Assembling a biological nanomotor on a nano-engineered surface

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« Because technology provides the tools and biology the problems, the two should enjoy a happy marriage »

S. Fields, S. Proc. Natl. Acad. Sci USA 2001

1- Nanotechnologies for Biology

Devices

Materials

« Biology offers a window into the most sophisticated collection of functional nanostructures that exists. »

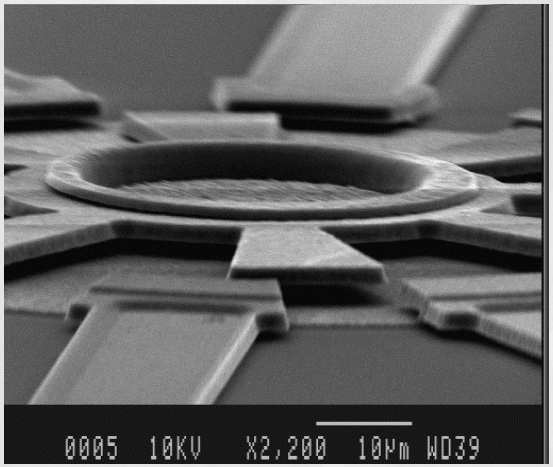
G.M Whitesides, Nature Biotech, The « right » size in Nanobiotech, 2003

2- Nanotechnologies from Biology

Self-Assembly

Bio-Nanomachines

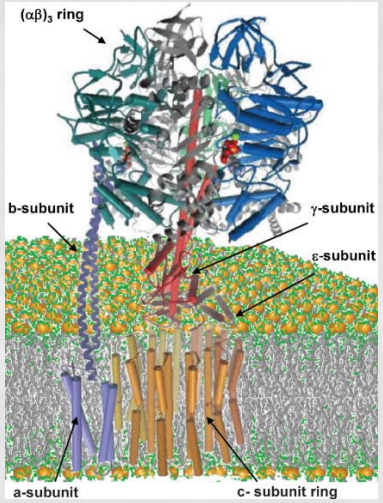
Nanotechnologies from Biology



LAAS Si Micromotor H. Camon et al 1999

- Size > 10 µm
- Hard material
- 2D
- Em actuation
- Poor efficiency
- Air or vacuum
- Fragile

•Techno-assembled : Alignment



D. Spetzler et al. Lab Chip, 2007, 7, 1633–1643

F1-FO ATP synthase

Myosin

Kinesin

Dynein

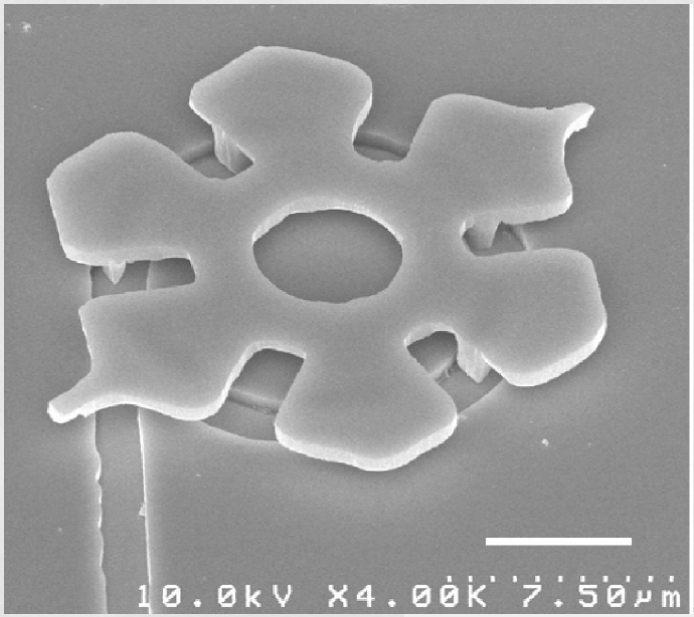
Flagellar nanomotor of bacteria

- Size : 5-50 nm
- Molecular material
- 3D
- Chemical actuation
- High efficiency
- Liquid
- Self-repairable

•Self-assembled : Stochastic

Nanotechnologies from Biology

Using the assembled biomachine inside a device



A microrotary motor composed of a 20-µm-diameter silicon dioxide rotor driven on a silicon track by the gliding bacterium Mycoplasma mobile : 2 rpm

Y. Hiratsuka et al, 13618–13623 PNAS**, 2006** vol. 103 no. 37

0.5 µm

Nanotechnologies from Biology

-Assembling Bionanomachines on chip from purified proteins

PROJET FLANAMO – ANR PNANO

Assembling the flagellar rotary nano-motor of *E-Coli* on a solid surface through Nanotechnologies

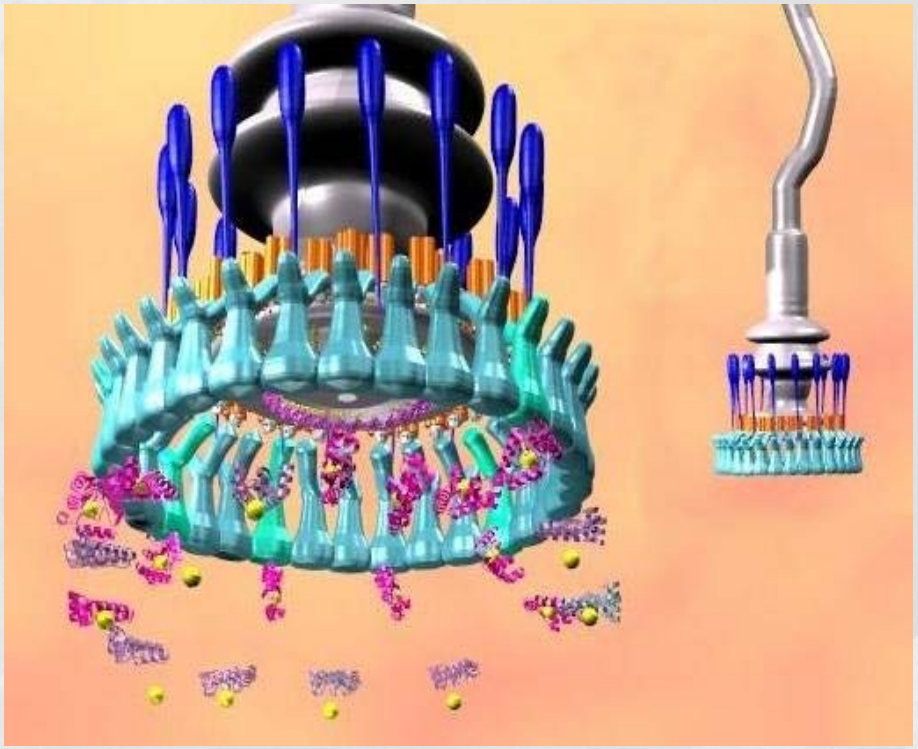
35 Proteins involved

Dimension : 45 nm

Speed : 20 000 rpm – 60 µm/s

Power : 1000 H^+/rotation

Reversible



Method: Engineering of a surface for

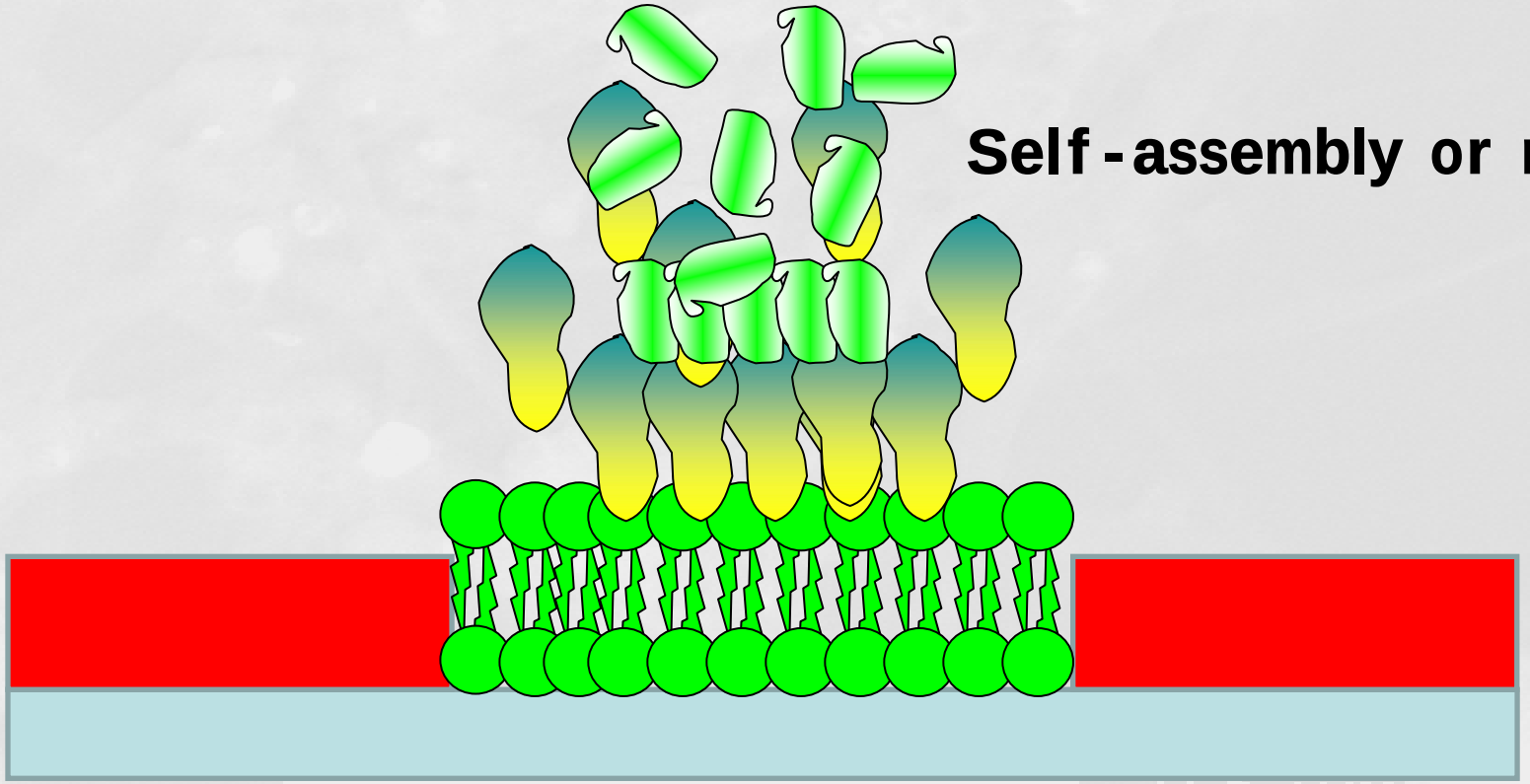
Re-creating the conditions of self-assembly

Observation using AFM

Objectives :

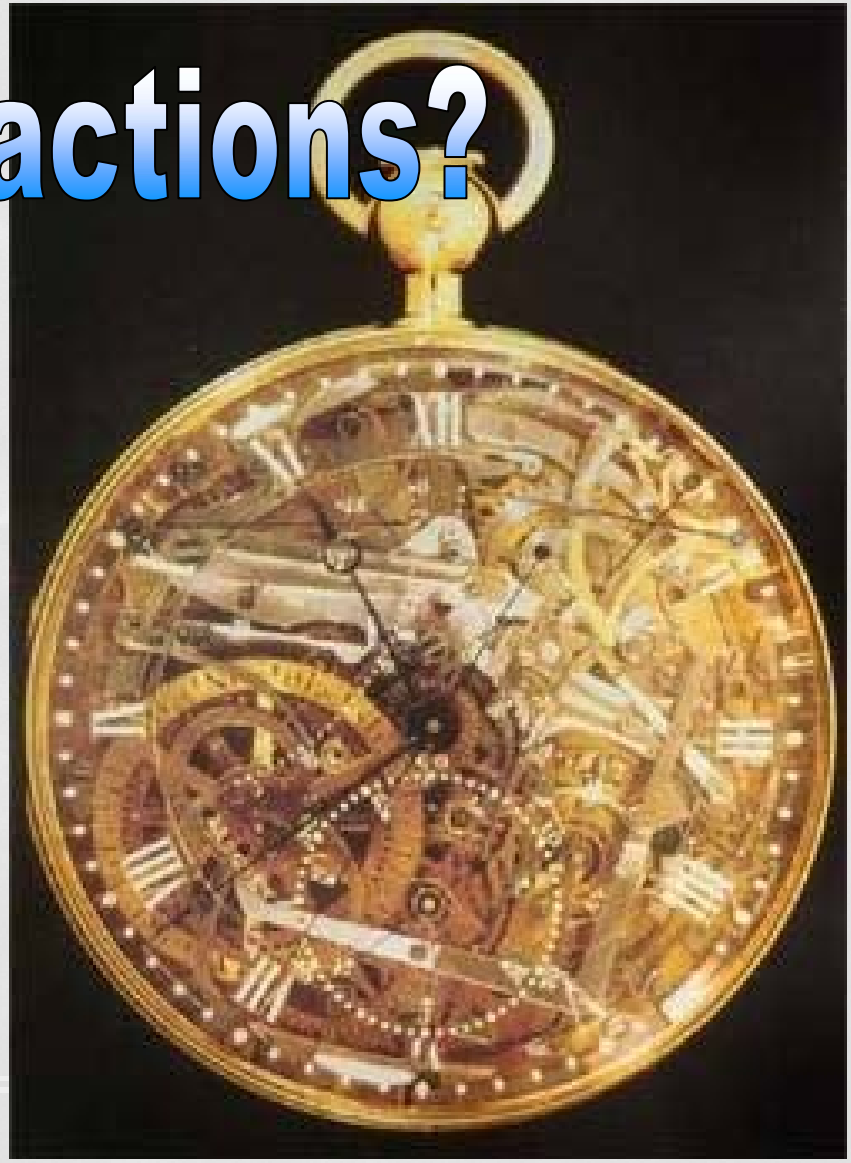
- **Understand the mechanism of the nano-motor**
- **Artificial Assembly of a bio-nanomachine from isolated proteins**

Assembling of purified proteins of the nanomotor on an artificial surface



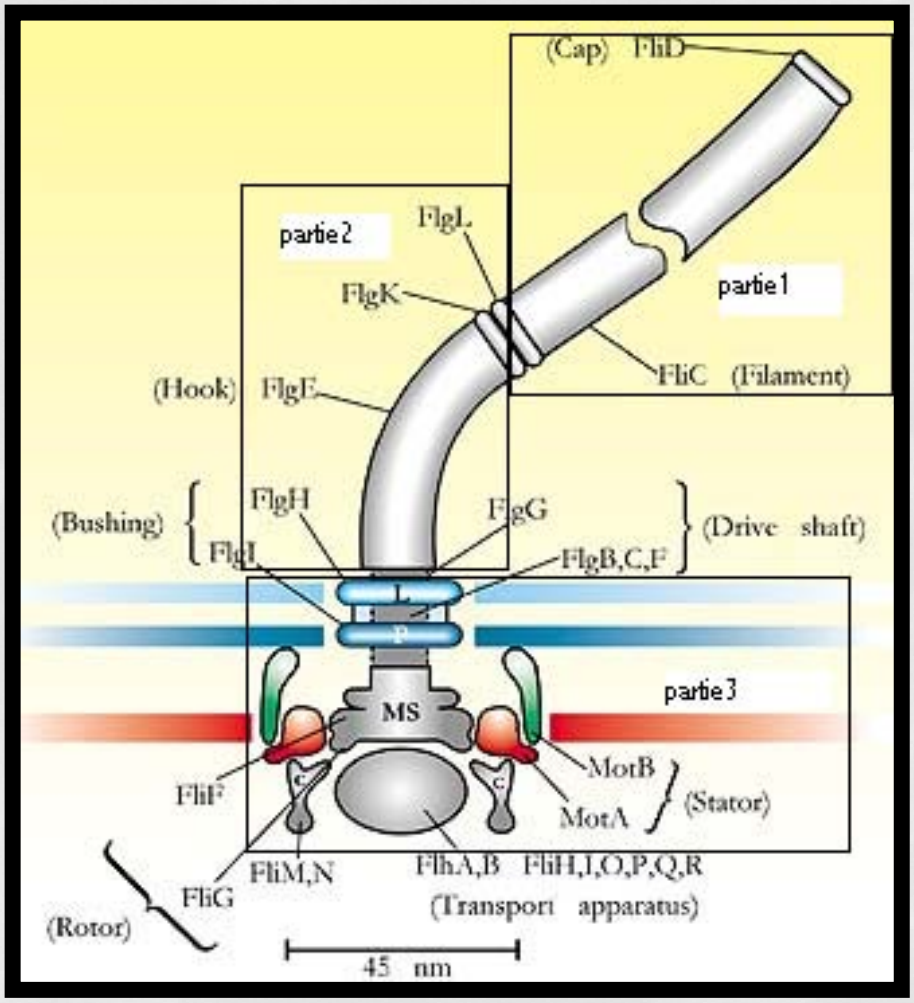
Self-assembly or not ?

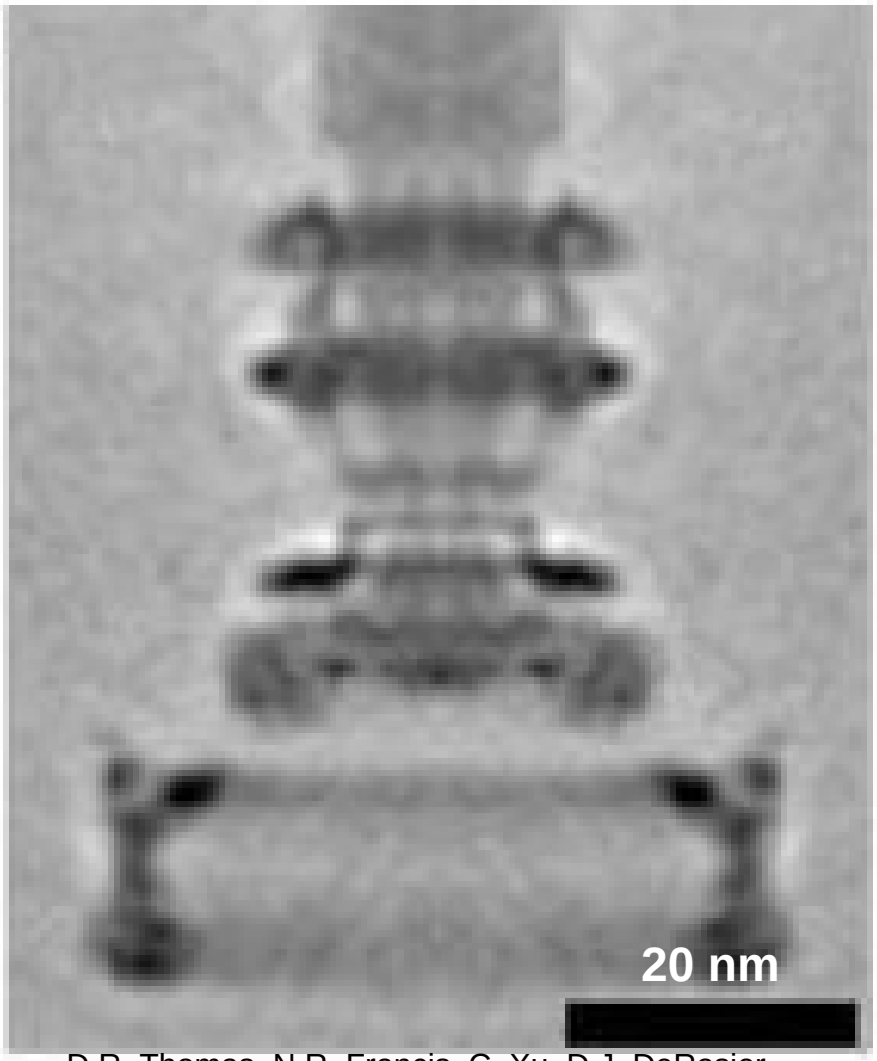
Interactions?



Interactions
Using a novel approach
based on the QCM-D technology

6 main proteins : MotA, MotB, FliF, FliG, FliM, FliN





D.R. Thomas, N.R. Francis, C. Xu, D.J. DeRosier, J. Bacteriol. 188 (2006) 7039.

How to investigate X/Y interaction?

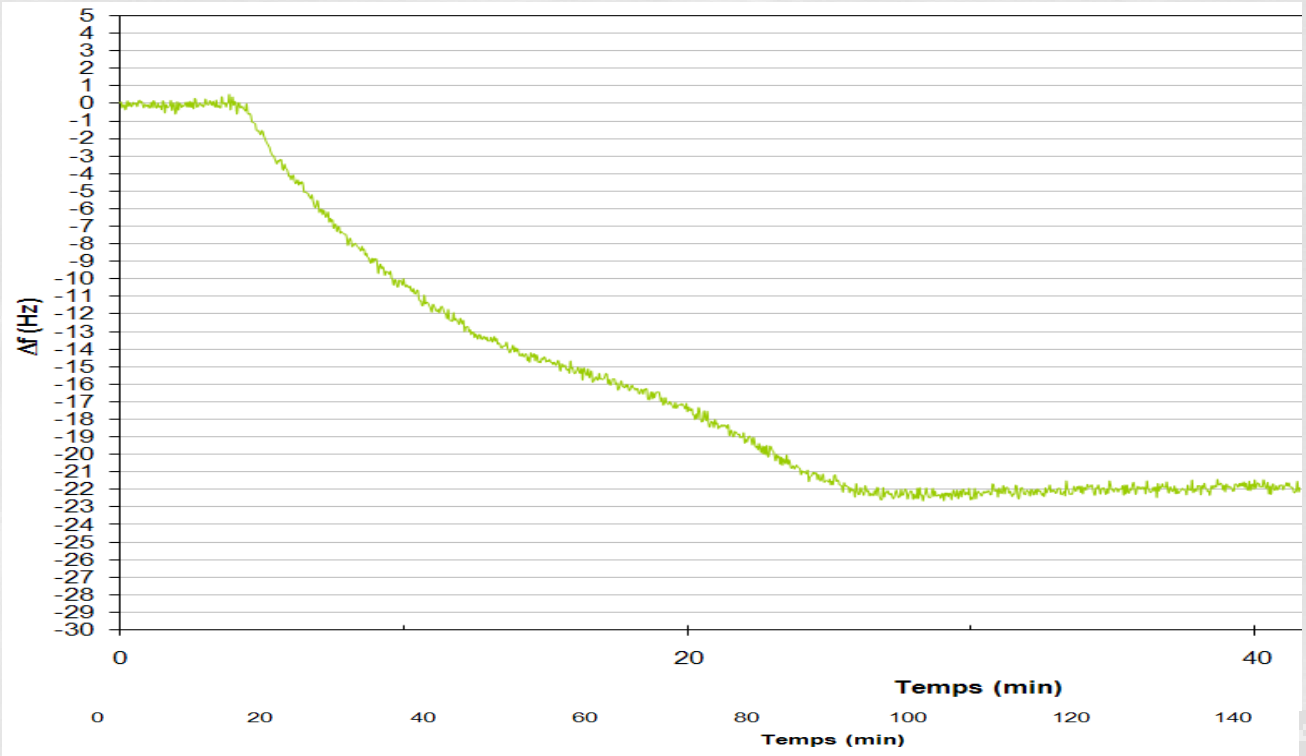
1/ Thiol layer Formation

2/ NHS/EDC activation

3/ Anti-body Anti-Gst Incubation

4/ Ethanolamine passivation

5/ Injection X-Gst protein



6/ Injection Y protein

How to evaluate the final interaction between X-Gst and Y?

$$\Delta m = C.\Delta f$$

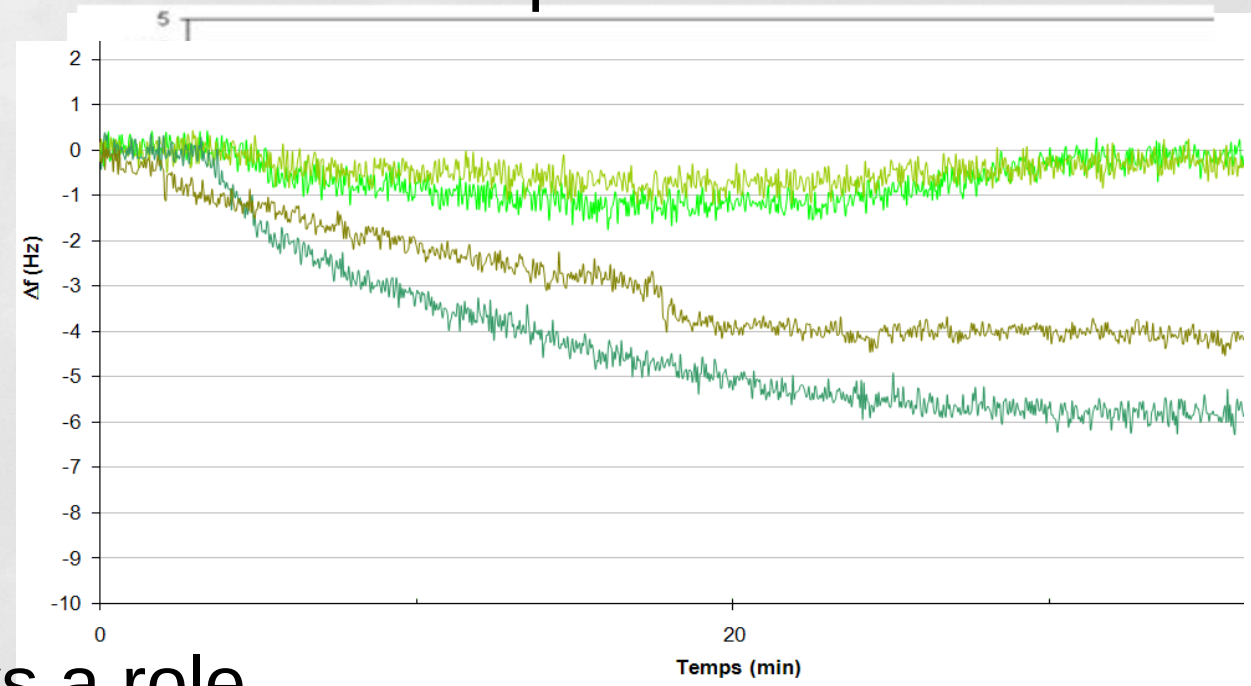
Sauerbey

$$d_{X\text{-}Gst} = (\Delta m_{X\text{-}Gst} * N) / Mw_{X\text{-}Gst}$$

$$d_{Y} = (\Delta m_{Y} * N) / Mw_{Y}$$

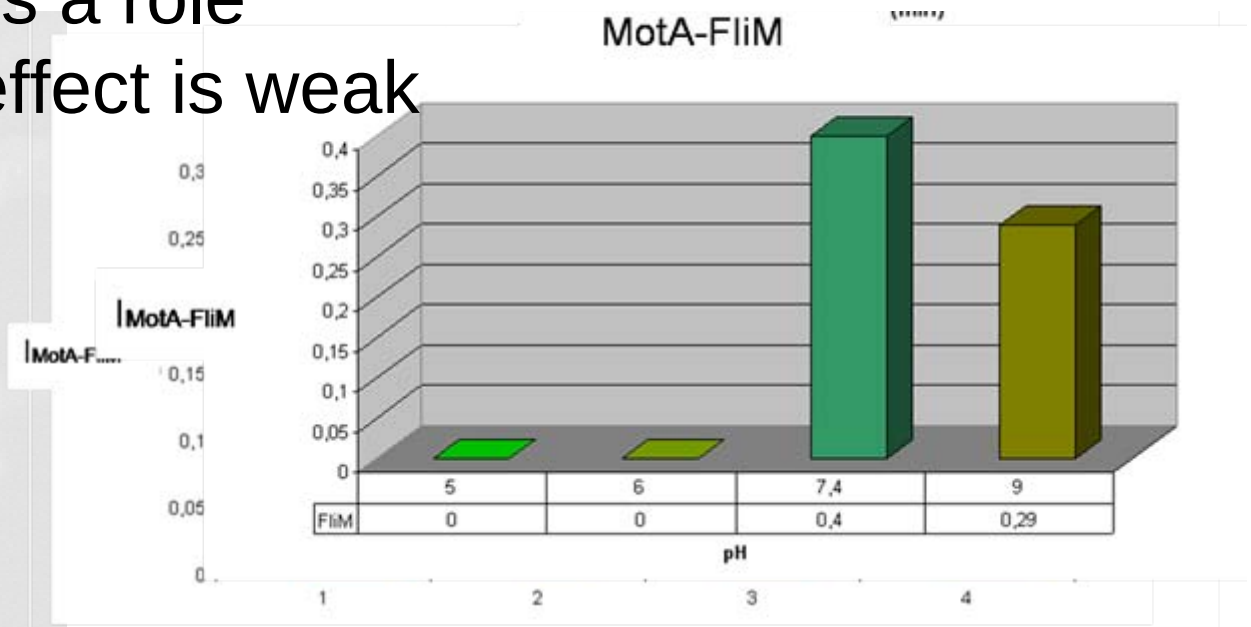
$$I_{Y/X\text{-}Gst} = d_Y / d_{X\text{-}Gst} = (\Delta f_Y / \Delta f_{X\text{-}Gst}) * (Mw_{X\text{-}Gst} / Mw_Y)$$

One example: MotA-Gst and FliM

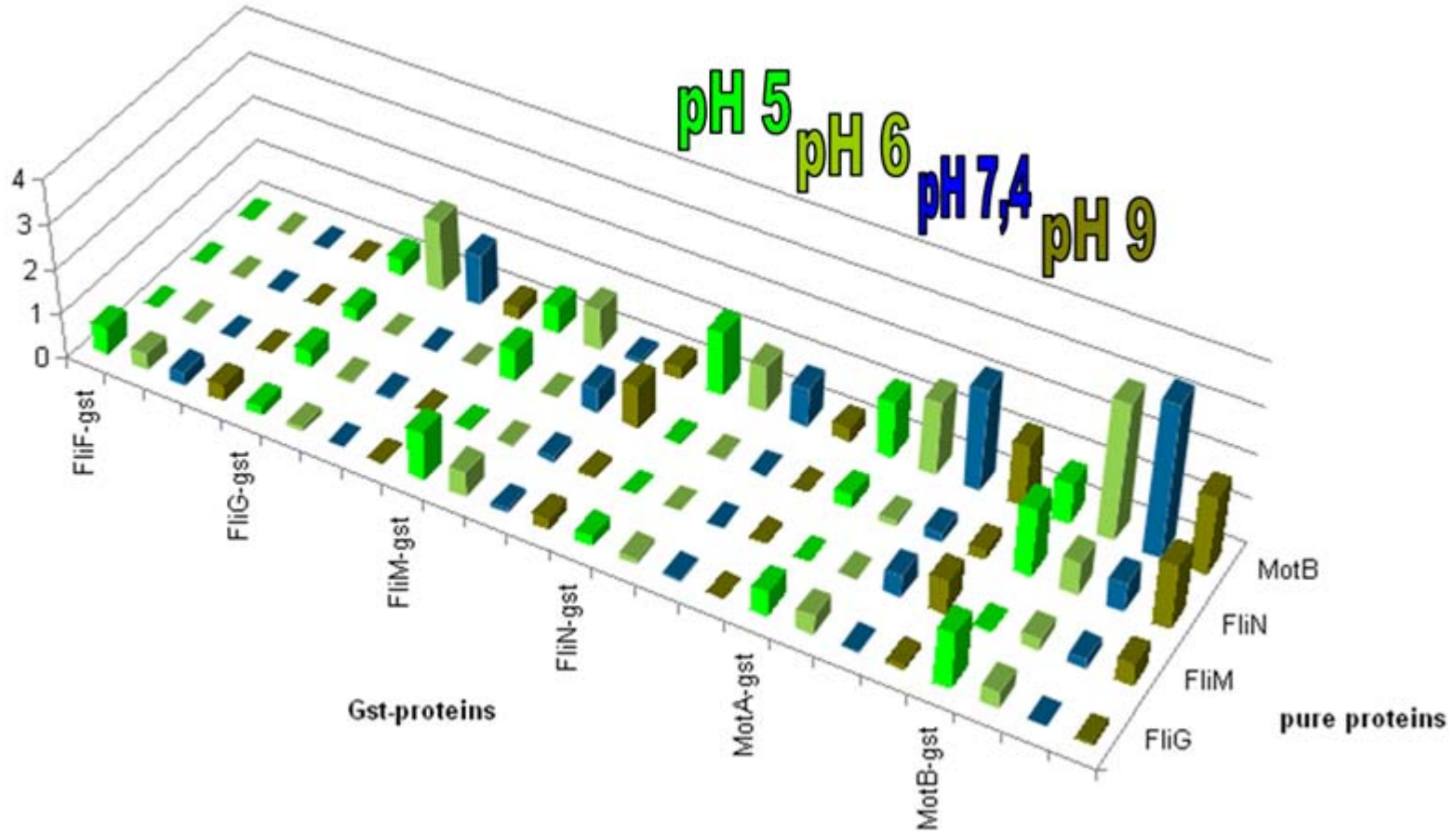


pH 5
pH 6
pH 9
pH 7,4

pH plays a role
Buffer effect is weak



Extensive data on the interactions



l factor

In

0.5 µm

Interactions conclusions

QCM-D was used for the first time and brings new data

Expected and not observed

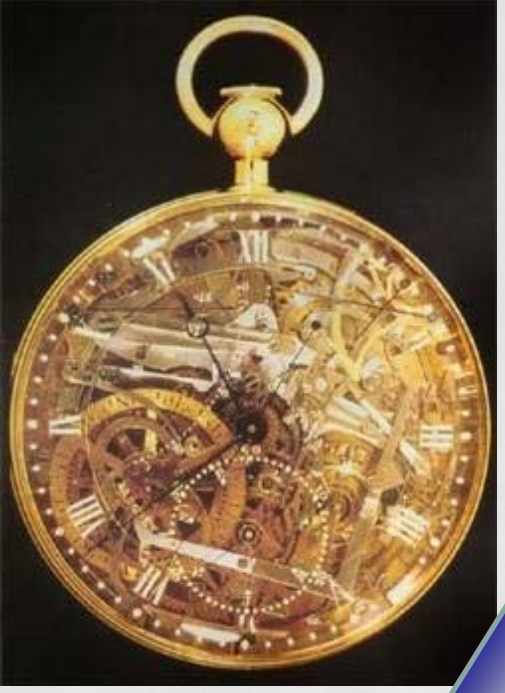
FliG/FliG
FliG/FliM
FliN/FliN

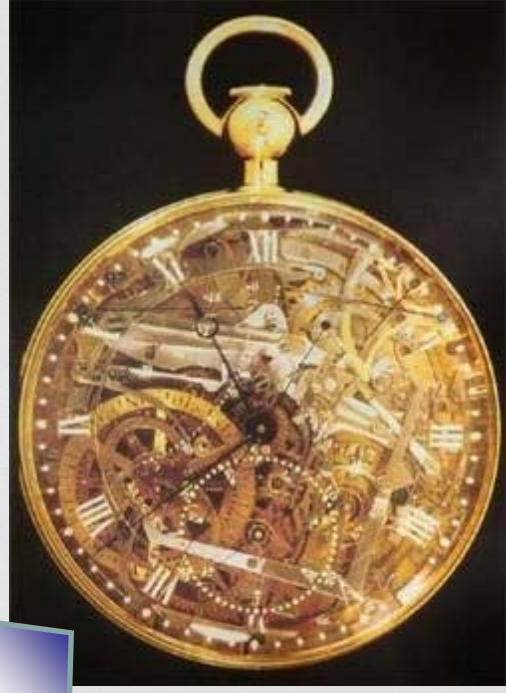
Expected and observed

MotA/MotA
MotA/MotB
FliM/FliN
FliF/FliG
MotA/FliG
FliM/FliM

Unexpected and observed

FliG/MotB
FliN/MotB
FliM/MotA
MotB/FliM

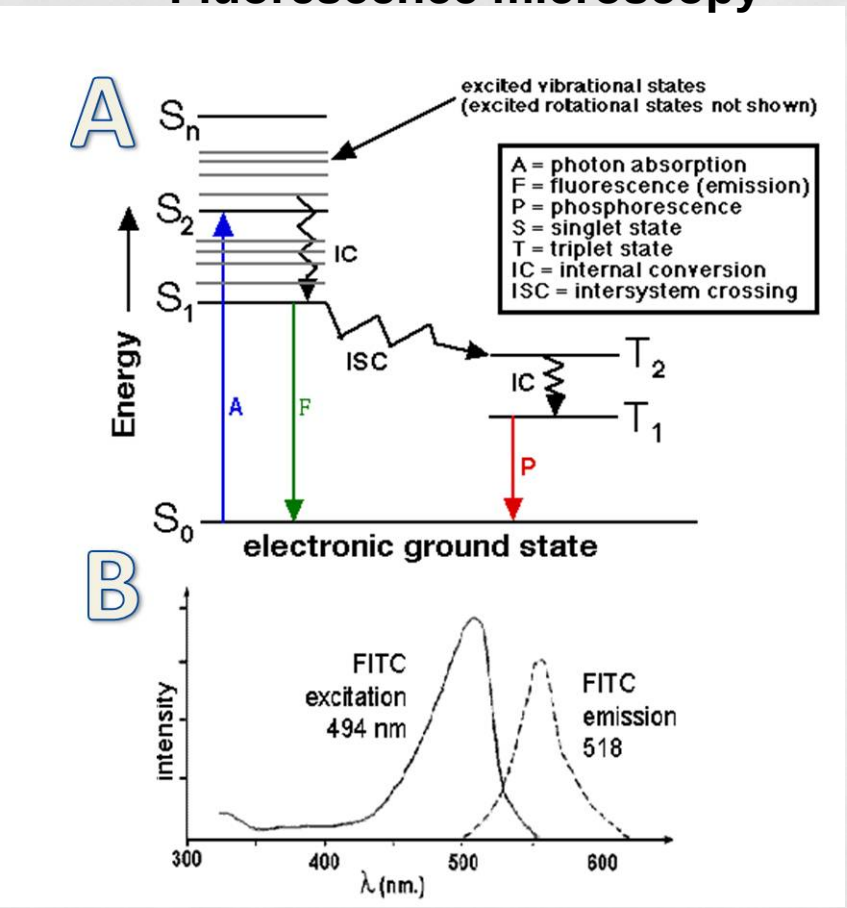




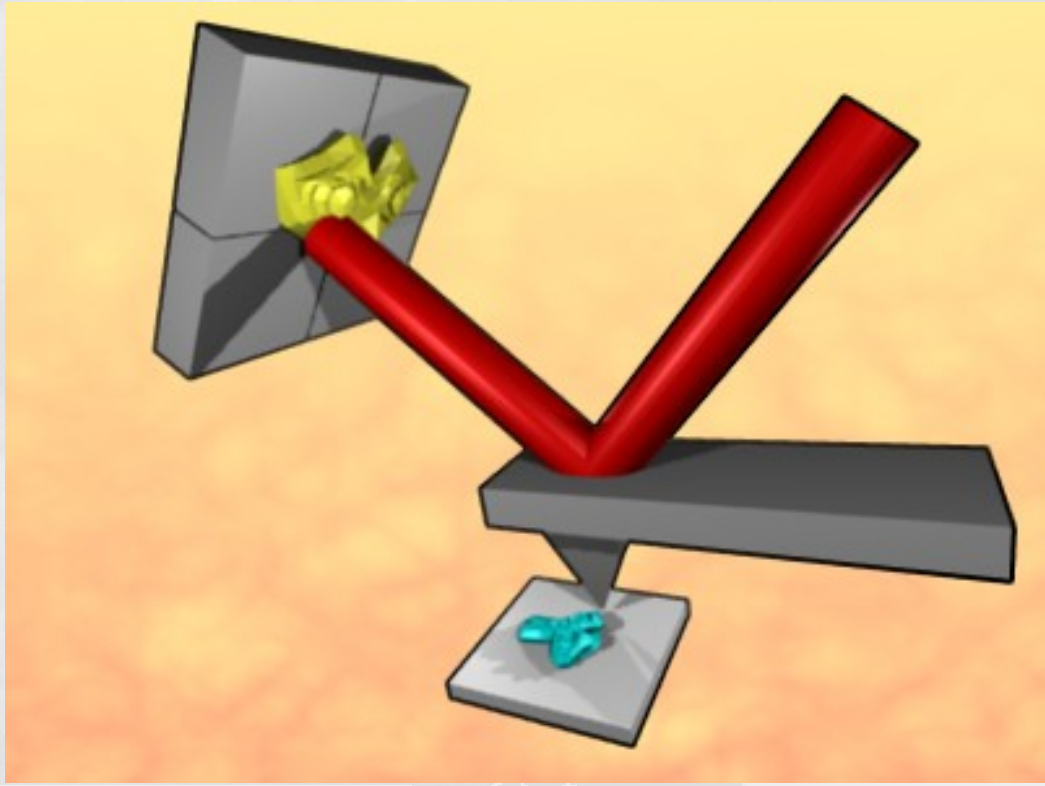
Building the watch…

Which tools?

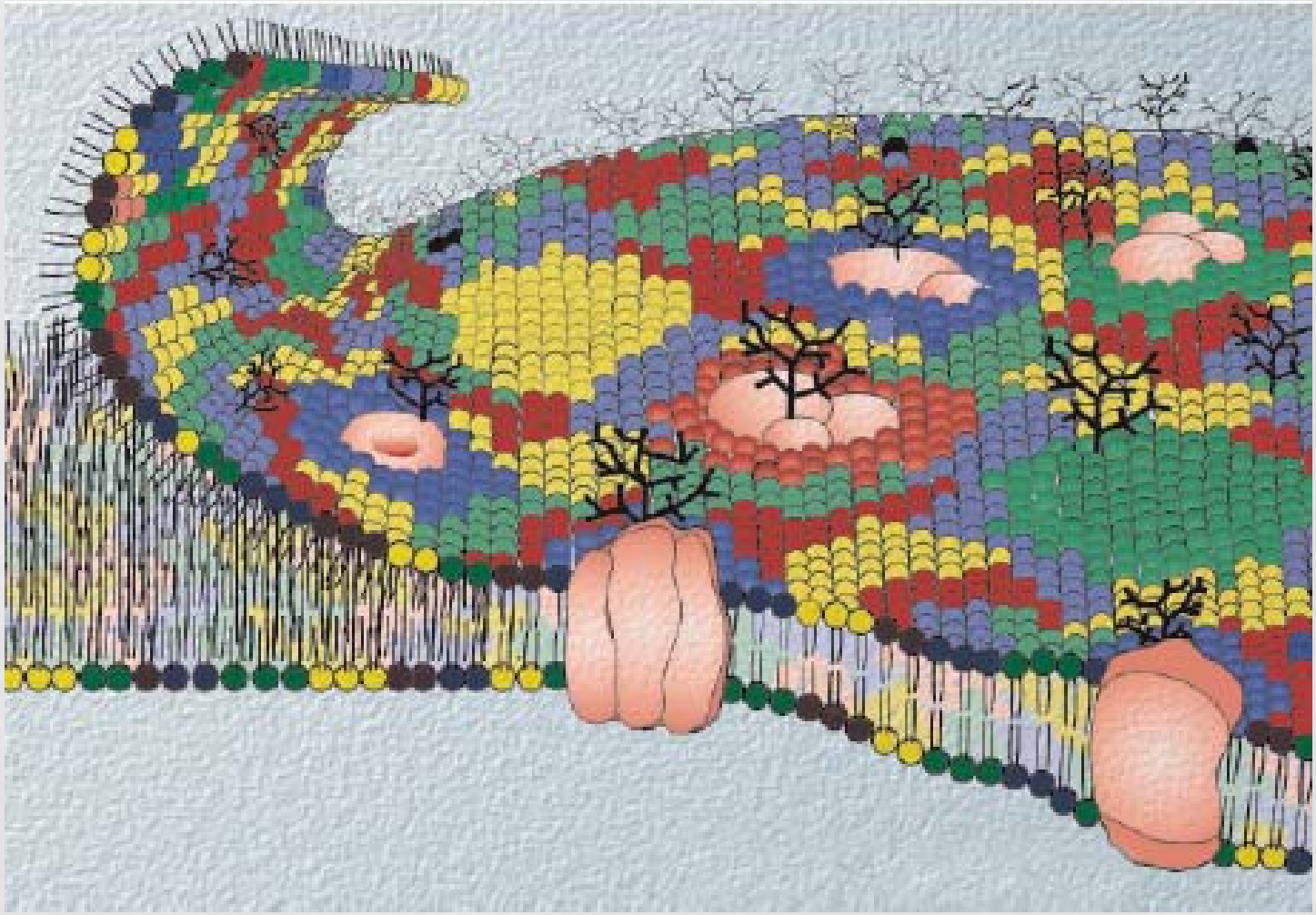
Fluorescence microscopy



Atomic Force Microscopy (AFM)



Which surface?



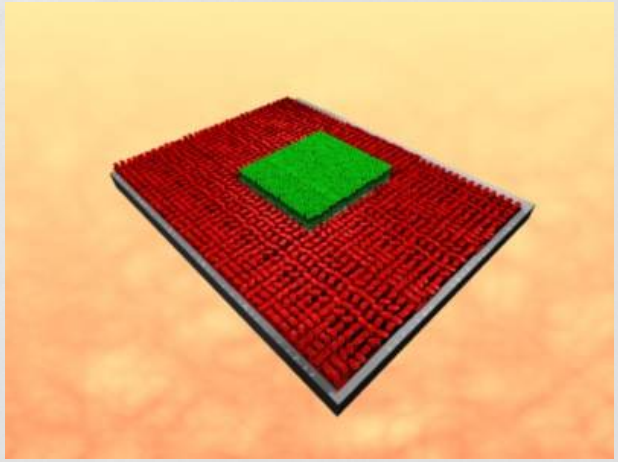
Lipids on the frontier: a century of cell-membrane bilayers
Nature Reviews Molecular Cell Biology 4, 414 - 418 (01 May 2003)

Lipidic Bilayers form a fluid where membrane proteins diffuse rapidly

Lipid diffusion coefficient 10^{-8} cm^2/s (1 µm per s!!!!)

A technological problem:

Build domains of lipidic bilayers (supported membranes) where the membrane proteins will be confined geometrically

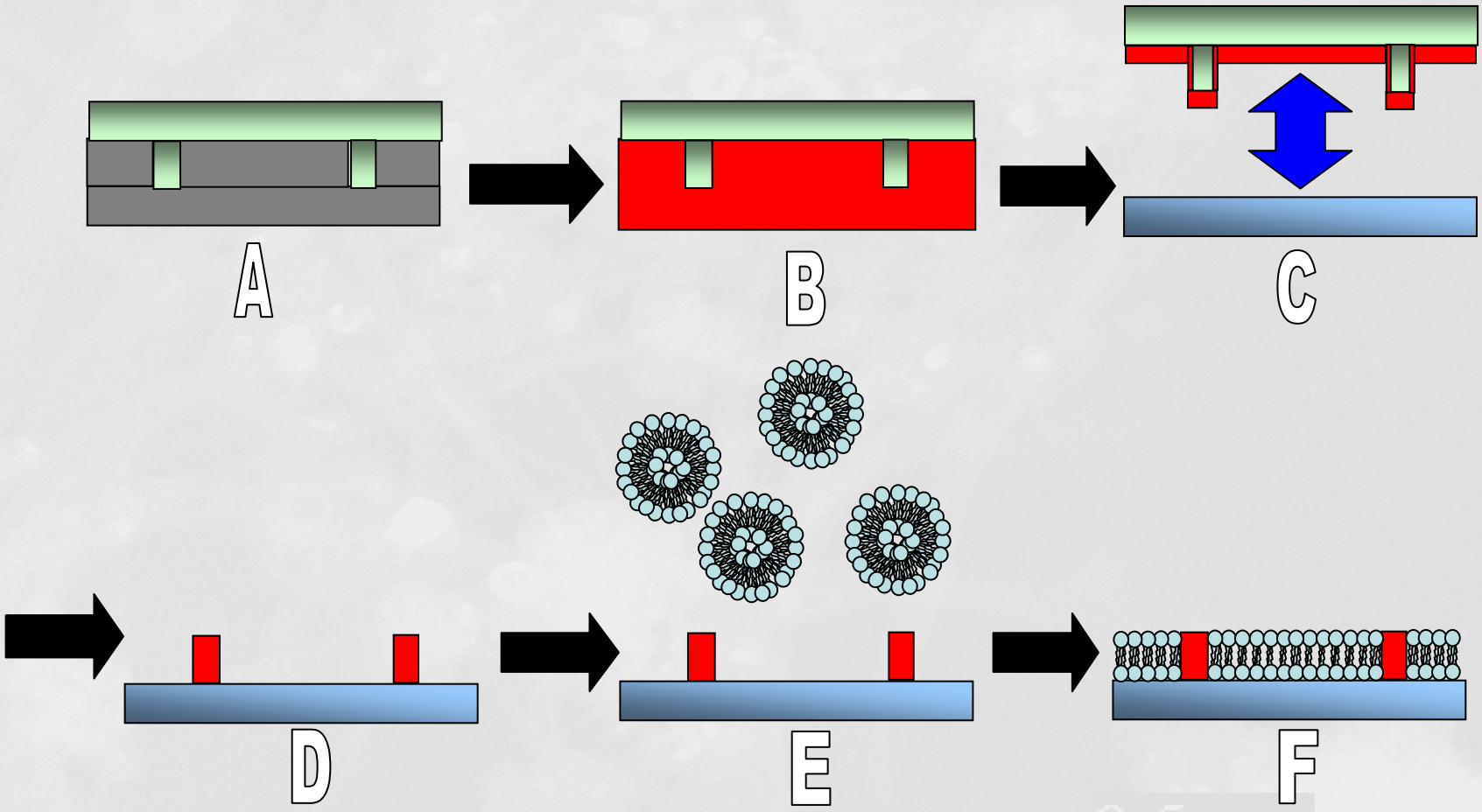


Use Soft-Lithography and self-assembly techniques for creating Micro and nanodomains of supported membranes of arbitrary shape and dimension

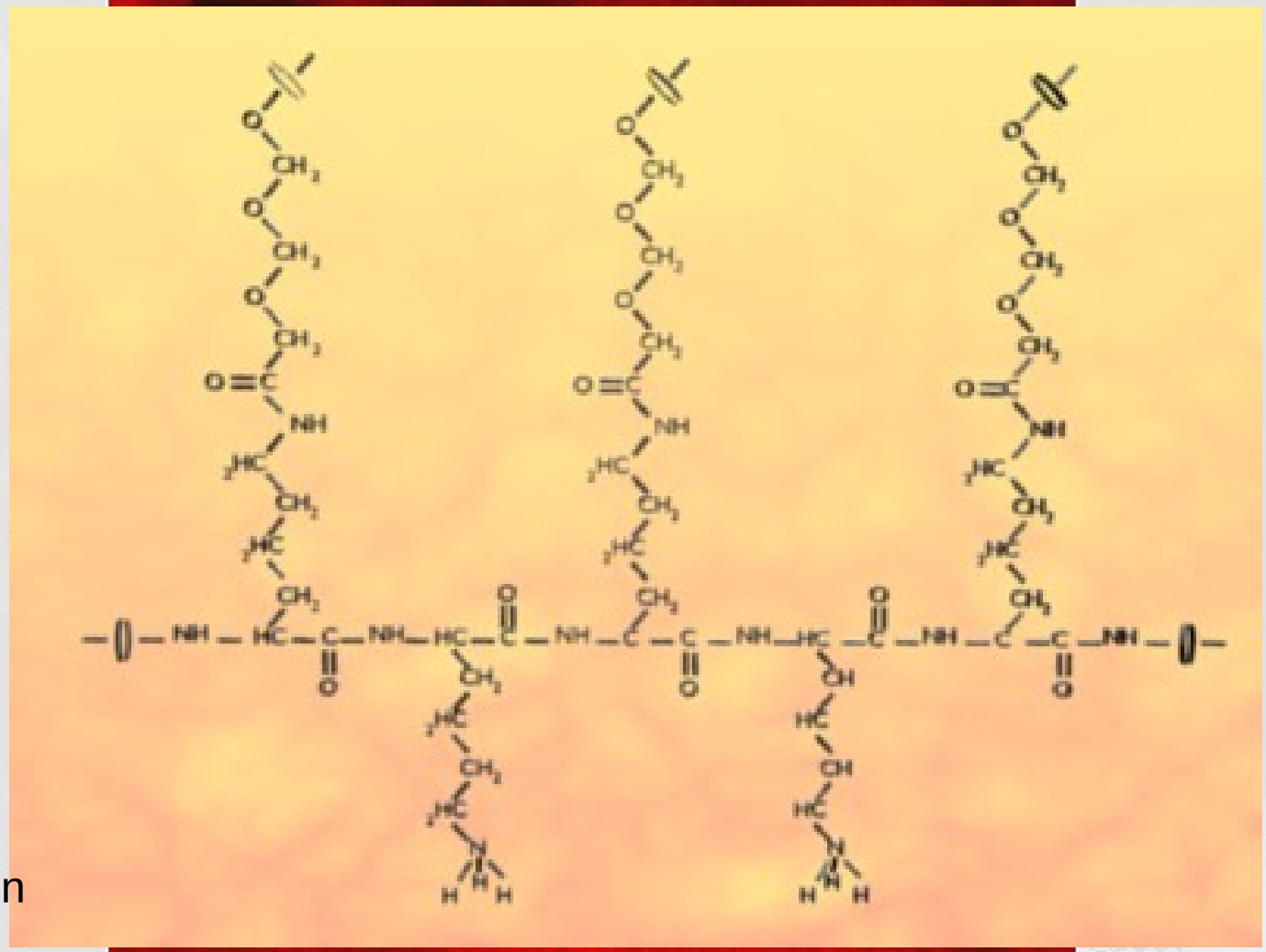
0.5 µm

SOFT LITHOGRAPHY/SELF ASSEMBLY PROCESS

J. Chalmeau et al, J. Microelec. Engin. 84, 1754 (2007)



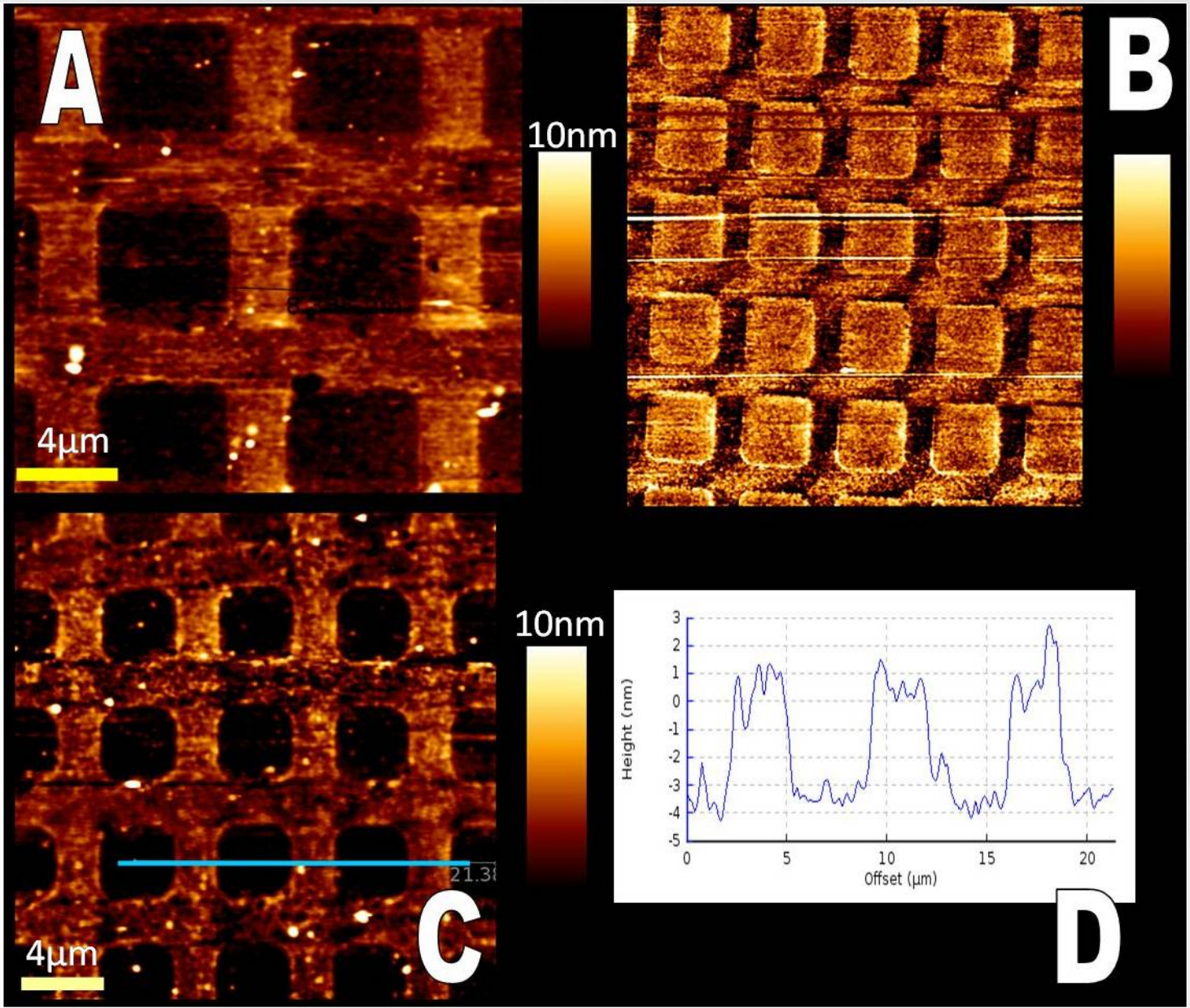
An antifouling molecule
The PolyL-Lysine-*grafted*-PolyethylenGlycol (Pll-*g*-PEG)



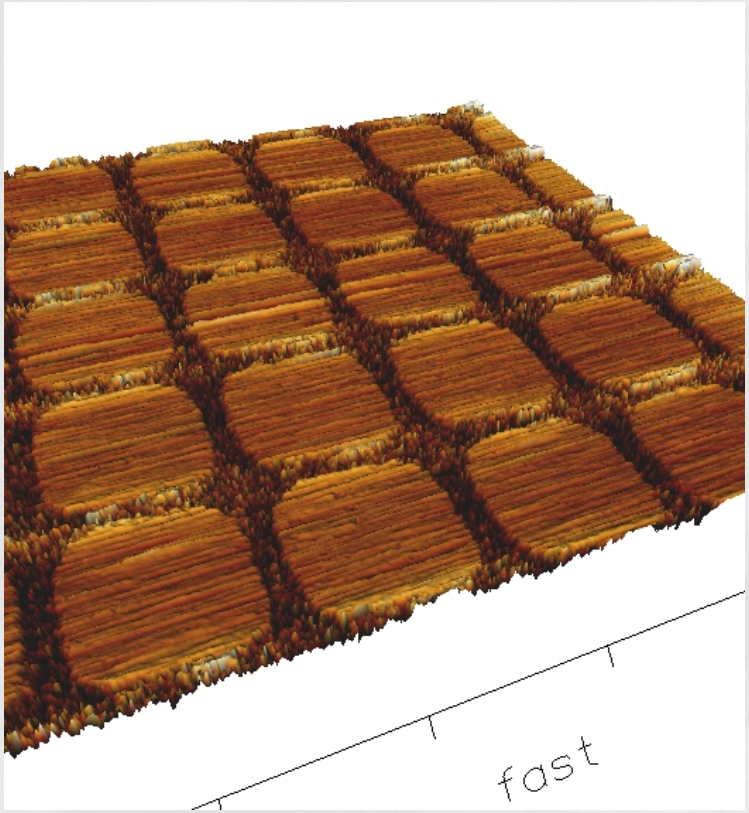
Fluorescence characterization

…What about Nanocharacterization?

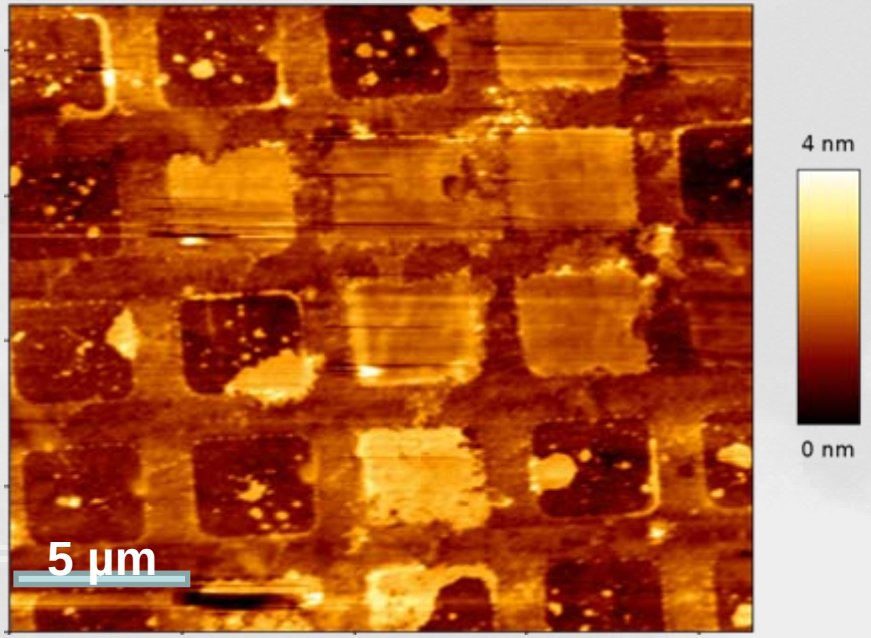
AFM images in liquid of Pll-g-PEG molecules deposited on the surface



Micro-domains of Supported Phospholipidic Membrane



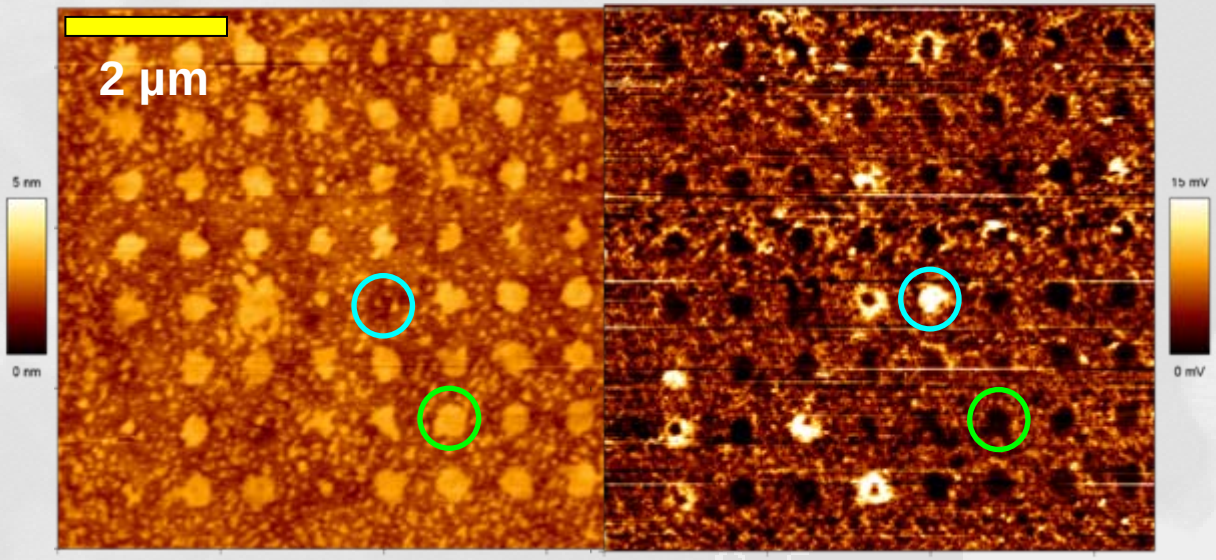
Micro-domains 4 µm Egg-PC



Micro-domains 4 µm E-Coli

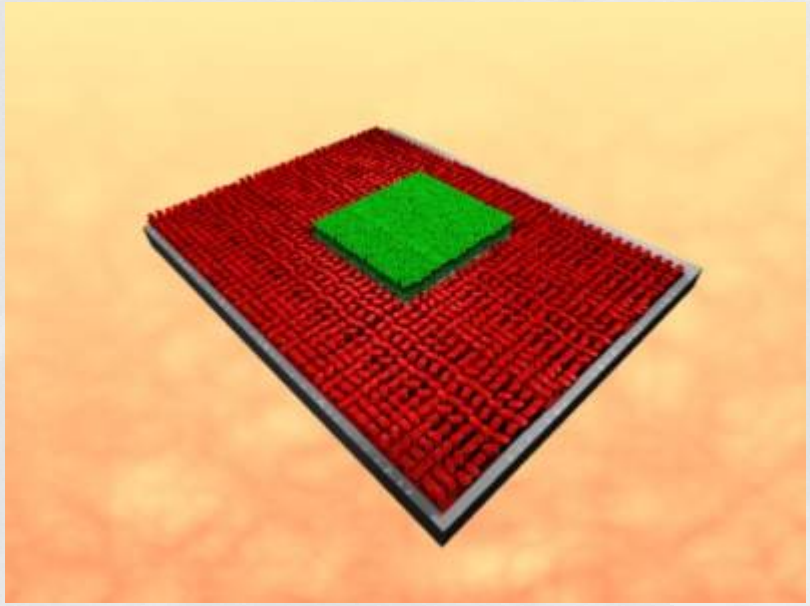
Nano-domains of Supported Phospholipidic Membrane

Nano-domains 200 nm Egg-PC

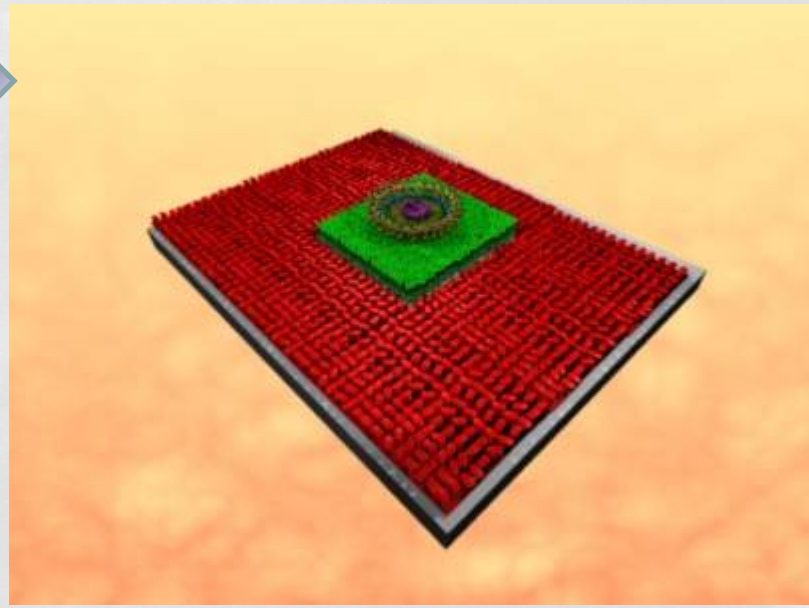


Nano-domains 400 nm E-Coli

Partitionned SPBM



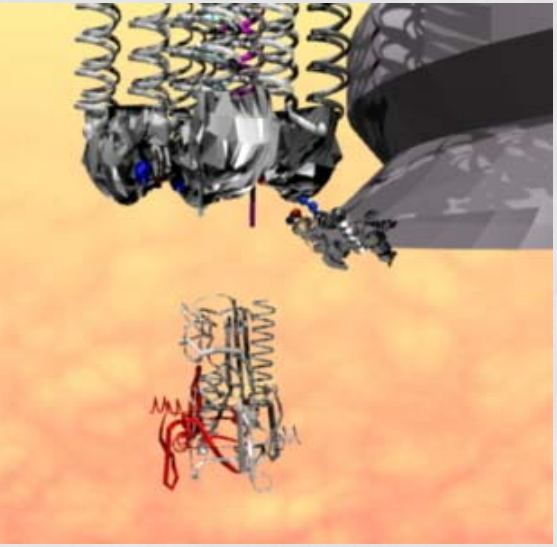
Motor Assembled into P-SPBM?



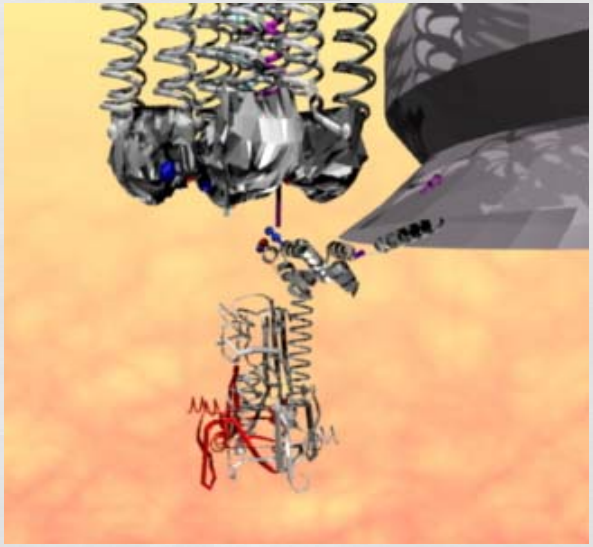
FliG
Localization crucial

3 hypotheses

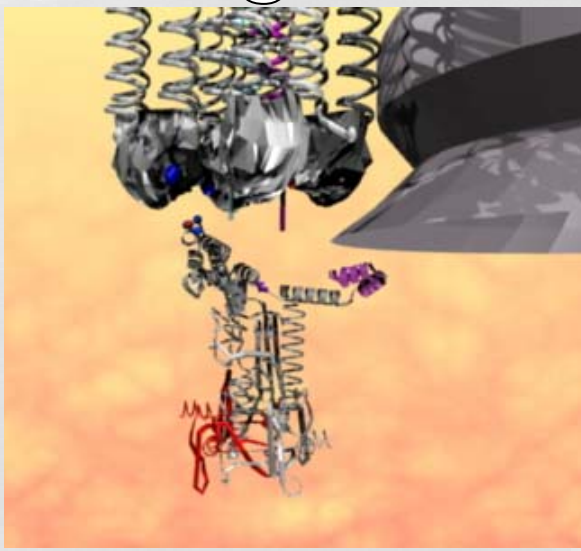
1



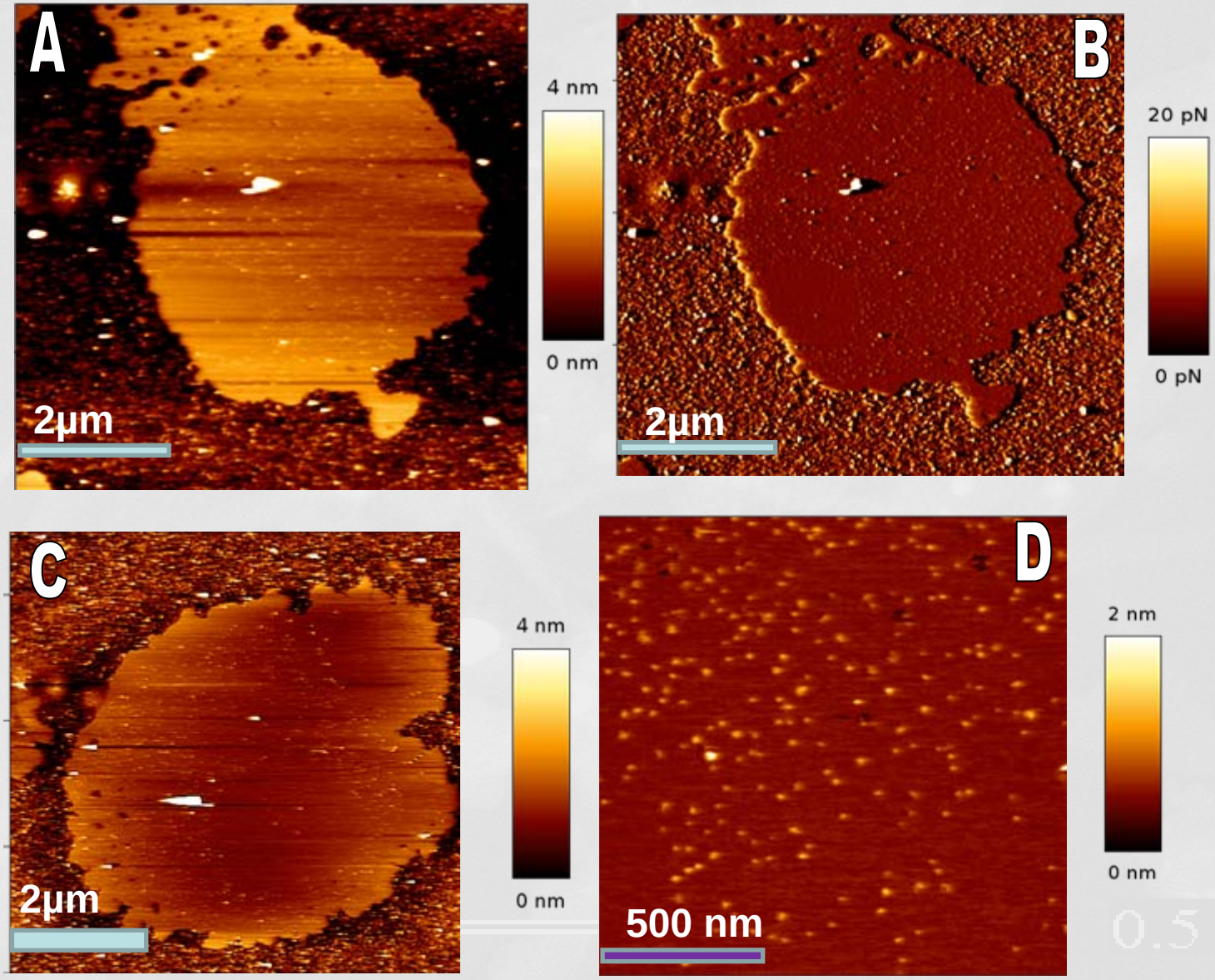
2



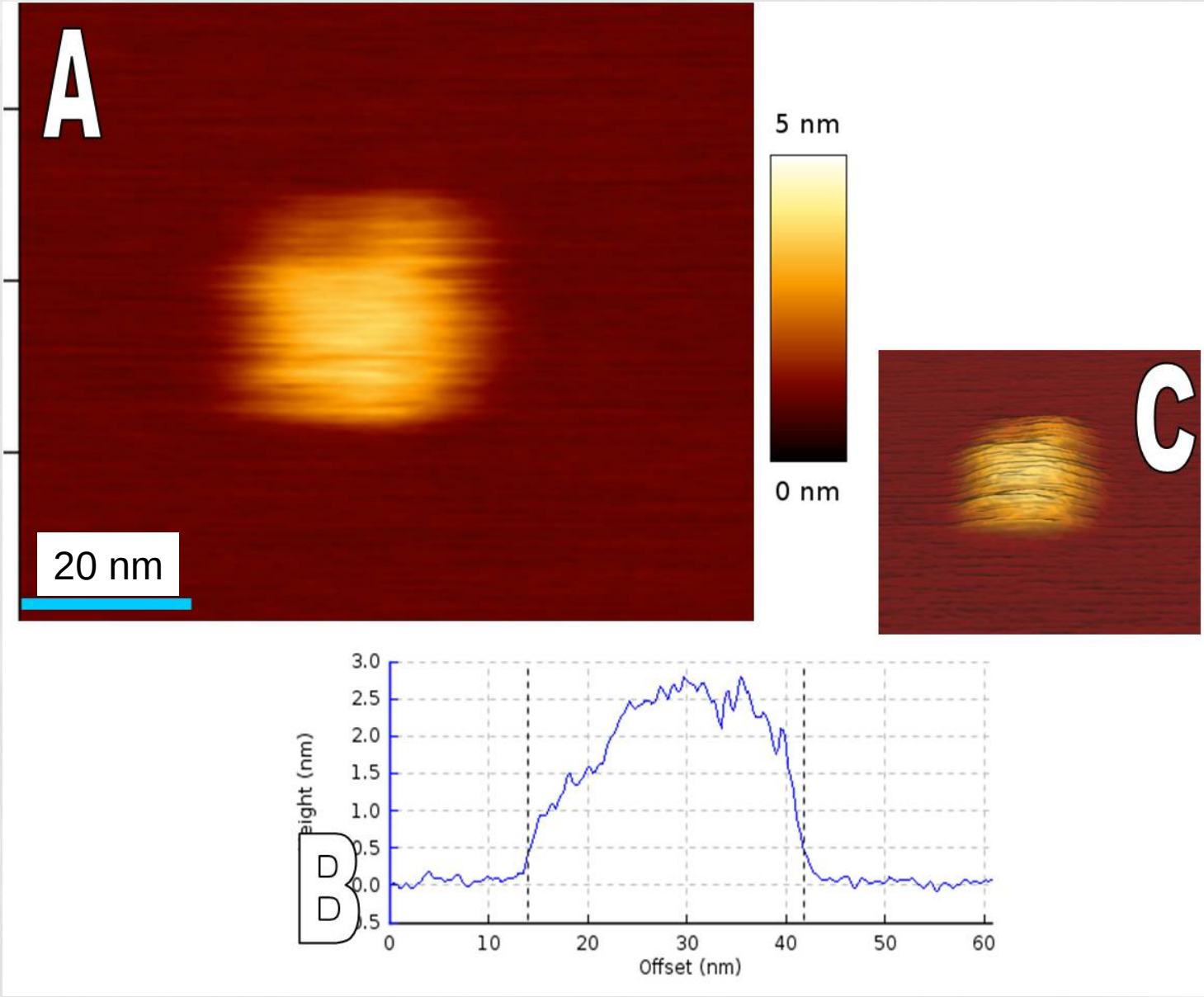
3



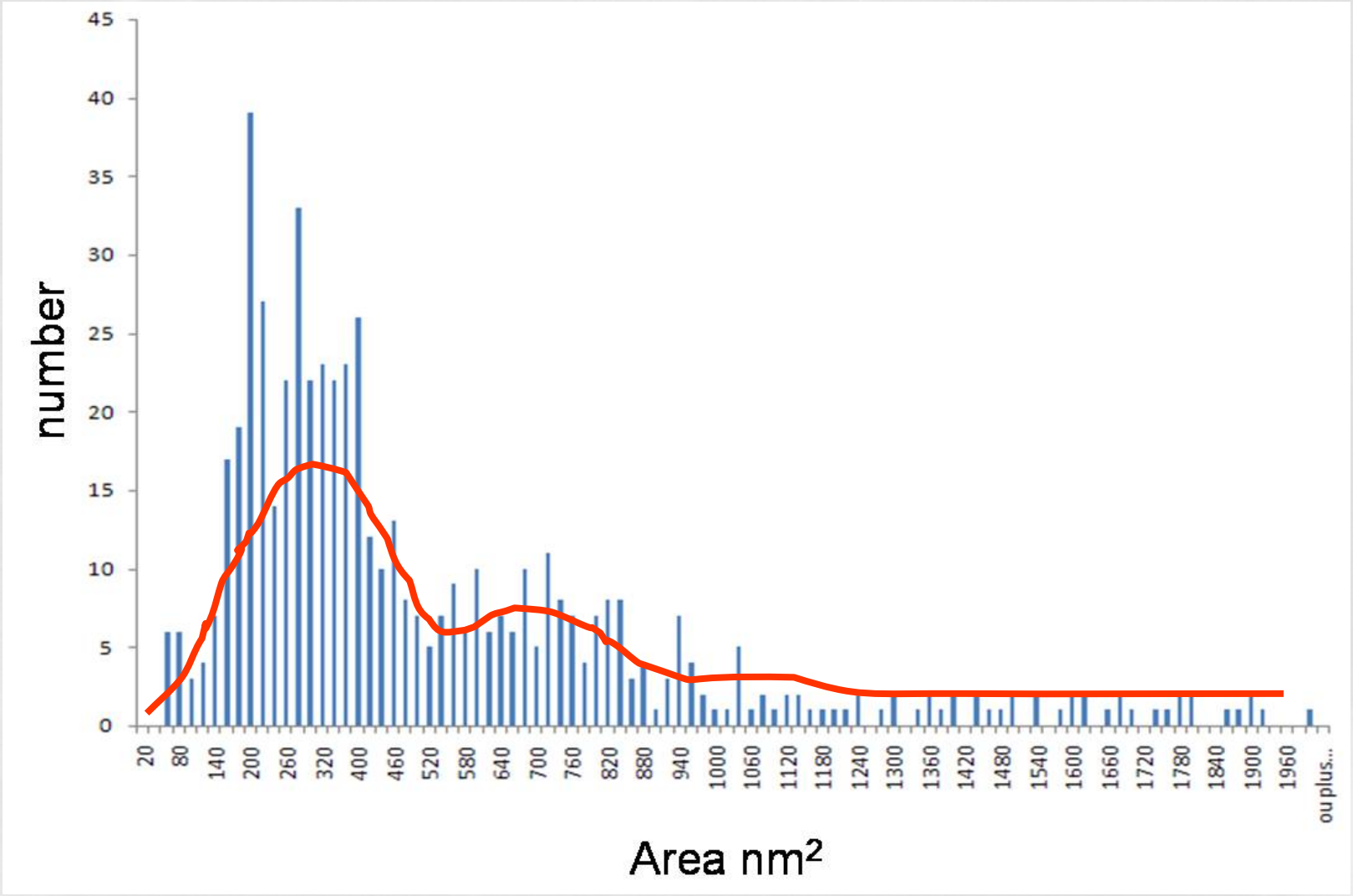
Assembly of the FliG protein on Microdomains of Supported Phospholipidic Membrane



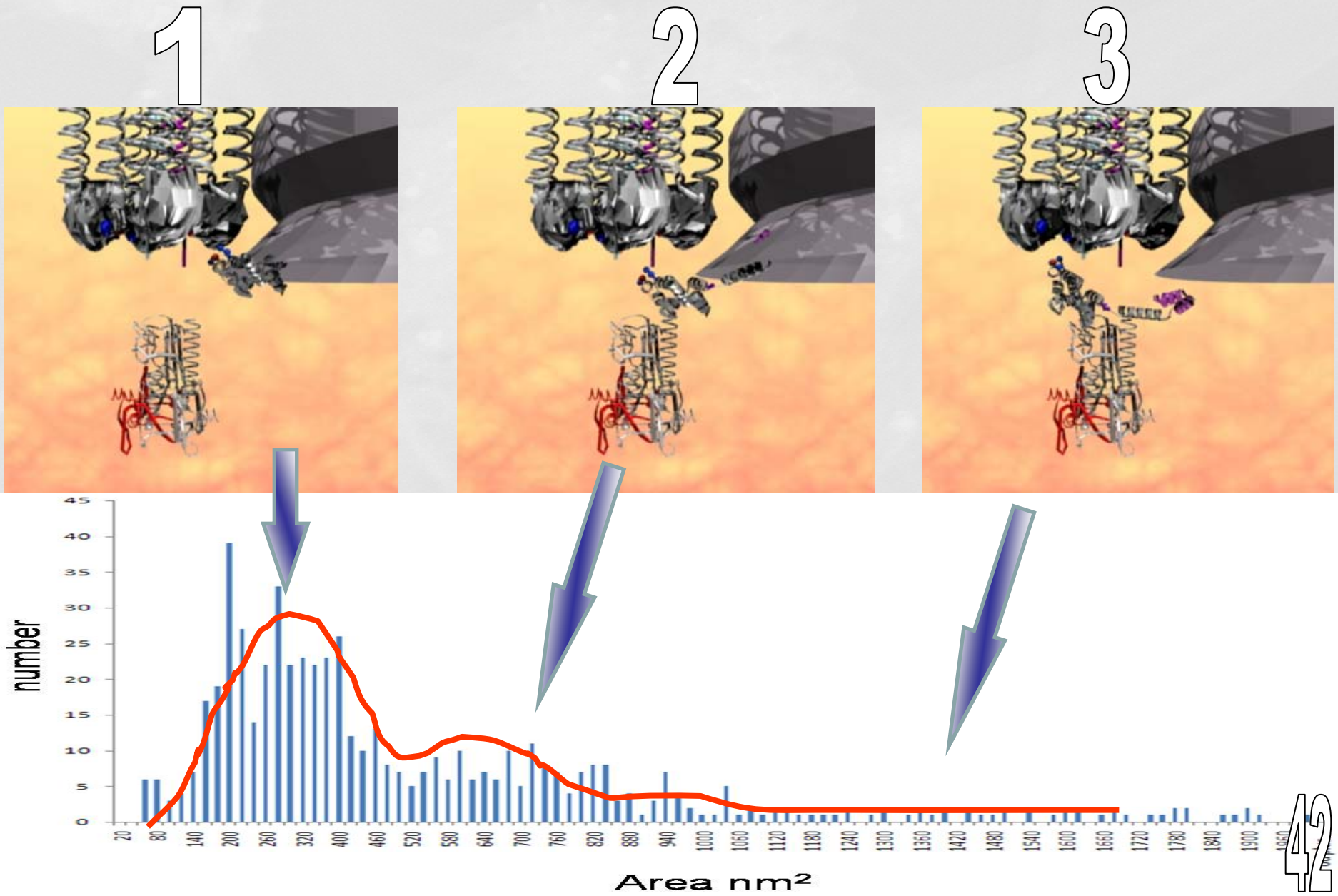
FliG proteins on PE-PG SPBM



Size distribution of FliG assembly



3 hypotheses



Conclusions

- We propose to assemble the flagellar nanomotor of bacteria from purified proteins using soft-lithography&self-assembly
- We have assembled micro and Nanodomains of E-coli lipidic bilayers
- On these Nano-Engineered surfaces we have observed the 2D assembly of part of the MS-ring (FliG)
- From AFM and QCM data, a new model for the nanomotor has been proposed
- Is the 3D self-assembly of the complete nanomotor possible (ex-vivo) on surfaces ?

ACKNOWLEDGEMENTS

Assembling a biological nanomotor on a nano-engineered surface

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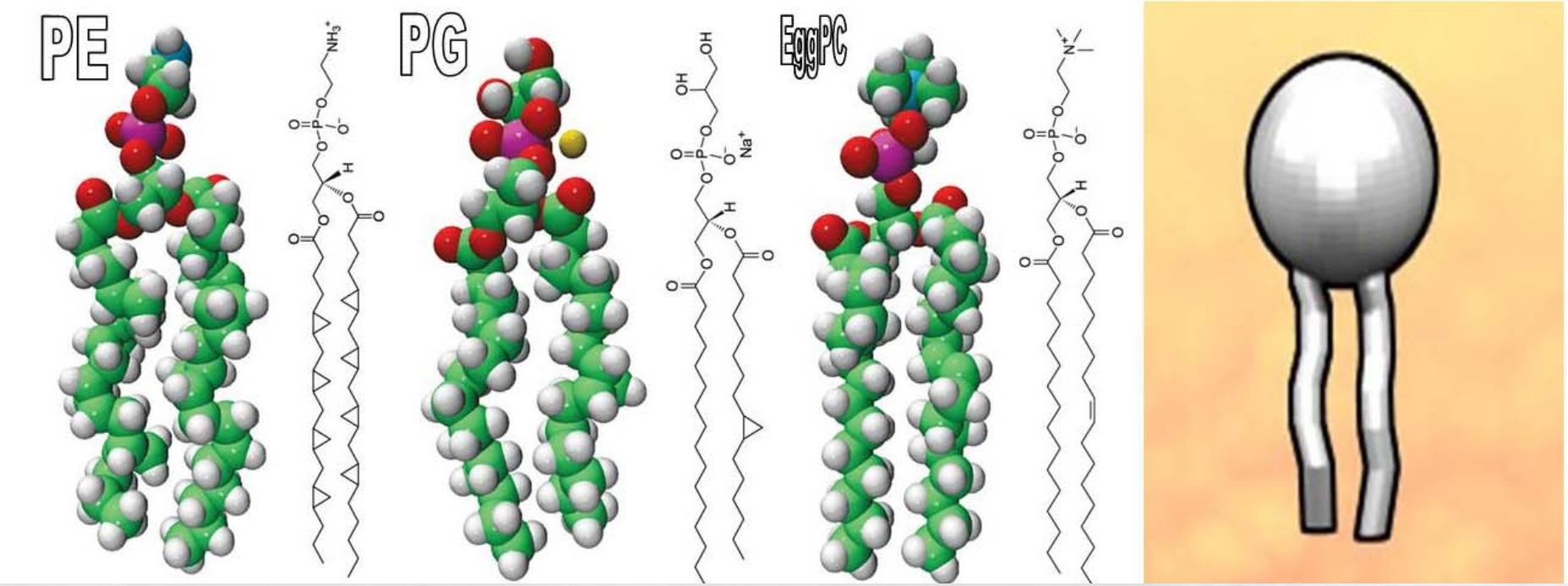
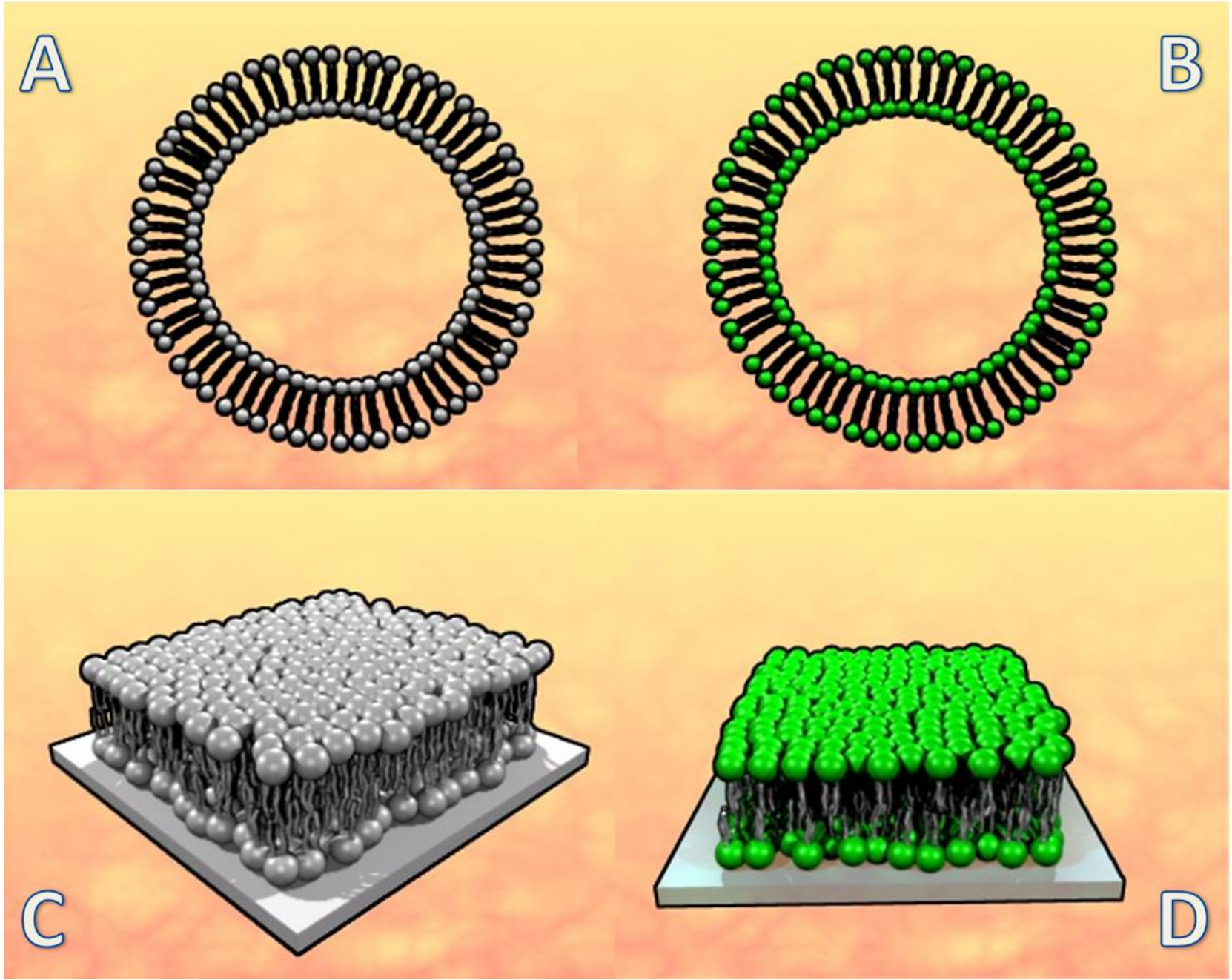
and J. Chalmeau

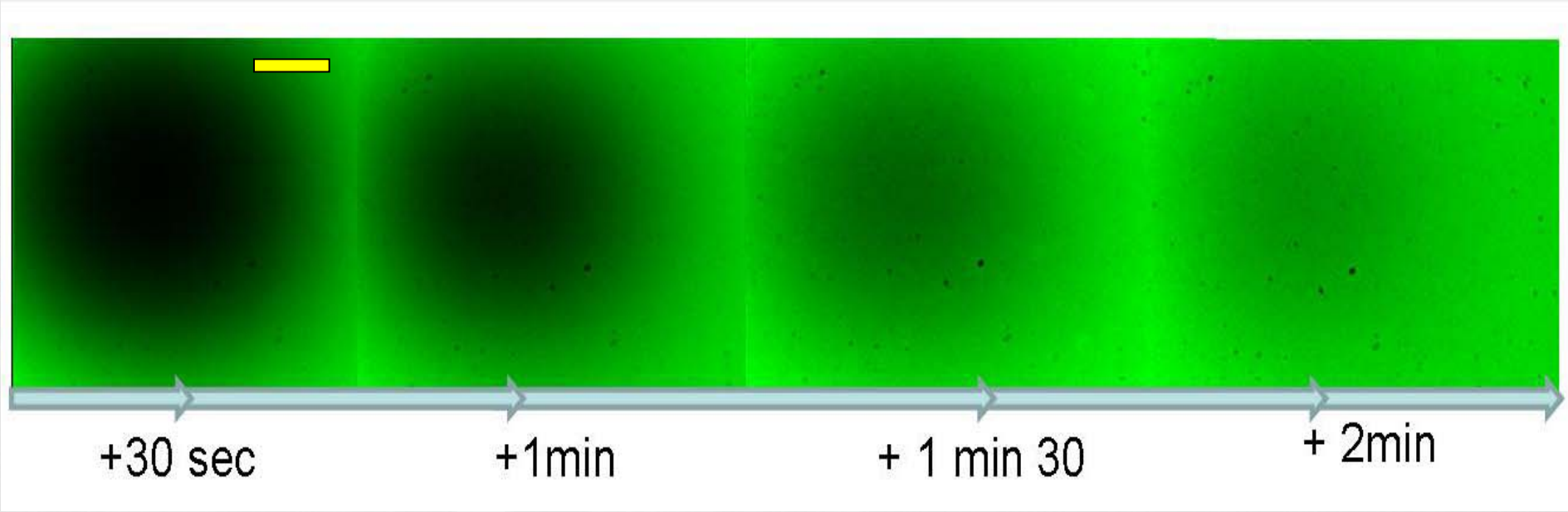
Which surface? Supported Phospholipid Bilayer Membrane (SPBM)



Re-assemble in vitro?



A dynamical surface….



1,5µm²/s

How to confine?