Active Membranes: Where we are and Where should we go

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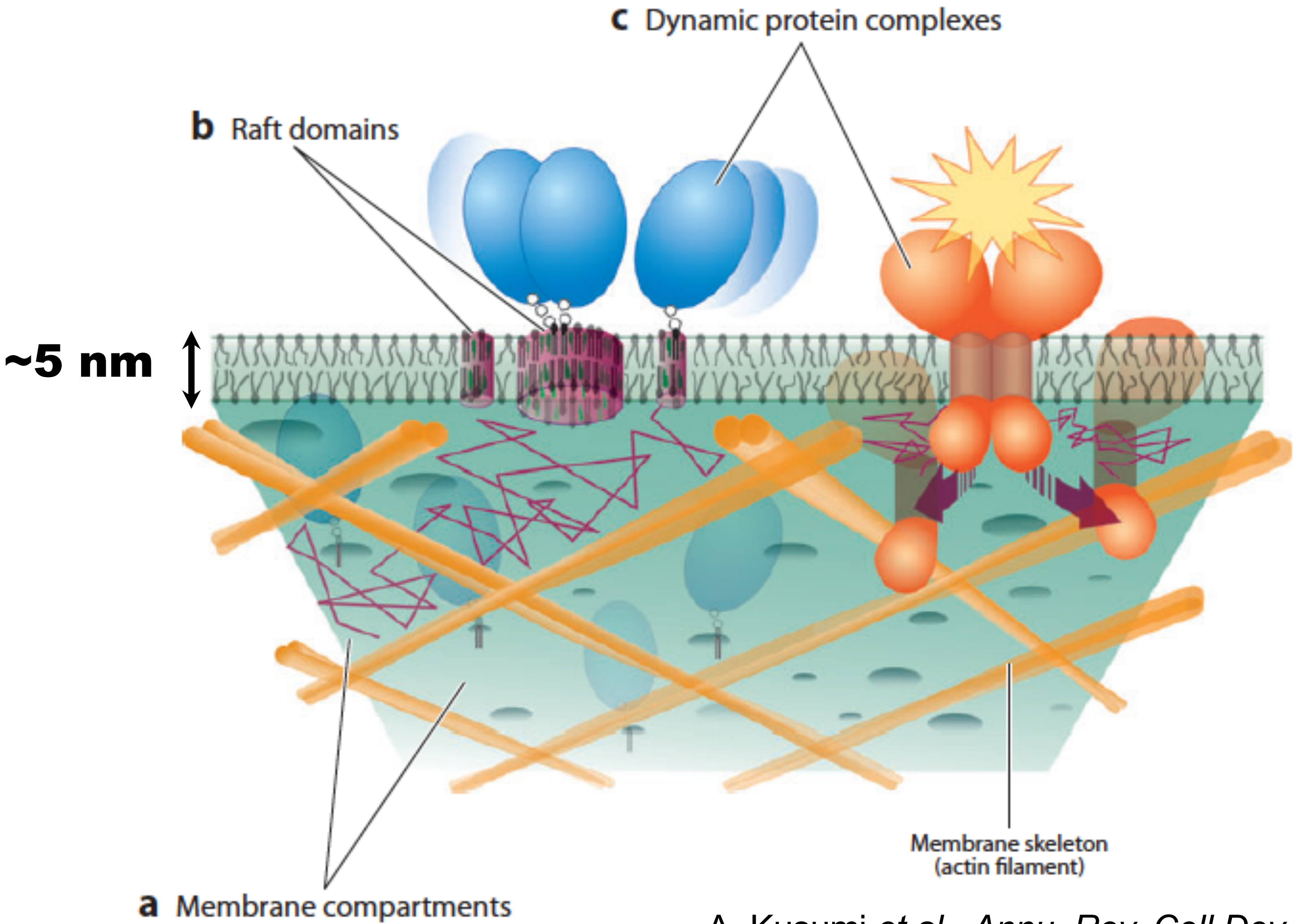
Institut Curie

Cell Plasma Membrane

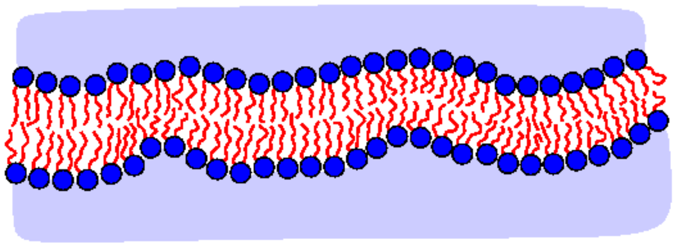


A. Kusumi *et al.*, *Annu. Rev. Cell Dev. Biol.* (2012)

Membrane: *Fluid* (viscosity ~ 100 times water)
+ many membrane proteins

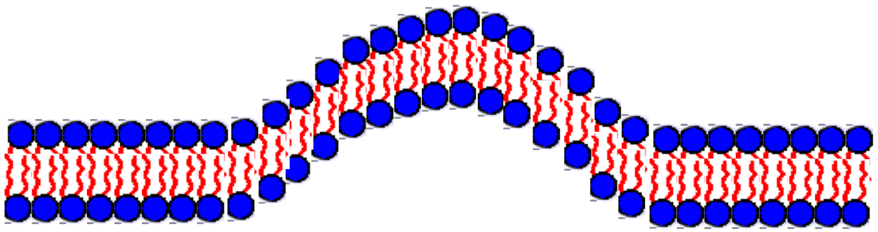
Confinement due to cortical actin filaments

Physics of Fluid Membranes



- Membrane mechanical properties described by 2 parameters:

κ: Bending rigidity modulus

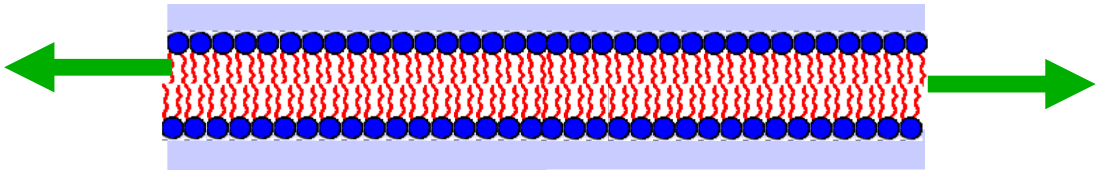


$$f_{bending} = \frac{1}{2}\kappa C^2$$

C: Mean curvature

W. Helfrich, *Zur Naturforschung* **28c**, 693 (1973)

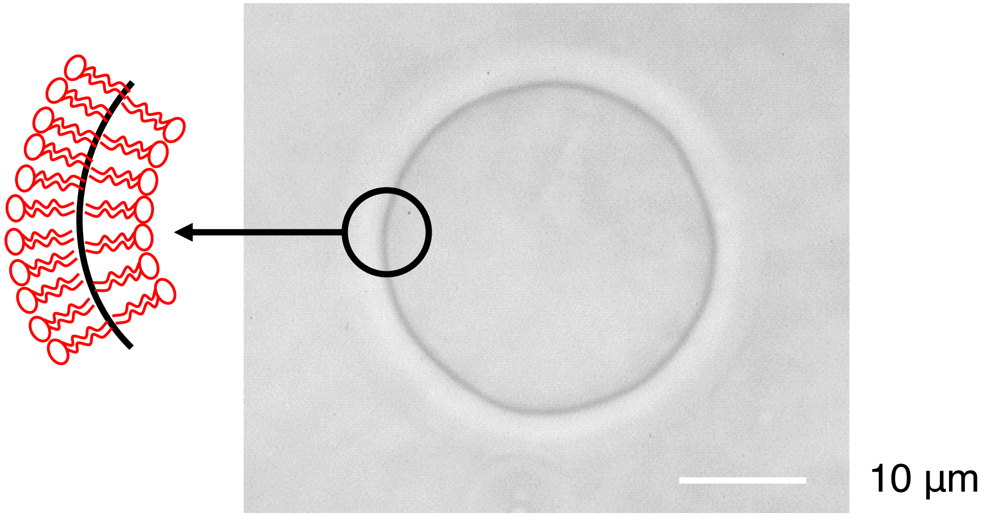
σ: Membrane tension

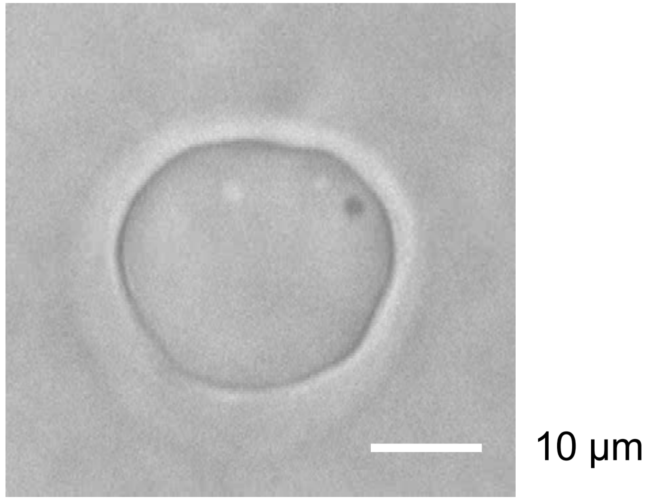


$$\sigma = \frac{\partial H_s}{\partial A}$$

Membrane Fluctuations

κ: from a few k_BT to ≈ 60 k_BT

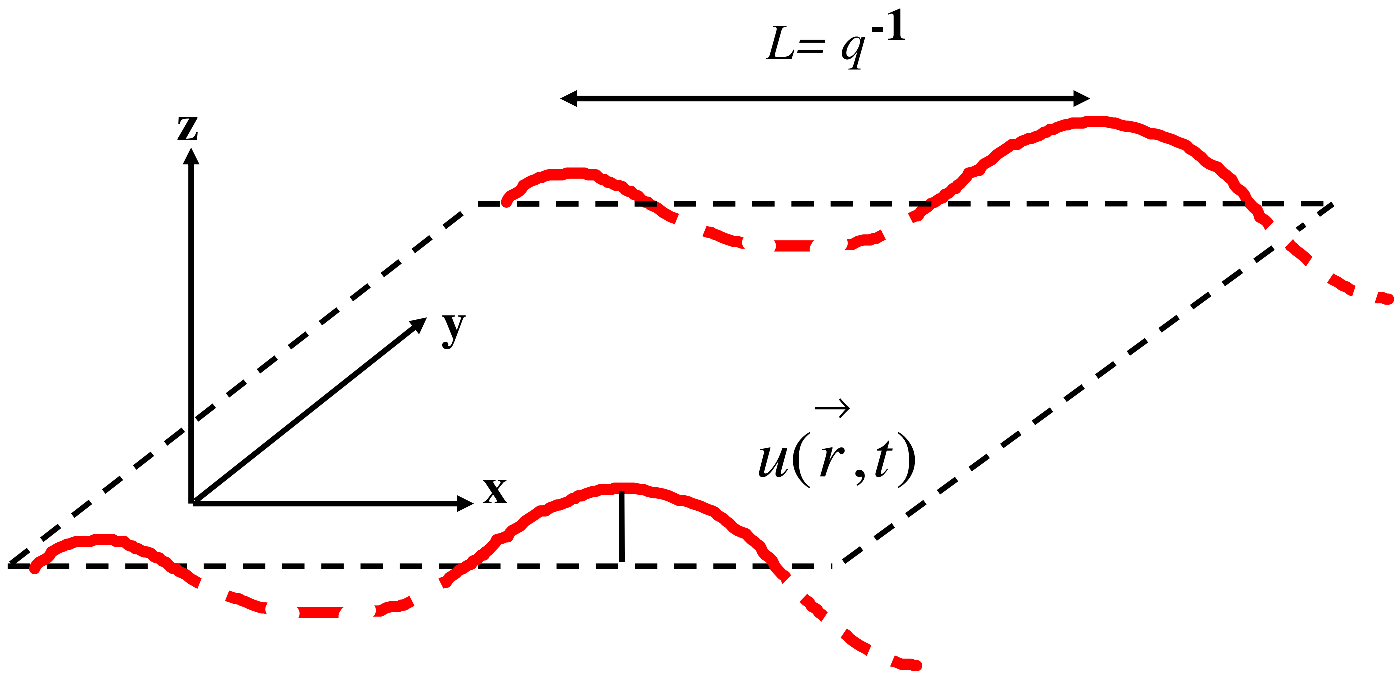




Optically visible membrane fluctuations, *thermally induced*

See Petia Vlahovska's talk

Membrane Fluctuation Spectrum

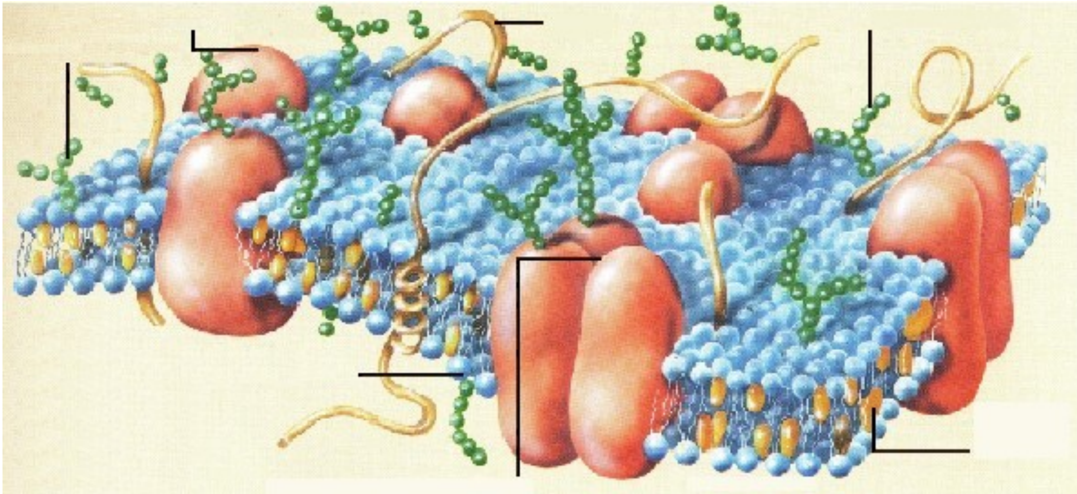


"Thermal noise" only (at equilibrium)

$$\left\langle \left| u(\vec{q}\,) \right|^2 \right\rangle = \frac{k_B T}{\sigma q^2 + \kappa q^4}$$

W. Helfrich, R.M. Servuss
Il nuovo Cimento 3**D** (1984)

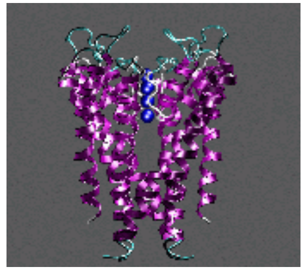
But, *Active* Transport of Ions through Membranes



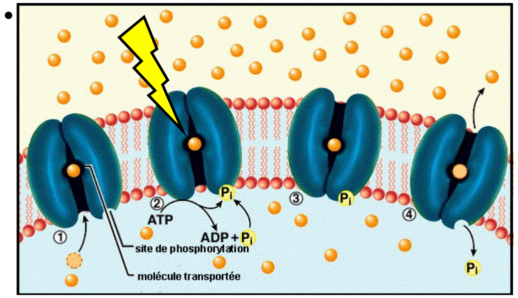
Lipid bilayer: quasi impermeable to ions

Transport of ions: Specialized proteins + source of energy

- Channels: Activable selective holes
  Fast response (1000 ions/ms) by gradient relaxation
  Muscle contraction, Action potential …
  Activated by: voltage, mechanical stretching…



- Pumps: Use energy to build up gradients
  Slow transport : 1 ion/ms
  Activated by: light, ATP hydrolysis...



1994 : Jacques Prost & Robjin Bruinsma @ KITP (Santa Barbara)

effect of the *non-equilibrium* activity of these proteins on the fluctuation spectrum?

EUROPHYSICS LETTERS 1 February 1996

Europhys. Lett., **33** (4), pp. 321-326 (1996)

Shape fluctuations of active membranes

J. PROST[1,2] and R. BRUINSMA[3]

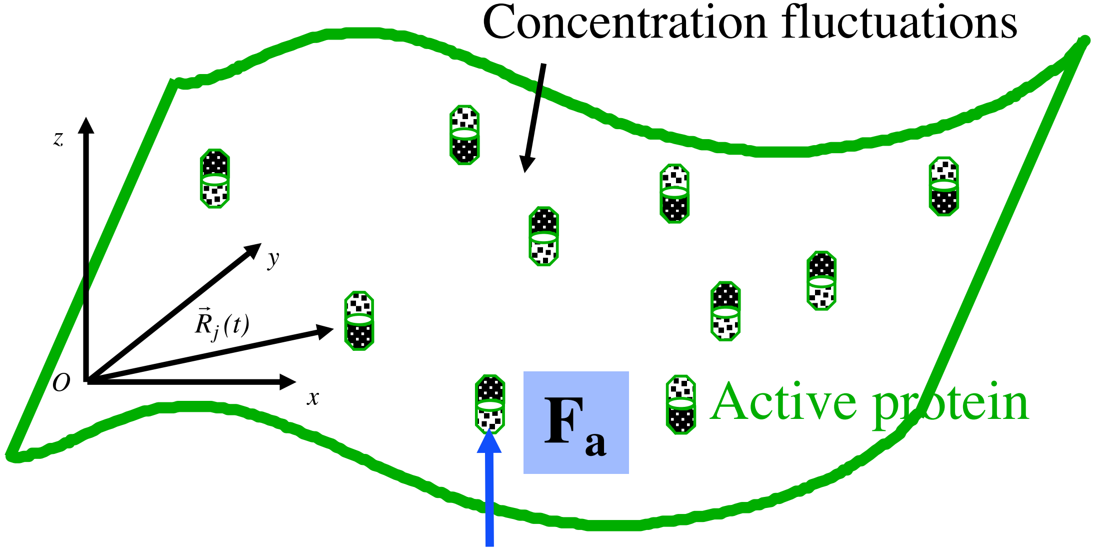
[1] *Institut Curie, Section de Recherche*
11 rue P. et M. Curie, 75231 Paris Cedex 05, France
[2] *ESPCI - 10 rue Vauquelin, 75231 Paris Cedex 05, France*
[3] *Physics Department, UCLA - Los Angeles, CA, 90024, USA*

Pioneer in a new area of biophysics (*active materials*)

Active Membranes: a Different Fluctuation Spectrum

J. Prost, R. Bruinsma *Europhys. Lett.*(1996)

Model initially for *ion channels*



F_a : active force

λ_P : permeation coefficient

η : solvent viscosity

ρ : pump/channel concentration

D: diffusion coefficient

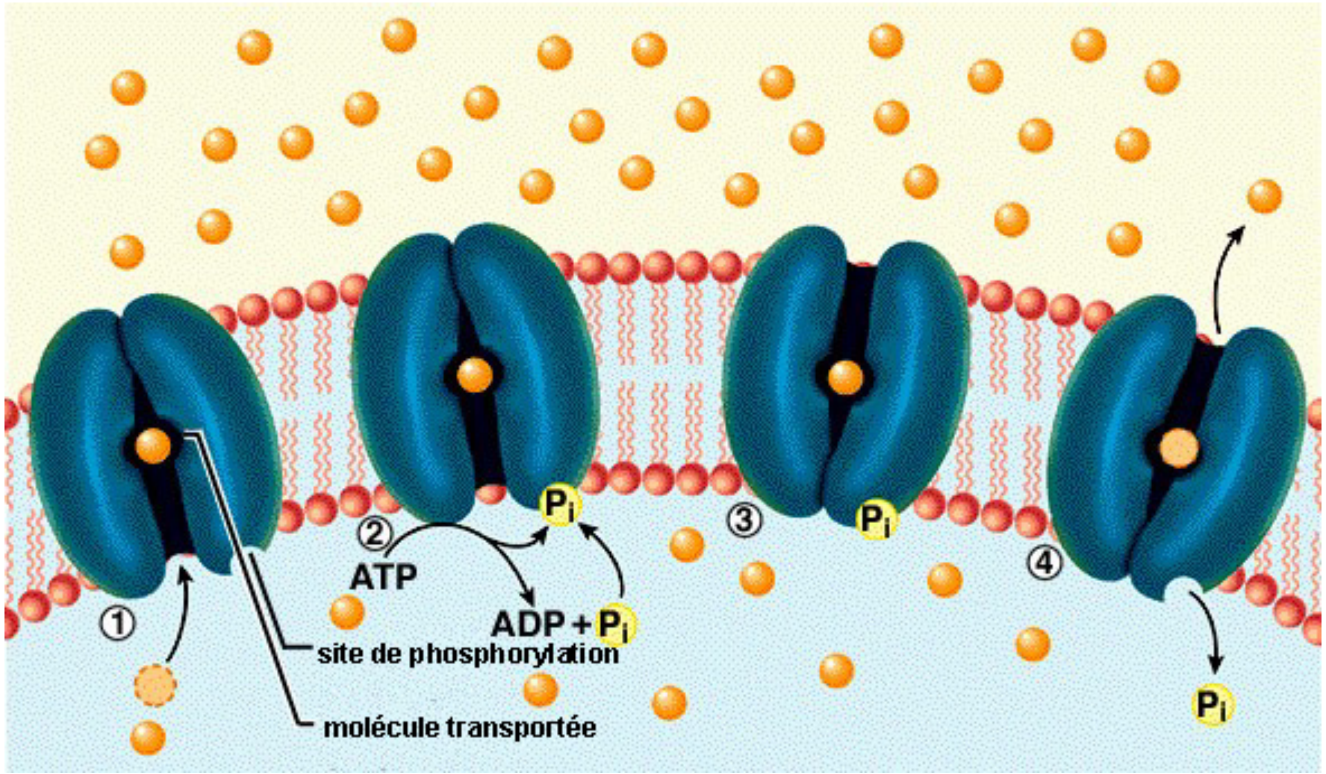
$$\sigma \longrightarrow 0 \qquad \left\langle |u(\vec{q})|^2 \right\rangle = \frac{kT}{\kappa q^4} + \frac{\rho \lambda_P^2 F_a^2}{\frac{\kappa q^3}{4\eta}} \times \frac{1}{\frac{\kappa q^3}{4\eta} + \frac{1}{4} D q^2}$$

Passive contribution

Active contribution

$$q \longrightarrow 0 \qquad \left\langle |u(\vec{q}_\perp)|^2 \right\rangle \approx \frac{\eta \lambda_P^2 F_a^2 \rho}{D\kappa} \frac{1}{q^5}$$

New spectrum + Fluctuation enhancement



Active Membranes

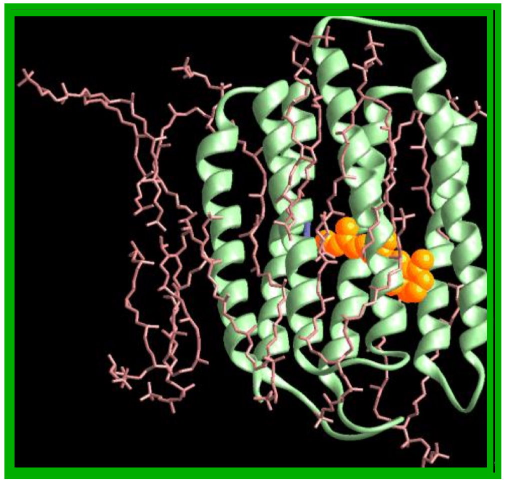
Jean-Baptiste Manneville, Philippe Girard, Jacques Pécréaux, Faris El Alaoui

Theory: J.F. Joanny, J.Prost (I.C.)
Coll: S. Ramaswamy (Bangalore), R. Bruinsma (UCLA),
J. Toner (Eugene), D. Lacoste (ESPCI, Paris)
Coll: J-L. Rigaud , D. Lévy (I.C.)
P. Falson, (CEA-Saclay), H.G. Döbereiner (MPI Golm), T. Salditt (Göttingen)

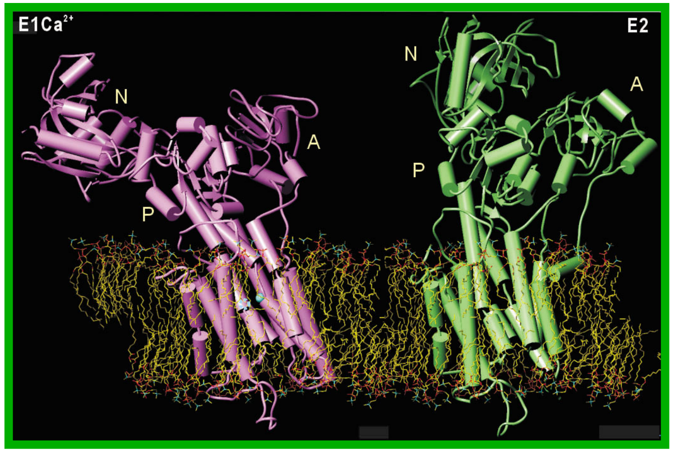
Experimental Systems

Coll: D. LévyJ.L Rigaud (Curie) - P. Falson (CEA Saclay

Ion pumps



or



Bacteriorhodopsin

H^+ pump

Activated by visible light (green)

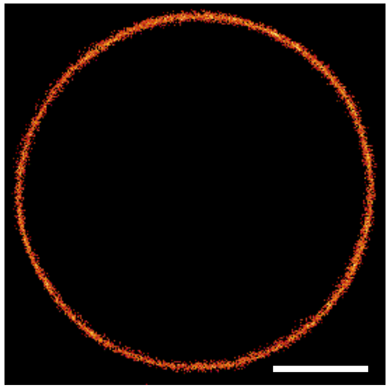
Ca^{2+}-ATPase (SERCA1a)

Ca^{2+} pump

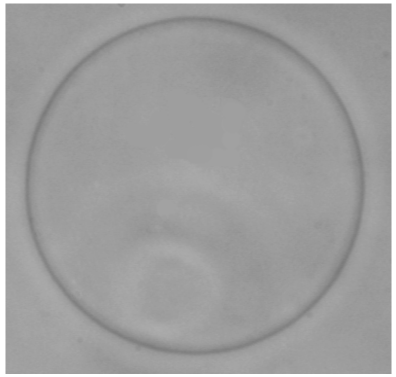
Activated by ATP hydrolysis

Reconstituted in lipid

Giant Unilamellar Vesicles



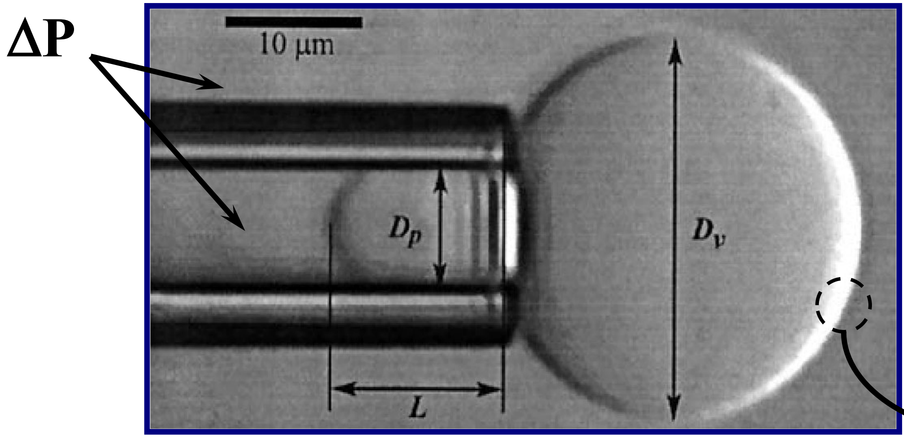
Ca^{2+}-ATPase 5 µm

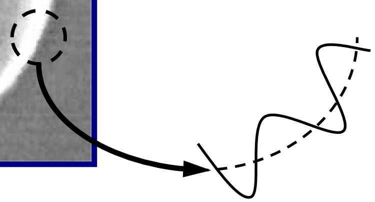


P. Girard et al *Biophys.J.* (2004)

Method

Micropipette aspiration (E. Evans) Kwok and Evans, *Biophys. J.* (1981)





Aspiration ΔP ⟷ Tension σ

Tongue length ΔL ⟷ Excess area Δα

At low tension, at equilibrium :

$$\ln\left(\frac{\sigma}{\sigma_0}\right) \cong \frac{8\pi\kappa}{k_B T}\Delta\alpha$$

Evans, E., W. Rawicz. *PRL* (1990)

Expected for active membranes (not possible to measure spectrum)

$$\ln\left(\frac{\sigma}{\sigma_0}\right) \cong \frac{8\pi\kappa}{k_B T_{eff}}\Delta\alpha$$

T_{eff} ⟷ Protein activity (F_A, λ_P)

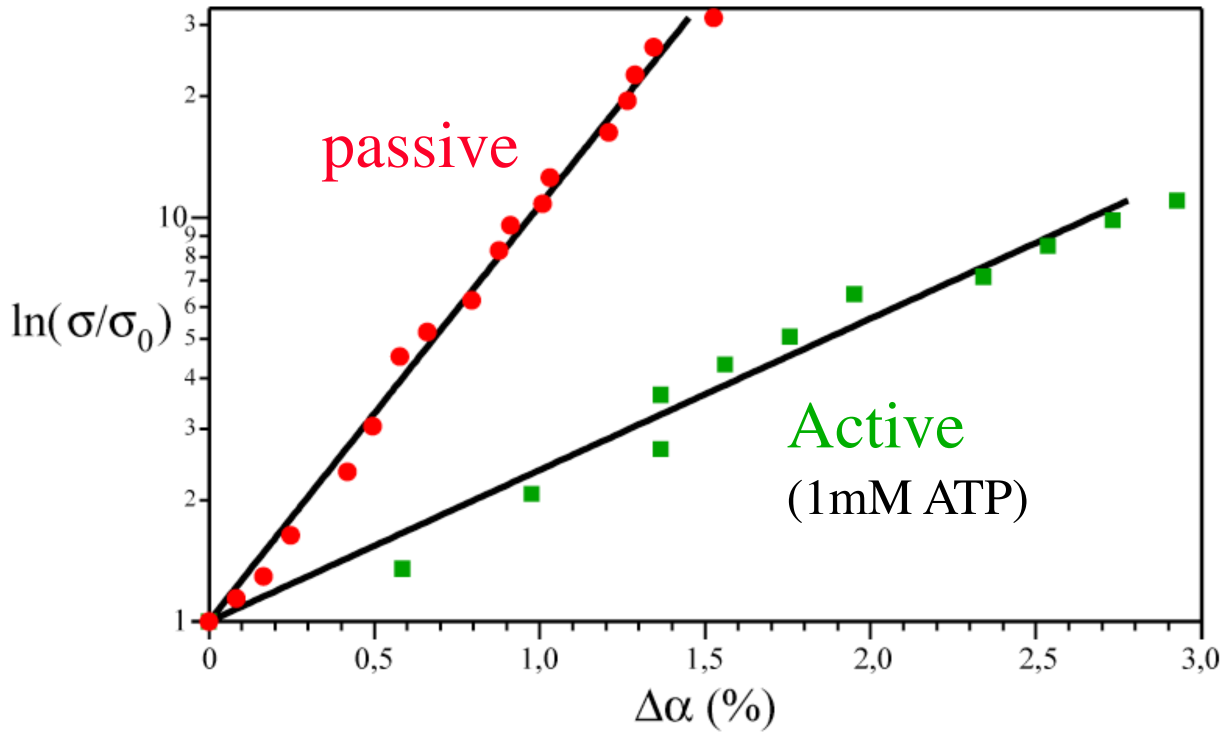
Effect of Protein Activity

Ca^{2+} -ATPase

Girard et al, *PRL* (2005)

$\phi = 3 \times 10^{15}$ m^{-2}

Slope: $\frac{8\pi\kappa}{k_B T}$ $\frac{8\pi\kappa}{k_B T_{eff}}$



• no ATP : **passive proteins**

$\frac{\kappa_p}{k_B T} = 9.4 \pm 0.3$

■ with ATP (1mM) : **active proteins**

$\frac{\kappa_p}{k_B T_{eff}} = 3.5 \pm 0.4$

$T_{eff} = 2.7$ T

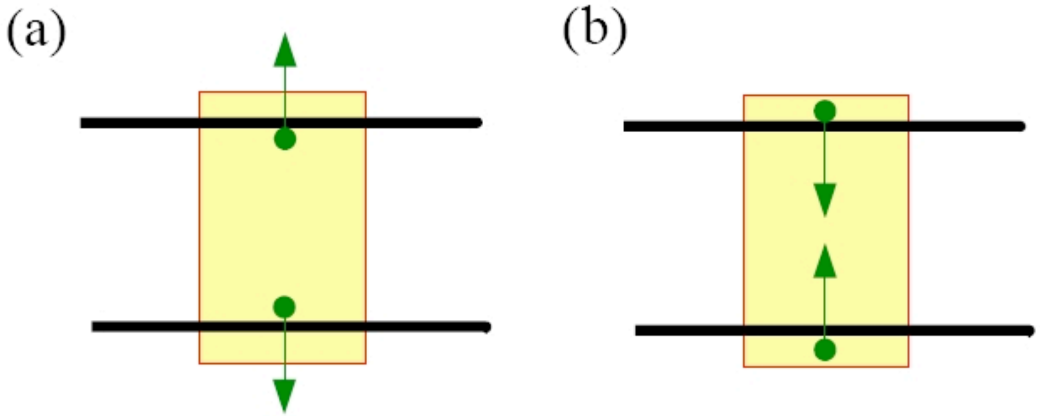
$T_{eff} \approx 1000$°K

Bacteriorhodopsin + light : **$T_{eff} = 2.0$ T**   Manneville et al, *PRL* (1999), *PRE* (2001)

Pump activity has a strong effect
on membrane fluctuations

- Linear term negligible for pumps (very low permeation)
- Consider the dipolar term

Force dipoles:



Change of conformation of the pump, but at that stage,
not possible to relate the molecular effect of activity and the force dipole

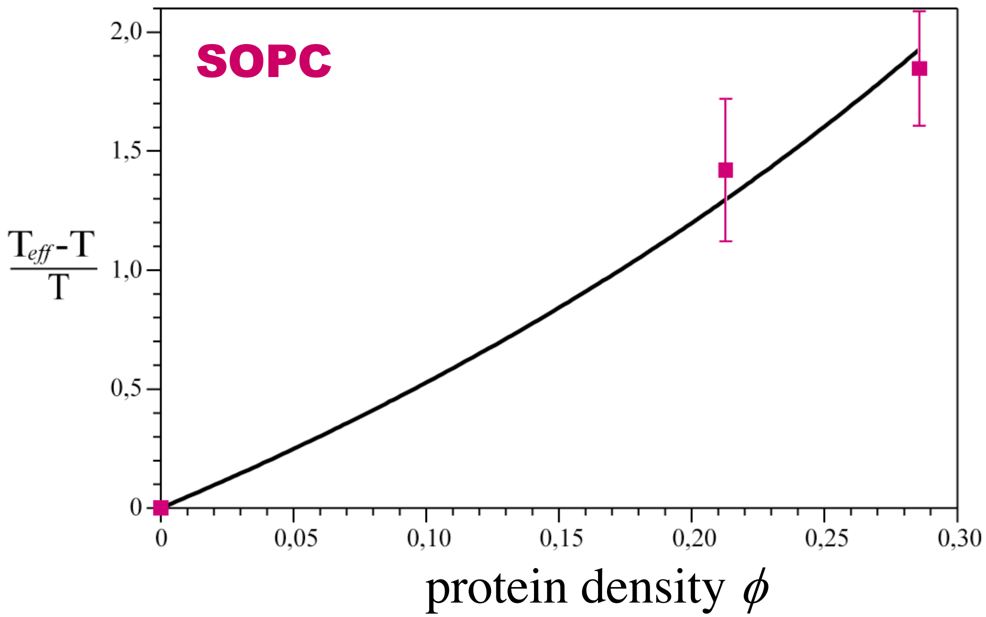
Manneville et al, *PRE* (2001)

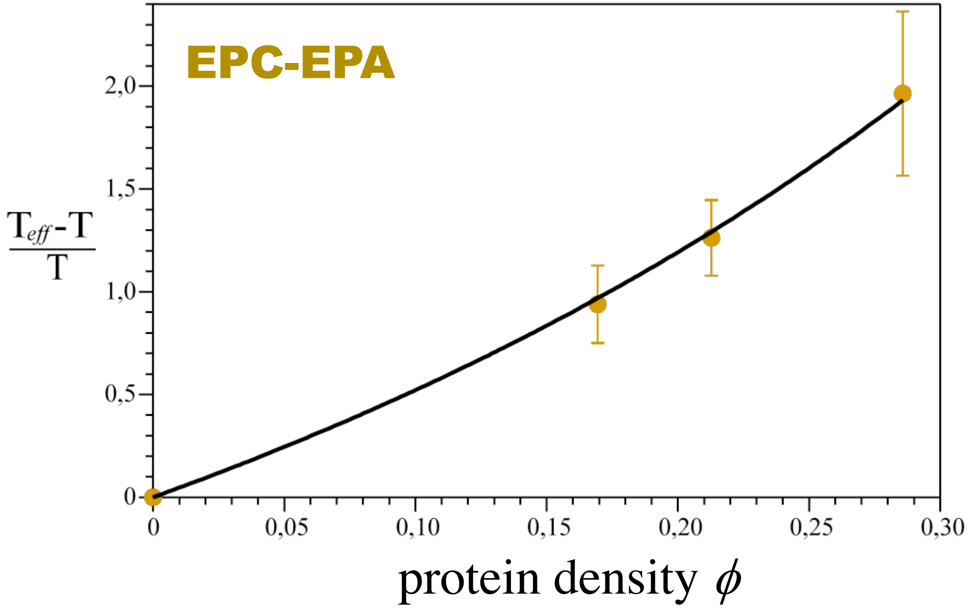
Theory : Activity ⟷ Force dipole ⟷ Effective temperature

$$\frac{T_{eff} - T}{T} = f(\mathcal{P}_a, \phi)$$

Manneville et al, *PRE* (2001)

Ca^{2+} -ATPase



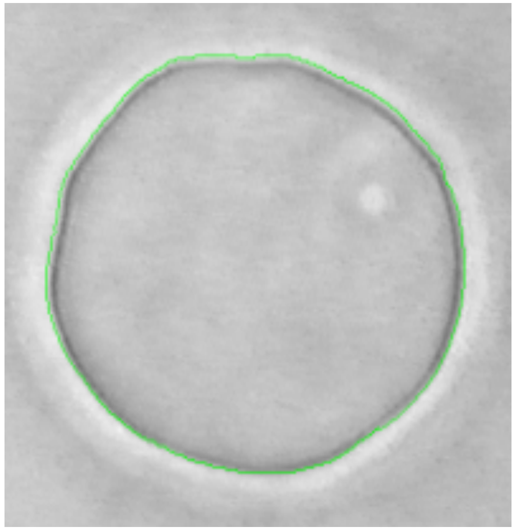


Force dipole : $\mathcal{P}_a$ = **8-10 k_BT**

1 ATP hydrolysis≈15 k_BT

Girard et al, *PRL* (2005)

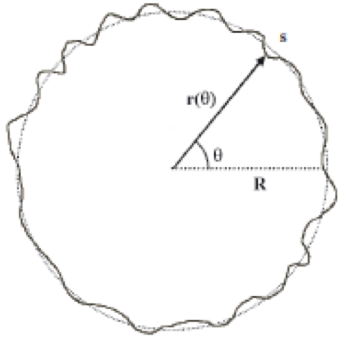
Fluctuation Spectrum



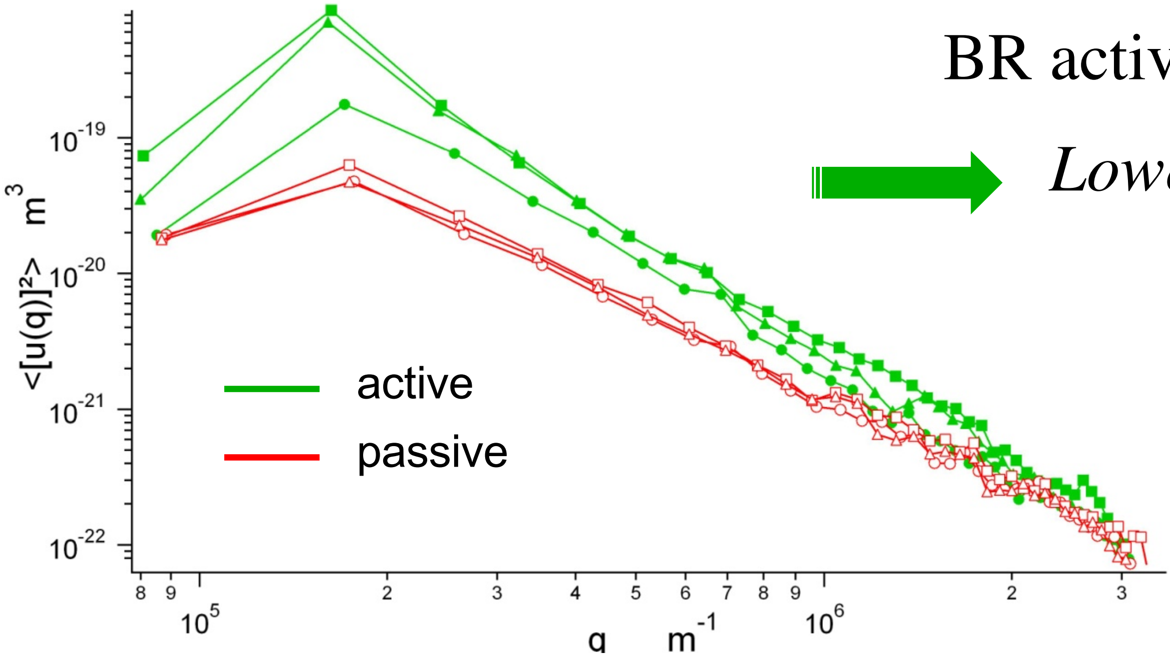
Flickering Spectroscopy:
Contour Fourier analysis

J. Pécréaux *et al.*, *EPJE* (2004)

Coll. H.G. Döbereiner



Same GUV (Bacteriorhodopin))



BR activity

→ *Lowering* of membrane *tension*

M. El Alaoui Faris et al., *PRL* (2009)

Fluctuation Spectrum

Cf. later model (M. Lomholt): *active effect on tension*:

M. Lomholt, *PRE* (2006)

$$\left\langle |u(q)|^2 \right\rangle = \boxed{\frac{k_B T}{2\tilde{\sigma}}\left[\frac{1}{q} - \frac{1}{\sqrt{q^2+\tilde{q}_c^2}}\right]} + \frac{F_{(2)}^2 n_\Sigma}{16\kappa^2}\frac{1}{\left(\sqrt{q^2+\tilde{q}_c^2}\right)^3}$$

Active correction

With: $\boxed{\tilde{\sigma} = \sigma + \sigma_{dip}}$

& $\sigma_{dip} = \int h F_{act}(h) dh$

$F_{(2)} \propto Q = \int h^2 F_{act}(h) dh \qquad \tilde{q}_c = \tilde{\sigma}/\kappa$

$\Rightarrow \quad \boxed{\sigma_{dip} \propto -10^{-7}\ N/m}$

M. El Alaoui Faris et al., *PRL* (2009)

Active Membranes : Some Conclusions

- Increase of the fluctuations due to protein activity

  Consequences at large scale of molecular conformational changes

- Some quantitative validation of the theoretical model

  Measurement of the force dipole: $P_a = 8\text{-}10\ k_BT$ (Ca^{2+}-ATPase)

  Effective tension due to activity $\sigma_{eff} < \sigma$

- Non-equilibrium membranes upon lipid addition/fluxes

  J. Solon *et al.*, *PRL* (2006)

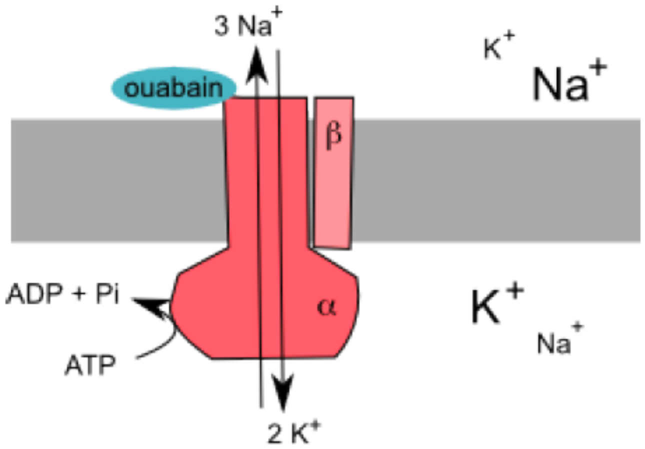
  Theor: P. Girard, F. Jülicher, J. Prost *EPJE* (2004)
  M. Rao, R. Sarasij, *PRL* (2001)

- Limited number of experiments so far

More Recent Work

Active Model Membranes

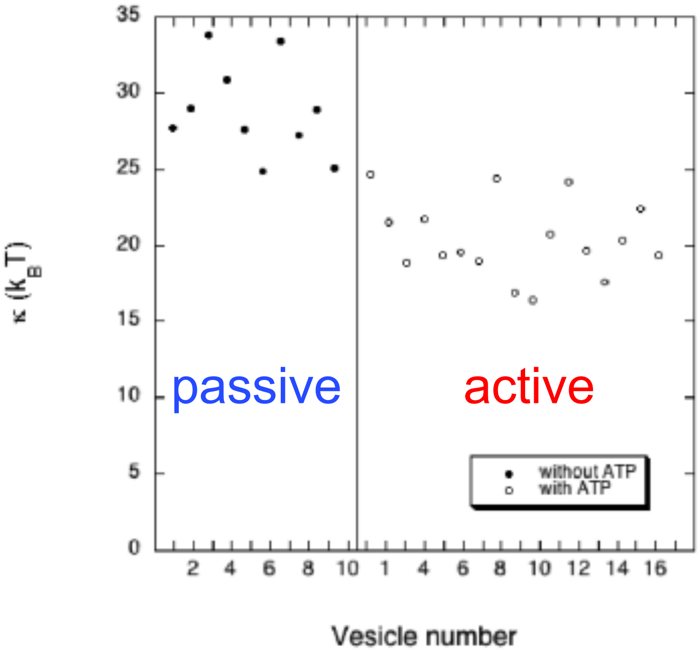
H. Bouvrais…. J.H. Ipsen, O. Mouritsen, *PNAS* **109**, 18442-18446, (2012)



Na^+, K^+ ATPase pump

Fluctuation spectrum

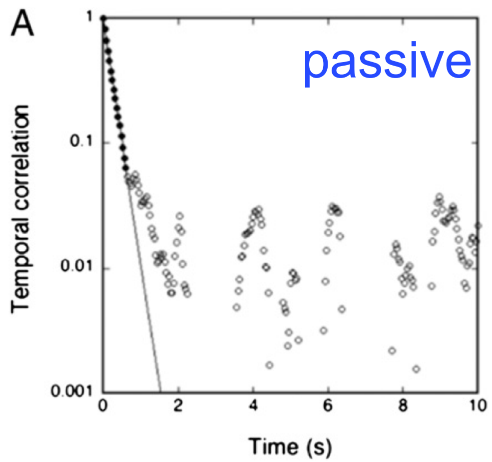
Bending modulus

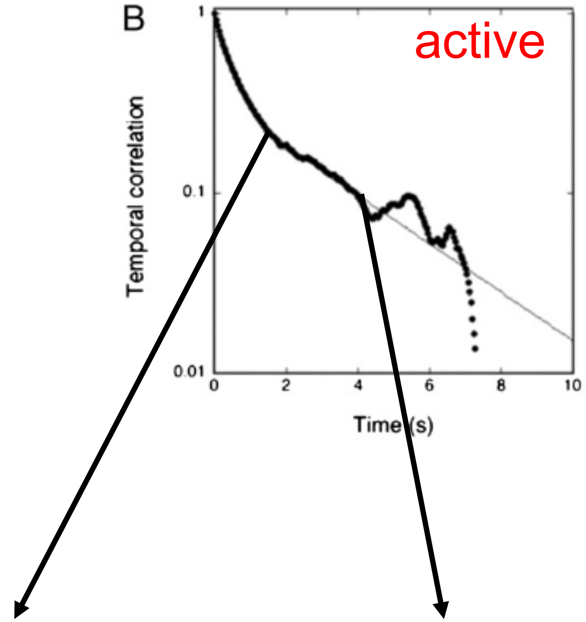


Membrane softening

Dynamics: Time correlation (n=5)

2 exponentials





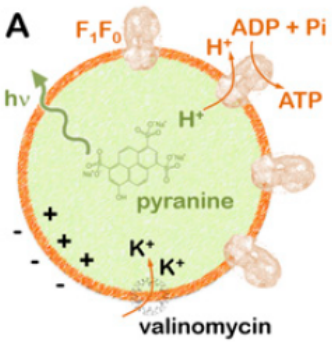
n^{-3}

~ passive membrane

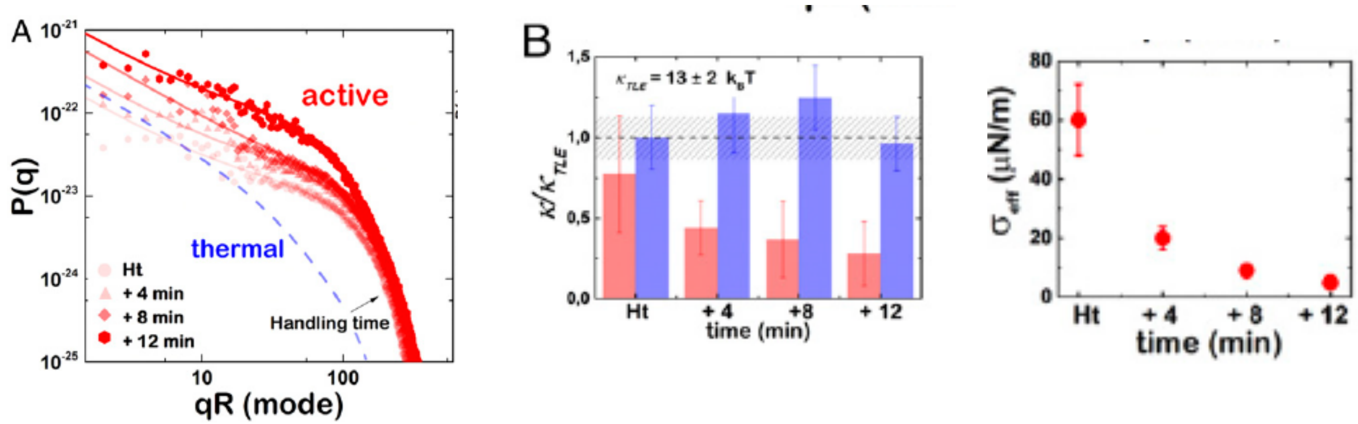
n^{0}

~ 0.5 sec
ATP turn-over

Active Model Membranes

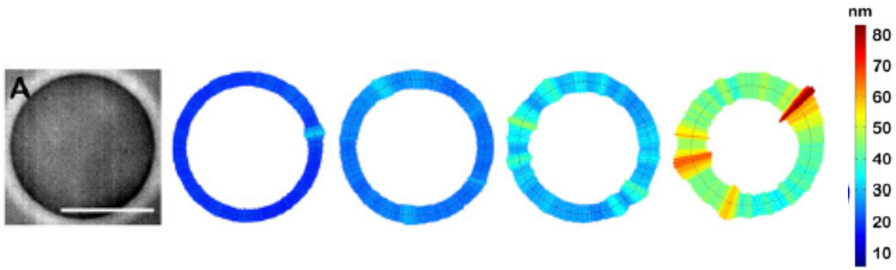


Rotating motor (F1-F0 ATP-synthase)



Activity ⟹ softening of the membrane
reduction of the tension

Localized membrane deformations



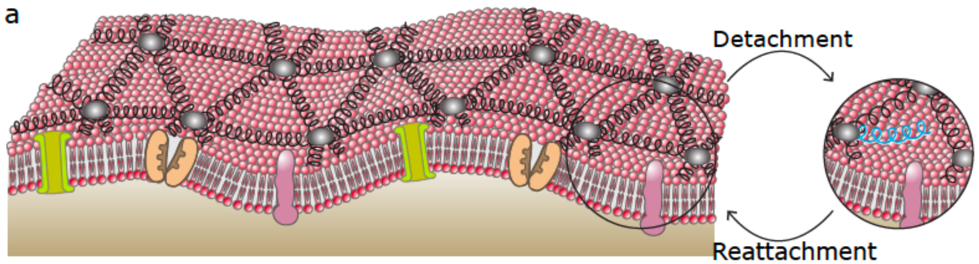
Active clustering?

V. Almendro-Vedia …I. López-Montero *PNAS* **114**, 11291-11296 (2017)

Active Cell Membranes

Red Blood Cell flickering

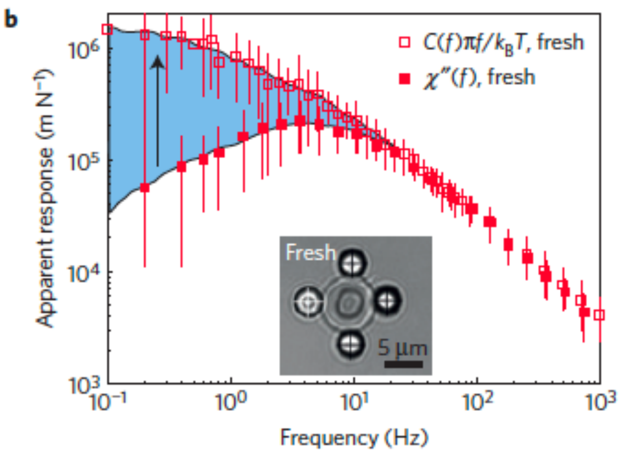
H. Turlier … T. Betz *Nat. Phys.* **12**, 513-519 (2016)
H. Turlier & T. Betz. *Annu. Rev. Condens. Matter Phys.* **10**, 213-232 (2019)



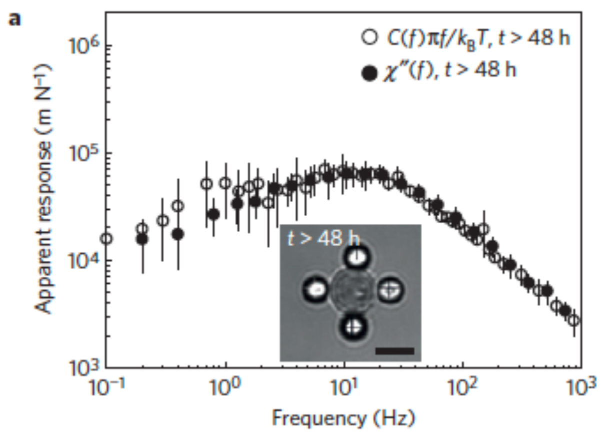
Membrane actively coupled to a cytoskeleton

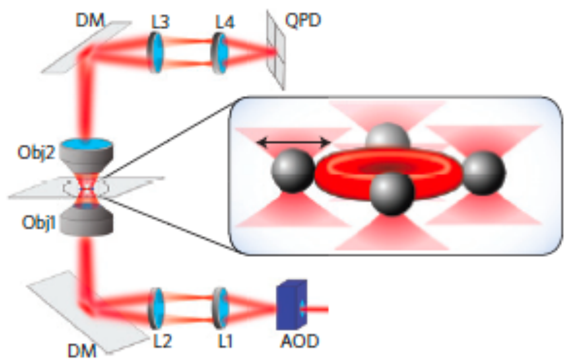
Measures fluctuations *C(f)* and mechanical response *χ(f)*

Fresh cells



"Old" cells





$$C(f)\pi f/k_B T \neq \chi''(f)$$

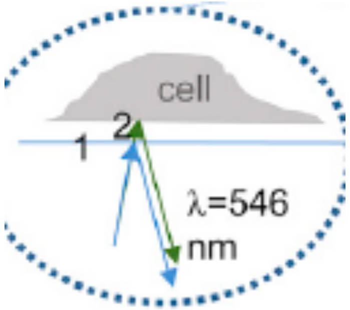
Violation of the fluctuation-dissipation theorem

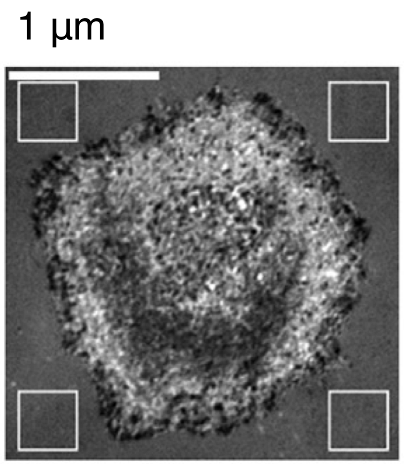
Non-equilibrium membrane

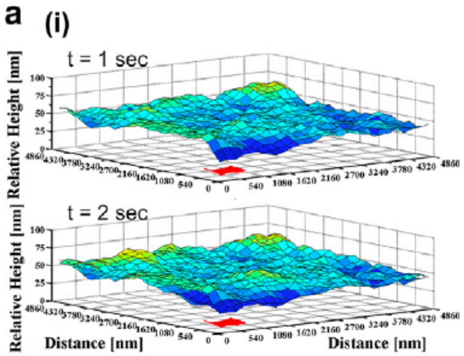
Active Cell Membranes

Cell fluctuating on a surface

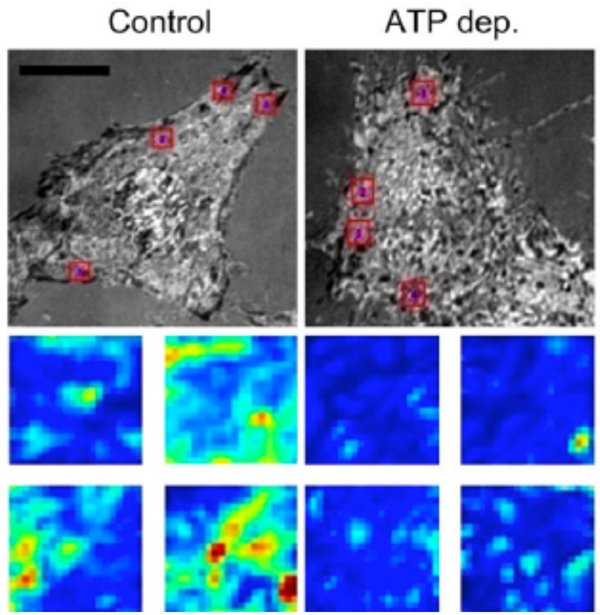
A. Biswas, A. Alex, B. Sinha, *Biophys. J.* **113**, 1768 (2017)







Interferometric image of the bottom part

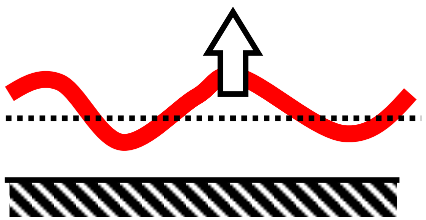


Fluctuations depend on ATP

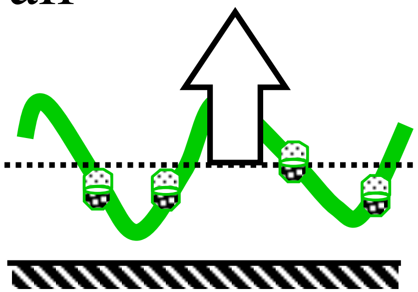
(active)

To be Studied (in more details):

- Active fluctuating membrane near a wall



"Helfrich" entropic pressure



Amplification of the fluctuations

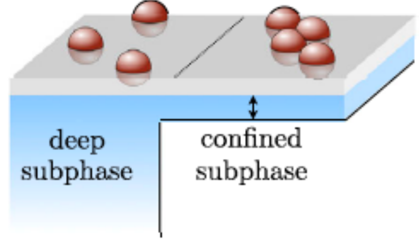
J. Prost, J.-B. Manneville, R. Bruinsma, *Eur. Phys. J. B* **1**, 465 (1998).

See T. Mukhina…T. Charitat, G. Fragneto *J. Colloid Interf. Sci.* on line

Future experiments?

- Effect on adhesion?

- *Clustering* predicted for active proteins if membrane close to a surface



H. Manikantan, *PRL* **125**, 268101 (2020)

Active Proteins in Membranes: Diffusion, Clustering

- Attractive Forces between 2 active inclusions

Theory J.-B. Fournier, K. S. Ronia, *J. Stat. Mech.* **2013**, P08005 (2013)

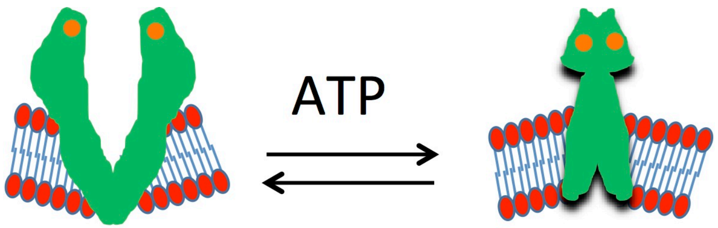
If clustering, size of the active domains?

Coupling membrane deformation, protein clustering and protein activity

- Mobility in a membrane depends on protein shape

F. Quemeneur *et al.*, *PNAS* **111** 5083 (2014)

If protein actively switches its shape, mobility affected?



More work still needed:

Theory, simulations and experiments

Thanks to:

Experiments

Philippe Girard
Jerome Solon
Jacques Pécréaux
Jean-Baptiste Manneville

Theory

Philippe Girard
J-B. Manneville
Frank Jülicher
J. François Joanny
Jacques PROST

Collaborations :

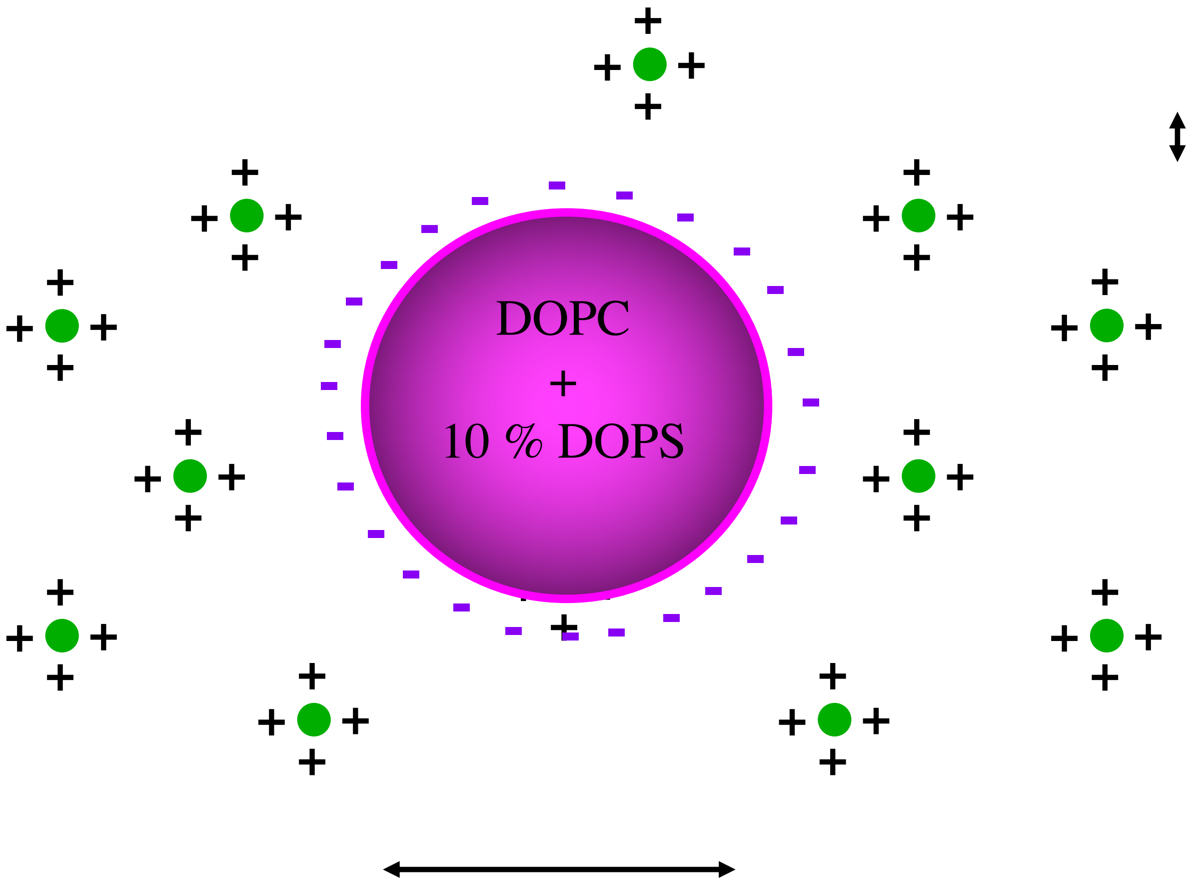
P. Falson, G. Lenoir,
P. Champeil (CEA-Saclay)
J-L. Rigaud , D. Lévy
(PCC, Institut Curie)
H.G. Döbereiner

S. Ramaswamy (Bangalore)
R. Bruinsmac(UCLA)
D. Lacoste (ESPCI, Paris)

Experimental System for Fast Lipid Addition

J. Solon

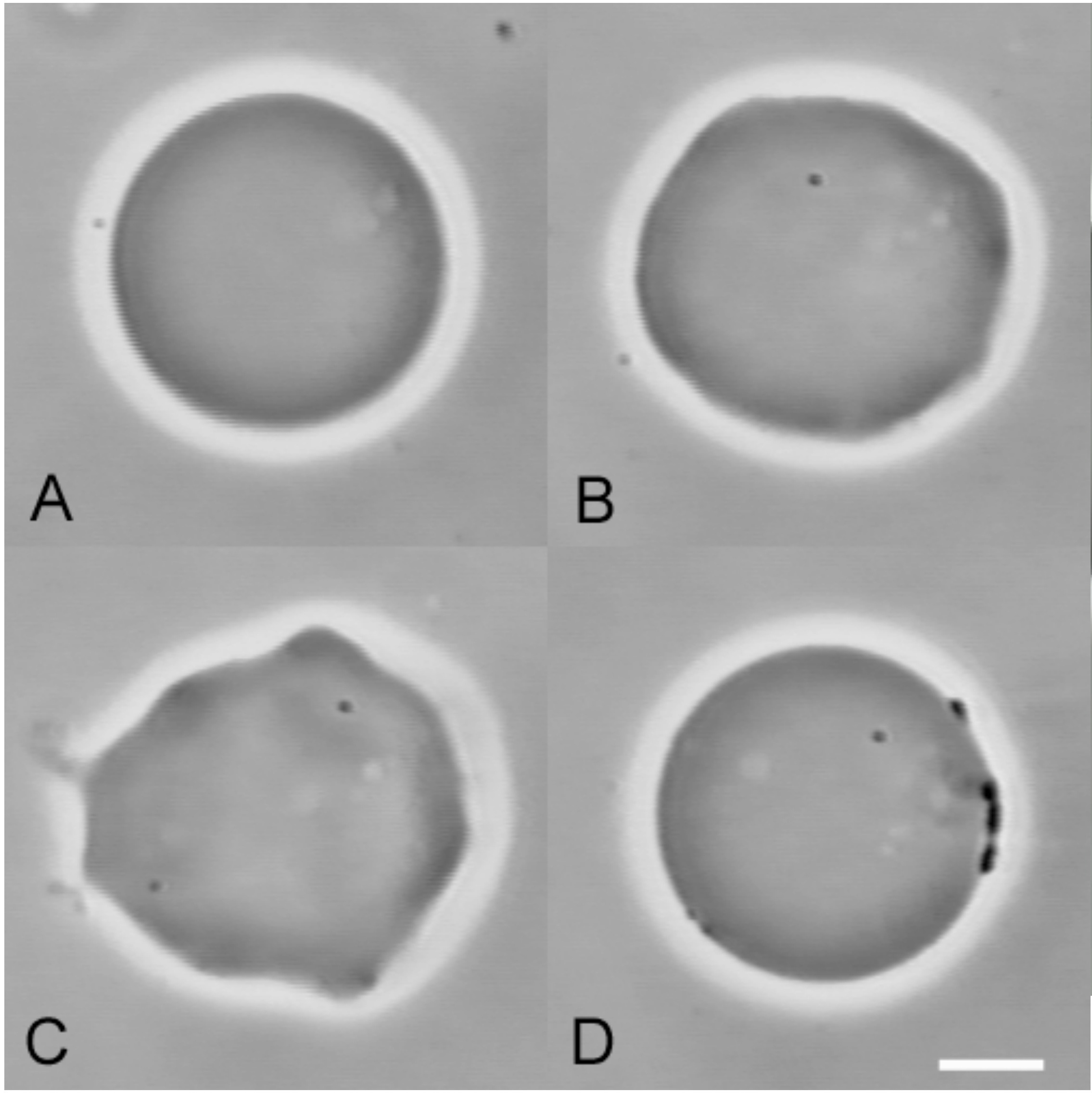
High fusion rate with charged systems

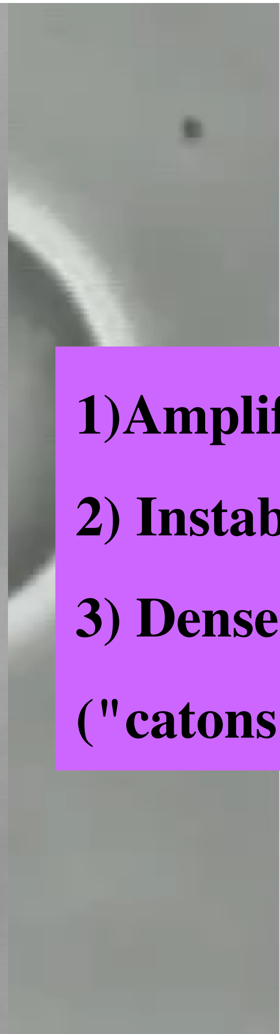


J. Solon *et al.*, *PRL* (2006)

If [DOTAP] > 7 %

Fusion and Shape Instabilities





1)Amplification of fluctuations

2) Instabilities.

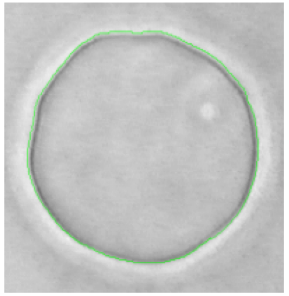
3) Dense lipid clusters ("catons") -tense vesicle.

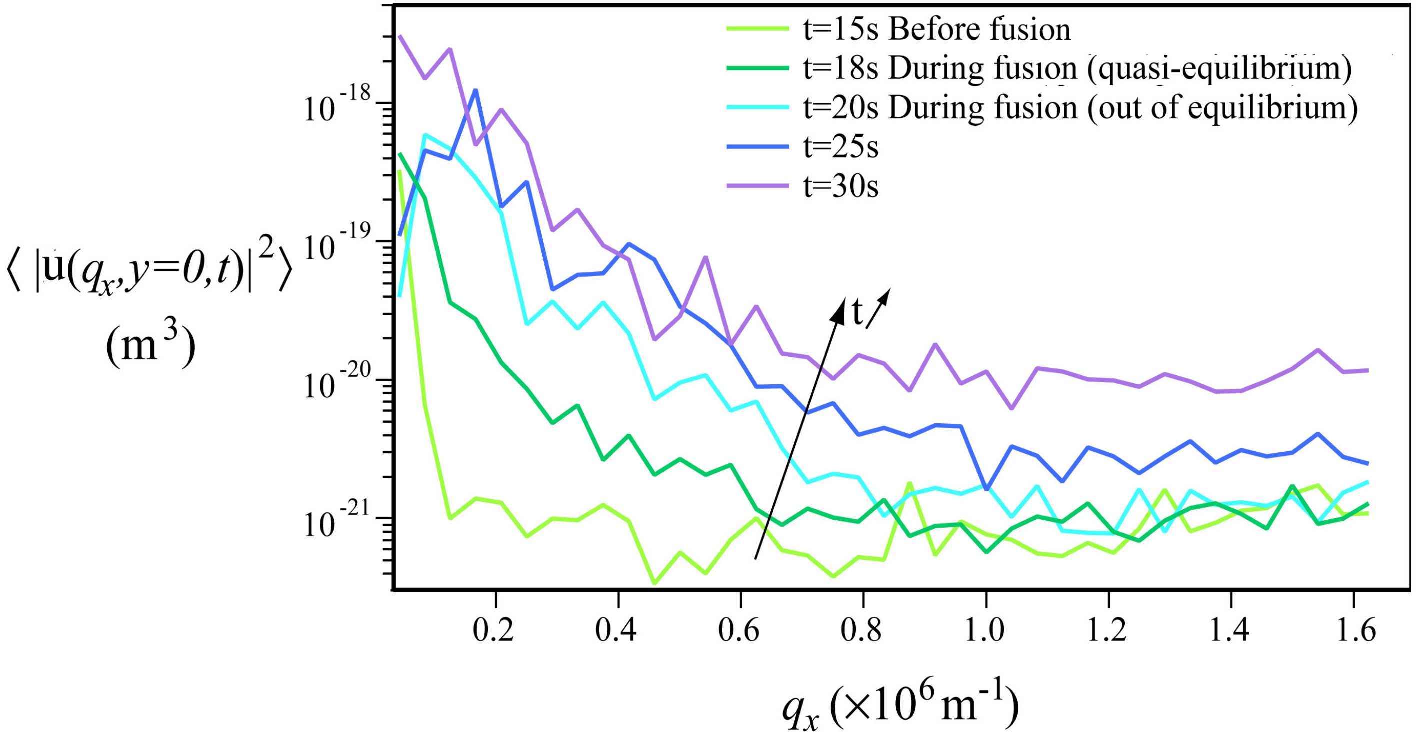
(Real Time)

10 µm

J. Solon *et al.*, *PRL* (2006)

Fusion Induces Large Amplification of Fluctuations





Theory(steady-state regime):

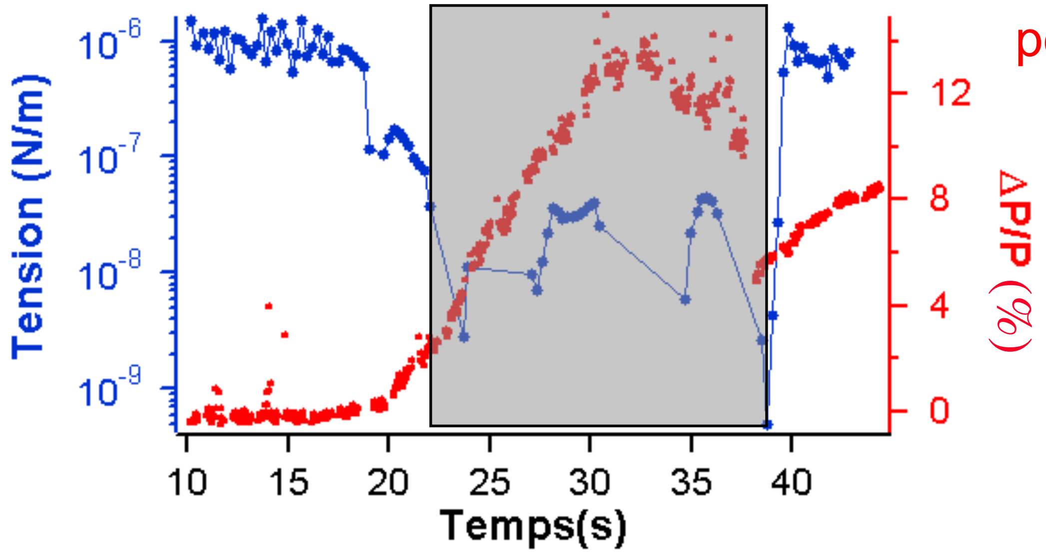
$$\left\langle u_{q_\perp}^2 \right\rangle = \frac{k_B T}{\sigma_{eff} q_\perp^2 + \kappa q_\perp^4}$$

$$\sigma_{eff} = \sigma - f(A)$$

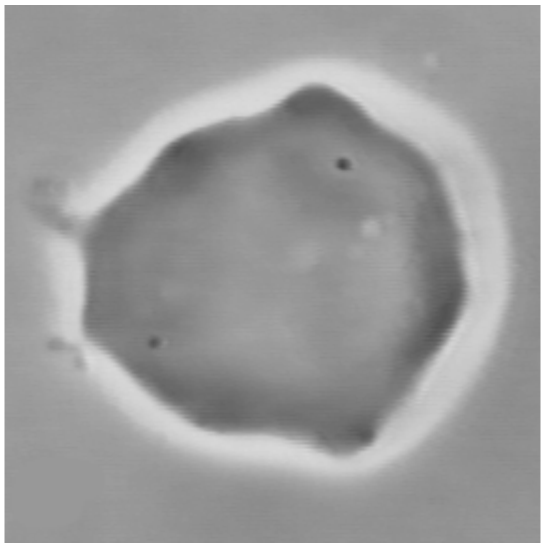
(P. Girard, F. Jülicher, J. Prost *EPJE* (2004)
(M. Rao, R. Sarasij, *PRL* (2001)

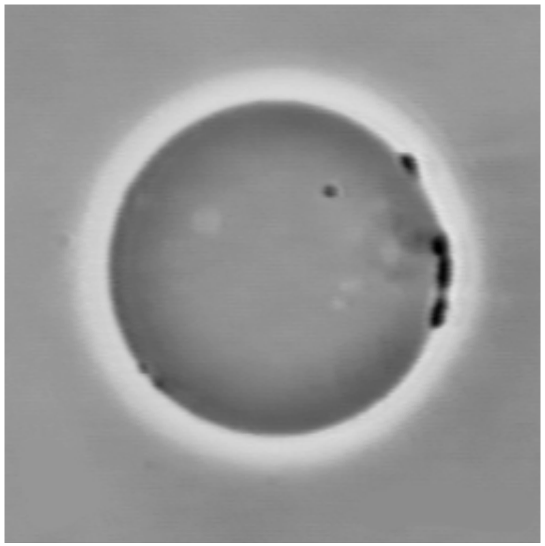
J. Solon *et al.*, *PRL* (2006)

Vanishing Tension and Instabilities



Fluctuation Regime → Large Deformation (instabilities) → Bilayer collapse





Dense lipid structures ("catons")

Equilibrium state

J. Solon *et al.*, *PRL* (2006)