Tuning the membrane permeability of polymersome nanoreactors developed by aqueous emulsion polymerization-induced self-assembly

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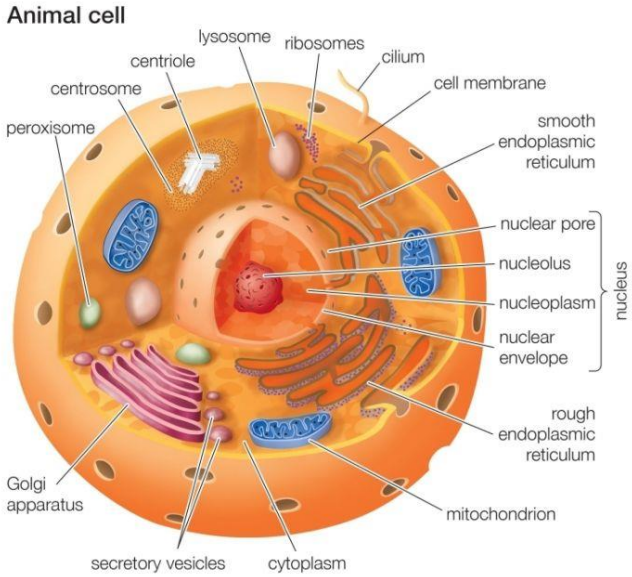
ACS Spring 2020 National Meeting & Expo

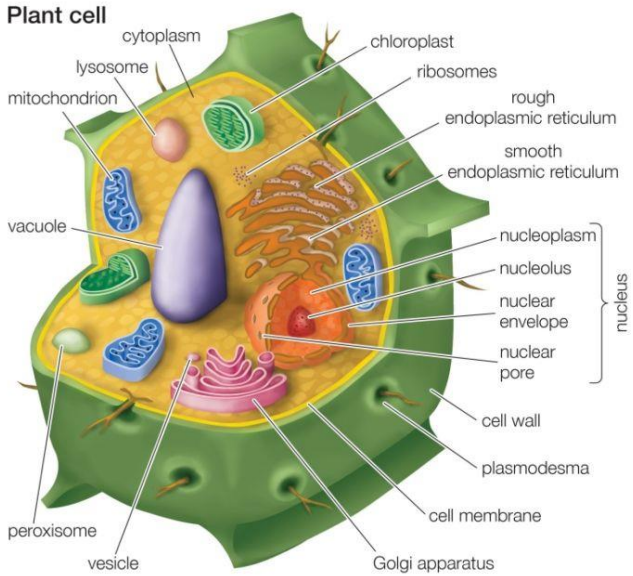
Excellence in Graduate Polymer Research Symposium

UNIVERSITY OF BIRMINGHAM

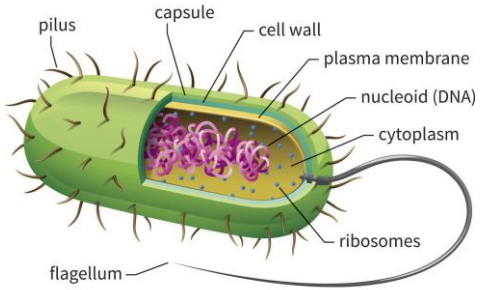
Compartmentalization and Confinement in Nature

Eukaryotic cells

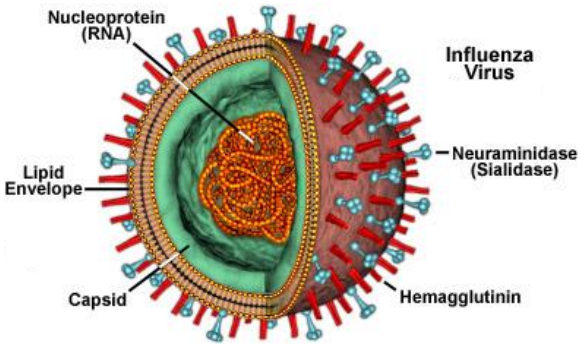




Bacteria

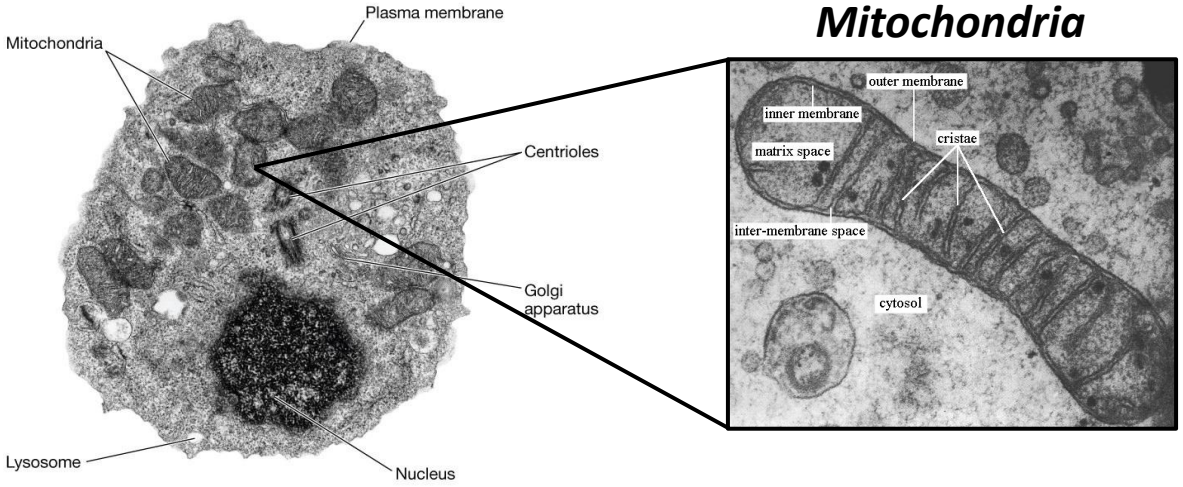


Viruses



In all living organisms:

- Structural and functional organization on both cellular and subcellular levels (*i.e*. organelles)
- Selective communication and transport of energy, nutrients and signaling molecules
- Vital biological reactions occur in confined compartments
- Protection from external detrimental conditions



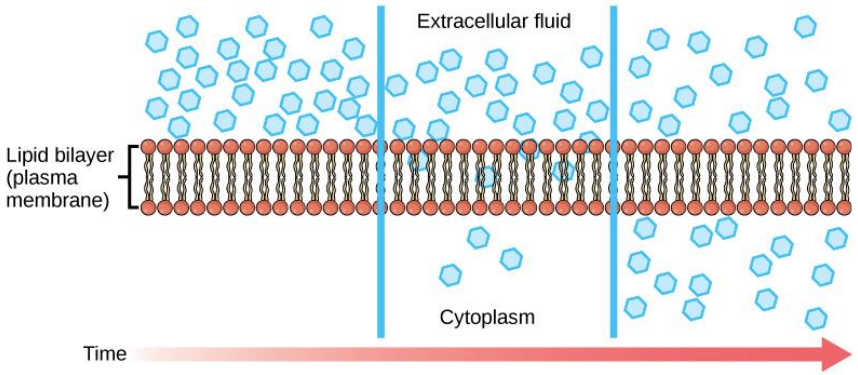
Mitochondria

Nobel Prize in Physiology or Medicine 1974
A. Claude, C. de Duve, and G. E. Palade
Nobel Prize in Chemistry 2003
P. Agre, and R. MacKinnon
Nobel Prize in Chemistry 2009
V. Ramakrishnan, T. A. Steitz, and A. E. Yonath

Transport of Molecules Across Cell/Organelle Membranes

Passive Transport

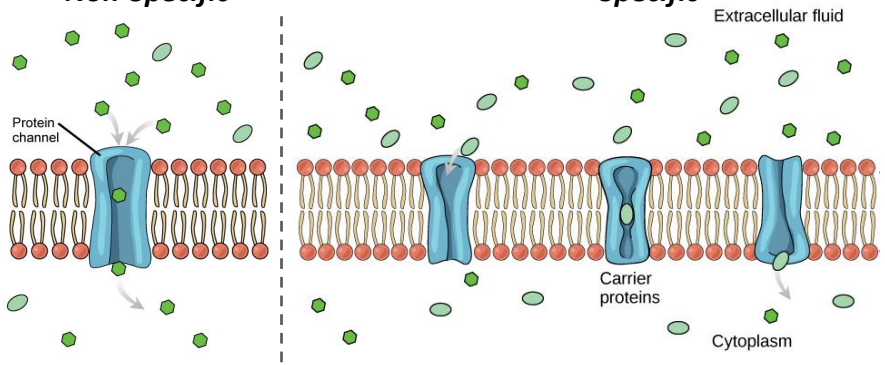
Simple Diffusion



Carrier-Mediated Diffusion

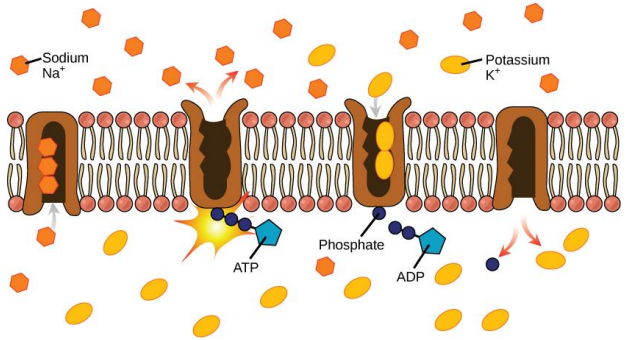
Non-Specific

Specific



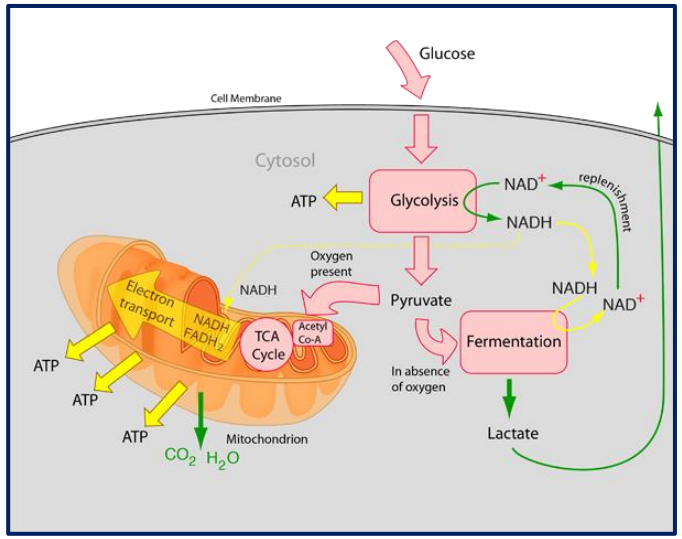
Active Transport

Energy-Promoted Diffusion



Application of Membrane Diffusion

(Glycolysis and Krebs Cycle for ATP production)



Two Main Pathways for Molecule Transport across Membranes:

1. ***Passive Transport:***

Either <u>non-specific</u> or <u>specific</u> permeability

Simple diffusion across semi-permeable membranes

Carrier-mediated transport *via* channel membrane proteins

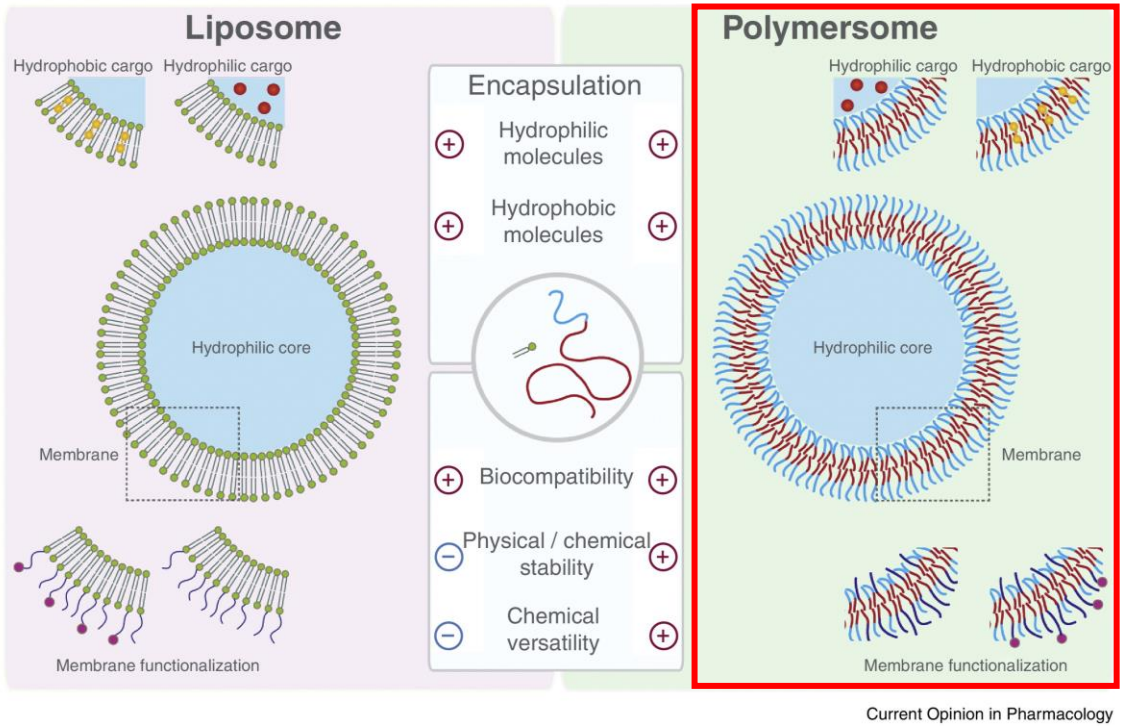
2. ***Active Transport:***

Diffusion across the membrane requires the consumption of energy as fuel

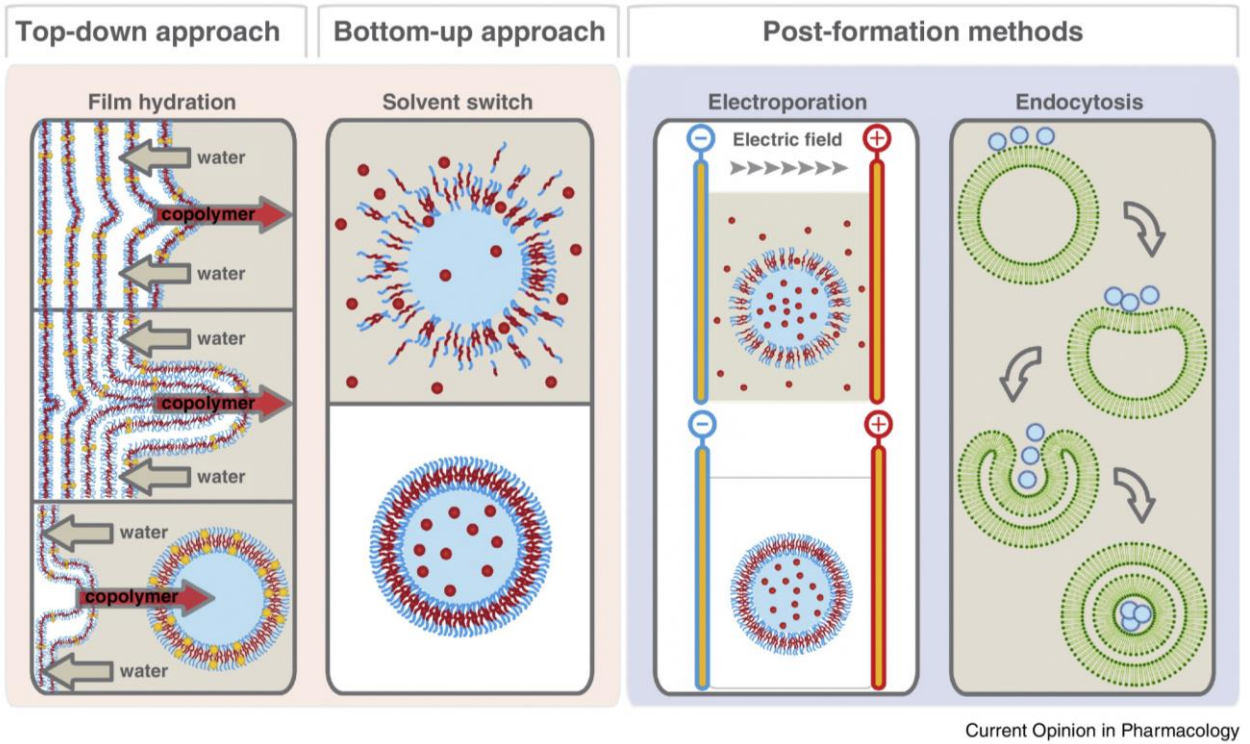
Different diffusion/transport mechanisms allow for vital enzyme-catalyzed cascade reactions (e.g. cellular respiration) to occur selectively in confined environments

Structural and Functional Cell-Mimicry

Platform Design



Conventional Methodologies for Small Molecule Encapsulation



Liposomes & Polymersomes:

Self-assembled bilayer nanostructures

Minimal cell-mimicking synthetic analogues

Able to incorporate both hydrophilic and hydrophobic cargo

Application as delivery vehicles, functional artificial organelles and catalytic nanoreactors

Polymersomes have superior physical and chemical stability

Preparation of polymersomes *via* conventional block copolymer self-assembly:

Laborious multi-step methodologies

Dilute conditions – low solids concentration (≤ 1% w/w)

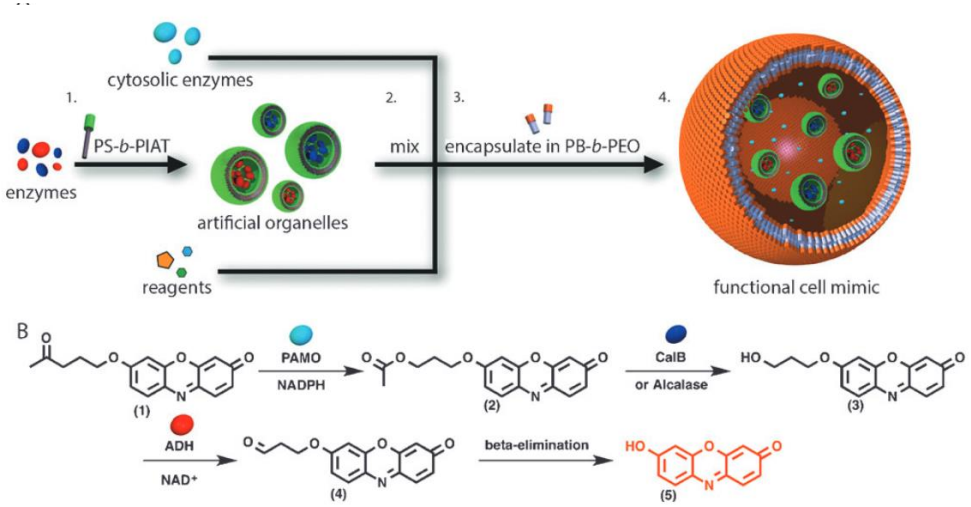
Require post-polymerization processing to target pure morphologies

Require the use of toxic organic solvents

Messager, *et al*. *Curr. Opin. Pharmacol.*, 2014, **18**, 104-111, Rideau, *et al*. *Chem. Soc. Rev.*, 2018, **47**, 8572-8610

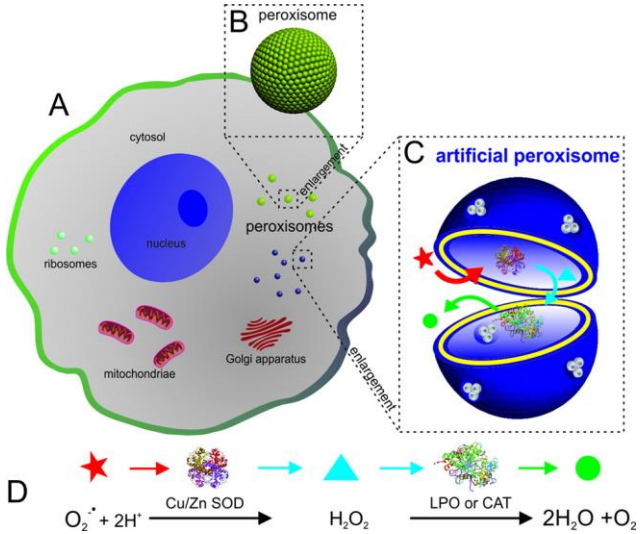
Catalytic Nanoreactors & Artificial Organelles

Inherent Permeability



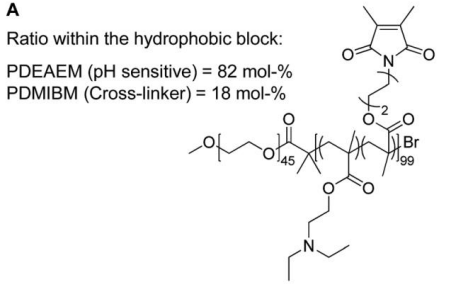
Peters, *et al*. *Angew. Chem. Int. Ed*., 2014, **53**, 146-150

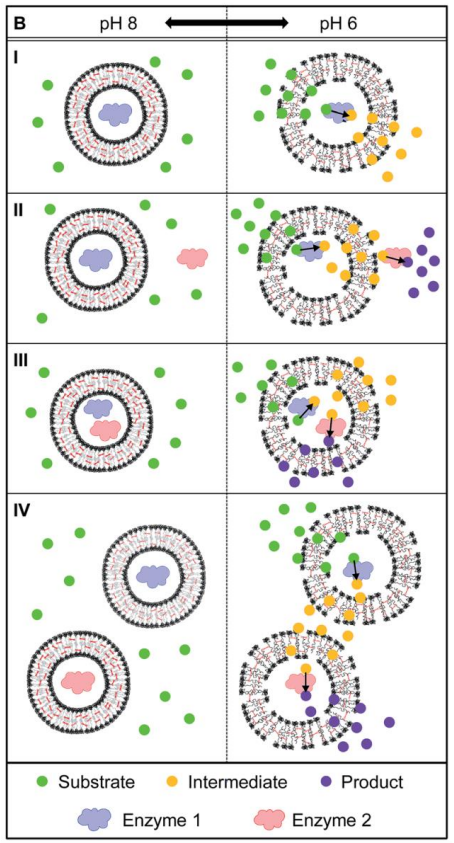
Membrane Protein Incorporation



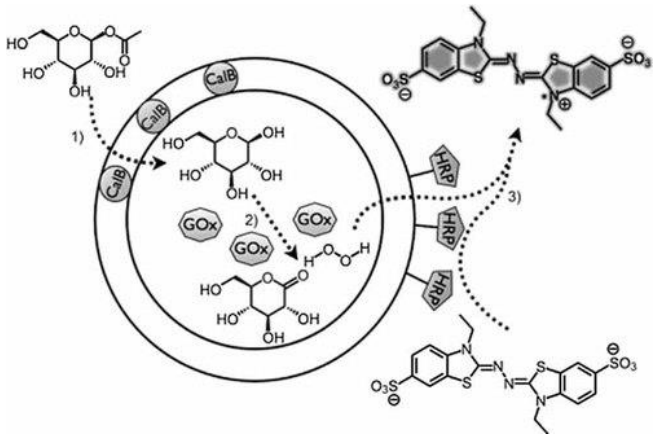
Tanner, *et al*. *Nano Lett*., 2013, **13**, 2875-2883

Stimuli-Responsive Membranes





Grafe, *et al*. *Nanoscale*, 2014, **6**, 10752-10761



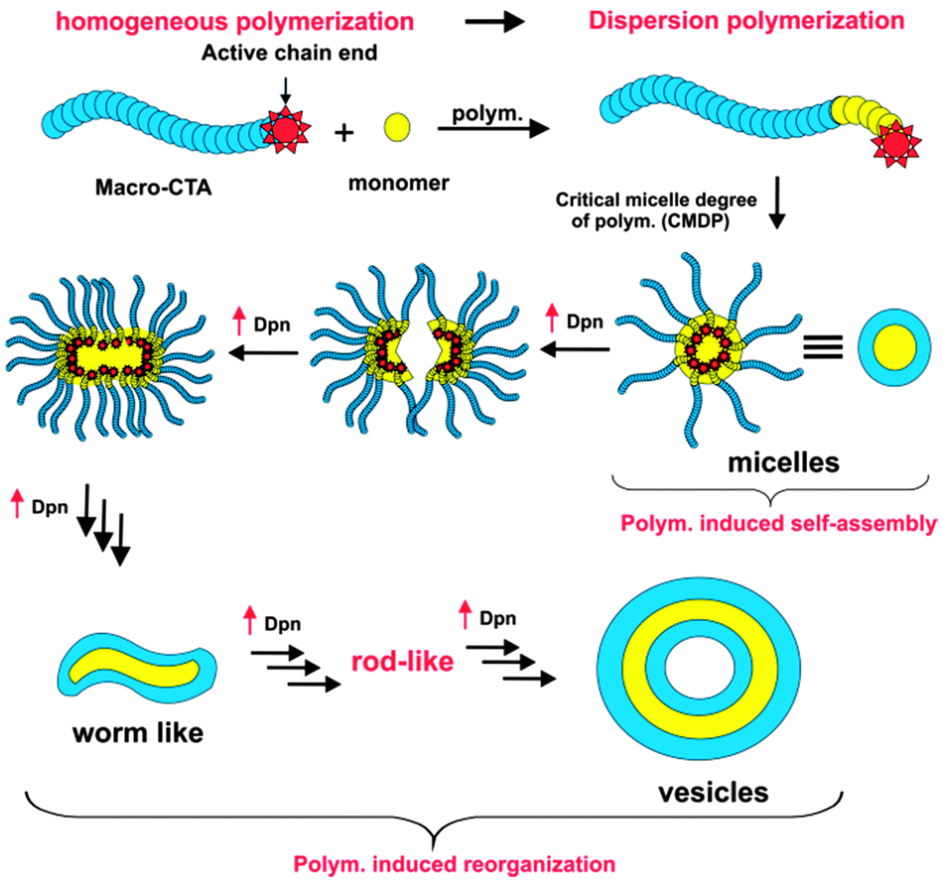
van Dongen, *et al*. *Chem. Eur. J.,* 2009, **15**, 1107-1114

Common methodologies for permeability enhancement of nanoreactors toward substrates and catalysis products involve:

- Incorporation of channel-forming transmembrane proteins (e.g. OmpF, aquaporins)
- Stimuli-responsive membranes
- Inherently permeable membranes

Polymerization-Induced Self-Assembly (PISA)

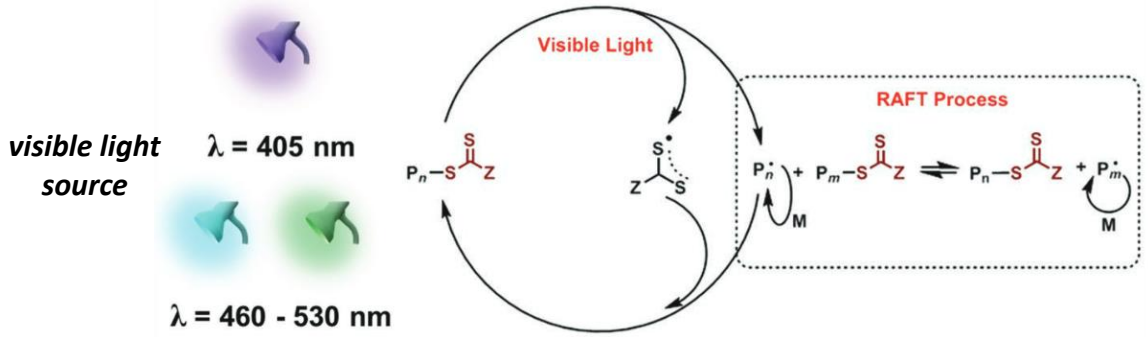
PISA Mechanism



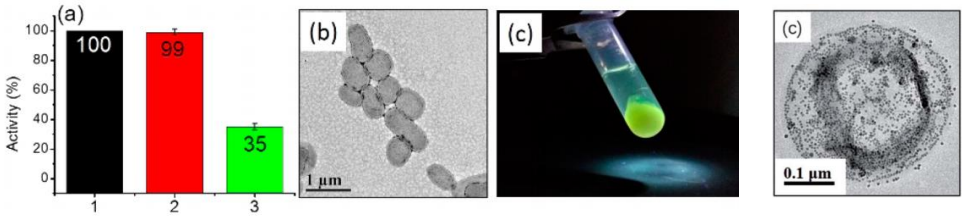
- One-pot polymerization and self-assembly process
- High solids concentration (10-30% w/w)
- Compatible with aqueous/organic media
- Reproducible
- Facile access to higher-order morphologies
- Radical (RAFT, ATRP, NMP) and non-radical methodologies (ROMP, ROP)

Karagoz, *et al*. *Polym. Chem*., 2014, **5**, 350-355
Canning, *et al*. *Macromolecules*, 2016, **49**, 1985-2001

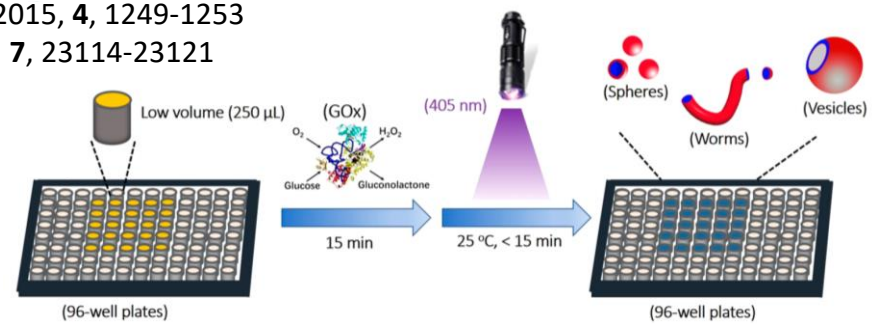
Photoinitiated RAFT-mediated PISA (Photo-PISA)



Yeow, *et al. Adv. Sci*., 2017, **4**, 1700137



Tan, *et al. ACS Macro Lett*., 2015, **4**, 1249-1253
Tan, *et al. RSC Adv*., 2017, **7**, 23114-23121



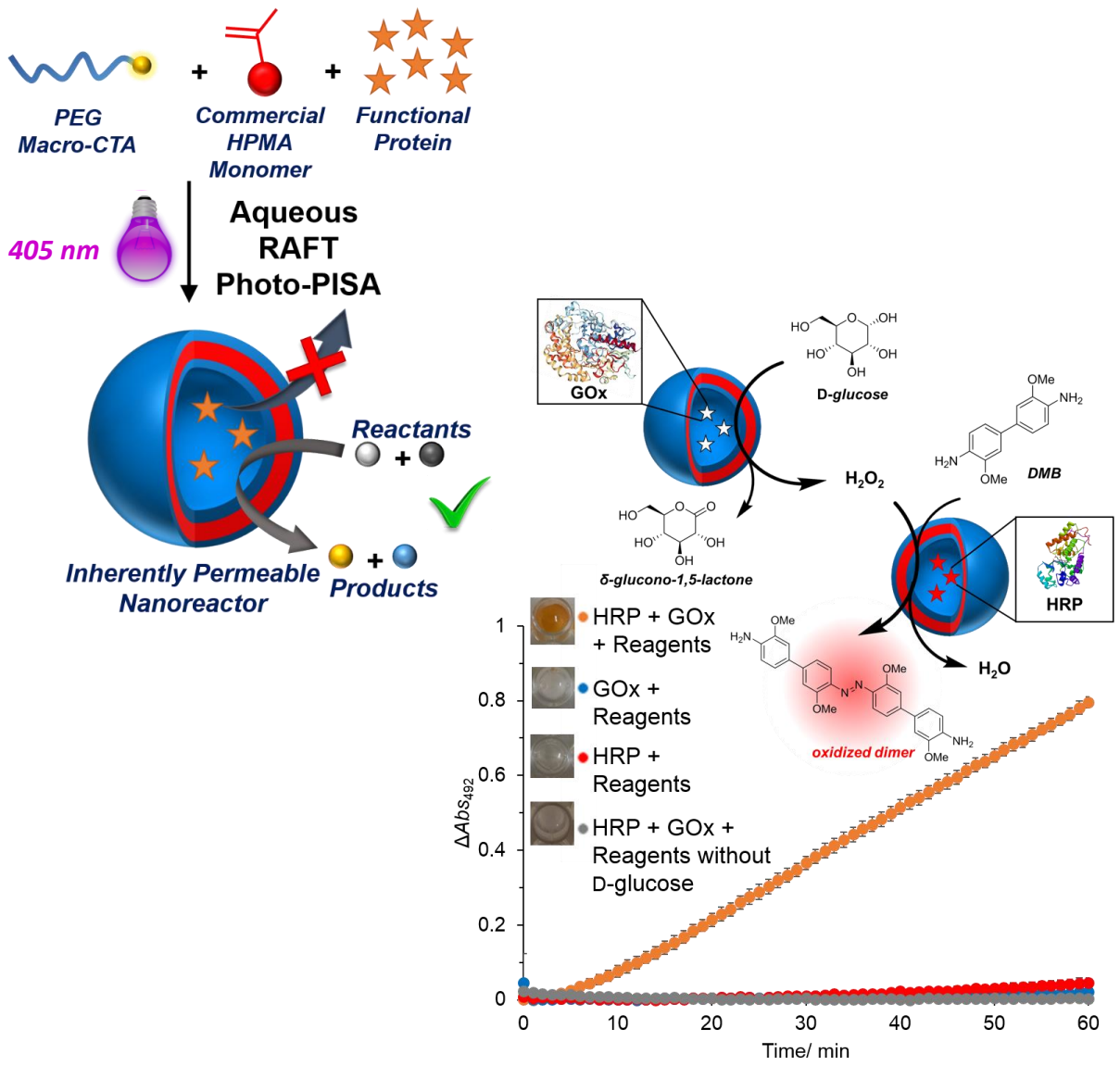
Tan, *et al. Macromolecules*, 2017, **50**, 5798-5806

Aqueous Photo-PISA:

- Ambient temperatures compared to thermally initiated PISA (50 - 70 °C)
- Oxygen-tolerant processes (under certain conditions)
- One-pot non-disruptive encapsulation of INPs, drugs, fluorophores and enzymes into polymersomes
- Biologically relevant applications

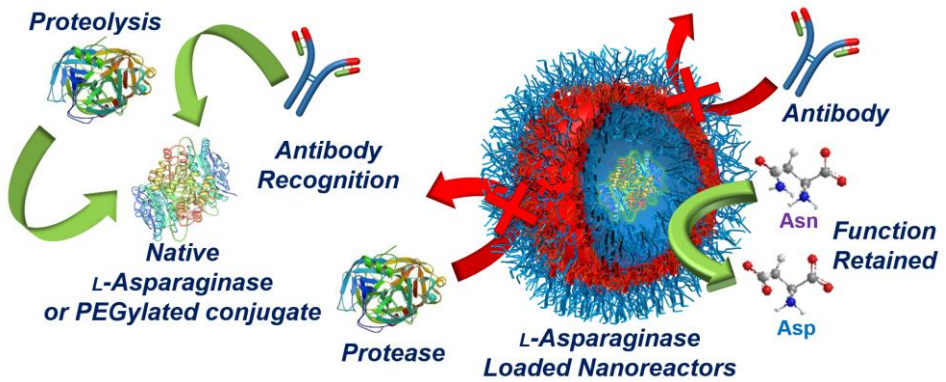
Functional Polymersome Nanoreactors Developed in the O'Reilly Group

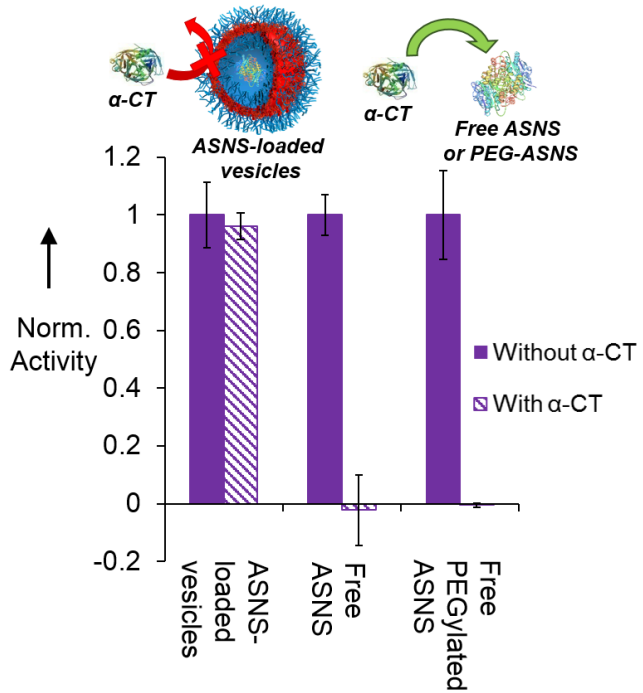
Semi-Permeable Cascade Nanoreactors

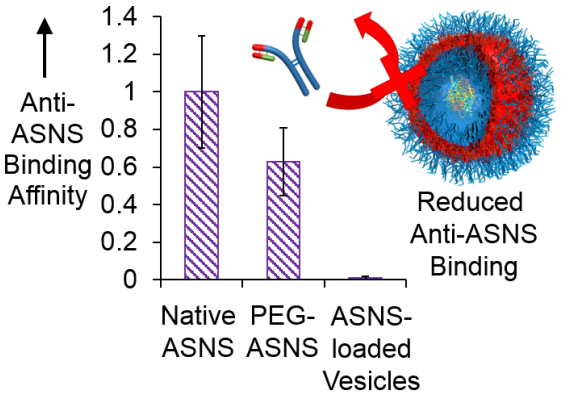


Blackman, *et al*. *ACS Macro Lett.*, 2017, **6**, 1263-1267

Therapeutic Nanoreactors



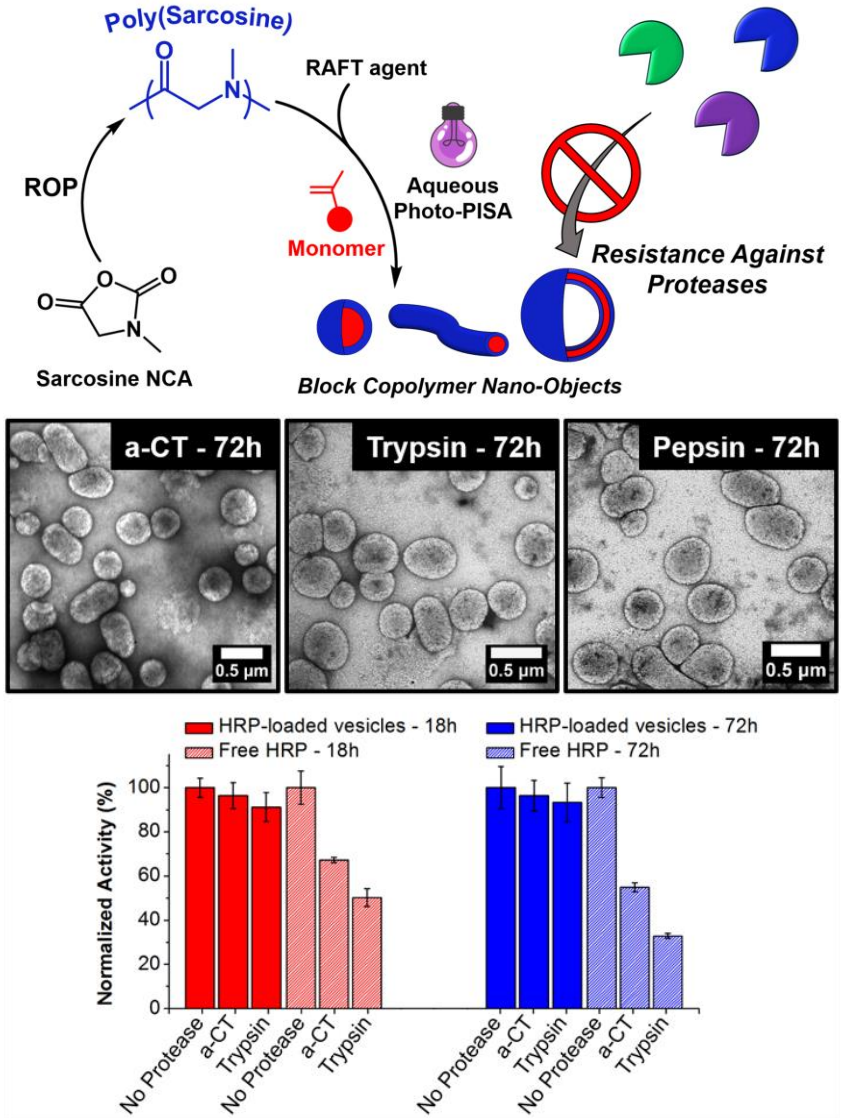




Blackman, *et al*. *ACS Cent. Sci.*, 2018, **4**, 718-723

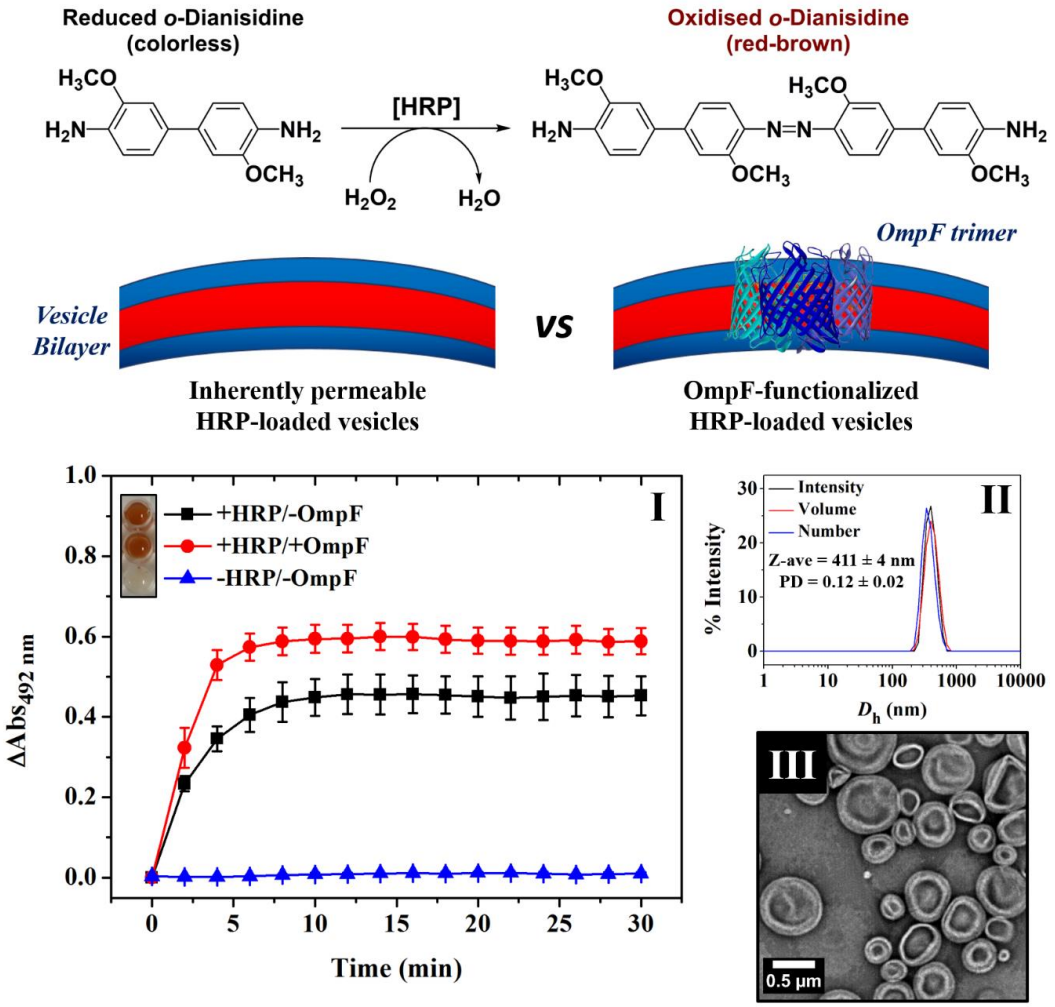
Functional Polymersome Nanoreactors Developed in the O'Reilly Group

New Corona Chemistries



Varlas, *et al*. *Biomacromolecules*, 2018, **19**, 4453-4462

Membrane Protein Incorporation



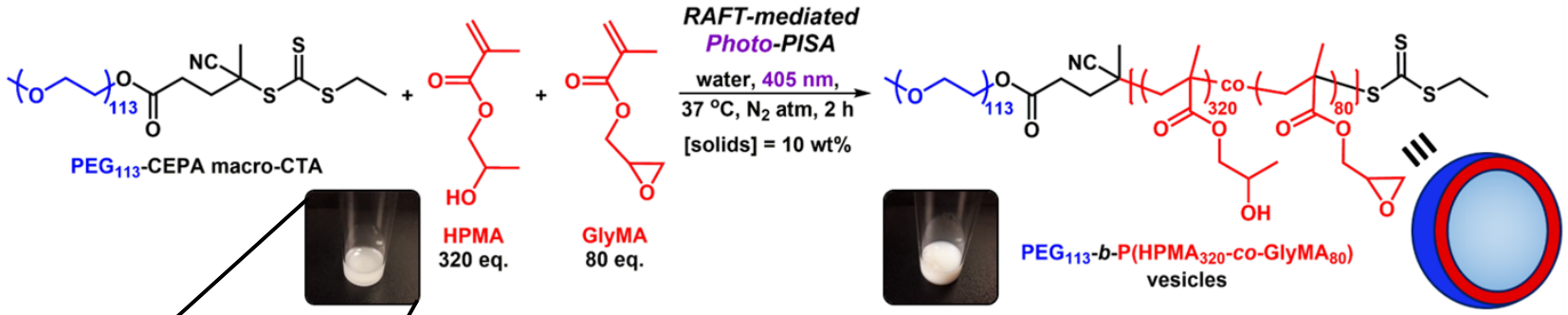
Varlas, *et al*. *Macromolecules*, 2018, **51**, 6190-6201

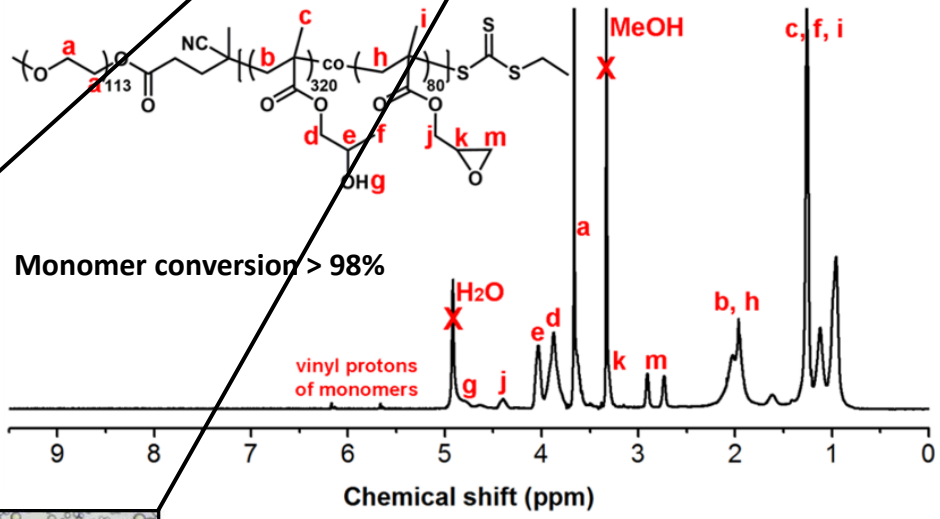
Emerging Challenge

Can we fabricate catalytic polymersome nanoreactors with programmed size-selective permeability exploiting the advantages of aqueous photo-PISA?

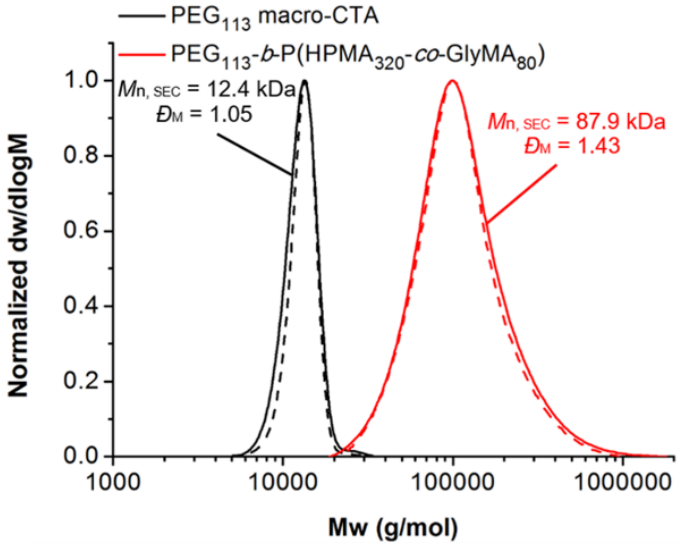
Polymersome Nanoreactors with Tunable Permeability *via* Emulsion Photo-PISA

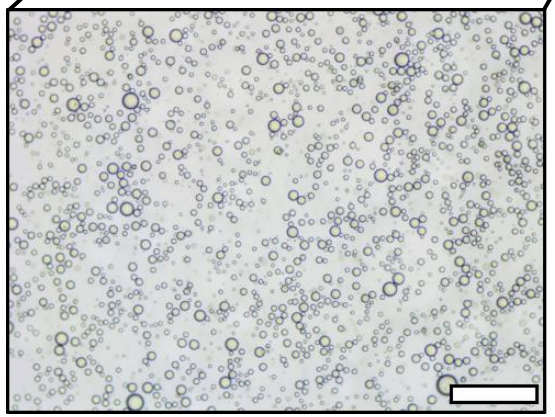
Experimental Design:





Monomer conversion > 98%



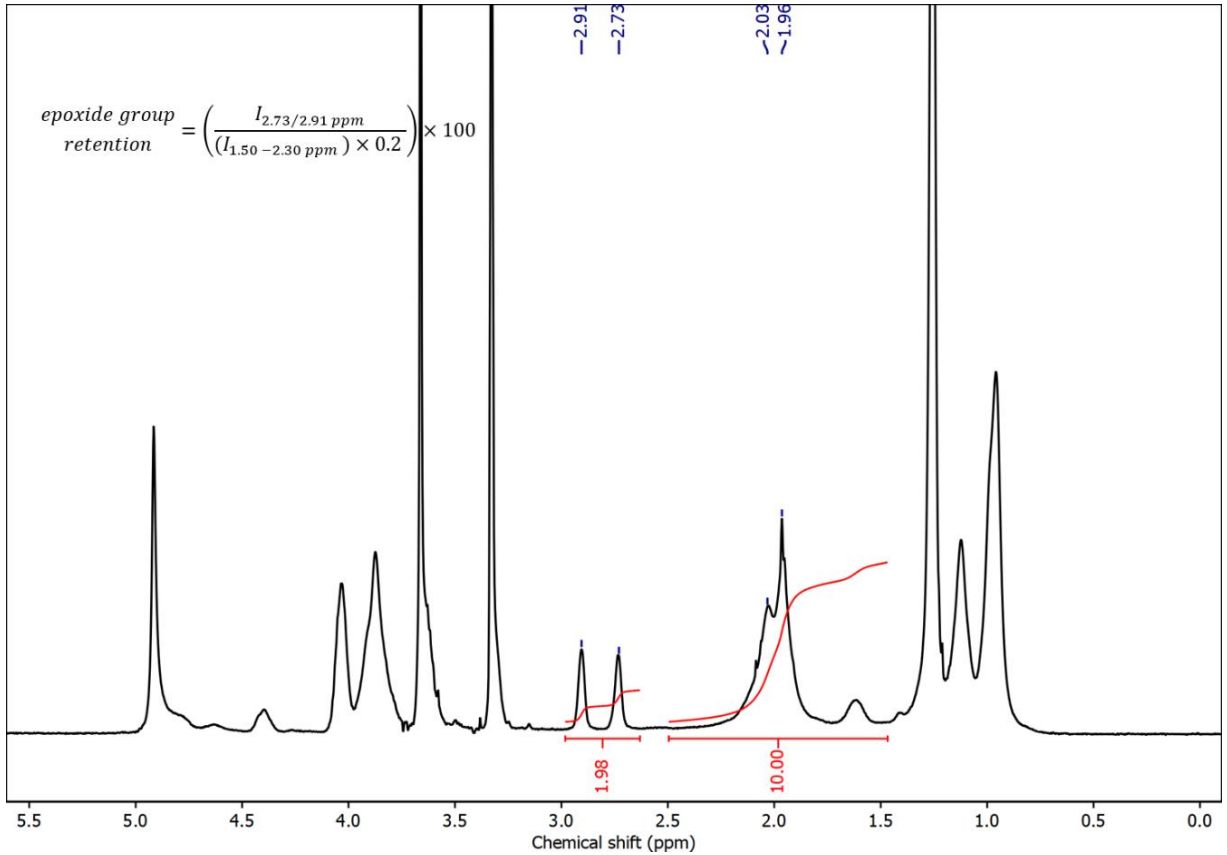


Quantitative monomer conversion (> 98%) after 2 hours

Optical microscopy – Heterogeneous monomer-*in*-water emulsion (HPMA is water-miscible, GlyMA is water-immiscible) prior to photo-PISA with monomer droplets of 5-30 µm

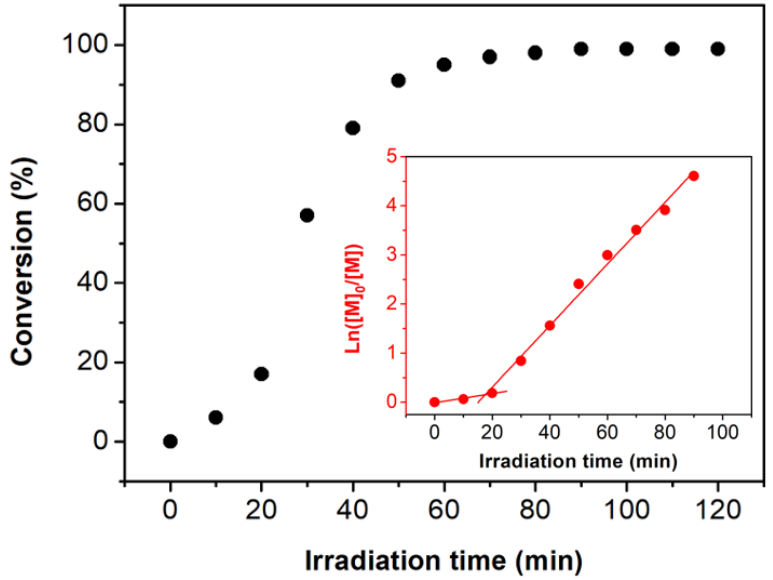
Varlas, *et al*. *Nanoscale*, 2019, **11**, 12643-12654

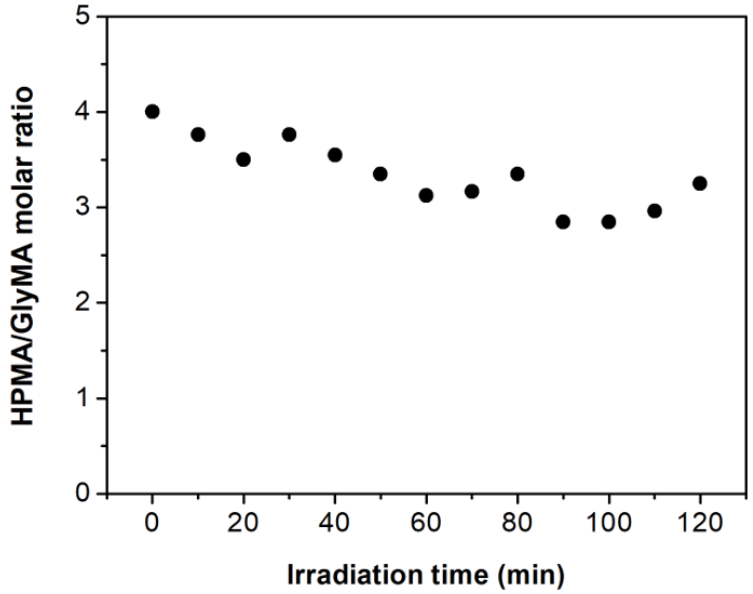
Polymersome Nanoreactors with Tunable Permeability *via* Emulsion Photo-PISA



- Quantitative retention of pendant epoxide groups (99%) after photo-PISA at 37 °C
  !!During thermally initiated PISA at elevated temperatures (60 - 100 °C), epoxides can undergo partial hydrolysis!!
- Increase in polymerization rate and onset of particle micellization after *ca*. 20 min (~17% monomer conversion)
- HPMA/GlyMA ratio between 3.0 - 4.0 indicates the formation of statistical copolymers

Kinetic Investigation:





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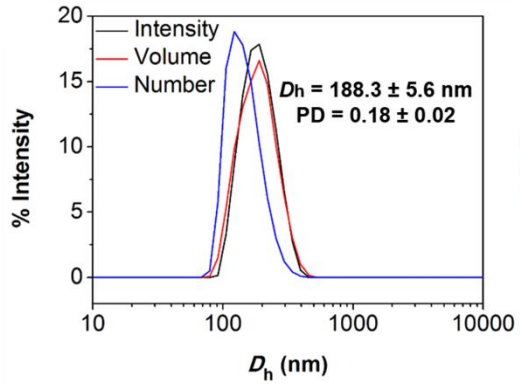
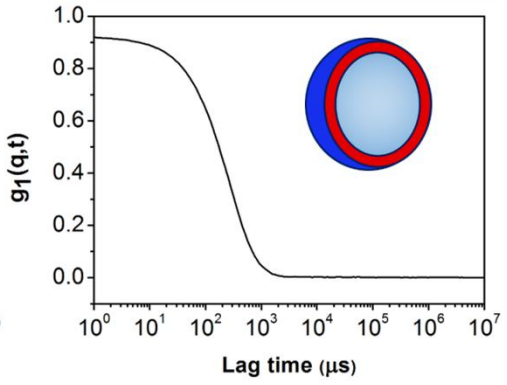
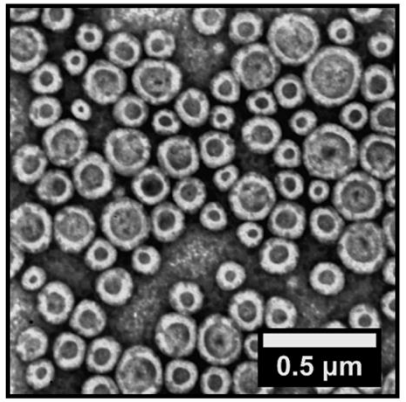
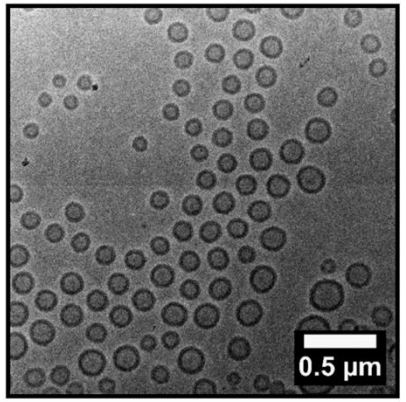
Polymersome Nanoreactors with Tunable Permeability *via* Emulsion Photo-PISA

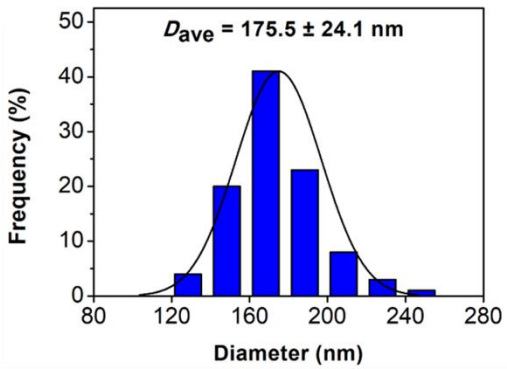
Empty Polymersomes:

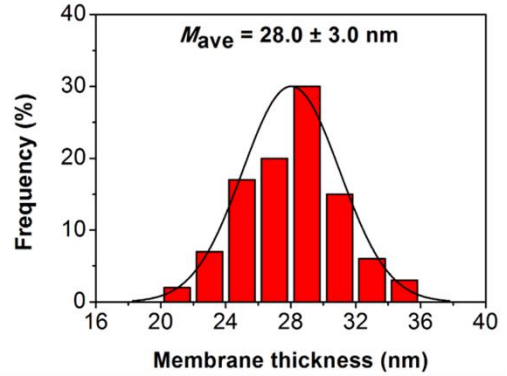




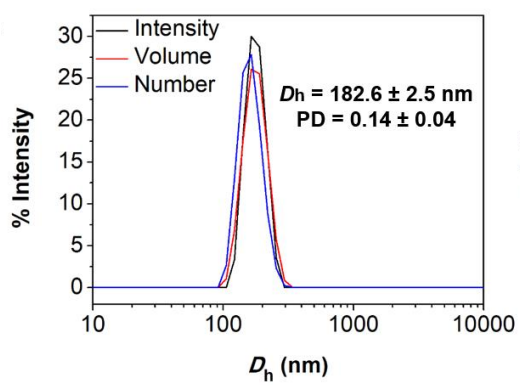


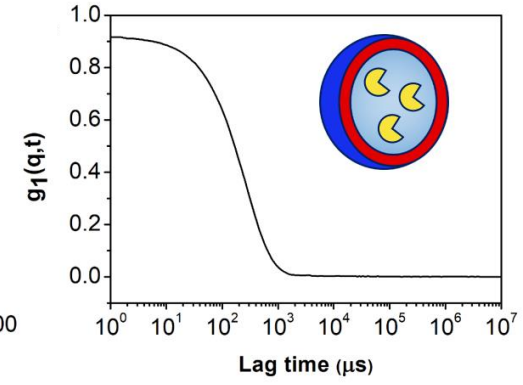


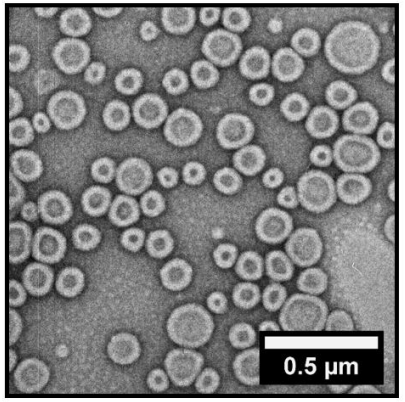


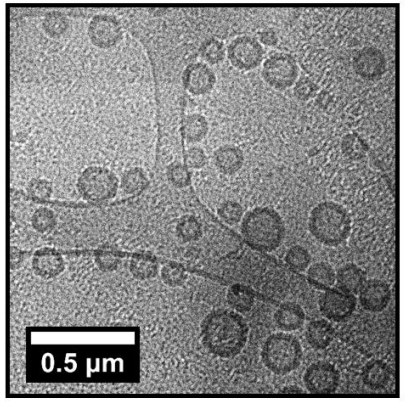


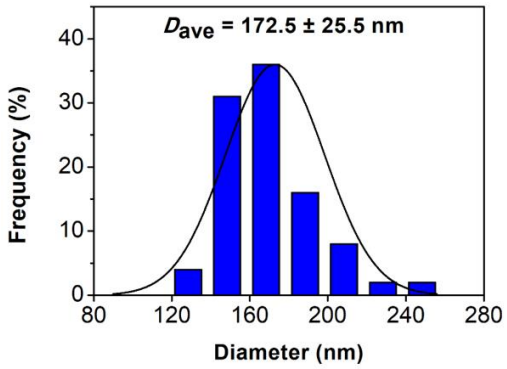
HRP-loaded Polymersomes:

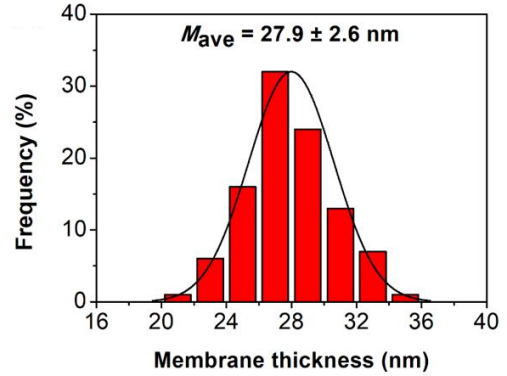






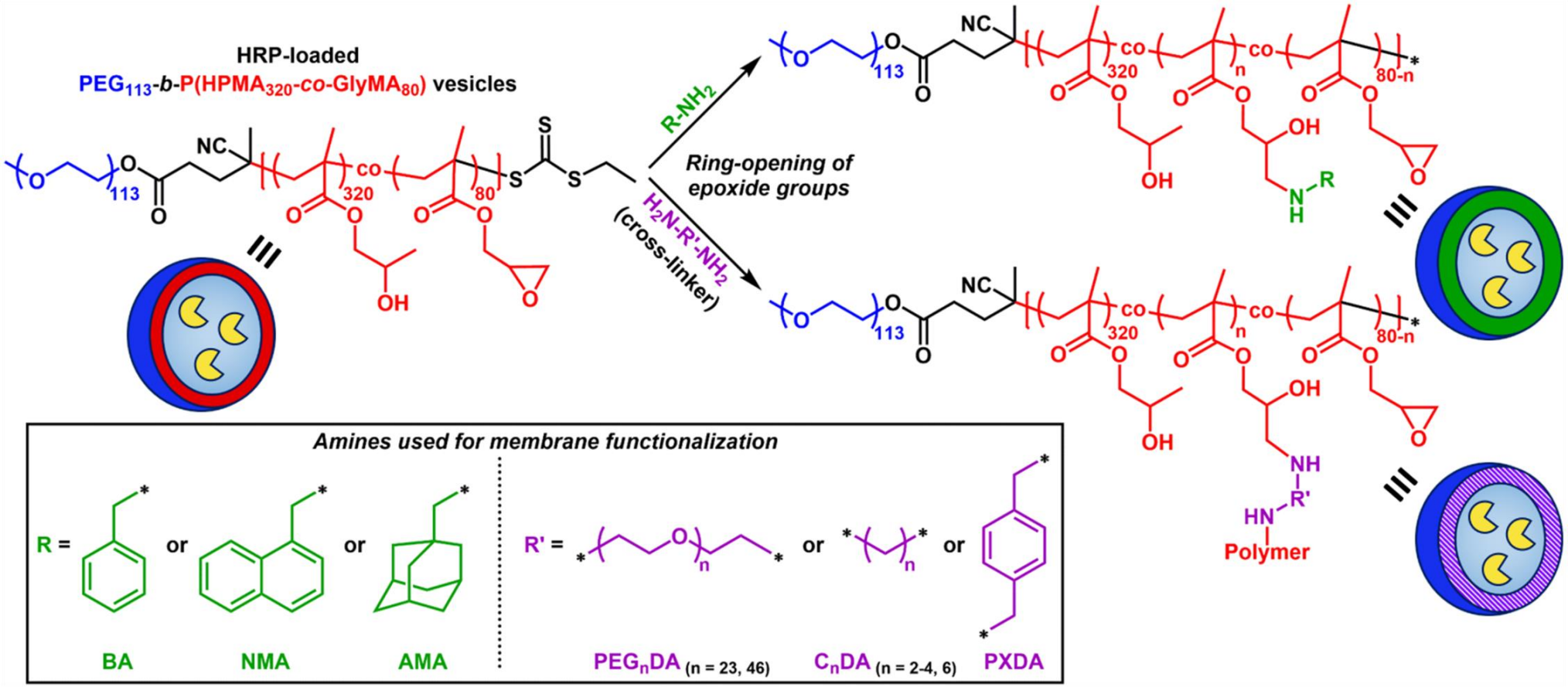






Polymersome Nanoreactors with Tunable Permeability *via* Emulsion Photo-PISA

Polymersome Membrane Modification Methodology:

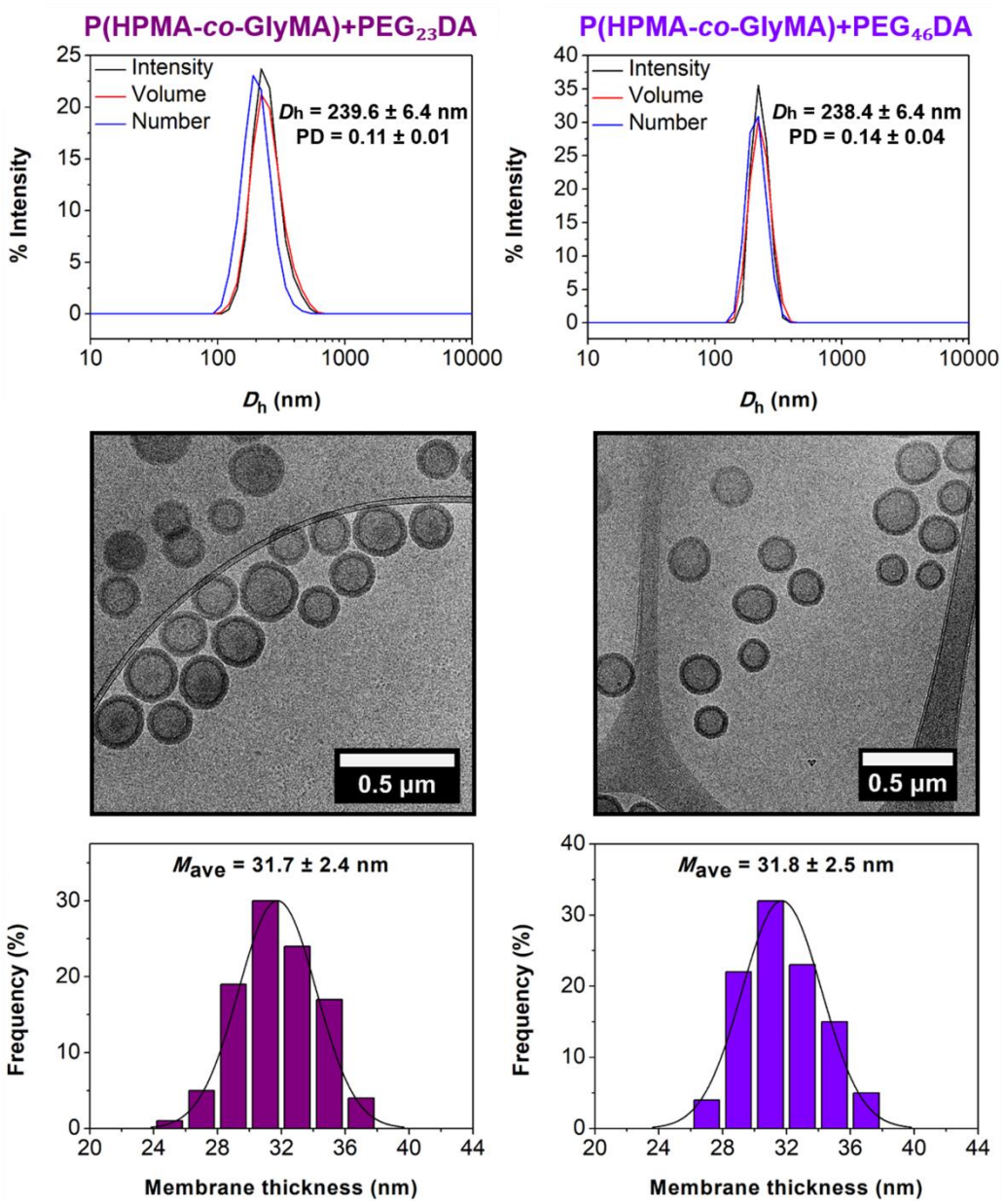


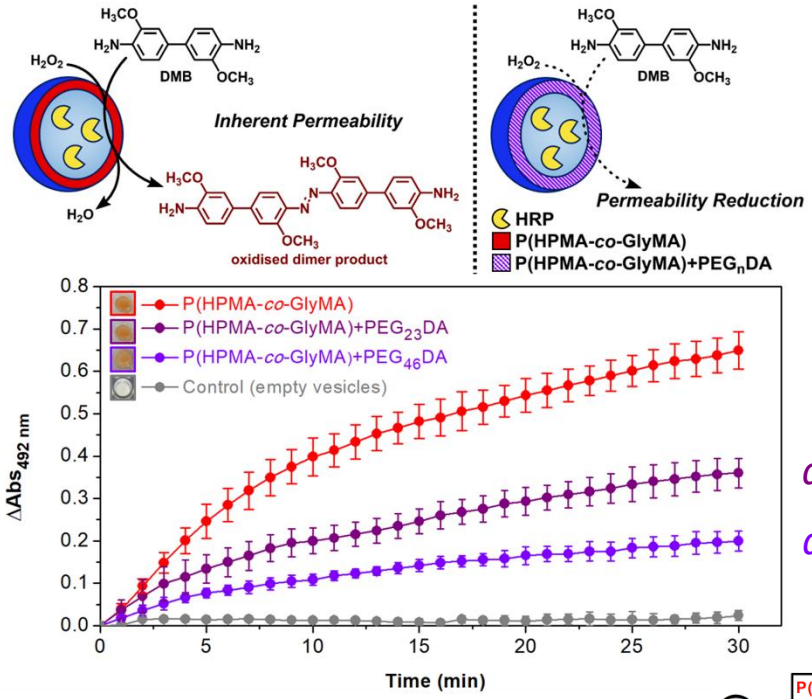
Involves ring-opening of pendant PGlyMA epoxide groups using a series of primary amines and cross-linking diamines of varying hydrophobicity as nucleophiles

Varlas, *et al*. *Nanoscale*, 2019, **11**, 12643-12654

Polymersome Nanoreactors with Tunable Permeability *via* Emulsion Photo-PISA

Hydrophilic Cross-linking Diamines:





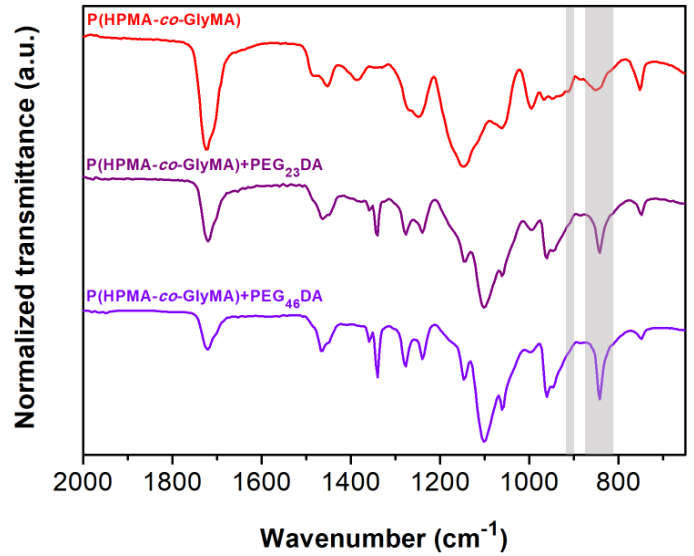
HRP-catalysed assay

*absorbance decrease of **45 ± 5%***

*absorbance decrease of **69 ± 4%***

FT-IR – Complete disappearance of epoxide ring characteristic peaks at 849 and 909 cm^{-1} indicating quantitative ring-opening

Permeability reduction due to thicker and more densely packed membranes as crosslinking reduces diffusivity



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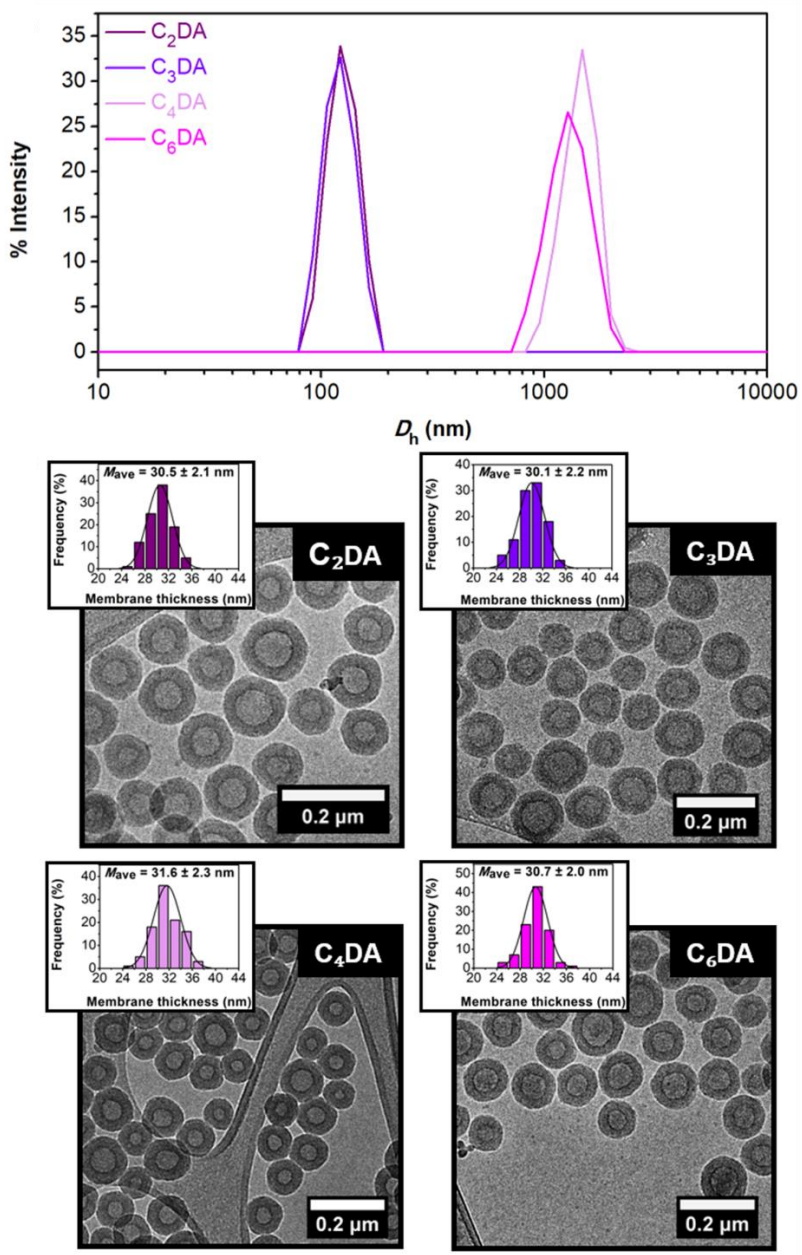
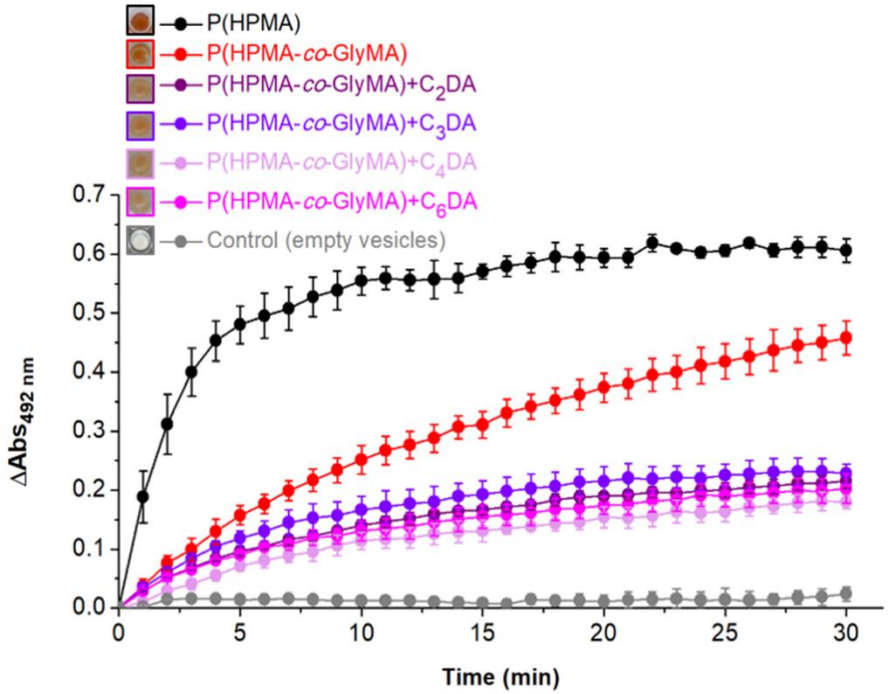
Polymersome Nanoreactors with Tunable Permeability *via* Emulsion Photo-PISA

Linear Aliphatic Cross-linking Diamines:





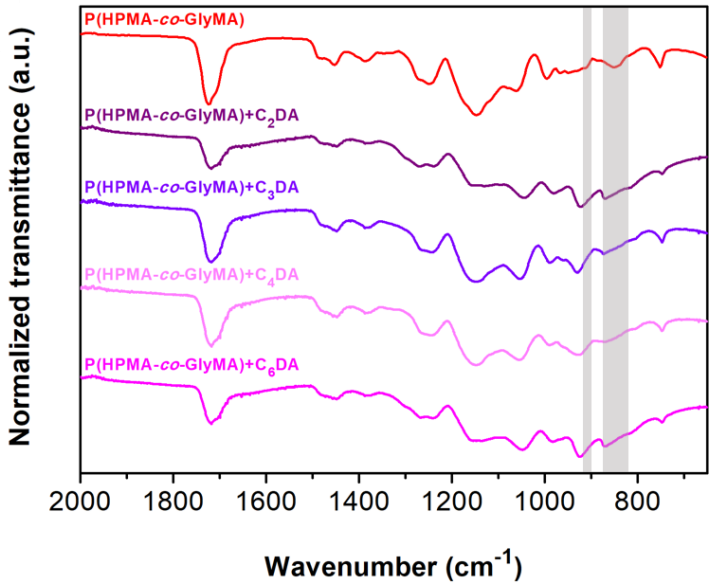
HRP-catalysed assay

*absorbance decrease ranging from **50%** to **61%** depending on membrane thickness increase*

Occurrence of particle agglomeration in the case of longer aliphatic C_4DA and C_6DA cross-linkers due to the development of inter-vesicular interactions

FT-IR indicates quantitative ring-opening of epoxide groups

Permeability reduction due to thicker and more hydrophobic (less hydrated) membranes that hinder diffusion



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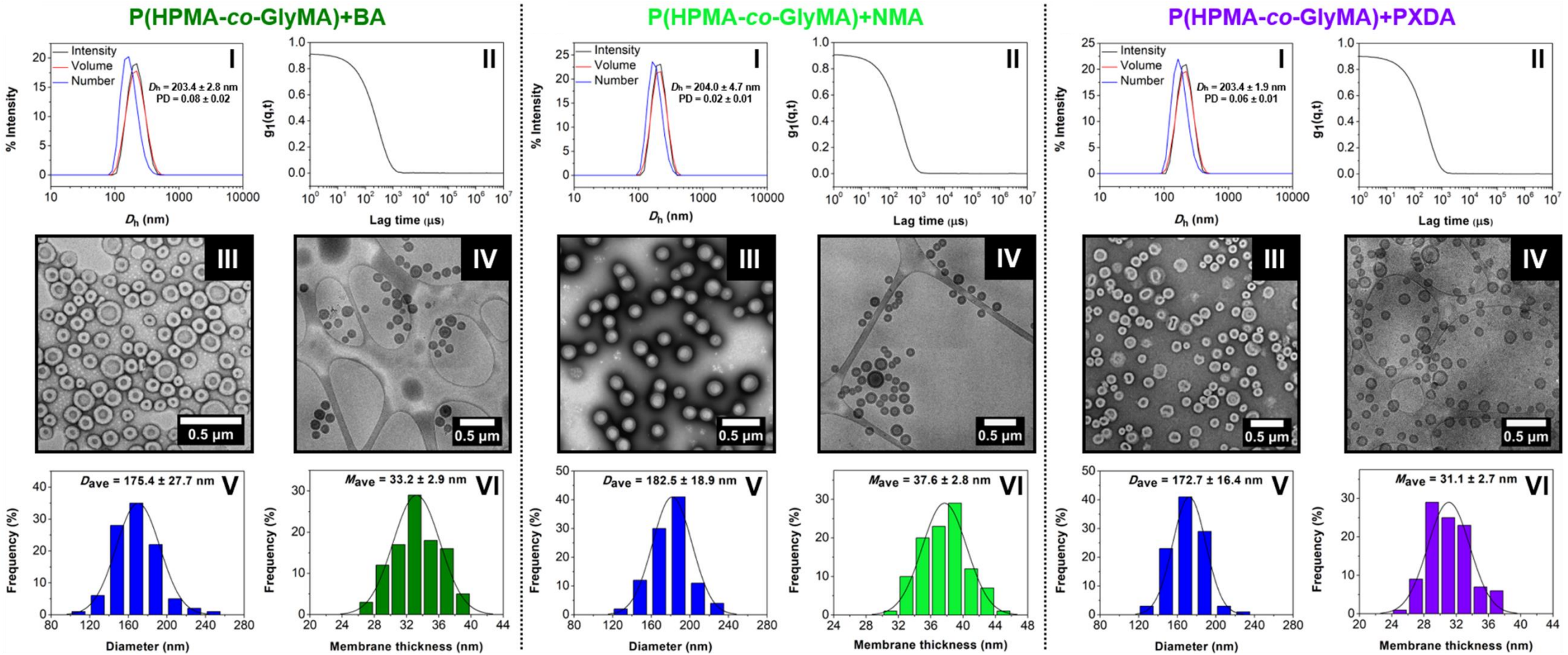
Polymersome Nanoreactors with Tunable Permeability *via* Emulsion Photo-PISA

Other Hydrophobic Primary Amines:



Significant increase in membrane thickness in all cases that is dependent on the hydrophobicity of the modifier (NMA > BA > PXDA)

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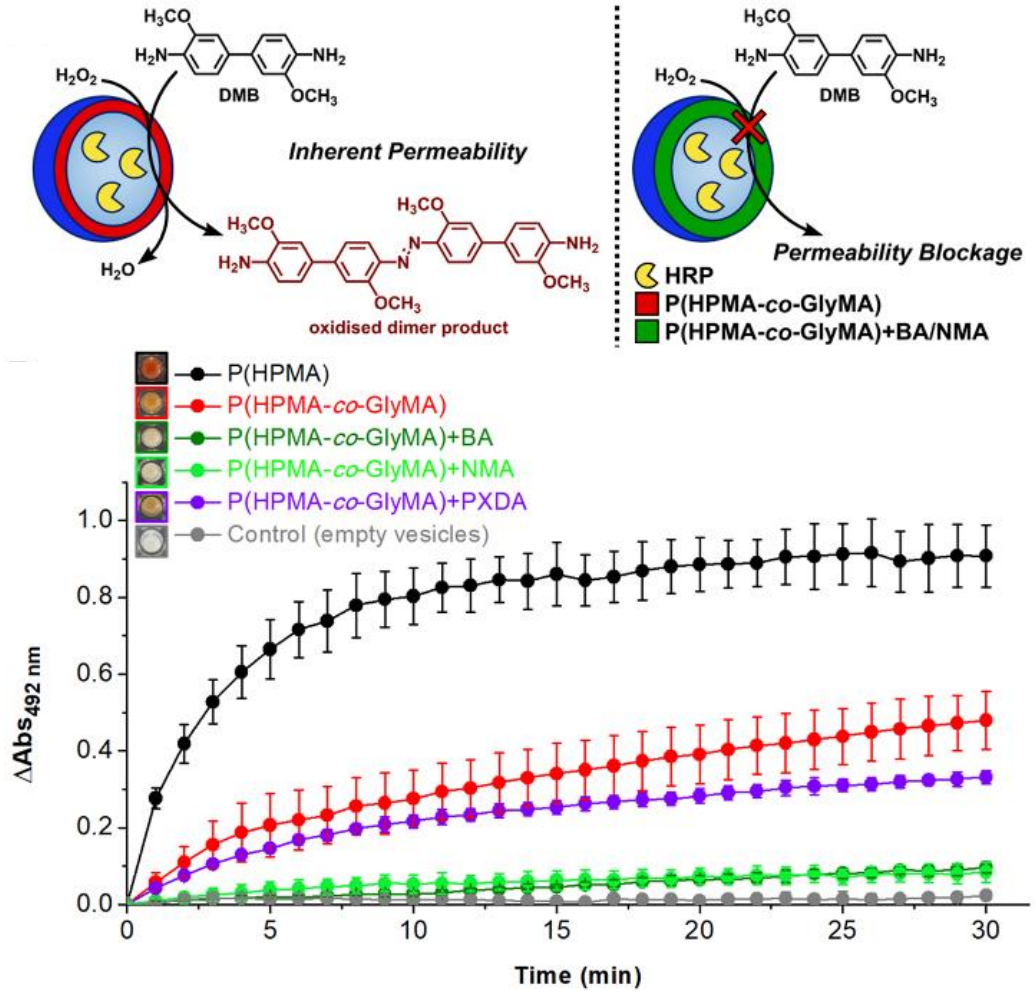
Polymersome Nanoreactors with Tunable Permeability *via* Emulsion Photo-PISA

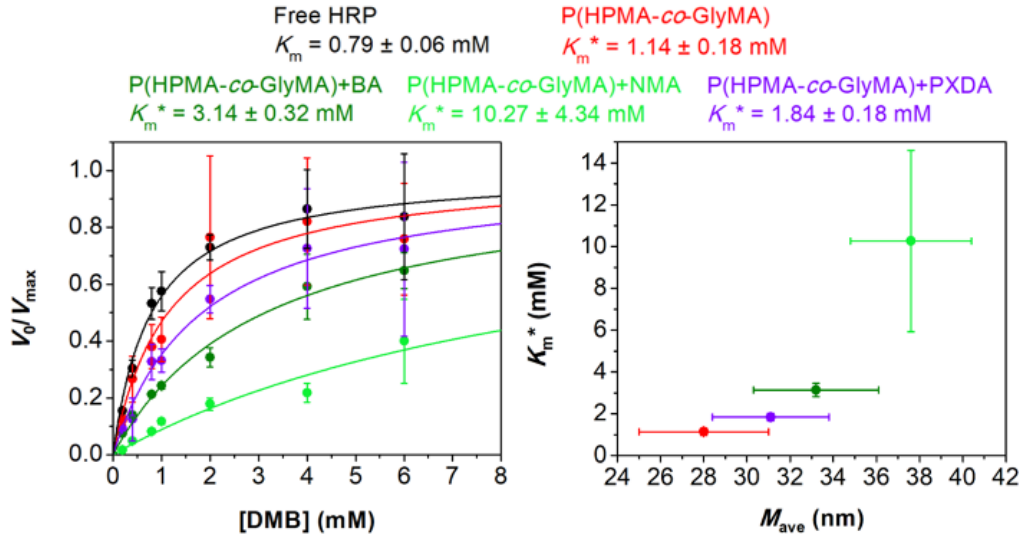


Kinetic colorimetric assay at fixed [substrate]:

~30% reduction in product formation for PXDA-crosslinked nanoreactors

>80% absorbance decrease was measured in the case of BA and NMA-functionalized polymersomes, showing near-complete permeability blockage

Detailed Permeability Investigation:



Effect of [DMB] and determination of observable permeability

Compared to K_m of free HRP (0.79 ± 0.06 mM):

K^*_m of PGlyMA nanoreactors increased by *ca*. 30%

K^*_m of PGlyMA+PXDA nanoreactors increased by *ca*. 60%

K^*_m of PGlyMA+BA nanoreactors increased by *ca*. 75%

K^*_m of PGlyMA+NMA nanoreactors increased by *ca*. 230%!!

Rate of substrate turnover was dependent of polymersome membrane thickness in a non-linear manner and trended based on the hydrophobicity of the utilized amine

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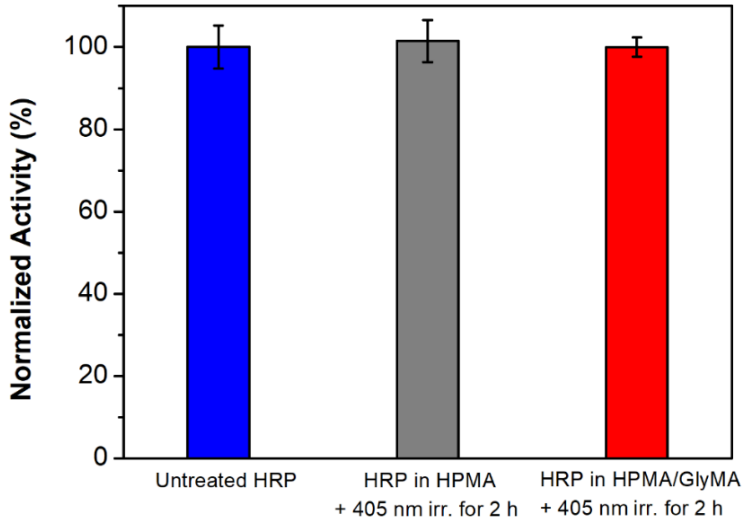
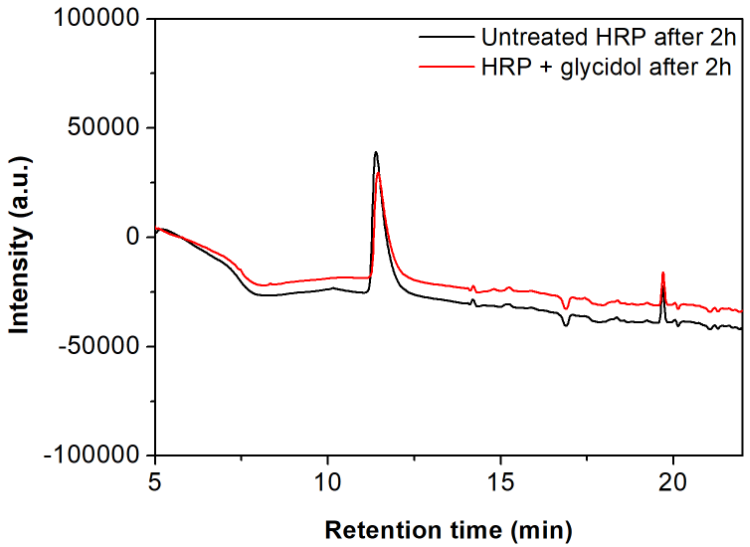
Polymersome Nanoreactors with Tunable Permeability *via* Emulsion Photo-PISA

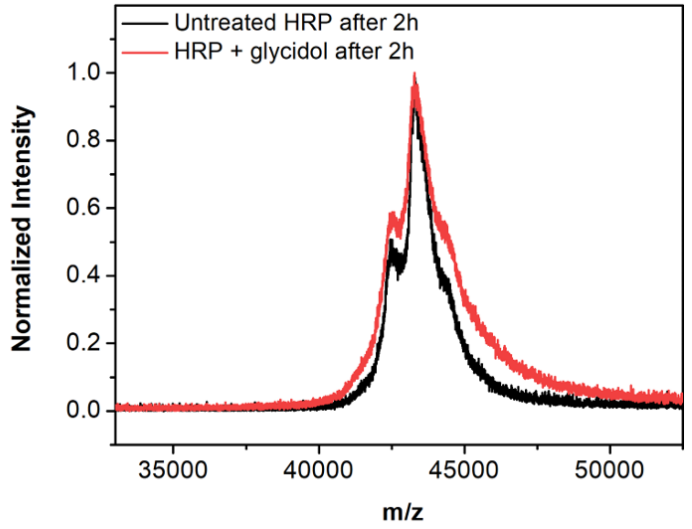
Control Experiments:

Effects of monomers and photo-PISA conditions on HRP activity:

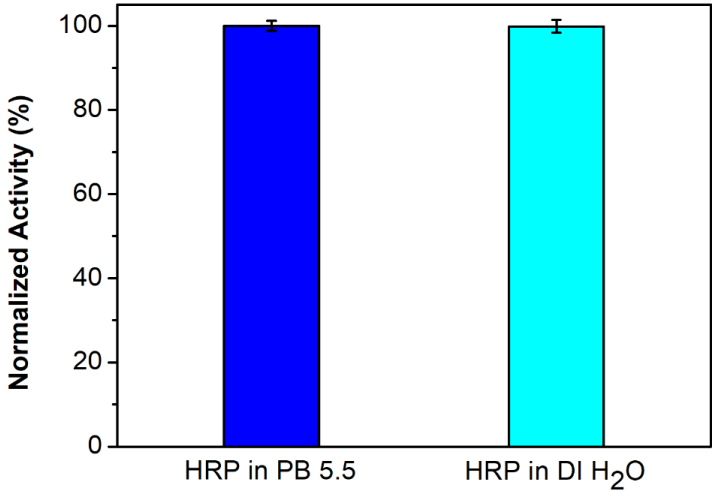


Assessment of epoxide ring-opening degree *via* HRP lysine residues:

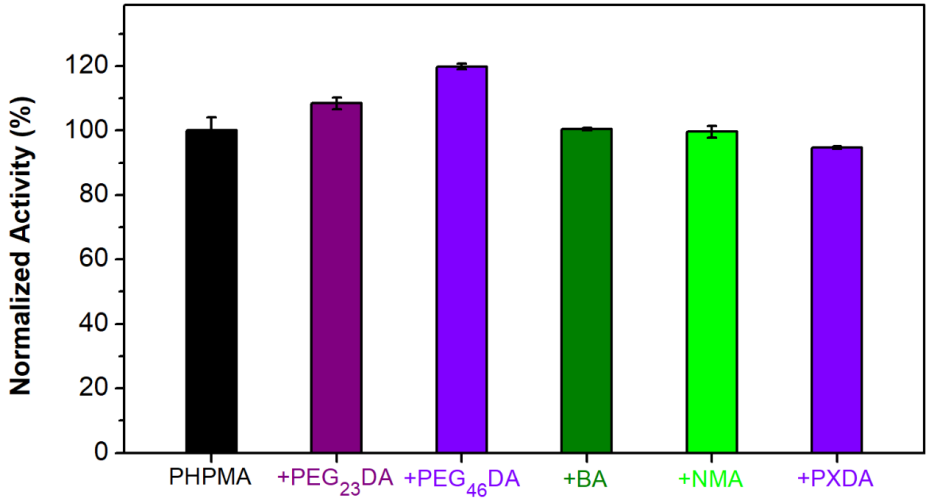




Effects of ring-opening conditions on HRP activity:



Effects of different amines on HRP activity:



A series of control experiments show no loss of enzyme activity or enzyme modification after photo-PISA and under applied ring-opening conditions

Summary

- Aqueous photo-PISA is a facile methodology to prepare epoxy-functionalized enzyme-loaded polymersome nanoreactors in one-pot
- Membrane modification is readily feasible using as series of amines as nucleophiles for ring-opening of pendant epoxide groups under mild reaction conditions
- Membrane modification resulted in increased thickness and reduced porosity/permeability relative to the non-functionalized particles
- Membrane thickness increase and blocking effect were more prominent as the hydrophobicity of utilized nucleophile increased
- Small molecule diffusion across nanoreactor membranes can be controlled by following our two-step methodology and by using appropriately selected amines

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