Title: Janus Microgels with Tunable Functionality, Polarity and Optical Properties

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Abstract:

A facile self-assembly method is presented to prepare poly (N-isopropylacrylamide) (pNIPAm)-based microgels modified anisotropically with gold nanoparticles (Au NPs) to yield Janus microgels. Transmission electron microscopy (TEM) is used to confirm that microgels are selectively coated on one or both sides with Au NPs. This approach is able to generate microgels with the same (monopolar) or different (bipolar) charge on either side of the microgel surface. The optical properties of the Au NPs adsorbed to the microgel surface are also characterized as a function of temperature and pH. We found that the plasmon absorption of the Au NPs depends on each, which could be explained by the microgel's solvation state dictating the distance between the Au NPs. The surface adsorption behavior of the monopolar and bipolar microgels is also investigated, and we demonstrate that the bipolar microgels exhibit enhanced surface adsorption compared to the monopolar microgels. Finally, we show that the Janus microgel assembly could be controlled by modifying the Au NPs of at least two different sets of Janus microgels with complementary DNA sequences. The work here could find utility for generating surface adsorbed materials with controllable optical properties, sensors, and for studying fundamental behavior of self-assembling materials.

1. Introduction

Micro and nanoparticles modified with two chemically/physically distinct regions are referred to as Janus particles, named after the two-faced Roman God Janus. Janus particles have been synthesized using a variety of novel approaches over the years since their first mention by de Gennes in his Nobel Prize lecture in 1991. Importantly, they have also been used for a variety of applications, e.g., as electronic paper (e-paper). Specifically, first generation e-paper utilized Janus particles that were coated on one side with white titanium oxide, and black polyethylene on the other. The different coatings resulted in optical and electronic anisotropy, which could be used to display black and white images that depended on the potential applied to the particles embedded in a transparent silicone film.[1] In another interesting demonstration, Crespi and coworkers showed that Janus nanorods with Pt and Au segments could be generated, and act as micro/nanomotors. This was due to the ability of the Pt portion of the nanorod to catalytically decompose hydrogen peroxide to generate oxygen bubbles, which propels them.[2]

In addition to the two examples above, there have been numerous other reports demonstrating Janus particle generation using toposelective modification,[3] Pickering emulsions,[4] microfluidics[5] and electrified jetting.[6] In most examples, the resulting particles simply have one side of the particle that is distinct from the other. Although, there are specific approaches that yield particles that have multiple distinct regions spatially isolated on a particle surface.[7] A number of approaches have been used to generate particles with complex and isolated surface chemistries, with sequential masking/unmasking approaches being among the most widely used and easiest to implement.[3, 8] Granick and coworkers reported a two-step μ-contact printing method to synthesize trivalent colloidal particles — these particles can be

modified from both sides while the central region of the particle is left unmodified, resulting in particles with three distinct regions (trivalent). To accomplish this, different silane "inks" were transferred onto two different polydimethylsiloxane (PDMS) stamps by spin coating. One PDMS stamp was brought into contact with one side of a layer of silica particles that were previously deposited on a flat substrate. With applied pressure, the silica particles adhered to the first PDMS stamp, which allowed them to be lifted off the flat substrate. Then, a second PDMS stamp containing another silane ink was brought into contact with the exposed, unmodified side of the silica particles, which allowed the ink to transfer to that side of the particle. Sonication allowed the modified particles to be released from the surface and isolated.[7] In another example, Möhwald's group developed an approach to generate particles with many multiple distinct regions spatially isolated on a particle surface. [9] This was accomplished using surface-adsorbed colloidal spheres as masks when evaporating layers of Au followed by reactive ion etching steps to yield microparticles with Au "dots" spatially arranged on particles similar to sp, sp^2, sp^3 molecular orbitals. These Au-modified regions of the resultant particles can be modified and treated as binding sites to form complex clusters, and offers a new route to creating complex assemblies with novel physiochemical properties. In yet another example, Huskens' group coated a monolayer of silica particles with a sacrificial layer of poly (methyl methacrylate) (PMMA). Then, the PMMA layer was partially removed by O_2 plasma, exposing some portion of the silica particles, which depended on the time of plasma exposure. Only the exposed portion of the particles could then be chemically modified. Later, the sacrificial layer could be dissolved and the unmodified part of the released particles can be further functionalized with another chemistry. [10] Finally, the Serpe Group showed that complex anisotropic particles could be generated using iron oxide microparticles suspended in a pre-gel droplet. The magnetic particles

could be induced to assemble into complex patterns, which could be locked into place by polymerizing the pre-gel solution. The advantage of the approach is that the number, direction and distance between the magnetic microparticle features could be tuned by manipulation of the external magnetic field that directs the organization of the microparticles.[11] Generally speaking, fabricating multi-region anisotropic particles is complex, needs multiple steps, and is time-consuming.

In this submission, we show that gold nanoparticles (Au NPs) can be used to generate anisotropic stimuli responsive particles. Au NPs have been utilized in a variety of applications such as for biological imaging,[12] sensing,[13] and cancer therapy[14] due to their unique optical properties resulting from localized surface plasmon resonance (LSPR).[15] Furthermore, Au NPs have also been used as Janus particle precursors, which have been shown to have tunable optical properties and sensing applications.[16] In many examples, the Au NPs have been adhered to spatially isolated regions of particle surfaces, to generate Janus particles. However, due to their high monodispersity and easy surface modification, most of the investigations used polystyrene or silica as core particles and the Au NPs were selectively adhered to one side of the core particles.[16a, 17] These traditional core particles are generally non-responsive and do not have the ability to modulate their properties (and the Au NP optical properties) in a dynamic and reversible fashion; if this was possible, new applications could be accessible. While there have been efforts to generate such responsive Janus particles, assembly of Au NPs on responsive polymer-based particle cores is much more complex, and not as well understood.

In this submission, we developed a self-assembly method to selectively coat one pole or both sides (poles) of poly (N-isopropylacrylamide) (pNIPAm)-based hydrogel particles (microgels) with Au NPs. Briefly, pNIPAm-based materials are among the most widely studied

stimuli (temperature) responsive polymers to date. PNIPAm-based microgels are well known to be water swollen (and large in diameter) at T < 32 °C, while they are deswollen (relatively small in diameter) at T > 32 °C; the transition is fully reversible over many cycles. The approach presented here is simple, template-free and requires no extra treatments, e.g., creating/removing sacrificial layers. As part of this investigation we synthesized two different sets of pNIPAm-based microgels — pNIPAm-co-N-(3-aminopropyl) methacrylamide hydrochloride (pNIPAm-co-APMAH) microgels, which have a pKa of ~9, and pNIPAm-co-acrylic acid (pNIPAm-co-AAc) microgels, which have a pKa of ~ 4.25.[18] The structures of the monomers are shown in **Figure 1**. Therefore, the pNIPAm-co-APMAH microgels are positively charged at pH<9, while the pNIPAm-co-AAc microgels are negatively charged at pH>4.25; they are neutral at all other pH values. It is important to note that most of the Au NPs, unless specifically mentioned, used in this investigation were capped with citrate. Therefore, microgels with different charges isolated on their surface could be generated with pNIPAm-co-APMAH microgels at pH<9 because one side of the microgels will be positively charged, while the Au NPs will be negatively charged. These microgels are referred to here as being "bipolar", as they are zwitterionic, with the charges isolated from one another on the microgel surface. Similarly, "monopolar" microgels could be generated with pNIPAm-co-AAc microgels at pH>4.25 because both sides of the microgels will be negatively charged. We go on to show that bipolar microgels adhere to surfaces in a manner that is dramatically different than monopolar microgels. Finally, we demonstrate that the anisotropic structure can be used for ordered DNA guided assembly.

2. Results and Discussion

We developed both a "top" and "bottom" modification protocol such that monopolar and bipolar Janus particles could be generated in a simple and straightforward manner, which has not been reported extensively in previous publications.[8] Initial experiments focused on demonstrating that AuNPs can be immobilized on a single side of pNIPAm-based microgels. To accomplish this, we used pNIPAm-co-APMAH microgels and the "top" modification approach, as shown schematically in Figure 2a. In this case, positively charged microgels were first immobilized onto the surface of glass slides, allowing the negatively charged Au NPs to attach to the exposed microgel surface via electrostatic interactions. For example, 1 mL of a 1 mg/mL pNIPAm-co-APMAH microgel solution (pH~5.5) was exposed to a piranha cleaned glass slide for 5 min followed by the addition of 70 nm citrate capped Au NPs. Exposure to EDC solution was then used to covalently link the Au NPs to the microgels. After rinsing and sonication, the modified microgels could be isolated via centrifugation. The "bottom" modification approach is similar to the "top" modification, except for the fact that Au NPs are first attached to the surfaces before microgel addition (Figure 2b). As can be seen from the TEM images in Figure 3 (a-d), Au NPs of different diameters can be immobilized on a single side of the microgels. UV-Vis of the resultant Janus microgels (Electronic Supporting Information (ESI) Figure S3) also revealed the characteristic LSPR absorption peak that is observed for Au NPs dispersed in solution, which provided further proof that the Janus microgels are indeed modified with Au NPs. Furthermore, as can be seen in the TEM images in Figure 3(e,f), Janus microgels with two different diameter Au NPs immobilized on two different sides of the microgels can be generated using a combination of both "top" and "bottom" modification (Figure 2c). This is particularly important when the microgels are modified with "small" Au NPs (15 nm and 30 nm), which are more prone to penetrate the microgels if the "top" modification approach is used as is shown in Figure S4.

We also determined that when the Au NPs size increases, the number of Au NPs per microgel decreases (Figure S5).

For all above experiments, the resultant microgels are referred to as being "bipolar", since the microgels were positively charged, while the Au NPs were negatively charged. In subsequent experiments, we generated Janus microgels by modifying negatively charged pNIPAm-co-AAc microgels with negatively charged Au NPs; these are referred to as "monopolar" microgels. When generating monopolar microgels, cysteamine must be used as a crosslinker since the like charges prevent the electrostatic immobilization of the Au NPs on the surface. To accomplish this, the primary amine group of cysteamine was coupled with the microgel's carboxylic acid group via EDC coupling,[19] leaving the thiol group available to attach to the Au NPs.[20] It should be mentioned here that by exploiting the thiol-Au bond, Au NPs with various chemistries could also potentially be immobilized on the microgel surface. Here, we show that polyvinylpyrrolidone (PVP)-modified Au NPs could also be attached to the surface of microgels. Table 1 shows the various Janus microgels we generated as part of this study, and the appropriate synthetic route.

Next, we go on to show that the temperature-dependent solvation state of pNIPAm-based microgels can influence the LSPR absorbance of the Au NPs attached to the microgel surface. We hypothesize that the change in refractive index of the microgels could have an influence on these properties.[21] Perhaps more importantly, the microgel solvation state should be able to modulate the distance between the Au NPs, which is well known to change the LSPR absorbance of the Au NPs.[22] For example, in the collapsed state, the distance between Au NPs is much smaller than in the swollen state, resulting in relatively strong plasmon coupling between the Au NPs.[21b, 23] In this investigation, we showed that temperature and/or pH could be used to

modulate the LSPR of the Au NPs attached to the microgels and use JM 50 to demonstrate this. First, we used DLS to measure the diameter of JM 50 and how it depended on solution temperature and pH. For the DLS measurements, 2 min was allowed for equilibration after changing the conditions. As can be seen in Table 2, the diameter of the Janus microgels was 643±13 nm at pH=6, T=25 ºC, while the diameter decreased to 414±2 nm at pH=6, T=55 ºC. This decrease in diameter was attributed to the thermoresponsivity of the pNIPAm-based microgels. We point out that the Janus microgels were not stable at high pH (pH=12) and high temperature, and large aggregates formed, and therefore the microgel diameter could not be accurately measured at these conditions. We also show that the diameter of JM 50 depended on pH, exhibiting a diameter of 546±6 nm at pH=12, T=25 ºC, compared to 643±13 nm at pH=6, T=25 ºC. The observed increase in diameter was attributed to the protonation of the microgels, and their resultant swelling. Furthermore, DLS was used to determine the microgel diameter as a function of temperature to determine the LCST for the Janus microgels. The results show that JM 50 has a well-defined volume transition at ~35 °C, pH=6, which is comparable to that of pure pNIPAm-co-APMAH microgels (Figure 5a). In Figure 5b, the heating-cooling cycles of JM 50 show the reversibility of the microgel diameter as the solution temperature is varied above and below the transition temperature. This diameter change can also be observed in the TEM images of microgels dried at 25 and 55 ºC, which clearly shows the smaller diameter of the microgels dried at elevated temperature. We acknowledge that in these experiments drying at both temepratures results in a fully dehydrated state, which should yield microgels with the same diameters. Although, the images clearly show that the deswollen state of the microgels dried at elevated temperature is preserved as is evident in the images.

The corresponding optical properties of JM 50 were subsequently monitored at the same conditions as above to show that the Au NP LSPR absorbance could be influenced by microgel solvation state. As can be seen in in Figure 5(c, d), a red shift and broadening of the LSPR peak at high pH (pH=12) and high temperature (T=55 ºC) was observed. This is consistent with our hypothesis that the change in refractive index and diameter of the microgels could influence the LSPR absorbance of the Au NPs. We also show in the figure insets that the optical properties were reversible over a number of cycles. We point out that since the microgels have a larger response to temperature (from 643±13 nm to 414±2 nm) than pH (from 643±13 nm to 546±6 nm), the response in more pronounced with (and dominated by) temperature.

Next, we investigated the ability of the generated Janus microgels to adsorb to surfaces. Our results revealed that compared to monopolar microgels, bipolar microgels had enhanced ability to adsorb to surfaces, which can be seen by comparing panels 1 and 2 in Figure 6a. Specifically, when we add JM 50 (bipolar particles, pH=6) to the inside of a glass vial, the vial's surface turns visually red, which indicates that the Janus microgels were adsorbed to the glass surface. Alternatively, when the same concentration of JM 50’ (monopolar particles) were exposed to the inside of a glass vial for the same amount of time as JM 50 above, there is minimal adsorption, as indicated by the minimal/no change in the color of the glass. To investigate this further, we collected AFM images of the respective surfaces. The images in Figure S7 revealed that JM 50 formed large clusters on the glass surface, which was distinctly different than the resultant surfaces that were exposed to JM 50'. We went on to show that this phenomenon could be used to coat a polystyrene Eppendorf tube (Figure 6a, panel 3) and a PDMS substrate (Figure 6a, panel 4). We also showed that other bipolar microgels generated as part of this study could also adsorb to surfaces, in a manner similar to JM 50. We point out that

the ability of the Janus microgels to adsorb to surfaces was greatly influenced by deposition pH, which modulates the Janus microgel charge. As a result, films at pH=12 or pH=3 were not stable.

In order to explain the observed phenomenon, we measured the zeta potential of JM 50 at pH=3, pH=6, and pH=12, and the results are shown in Table 2. The results revealed that at pH 6, the whole Janus microgel was neutral, even though both sides of the microgels should be highly charged at this pH. This is a result of the charges on the two halves of the microgel surface cancelling one another out, which can lead to strong electrostatic attraction between the highly charged halves, with minimal repulsive forces between the microgels as a whole. Although, at pH=3/pH=12 this is not the case, as one side of the Janus microgel has excess charge relative to the other rendering the microgel as a whole charged. This adds more repulsion between the microgels, which we hypothesize greatly influences the surface adsorption ability. Granick's group also showed that Janus particles could form large clusters at their electroneutral state, which supports our hypothesis and observations here.[24]

This ability of these Janus microgels to adsorb to surfaces makes them perfect for generating surface coatings with switchable optical properties in a manner that doesn't require any surface pre-treatment. To demonstrate this potential, we added water (pH 6) to glass vials coated with Janus microgels and evaluated the optical properties via UV-Vis as a function of temperature. As can be seen in Figure 6 (b, c), the optical properties of the films/vial change as the solution temperature is varied from below to above the microgel transition temperature. Specifically, the color of the vial changes from red (T=25 ºC) to purple (T=55 ºC). We point out that the opaqueness of the microgels at elevated temperature had little influence on the film's optical properties in the region of the spectrum where the Au NPs absorb (Figure S8).

Finally, we demonstrated that the Janus microgels generated here could be used as building blocks for self-assembled structures. In this case, thiolated DNA was coupled with the Au NPs on JM 70' microgels; one set of microgels was modified with a sequence of DNA that was fully complementary to DNA that was attached to another set of JM 70' microgels. The full sequences are shown in the experimental section. Figure 7a shows the process schematically, while Figure 7b shows the TEM images of the mixed microgels, and reveals that dimer structures could be achieved.

3. Conclusion

In this investigation, we demonstrated that a simple self-assembly method can be used to modify one or both sides of pNIPAm-based microgels with Au NPs. The Au NPs are extremely versatile, as their surface chemistry can be easily changed by attachment of functional thiols to their surfaces. In this submission, we showed that Janus microgels with the same (monopolar) or different (bipolar) charge on their surface could be generated, and that the optical properties of the Janus microgels could be modulated with temperature and pH, which was related to the solvation state of the microgels. We went on to show that bipolar microgels have enhanced surface adsorption capacity compared to monopolar Janus microgels. Interestingly, the resultant films exhibited tunable optical properties, which could be used for a variety of applications. Finally, we modified the Janus particles with DNA and showed that this property could be used to direct particle self-assembly. Due to the versatility of this system, we feel that these materials could find their way into sensors, and adaptive/responsive optical thin films.

4. Experimental Section

Materials: N-isopropylacrylamide (NIPAm) was purchased from TCI (Portland, Oregon) and purified by recrystallization from hexanes (ACS reagent grade, EMD, Gibbstown, NJ) prior to use. N,N'-methylenebisacrylamide (BIS) (99%), acrylic acid (AAc) (99%) , ammonium persulfate (APS) (98+%), hydrogen peroxide and sulfuric acid (99.999%) were obtained from Aldrich (St. Louis, MO) and were used as received. N-(3-aminopropyl) methacrylamide hydrochloride (>98%) was purchased from Polysciences (Warrington, PA). Deionized (DI) water with a resistivity of 18.2 MΩ·cm was used. All of Au NPs used in the manuscript were purchased from Nanocomposix (San Diego, CA) and concentration is 0.05 mg/mL. Microscope glass slides were and obtained from Fisher Scientific (Ottawa, Ontario) and cut into pieces (25 × 25 mm). All DNA was purchased from Integrated DNA Technologies (Coralville, IA). Tris(2-carboxyethyl) phosphine hydrochloride (TCEP•HCl) and 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) were purchased from Thermo Fisher Scientific (Rockford, IL).

Microgel Synthesis: Microgels were synthesized following previously described protocols.[18] Briefly, a 3-necked round bottom flask was fitted with a reflux condenser, nitrogen inlet, and thermometer, and charged with a solution of NIPAm (11.9 mmol) and BIS (0.703 mmol) in 99 mL deionized water, previously filtered through a 0.2 μm filter. The solution was purged with N_2 and allowed to heat to 70 °C, over ~1 hour. AAc (1.43 mmol) was added to the heated reaction mixture in one aliquot. The reaction was then initiated with a solution of APS (0.2 mmol) in 1 mL of deionized water. The reaction was allowed to proceed at 70 °C for 4 hours under a blanket of nitrogen gas. The resulting suspension was allowed to cool overnight, and then it was filtered through a Whatman #1 paper filter to remove any large aggregates. The microgel solution was then distributed into centrifuge tubes and purified via centrifugation at ~8300 rcf to form a pellet,

followed by removal of the supernatant and resuspension with deionized water; this process was completed 6 times. PNIPAm-co-APMAH microgels were composed of NIPAm (90%), BIS (5%), and APMAH (5%) and synthesized in the same manner as the microgels above. After their synthesis, the microgels were lyophilized and redispersed in water to yield a concentration of 1 mg/mL.

Fabrication of Janus Microgels: In this investigation, two different modification approaches ("bottom" and "top" modification) were used to generate Janus microgels. For both approaches, glass microscope slides were previously cleaned by soaking in piranha solution (H_2SO_4/H_2O_2 7:3 V/V) for 4 h, to remove any impurities from the substrate surface. (***Caution****: piranha solutions react violently with organic materials and should not be stored in closed containers*). The substrates were then rinsed copiously with H_2O followed by 95% ethanol, and immediately used. Using the "bottom" modification approach, piranha-cleaned substrates were immersed in an ethanolic (absolute ethanol) solution containing 1% APTMS for at least 2 h. After 2 h, the substrates were removed from the APTMS solution and again rinsed copiously with 95% ethanol. Then, the substrates were rinsed with H_2O and dried under a stream of nitrogen gas and placed into a Petri dish. Next, 0.5 mL 15 nm Au NPs were added to the glass slides and the Petri dish was sealed to avoid solution evaporation for at least another 5 h and then the substrate was again rinsed copiously with DI water and dried under a stream of nitrogen gas. 20 mg of EDC was added to 1 mL of a 1 mg/mL pNIPAm-co-APMAH microgel solution, and after shaking, the mixture was added to the top of the Au NP-functionalized glass slide and left overnight. The excess microgels were subsequently rinsed off the surface with DI water, 95% ethanol and dried with nitrogen gas. The glass slides were then immersed in DI water and sonication was used to

release the microgels from the surface, yielding microgels in solution with Au NPs attached to one side of the microgels. For the 30 nm Au NPs, all the procedures above were the same except that the volume of Au NPs added onto the glass side was 1 mL.

For the "top" modification method, piranha-cleaned glass slides were soaked in 1 mL 1 mg/mL (pNIPAm-co-APMAH) microgel solution for 5 min. After 5 min, the slides were rinsed with H_2O and 95% ethanol and dried under a stream of nitrogen gas. 0.5 mL of a solution of 70 nm Au NPs were added to the top of the glass slides and the glass slides were placed inside a sealed Petri dish for 5 h. Then 1 mL 20 mg/mL EDC solution was added onto the gold modified glass slides and left overnight. The slides were then rinsed with H_2O and 95% ethanol and dried under a stream of nitrogen gas. The glass slides were subsequently immersed in DI water and sonication was used to release the microgels from the surface, yielding microgels in solution with Au NPs attached to one side of the microgels. Modification with the 50 nm Au NPs was done in the same way, although exposure to the Au NPs solution was reduced to 1 h.

Both the "bottom" and "top" modification approaches were used in conjunction to yield microgels with AuNPs immobilized on two different sides of their surface. To accomplish this, piranha-cleaned glass slides were immersed in an ethanolic (absolute ethanol) solution containing 1% APTMS for at least 2 h. The substrates were removed from the APTMS solution and rinsed copiously with 95% ethanol and H_2O and dried under a stream of nitrogen gas. Next, 1 mL of a solution of 30 nm Au NPs was added on top of the glass slides for at least another 5 h. 20 mg of EDC was added to 1 mL of the 1 mg/mL pNIPAm-co-APMAH microgel solution, and after shaking, the mixture was added to the top of the Au NP-functionalized glass slide and left overnight. The glass slide was then washed by rinsing copiously with DI water and 95% ethanol and dried with nitrogen gas. 0.5 mL of a solution of 70 nm Au NPs was added to the top of the

glass slides for at least 5 h. The slides were subsequently rinsed with H_2O and 95% ethanol and dried under a stream of nitrogen gas. 20 mg EDC was added to 1 mL of MES buffer and was added to the glass slides and allowed to react overnight. The slide was again rinsed copiously with DI water and 95% ethanol and dried with nitrogen gas.

In above case, the pNIPAm-co-APMAH microgels were positively charged, while the Au NPs were negatively charged, therefore all Janus microgels mentioned above are referred to as being "bipolar". "Monopolar" Janus microgels have the same charge on both sides of their surface, and are made in a similar manner as above, except for the use of negatively charged pNIPAm-co-AAc microgels and the use of cysteamine for the microgel modification. For example, consider JM 50' in Table 1; 1 mL of 1 mg/mL pNIPAm-co-AAc microgel solution was added onto APTMS modified glass slides for 5 min (chosen because it yielded the desired surface coverage). After 5 min, the glass slides were rinsed copiously with DI water and 95% ethanol and dried with nitrogen gas. Then the microgel modified glass slides were soaked in 20 mL 30 mg/mL cysteamine solution and left overnight. The slides were then rinsed copiously with DI water and 95% ethanol and dried with nitrogen gas. 1 mL of a 20 mg/mL EDC solution was added on top of the slide and allowed to react overnight. The slides were again rinsed copiously with DI water and 95% ethanol and dried with nitrogen gas. Finally, 0.5 mL of a solution of 50 nm Au NPs solution (citrate surface or PVP surface) was added on top of the glass slides and allowed to react for 1 h.

All the above Janus particles were removed from glass slides by sonication in DI water for further experiments. The concentration of the Janus microgels in DI water was ~ 0.03 nM (by calculation). This calculation was done by imaging a 3 μm × 3 μm area via atomic force microscopy (AFM) and the number of particles in this area was counted. This number was then

used to calculate the approximate number of particles on the whole 1 inch × 1 inch glass slide area. This calculation yielded ~ 2.58×10^{9} particles • $inch^{-2}$. For each Janus microgel solution, the particles were collected from eight 1-$inch^2$ slides via sonication in a total of 1 mL DI water.

DNA Guided Self-Assembly: The DNA functionalization process used here was slightly modified from a previous publication[17b]. In detail, 3 µL of 600 µM thiolated DNA solution was first exposed to 1 µL of 10 mM TCEP solution for 1 h. This was done to reduce the DNA disulfide groups to thiols. The thiolated DNA solution was then mixed with 1 mL of the resultant 0.28 nM Janus microgel solution (from above) and incubated for 12 h. 500 mM PBS buffer (pH=7.4) and 1 % SDS were added to the mixture solution to bring the final concentration to 10 mM PBS and 0.01 % SDS, respectively. To this mixture, 20 µL of 2 M NaCl was added, followed by sonication for 10 s. The salt addition was repeated five times every 20 min and the solution allowed to incubate for 24 h. The sequences of the DNA used here were complementary, and are: 5'- /5ThioMC6-D/ TTT TTT TTT TTT TTT GGT TTG AGT TCT GCT -3' and 5'- /5ThioMC6-D/ TTT TTT TTT TTT TTT AGC AGA ACT CAA ACC-3'. The microgels in solution were then isolated by centrifuging at 8000 rpm for 10 min. The microgels were then resuspended in PBS buffer (10 mM, pH=7.4, NaCl=100 mM, SDS=0.01 %) and the entire centrifugation/resuspension process was repeated a total of 3 times. This was done to separate the DNA modified Janus microgels from the free DNA. The two sets of DNA-modified Janus microgels were then mixed together and allowed to incubate for 24 h for hybridization.

Characterization : UV-Vis spectra were obtained using an Agilent 8453 UV−Vis spectrophotometer equipped with an 89090A temperature controller and Peltier heating device

(Agilent Technologies Canada Inc., ON, Canada). Transmission electron microscope (TEM) images were acquired using a JEOL, JEM 2100 (JEOL USA, Inc., MA, USA) with an accelerating voltage of 200 kV. The specimens were prepared by drying 5 μL solutions of highly diluted samples on carbon coated copper grids. Non-contact mode atomic force microscopy was used to image surfaces (Digital Instrument, Dimension 3100, Veeco Instruments Inc. NY, USA). The microgel diameter and zeta potential was measured using a Malvern Zetasizer Nano Series (Malvern Instruments Ltd, Malvern, UK).

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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