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Photo-polymerization induced polymersome rupture

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£ equal contributions

ABSTRACT

Poly(butadiene)-*b*-poly(ethylene oxide) (PBut_{2.5}-*b*-PEO_{1.3}) giant polymersomes were prepared using an emulsion-centrifugation method. The impact of a fast decrease of the osmotic pressure inside the lumen of giant PBut-*b*-PEO vesicles was studied by confocal microscopy. This osmotic imbalance was created by performing the photo-induced polymerization of acrylamide inside these giant polymersomes, mimicking cell-like confinement. Experimental conditions (irradiation time, relative concentration of monomer and photo-initiator) were optimized to

induce the fastest and highest osmotic pressure difference in bulk solution. When confined inside polymersomes with low permeability membrane made of PBut-*b*-PEO copolymers, this hyper-osmotic shock induced a fast disruption of the membrane and polymersome burst. These findings, complementary to hypo-osmotic shock approaches previously reported, are demonstrating the versatility and relevance of controlling and modulating osmotic pressure imbalance in self-assembled artificial cell systems and protocells.

INTRODUCTION

Polymersomes are vesicles formed by the self-assembly of amphiphilic copolymers. Since they were first reported by Meijer¹ and Eisenberg² in 1995, extensive research has been carried out, and they have found applications as nanoreactors³ and cell mimics⁴ as well as in encapsulation technologies and drug delivery.^{5, 6} Because of their inherent structure that makes them able to carry cargoes, control over the release of encapsulated compounds is of primordial importance. A plethora of stimuli-responsive polymersomes have been developed to satisfy the need for controlled release.^{5, 7-12} The variety of potential stimuli can be divided into two main categories: the chemical/biological stimuli (e.g. pH, redox, enzymatic activity) and the physical stimuli (e.g. temperature, ultrasound, light). The controlled release of the vesicular content may then have two main causes: i) a direct degradation or increased permeability of the membrane, or ii) an indirect membrane loss of integrity (collapse or bursting) as a consequence of an osmotic imbalance between the lumen of the vesicle and the outer medium which is generated by the stimulus.⁹ In cells, aquaporin channels, caveolar invaginations and the exo/endocytosis machinery are at play to accommodate cell swelling or deswelling.^{13, 14} In lipid or polymer vesicles lacking such

regulatory mechanisms, acute osmotic imbalance usually leads to membrane permeabilization. Upon hypo-osmotic shock, vesicles swell and burst, while hypertonic shock leads to vesicle shrinkage or ultimately to irreversible collapse.¹⁵⁻²² In particular, light can be used with vesicles containing dyes that may undergo photo-cleavage, and thus increase the internal osmotic pressure. In previous work, we showed that polymersome rupture could be triggered under irradiation with a spectral control, depending on the chemical nature of photo-cleavable molecules and the wavelength of the excitation light.¹⁸ The present study aims at describing the opposite scenario, and elucidate the effect of a light-induced hypertonic shock on the morphology and fate of polymersomes. A key challenge was to design a chemical process that allowed fast osmotic change under microscope irradiation.

As depicted in Figure 1, an experiment was designed where acrylamide and a hydrophilic radical photo-initiator are co-encapsulated inside the lumen of giant poly(butadiene)-*b*-poly(ethylene oxide) (PBut-*b*-PEO) vesicles. Upon UV irradiation, poly(acrylamide) is produced in the vesicles interior. As polymerization progresses, acrylamide monomers are consumed, thus leading to a decrease of the internal osmotic pressure concomitantly with an increase of viscosity.²³⁻²⁵ Our working hypothesis is that the osmotic pressure resulting from the photo-polymerization reaction could be reduced at such a rate that weakly permeable polymersome membranes inhibit fast equilibration and induce irreversible membrane collapse and release of the vesicular content.

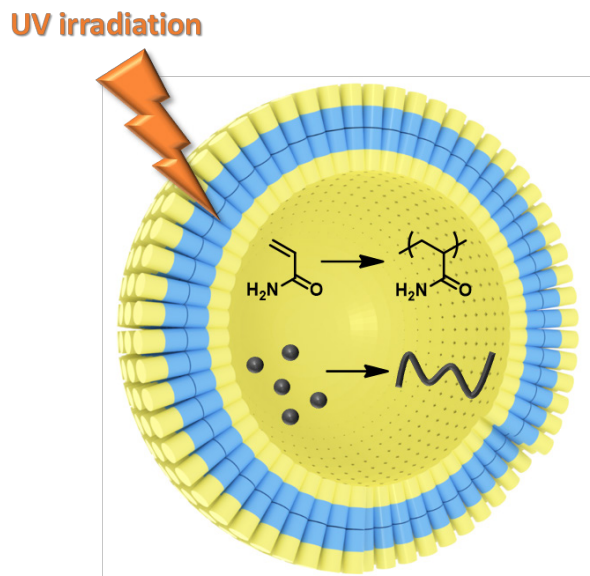


Figure 1. Schematical representation of UV photo-induced polymerization of acrylamide inside the lumen of a giant polymersome.

EXPERIMENTAL SECTION

Materials

Poly(butadiene)-*b*-poly(ethylene oxide) PBut₄₆-*b*-PEO₃₀ (Mn x 10³ PBut-*b*-PEO: 2.5-*b*-1.3; Mw/Mn 1.04) was ordered from Polymer Source (P18422-BdEO, 89% 1,2-addition of butadiene). 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959), acrylamide monomer, sucrose and glucose were purchased from Sigma Aldrich and used as received.

Methods

Electronic absorption experiments were carried out on a Varian Cary 5000 UV-vis-NIR spectrophotometer. Osmolarity measurements were performed with a freezing point automatic osmometer (Type-15M, Löser, Berlin, Germany). Irradiation experiments were carried out with a Mercury-Xenon 200 W lamp. A filter was used to cut UV light below 300 nm and over 480 nm. Samples (≈ 1 -2 mL) were placed 1 cm from the light guide output end and irradiated in the dark for a defined time. Osmolarities and viscosities were measured before and after irradiation. Viscosities were determined with a density meter (DMA generation M from Anton Paar) equipped with a rolling ball micro-viscometer (Lovis 2000 M/ME from Anton Paar) at 20°C. Laser scanning confocal microscopy images were acquired on an inverted Leica TCS SP5 microscope equipped with an HCX PL APO 63 \times , NA 1.4 oil immersion objective in transmission mode. Samples (≈ 20 μ L) were injected in a homemade chamber that was sealed to prevent evaporation. The light guide output end of the UV lamp was placed 1 cm away from the chamber for optimized irradiation. Images were collected in simultaneous mode using a resonant scanner at 8000 Hz in bidirectional mode and processed with the ImageJ freeware. Size exclusion chromatography in water was performed on a Wyatt apparatus. Samples (≈ 1 mL) were prepared from the dissolution of 5 mg lyophilised polymer in a 0.1 M NaNO₃ + 0.1 M H₂O₄P buffer and were filtered (0.45 μ m) before injection. ¹H NMR experiments were performed in D₂O at 25 °C on a Bruker Advance 400 spectrometer operating at 400 MHz calibrated to the solvent peak.

Vesicle preparation

Poly(butadiene)-*b*-poly(ethylene oxide) (PBut_{2.5}-*b*-PEO_{1.3}) giant polymersomes were prepared from the previously discussed emulsion-centrifugation method.²⁶⁻²⁸ This method is rather simple

and allows for the preparation of complex and eventually compartmentalized polymersomes with accurate control of the content and good reproducibility.^{27, 28} Nevertheless, the formed vesicles present some dispersity. The use of a double emulsion approach in microfluidic may overcome this issue if needed but presents some limitations in terms of solvent removal.²⁹⁻³² Briefly, 5 μL of sucrose 0.38 M was poured into 3 mg/mL PBut_{2.5}-*b*-PEO_{1.3} in 500 μL toluene. The solution was vigorously hand-shaken for 30 seconds to create a water-in-oil emulsion. An interface was prepared by pouring 30 μL of PBut_{2.5}-*b*-PEO_{1.3} (3 mg/mL) in toluene over 30 μL glucose 0.38 M and allowed to stabilize for 30 minutes. 60 μL of the above emulsion was slowly poured over the interface and the sample was immediately centrifuged (3 min, 500 g, ambient temperature). The resulting polymersomes were recovered in the lower phase. In the case of acrylamide and Irgacure-loaded polymersomes, the monomer and initiator were dissolved in a sucrose solution to reach the desired concentration and 5 μL of this solution was used to form the emulsion. The concentration of glucose was adjusted to the same osmotic pressure as the sucrose solution. Samples were kept in the dark prior to irradiation.

RESULTS AND DISCUSSION

By contrast with the developed strategy that aims at generating an hypo-osmotic shock inside vesicles by exploiting the cleavage of photo-sensitive species enclosed in the vesicle lumen,¹⁸ we investigated the impact of a fast decrease of the osmotic pressure inside the lumen of giant PBut-*b*-PEO vesicles. Since osmotic pressure is directly related to the number of molecules at a defined time and in a given volume, polymerization reactions appear to be ideal candidates since the formation of one polymer chain depletes the reservoir from monomers. More specifically,

polymerization of acrylamide was chosen as a model reaction (Figure 1). Indeed, poly(acrylamide) is obtained from the polymerization of an acrylamide monomer, which is soluble in water at room temperature and possesses a high constant of propagation (k_p) in water.²⁴

2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure, Figure 2A) was chosen as a photo-initiator because it is fairly soluble in water and has proven to be effective in photo-initiated polymerization reactions.³³ The absorbance spectra for different Irgacure concentrations were monitored with a spectrophotometer to verify that the initiator could be activated in the wavelength range of a mercury-xenon 200 W UV lamp (300-480 nm) (Figure 2B). While 0.10 and 0.25 wt/v (%) Irgacure only show low signal in this range of wavelengths, a significant increase in absorbance is observed for 0.5 wt/v (%) Irgacure.

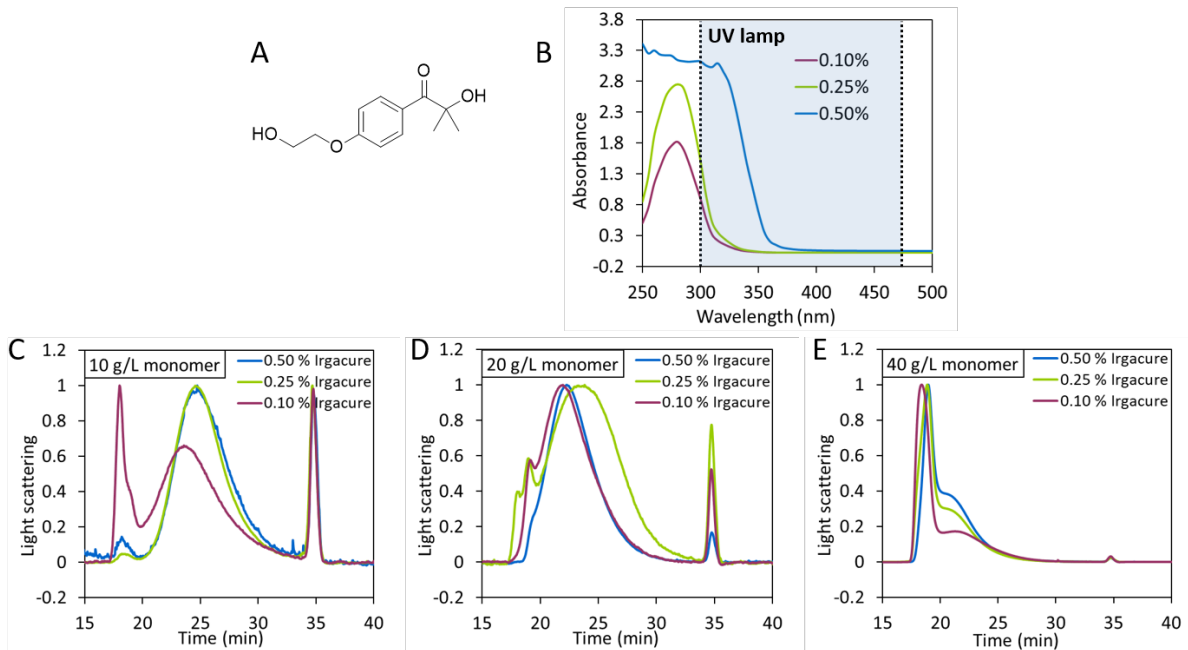


Figure 2. A) Chemical structure of Irgacure photo-initiator; B) Absorbance spectra of Irgacure with varying wt/v (%); C,D,E) Size exclusion chromatograms (as measured by light scattering)

of generated poly(acrylamide) for different Irgacure (0.10, 0.25 and 0.50 wt/v (%)) and monomer (10, 20 and 40 g/L) concentrations after 5 min UV irradiation (300 – 480 nm) in bulk solution with a mercury-xenon 200 W lamp in 0.3 M sucrose.

In photo-polymerization processes, both monomer and initiator concentration have an impact on the rate of the reaction.³⁴ Therefore, the photo-polymerization was first performed in bulk solution using different monomer-to-initiator ratio to verify the effective polymerization of acrylamide under these conditions and to monitor the changes in viscosity and osmolarity. In each experiment, the monomer and initiator were mixed in a 0.3 M sucrose solution (used for polymersome formation) and irradiated 5 minutes with a mercury-xenon 200 W lamp between 300 and 480 nm. Figures 2C, 2D and 2E show the chromatograms obtained for the size exclusion chromatography in water of the poly(acrylamides) generated after photo-polymerization with different concentrations of monomer and initiator.

The large peaks indicate the high polydispersity of the samples, as typically observed for free radical polymerization reactions. However, a net shift between the peaks indicates the influence of the concentration of the monomer on the final molar masses of the polymers. Indeed, higher molar masses are obtained with higher monomer concentrations, also suggesting higher changes in osmolarity and viscosity. To test this hypothesis, osmolarity and viscosity measurements were performed before and after irradiation of the samples (Figure 3). As expected, a decrease in osmolarity (Figure 3A) and an increase in viscosity (Figure 3B) were observed for all monomer-to-initiator ratios with the most prominent differences obtained for 0.5 wt/v (%) Irgacure concentration. In the absence of initiator, no changes in either osmolarity or viscosity were observed, showing that polymerization cannot occur in the absence of Irgacure as a source of radicals.

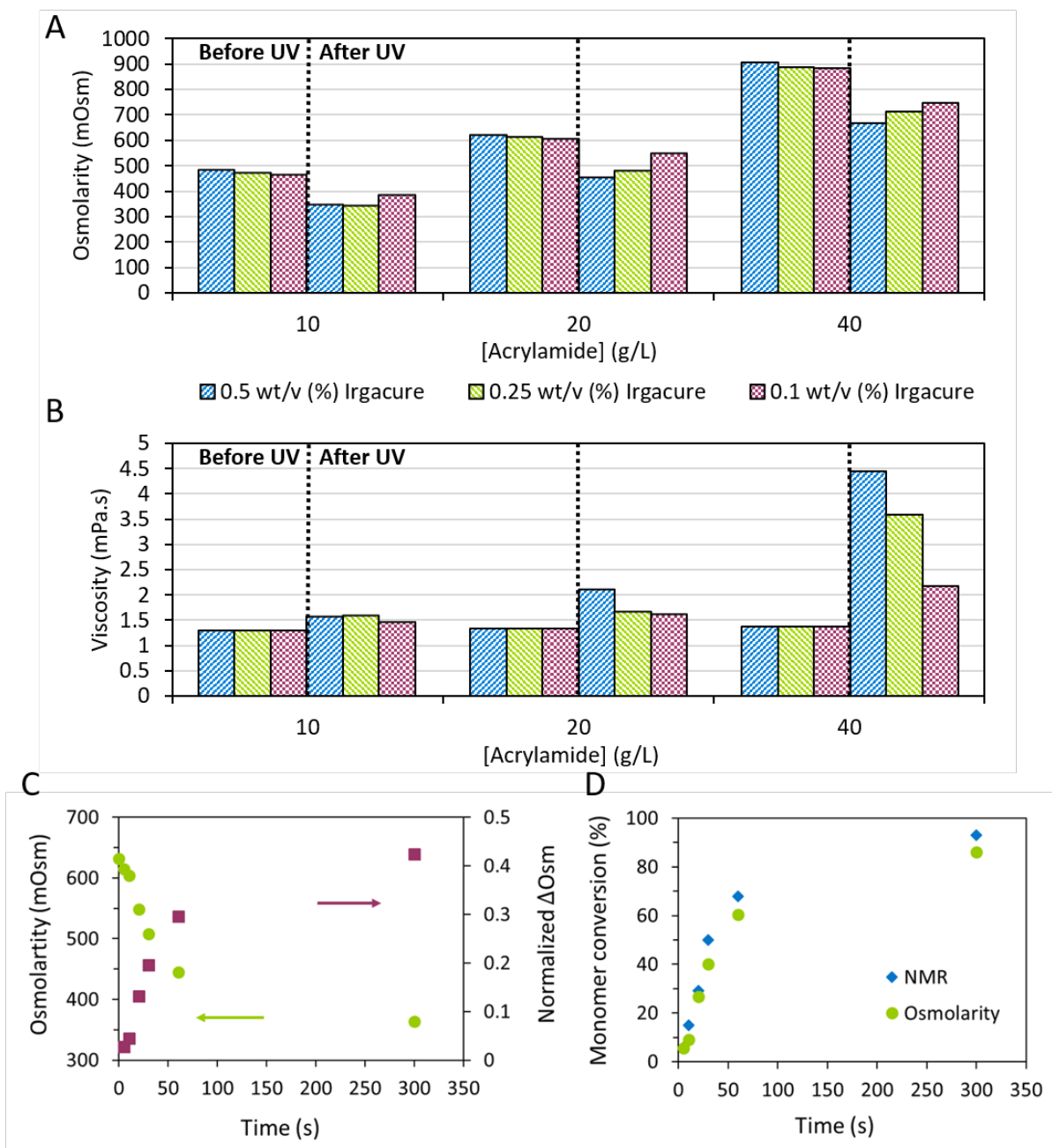


Figure 3. A) Osmolarity and B) viscosity measurements on obtained poly(acrylamides) for different monomer and initiator concentrations before (left of dotted line) and after (right of dotted line) 5 min UV irradiation (300 – 480 nm) with a mercury-xenon 200 W lamp in 0.3 M sucrose. C) Osmolarity and normalized Δ Osm vs time during the polymerization of acrylamide

at 20 g/L, using 0.5 wt/v (%) Irgacure, under UV irradiation (300 – 480 nm) with a mercury-xenon 200 W lamp in 0.3 M sucrose. D) Monomer conversion vs time, using two methods.

Kinetic measurements were also performed to confirm the fact that the polymerization, hence the change in osmolarity, was occurring quickly. Figure 3C shows the evolution of the osmolarity and the normalized osmolarity difference ΔOsm with time when irradiating a mixture containing 20 g/L acrylamide and 0.5 wt/v (%) Irgacure in 0.3 M sucrose. Figure 3D displays the corresponding monomer conversion (details on the determination of conversion using NMR and osmolarity can be found in supporting information). We observed that osmolarity decreased by 20 % after only 30 s, and 30 % after 60 s, which corresponds to 45 ± 5 % monomer conversion, and 64 ± 4 % monomer conversion, respectively.

Having determined the optimal concentration conditions to induce the highest osmotic shock in a rapid way, the photo-initiated reaction was then triggered inside the lumen of the giant PBut-*b*-PEO polymersomes as schematically represented in Figure 1. Different ratios of monomer and initiator solubilized in sucrose were loaded in giant PBut-*b*-PEO vesicles using an emulsion-centrifugation protocol initially developed by Pautot et al.²⁶ and further adapted.²⁷ The light guide output end of the mercury-xenon 200 W UV lamp was placed over the hermetic chamber containing the polymersomes on the stage of a confocal microscope as shown in Figure 4A. The behavior of vesicles could thus be monitored by confocal microscopy under white light observation during irradiation.

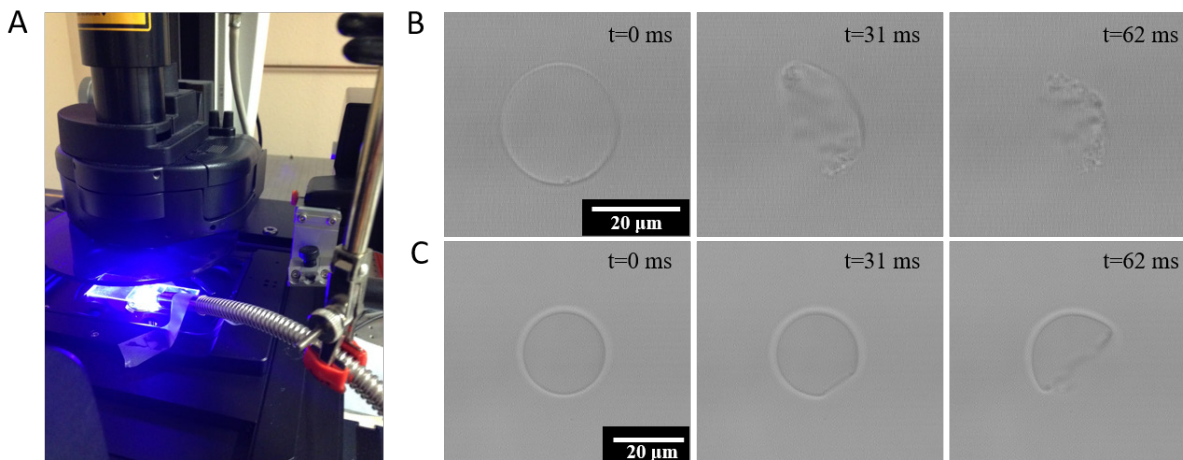


Figure 4. A) Confocal setup. The light guide output end of a mercury-xenon UV lamp is placed over the hermetic chamber containing the sample. B) and C) Confocal images of vesicle following in situ polymerization of acrylamide in the presence of an Irgacure initiator under UV irradiation (300 – 480 nm). The images are acquired from a video. B) 0.50 wt/v (%) Irgacure, 20 g/L monomer and C) 0.25 wt/v (%) Irgacure and 40 g/L monomer.

Vesicles containing 0.1 wt/v (%) Irgacure did not show any change, even after 10 min irradiation at maximum lamp intensity, suggesting that the build-up in osmotic pressure is too small and too slow in these conditions (see supporting information). However, the outcome was drastically different for vesicles loaded with either 0.25 or 0.50 wt/v (%) initiator with respectively 40 and 20 g/L monomer. We observed rapid vesicle rupture (within less than 1-minute UV irradiation) for most vesicles. Figure 4B and 4C are snapshots of vesicles during the irradiation sequence. Controls were performed to verify that no explosion occurred in empty (sucrose-loaded) vesicles under the same conditions, confirming that the observed bursting was not due to any possible heating effect during UV irradiation.

Remarkably, the membrane rim seems to curl towards the inner medium, where the viscosity is higher, in agreement with previous studies reporting polymersome bursting.^{18, 35} Altogether, these results confirm our hypothesis that the low membrane permeability of polymer vesicles drastically restricts effective water diffusion and therefore prevents a re-equilibration of the osmotic pressure difference between both sides. More generally, these results evidenced the importance of controlling osmotic pressure variations in the context of cell membrane mimics. Subtle equilibrium of forces, surface-area–volume ratio, swelling, pore formation, mechanical tension and their dynamics are leading to intriguing phenomena from popping to membrane deformation and quasi-homeostatic self-regulatory behavior.^{18, 36-38}

CONCLUSIONS

While a classical approach to cause membrane rupture of a vesicle is to produce a hypo-osmotic shock, which swells the vesicle and increase its membrane tension, the effect of a hyper-osmotic shock is less intuitive and has been overlooked. In the present work, we have investigated the impact of light-induced hypertonic shocks on giant polymer vesicles. Light was used as an external trigger to induce a fast polymerization reaction inside the lumen of the vesicles. Here, we demonstrate that, under optimal conditions, this reaction generates a fast decrease of the internal osmotic pressure and leads to vesicle rupture due to the low water diffusion conferred by the weakly permeable membrane. This mechanism offers a new route to control directed delivery in nanomedicine. Instead of producing low small molecular fragments during the hypo-osmotic shock that can eventually create ROS that may be harmful for neighboring cells, the release of polymer chains together with the active species may be envisioned as a more gentle and harmless

strategy. In addition, the approach can be extended to any other monomers, included charged ones, if the polymerization process is fast and the obtained polymers do not interact with the polymersome membrane. After demonstrating the feasibility of the approach, future works should be dedicated to reach a proof of concept within the context of a relevant nanomedical application and in contact with cells.

ASSOCIATED CONTENT

Supporting Information. Details on the calculation of monomer conversion, SEC traces of obtained polymer and additional confocal images are provided in supporting information.

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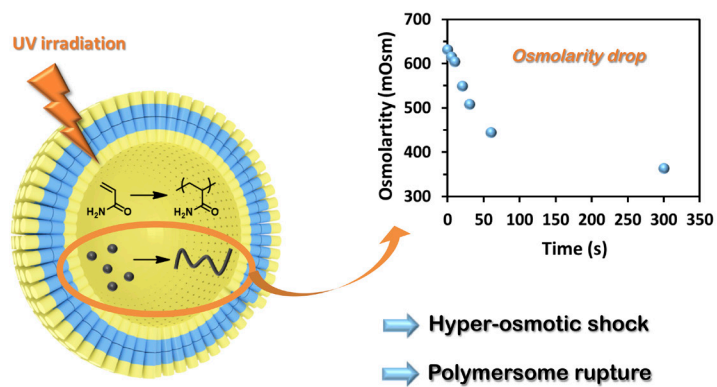
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Table of Content Graphic



Supporting Information

Photo-polymerization induced polymersome rupture

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Determination of monomer conversion

1. Osmolarity method

The measured osmolarity (Osm) is the addition of contributions from concentrations in sucrose, monomer and initiator:

$$\text{Osm}_{\text{tot}} = \text{Osm}_{\text{sucrose}} + \text{Osm}_{\text{initiator}} + \text{Osm}_{\text{mon}}$$

UV irradiation does not influence the concentration of sucrose over time, and the evolution of concentration in initiator can be considered negligible. Therefore the evolution of osmolarity can be imputed to the decrease of monomer concentration, which corresponds to the conversion (cvs):

$$\text{Osm}_{\text{sucrose}} \text{ constant and } \text{Osm}_{\text{initiator}} \sim \text{constant} \Rightarrow \Delta\text{Osm}_{\text{tot}} \sim \Delta\text{Osm}_{\text{mon}}$$

$$\Rightarrow \text{cvs} = ([\text{mon}]_0 - [\text{mon}]_t) / [\text{mon}]_0 = \Delta\text{Osm}_{\text{mon}} / [\text{mon}]_0 = \Delta\text{Osm}_{\text{tot}} / [\text{mon}]_0$$

2. NMR method

As shown in Figure S1, monomer conversion was also determined more precisely by comparing the integrations of the vinyl protons corresponding to the remaining monomer with the integration of polymer backbone signals.

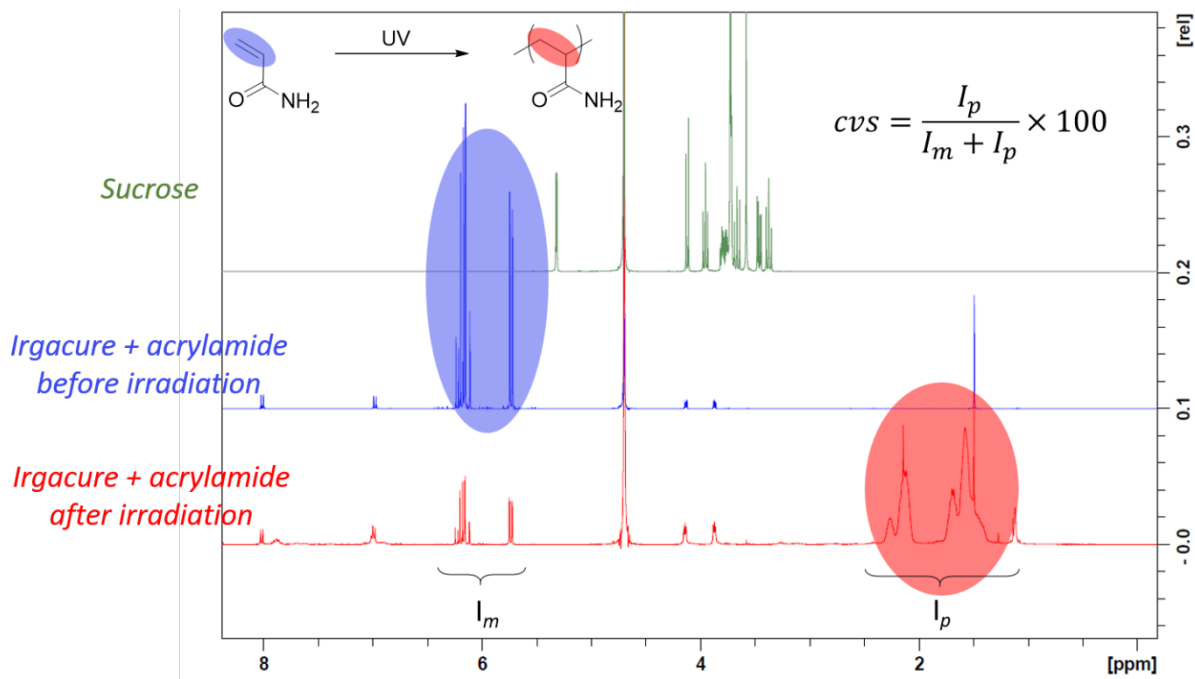


Figure S1: Determination of monomer conversion by ^1H NMR.

Table S1 provides all kinetic data and shows the comparison between the two methods.

Table S1: Kinetic measurements of the polymerization of acrylamide at 20 g/L, using 0.5 % wt/v Irgacure, under UV irradiation (300 – 480 nm) with a mercury-xenon 200 W lamp in 0.3 M sucrose.

Time (s)	Osmotic pressure (mOsm)	Norm ΔmOsm (%)	Conversion (%)	
			NMR	Osmolarity
0	632	/	/	/
5	615	2.7	5.9	5.5
10	604	4.4	15	9.0
20	549	13.1	29	27
30	508	19.6	50	40
60	445	29.6	68	60
300	364	42.4	93	86

SEC traces of obtained polymers

Fig. S2 shows typical SEC traces obtained during irradiation.

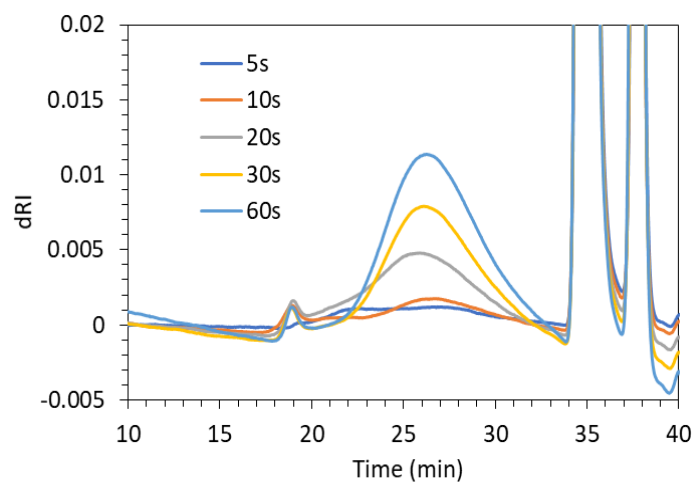


Figure S2: SEC traces of the formed polymers during kinetic experiments.

Confocal images

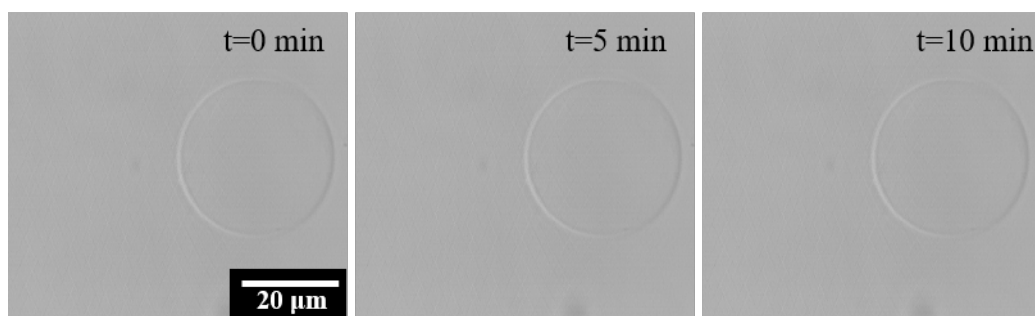


Figure S3: Confocal images of vesicle following in situ polymerization of 40g/L acrylamide in the presence of 0.1 wt/v (%) Irgacure initiator under UV irradiation (300 – 480 nm). The images are acquired from a video.