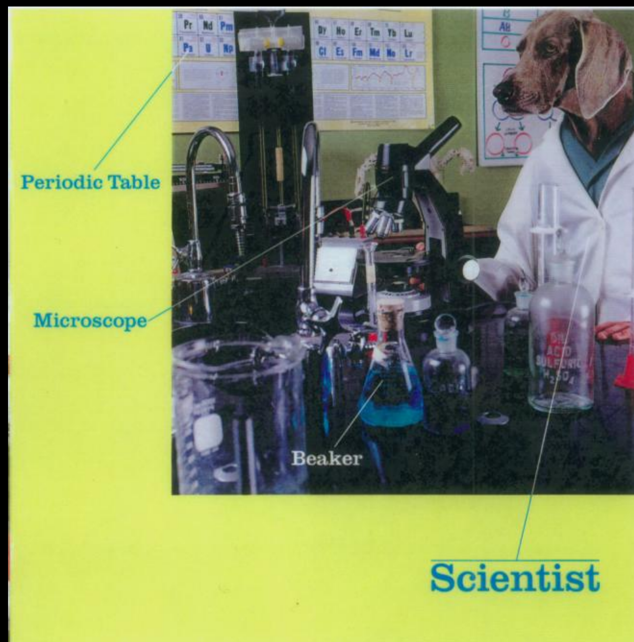
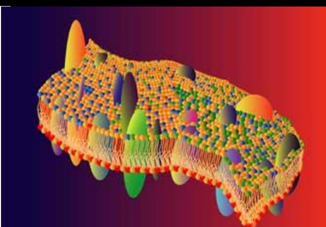
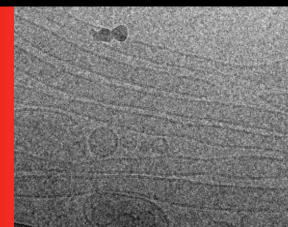
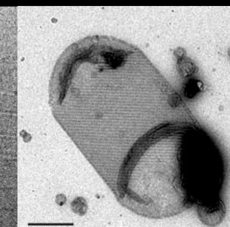
International Workshop on Electron Crystallography of membrane proteins 2010-Basel

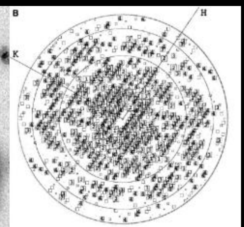


2D crystallisation: BB and monolayer
D. Lévy (Institut Curie Paris)

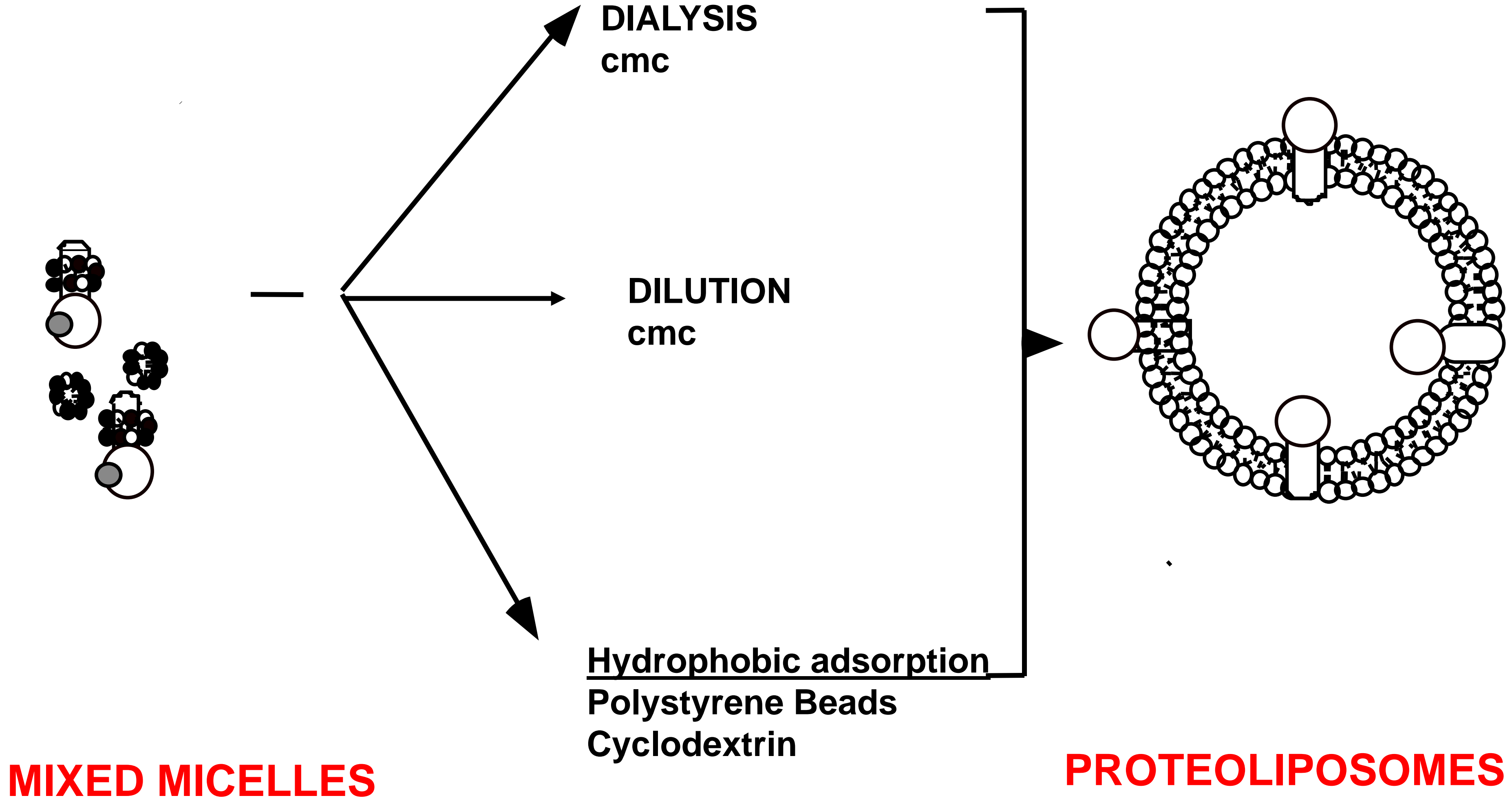






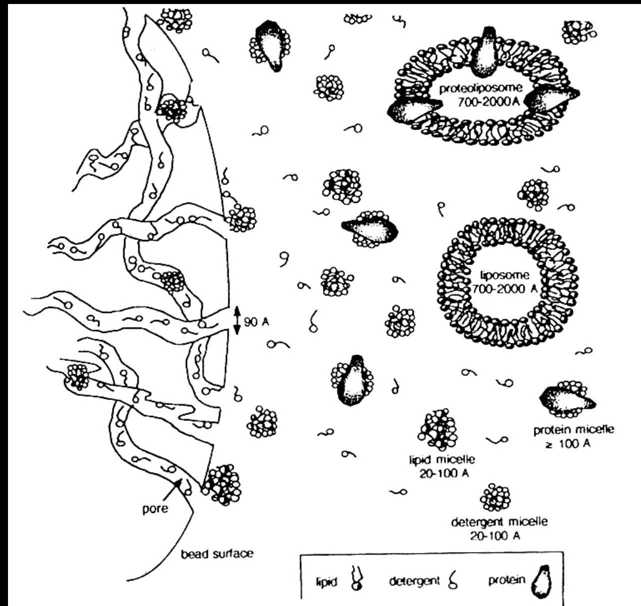


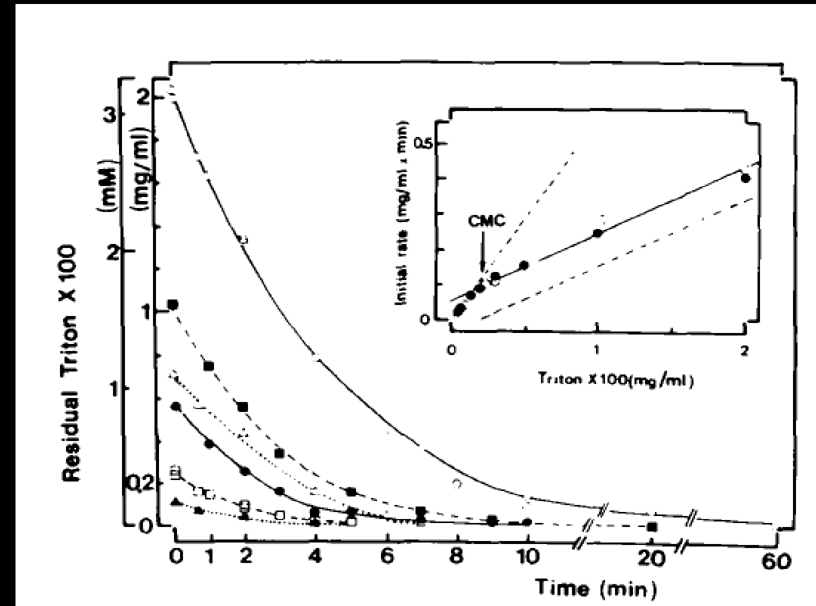
DETERGENT-MEDIATED RECONSTITUTIONS

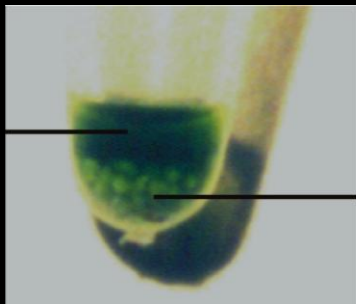


Hydrophobic adsorption of detergent by Bio-Bead

(several publications from 1990 to 2000 Rigaud, J.L.)

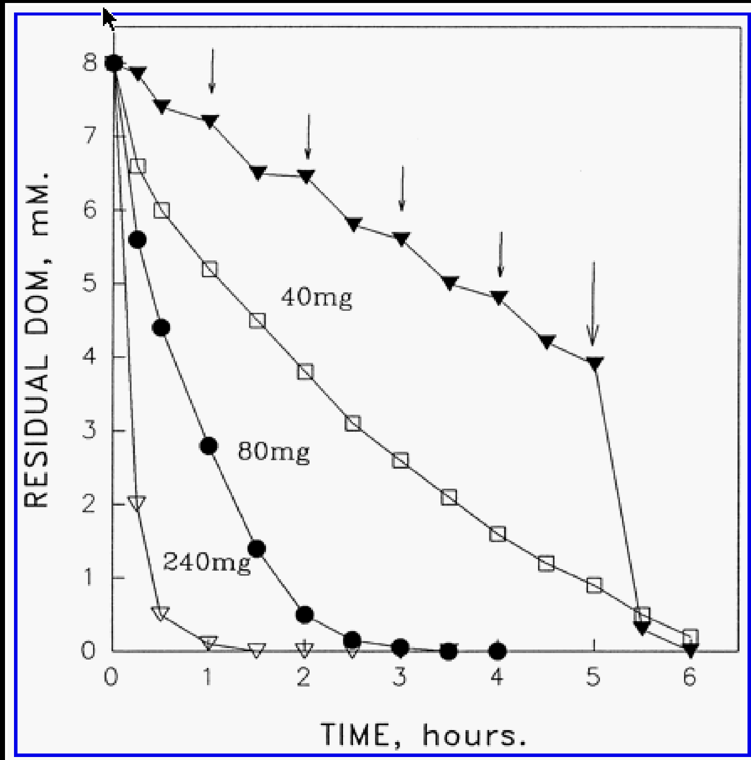


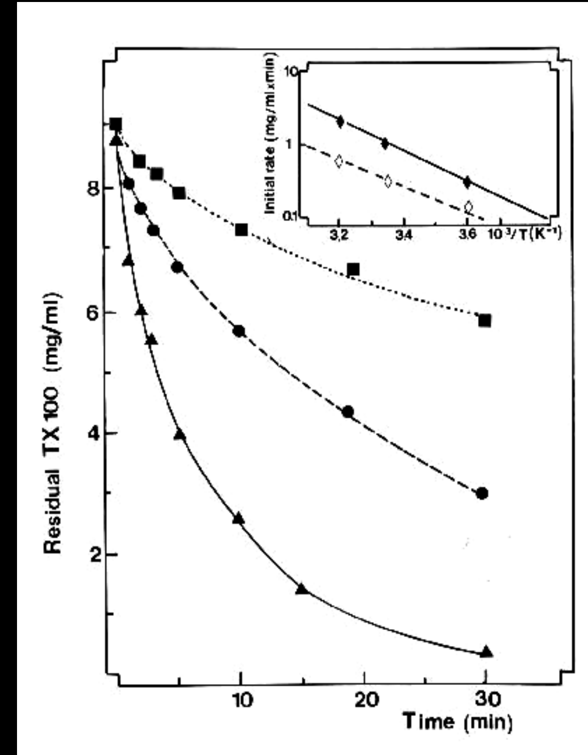




Monomers and pure detergent micelles are adsorbed
No absorption of protein and negligeable adsorption of lipids

Complete detergent removal using Bio-Beads and control of the rate detergent removal





4°C

20°C

37°C

Reconstitution in 2D trials from 1H to 48H

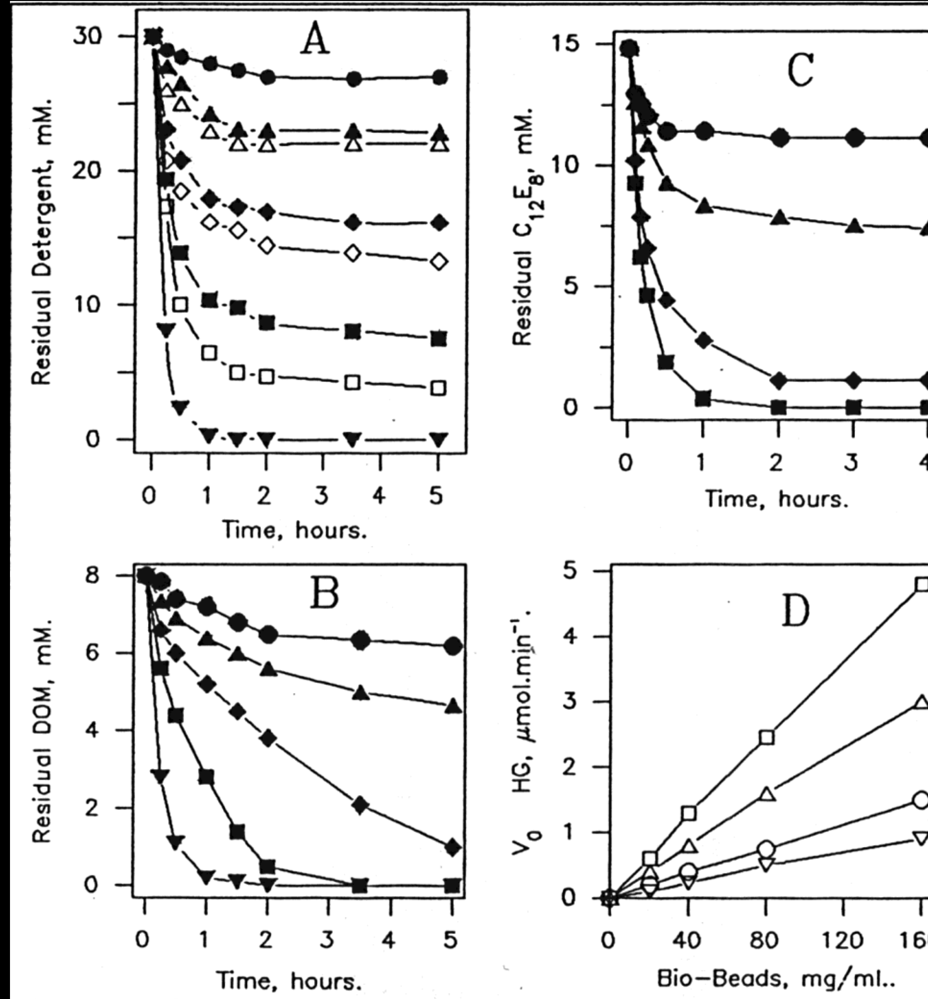
The rate of detergent removal depends on the BioBeads /detergent ratio, i.e., the rate is the same to remove DDM at 0.1 % from 1 ml solution with 50 mg Bio beads than to DDM at 0.1% from 100 microl solution with 5 mg of Bio Beads

Bio-Beads adsorb low and high cmc detergents

OG (17mM)
Hecameg (16 mM)

DDM
(0.2mM)

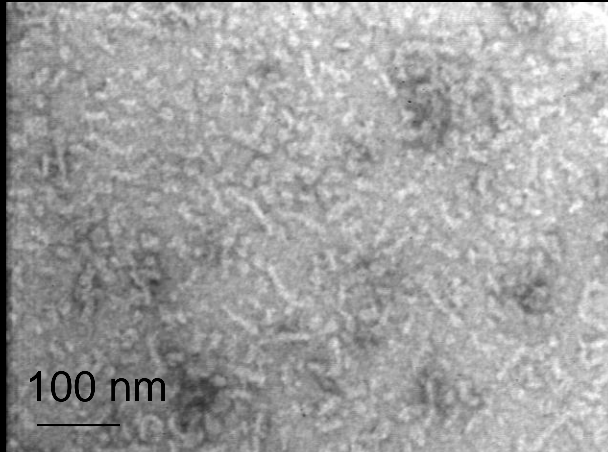
C12E8
(0.2mM)



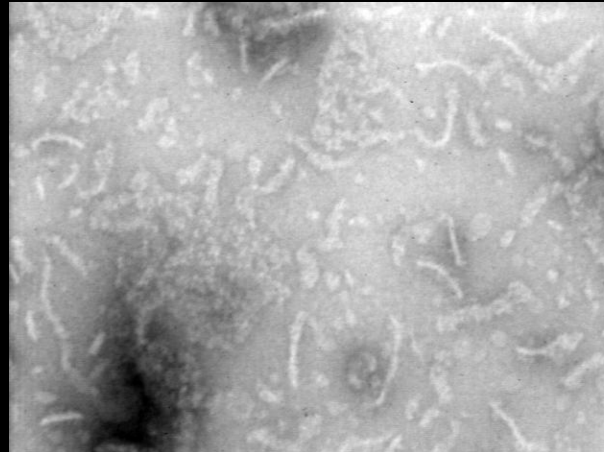
J.Struct.Biol (1997) 118, 226

Screening of reconstitution

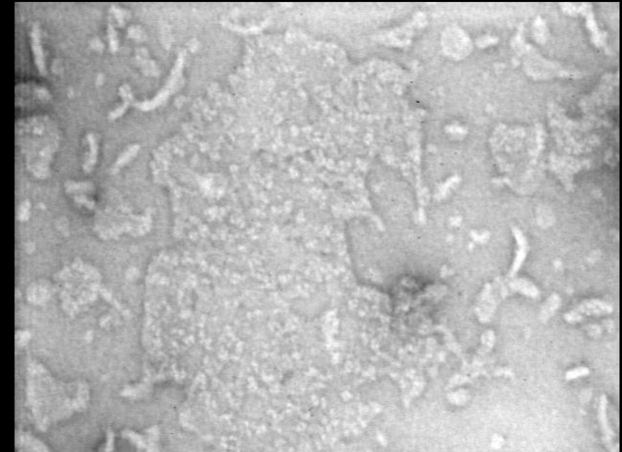
t=16h

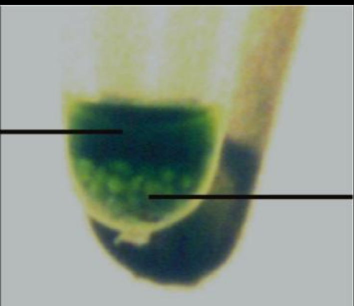


t=18h

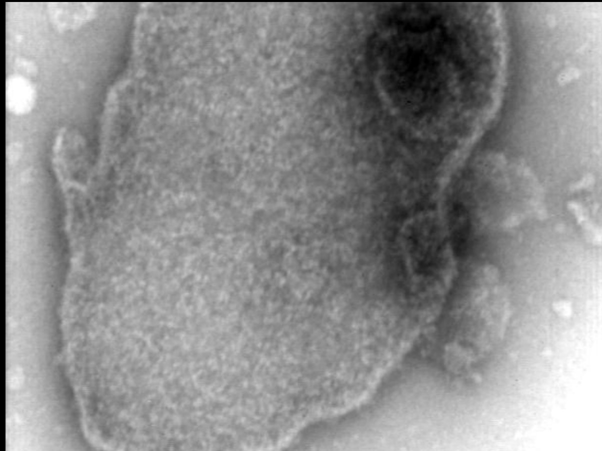


t=24h

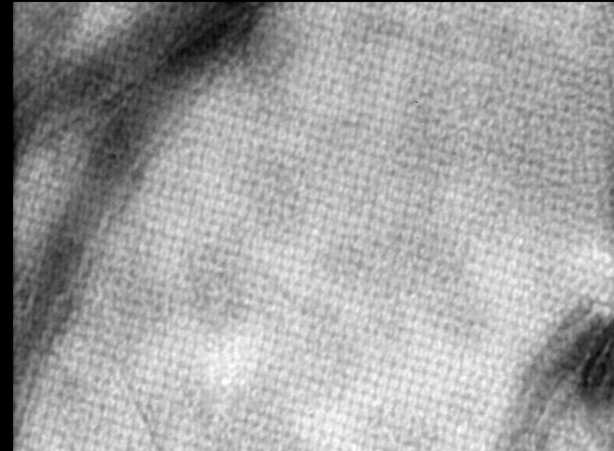




t=36h



t=40h



Detergents adsorbed by Bio Beads and 2D crystals (non complete)

Ionic	**Zwitterionic**	**Sugar-based**	**Others**
SDS	Chaps	Glucoside (7,8, 9, 10)	TX100
Cholic acid	Chapso	Maltoside (8, 9, 10, 11, 12, 13),	C12E8
	Fos-Choline (12, 14, 16)	Thiomaltoside (10, 11,12)	
	LDAO		

KirBac 3.1 Chanel
9 Å (Venien, 2005)
Secondary transporters
MelB (10 Å, Herbert, 2005)

P type ATPase
Ca-ATPase (8 Å, H. Young, pers.com.)
H-ATPase (9Å, Kuhlbrandt, 2001)

Porins
FhuA (8 Å, Lambert 1999)
FepA (8 Å, Célia pers.com.)

Wza (20 Å, Beis, 2004)

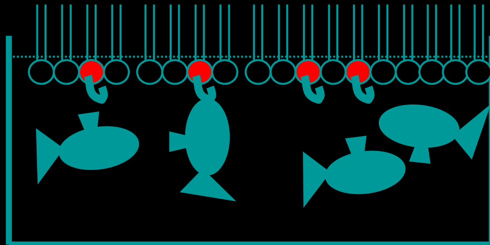
Photosynthetic apparatus
cytB6f (9 Å, Mosser, 2001)
LH2 (10 Å, Lévy, 2003)
LH1-RC (25 Å, Lévy, 2003)
PSII (9 Å, Hankamer, 1999)

TF1F0 (25 Å, Lévy, 1999)
F1FO (yeast, 25 Å, Fotiadis, 2007)

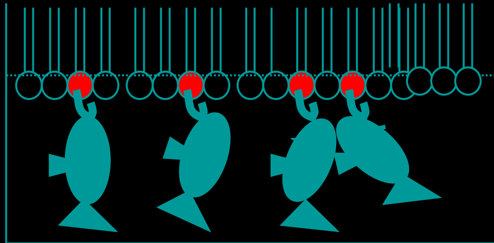
ABC transporters
PgP (25 Å, Wilkens 2007)
MRP1 (20 Å, Rosenberg, 2001)
BmrA (18 Å, Lévy)

2D crystallization on functionalyzed lipid layer

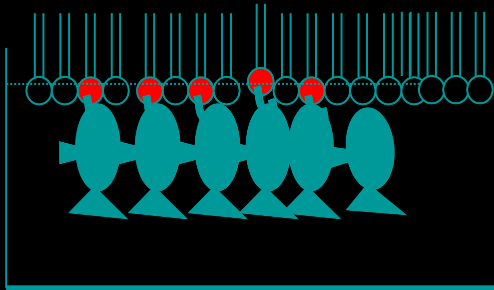
Binding



Diffusion of lipid/protein complexes



2D crystallization



Higly specific interactions
- Lipid Biot/streptavidin
- Toxins receptor:
  GM1 (CTB) (A. Brisson)
  Gb3 (StxB)
- novobiocin-lipid (DNA girase) (P. Schulz)
- NiNTA lipid (Kubalek, 1994)

Electrostatic interactions

PS -

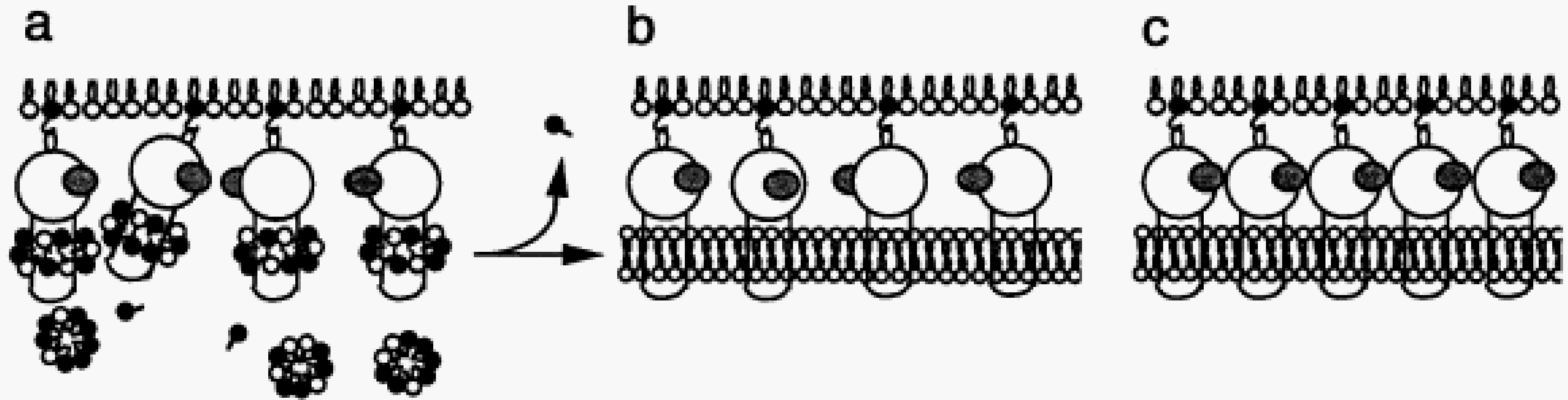
(anexins, a toxin) (H. Hebert)

Stearylamaine +

(DNA, actin, RNA pol) R. Kornberg, Taylor)[2]

- 2D crystallogenesis:
- Resolution 3 Å in plane (Kubalek, 1991)
- Crystallization in presence of contaminants (StB/GM1, BSA 90 %)(Mosser, JSB. 1991)
- Improvement of the transfer onto EM grid (Norville, Walz JSB, 2007)

2D crystallization on lipid layer of membrane proteins

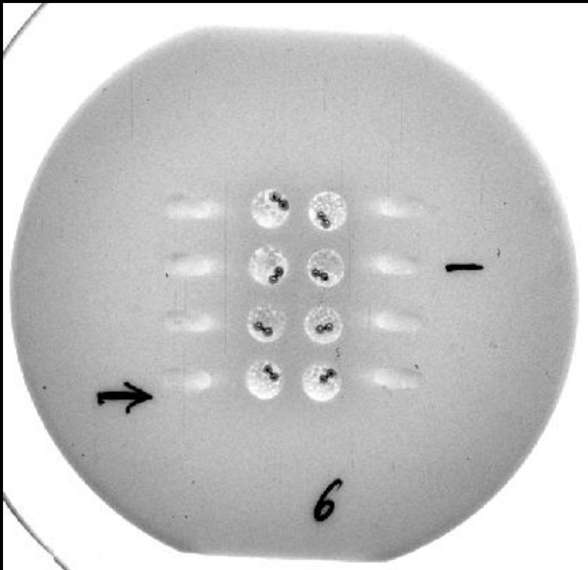


BINDING
Ternary micelles

RECONSTITUTION
BILAYER

CRYSTALLIZATION

Experimental set-up



1) Preparation of the lipid/protein/detergent mixture in a eppendorf as for a 2D trial in bulk but in a low cmc detergent (DDM, DOTM, C12E8, TX100 etc..)

2) Spreading of the lipid layer onto the surface of the well

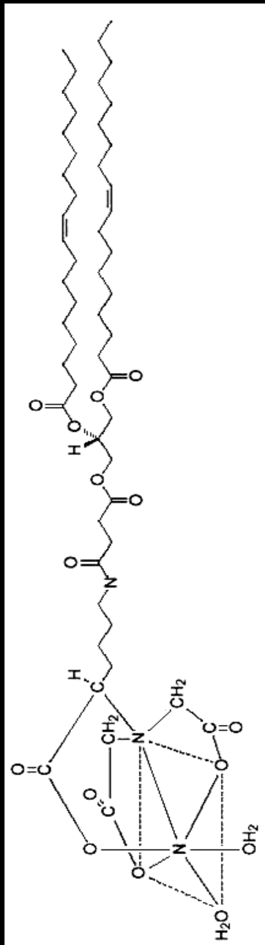
3) Injection of the lipid/detergent/prot mix
The final detergent concentration is above the cmc. Prot conc 10-50 ugr/ml

4) Binding upon incubation (1-24 h)

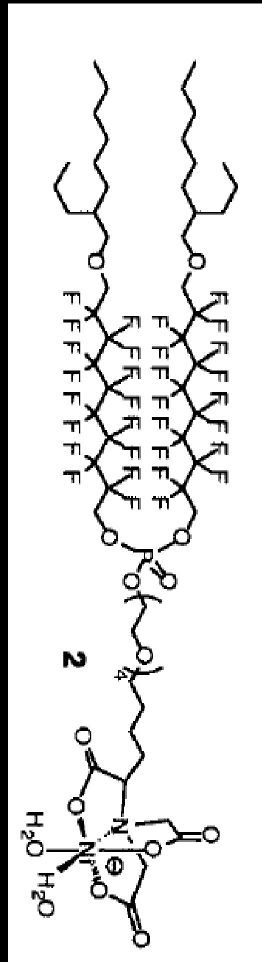
5) reconstitution by detergent removal (Bio-Bead or cyclodextrins)

6) Transfer onto EM grid

Ni-NTA lipids to bind Hist-prot



NiNTA DOGS
Avanti Polar



NiNTA Fluor Lipid
Lebeau, 2001

Charged lipids

Negative
EPA, PS

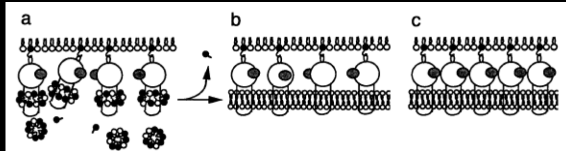
Positive

DOTAP
Stealyamine
DDAB

Membrane proteins crystallized by the lipid layer method

Proteins	Surface lipids	Resolution
FhuA	NiNTA	15 Å (Lévy, 1999)
TF1FO	NiNTA	25 Å (Levy, 1999)
EF1FO	NiNTA	Nd (Arachega, 2007)
Pgp	NiNTA	25 Å (Senior, 2008)
Aqp1	NiNTA	Nd (S.Scheuring)
H+-ATPase	Ni-NTA Flipid	8 Å (lebeau, 2001)
Anc2	Ni-NTA/lipid +	17 Å (Lévy, unpublished)
OmprN	Ni-NTA	Nd (M.Chami)
Br	EPA (-)	10 Å (Lévy, 2001)
BmrA	Ni-NTA	17 Å
Wza	Ni-NTA	20 Å (Nesper,2003)
Ryanodin receptor	Ni-NTA	20 Å (Lai, 2005)

Characteristics of the lipid layer method



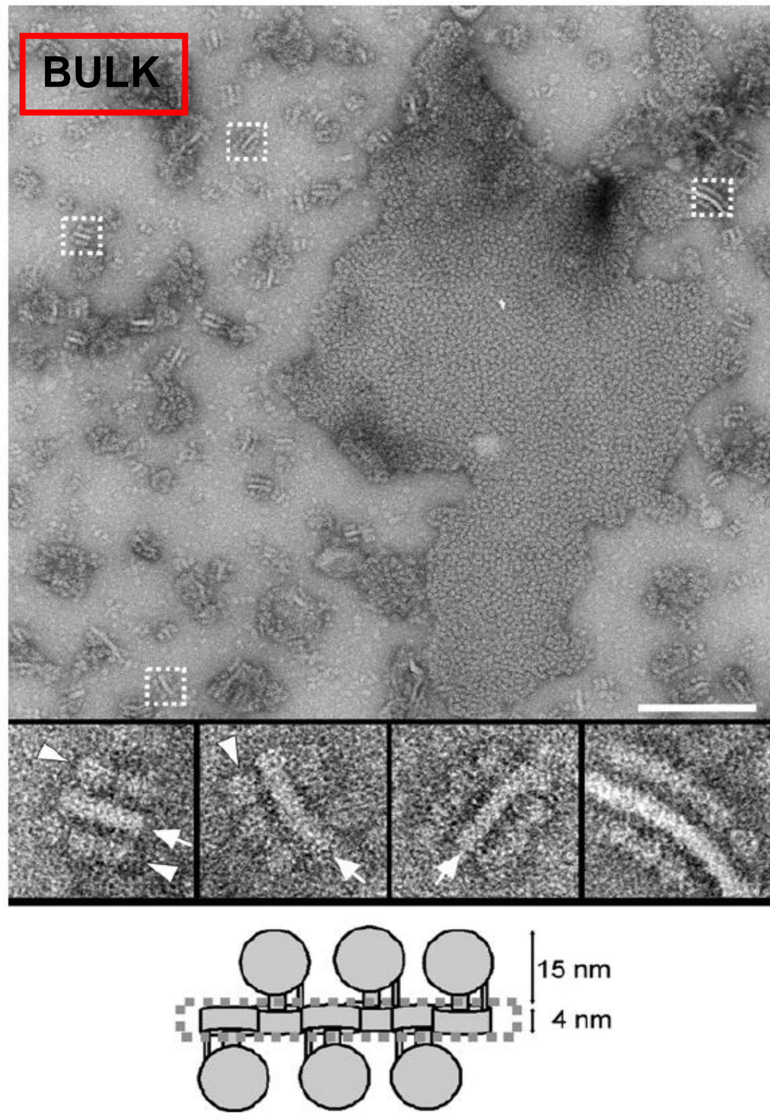
1. Protein concentration up to 10μgr/ml (1μg/trial)
2. Unic orientation of the proteins

Lipid layer, p2

In volume, p22121

2D crystals of FhuA (Levy, JSB, 1999)

Large extramembraneous domains e.g. FOF1, ABC transporters



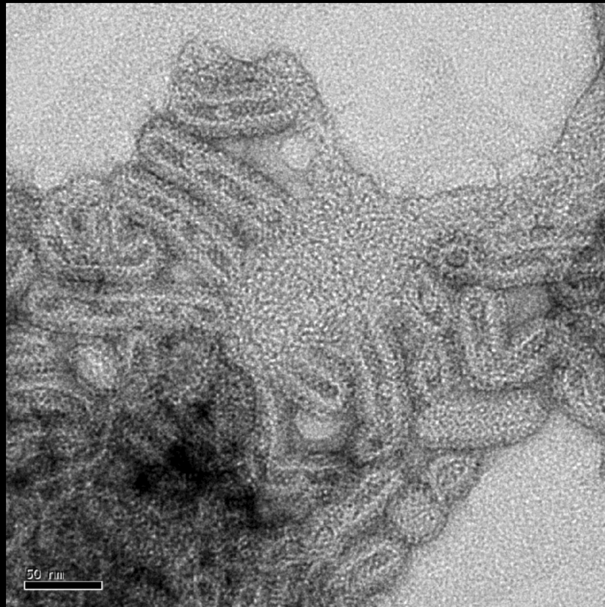
A

Monolayer

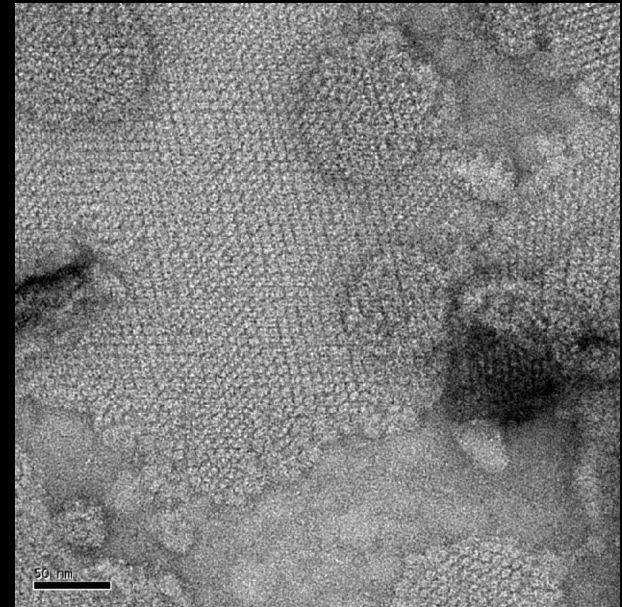
B

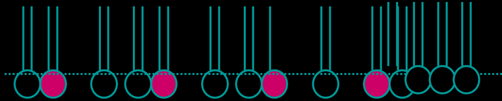
2D crystallization in bulk lead to stacked membranes
And no crystal are formed. In lipid layer, proteins interact
In a single orientation and form 2D crystals
(Arechaga, Fotiadis, JSB, 2007)

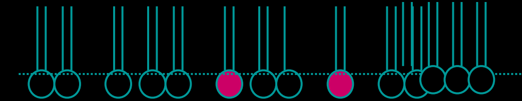
Conformational change of BmrA, a bacterial ABC transporter induced a change in membrane morphology

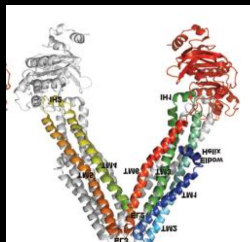


ATP/vi

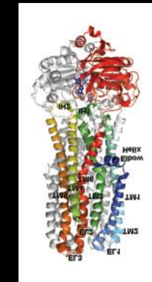






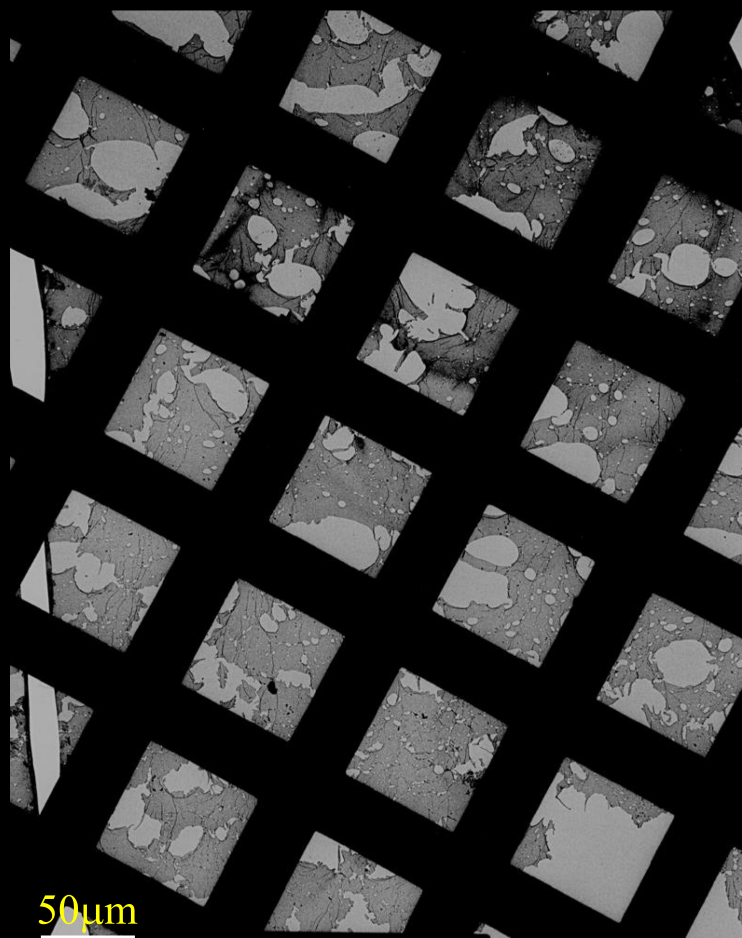


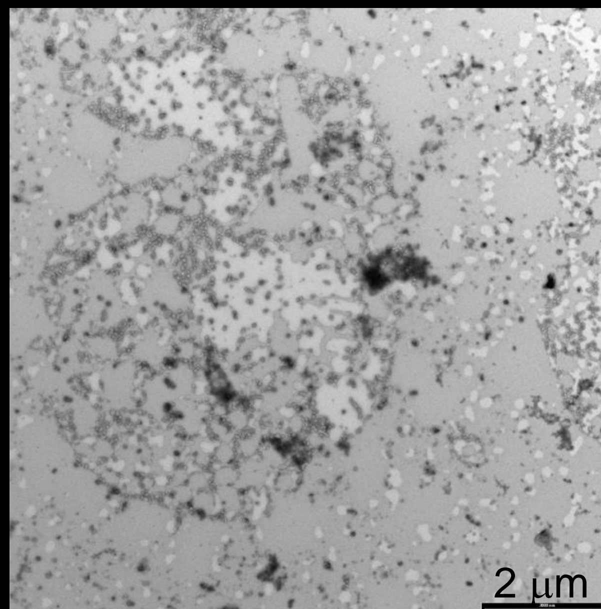
Due to the single orientation in the bilayer,
the V shaped of the protein in absence of ATP leads
the formation of tubes and planar sheets
after ATP binding. No crystals are obtained
in 2D crystallization in volume

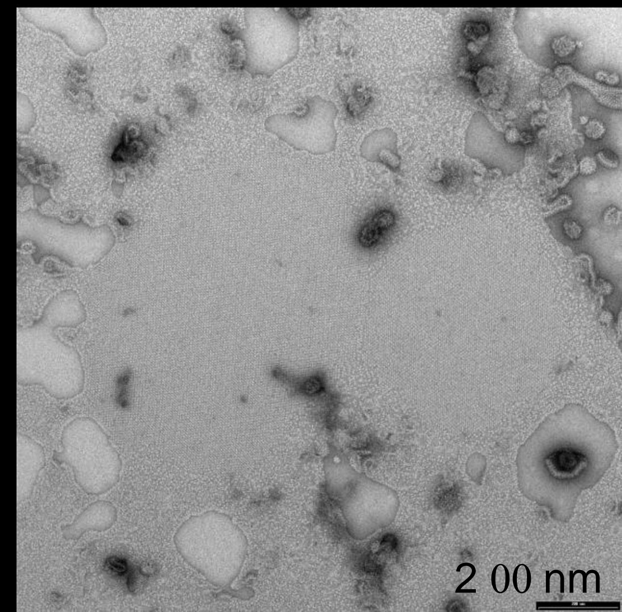


MsbA, Chang, 2007

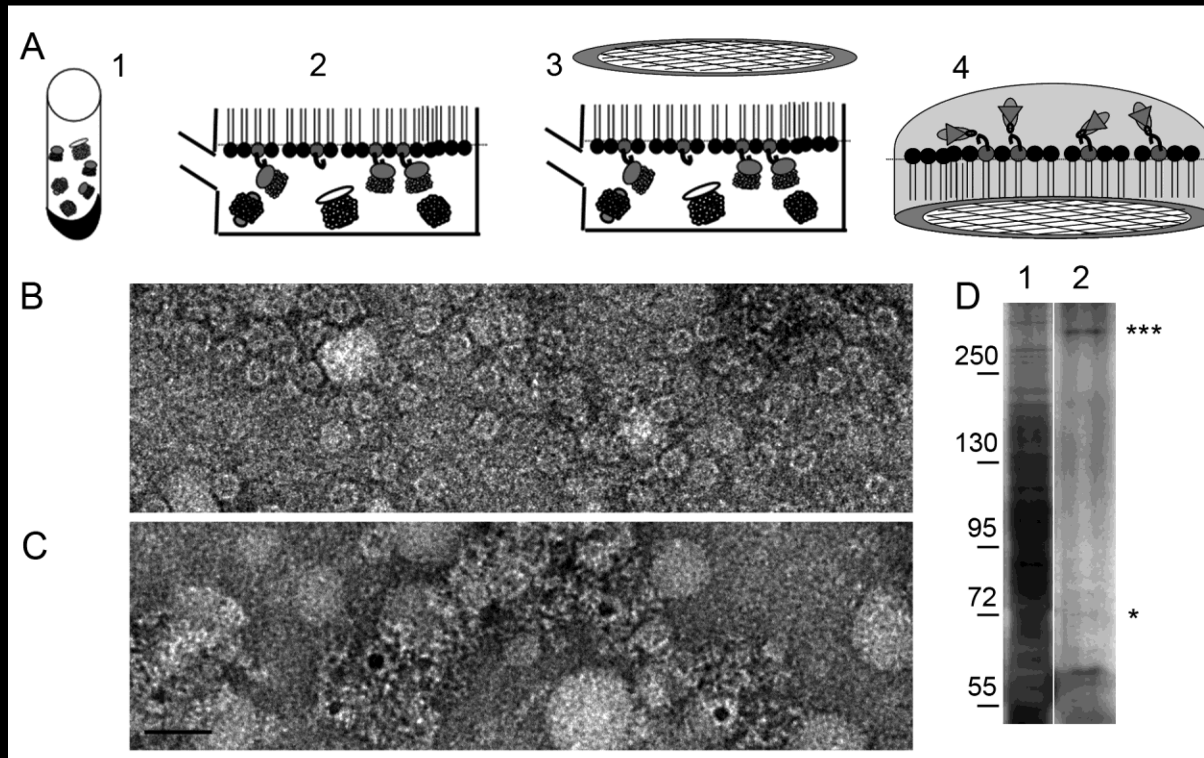
Large and connected membranes

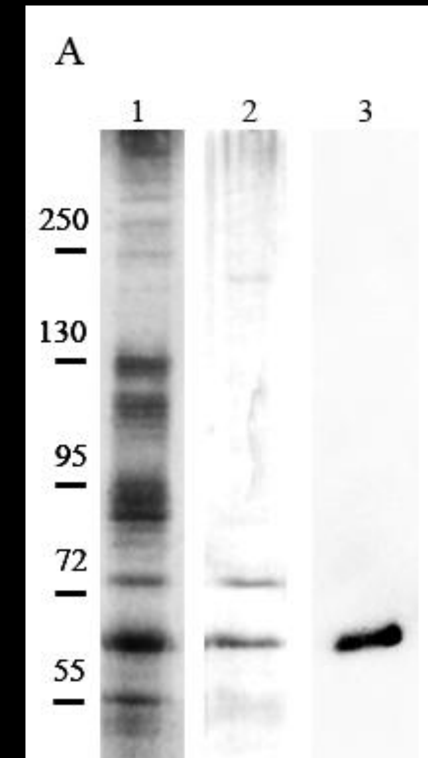






Improved purification at functionalised lipid surface even starting from non purified proteins (see also Walz 2007, 2008)

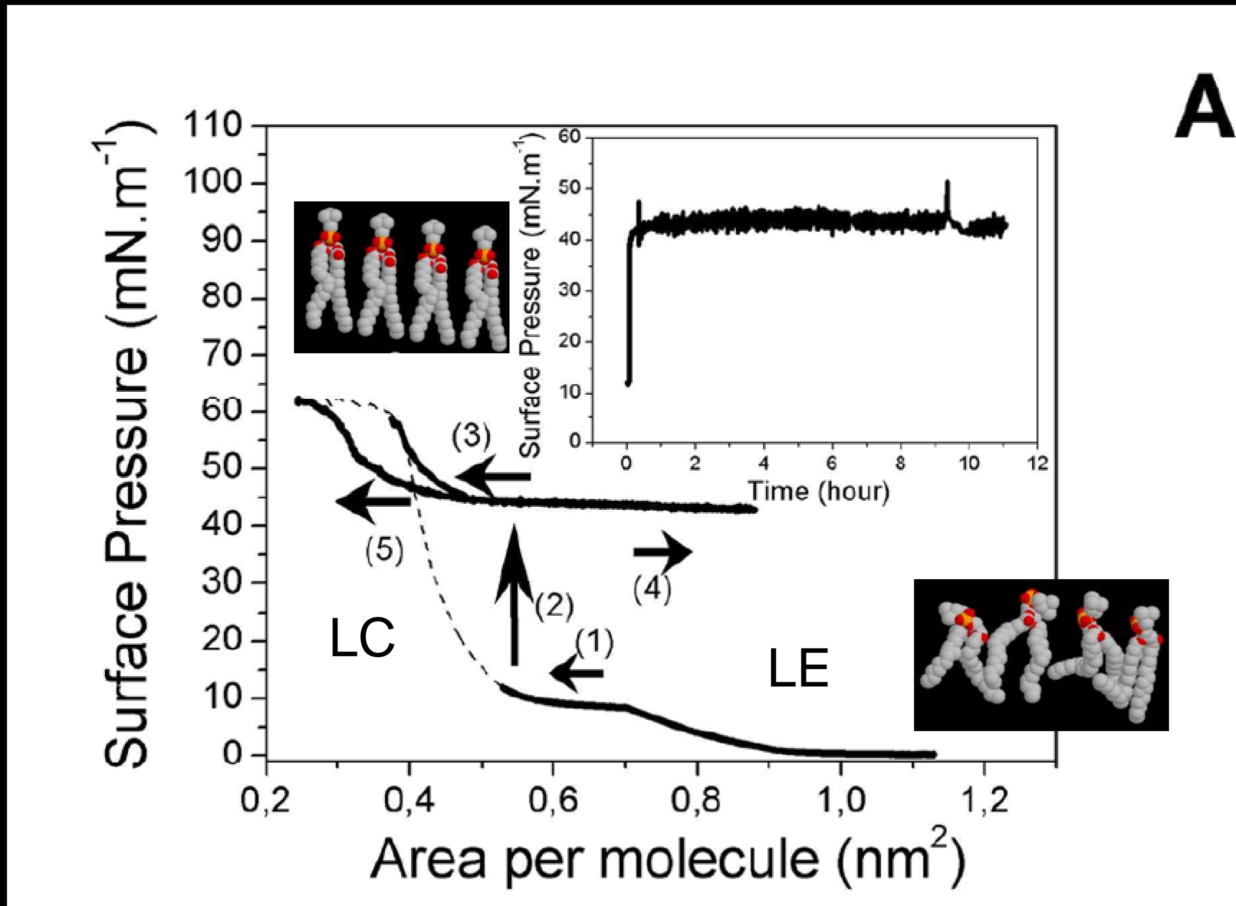




His-ABCG2 expressed in insect cells, (1) solubilized in DDM and (2) purified at the surface

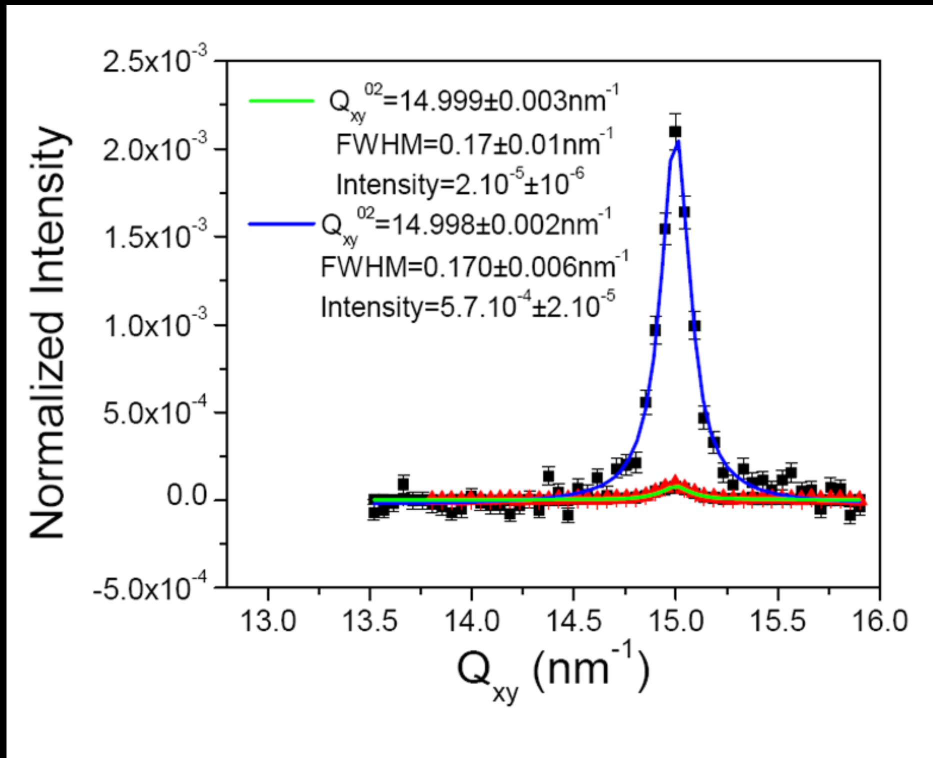
His-BmrA expressed in E.coli, (1) solubilized in DOTM and (2) purified at the surface

Stability of the hydrogenated lipid layer in presence of detergent (Fontaine, Langmuir 2009)



DMPE, injection of TX100 at 1 mM (cmc = 0.2 mM)

Grazing Incidence x-ray Diffraction

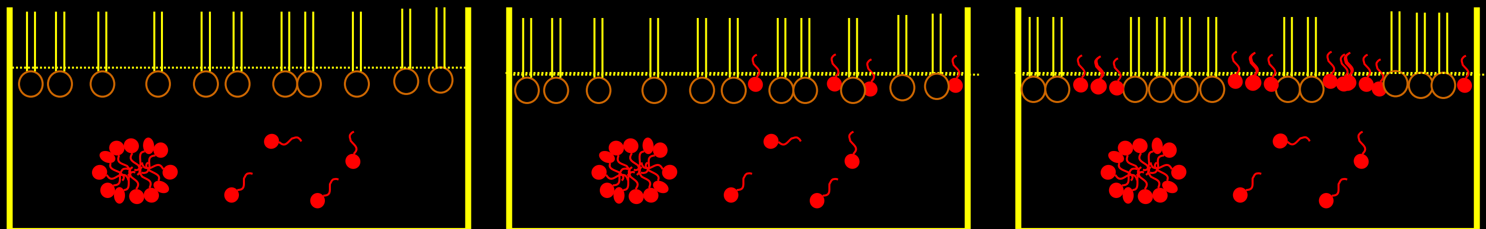


DMPE, TX100 1 mM

- 6 Hours (blue peak)
- Peak a=0.48nm, B=0.84 nm

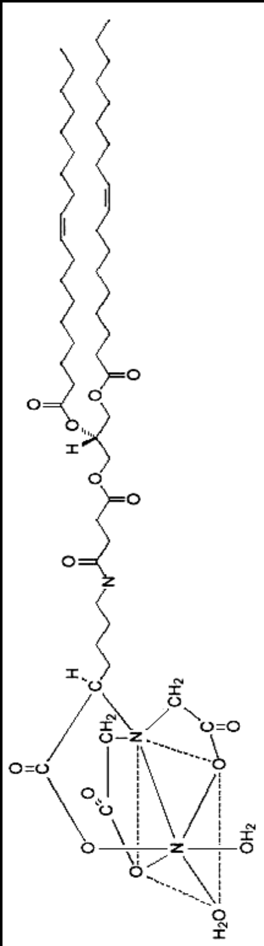
Hexagonal untilted chains

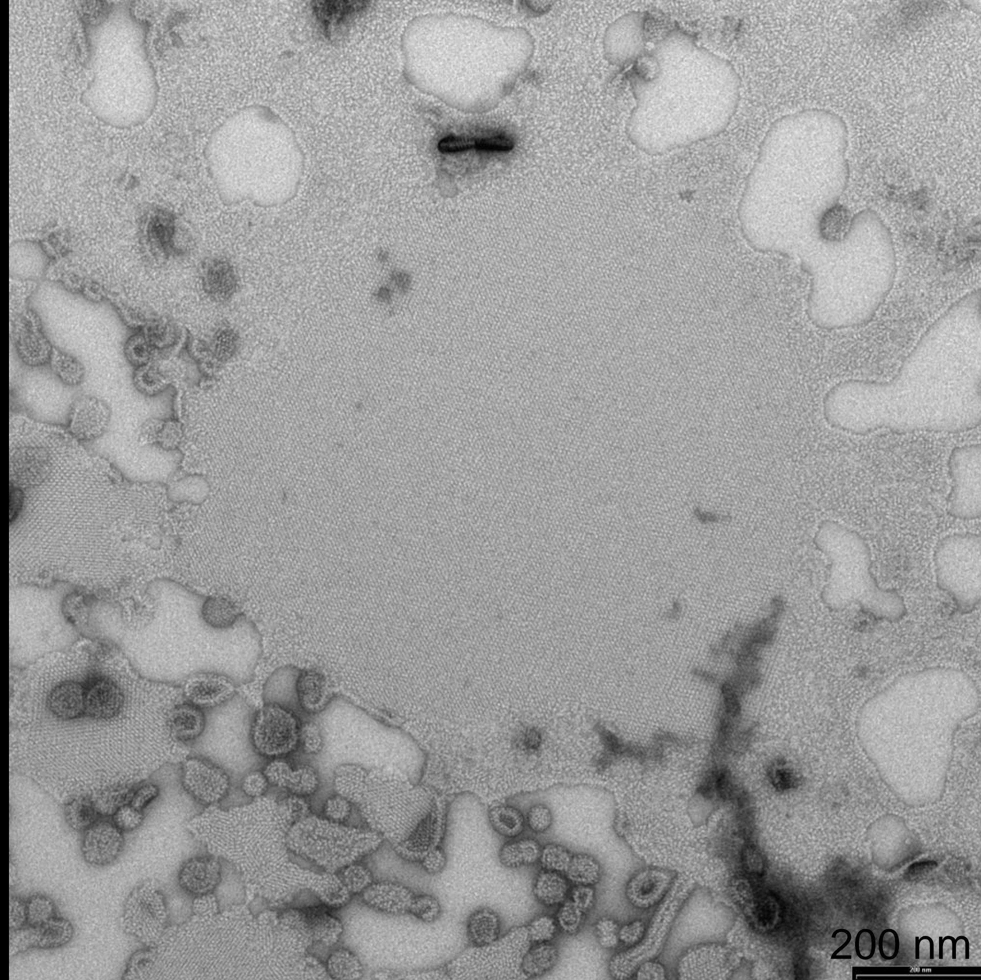
The surface is made of
lipid and detergent separated domains



Fast insertion of TX100 followed by compression of DMPE to form a stable film

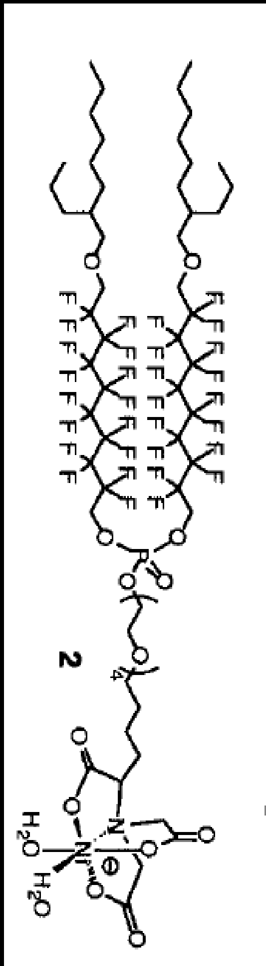
Fluorinated versus hydrogenated lipids
1. Hydrogenated lipid

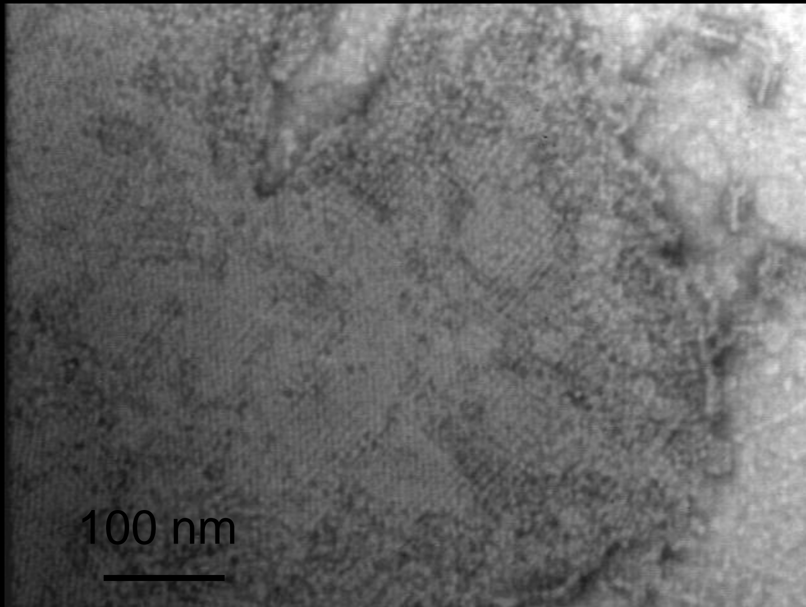


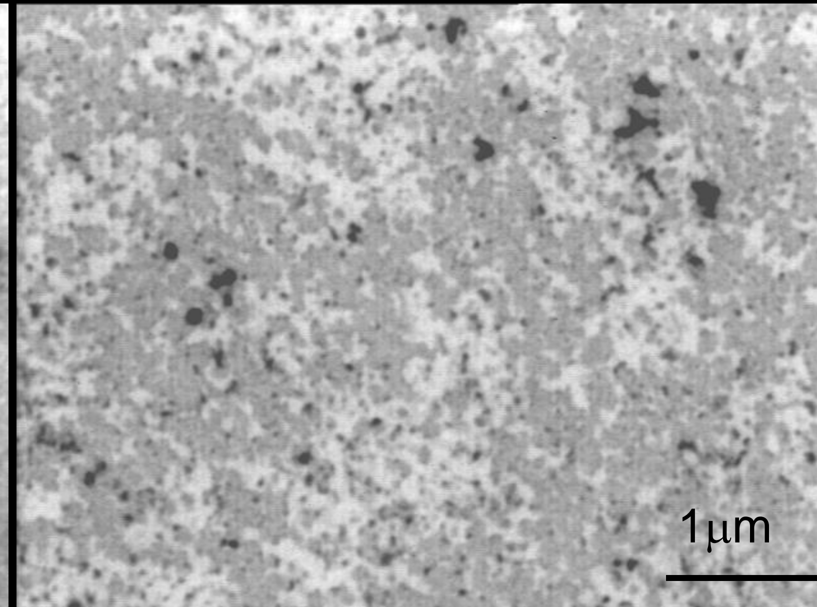


NiNTA DOGS
Avanti Polar

Fluorinated versus hydrogenated lipids (Hussein, JOC, 2009)
2. Fluorinated lipid







Membranes were smaller, more fragmented and less crystallized than with H lipids
But much more trials have been done with H than with F lipids
(or other functionalized templates and this could be improved)

Optical set-up for in situ screening the binding and the reconstitutio

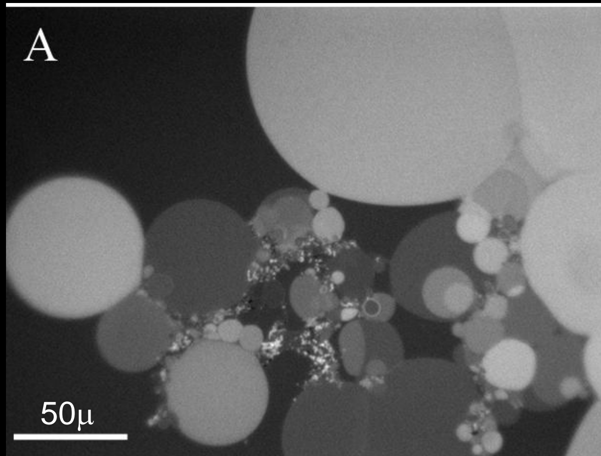
Upright microscope in reflection mode (no fluorescence)

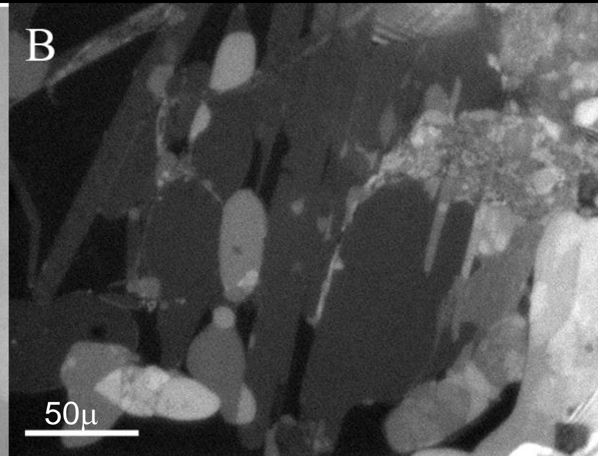
Binding of proteins observed by optical microscopy

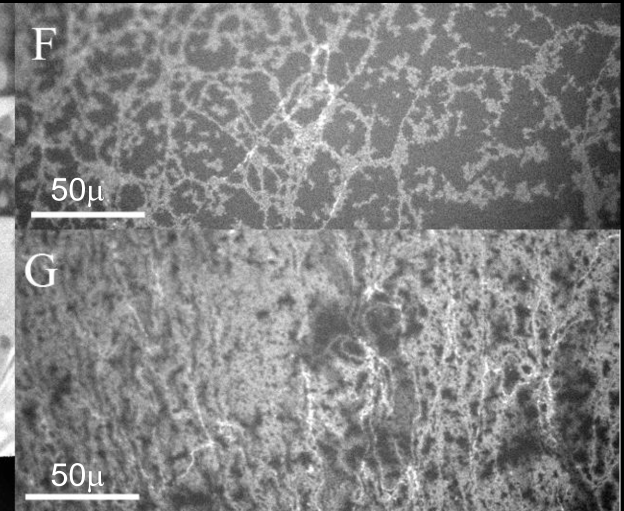
(A) GB3/DOPC
(optical microscopy)

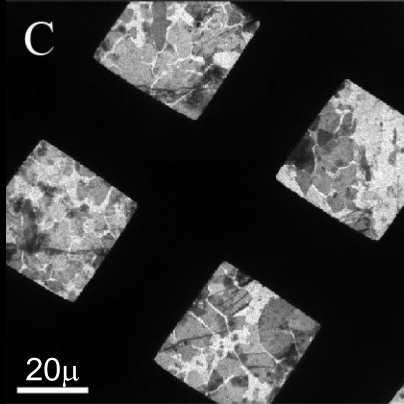
(B) Addition of Shiga StxB
(optical microscopy)

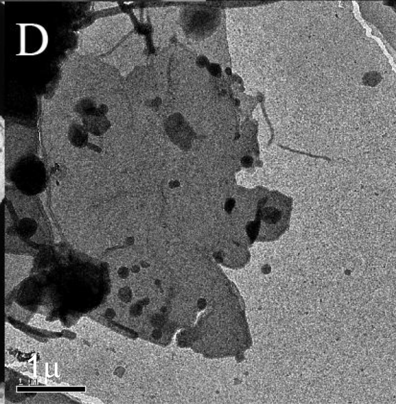
(F) DOTAP
(G) Addition of Purple Membrane (optical microscopy)

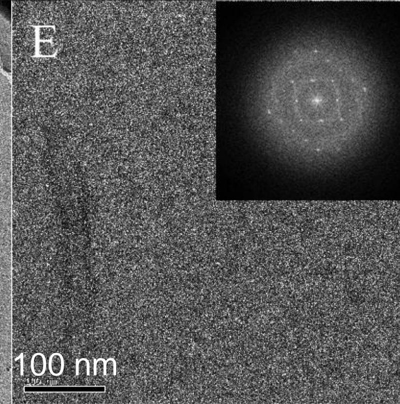


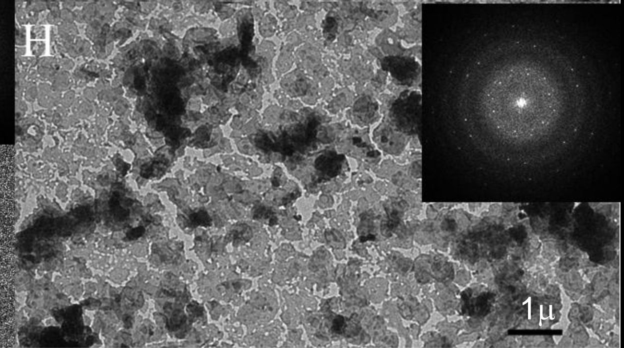








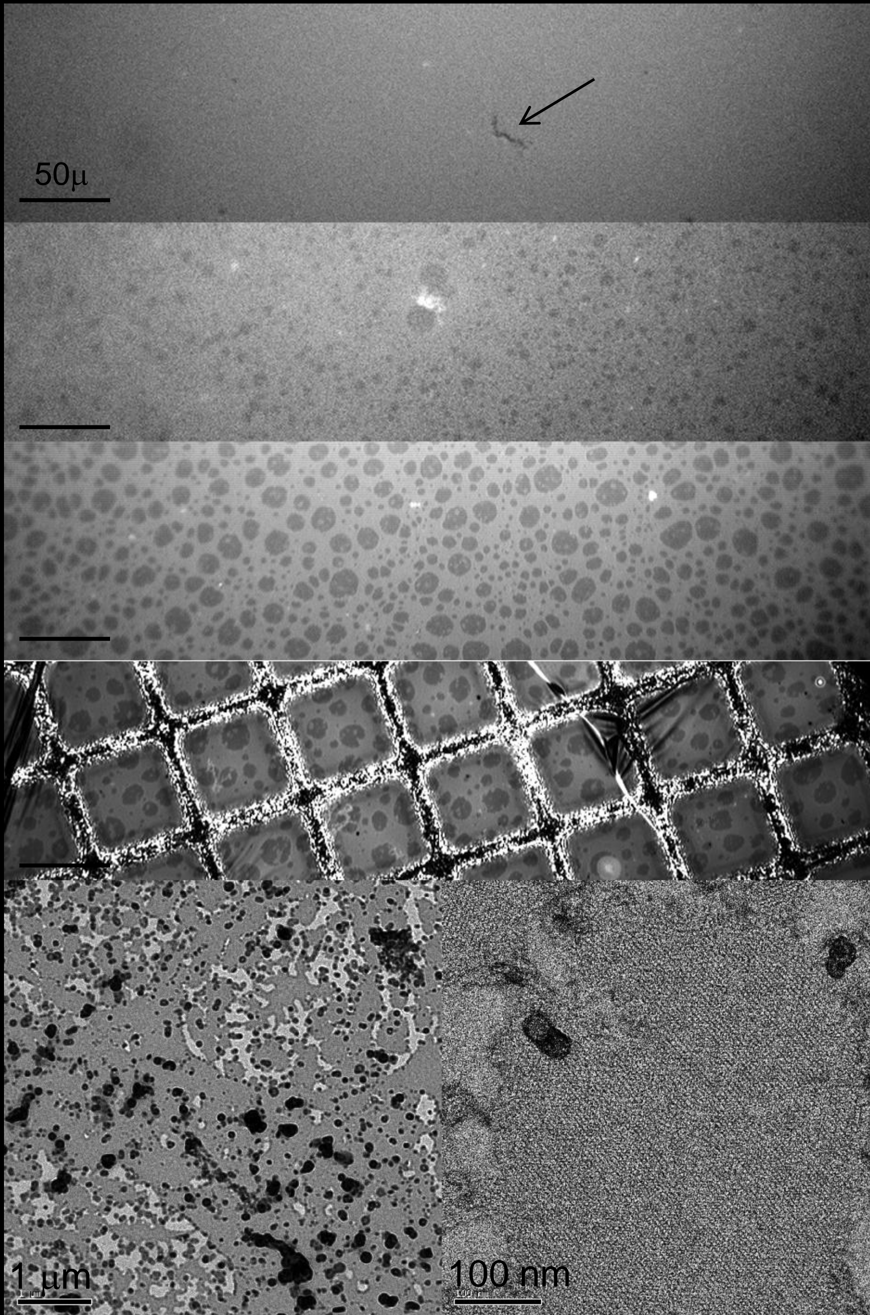




Transfert onto EM grid
(EM)

Transfert onto EM grid
(EM, trealose embebding)

Screening of Membrane reconstitution at the lipid layer



Binding of micellar protein (optical microscopy)
t=0 the dark crevasse showed a free protein area (arrow)

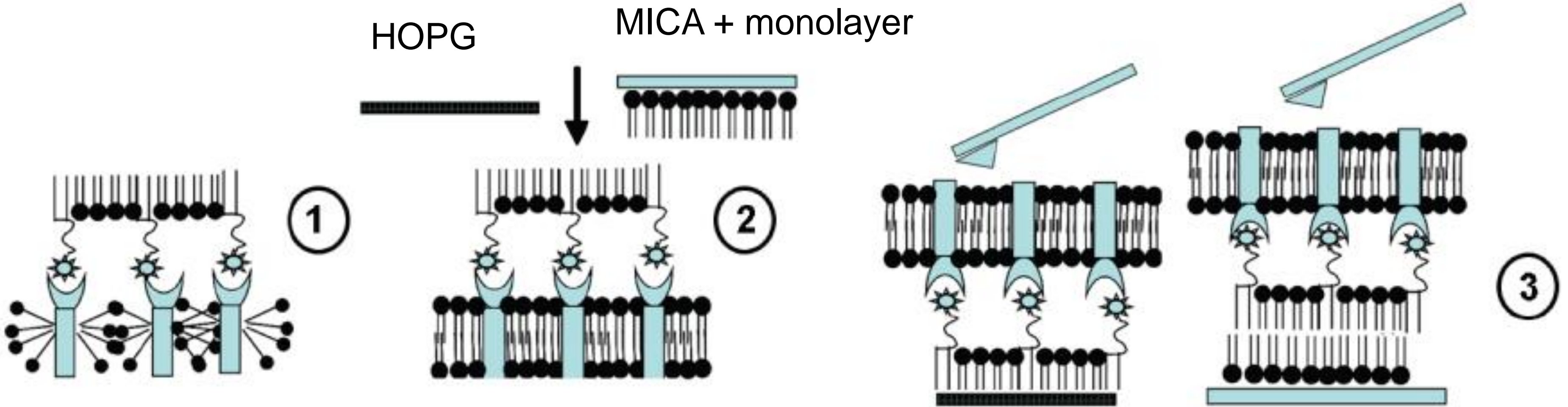
Reconstitution of into lipid bilayer (optical microscopy)
t=24 h BioBeads

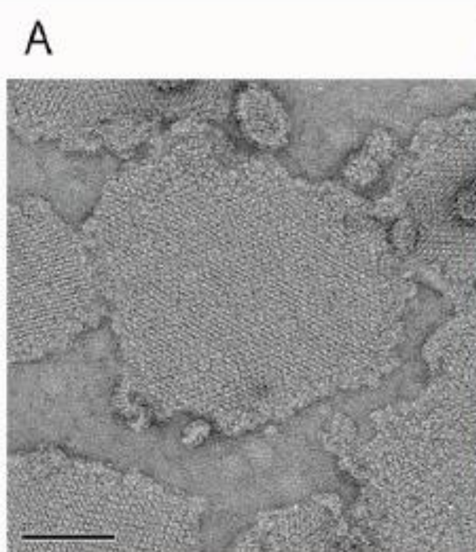
t=36h

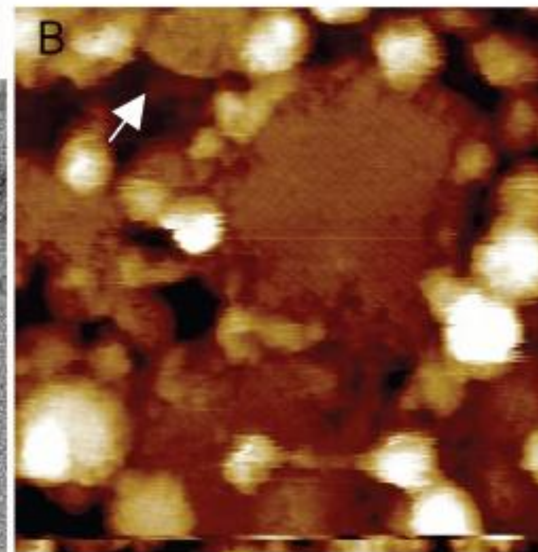
Transfert onto EM grid (optical microscopy)

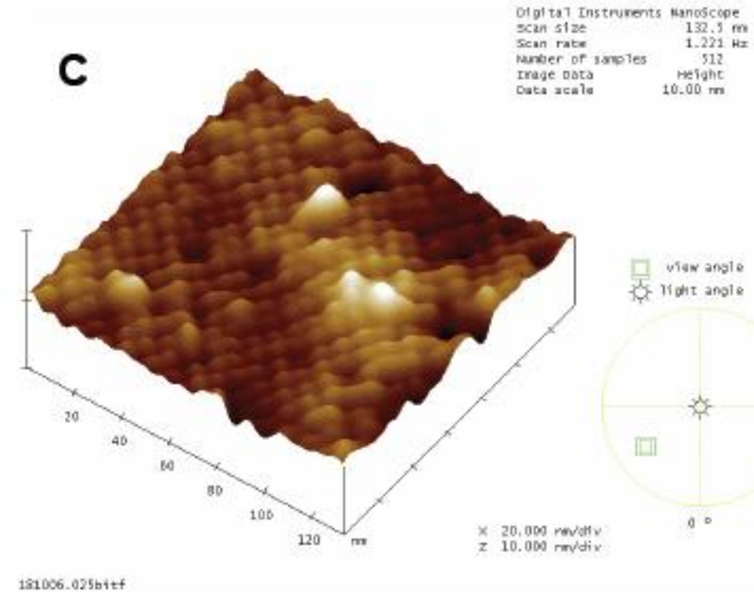
EM analysis

Transfer onto hydrophobic surface for AFM imaging









Sentier, J. Mol.Recog. 2010

BioBeads
- All detergents
- Screening of reconstitution
- Possibly high resolution

Monolayer
- Low amount of proteins
- Unique orientation
- Large membranes
- Possibly high resolution
- Other applications, SPA

Thanks again to the organizers and the Cina team