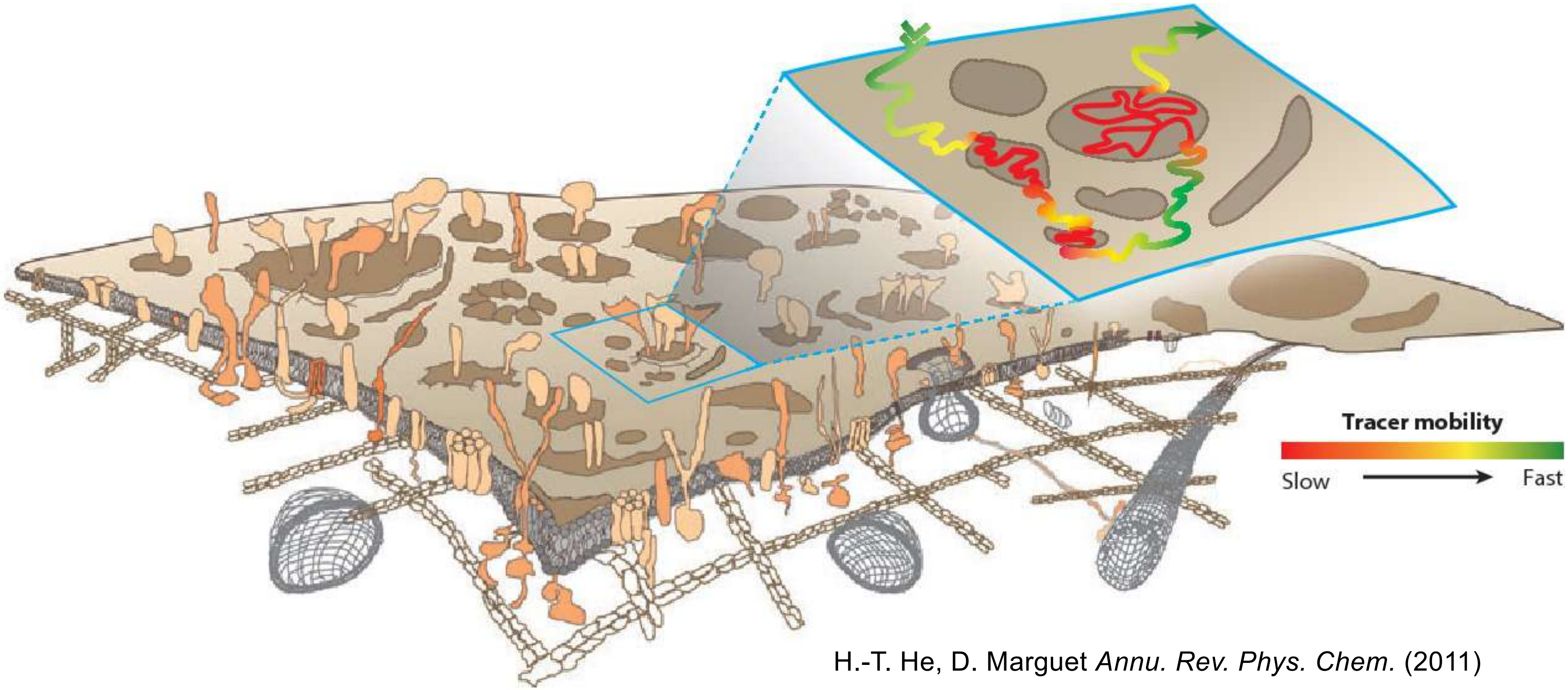


H.-T. He, D. Marguet *Annu. Rev. Phys. Chem.* (2011)

4. Active Protein Clustering

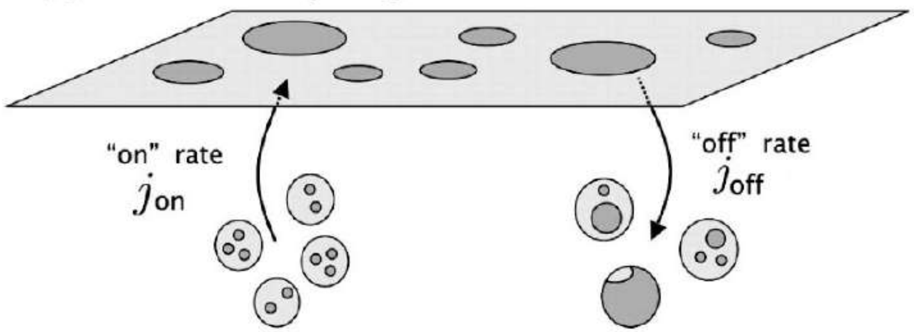
Previous lecture: Thermodynamic equilibrium

Why clusters of finite, small size in cells?

→ *Non-equilibrium* considerations to be added

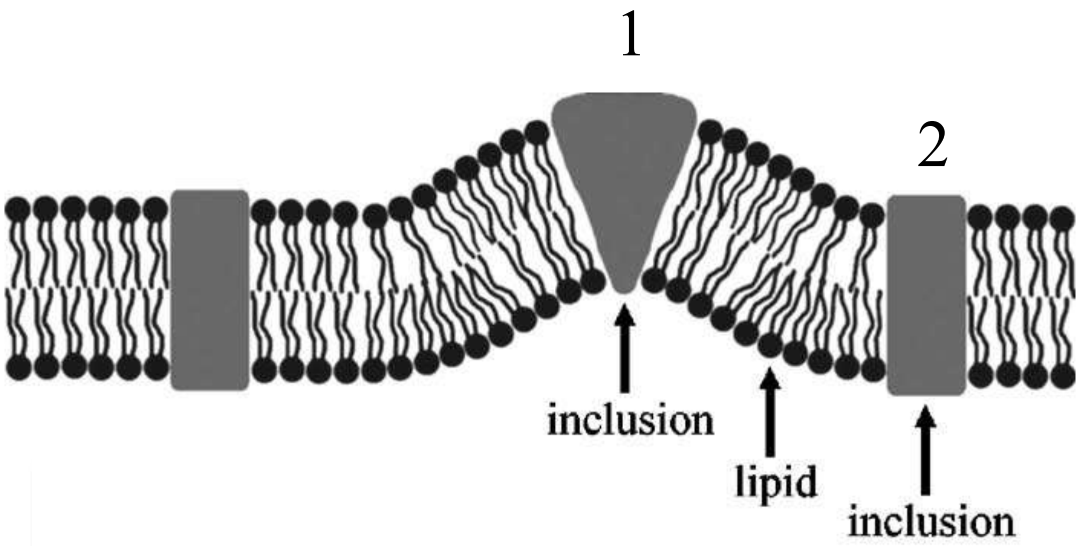
- Active remodeling of the cortical cytoskeleton
- Rapid traffic of membrane components

(b) Membrane recycling

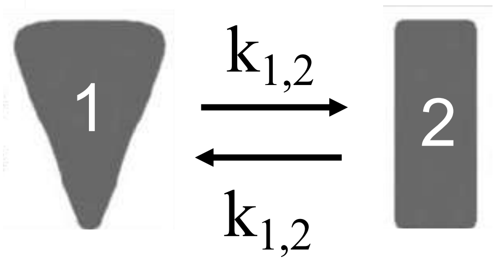


- Transfer of energy to the membrane by active "machines"

Active Inclusions: Finite Size of the Clusters



Monte-Carlo simulations



1 attracts 1

G: inclusion (1) coupling to curvature

2/1, 2/2 or 2/lipids: no interaction

C.H. Chen, H.Y. Chen *Phys. Rev. E* (2006)

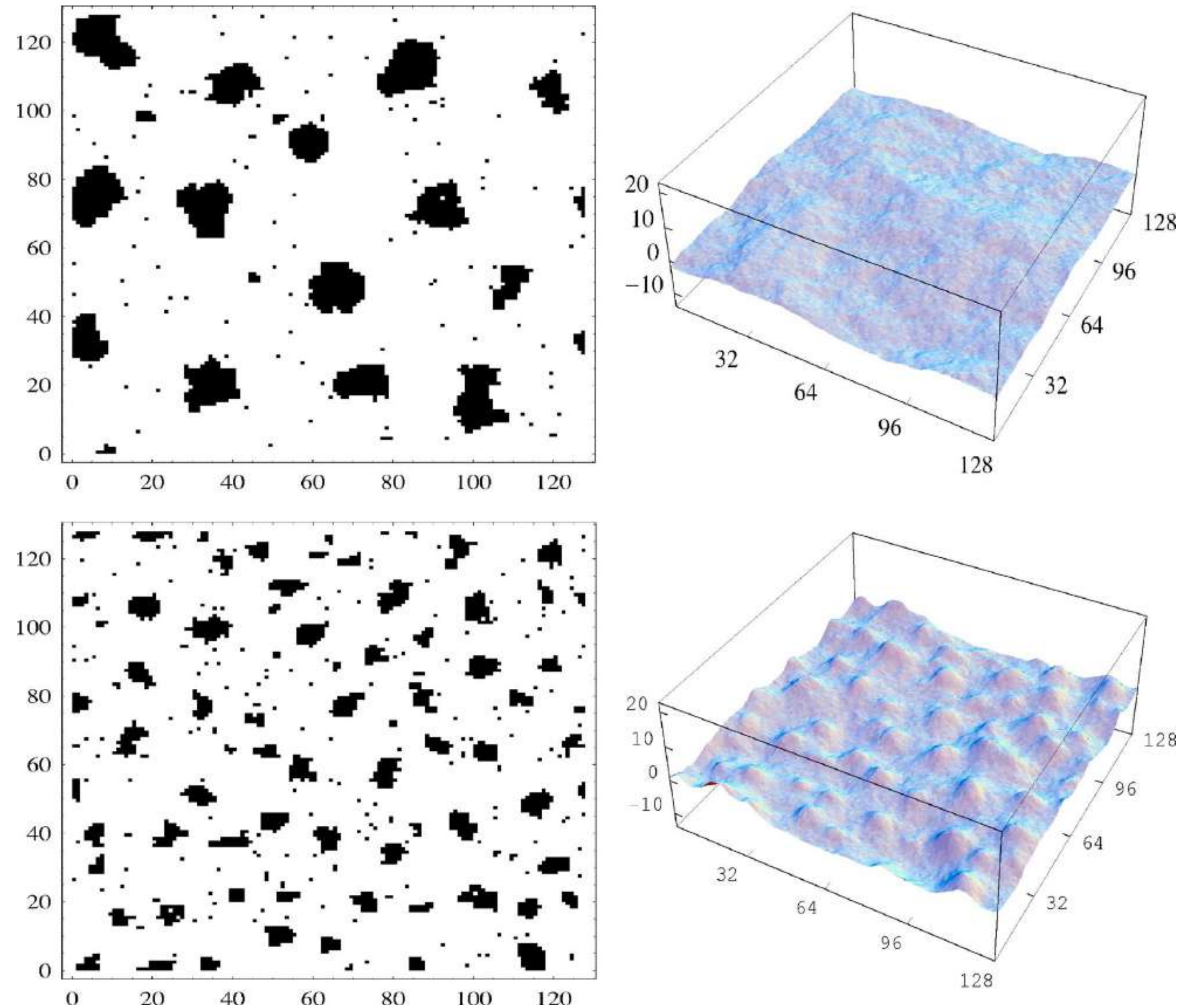
Active Inclusions: Finite Size of the Clusters

Steady state

Inclusions | Membrane

G=0

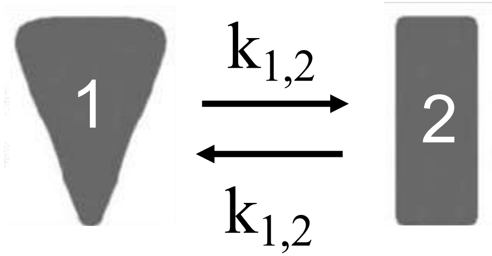
G=2



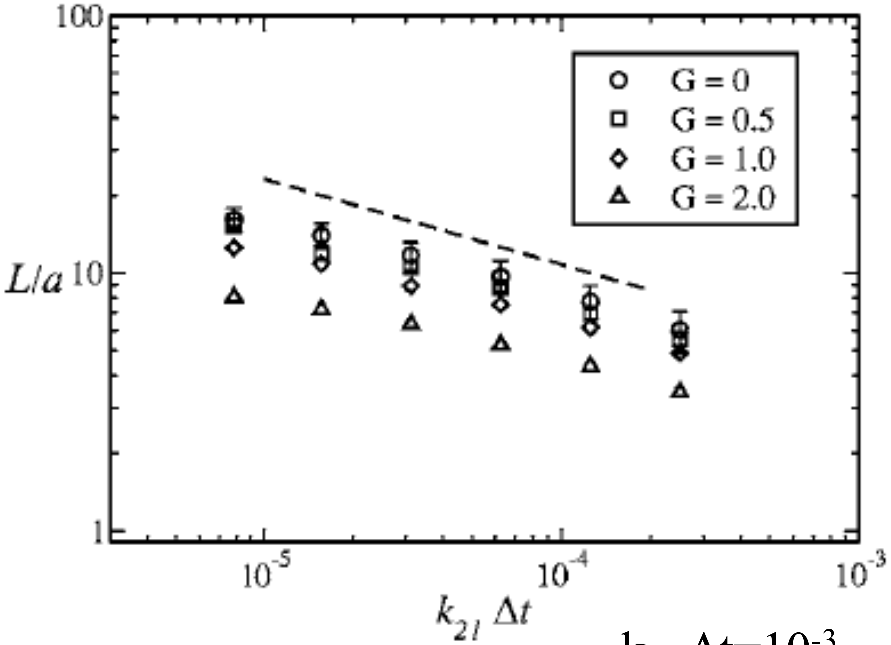
Adimensional length (/a =lattice Ct)

C.H. Chen, H.Y. Chen *Phys. Rev. E* (2006)

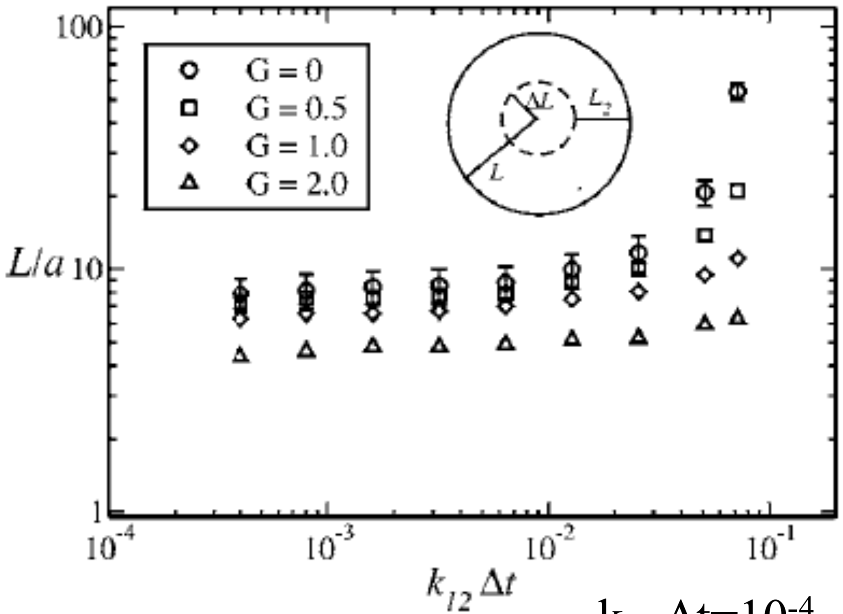
Active Inclusions: Finite Size of the Clusters



Cluster size



$k_{1,2}\Delta t=10^{-3}$



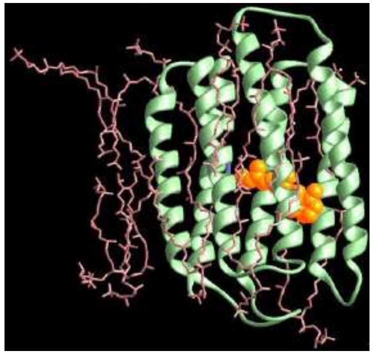
$k_{2,1}\Delta t=10^{-4}$

$L \sim k_{2,1}{}^{1/3}$

Depends weakly on $k_{1,2}$

C.H. Chen, H.Y. Chen *Phys. Rev. E* (2006)

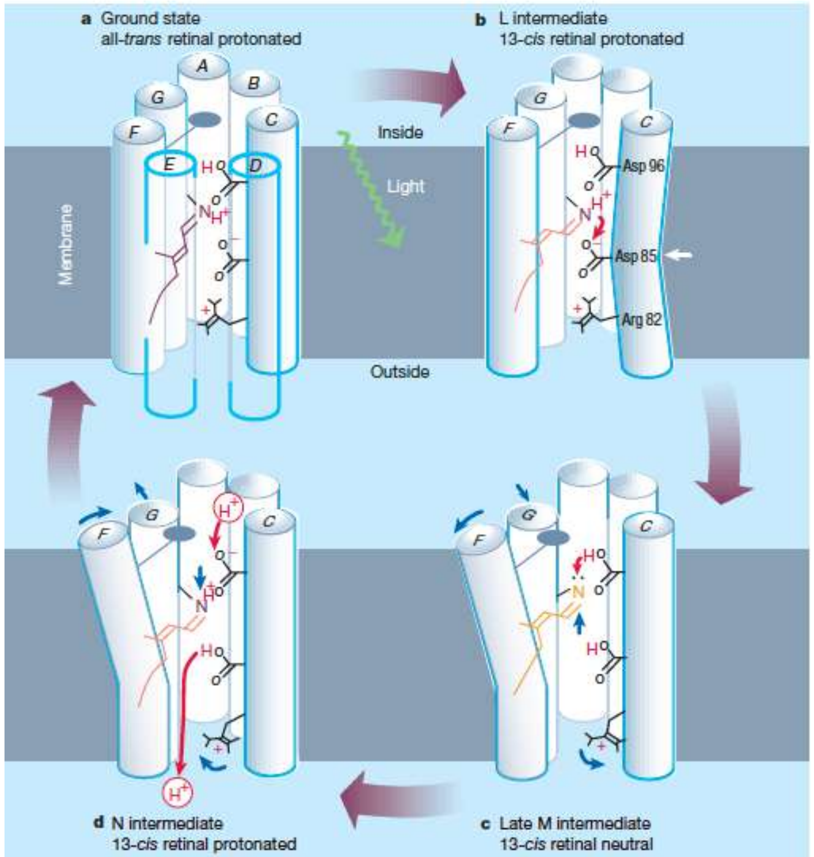
Bacteriorhodopsin Clustering due to Light Activation

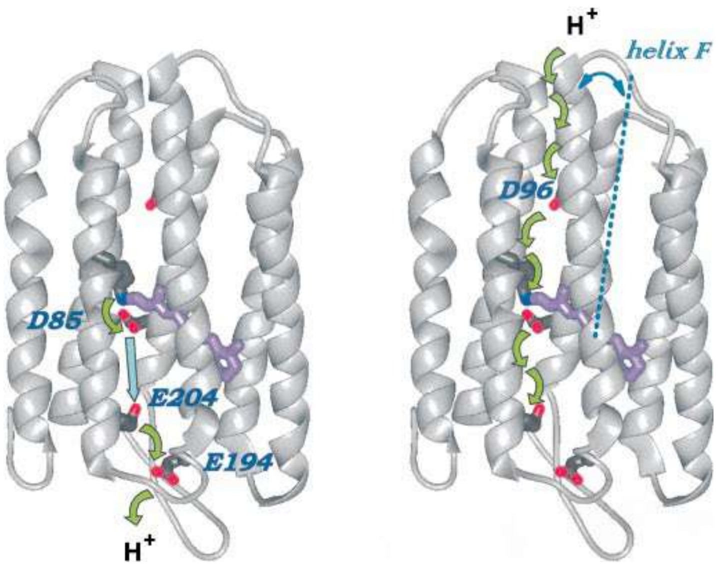


Proton pump

Activated by green light

Conformational changes during transport cycle

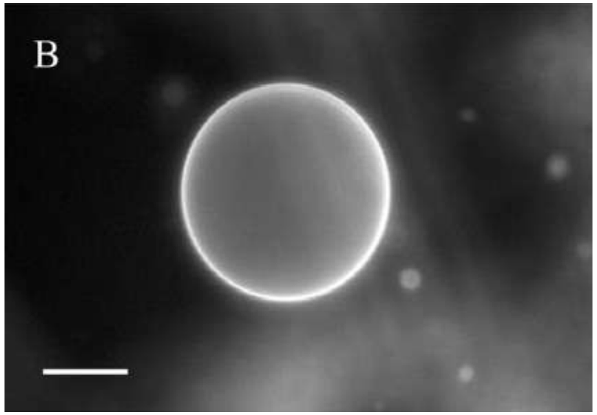




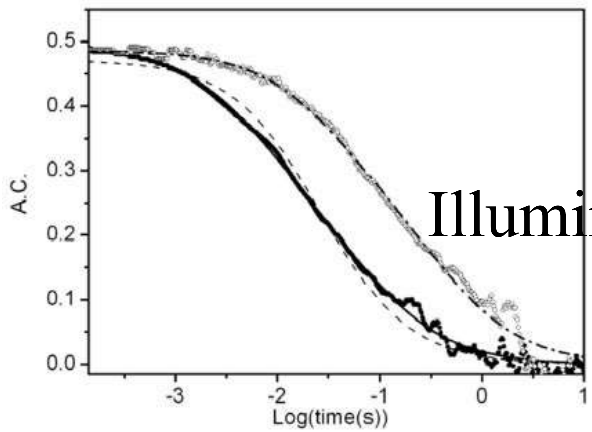
J.K. Lanyi *J. Biol. Chem.* (1997)

W. Kühlbrand *Nature* (2000)

Bacteriorhodopsin Clustering due to Light Activation



Low protein density



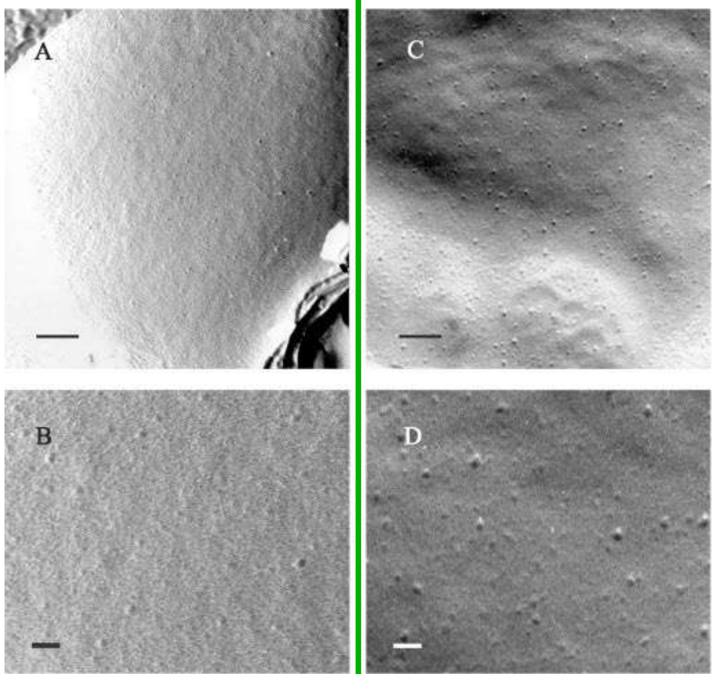
FCS

Illumination (Activation)

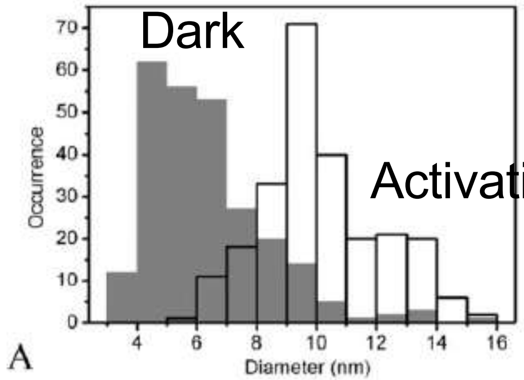
Dark | Activation

100 nm

35 nm



Clustering
Finite size

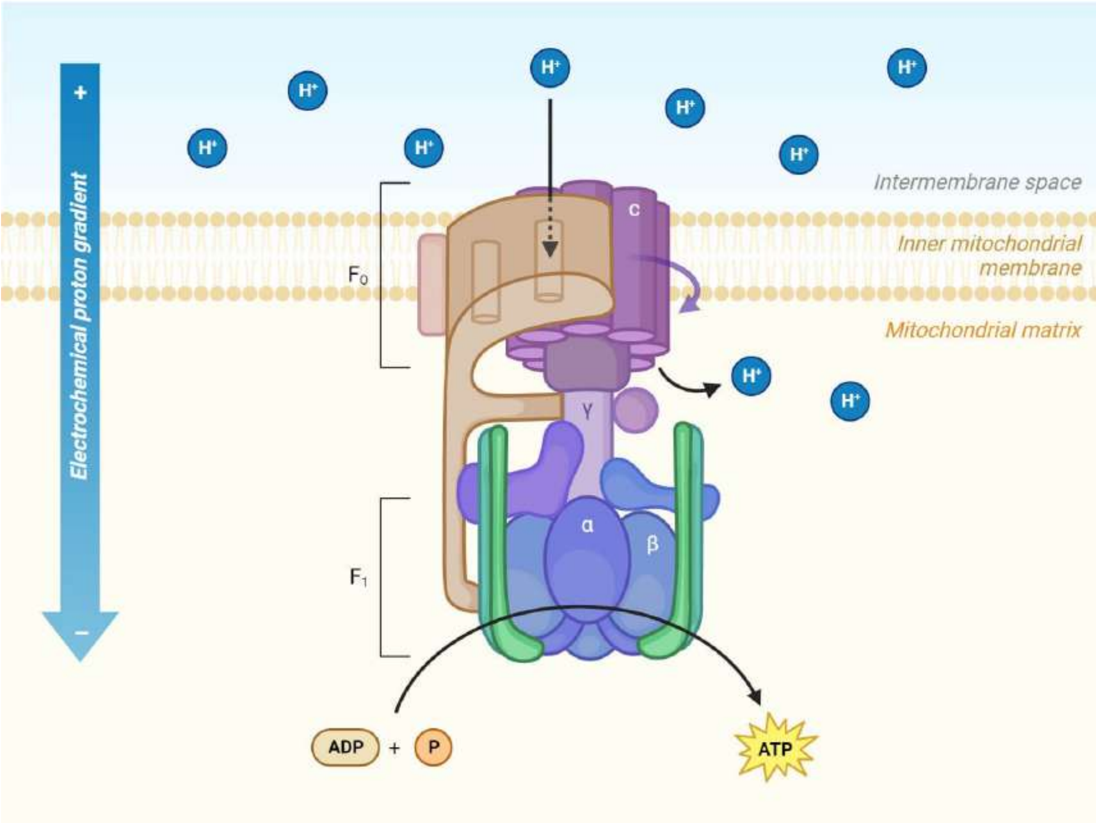


Dark

Activation

N. Kahya... D. Hoekstra *J. Biol. Chem.* (2002)

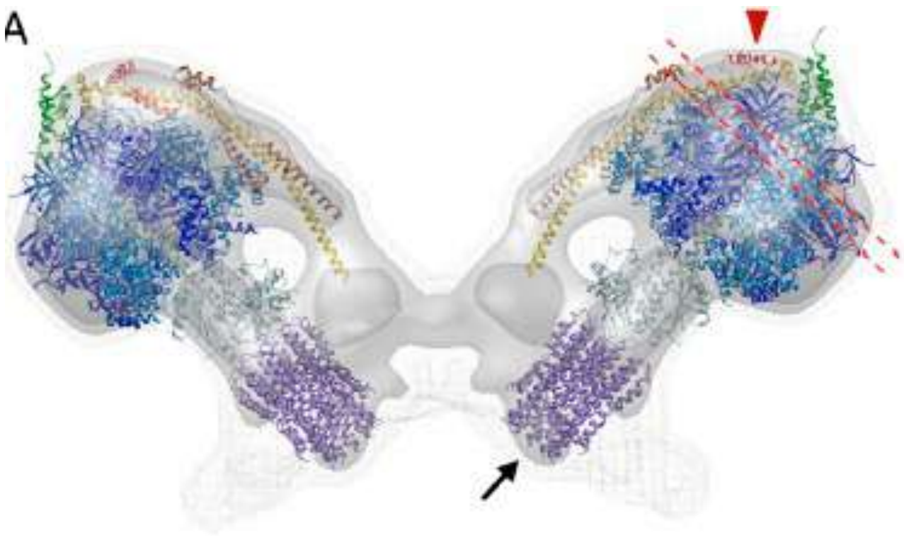
Clustering of Active ATP-Synthase (F_1F_0)

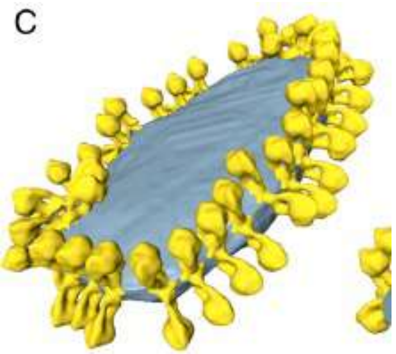


https://microbenotes.com/atp-synthase/#mechanism-of-atp-synthesis

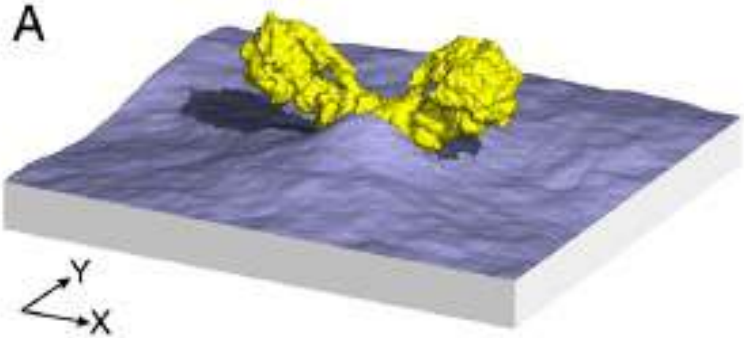
ATP production in mitochondria, induced by H+ gradient

ATP-Synthase: Dimers that Bend Membrane



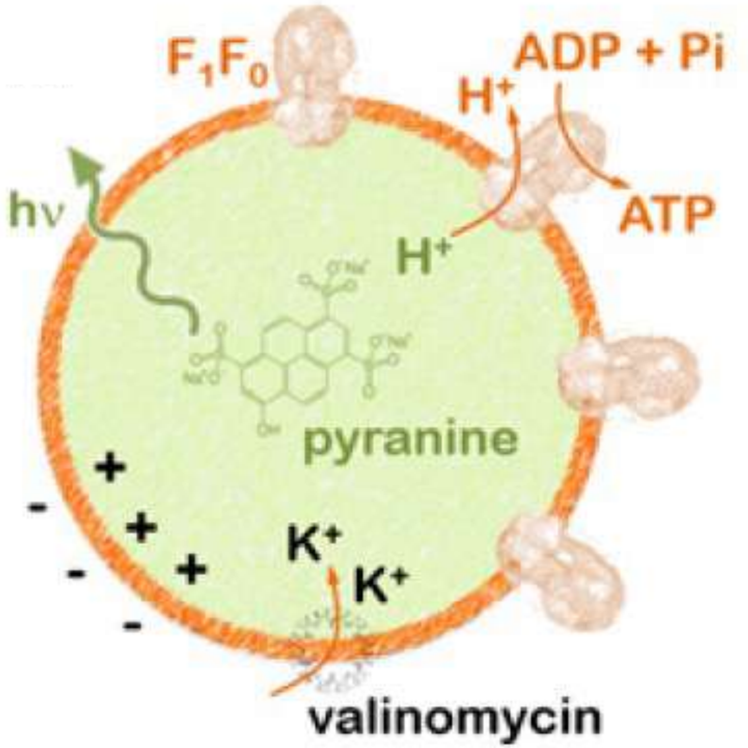


K.M. Davies et al. *PNAS* (2012)



Clustering of Active ATP-Synthase (F_1F_0)

In vitro system



Valinomycin

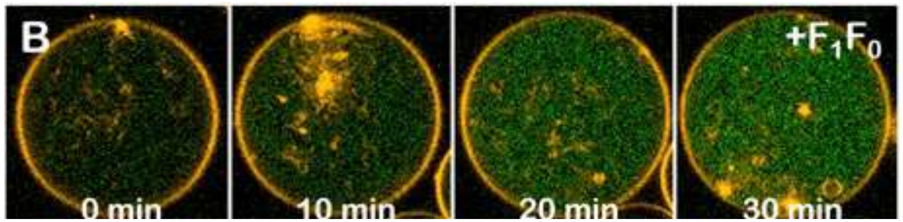
➡ K+ entry

➡ H+ exit

➡ Activates F_0

➡ ATP production

Pyranine: pH sensor

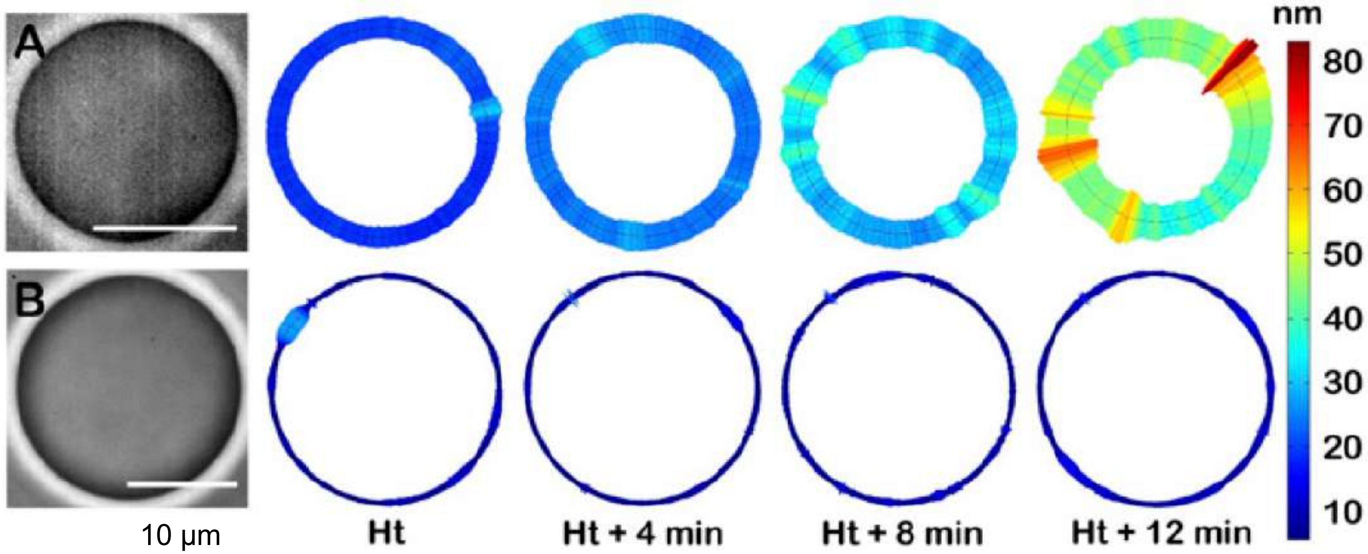


V.G. Almendro-Vedia…**I. López-Montero** *PNAS* (2017)

Localized Enhanced Fluctuations of Active F_1F_0

active

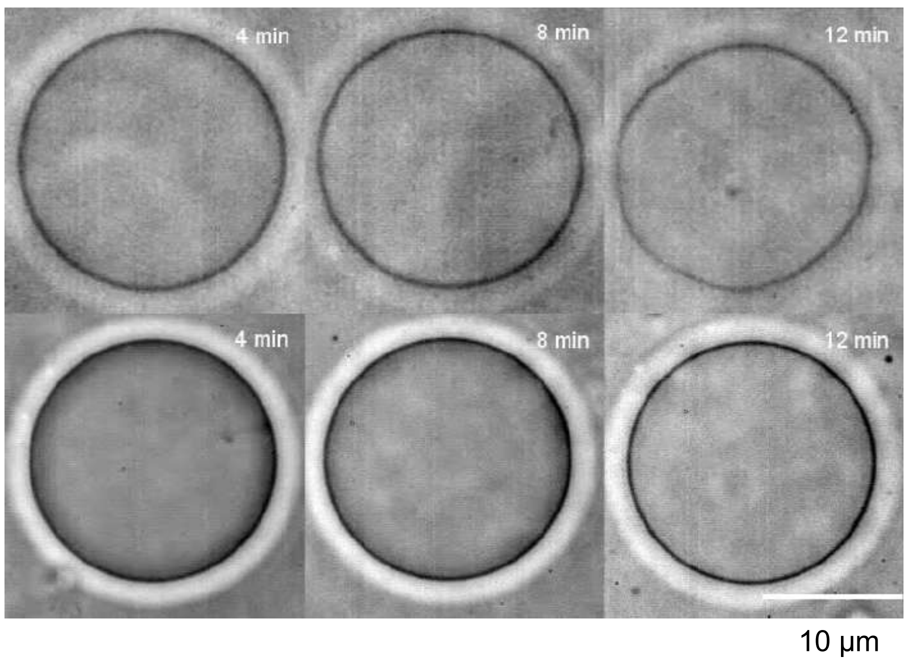
passive



Fluctuation amplitude

active

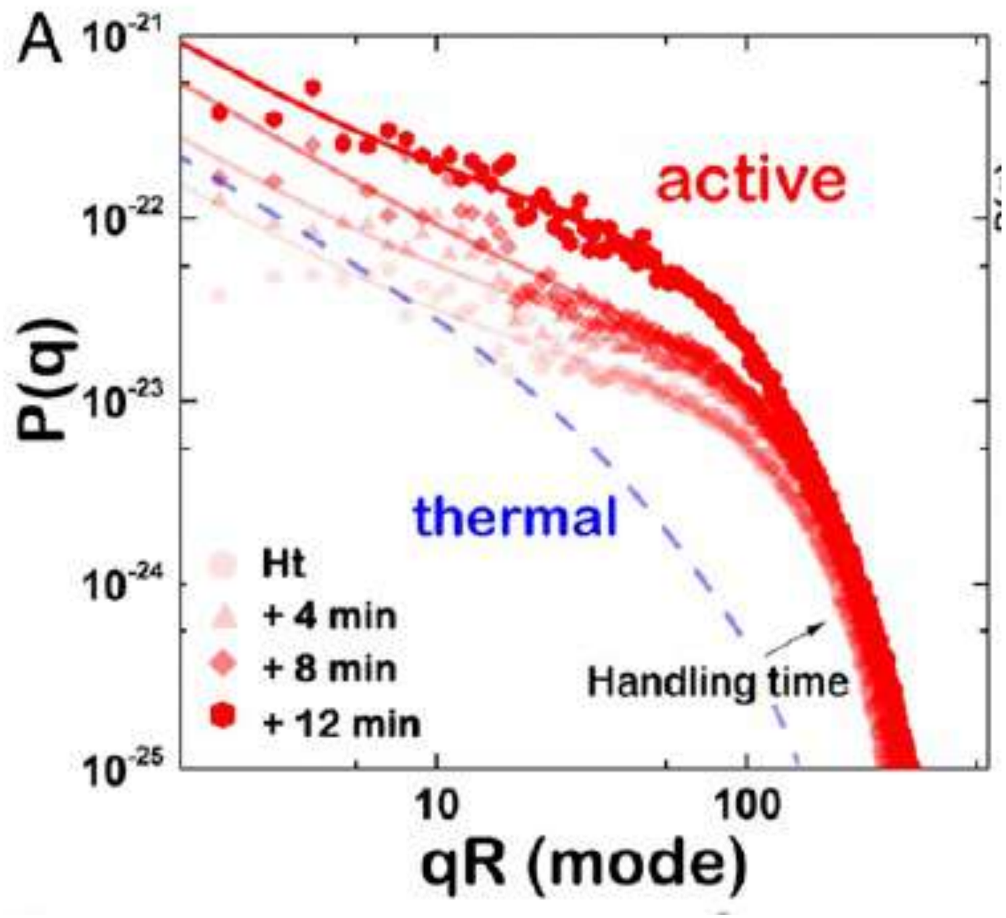
passive



Protein clusters?

V.G. Almendro-Vedia…
I. López-Montero *PNAS* (2017)

Activity Changes Fluctuation Spectrum



V.G. Almendro-Vedia…**I. López-Montero** *PNAS* (2017)

Clustering of an Active Transporter

Raju Regmi

institut Curie

Daniel Lévy

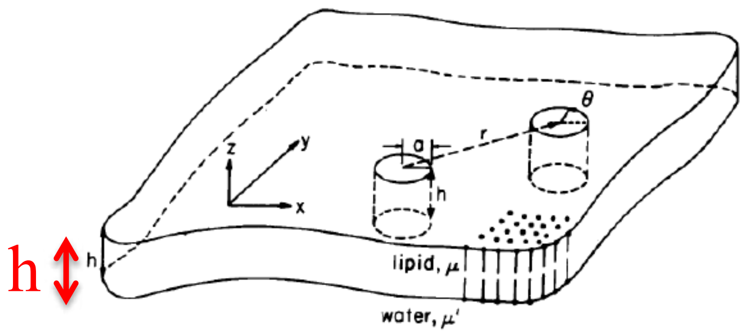
UNIVERSITY OF COPENHAGEN

Jacob Kaestel-Hansen

Nikos Hatzakis

Diffusion of a Protein in a Membrane: "Classical" Hydrodynamic Model

- Brownian motion in membranes

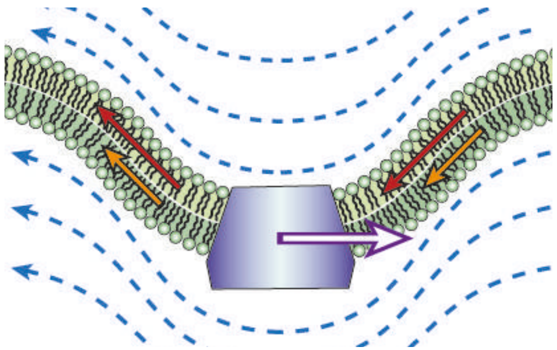


Saffman and Delbrück, PNAS 1975

Finite membrane size R_{mem}

$$D = \frac{kT}{4\pi \mu_{mem} h} \ln\left(\frac{R_{mem}}{R_{prot}}\right)$$

Depends on *membrane viscosity* - Weak dependence on the size R_{prot}



- Curvature-coupled protein
  Deforms the bilayer

→ Additionnal dissipations

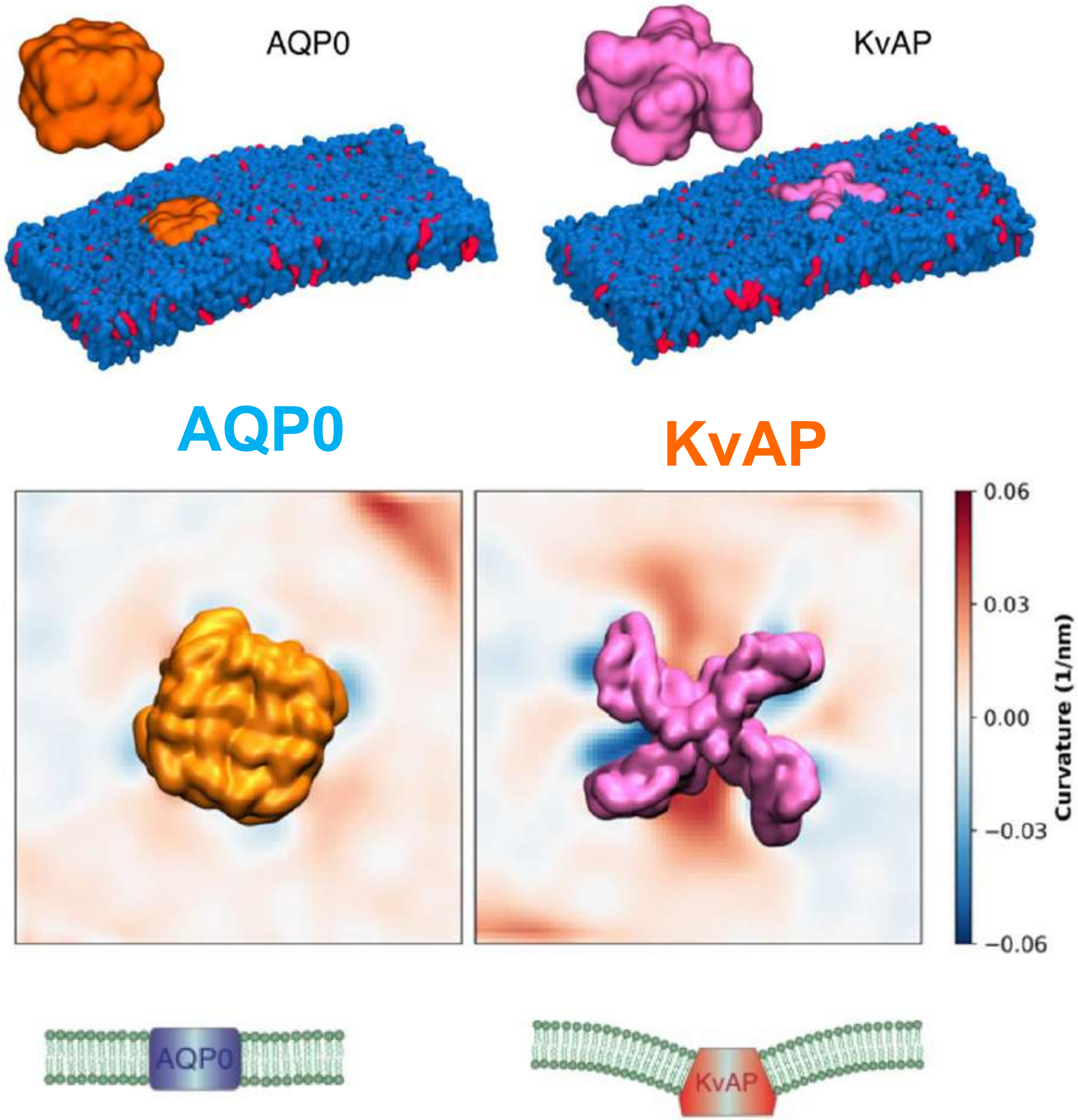
A. Naji, A. J. Levine, P. Pincus, *Biophys. J.* (2007)

KvaP Bends Membrane - AQP0 Not

Coarse-grained simulations

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

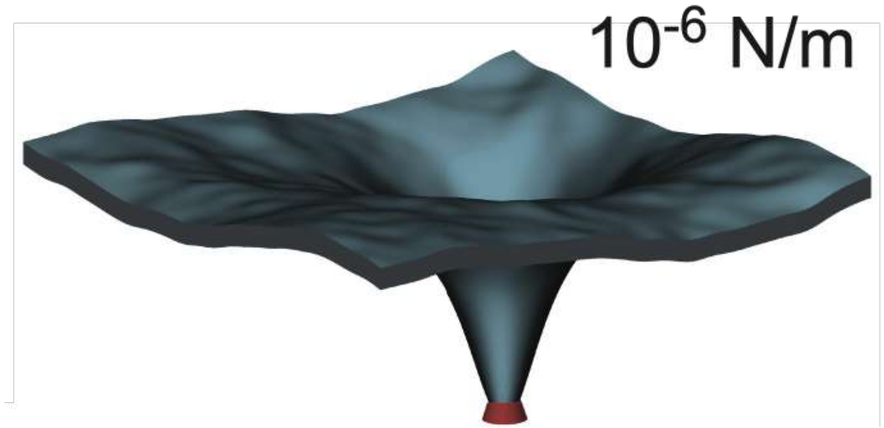
Cylindrical



Conical

Membrane Deformation Depends on Tension

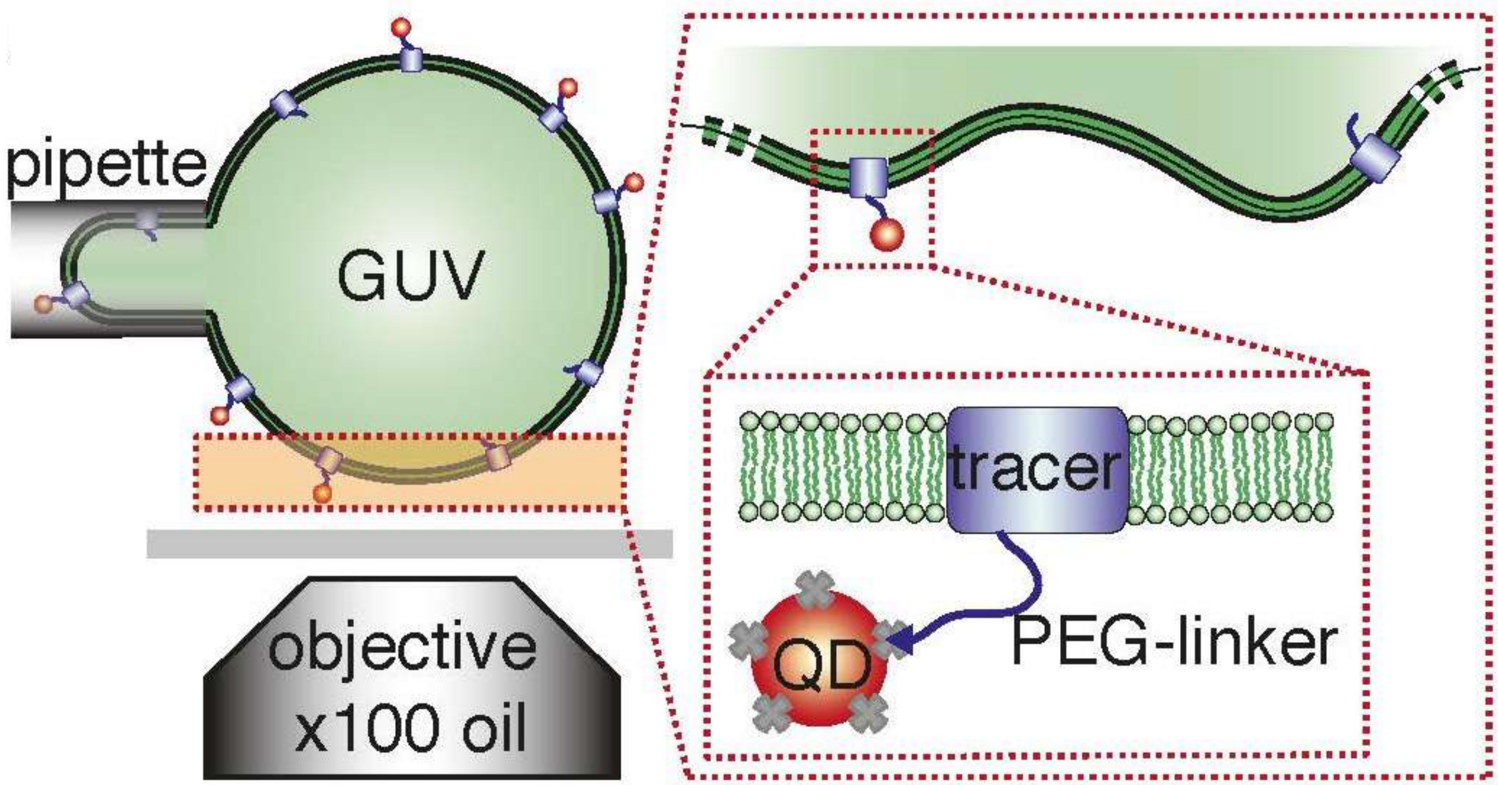
conical

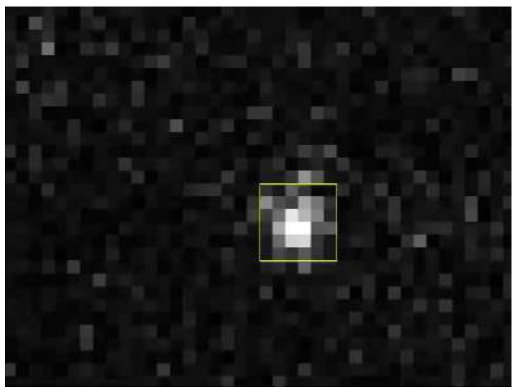


Simulations: 135x135 nm

J. Sigurdsson and P. Atzberger (UCSB)

Single Particle Tracking on GUVs

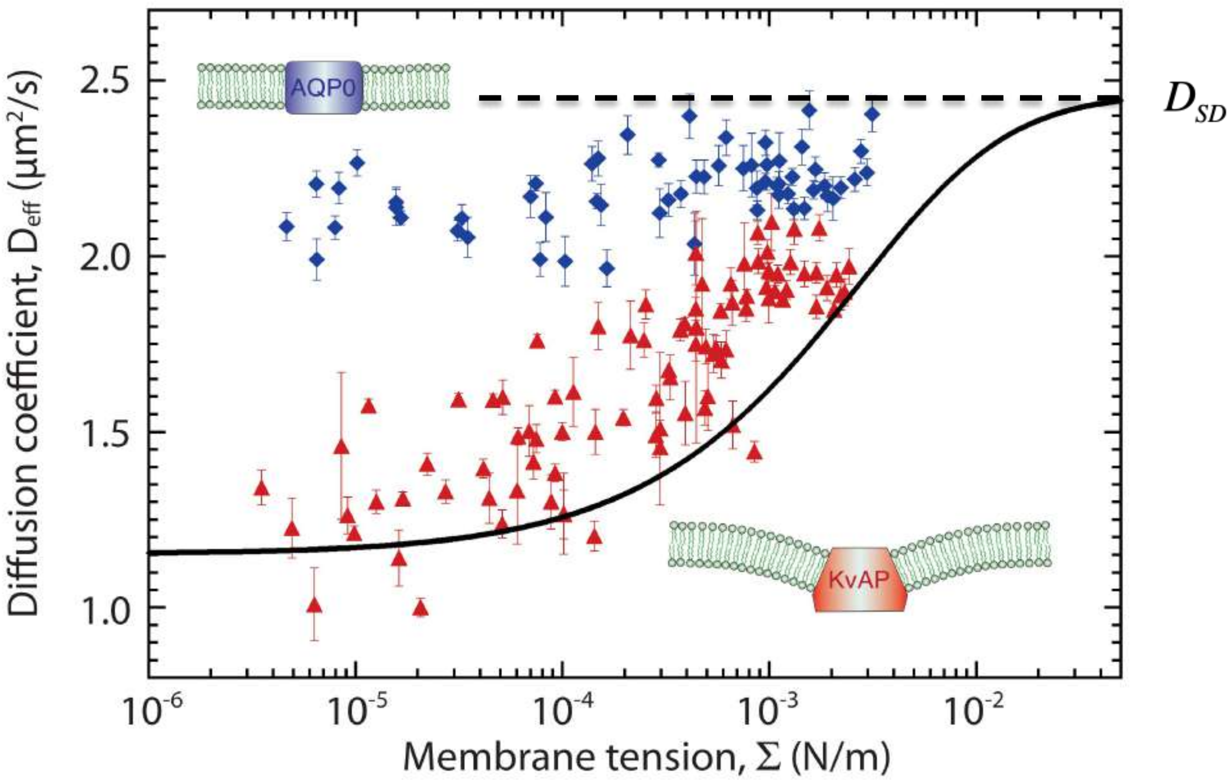




Low density:
100 proteins/μm^2

Quemeneur *et al.*,
PNAS (2014)

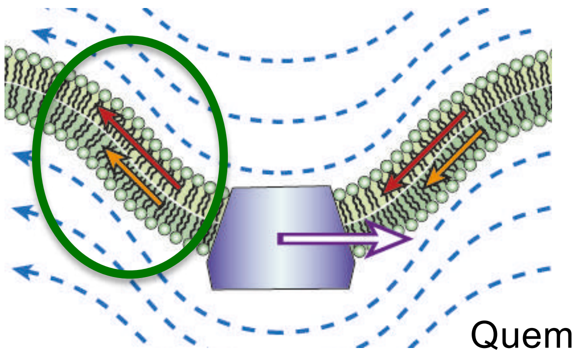
Effect of Membrane Tension on Diffusion



Model: *dissipation* mechanisms with the bulk and inside the bilayer

Dominant: *interleaflet friction*

D. Lacoste (ESPCI)
J. Sigurdsson, P. Atzberger (UCSB)



Quemeneur *et al.*, *PNAS* (2014)

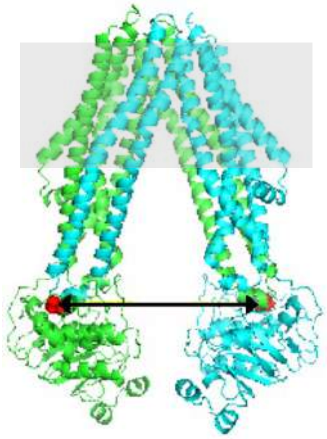
BmrA

Bacterial ABC Transporter (*B subtilis*)

Apo (open)

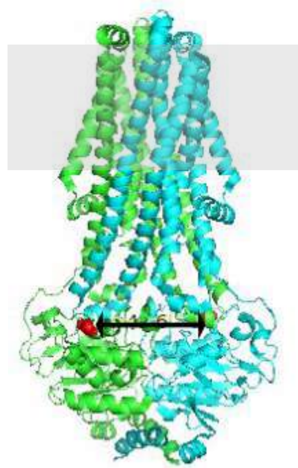
Post-Hydrolytic (closed)

Membrane



ATP binding

ATP hydrolysis



M. Di Cesare et al
J. Biol. Chem. (2024)

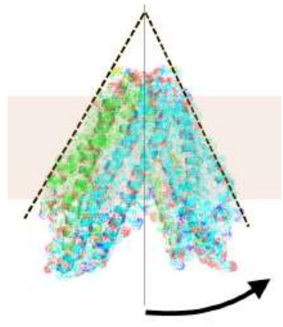
V. Chaptal et al
Sci. Adv. (2022)

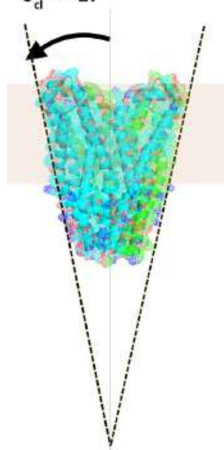
6.85 nm

4.3 nm

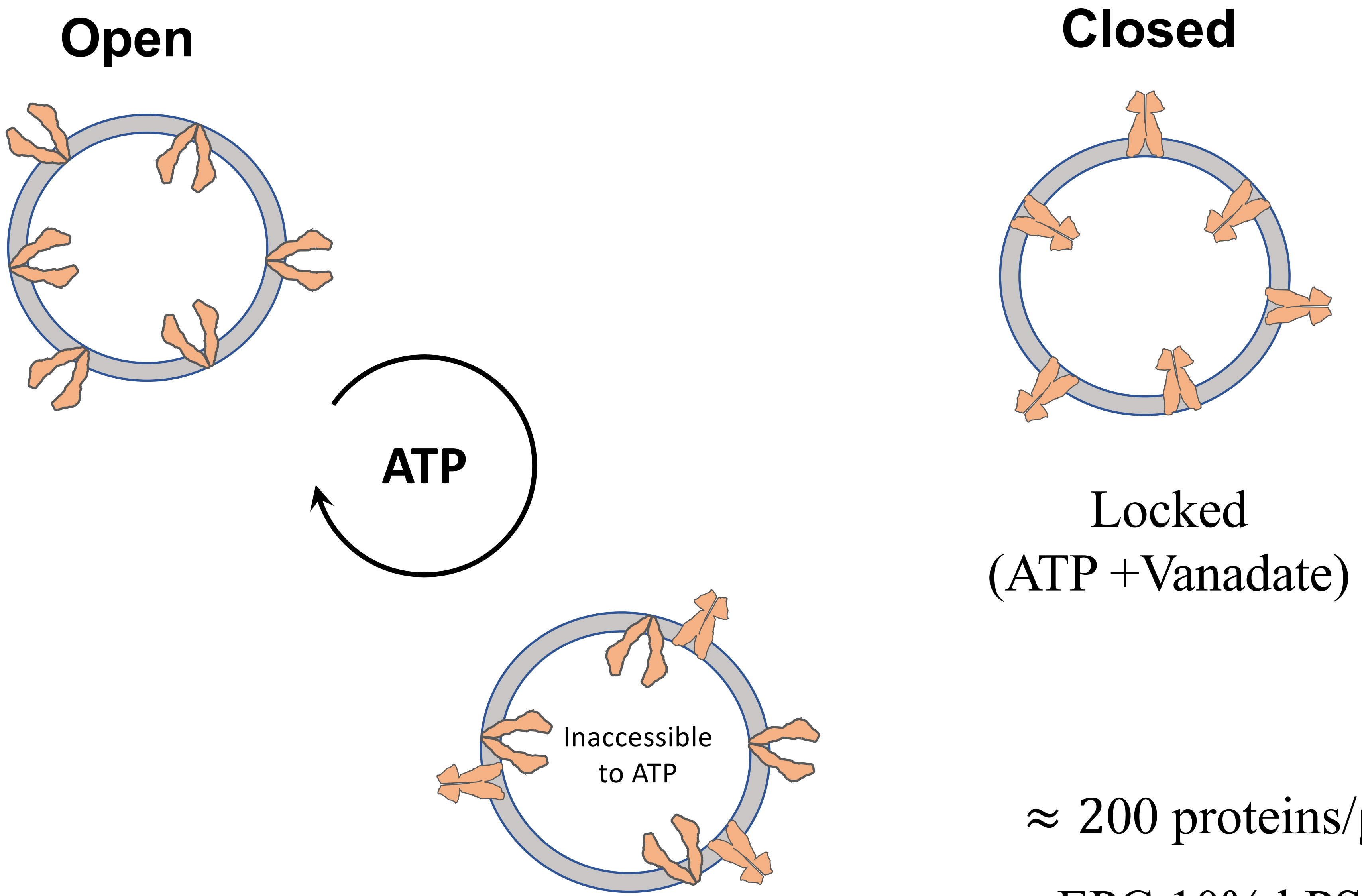
Large conformational changes

Also in the trans-membrane domain





Single BmrA Diffusion in GUVs



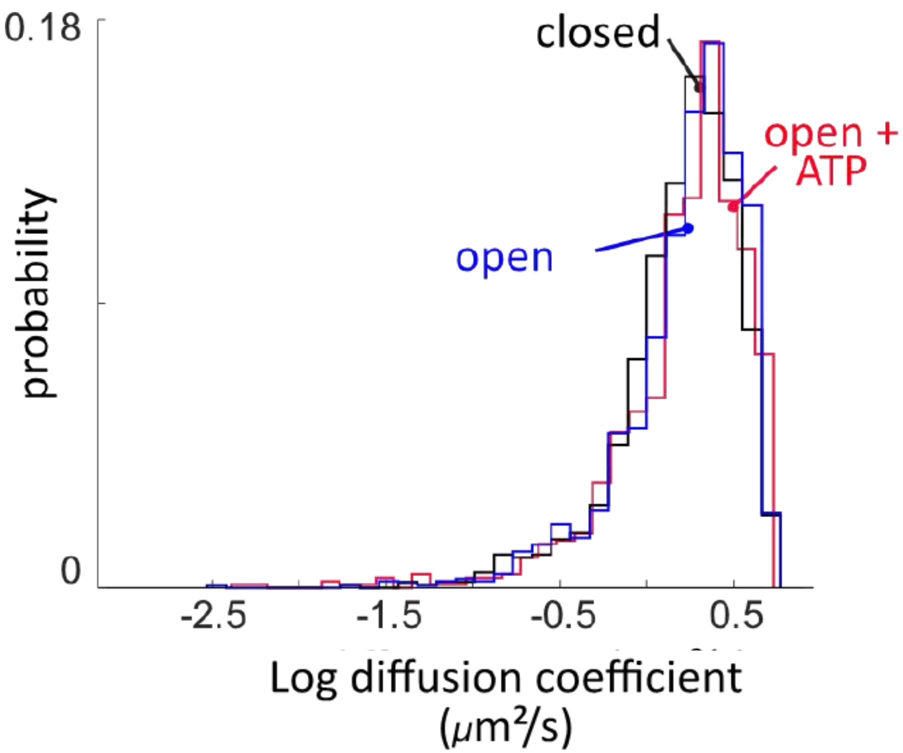
≈ 200 proteins/µm^2

EPC:10% bPS

T=20°C

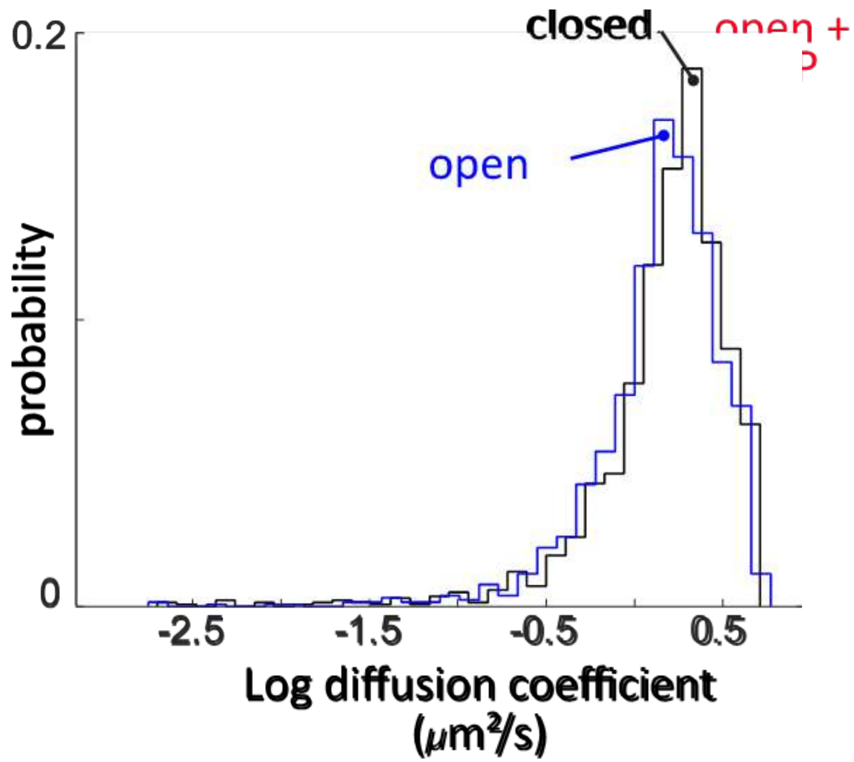
MSD Analysis : Diffusion Depends upon Activity at Low Tension

High tension (~10^{-3} N/m)



No difference

Low tension (~ 10^{-5} N/m)



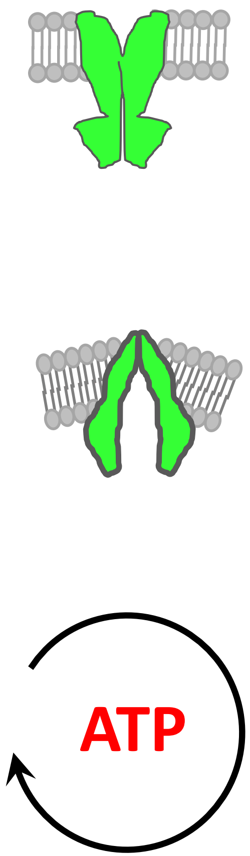
+ATP: Non trivial

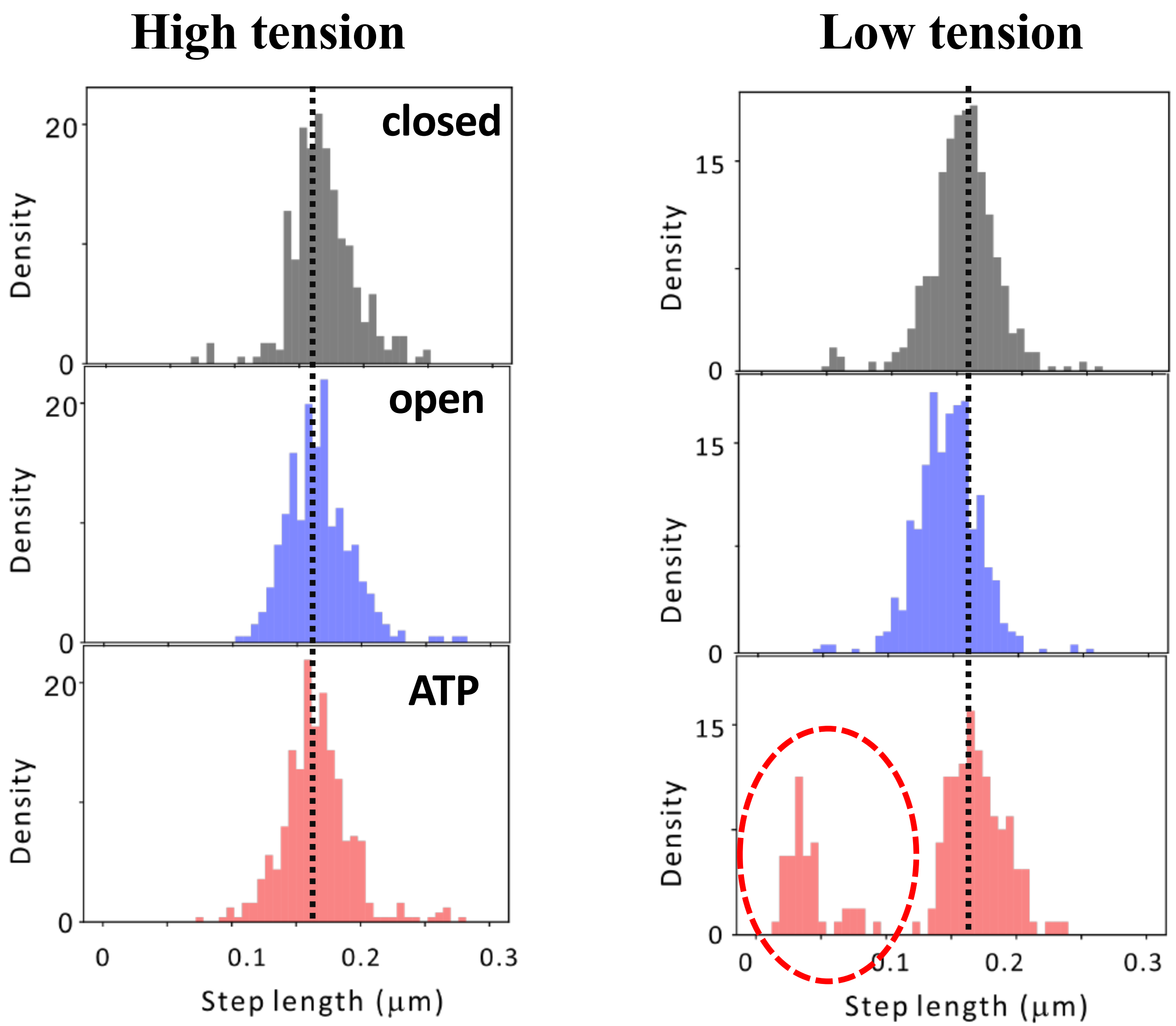
Fast and slow diffusion

ATP-Dependent Clustering at Low Tension ?

J. **Kæstel-Hansen, N. Hatzakis**
Machine learning approach

Δt= 5 ms

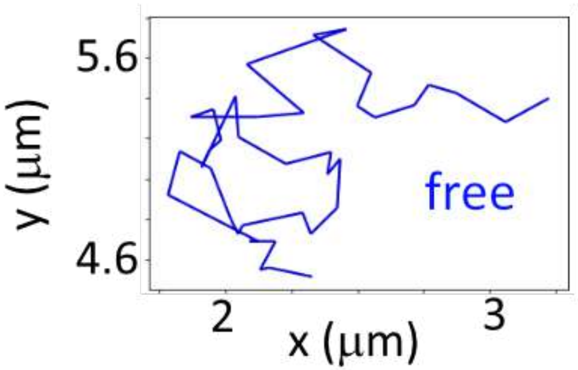


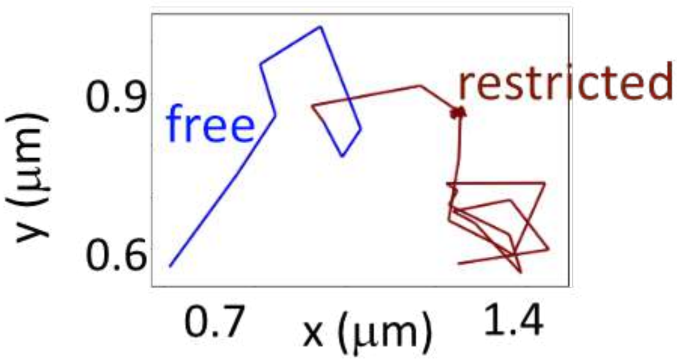


Upon ATP Activation, Restricted Motion

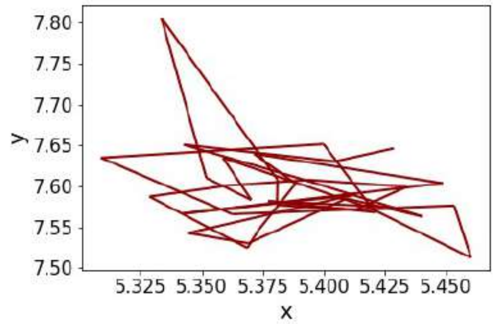
Segmentation (Deep Learning)

Trajectories ≤ 1 sec

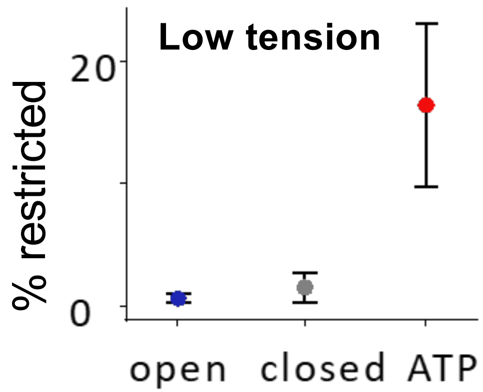


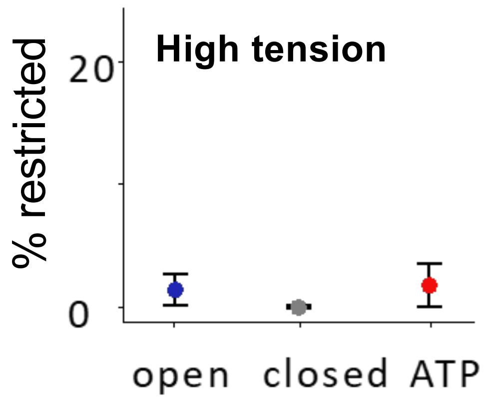


only restricted



Restricted area ~ psf



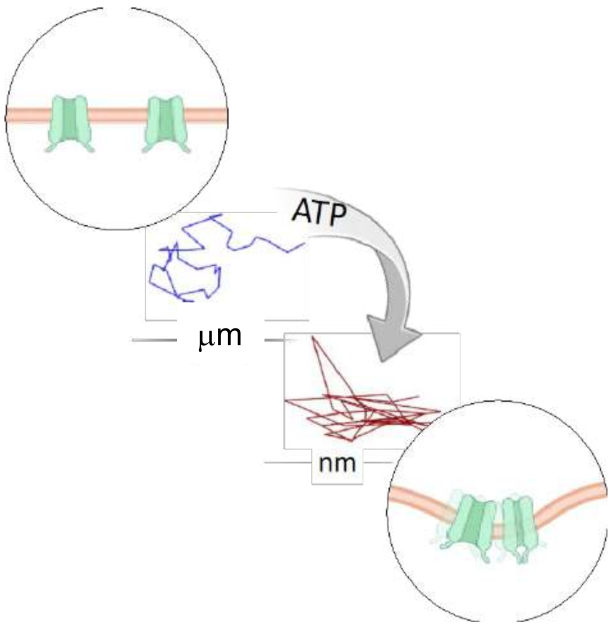


Restricted motion ⟷ Higher local density of proteins

Protein clusters

- Protein *activity* induces *clustering*
- Significant at low membrane tension, disappears at high tension

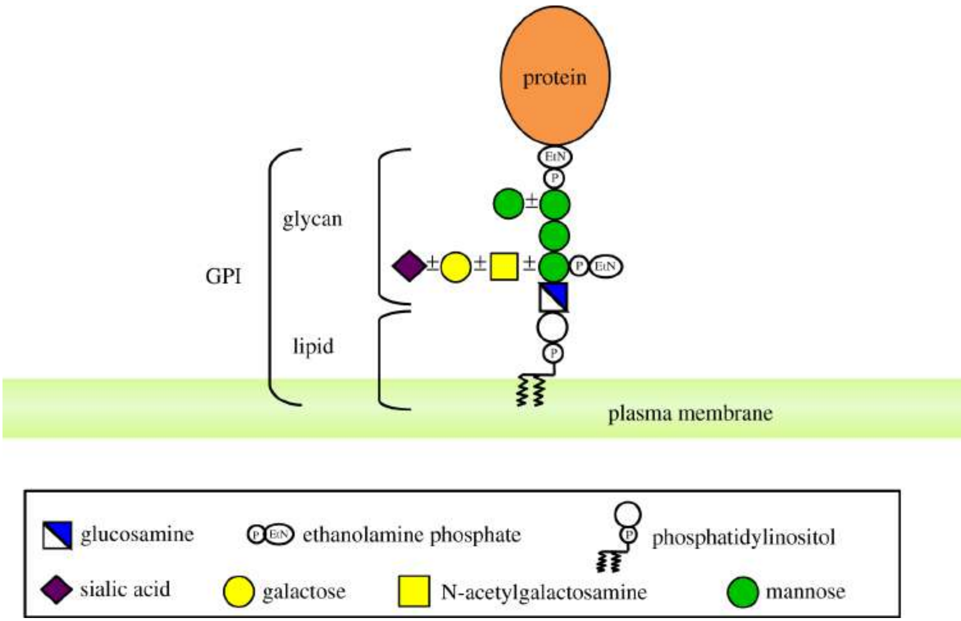
⇨ membrane-mediated



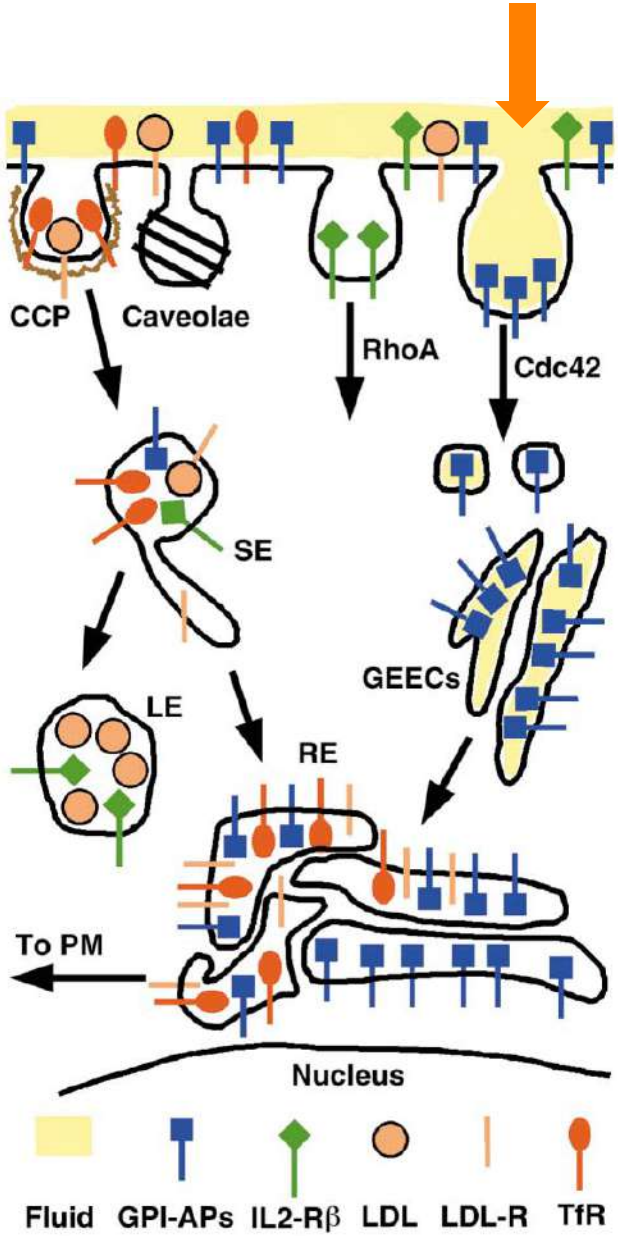
Mechanism ????

Clustering via Cytoskeleton and Motors

GPI-Anchored Proteins: Clathrin-Independent Endocytosis



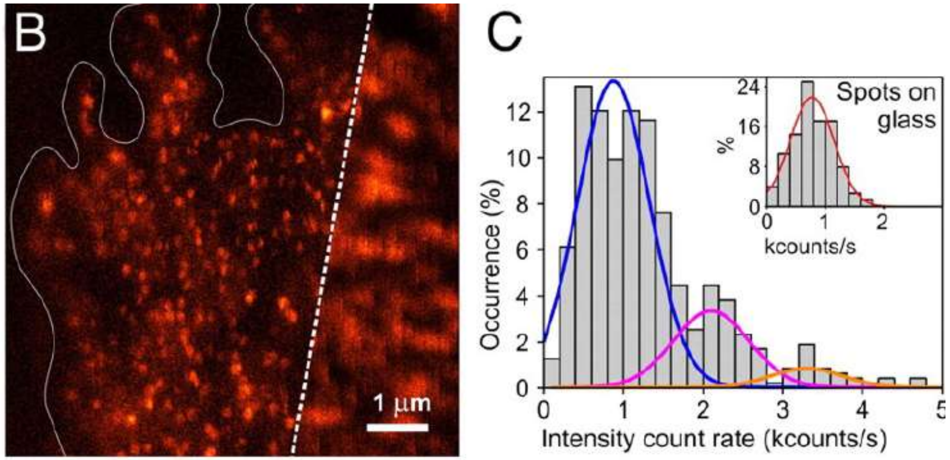
T. Kinoshita *Open Biol* (2020)



S. Sabharanjak… **J. Mayor** *Dev. Cell* (2002)

GPI-Anchored Protein Clustering

Single-molecule Near-field Optical Microscopy – Non activated monocytes

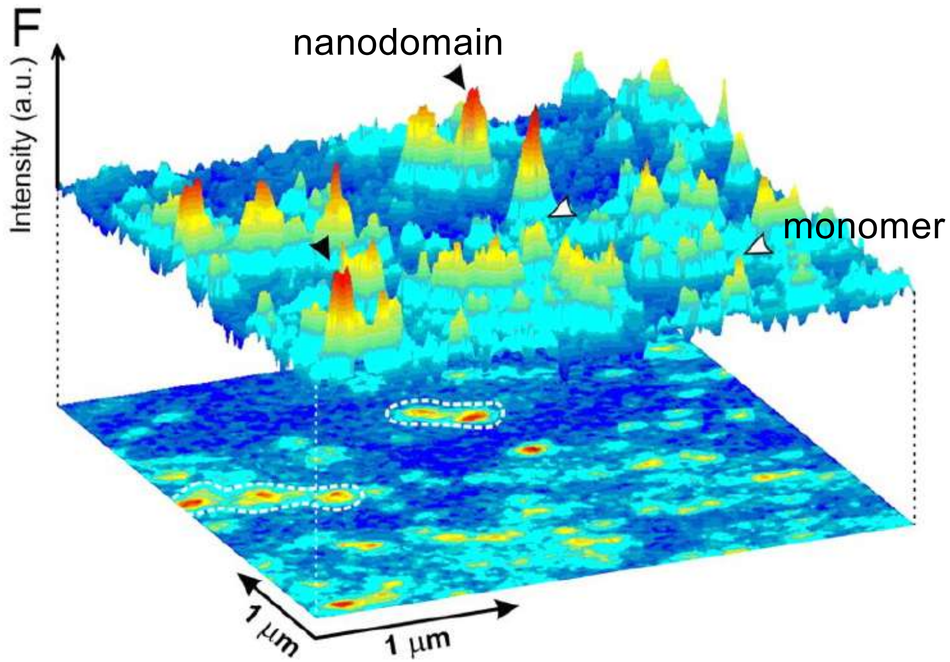


Nanodomains

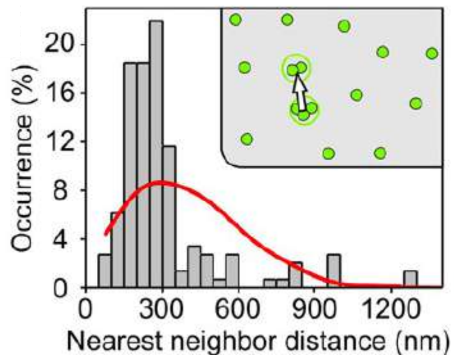
Dimers: 19%

Trimers 5%

Oligomers: 5%



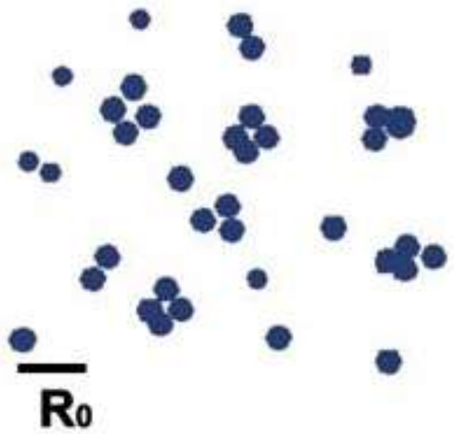
Nano-domains (4-5 nm) not randomly distributed



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

GPI-Anchored Protein Clustering

Also: GPI-AP nanodomains on CHO cells (fluorescence anisotropy)



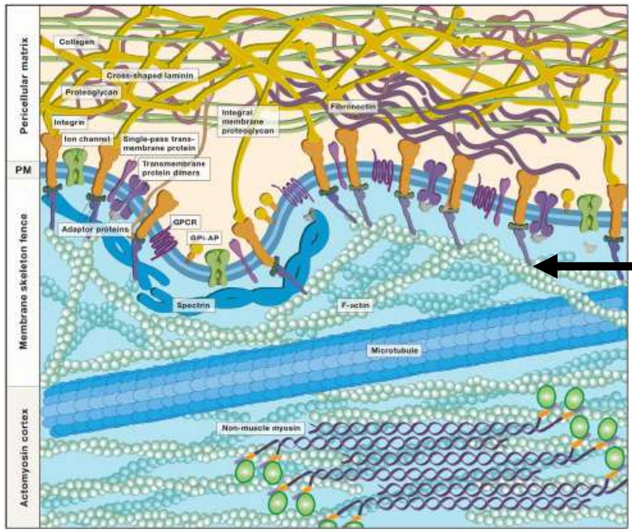
R_0=4.65 nm
(Förster radius/GFP)

+ Role of Cholesterol and Sphingolipids

Not randomly mixed: *active* process?

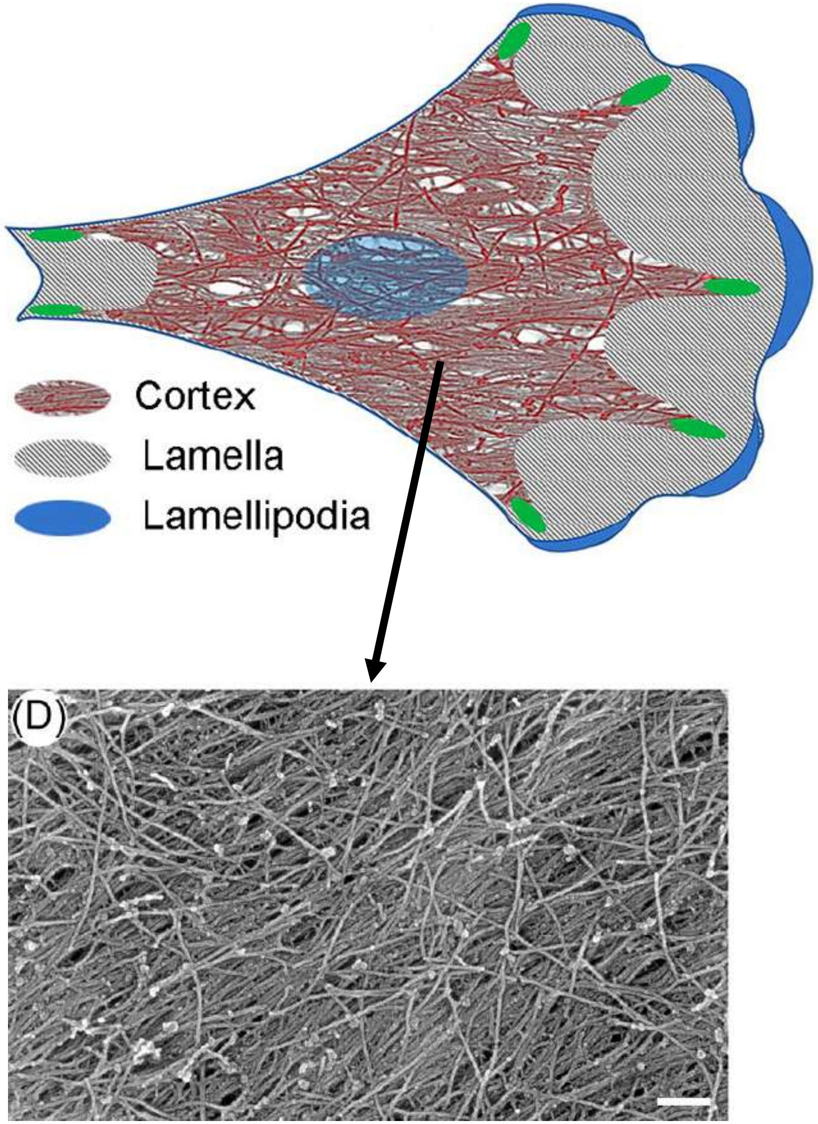
P. Sharma… **M. Rao, S. Mayor**, *Cell* (2004)

Correlation between Distribution and Dynamics of Nanoclusters and Cortical Actin



Cortical actin filaments

K. Jacobson et al *Cell* (2019)



T.M. Svitkina *Trends Cell Biol.* (2020)

200 nm

Correlation between Distribution and Dynamics of Nanoclusters and Cortical Actin

Nanoclusters: spatial distribution and dynamics (formation, break-up)

⟷ Local cortical Actin active remodeling ((de)polymerization, acto-myosin contractility)

Different signs of an *active process*

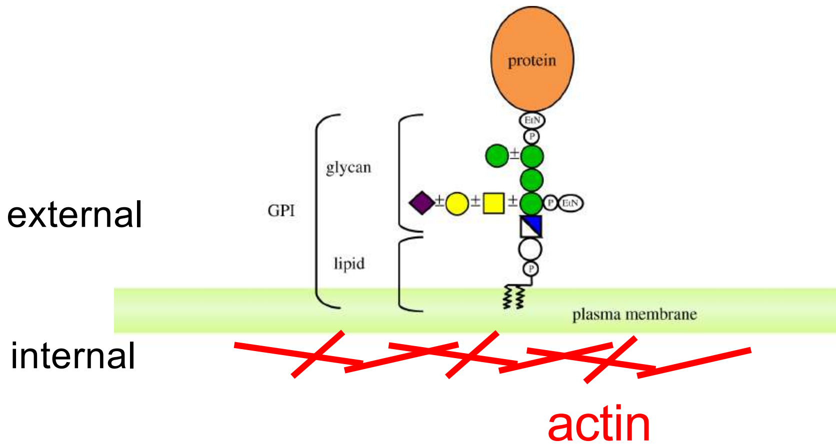
(i.e. exponential tails of distributions)

2 scales: nanodomains (<10 nm)

larger domains (450 nm) where nanodomains concentrate (Cf rafts)

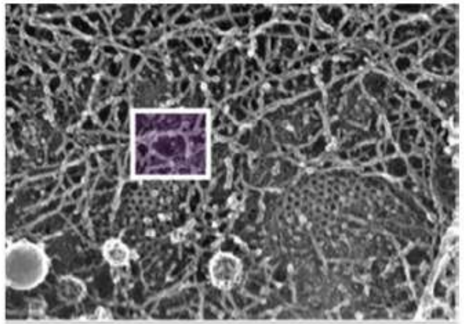
Coupling between GPI-AP and actin??

Cholesterol important

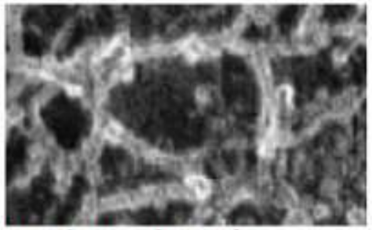


D. Goswami … **M. Rao, S. Mayor** *Cell* (2008)

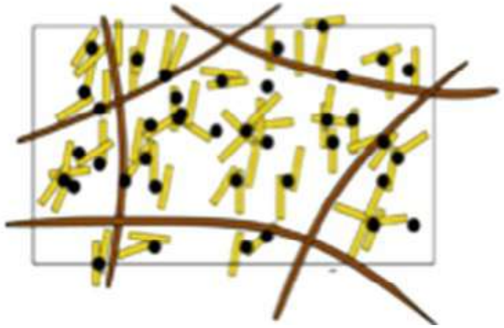
Active Clustering (Model)

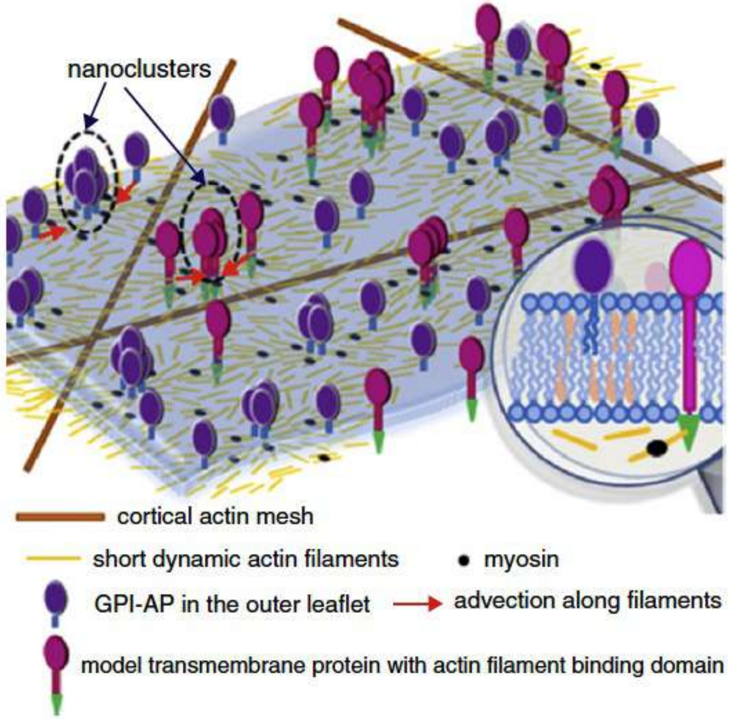


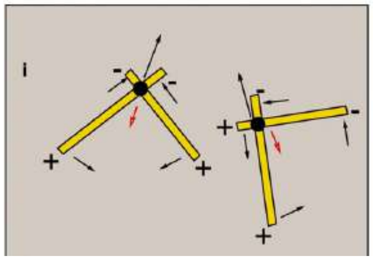
Stable crosslinked actin filaments



Short dynamic actin







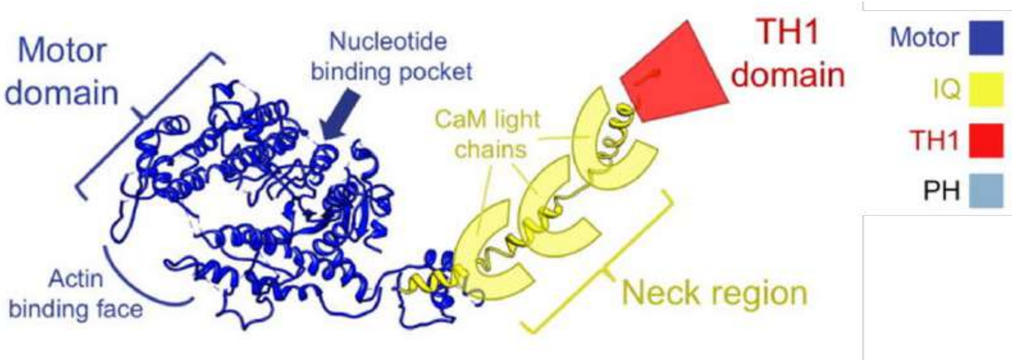
Myosin motors crosslink
short actin filaments
+ reorganize them (asters)

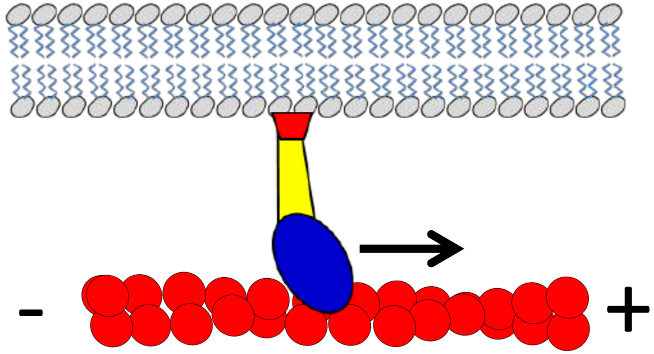
K. Gowrishankar … **S. Mayor, M. Rao** *Cell* (2012)

M. Rao, S. Mayor, *Curr. Opin. Cell Biol.* **29**, 126 (2014)

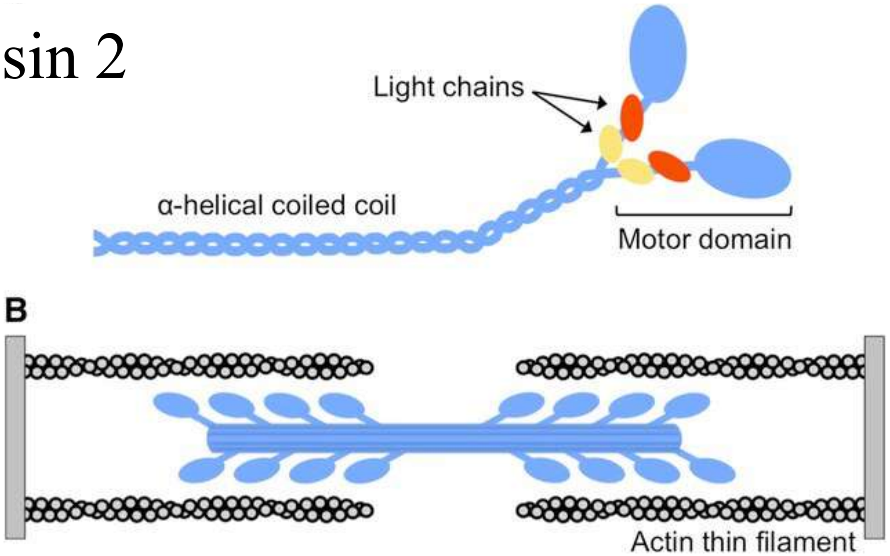
Myosins

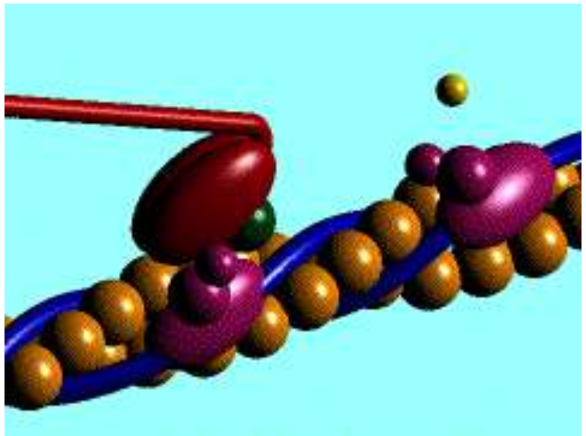
Myosin 1





Myosin 2

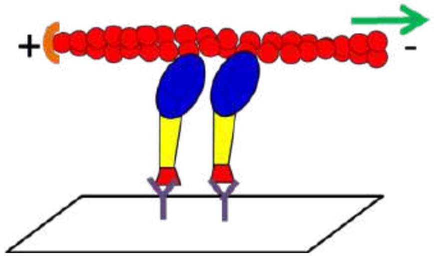




Can for short bundles

Gliding Assays

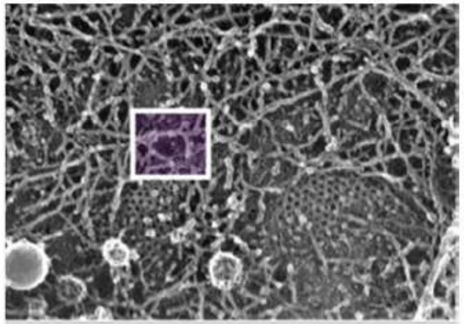
Stabilized F-actin



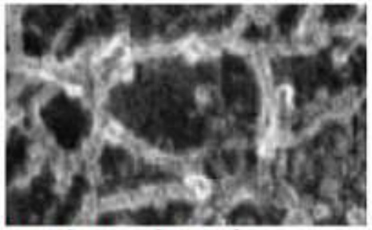
J. Pernier

X 200 times

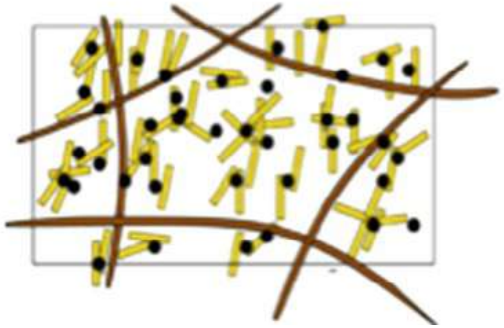
Active Clustering (Model)

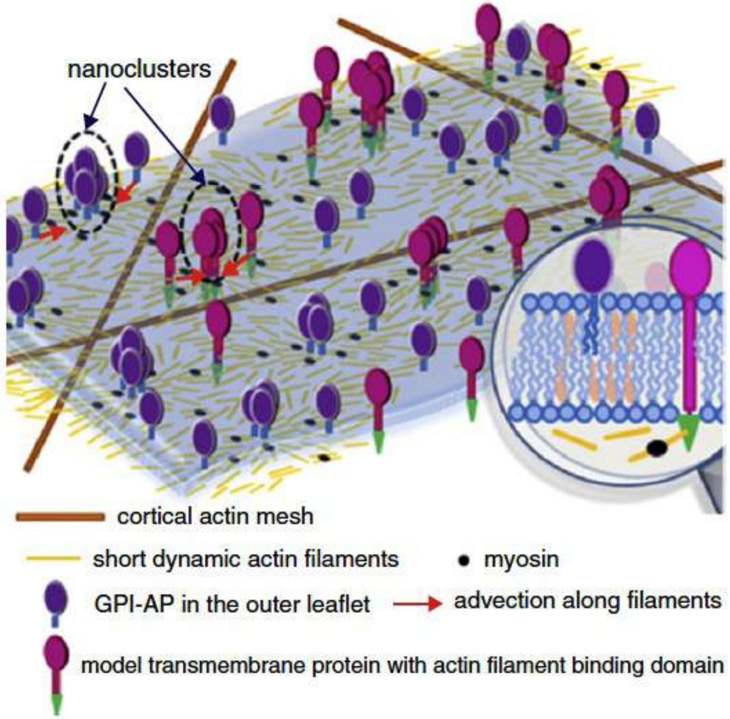


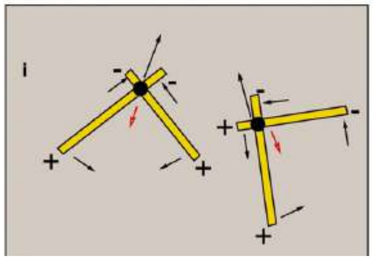
Stable crosslinked actin filaments

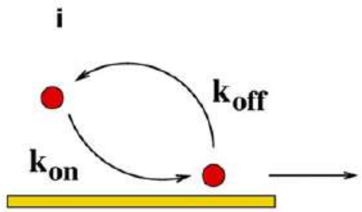


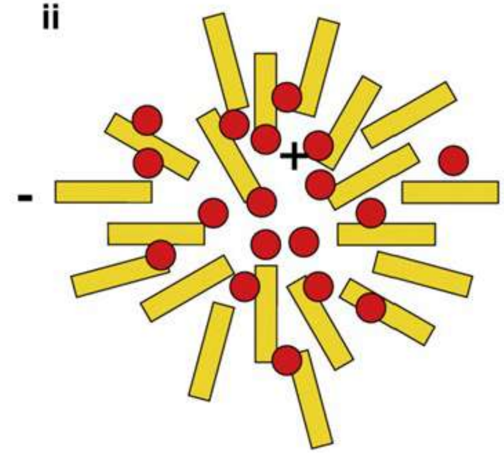
Short dynamic actin











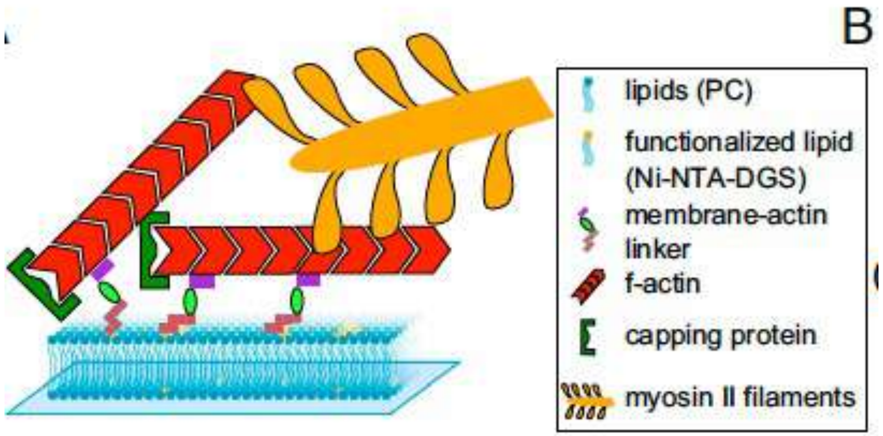
Myosin motors crosslink short actin filaments + reorganize them (asters)

Passive molecules (GPI-AP) bind, advected to aster/core

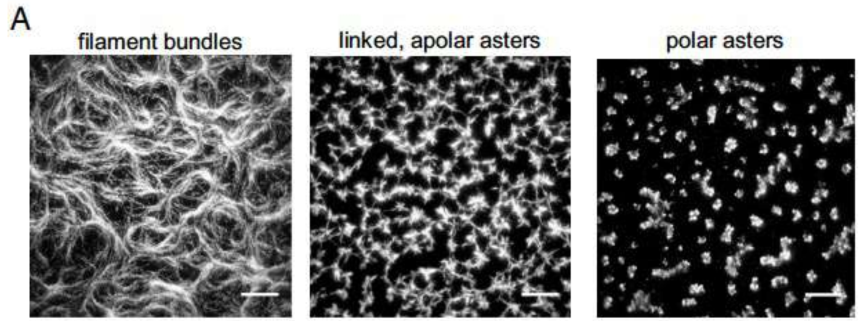
K. Gowrishankar … **S. Mayor, M. Rao** *Cell* (2012)

M. Rao, S. Mayor, *Curr. Opin. Cell Biol.* **29**, 126 (2014)

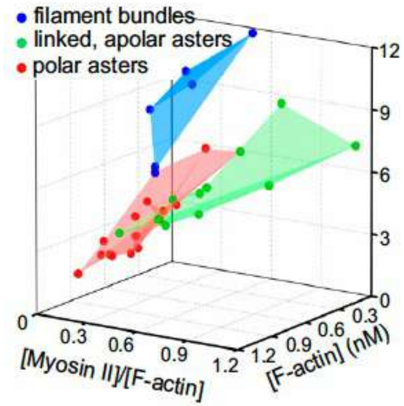
Active Clustering (*in vitro* Exp.)



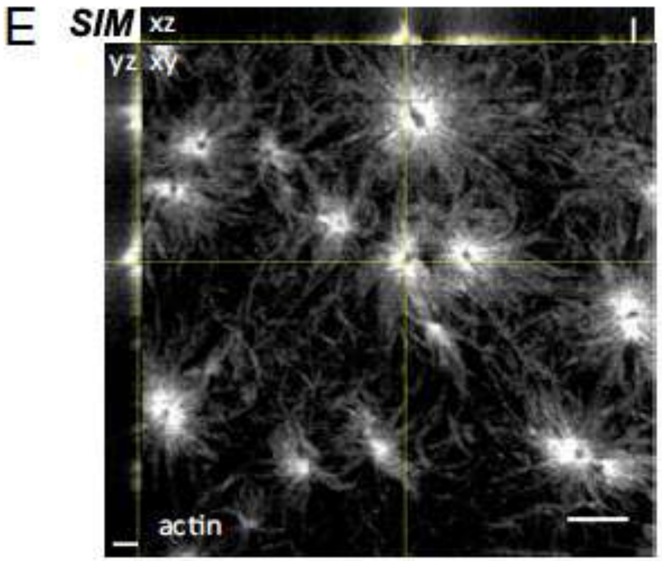
3 actin organizations

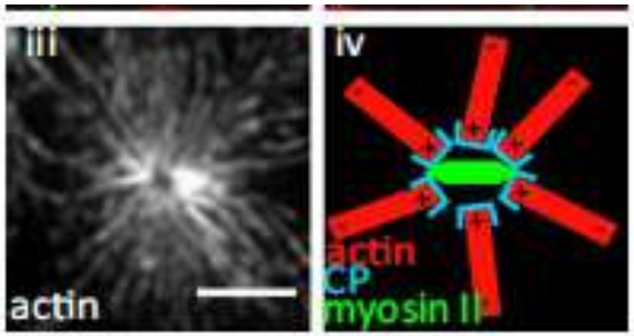


10 µm



Polar asters



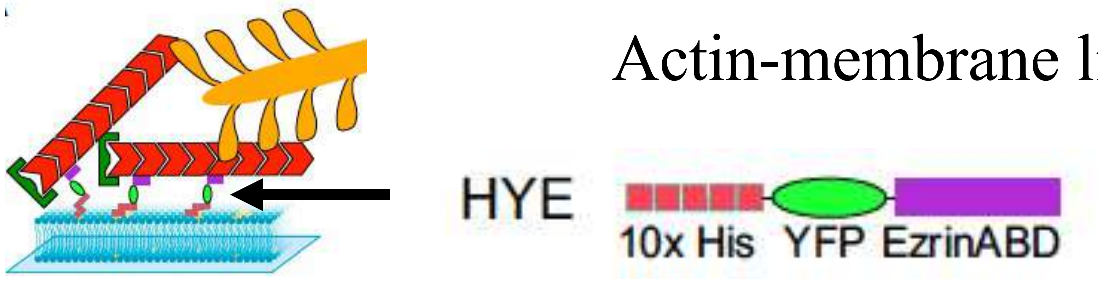


2 µm

2 µm 5 µm

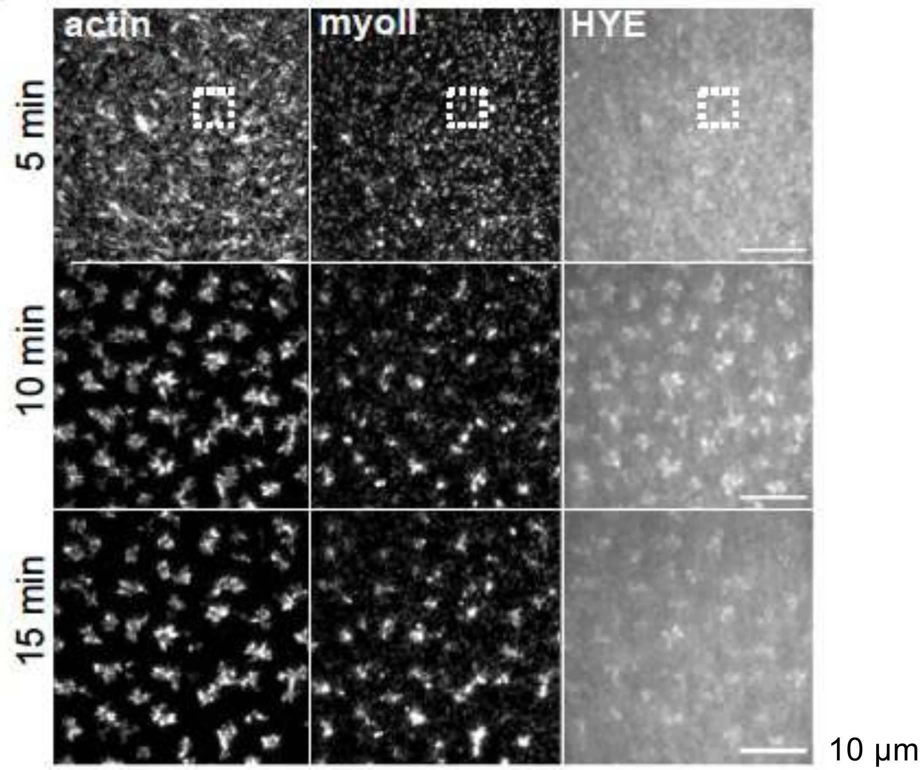
D.V. Köster … **M. Rao, S. Mayor** *PNAS* (2016)

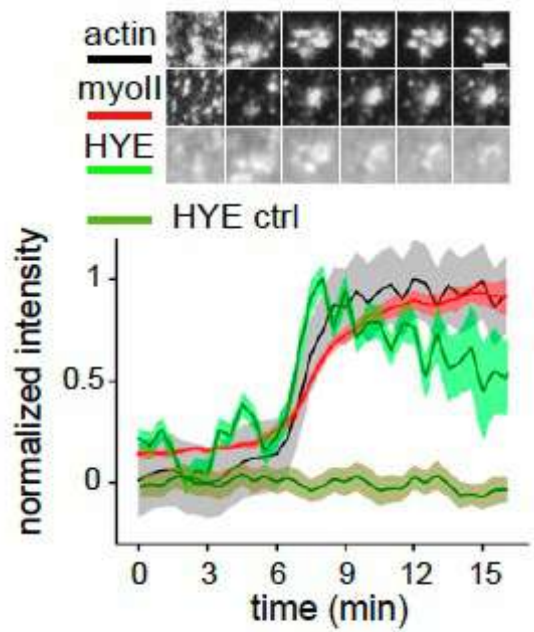
Active Clustering (*in vitro* Exp.)



HYE clustering upon aster formation

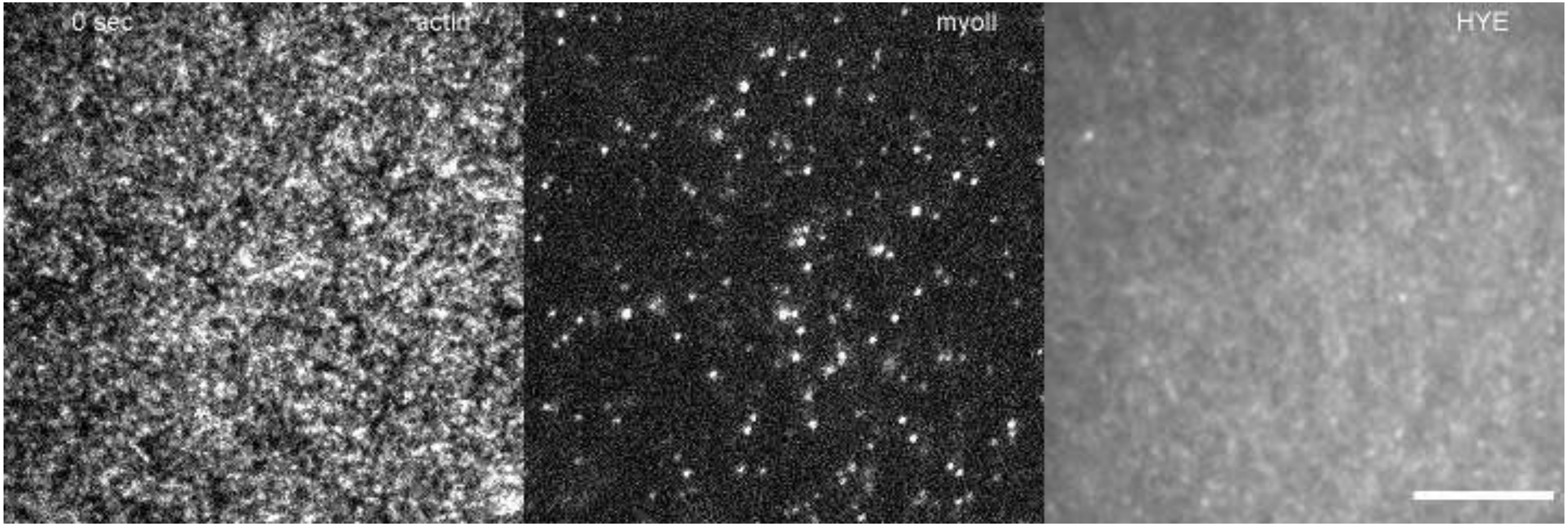
Only when actomyosin dynamical





D.V. Köster … **M. Rao, S. Mayor** *PNAS* (2016)

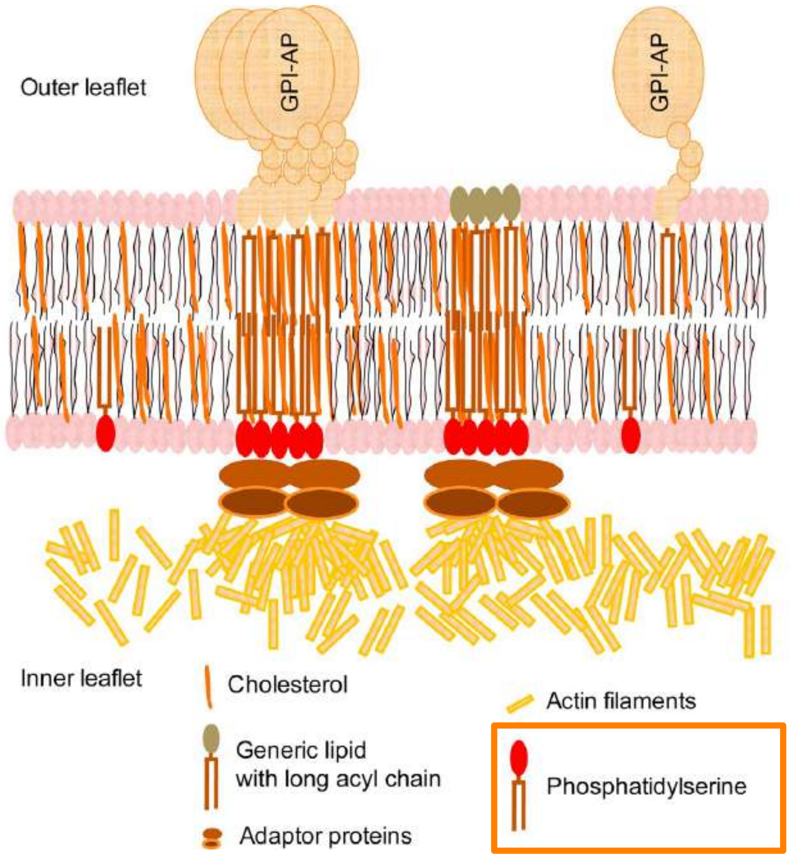
Active Clustering (*in vitro* Exp.)



10 μm

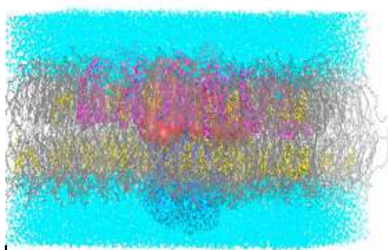
D.V. Köster … **M. Rao, S. Mayor** *PNAS* (2016)

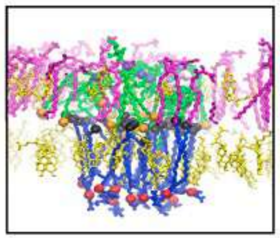
Trans-Bilayer Coupling

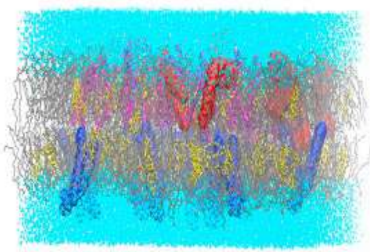


Requires:

- Long chain PS lipids
- Long saturated GPI lipids
- Cholesterol







GPI or GPI

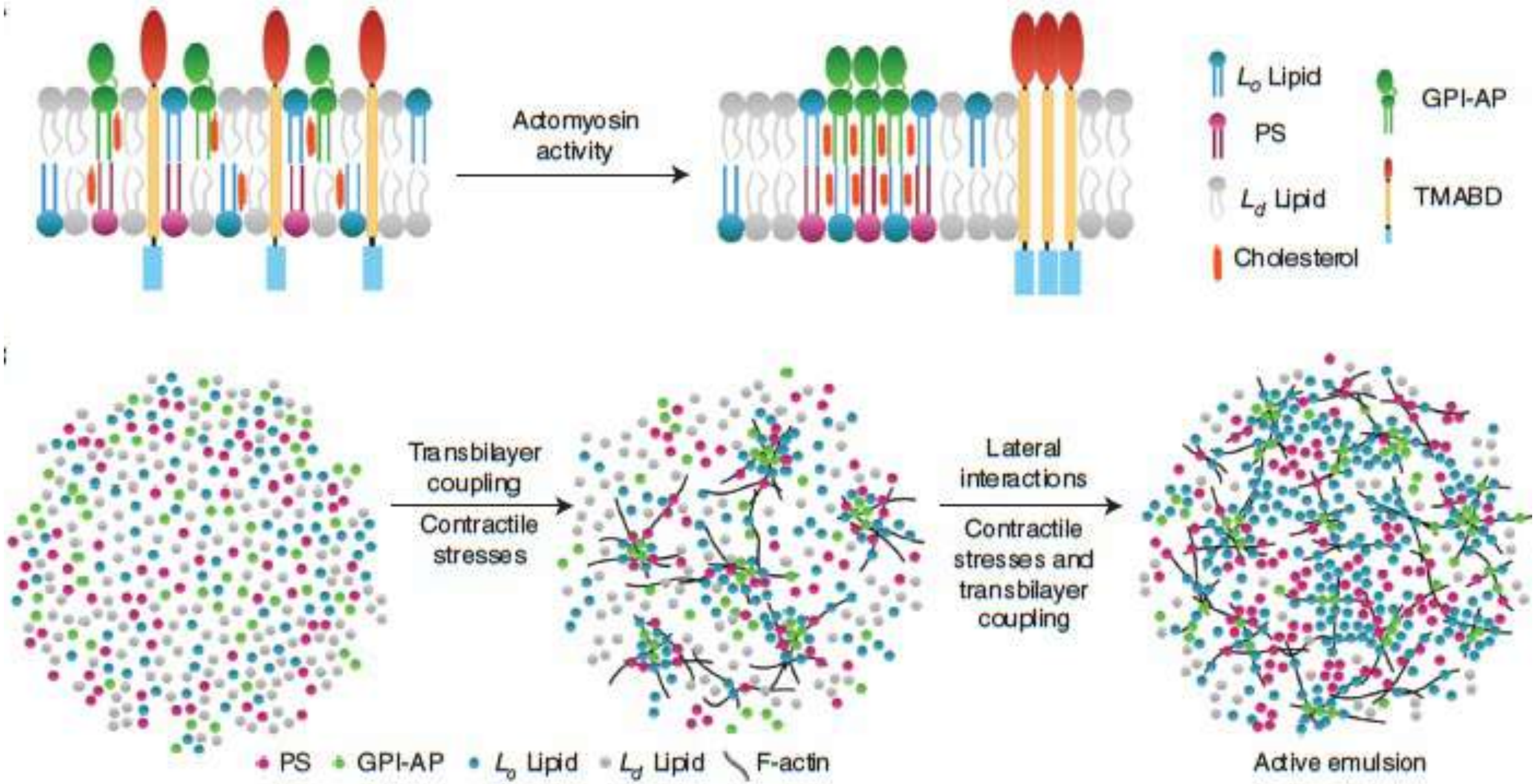
PS Chol

Sphingomyelin

PS immobilized

D.V. Köster … **M. Rao, S. Mayor** *PNAS* (2016)

New Vision of the PM: Active Emulsion



S. Saha… **S. Mayor, M. Rao** *PNAS* (2022)

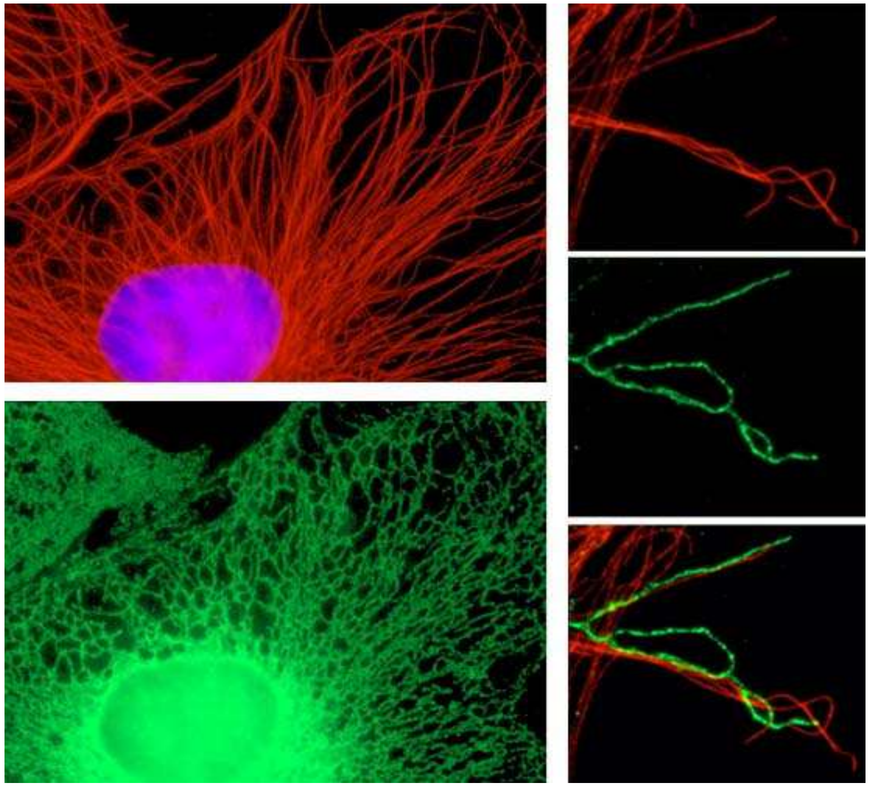
S. Mayor et al. *Cold Spring Harb. Perspect. Biol.* (2023)

Dynamical Clustering of Molecular Motors to form Membrane Nanotubes in Cells

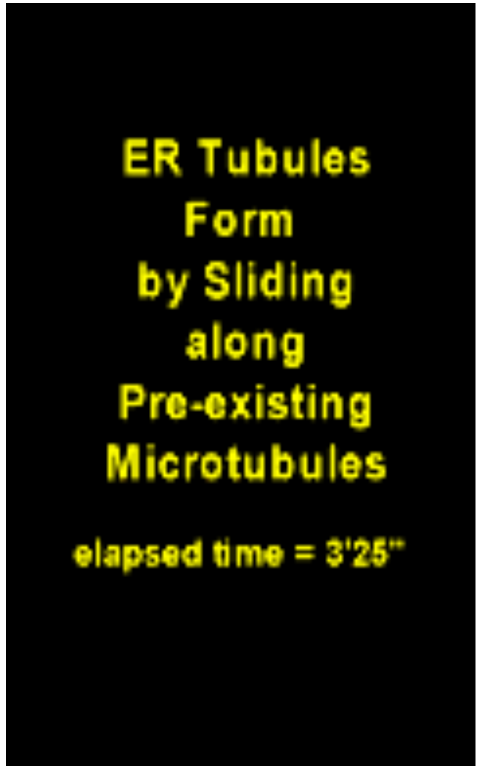
Endoplasmic Reticulum Dynamics

MT

ER



Lane & Allan, *MBC* (1999)

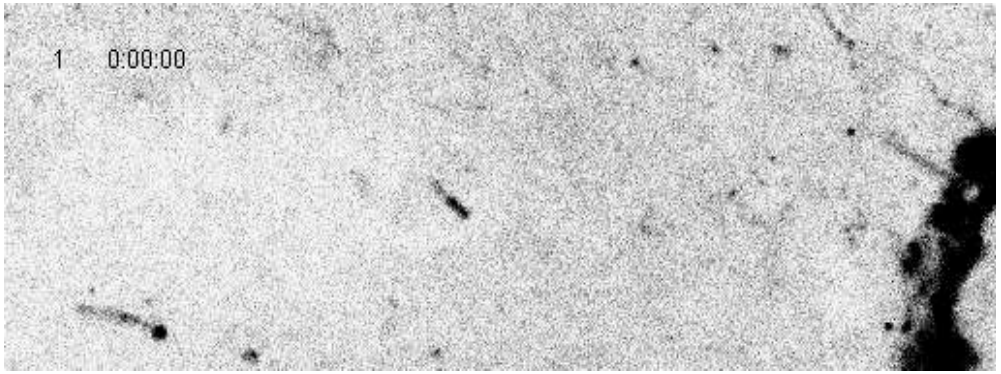


Waterman-Storer & Salmon, *Curr. Biol.* (1998)

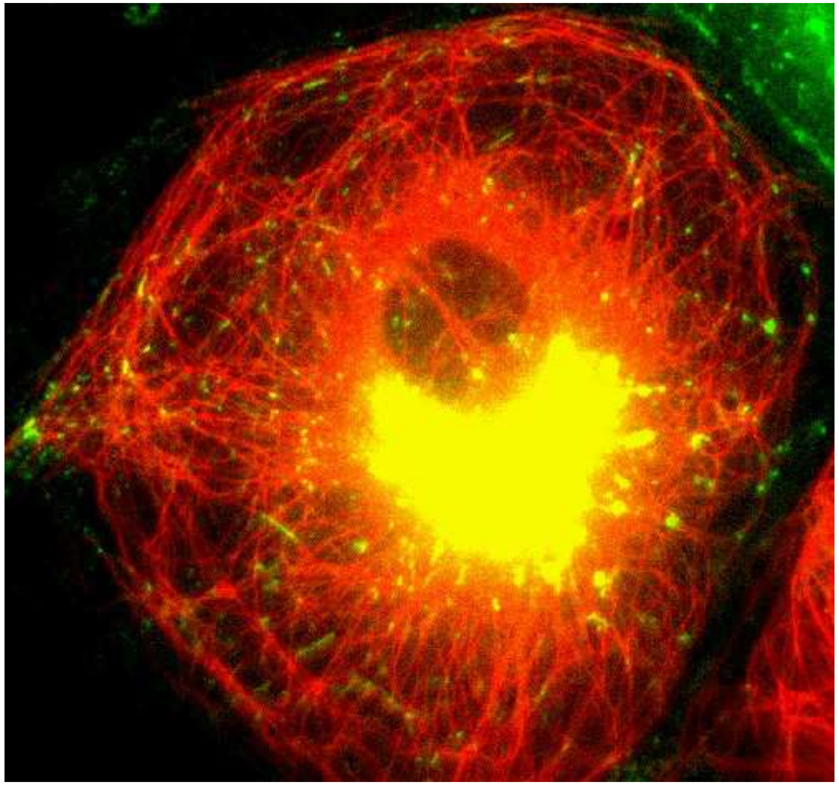
Microtubules

ER Membrane

Tubular Transport Intermediates

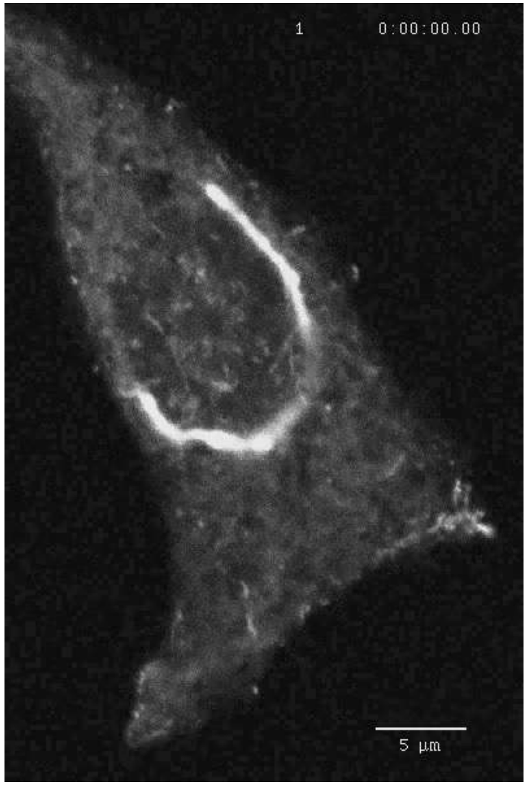


VSVG-GFP *J. Lippincott Schwartz (CBMB-NIH)*



Toomre et al
J. Cell Sci. (1999)

Golgi



Trafficking of Rab6 in HeLa cell

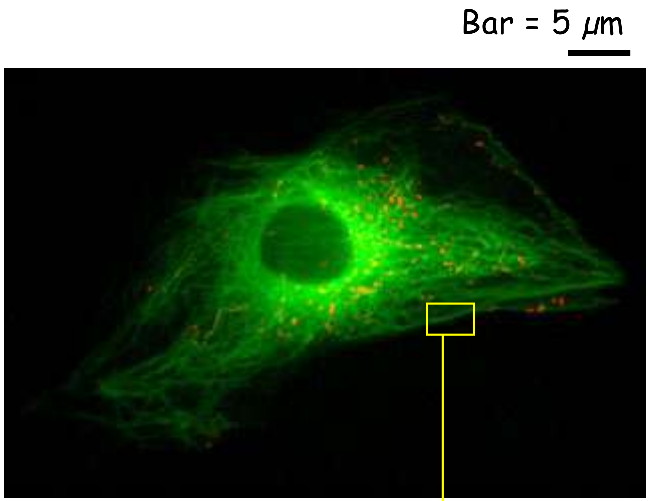
White & al. *JCB (1999)*

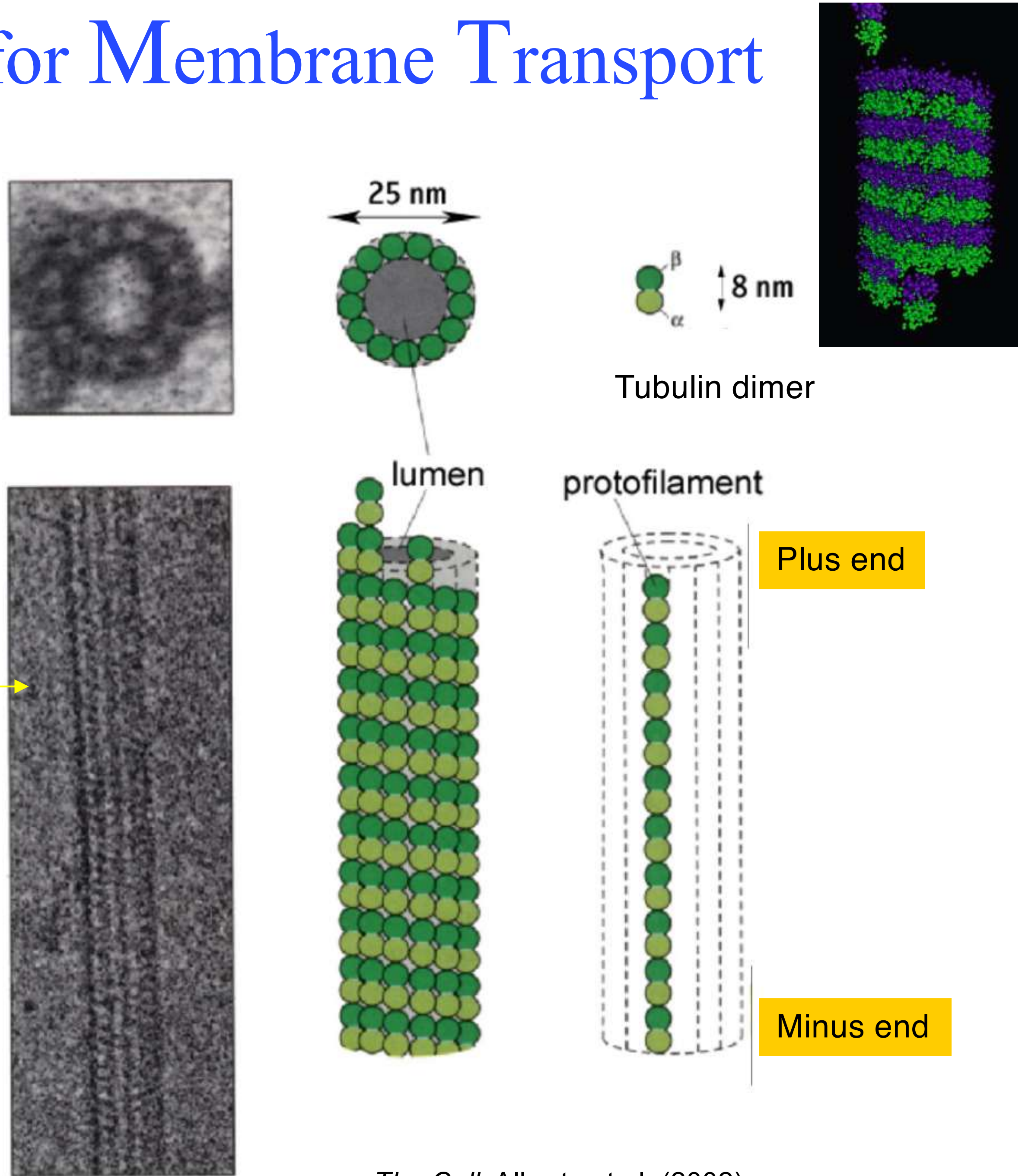
No Microtubules
or Motor (kinesin) inhibition

⇨ NO TUBE

⇨ **Required :**
Microtubules + Motors

Microtubules: Rails for Membrane Transport





- Tubulin dimers self-assembled in *parallel protofilaments*
- *Hollow* rigid cylinders
- Dynamics: growth by + end but *fast depolymerization* (catastrophy)

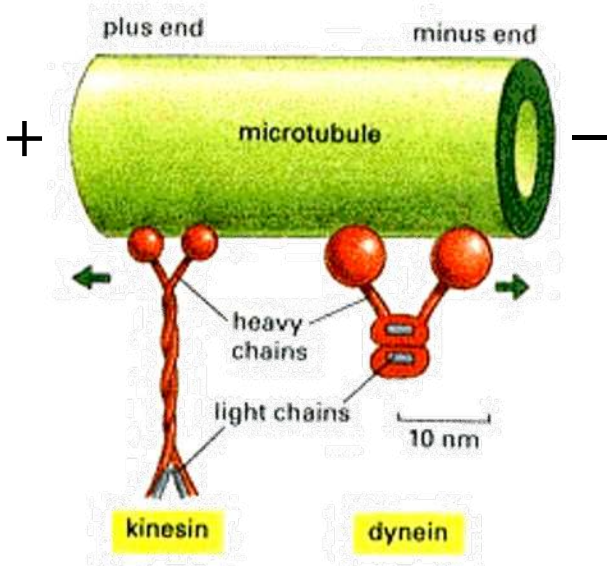
The Cell, Alberts et al, (2002)

Molecular Motor Toolbox for Transport

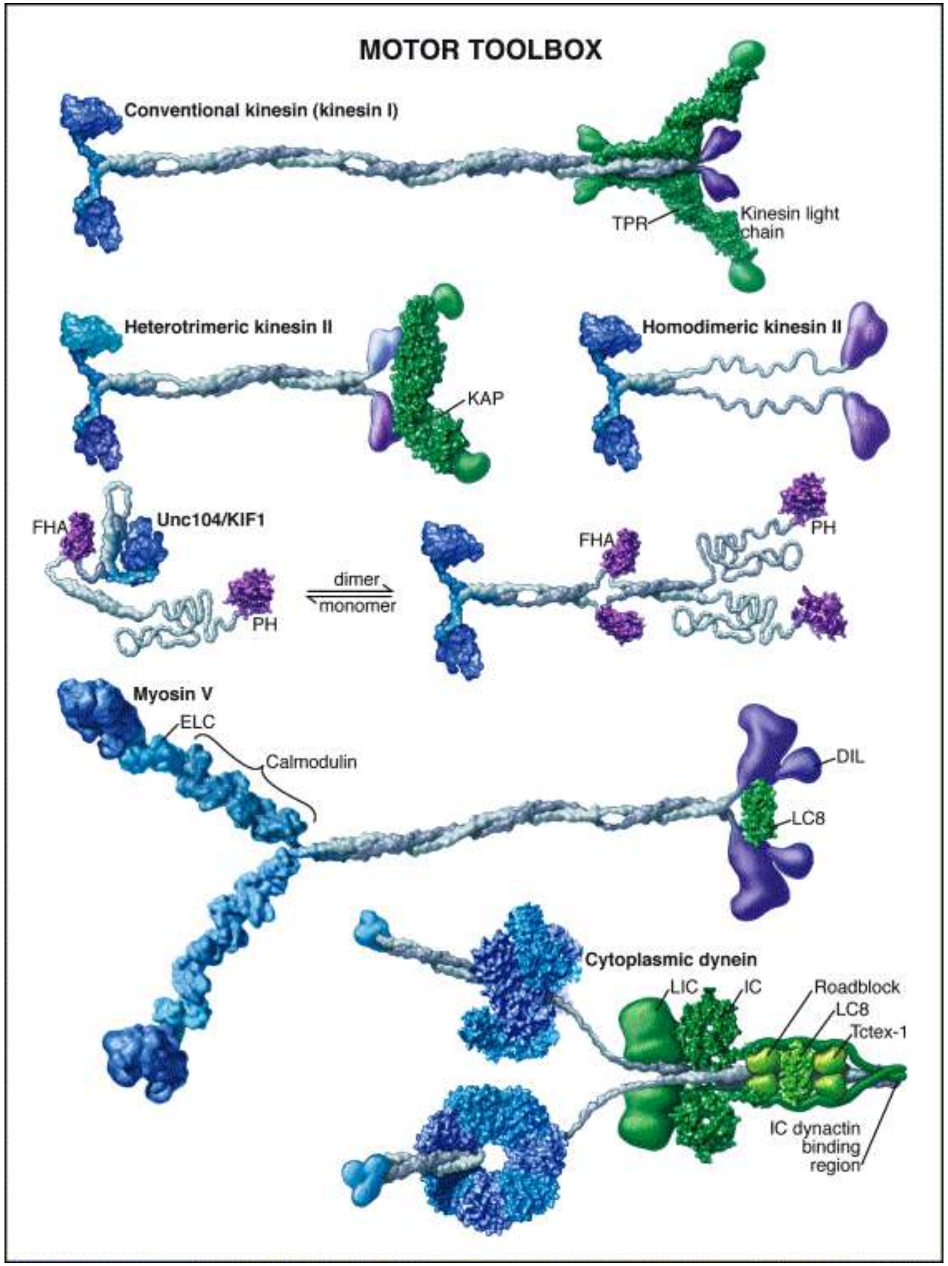
3 motor families

- Kinesins
- Dyneins

➡ Microtubules

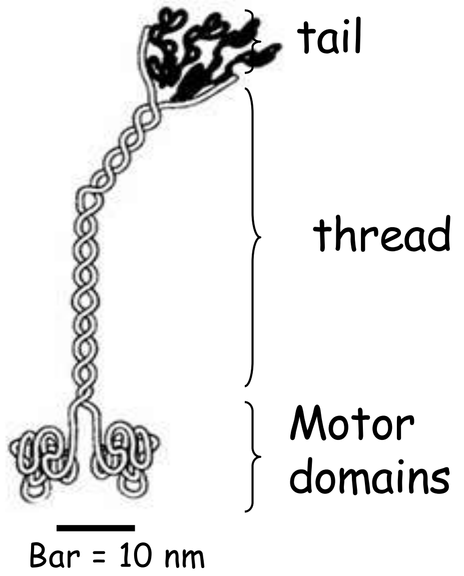
- 

- Myosins ➡ Actin

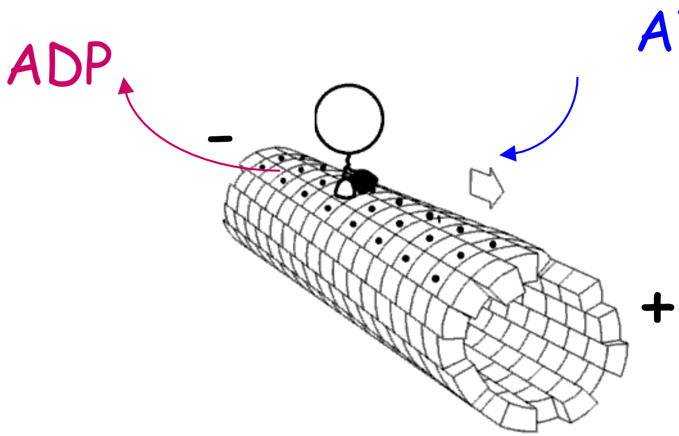


R. Vale, Cell (2003)

Kinesin-1 (Conventional): the best characterized kinesin



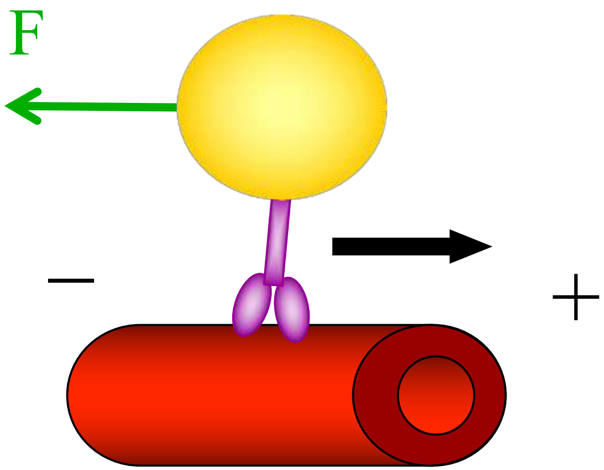
Movement along a Single Protofilament



Linear displacement

Processive motor: ≈ 100 steps before detachment

Kinesin Dynamics



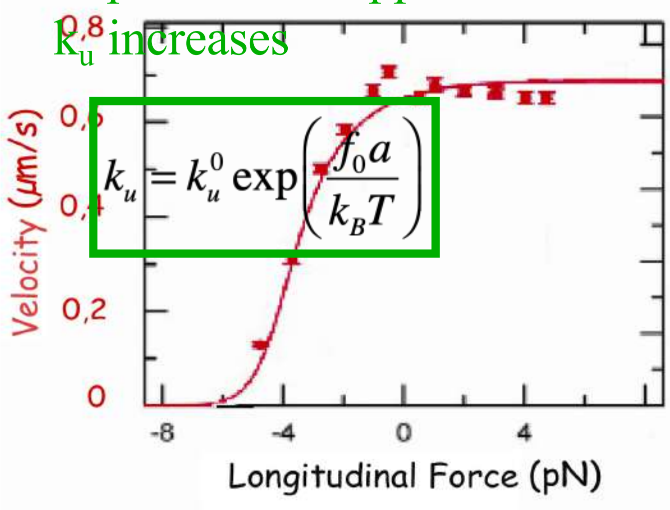
Bead assay

V_0: velocity of kinesin in absence of external load

$V_0 = 0.6 \pm 0.1\ \mu m/s$

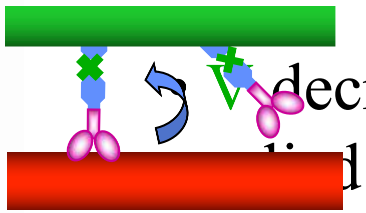
In presence of applied force

k_u increases



$$k_u = k_u^0 \exp\left(\frac{f_0 a}{k_B T}\right)$$

Block et al., *PNAS* (2003)



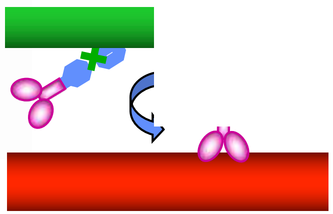
k_u^0: unbinding rate

V decreases with load

$k_u^0 = 0.42\ s^{-1}$

Vale et al., *Nature* (1996)

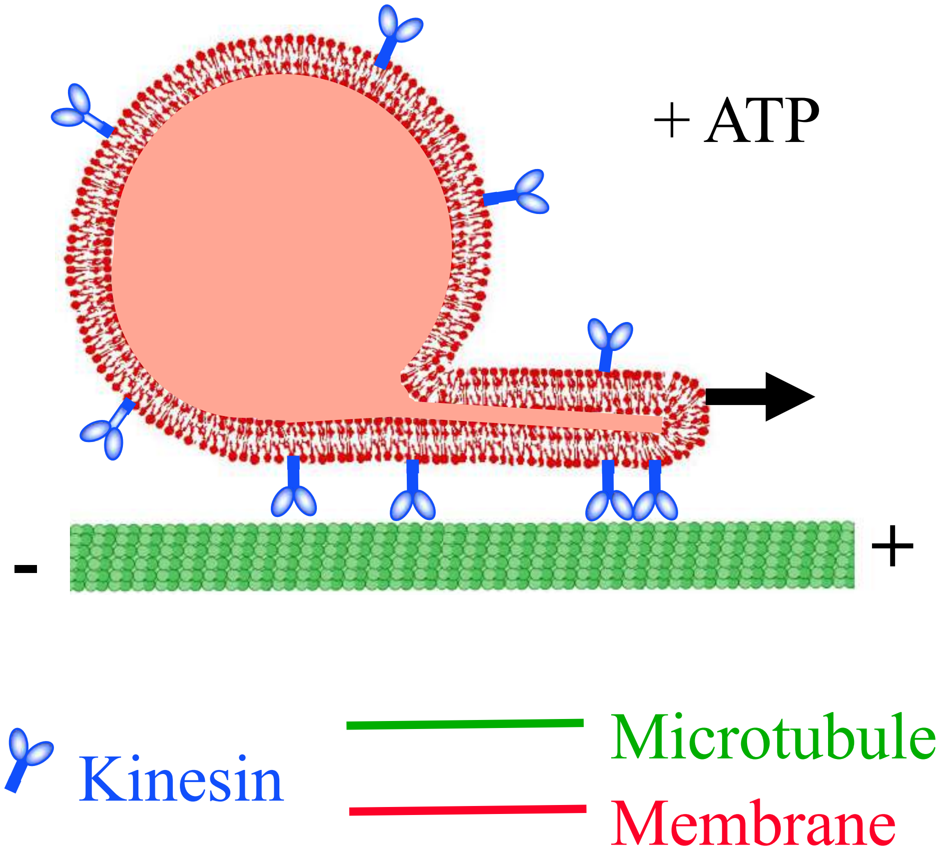
• Stall force:

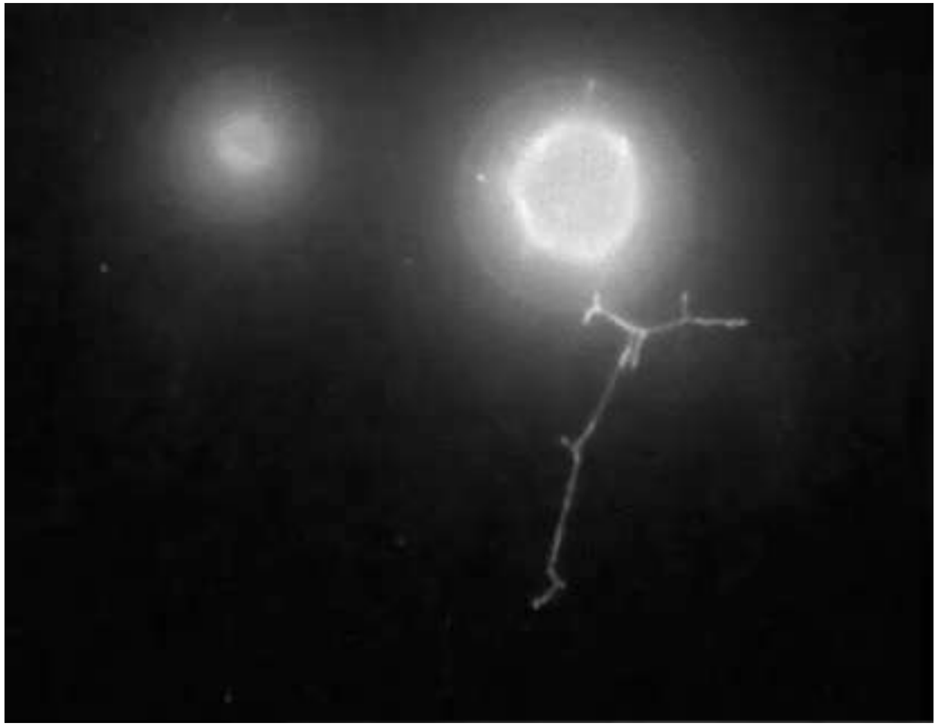


$F_S = 6\ pN$

kinesin onto MT

Minimal Biomimetic System



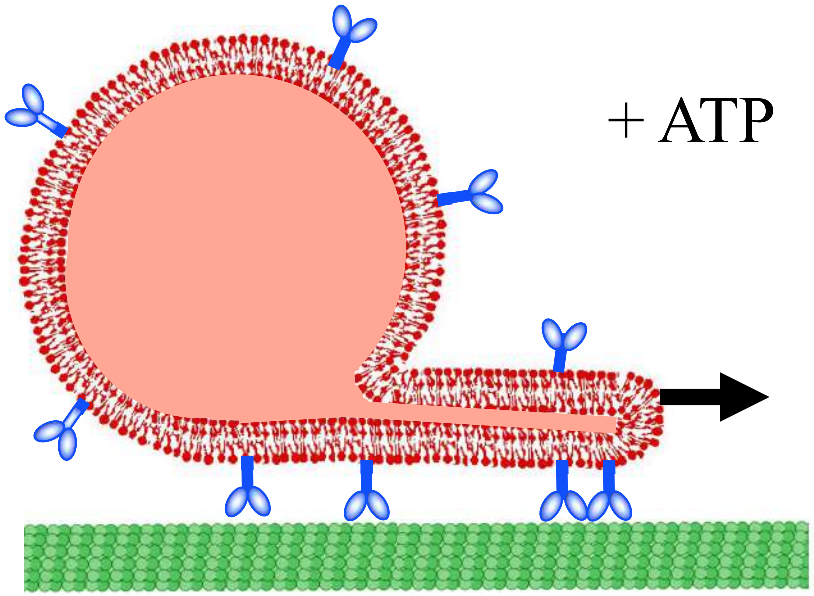


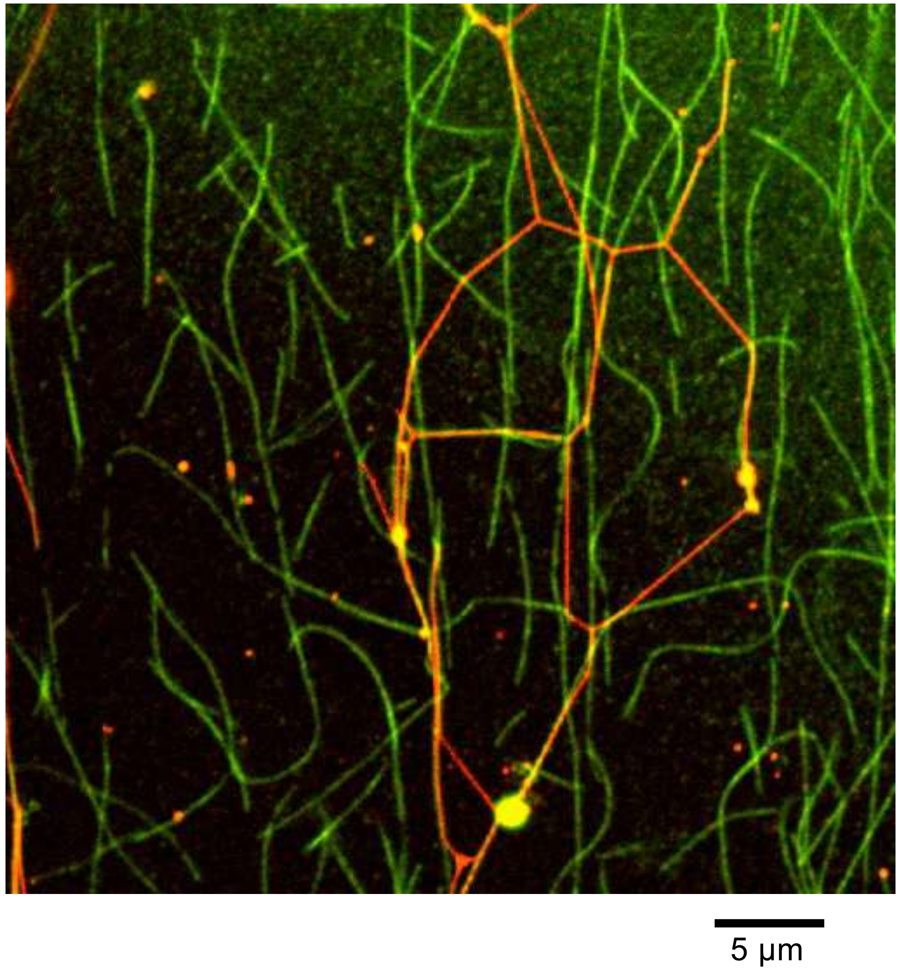
C. Leduc et al *PNAS* (2004)

X40 accelerated

A. Roux et al, *PNAS* (2002)
C. Leduc, O. Campàs et al, *PNAS* (2004)

Minimal Biomimetic System





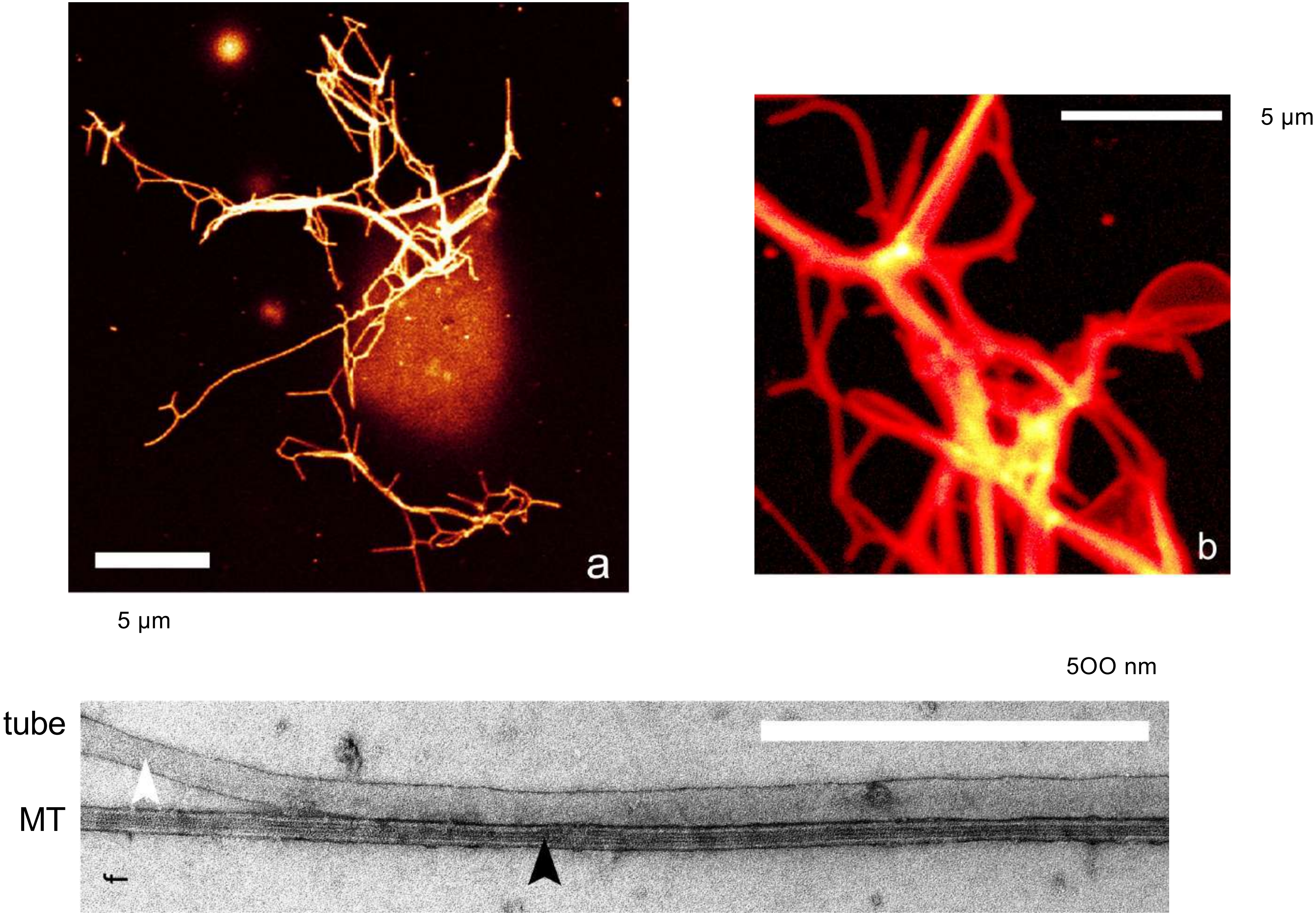
Kinesin

Microtubule

Membrane

A. Roux et al, *PNAS* (2002)

C. Leduc, O. Campàs et al, *PNAS* (2004)

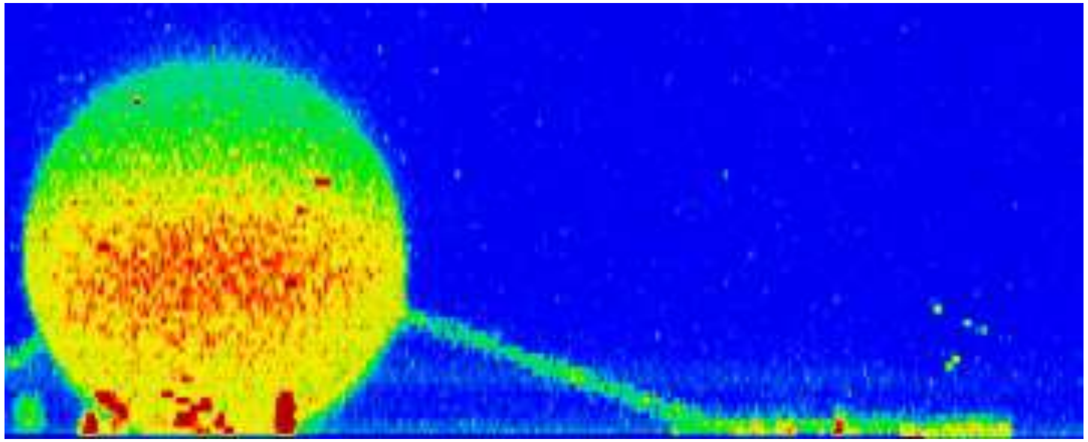


Roux A. et al *PNAS* (2002)

10 μm

Membrane labeling

Side view



10 nm/s < V< 300 nm/s

for σ= 2.10^{-4} N/m

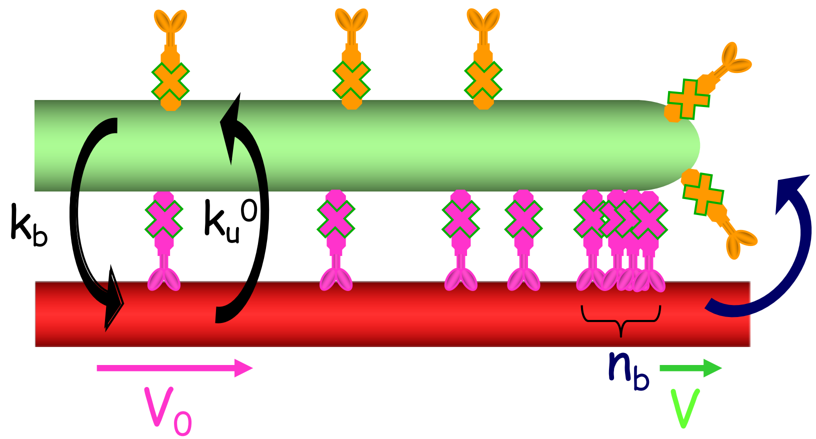
X40
accelerated

C. Leduc, O. Campàs et al, *PNAS* (2004)

Mechanism for Tube Formation by Molecular Motors

- With our optical tweezers: F=30 pN for pulling these tubes
- Force max/1 motor = 6 pN

Motors have to work *COLLECTIVELY* to pull these tubes



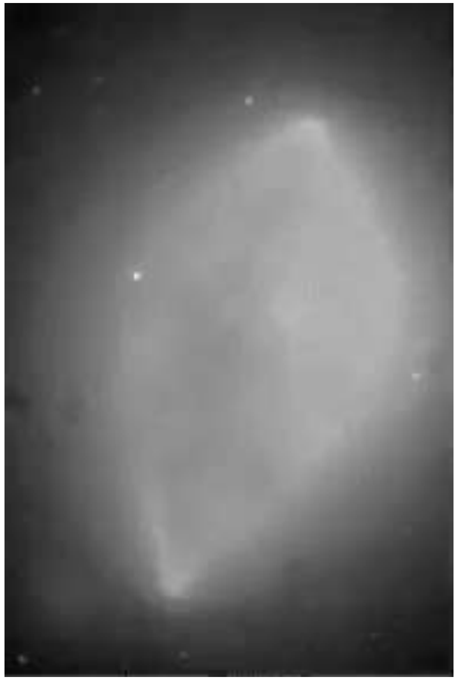
- Full theoretical model describing motor fluxes

(O. Campàs, JF Joanny, J. Prost)

G. Koster ….. M. Dogterom *PNAS* (2003)
C. Leduc, O. Campàs et al, *PNAS* (2004)

Conditions for Tube Extraction

• Fixed **motor concentration ρ_∞** :



High tension

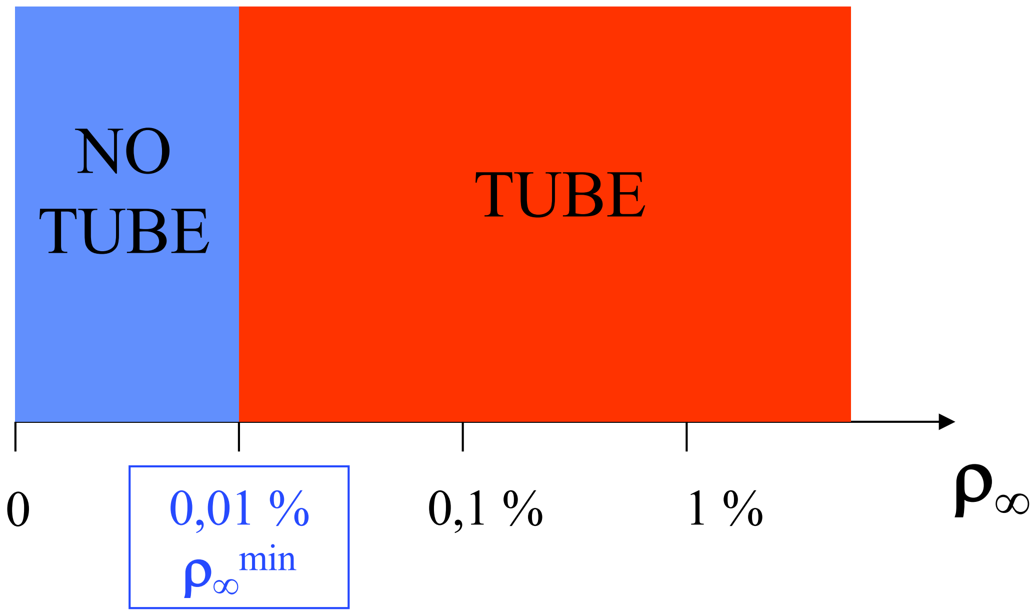
↘ σ , ↘ F_0

Lower tension

Tension Threshold for a given motor concentration

C. Leduc, O. Campàs et al, *PNAS* (2004)

• Fixed **membrane tension σ**



<u>Quantitative measurements</u>

For $\sigma = 2.10^{-4}$ N/m,

$\rho_\infty^{min} = 200$ motors/μm^2

Threshold in motor concentration for a given tension

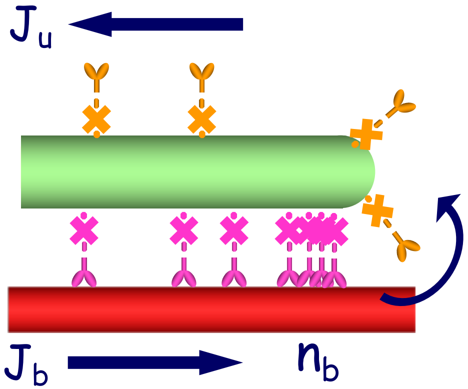
• **Theoretical analysis** effectively predicts:

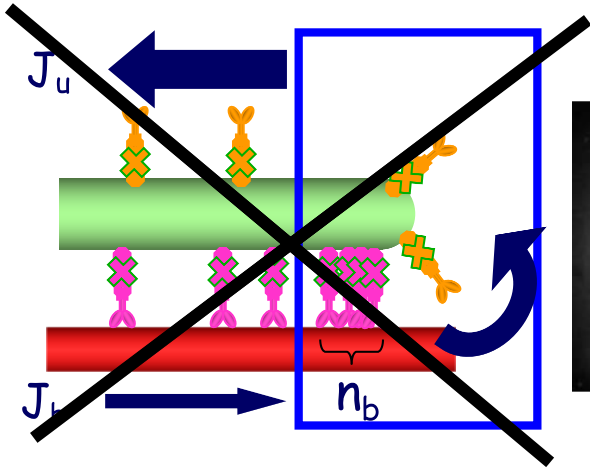
$$\rho_\infty^{min} = \text{cste} \ . \ \sigma^{max}$$

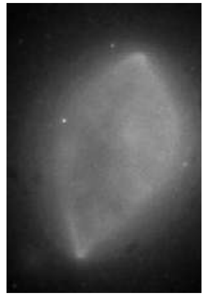
C. Leduc, O. Campàs et al, *PNAS* <u>101</u>, 17096 (2004)

Theoretical Analysis

O. Campàs, J.-F. Joanny and J. Prost

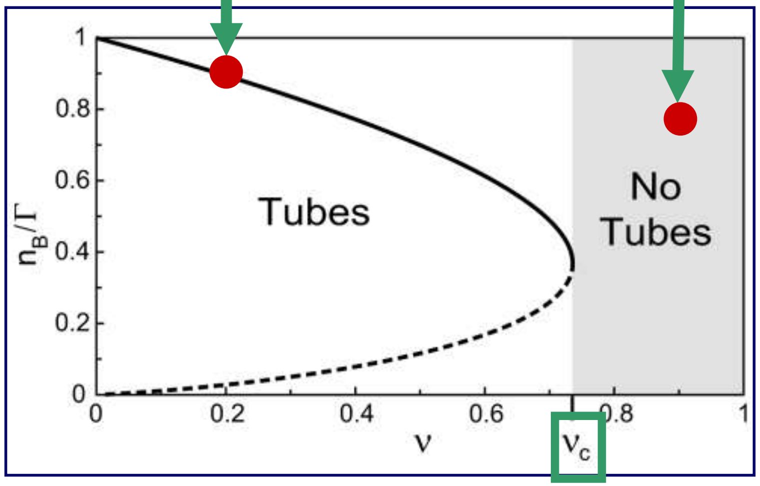






Analytical solutions

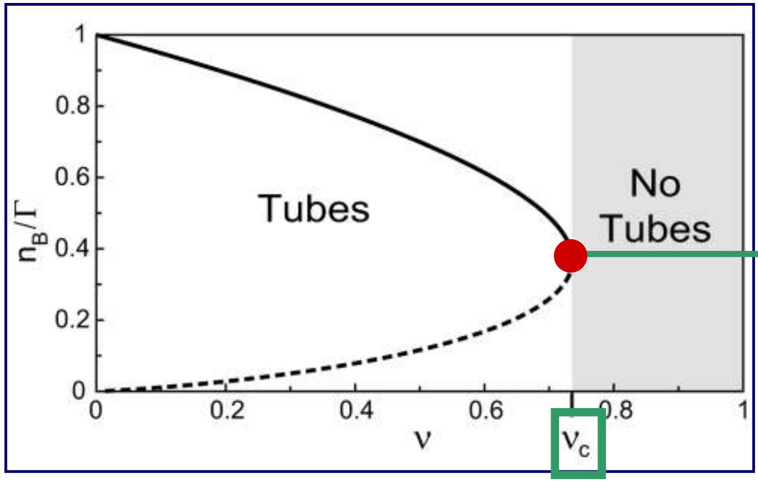
Bifurcation diagram



$$\nu \sim \sqrt{\frac{\sigma}{\rho_\infty}}$$

C. Leduc, O. Campàs et al, *PNAS* 101, 17096 (2004)

Condition for Tube Formation at the Threashold



$$\rho_\infty > \frac{e^2}{2} f_S \left(\frac{a}{K_B T}\right)^2 \frac{k_b + k_u^0}{k_b} \frac{k_u^0}{V_0} \equiv \rho_\infty^{min}$$

$$n_b^{\ min} = \frac{f_0 a}{2K_B T}$$

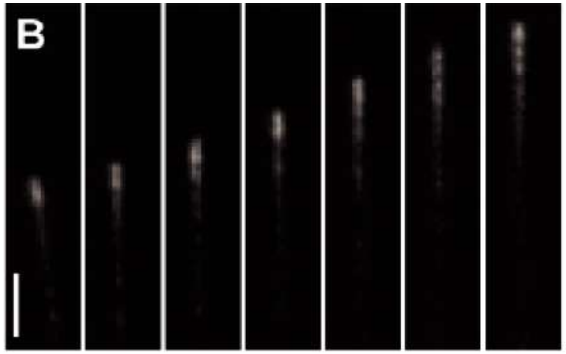
At the threashold:

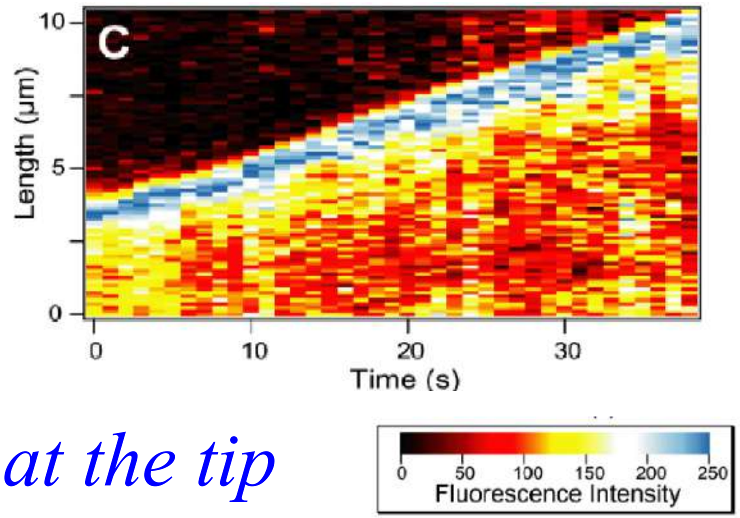
$\rho_\infty^{min,\,th} = 400 \pm 200$ motors/μm^2 — Theory

$\rho_\infty^{min,\,exp} = 200 \pm 100$ motors/μm^2 — Experiments

$n_b^{min} \sim 5$ motors

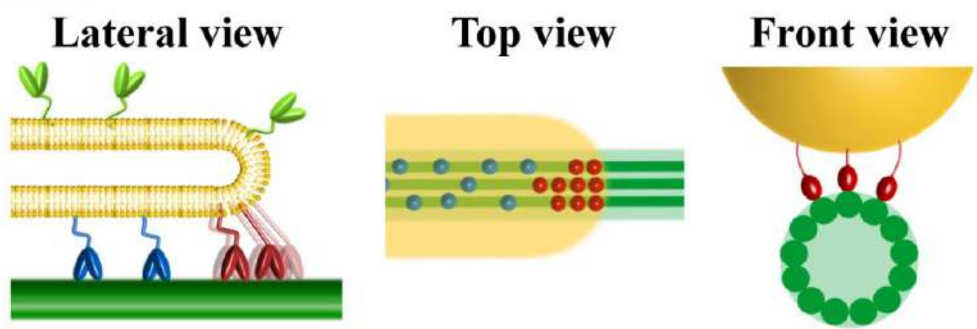
Motor Distribution along the Tubes





Motor accumulation at the tip

C. Leduc, O. Campàs et al, *PNAS* (2004)



9 motors pull

O. Campàs, C. Leduc et al, *Biophys. J.* (2008)

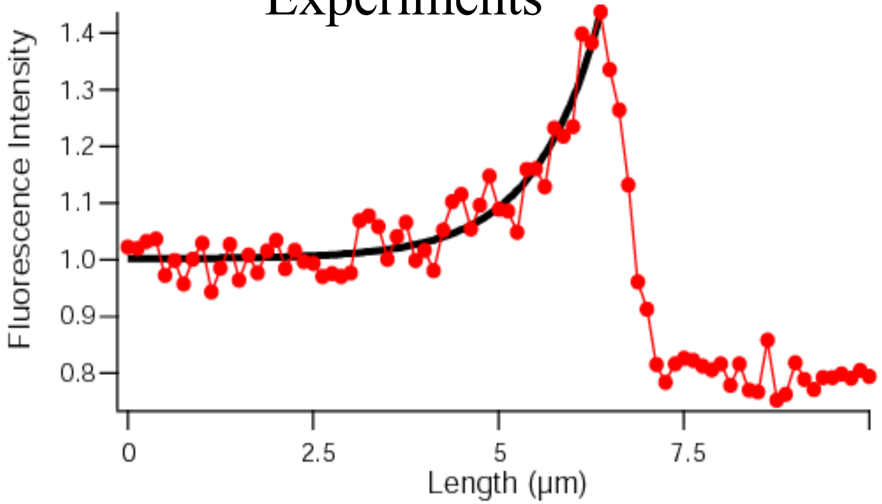
Motor labeling

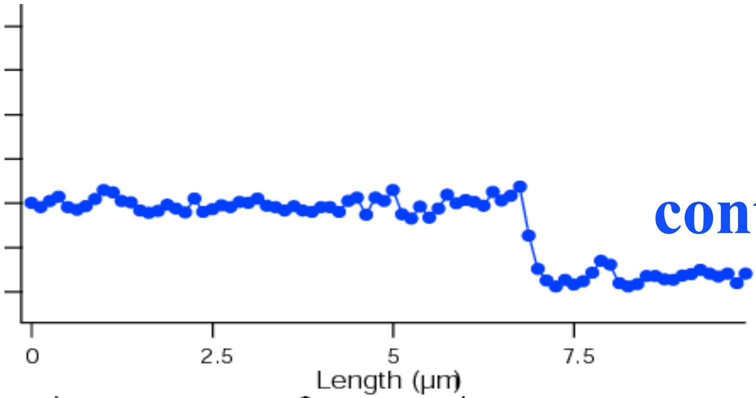
Bar : 1 µm

speed x 60

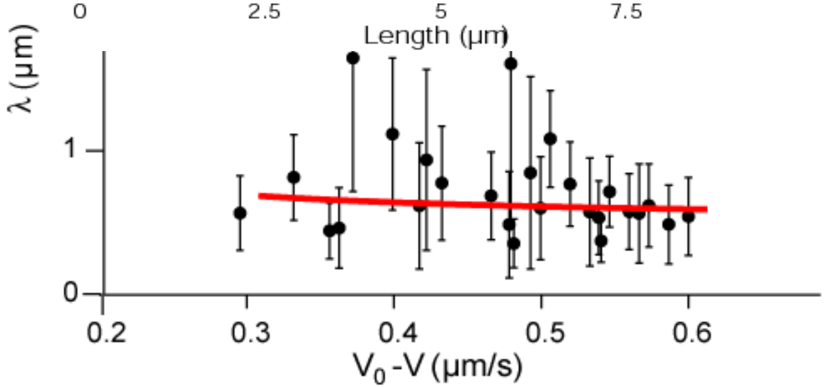
Experiments vs. Theory

Experiments





control



Theory

Exponential distribution

$$\lambda = \frac{k_u^0 D}{2k_B(V_0 - V)}\left(1 + \sqrt{1 + \frac{4k_B}{k_u^0}\frac{(V_0 - V)^2}{k_u^0 D}}\right)$$

With

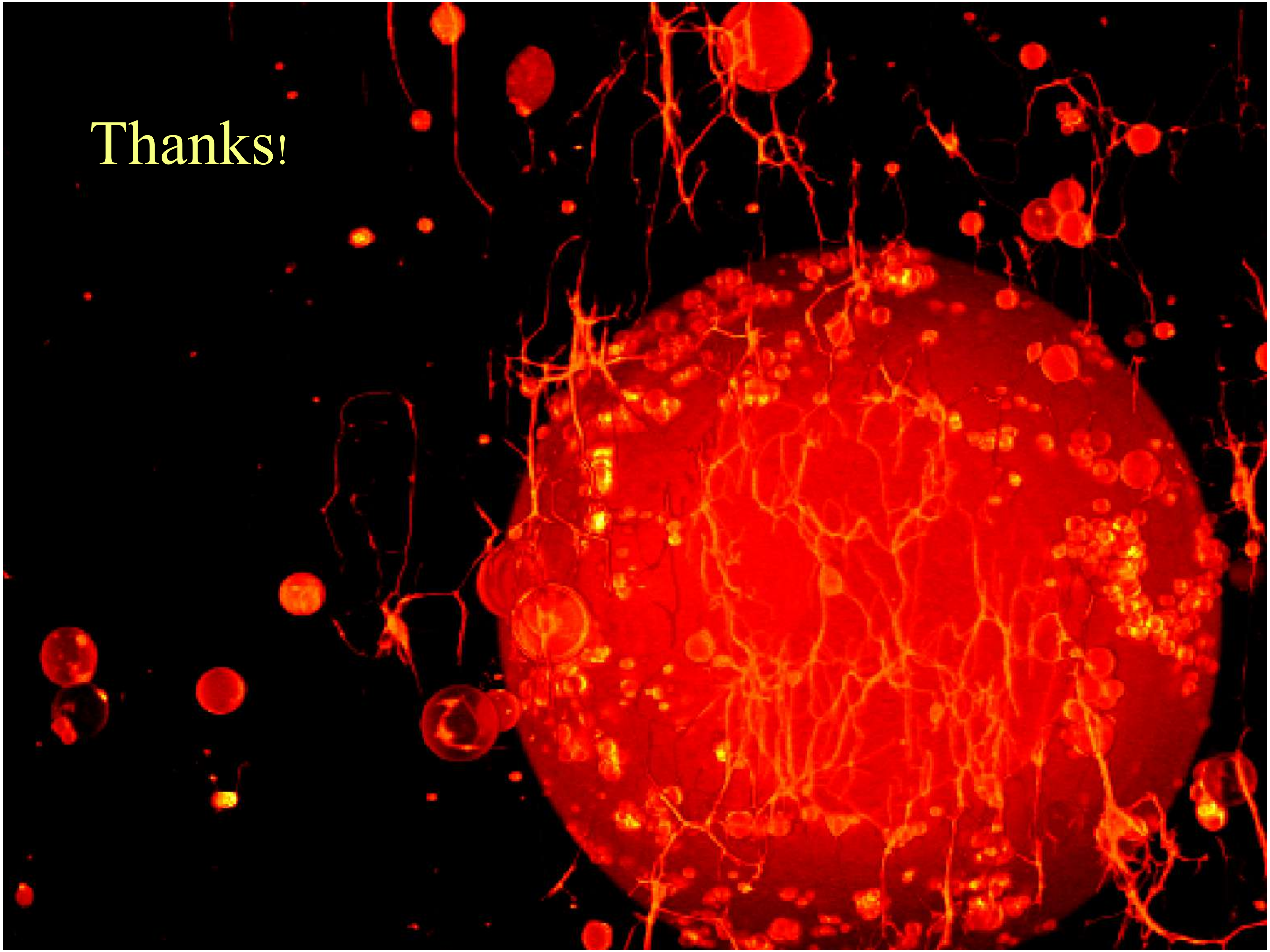
- $k^0_u = 0.42\ s^{-1}$
- $D = 1{,}0 \pm 0.5\ \mu m^2/s$ (FRAP)
- $V_0 = 0.6 \pm 0.1\ \mu m/s$

$n_B \approx 20$ motors

pulling the tube

One parameter fit: $\mathbf{k_b = 4.7 \pm 2.4\ s^{-1}}$

C. Leduc, O. Campàs et al, *PNAS* (2004)


Thanks!