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Design and Synthesis of Core-Shell Microgels with One-step Clickable Crosslinked Cores and Ultra-low Crosslinked Shells

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Abstract

The present study was conducted to explore the engineering of core-shell microgels such that the core can be rapidly labeled with a variety of fluorophores, while the shell retains the softness needed in specific biomedical applications. We synthesized azide containing crosslinked core particles based on a crosslinked poly (N-isopropylacrylamide) particle, using a one-pot, multi-step polymerization. A core-shell microgel was then synthesized by growing a crosslinker-free poly (N-isopropylacrylamide)-co-acrylic acid (ULC10AAc) shell through a two-step seed and feed polymerization. We further demonstrate a simple ‘click’ reaction between the azide present on the core and dibenzocyclooctyne containing fluorophores to make dyed core-shell microgels. This approach to dyed core-shell microgels eliminates the difficulty of cross-reactivity between shell-localized chemoligation sites and the core-localized functionalities. The use of strained-ring systems to achieve the click reaction eliminates the use of cytotoxic

catalysts common in traditional click chemistry. This approach allows us not only to label the microgel core with different fluorophores but also allows us to synthesize multi-probe core-shell microgel by simultaneously labeling a microgel core with multiple different fluorophores.

TOC:

Engineering Dyed Core-Shell Microgel

Keywords:

Core-Shell, Microgel, ULC, Strain-Promoted Click Reaction

Introduction

Microgels are colloidally stable, porous, soft microparticles composed of three-dimensionally crosslinked polymer chains swollen in a solvent. Depending on their architecture, microgels can retain many of the physical properties of the parent polymer, while also enabling new properties of potential utility in a variety of applications.[1-4] In particular, the potential for tuning their properties by incorporating a variety of chemical and structural features, along with their ease of synthesis makes them potentially outstanding materials for various biomedical applications. These potential advantages have led to our group's continued interest in the most widely studied class of responsive polymers: those based on temperature-responsive poly(N-isopropylacrylamide) (pNIPAm).[5-10] PNIPAm-based microgels continue to enjoy tremendous popularity due to lower critical solution temperature ($\sim 32^\circ\text{C}$) behavior near physiological temperatures, along with the aforementioned ease of physical and chemical property tuning.[2-4, 11-21] Indeed, by virtue of this broad tunability, multi-responsive pNIPAm microgels have found applications across many biomedical applications, including sensing, drug delivery, and diagnostics.[22-32]

For many years now, we and others have illustrated that core-shell microgel structures solve enable the precise segregation of mechanical and/or chemical properties into separate "compartments".[5, 6, 18, 19, 33-40] However, a synthetic challenge remains if we are not able to selectively and controllably use and modify the functionalities within those compartments to create multi-responsive particles for drug delivery or therapeutic applications. In particular, are interested in particles with a core compartment containing

chemical handles that allow for facile, rapid labeling with NIR dyes for tracking and visualization. However, in addition to those optical properties, our target particles should also display a soft, ULC-like shell that could be coupled to fibrin-binding proteins for artificial platelet applications.[27, 41] Furthermore, the two chemistries (protein and dye coupling) should be chemo-orthogonal, thereby removing the need for protection/deprotection chemistries.

Advancements in bioconjugation chemistry have opened a plethora of options for precise coupling of a variety of structures onto synthetic and natural macromolecules. Among the most popular of these are ‘click’ reactions using functional groups like primary amines, acids, ethynyl, sulfhydryl, ester, azide, and strained cycloalkene and cycloalkyne groups to achieve selective reactivity.[26, 30, 42-57] Previously we reported[58] the synthesis of multifunctional core-shell microgels containing acid, azide, and alkyne groups to enable fluorophore attachment by EDC coupling and a copper-catalyzed click reaction. Unfortunately, this approach required a buffered metal catalyst solution, which has drawbacks due to the cytotoxicity of the catalyst. Therefore, we are now focused on catalyst-free, strain promoted cycloaddition reactions between alkynes and azides for our synthetic approach. Thus, by designing a core-shell microgel with segregated ULC and click compartments, a NIR-dyed, platelet-like particle should obtain.

In this paper, we describe the development of a one-pot, multi-feed synthesis to make azide-containing crosslinked poly(N-isopropylacrylamide) (pNIPAm) core particles. We

further used these azide containing cores to grow ultralow crosslinked (ULC) poly (N-isopropylacrylamide-co-acrylic acid) (pNIPAm-co-AAc) microgel shells by seed and feed polymerization. This results in a thermoresponsive, click-able core, and a shell that is both pH and thermoresponsive. Finally, a click reaction was used between the azide containing core and cyclic alkynyl-containing fluorophores to form dyed core-shell microgels with a range of different emission wavelengths. This approach allows for the trivial synthesis of multiprobe core-shell microgel by simultaneously labeling a microgel core with multiple different fluorophores.

Experimental

Materials

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless stated otherwise. *N*-Isopropylacrylamide monomer (NIPAm) was recrystallized from hexanes and vacuum dried before use. Acrylic acid (AAc), *N,N'*-methylenebis(acrylamide) (BIS), glycidyl methacrylate (GMA), sodium azide (NaN₃), DMSO, sodium dodecyl sulfate (SDS) and ammonium persulfate (APS) were all used as received. Dibenzocyclooctyne derivatives borondipyrromethene FL (BDP FL), cyanine3 dibenzocyclooctyne (Cy3 DBCO), cyanine5 dibenzocyclooctyne (Cy5 DBCO), and cyanine7 dibenzocyclooctyne (Cy7 DBCO) were purchased from Lumiprobe (Maryland, USA) and used as received. Deionized water (DI) was obtained from a Thermo Scientific GenPure UV xCAD system (GmbH, Germany) and was filtered to have a minimum resistivity of 18.2 MΩ·cm. Dialysis tube Biotech CE, 1000 kDa MWCO, 31 mm Flat-width was purchased from VWR.

General Method of Microgel Synthesis

Precipitation polymerization was used to prepare core microgel particles, and core-shell microgel particles were prepared through a two-step seed and feed method. A detailed description of this approach may be found elsewhere. [5, 6, 34] After synthesis, all particles were filtered through glass wool and purified via dialysis (1000 kDa MWCO) against frequent changes of DI H₂O until the conductivity of the dialysate matched the conductivity of the DI water.

Poly(NIPAm-BIS-Azide) Core Synthesis

A variety of synthetic approaches were used in order to determine how various parameters influenced the resultant particle structure and function. First, azide containing core particles were synthesized by a three-step, one-pot reaction without SDS in a manner similar to that reported previously.[58] Monomers NIPAm (1.570 g) and BIS (0.112 g) were dissolved in 85 mL of H₂O and then filtered twice through a membrane filter (0.2 μ m, Pall Gelman Metrice). Nitrogen gas was bubbled through the solution for 30 min to remove dissolved oxygen. DI water (10 mL) was used to transfer and wash throughout filtration, and the total monomer concentration was adjusted to be 146 mM. The solution was placed into a 250 mL three-neck, round-bottom flask and heated for 1 h with a heating mantle under a constant nitrogen purge and maximum stir rate. After stabilizing for 15 min at 70 °C, polymerization was initiated via the addition of deoxygenated and filtered APS solution (0.023 g dissolved in 5 mL of DI H₂O). The reaction was continued at 70 °C for 45 min after which GMA (0.143g dissolved in 5 mL

acetone) was added in four aliquots over 5 minutes. The reaction was continued at 70 °C for 1 h, and NaN₃ solution (0.098 g in 5 mL H₂O) was added. The azide solution was added in multiple portions over 30 min, and the reaction was continued for another 6 h. The reaction mixture was cooled to room temperature and filtered through glass wool and later purified by dialysis (1000 kDa MWCO) until the conductivity of the dialysate matched the conductivity of DI water.

Core microgels were also prepared with the presence of SDS using a similar procedure to that detailed above. In this case, the total monomer concentration was kept at 70 mM. SDS (0.029 g) was added to the monomer solution, stirred for 15 min, and filtered through a 0.2 µm membrane filter before the reaction. A third type of core microgel particle was synthesized without any SDS with a slight modification of the rate of addition of GMA to the reaction mixture, maintaining the same composition of monomers as in the first reaction, with a total concentration of 146 mM. In this case, however, the entire GMA solution was added into the reaction at once. The % mass conversion (monomer to microgel shell) was found to be ~70 to 80% in all syntheses. The presence of the azide groups was confirmed by the FTIR. Figure S1 shows the presence of azide with a very strong peak characteristic of azide group around 2360 cm⁻¹.

Poly(NIPAm-co-AAc) Shell Solution

The shell solution was prepared with a 90:10 ratio of comonomers (NIPAm: AAc). NIPAm (1.49 g), and AAc (100 µL) were dissolved in 85 mL of DI H₂O. The solution was stirred

for 30 minutes before filtering the solution through a 0.2 μm syringe filter. An additional 10 mL DI water was used to transfer and wash the beaker and filter into the shell solution.

Core-Shell Microgel Synthesis

The purified core microgel particles were heated to 70 °C while stirring and passing N_2 through the solution for ~ 1 h. Once the temperature had stabilized at 70 °C, 15 mL of degassed shell solution was injected through a 0.2 μm syringe filter to the core solution, and heating was continued to reach 70 °C. The reaction was initiated by adding APS solution (0.026 g dissolved in 5 mL DI H_2O). After 30 min, the rest of the shell solution was added in 5 mL increments over the next 30 min. The reaction was continued for 6 h under N_2 . The mixture was cooled to room temperature, filtered through glass wool, and dialyzed (1000 kDa MWCO) against a change of DI water twice daily until the conductivity of the dialysate matched the DI water.

This same protocol was used to synthesize core-shell microgels when starting with smaller seed particles synthesized in presence of SDS. However, we found that the amount of the coagulum was very high in those syntheses. We believe that this is due to the particle aggregation during shell addition, perhaps brought about by significant gelation in the continuous phase as opposed to the deposition of shell polymer upon pre-existing seed particles.

Labeling of Core-Shell Microgels

Core-shell microgels were labeled with different fluorescent dyes by utilizing the azide-DBCO click reaction in aqueous media. Microgel suspension was mixed with DMSO-water-solution (65:35, v/v) of different DBCO dyes at a molar ratio of 1.5 (dye to azide based on feed ratio of azide) in a centrifuge tube. The reaction solution was agitated on a shaker table for 2 h. The reaction solution was then centrifuged and the pellet was resuspended in DI water. The centrifugation was repeated for six times and the concentrated dyed core-shell microgel was stored in glass vial until use.

Microgel Surface Deposition

For AFM and SEM imaging, microgels were deposited on 22 mm x 22 mm functionalized glass coverslips (VWR). The details of the coverslip functionalization can be found elsewhere.[59] Briefly, glass slides were sonicated in different solvents for 30 min in each case: dilute Alconox solution, acetone, ethanol, and isopropanol. The coverslips were then incubated in ethanol for 30 min before being incubated in 1 % (v/v) APTMS in absolute ethanol. The slides were then agitated in the APTMS solution on a table shaker for 2 h to silanize the surface, thereby rendering it positively charged. The functionalized coverslips were stored in absolute ethanol until further use (maximum storage time of 2 weeks). Before microgel deposition, the glass slides were rinsed with DI water, dried with nitrogen gas, and individually placed into wells of 24-well plate containing microgel solution (0.01 mg/mL). Microgels were centrifugally deposited (active deposition) onto the

glass surface at 3700 rpm (2250 x g) for 10 min using an Eppendorf 5804 R centrifuge equipped with a plate rotor at 20 °C. Once the microgels were deposited, the coverslip was washed with a gentle stream of water to remove excess microgel, dried by blowing N₂, and analyzed by AFM and SEM.

Particle Characterization

Dynamic Light Scattering (DLS)

DLS (Mobius, Wyatt Technology, Santa Barbara, CA) was used to determine the average hydrodynamic radius (R_H) of microgel particles. The concentration of the microgel solution was kept ~ 0.1 mg/mL. Scattering data were collected for 20 s per acquisition with a total of 5 acquisitions.

Infrared Spectrophotometer (FTIR)

IRAffinity-1S Fourier Transform Infrared (FTIR) Spectrophotometer (Shimadzu, Maryland, U.S.A.) was used to characterize the azide functionalized core microgels. Lyophilized microgels were analyzed using an attenuated total reflection (ATR) sample geometry. The collected data was analyzed by LabSolutions IR software provided by Shimadzu. Figure S1 shows a representative FTIR spectrum of the lyophilized microgel.

Atomic Force Microscopy (AFM)

AFM was used to study the size and morphology of actively deposited microgels in their dried state. Microgel monolayers were assembled on amine-functionalized (APTMS)

glass coverslips by the method described above. An MFP-3D origin AFM (Asylum Research, Santa Barbara, CA) was used to analyze microgels on a substrate. Conical-shaped silicon AFM probes with Al reflex coating ($k = 42 \text{ N m}^{-1}$) were mounted on the cantilever holder and operated in AC mode. AFM data were processed using the supplied software written in an IgorPro environment (Wavemetrics, Inc.).

Optical Microscopy (OM)

Microgels were imaged in the swollen state using optical microscopy on a Zeiss Axio Imager 2 (Carl Zeiss Microscopy, LLC White Plains, NY). The solution of microgel was dropped onto a microscope slide and covered with a 1.5 mm coverslip before adding a drop of immersion oil (refractive index 1.515) to the top of the coverslip. All the images were captured using plan Apochromat 100x/1.4 oil objective in differential interference contrast mode. The same objective was used to capture fluorescence images. Optical micrographs were recorded and processed with ZEN imaging software (ZEN 2.5) provided by Zeiss.

Osmotic Stress Experiments

A microgel solution was confined in a dialysis tube (Biotech CE Dialysis Tubing, 8 kDa MWCO, 18 mm Flat-width) and placed into a solution of polyethylene glycol (MW 20000, molecular weight distribution ranging from $\sim 0.5x$ to $1.5x$, Polysciences, Inc, Warrington, PA). The concentration of the PEG solution was varied from 0.4 wt% to 5 wt%, allowing the osmotic pressure to vary over the range of 1 kPa to 23 kPa. The microgel solution

was kept under osmotic compression for 1 week, and the concentrated microgel was squeezed into micro centrifuge tube for storage.

Steady State Fluorescence Spectroscopy

The fluorescence spectra of microgel solutions were recorded by PTI QuantaMaster 8075 11C Fluorometer by Horiba (Horiba Canada, Inc. Burlington, Ontario). The data was recorded and processed by FelixGX 4.9.0.10206 provided by Horiba. Figure S3 shows the steady state fluorescent spectra of pNIPAmAzideCore1-ULC shell microgels dyed with BDP FL DBCO, Cy3 DBCO, Cy5 DBCO, and Cy7 DBCO fluorophores.

Scanning Electron Microscopy (SEM)

SEM images were taken on a Sigma 300 Electron microscope by Zeiss (Carl Zeiss Microscopy, LLC White Plains, NY). Microgels deposited by active deposition on coverslips were coated with a thin layer of gold/palladium (Au/Pd 80/20%) using a sputter coater model SC7620 by Quorum. Argon gas was used in the vacuum chamber (Zero Grade: 99.999%) fitted with a two-stage regulator with pressure around 5-10 psi (0.5 bar). The sputter coater was run for 45 s at 20 mA to achieve a thickness of ~3-4 nm. Depositing conductive thin films helps inhibit "charging," reduces thermal damage, and enhances secondary electron emission for better imaging. The SEM operating voltage was maintained within 10-25 kV, and the SE2 detector was used with a WD of 10.5 mm. Electron micrographs were recorded and processed with SmartSEM imaging software provided by Zeiss.

Scanning Transmission Electron Microscopy (STEM)

A microgel solution was drop cast onto the TEM grid (Formvar film covered with a thin layer of carbon, 300 mesh TH, Gold, product # 01804G from Ted Pella, Inc) and dried. The operating voltage was set at 2 kV with a working distance of 2.5 mm. The Zeiss 300 Sigma (Carl Zeiss Microscopy, LLC White Plains, NY) was used in STEM mode. Images were recorded and processed with SmartSEM imaging software provided by Zeiss.

Confocal Laser Scanning Microscopy

Microgel solutions were imaged using LSM 880 Confocal microscopy by Zeiss (Carl Zeiss Microscopy, LLC White Plains, NY). The microscope is equipped with the following laser lines: Argon 458, 488 and 516 nm, Diode 405-30 405 nm, DPSS 561-10 561 nm, HeNe 633 nm. A solution of microgels was dropped onto a microscope slide and covered with a 1.5 mm coverslip before adding a drop of immersion oil (refractive index 1.515) to the top of the coverslip. All the images were captured using plan-Apochromat 63x/1.4 Oil DIC M27 objective using the 488, 516, 561, and 633 nm excitation wavelengths. Confocal micrographs were recorded by the Airyscan technique in Superresolution Microscopy (SR) mode and processed with ZEN 2.3 imaging software provided by Zeiss.

Results and Discussion

The crosslinked core microgel was synthesized with different sizes in the presence or absence of SDS, and by slightly changing the procedure of adding GMA into the reaction feed. We also found that slight variations in the addition of GMA during the synthesis of the microgel resulted in changes in the core morphology and size. Scheme 1 shows the three-step, multi-feed polymerization reaction for core synthesis. Initially, the copolymerization of NIPAm and *N,N'*-methylenebis(acrylamide) (BIS) was initiated by ammonium persulfate (APS) in deionized (DI) water at 70 °C and continued for 1 h. Then, the GMA solution was added into the microgel copolymerization mixture. The late addition of GMA allows us to distribute those groups close to the surface of the core by copolymerization with the remaining NIPAm and BIS in the reaction mixture. After another hour of copolymerization, NaN₃ was added to the copolymerization mixture for the azidation of the GMA moieties in situ. The detailed mechanism of this polymerization is currently under further investigation in our group.

Scheme 1. One-pot, three-step synthesis of azide-containing crosslinked cores.

Generally, we found that the slow addition of GMA results in microgel particles that display nanoscale protrusions on their surface and overall hydrodynamic diameters of $\sim 470\text{ nm}$ and $\sim 220\text{ nm}$ without or with added SDS, respectively. Adding GMA in one aliquot in the absence of SDS results in microgels with much larger hydrodynamic diameters ($\sim 900\text{ nm}$) and relatively smooth surfaces. Purified core microgels were characterized by DLS (0.1 mg/mL), and the variation of their size is given in Table 1. The ability to tune the morphology of the microgels is significant as this suggests the ability to tailor the structure of the particles, if needed.

Table 1. DLS data of the crosslinked core microgels in solution

Microgel Name	Hydrodynamic Diameter (nm)
pNIPAmAzideCore1	470 ± 7
pNIPAmSDSAzideCore2	220 ± 12
pNIPAmAzideCore3	880 ± 23

Figure 1. SEM (A) and AFM images (B and C) of the pNIPAmAzideCore1 microgel deposited on functionalized glass coverslips by active deposition. Panel C is the 3D rendering of the AFM data. All images clearly indicate the presence of nanoscale features on the surface of the microgels.

Figure 1 shows the morphology of the core microgel pNIPAmAzideCore1. The microgel contains nanoscale features homogeneously distributed on the surface of the microgels, presumably due to phase separation of the hydrophobic GMA. The size of the nanostructures is around 30-40 nm. When synthesized in the presence of SDS (pNIPAmSDSAzideCore2), we find that the overall particle size decreases to ~220 nm, with the nanoscale heterogeneities remaining, as shown in Figure 2. Finally, Figure 3 shows core microgel prepared without any SDS and with a single-shot addition of GMA. Under these conditions, the pNIPAmAzideCore3 microgel shows a smooth surface, suggesting that the rate of GMA addition can provide some control over phase separation. The precise mechanisms associated with this nanoscale phase separation are currently under investigation and will be discussed in a future publication.

Figure 2. AFM (A and B) and SEM (C) images of the pNIPAmSDSAzideCore2 microgel. Panel B is the 3D rendering of the AFM data. Nanoscale features are clearly visible in all the images. Samples were prepared by depositing the microgel on functionalized coverslips by active deposition.

Figure 3. AFM (A) and an associated 3D rendering of the AFM data (B) and (C) SEM of the pNIPAmAzideCore3 microgel. Samples were prepared by depositing the microgel on functionalized coverslips by active deposition. Note that the image appearance is smooth under a variety of imaging conditions, suggesting that any phase separation present in the particles is not visible via AFM or SEM.

Core-shell microgels were synthesized using pNIPAmAzideCore1 and utilizing seed and feed polymerization. Scheme 2 shows the general procedure, where core particles are collapsed at 70 °C and serve as nuclei for further polymerization, allowing the shell to

grow preferentially on the core particle surface as opposed to forming new particles in the continuous phase. Since the core is highly crosslinked (10% BIS) and the ULC (0% BIS) shell is added under conditions when both phases are collapsed, monomer and polymer chain penetration into the core should be inhibited during shell addition. As described in numerous publications previously, this affords microgels with well-defined core and shell structures.[5, 19, 33-35, 37, 39] Figure 4 shows the DIC micrographs of the parent core and daughter core-shell microgels; the presence of the shell is clearly visible in Figure 4B; the very different crosslinking densities present in the core and shell components provides for excellent contrast between those two particle regions. The particle size of the core-shell microgels in the hydrated state is $\sim 2.2\ \mu\text{m}$

Scheme 2. The synthesis of core-shell microgel through a seed-mediated feed polymerization.

Figure 4. OM images of pNIPAmAzideCore1 (A) and core-shell microgels (B) in aqueous media.

The core-shell microgel was further characterized by electron and probe microscopies, as shown in Figure 5. The SEM images in Figures 5A (backscattering) and 5B (transmission) show the presence of a low-density shell surrounding the highly crosslinked core. Similar features are observed via AFM, as shown in Figures 5C (height) and 5D (phase). Previous studies suggested that the height of ULC microgels are only on the order of a few nm when deposited by active deposition and then dehydrated, as a result of their extremely low polymer segment density. The AFM data presented here are aligned with those previous results, as the height of the core is ~ 80 nm in Figure 5, which is similar to that observed in Figure 1, while the shell region is ~ 3 nm in height. In other words, the amount of dehydrated polymer thickness added to the core is not easily discerned when looking at the core component, and is only directly measurable when visualizing the shell portions that are spread on the substrate away from the core.

Figure 5. Morphology of the core-shell microgels as determined by SEM (A), STEM (B), and AFM (C, D and E). Here C and D show the height and E shows phase images,

respectively. In C, the shell is clearly visible due to the small z-scale. However, rescaling the image (D) to observe the core surface does not permit direct observation of the shell. The images in A, C, D and E were taken from core-shell microgel deposited by active deposition. A dilute microgel solution (0.01 mg/mL) was drop cast onto the TEM grid and dried before taking image B by STEM.

Thus far, we have described the design of a core-shell microgel where the core is highly crosslinked, whereas the shell is based on a ULC synthesis, allowing us to have a core-shell construct with vastly different mechanical properties in each component. Furthermore, as described previously, the thermoresponsive nature of the core and the dual pH and thermoresponsive properties of the shell results in somewhat independent tuning of the collapse of each component. Finally, the presence of azide units in the core gives us the additional potential of localizing alkyne-containing moieties to the core via click reactions, while the AAc groups in the shell can act as additional handles for immobilization of other functionalities such as proteins.

To illustrate the facile nature of the core-localized click reactions, core-shell microgels were functionalized with fluorophores containing the strained alkyne dibenzocyclooctyne (DBCO). Others have shown the cycloaddition of DBCO with azides to be rapid at room temperature with very high conversion and without the need for catalyst or activating agent.[60, 61] Scheme 3 shows the general functionalization of microgels with fluorophore Cy7 DBCO as a representative synthon. Figure 6A shows the DIC, and Figure

6B shows the epi-fluorescence image of the same microgel in the same area. One can see the core of the microgel labeled with the fluorophore, which indicates the localization and coupling of the dye to the core, demonstrating the specificity and control of this method. When we further visualized the dyed core-shell microgels by confocal microscopy (excitation wavelength 633 nm) using airy scan technique in superresolution mode not only are we able to visualize the dyed core, but the internal nanostructure of the core can also be imaged (Figures 6C and 6D). Note however, that quantitative determination of the sizes of the nanostructures is not possible via confocal. For example, Figure 1 seems to suggest that the nanostructures on the order of 30-40 nm, while we see ~200 nm features via confocal. Nonetheless, with a reported lateral resolution of 140 nm and an axial resolution of 400 nm, it is impressive that we can see these features via the airyscan technique. Epi-fluorescence and confocal images also suggest that the attachment of the fluorophores is selective to the core, and we do not observe dye in the shell region under any imaging conditions.

Scheme 3. Functionalization of the core of the core-shell microgel with Cy 7 dye by the DBCO-azide reaction.

Figure 6. Optical microscopy images of core-shell microgels. A is the DIC micrograph, and B is the epi-fluorescence micrograph of the core-shell microgel in aqueous solution. The DIC image clearly shows the appearance of a low refractive index shell surrounding a dense core. C and D are the confocal microscopy images obtained by the airy scan technique. The nanostructured core can be visualized in this manner; panel D clearly shows that the core fluorescence is not uniform, presumably due to the non-uniform distribution of azide groups.

Given the extremely soft and deformable nature of the shell, we sought to interrogate how core-shell microgels packed under osmotic compression. Figures 7 and 8 show the DIC and confocal microscopy images of dyed core-shell microgels under increasing osmotic pressures. The inset to Figure 7D shows that the dyed core-shell microgels display iridescence under high packing pressures (23 kPa), indicating crystalline packing of the core-shell microgels. However, the associated DIC image does not clearly show this crystalline arrangement, perhaps due to disruption of the long-range order following transfer from the dialysis tube to the microscopy cell. It should be noted that the presence of the shell becomes less evident with increasing applied osmotic pressures (Figure 7). The origin of this change in contrast is not clear at this point and is the topic of further investigation. It is also important to point out that the shell cannot be collapsed completely, even at the highest pressure of 23 kPa. Similar behavior is observed via confocal microscopy, as shown in Figure 8. Together, these results illustrate the mechanical robustness of the ULC shell in the swollen state despite its extremely low segment density.

Figure 7. OM images of core-shell microgels dialyzed against different osmotic pressure PEG solutions. Here, A, B, C, and D show core-shell microgels subject to 1, 2.6, 7 and 23 kPa, respectively.

Figure 8. Confocal microscopy images of core-shell microgels dialyzed against (A) 1 (B) 2.6 (C) 7 and (D) 23 kPa osmotic pressure PEG solutions.

To show the versatility and ease of one-step click reactions for core labeling, we employed several dyes that cover the entire visible range. The same core-shell microgels were labeled with fluorophores displaying 509, 570, and 662 nm emission wavelengths using the identical click protocol to that described above. The chemical structures of all the

fluorophores used in these studies are shown in Figure S2. Figure S4 shows the DIC images of the core-shell microgels labeled with the different dyes, while Figure 9 shows their confocal micrographs using different emission channels. Figure 9E shows the confocal image obtained from a solution of all four differentially labeled core-shell microgels in a mixture. We also functionalized pNIPAmSDSAzideCore2 and pNIPAmAzideCore3 cores with different fluorophores separately without any shell added. Figures S5 and S6 show the confocal micrographs of the cores labeled with different dyes.

Figure 9. Confocal micrographs of dyed core-shell microgels labeled with (A) BPD FL DBCO, (B) Cy3 DBCO, (C) Cy5 DBCO, and (D) Cy7 DBCO. The images from A-D were

captured individually using excitation wavelengths 488, 516, 561, and 633 nm, respectively. E is the confocal micrograph of all four dyed core-shell microgel solutions mixed together and captured by irradiating with all the wavelengths simultaneously. The scale bar is the same for all images.

The synthetic method we have presented allows for functionalization of the core with single dyes without labeling of the shell. In principle, this method should also allow us to create core-shell microgels with multiple different probes attached. To further explore the possibility of designing multi-probe core-shell microgels, we synthesized a core-shell microgel with all the fluorophores attached to the core. This was achieved by simply allowing the core-shell microgel to react with all the fluorophores simultaneously under equimolar conditions. Figure 10 shows the confocal images of the multi-fluorophore core-shell microgels. Figures 10A-D were obtained via illumination in the different individual channels, while 10E was obtained by illuminating the microgels with all the excitation wavelengths simultaneously. It is clear from these images that robust coupling of all dyes simultaneously is possible, but it also appears that there is a significant distribution of labeling densities, as the different particles show different fluorescent properties under broadband illumination (Figure 10E). The control of multi-dye labeling reactions is currently under investigation.

Figure 10. Confocal micrographs of multiply-dyed core-shell microgels captured at excitation wavelengths (A) 488, (B) 516, (C) 561, and (D) 633 nm. E is the confocal

micrograph of multiply-dyed core-shell microgels captured by irradiating with all the wavelengths simultaneously. The scale bar is the same for all images.

Conclusions

In summary, we report a simple approach for the synthesis of azide-containing core microgels upon which a ULC shell can be deposited. The presence of azide groups in the core was used to attach dyes with alkyne-containing moieties via a simple room temperature catalyst-free click reaction. This eliminated the problem of cross-reactivity of different functional groups present in microgels for different applications. The versatility and ease of one-step click reaction for core labeling were tested with several dyes covering the entire visible and near IR range. We find that the presence of ULC shell does not interfere with the dye addition, and the selectivity of the click reaction retains the ULC shell unmodified, thus allowing us to use AAc groups in the shell as additional handles for immobilization or sensing. Additionally, we found that these extremely soft and deformable shells retain their network integrity under high osmotic stress confirming the mechanical robustness of the attached shell. The utility of the azide-alkyne cycloaddition reaction was further explored to make multi-probe core-shell microgels containing all the fluorophores attached to the same core. This core-shell construct allows us to design systems for applications that warrant the same microgel to have distinct compartments and characteristics for manipulating them individually without sacrificing the uniqueness of their properties.

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Conflicts of interest

Dr. Lyon is a co-founder and holder of an equity stake in SelSym, Inc., an early-stage biotechnology company aimed at the commercialization of artificial platelet technologies based on ULC microgels.

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Supporting Information

Design and Synthesis of Core-Shell Microgels with One-step Clickable Crosslinked Cores and Ultra-low Crosslinked Shells

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Figure S1. The FTIR spectrum of Azide containing pNIPAmAzideCore1 microgels.

BDP FL DBCO

Cy3 DBCO

Cy5 DBCO

Cy7 DBCO

Figure S2. The FTIR spectrum of Azide containing pNIPAmAzideCore1 microgels.

Figure S3. Steady-state fluorescence spectra of the pNIPAmAzideCore1-ULC shell microgels dyed with (A) BDP FL DBCO, (B) Cy3 DBCO, (C) Cy5 DBCO, and (D) Cy7 DBCO fluorophores.

Figure S4. DIC images of dyed core-shell microgels labeled with (A) BPD FL DBCO, (B) Cy3 DBCO, (C) Cy5 DBCO, and (D) Cy7 DBCO. The scale bar is same for all images.

Figure S5. Confocal micrographs of dyed pNIPAmSDSAzideCore2 labeled with (A) BPD FL DBCO, (B) Cy3 DBCO, (C) Cy5 DBCO, and (D) Cy7 DBCO. The aggregates of the pNIPAmSDSAzideCore2 is clearly visible in the confocal images. The images from A-D were captured individually using excitation wavelengths 488, 516, 561, and 633 nm, respectively. The scale bar is the same for all images.

Figure S6. Confocal micrographs of dyed pNIPAmAzideCore3 labeled with (A) BPD FL DBCO, (B) Cy3 DBCO, (C) Cy5 DBCO, and (D) Cy7 DBCO. The images from A-D were captured individually using excitation wavelengths 488, 516, 561, and 633 nm, respectively. The scale bar is the same for all images.