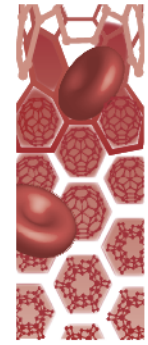
Perspective

For reprint orders, please contact: reprints@futuremedicine.com

Nanomedicine



Toward enzyme-responsive polymersome drug delivery

Bipin Chakravarthy Paruchuri[1], Varun Gopal[1,2], Sapna Sarupria[1,3,4] & Jessica Larsen*[,1,5]

[1]Department of Chemical & Biomolecular Engineering, Clemson University, Clemson, SC 29631, USA
[2]Department of Chemical Engineering & Material Science, University of Minnesota, Minneapolis, MN 55455, USA
[3]Center for Optical Materials Science & Engineering Technologies (COMSET), Clemson University, Clemson, SC 29670, USA
[4]Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, USA
[5]Department of Bioengineering, Clemson University, Clemson, SC 29631, USA
*Author for correspondence: larsenj@clemson.edu

In drug delivery, enzyme-responsive drug carriers are becoming increasingly relevant because of the growing association of disease pathology with enzyme overexpression. Polymersomes are of interest to such applications because of their tunable properties. While polymersomes open up a wide range of chemical and physical properties to explore, they also present a challenge in developing generalized rules for the synthesis of novel systems. Motivated by this issue, in this perspective, we summarize the existing knowledge on enzyme-responsive polymersomes and outline the main design choices. Then, we propose heuristics to guide the design of novel systems. Finally, we discuss the potential of an integrated approach using computer simulations and experimental studies to streamline this design process and close the existing knowledge gaps.

Tweetable abstract: How can we optimize the design of enzyme-responsive polymersomes to better treat disease? In this perspective, three common modes of enzymatic action in these nanoparticles are identified.

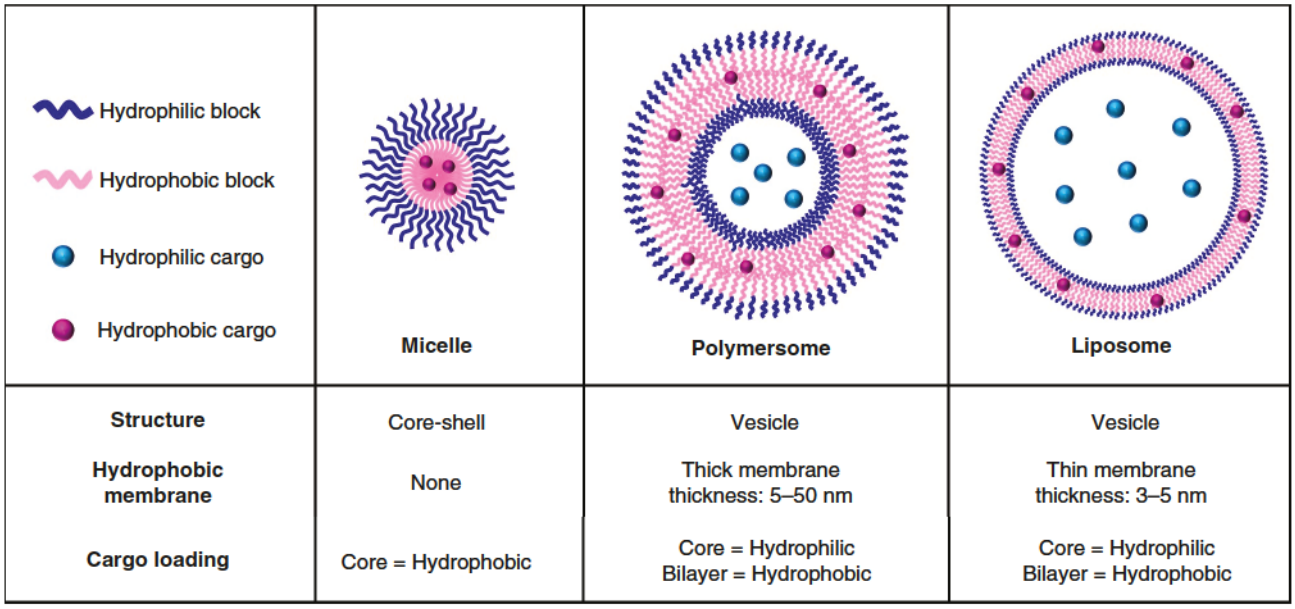
First draft submitted: 21 May 2021; Accepted for publication: 26 October 2021; Published online: 6 December 2021

Keywords: computer simulations • drug delivery • enzyme responsiveness • morphology phase diagrams • nanomedicine • nanoparticles • polymersomes • stimuli responsiveness

Driven by the continued improvement in our understanding of disease pathology, the potential for disease-driven drug-vehicle design is rapidly increasing. Recently, many diseases have been associated with alterations in enzymatic activity [1–4], bringing the focus on developing enzyme-responsive nanoparticles. Specifically, various enzymes native to the diseased tissue have shown higher activity in cancers [1,4], inflammatory diseases like osteoarthritis [3], neurodegenerative conditions [5–7] and idiopathic pulmonary fibrosis [2]. This pathological upregulation of enzymes presents an opportunity to devise enzyme-responsive delivery vehicles to achieve controlled drug release. Currently, most enzyme-responsive systems comprise of mesoporous silica nanoparticles [8–11], which are plagued by limited drug loading due to the small size of pores and the necessity of capping agents to introduce stimuli-responsive behavior [12]. Self-assembled nanoparticles can overcome these pitfalls, with larger drug reservoirs, structurally driven stimuli-responsive behavior and the ability to encapsulate both hydrophilic and hydrophobic drugs.

One such class of self-assembled nanoparticles, polymersomes, are a polymeric counterpart to liposomes with improved functionalities as drug carriers. The bilayer of these self-assembled polymer vesicles contains a hydrophobic membrane surrounded by outer and inner hydrophilic brushes (Figure 1). Due to the higher molecular weight of the constituent polymers compared to lipids, polymersome membranes are thicker than liposomes (Figure 1). The thick membranes improve the stability and drug retention capability of polymersomes [13]. Within the class of polymer-based nanoparticles, polymersomes have added value compared to micelles. With a core-shell structure, micelles are limited to carrying hydrophobic payloads. Polymersomes can carry both hydrophobic and hydrophilic payloads, enabling them to deliver multiple drugs with distinct solubilities simultaneously in combination therapy [14,15].

Future Medicine



	Micelle	Polymersome	Liposome
Structure	Core-shell	Vesicle	Vesicle
Hydrophobic membrane	None	Thick membrane thickness: 5–50 nm	Thin membrane thickness: 3–5 nm
Cargo loading	Core = Hydrophobic	Core = Hydrophilic Bilayer = Hydrophobic	Core = Hydrophilic Bilayer = Hydrophobic

Figure 1. A comparison of the properties and 2D structure of three different self-assembled nanoparticles: micelles, polymersomes and liposomes.

Polymersomes that are stimuli-responsive have an advantage of triggered drug release in response to their environment. Enzymes as stimuli are of interest due to pathological enzyme dysregulation. The high substrate specificity of enzymes reduces the likelihood of off-target release and could alleviate the potential side effects of the drug. Enzyme-responsive polymersomes also show potential for control over drug release rates, which can be achieved by tailoring the synthetic polymers.

Polymersome morphology is usually controlled through different design choices pertaining to the properties of the synthetic amphiphile. Three common design choices are the hydrophilic fraction (*f*) of the polymer chain, the polymer molecular weight and the monomer chemistry. However, the large parameter space associated with these choices can make nanoparticle design difficult. Currently, the design rules stating the optimal parameter ranges are well understood for poly(ethylene glycol) (PEG)-based polymersomes [16], but similar rules are lacking for enzyme-responsive polymersomes. Without a systematic approach for selecting the parameter values, designing new enzyme-responsive systems requires a trial and error approach and is resource intensive. In this perspective, we outline the current knowledge of enzyme-responsive polymersome systems and provide heuristics for designing novel polymersome systems using a combination of experiments and computer simulations.

Enzyme-responsive polymersomes

Enzyme-responsive polymersomes undergo structural or functional changes by the direct action of enzymes. These changes can disrupt the hydrophobic driving forces maintaining the self-assembled structures, resulting in a change in the morphology of the polymersomes. Currently, the literature reports few enzyme-responsive polymersome systems, summarized in Table 1, but the underlying mechanisms of disassembly are not well understood. The most frequent enzymatic action noted is bond cleavage, which can occur at either a) the hydrophobic membrane, b) the link between the hydrophobic membrane and the hydrophilic brush, or c) the hydrophilic brush of the polymersome (Figure 2). The enzyme can either act on the polymer backbone or a sidechain. Next, we discuss the enzymatic action at each location in detail.

Enzymatic cleavage in the hydrophobic membrane

The hydrophilic fraction (*f*) of an amphiphile is a design choice that determines the morphology of the self-assembled nanoparticle. Enzymatic cleavage of a bond within the hydrophobic block of the copolymer increases the *f* of the amphiphile. In systems based on PEG, with low molecular weight versions known as poly(ethylene oxide) (PEO),

Table 1. A list of enzyme-responsive polymersome systems.

Location of enzymatic action	Material	Enzyme stimuli	Target disease or tissue	Morphology change	Ref.
Hydrophobic membrane	PEO-*b*-PTMQ	NQO1	Breast, pancreatic, colorectal, cervical, and Lung cancers	Vesicle to core-crosslinked micelles	[17]
	PEG-*b*-PC	MMP-2	MRSA infection	Vesicle to core-crosslinked micelles	[18]
	PEG-*b*-PLLNA	Esterase	Tumor tissue	Bilayer is permeabilized and vesicle structure is retained	[19]
The link region between the hydrophobic membrane and the hydrophilic brush	PGA_{15}-*b*-PVGLIG-*b*-$PTMC_{50}$	MMP-2	Tumor tissue	Loss of vesicular structure	[20]
	PEG-*b*-PVGLIG-PLA	MMP-2	Tumor tissue	Loss of vesicles, solid aggregates were observed after enzyme treatment for 24 h	[21]
	mPEG-GFLGF-PDLLA	Cathepsin B	Tumor tissue	Complete loss of vesicles at seven days, formation of aggregates	[22]
Hydrophilic brush	PVGLIG-*b*-PTMC	MMP-2	Tumor tissue	Loss of vesicular structure	[23]
Dual-responsive polymersomes	HYA-*b*-PCL	Hyaluronidase and lipases	Bacterial infection	Size reduction upon addition of enzyme	[24]
	HYA-*b*-PLA	Hyaluronidase and proteinase K	Bacterial infection	Size reduction upon addition of enzyme	[25]
Surface of polymersomes	PDMS-PMOXA	MMP-9	Tumor tissue (breast cancer)	NA	[26]

Systems are primarily organized by the location of enzymatic action and, therefore, the location of the enzymatic substrate. Target disease and corresponding pathologic, enzymatic stimuli being targeted as delivery catalysts are highlighted. It is important to note that morphologic changes observed are not necessarily the same within each category.
GFLGF: Gly-Phe-Leu-Gly-Phe; HYA: Hyaluronic acid; mPEG: Methyl poly(ethylene glycol); MRSA: Methicillin-resistant *staphylococcus aureus*; NA: Not applicable; PC: Polymer backbone with cephalosporin terminal groups; PCL: Poly(caprolactone); PDLLA: Poly(D,L-lactide); PDMS: Poly(dimethylsiloxane); PEG: Poly(ethylene glycol); PEO: Poly(ethylene oxide); PGA: Poly(glutamic acid); PLA: Poly(lactic acid); PLLNA: Poly-L-lysine (N-acetoxybenzylacetate); PMOXA: Poly(methyloxazoline); PTMC: Poly(trimethylene carbonate); PTMQ: Polytrimethyl quinone; PVGLIG: Pro-Val-Gly-Leu-Ile-Gly.

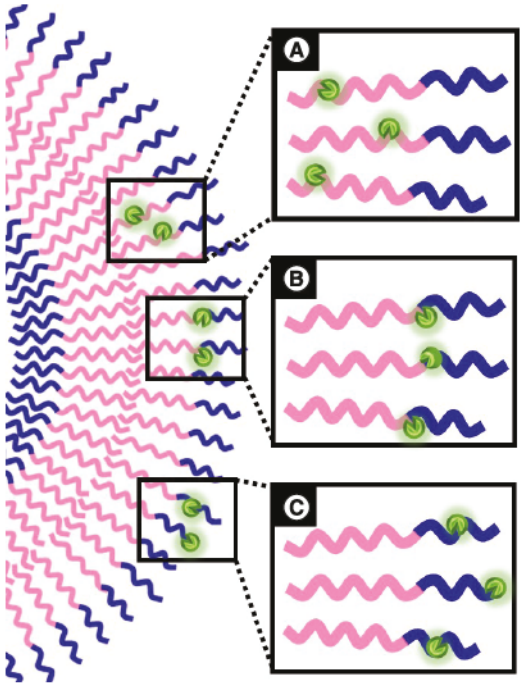


Figure 2. Common locations of enzymatic action leading to polymersome degradation. Within the polymersome structure, an enzyme molecule (depicted in green) can cleave the polymer chain in one of the three possible locations: **(A)** the hydrophobic membrane, **(B)** the link region between the hydrophobic membrane and the hydrophilic brush and **(C)** the hydrophilic brush. The insets depict the cleavage site within a polymer chain.

10.2217/nnm-2021-0194

copolymers with a higher *f* self-assemble into micelles [16]. In theory, an increasing *f* value due to the enzymatic cleavage in the hydrophobic membrane of polymersomes should drive the nanoparticle morphology from vesicles to micelles. Experimental evidence suggests that this transition in PEO-based systems can sometimes be coupled with cross-linking of polymer chains. For example, Yao *et al.* synthesized a quinone derivative N-(4-(hydroxylmethyl)phenyl)-3-methyl-3-(2,4,5-trimethyl-3,6-dioxocyclohexa-1,4-dien-1-yl)butanamide (TMQH) that is responsive to an enzyme upregulated in tumor cells called NAD(P)H: quinone oxidoreductase isozyme 1 (NQO1) [17]. TMQH was incorporated into the side chains of a PEO copolymer, resulting in an amphiphilic PEO-*b*-PTMQ. When incubated with NQO1, the quinone moieties in the hydrophobic block were cleaved, increasing the *f* of the copolymer. When PEO_{45}-*b*-$PTMQ_{20}$ polymersomes were treated with NQO1, the nanoparticle morphology changed from a vesicle to a core-crosslinked micelle. Cross-linking of the hydrophobic chains in core-crosslinked micelles can hinder further enzymatic degradation of the chains [27]. Additionally, the amphiphile PEO_{45}-*b*-$PTMQ_{12}$ with a higher *f* self-assembled into spherical micelles. No cross-linking was observed in the absence of NQO1. This result indicates that cross-linking is triggered by the enzymatic cleavage of quinone moieties. In another study, Li *et al.* designed enzyme-responsive vesicles to overcome antimicrobial resistance using a block copolymer called PEG-*b*-PC by the authors [18]. Drug-resistant bacterial strains such as MRSA produce the enzyme β-lactamase (Bla). The amphiphile PEG-*b*-PC contains Bla-responsive cephalosporin as terminal groups in the side chains of the hydrophobic block PC. The cephalosporin terminal groups were cleaved when exposed to Bla, triggering a cross-linking reaction followed by a morphology change from vesicles to core-crosslinked micelles. Additionally, the Bla-responsive polymersomes selectively inhibited the growth of MRSA by releasing the hydrophilic drug vancomycin. These studies suggest that factors other than the *f* can dictate the morphology change of enzyme-responsive polymersomes. One major concern in the enzymatic degradation of a substrate in the hydrophobic membrane is the enzyme's accessibility to the substrate, i.e. the diffusion of the large enzyme molecule into the densely packed hydrophobic membrane. A proposed explanation is that the enzyme degrades the hydrophobic substrate of the free polymer chains which are in equilibrium with the polymersomes [19]. Cross-linking in the hydrophobic membrane triggered by the enzymatic action can trap the polymer chains in the self-assembled structure, thereby disrupting the equilibrium. This may prevent the polymersomes from attaining predicted morphologies following the enzymatic degradation. Future work should explore the effect of monomer chemistry on achieving or avoiding cross-linking upon enzymatic action and the effect of cross-linking on drug release.

Enzymatic cleavage in the link region

In polymersomes, enzymatic cleavage can also occur at the link between the hydrophilic and hydrophobic blocks of the amphiphile. This cleavage could disrupt the vesicular structure of the nanoparticle, creating aggregates of the insoluble polymer. Polymersome systems with peptide linkers such as Pro-Val-Gly-Leu-Ile-Gly (PVGLIG) and Gly-Phe-Leu-Gly (GFLG) are commonly used in delivery systems for tumor-specific drug release. Tumor-associated proteases like MMPs and cathepsin B (Cath B) degrade PVGLIG and GFLG, respectively [28,29]. For example, Bacinello *et al.* synthesized a poly(glutamic acid)-*b*-PVGLIG-*b*-poly(trimethylene carbonate) (PGA-*b*-PVGLIG-*b*-PTMC) copolymer [20]. The resulting polymersomes showed a complete release of encapsulated imipramine hydrochloride within 4 days in the presence of 10 nM MMP-2 and 16 days without the enzyme. This study suggests that enzymatic cleavage of the link region resulted in the loss of vesicular structure and rapid release of the cargo relative to the control. Similarly, Ramezani *et al.* obtained a sevenfold higher release of the cargo, 7-ethyl-10-hydroxy camptothecin, from PEG-PVGLIG-poly(lactic acid) (PEG-*b*-PVGLIG-PLA) polymersomes when treated with 10 nM MMP-2 [21]. The enzymatic action resulted in a complete release of the encapsulated molecule in 10 days. The faster drug release from PGA-*b*-PVGLIG-*b*-PTMC polymersomes compared to PEG-*b*-PVGLIG-PLA might indicate a rapid degradation of the PGA-based vesicles. The higher hydrophilicity of PGA could increase the hydration of the hydrophilic brush and enhance the accessibility of MMP-2 to the peptide linker [21]. Lee *et al.* developed a methoxy poly(ethylene glycol)-GFLGF-poly(D,L-lactide) (mPEG-GFLGF-PDLLA) polymersome system which is responsive to Cath B. In the presence of the enzyme, complete release of the model drug acridine orange was observed within 3 days compared to a 40% release during the same time without the enzyme [22]. Similar to the studies mentioned above, both a complete loss of vesicles and aggregate formation was observed after 7 days of incubation with Cath B. Such peptide links can be beneficial for systems requiring rapid drug release.

In amphiphiles without a dedicated linker group, the bond connecting the hydrophobic and hydrophilic components can be enzymatically cleaved to achieve disassembly. For example, Pramod *et al.* synthesized an

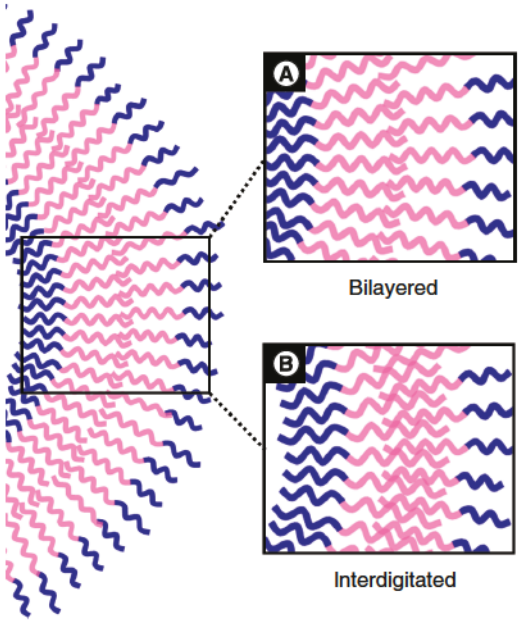


Figure 3. Common types of hydrophobic membranes in polymersomes.

amphiphile with an aliphatic ester bond linking hydrophobic 3-pentadecylphenol (PDP) to a dextran backbone [30]. In the presence of esterase, the vesicles disassembled and released the hydrophobic drug camptothecin (CPT). The release occurred in two phases – an initial rapid release of 60–70% of the encapsulated CPT followed by a steady release of the remaining drug. A precipitate of PDP was observed in the presence of esterase after 24 h, indicating its separation from the backbone. These studies agree with the expected morphology loss upon enzymatic bond cleavage at the link region.

For the enzymatic degradation of the hydrophobic membrane or at the link region, a potential concern is the diffusion of enzymes into the polymersome structure. The diffusivity of large molecules like enzymes into the hydrophobic membrane is lower than small molecules, limiting the access of the substrate to the enzyme [17]. The permeability of the vesicles is determined by the polymersome membrane structure [31], which is in turn governed by the molecular weight of the copolymer. A lower molecular weight amphiphile results in a bilayer structure with minimal chain entanglement across the bilayer (Figure 3A). A higher molecular weight copolymer results in an interdigitated structure with a higher degree of chain entanglement (Figure 3B). In general, interdigitated structures result in thinner membranes [32], thereby increasing the membrane packing density and potentially limiting the diffusivity of the enzyme. Future studies should explore the effects of polymersome membrane density and packing style on membrane fluidity. This information could improve the drug retention capability of polymersomes. Additionally, the effects of membrane fluidity on the permeability of enzymes should also be studied. This knowledge could provide potential control over the drug release rates.

Enzymatic cleavage at the hydrophilic brush

Designing polymersomes with enzyme-degradable hydrophilic blocks has a few challenges. The outer hydrophilic brush is preferentially degraded by enzymes compared to the inner brush due to the limited enzyme diffusion through the hydrophobic membrane. The composition of the outer hydrophilic brush affects the nature of proteins adsorbed on the vesicle surface. The protein corona of nanoparticles plays an important role in avoiding systemic clearance and increasing the circulation half-life of the nanoparticles [33]. This phenomenon, combined with biocompatibility and safety requirements, greatly limits the available options of enzyme-responsive hydrophilic polymers. Despite these limitations, polymersomes with an enzyme-responsive hydrophilic brush can be designed with hydrophilic biomolecules. Bacinello *et al.* synthesized a PVGLIG-PTMC hybrid amphiphile, comprised of hydrophilic peptide PVGLIG and a hydrophobic synthetic polymer PTMC, that can self-assemble into vesicles and micelles [23]. The disassembly of these vesicles in the presence of MMP-2 was demonstrated by dynamic light scattering analysis; a reduction of relative scattering intensity to 60% was observed in 2 days. When located in the hydrophilic brush, PVGLIG is more accessible to MMP-2, which allows a faster degradation compared to PVGLIG as a linker where a similar loss in scattering intensity was observed in 5 days [20]. This effect of the

10.2217/nnm-2021-0194

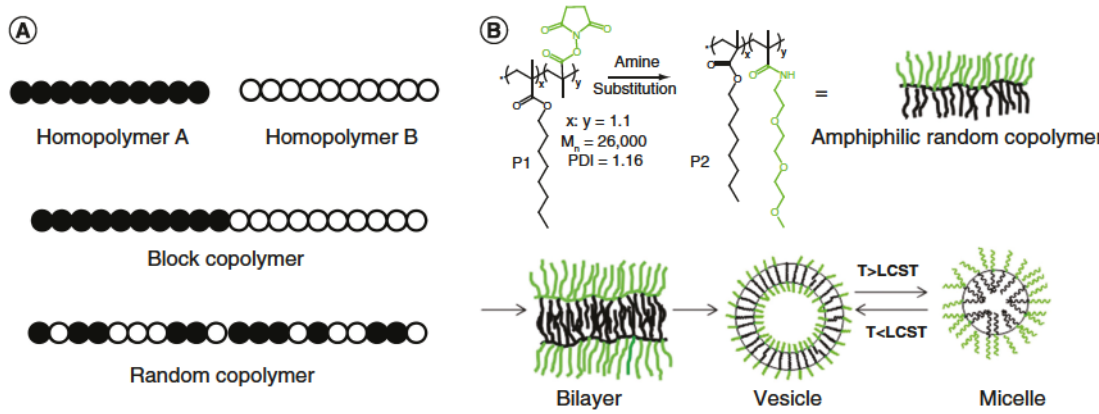


Figure 4. Self-assembly of random copolymers. (A) Comparison between block copolymers and random copolymers. **(B)** The synthesis route of a random copolymer, P2, shown in standard notation along with a schematic of the resulting self-assembled morphologies.
LCST: Lower critical solution temperature; Mn: Number average molecular weight; PDI: Polydispersity index; T: Temperature.
Reproduced with permission from [36], © 2015 American Chemical Society.

hydrophilic component on the enzyme's accessibility to the substrate can be explored further to achieve higher release rates. Moreover, the pool of suitable hydrophilic polymers could be expanded through polymersome surface modifications. For example, the protein adsorption on nanoparticles can be altered by coating the nanoparticle surface with hydrophilic polymers such as PEG and poly(phosphoester)s [33]. The effect of such coatings on the enzyme degradability of polymersomes should be further studied.

Dual enzyme-responsive polymersomes with multiple responsive units have also been reported. In hyaluronic acid-*b*-poly(ε-caprolactone) (HYA-*b*-PCL) polymersomes, the hydrophilic HYA is degraded by the hyaluronidase enzyme secreted by pathogenic bacteria, and the hydrophobic PCL can be degraded by certain lipases [24]. In another study, Tücking *et al.* demonstrated the selective degradation of HYA-b-PLA polymersomes by both hyaluronidase and proteinase K [25]. Apart from the locations on the amphiphilic copolymer chain, another plausible location for an enzyme-responsive unit is the surface of the polymersome. Porta *et al.* synthesized poly(dimethylsiloxane)-*b*-poly(methyloxazoline) (PDMS-*b*-PMOXA) polymersomes with an enzyme-digestible peptide shell [26]. The authors proposed that when MMP-9 digests the crosslinked peptide shell, the encapsulated drug permeates through the vesicle membrane.

Random copolymers

Until now, we have discussed enzyme-responsive polymersomes based on block copolymers. There is also a growing interest in the self-assembly of random copolymer amphiphiles due to the relative ease of their synthesis compared to block copolymers [34]. In the random copolymer chain, the hydrophilic and/or hydrophobic monomers are randomly arranged (Figure 4A). The random copolymer-based self-assemblies such as micelles and polymersomes can respond to stimuli like pH [35], temperature [36] and light [37]. Figure 4B highlights one such random co-polymer used to create thermally-responsive polymersomes made from octyl methacrylate and *N*-hydroxysuccinimide methacrylate monomers with the addition of a methoxy group [36]. But enzymes as stimuli can present a challenge due to their substrate specificity and binding requirements. For example, β-chitin is a random copolymer that can be degraded by lysozyme. The binding of the active site onto the copolymer chain requires a sequence of six contiguous monomers with acetamido side groups [38]. Consequently, a high degree of deacetylation of the polymer chain reduces the extent of enzymatic degradation [39,40]. Similarly, in a poly(D,L-lactide) (PDLLA) stereocopolymer, proteinase K preferentially cleaves the ester bonds between two L-lactyl units. As a result, the degradation rate of the copolymer chain by proteinase K decreases with a decreasing percentage of L-lactyl units, and poly(D-lactic acid) remains undegradable [41]. These results suggest that the sequence of the monomers in a random copolymer can impact the enzyme-substrate binding and the number of cleavable sites. Further studies are required to explore this phenomenon to potentially achieve a tunable release from nanoparticles.

While there are many aspects of enzyme-responsive polymersome systems that require further study, a significant challenge in developing novel systems is a lack of optimal design parameter values for obtaining vesicles upon self-assembly. The parameter space created by the hydrophilic fraction (f), the polymer molecular weight, and the chemistry of the amphiphile is large, rendering nanoparticle design difficult. Current design strategies are primarily guided by intuition which may not always generate vesicular structures. As a result, extensive experimental testing is necessary, which can be time consuming and laborious. In the next section, we propose a set of heuristics for designing enzyme-responsive polymersomes to address this issue. These heuristics, in combination with computational techniques can optimize the design process.

Designing enzyme-responsive polymersomes

The key requirement in creating enzyme-responsive polymersomes is designing an amphiphile that contains the enzyme's substrate and will self-assemble into a vesicular morphology. Here, we define a set of design choices to streamline this process.

The first design choice to construct enzyme-responsive polymersomes is the selection of a suitable enzyme stimulus. This choice will be guided by the disease pathology and the desired enzymatic activity. Enzymes with endolytic activity can induce random scission in the polymer chain, resulting in a rapid decline in the molecular weight of the chain. Enzymes with exolytic activity cleave the terminal groups on the chain, causing a slower reduction in the molecular weight. The choice of enzyme will define the substrate(s) that can be incorporated into the amphiphilic copolymer. The location of the substrate in the amphiphile can influence the enzymatic degradation of the polymersome, as discussed in the previous section.

The subsequent design choices that could be used to alter the nanoparticle morphology include the chemistry of the unresponsive portion of the amphiphile, the polymer shape (i.e., linear, circular, hyperbranched, etc.), the f value and the degree of polymerization (DP) of the polymer. DP is the total number of monomer units in a polymer chain. These choices reflect polymer characteristics that are easily controlled. Even with these few design choices, the parameter space is large. For example, there are a vast number of monomers that could be selected for the non-responsive portion of the amphiphile. Searching this space for the optimal conditions to create enzyme-responsive polymersomes can be difficult. To improve this search, we have developed a few design heuristics based on the previous work in the field.

Heuristic 1: Select a linear copolymer shape

The first heuristic we suggest corresponds to the design choice of the polymer shape. Polymer shapes such as star copolymers [42–44], linear-dendrite block copolymers [45] and hyperbranched copolymers [46] are known to self-assemble into polymersomes. However, the linear copolymer shape is more frequently used for synthesizing enzyme-responsive polymersomes [17,18,20–25,47]. The behavior of linear diblock copolymers in aqueous solutions has been studied, and general correlations between the design choices and the nanoparticle characteristics have been proposed [16,48].

One empirical relation correlates the f value of the amphiphile with the aggregate morphology of PEO-based systems. The hydrophilic fraction can predict whether spherical micelles ($f > 50\%$), worm micelles ($50\% > f > 40\%$), or polymersomes ($40\% > f > 25\%$) are formed [16]. The desired f value can be achieved by controlling the DP of the hydrophobic and hydrophilic blocks in the copolymer chain. Similar trends have been observed for other block copolymers [49–52] and other polymer shapes [45]. Another correlation relates the polymersome membrane thickness (d) to the hydrophobic block DP [48]:

$$d \sim M_h^{\,b} \; (b = 0.55)$$

where M_h is the molecular weight of the hydrophobic block, which monotonically increases with the hydrophobic block DP. This scaling relation was developed for PEO-*b*-poly(butadiene) (PBD) and PEO-*b*-poly(ethylethylene) (PEE) polymersome systems. Future work should focus on developing such relations for novel systems and explore the generalizability of these relations.

While the hydrophilic fraction of the amphiphile can predict equilibrium morphologies, the predicted morphologies may not always be observed due to kinetic effects [50,53]. The extent of these effects could be dependent on the nanoparticle preparation method. Barnhill *et al.* observed that the choice of cosolvent prior to dialysis by water in the solvent-switch method resulted in different nanoparticle morphologies [53]. The proposed explanation

10.2217/nnm-2021-0194

is that the polymer chains experience different degrees of kinetic trapping which is influenced by polymer-solvent interactions. Kinetic trapping results in a slow reorganization rate of the polymer chains. This effect could prevent the thermodynamically favored nanoparticle morphology from being observed at an experimental timescale. Blanazs *et al.* found that the DP of a diblock copolymer could affect the kinetics of chain reorganization in the polymerization-induced self-assembly (PISA) method of nanoparticle formation [50]. Polymer chains with higher DP were kinetically trapped, preventing polymersomes from forming. These results suggest that kinetic factors should be considered when predicting the self-assembled morphologies of novel amphiphiles.

Heuristic 2: Use morphological phase diagrams

This heuristic suggests using morphological phase diagrams of known copolymer systems to design polymersomes from novel amphiphilic polymers. Phase diagrams for amphiphilic copolymers reflect the effects of multiple parameters such as the polymer DP, polymer concentration, and f on nanoparticle morphology. Typically, phase diagrams are presented as phase planes displaying the effect of two variables on morphology.

Phase diagrams can be experimentally or computationally obtained. To our knowledge, most experimentally obtained phase diagrams are for linear copolymers [49–51,53,54], further emphasizing the benefits of our first heuristic. Figure 5 shows one such example of experimental phase diagrams for two linear diblock copolymers. The diagrams reflect the observed nanoparticle morphology at various DP values and hydrophilic fractions. Phase diagrams represent phase boundaries between morphologies and allow one to effectively search the parameter space to find suitable conditions for generating polymersomes.

Synergy between experiments & simulations

Design of polymersomes includes several aspects such as identifying the appropriate monomers and conditions to obtain the desired morphology, engineering the desired enzyme responsiveness and achieving the required drug loading and delivery. Understanding the interplay of the various factors that govern each of these aspects becomes important for optimizing the parameters involved to obtain the desired enzyme-responsive polymersome. Acquiring this knowledge only through experiments can be rather challenging due to both resource intensiveness and limitation of accessible time and length scales. Computer simulations can be a powerful tool to complement experimental studies. For example, developing experimental phase diagrams for novel amphiphiles is resource-intensive due to the large parameter space of design choices. For such systems, *in silico* generated phase diagrams can be an effective alternative. Factors like monomer choice, DP, polymer shape, and solvent type can be explicitly controlled in computer simulations, allowing the prediction of the individual effects of these design choices on particle morphology. A variety of morphology phase diagrams have been computationally obtained for linear block copolymers [55–57], linear-dendrite copolymers [58], hyperbranched copolymers [59,60], and cyclic copolymers [61]. Figure 6 shows simulated phase diagrams for a linear copolymer [57] and a hyperbranched copolymer [60]. Phase diagrams are obtained using simulation techniques such as coarse-grained molecular dynamics (CG-MD) and dissipative particle dynamics (DPD). In both techniques, groups of atoms are represented by single interaction sites (Figure 6B), and the motion of each site is governed by Newton's equations of motion. Typical software for conducting these simulations include AMBER [62], OpenMM [63], HOOMD [64], GROMACS [65,66], and LAMMPS [67,68]. Computer simulations of these systems are complex, require careful development of the system and simulation parameters to produce physically reasonable results, and are often resource-intensive. In recent years, the combination of machine learning with molecular simulations has proven to be very effective in reducing the parameter search space. Using similar approaches utilizing active learning techniques to optimize the parameter search space for polymersome design can be transformative.

In addition to equilibrium structures, simulations also enable studies of the dynamics of morphology changes. In this regard, the integration of simulations and experiments shows promise in aiding the study of enzyme-responsive systems. Wright *et al.* used this combined approach to study the morphology change of enzyme-responsive micelles upon enzyme action [69]. Their experiments indicated that the morphology changes were affected by two characteristics of the amphiphile's hydrophobic group: the glass transition temperature and the relative solvophobicity. Simulations provided mechanistic insight to suggest that these characteristics influence the kinetics of the morphology change. If the kinetically accessible pathway of a morphology change is the same as the thermodynamically favored pathway, the resultant structure is an equilibrium morphology. However, when these pathways differ, the result is a kinetically-trapped morphology. A similar approach has been extended to study enzyme-responsive polymersomes. Li *et al.* used simulations to explain their experimental results that enzymatic action could cause

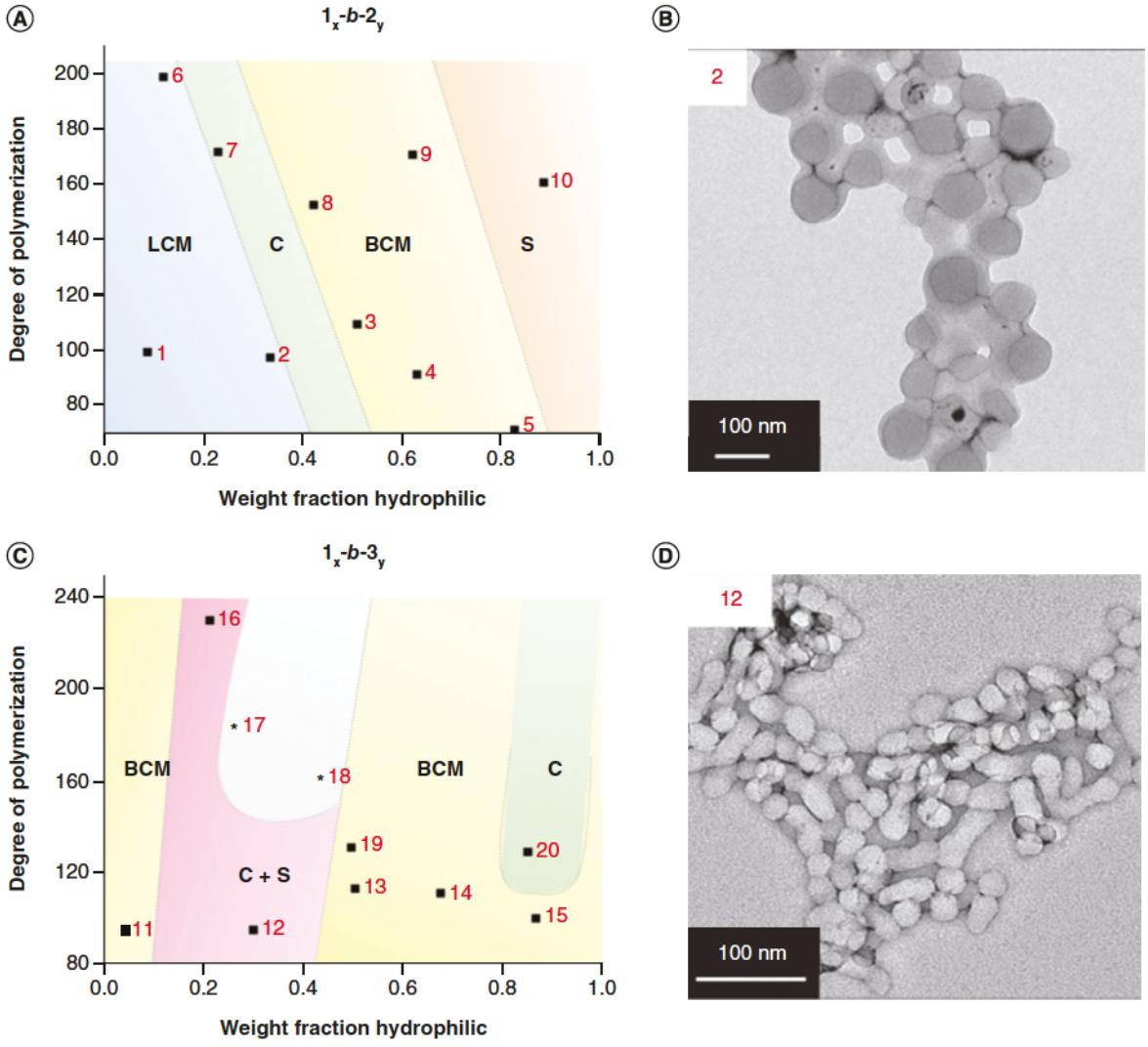


Figure 5. Experimentally obtained morphology phase diagrams for two diblock copolymers and corresponding TEM images of nanoparticles. (A & C) Phase diagrams reflect aggregate morphologies of amphiphiles 1-*b*-2 and 1-*b*-3 where 1, 2, and 3 correspond to norbornene-phenyl, norbornene-PEG, and norbornene-ethanolamide, respectively. Component 1 is hydrophobic. Components 2 and 3 are hydrophilic. Subscripts x and y are the numbers of monomer units. Experimental data points are denoted with numbered points on the phase diagram. Areas of the phase diagram are shaded in different colors corresponding to the nanoparticle morphology observed. TEM images **(B)** and **(D)** correspond to points number 2 and 12, respectively, in **(A & C)**.
*nanoparticles were not observed.
BCM: Bicontinuous micelle; C: Cylindrical micelle; C + S: Cylindrical micelles + spherical micelles; LCM: Large compound micelle; S: Spherical micelle; TEM: Transmission electron microscope.
Reproduced with permission from [53], © 2015 American Chemical Society.

polymersomes to transition into either multicavity vesicles or small nanoparticles [70]. The simulations suggest that the transition mechanism and kinetics are affected by the local concentration of nanoparticles; secluded particles slowly transition to modified vesicles and small micelles while concentrated nanoparticles merge more quickly to form multicavity vesicles. Furthermore, the simulations also suggest a mechanistic hypothesis for the release of the encapsulated molecules from the polymersomes. These studies indicate that an integrated approach can provide high levels of mechanistic insight into experimentally observed phenomena. Another exciting avenue where simulations can complement experiments is illustrated in the recent work where machine-learning models have been designed to predict nanoparticle characteristics from experimental small-angle neutron scattering (SANS) data [71,72]. This technique could improve the accuracy in measuring the dimensions of nanoparticles, advancing

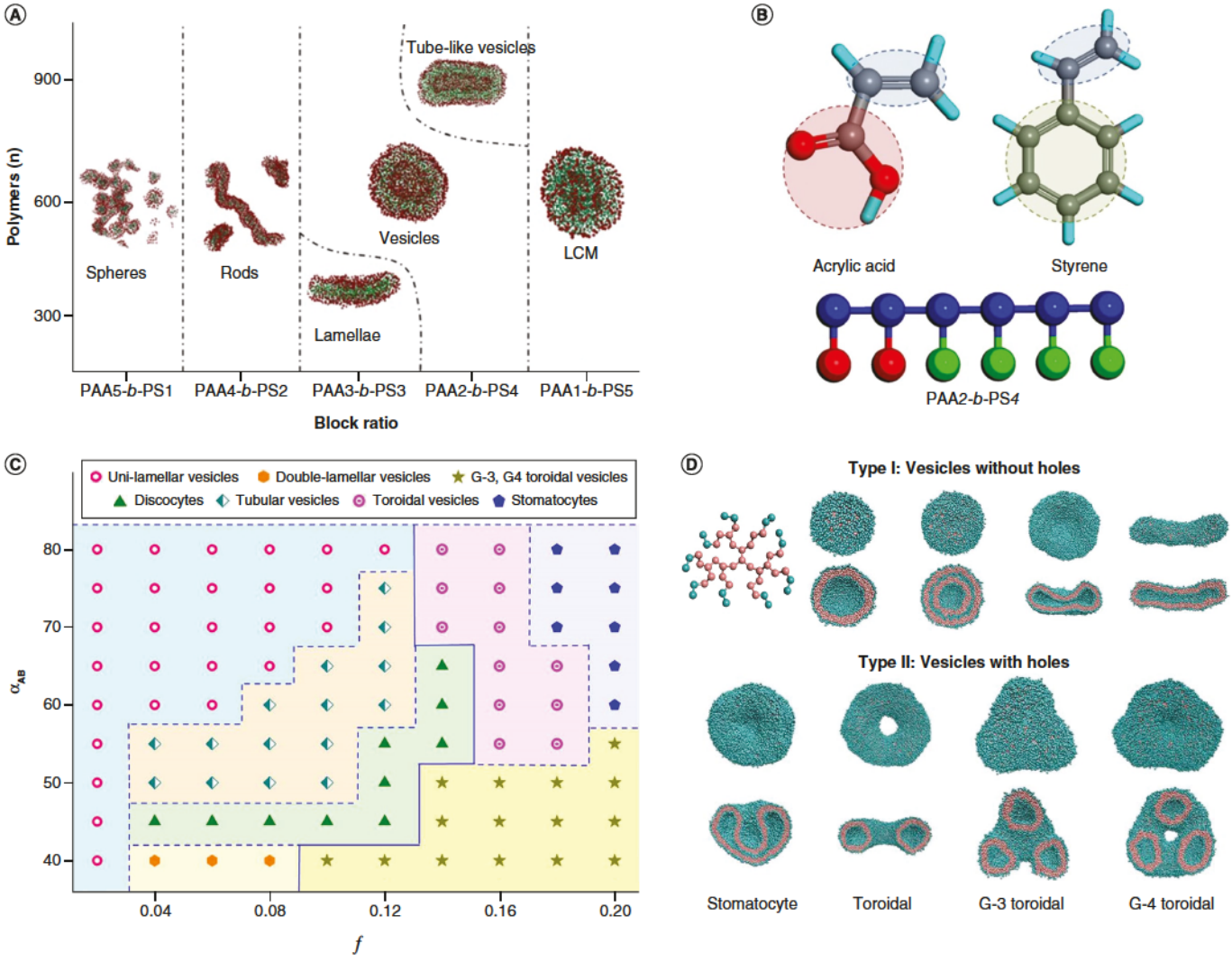


Figure 6. Computationally obtained phase diagrams for two amphiphilic polymers. The phase diagram **(A)** was obtained from coarse-grained molecular dynamics and a representation of the amphiphile simulated is shown in **(B)**. **(B)** Provides an atomistic representation of two monomers and a corresponding coarse-grained representation of the monomers in a linear amphiphile. **(C)** Shows a phase diagram for a hyperbranched polymer obtained from dissipative particle dynamics. Visual representations of the morphologies in the phase diagram are shown in **(D)**, along with a coarse-grained representation of the hyperbranched polymer. The interaction parameter (α) between A (pink - hydrophobic) and B (cyan - hydrophilic) and the hydrophilic fraction (*f*) of the polymer were varied to evaluate their effects on nanoparticle morphology.
LCM: Large compound micelle.
(A & B) Reproduced with permission from [57], © 2017 Wiley Periodicals, Inc. **(C & D)** Reproduced with permission from [60], © 2018, American Chemical Society.

the ability to target specific nanoparticle sizes. The detailed mechanistic insights from the synergy of experiments and simulations lays the foundation for building predictive models in the future. Thus, *in silico* studies can narrow the polymersome design space that needs to be explored experimentally to achieve the desired characteristics. It has been suggested that such synergistic use of experiments and simulations can significantly speed up the discovery of new materials [73].

In addition to polymersome morphology prediction, molecular simulations can provide the insights required to control drug encapsulation and release. In a recent study, Zatorska-Płachta *et al.* provided insight into the details of drug encapsulation within nanoparticles [74]. Specifically, they examined the interactions between drug molecules and the nanoparticle core using experimental and simulation techniques. One simulation system was designed by randomly placing drug molecules and polymer strands of the hydrophobic monomer in a box filled

with water. During the simulation, they observed that the drug molecules formed an aggregate with the polymer chains, which mimicked the encapsulation process of drugs within micelle cores. The timescale for this association reflects the affinity of the drug for the core. This methodology could be extended to polymersome design by screening hydrophobic monomers based on the aggregation propensity of a hydrophobic drug to the core. In another work, Zhu *et al.* developed a new method for simulating the deformation of vesicles due to reactive events such as enzymatic action [75]. The rate constants of the enzymatic reaction can be altered, providing the chance to investigate the effects of turnover rates on polymersome deformation. These results could inform design choices for achieving controlled release from polymersomes. Molecular simulations have been used extensively to elucidate the mechanisms of enzymatic reactions. Usually, these studies use quantum mechanical-molecular mechanics (QM/MM) types of approaches combined with advanced sampling techniques such as metadynamics [76,77], transition path sampling [78–80] and transition interface sampling [81]. These studies have been used widely to comment on the role of mutations on enzyme activity, motivated either through enzyme engineering or disease pathology. In the design of polymersomes, these insights can instead be used to fine-tune the polymersomes. For example, the mechanistic insights into the binding and reaction of the enzyme to a given substrate can be used to design substrates that might slow down or increase the rate of the enzymatic reaction and thus control the rate of polymersome degradation.

The combined simulation-experimental approach has the potential of exploiting a broad variety of polymeric structures and chemistry for polymersome design while reducing the developmental time. For the immediate future, however, we recommend using linear diblock copolymers due to the availability of empirical morphology relations and phase diagrams. We encourage using *in silico* experiments to find a range of conditions optimal for generating polymersomes. This narrowed design parameter space can then be explored effectively with new experimental techniques [54] developed for the rapid generation of morphology phase diagrams.

Conclusion

Enzyme-responsive polymersomes are predominantly studied because of their potential to treat infections and cancers. Their use as drug carriers and diagnostic tools can be expanded to other diseases associated with an enzyme overexpression, but this expansion requires the mechanistic understanding of enzyme-induced polymersome disassembly. In this perspective, we discussed the possible locations to incorporate enzyme-responsiveness in a polymersome: the hydrophilic brush, the hydrophobic membrane, the link region between the brush and the membrane and the surface of the polymersome. We have also provided heuristics based on available studies to guide the design of novel enzyme-responsive polymersomes. These heuristics include using a linear block copolymer shape, designing amphiphilic copolymers to meet empirically suggested hydrophilic fraction values and referring to nanoparticle morphology phase diagrams to guide design choices. We provided examples of such morphology phase diagrams obtained experimentally and *in silico*. We highlighted the role that computer simulations can play in screening a broad parameter space to identify the optimal conditions for polymersome self-assembly.

Future perspective

Polymersomes have continued to be refined since their advent in 2002, a discovery published by Dennis Discher and Adi Eisenberg in Science [82]. Although polymersomes of varying makeups demonstrate preclinical success, they have yet to translate to clinical application. There are many potential reasons for this, one being the biological variability from patient to patient leading to deviations in the pharmacodynamic profile of the polymersomes. Future research in the space of enzyme-responsive polymersomes will need to focus on translation to personalized medicine, increasing their applicability as drug products.

Many disorders are beginning to be associated with lysosomal changes, which increases the role of enzyme levels in disease diagnosis and treatment. Enzyme-responsive polymersomes can play a major role in this space if they are studied in-depth and carefully designed with the patient in mind. Simultaneously, combining benchtop and *in silico* methods leads to a more rigorous and less trial and error based approach to polymersome design. Enzyme-responsive polymersomes should be studied with a focus on understanding the interactions between the enzyme and the amphiphile and the driving forces behind polymersome disassembly. With this information, the field will be able to design the right system for each given enzyme activity level, leading to the most effective therapy.

Future research will need to address key knowledge gaps before enzyme-responsive vesicles can be utilized in personalized medicine. Tailoring drug dosage for individual patients is an important feature of personalized medicine and can be better understood by constructing drug-release models. Currently, enzyme-responsive polymersomes

10.2217/nnm-2021-0194

lack accurate models of drug release due to a paucity of mechanistic understanding of enzyme-induced polymersome disassembly. Therefore, understanding this degradation mechanism can enable greater control over drug release profiles. In the next decade, we anticipate strides on this front, centered around polymersomes from linear-polymer shapes due to their widespread use in the field. We can also expect to see an increase in the use of random copolymers due to their ease of synthesis relative to block copolymers.

However, this knowledge alone does not guarantee translational success. Interactions between enzyme-responsive polymersomes and target tissues must be studied to ensure the safety and efficacy of nanoparticle drug carriers [83]. For example, enzyme-polymersome interactions at the target tissue can be affected by protein opsonization during transport and should be studied. Additionally, with a better understanding of the pathology of the diseases and the associated enzymes, suitable enzyme-responsive polymersomes could be designed to efficiently target a specific disease.

Given the number of design parameters and interactions at play in governing the drug-delivery characteristics of polymers, we believe that designing novel enzyme-responsive polymersomes will be driven by an interdisciplinary approach. Computational and experimental methods will be used synergistically to achieve optimal polymer chemistry selection to obtain the desired morphology, enzyme responsiveness and drug delivery. In combination with machine learning, this discovery process can be accelerated even further.

Executive summary

Current knowledge of enzyme-responsive polymersomes

- The pathological overexpression of enzymes renders enzyme-responsive polymersomes a disease-specific therapy.
- Enzyme-responsive polymersomes undergo morphological changes by the direct action of enzymes to release their cargo.
- The common structural locations of enzyme-responsive units in polymersomes are the hydrophobic membrane, the hydrophilic brush or the link between the hydrophobic membrane and the hydrophilic brush.

Challenges of designing novel enzyme-responsive polymersomes

- A major challenge is designing novel amphiphiles that form vesicles and are enzyme degradable.
- The key design choices are the chemistry of the unresponsive portion of the amphiphile, the polymer shape, the hydrophilic fraction of the amphiphile and the degree of polymerization.

Proposed design heuristics

- A linear copolymer shape is suggested for the amphiphile. General design rules obtained from the literature can be utilized to target polymersome morphologies.
- Experimental and/or *in silico*-obtained phase diagrams can be used to determine the optimal parameters to generate polymersome morphologies.

Financial & competing interests disclosure

This work was supported primarily by the National Science Foundation EPSCoR Program under NSF Award #OIA-1655740. Any opinions, findings, conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect those of the National Science Foundation. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Das A, Monteiro M, Barai A, Kumar S, Sen S. MMP proteolytic activity regulates cancer invasiveness by modulating integrins. *Sci. Rep.* 7(1), 1–13 (2017).
2. McKeown S, Richter AG, O'Kane C, McAuley DF, Thickett DR. MMP expression and abnormal lung permeability are important determinants of outcome in IPF. *Eur. Respir. J.* 33(1), 77–84 (2009).
3. Roach HI, Yamada N, Cheung KSC *et al.* Association between the abnormal expression of matrix-degrading enzymes by human osteoarthritic chondrocytes and demethylation of specific CpG sites in the promoter regions. *Arthritis Rheum.* 52(10), 3110–3124 (2005).
4. Wang S, Jia J, Liu D *et al.* Matrix metalloproteinase expressions play important role in prediction of ovarian cancer outcome. *Sci. Rep.* 9(1), 1–11 (2019).

5. Wang C, Telpoukhovskaia MA, Bahr BA, Chen X, Gan L. Endo-lysosomal dysfunction: a converging mechanism in neurodegenerative diseases. *Curr. Opin. Neurobiol.* 48, 52–58 (2018).
6. Smith S, Larsen J. Linking enzyme upregulation to autophagic failure: a potential biomarker for GM1 gangliosidosis. *bioRxiv.* doi:10.1101/2020.10.28.359083 (2020) (Epub ahead of print).
7. Bonam SR, Wang F, Muller S. Lysosomes as a therapeutic target. *Nat. Rev. Drug Discov.* 18(12), 923–948 (2019).
8. Liu J, Zhang B, Luo Z *et al.* Enzyme responsive mesoporous silica nanoparticles for targeted tumor therapy *in vitro* and *in vivo*. *Nanoscale* 7(8), 3614–3626 (2015).
9. Liu Y, Ding X, Li J *et al.* Enzyme responsive drug delivery system based on mesoporous silica nanoparticles for tumor therapy *in vivo*. *Nanotechnology* 26(14), 145102 (2015).
10. Kumar B, Kulanthaivel S, Mondal A *et al.* Mesoporous silica nanoparticle based enzyme responsive system for colon specific drug delivery through guar gum capping. *Colloids Surfaces B Biointerfaces* 150, 352–361 (2017).
11. Li E, Yang Y, Hao G *et al.* Multifunctional magnetic mesoporous silica nanoagents for *in vivo* enzyme-responsive drug delivery and mr imaging. *Nanotheranostics* 2(3), 233–242 (2018).
12. Manzano M, Vallet-Regí M. Mesoporous silica nanoparticles in nanomedicine applications. *J. Mater. Sci. Mater. Med.* 29(5), 65 (2018).
13. Rideau E, Dimova R, Schwille P, Wurm FR, Landfester K. Liposomes and polymersomes: a comparative review towards cell mimicking. *Chem. Soc. Rev.* 47(23), 8572–8610 (2018).
14. Ahmed F, Pakunlu RI, Brannan A, Bates F, Minko T, Discher DE. Biodegradable polymersomes loaded with both paclitaxel and doxorubicin permeate and shrink tumors, inducing apoptosis in proportion to accumulated drug. *J. Control Release* 116 (SPEC. ISS. 2), 150–158 (2006).
15. Thambi T, Deepagan VG, Ko H, Lee DS, Park JH. Bioreducible polymersomes for intracellular dual-drug delivery. *J. Mater. Chem.* 22(41), 22028–22036 (2012).
16. Discher DE, Ahmed F. Polymersomes. *Annu. Rev. Biomed. Eng.* 8(1), 323–341 (2006).

•• **A seminal paper on polymersomes that establishes design rules for PEG-based amphiphiles.**

17. Yao C, Li Y, Wang Z, Song C, Hu X, Liu S. Cytosolic NQO1 enzyme-activated near-infrared fluorescence imaging and photodynamic therapy with polymeric vesicles. *ACS Nano.* 14(2), 1919–1935 (2020).

• **Demonstrates enzymatic action in the hydrophobic membrane of polymersomes.**

18. Li Y, Liu G, Wang X, Hu J, Liu S. Enzyme-responsive polymeric vesicles for bacterial-strain-selective delivery of antimicrobial agents. *Angew. Chemie Int. Ed.* 55(5), 1760–1764 (2016).
19. Duan W, Ji S, Guan Y *et al.* Esterase-responsive polypeptide vesicles as fast-response and sustained-release nanocompartments for fibroblast-exempt drug delivery. *Biomacromolecules* 21(12), 5093–5103 (2020).
20. Bacinello D, Garanger E, Taton D, Tam KC, Lecommandoux S. Tailored drug-release from multi-functional polymer-peptide hybrid vesicles. *Eur. Polym. J.* 62, 363–373 (2015).
21. Ramezani P, Abnous K, Taghdisi SM, Zahiri M, Ramezani M, Alibolandi M. Targeted MMP-2 responsive chimeric polymersomes for therapy against colorectal cancer. *Colloids Surfaces B Biointerfaces.* 193, 111135 (2020).

• **Reports the enzymatic degradation of the link region between the hydrophobic membrane and the hydrophilic brush of vesicles.**

22. Lee JS, Groothuis T, Cusan C, Mink D, Feijen J. Lysosomally cleavable peptide-containing polymersomes modified with anti-EGFR antibody for systemic cancer chemotherapy. *Biomaterials* 32(34), 9144–9153 (2011).
23. Bacinello D, Garanger E, Taton D, Tam KC, Lecommandoux S. Enzyme-degradable self-assembled nanostructures from polymer-peptide hybrids. *Biomacromolecules* 15(5), 1882–1888 (2014).

• **Establishes that enzymatic cleavage can occur in the hydrophilic membrane of polymersomes.**

24. Haas S, Hain N, Raoufi M *et al.* Enzyme degradable polymersomes from hyaluronic acid-block-poly(ε-caprolactone) copolymers for the detection of enzymes of pathogenic bacteria. *Biomacromolecules* 16(3), 832–841 (2015).

• **A polymersome system that contains enzyme-responsive groups in both the hydrophilic brush and the hydrophobic membrane.**

25. Tücking KS, Grützner V, Unger RE, Schönherr H. Dual enzyme-responsive capsules of hyaluronic acid-block-poly(lactic acid) for sensing bacterial enzymes. *Macromol. Rapid Commun.* 36(13), 1248–1254 (2015).
26. Porta F, Ehrsam D, Lengerke C, Meyer Zu Schwabedissen HE. Synthesis and characterization of PDMS-PMOXA-based polymersomes sensitive to MMP-9 for application in breast cancer. *Mol. Pharm.* 15(11), 4884–4897 (2018).
27. Yang L, Li J, Jin Y, Li M, Gu Z. *In vitro* enzymatic degradation of the cross-linked poly(ε-caprolactone) implants. *Polym. Degrad. Stab.* 112, 10–19 (2015).
28. Chau Y, Padera RF, Dang NM, Langer R. Antitumor efficacy of a novel polymer-peptide-drug conjugate in human tumor xenograft models. *Int. J. Cancer.* 118(6), 1519–1526 (2006).
29. Zhong Y, Shao L, Li Y. Cathepsin B-cleavable doxorubicin prodrugs for targeted cancer therapy. *Int. J. Oncol.* 42(2), 373–383 (2013).

30. Pramod PS, Takamura K, Chaphekar S, Balasubramanian N, Jayakannan M. Dextran vesicular carriers for dual encapsulation of hydrophilic and hydrophobic molecules and delivery into cells. *Biomacromolecules* 13(11), 3627–3640 (2012).

31. Zhu Y, Yang B, Chen S, Du J. Polymer vesicles: mechanism, preparation, application, and responsive behavior. *Prog. Polym. Sci.* 64, 1–22 (2017).

32. Battaglia G, Ryan AJ. Bilayers and interdigitation in block copolymer vesicles. *J. Am. Chem. Soc.* 127(24), 8757–8764 (2005).

33. Schottler S, Becker G, Winzen S *et al.* Protein adsorption is required for stealth effect of poly(ethylene glycol)- and poly(phosphoester)-coated nanocarriers. *Nat. Nanotechnol.* 11(4), 372–377 (2016).

34. Li L, Raghupathi K, Song C, Prasad P, Thayumanavan S. Self-assembly of random copolymers. *Chem. Commun.* 50(88), 13417–13432 (2014).

• **A review of nanoparticles self-assembled from random copolymers.**

35. Zhang CY, Yang YQ, Huang TX *et al.* Self-assembled pH-responsive MPEG-b-(PLA-co-PAE) block copolymer micelles for anticancer drug delivery. *Biomaterials* 33(26), 6273–6283 (2012).

36. Dan K, Bose N, Ghosh S. Vesicular assembly and thermo-responsive vesicle-to-micelle transition from an amphiphilic random copolymer. *Chem. Commun.* 47(46), 12491–12493 (2011).

37. Tian F, Yu Y, Wang C, Yang S. Consecutive morphological transitions in nanoaggregates assembled from amphiphilic random copolymer via water-driven micellization and light-triggered dissociation. *Macromolecules* 41(10), 3585–3588 (2008).

38. Pangburn S, Trescony P, Heller J. Lysozyme degradation of partially deacetylated chitin, its films and hydrogels. *Biomaterials* 3(2), 105–108 (1982).

39. Kurita K, Kaji Y, Mori T, Nishiyama Y. Enzymatic degradation of β-chitin: susceptibility and the influence of deacetylation. *Carbohydr. Polym.* 42(1), 19–21 (2000).

40. Hirano S, Tsuchida H, Nagao N. N-acetylation in chitosan and the rate of its enzymic hydrolysis. *Biomaterials* 10(8), 574–576 (1989).

41. Li S, Girard A, Garreau H, Vert M. Enzymatic degradation of polylactide stereocopolymers with predominant D-lactyl contents. *Polym. Degrad. Stab.* 71(1), 61–67 (2000).

42. Schacher FH, Elbert J, Patra SK, Yusoff SFM, Winnik MA, Manners I. Responsive vesicles from the self-assembly of crystalline-coil polyferrocenylsilane-block-poly(ethylene oxide) star-block copolymers. *Chem. - A Eur. J.* 18(2), 517–525 (2012).

43. Croitoru-Sadger T, Leichtmann-Bardoogo Y, Mizrahi B. A flexible polymersome system with tunable morphology and release profiles for efficient intracellular delivery. *Int. J. Pharm.* 508(1–2), 34–41 (2016).

44. Wang K, Dong HQ, Wen HY *et al.* Novel vesicles self-assembled from amphiphilic star-armed PEG/polypeptide hybrid copolymers for drug delivery. *Macromol. Biosci.* 11(1), 65–71 (2011).

45. Del Barrios J, Oriol L, Sánchez C *et al.* Self-assembly of linear-dendritic diblock copolymers: from nanofibers to polymersomes. *J. Am. Chem. Soc.* 132(11), 3762–3769 (2010).

46. Jiang W, Zhou Y, Yan D. Hyperbranched polymer vesicles: from self-assembly, characterization, mechanisms, and properties to applications. *Chem. Soc. Rev.* 44(12), 3874–3889 (2015).

47. Tücking KS, Handschuh-Wang S, Schönherr H. Bacterial enzyme responsive polymersomes: a closer look at the degradation mechanism of PEG-block-PLA vesicles. *Aust. J. Chem.* 67(4), 578–584 (2014).

48. Discher DE, Ortiz V, Srinivas G *et al.* Emerging applications of polymersomes in delivery: from molecular dynamics to shrinkage of tumors. *Prog. Polym. Sci.* 32(8–9), 838–857 (2007).

49. Sugihara S, Blanazs A, Armes SP, Ryan AJ, Lewis AL. Aqueous dispersion polymerization: a new paradigm for in situ block copolymer self-assembly in concentrated solution. *J. Am. Chem. Soc.* 133(39), 15707–15713 (2011).

50. Blanazs A, Ryan AJ, Armes SP. Predictive phase diagrams for RAFT aqueous dispersion polymerization: effect of block copolymer composition, molecular weight, and copolymer concentration. *Macromolecules* 45(12), 5099–5107 (2012).

51. Tan J, He J, Li X *et al.* Rapid synthesis of well-defined all-acrylic diblock copolymer nano-objects: via alcoholic photoinitiated polymerization-induced self-assembly (photo-PISA). *Polym. Chem.* 8(44), 6853–6864 (2017).

52. Shae D, Becker KW, Christov P *et al.* Endosomolytic polymersomes increase the activity of cyclic dinucleotide STING agonists to enhance cancer immunotherapy. *Nat. Nanotechnol.* 14(3), 269–278 (2019).

53. Barnhill SA, Bell NC, Patterson JP, Olds DP, Gianneschi NC. Phase diagrams of polynorbornene amphiphilic block copolymers in solution. *Macromolecules* 48(4), 1152–1161 (2015).

54. Touve MA, Wright DB, Mu C, Sun H, Park C, Gianneschi NC. Block copolymer amphiphile phase diagrams by high-throughput transmission electron microscopy. *Macromolecules* 52(15), 5529–5537 (2019).

•• **Uses a novel method for the rapid generation of nanoparticle morphology phase diagrams.**

55. Liao M, Liu H, Guo H, Zhou J. Mesoscopic structures of poly(carboxybetaine) block copolymer and poly(ethylene glycol) block copolymer in solutions. *Langmuir.* 33(30), 7575–7582 (2017).

56. Wang Z, Yin Y, Jiang R, Li B. Morphological transformations of diblock copolymers in binary solvents: A simulation study. *Front. Phys.* 12(6), 128201 (2017).

57. Sun X, Pei S, Wang J, Wang P, Liu Z, Zhang J. Coarse-grained molecular dynamics simulation study on spherical and tube-like vesicles formed by amphiphilic copolymers. *J. Polym. Sci. Part B Polym. Phys.* 55(16), 1220–1226 (2017).

58. Lin Y-L, Chang H-Y, Sheng Y-J, Tsao H-K. Photoresponsive polymersomes formed by amphiphilic linear–dendritic block copolymers: generation-dependent aggregation behavior. *Macromolecules* 45(17), 7143–7156 (2012).

59. Tan H, Yu C, Lu Z, Zhou Y, Yan D. A dissipative particle dynamics simulation study on phase diagrams for the self-assembly of amphiphilic hyperbranched multiarm copolymers in various solvents. *Soft Matter.* 13(36), 6178–6188 (2017).

60. Tan H, Li S, Li K, Yu C, Lu Z, Zhou Y. Shape transformations of vesicles self-assembled from amphiphilic hyperbranched multiarm copolymers via simulation. *Langmuir.* 35(21), 6929–6938 (2019).

•• **Demonstrates the capability of computer simulations in predicting nanoparticle morphology phase diagrams.**

61. Song Y, Jiang R, Wang Z, Yin Y, Li B, Shi A-C. Formation and regulation of multicompartment vesicles from cyclic diblock copolymer solutions: a simulation study. *ACS omega.* 5(16), 9366–9376 (2020).

62. Salomon-Ferrer R, Case DA, Walker RC. An overview of the amber biomolecular simulation package. *Wiley Interdiscip. Rev. Comput. Mol. Sci.* 3(2), 198–210 (2013).

63. Eastman P, Swails J, Chodera JD *et al.* OpenMM 7: Rapid development of high performance algorithms for molecular dynamics. *PLoS Comput. Biol.* 13(7), 1–17 (2017).

64. Anderson JA, Glaser J, Glotzer SC. HOOMD-blue: A python package for high-performance molecular dynamics and hard particle Monte Carlo simulations. *Comput. Mater. Sci.* 173, 109363 (2020).

65. Abraham MJ, Murtola T, Schulz R *et al.* Gromacs: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX.* 1–2, 19–25 (2015).

66. Páll S, Abraham MJ, Kutzner C, Hess B, Lindahl E. Tackling exascale software challenges in molecular dynamics simulations with GROMACS. In: *Solving Software Challenges for Exascale.* Markidis S, Laure E (Eds). Springer International Publishing, Cham, Switzerland, 3–27 (2015).

67. Plimpton S. Fast parallel algorithms for short-range molecular dynamics. *J. Comput. Phys.* 117(1), 1–19 (1995).

68. LAMMPS (2021). www.lammps.org/

69. Wright DB, Ramírez-Hernández A, Touve MA *et al.* Enzyme-induced kinetic control of peptide-polymer micelle morphology. *ACS Macro Lett.* 8(6), 676–681 (2019).

70. Li J, Xiao S, Xu Y *et al.* Smart asymmetric vesicles with triggered availability of inner cell-penetrating shells for specific intracellular drug delivery. *ACS Appl. Mater. Interfaces* 9(21), 17727–17735 (2017).

71. Beltran-Villegas DJ, Wessels MG, Lee JY *et al.* Computational reverse-engineering analysis for scattering experiments on amphiphilic block polymer solutions. *J. Am. Chem. Soc.* 141(37), 14916–14930 (2019).

72. Wessels MG, Jayaraman A. Computational reverse-engineering analysis of scattering experiments (CREASE) on amphiphilic block polymer solutions: cylindrical and fibrillar assembly. *Macromolecules* 54(2), 783–796 (2021).

73. NSTC. Materials genome initiative for global competitiveness. *Genome* (June), (2011).

74. Zatorska-Płachta M, Łazarski G, Maziarz U *et al.* Encapsulation of curcumin in polystyrene-based nanoparticles-drug loading capacity and cytotoxicity. *ACS Omega.* 6(18), 12168–12178 (2021).

75. Zhu Q, Scott TR, Tree DR. Using reactive dissipative particle dynamics to understand local shape manipulation of polymer vesicles. *Soft Matter.* 17(1), 24–39 (2021).

76. Nin-Hill A, Rovira C. The catalytic reaction mechanism of the β-galactocerebrosidase enzyme deficient in Krabbe disease. *ACS Catal.* 10(20), 12091–12097 (2020).

77. Morais MAB, Coines J, Domingues MN *et al.* Two distinct catalytic pathways for GH43 xylanolytic enzymes unveiled by x-ray and QM/MM simulations. *Nat. Commun.* 12(1), (2021).

78. Burgin T, Mayes HB. Mechanism of oligosaccharide synthesis: via a mutant GH29 fucosidase. *React. Chem. Eng.* 4(2), 402–409 (2019).

79. Bharadwaj VS, Knott BC, Ståhlberg J, Beckham GT, Crowley MF, Hart GW. The hydrolysis mechanism of a GH45 cellulase and its potential relation to lytic transglycosylase and expansin function. *J. Biol. Chem.* 295(14), 4477–4487 (2020).

80. Silveira RL, Knott BC, Pereira CS, Crowley MF, Skaf MS, Beckham GT. Transition path sampling study of the feruloyl esterase mechanism. *J. Phys. Chem. B.* 125(8), 2018–2030 (2021).

81. Bonk BM, Weis JW, Tidor B. Machine learning identifies chemical characteristics that promote enzyme catalysis. *J. Am. Chem. Soc.* 141(9), 4108–4118 (2019).

82. Discher DE. Polymer vesicles. *Science* 297(5583), 967–973 (2002).

83. Murphy CJ, Vartanian AM, Geiger FM *et al.* Biological responses to engineered nanomaterials: needs for the next decade. *ACS Cent. Sci.* 1(3), 117–123 (2015).