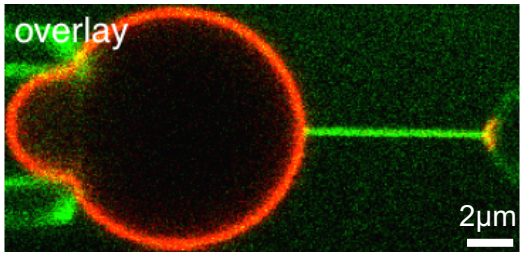
B. Sorre B., A. Callan-Jones et al, *PNAS* (2012)
F.C. Tsai et al *Soft Matter* (2021)



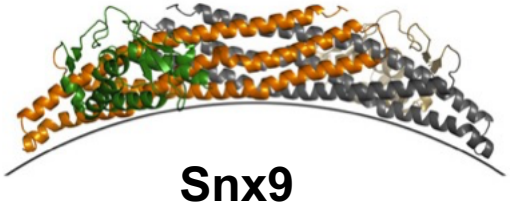
Endophilin

M. Masuda et al *EMBO J.* (2006)

$\overline{C_p} \sim 1/10nm$



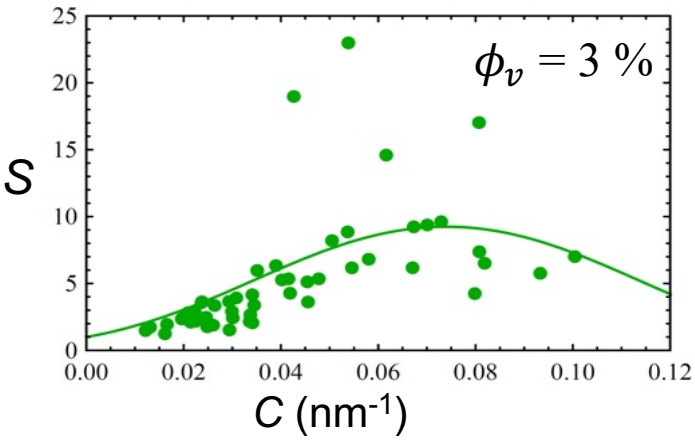
M. Simunovic

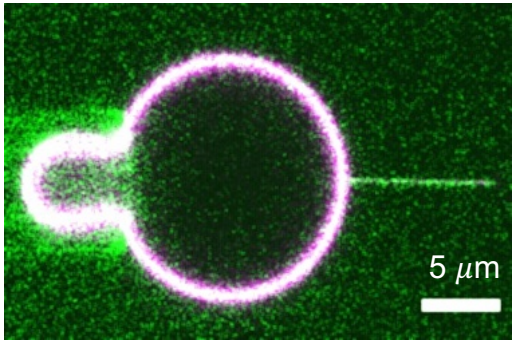


Snx9

O. Pylypenko et al *EMBO J.* (2007)

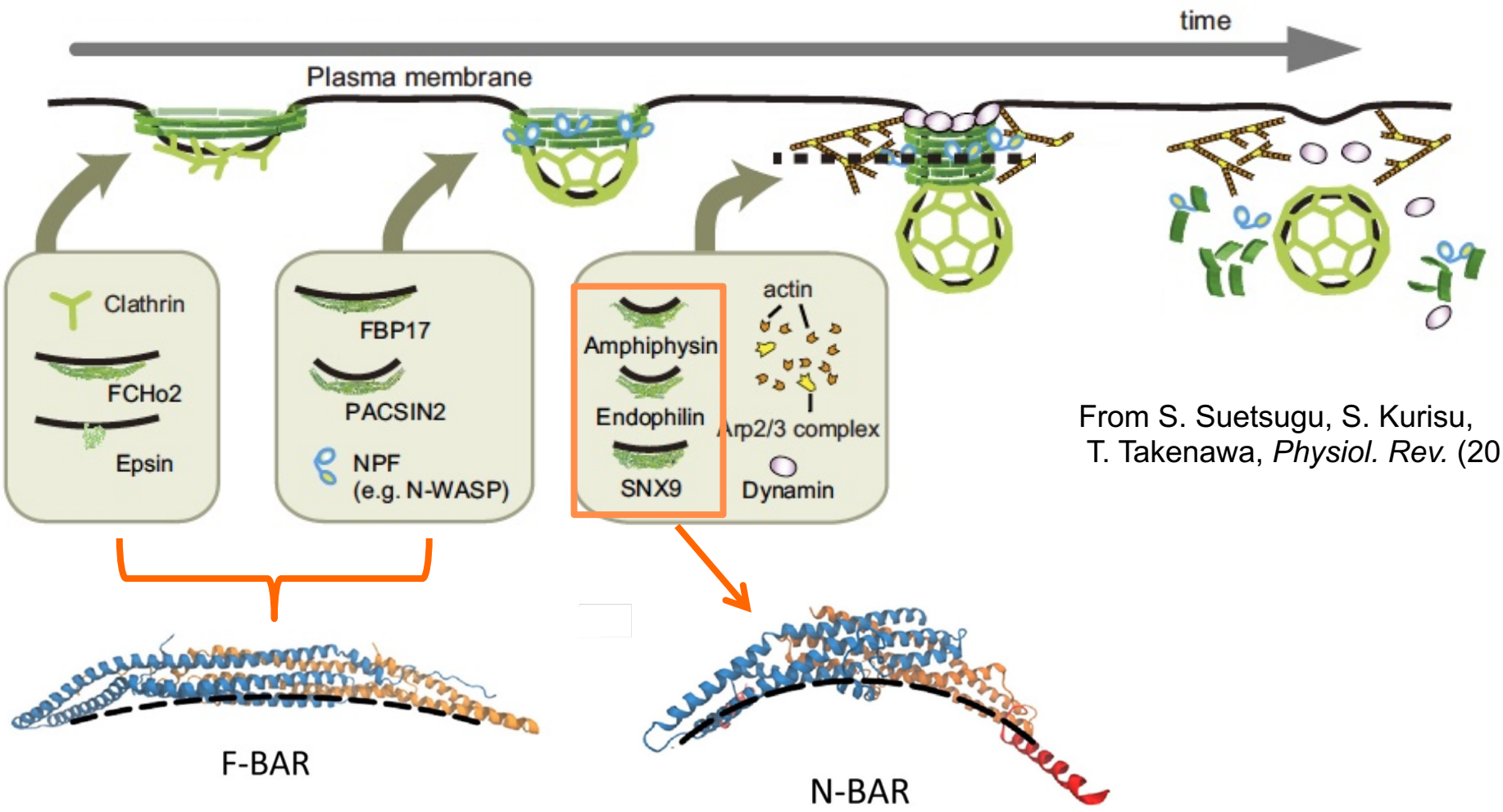
$\overline{C_p} = 1/13nm$





F.C. Tsai et all (in preparation)

BAR-Domain Proteins and Endocytosis



From S. Suetsugu, S. Kurisu, T. Takenawa, *Physiol. Rev.* (2014)

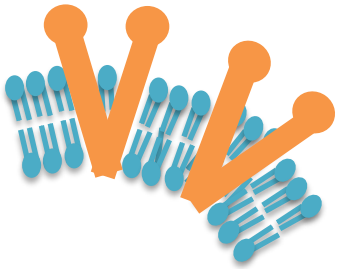
Very "curved" BAR proteins : last stage of endocytosis when *neck constricted* (R≈10 nm)

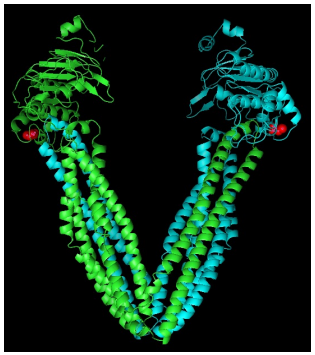
Curvature matching mechanism

Curvature-Induced Sorting of Transmembrane Proteins

Liposome Tubulation

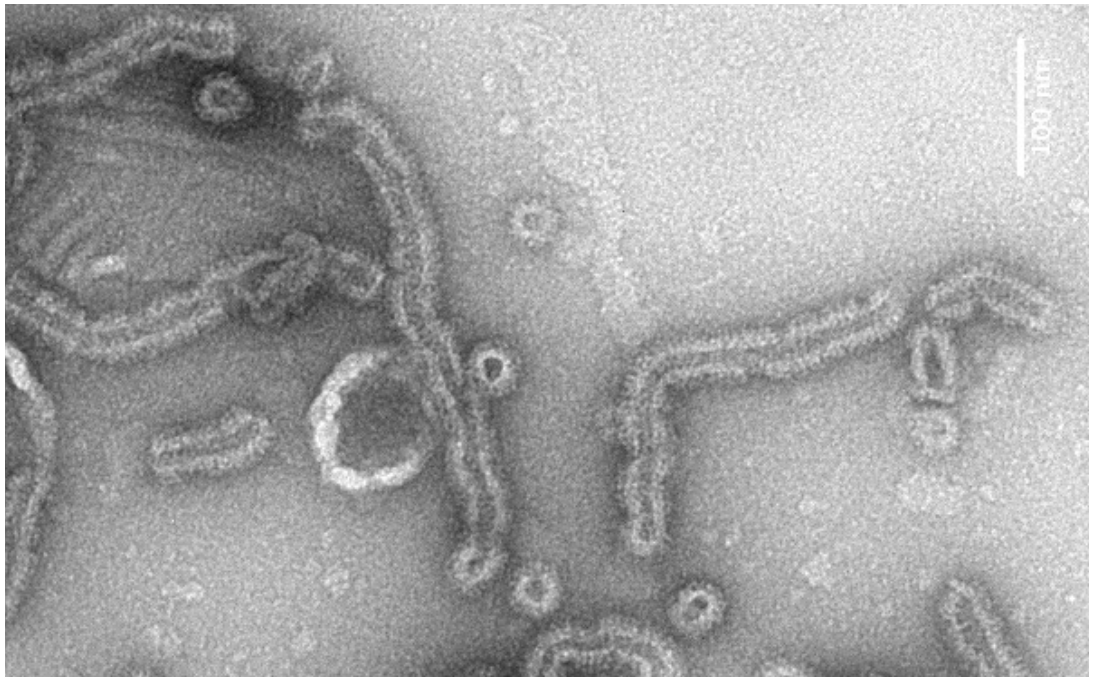
BmrA (ABC transporter)





Conical shape

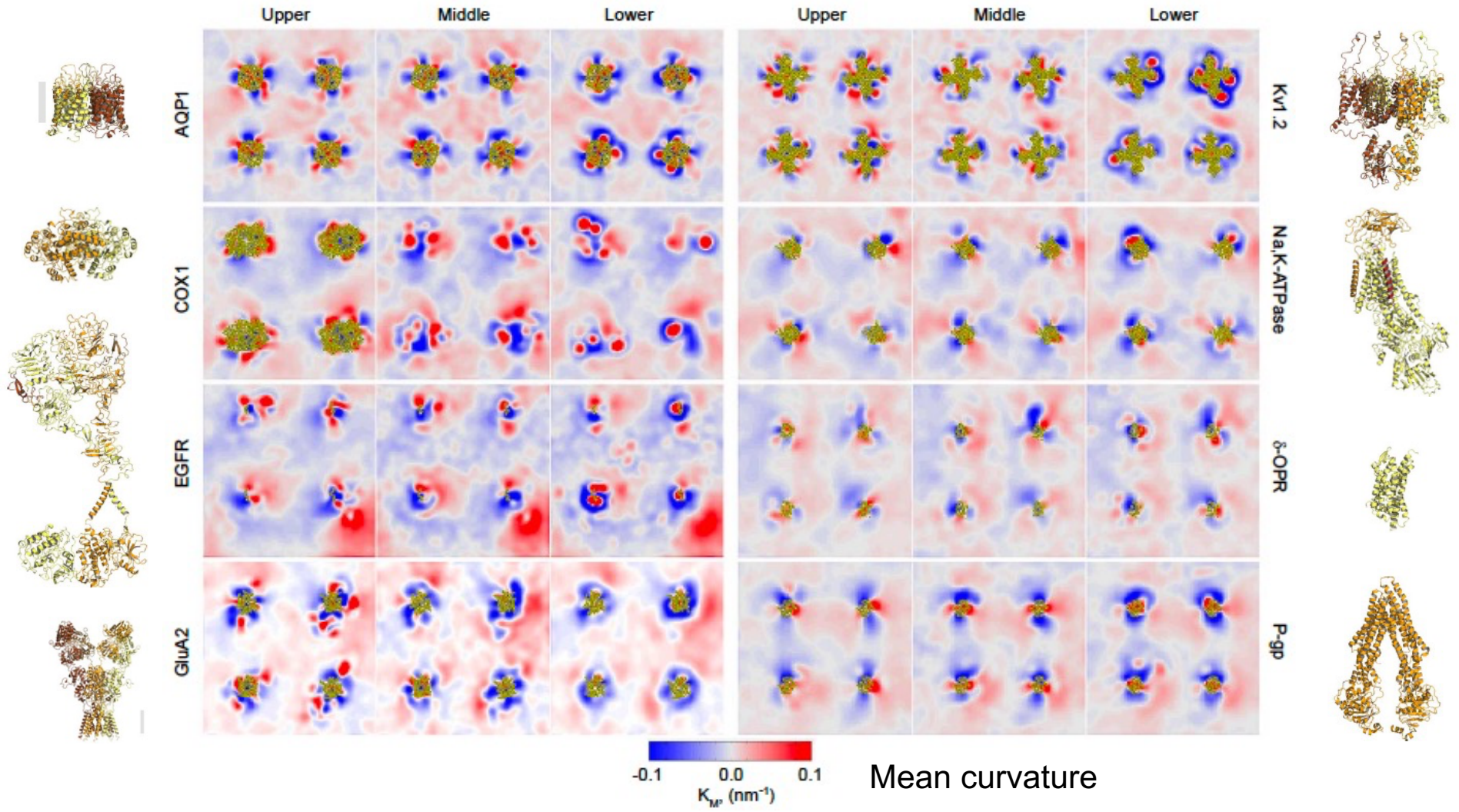
Reconstituted in liposomes



100 nm

P. Fribourg … D. Levy *J. Mol. Biol.* (2014)

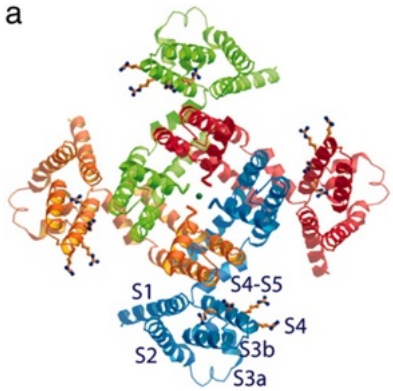
Local Membrane Bending: "Fingerprint" of Membrane Proteins

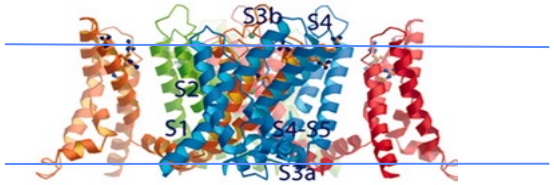


V. Corradi….**D. P. Tieleman** *ACS Cent. Sci.* (2018)

Distinguishing Transmembrane Proteins Shape

- *Voltage-gated K^+ channel: KvAP*

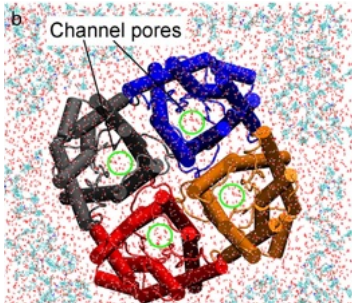


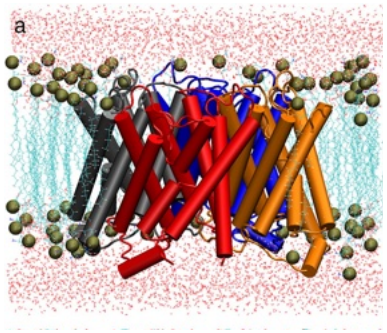


Lee et al *PNAS* (2006)

conical

- *Aquaporin 0 (from eye lenses): AQP0*





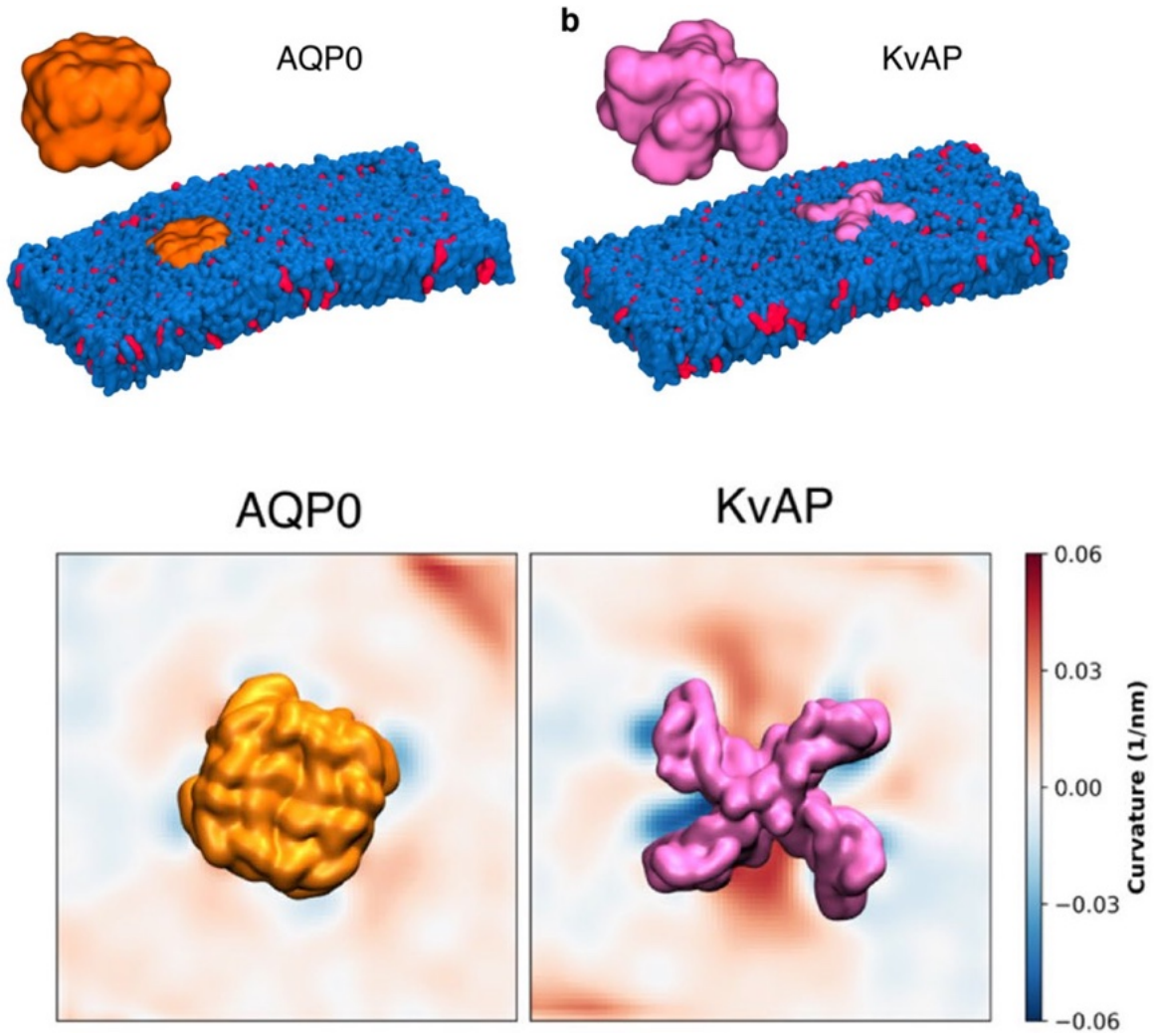
Qiu et al, 2010

cylindrical

Aimon S. *et al, Dev. Cell* (2014)

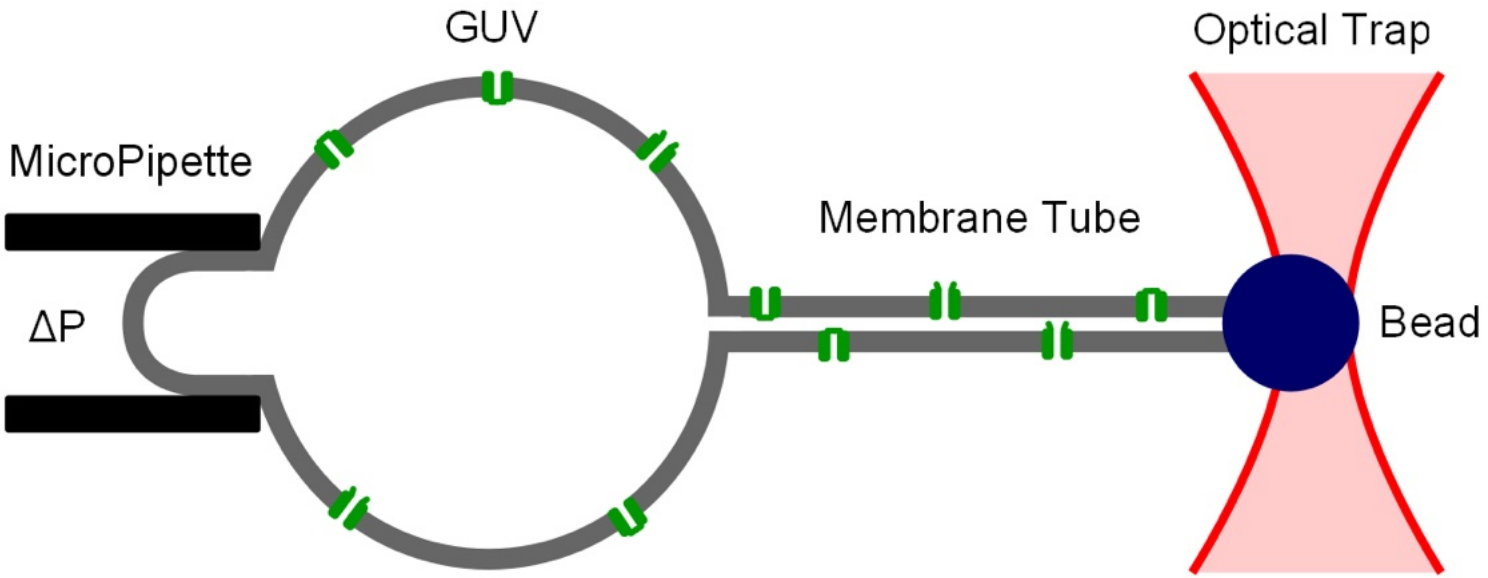
KvaP Bends Membrane - AQP0 Not

Coarse-grained simulations



D. P. Tieleman et al *Biophys. Rev.* (2021)

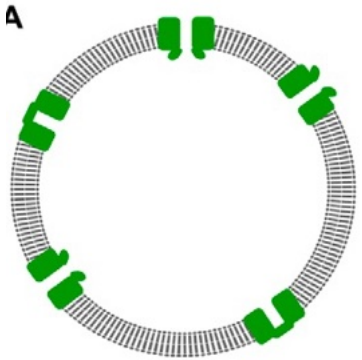
Curvature-Induced Trans-Membrane Protein Sorting



KvAP Sorting

Theory **A. Callan-Jones**
G. Toombes

$$F_{bending} = \frac{\kappa(\phi)}{2} \times \left(C - C_0(\phi)\right)^2$$



- 2 orientations (equal)

$$C_0(\phi) = \sum_i \overline{C_{p,i}} \phi_i \quad \text{If} \quad \phi^+ = \phi^- \quad C_0(\phi) = 0$$

Requires higher order term

- If proteins stiffer than the lipid membrane
  ➡ limits enrichment

$$\frac{1}{\kappa(\phi)} = \frac{1-\phi}{\kappa_{lipid}} + \frac{\phi}{\kappa_{protein}}$$

- Mixing entropy

Aimon S. *et al, Dev. Cell* (2014)

In the limit of low concentration and low curvature:

$$S \approx 1 + \frac{\left(\frac{A_p \kappa_l \overline{C}_p}{k_B T}\right)^2 - \frac{A_p \kappa_l \left(1 - \kappa_l / \kappa_p\right)}{k_B T}}{\left(1 + \frac{\kappa_l \overline{C}_p^{\,2} \phi_v^2}{k_B T}\right)^2} \times \frac{C^2}{2}$$

A_p= 45 nm^2 (KvAP molecular area)

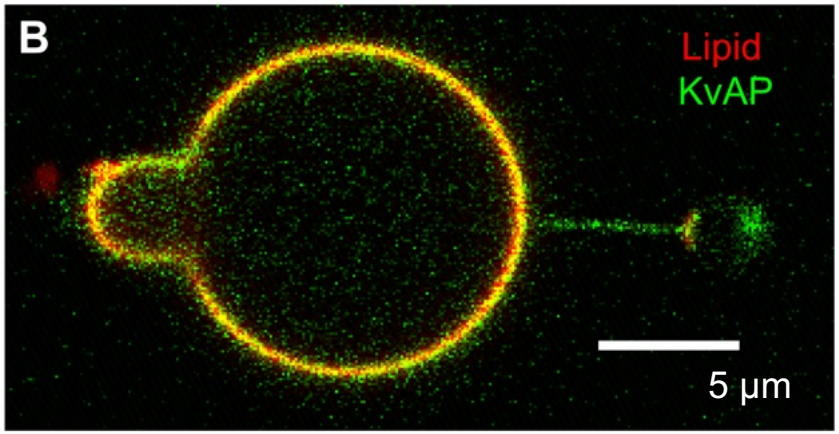
κ_l: bending rigidity pure *lipid* membrane

κ_p: effective bending ridity of the *proteins*

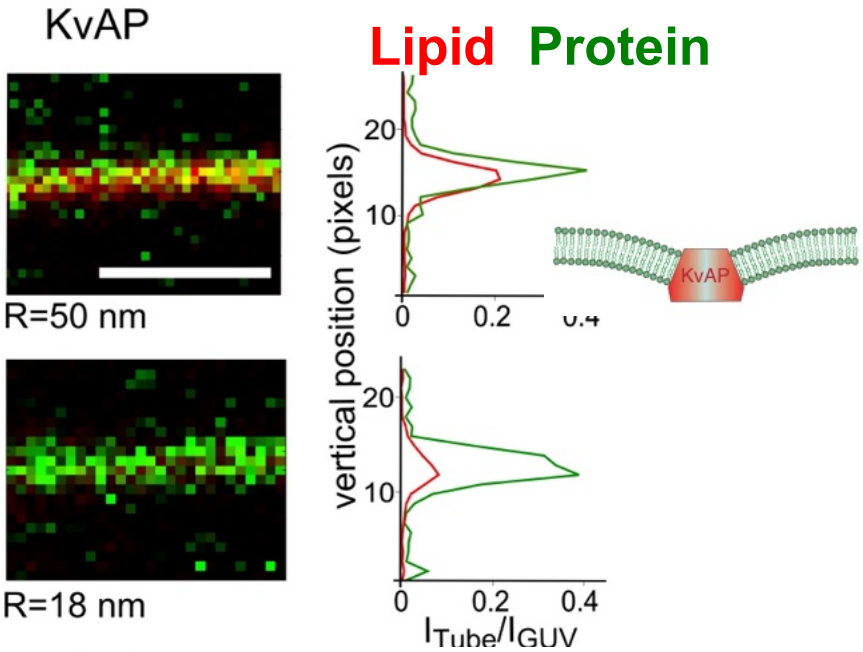
Aimon S. *et al, Dev. Cell* (2014)

Curvature and Protein Distribution

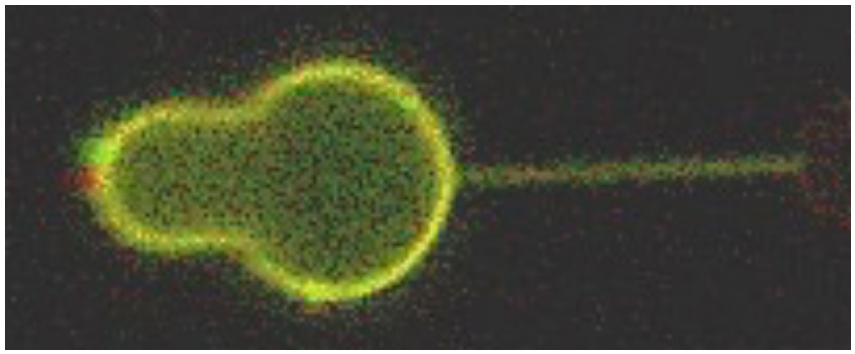
KvAP: *enriched in tubes*



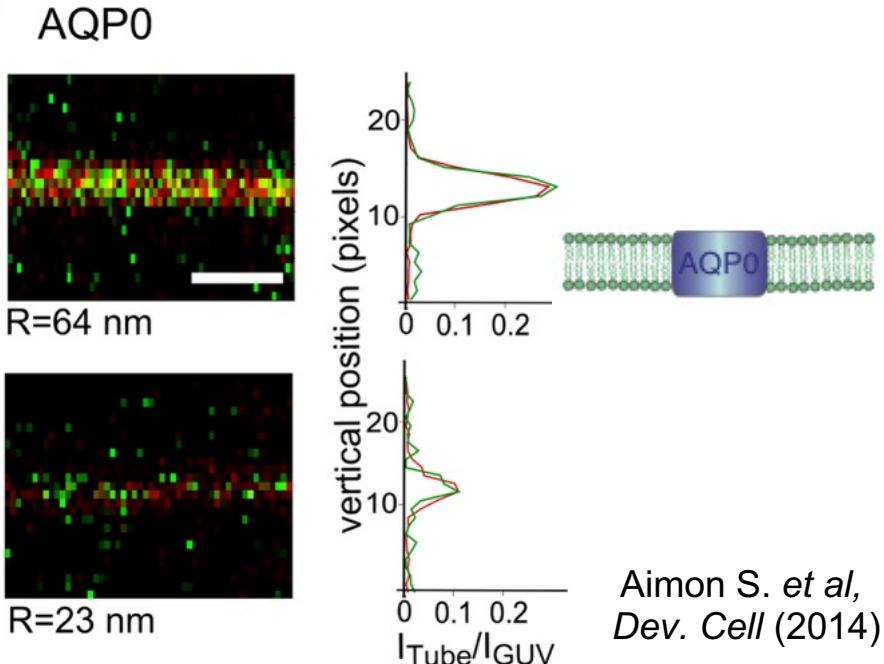
R=14 nm $\Phi_v = 70$ prot/μm^2



AQP0: *no enrichment*

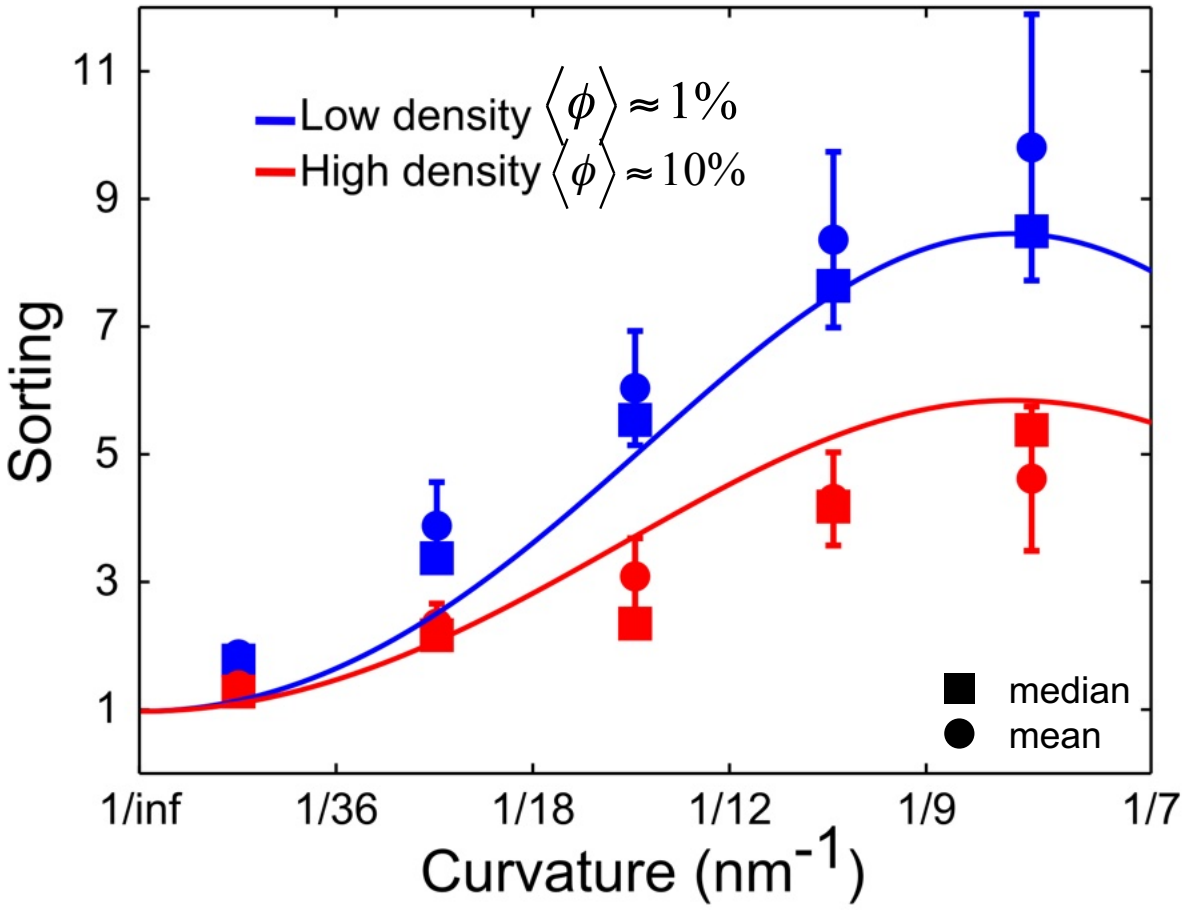


R= 20 nm $\Phi_v \approx 100$ prot/μm^2



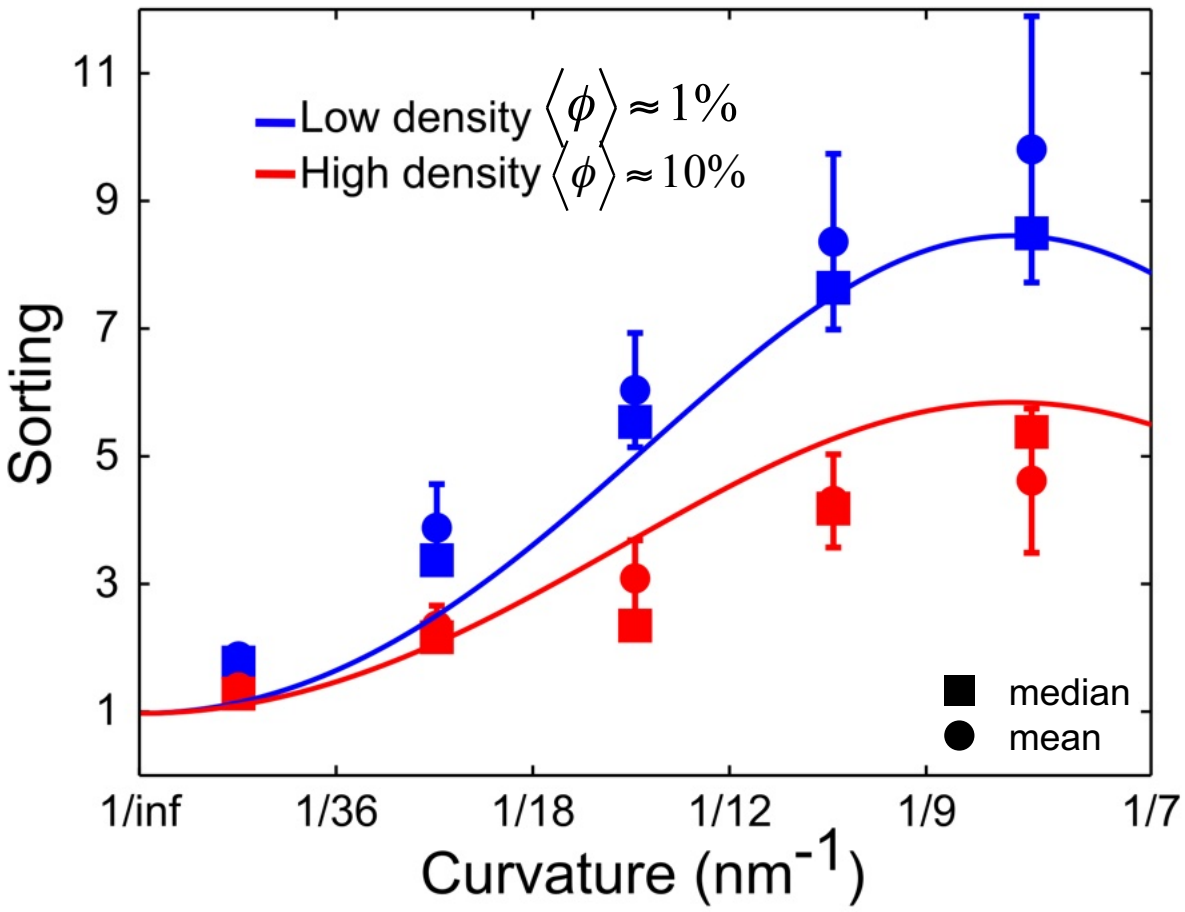
Aimon S. *et al*, *Dev. Cell* (2014)

KvAP Sorting

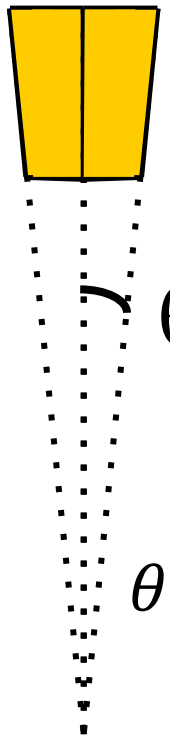


Aimon S. *et al, Dev. Cell* (2014)

KvAP Sorting



$$\overline{C_p} = 1/25nm$$



$$\theta \approx 5^\circ \ (\frac{\pi}{40})$$

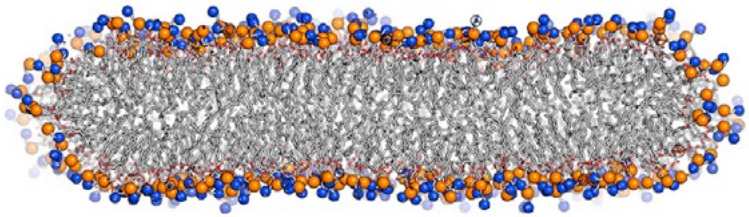
$$\kappa_{protein} \approx 1.5\ \kappa_{lipid}$$

Aimon S. *et al, Dev. Cell* (2014)

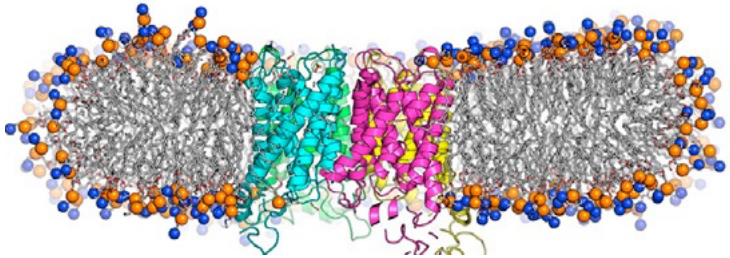
Rainer Böckmann

C. Kluge et al *Biophys. J.* (2022)

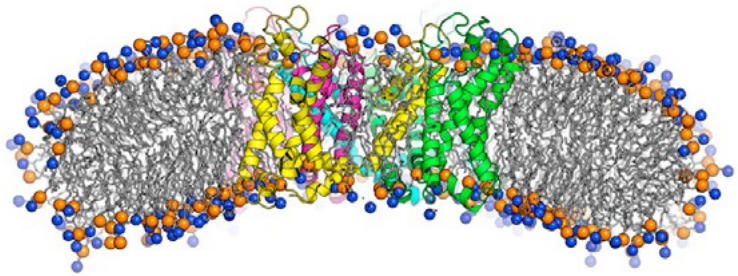
POPC bicelle



AQP0



KvAP



$\overline{C_p} \sim 1/27nm$

MD + coarse-grained simulations

Message

Membrane *curvature* can induce membrane protein *sorting*

$\longleftrightarrow$ protein "*effective shape*"

Linear coupling for insertions

Curvature-matching model for "curved" bound proteins

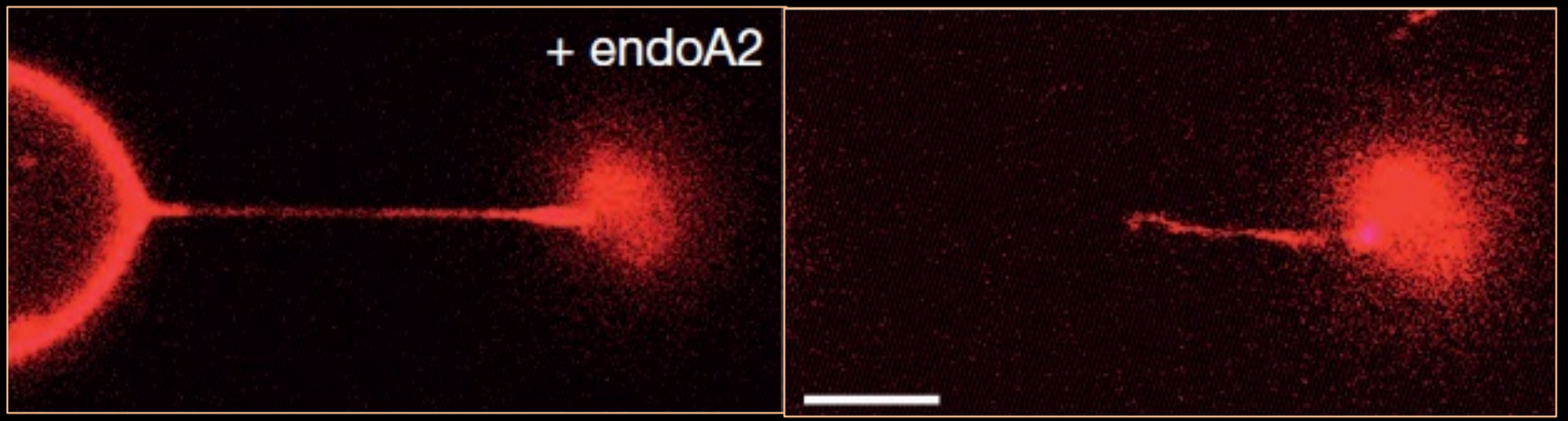
Molecular information (effective shape) can be deduced

from *macroscopic* measurements

in a *lipid* membrane environment

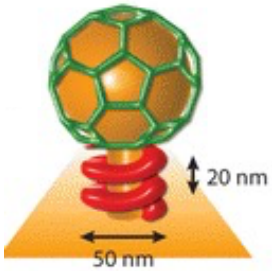
(not in detergent, or nanodisc)

Scissioning Fluid Membranes

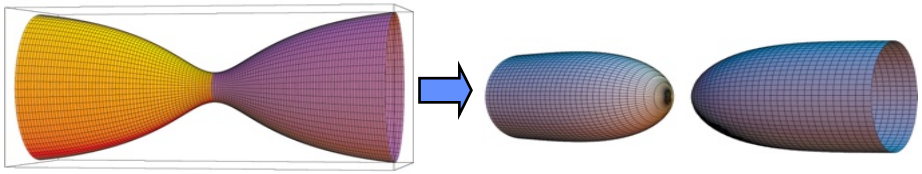


Different Modes of Membrane Scission

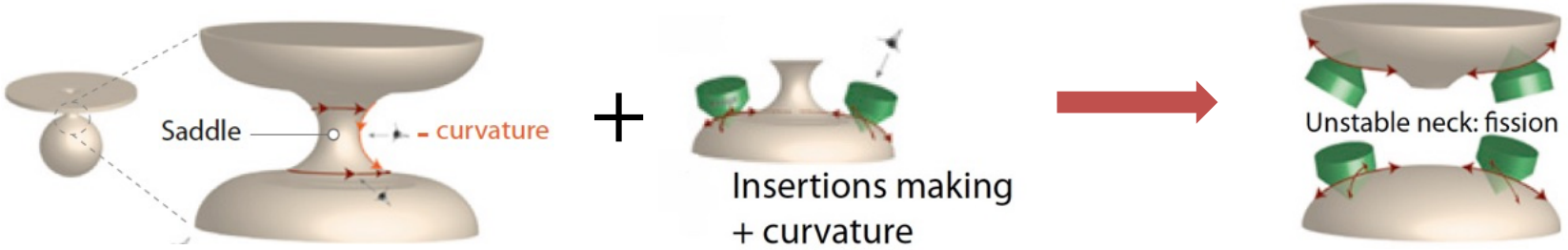
1. *Constriction* by GTPases (i.e. dynamin)



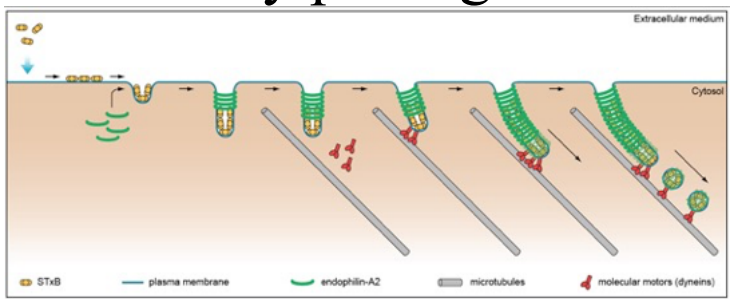
2. *Constriction* by line tension



3. *Insertion* of amphipathic helices (spherical buds)

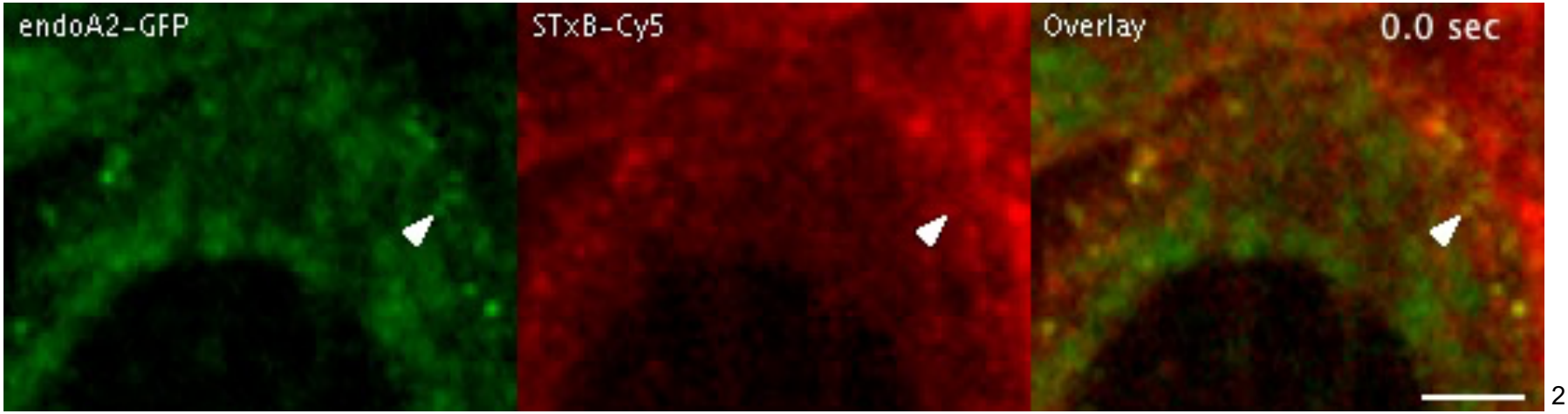


4. *Friction*-Driven Scission by pulling on scaffolded tubes (FDS)

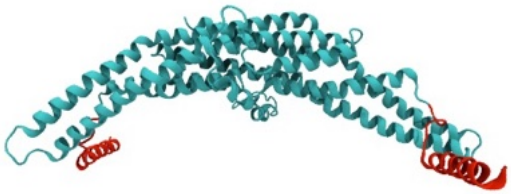


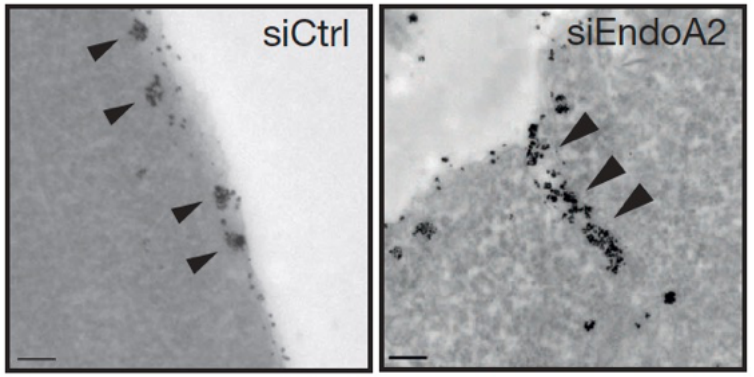
Endophilin A2 and Shiga Toxin Endocytosis

H.F. Renard, L. Johannes

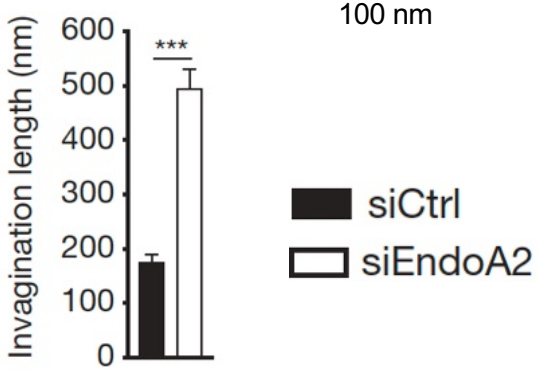


Endophilin A2



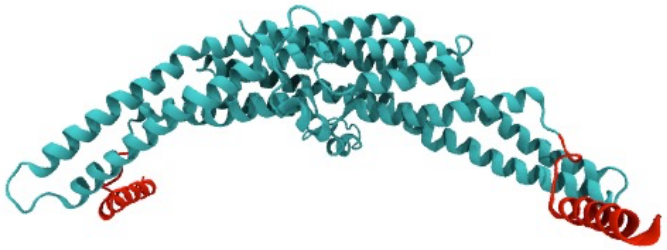


- Colocalizes with STxB tubules
- Involved in scission !!!!!



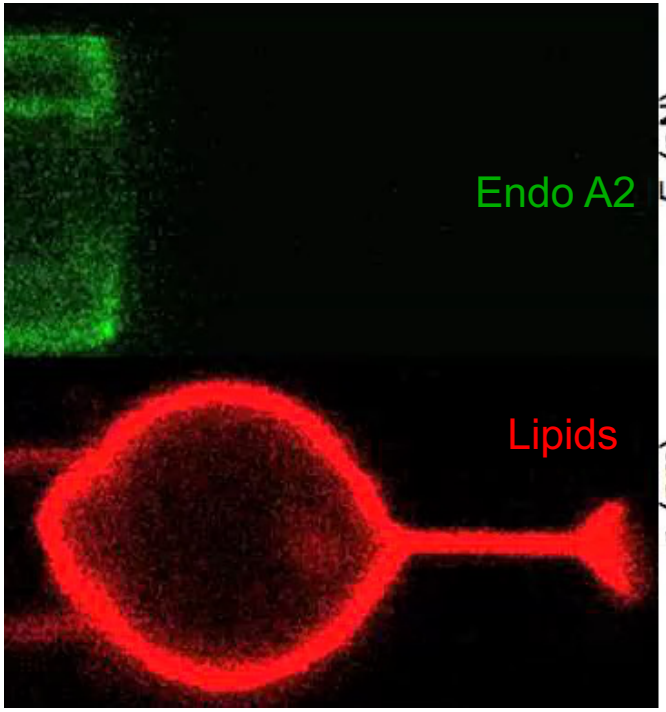
H.-F. Renard, M. Simunovic, J. Lemière *et al.*, *Nature* **517**, 493 (2015)

Scaffolding by Endophilin A2 (4 helices)

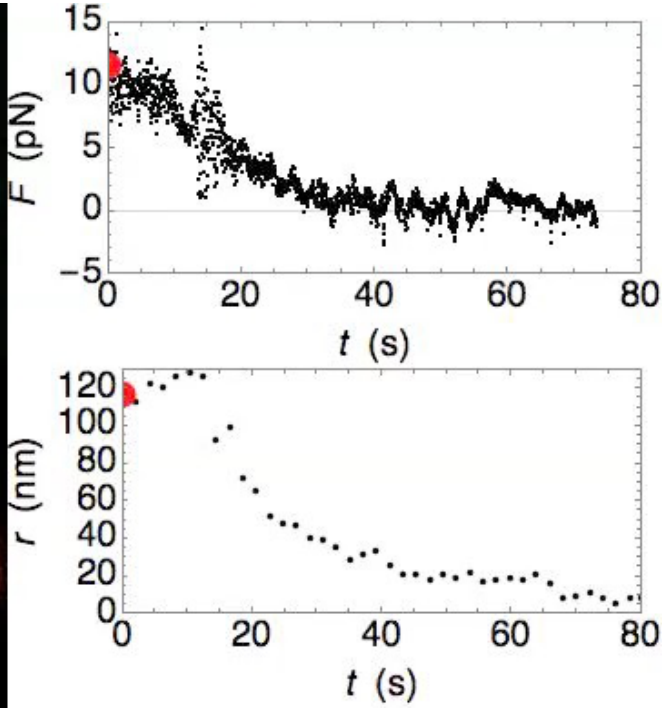


High density on the tube

$$\phi_t \approx 30 - 40\%$$



Total brain extract + 5% PIP_2



Force ≈ 0

$R_s = 10\ nm$

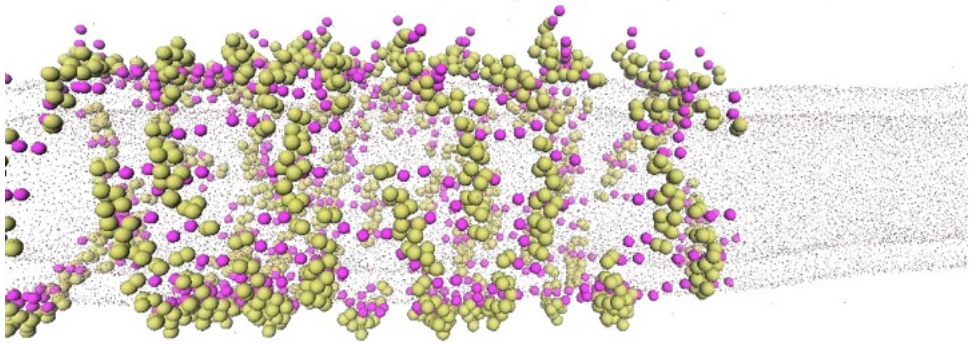
STABLE SCAFFOLD

H.-F. Renard, M. Simunovic, J. Lemière *et al.*, *Nature* (2015)
M. Simunovic et al, *PNAS* (2016)

Protein Scaffolds at Molecular Resolution

Coarse-grained MD simulation (endophilin) on a tube (ø 20 nm)

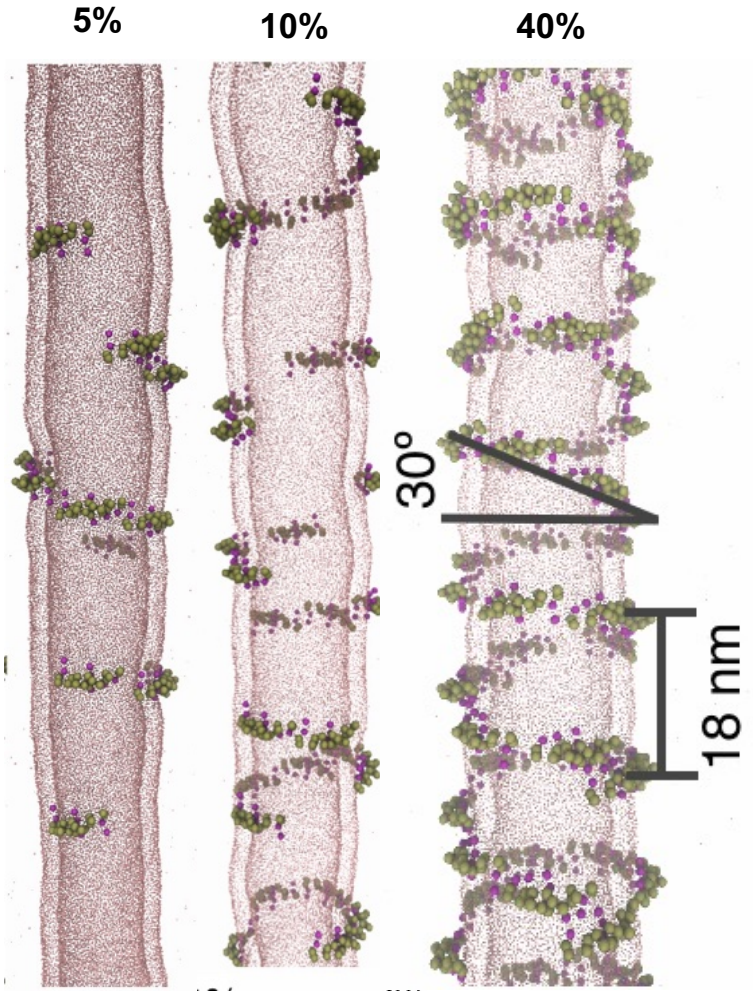
$\phi_t \approx 40\%$



Spontaneous helical arrangement

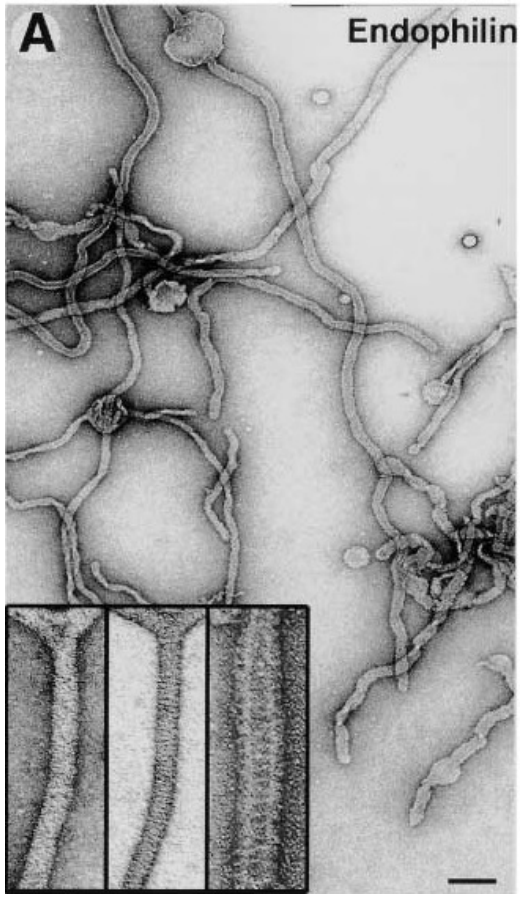
"Loose" scaffold constraining tube

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



M. Simunovic et al, *PNAS* (2016)

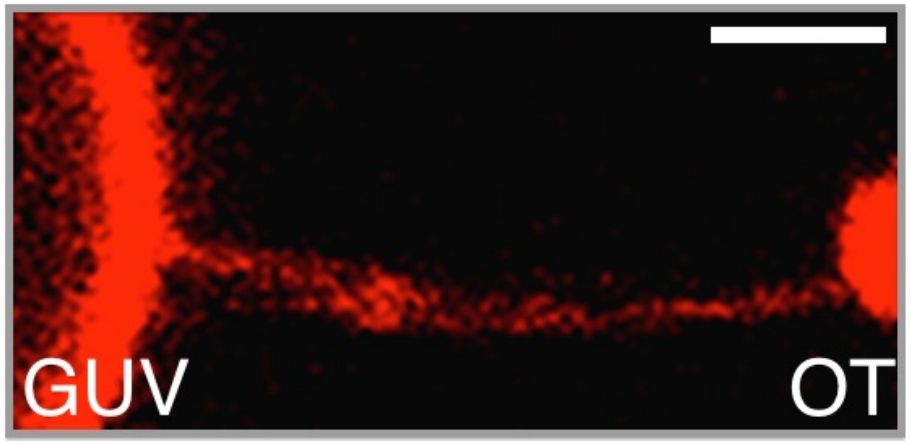
Forms Stable Tubular Scaffolds from Liposomes

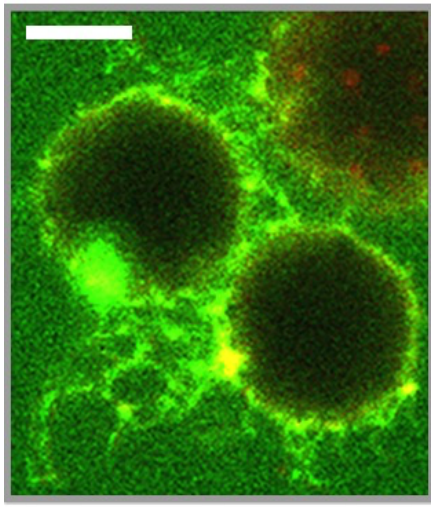


100 nm

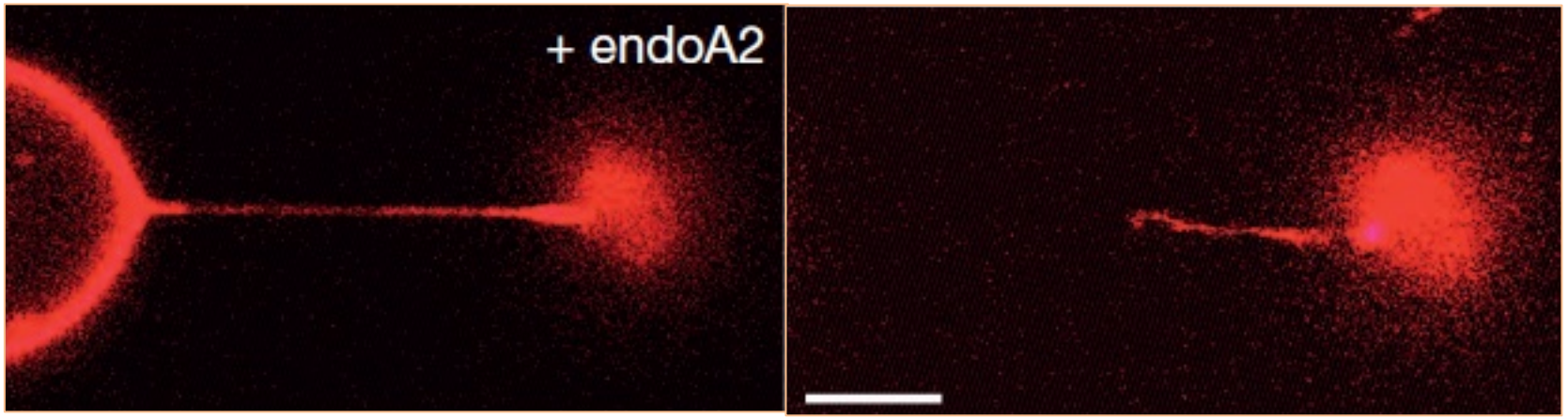
K. Farsad …P. de Camilli *J. Cell Biol*. (2001)

How proteins that STABILIZE membrane tubes





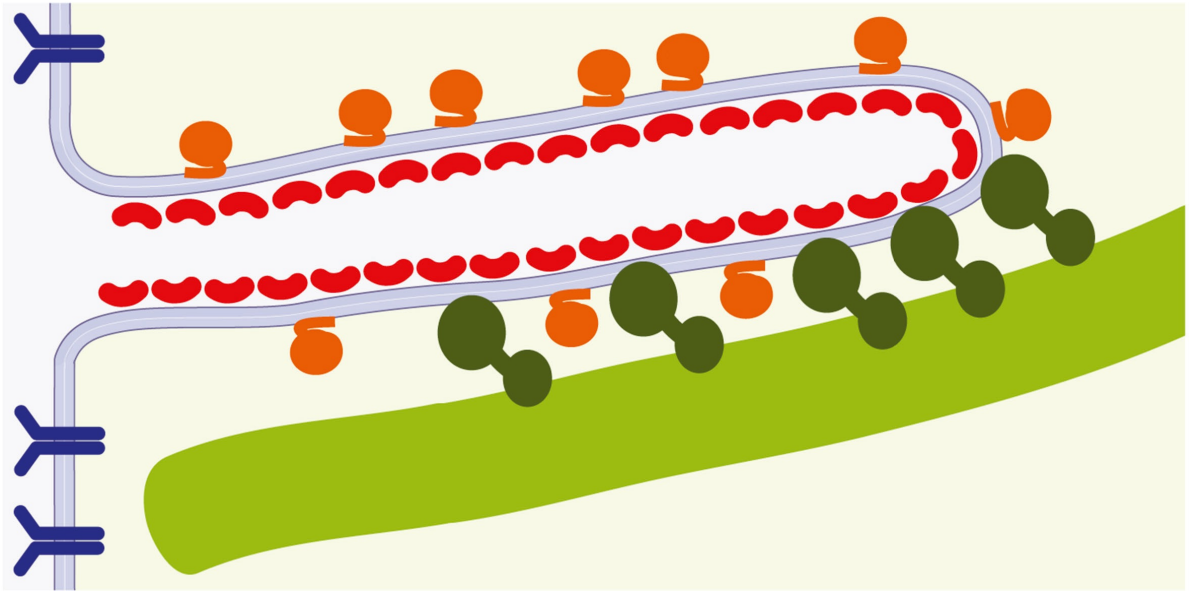
Can SCISSION membrane tubes ????

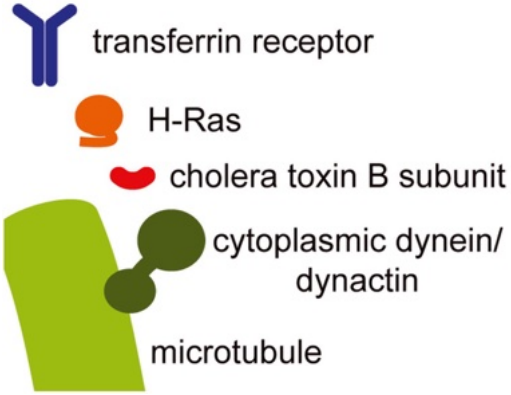


Role of Molecular Motors

In vivo, *microtubule motors* contribute to STxB *tubule extension*

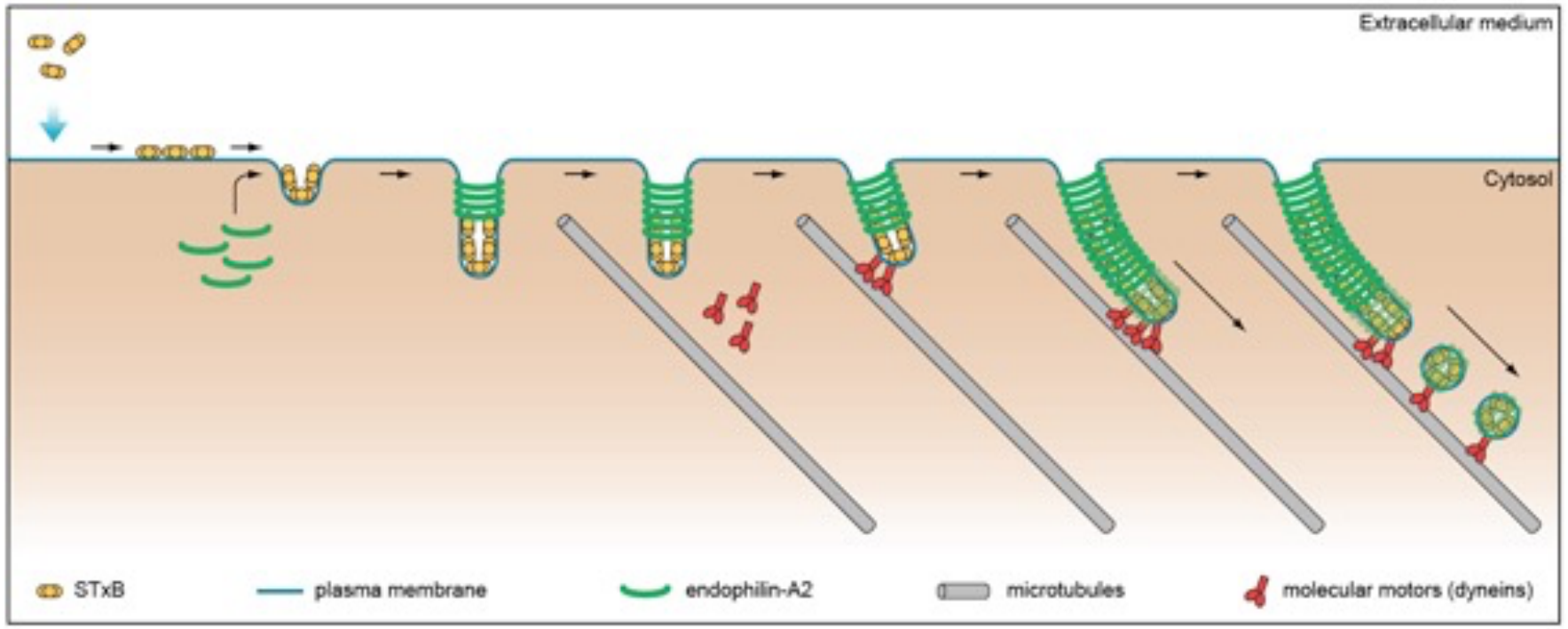
A. Kenworthy
(Vanderbilt U.)





C. A. Day *et al.*, *Traffic* (2015)

STxB/CTxB Endocytosis

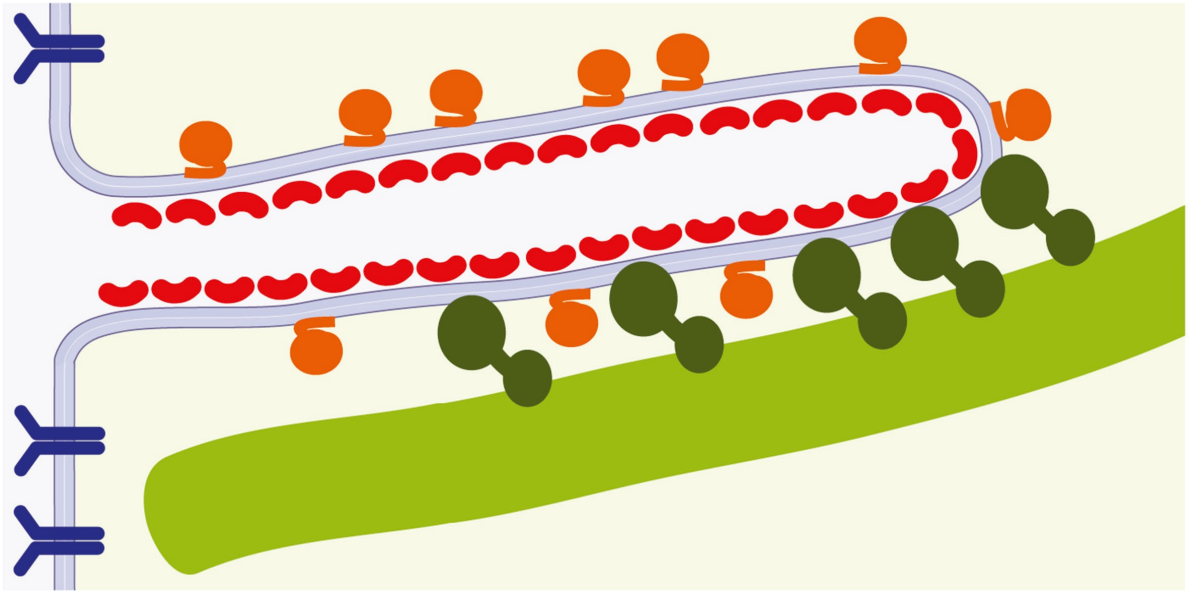


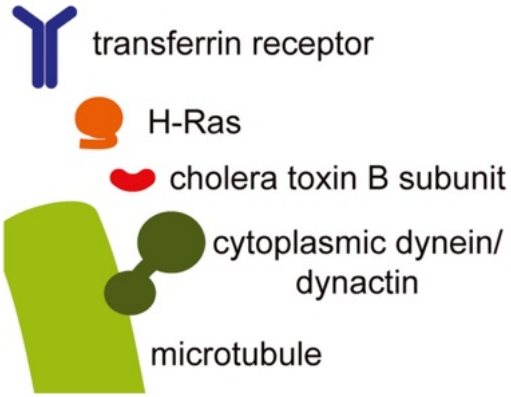
Scheme from L. Johannes

Role of Molecular Motors

In vivo, *microtubule motors* contribute to STxB *tubule extension*

A. Kenworthy
(Vanderbilt U.)



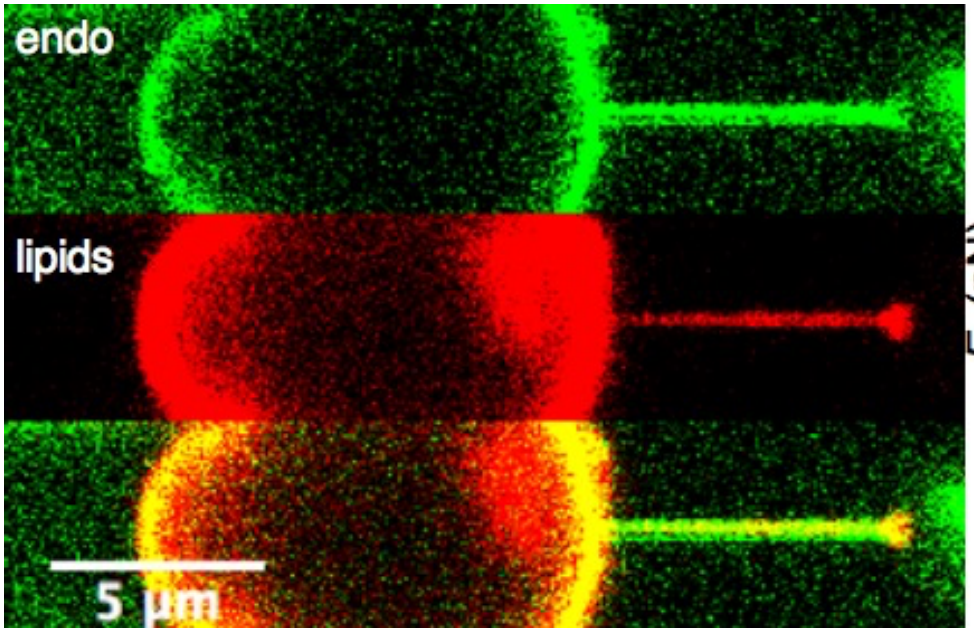


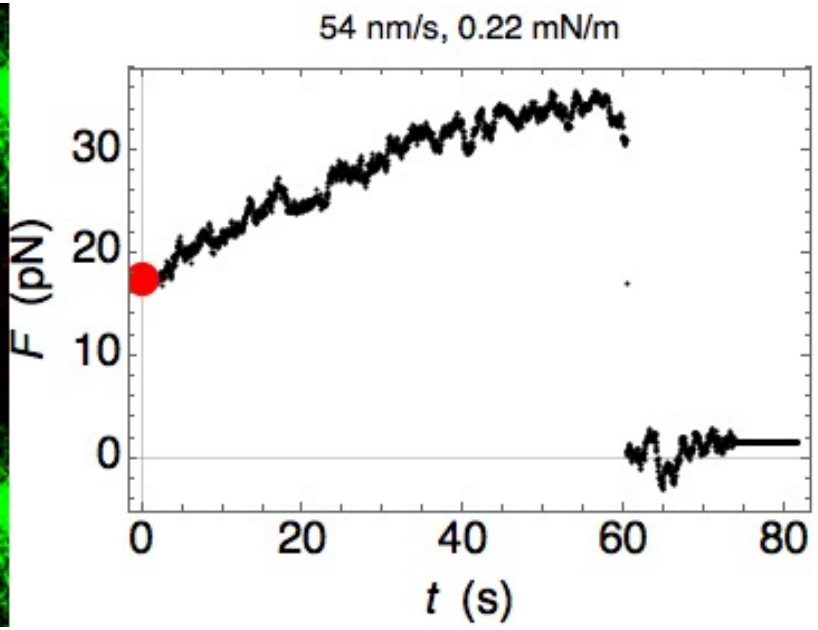
C. A. Day *et al.*, *Traffic* (2015)

→ Pulling on endophilin-coated tubes *in vitro*

Dynamic Scission of EndoA2-Scaffolded Tube

Elongating a tubule coated with EndoA2



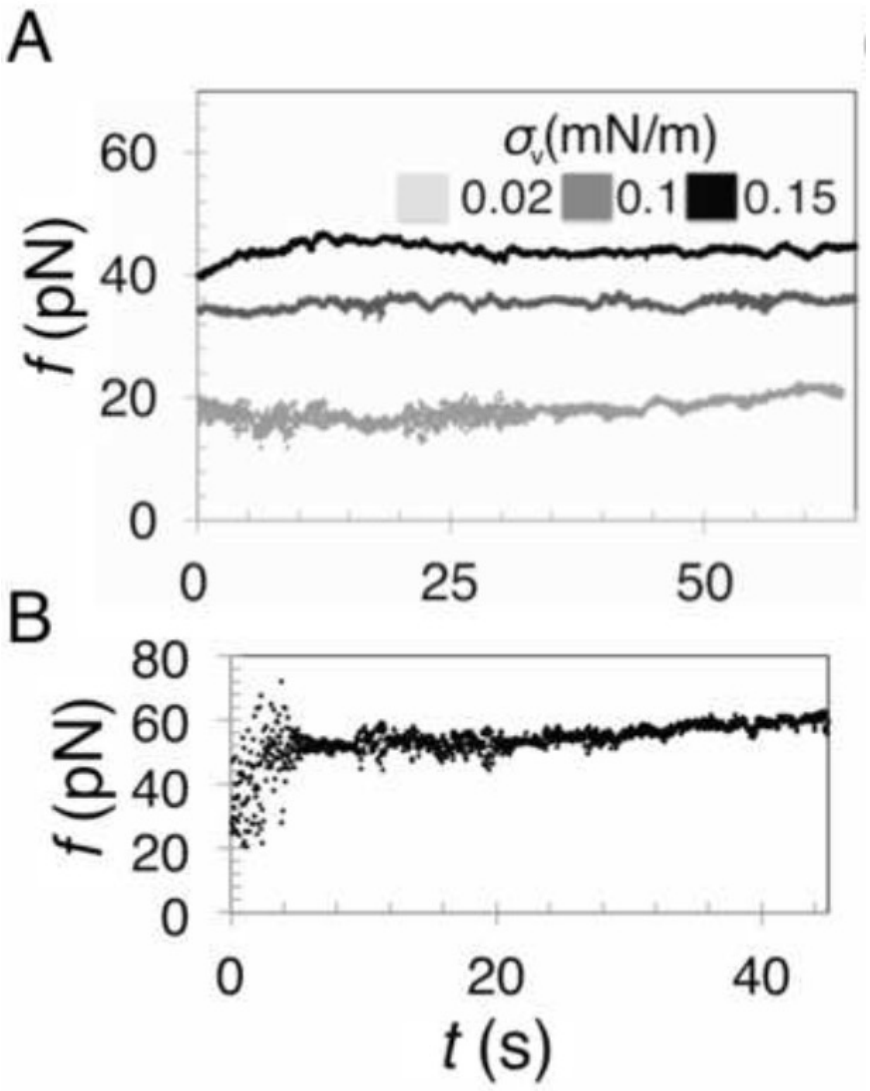


Scission if V> 20-30 nm/s

H.-F. Renard, M. Simunovic, J. Lemière *et al.*, *Nature* (2015)
M. Simunovic et al, *Cell* (2017)

Pulling on a Bare Tube

V=300 nm/s



V=1500 nm/s

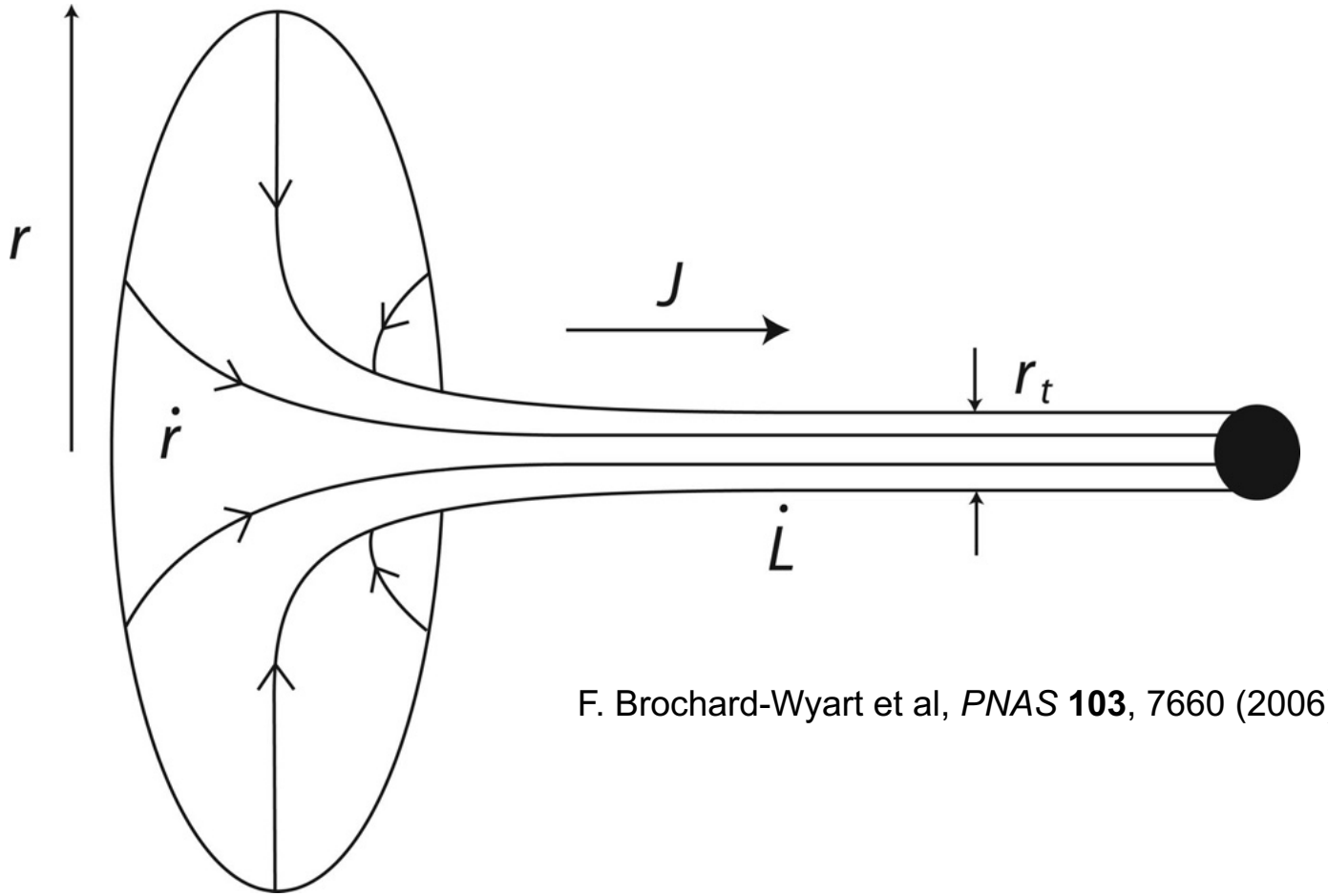
σ =0.08 mN/m

Stable tube

No scission

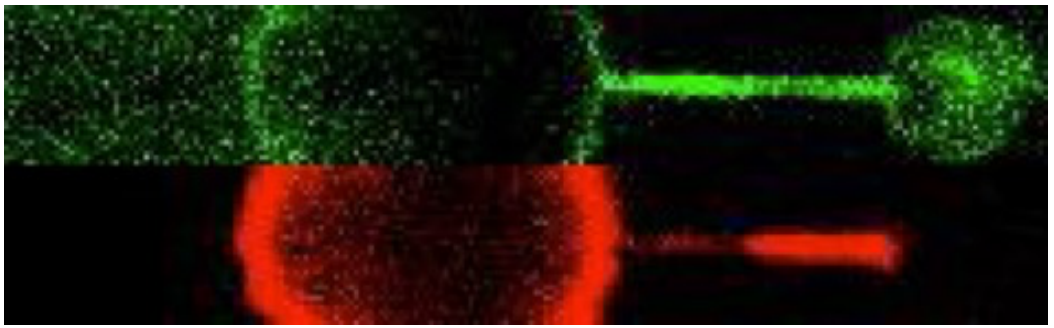
M. Simunovic et al, *Cell* (2017)

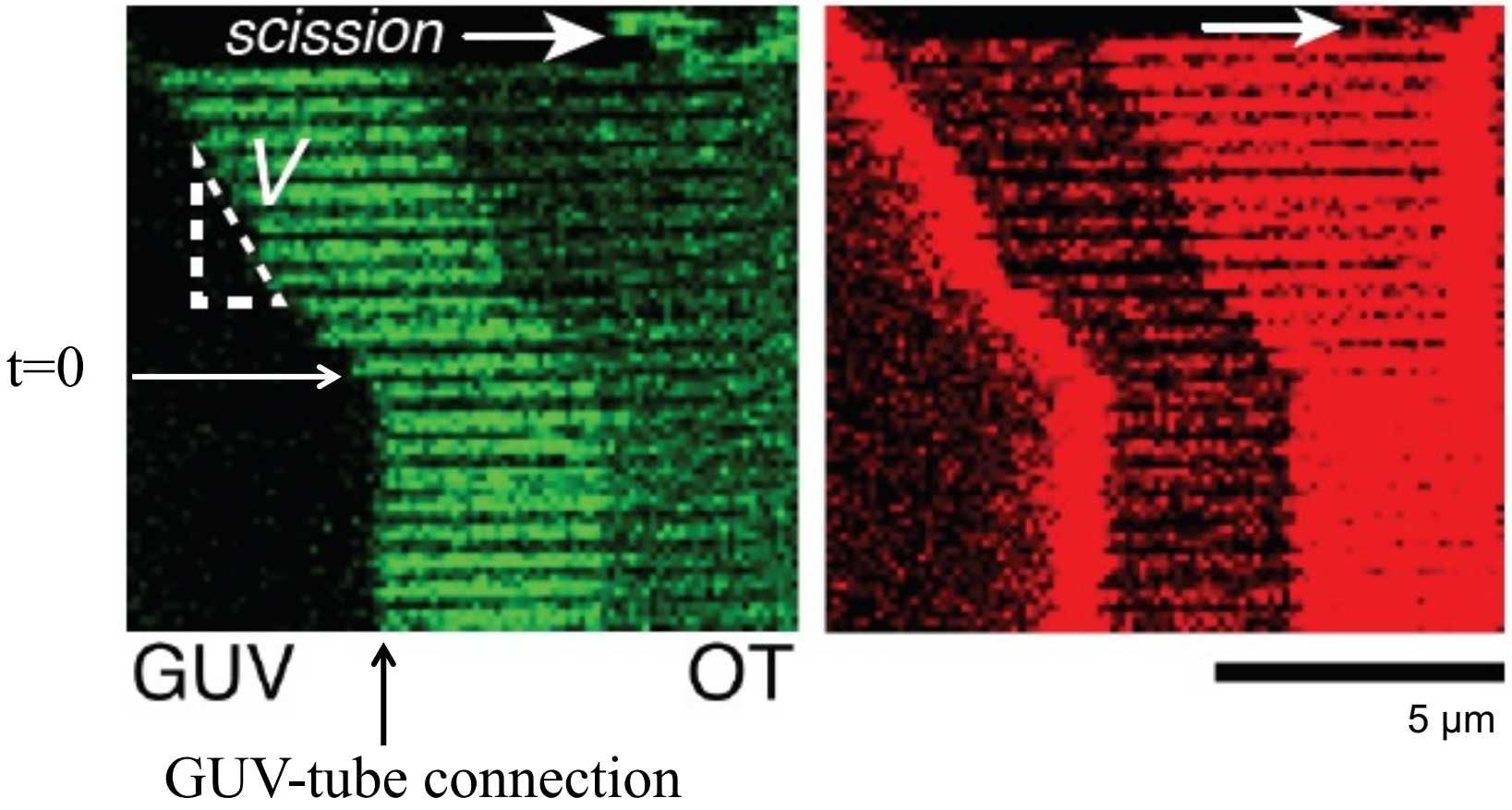
Flow of Lipids upon Tube Elongation



F. Brochard-Wyart et al, *PNAS* **103**, 7660 (2006)

Often, only a *partial* scaffold on the tube





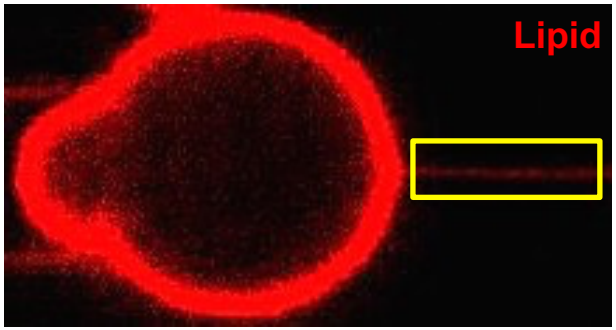
Important: scaffold remains *connected* to the GUV

M. Simunovic et al, *Cell* (2017)

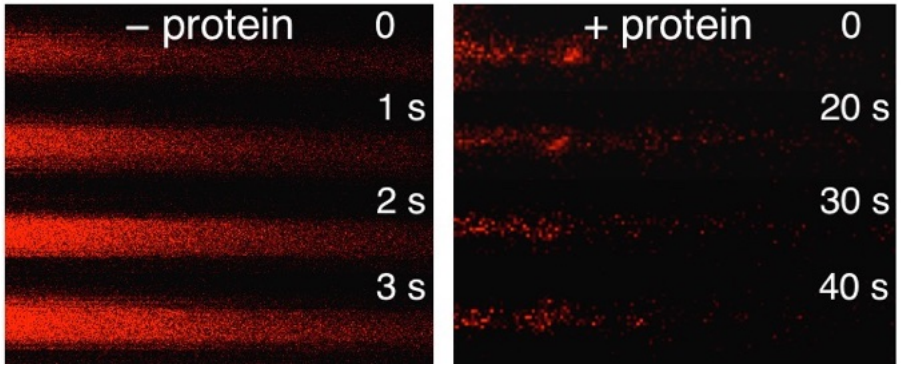
Endo A2 Scaffold: Barrier for Lipids

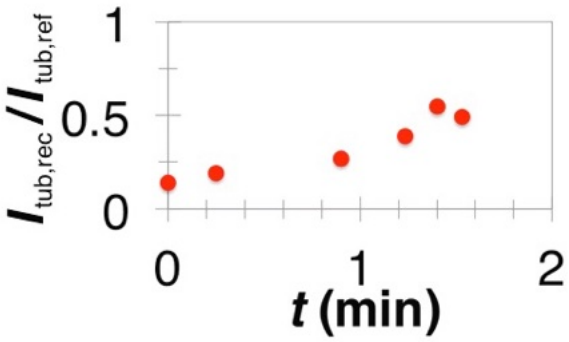
- *Diffusion blocked*

FRAP

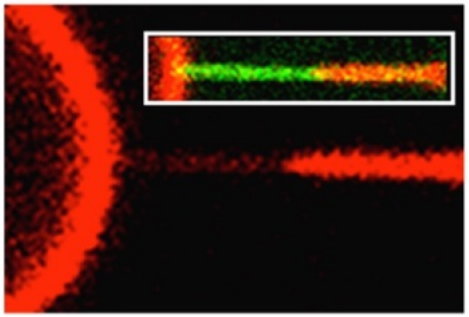


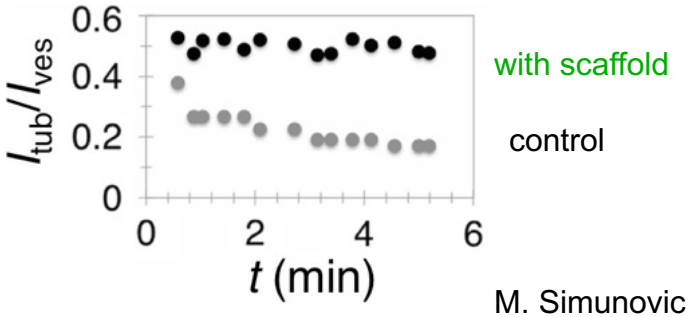
Fluorescence recovery



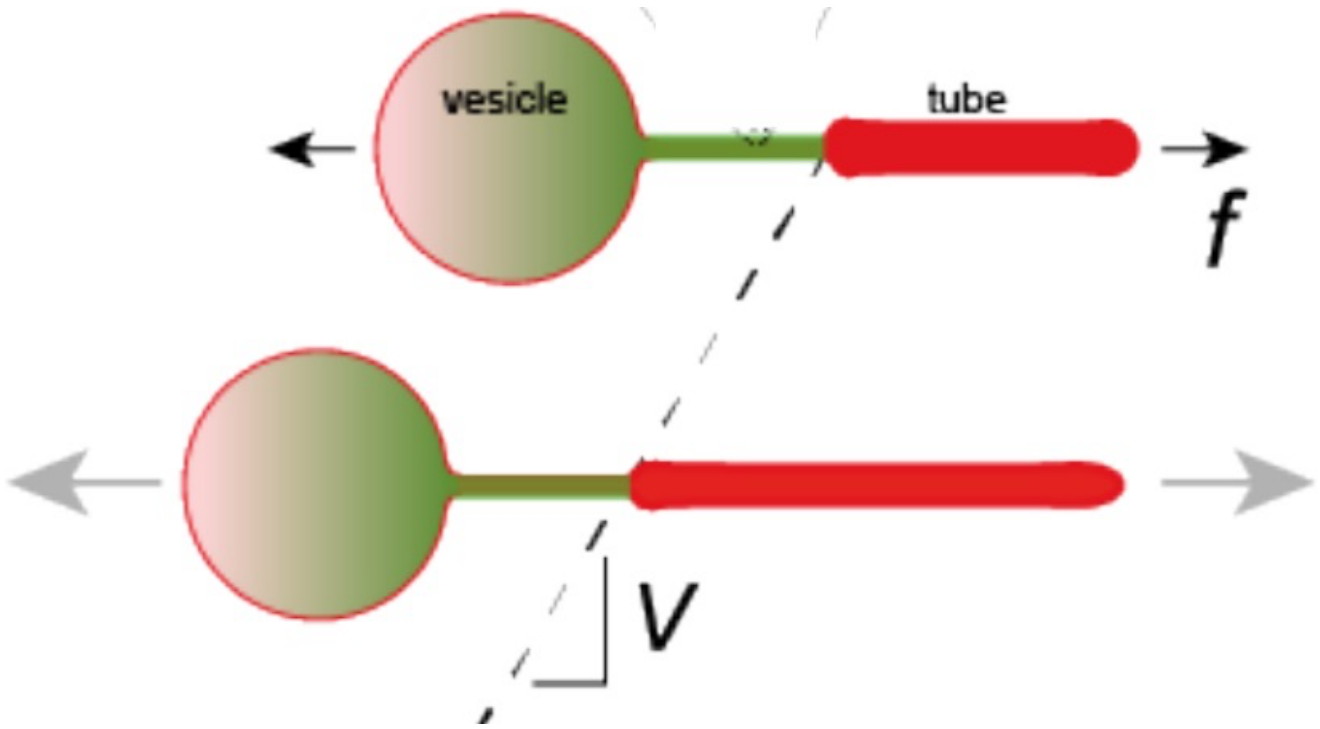


- *Tension* between GUV and tube *not equilibrated*





M. Simunovic et al, *Cell* (2017)



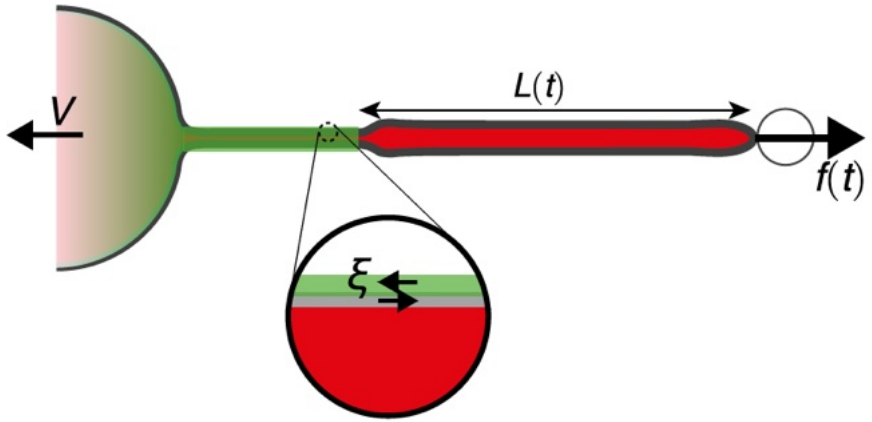
Endo A2 scaffold limits advection of new lipids in the tube

M. Simunovic et al, *Cell* (2017)

Friction-Driven Scission (FDS)

Theoretical model:
A. Callan-Jones
J. Prost

Friction ξ *between Endo scaffold and the membrane*



Creates *a difference of tension* between tube and GUV

$$\sigma(t) = \sigma_0 + \xi\, v_l$$

σ ↔ Force

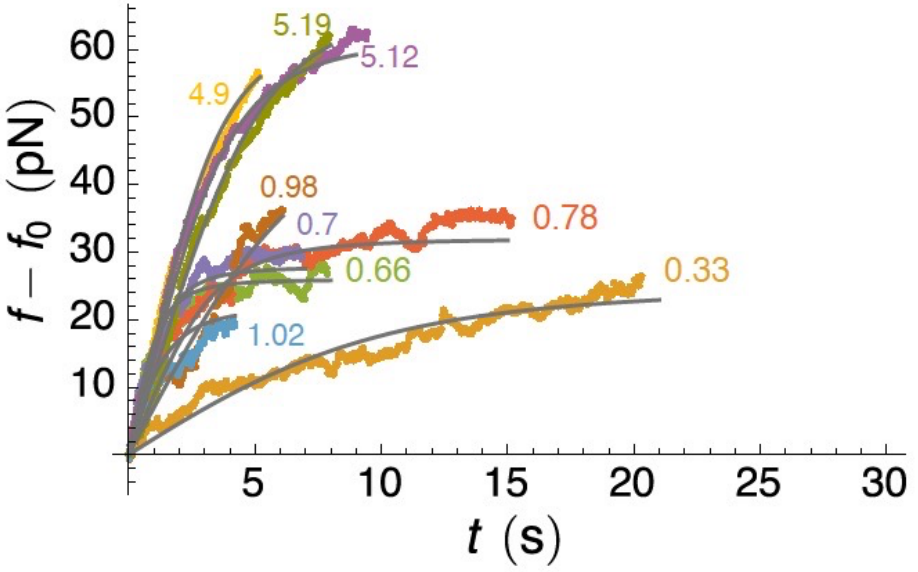
M. Simunovic et al, *Cell* (2017)

Friction-Driven Scission (FDS)

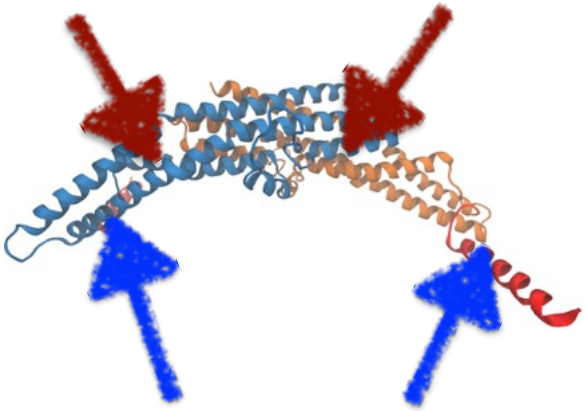
Theoretical model:
A. Callan-Jones
J. Prost

Force increase depends on *elongation speed*

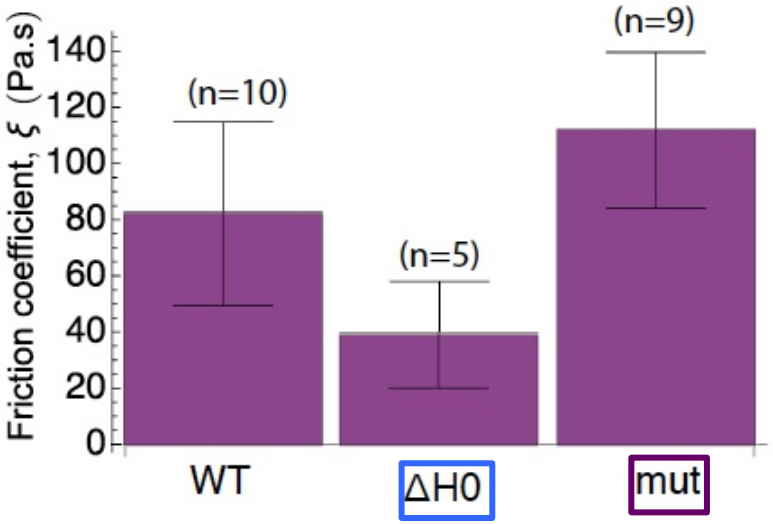
- Short time: *elastic*
- Long time: *viscous*



Mutations

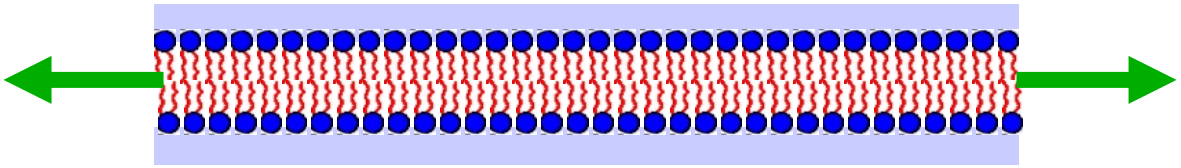


2 mutants : Endo ΔH0
mut (E37K, D41) (Stronger binder)

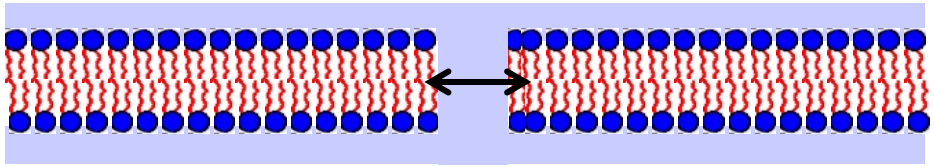


M. Simunovic et al, *Cell* (2017)

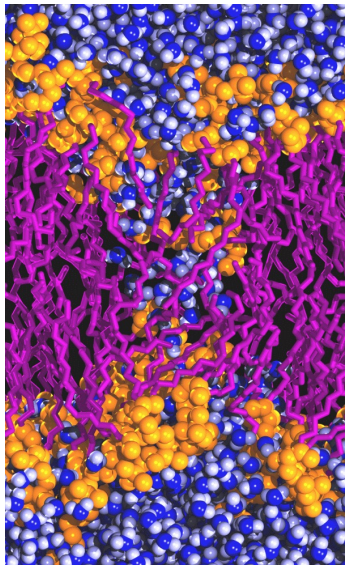
High Tension: Membrane Lysis



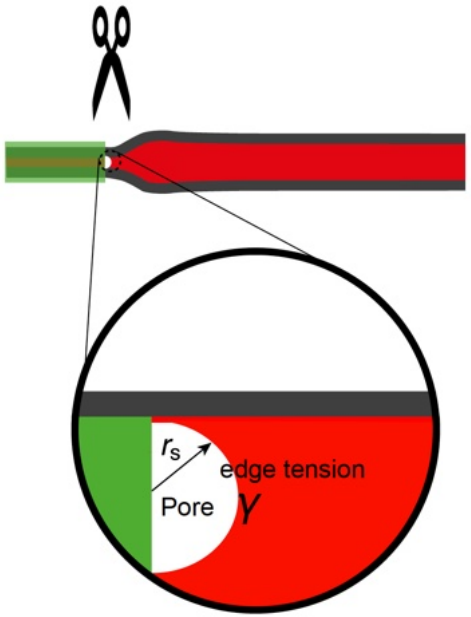
HOLE



Numerical simulations



Tension Increases up to Membrane Lysis



Pore nucleation

Rate-dependent pore nucleation at the edge

Cf. **E. Evans**, et al, *Biophys. J.* **85**, 2342 (2003)

$$W(t) \simeq \pi \gamma r_s - \pi r_s^2 \sigma(t)$$ Energy barrier

Kramer's theory
Pore nucleation probability

$$\nu(t) = \nu_0 \exp[-W(t)/(k_B T)]$$

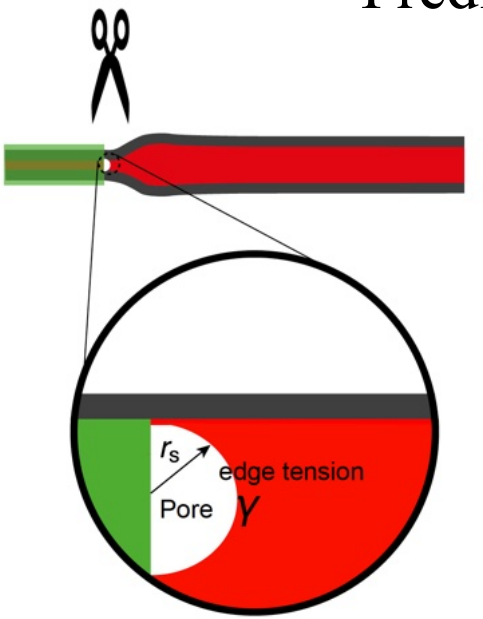
Prediction: $$f_{break} - f_0 \simeq (16\pi^3 \kappa^2 \xi / r_s)^{1/3} V^{1/3}$$

$$Ln(t_{break}) \propto V^{2/3}$$

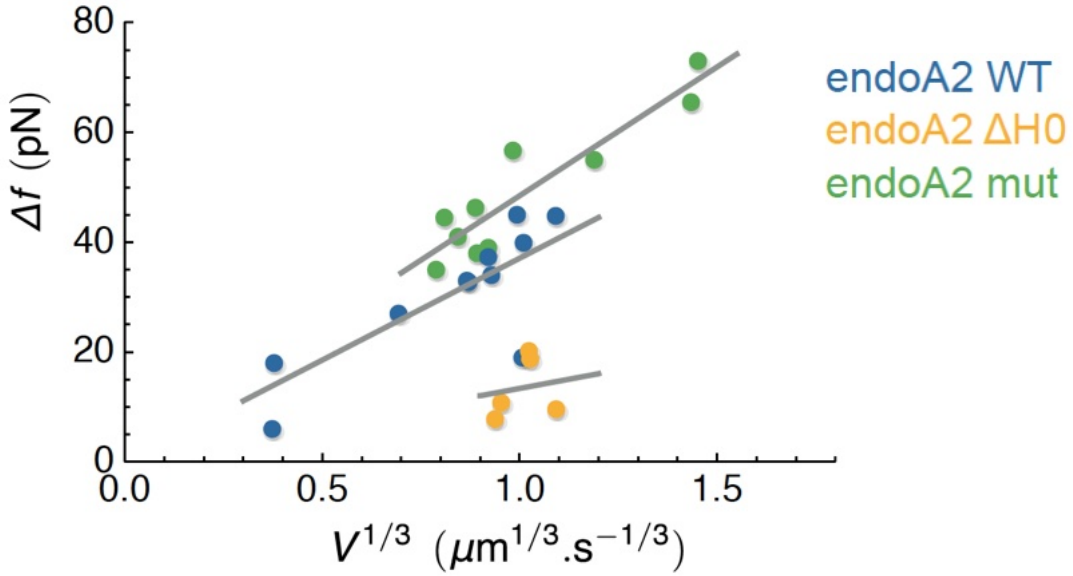
M. Simunovic et al, *Cell* (2017)

Tension Increases up to Membrane Lysis

Prediction: $f_{break} \propto V^{1/3}$ at low pulling speed



Pore nucleation

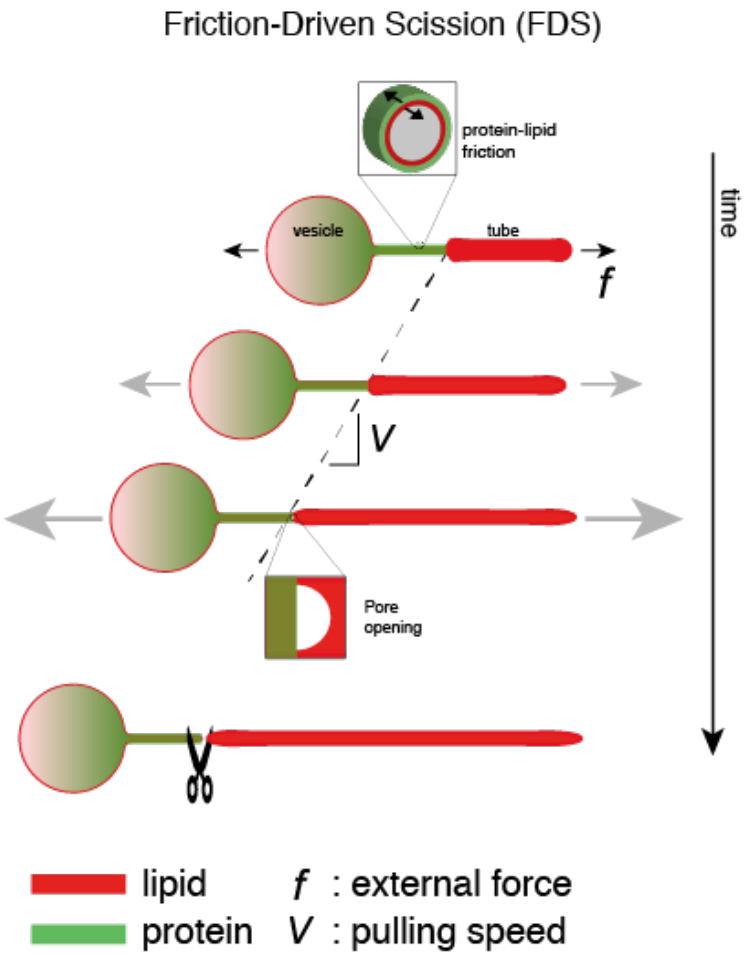


	ξ (Pa.s) - WT	ΔH0	mut
f increase	*80*± 30	*39*± 19	*112*± 27
f break	*30*± 12	*1.4*± 2	*66*± 6

M. Simunovic et al, *Cell* (2017)

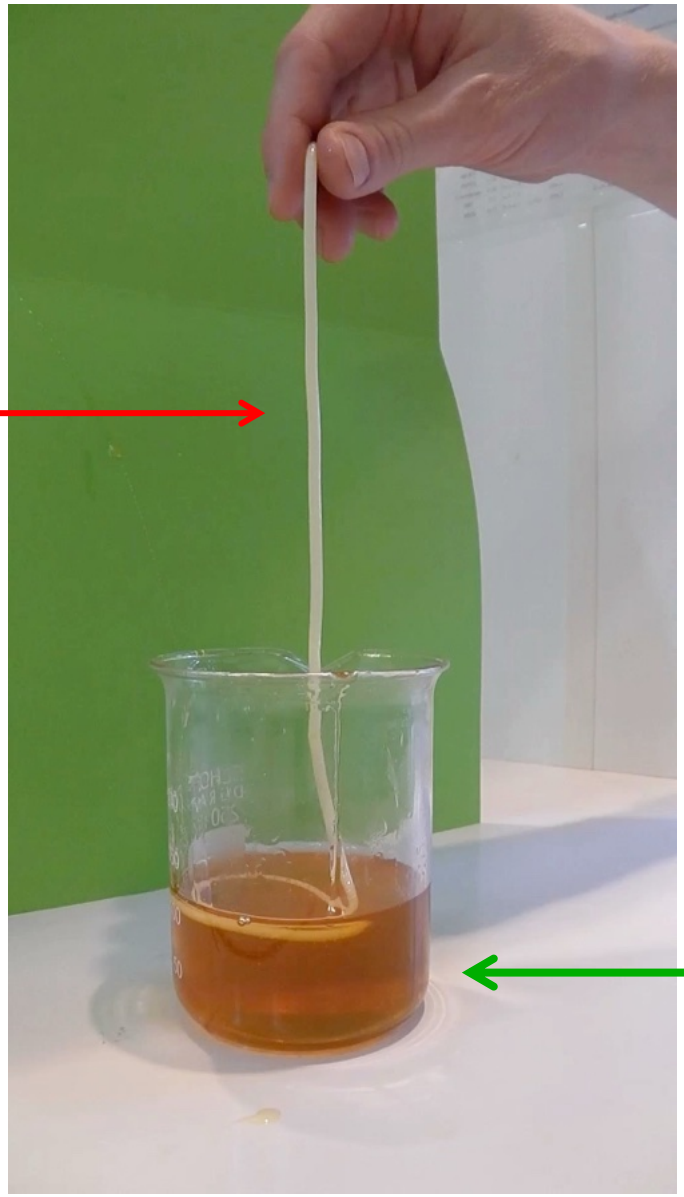
Conclusion

Friction pull coat/membrane + GUV-coat linked → Tension builds up (Force increases) → Pore Nucleation → Break



Mechanical Analogy

Elastic material (overcooked spaghetti)



Viscous medium (honey)

(Speed 1:6)

M. Simunovic et al, *Cell* (2017)