Stimuli-responsive self-regulating chiral colloidal self-assembly for robust size and shape control

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In most synthetic self-assembly processes the size of the final structure grows unbound and is only limited by the number of accessible microscopic building blocks. In comparison, biological assemblies can autonomously regulate their size and shape. One mechanism for such self-regulation is based on the chirality of microscopic units. Chirality induces a twisted geometry of building blocks that is incompatible with long-ranged crystalline packing, thereby stopping the assembly's growth at a given stage. Chiral self-regulating self-assemblies, based on thermodynamic equilibration rather than kinetic trapping, remain an elusive target that has attracted considerable attention. So far studies of chiral self-assembly processes have focused on non-responsive systems, whose equilibrium points are not easily shifted *in situ,* which limits their versatility and applicability. Here, we demonstrate stimuli-responsive self-regulating self-assembly. This assembly is composed of chiral and magnetically alignable nanorods, where the effective chirality is modulable by balancing chirality-induced twisting with magnet-induced untwisting alignment. Changing the magnetic field intensity, controls the strength of self-regulation, leading to assemblies whose sizes and shapes are rationally controlled. The described size/shape control mechanism is tunable, reversible, robust, and widely applicable, opening up new possibilities for generating biomimetics structures with desirable functions and properties.

From biological tissues to synthetic materials, self-assembly is critical for constructing functional structures from microscopic building blocks[1]. In most self-assembly processes, identical building blocks repeat the same packing pattern indefinitely to form size-unlimited structures (Fig. 1a, right)[2]. In nature, however, some assemblies autonomously regulate their size and shape, in a self-regulating process[3]. A well-known, intuitive example is the assembly regulated by self-closing, represented by a viral outer shell with a defined diameter[3a], which

grows along a curvature and eventually closes into a sphere or tube. Another intriguing example is the assembly regulated by chirality-induced frustration, as exemplified by cytoskeletal bundles of defined thickness[3b]. The chirality of the filamentous building blocks induces their mutual local twisting, which is incompatible with long-ranged ordered packing. Thus, bundle growth terminates beyond a critical diameter (Fig. 1a, left). This example involves a complex feedback mechanism wherein the building blocks sense the assembly's size which is much larger than that of individual units, and reflect it on the assembly pathways. The ability to systematically design assemblies with self-regulating sizes and shapes could lead to diverse but highly desirable functions, such as selective mass confinement/transport or precise stiffness/strength control[3].

Self-regulating assemblies in nature yield size/shape-regulated structures that are not kinetically trapped intermediates but are thermodynamically equilibrated products. Therefore, this regulation is robust even in complicated and uncontrollable environments such as *in vivo*. This is in sharp contrast to the size/shape regulation in synthetic self-assembly, which relies exclusively on the kinetic trapping of growing intermediates[4], and is therefore feasible only when conditions such as temperature, solvent, and component concentration are precisely controllable. Inspired by biology and above reasoning there are surging efforts to develop self-regulating assemblies[3,5]. However, previous studies have focused on systems whose thermodynamic equilibrium points are fixed by external parameters such as species concentrations, so that their size and shape cannot be easily changed[3,5]. Here, we demonstrate the first example of a self-regulating assembly that can respond to external stimuli to change its thermodynamic equilibrium point, allowing for rational *in-situ* control of its size and shape.

To study an assembly mechanism with both self-regulation and stimuli-responsiveness, we focused on the previously described feedback mechanism of chirality-induced frustration (Fig. 1a, left)[5]. This self-regulation is expected to operate more intensely at higher degrees of

chirality in the assembly. On the other hand, in some self-assemblies with chirality such as cholesteric liquid crystals, the net chirality expressed in the system can be modulated by an external physical field, such as an electric or magnetic field, which forces the building blocks to align unidirectionally against their twisting tendency (Fig. 1b)[6]. We envisioned that, by introducing this chirality modulation mechanism, a self-regulating assembly would acquire stimuli-responsiveness.

Realizing the above idea requires chiral and field-orientable building blocks that pack into ordered structures. While several candidates satisfy these requirements[7], we chose rod-shaped viruses[7d], M13 bacteriophage[8]. This highly monodisperse with 880 nm contour length and 6.6 nm diameter is readily purified. When mixed with a non-adsorptive polymer, the virus rods pack side by side due to the polymer's entropic demand to minimize their exposed surface, a phenomenon known as the depletion effect[9]. In addition, the virus's outer shell is composed of amino acids, whose chirality causes a twisted geometry between the virus rods (Fig. 1b, left)[7d,9f,g]. Furthermore, applying a magnetic field, aligns the virus rods unidirectionally in a non-contact, non-destructive, and non-invasive manner (Fig. 1b, right)[10]. We show that the virus rods form a self-regulating assembly capable of controlling its size and shape in response to magnetic stimuli. When the depletion-induced assembly is performed without a magnetic field, the virus's intrinsic chirality is unhated maximizing the self-regulating mechanism, thereby resulting in assemblies with a uniform small diameter. In comparison, under a strong magnetic field, the net chirality and concomitant self-regulation are attenuated, resulting in assemblies with larger diameters (Fig. 1c and Supplementary Video 1). By adjusting the magnetic field intensity, the thermodynamic equilibrium point of the system is changed accordingly, allowing for the rational control of the assembly diameter. Furthermore, when the magnetic field is switched on/off for a post-formed assembly, the thermodynamic equilibrium abruptly shifts, so that the assembly undergoes a drastic shape change (Fig. 1d and

Supplementary Video 2). These phenomena are explained by the theoretical model, which further rationalizes our findings (Supplementary Method 10).

The described self-regulating and stimuli-responsive self-assembly mimics biological processes. Its precise and yet responsive size/shape control is driven by thermodynamic equilibrium, in contrast to the conventional synthetic assemblies whose size is controlled by kinetic trapping[4]. The tuning of size and shape is possible in a clean, remote, and reversible manner, by simply changing the magnetic field. The elucidated self-assembly principles apply to various non-spherical particles that simultaneously possess chirality and field-induced orientability[7].

Results

Effects of a magnetic field on self-regulation during the assembly formation

We started by investigating the depletion-induced assembly of the above-mentioned virus without a magnetic field. When colloidally dispersed rods of the virus (6.5 mg/mL) were mixed with dextran (20 mg/mL) in tris-buffered saline, we observed the formation of micrometer-sized cylindrical objects (Fig. 2a, i and Supplementary Fig. 1a). Cylinders exposed from their side exhibited a stripe pattern with a periodicity of 1.2 μm (Fig. 2a, ii, upper), while cylinders viewed along their long axis (from the top) exhibited no periodicity (Fig. 2a, ii, lower). Polarized optical microscopy (POM) observation indicated that the virus rods aligned parallel to the cylinder (Supplementary Fig. 2a). The 3D confocal laser scanning microscopy (CLSM) reconstruction revealed that the stripe pattern persisted throughout the cylinder (Fig. 2a, iii and Supplementary Video 1), indicating that this assembly was a lamellar stack of disks with a uniform thickness of ~1 μm. The formation of individual disks is attributed to the side-by-side packing of virus rods to form a one-rod-length thick monolayer assembly caused by the depletion effect of dextran, as reported[9]. Meanwhile, the stacking of thus formed disks is driven by the inter-disk bridging due to the small fraction of dimer viruses that have twice the contour

length of the native phase (Supplementary Fig. 3a) [9e]. Since the virus monomer and dimer are separable through the phase separation of their mixture dispersion (Supplementary Fig. 4)[9e], the number of stacked disks in a lamellar cylinder can be controlled by controlling the ratio of dimers to monomers (Supplementary Fig. 5). The lamellar cylinder was assembled over a wide range of the virus (4.0~6.5 mg/mL) and dextran (18~22 mg/mL) concentrations (Fig. 2b). Notably, the self-assembled cylinders had a narrow diameter polydispersity around ~4 μm with (Fig. 2c), suggesting presence of a self-regulating mechanism due to virus chirality.

Motivated by this hypothesis we studied the assembly process in a strong magnetic field that aligned viruses and thus minimized the chiral effects. Notably, the strength of the employed magnetic field (10 T) was enough to align virus rods almost perfectly[10]. Under a 10-T magnetic field applied in-plane direction of the sample container, viruses again assembled into the lamellar cylinder (Supplementary Fig. 1b). The long axis of the assembled cylinders waswere aligned parallel to the magnetic field (Fig. 2d, i), and could be re-oriented by changing the magnetic-field direction (Supplementary Fig. 6). Optical microscopy (Fig. 2d, ii), 3D CLSM (Fig. 2d, iii and Supplementary Video 1), and POM (Supplementary Fig. 2b) revealed that the assembled structure was similar to that without a magnetic field. The range of concentration of the virus and dextran that yielded the lamellar cylinders was also identical to those without a magnetic field. Interestingly, however, the magnetic field significantly affected the diameter of the lamellar cylinders, which were widely distributed and on average ~2 times larger (Fig. 2f), when compared to those assembled without a magnetic field (Fig. 2c). Such comparison suggests that the self-regulation was attenuated by the magnetic field by minimizing the twisting of chiral rods.

Mechanism for the size control by the magnetic modulation of self-regulating assembly

To identify the mechanism of how chirality regulates the assembly's size and how the magnetic field modulates this regulation, we developed a theoretical model to calculate the free energy

of the lamellar cylinder. The model considers depletion energy, Frank free energy (equivalent here to twist energy), and interactions of rods with the magnetic field (Supplementary Method 10)[11]. Given the cylindrical symmetry, the lamellar cylinders can be described by the tilt angle of the virus rod from the normal of the disks that stack in the cylinder, denoted as θ, as a function of the distance from the disk center denoted as r (Supplementary Fig. 7a). The cylinder's radius, corresponding to the maximum of r, is denoted as R. This model affords the energy-optimized structures of the lamellar cylinder with varying its radius R from 1 to 10 μm, as well as the dependency of the free energy on the radius R, under conditions without and with a magnetic field (Fig. 3a,b).

Virus rpreferefes to twist continuously with a pitch whose magnitude is determined by their intrinsic chiral structure[7d]. Meanwhile, the depletion effect demands that virus rods minimize their total exposed surface. Consequently, virus rods orient parallel to each other and normaly to the monolayer disk. At the disk edge, the rod-polymer surface tension enforces that semi-circular profile that is accommodated by the virus rods tilt locally away from the disk normal[9f]. Without a magnetic field, the tilt angle θ increases continuously from the center (r = 0) and eventually becomes close to 90° at the edge ($r = R$), as a common feature regardless of the cylinder's radius R (Fig. 3a, i). In contrast, under a 10-T magnetic field, the preference for continuous twisting due to the virus's intrinsic chirality is excluded, while the depletion effect still demands the virus rods to orient vertically at the center and horizontally at the edge to minimize their exposed surface area. Therefore, upon increasing r from 0 to R, the tilt angle θ remains close to 0° over a wide range and abruptly increases only at the edge, which is common regardless of the cylinder's radius R (Fig. 3b, i). The theoretically predicted structures without and with a magnetic field are in good agreement with their top-view POM observation (Supplementary Fig. 2, i), which can selectively visualize the domains with tilt virus rods as bright. For both without and with a magnetic field, most parts of the lamellar cylinder appeared

as dark, while only the cylinder's edge appeared as bright. Furthermore, at the edge region, the lamellar cylinder with a magnetic field showed a sharper brightness increase than without a magnetic field.

As clarified above, the presence or absence of a magnetic field brings critical effects on the twisting pitch of the virus rod array, which is represented by the slope of the θ–r curve, i.e. how quickly θ increases upon increasing r. Without a magnetic field, the θ–r curves for various R values show different slopes (Fig. 3a, i), so that the free energy is dependent on R and has a sharp minimum at R = 2 μm (Fig. 3a, ii). Meanwhile, with a magnetic field, the θ–r curve can be divided into a flat region at smaller r and a steep region at larger r. At both the flat and steep regions, the θ–r curves for various R values show similar slopes to each other (Fig. 3b, i). The free energy curve becomes shallow and the minimum positions shift to larger r (Fig. 3b, ii). This result agrees with our observation that the lamellar cylinders prepared without a magnetic field have diameters of ~4 μm with a narrow dispersity (Fig. 2c), while those prepared under a magnetic field grow beyond the diameter of ~4 μm with a wide distribution (Fig. 2f).

The present model also allows for predicting how the cylinder's diameter changes when the net chirality expressed in the assembly is gradually tuned. Thus, we calculated the free energy–R curves of the lamellar cylinder under various magnetic-field intensities (Fig. 3c and Supplementary Fig. 8). Upon increasing the magnetic-field intensity from 0 to 10 T, the minimum point of the free energy–R curve progressively shifts to the larger R region and becomes shallower in depth (Fig. 3c). In good accordance with this theoretical prediction, when the assembly formation experiment was performed under various magnetic-field intensities, the diameters of the resultant lamellar cylinders became larger with wider distribution as the magnetic field became stronger (Fig. 3d).

Effects of a magnetic field on the self-regulation of post-formed virus assembly

Considering the dynamic nature of virus rods in assembled cylinders, their effective twisting might be modulable even after the self-assembly process is completed. Therefore, we switched the magnetic field for already formed lamellar cylinders, so that the strength of net chirality and concomitant self-regulation are abruptly changed. We first assembled virus rods under a 10-T magnetic field, which generated larger-diameter lamellar cylinders, as described above (Fig. 2d). Consequently, we removed the magnetic field. Surprisingly, we observed the growth and continued elongation of many fibers with a uniform thickness of ~1.2 μm from the cylinder's periphery (Fig. 4a, i and Supplementary Video 3, former half). Accompanying this, the cylinder at the center decreased in diameter (Fig. 4b, i). This branching transformation took place regardless of the alignment direction of the lamellar cylinders (Supplementary Fig. 9, i–iii and Supplementary Video 2). According to 3D CLSM (Fig. 4c, i), the emerging filaments were twisted ribbons with the thickness of a virus-rod monolayer, similar to those reported previously[9f,12]. In the reflection-mode CLSM image without a fluorescent dye bright regions appeared periodically in synchronization with the twisted shape of the bright field image (Fig. 4c, ii), which further verified that the emerging filaments were twisted ribbons of virus-rod monolayer.

Of further interest, when a 10-T magnetic field was applied to this branched assembly so that chirality transfer was switched off again, a reverse phenomenon occurred. The twisted ribbons gradually converged towards the central lamellar cylinder (Fig. 4a, ii and Supplementary Video 3, latter half). Consequently, the cylinder continued thickening eventually recovering close to its original size (Fig. 4b, ii). The converging transformation did not depend on the orientation of the cylinders (Supplementary Fig. 9, iii–v). Overall, these experiments show that on/off switching of the magnetic field controls the reversible polymorphic shape change of virus assembly between the lamellar cylinder and the twisted ribbon.

We hypothesized that the drastic shape changes of the virus rod assemblies are controlled by the structural switching of the net chirality, i.e. the degree of twist between neighboring virus rods. To confirm this, the larger-diameter lamellar cylinder, just after being prepared in a 10-T magnetic field, was monitored under a magnet-free condition from the cylinder's top, using retardation imaging (Fig. 5a, i and Supplementary Fig. 10) and director field mapping (Fig. 5a, ii)[13]. Initially, only the edge of the cylinder showed weak retardation (Fig. 5a, i/ii, 0 min). As time passed, the retardation increased at the edge and expanded towards the cylinder interior (Fig. 5a, i/ii, 10~30 min), indicating that the tilting of the virus rods progressed from the edge to the center. The time-course plot of the retardation against the distance from the cylinder's center revealed a quantitative change in the local rod tilt (Supplementary Fig. 11).

Tilting of edge-bound virus rods impacts the microscopic dynamics of virus rods, as revealed by fluorescence optical microscopy of the lamellar cylinder containing 0.02% fluorescent-labeled virus rods[9e]. When viewed from the cylinder's top, the individuals of the labeled virus rods were observed as spots migrating within a disk in a cylinder. We classified these spots into two groups based on their diffusion being higher and lower than a threshold of 0.09 μm s^{-1}. We found that the spots of the higher-mobility group were predominantly located at the cylinder's edge (Fig. 5a, iii, yellow), while those of the lower-mobility group were at the cylinder's center (Fig. 5a, iii, red). The diffusion coefficient of the former group was more than an order of magnitude higher than that of the latter group (Supporting Fig. 12)[14].

For further rationalization of this shape-change mechanism driven by tuning the net chirality in the post-formed assembly, we employed the theoretical model constructed in the previous section, where the free energy diagram of the lamellar cylinder without (Fig. 5b, yellow) and with a magnetic field (Fig. 5b, red) has already been obtained. We also calculated the optimized structure of the twisted ribbon and its free energy (Supplementary Method 10 and Supplementary Fig. 13)[12], where its radius R should be constant at 0.6 μm as experimentally

observed (Fig. 4c), so that the free energy is depicted as a horizontal line in the diagram (Fig. 5b, green). Under the minimized net chirality by applying a magnetic field, the depletion effect urges virus rods to assemble according to the corresponding free energy curve (Fig. 5b, red) to form the larger-diameter lamellar cylinder with little tilt of virus rod (Fig. 5b, i). When the net chirality is maximized by removing the magnetic field, the assembly transitions to a new free energy curve (Fig. 5b, yellow), rendering the large cylinder diameter energetically unstable (Fig. 5b, ii). To release this instability, the lamellar cylinder begins to reduce its diameter by sprouting twisted ribbons (Fig. 5b, iv). This transition continues until the assembly reaches the free energy minimum (Fig. 5b, iii). When the net chirality is again minimized by applying a magnetic field, the assembly returns to the original free energy curve (Fig. 5b, red), the cylinder's edge converges, and the twisted ribbons re-form the larger-diameter lamellar cylinder, as demanded by the depletion effect (Fig. 5b, i).

Discussion

Using a rod-shaped virus as a simple model[8], we demonstrated a self-regulating assembly whose size and shape can be modulated by external stimuli. The key to this achievement is the introduction of the chirality-modulation mechanism[6] into a self-assembly regulated by chirality-induced frustration[5]. In conventional chiral self-regulating assemblies the degree of chirality is fixed, so that the size and shape of the assembled structure are predetermined and not controllable. In contrast, our assembly composed of the viruses, which possess both chirality[7d] and magnetic orientability[10], modulate the net twisting by the magnetic-field intensity (Fig. 1b), so that the intensity of self-regulation is modulated accordingly. When the net chirality is modulated during the assembly formation, allows for the rational control of the size and shape of final structures (Fig. 1c). In comparison, when the effective twisting is

switched on/off in already formed assemblies, the structures undergo drastic shape change (Fig. 1d).

The *in-situ* self-regulating self-assembling mechanisms described here have widespread significance. From the viewpoint of biomimetic science, the self-assembly presented here is an important example that reproduces biological structures that respond to their environment while regulating their size and shape. From a materials science perspective, the thermodynamic equilibrium-based self-assembly could solve the limitation of conventional synthetic self-assemblies, whose size/shape control is based solely on the kinetic trapping of growing intermediates and can only be performed in precisely controllable environments and cannot be post-modulated[4]. From the standpoint of engineering, the present size/shape control can be induced simply by changing the magnetic field intensity. Therefore, it is clean, remote, and reversible without changing the properties of the building blocks. Our approach, based on the balance between chirality-induced twisting and field-induced alignment applies to diverse non-spherical chiral and orientable colloids[7].

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Supplementary Information is available in the online version of the paper.

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Author contributions

S.W. and Y.I. conceived the project. S.W. designed and performed all experiments. X.W. and N.U. co-designed the experiments. S.W. and L.K. conducted the theoretical studies. P.S. and F.A. analyzed the data of retardation imaging. S.W., T.A., Z.D., and Y.I analyzed the data and wrote the manuscript with the input of all other authors. The manuscript reflects the contributions of all authors.

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Figure 1 | Design of an assembly with self-regulation and stimuli-responsiveness and rational control of its size and shape.

a, Left: self-assembly with the regulation of chirality-induced frustration, where the chirality of the building blocks induces their mutual twist, which is incompatible with their ordered packing and stops the assembly's growth over a certain size. Right: self-assembly without the regulation of chirality-induced frustration, where the building blocks devoid of chirality tend to repeat the same aggregation infinitely to form an unlimited bulk. **b**, Strategy for tuning the degree of chirality expressed in the system, i.e. net chirality, by the balance between chirality-induced twisting and magnet-induced flattening. **c**, Size control of the present self-regulating assembly by tuning net chirality during the assembly is forming. **d,** Shape change of the present self-regulating assembly by switching net chirality after the assembly is formed.

Figure 2 | Effects of a magnetic field on the self-regulation during the assembly formation.
a,**d**, Lamellar cylinders composed of M13 bacteriophage (6.5 mg/mL) in the presence of dextran (20 mg/mL) without (**a**) and with (**d**) a 10-T magnetic field; (i) wide-view optical microscopy image, (ii) magnified optical microscopy image, and (iii) 3D reconstruction of confocal laser scanning microscopy (CLSM) images with negative fluorescent contrast. **b**,**e**, Phase behavior of the virus assembly upon changing the concentrations of the virus and dextran without (**b**) and with (**e**) a 10-T magnetic field. **c**,**f**, Diameter distributions of the lamellar cylinders at the equilibrated state, formed from [virus] = 6.5 mg/mL and [dextran] = 18~22 mg/mL, without (**c**) and with (**f**) a 10-T magnetic field. All assembly experiments were conducted in a tris-buffered saline (pH 7.5, 50 mM of tris, 150 mM of NaCl) within a glass sandwich cell at 20 °C.

Figure 3 | Mechanism for the size control by the magnetic modulation of self-regulating assembly. **a**,**b**, Theoretically predicted structure and free energy of the lamellar cylinder without (**a**) and with (**b**) a 10-T magnetic field: (i) energy-optimized structures with various cylinder radii R and (ii) free energy as a function of cylinder radius R. **c**, Theoretically predicted structure of the lamellar cylinder at various magnetic-field intensities. Main: free energy as a function of cylinder diameter $2R$, where the plots are offset for easy comparison of their shapes (for plots without offsetting, see Supplementary Fig. 8). Inset: cylinder diameter $2R$ at the energy-minimal as a function of magnetic-field intensity. **d**, Experimental control of the size of lamellar cylinder by changing the intensity of magnetic field applied during the assembly formation: (i) optical microscopy images and (ii) diameter distributions.

Figure 4 | Effects of a magnetic field on the self-regulation of post-formed virus assembly.
a, Shape change of the virus-rod assembly monitored by top-view optical microscopy (upper) and 3D CLSM (lower): (i) branching from the lamellar cylinder to the twisted ribbons upon removal of the magnetic field and (ii) converging from the twisted ribbons back to the lamellar cylinder upon reapplication of the magnetic field. **b**, Time-course changes in the diameters of the lamellar cylinders during (i) branching upon removal of the magnetic field and (ii) converging upon reapplication of the magnetic field. **c**, Characterization of the twisted ribbon branched from the lamellar cylinder: (i) 3D CLSM image and (ii) reflection-mode CLSM (mid), bright-field (upper), and overlapping (lower) images. The original lamellar cylinders were prepared under similar conditions to those in Fig. 2b and then reoriented by a 10-T magnetic field in the out-of-plane direction for their top-view observation. To induce the branching, the magnetic field was removed. To promote converging, a 10-T magnetic field was reapplied in the out-of-plane direction.

Figure 5 | Mechanism of the shape control by the magnetic modulation of self-regulating assembly. **a**, Structural and dynamics changes of the larger-diameter lamellar cylinder just after removal of the magnetic field: (i) time-course changes of the retardation image viewed from the top, (ii) time-course changes of director field of the area highlighted by the dotted square in (i), and (iii) trajectories of fluorescently labeled virus rods monitored by top-view fluorescence optical microscopy in which 0.02% of virus rods are fluorescently labeled. **b**, Theoretically predicted energy diagram of the virus rod assembly: (i) the larger-diameter lamellar cylinder prepared with a magnetic field, (ii) the lamellar cylinder just after the removal of the magnetic field, (iii) the lamellar cylinder after reducing its radius through branching of the twisted ribbon, and (iv) the twisted ribbon branched from the lamellar cylinder.