Construction from Destruction: Hydrogel Formation from Triggered Depolymerization-based Release of an Enzymatic Catalyst

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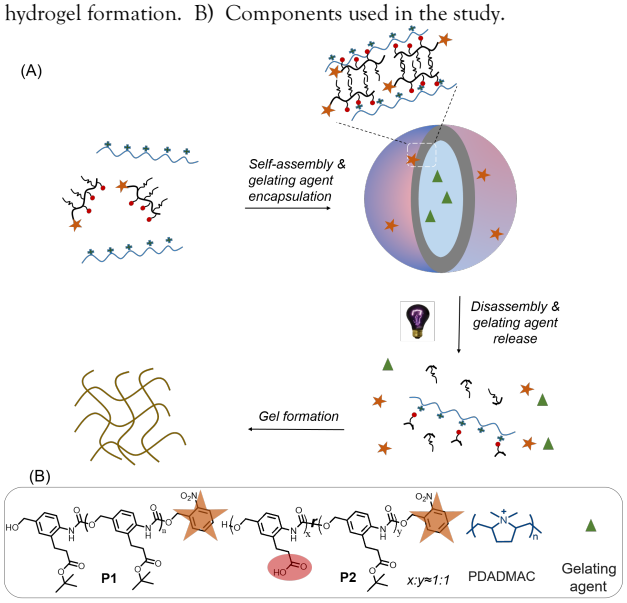
ABSTRACT: Biomimetic systems that can undergo macroscopic phase transformation by transducing and amplifying external cues are highly desirable for applications such as self-healing. Here, we report self-assembly of polyelectrolyte complexes into a vesicular structure that can accommodate hydrophilic guest molecules, including enzymes. Triggered depolymerization of one of the polyelectrolyte molecules in the complex causes the vesicle to disassemble and release its contents. Such a triggered release of enzymes causes molecular-scale events to be amplified due to the enzyme's catalytic properties. This feature has been utilized to demonstrate construction of hydrogels from the destruction of nanoscopic polymeric vesicles. The design principles developed here are broadly adaptable to other triggerable depolymerization motifs reported in the literature.

There has been significant recent focus in depolymerizations because of potential applications in diverse areas from biomedicine to environmental sustainability.[1–4] In many of these polymer designs, an unstable state of the polymer is kinetically trapped using a stabilizing functionality at a chain terminus.[5] When this functionality is removed using a stimulus, the polymer unravels through sequential removal of monomeric building blocks from the chain, *i.e.* the chain unzips. This depolymerization capability offers opportunities in many fields. For example, mechanically robust solid-state materials have been shown to degrade rapidly upon triggering into small molecular components.[6,7] Similarly, the process has been utilized in amplification schemes, where a single stimulus-induced input event causes the release of multiple molecular components that are embedded in the repeat units of a dendrimer or a polymer.[8–10] These processes take advantage of the triggered destruction of polymers to small molecule components.

We were interested in developing strategies for constructing crosslinked networks from a polymer destruction process, as such a capability could offer advantages in applications such as in self-healing materials which require on-demand network-like species formation. We were particularly inspired by 'hagfish slime', where a rapid hydrogel formation occurs due to release of proteins from vesicles in response to their mechanically triggered destruction.[11–13] This gel formation is used as a self-defense mechanism by the hagfish eel against predatory encounters, such as from a shark attack. In this manuscript, we describe an approach in which a depolymerization process forms the basis for disassembly of vesicles, where the ensuing molecular release is utilized to trigger gel formation.

We envisaged the use of polyvalent interactions[14] as the basis for forming vesicles and its triggered disassembly. Briefly, complexes between two polyelectrolytes can be used to form a variety of nanoscale and mesoscale assemblies.[15–18] Because the basis for forming these assemblies is polyvalency, we hypothesized that the depolymerization process can be leveraged to alter the strength of interaction between the polyelectrolytes and thus the stability of the assembly. One of the polyelectrolytes is designed to undergo chain unzipping in response to a trigger. As the degree of polymerization reduces towards the monomeric state, the polyvalent interaction weakens. To achieve gel formation from this disassembly process, we envisioned encapsulation of gelating agents into the vesicles, which upon release into bulk aqueous phase would result in the formation of hydrogel. Here, the components of the vesicular aqueous lumen and the bulk aqueous phase have to be such that the latter encourages gel formation. The polyvalent assembly, triggered disassembly, and the gel formation processes are illustrated in Scheme 1.

Scheme 1. A) Self-assembly of negatively charged UV sensitive polymer in presence of PDADMAC and gelating agent encapsulation. UV triggered polymer degradation leading to release of gelating agent and hydrogel formation. B) Components used in the study.



Poly(benzylcarbamate) was used as the depolymerizable anionic polymer,[19] where the chain end is capped with a *o*-nitrobenzyl unit. The nitrobenzyl moiety has been widely used for its photodeprotection properties.[20,21] Light-induced removal of this functionality from the chain end liberates the aniline moiety, which causes an electronic cascade to unzip the polymer chain. The targeted polymer structure is shown as **P2** in Scheme 1**.** First, we synthesized polymer **P1**, where the anionic carboxylic acid moie-

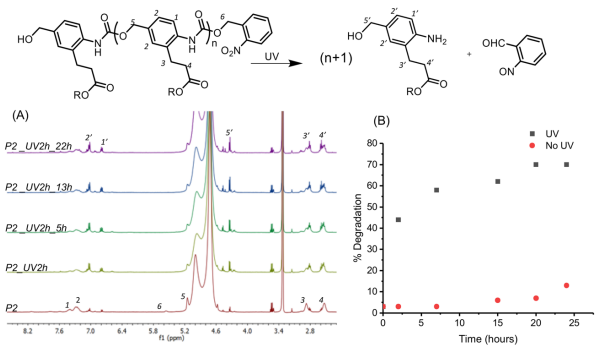


Figure 1. Depolymerization of polymer **P2** after UV irradiation. A) ^{1}H NMR spectra of **P2** at different time intervals after UV exposure for 2 h. B) Black: Percentage of polymer degraded at different time intervals. Red: Degradation due to hydrolysis of P2.

ties were protected as the corresponding *t*-butyl ester. 2-Nitrobenzyl-capped **P1** was synthesized using a literature-reported condensation reaction (see SI for synthetic details).[19] Some of the *t*-butyl groups in **P1** were then removed using trifluoroacetic acid deprotection in a controlled fashion to achieve **P2,** which contained random and equal distribution of carboxylic acid and *t*-butyl ester groups in the polymer side chains (Figure S1). Partial deprotection of the ester moieties proved to be important in obtaining the right hydrophilic-hydrophobic balance in the polymer to form the targeted vesicles.

Prior to polyelectrolyte-based assembly formation, we first studied the light-triggered depolymerization. A solution of **P2** in MeOD: D_2O mixture (2:1) was irradiated at 350 nm (2.2 mW/cm^2) for 2 h. The resulting solution was monitored using ^{1}H NMR spectroscopy, where a temporal increase in the intensity of the proton signals corresponding to the small molecule products from depolymerization was observed (Figure 1A). About 70% of the polymer unzipped to form the corresponding monomer in ~24 h, after 2 h of UV illumination (Figure 1B).

Since **P2** is inherently amphiphilic, we examined its potential for self-assembly. The polymer was found to exhibit poor self-assembly characteristics under aqueous conditions, as discerned by the poor correlation coefficient and multimodal distribution in dynamic light scattering (DLS) with a dispersity of 0.587 (Figure 2B and Figure S2). Interestingly however, **P2** assembled into stable colloidal nanoparticles in the presence of a stoichiometrically equivalent (based on charge) poly(diallyl)dimethylammonium chloride (PDADMAC) (Figure 2A, Figure 2B and Figure S2). Note that a charge equivalent amount of PDADMAC was required to achieve stable nanoparticles (Figure S3). The resultant nanoparticles were found to be ~250 nm, with an excellent correlation function in DLS (Figure 2B and Figure S2) and significantly less dispersity (0.206). Particle morphology was examined using cryogenic and dry state transmission electron microscopy (TEM) as well as atomic force microscopy (AFM) which revealed a vesicle like morphology and spherical nature of the particles (Figure 2C,Figure S4 and Figure S5). We reasoned that this assembly behaviour was driven by the hydrophobic interactions between the *t*-butyl groups and the electrostatic interaction between **P2** and PDADMAC.

Since these nanoparticles were formulated in aqueous solution, the lumen of vesicles should be aqueous and therefore should be able to accommodate water-soluble dyes such as rhodamine 6G. Accordingly, the dye molecule (35 µM) was incorporated into the vesicles during formation, followed by removal of unincorporated dye molecules through dialysis. We then used the self-quenching features of rhodamine 6G to investigate its location. When the local concentration of the dye is high (e.g., inside the vesicle), it exhibits substantially lower fluorescence compared to the same amount of the free dye in water (Figure S6). Indeed, the observed reduced-fluorescence supports dye incorporation into the vesicle (Figure 2D and Figure S6).

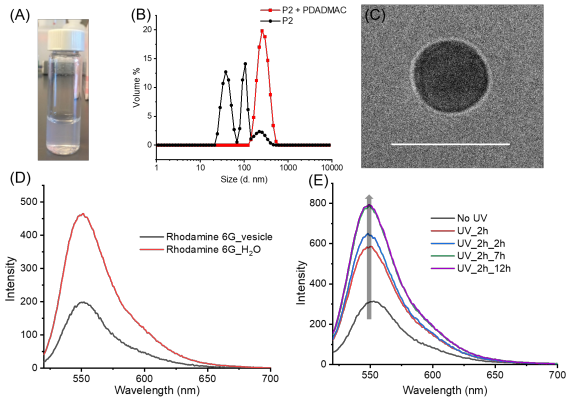


Figure 2. Assembly of **P2** with PDADMAC. A) Colloidal dispersion after mixing **P2** with charge equivalent PDADMAC. B) Dynamic light scattering profile of polymer **P2** (black); **P2** + PDADMAC (red). C) CryoTEM image of **P2** + PDADMAC nanoparticle (scale bar 90 nm). D) Fluorescence comparison of rhodamine 6G dye molecules in aqueous bulk (red) vs encapsulated in vesicles (black) using an excitation wavelength of 510 nm. E) Fluorescence profile of rhodamine 6G dye molecules encapsulated in vesicles after UV exposure for 2 h and subsequent time intervals

The possibility of light-triggered disassembly was subsequently investigated. Upon irradiating the solution containing vesicles at 350 nm, an increase in the fluorescence of rhodamine 6G was observed, where the increase is commensurate with that expected for free dye molecules in the aqueous phase (Figure 2E). Disassembly of vesicles was also further studied by TEM and DLS (Figure S7, Figure S8 and Figure S9). TEM images revealed that the spherical morphology is lost and the product morphology is ill-defined. The poor correlation function in DLS also supported

the notion that robust nanoassemblies do not exist in solution after UV irradiation.

Successful encapsulation and triggered release of rhodamine 6G laid the foundation for encapsulating a progelator or gelating agent into the vesicles, which can then be released into the bulk aqueous phase for gel formation. We were intrigued by literature-reported peptide molecules, which have the propensity to form hydrogels after enzymatic cleavage.[22] To test this approach in the

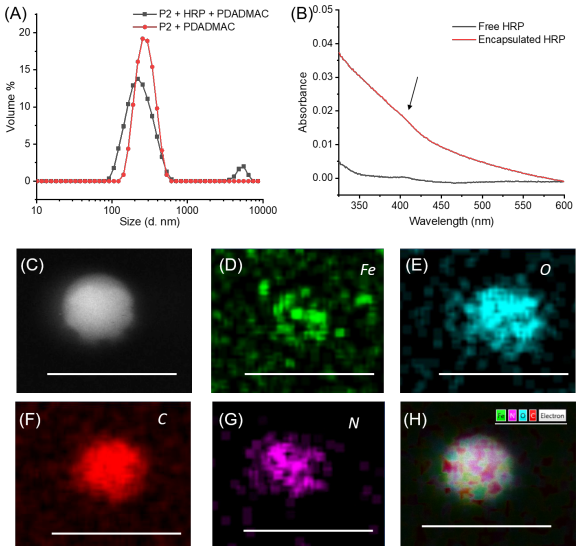


Figure 3. HRP encapsulation in **P2** + PDADMAC vesicles. A) Dynamic light scattering profile of **P2** + HRP + PDADMAC (Black); **P2** + PDADMAC (Red). B) UV- Vis spectra of vesicles with HRP encapsulated (Red); Free enzyme after repeated washing (Black). C) Bright field electron microscopy image of **P2** + HRP + PDADMAC. Energy dispersive X-Ray Spectroscopy (EDS) electron mapping profile of particle in figure C for different elements mentioned as D) Iron (Fe). E) Oxygen. F) Carbon. G) Nitrogen. H) combined electron mapping for all the elements. Scale bar is 500 nm.

current system, we encapsulated Fmoc-protected tyrosine ethyl ester peptides into the vesicular assembly. When the depolymerization is triggered with light, the Fmoc-protected peptide is released and the ester group can be cleaved by the enzyme, chymotrypsin, causing gel formation. Our attempts here proved futile. The challenge arose from mismatch between the loading capacity of the peptide molecules within the vesicles and their critical gelation concentration. Therefore, we targeted encapsulation of the enzyme instead. Note also that the process of encapsulating a catalyst and then releasing it in response to a trigger offers an inherent amplification capacity because a single enzyme molecule can react with multiple substrate species to bring about the desired change.

As the self-assembly is driven by electrostatic interactions and enzymatic activity can be modulated through electrostatic interactions,[23] we chose a charge-neutral enzyme with an isoelectric point (pI) close to the pH of the experimental conditions. Accordingly, we used horse radish peroxidase (HRP) (pI = ~9.0; MW = 44 kDa) as the enzyme to encapsulate in the aqueous lumen of the vesicles. In the presence of H_2O_2, HRP oxidizes tyrosine methyl ester into its radical form which can cross-link to form dimer or higher order polymeric products.[24–26] We therefore envisioned the possibility of HRP-catalyzed hydrogel formation through crosslinking 4-arm-polyethyleneglycol polymers, which has previously been shown to form hydrogels.[27] Thus, we modified commercially available 4-arm-PEG-succinimidyl carbonate with tyrosine methyl ester to obtain the branched polymer **P3** containing four tyrosine molecules (see SI for structure and synthetic details). Exposure of this polymer substrate to HRP causes gelation in the presence of H_2O_2 within two minutes, presumably due to the radical dimerization of the tyrosine moieties (Figure S10).

Compartmentalization of HRP inside the vesicles should physically separate the enzyme from polymer **P3** that is present in the aqueous bulk. Therefore, there should not be any gel formation. We hypothesized that vesicle disruption due to the light-triggered depolymerization of **P2** should cause HRP to be released from the vesicles and crosslink the branched **P3** to form gels .

Prior to testing this triggered gelation process, we characterized the encapsulation of the enzyme inside the vesicles. HRP was incorporated during the polyelectrolyte complexation-based vesicle

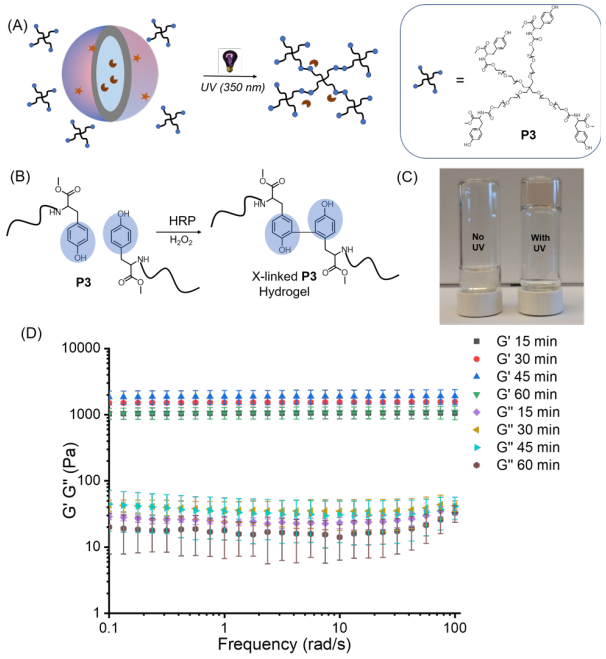


Figure 4. A) Illustration of HRP encapsulation in the vesicles and its release upon UV irradiation to initiate cross-linking of **P3** in the presence of H_2O_2. B) Illustration of HRP mediated cross-linking of **P3**. C) Images of vial containing solution of **(P2** + PDADMAC) dispersed in the substrate solution with H_2O_2 with and without UV illumination. D) Storage (G') and loss (G") modulus of hydrogels with 15, 30, 45 and 60 min of UV irradiation (the values represent the average of two measurements).

formation (see SI for details). As the presence of unencapsulated enzyme could prematurely cause gelation, the vesicular solution was washed multiple times using a centrifuge filter with 100 kDa MW cut off. DLS studies revealed that the enzyme-containing particles have the same size distribution as empty particles (Figure 3A). The encapsulated HRP was characterized using UV-Vis spectroscopy in which we observed the characteristic absorbances corresponding to the Soret band of the porphyrin ring in HRP at 403 nm[28] (Figure 3B). The concentration of enzymeinside the vesicles was 4.2 μM, which was determined using a calibration curve (Figure S11). The enzyme loading capacity of the nanoparticles was ~20 %. Because the shoulder from the Soret band is rather weak, we further confirmed its presence using elemental mapping in energy dispersive X-ray spectroscopy (EDS). HRP is a metalloenzyme containing an iron-based porphyrin co-factor. Indeed, EDS elemental distribution analysis of the nanoparticles clearly showed the presence of iron and thus the enzyme within the vesicles (Figure 3C-3H, Figure S12 and Figure S13).

The possibility of light triggered release of the enzyme and catalyzed amplification to form hydrogels was then investigated. The vesicles containing HRP were dispersed in a solution containing substrate **P3** (5 wt. %) (Figure 4A). In the presence of H_2O_2, no gelation was observed, presumably due to the fact that HRP is compartmentalized inside the vesicle. When irradiated with UV light, gelation was observed in an hour after UV illumination as evidenced by the inverted vial test due to the cross-linking of tyrosine moieties (Figure 4B and Figure 4C). These results suggest that the HRP was released into the aqueous solution after vesicle disassembly, then reacting with **P3** to form crosslinks .

Next, we investigated the dependence of irradiation time on the extent of gel formation. As the extent of activation of depolymerization should depend on the irradiation time, we surmised that the extent of enzyme release from the vesicle and thus the gel formation should also depend on the irradiation time. To test this hypothesis, irradiation time was varied for 15, 30, 45 and 60 min. Oscillatory shear rheology was utilized to characterize the storage (G') and loss (G'') modulus of hydrogels. Frequency sweeps show that G' is greater than G'' from 0.1-100 rad/s and is nearly independent of frequency, suggesting that the gels are elastic, cross-linked materials (Figure 4D). The G' value of hydrogels increased with irradiation time demonstrating that gels were stronger with more UV exposure (Figure 4D and Figure S14). Interestingly, the G' value upon 60 min of light exposure was lower than that for 45 min. This is attributed to the inherent decrease in enzyme activity upon prolonged light exposure (Figure S15).

In summary, we utilize a depolymerization-based modulation of polyvalent interactions to disassemble a vesicle and utilize this process to trigger gel formation. We show that anionic poly(benzylcarbamate) polymers can form vesicles on complexation with cationic PDADMAC. When the former polymer is triggered for depolymerization, the vesicle is destabilized, presumably due to the weaker interaction strength between the polyelectrolytes with decreasing polymer length. This process has been used to encapsulate an enzyme and then release it in the presence of the specific trigger. The catalytic nature of the enzyme amplifies the signal, as a single enzymatic molecule can cause multiple crosslinking reactions. The fact that the resultant product causes a phase change in the form of a self-supporting hydrogel, the amplification event is even macroscopically visualized. This is a demonstration of a triggered "destruction" of a polymeric molecule to "construct" a different species. This construction from destruction principle has been previously explored in the context of mechanical disruption of microcapsules. Extending this idea to a molecularly addressable depolymerization process through polyvalency significantly expands its scope in many applications that require controlled or triggered release.

ASSOCIATED CONTENT

Supporting Information. "This material is available free of charge via the Internet at http://pubs.acs.org."

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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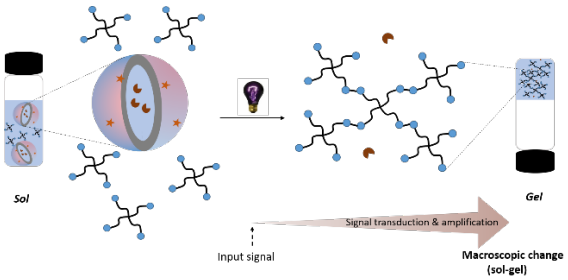
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Signal transduction & amplification
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