Workshop: Applications of Amphipols to Membrane protein Studies

March 8, 2010 – Paris

Trapping membrane proteins with amphipols.

Structure and properties of membrane protein/amphipol complexes

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- Direct extraction of MPs from the biological membrane has been observed in a very few cases

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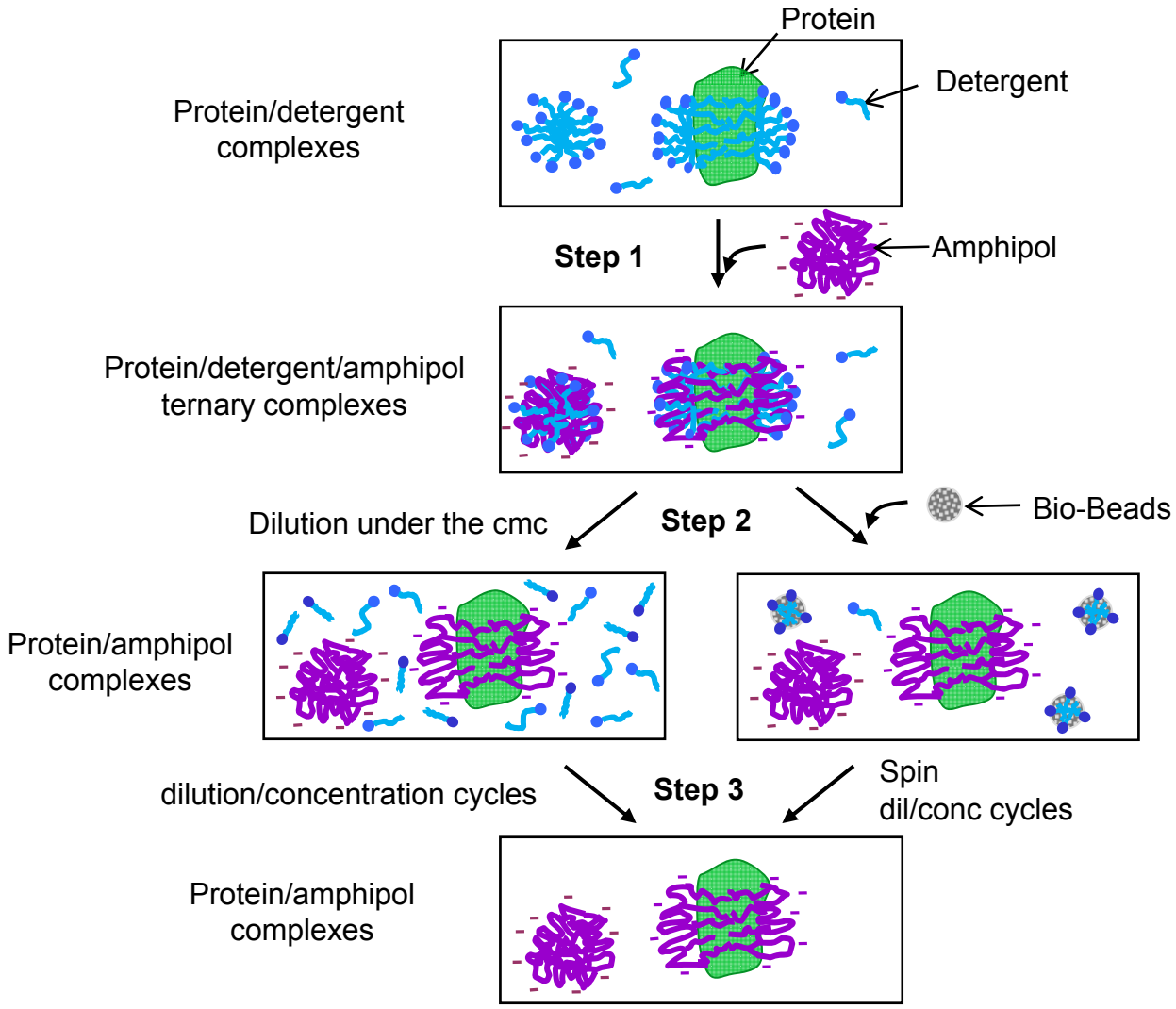
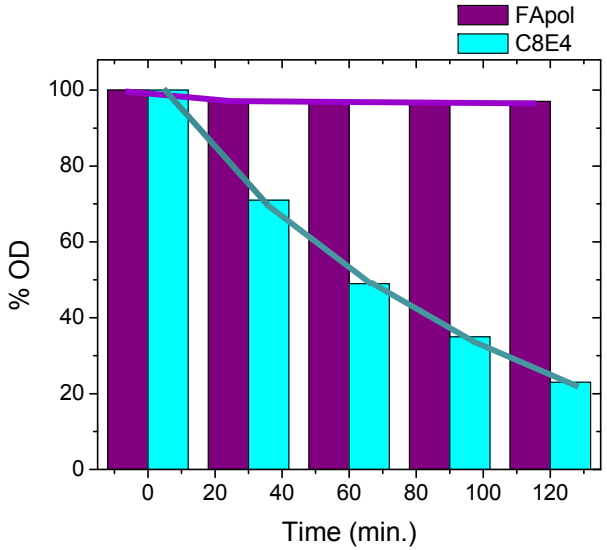
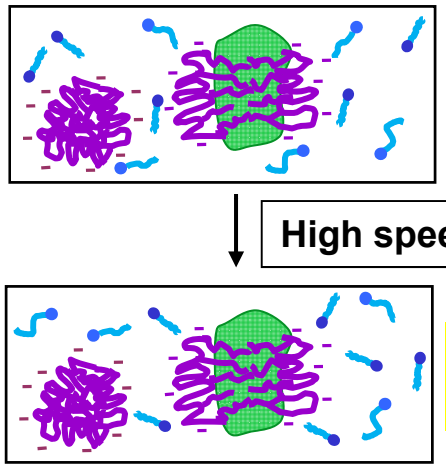




1) How to define conditions for trapping a MP in APols?

- Estimation of the best [protein:APol] w/w ratio

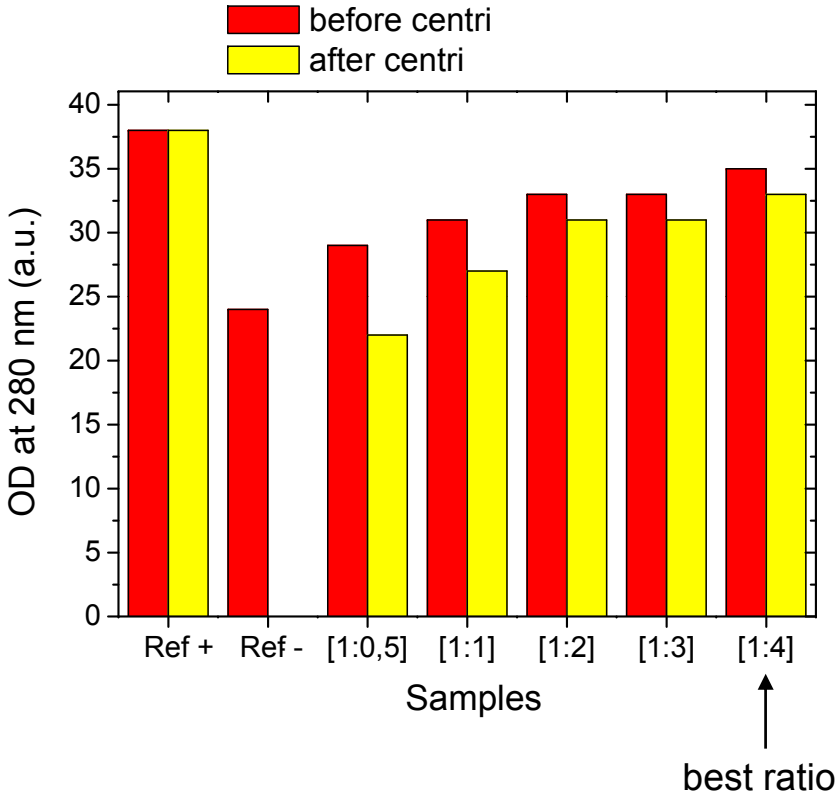
Trapping after dilution under the cmc



Measure the absorbance at 280nm

High speed centrifugation

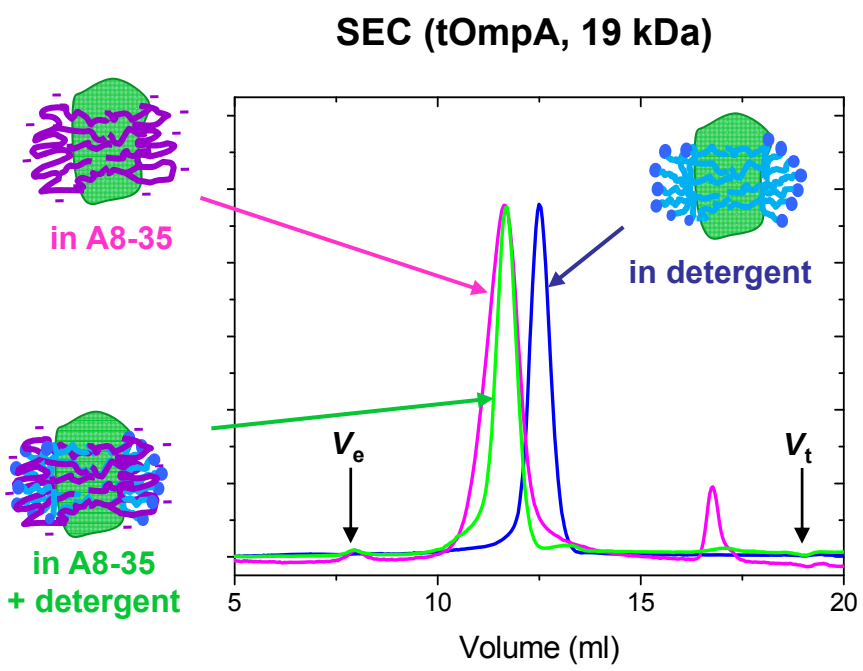
Measure the absorbance at 280nm



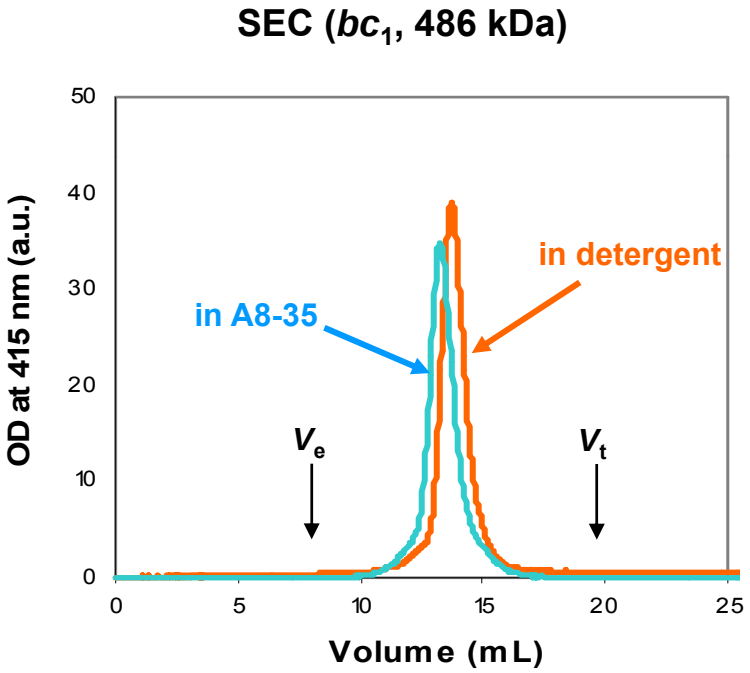
- Analyze the resulting complexes by size exclusion chromatography (SEC)

2) What do MP/APol complexes look like after trapping?

1. MP/APol complexes are slightly larger than MP/detergent complexes (SEC, SANS, AUC, NMR)



Zoonens *et al., Biochemistry*, 2007

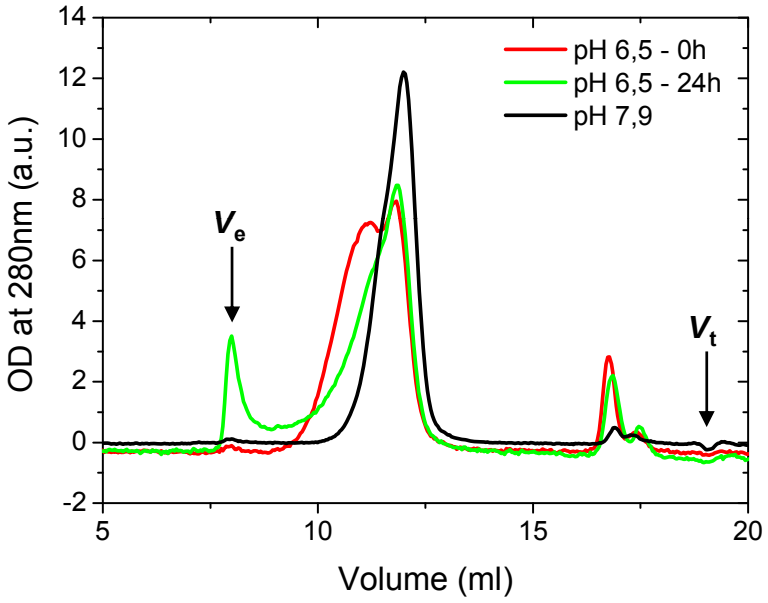


Charvolin *et al., in preparation*

2) What do MP/APol complexes look like after trapping?

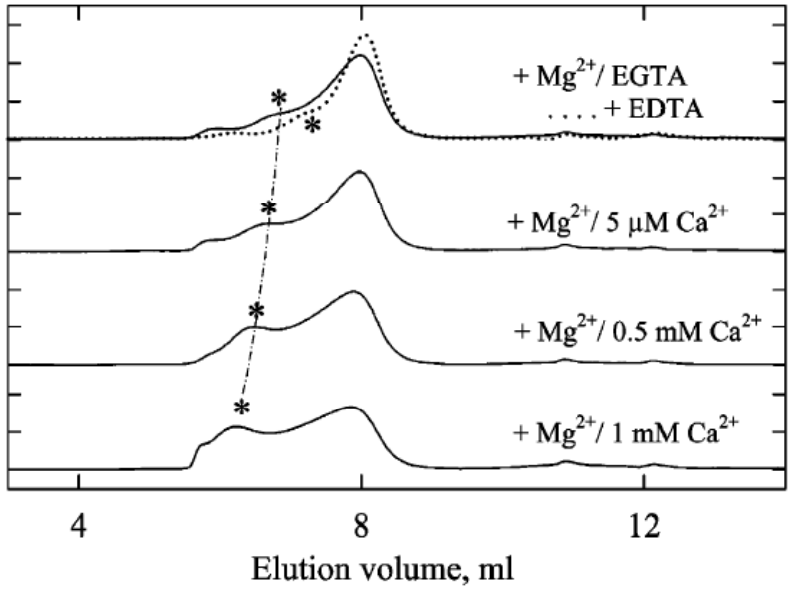
1. MP/APol complexes are slightly larger than MP/detergent complexes
2. The monodispersity of the complexes depends on several factors

pH effect



Zoonens *et al., Biochemistry*, 2007

Presence of divalent cations (Ca^{2+})



Picard *et al., Biochemistry*, 2006

Hypothesis:

Because the solubility of A8-35 is conferred by its charges, lowering pH or the presence of divalent cations reduces the electrostatic repulsions between particles. Divalent cations could also link up particles together and lead to aggregation.

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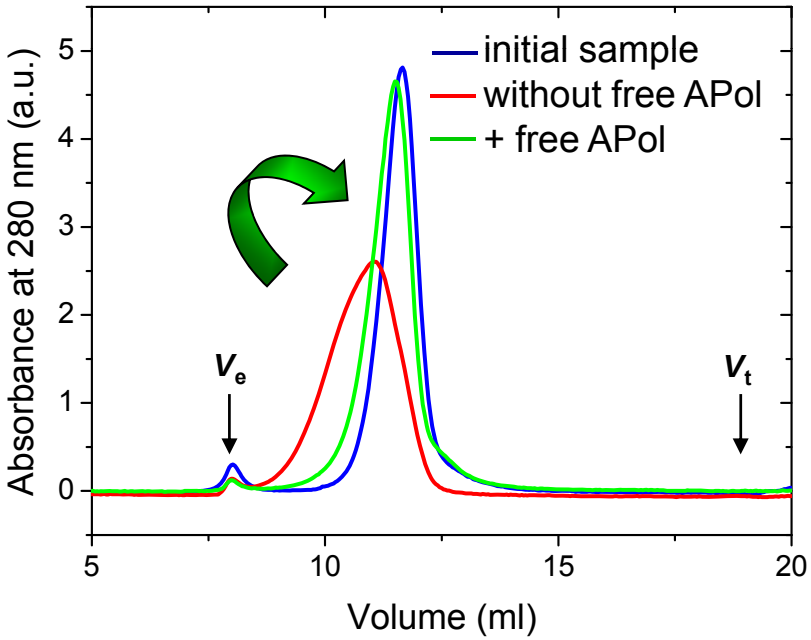
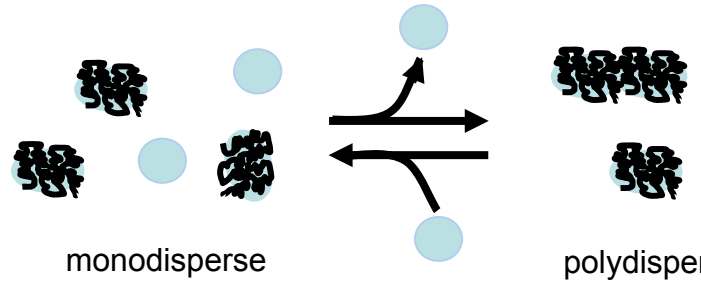
Separating the complexes from extra free APol



Hypothesis:

Because A8-35 is poorly dissociating, it has to be present in excess to efficiently compete with protein/protein interactions

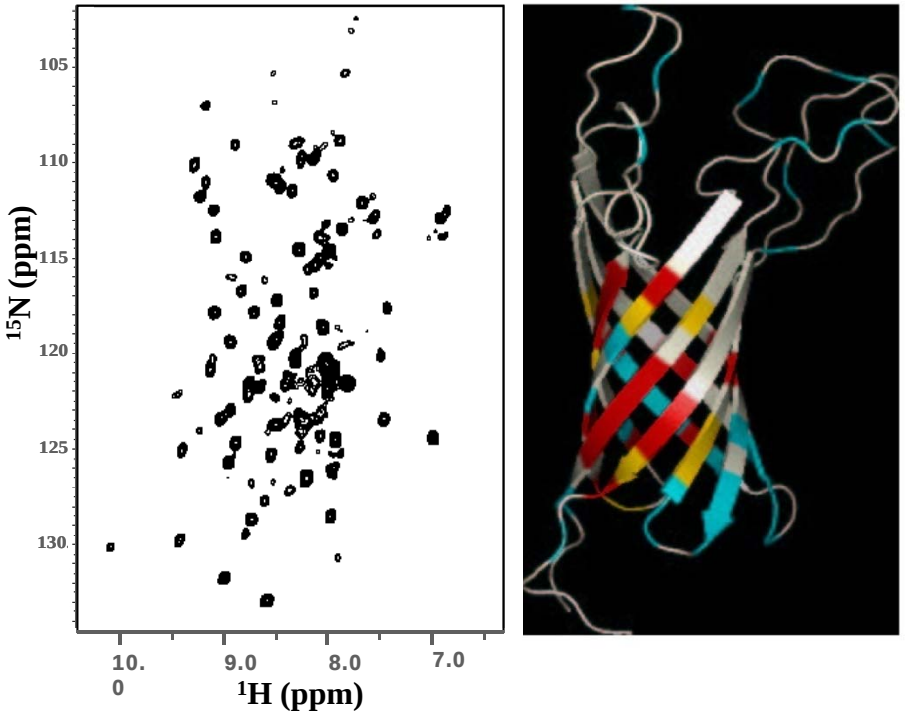


Zoonens *et al.*, *Biochemistry*, 2007

2) What do MP/APol complexes look like after trapping?

1. MP/APol complexes are slightly larger than MP/detergent complexes
2. The monodispersity of the complexes depends on several factors
3. Amphipols form a **compact layer** (1.5-2 nm) around the **transmembrane surface** of the protein (**no diffuse corona**; SANS, NMR, AUC)

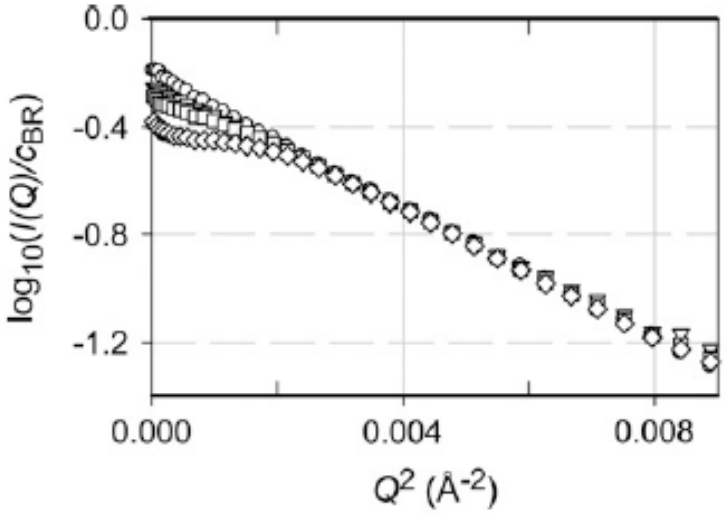
NMR (tOmpA, 19 kDa)



Zoonens *et al*., *PNAS*, 2005

Catoire *et al*., *Eur Biophys J,* 2010

SANS (BR, 27 kDa)



Gohon *et al*., *Biophys. J.*, 2008

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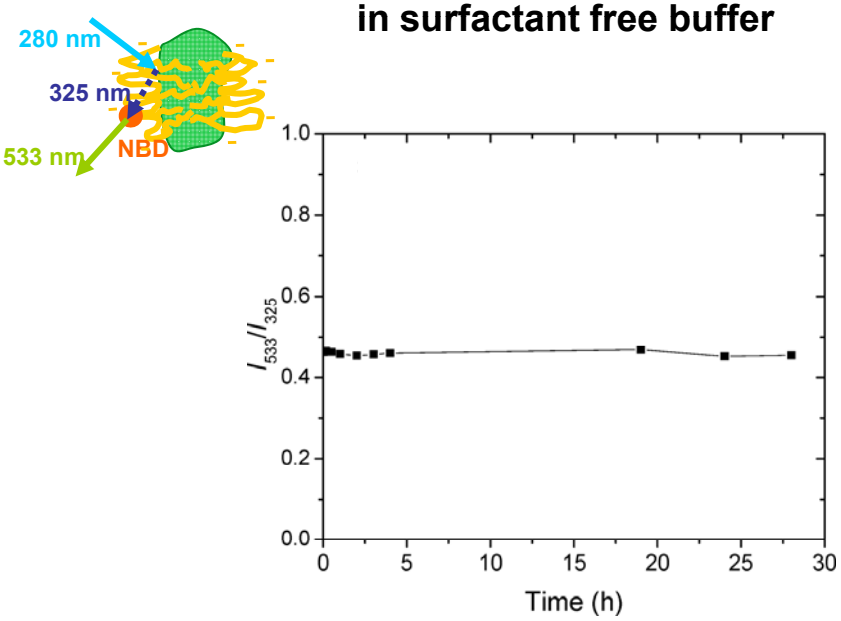
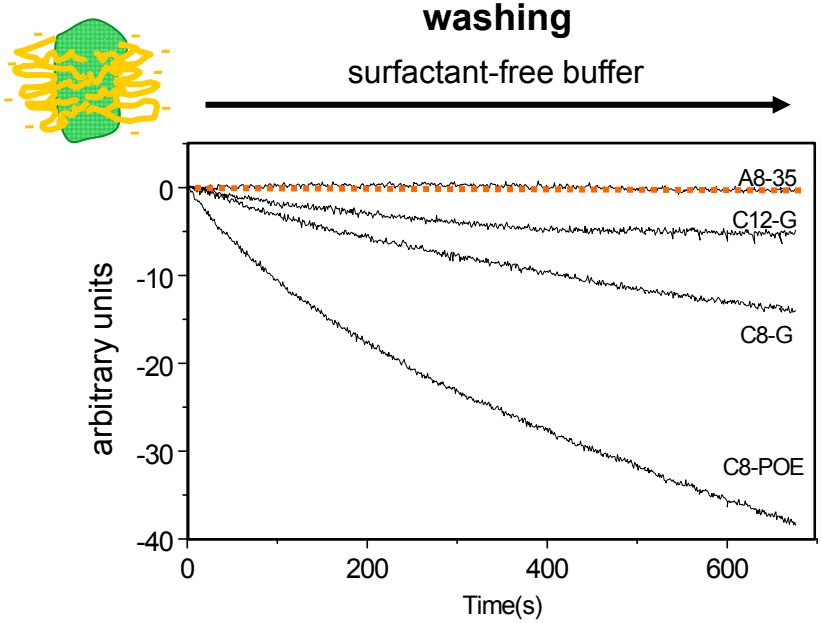
3. Amphipols form a compact layer around the transmembrane surface of the protein
4. Binding is non-covalent, but irreversible in the absence of a competing surfactant

FRET measurements after 1000-fold dilution in surfactant free buffer



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

Surface Plasmon Reasonance measurements upon extensive washing



Hong & Lakey; *in* Popot *et al*., *CMLS*, 2003

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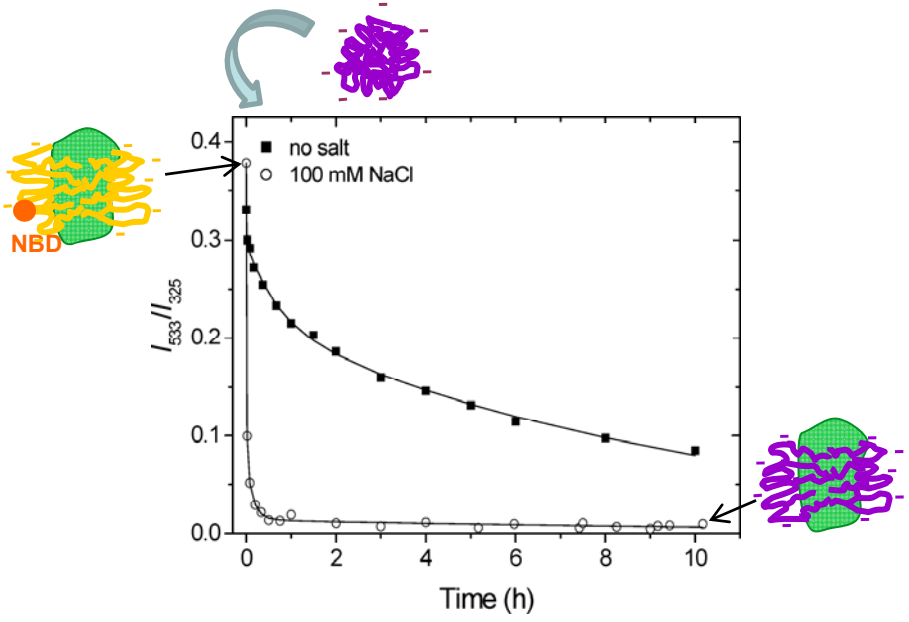
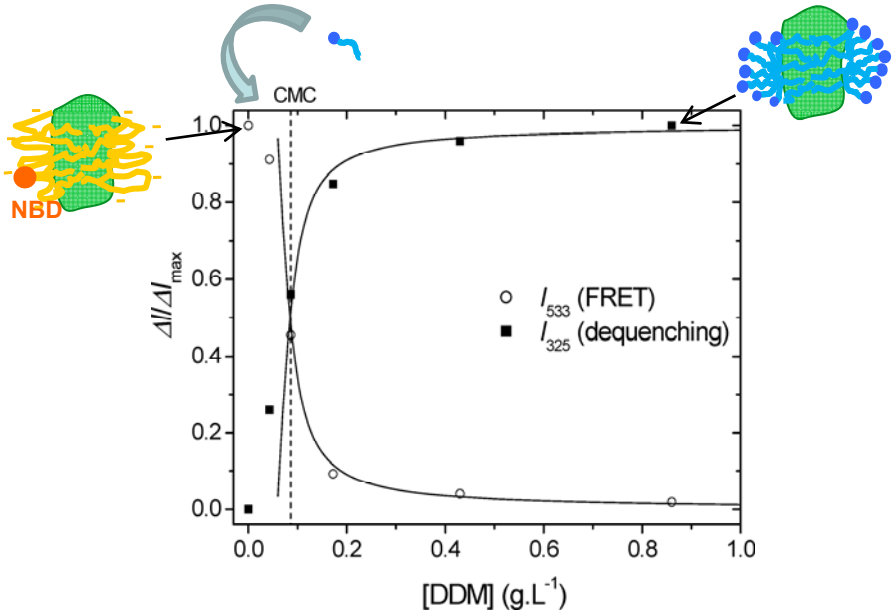
3. Amphipols form a compact layer around the transmembrane surface of the protein
4. Binding is non-covalent, but irreversible in the absence of a competing surfactant
5. Bound amphipols can be displaced by free amphipols, detergents, or lipids (Tribet *et al.*, *Langmuir*, 1997; Nagy *et al*., *FEBS Lett.*, 2001; Pocanschi *et al*., *Biochemistry*, 2006; Zoonens *et al*., *Biochemistry*, 2007; Tribet *et al., Langmuir*, 2009)

Exchange for free amphipol



Displacement by detergent

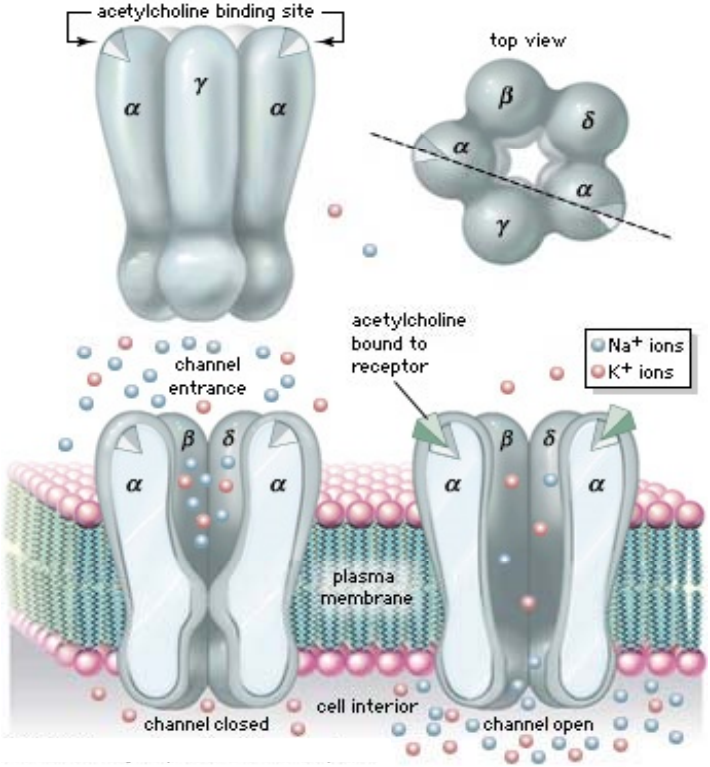


Zoonens *et al*.,*Biochemistry*, 2007

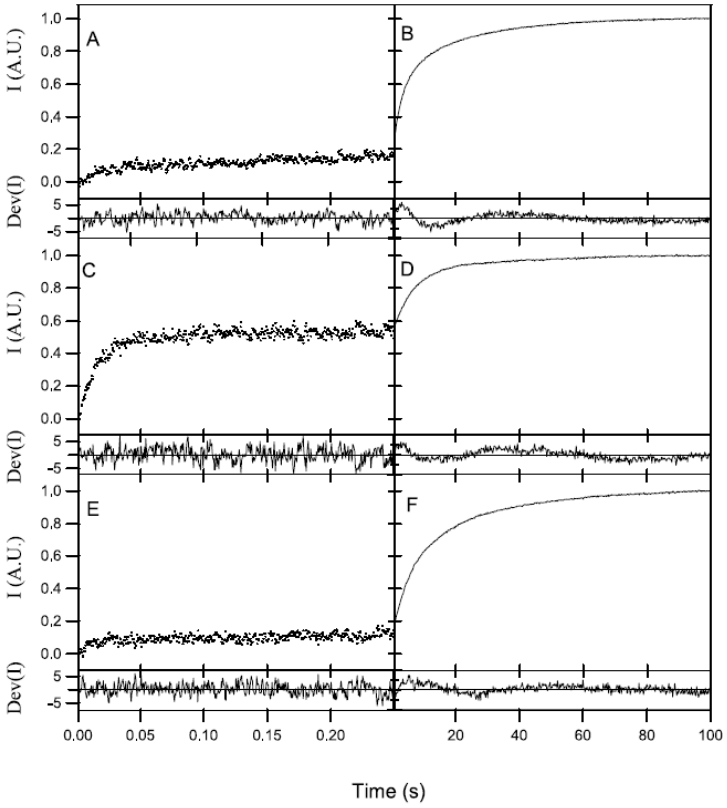
3) How do MP/APol complexes behave in term of activity ?

1. Amphipols may affect the dynamics –and, thereby, the activity– of the proteins they bind to

• Nicotinic acetylcholine receptor: no transmembrane movement; allosteric transitions unaffected



Kinetics of binding



In native membranes

In detergent (CHAPS)

In A8-35

Martinez *et al.*, *FEBS Lett.,* 2002

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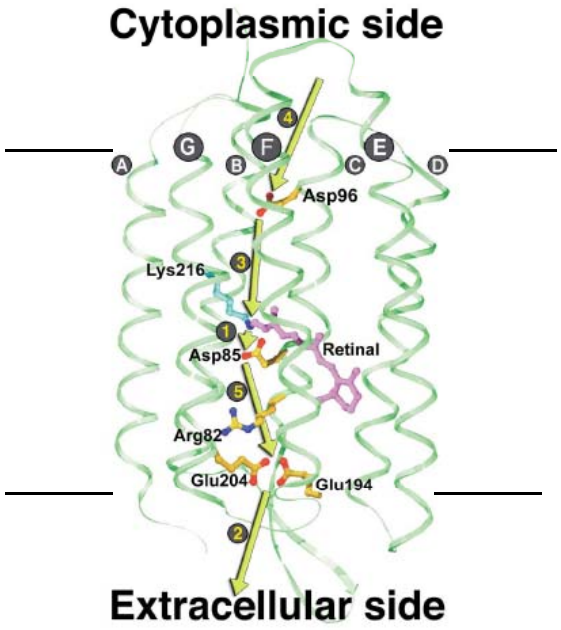
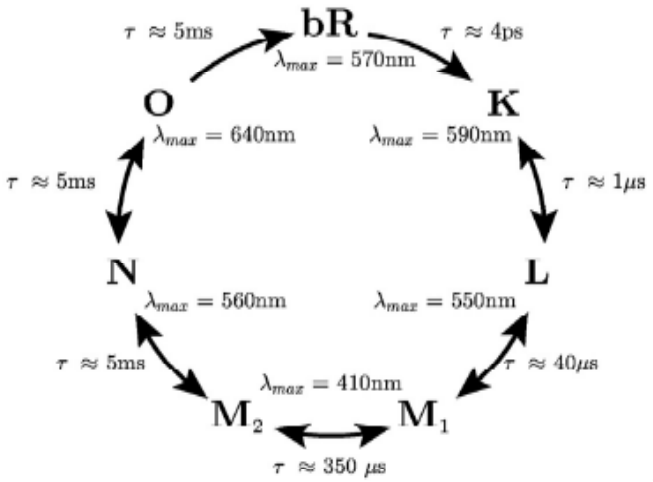
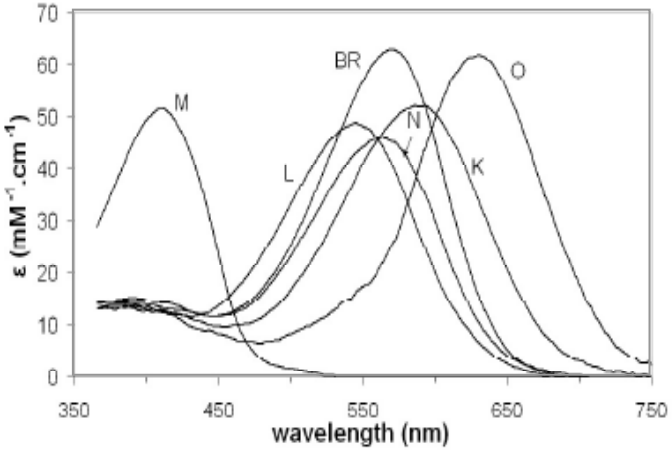


Neutze *et al.*, *BBA*, 2002

Photocycle of BR



Spectral changes



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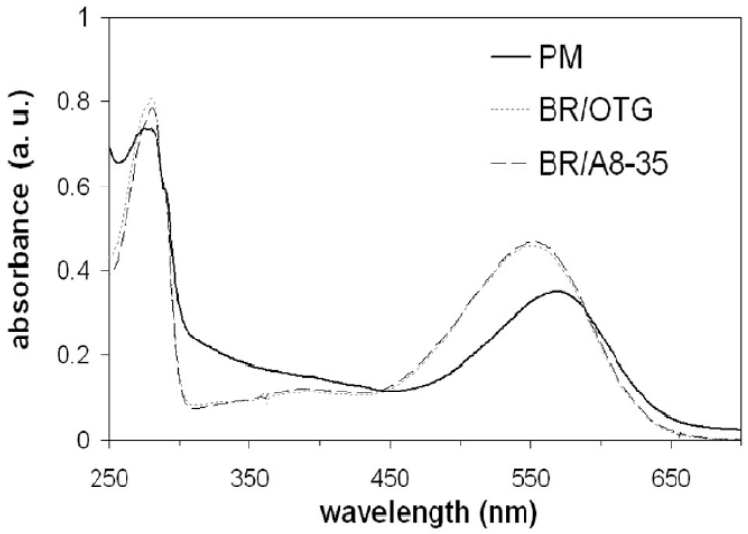
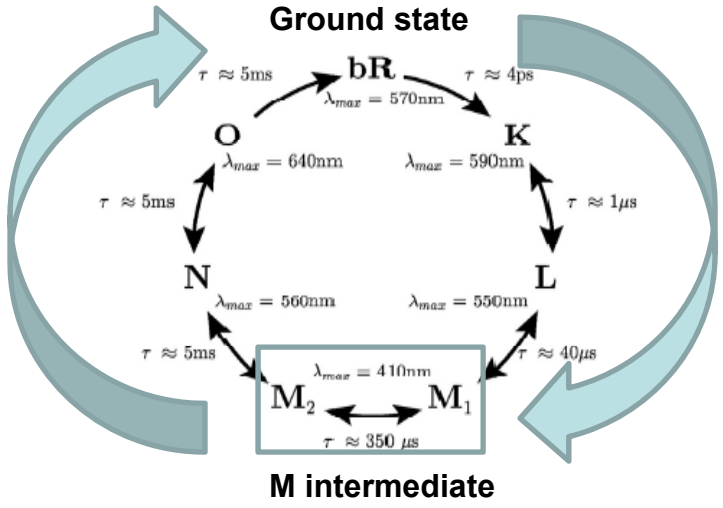
UV-visible spectra of BR



Gohon *et al.*, *Biophys. J.*, 2008



	DAS1 (ns)	DAS2 (μs)	DAS3 (μs)	DAS4 (ms)	DAS5 (ms)
PM	650	21	84	1.4	4.7
BR/OTG	375	4.3	23	0.53	9
BR/A8-35	480	5.8	53	1.0	6.3

M's rise | M's decay

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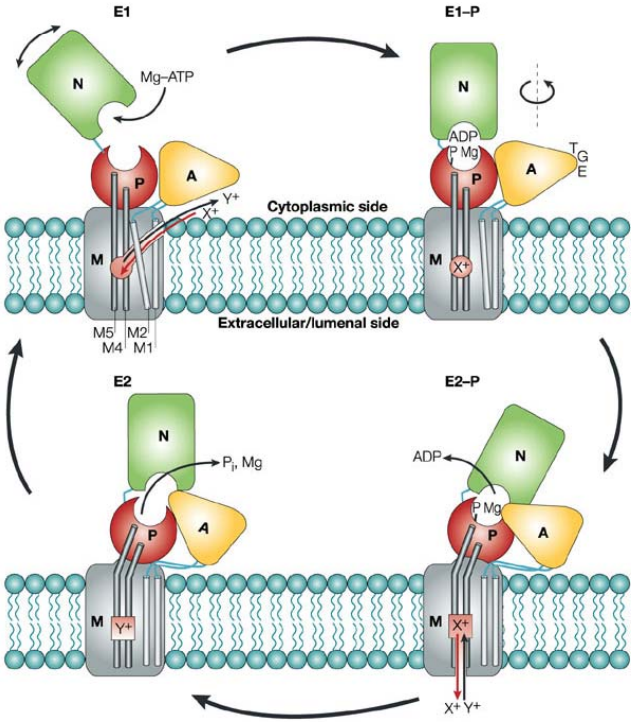
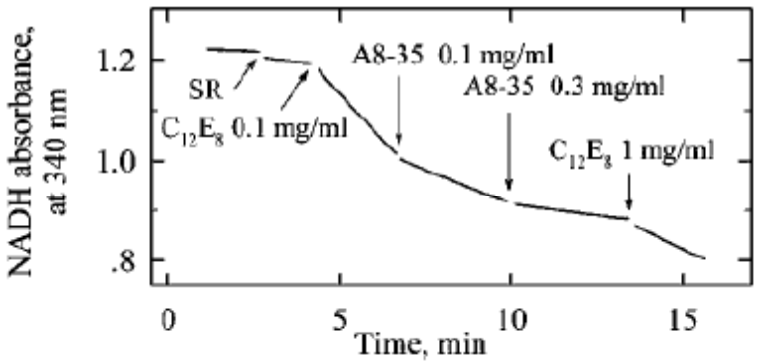
- Sarcoplasmic calcium ATPase: large-scale transmembrane rearrangements; ATP hydrolysis and Ca^{2+} release reversibly inhibited Champeil et al., JBC 2000; Picard *et al*., *Biochemistry* 2006

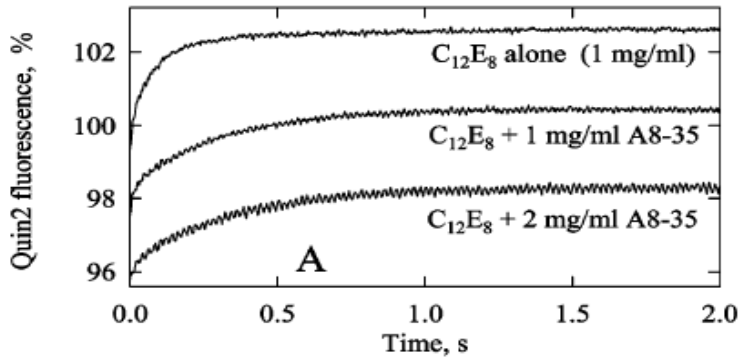


Kühlbrandt, *Nature Reviews Molecular Cell Biology*, 2004

Hydrolytic activity of Ca^{2+} ATPase



Ca2+ dissociation experiment



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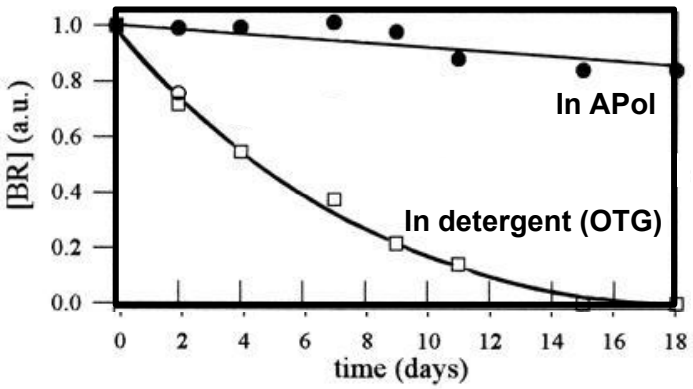
⇒ damping of large-scale transmembrane movements ('Gulliver effect')?

3) How do MP/APol complexes behave in term of activity ?

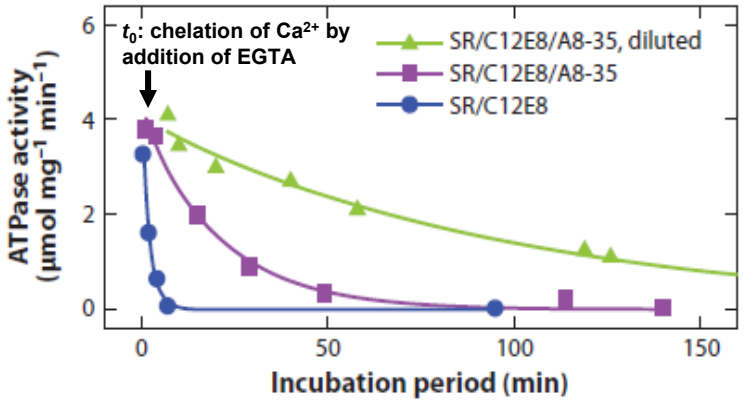
1. Amphipols may affect the dynamics –and, thereby, the activity– of the proteins they bind to
2. Damping of dynamics may contribute to membrane protein stabilization by amphipols

Bacteriorhodopsin



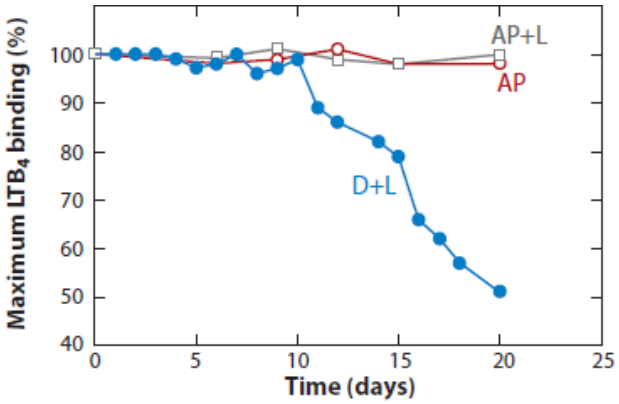
Tribet *et al.,* (1996) *PNAS* **93,** 15047

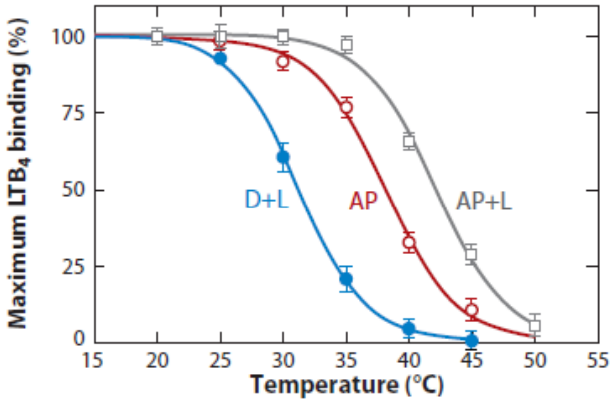
Calcium ATPase



Champeil *et al*., *JBC* 2000

Leukotriene receptor BLT1 (GPCR)





Dahmane *et al*., *Biochemistry,* 2009

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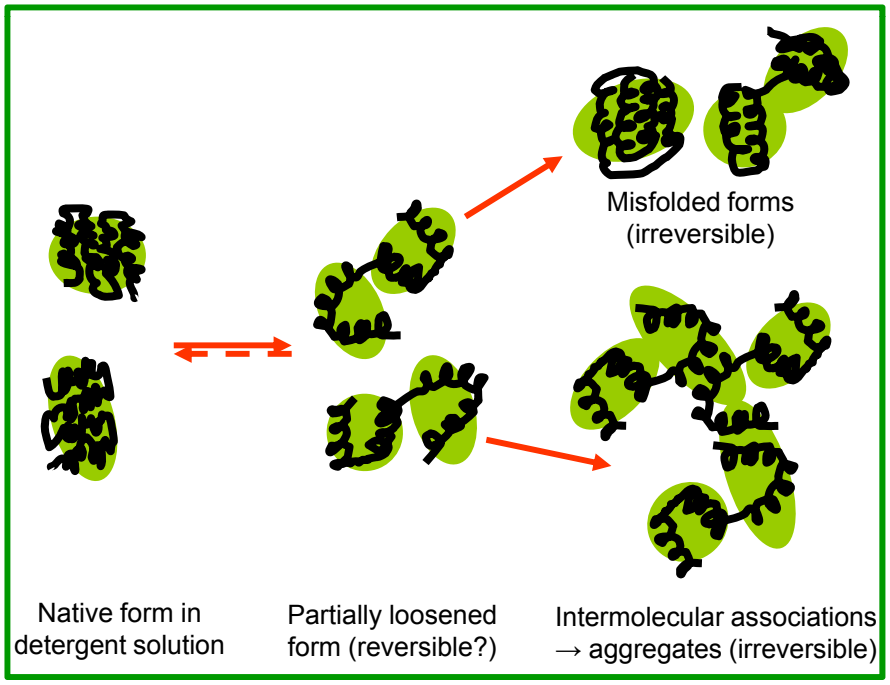
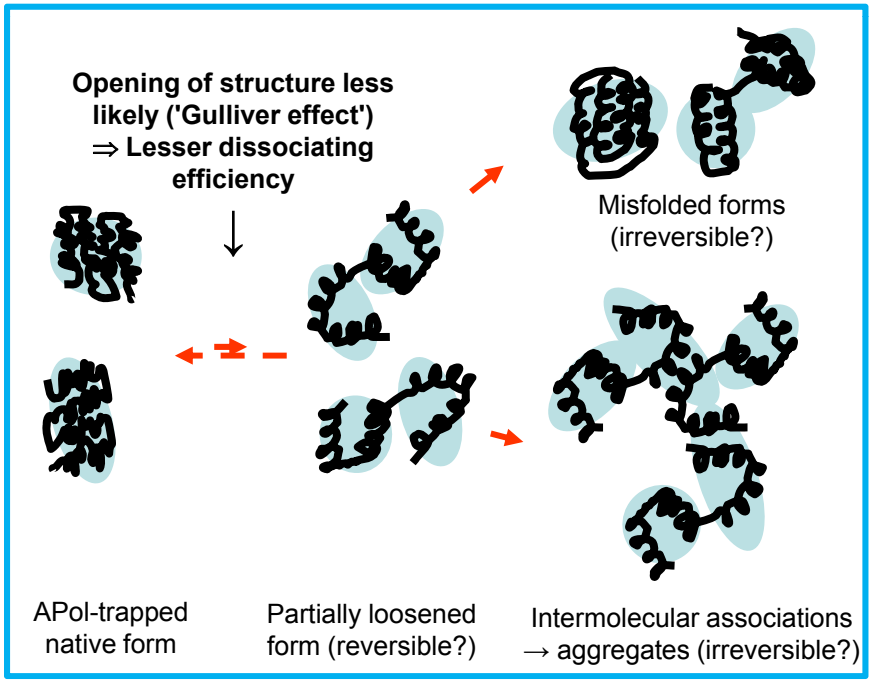
1. Amphipols may affect the dynamics –and, thereby, the activity– of the proteins they bind to
2. Damping of dynamics may contribute to membrane protein stabilization by amphipols

⇒ Could the 'Gulliver effect' contribute to stabilizing APol-trapped MPs against inactivation ?

Denaturation by detergents



Stabilization by amphipols



For discussion, see Popot *et al.*, *CMLS*, 2003; Picard *et al.*, *Biochemistry*, 2006.

In conclusion

• Protocol of trapping:	Usually, we transfer MPs from detergent solution to APols
• Solution properties:	MP/APol complexes are essentially homogeneous but the monodispersity depends on the pH (higher than 7), the absence of divalent cations, and the presence of extra free APols
• Structural organization:	APols interact exclusively with the hydrophobic transmembrane surface of MPs and form a compact layer of 1.5 to 2nm thickness
• Dynamcis of association:	APols do not desorb from MPs but they exchange for other surfactants (detergent, APols, or lipids)
• Activity:	It seems to depend on the amplitude of the transmembrane conformational changes occurring during the catalytic cycle of the protein of interest
• Stability:	MPs trapped in APols are generally more stable than in detergent solution
• Ligand binding:	Generally unaffected by Apol trapping