Wet-Chemical Synthesis of Chiral Colloids

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ABSTRACT: We disclose a method for the synthesis of chiral colloids from spontaneously formed hollow sugar-surfactant microtubes with internally confined mobile colloidal spheres. Key feature of our approach is the grafting of colloid surfaces with photosensitive coumarin moieties, which allow for UV-induced, covalent clicking of colloids into permanent chains, with morphologies set by the colloid-to-tube diameter ratio. Subsequent dissolution of the template yields aqueous suspensions that comprise bulk quantities of a variety of linear chains, including single helical chains of polystyrene colloids. These colloidal equivalents of chiral (DNA) molecules are intended for microscopic study of chiral dynamics on a single-particle level.

KEYWORDS: colloidal synthesis, chirality, helices, UV irradiation, confined assembly

Among the captivating consequences of symmetry-breaking chirality is the coupling of rotational and translational degrees of freedom manifested in the Baranova and Zel’ dovich propeller effect.1−4 Since it is not possible to visualize this intriguing coupling, and other phenomena such as chiral liquid crystal formation, on a single-particle level for chiral molecules, we set out to synthesize chiral colloids. Colloids are similar to molecules in that they are sufficiently small to exhibit Brownian motion in low-Reynolds number regime, but unlike their molecular counterparts they are in situ observable by optical microscopy.5,6 A drawback of reported synthesis methods of chiral colloids is the need for complex, anisotropic building blocks with directional interactions. For example, colloidal helical structures that mimic the DNA helix have been assembled from anisotropic magnetic colloids and amphiphilic Janus spheres.7 Furthermore, template-based (e.g., DNA) and lithographic techniques are often employed.8,9−11 These methods have several downsides such as a low yield,9,9 fragile helical products prone to dissociation,8 clusters subsisting only in an external magnetic field,9 or the need for expensive chemicals and multiple purification steps.10−12

Inspired by a computational study of Pickett et al.,12 predicting that spheres can assemble into helical chains upon cylindrical confinement, spherical particles have been used as building blocks for complex structures.13,14 Yin and Xia15 for example, experimentally explored the preparation of helical chains from simple colloidal spheres confined in V-grooves by capillary forces. However, the obtained structures are immobile and produced in low yield with only a limited number of particles available through slow and batch processes.

Here, we report a synthetic strategy for chiral colloids that is not limited to certain materials or particle properties and that does not require DNA, anisotropic particle shapes or directional interactions. Robust colloids have been prepared with a yield not attainable with conventional (lithography) techniques. Our method exploits the coassembly of microtubes and colloidal particles, developed in our group, to synthesize helical, zigzag and zipper colloidal chains in bulk.16−18 To this end, we designed photosensitive colloids that form covalent bonds upon irradiation with UV-light inside the cylindrically confining environment created by the microtubes. Upon removal of the template, a variety in assemblies is obtained in aqueous suspension. The resulting colloidal chains depend sensitively on the colloid-to-tube diameter ratio.

RESULTS

Coassembly of Colloids and Microtubes. Cyclodextrins (CDs) are donutlike sugar molecules with a hydrophilic exterior and a hydrophobic interior.19 In water, the cavities of CDs can minimize undesirable interactions by incorporating the hydrophobic tails of surfactants. Mixtures of β-cyclodextrin (β-CD) and sodium dodecyl sulfate (SDS) thus form host–guest inclusion complexes in a 2:1 molar ratio at temperatures

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above 40 °C in aqueous media. Upon cooling to room temperature these SDS@2β-CD complexes self-assemble into multiple equally spaced curved bilayers forming a set of concentric hollow cylinders.20 These cylinders are straight and rigid tubes that can be several tens of micrometers long with a pore diameter of 0.9 μm ± 10%. On addition of colloidal spheres, the tubular structures act as a cylindrically confining environment and ordered, chainlike colloid-in-tube assemblies are obtained. As the microtubes form a space-filling structure, all colloidal particles with a size smaller than the microtube diameter appear confined in the tubes. Depending on the size ratio of the colloid-to-tube diameters (see Figure S1 in the Supporting Information), various structures, including helical sphere chains, are formed; three distinct size ratios were chosen for further investigation.

Photoresponsive Particles. Upon removal of the confinement, the formed colloid-in-tube assemblies dissociate. To secure the morphology of colloid structures prior to dissociation, we designed photoresponsive polystyrene spheres for UV-induced cross-linking; see Figure 1. The desired sterically stabilized photoactive colloids in water were obtained by utilizing surface-initiated atom transfer radical polymerization (SI-ATRP) to graft well-defined hydrophilic polymeric hairs from the surface of polystyrene spheres.21,22 The ATRP reaction was quenched to obtain polymer brushes of desired length (≈30 nm), end-functionalized with bromine atoms. These halogen atoms could then be substituted with azides via a nucleophilic substitution reaction.24,25 Alkyne-coumarin molecules could be attached via the efficient and robust click chemistry pathway27 (see Figure 2).

Having successfully synthesized photo-cross-linkable colloids, as characterized with infrared spectroscopy (see Figure S2), dynamic light scattering, and microscopy techniques, the behavior in bulk was investigated. Covalent bonds between neighboring colloids were evidently formed, as the photoresponsive colloids clearly showed the expected photoinduced aggregation in bulk (see Supporting Information). The assembly into chiral structures was subsequently studied.

Assembly into Sphere Chains. Polystyrene spheres with surface-immobilized coumarin derivatives were assembled inside the microtubes with a particle loading of 10 wt %. Upon irradiation of the photoresponsive colloids inside the
microtubes with UV-light with a wavelength of 365 nm, coumarin molecules on adjacent particles photo-dimerized and the colloid-in-tube assemblies were affixed. The cylindrical confinement was subsequently removed by dilution of the system with water, causing the microtubes to disassemble (see Figure 3). The obtained structures exhibit Brownian motion and are mechanically robust as the assemblies can withstand hours of exposure to ultrasonication without disintegrating. Per synthesis cycle about $10^9$ chains are obtained (see the Supporting Information for calculation of overall yield).

Analysis of Formed Structures. The size ratio of the colloid-to-tube diameters ultimately determines the structures that can be formed. To investigate the effect of the size ratio on the assemblies, we employed colloids of different sizes. Figure 4 shows the distribution of structures that are obtained as a function of the colloid-to-tube diameter size ratio. As the particle diameter approaches that of the microtubes (colloid-to-tube diameter ratio of about one), a clear preference for assembly in linear, rod-like chains is observed. Particles that are much smaller than the microtube diameter, do not feel the confinement and are thus randomly distributed throughout the cylindrical tubes forming small and random aggregates. Conversely, particles that are much larger than one micron cannot be incorporated inside the tubes. The structures in bulk resulting from particles that have a colloid-to-tube diameter ratio of about 0.5 vary from linear chains, zigzag and zipper chains to helical assemblies; see Figure 5, Figure S7, and (see Supporting Videos)

Supporting Videos. Typically, a racemic mixture is formed and the chain lengths vary from 2 (dimers) to 30 spheres per chain, with an average chain length of 10 spheres. The average length of the chains can be varied by changing the weight percentage of particles inside the microtubes. An increase in the particle concentration leads to an increase in the number of spheres per chain, but the length is ultimately limited by the extent of the tubes, typically in the range of tens of microns.

DISCUSSION

By utilizing microtubes and photoresponsive polystyrene spheres, freely dispersed complex structures in bulk are obtained. As microtubes tend to be sensitive to changes in concentration, pH, and ionic strength, among others, we developed photoresponsive colloids since light is an external and nondisruptive trigger toward the confinement. The advantage of employing coumarin as the photosensitive glue is that no photoinitiator was required. However, coumarin is hydrophobic and grafting densities had to be kept to a minimum to safeguard colloidal stability and to prevent intraparticle coumarin cross-linking. On the other hand, the surface number density of coumarin molecules had to be sufficiently high to induce colloid attachment.

A wide range of structures is produced for a colloid-to-tube diameter ratio of about 0.5. According to theory, the yield of each species and the pitch of the chiral chains could be...
controlled by fine-tuning the ratio of the colloid-to-tube diameter. As a consequence of the inherent polydispersity in particle size and microtubule diameter, however, it is difficult to fall within these narrow ranges experimentally. Therefore, postsynthesis separation techniques were explored. Established chiral separation methods for molecules, such as chromatography and capillary electrophoresis, are not suitable for colloidal particles as they utilize chirality specific chemical interactions. We employed density gradient centrifugation (DGC) to successfully separate small clusters and single particles from the desired chains. Unfortunately, DGC cannot distinguish between a linear or helical chain composed of 10 spheres. Our chiral colloids might be isolated in a helical flow fied; Aristov et al. demonstrated the separation of left- and right-handed micron-sized chiral particles in a helical fluid flow inside a microfluidic device.

Since the colloidal assemblies show variations in size, geometry, and type of enantiomers, they are ideal for single-particle studies of the dynamics and Brownian motion of colloids with chiral and other geometries, which is currently ongoing work. Furthermore, preliminary results have shown that the synthesis procedure as outlined in this Article is not limited by the chemical nature of the colloids. Photoresponsive silica spheres have been prepared in a similar fashion yielding colloidal silica assemblies. By using metallic functionalization, chiral plasmonic properties can be achieved for the fabrication of optically active media. In addition, our wet synthesis method can easily be performed on larger scales further increasing the yield. Finally, the chiral structure of the microtubes, as a result of the constituent chiral cyclodextrin molecules, could be exploited to synthesize homochiral chains. However, the chiral coupling is currently too weak to influence the confined colloids. If the chirality on the nanoscale could be enhanced, this might lead to homochiral chains but this is a topic for future research.

CONCLUSIONS

In conclusion, we have developed a synthesis method to produce robust colloidal assemblies, including chiral colloids, in high yield. Employing the coassembly of colloidal particles and microtubules, colloidal helices can be assembled from spherical building blocks. Our synthesis route yields photo-cross-linkable colloidal polystyrene spheres that are sterically stabilized with polymer hairs and end-functionalized with coumarin molecules. By exposure to UV-light, the coumarin molecules act as a chemical glue holding the spheres in place even after dissolution of confining tubes. Eventually, a library of ordered structures, such as linear chiral assemblies, zigzag, and zipper configurations of isotropic colloidal spheres, has become available in bulk. These colloidal analogues of chiral molecules are a promising model system to study the role of chirality in the self-assembly and the diffusion of biological macromolecules, such as the DNA helix. Further insight into the functionality of chirality on the molecular scale could aid in the design of chiral materials.

METHODS

Materials. Styrene (St, 99%), divinylbenzene (DVB, 55% mixture of isomers, tech. grade), 2-bromoisobutyl bromide (BiBB, 98%), 2-hydroxyethyl acrylate (HEA, 96%, contains 20–65 ppm mono-methyl ether hydroquinone as inhibitor), sodium sulfate (Na2SO4, ACS reagent, ≥99%), anhydrous), copper bromide (CuBr, 98%, stored under inert atmosphere), N,N,N',N'-pentamethyldiethyle-
0.60 mmol) were mixed with MeOH/H₂O mixture (7:3, v/v) (0.5 mL) in an oven-dried Schlenk flask, resulting in a light green mixture (note: the copper bromide salt does not completely dissolve in the reaction medium). The obtained mixture was degassed by evacuation and refilling with nitrogen (three cycles). Subsequently, PMDTA (29 µL, 0.14 mmol) was injected, resulting in the appearance of a blue/green color and complete solubilization of the copper bromide salt. The degassing procedure was repeated once more to further exclude the presence of oxygen in the obtained catalyst monomer reaction mixture.

In a separate Schlenk flask, the CPs-Br colloids dispersed in a 7:3 (v/v) MeOH/H₂O mixture (0.5 mL, 2 wt %) were degassed by evacuation and refilling with nitrogen (three cycles). After degassing, the dispersion was injected into the monomer catalyst mixture under inert atmosphere. The resulting reaction mixture had a white/green appearance. The ATRP reaction was allowed to run for 40 min at room temperature, after which the reaction was terminated by exposure to air, yielding an intense blue color. The particles were washed three times with the MeOH/H₂O mixture, 10 times with a 50 mM aqueous NaHSO₃ solution and finally three times with water. The NaHSO₃ solution was used to facilitate the removal of the copper bromide salt. The presence of p(HEA) was probed using IR spectroscopy (see Figures S2; 1732 cm⁻¹, C=O vibration of extending functional groups of grafted polymers). Furthermore, DLS showed a clear increase in apparent hydrodynamic diameter (529 nm), providing additional evidence for successful polymer grafting.

**Nucleophilic Substitution of Halogen Chain Ends of Grafted p(HEA) Hairs with Sodium Azide (CPs-p(HEA)-N₃).**

Na₂S (4.5 mg, 0.07 mmol) was dissolved in DMF (0.5 mL). To this solution, a dispersion containing the p(HEA) grafted colloids in DMF was added (0.5 mL, solid content = 1%). The obtained reaction mixture was allowed to stir for 24 h at 70 °C. After this period, the particles were washed with DMF (three times) and water (three times) to remove excess Na₂S. IR spectroscopy was used to verify successful formation of azido-end-functionalized polymers (see Figures S2; 2096 cm⁻¹).

**Synthesis of 4-Methyl-7-(prop-2-yn-1-yl oxy)-coumarin (Alkyne-Coumarin).**

The synthesis was adapted from ref 34. 4-Methyl-7-hydroxy-coumarin (2.5 g, 14.3 mmol) and K₂CO₃ (8.18 g, 59 mmol) were transferred into a 100 mL Schlenk flask containing dry DMF. The dispersion was heated in an oil bath at 85 °C for 24 h. Control samples were enclosed in aluminum foil to prevent the penetration of UV-light.

**Characterization.** IR spectra were obtained using a PerkinElmer FT-IR/FIR Frontier spectrometer in attenuated total reflectance (ATR) mode. The measurements were carried out on powders (obtained by drying the corresponding particle dispersion).

Transmission electron microscopy (TEM) pictures were taken with a Philips Tecnai10 electron microscope typically operating at 100 kV. Bright field images were recorded using a SIS Megaview II CCD camera. The samples were prepared by drying a drop of diluted aqueous particle dispersion on top of polymer coated copper grids.

Scanning electron microscopy (SEM) pictures were taken with a FEI XL30 FEG scanning electron microscope operating at 5–15 kV. SEM samples were prepared by drying a drop of diluted aqueous particle dispersion on top of polymer coated copper grids and sticking these grids on a stub using a conductive carbon sticker, which was coated with a platinum layer of typically 6 nm.

Dynamic light scattering (DLS) was performed using a Malvern Zetasizer Nano instrument using highly diluted aqueous dispersions at 25 °C. The DLS measurements were taken in ten runs of 10–15 individual measurements in backscatter mode (173°). The hydrodynamic particle dimensions are reported as number-average diameters with their corresponding polydispersity index (PDI).

**H NMR spectra** were recorded using a Varian MRF400 400 MHz NMR machine. CDCl₃ was employed as solvent.

**UV-vis spectra** were recorded on a PerkinElmer Lambda-35 spectrophotometer from λ = 450 nm to λ = 250 nm with 1.0 nm intervals. Helma QS quartz cuvettes with a path length of 10 mm were used. Sample concentrations were chosen such that the maximum absorption remained below 2.5.

Optical microscopy images were obtained using a Nikon Eclipse Ti-E inverted microscope equipped with a Hamatsu Orca Flash 4.0 V2 digital camera. A Nikon CFi Apo TIRF objective (100x magnification, N.A. 1.49) was used. Pictures were recorded in bright field mode. For fluorescence microscopy, a Nikon Intensilight CHGFI light source was used with a TRITC filter cube.
Real-time optical microscopy video illustrating Brownian motion of a linear chain (AVI)
Real-time optical microscopy video illustrating Brownian motion of a zigzag chain (AVI)
Real-time optical microscopy video illustrating Brownian motion of a zipper chain (AVI)
Real-time optical microscopy video illustrating Brownian motion of a helical chain (AVI)
Colloids at microtubes, IR spectra, behavior of the photoresponsive colloids in bulk, photochemistry of alkyne-coumarin, calculations of the estimated yield, morphology of the photoresponsive colloids, fluorescence microscopy images, SEM images (PDF)

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Notes
The authors declare no competing financial interest.

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