Hydrophilic-Hydrophobic

TEL AVIV UNIVERSITY

Designing delivery systems based on dendrimers and dendritic hybrids (the power of molecular precision)

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Department of Organic Chemistry - School of Chemistry,
The Adama Center for Novel Delivery Systems In Crop Protection,
TAU-Nano Center
and The Blavatnik Center for Drug Discovery

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ADAMA

BLAVATNIK CENTER for Drug Discovery

Tel Aviv University Center for Nanoscience & Nanotechnology
Powering Innovation

HORIZON 2020

Why using polymeric amphiphiles based nano-carriers?

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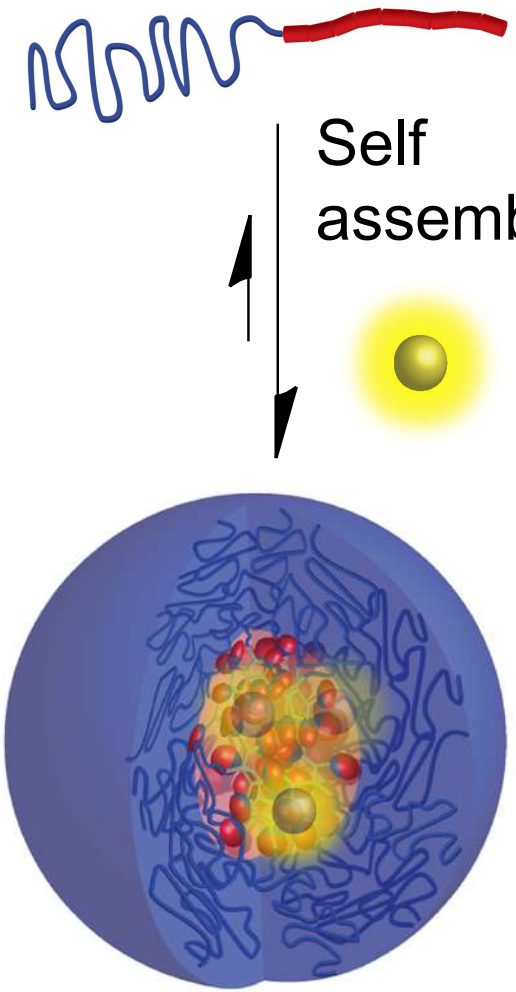
Blue = hydrophilic
Red = hydrophobic



Common concerns about small drug molecules:

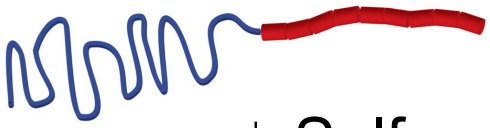
- Poor solubility
- Get cleared quickly
- Degradation
- Non-specific

Benefits of delivery platforms:

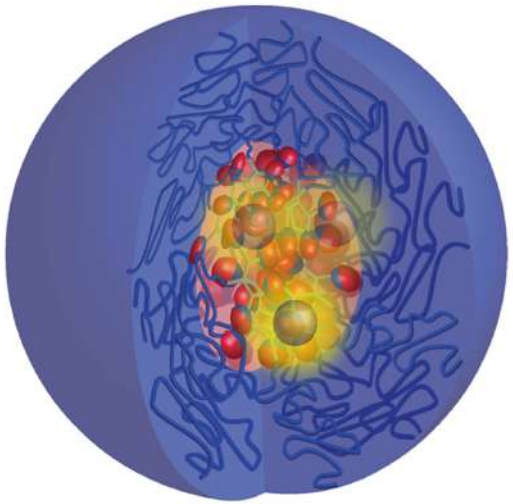
- Improved solubility
- Long circulation times
- Protect drugs from degradation
- Can be targeted

Delivery platforms require selective release mechanisms

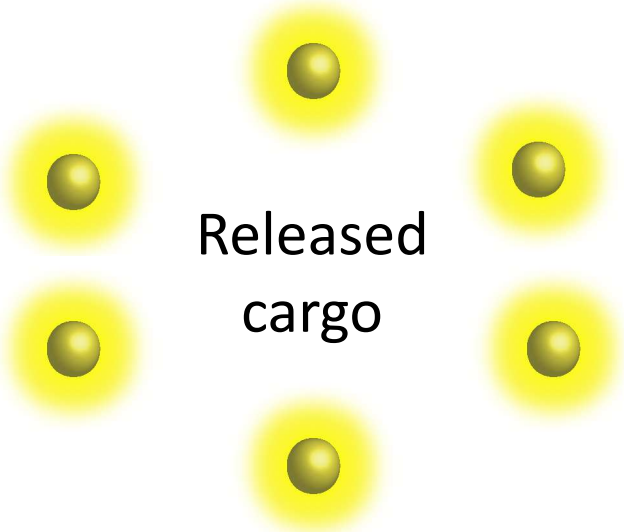
Blue = hydrophilic
Red = hydrophobic



Self assembly



Release mechanism



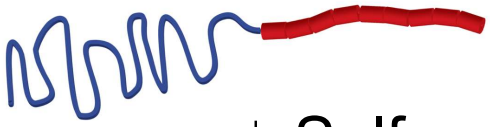
Released cargo

Structural change

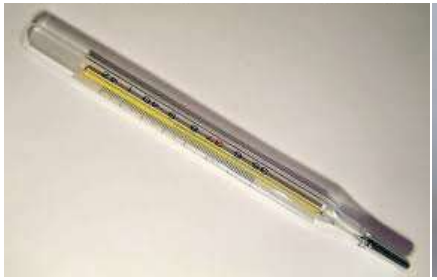
hydrophilic-hydrophilic

Stimuli-responsive polymers

Blue = hydrophilic
Red = hydrophobic



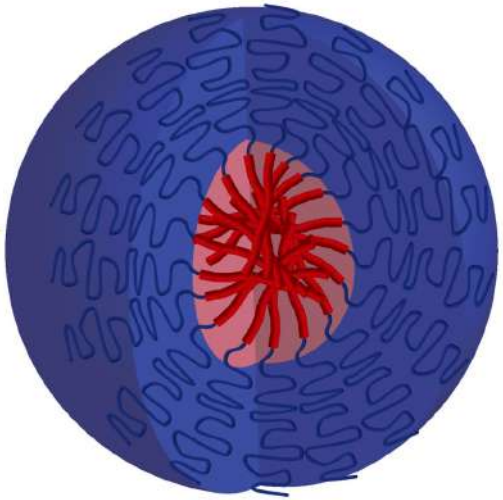
Self assembly



Thermal Activation

Photochemical Activation

pH Activation



External stimuli

Structural change

hydrophilic-hydrophilic

Polymers can respond to various types of stimuli

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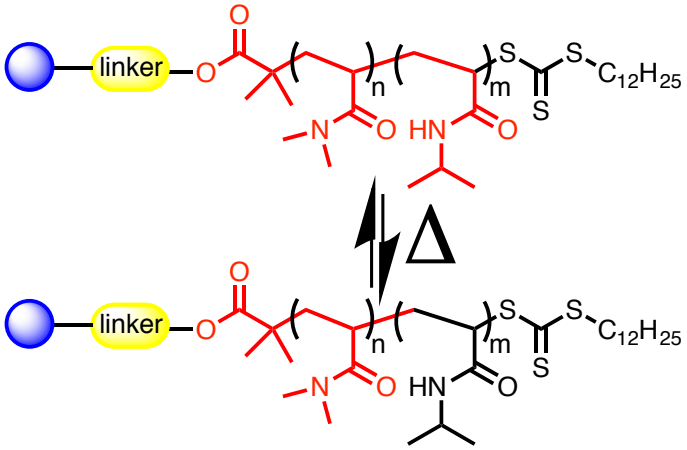
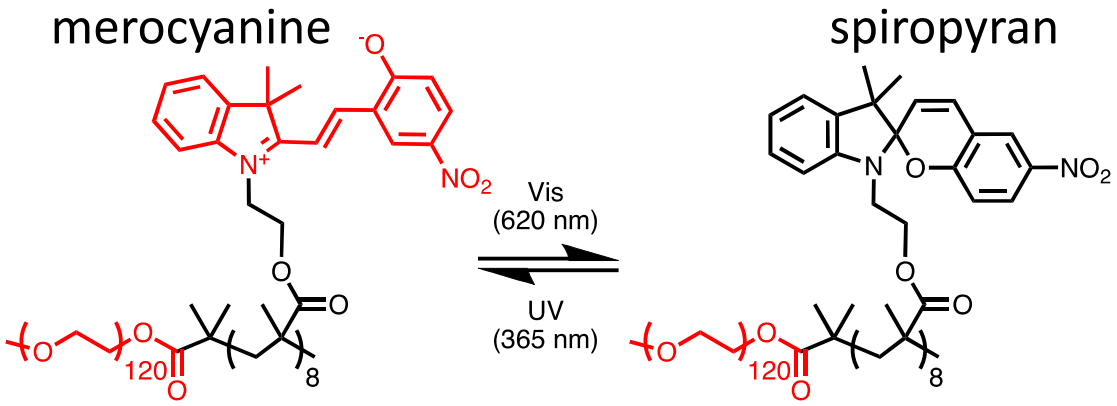
Thermal Activation:



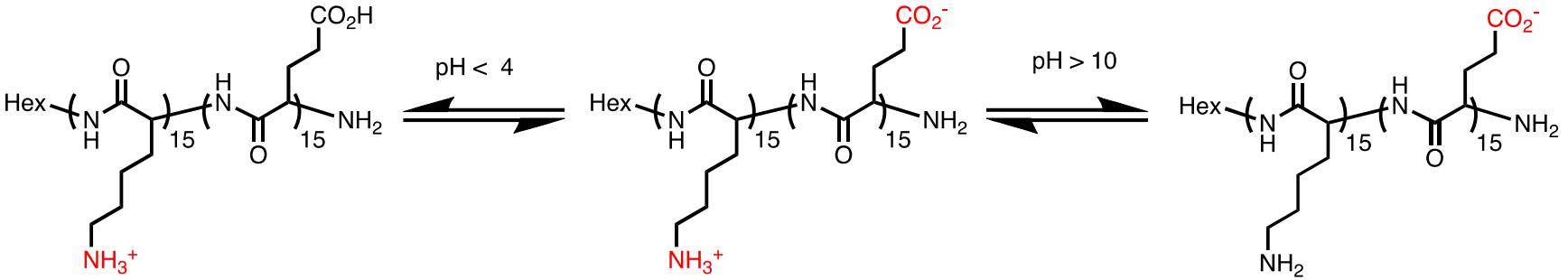
P. De, S. R. Gondi, B. S. Sumerlin, *Biomacromolcules*, **2008**, 9, 1064.

Photochemical Activation:



H.-I. Lee, W. Oh J. K Wu, L. Muller, G. Sherwood, L. Peteanu, T. Kowaleski, K. Matyjaszewski, *Angew. Chem. Int. Ed.* **2007**, 46, 2453.

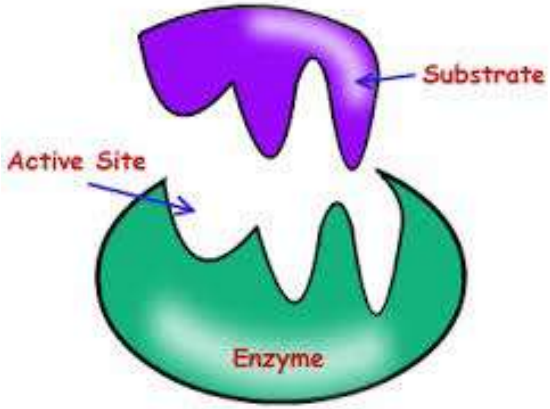
pH Activation:

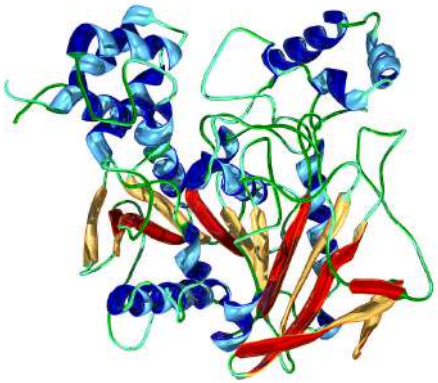


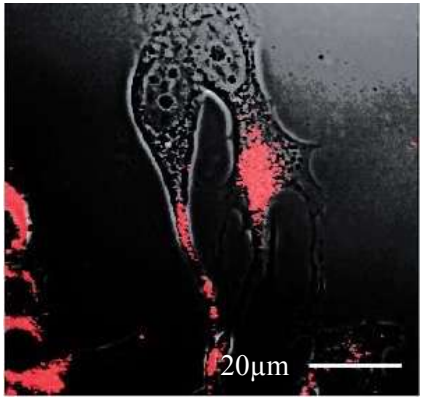
J. Rodriguez-Hernandez, S. Lecommandoux, *JACS* **2005**, 127, 2026.

Enzymes: selective release and biodegradability

- High Selectivity
- Catalytic capabilities
- Naturally present in the body
- **Often over-expressed in diseased tissues**



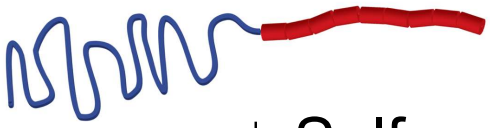




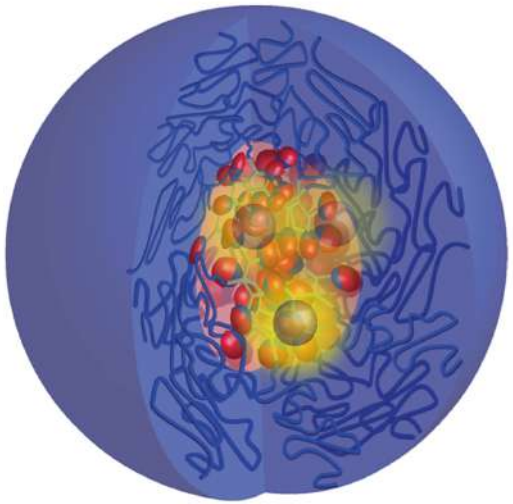
Cathepsin B in breast cancer tumor

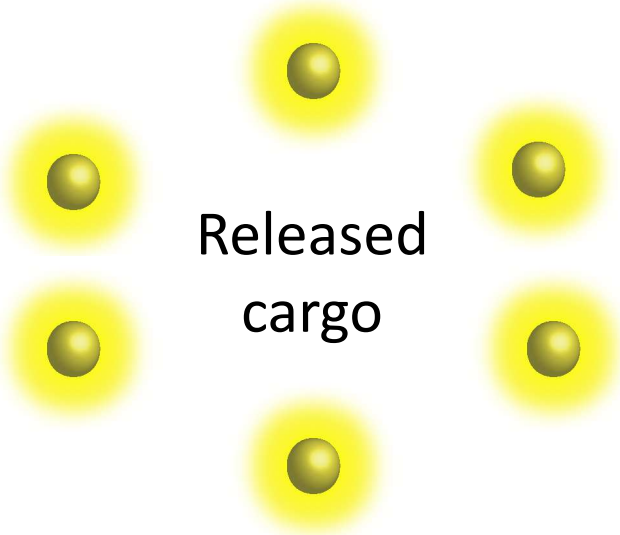
Can we use enzymes to trigger disassembly of micelles?

Blue = hydrophilic
Red = hydrophobic



Self assembly





Released cargo

Structural change

hydrophilic-hydrophilic

Inspiration: Polymer-dendrimers hybrids combine the best of two worlds

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J. M. J. Fréchet

C. J. Hawker

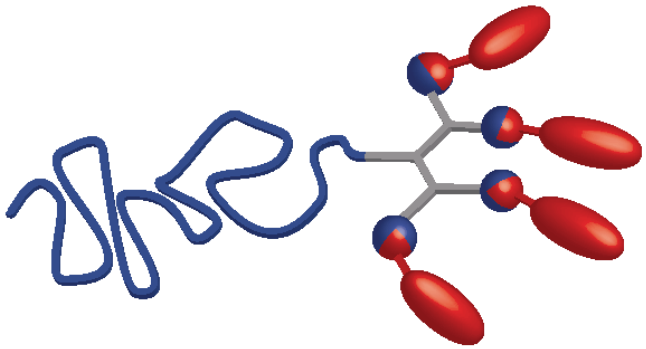
I. Gitsov

K. Wooley

P. Hammond

A. Hult

E. Malmström



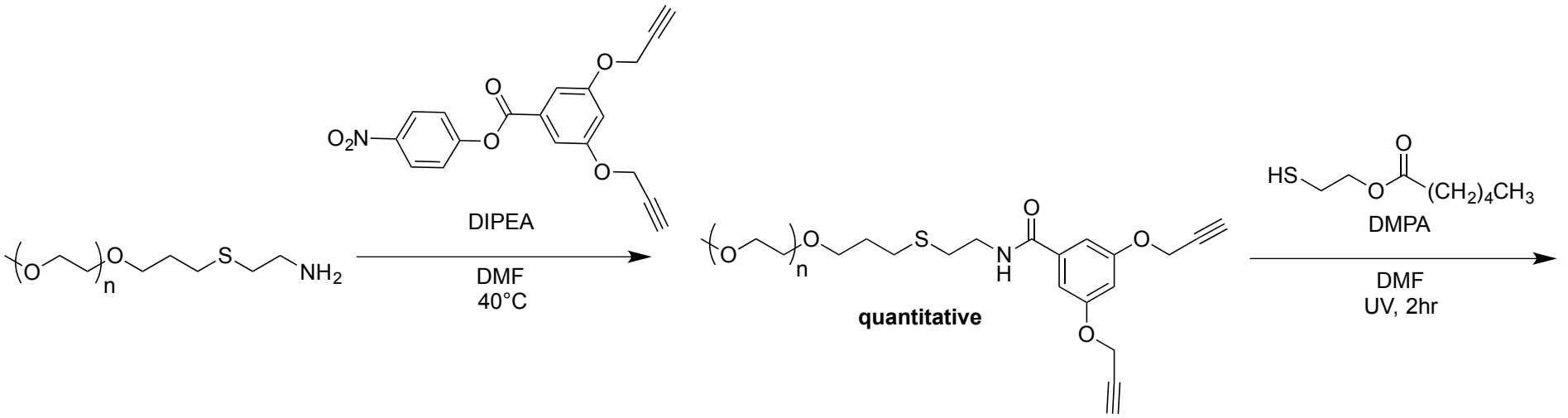
Dendron gives molecular precision

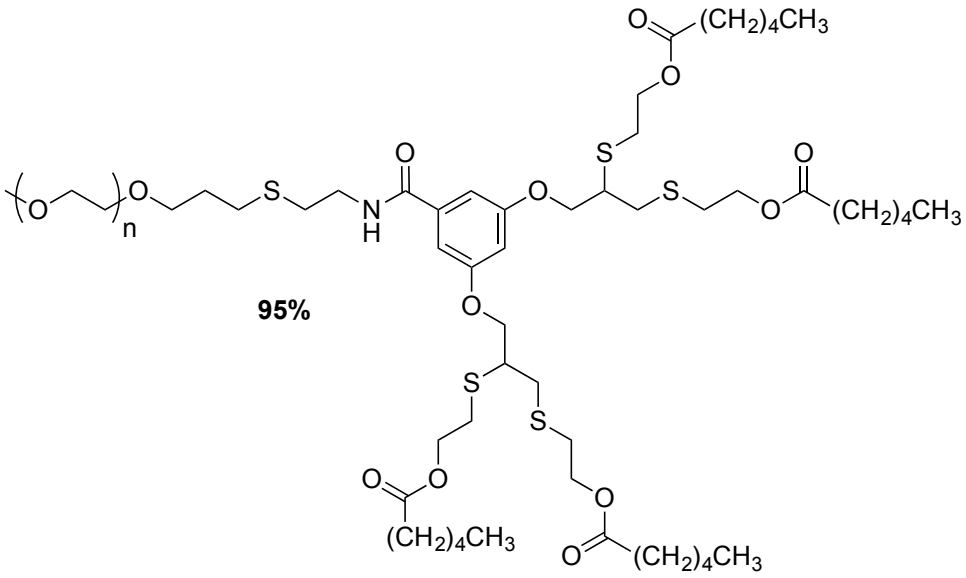
T. Aida

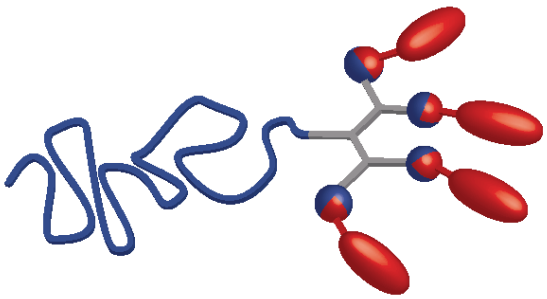
M. Malkoch

A. Nyström

High yielding synthesis of the polymeric hybrids

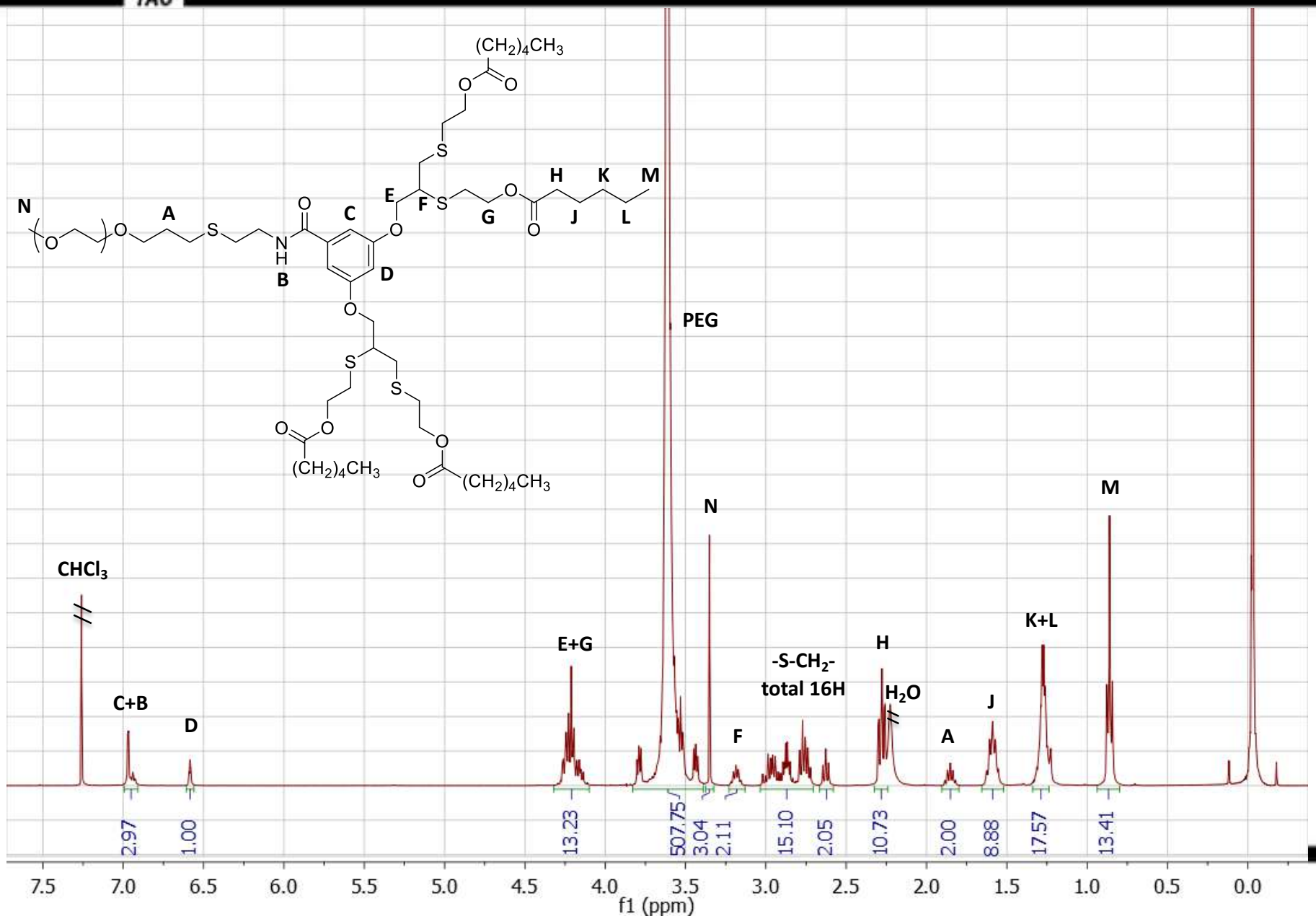






Hydrophilic-Hydrophobic

^{1}H-NMR Spectrum of a PEG_{5KDa}-G2-dendron hybrid



Low PDI doesn't mean it's a single molecule

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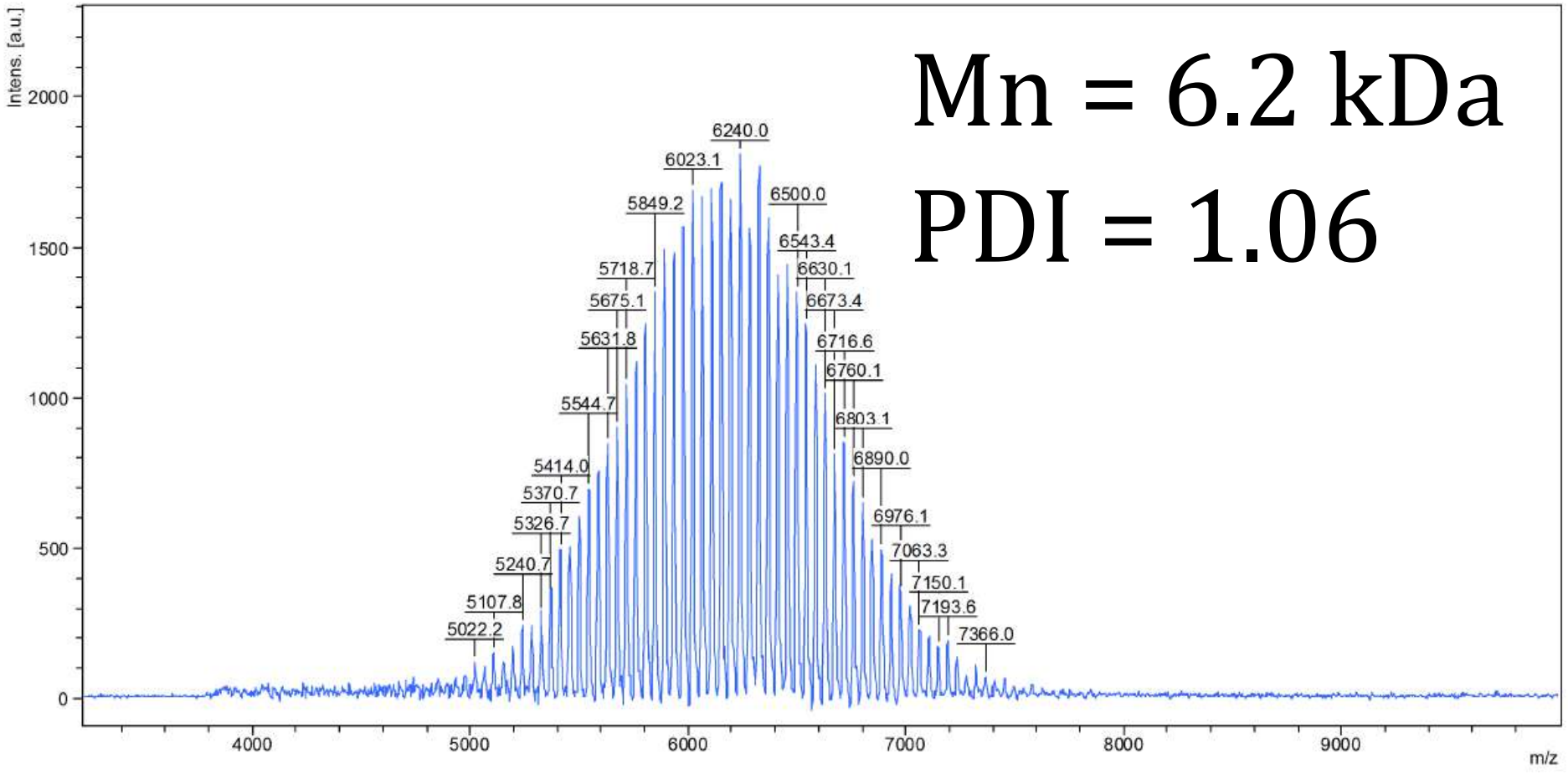
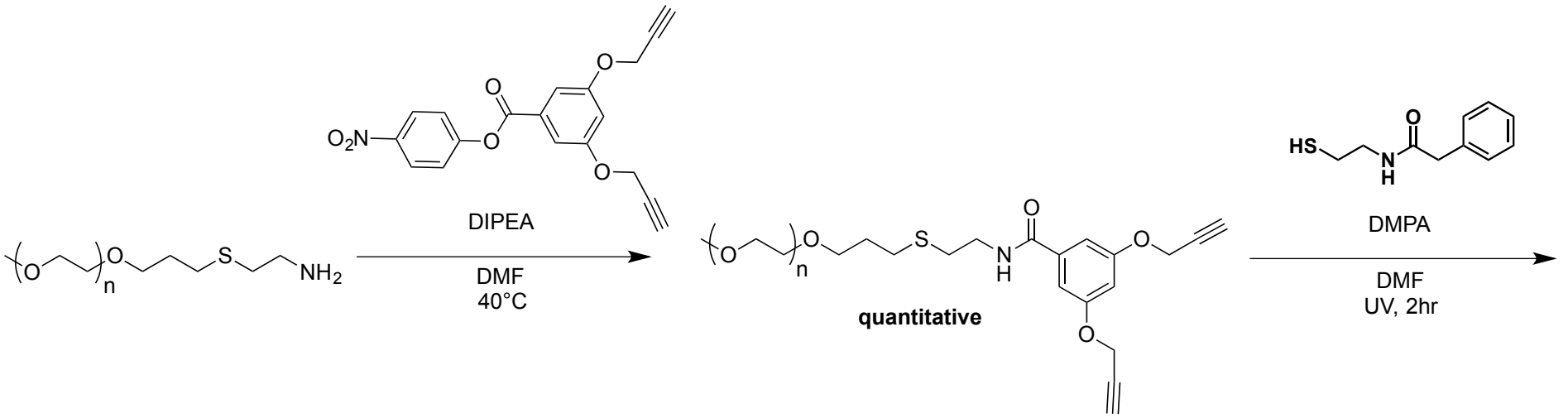
BUT all the hydrophobic dendrons are the same

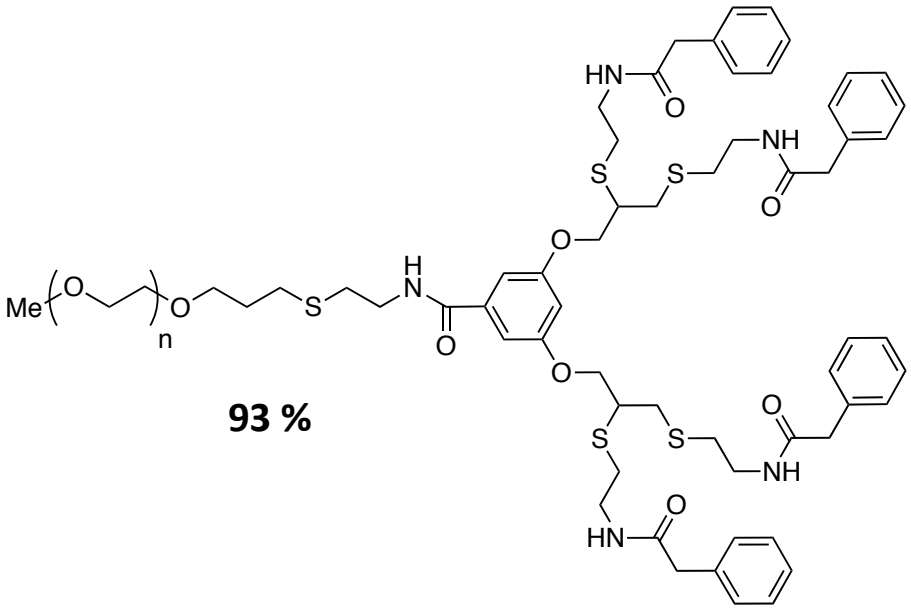


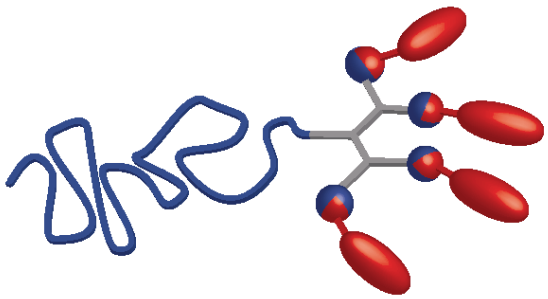
Mn = 6.2 kDa

PDI = 1.06

We can also link the end-groups by amide bonds

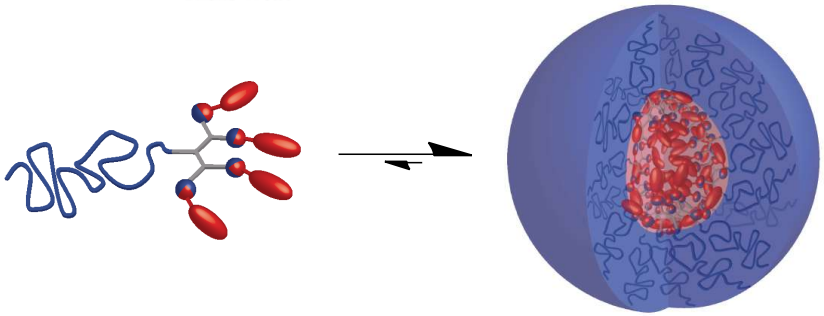






Hydrophilic-Hydrophobic

Would these PEG-dendron hybrids self-assemble?



Fluorescence, DLS, TEM and ^{1}H-NMR confirm formation of micelles

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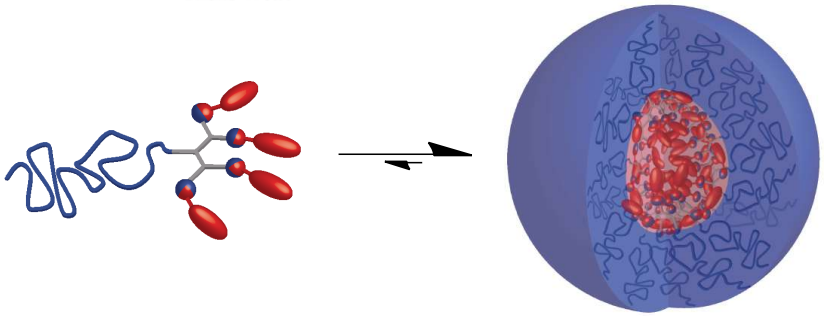
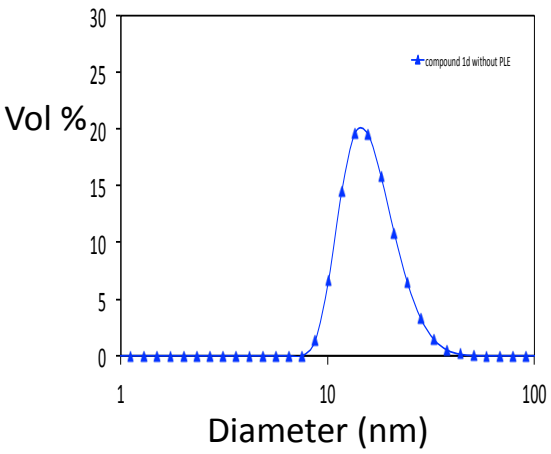
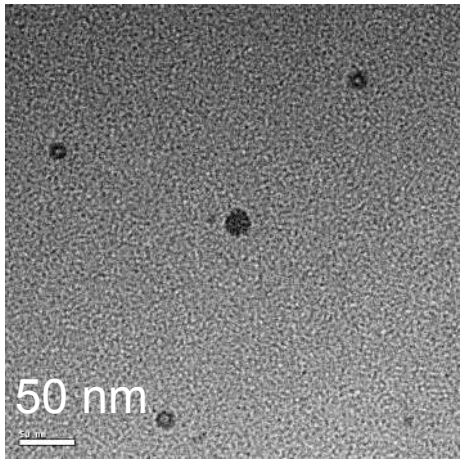
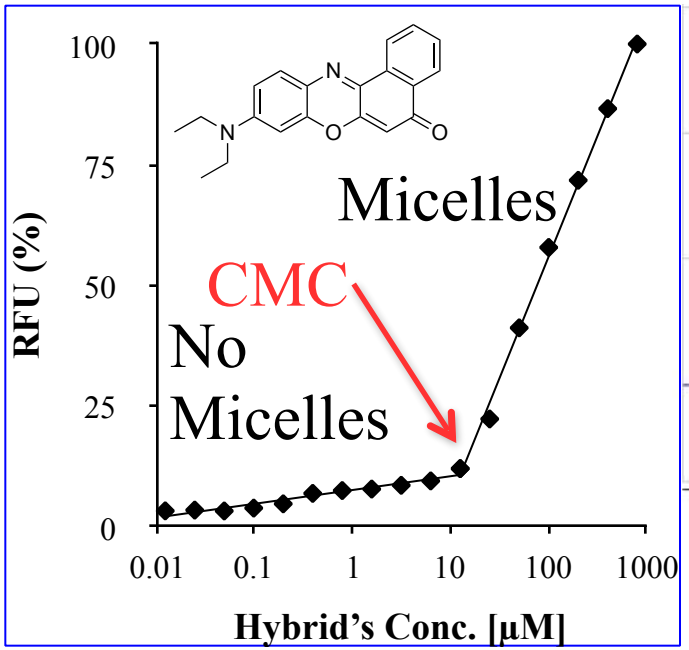
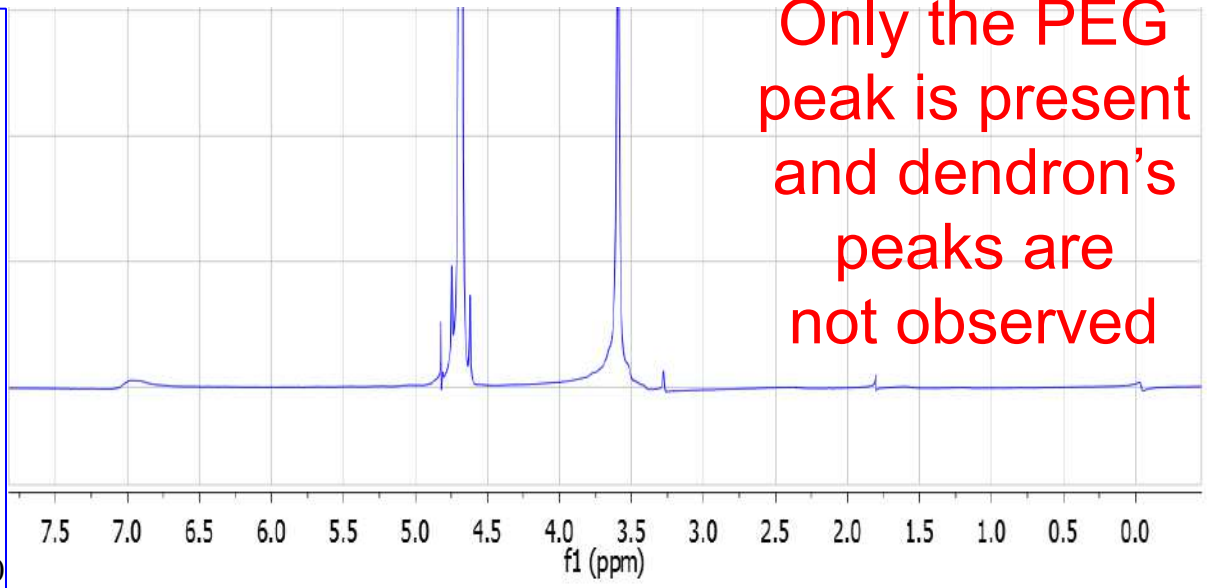






CMC = critical micelle concentration

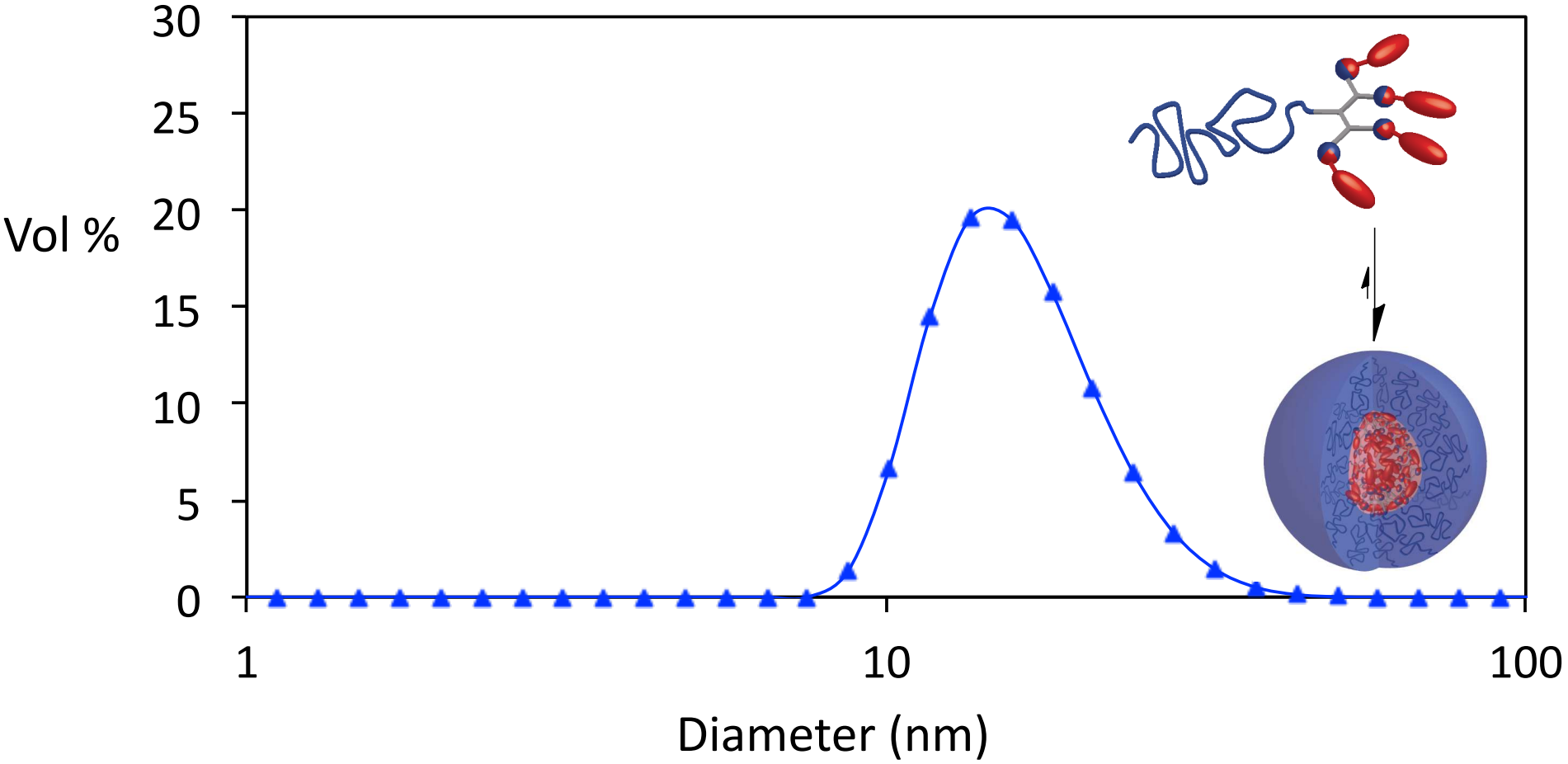




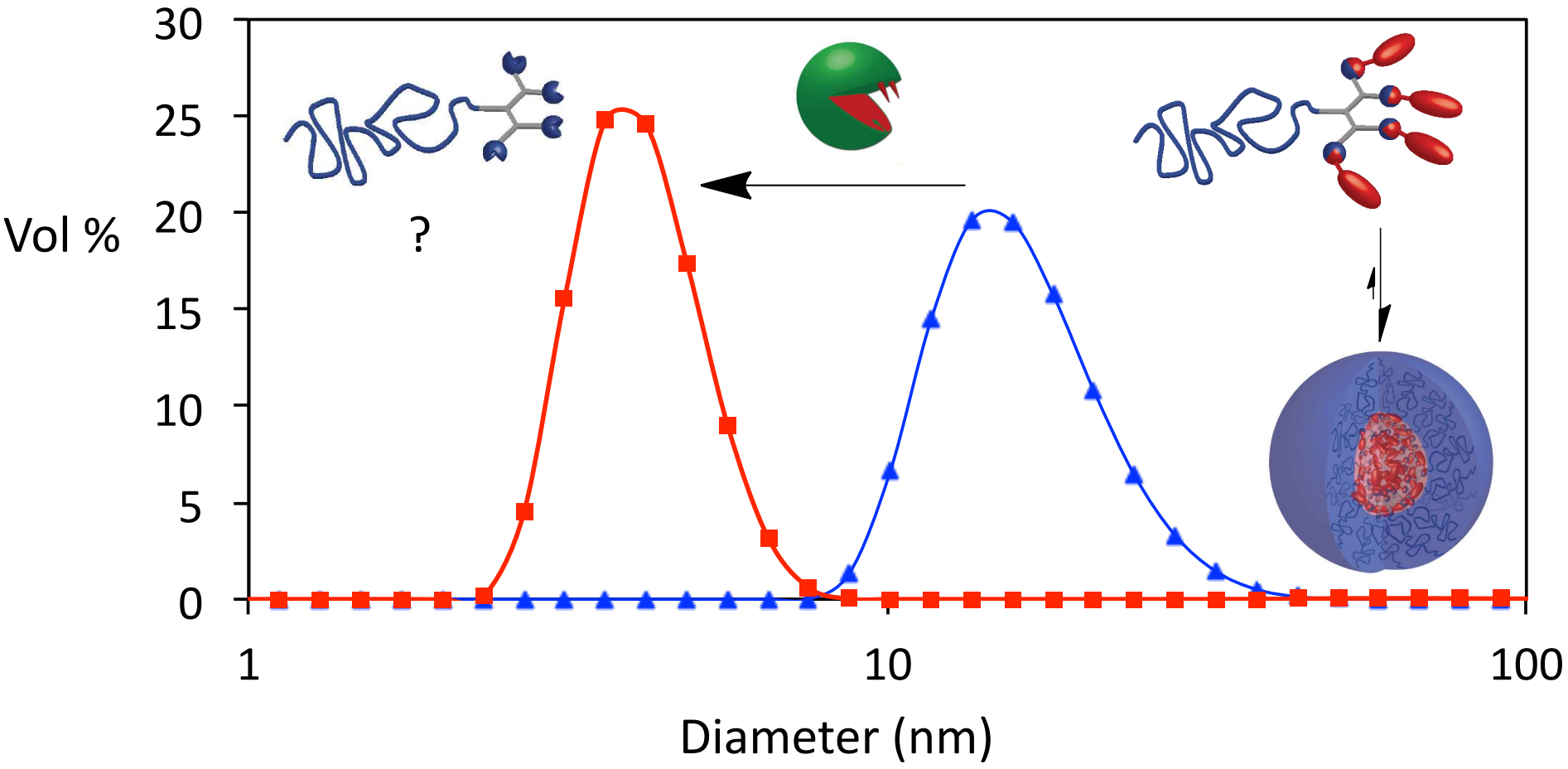
Only the PEG peak is present and dendron's peaks are not observed

* Nile Red Conc. = 1.25µM

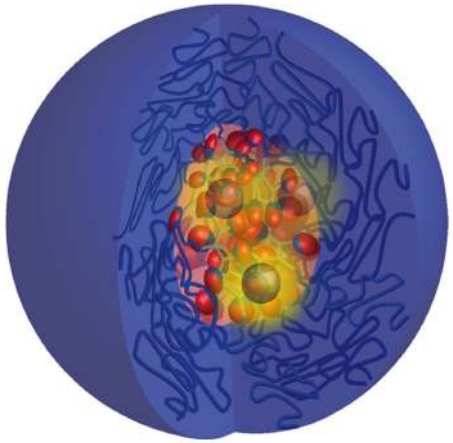
Using DLS to follow micellar disassembly

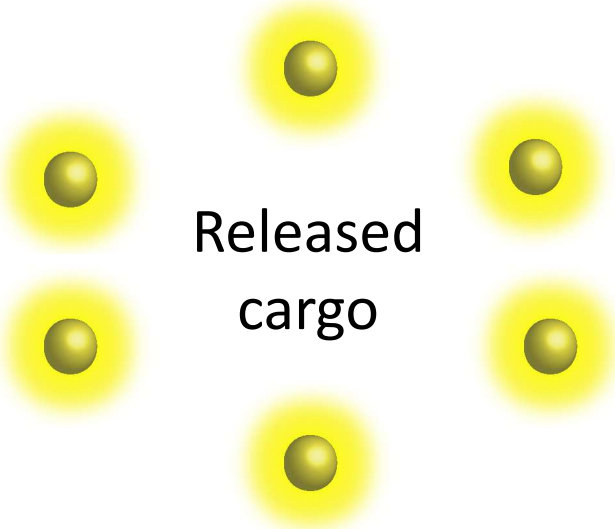


DLS show reduction in sizes after addition of the activating enzyme



Can the enzyme trigger the release of hydrophobic guests?





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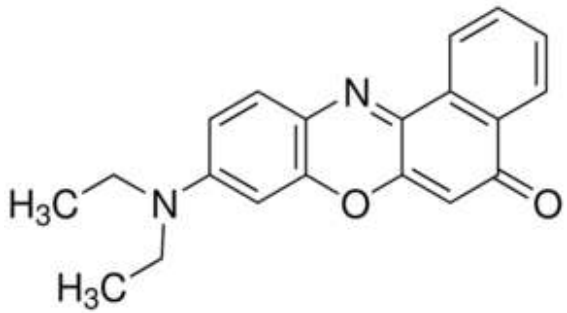
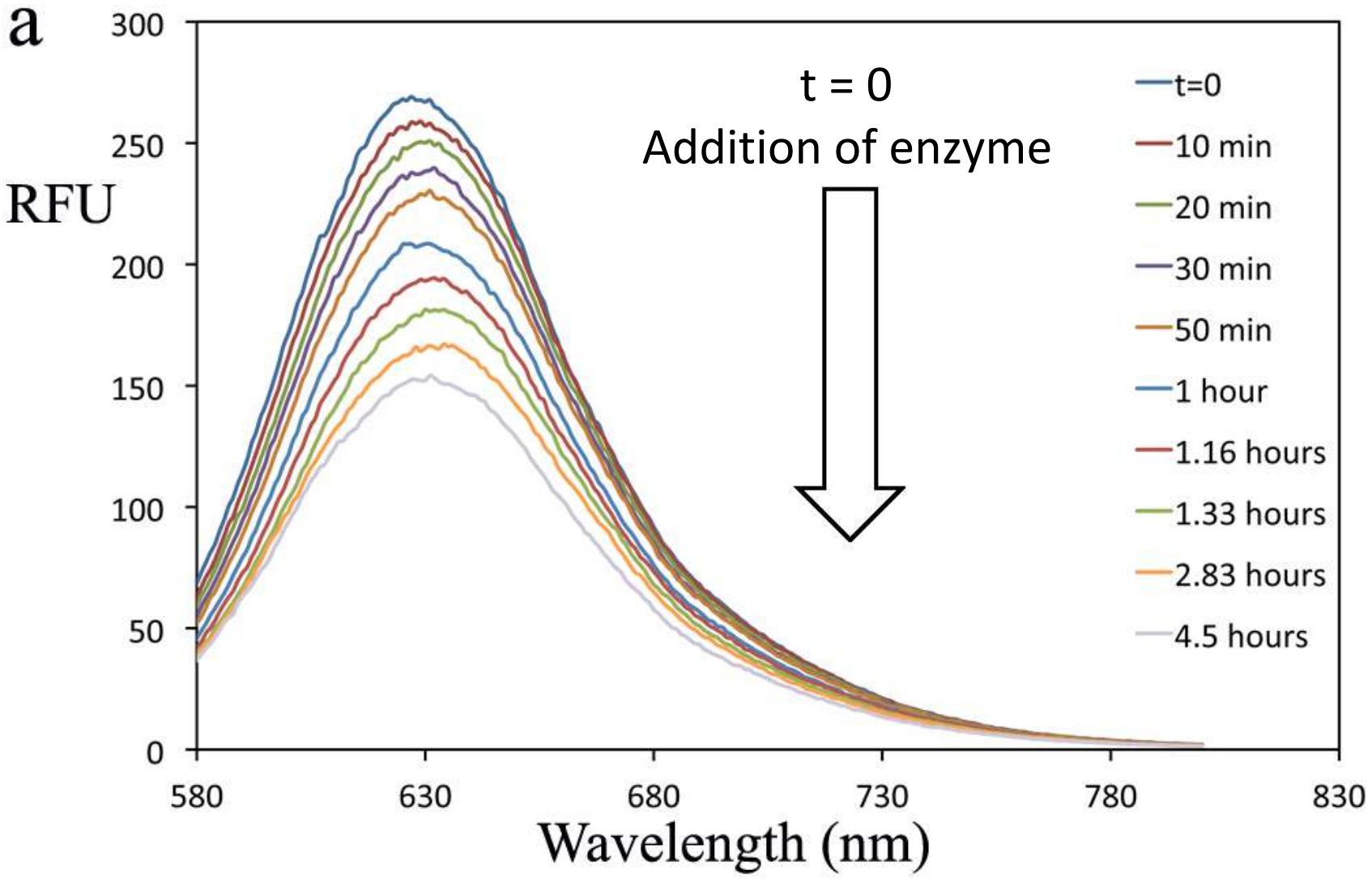
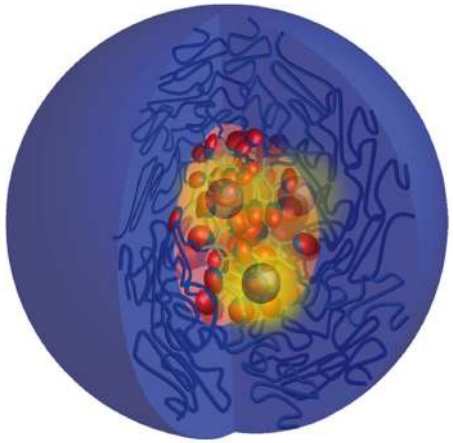
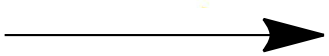


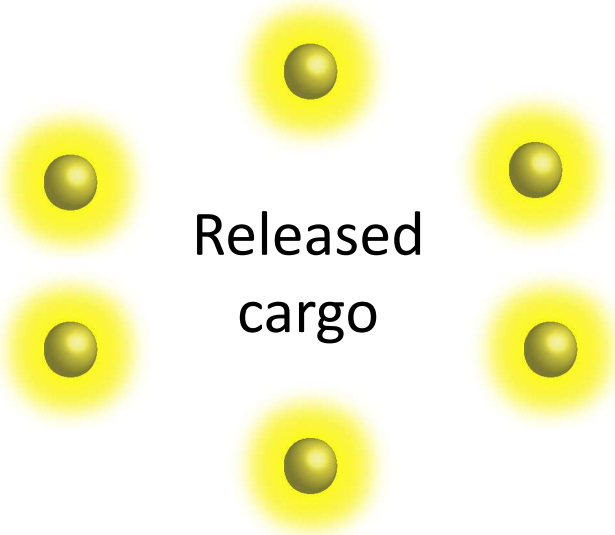
Nile red's fluorescence depends on its environment



The enzyme released the dyes but did it cleave the hydrophobic groups?





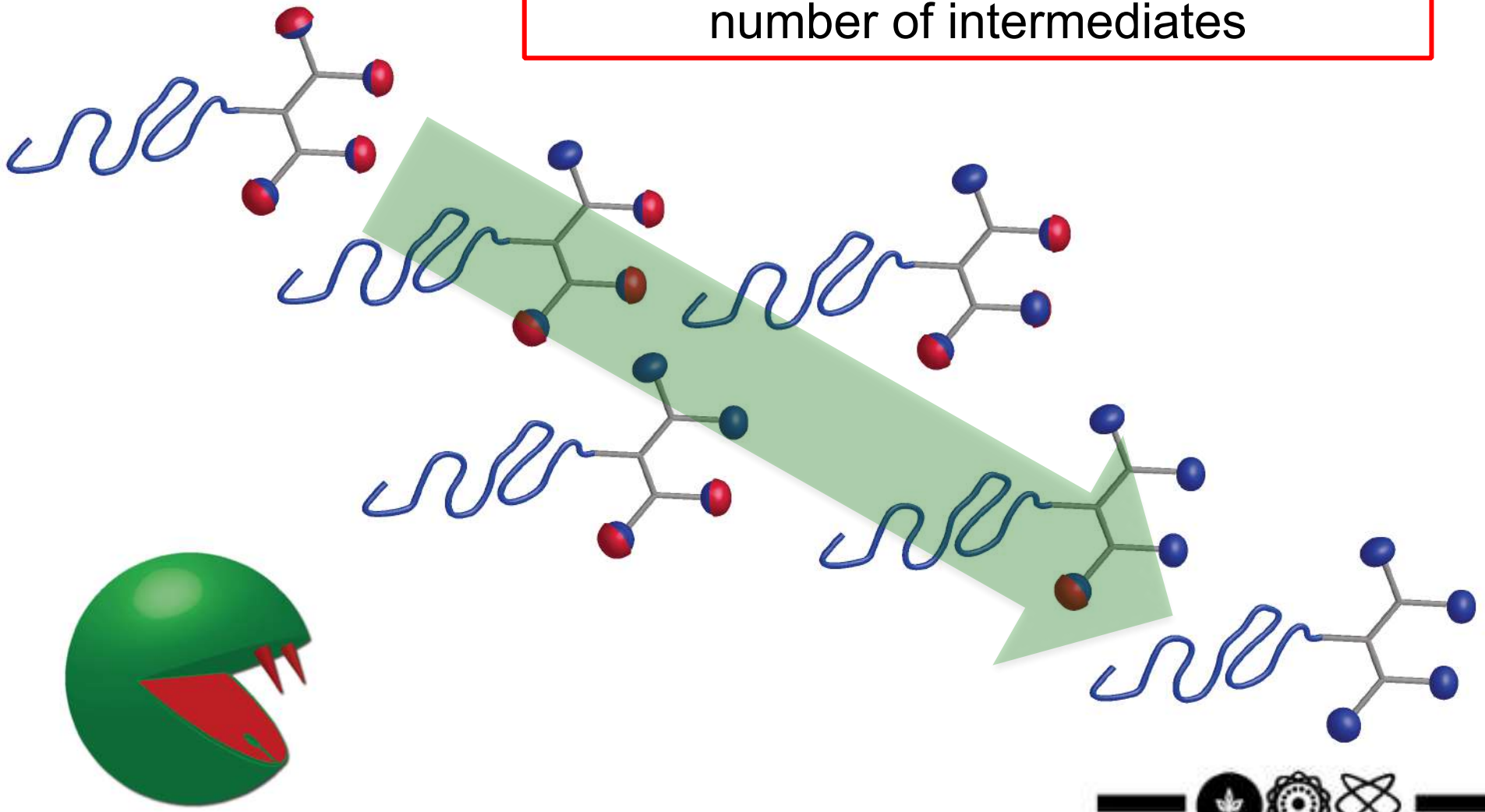


Released cargo

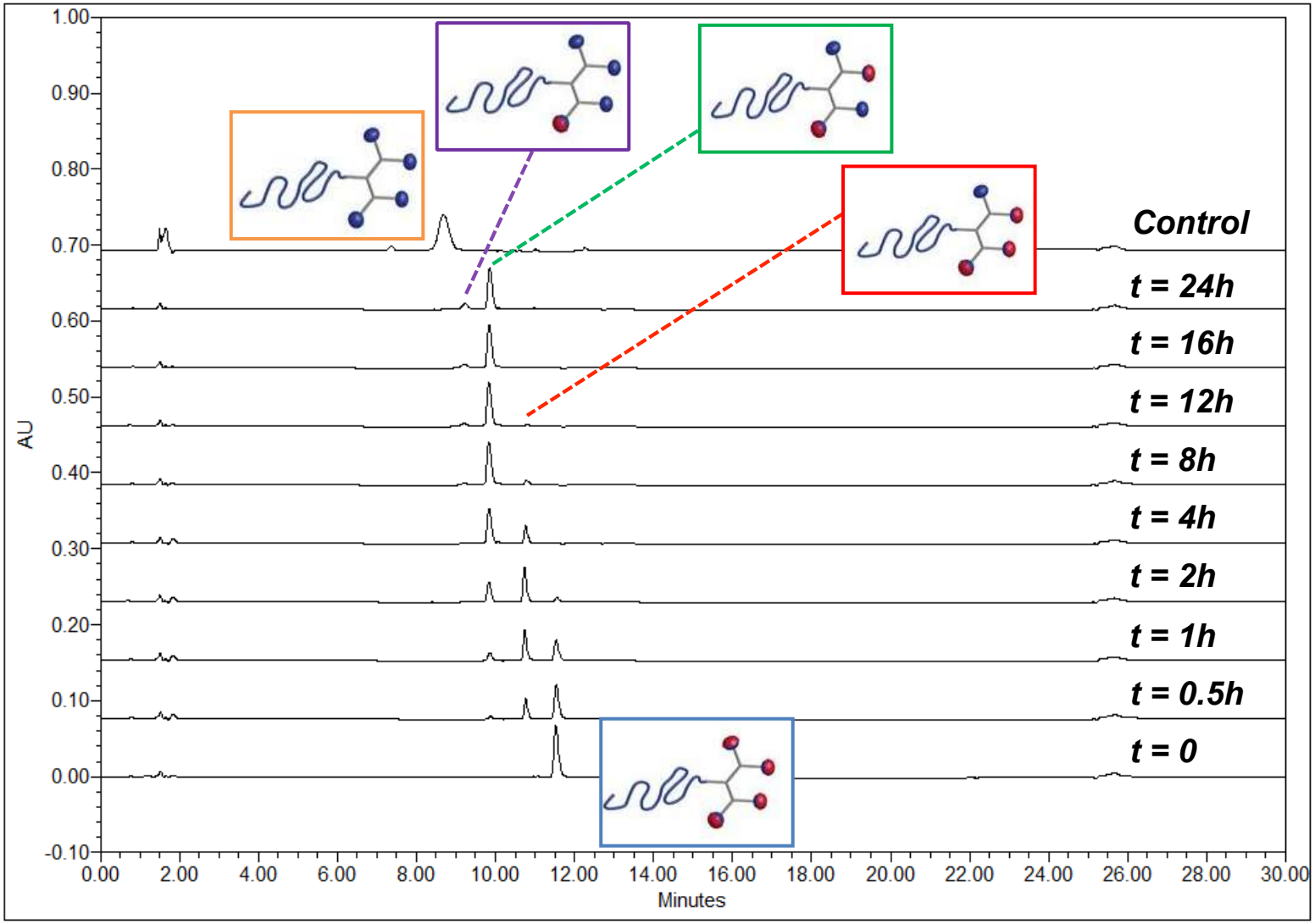
?

Possible intermediates in the enzymatic degradation

Dendron symmetry leads to distinctive number of intermediates



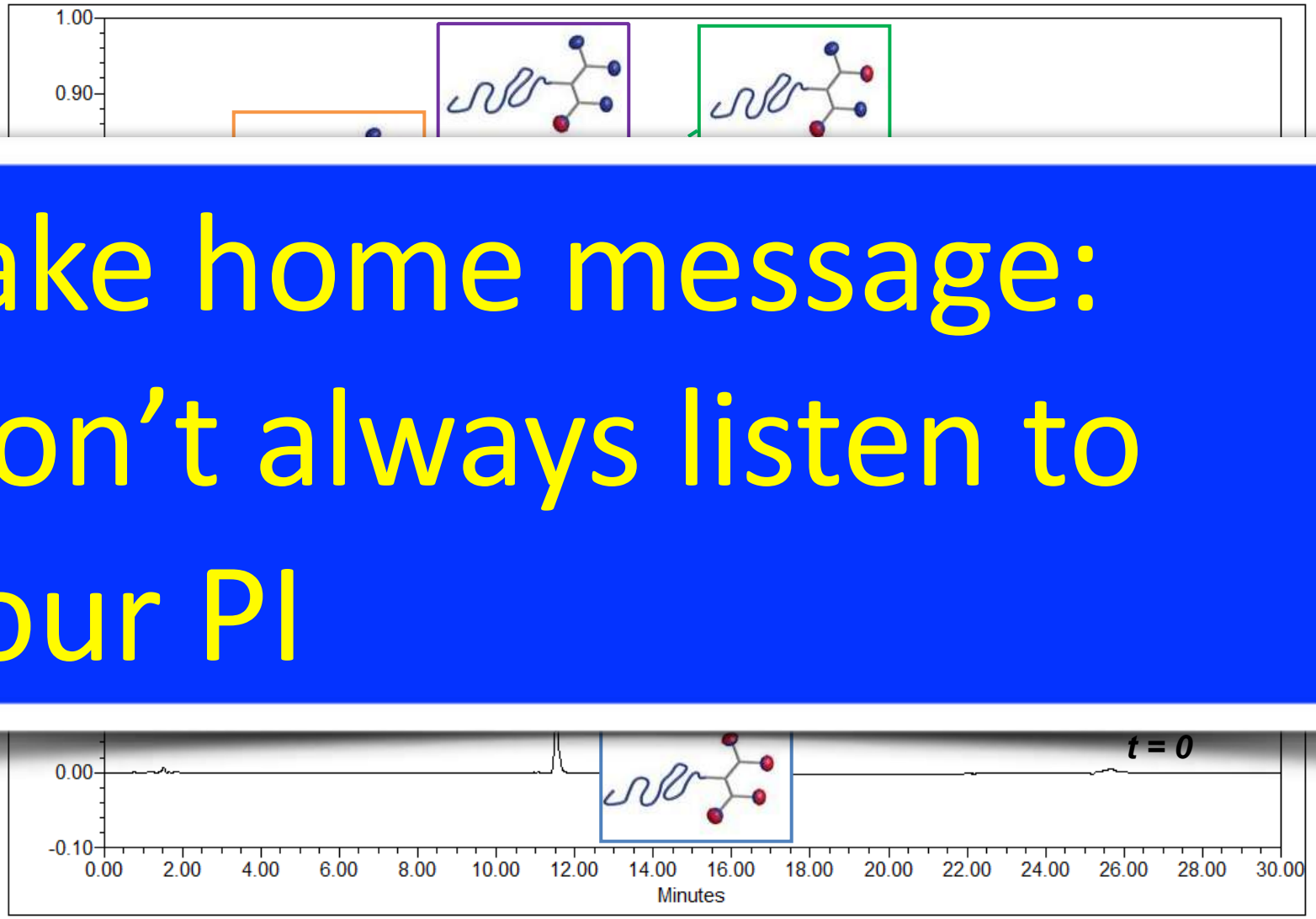
How many groups the enzyme has to cleave to break a micelle?



HPLC data - C18 column

How many groups the enzyme need to cleave to break a micelle?

TAU

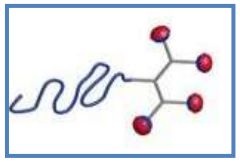


Take home message:
Don’t always listen to your PI

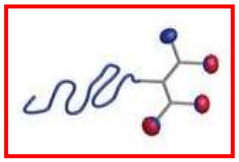
HPLC data - C18 column

Intermediates were confirmed by MALDI-TOF MS

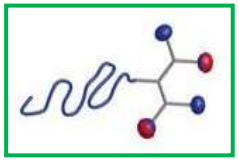
5kDa Hybrid
6.3kDa



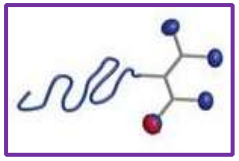
Intermediate 1
6.2kDa



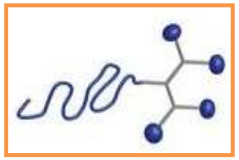
Intermediate 2
6.0kDa

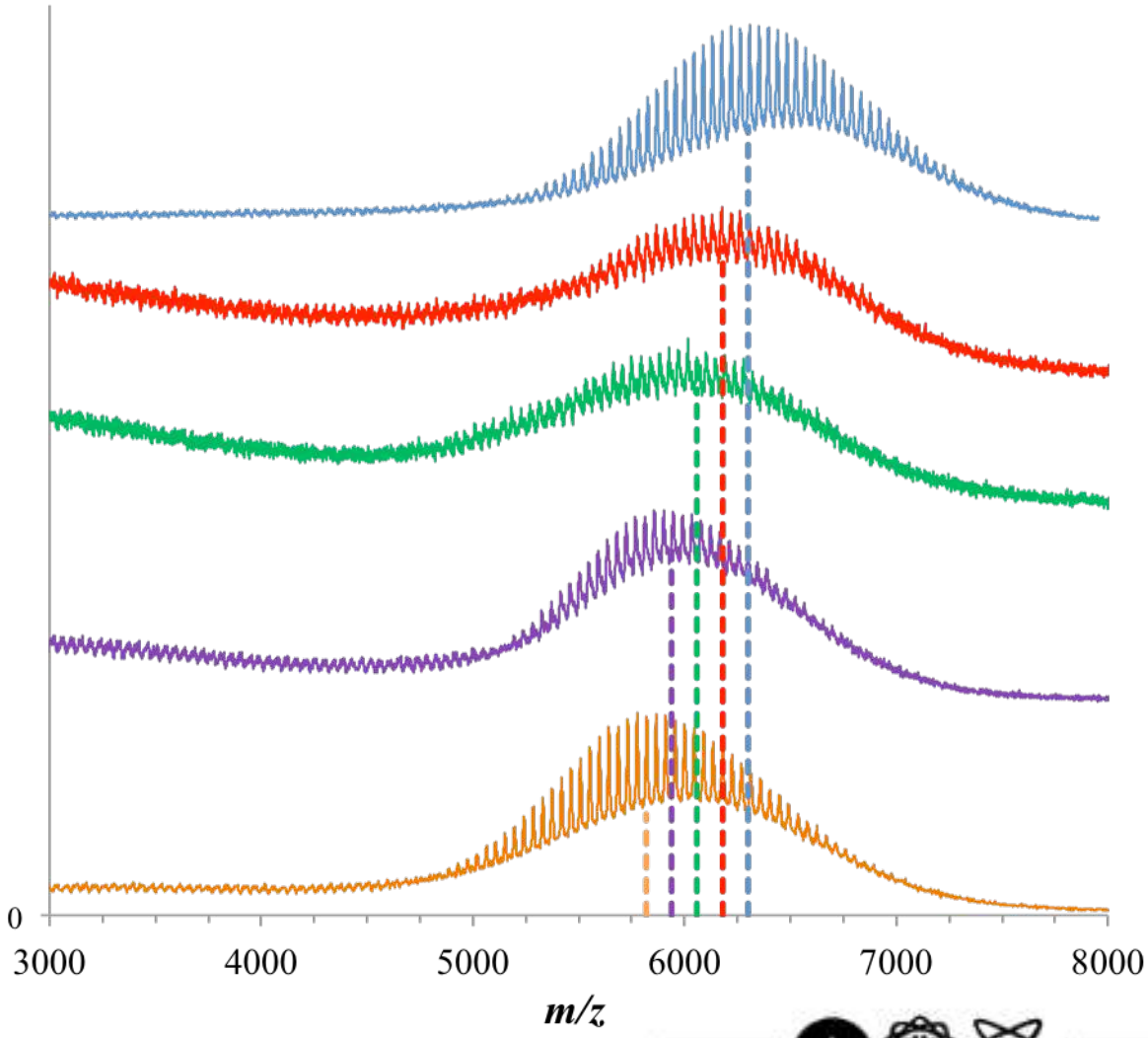


Intermediate 3
5.9kDa



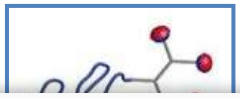
5kDa-4NH_3^+
5.8kDa





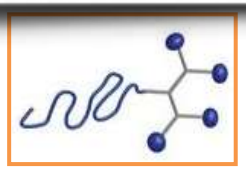
Intermediates were confirmed by MALDI-TOF MS

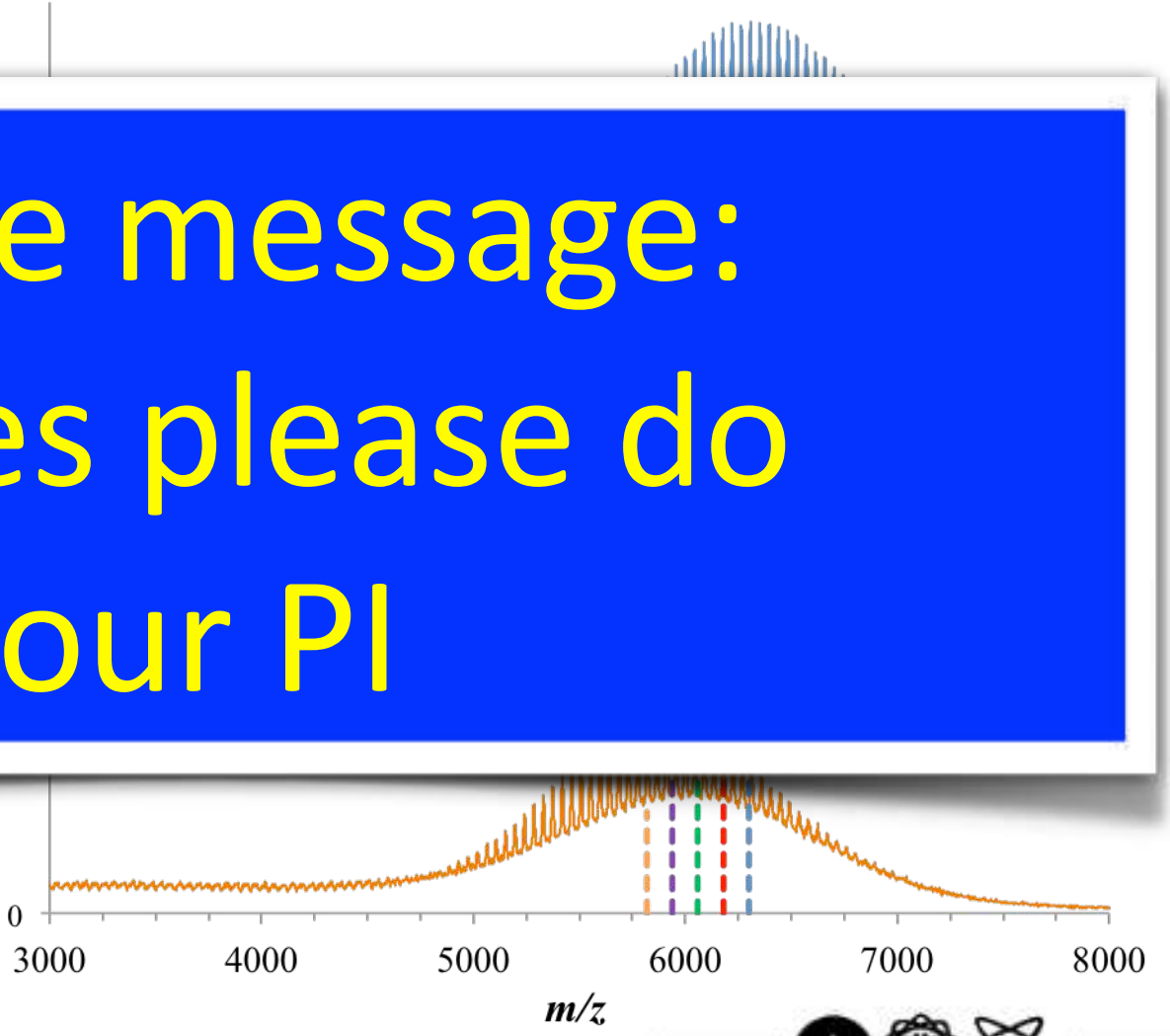
5kDa Hybrid



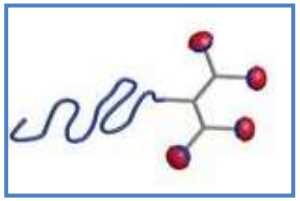
Take home message:
Sometimes please do listen to your PI

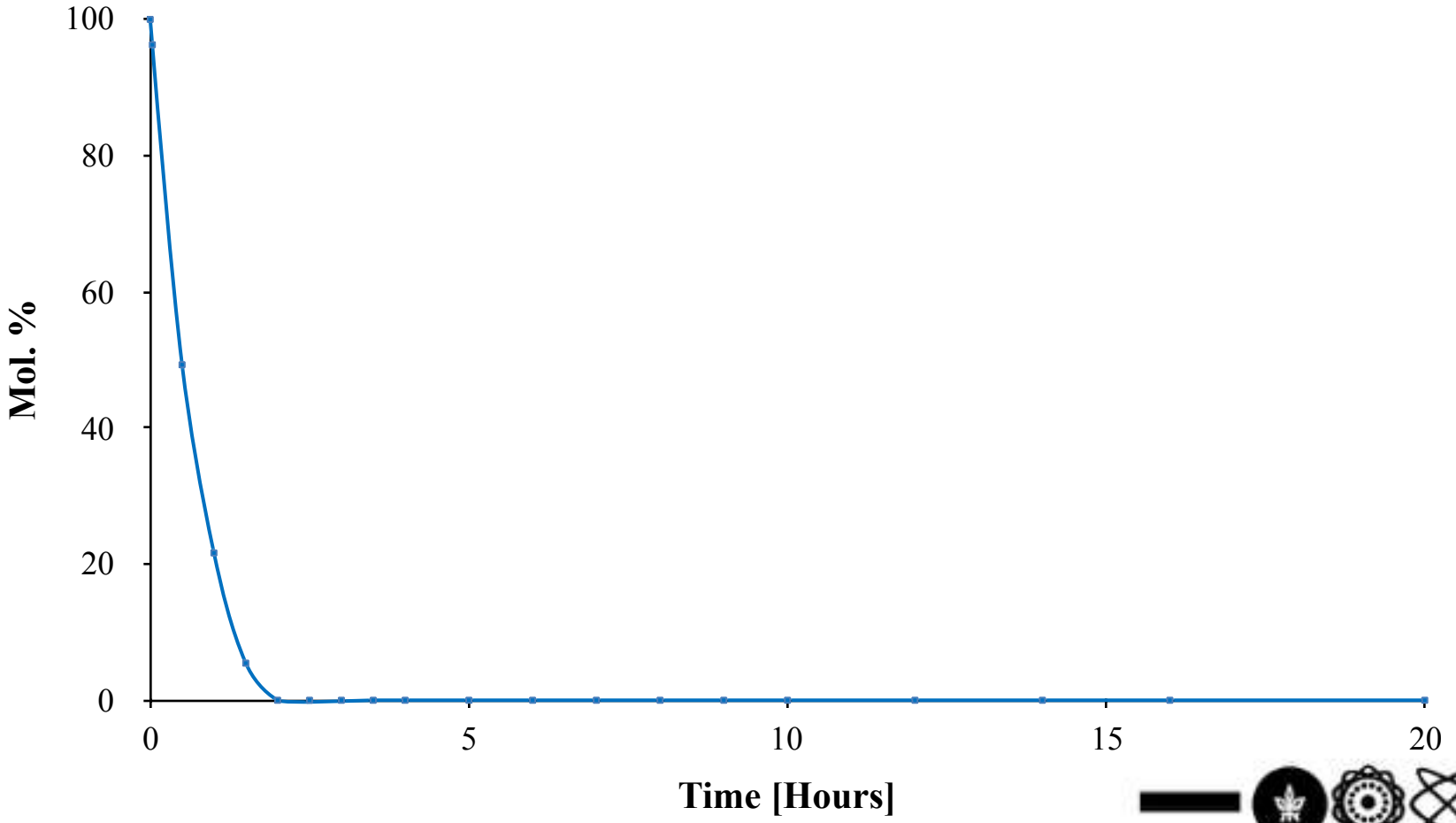
$5kDa\text{-}4NH_3^+$
5.8kDa



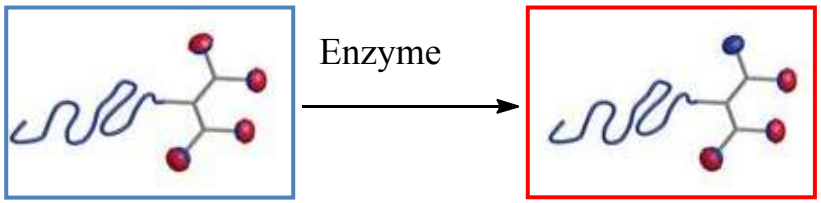


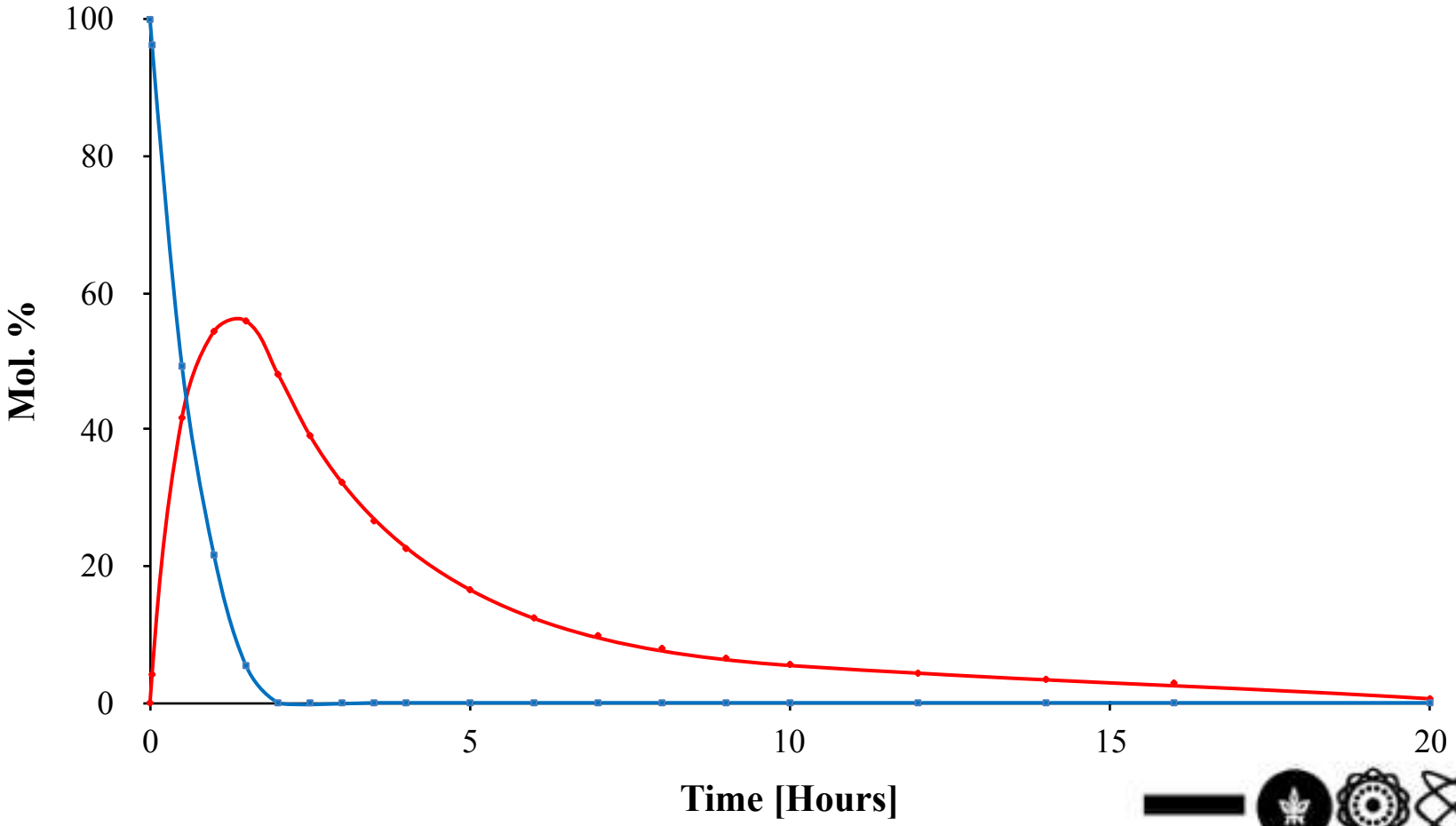
HPLC: revealing the molecular details of the disassembly



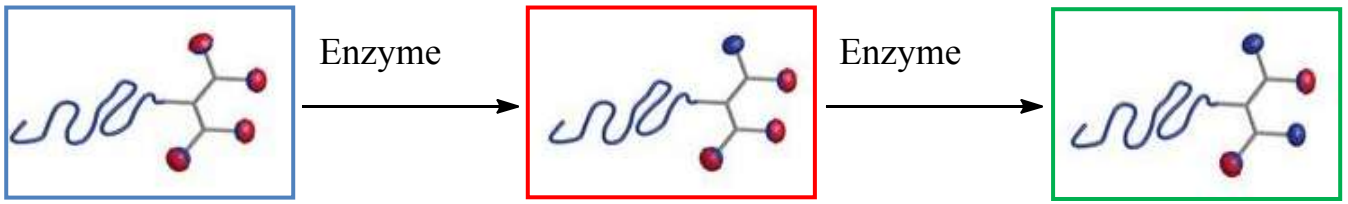


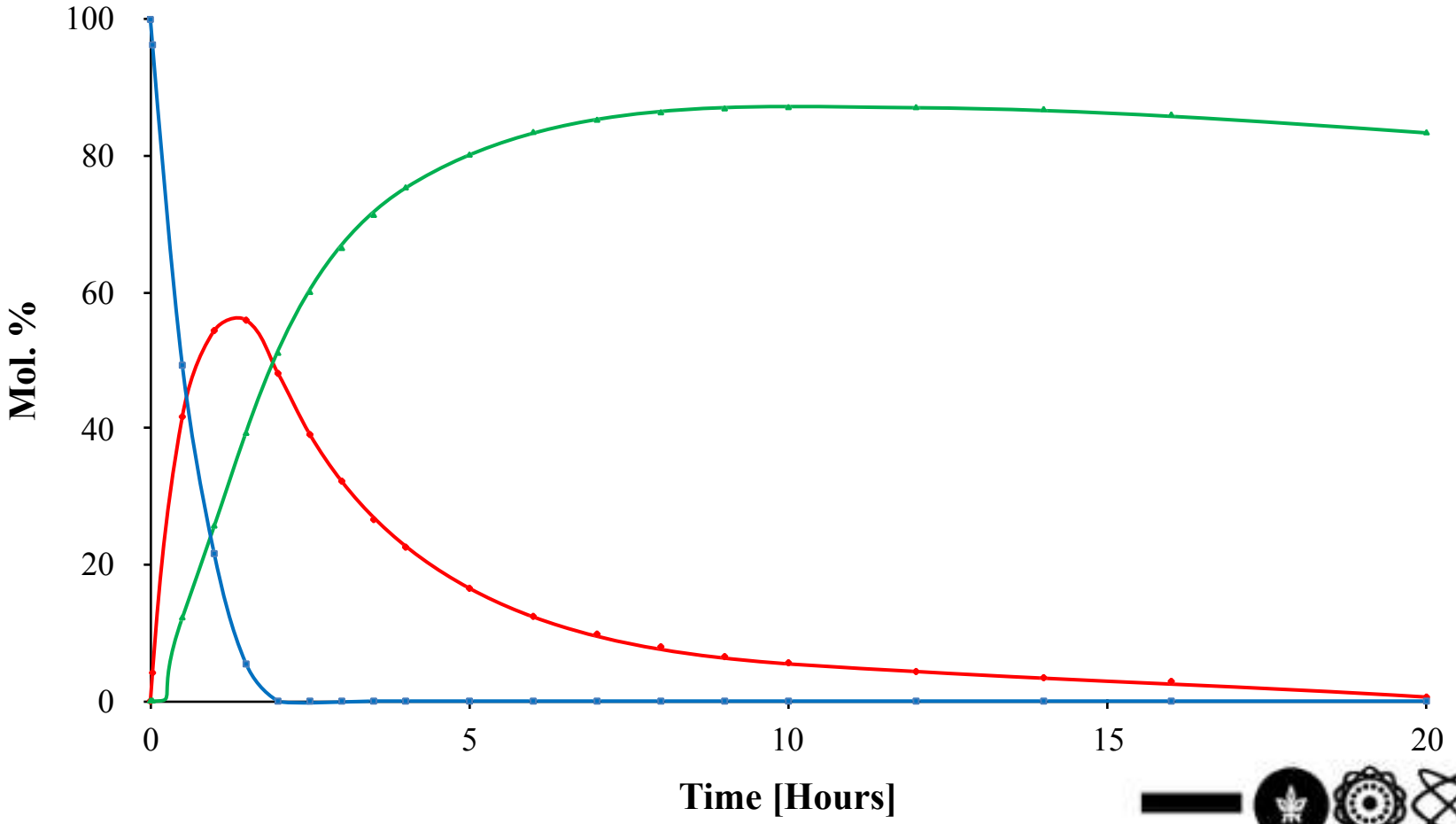
HPLC: revealing the molecular details of the disassembly



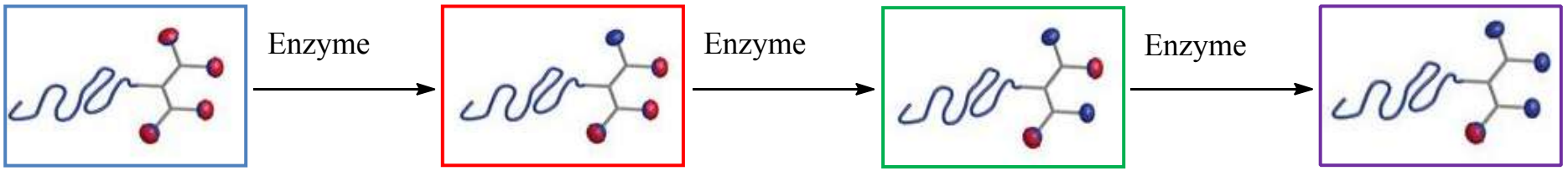


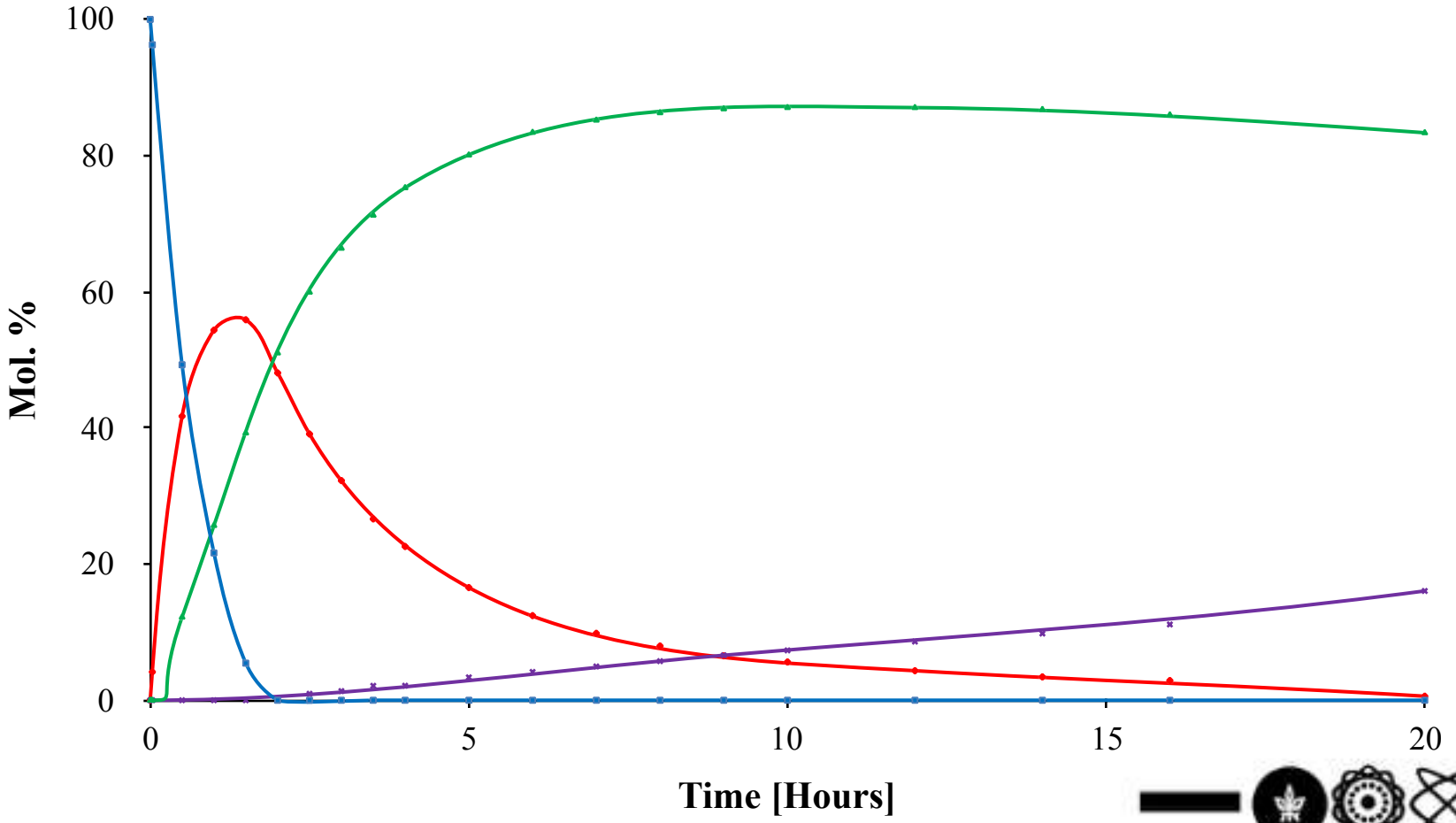
HPLC: revealing the molecular details of the disassembly



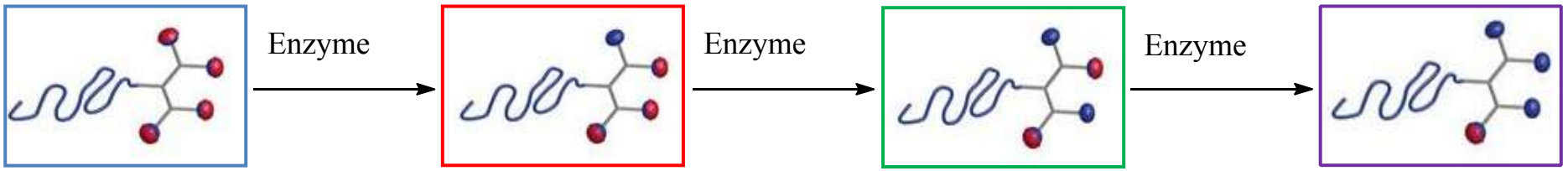


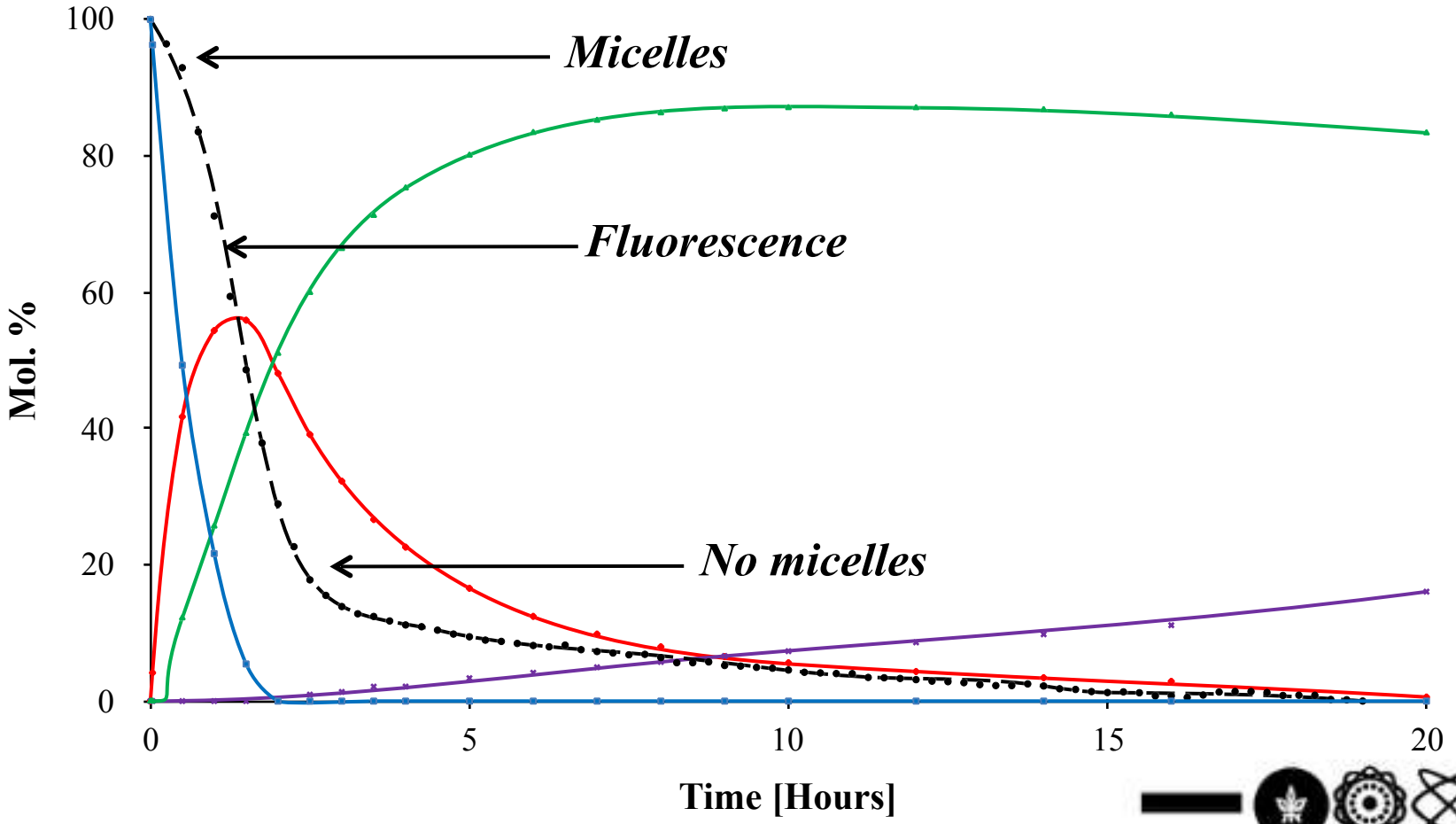
HPLC: revealing the molecular details of the disassembly



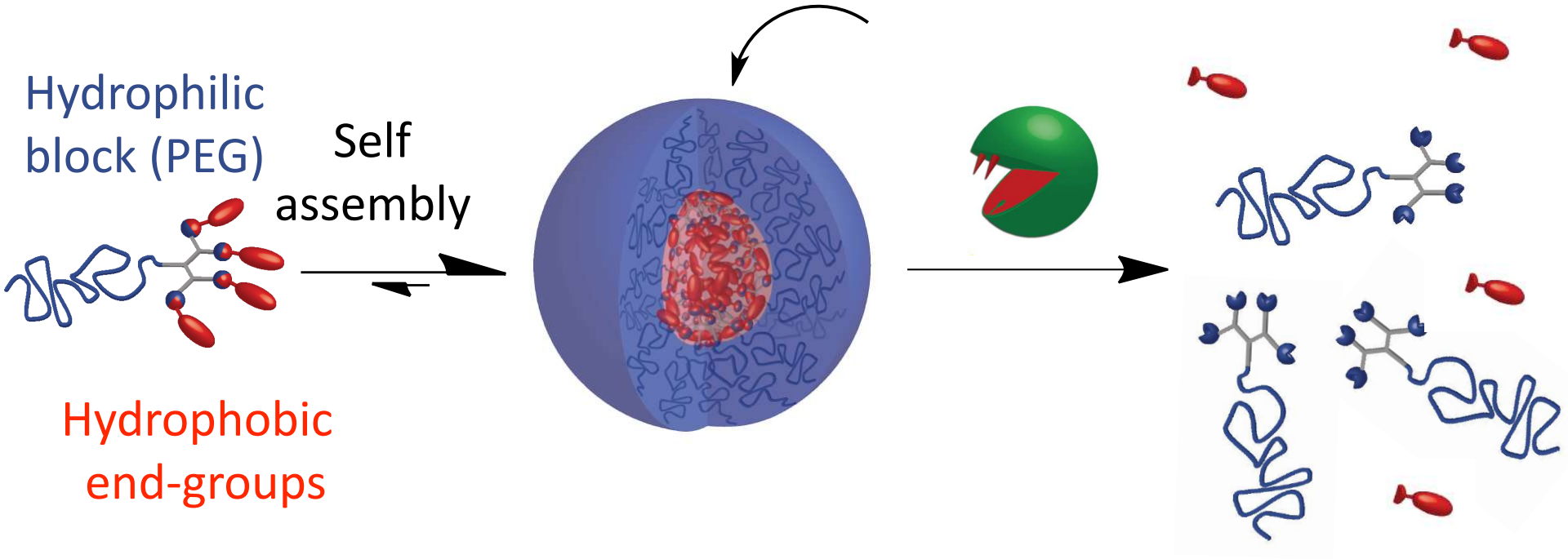


HPLC: revealing the molecular details of the disassembly

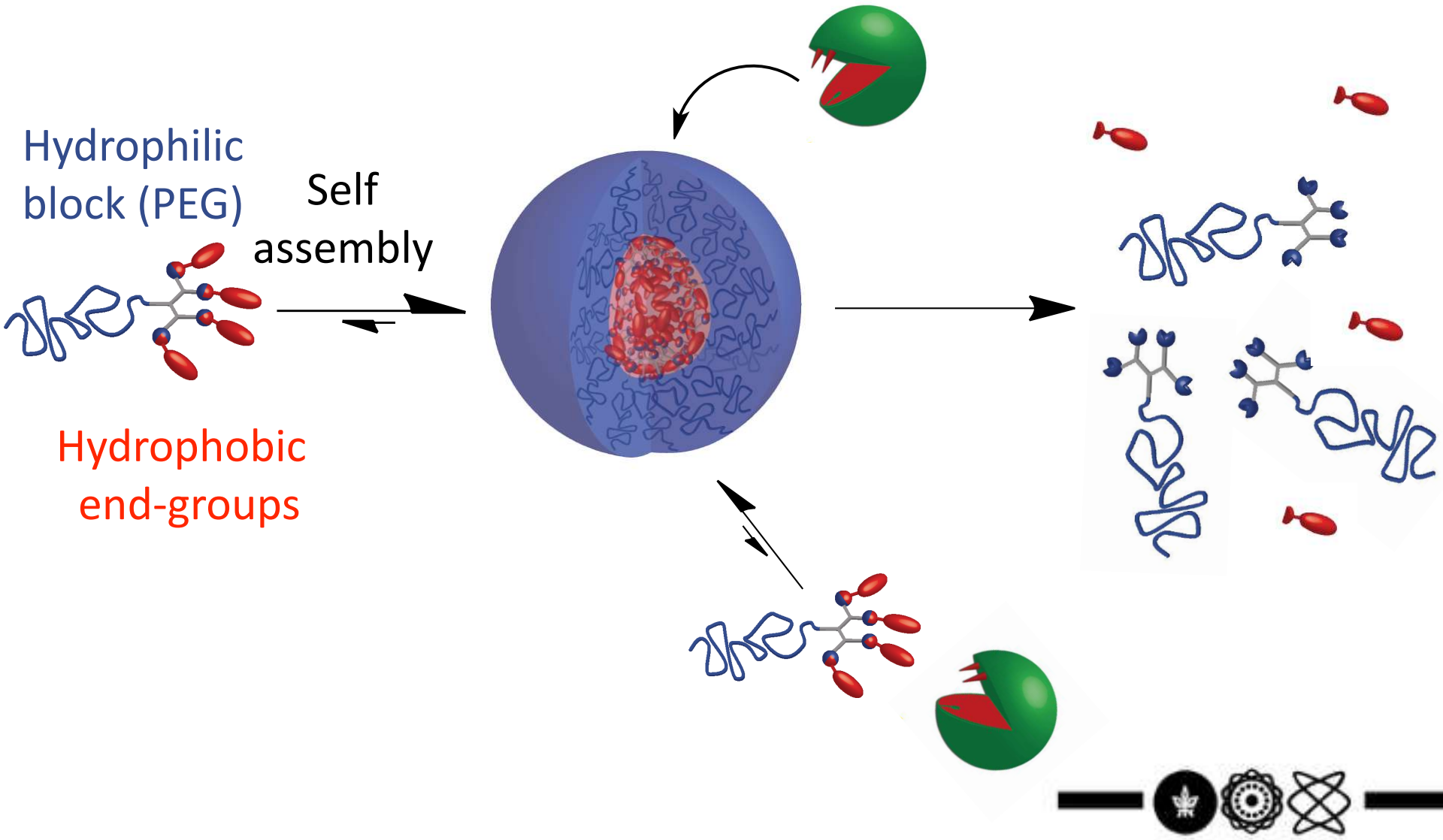




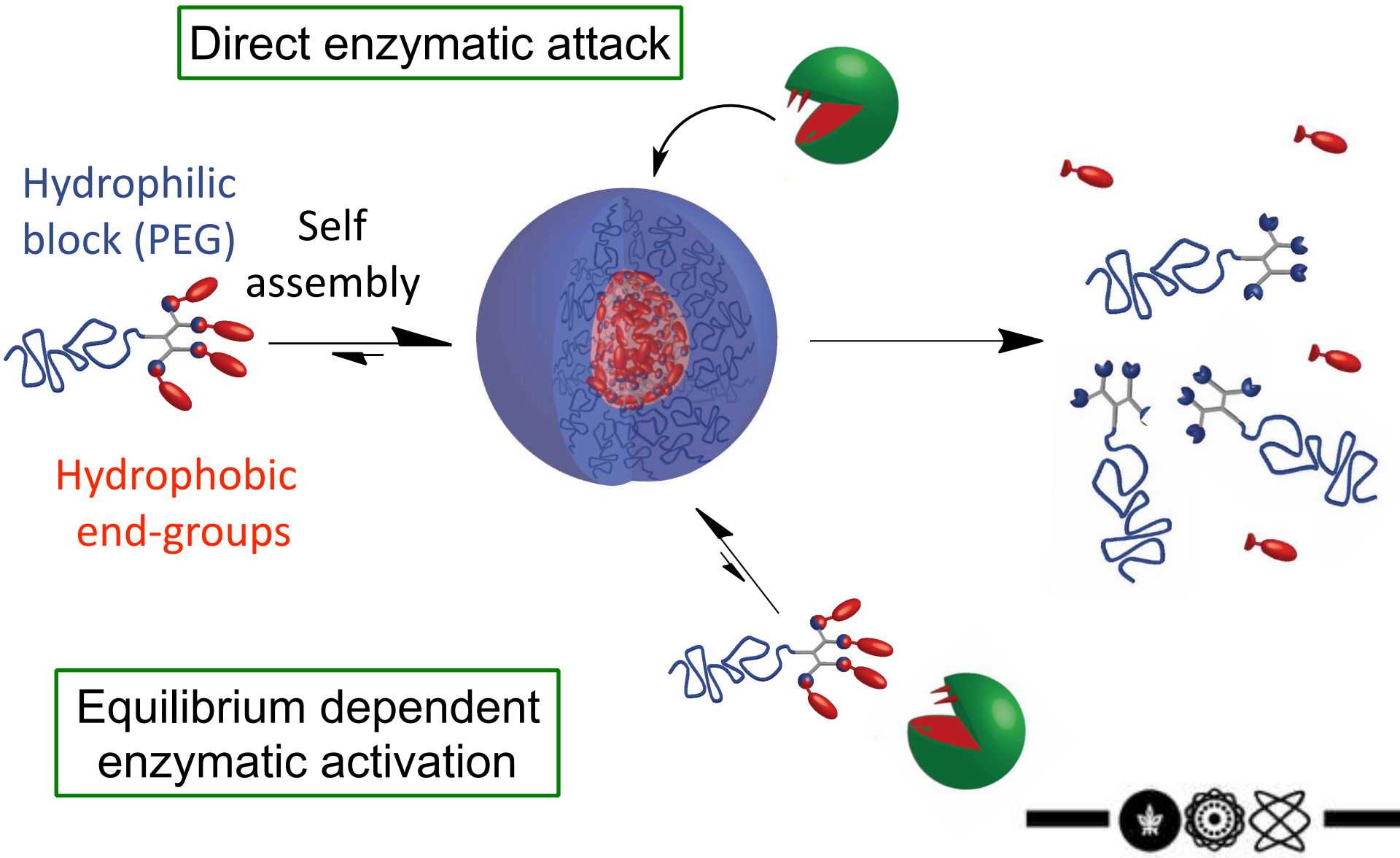
How can an enzyme break a micelle?



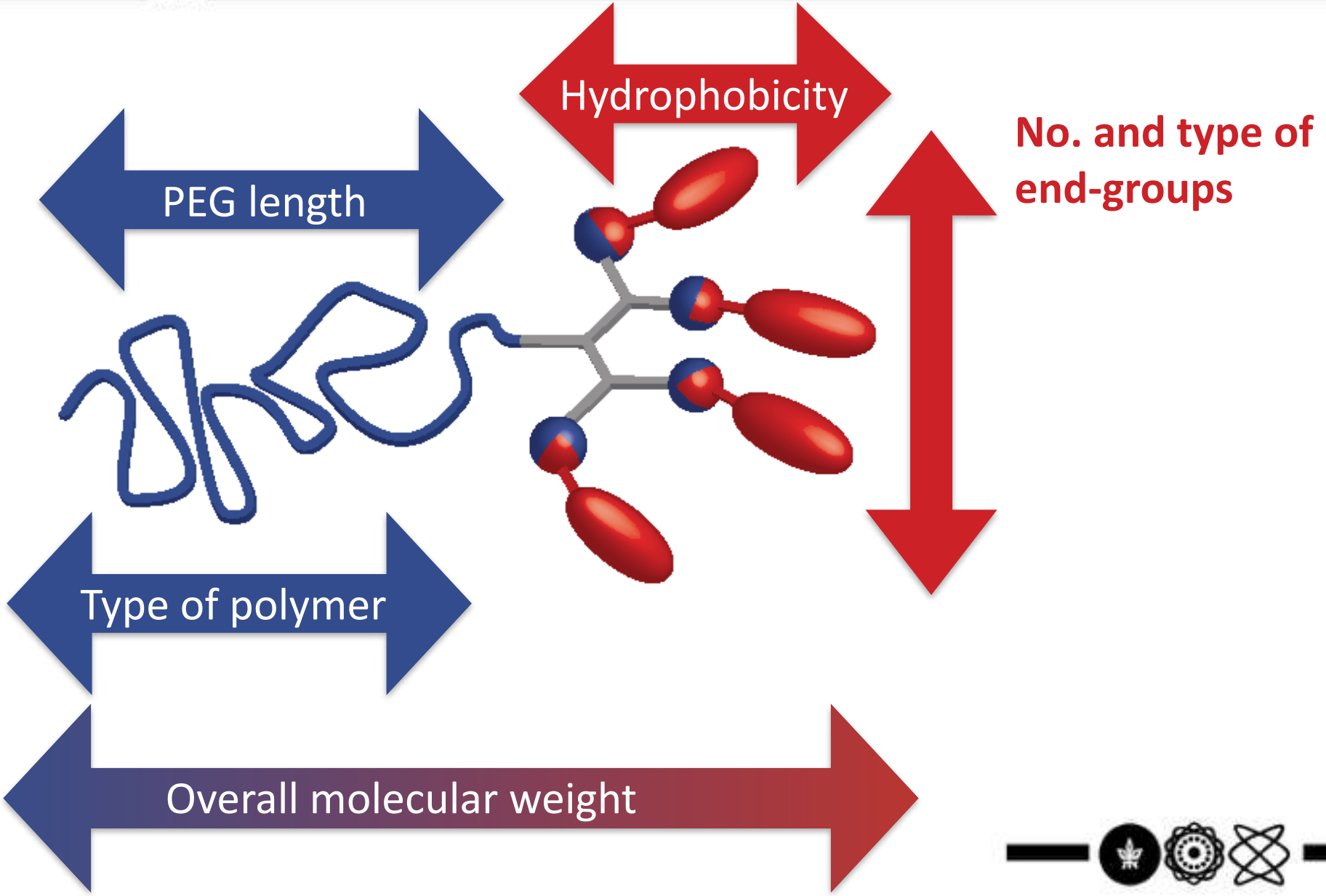
How can an enzyme break a micelle?



Two possible mechanisms



Tuning amphiphilicity with high molecular precision



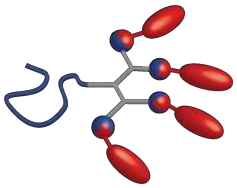
How would the PEG length affect the disassembly rate?

TAU

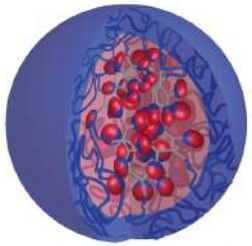
PEG Mn

CMC values

2 kDa

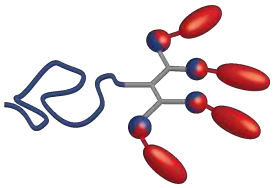


7 μM

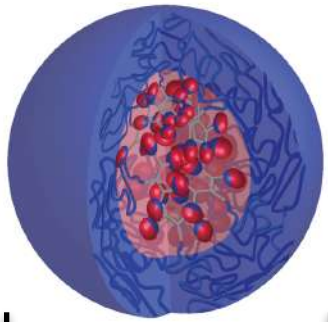


11 nm

5 kDa

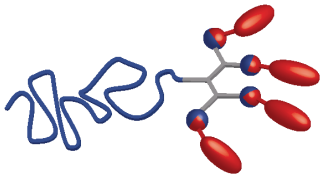


12 μM

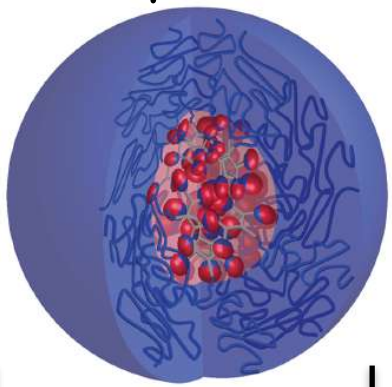


14 nm

10 kDa



21 μM



18 nm

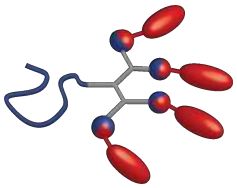
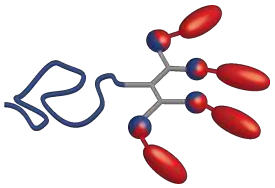
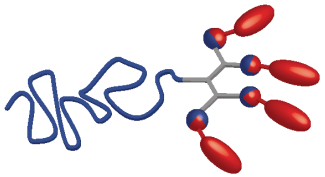
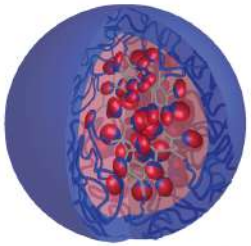
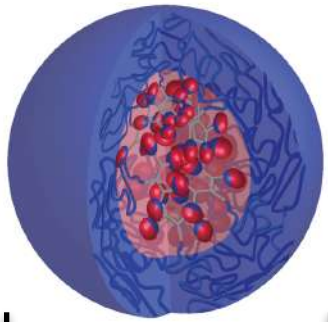
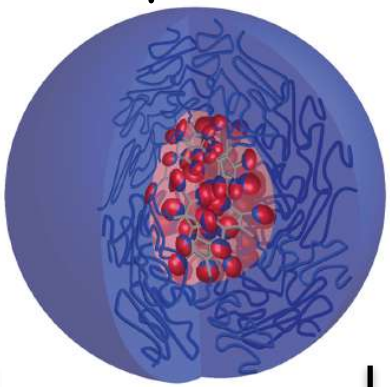
Diameter, shell thickness and CMC

How would the PEG length affect the disassembly rate?

TAU

PEG Mn

CMC values

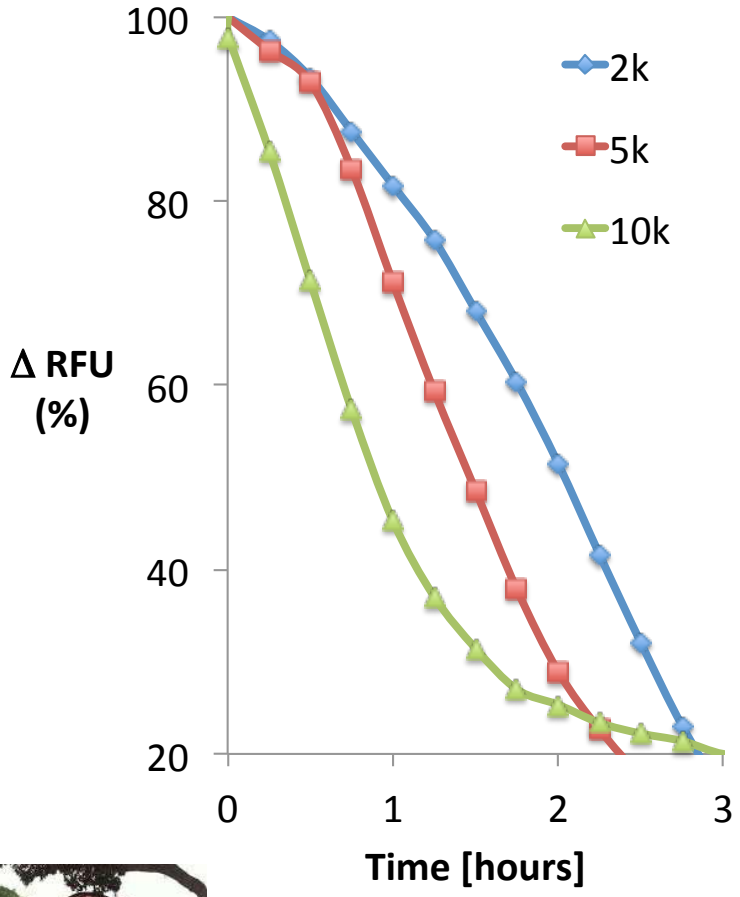
2 kDa	5 kDa	10 kDa
		
7 μM	12 μM	21 μM
		
11 nm	14 nm	18 nm

Direct enzymatic attack

Rate

Equilibrium dependent enzymatic activation

Micelles with longer PEG blocks disassemble faster

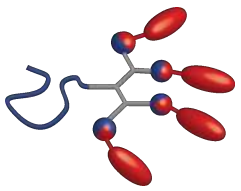


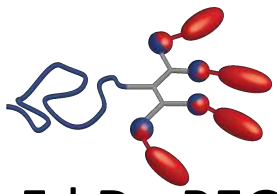
CMC values

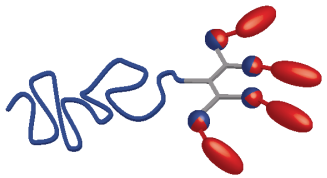
7 μM

12 μM

21 μM



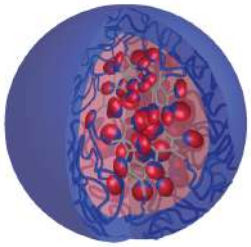


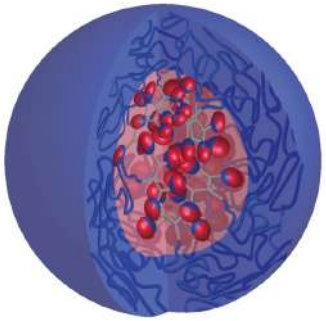


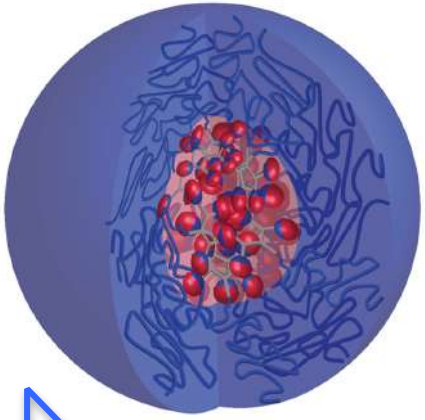
2 kDa PEG

5 kDa PEG

10 kDa PEG



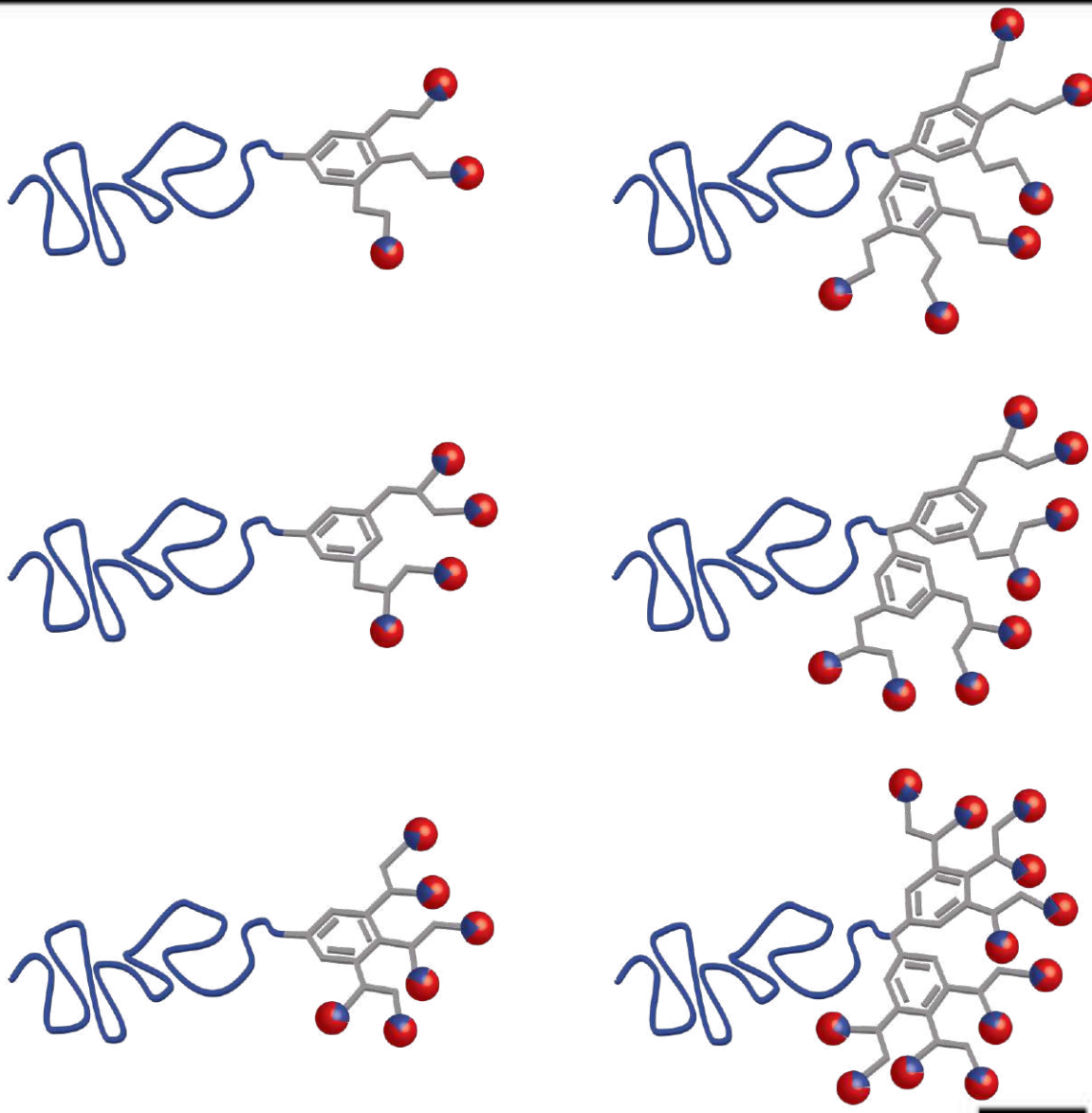




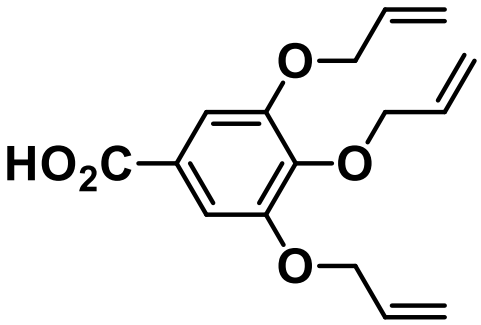
CMC and enzymatic degradation rate

Asi Harnoy et al. JACS **2014**

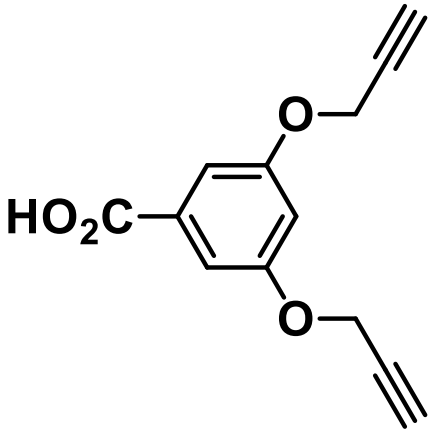
Controlling the number of hydrophobic end-groups with high precision



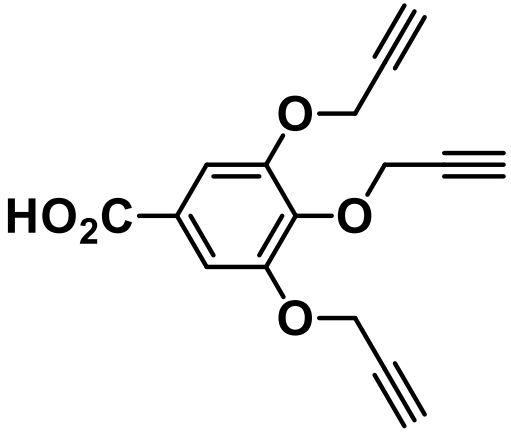
The multi-valency of the branching unit can be tuned



AB_3



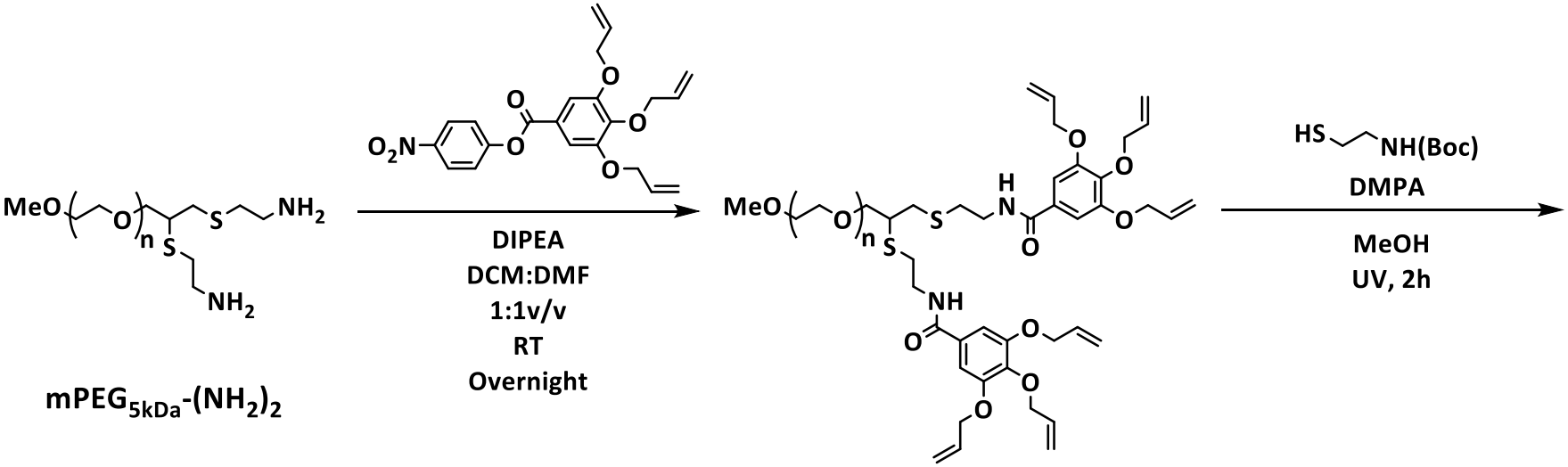
AB_4

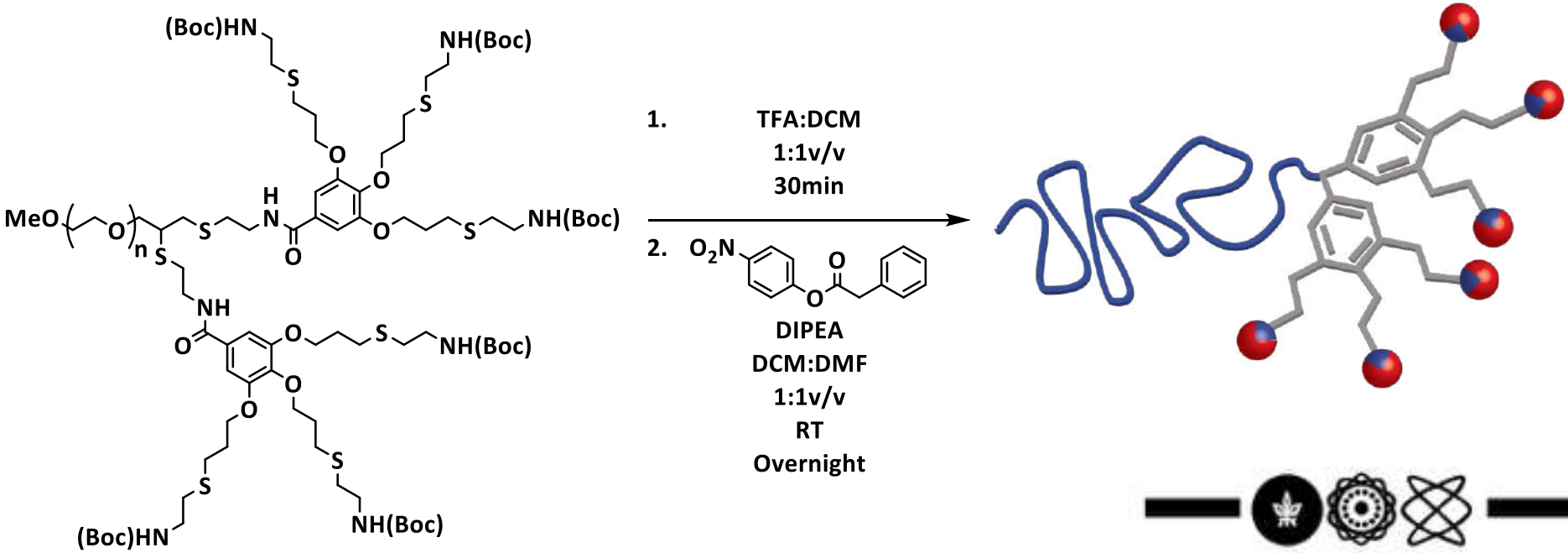


AB_6

Harnoy et al. Biomacromolecules **2017**

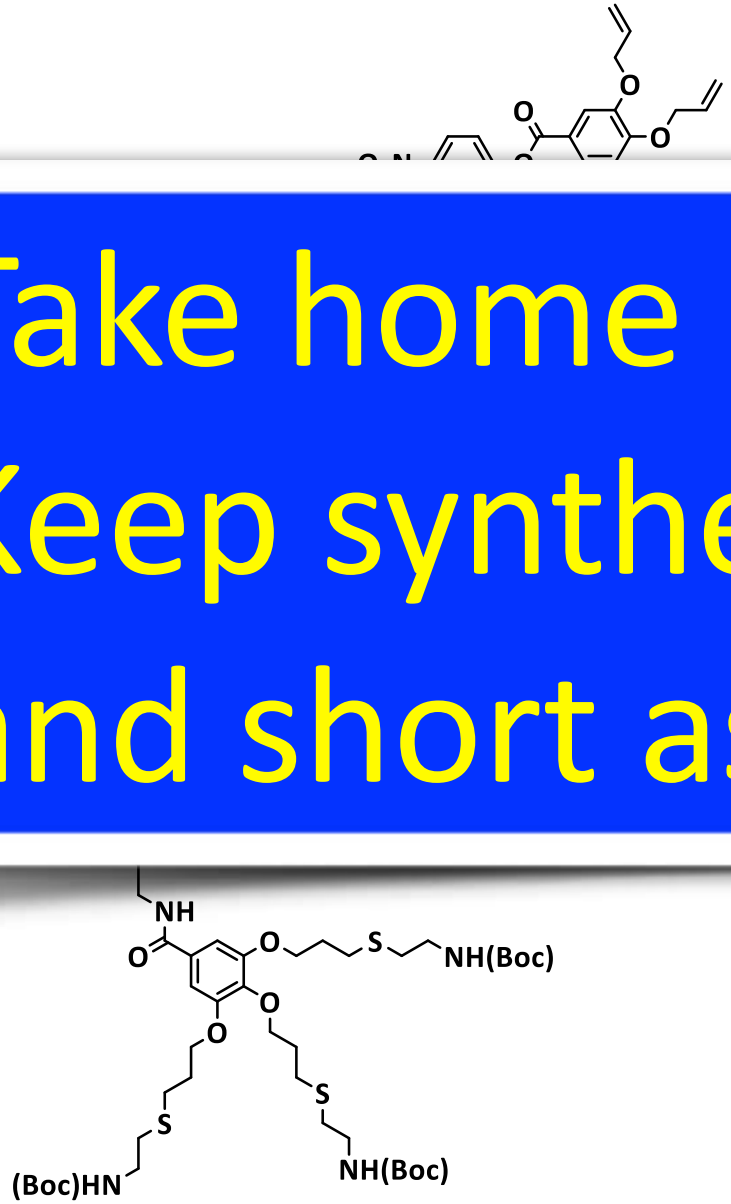
Simple synthesis with overall quantitative yields





Simple synthesis with overall quantitative yields

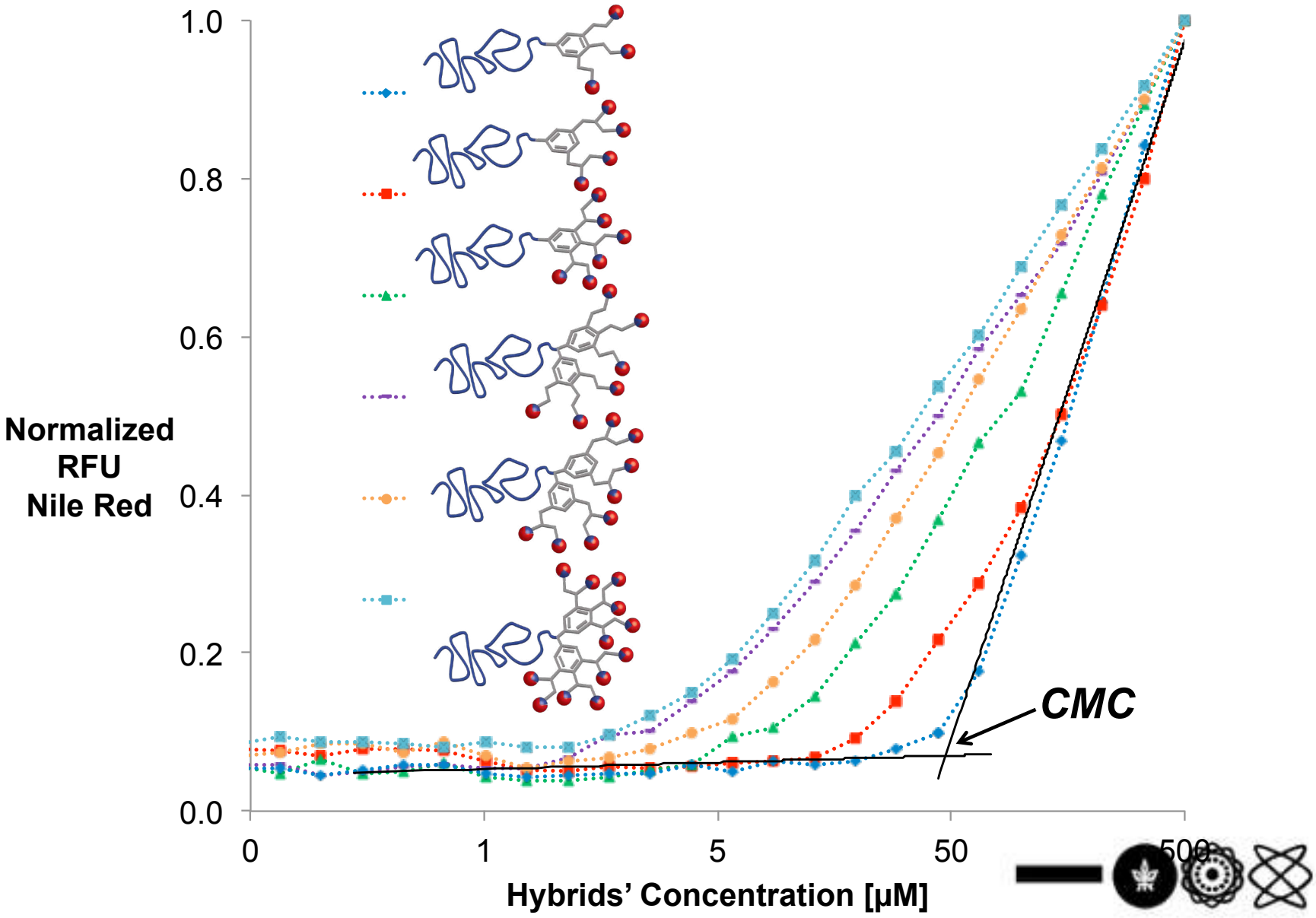
TAU



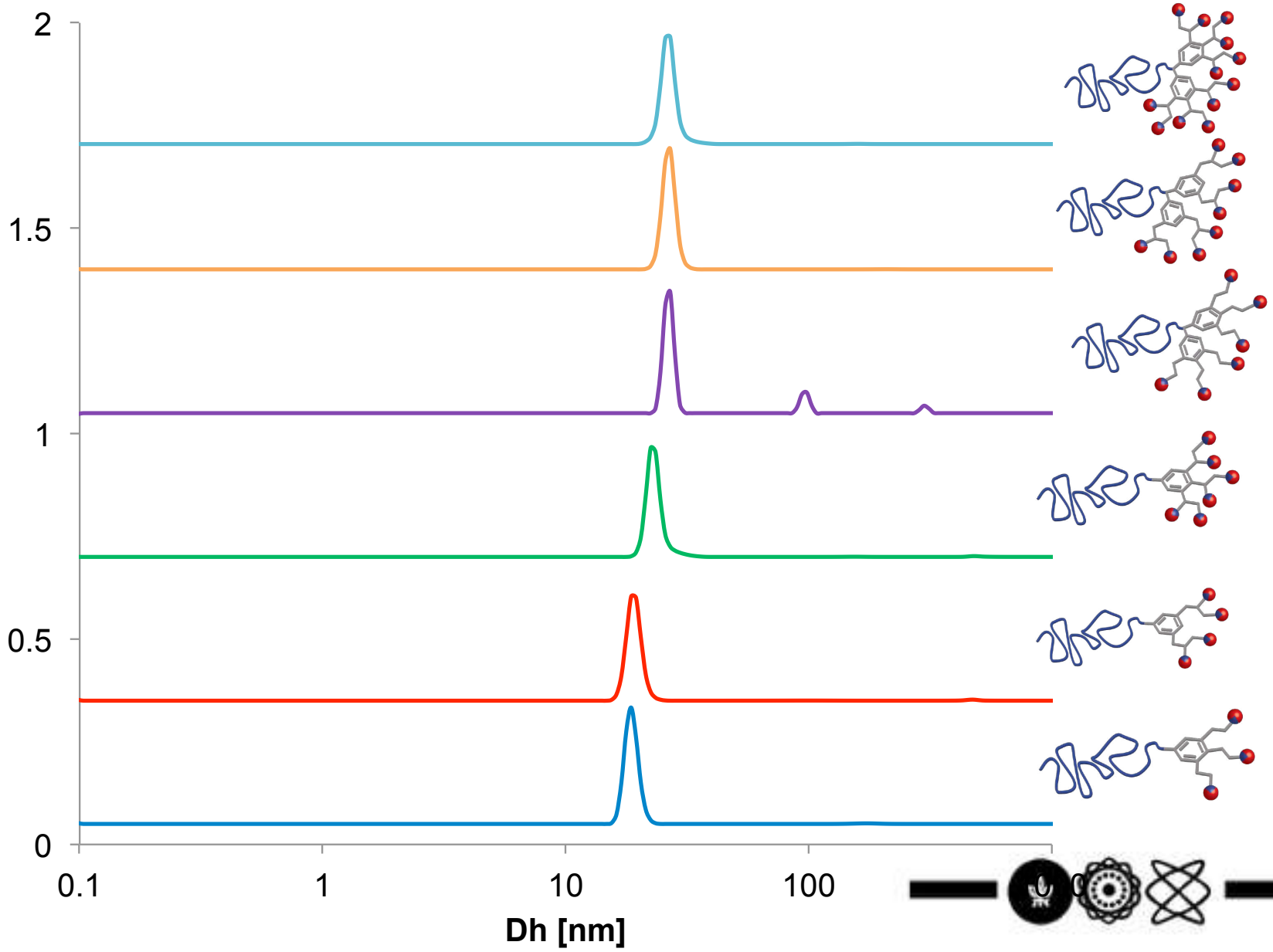
Take home message:

Keep synthesis as simple and short as possible

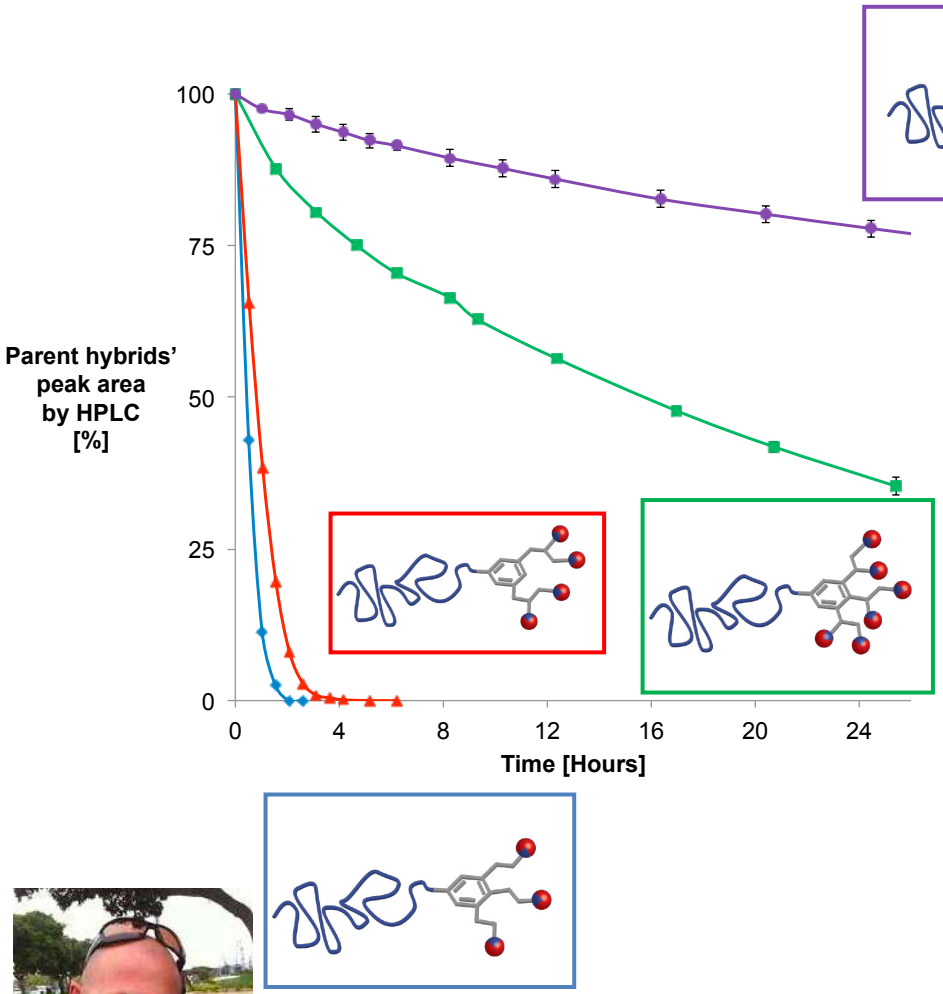
Increasing the hydrophobicity lower the CMC

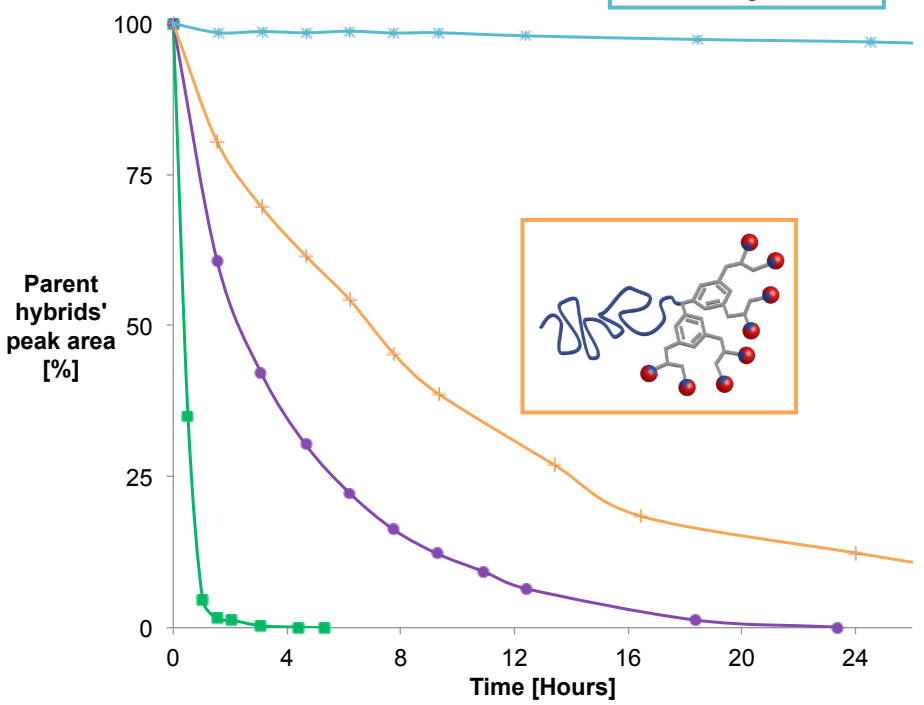


(Almost) All hybrids self-assemble into micelles



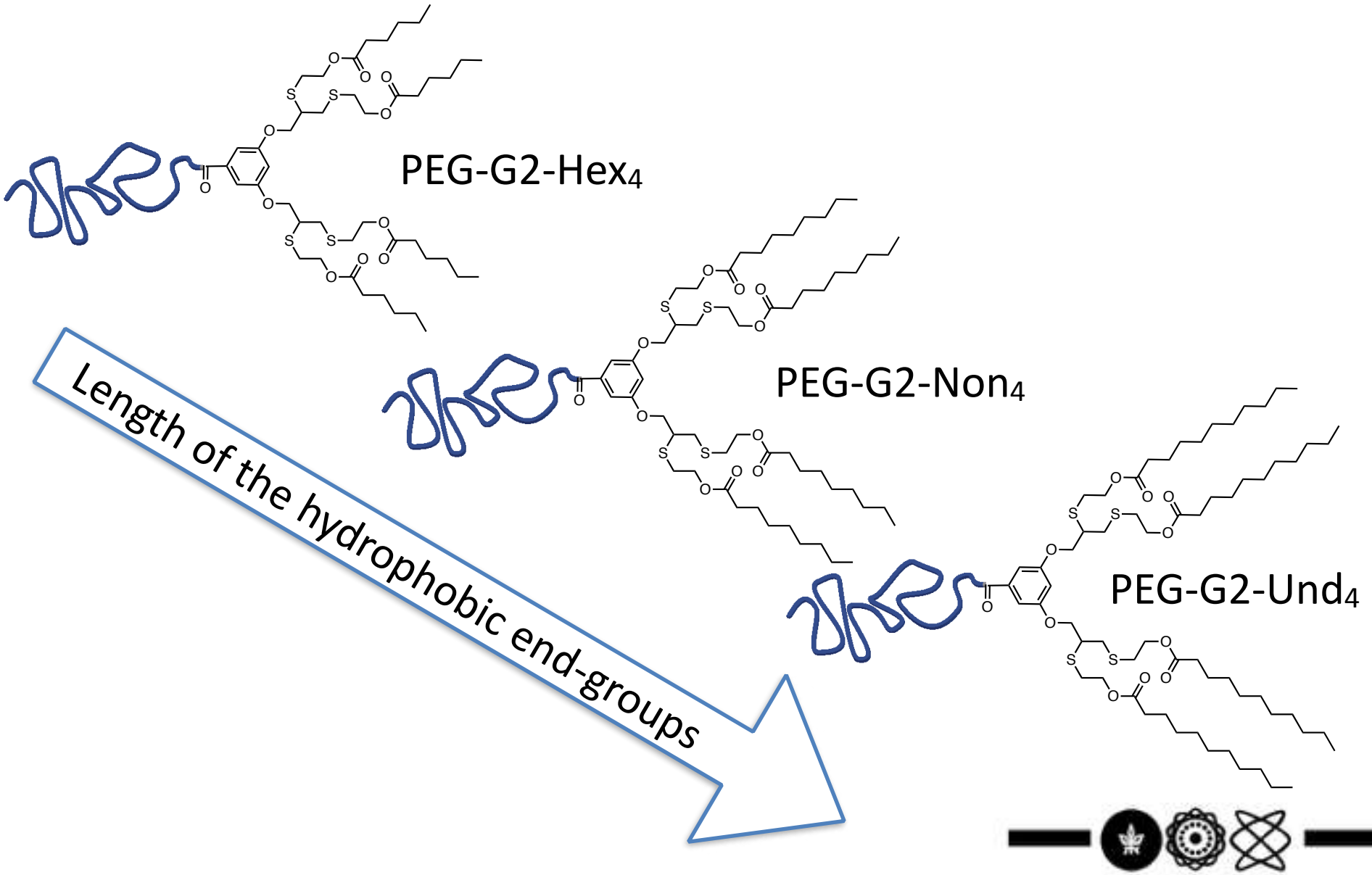
Adjusting the number of end-groups tunes the degradation rate



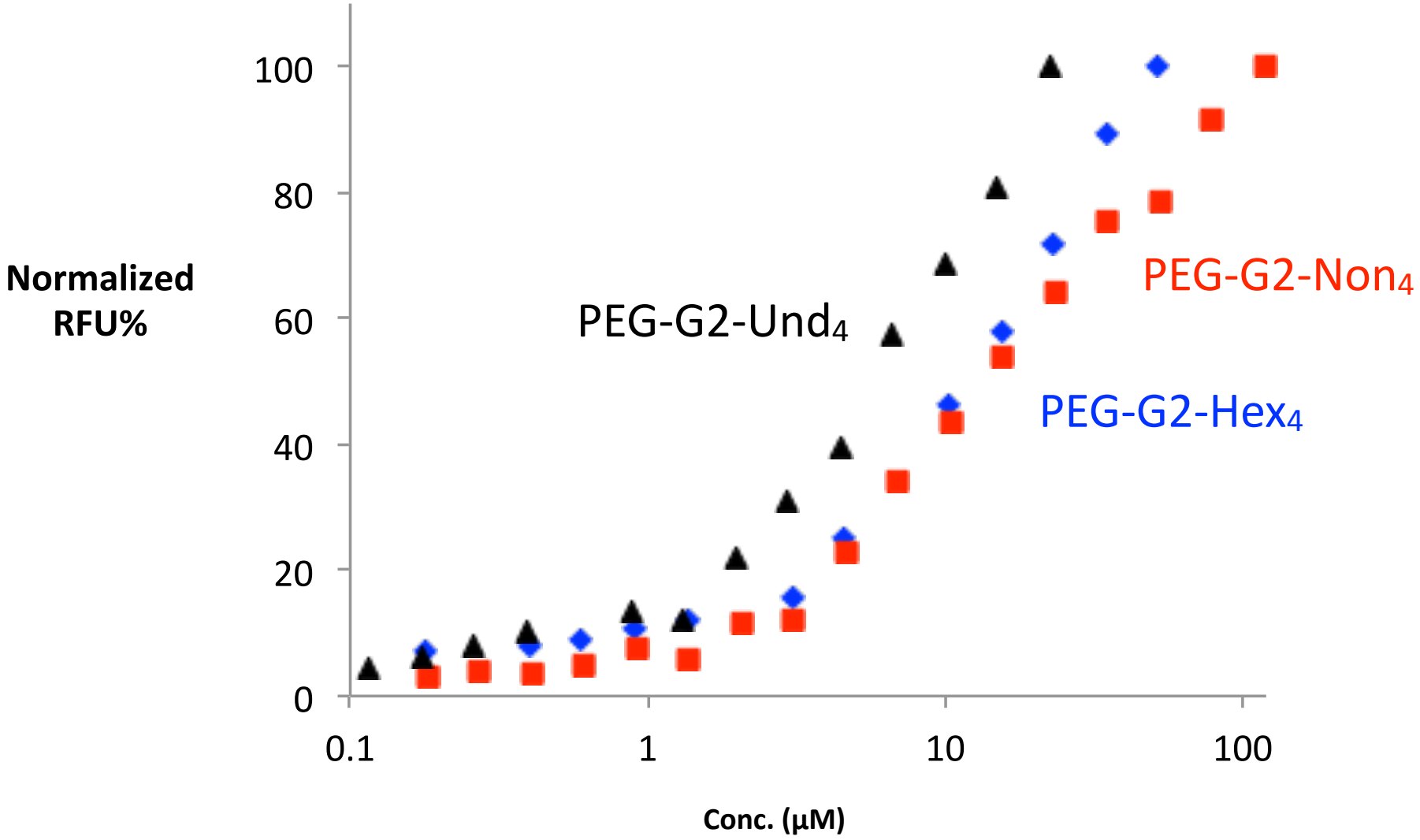


Asi Harnoy et al. Biomacromolecules **2017**

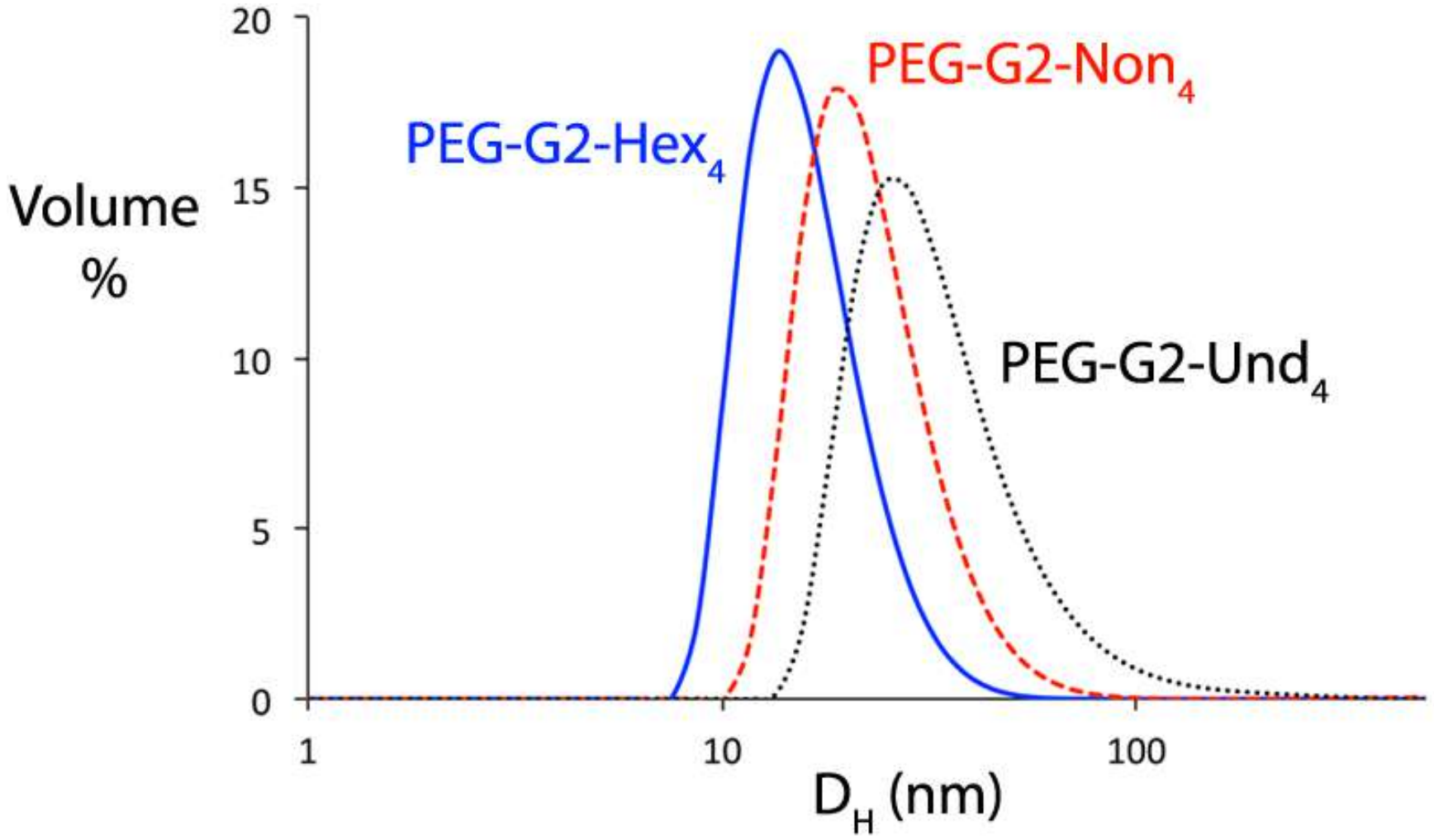
What about changing the length of the hydrophobic end-groups?



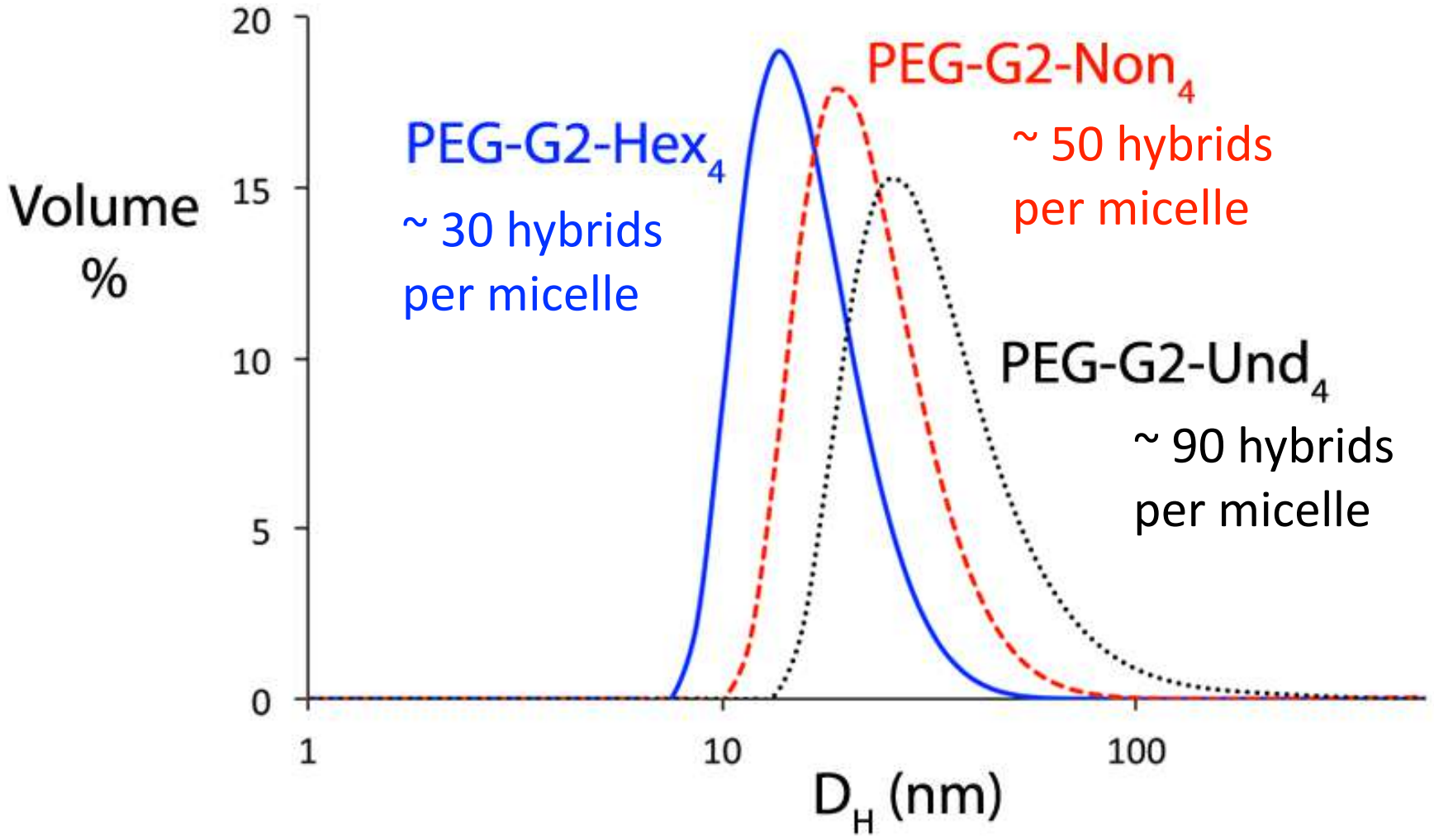
All three hybrids show similar CMC values



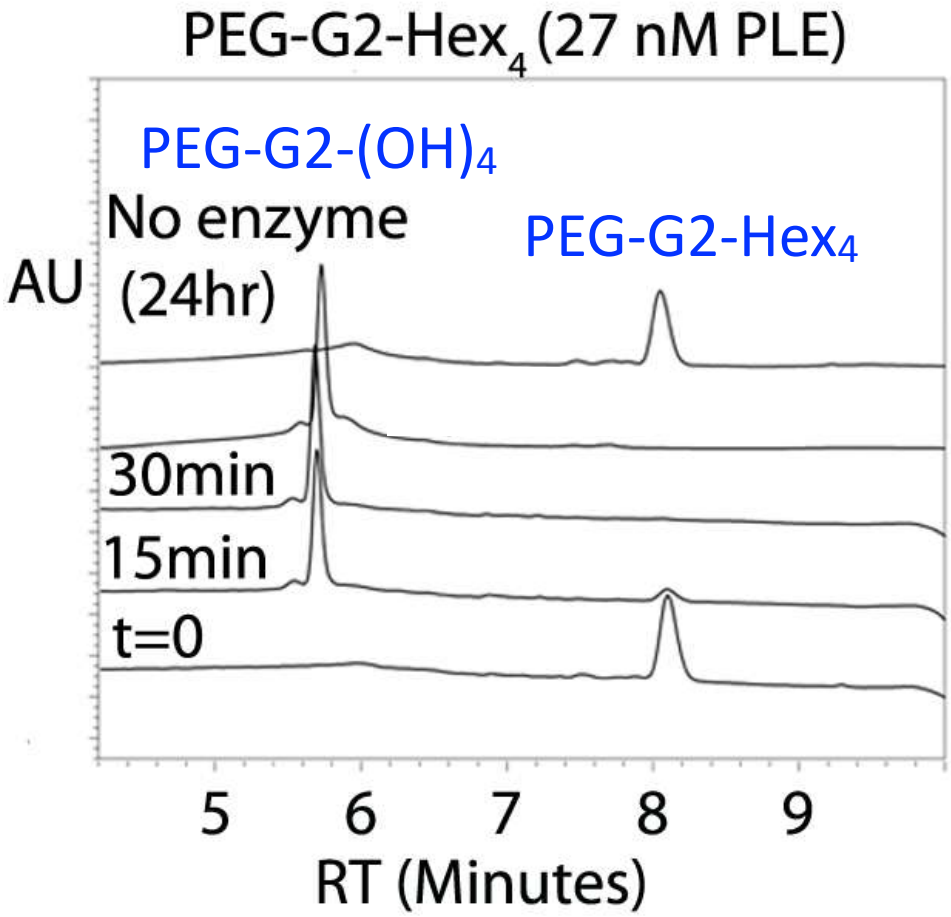
DLS shows big differences in the sizes of the assembled micelles

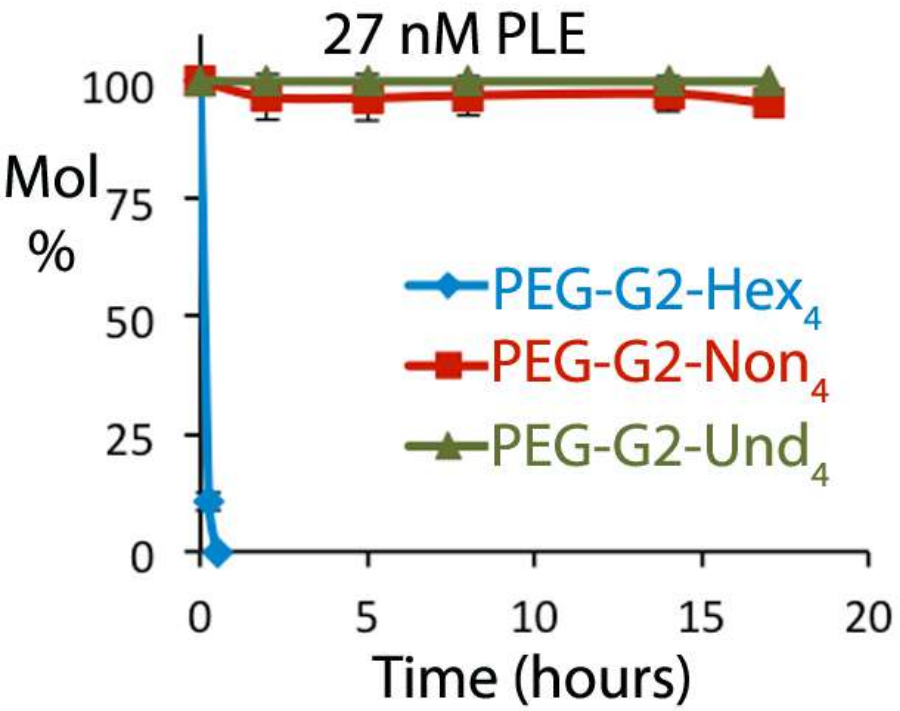


SAXS was used to estimate the aggregation numbers

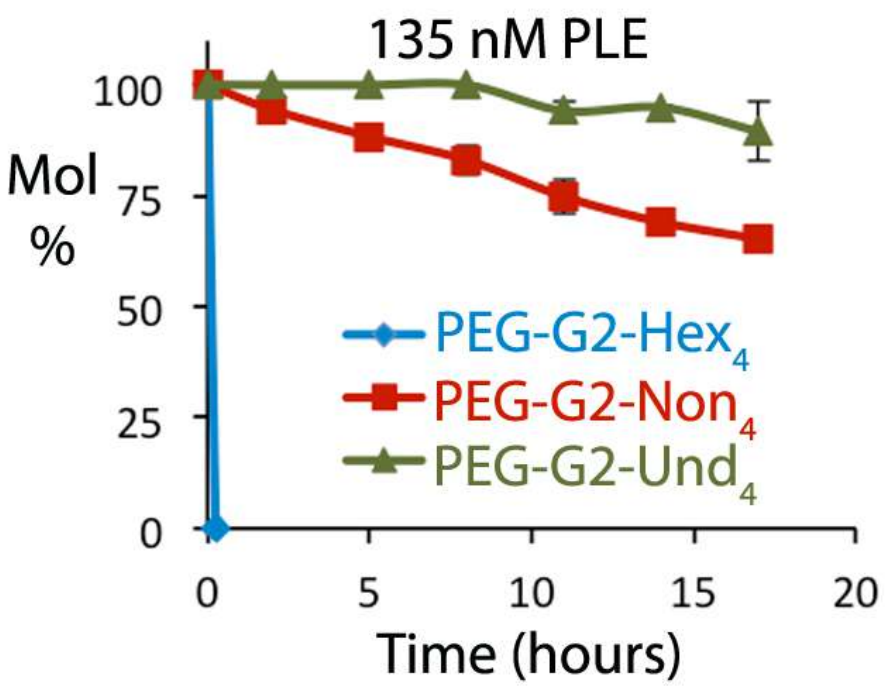


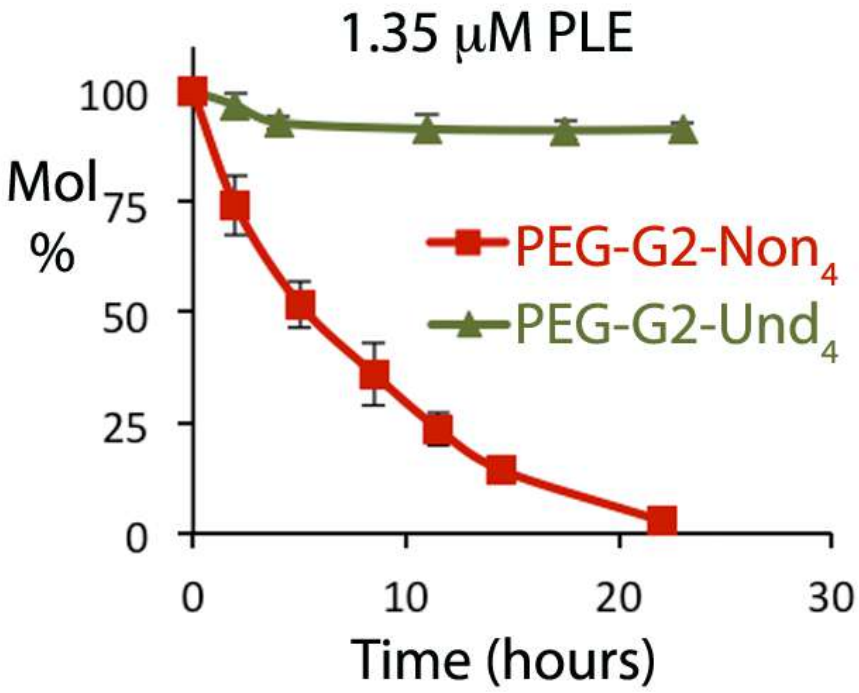
Adjusting the number of end-groups tunes the degradation rate





Adjusting the number of end-groups tunes the degradation rate



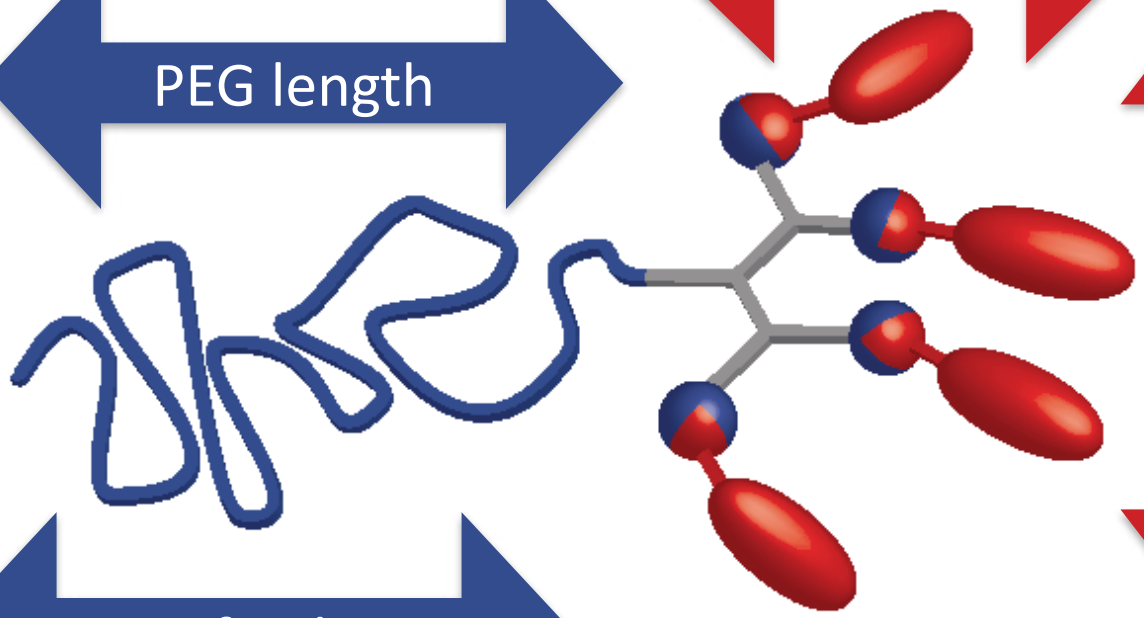


Tuning amphiphilicity with high molecular precision

Harnoy et al. *JACS* **2014**

Hydrophobicity

PEG length



No. and type of end-groups

Rosenbaum et al. *JACS* **2015**

Harnoy & Slor et al. *OBC* **2016**

Harnoy et al. Biomacromolecules **2017**

Segal et al. *JACS* **2017**

Harnoy et al. Synlett **2018**

Type of polymer

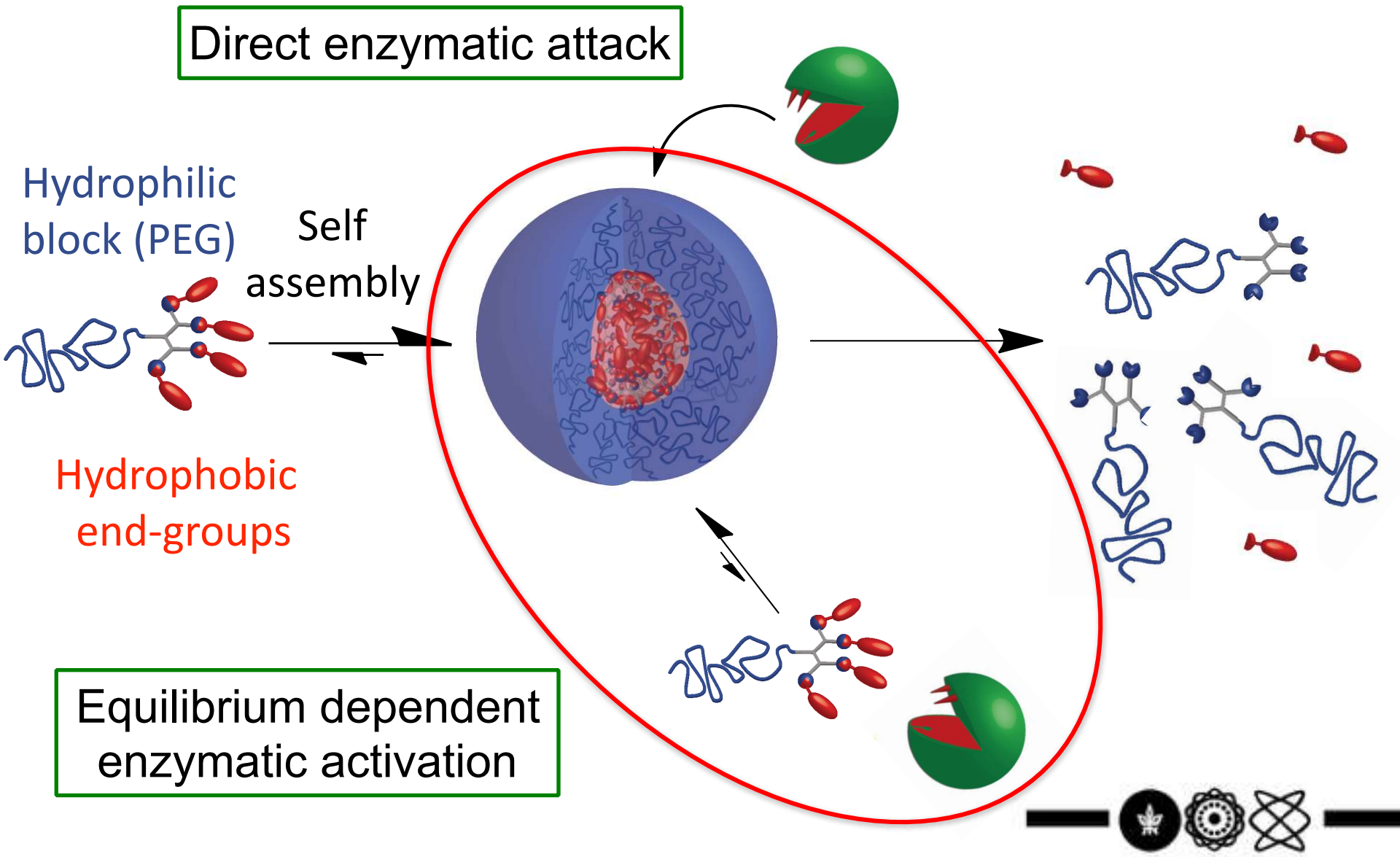
Slor et al. *In preparation*

Slor et al. *ChemComm* **2018**

Overall molecular weight

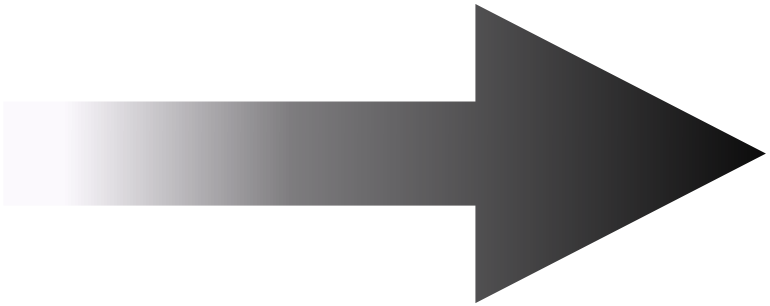
Rosenbaum et al. *Biomacromolecules* **2017**

The kinetic trends point to an equilibrium based mechanism

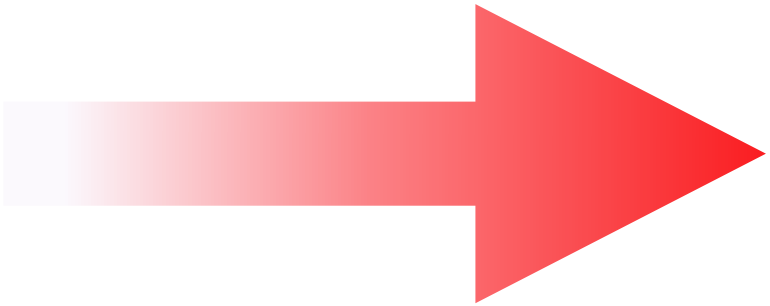


How sensitive is the enzymatic responsiveness?

Micellar stability

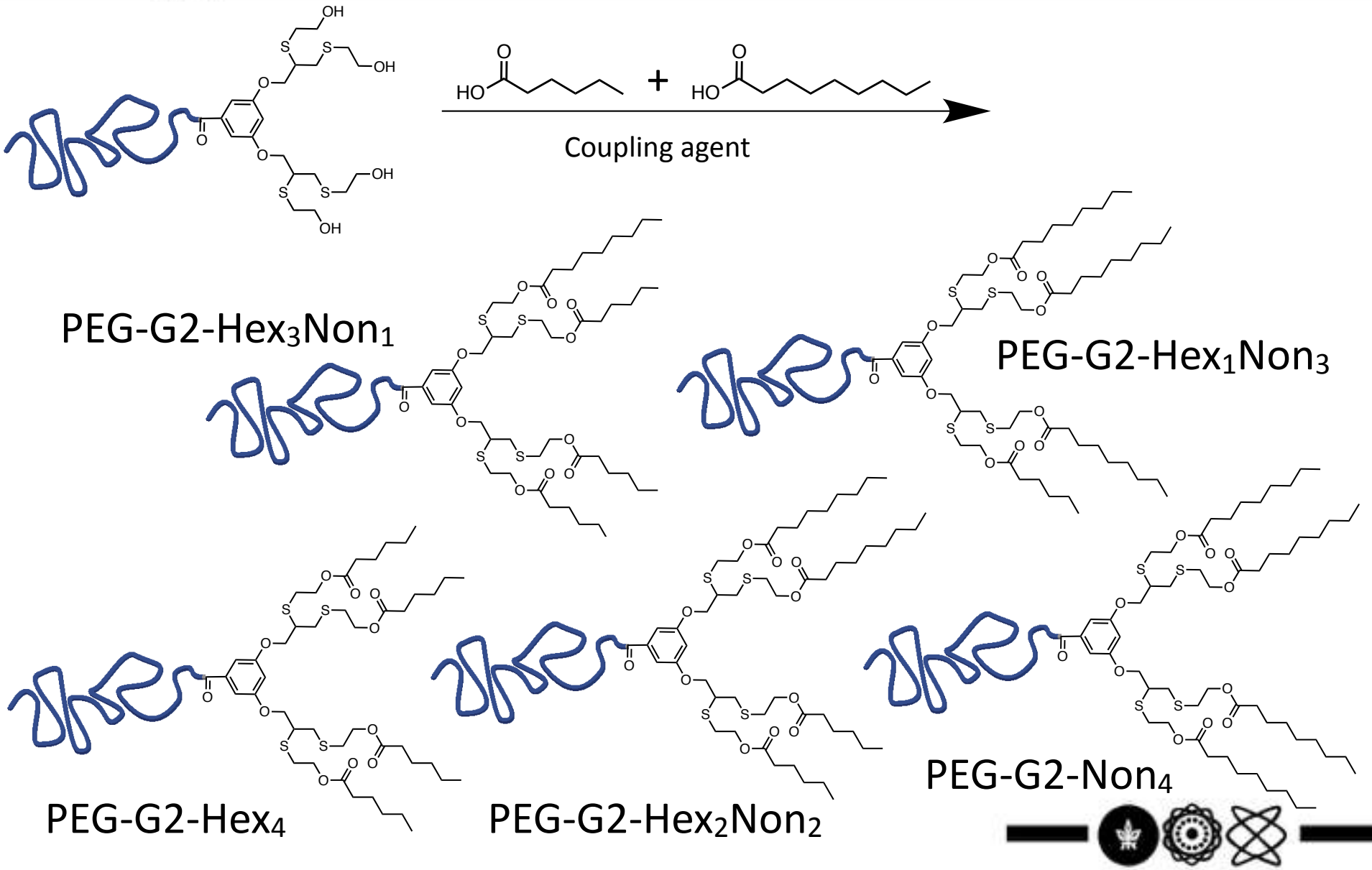


Hydrophobicity (and/or MW)

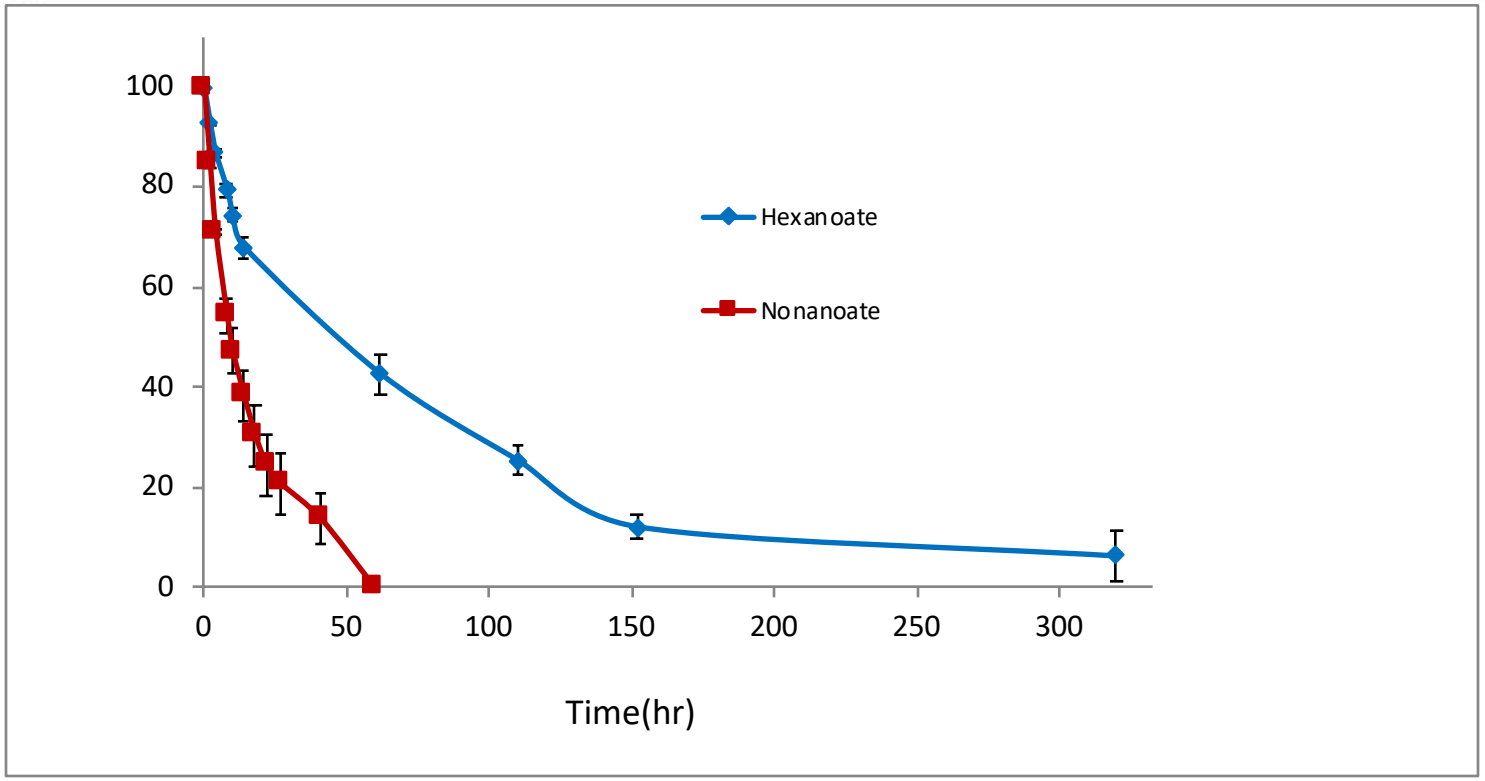


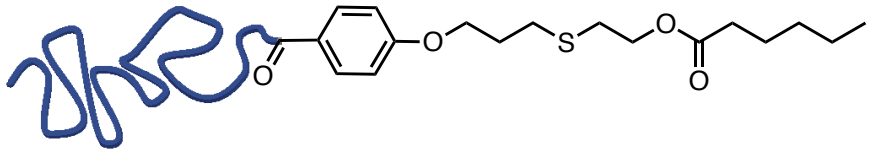
Enzymatic responsiveness

Mixing two end-groups yields a mixture of mixed hybrids

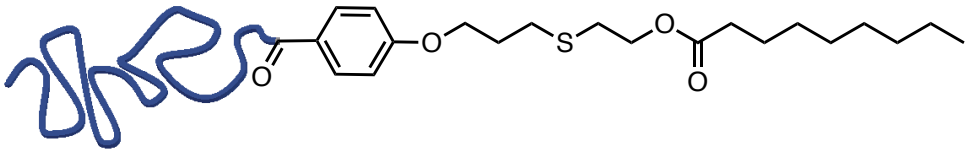


Checking substrate specificity below the CMC



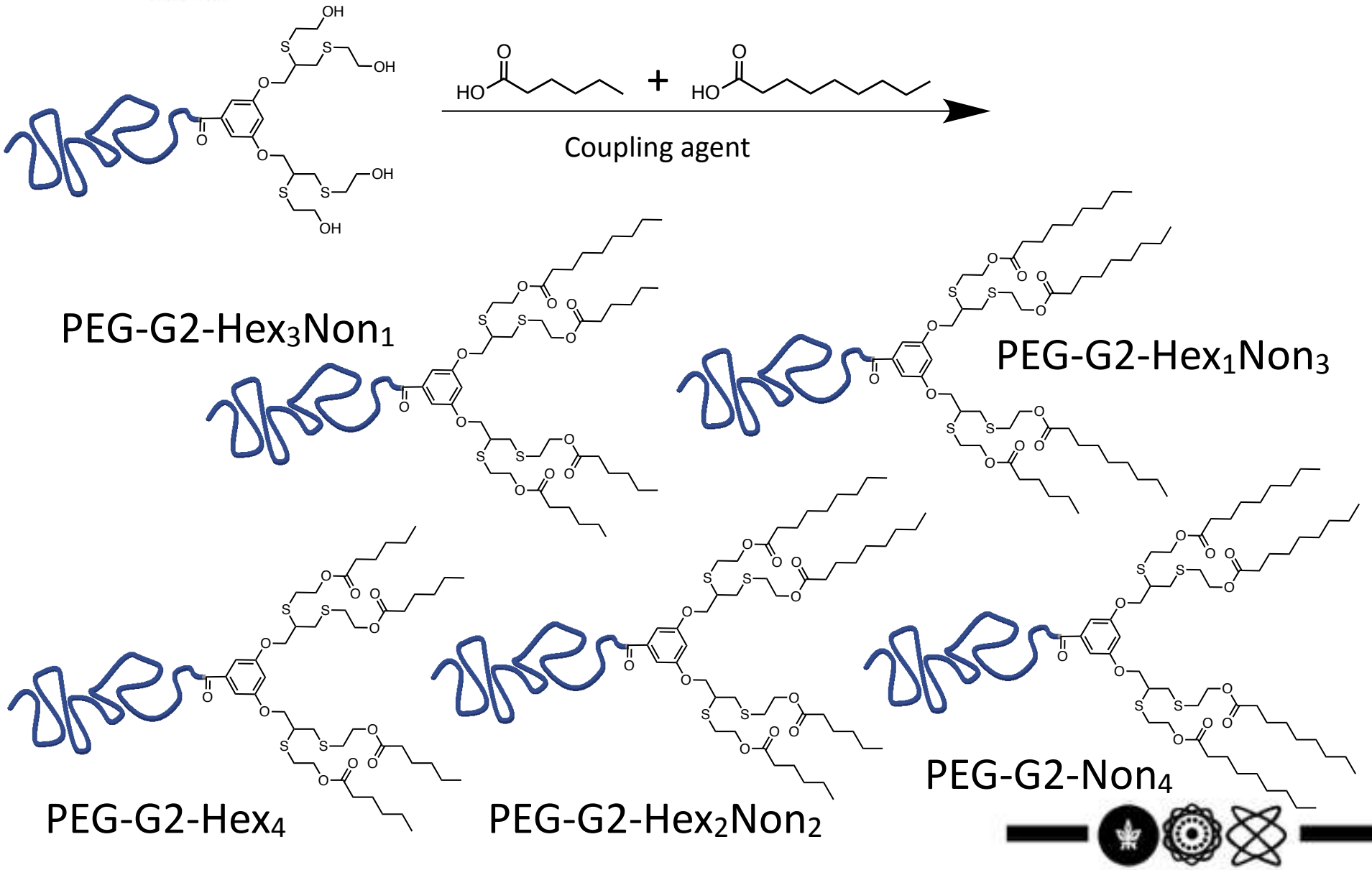


PEG-G0-Hex$_1$

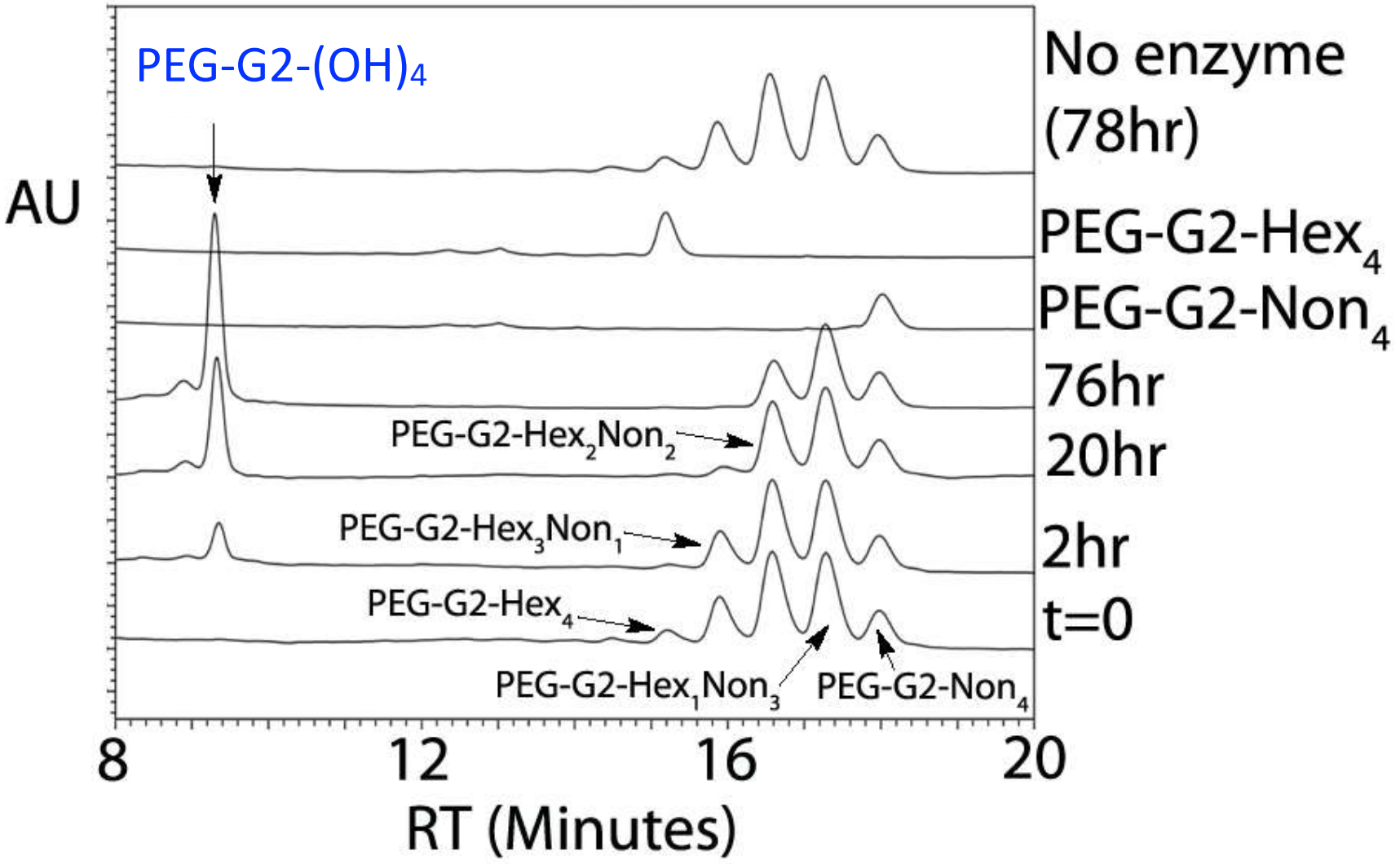


PEG-G0-Non$_1$

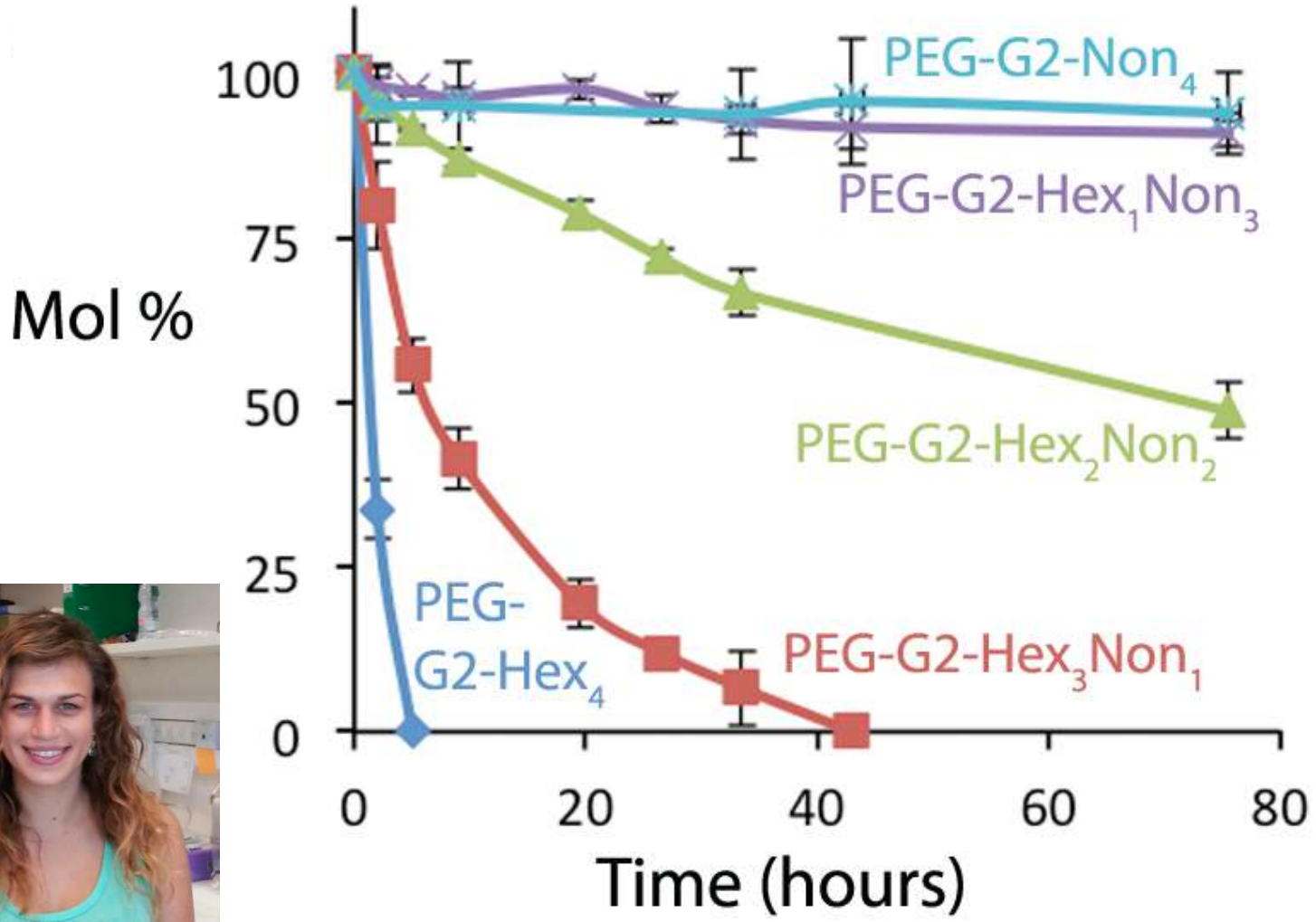
Mixing two end-groups yields a mixture of mixed hybrids



HPLC enables tracking of each hybrid in the mixture



The difference between the hybrids are only 3 CH_2 units!!!



Merav Segal et al. *JACS* **2017**

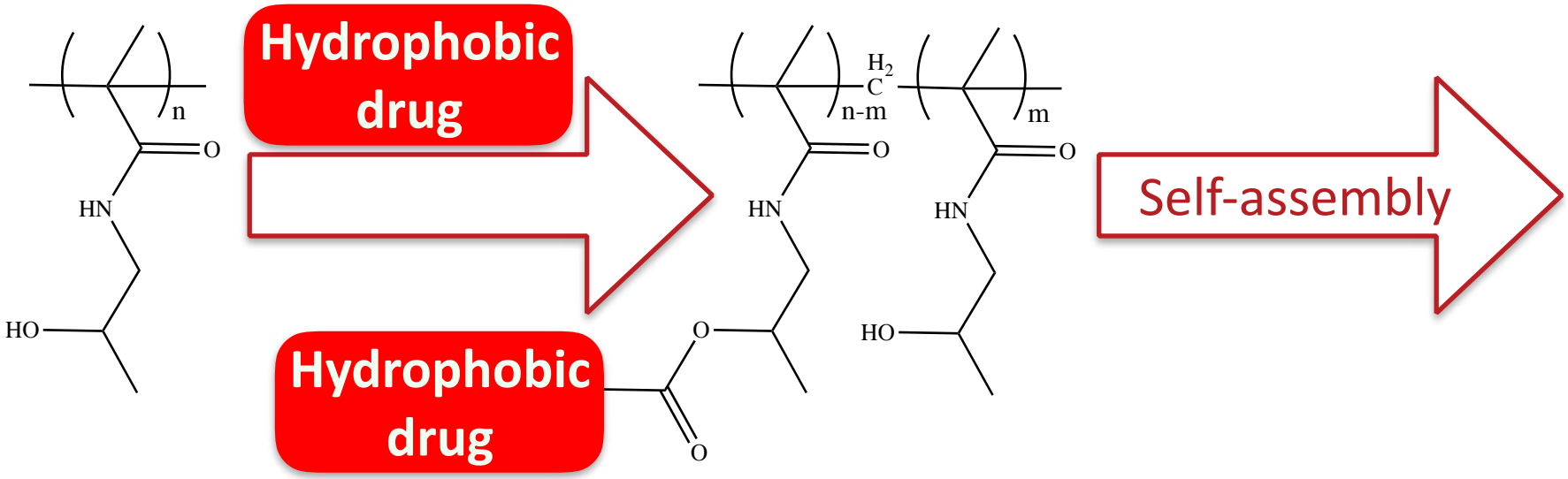
The difference between the hybrids are only 3 CH_2 units!!!

Take home message:
Small changes in the hydrophobic block can make a big difference

Segal et al. *JACS* **2017**

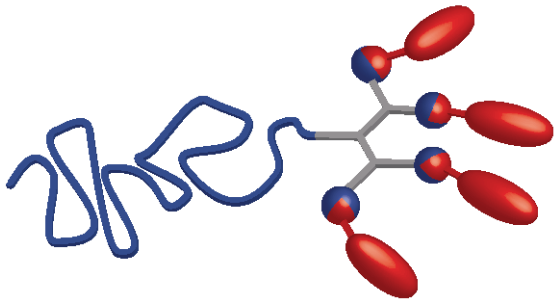
The increased hydrophobicity may be the reason for poor drug release

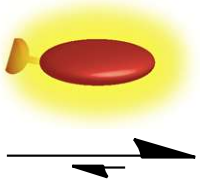
Polymers loaded with “too many” drugs might become not accessible to the releasing enzyme

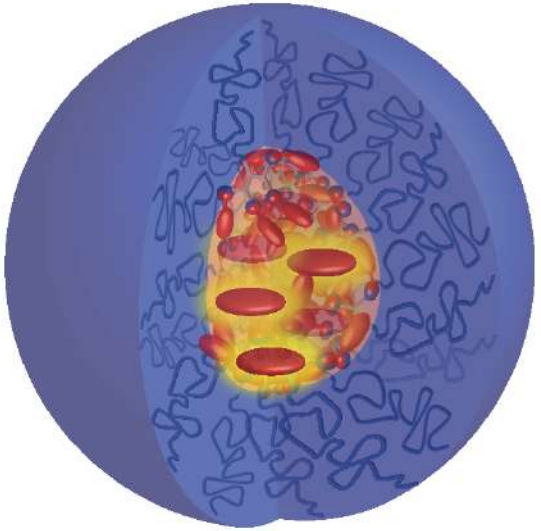


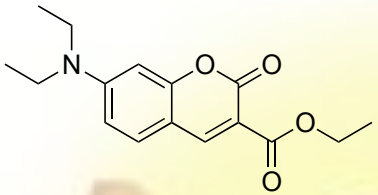
Random number and position of the loaded drugs

How would the loading and release rates be affected by guest hydrophobicity?

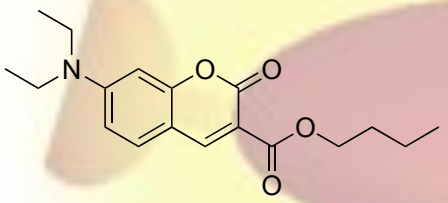




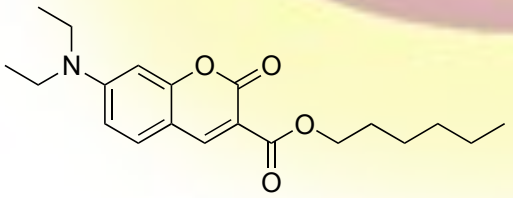




Ethyl coumarin

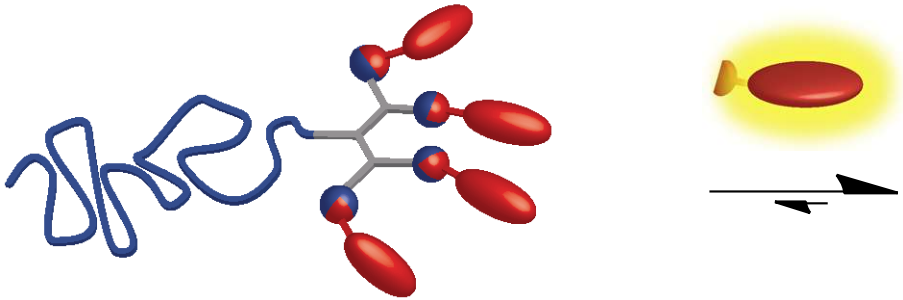


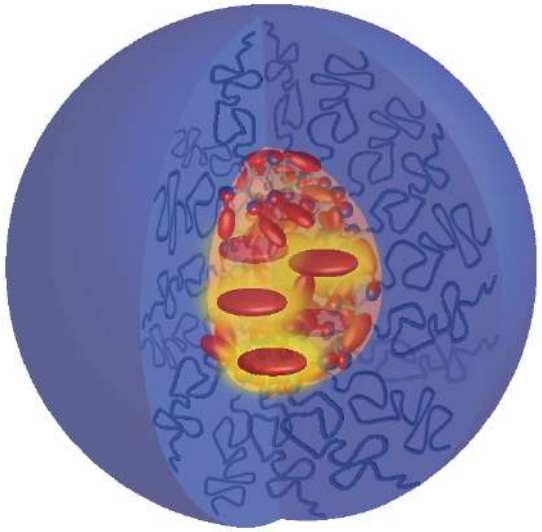
Butyl coumarin

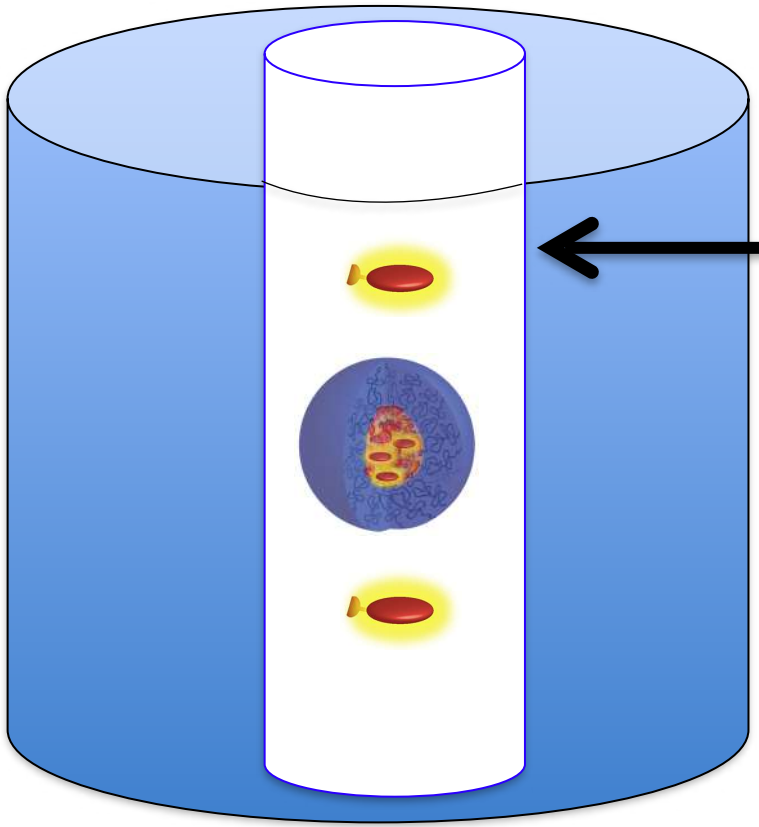


Hexyl coumarin

A dialysis setup was used to study loading and release

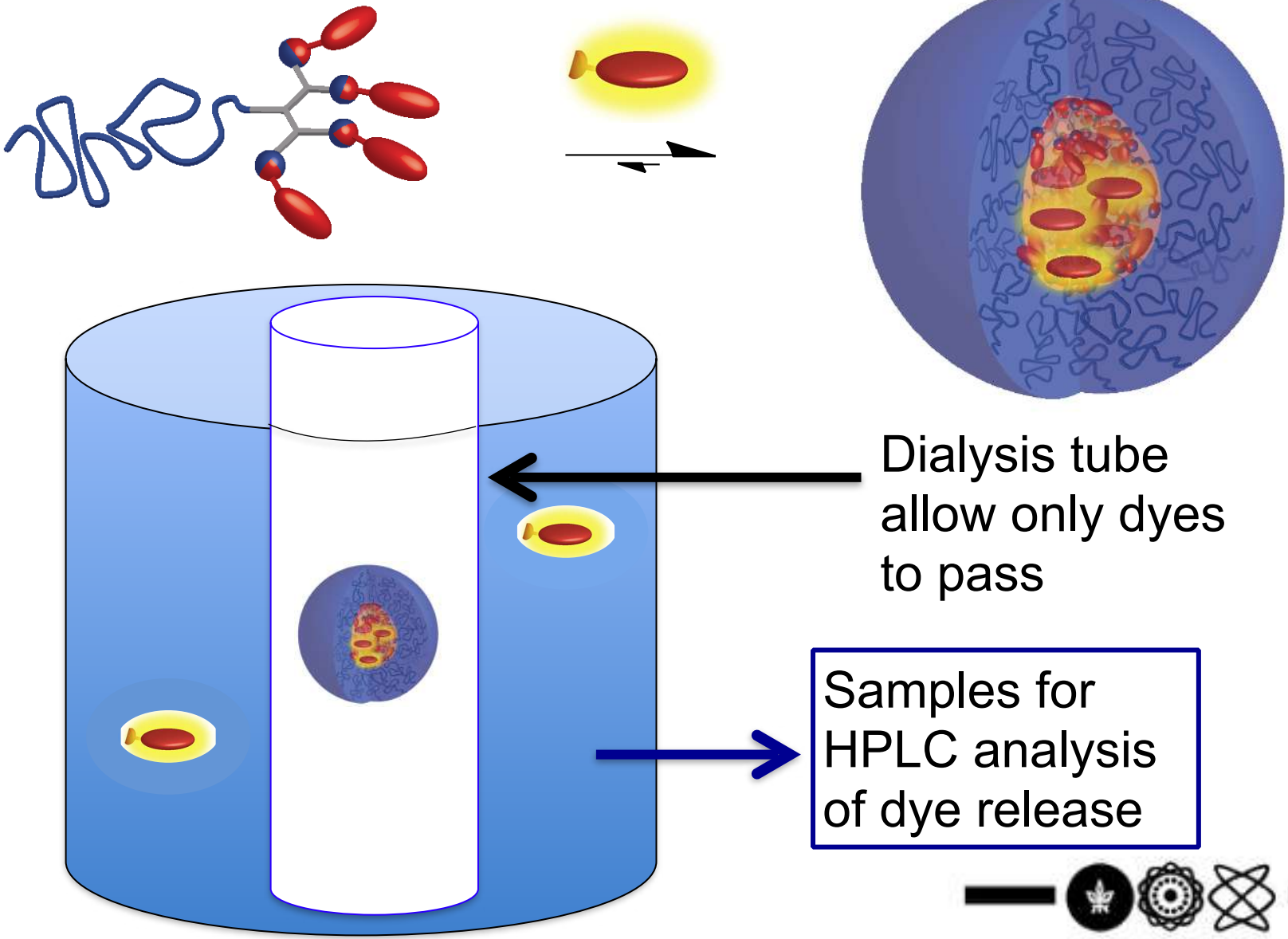




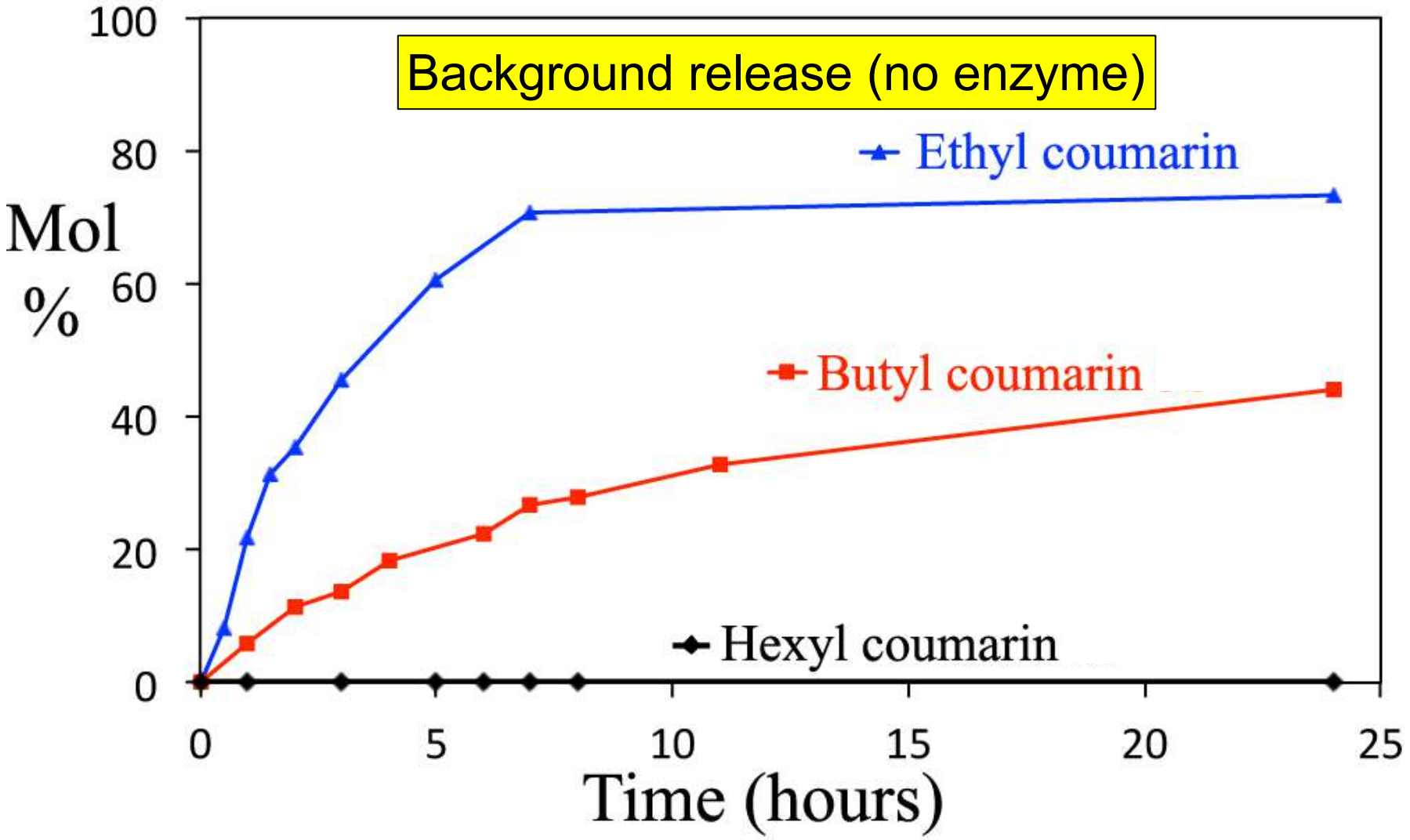


Dialysis tube allow only dyes to pass

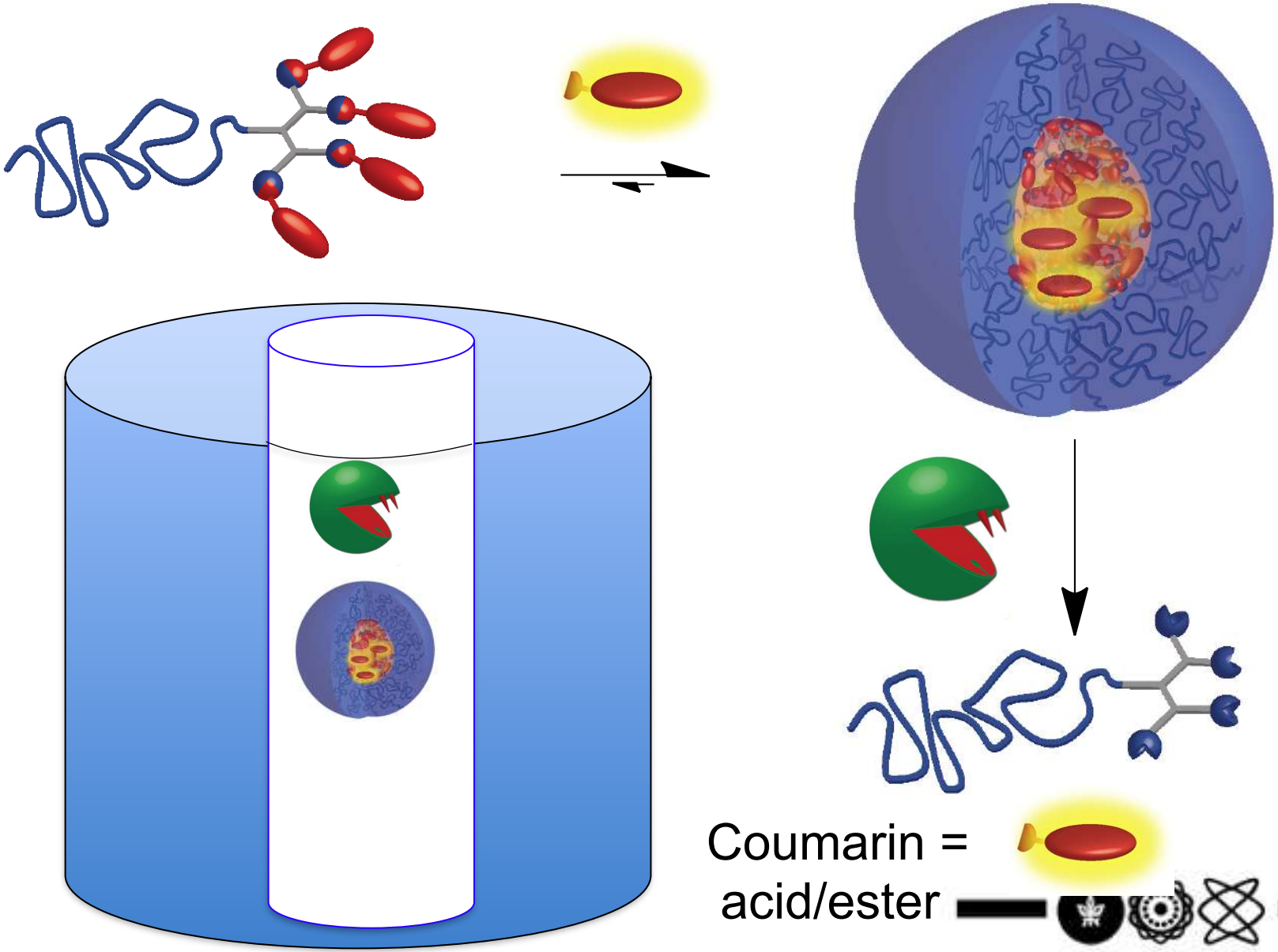
A dialysis setup was used to study loading and release



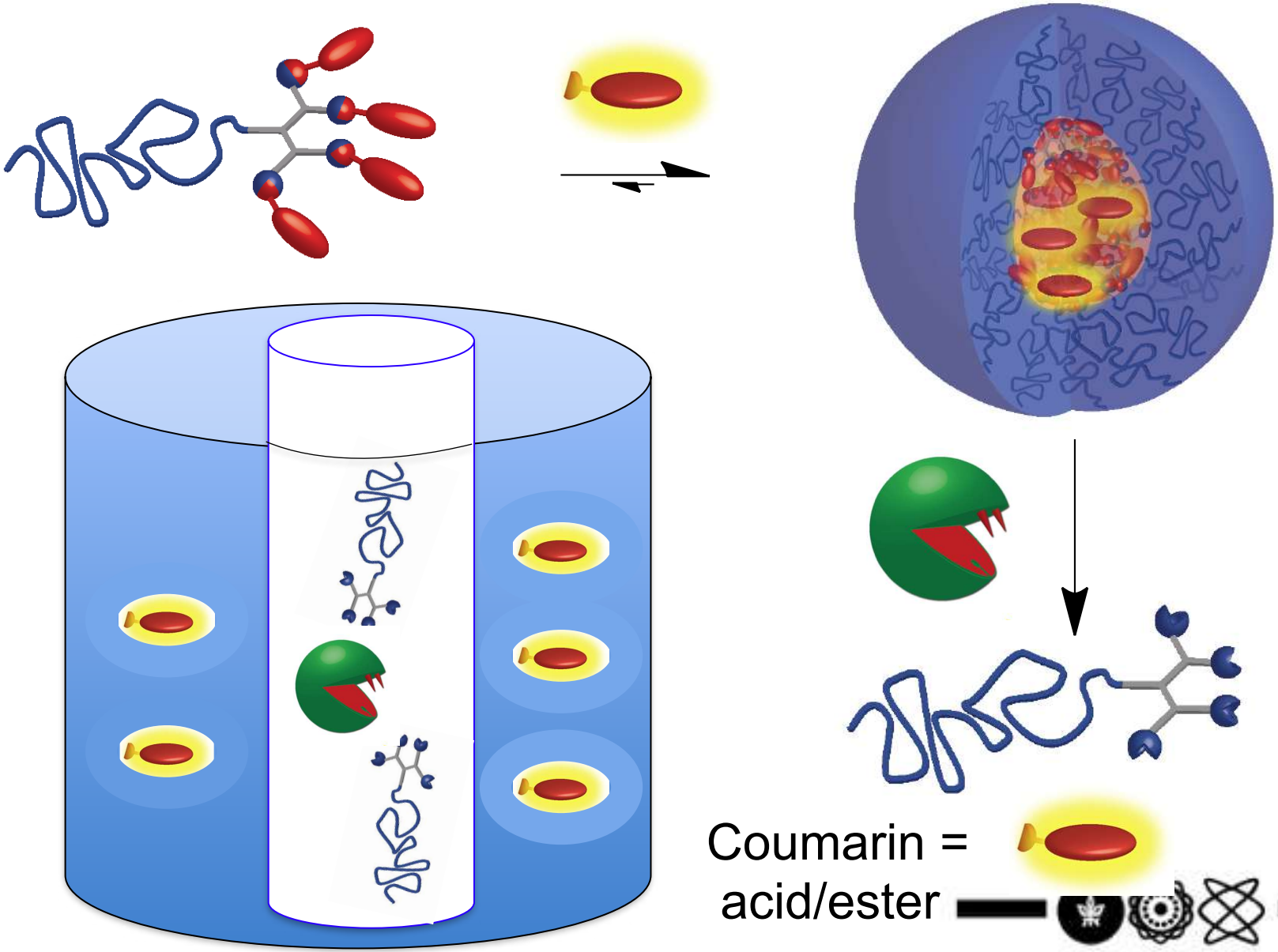
Loading capacity increases with increase in hydrophobicity



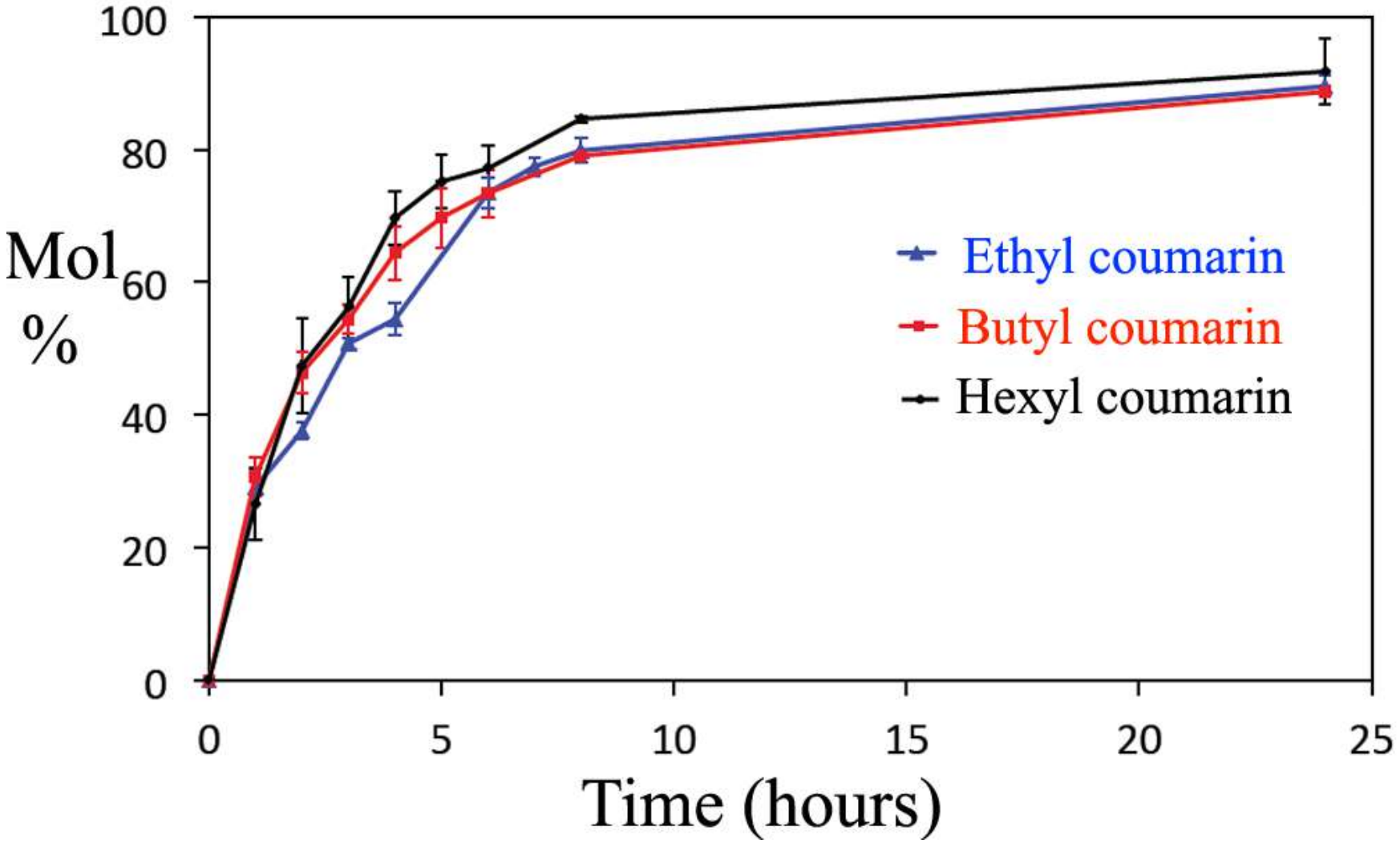
A dialysis setup was used to study loading and release



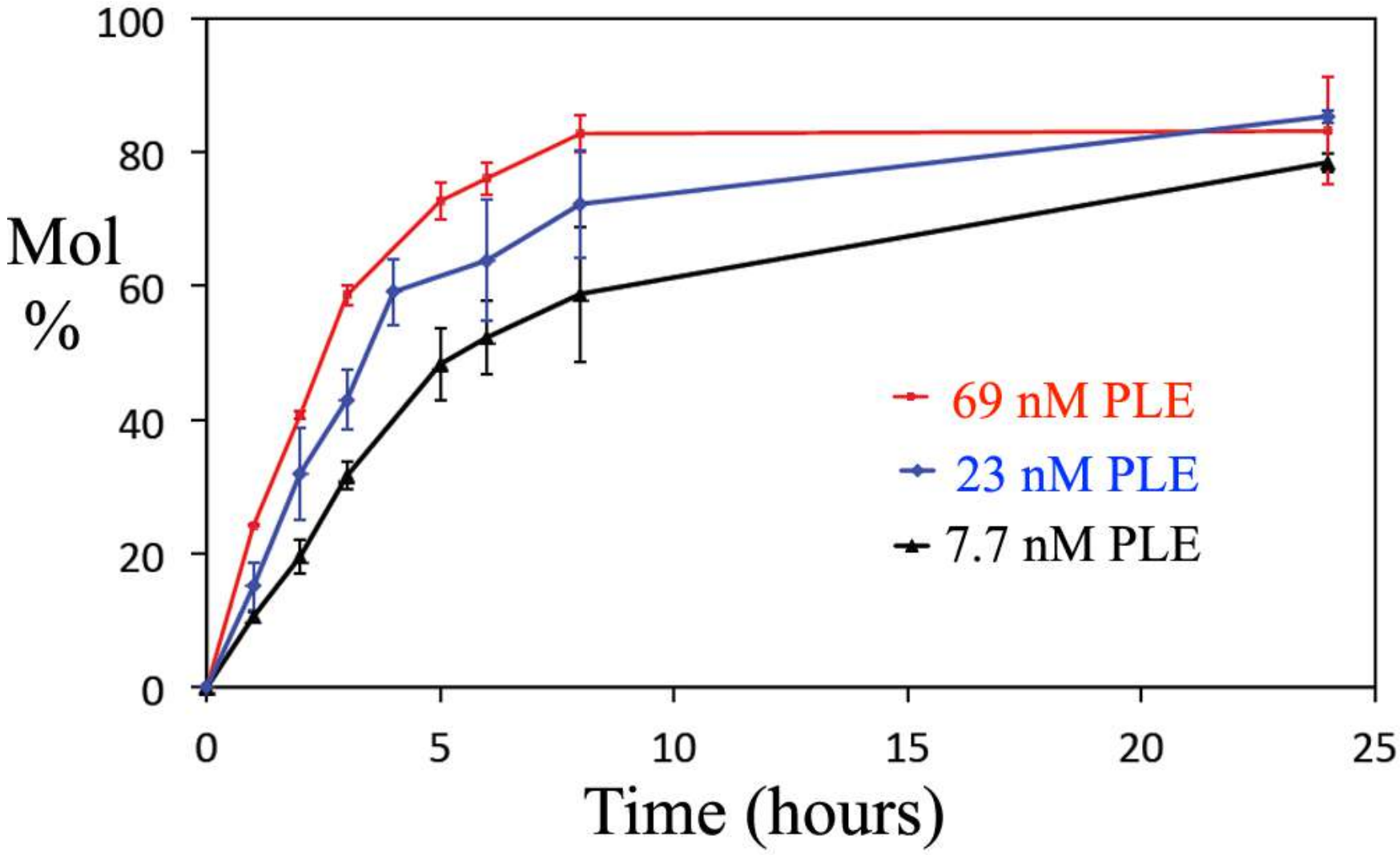
A dialysis setup was used to study loading and release



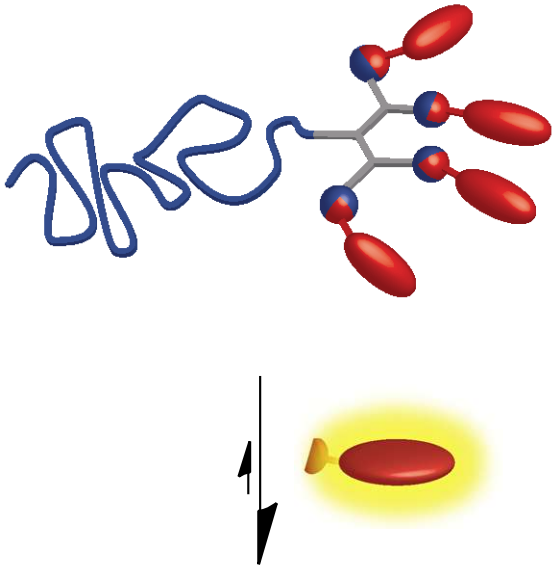
Release rates are not affected by guest's hydrophobicity



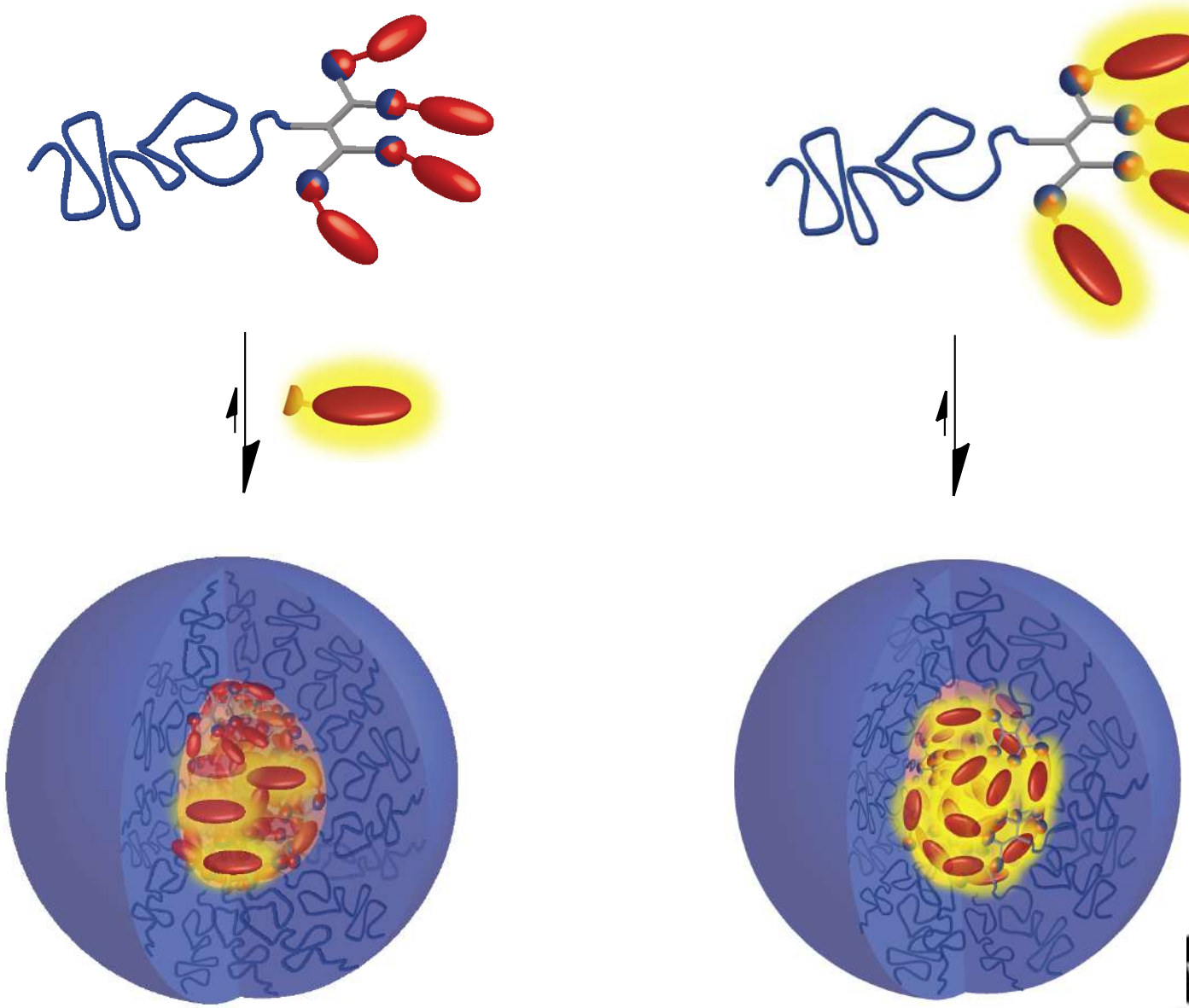
The concentration of the enzyme determines the release rates



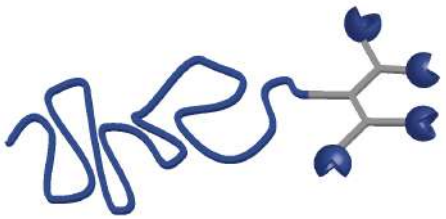
Can we get better control over the amount of loaded cargo molecules?

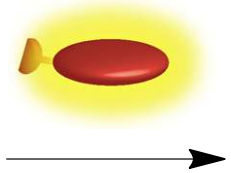


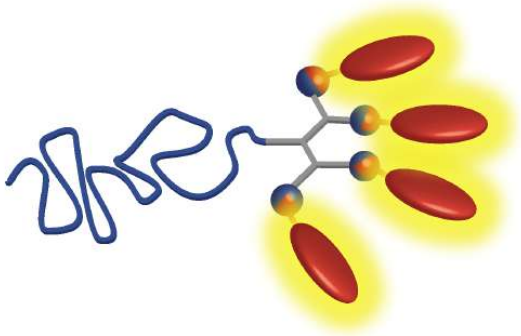
Covalent binding should allow to control the number of cargo molecules

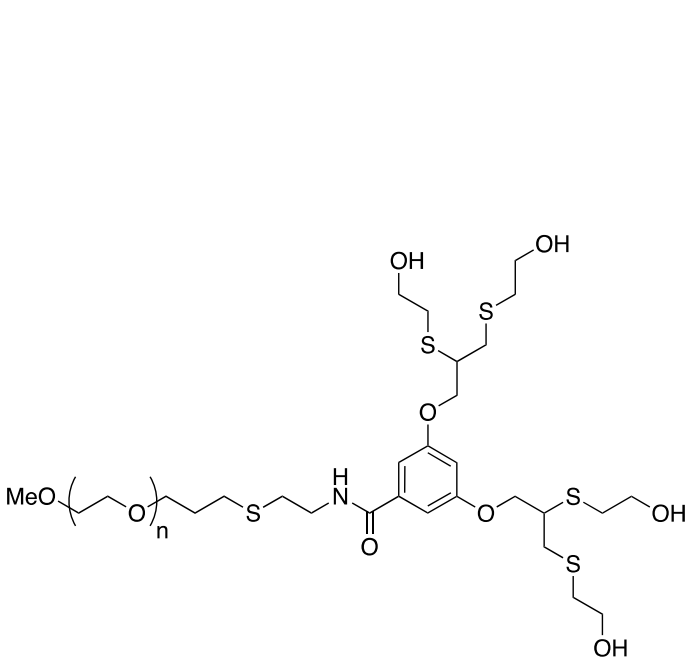


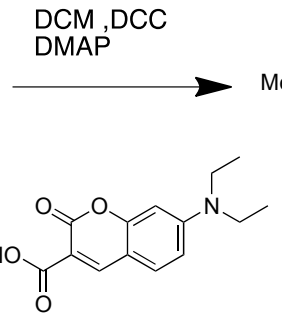
The hybrids can be covalently loaded at the last step of the synthesis

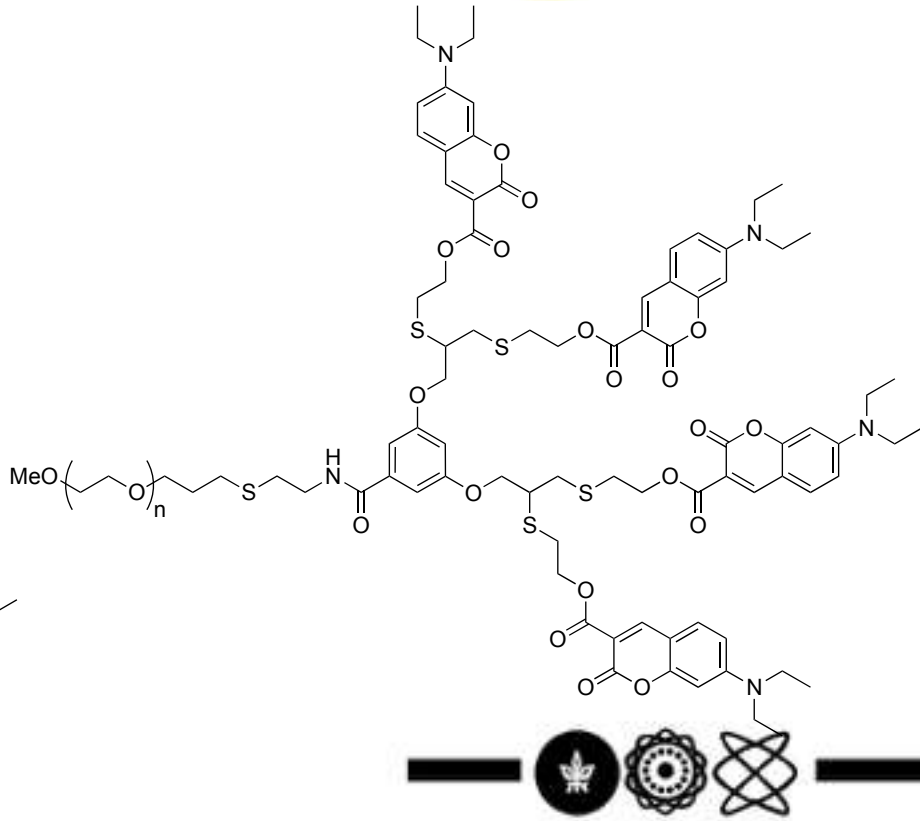




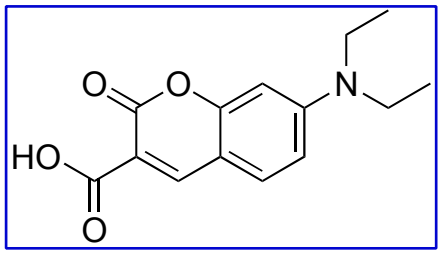


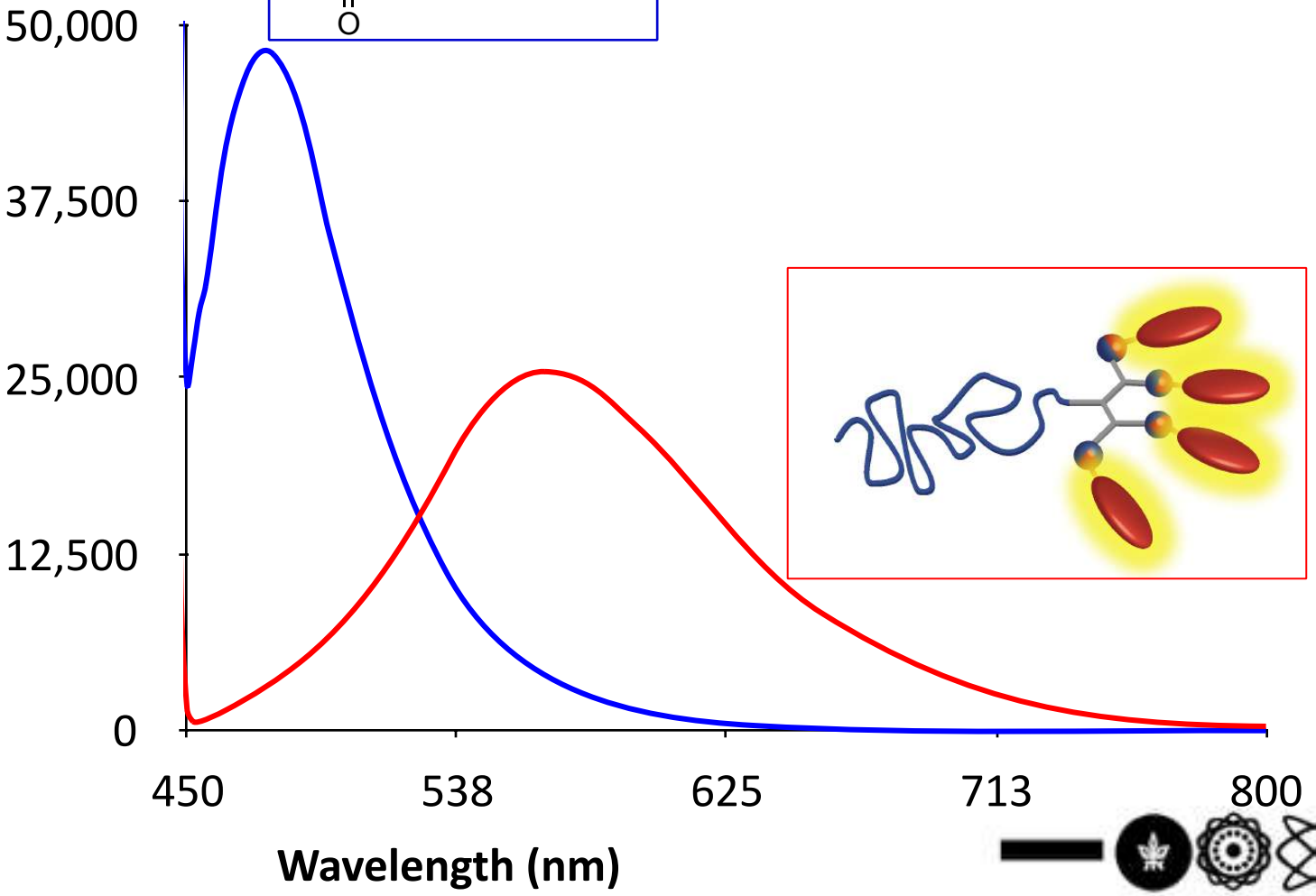






A significant red-shift is observed although the conjugation was not changed





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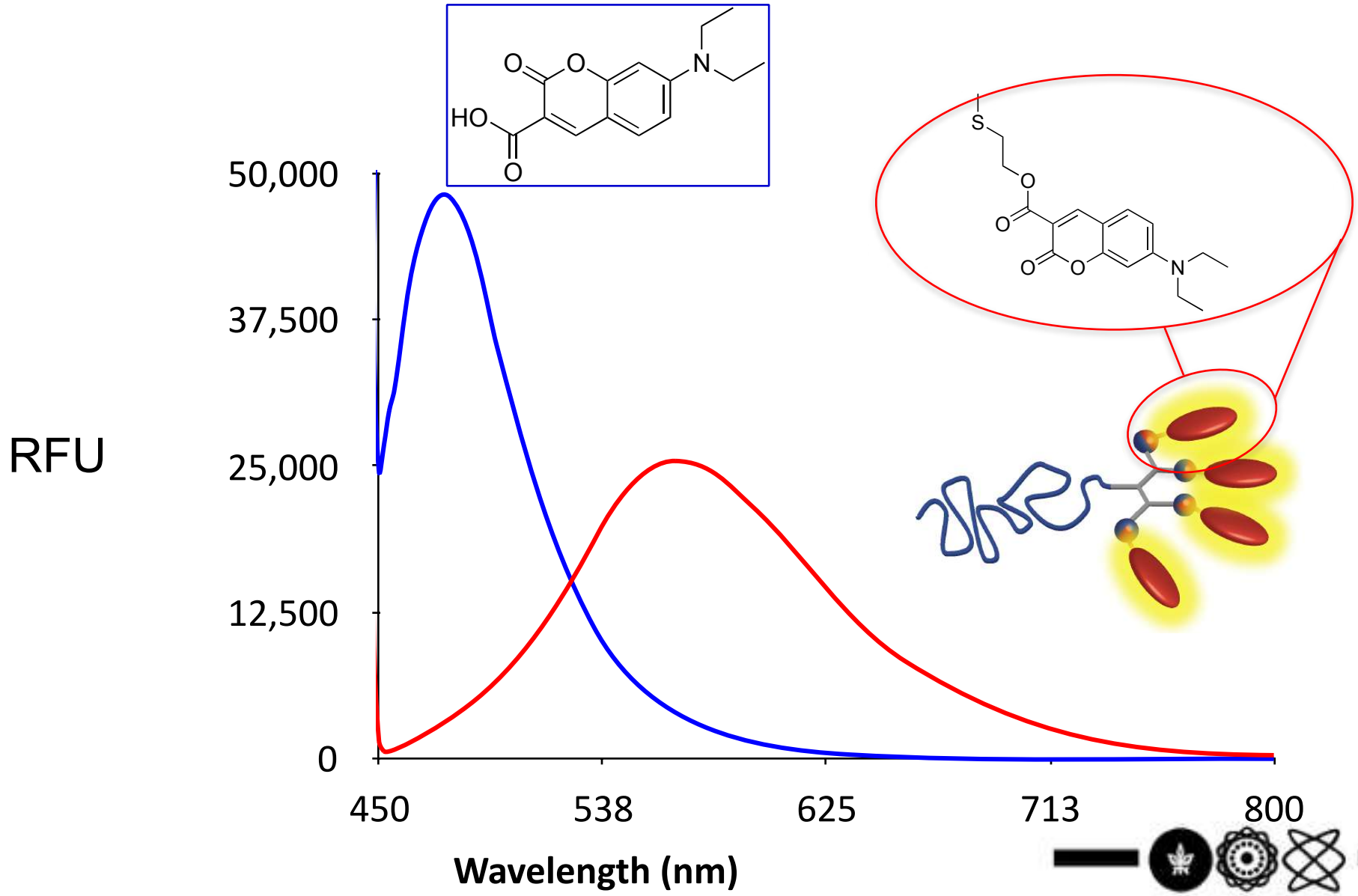
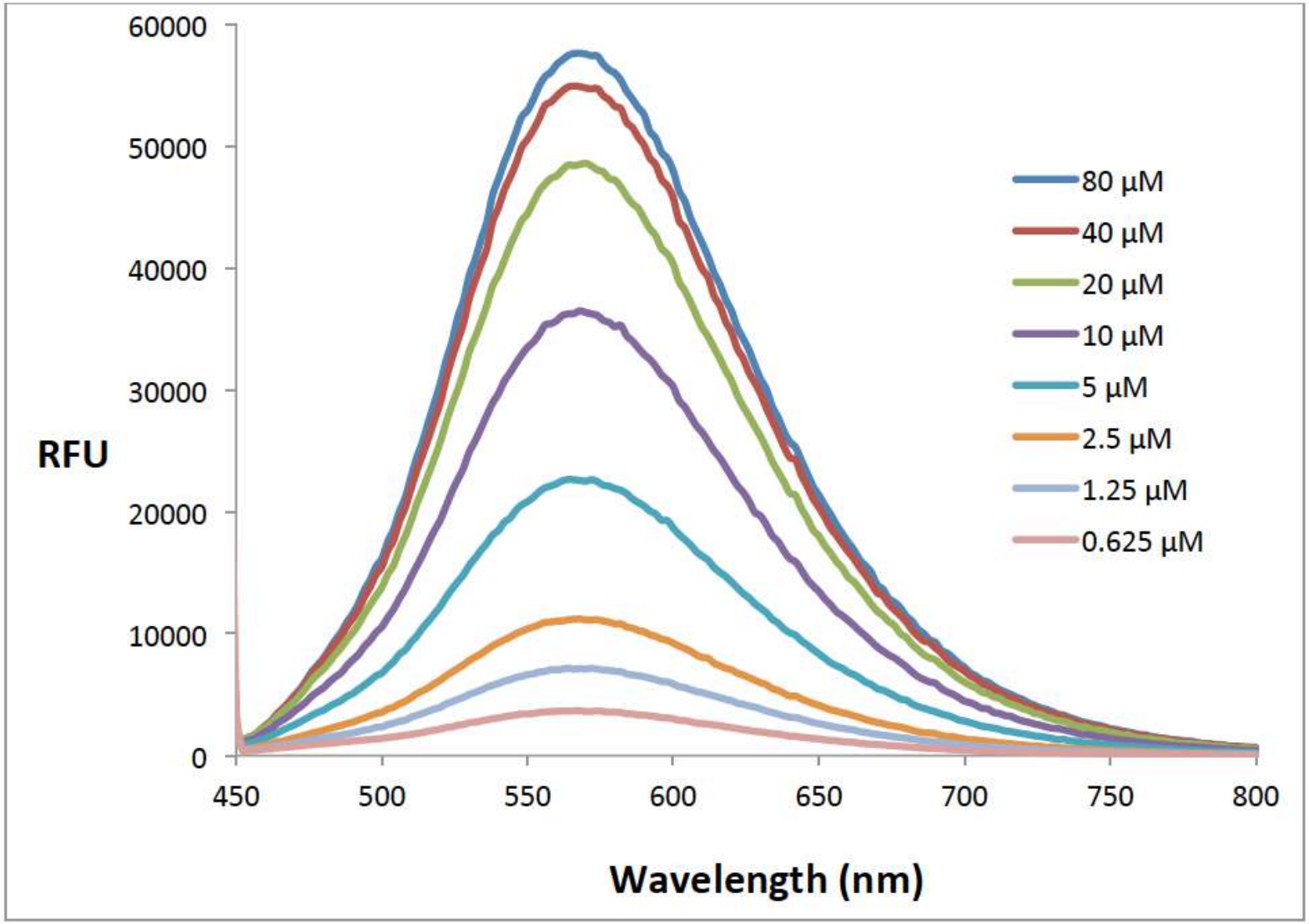
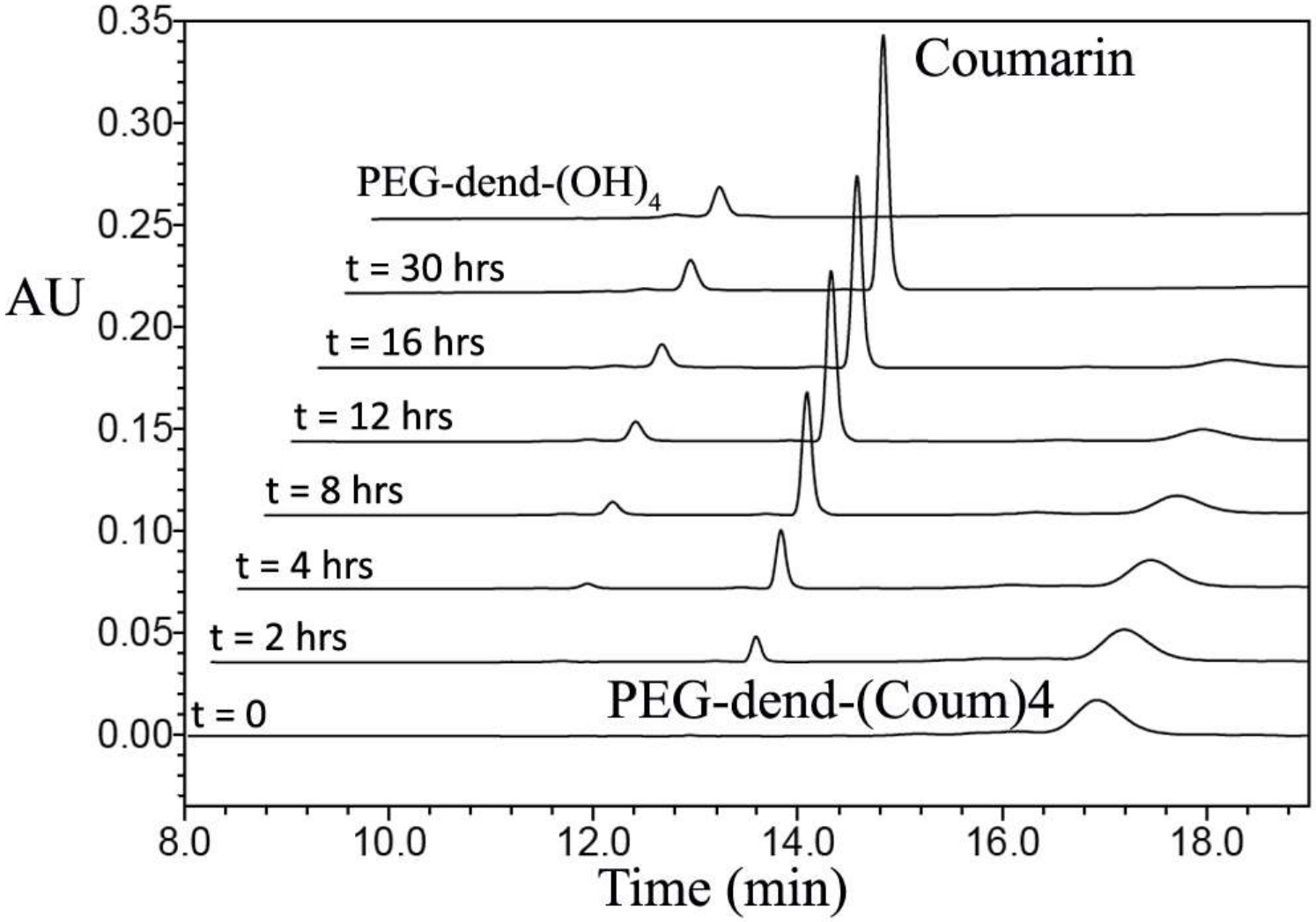


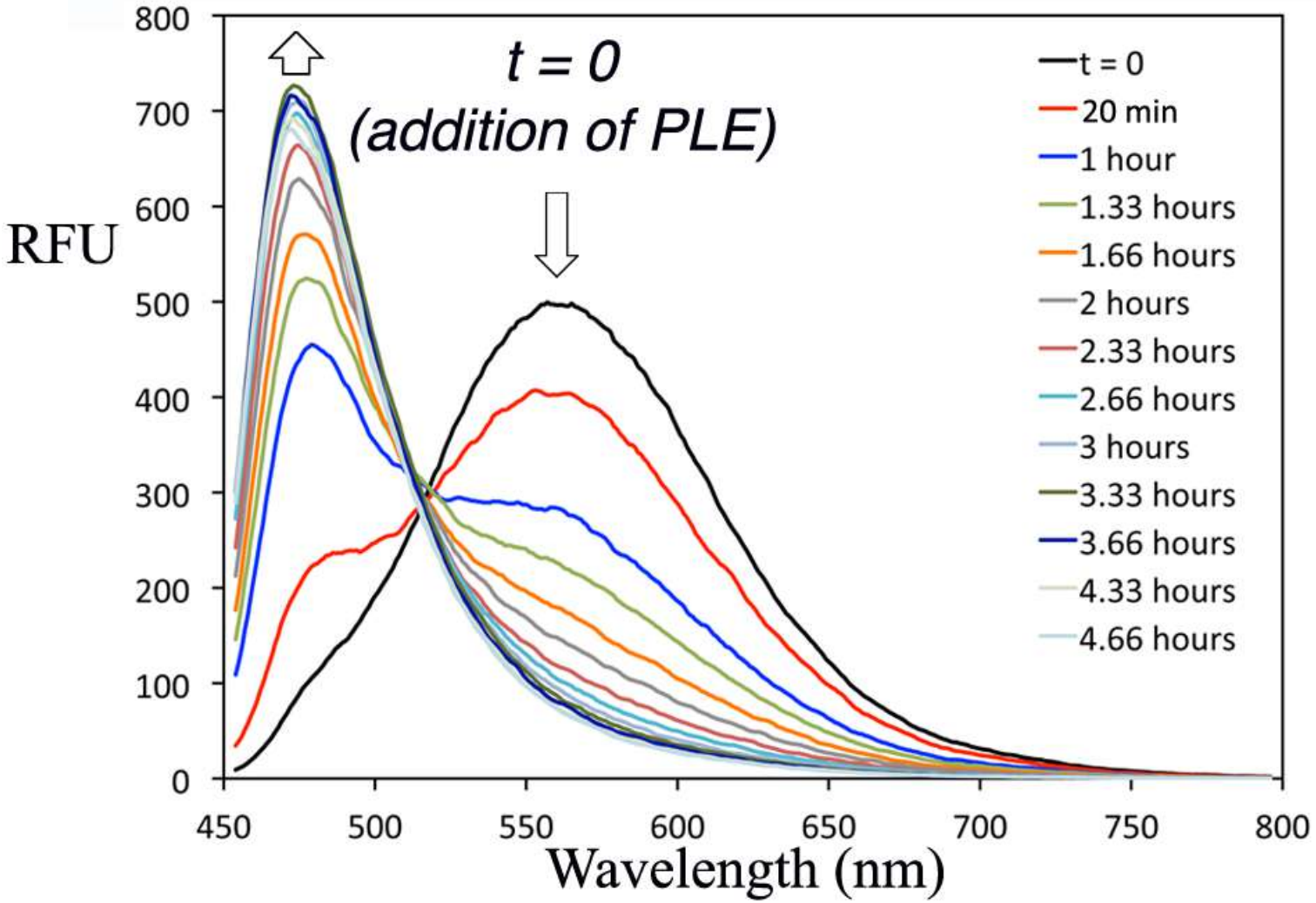
The observed red-shift below the CMC indicates on intramolecular interactions



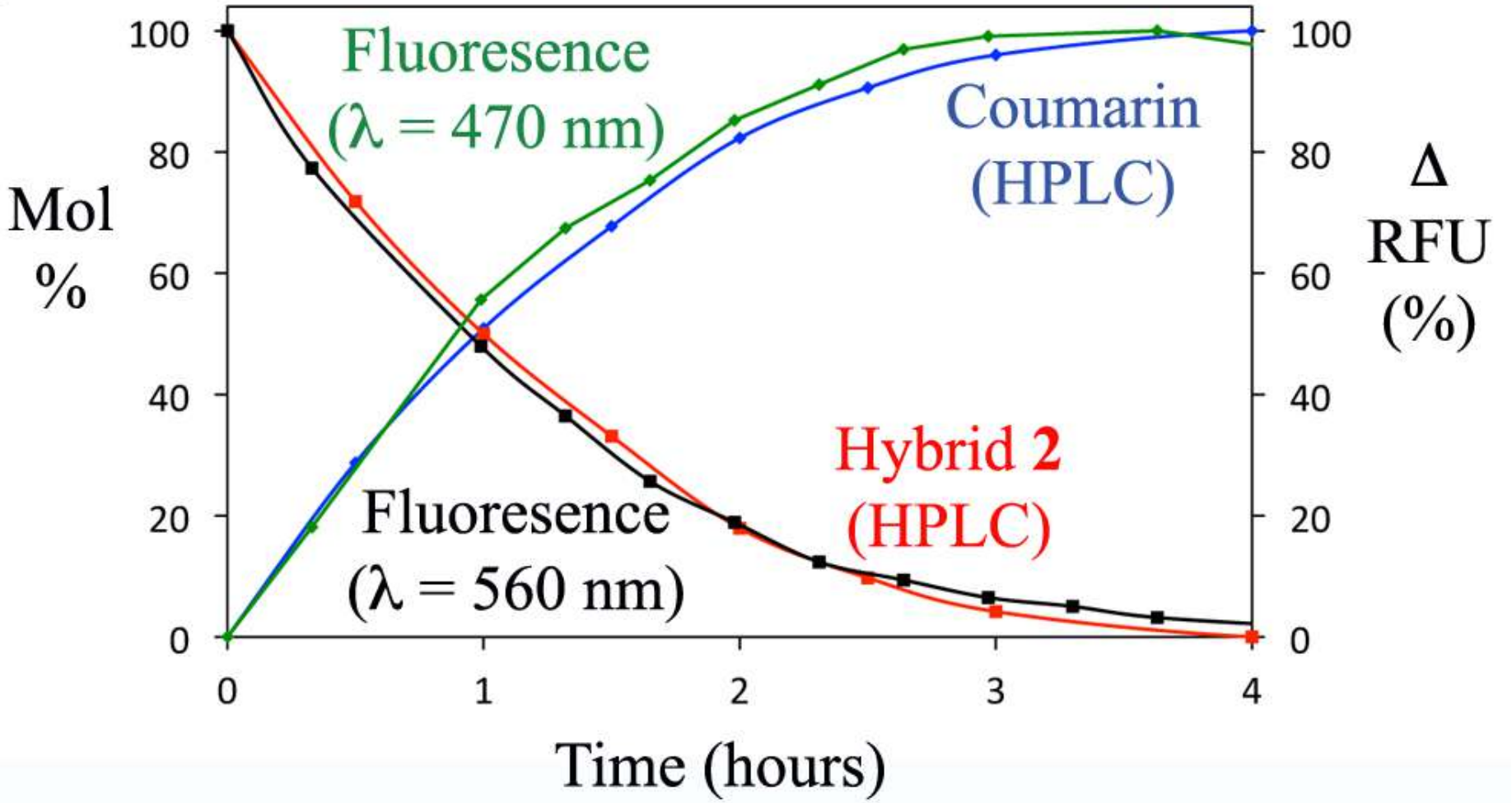
HPLC show complete cleavage of the coumarin end-groups



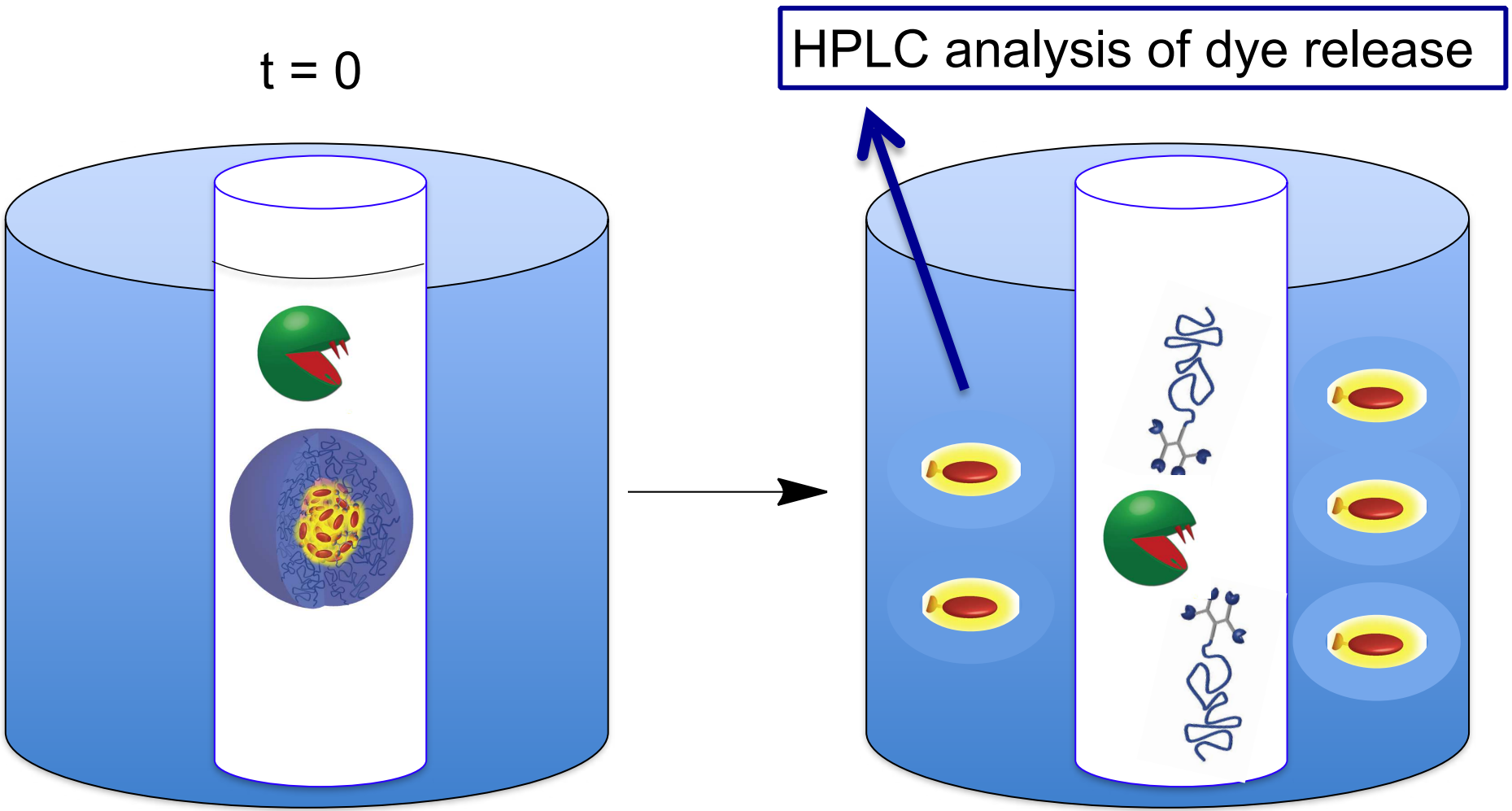
The change in emissions wavelength leads to intrinsic spectral response



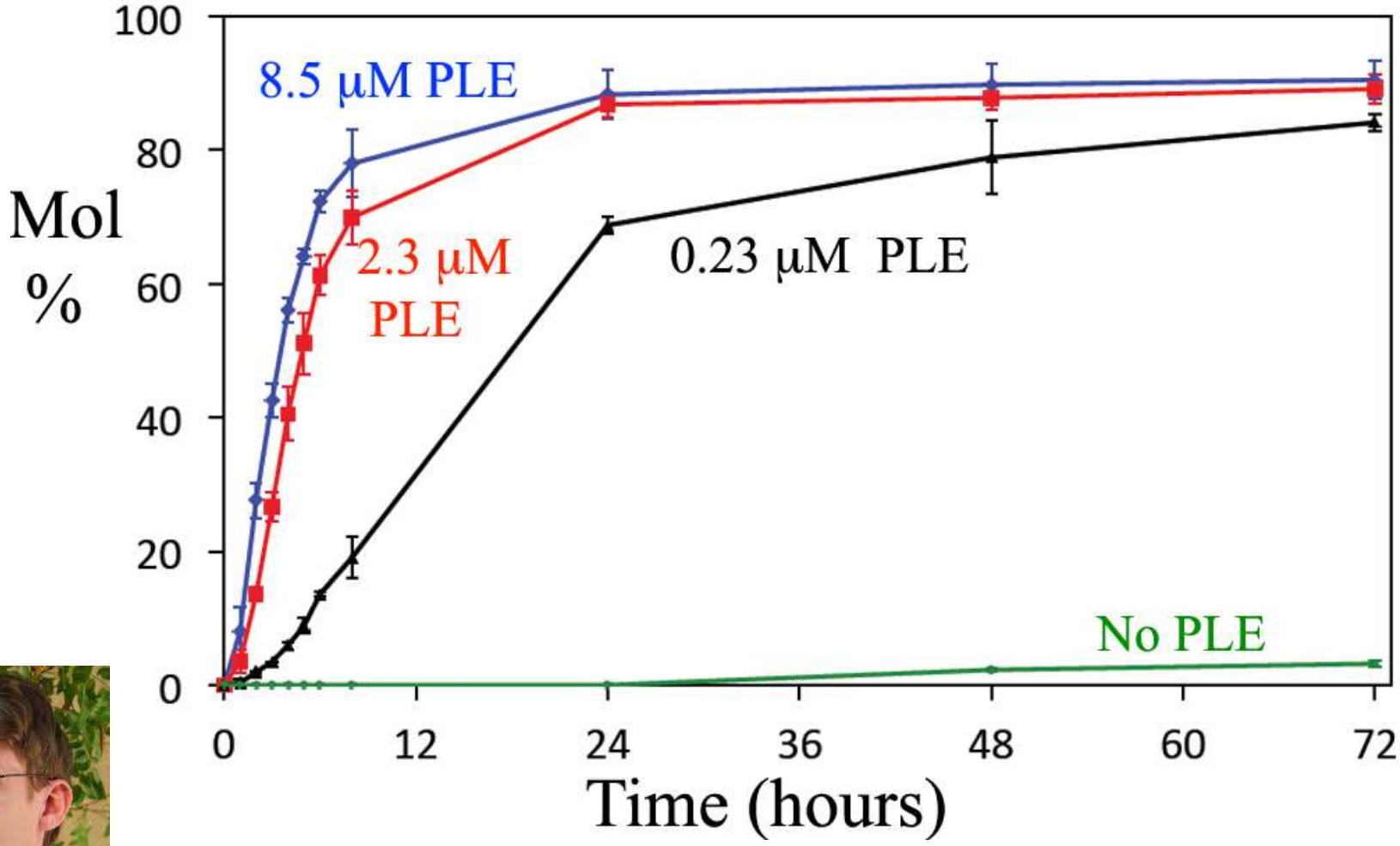
Excellent correlation between the enzymatic activation and the spectral response



A dialysis setup was used to study dye release



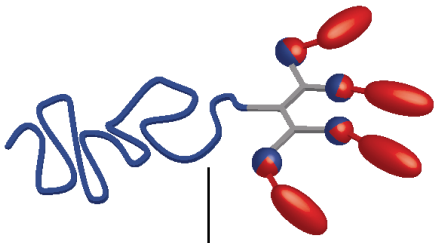
Covalently loaded micelles showed slower release and lower background release



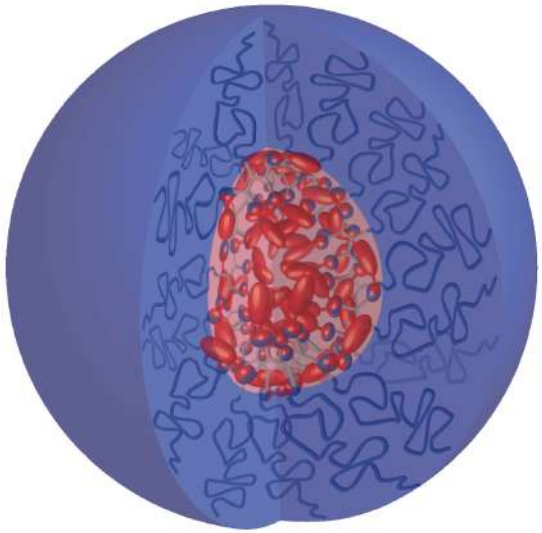
Ido Rosenbaum et al. *JACS* **2015**

Programming micelles to report their disassembly

Blue = hydrophilic
Red = hydrophobic

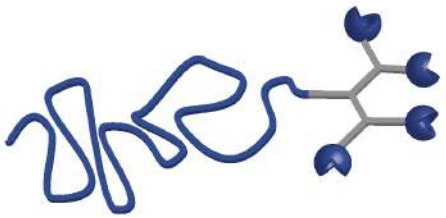


Self assembly



Enzymatic stimuli

hydrophilic groups



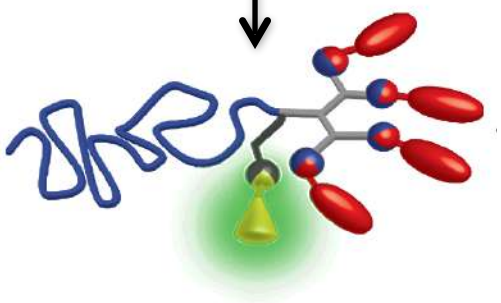
Structural change

Supramolecular translation mechanism

Spectral response

Designing labeled molecular building blocks

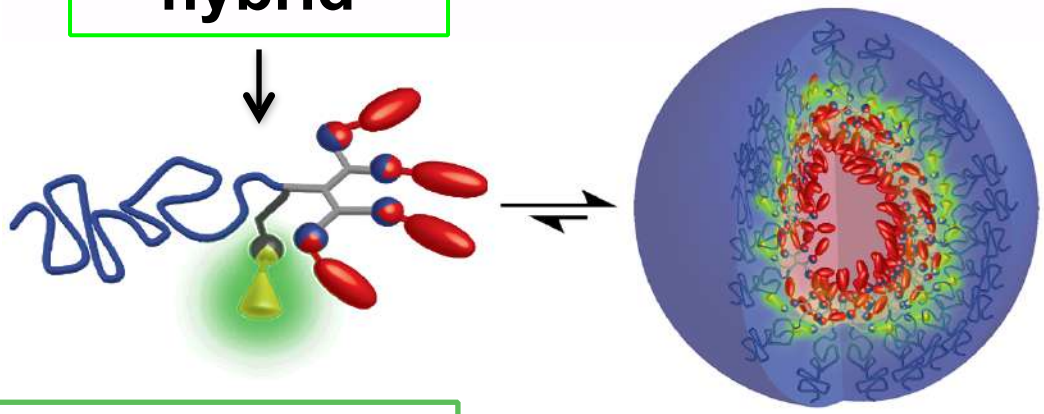
Labeled hybrid



Intrinsic spectral properties

Self-assembly leads to altered supramolecular spectral properties

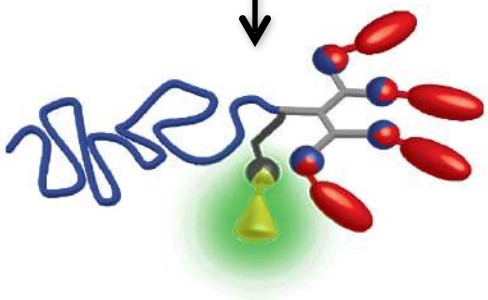
Labeled hybrid



Intrinsic spectral properties

Self-assembly leads to altered supramolecular spectral properties

Labeled hybrid



Intrinsic spectral properties

Supramolecular spectral properties (dye-dye interactions)

Turn Off

1. Self-quenching (FRET)
2. FRET with dark quencher

Spectral Switch

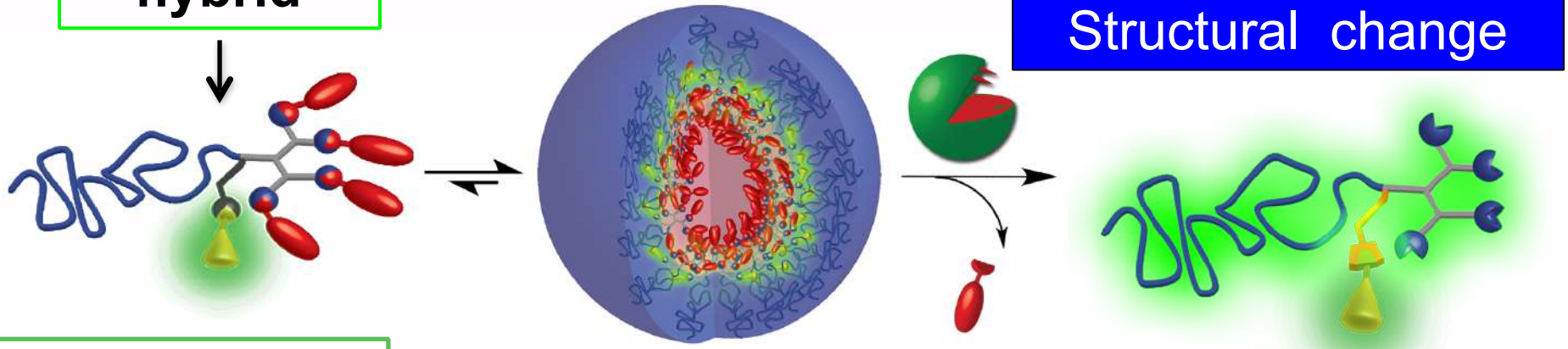
3. FRET pairs
4. Excimer formation

The enzymatically induced structural change causes a spectral response

Spectral response

Structural change

Labeled hybrid



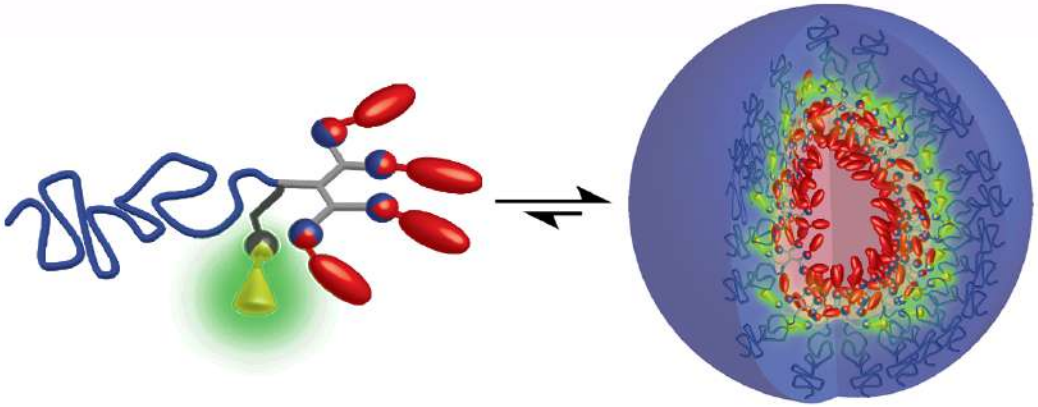
Intrinsic spectral properties

Supramolecular spectral properties (dye-dye interactions)

Intrinsic spectral properties

Marina Buzhor

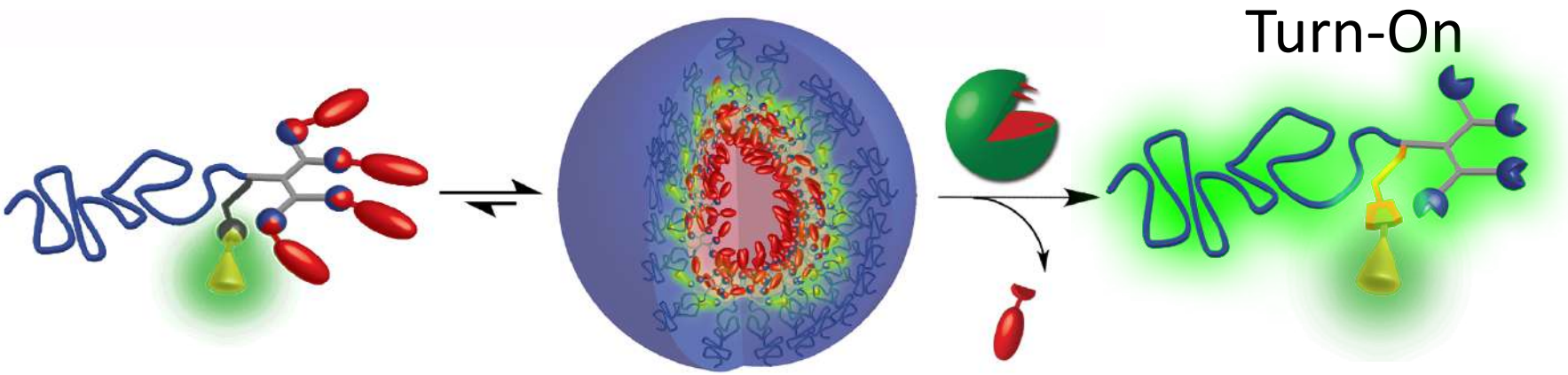
Supramolecular Turn-Off of fluorescence

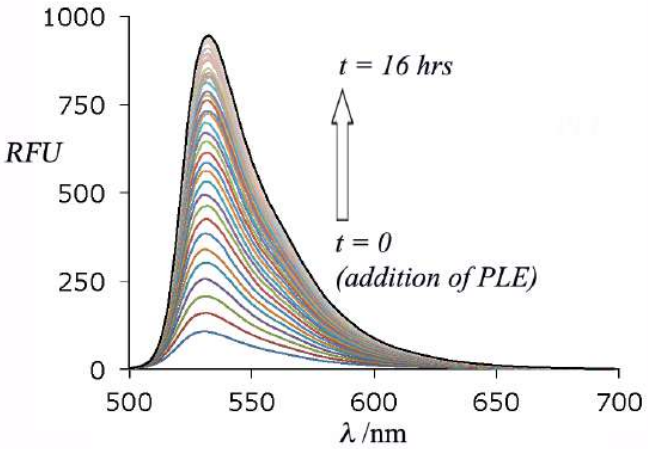


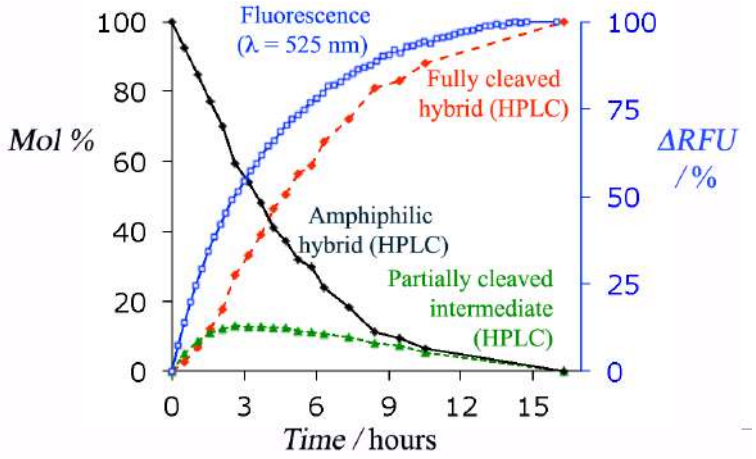
Fluorescein

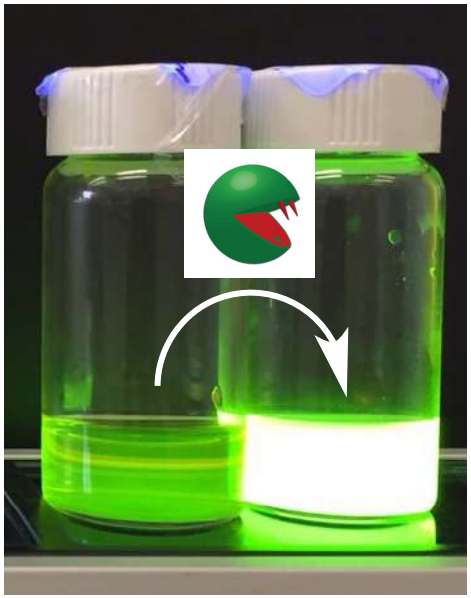
Turn-Off

Supramolecular Turn-Off and enzymatic Turn-On of fluorescence

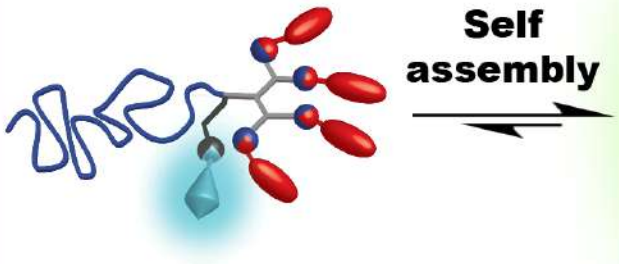




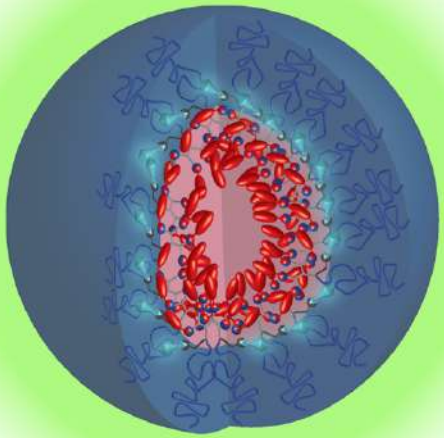




Excimer based supramolecular Spectral-Switch



Self assembly

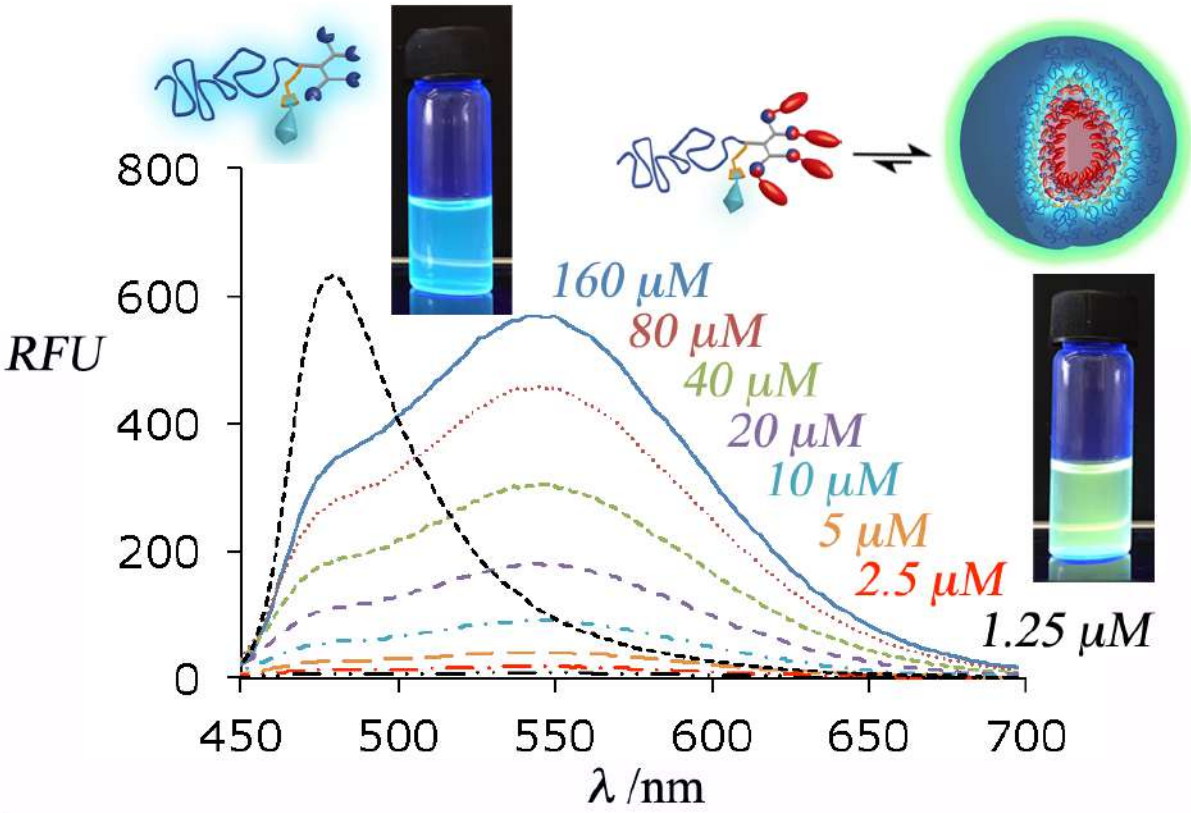


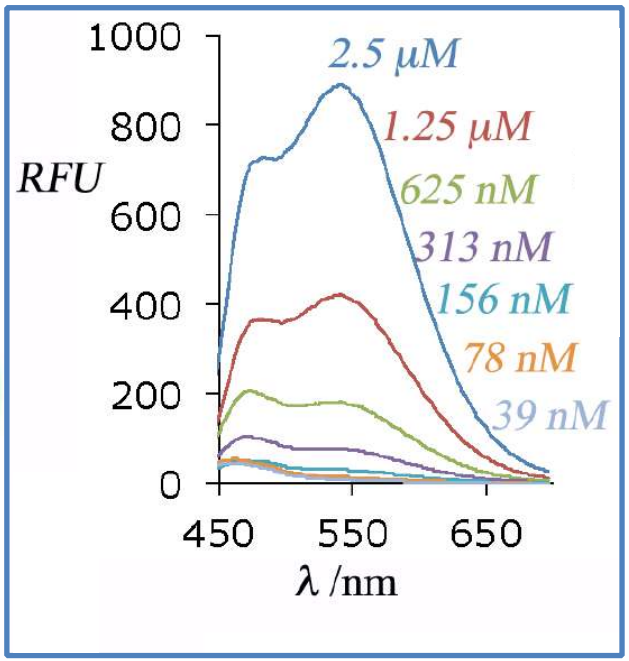
7-DACC

Excimer based supramolecular Spectral-Switch

TAU

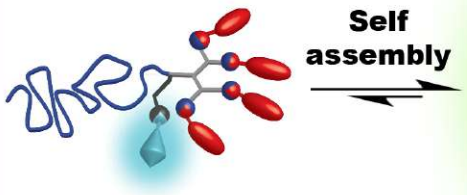
The labeled micelles show a 70 nm red-shift due to formation of excimers



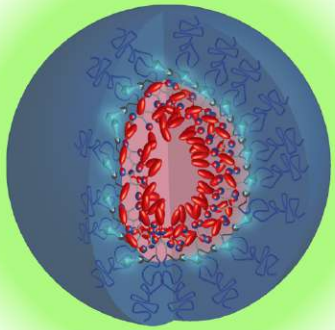


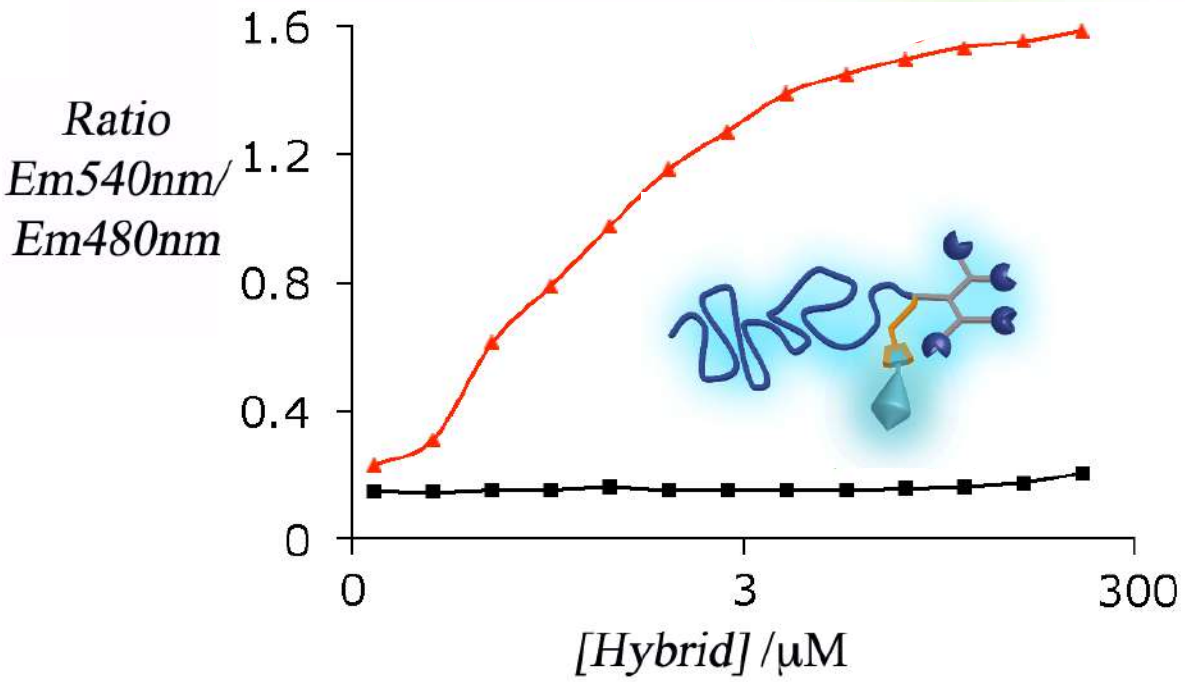
CMC = 3 μM

Excimer based supramolecular Spectral-Switch



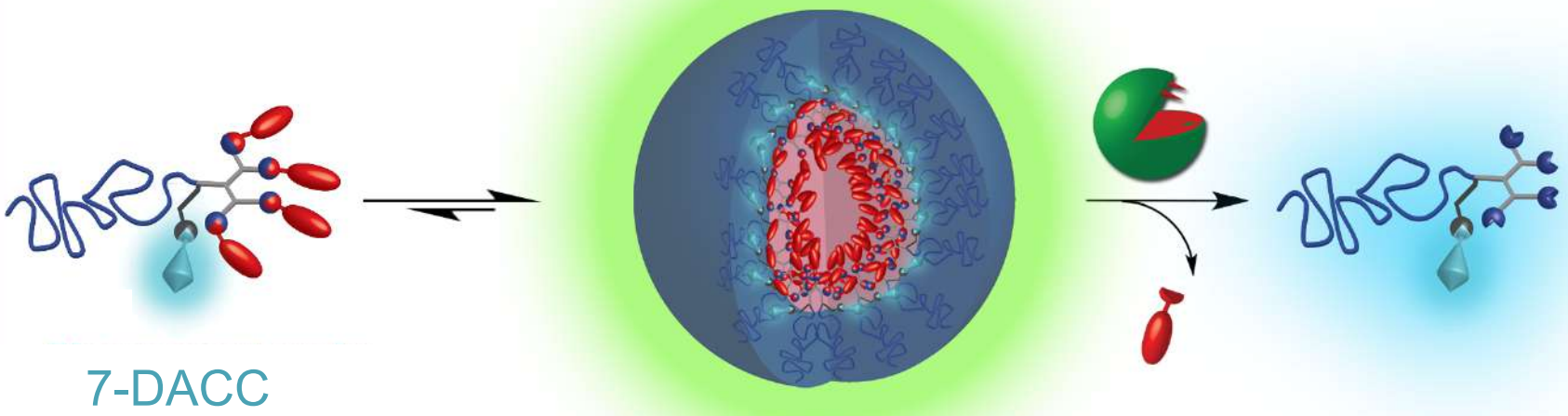
Self assembly



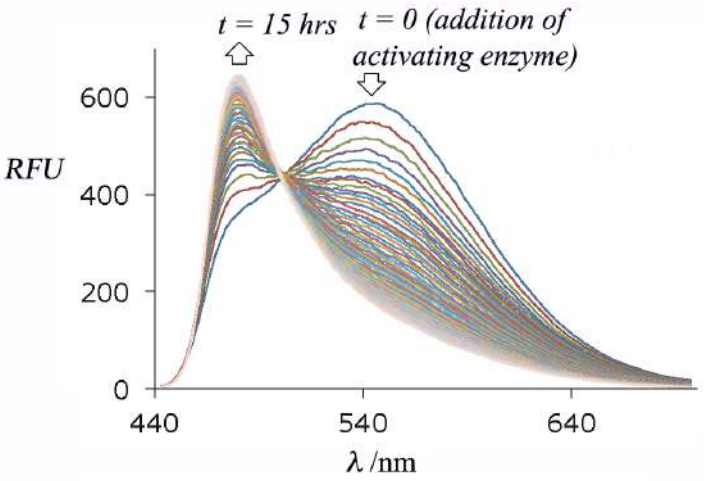


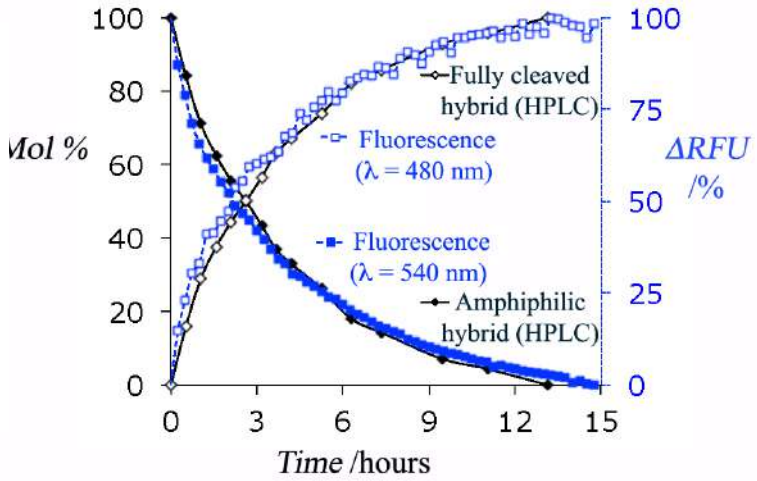
Dye-dye interactions occur at significantly lower concentrations than the CMC (3 μM).

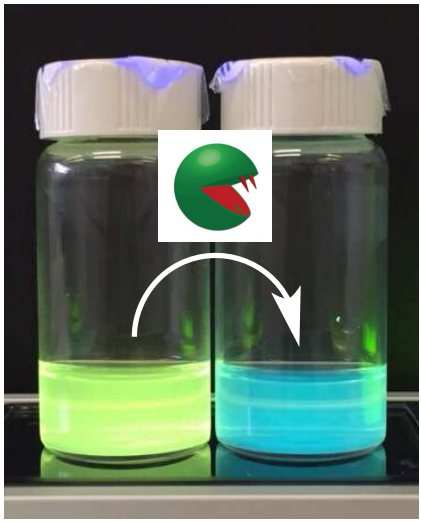
Supramolecular Spectral-Switch of fluorescence



7-DACC

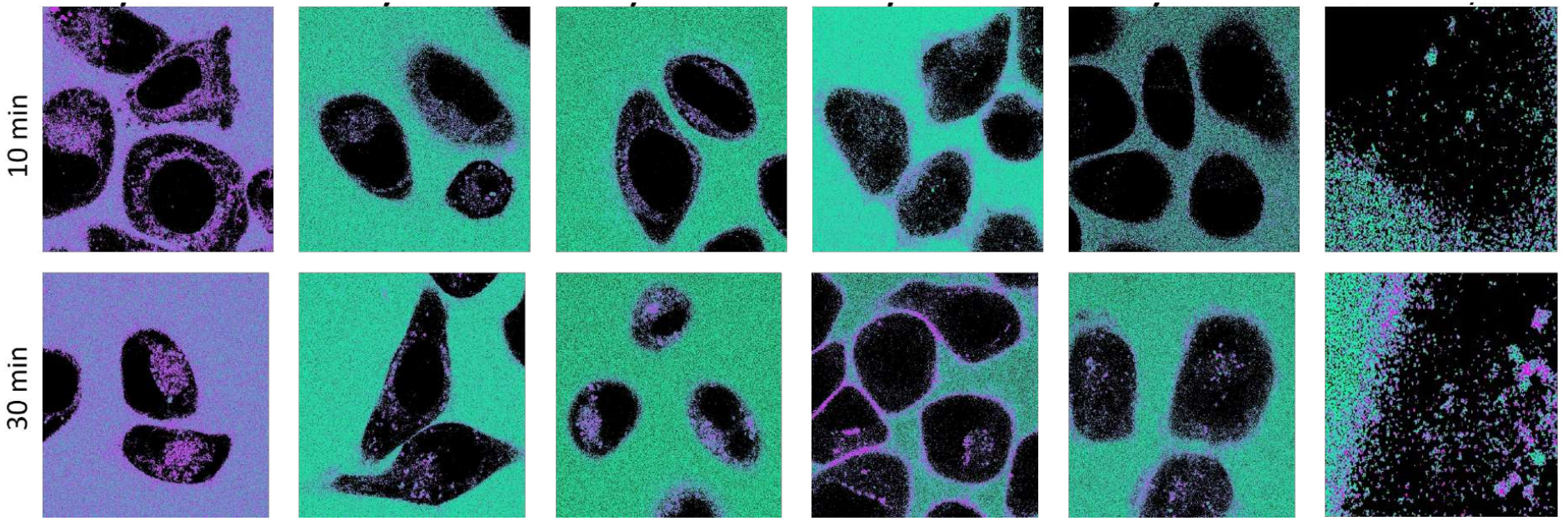






Buzhor et al., *Chem. Eur. J,* **2015**, 21, 15633.

The degree of hydrophobicity dictates the internalisation pathway



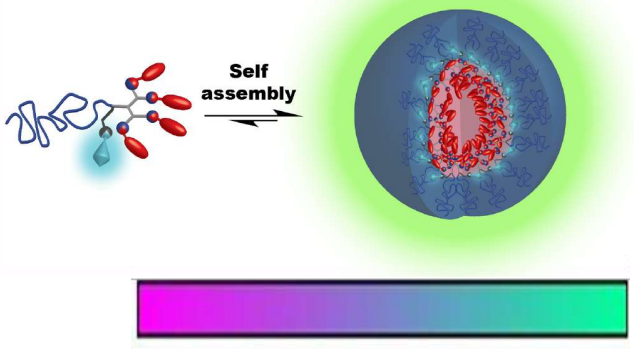
Hydrophilic hybrid

Increasing hydrophobicity of end-groups

Zoom in

Natalia Feiner

Marina Buzhor



monomer

micelle

Feiner & Buzhor JACS **2017**

The degree of hydrophobicity dictates the internalisation pathway

Take home message:
Small changes in the hydrophobic block affect the internalization pathway

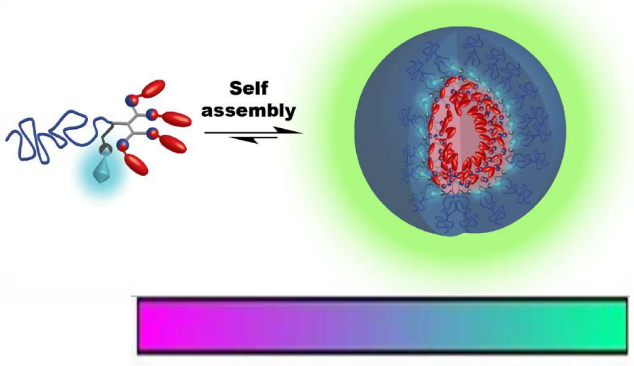
Feiner & Buzhor JACS **2017**

The degree of hydrophobicity dictates the internalisation pathway

TAU

Take home message:
Real life is different from a vial with buffer

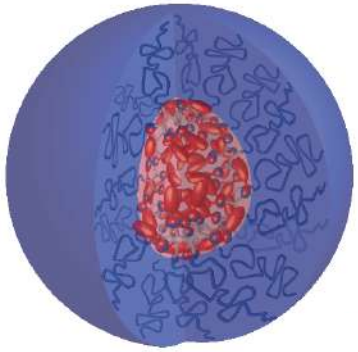
hybrid



Feiner & Buzhor JACS **2017**

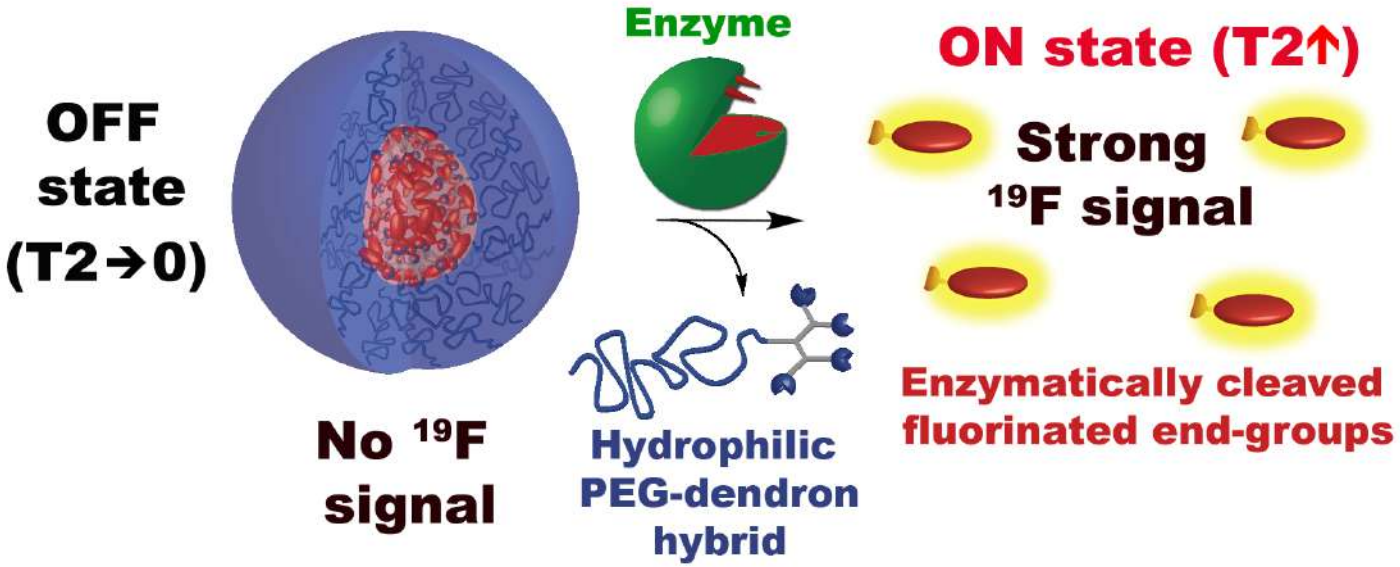
Translating the structural change into a magnetic resonance response

OFF state (T2→0)

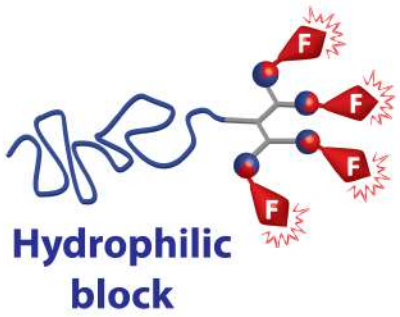


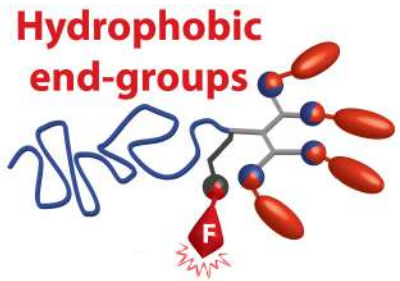
No ^{19}F signal

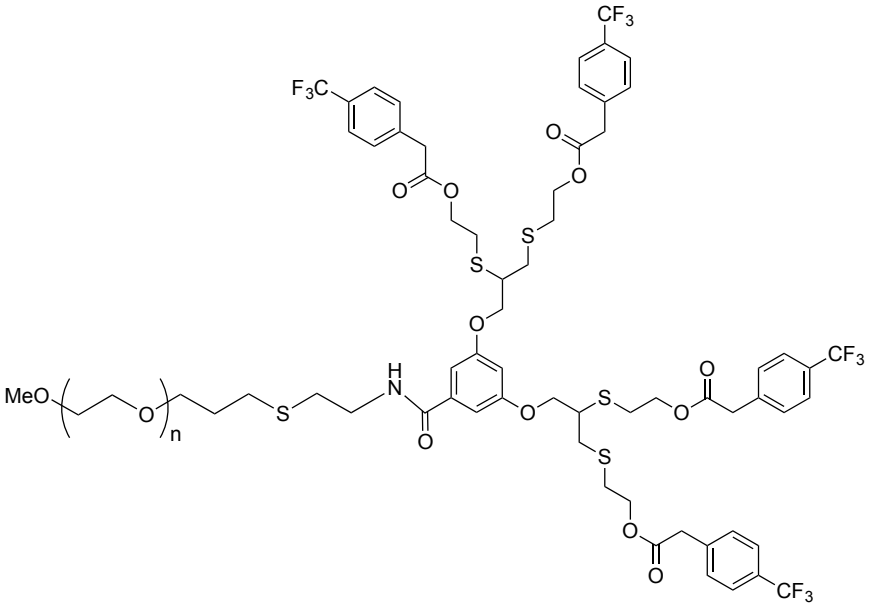
Translating the structural change into a magnetic resonance response

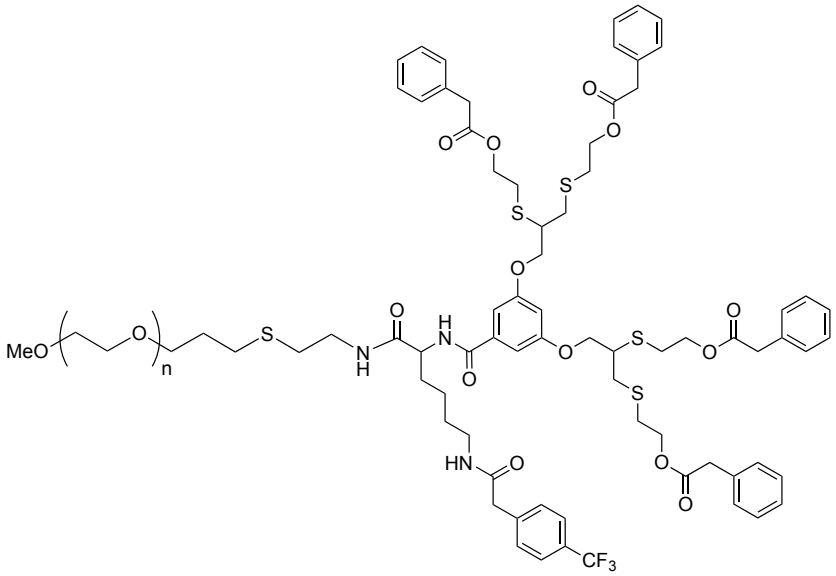


Translating the structural change into a magnetic resonance response

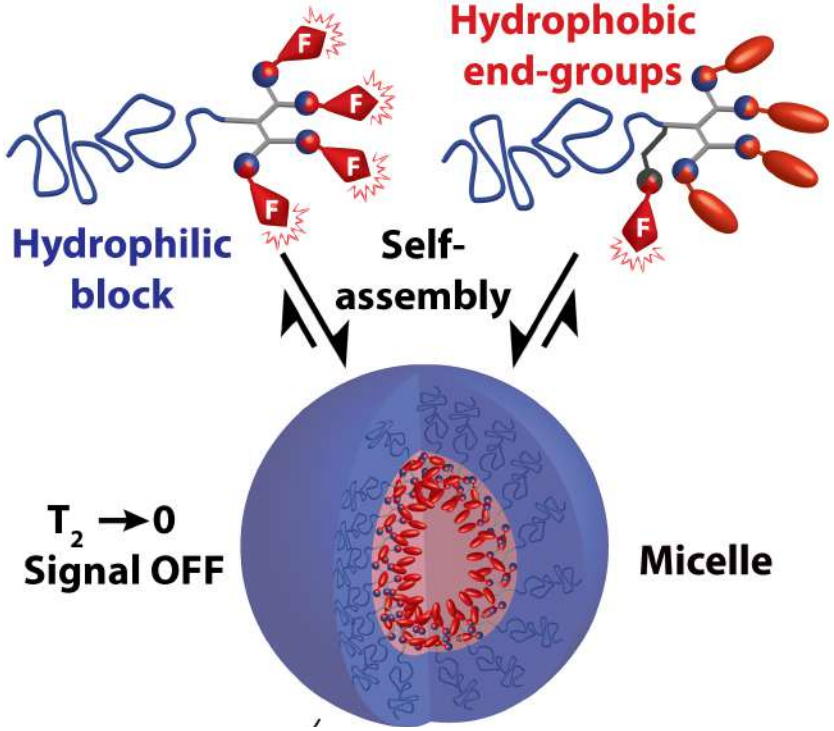




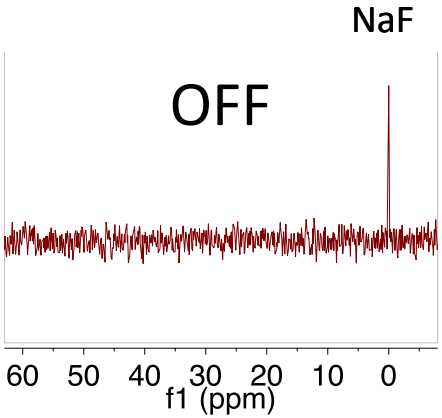


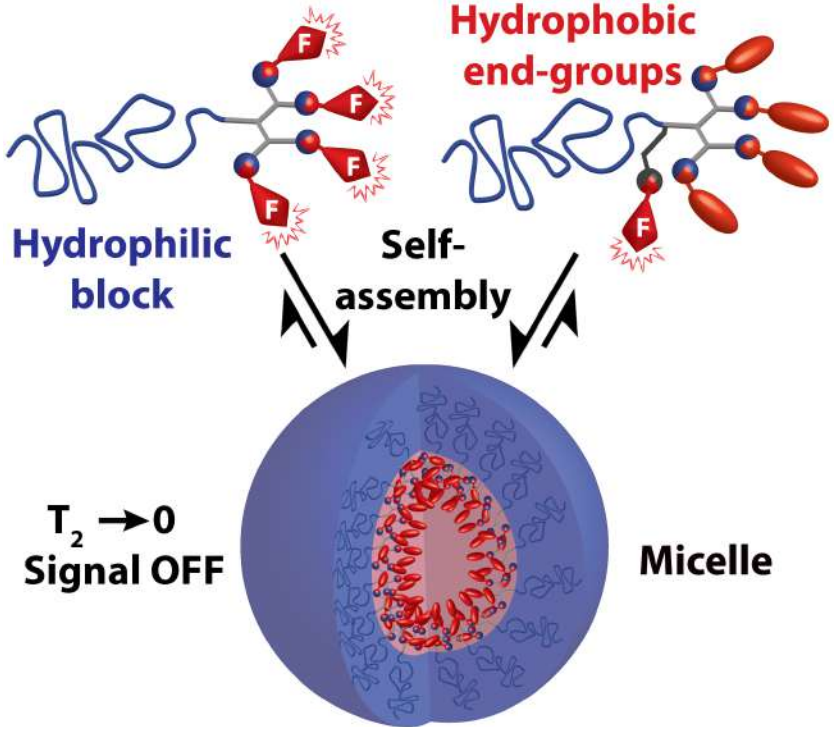


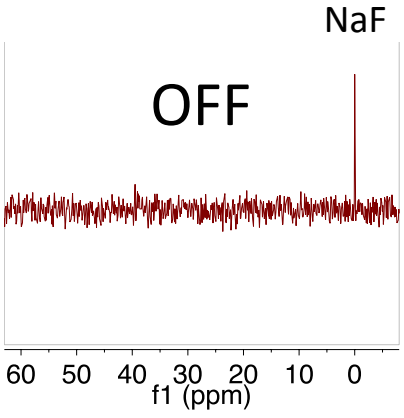
Translating the structural change into a magnetic resonance response



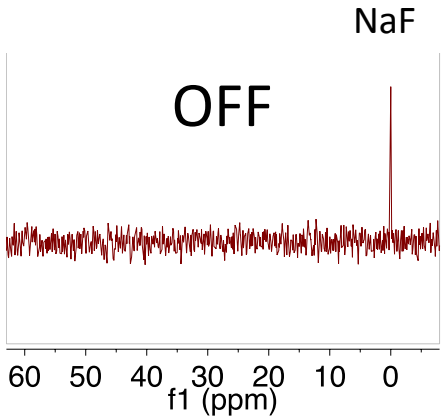
Translating the structural change into a magnetic resonance response

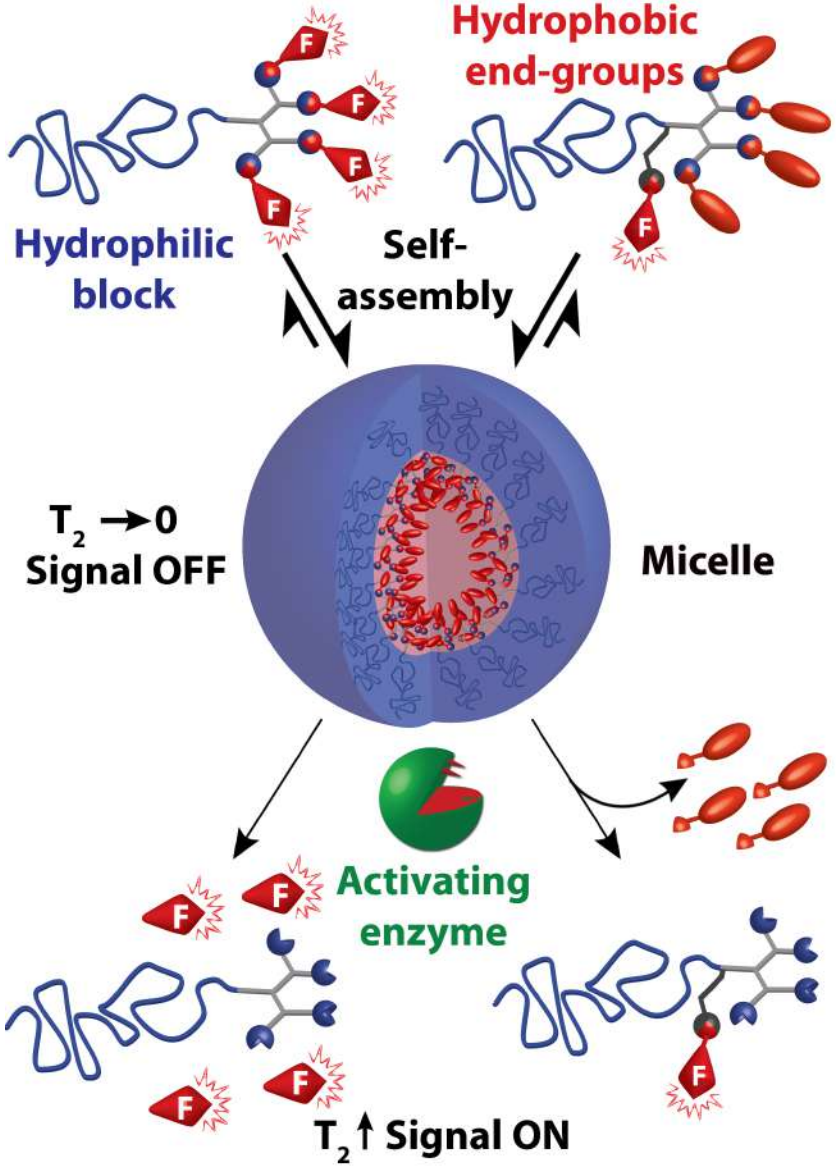


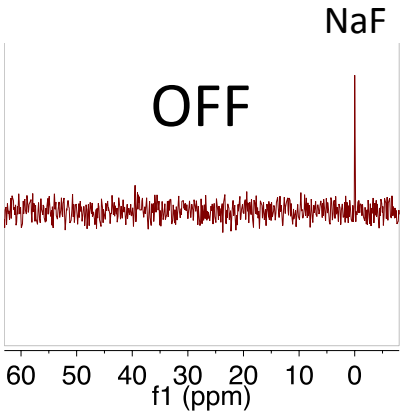




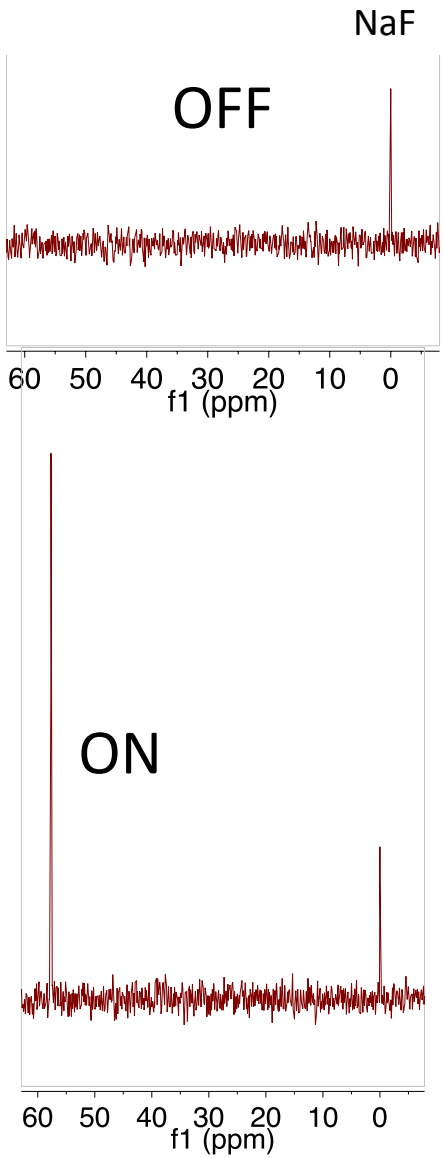
Translating the structural change into a magnetic resonance response

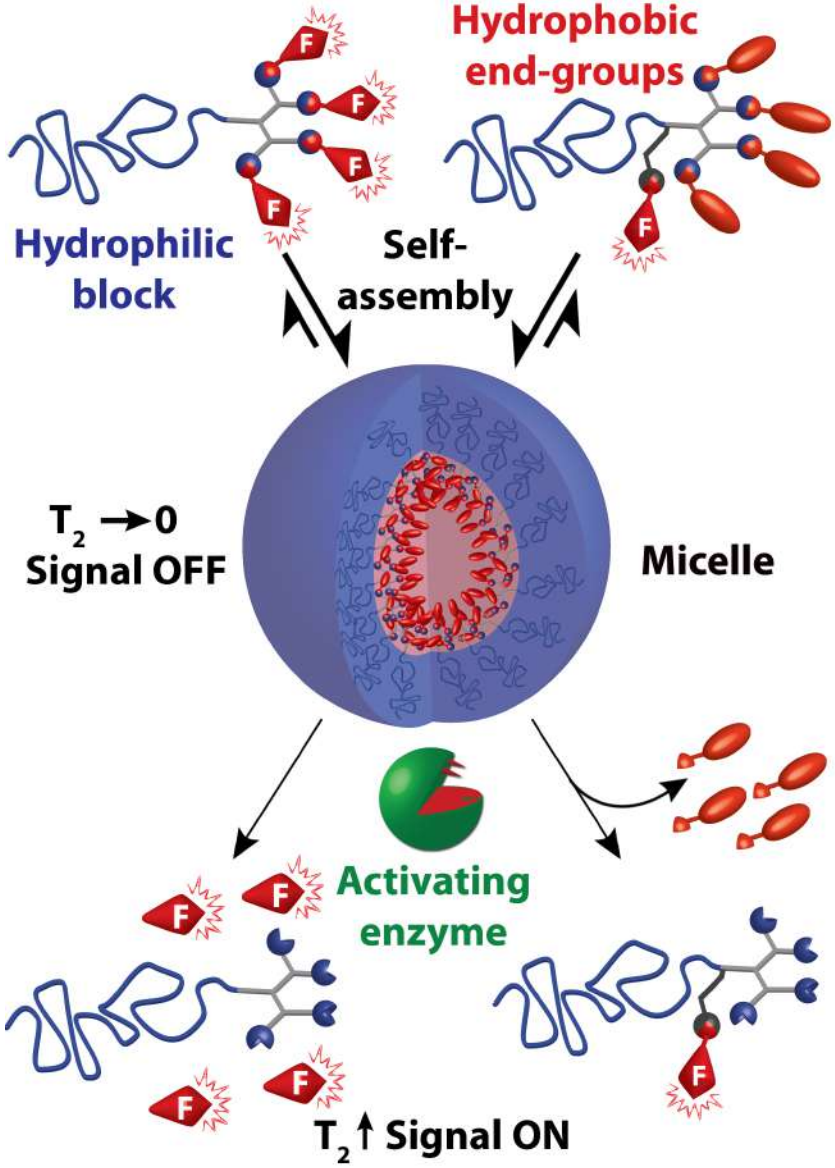


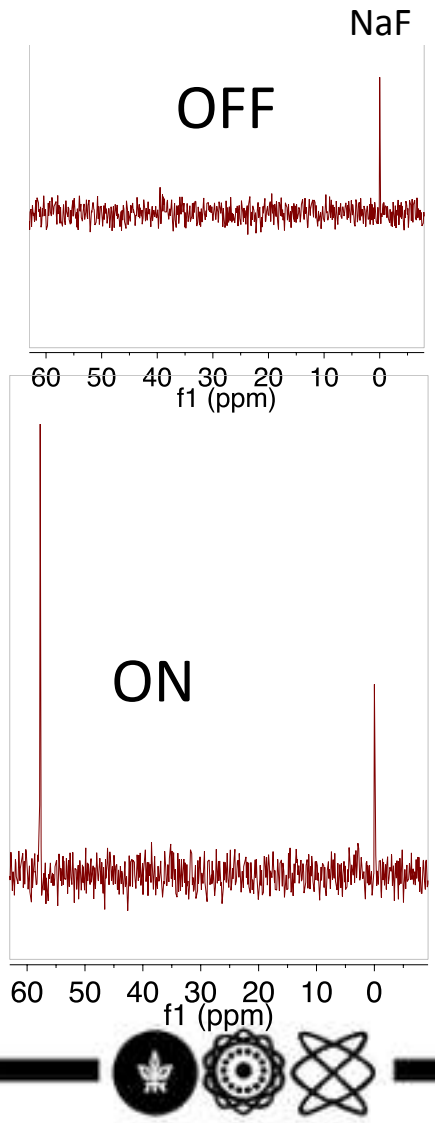




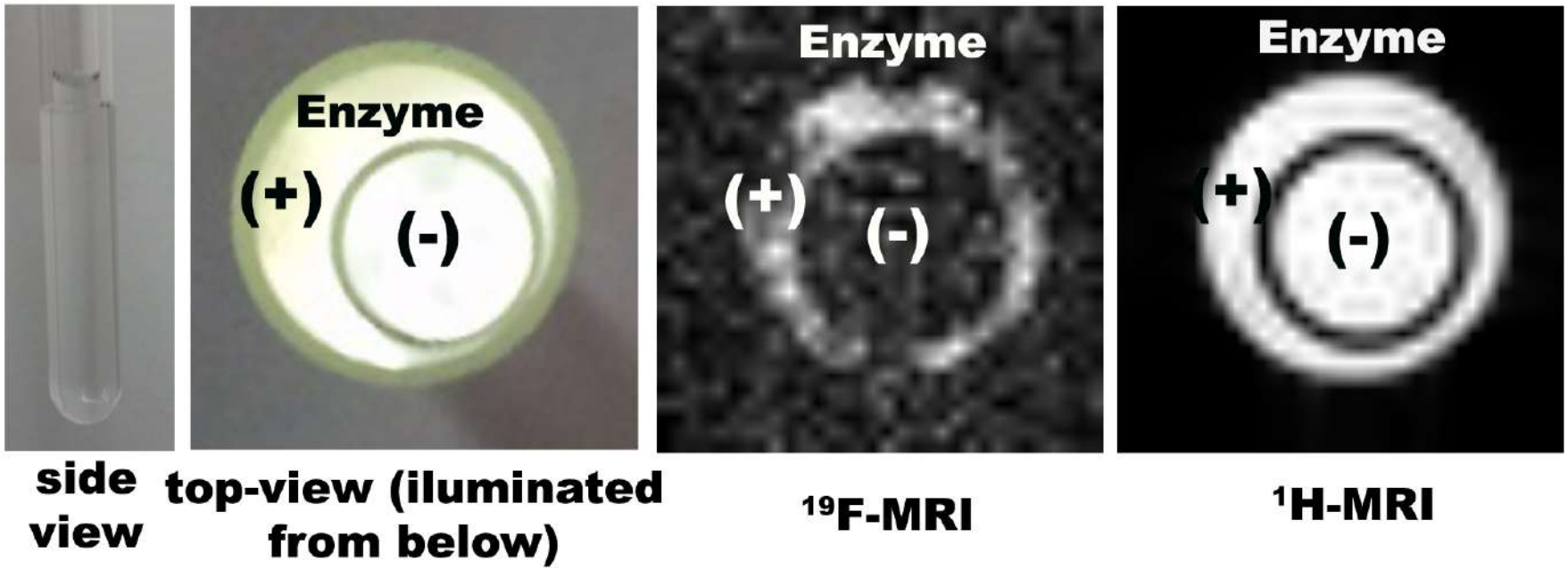
Translating the structural change into a magnetic resonance response







Enzyme responsive ^{19}F-MRI probe

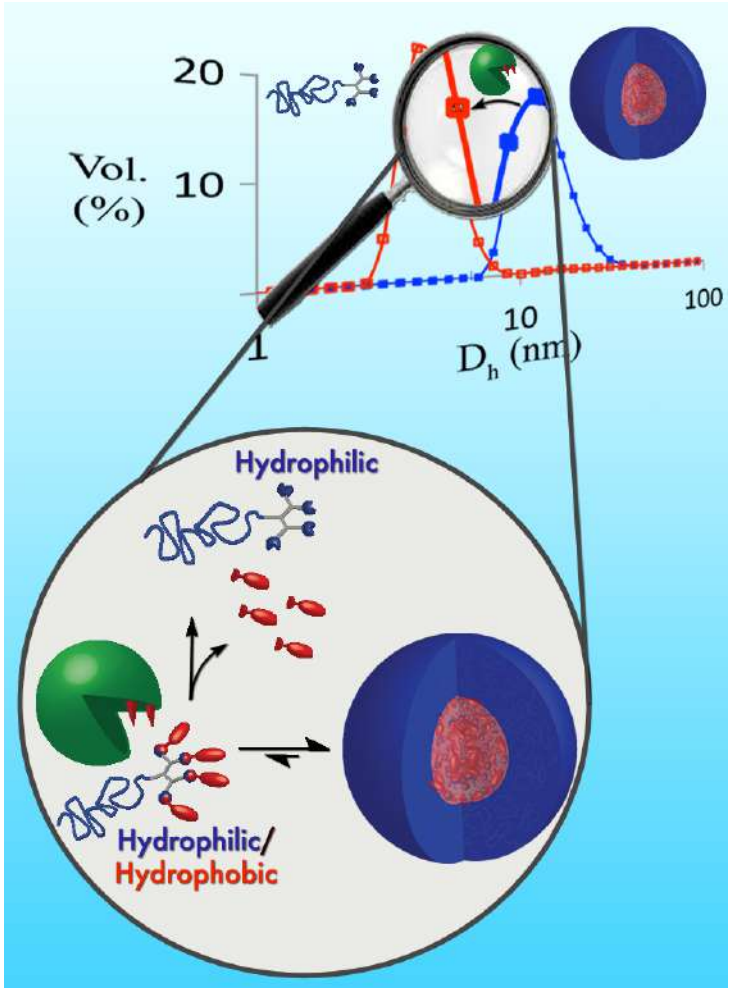


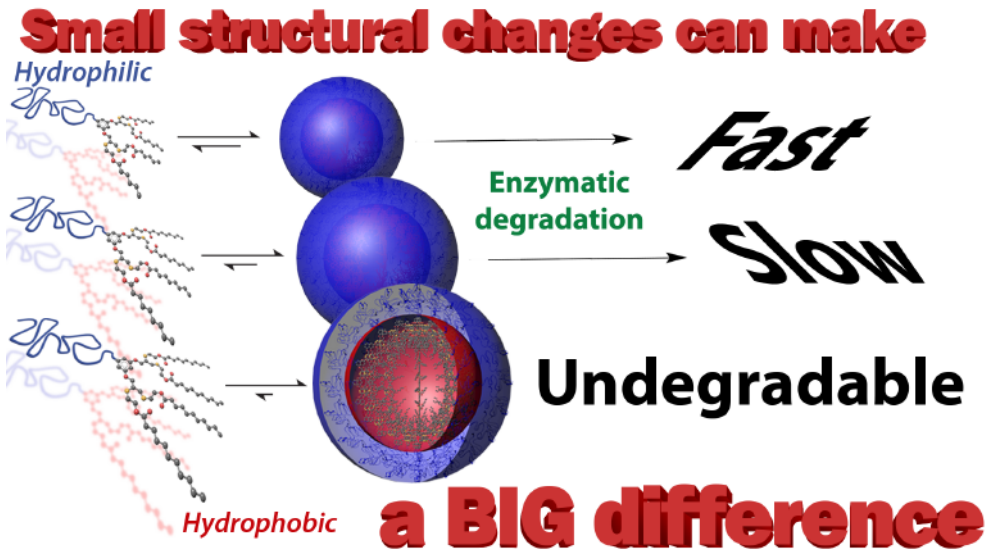
Buzhor et al., *J. Mat. Chem. B.* ***2016*** 4, 3037.

The power of molecular precision

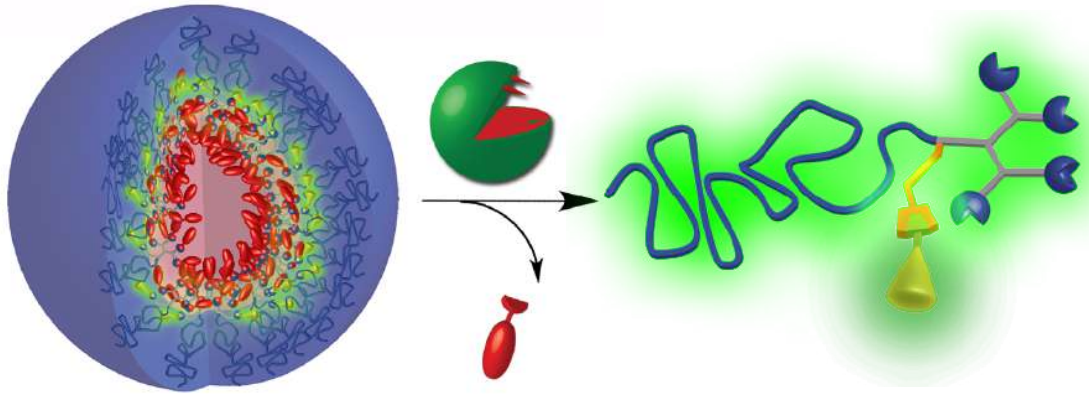
TAU

Detailed mechanistic studies





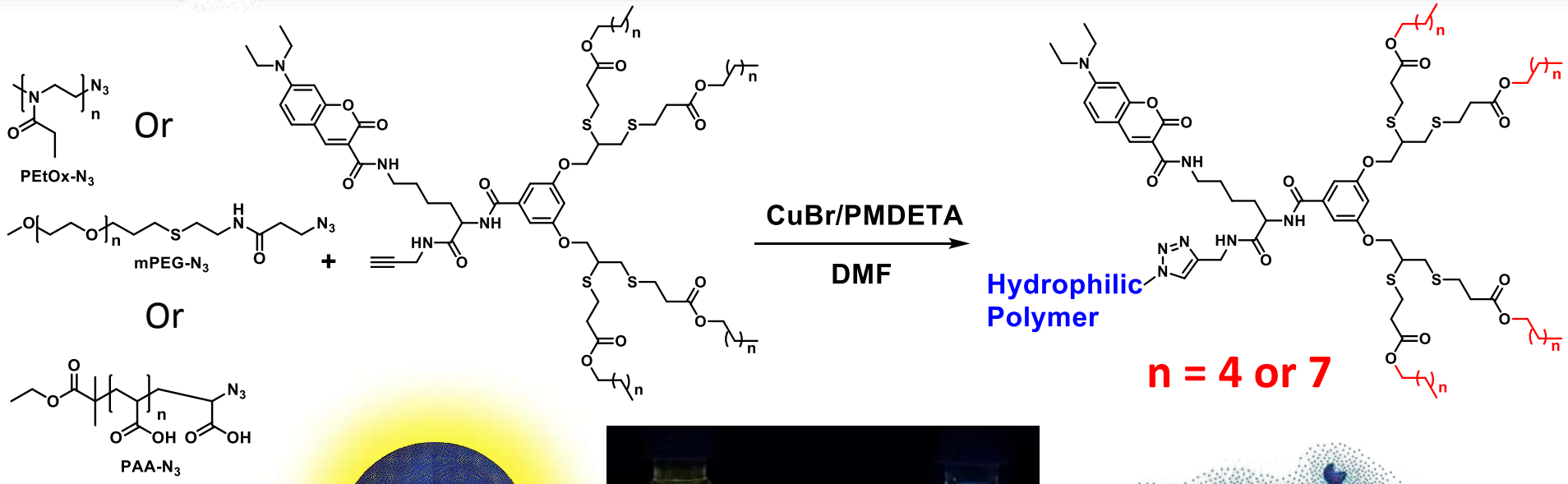
Tunable disassembly rates

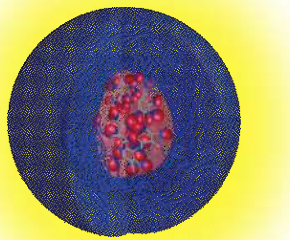


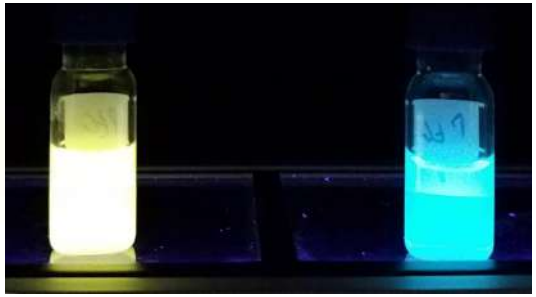
Self-reporting micelles

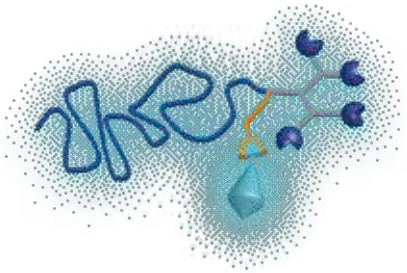
Using click chemistry to form amphiphiles with different hydrophilic blocks

TAU





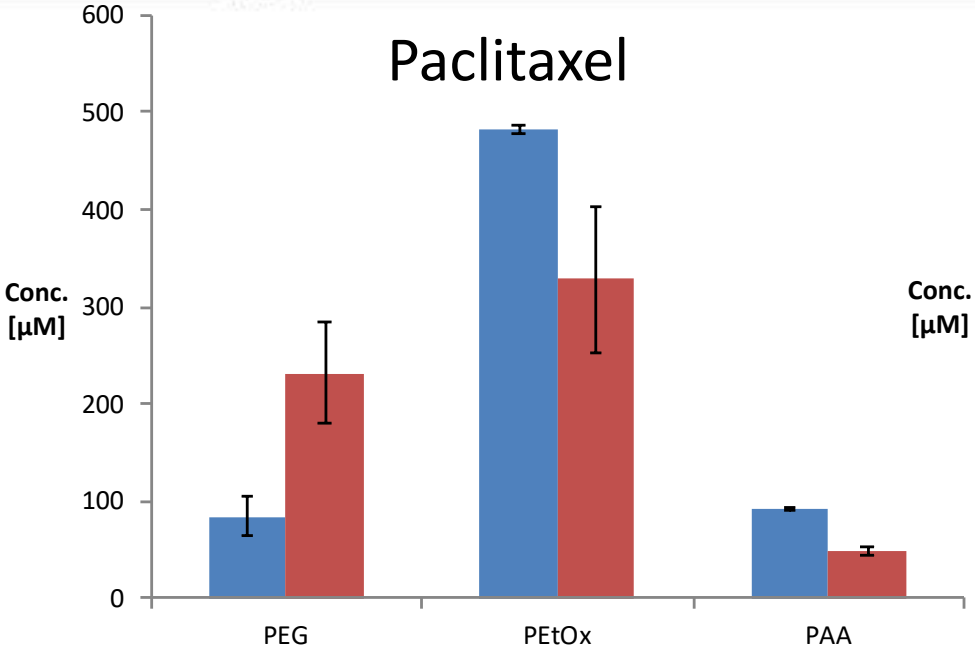


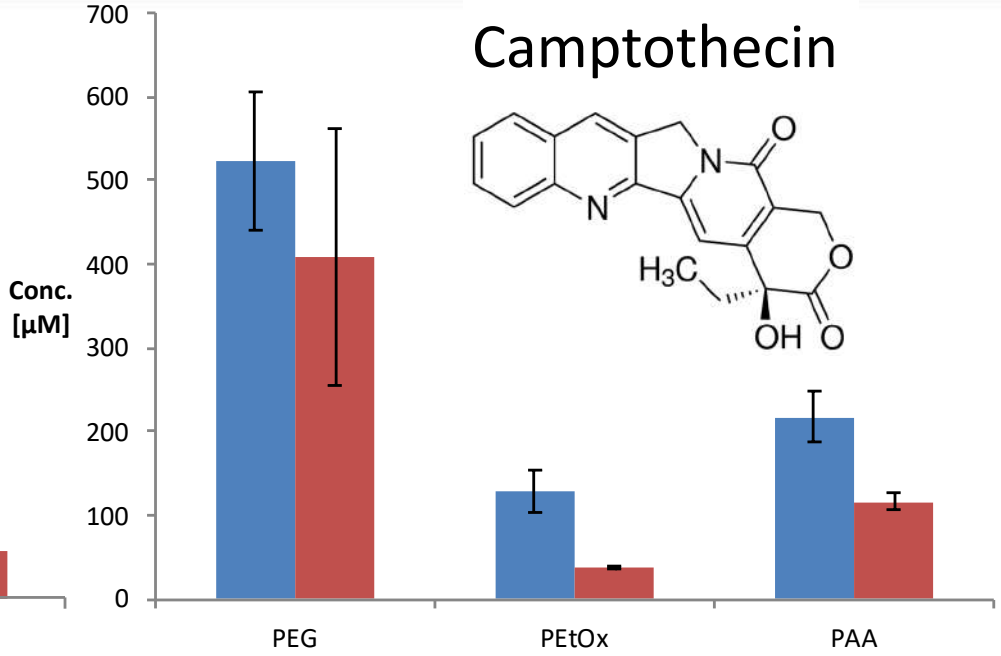


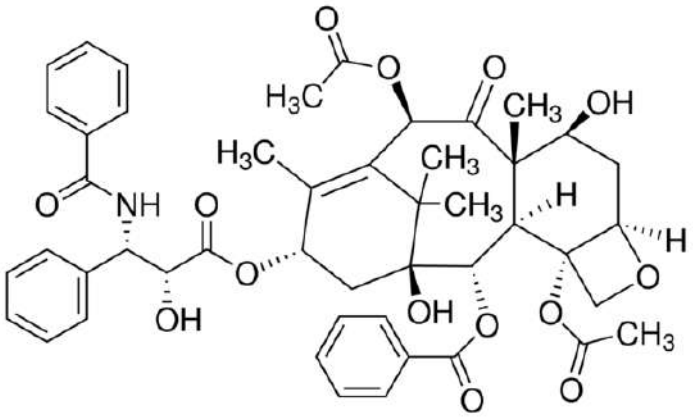
Rodica
Alis Olea Gadi Slor

	Micelle 1	Micelle 2	Micelle 3	Micelle 4	Micelle 5	Micelle 6
	PEtOx-Hex	PEtOx-Non	PEG-Hex	PEG-Non	PAA-Hex	PAA-Non
CMC (μM)	5±1	3±1	6±1	5±1	9±3	9±1

Are the drugs located in the micellar core? the shell? both?







Dendron with four Hexanoate end-groups

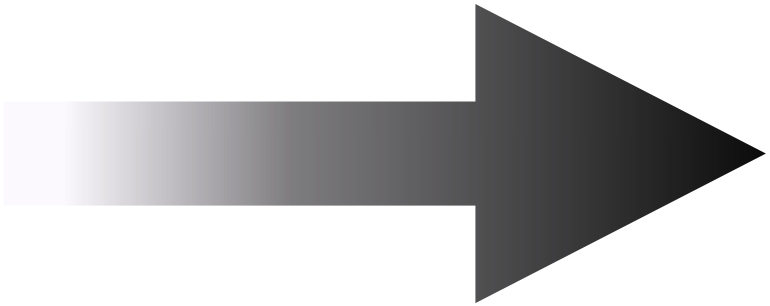
Dendron with four Nonanoate end-groups

Are the drugs located in the micellar core? the shell? both?

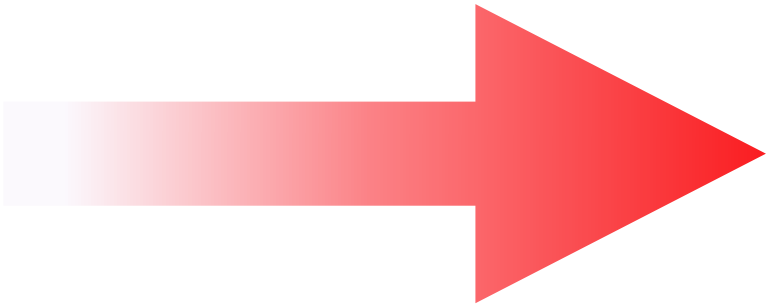
Take home message:
Both the micellar shell and core seems to contribute to drugs' encapsulation

How to overcome the stability-responsiveness challenge?

Micellar stability

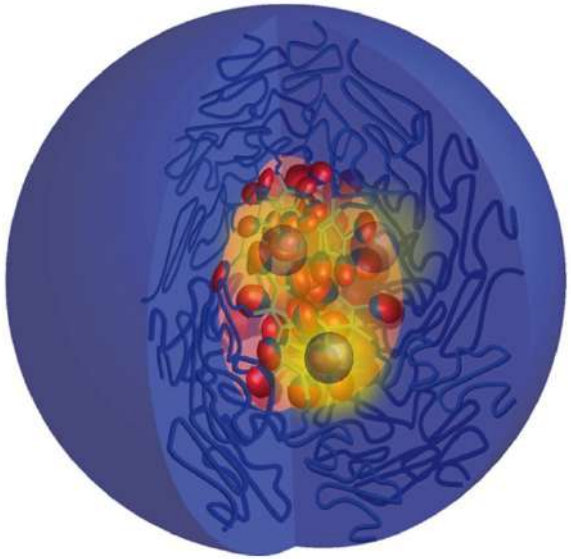


Hydrophobicity (and/or MW)



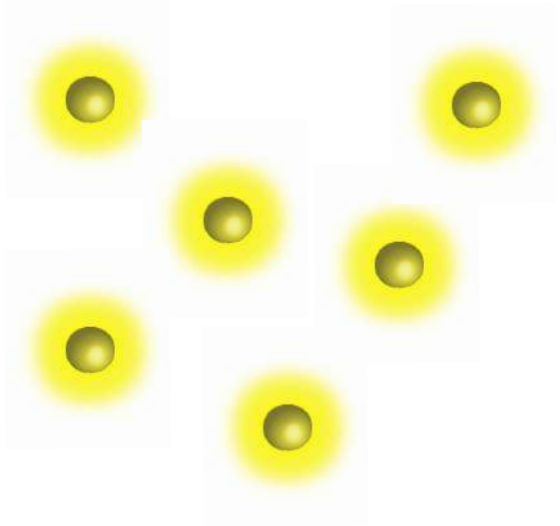
Enzymatic responsiveness

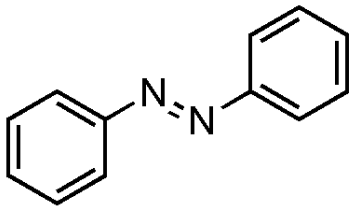
Can we turn-on the enzymatic activation?



Enzyme/Irradiation

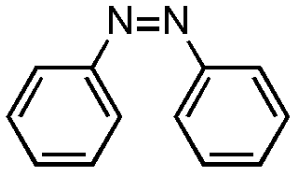
/





UV

Vis.



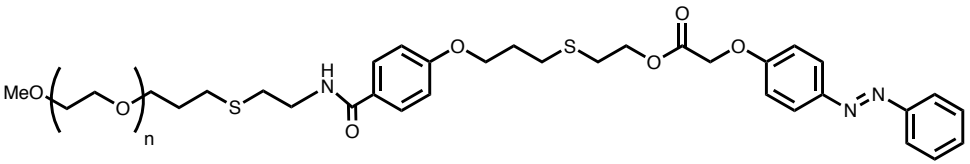
trans-azobenzene

NON-POLAR

cis-azobenzene

POLAR

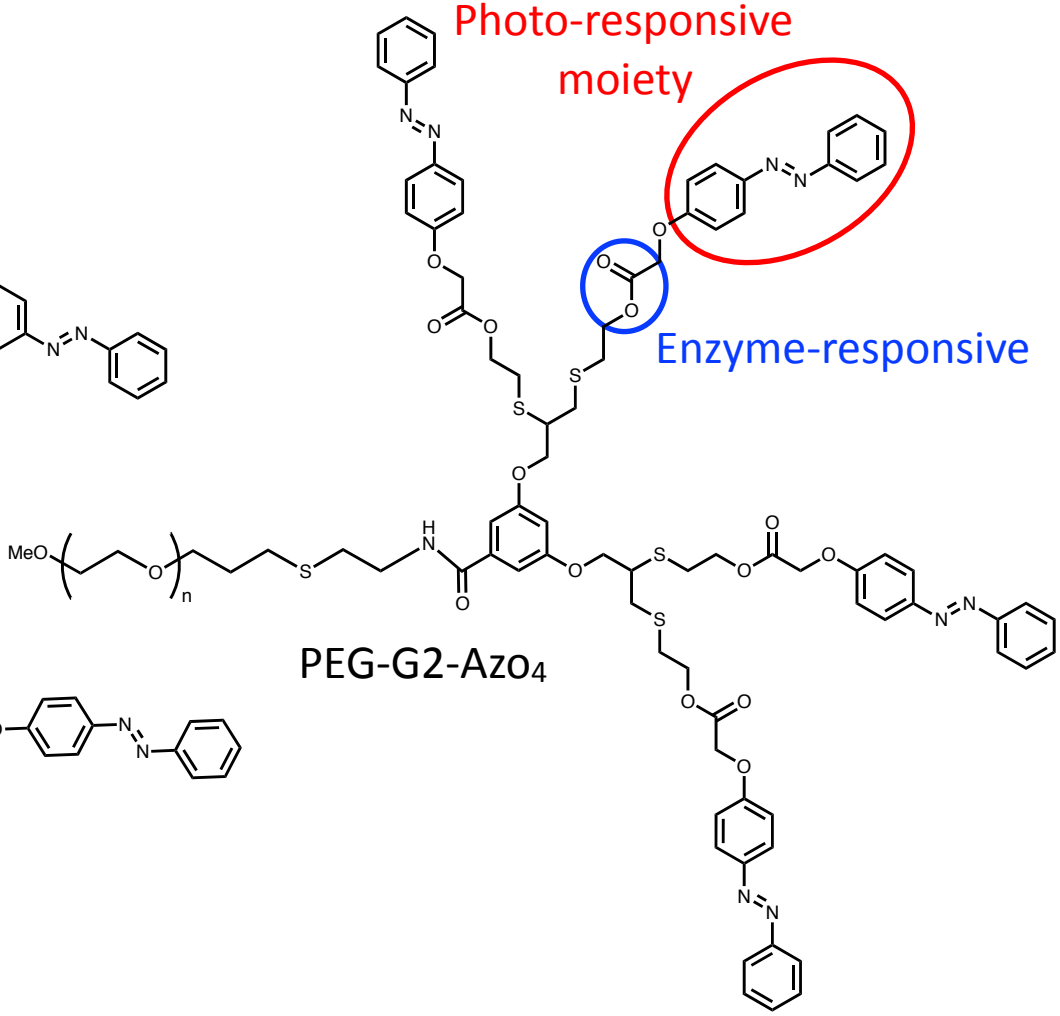
Designing photo/enzyme responsive polymers



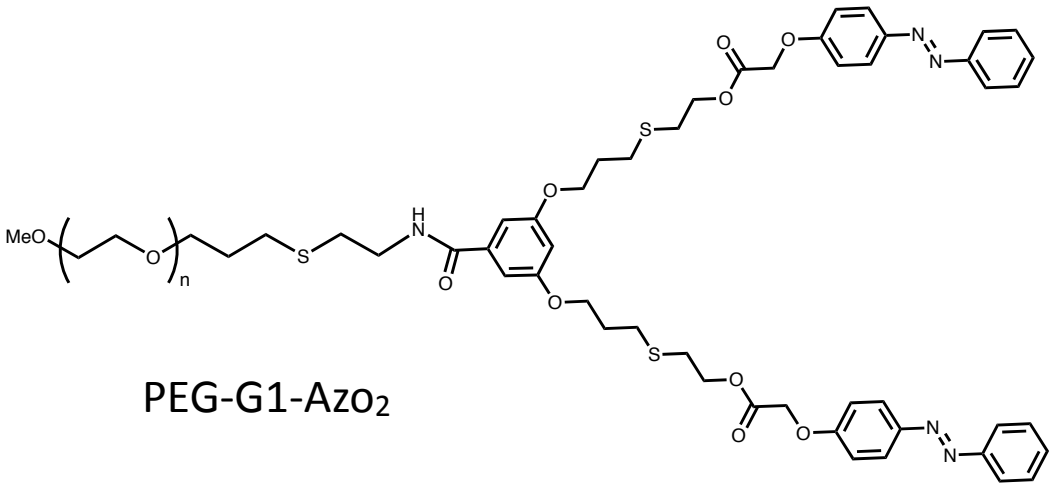
PEG-G0-Azo$_1$

Photo-responsive moiety

Enzyme-responsive

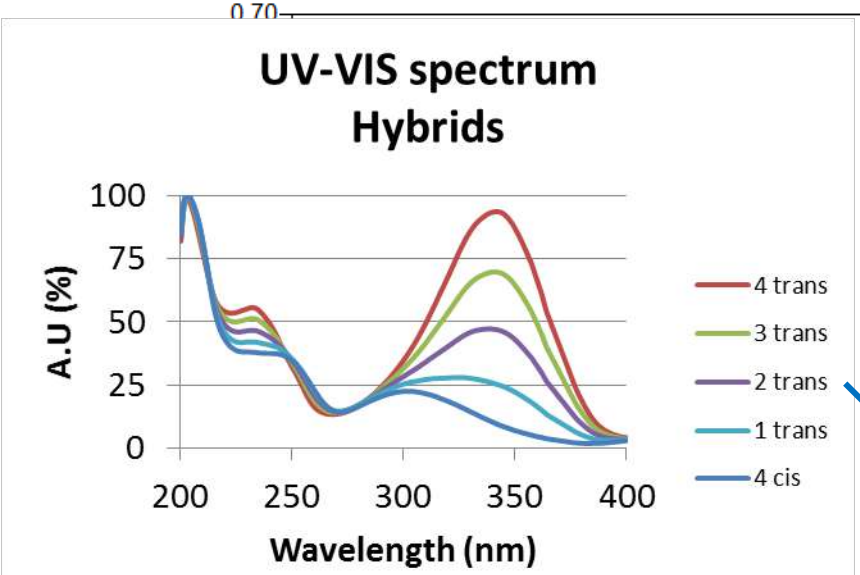


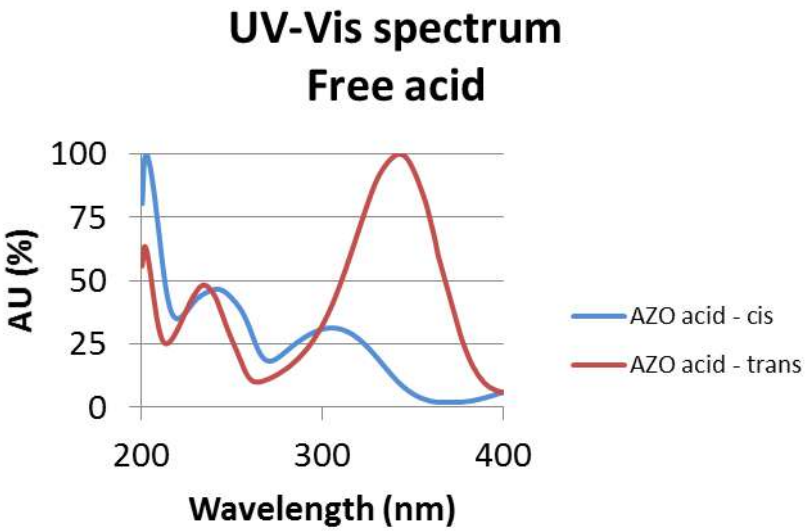
PEG-G2-Azo$_4$

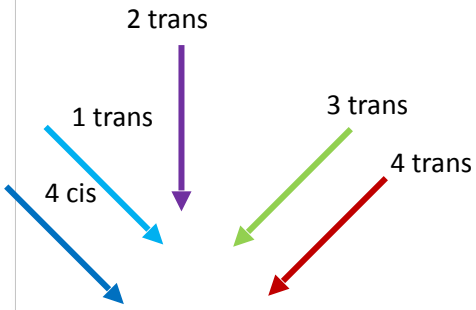


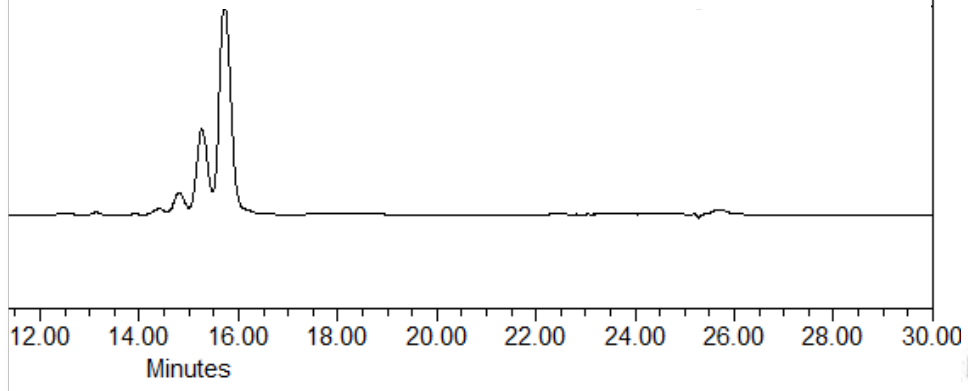
PEG-G1-Azo$_2$

HPLC – uncompleted reaction or high molecular resolution?

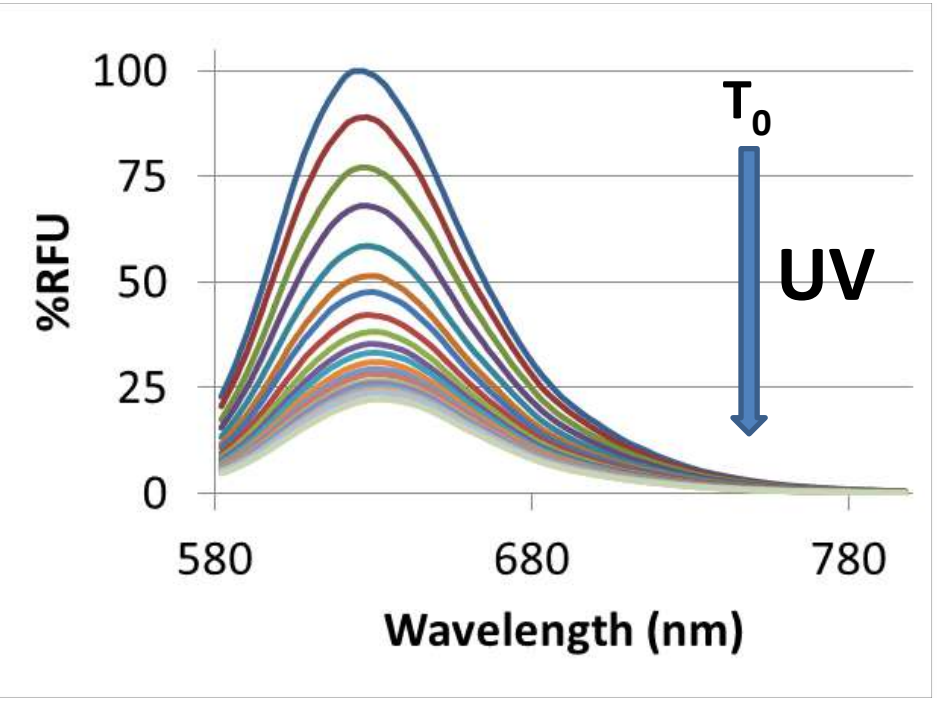


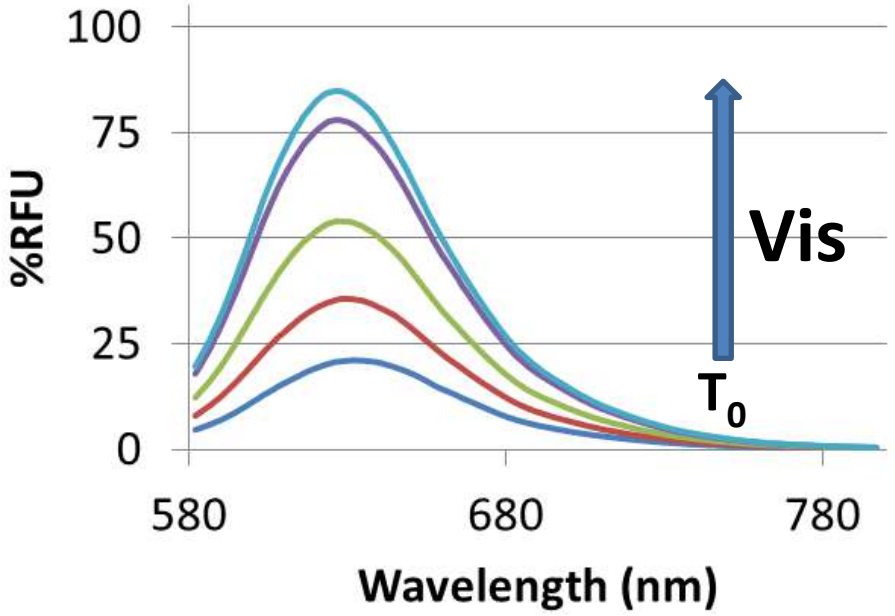


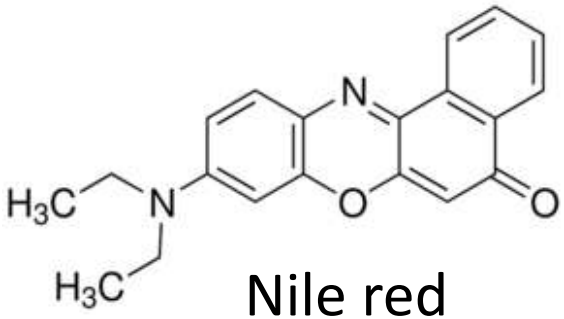




Fluorescence of Nile Red indicates significant polarity changes

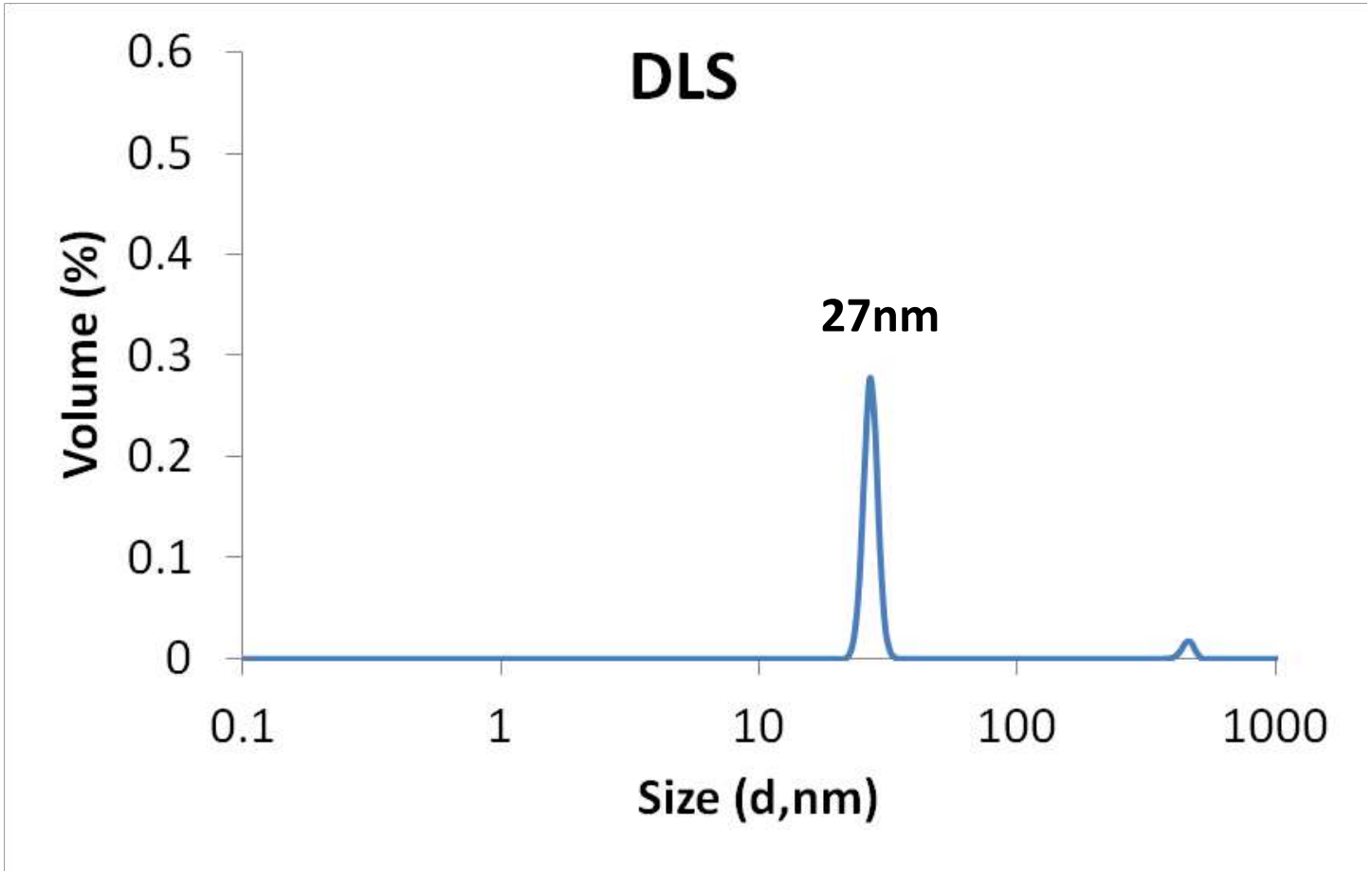




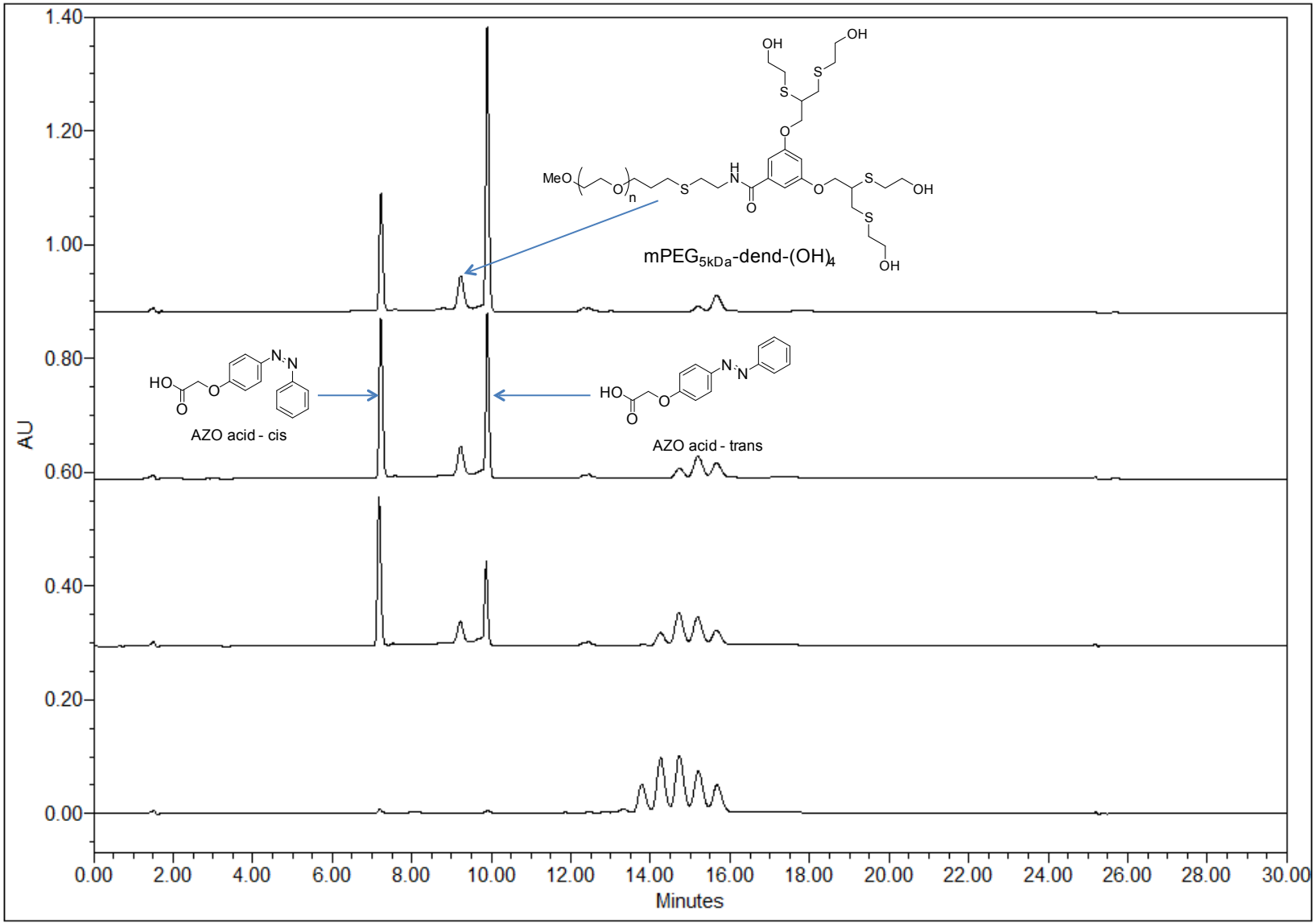


Nile red

How would UV light effects the micelles?

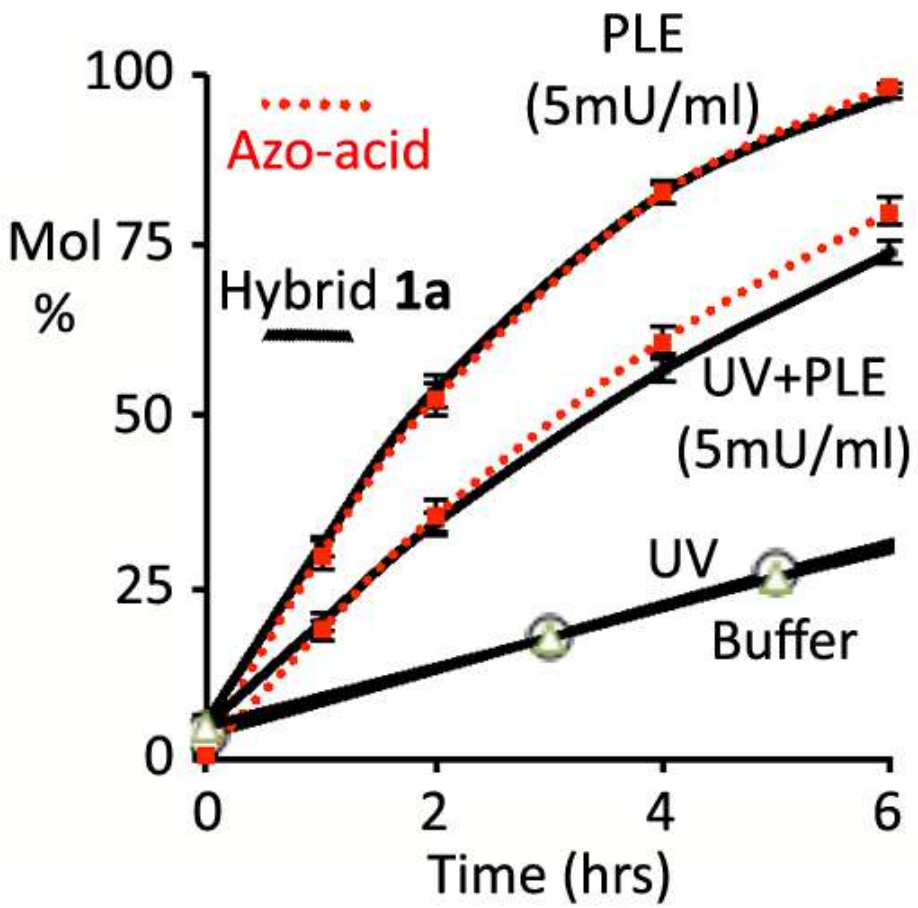


HPLC allows direct monitoring of the enzymatic activation

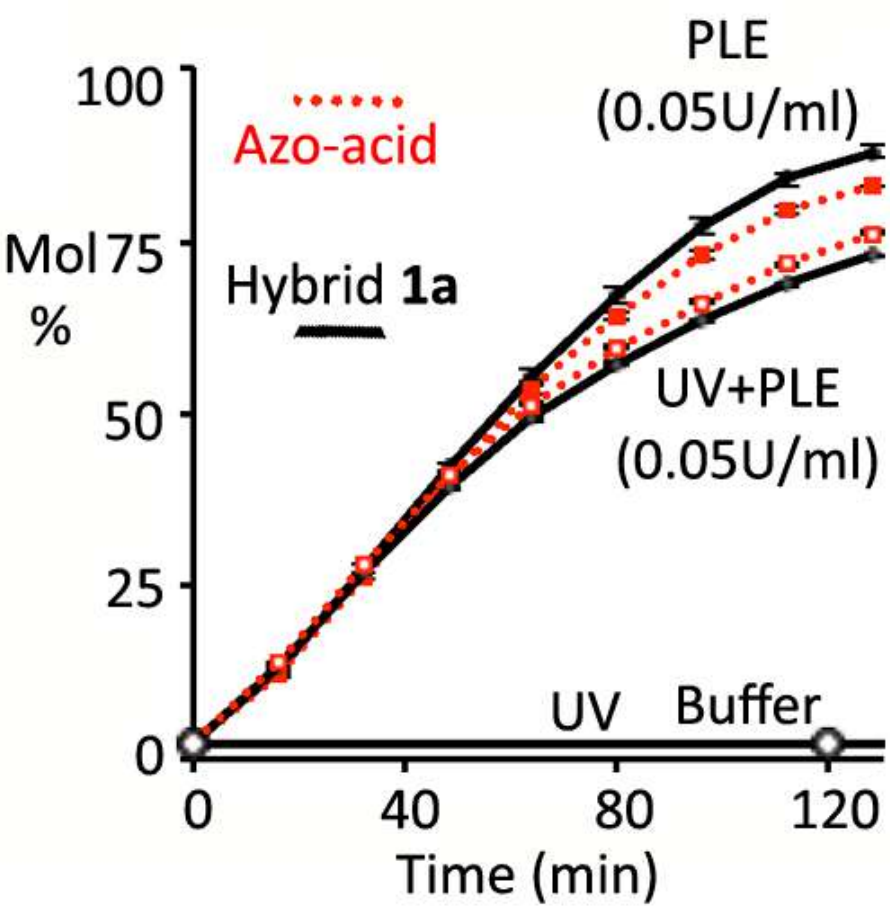


Substrate quality Vs. Availability (due to self-assembly)

PEG-G0-Azo$_1$ below CMC



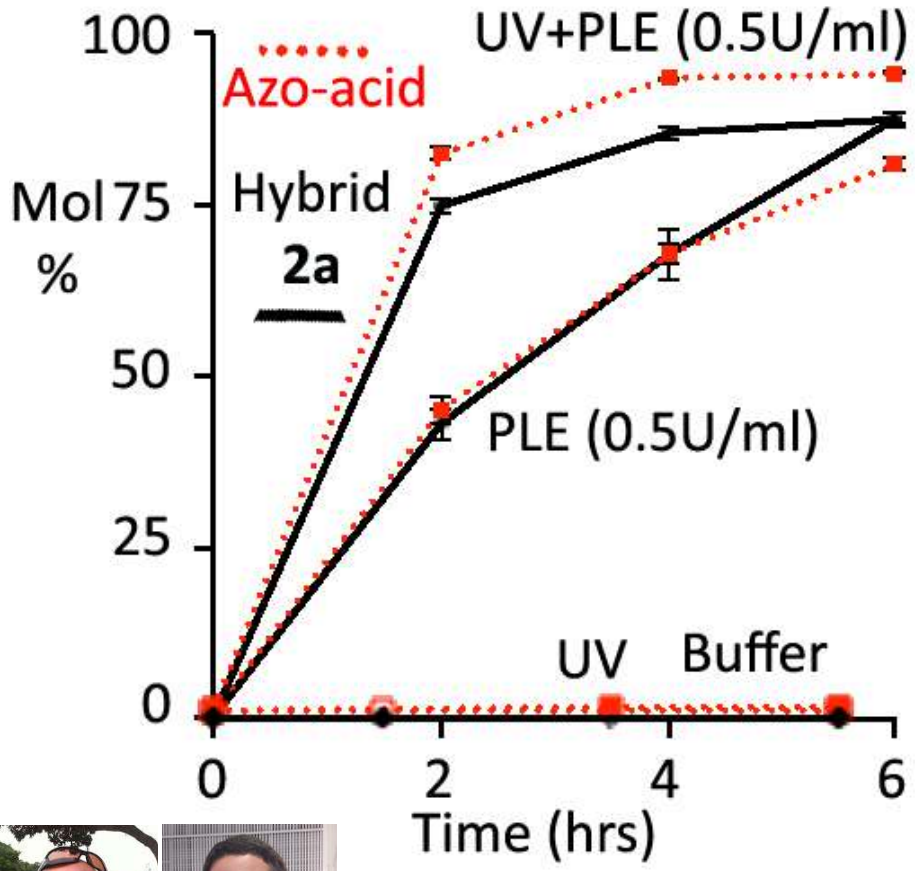
PEG-G0-Azo$_1$ above CMC



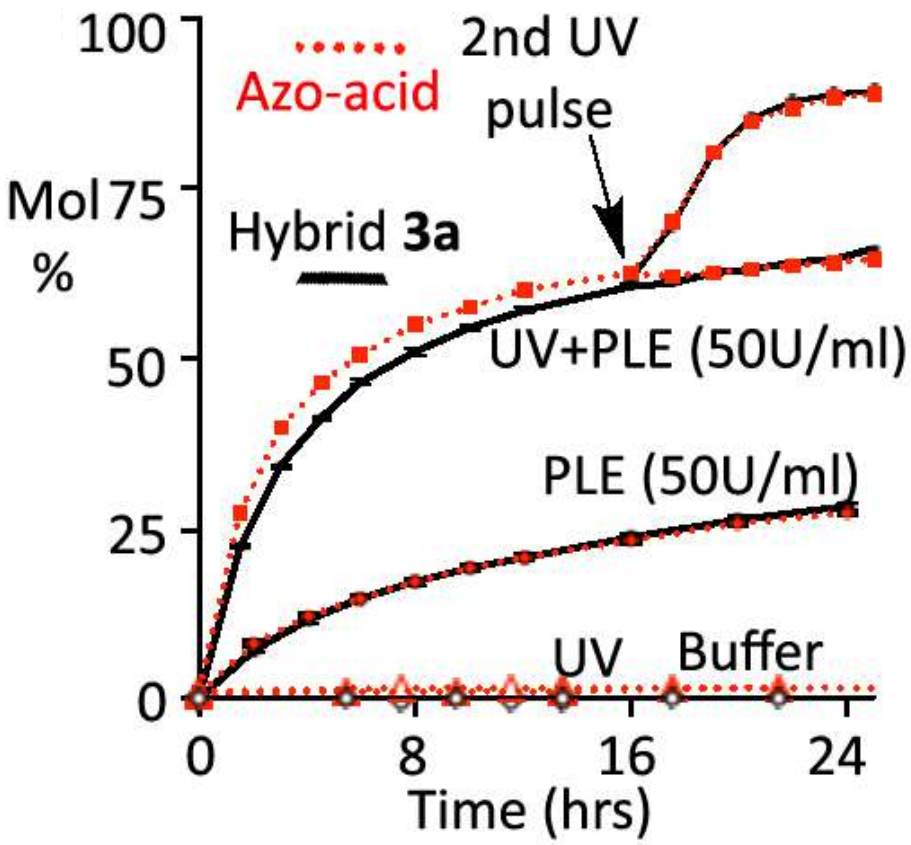
Self-assembly governs the kinetics as hydrophobicity increases

TAU

PEG-G1-Azo_2 above CMC



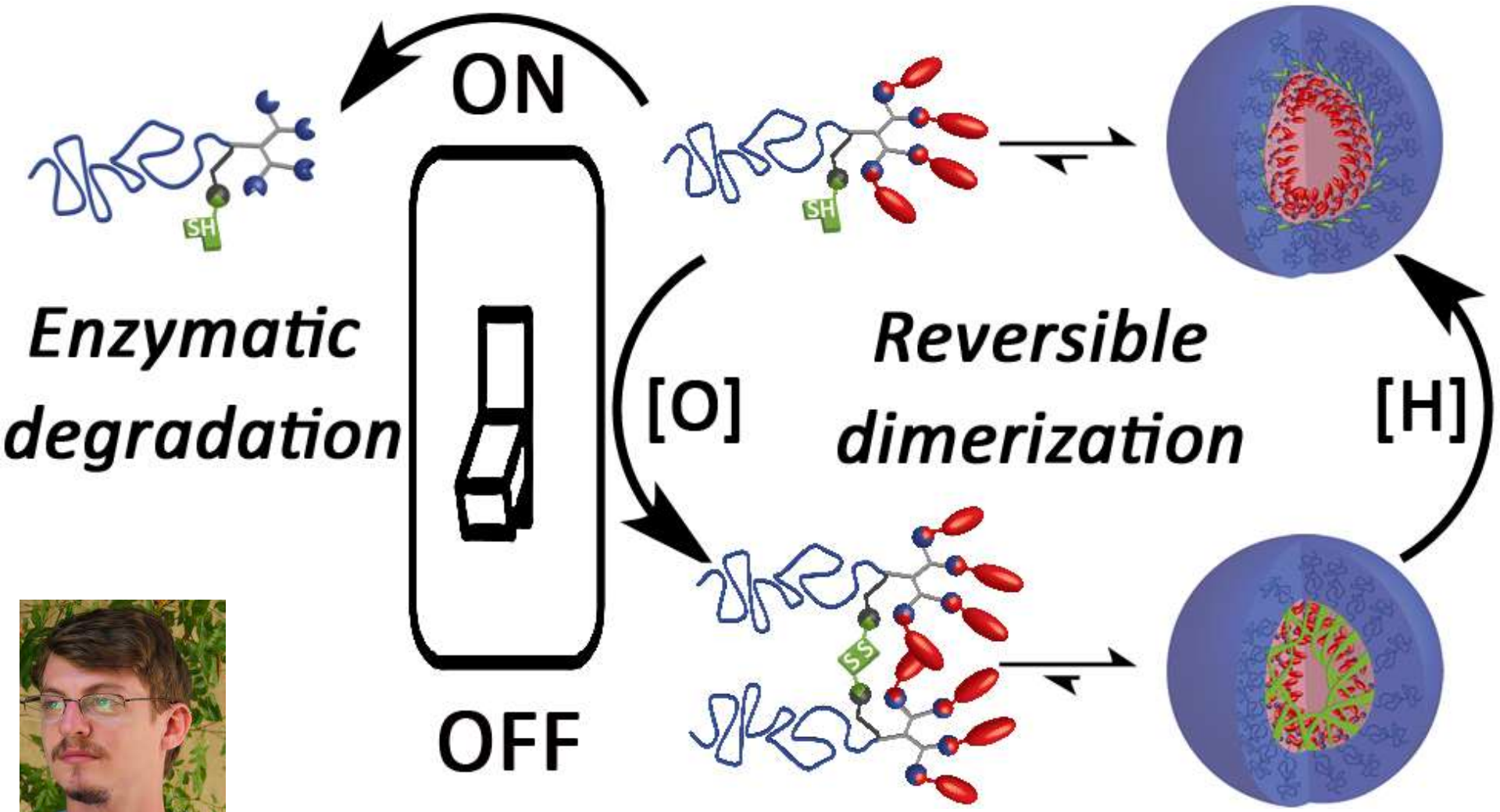
PEG-G2-Azo_4 above CMC



Harnoy & Slor et al. *Org. & Biomol. Chem.* ***2016***

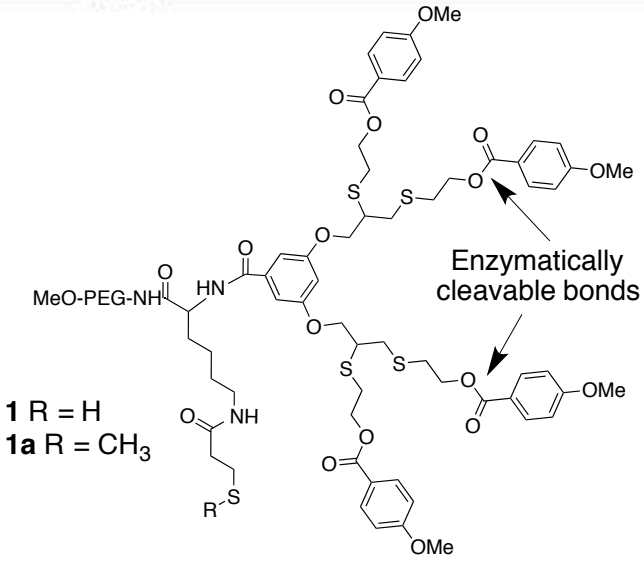
Controlling micellar stability by reversible dimerization

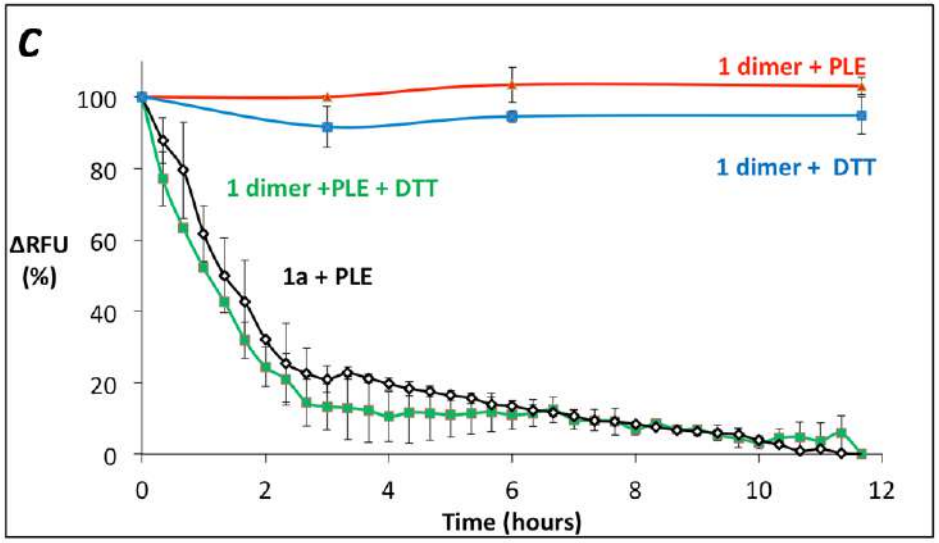
TAU

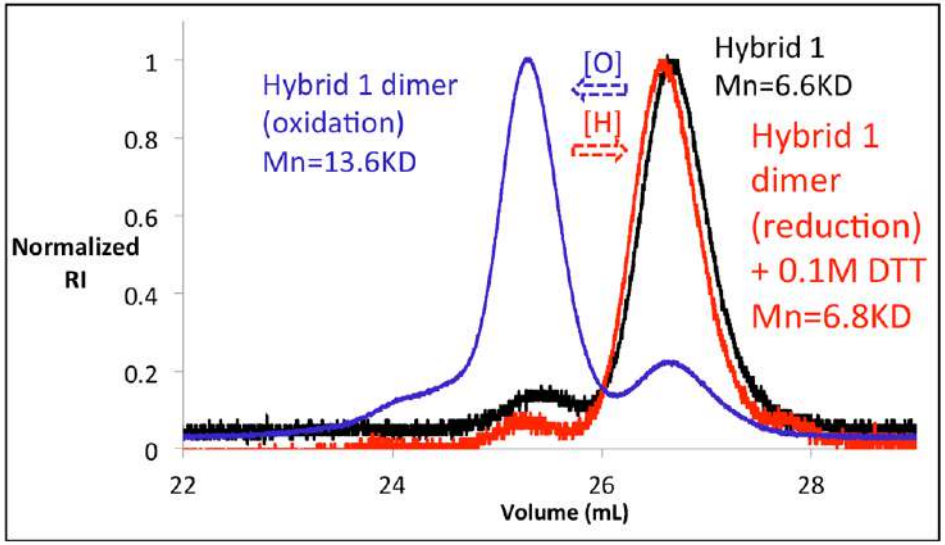


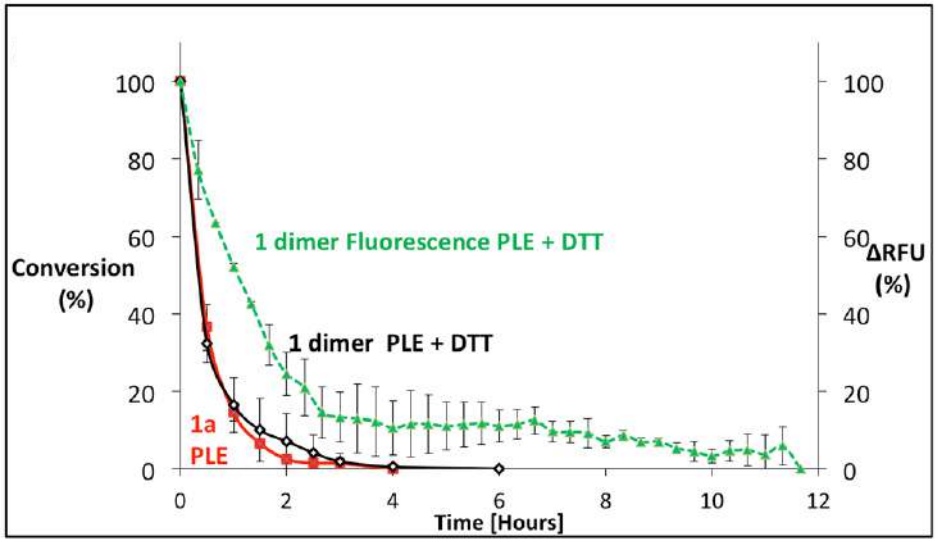
Ido Rosenbaum et al. Biomacromolecules **2017**

Highly stable dimers are reduced into responsive monomers

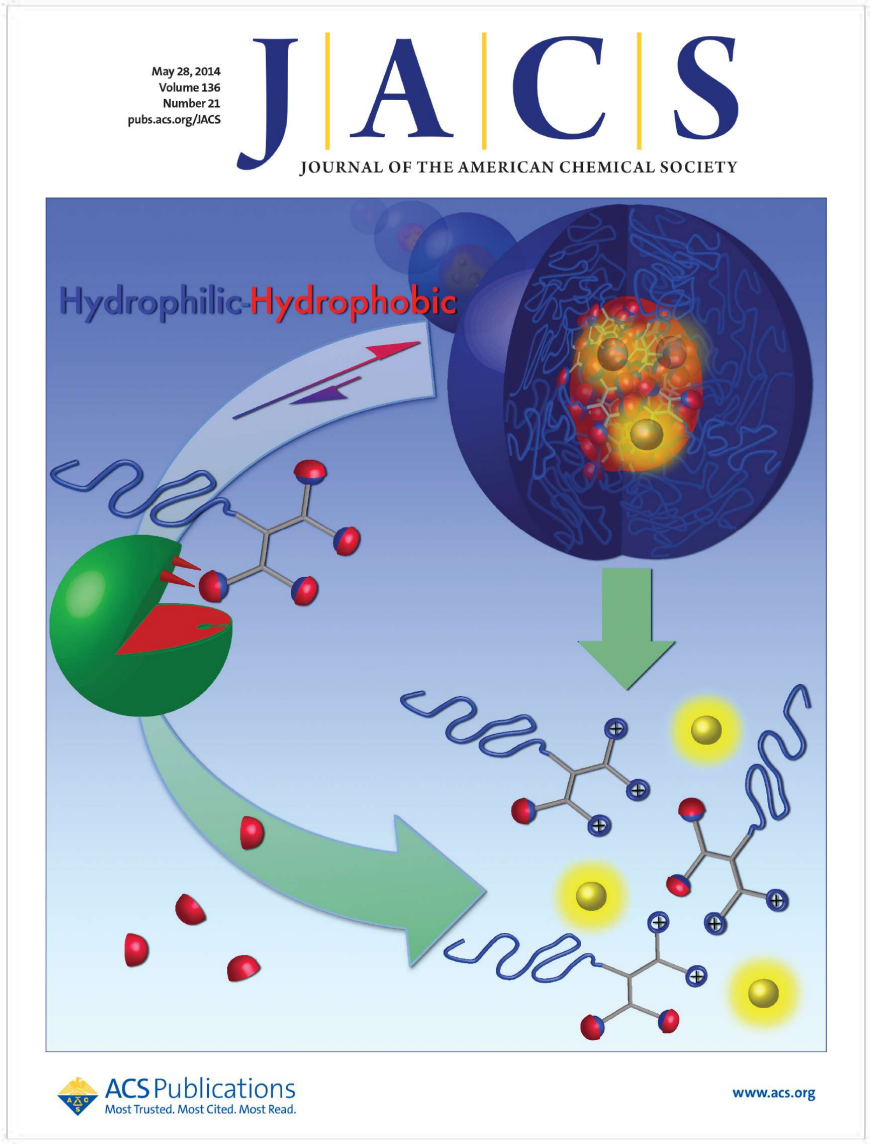


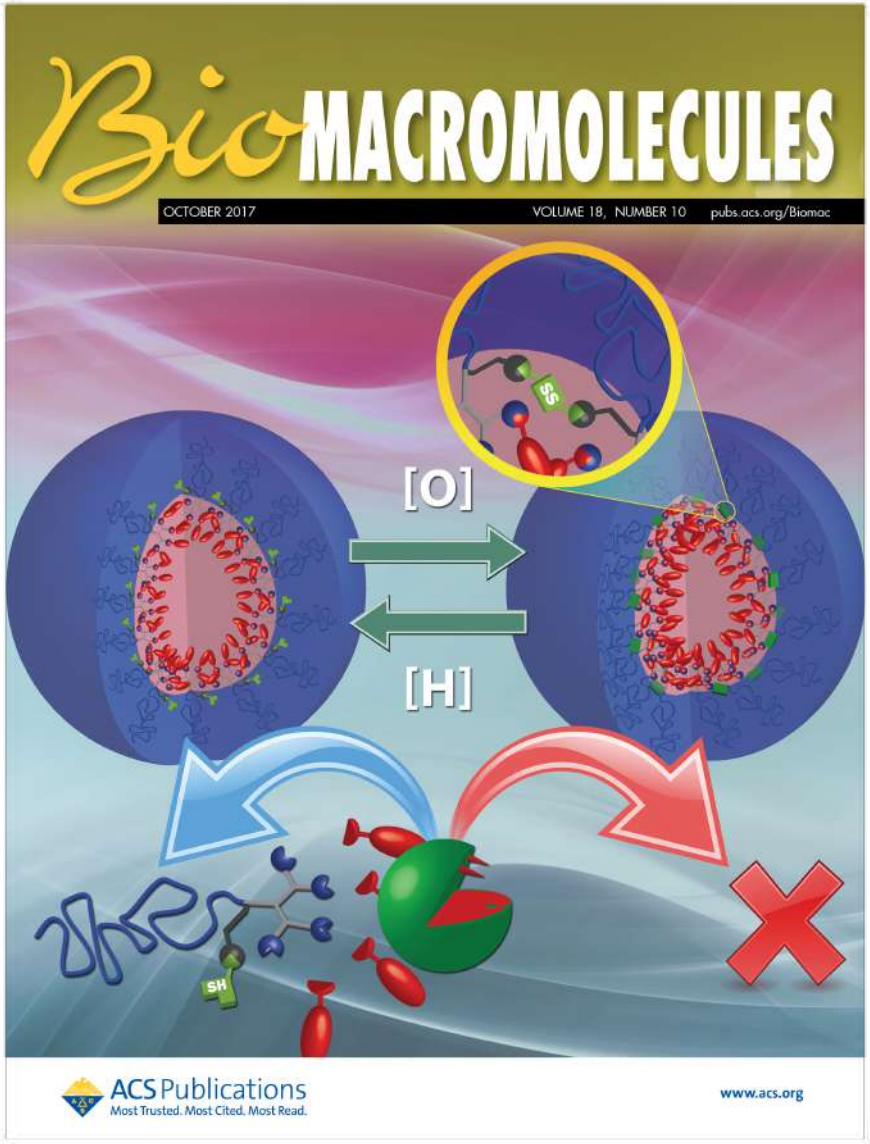






Understanding the challenges allows to suggest possible solutions





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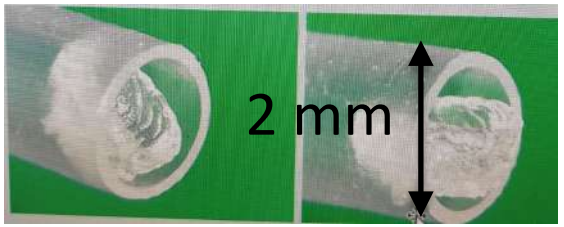
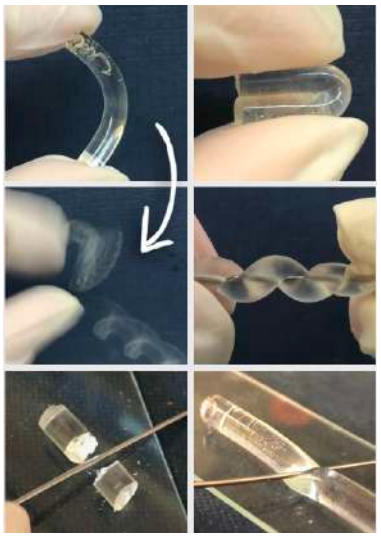
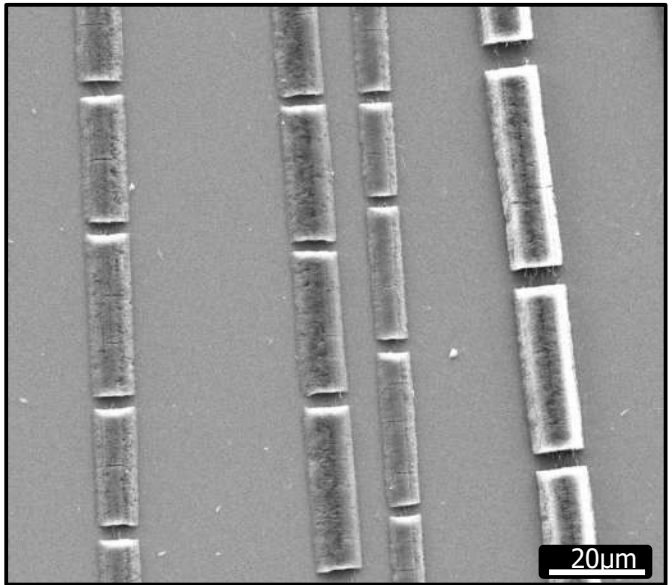
Thank you

Polymeric inks for 3D and 4D printing





Nano & Micro fabrication



Micellar catalysts