

# Oil-in-Oil Emulsions Stabilized by Asymmetric Polymersomes Formed by AC + BC Block Polymer Co-Assembly

Itaru Asano,<sup>†,§</sup> Soonyong So,<sup>‡</sup> and Timothy P. Lodge<sup>\*,†,‡</sup>

<sup>†</sup>Department of Chemistry and <sup>‡</sup>Department of Chemical Engineering & Materials Science, University of Minnesota, Minneapolis, Minnesota 55455, United States

<sup>§</sup>Chemicals Research Laboratories, Toray Industries, Inc., 9-1, Oe-cho, Minato-ku, Nagoya 455-8502, Japan

**S** Supporting Information

**ABSTRACT:** We demonstrate a facile route to asymmetric polymersomes by blending AC and BC block copolymers in oil-in-oil emulsions containing polystyrene (PS) and polybutadiene (PB) in chloroform (CHCl<sub>3</sub>). Polymersomes were prepared by mixing polystyrene-*b*-poly(ethylene oxide) (SO) and polybutadiene-*b*-poly(ethylene oxide) (BO) in the oil-in-oil emulsion, where the droplets and continuous phase are PS- and PB-rich, respectively. The polymersome structure was directly visualized using dye-labeled SO and BO with confocal fluorescence microscopy; SO and BO with a high O block fraction co-assemble to produce asymmetric polymersomes. As the O block is insoluble in both PS and PB, we infer that the detailed structure of the polymersomes is a bilayer in which the S and B blocks face the PS-inner and PB-outer phases, respectively, while the common O blocks form the core membrane. This structure is only observed for sufficiently long O blocks. It is remarkable that although all the polymers are soluble in CHCl<sub>3</sub>, such elaborate structures are created by straightforward co-assembly. These asymmetric polymersomes should provide robust bilayer membranes around emulsion droplets, leading to stable nanoscopic dispersions of two fluids.

Polymeric vesicles, also known as polymersomes, offer an attractive route to stabilizing nanoscopic emulsions, by forming a bilayer surrounding droplets of the interior fluid.<sup>1</sup> Polymersomes in aqueous media are commonly composed of amphiphilic AB diblock copolymers having relatively short hydrophilic blocks and longer hydrophobic blocks.<sup>2</sup> The hydrophilic blocks form swollen corona layers facing both the interior and exterior fluids, whereas the hydrophobic blocks form dense core membranes. It is of general interest to also prepare “asymmetric” polymersomes, in which the interior and exterior coronas are different; such a structure could be used, e.g., to stabilize dispersions of two distinct fluids. One approach is to employ more elaborate block architectures, such as ABC triblocks<sup>3</sup> and ABCA tetrablock terpolymers.<sup>4</sup> On the other hand, asymmetric vesicles formed by co-assembly of two diblock copolymers (AC + BC) would be a particularly attractive methodology because it is straightforward to prepare diblocks, and their stoichiometry can be readily tuned. In this case the common C blocks would form the solvophobic

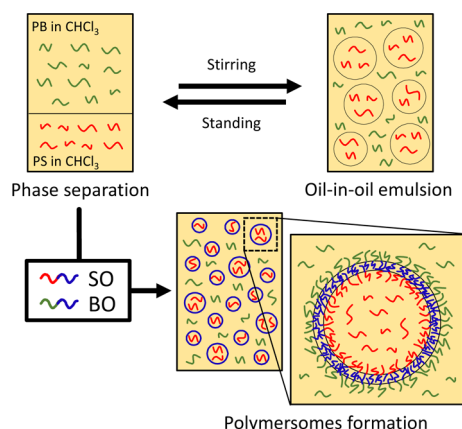
membrane, while the (mutually immiscible) A and B blocks form distinct interior and exterior coronas. There have been relatively few reports of blending amphiphilic block copolymers in water, and these strategies were aimed primarily at preparing more elaborate, multicompartments structures.<sup>5,6</sup> Block copolymers have successfully been used to prepare polymersomes with distinct inner- and outer-fluids, e.g., with ionic liquid interiors dispersed in an aqueous phase.<sup>7–11</sup> In this case poly(ethylene oxide) (PEO) blocks formed both interior and exterior coronas, with either polystyrene (PS) or poly(butadiene) (PB) as the solvophobic interior. While this strategy enables potential applications as nanoreactors,<sup>7</sup> it requires that the AB diblock exhibits the same “amphiphilicity” with respect to both immiscible solvents, which is a significant constraint. In contrast, successful blending of AC + BC diblocks could, in principle, allow access to vesicles containing any “A-philic” or “B-philic” fluid. Furthermore, as we demonstrate here, it is not even necessary that the membrane-forming C block be insoluble in the two fluids.

Oil-in-oil and water-in-water polymeric emulsions are an interesting class of emulsions, both from a fundamental<sup>12</sup> and an application perspective.<sup>13</sup> Generally, these emulsions are formed by mixing A and B homopolymers in a common solvent. Beyond a certain concentration of A and B, liquid–liquid phase separation is induced by weak unfavorable interactions between A and B; the resulting phases therefore are A- and B-rich. Interestingly, although both phases contain the same solvent, distinct A- and B-rich phases enable selective molecular capture, as exemplified by biopolymer partitioning in separation science.<sup>13</sup> Recently, attention has been directed toward the construction of stable oil-in-oil emulsions using block polymers. Thus, AB diblock<sup>14,15</sup> or ACB triblock<sup>16</sup> copolymers have been proposed to stabilize emulsions by monolayer adsorption at interface. In the ACB case, a hydrophobic C block formed a stabilizing membrane between two different aqueous polymer phases, with A and B being distinct hydrophilic blocks. Furthermore, ABC triblock terpolymers have been used to prepare oil-in-water emulsions<sup>17</sup> and to stabilize air nanobubbles in water.<sup>18</sup>

Here, we demonstrate the first observation of bilayer asymmetric polymersomes formed by co-assembly of AC and BC block copolymers, as illustrated in the cartoon shown in Figure 1. In this case PS-*b*-PEO (SO) and PB-*b*-PEO (BO)

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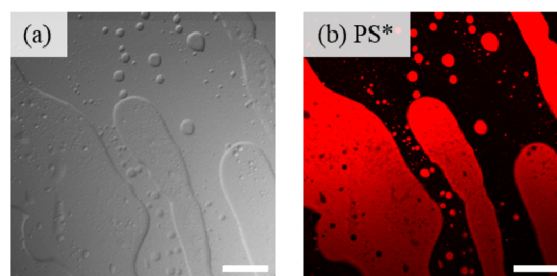


**Figure 1.** Schematic illustrations of polymersomes formed by co-assembly of SO and BO diblocks in oil-in-oil emulsions composed of PS/PB/CHCl<sub>3</sub>. All polymers are soluble in CHCl<sub>3</sub>.

diblocks are blended in an oil-in-oil emulsion containing PS and PB homopolymers in chloroform (CHCl<sub>3</sub>). Thus, PEO represents the common block “C”.

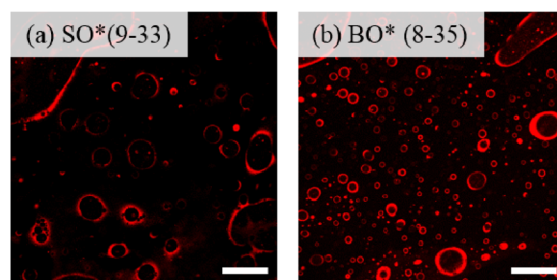
All polymers were prepared by living anionic polymerization by methods described previously;<sup>7,15</sup> CHCl<sub>3</sub> was purchased from Aldrich (Supporting Information, Figure S1, Table S1). For PS and PB,<sup>19</sup> the number-average molecular weight ( $M_n$ ) and dispersity ( $\mathcal{D}$ ) determined by <sup>1</sup>H NMR spectroscopy and size exclusion chromatography (SEC) are  $M_n = 9$  kg/mol and  $\mathcal{D} = 1.02$  and  $M_n = 8$  kg/mol and  $\mathcal{D} = 1.06$ , respectively. Diblock copolymers are denoted SO( $x$ - $y$ ) and BO( $x$ - $y$ ), where  $x$  indicates  $M_n$  (kg/mol) of S or B block, and  $y$  indicates  $M_n$  (kg/mol) of O block, respectively. The volume fraction of the O block ( $f_{PEO}$ ) is estimated from the bulk density of each polymer.<sup>20</sup> All S and B blocks were 9 and 8 kg/mol, respectively, which were designed to match the  $M_n$  of PS (9 kg/mol) and PB (8 kg/mol) homopolymers used for the oil-in-oil emulsions. A series of three values of  $M_n$  of the O blocks was prepared, to yield SO(9-2), SO(9-13), SO(9-33) and BO(8-2), BO(8-10), BO(8-35);  $f_{PEO} = 0.14, 0.55, 0.75$  and  $0.15, 0.48, 0.75$ , respectively.<sup>21</sup> Rhodamine B labeled polymers (denoted as PS\*, SO\*, and BO\*) were prepared via esterification of the hydroxyl-end group of each polymer with rhodamine B acid chloride, following previous reports.<sup>7,15</sup> The primary location of PS, SO, and BO in the oil-in-oil emulsions was imaged separately by confocal fluorescence microscopy (CFM), using the corresponding dye-labeled polymer.

A representative oil-in-oil emulsion composed of PS/PB/CHCl<sub>3</sub> with 15/15/70 (wt %) of each component is shown in the optical image in Figure 2a.<sup>22</sup> To locate the PS, a small amount of PS\* was added. In CFM, the Rhodamine B is excited at 536 nm, and the emission around 580 nm is observed as red. Since at most 0.002 wt % of unreacted RhoB remained in the PS\*, free dye is a negligible factor (Table S1). Therefore, as shown in Figure 2b, PS is primarily located in the droplet phase. The composition of the upper and bottom layers was quantified after macroscopic phase separation. The upper layer included 98 wt % of the total PB, whereas the bottom layer included 98 wt % of the PS (Table S2). As the droplets are the PS-rich phase, the continuous phase is PB-rich, and a PS-in-PB emulsion is formed. The fact that the PB phase forms the matrix presumably reflects the greater volume of PB relative to PS. The ternary phase diagram is also presented in Figure S2.



**Figure 2.** (a) Representative optical and (b) CFM images of oil-in-oil emulsions composed of PS/PB/CHCl<sub>3</sub> at 15/15/70 weight ratio. In the CFM image, red (PS\*) indicates the PS-rich phase. Scale bars = 100  $\mu$ m.

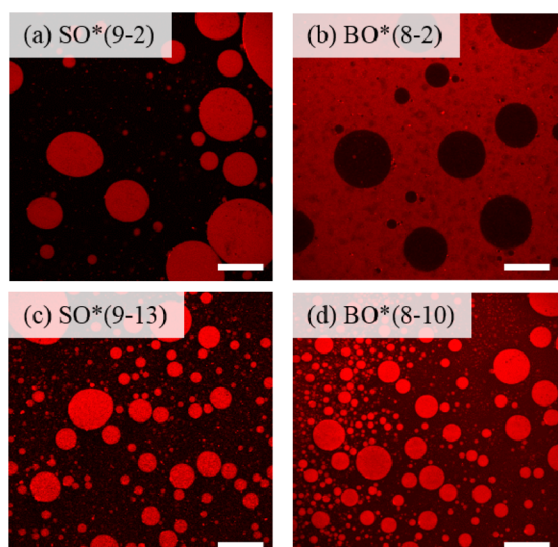
To investigate the effect of SO and BO blending on the oil-in-oil emulsion, 0.5 wt % of both SO(9-33) and BO(8-35), each with  $f_{PEO} \approx 0.75$ , were added. As shown in Figure 3a,b, the droplet size (mean diameter  $D_n$ ) decreased significantly to 15  $\mu$ m, and moreover, the resulting CFM images indicate that both SO(9-33) and BO(8-35) are primarily located at the interface, while emulsion droplets and continuous phase remain PS- and PB-rich, respectively (see Figure S3a). These images demonstrate the first visualization of polymersomes formed by co-assembly of two diblocks, in this case SO and BO, between PS- inner and PB-rich outer phases. In the absence of either SO or BO, the emulsion is very unstable, rapidly coarsening into many huge elliptical domains over 200  $\mu$ m in size (Figure 2a). It is evident that the polymersomes stabilize small droplets and prevent coalescence, over a time scale of at least 1 week.



**Figure 3.** (a,b) CFM images obtained from PS-in-PB emulsions with 0.5% SO(9-33) and BO(8-35). Red indicates the primary location of SO\* (a) and BO\* (b), respectively. Scale bars = 100  $\mu$ m.

It is quite remarkable that polymersome formation was induced when SO(9-33) and BO(8-35) were present concurrently in the emulsion. As the common O block is immiscible with both PS and PB, while the S and B blocks are miscible with PS and PB homopolymers, respectively, we infer that the polymersome structure features the S block in SO as the “inner” corona, and the B block in BO as the “outer” corona, while the membrane core comprises the two O blocks. Consequently, as depicted in Figure 1, SO and BO behave as a pseudo SOB triblock copolymer. Energetically, this structure is also expected to be the most stable state; that is, all blocks can interact with each miscible polymer, meaning no energy penalty. However, the balance of free energy contributions is delicate; e.g., the SO and BO diblocks themselves have a preferred curvature, which could easily lead to, e.g., micelles in one or other of the two phases. Furthermore, the O blocks could also prefer to dissolve in the solvent.

To explore this issue further, we studied the effect of added SO and BO as a function of  $f_{\text{PEO}}$ . With 0.5/0.5 wt % of SO(9–2)/BO(8–2) for which  $f_{\text{PEO}} \approx 0.15$ , and SO(9–13)/BO(8–10) for which  $f_{\text{PEO}} \approx 0.50$ ,  $D_n$  decreased to 73 and 40  $\mu\text{m}$ , respectively. SOs and BOs tend to reduce droplet size, presumably by reducing the (already small) interfacial tension, although their efficacy is much lower than with  $f_{\text{PEO}} \approx 0.75$ . However, crucially, as displayed in Figure 4a–d, neither SO nor



**Figure 4.** CFM images obtained from PS-in-PB emulsions with (a) 0.5% SO(9–2) and (b) 0.5% BO(8–2) and with (c) 0.5% SO(9–13) and (d) 0.5% BO(8–10). Red in (a) and (c) indicates the primary location of SO\*, while in (b) and (d) red indicates the primary location of BO\*. Scale bars = 100  $\mu\text{m}$ .

BO is preferentially observed at the interface, but rather either in the PS-rich droplets or the PB-rich continuous phase (see also Figures S3b,c). In diblock self-assembly, bilayer formation is generally favored when the fraction of the core block is large, and thus in this context it is perhaps not surprising that reducing  $f_{\text{PEO}}$  destroys the stabilizing bilayer.

From these results, it is reasonable to infer that the driving force for asymmetric polymersome formation is the unfavorable interaction between the O blocks and the PS or PB homopolymers. To examine this hypothesis further, only one of SO(9–33), BO(8–35), or a PEO homopolymer (i.e.,  $f_{\text{PEO}} = 1$ , Fuluka,  $M_n = 32$  kg/mol,  $D = 1.03$ ) was added at 1 wt %, and the primary location was visualized by CFM.<sup>23</sup> By itself, SO(9–33) forms new droplets inside the PS-rich droplets, while BO(8–35) is partially located at the interface. The PEO homopolymer forms a separate phase within PS-rich droplets (Figures S4a–c). These primary locations may be rationalized in terms of the relative polymer–polymer interaction parameters ( $\chi_{ij}$ ). Estimated  $\chi_{ij}$  values are  $\chi_{\text{PS-PB}} \approx 0.09$ ,  $\chi_{\text{PS-PEO}} \approx 0.07$ , and  $\chi_{\text{PB-PEO}} \approx 0.21$ , respectively.<sup>24</sup> For SO and PEO, inside the PS-rich droplets is the most preferable state because although this incurs S/O contacts, the resulting penalty is much less than in the PB-rich phase. For BO, one might initially assume that the PB-rich phase would be the most preferable. However, much of the BO resides at the interface. In this case, the B and O blocks should face the PB- and PS-rich phases, respectively, as  $\chi_{\text{PS-PEO}}$  is only half  $\chi_{\text{PB-PEO}}$ . Therefore, the interface is the most preferable location for BO.

In summary, we demonstrated the first asymmetric polymersomes formed by co-assembly of two distinct diblock copolymers. In this system, SO and BO diblocks were blended in an oil-in-oil emulsion containing PS/PB/ $\text{CHCl}_3 = 15/15/70$  by weight, as a function of diblock copolymer composition  $f_{\text{PEO}}$ . Only SO and BO with a high  $f_{\text{PEO}}$  formed a bilayer at the interface. It is inferred that the detailed structure of polymersomes is a bilayer in which the S and B blocks face the PS-rich inner and PB-rich outer phases, respectively, while the common O blocks embody the membrane cores. It is intriguing that although all the polymers are soluble in the solvent,  $\text{CHCl}_3$ , such highly refined self-assembly occurs. Furthermore, this very straightforward methodology holds the potential to tune block copolymer co-assembly extensively. This new kind of polymersome provides a potentially robust membrane around emulsion droplets, leading to stable nanoscale oil-in-oil emulsions.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b01697.

Experimental details and data (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Author

\*lodge@umn.edu

### Notes

The authors declare no competing financial interest.

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- (19) Content of 1,2-addition in PB and BO determined by  $^1\text{H}$  NMR spectroscopy is 90%.
- (20) Densities are taken as  $\rho_{\text{PS}} = 1.06$ ,  $\rho_{\text{PB}} = 0.91$ , and  $\rho_{\text{PEO}} = 1.27$  g/cm<sup>3</sup>, respectively.
- (21) The range of  $\mathcal{D}$  is 1.01–1.13.
- (22) Phase separation was observed above 28 wt % total polymer concentration, whereas below 26 wt%, the solution remained clear.
- (23) In PEG imaging, fluorescein was added to solvate with PEG in  $\text{CHCl}_3$ . Then the green color reflecting fluorescein emission indicates the primary location of PEG.
- (24) Estimates for  $\chi_{ij}$  can be calculated from  $\chi_{ij} = V(\delta_i - \delta_j)^2/RT$ , where  $\delta$  is the solubility parameter (PS; 9.1, PB; 8.6, PEO; 9.9, (cal/cm<sup>3</sup>)<sup>1/2</sup>),<sup>14,15</sup>  $V$  is the segment molar volume given by the average value of  $i$  and  $j$  components,  $R$  is the gas constant (= 1.987 cal/mol·K), and  $T$  is the absolute temperature (here  $T = 298$  K), giving the estimated interaction parameter as  $\chi_{\text{PS-PB}} = 0.09$ ,  $\chi_{\text{PS-PEO}} = 0.07$ , and  $\chi_{\text{PB-PEO}} = 0.21$ , respectively.