Com estudiar interaccions entre lípids i proteïnes de membrana amb tècniques nanomètriques ?

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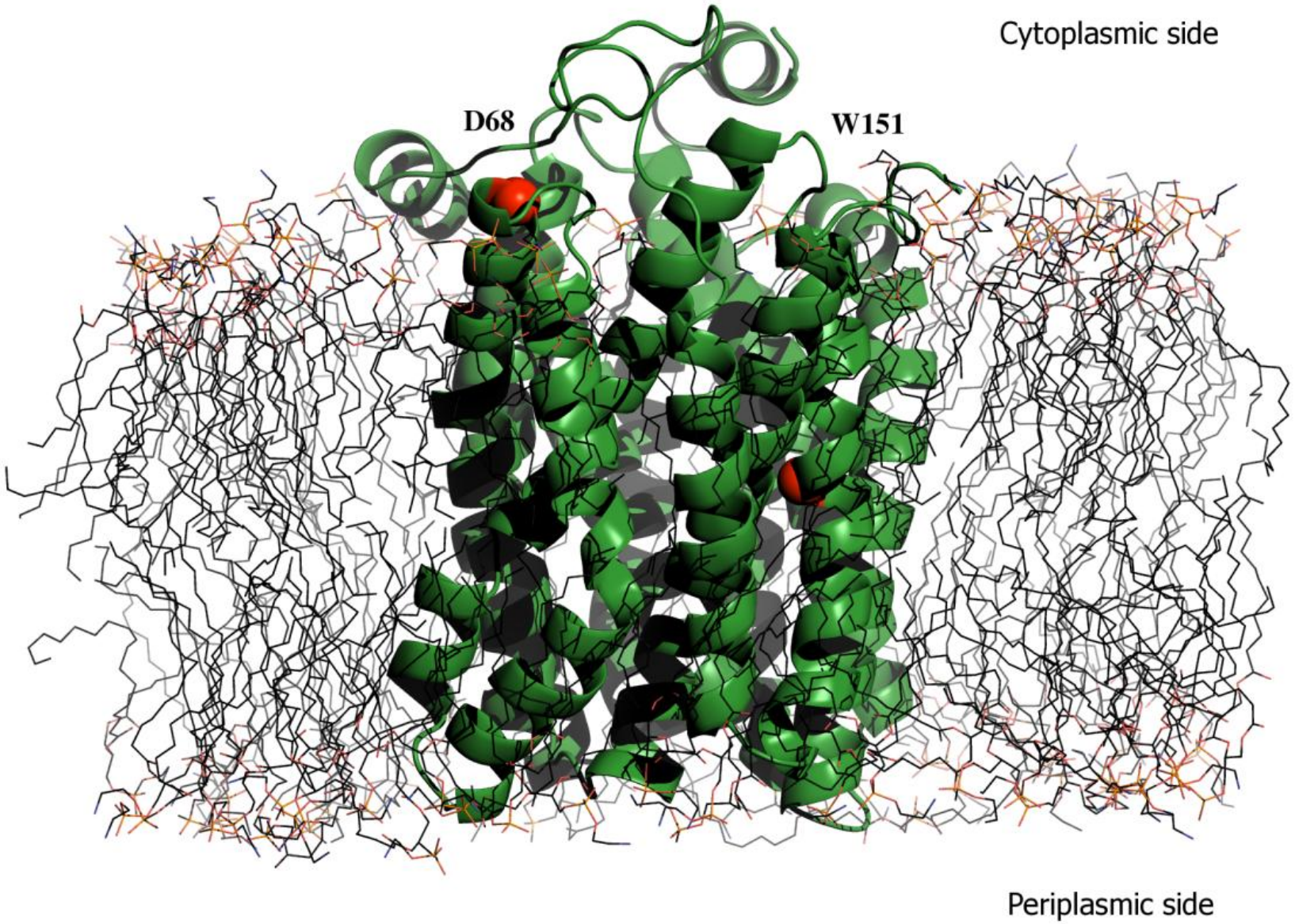
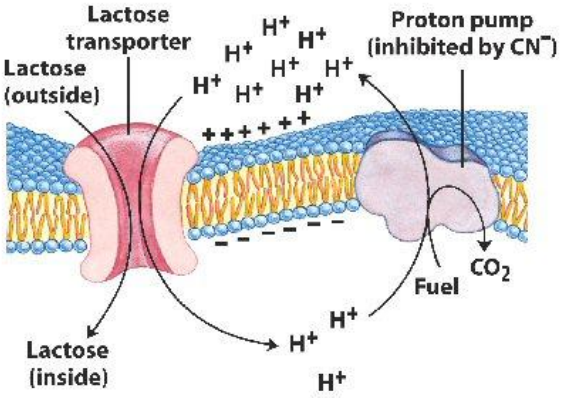
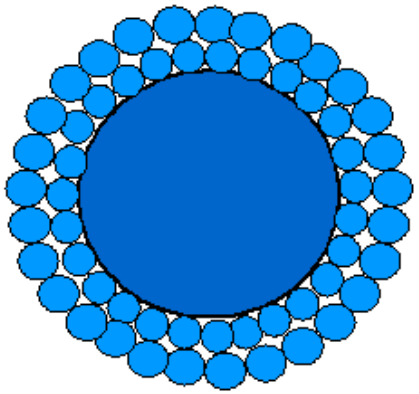
Seminari tecnològic Facultat de Farmàcia 10-3-2015

1. Proteïnes transmembranàries: el paradigma LacY
2. El microscopi de força atòmica (AFM)
3. Lípids biomimètics de Escherichia coli
4. Com caracteritzem bicapes amb l'AFM?
   –topografia i nanomecànica-
5. Que ens diu la topografia quan insertem LacY en una bicapa ?
6. Perquè no despleguem una proteïna ?
7. Te afinitat la proteïna per algún lípid ? –FRET-
8. La regió periférica de la proteïna
   – un problema de posició i temps-

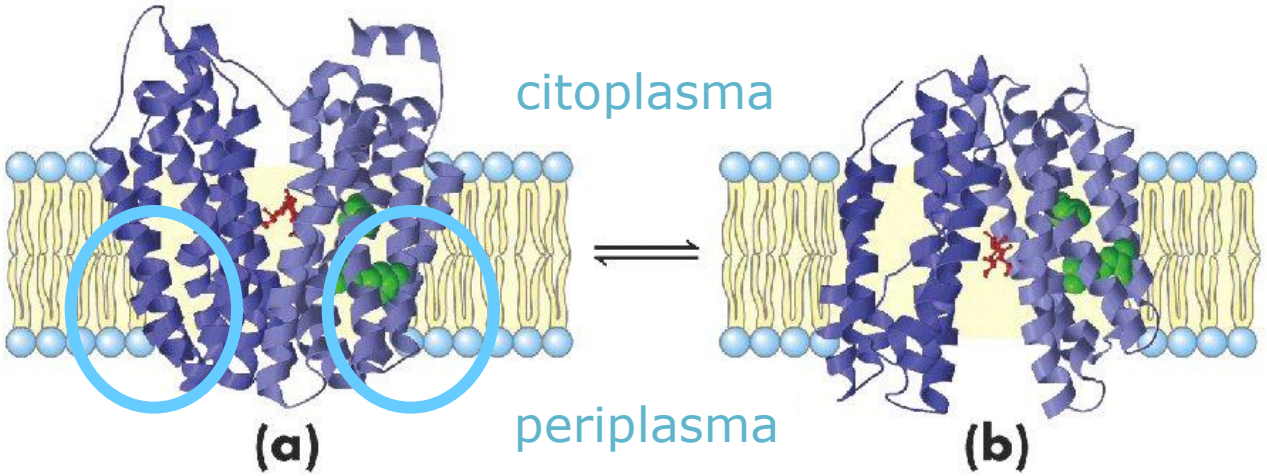

Cytoplasmic side
D68
W151
Periplasmic side

Lactosa permeasa de *Escherichia coli*

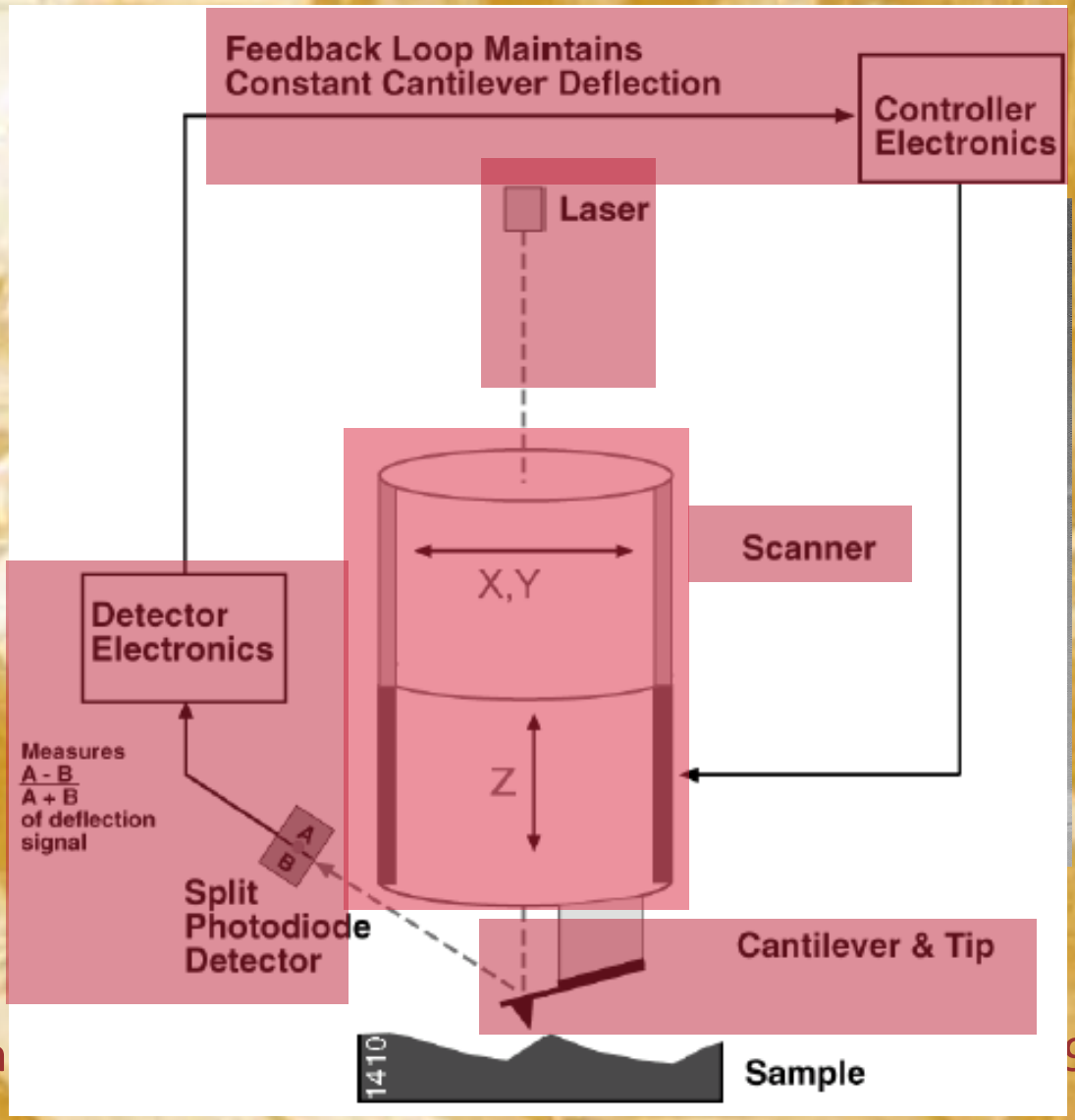




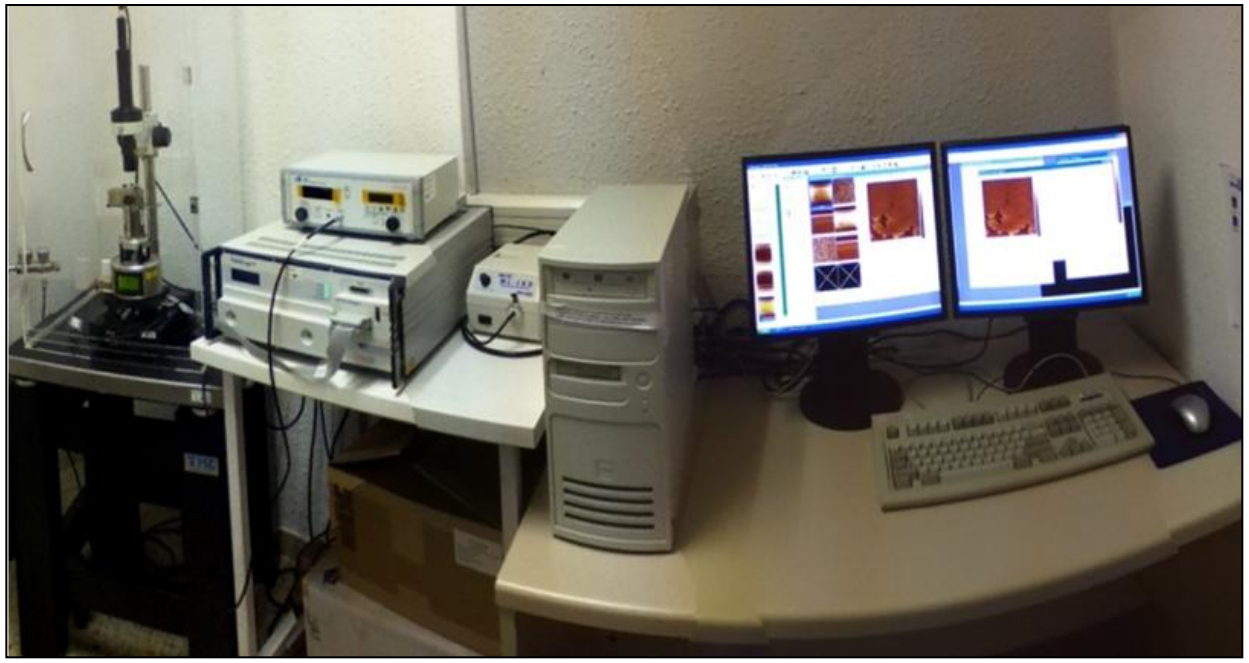
región anular

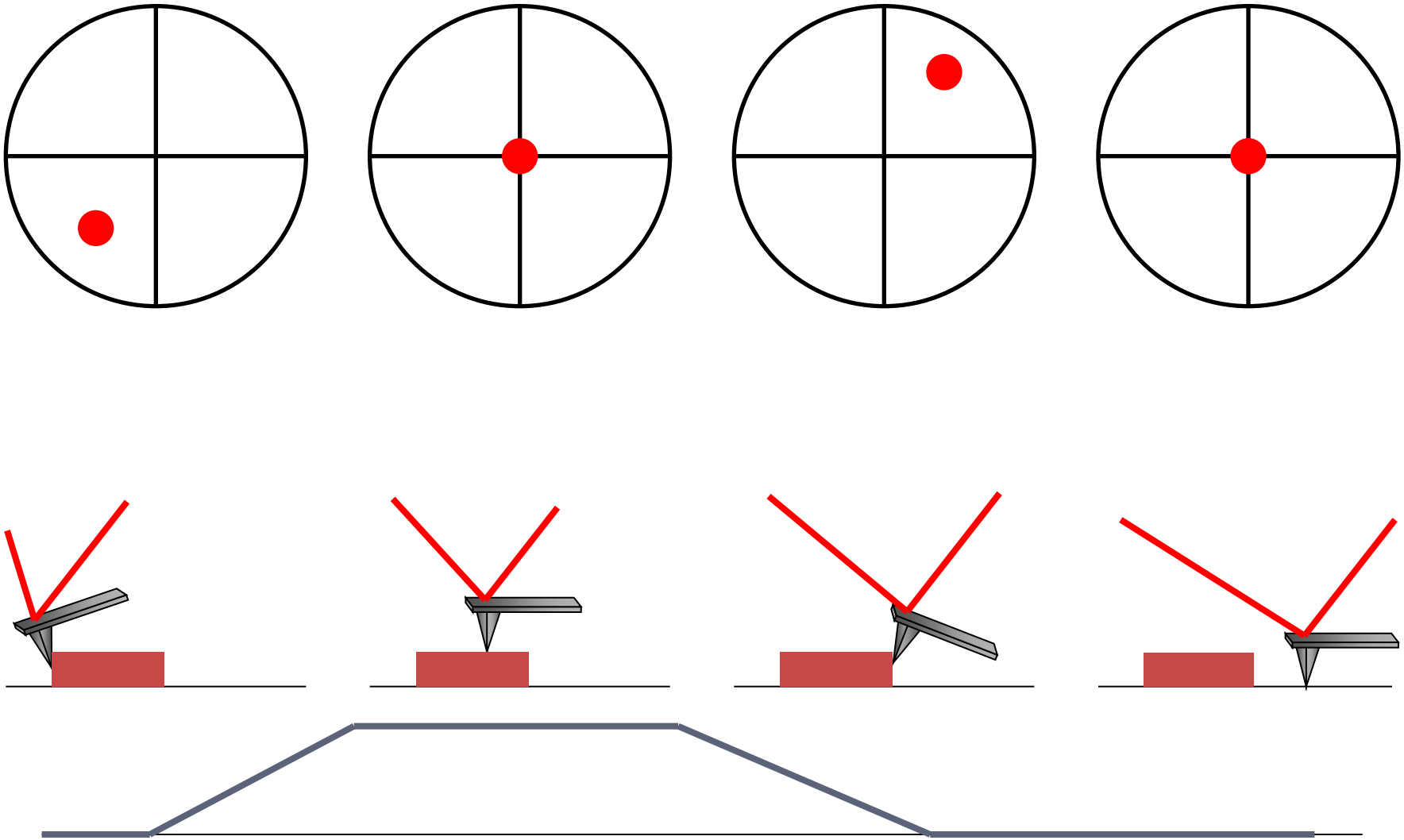


Key components of an SPM

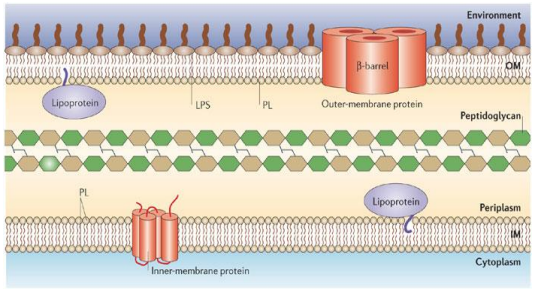


G. Binn [illegible] 930 (1986)





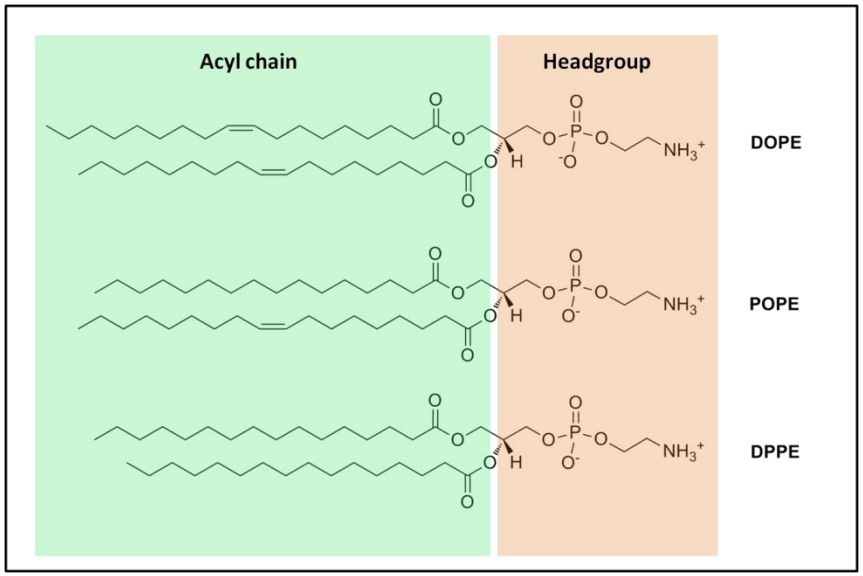
Inner membrane of *E. coli*

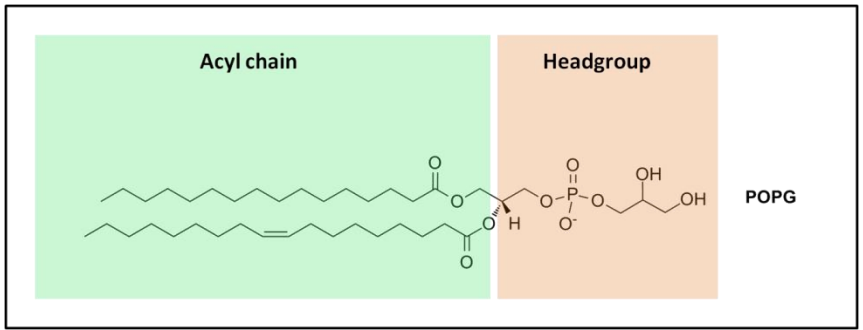


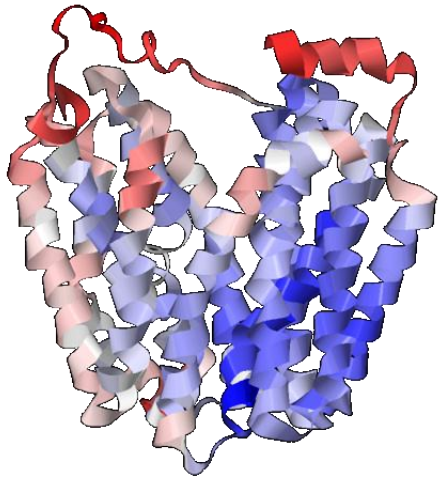
- Phosphatidylethanolamine (PE) = 74 %
- Phosphatidylglycerol (PG) =19 %
- Cardiolipin (CL) = 3 %

- MFS family, 12 TMS
- Paradigm of secondary transporters
- H^+/galactoside symport

Binary biomimetic model system PE:PG (3:1, mol/mol)

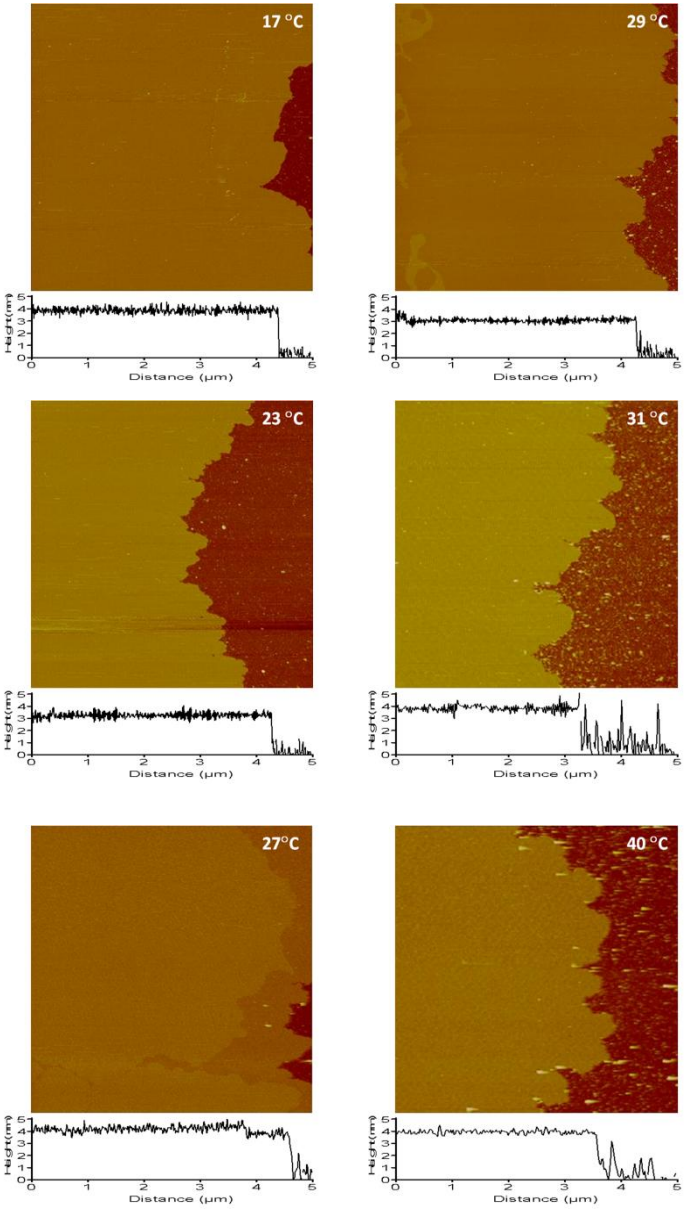




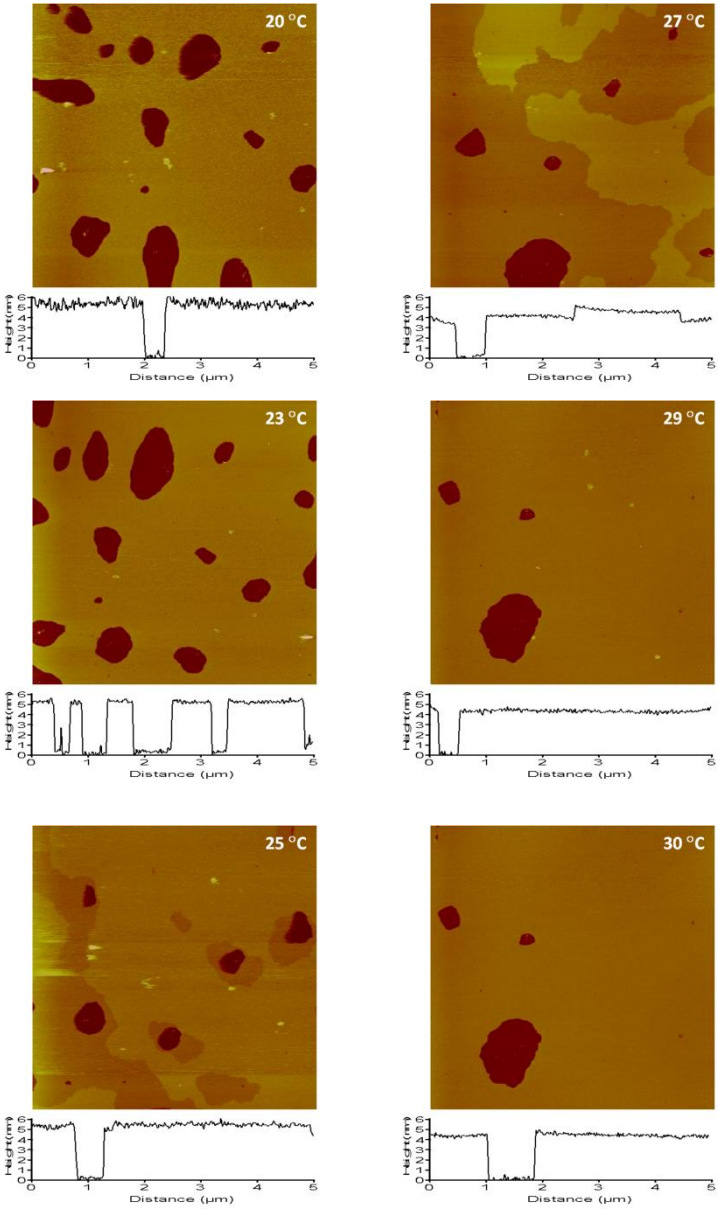


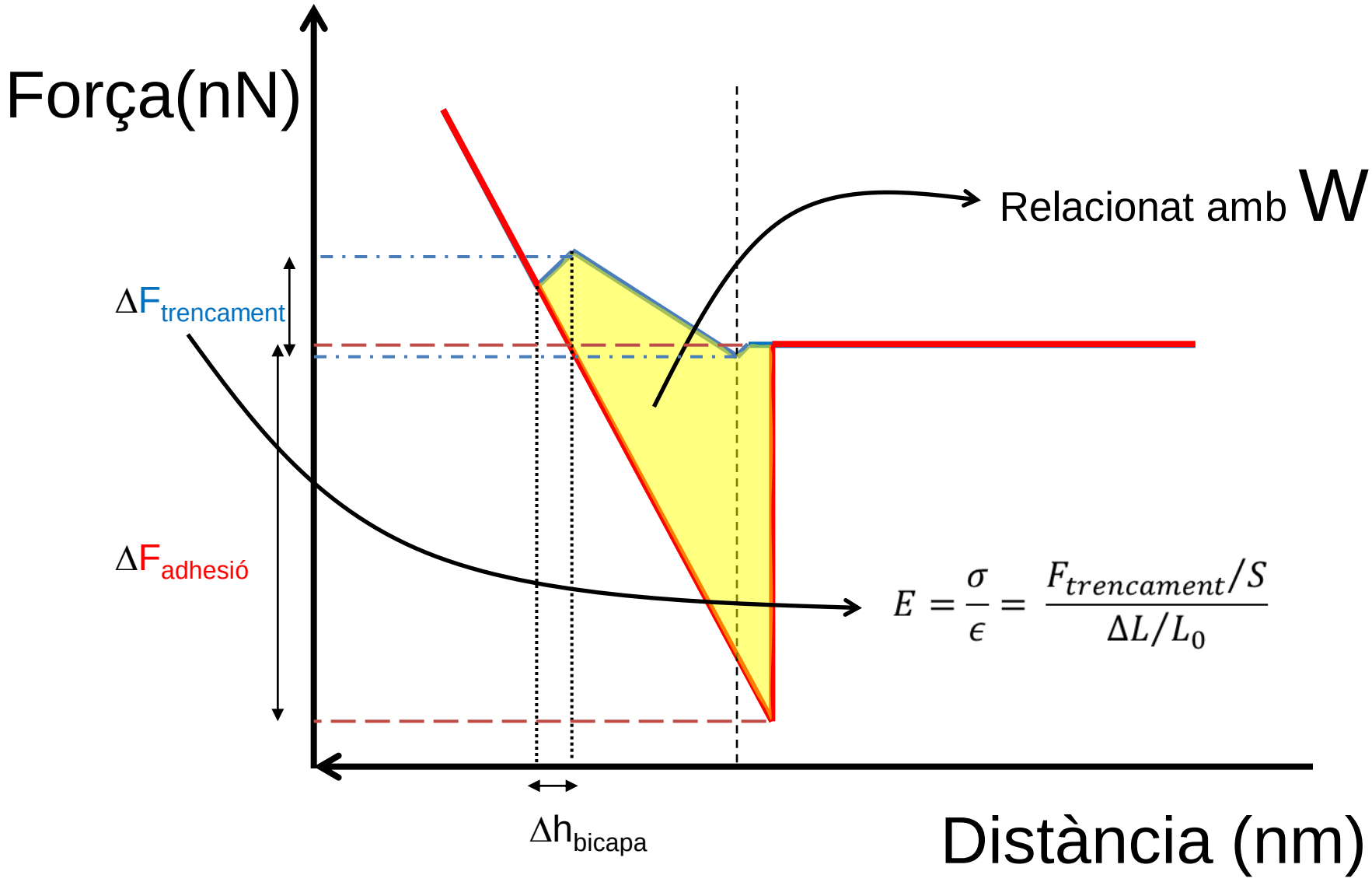
LacY mutant: C154G

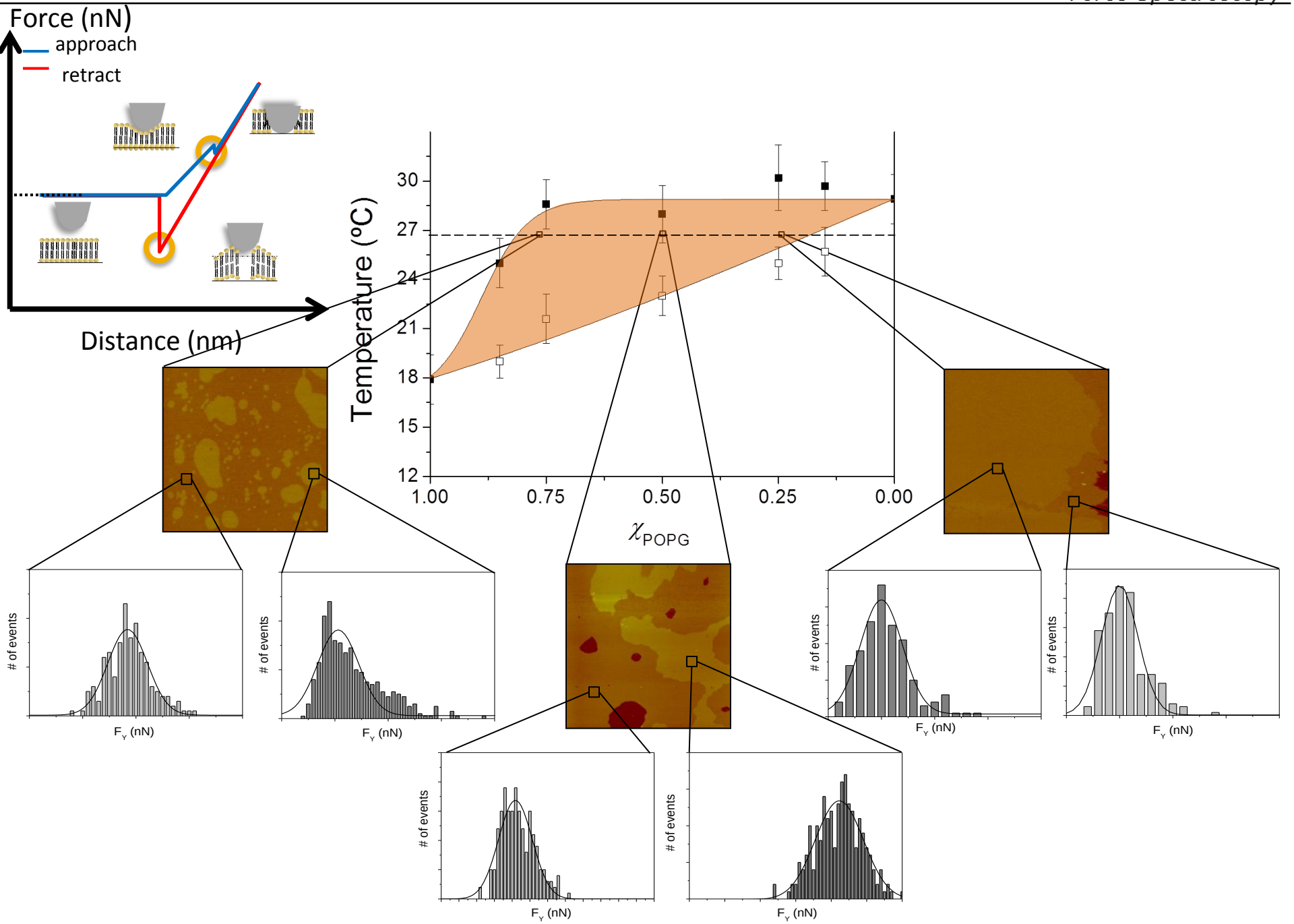
POPE:POPG (3:1)

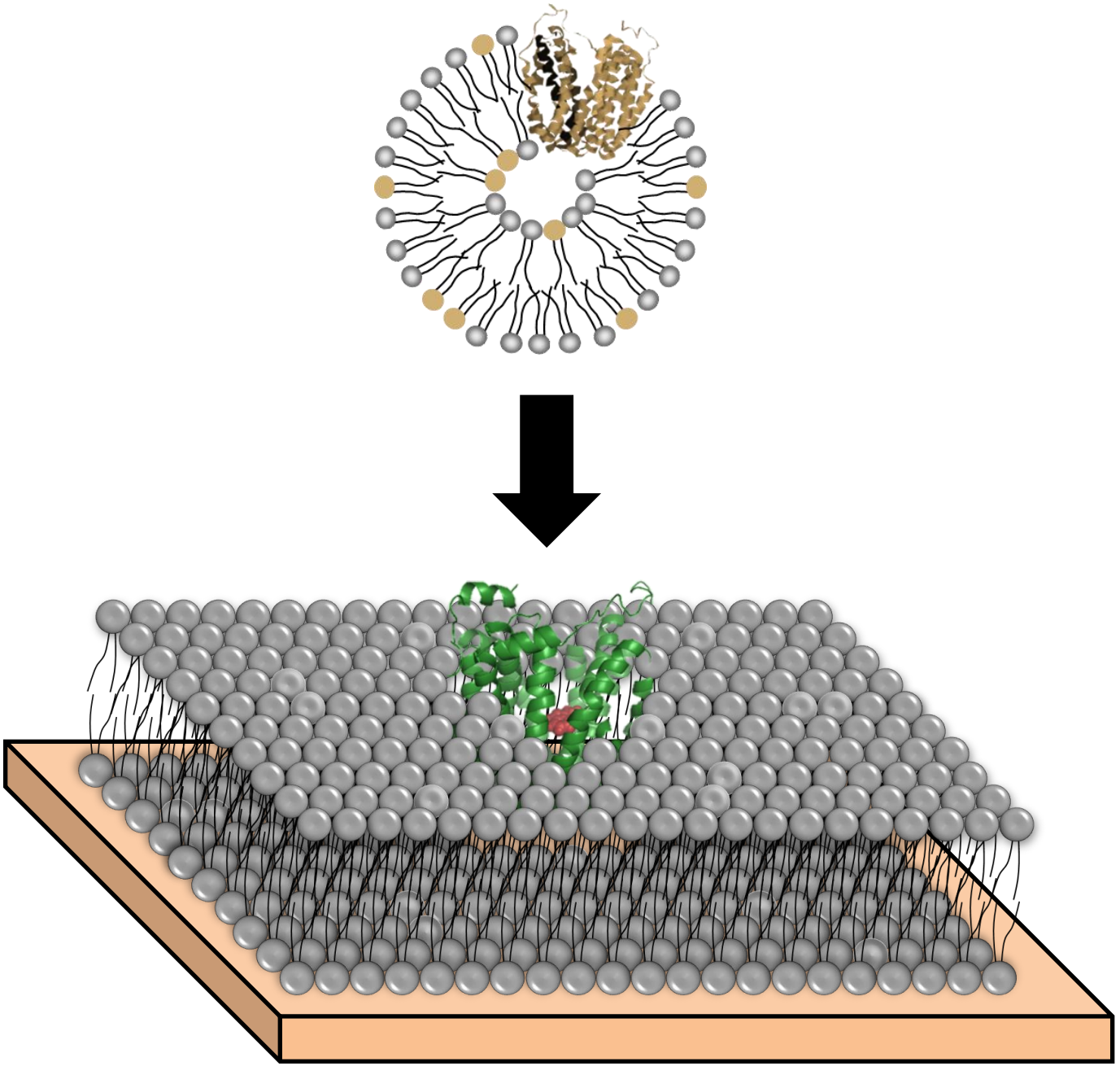


POPE:POPG (1:1)



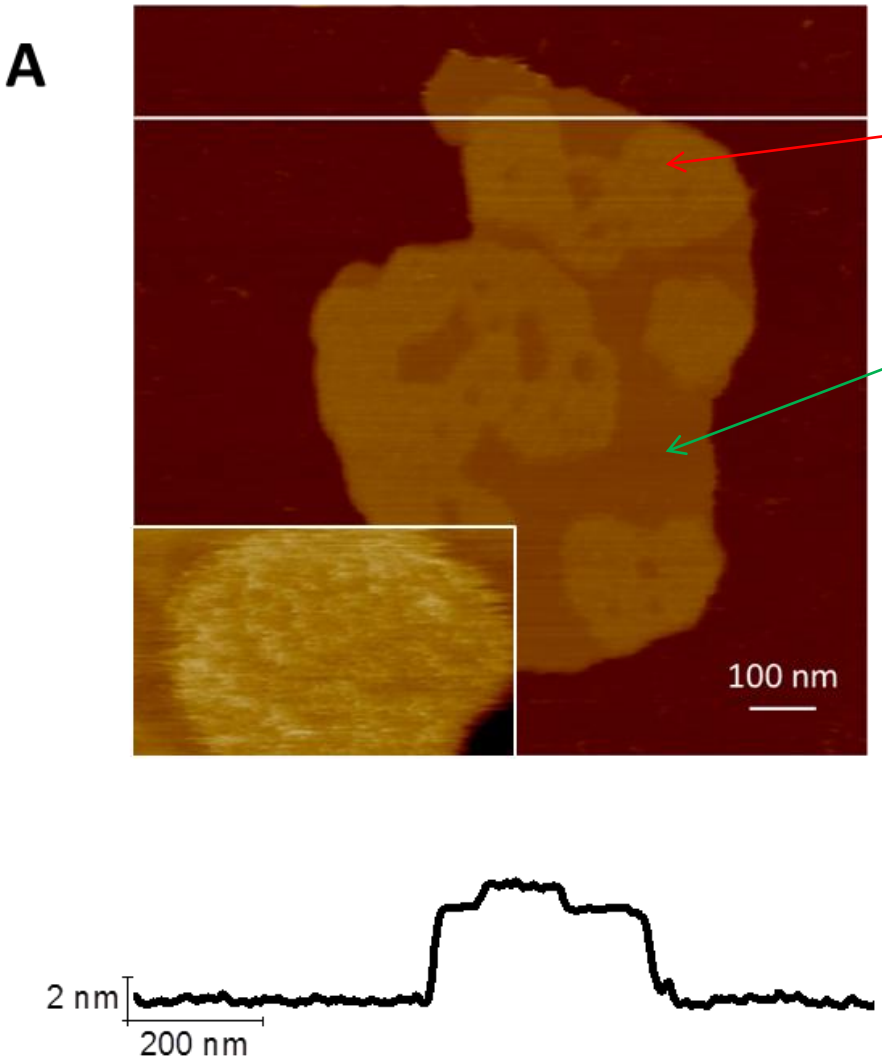

Força(nN)
Relacionat amb W
ΔF trencament
ΔF adhesió
$E = \frac{\sigma}{\epsilon} = \frac{F_{trencament}/S}{\Delta L/L_0}$
Δh bicapa
Distància (nm)


Force (nN)
approach
retract
Distance (nm)
Temperature (ºC)
30
27
24
21
18
15
12
1.00
0.75
0.50
0.25
0.00
χ_{POPG}
# of events
F_Y (nN)

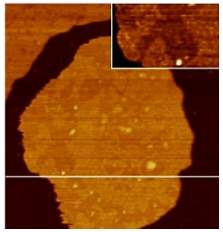


DPPE:POPG (3:1)

LPR = 0.5



POPE:POPG (3:1)



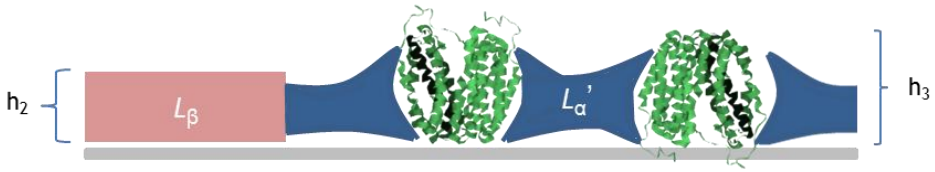
LPR = 0.5

SLB

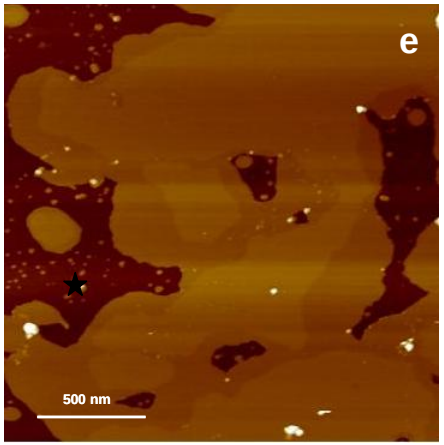
SLB + lactose permease

Step height between both phases

- SLB → 0.9 ± 0.1 nm
- SLB + lactose permease → 0.4 ± 0.4 nm



Protrusions = 1.4 ± 0.5 nm

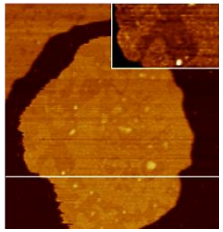


LPR = 40

POPE:POPG (3:1)

SLB

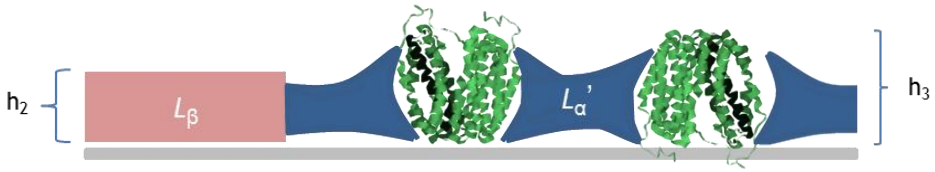
SLB + lactose permease



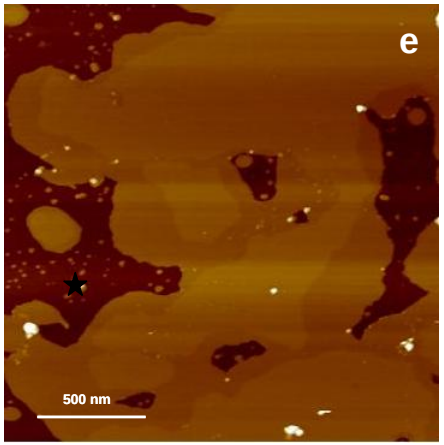
LPR = 0.5

Step height between both phases

- SLB → 0.9 ± 0.1 nm
- SLB + lactose permease → 0.4 ± 0.4 nm

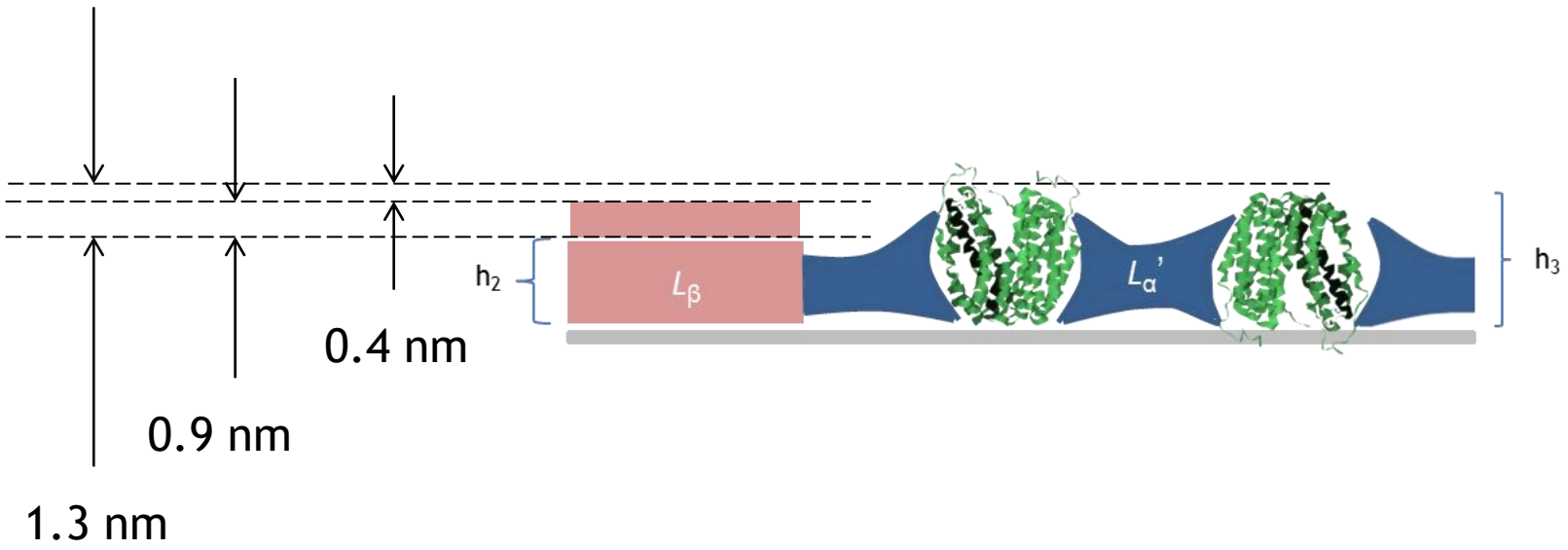


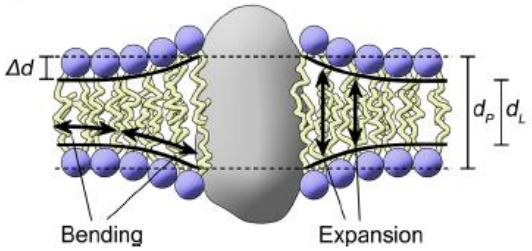
Protrusions = 1.4 ± 0.5 nm

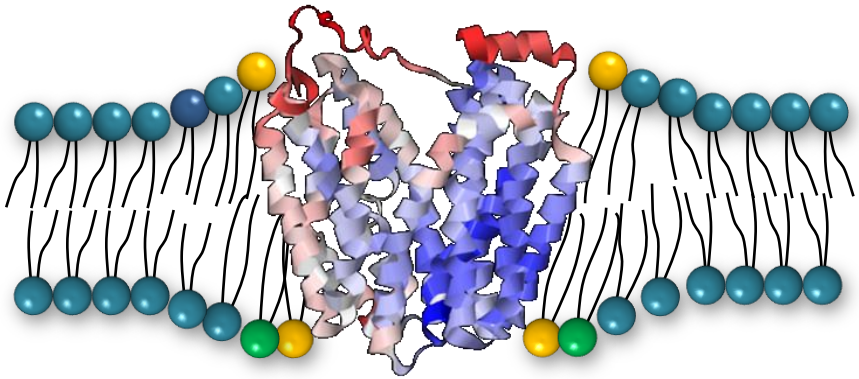


LPR = 40

POPE:POPG (3:1)

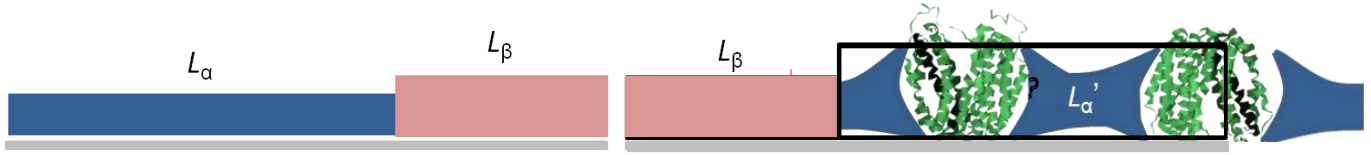






Brown, M.F (2012) Biochemistry 51,9782-9795

DPPE:POPG (3:1)



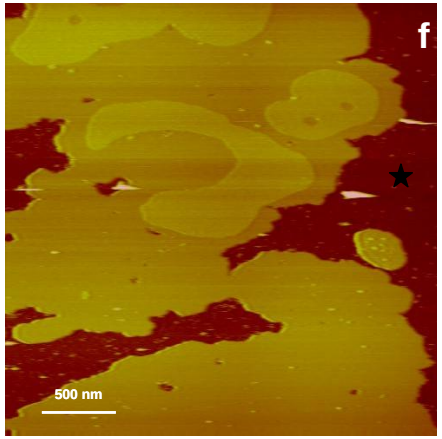
SLB

SLB + lactose permease

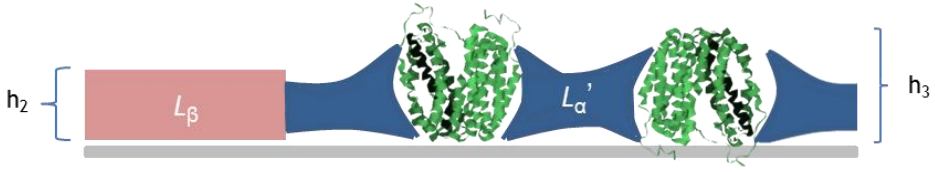
Step height between both phases

- SLB → 0.7 ± 0.4 nm
- SLB + lactose permease → 1.0 ± 0.4 nm

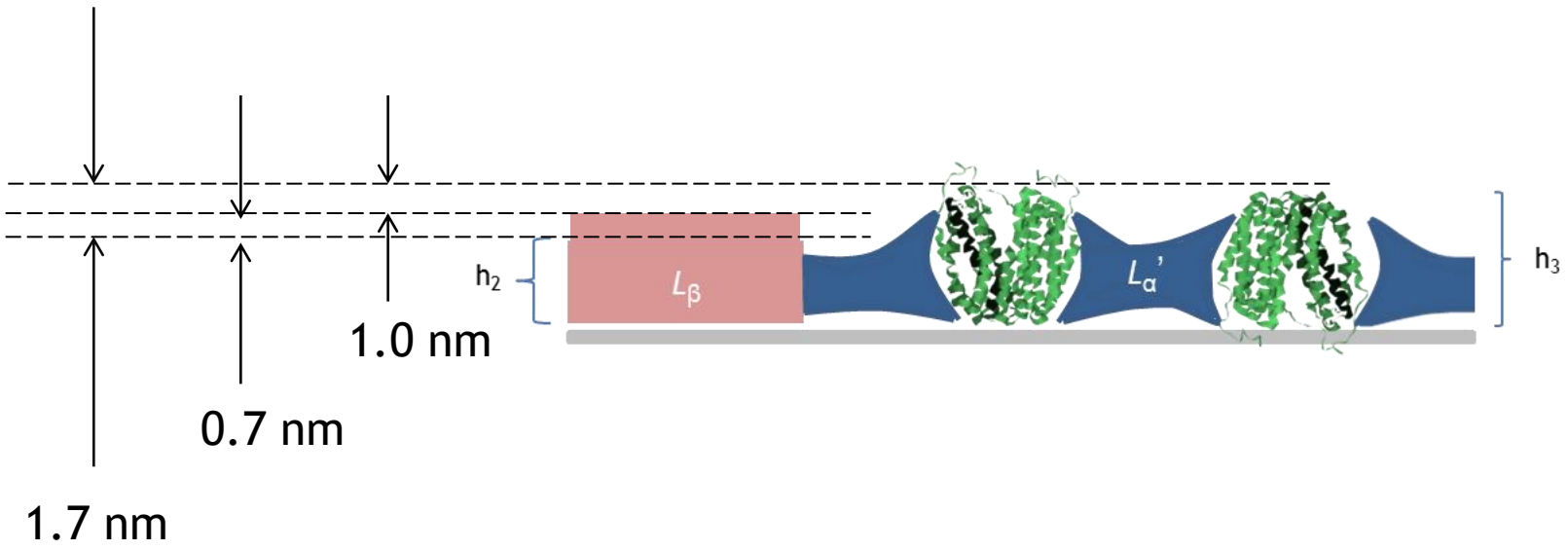
LPR = 40

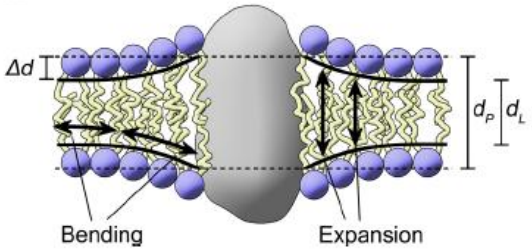


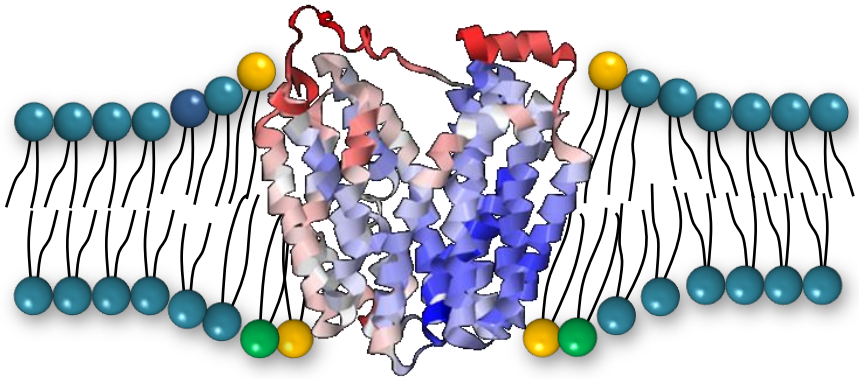
Protrusions = 2.0 ± 0.7 nm



DPPE:POPG (3:1)







Brown, M.F (2012) Biochemistry 51,9782-9795

Estirament de proteïnes

Força (nN)

Distància (nm)

Relacionat amb W

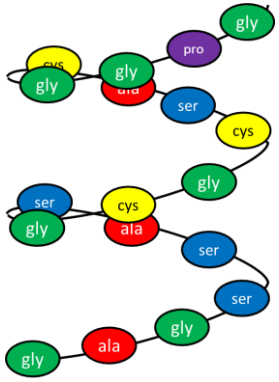
L_{C1}

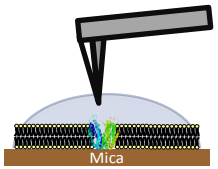
L_{C2}

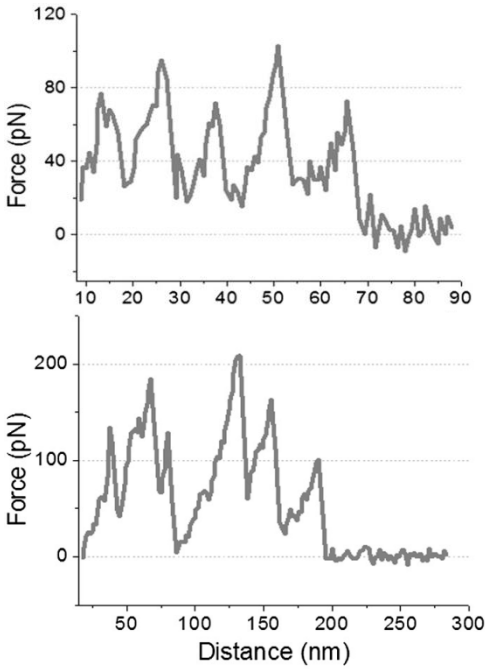
L_{C3}

Model *Worm Like Chain*

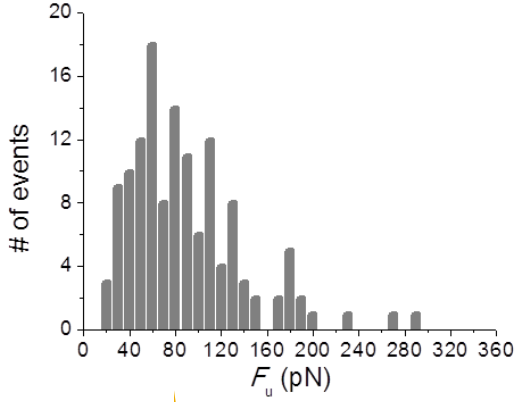
$$F(x) = \frac{k_B T}{p}\left[\frac{1}{4\left(1-\frac{x}{L_C}\right)^2} - \frac{1}{4} + \frac{x}{L_C}\right]$$

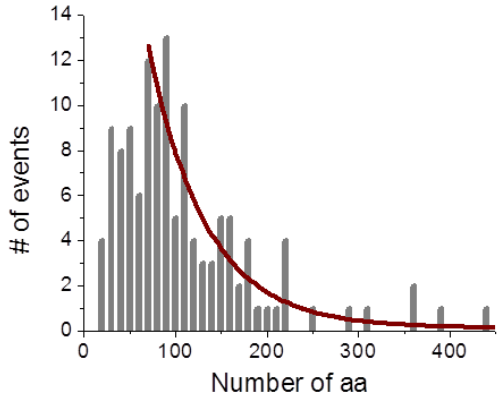




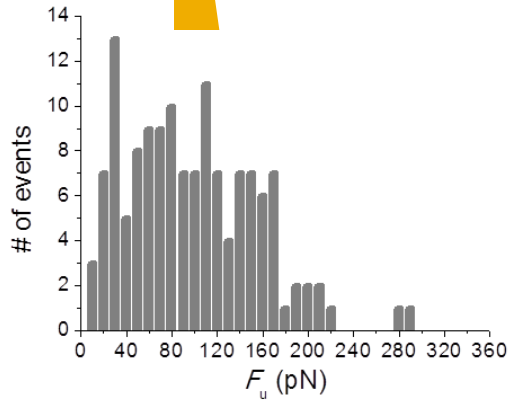


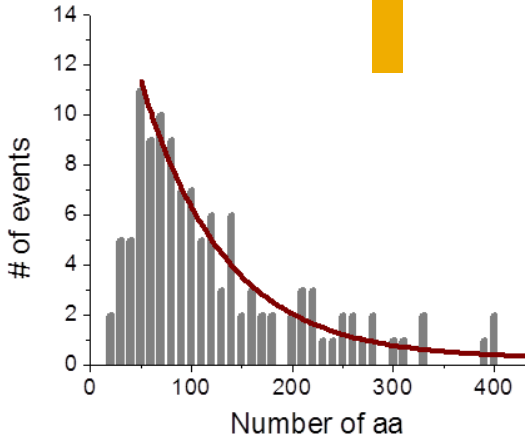
POPE:POPG





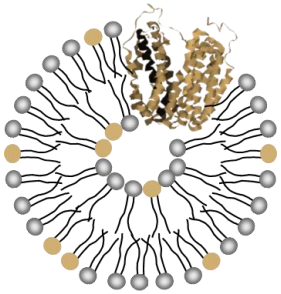
✓ Modification of the pattern of unspecific pulling



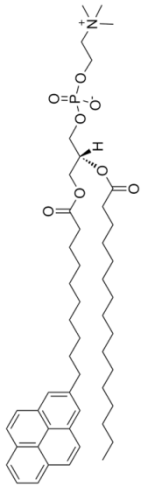


DPPE:POPG

LacY mutant: single-W 151 C154G and D68C

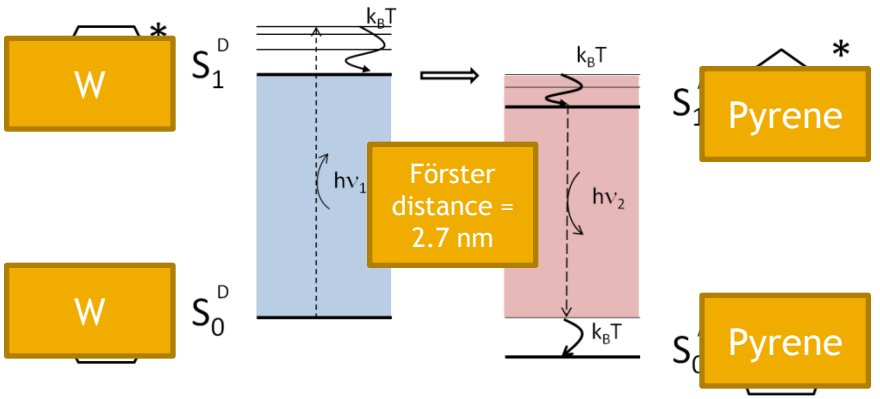


+



Proteoliposomes
LPR = 40

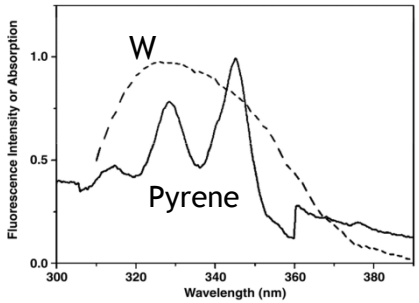
Acyl chain labeled phospholipids
x_{probe} = 0.015



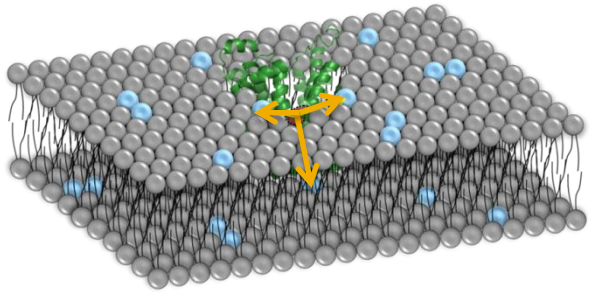
STUDY OF THE ANNULAR REGION

W

Pyrene



W-Pyrene FRET

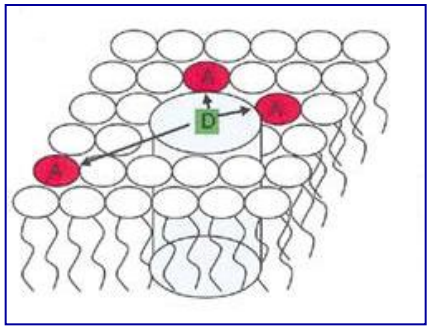


FRET data analysis

W

Pyrene

1 Donor
&
Several Acceptors



Model based on binomial distribution

$$i_{DA}(t) = i_D(t)\rho_a(t)\rho_r(t)$$

$$\rho_a(t) = \sum_{n=0}^{m} e^{-n\kappa_t t}\binom{m}{n}\mu^n(1-\mu)^{m-n}$$

$$\rho_r(t) = \exp\left\{-4n_2\pi l^2\int_0^{\frac{1}{\sqrt{l^2+R_e^2}}}\frac{1-\exp\left(-tb^3\alpha^6\right)}{\alpha^3}d\alpha\right\}$$

$$b = \left(R_o^2/l\right)^2\tau_D^{-1/3}$$

$$k_\tau = \frac{1}{\tau_D}\left(\frac{R_o}{d}\right)^6$$

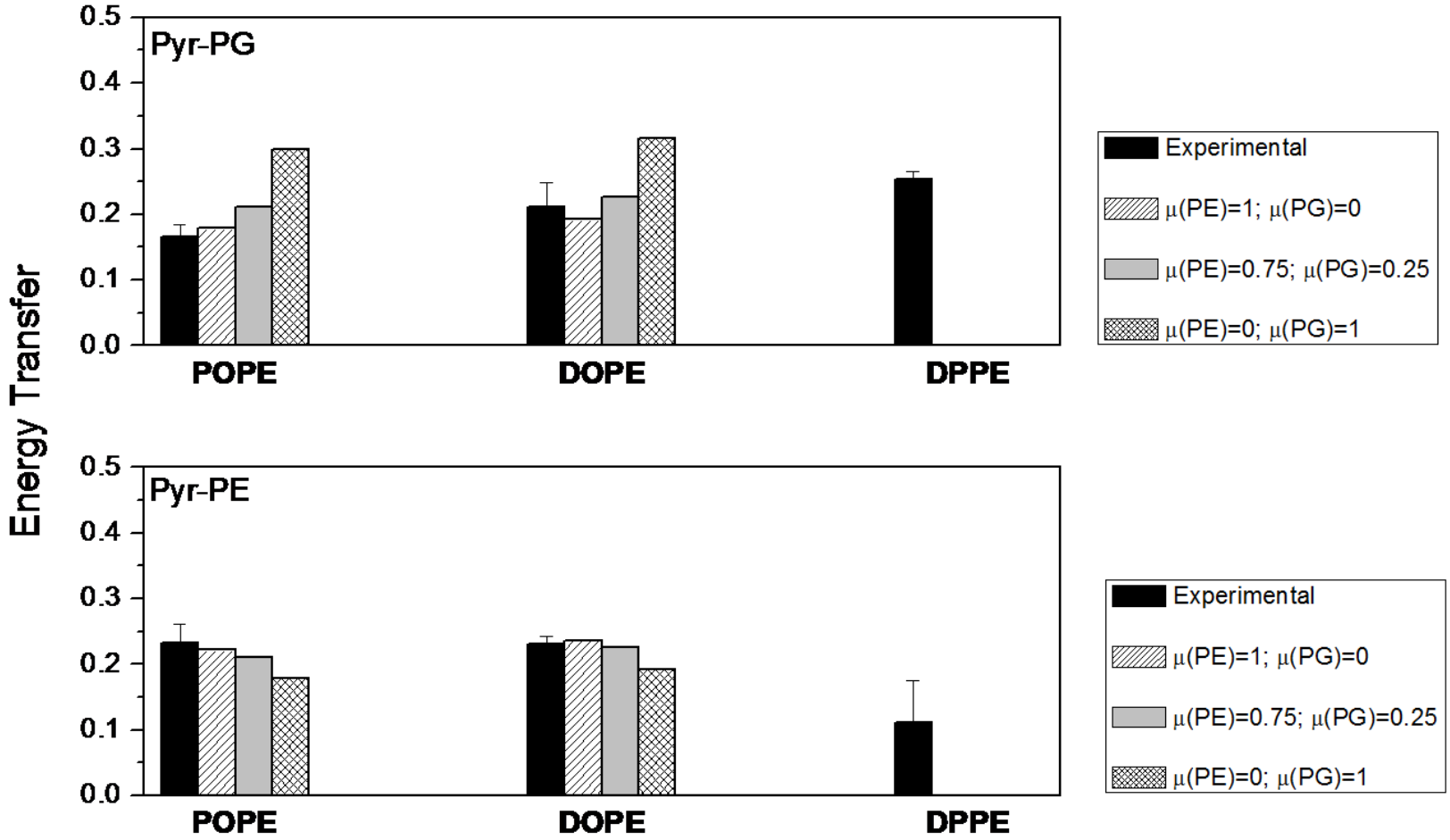
$$\mu = K_s\frac{n_{pyr}}{n_{pyr}+n_{PL}} = K_s X_{pyr}$$

μ

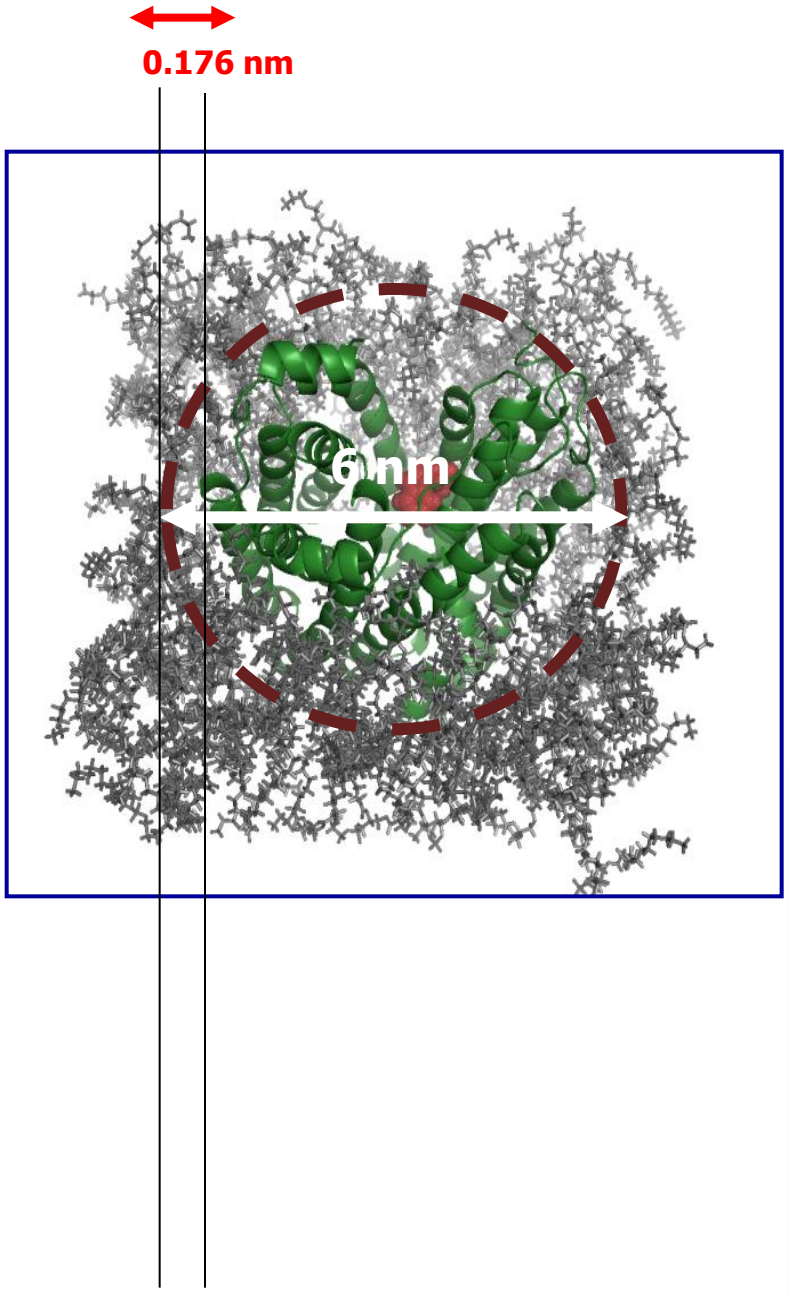
Probability of each site in the annular ring being occupied by a labeled pyrene

K_s

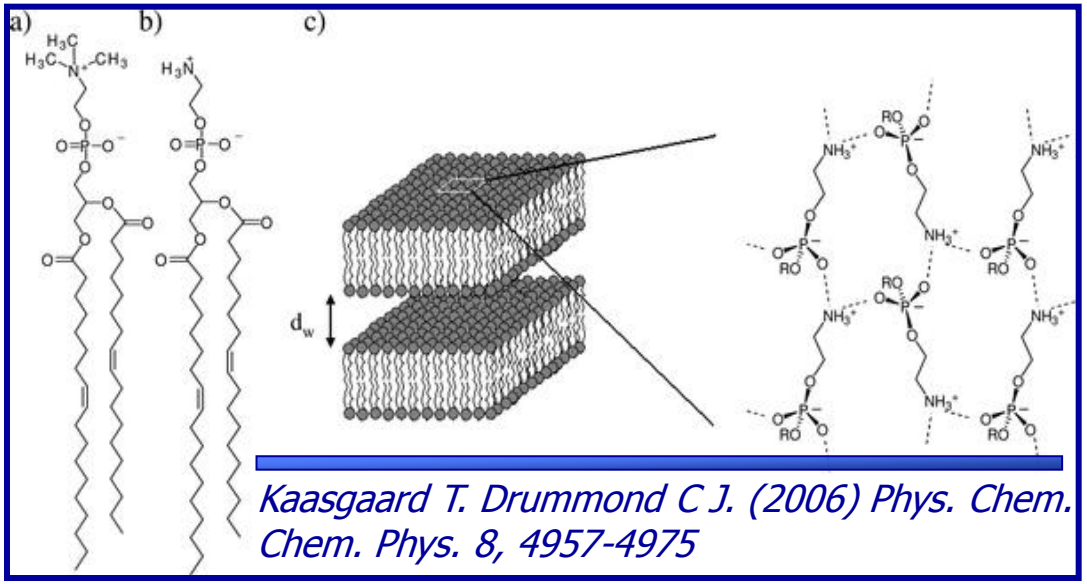
Ratio between the acceptor mole fractions in the annular region and in the overall system



Comparison of experimental and theoretical values of FRET efficiency between W151 and Pyr-PG (top) and Pyr-PE (bottom) at 37 °C in POPE/POPG (3:1, mol/mol) (left), DOPE/POPG (3:1, mol/mol) (center), and DPPE/POPG (3:1, mol/mol) (right) proteoliposomes (1.5 μM LacY). The error bars stand for $\sigma/\sqrt{n}$, σ being the standard deviation and n the number of measurements performed.

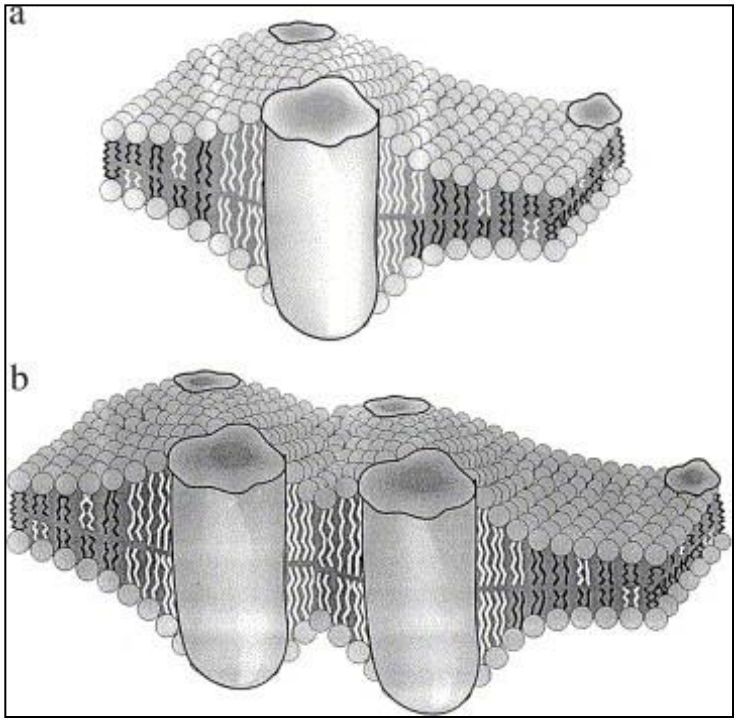


T	Distance, *R* (nm)	
	Pyr-PE	Pyr-PG
10 ºC	2.77	2.93
26 ºC	2.69	2.83

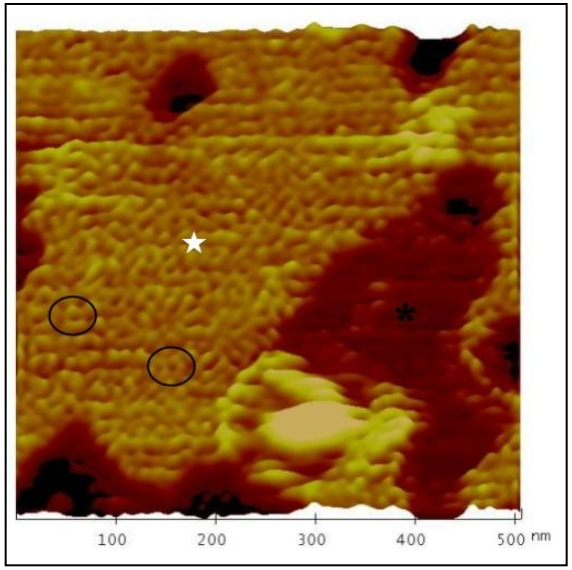


Kaasgaard T. Drummond C J. (2006) Phys. Chem. Chem. Phys. 8, 4957-4975

- are located at a defined position
- are time dependent



Jensen M Ø, Mouritsen O G. (2004) Biochim. Biophys. Acta- 3, 205-226



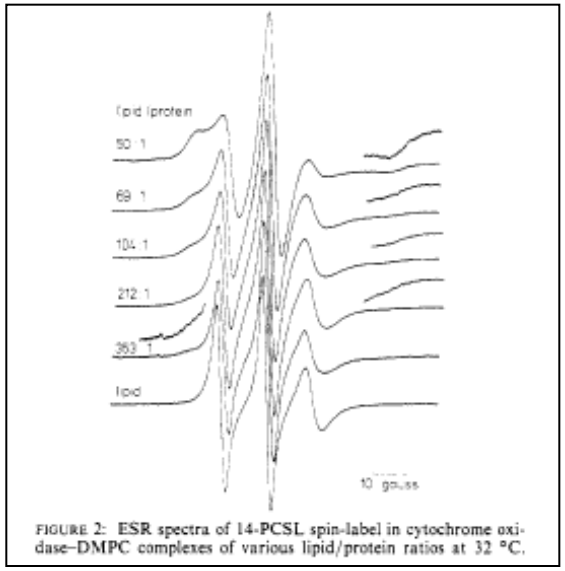


FIGURE 2: ESR spectra of 14-PCSL spin-label in cytochrome oxidase–DMPC complexes of various lipid/protein ratios at 32 °C.

$$\Delta p\ \Delta x \geq \frac{1}{2}\hbar$$

$$\Delta E\ \Delta t \geq \frac{1}{2}\hbar$$

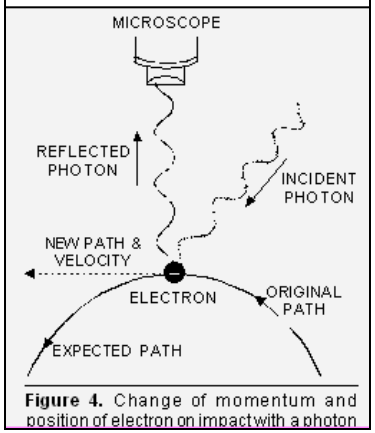
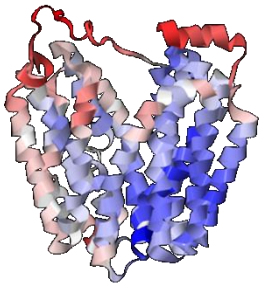


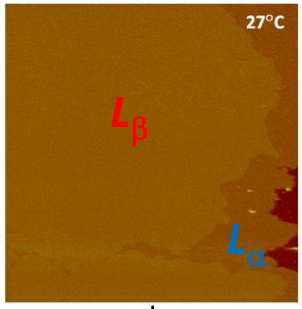
Figure 4. Change of momentum and position of electron on impact with a photon

Impossible to know exactly:
- where something is
- how fast it is going

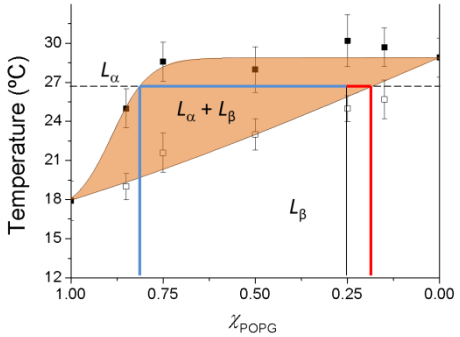
POPE:POPG (3:1)

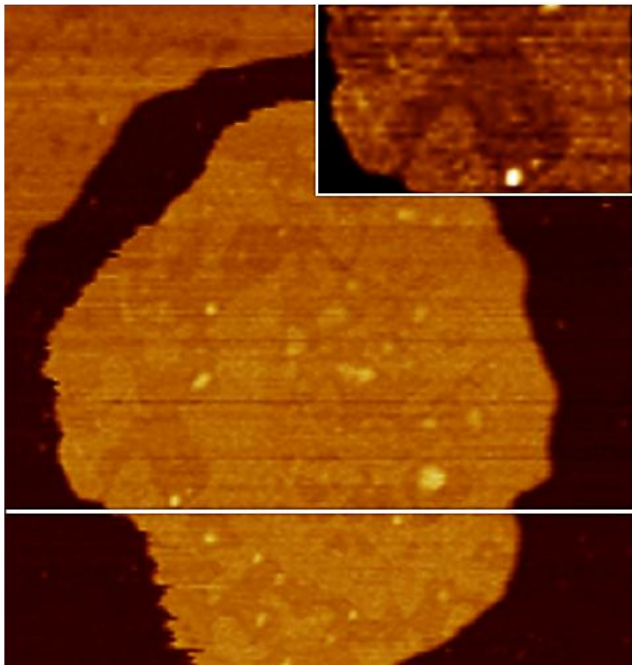


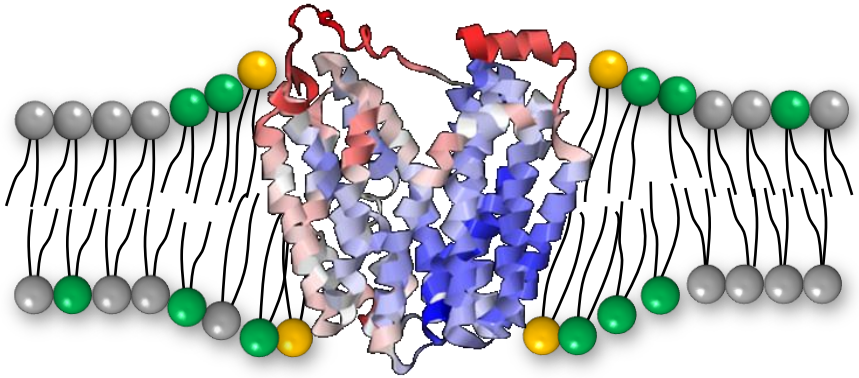
Enriched in POPE



Enriched in POPG





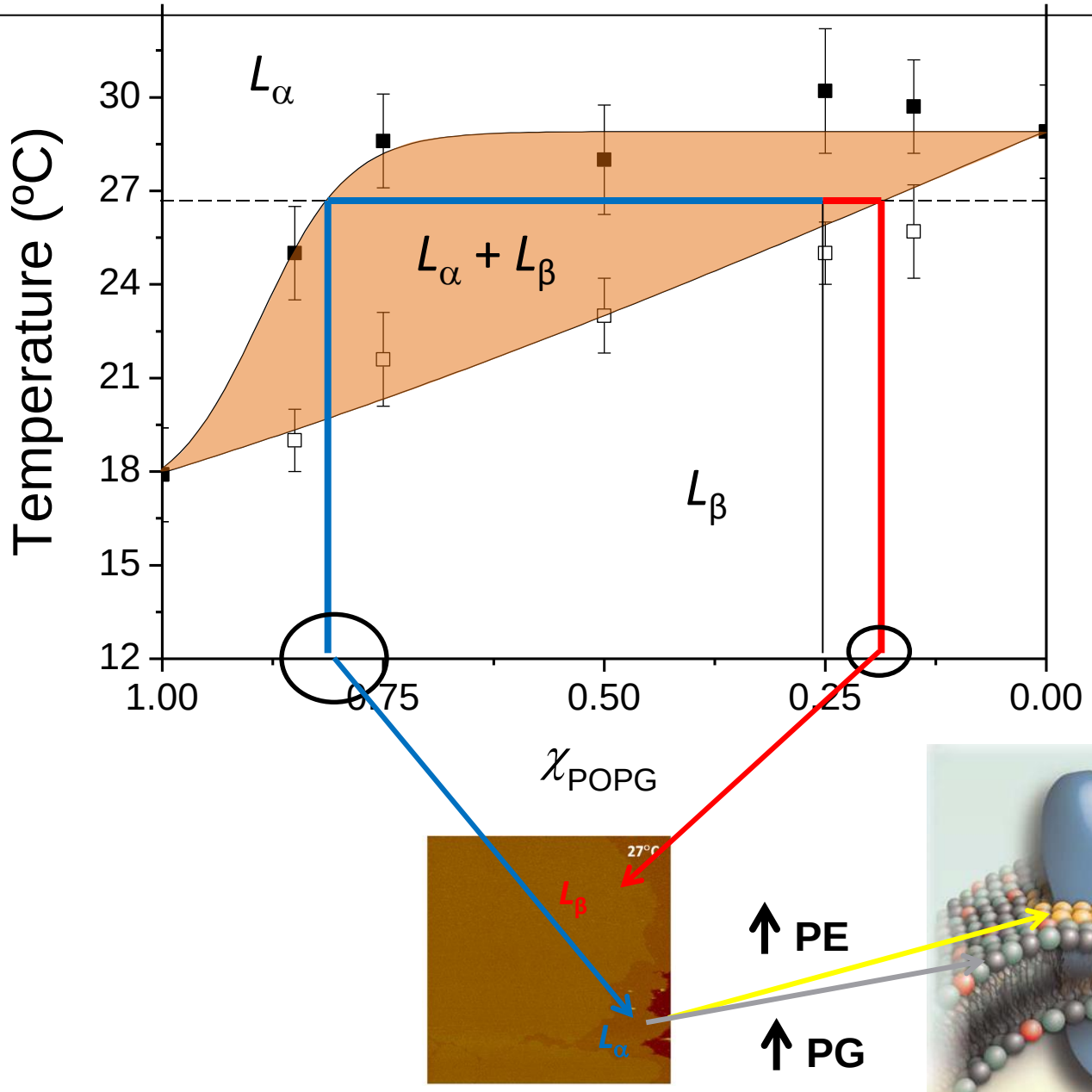


POPE

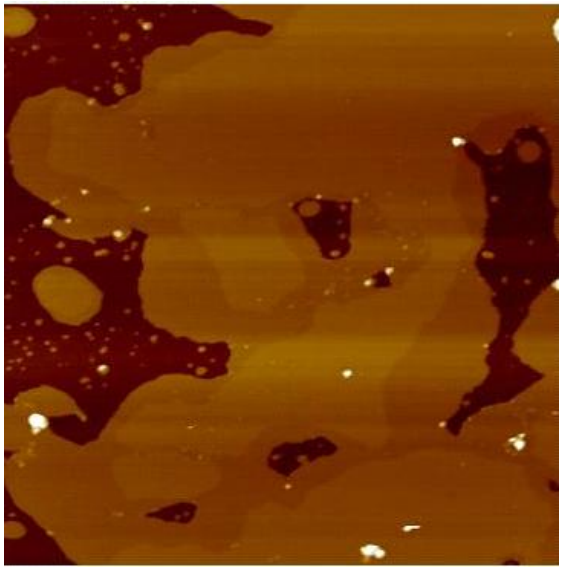
POPG

????

POPE ← FRET

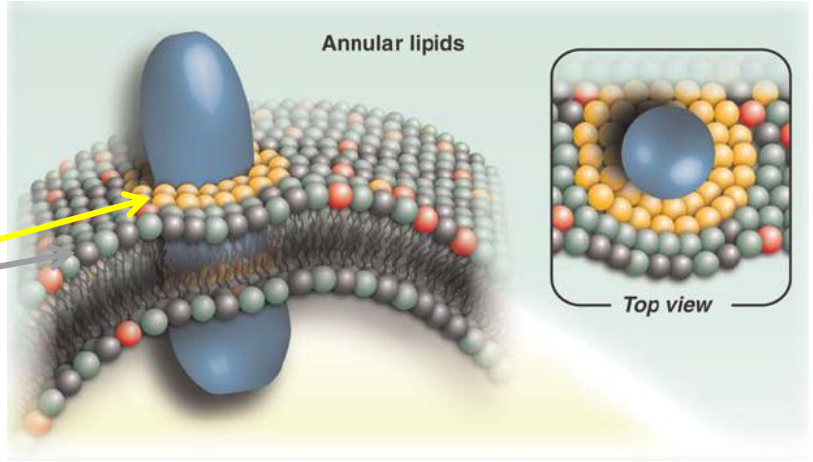


LacY is inserted in fluid phases



PE

PG



Contreras et al., (2011) Cold Spring Perspect. Biol. 4: a900475