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Multidentate Polysarcosine-Based Ligands for Water-Soluble Quantum Dots

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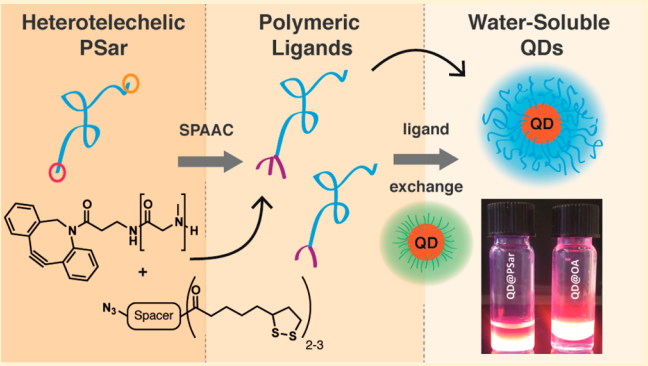
Supporting Information

ABSTRACT: We describe the synthesis of heterotelechelic polysarcosine polymers and their use as multidentate ligands in the preparation of stable water-soluble quantum dots (QDs). Orthogonally functionalized polysarcosine with amine and dibenzocyclooctyl (DBCO) end groups is obtained by ring-opening polymerization of *N*-methylglycine *N*-carboxyanhydride with DBCO amine as initiator. In a first postpolymerization modification step, the future biological activity of the polymeric ligands is adjusted by modification of the amine terminus. Then, in a second postpolymerization modification step, azide functionalized di- and tridentate anchor compounds are introduced to the DBCO terminus of the polysarcosine via strain-promoted azide−alkyne cycloaddition (SPAAC). Through the separate synthesis of the anchor compounds, it is possible to ensure reproducible introduction of a well-defined number of multiple anchor groups to all polymers studied. Finally, the obtained multidentate polymeric ligands are successfully used in the ligand exchange procedures to yield stable, water-soluble QDs. As polysarcosine-based ligands can provide biocompatibility, prevent nonspecific interactions, and simultaneously enable specific targeting, the systems presented here are promising candidates to provide QDs well suitable for *ex vivo* analytics or bioimaging.



INTRODUCTION

Quantum dots (QDs) have been intensively investigated as fluorophores in biological applications such as cellular labeling, *in vivo* and deep tissue imaging.[1−4] QDs are a promising alternative to organic fluorophores due to their superior optical properties such as high quantum yields, broad absorption, narrow emission, and easily tunable emission wavelength.[5] To synthesize water-soluble QDs that are suitable for use in biological imaging, hydrophilic polymers as surface ligands have been widely studied.[6,7] Directly after synthesis, the surface of commonly used high quality QDs is covered by hydrophobic ligands leading to QDs, which are insoluble in aqueous media.[8] Therefore, an additional surface modification step is required to obtain water-soluble QDs applicable in biological settings. Generally, the ligand exchange procedure provides an efficient way to achieve surface modification, and new ligands can versatilely modify solubility or introduce specific functionalities to the inorganic nanoparticles of interest.[9,10] For the case discussed here, the replacing ligands should possess an anchoring group (or groups) to facilitate a strong coordination to the QD surface as well as a hydrophilic part, enabling QD solubility in aqueous media. Polymeric ligands have proven to be successful candidates to synthesize stable, water-soluble QDs by the ligand exchange procedure.[11] In addition to the hydrophilicity and anchor groups, polymers used as surface ligands should possess further functional groups for subsequent conjugation with biologically active species. This is necessary for customized modification of QD surfaces and enables QD-specific interactions with various target biomolecules. Furthermore, polymers used should exhibit negligible nonspecific interactions with surrounding biological medium.[12]

While a lot of work has been devoted to anchor group modification of the polymeric ligands, only few variations have been reported concerning the hydrophilic part of the polymer.[13−17] The vast majority of published studies have so far solely used poly(ethylene glycol) (PEG).[18−24] In order to minimize

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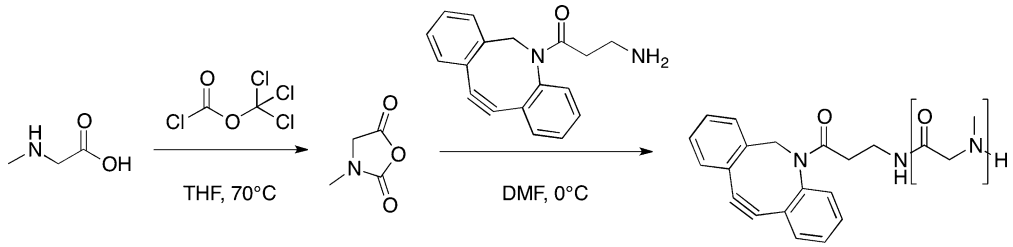
Scheme 1. Reaction Scheme of Orthogonally Functionalized Polysarcosine



nonspecific interactions of the functionalized QDs in the biological environment, polymers with *stealth*-like properties are needed. This explains why PEG is such a prominent candidate as hydrophilic ligand for QD modification. Polysarcosine (poly((*N*-methyl)glycine)) has been proposed as a promising alternative to PEG.[25−30] Polysarcosine belongs to the polymer class of polypeptoids and displays comparable properties to PEG, including zero net charge, hydrophilicity, and inability to act as hydrogen bond donor. However, unlike PEG, polysarcosine consists of the endogeneous amino acid sarcosine and is thus expected to be not only biocompatible but also potentially biodegradable. The degradability of polypeptoids by reactive oxygen species (ROS) has already been reported by Luxenhofer and co-workers.[31]

For the coordination to QDs with ZnS or CdZnS shell, various functionalities have been investigated as anchor groups of polymeric ligands. Alkylphosphines, phosphine oxides, alkylamines, imidazole, lipoic acid (LA), and dihydrolipoic acid (DHLA) functionalized polymers have been used to carry out ligand exchange procedures to obtain water-soluble quantum dots.[21,32−35] Especially sulfur-based ligands show high ligand-to-QD surface affinity and have been shown to efficiently replace initial ligands.[16,17,23,36,37] So the LA functionality alone is sufficient to enable ligand exchange on a QD surface. In addition, its grafting ability can be enhanced by the reduction of LA to DHLA or as the result of a photoinduced ligation process.[38,39] It has been recently reported that UV light or sunlight-assisted photoligation of QDs provides a highly efficient method for the direct grafting of LA-modified ligands to QD surfaces, thus, eliminating the need of an additional reduction step.[40]

Because of the dynamic behavior of interactions between the QD surface and the ligand, polymeric ligands with only one anchor group can, however, desorb from the QD surface on dilution, resulting in QD precipitation. Previous reports which are focused on multidentate ligands show that the presence of multiple anchoring groups substantially improves coordination of a polymer to the quantum dot surface.[15,32,41−43] The multidentate nature of polymeric ligands with multiple anchor groups diminishes the desorption probability and leads to more stable QD solutions.[16] Multiple anchor functionalities can be introduced by block copolymer approach.[15,32]However, the better control of the number of the introduced anchor groups can be achieved by the use of more defined structures, e.g., peptides or dendrimers.[44−46]

In this study, we present a modular approach for an effective and easily variable synthesis of new hydrophilic polymeric ligands consisting of heterotelechelic polysarcosine with di- or trilipoic acid-based anchoring groups and an additional functionality for a specific ligand modification. The developed polymers are then tested as multidentate ligands in the preparation of water-soluble QDs.

RESULTS AND DISCUSSION

Orthogonally Functionalized Polysarcosine. The synthesis of DBCO-functionalized polysarcosine was carried out as reported previously by primary amine-initiated ring-opening polymerization of SarNCA using DBCO amine (Scheme 1).[47] The polymerization was conducted in absolute DMF at 0 °C to avoid premature degradation of the reactive cyclooctyne moiety. Analytical data of the end group-functionalized polymer are summarized in Table 1.

Table 1. Analytical Data of DBCO-PSar

polymer	M/I	$X_{n(NMR)}$ [a]	$M_{n(NMR)}$ [a]	$M_{n(GPC)}$ [b] (g mol^{-1})	$Đ_{GPC}$ [b]
DBCO-PSar	200	198	14 350	26 055	1.2

[a]Determined by ^{1}H NMR in DMSO-d_6 by relating end group signals to those of the repeating unit. [b]Relative to PMMA standards.

The resulting polymer displayed considerably narrow dispersity ($Đ$ = 1.2); the degree of polymerization (X_n) was determined by ^{1}H NMR by comparing the signal originating from the eight aromatic protons of the initiator with the backbone protons of PSar (*N*-methyl and methylene, respectively). The determined degree of polymerization was close to the calculated one (M/I), which was expected for a controlled ring-opening polymerization. In a postpolymerization reaction, the cyclooctyne-functionalized polysarcosine was reacted in a strain-promoted azide−alkyne cycloaddition (SPAAC) with a di- and trilopoic acid linkers to produce hydrophilic multidentate polymeric ligands.

Lipoic Acid-Based Multidentate Anchor Compounds. To obtain anchor groups with exactly 2 or 3 binding sites, **A1** and **A2** were synthesized (Scheme 2). The bidentate anchor **A1** was obtained in a two-step synthesis. Two lipoic acid functionalities were coupled to the amine groups of 1,3-diamino-2-propanol **1** via a *N*,*N*′-dicyclohexylcarbodiimide (DCC) mediated coupling reaction leading to the formation of the compound **2**. The following DCC coupling of the hydroxy group of **2** with 6-azidohexanoic acid yielded the desired product **A1**. The tridentate anchor (**A2**) was synthesized in four steps. First, the amine functionality of tri(hydroxymethyl)aminomethane **3** was protected with a *tert*-butyloxycarbonyl (BOC) protecting group. The DCC coupling of the three hydroxy groups of the compound **4** with lipoic acid was carried out resulting in the formation of a triester (**5**). Amine deprotection and subsequent DCC coupling of the amine group with 6-azidohexanoic acid led to the tridentate anchor **A2**.

In contrast to the commonly applied block copolymer approach to introduce multiple anchor units, compounds **A1** and **A2** allow the precise control over the amount of the incorporated anchor groups. In addition, introduction of the multiple anchor groups to the polymer becomes highly reproducible. An azide functionality enables the efficient addition of the anchoring groups to the polymer via click chemistry, which has been widely shown to be

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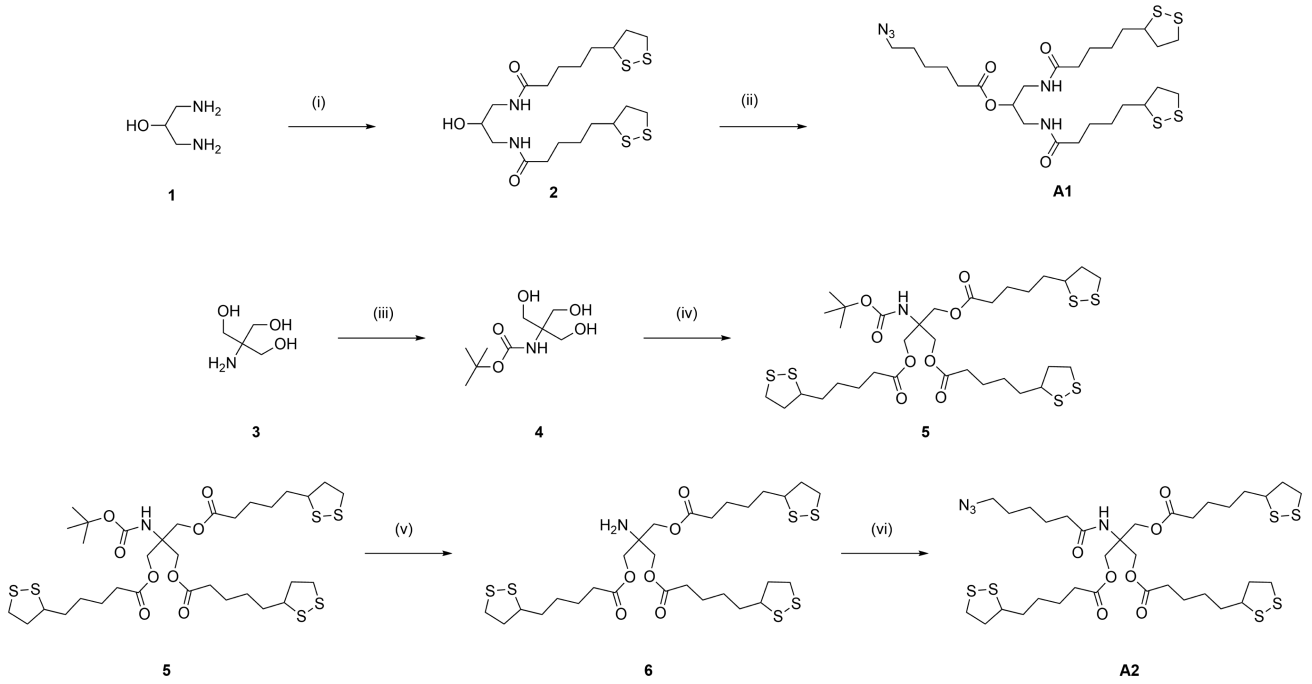
Scheme 2. Synthesis of Di- and Trivalent Lipoic Acid Compounds A1 and A2[a]



[a](i) Lipoic acid, DCC, DMAP, DCM; (ii): 6-azidohexanoic acid, DCC, DMAP, DCM; (iii): Boc anhydride, methanol; (iv): DCC, DMAP, HOBt, DMF; (v) TFA, DCM; (vi) 6-azidohexanoic acid, DCC, DMAP, HOBt, DCM.

a nearly quantitative synthetic step under the conditions applied. Furthermore, the presence of two (**A1**) or three (**A2**) anchoring groups facilitates an efficient grafting of a polymer onto the quantum dot surface.

Polymeric Multidentate Ligands. In order to obtain polysarcosine polymeric ligands, orthogonally functionalized polysarcosine was applied to postpolymerization modification reactions. First, the amine was capped with acetic anhydride or biotin *N*-hydroxysuccinimide (NHS) ester or was kept unmodified. For the *N*-terminus modification, polymer and the corresponding capping agent were dissolved in dry DMF and stirred at room temperature for 24 h. The functionalization of the amine end group determines later the biological activity of the modified QDs. Second, the SPAAC reaction was carried out between the polymeric dibenzocyclooctyne group and the azide groups of bidentate lipoic acid anchor **A1** or the tridentate anchor **A2** leading to **PL1a−c** and **PL2a−c**, respectively (Scheme 3 and Table 2). The successful polymer functionalization was confirmed with ^{1}H and ^{1}H DOSY NMR spectroscopy (Figure 1 and Figure S7a).[47]

In DOSY spectra, the signals corresponding to the polymer backbone (2.5−2.9 and 3.6−4.2 ppm), acetyl (2.0 ppm), and lipoic acid-based anchor end groups (1.3−1.7, 3.2, and 3.6 ppm) are visible as one single diffusing species, indicating the successful polymer functionalization. The negative control consisting of a mixture of DBCO-initiated PSar and trilipoic acid linker without azide functionality clearly shows two well-distinguishable diffusing species (see Figure S7b). According to the GPC data, only a slight change in molecular weight and dispersity occurs after subsequent postpolymerization modification steps (see Figure S18). It needs to be mentioned, though, that GPC traces show the formation of a small fraction of polymers with higher molecular weight than initial DBCO-PSar, visible in the GPC elugram as peaks at lower elution volumes. This can be explained by cross-linking reactions between individual polymer chains due to the dynamic character of the disulfide bonds of the lipoic acid anchor group.[48]

The *stealth*-like properties of the polysarcosine backbone are advantageous to prevent the nonspecific interactions of QDs with surrounding medium. Additionally, the presence of the second functional group enables the synthesis of ligands, which are able to undergo specific interactions with target compounds in a biological setting. The choice of the reactants in the first postpolymerization reaction step determines the future biological activity of the ligands. The modification of the amine group with biotin *N*-hydroxysuccinimide ester (biotin NHS ester) reflects only one example of a highly efficient way to incorporate of a huge variety of biologically active components to the polysarcosine polymer through the NHS ester coupling method (**PL1b** and **PL2b**). The use of such premodified polysarcosine as a ligand would lead to QDs with biologically active surface. On the other hand, the ligands **PL1a** and **PL2a** with the inert acetamide end group are not expected to interact with surrounding biomolecules. Moreover, if the functionalization of QDs is carried out with polymeric ligands **PL1c** and **PL2c**, the coupling with biologically active components could be performed in the later stage directly on the quantum dot surface.

Polysarcosine-Functionalized Water-Soluble Quantum Dots. The ligand exchange procedure was carried out to obtain water-soluble quantum dots. Polysarcosine polymeric ligands (3 mg) and quantum dots (1 mg) were dissolved in chloroform (200 μL) and immerged into a sonication bath for 120 min. Afterward, an excess of water was added to the reaction mixture, and the now water-soluble quantum dots were extracted from the organic to the aqueous phase. The excess polymer was removed by repeated centrifugation with Amicon Ultra centrifugal filters (100 kDa MWCO). The water-soluble QDs (with **P1a−c** and **P2a−c** as new hydrophilic ligands) retained the optical properties of the original oleic acid-modified QDs with fluorescence maxima at 623 nm and fwhm of 28 nm (Figure 2b).

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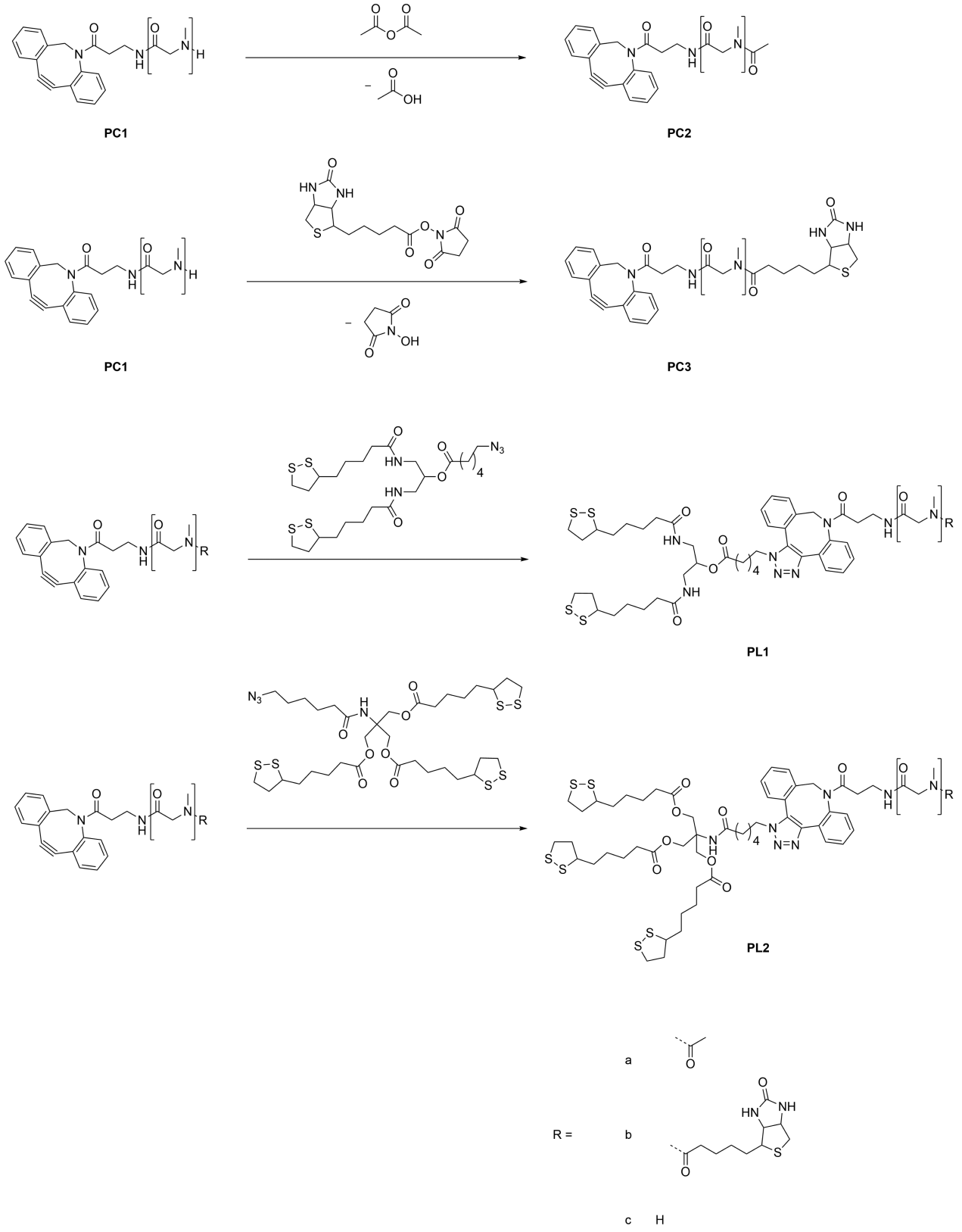
Scheme 3. Synthetic Scheme of Polysarcosine Multidentate Ligands PL1a−c and PL2a−c



Moreover, QDs functionalized with ligands **PL1a** and **PL2a** are stable at high salt concentrations (1 M NaCl) and in a physiologically relevant pH range between 4.6 and 8.1. The photoluminescence quantum yield of hydrophilic QDs in water was decreased by 25% for tridentate ligand **PL2a** and by 23% for bidentate ligand **PL1a** compared to the pristine QDs with oleic acid ligands in toluene. Such a decrease in photoluminescence, however, was expected and is well in agreement with previously published results.[14,39]

In addition, the formation of individually dispersed QDs with narrow size distributions was confirmed by transmission electron

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Table 2. Synthesized Polymeric Ligands by Postpolymerization Modification

polymer	anchoring group	functional group
PL1a	dilipoic amide	acetyl
PL1b	dilipoic amide	biotin
PL1c	dilipoic amide	amine
PL2a	trilipoic ester	acetyl
PL2b	trilipoic ester	biotin
PL2c	trilipoic ester	amine

microscopy (TEM) and atomic force microscopy (AFM) measurements (Figure 3, Figures S21–S23). TEM images provided information about the size of the inorganic sphere, while the QDs with the outer polysarcosine corona were detected in AFM.

CONCLUSIONS

Because of their high biocompatibility and protein resistance, polysarcosine-based ligands bear the potential to provide a promising platform for the development of QDs for applications in biological systems, *ex vivo* and *in vitro*. In this study, orthogonally functionalized polysarcosine-based hydrophilic ligands have been synthesized and, for the first time, utilized as ligands to obtain water-soluble QDs. Small molecules containing a defined number of anchoring functionalities were separately synthesized and sequentially covalently linked to the polysarcosine backbone using highly efficient SPAAC. It needs to be stressed here that this approach allows for the reproducible attachment of the same amount of anchoring units to various polymers. Moreover, the free amine group of polysarcosine was prefunctionalized with biotin as an example of a target molecule or capped with acetic anhydride to prevent unspecific interactions with either the biological environment or with the QD itself. Alternatively, the amine group was left unmodified to produce QDs available for further modifications with biologically active species directly on water-soluble quantum dots. Finally, stable, water-soluble QDs using the above-mentioned polymeric ligands were prepared in the ligand exchange procedure retaining their optical properties.

EXPERIMENTAL SECTION

Materials and Methods. Solvents used in the reactions were purchased from Sigma-Aldrich and used without further purification unless stated otherwise. Dry DMF was purchased from Acros Organics (99.8% Extra). For NCA polymerization, dry DMF was further dried over BaO and fractionally distilled *in vacuo*. Chemicals were purchased from Sigma-Aldrich, Acros Organics, Alfa Aesar, or Fluka. Quantum dots with CdSe core, $Cd_xZn_{1-x}S$ shell (core diameter 4 nm, total diameter 16 nm/9 nm with oleic acid surface ligands), were synthesized according to the literature.

Millipore water was prepared using a MILLI-Q Reference A+ System. Water was used at a resistivity of 18.2 MΩ cm and total organic carbon <5 ppm.

NMR spectra were recorded in $CDCl_3$ (Deutero GmbH) and DMSO-d_6 (Deutero GmbH) with a Bruker AV 400 or Bruker AC 300 spectrometer at room temperature. Chemical shifts are reported with respect to residual solvent signal as internal standards $CDCl_3$ (^1H, ^{13}C) 7.26 and 77.16 ppm and DMSO-d_6 (^1H) 2.50 ppm. Infrared spectroscopy to verify complete consumption of SarNCA during NCA polymerization was performed on a Jasco FT/IR-4100 with an ATR sampling accessory (MIRacle, Pike Technologies) using 16 scans per measurement. IR spectra were analyzed using Spectra Manager 2.0 (Jasco). Polymer molecular weight was determined by gel permeation chromatography (GPC). GPC in HFIP was performed with 3 g L^{-1} potassium trifluoroacetate at 40 °C. The columns were packed with modified silica (PFG columns, particle size: 7 μm; porosity: 100 and 1000 Å). A refractive index detector (G 1362A RID, Jasco) and a UV/vis detector (UV-2075 Plus, JASCO) were used to detect the polymer.

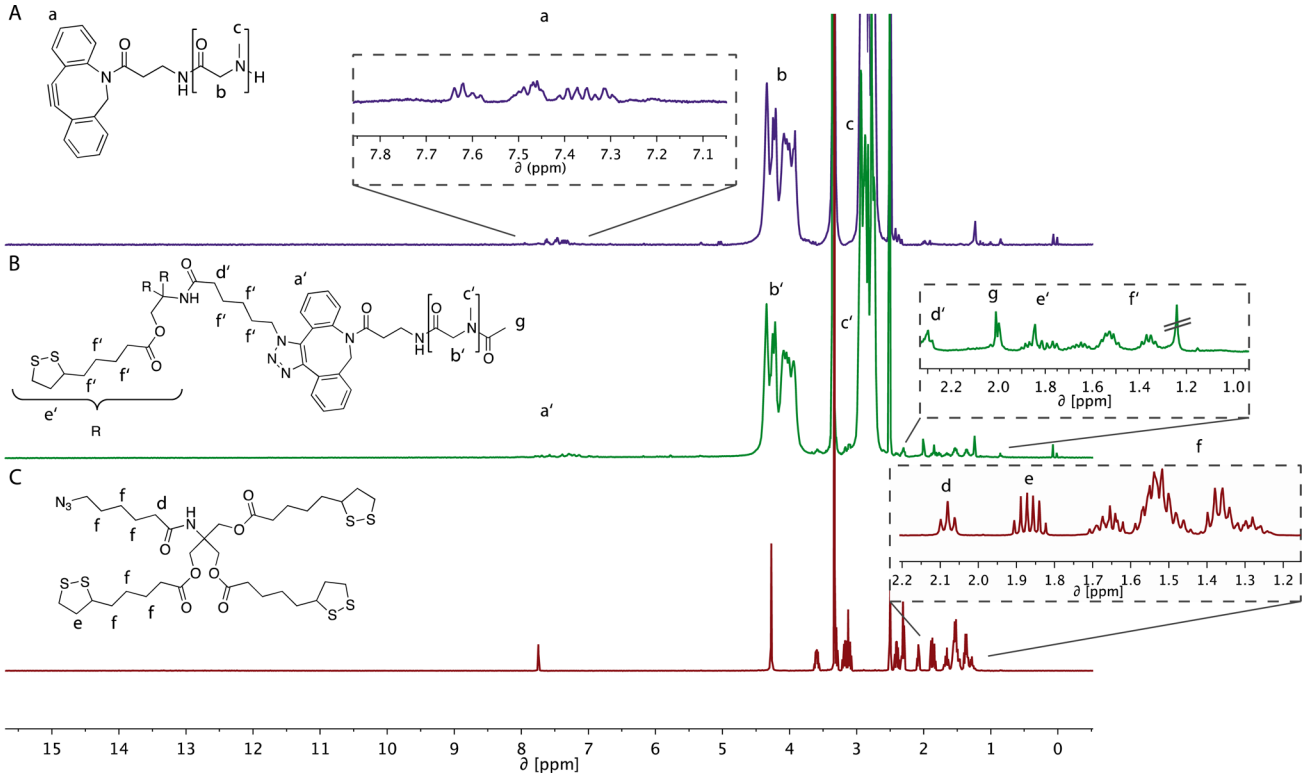

Figure 1. ^1H NMR in DMSO-d_6 (400 MHz) of (A) DBCO-amine-initiated PSar (**PC1**), (B) trilipoic acid-functionalized PSar capped with acetyl (**PL2c**), and (C) trilipoic acid anchoring group (**A2**), demonstrating successful postpolymerization modification of polymeric ligands.

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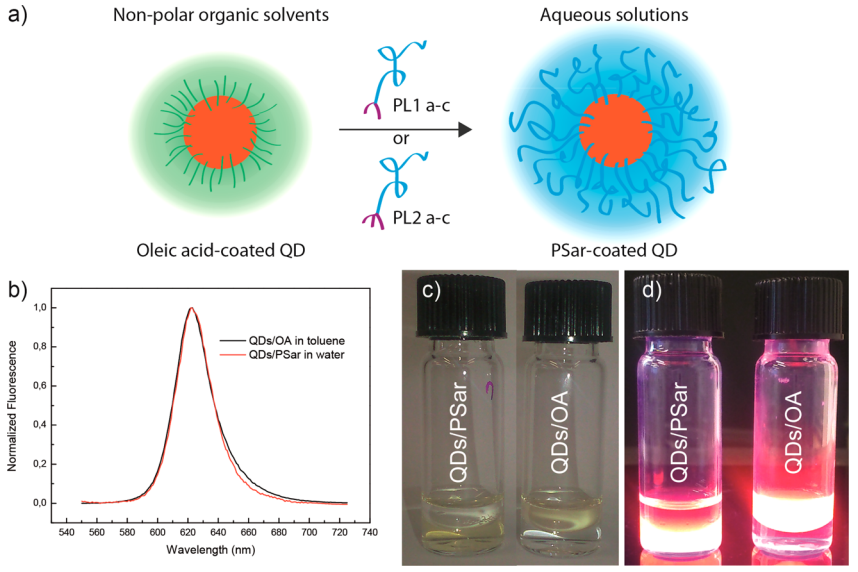

Figure 2. Schematic representation of the ligand exchange procedure to obtain water-soluble QDs (a), normalized fluorescence spectra of QDs functionalized with **PL1a** ligands (QDs@**PL1a**) in water and QDs@OA in toluene (b), and QDs@**PL1a** and QDs@OA in water/hexanes mixture under ambient (c) and UV light (d).

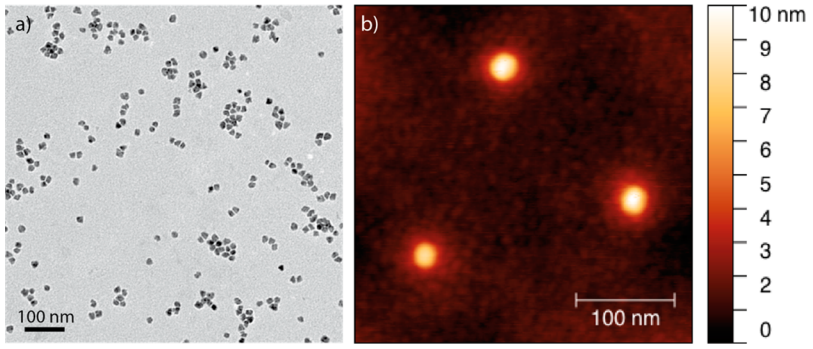

Figure 3. TEM image of QD@**PL1a** (a) and AFM height image of QDs@**PL1a** (b).

Molecular weights were calculated using calibration performed with PMMA standards (Polymer Standards Services GmbH). As the internal standard, toluene was used. Melting points were determined on a Mettler Toledo FP62 melting point apparatus at a heating rate of 5 °C min^{-1}. UV−vis spectra were recorded on a Varian Cary 5000 spectrometer. Emission spectra were recorded on a Varian Cary Eclipse spectrometer. Transmission electron microscopy (TEM) images were obtained with a JEOL JEM-1400 electron microscope. Atomic force microscopy (AFM) was performed on a Cypher (Asylum Research) in ac mode on freshly cleaned mica at a scan rate of 1 Hz. Images were treated using Gwyddion and particles were counted using ImageJ. For particle size distributions, >70 particles were counted. Lateral dimensions were used.

Sarcosine-*N*-Carboxyanhydride. The synthesis of sarcosine NCA was adapted from the literature and modified.[49]

Sarcosine (15.16 g, 170.2 mmol, 1 equiv) was weighed into a predried three-necked flask and dried under vacuum for 1 h. 300 mL of absolute (abs) THF was added under a steady flow of nitrogen. The apparatus was connected to two gas washing bottles filled with aqueous sodium hydroxide solution. Diphosgene (16.26 mL, 134 mmol, 0.8 equiv) was added slowly via syringe. The colorless suspension was heated to 70 °C yielding a clear solution after 3 h of stirring. The solvent was evaporated under reduced pressure yielding a brown oil as crude reaction product. The oil was heated to 50 °C and dried under reduced pressure to obtain an amorphous solid. The crude reaction product was redissolved in 60 mL of THF and precipitated with 300 mL of abs *n*-hexane. The precipitate was filtered off under a N_2 atmosphere and dried with a stream of dry nitrogen for 60−90 min to remove residual traces of solvents. The next day, the product was dried in high vacuum for 2 h in the sublimation apparatus and subsequently sublimated at 80−85 °C and $<1 \times 10^{-2}$ mbar. The product was collected from the sublimation apparatus in a glovebox on the same day. Colorless crystals were obtained (50−67%); mp =104.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 2.86 (s, 3H, NH−**CH_3**), 4.22 (s, 2H, NH−**CH_2**−CO).

Synthesis of Compound PC1, DBCO-Amine-Initiated Polysarcosine. 551.1 mg of SarNCA (4.788 mmol) was transferred under nitrogen counter flow into a predried Schlenk-tube, equipped with a stir bar, and again dried under vacuum for 30 min. The NCA was then dissolved in 4 mL of dry DMF. A stock solution of 13.23 mg (0.024×2 mmol, 1/200 equiv, M/I = 200) of DBCO-amine in 2 mL of DMF was prepared, and 1 mL of this stock solution was added to the monomer solution via syringe. The solution was stirred at 0 °C and was opened to the Schlenk line to prevent impurities from entering the reaction vessel while allowing CO_2 to escape. Completion of the reaction was confirmed by IR spectroscopy (disappearance of the NCA peaks (1853 and 1786 cm^{-1})). Directly after completion of the reaction, the polymer was precipitated in cold diethyl ether and centrifuged (4500 rpm at 4 °C for 15 min). After discarding the liquid fraction, new ether was added and the polymer was resuspended using sonication. The suspension was centrifuged again, and the procedure was repeated. The polymer was then dissolved in H_2O and lyophilized to obtain 314.7 mg of a fluffy powder (4.429 mmol, 93%). ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm = 2.58−3.11 (br, 3nH, N−CH_3), 3.73−4.57 (br, 2nH, −CO−CH_2−N) 7.86−7.10 (m, 8H, −C_4H_4 (2×)).

Synthesis of the Compound 2. DL-α-Lipoic acid (2.03 g, 9.86 mmol), *N*,*N*′-dicyclohexylcarbodiimide (DCC) (2.34 g, 10.85 mmol)

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and 4-(dimethylamino)pyridine (DMAP) (120 mg, 0.98 mmol) were dissolved in dry DCM (55 mL). 1,3-Diamino-2-propanol (400 mg, 4.44 mmol) was separately dissolved in dry DCM (5 mL) and slowly added to the reaction mixture. The reaction mixture was stirred at room temperature and under protectection from light for 40 h. The colorless residue formed was separated via filtration through Celite. The solvent of the remaining yellow filtrate was removed by rotary evaporation. The residue was purified via column chromatography (eluent dichloromethane/methanol) to yield compound **2** as yellow solid (1,23 g, 2.64 mmol, 59%). ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 6.81 (t, J = 6 Hz, 2H), 3.73 (p, J = 5.1 Hz, 1H), 3.57−5.51 (m, 2H), 3.39−3.32 (m, 2H), 3.24−3.05 (m, 6H), 2.47−2.39 (m, 2H), 2.21 (t, J = 7.5 Hz, 4H), 1.92−1.84 (m, 2H), 1.73−1.56 (m, 8H), 1.51−1.36 (m, 4H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ/ppm: 174.71, 69.96, 56.48, 42.53, 40.34, 38.57, 36.36, 34.67, 28.94, 25.48. HRMS (ESI$^+$) m/z calcd 467.1531 $[M + H]^+$; found 467.1525.

Synthesis of the Bidentate Anchor Compound A1. 6-Azidohexanoic acid (298 mg, 1.89 mmol) was dissolved in DCM (30 mL), and DCC (426 mg, 2.06 mmol) and DMAP (42 mg, 0.34 mmol) were added. The compound **2** (800 mg, 1.72 mmol) was separately dissolved in DCM (30 mL) and subsequently added to the first solution. The reaction solution was stirred for 30 h at room temperature and under protection from light. The colorless residue formed was separated via filtration through Celite. The solvent of the remaining yellow filtrate was removed by rotary evaporation. The residue was purified via column chromatography (eluent dichloromethane/acetone) to yield compound **A1** as yellow solid (930 mg, 1.54 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 6.40 (t, J = 6.5 Hz, 2H), 4.92−4.76 (m, 1H), 3.60−3.52 (m, 2H), 3.51−3.45 (m, 2H), 3.37−3.32 (m, 2H), 3.29 (t, J = 6.8 Hz, 2H), 3.20−3.07 (m, 4H), 2.45 (dtd, J = 13.1, 6.6, 5.4 Hz, 2H), 2.32 (t, J = 7.4 Hz, 2H), 2.23 (t, J = 7.5 Hz, 4H), 1.90 (dtd, J = 13.1, 6.6, 5.4 Hz, 2H), 1.75−1.57 (m, 12H), 1.53−1.37 (m, 6H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 (t, J = 6.0 Hz, 2H), 4.81−4.76 (m, 1H), 3.63−3.56 (m, 2H), 3.31 (t, J = 6.8 Hz, 2H), 3.29−3.25 (m, 2H), 3.21−3.05 (m, 6H), 2.44−2.37 (m, 2H), 2.26 (t, J = 7.4 Hz, 2H), 2.05 (t, J = 7.3 Hz, 4H), 1.90−1.82 (m, 2H), 1.70−1.43 (m, 12H), 1.38−1.27 (m, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 173.88, 172.75, 71.11, 56.54, 51.34, 40.39, 39.01, 38.61, 36.51, 34.72, 34.13, 28.97, 28.63, 26.29, 25.49, 24.40. HRMS (ESI$^+$) m/z calcd 606.2276 $[M + H]^+$; found 606.2287.

Synthesis of the Compound 5. Tris(hydroxymethyl)-BOC-aminomethane (BOC-TRIS) (0.91 g, 4.09 mmol) and DL-α-lipoic acid (3.80 g, 18.42 mmol) were dissolved in dry DMF (20 mL). DCC (3.80 g, 4.95 mmol), DMAP (450 mg, 3.68 mmol), and 1-hydroxybenzotriazole hydrate (HOBt hydrate) (83 mg, 0.61 mmol) were separately dissolved in dry DMF and subsequently added to the first solution. The reaction was stirred at room temperature and under protection from light for 90 h. The colorless residue formed was separated via filtration through Celite. The solvent of the remaining yellow filtrate was removed by rotary evaporation. The residue was purified via column chromatography (eluent hexanes/ethyl acetate) to yield compound **5** as a yellow waxy oil (3.16 g, 4.02 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 4.35 (s, 6H), 3.60−3.53 (m, 3H), 3.21−3.53 (m, 6H), 2.50−2.43 (m, 3H), 2.35 (t, J = 7.5 Hz, 6H), 1.95−1.87 (m, 3H), 1.74−1.60 (m, 12H), 1.55−1.43 (m, 15H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ/ppm 173.11, 156.26, 62.50, 58.44, 56.46, 40.40, 38.63, 34.68, 33.95, 28.84, 28.57, 28.51, 24.68. HRMS (ESI+) m/z calcd 808.2156 $[M + Na]^+$; found 808.2158.

Synthesis of the Compound 6. The compound **5** (3.16 g, 4.02 mmol) was dissolved in DCM (90 mL), and trifluoroacetic acid (TFA) (9 mL) was slowly added to reaction solution. After stirring for 3 h at room temperature 1 M Na_2CO_3 solution (90 mL) was added to the reaction solution. The phases were separated, and the organic phase was extracted once with 1 M Na_2CO_3 solution, twice with water, and once with brine. Finally, the organic phase was dried with Mg_2SO_4, and the solvent was removed by rotary evaporation. The remaining crude product (compound **6**, 2.90 g,) was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 4.03 (s, 6H), 3.60−3.53 (m, 3H), 3.21−3.08 (m, 6H), 2.50−2.40 (m, 3H), 2.35 (t, J = 7.4 Hz, 6H), 1.95−1.86 (m, 3H), 1.72−1.41 (m, 18H). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ/ppm 173.04, 65.50, 56.45, 54.21, 40.38, 38.63, 34.71, 34.00, 28.86, 24.73. HRMS (ESI+) m/z calcd 686.1800 $[M + H]^+$; found 686.1798.

Synthesis of the Tridentate Anchor Compound A2. 6-Azidohexanoic acid (367 mg, 2.33 mmol) was dissolved in DCM (20 mL), and DCC (451 mg, 2.19 mmol), DMAP (36 mg, 0.29 mmol), and HOBt hydrate (20 mg, 0.15 mmol) were added. Compound **5** (1.00 g, 1.46 mmol) was separately dissolved in DCM (20 mL) and subsequently added to the first solution. The reaction solution was stirred for 50 h at room temperature and under protection from light. The colorless residue formed was separated via filtration through Celite. The solvent of the remaining yellow filtrate was removed by rotary evaporation. The residue was purified via column chromatography (eluent dichloromethane/cyclohexanes/acetone) to yield compound **A2** as yellow waxy oil (580 mg, 0.70 mmol, 48%). ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm 5.93 (s, 1H), 4.42 (s, 6H), 3.60−3.53 (m, 3H), 3.27 (t, J = 6.8 Hz, 2H), 3.1−3.08 (m, 6H), 2.46 (dtd, J = 13.1, 6.6, 5.4 Hz, 3H), 2.35 (t, J = 7.4 Hz, 6H), 2.17 (t, J = 7.5 Hz, 2H), 1.91 (dtd, J = 13.0, 6.7, 5.4 Hz, 3H), 1.74−1.57 (m, 16H), 1.52−1.38 (m, 8H). ^{1}H NMR (400 MHz, DMSO-d_6): δ/ppm 7.74 (s, 1H), 4.27 (s, 6H), 3.63−3.56 (m, 3H), 3.30 (t, J = 6.9 Hz, 2H), 3.21−3.08 (m, 6H), 2.41 (dtd, J = 12.8, 6.5, 5.5 Hz, 3H), 2.31 (t, J = 7.3 Hz, 6H), 2.08 (t, J = 7.3 Hz, 2H), 1.86 (dtd, J = 12.7, 6.5, 5.4 Hz, 3H), 1.71−1.44 (m, 16H), 1.40−1.24 (m, 8H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ/ppm 173.16, 173.03, 62.67, 58.45, 56.46, 51.33, 40.39, 38.63, 37.01, 34.69, 33.97, 28.85, 28.72, 26.32, 25.05, 24.69. HRMS (ESI$^+$) m/z calcd 847.2371 $[M + Na]^+$; found 847.2355.

Synthesis of PC2, Amine End Group Modification with Acetic Acid Ester Anhydride. Polysarcosine (48.0 mg, 0.0031 mmol), acetic anhydride (3.16 mg, 3.0 μL, 0.0309 mmol), and triethylamine (6.27 mg, 8.6 μL, 0.062 mmol) were dissolved in DMF (1 mL) and stirred at 25 °C for 24 h. Subsequently, the polymer was precipitated in diethyl ether, redissolved in water, and lyophilized.

Synthesis of PC3, Amine End Group Modification with Biotine. Polysarcosine (60.0 mg, 0.0039 mmol), (+)-biotine *N*-hydroxysuccinimide ester (13.2 mg, 0.0387 mmol), and triethylamine (7.83 mg, 11.0 μL, 0.077 mmol) were dissolved in DMF (1 mL) and stirred at 25 °C for 24 h. Subsequently, the polymer was precipitated in diethyl ether, redissolved in water, and lyophilized.

General Procedure of Dibenzocyclooctyl End Group Modification via Strain-Promoted Azide−Alkyne Cycloaddition (SPAAC). One equiv of polysarcosine and 3 equiv of azide modified anchor compounds (**A1** or **A2**) were dissolved in DMF (polymer concentration ca. 0.001 mmol/mL) and stirred for 24 h at 25 °C. Subsequently, the polymer was dialyzed against methanol and water and lyophilized.

Synthesis of Water-Soluble QDs with PSar-Based Ligands. PSar-based hydrophilic polymeric ligands **PL1a−c** and **PL2a−c** (3 mg) and quantum dots (red, CdSe core, core diameter 4 nm, $Cd_xZn_{1-x}S$ shell, total diameter 16 nm, oleic acid ligands, 1 mg, synthesized according previously published procedure,[50] photoluminescence quantum yield in hexanes 80%) were separately dissolved in chloroform (each 100 μL) and subsequently combined. The reaction mixture was sonicated for 2 h, and water (1.0 mL) was added. The water-soluble quantum dots were extracted from the organic to the aqueous phase. The aqueous phase was separated and the extraction repeated two more times. The excess polymer was removed by repeated centrifugation with Amicon Ultra centrifugal filters (100 kDa MWCO). After repeated extraction steps, the organic phase exhibited no visible photoluminescence from QDs under irradiation with a UV lamp at 312 nm.

QD@PL Stability Studies. For stability studies of QD@PL, 1 mM NaOAc/AcOH buffer (pH = 4.6), PBS buffer (pH = 8.1), and 1 M NaCl solutions were prepared, and QD@PL was incubated with these solutions for 3 days.

■ ASSOCIATED CONTENT

Supporting Information

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NMR spectra of anchor compounds, NMR spectra and GPC traces of polymers, additional AFM images of functionalized QDs, and statistical data on partical size distributions (PDF)

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Notes

The authors declare no competing financial interest.

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