Biotin-Terminated Ruthenium Bipyridine Ring-Opening Metathesis Polymerization Copolymers: Synthesis and Self-Assembly with Streptavidin

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ABSTRACT: We present the synthesis of ring-opening metathesis polymerization block polymers containing a ruthenium bipyridine luminescent block and a hydrophobic block and end-terminated with a biologically active biotin molecule. These copolymers undergo self-assembly in water into star micelles containing a large number of luminescent Ru(II) centers, with the hydrophobic block in their core and the molecular recognition unit biotin at their periphery. Addition of the protein streptavidin results in the cross-linking of these polymer nanospheres, through biotin−steptavidin binding, into extended networks. In addition to its usefulness as a method to organize polymeric nanostructures into biochemically addressable networks, this study has generated luminescent micellar aggregates capable of binding to biological compounds, and thus useful for the luminescence detection and signal amplification of biomolecules.

Introduction

Ruthenium bipyridine-containing polymers have recently been the subject of increasing interest, due to their numerous potential applications, such as photoconductive materials, photocatalysts, solar energy conversion materials, sensors, and supramolecular building blocks.[1−10] One interesting and underexplored application for this class of polymers is in the luminescence detection and labeling of biological molecules.[11] Ruthenium bipyridine complexes present a number of distinct advantages as chromophores for biological assays, including long excited-state lifetimes, chemical inertness and photostability, tunability of their photophysical characteristics, and large Stokes shifts.[12] The incorporation of many of these chromophores into a polymeric backbone can provide a facile method to amplify a luminescence signal triggered by the recognition of a biological molecule.[13] To achieve an even greater degree of luminescence amplification, we have been interested in the construction of diblock copolymers containing ruthenium(II) bipyridine chromophores. Self-assembly of these copolymers is expected to yield luminescent nanoscale micellar aggregates, containing a large number of Ru(II) chromophores. When labeled with molecular recognition units, these micelles can act as a strong luminescence marker for specific biological molecules.

We have recently reported the synthesis of ruthenium(II) bipyridine-containing homopolymers and diblock copolymers using ring-opening metathesis polymerization (ROMP).[14] To access block copolymers suitable for biomolecule detection, we needed to devise a method to end-functionalize these ROMP polymers with a molecular recognition unit. While the end-termination of ROMP polymers generated using the Schrock molybdenum-based catalyst is relatively straightforward,[15] fewer reports have described this process for the more functional group tolerant Ru-based catalysts. The group of Grubbs has reported the creation of telechelic ROMP polymers with functional groups at both ends, by carrying out the ROMP reaction in the presence of disubstituted olefins.[16] Kiessling et al. have also generated end-functionalized neoglycopolymers by quenching the active ROMP polymer chain with functionalized enol ethers.[17] We here report a facile method to create ROMP diblock copolymers containing ruthenium(II) bipyridine units and end-functionalized with a biotin molecule. Self-assembly of these copolymers yields luminescent micellar aggregates, with biotin at their periphery. Transmission electron microscopy (TEM) studies reveal their ready association and cross-linking with the tetravalent protein streptavidin.

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Results and Discussion

Synthesis of Chain-Transfer Agent 4. To generate biologically compatible ROMP polymers, we chose to attach the molecule biotin, which has found widespread applications in bioassays,[18] to the end of our polymers. Biotin is known to bind to the protein avidin or streptavidin with very high affinities ($K_d \approx 10^{-15}$ M). In addition, (strept)avidin can bind up to four biotin units, thus allowing it to act as a linker between two (or more) biotinylated molecules.[18] We have recently shown that biotinylated ruthenium(II) bipyridine units can be used as effective luminescence markers for the protein avidin.[19] To create ROMP polymers that are end-functionalized with biotin, we used enol ether **4** (Scheme 1) to quench the active ROMP polymer chain. This molecule was accessed by a facile and relatively high yield four-step synthesis, starting from the commercially available 6-bromohexanol (Scheme 1). PCC oxidation of this compound yielded aldehyde **1**,[20] which was subjected to a Wittig reaction with $PPh_3{=}CHOCH_3$ to give enol ether **2**. Heating **2** with a large excess of piperazine and potassium carbonate in acetonitrile gave amino compound **3**. Biotinylated molecule **4** was then obtained by coupling **3** with the *N*-hydroxysuccimide ester[21] of biotin. All compounds were characterized by ^{1}H NMR,

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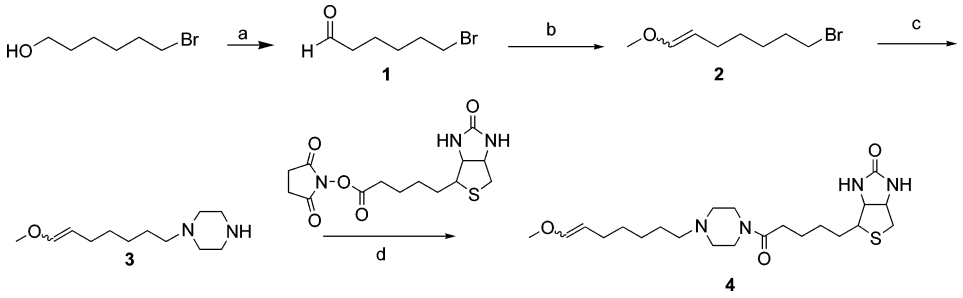
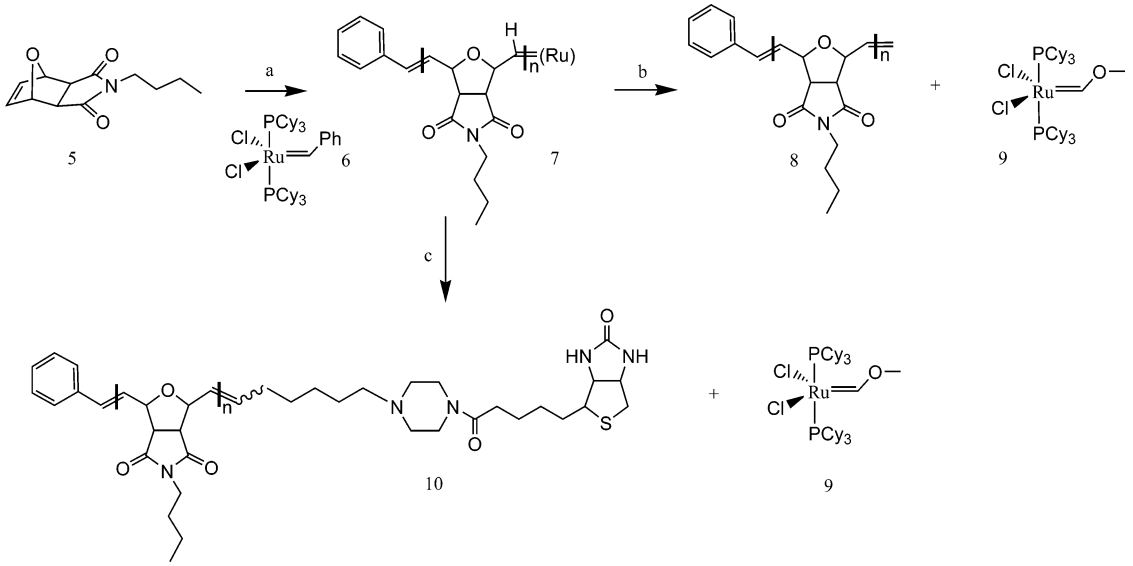
Scheme 1. Synthesis of Biotinylated Chain-Transfer Agent 4[a]



[a] Reagents and conditions: (a) PCC, CH_2Cl_2, room temperature, 1 h, 80%; (b) $PPh_3CH_2OCH_3Cl$, KO-t-Bu, THF, room temperature, 56% (two steps); (c) piperazine, K_2CO_3, CH_3CN, reflux, 12 h, 95%; (d) Et_3N, $CHCl_3$/2-propanol, room temperature, 1 h, 83%.

Scheme 2. Synthesis of Homopolymers 8 and 10[a]



[a] Reagents and conditions: (a) d_6-acetone, room temperature, 20 min; (b) ethyl vinyl ether, 20 min; (c) compound **4**, d_6-acetone, 3 h.

^{13}C NMR, and HR-ESI-MS. The ^{1}H NMR spectrum of compound **4** showed a double bond cis:trans ratio of 1:3.

End-Termination of ROMP Polymers with Biotin. To examine the ROMP quenching efficiency with chain-transfer agent **4**, we first carried out the polymerization reaction of *N*-butyloxanorbornenimide (**5**) with the Grubbs catalyst $(PCy_3)_2Cl_2Ru{=}CHPh$ (**6**) (monomer:initiator = 20:1). After monomer conversion (^{1}H NMR), a solution of chain-transfer agent **4** in acetone (30 equiv) was added to half of the reaction mixture at room temperature. ^{1}H NMR showed the complete disappearance of the alkylidene signal corresponding to the propagating polymer chain at 18.8 ppm after 3 h, and the appearance of a new signal at 14.5 ppm, which we have assigned as the carbene proton of the Fisher-type complex **9** (Scheme 2; see the ^{1}H NMR spectra in the Supporting Information), on the basis of literature precedent.[22] Biotinylated polymer **10** was then isolated and purified from the excess chain-transfer agent **4** by repeated precipitation from ether. The second half of this reaction was quenched with excess ethyl vinyl ether, and polymer **8** was purified by repeated precipitation from hexane (Scheme 2). Comparison of the ^{1}H NMR spectra of polymers **8** and **10** revealed that the end-termination of **10** with biotin was successful. The ^{1}H NMR spectra show the feature signals of the biotin moiety at 2.7 ppm (d) and 2.9 ppm (dd) (SCH_2) (Figure 1). Integration of the end phenyl peaks of **10** at 7.2−7.5 ppm versus these characteristic biotin peaks showed a ratio of 5:1:1. This corresponds to termination efficiency above 95% for this ROMP reaction.

We have previously reported the synthesis of diblock copolymer **14** (Scheme 3), containing a hydrophobic block, as well as a block of ruthenium(II) bipyridine units.[14] With an efficient method to end-functionalize ROMP polymers in hand, we proceeded to attach a terminal biotin moiety to the Ru(II)-containing diblock copolymers. Thus, monomer **5** was reacted with catalyst **6** (predissolved in a minimum amount of CD_2Cl_2) in d_6-acetone at room temperature. After complete consumption of **5** (monitored by ^{1}H NMR), an average degree of polymerization of ∼10 for poly(**5**) was deduced (^{1}H NMR integration of the methyl signal of **5** (0.95 ppm) vs the terminal phenyl group (7.2−7.5 ppm)). Ruthenium(II) bipyridine monomer **11**[14] was then added to the reaction mixture. ^{1}H NMR showed complete conversion of the monomer after 2 h at room temperature, and the ratio of poly(**5**) to poly(**11**) in block copolymer **12** was calculated as ∼1:1 (^{1}H NMR integration of the methyl signal at 0.95 ppm in poly(**5**) vs the 6- and 6′-bpy signal at 8.06 ppm in poly(**11**)). The reaction mixture was divided into two portions, biotin quenching agent **4** (30 equiv) was added to one of the portions, and the mixture was stirred for 3 h at room temperature. Again, complete disappearance of the alkylidene proton of the active ROMP chain at 18.8 ppm and appearance of a new carbene signal at 14.5 ppm, corresponding to the Fischer-type complex **9**, were observed by ^{1}H NMR. The

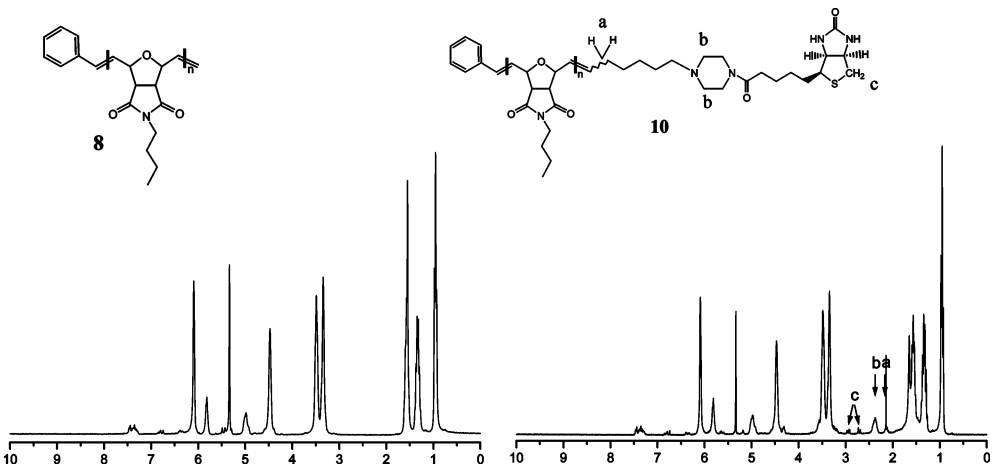
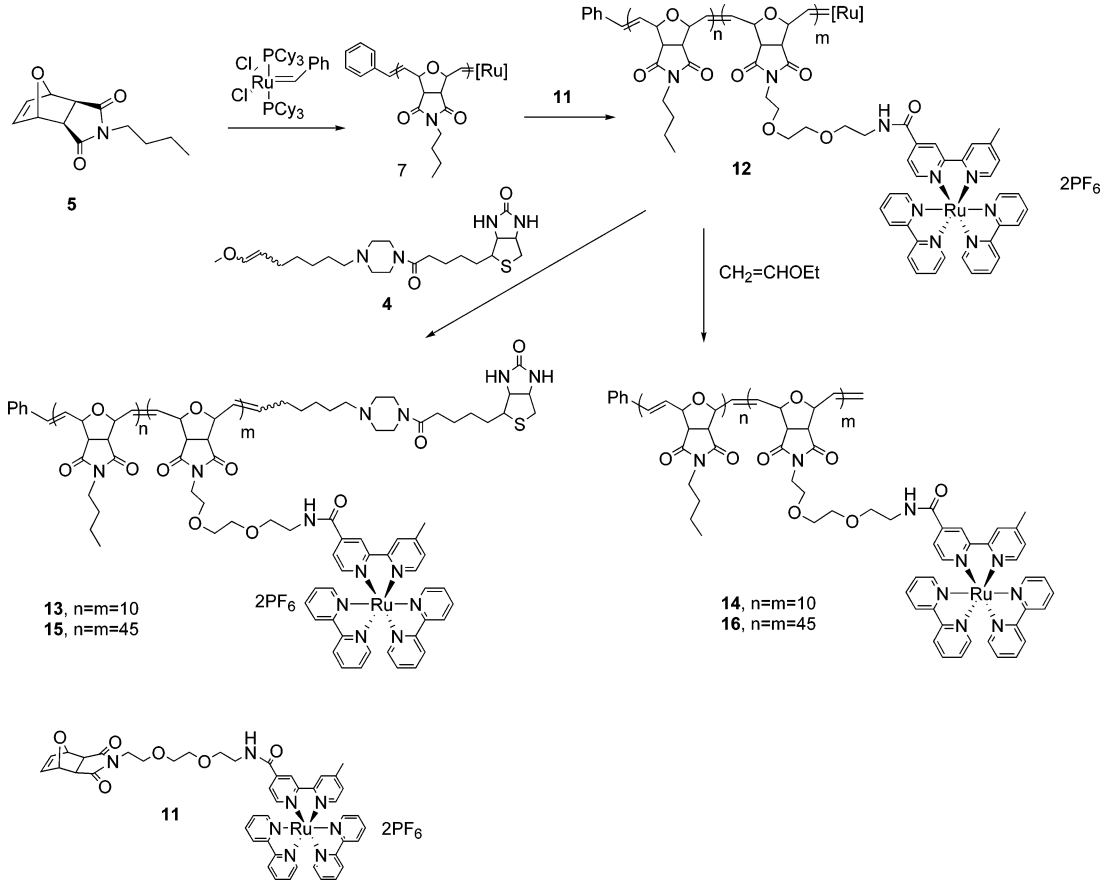


Figure 1. ^{1}H NMR spectra of homopolymers **8** and **10** in CD_2Cl_2.

Scheme 3. Synthesis of Copolymers 14 and 16 and End-Biotinylated Copolymers 13 and 15[a]

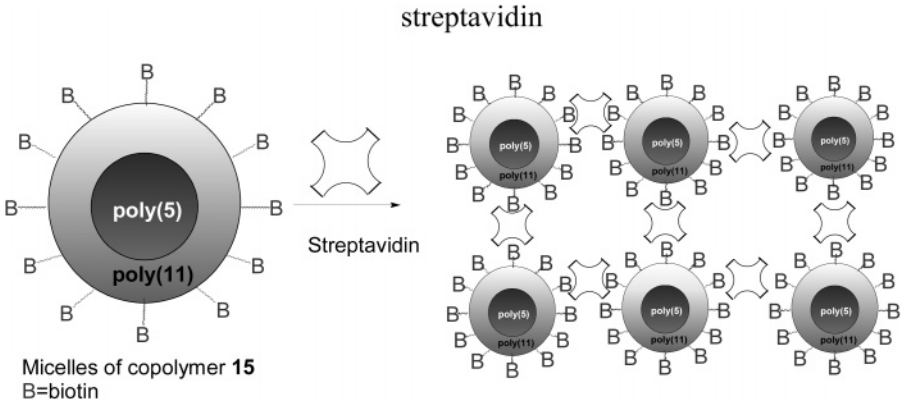


[a] Reagents and conditions: (a) d_6-acetone/CD_2Cl_2, 20 min, room temperature; (b) monomer **11**, d_6-acetone, 2 h, room temperature; (c) compound **4** (30 equiv), d_6-acetone, room temperature, 3 h; (d) ethyl vinyl ether, room temperature, 20 min.

resulting copolymer **13** was purified by repeated precipitations from ether. The second portion was quenched with ethyl vinyl ether, and a similar alkylidene proton shift was observed. Copolymer **14** was purified by repeated precipitations from ether. Comparison of the ^{1}H NMR spectra of copolymers **13** and **14** again showed successful end-functionalization with biotin. The ^{1}H NMR integration ratio for the biotin peaks (2.9 and 2.7 ppm, biotin SCH_2) to the poly(**5**) block (0.95 ppm, $-$CH_3) was found to be ~2:30, again indicating a near-quantitative end-functionalization efficiency for the chain-transfer agent **4**. Thus, the above one-pot synthesis resulted in the efficient formation of a ROMP diblock copolymer, containing luminescent and redox-active ruthenium(II) bipyridine units, and end-terminated with the protein binding molecule biotin. We also synthesized longer diblock copolymers **15** and **16**, with a ratio of poly(**5**) to poly(**11**) of 45:45, and we carried out the end-functionalization of copolymer **15** with biotin as above (Scheme 3).

Micelle Formation. The self-assembly of diblock copolymer **15** was examined in an acetonitrile/water solvent mixture. In this copolymer, the hydrophobic block poly(**5**) is soluble in acetonitrile, and completely insoluble in water, while the Ru(II)-containing block poly(**11**) is soluble in acetonitrile and somewhat soluble

Scheme 4. Schematic Representation of Aggregation of Biotinylated Micelles by Streptavidin



in water. Thus, the self-assembly of copolymer **15** in acetonitrile/water is expected to be driven by the incompatibility of poly(**5**) with water, and would yield micellar aggregates with poly(**5**) in the core, and poly(**11**) as the corona. Because it is attached to the end of the poly(**11**) block, the biotin functionality in these polymers would then reside at the periphery of these micelles (Scheme 4). Copolymer **15** was first dissolved in acetonitrile, and water was added dropwise under vigorous stirring, until the solution became turbid, indicating the onset of aggregation. To ensure the stability of the obtained morphology, a large excess of water was then added, and the solution was dialyzed several times against water to remove acetonitrile. With excess water, the decreased solubility of the poly(**5**) block is expected to result in decreased chain mobility, and structural rearrangement of the morphology is expected to be slow (the micelles are "frozen").

The morphology of the copolymer micelles was examined by TEM. A drop of dilute micellar solution was deposited on a carbon-coated TEM grid, and water was evaporated overnight at room temperature. Parts A and C of Figure 2 show representative transmission electron micrographs of the micellar aggregates of copolymer **15** in pure water. Due to the presence of a large number of ruthenium centers in their corona, the copolymer affords sufficient electronic contrast for direct observation of the micelles without any further staining. Small spherical star micelles, with an average diameter of 41 nm (standard deviation 11 nm) constitute the majority of these aggregates. Similar TEM results were obtained from the self-assembly of copolymer **16**, which contains the same composition of poly(**5**) and poly(**11**), but does not possess a biotin end group (Figure 2E).

Emission spectra for copolymer **15** in water (where micelle formation is expected) and acetonitrile (where **15** is not aggregated) were obtained upon excitation at 455 nm (298 K), and the emission spectra are shown in Figure 3. All samples were purged with argon for 30 min prior to use. The emission peak of copolymer **15** in acetonitrile is centered at 627 nm, and shifts to 641 nm in pure water, likely due to the stabilization of the metal-to-ligand charge transfer (MLCT) state in polar solvents. The luminescence intensity is partially retained upon micelle formation in water, compared to acetonitrile. The observed reduction in intensity is consistent with the decrease in quantum yield of $Ru(bpy)_3^{2+}$ with increasing solvent polarity.[24]

Self-Assembly of Copolymer 15 Micelles with Streptavidin. With the preparation and characterization of biotinylated Ru(II) micelles, we examined their association with streptavidin. This protein possesses four biotin binding sites; thus, it is expected to cross-link, and initiate the aggregation of these biotin-labeled micelles (Scheme 4).

A solution of the micellar aggregates of copolymer **15** (1.5 mL, 0.25 mg/mL) was incubated with 260 μL of streptavidin (1.0 μM in phosphate buffer) for 3 h. The ratio of biotin to streptavidin was estimated to be ~20:1. Transmission electron microscopy studies on the micellar solution of copolymer **15** with streptavidin (Figure 2B,D) were then carried out. These show the predominance of networks of interconnected particles, with a size range from 100 to 1000 nm, consistent with cross-linking of the star micelles of copolymer **15**.[27] This is likely the result of streptavidin-induced aggregation of the individual micelles into larger particles. In a control experiment, streptavidin was added to micellar solutions of the nonbiotinylated copolymer **16**. In contrast to copolymer **15**, TEM (Figure 2F) showed no aggregation of the individual micelles upon addition of streptavidin. Thus, the observed aggregation of the micelles from **15** is due to binding of streptavidin to the biotin at the periphery of these micelles, and cross-linking of these micelles by the protein (Scheme 4). This demonstrates the accessibility of these biotin units, and suggests the usefulness of these micelles as luminescence markers for biotinylated biomolecules. In addition, our streptavidin-induced cross-linking approach represents a new method to mediate the controlled association of polymeric micellar aggregates into higher order networks. There has been increasing interest in the biomolecule-mediated association of metal or semiconductor nanoparticles as a method to provide biochemically tunable materials.[25,26] To our knowledge, this is the first demonstration of the biotin−streptavidin-mediated association of *polymeric nanostructures*. Considering the versatility of ROMP, this approach should yield a large variety of networks of polymeric nanoparticles, which can be switched through biochemical interactions.

Conclusion

We have shown the ready synthesis of ruthenium(II) bipyridine-containing block copolymers, which are end-functionalized with the molecular recognition unit biotin. Self-assembly of these copolymers in acetonitrile/water yields luminescent star micelles with a hydrophobic core, a Ru(II)-containing corona, and biotin units at their periphery. Addition of streptavidin to these micelles induces their cross-linking into larger networks, through biotin−streptavidin binding. In addition to its useful-

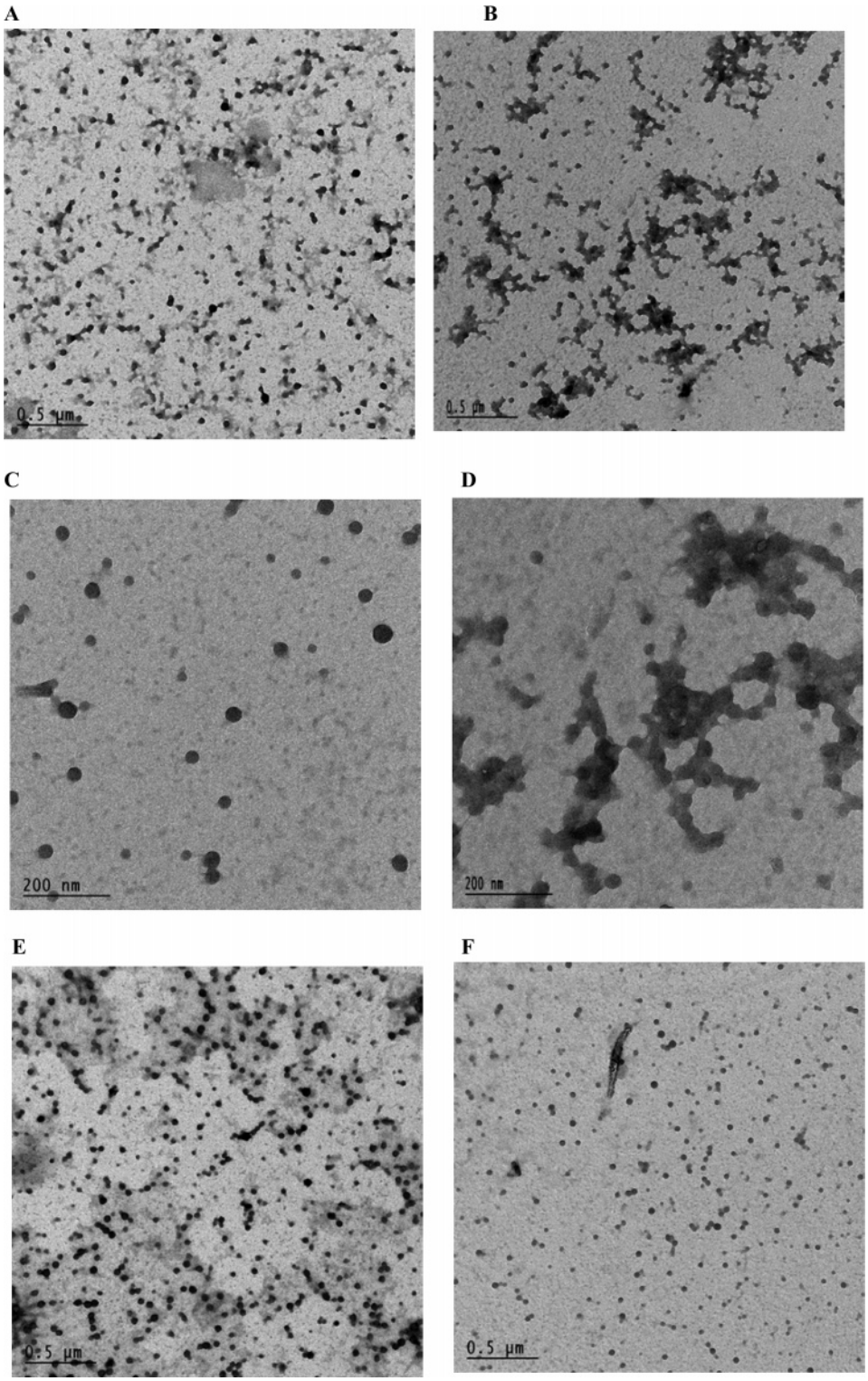


Figure 2. (Left column images (A, C)) TEM of micelle solutions of copolymer **15** in water before addition of streptavidin with different magnifications. (Right column images (B, D)) TEM of micellar solutions of **15** in water after addition of streptavidin. (E) TEM of micelle solutions of copolymer **16** in aqueous solution before addition of streptavidin. (F) TEM of micellar copolymer **16** in aqueous solution after addition of streptavidin (3 h).

ness as a method to organize polymeric nanostructures into networks, the end-conjugation of these ROMP polymers with biotin has created micellar aggregates containing a large number of luminescent Ru(II) centers, and surface-accessible biotin units. We are currently exploring the potential of these micelles for the luminescence detection and signal amplification of biomolecules.

Experimental Procedures

General Considerations. All chemicals were purchased from Aldrich (streptavidin was purchased from Sigma) and used without further purification. Monomers **5**[28] and **11**[14] were synthesized according to published procedures. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian M300 spectrometer operated at 300.140 MHz. Chemical shifts are reported in parts per million relative to the deuterated solvent reso-

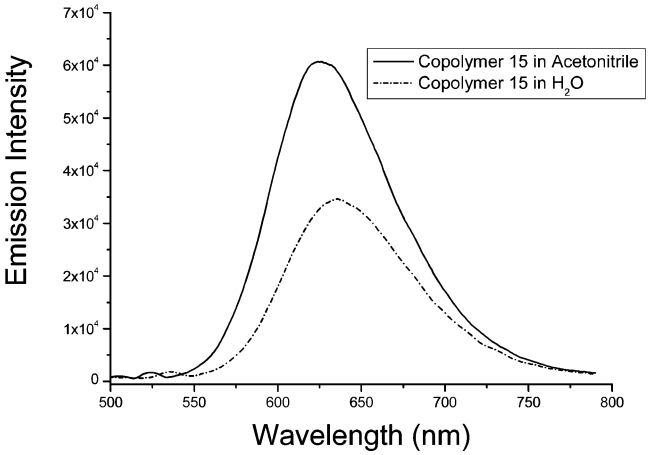


Figure 3. Emission spectra of copolymer **15** in acetonitrile and water.

nance. Fluorescence experiments were carried out on a PTI (Photon Technology International) TimeMaster model C-720F spectrofluorimeter. Compound **1** was synthesized according to the literature method.[20]

Micelle Formation and Transmission Electron Microscopy. Doubly distilled water was added dropwise into the block copolymer **15** or **16** solution in CH_3CN (the initial concentration is 5 mg/mL) with stirring to reach a final volume of 5 mL. The micelle solution was dialyzed against pure water several times over 36 h to remove CH_3CN, and the final concentration was adjusted to 0.25 mg/mL. Samples were prepared by placing a drop of this solution onto TEM copper grids (400 mesh, carbon-coated, purchased from Electron Microscopy Sciences), and the excess of solution was blotted with a filter paper to form a thin aqueous film on the grids. The grids were air-dried for 12 h. The aggregates were then examined using a JEOL 2000FX electron microscope operated at 80 kV.

Synthesis of 2 and 3. Potassium *tert*-butoxide (1 M in THF, 3.35 mL, 3.35 mmol) was added to a suspension of (methoxymethyl)triphenylphosphonium chloride (1.15 g, 3.35 mmol) in dry THF at 0 °C. The dark red solution was stirred at 0 °C under N_2 for 5 min. The solution was then transferred to a flask containing 0.6 g (3.35 mmol) of 6-bromo-1-hexanal (**1**) in 10 mL of THF. The orange color disappeared rapidly, and a gray precipitate formed. The reaction was quenched with saturated aqueous NaCl (20 mL) and extracted with CH_2Cl_2 (3×15 mL). The combined organic solution was washed with water and brine, and dried over Na_2SO_4. After removal of the solvent, the crude product was obtained (56%) and used for the next step without further purification. A 0.86 g (10 mmol) sample of piperazine and 0.4 g of compound **2** were then dissolved in 10 mL of dry CH_3CN, and K_2CO_3 (0.4 g) was added. After being refluxed overnight and cooled to room temperature, the mixture was filtered, and the solvent and excess piperazine were removed in vacuo. The pure product **3** was obtained by chromatography on alumina with CH_2Cl_2/methanol (100:5). ^{1}H NMR ($CDCl_3$): δ (ppm) 1.32 (4H, m), 1.49 (2H, m), 1.93 (2H, q, J = 6.9 Hz), 2.30 (2H, t, J = 7.8 Hz), 2.42 (br s, 4H), 2.91 (4H, t, J = 5.0 Hz), 3.52 (2H, s, *trans*-OCH_3), 3.57 (1H, s, *cis*-OCH_3), 4.32 (0.33H, dt, J = 7.1, 7.0 Hz, *cis*-OCH=C*H*), 4.71 (0.66H, dt, J = 12.3, 7.5 Hz, *trans*-OCH=C*H*), 5.85 (0.33H, dt, J = 6.2, 1.5 Hz, *cis*-OC*H*=CH), 6.26 (0.66, d, J = 11.1 Hz, *trans*-OC*H*=CH). ^{13}C NMR ($CDCl_3$): δ (ppm) 24.14, 26.83, 27.31, 27.58, 27.96, 30.08, 31.05, 46.26, 54.70, 56.21, 59.66, 103.23, 107.08, 146.21, 147.16. HR-FAB-MS: *m/z* calcd for $C_{12}H_{24}N_2O + H^+$ 213.1967, found 213.1967.

Synthesis of Compound 4. Compound **3** (200 mg, 0.94 mmol) and biotinyl-*N*-hydroxylsuccinimide (350 mg, 1.0 mmol) were dissolved in $CHCl_3$/2-propanol (2:1) (20 mL), and triethylamine (200 μL) was added. Slight warming and ultrasonication dissolved this mixture into a clear solution. The mixture was stirred for 1 h at room temperature to complete the reaction. The solvents were removed under reduced pressure. Pure product (83%) was obtained by chromatography on alumina eluted with CH_2Cl_2/methanol (98:2). ^{1}H NMR ($CDCl_3$): δ (ppm) 1.33 (4H, m), 1.49 (4H, m), 1.68 (4H, m), 1.93 (2H, m), 2.09 (1.2H, *trans*-OCH=CHC*H*$_2$), 2.38 (8H, $-CH_2N(CH_2-)_2$), 2.73 (1H, br d, J = 10.8 Hz, SC*H*$_2$), 2.91 (1H, dd, J = 12.9, 4.8 Hz, SC*H*$_2$), 3.16 (1H, m, C*H*S), 3.20−3.60 (7H, m, *cis*- and *trans*-OC*H*$_3$, $-CON(CH_2)_2$), 4.31 (1.33H, m, NC*H* and *cis*-OCH=C*H*), 4.5 (1H, m, NC*H*), 4.71 (0.66H, dt, J = 12.3, 7.5 Hz, *trans*-OCH=C*H*), 5.2 (1H, N*H*), 5.8 (1H, N*H*), 5.85 (0.33H, dt, J = 6.2, 1.5 Hz, *cis*-OC*H*=CH), 6.26 (0.66, d, J = 11.1 Hz, *trans*-OC*H*=CH). ^{13}C NMR ($CDCl_3$): δ (ppm) 24.12, 25.44, 27.02, 27.20, 27.47, 27.96, 28.64, 28.69, 30.04, 31.01, 33.00, 40.91, 41.89, 45.97, 53.23, 53.84, 55.65, 56.23, 58.89, 60.44, 62.11, 103.17, 106.99, 146.27, 147.21, 163.53, 171.42. HR-FAB-MS: *m/z* calcd for $C_{22}H_{38}N_4O_3S + H^+$ 439.2743, found 439.2743.

Synthesis of Homopolymers 8 and 10. Monomer **5** (22.1 mg, 0.1 mmol) and catalyst **6** (4.1 mg, 0.005 mmol) were dissolved in CD_2Cl_2, and transferred to an NMR tube under argon. The polymerization was monitored by ^{1}H NMR, and monomer consumption was observed after 20 min. The reaction mixture then was divided into two portions. One of these was mixed with ethyl vinyl ether (large excess) for 20 min and precipitated (3×) from hexanes to obtain **8**. Chain-transfer agent **4** (30 equiv) was added to the other portion of the reaction. After 3 h, the end-biotinylated homopolymer **10** was obtained by precipitation (6×) from ether.

Homopolymer 8. ^{1}H NMR (CD_2Cl_2): δ (ppm) 0.95 (60H, CH_3), 1.32 (40H, CH_2), 1.57 (40H, CH_2), 3.34−3.48 (80H, NCH_2 and OCCHCHCO), 4.47 (30H, *trans*-CHO), 4.95 (10H, *cis*-CHO), 5.82 (10H, *cis*-CH=CH), 6.09 (30H, *trans*-CH=CH), 7.2−7.5 (5H, phenyl H); ^{13}C NMR ($CDCl_3$): δ (ppm) 13.77, 20.38, 30.01, 38.92, 77.80, 81.25, 131.21, 132.23, 175.71.

Homopolymer 10. ^{1}H NMR (CD_2Cl_2): δ (ppm) 0.95 (60H, CH_3), 1.32 (40H, CH_2), 1.57 (40H, CH_2), 2.08 (1H, OCH=C*H*CH_2), 2.73 (1H, br d, J = 12.9 Hz, SC*H*$_2$), 2.91 (1H, dd, J = 12.9, 4.8 Hz, SC*H*$_2$), 3.34−3.48 (84H), 4.47 (30H, *trans*-CHO), 4.95 (10H, *cis*-CHO), 5.82 (10H, *cis*-CH=CH), 6.09 (30H, *trans*-CH=CH), 7.2−7.5 (5H, phenyl H). ^{13}C NMR (CD_3Cl): δ (ppm) 13.77, 20.38, 30.01, 38.94, 77.80, 81.30, 131.22, 132.23, 175.73.

Synthesis of Copolymers 13−16. Monomer **5** (2.0 mg, 0.009 mmol) was dissolved in 0.5 mL of d_6-acetone. The solution of monomer **5** was added to a catalyst **7** (0.0003 mmol) solution in 0.2 mL of CD_2Cl_2. The mixture was allowed to stir at room temperature. After the complete consumption of monomer **5** (monitored by ^{1}H NMR), monomer **11** (10.7 mg, 0.009 mmol)[29] in 1 mL of d_6-acetone was added to the remaining solution. After complete consumption of monomer **11** (monitored by ^{1}H NMR), the reaction mixture was divided into two portions. One of the portions was added to a d_6-acetone solution of excess biotin quencher **4** (30 equiv), and stirred for 3 h. The copolymer **13** was obtained by precipitation from ether (6×). The other half was quenched by addition of ethyl vinyl ether and precipitation from ether to obtain copolymer **14**. Similar procedures were used to obtain **15** and **16**.

Copolymer 13. ^{1}H NMR (CD_3CN): δ (ppm) 0.94 (br, 3H), 1.31 (br, 2H), 1.54 (br, 2H), 2.53 (br, 3H), 2.8−3.0 (0.2H, biotin SC*H*$_2$), 3.1−3.2 (0.4H), 3.2−3.6 (br, 18H), 4.2−4.88 (br, 4H), 5.5−6.1 (br, 4H), 7.28 (br, 1H), 7.40 (br, 4H), 7.60 (br, 1H), 7.66 (br, 1H), 7.75 (br, 4H), 7.86 (br,1H), 8.06 (br, 4H), 8.50 (br, 4H), 8.60 (br, 1H), 8.90 (br, 1H).

Copolymer 14. ^{1}H NMR (CD_3CN): δ (ppm) 0.94 (br, 3H), 1.31 (br, 2H), 1.54 (br, 2H), 2.53 (br, 3H), 3.2−3.6 (br, 18H), 4.2−4.88 (br, 4H), 5.5−6.1 (br, 4H), 7.28 (br, 1H), 7.40 (br, 4H), 7.60 (br, 1H), 7.66 (br, 1H), 7.75 (br, 4H), 7.86 (br,1H), 8.06 (br, 4H), 8.50 (br, 4H), 8.60 (br, 1H), 8.90 (br, 1H).

Copolymer 15. ^{1}H NMR (CD_3CN): δ (ppm) 0.92 (br, 3H), 1.33 (br, 2H), 1.54 (br, 2H), 2.53 (br, 3H), 3.2−3.6 (br, 18H), 4.2−4.5 (br, 2.6H), 4.88 (br, 1.4H), 5.5−6.1 (br, 4H), 7.28 (br, 1H), 7.40 (br, 4H), 7.60 (br, 1H), 7.66 (br, 1H), 7.75 (br, 4H), 7.86 (br,1H), 8.06 (br, 4H), 8.50 (br, 5H), 8.90 (br, 1H).

Copolymer 16. ^{1}H NMR (CD_3CN): δ (ppm) 0.92 (br, 3H), 1.33 (br, 2H), 1.54 (br, 2H), 2.53 (br, 3H), 3.2−3.6 (br, 18H), 4.2−4.5 (br, 2.6H), 4.88 (br, 1.4H), 5.5−6.1 (br, 4H), 7.28 (br, 1H), 7.40 (br, 4H), 7.60 (br, 1H), 7.66 (br, 1H), 7.75 (br, 4H), 7.86 (br,1H), 8.06 (br, 4H), 8.50 (br, 5H), 8.90 (br, 1H).

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, HR-FAB-MS of compound **3** and monomer **4**, ^{1}H NMR and ^{13}C NMR of polymers **8** and **10**, ^{1}H NMR monitoring of the polymerization of **10**, ^{1}H NMR of copolymers **13**−**16**, ^{1}H NMR monitoring of the polymerization of **13**, and additional TEM pictures of copolymer **15** micelle solutions before and after streptavidin addition (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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