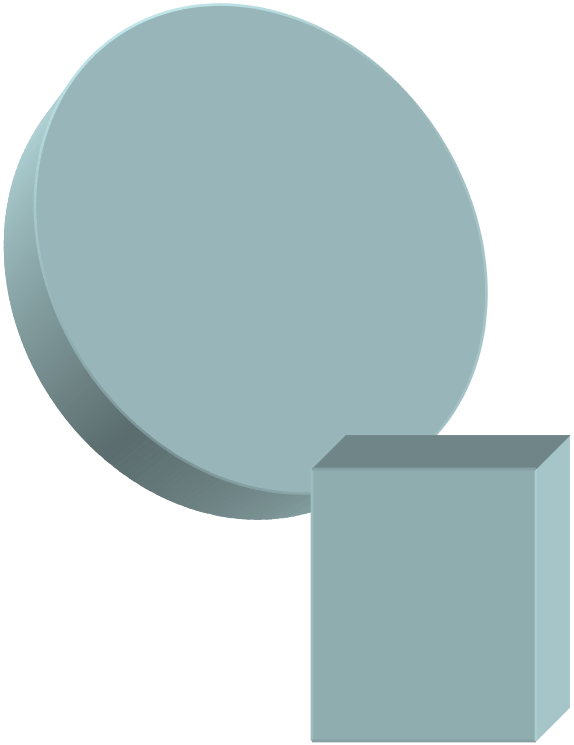
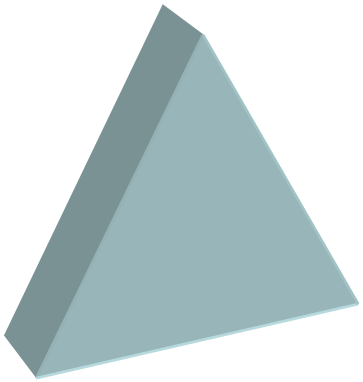
Cellular and Synthetic Membranes



James L. Thomas
University of New Mexico
Biophysics Seminar
February 18, 2004



Timeline | **A century of cell-membrane bilayers**

Lord Rayleigh, Agnes Pockels and many others begin to investigate the spreading of oil on water.

Langmuir[7] publishes a model of how oil molecules are orientated at the water/air interface, which is based on t experiments of Agn Pockels but with an improved apparatu (BOX 1).

After considerable

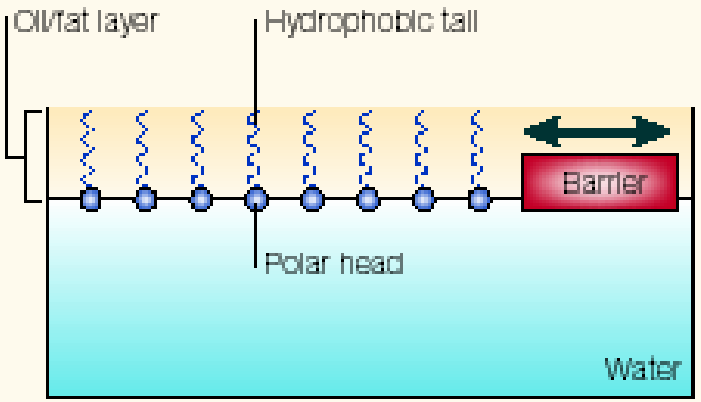
1880s 1899 1917

Overton[1] describes a lipid barrier between the eukaryotic cell cytoplasm and the outside world. This work also focuses attention on the cell-surface membrane as the membrane that is most accessible to experimental study.

Gort use meti eryt mer bilay

Box 1 | **Oil spreading on water, Ms Agnes Pockels and the Langmuir trough**

A Langmuir trough is a simple device for controlling the spreading of an oil or fat on a water surface (see figure). The molecules in the film become orientated so that their hydrophobic tails are in the air and their polar heads are in the water. A key part of this device is a method for moving the barrier to cause a defined lateral pressure against the oil layer. This was Langmuir's great improvement on Ms Agnes Pockels' apparatus (see below).



Oil films on water have been used and characterized in many different ways:

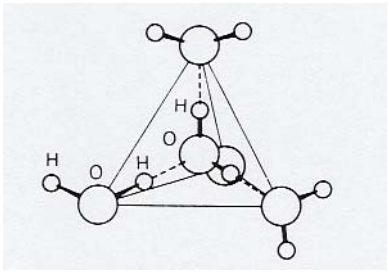
- Eighteenth century BC: Babylonians spread oil for divination.
- 1770: Benjamin Franklin experimented with the damping of surface waves by spreading olive oil on the surface of an English pond.
- Late nineteenth century: Lord Rayleigh worked on surface tension and received a letter from Agnes Pockels who developed the Langmuir trough in her family's kitchen. You can read more about Ms Pockels at the Contributions of 20th century women to physics web site (see Online links).
- Early twentieth century: Langmuir[7] provided detailed explanations of the thickness of oil layers and the orientation of molecules. He developed the Langmuir film balance to measure surface tension.

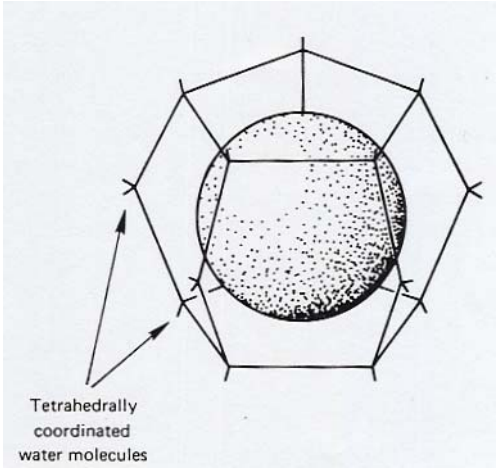
The diagram and information in this box were provided courtesy of M. Dennin, Department of Physics and Astronomy, University of California, Irvine, USA.

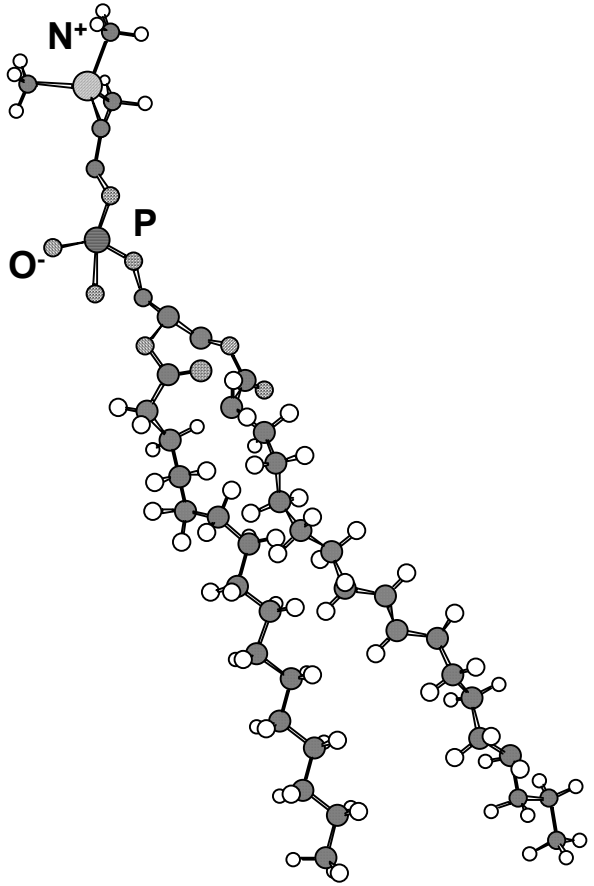
Michael Edidin

Johns Hopkins University

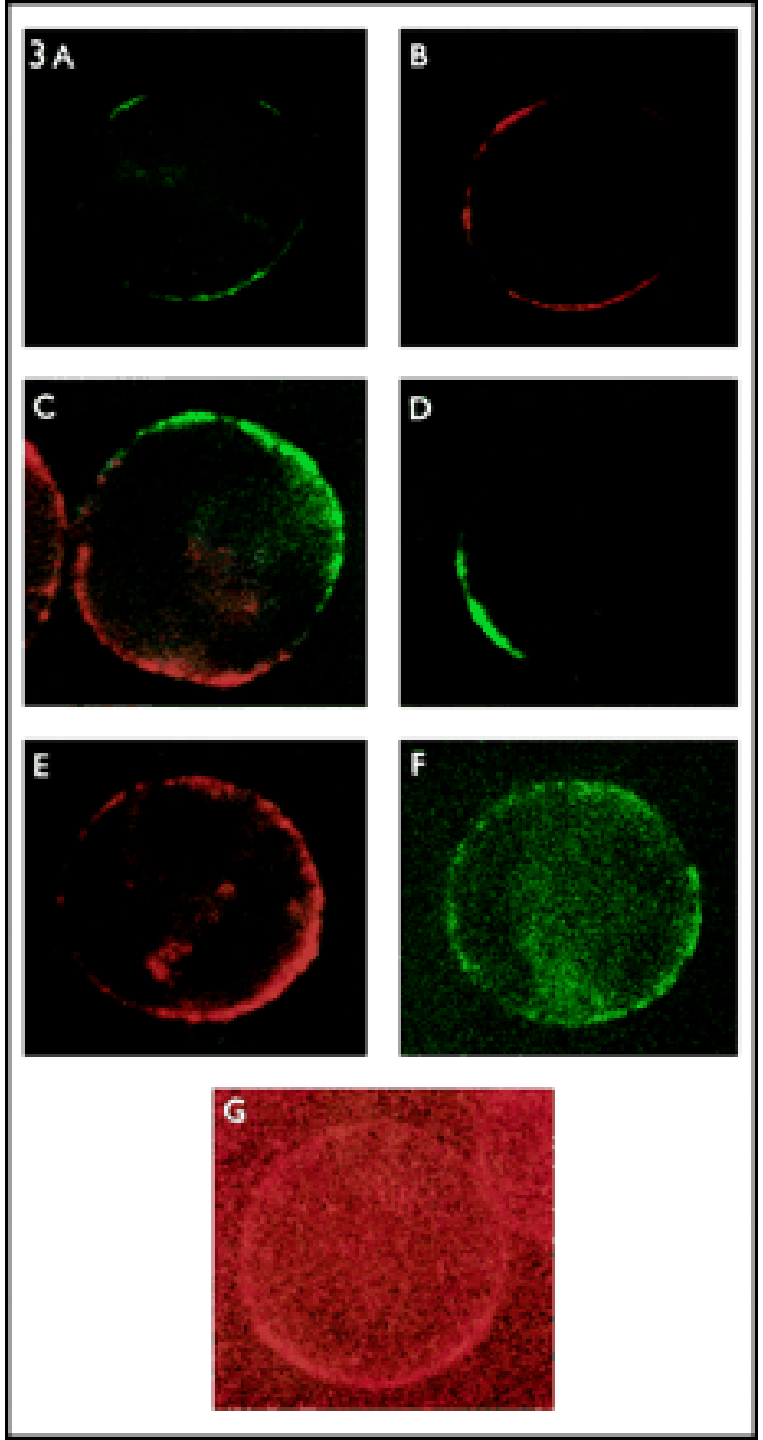
Amphiphilic Molecules Self-Assemble (in water)
By Hydrophobic Forces





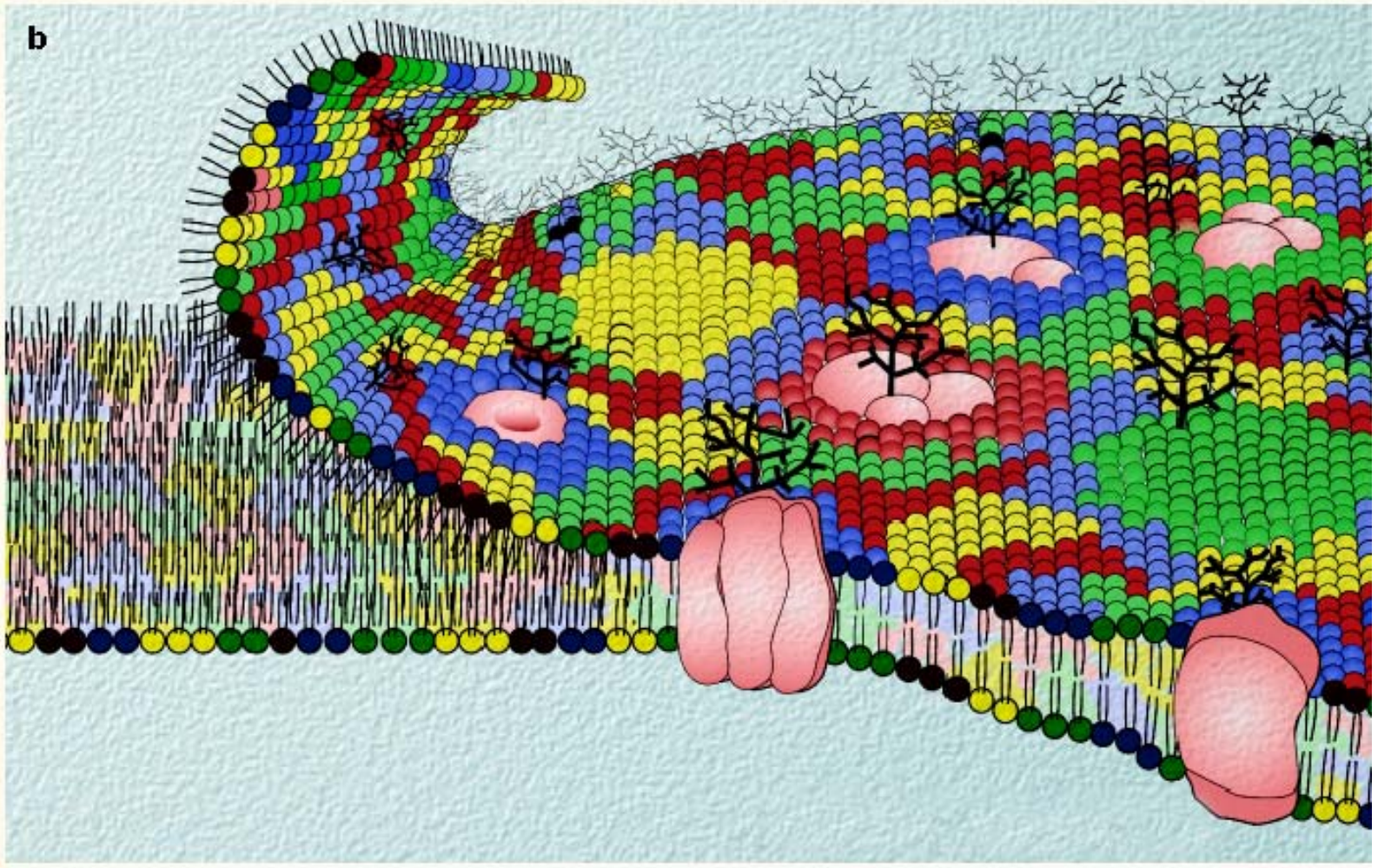


Palmitoyl Oleoyl Phosphatidylcholine
POPC
(a common cell membrane lipid)



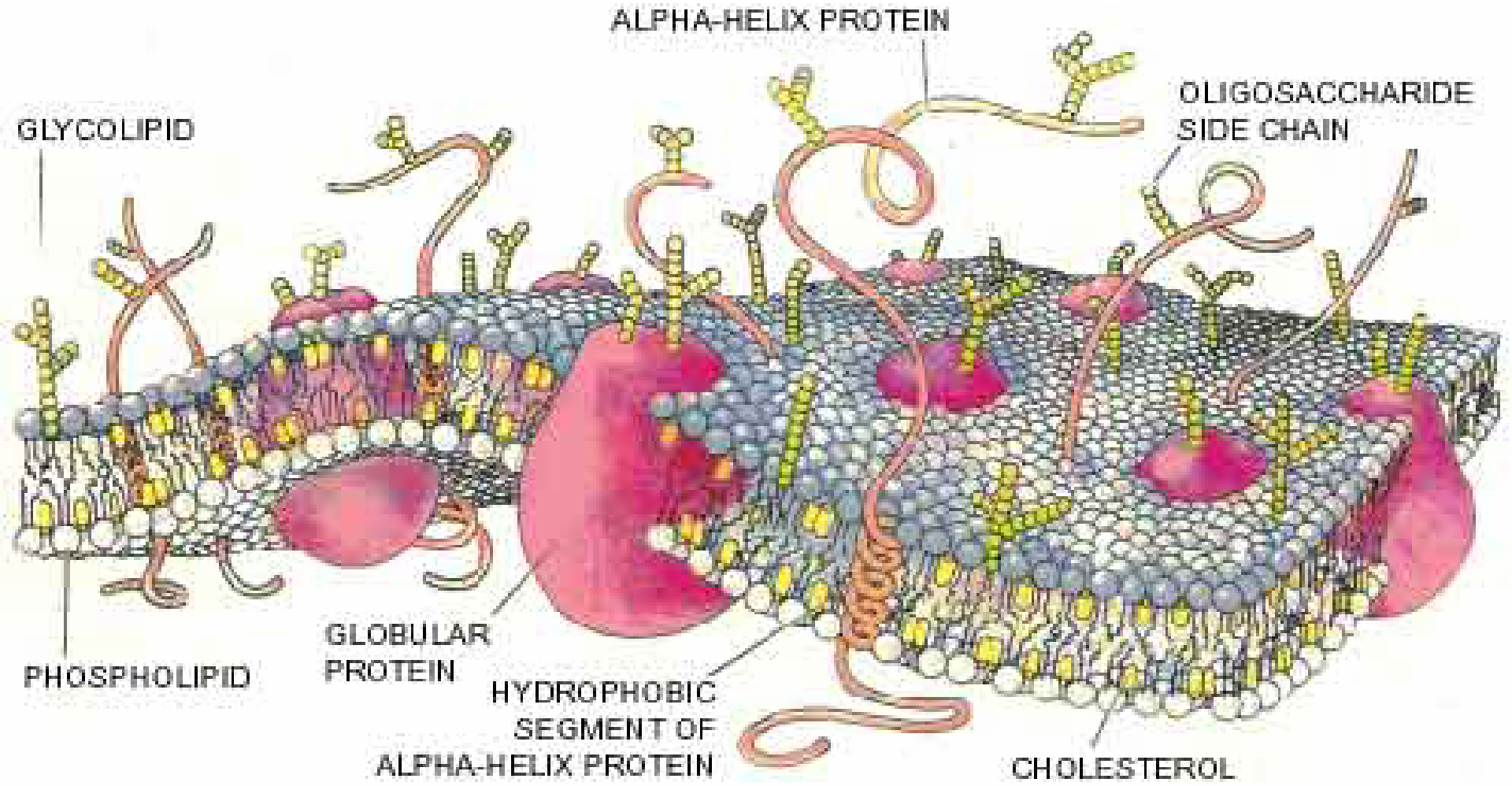
Thirty years ago, Frye and Edidin demonstrated "the rapid intermixing of cell surface antigens after formation of mouse-human heterokaryons" (1970, J. Cell. Sci. *7*, 319-335).

In 1970, Frye and Edidin fused mouse and human cells and followed by immunofluorescence the redistribution of antigens on the surface of the resulting hybrids (see Cover). They first showed that in established hybrid cells "infinite time" controls both the mouse (green; panel A) and the human (red; panel B) surface antigens were in circumferential rings, hence, more or less uniformly distributed. In contrast, these antigens were located in separate hemispherical poles when viewed immediately after fusion (panel C). The redistribution of these proteins then proceeded briskly, with the mouse antigen (panel D) moving somewhat more slowly than its human counterpart (panel E). Within 40 min, both markers covered the entire surface of almost every fused cell pair (panels F and G). That the proteins diffused freely in a fluid phospholipid bilayer was inferred from a simple kinetic analysis and from the complete arrest of antigen redistribution below 15°C



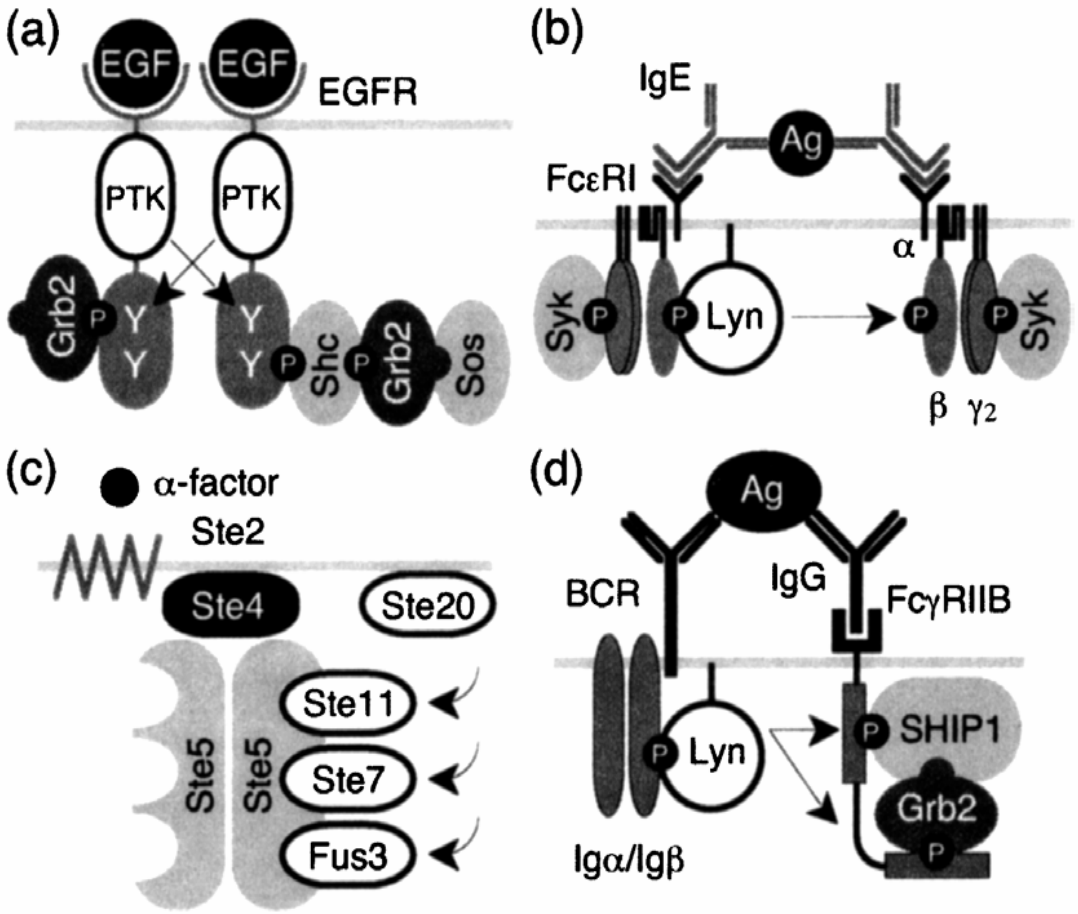
Fluid Mosaic Model of Cell Membranes:
Singer S. J. & Nicolson, G. L. The fluid mosaic model of cell membranes. *Science* **175**, 720–731 (1972).

The molecules of the cell membrane



(Bretscher, 1985. Scientific American)

Why Do We Care About Protein Mobility?



Hlavacek, 2003.

Figure 1. Multicomponent complexes that can form during receptormediated signal transduction.

(a) Complex formation around EGFR (Jorissen et al., 2003; Schlessinger, 2000). The cytosolic adapters Grb2 and Shc are recruited to the membrane when EGFR tyrosines are autophosphorylated. Grb2 also binds phosphorylated Shc and interacts constitutively with Sos, a guanine nucleutide exchange factor.

(b) Complex formation around FceRI (Kinet, 1999; Turner and Kinet, 1999). Syk, a cytosolic protein tyrosine kinase (PTK), is recruited to the g chain of FceRIafter phosphorylation of receptor tyrosines by the Src-family PTK Lyn, which is tethered to the membrane and interacts with the h chain of FceRI via constitutive low-affinity and phosphorylation-dependent high-affinity interactions.

(c) Complex formation around Ste5p (Elion, 2001). The kinases Stel1p, Ste7p, and Fus3p constitute a MAPK cascade involved in the mating response of yeast and interact with the scaffold protein Ste5p, which forms homodimers. When a-factor pheromone binds Ste2p, Ste4p, a G protein component, is liberated to interact with Ste5p. Recruitment of Ste5p to the membrane enables membrane-associated kinase Ste20p to phosphorylate Ste11p, which initiates the MAPK cascade.

(d) Complex formation around FcgRIIB (March and Ravichandran, 2002). SHIP1, a cytosolic inositol phosphatase, is recruited to the membrane after phosphorylation of FcgRIIB tyrosines. Recruitment of SHIP1 depends on Grb2, which interacts constitutively with SHIP1 and, like SHIP1, interacts with FcgRIIB.

Methods of Measuring Membrane Protein Mobility
1. Post-field Relaxation

Biophysical Journal, Vol 26, 1-21, 1979 **Electrophoresis and diffusion in the plane of the cell membrane**

- **M Poo, JW Lam, N Orida and AW Chao**

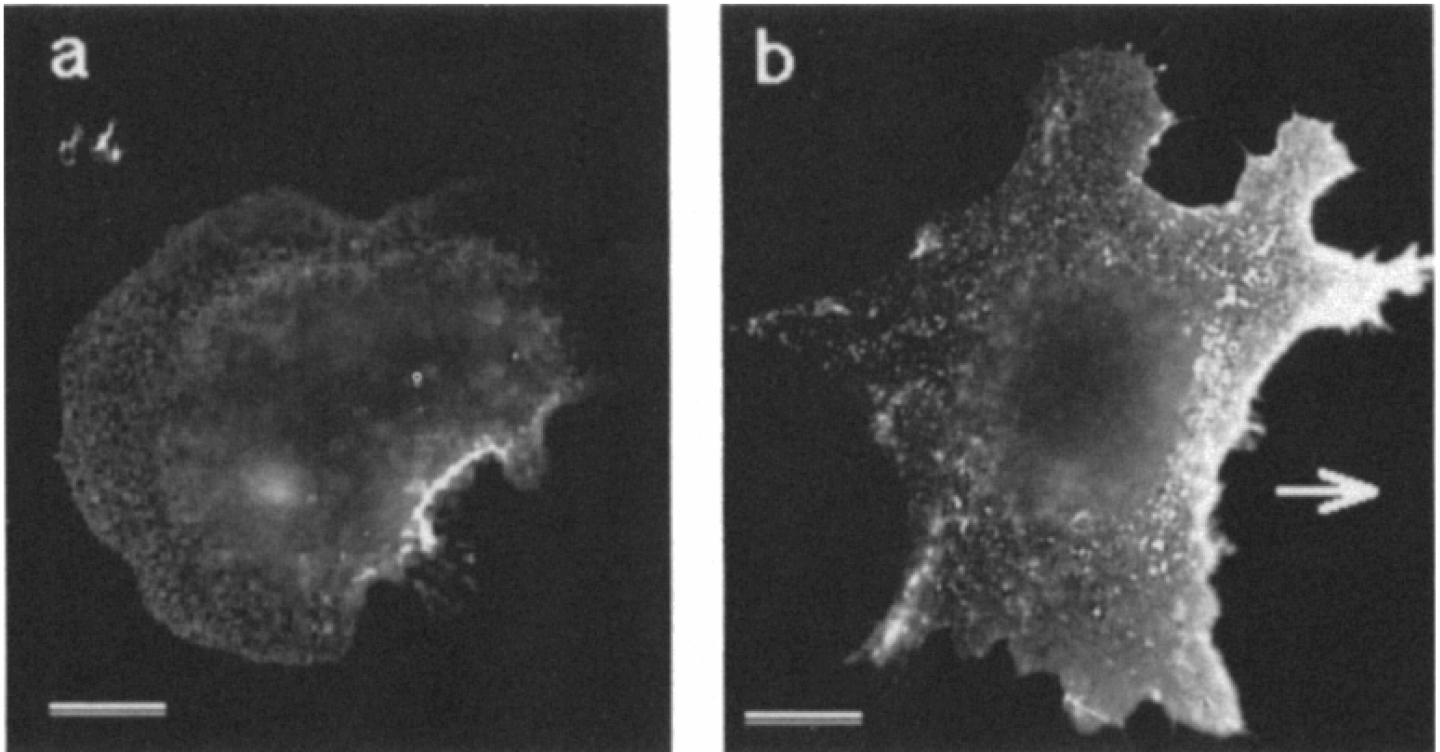
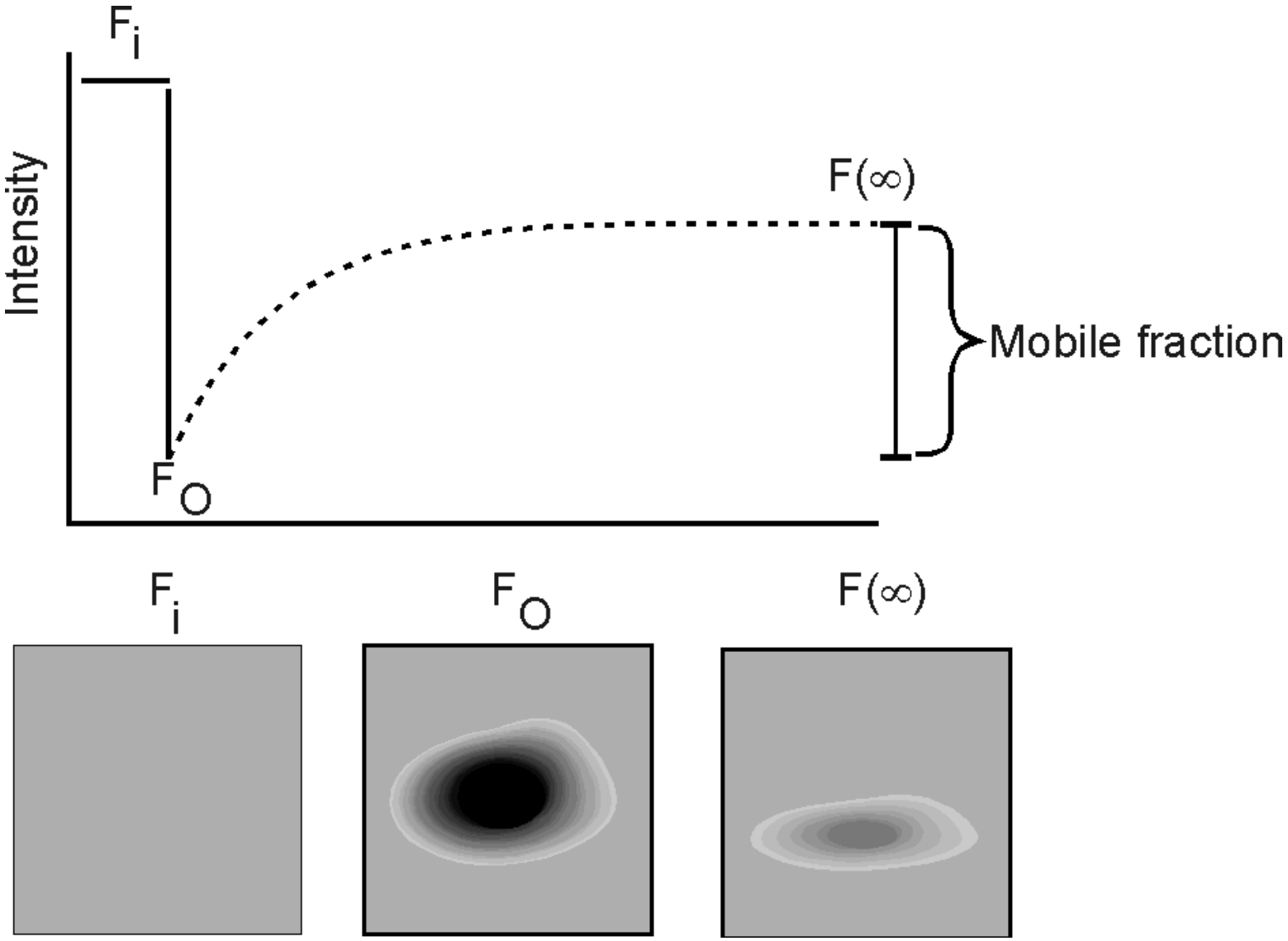
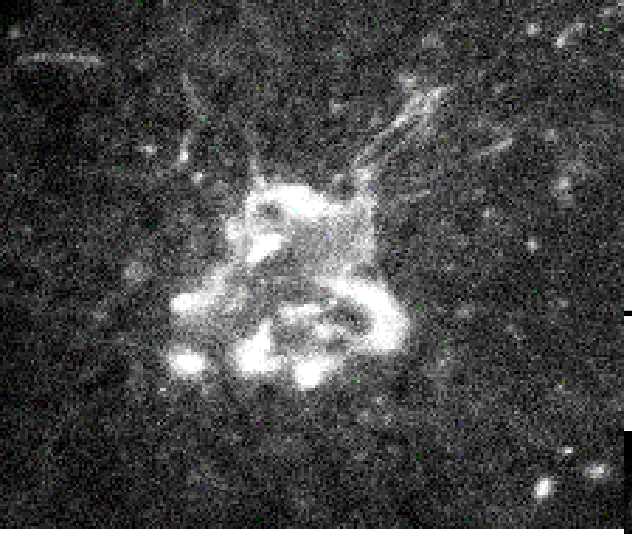
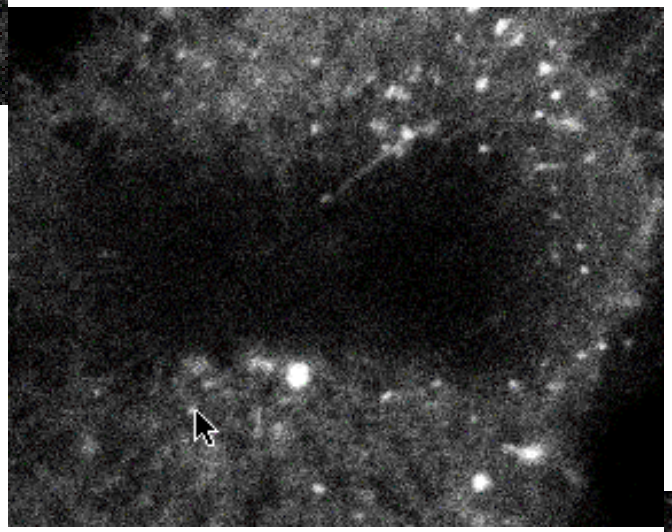


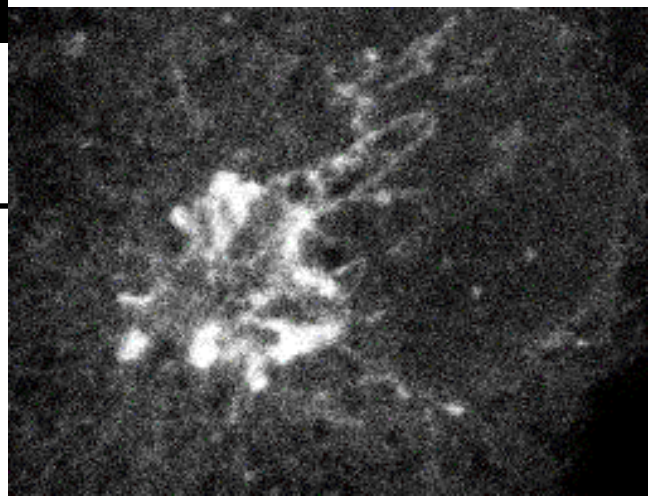
Figure 6. The electric field induced, cathodal accumulation of plasma membrane Con A receptors in NIH 3T3 plated on Con A–coated substratum. (*a*) Pre-field label with 100 μg/ml FITC-Con A. (*b*) FITC-Con A label following 40-min exposure to a 4 V/cm electric field. Arrow in *b* indicates the direction of the cathode. Bar, 10 μm.

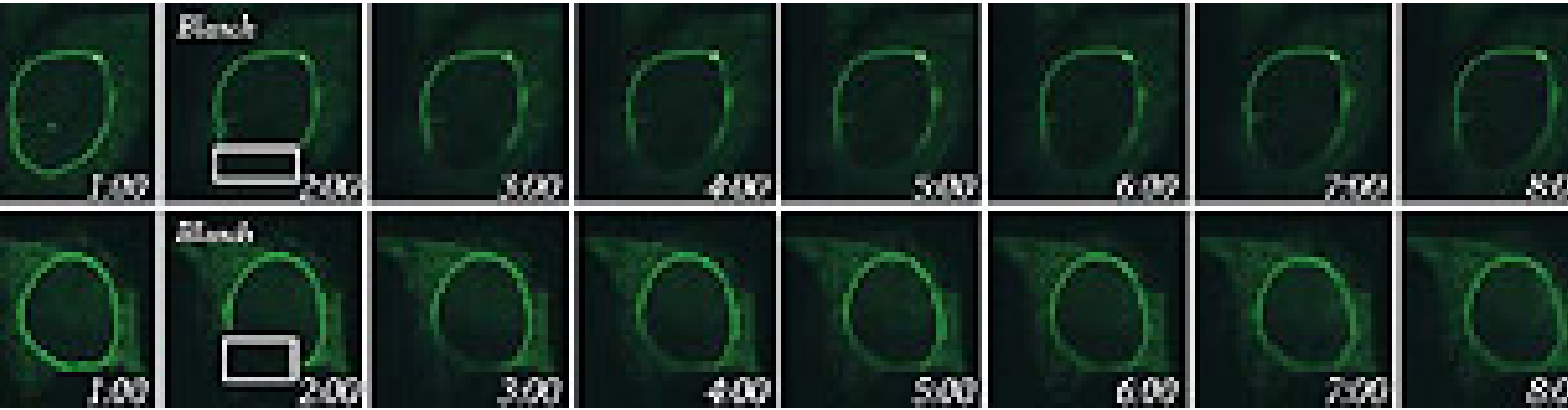
2. Fluorescence Recovery after Photobleaching (FRAP)







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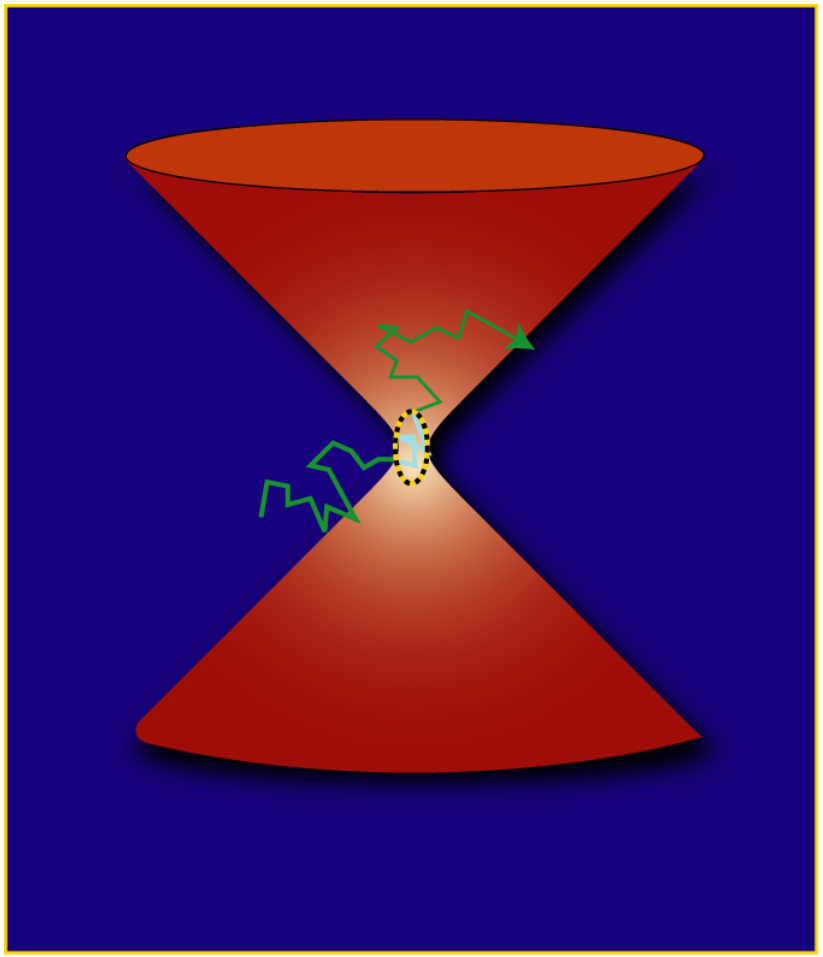
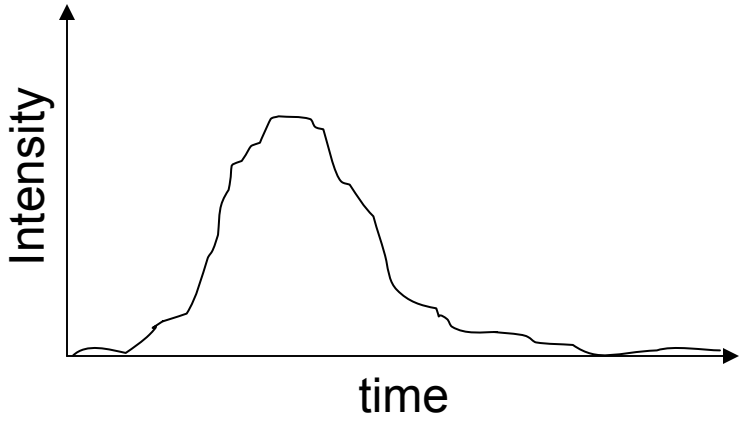
3. Fluorescence Correlation Spectroscopy

Single Molecule Dynamics

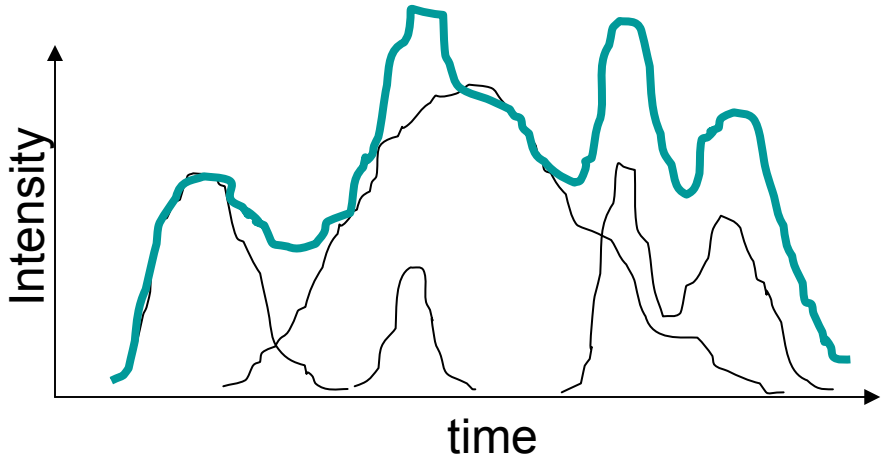


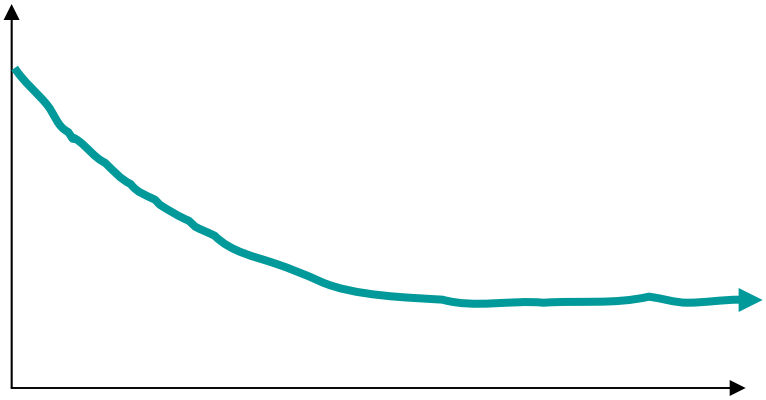


A single molecule: a burst of fluorescence, the duration indicative of the residence time in the sample volume.

Faster Diffusion → Longer **AVERAGE** residence time

Fluorescence with Several Molecules





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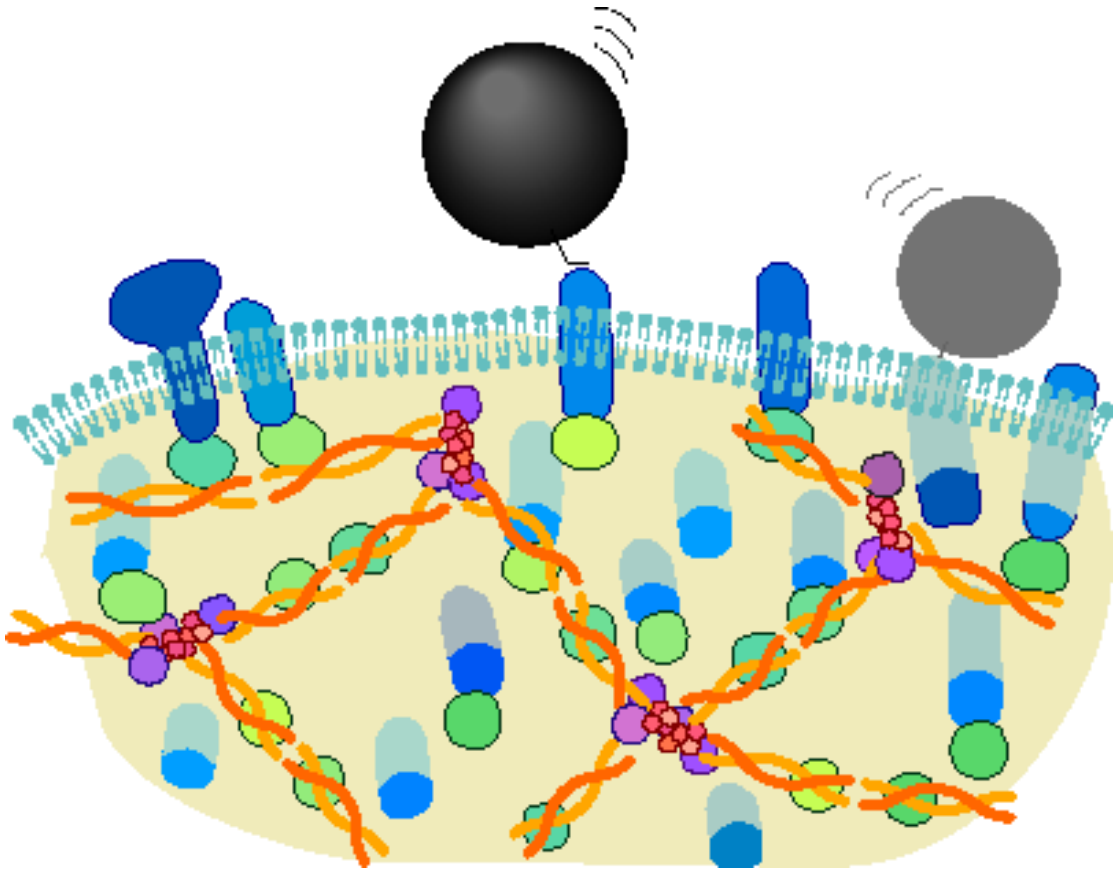
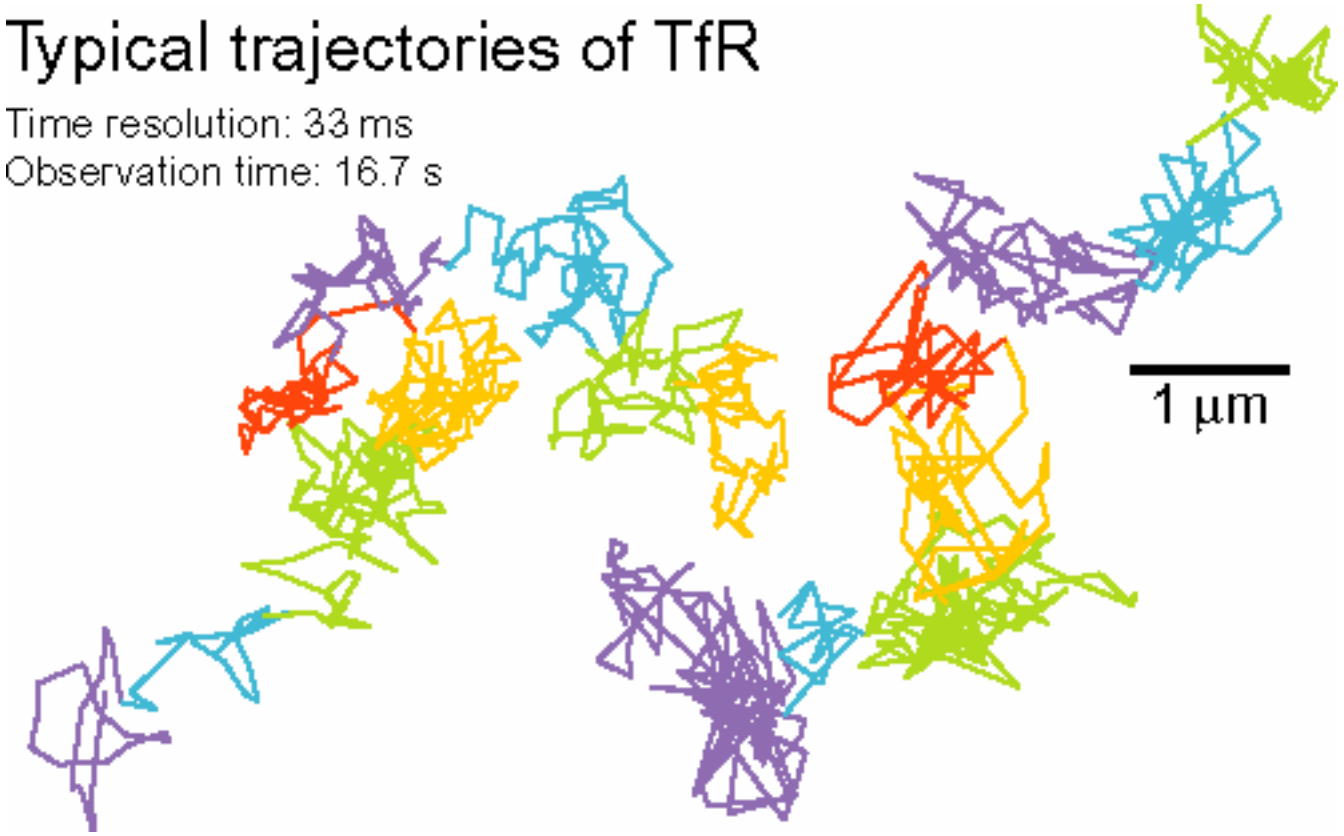
Decay Time Reveals Dynamics

Amplitude Reveals #

Fluctuation Size ~ $\sqrt{n}$

4. Single-particle tracking





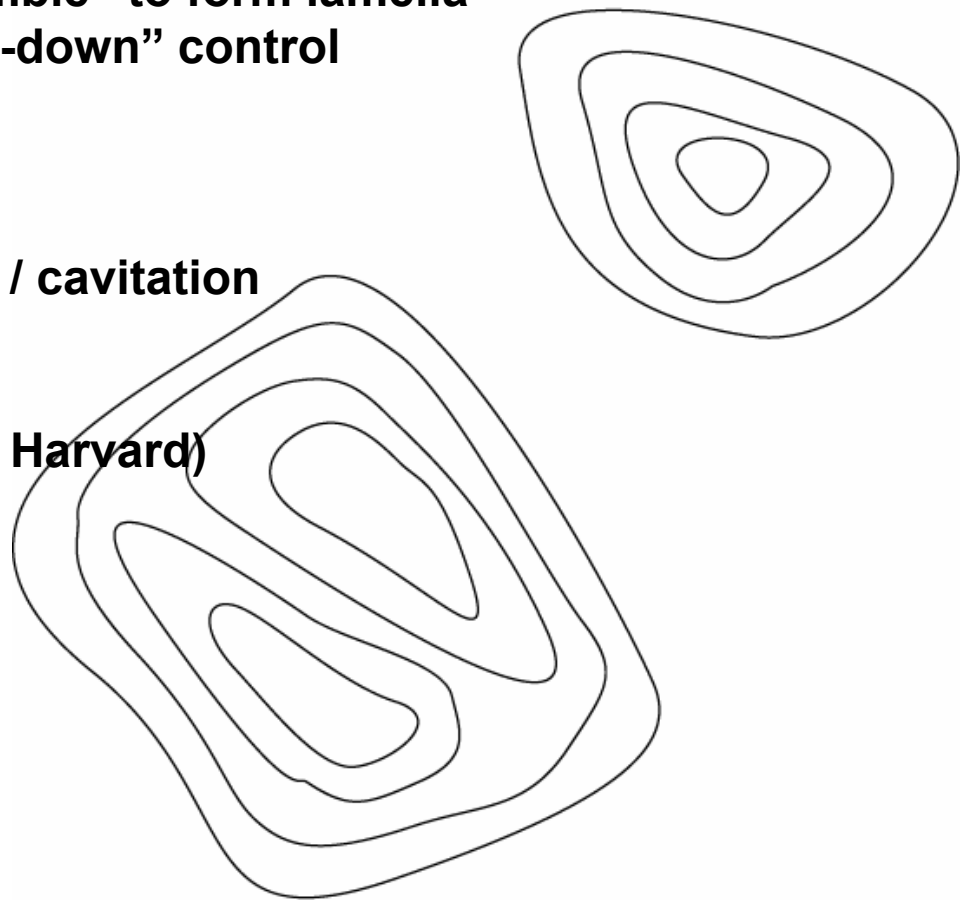
Plausible compartments are shown in different colors.

“Artificial Membranes”

Biophysics

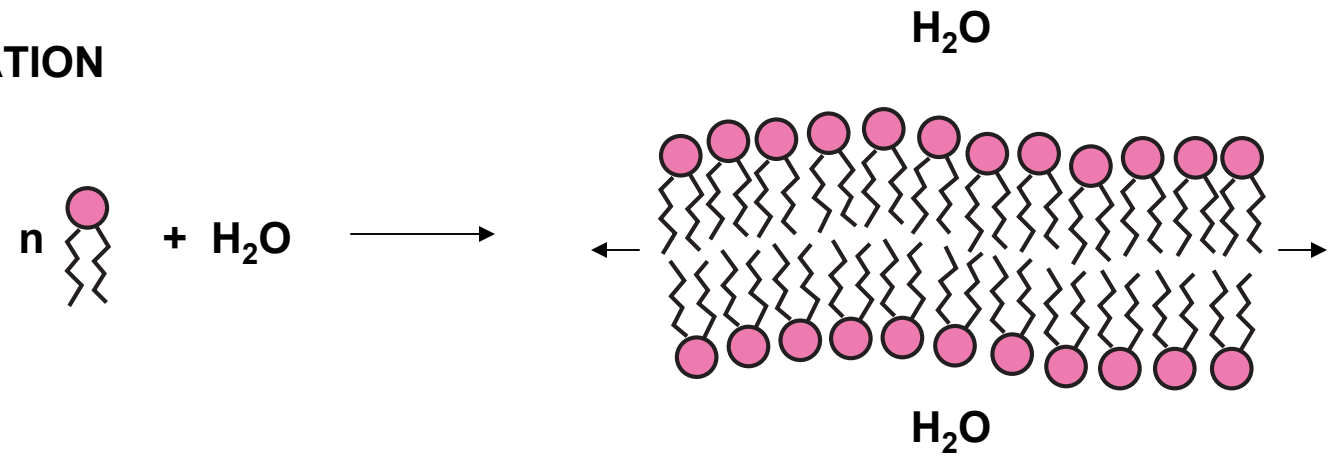
Drug Delivery

Phospholipid molecules “self-assemble” to form lamella
Mesoscopic Structure requires “top-down” control



- **Multilamellar liposomes**
- **Low frequency (20 kHz) ultrasound / cavitation**
- **Extrusion of liposomes**
- **Electroformation**
- **A new interfacial method (D. Weitz, Harvard)**

HYDRATION



Lamellar Structure Depends on the Balance of Headgroup Area & Tail Volume

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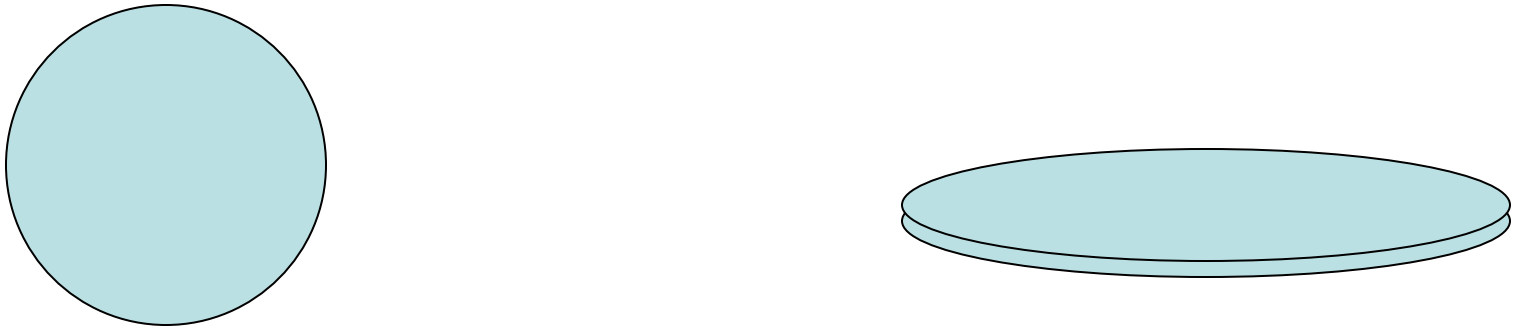
Sonication (Ultrasound Cavitation)

Energy of a sphere (liposome) vs. energy of a pancake disk



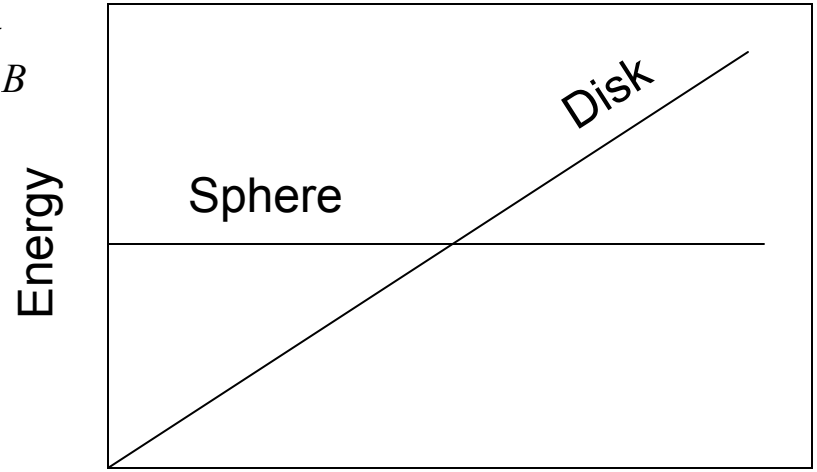
Ultimate sizes of sonicated liposomes can be predicted from a simple energy analysis.

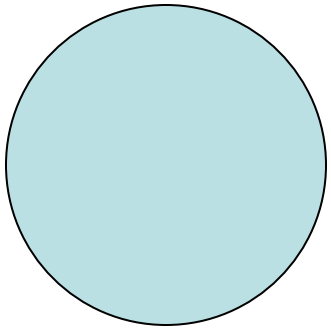
$$E_c = K_B \oint \frac{1}{r_1^2} + \frac{1}{r_2^2} dA$$

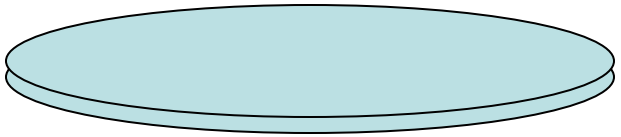
$$= 8\pi K_B$$

$$E_d = \sigma P t = 2\pi r_d t \sigma = 4\pi r_s t \sigma$$

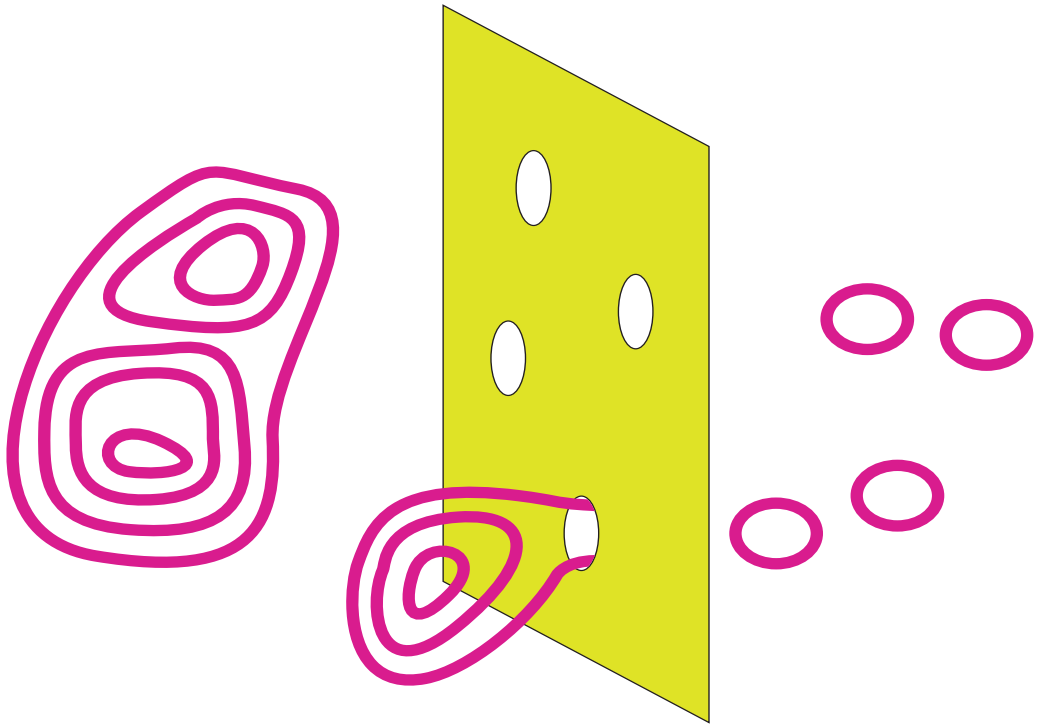
The bending modulus is about 20kT







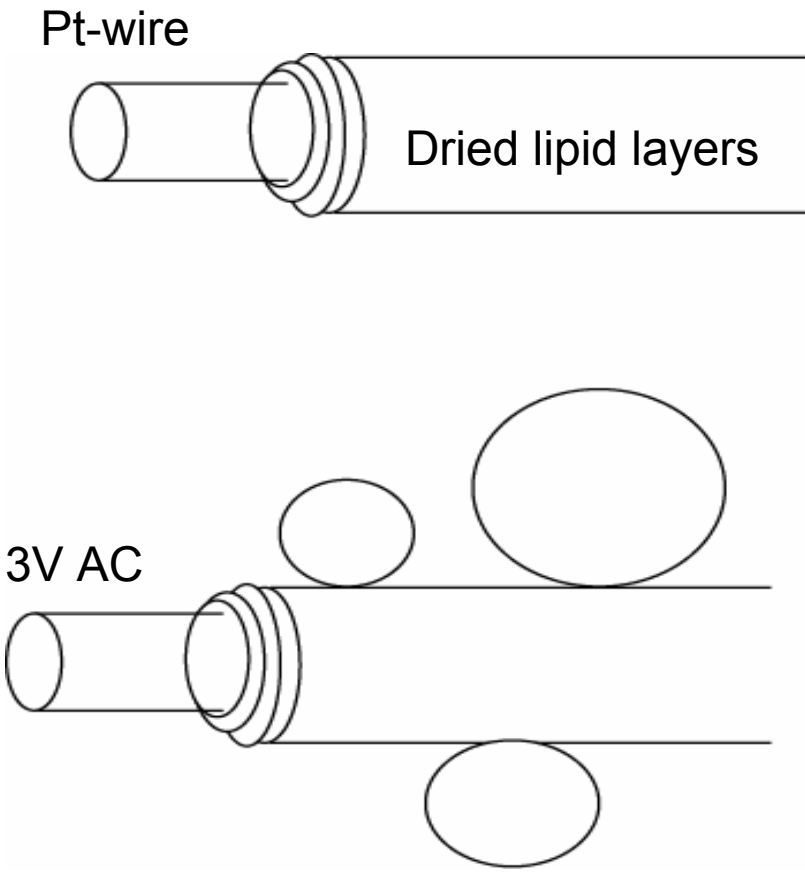
EXTRUSION



Nuclear track-etch 100 nm filter

Details of mechanism are not understood. What is the role of the membrane bending stiffness here?

ELECTROFORMATION



The following steps were used to prepare the GUVs: (i) 3 ml of the lipid stocks solution (0.2 mg:ml) were spread on each Pt wire under a stream of N2. To remove the residues of organic solvent the samples were lyophilized for about 2h; (ii) To add the aqueous solvent inside the chamber (Millipore water 17.5 MV:cm), the bottom part of the chamber was sealed with a coverslip.The Millipore water was previously heated at the desired temperature (50°C for DPPC and 30°C for DLPC and POPC) and then sufficient water was added to cover the Pt wires. Just after this step the Pt wires were connected to a function generator (Hewlett-Packard, Santa Clara, CA), and a low frequency AC field (sinusoidal wave function with a frequency of 10 Hz and amplitude of 3 V) was applied for 90 min.

"Details" of this mechanism are not understood.

- Chemistry and Physics of Lipids 105 (2000) 135–147 Giant phospholipid vesicles: comparison among the whole lipid sample characteristics using different preparation Methods: A two photon fluorescence microscopy study L.A. Bagatolli , T. Parasassi , E. Gratton

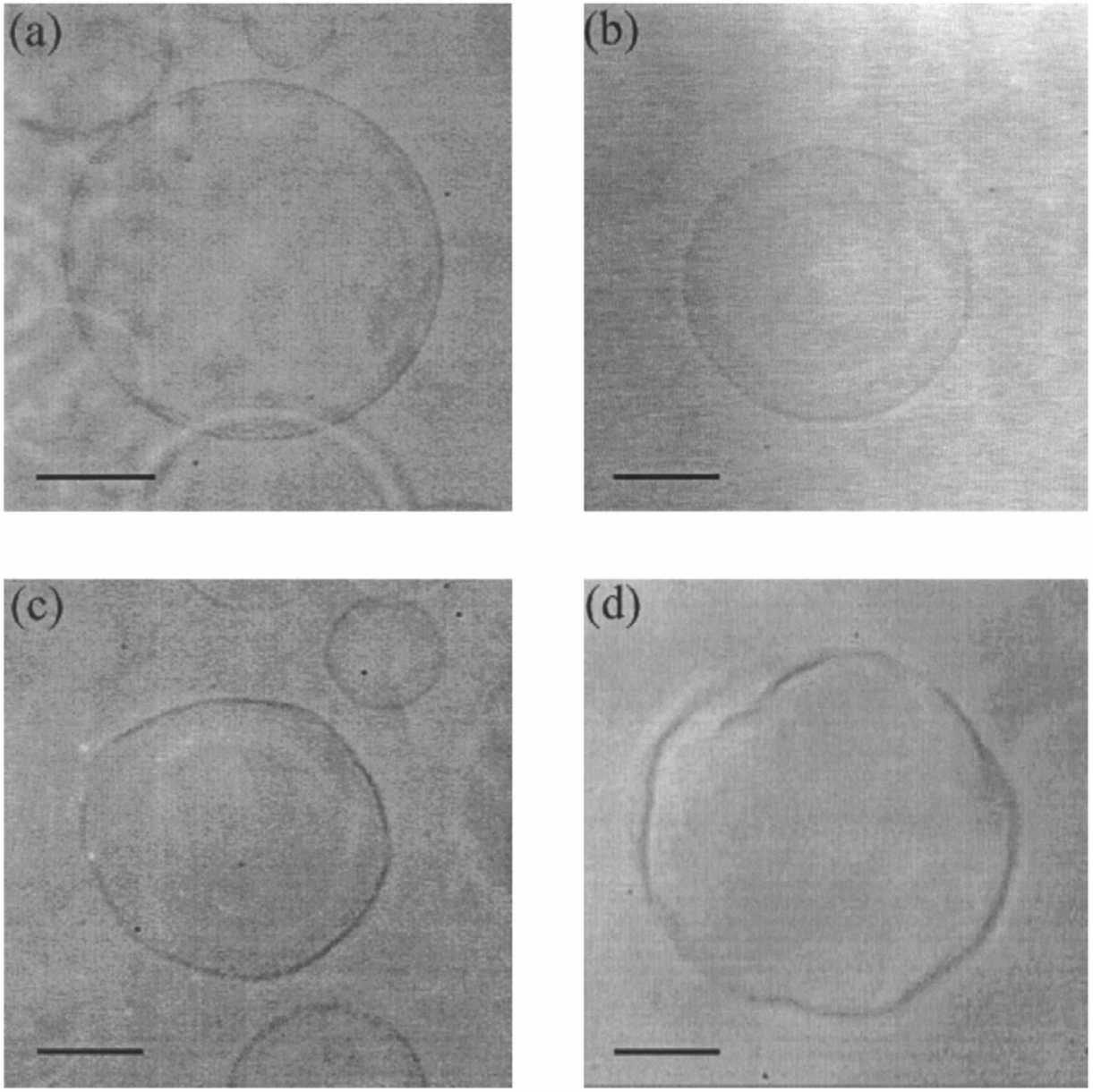
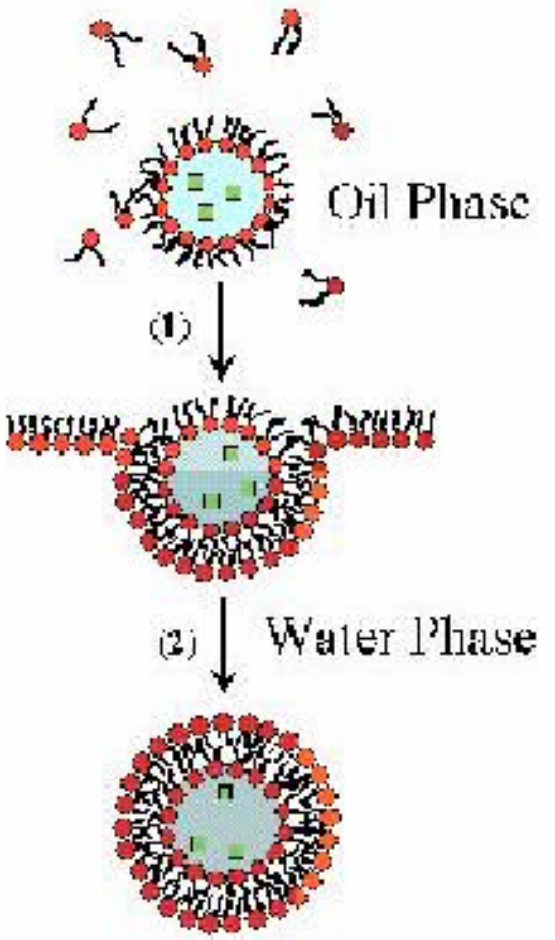


Fig. 2. (a) DMPC vesicles prepared in distilled water at 43°C, this picture also represents the equilibrium "molten" shapes of GUV prepared in water/ethanol solutions; (b) DMPC vesicles in distilled water cooled to 18°C; (c) DMPC vesicles prepared in distilled water with 8 vol% of ethanol added subsequently at 18°C; (d) DMPC vesicles prepared in distilled water with 18 vol% of ethanol added subsequently at 18°C. Scale bar is equal to 20 μm.

Inverse Emulsion Technique – Sophie Pautot and David Weitz, Harvard



-**Inverted** emulsion:

high **encap**sulation yield

size **distri**bution

membrane **compo**sition

- ρ**water** > ρ**oil**

inverted emulsion **sedi**ments

-**Liposomes** in the final continuous **phase**

Can form asymmetric liposomes.

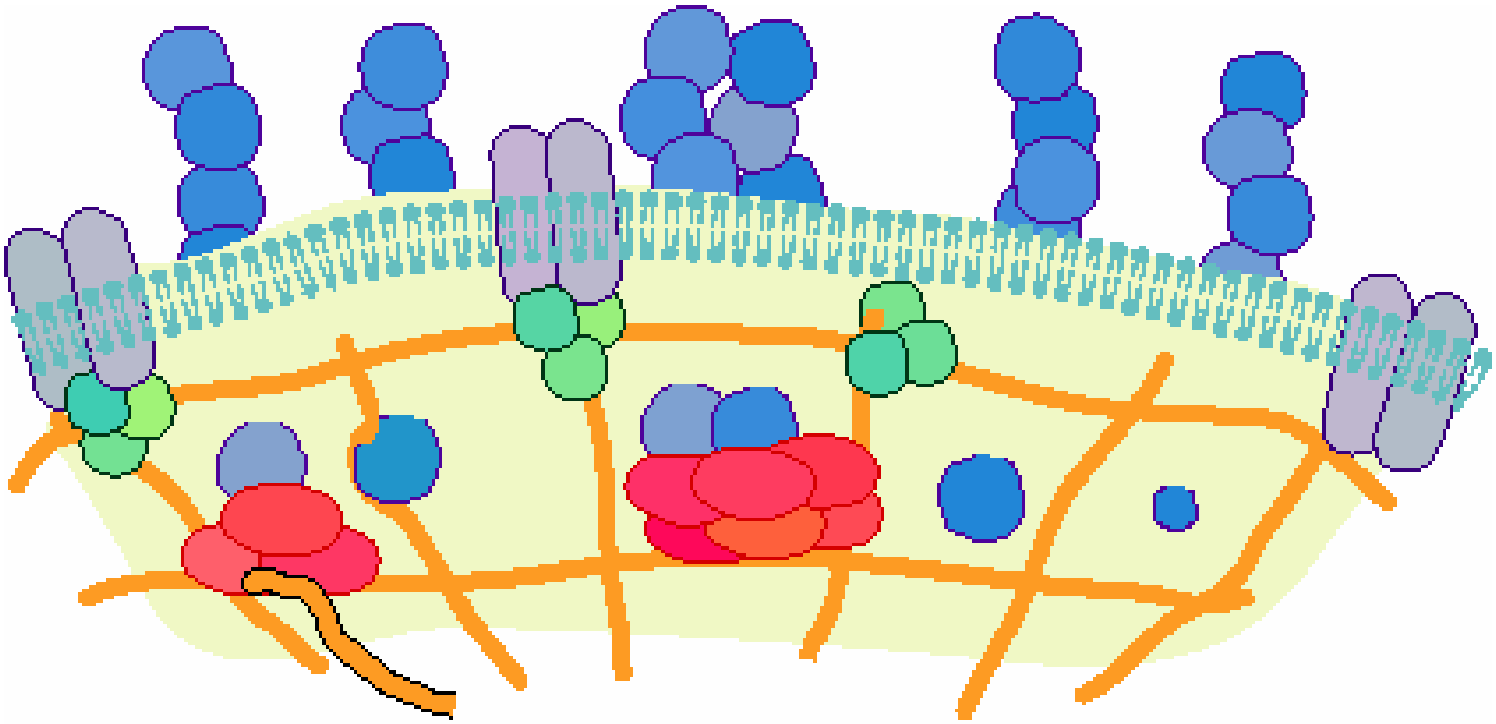
Diffusion of membrane proteins

Artificial membranes

Protein	Temp (°C)	D (10^{-8} cm^2s^{-1})
Band3-DMPC	30	1.6
Rhodopsin-DMPC	36	3.3
Acetylcholine receptor-DMPC	36	2.4
ATPase-lipids	36	1.8

Plasma membranes

Protein	Temp (°C)	D (10^{-8} cm^2s^{-1})
Band3 (human blood cell)	26	0.0038
FcεRI (mouse mast cell)	25	0.023
Insulin receptor (mouse fibroblast)	37	0.001-0.01
LDL receptor (human fibroblast)	10	0.0005-0.003



Membrane Skeleton Fence Structure

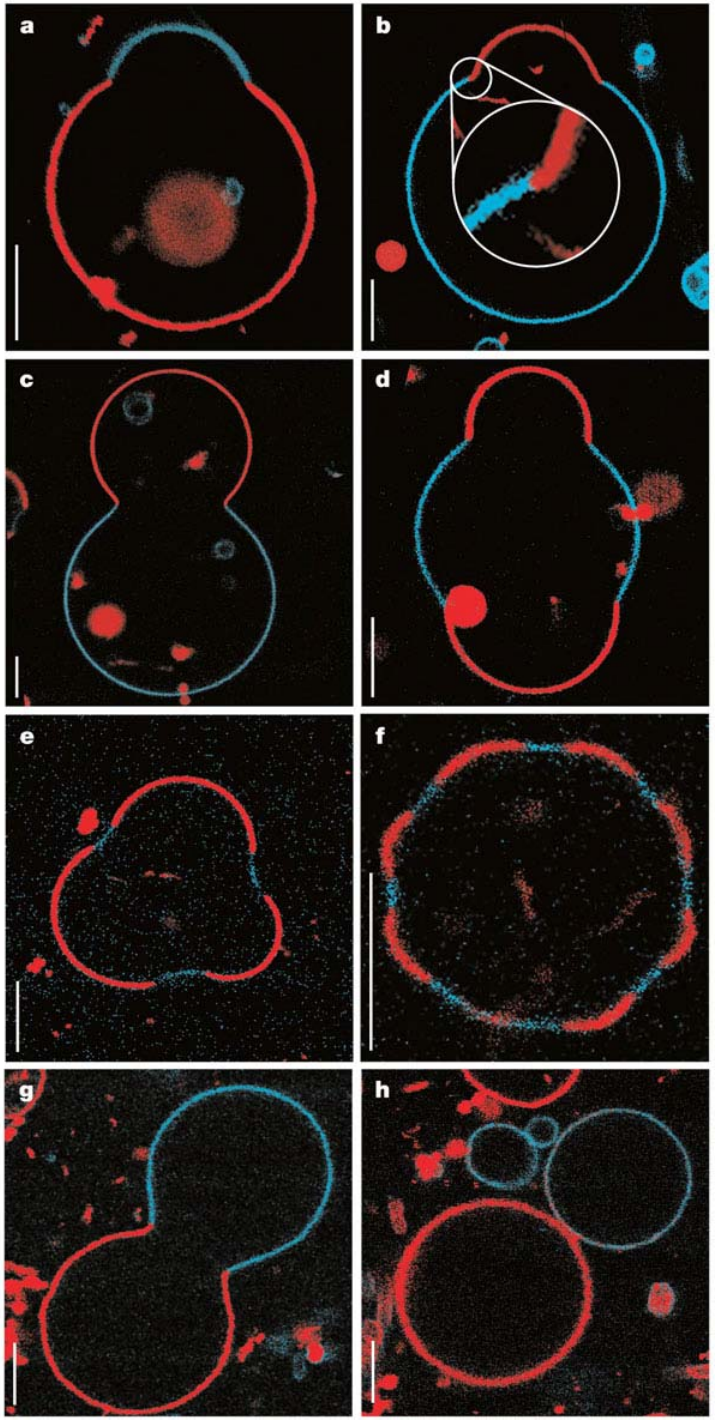
- A Basic Feature of the Plasma Membrane
- Compartment Size : 600 nm
- Elastic : 0.1 ~10 pn / μm

When liposomes are formed from mixtures of lipids with different bending stiffness, they may segregate into regions of higher and lower curvature.

2-D phase behavior

↓

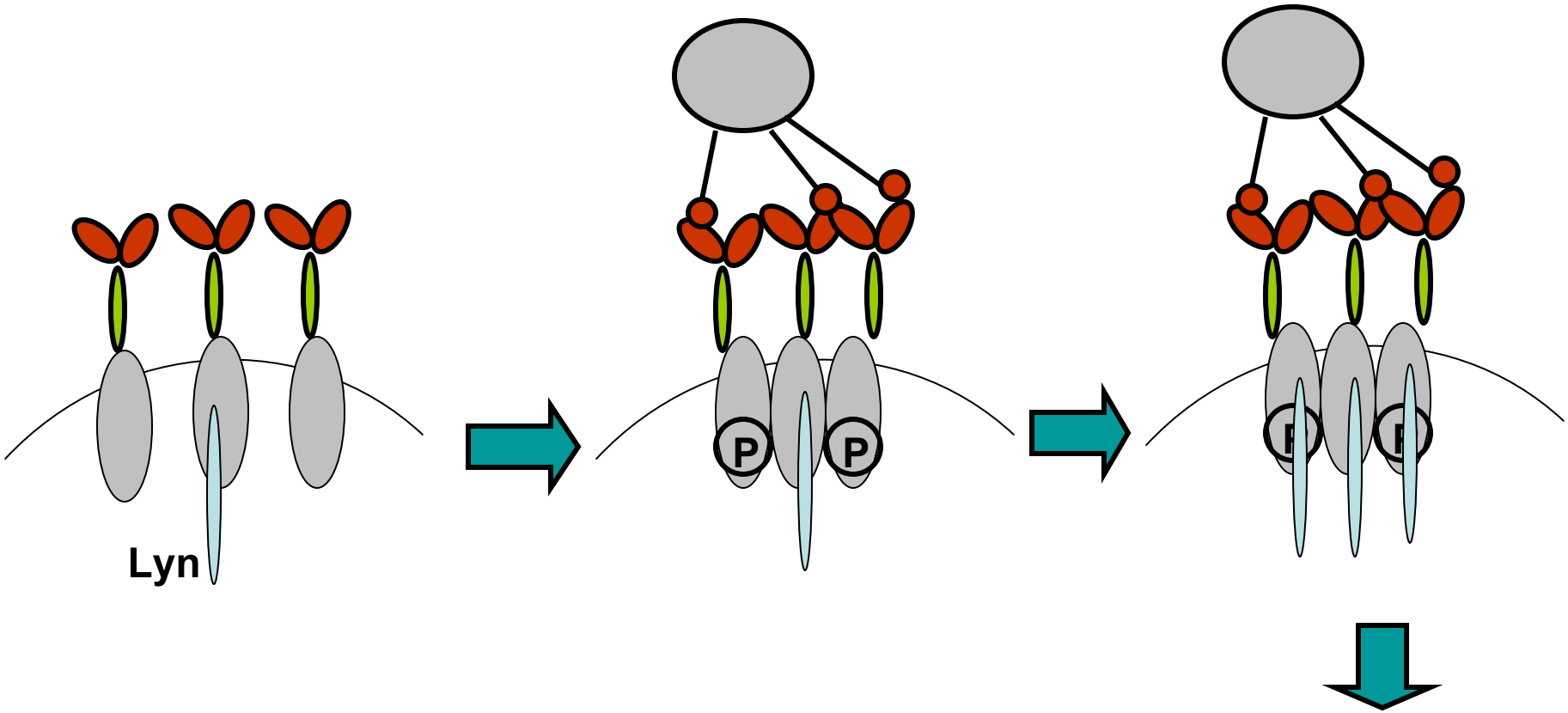
3-D structure



Webb group, Cornell Univ.

Initiation of FcεRI signaling

Transphosphorylation model



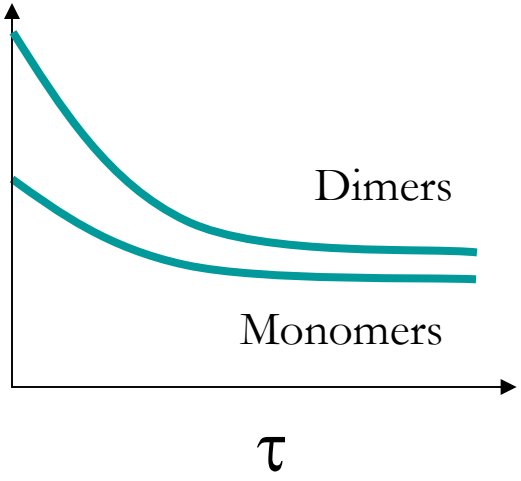
Fluorescence Correlation Spectroscopy Can Reveal Molecular Interactions (e.g. Dimerization)

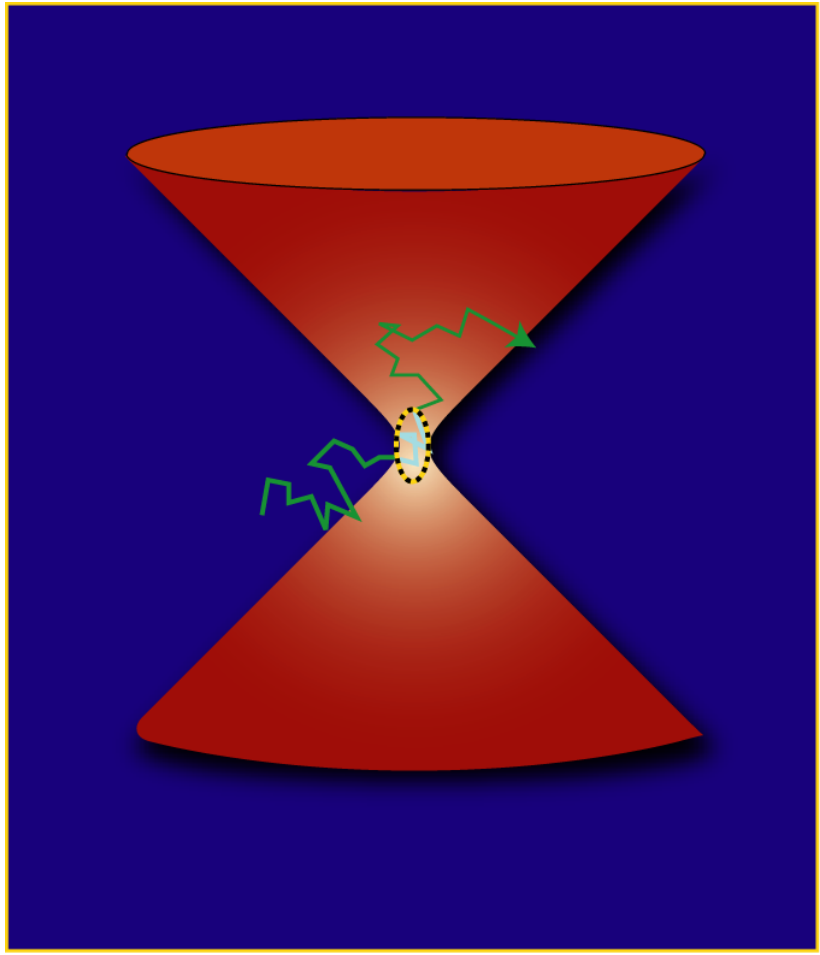
- **Small change in D (hard to detect)**
- **Large change in amplitude**

$$\langle n^2 \rangle - \langle n \rangle^2 = \langle n \rangle$$

or

$$\frac{\langle n^2 \rangle}{\langle n \rangle^2} - 1 = \frac{1}{\langle n \rangle}$$



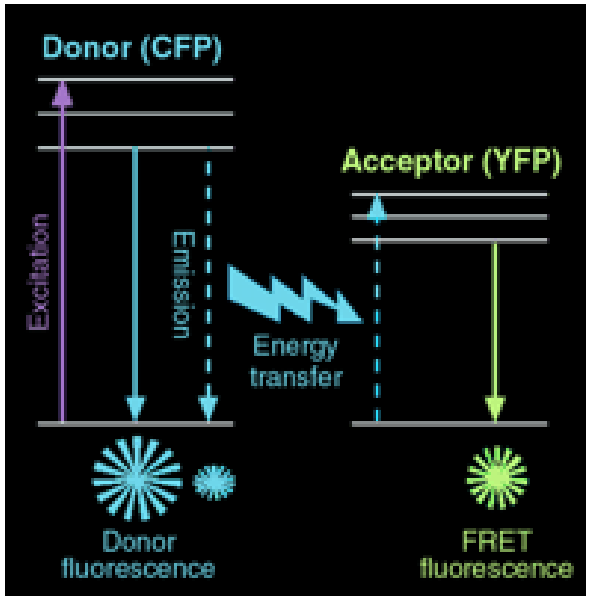


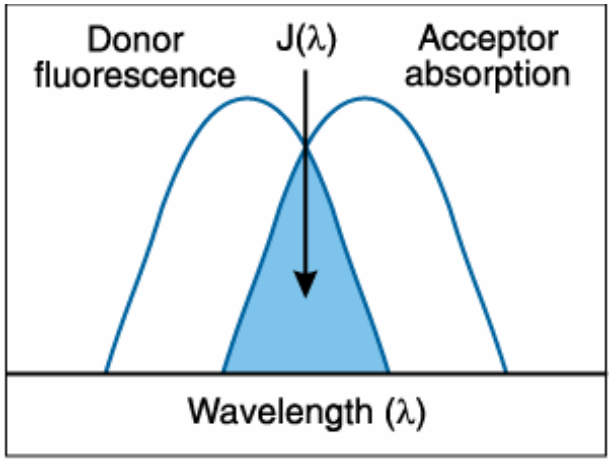
If <n> = 2, $\frac{\langle n^2 \rangle}{\langle n \rangle^2} = 3/2$

If <n> = 1, $\frac{\langle n^2 \rangle}{\langle n \rangle^2} = 2$

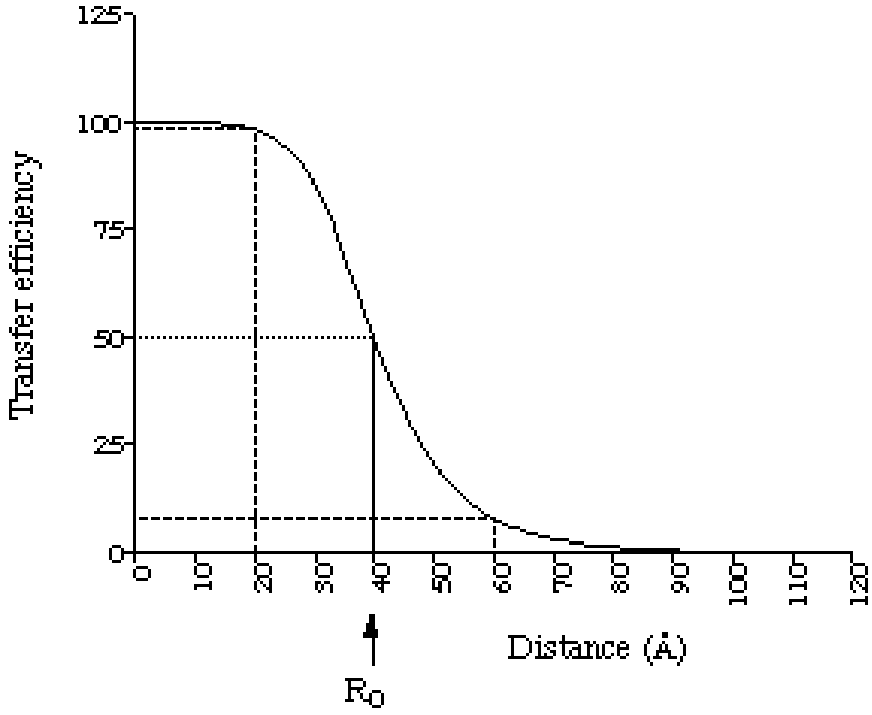
You can use FCS to determine the concentration of objects, *without calibrating the fluorescence per object.*

Forster Resonance Energy Transfer – A "Molecular Ruler"





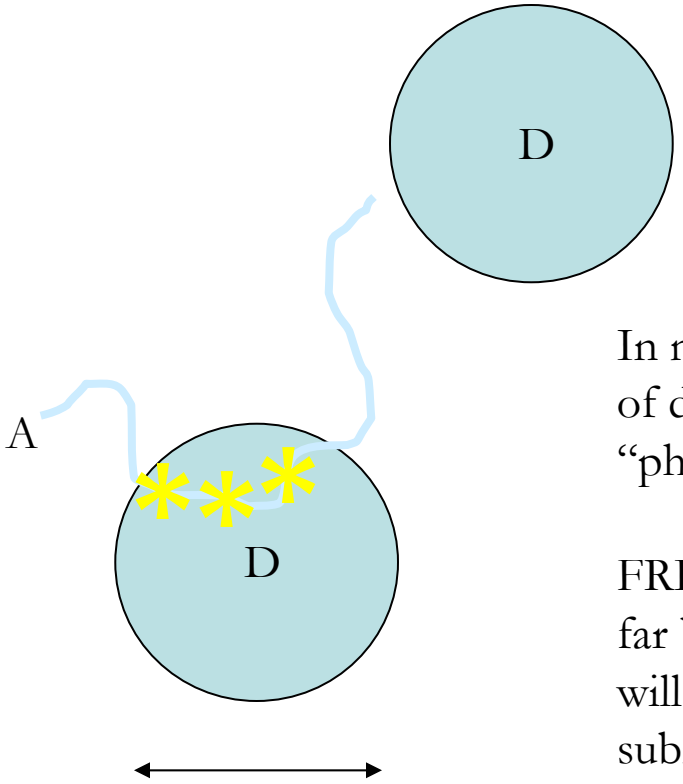
Efficiency of energy transfer = $R_0^6/(R_0^6+R^6)$



Determination of the distance between fluorescent molecules

Reducing the Sampled Volume with FRET

Forster Resonant Energy Transfer



In membranes, we can study the dynamics of donor-acceptor pairs in different "phases" (i.e. in and out of rafts.)

FRET/FCS allows mmts at length scales far below the optical resolution limit, and will allow us to study phase dynamics of submicroscopic domains.

Other applications:
Transient molecular partnerships
Intramolecular conformational fluctuations

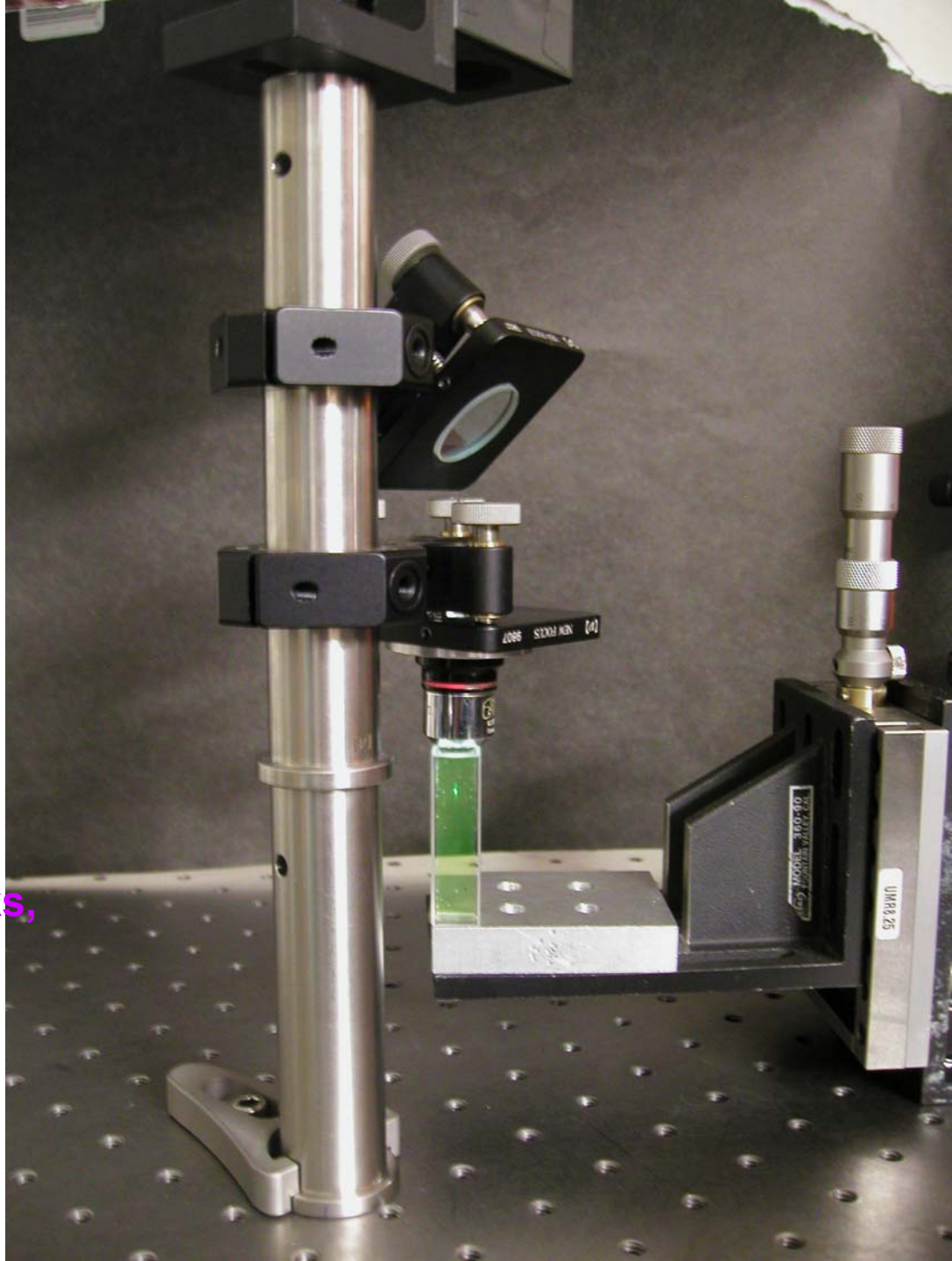
Multiphoton Excitation with
Femtosecond laser pulses
Confines sample volume.

There are great opportunities for physicists to contribute:

Experimental / Optics

Membrane modeling, structures, phases, and transformations

Cellular modeling: reaction networks, spatial and chemical coupling



There's more to biology than information!