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Compartmentalization Technologies via Self-Assembly and Cross-Linking of Amphiphilic Random Block Copolymers in Water

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Supporting Information

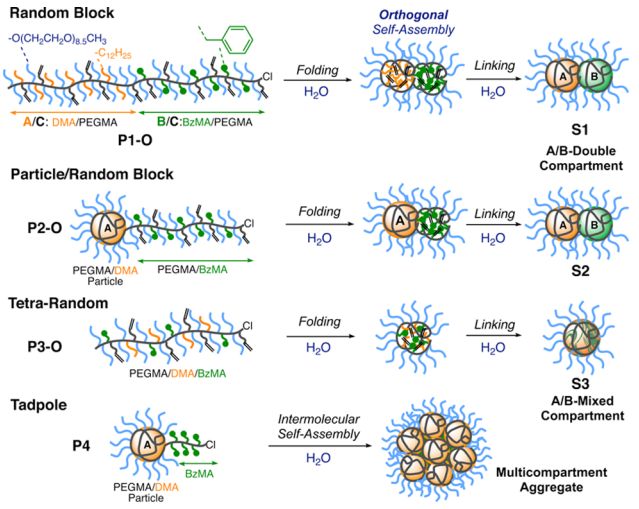
ABSTRACT: Orthogonal self-assembly and intramolecular cross-linking of amphiphilic random block copolymers in water afforded an approach to tailor-make well-defined compartments and domains in single polymer chains and nanoaggregates. For a double compartment single-chain polymer, an amphiphilic random block copolymer bearing hydrophilic poly(ethylene glycol) (PEG) and hydrophobic dodecyl, benzyl, and olefin pendants was synthesized by living radical polymerization (LRP) and postfunctionalization; the dodecyl and benzyl units were incorporated into the different block segments, whereas PEG pendants were statistically attached along a chain. The copolymer self-folded via the orthogonal self-assembly of hydrophobic dodecyl and benzyl pendants in water, followed by intramolecular cross-linking, to form a single-chain polymer carrying double yet distinct hydrophobic nanocompartments. A single-chain cross-linked polymer with a chlorine terminal served as a globular macroinitiator for LRP to provide an amphiphilic tadpole macromolecule comprising a hydrophilic nanoparticle and a hydrophobic polymer tail; the tadpole thus self-assembled into multicompartment aggregates in water.

Self-assembly and/or cross-linking of amphiphilic copolymers in water is fundamental technologies to create compartmentalized materials (e.g., micelles, vesicles, and nanogels).[1−7] Among them, constructing microdomains within nanoaggregates, i.e., multicompartmentalization, allows one to introduce different functional units into discrete but closely located nanospaces; this attracts attention to create selective, unique, and bioinspired functions.[2−4] Such multicompartmentalized materials have been prepared by intermolecular self-assembly of multiple block copolymers whose block segments carry different properties, solubility, and miscibility (e.g., ABC triblock copolymers). In natural biopolymers, proteins and enzymes consist of amphiphilic polymers with inherent primary structure, where hydrophilic and hydrophobic monomer units are sequenced along a chain. As a result, they site-specifically self-fold in water via the orthogonal self-assembly of pendant functional groups, according to the programmed monomer sequences, to form single-chain compartmentalized structures that carry precision cavities and/or discrete domains.

For compartmentalized synthetic single-chain polymers, self-folding of random copolymers is recognized as a promising strategy.[7−14] Typically, amphiphilic random copolymers of hydrophilic poly(ethylene glycol) methyl ether methacrylate (PEGMA; M_n = 475) and hydrophobic dodecyl methacrylate (DMA) autonomously self-fold into unimer micelles with dynamic, hydrophobic compartments in water (∼10 nm).[12a] Uniquely, the copolymers have threshold chain length (degree of polymerization: DP_{th}) suitable for single-chain folding, dependent on the hydrophobic DMA composition (e.g., 40 mol % DMA; DP_{th} = ∼200). Thus, PEGMA-based amphiphilic random block copolymers, where hydrophobic units with different miscibility are respectively incorporated into the block segments of DP_{th}, potentially induce double self-folding via selective self-assembly of the hydrophobic pendants in water to give single-chain double compartment polymers.

We herein report the self-assembly and cross-linking of amphiphilic random block copolymers in water to design precision multicompartments in single polymer chains or nanoaggregates (Scheme 1). Typically, distinct but connected domains are successfully built in single polymer chains via the orthogonal intramolecular self-assembly of the hydrophobic

Scheme 1. Nanocompartment Polymers Created via the Self-Folding and Cross-Linking of Amphiphilic Random/Block Copolymers in Water



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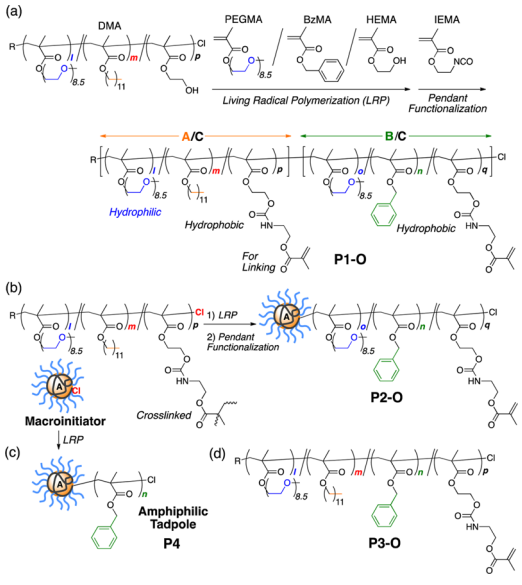
pendants (A, B) of a A/C−B/C random block copolymer (C: hydrophilic). This is a strategy to tailor-make small domains (<10 nm) in macromolecules, distinct from multicompartment micelles via the intermolecular self-assembly of ABC triblock copolymers.[4]

For this, we designed an amphiphilic random block copolymer (**P1-O**) bearing different hydrophobic pendants (A: dodecyl; $-C_{12}H_{25}$, B: benzyl: $-CH_2Ph$) in respective block segments, whereas hydrophilic poly(ethylene glycol) methyl ether (PEG) pendants (C) were statistically distributed along a chain. **P1-O** induced double self-folding via the "orthogonal" self-assembly of the hydrophobic pendants (A, B) in water, followed by intramolecular cross-linking,[12b] to provide a single-chain cross-linked polymer (**S1**) with distinct hydrophobic dodecyl (A) and benzyl (B) cores both of which are covered by PEG. It should be noted A/B double domains are constructed via the phase separation between simple hydrophobic pendants. In contrast, an amphiphilic random copolymer statistically bearing both dodecyl and benzyl pendants without segmentation (**P3-O**) gave a single-chain cross-linked polymer with a dodecyl and benzyl-mixed core. Single-chain cross-linked polymers obtained with ruthenium-catalyzed living radical polymerization (LRP) carry a chlorine terminal originating from the precursors. Thus, the cross-linked polymers served as "globular" macroinitiators for LRP, giving an amphiphilic tadpole macromolecule consisting of a hydrophilic nanoparticle and a hydrophobic linear polymer (**P4**). The tadpole intermolecularly self-assembled into multicompartment aggregates in water. Thus, amphiphilic random block copolymers opened ways to design compartmentalized materials structurally close to proteins.

Two synthetic approaches were investigated for single-chain A/B-double compartment polymers (**S1**, **S2**): (1) direct intramolecular cross-linking of double self-folded amphiphilic random block copolymers in water and (2) iterative synthesis via random copolymerization with a single-chain cross-linked macroinitiator, self-folding, and intramolecular cross-linking (Schemes 1 and 2). To achieve orthogonal self-folding of the respective block segments, the content of hydrophobic monomers including olefin pendants were set as 40 mol % (including 20 mol % hydrophobic olefin), whereas degree of polymerization on respective block segments was targeted to 200.

Scheme 2. Synthesis of Amphiphilic Random/Block Copolymers (a: P1-O, b: P2-O, c: P3-O, and d: P4) via Living Radical Polymerization and Pendant Functionalization



For the first block segment including dodecyl units, PEGMA, DMA, and 2-hydroxylethyl methacrylate (HEMA) were copolymerized with a ruthenium catalyst [RuCp*Cl(PPh_3)$_2$/4-(dimethylamino)-1-butanol] and a chloride initiator (ethyl 2-chloro-2-phenylacetate) in ethanol at 40 °C to give a well-controlled PEGMA/DMA/HEMA random copolymer with narrow molecular weight distribution [Conv. >80%, 31 h, M_n = 52 000, M_w/M_n = 1.14, by size exclusion chromatography (SEC) in DMF with PMMA calibration, Figure S1, Table S1]. The degree of polymerization was determined by ^{1}H nuclear magnetic resonance (NMR) spectroscopy: PEGMA/DMA/HEMA ($l/m/p$) = 99/33/33; total DP = 165 (Figure S1). Subsequently, block copolymerization of PEGMA, benzyl methacrylate (BzMA), and HEMA was conducted with the random copolymer as a macroinitiator to provide an amphiphilic random block copolymer (**P1**: M_n = 115 000, M_w/M_n = 1.13 by SEC, Table S1, Figure S2). Confirmed by ^{1}H NMR (Figure S3), **P1** consisted of a random A/C segment including DMA ($l/m/p$ = 99/33/33) and a random B/C segment including BzMA ($o/n/q$ = 115/35/35). Then, **P1** was treated with 2-isocyanatoethyl methacrylate (IEMA) and dibutyltin dilaurate in CH_2Cl_2 at 25 °C ([OH]$_0$/[IEMA]$_0$ = 1/10) to quantitatively give an olefin-bearing random block copolymer (**P1-O**: M_n (NMR) = 137 000, Table S2, Figure S3).

The self-folding of **P1-O** by hydrophobic effect in water was confirmed by SEC and ^{1}H NMR (Figure 1a and S4). Analyzed by

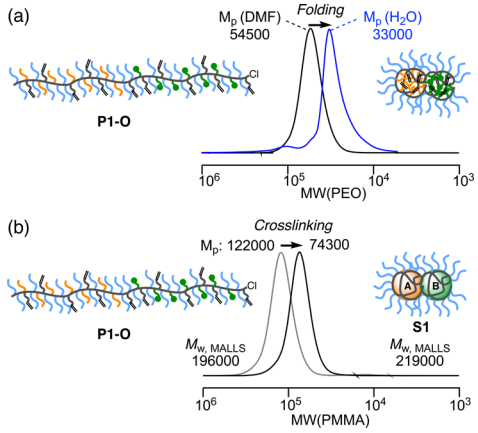


Figure 1. (a) SEC curves (with PEO calibration) of **P1-O** in DMF (black) or water (blue). (b) SEC curves (in DMF with PMMA calibration) of **S1** (black) obtained via the intramolecular cross-linking of **P1-O** (gray) with AIBN in water under UV irradiation (λ = 375 nm) at 25 °C: [polymer]/[AIBN] = 10/0.6 mg/mL.

SEC with poly(ethylene oxide) calibration, SEC peak-top molecular weight of **P1-O** in water was smaller than that in DMF (M_p = 33 000 in water, 54 500 in DMF). ^{1}H NMR measurement of **P1-O** in D_2O further revealed that the proton signals of hydrophobic dodecyl, benzyl, and olefin pendants and methacrylate backbones were broad (Figure S4).

The intramolecular cross-linking of **P1-O** was carried out with 2,2′-azodiisobutyronitrile (AIBN) in water under UV irradiation (375 nm) at 25 °C ([polymer]$_0$ = 10 mg/mL). The reaction proceeded without macroscopic gelation up to 94% olefin conversion in 82 h to give a cross-linked **S1** (Figure S4). **S1** had smaller peak-top molecular weight in DMF than **P1-O** [M_p =

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74 300 (**S1**), 122 000 (**P1-O**), M_w/M_n (**S1**) = 1.11 by PMMA calibration, Figure 1b], whereas absolute weight-average molecular weight of **S1** by SEC-MALLS almost agreed with that of **P1-O** [M_W = 219 000 (**S1**), 196 000 (**P1-O**)]. These results support that **S1** has self-folded and compact structure even in organic solvents.

Another synthetic approach involves use of a single-chain cross-linked polymer as a macroinitiator (Scheme 2b). The chlorine-capped macroinitiator (M_n = 38 000, M_w/M_n = 1.17) was prepared by intramolecular cross-linking of an olefin-bearing PEGMA/DMA/HEMA random copolymer in water (Figure S5). Then, ruthenium-catalyzed living radical copolymerization of PEGMA, BzMA, and HEMA was conducted with the macroinitiator, giving a block copolymer consisting of a globular nanoparticle and linear amphiphilic random copolymer bearing benzyl units (**P2**). An olefin-bearing **P2-O**, obtained via the postfunctionalization of **P2** with IEMA, was cross-linked with AIBN in water to provide a single-chain cross-linked **S2** (M_w = 240 000, M_w/M_n = 1.20, Figure S6). The structure was similarly confirmed by SEC-MALLS (Tables S2 and S3).

A cross-linked polymer bearing both dodecyl and benzyl units in a single core (**S3**) was also designed, as a controlled sample against double compartment polymers (**S1**, **S2**). **S3** was obtained from the intramolecular cross-linking of a self-folded **P3-O** in water [**S3**: M_w = 98 900 by SEC-MALLS, $l/m/n/p$ = 95/15/16/31, Figure S6]; **P3-O** was similarly synthesized by LRP and postfunctionalization (SI).

The double compartment structures of **S1** and **S2** were evaluated by small-angle X-lay scattering (SAXS) and transmission electron microscopy (TEM) (Figure 2 and S7). **S1** and

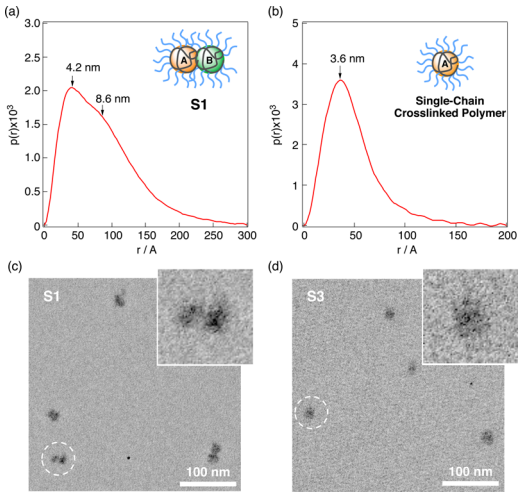

Figure 2. (a, b) Pair-distance distribution function $P(r)$ of the SAXS profiles of (a) **S1** and (b) a single-chain cross-linked polymer with a DMA core in DMF at 25 °C. (c, d) TEM images of (c) **S1** and (d) **S3** cast from the aqueous solutions ([**S1** or **S3**] = 10 mg/mL) to carbon-coated grid. TEM samples were stained with 1% OsO_4 aqueous solution. Right top: magnified images of the circled parts.

S2 were analyzed by SAXS in DMF or water ([polymer] = 10 mg/mL), compared with a single-chain cross-linked polymer with a DMA core (macroinitiator for **P2-O**). All of the cross-linked polymers had globular structures in the solvents, whereas the pair-distance distribution functions [$P(r)$] were dependent on the monomer sequences. **S1** showed a peak with a shoulder at large size region (r = 4.2 nm, 8.6 nm),[15] whereas the single-chain cross-linked polymer only exhibited a unimodal peak without such a shoulder (r = 3.6 nm). **S2** obtained via an iterative synthetic approach also had almost identical $P(r)$ to **S1** in both DMF and water (Figure S7). These results strongly support that, independent of synthetic pathways, both **S1** and **S2** had double domains comprising dodecyl or benzyl units in single polymer chains. Thus, an amphiphilic random block copolymer bearing dodecyl and benzyl pendants actually double self-folded in water via the orthogonal phase separation of dodecyl and benzyl pendants, resulting in a single-chain double compartment polymer. Additionally, the double compartment structure of **S1** was observed by TEM (Figure 2c). Such double domains were not observed for **S3** with a dodecyl and benzyl-mixed core (Figure 2d).

To evaluate the local distance of pendants, **S1**, **S2**, and **S3** were analyzed by ^{1}H nuclear Overhauser effect (NOE) difference spectroscopy in $CDCl_3$ and D_2O at 30 °C (Figures 3, S8, and S9).

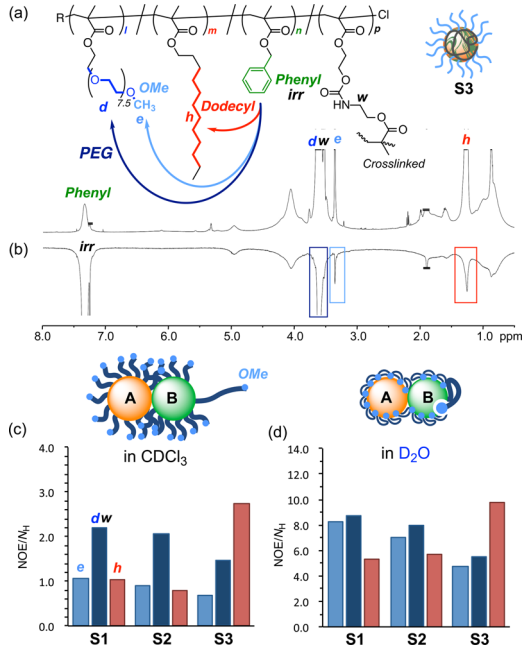

Figure 3. (a) ^{1}H NMR and (b) ^{1}H NOE difference spectroscopies of **S3** in $CDCl_3$ at 30 °C. (c, d) ^{1}H NOE signal intensity for one proton (NOE/N_H) of poly(ethylene glycol) methyl ether [d: $-CH_2(OCH_2CH_2)_{7.5}-$, e: $-OCH_3$], dodecyl (h), and cross-linked (w) units in **S1**, **S2**, and **S3** in $CDCl_3$ and D_2O at 30 °C.

These polymers showed the aromatic protons of BzMA units at 7.4−7.3 ppm without overlapping with the other protons (Figure 3: **S3** in $CDCl_3$). In general, the intensity of ^{1}H NOE signals depends on the distance between the proton and an irradiated proton (NOE/$N_H \propto r^{-6}$, N_H: proton numbers, r: distance). Thus, by irradiating the BzMA aromatic protons, the area ratio for NOE signals of dodecyl protons (***h***) and PEG protons (***d***, ***e***) per one proton (NOE/N_H) were estimated, to evaluate the distance between the respective NOE signal segments and the irradiated proton. The one aromatic proton of irradiated BzMA (***irr*** in Figure 3) was set to 100 as integral normal, and the proton number of respective NOE peaks was given: N_H (***e***) = 3 × (I_{obsd} + o_{obsd}); N_H (***d***, ***w***) = 32 × (I_{obsd} + o_{obsd}) + 2 × (p_{obsd} + q_{obsd}); N_H (***h***) = 18 × m_{obsd}.

In $CDCl_3$, the NOE signal for the dodecyl pendants (***h***) of both **S1** and **S2** was much smaller than that of **S3**, whereas that for PEG chains (***d***, ***e***) was close among the three samples (Figure 3c).

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NOE signal intensity for the dodecyl and PEG pendants of **S1** was virtually identical to that of **S2**. Non-cross-linked **P3-O** hardly showed NOE signals of dodecyl and PEG chains in $CDCl_3$ (Figure S9). These results importantly indicate the following facts: (1) Local distance between benzyl pendants and dodecyl or PEG pendants within a single polymer turns close via intramolecular cross-linking. (2) Even after intramolecular cross-linking, dodecyl pendants in **S1** and **S2** are yet spatially separated from benzyl counterparts, in contrast to the close location of dodecyl and benzyl pendants in **S3**. (3) **S1** has almost the same inner domain structure as **S2** in spite of different synthetic processes.

In D_2O, similar tendency was observed for the signal intensity of the dodecyl pendants (Figure 3d). The absolute NOE signal intensity in D_2O was much larger than that in $CDCl_3$. This is owing to the shrunk structures of **S1**, **S2**, and **S3** in water by hydrophobic effect; the compact structures in water were confirmed by SEC and DLS [Figure S10, e.g., **S1**: R_h = 7.5 nm (H_2O), 8.9 nm (DMF)]. However, **S1** and **S2** still maintain remote position between dodecyl and benzyl pendants, compared with **S3**. In all of the samples, the absolute signal intensity for PEG chains (***d***, ***e***) increased in D_2O, whereas the intensity of the tip methyl group (***e***) turned close to that of the middle ethylene oxide segments (***d***). This result suggests two facts: (1) Inner PEG pendants, partially included within the hydrophobic spaces, become close to hydrophobic benzyl pendants by shrunk structure in water. (2) Outer PEG chains effectively cover the hydrophobic cores in water and all of the segments are uniformly close to inner benzyl units.

In contrast to **S1**−**S3**, an amphiphilic tadpole comprising a hydrophilic nanoparticle and a hydrophobic poly(BzMA) (**P4**, M_n = 46 700, M_w/M_n = 1.28, DP of BzMA = 53) formed large aggregates in water (R_h = 53 nm by DLS, Figure 4a). The TEM

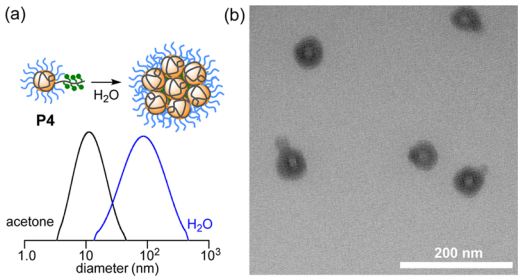

Figure 4. (a) DLS intensity size distribution of **P4** in acetone or water: [**P4**] = 10 mg/mL at 25 °C. (b) A TEM image of **P4** cast from its aqueous solution (10 mg/mL) on a carbon coat grid and stained with OsO_4.

image showed unique spherical aggregates via self-assembly of multiple **P4** (Figure 4b). The aggregates contain multiple **S1** bearing a hydrophobic core as distinct compartments. Thus, living radical polymerization with single-chain cross-linked polymers as macroinitiators is effective to design multi-compartmentalized materials.

In conclusion, amphiphilic random block copolymers with distinct hydrophobic pendants successfully provided single-chain cross-linked polymers bearing double domains. The compartmentalization is effectively achieved via the phase separation of the hydrophobic pendants within self-folded polymers in water. This is indeed an innovative technique to create nanospaces within single polymer chains. LRP with a chlorine-capped single-chain polymer nanoparticle as a macroinitiator was further effective for the design of multicompartmentalized materials. The precision compartmentalization technologies with amphiphilic random block copolymers, developed herein, would open new vistas in polymer, organic, bio, and material chemistries and related research fields. Distinct but closely connected nano-compartment materials would be useful to create biomimetic, cooperative functions, tandem catalysis, among many others.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.7b03152.

Experimental details, SEC, ^{1}H NMR, DLS, and SAXS of polymers (PDF)

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Notes

The authors declare no competing financial interest.

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