Vol. 36 · No. 5 · May 2019

Particle & Particle Systems Characterization

Special Issue: Nanoscale Chirality

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Particle & Particle Systems Characterization

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Janus Nanoparticle Emulsions as Chiral Nanoreactors for Enantiomerically Selective Ligand Exchange

*Jia En Lu, Yi Peng, and Shaowei Chen**

Janus nanoparticles capped with a hydrophobic and hydrophilic hemisphere of mercapto ligands can self-assemble into hollow, emulsion-like nanostructures in controlled media. As the nanoparticle emulsions are chiroptically active exhibiting a plasmonic circular dichroism absorption in the visible range, they can be exploited as a unique chiral nanoreactor by selective encapsulation of D-enantiomer into the water phase of the water-in-oil emulsions for directional functionalization of the nanoparticles and endow the resulting nanoparticles with select chirality. This is demonstrated in the present study with gold Janus nanoparticles functionalized with (hydrophobic) hexanethiolates and (hydrophilic) 3-mercapto-1,2-propandiol, and D,L-cysteine is used as the molecular probe. Experimental results demonstrate that D-cysteine is the preferred enantiomers entrapped within the nanoparticle emulsions, where the ensuing ligand exchange reaction is initially confined to the hydrophilic face of the Janus nanoparticles. This suggests that with a deliberate control of the reaction time, chiral Janus nanoparticles can be readily prepared by ligand exchange reactions even with a racemic mixture of ligands.

Chiral metal nanoparticles represent a unique family of functional nanomaterials that has been attracting extensive interest because of their potential applications in diverse areas, such as sensing and catalysis for a specific enantiomer of interest.[1] The nanoparticle chiroptical properties have been observed to arise from the formation of a chiral metal core and/or chiral organic capping ligands. The former can be achieved by using chiral templates (e.g., amino acids, DNA, etc) to direct the asymmetrical growth of the nanoparticle cores,[2] such that the resulting nanoparticles inherit the morphology and handedness from the chiral scaffolds. In the latter, chirality is produced by capping the nanoparticle cores with enantiomerically pure organic ligands. Yet, one may ask, is it possible to selectively functionalize nanoparticles with an enantiomer from a racemic mixture of ligands? This will significantly simplify the preparation procedure of chiroptically active nanoparticles. Such a deliberate selection of the organic capping ligands can be achieved by taking advantage of a chiral nanoreactor. In a previous study,[3] we observed that with hydrophobic (hexanethiolate, C6) ligands on one hemisphere and hydrophilic (3-mercapto-1,2-propandiol, MPD) ligands on the other, gold Janus nanoparticles (JNPC6-MPD) behaved analogously to conventional surfactant molecules and could self-assemble into hollow emulsions. Significantly, the resulting nanoparticle ensembles exhibited positive circular dichroism absorption in the visible range that corresponded to the surface plasmonic resonance (SPR) region of the ensemble structures (600–800 nm),[3] which was accounted for by the vortex arrangements of nanoparticle dipoles on a spherical surface, in sharp contrast to individual Janus nanoparticles that were achiral. This led to preferred encapsulation of select enantiomers into the nanoparticle emulsion cavities, such as D-alanine over L-alanine.[3]

Such a unique property may be exploited for controlled functionalization of nanoparticles with select enantiomers from a racemic mixture, where the nanoparticle emulsions serve as an enantiomerically selective nanoreactor. In the present study, cysteine will be used as the illustrating example. Experimentally, JNPC6-MPD (core dia. 2.7 ± 0.4 nm) were prepared by interfacial engineering based on the Langmuir method, where a monolayer of hexanethiolate-capped gold (AuC6) nanoparticles was formed on the water surface of a Langmuir–Blodgett trough and underwent ligand exchange reactions with MPD dispersed in the water subphase (details in the Experimental Section).[4] The obtained JNPC6-MPD nanoparticles were then dispersed in $CHCl_3$ at a concentration of 1 mg mL^{-1}, and mixed with an equal volume of an aqueous solution of D,L-cysteine (Cys, concentration 10 mM), where the Janus nanoparticles underwent template-less self-assembly into water-in-oil emulsions (**Scheme 1**). As the Cys ligands were encapsulated within the water phase and in direct contact with the MPD hemisphere,[5] ligand exchange reaction would occur, but mostly initiated with the MPD face. The resulting particles were denoted as JNPC6-MPD/$_{DL}$Cys. Fourier-transform infrared (FTIR) spectroscopic measurements show a broad peak between 3500–3200 cm^{-1} (O—H stretch), 2950–2800 cm^{-1} (sp^3 C—H), 1725 cm^{-1} (C=O stretch), and 1065 cm^{-1} (C—O stretch) (Figure S1, Supporting Information),[6] suggesting the successful incorporation of the Cys ligands onto the nanoparticle surface, and the lack of a vibrational peak at 2550 cm^{-1} indicates the absence of free thiol ligands (e.g., MPD and cysteine molecules) in the samples.

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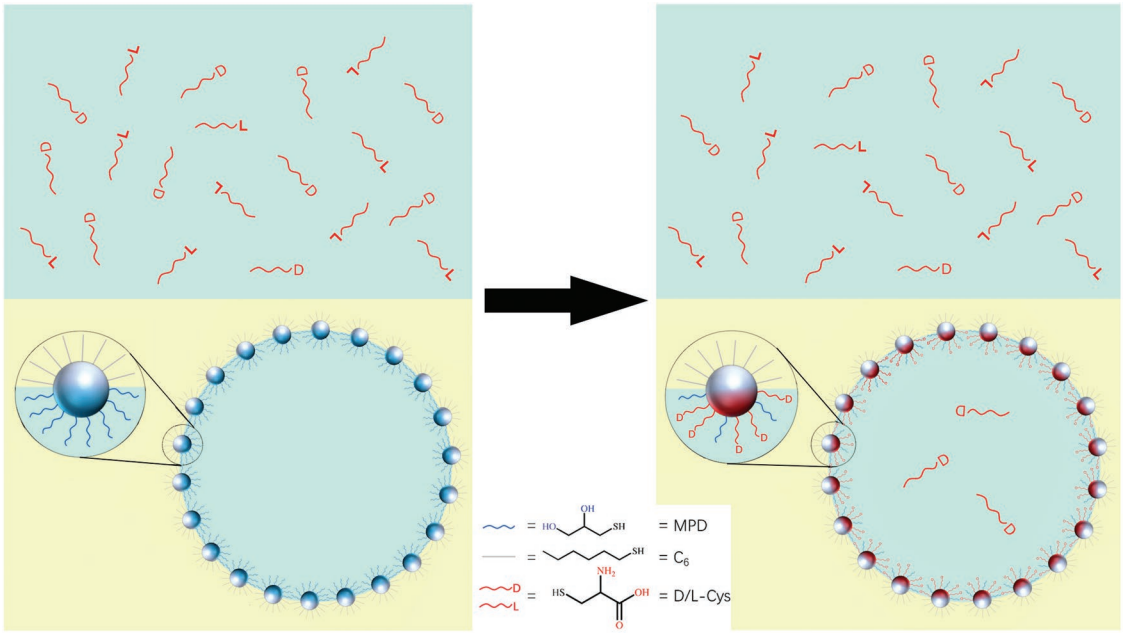
The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/ppsc.201800564.

DOI: 10.1002/ppsc.201800564



Scheme 1. Schematic of enantiomerically selective interfacial ligand exchange of nanoparticles by encapsulation of an optical enantiomer from a racemic mix of ligands.

The ligand composition on the nanoparticle surface was then quantified by ^{1}H NMR measurements (Figure S2a, Supporting Information), whereby the ligands were desorbed from the nanoparticle surface by the addition of iodine. The percent composition of each ligand (Table S1, Supporting Information) was calculated based on the integrated peak areas of the terminal methyl protons of C6 (0.88 ppm), methylene protons of MPD (3.35 ppm), and methine protons of Cys (4.35 ppm).[6] From **Figure 1**, one can see that the as-prepared JNPC6-MPD nanoparticles consisted of about 52% C6 and 48% MPD ligands. That is, the hydrophobic and hydrophilic ligands each accounted for roughly half of the capping ligands on the nanoparticle surface. Within the initial four hours of mixing with D,L-Cys, one can see that the C6 ligand fraction remained virtually unchanged, whereas MPD concentration diminished rapidly to only 15.7%

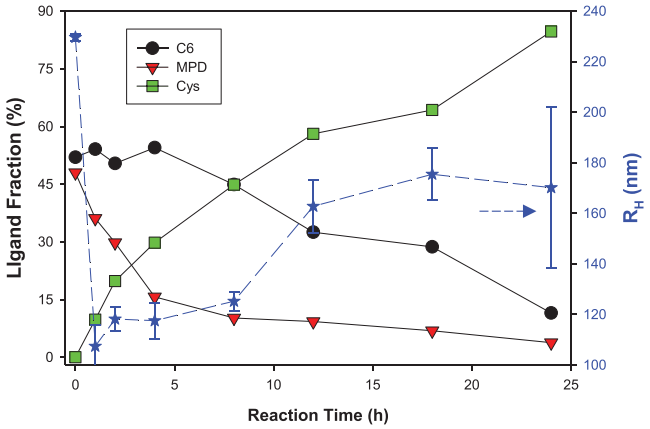


Figure 1. Ligand composition and average hydrodynamic radius of JNPC6-MPD nanoparticle ensembles after exchange reaction with an enantiomeric mixture of D,L-cysteine for different periods of time.

and concurrently Cys became detectable with the concentration increasing accordingly to 29.8%. This suggests that within this time frame, ligand exchange reaction occurred between the nanoparticles and Cys ligands, and the reaction was largely confined to the MPD hemispheres. This can be ascribed to the formation of water-in-oil reverse emulsions by the Janus nanoparticles, where Cys was encapsulated within the water phase, leading to direct contact of the MPD hemisphere with the Cys ligands (Scheme 1). These observations suggest that the Janus nanoparticle emulsions can indeed be exploited as a unique nanoreactor for directional functionalization of nanoparticles.[7] At longer reaction times, the surface concentrations of both C6 and MPD ligands can be found to diminish appreciably, suggesting extensive replacements of both types of ligands by the Cys ligands. For instance, after mixing for 24 h, the surface concentration of the C6 ligand decreased to only 11.5%, MPD to 3.8%, whereas Cys increased to 84.7%.

Interestingly, the nanoparticle ensemble structures remained largely intact during the initial four hours of exchange, as manifested in transmission electron microscopic (TEM) measurements. From **Figure 2**a, one can see that the as-prepared JNPC6-MPD nanoparticles indeed formed hollow emulsions of up to 500 nm in diameter (Figure S3, Supporting Information), as observed previously,[3] and after four hours of exchange reactions with Cys, whereas the shape was almost retained, the size did diminish somewhat (Figure 2b). However, at even longer reaction times, the nanoparticle ensembles appeared to be dismantled (Figure 2c). This is consistent with the variation of the ligand surface concentration (Figure 1). In the first four hours of ligand exchange, the Janus characters of the nanoparticles were maintained except for a different hydrophilic ligand composition. Thus, the hollow structure of the nanoparticle emulsions was unchanged. Yet, at prolonged ligand exchange reactions with Cys, the surface capping ligands of

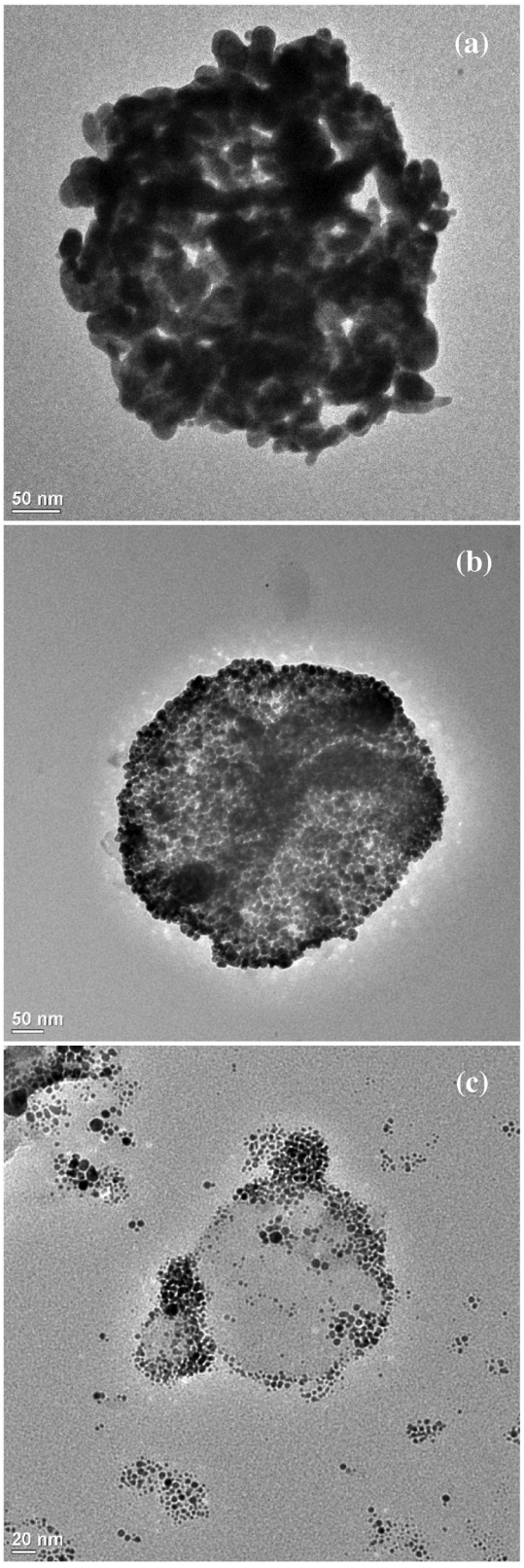


Figure 2. Representative TEM images of JNPC6-MPD Janus nanoparticle ensembles a) before and after exchange reaction with Cys for b) 4 and c) 24 h. Nanoparticle concentration of 0.15 mg mL^{-1} in $CHCl_3$. Scale bars are A,B) 50 nm and C) 20 nm.

the nanoparticles became dominated with Cys. The lack of an amphiphilic character of the nanoparticle surface rendered it difficult to maintain the emulsion arrangement. In fact, one can see that some small, compact ensembles of nanoparticles were formed instead, because of decreasing solubility of the resulting nanoparticles in $CHCl_3$.

Consistent structural insights were obtained in dynamic light scattering (DLS) measurements (Figure S2b, Supporting Information). Figure 1 (blue dashed curve) shows the variation of the hydrodynamic radius (R_H) of the JNPC6-MPD nanoparticles in $CHCl_3$ upon mixing with Cys in water. It can be seen that prior to mixing with the Cys ligands, the JNPC6-MPD nanoparticles showed an average hydrodynamic radius of ≈230 nm, which decreased significantly to ≈107 nm after mixing with Cys for 1 h and remained virtually invariant for up to 4 h. This is most likely due to the partial replacement of MPD ligands on the nanoparticle surface by Cys where the strong hydrogen bonding between Cys of neighboring nanoparticles enhanced packing within the nanoparticle ensembles.[8] In fact, one can see that the nanoparticle ensembles became less porous than the initial ones (Figure 2a,b). However, at longer reaction times, the hydrodynamic radius can be seen to actually increase slowly, although it became increasingly difficult to form nanoparticle ensembles, as revealed in TEM measurements (Figure 2c). This is likely due to the formation of Au(I)-SR polymeric complexes as a result of thiol (cysteine) etching of the gold cores (more discussion below),[9] as manifested by the dark grey enclosure surrounded by nanoparticles in Figure 2c.

UV–vis and circular dichroism (CD) measurements were then carried out to examine the chiroptical response of the nanoparticle ensembles during ligand exchange reactions with Cys. From **Figure 3**a, one can see that prior to mixing with Cys, the JNPC6-MPD nanoparticles in $CHCl_3$ exhibited a broad absorption peak centered around 600 nm, consistent with the formation of nanoparticle ensembles where dipolar coupling between neighboring nanoparticles led to a significant red-shift of the SPR absorption, as compared to that (510 nm) of individual nanoparticles.[3] Upon mixing with Cys for up to 24 h, a significant red-shift of the SPR absorption remained apparent, in good agreement with results from TEM measurements (Figure 2), where formation of ensembles/aggregates of nanoparticles can be clearly seen. In addition, after prolonged ligand exchange reaction, a new absorption peak emerged at ≈370 nm. This likely arose from the combined contributions of interband transitions from Au $5d^{10}$ to 6sp as well as ligand-metal charge-transfer transitions of Au(I)-thiolate polymeric complexes produced in the presence of excess thiol ligands (Cys), as observed previously.[9,10]

The CD absorption profiles varied accordingly. From Figure 3b, it can be seen that for the JNPC6-MPD nanoparticles, positive CD signals (≈10 mdeg) appeared in the wavelength range of 550–800 nm, which was coincidental to the broad SPR absorption observed in Figure 3a. This has been ascribed to plasmonic circular dichroism (PCD) absorption and accounted for by the vortex arrangement of the nanoparticle dipoles when they self-assembled onto a spherical surface forming hollow emulsion-like nanostructures.[3] Upon

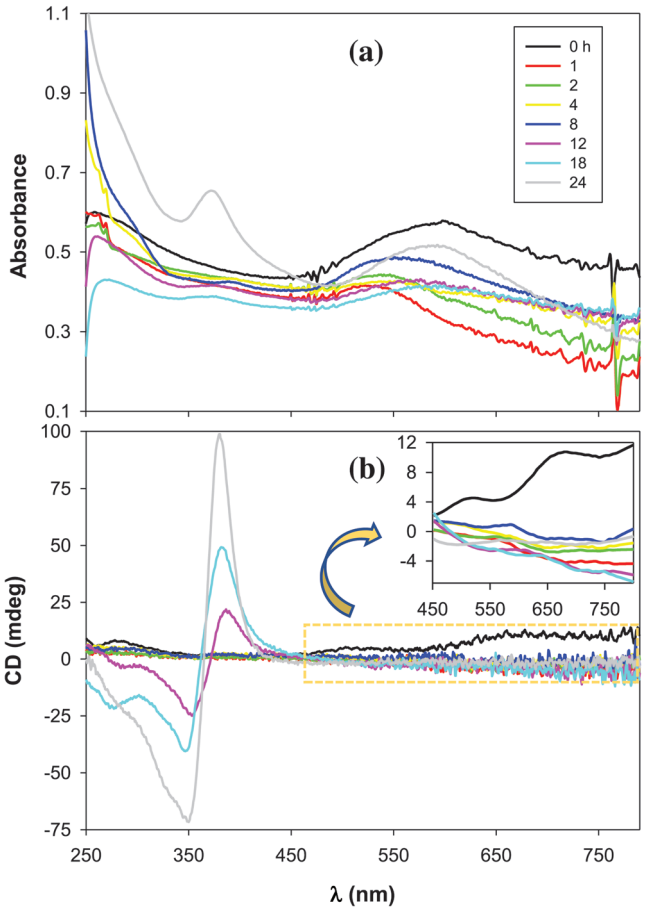


Figure 3. a) UV–vis and b) CD spectra of JNPC6-MPD nanoparticles after exchange reactions with D,L-cysteine for different periods of time. Nanoparticles concentration of 0.15 mg mL^{-1} in $CHCl_3$. Inset to panel (b) is the zoom in of the region between 450 and 800 nm.

mixing with Cys, this PCD absorption actually changed sign and became slightly intensified with prolonging reaction time (Figure 3b inset). In the previous study,[3] a similar transition was observed when JNPC6-MPD emulsions were mixed with D,L-alanine and the change of PCD sign was accounted for by the preferred encapsulation of D-Ala into the emulsion interior because the nanoparticle emulsions behaved analogously to an L enantiomer. This suggests that, in the present study, D-Cys was the preferred enantiomers that were entrapped within the nanoparticle emulsions, and incorporated onto the nanoparticle surface by interfacial ligand exchange reactions. From these results, one can see that with a proper control of the reaction time (e.g., 4 h within the present experimental context), chiral gold Janus nanoparticles could actually be produced due to selective encapsulation and surface functionalization with the D-Cys ligands, in contrast to JNPC6-MPD that were achiral.[3] That is, the Janus nanoparticle emulsions can be exploited as a chiral nanoreactor for selective uptake of a certain enantiomer from a racemic mixture, leading to controlled chirality of the resulting nanoparticles.

In addition, one can see that a CD response started to appear at 300–400 nm with an apparent positive cotton effect after ligand exchange with Cys for 8 h, and the absorption became intensified at longer reaction times. It should be noted that this is completely different from the CD characteristics of free Cys monomers, which only appear in the far UV region (λ < 250 nm) for either enantiomers (Figure S4, Supporting Information). A similar response has been observed previously with Au(I)-thiolate polymeric complex formed by reaction of $HAuCl_4$ with D-Cys.[9] This further confirms that D-Cys was indeed the preferred enantiomers that were encapsulated into the nanoparticle emulsions. The fact that this CD peak only appeared after prolonged ligand exchange reactions with Cys was due to etching of the gold nanoparticle cores by the Cys ligands producing the corresponding chiral polymeric complexes, in agreement with results obtained from TEM (Figure 2) and UV–vis (Figure 3a) measurements. Consistent optical and PCD responses were observed when enantiomerically pure D-Cys ligands were used (not shown).

With D-Cys preferentially encapsulated by the JNPC6-MPD nanoparticle ensembles (in $CHCl_3$) and incorporated onto the nanoparticle surface, L-Cys indeed was found to be in 10.2% excess in the initially racemic mixture of D,L-Cys in water after ligand exchange reaction with JNPC6-MPD nanoparticles for 24 h (Figure S5, Supporting Information). Of the 10.2% D-Cys that was transferred into the organic phase, ≈6.7% was incorporated onto the nanoparticle surface (Figure S6, Supporting Information), and the rest (3.5%) likely in the forms of Au(I) complex and free monomers within the nanoparticle emulsions.

In summary, in the present study we demonstrate that as Janus nanoparticle could self-assemble into hollow, emulsion-like nanostructures that exhibited apparent PCD characteristics, selective encapsulation of a certain enantiomer from a racemic mix of ligands can be readily achieved. This suggests that the nanoparticle emulsions can be exploited as a chiral nanoreactor for directional functionalization of the nanoparticle surface, leading to the formation of controlled chirality of the (individual) nanoparticles, as demonstrated in the present study with D,L-cysteine.

Significantly, it has been previously demonstrated that the chirality of mesophases of achiral monomers can be induced and manipulated by external stimuli, such as vortex agitation and chiral dopants.[11] Such a mechanism may also be exploited for a more deliberate control of the chirality of the nanoparticle emulsions and hence the resulting individual nanoparticles after directional ligand exchange. This will be pursued in further studies.

Experimental Section

Chemicals: Hydrogen tetrachloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$, Fisher, 99%), tetra-n-octylammonium bromide (TOABr, Alfa Aesar, 98%), 1-hexanethiol (C6SH, Acros, 96%), sodium borohydride ($NaBH_4$, Acros, 99%), racemic MPD (Aldrich, 95%), D-cysteine (D-Cys, CHEM-IMPEX INT'L INC., 99.12%), and L-cysteine (L-Cys, MATHESON COLEMAN & BELL) were used as received. All solvents used were purchased from

commercial sources at their highest purities and used without further treatment. Water was supplied by a Barnstead Nanopure water system (18.3 MΩ cm).

Sample Preparation: The preparation of JNPC6-MPD Janus nanoparticles (average core diameter 2.7 ± 0.4 nm) has been detailed previously.[3] For ligand exchange reaction with D,L-Cys, the as-prepared JNPC6-MPD nanoparticles are dispersed in $CHCl_3$ at a concentration of 1 mg mL^{-1}, and a cysteine aqueous solution was prepared at a concentration of 10 mM (5 mM each for D-Cys and L-Cys). The two solutions were then mixed under magnetic stirring for ligand exchange reactions. An aliquot of the organic phase was removed at select time intervals for UV–vis and CD measurements. The nanoparticles were then precipitated by the addition of an excess of methanol and collected by centrifugation.

Characterization: TEM images were acquired with a FEI monochromated F20 UT Tecnai transmission electron microscope operated at 200 kV. UV–vis absorption spectra were obtained with a PerkinElmer Lambda 35 UV-vis spectrometer, and CD measurements were carried out with a JASCO J1500 CD spectrometer. FTIR measurements were performed with a PerkinElmer Spectrum One spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) measurements were conducted with a Varian Unity 500 MHz spectrometer, where the samples were dissolved in $CDCl_3$ and ligands were desorbed from the nanoparticle surface by the addition of a small amount of iodine. DLS measurements were carried out with a Wyatt DynaPro NanoStar temperature-controlled micro-sampler at room temperature. Thermogravimetric analysis (TGA) was performed with a Perkin-Elmer Pyris 1 instrument at the heating rate of 10 °C min^{-1} under a nitrogen atmosphere.

Supporting Information

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

Acknowledgements

This work was supported in part by the National Science Foundation (CHE-1710408 and CBET-1848841). TEM studies were conducted as part of a user project at the National Center for Electron Microscopy of the Lawrence Berkeley National Laboratory, which is supported by the US Department of Energy.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

emulsion, enantiomeric selectivity, Janus nanoparticle, ligand exchange, plasmonic circular dichroism

Received: December 30, 2018
Revised: February 14, 2019
Published online:

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