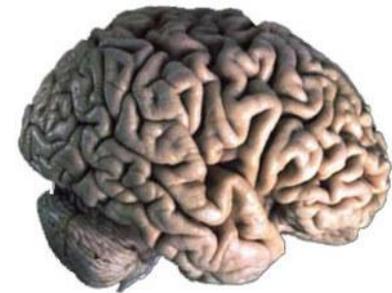
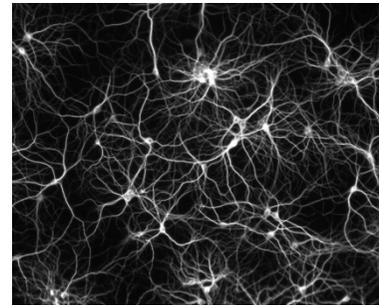
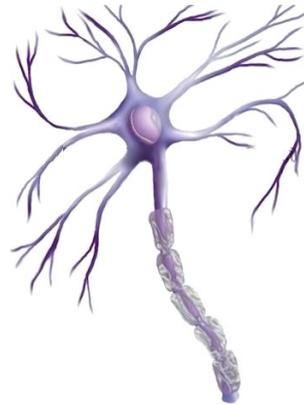
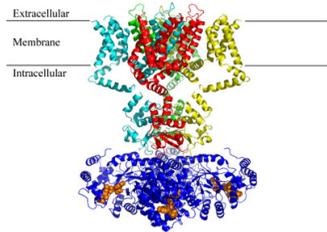


Imagerie et nanoscience pour l'étude des cellules nerveuses

Maxime Dahan

De la molécule, au neurone, au réseau ... au cerveau



1 nm

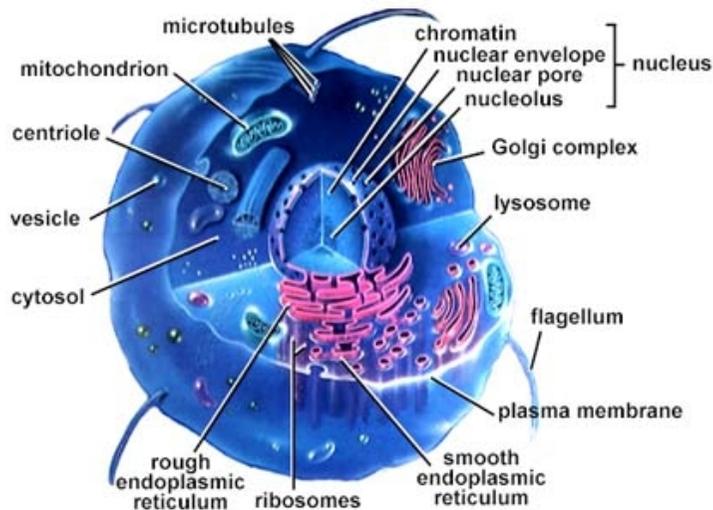
1 μm

1 mm

1 cm

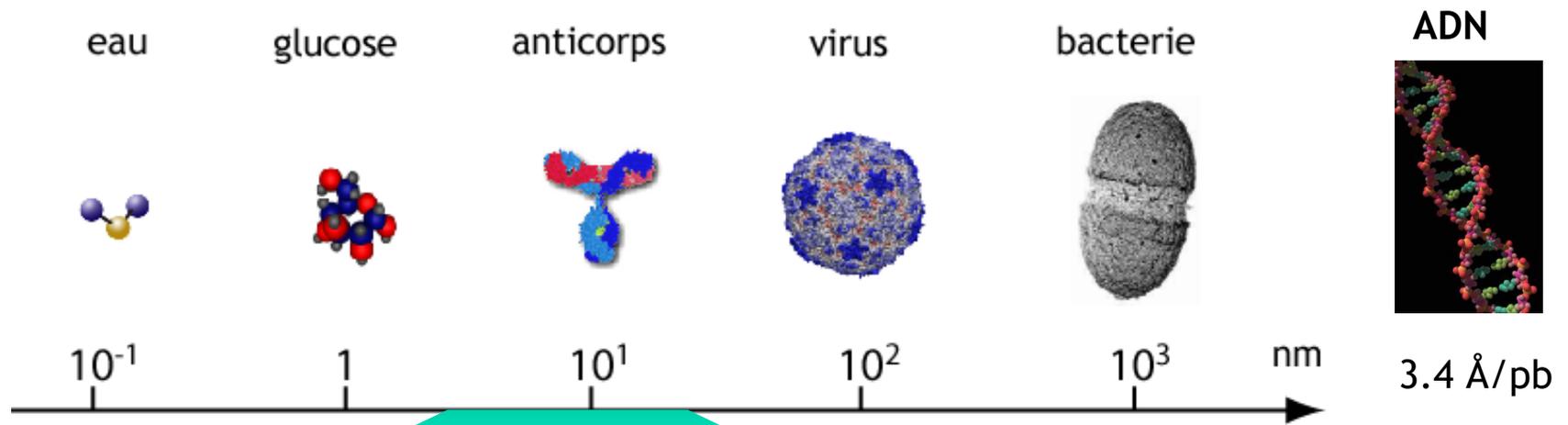
électrophysiologie, imagerie optique, EEG, PET, IRM fonctionnelle

La cellule est un milieu spatialement organisé



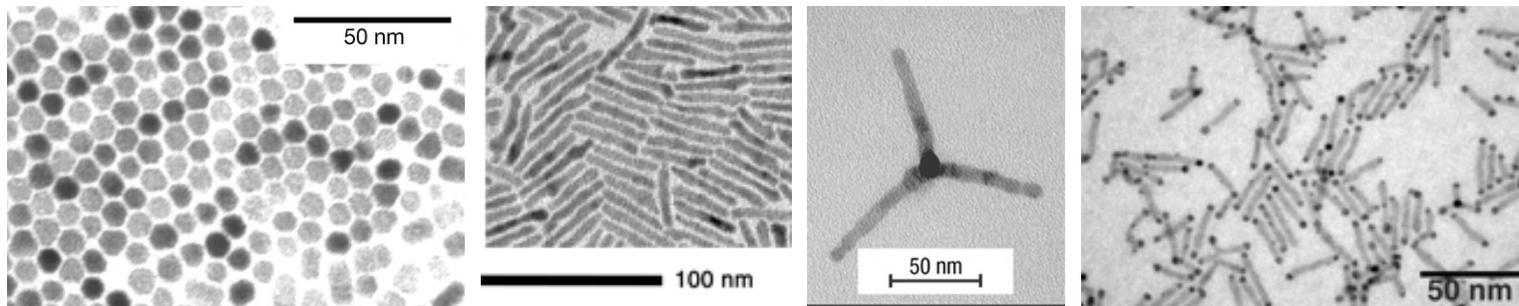
- **Génomique.** A pour but d'identifier les gènes et leurs fonctions.
- **Protéomique:** concerne l'étude des ensembles de protéines: leur rôle, leur structure, leurs interactions, ...

Comment peut-on comprendre l'émergence d'une organisation spatiale des cellules vivantes ?



New nanomaterials

- **Composition** : metallic (gold, silver,...), semiconductor (CdSe, CdTe, CdS, PbSe,...), carbon nanotubes,...
- **Size** : controled at the nanometer scale
- **Shape** : nanosphere, nanorod, nanowire, tetrapod, dumbbell...

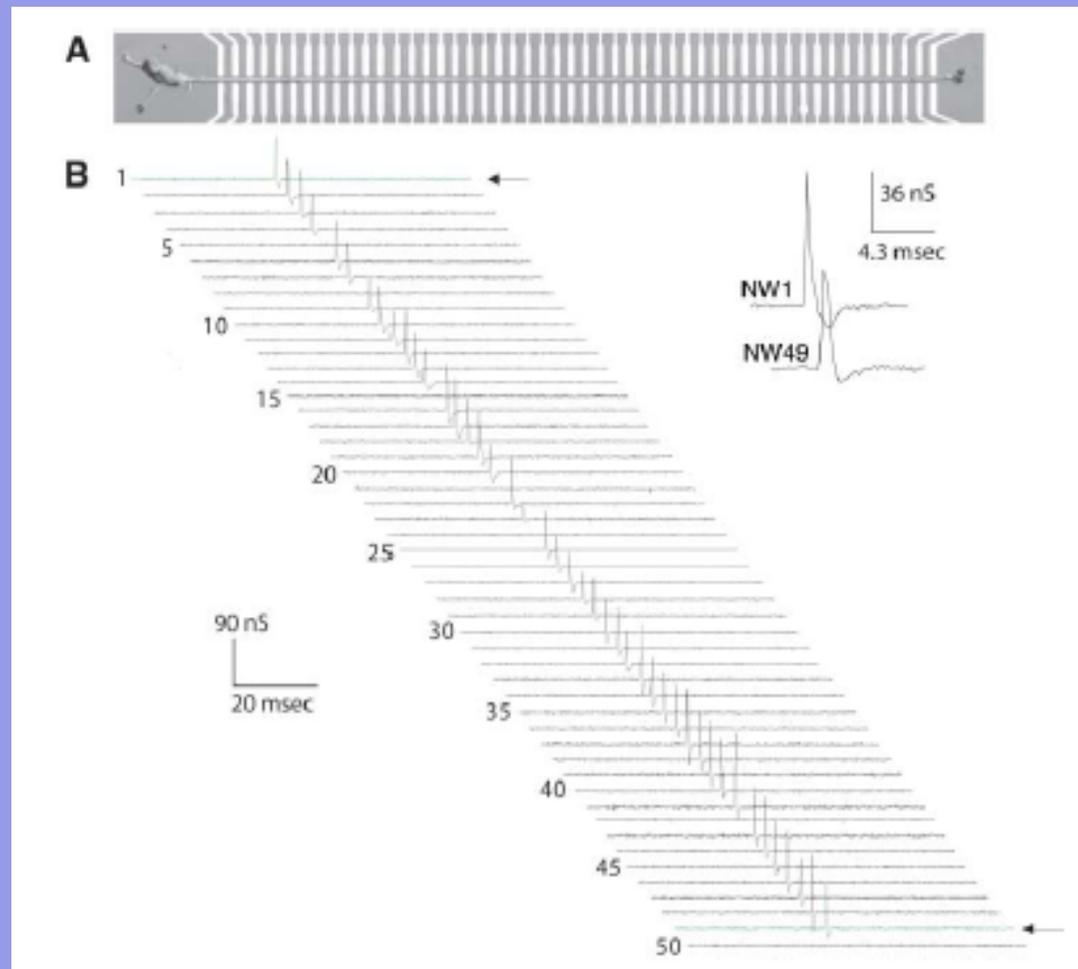
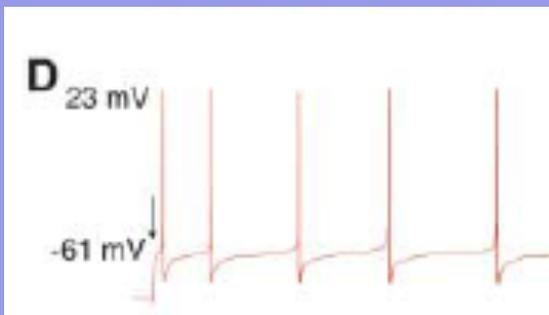
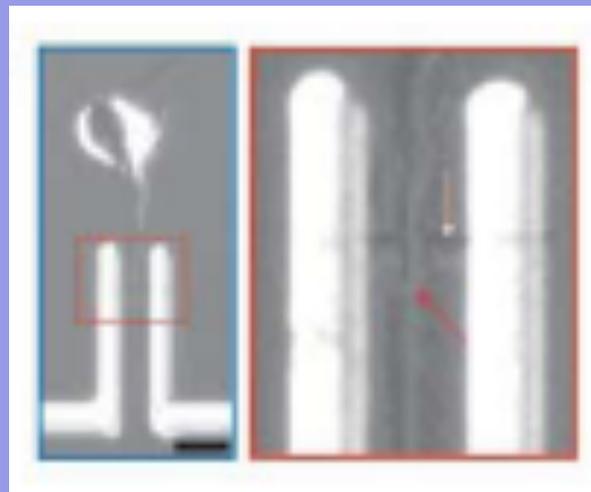


- **Physical properties** : optical, electrical, mechanical, magnetic...

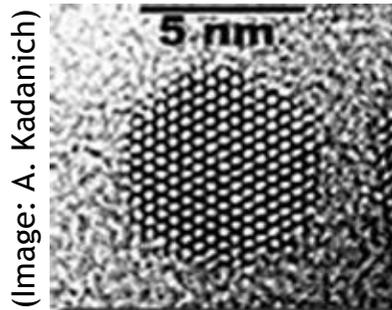
Detection, Stimulation, and Inhibition of Neuronal Signals with High-Density Nanowire Transistor Arrays

Fernando Patolsky,^{1*} Brian P. Timko,^{1*} Guihua Yu,¹ Ying Fang,¹ Andrew B. Greytak,¹
Gengfeng Zheng,¹ Charles M. Lieber^{1,2}†

SCIENCE VOL 313 25 AUGUST 2006



Semiconductor nanocrystals: structure and synthesis

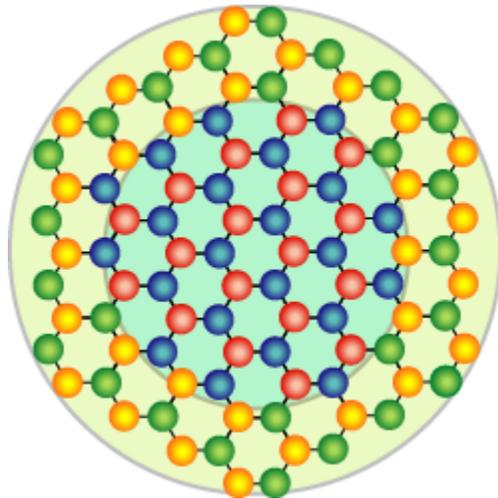


Colloidal nanoparticles composed of
100 to 100000 atoms

Size and shape can be controlled
during the colloidal synthesis

Core :
CdSe

Shell :
ZnS



Murray CB, Norris DJ, Bawendi MG *JACS* (1993)

Hines, M. A.; Guyot-Sionnest, P. *J. Phys. Chem.* (1996)

Dabbousi BO, *et al.* *J. Phys. Chem. B* (1997)

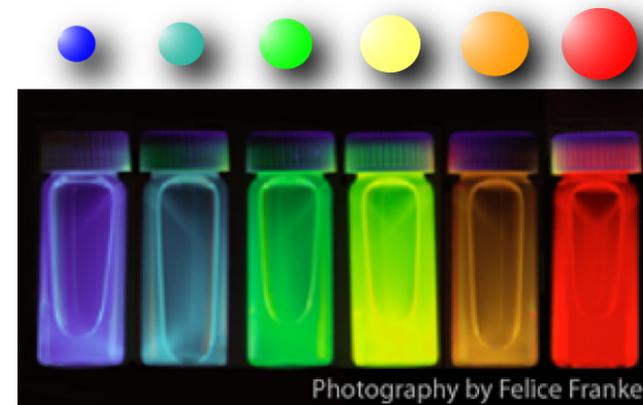
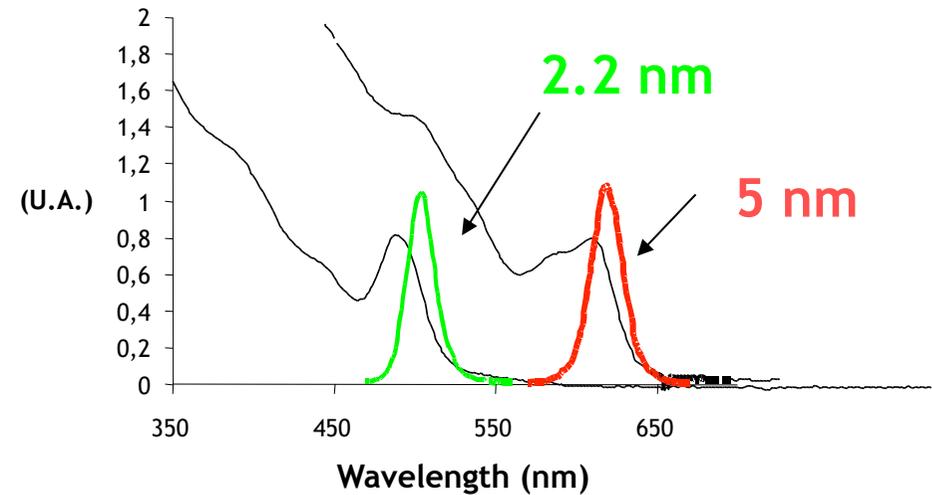
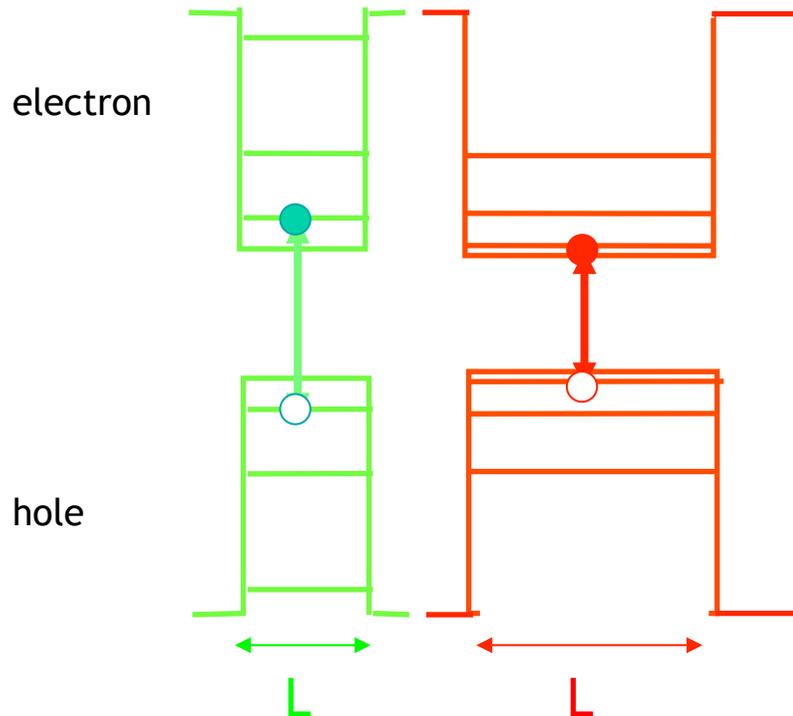
Peng X, *et al.* *JACS* (1997)

optical properties are dominated by the quantum confinement

a « particle-in-a-box » when $L < a_B \sim 6 \text{ nm}$

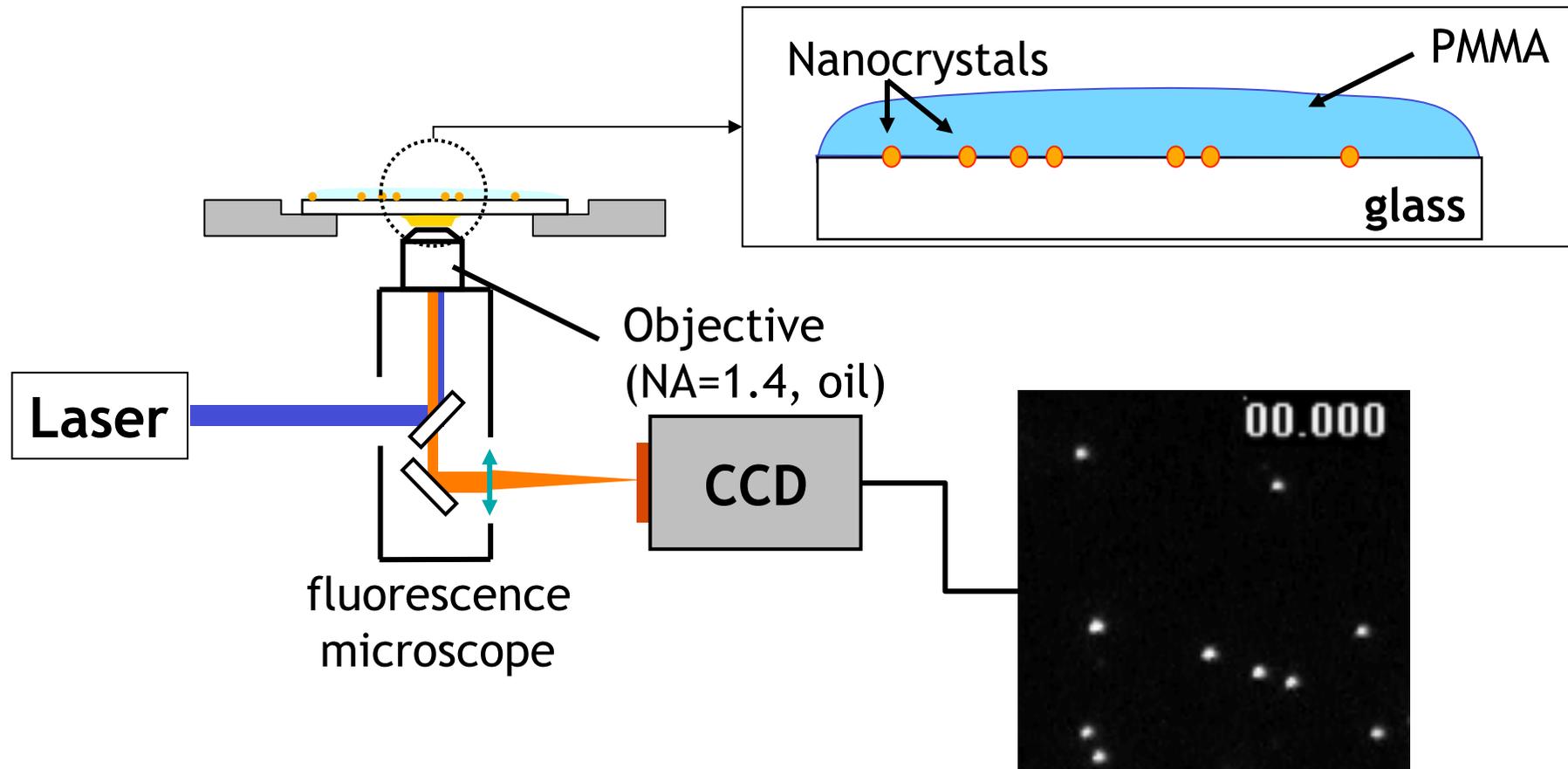
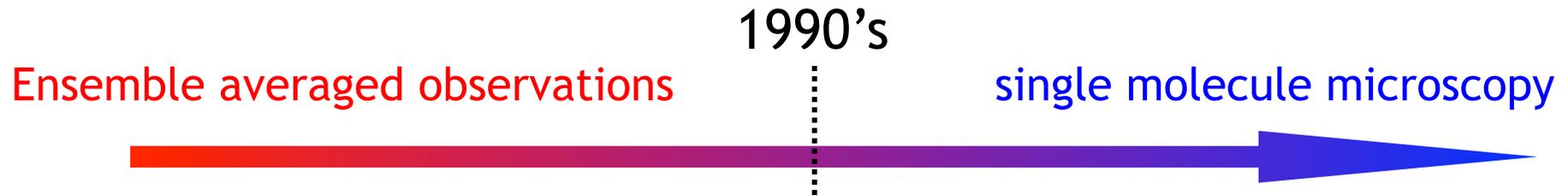
$$\frac{\hbar^2}{2m} \frac{\partial^2 \Psi(x)}{\partial x^2} + E\Psi(x) = 0$$

$$E_s = \frac{\hbar^2 \pi^2}{2mL^2}$$



Detecting single nanoparticles by optical means

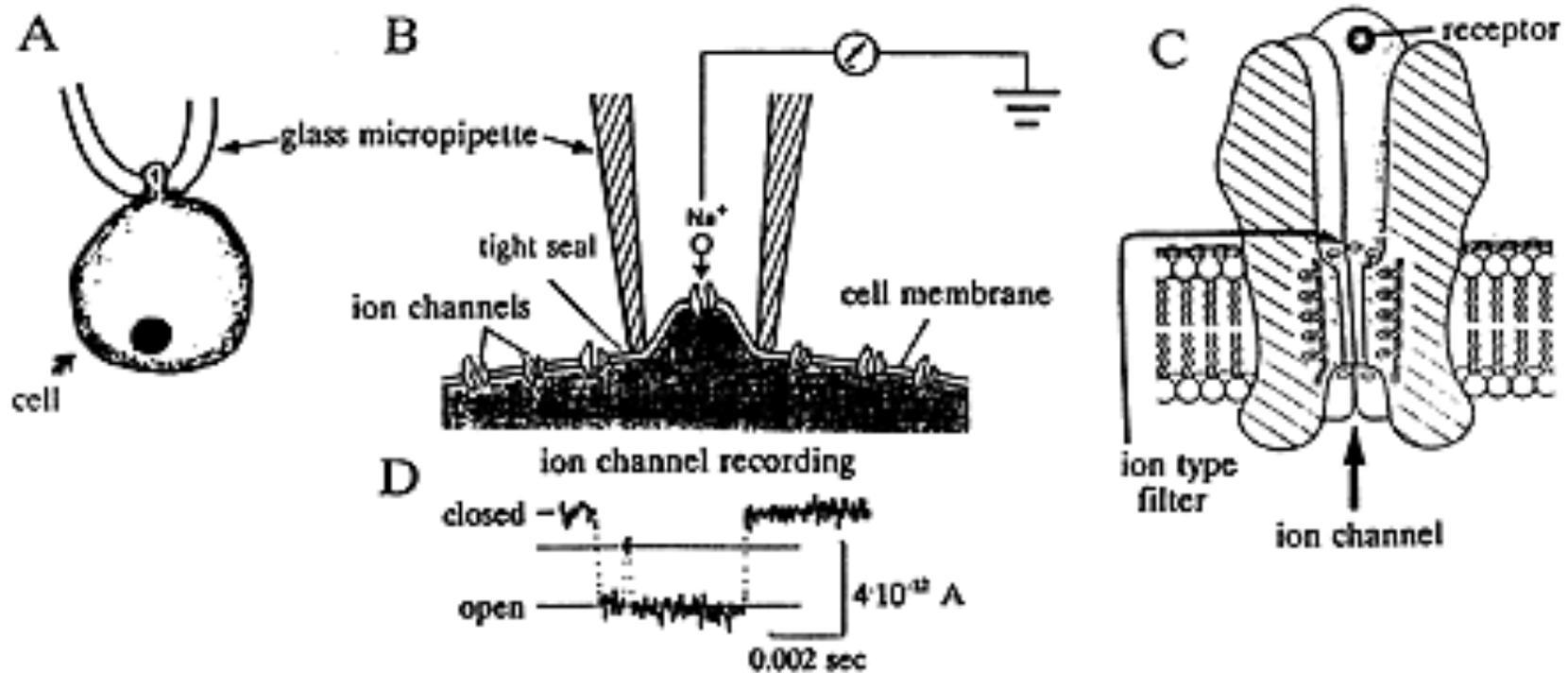
Single molecule imaging and spectroscopy



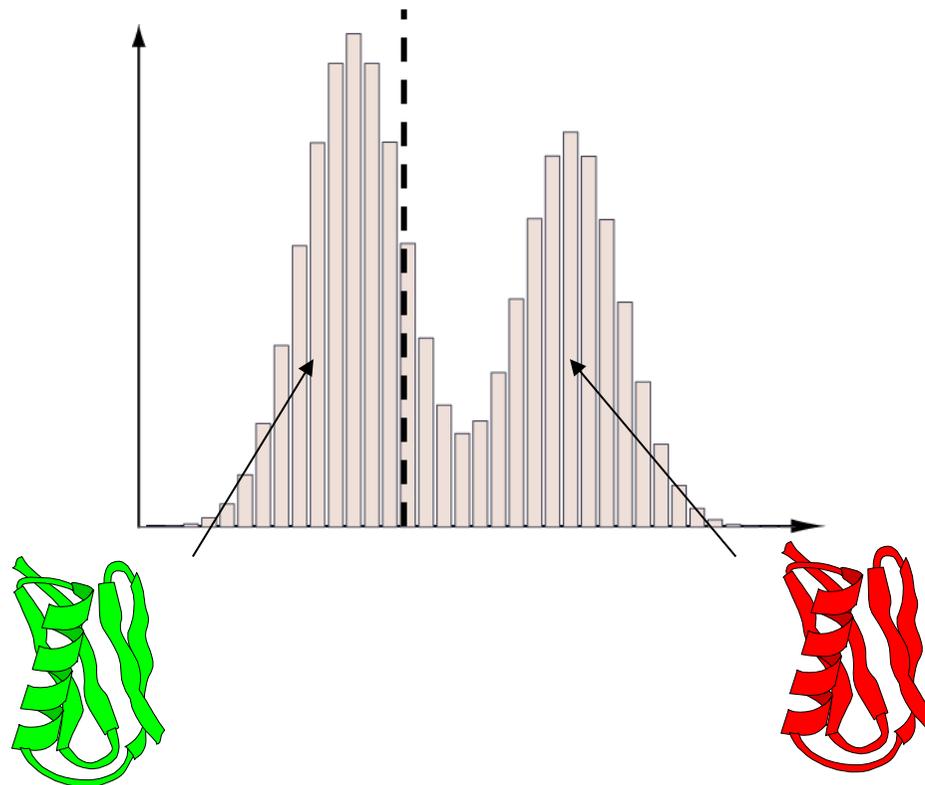
Single molecule biophysics

Ion channels

Neher and Sackmann (1976) [Nobel Prize 1991]

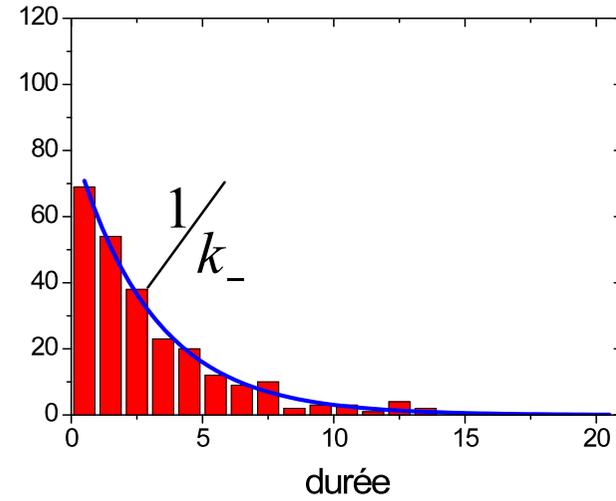
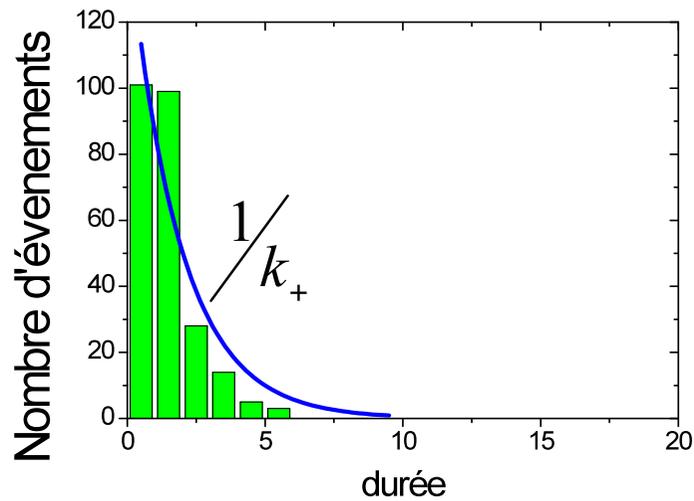
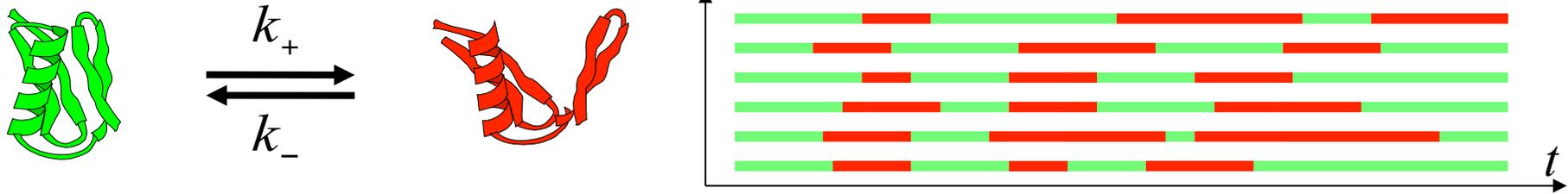


Static fluctuations



**Identify heterogeneities in a sample
by examining molecules one at a time**

Dynamic fluctuations

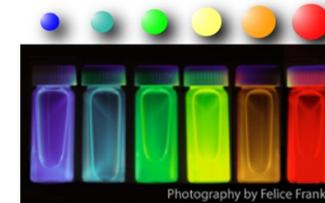
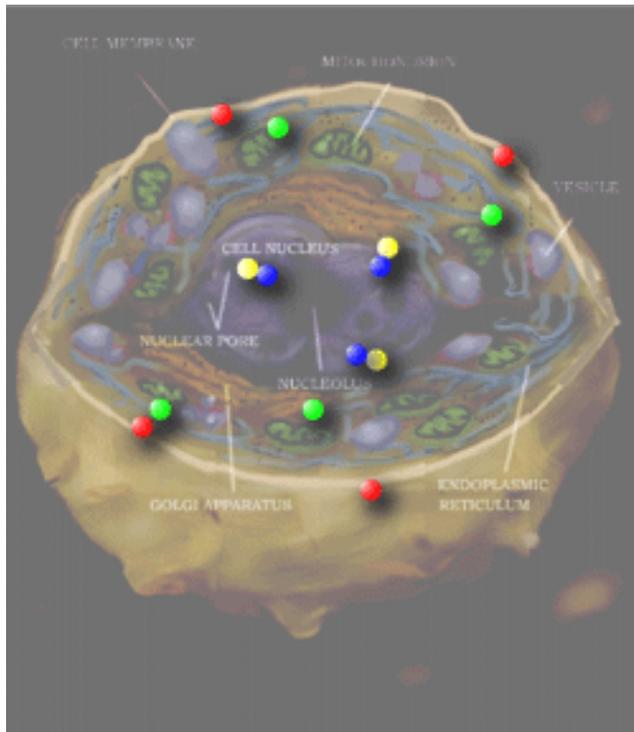


Access kinetic parameters, even at equilibrium

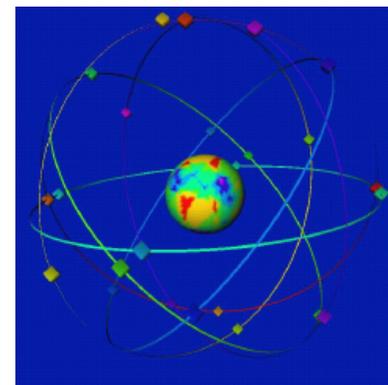
Single molecule measurements

- Static fluctuations : probing heterogeneities
- Dynamic fluctuations : investigating the kinetics of stochastic processes
- Some cellular processes are mediated by few molecules :
Ex: Gene regulation by transcription factors
Signal transmission at synapses

Towards a single-molecule positioning system in live cells

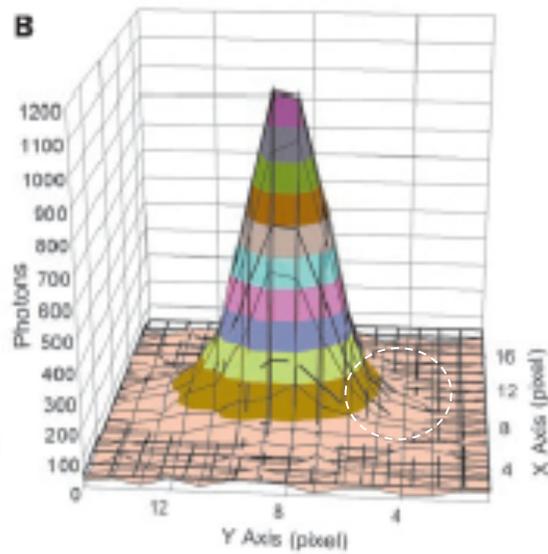


- Multicolor tagging and detection
- Real-time observation
- 3D Imaging and nanometer localization

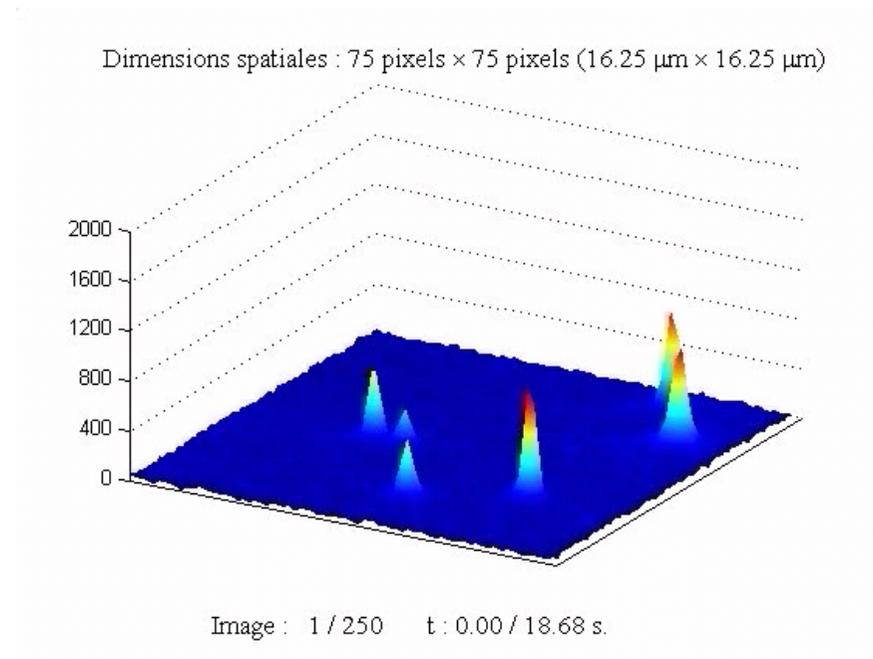


« cellular GPS »

The localization accuracy is in the nanometer range



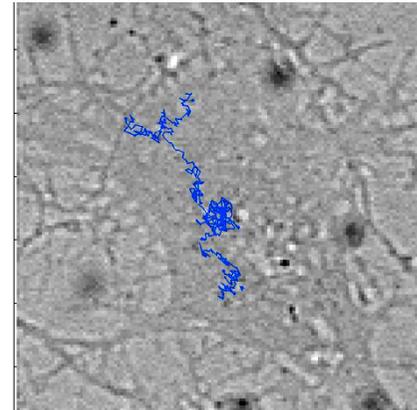
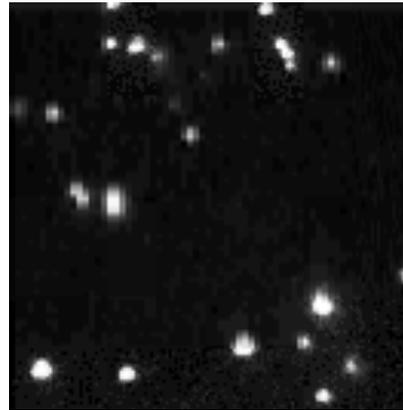
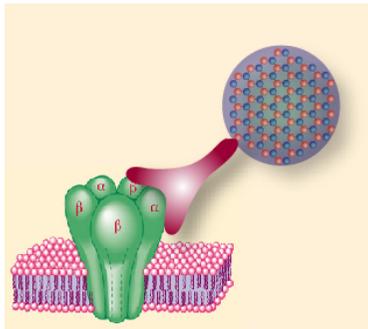
Yildiz et al. Science (2003)



Localization:

$$\sigma \approx \frac{(\lambda/2)}{\sqrt{N}} \sim 5 - 10 \text{ nm}$$

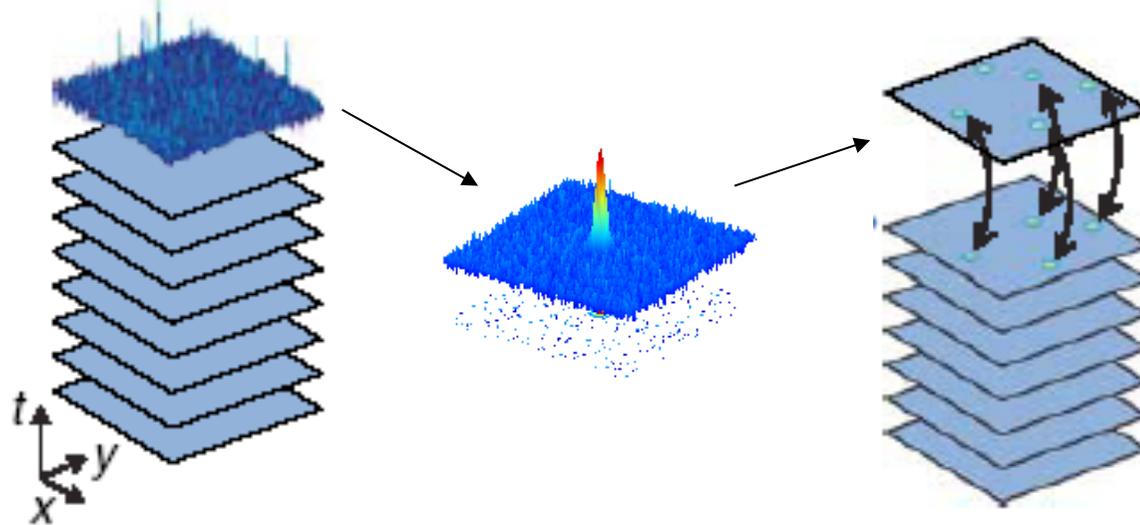
Tracking individual molecules



acquisition

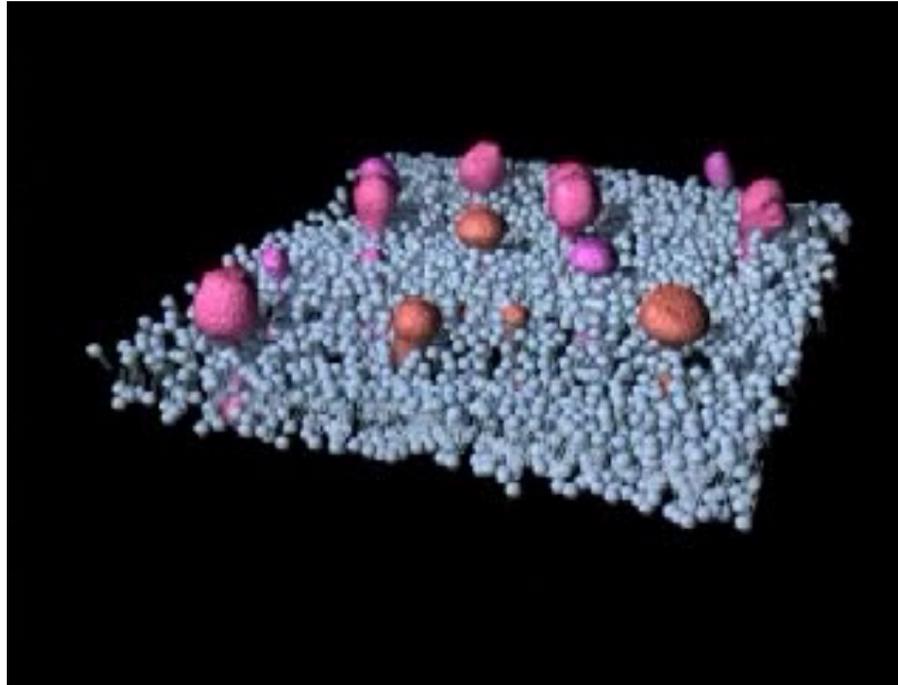
localization

tracking



At the molecular scale, the biological world is intrinsically brownian

Mobility in the membrane



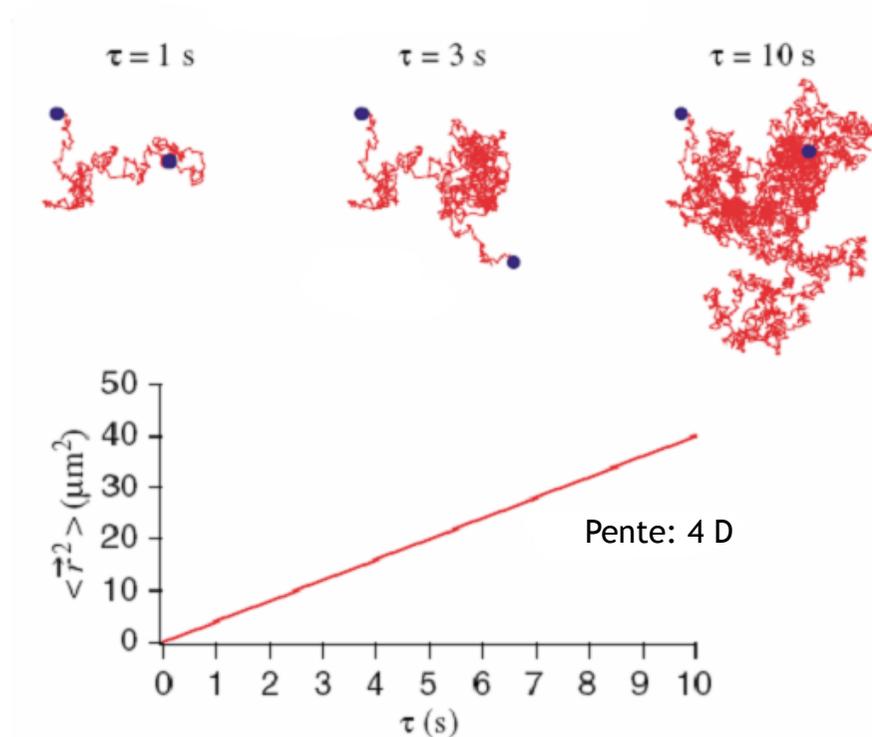
(movie from A. Kusumi's lab)

« Fluid mosaic model »
Singer and Nicolson (1972)

« fast » proteins
diffuse with
 $D \sim 0.1 \mu\text{m}^2/\text{s}$

How to achieve a dynamic organization in the membrane ?

Analyse d'un mouvement en deux dimensions



$\langle r^2 \rangle$: déplacement quadratique moyen
(*mean square displacement: MSD*)

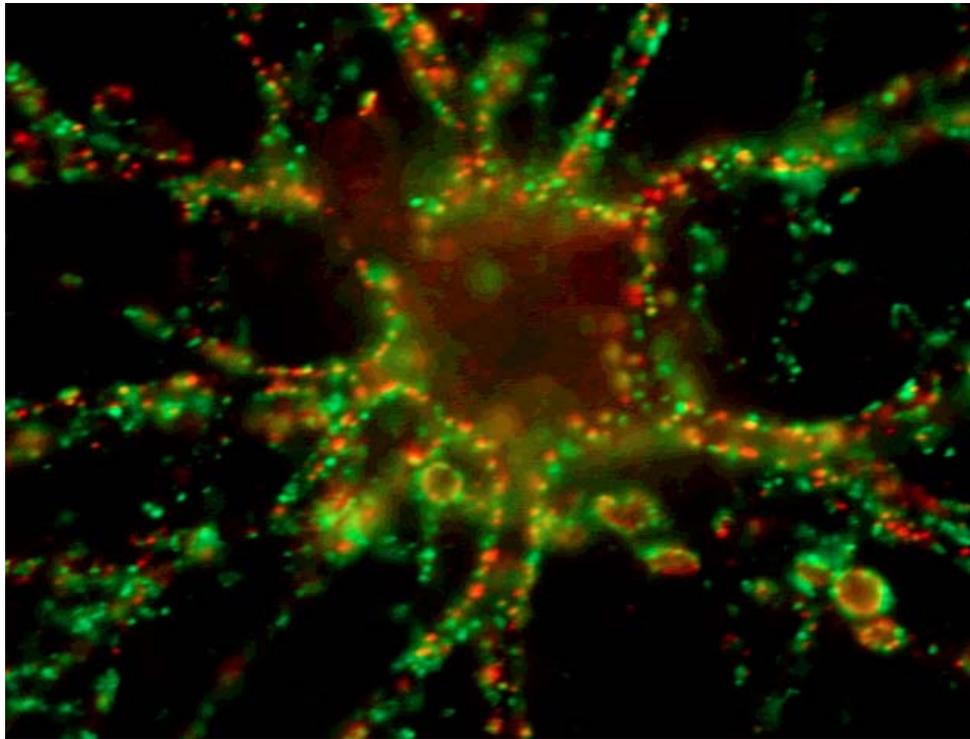
$$\langle r^2 \rangle = 4 D \tau$$

D : coefficient de diffusion

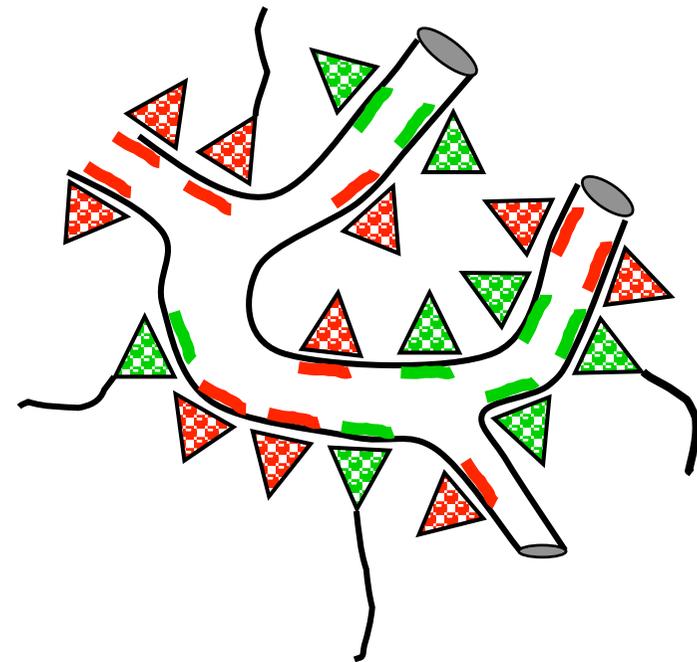
Mouvement brownien : D = constant

**Membrane compartmentalization:
Diffusion dynamics of membrane
receptors in live neurons**

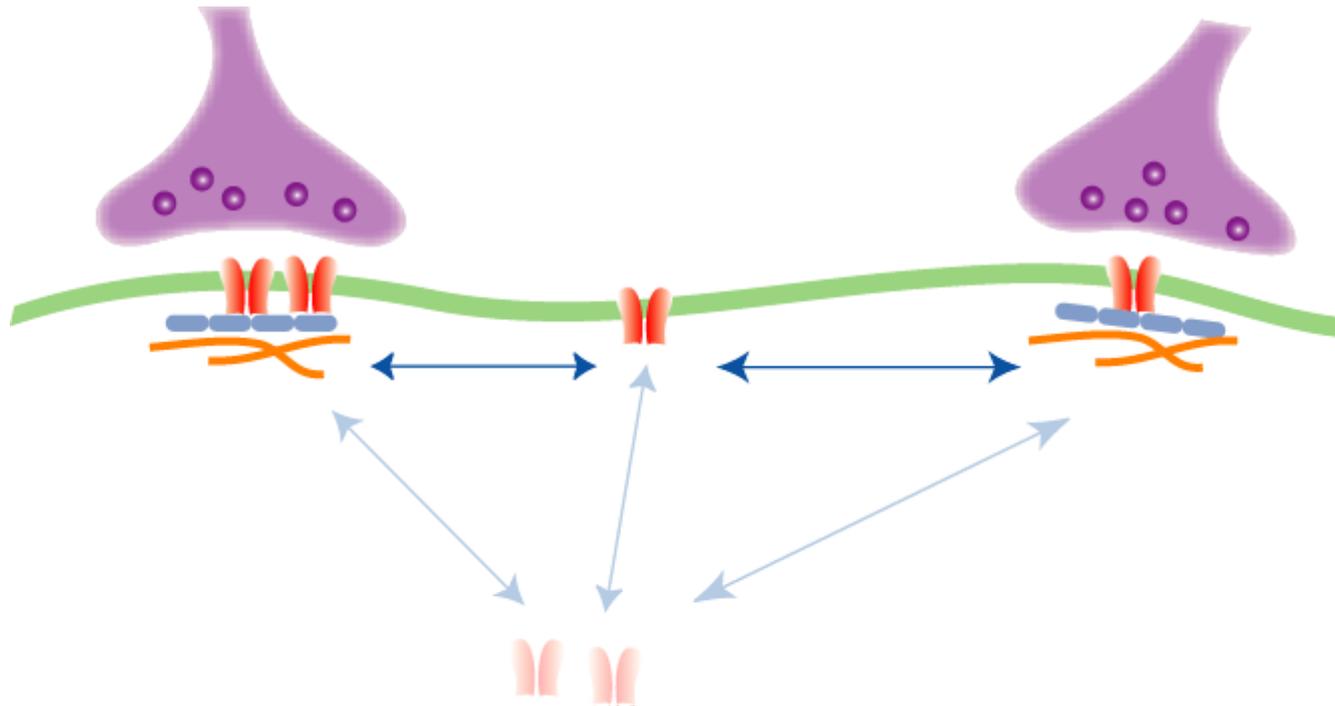
Le « manteau d'arlequin » d'un neurone en culture



(Image : Levi/Triller)



Lateral dynamics of Glycine receptors

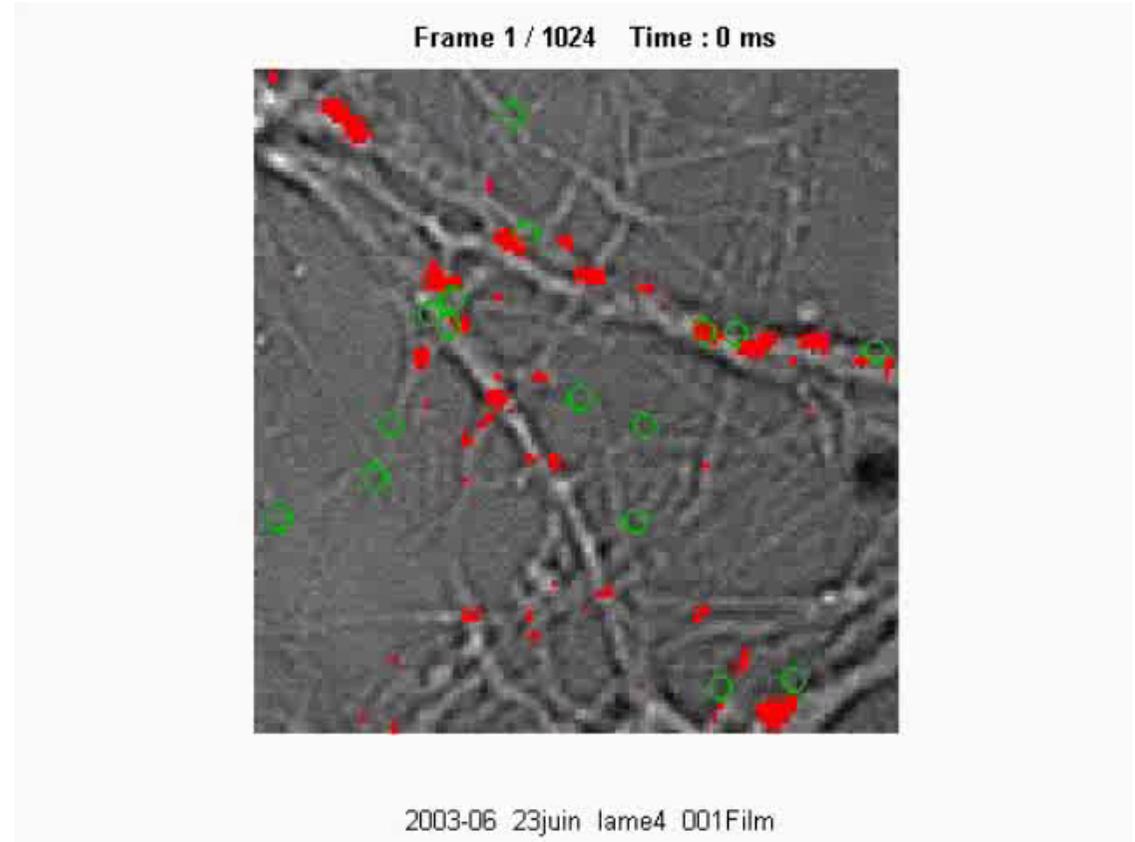


Change in the number of receptors at synapses during **synaptic plasticity** and **synaptogenesis**

Single quantum dot tracking of glycine receptors in live neurons



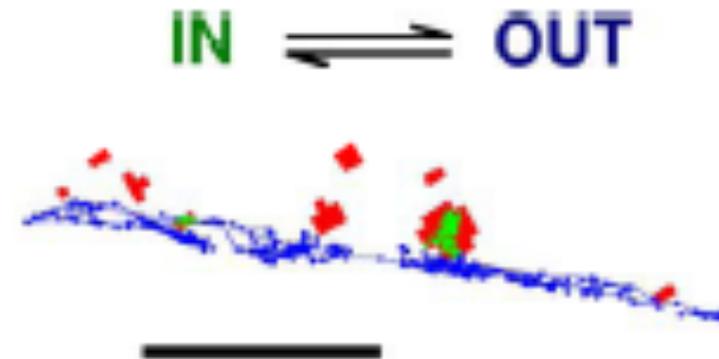
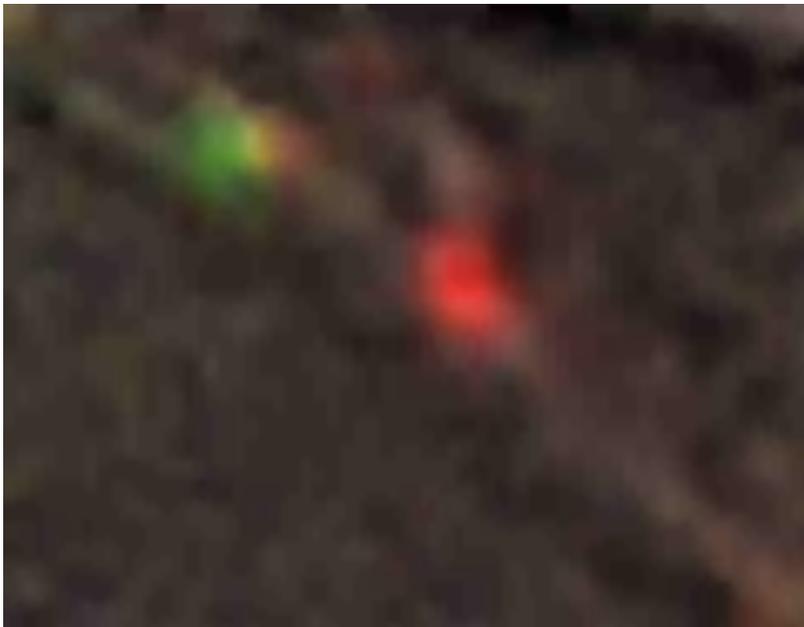
Acquisition time: 75 ms
Total time : 65 s



Red : marker for synapses

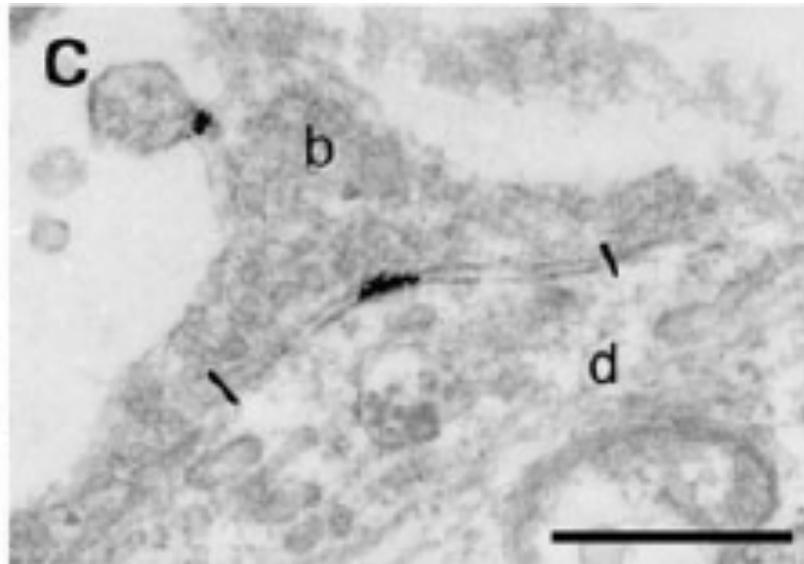
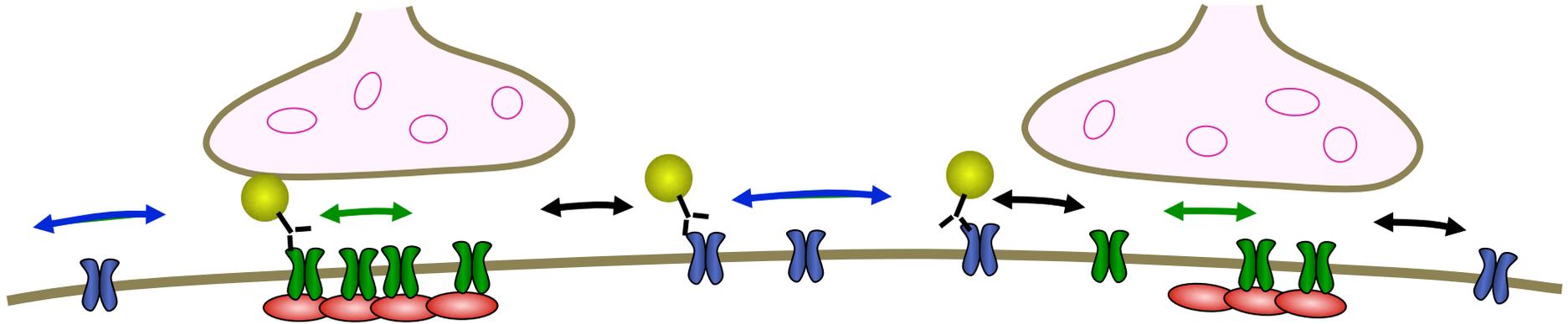
Dahan et al., Science (2003)
Ehrensperger et al. Biophys. J. (2007)

Single QD tracking directly reveals entry and exit of receptors at synapses



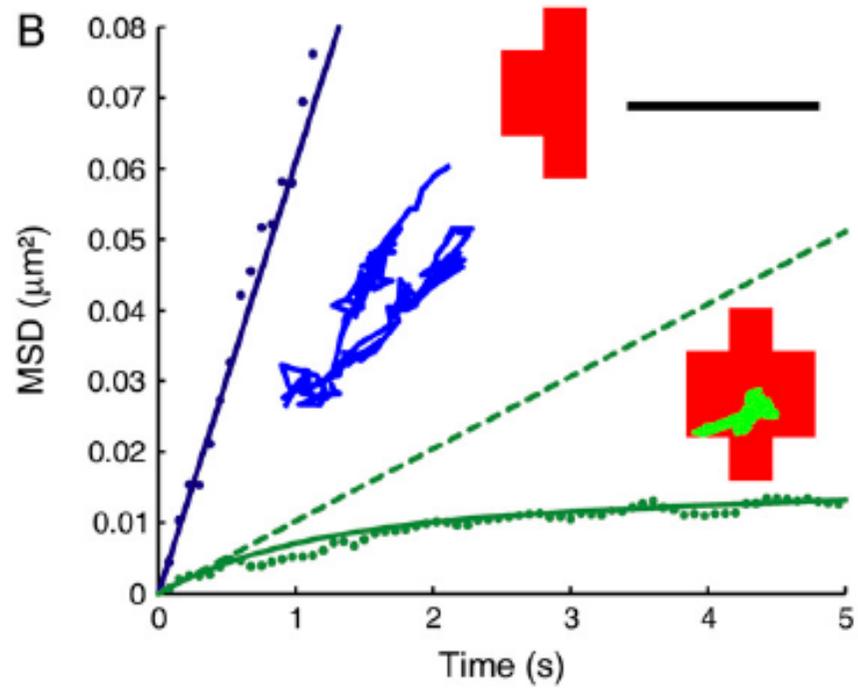
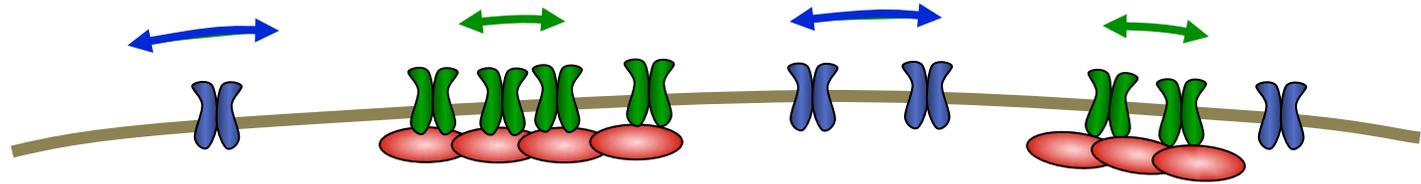
In red : marker for presynaptic vesicles (FM 4-64)

Extra ↔ Syn

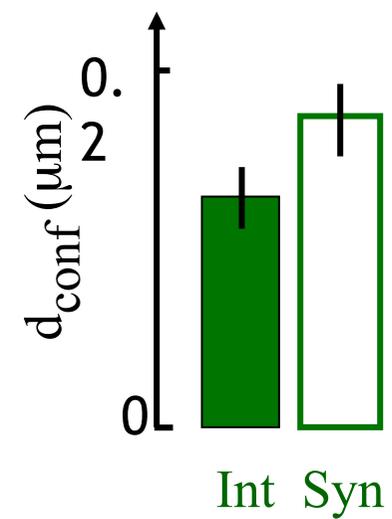
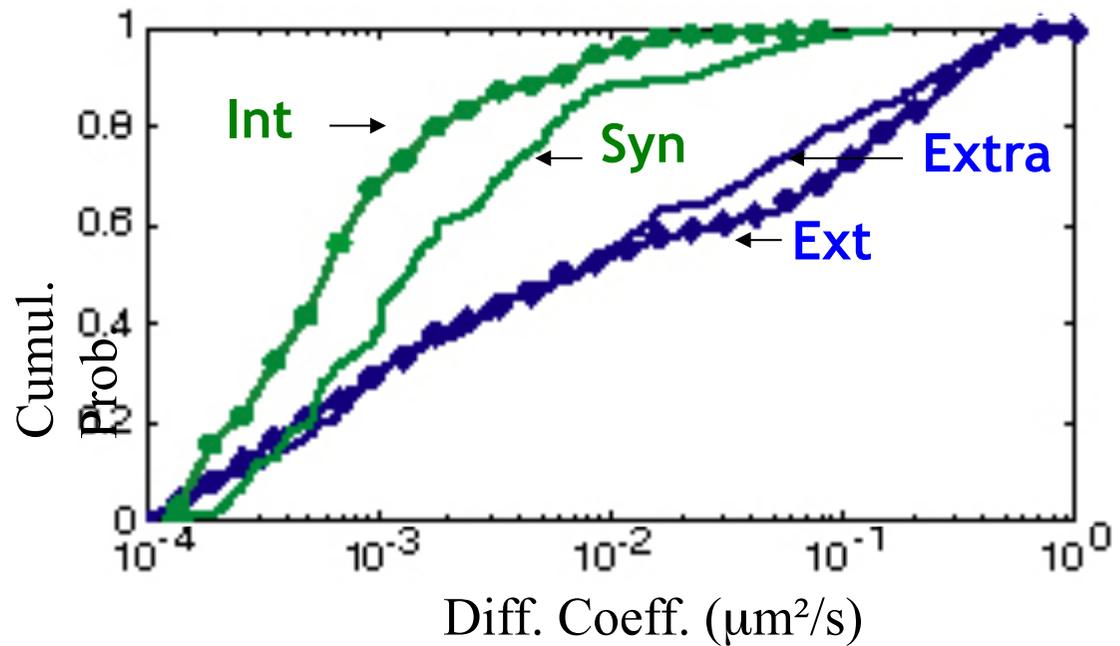
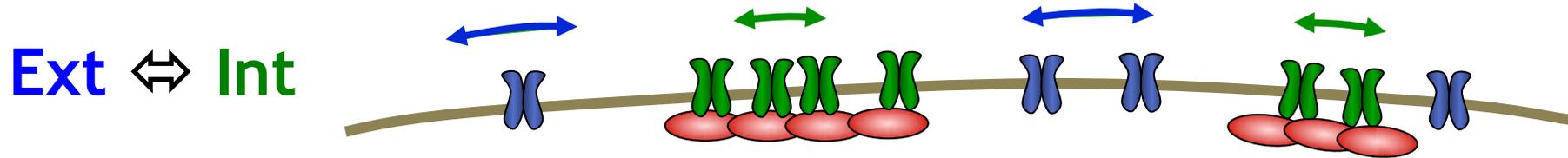


Diffusion properties of RGly

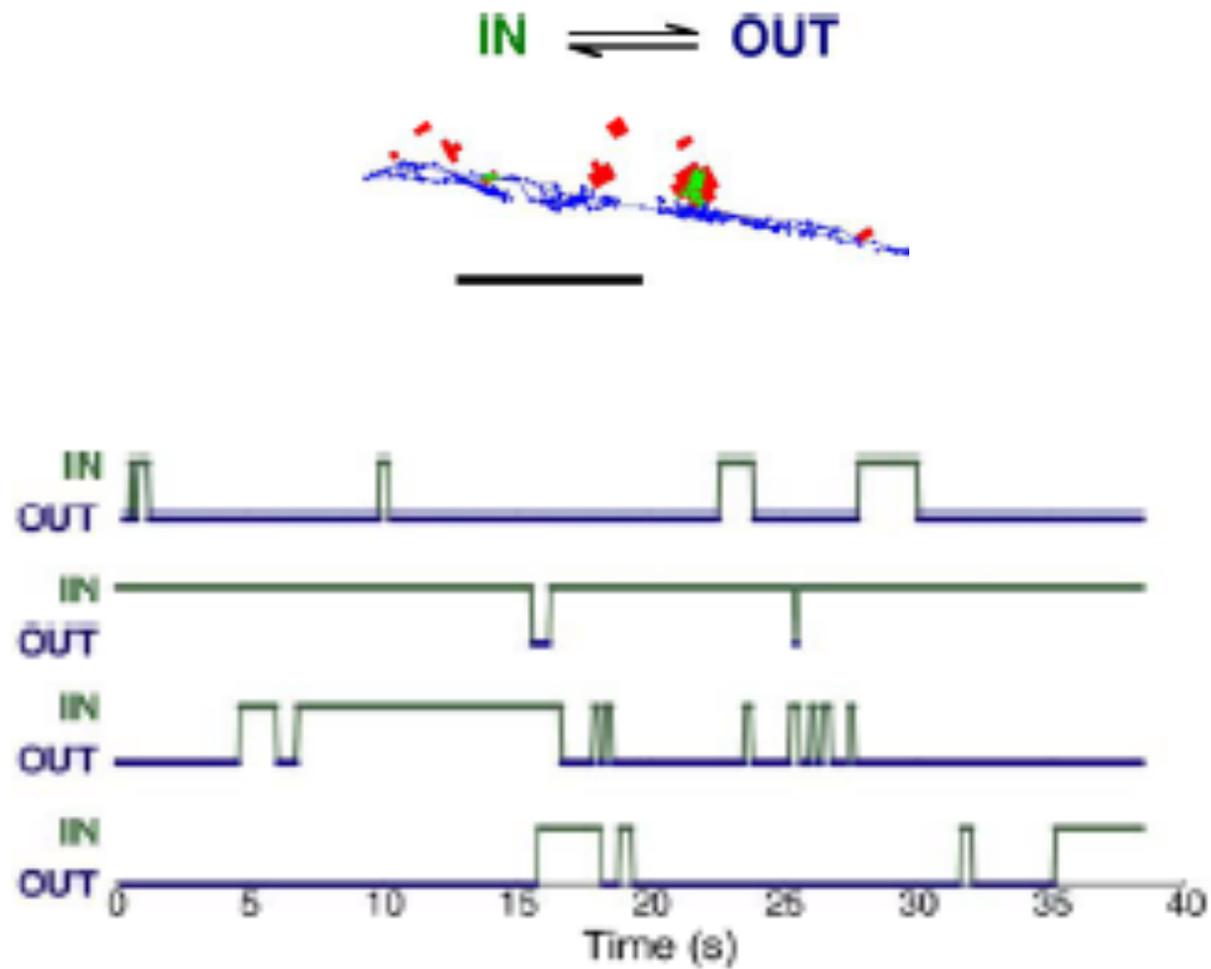
Ext \leftrightarrow Int

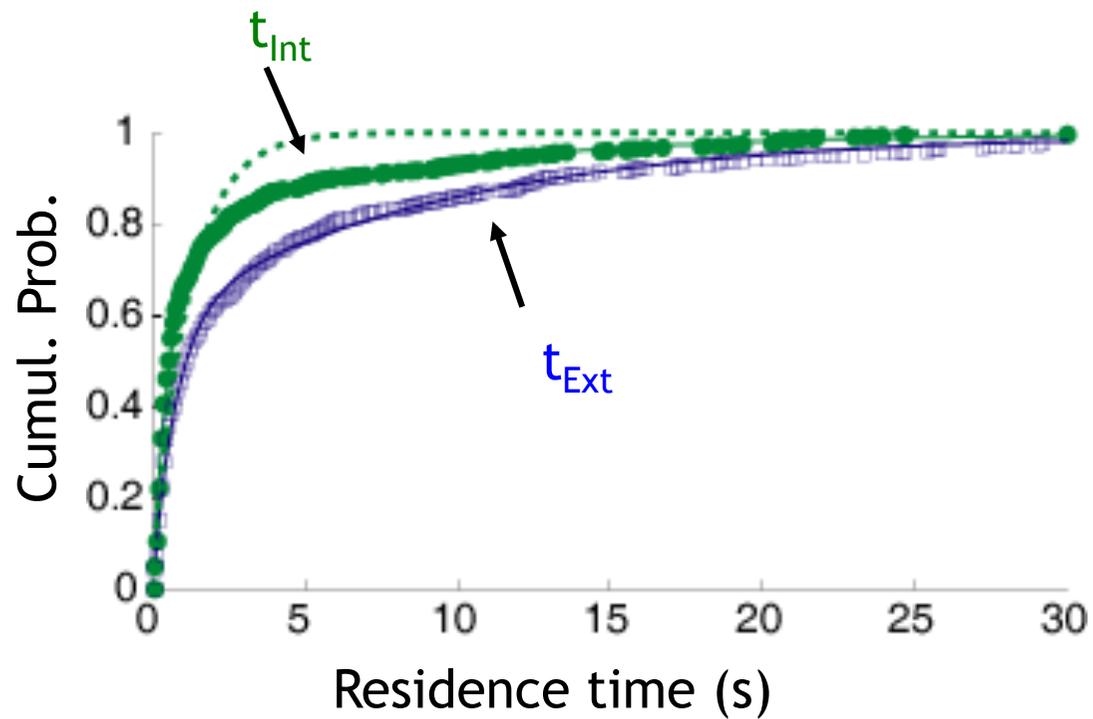


Diffusion properties of RGly



Kinetic properties of stabilization by gephyrin clusters



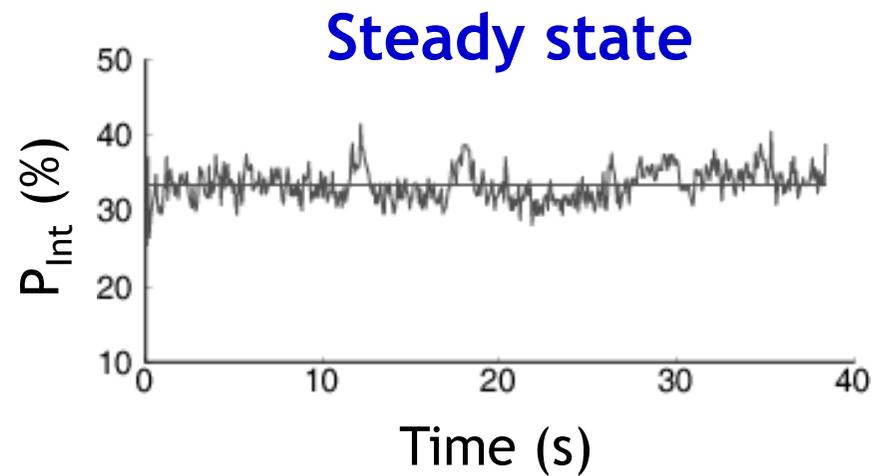


$$\langle t_{Int} \rangle = 2.3 \text{ s}$$

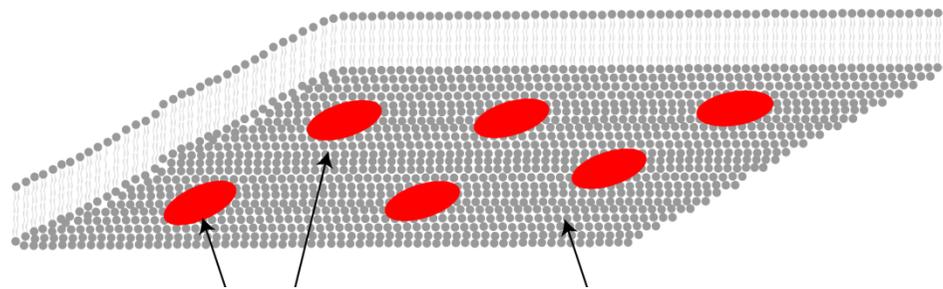
$$\langle t_{Ext} \rangle = 4.3 \text{ s}$$

$$F_{Int} \approx 35\%$$

$$F_{Int} \approx P_{Int}$$



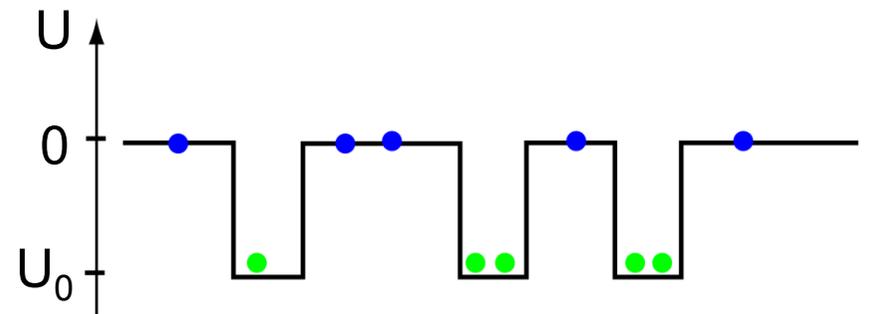
$$P_{\text{IN}} = \frac{\alpha \times \exp(-U_0/kT)}{1 - \alpha + \alpha \times \exp(-U_0/kT)}$$



Gephyrin cluster

$U_{\text{Ext}}=0$

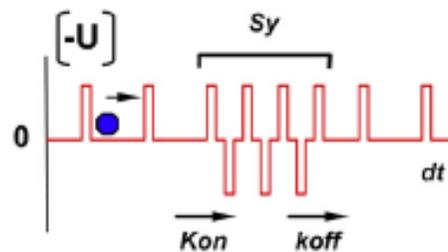
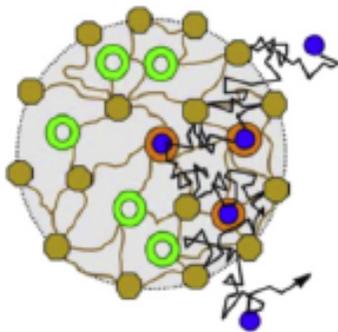
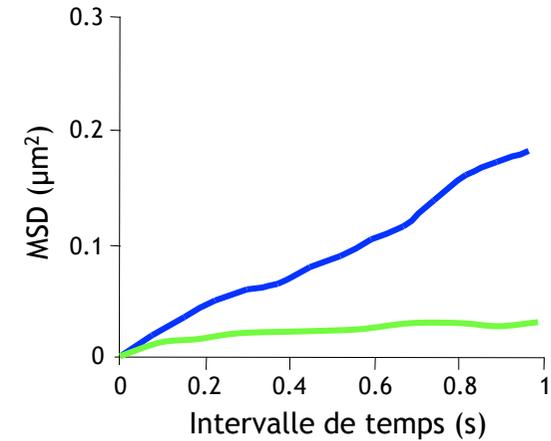
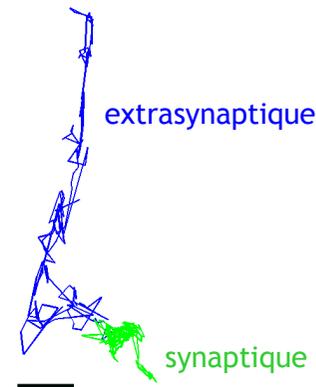
$U_{\text{Int}}=U_0$



$U_0 \sim -2kT$

- Une partie des récepteurs s'échange rapidement entre la membrane synaptique et extrasynaptique.

- La diffusion de récepteurs est ralentie à la synapse par la présence des interactions mais aussi par la présence des obstacles, car la membrane synaptique est encombrée.

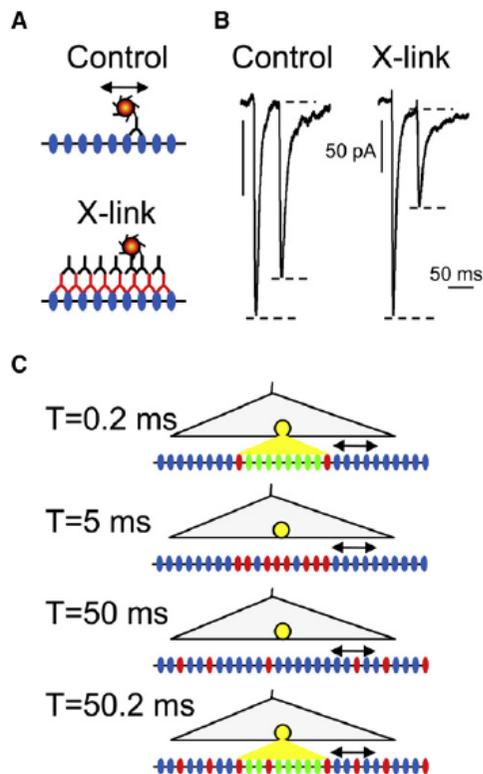


Quelles sont les conséquences de cette dynamique sur la physiologie synaptique?

Surface Mobility of Postsynaptic AMPARs Tunes Synaptic Transmission

Martin Heine,^{1*} Laurent Groc,¹ Renato Frischknecht,⁴ Jean-Claude Béïque,³ Brahim Lounis,² Gavin Rumbaugh,³ Richard L. Huganir,³ Laurent Cognet,² Daniel Choquet^{1†}

Science 320:201-205



Dans des conditions de stimulation excitatrice rapide, les récepteurs de type AMPA désensibilisés sont échangés avec des récepteurs naïfs par diffusion latérale.



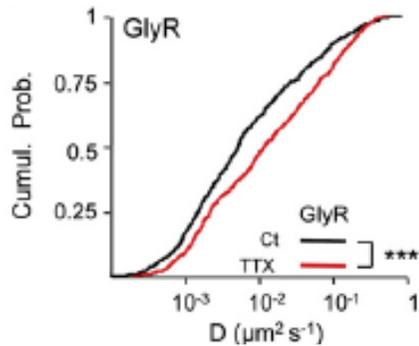
La régulation de la mobilité des récepteurs peut affecter la fidélité de la transmission synaptique

Homeostatic Regulation of Synaptic GlyR Numbers Driven by Lateral Diffusion

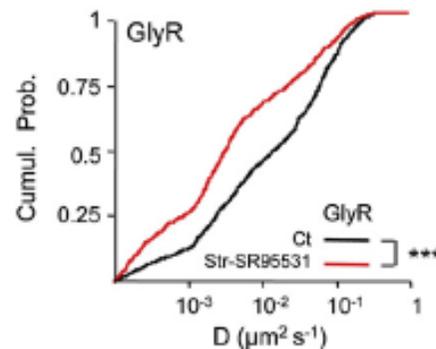
Sabine Lévi,^{1,2} Claude Schweizer,¹ Hiroko Bannai,^{1,3} Olivier Pascual,¹ Cécile Charrier,¹ and Antoine Triller^{1,*}

Neuron 59:261-273

Inhibition de l'activité synaptique

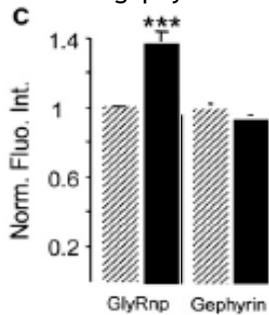


Augmentation de l'activité synaptique

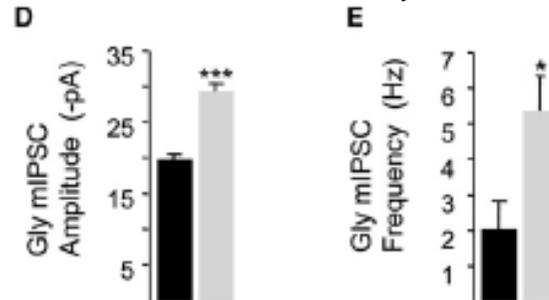


L'activité synaptique excitatrice modifie la mobilité des GlyR (récepteur inhibiteur). En conséquence, la quantité des GlyR synaptiques est modifiée de manière homeostatique.

Quantité de GlyR et gephyrine



Activité GlyR



Conclusion:

« la force des liens faibles »

- on a accès par des méthodes combinant imagerie optique et nanosciences aux comportements moléculaires : vers la biochimie in situ
- les interactions entre protéines semblent être souvent de faible affinité
- comment comprendre à partir de là le saut d'échelle entre les comportements microscopiques et la dynamique « macroscopique » de la cellule ?

The Strength of Weak Ties

Mark S. Granovetter

The American Journal of Sociology, Vol. 78, No. 6. (May, 1973), pp. 1360-1380.

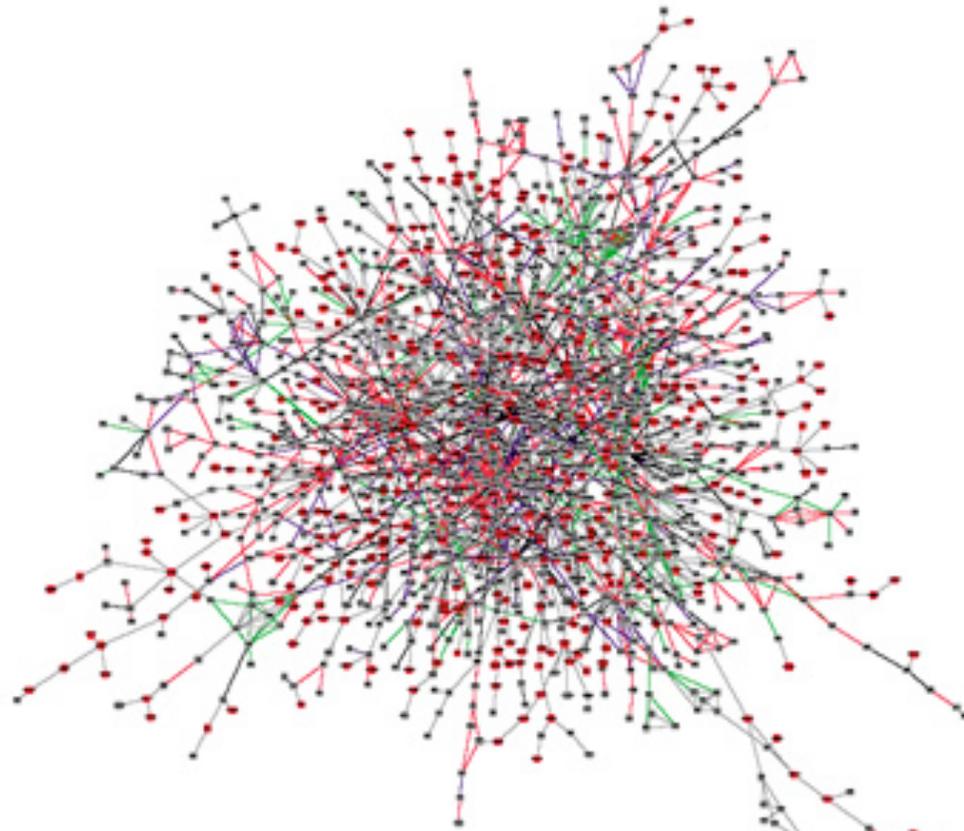
Stable URL:

<http://links.jstor.org/sici?sici=0002-9602%28197305%2978%3A6%3C1360%3ATSOWT%3E2.0.CO%3B2-E>

The American Journal of Sociology is currently published by The University of Chicago Press.



Les réseaux de protéines



Carte d'interactions dans le protéome de la levure

La compréhension de ces réseaux/circuits d'interaction a conduit au développement d'une nouvelle discipline:
la biologie synthétique