pH-responsive polymersomes prepared using polymerization-induced self-assembly for biotherapeutic delivery

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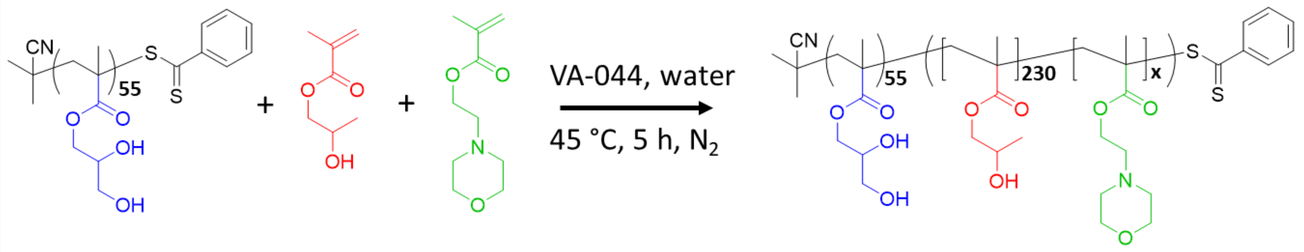
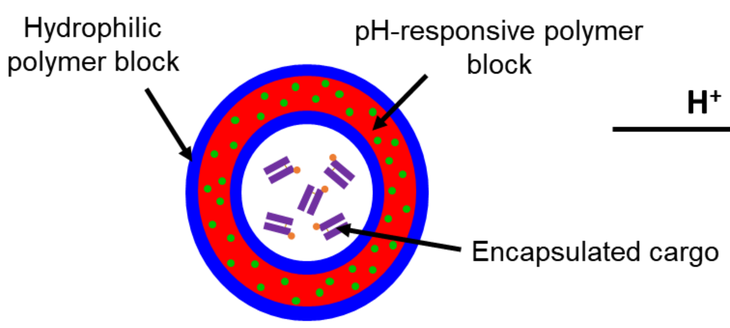
Engineering and Physical Sciences Research Council

Biotechnology and Biological Sciences Research Council

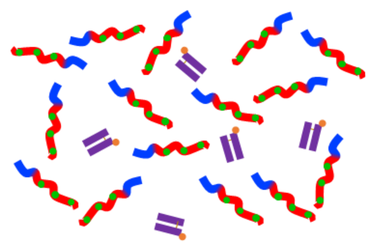
1. In one step: Polymer synthesis, self-assembly, and payload encapsulation



pH 7: polymersomes



pH ≤ 5.25: dissolved polymer chains

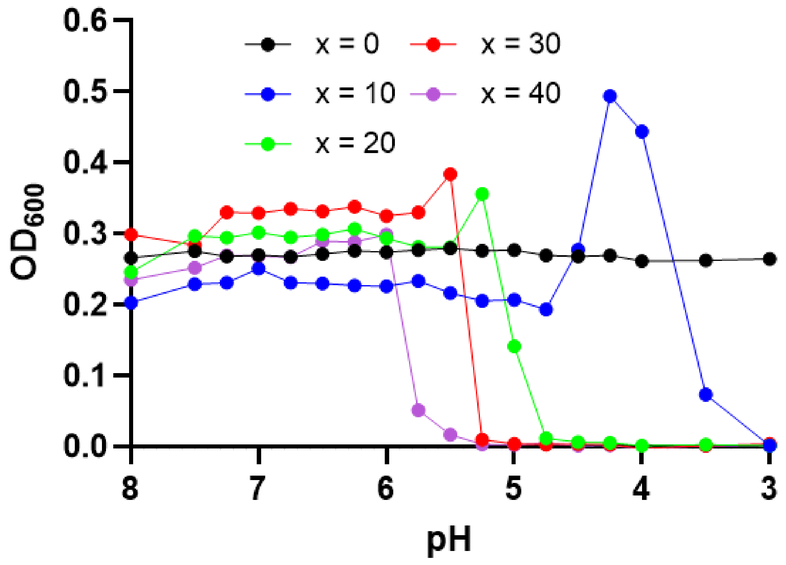


Dissociation and release of encapsulated cargo

- pH-responsive copolymers synthesized using controlled radical polymerisation
- Polymers spontaneously assemble into vesicle structures (“polymersomes”) during synthesis
- Reaction is performed under mild conditions in water
- Biomolecules can be encapsulated during reaction (polymersomes assemble around them)
- Polymersomes dissociate and release their cargo at low pH

2. Tunable pH-responsiveness

- Critical pH for polymersome dissociation release can be tuned
  - x = 40: Dissociation at pH 5.75 - 6
  - x = 10: Dissociation at pH 3.5 - 4
- Polymersomes can also be non-pH responsive (x = 0)



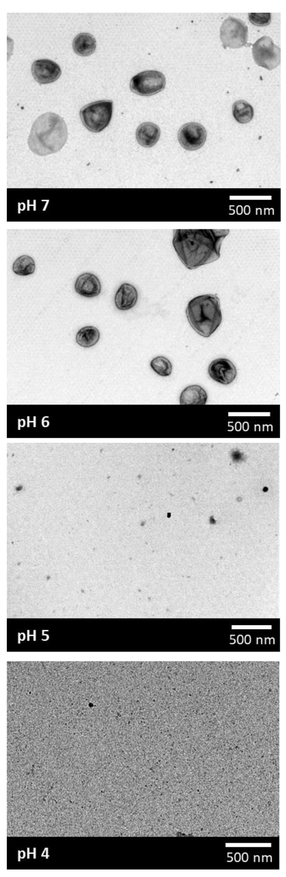
Polymersome dispersions were prepared at 0.1 % w/v in buffers of varying pH before measuring turbidity (by optical density as 600 nm). Polymersome dissociation is detected as a decrease in turbidity.

3. Tuned for dissociation at pH 5

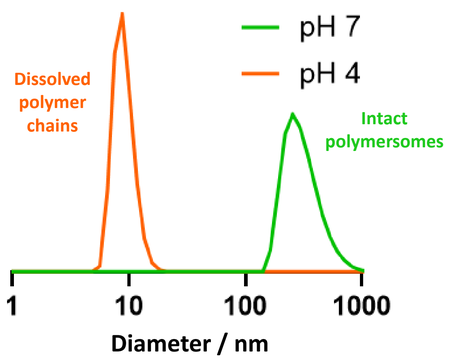
Dissolved polymer chains at low pH:

Dispersion of polymersomes at neutral pH:

Transition electron microscopy:

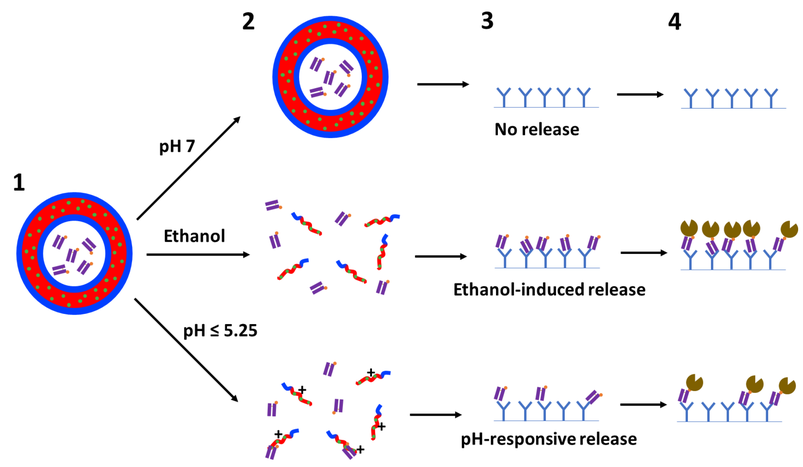


Dynamic light scattering at neutral and low pH:

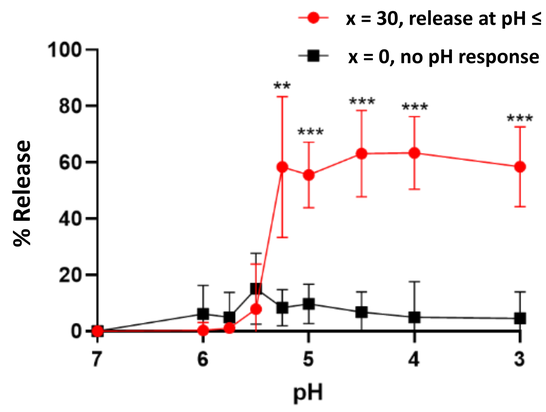


Polymer composition as section 1, with x = 30

4. Antibody fragment: encapsulation and pH-responsive release



1. Antibody fragments are encapsulated during reaction (encapsulation efficiency = 42 ± 4 %)
2. Polymersomes dissociate and release the antibody fragments at low pH or in ethanol
3. Released (and biologically functional) antibody fragments bind to surface-immobilised antigen
4. Functional antibody concentration is measured by enzyme-linked immunosorbent assay



Polymersomes release their payload at the same pH at which they dissociate. Polymersomes were mixed with buffers of varying pH for 5 seconds before diluting in PBS and measuring antibody fragment concentration by ELISA.

Summary

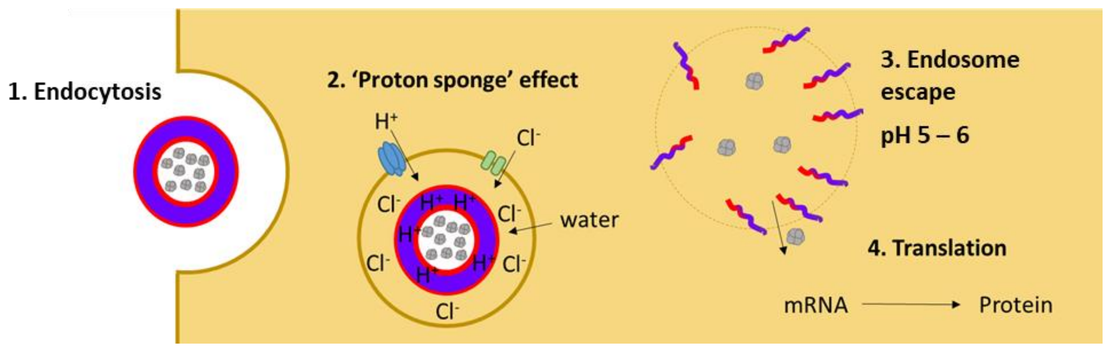
- Polymersomes offer many potential advantages for the intracellular delivery of biological payloads:
  - High capacity, tuneable release, targeted uptake, high mechanical strength, ability to encapsulate many different cargoes
- pH-responsive polymersomes can be designed to dissociate at endosomal pH (pH ~5) to release and deliver their cargo to the cytoplasm
- However, the encapsulation of biopolymers (DNA, RNA, and proteins) is challenging for polymersomes
- We have developed a novel platform technology for encapsulating biopolymers by using polymerization-induced self-assembly (PISA) to synthesize polymers, assemble them into polymersomes, and encapsulate biopolymer cargoes in a single step
- In this work we demonstrate that PISA can be used to load biologically functional antibody fragments or DNA and release in response to endosomal pH

Full article:
Edmans (2025), *ACS Appl. Mater. Interfaces*, **17**, 24

5. Plasmid DNA: encapsulation and intracellular delivery

- Polymersomes were loaded with plasmid DNA
- Encapsulated DNA was stained using PicoGreen
- Encapsulation was confirmed using nano flow cytometry
- Oral keratinocyte cells treated with DNA-polymersomes
- Visualised with confocal microscopy
- DNA was successfully delivered into the cytoplasm

Future work: mRNA vaccine delivery



- We are developing polymersomes to deliver **mRNA therapeutics**
- The delivery of mRNA-polymersomes to the oral cavity using **mucoadhesive patches** is expected to enable new needle-free vaccines against cancers and infectious diseases