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Modular Synthesis of Biodegradable Diblock Copolymers for Designing Functional Polymersomes

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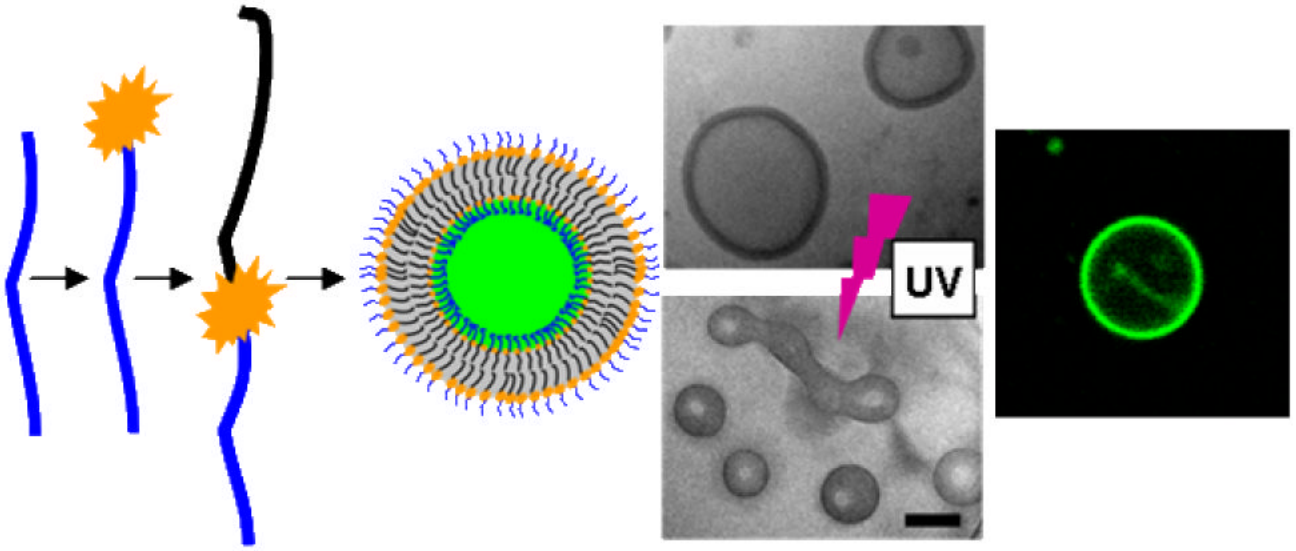
Abstract



Polymer vesicles, or polymersomes, are promising candidates for applications in drug delivery and tissue imaging. While a vast variety of polymers have been explored for their ability to assemble into polymersomes, relatively little research has been reported for studies into the functionalization of these polymers. We present here a novel route to synthesizing poly(caprolactone)-b-poly(ethylene glycol) diblock copolymers that allows for the insertion of functional groups at the blocks' junction and the assembly of functional membranes. This modular synthesis has been developed based on solid phase peptide synthesis techniques and is accomplished through the formation of two peptide bonds—between an amine terminated PEG and the carboxyl of the functional group and between the functional group amine and a carboxy-terminated PCL. As a demonstration of the potential utility of the resulting vesicles, we incorporated two different amino acid functional groups at the junction. 2-Nitrophenylalanine was utilized to create UV-responsive membranes in which the vesicles destabilized and released encapsulated contents upon irradiation. A fluorescein-conjugated lysine was also utilized to create stable fluorescent membranes in which the fluorescence was built into the polymer. This method should contribute to our ability to further develop smart, functional membranes.

Polymeric vesicles or polymersomes comprise a relatively new class of materials based on the self-assembly of amphiphilic block copolymers.[1] Polymersomes offer several distinct advantages over liposomes, including increased mechanical robustness, the ability to solubilize large quantities of hydrophobic and hydrophilic molecules, and typically a complete PEG surface-functionality, offering a stealth character *in vivo*. Polymersomes are useful for a range of applications including drug delivery, *in vivo* imaging, and as cell mimetics.[2-6] The majority of application-based research has focused on systems using existing diblock polymers. However, new synthetic routes that introduce additional functionality may be beneficial for other applications or towards increasing polymersome efficacy.[7] We present here a novel synthetic route that utilizes a modular building of biodegradable block polymers, allowing for the incorporation of a wide variety of chemical groups in the membrane. As two examples, we developed polymersomes that are susceptible to UV-induced degradation or include fluorescence directly in the hydrophobic core of the membrane.

This method for diblock synthesis is inspired by chemistry commonly used for solid phase peptide synthesis and utilizes amino acids with any desired side groups.[8] In the first step, an FMOC-protected amino acid is conjugated to an amine-terminated PEG through an amidation reaction (Scheme 1; for complete Materials and Methods, see the Supporting Information). Following FMOC removal and purification, the functional PEG (still with an amine terminus) is coupled in excess to a carboxy-PCL through a second amidation reaction. Precipitation of the resulting polymer into methanol selectively precipitates the diblock copolymer, yielding the desired product.

As a first example, we incorporated photo labile 2-nitrophenylalanine (2NPA) as the amino acid joining the two blocks. Incorporation of this amino acid into the backbone of poly(peptides) has enabled site-specific cleavage of the peptide bond between the 2NPA and the next amino acid towards the N-terminus.[9] Initial characterization of the polymer via NMR confirms coupling of the 2NPA to PEG and subsequent coupling to the PCL (Figure S1). To rule out the possibility that PEG-2NPA co-precipitated with the PCL but was not actually coupled, GPC was performed on the resulting diblock copolymer (Figure S2). A shift to higher molecular weights was observed for the PCL peak owing to the PEG coupling and a second peak corresponding to free PEG was notably absent. Exposure of the GPC sample with a trace amount of water as a proton source to 365nm light for 2 hours induced a shift back towards the lower molecular weight PCL and the evolution of a second peak corresponding to liberated PEG was observed.

Assembly of polymersomes was accomplished through film rehydration, sonication, and extrusion to remove any aggregated material. Polymersomes were exposed to UV light for up to 6 hours. A visible precipitate became apparent after approximately 3 hours of exposure. High resolution NMR of polymersomes after exposure shows a complete disappearance of the 4 characteristic aromatic peaks corresponding to the 2NPA moiety (Figure S3). GPC of exposed polymersomes reconstituted in THF following lyophilization shows a steady increase in the normalized area of the PEG peak (Figure 1a). Interestingly, a shoulder corresponding to higher molecular weight species also appears in the GPC chromatograph of UV-exposed polymersomes (Figure S4). Peak deconvolution suggests the shoulder corresponds to a species of approximately double the molecular weight of the starting polymer. Such dimerization could be due to side reactions that occur during the UV rearrangement,[9] though the exact mechanism is beyond the scope of this report.

As a demonstration of the potential utility of polymersomes that undergo UV-induced cleavage, release of encapsulated biocytin was monitored as a function of exposure time through a fluorescent capture assay (Figure 1b).[6] Release is observed over the entire 6 hour

exposure, while negligible release was observed for controls lacking the 2NPA group. To explore the possible mechanism of release, the change in size of polymersomes was monitored by DLS. Intriguingly, only a slight decrease in the size of the species present was observed (Figure S5). To explore this phenomenon further, polymersomes were observed by cryo-TEM imaging before and after UV exposure (Figure 1c,d; S6). Images suggest the decrease in size is caused by a thickening and gradual collapse of the membrane coupled with the expulsion of aqueous contents which was not observed for control polymer lacking the 2NPA group (Figure S6,7). After sufficient PEG liberation, the remaining PCL aggregates (i.e. Figure 1d, top) and precipitates. The lower polymer density relative to solution could explain the lack of corroboration via DLS; photolysed samples float out of the DLS light path. While polymersome systems have been reported that are responsive to UV light,[6,10] and cleavable polymers have been applied to other self-assembled systems,[11] to the best of our knowledge, this is the first example of a polymersome where UV-induced release is affected by chemical cleavage of the polymer.

As a second example of the utility of modular synthesis of PCL-PEG diblock copolymers, we incorporated a fluorescent amino acid (fluorescein-conjugated lysine) between the blocks. Fluorescent polymersomes have been useful as imaging agents, but in all cases the fluorophore has been dissolved in the polymersome rather than conjugated to it.3,12 In the present study, polymersomes assembled from blends of fluorescein-labeled polymer and regular PCL-PEG exhibited membrane-localized fluorescence, confirming conjugation of the fluorophore (and PEG) to the PCL (Figure 2a, inset). Absence of a PEG peak in the GPC of the polymer further rules out the possibility that free fluorophore-conjugated PEG could be partitioning into the membrane (Figure S8). To ensure uniform distribution of the fluorescent polymer in polymersomes, we performed flow cytometry on polymersomes containing various amounts of the fluorescent polymer (Figure 2a). Narrow distributions of fluorescence were observed and the peak value increased with increasing amounts of fluorescent polymer. Finally, fluorescent polymersomes were fed to immature dendritic cells for 5 hours and observed following washing to remove unassociated polymersomes (Figure 2b). While negative controls exhibited no fluorescence, intense fluorescence was observed in cells incubated with fluorescent polymersomes. These results suggest that modularly-synthesized fluorescent polymers could be useful for studies that require labeling or tracking of cells.

We have presented here a novel approach to synthesizing biodegradable block copolymers that allows for the incorporation of a variety of functional groups at the junction of the two blocks. Photocleavable and fluorescent moieties are just two possible functionalities that could be incorporated. As our method relies on separate synthesis of the two blocks, this method could also be useful for applications in which systematic variation of only one block of the polymer is desired, such as in blended systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Discher BM, Won YY, Ege DS, Lee JCM, Bates FS, Discher DE, Hammer DA. Science 1999;284:1143–1146. [PubMed: 10325219]
2. Ahmed F, Pakunlu RI, Brannan A, Bates F, Minko T, Discher DE. J. Cont. Rel 2006;116:150–158.
3. Ghoroghchian PP, Frail PR, Susumu K, Blessington D, Brannan AK, Bates FS, Chance B, Hammer DA, Therien MJ. Proc. Natl. Acad. Sci. U.S.A 2005;102:2922–2927. [PubMed: 15708979]
4. Hammer DA, Robbins GP, Haun JB, Lin JJ, Qi W, Smith LA, Ghoroghchian PP, Therien MJ, Bates FS. Faraday Disc 2008;139:129–141.
5. Ghoroghchian PP, Li GZ, Levine DH, Davis KP, Bates FS, Hammer DA, Therien MJ. Macromol 2006;39:1673–1675.
6. Robbins GP, Jimbo M, Swift J, Therien MJ, Hammer DA, Dmochowski IJ. J. Am. Chem. Soc 2009;131:3872–3874. [PubMed: 19249827]
7. Cerritelli S, Velluto D, Hubbell JA. Biomacromol 2007;8:1966–1972.
8. Fields GB, Noble RL. Int. J. Pept. Protein Res 1990;35:161–214. [PubMed: 2191922]
9. Peters FB, Brock A, Wang JY, Schultz PG. Chem. Biol 2009;16:148–152. [PubMed: 19246005]
10. Mabrouk E, Cuvelier D, Brochard-Wyart F, Nassoy P, Li MH. Proc. Natl. Acad. Sci. U.S.A 2009;106:7294–7298. [PubMed: 19383800]
11. Zhao Y. J. Mater. Chem 2009;19:4887–4895.
12. Christian NA, Milone MC, Ranka SS, Li GZ, Frail PR, Davis KP, Bates FS, Therien MJ, Ghoroghchian PP, June CH, Hammer DA. Bioconj. Chem 2007;18:31–40.

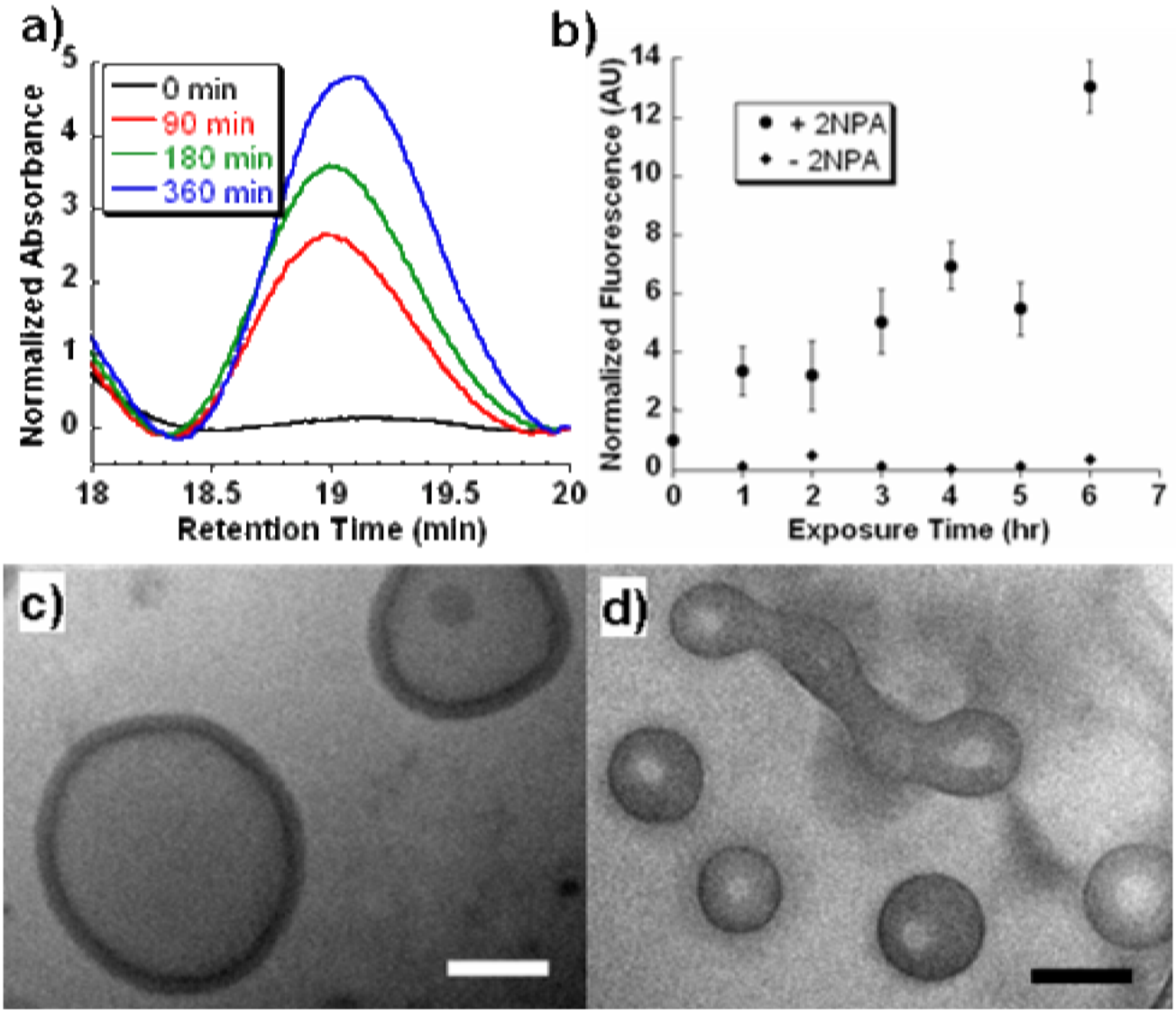


Figure 1.
(a) GPC of eluted PEG peak shown with increasing UV exposure times. (b) Release of encapsulated biocytin. (c), (d) Cryo-TEM images of 2NPA-polymersomes before (c) and after (d) 6 hours of UV exposure. Scale bars = 100 nm.

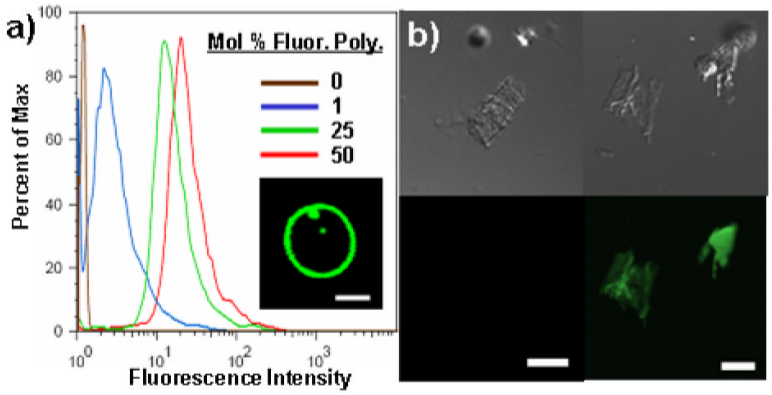
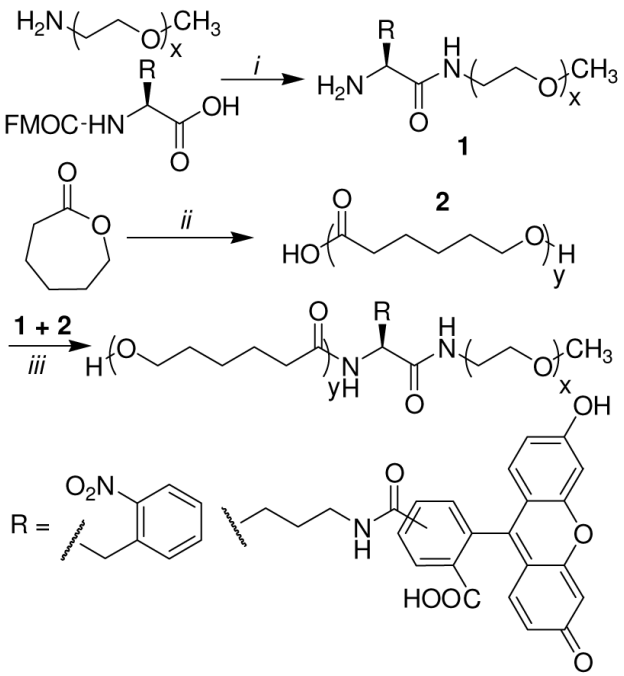


Figure 2.

(a) Flow cytometry of PCL-PEG polymersomes blended with the noted amount of fluorescent polymer. Inset: Confocal image of a 50 mol% fluorescent polymer polymersome. Scale bar = 5 μm. (b) DIC (top) and fluorescent (bottom) microscopy images of immature dendritic cells following 5 hour incubation with PCL-PEG (left) and 50% fluorescent polymer (right) polymersomes. Scale bars = 20μm.



i. 1: HBTU, Methyl Morpholine, 2: Piperidine *ii*. H_2O, $SnOct_2$ *iii*. HBTU, Methyl Morpholine

Scheme 1.
Modular Synthesis of Diblock Copolymers