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Tailoring Micrometer-Long High-Integrity 1D Array of Superparamagnetic Nanoparticles in a Nanotubular Protein Jacket and Its Lateral Magnetic Assembling Behavior

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Supporting Information

ABSTRACT: Tailoring of a micrometer-long one-dimensional (1D) array of superparamagnetic iron oxide nanoparticles (SNPs) was achieved by Mg^{2+}-mediated supramolecular polymerization of a SNP-containing chaperonin protein ($GroEL_{MC}$⊃SNP). The inclusion complex $GroEL_{MC}$⊃SNP formed when ligand-modified SNPs were mixed with $GroEL_{MC}$, a GroEL mutant having multiple merocyanine (MC) units at its apical domains. Upon mixing with $MgCl_2$ in phosphate buffer, $GroEL_{MC}$⊃SNP polymerized via the formation of multiple MC−Mg^{2+}−MC coordination bonds, yielding thermodynamically stable micrometer-long nanotubes encapsulating 1D-arrayed SNPs (NT_{GroEL}⊃SNP). When the NT_{GroEL}⊃SNP nanotubes in phosphate buffer were incubated in a 0.5 T magnetic field, they began to assemble laterally and then organized into thick 1D bundles, where longer nanotubes were more preferentially incorporated. When the applied magnetic field was turned off, such bundles disassembled back to the individual 1D nanotubes. Lateral assembly of 1D SNP arrays in a magnetic field has been theoretically predicted but never been proven experimentally.

Superparamagnetic nanoparticles (SNPs) have attracted broad scientific attention because of their unique properties such as a large magnetic susceptibility in a small dimension, which allows SNPs to assemble in a moderate magnetic field.[1] In sharp contrast with ferromagnetic nanoparticles,[2] SNPs possess a thermally fluctuating magnetic spin under ambient conditions and are therefore magnetically isotropic. In this context, one of essential issues to consider is how SNPs behave magnetically if connected together one-dimensionally (1D). When a magnetic field is applied to 1D-arrayed SNPs, a magnetic moment is generated temporarily in the individual SNPs. The 1D architecture allows a tip-to-tip interaction of these magnetic moments, resulting in the induction of a large magnetic moment along a longer axis of the 1D SNP array in a direction parallel to the applied magnetic field. Here, one may conjecture that 1D arrays of SNPs could repel each other laterally in a magnetic field because their magnetic moments induced along the longer axis are mutually parallel (Figure 1a). Contrary to such a intuitive recognition, theoretical models

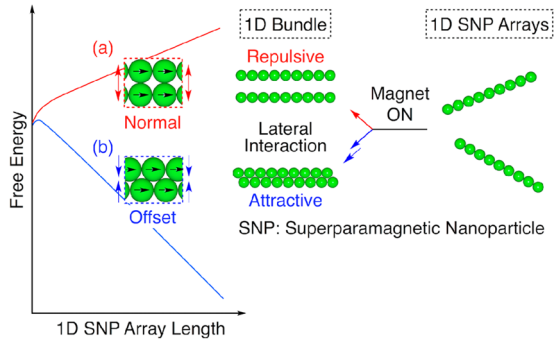

Figure 1. Schematic illustrations of two possible lateral interactions of 1D SNP arrays upon bundling in an applied magnetic field and their array length-dependent theoretical energy diagrams.

have predicted that 1D SNP arrays could laterally attract one another if their parallel orientation adopts an offset geometry as shown in Figure 1b.[3] However, because of the absence of long 1D SNP arrays with a high structural integrity,[4] this theoretical prediction has not been experimentally proven (Figure 1). Desired 1D SNP arrays should also be thermodynamically stable and well dispersed in solution without entanglement. We herein report successful tailoring of such 1D-arrayed SNPs jacketed with a protein nanotube by supramolecular polymerization of a SNP-including chaperonin GroEL mutant. Owing to the noncurvy/nonwavy rigid architecture of this 1D SNP array, we obtained the first unambiguous experimental proof that 1D-arrayed SNPs magnetically assemble laterally to form bundles. Also noteworthy, those bundles disassemble back to the individual 1D SNP arrays when the applied magnetic field is turned off.

Our nanotubular protein jacket is composed of a chaperonin GroEL mutant, which bears zwitterionic merocyanine (MC) units at its apical domains ($GroEL_{MC}$). Recently, we reported that $GroEL_{MC}$, upon mixing with Mg^{2+} in phosphate buffer,

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assembles into a high aspect-ratio 1D nanotubular object by supramolecular polymerization via the formation of multiple MC···Mg^{2+}···MC coordination bonds.[5] This nanotubular assembly is thermodynamically stable and even tolerant under chromatographic conditions. GroEL is a cylindrical double-decker protein assembly having two partially separated identical cavities with an inner diameter of 4.6 nm. It is also known that GroEL can accommodate hydrophobic guests in its cavity.[6,7] We envisaged that, if $GroEL_{MC}$ entraps a properly tailored SNP and the resulting inclusion complex ($GroEL_{MC}$⊃SNP) can be likewise polymerized by the action of Mg^{2+}, a 1D SNP array with a rigid nanotubular protein jacket could be prepared (Figure 2a) as an ideal motif for investigating the magnetic behavior of 1D-arrayed SNPs (Figure 1).

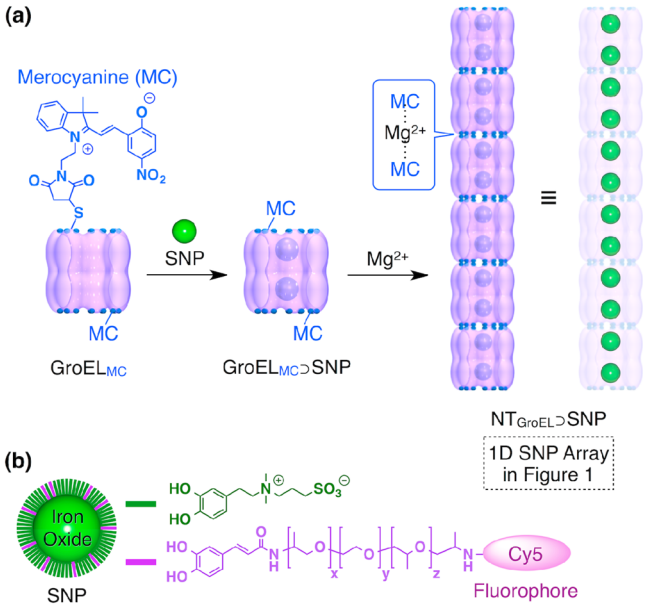

Figure 2. Schematic illustrations of (a) the synthesis of a micrometer-long nanotubular protein-jacketed one-dimensional (1D) array (NT_{GroEL}⊃SNP) of SNPs by Mg^{2+}-mediated supramolecular polymerization of a SNP-containing chaperonin GroEL mutant ($GroEL_{MC}$⊃SNP) and (b) a SNP with a dopamine sulfonate zwitterionic ligand (green bar) shell containing 15% of a catechol-modified hydrophobic dye (purple bar).

The first step toward this goal was to tailor a proper SNP such that it can efficiently be taken up into the GroEL cavity in aqueous media (Figure 2b). We chose an iron oxide SNP because of its high colloidal stability together with well-established chemistry for the size-selective synthesis and surface modification. Because GroEL intrinsically prefers hydrophobic guests such as denatured proteins,[6,7] the guest SNP should be hydrophobic to some extent, while holding a sufficient dispersibility in aqueous media. After numerous trials, we successfully tailored optimal SNPs with a fluorescence reporter function (λ_{em} = 670 nm) by a method using a zwitterionic dopamine sulfonate in conjunction with a dye-appended lipophilic catechol ligand[8] (Figure 2b). Their surface properties were elaborately optimized by changing the mole ratio of two ligands for better hybridization with GroEL and $GroEL_{MC}$.[9,10]

Fluorescent SNPs thus synthesized were mixed in excess with GroEL (2.4 μM) for 4 h in 10 mM phosphate buffer at 37 °C. As shown by the size-exclusion chromatographic (SEC) traces in Figure 3a, the GroEL fraction in the reaction mixture

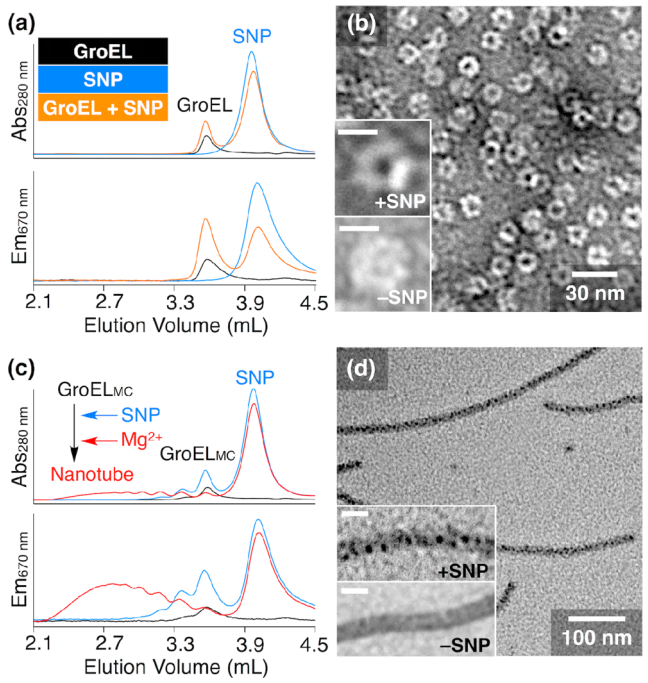

Figure 3. (a) SEC traces, monitored by UV (λ = 280 nm) and FL (λ_{ext} = 647 nm, λ_{em} = 670 nm) detectors, of a mixture of GroEL and SNPs incubated at 37 °C for 4 h (orange), together with those of intact GroEL (black) and SNP (blue) as references. (b) TEM of a mixture of GroEL and SNPs incubated at 37 °C for 4 h. Excess SNPs were removed by ultracentrifugation (scale bar in the inset; 10 nm). (c) SEC traces of Mg^{2+}-mediated supramolecular polymerization of $GroEL_{MC}$⊃SNP, monitored by UV (λ = 280 nm) and FL (λ_{ext} = 647 nm, λ_{em} = 670 nm) detectors. $GroEL_{MC}$ (black) was incubated with SNPs (blue) for 4 h in phosphate buffer (10 mM) at 37 °C to allow the formation of $GroEL_{MC}$⊃SNP, which was then polymerized by the incubation with $MgCl_2$ at 37 °C for 2 h (8 mM) to obtain NT_{GroEL}⊃SNP (red). (d) TEM images of air-dried NT_{GroEL}⊃SNP after the removal of excess SNPs by ultracentrifugation (scale bar in the inset; 20 nm).

gradually became luminescent at 670 nm because of the hybridization with the fluorescent SNP. The formation of inclusion complex GroEL⊃SNP was supported by transmission electron microscopy (TEM; Figure 3b), where the entrapped SNP by GroEL was clearly visualized as a black dot. When $GroEL_{MC}$ having merocyanine units at each apical domain (Figure 2a) was used instead of GroEL, short-chain oligomers spontaneously formed as observed by SEC (Figure 3c, blue). This oligomeric fraction was luminescent, although $GroEL_{MC}$ itself is scarcely luminescent (Figure 3c, black), indicating that the oligomers in this fraction contained the fluorescent SNP. Note that the GroEL family, intrinsically, is prone to a tip-to-tip attractive interaction at the hydrophobic apical domains in aqueous media.[6] We suppose that $GroEL_{MC}$, upon inclusion of hydrophobic SNPs ($GroEL_{MC}$⊃SNP), would have a higher tendency to undergo such a hydrophobic tip-to-tip interaction. At any rate, when $MgCl_2$ was added to the system (Figure 3c), micrometer-long nanotubes formed. The presence of SNPs in the nanotube interior was clearly confirmed by TEM (Figure 3d), where black dots assignable to SNPs align with a high structural integrity along the nanotube. Subsequent ultracentrifugation to remove excess SNPs enabled successful isolation of inclusion complex NT_{GroEL}⊃SNP (Figure S4). By manual counting on multiple TEM snapshots, we figured that

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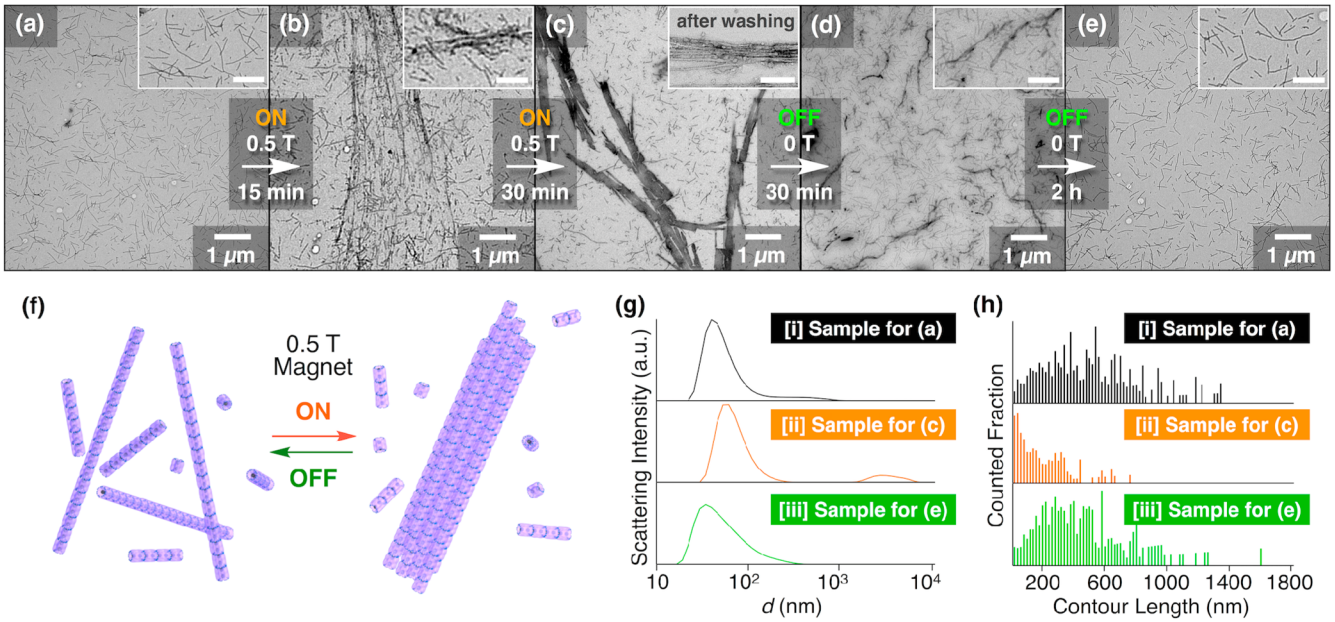

Figure 4. (a–e) TEM images of air-dried NT_{GroEL}⊃SNP samples stained with uranyl acetate. A phosphate buffer dispersion of NT_{GroEL}⊃SNP [sample for (a), scale bar in the inset; 500 nm] was placed inside the bore of a 0.5 T magnet and incubated for 15 [sample for (b), scale bar in the inset; 500 nm] and 30 min [sample for (c), scale bar in the inset; 200 nm]. Then, the resultant dispersion was taken out from the magnet bore and incubated for 30 min [sample for (d), scale bar in the inset; 500 nm] and 120 min [sample for (e), scale bar in the inset; 500 nm] under ambient conditions otherwise identical to the above. For better visualization of the individual NT_{GroEL}⊃SNP filaments in the inset for (c), the sample was washed extensively with water for the removal of minerals after the protein fixation with 2.5% glutaraldehyde. (f) Schematic illustration of the magneto-induced lateral assembly of superparamagnetic NT_{GroEL}⊃SNP. (g) DLS profiles of the NT_{GroEL}⊃SNP samples for (a) [i], (c) [ii], and (e) [iii]. (h) Counted distribution histograms of nonassembled NT_{GroEL}⊃SNP objects for (a) [i], (c) [ii], and (e) [iii].

~80% of GroEL proteins employed are filled at their cavity with SNPs.

Next, we investigated the behaviors of NT_{GroEL}⊃SNP in a static magnetic field. As shown by the dynamic light scattering (DLS) profiles at 25 °C, larger objects (Figure 4g [ii]) formed when a 0.5 T magnetic field was applied for 30 min to a 0.4 μM phosphate buffer dispersion of NT_{GroEL}⊃SNP (Figure 4g, [i]). For atomic force microscopy, an aliquot of the resultant sample was treated with glutaraldehyde for protein fixation on a mica substrate. As shown in Figure S5, thick 1D bundles formed by the lateral assembly of NT_{GroEL}⊃SNP (Figure 4f). When the bundling process was carefully traced by TEM (Figure 4a–c), single nanotubes of NT_{GroEL}⊃SNP were revealed to gather gradually with time and form larger and thicker bundles. Of interest, the number of unassembled NT_{GroEL}⊃SNP residues, counted based on their contour lengths (Figure 4h), showed a clear tendency that longer nanotubes are bundled more preferentially. This chain length-dependent bundling behavior of NT_{GroEL}⊃SNP is consistent with a theoretical model in Figure 1 for the lateral assembly of 1D-arrayed SNPs.[2] As a control experiment, a 0.4 μM dispersion of guest-free GroEL nanotubes (NT_{GroEL}), comparable in length to that of NT_{GroEL}⊃SNP, was likewise incubated in a 0.5 T magnetic field. However, bundling of NT_{GroEL} was not detected (Figures S6 and S7).

To our surprise, when the magnetic field was turned off for 2 h, the bundles of NT_{GroEL}⊃SNP became completely unbundled as observed by DLS (Figure 4g, [ii] and [iii]) and TEM (Figures 4d and 4e). Accordingly, the sample recovered its original counted length population profile (Figure 4h, [iii] and [i]). Furthermore, in response to turn-on and off the magnetic field, NT_{GroEL}⊃SNP was bundled and unbundled repeatedly without denaturation, respectively. In the absence of the nanotubular protein jacket, such a reversible assembling behavior would not emerge for 1D-arrayed SNPs. We incubated a phosphate buffer dispersion of SNPs for 0.5 h in the 0.5 T magnetic field and then 2 h after the magnetic field was turned off. As reported for other magnetic NPs, our SNPs likewise gathered magnetically, and the resultant agglomerate no longer disassembled after turn-off the mangetic field (Figure S8).

In conclusion, by taking advantage of the supramolecular polymerization of GroEL⊃SNP (Figure 2), we successfully tailored micrometer-long high-integrity 1D-arrayed SNPs with an excellent thermodynamic stability due to the chaperonin-based nanotubular protein jacket. Because of the noncurvy/nonwavy rigid architecture, we obtained the first unambiguous experimental proof that 1D SNP arrays, as predicted by the theory (Figure 1), laterally attract one another to form bundles, where longer arrays are more preferentially incorporated. Due to physical separation of the incorporated SNPs by the nanotubular protein jacket, the bundles can disassemble back to the original SNP arrays upon turning off the magnetic field. Hierarchical assembly of long 1D objects may lead to the development of conceptually new soft materials.[11] This concept is reminiscent of the biological role of Lamin protein that structurally supports the nucleus by forming mechanically tough filaments through longitudinal growth and subsequent lateral attraction.[12] Our SNP-based magneto-responsive 1D protein filament may spatiotemporally control biological events, since its bundling and unbundling events are noninvasively directed by a magnetic field.

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■ ASSOCIATED CONTENT

Supporting Information

Details of experimental procedures and micrographs including synthesis of NT_{GroEL}⊃SNP. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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