Formation of Recognition-Induced Polymersomes Using Complementary Rigid Random Copolymers

Ulf Drechsler, Raymond J. Thibault, and Vincent M. Rotello*

Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003

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The hollow structure of vesicular systems provides a versatile architecture for a variety of applications, including cell mimics,[1] biosensors,[2] and encapsulating agents. Furthermore, the ability to control composition, morphology, and function of vesicles allows for the formation of novel materials with diverse functions, ranging from microreactors[3] to drug delivery.[4] In recent studies, extension of this self-assembly process to diblock copolymers has been shown to provide polymer-based vesicles (polymersomes), affording access to a variety of new vesicular systems.[5,6]

Applying the concepts of molecular recognition to the self-assembly of macromolecules provides a powerful tool for the creation of higher-order supramolecular assemblies.[7,8] In prior studies, we reported the self-assembly of complementarily functionalized polystyrenes into giant (∼3 μm in diameter) vesicular recognition-induced polymersomes (RIPs).[9] The unprecedented formation of vesicles from these random copolymers lacking defined headgroups presents a new method for the creation of supramolecular assemblies. Moreover, these RIPs interact specifically with guests featuring appropriate recognition functionality, providing a novel means for the control of self-assembled structures.[10]

To extend the versatility of the RIP assembly process, we have synthesized polynorbornene-based copolymers featuring complementary side chains. Specific molecular recognition in these polymers is provided by three-point hydrogen bonding between 2,6-diacyldiaminopyridine and uracil side chains on the respective polymer chains. These complementary units typically exhibit association constants $K_a \sim 200$ M^{-1} in chloroform. The required polymers were prepared from a randomly substituted norbornene copolymer (M_w = 10 500, PDI = 1.6[11]) obtained through ring-opening metathesis polymerization of a mixture of methyl norbornene-2-carboxylate (to enhance solubility in organic solvents) and amine-reactive succinimidyl norbornene-2-carboxylate using Grubbs' catalyst ($Cl_2(PCy_3)_2Ru{=}CHPh$).[12] The resulting polymer features 30% succinimdyl ester subunits. This parent polymer was then reacted with 6-(5-aminopentyl)uracil and *N*-(6-aminohexanoyl)-*N*′-propionyl 2,6-diaminopyridine, respectively, to give the random complementarily functionalized copolymers **1** and **2** (Figure 1a; see Supporting Information for all synthesis and preparations).

Self-assembly of polymers **1** and **2** was explored using differential interference contrast (DIC) and laser confocal scanning (LCSM) microscopy. In these studies, the individual polymers were dissolved in chloroform to 5 × 10^{-3} mol/L based on recognition elements. Mixing of

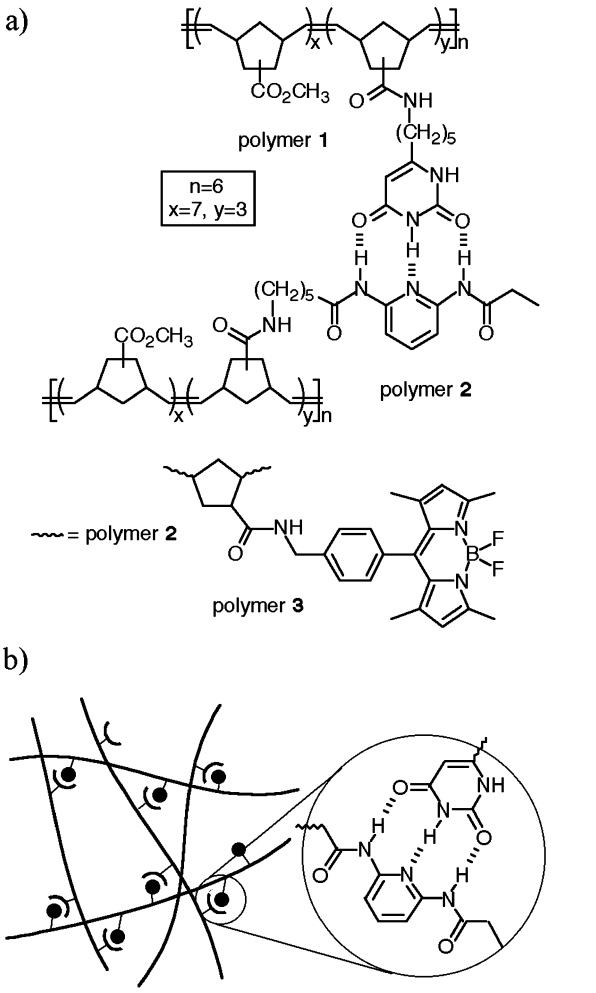

Figure 1. (a) Structures of polymers **1** and **2** and fluorophore-tagged polymer **3**, a derivative of polymer **2**. (b) Schematic representation of recognition-mediated self-assembly of complementary polymers **1** and **2** within the polymersome wall.

the two homogeneous solutions resulted in an instantaneous formation of recognition-induced aggregates (Figure 1b), as evidenced by the immediate appearance of turbidity. IR spectroscopy on these aggregates clearly confirms hydrogen bonding; absorptions at 3274 and 3211 cm^{-1} are attributed to hydrogen-bound N−H stretching vibrations. DIC microscopy of this solution revealed spherical structures. (Figure 2). Initially, these spheres were an average of 3.43 μm in diameter; however, over the course of 20 min fusion of smaller aggregates increased the average diameter to 4.49 μm (Figure 3) (see Supporting Information for size distributions). Continued fusion ultimately led to the formation of a solvent-swollen gel after 2 h, indicating the metastable nature of these vesicles. It should be noted that heating of a sample to 61 °C led to an unexpected stabilization of some of the spheres but had virtually no effect on the size distribution.

Further insight into the nature of the self-assembled systems was obtained using polymer **3**, a fluorescent derivative of polymer **2** (Figure 1a). Because of the high fluorescence quantum yield of the boron dipyrromethene fluorophore employed, only ∼1 tag per polymer strand was needed to provide efficient labeling. Figure 4 shows

* Corresponding author. E-mail: rotello@chem.umass.edu.

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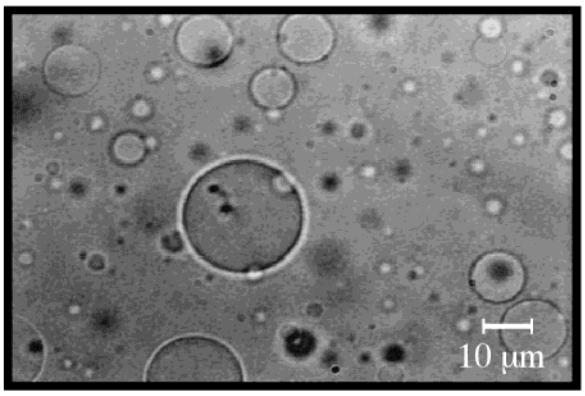

Figure 2. Representative DIC micrograph of aggregates of polymers **1** and **2** (after 1 min).

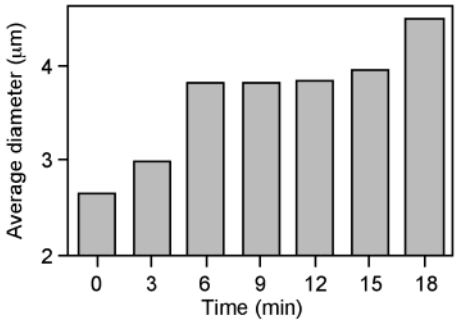

Figure 3. Average RIP diameter over time (sampled from ~200 vesicles).

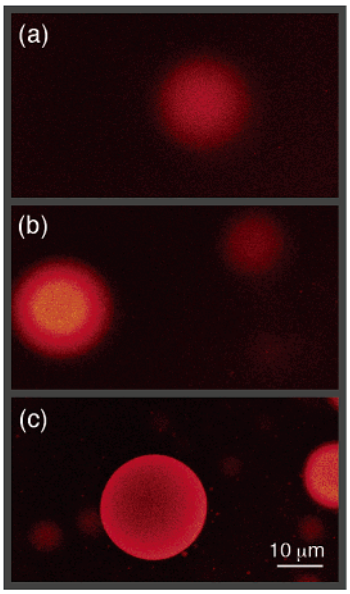

Figure 4. LCSM images of ~100 nm cross sections through one individual RIP at different heights (not centered due to motion between scans): (a) top of vesicle; (b) midsection; (c) center.

LCSM cross-sectional analysis through one individual sphere at different heights. These images clearly show the hollow interior diagnostic of vesicular morphology, as evidenced by a significant decrease in fluorescence intensity toward the center of the spheres.

In summary, we have prepared rigid ROMP polymers bearing complementary diamidopyridine and uracil recognition elements. These random copolymers self-assemble into spherical polymersomes that slowly fuse to form larger structures. The ability of this system to form RIPs demonstrates the generality of recognition-based formation of vesicular structure. Moreover, the metastable nature of these assemblies demonstrates that the polymer structure influences the dynamics of these systems. Further investigation on the mechanism of RIP formation and the molecular arrangement of polymers within the vesicle walls is underway and will be reported in due course.

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Supporting Information Available: Experimental procedures for all polymers and precursor compounds; size distribution histograms at multiple time points. This material is available free of charge via the Internet at http://pubs.acs.org.

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