ABSTRACT: Here, we demonstrate the novel double-component liquid crystalline colloids composed of mesogenic inorganic nanosheets and the rods with dynamically variable length controlled by temperature. As the length-controllable rod, stiff biopolymer microtubule is used, which was successfully polymerized/depolymerized from tubulin proteins through a biochemical process even in the presence of the nanosheets. The mesoscopic structure of the liquid crystal phase was reversibly modifiable as caused by the change of the rod length.

INTRODUCTION

Anisotropic and hierarchical structures composed of inorganic and organic nanocomponents, such as abalone shell (protein + calcium carbonate) and human body (muscle + bone), are key components for sophisticated functions of living organisms.\(^1\) The complex architectures of living organisms are assembled through consumption of energy and exchange of molecules. Inspired by living systems, many kinds of artificial materials with regulated superstructures and functions have been fabricated by controlling electrostatic force, hydrogen bond, van der Waals interactions, and coordination bond: DNA-origami,\(^2\) mesophases of liquid crystals (LCs), surfactant micelles, and metal–organic-framework\(^3\) are old and new examples. In addition to those interactions, entropic interactions are also important to regulate the assembly of the nanocomponents. Prominent examples are the ordered structures found in colloidal systems\(^4\) such as colloid crystals and colloidal LCs.\(^5\) Further, combination of components with dissimilar morphologies such as rod/plate,\(^6\) rod/sphere,\(^7\) plate/sphere,\(^8\) nanosheet/rod,\(^9\) nanosheet/nanosheet,\(^10\) and plate/polymer\(^11\) types of particles also facilitates formation of variety of well-ordered structures. Thus, complexity of the system is a key to develop highly organized and hierarchical structures.

Herein, we demonstrate a new type double-component colloidal system composed of two types of colloidal LC mesogens (Scheme 1): one is a thin platy particle and the other is a rigid hollow rod with the length reversibly controllable in situ in response to temperature. As the platy particle, we use inorganic nanosheets obtained by exfoliation of a layered clay mineral fluorohectorite (FHT).\(^12\) Among several kinds of anisotropic particles, nanosheets\(^13\),\(^14\) are distinguished by the ultralarge anisotropy and 2D shape: uniform thickness of ca. 1 nm and the lateral size of up to 100 μm. Because of the large anisotropy, the LC phase is formed at very low concentration as low as <0.2 wt %.\(^15,16\) Many applications of...
liquid crystalline nanosheets for anisotropic composite gel, fibers, optical devices, and anisotropic medium for spectroscopy have been investigated. High biocompatibility of clay nanosheets allows us to choose a living biopolymer, microtubule (MT), as the counterpart. A MT is a hollow rod with a diameter of 25 nm, length of up to several tens of μm, and high rigidity (the persistent length on the order of millimeters). The MT is actively formed by polymerization and high rigidity (the persistent length on the order of millimeters). The MT is formed by polymerization of tubulin dimers (TBs) through a biochemical reaction that consumes biochemical energy obtained from hydrolysis of guanosine triphosphate (GTP) at ~37 °C, whereas it reversibly dissociates to TBs by cooling. Because of the large excluded volume of the rigid hollow tube, the solution of MTs forms a nematic LC phase. Organization of MTs into well-defined structure is valuable for future applications as artificial muscles, sorting devices, and biosensors. The present system will not only give new insights into mixed colloidal systems controlled by entropic interactions but also lead to various active molecular devices of which structures are hierarchically regulated both by mesogenic nanosheets and MT rods.

### RESULTS AND DISCUSSION

FHT and MT were uniformly mixed and polymerization/depolymerization of the MT proceeded without being disturbed by the presence of nanosheets. The single-component colloids of FHT (2 wt %) (Figure 1a) and MT (120 μΜ) (Figure 1b) showed permanent birefringence because of the LC phase in which nanosheets or MTs are oriented. Because of the flow of the sample when it was introduced into the capillary, the optical axis is preferentially oriented along the capillary wall. No specific textures like Schlieren texture were observed. The double-component system containing both FHT and MT (Figure 1c) showed a birefringent image similar to the single-component FHT system. It is notable that we did not observe phase separation or aggregation of the nanosheets, confirming that the two components are mixed homogeneously. Generally, in double-component colloidal liquid crystalline systems, macroscopic phase separations are observed after storing the sample for sufficiently long time or by centrifugation, which is valuable to investigate the system more in detail. However, this phenomenon was not observed in our system, although we stored the sample up to 24 h; we cannot store the sample longer at the present condition because the MT dissociate into TBs when GTP is exhausted. Gravity-induced sedimentation also sometimes prevents us from clear observation of the phase separation.

The birefringence was weakened when the double-component sample was cooled at 4 °C for 30 min (Figure 1d). This indicates depolymerization of MTs to TBs. The birefringence of the double-component system was weaker than the single-component nanosheet colloid containing same concentration of the nanosheet, suggesting that TBs interact with the nanosheets and partly disturbed the formation of the LC phase of the nanosheets.

Effects of the concentrations of FHT and MT were then examined. Figure 2 shows the polarized optical microscopy (POM) images of the solutions containing various concentrations of FHT and MT. Single-component FHT systems showed the LC phase at above 1 wt %, and the birefringence increased with increasing FHT concentration. They did not show temperature dependence. Meanwhile, the single-component MT system showed an LC phase at above 90 μΜ, and birefringence increased with increase in MT concentration. In the double-component system, stronger birefringence was observed at higher MT or FHT concentrations. The samples containing the MT showed response to temperature with or without nanosheets.

Small angle X-ray scattering (SAXS) measurements revealed the structural change on the mesoscale induced by temperature change, corresponding to the macroscopic change observed by POM. The profile of the MT solution (Figure 3a) agrees with the theoretical form factor as shown by eq 1 (Figure 3a’).

$$ P(q) = \frac{A}{V_{shell}} \int_0^{2/α} f^2(α, q) \sin(α) dα $$

where

$$ f(q, α) = 2ΔρV_{core}J_0(qH \cos α) \cdot \frac{J_1(qr \sin α)}{(qr \sin α)} $n + 2ΔρV_{core}J_0(q(H + t) \cos α) \cdot \frac{J_1(q(r + t) \sin α)}{(q(r + t) \sin α)} $$

$$ V_{core} = πr^2L, \quad V_{shell} = π(r + t)^2L $$

Figure 1. POM images of (a) FHT colloid, (b) MT solution, (c) as prepared FHT/MT mixture (37 °C), and (d) FHT/TB mixture after cooling (4 °C). FHT concentration is 2 wt % and TB concentration is 120 μΜ.
Here, \( q \) is the scattering vector, \( r \) is the core radius, \( r + t \) is the shell radius, \( L \) is the length of the MT, \( \Delta \rho \) is the scattering contrast between the core and the shell, \( A \) is the factor related to the particle volume and concentration, and \( J_1 \) is the first order Bessel function. This geometrical size corresponds to MTs composed of 15 TBs in one lap, whereas it is known that the MTs with various diameters are formed depending on the condition.\(^{29}\) The nanosheet colloid (Figure 3c) and mixture of the nanosheet and MT (Figure 3b) showed profiles with the slope of \( q^{-2} \). This indicates that most of the nanosheets are present as single layers because the theoretical form factor (eq 2) (Figure 3c') of the nanosheets is approximated as \( q^{-2} \) for nanosheets larger than 100 nm in the range of \( q \) in the present measurement.\(^{30}\)

\[
P(q) = \left( \frac{2}{q^2 R^2} \right) \left[ 1 - \frac{J_1(2qR)}{qR} \right] \sin^2(qL/2) \left( qL/2 \right)^2
\]

(2)

Note that the profile in the double-component system mainly reflects the scattering from nanosheets because scattering capability of the inorganic nanosheets is more than one order of magnitude higher than MTs which is composed only of organic atoms.

From SAXS patterns, the superstructures in double component colloids were evaluated after eliminating the effect of the form factor. The as-observed scattering profile \( I(q) \) is expressed as

\[
I(q) = AS(q)P(q)
\]

(3)

where \( S(q) \) is the structure factor, \( P(q) \) is the form factor, and \( A \) is the factor determined by scattering power and number of the particles.

Because the form factor \( P(q) \) of the nanosheets is approximated as \( q^{-2} \), \( S(q) \) is calculated as \( S(q) = I(q)q^2 \) with \( A = 1 \). As a consequence, we show \( I(q)q^{-2} \) versus \( q \) plots in Figure 4 to discuss the structure of the colloid. Single-component FHT colloids (Figure 4a) showed the peaks because of the structure with the basal spacing \( d = 34.1 \text{ nm} \).\(^{12}\) Because the peaks are not so sharp, the LC phase is supposed as the nematic phase with rather low positional order.\(^{31}\) In the double-component system of FHT and MT (Figure 4b), the peak is shifted to higher \( q \) (\( d = 13.1 \text{ nm} \)).\(^{32}\) Although basal spacing significantly changes by the salt effect (Figure S2), we have adjusted the concentration of buffer constituents in all the samples examined. Hence, the observed peak shift is explained that the coexisting MTs deprive the free volume for nanosheets so that the nanosheet LC phase was compressed. After cooling, the peak shifted to lower \( q \) (\( d = 21.3 \text{ nm} \)) again. The temperature-triggered change is reversible, and the peak shifted to higher \( q \) (\( d = 15.3 \text{ nm} \)) after heating again. The 2D-SAXS patterns (right side of Figure 4) are more or less anisotropic for all cases, indicating the presence of anisotropic liquid crystalline domains.

Figure 2. POM images of the FHT/MT mixtures at various mixing ratios. The observation was made (a) as-prepared (37 °C), (b) after cooling (4 °C), and (c) after heating again (37 °C).

Figure 3. SAXS patterns of (a) MT solution (from 75 μM of the TB), (b) the mixture of FHT (4 wt %) and MT (from 75 μM of the TB), and (c) FHT colloid (4 wt %). The theoretical form factors for (a') hollow cylinder and (c') thin disk are also shown as dotted lines.

\[
P(q) = \left( \frac{2}{q^2 R^2} \right) \left[ 1 - \frac{J_1(2qR)}{qR} \right] \sin^2(qL/2) \left( qL/2 \right)^2
\]

(2)
Structural formation in response to temperature found in the double-component system is driven by entropic interactions explained based on Onsager theory. Onsager theory considers hard core repulsive force between the cylindrical particles \( D \gg L \) is disk or \( D \ll L \) is rod of diameter \( D \) and length \( L \). The particles occupy considerably larger excluded volume \( b \) as compared with their own actual volume \( v_p \) \( (= \pi D^2 L / 4) \) when the anisotropic particles are freely rotating. The \( b/v_p \) is calculated by the following equation

\[
\frac{b}{v_p} = \frac{1}{4} \pi D \left( \frac{L^2}{2} + \frac{1}{2} (\pi + 3) DL + \frac{1}{4} \pi D^2 \right)
\]

When the sum of the excluded volume \( b \) becomes larger than a certain value, the particles cannot keep free rotation so that the LC phase is formed. It has been reported so far that the formation of the LC phase of nanosheet colloids can roughly be explained by this theory. Because both FHT nanosheets and MTs are negatively charged, repulsive force is dominant in the system, so that Onsager theory can be applied in this double-component system. Usually, when treating with Onsager theory, electrostatic repulsion between the charged particles is taken into account, for example, by considering effective size of particles defined as the sum of the size of the particle core and the additional size related to Debye length and charge density. However, here, we ignore this effect because ionic strength is adjusted at a high value (>0.2 M) and the high ionic strength screens the electrostatic interaction.

If the MT is regarded as a cylinder with \( D_{MT} = 29 \text{ nm} \) and \( L_{MT} = 10^3 \text{ nm} \), the excluded volume \( b \) per one MT unit is calculated as \( 2.5 \times 10^9 \text{ nm}^3 \) from eq 4. Meanwhile, \( b \) for one TB dimer is \( 5.5 \times 10^2 \text{ nm}^3 \) if we model the dimer as a cylinder with \( D_{TB} = 4 \text{ nm} \) and \( L_{TB} = 8 \text{ nm} \). The number of dimers consisting one MT is calculated as \( L_{MT} / 8 \times 15 = 1.9 \times 10^4 \), considering that the dimer is 8 nm in height, and there are 15 dimers per lap. Thus, when one MT is cooled and dissociates into TBs, the total excluded volume reduces to \( (1.9 \times 10^4) \times (5.5 \times 10^2) = 1.0 \times 10^7 \text{ nm}^3 \), that is 250 times smaller than the value for the MT. Therefore, when TBs polymerize to MTs, the free volume for nanosheets is largely deprived. Considering the SAXS result that the spacing between the nanosheets in the double-component system is smaller than the diameter of the MT (29 nm), MTs cannot be present between the nanosheets forming the LC phase. This indicates that nanosheets and MTs are forming separated LC domains although they seem to be homogeneously mixed on the macroscopic scale as observed by POM. The decrease in the basal spacing in the double-component system compared to the single-component FHT colloid is ascribable to the compression of the LC phase of the nanosheet by coexisting MTs.

### CONCLUSIONS

FHT and MT were mixed uniformly, and biochemical polymerization/depolymerization of the MT proceeded without being disturbed by the presence of nanosheets. The present system is highlighted as the first system that nanosheets are mixed with rods that reversibly change their length triggered by external factors. Such a system will be a good model system for fundamental studies of anisotropic colloidal systems composed of multiple mesogenic components. Because MTs constitute the molecular motor system with kinesin, the present system will be further developed as a new molecular motor system or artificial muscle with a hierarchical ordered structure formed by synergy of mesogenic nanosheets and biological MTs.

### EXPERIMENTAL SECTION

The liquid crystalline nanosheet colloid of synthetic FHT was prepared, according to the previously reported process from NHT-B2 sol supplied from Topy Industries. This colloidal solution was diluted with pure water to 2 wt %. The average particle size of the nanosheets was 1.2 \( \mu \text{m} \) with a standard deviation of 0.14 \( \mu \text{m} \) as confirmed by dynamic light scattering (Figure S1). The TB was extracted and purified from porcine brain according to the previously reported process, using an aqueous buffer solution (pH 6.8) containing 1 M of 1,4-piperazine diethanesulfonic acid (PIPES), 20 mM of ethylene glycol tetracetic acid (EGTA), and 10 mM of MgCl\(_2\). To polymerize the TB to MT, the TB solution was added with GTP and dimethylsulfoxide (the final concentrations are 0.1 mM and 5 vol %, respectively) and incubated at 37 °C for 30 min. The nanosheet colloid and MT solution were mixed at various ratios to obtain the samples for evaluation. For all samples, including the single-component FHT colloid, the concentrations of the buffer constituents were adjusted to 0.08 M of PIPES, 0.001 M of EGTA, and 0.001 M of MgCl\(_2\) for the POM observation (Nikon ECLIPSE LV100POL), the samples were sealed in a glass capillary. Structural analyses by SAXS were performed with the Bruker NANOSTAR (Cu Ka radiation at 30 V and 40 mA) with a camera distance of 1070 mm or with the Rigaku NANOPIX (Cu K\(\alpha \) radiation at 30 V and 40 mA) with a camera distance of 750 mm. For SAXS measurements, the samples were introduced into a thin glass capillary or a cell with a thickness of 1.5 \( \mu \text{m} \). The SAXS and POM observations were conducted under a controlled temperature of 4 or 37 °C.
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b01050.

Particle size distribution of the nanosheets and SAXS patterns of the nanosheet colloids in the buffer solution (PDF)

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R.K. conducted most of the experiments, data analyses, and wrote the manuscript. N.M. and A.K. designed the research and cowrote the manuscript. K.S., Y.O., and A.M.R.K. conducted experiments regarding tubulin treatments.

**Notes**
The authors declare no competing financial interest.

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