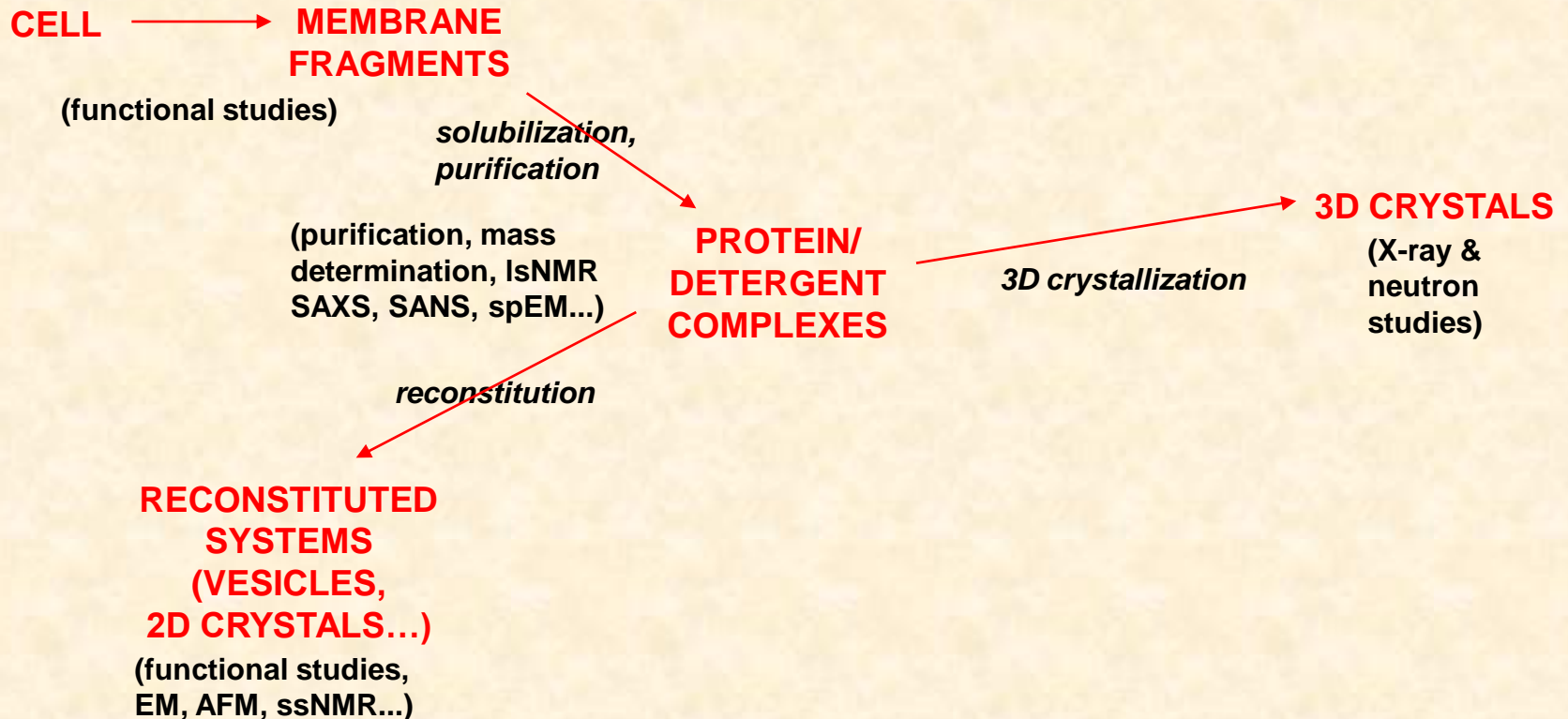


# **Membrane protein stability in aqueous solutions; destabilization by detergents**

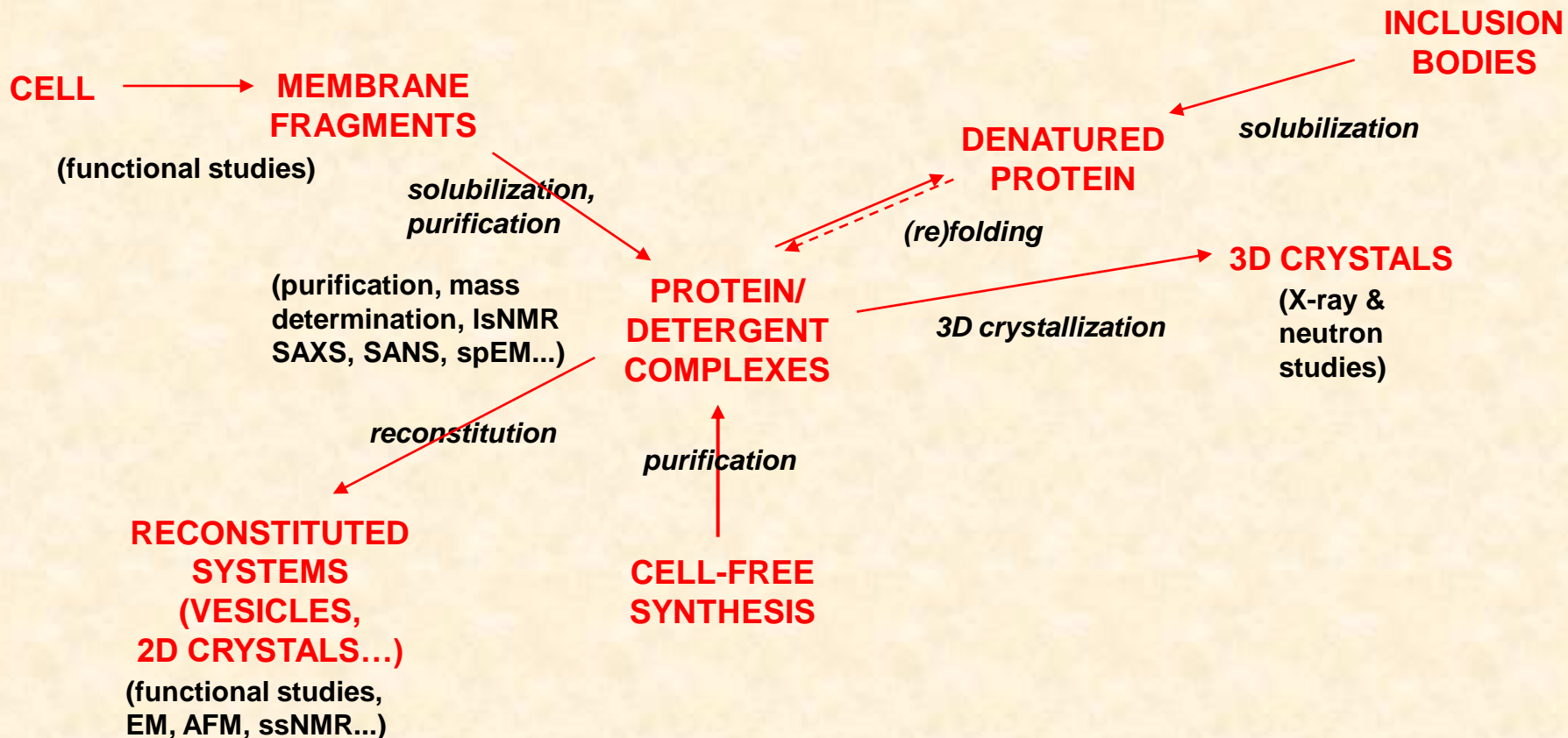
**Jean-Luc Popot**

CNRS/Université Paris-7  
Institut de Biologie Physico-Chimique  
Paris, France.

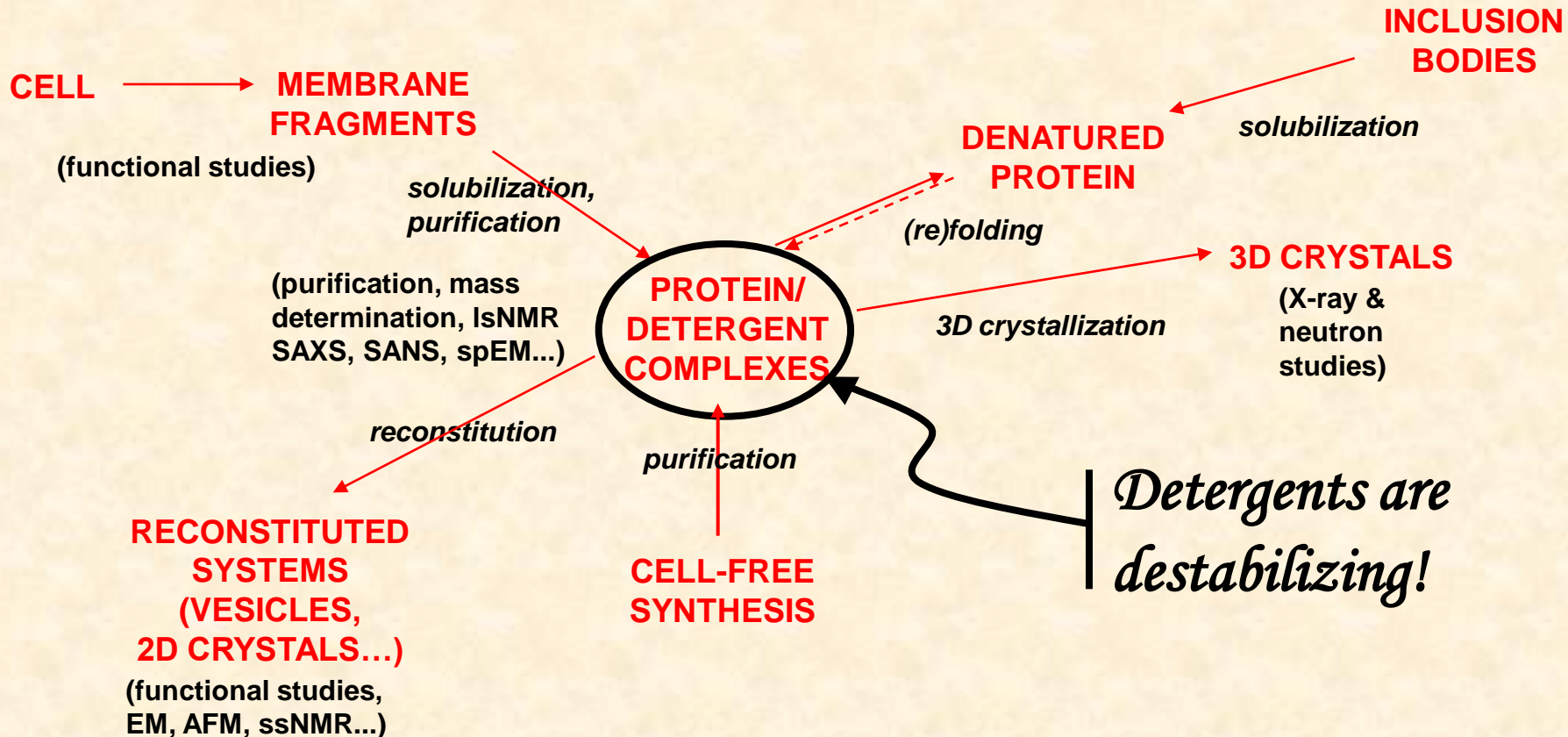
# Experimental systems for studying membrane proteins



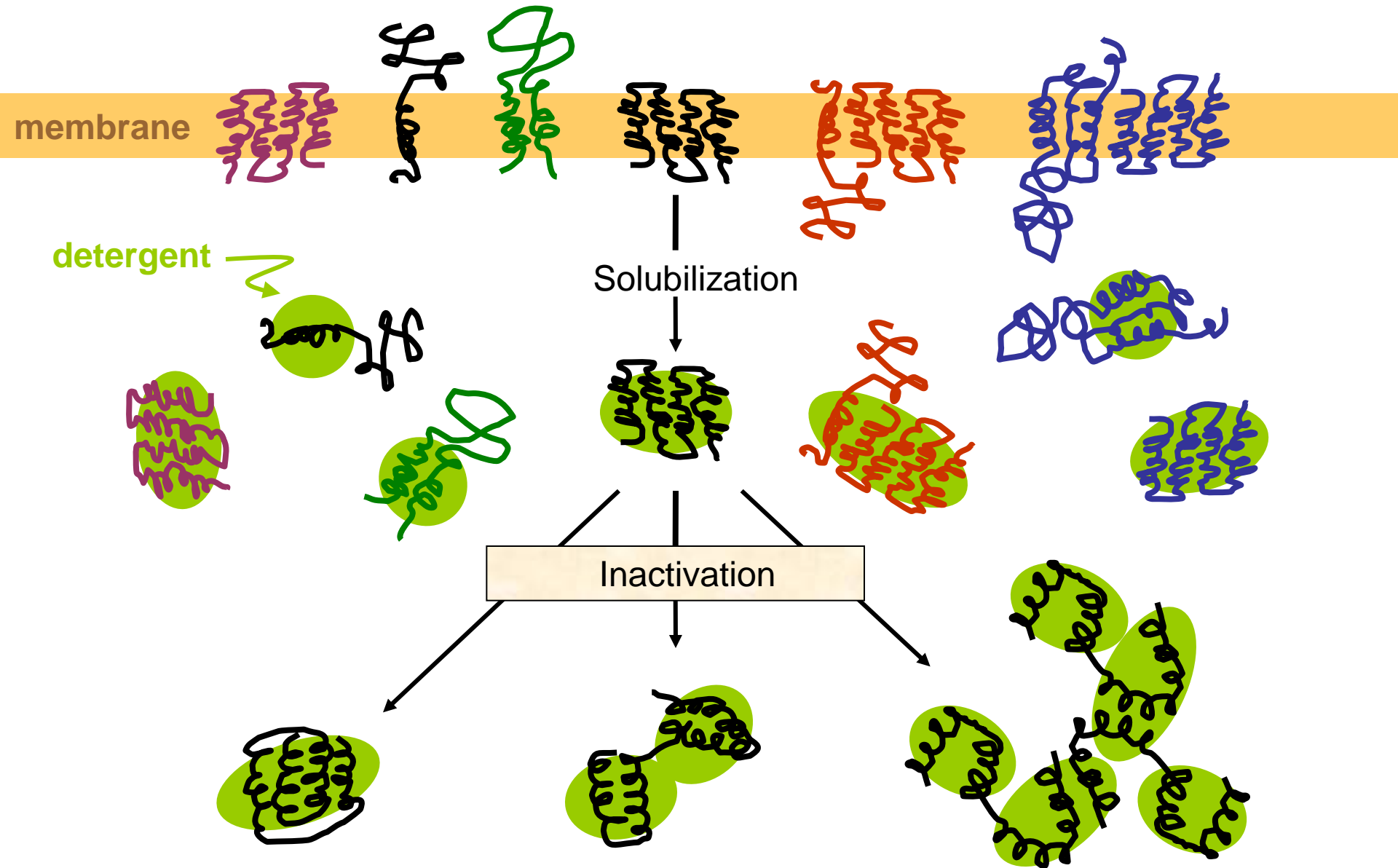
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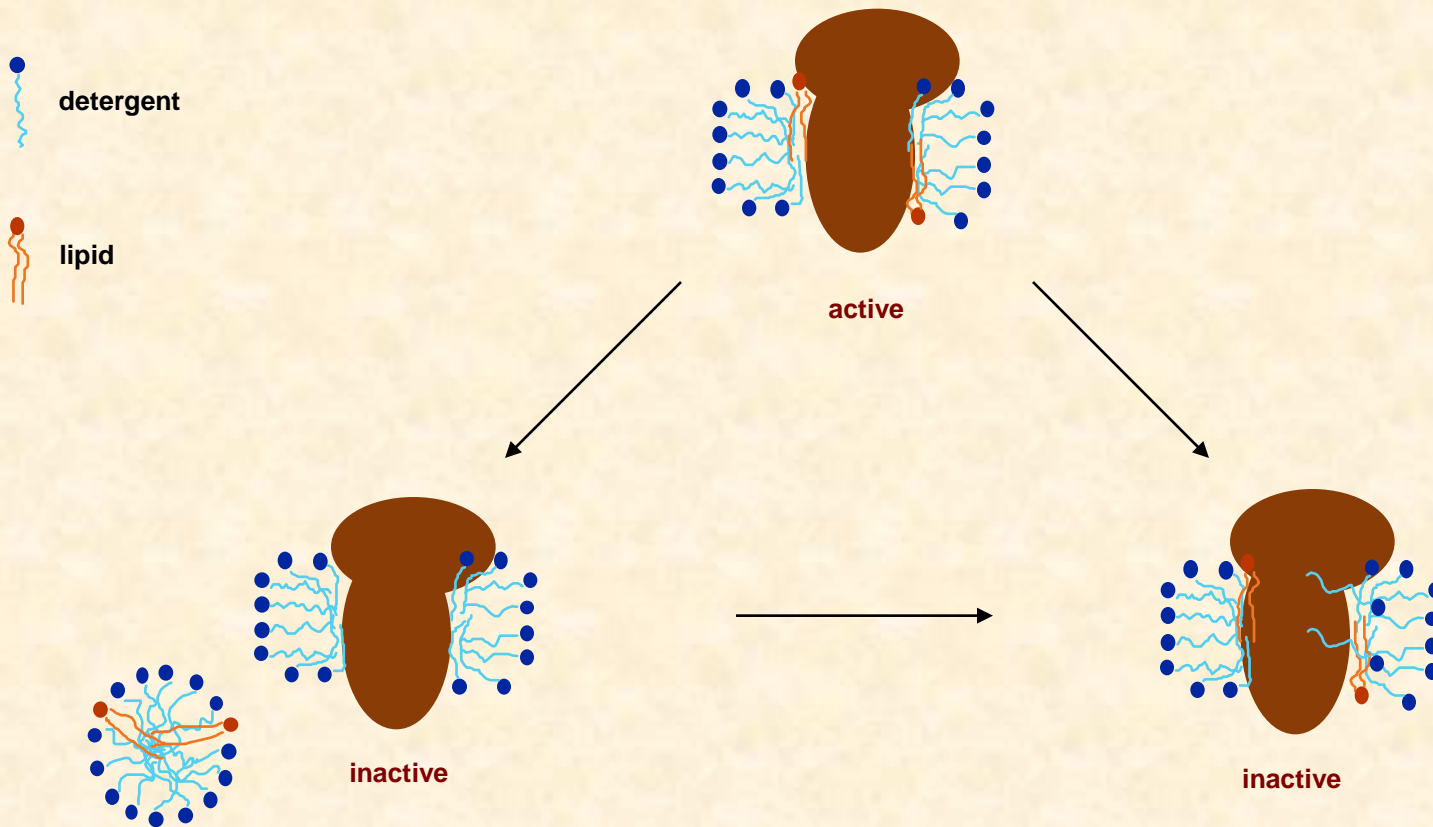


# From solubilization to inactivation



# Molecular mechanisms of membrane protein inactivation by detergents

## Two non-exclusive hypotheses



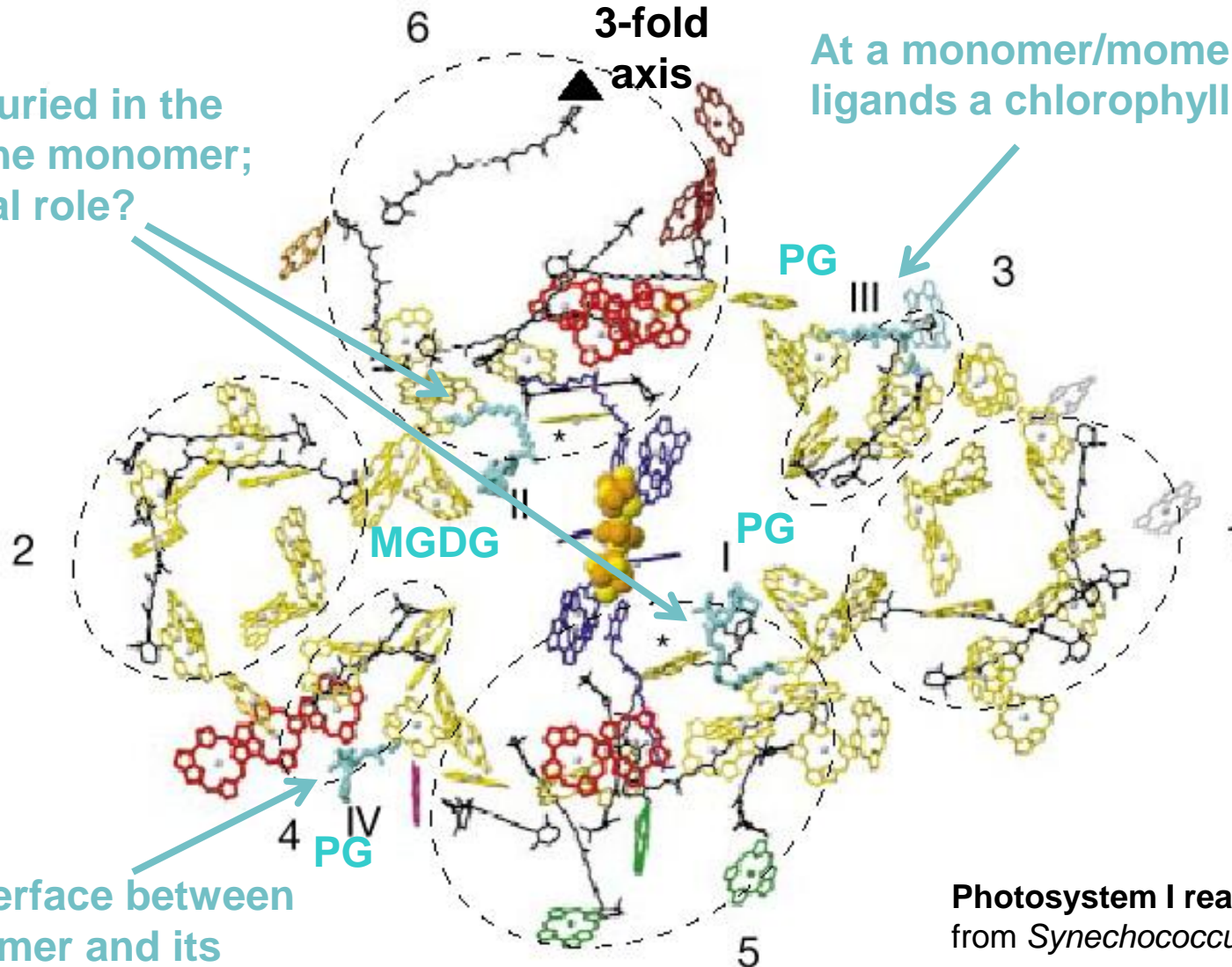
**1. Loss of stabilizing cofactors  
(lipids, subunits, prosthetic groups...)**

**2. Direct perturbation of the  
transmembrane region of the protein**

# Lipids are not just a fluid continuum: some of them occupy well-defined positions and can be considered as authentic cofactors

Deeply buried in the core of the monomer;  
functional role?

At a monomer/monomer interface;  
ligands a chlorophyll



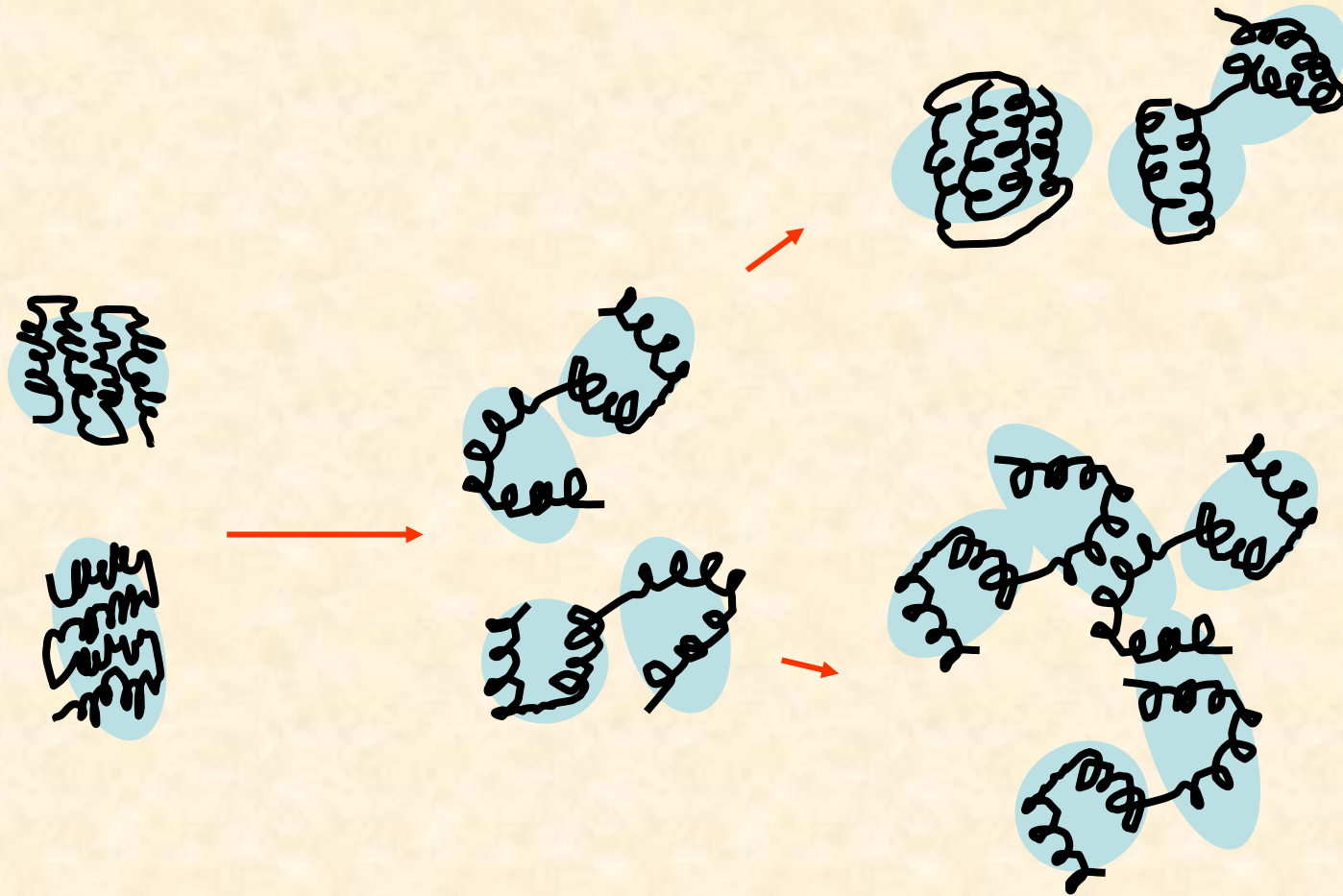
At the interface between  
the monomer and its  
membrane environment

Photosystem I reaction center  
from *Synechococcus elongatus*

Jordan et al., Nature 411:909-917, 2001

# Partial opening of the structure may initiate misfolding and/or aggregation events leading to inactivation

(cf. calcium ATPase)



# Stabilizing membrane proteins in aqueous solution

## Classical approaches

- Transfer to "weak" detergents (Tween, digitonin...)
- Work close to the cmc (*i.e.*, limit the volume of micellar phase)
- Supplement micelles with lipids, cofactors, ligands...
- Lower the temperature
- Add glycerol
- Work fast!

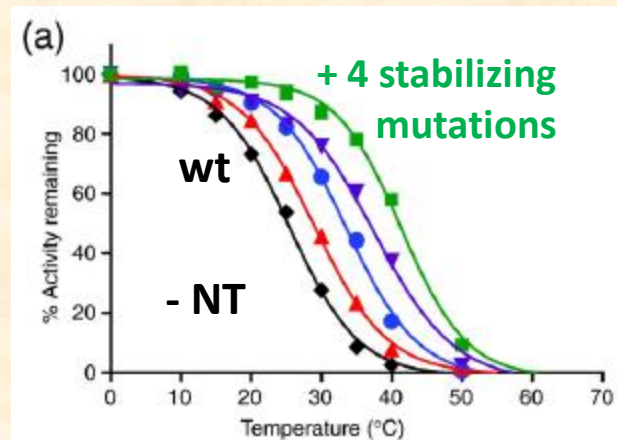
For references, see *e.g.*:

- Garavito & Ferguson-Miller, *J. Biol. Chem.* **276**:32403-32406, 2001.
- Bowie, *Curr. Opin. Struct. Biol.* 11:397-402, 2001.
- Gohon & Popot, *Curr. Opin. Colloid Interface Sci.* **8**:15-22, 2003.

# Stabilizing membrane proteins in aqueous solutions

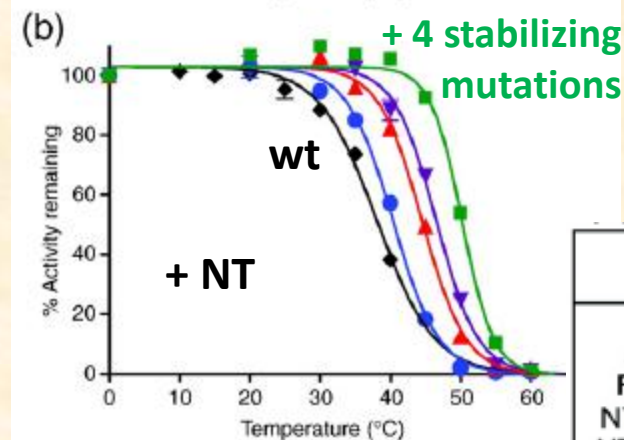
## Novel approaches

- Select or engineer more stable proteins (ex. DAGK, GPCRs) (J.U. Bowie, C.G. Tate)



Example: Stabilization of the neurotensin receptor  
(Shibata *et al.*, *J. Mol. Biol.*, 2009):

Thermostabilization by 17°C  
(no NT) or 13°C (+ NT).

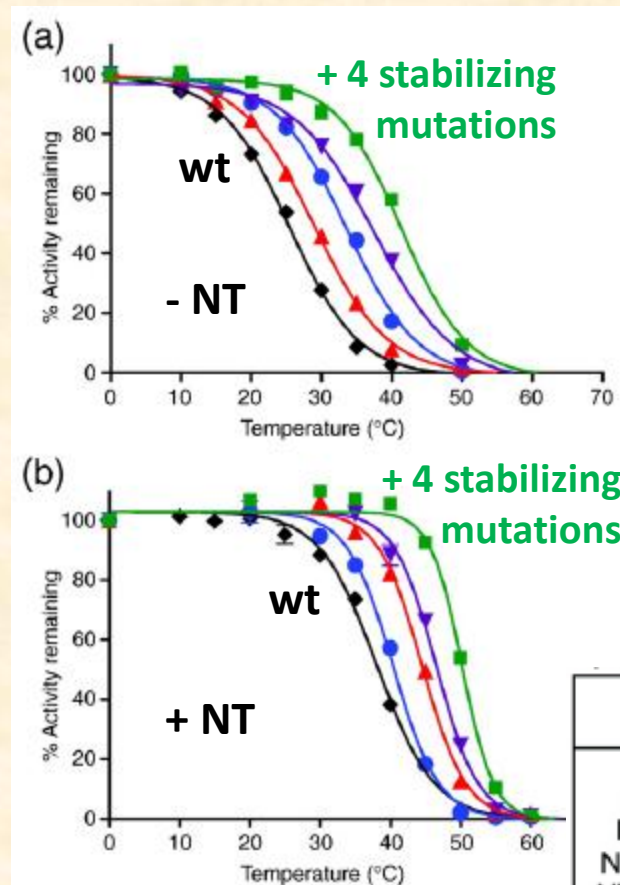


	-NT assay		+NT assay	
	$T_m$ (°C)	$\Delta T_m$ (°C)	$T_m$ (°C)	$\Delta T_m$ (°C)
wt	25	-	37	-
A86L	33	8	40	3
F358A	28	3	45	8
NTS1-7a	36	11	47	10
NTS1-7m	42	17	50	13

# Stabilizing membrane proteins in aqueous solutions

## Novel approaches

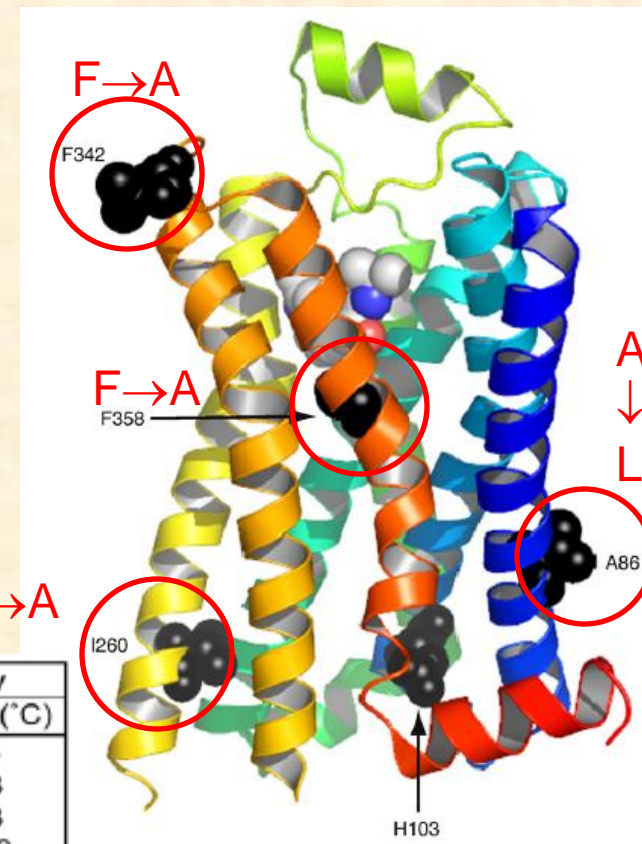
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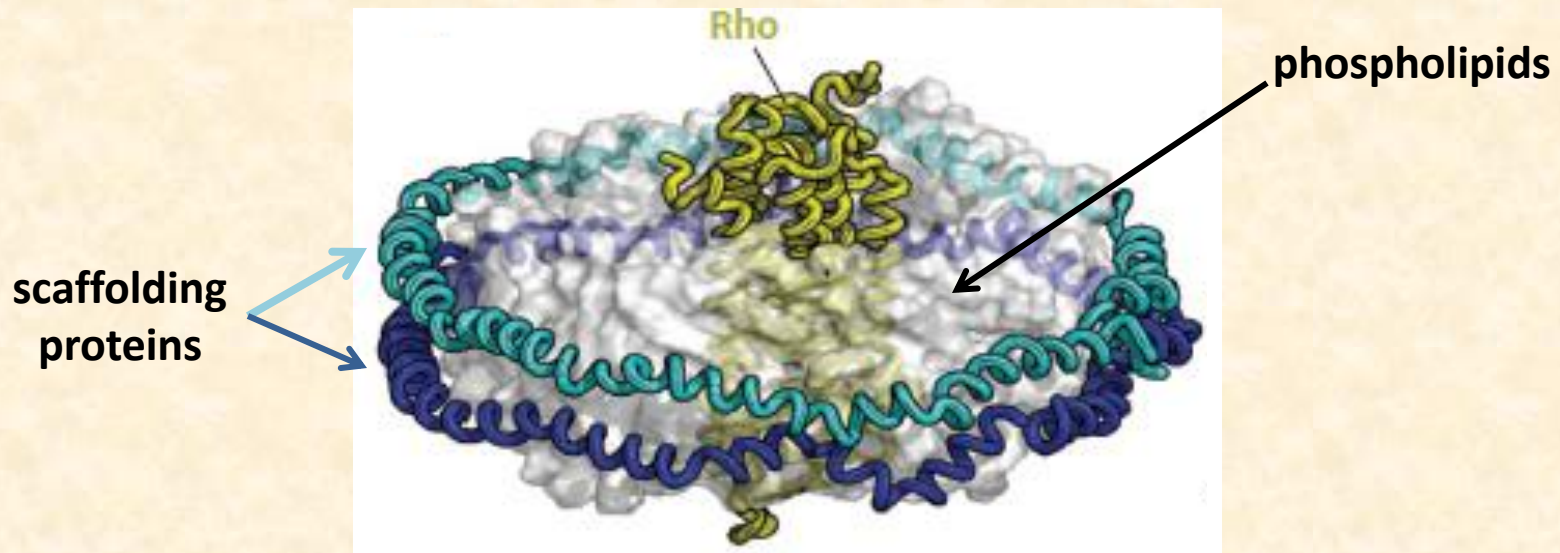
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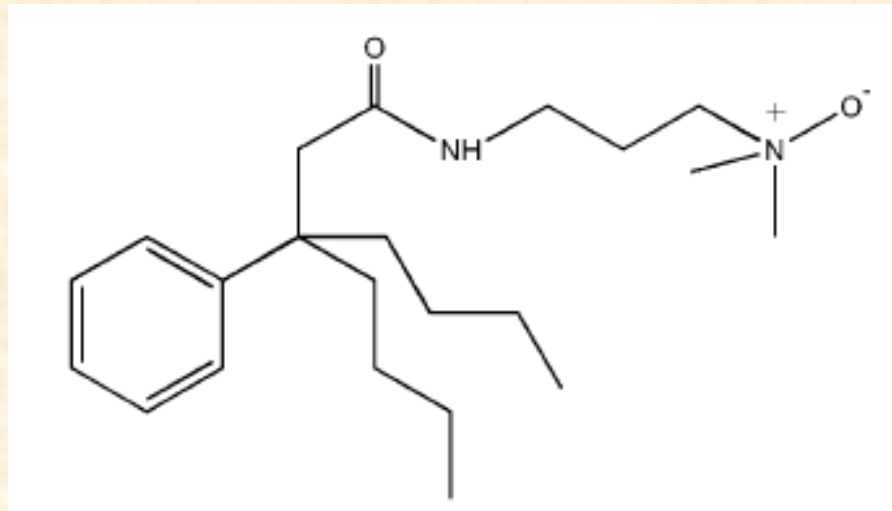


~10 nm

# Stabilizing membrane proteins in aqueous solutions

## Novel approaches

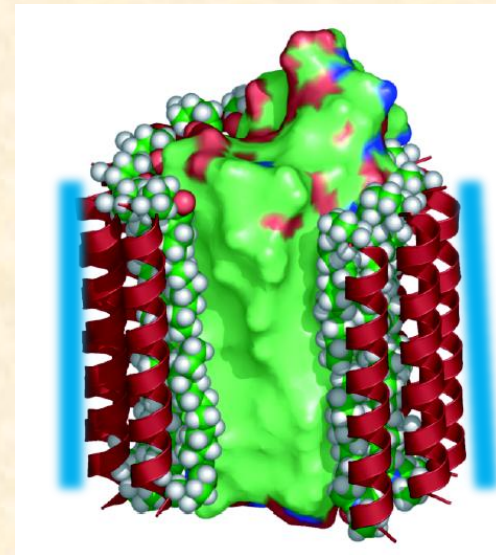
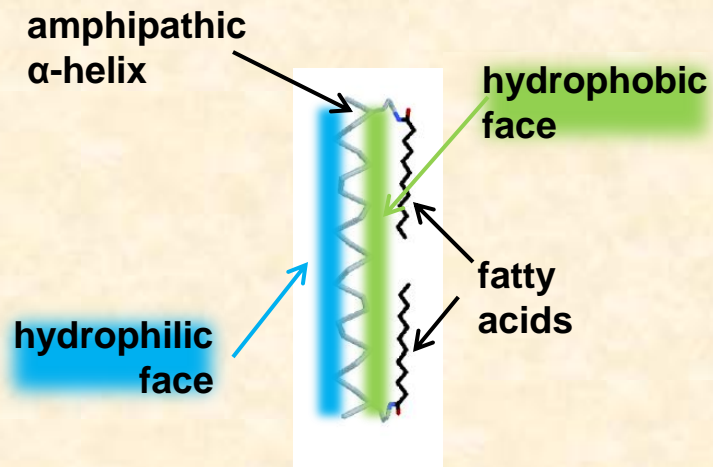
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# Stabilizing membrane proteins in aqueous solutions

## Novel approaches

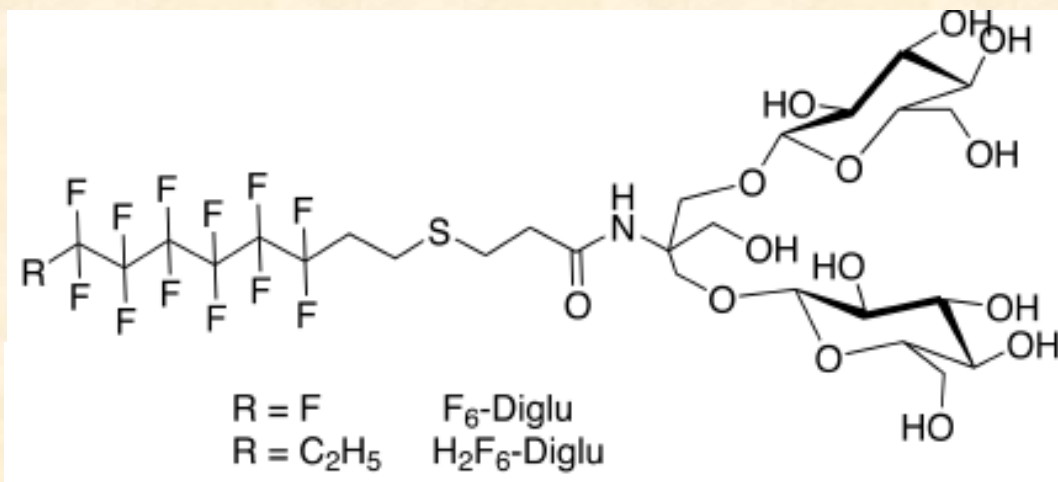
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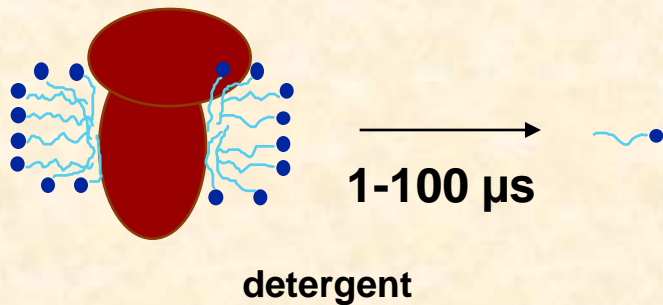
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  - Fluorinated surfactants (B. Pucci & J.-L. Popot)
  - "Amphipols" (C. Tribet, R. Audebert & J.-L. Popot)

# Rationales for the design of amphipols

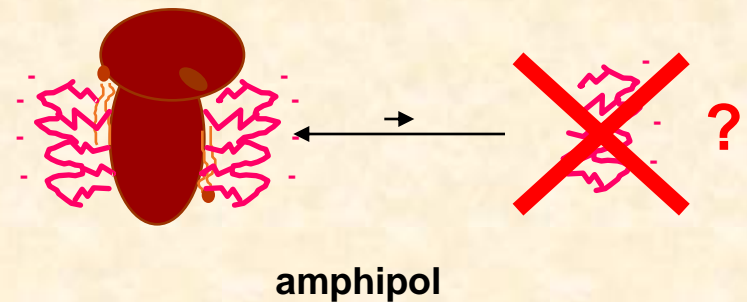
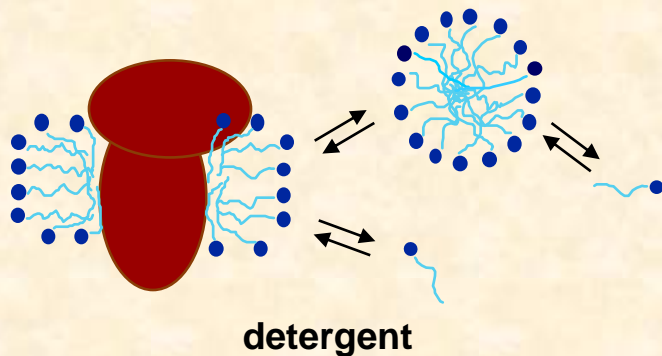
## Principle

Non-covalent multipoint attachment of an **amphipathic polymer** onto the transmembrane surface of a membrane protein ought to result in a soluble complex featuring:

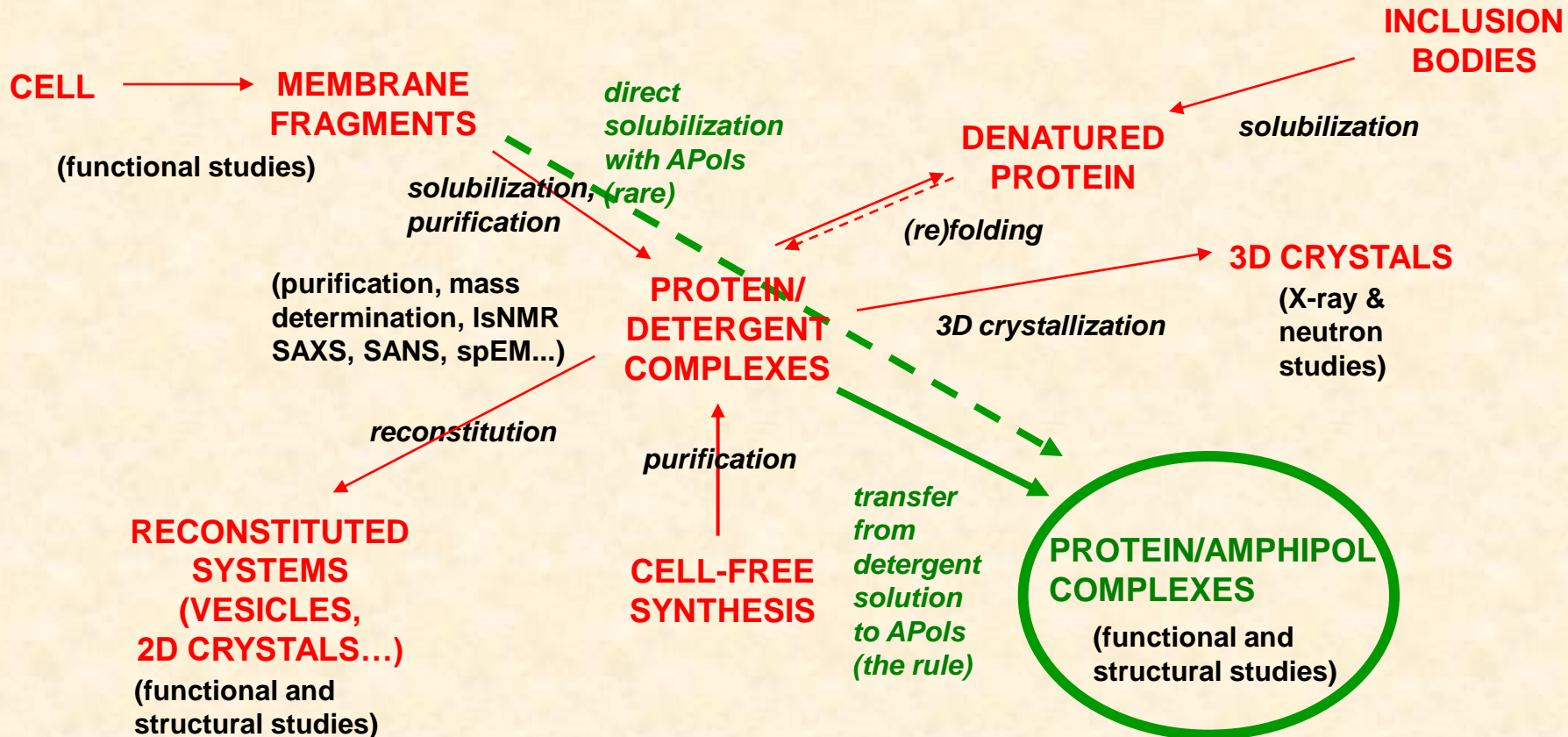
- low  $k_{\text{off}}$   $\rightarrow$  quasi-irreversible attachment



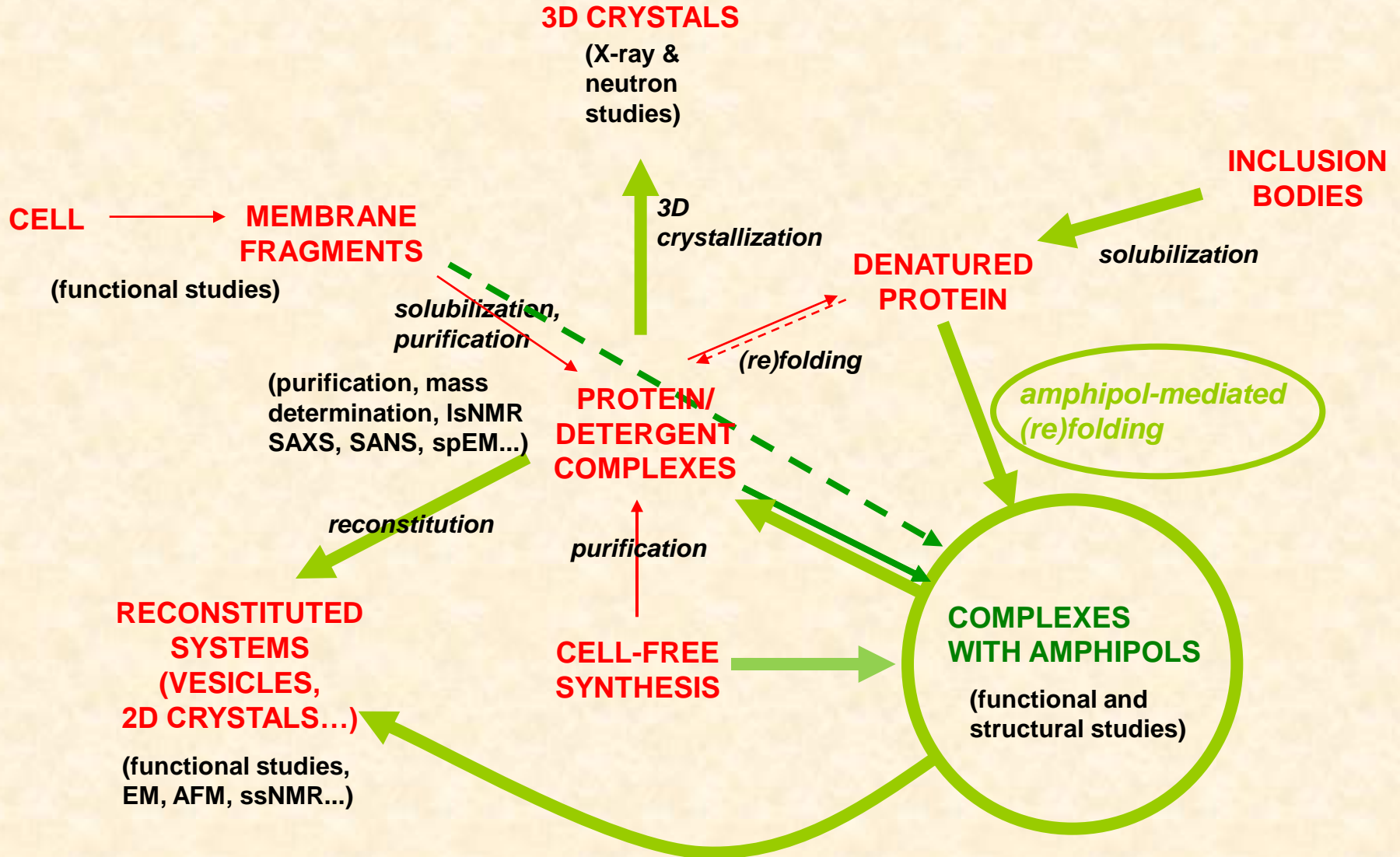
- small  $K_D$   $\rightarrow$  very low equilibrium concentration of free surfactant



# Experimental systems for studying membrane proteins



# Experimental systems for studying membrane proteins



# Opportunities and constraints associated with the use of amphipols, nanodiscs, and fluorinated surfactants

Technology	Amphipols	Nanodiscs	Fluorinated surfactants
<b>Membrane protein (MP) stabilization</b>	+	+	+
<b>Functional studies</b>	+	++	+
<b>Mediating MP immobilization for ligand binding measurements</b>	+	+	-
Optical spectroscopy (visible absorption spectrum)	+	+	+
Optical spectroscopy (UV, intrinsic MP fluorescence, circular dichroism)	+	±	+
Fluorescence spectroscopy using probes	+	+	+
Infrared spectroscopy	-	±	-
MP solution studies by AUC, SEC, SAXS, SANS	+	±	+
<b>Solution NMR</b>	+	±?	?
Solid-state NMR	+?	+	-
Three-dimensional crystallization	±	-?	+?
Two-dimensional crystallization	-	-?	+?
Trapping MP supercomplexes	+	±	+
EM, AFM (single particles)	+	+	+
Transferring MPs to preformed membranes	+	?	+
<b>Folding full-length MPs to native state</b>	+	?	+
<b>MP cell-free translation</b>	+	+	+
<b>Isoelectrofocusing and two-dimensional gels</b>	+	+?	?
<b>MP mass spectrometry</b>	+	?	?

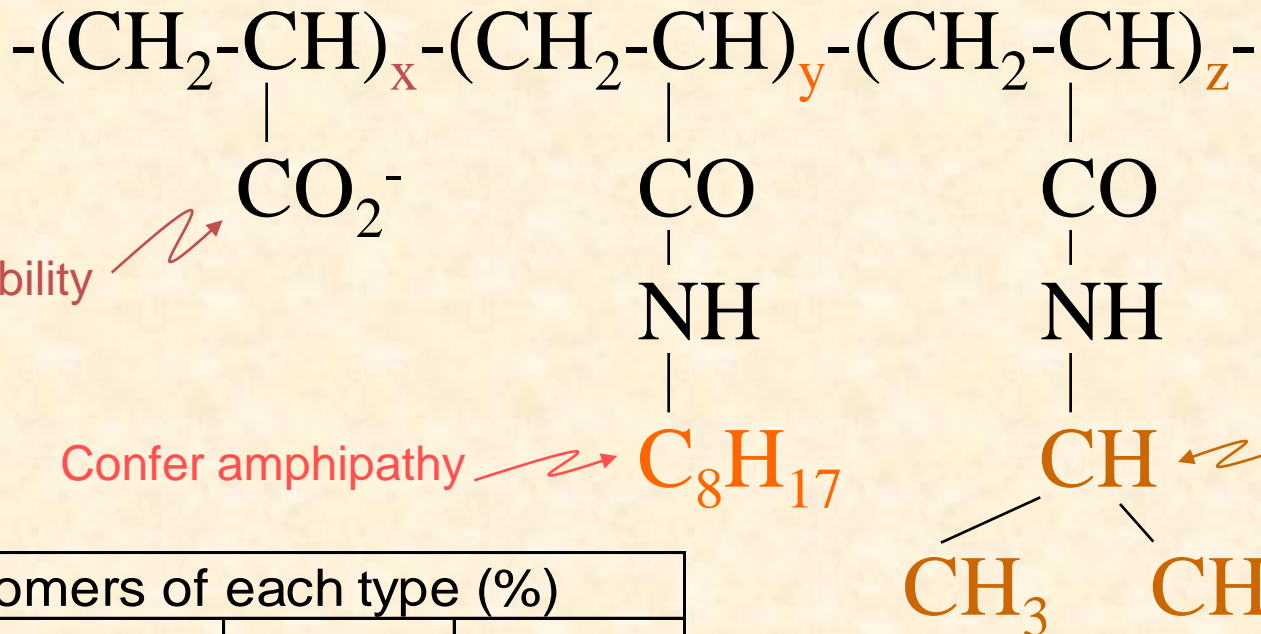
# **Structure and properties of amphipols; functionalized amphipols**

**Jean-Luc Popot**

CNRS/Université Paris-7  
Institut de Biologie Physico-Chimique  
Paris, France.

# Polyacrylate-based amphipols

Synthesis: graft at random long- and short-chain amines onto polydisperse polyacrylic acid



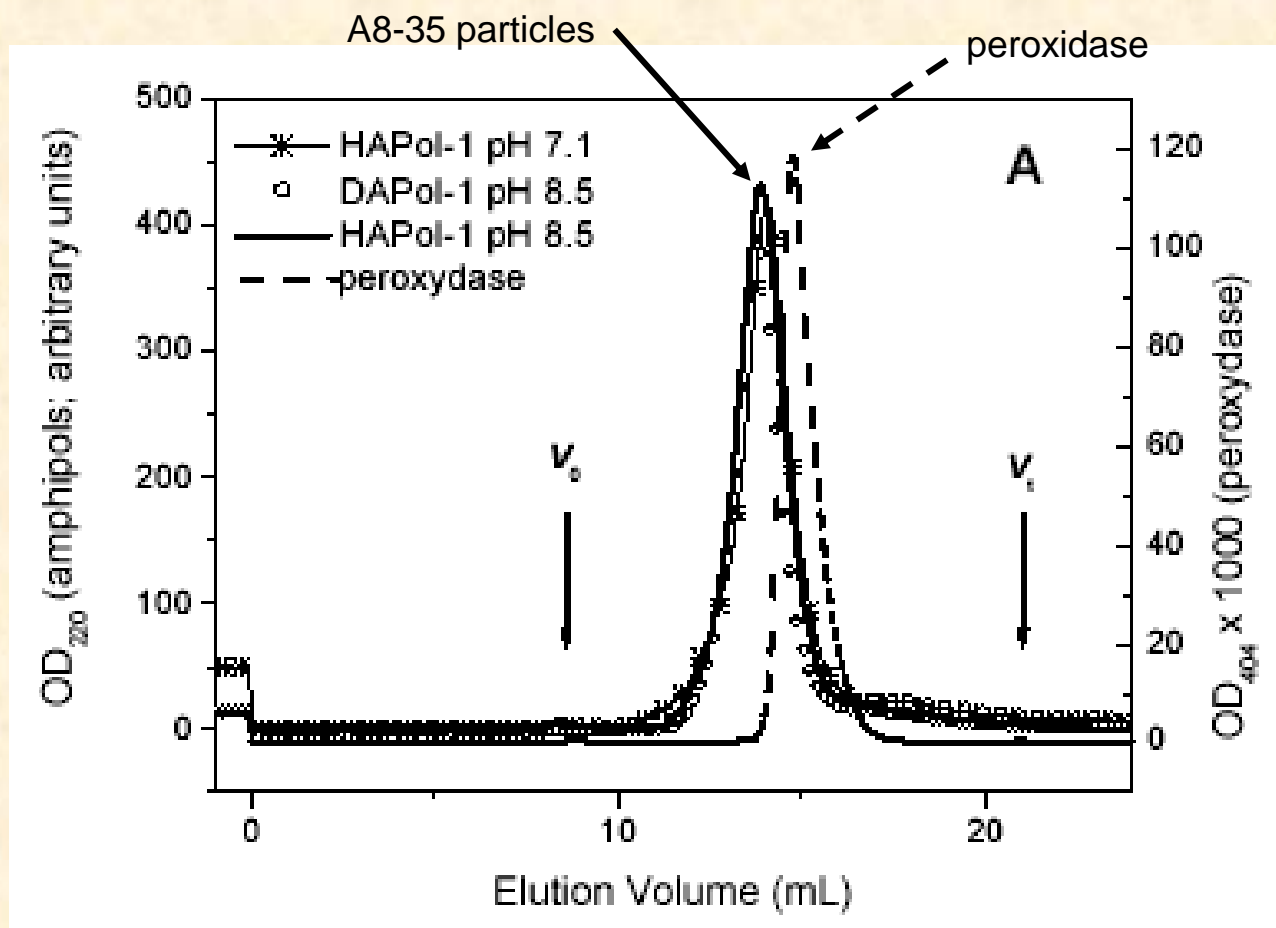
Monomers of each type (%)			
	x	y	z
A8-35	35	25	40

**Amphipol A8-35 :  $\langle \text{MW} \rangle \approx 9\text{-}10$  kDa, 35% of underivatized carboxylic groups; on average  $\sim 70$  monomers,  $\sim 18$  of which bear an octyl chain.**

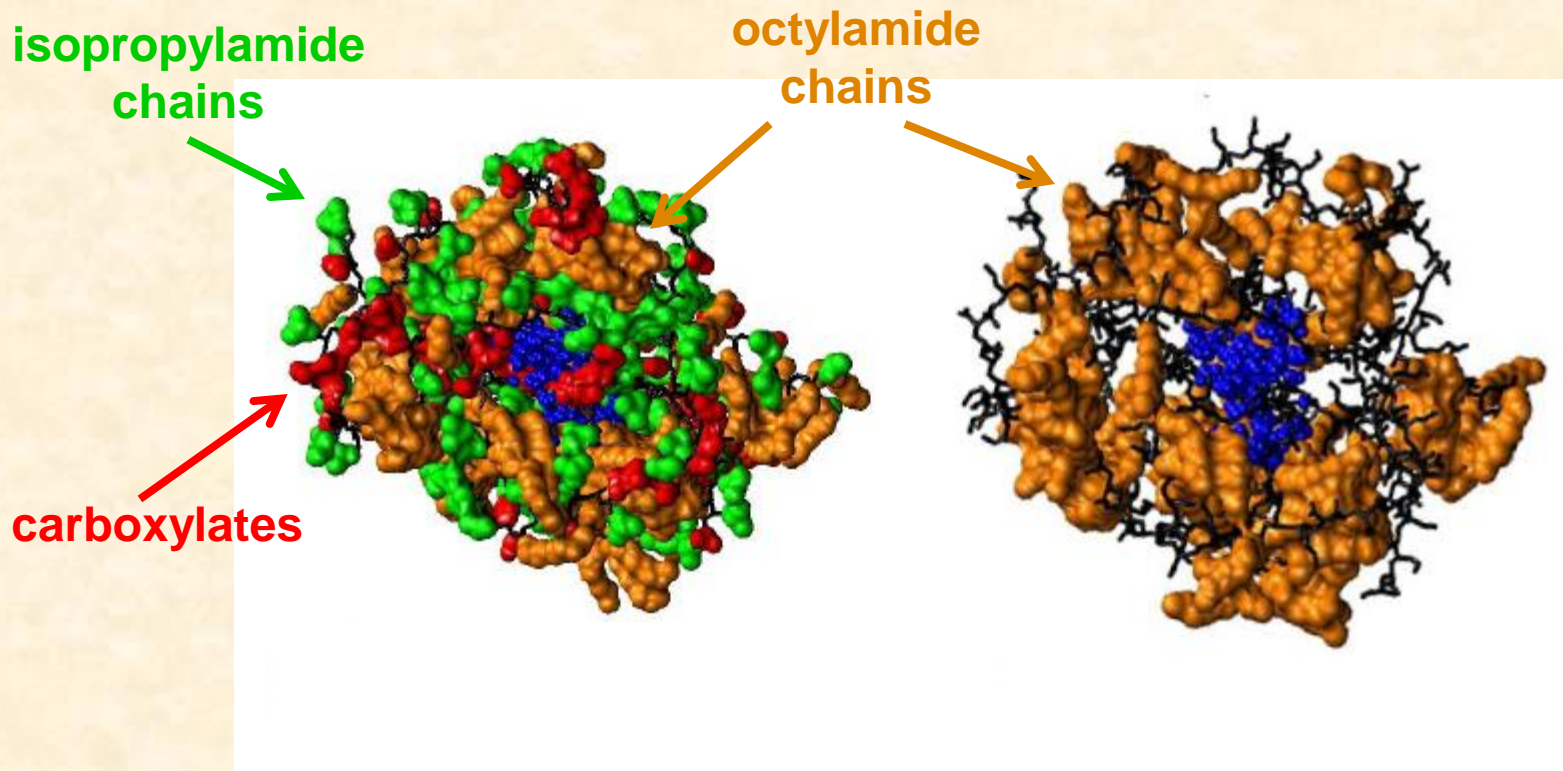
# Solution properties of A8-35

(as analyzed by SANS, SEC, AUC, DLS...)

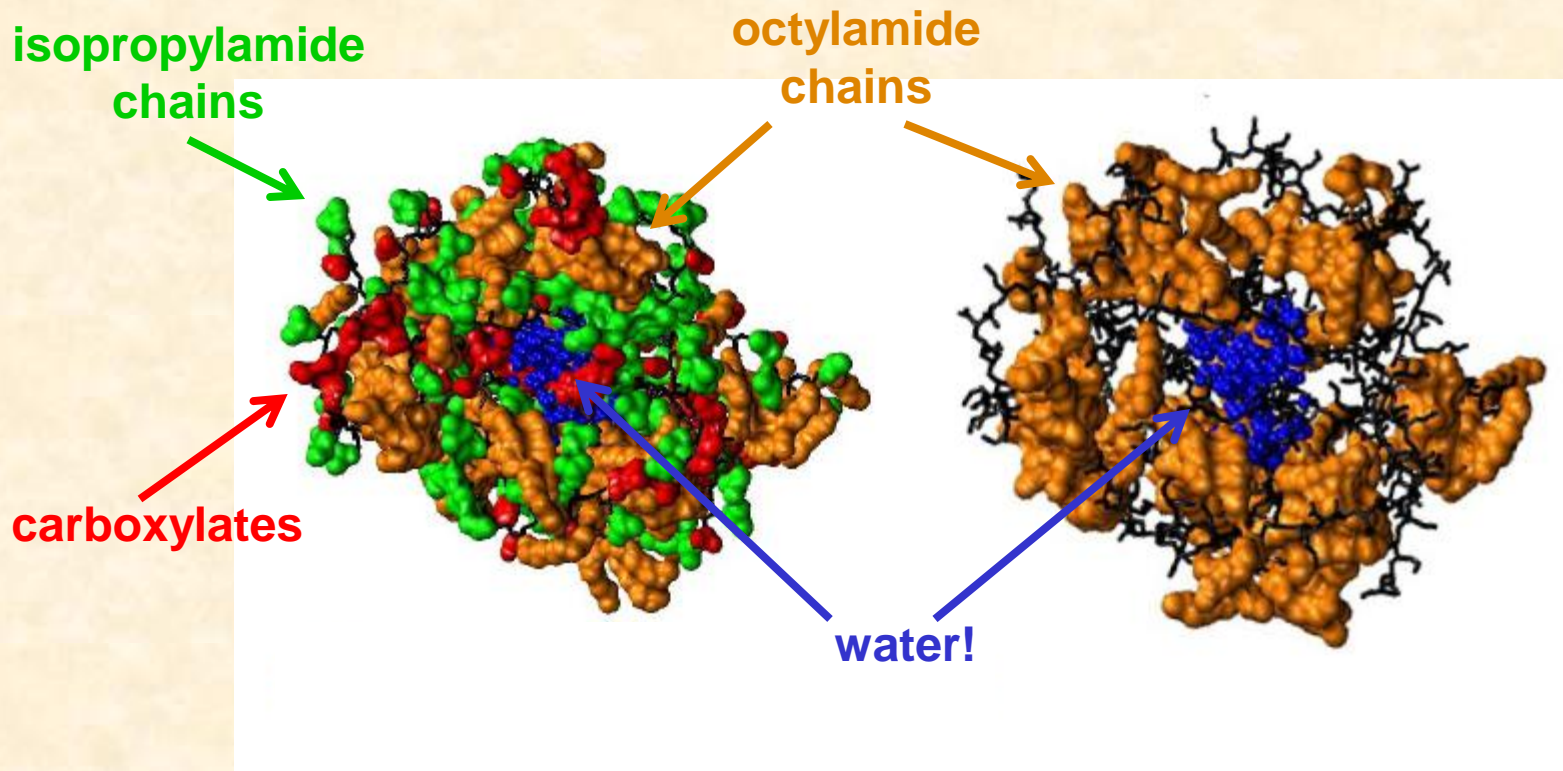
- Fully ionized at pH  $\geq 7$
- Highly soluble in water ( $>200$  g/l)
- Self-associates into small, compact, hydrated particles comprising  $\sim 4$  molecules ( $M \approx 40$  kDa,  $R_S \approx 3.15$  nm)



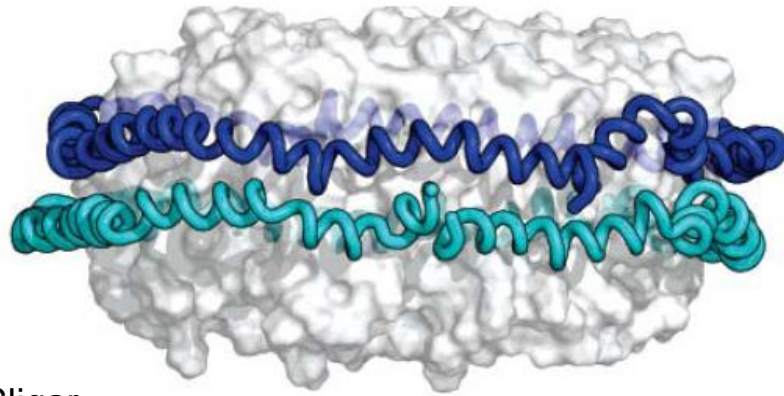
# Molecular dynamics of A8-35 particles



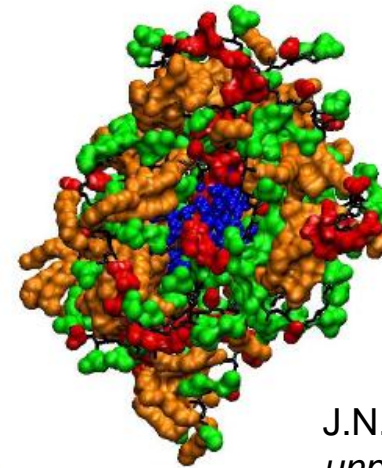
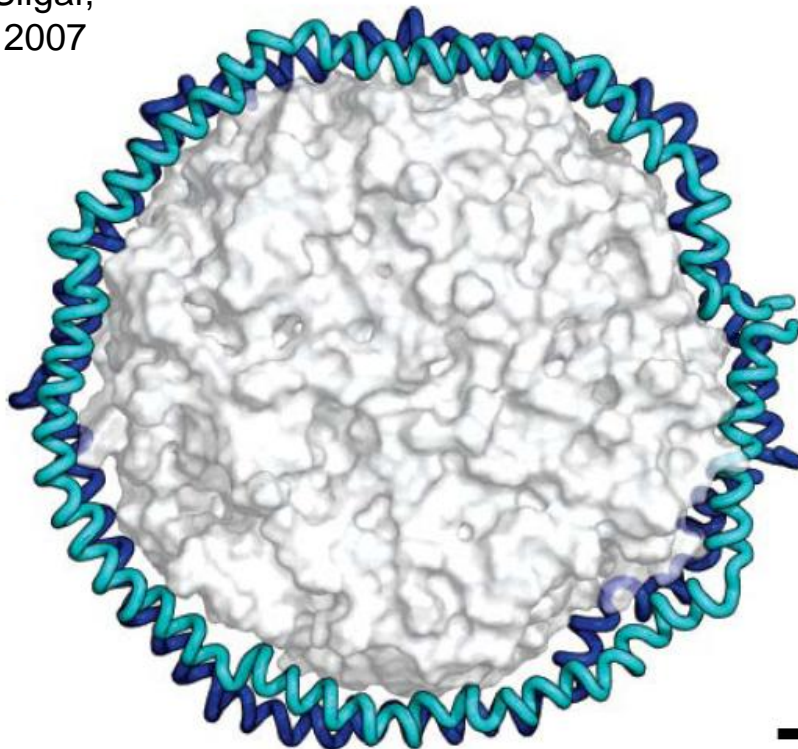
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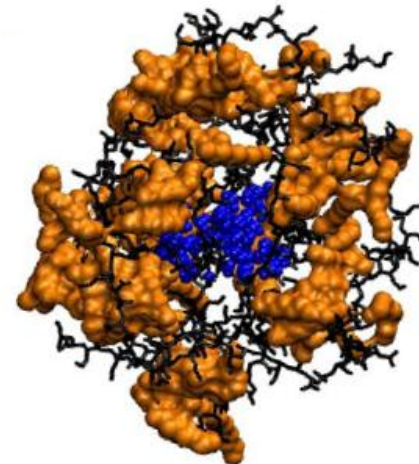
# A8-35 particles vs. nanodiscs



From Nath & Sligar,  
*Biochemistry*, 2007



J.N. Sachs,  
*unpublished data*



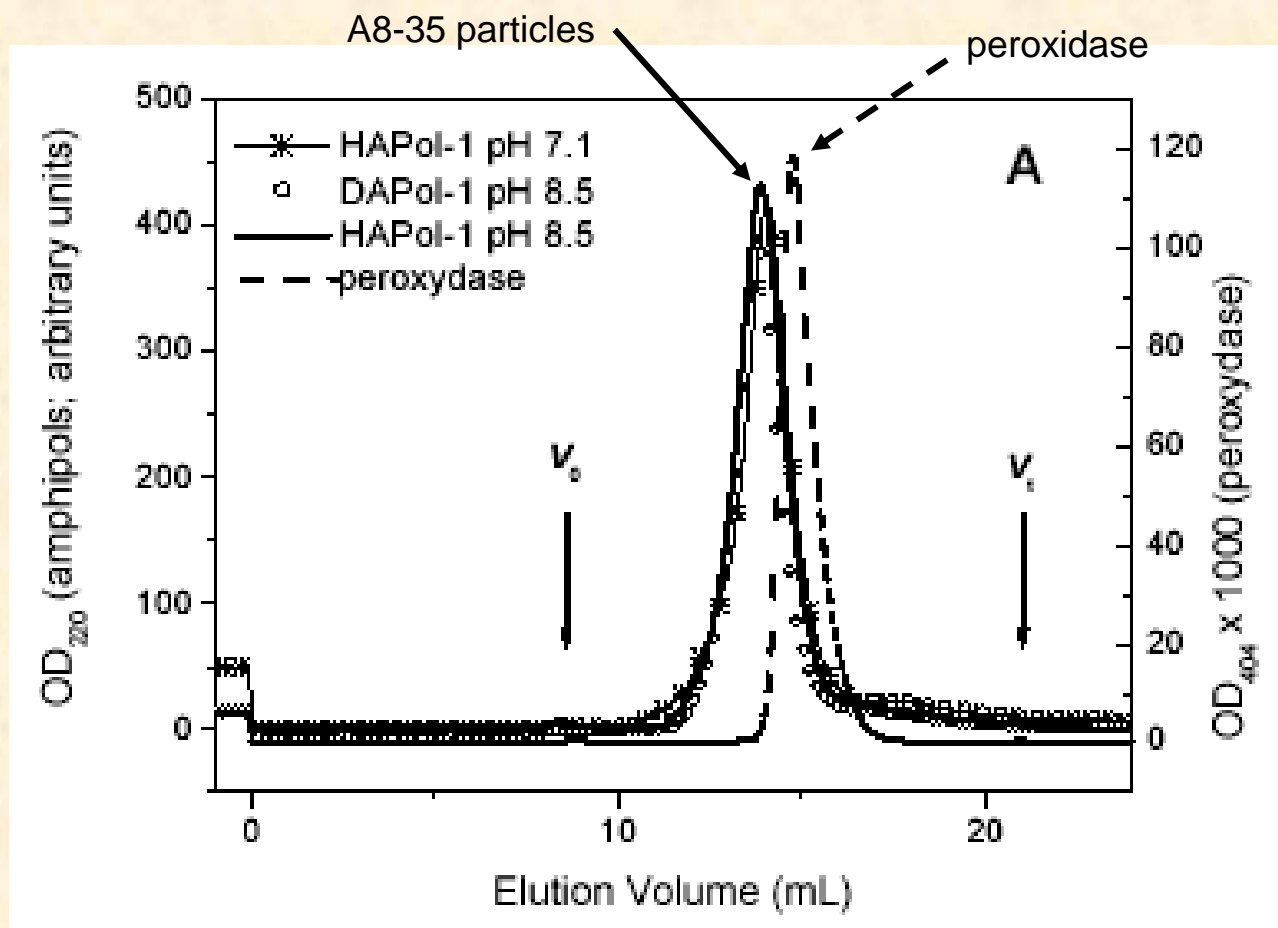
4 nm



# Solution properties of A8-35

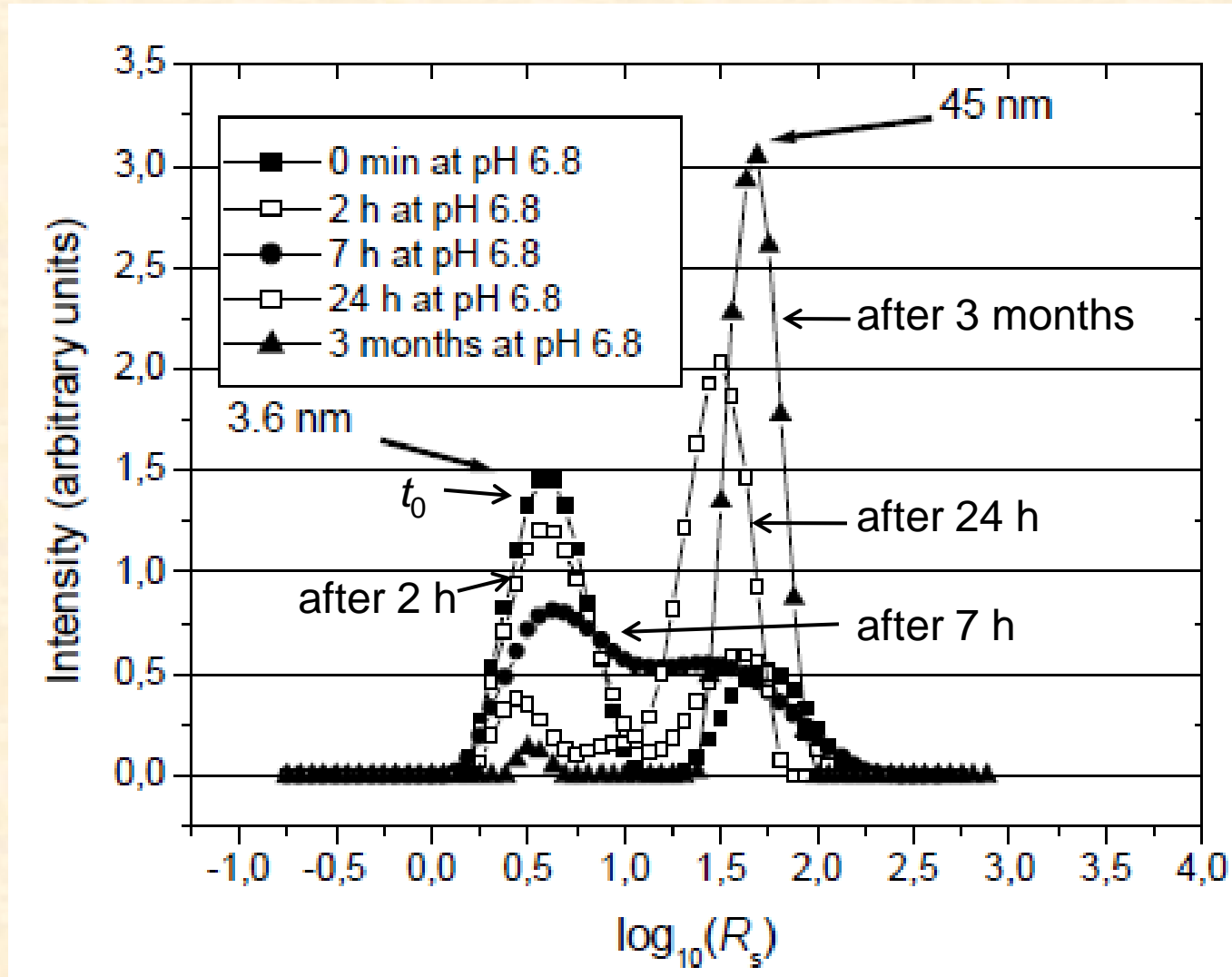
(as analyzed by SANS, SEC, AUC, DLS...)

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- Self-associates into small, compact, hydrated particles comprising  $\sim 4$  molecules ( $M \approx 40 \text{ kDa}$ ,  $R_S \approx 3.15 \text{ nm}$ )
- **pH- and  $\text{Ca}^{2+}$ -sensitive**



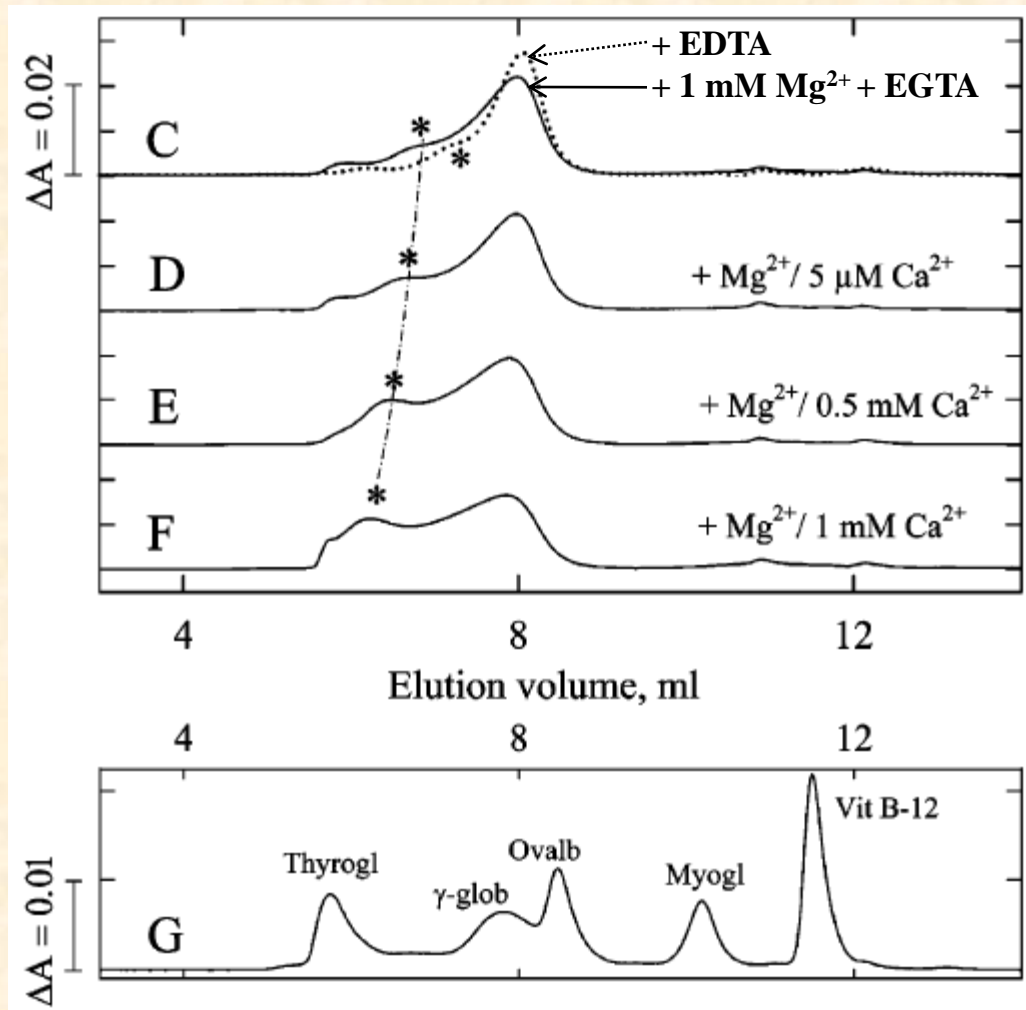
# Aggregation of A8-35 at pH < 7

(light scattering analysis)



# Calcium-induced aggregation of A8-35

(SEC analysis)



# Interaction of amphipols and other surfactants

## Amphipols and detergents are freely miscible

- As free mixed particles:
  - ITC : Diab *et al.*, *Langmuir*, 2007
- At the surface of membrane proteins:
  - FRET: Zoonens *et al.*, *Biochemistry*, 2007
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⇒ **exchange is possible! (in both directions)**

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## Amphipols bind to lipid vesicles (and, in some cases, can fragment them):

- light scattering and electron microscopy: Ladavière *et al.*, *J. Colloid Interface Sci.*, 2001
- fluorescence microscopy: Ladavière *et al.*, *Langmuir*, 2002

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⇒ **delivering membrane proteins to preformed membranes is possible!**

# Diversifying amphipols

## 1. Functionalized or labeled versions of A8-35

- isotopically labeled amphipols ( $^2\text{H}$ ,  $^3\text{H}$ ,  $^{14}\text{C}$ ) (Tribet *et al.*, 1997; Gohon *et al.*, 2004, 2006, 2008)
- fluorescently labeled A8-35 (NBD, fluoresceine, rhodamine...) (Zoonens *et al.*, 2007)
- tagged A8-35 (biotinylated (Charvolin *et al.*, 2009); in the tube: oligonucleotide-tagged, histidine-tagged...)
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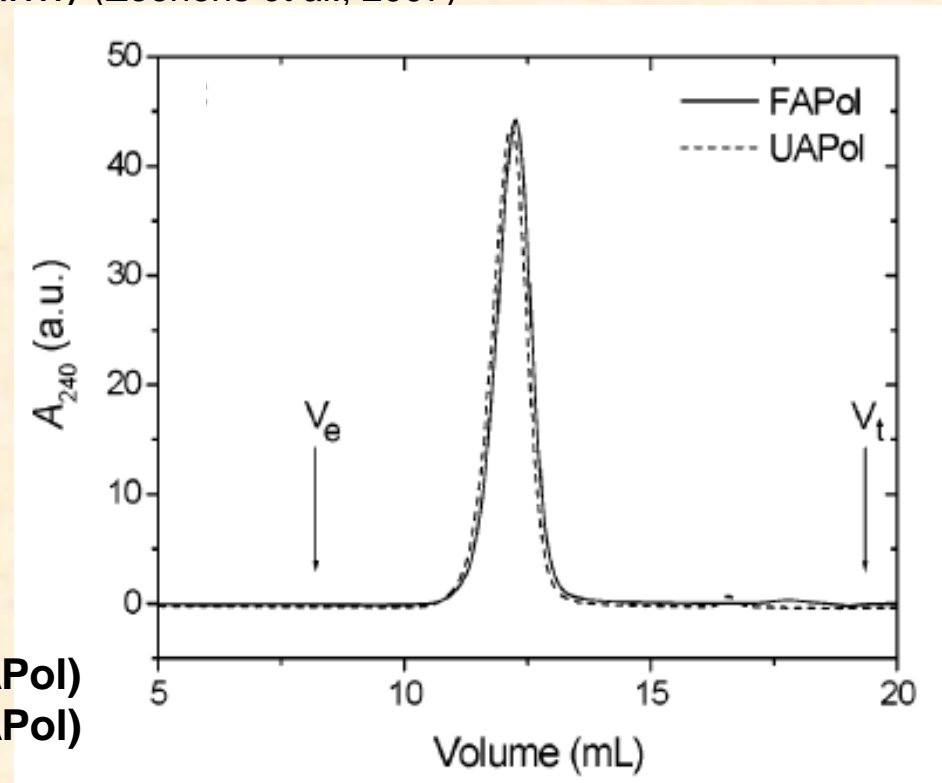
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**Same physical-chemical properties!**

SEC analysis of "universal" (UAPol) vs. NBD-labeled A8-35 (FAPol)



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- A8-75, A34-35, A34-75, A2-75... (Tribet *et al.*, 1996, 1997; Gohon, 1996)
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- **Similar but possibly different physical-chemical and/or biochemical properties**
- **Potential remains to be fully exploited**

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## 3. Amphipols with radically different chemical structures

- non-ionic amphipols (Prata *et al.*, 2001; Sharma *et al.*, 2008; Bazzacco *et al.*, 2009) (B. Pucci)
- zwitterionic/cationic amphipols (PMAL-B-100) (Gorzelle *et al.*, 2002) (C.R. Sanders/*Anatrace*)
- amphipols with phosphorylcholine polar heads (Diab *et al.*, 2007) (F. Winnik/C. Tribet)
- sulfonated amphipols (Picard *et al.*, 2006; Dahmane *et al.*, *in preparation*) (*Anatrace*; F. Giusti)

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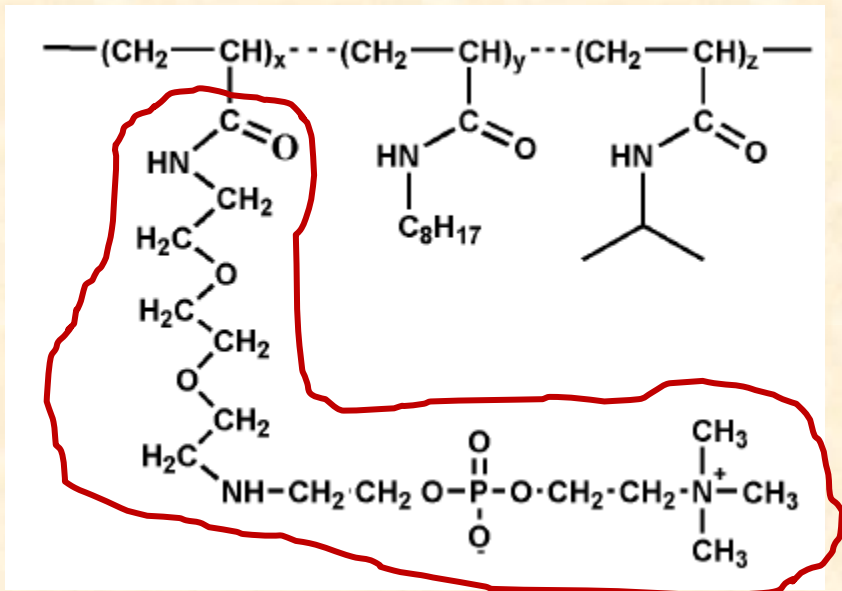
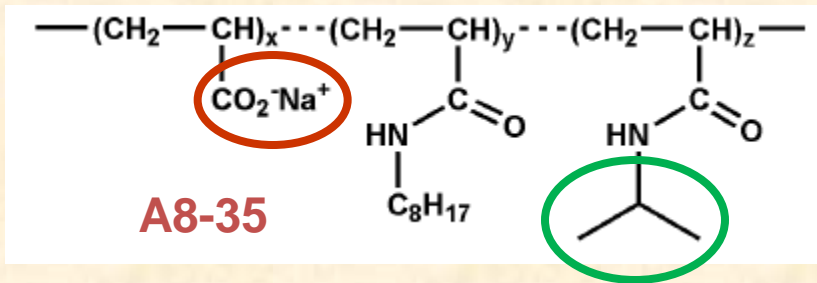
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- sulfonated amphipols (Picard *et al.*, 2006; Dahmane *et al.*, *in preparation*) (*Anatrace*; F. Giusti)



**Different physical-chemical properties**

**⇒ back to square one!**

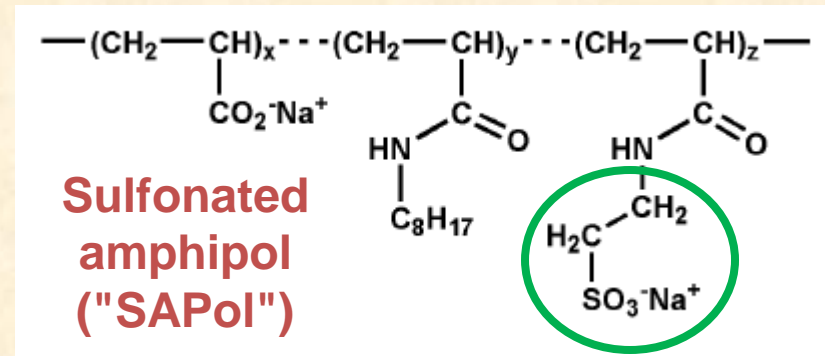
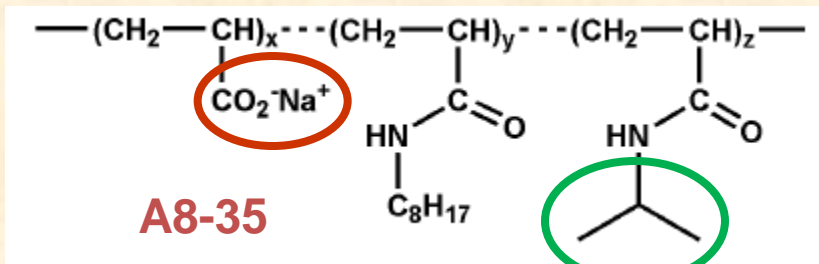
# Most heavily studied amphipols



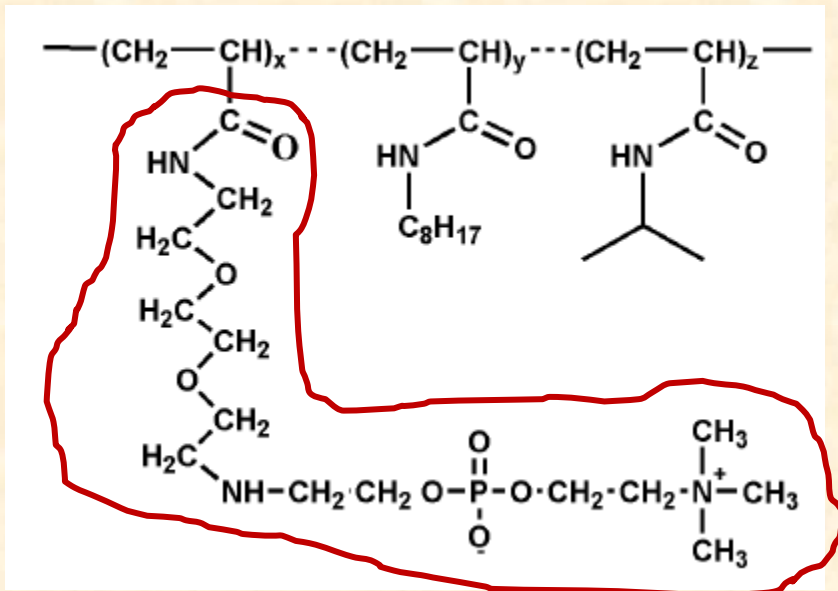
Developed by F. Winnik (U. Montreal)  
and C. Tribet (ENS, Paris)

Phosphocholine-based amphipol  
("PC-APol")

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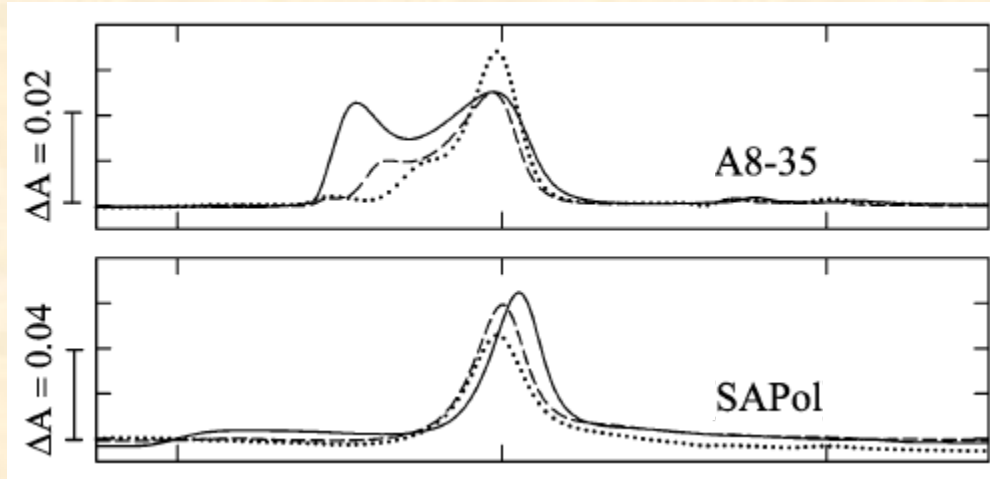
Developed by **F. Giusti**  
(IBPC, Paris)



**Phosphocholine-based amphipol**  
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# SAPols, PC-APols and NAPols are insensitive to low pH or calcium



**SEC in the presence of:**

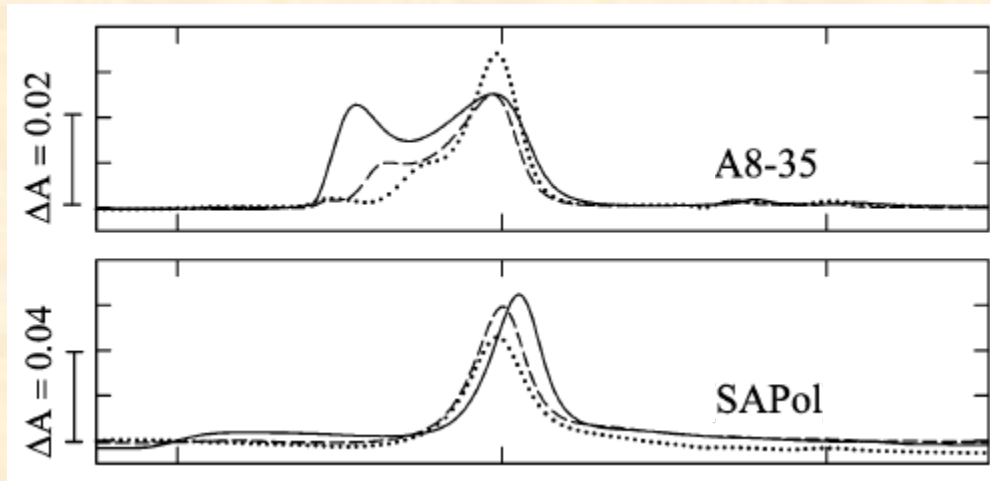
..... 0.5 mM EDTA

----- 1 mM  $Mg^{2+}$

————— 1 mM  $Mg^{2+}$  + 0.5 mM  $Ca^{2+}$

From Picard *et al.*, *Biochemistry*, 2006

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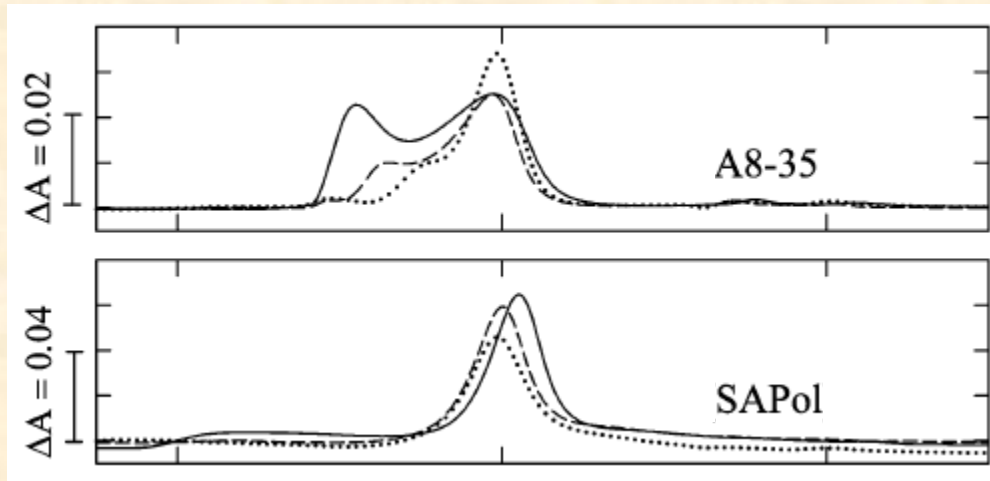
From Picard *et al.*, *Biochemistry*, 2006

Conditions	NaCl	pH	Ca <sup>2+</sup>	NaCl	pH	Ca <sup>2+</sup>
	1 M	5	12 mM	1 M	5	12 mM
Amphipol	A8-35			C22-43 (PC-APol)		
Turbidity at <i>t</i> =0	+	+	+	-	-	+ <sup>a</sup>
% BR in supernatant <i>t</i> =15 min	80	0	22	91	97	94

**Stability of bacteriorhodopsin/  
amphipol complexes in the  
presence of 1 M NaCl, pH 5,  
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**Similar results with non-ionic amphipols** (Bazzacco, 2009, and articles in preparation).

## **New avenues opened by novel amphipols**

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- **Non-ionic amphipols give access to isoelectrofocusing** (*cf.* presentation by P. Bazzacco)
- **Other applications remain to be explored** (ion exchange chromatography, membrane protein crystallization...)

# Main contributors to the amphipol project within our laboratory

(1995-2010)

● present

● past

**Paola Bazzacco** (NAPols, immobilization, folding GPCRs)

**Laurent Catoire** (solution NMR, SAPols)

**Delphine Charvolin** (crystallization, immobilization)

**Tassadite Dahmane** (folding BR and GPCRs, SAPols, Ca<sup>2+</sup>-ATPase...)

**Fabrice Giusti** (chemistry chief cook)

**Yann Gohon** (BR, NAPols, PC-APols...)

**Christel Le Bon** (functionalized APols)

**Kyu-Ho Park** (cell-free synthesis)

**Martin Picard** (crystallization)

**Florent Rouvière** (chemistry)

**Manuela Zoonens** (tOmpA, FRET, NMR)

## Major collaborations

<b>C. Tribet</b> (ESPCI → ENS, Paris)	<b>polyacrylate- &amp; PC-based amphipols</b>
<b>B. Pucci &amp; coworkers</b> (University of Avignon)	<b>non-ionic amphipols</b>
<b>F. Winnik, C. Diab</b> (U. de Montréal)	<b>phosphorylcholine-based amphipols, ITC</b>
<b>C. Ebel, F. Gabel, P. Timmins</b> (IBS & ILL, Grenoble)	<b>ultracentrifugation, small angle neutron scattering</b>
<b>D.M. Engelman</b> (Yale)	<b>small angle neutron scattering</b>
<b>J.N. Sachs</b> (U. Minnesota)	<b>molecular dynamics</b>
<b>E.A. Berry</b> (University of Berkeley)	<b>3D crystallization of <i>bc<sub>1</sub></i>/amphipol complexes</b>
<b>J.H. Kleinschmidt, C. Pocanschi</b> (U. of Constance)	<b>renaturation of <math>\beta</math>-barrel membrane proteins</b>
<b>F. Rappaport</b> (IBPC, Paris)	<b>BR photocycle</b>
<b>P. Champeil, M. Picard</b> (CEA, Saclay)	<b>sarcoplasmic calcium ATPase activity &amp; stability</b>
<b>K. Leonard, M. Flötenmeyer</b> (EMBL, Heidelberg)	<b>EM of Complex I/APol complexes</b>
<b>J.-L. Banères &amp; coworkers</b> (CNRS, Montpellier)	<b>folding of GPCRs</b>
<b>K. Martinez, J.-B. Perez</b> (U. of Copenhagen)	<b>ligand binding to nAChR immobilized onto beads</b>

## Funding

**CNRS**

**Human Frontier Science Program Organization**

**EC (Biotech, IMPS)**

**Paris-7 University**

**Ministère de la Recherche et de la Technologie**

**Fondation Rothschild**

**ANR**