Non-Spherical Janus Microgels Driven by Thiolated DNA Interactions

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Abstract. Janus poly (*N*-isopropylacrylamide)-*co*-acrylic acid/Au microgels that resemble a "snowman", "dumbbell", and an "abalone" were prepared by thermally evaporating a layer of Au on half of the microgel surface, followed by exposure to thiolated single-stranded DNA with complementary sequences. We hypothesize that when the complementary single-stranded DNA attached to the Au forms the more stable double strand, the Au reorganizes on the microgel surface, yielding the observed unique Janus particle structures.

Keywords: Janus microgels, DNA interactions, non-spherical particles

1. **Introduction**.

Named after the two-faced Roman God Janus, the simplest Janus particles[1-2] have one half of their structure chemically and/or physically modified independent of their other half. Additionally, more complex "Janus-like" structures have also been generated.[3-17] The anisotropy of Janus particles makes them suitable for unique applications[3-8] such as spatially-controllable chemical reactions,[6b,c] asymmetric catalytic systems,[3c,4b,c] self-assembled hierarchical structures,[3a-b,6a,7a] and biomimetic colloidal building blocks.[5a,b,7b,8b] During the past decade, numerous techniques have been established to synthesize Janus particles,[9-17] including microfluidic assembly,[9b,c,10a] emulsion polymerization,[11-12] masking coating,[10b,c] and block copolymer self-assembly.[14-17] For example, Xu et al.[9c] generated monodisperse Janus particles (such as rods, disks, ellipsoids) via a microfluidic technique. Weitz et al.[11b] prepared triple rod, triangle, cone, diamond, and snowman-like particles in a well-controlled manner from emulsion polymerization. Granick[10b] achieved "matchstick" particles via asymmetric deposition. Although a plethora of Janus particles with controllable size/shape have been successfully achieved, it is still challenging to fabricate Janus particles with advanced functionality, using a facile protocol.

DNA has found its way into numerous applications due to its ability to be "engineered" via tailoring of the base sequences.[8a,b] DNA has been used as a template to position nanoscale components on the DNA double-strand backbone in well defined 1-D,[18a] 2-D,[18b] and 3-D patterns[18c] or as a linker to attach nanoparticles, proteins, and quantum dots to direct the formation of large periodic structures.[19] However, DNA isn't as prominent in applications involving the design and assembly of asymmetric nanostructures.[8c-f] Herein, we present a simple method to prepare functional hybrid Janus poly (*N*-isopropylacrylamide)-*co*-acrylic acid (pNIPAm-*co*-AAc)/Au microgels. To accomplish this, a thin layer of Au was thermally evaporated on microgels attached to a solid surface as shown in Scheme 1, which yielded microgels half coated with Au. The microgels were then removed from the surface and exposed to a mixture of two thiolated ssDNA molecules, which had complementary sequences. The thiol group is well known to attach to the Au layer, anchoring the DNA to the Au, and the presence of the complementary ssDNA chains induces the aggregation of the DNA. We found that these interactions were strong enough to re-arrange Au on the microgels to yield complex Janus

microgels. Specifically, this treatment yielded particles that exhibited snowman-like, dumbbell-like and abalone-like morphologies.

2. Experimental.

2.1. Synthesis of poly (*N*-isopropylacrylamide)-*co*-acrylic acid (pNIPAm-co-AAc) microgels. Microgels were synthesized following a previously published procedure.[20a] *N*-isopropylacrylamide (NIPAm) (11.9 mmol) and *N, N*-methylenebisacrylamide (BIS) (0.703 mmol) and deionized water (99 mL) were charged into a three-necked round bottom flask. After 1 h of heating to 70 ^{o}C while bubbling N_2 gas through the solution, acrylic acid (AAc) (1.43 mmol) was added into the solution and immediately initiated by adding 0.2 mmol ammonium persulfate (APS), in 1 mL deionized water to the solution. The reaction proceeded for 4 h, and the resultant microgels were purified via centrifugation and resuspension in water 6x.

2.2. Coating of the Al-coated substrate with microgels. A single layer of pNIPAm-co-AAc microgels was deposited on the Al-coated substrate by "painting" a *dilute* solution of microgels onto the substrate. This was a slight modification to what was previously published.[20b] Following painting, the film was soaked overnight in deionized water to remove any microgels not directly adhered to the Al substrate. Subsequently, the film was washed copiously with deionized water to remove any excess microgels from the surface.

2.3 Functionalization of microgels with thiol. Functionalization was accomplished following previously published protocol.[20c] A layer of pNIPAm-co-AAc microgels attached to an Al-coated glass substrate were placed in pH 4.7 MES buffer (Pierce) and 9 mg cysteamine (Sigma-Aldrich, Oakville, Ontario) was added to the buffer. The solution and sample were allowed to mix while gently shaking for 1 h. Subsequently, 20 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) was added to the system, and the reaction was allowed to proceed at 4 °C for 5 h. All samples were then rinsed with deionized water and soaked in pH 7.2 10 mM phosphate buffer saline solution (with 150 mM ionic strength from NaCl) for several hours to remove any unreacted reagents. The samples were again rinsed with H_2O and dried.

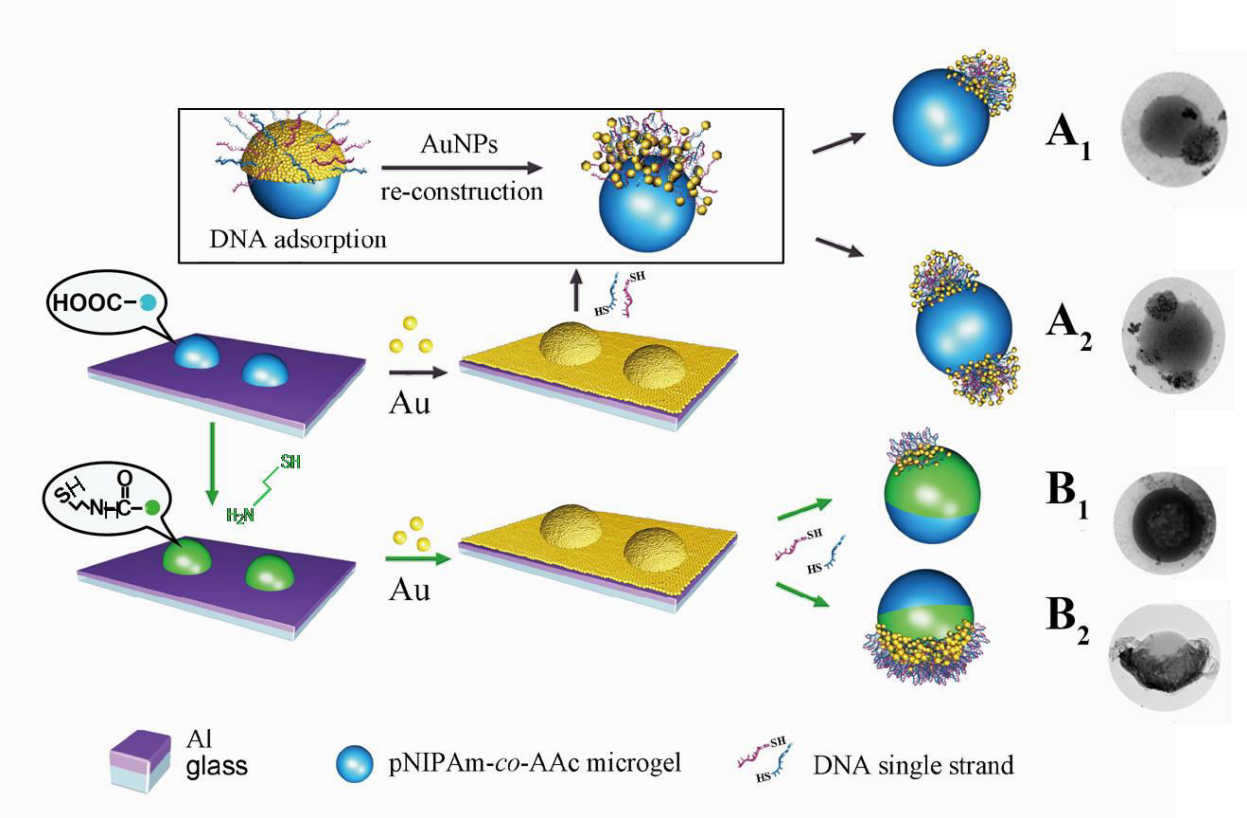
2.4 Coating microgels with Au. Au was added to the microgel via thermal evaporation (Torr International Inc., Model THEUPG, New Windsor, NY) at a rate of 0.1 Å s^{-1}. The thickness of

the Au layer was varied from 0.6, 1.2, 1.5, 2.0 to 5 nm by controlling the evaporation time, and monitored via a quartz crystal microbalance.

2.5 Modification of microgels with DNA.[18d] All DNA was purchased from Integrated DNA Technologies. Prior to use, the disulfide functionality on the oligonucleotides was cleaved by dissolving lyophilized DNA in a DL-Dithiothreitol (DTT, obtained from Sigma-Aldrich) solution (0.1 M DTT, 0.18 M phosphate buffer (PB), pH=8.0) at a mole ratio of 1:2 for DNA:DTT, and incubating at room temperature for 1h. The cleaved DNA was purified using a PD-25 column, obtained from GE Healthcare Life Sciences. Subsequently, the purified thiolated DNA was added to the Au-coated microgels. We added ~10 nmol DNA_a in 250 μL buffer solution (0.01M PBS, 0.01% SDS), and 10 nmol DNA_b in 250 μL buffer solution (0.01M PBS, 0.01% SDS) to a solution containing Au-coated microgels (250 μL buffer solution, 0.01M PBS, 0.01% SDS) according to the timing in the manuscript, and 20 min was allowed to pass before increasing the concentration of NaCl to 0.05 M using 2M NaCl in 0.01M PBS and SDS 0.01% while maintaining an SDS concentration of 0.01%. This process was repeated once more and then increased at 0.1 M NaCl increments until a concentration of 0.7 M NaCl was reached. The salting process was followed by incubation for 12 h at room temperature. To remove excess DNA, the Au-coated microgels were centrifuged and the supernatant solution removed, leaving a pellet of microgels at the bottom of the centrifuge tube. The microgels were then resuspended in fresh PB solution with 0.01% SDS. This washing process was repeated for a total of five times.

Control experiments were done by charging the as-obtained microgels coated with Au with or without Au-S bond into a buffer solution containing 0.01M PBS, 0.01% SDS and 0.01 M NaCl, and then NaCl was gradually added until the concentration reached 0.7 M, the mixture was incubated at room temperature for 12 h. The as-prepared samples were characterized by TEM (JEOL, JEM 2100) and SEM (S-4800, Japan Hitachi, operating voltage of 5.0 kV) with no prior treatment of the samples.

3. Results and Discussion.



Scheme 1. Schematic of the formation process of the hybrid Janus pNIPAm-*co*-AAc microgels. The microgels were either (A1, A2) directly coated with Au or (B1, B2) were coated with Au after modifying the microgels with a thiol group. The process includes directional coating of a Au layer on a single side of microgel and subsequent DNA modification. (Far right), SEM images of the corresponding Janus particles.

Scheme 1 presents the possible formation process for the hybrid pNIPAm-*co*-AAc/Au Janus microgels, along with the proposed mechanism that leads to the Janus microgels with unique morphologies. Firstly, the as-synthesized pNIPAm-*co*-AAc microgels were painted on an Al coated glass substrate to yield a single microgel layer.[20a] Subsequently, a thin layer of Au was then deposited on the microgels using a thermal metal evaporator. Followed by the introduction

of ssDNAa and ssDNAb into the peeled microgel, the unique non-spherical morphologies can be obtained. In this process, the presence or absence of the S group n the microgel surface plays an key role on the resultant morphologies of the Janus microgel.

3.1 Fabrication of Non-spherical microgels in the absence of thiol on the microgel

PNIPAm-*co*-AAc microgels were synthesized by free radical precipitation polymerization[20b] . The morphology of the as-synthesized microgels was characterized by transmission electron microscopy (TEM) as seen in Figure 1a, presenting uniform dimensions and shape. After the microgel is single-layer painted on the substrate, the microgels flatten out on the Al-coated glass substrate as shown in scanning electron microscope (SEM) image in Figure 1b, which is favorable to protecting the one side from Au deposition. Subsequently, a thin layer of Au was then deposited on the microgels using a thermal metal evaporator. In this case, only the side of the microgel that is not adhered to the Al surface is coated with Au. Following Au deposition, the microgels were removed from the substrate by dissolving the Al by exposure to an aqueous solution with a pH of ~3. The as-obtained microgel solution was centrifuged and resuspended in deionized water until a neutral pH was achieved.

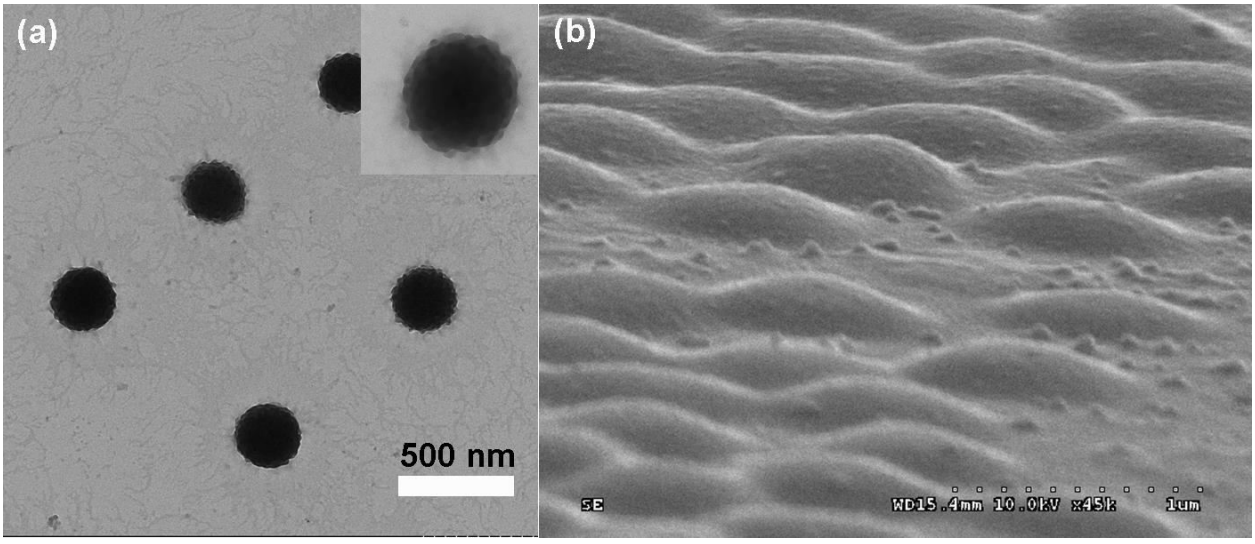


Figure 1. (a) TEM image of the as-synthesized pNIPAm-*co*-AAc microgels, (b) SEM image of a single layer of microgels. As can be seen, the microgels flatten out on the Al substrate, protecting one side from Au deposition. The microgels are the large, rounded features in the SEM.

Figure 2 is TEM images of the resultant microgels after deposition of Au layer with evaporation times of 6 s, 12 s, 20 s and 30 s on the microgels. The Au layer was obtained by thermally evaporating Au on a single-layer of microgels at a deposition rate of *ca.* 0.1 $Å s^{-1}$. As can be seen, Au was coated only on a single side of the microgel, leaving the other side exposed. Furthermore, when the evaporation time is short, it is difficult to form a uniform Au layer, but yields a dispersed island of Au "nanoparticles" (AuNPs) on the surface of the microgels. For example, when the evaporation time is 6 s, only a sparse deposition of "AuNPs" on the microgel surface is observed in Figure 2a. The density and number of AuNPs grow with the increased evaporation time of Au layer, until aggregation of AuNPs occurs at the microgel's surface in Figure 2c with evaporation time of 20 s. Finally, a homogeneous and continuous Au film is formed when the evaporation time of the Au layer approaches 30 s in Figure 2(d). In this case, the microgel's surface is completely covered by the Au layer on a single side. The asymmetric coating of Au on the microgels can be further verified by varying the imaging angle in the TEM. As can be seen in Figure 2(e), changing the observation angle from -20° to 20° allows the asymmetric coating to be unambiguously determined.

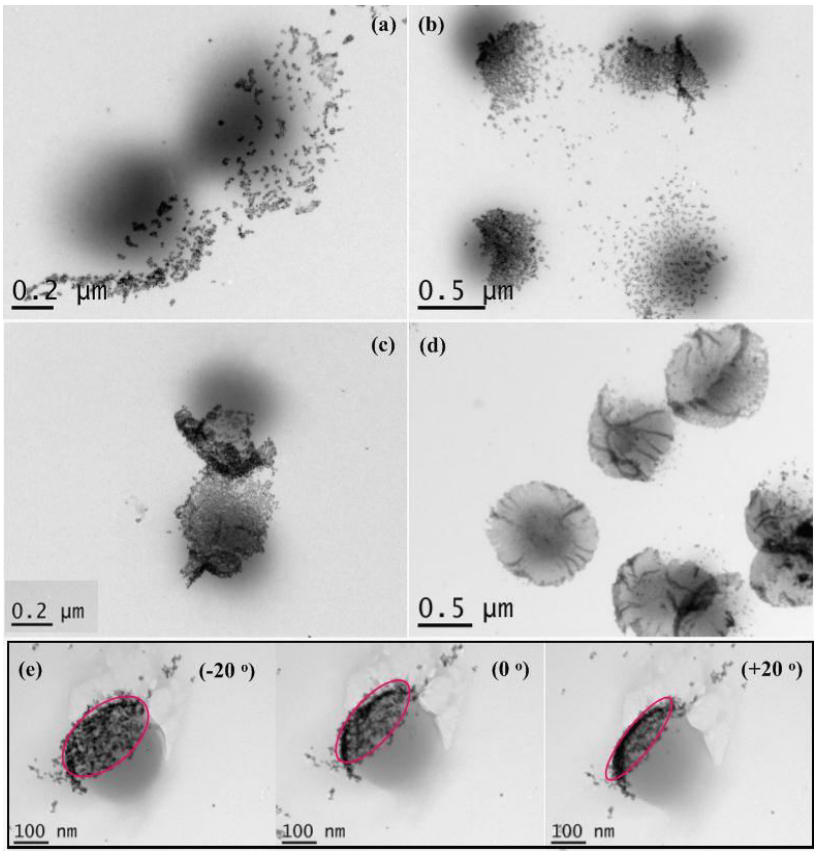


Figure 2. PNIPAm-*co*-AAc microgels half-covered with a Au layer with varying thickness due to the different evaporation times of (a) 6, (b) 12, (c) 20 and (d) 30 s. (e) TEM images of microgels being evaporated for 12 s when varying the observing angles from -20°, 0 ° to 20°. This indicates successful directional modification of the microgels by Au in a high yield. The sample is obtained after removal of the microgels from the Al, but before DNA addition. The red circle indicates the position of the Au cap.

The Janus microgels were then used to selectively deposit DNA on the Au coated half of the microgels. That is, the Au surface of microgel can be functionalized with a thiolated DNA linker, $ssDNA_a$, while the other pNIPAm-*co*-AAc surface remains unmodified. To accomplish this, a solution (250 μL) containing freshly prepared thiol modified ssDNA (5'HS-GCC GAT GGC CGC CG-3') ($ssDNA_a$) was added into a solution of the Janus microgels(250 μL), the process is

followed by the addition of the complementary ssDNA (5'HS-CGG CGG CCA TCG GC-3') ($ssDNA_b$) (250 μL) 2 min later. Specifically, the mole ratio of DNA_a:$DNA_b$was approximately 1:1. Subsequently, the mixture (750 μL) was charged into a buffer solution with volume of 750 μL containing 0.01M PBS, 0.01% SDS and 0.01 M NaCl, and then NaCl was gradually added until the concentration reached 0.7 M to increase DNA loading, according to the literature.[18d] The solution was incubated at room temperature for 12 h. In the process, we hypothesize that the thiolated DNA will attach to the Au layer on the microgels, and since the complementary strands of DNA prefer to bond with their complement, the Au layer restructures to facilitate this interaction. It is expected that thiolated DNA is bound to the Au layer on the microgels, while the pNIPAm-*co*-AAc hemisphere remained largely clear of any DNA, resulting in a asymmetric structure both chemically and physically. As a result, two distinct morphologies either a "snowman" or a "dumbbell" were achieved as depicted in Scheme 1 (A1, A2), which depended on the thickness of the Au layer initially deposited on the microgels. As can be seen in Figure 3, when Au is deposited for less than 8 s, the DNA chain will induce the Au layer to deform and aggregate into a spherical structure on one side of the microgel, forming a snowman-like structure in Figure 3a and 3b. We point out that in all cases, the TEM grid was filled with microgels of similar morphologies, see ESI. When further increasing the Au evaporation time to 20 s, two spherical aggregates of Au can be seen on either side of the microgels, adopting a dumbbell structure in Figure 3c and 3d. We hypothesize that initially ssDNA interacts with Au based on Au-S bond, and then base pair interactions of complementary chains of ssDNA bound to the Au portion of the microgel results in the aggregation of complementary DNA chains. The aggregation process may peel Au from the surface of the microgel owing to the relatively weak

linkage between Au and microgel since there is no adhesion layer, which induces the re-arrangement of Au onto the pole of the microgel for thin AuNPs in Scheme 1 A1.

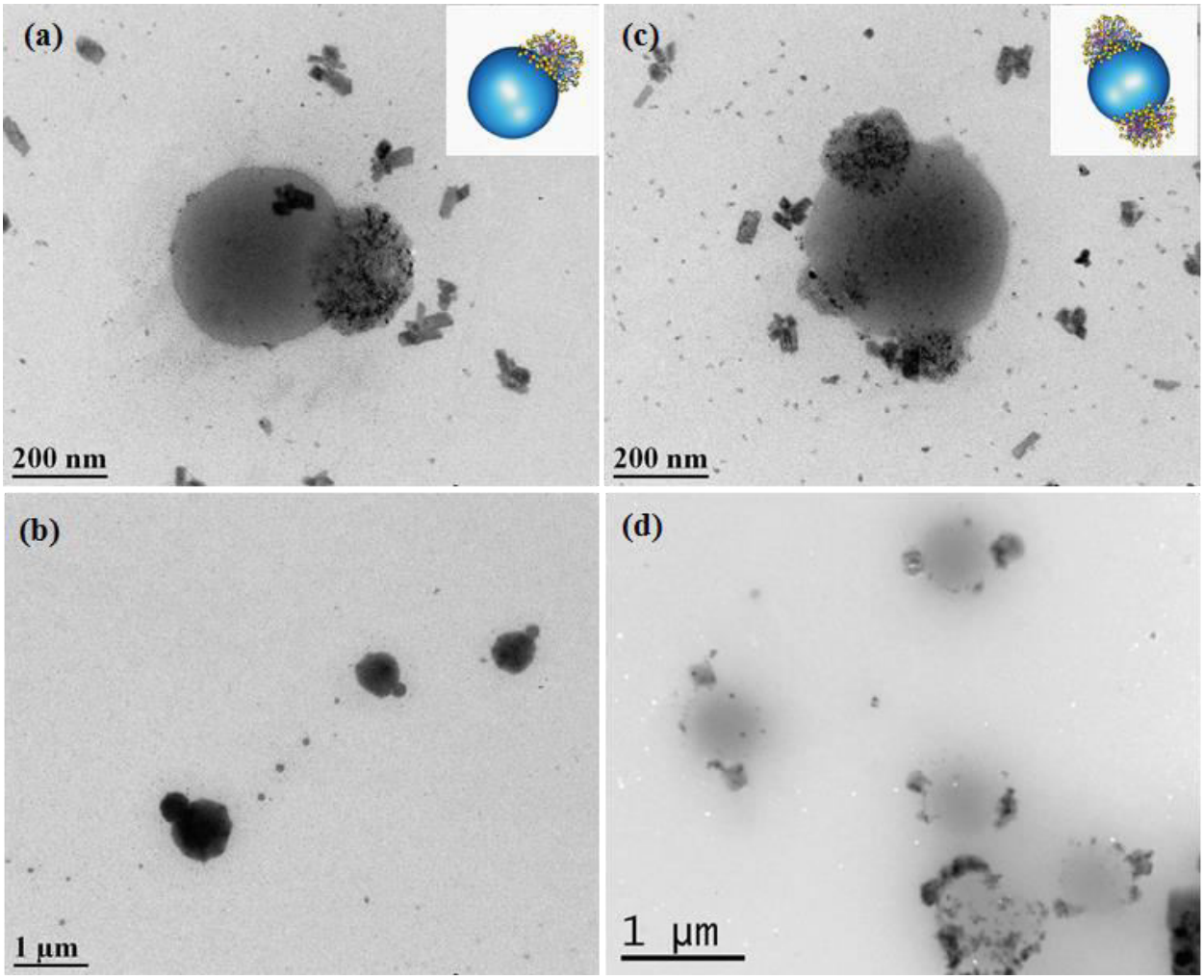


Figure 3. TEM images of Janus microgels after exposure to DNA. The microgels coated with Au with different evaporation time (a,b) less than 8 s, and (c,d) 20 s. The insets show cartoons of the related particles.

3.2 Fabrication of Non-spherical microgels in the presence of thiol on the microgel

If it is the case that this restructuring is allowed due to the weak Au-microgel bond, it should be hindered if we strengthen the Au-microgel bond. To accomplish this, we chemically modified the microgels with a thiol group by exposing the AAc-modified microgels to cysteamine in an aqueous solution containing 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.[19c] The process results in the modification of microgel's COOH groups to SH group as depicted in Scheme 1 (B1, B2). Following thiol modification, we coated the microgels with a Au layer and introduced thiol-modified DNA into the system, using the same procedure as detailed above for the un-thiolated microgels. The introduction of SH group onto the microgel surface offers a strong interaction between the microgel and the Au, which appears to lock the Au onto the microgels surface even if after the addition of thiolated DNA that ever induced the Au's rearrangement without the thiol present on the microgel. Specifically, when DNA is introduced to the microgels with Au deposited (3 s deposition), a prominent "bump" on a single side of microgel can be observed in Figure 4a and 4d, while an abalone-like structure could be formed with on microgels with a thicker layer of Au deposited (8 s) in Figure 4b and 4e. Additionally, a flower-like structure was obtained with microgels with Au deposited for 20 s, see Figure 4c and 4f. The morphologies obtained in this case are much different than what is observed in Figure 3. This was attributed to the presence of S strengthening the microgel-Au bond, which doesn't allow the AuNPs to rearrange on the microgel surface after DNA addition. Thus, the "thin" AuNP layer resulted in a prominent "bump", while the "thick" AuNP layer led to a flower-like structure. These different morphologies indicate enhanced spreading of DNA around the microgel from the increased dosage of AuNPs from longer evaporation time. Therefore, we concluded that enhancement of linkage between microgel and Au prevents the DNA induced reorganization of the Au on the microgels without the thiols present.

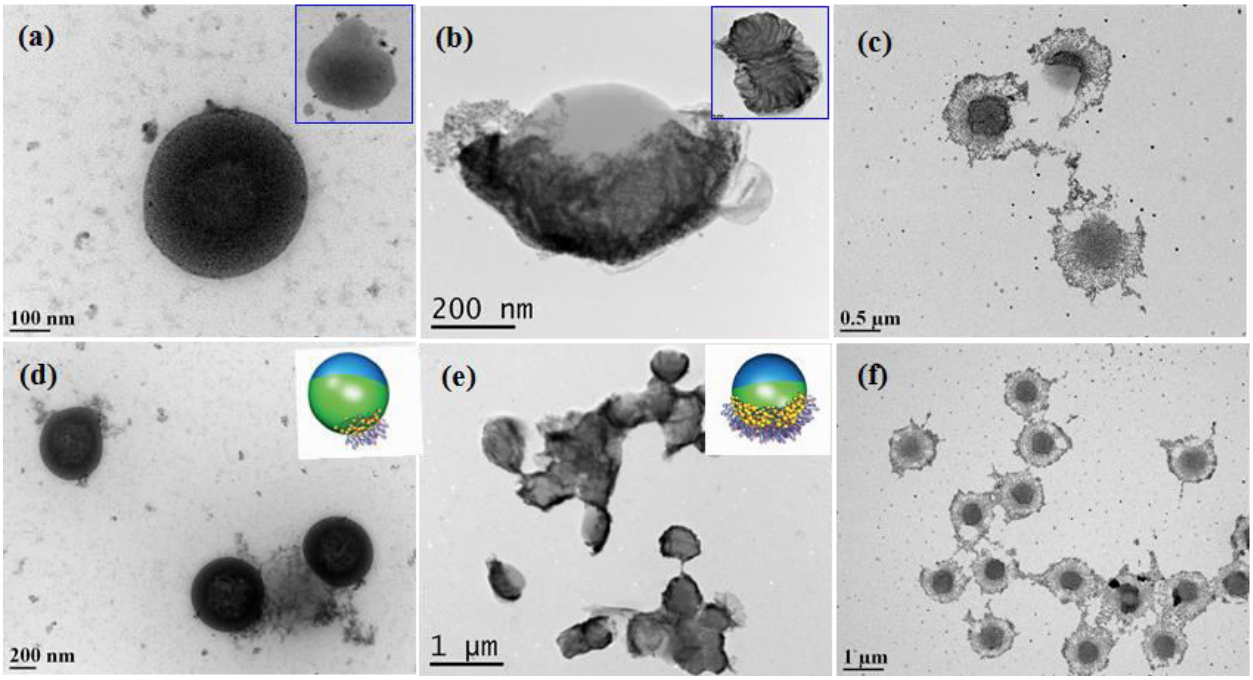


Figure 4. TEM images of thiol-modified microgels after exposure to DNA strand with varying Au thickness achieved using different evaporation times. Au was deposited on the microgels for (a,d) 3 s (b,e) 8 s Au (c,f) 20 s. The inset in (a) and (b) are the microgels observed at different angles, while the insets in (d) and (e) are the cartoon images of the corresponding microgels.

3.3. DNA-mediated microgel aggregation

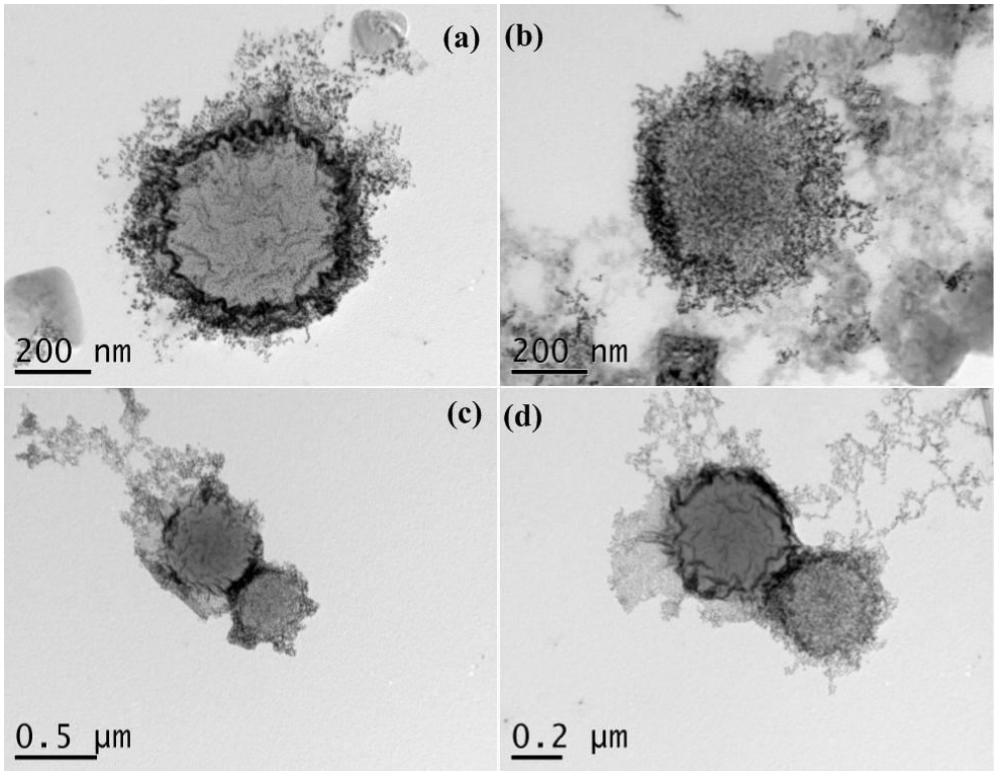


Figure 5. TEM images of the Janus microgels with (a) ssDNAa and (b) ssDNAb bound to their respective Au surfaces. (c, d) assemblies formed after mixing two different sets of Janus microgels containing $ssDNA_a$ and $ssDNA_b$.

To investigate whether DNA base pairing could induce microgel aggregation, we generated one batch of Janus microgels with ssDNAa and another batch with ssDNAb. For this experiment, we utilized the approach outlined in Scheme 1B. Microgels with different diameters were modified with the different DNA strands, for example, microgels with an average diameter of ~760 nm (by TEM) were modified with ssDNAa, while microgels with an average diameter of ~440 nm (by TEM) were modified with ssDNAb (TEM images are shown in Figure S10). Following the microgel generation, we mixed the two sets of microgels and characterized the

resultant assemblies via TEM. Figure 5a and b shows the TEM images of the Janus microgels modified by $ssDNA_a$ and $ssDNA_b$, respectively. After mixing the two different sets of microgels together, aggregates could be observed, similar to what is seen in Figure 5c and d. We note that the two sets of microgels have slightly different diameters, giving us confidence that the two different complementary sets of microgels are linked together. This phenomenon allows for a novel assembly procedure for generating higher ordered structures.

4. Conclusions

In conclusion, we have shown a simple approach to fabricate hybrid pNIPAm-*co*-AAc Janus microgels. This method only requires deposition of a microgel layer on an Al substrate, followed by deposition of a Au overlayer via thermal evaporation. After Al dissolution at pH 3.0, spherical hybrid pNIPAm-*co*-AAc/Au microgels can be obtained. Furthermore, exposure of these particles to thiolated DNA allowed the Au to restructure, leading to uniquely shaped Janus microgels, which could be controlled by changing the strength of the microgel/Au bond by thiolating the microgel prior to Au deposition. This fabrication approach not only provides a new method for generation of functional Janus microgels with variable surface chemistry (DNA), but also is of great importance for the construction of novel building blocks for directed assembly of higher order structures.

Supporting Information. Materials, experimental section, characterization of Au layer, and microgel covered by Au are included.

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