Biologie Physico-Chimique
des Protéines Membranaires

UMR 7099

Solubilization and stabilization of membrane proteins by cycloalkane-modified amphiphilic polymers

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SMALP meeting

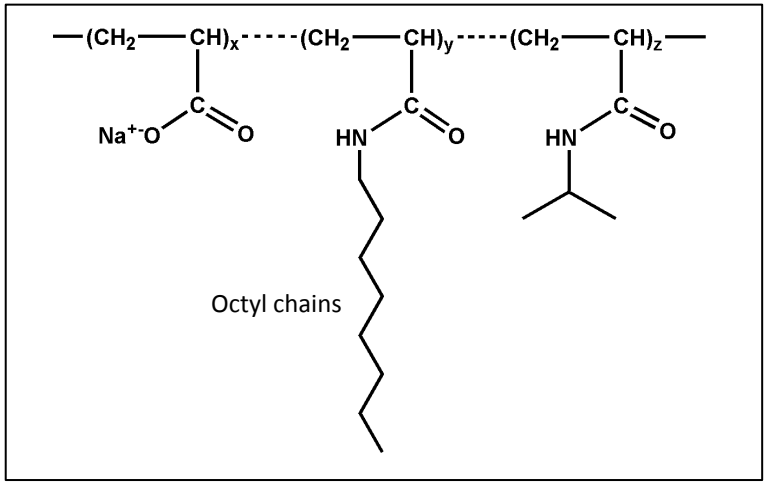
Septembre 18, 2020

Design of the cycloalkane-modified amphiphilic polymers

⇒ Different hydrophobic groups between A8-35 and SMA

A8-35

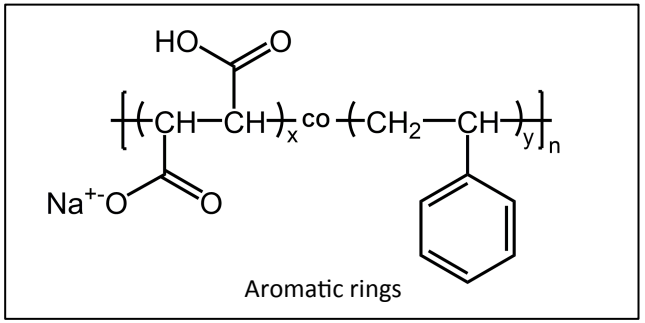
J.-L. Popot & co-workers



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

SMA

M. Overduin & T. Dafforn



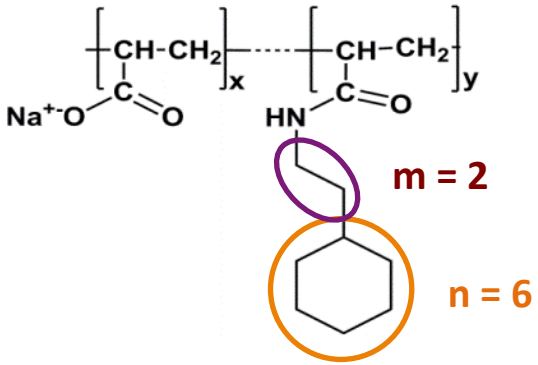
Knowles *et al.* (2009) *JACS,* **131,** 7484-5

What role the hydrophobic cycles play in the membrane-solubilizing properties of polymers?

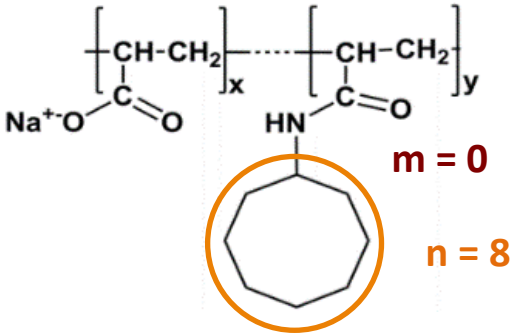
Design of the cycloalkane-modified amphiphilic polymers

⇒ Synthesis of a new family of polymers, called "**CyclAPols**"

C_6-C_2-50

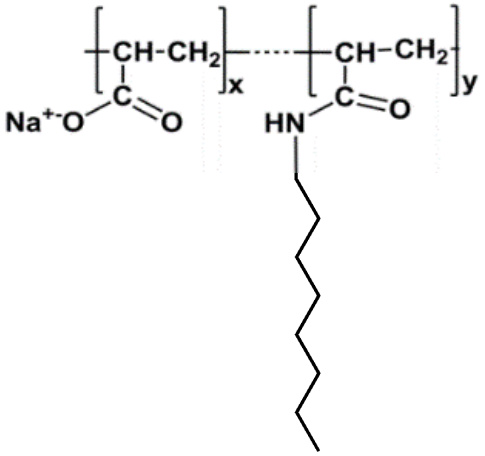


C_8-C_0-50



APol of reference:

A8-50



Marconnet *et al.* (2020) *Biomacromolecules,* **21,** 3459-67

Nomemclature used for the CyclAPols:

C_n-C_m-y

n = number of carbon atom forming the ring

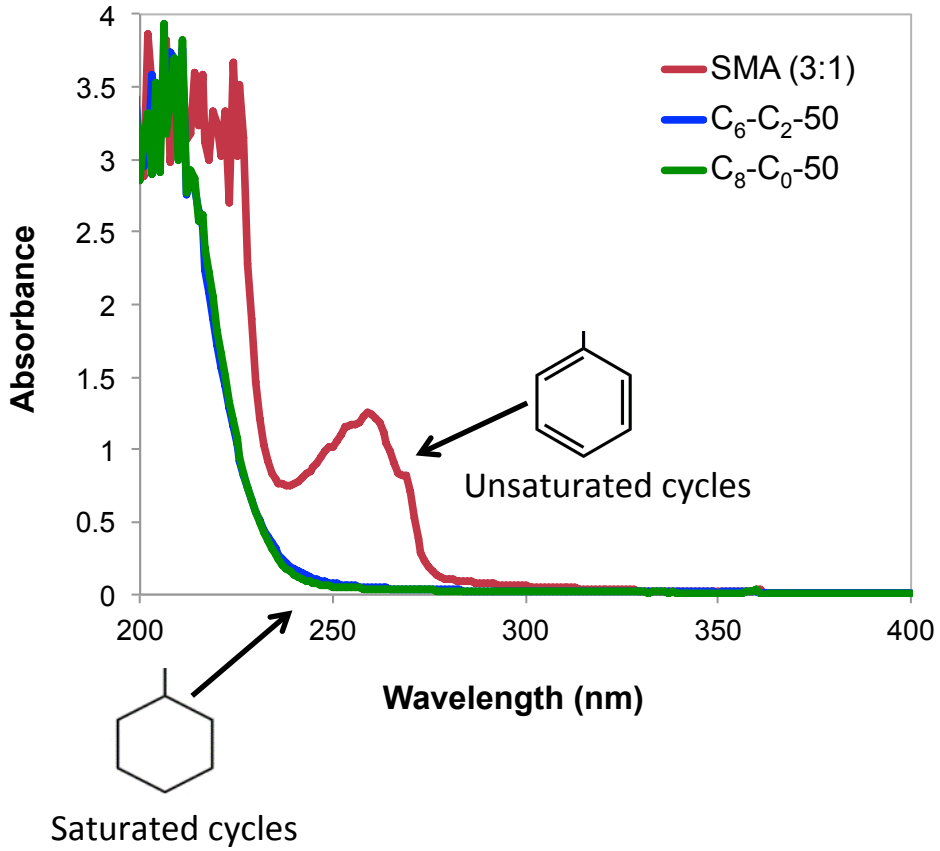
m = number of carton atoms between the cycle and the amid bound

Y = level of grafting of hydrophobic moieties

Characterization of the CyclAPols

⇒ Higly soluble

⇒ UV-compatibility



Characterization of the CyclAPols

⇒ Higly soluble

⇒ UV-compatibility

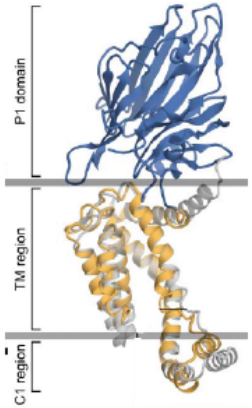
⇒ Membrane-solubilizing properties

- **Biological membranes:**
  - bacterial membranes (*Escherichia coli*)
  - purple membrane (*Halobacterium salinarum*)

- **Target membrane proteins:**
  - YidC
  - Bacteriorhodopsin (BR)
  - BR

Characterization of the CyclAPols

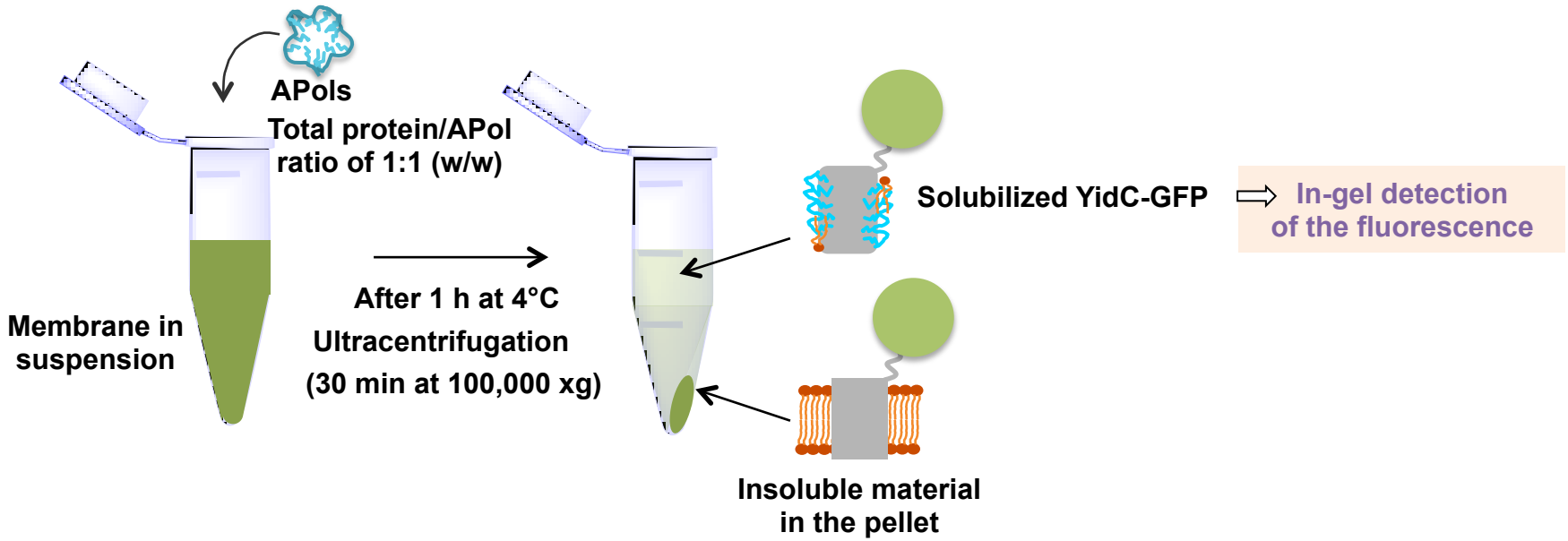
1. Extraction performed with the plasma membranes of *E. coli*
    1.1. Overexpression of YidC-GFP



YidC-GFP overexpressed in E. coli plasma membrane
(62 kDa + 27 kDa)
TM domain: 6 α helix
Function: insertase

Kumazaki et al., *Scientific Reports* (2014)

Protocol:



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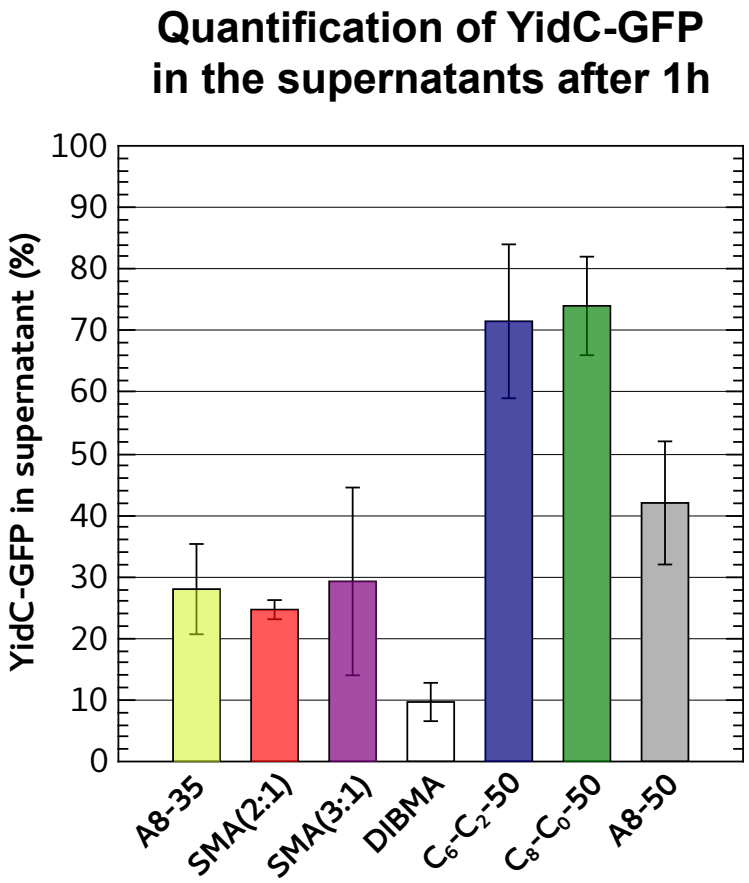
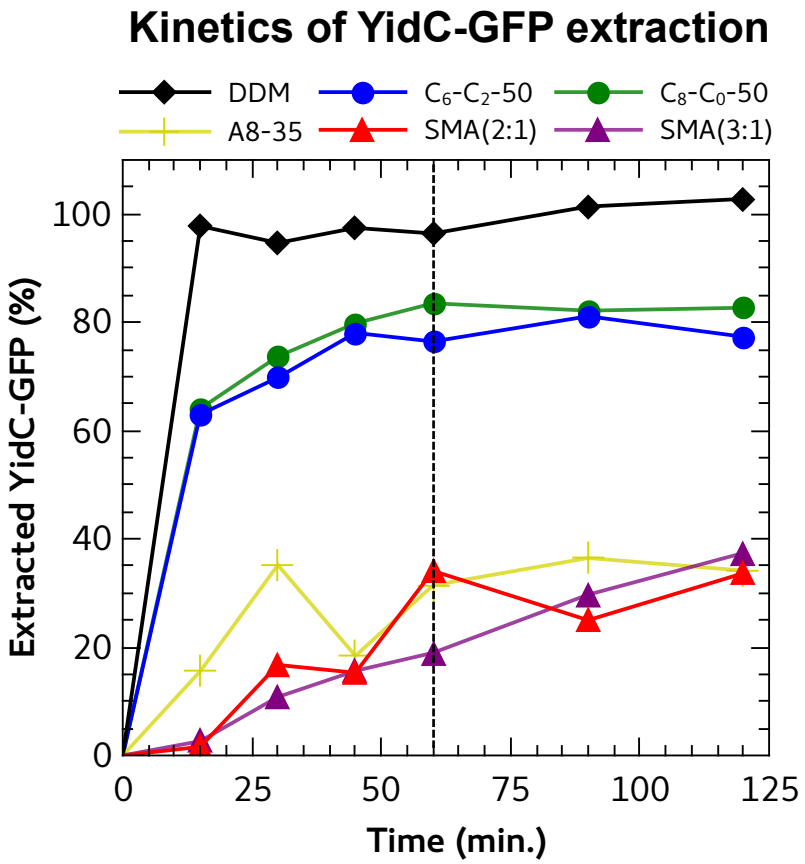


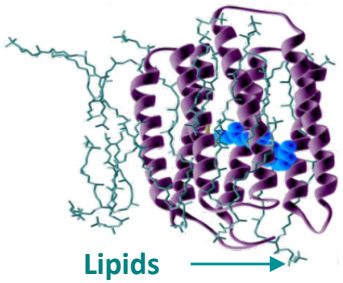


Experimental conditions
[polymer] = 0.2%
Buffer: 20 mM Tris-HCl, 150 mM NaCl pH 8.0
Incubation 1 hour at 4°C
Ultracentrifugation 100,000 xg for 30 min

Direct extraction using CyclAPols is the most efficient as compared to the other polymers

Characterization of the CyclAPols

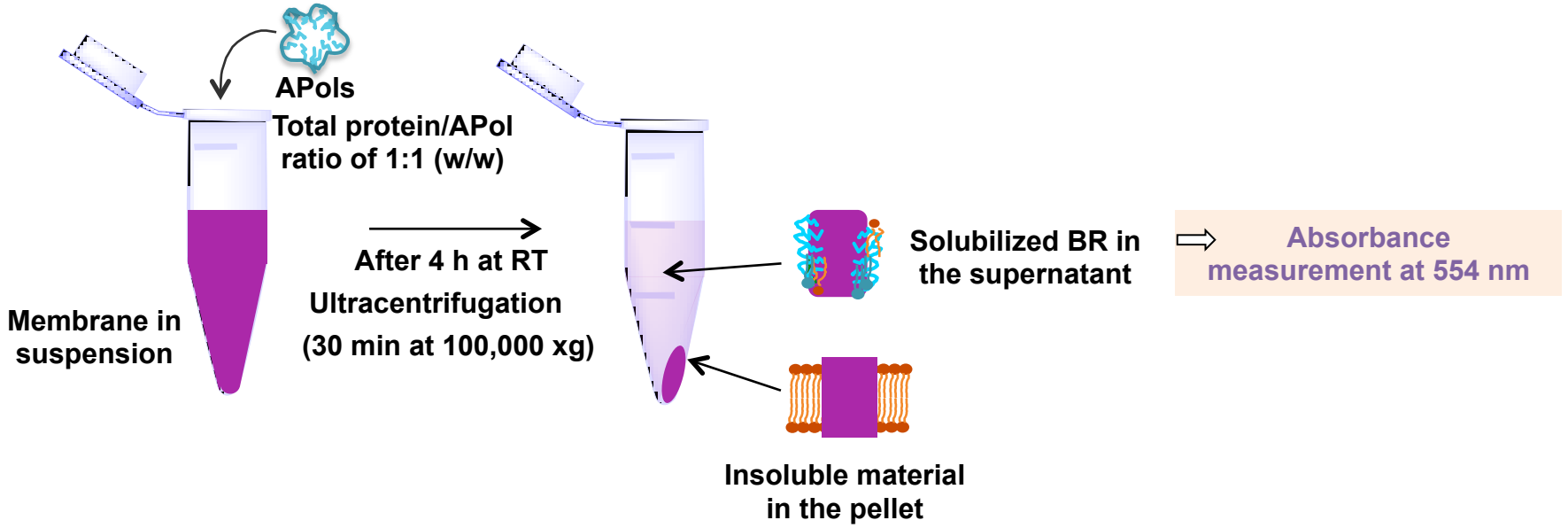
1. Extraction performed with the plasma membranes of *E. coli*
    1.2. Overexpression of BR from *Haloquadratum walsbyi* (*Hqw*BR)



BR

Bacteriorhodopsin (27 kDa)
TM domain: 7 α helices
Function: Proton pump
Cofactor: retinal

Protocol:



Characterization of the CyclAPols

1. Extraction performed with the plasma membranes of *E. coli*
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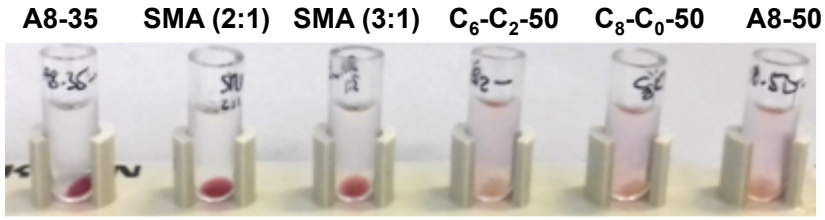
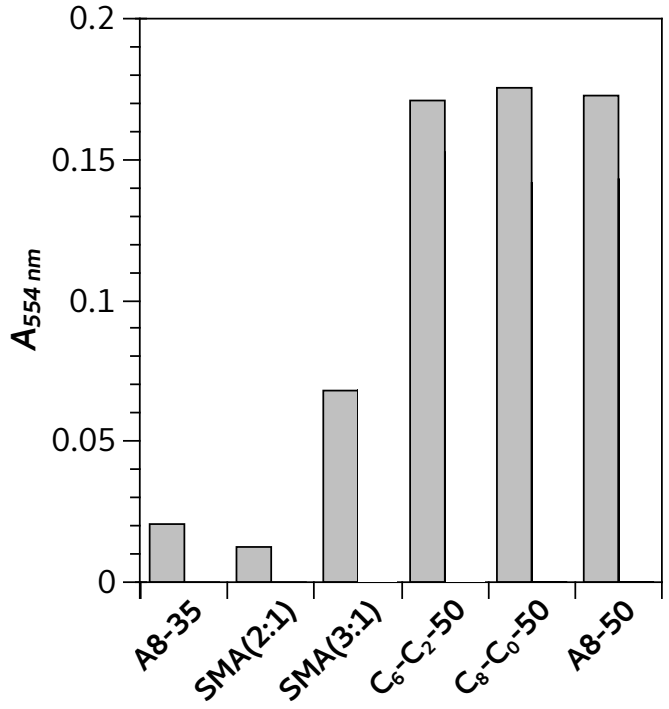
Quantification of *Hqw*BR in the supernatants





Experimental conditions
[polymer] = 0.2%
Buffer: 20 mM Tris-HCl, 150 mM NaCl pH 8.0
Incubation 4 hour at RT
Ultracentrifugation 100,000 xg for 30 min

Characterization of the CyclAPols

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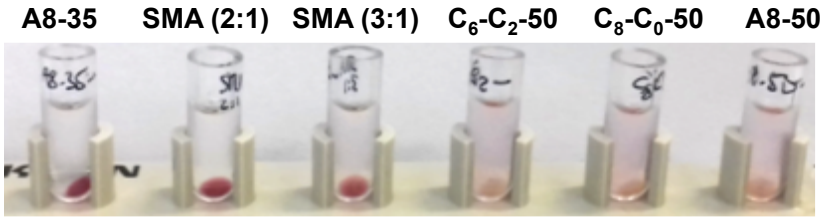
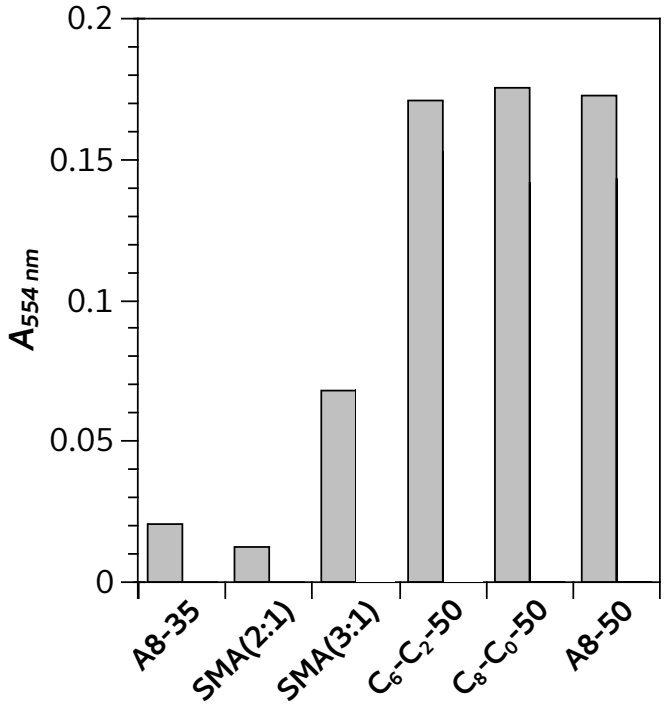
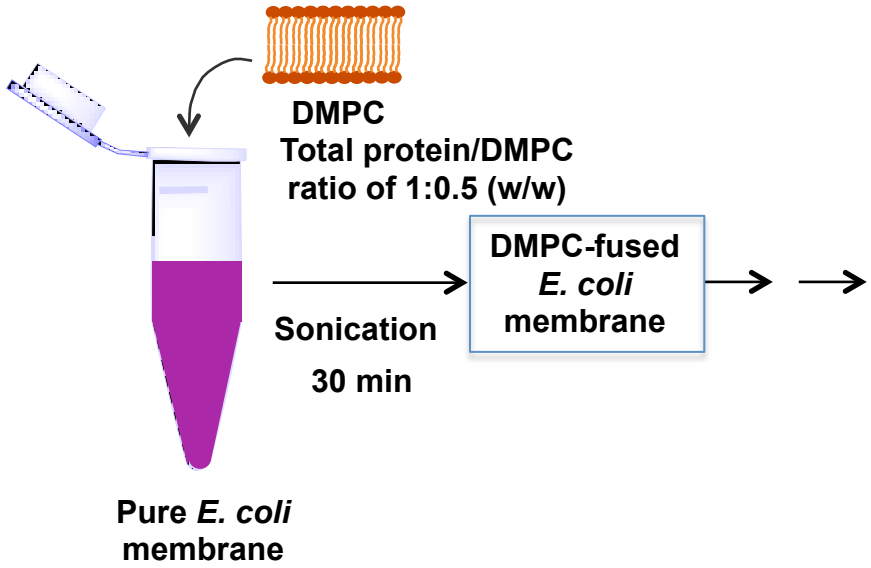




Protocol 2:

Broecker et al., *Structure* (2017)



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    1.2. Overexpression of BR from *Haloquadratum walsbyi* (*Hqw*BR)

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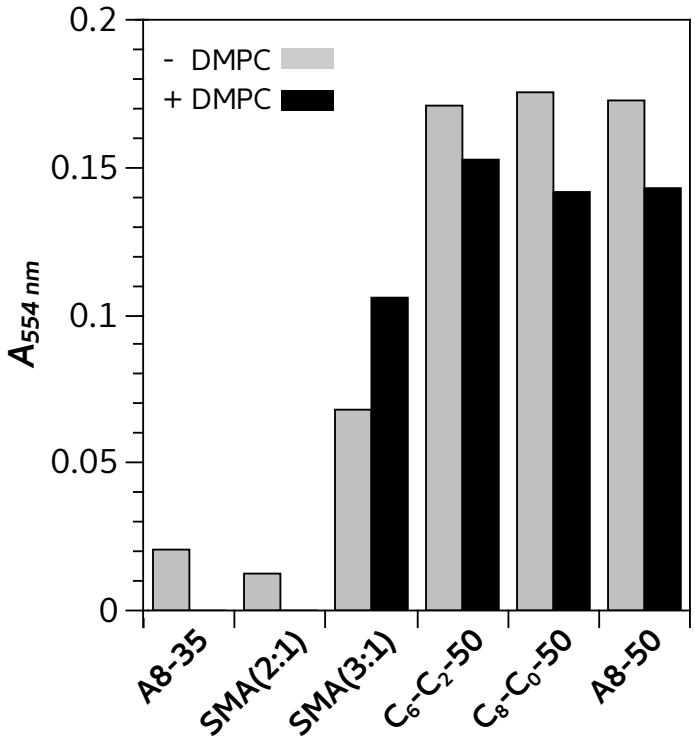
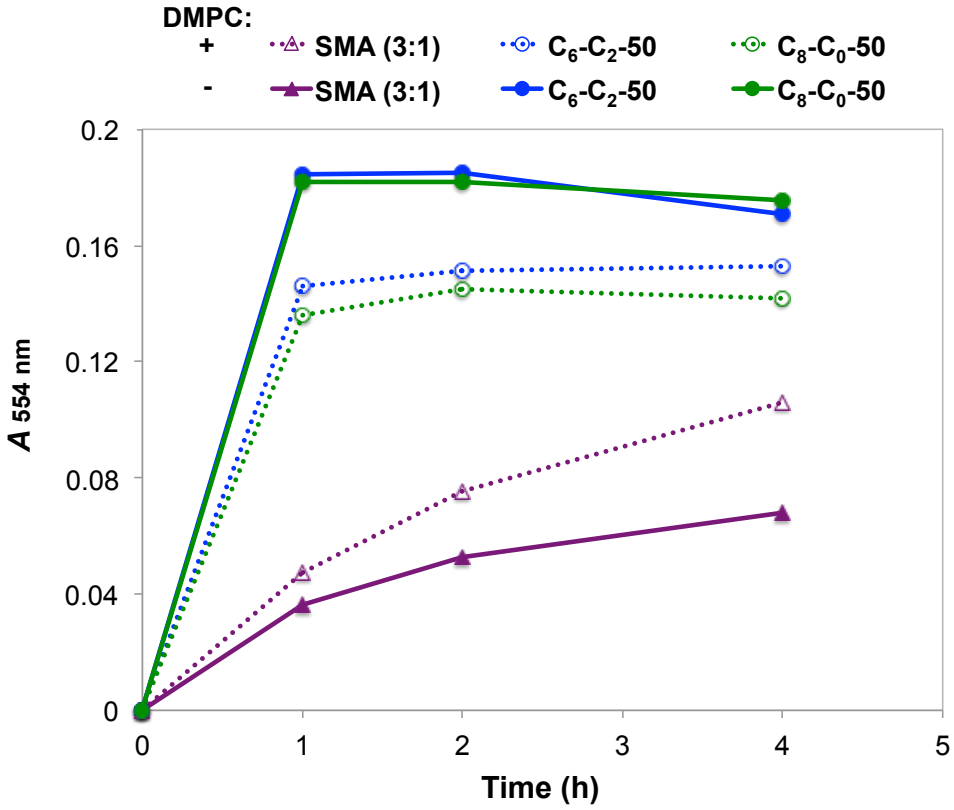


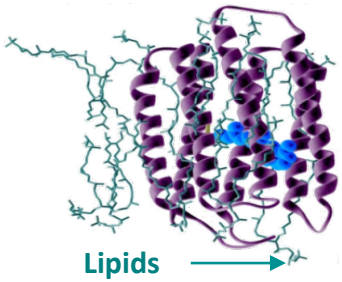
Kinetics of *Hqw*BR extraction



Unlike SMA (3:1), the CyclAPols are not better solubilizers in the presence of exogeneous lipids

Characterization of the CyclAPols

2. Extraction performed with the purple membranes of *Halobacterium salinarum*
   2.1. Natural expression of BR (*Hbs*BR)



BR

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TM domain: 7 α helices
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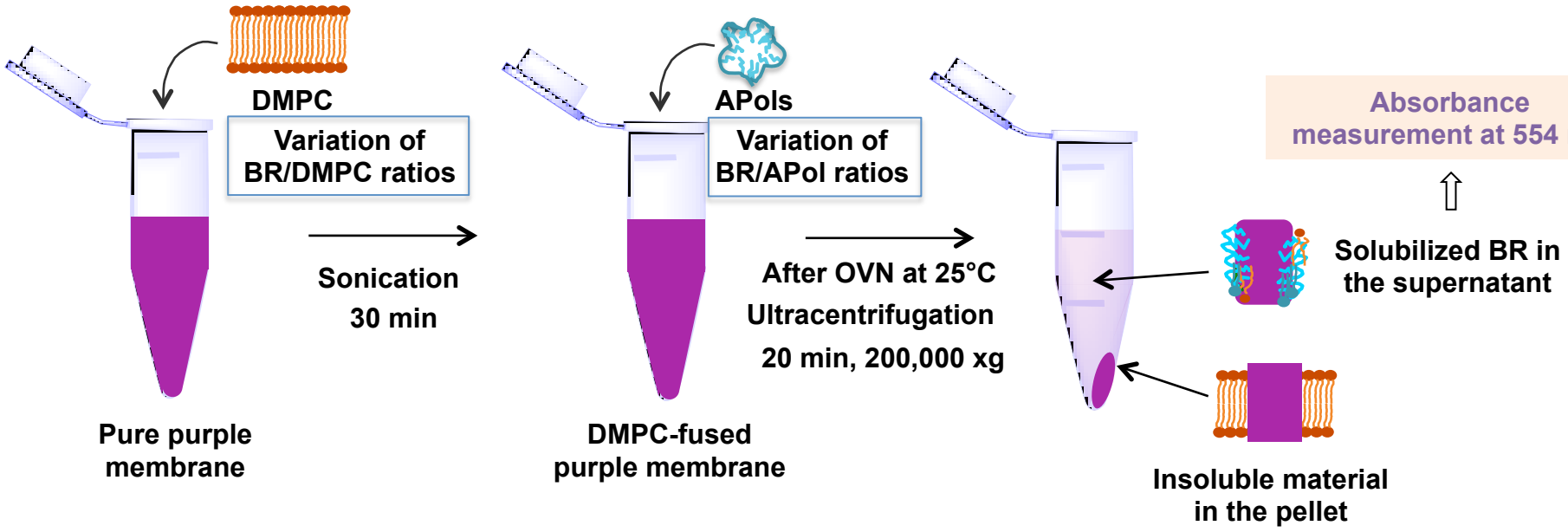
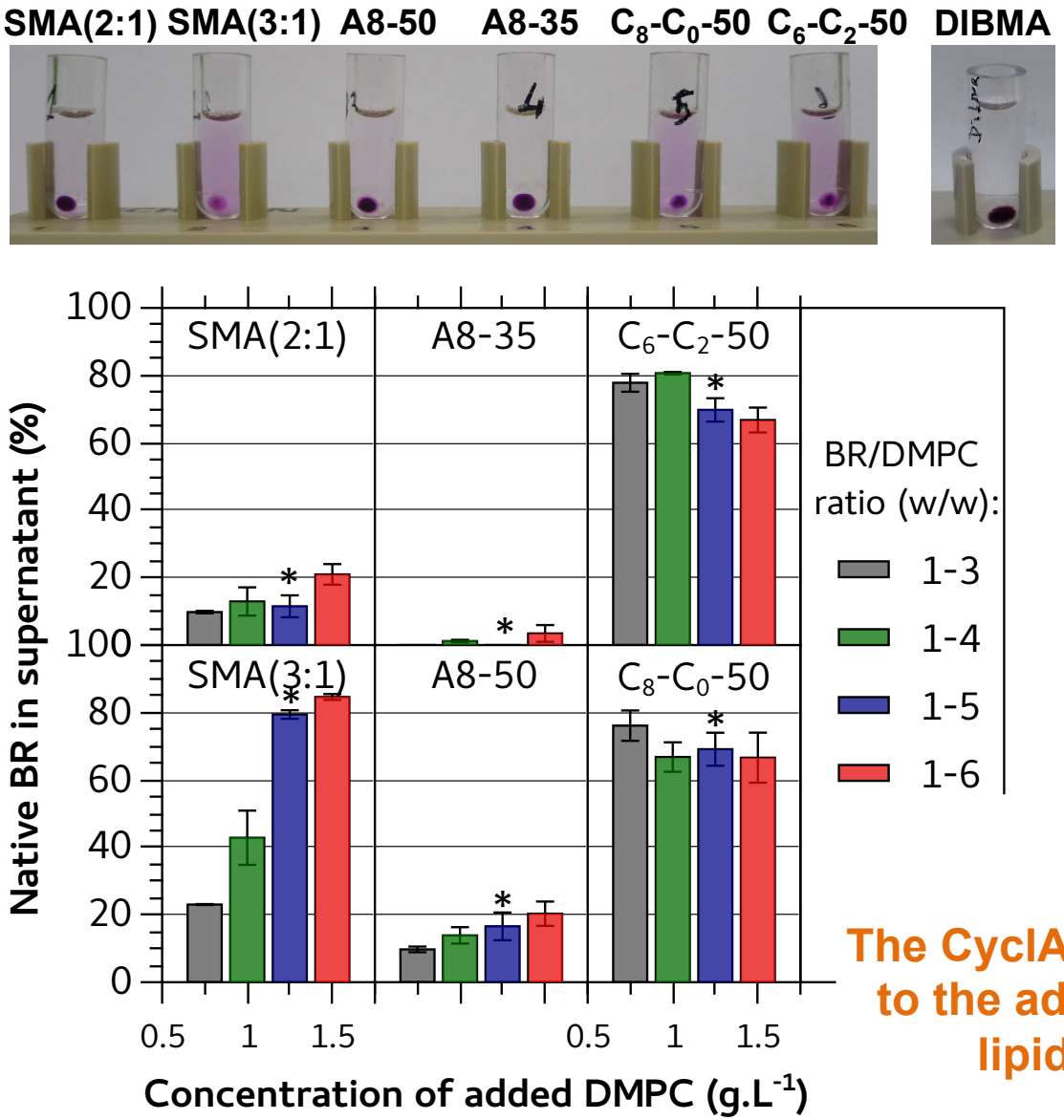
Protocol 2:

Knowles et al., *JACS* (2009)



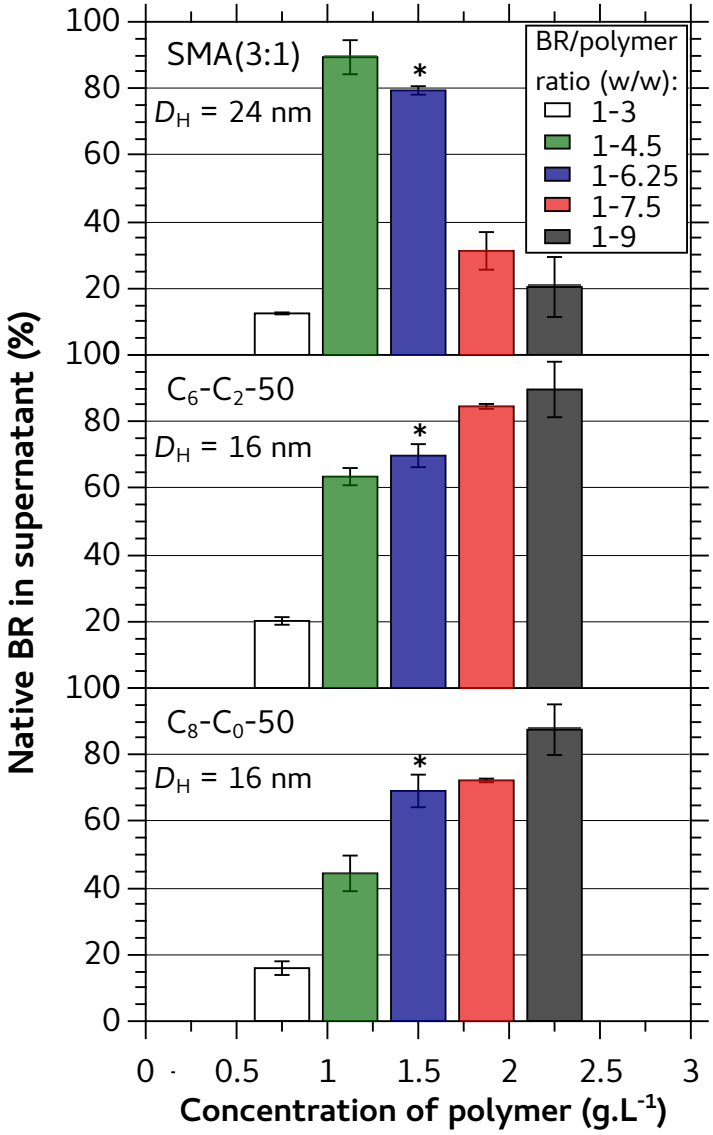
Characterization of the CyclAPols

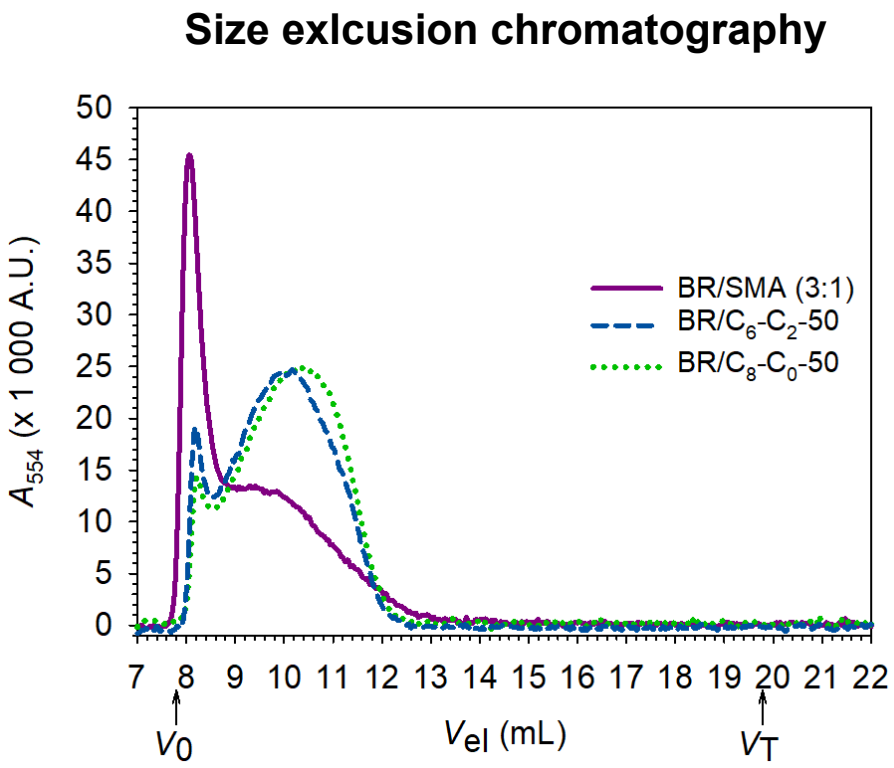
Extraction of *Hbs*BR from DMPC-fused purple membrane upon addition of the polymers



The CyclAPols are less sensitive to the addition of exogeneous lipids than SMA (3:1)

Characterization of the CyclAPols





The CyclAPol-extracted BR is smaller and less heterogeneous than the objects formed with SMA (3:1)

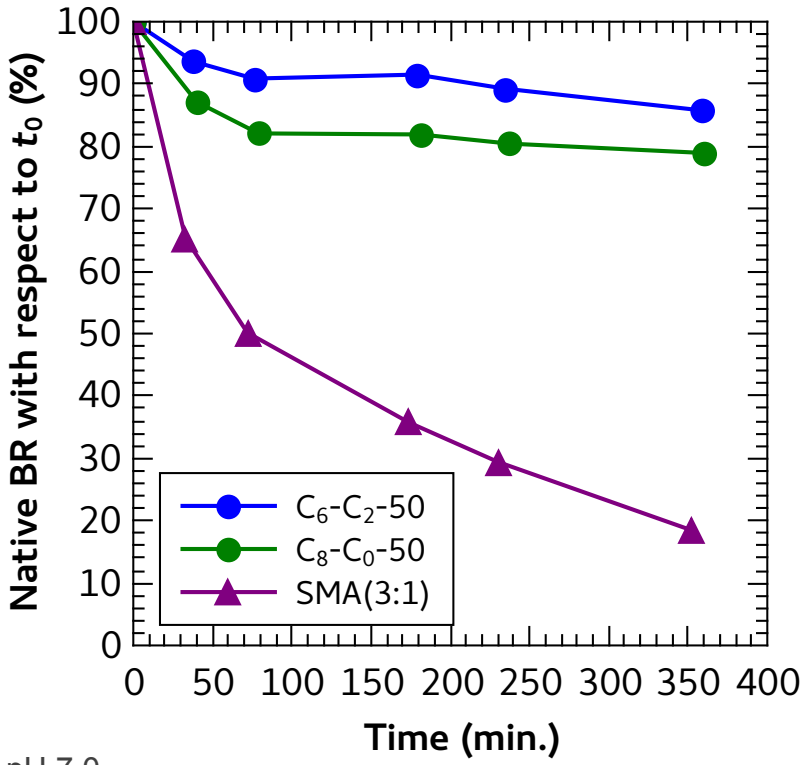
Characterization of the CyclAPols

⇒ Higly soluble

⇒ UV-compatibility

⇒ Membrane-solubilizing properties

⇒ Membrane protein stability



Experimental conditions
Buffer: 20 mM NaPi, 100 mM NaCl pH 7.0
Incubation at 50°C

The CyclAPol-extracted BR is more stable than in SMA (3:1)

Conclusions

✓ Cyclic hydrophobic moieties in APols enable to solubilize all types of protein and membranes

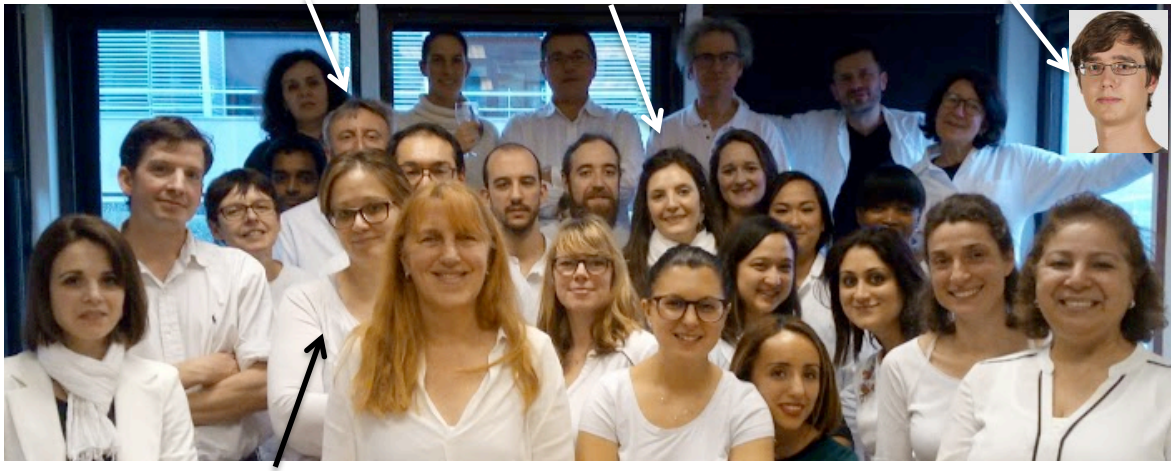
=> This would result most likely from a lower propensity of the polymer to self-assemble, making the hydrophobic groups more available to interact with the membranes

✓ The CyAPols present the advantages over SMAs to be:
- UV-transparent
- efficient at lower polymer concentrations
- less sensitive to the addition of exogeneous lipids
- better stabilizer for some membrane proteins

Acknowledgments

UMR 7099

Fabrice Giusti Anaïs Marconnet Baptiste Michon



Christel Le Bon

Collaborator

ENS Chimie
Christophe Tribet

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