Trapping membrane proteins with amphipols.
Structure and properties of membrane protein/amphipol complexes

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1) How to trap a membrane protein (MP) in amphipols (APols)?

- Direct extraction of MPs from the biological membrane has been observed in a very few cases
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![Diagram showing steps for trapping membrane proteins in amphipols](image-url)
1) How to define conditions for trapping a MP in APols?

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

![Diagram of trapping after dilution under the cmc]

- High speed centrifugation

![Graph showing absorbance at 280nm before and after 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)


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

**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)**

**SANS (BR, 27 kDa)**


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

Catoire *et al.*, *Eur Biophys J*, 2010
2) What do MP/APol complexes look like after trapping?

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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**

**Surface Plasmon Reasonance measurements upon extensive washing**

Zoonens et al., Biochemistry, 2007

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

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**Exchange for free amphipol**

![Graph showing exchange for free amphipol](image1)

**Displacement by detergent**

![Graph showing displacement by detergent](image2)

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

Martinez et al., FEBS Lett., 2002
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Photocycle of BR

Spectral changes

Neutze et al., BBA, 2002
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![UV-visible spectra of BR](image)

Gohon et al., Biophys. J., 2008

<table>
<thead>
<tr>
<th></th>
<th>DAS1 (ns)</th>
<th>DAS2 (μs)</th>
<th>DAS3 (μs)</th>
<th>DAS4 (ms)</th>
<th>DAS5 (ms)</th>
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<tr>
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<td>6.3</td>
</tr>
</tbody>
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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

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\[\Rightarrow \text{damping of large-scale transmembrane movements ('Gulliver effect')}\]
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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

**Bacteriorhodopsin**


**Calcium ATPase**

Champeil *et al.*, *JBC* 2000

**Leukotriene receptor BLT1 (GPCR)**

Dahmane *et al.*, *Biochemistry*, 2009
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⇒ Could the 'Gulliver effect' contribute to stabilizing APol-trapped MPs against inactivation?

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

• Dynamics 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