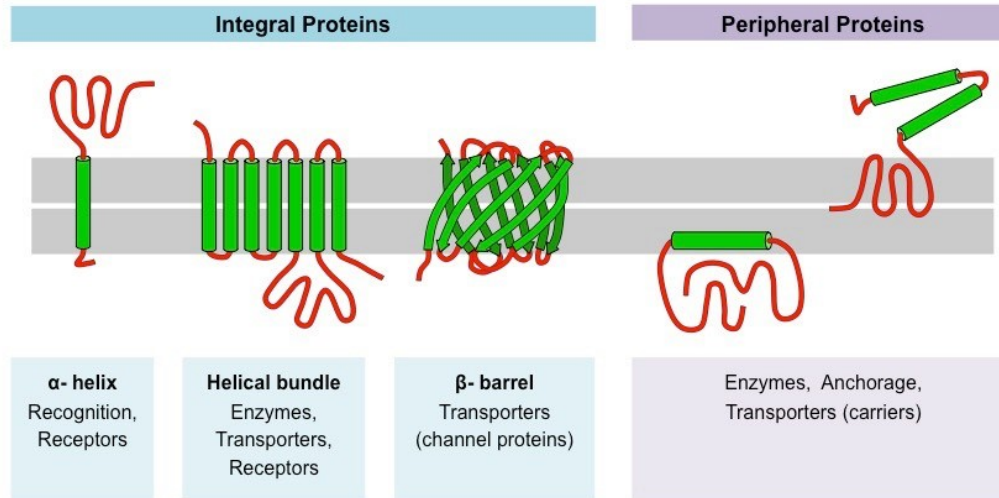
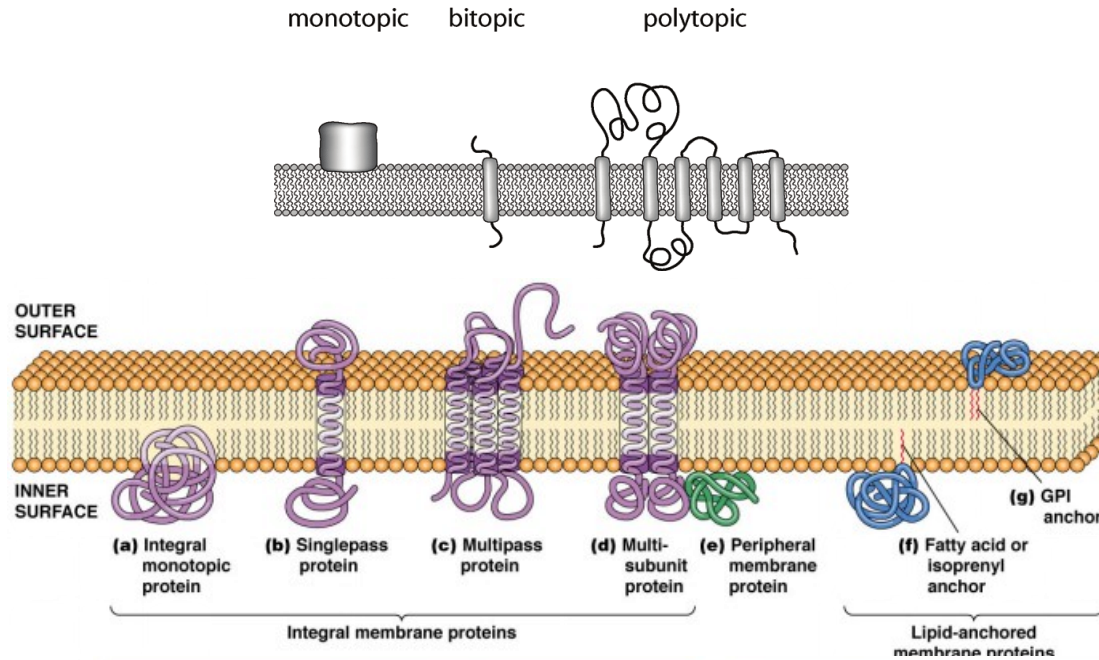


Proteine ricombinanti per studi  
strutturali:  
proteine di membrana

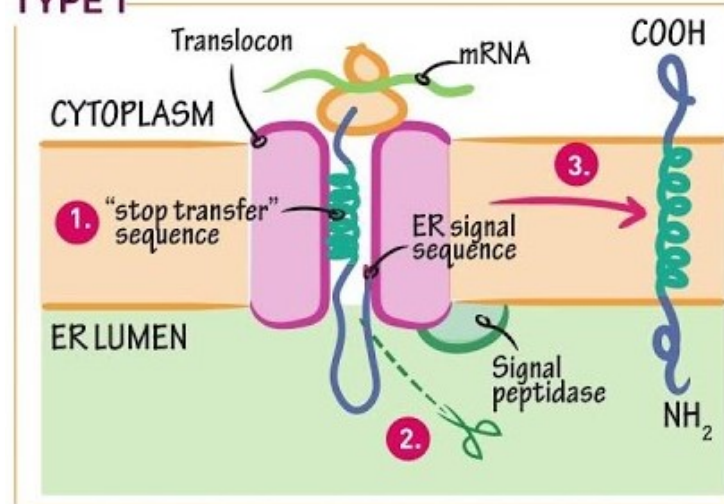
# Proteine di membrana



# Proteine di membrana: topologie

- ✓ Type I —
  - ✓ Signal & stop-transfer sequences
- ✓ Type II —
  - ✓ Internal signal sequence
- ✓ Type III —
  - ✓ Internal signal sequence
- ✓ Type IV (multipass) —
  - ✓ Internal signal and stop-transfer sequences

**TYPE I**



**Step 1**

Stop-transfer sequence "stops" the "transfer" of nascent peptide through translocon.

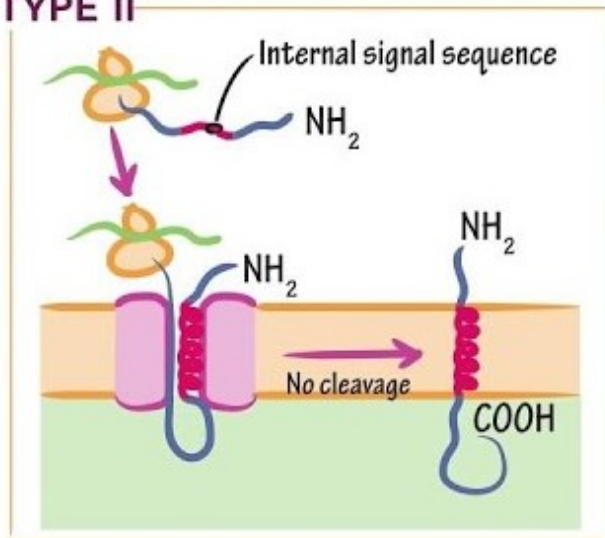
**Step 2**

Signal peptidase cleaves the signal sequence.

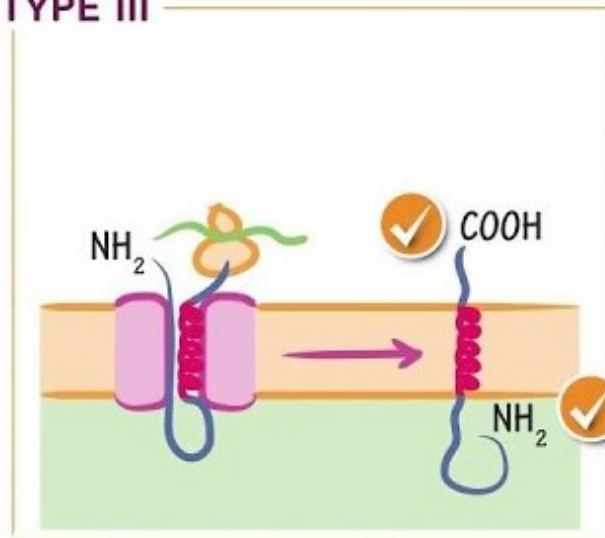
**Step 3**

Stop-transfer sequence exits the translocon laterally. Ribosome translates remainder of protein.

**TYPE II**

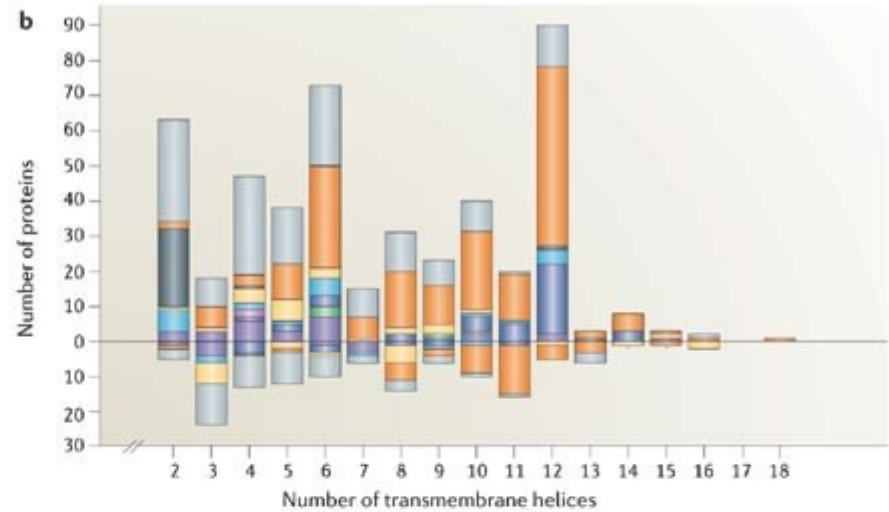
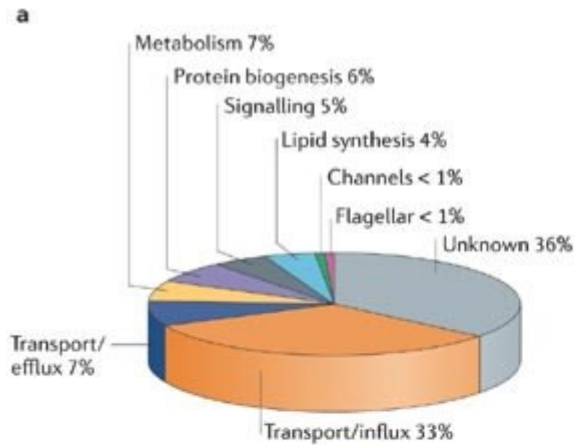


**TYPE III**

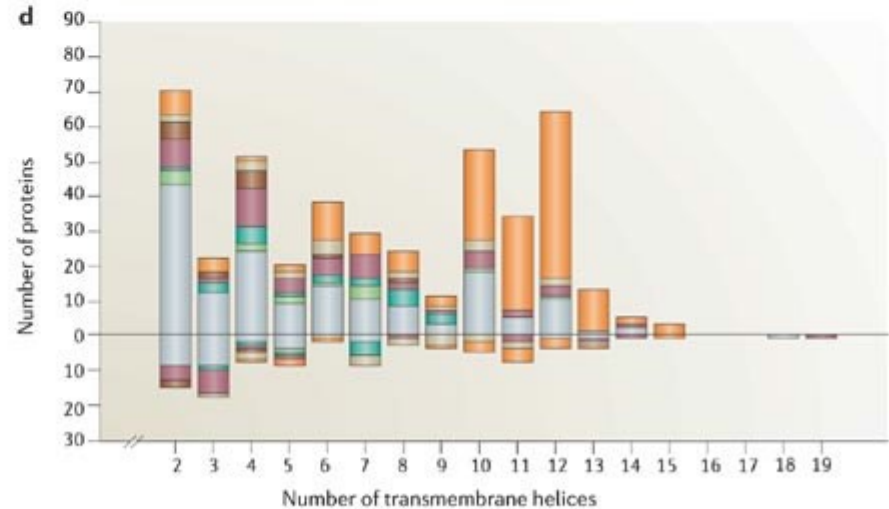
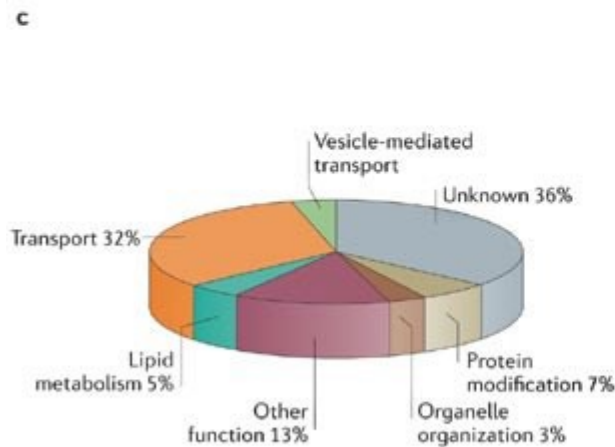


# Proteine di membrana: funzioni e rilevanza fisiologica

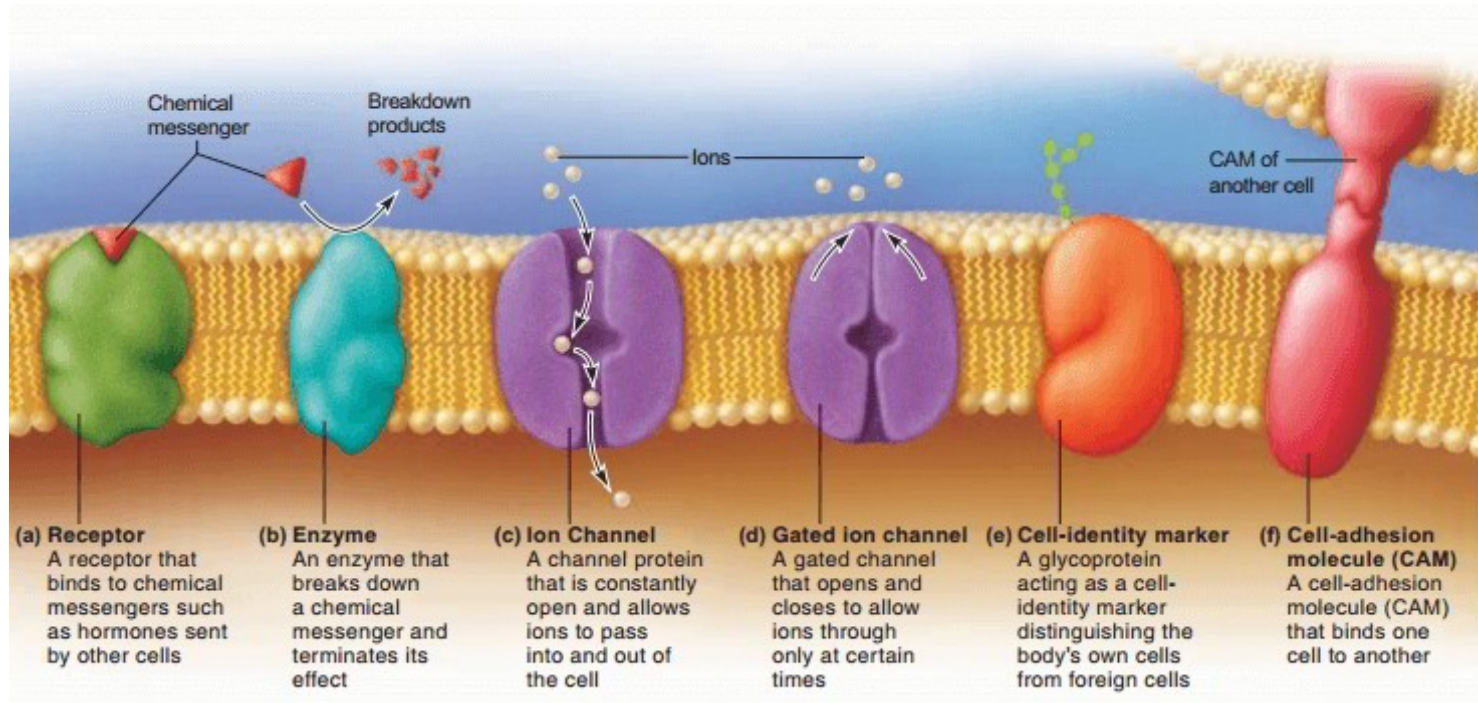
*Escherichia coli*



*S. cerevisiae*



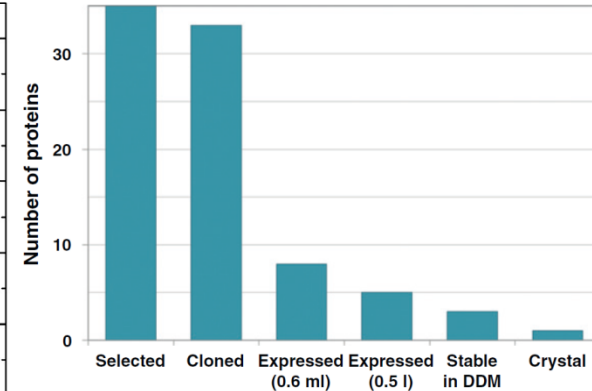
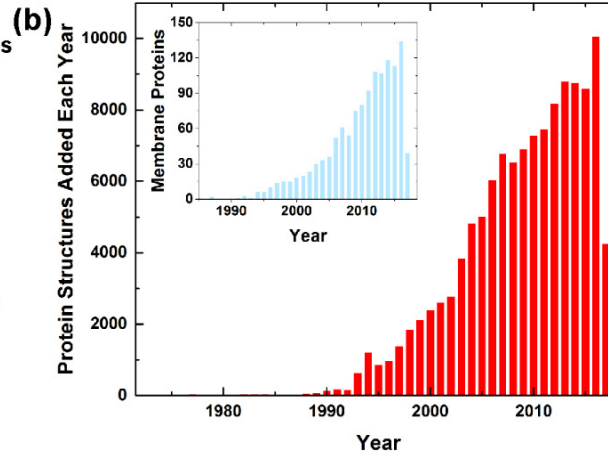
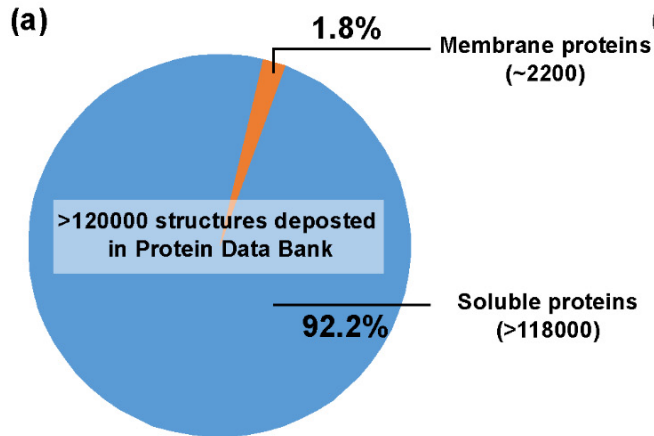
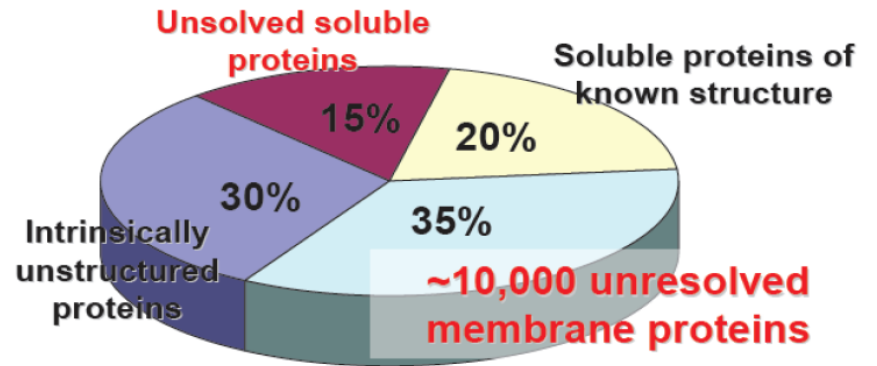
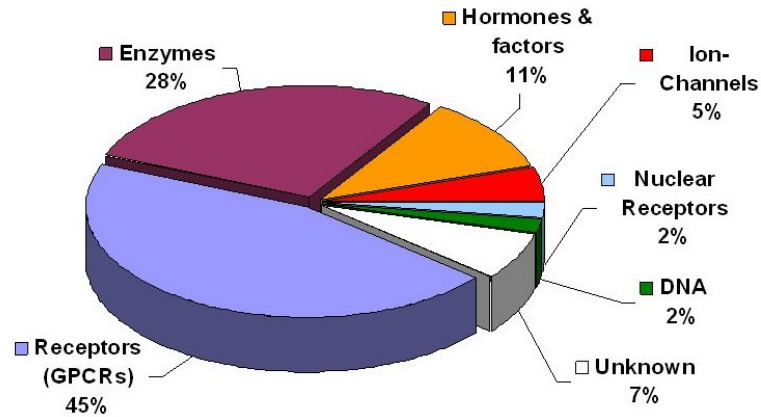
# Proteine di membrana: funzioni e rilevanza fisiologica



Mutant Membrane Protein	Description	Target	Disease Relevance	Readout
<a href="#">ADRB2(W158A)</a> **	Adrenergic receptor $\beta 2$	GPCR		Endosome - EA
<a href="#">AVPR2(S167T)</a>	Vasopressin receptor 2	GPCR	Nephrogenic diabetes insipidus	Endosome - EA
<a href="#">CFTR-<math>\Delta</math>F508</a>	Cystic fibrosis transmembrane conductance regulator	Ion Channel	Cystic fibrosis	Membrane - EA
<a href="#">KCNH2(G601S)</a>	Potassium voltage-gated channel, subfamily H (eag-related), member 2	Ion Channel	Long QT syndrome (Cardiac arrhythmias)	Membrane - EA
<a href="#">MC4R(T162I)</a>	Melanocortin 4 receptor	GPCR	Obesity	Endosome - EA
<a href="#">mRHO(P23H)</a>	Rhodopsin	GPCR	Retinitis pigmentosa	Endosome & Membrane - EA
<a href="#">SMO(W535L)</a>	Smoothened frizzled family receptor	GPCR	Basal skin cell carcinomas	Membrane - EA

# Proteine di membrana: rilevanza farmacologica

più di META' dei TARGET FARMACOLOGICI sono proteine di membrana



# Proteine di membrana: studi strutturali

Cell. Mol. Life Sci. 63 (2006) 36–51  
1420-682X/06/010036-16  
DOI 10.1007/s00018-005-5350-6  
© Birkhäuser Verlag, Basel, 2006

Cellular and Molecular Life Sciences

## Review

### The Membrane Protein Data Bank

P. Raman<sup>a</sup>, V. Cherezov<sup>a,c</sup> and M. Caffrey<sup>a,b,c,d</sup>

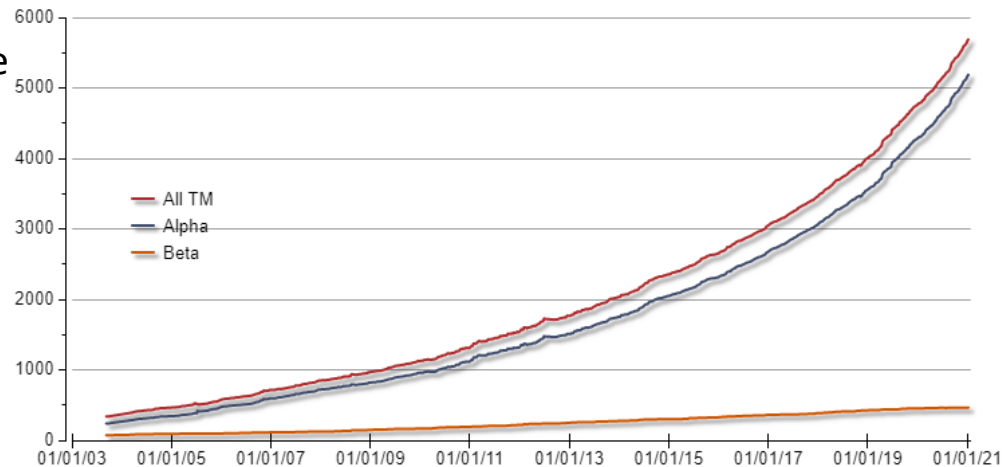
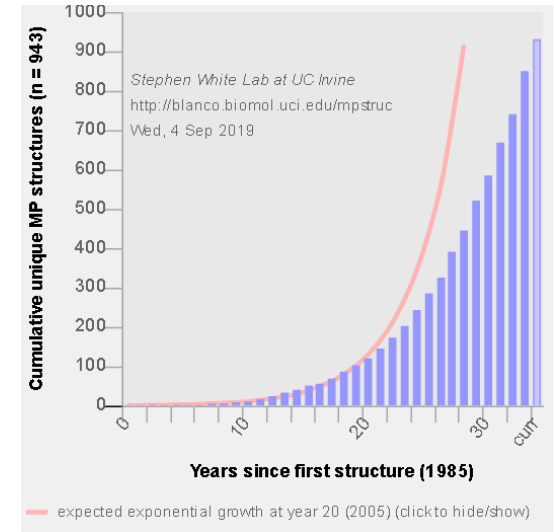
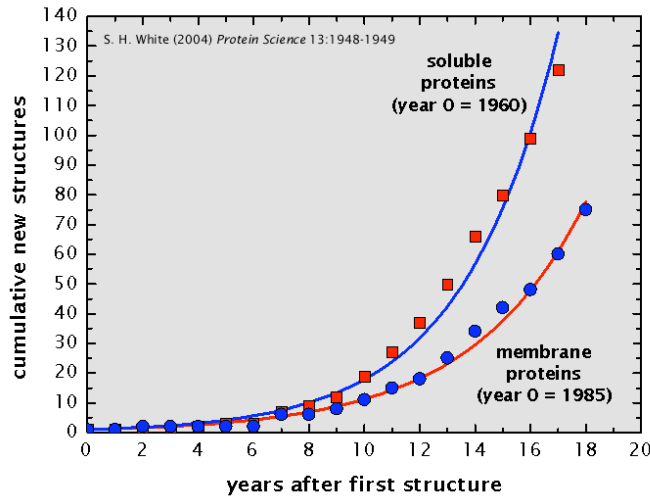
Last database update:  
30 ago 2019

Unique proteins in database = 943  
Coordinate files in database = 2839

PDBTM (transmembrane protein selection of the  
[Protein Data Bank \(PDB\)](#))

PDBTM version: 2021-01-08

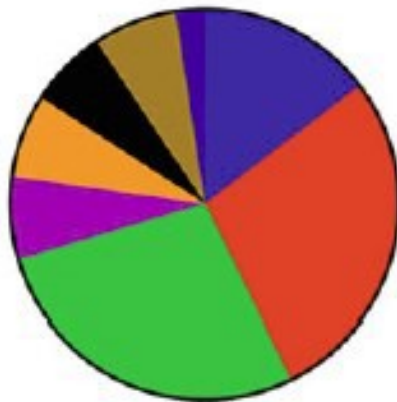
Number of transmembrane proteins:  
5685 (alpha: 5184 , beta: 464 )



# Proteine di membrana: studi strutturali su proteine di membrana ad alfa-elica



2008



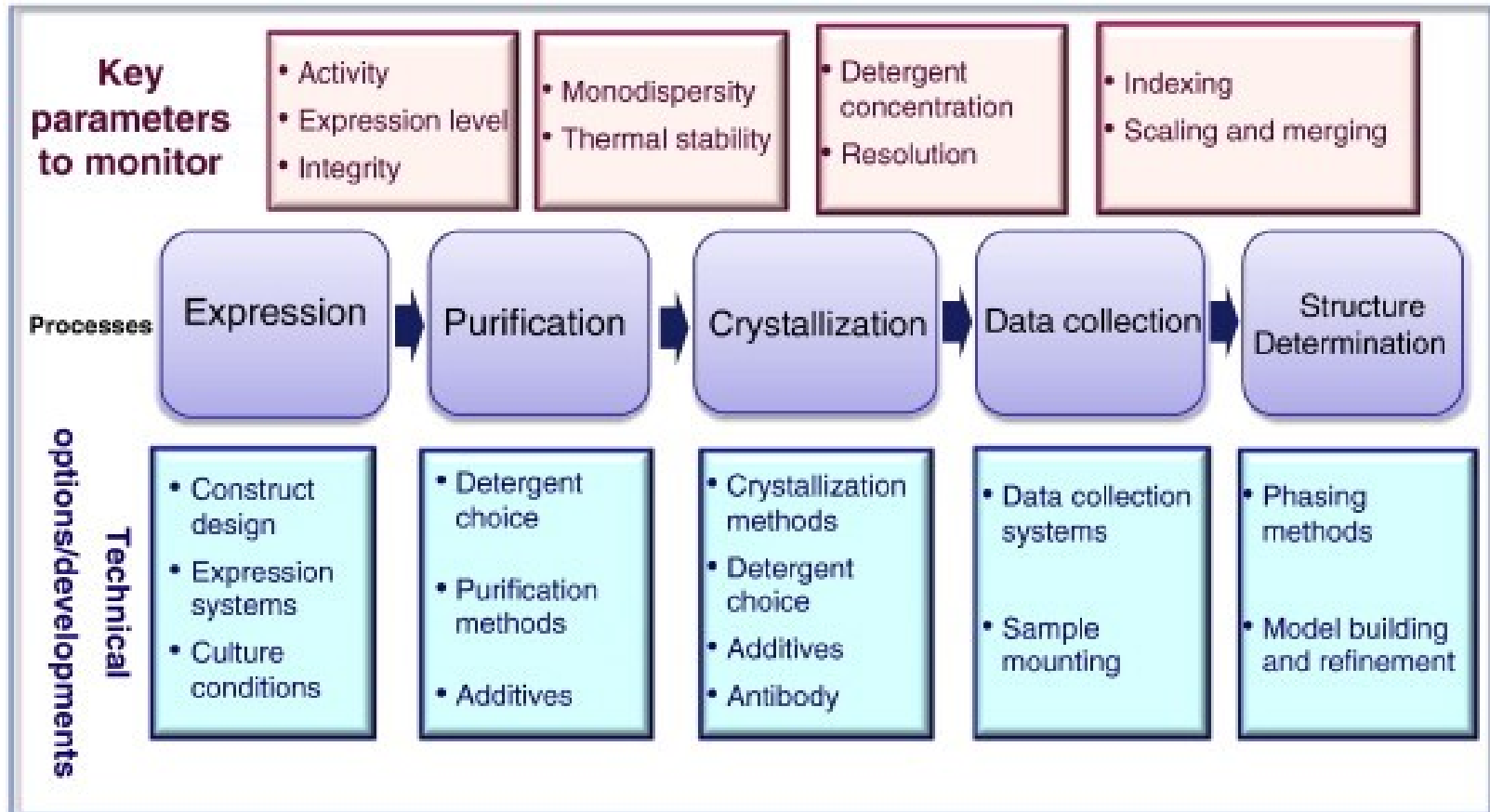
2012



2015



# Studi strutturali di proteine di membrana: flowchart



# Produzione di proteine di membrana per studi strutturali: espressione

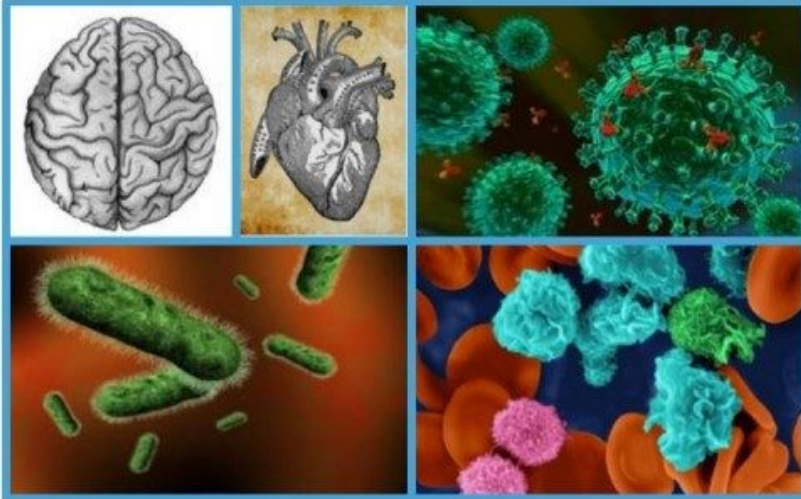
## Fonti naturali

## Sistema di espressione

### ENDOGENOUS SYSTEMS

Organ

Virus



Bacteria

Primary cells

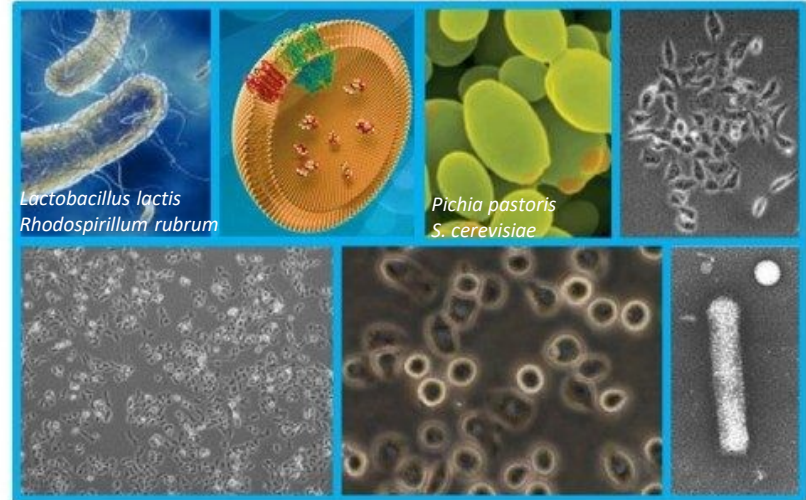
### RECOMBINANT SYSTEMS

E.coli

Cell-free

Yeast

HEK



*Lactobacillus lactis*  
*Rhodospirillum rubrum*

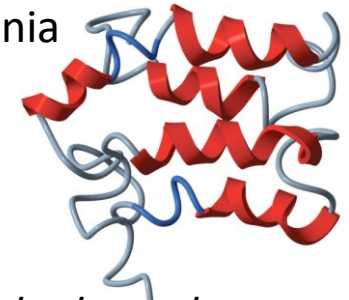
*Pichia pastoris*  
*S. cerevisiae*

CHO

Insect cells / Baculovirus

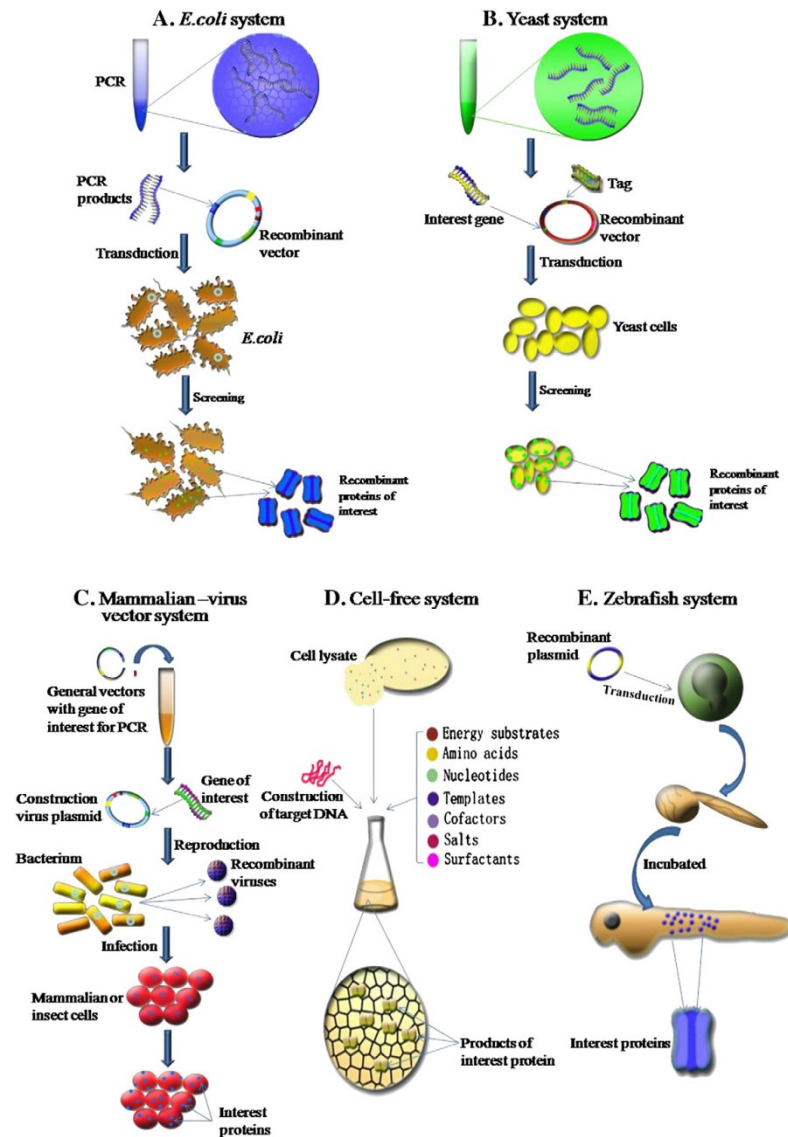
alghe (chlorella), *Leishmania tarentolae*

In fusione con **MISTIC** (Membrane Integrating Sequence for Translation of Integral membrane protein Constructs)



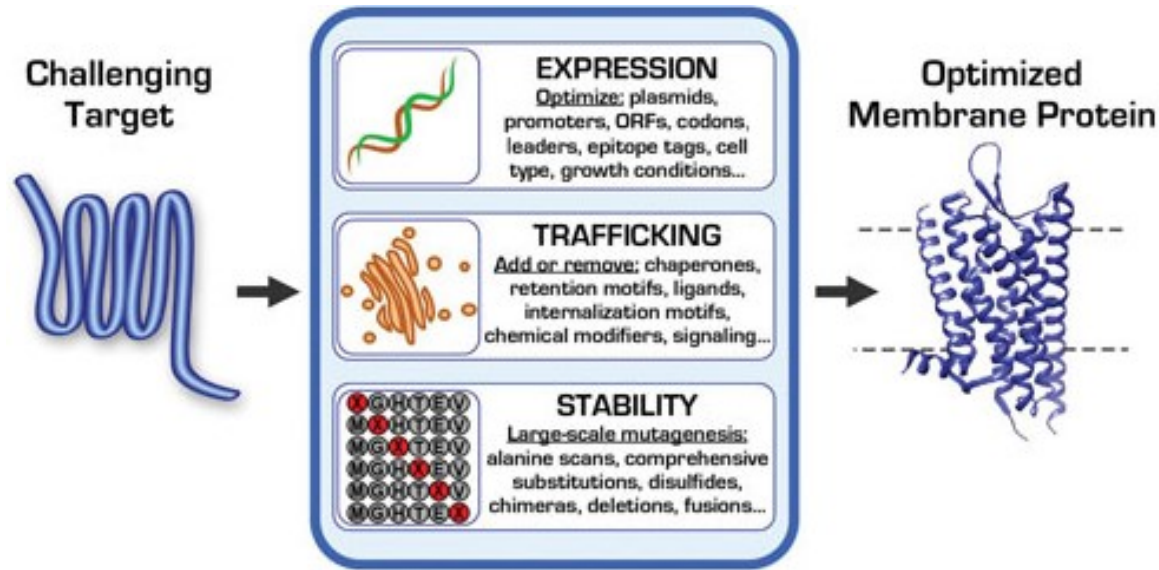
*B. Subtilis* dual-topology protein

# Produzione di proteine di membrana per studi strutturali: espressione



in diversi tessuti dell'embrione  
(testa, pinne pettorali, cellule  
endoteliali aortiche cellule  
muscolari lisce e del tratto  
gastrointestinale)

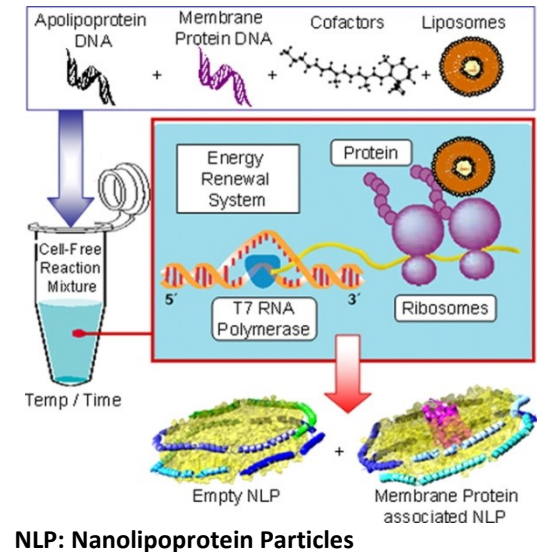
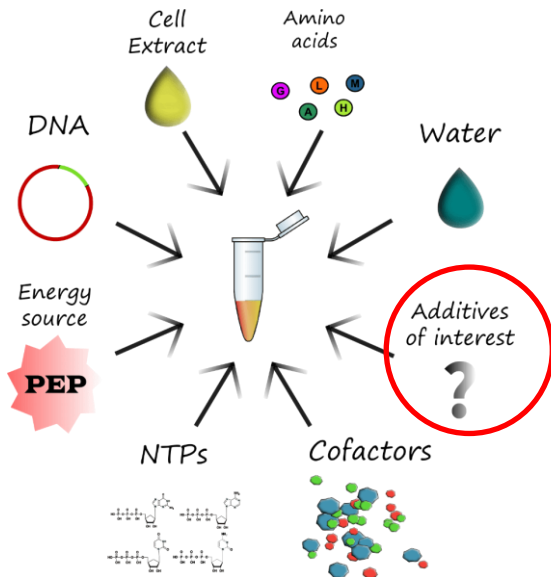
# Produzione di proteine ricombinanti di membrana per studi strutturali:



## Espressione in sistemi cell-free:

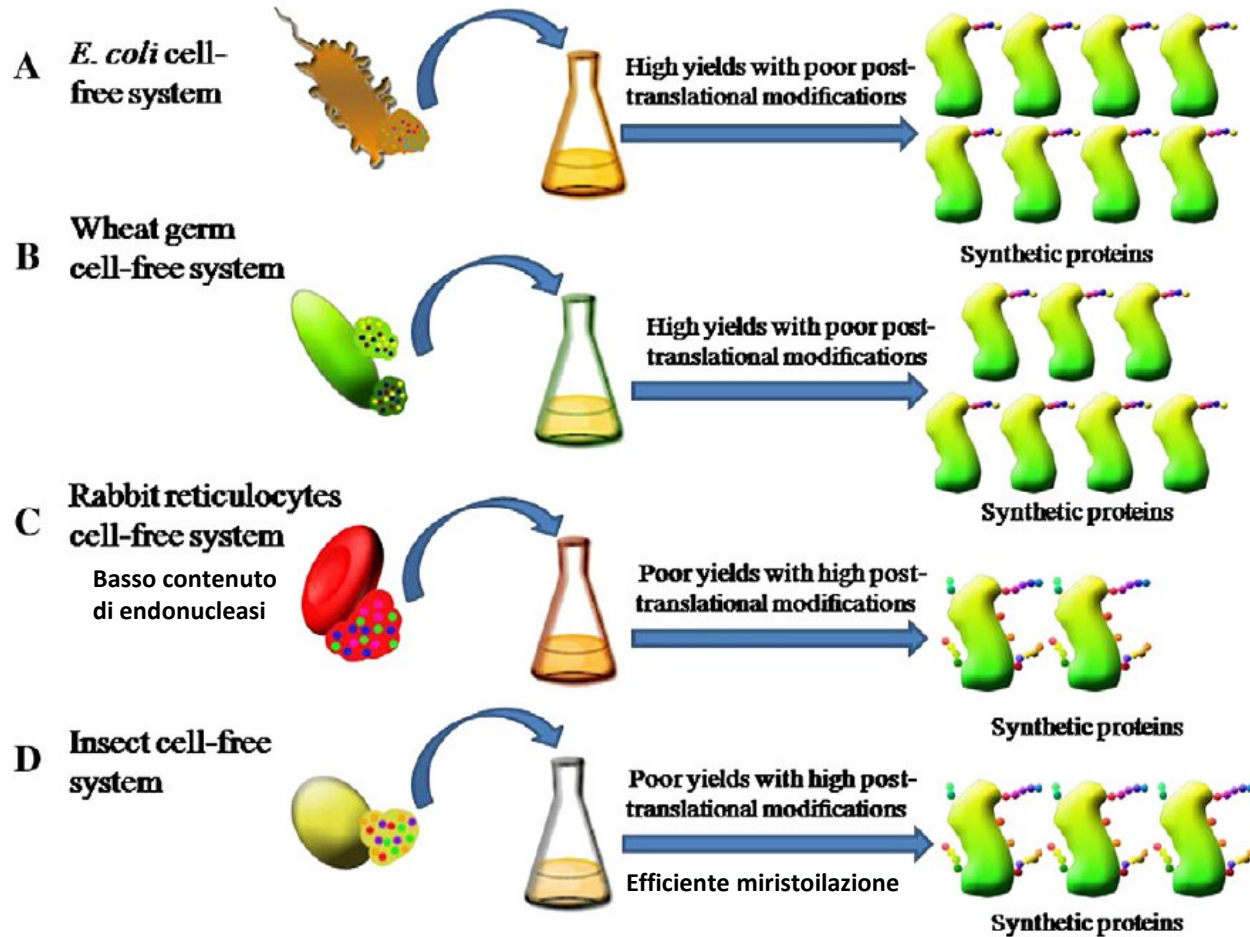
Co-espressione con membrane scaffold proteins (apolipoproteina) in presenza di liposomi

HT format



# Produzione di proteine ricombinanti di membrana per studi strutturali:

## Espressione in sistemi cell-free:



# Produzione di proteine di membrana per studi strutturali: espressione eterologa

TABLE 1. List of epitope tags used for detection and purification of recombinantly expressed proteins

Tag	Suitable for detection?	Suitable for purification?
His	Yes	Yes
Haemagglutinin	Yes	No
Strep	Yes	Yes (only <i>Escherichia coli</i> )
Strep II	Yes	Yes
Biotin	Yes	Yes
Flag	Yes	No
Protein C	Yes	Yes
C-myc	Yes	No
V5	Yes	No
GST	Yes	Yes

## Chiave: approccio HT

- Serie di costrutti


- Serie di ortologhi della stessa proteina (**Homology screening**):  
ex: SecY da 10 specie: solo una struttura ad alta risoluzione,  
da *Methanococcus Jannaschii*

- varianti sintetiche

**nature**  
International journal of science

Article | Published: 03 December 2003

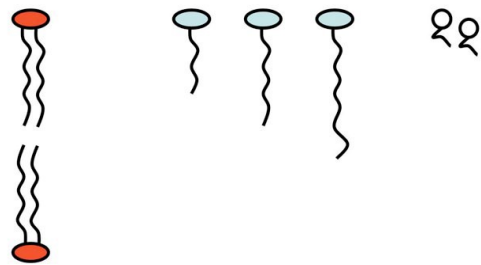
## X-ray structure of a protein-conducting channel

Bert van den Berg, William M. Clemons Jr, Ian Collinson, Yorgo Modis, Enno Hartmann, Stephen C. Harrison & Tom A. Rapoport 

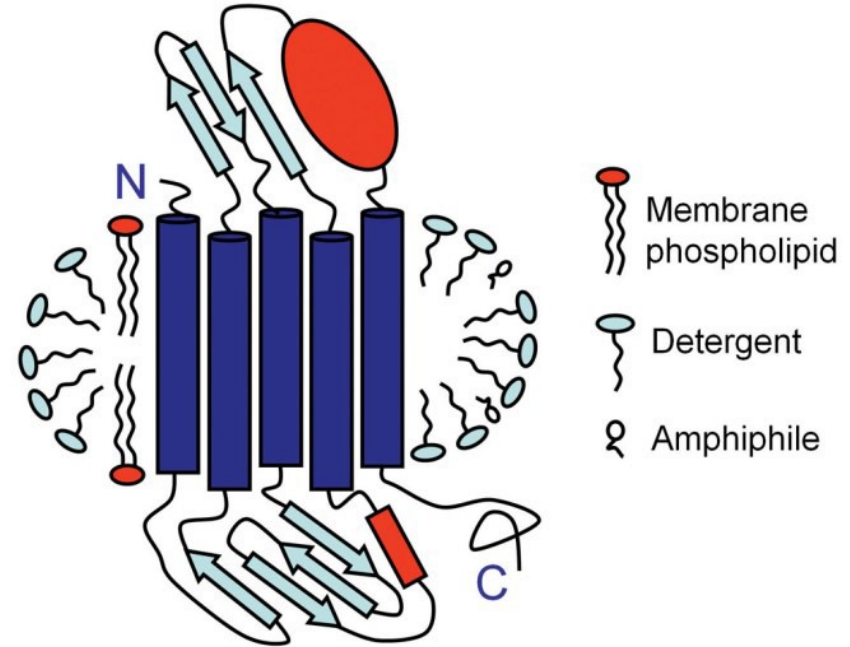
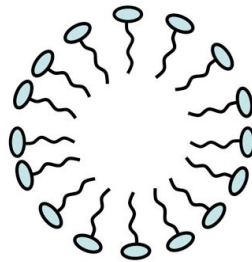
Nature 427, 36–44 (2004) | [Download Citation](#) 

# Produzione di proteine ricombinanti di membrana per studi strutturali:

## Solubilizzazione :



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FOSPOLIPIDI

DETERGENTI

MOLECOLE ANFIFILICHE

LIPOSOMI (da detergenti naturali o artificiali)

MICELLE (si formano superata la **CMC** –critical micelle concentration-)

NANODISCHI (mediate scaffold membrane proteins)

Protocolli specifici di screening nella fase di

**Solubilizzazione**

**Stabilità**

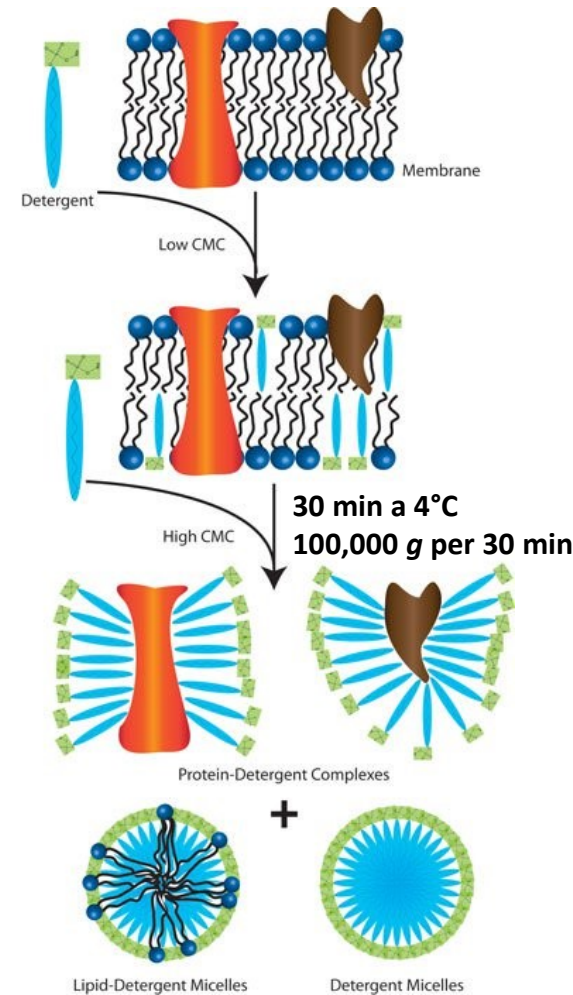
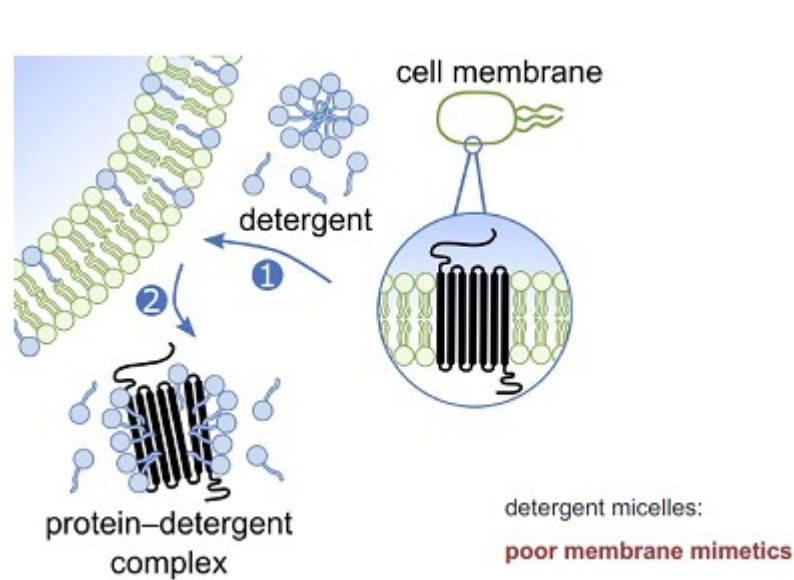
**Cristallizzazione**

NB: Ristretto subset di detergenti più efficaci (n-Dodecyl- $\beta$ -D-Maltoside/fos colina)

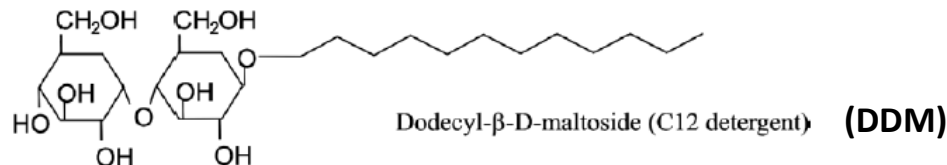
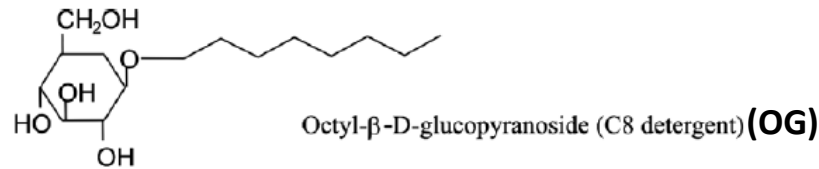
# Produzione di proteine ricombinanti di membrana per studi strutturali:

## Estrazione & solubilizzazione:

DETERGENTI formano MICELLE (superata la CMC che dipende da T, forza ionica)



## Screening: Trial and error

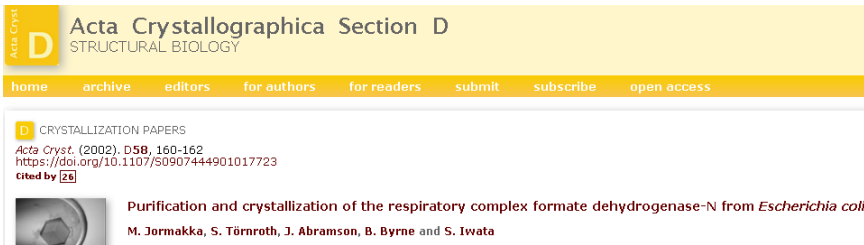




# Produzione di proteine ricombinanti di membrana per studi strutturali:

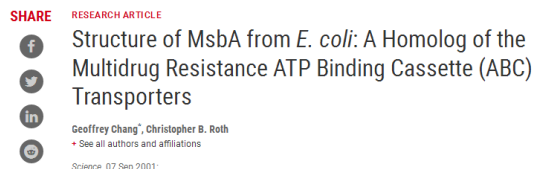
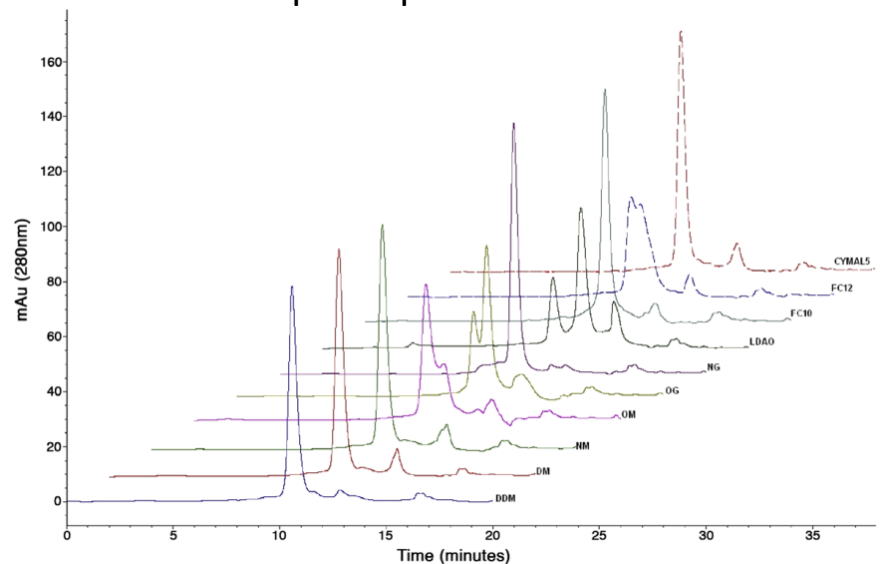
## Purificazione:

### A) Fonti naturali: strategie specifiche di purificazione



1. prefrazionamento per eliminare DNA, RNA o altre proteine non correlate
2. Refinement per migliorare la risoluzione.

### B) Proteine ricombinanti: IMAC + SEC (metodo predittivo informativo per la probabilità di cristallizzazione)

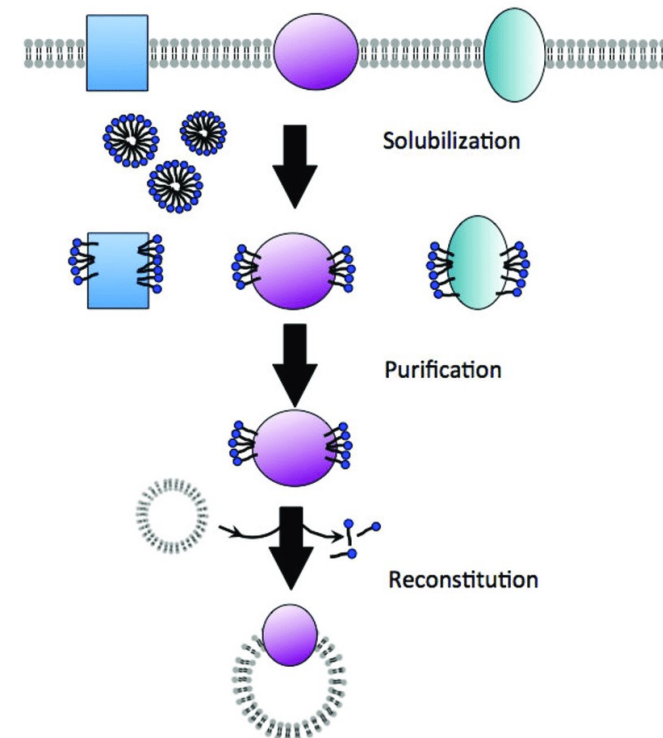
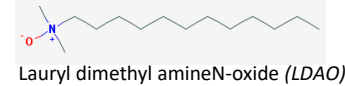


# Produzione di proteine ricombinanti di membrana per studi funzionali:

## Ricostituzione

Summary of different reconstitution methods for membrane proteins

Reconstitution method	Strengths	Weaknesses	Examples
<i>Detergent micelles</i>			
Ionic detergents, e.g. SDS	Excellent at solubilizing membrane proteins	Generally denaturing to some extent	bR, LCHIL, DAGK, Ca <sup>2+</sup> -ATPase
Bile acid salts	Mild and not generally deactivating.		Ca <sup>2+</sup> -ATPase
Nonionic detergents, OG, DM, DDM	Mild and non-denaturing	Short chain can be deactivating	Ca <sup>2+</sup> -ATPase,
Zwitterionic detergents	Uses in structural studies	Generally more deactivating than nonionic detergents	<i>Rhodospseudomonas sphaeroides</i> reaction centre, rhodopsin
Tripod amphiphiles	Shown to be successful at solubilizing certain proteins, removes need for traditional detergents	Limited use so far	Rhodopsin, bR
Amphipols	No micelles formed therefore less problems with viscosity or phase separation, removes need for traditional detergents	Limited use so far	Bacteriorhodopsin, reaction centre, OmpF, cytochrome <i>b<sub>6</sub>f</i>
<i>Lipid-detergent micelles</i>			
Detergent solubilization	Facile incorporation of protein into the bilayer,	Protein must be stable in detergent, detergent removal must be chosen carefully to suit specific properties of detergent.	bR, LmrP, LacS,
Dilution	Good for detergents with high cmc's	Leaky proteosomes, inhomogeneity in protein distribution.	OmpF
Organic solvent mediated	Preparation of liposomes with a large internal volume	Exposure to organic solvent can denature membrane proteins, resulting liposomes often fragile	Rhodopsin, cytochrome <i>c</i> oxidase, acetyl choline receptor, bR
Sonication	Freeze thawing improves liposome quality—good technique for proteins that cannot withstand detergent.	Small liposomes—often deactivated protein	D-glucose carrier from red blood cells
<i>Bicelles</i>	May be a better mimic of the lipid bilayer. Useful in structural studies such as NMR	Limited application so far	DAGK



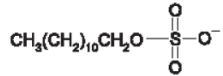
# Produzione di proteine ricombinanti di membrana per studi strutturali:

## Cristallizzazione:

### TIPI di DETERGENTI:

#### a. Ionic detergents

Sodium dodecyl sulfate (SDS)

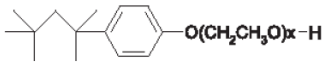


#### c. Non-ionic Detergents

R = glucose, x = 7, n-octyl-β-D-glucopyranoside  
 R = maltose, x = 9, decyl-β-D-maltoside  
 x = 11, dodecyl-β-D-maltoside

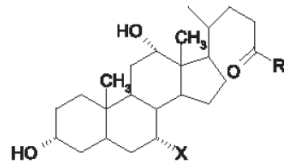


x = 9, Triton® X-100  
 x = 7-8, Triton® X-114



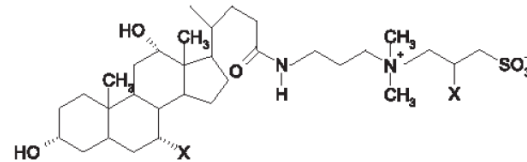
#### b. Bile Acid Salts

X=H, R = O-Na<sup>+</sup>, sodium deoxycholate  
 X=OH, R = O-Na<sup>+</sup>, sodium cholate

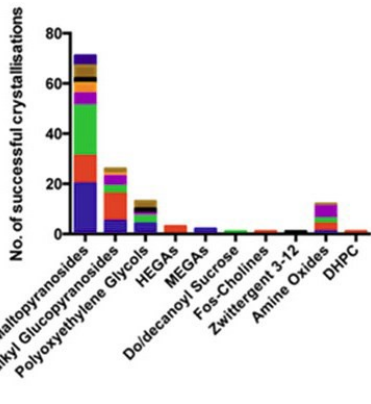
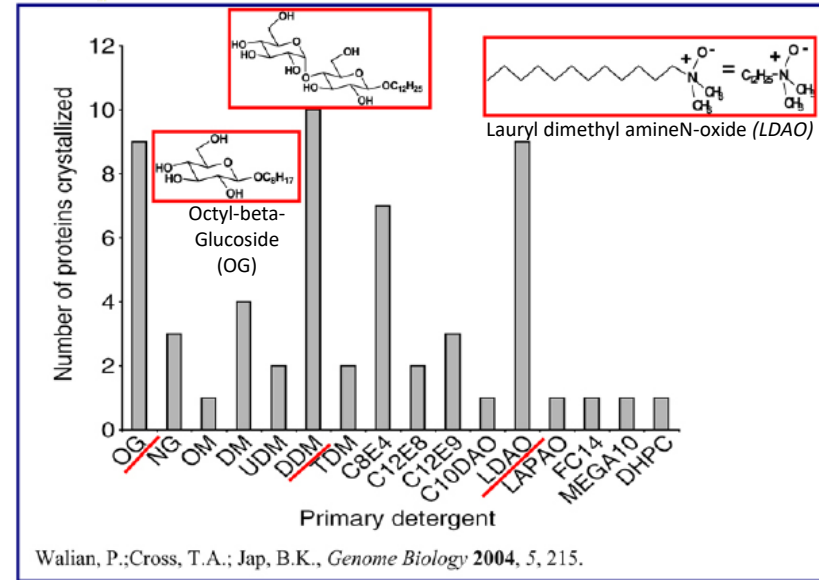
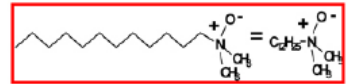
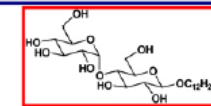


#### d. Zwitterionic Detergents

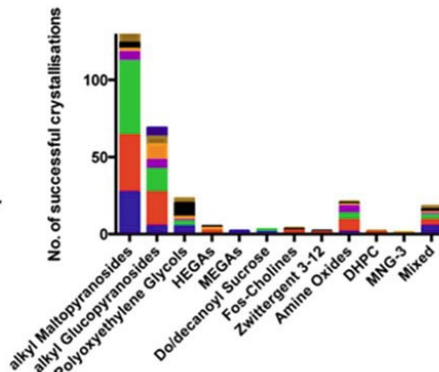
x = H, CHAPS  
 x = OH, CHAPSO



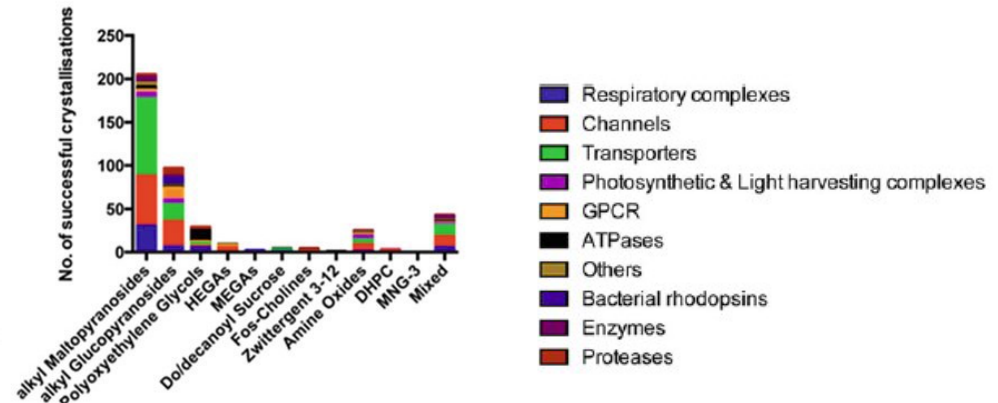
n-dodecyl-β-D-maltoside (DDM)



2008



2012

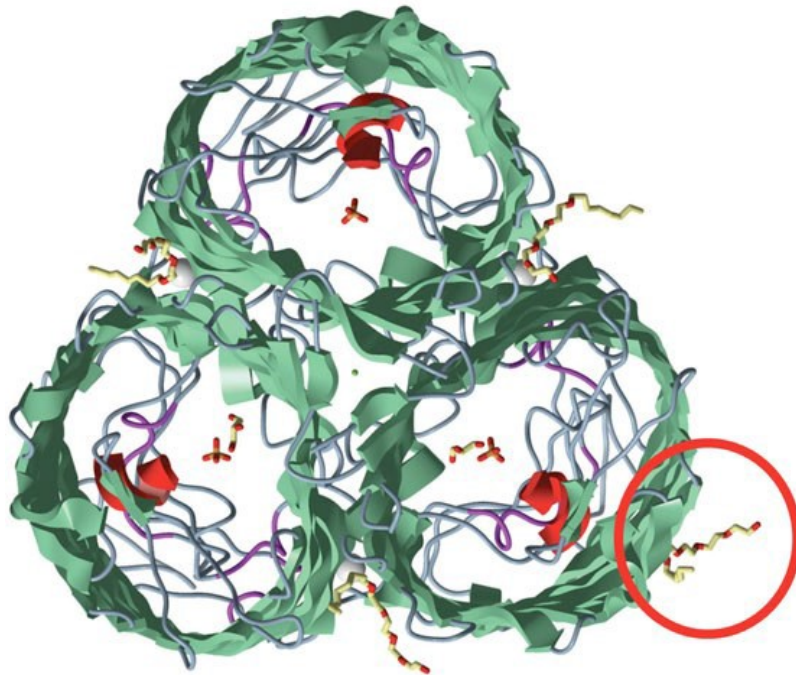


2015

Produzione di proteine ricombinanti di membrana per studi strutturali:

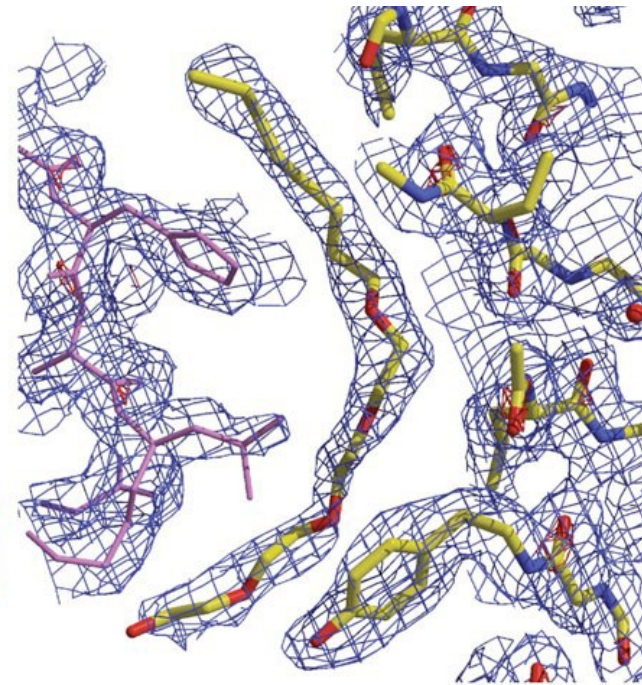
### Cristallizzazione:

Ruolo positivo dei DETERGENTI a catena corta



© Garland Science 2010

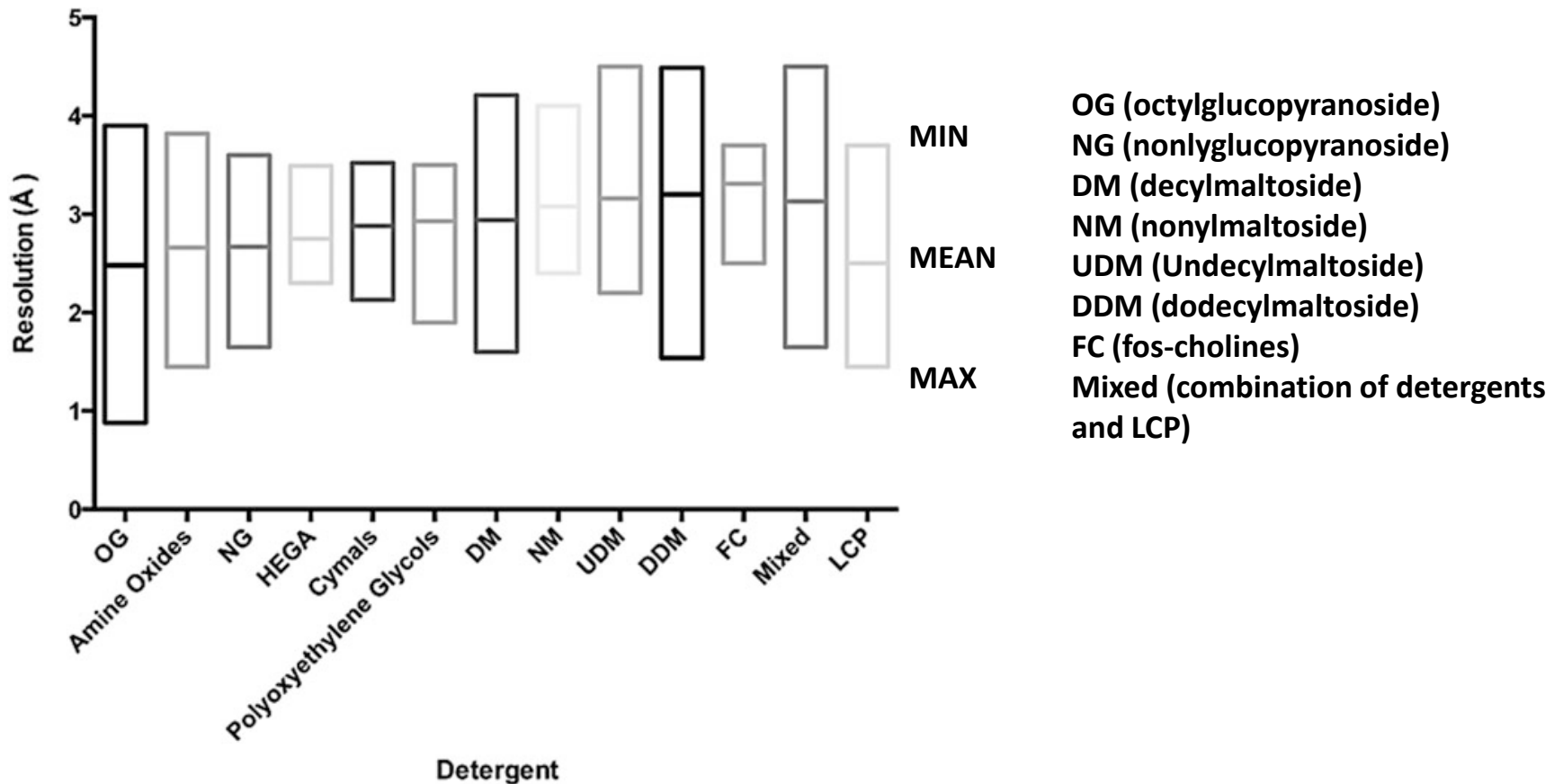
**Hydroxy-ethyl-oxy-tri(ethyloxy)-octane**



outer membrane protein OprP di *P. aeruginosa*

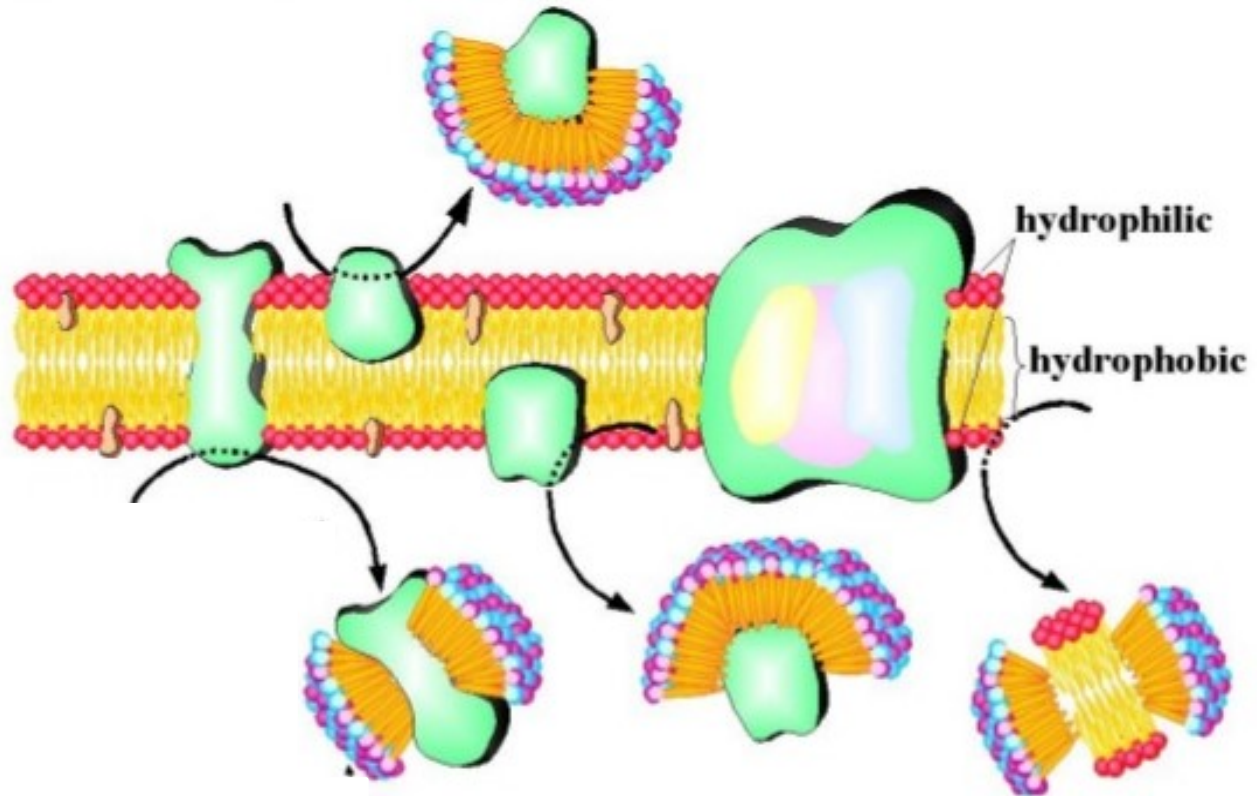
# Produzione di proteine ricombinanti di membrana per studi strutturali:

## Cristallizzazione:



Produzione di proteine ricombinanti di membrana per studi strutturali:

**CRITERI per un buon DETERGENTE:**



Non denaturanti

Stabilizzanti

Complessi piccoli

Impaccamento stretto

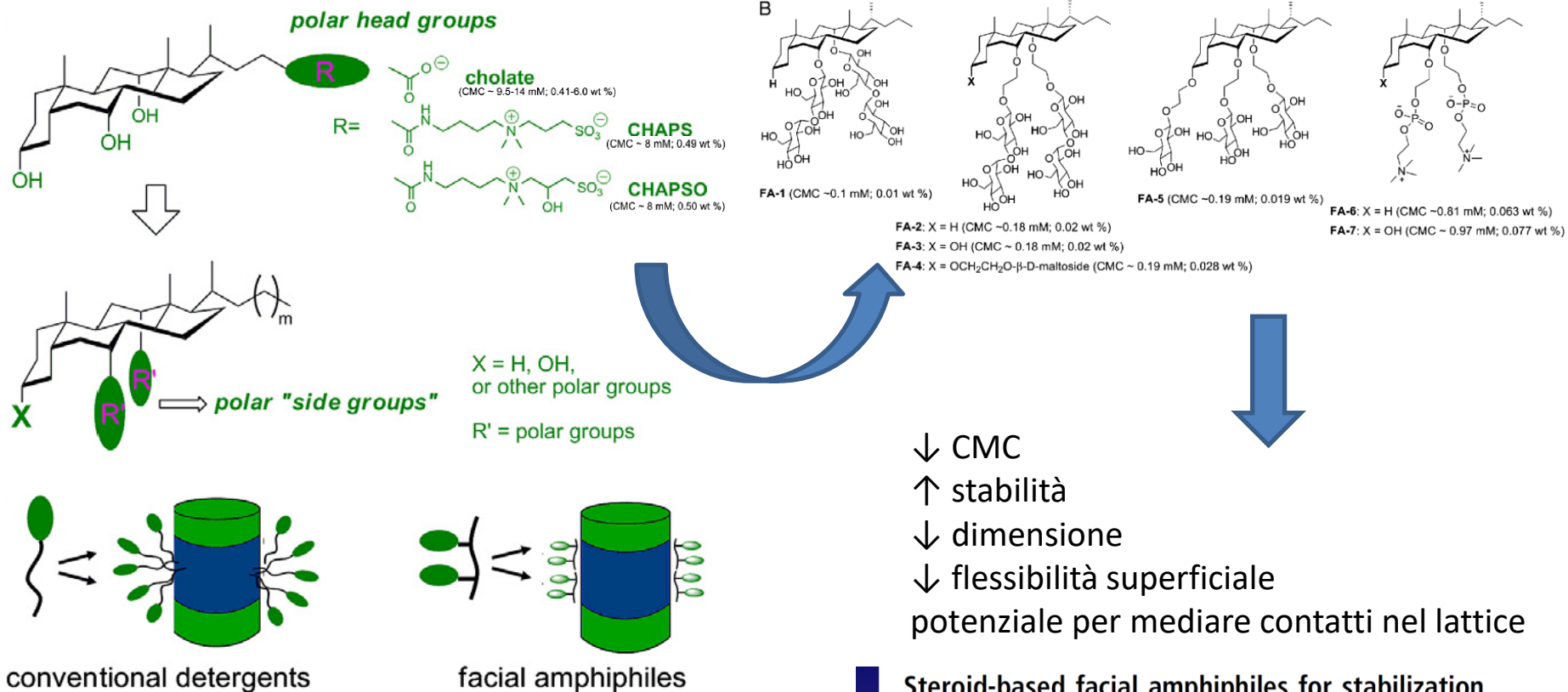
Grandi superfici esposte per contatti proteina/proteina

Simili alla struttura naturale delle membrane (?)

# Produzione di proteine ricombinanti di membrana per studi strutturali:

## DESIGN di un DETERGENTE:

A partire dai "facial amphiphiles": Più simili alla struttura naturale delle membrane



### Steroid-based facial amphiphiles for stabilization and crystallization of membrane proteins

Sung Chang Lee<sup>a</sup>, Brad C. Bennett<sup>b</sup>, Wen-Xu Hong<sup>a</sup>, Yu Fu<sup>a</sup>, Kent A. Baker<sup>c</sup>, Julien Marcoux<sup>d</sup>, Carol V. Robinson<sup>d</sup>, Andrew B. Ward<sup>e</sup>, James R. Halpert<sup>e</sup>, Raymond C. Stevens<sup>a</sup>, Charles David Stout<sup>a</sup>, Mark J. Yeager<sup>b,c</sup>, and Qinghai Zhang<sup>a,1</sup>

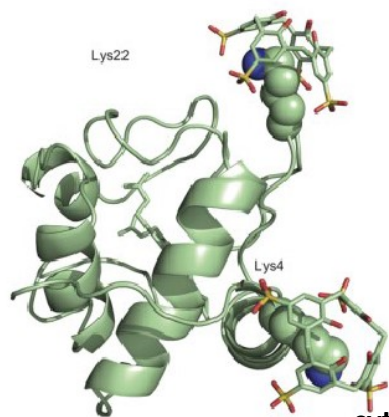
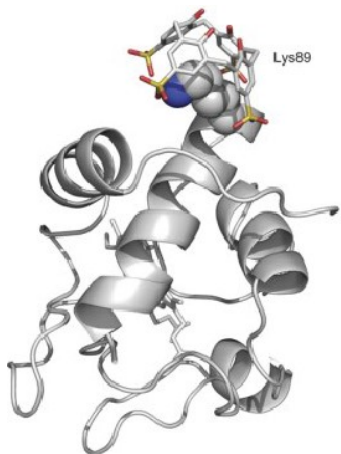
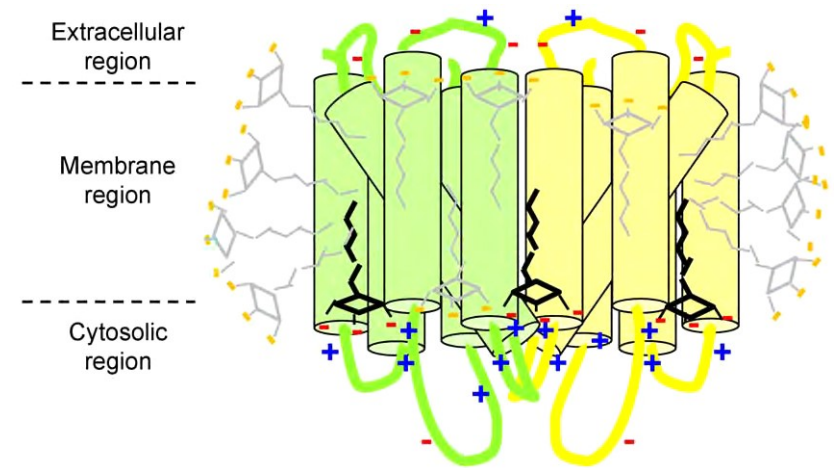
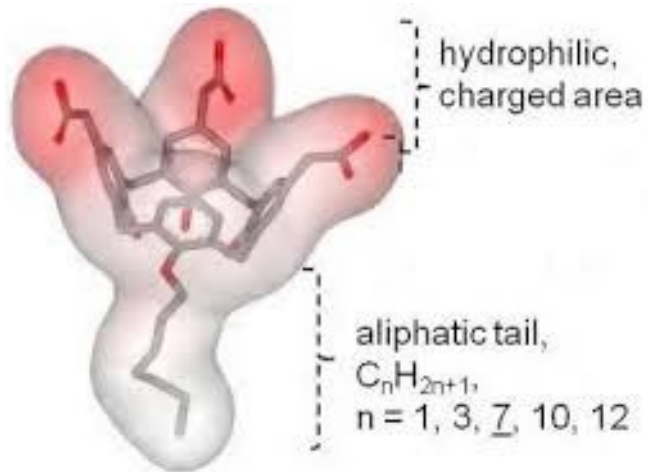
Departments of <sup>a</sup>Molecular Biology and <sup>c</sup>Cell Biology, The Scripps Research Institute, La Jolla, CA 92037; <sup>b</sup>Department of Molecular Physiology and Biological Physics, University of Virginia School of Medicine, Charlottesville, VA 22908; <sup>d</sup>Department of Chemistry, University of Oxford, Oxford OX1 3QZ, United Kingdom; and <sup>e</sup>Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego, La Jolla, CA 92093

Edited\* by H. Ronald Kaback, University of California, Los Angeles, CA, and approved February 13, 2013, 2013 (received for review December 17, 2012)

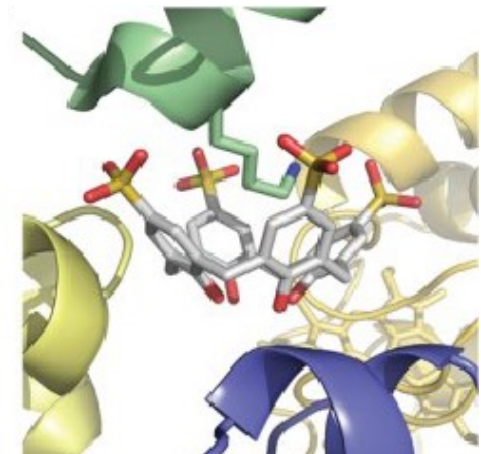
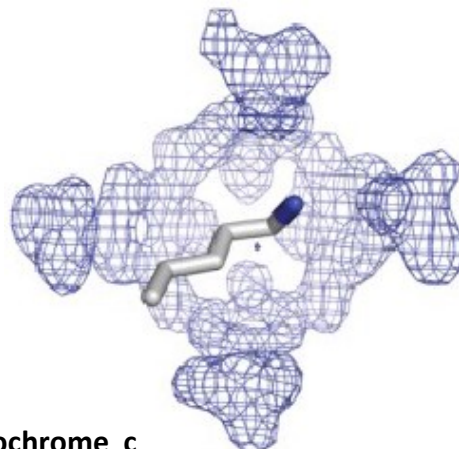
Produzione di proteine ricombinanti di membrana per studi strutturali:

## DETERGENTE VERSATILE:

## CALIXARENI



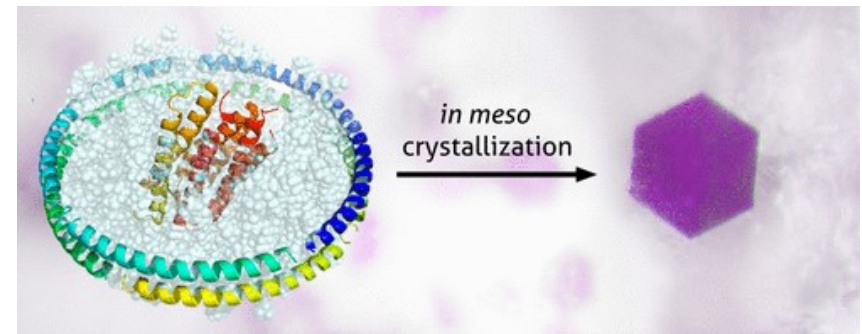
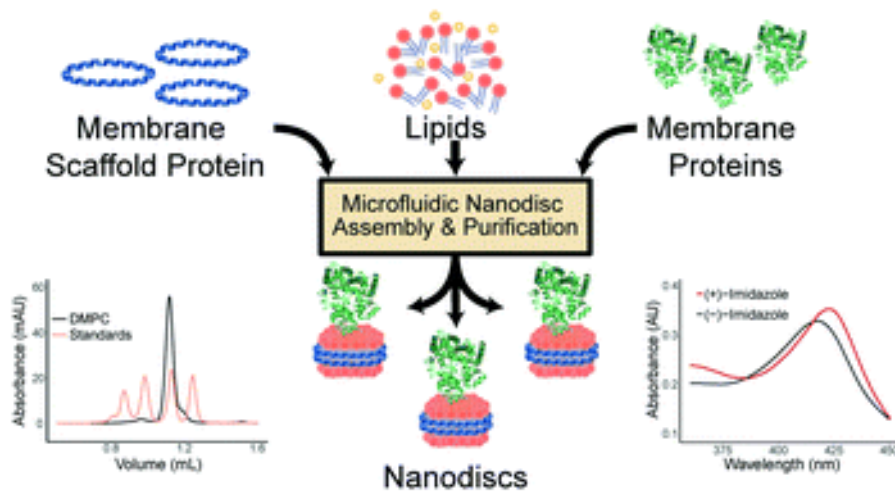
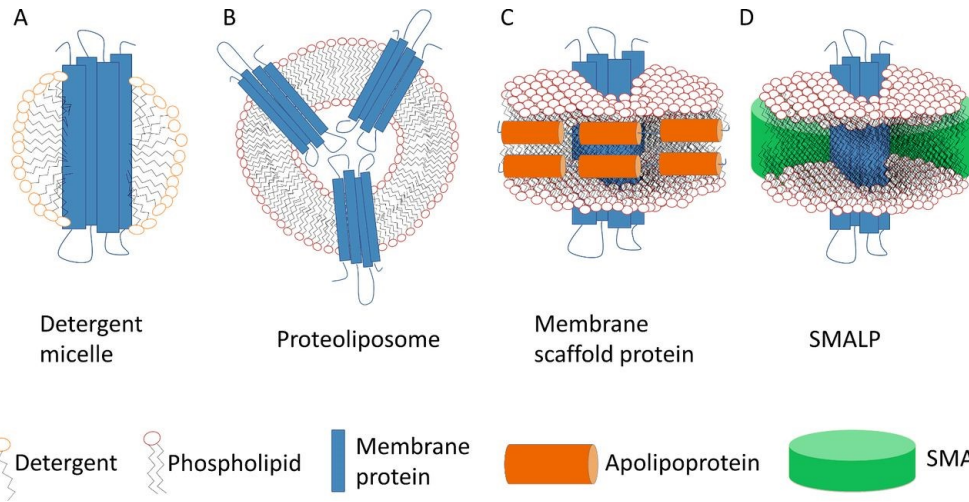
cytochrome c





# Produzione di proteine ricombinanti di membrana per studi strutturali:

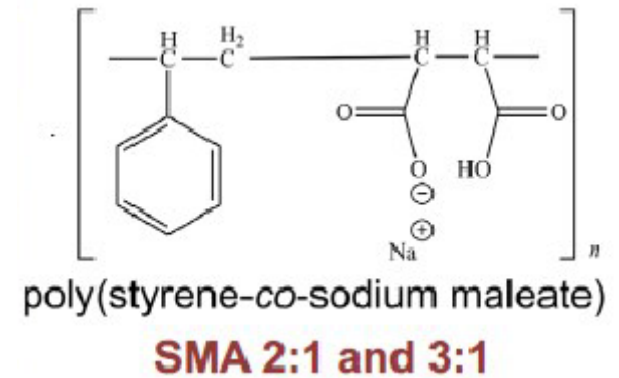
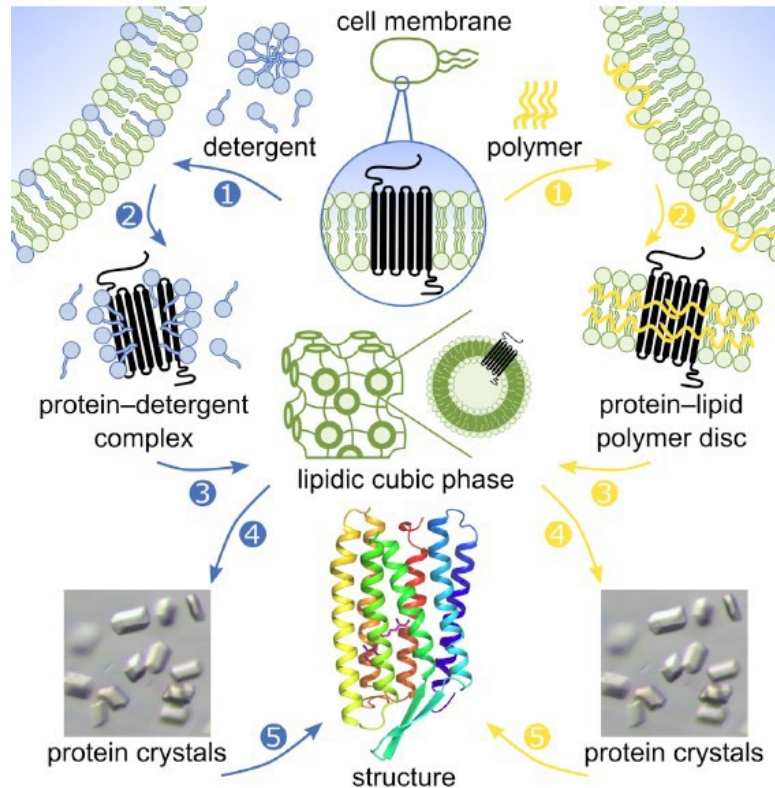
## NANODISCHI:



# Studi strutturali di proteine di membrana: metodi di cristallizzazione in meso

## NANODISCHI

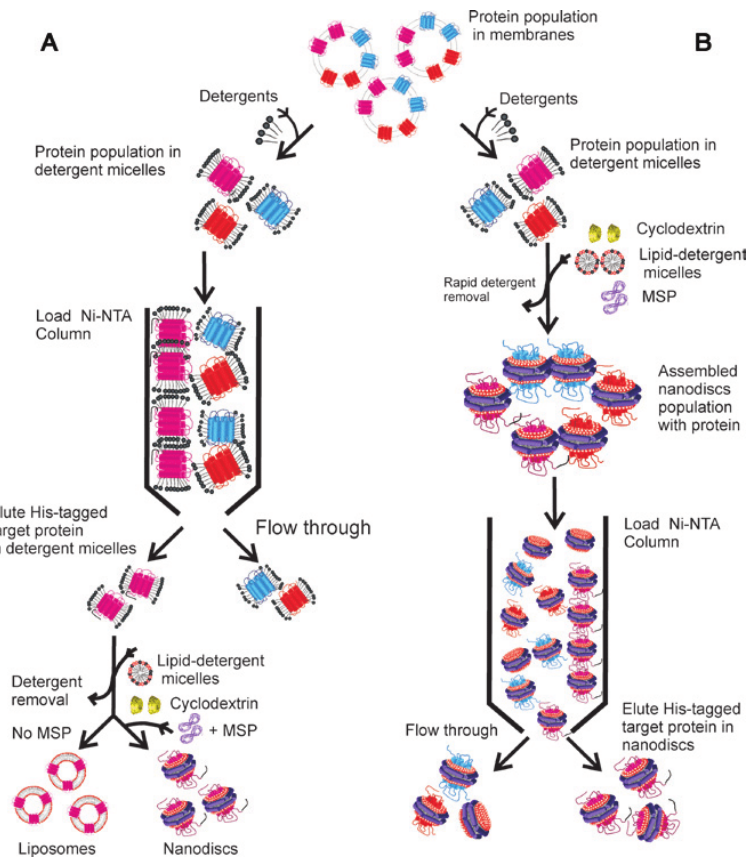
Formati dall'estrazione delle proteine di membrana mediante copolimeri di acido maleico e stirene.



Membrane native e termostabili di lipidi nativi (senza detergenti) per la cristallizzazione in meso.

# Produzione di proteine ricombinanti di membrana per studi strutturali:

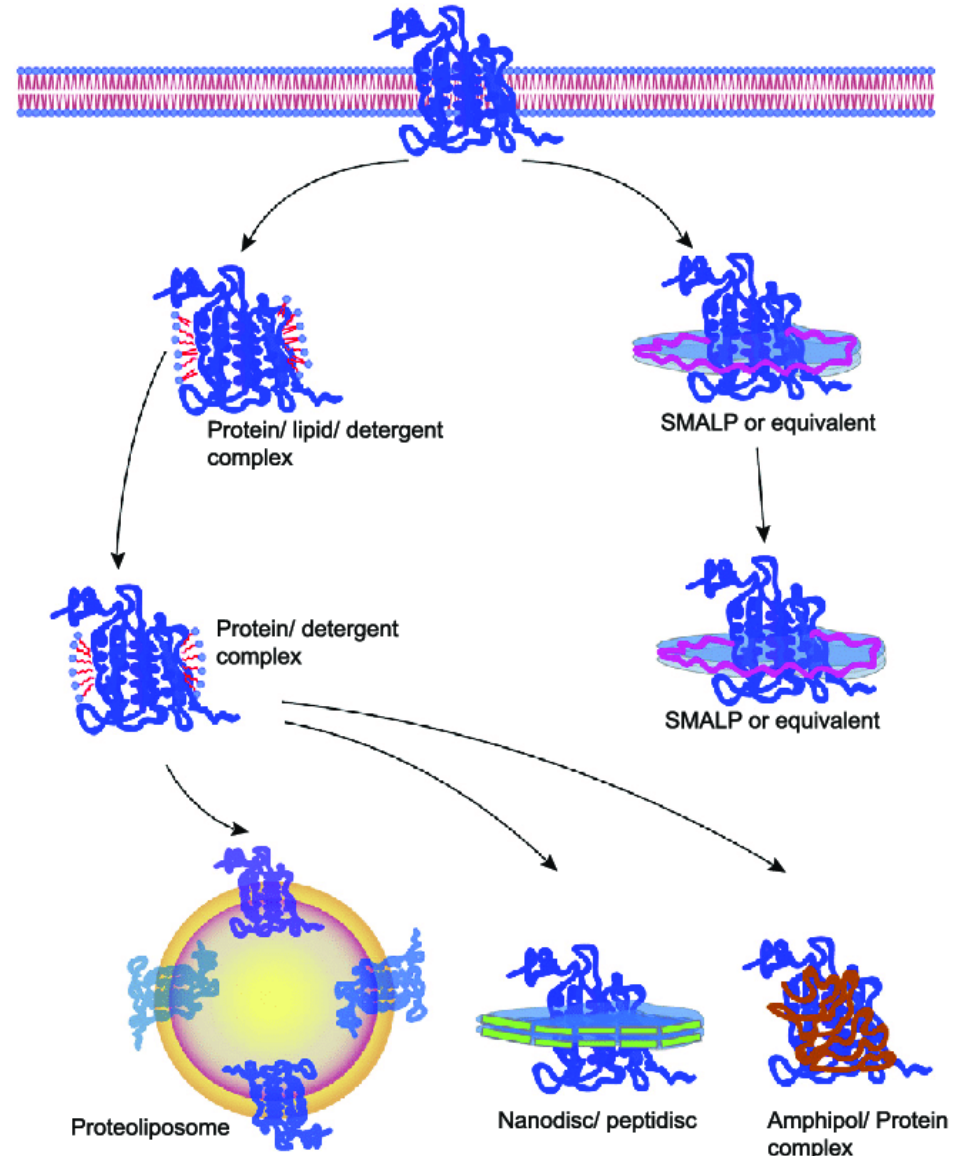
## NANODISCHI:



Solubilization

Purification

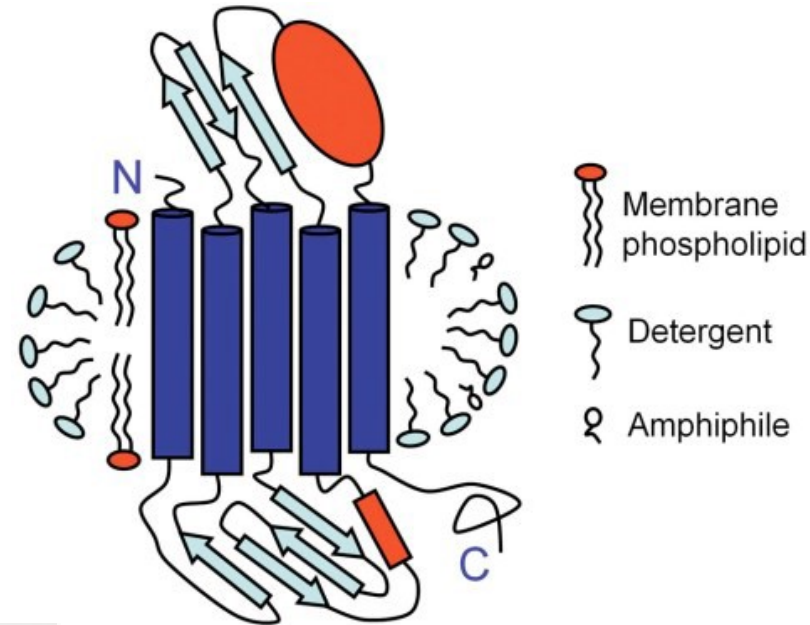
Reconstitution



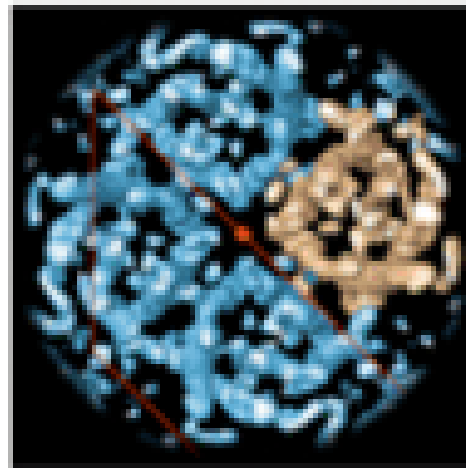
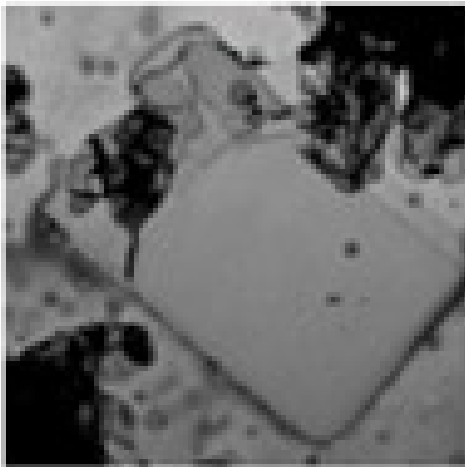
# Studi strutturali di proteine di membrana: tipi di cristalli

## 1) Cristalli 2D:

biomembrane ricostituite formate da interazioni idrofobiche



## 2D crystal



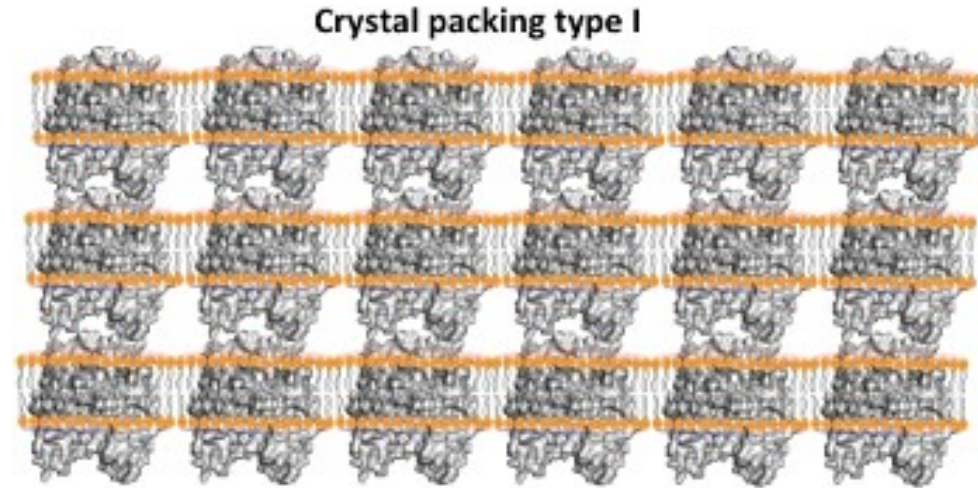
nd Science 2010

**EM**

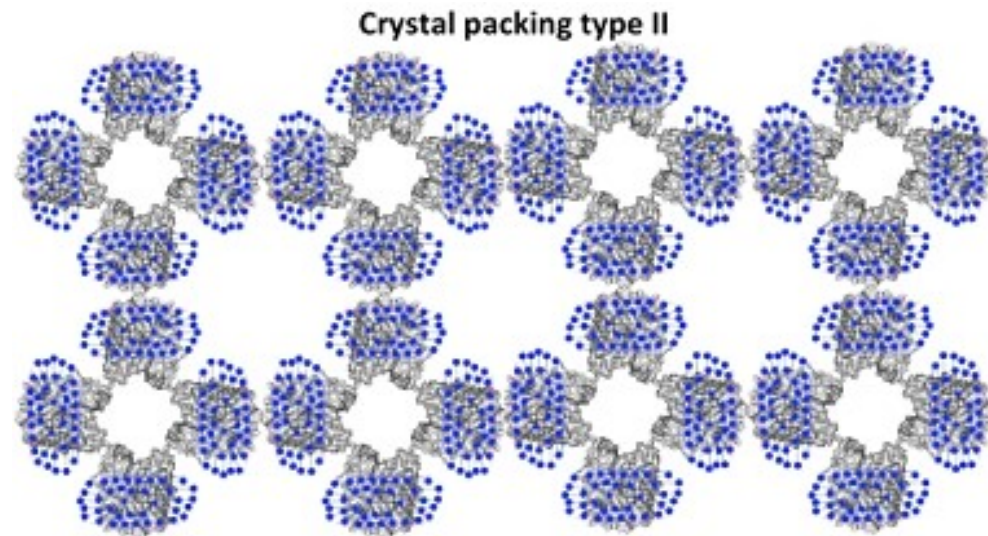
# Studi strutturali di proteine di membrana: tipi di cristalli

## 2) Cristalli 3D:

a) di tipo I (da metodi in meso): **fogli planari**



b) di tipo II (da metodi in surfo): il lattice dipende dalle interazioni tra le regioni idrofile.



# Studi strutturali di proteine di membrana: metodi di cristallizzazione in surfo

## Antibody fragment crystallization

- ↑ solubilizzazione
- ↑ stabilità
- ↑ superficie idrofilica
- ↑ contatti del reticolo

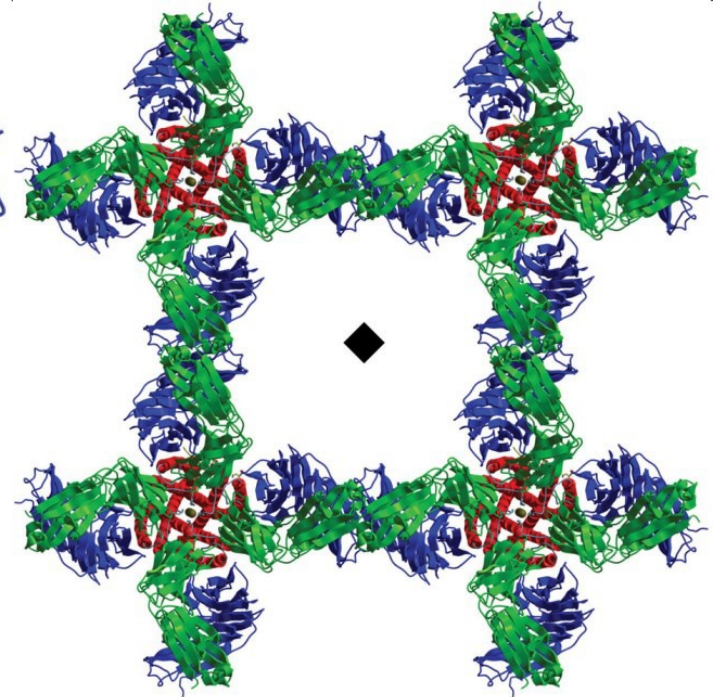
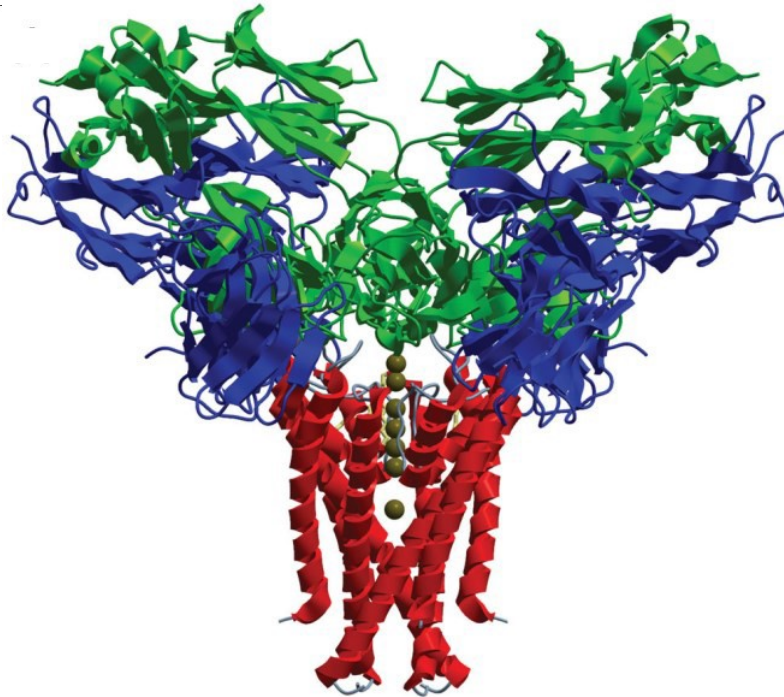
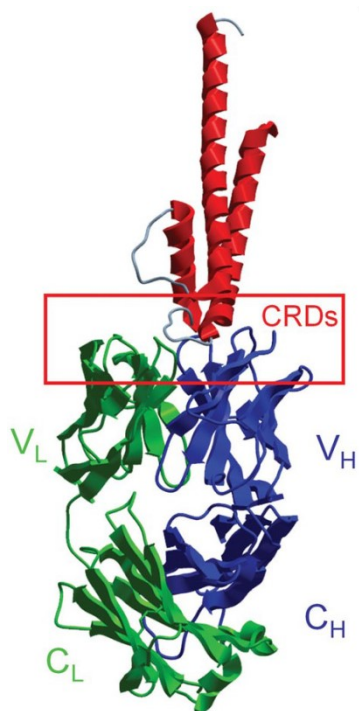
**nature**  
International journal of science

Article | Published: 01 November 2001

### Chemistry of ion coordination and hydration revealed by a $K^+$ channel–Fab complex at 2.0 Å resolution

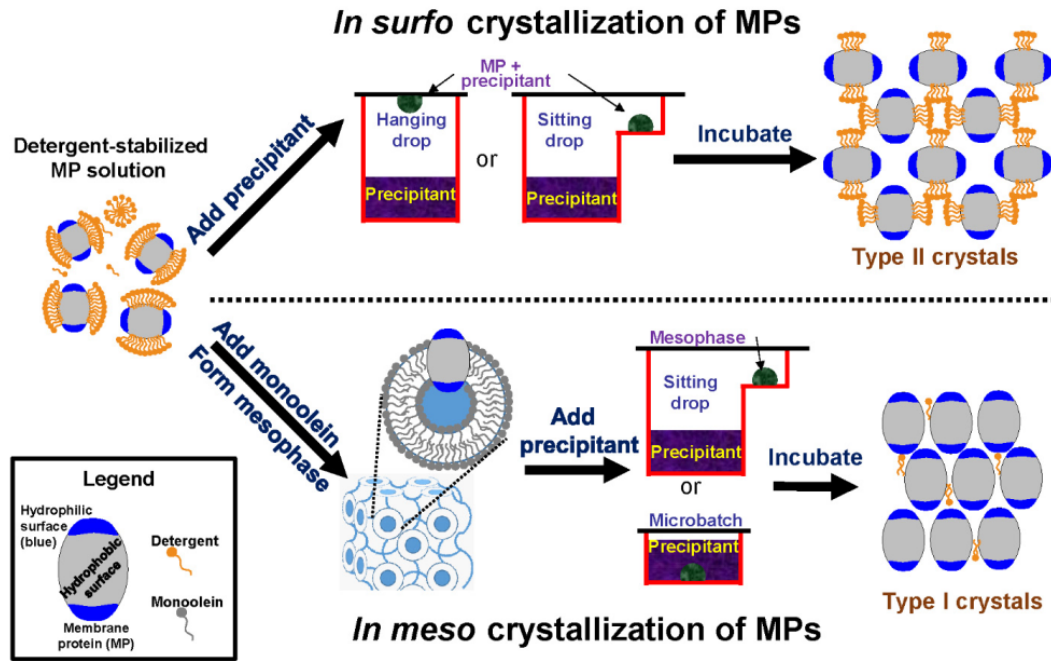
Yufeng Zhou, João H. Morais-Cabral, Amelia Kaufman & Roderick MacKinnon

*Nature* **414**, 43–48 (2001) | [Download Citation](#) 



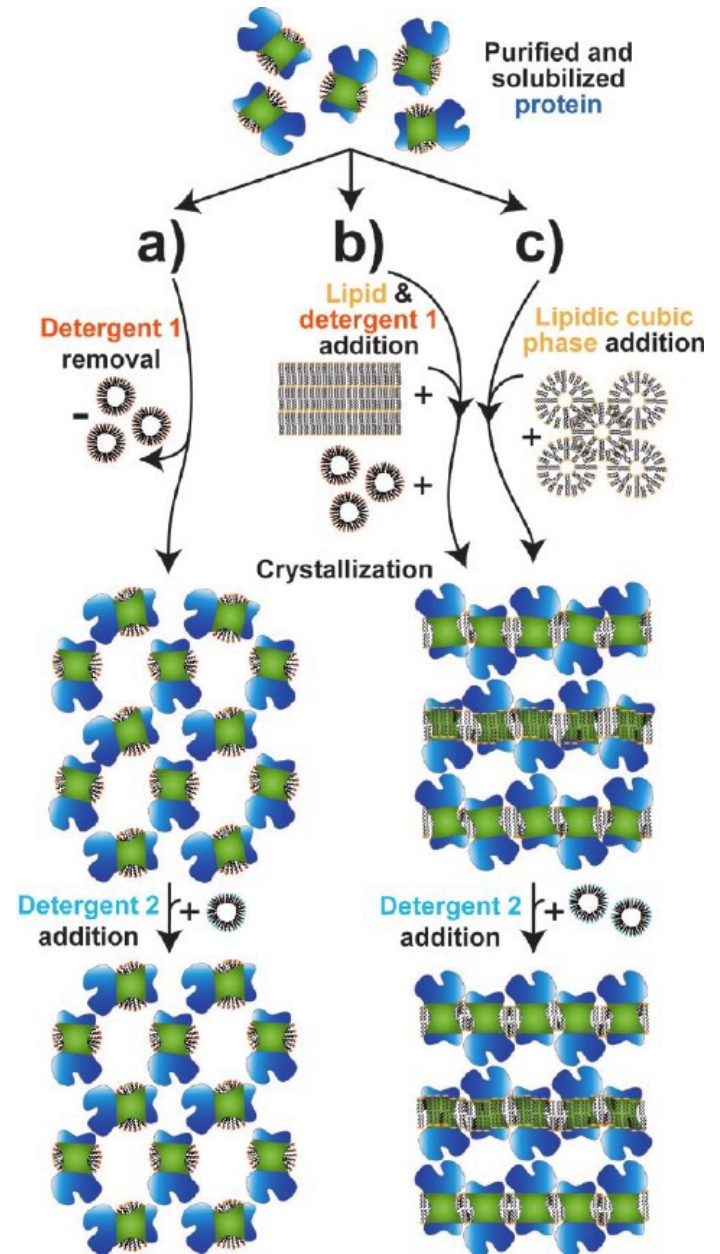
# Studi strutturali di proteine di membrana: metodi di cristallizzazione

## 1) cristallizzazione tradizionale della proteina delipidata

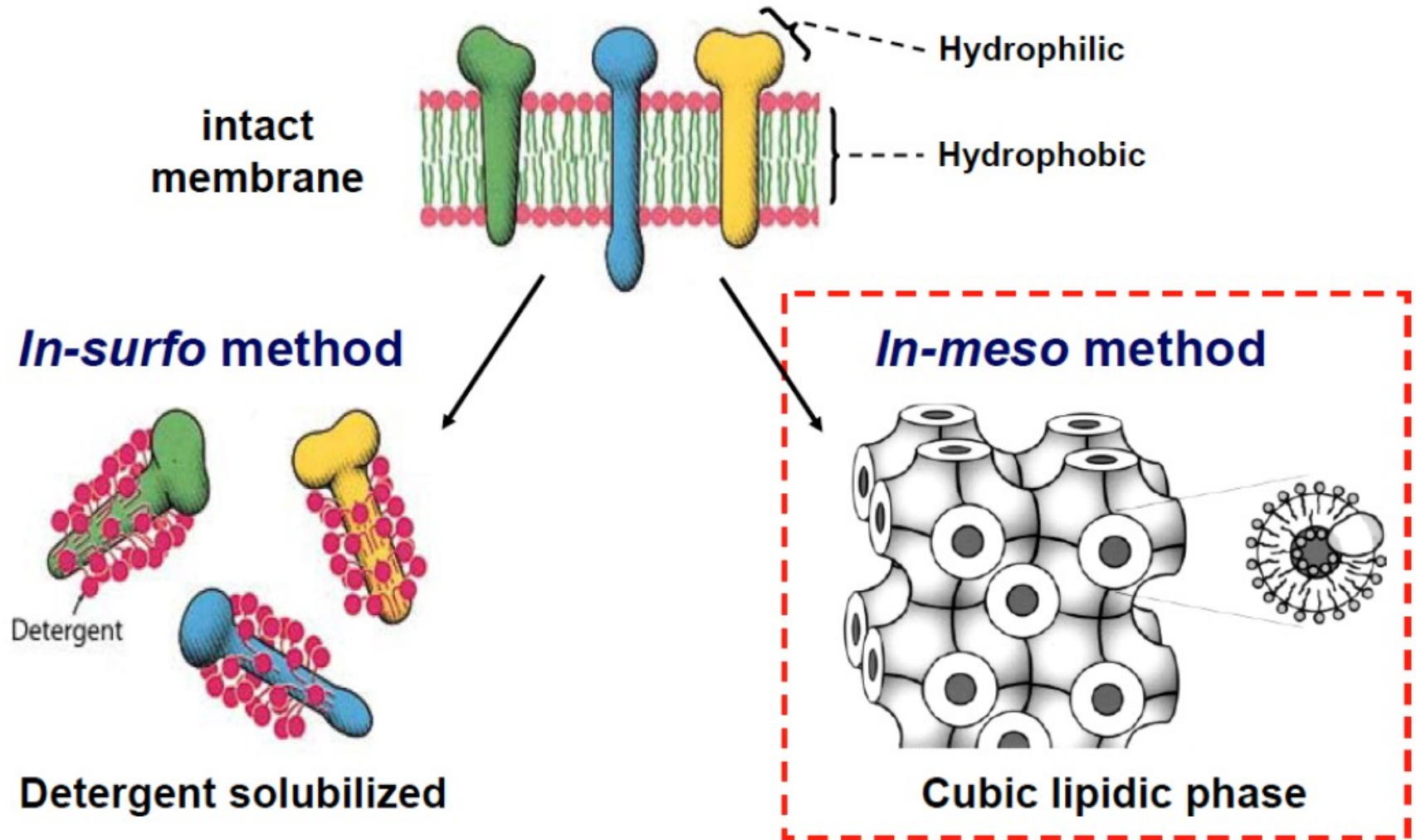


## 2) cristallizzazione della proteina relipidata

## 3) lipid cubic phase crystallization

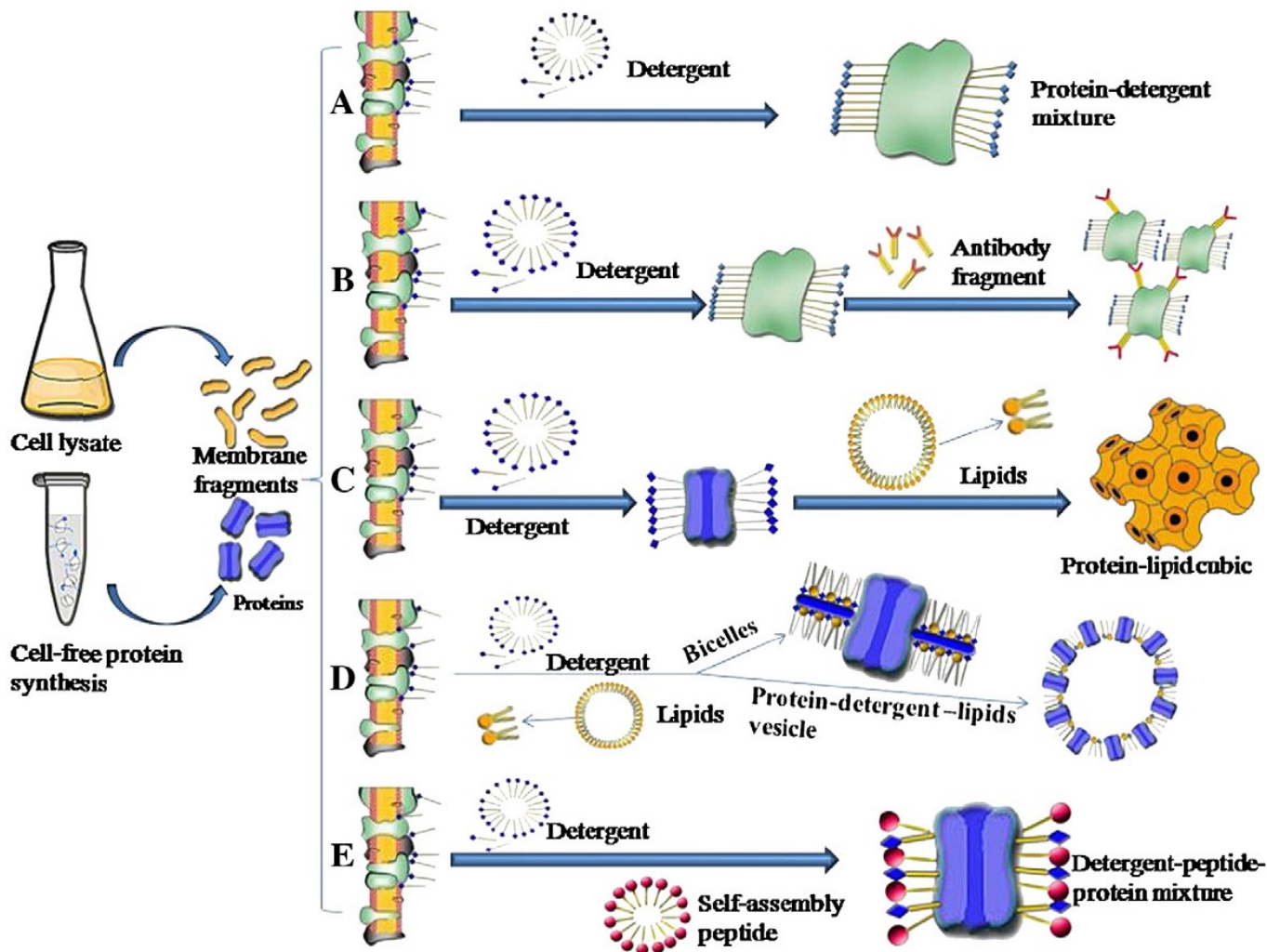


# Studi strutturali di proteine di membrana: metodi di cristallizzazione

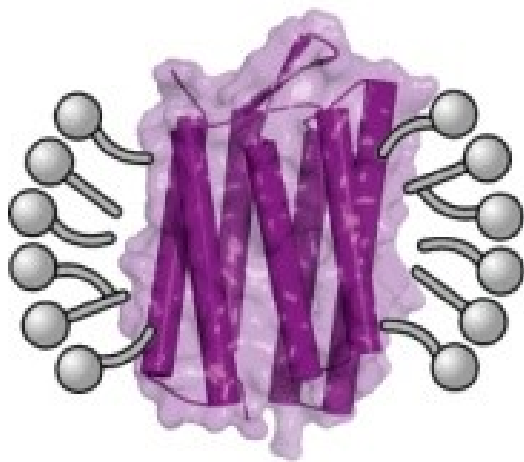




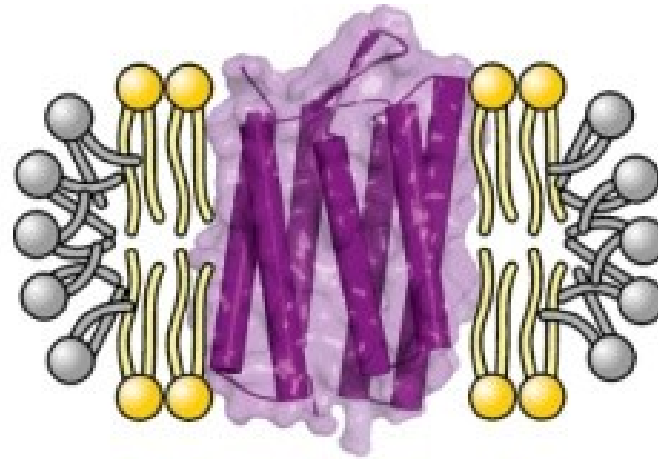
# Studi strutturali di proteine di membrana: metodi di cristallizzazione



Studi strutturali di proteine di membrana:  
metodi di cristallizzazione in meso



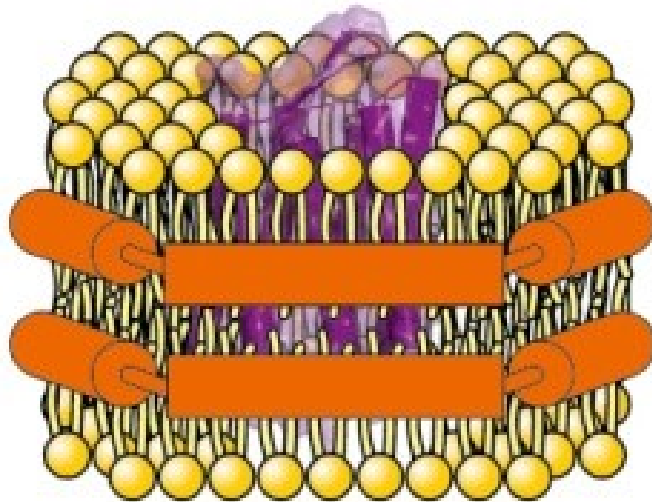
Micelle



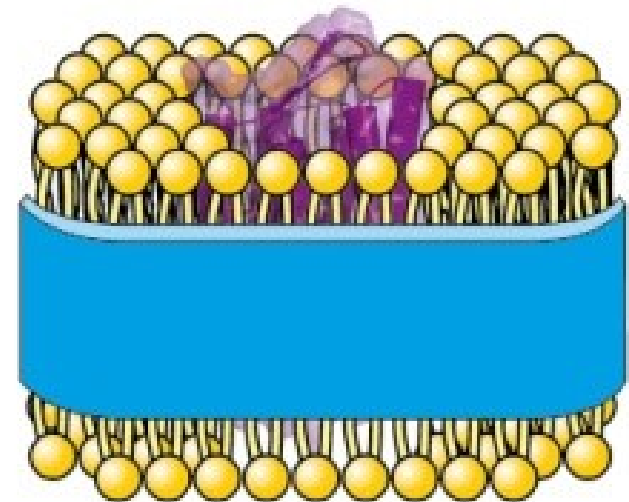
Bicelle



Amphipol



LPN



SMALP

# Studi strutturali di proteine di membrana: metodi di cristallizzazione in meso

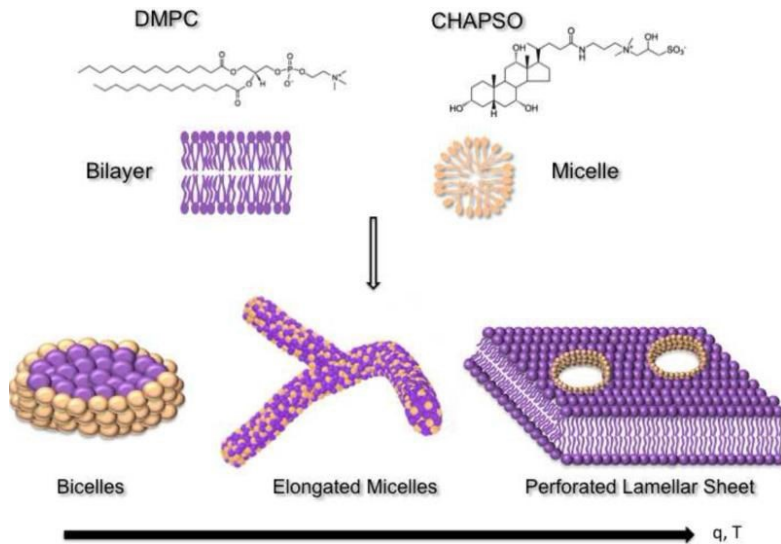
## Bicelles (bilayered mixed micelles)

piccoli dischi a doppio strato formati da fosfolipidi a catena lunga (12-18 C) che formano una regione planare  
fosfolipidi a catena corta (6-8 C)/detergenti che compongono i bordi laterali.

La dimensione dipende da lipidi / detergente

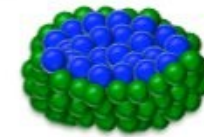
La carica superficiale può essere regolata sostituendo i lipidi neutri  
con fosfolipidi carichi negativamente.

Ambiente a doppio strato nativo, migliora la crescita dei cristalli di tipo I.



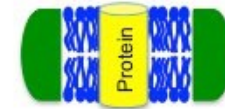
La viscosità delle miscele proteine / bicelle simile alla goccia con il detergente  
consente di utilizzare setup tradizionali

### Step 1: Preparation of Bicelles



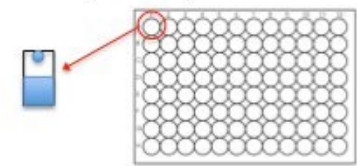
process of repeated  
vortex mixing,  
ultrasonic,  
heating and cooling

### Step 2: Incorporation of protein into bicelles



on ice incubation

### Step 3: Setup of crystallization trials



### Step 4: Visualization and Crystal Extraction

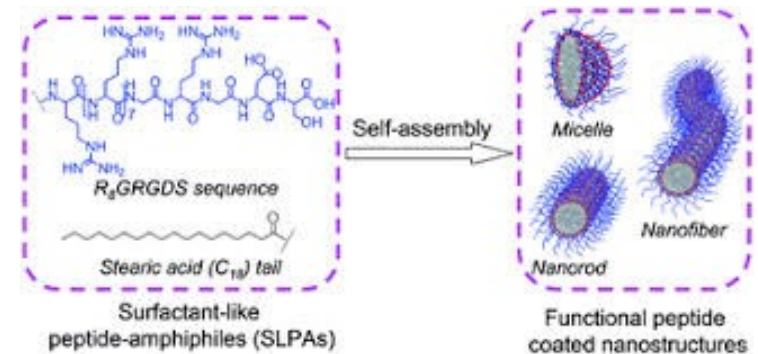


# Studi strutturali di proteine di membrana: metodi di cristallizzazione in meso

## Self-assembly peptide surfactants and lipid-like peptide surfactants

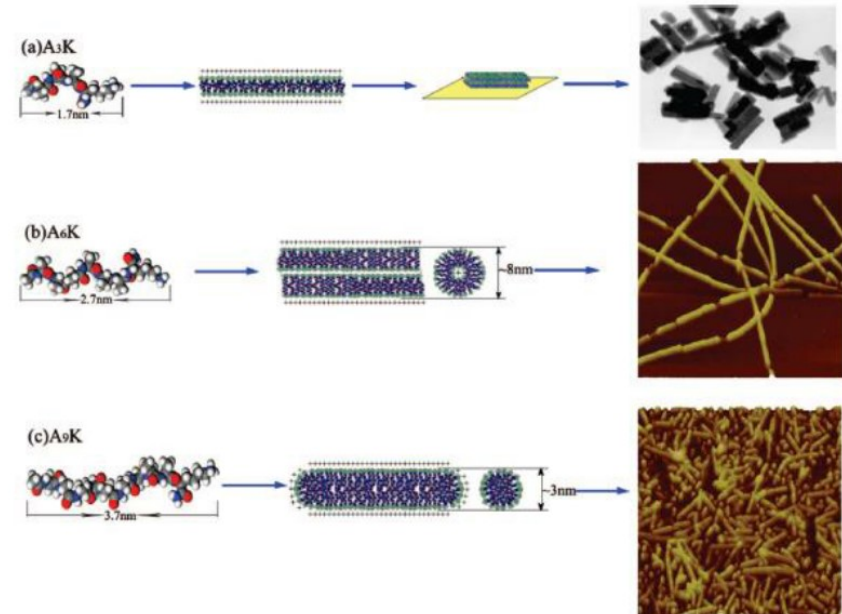
### Self-assembly peptide surfactants

testa polare (ripetizioni di aa)  
breve coda idrofobica

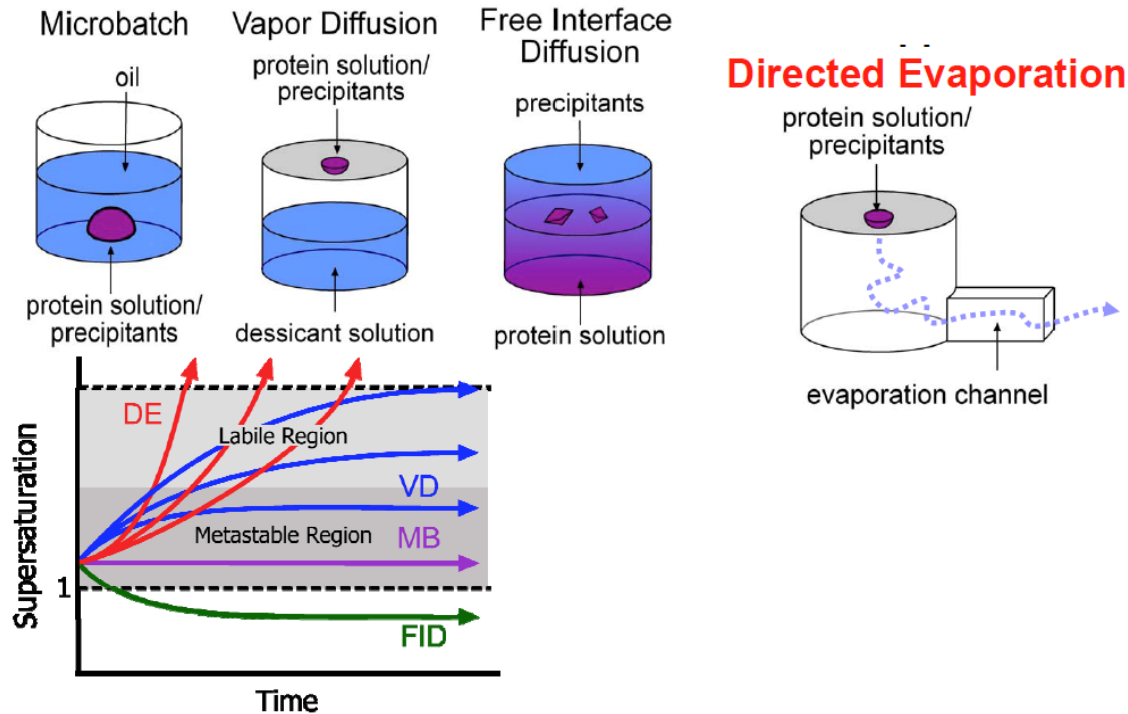


### Lipid-like peptide surfactants

code idrofobiche modificabili: aa con vari gradi di idrofobicità  
(glicina, alanina, isoleucina e leucina)  
testa polare: aa positivi (lisina e arginina) o negativi  
(acido aspartico glutammico)



# Studi strutturali di proteine di membrana: metodi di cristallizzazione in surfco



## 1) SCELTA del DETERGENTE

forma e dimensioni delle micelle:

Octyl / nonil- $\beta$ -d-maltoside (8 M e 9 M): micelle piccole

tridecyl- $\beta$ -d-maltoside (13 M): micelle grandi

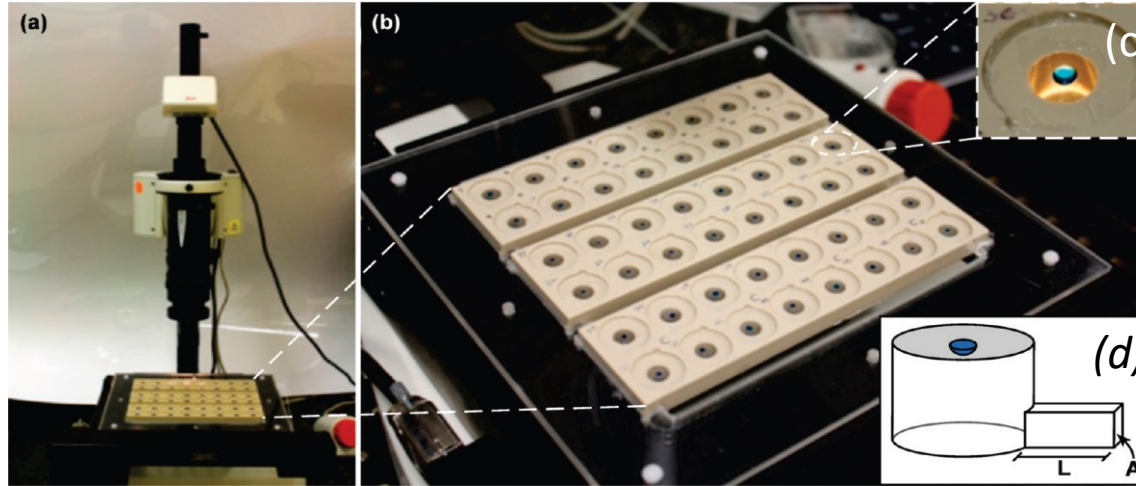
## 2) ADDITTIVI

piccoli anfifili (eptano-1,2,3-triolo e benzamidina): micelle piccole

metalli pesanti, sali e solventi organici

# Studi strutturali di proteine di membrana: metodi di cristallizzazione in surfco

**Directed evaporation:**  
preciso controllo del tasso di evaporazione:



Il tasso volumetrico di evaporazione dalla goccia  $J \sim \Delta P \left( \frac{A}{L} \right)$

$\Delta P$  = differenza di umidità o pressione fra la fase di vapore intorno alla goccia e l'ambiente

A = cross-sectional area

L = lunghezza

4432

*J. Phys. Chem. B* 2010, 114, 4432–4441

Determination of the Phase Diagram for Soluble and Membrane Proteins

Sameer Talreja, Sarah L. Perry, Sudipto Guha, Venkateswarlu Bhamidi, Charles F. Zukoski,\*  
and Paul J. A. Kenis\*

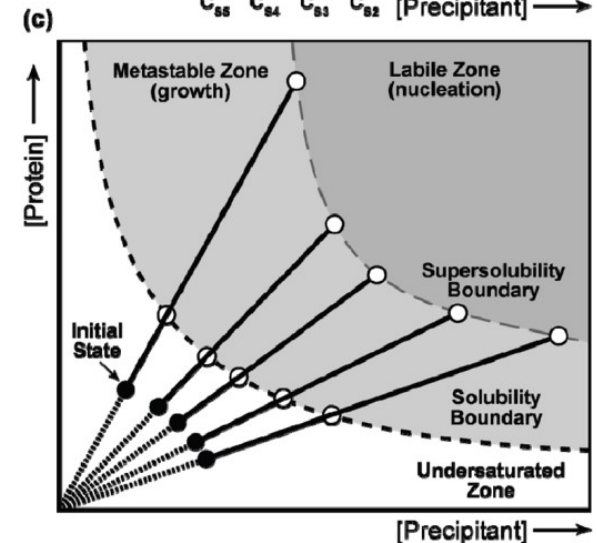
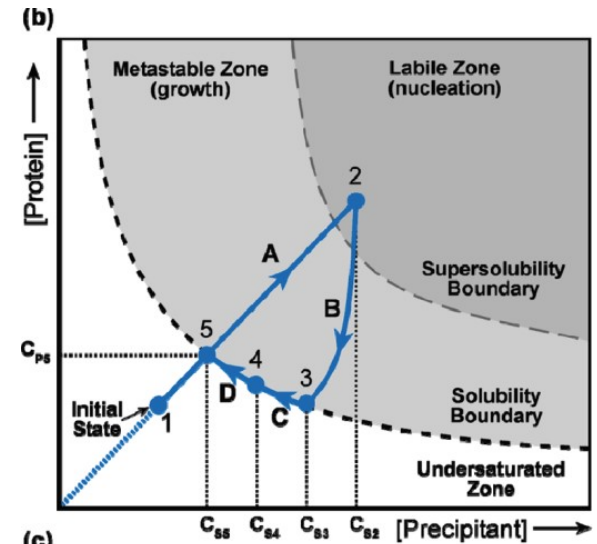
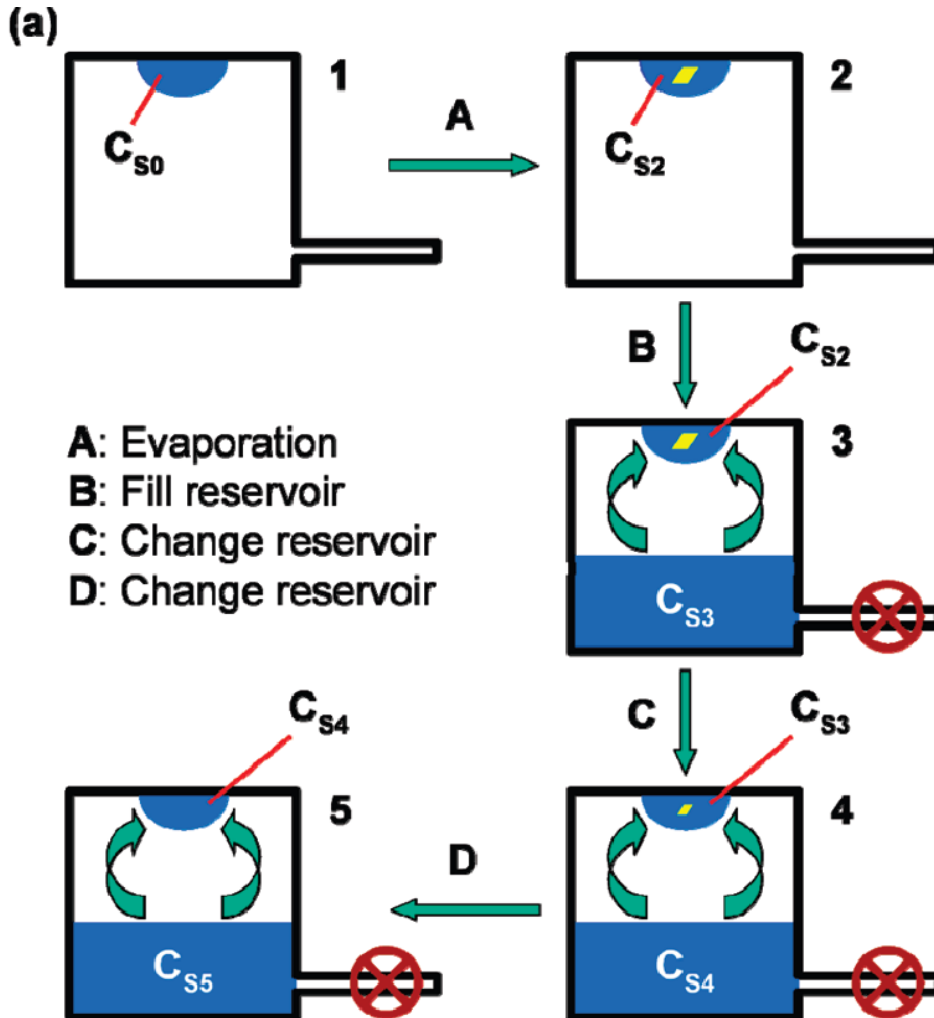
*Department of Chemical & Biomolecular Engineering, University of Illinois at Urbana–Champaign,  
Urbana, Illinois 61801*

*Received: December 13, 2009; Revised Manuscript Received: February 23, 2010*

# Studi strutturali di proteine di membrana: metodi di cristallizzazione in surfco

## Directed evaporation:

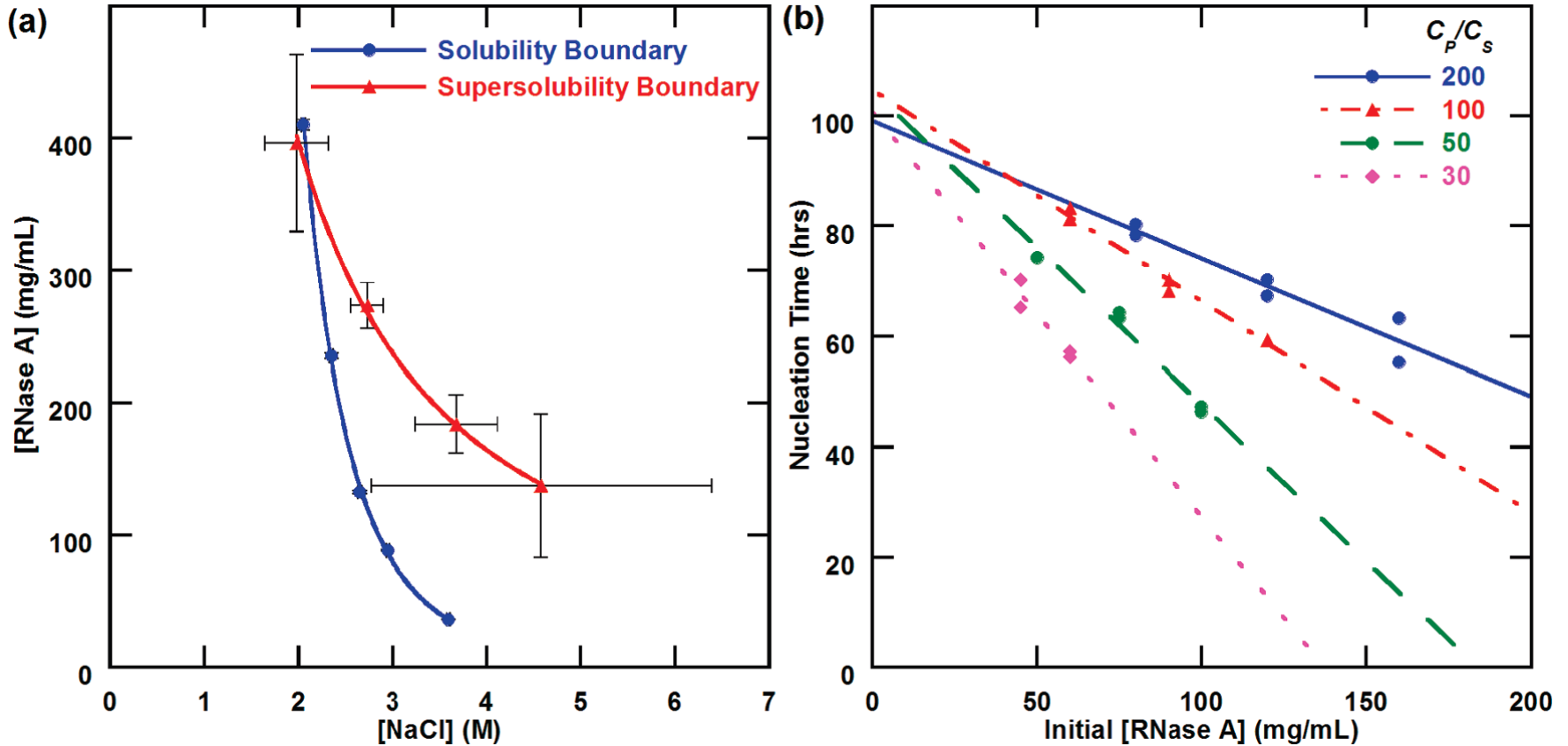
Metodo dinamico che consente di mappare il **limite di solubilità** del sistema proteina/precipitante mediante evaporazione (step A) seguita da un processo iterativo di reverse vapor diffusion (steps B-D).



# Studi strutturali di proteine di membrana: metodi di cristallizzazione in surfco

## Directed evaporation:

Esempio di limite di solubilità sperimentale del sistema RNaseA/NaCl.





# Studi strutturali di proteine di membrana: metodi di cristallizzazione in surfo

Efficacia del metodo di Directed evaporation:

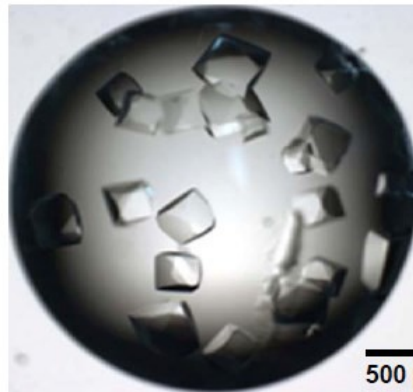
## RNase A

$$C_P/C_S = 10 \text{ mg}/(\text{mL}\cdot\text{M})$$

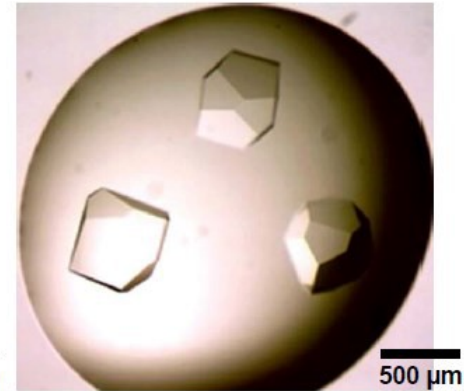
$$C_{P0} = 20 \text{ mg/mL}$$

$$C_{S0} = 2 \text{ M}$$

X-ray 1.12Å



Without Dilution

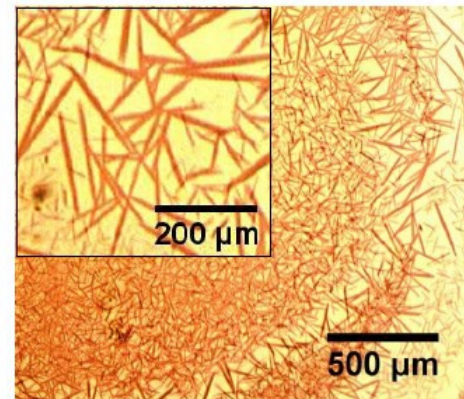
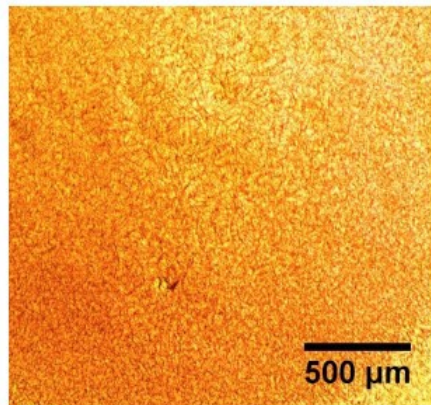


With Dilution

## Bacteriorhodopsin (a membrane protein)

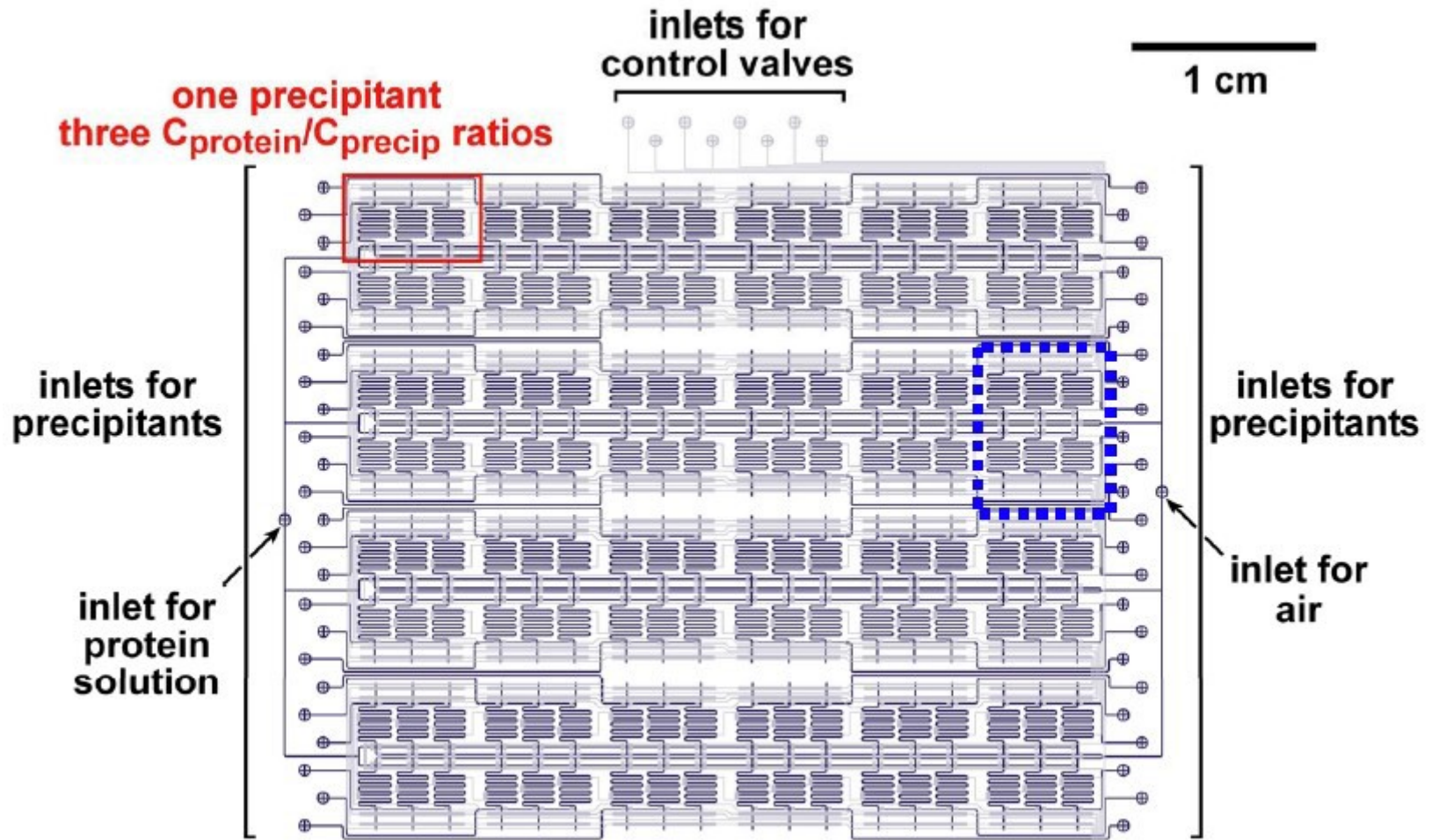
$$C_{P0} = 5 \text{ mg/mL}$$

X-ray 7Å



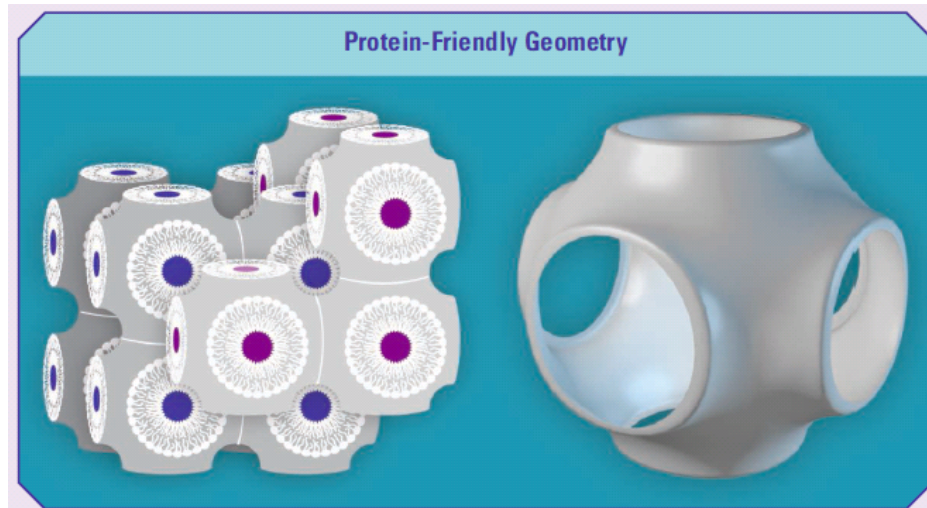
# Studi strutturali di proteine di membrana: metodi di cristallizzazione in surfo

Sistema HT:  
Microfluidic screening chip

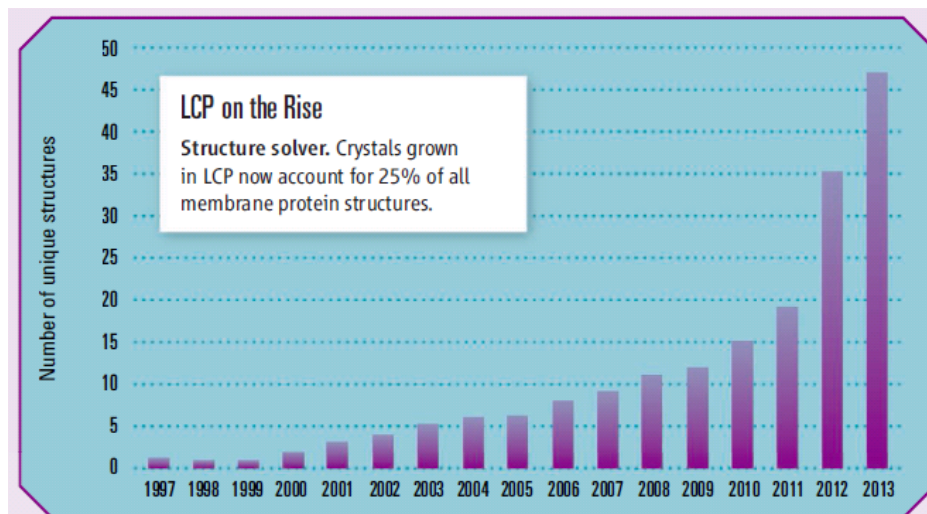


# Studi strutturali di proteine di membrana: metodi di cristallizzazione in meso

Lipidic cubic phases basato sulla mesofase (in meso)

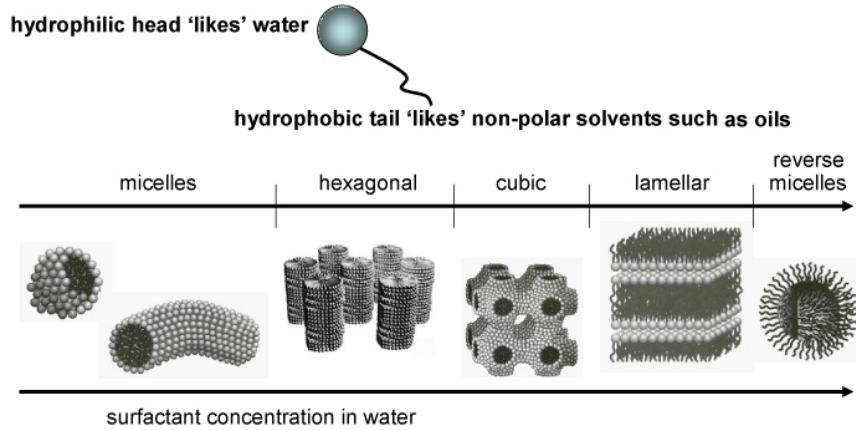


**Shapely.** In a lipidic cubic phase structure, lipid molecules form a hollow framework (*right*) that extends to form a 3D grid around water channels (*left*, purple and blue).



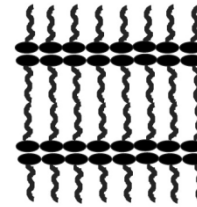
# Studi strutturali di proteine di membrana: metodi di cristallizzazione in meso

## Fasi dei lipidi



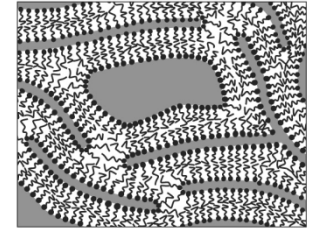
### SOLIDO

Lamellar crystal - Lc



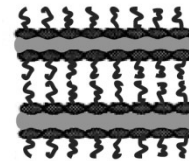
### LIQUIDO

Fluid isotropic - FI

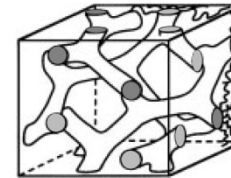


### LIQUIDO/CRISTALLINO (MESOFASE)

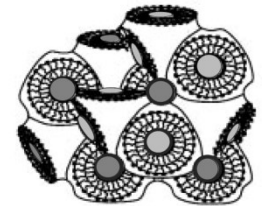
Lamellar liquid crystal - L $\alpha$



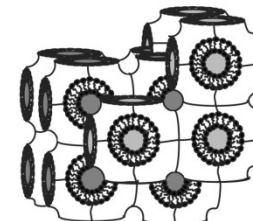
Cubic - Ia3d



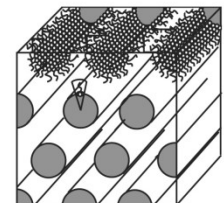
Cubic - Pn3m



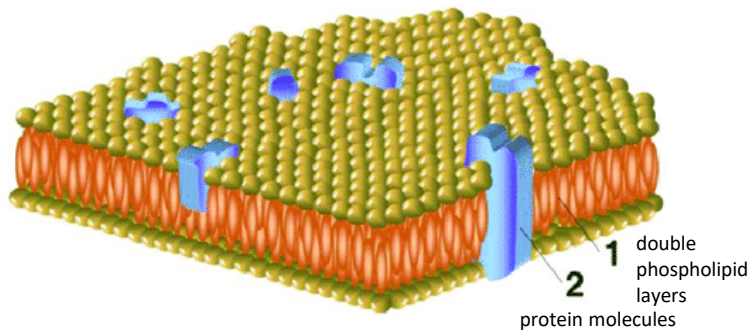
Cubic - Im3m



Inverted hexagonal - H $_{II}$



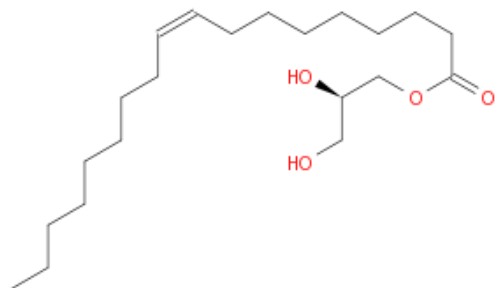
### cell membrane



# Studi strutturali di proteine di membrana: metodi di cristallizzazione in meso

## Lipidic cubic phases (LCP)

Monooleina



fasi cristalline liquide osservate

**fasi cubiche**

- 1) a spugna
- 2) a doppio diamante (Pn3m)
- 3) doppio giroide (Ia3d)

**fase lamellare  $L\alpha$**

struttura  $\beta$  barrel dell' intimina

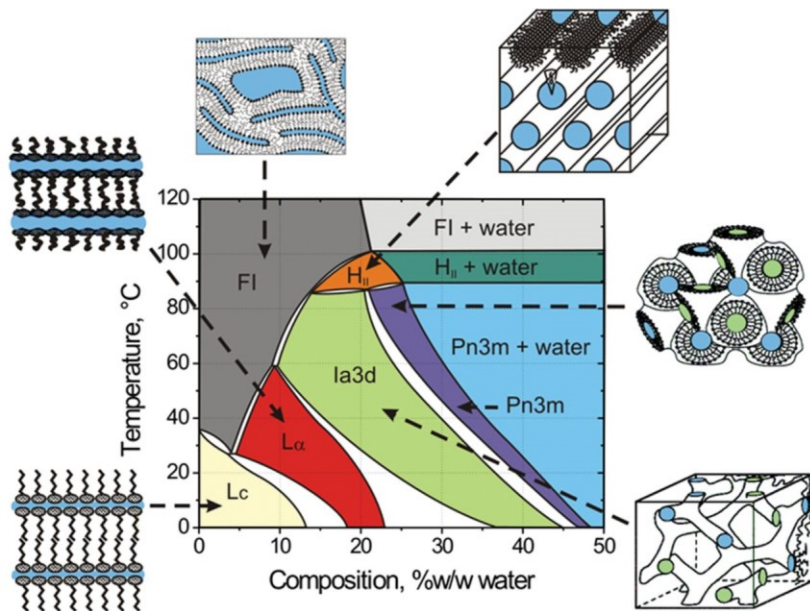
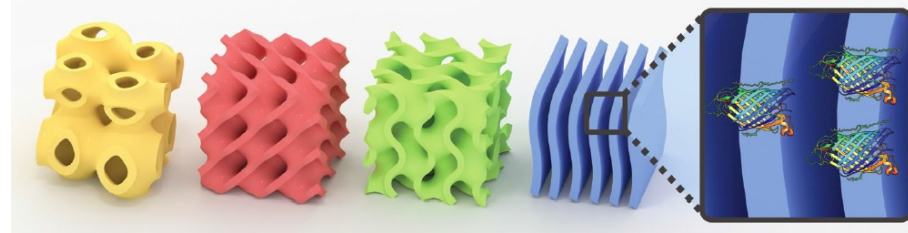


diagramma di fase

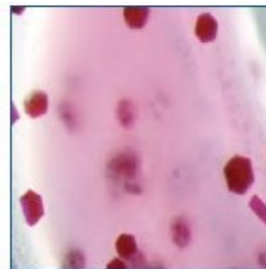
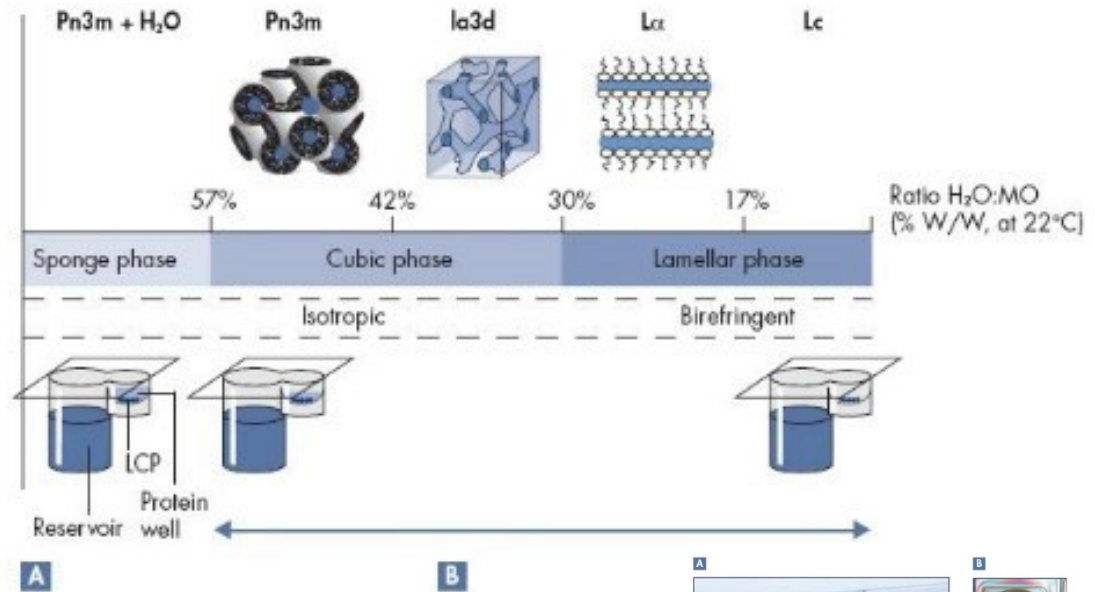
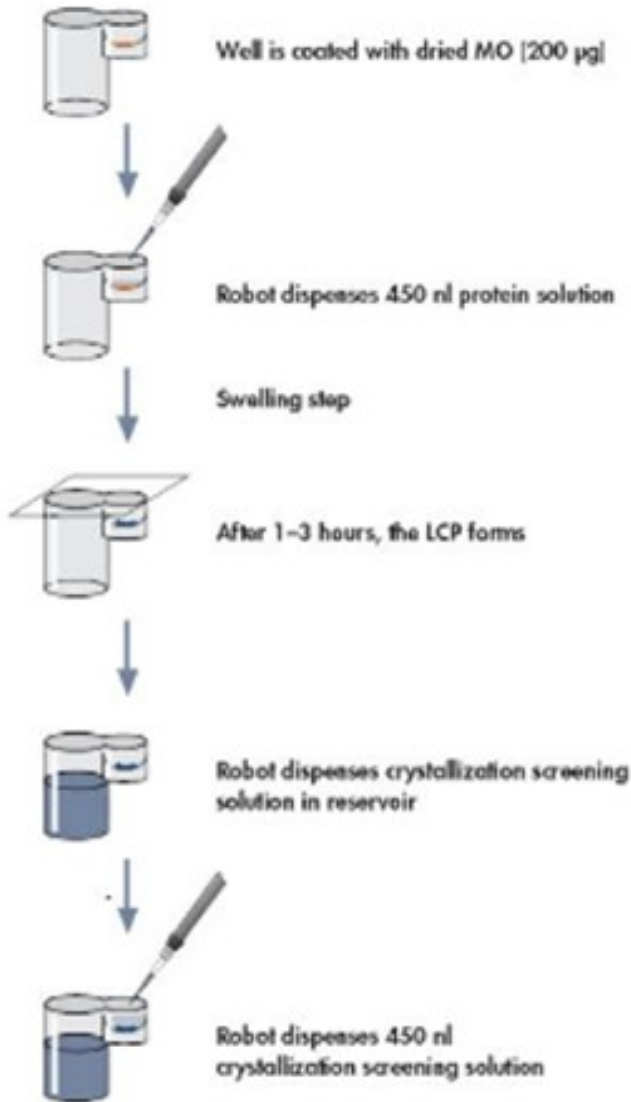


Pn3m la fase più efficace per il sistema  
monooleina/acqua:

- a) disidratata mediante sali, PEG ad alto MW, lipidi e caotropi
- b) idratata mediante MPD, PEG ad basso MW, caotropi

# Studi strutturali di proteine di membrana: metodi di cristallizzazione in meso

## Lipidic cubic phases (LCP): crystallization trials setup



SHARE REPORT

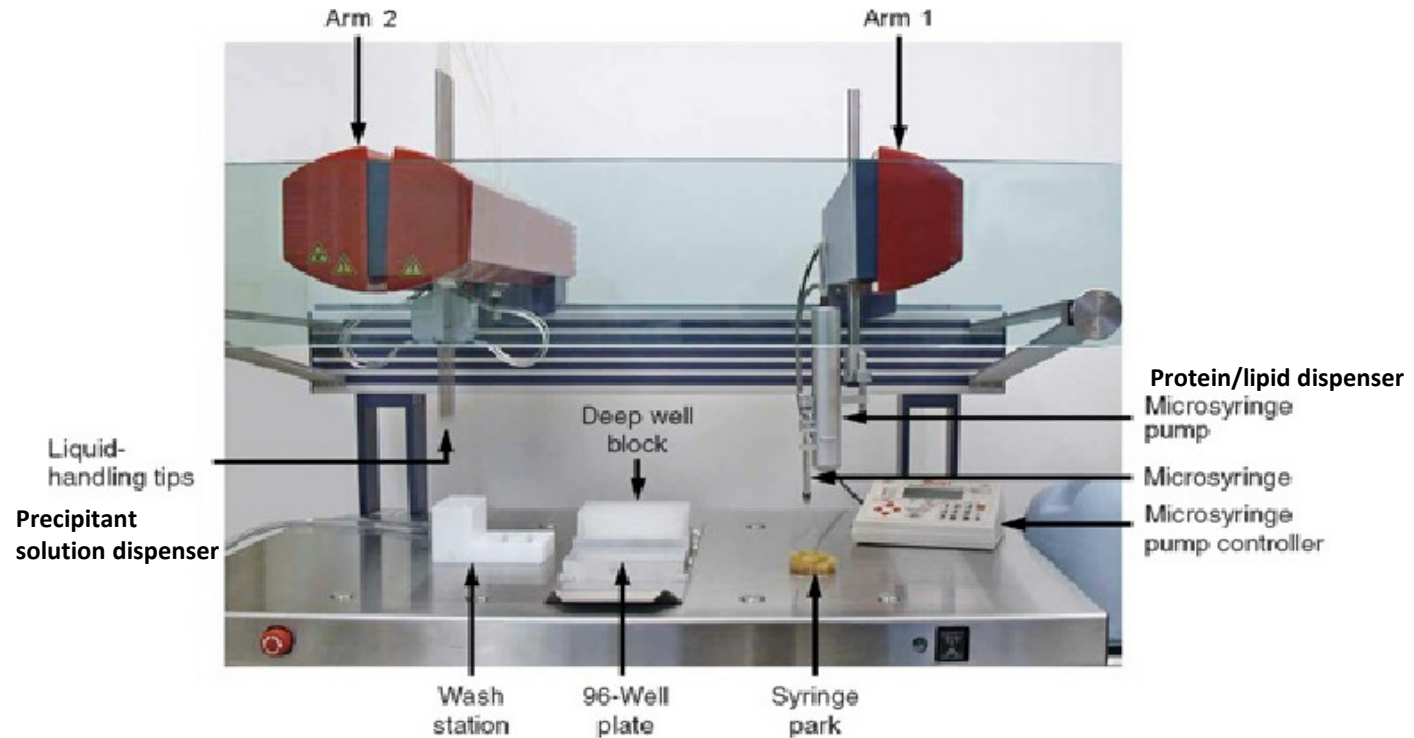
### X-ray Structure of Bacteriorhodopsin at 2.5 Angstroms from Microcrystals Grown in Lipidic Cubic Phases

Eva Pebay-Peyroula, Gabriele Rummel, Jurg P. Rosenbusch, Ehud M. Landau  
+ See all authors and affiliations

Science 12 Sep 1997:  
Vol. 277, Issue 5332, pp. 1676-1681  
DOI: 10.1126/science.277.5332.1676

# Studi strutturali di proteine di membrana: metodi di cristallizzazione in meso

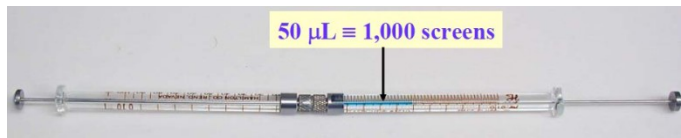
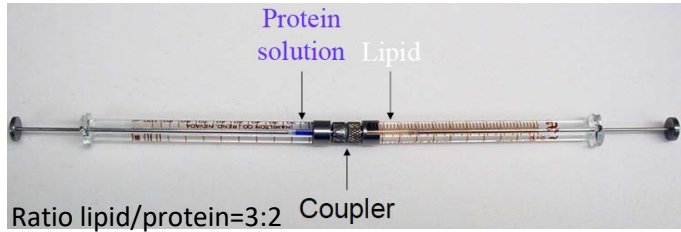
## Robotic setup



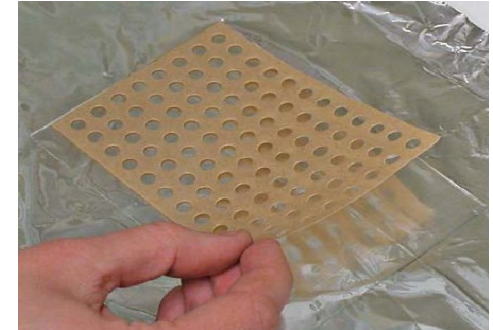
# Studi strutturali di proteine di membrana: metodi di cristallizzazione in meso

## Robotic crystallization trials setup

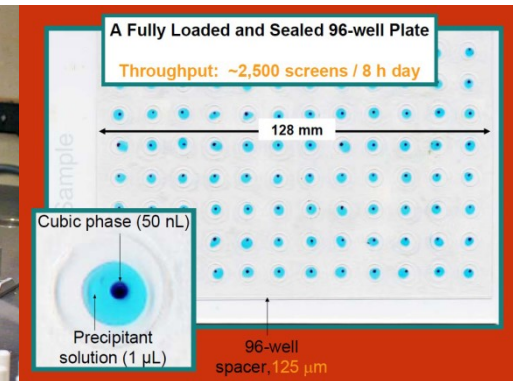
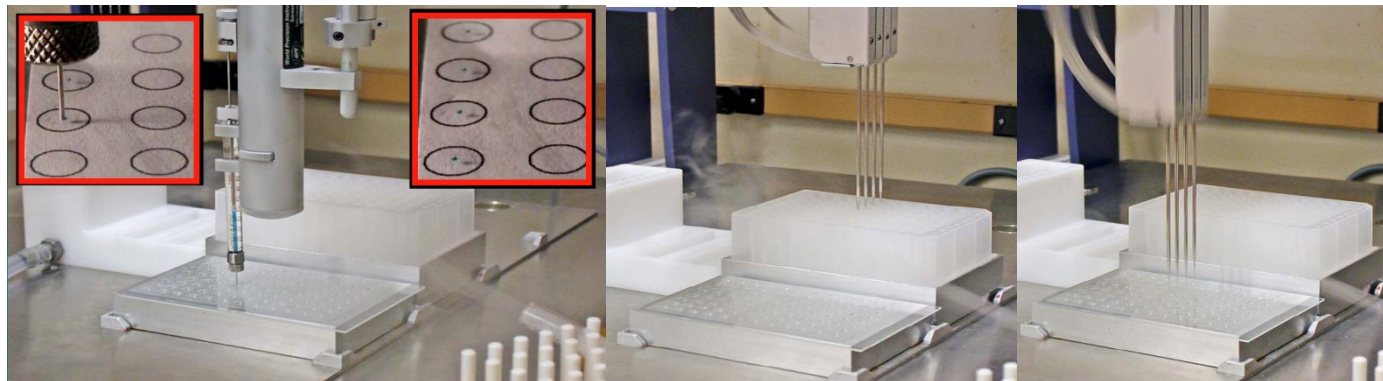
### 1) Preparazione della mesofase cubica



### 2) Preparazione della piastra



### 3) Crystallization set up

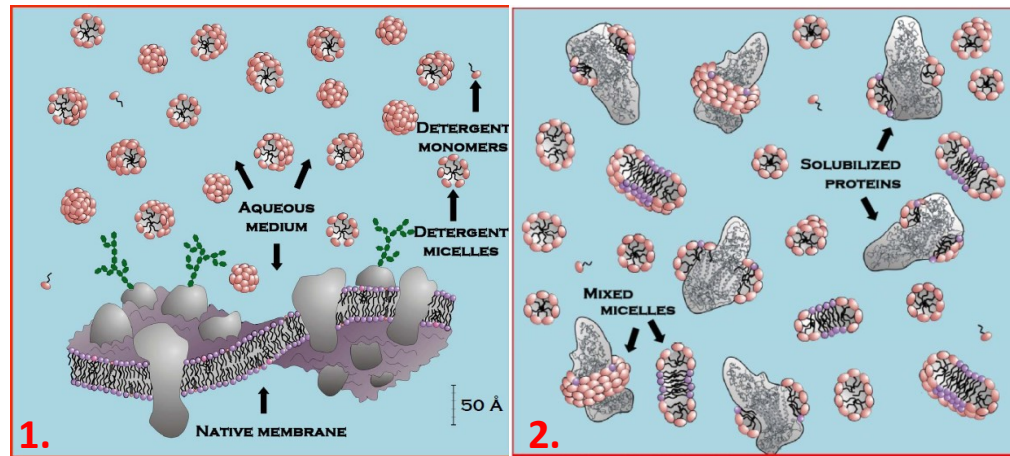




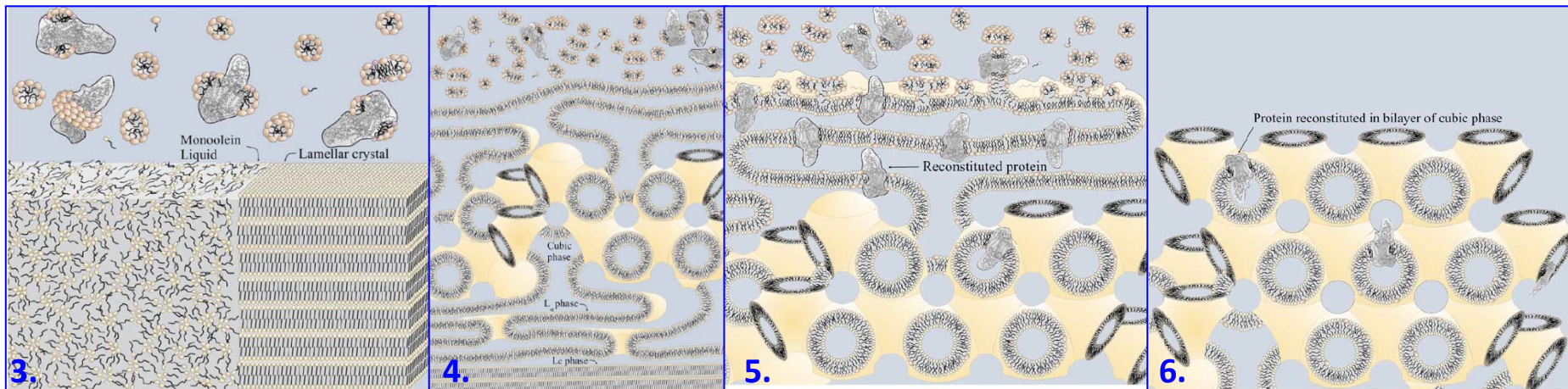
# Studi strutturali di proteine di membrana: metodi di cristallizzazione in meso

Ipotesi: eventi

durante la **Solubilizzazione**



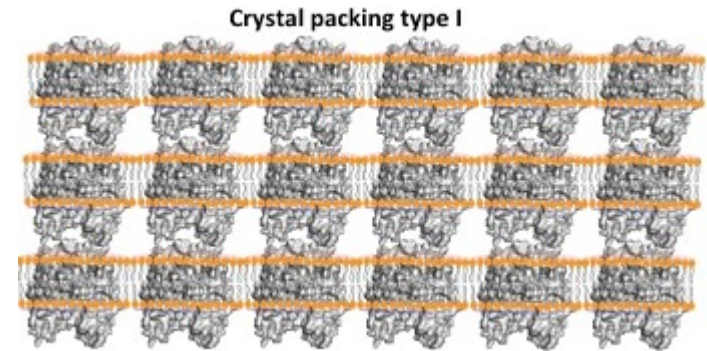
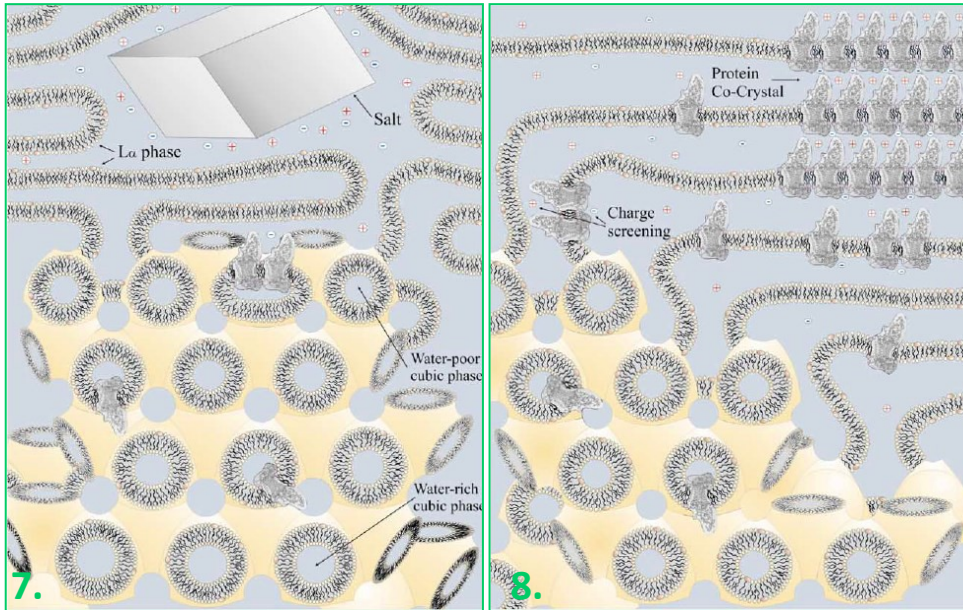
durante la **Ricostituzione**



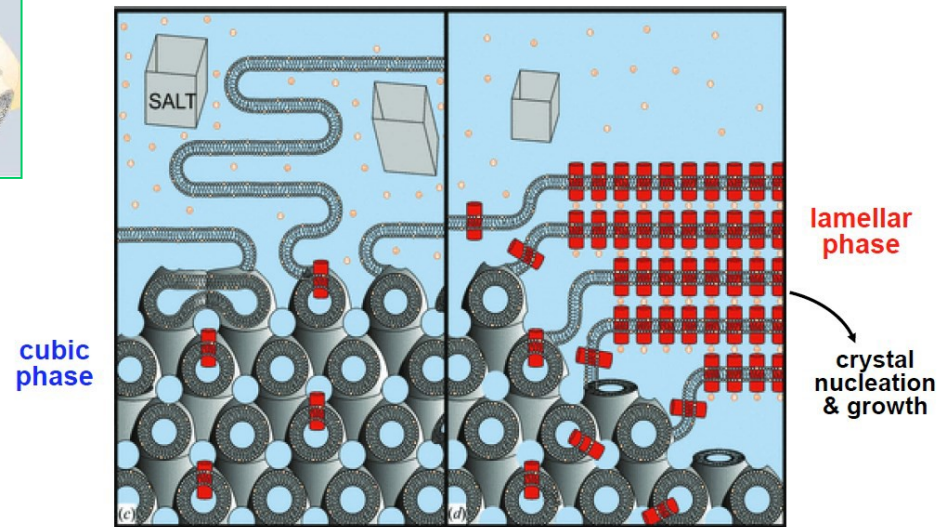
# Studi strutturali di proteine di membrana: metodi di cristallizzazione in meso

Ipotesi: eventi

durante l'Aggiunta del precipitante (sale)



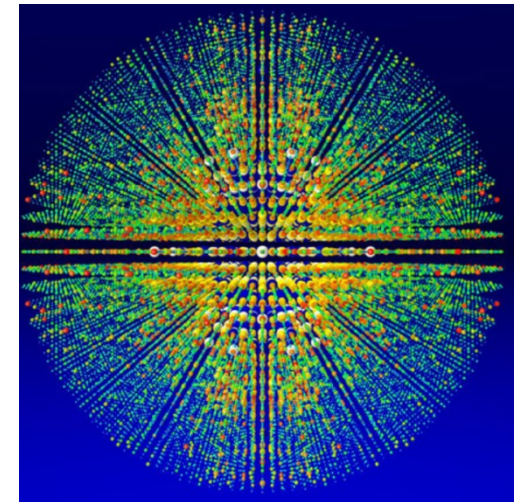
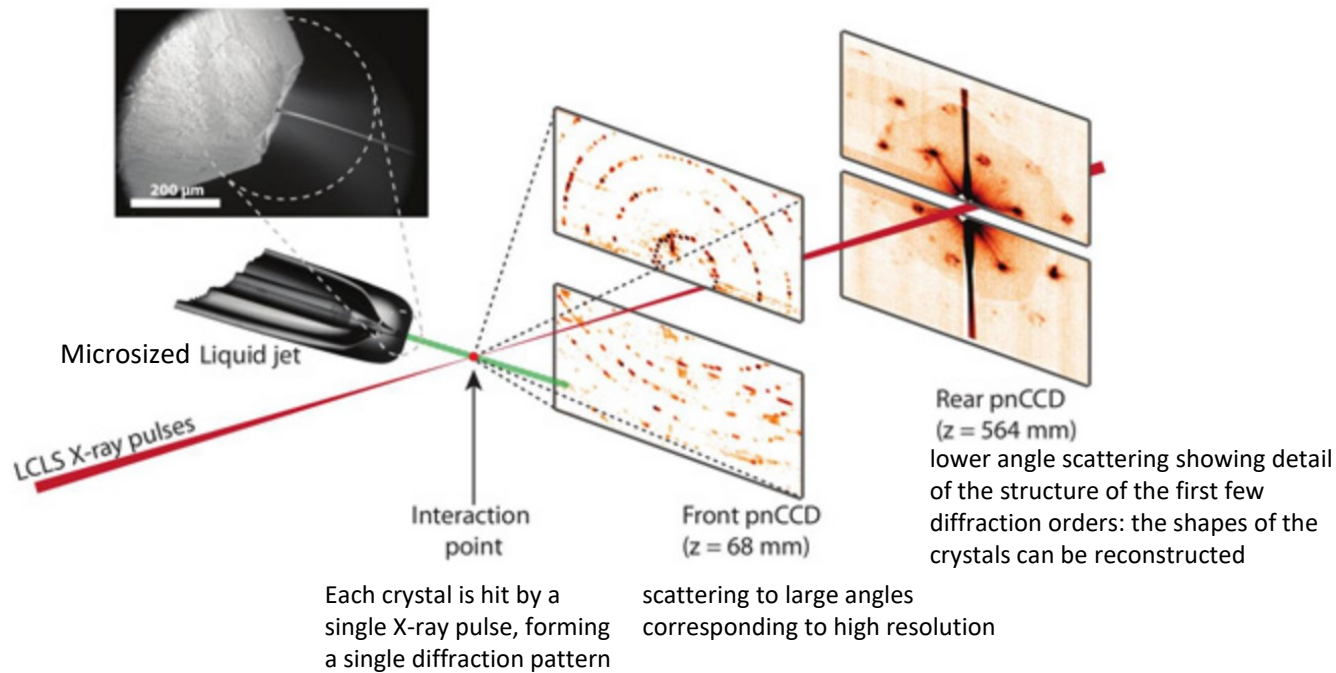
Addition of salt  $\rightarrow$  phase transition (dehydration of cubic phase)



# Studi strutturali di proteine di membrana: metodi di cristallizzazione in meso

## Serial Femtosecond Crystallography

utilizza impulsi di un laser a elettroni liberi a raggi X (XFEL) della scala dei femtosecondi:

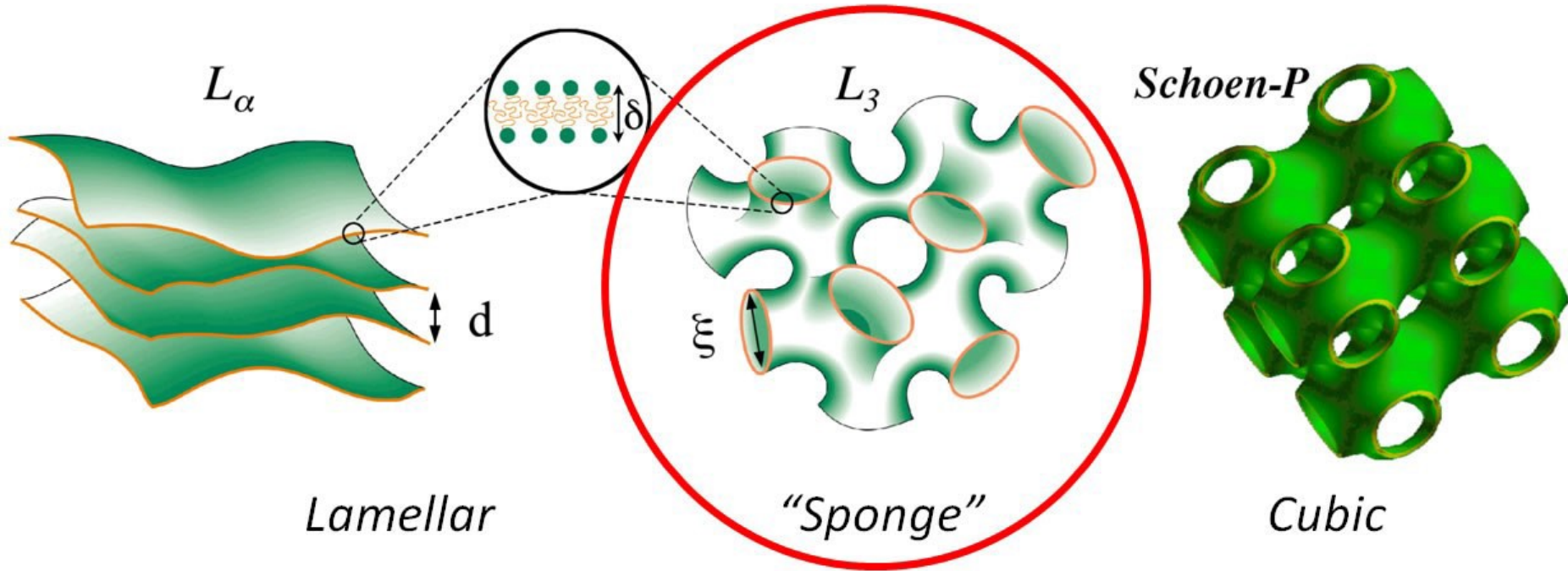


Visualizzazione tridimensionale delle intensità di diffrazione determinate combinando oltre 15.000 singoli spettri di diffrazione del complesso proteico del fotosistema I

Studi strutturali di proteine di membrana:  
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**Serial Femtosecond Crystallography**

Flusso di dimensioni micrometriche



*Lamellar*

*'Sponge'*

*Cubic*

**LSP**

Jeffamine o PEG che idrata la fase cubica in una fase liquida  
Può essere adattato al flusso micrometrico.

**LCP**

a causa della sua natura semisolida, non è adatto a un flusso micrometrico

# Studi strutturali di proteine di membrana: attuali risultati



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[Raman, P., Cherezov, V., Caffrey, M. 2006.  
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# Studi strutturali di proteine di membrana: attuali risultati

MEMBRANE PROTEIN COUNT VS SOURCE ORGANISM		
SOURCE ORGANISM	COUNT	
ESCHERICHIA COLI	231	
HOMO SAPIENS	124	
HALOBACTERIUM SALINARUM	78	
RHODOBACTER SPHAEROIDES	85	
BOS TAURUS	53	
ORYCTOLAGUS CUNICULUS	33	
STREPTOMYCES LMDANS	21	
BACILLUS BREVIS	21	
RATTUS NORVEGICUS	20	
OVIS ARIES	20	
AQUIFEX AEOLICUS	19	
SYNTHETIC	16	
GALLUS GALLUS	16	
ALICYCLOBACILLUS ACIDOCALDARIUS	16	
PSEUDOMONAS AERUGINOSA	15	
ESCHERICHIA COLI K12	15	
MUS MUSCULUS	14	
BLASTOCHLORIS MRIDIS	14	
BACILLUS CEREUS	13	
THERMUS THERMOPHILUS	10	
SACCHAROMYCES CEREVISIAE	10	
MAGNETOSPIRILLUM MAGNETOTACTICUM	10	

MEMBRANE PROTEIN COUNT VS EXPRESSION SYSTEM		
EXPRESSION SYSTEM	COUNT	
ESCHERICHIA COLI	492	
NATIVE (NOT RECOMBINANT)	311	
SYNTHETIC	90	
PICHA PASTORIS	49	
HALOBACTERIUM SALINARUM	46	
RHODOBACTER SPHAEROIDES	38	
SPODOPTERA FRUGIPERDA	32	
PSEUDOMONAS AERUGINOSA	11	
THERMUS THERMOPHILUS	7	
MOLINELLA SUCCINOGENES	3	
SACCHAROMYCES CEREVISIAE	3	
CELL FREE SYNTHESIS	2	
TRICHOPLUSIA NI	1	
SCHIZOSACCHAROMYCES POMBE	1	
SALMONELLA TYPHIMURIUM	1	
RHODOBACTER CAPSULATUS	1	
NATRONOBACTERIUM PHARAONIS	1	
DROSOPHILA MELANOGASTER	1	
CHLAMYDOMONAS REINHARDTII	1	

MEMBRANE PROTEIN COUNT VS LIPID USED IN BILAYER CRYSTALLIZATION		
LIPID USED IN BILAYER CRYSTALLIZATION/NMR	COUNT	
NOT USED	274	
MONOLEIN	64	
1,2-DIMYRISTOYL-SN-GLYCERO-3-PHOSPHOCHOLINE (DMPC)	35	
N/A	21	
CHOLESTEROL	15	
PURPLE MEMBRANE LIPIDS	9	
1,2-DIOLEOYL-SN-GLYCERO-3-PHOSPHOCHOLINE (DOPC)	5	
1-PAUMITOYL-2-OLEOYL-SN-GLYCERO-3-PHOSPHOCHOLINE (POPC)	4	
MONOVACCENIN	3	
1,2-DIPALMITOYL-SN-GLYCERO-3-PHOSPHOCHOLINE (DPPC)	3	
1,2-DIOLEOYL-SN-GLYCERO-3-PHOSPHATIDYLGLYCEROL (DOPG)	3	
1,2-DI-O-HEXYL-SN-GLYCERO-3-PHOSPHOCHOLINE	3	
PHOSPHATIDYLCHOLINE	2	
E. COLI TOTAL LIPID EXTRACT	2	
DICETYL PHOSPHATE	2	
ASOLECTIN	2	
1,2-DI-O-TETRADECYL-SN-GLYCERO-3-PHOSPHOCHOLINE	2	
1,2-DILAUROYL-SN-GLYCERO-3-PHOSPHOCHOLINE (DLPC)	2	
1,2-DIHEXANOYL-SN-GLYCERO-3-PHOSPHOCHOLINE (DHPC)	2	
PHOSPHATIDYLGLYCEROL	1	
PHOSPHATIDYLETHANOLAMINE	1	
NOT	1	
LIPOPEPTIDOPHOSPHOGLYCAN (LPPG)	1	
E. COLI POLAR LIPIDS	1	
CLARET MEMBRANE LIPIDS	1	
CHAPSO	1	
CARDIOLIPIN	1	
BOVINE LIVER PHOSPHATIDYLCHOLINE	1	
BOVINE LIVER LECITHIN	1	
1,2-DIPALMITOYL-SN-GLYCERO-3-PHOSPHATIDYLGLYCEROL (DPPG)	1	
1,2-DI-O-HEXADECYL-SN-GLYCERO-3-PHOSPHOCHOLINE	1	

# Studi strutturali di proteine di membrana: attuali risultati

MEMBRANE PROTEIN COUNT VS DETERGENT USED IN SOLUBILIZATION/NMR	
DETERGENT USED IN SOLUBILIZATION/NMR	COUNT
N-OCTYL-β-D-GLUCOPYRANOSIDE (OG)	158
N-DODECYL-β-D-MALTOPIRANOSIDE (DDM)	149
N,N-DIMETHYLDODECYLAMINE-N-OXIDE (LDAO)	126
N-DECYL-β-D-MALTOPIRANOSIDE	79
OCTYL-TETRAOXYETHYLENE (C8E4)	66
DODECYL PHOSPHOCHOLINE (DPC)	44
DODECYLOCTAOXYETHYLENE (C12E8)	34
SODIUM DODECYL SULFATE (SDS)	28
N-NONYL-β-D-GLUCOPYRANOSIDE	28
TRITON X-100	27
N/A	25
CHAPS0	23
ZWITTERGENT 3-12	21
N-UNDECYL-β-D-MALTOPIRANOSIDE	20
DODECYLNONAOXYETHYLENE (C12E9)	19
OCTYLPOLYOXYETHYLENE	18
N-OCTYL-2-HYDROXYETHYL SULFOXIDE	16
1,2-DIHEXANOYL-SN-GLYCERO-3-PHOSPHOCHOLINE (DHPC)	12
N-OCTYL-β-D-THIOGLUCOPYRANOSIDE	10
N,N-DIMETHYLDECYLAMINE-N-OXIDE	10
CYMAL-6	10
CYMAL-5	10

MEMBRANE PROTEIN COUNT VS PRECIPITANT USED IN CRYSTALLIZATION	
PRECIPITANT USED IN CRYSTALLIZATION	COUNT
POLYETHYLENE GLYCOL 4000	171
POLYETHYLENE GLYCOL 400	164
AMMONIUM SULFATE	85
POLYETHYLENE GLYCOL 2000	54
N/A	51
POLYETHYLENE GLYCOL 3350	37
SODIUM/POTASSIUM PHOSPHATE	36
POLYETHYLENE GLYCOL MONOMETHYLETHER 2000	36
POTASSIUM PHOSPHATE	35
POLYETHYLENE GLYCOL MONOMETHYLETHER 500	33
POLYETHYLENE GLYCOL 600	25
POLYETHYLENE GLYCOL 300	24
SODIUM CHLORIDE	22
POTASSIUM CHLORIDE	21
POLYETHYLENE GLYCOL 8000	21
2-METHYL-2,4-PENTANEDIOL	20
SODIUM PHOSPHATE	19
MAGNESIUM CHLORIDE	18
POLYETHYLENE GLYCOL 3000	17
POLYETHYLENE GLYCOL 1000	15
JEFFAMINE-M600	15
POLYETHYLENE GLYCOL 8000	14
SODIUM CITRATE	13
LITHIUM SULFATE	12
POLYETHYLENE GLYCOL 1500	10

MEMBRANE PROTEIN COUNT VS CRYSTALLIZATION METHOD	
CRYSTALLIZATION METHOD	COUNT
VAPOR DIFFUSION	744
HANGING DROP	339
SITTING DROP	338
BATCH	80
LIPID CUBIC PHASE	64
ANTIBODY	50
DIALYSIS	49
N/A	45
BICELLE	24
VESICLE FUSION	11
MICROSEEDING	9
MACROSEEDING	5
UNDER OIL	3
MICROFLUIDIC	3
LIPID SPONGE PHASE	3
EPITAXIAL NUCLEATION	2

MEMBRANE PROTEIN COUNT VS ADDITIVE USED IN CRYSTALLIZATION	
ADDITIVE USED IN CRYSTALLIZATION	COUNT
SODIUM CHLORIDE	179
GLYCEROL	115
1,2,3-HEPTANETRIOL	73
MAGNESIUM CHLORIDE	64
N/A	57
CALCIUM CHLORIDE	43
2-METHYL-2,4-PENTANEDIOL	43
SODIUM AZIDE	41
LITHIUM SULFATE	37
DITHIOTHREITOL	36
POTASSIUM CHLORIDE	33
EDTA	30
MAGNESIUM ACETATE	27
1,6-HEXANEDIOL	26
NOT USED	25
AMMONIUM SULFATE	23
SODIUM ACETATE	21
ETHYLENE GLYCOL	16
DIOXANE	16
LITHIUM CHLORIDE	12
IMIDAZOLE	12
EGTA	12
BENZAMIDINE	12
SODIUM POTASSIUM TARTRATE	11
TREHALOSE	10
POLYETHYLENE GLYCOL 400	10
MAGNESIUM SULFATE	10

# Studi strutturali di proteine di membrana: attuali risultati

MEMBRANE PROTEIN COUNT VS pH

pH	COUNT
7	105
6.6	105
7.6	101
8	85
8	68
6.6	59
8.5	49
N/A	48
6.8	32
4.6	31
4	27
6.6	26
6.7	25
7.2	23
5	23
4.5	22
6.1	19
4.8	18
6.2	17
9.5	13
6.6	13
6.4	13
9.8	12
7.4	12
3.7	12
6.3	11

MEMBRANE PROTEIN COUNT VS TEMPERATURE

TEMPERATURE	COUNT
20	266
4	146
25	106
N/A	99
18	72
22	70
30	44
37	28
19	28
10	23
27	21
40	20
23	20
5	17
16	13
15	13
24	12
46	11
55	9
21	9
50	7
17	7
14	6
25.0	5
12	5
11	5