Membrane protein stability in aqueous solutions; destabilization by detergents

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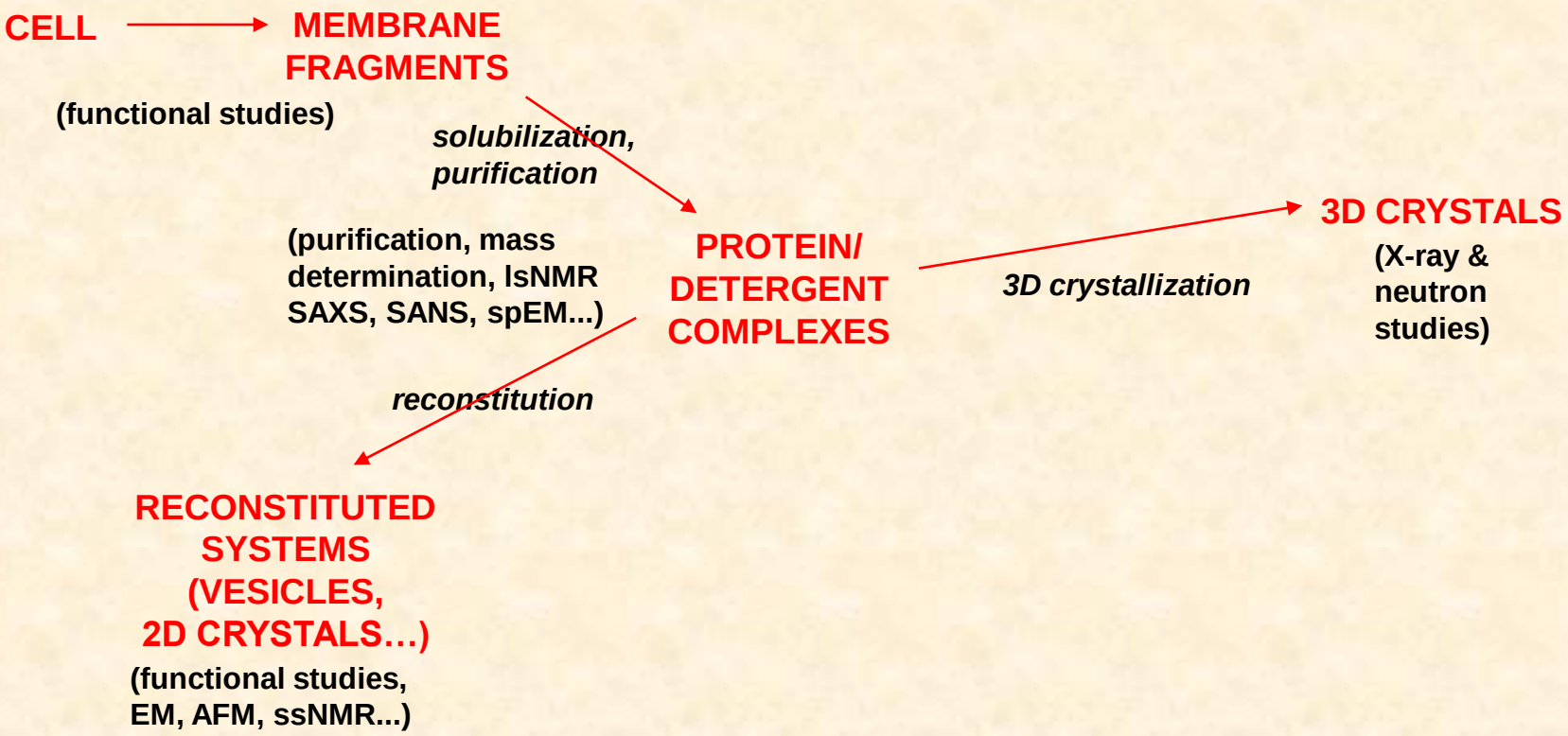
Experimental systems for studying membrane proteins



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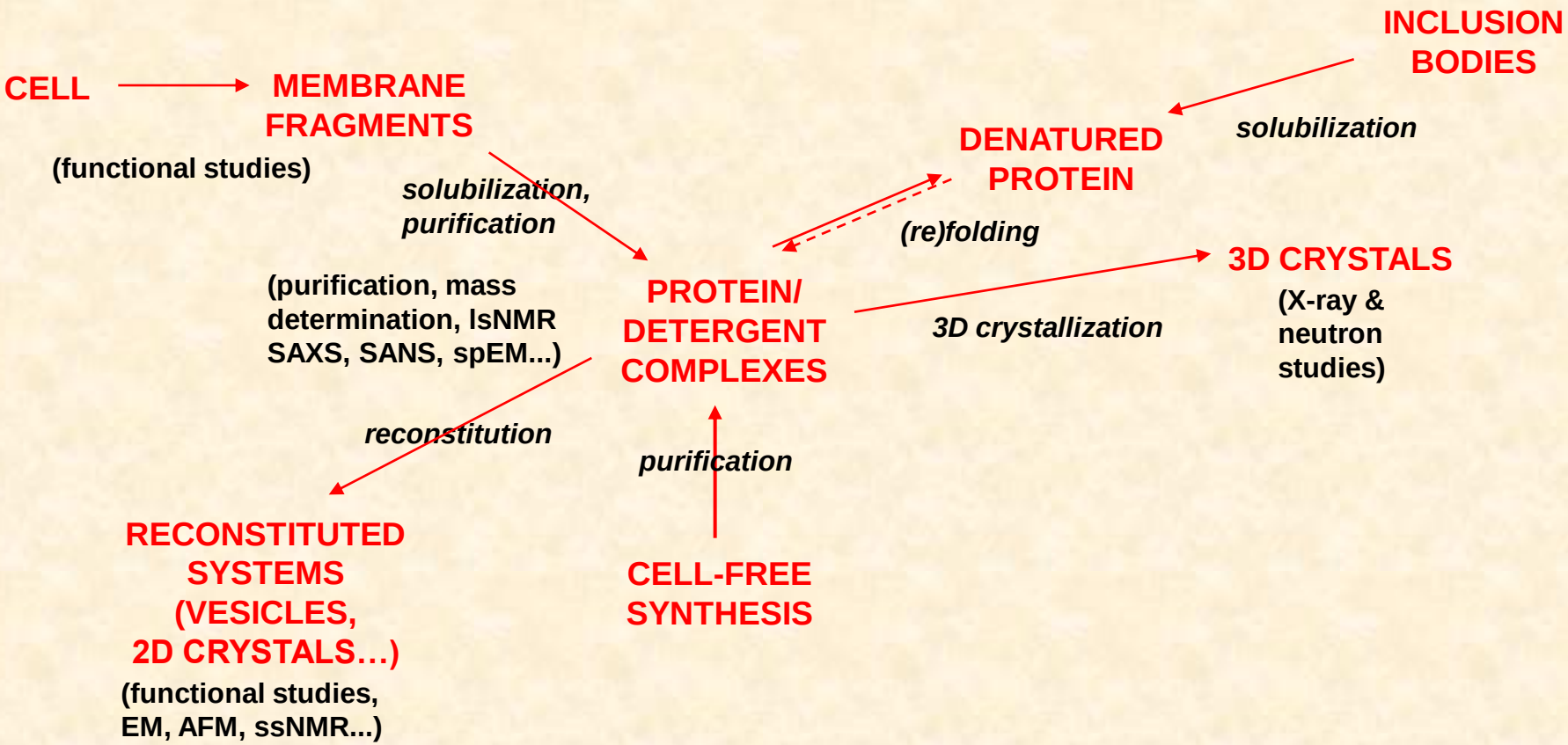

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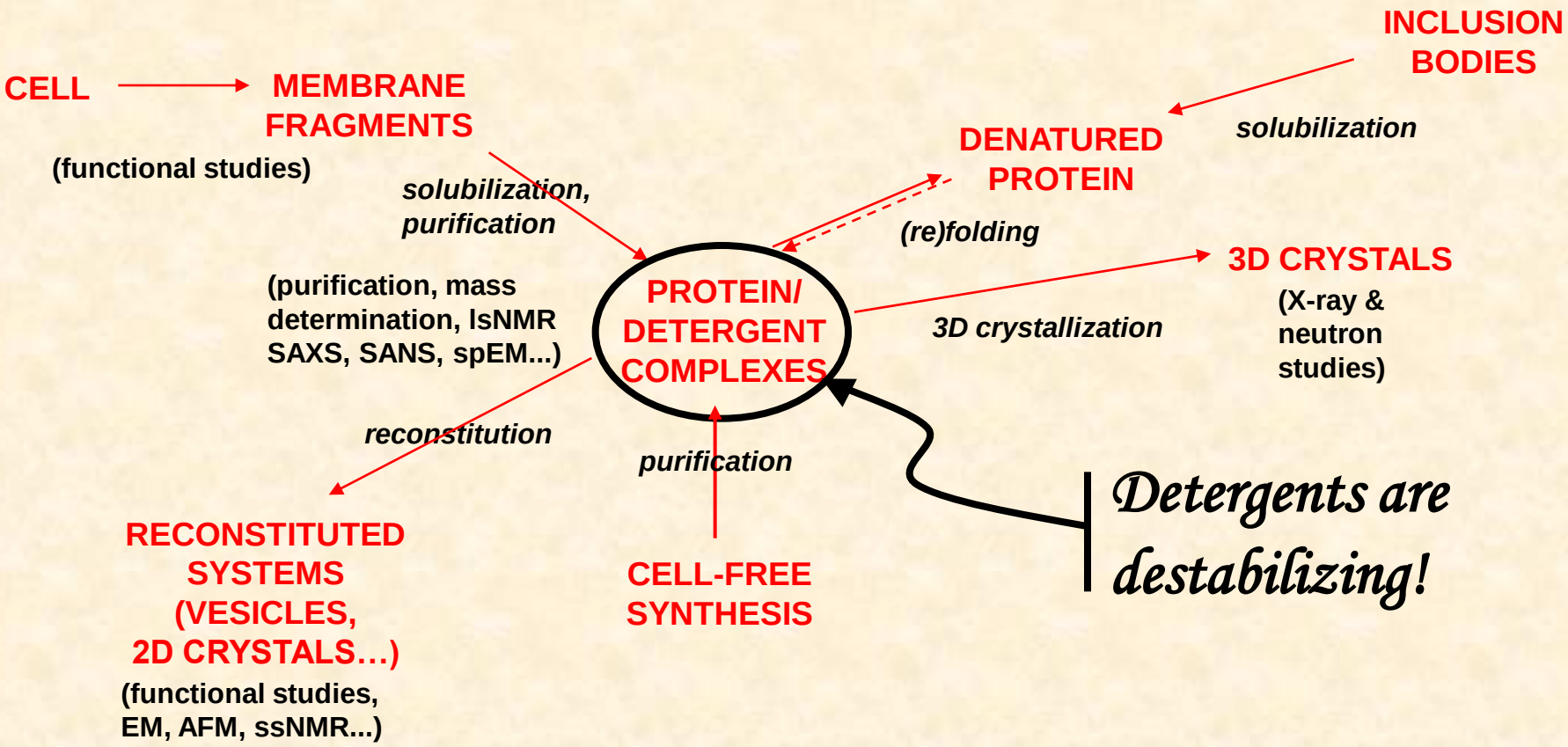
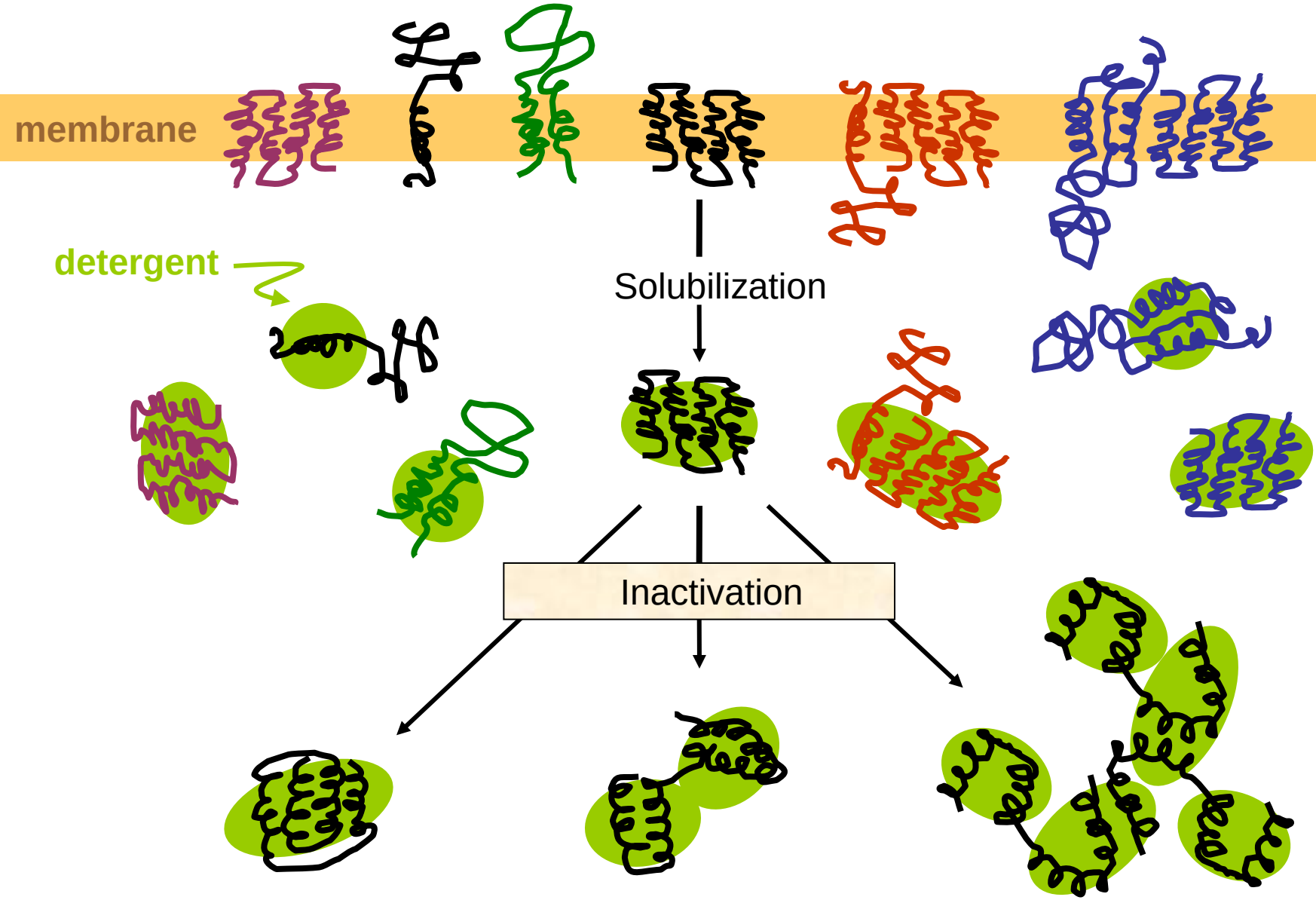
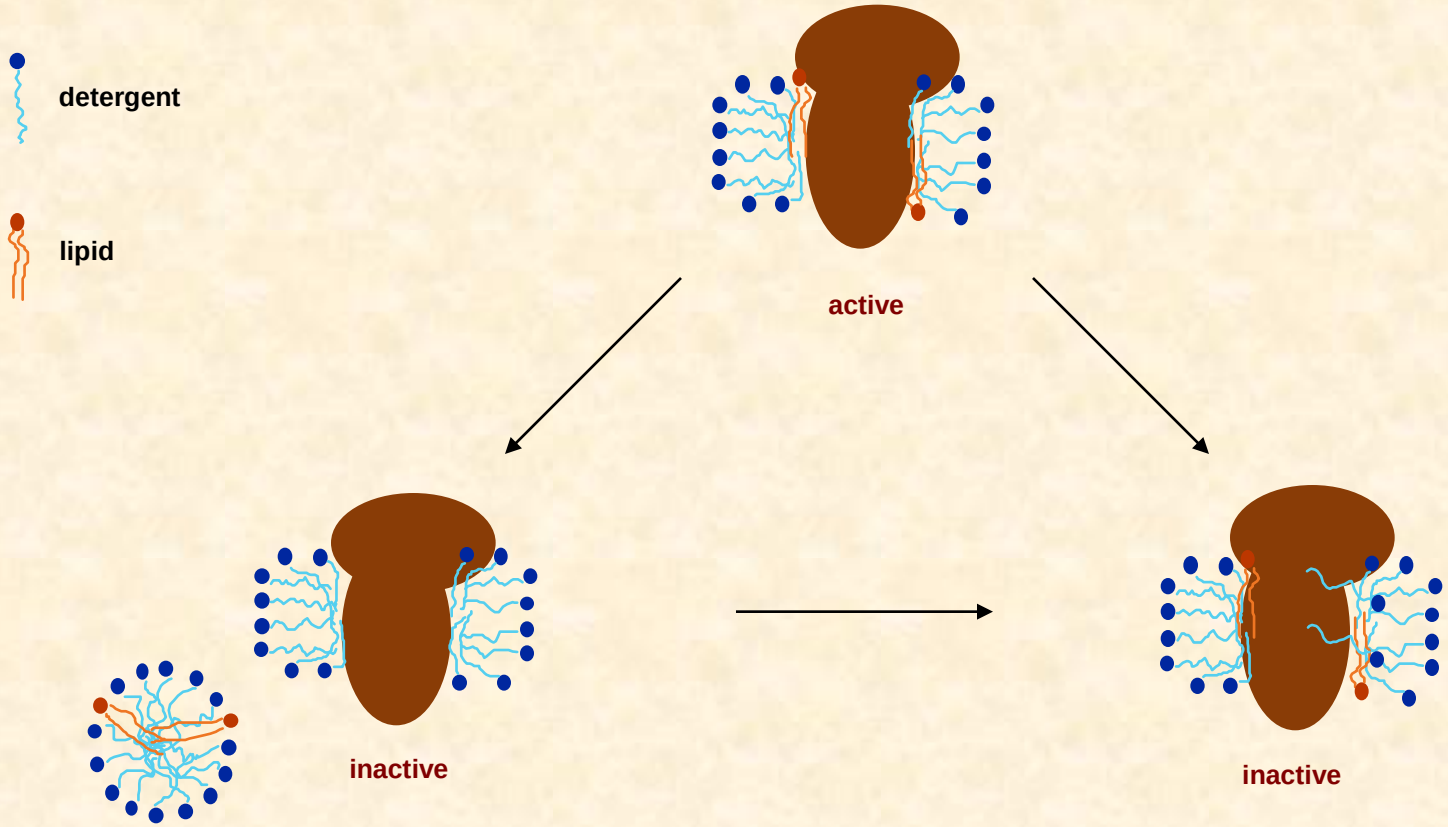


From solubilization to inactivation



Molecular mechanisms of membrane protein inactivation by detergents

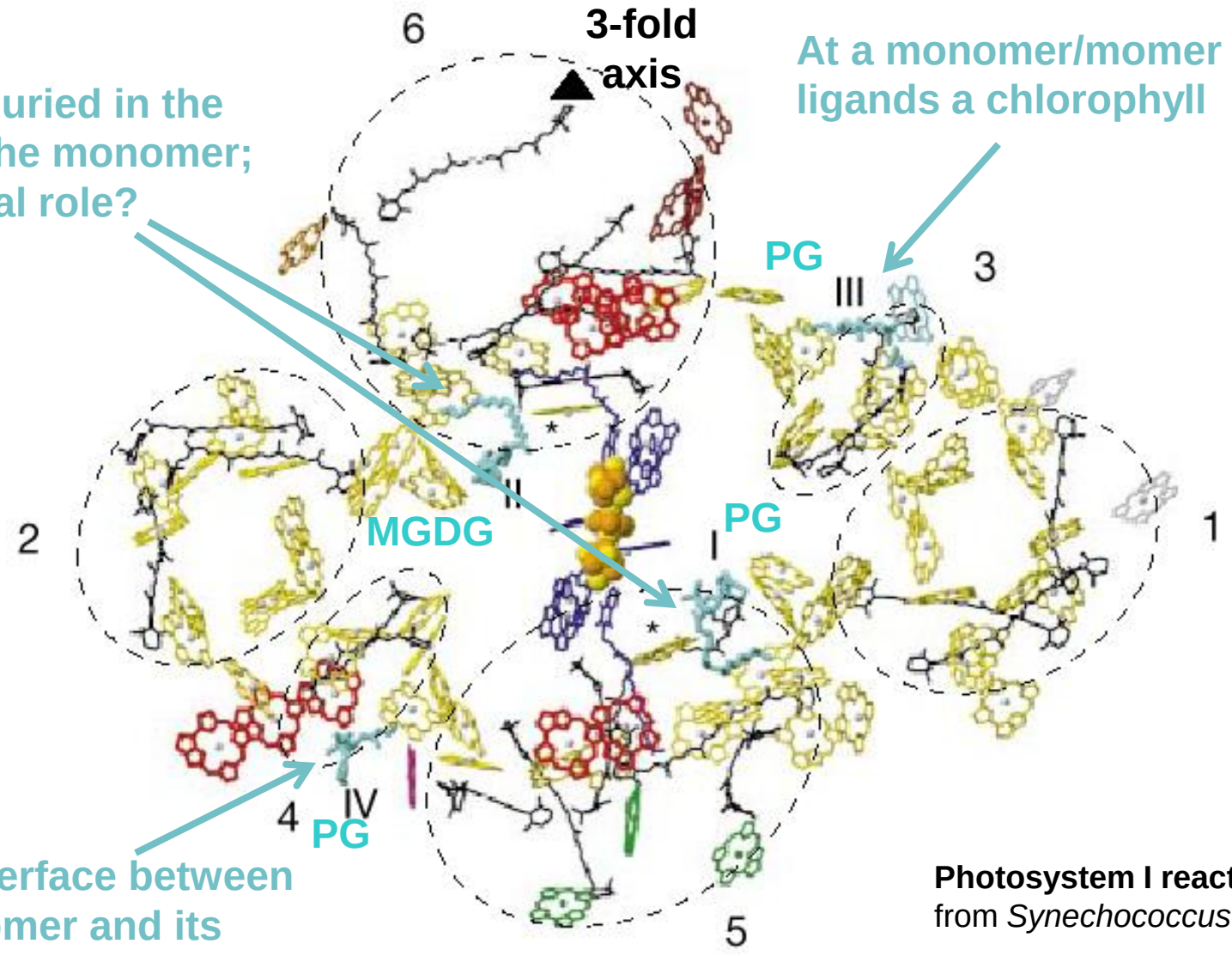
Two non-exclusive hypotheses



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

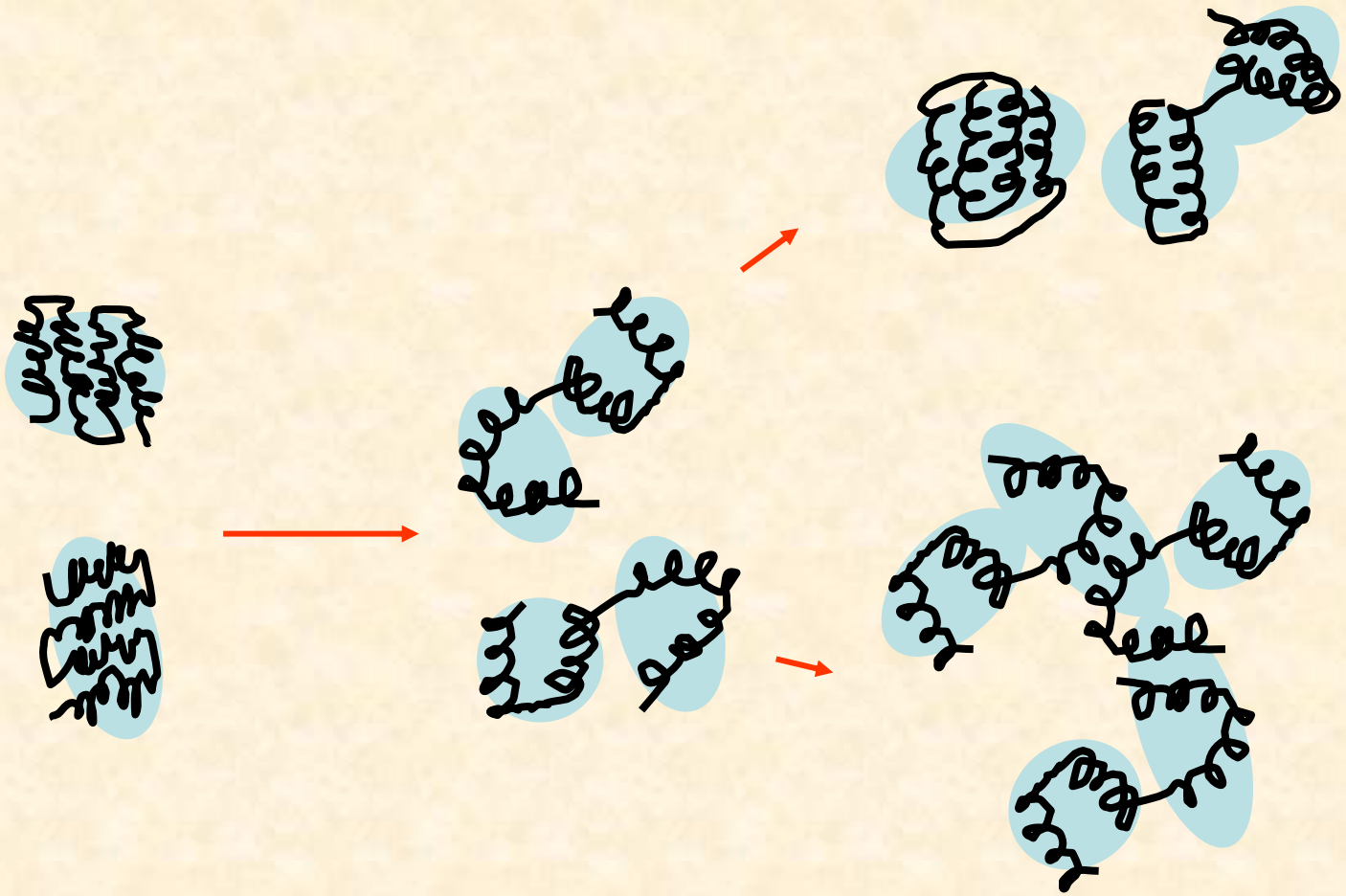


Photosystem I reaction center
from *Synechococcus elongatus*

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!

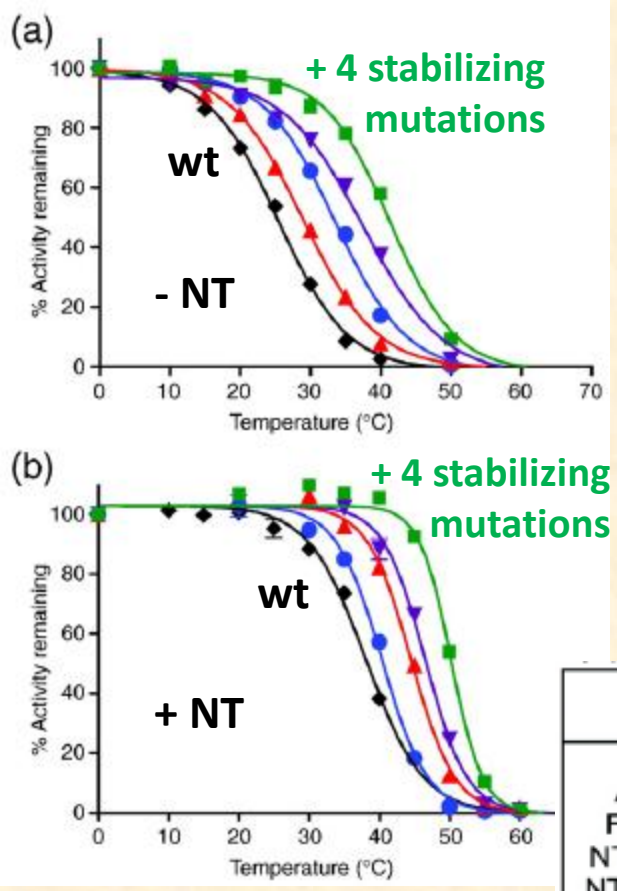
For references, see *e.g.:*

- 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)	ΔT_m (°C)	T_m (°C)	ΔT_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

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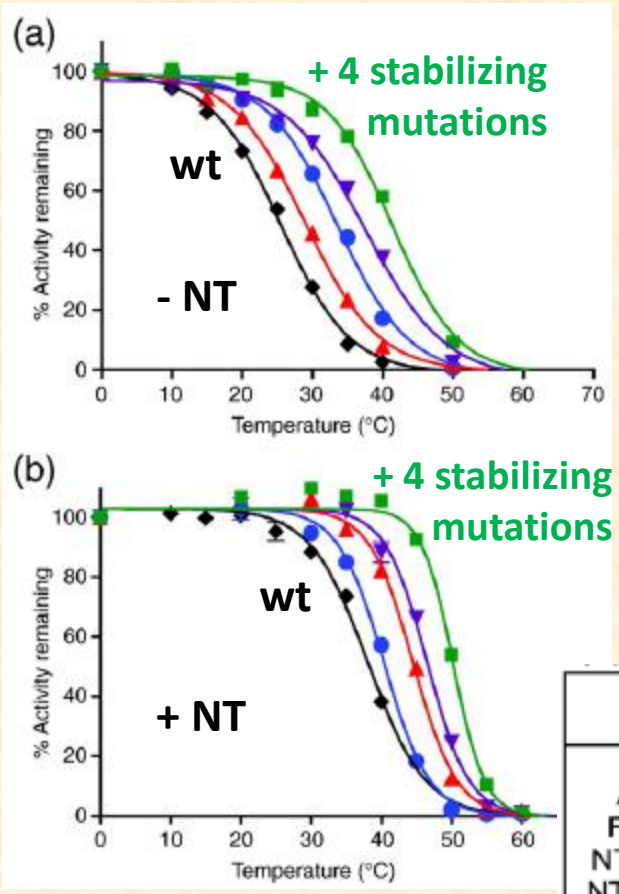

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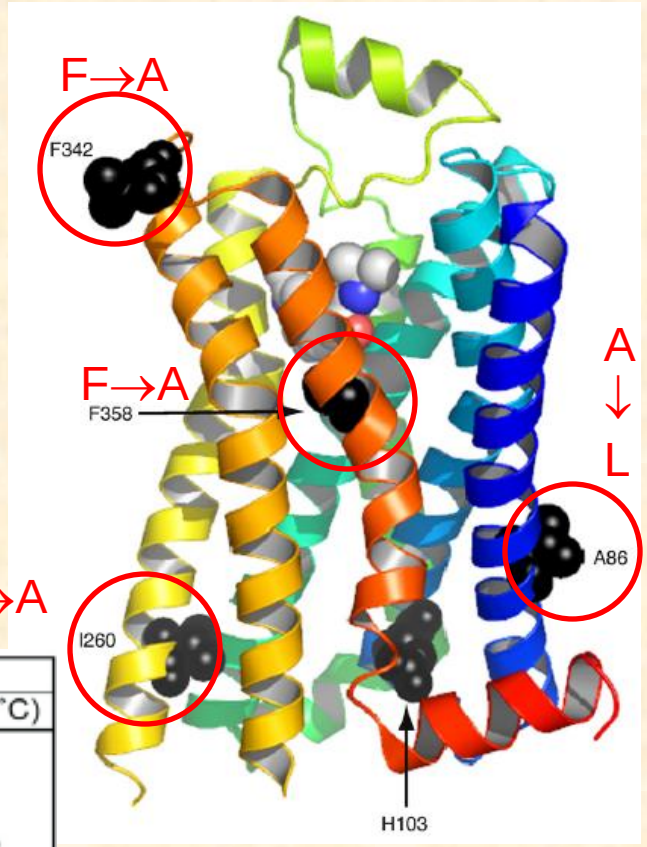

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- Select or engineer more stable proteins (ex. DAGK, GPCRs) (J.U. Bowie, C.G. Tate)
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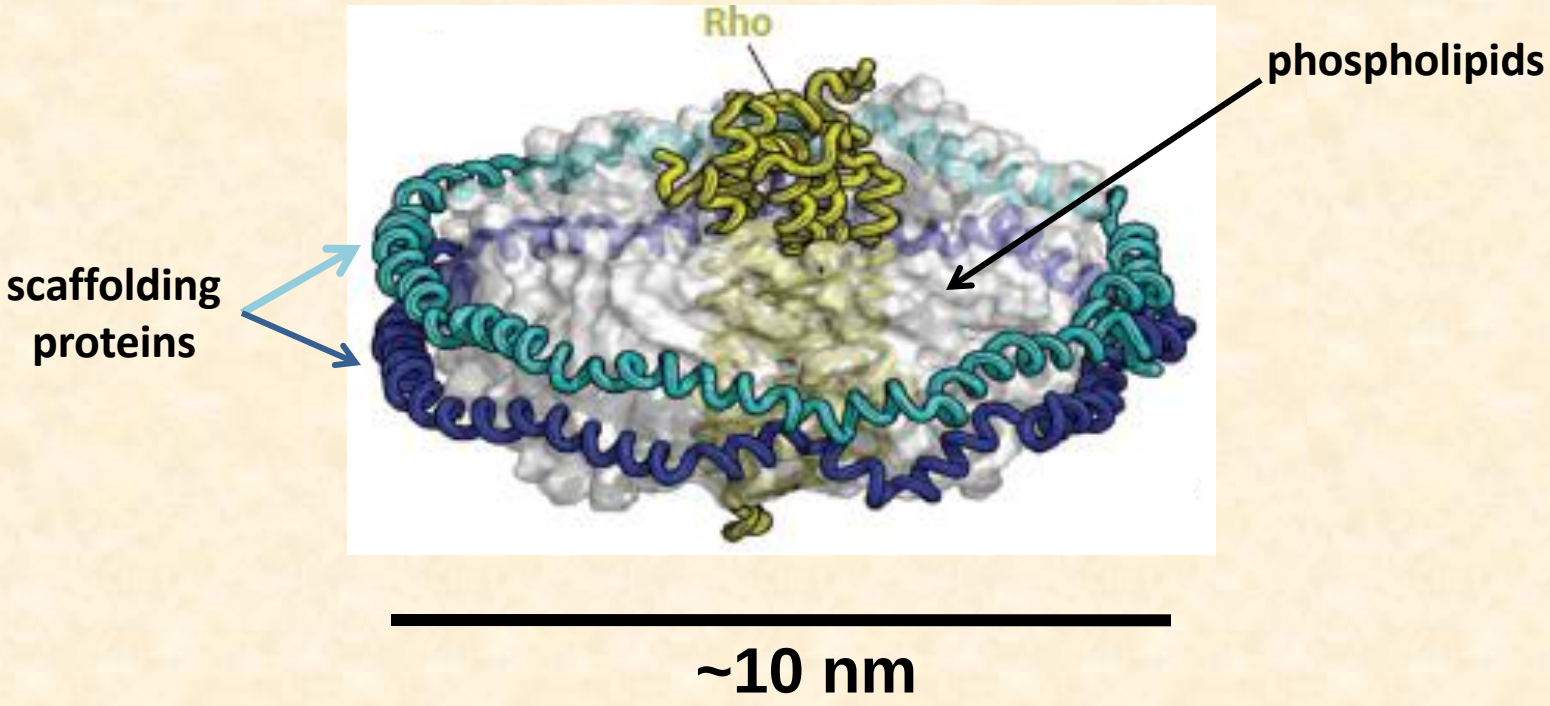

From Nath & Sligar, *Biochemistry*, 2007

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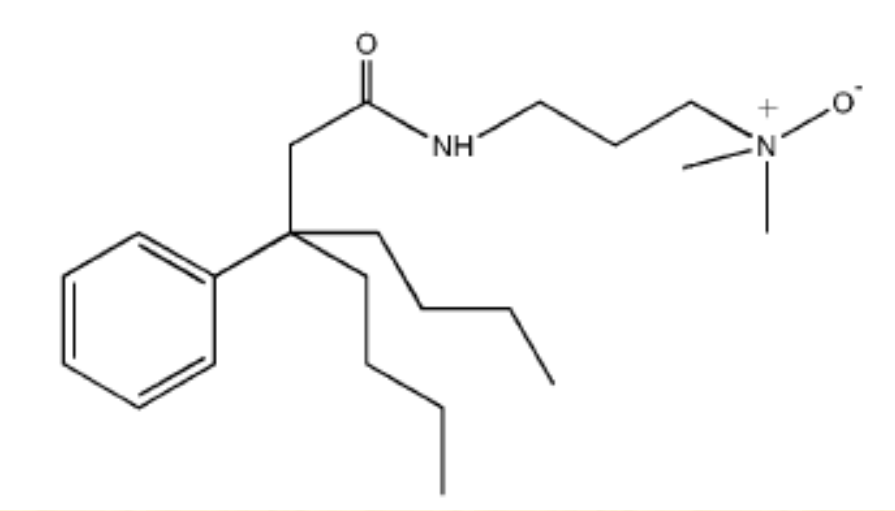

From Yu *et al*., *Protein Sci.*, 2000

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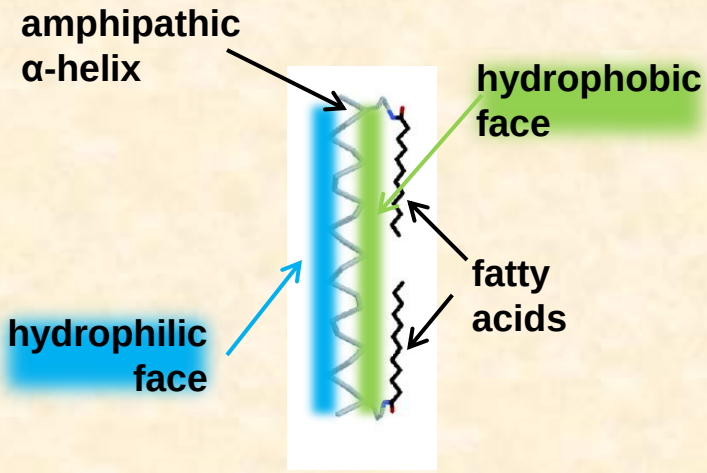

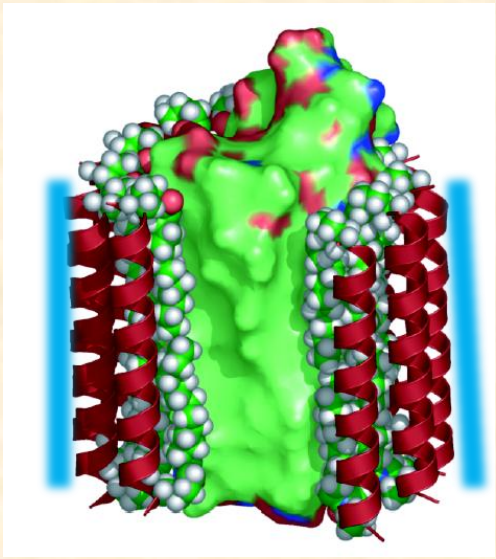

McGregor *et al., Nat. Biotechnol.*, 2003

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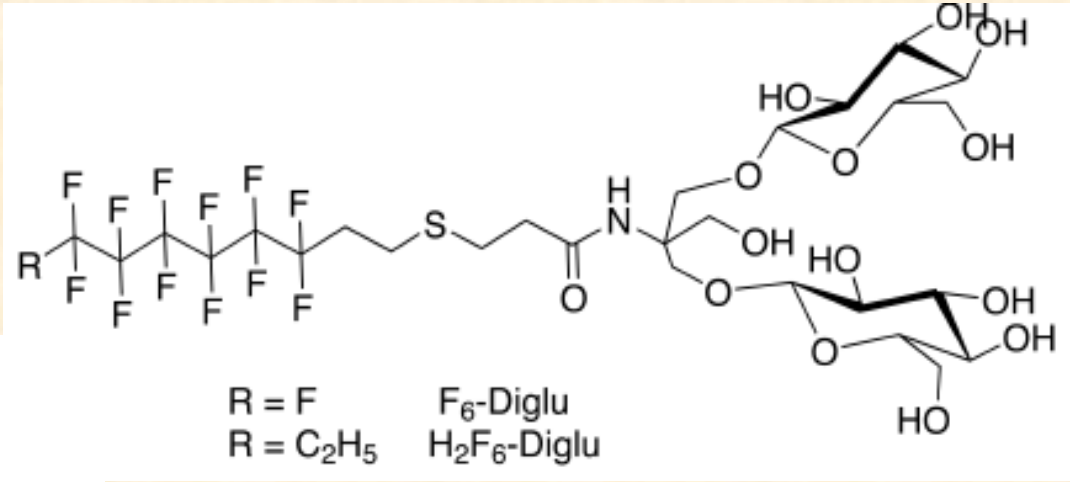

Breyton *et al.*, *Biophys. J.*, 2009

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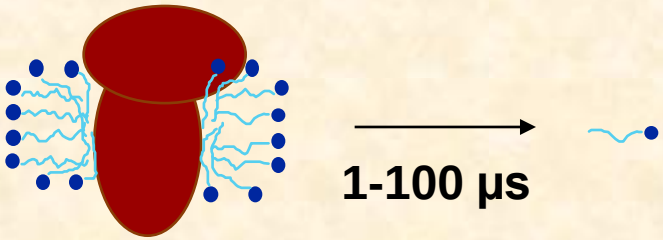
  - Fluorinated surfactants (B. Pucci & J.-L. Popot)
  - "Amphipols" (C. Tribet, R. Audebert & J.-L. Popot)

Rationales for the design of amphipols

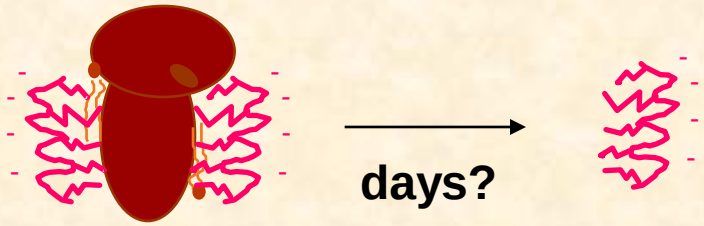
Principle

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_{off} → quasi-irreversible attachment

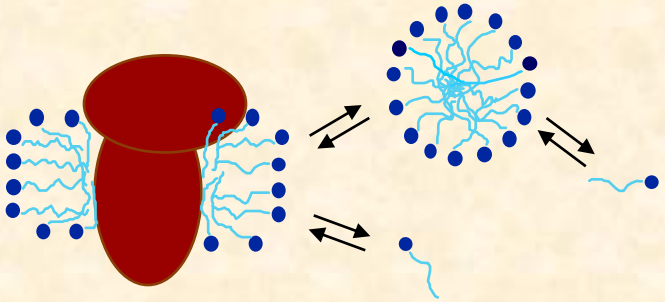


detergent



amphipol

- small K_D → very low equilibrium concentration of free surfactant



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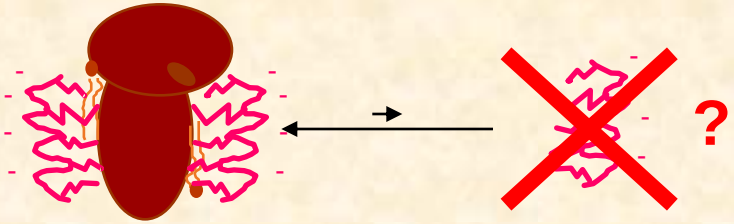
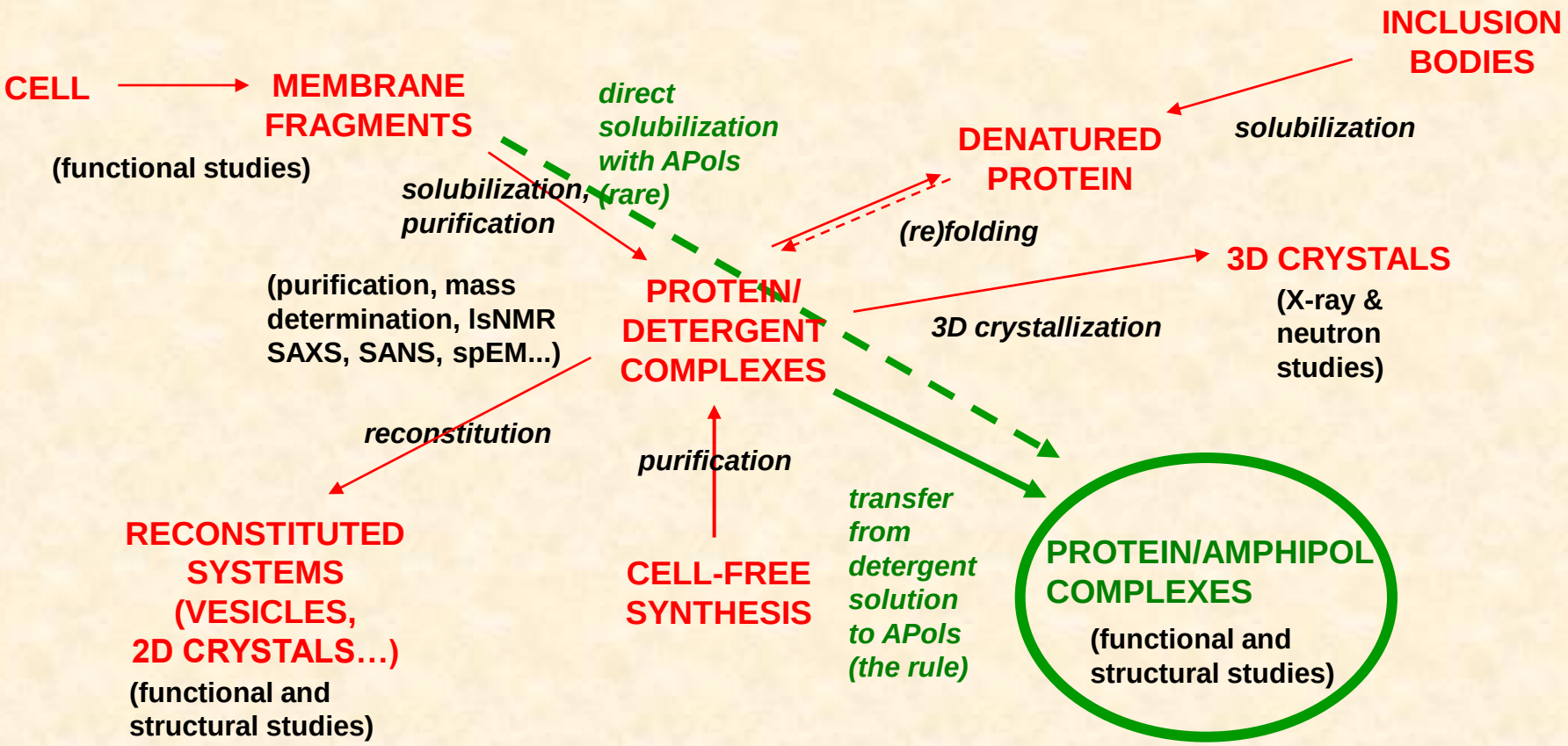


amphipol

Experimental systems for studying membrane proteins



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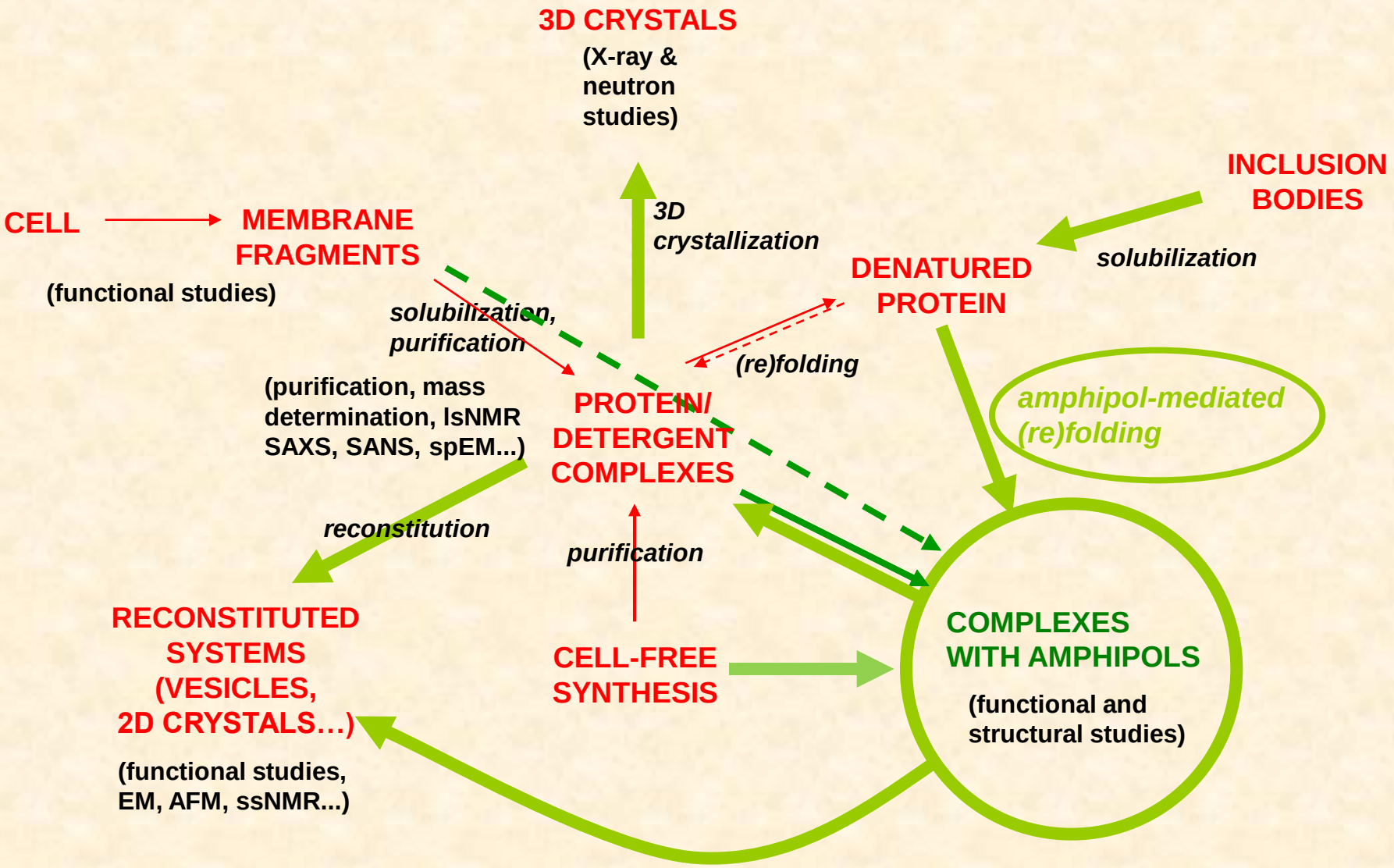

Opportunities and constraints associated with the use of amphipols, nanodiscs, and fluorinated surfactants

Technology	Amphipols	Nanodiscs	Fluorinated surfactants
Membrane protein (MP) stabilization	+	+	+
Functional studies	+	++	+
Mediating MP immobilization for ligand binding measurements	+	+	–
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	+	±	+
Solution NMR	+	±?	?
Solid-state NMR	+?	+	–
Three-dimensional crystallization	±	–?	+?
Two-dimensional crystallization	–	–?	+?
Trapping MP supercomplexes	+	±	+
EM, AFM (single particles)	+	+	+
Transferring MPs to preformed membranes	+	?	+
Folding full-length MPs to native state	+	?	+
MP cell-free translation	+	+	+
Isoelectrofocusing and two-dimensional gels	+	+?	?
MP mass spectrometry	+	?	?

From Popot, *Annu. Rev. Biochem., in the press*

Structure and properties of amphipols; functionalized amphipols

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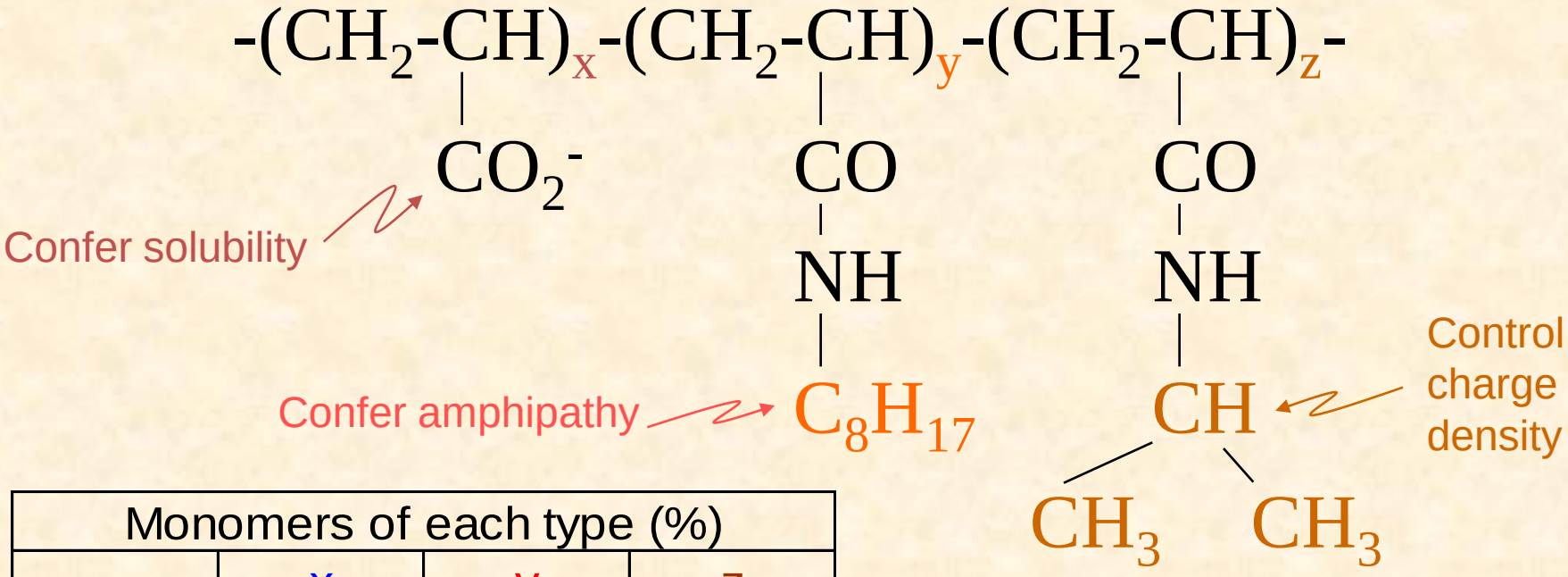
Polyacrylate-based amphipols

Synthesis: graft <u>at random</u> long- and short-chain amines onto <u>polydisperse</u> polyacrylic acid



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

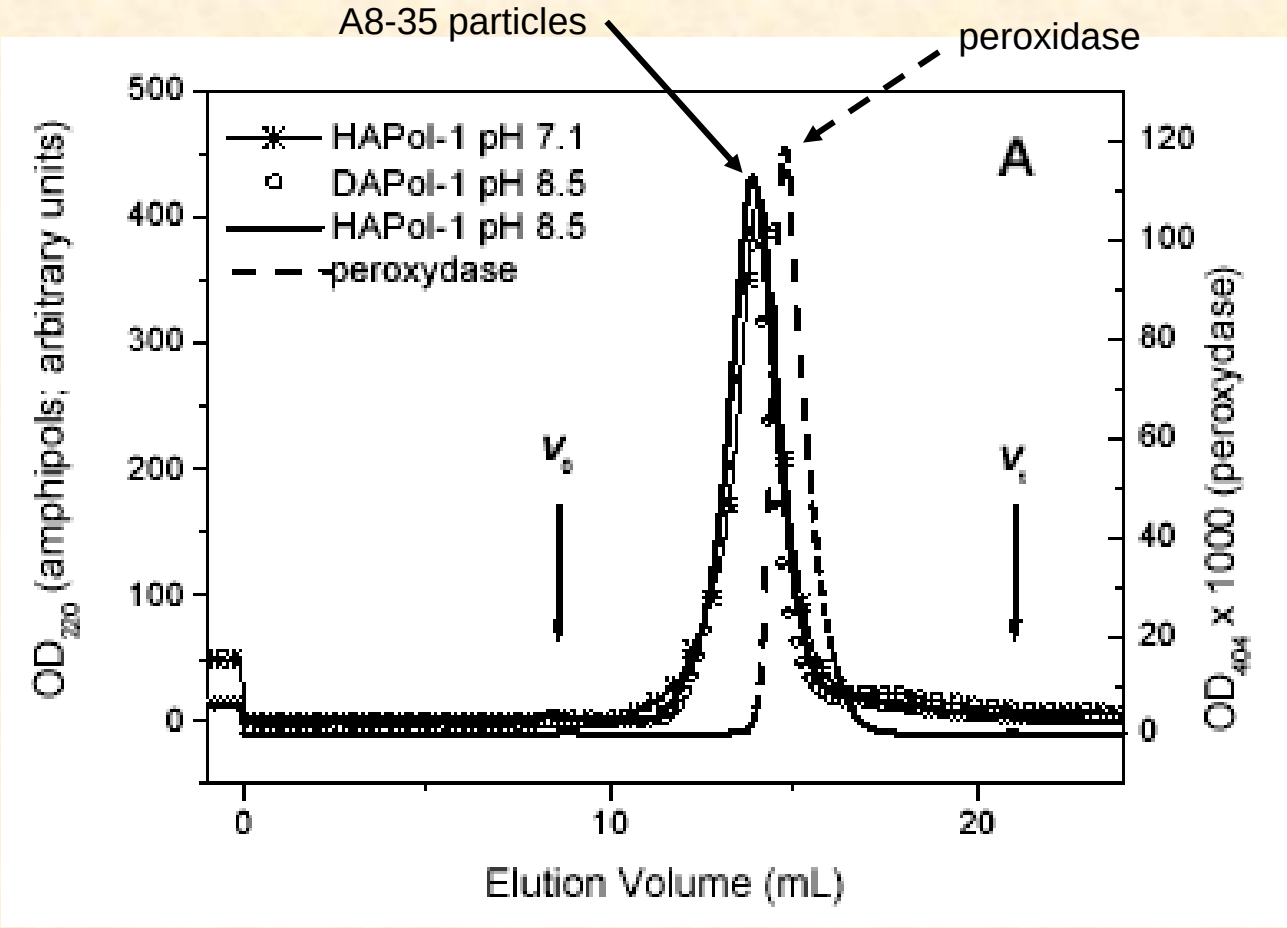
Amphipol A8-35 : <MW> ≈ 9-10 kDa, 35% of underivatized carboxylic groups; on average ~70 monomers, ~18 of which bear an octyl chain.

Tribet *et al.*, *PNAS*, 1996; Gohon *et al.*, *Langmuir*, 2006.

Solution properties of A8-35

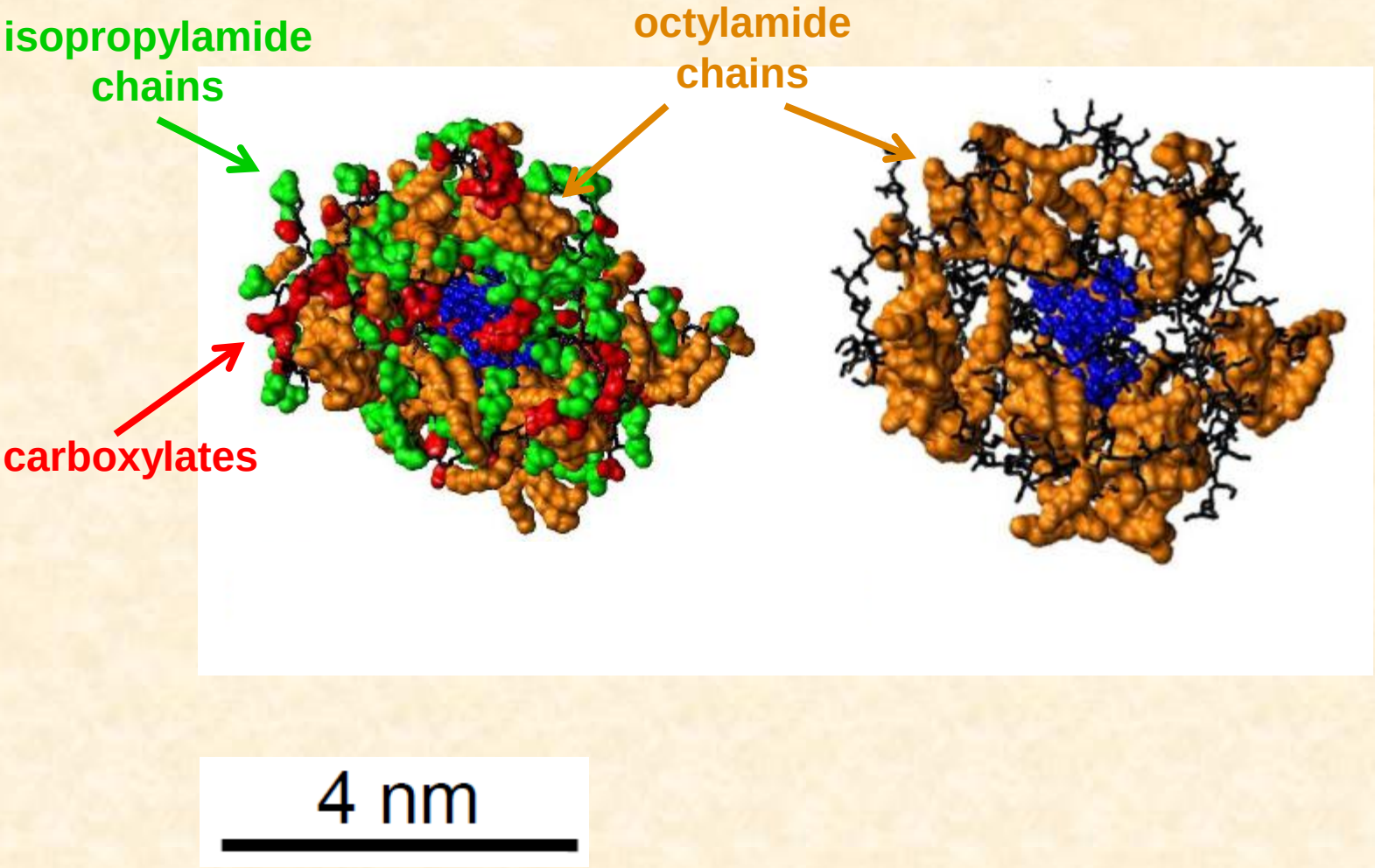
(as analyzed by SANS, SEC, AUC, DLS...)

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



Gohon *et al. Anal. Biochem.*, 2004; *Langmuir*, 2006.

Molecular dynamics of A8-35 particles



J.N. Sachs, *unpublished data*

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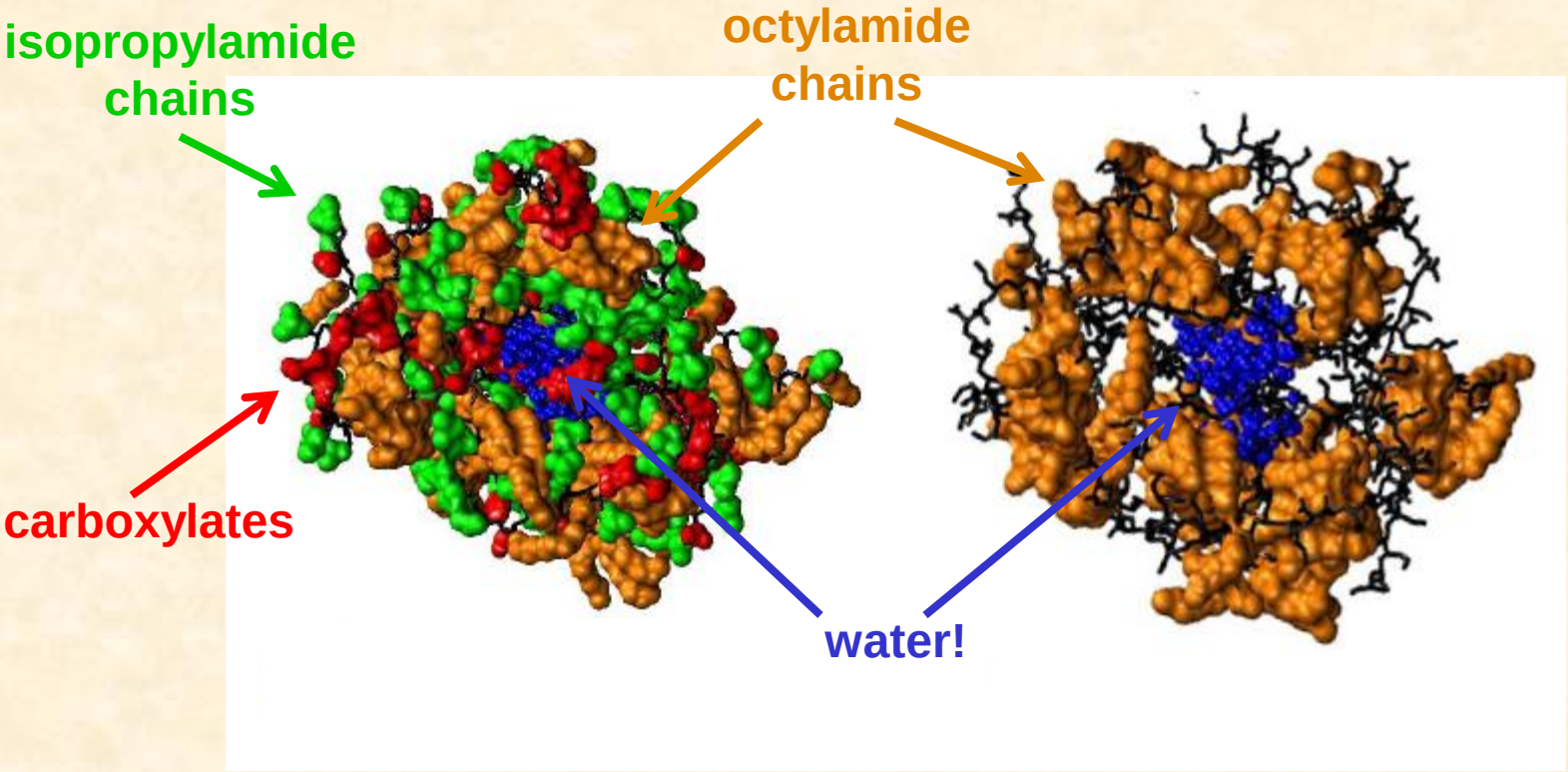
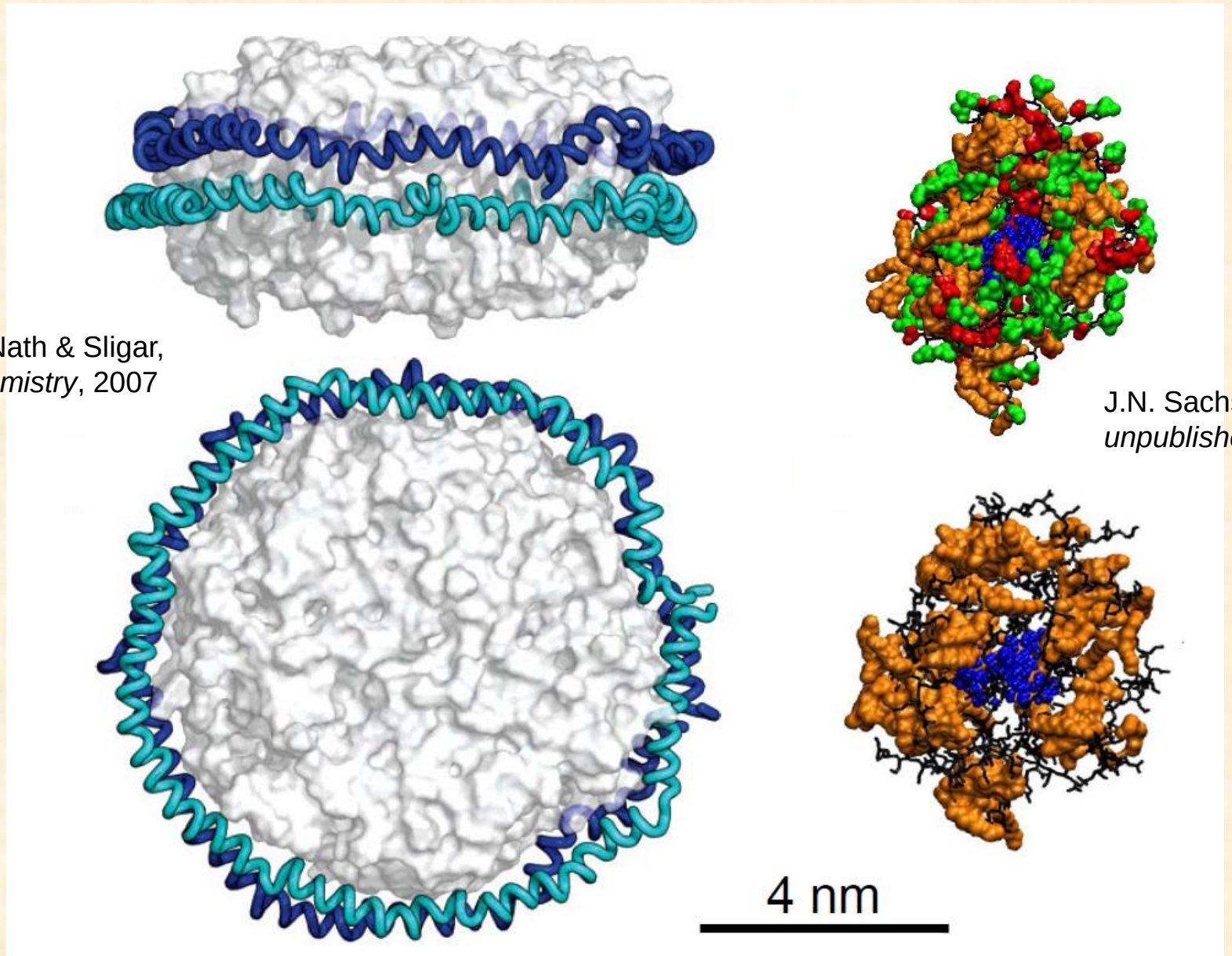


J.N. Sachs, *unpublished data*

A8-35 particles *vs.* nanodiscs



From Nath & Sligar, *Biochemistry*, 2007

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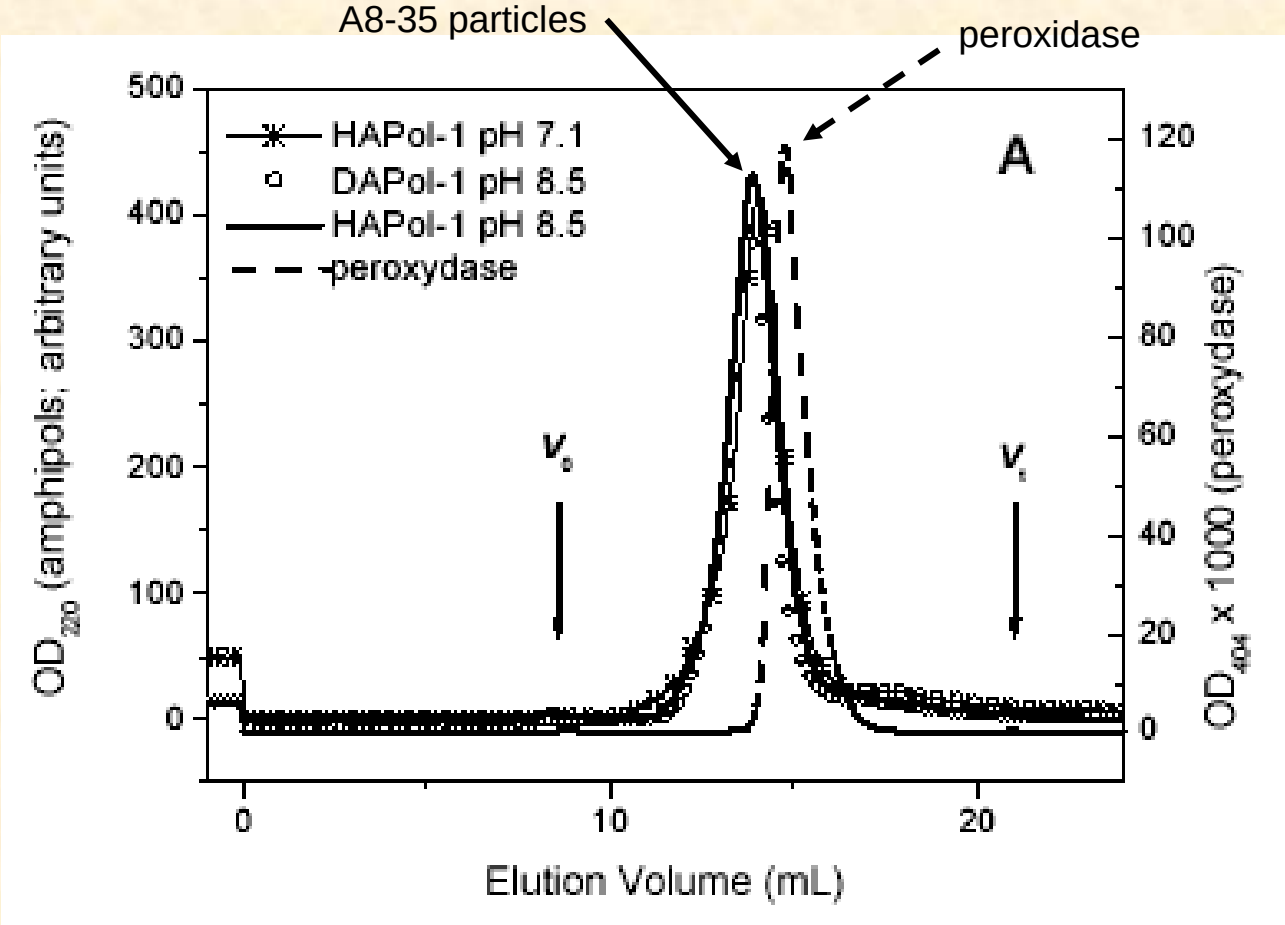
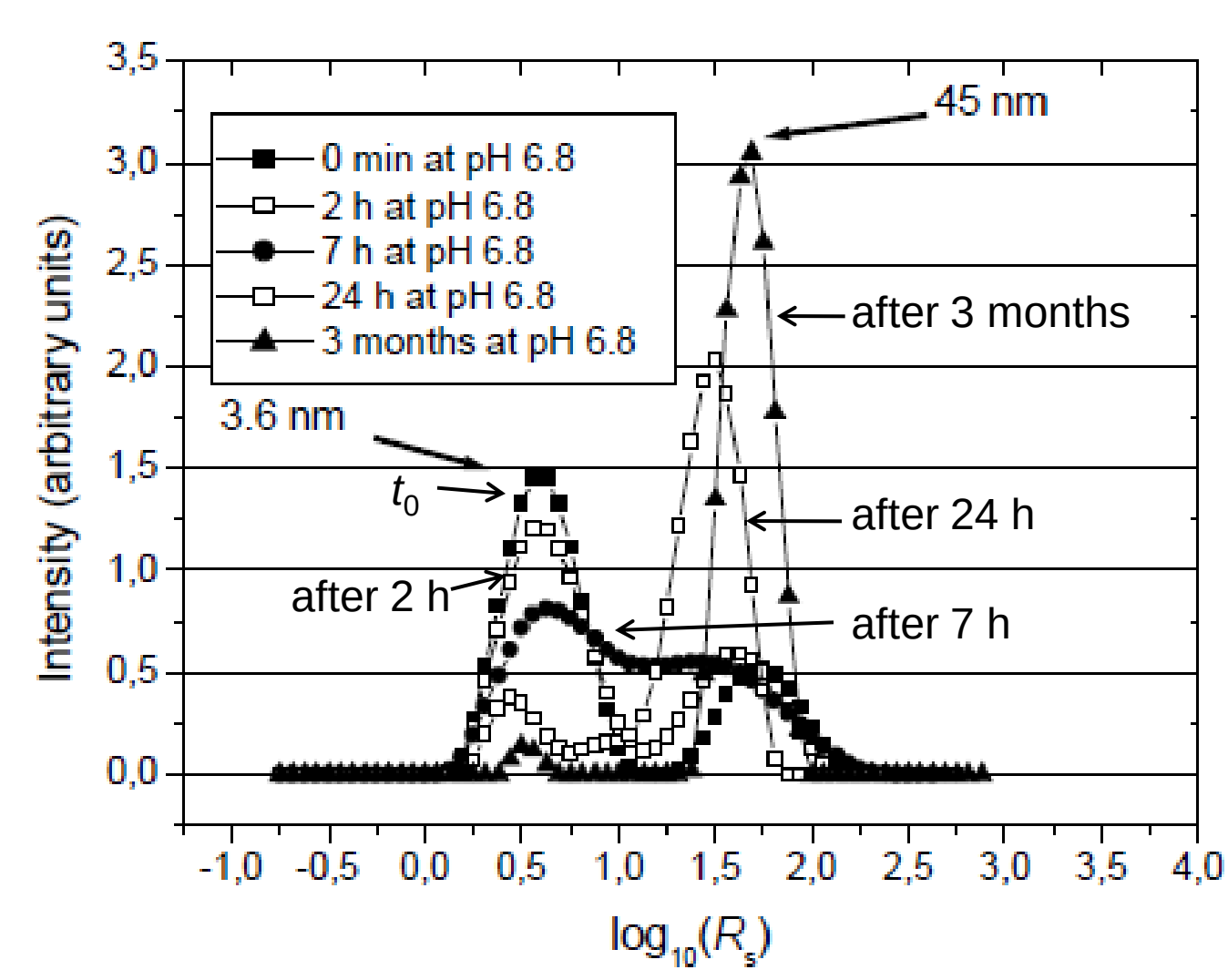
- **pH- and Ca^{2+}-sensitive**



Gohon *et al. Anal. Biochem.*, 2004; *Langmuir*, 2006.

Aggregation of A8-35 at pH < 7

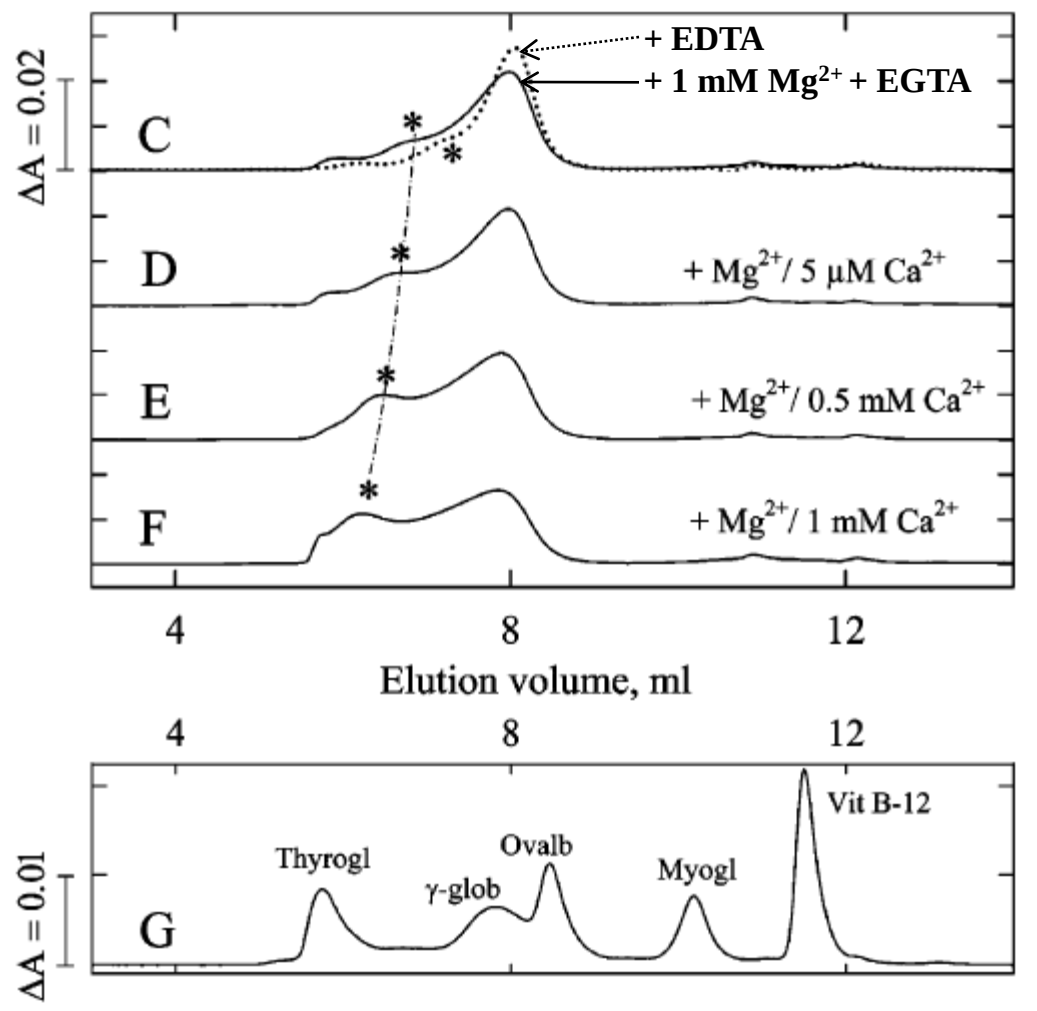
(light scattering analysis)



From Gohon *et al*., *Langmuir*, 2006

Calcium-induced aggregation of A8-35

(SEC analysis)



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

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
  - ITC: Tribet *et al*., *Langmuir*, 2009

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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}H, ^{3}H, ^{14}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...)
- "universal" (carrying a reactive amine arm) (Zoonens *et al.*, 2007)

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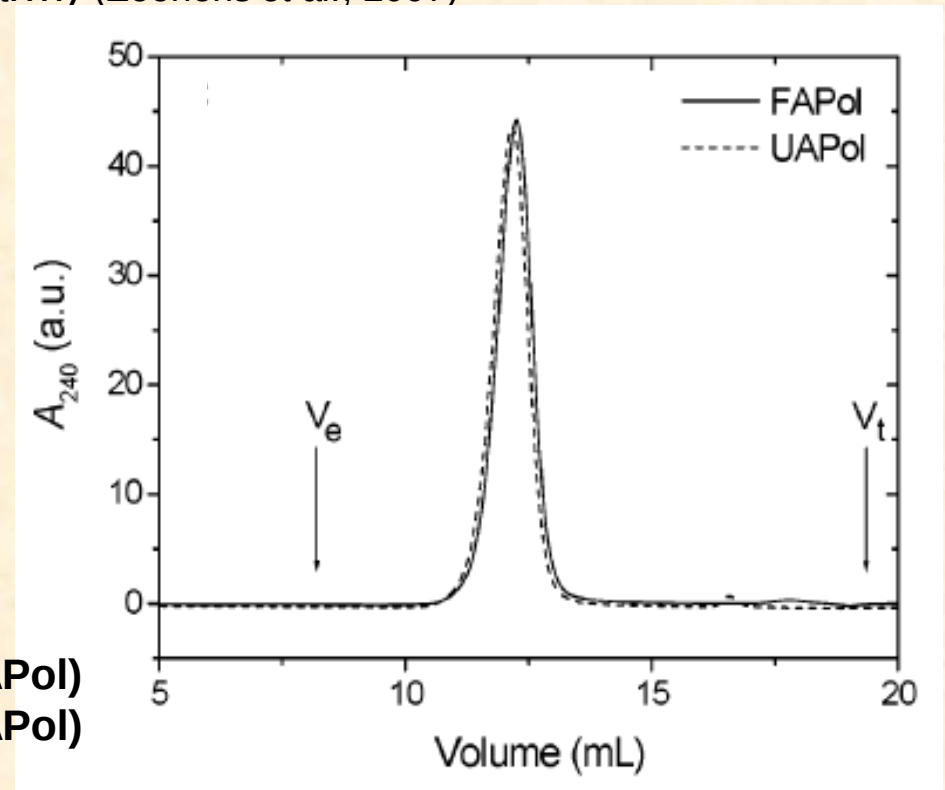
Same physical-chemical properties!



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

From Zoonens *et al*., *Biochemistry*, 2007

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2. Variants of A8-35

- A8-75, A34-35, A34-75, A2-75... (Tribet *et al.*, 1996, 1997; Gohon, 1996)
- C_{12}-A8-75 (C. Tribet; unpublished)
- OAPA-20 etc. (Nagy *et al.*, 2001) (C.R. Sanders/*Anatrace*)

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- **Similar but possibly different physical-chemical and/or biochemical properties**
- **Potential remains to be fully exploited**

Diversifying amphipols

1. Functionalized or labeled versions of A8-35

- isotopically labeled amphipols (^{2}H, ^{3}H, ^{14}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.*, *in preparation*); in the tube: his-tagged, oligonucleotide-tagged...)
- "universal" (carrying a reactive arm) (Zoonens *et al.*, 2007)

2. Variants of A8-35

- A8-75, A34-35, A34-75, A2-75... (Tribet *et al.*, 1996, 1997; Gohon, 1996)
- C_{12}-A8-75 (C. Tribet; unpublished)
- OAPA-20 etc. (Nagy *et al.*, 2001) (C.R. Sanders/*Anatrace*)

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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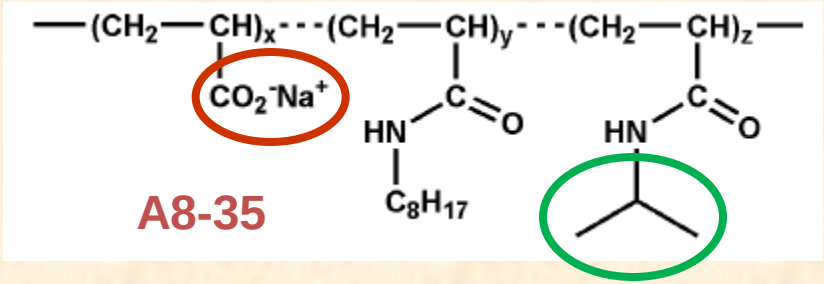
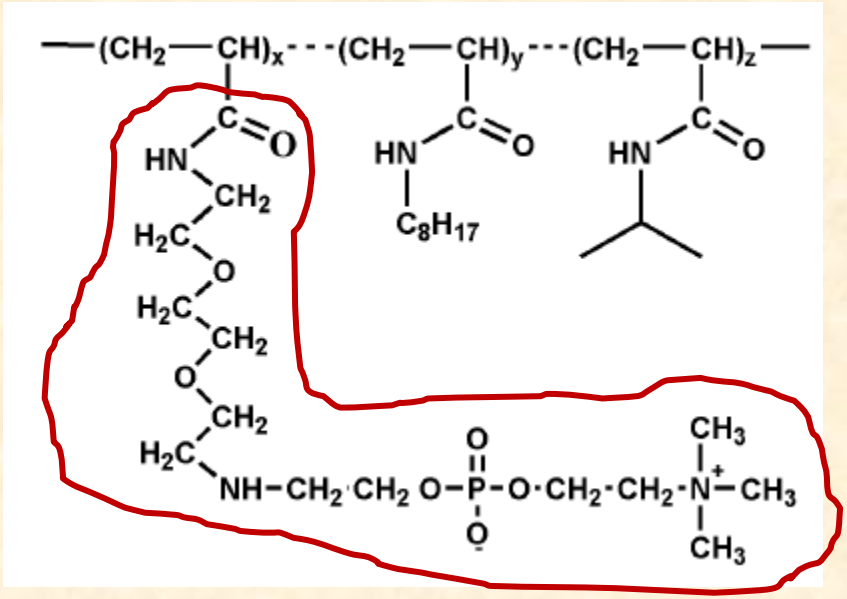
Different physical-chemical properties

⇒ back to square one!

Most heavily studied amphipols





Phosphocholine-based amphipol ("PC-APol")

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

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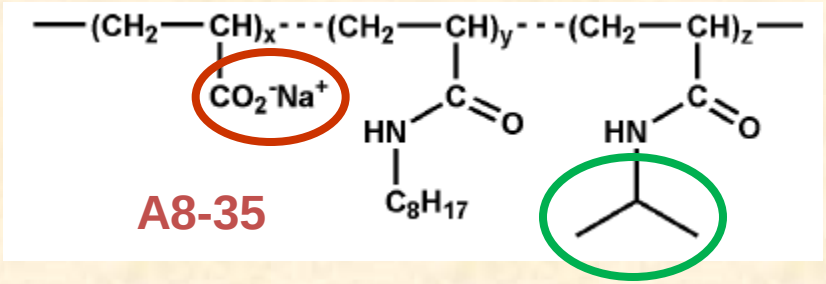
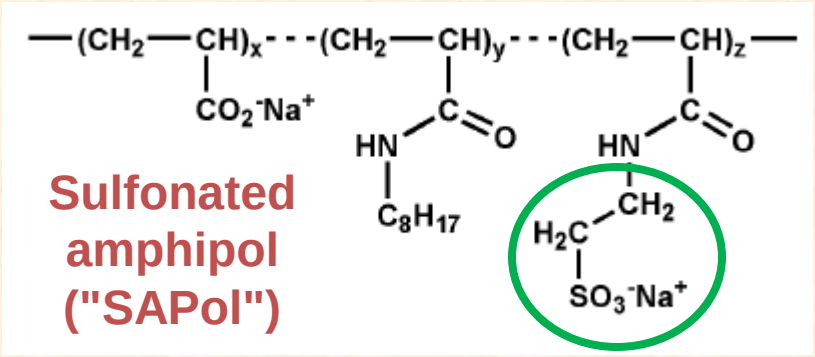
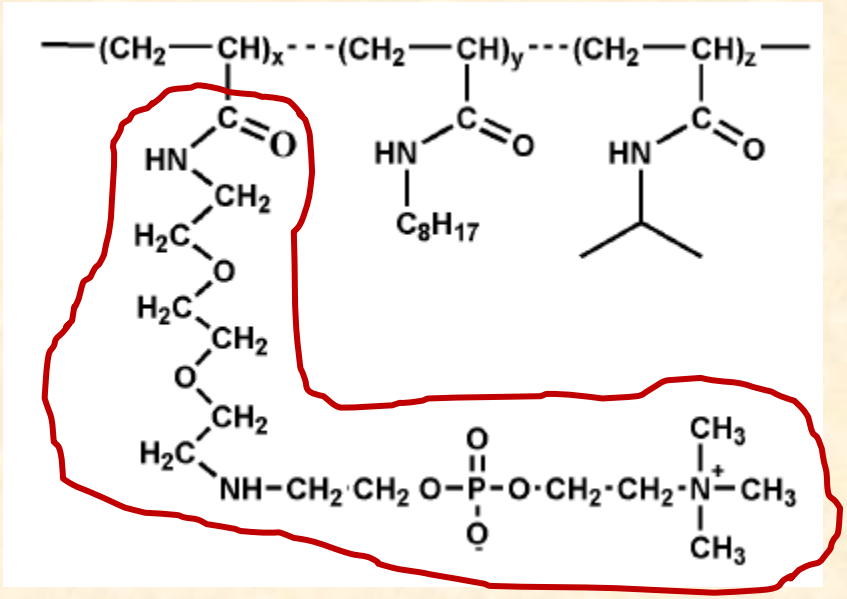




Developed by F. Giusti (IBPC, Paris)



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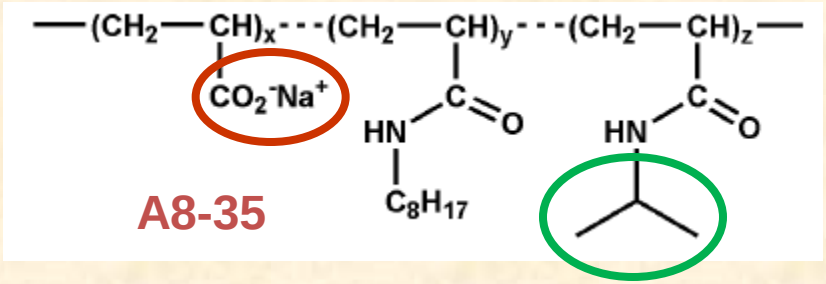
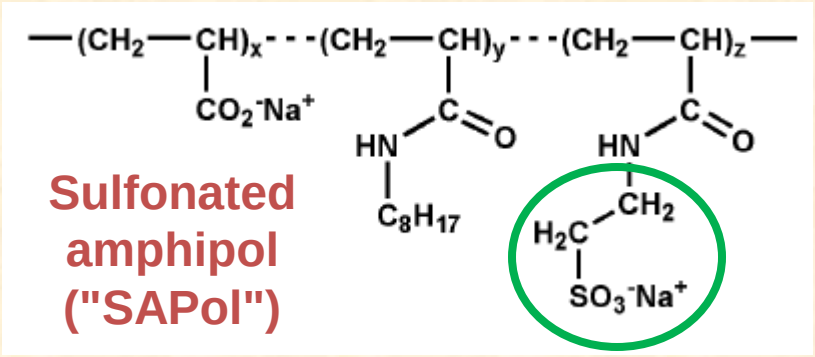
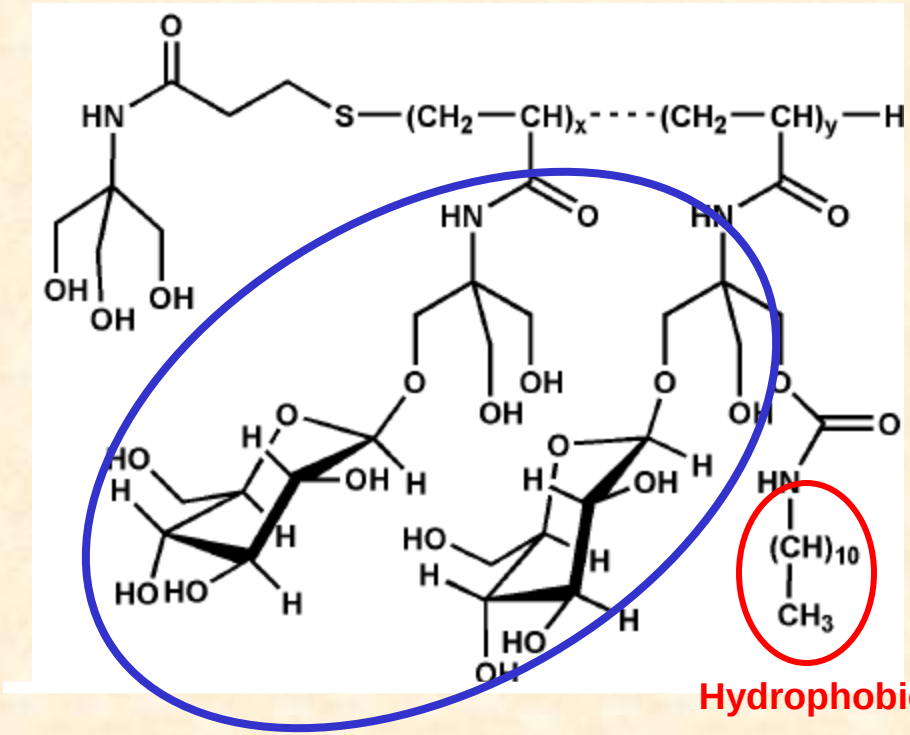


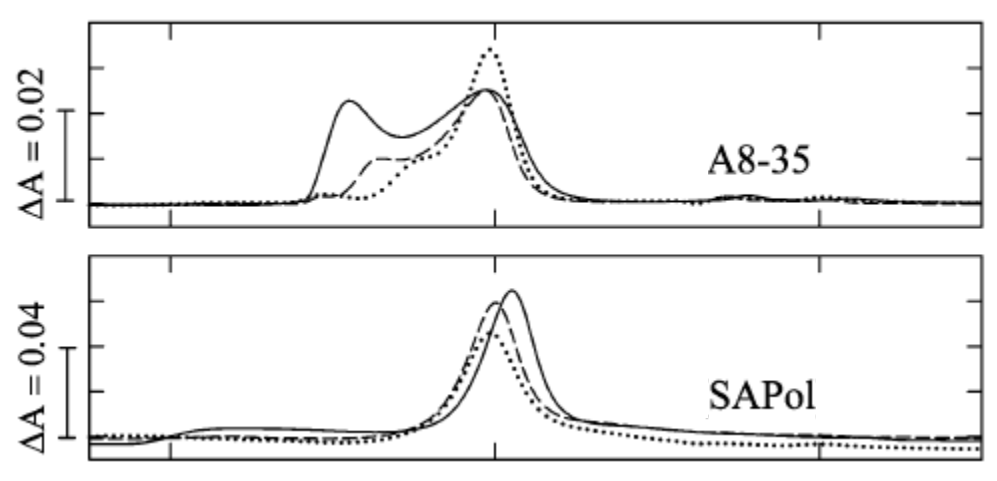


Non-ionic, glucosylated amphipols ("NAPols")

Developed in collaboration with the laboratory of B. Pucci (U. of Avignon)



SAPols, PC-APols and NAPols are insensitive to low pH or calcium



SEC in the presence of:

..... **0.5 mM EDTA**

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

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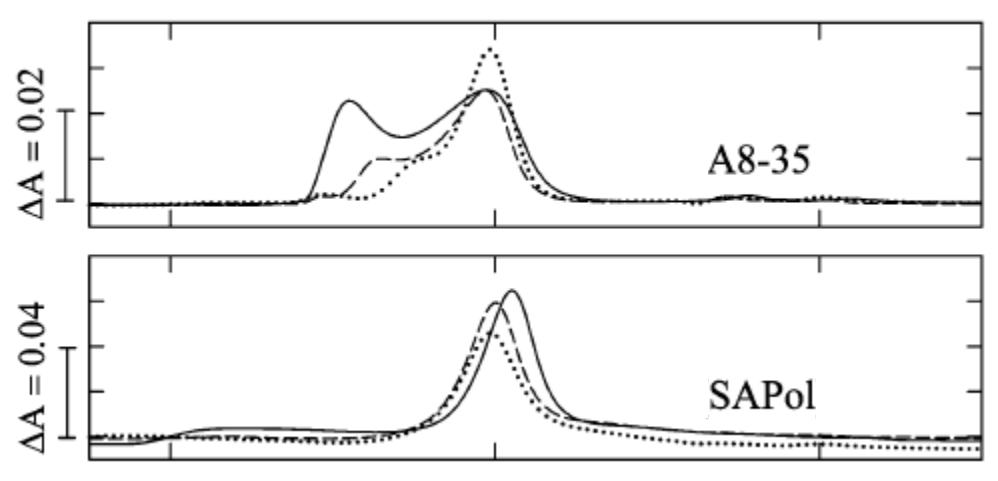

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Conditions	NaCl 1 M	pH 5	Ca^{2+} 12 mM	NaCl 1 M	pH 5	Ca^{2+} 12 mM
Amphipol	A8-35			C22-43 (PC-APol)		
Turbidity at $t=0$	+	+	+	–	–	+[a]
% BR in supernatant $t=15$ min	80	0	22	91	97	94

Stability of bacteriorhodopsin/ amphipol complexes in the presence of 1 M NaCl, pH 5, or 12 mM Ca^{2+}

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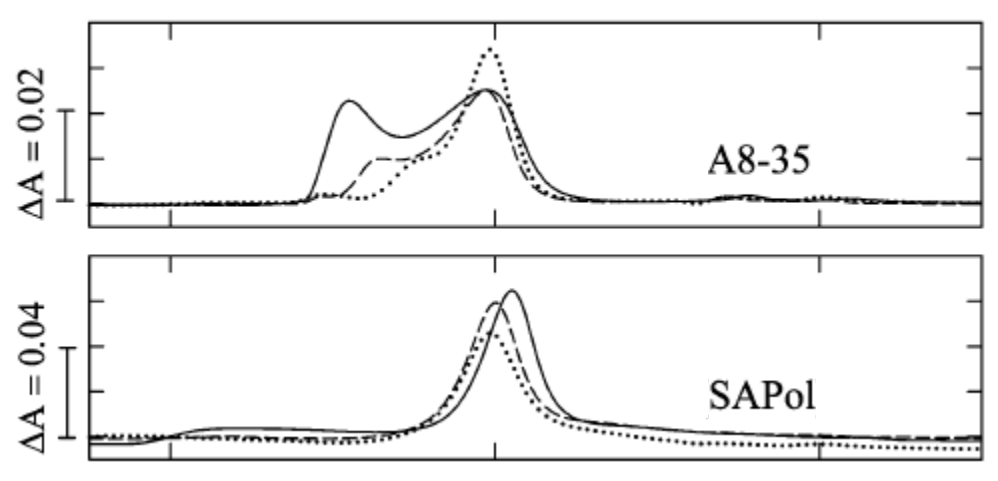

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Similar results with non-ionic amphipols (Bazzacco, 2009, and articles in preparation)**.**

New avenues opened by novel amphipols

• **Non-ionic and sulfonated amphipols validated for solution NMR** (L.J. Catoire, unpublished data) ⇒ **access to low pH for studying amide protons.**

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• **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^{2+}- 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

C. Tribet (ESPCI → ENS, Paris)	**polyacrylate- & PC-based amphipols**
B. Pucci & coworkers (University of Avignon)	**non-ionic amphipols**
F. Winnik, C. Diab (U. de Montréal)	**phosphorylcholine-based amphipols, ITC**
C. Ebel, F. Gabel, P. Timmins (IBS & ILL, Grenoble)	**ultracentrifugation, small angle neutron scattering**
D.M. Engelman (Yale)	**small angle neutron scattering**
J.N. Sachs (U. Minnesota)	**molecular dynamics**
E.A. Berry (University of Berkeley)	**3D crystallization of *bc*$_1$/amphipol complexes**
J.H. Kleinschmidt, C. Pocanschi (U. of Constance)	**renaturation of β-barrel membrane proteins**
F. Rappaport (IBPC, Paris)	**BR photocycle**
P. Champeil, M. Picard (CEA, Saclay)	**sarcoplasmic calcium ATPase activity & stability**
K. Leonard, M. Flötenmeyer (EMBL, Heidelberg)	**EM of Complex I/APol complexes**
J.-L. Banères & coworkers (CNRS, Montpellier)	**folding of GPCRs**
K. Martinez, J.-B. Perez (U. of Copenhagen)	**ligand binding to nAChR immobilized onto beads**

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CNRS

Human Frontier Science Program Organization

EC (Biotech, IMPS)

Paris-7 University

Ministère de la Recherche et de la Technologie

Fondation Rothschild

ANR