

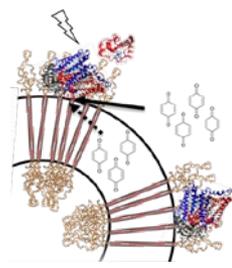
# Functional reconstitution of photosynthetic reaction centres in polymersomes

Francesco Milano, Rocco Roberto Tangorra, Omar Hassan Omar, John Henrard, Roberto Comparelli, Francesca Italiano, Alessandra Operamolla, Angela Agostiano, Gianluca M. Farinola, Massimo Trotta

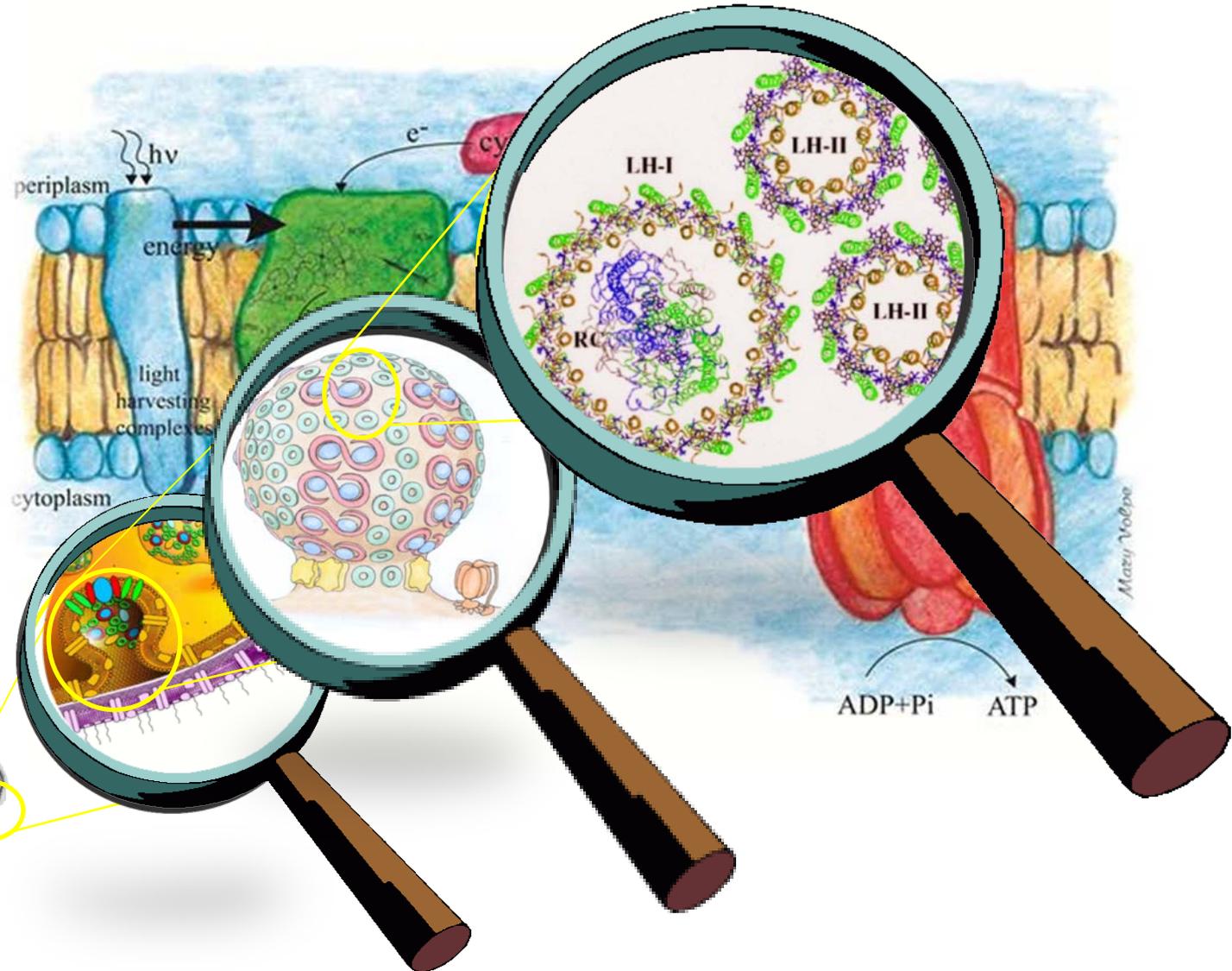
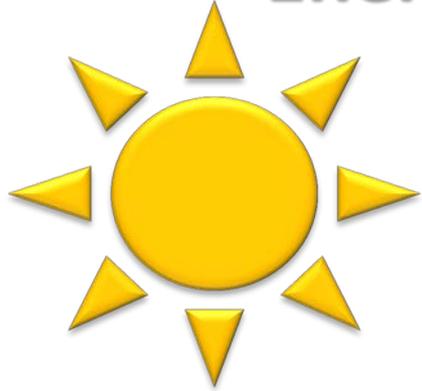
*University of Bari- Chemistry Department  
Institute of Physico-Chemical Processes – CNR Bari*



*Bari - Italy*

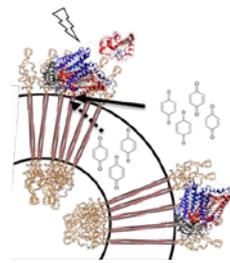


# Energy conversion in photosynthesis



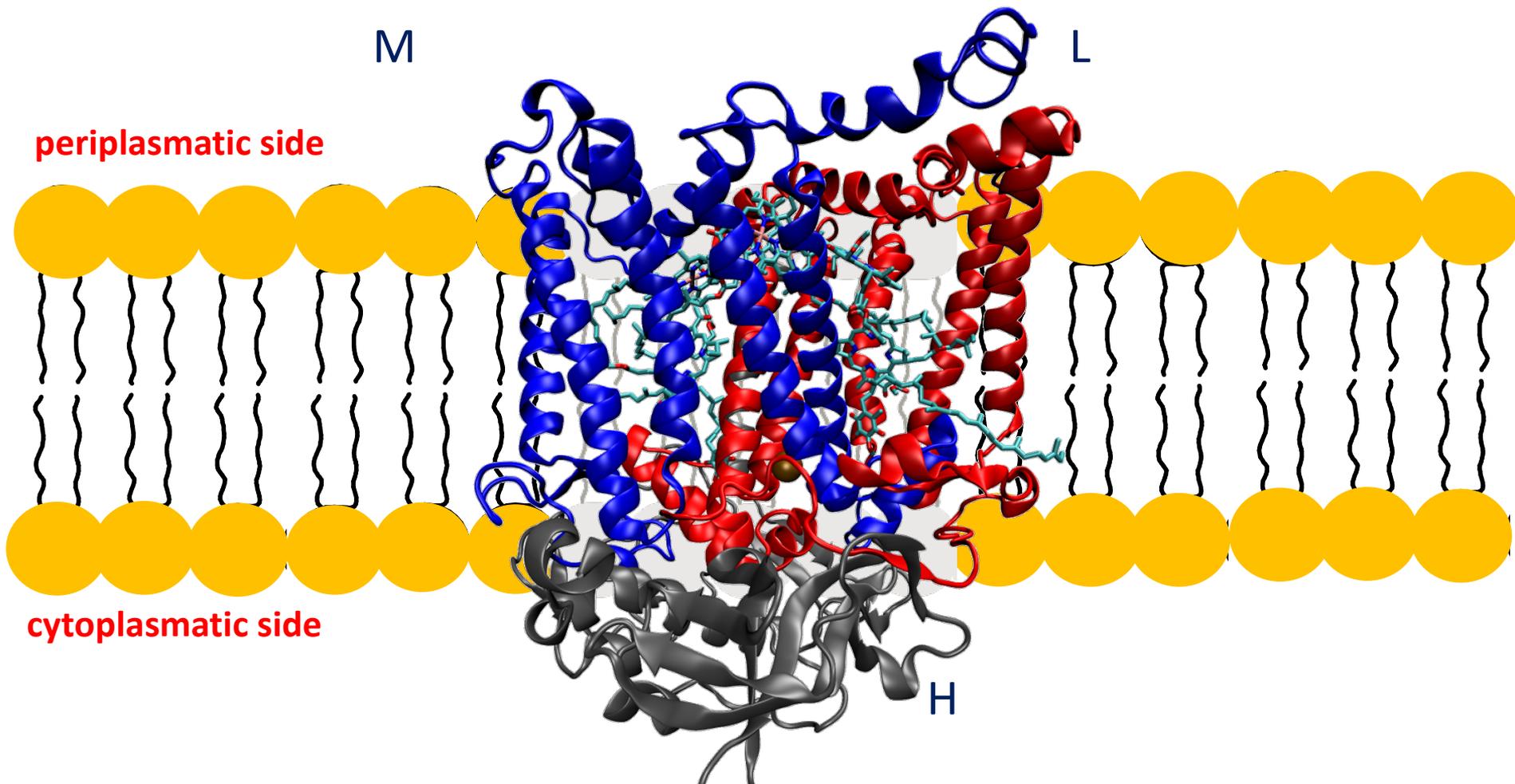
*Rhodobacter sphaeroides* R26

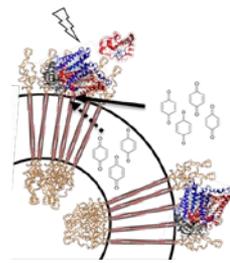




# Bacterial photosynthetic photoconverter

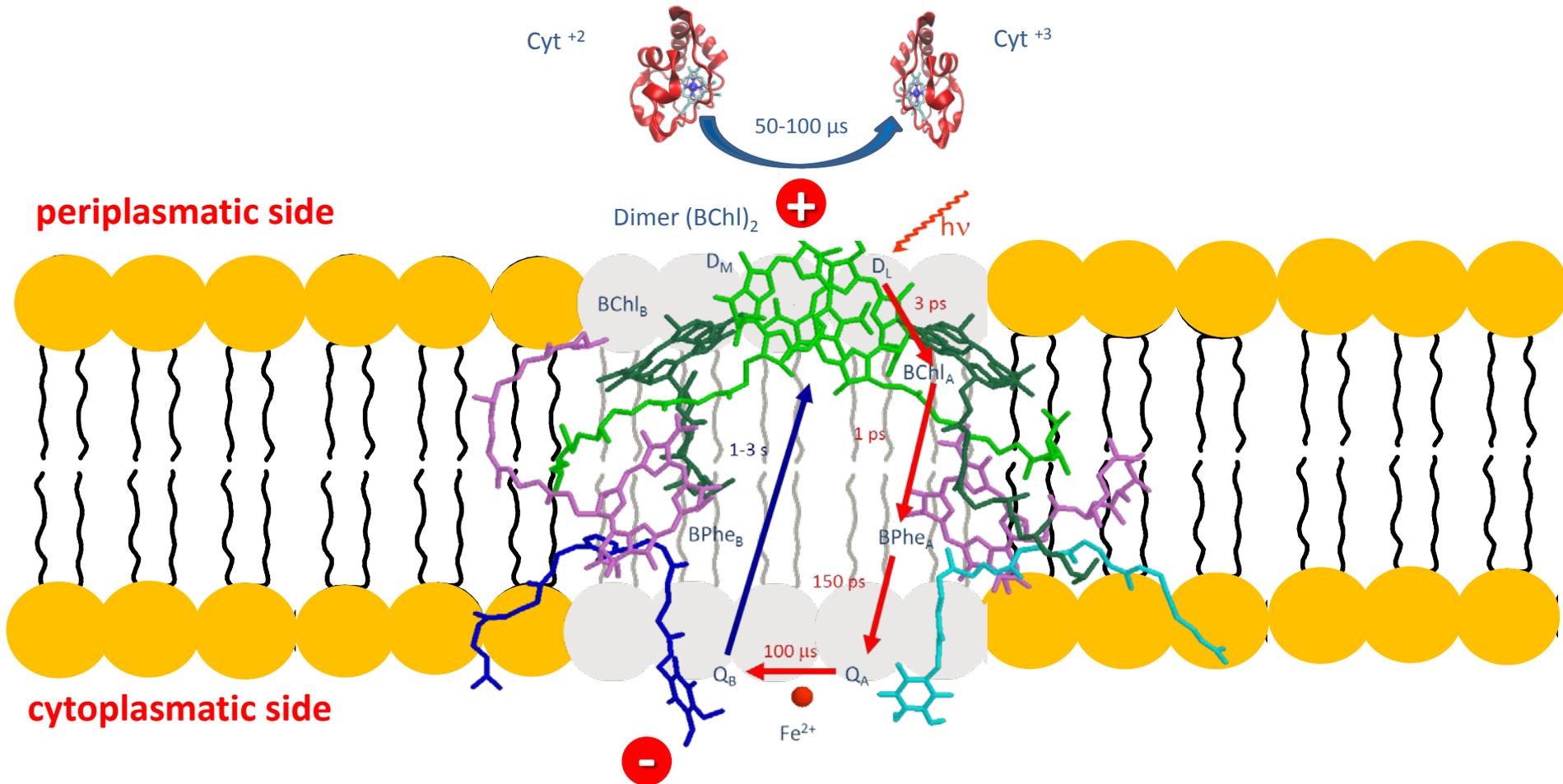
Reaction centre of the purple bacterium *Rhodobacter sphaeroides* strain R26

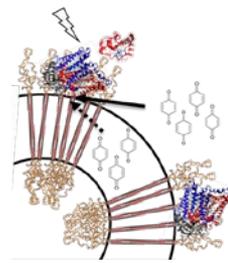




# Bacterial photosynthetic photoconverter

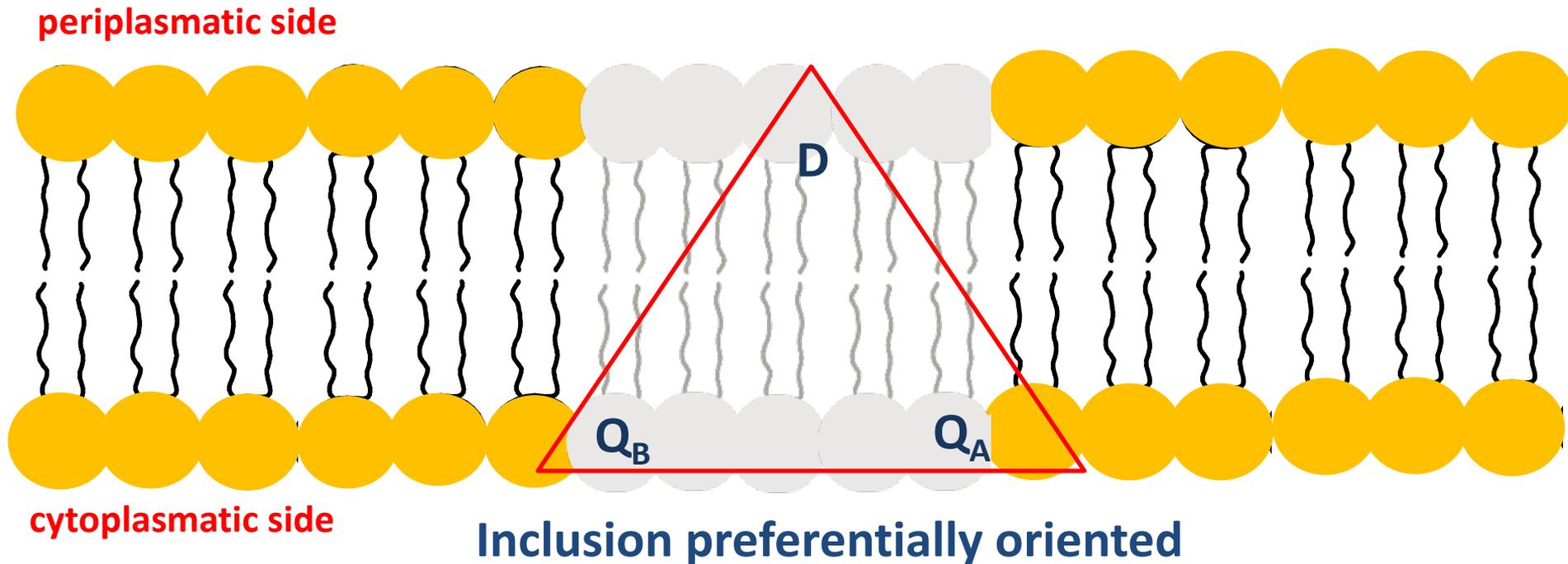
Reaction centre of the purple bacterium *Rhodobacter sphaeroides* strain R26

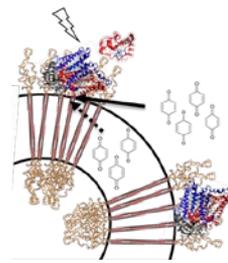




# Bacterial photosynthetic photoconverter

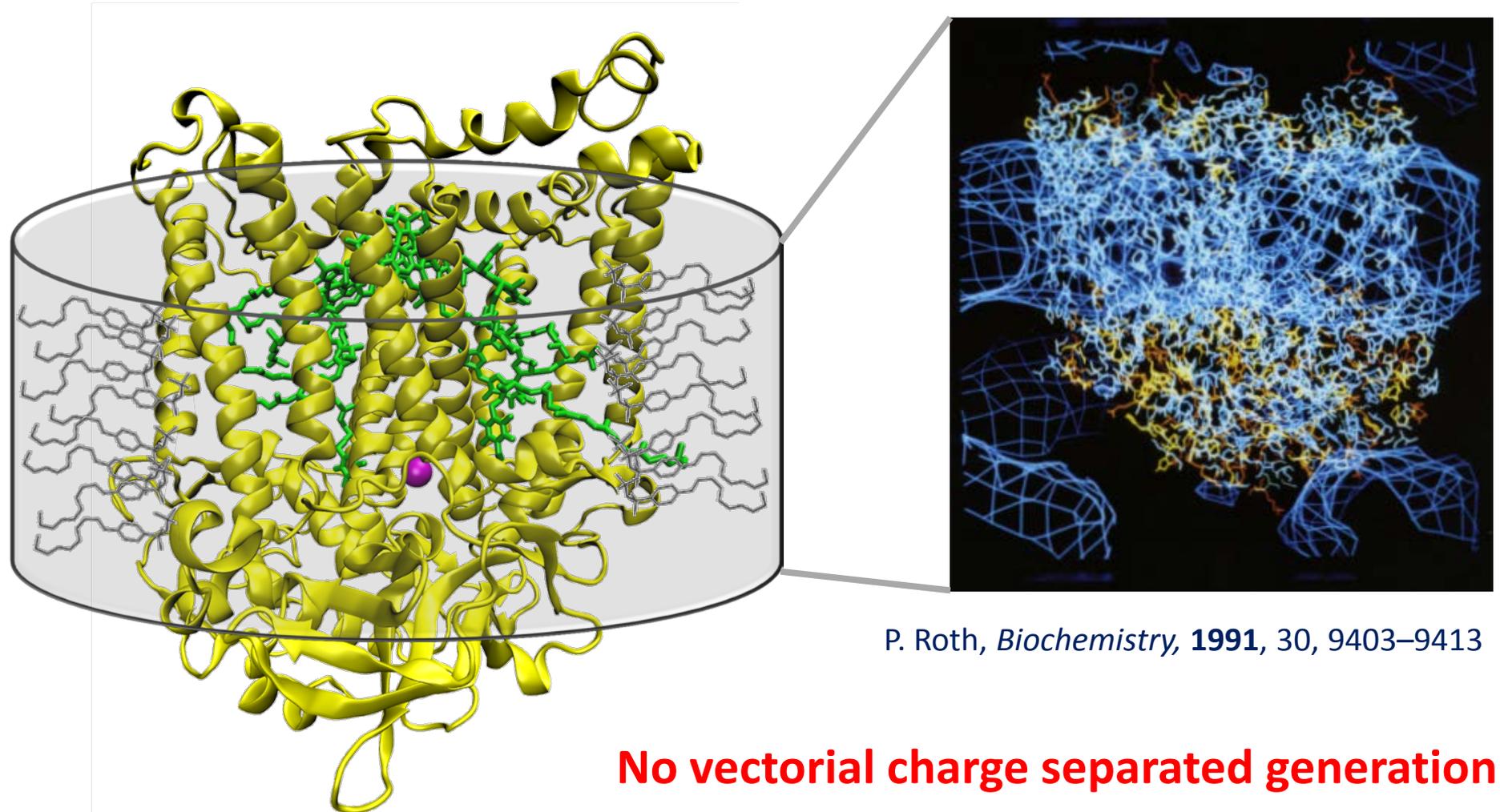
## The natural driving force





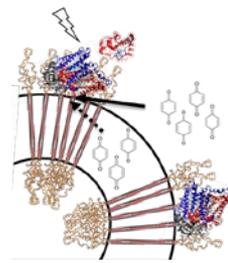
# Bacterial photosynthetic photoconverter

Reaction centre in detergent solution

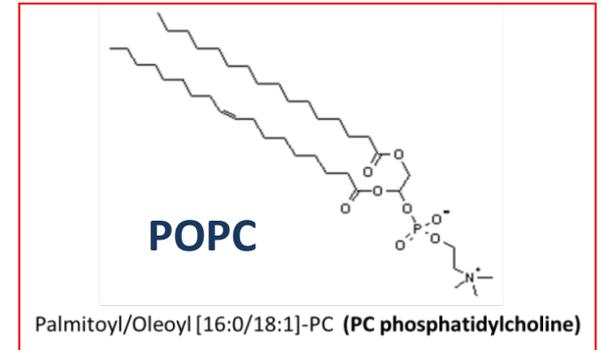
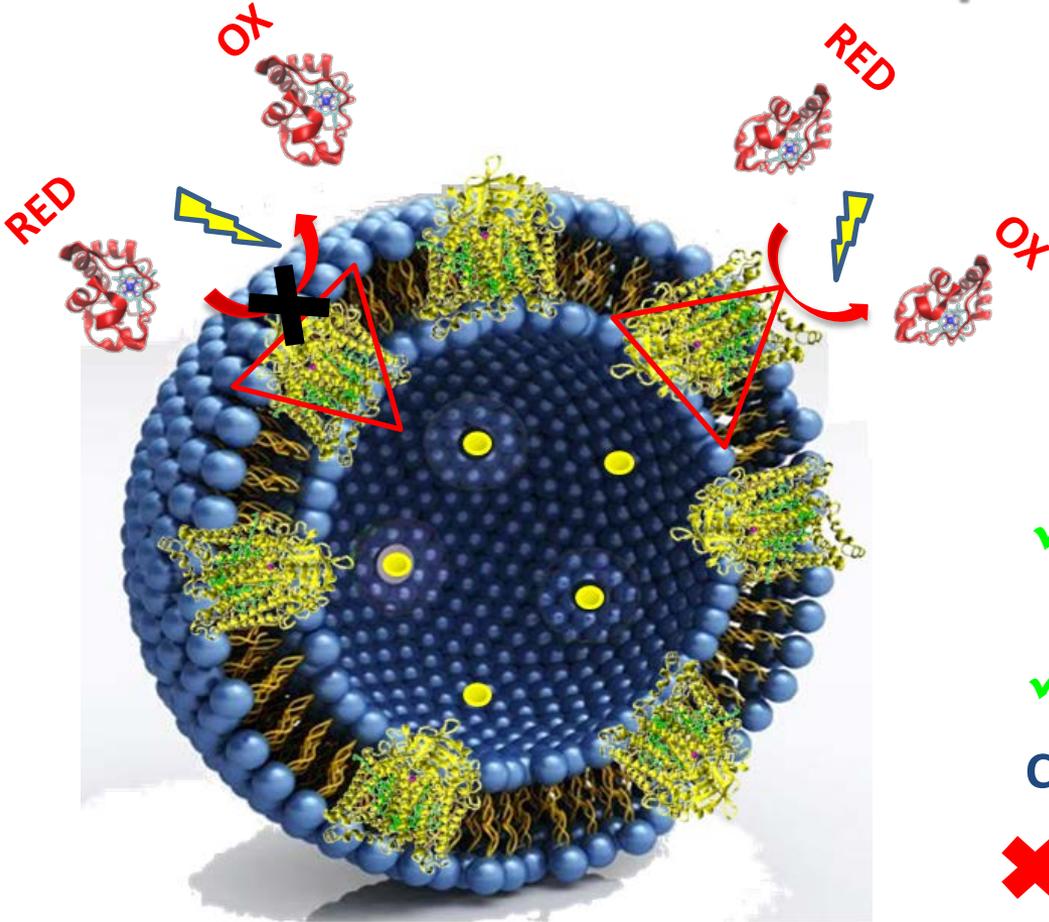


P. Roth, *Biochemistry*, 1991, 30, 9403–9413

**No vectorial charge separated generation**



## RC inclusion in liposomes by MVT

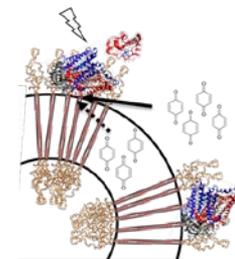


### Pros

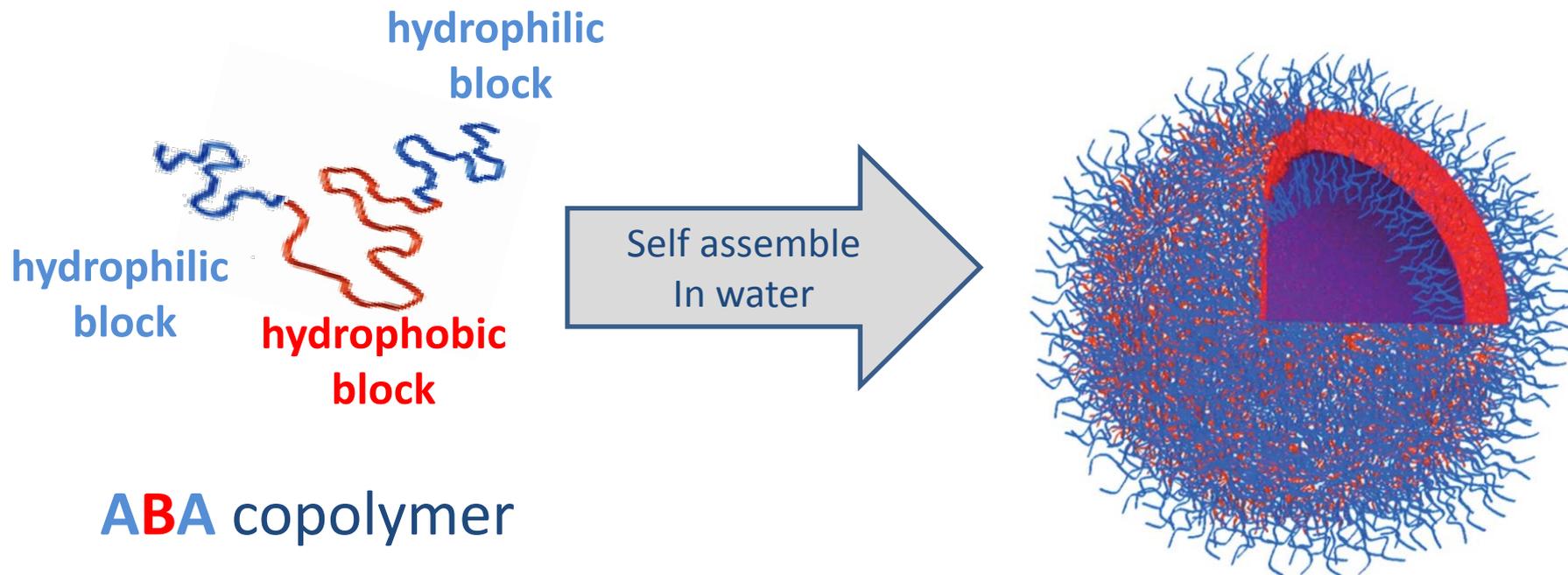
- ✓ natural phospholipid able to self assemble in vesicle
- ✓ fluid bilayer

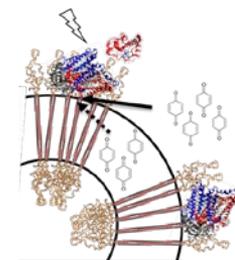
### Cons

- ✗ mechanical and chemical instability
- ✗ RC randomly oriented

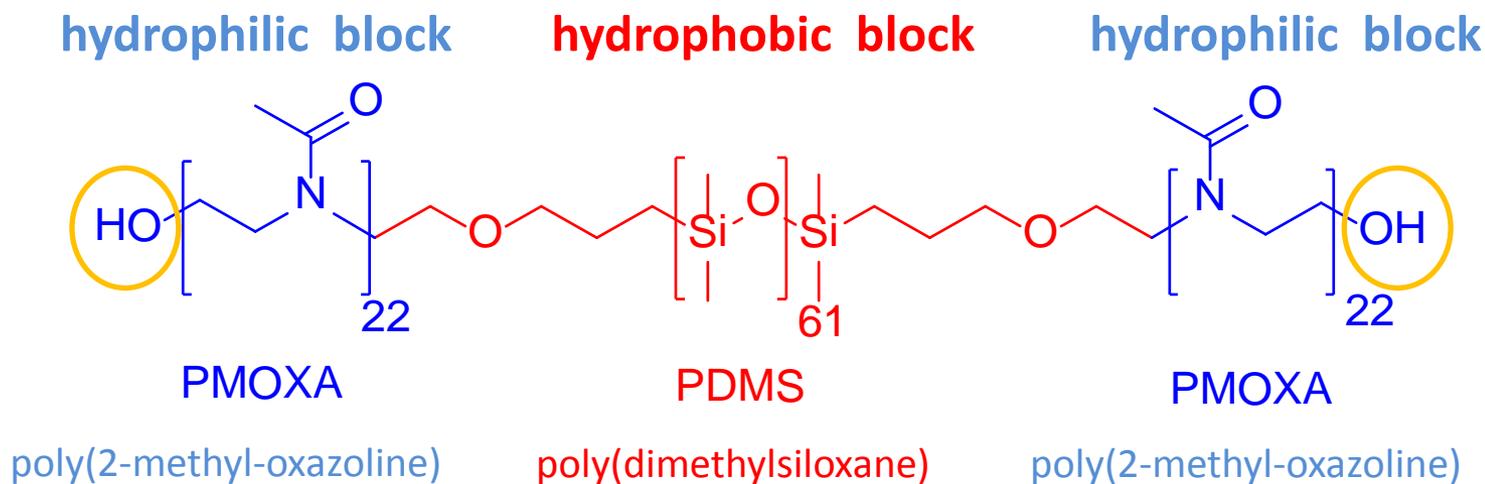


## Block copolymer: amphiphilic organic scaffoldings for biomimetic vesicles

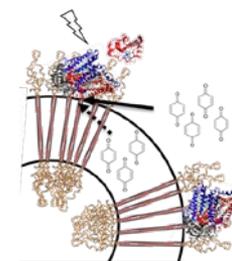




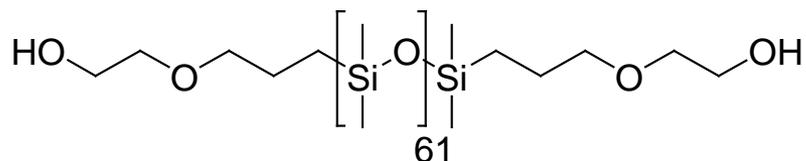
## Block copolymer: amphiphilic organic scaffoldings for biomimetic vesicles



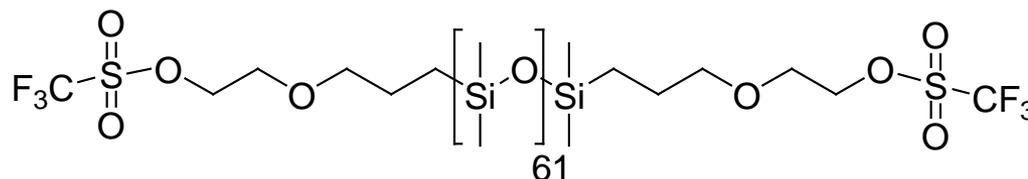
- ✓ tunable properties by suitable organic synthetic strategy
- ✓ possible modification of ending groups to obtain supramolecular assemblies



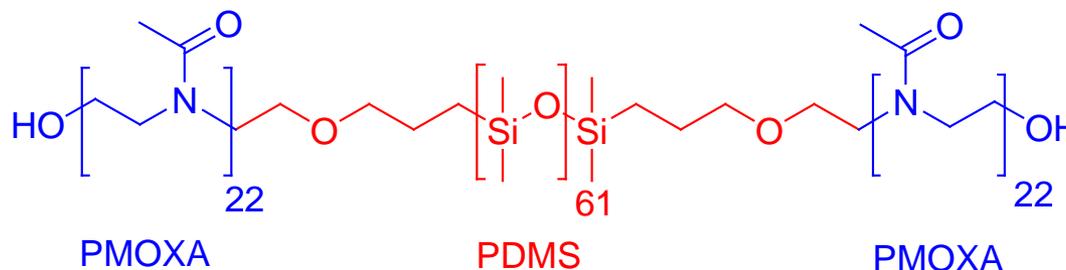
## PMOXA-PDMS-PMOXA triblock copolymers



$(\text{CF}_3\text{SO}_2)_2$  7 eq  
Pyridine 7 eq  
Hexane, 0°C, 17h  
55%



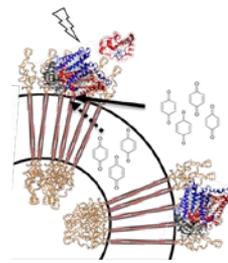
1) 2-methyloxazoline 42 eq.  
2) KOH, 1,2-dichloroethane, 50°, 48 h  
78%



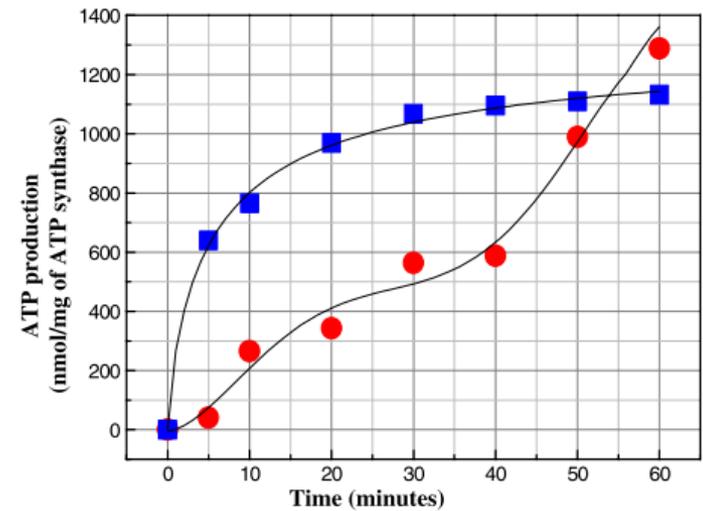
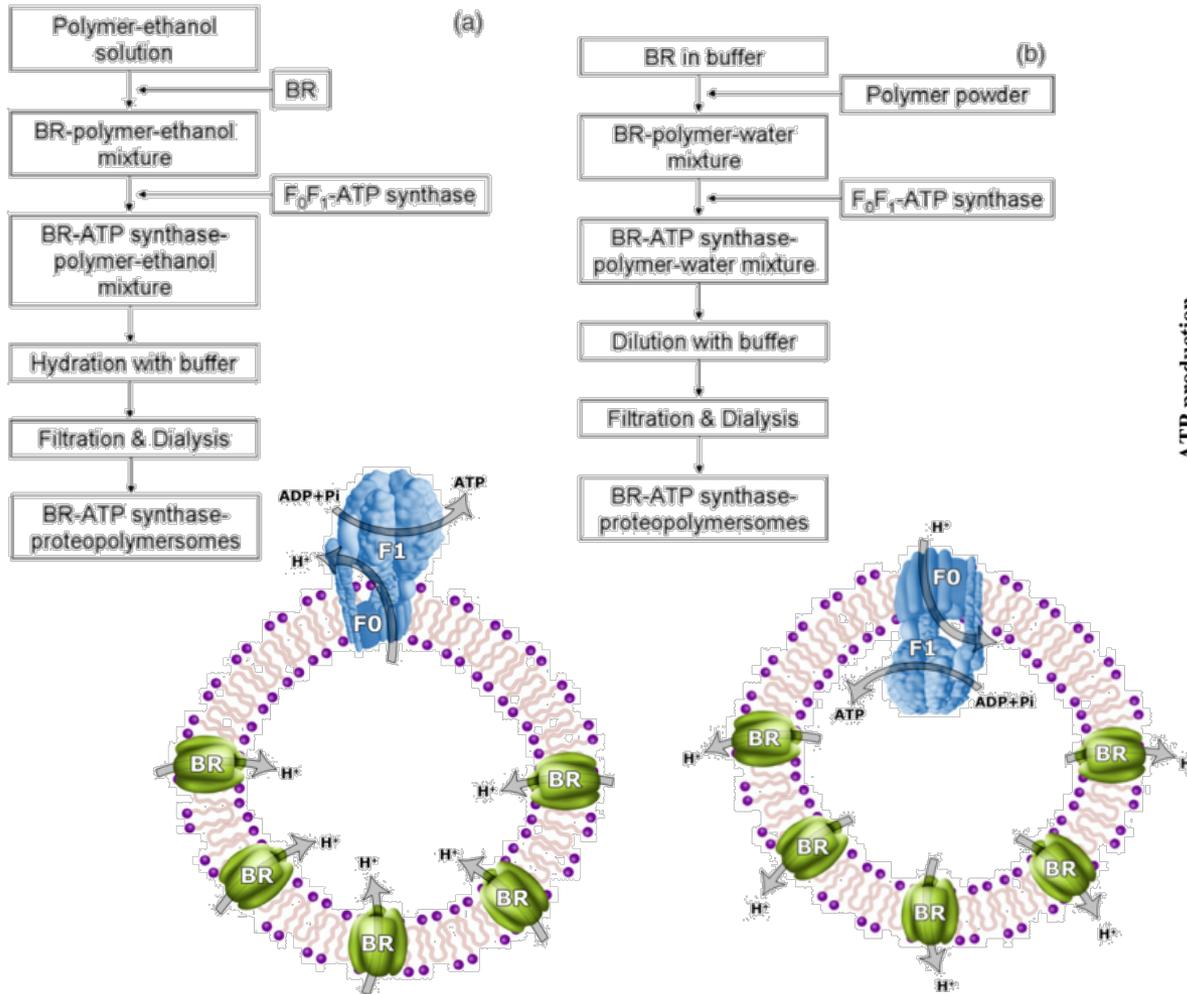
PMOXA

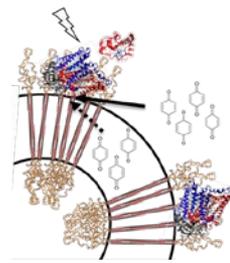
PDMS

PMOXA



# State of the art (1)





## State of the art (2)

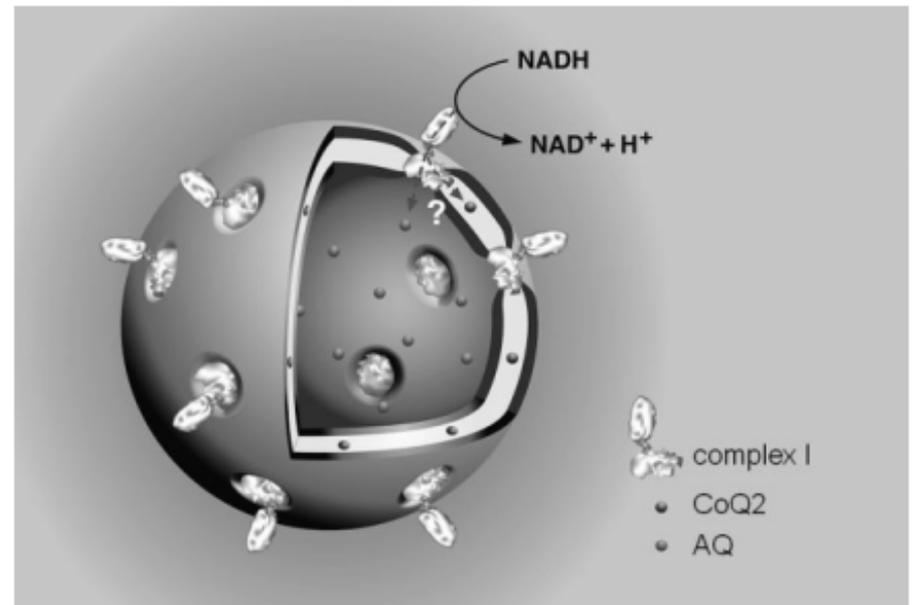
Polymersomes preparation  
by hydratation in water

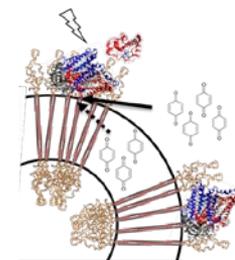
Doping the vesicles with  
triton 0.5%

Addition of DM-solubilized protein

Detergent removal by bio-beads

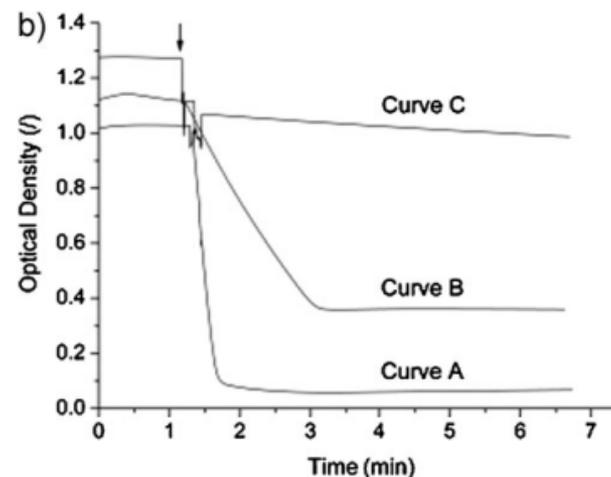
Not incorporated protein removal  
by Sepharose 4B cromatography



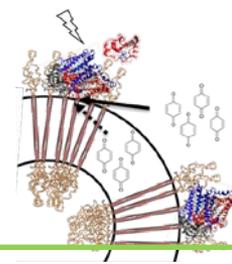


## State of the art (2)

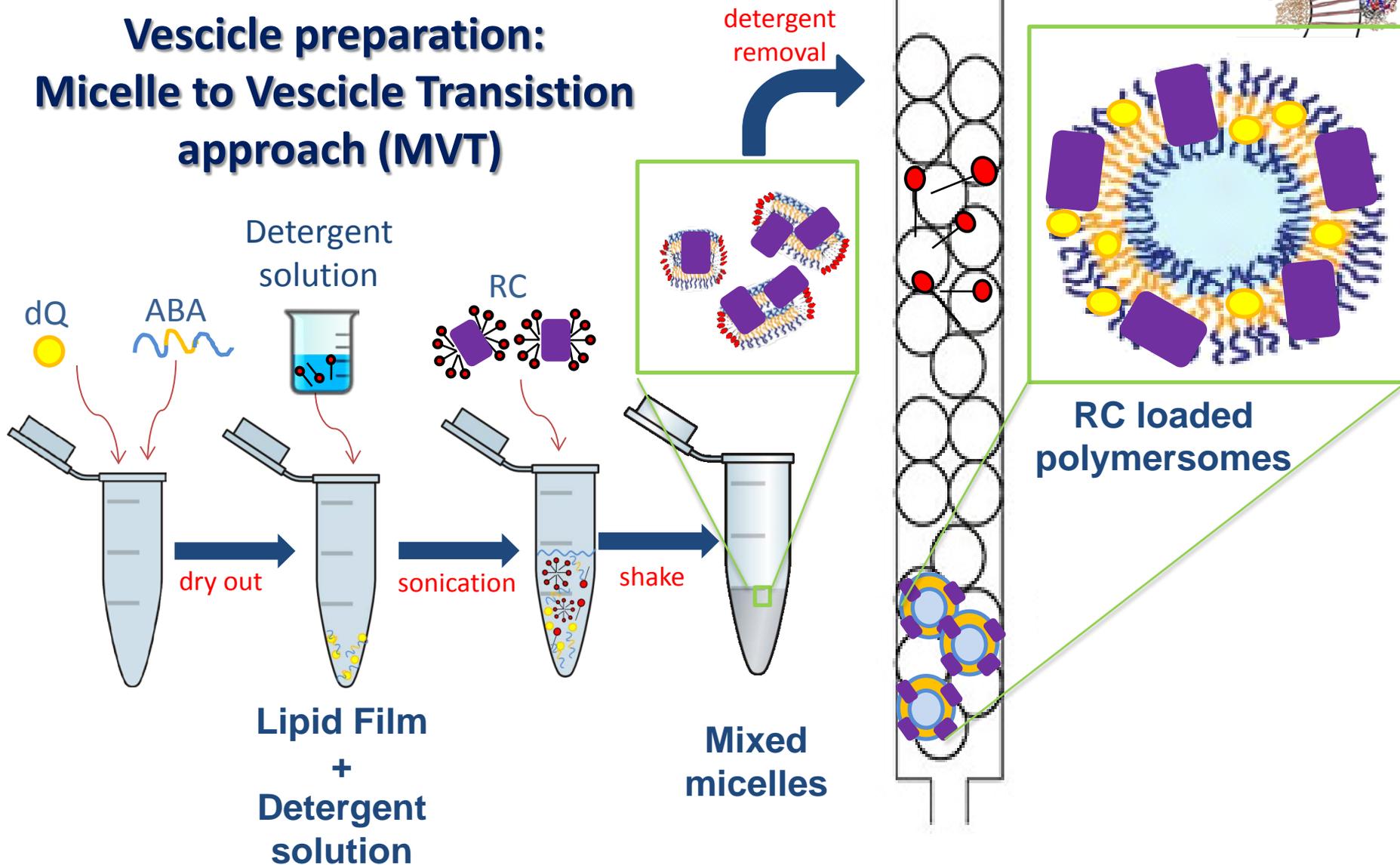
Polymer composition <sup>a)</sup>	$\bar{M}_w$ <sup>a)</sup>	Polymer composition <sup>b)</sup>
A <sub>9</sub> B <sub>106</sub> A <sub>9</sub> – 1	9 486	1.38
A <sub>13</sub> B <sub>62</sub> A <sub>13</sub> – 2	6 938	1.47
A <sub>15</sub> B <sub>62</sub> A <sub>15</sub> – 3	7 276	1.50
A <sub>21</sub> B <sub>69</sub> A <sub>21</sub> – 4	8 816	2.00
A <sub>13</sub> B <sub>23</sub> A <sub>13</sub> – 5	4 052	Insoluble in THF
A <sub>65</sub> B <sub>165</sub> A <sub>65</sub> – 6	23 372	1.63
A <sub>13</sub> B <sub>110</sub> A <sub>13</sub> – 7	10 462	1.44
A <sub>14</sub> B <sub>110</sub> A <sub>14</sub> – 8	10 632	1.36

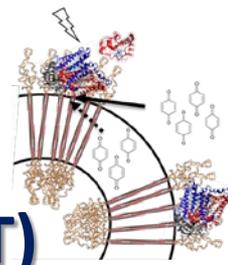


NADH/ferricyanide oxidoreductase activity measured at 410 nm and 25 °C. The reaction was started by an addition of NADH (represented by the arrow) to: complex I in solution (curve A), complex I incorporated in polymer vesicles (curve B), protein-free vesicles solution (curve C).



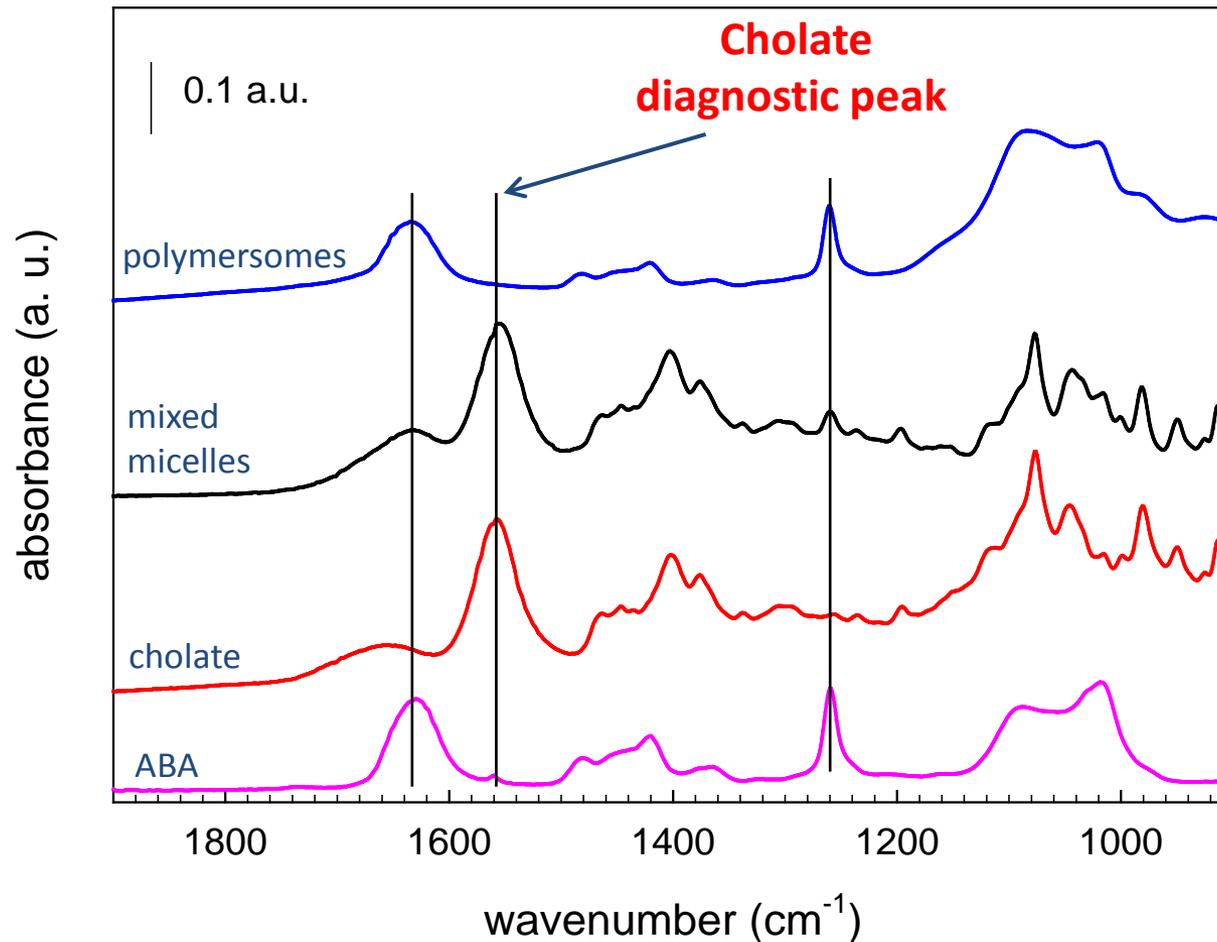
# Vesicle preparation: Micelle to Vesicle Transition approach (MVT)

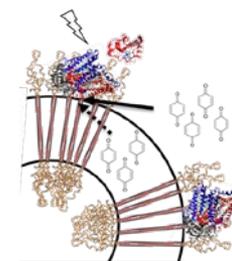




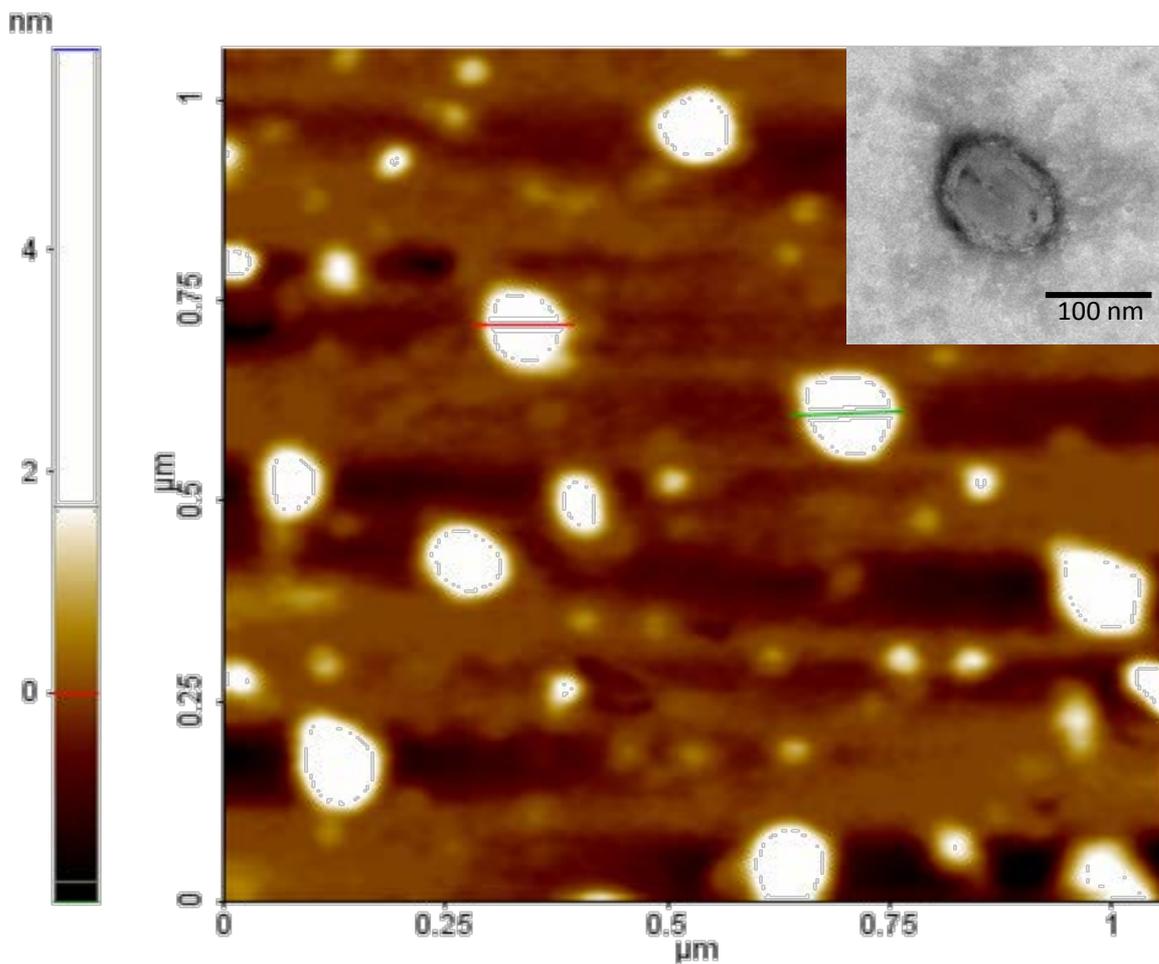
# Micelle to Vesicle Transition approach (MVT)

## Detergent removal analysis by IR-ATR spectra analysis



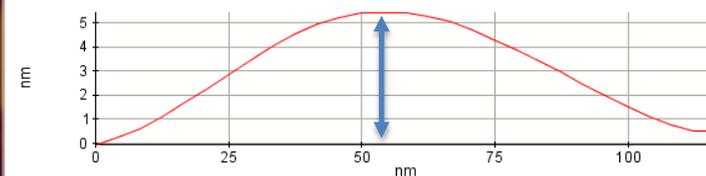


# ABA polymersomes characterization

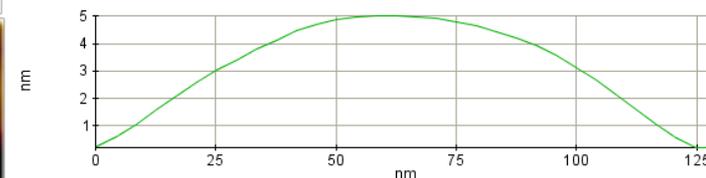


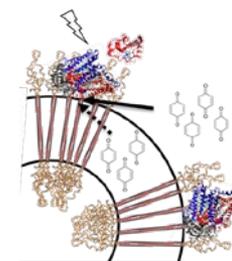
Average diameter  
 **$70 \pm 7$  nm**  
Height  $\approx 5$  nm

Line Profile: Red



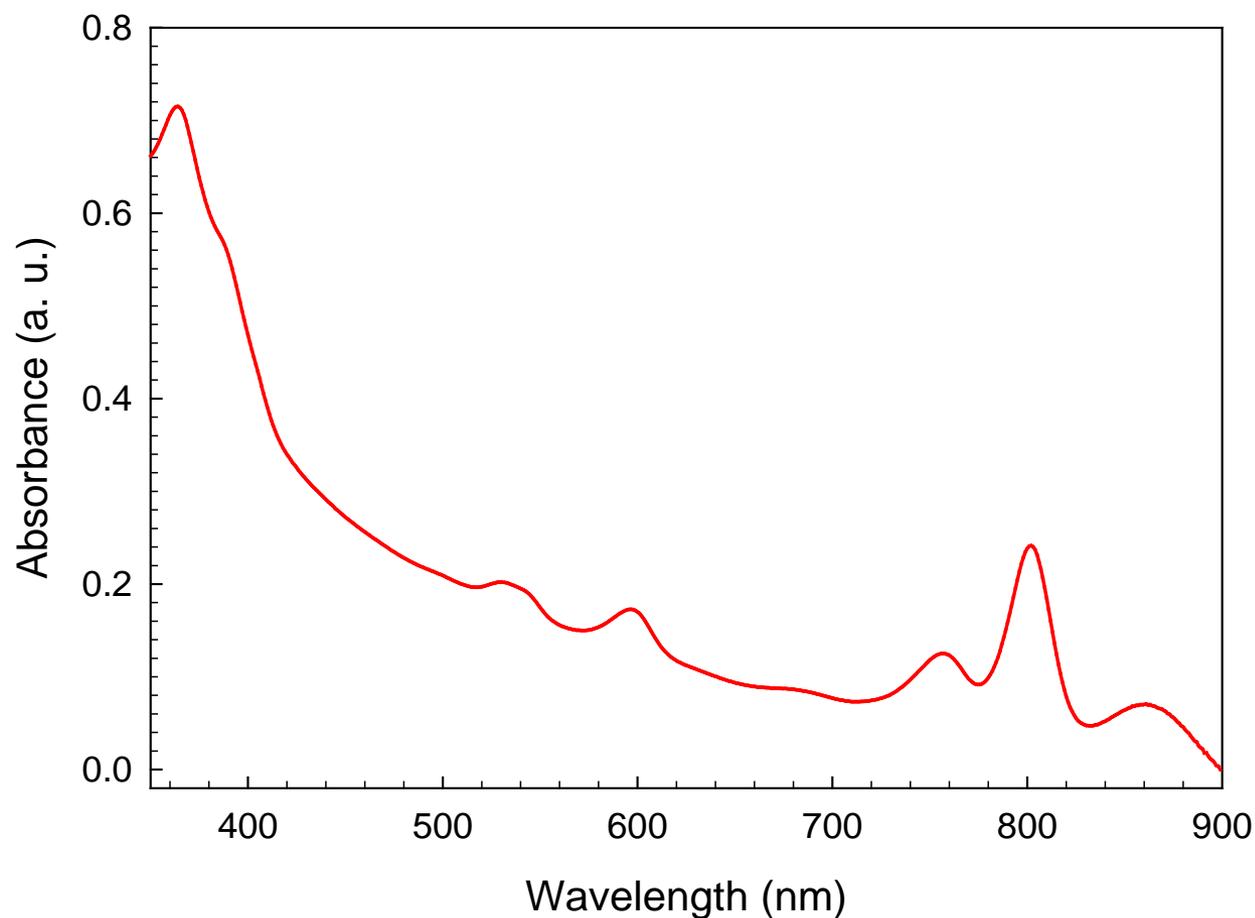
Line Profile: Green

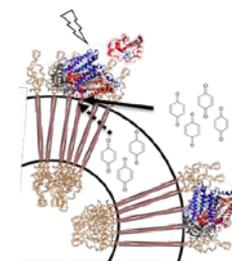




## RC loaded in ABA polymersomes

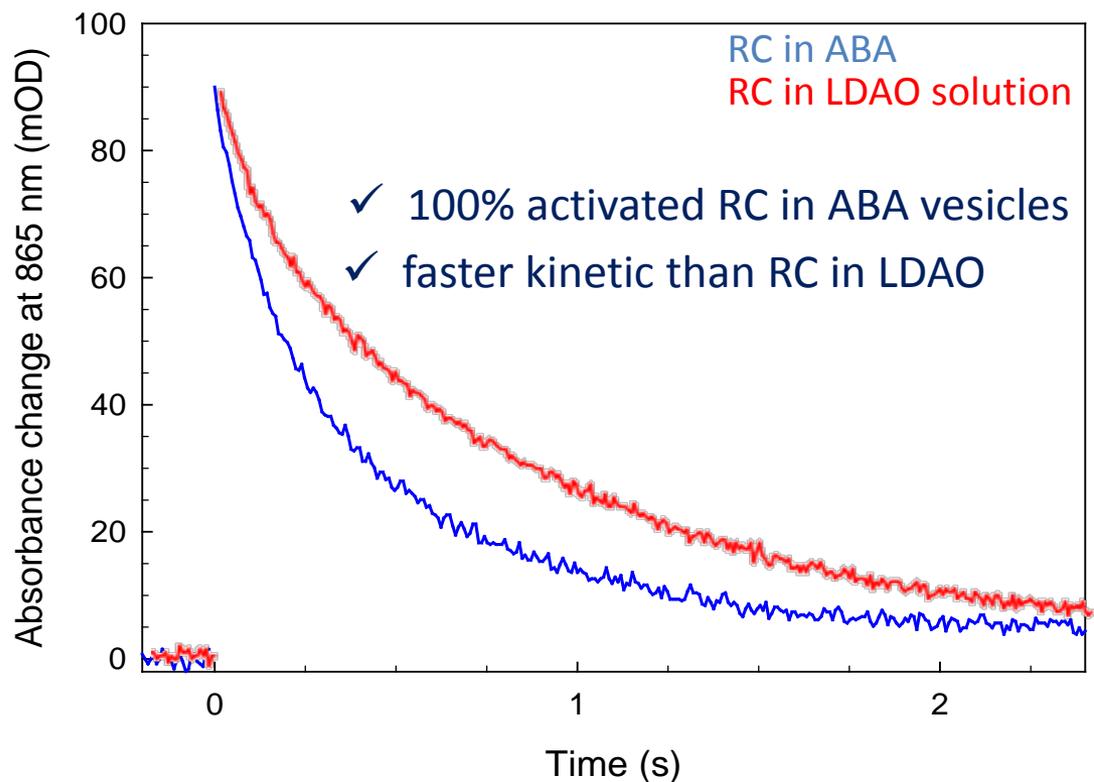
### Absorbance spectrum of RC embedded in ABA polymersomes



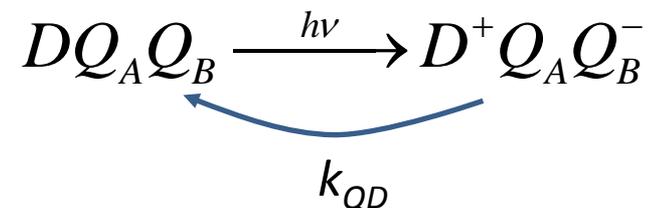


## RC loaded in ABA polymersomes

### Photochemical assay for checking protein integrity

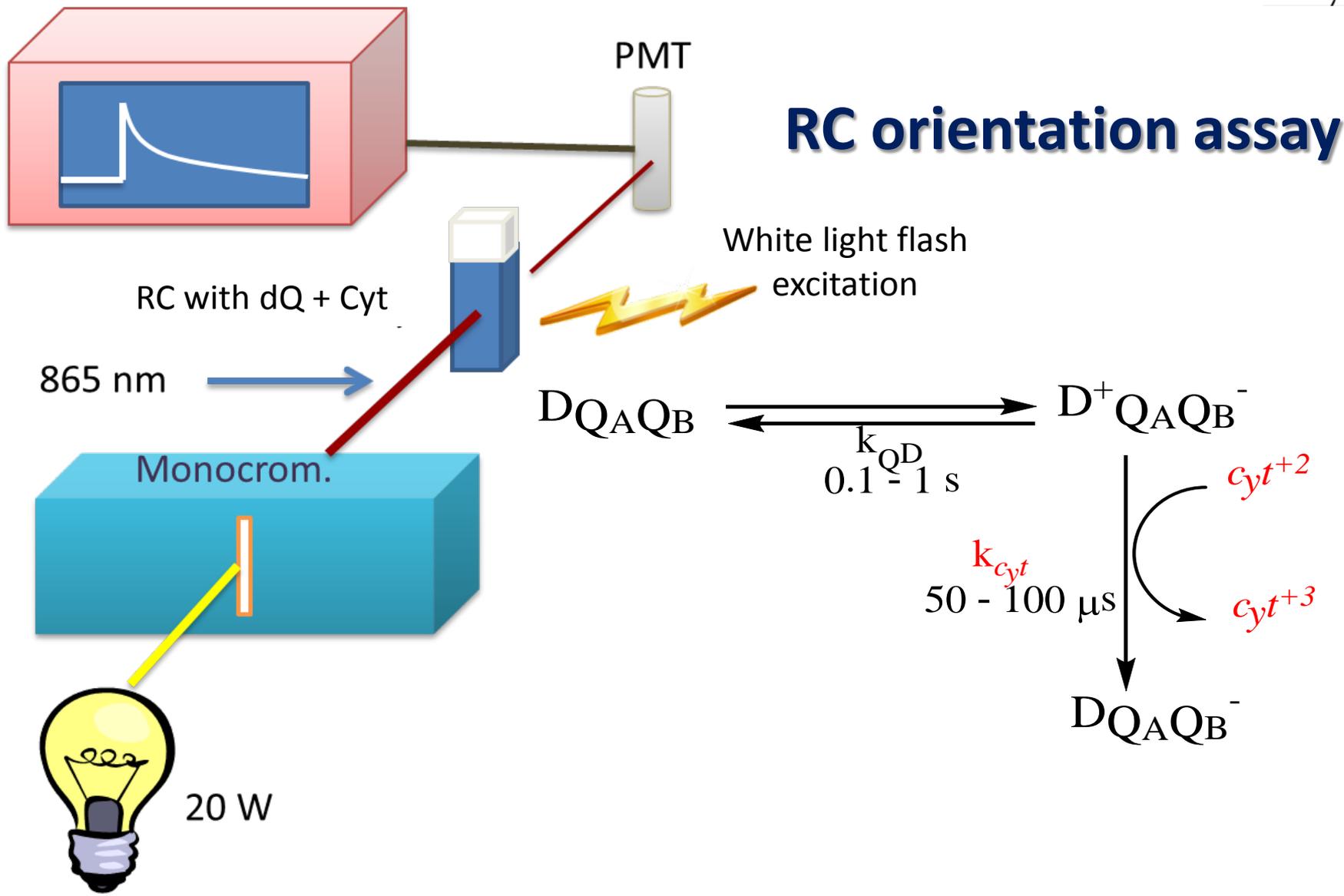
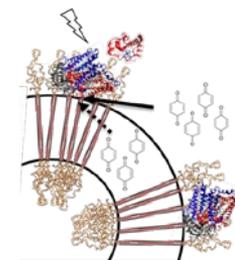


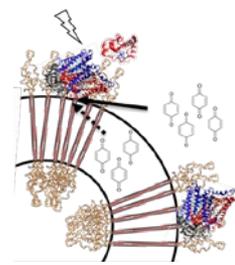
Charge recombination reaction



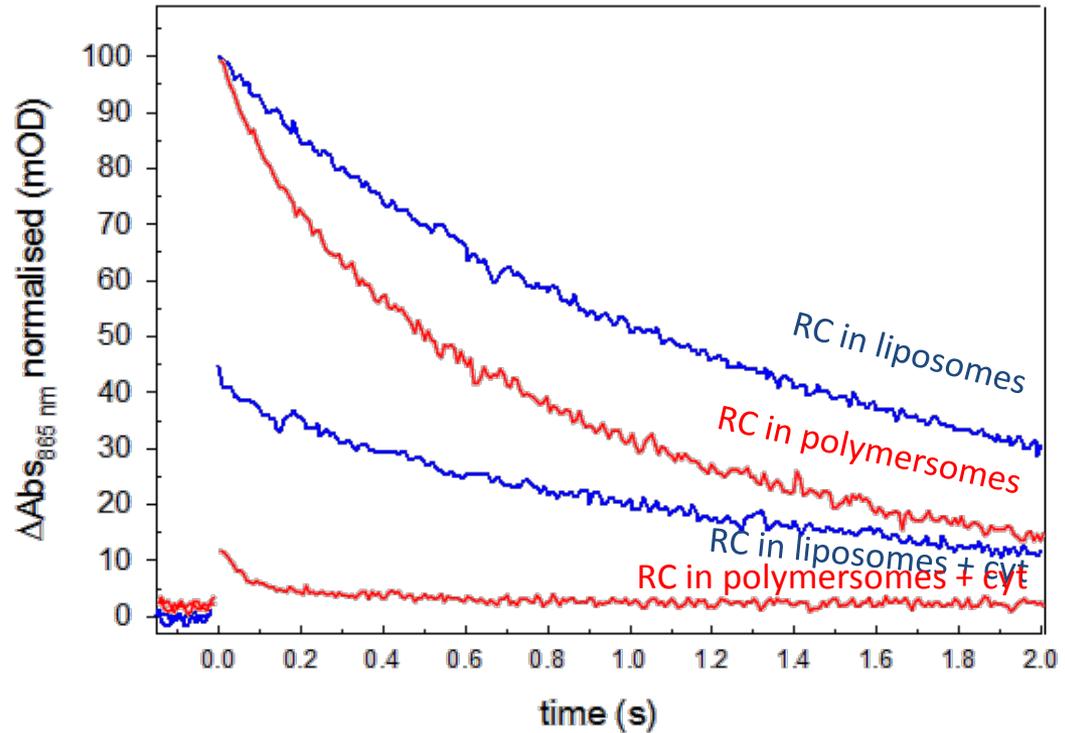
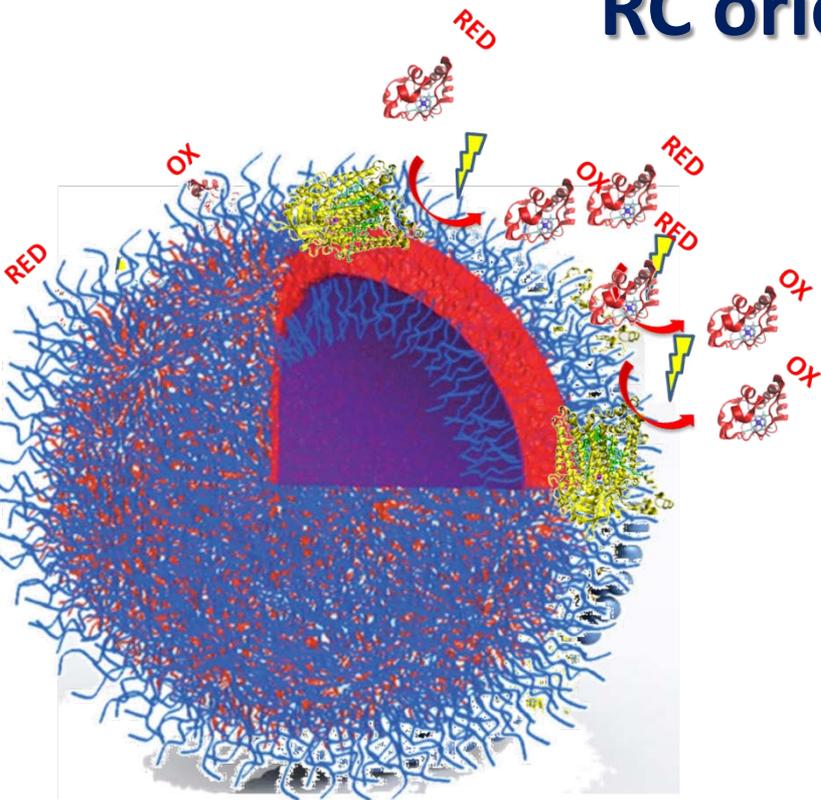
*in ABA*  $k_{QD} = 1.8 \pm 0.1 \text{ s}^{-1}$

*in detergent solution*  $k_{QD} = 1.0 \pm 0.1 \text{ s}^{-1}$



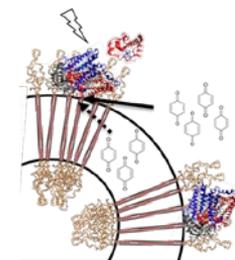


## RC orientation assay



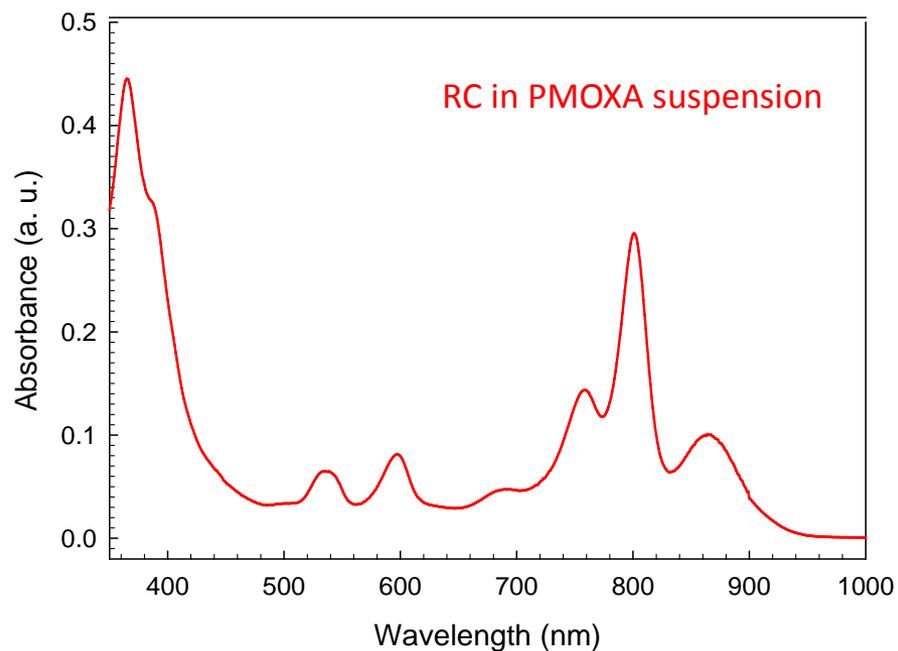
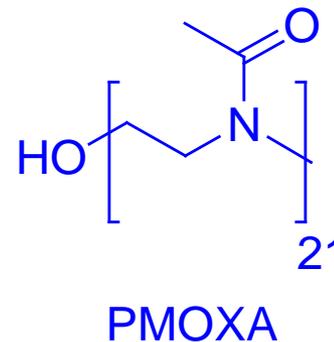
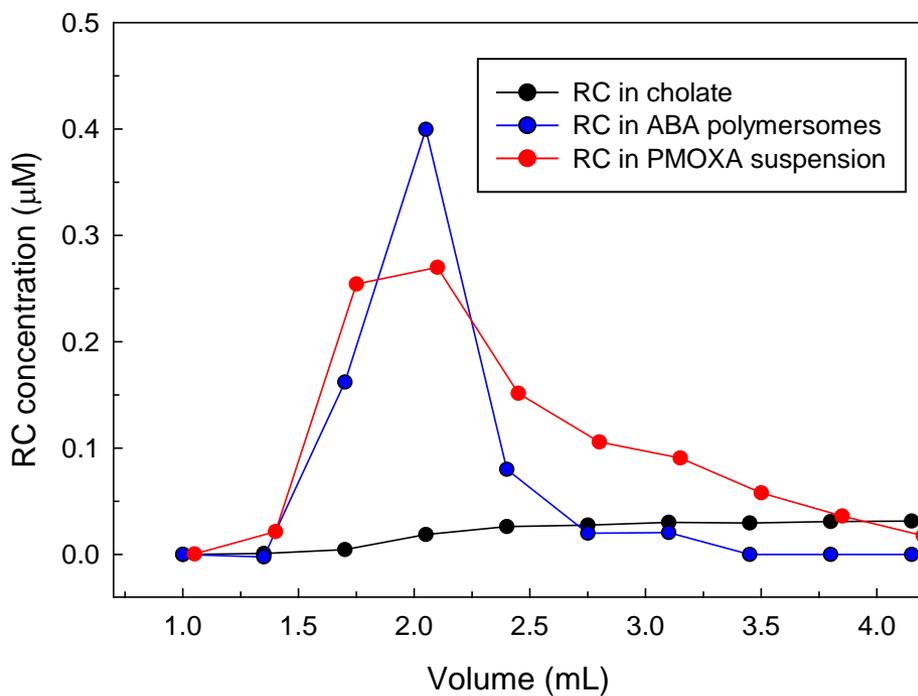
**100 % RC Dimers oriented towards the vesicle outer face**  
**or**  
**RC included into the external PMOXA block**

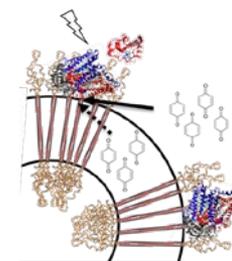




# RC in PMOXA moiety

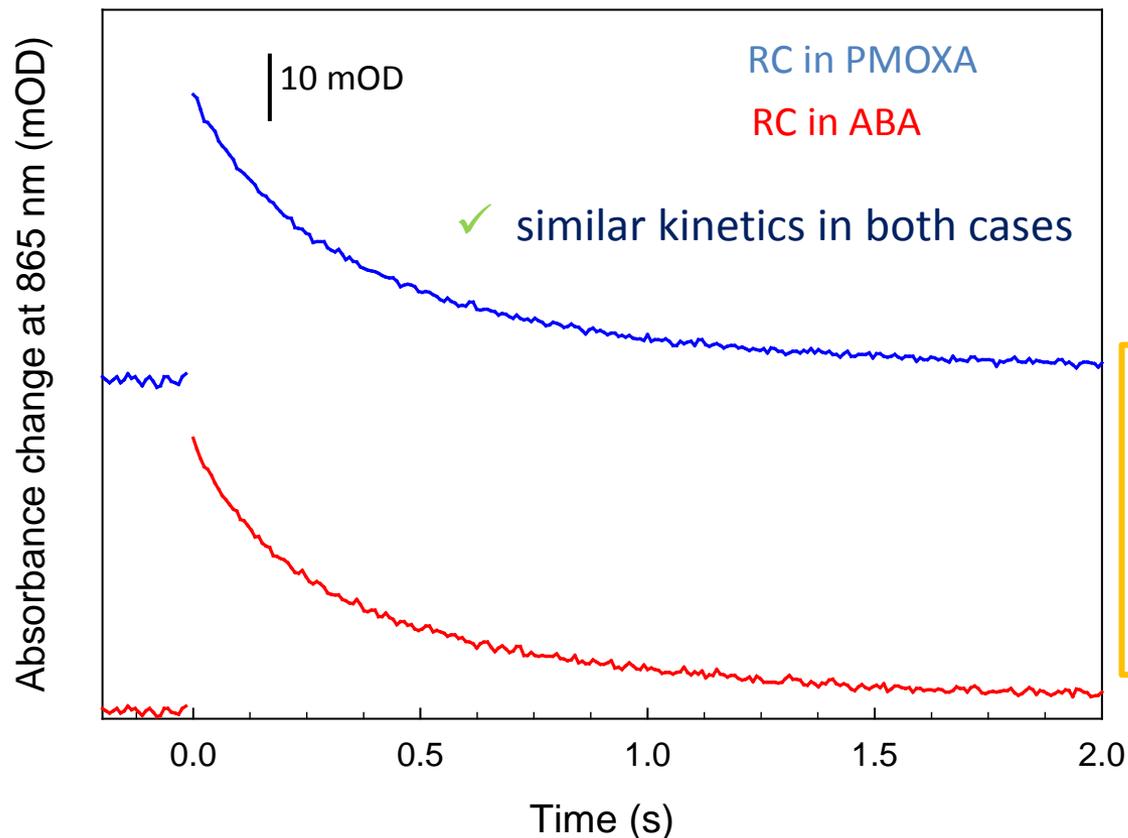
Sepharose 4B SEC elution profiles



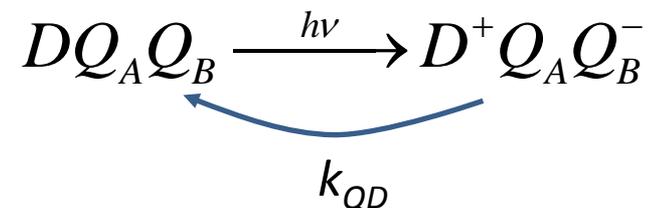


## RC in PMOXA moiety

Comparison between the RC charge recombination reaction in ABA  
and in PMOXA environment



Charge recombination reaction



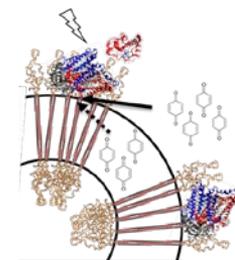
*in PMOXA  
suspension*

$$k_{QD} = 1.9 \pm 0.1 \text{ s}^{-1}$$

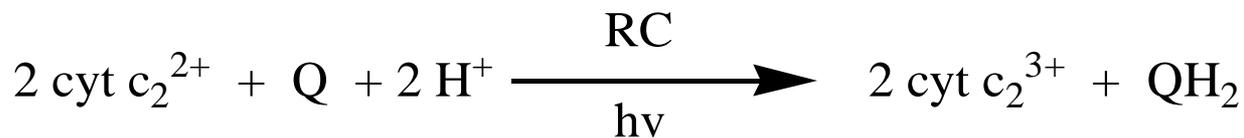
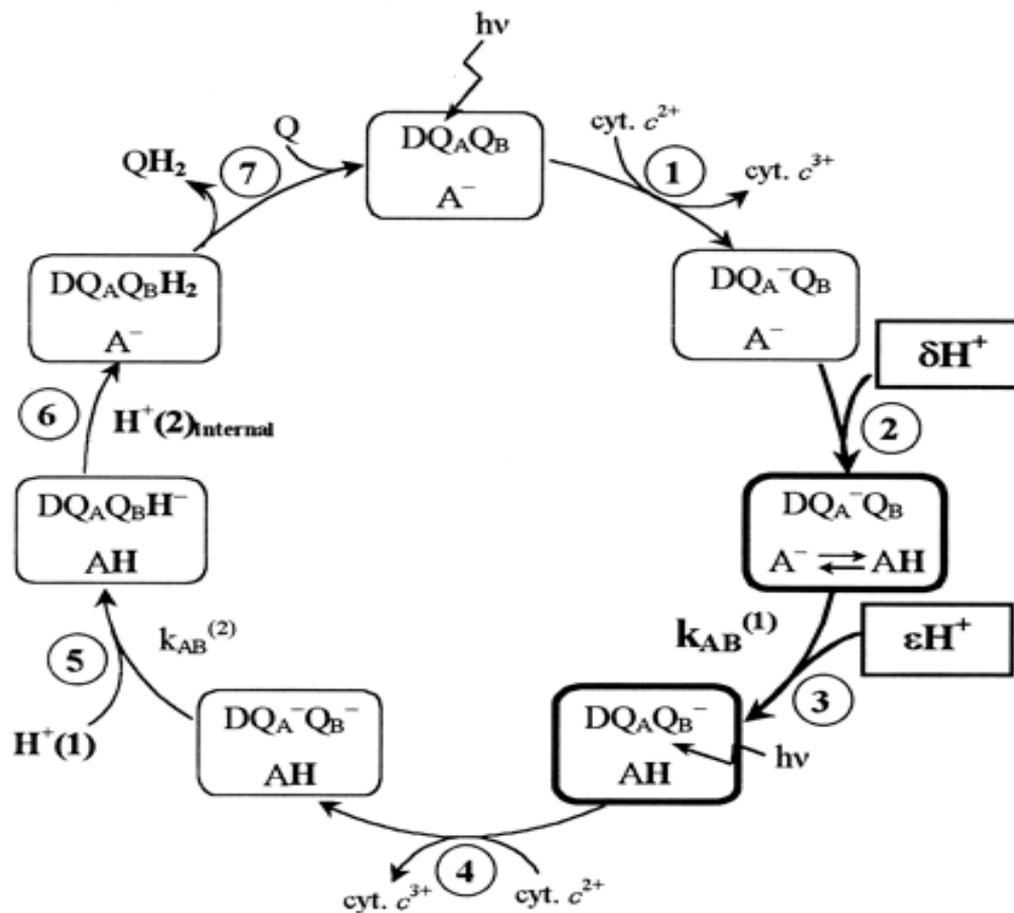
*in ABA*

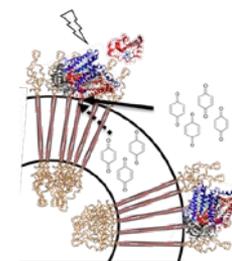
$$k_{QD} = 1.8 \pm 0.1 \text{ s}^{-1}$$





# Photocycle of quinone reduction



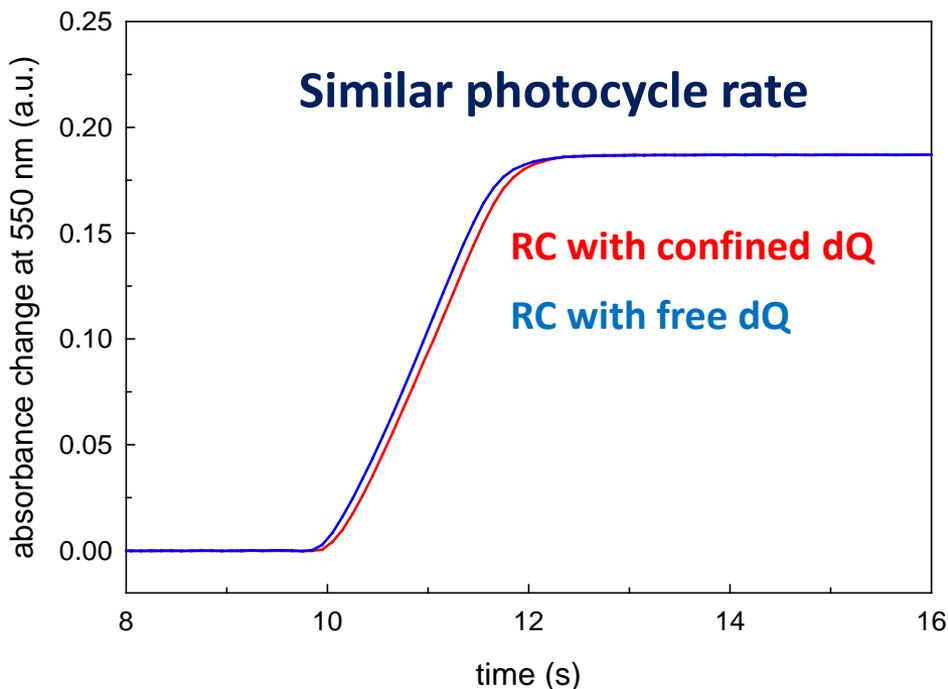


# RC photochemical activity

## Cytochrome turn over in the RC photocycle

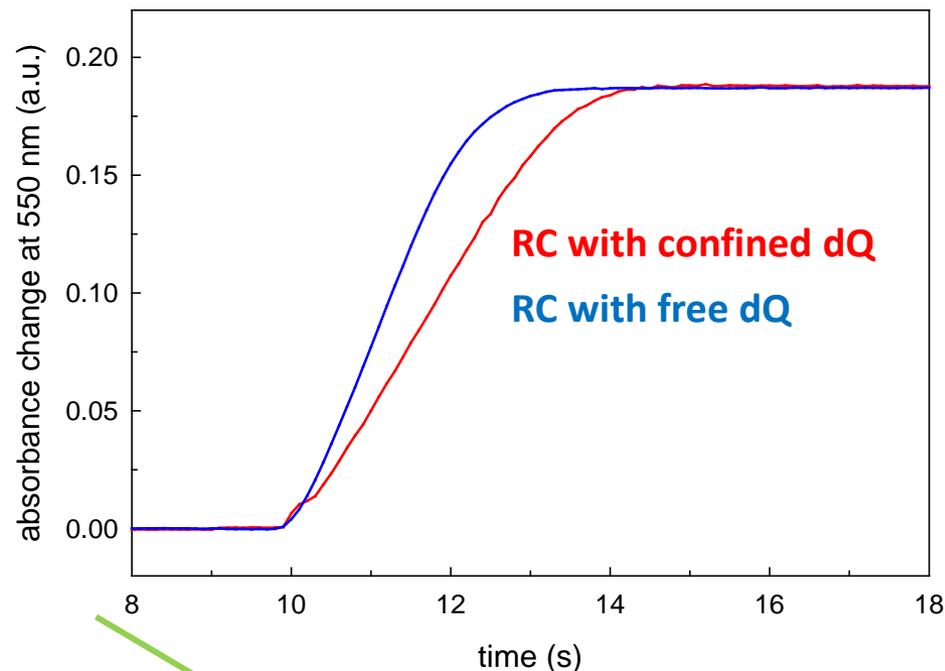
RC in **PMOXA** suspension

RC embedded in **ABA** polymersomes



$$k_{dQ}^{confined} = 0.56 \pm 0.08 \text{ s}^{-1}$$

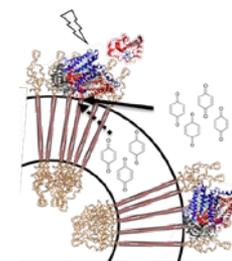
$$k_{dQ}^{free} = 0.64 \pm 0.01 \text{ s}^{-1}$$



$$k_{dQ}^{confined} = 0.28 \pm 0.02 \text{ s}^{-1}$$

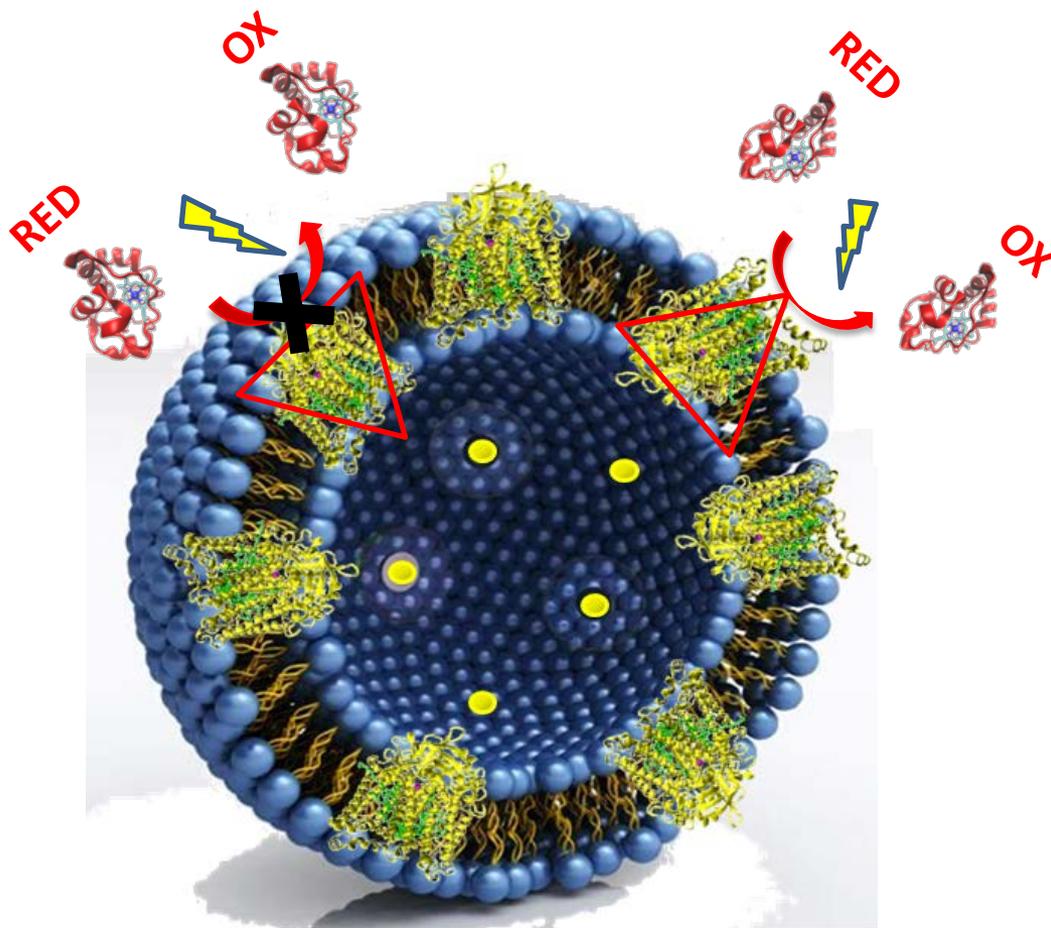
$$k_{dQ}^{free} = 0.59 \pm 0.06 \text{ s}^{-1}$$

Conditions: RC 1  $\mu\text{M}$ ,  $\text{cyt}^{2+}$  10  $\mu\text{M}$ , dQ 20  $\mu\text{M}$  or dQ 20  $\mu\text{M}$  added in the bulk solution. Excitation at 550 nm under continuous illumination with red-filtered light



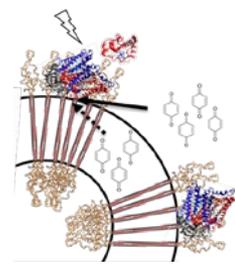
# RC photochemical activity

## Cytochrome turn over in the RC photocycle in liposomes

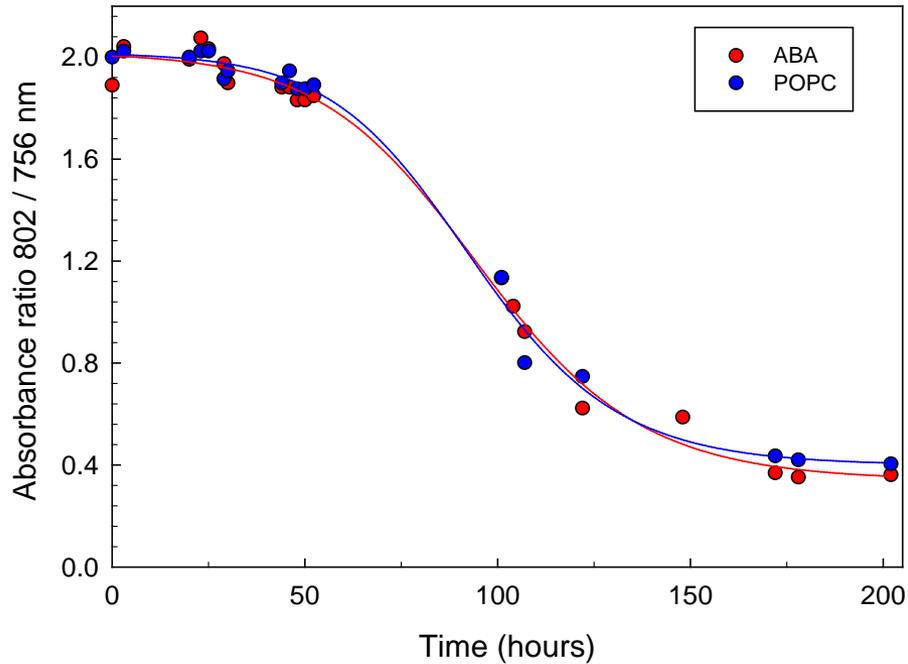


$$k_{dQ}^{confined} = 1.23 \pm 0.01 \text{ s}^{-1}$$

$$k_{dQ}^{free} = 0.90 \pm 0.01 \text{ s}^{-1}$$



## RC time stability in ABA vs POPC vesicles

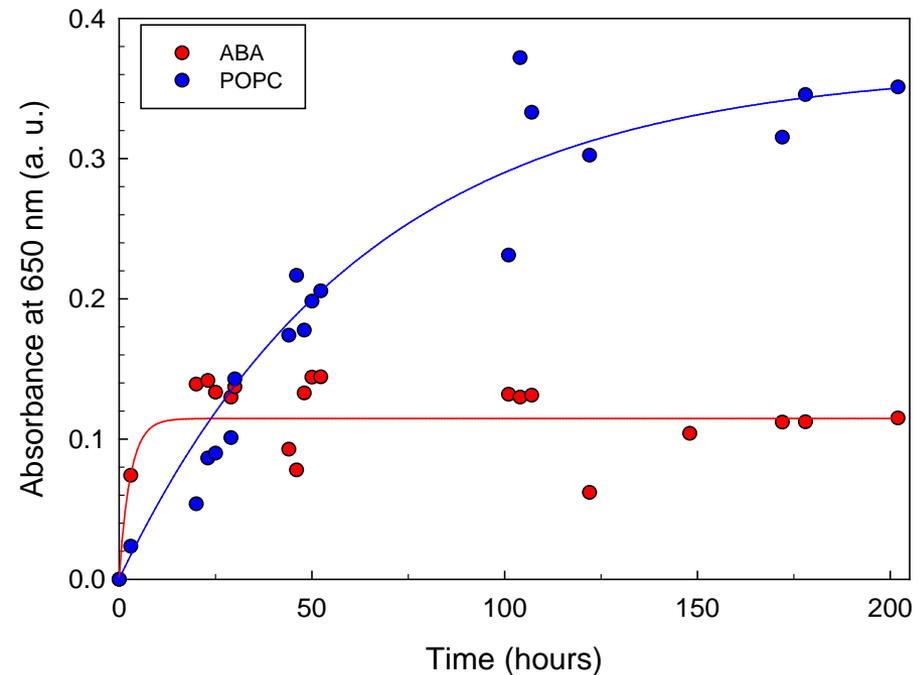


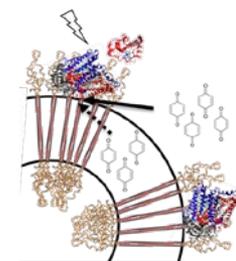
Preserved RC integrity in ABA  
as in POPC vesicles

Higher stability of ABA vesicles

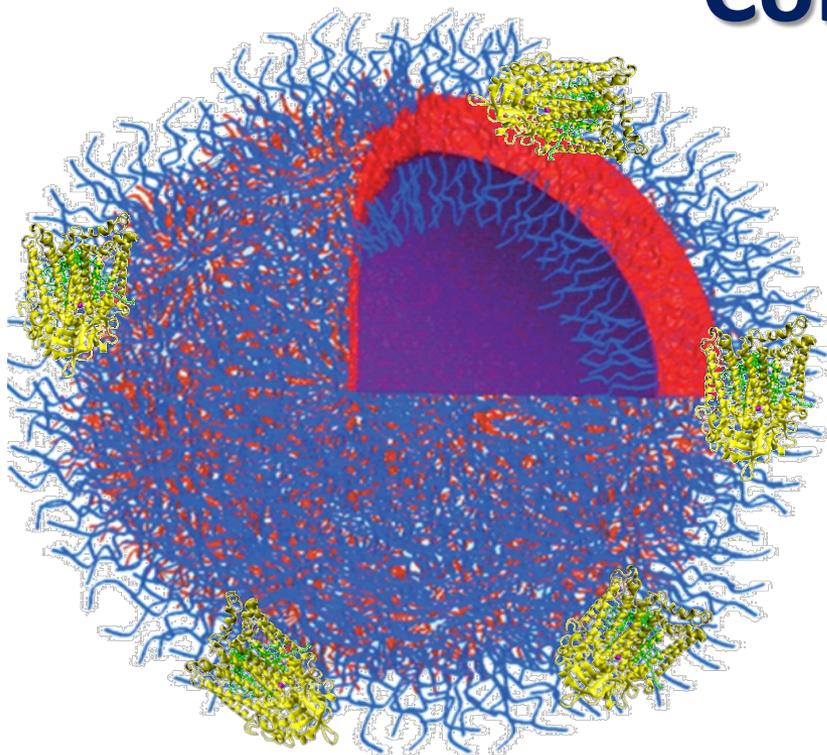


no aggregation



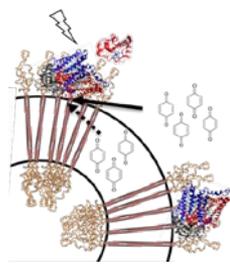


# Conclusions

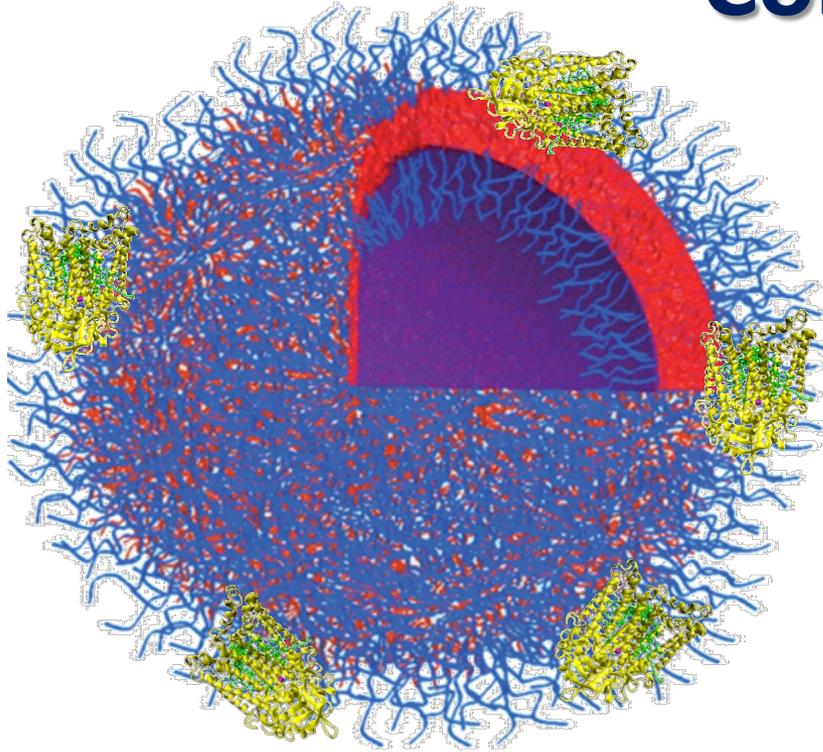


## RECONSTITUTION OF FULLY FUNCTIONAL REACTION CENTER IN ABA POLYMERSOMES

- First example of RC-ABA polymersomes made by MVT technique
- 100% photoactive RC and unperturbed photoenzymatic activity in polymersomes
- Improved mechanical and chemical properties



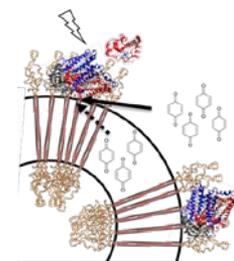
# Conclusions



## Localization of RC in the PMOXA palisade:

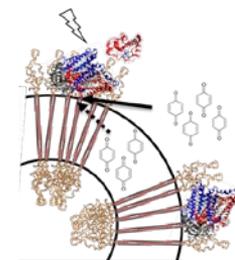
- ✓ Full interaction with cytochrome
- ✓ Same charge recombination rate
- ✓ Same cythochrome turnover rate

➤ First example of detailed characterization of protein positioning

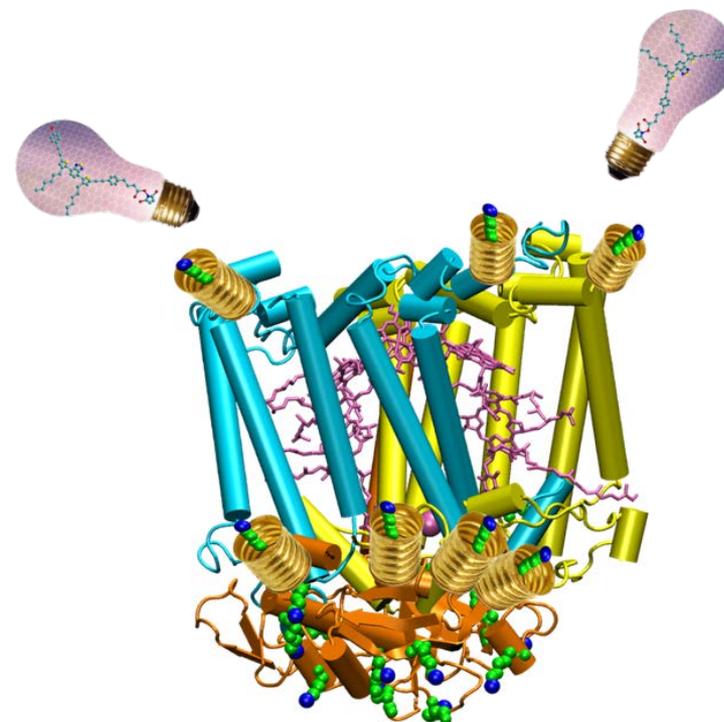
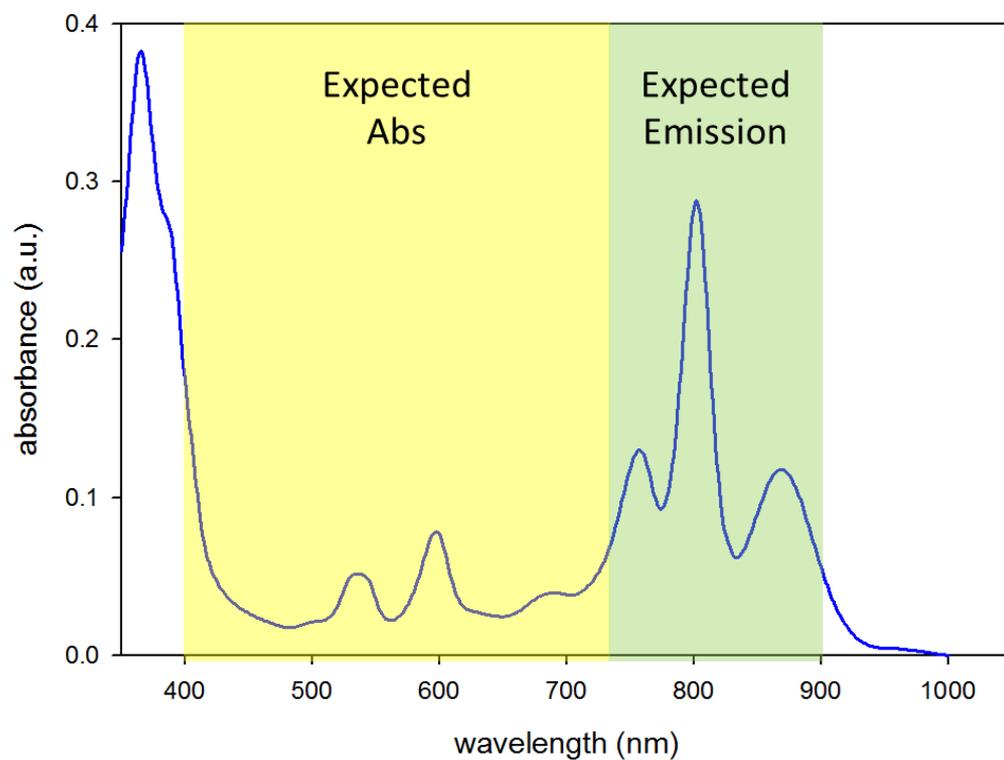


# Outlook

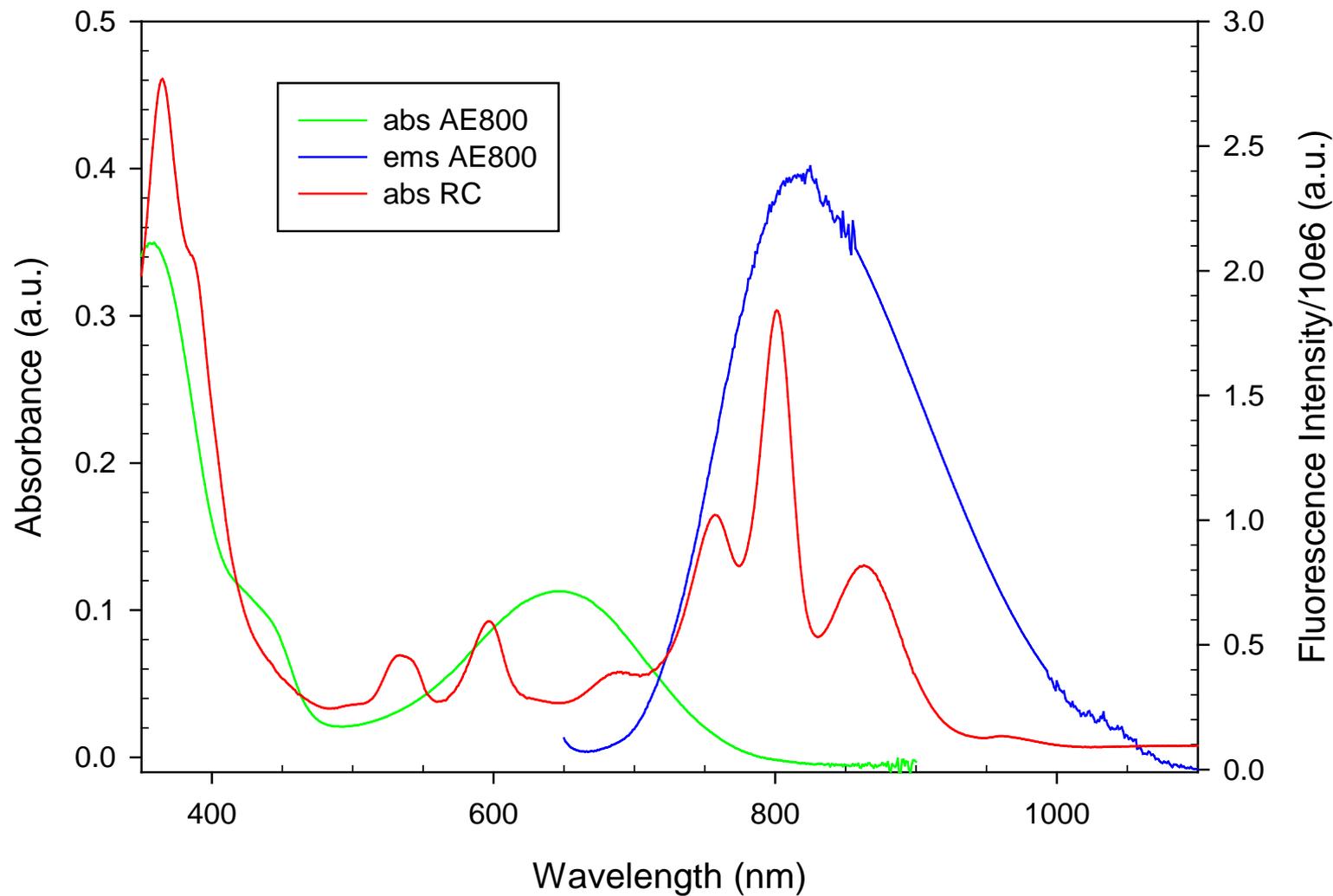
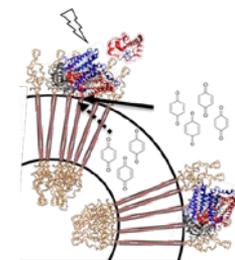
- **Functionalization of ABA ending groups with opportune organic moieties to form supra-molecular assemblies**
- **Employing these functionalized RC-ABA vesicles as building blocks for the design of hybrid bio-organic optoelectronic devices**

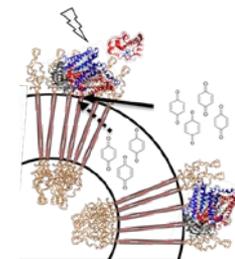


# Enhancing RC photoactivity





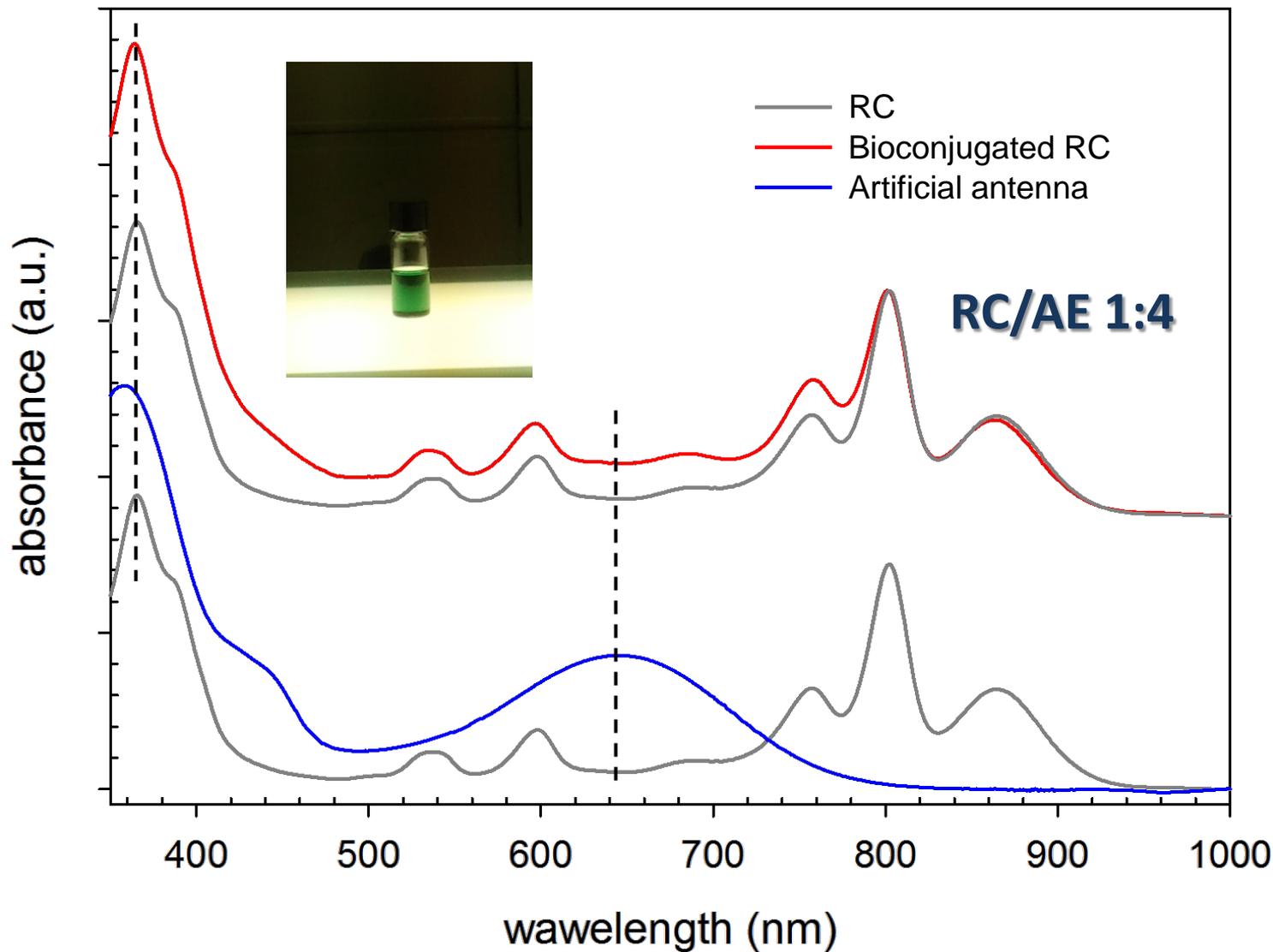
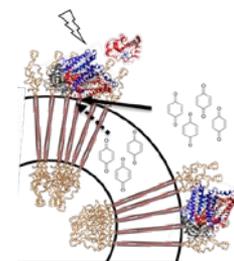


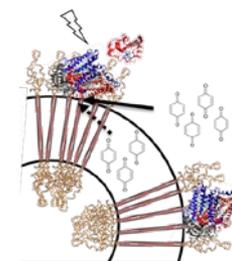


## **Characterization of AE800 in Triton X-100 3%**

- ✓ Fluorescence QY: **5.6%**
- ✓ Fluorescence lifetime: **1.2 ns**
- ✓ Molar extinction coefficient: **9800 M<sup>-1</sup>cm<sup>-1</sup>**

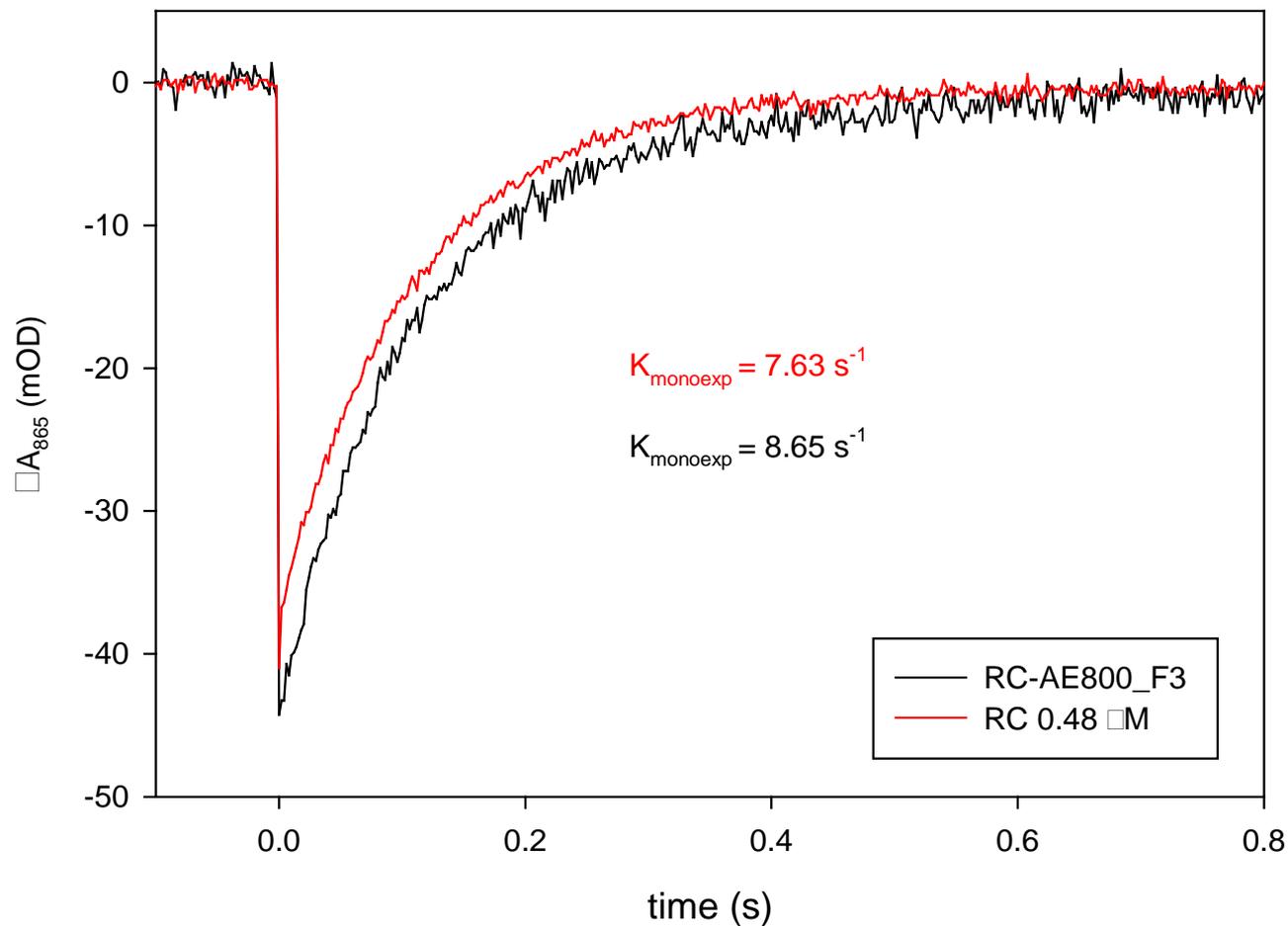
## AE-800 bioconjugation to RC

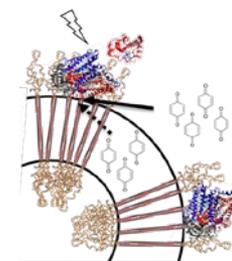




## AE-800 bioconjugation to RC

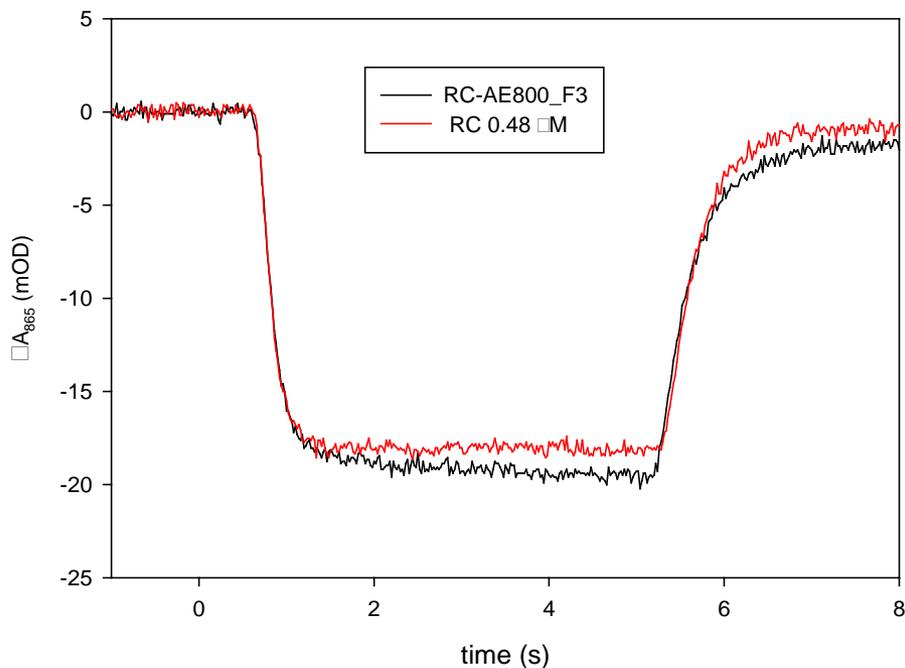
### Charge recombination kinetics



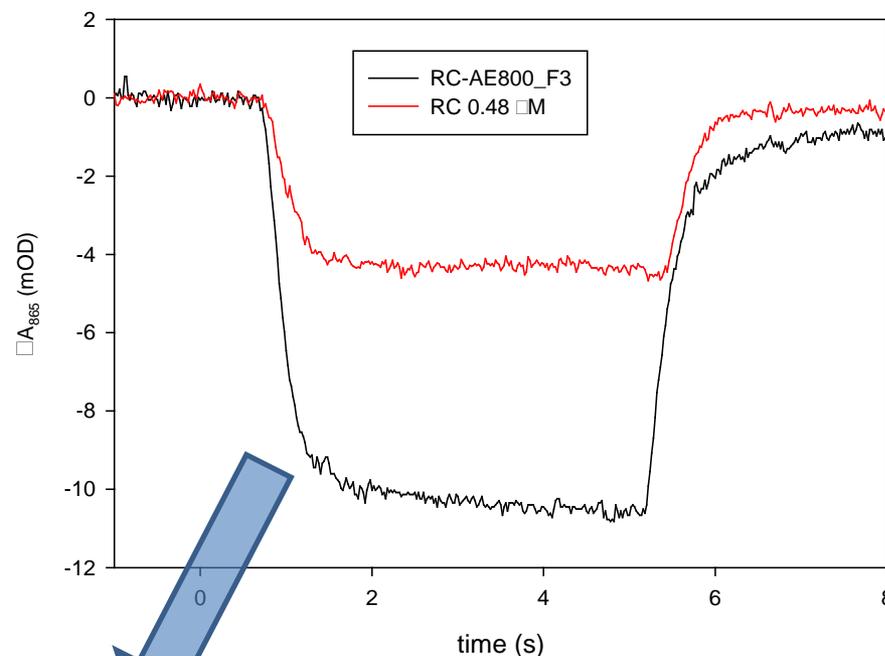


# Antenna effect AE800: single wavelength

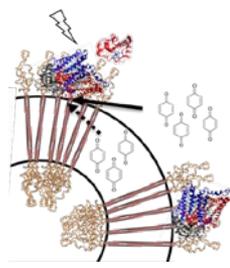
Charge separation with  $\lambda_{\text{ex}} = 860 \text{ nm}$



Charge separation with  $\lambda_{\text{ex}} = 650 \text{ nm}$

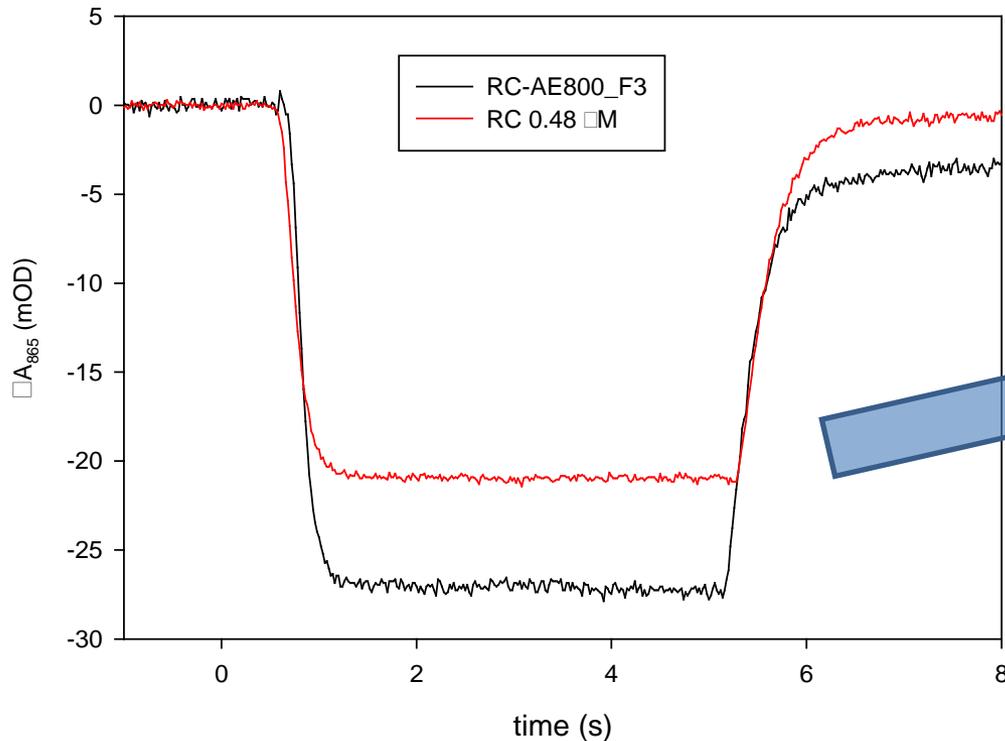


2.3 –fold increase



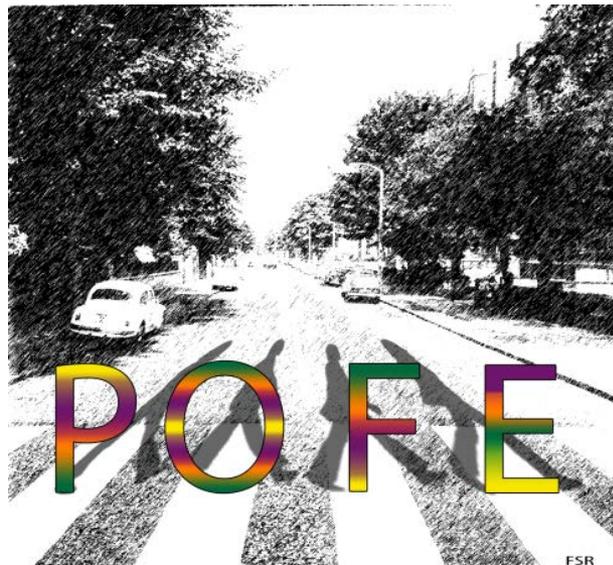
# Antenna effect AE800: white light

Charge separation with  $\lambda_{\text{ex}} < 668 \text{ nm}$ ,  $T=25\%$



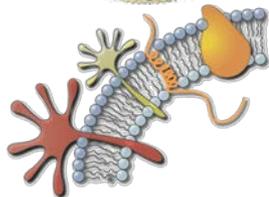
30% activity increase

## Co-workers



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COST Action CM0902  
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Francesco Milano



**THANK YOU FOR YOUR KIND ATTENTION**