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Design, synthesis and charactrization of protease-sensitive polymeric vesicles

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Design, Synthesis and Characterization of Protease-sensitive Polymeric vesicles

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Joint ICTP-KFAS Conference on
Nanotechnology for Biological and
Biomedical Applications (Nano-Bio-Med)
13 October 2011

Why Protease?

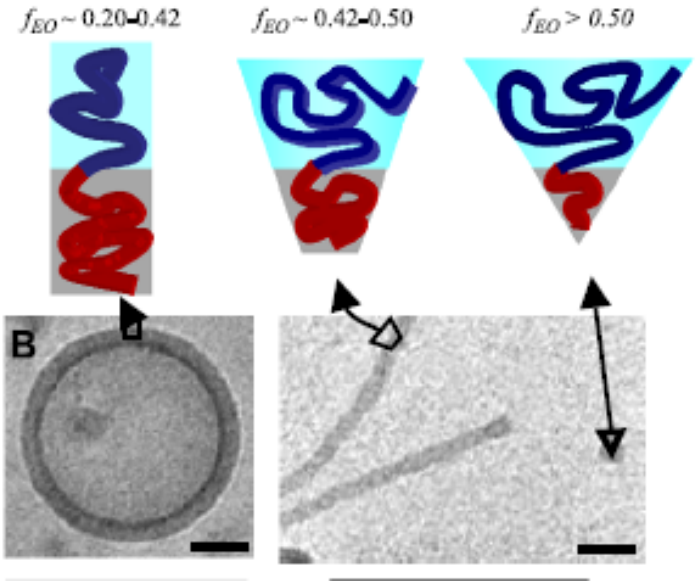
- Proteases are responsible for different biological events: digestion, inflammation and tumoral growth
- Protease has specific preference on the peptide substrates
- Protease is highly regulated inside the body
- Protease can be used as specific biological stimuli to target at the tissues that express it

Polymer vesicles for Drug Delivery

- Mechanically stable
- Able to carry therapeutics without chemical modification (by physical entrapment)
- Able to actively target to specific cells by tagging moieties on vesicle surface
- Possible to control release mechanism by engineering the chemistry of the polymer

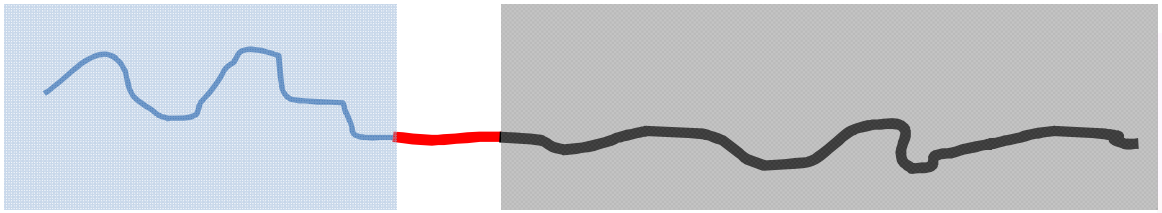
Principle of Responsiveness

- Hydrophilic-hydrophobic balance in the unimer determines the self-assembled morphology
- Disruption of vesicles can be effected by shifting the hydrophilic-hydrophobic balance

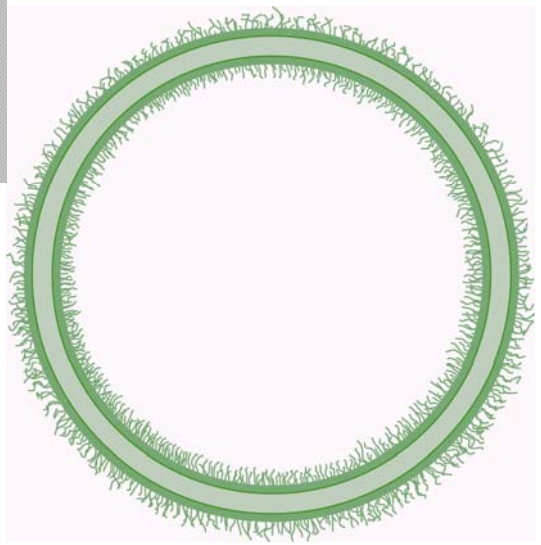


Ahmed et al. J Control. Release 2004

Protease-Sensitive Polymer Vesicle



Hydrophilic block (PEG) Peptide Hydrophobic block (PCL)

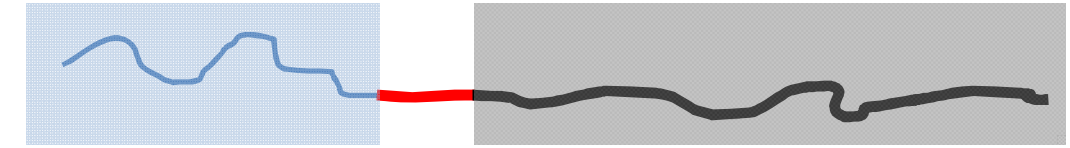


Weight fraction of PEG = 0.24

Material Design & Rationales

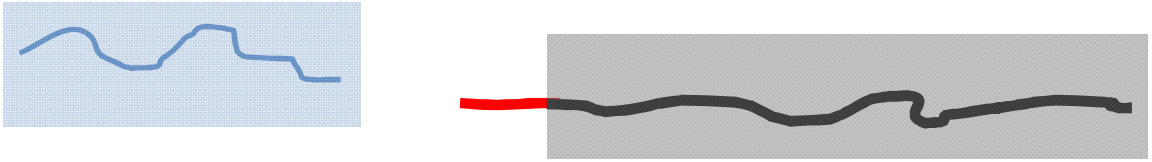
- Peptide
  - Tetrapeptide sequence
  - Labile to chymotrypsin digestion
- Polycaprolactone- 15 KDa
  - Biocompatible
  - Slow and chain-end degradation by hydrolysis
- Polyethylene Glycol- 5 KDa
  - Biocompatible
  - Render the resistance to protein and cell adhesion
  - Prolong circulation time
  - Could allow penetration of protease

Protease-sensitivity and morphology change

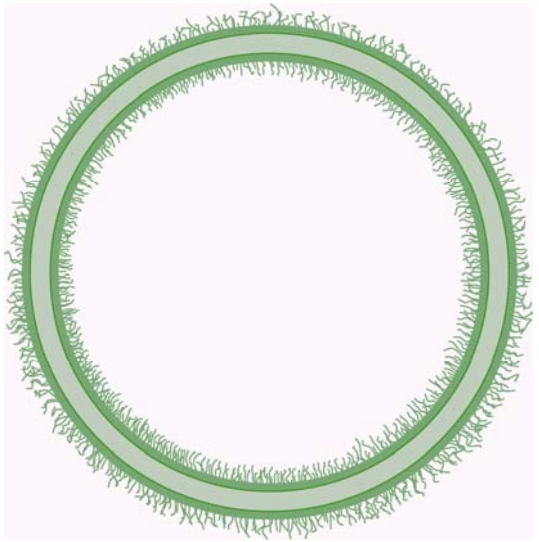


PEG Peptide PCL

Chymotrypsin



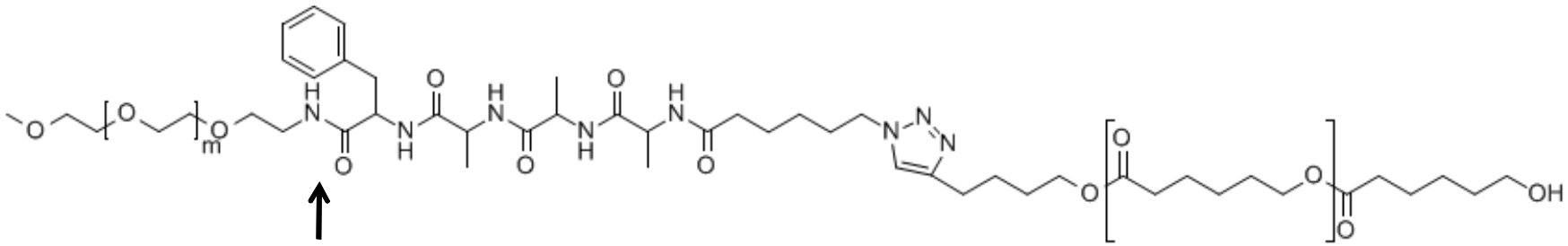
PEG Peptide PCL



Synthesis of Polymer and Peptide components

Synthesis Strategy of Polymer

- Orthogonal chemistry to link the components
  - Couple PEG and peptide by amide bond
  - Conjugate peptide-PEG and PCL by azide-alkyne “click” chemistry
  - Simple purification steps



Characterization of the polymer-peptide hybrid

	Mn	Polydispersity
PCL	15650	1.19
PEG	4950	1.09
PEG-peptide-PCL	21050	1.3

Vesicle preparation

PBS

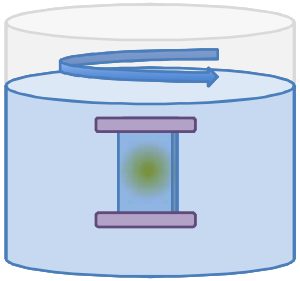
THF,
10mg/ml

Ultra-sound

Evaporation
under vacuum

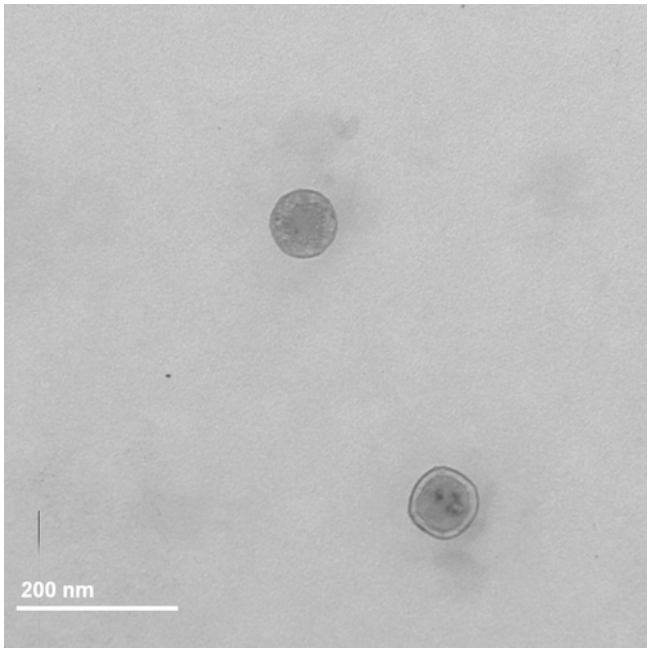
Centrifugation

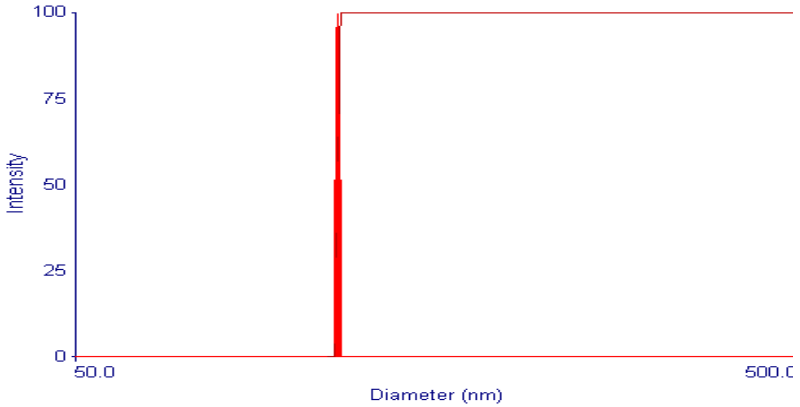
Extrusion



Dialysis to remove free dye

Size of the vesicles



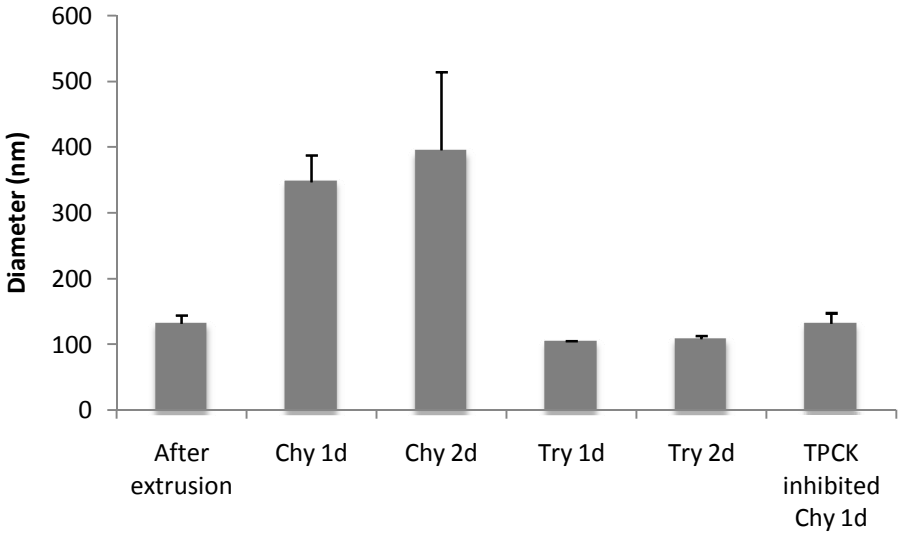


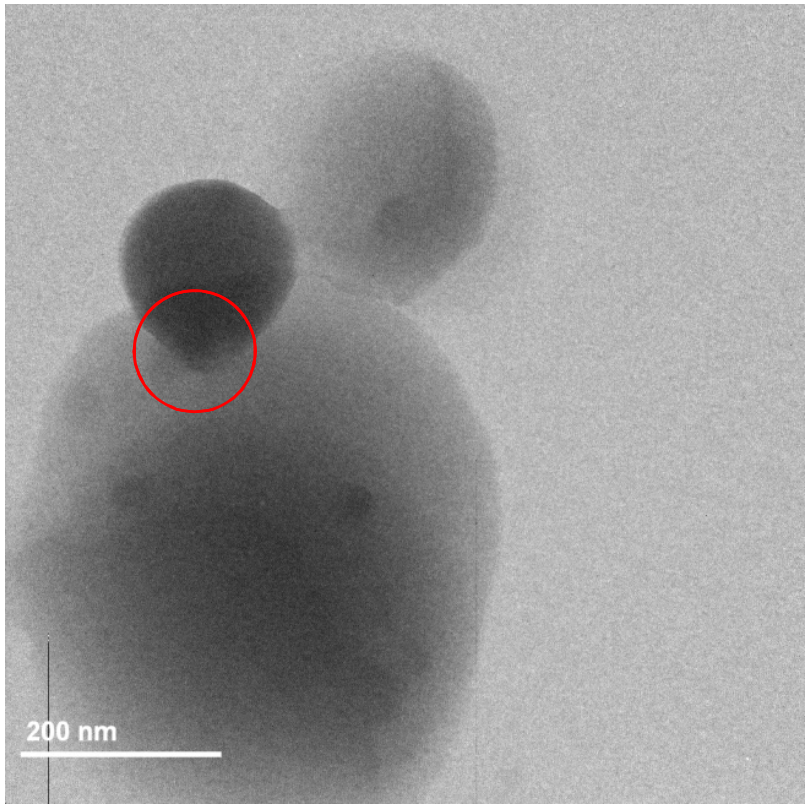
After extrusion

135.8 nm

Size changes induced by chymotrypsin

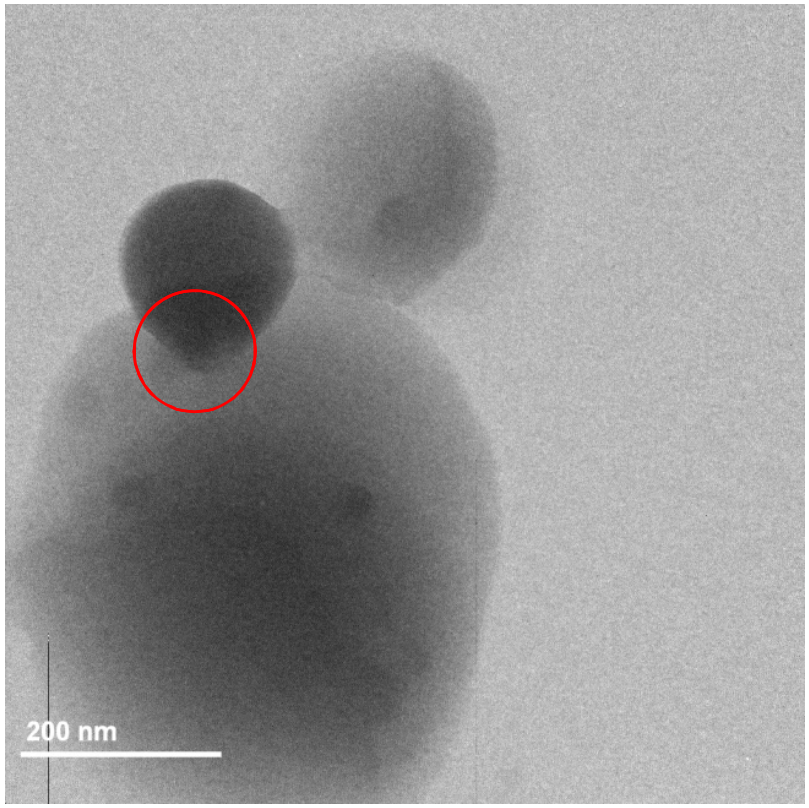
Chymotrypsin treated vesicles after 0.5 hr 1μM





Vesicles incubated with chymotrypsin for 45 min

- Generally spherical shape,…
- but budding grew on the surface



Vesicles incubated with chymotrypsin for 45 min

- Generally spherical shape,…
- but budding grew on the surface
- After expanding to 400 nm in diameter, the morphology changes stopped thereafter

Any triggered release?

- Carboxyfluorescein
- There was no enhanced release for 48 hours, compared to the controls (trypsin and TPCK-inhibited chymotrypsin)

Conclusion

- PEG-peptide-PCL was prepared that self-assembles into nano-sized polymeric vesicles
- By incorporating peptide sequence that was cleavable by chymotrypsin, the vesicles expanded in diameter as fast as in 30 minutes
- The morphology change stopped after the expansion in size and there was no triggered release in the first 48 hours

Acknowledgements

- Prof. Ying Chau
- Dr. Zhongyu Li
- Mr Tze Kin Cheung
- Bioengineering Graduate Program
- Hong Kong Research Grant Council, RGC 600207

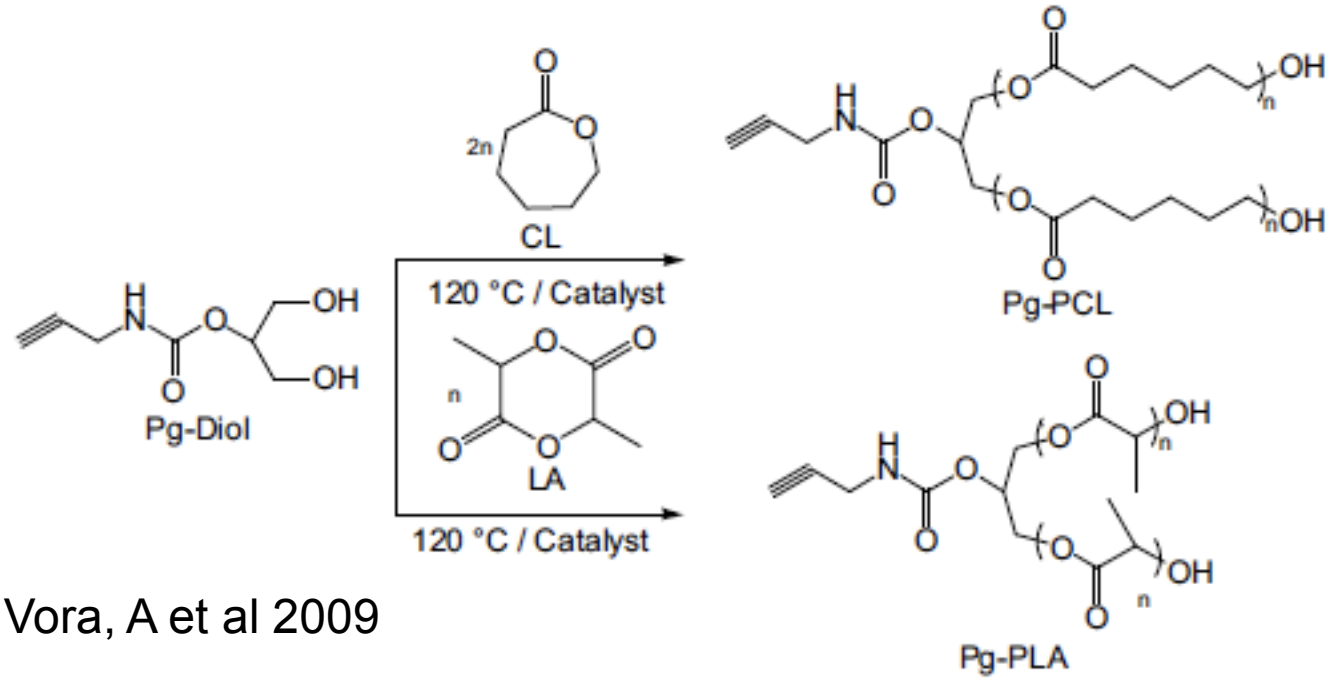
Molecular weights of PEG and Protease sensitivity

Anti-fouling, Anti-protein absorption ~ MW_{PEG}
Starts to be constant > 2000 Da

MW_{PEG} (Da)	MW_{HB} (Da)	MW_{TOT} (Da)	f
1400	4800	6200	0.23
2200	4800	7000	0.31
3000	4800	7800	0.38

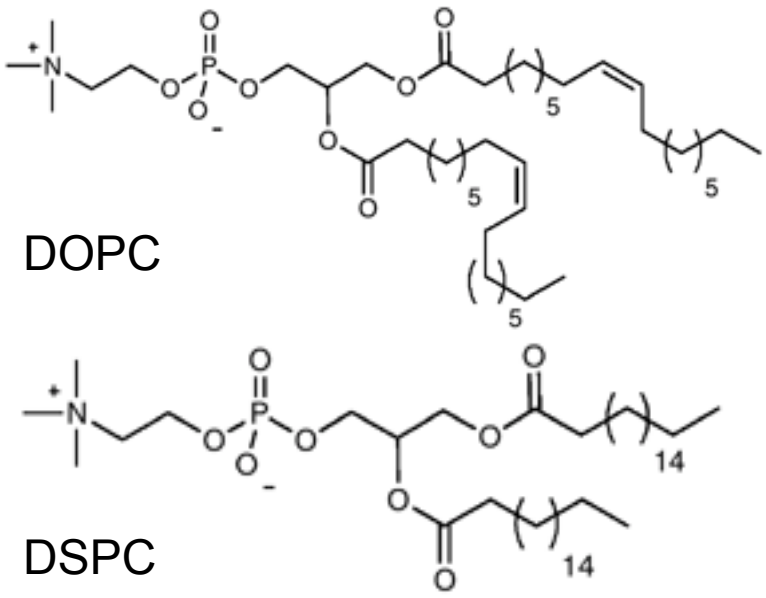
Glassy Hydrophobic Polymer

- Tg of PCL : -62°C
- Induced morphology change at rt possible
- Release retardation by glassy HB polymer
- Tg of PLA : 50°C



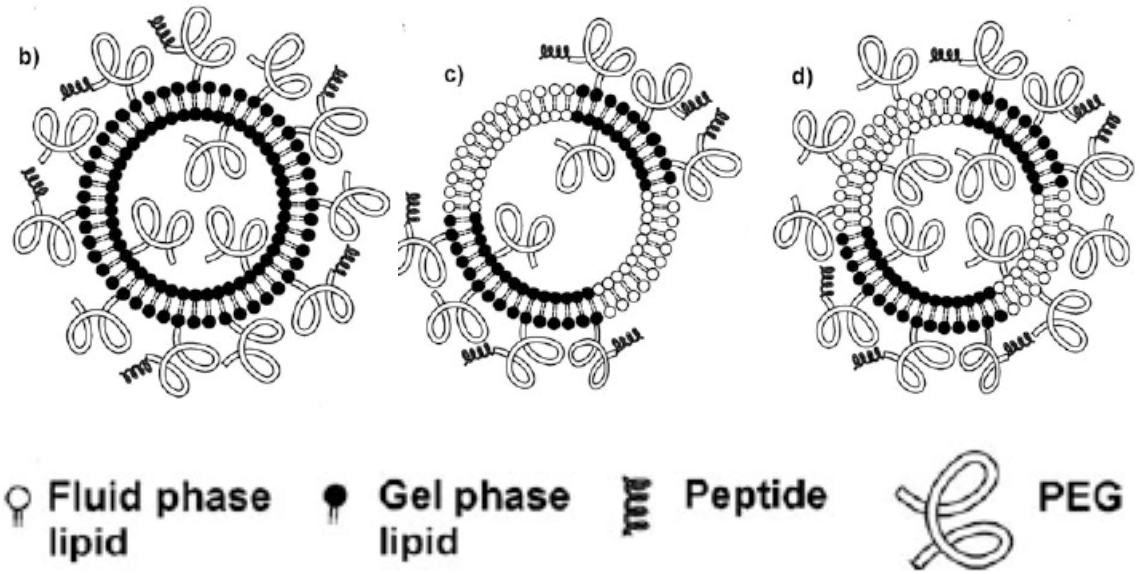
Vora, A et al 2009

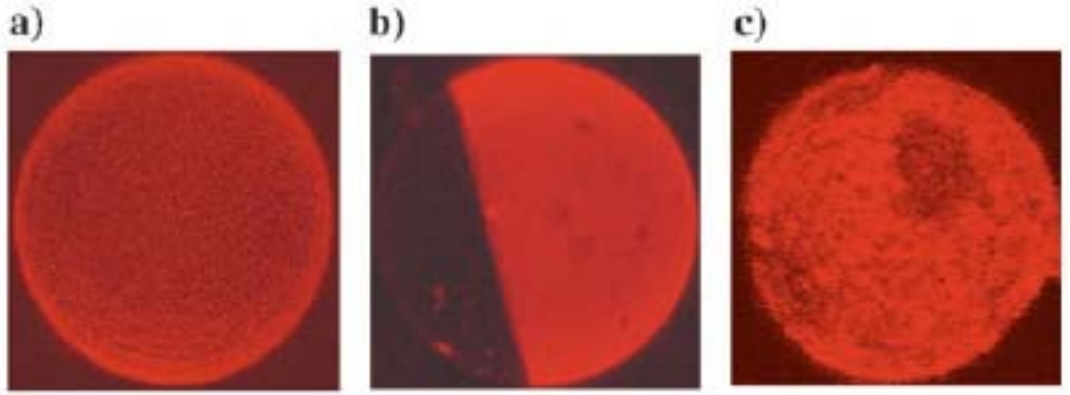
Lipid Rafts



DOPC

DSPC



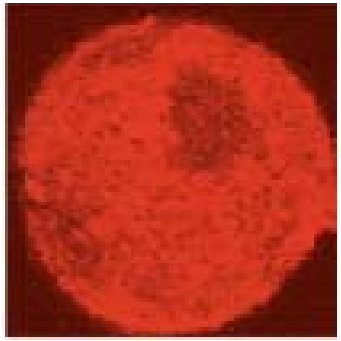


Rei, P et al 2008

“Rafts” on Polymer Vesicles

	Lipid	Polymer	Mole %
Fluid Phase	DOPC	PCL	75
Gel Phase	DSPC	PLA	25

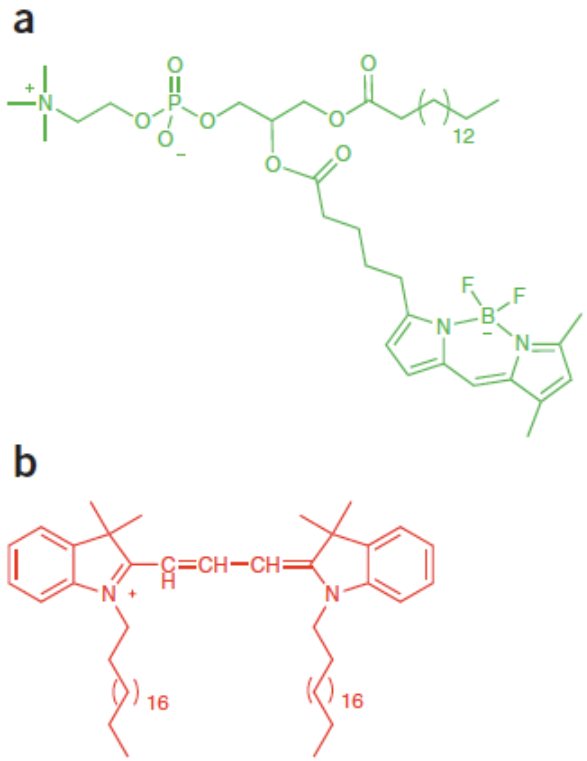
- PEO(2200)-PCL(6600)/ PEO(2200)-PD-PLA(6600)
- Localizing PD substrate
- Creating large defects
- Enhancing release rate



Rei, P et al 2008

Translation to MMP-2 sensitive

- Replace PD linker by PVGLIG (Proline-Valine-Glycine-Leucine-Isoleucine-Glycine)
- Same methodology of synthesis and release characterization
- Recombinant human MMP-2 from commercial sources



Bodipy-PC, prefer fluid phase

C20:0-Dil, prefers gel phase

Feigenson (2006)

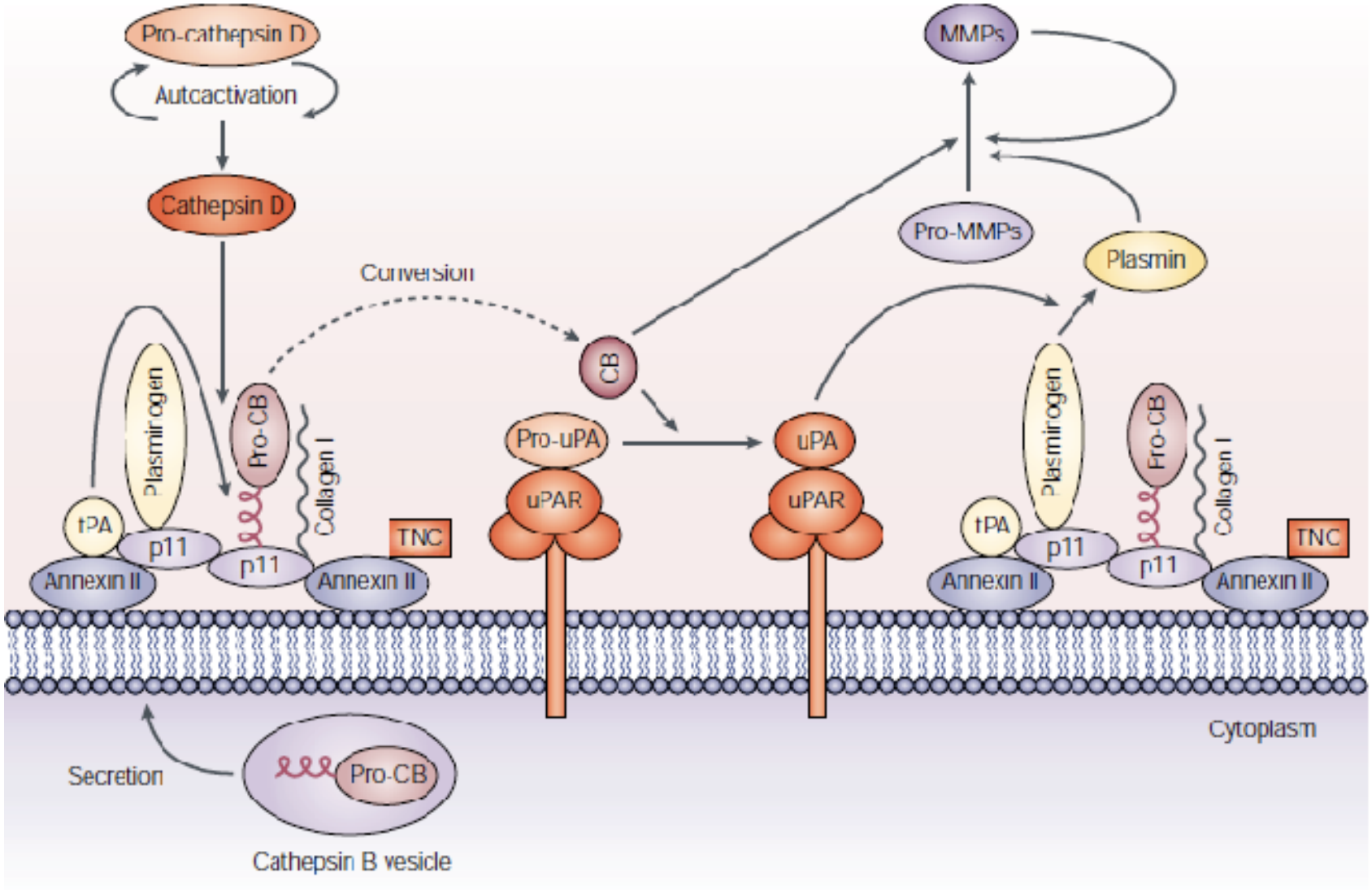
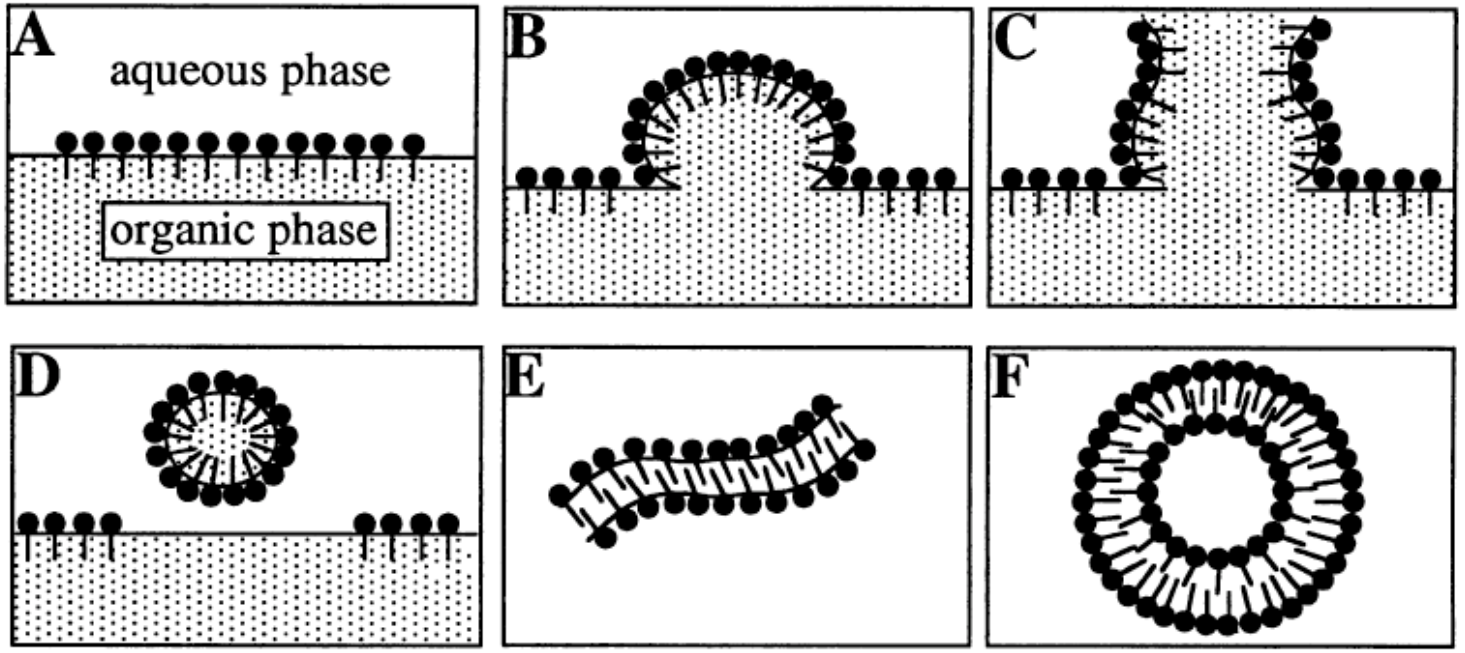


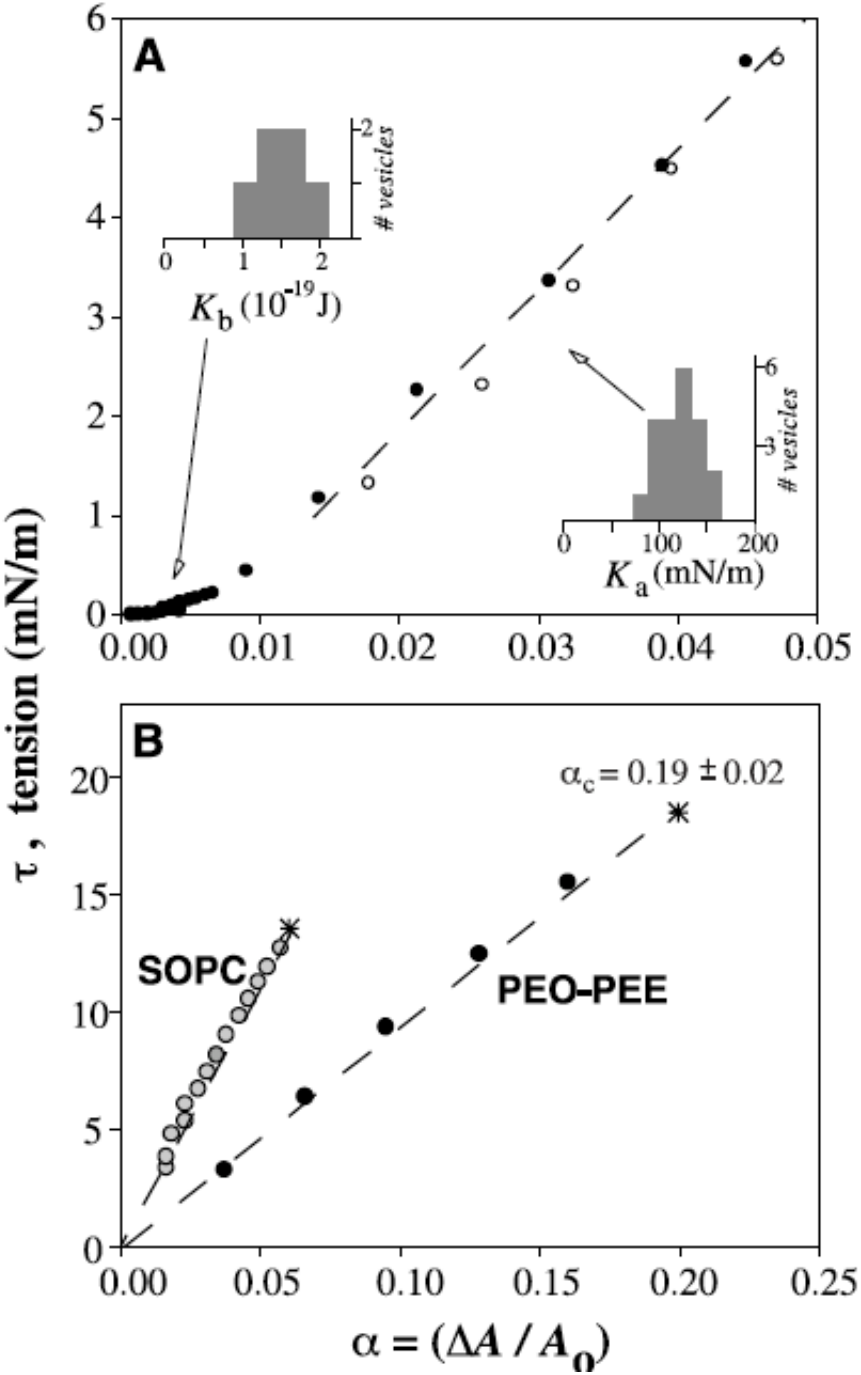
Figure 5 | **The cathepsin-B proteolytic cascade.** Pro-cathepsin B (Pro-CB) is activated by tissue-type plasminogen activator (tPA) and cathepsin D, initiating a proteolytic cascade that results in the activation of urokinase-type plasminogen activator (uPA), matrix metalloproteinases (MMPs) and plasmin. Pro-CB is situated on the annexin II tetramer (AIIt). Binding of annexin II to the plasma membrane is Ca^{2+}-dependent. Although the mechanism is unclear, AIIt is known to interact with tPA, collagen I and tenascin C (TNC). p11, which binds to AIIt on the cell surface, has been shown to interact with plasminogen and pro-CB at different binding sites. Co-localization of proteases with AIIt would facilitate the activation of pro-enzymes in a proteolytic cascade and the selective degradation of matrix components. Collectively, active proteases can degrade all of the components of the extracellular matrix.

Direct dissolution	Phase inversion	Film rehydration
Solvent free	Disperse organic solution, hydration	Evaporate organic solvent, hydration, sonication, extrusion
	Micron sized	Nano sized



Moscho et al, 1996

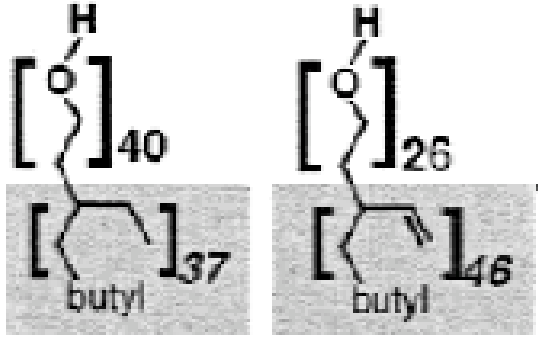
- Good drug delivery vehicle
  - Stable against high dilution
  - Mechanical strength
  - High loading possible
  - Easy decoration for targeting moieties on surface
  - Responsive to stimuli



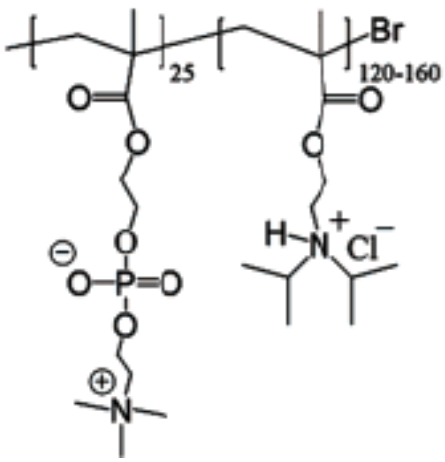
Discher et al (1999)

Method of Preparation

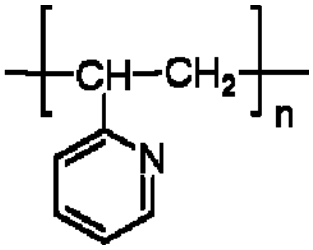
- Diblock amphiphilic copolymer
- PEO-PEE, PEO-PBD
- PEO-PLA, PEO-PCL
- PEO-P2VP, PMPC-PDPA



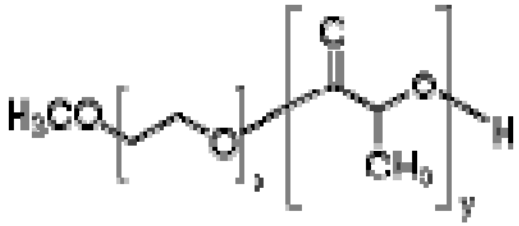
PEG-PEE PEG-PBD



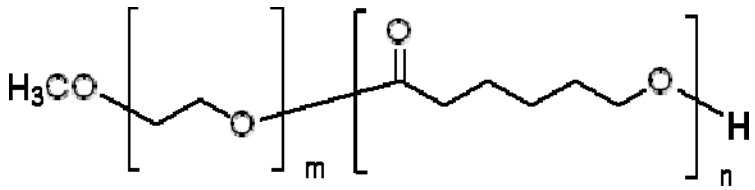
PMPC-PDPA



P2VP



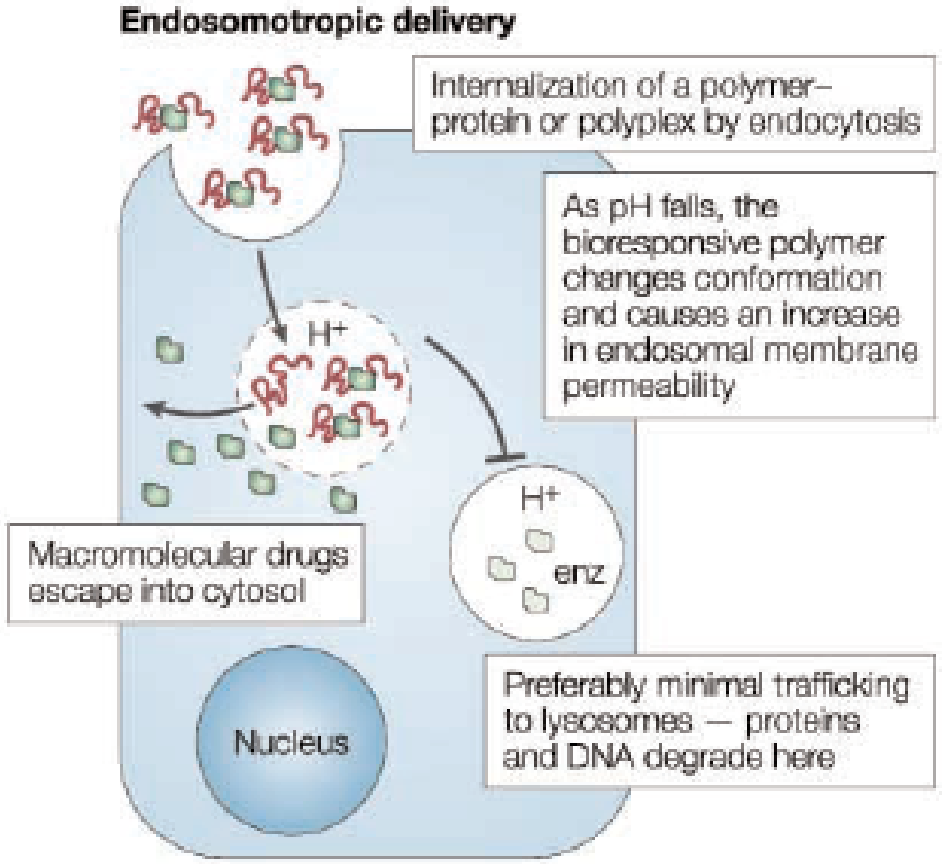
mPEG-PLA



mPEG-PCL

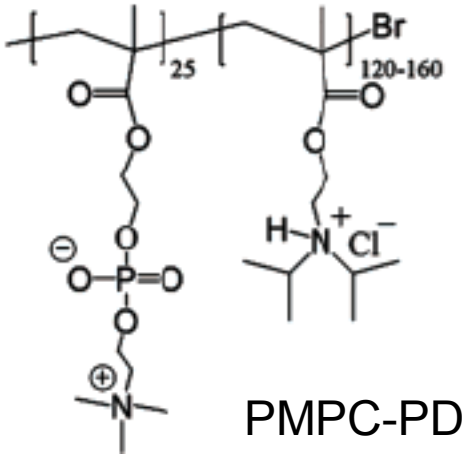
Stimuli responsive vesicles

- Hydrolysis
- pH
- Redox
- Temperature
- Light
- Magnetic field

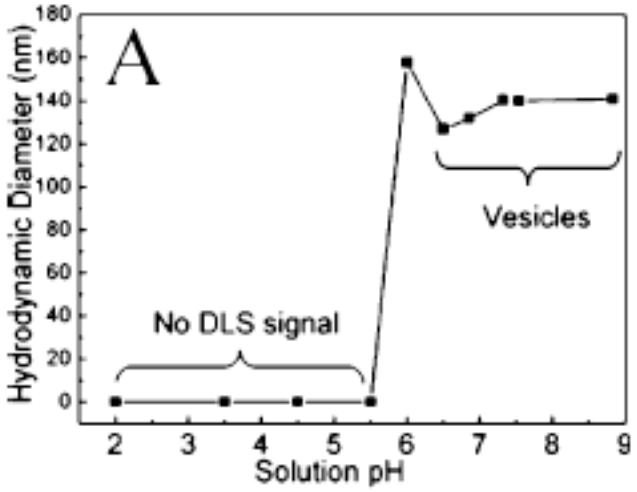


Duncan, R. 2003

pH responsive vesicles



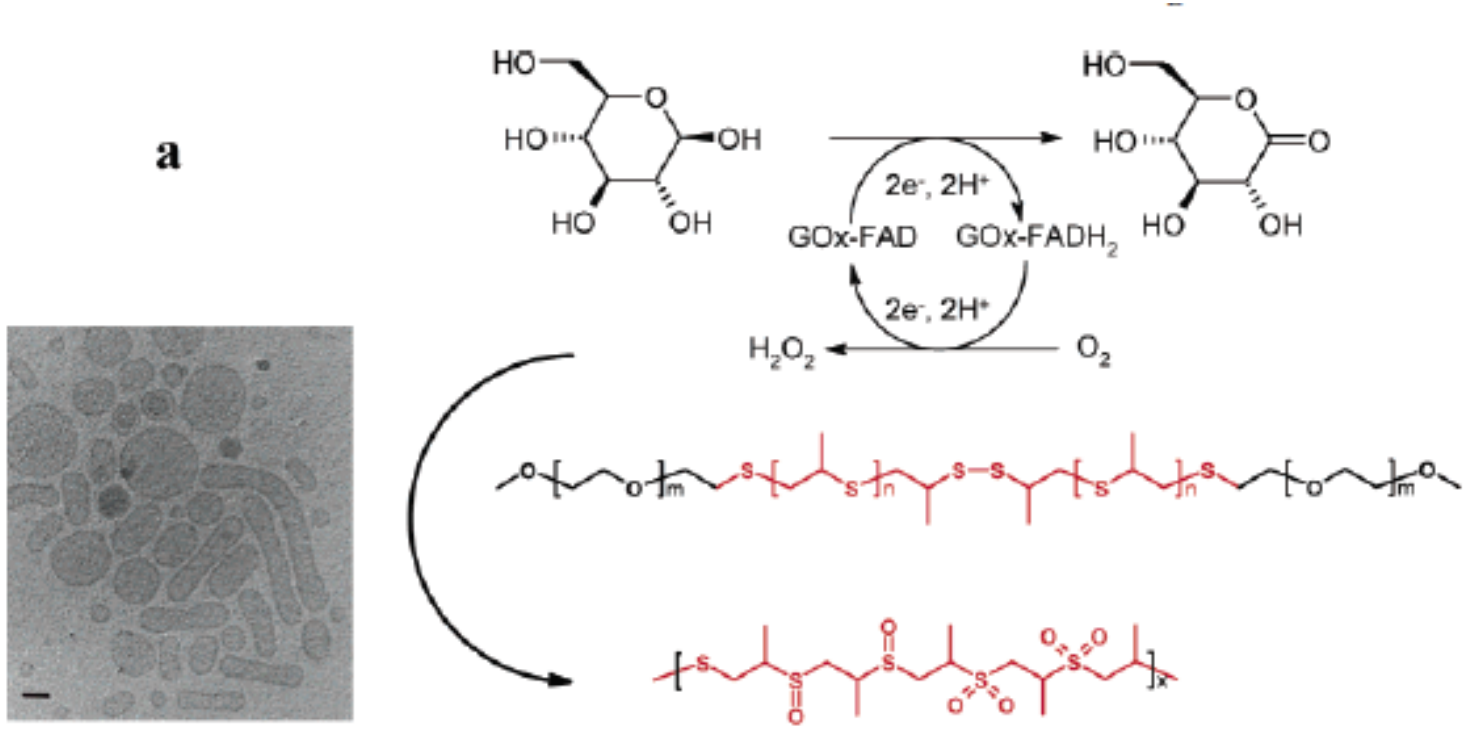
PMPC-PDPA



Du et al 2005

Lomas et al 2007

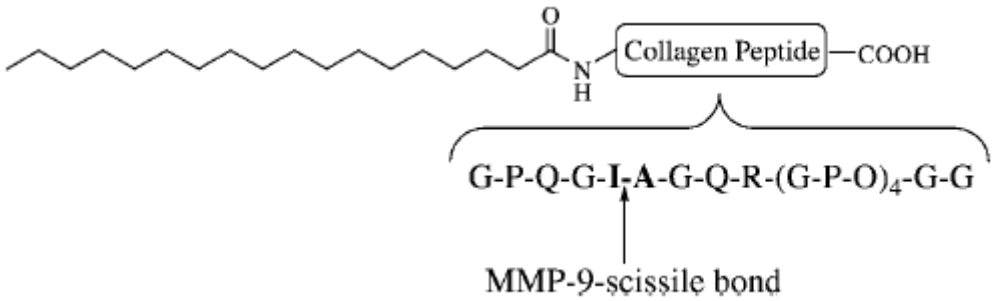
Oxidation responsive vesicles



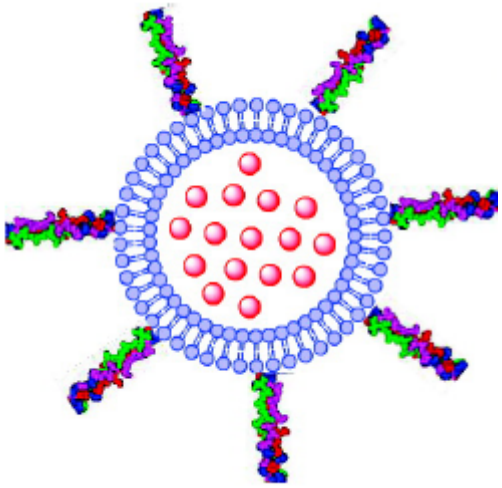
The GOx-catalyzed conversion of â-D-glucose into gluconolactone with production of H_2O_2 in the presence of oxygen. Below the symmetric PEG-PPS-PEG block copolymer under study and a possible repeating unit of PPS after oxidation by H_2O_2,
m=16, n=25. In the inset, a cryo-TEM micrograph of the polymersomes formed in buffer after extrusion (the bar represents100 nm).

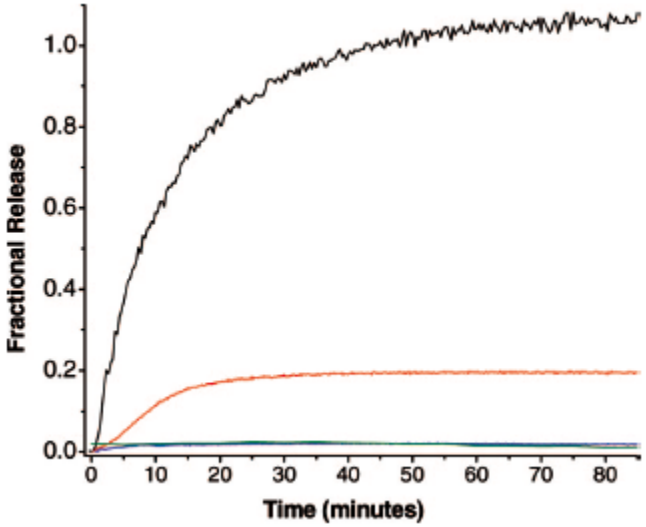
Napoli et al. Langmuir 20:3487

Triggered release of Liposomal Contents by MMP-9



Structure of the lipopeptide **LP1**; the cleavage site for MMP-9 is shown.





MMP-9-triggered leakage of encapsulated carboxyfluorescein from liposomes formulated with POPC and MMP-9-cleavable lipopeptides. The kinetic trace of carboxyfluorescein fluorescence (λ_{ex} = 480 nm, λ_{em} = 518 nm) was monitored for 85 min for liposomes formulated with 70 mol % POPC and 30 mol % lipopeptide **LP1** in the presence of 2.3 μM MMP-9. The reactions were conducted at 25 °C in 25 mM HEPES buffer (pH 8.0) in the (black) presence or (red) absence of 10 mM $CaCl_2$ and 10 μM $ZnCl_2$. The blue trace shows the control experiment using liposomes formulated with 100 mol % POPC. The green trace (which partially overlaps the blue trace) represents the leakage from liposomes formulated with 70 mol % POPC and 30 mol % lipopeptide **LP3**. Each trace shown is the average of three experiments.

Elegbede et al. J Am. Chem. Soc. 130:10633

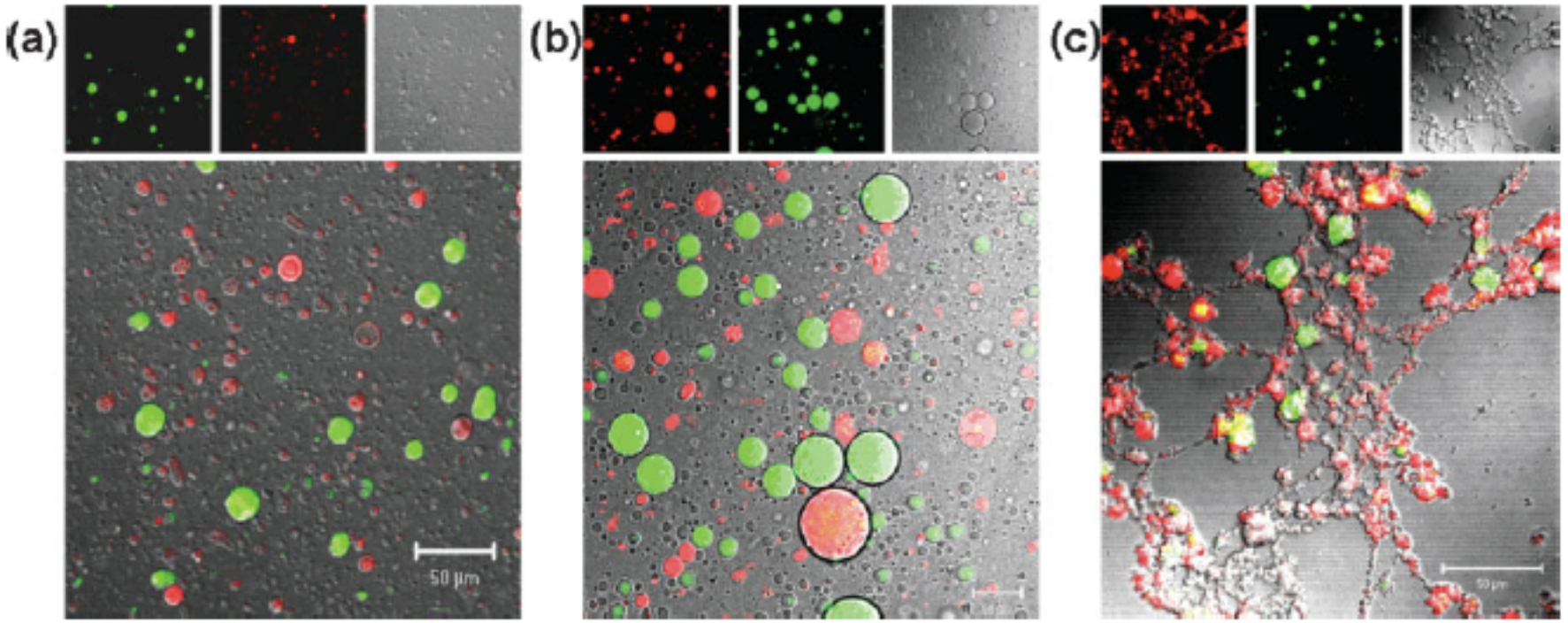


Figure 2. Confocal laser scanning micrographs of giant EB1 polymersomes before (a) and after the addition of 600 Da (b) and 20 000 Da (c) homopolymer PEO. Polymersomes were stained in two different batches with green fluorescein and red rhodamine so as to highlight vesicle–vesicle interactions. The yellow areas show membrane fusion between the two batches.

Smart et al 2008