Shape matters in protein mobility within membranes

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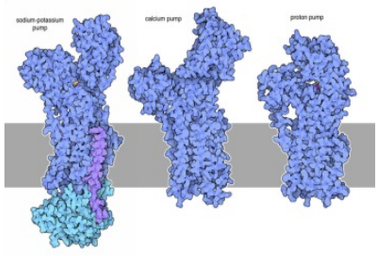
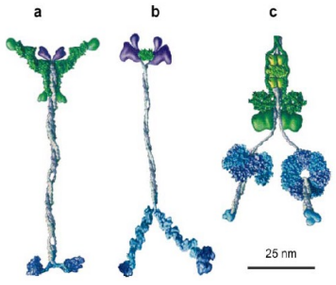
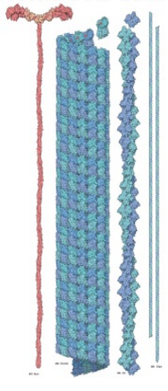
Emergent complexity of bio-chemical systems



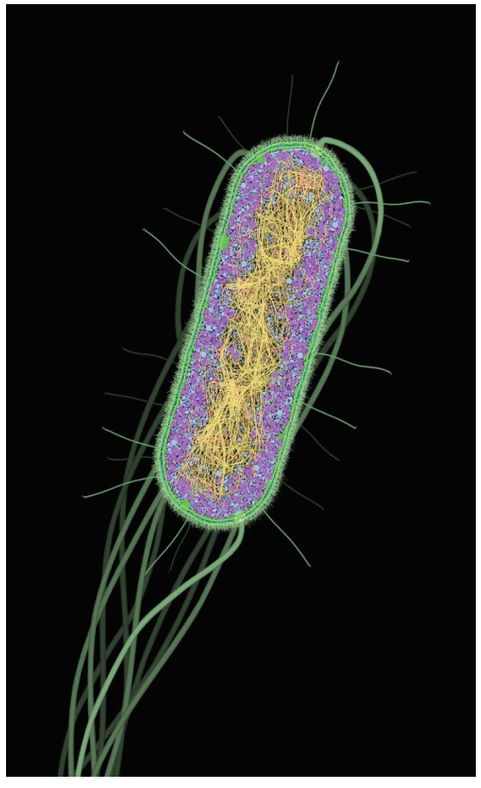
Membrane proteins



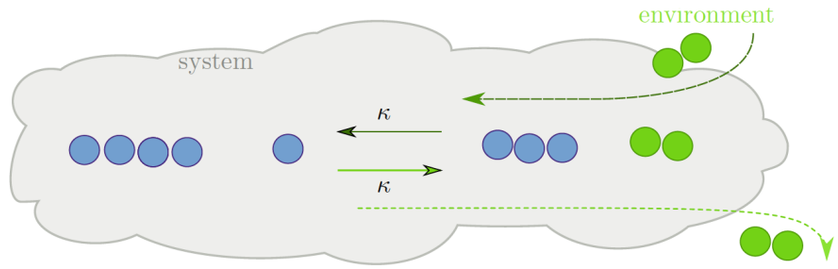
Molecular motor



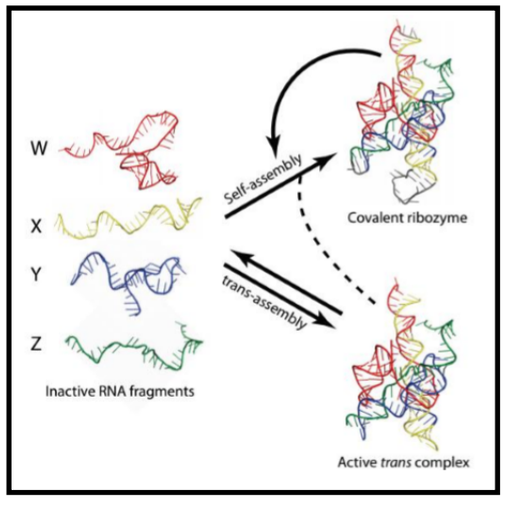
Biofilaments



« Machinery of life »

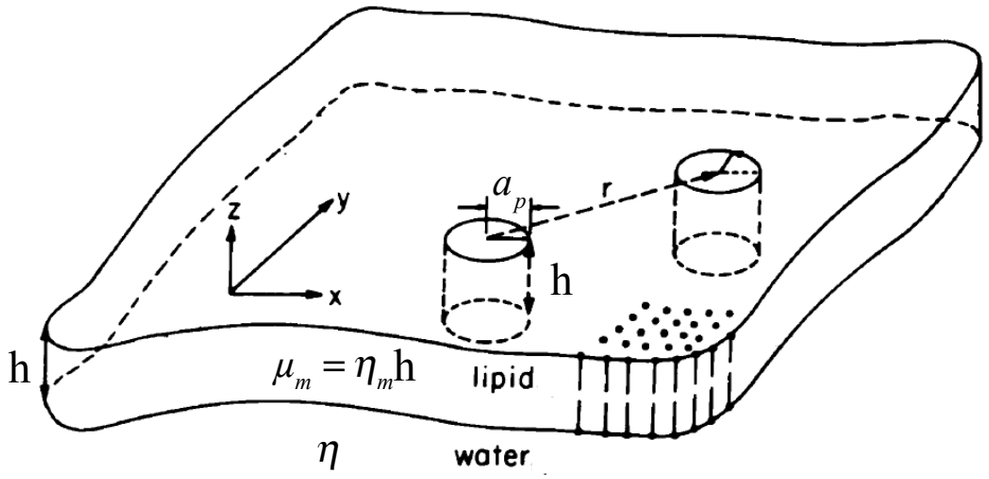


Open bio-chemical networks



Self-assembly of RNA replicators
(from N. Lehman, Nature, 2012)

Protein mobility in a membrane



Saffman and Delbrück, PNAS 1975

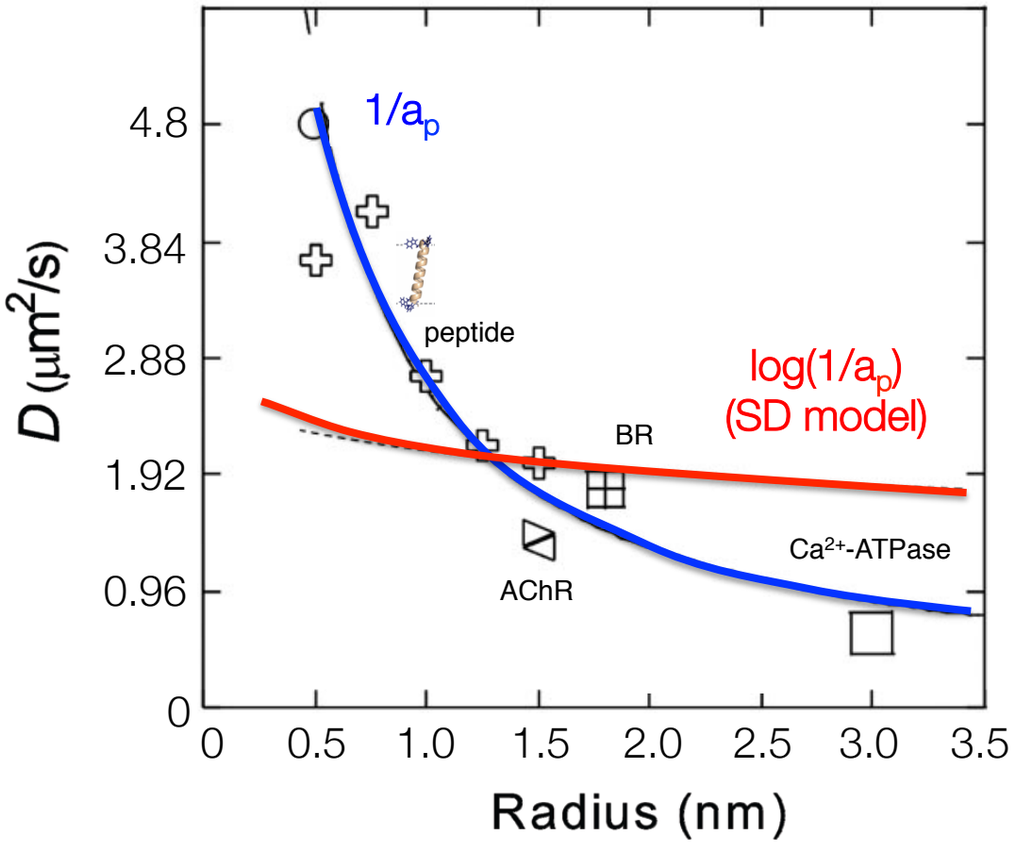
$$\eta_m / \eta \sim 10^2$$

$$l_{SD} = \frac{\mu_m}{\eta} \approx 500\text{nm}$$

$$D_{SD} = \frac{kT}{4\pi\mu_m}\left[\log\left(\frac{l_{SD}}{a_p}\right) - 0.577\right]$$

- Hydrodynamic resistance in the membrane $2\pi a_p h \times \frac{v\eta_m}{a_p}$ (area x stress)
- Hydrodynamic resistance in fluid above or below $\pi a_p^2 \times \frac{v\eta}{a_p}$
- Ratio of the two is $\frac{l_{SD}}{a_p}$ and the SD calculation holds when $\frac{l_{SD}}{a_p} >> 1$

Experimental deviations from the SD model



Gambin et al., PNAS 2006

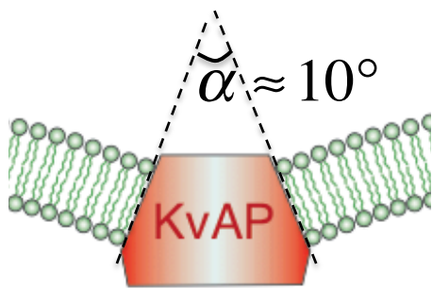
Possible origin of the $1/a_p$ behavior rather than $\log(1/a_p)$ could be due to
(i) strong local deformation of the membrane near the protein, or
(ii) Internal dissipation mecanisms

A. Naji et al., Biophys. J. 2007

A tale of two trans-membrane proteins

Voltage-gated K^+ (KvAP)

- Ion channel found in bacteria



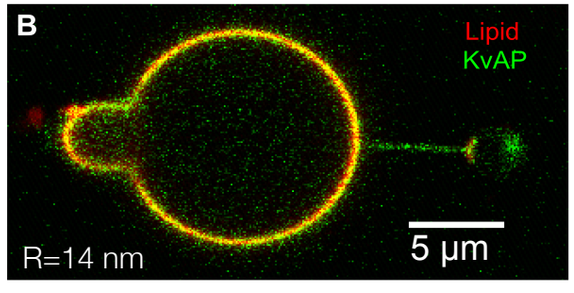
$a_p = 4$ nm $C_p \approx 0.04$ nm^{-1}

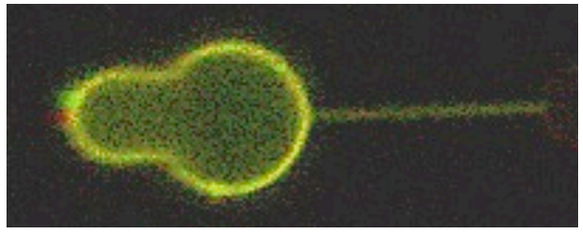
Aquaporine-0 (AQP0)

- Water channel found in the eye lens

$a_p = 4$ nm $C_p = 0$

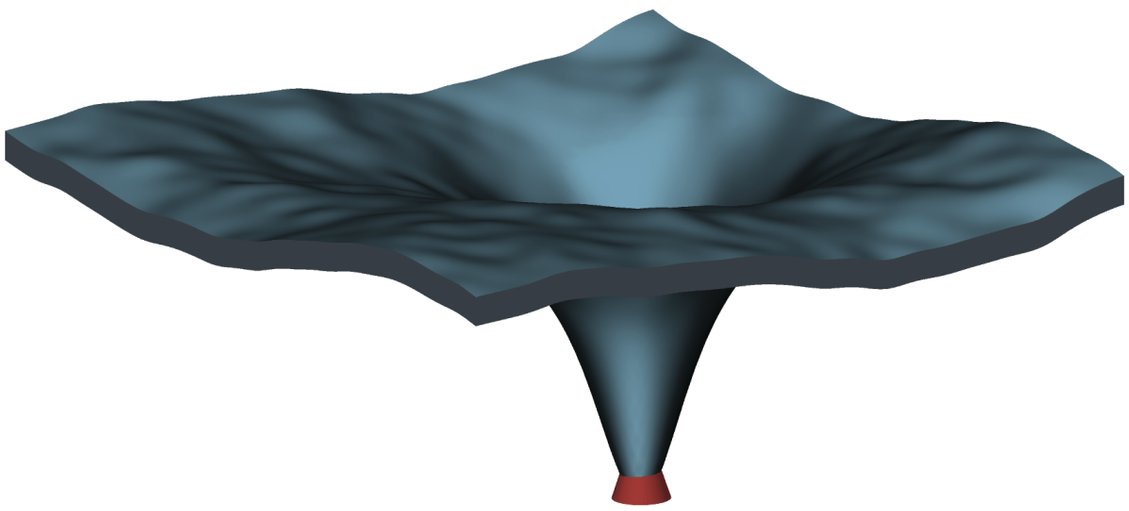
- KvAP is enriched in nanotubes as compared to AQP0 (S. Aimon et al. Dev. Cell. 2013)



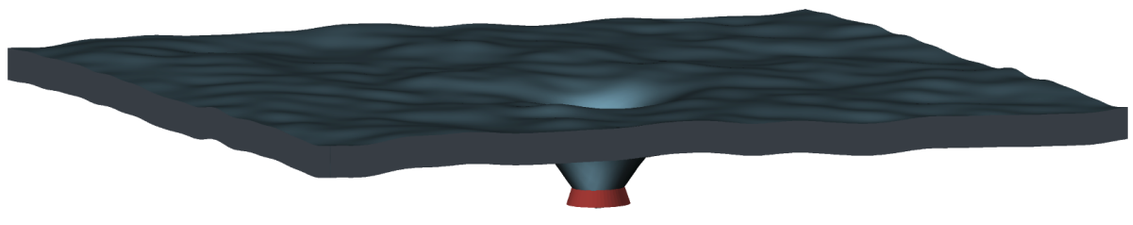


Membrane local deformation depends on tension

$\Sigma = 10^{-5}$ N/m



$\Sigma = 10^{-2}$ N/m



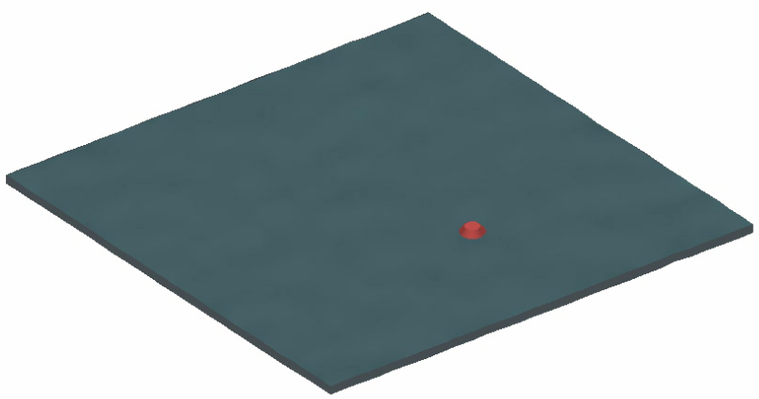
Simulations: 135x135 nm

Membrane tension ↘ ➡ larger deformation

Protein mobility depends on tension

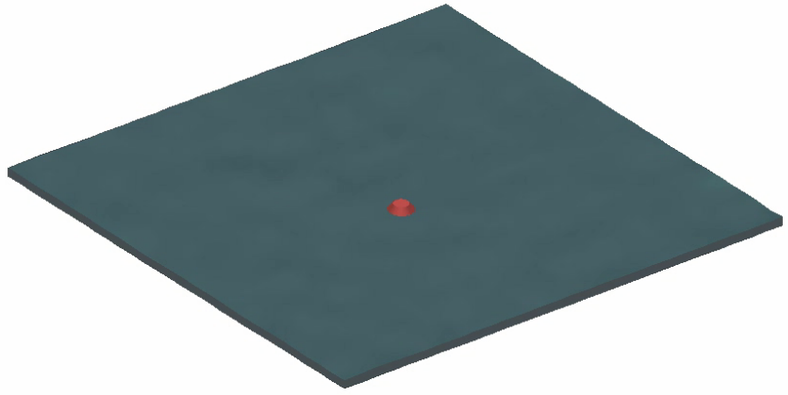
High tension

$\Sigma = 10^{-2}$ N/m



Low tension

$\Sigma = 10^{-5}$ N/m

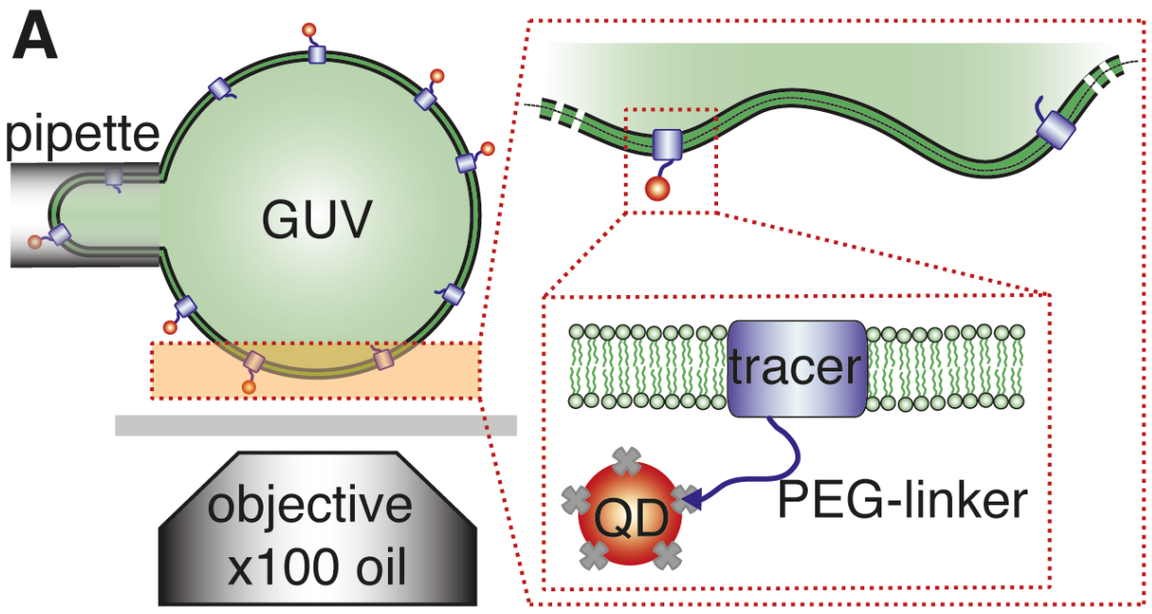


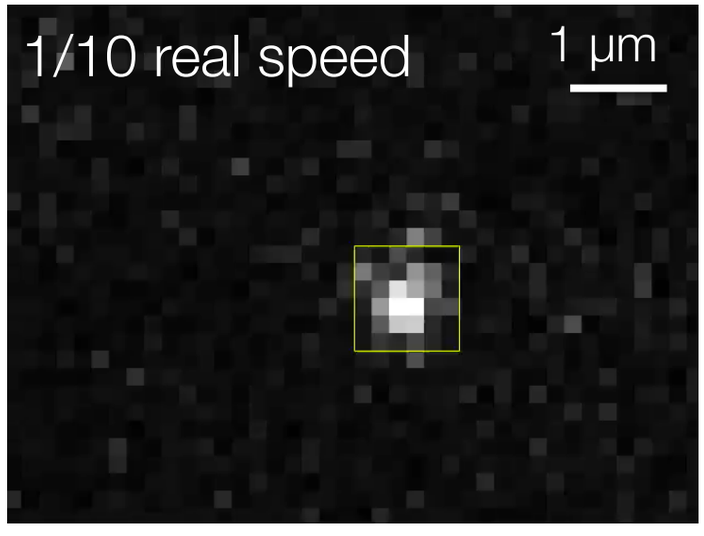
Simulations: 135x135 nm, 620 μs

Membrane tension ↘ ➡ larger membrane deformation ➡ Mobility decreased

Experimental setup using artificial lipid membranes

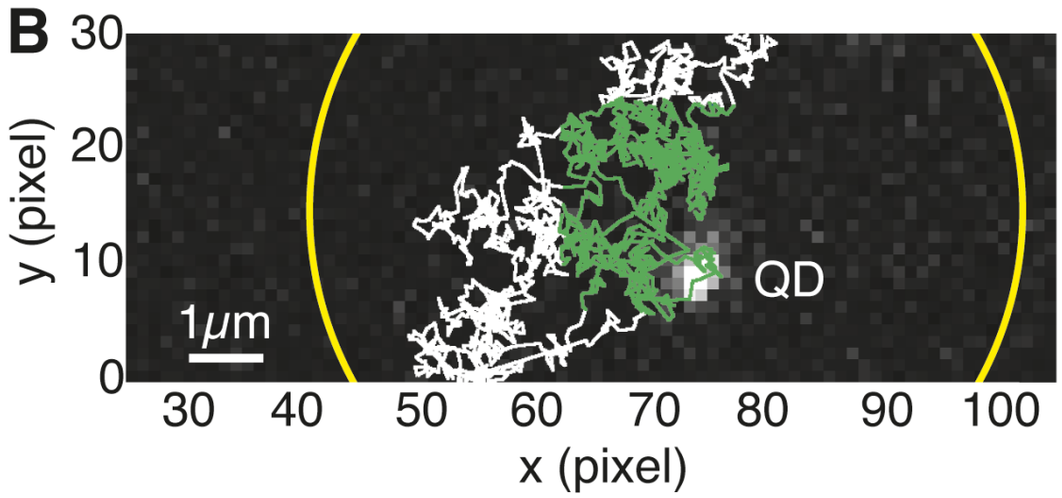
Membrane tension of a *vesicle* controlled by micropipette aspiration





Detection and tracking of single quantum dot (QDs) coupled to tracers (lipid or protein)

spatial resolution ~ 10 nm



Particle trajectories

⬇

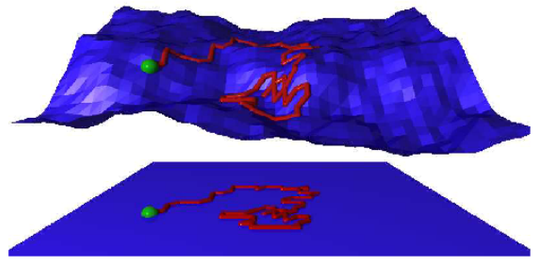
Mean Square Displacement

⬇

Diffusion coefficient versus tension

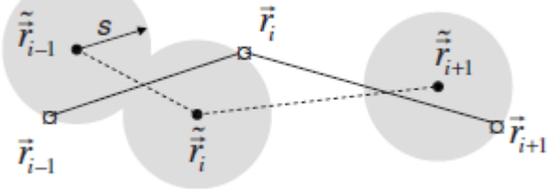
Some relevant issues in single particle tracking

- Difference between diffusion in 3D in the membrane and diffusion in the projected trajectories



Courtesy of U. Seifert, Univ. Stuttgart

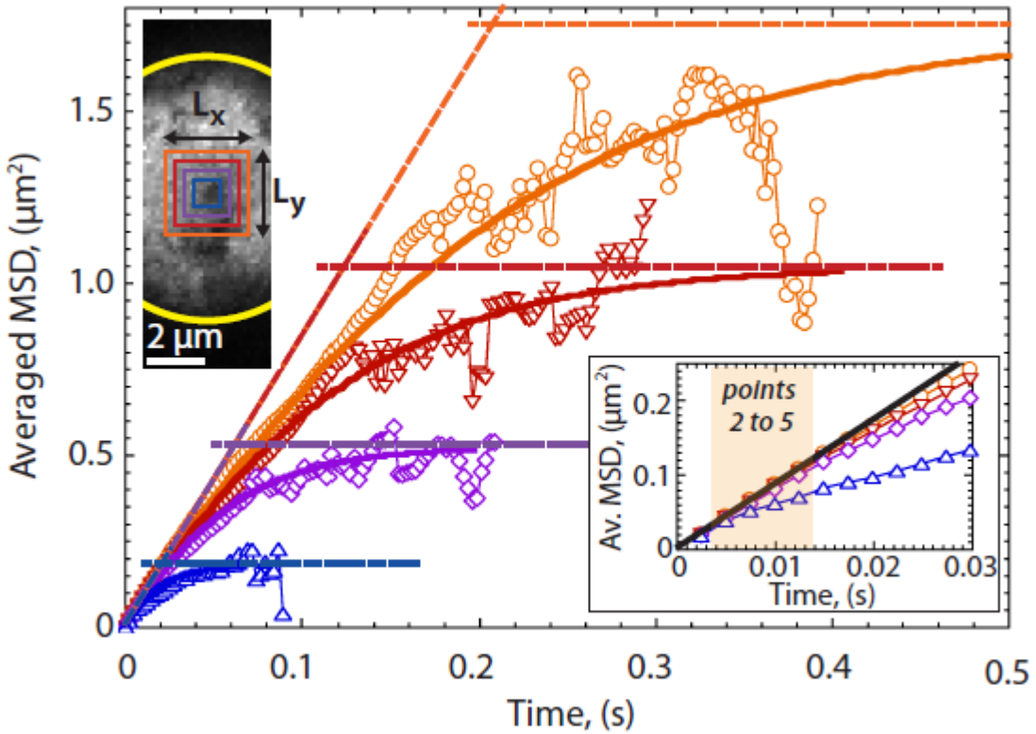
- Difference between real and measured trajectories (error in the localization, finite integration time..)



Michalet 2010

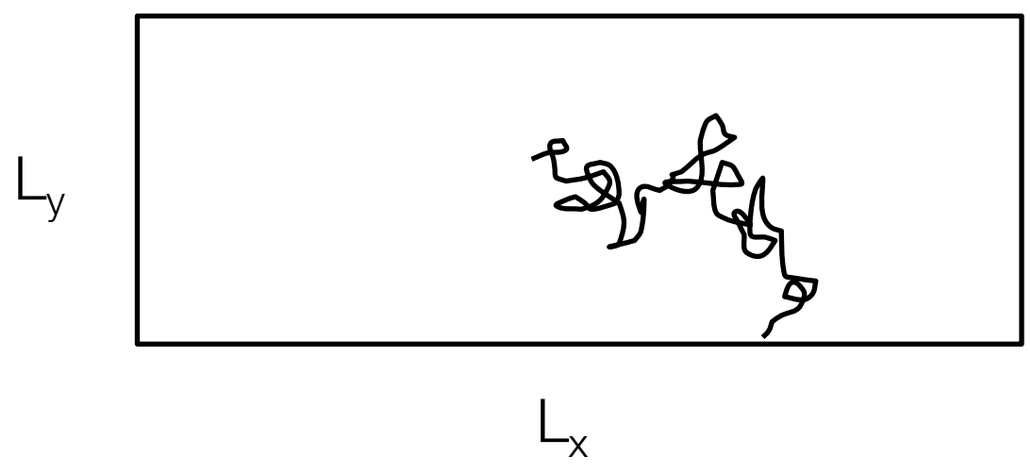
- When multiple tracers are tracked simultaneously, proper assignment of trajectories among the tracers is critical.

⟶ need for least-squared estimators or bayesian inference techniques



- Typical MSD trajectories are diffusive at short times but reach plateaux at long times. Such plateaux are a priori unexpected for the free diffusion of a tracer in an artificial homogeneous membrane

Role of the size of the observation window in SPT

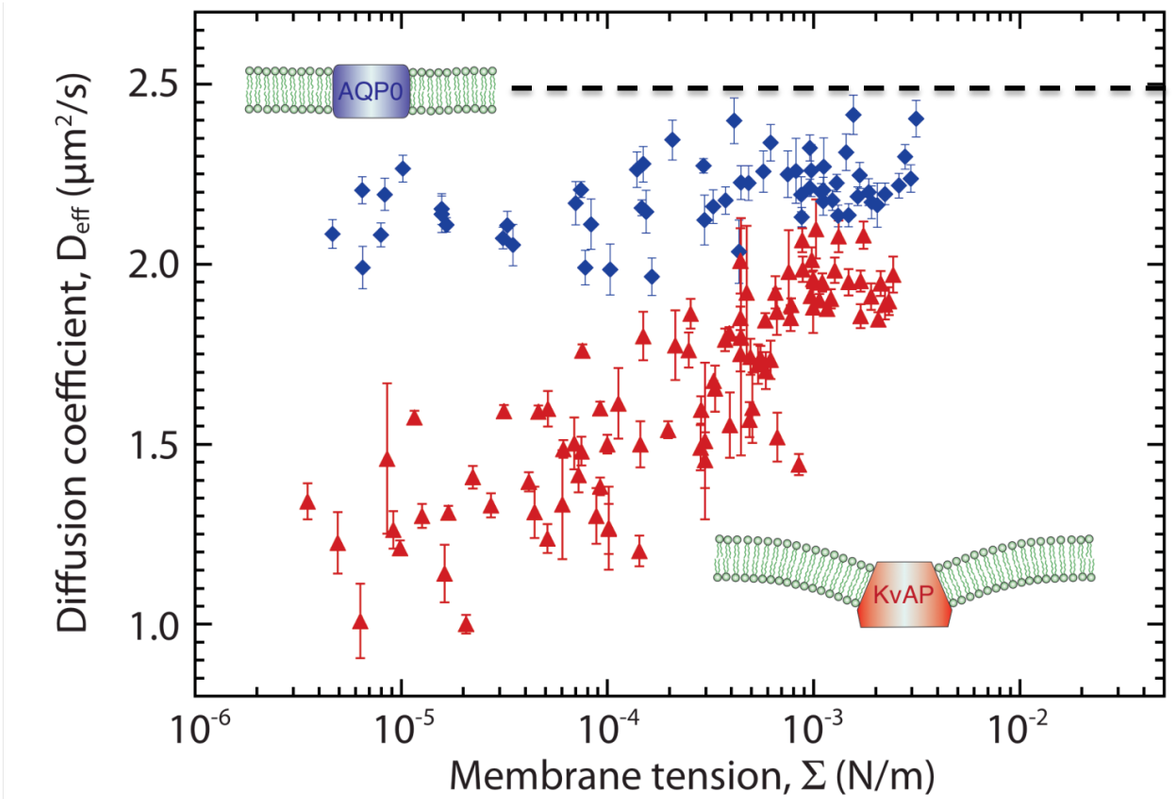


- One needs to consider the conditional MSD to start from an arbitrary point within the domain and reach the boundaries (with absorbing BC).

- Explicit analytical expression for conditional MSD is for a rectangular box:

$$\left\langle \Delta r^2(t) \right\rangle_{cond} \xrightarrow[t\to\infty]{} \frac{\pi^2 - 8}{2\pi^2}\left(L_x^2 + L_y^2\right) \simeq 0.0947\left(L_x^2 + L_y^2\right)$$

Membrane tension affects lateral diffusion



D_{SD}

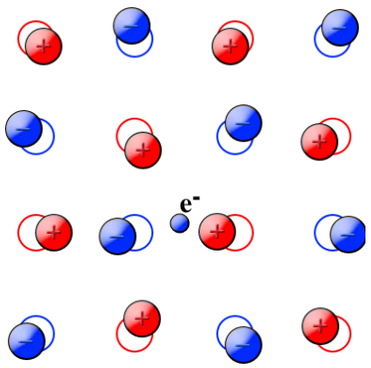
$a_p = 4$ nm

AQP0 $C_p = 0$

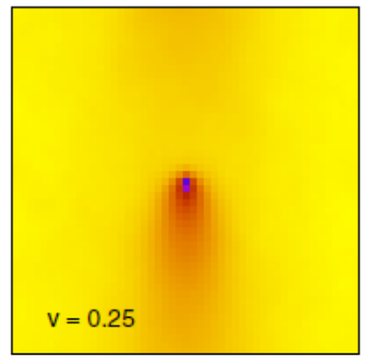
KvAP $C_p \approx 0.04\ \text{nm}^{-1}$

- SD model implicitly assumes that the membrane remains flat and unaffected by the diffusing protein. This works for AQP0 but not for KvAP.
- For KvAP, the feedback of the protein on the membrane leads to a reduction of the mobility, visible at low tension.

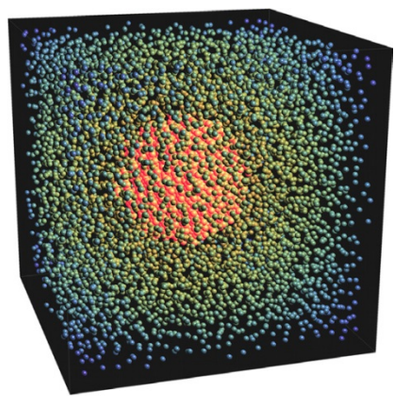
Active-tracer diffusion in fluctuating fields



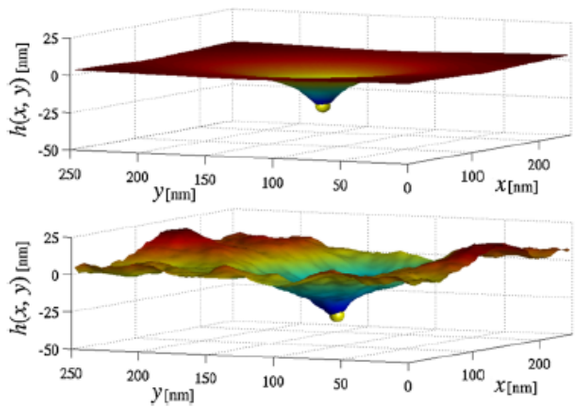
Polaron
(L. Landau, 1933)



Moving local magnetic field
in the 2D Ising model
(V. Démery et al., 2010)



Hot brownian motion
(D. Rings et al., 2010)



Diffusion of a protein on a membrane
(A. Naji al, 2009)

Model for the local membrane curvature coupling

$$H[h,\mathbf{R}]=\frac{\kappa}{2}\int d^2\mathbf{r}\left[\left(\nabla^2 h\right)^2+\frac{\Sigma}{\kappa}(\nabla h)^2-\Theta G(\mathbf{r}-\mathbf{R})\nabla^2 h\right]$$

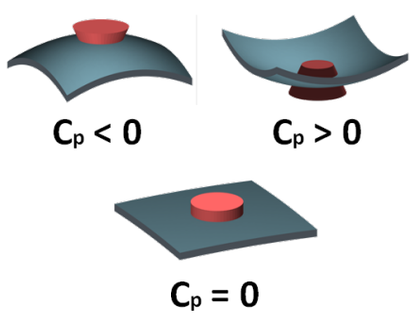
coupling constant

Gaussian weight function

By comparison with $H_{\text{int}}[h,\mathbf{R}]=\frac{\kappa}{2}\int d^2\mathbf{r}\tilde{G}(\mathbf{r}-\mathbf{R})\left[\nabla^2 h-2C_p\right]^2$

imposing $\tilde{G}(\mathbf{r}-\mathbf{R})=\pi a_p^2 G(\mathbf{r}-\mathbf{R})$ with $\int d^2\mathbf{r}G(\mathbf{r}-\mathbf{R})=1$

Protein area



Relation coupling constant-spontaneous curvature

$$\Theta=4\pi a_p^2 C_p$$

Dynamics described by coupled Langevin equations

$$\dot{\mathbf{R}} = -\mu \nabla_{\mathbf{R}} H[h, \mathbf{R}] + \eta,$$

$$\frac{\partial h(\mathbf{r},t)}{\partial t} = -\int d\mathbf{r}' \Lambda(\mathbf{r}-\mathbf{r}') \frac{\partial H}{\partial h(\mathbf{r}',t)} + \xi(\mathbf{r},t),$$

with $\Lambda(\mathbf{r}) = \dfrac{1}{8\eta|\mathbf{r}|}$ and $\langle \eta(t)\eta(t') \rangle = 2D_0 \delta(t-t'),$

$$\langle \xi(\mathbf{r},t)\xi(\mathbf{r}',t') \rangle = 2k_B T \delta(t-t')\Lambda(\mathbf{r}-\mathbf{r}')$$

Numerical simulations use these equations of motion — A. Naji et al., PRL (2009)

Analytical solution by path integral formulation — E. Reister et al., PRE (2010)

or by an operator formalism — V. Démery et al., PRL (2010)

A simplifying assumption: the adiabatic limit

At every instant, the hamiltonian is minimized with respect to the membrane height field evaluated at the current position of the inclusion **R**(t).

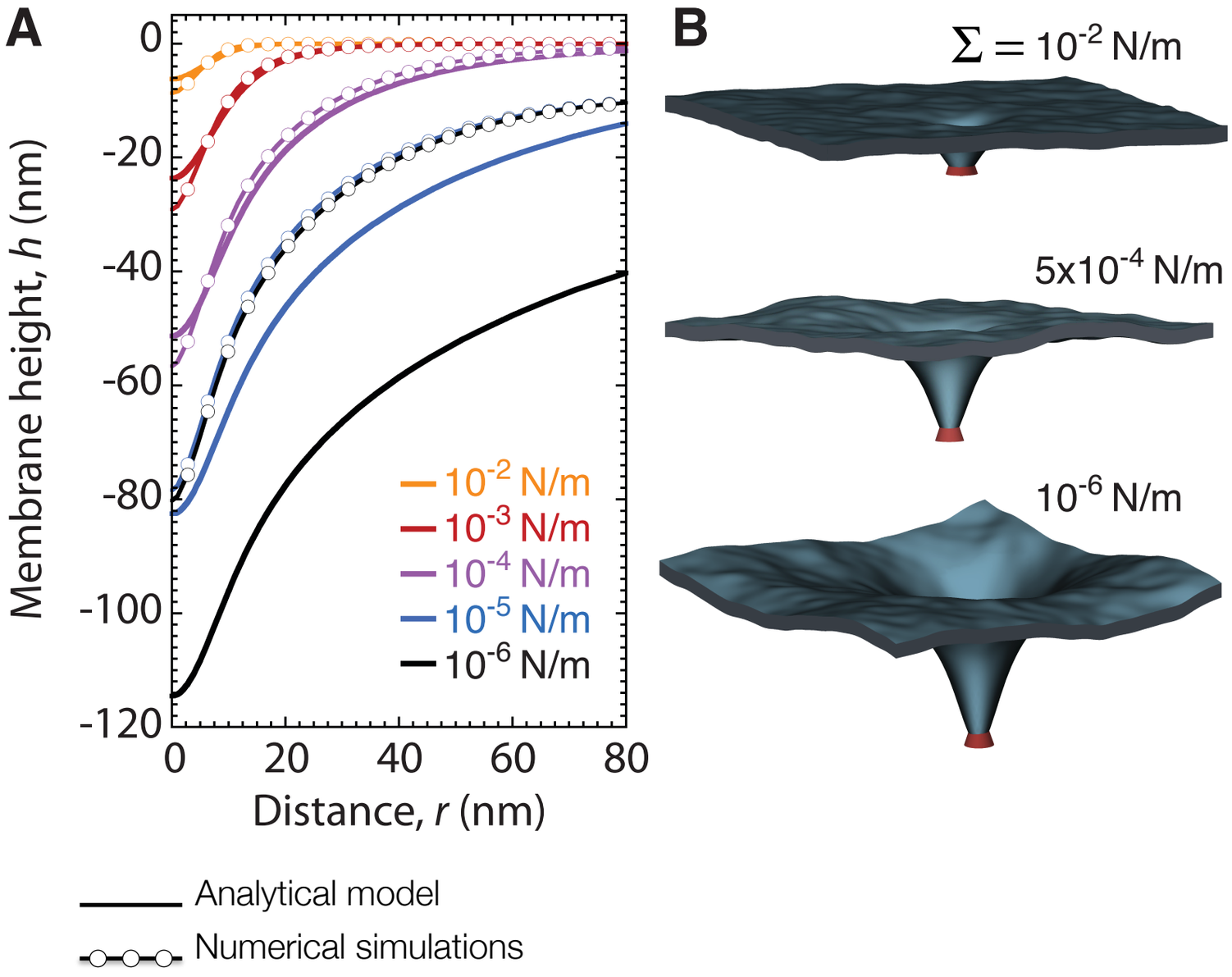
Slowest mode of membrane relaxation (n=2) for a quasi-spherical vesicle has a caracteristic time of

$$\tau_n = 4\eta R\left(n\Sigma + \frac{\kappa n^3}{R^2} \right)^{-1}$$

much shorter than the diffusion time $\tau_D = \frac{R^2}{D_0 n^2}$

One has $\frac{\tau_n}{\tau_D} = 0.02$ at R=6μm and $\frac{\tau_n}{\tau_D} = 0.008$ at R=20μm at the lowest tension.

Theoretical membrane profiles vs. simulations



➤ Height profile controlled by the coupling constant, width profile by $\xi = \sqrt{\frac{\kappa}{\Sigma}}$

Force acting on the inclusion $\bar{f} = -\partial_{\mathbf{R}} H\left[h, \mathbf{R}\right]$ V. Démery et al., PRL (2010)

Expanding at low v, one obtains $\bar{f} = -\lambda \mathbf{v}$

$$\lambda = \frac{2\eta}{(2\pi)^2} \int d^2\mathbf{k} \left|\mathbf{k}\right|^3 \bar{h}(\mathbf{k}) \bar{h}(-\mathbf{k})$$

Total drag (pure hydrodynamics + additional power losses due to membrane coupling)

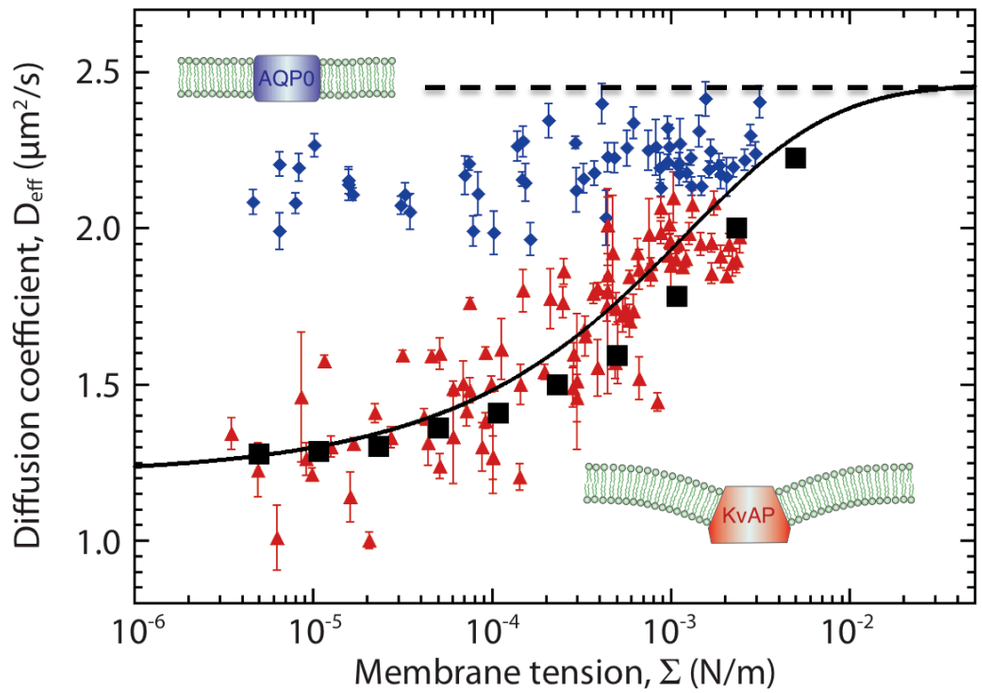
$$\lambda_{eff} = \lambda_0 + \lambda$$

Using the Fluctuation-dissipation theorem $D_{eff} = \frac{k_B T}{\lambda_{eff}}$

<u>Remarks:</u>

- In the regime $\xi \gg a_p$, one has $D_{eff} \approx \frac{k_B T}{a_p}$

Tension dependence of the diffusion coefficient



D_{SD}

Analytical model

Numerical simulations

- Cross-over to the SD limit occurs when $\xi = \sqrt{\frac{\kappa}{\Sigma}} \approx a_p$, i.e. $\Sigma \approx 5.10^{-3}\,\mathrm{N/m}$
- One parameter fit of the model gives $\Theta = 4\pi a_p^{\,2}\, C_p \approx 3.5\ 10^{-7}\,\mathrm{m}$
- <u>But:</u> major discrepancy with thermodynamic measurements: $C_p \approx 1.8\ \mathrm{nm}^{-1} \gg 0.04\ \mathrm{nm}^{-1}$

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

Discussion of the value of the coupling constant

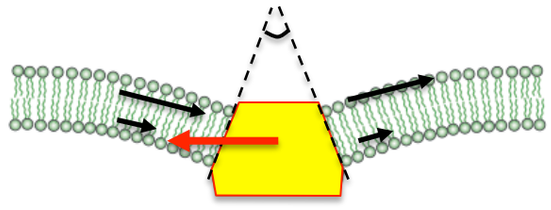
- Scenario 1: a correlated layer of lipids is advected with the protein

B. Camley et al., PRE (2012)

But, this layer should be of the order of 47nm, much larger than protein size.

- Scenario 2: there is an hidden mechanism of dissipation

Internal friction between the leaflets



➢ The monolayer friction has the largest contribution to the friction coefficient

Reduced coupling constant $\Theta \approx 3.4\,10^{-8}\,\mathrm{m}$ in better agreement with static measurements

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

Other order parameters

- General order parameter:

$$\mathcal{H}[\phi(\mathbf{r}),\mathbf{R}] = \frac{1}{2}\int \phi(\mathbf{r})\Delta * \phi(\mathbf{r}) d^2\mathbf{r} - K * \phi(\mathbf{R}),$$

$$\frac{\partial \phi}{\partial t}(\mathbf{r},t) = -R * \frac{\delta \mathcal{H}}{\delta \phi(\mathbf{r},t)} + \eta(\mathbf{r},t),$$

- Coupling to composition: in a lipid mixture, proteins may be wetted differently by one kind of lipids. The strongest effect on the mobility is expected near demixion point

- Coupling to thickness or to a nematic order parameter field:

➢ No observable local membrane deformation but a perturbed order parameter

V. Demery and D. L., chapter in book edited by Springer Netherlands (2016)
on « Mechanical factors affecting the mobility of membrane proteins »

Interplay between the conformation changes and physical properties of the surrounding membrane

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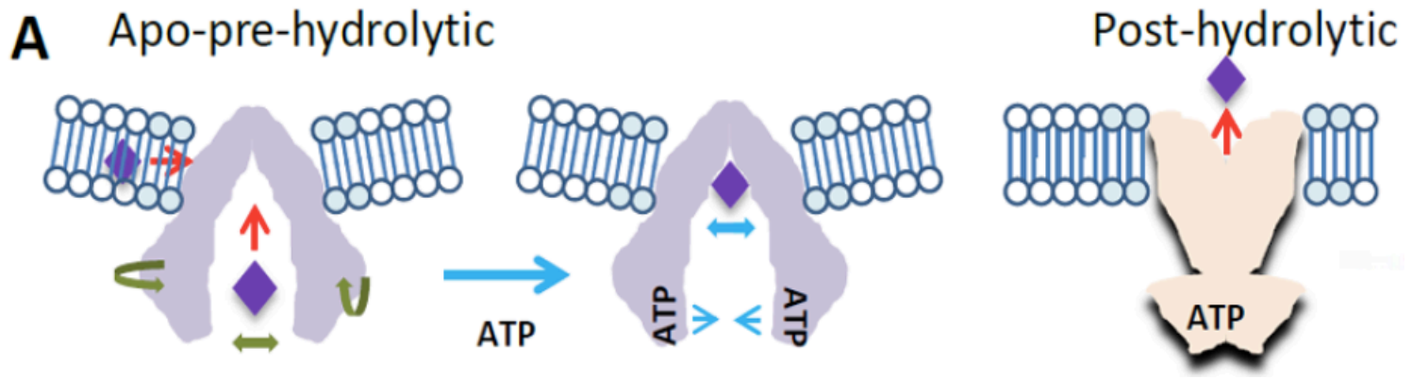
(i) How does the conformation dynamics depend on membrane lipid composition ?

(ii) How is it modified by the tension and curvature of the membrane ?

(iii) How does the conformation dynamics affect membrane protein mobility and binding affinity ?

ABC transporter:



Project participants: P. Bassereau, D. Levy, D. L., V. Démery, P. Tieleman, M. Dahan
funded by ANR

Conclusion

- The mobility of a protein depends on its coupling to the local environment, and not just on its size.

- To obtain a simplified description, one should not focus on the details of the local environment but on the coupling between the membrane and the protein

- This question goes beyond the issue of mobility but also concerns the function of membrane proteins (i.e. in mechano-sensitive channels, voltage gated channels, ABC transporters…)