Supramolecular Assembly Mediates the Formation of Single-Chain Polymeric Nanoparticles

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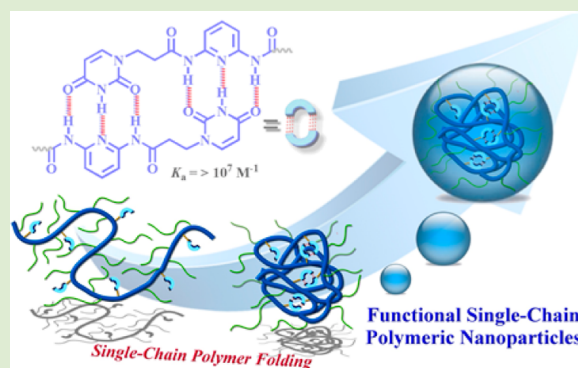
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S *Supporting Information*

ABSTRACT: A breakthrough innovation in water-based polymeric nanoparticles has enabled significant progress in mimicking the folding of natural proteins by generating novel "single-chain polymeric nanoparticles" (SCPNs) via supramolecular interactions. In this study, a practical approach to the living polymerization of functionalized oligo(ethylene glycol) methacrylate monomers allows the incorporation of self-constituted multiple hydrogen-bonded groups into physically cross-linked polymer networks, which enables the formation of highly functionalized SCPNs in an aqueous environment. The newly developed materials are particularly attractive from a practical point of view since they have a very low critical micellization concentration and uniform particle diameters of ca. 25 nm, making them extremely stable under dilute conditions. Concentration-dependent experiments showed that SCPNs formed at polymer concentrations up to 40 mg/mL with no significant change in morphology observed. Moreover, the formed SCPNs had a very high stability in an aqueous solution containing surfactant, suggesting potential for a wide variety of applications as a promising candidate nanocarrier for bioimaging, controlled release, and drug delivery systems.

As globular tertiary structures can be formed through the self-folding of single-stranded polypeptide chains in water, protein self-assembly has attracted significant attention recently.[1−3] Undoubtedly, the reversibility of this self-folding/unfolding process enables proteins to adopt a variety of biological conformations and exert different functions. Nature's method of self-assembly mediated by hydrogen bonding[4−9] and dynamic covalent bonding[10−17] has inspired the design of functional polymer chains that rapidly form single-chain polymeric nanoparticles (SCPNs) and which exhibit unique physical properties, such as low viscosity, controlled affinity, and superior catalytic ability.[18−22] Nevertheless, these processes need to be performed under dilute conditions to ensure the single polymer chains preferentially undergo a coil-to-particle transition via intrachain interactions. Indeed, the preparation of well-defined SCPNs is currently a relatively inefficient process compared to the level of control observed for natural proteins, which efficiently program the formation of higher-order structures at the macromolecular level. Therefore, there is an urgent need to find appropriate methods to improve the formation of synthetic SCPNs at high polymer concentrations.

Recently, Voets et al.,[23] Swamoto[24] et al., and Qiao[25] et al. demonstrated that poly[oligo(ethylene glycol) methyl methacrylate] (POEGMA) derivatives can significantly improve the formation of SCPNs at high polymer concentrations (10−100 mg/mL). These results may inform the rational design of POEGMA copolymers; the ability to form SCPNs at high polymer concentrations could have a wide variety of potential applications. However, the exact mechanisms and stability of SCPN formation remain largely unknown. Highly self-complementary supramolecular interactions (e.g., electrostatic, ionic, hydrogen bonds) have been strongly suggested to promote polymer self-assembly and directly interact with both hydrophilic and hydrophobic segments to promote the
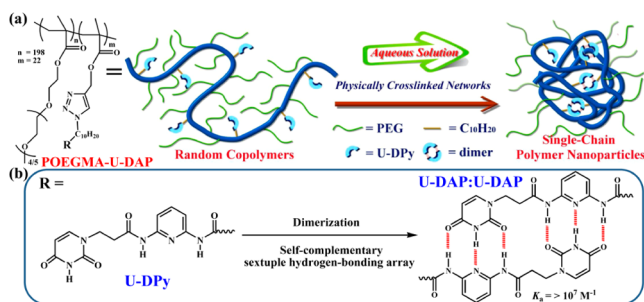
formation of a variety of amphiphilic micelles in aqueous solution.[26,27] Thus, we speculated that the introduction of supramolecular interactions into POEGMA backbones may alter the features of the micelles and initiate a self-organized process that effectively induces the formation of SCPNs in aqueous environments. Micellar stability is expected to be greatly improved by physical cross-linking by intramolecular noncovalent interactions among the polymer network.

N-(6-(3-(2,4-Dioxo-3,4-dihydropyrimidin-1(2H)-yl)-propanamido)pyridin-2-yl)undec-10-enamide (U-DPy) is a particularly interesting class of supramolecules, as it has an extremely high association constant ($K_a > 10^7$ M^{-1}) and is able to dimerize through self-complementary sextuple hydrogen-bonding arrays (Scheme 1).[28] Incorporation of U-DPy units

Scheme 1. (a) Schematic Representation of the Physically Cross-Linked SCPNs Formed by the Self-Complementary Multiple Hydrogen Bond Motifs of Water-Soluble Supramolecular POEGMA-U-DPy and (b) Structural Dimerization of U-DPy via Sextuple Hydrogen Bonding

into different polymer systems substantially enhanced thermal stability and induced unique morphological changes within these polymers in both solution and the solid state.[29] This provides evidence of the straightforward adaptability of incorporating U-DPy motifs as side chains into functional polymers. Herein, we report a novel POEGMA containing pendant U-DPy groups (POEGMA-U-DPy) in aqueous solution that exhibits an extremely low critical micelle concentration and viscosity and excellent self-assembly behavior owing to U-DPy-induced intramolecular physical cross-linking. More importantly, light scattering and morphological studies showed no apparent change in the particle size of the resulting SCPNs in aqueous solution across a broad concentration range (10^{-3}–10^2 mg/mL). In addition, the formed SCPNs possessed a high stability during prolonged treatment with the alkyl sulfate surfactant sodium dodecyl sulfate (SDS), which indicates POEGMA-U-DPy SCPNs may represent a potential drug carrier system for the safe, effective, and reliable delivery of anticancer drugs.

Our general strategy of producing SCPNs involves the design of synthetic structures and routes and is divided into two separate steps, as shown in Scheme S1 (see Supporting Information for further details). First, we synthesized well-defined alkyne side-chain copolymers (POEGMA-PgMA) by atom transfer radical polymerization of oligo(ethylene glycol) monomethyl ether methacrylate (average molecular weight = 300, ca. 4–5 repeat units) and (trimethylsilyl)propargyl methacrylate and then subsequently removed the trimethylsilyl-protecting groups.[30] Subsequently, alkyne-grafted PEGMA-PgMA was functionalized via an azide–alkyne click reaction with azide-labeled N₃-U-DPy[28,29] to produce high yields (92%)

of POEGMA-U-DPy, the structure of which is shown in Scheme 1. In all cases, an incorporation ratio of 4.8 mol % U-DPys into the polymer backbone was chosen; this was the optimal compromise between sample solubility and sufficient optical transparency in aqueous solution for further studies of SCPN formation (Figure S4). To evaluate the influence of intra- and intermolecular hydrogen-bonded interactions in solution, the molecular weight and polydispersity index (PDI) for a POEGMA-PgMA precursor and POEGMA-U-DPy copolymer were determined by gel-permeation chromatography (GPC) in various solvents [tetrahydrofuran (THF) and water] at 40 °C. In THF, the wide distribution of the GPC trace for PNI-U-DPy can be attributed to strong intermolecular aggregation in a typical organic solvent, as compared to a relatively low molecular weight and sharp distribution of POEGMA-PgMA, as indicated in Figure S5.[31] In contrast, POEGMA-U-DPy has a smaller hydrodynamic volume in water than the same concentration of POEGMA-PgMA (5 mg/mL), as the GPC trace for POEGMA-U-DPy shifted to a higher retention time (Figure 1a). This observation is very important, as steric repulsion by hydrophobic long-chain alkyl groups ($C_{10}H_{20}$) and intramolecular hydrogen bonding interactions from the pendant U-DPy units successfully isolate a single polymer chain and fold it in water, which hinders intermolecular aggregation (Scheme 1). It is also noteworthy
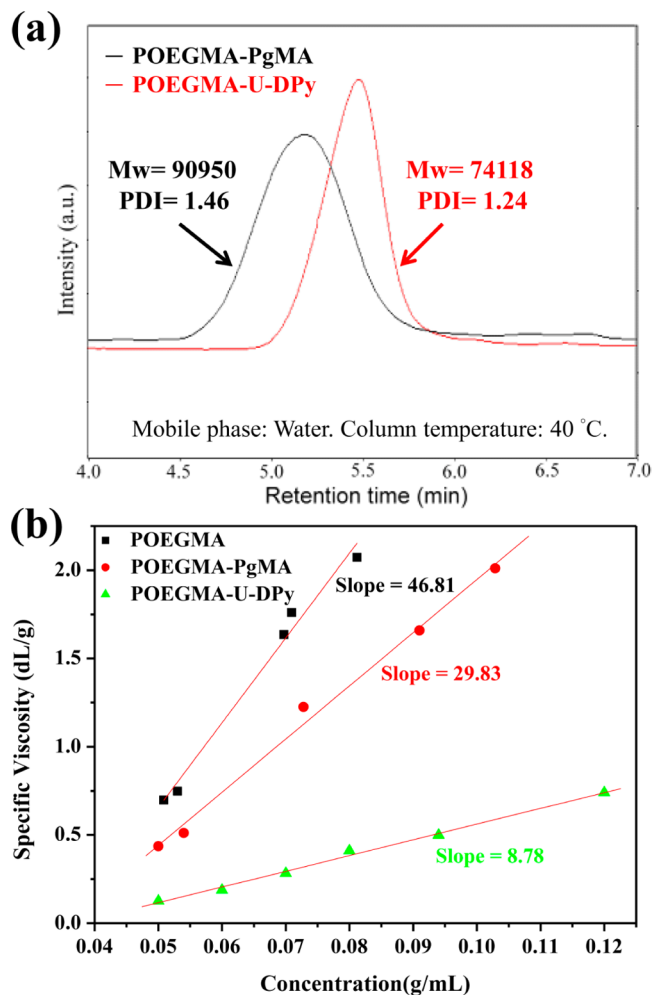

Figure 1. (a) GPC traces and (b) specific viscosity of POEGMA-PgMA and POEGMA-U-DPy in water.

that the POEGMA-U-DPy copolymer exhibited a significant decrease in PDI compared to POEGMA-PgMA (from 1.46 to 1.24), indicative of specific structures with a relatively regular arrangement. To further investigate these structural features, proton nuclear magnetic resonance spectra of all three polymers were measured in deuterated water (Figure S6). The peaks of the oligo(oxyethylene) units of POEGMA-U-DPy shifted slightly upfield and were broader, whereas control POEGMA and POEGMA-PgMA remained unchanged without wavelength shifts or peak shape changes. This indicates that chemical shift displacements occur via strong self-complementary hydrogen bonding between U-DPy units, resulting in highly specific conformational changes in aqueous solution, even though POEGMA-U-DPy has a high molecular weight (Figure 1a).

To confirm the natural properties of the physically cross-linked SCPNs in water, solution viscosity and dynamic light scattering (DLS) measurements were conducted. The viscosities of POEGMA, POEGMA-PgMA, and POEGMA-U-DPy were measured in water at 25 °C using an Ubbelohde viscometer (Figure 1b). The viscosity of each sample increased linearly with concentration. However, when the concentration reached 80 mg/mL, the viscosity of POEGMA-U-DPy significantly reduced compared to POEGMA and POEGMA-PgMA. This suggests that the dominance of intramolecular hydrogen bonds within the polymeric chain reduces interactions with the aqueous medium and prevents further increases in solution viscosity. In other words, the formation of SCPNs is expected to increase as the polymer concentration increases due to the covalent attachment of U-DPy units to the inner core of the particles, which ensures that single polymer chains preferentially favor intramolecular hydrogen bonding interactions at high concentrations. However, the viscosity value increased slowly with the increase in POEGMA-U-DPy concentration, and we speculate this phenomenon may be related to the formation of a multichain polymeric nanoparticle, which is an aggregation of a number of polymers.

Next, we performed a series of DLS measurements in aqueous solution at 20 °C. At sample concentrations higher than 40 mg/mL, POEGMA-U-DPy exhibited an average hydrodynamic diameter (d_H) of 26 nm, implying the formation of isolated SCPNs is mediated by a strong intramolecular hydrogen bonding network (Figure 2a). This observed diameter value is significantly higher than the theoretical value of SCPNs (5−20 nm),[18−20] which is possibly attributed to the water swelling effect and extension of flexible PEG chains in water (Scheme 1). In contrast, the d_H values of POEGMA and POEGMA-PgMA showed little variation and ranged from 5.3 to 5.8 nm. These values correspond to the random coil conformation of the polymers,[32] as long polymer chains in aqueous solution with high mobility in anisotropic diffusion would cause the translational motion to couple, leading to an apparent d_H in the range of several nanometers. In other words, PEGMA and PEGMA-PgMA cannot form particles but can form an "extended chain" conformation in water.[32] In order to investigate the formation and stability of the generated SCPNs, concentration-dependent DLS experiments were performed using various concentrations of each sample in aqueous solution, as shown in Figures 2a and 2b. The correlation functions of POEGMA-U-DPy clearly indicate the presence of single relaxation processes at concentrations between 0.004 to 40 mg/mL, which suggests the presence of only a single species in aqueous solution (Figure 2b). Interestingly, reducing the
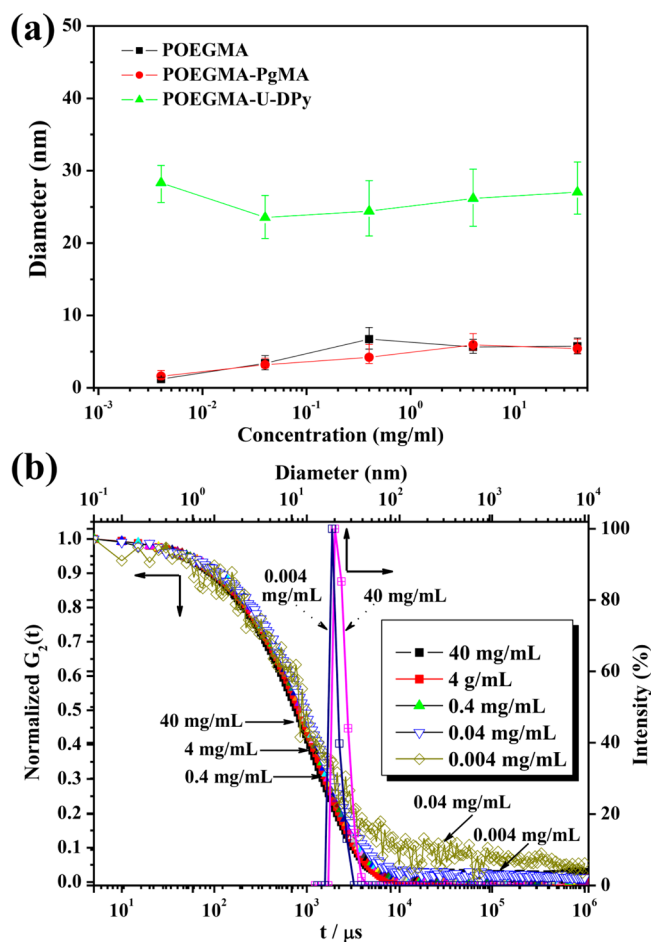

Figure 2. DLS analysis of various concentrations of POEGMA-U-DPy in water at 20 °C: (a) concentration dependence of particle size and (b) correlation functions and size distributions at an angle of 90°.

concentration of POEGMA-U-DPy did not affect the d_H value over a wide range of concentrations, whereas the average d_H values of POEGMA and POEGMA-PgMA gradually decreased from 5.8 to 1.2 nm as the concentrations reduced. These observations indicate that the hydrogen bond strength of the U-DPy:U-DPy complexes is sufficient to undergo stable intramolecular folding even at very high or dilute concentrations, which enables the formation of highly stable nanoparticles with precise low-dimensional nanostructures in an aqueous environment.

To further evaluate the apparent concentration independence of the generated SCPNs, scanning electron microscope (SEM) morphological studies were undertaken to determine the particle size distribution of POEGMA-U-DPy at different concentrations. At 0.004 mg/mL (Figure 3a), SEM confirmed self-assembly into nanoparticles with a diameter of 25−30 nm. This implies that single polymer chains can be folded to form nanoparticles via intramolecular chain-folding and U-DPy hydrogen bonding interactions (Scheme 1). More surprisingly, when the concentration increased from 0.004 to 40 mg/mL, a gradual increase in the number of particles with the same particle size was observed (Figures 3a−3d). Therefore, the formation of SCPNs is favored in concentrated samples, in a similar manner to previous reports of fibrous or higher hierarchical structures.[5,33,34] Furthermore, TEM and AFM experiments were performed to validate the SEM experiments
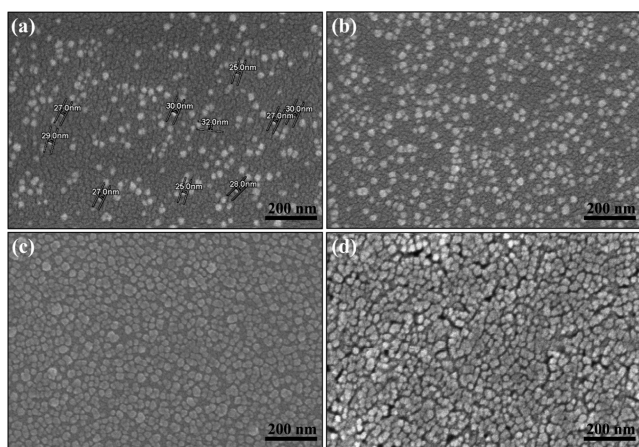
Figure 3. SEM images of different concentrations of POEGMA-U-DPy on the surface of the wafer: (a) 0.004, (b) 0.04, (c) 4, and (d) 40 mg/mL. All aqueous samples were prepared using distilled water. SEM samples were prepared by spin coating the sample solutions onto silicon substrates and then evaporating the solvent under ambient conditions. The bright regions visible in the image correspond to areas of the sample.

and confirmed that the formed nanoparticles have a small diameter of ca. 25 nm (Figure 4a–c). These results also
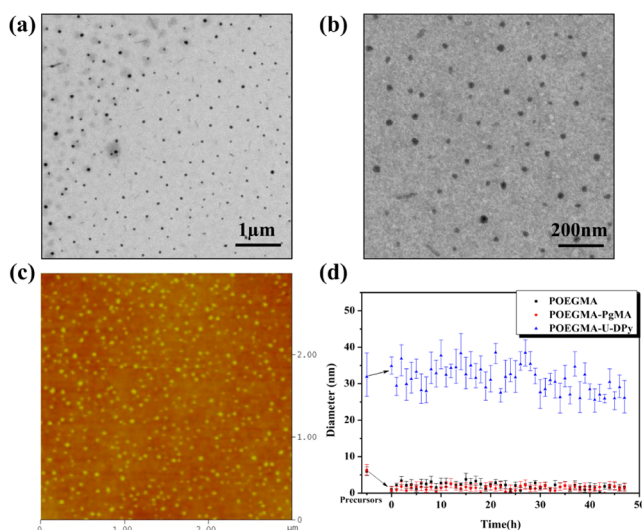

Figure 4. (a,b) TEM and (c) AFM images showing the characteristic morphologies of SCPNs in the dry state. (d) Particle size distribution measured at 90° for POEGMA-U-DPy nanoparticles over time after the addition of SDS. TEM samples were prepared by dripping several drops of the sample solution onto carbon-coated copper grids, and then the samples were stained with ruthenium tetroxide (RuO_4). The darker region of the image is the POEGMA-U-DPy segment, while the brighter region is the substrate. AFM images of all samples were spin-coated onto a silicon substrate and then dried under ambient conditions. The yellow region represents the morphological structure of the sample.

demonstrate the feasibility and utility of the effect of U-DPy modules on the morphological changes in SCPNs. In addition, the formation of SCPNs was independent of the concentration of PEGMA-U-DPy, indicating that intermolecular interactions do not occur between the pendent U-DPy units.

In order to more accurately assess the stability of these materials, kinetic experiments were performed using DLS in the presence of sodium dodecyl sulfate (SDS), which acts as a destabilizing agent (Figure 4d).[35] All measurements agreed well with the concentration-dependent DLS results in the absence of SDS: after 48 h of monitoring at 20 °C, the d_H value of POEGMA-U-DPy particles did not appreciably change in the presence of SDS, indicating that the formation of intra-molecular hydrogen bonds between the U-DPy groups was not appreciably affected by prolonged SDS treatment. Conversely, SDS-treated POEGMA and POEGMA-PgMA samples under-went drastic reductions in d_H (to below 1 nm) within 10 min of the addition of SDS. These findings demonstrate that POEGMA-U-DPy possesses long-term stability under such harsh aqueous conditions. While the strong intramolecular hydrogen bonds within the SCPNs preserve spherical integrity, foreign materials (and impurities) hardly disrupted the physical hydrogen-bonding network within the inner core of the particles. Therefore, this newly discovered material has potential as an advanced derivate for the development of superior nanocarriers for drug delivery.

In summary, we have successfully demonstrated a simple and efficient synthetic route to obtain well-defined SCPNs based on the presence of self-complementary sextuple hydrogen bonds and functionalized POEGMA. In aqueous solution, POEGMA-U-DPy can spontaneously self-assemble into well-defined, precise nanoparticles smaller than 30 nm. Concentration-dependent experiments showed that the hydrogen bond strength of the U-DPy groups is sufficient to promote intramolecular folding, even at very high or low concentrations, which enables the formation of highly functionalized micelles in an aqueous environment. Moreover, the formed SCPNs had a very high stability in aqueous solutions containing SDS, maintaining a practically unchanged hydrodynamic diameter over a period of 48 h, suggesting the potential of POEGMA-U-DPy for application as a suitable candidate carrier for drug delivery and cancer therapy. Our efforts to incorporate anticancer drugs into PEGMA-U-DPy elucidate the factors affecting the performance of controlled drug release *in vitro* and develop SCPNs for cancer treatment are continuing and will be reported in due course.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmacro-lett.5b00556.

Experimental details and characterization data (PDF)

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Author Contributions

C.-C. conceived the project, designed the research, and wrote the paper. C.-C. and H.-C. performed all experiments. All authors discussed the results and commented on the manuscript.

Notes

The authors declare no competing financial interest.

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