Electronic Supplementary Material (ESI) for Nanoscale.

Electronic Supplementary Information

Twisting Bio-Nanorods Serving as Template for Constructing Chiroptically Active Nanoflowers

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Experimental Section

Materials. Cellulose microcrystals (CMCs) with average length about 25 μm were purchased from Aldrich. $CuSO_5 \cdot H_2O$ and ammonia solution were purchased from Beijing Chemical Reagent Co. All chemicals were used as received without further purification.

Preparation of desulfated cellulose nanocrystals (CNCs). Sulfated CNCs were obtained from the acid hydrolysis of CMCs, as described previously.[1] Briefly, the CMCs/water-suspension (8.23 g/36.5 mL) was put in an ice bath and stirred at room temperature, in which 95~98 wt% sulfuric acid (35.5 mL) was added dropwise. Within 5 min, the temperature was heated to 45 °C, at which the suspension was stirred vigorously for 2 hours. Subsequently, the suspension was diluted 10-fold by water to stop the reaction, neutralized with sodium hydroxide aqueous solution, and purified by dialyzing (two weeks) until the effluent remained at neutral pH. The suspension was lyophilized to produce a white powder with a yield of 28% (of initial weight).

Then the sulfate ester groups were removed as reported with minor changes.[2] Sulfated CNCs (2g) were dispersed in 1 M NaOH (100 mL), and the mixture was stirred at 60 °C. After 5 h, the reaction was stopped, and the suspension was purified by dialyzing until the effluent remained at neutral pH. Then the product was lyophilized to produce desulfated CNCs powder with a yield of 80% (1.6 g). The amount of sulfate groups was determined by conductometric titration.[3] The sulfur content is 0.04 mmol/g cellulose in desulfated CNCs and 0.32 mmol/g cellulose in sulfated CNCs.

Preparation of CuO/CNC composite nanoflowers (Flower-2) and calcined CuO Flower-2. In a typical synthesis, 0.02 g CNCs was dispersed in 100 mL deionized water. Then the system was purged with nitrogen for 30 min to remove oxygen. After that, 200 μL ammonia solution was added to the CNCs suspension to control the pH of the system and then 1.67 mL of 75 mM $Cu(NH_3)_4^{2+}$ was added into the suspension.

Keeping the system's initial pH at 12.0 by adjusting the dosage of ammonia solution, the system was heated at 40 °C for 4 h. The CuO/CNC nanoflowers were successively washed with distilled water and absolute ethanol each for three times by using centrifuge (10 min at 10,000 rpm, GL–22MS). Then the CuO/CNC nanoflowers were dispersed in ethanol and used for SEM, TEM, STEM and AFM characterizations. The nanoflowers were dispersed in water for CD and UV characterizations. The CuO/CNC nanoflowers were freeze-dried to obtain powder for XRD, XPS and FTIR characterizations.

For removing cellulose nanocrystals, the freeze-dried composite powder was heated under flowing air according to the literature.[4] The solid sample (20 mg) was heated at a rate of 120 °C/h to 100 °C, held at the temperature for 2 h, then heated to 540 °C at a rate of 120 °C/h and held at the temperature for 6 h. After slowly cooling to room temperature, calcined CuO (12 mg) was obtained.

Enantioselective crystallization experiments. All crystallization processes were carried out from the racemic solutions of threonine. Taking Flower-2 as an example, the major procedure is stated below. DL-threonine (800 mg) were added into 3 mL deionized water at 50 °C under stirring. After threonine was completely dissolved, a certain amount of Flower-2 (approx. 20 mg) was added into the solution. The mixture was maintained at 50 °C for 10 min and cooled down spontaneously to room temperature. After 12 h, the crystals were taken out, dried and subjected to characterizations. The obtained crystals were dissolved in deionized water to achieve a certain concentration (5 mM), and subjected to CD measurement. The enantiomeric excess (ee) of induced crystals was calculated according to the following equation[5]:

$$e.e.(\%)=\theta 2/\theta_{max} \quad (1)$$

where θ2 is the maximum CD value of the induced crystals from the racemic solutions, θ_{max} is the maximum CD value of D-threonine or L- threonine at the same concentration.

Scanning Electron Microscope. The washed CuO/CNC nanoflowers were dispersed in ethanol and casted on a clean silicon wafer, then dried under N_2 flow. The samples were observed with scanning electron microscope (SEM, Hitachi S-4800).

Transmission Electron Microscope. The washed CuO/CNC nanoflowers were dispersed in ethanol and two microliters of the sample solution was deposited on a carbon TEM grid. The grid was left 24 h to dry for TEM imaging. TEM images were obtained from JEM-1400ST-FC electron microscope at 120 kV. HAADF-STEM and HRTEM images were performed on a TECNAIF30 microscope at 300 kV.

Atomic Force Microscope. The washed CuO/CNC nanoflowers were dispersed in ethanol and dropcast onto a Si substrate. The samples were dried under ambient conditions. Then AFM images were taking using the tapping model on a DMFASTSCAN2–SYS.

Circular Dichroism Spectroscopy. The washed nanoflowers were dispersed in water for CD measurements by using a Jasco-810 spectropolarimeter. The dried nanoflowers were measured in the solid state by diffuse reflectance circular dichroism (JASCO J-815). The anisotropy (g) factor was calculated according to the following equation[6,7]:

$$g = \frac{CD}{32980 \times abs.} \quad (2)$$

Other Apparatus. FTIR spectra were recorded on a Nicolet NEXUS 670 spectrophotometer (in KBr tablets). XPS measurements were performed with an ESCALAB 250Xi electron spectrometer. The PXRD patterns were recorded on a Rigaku D/Max 2000 powder diffractometer.

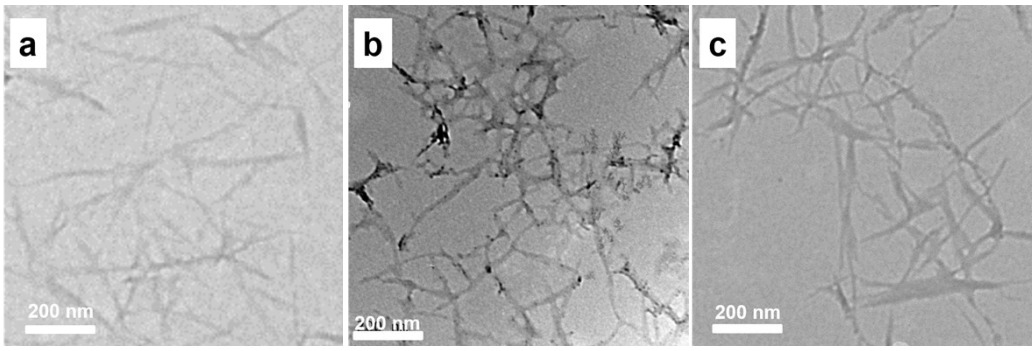

Fig. S1. TEM images of CNCs. **a**, pristine CNCs. **b**, CNCs coordinated with $Cu(NH_3)_4^{2+}$ ions. **c**, CNCs obtained from the CuO/CNC nanoflowers (Flower-2) by washing off the CuO with 1 M HCl.

The length of CNCs is about 200~500 nm, and the width of CNCs is about 10~30 nm (**Fig. S1a**). By mixing CNCs with $Cu(NH_3)_4^{2+}$ ions, the $Cu(NH_3)_4^{2+}$ ions formed coordination with the –OH groups on CNC surface, as showed in **Fig. S1b**. After the treatment by washing off CuO from CuO/CNC nanoflowers, the residual CNC rods changed little, compared with the pristine CNCs, indicating that CuO grew on the surface of CNC rods.

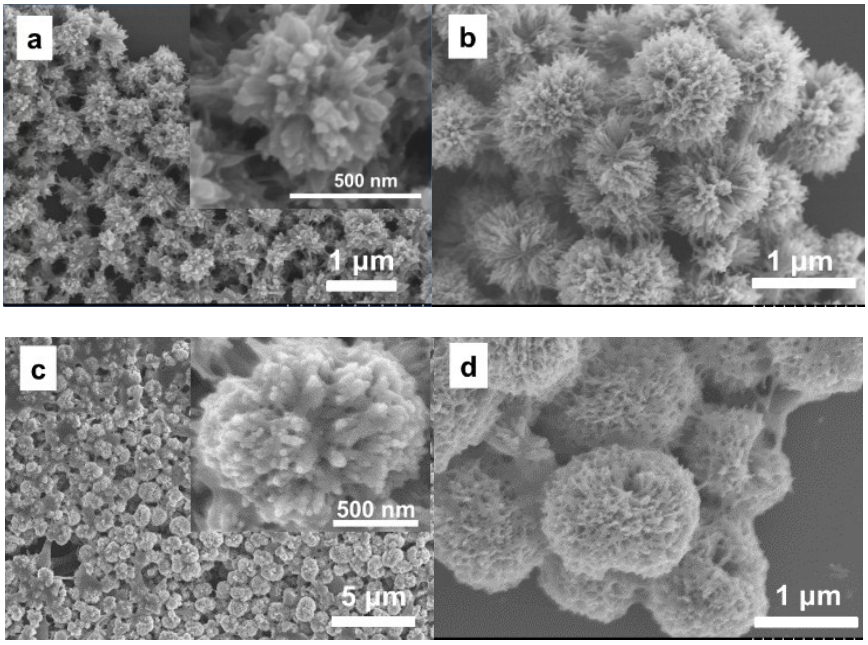

Fig. S2. Four kinds of chiroptical nanoflowers prepared at different conditions. **a–d**, Flowrer-1, Flowrer-2, Flowrer-3 and Flowrer-4, respectively. The corresponding TEM images are demonstrated in **Fig. S3**†.

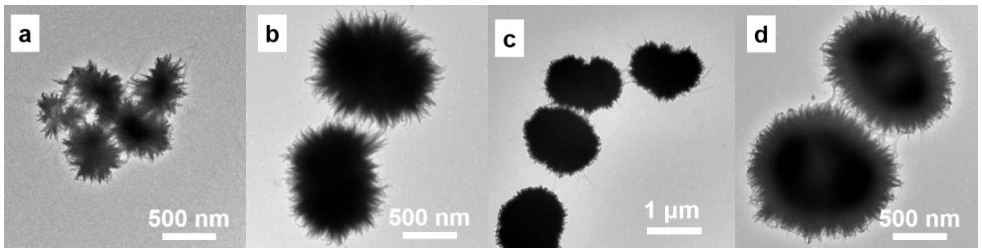

Fig. S3. TEM images of four kinds of chiroptical nanoflowers prepared at different conditions. **a–c**, TEM images of CuO/CNC composite nanoflowers are synthesised with different $Cu(NH_3)_4^{2+}$/glucose unit ratio. Keep the pH value at 12.0 and temperature 40°C, the $Cu(NH_3)_4^{2+}$/glucose unit/H_2O ratio is 0.75/1.2/56000 (**a**, Flower-1), 1.25/1.2/56000 (**b**, Flower-2), and 1.5/1.2/56000 (**c**, Flower-3), respectively. **d**, TEM image of Flower-4 prepared at 20°C, with other conditions same as in Flower-2.

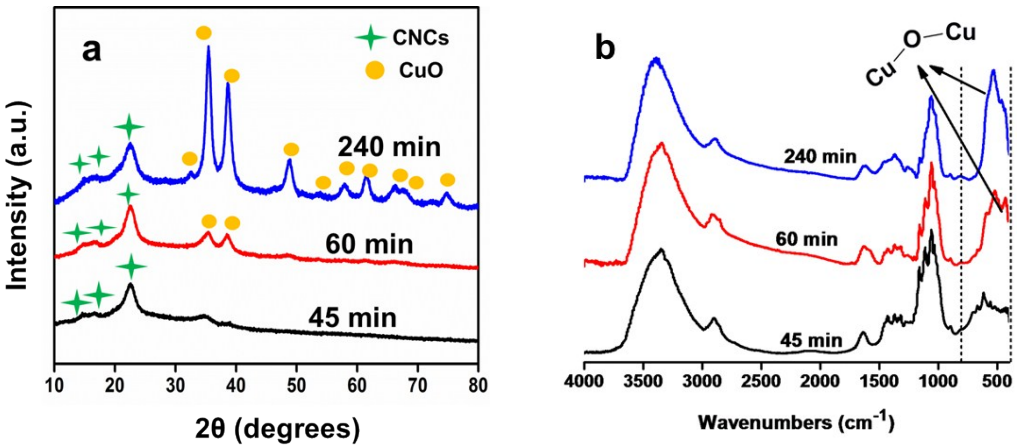

Fig. S4. PXRD patterns (**a**) and FTIR spectra (**b**) of CuO/CNC nanoflowers (Flower-2) growing at different time.

The PXRD patterns varied with time. At initial stage, it mainly demonstrated a typical cellulose I reflection.[8,9] At the second stage, we observed several PXRD diffraction peaks of CuO (corresponding to 002, 111, 200 crystallographic planes, see ***Fig. S4***). The CuO structures were increasing at this stage and formed composite structures.

Then the CuO further grew and the peak intensity of CuO increased at the last stage. At the growing process of Flower-2, the PXRD diffraction peaks of CNCs changed little, indicating that the structure of CNCs was maintained. Therefore, the Cu(II), such as $Cu(NH_3)_4^{2+}$ ions, $Cu(OH)_2$ and CuO, interacted with the surface –OH groups of CNCs without influencing the crystal structures of CNCs. These phenomenona are consistent with the results shown in the FTIR spectra growing at different time.

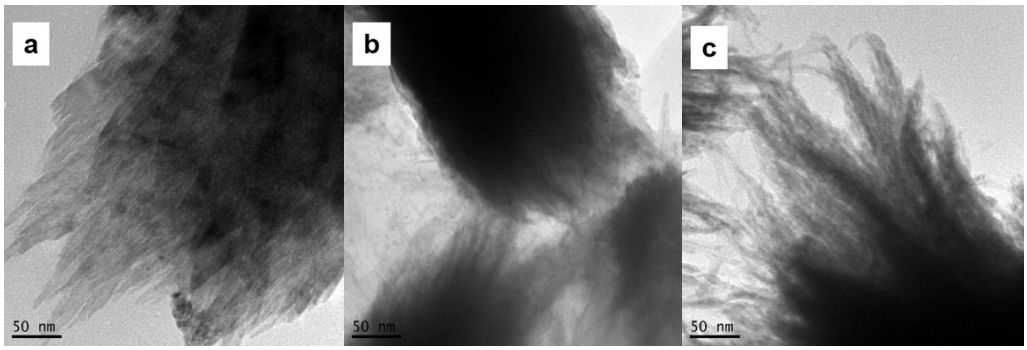

Fig. S5. TEM images of nanosheets (**a**) and nanofibers (**b, c**) in three samples. **a**, CuO nanosheets prepared without cellulose. CuO nanofibers are induced by cellulose microcrystals (CMCs, **b**) and CNCs(**c**).

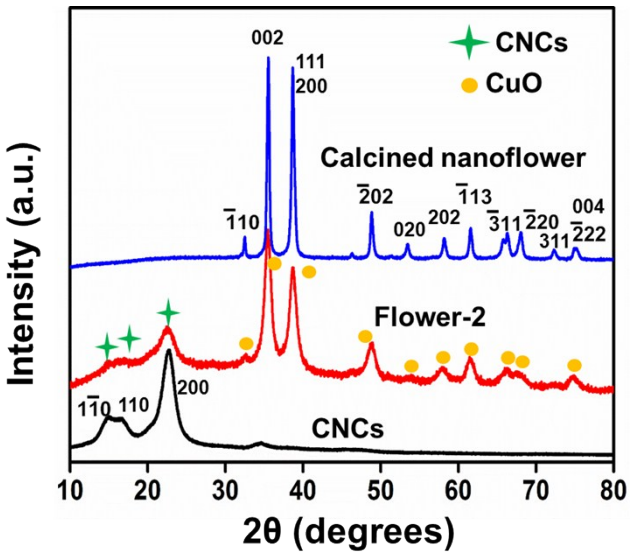

Fig. S6. PXRD patterns of CNCs, CuO/CNC composite nanoflowers (Flower-2) and calcined CuO from Flower-2.

The PXRD pattern of CNCs showed a typical cellulose I reflections, corresponding to three crystallographic planes of the monoclinic cellulose I lattice, according to the reported literatures.[8,9] In the composite nanoflowers (Flower-2), the PXRD diffraction peak shape and corresponding degrees of CNCs were similar to that in pure CNCs, confirming that crystal structure of CNCs did change after the formation of CuO. Therefore, it further confirmed that $Cu(NH_3)_4^{2+}$ ions formed coordination with the –OH groups on CNC surface, which contributed to the CuO growing on the surface of CNCs. However, the peak intensity of CNCs in Flower-2 decreased because of the presence of CuO, indicating the flower-like nanocomposites consisted of CNCs and CuO. Besides, no other peak reflecting impurities (such as Cu_2O) was observed. After removing cellulose nanocrystals in Flower-2 by calcination, the calcined CuO showed increased diffraction peaks and narrowed peak width, indicating that both the crystallinity of CuO and particles size increased, which was consistent with the electron diffraction patterns in ***Fig. S9***.

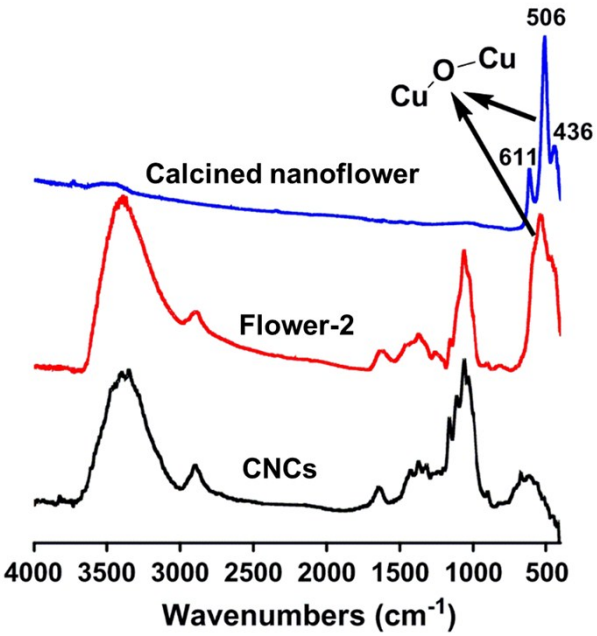

Fig. S7. FTIR spectra of CNCs, CuO/CNC composite nanoflowers (Flower-2) and calcined CuO from Flower-2.

The typical vibration characteristics of CNCs were observed in Flower-2, and the Cu-O vibration bands also existed, indicating the presence of CNCs and CuO in Flower-2. The result also confirmed that flower-like nanocomposites consisted of CNCs and CuO. By calcination treatment from Flower-2, calcined CuO was obtained. There were three infrared peaks observed at 436, 506 and 611 cm^{-1}, which were assigned to the Au mode, Bu mode and another Bu mode, respectively. The peak at 611 may be Cu-O stretching along the [-101] direction, and the peak at 506 may be Cu-O stretching along the [101] direction.[10,11]

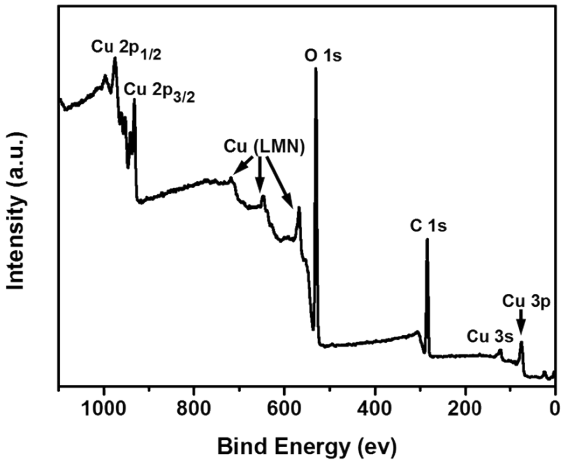

Fig. S8 Representative XPS survey spectrum of CuO/CNC composite nanoflowers (Flower-2). Further evidence for the purity and composition of the composite nanoflowers was obtained by XPS, and the result showed that the indexed peaks corresponded to Cu, O and C.

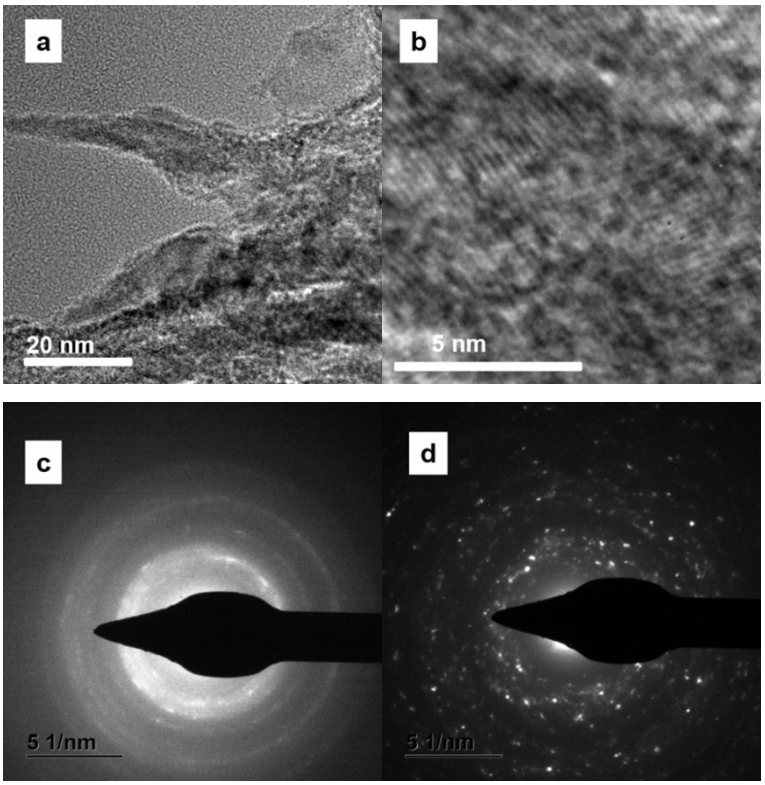

Fig. S9. HRTEM images (**a, b**) of nanofibers and electron diffraction patterns of composite nanoflowers (**c**) and calcined CuO from composite nanoflowers (**d**).

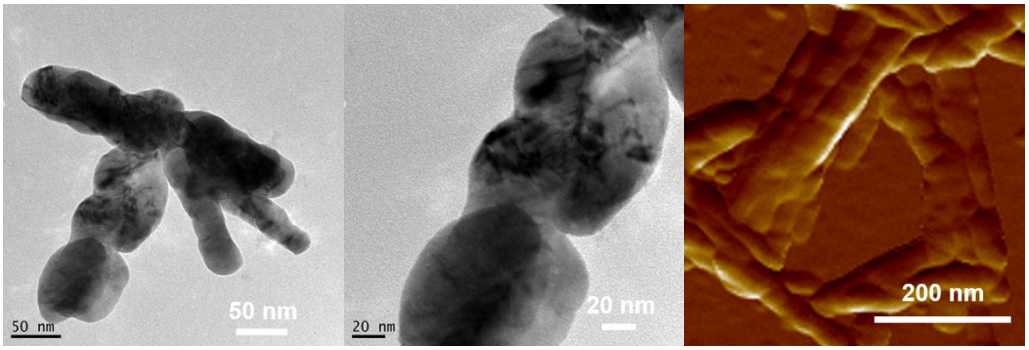

Fig. S10. TEM and AFM images of helical nanopetals from calcined CuO nanoflowers. To clearly observe one single right-handed nanopetal of the calcinated nanoflower, the aqueous dispersion of the sample was ultrasounded about 1 h and the supernatant was used.

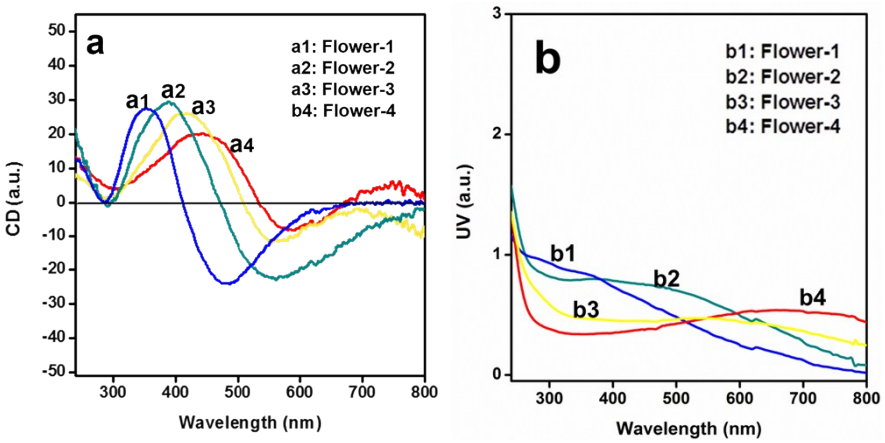

Fig. S11. CD and UV spectra of Four kinds of chiroptical nanoflowers prepared at different conditions.

Increasing the molar ratio of $Cu(NH_3)_4^{2+}$ to cellulose glucose units from Flower-1 to Flower-3, resulting in a redshift in the CD effects of the nanoflowers, as shown in **Fig. S11-a1**, **a2**, **a3**. This is mainly ascribed to the increased dimension of nanoflowers (**Fig. S2-a**, **b**, **c**), which also indicates the increased length of nanopetals. Size-dependent chiroptical redshifts have been observed in literatures.[6,12] Besides, the CD signal of Flower-3 at near-infrared wavelength was different compared with Flower-1 and Flower-2, resulting from the relatively stronger interaction of nanopetals in

Flower-3 (**Fig. S2-b**, **c**). The nanopetals in Flower-3 were packed more closely and tended to arrange helically, while in Flower-1 and Flower-2, the nanopetals were more randomly arranged. This phenomenon is more evident in Flower-4, which was prepared at 20 °C with other synthesis conditions keeping the same as in Flower-2. The CD signal had a redshift and rose from negative to positive at ≈670 nm compared with Flower-2 in **Fig. S11-a4**. This may be ascribed to the nanopetals in Flower-4 formed at a slower rate and arranged more orderly (**Fig. S2-d**). The CD signal difference at near-infrared wavelength possibly indicates that higher level chirality occurred in the nanoflowers by helically arranged nanopetals. Accordingly, an effective method was established for constructing chiroptical CuO/CNC nanoflowers using CNCs as chiral template.

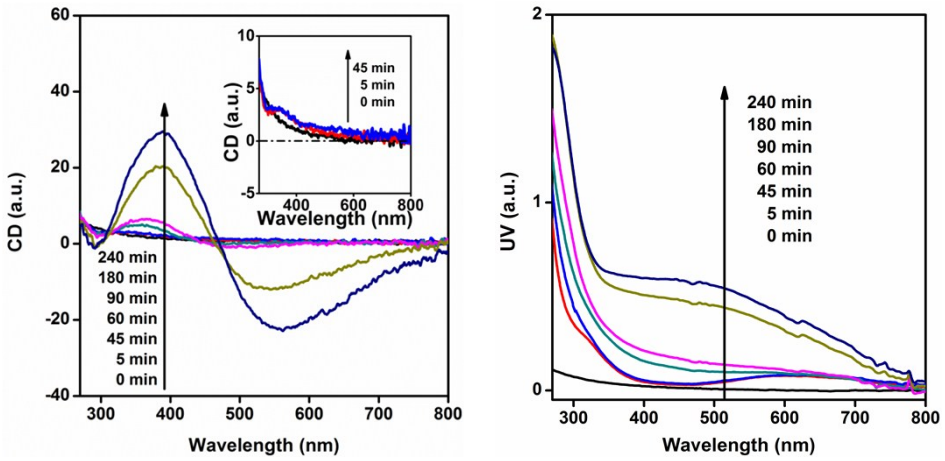

Fig. S12. CD and UV-vis spectra of CuO/CNC nanoflowers (Flower-2) growing at different time. In order to get the real-time chirality in the process of forming Flower-2, the spectra were measured with the reaction solution.

At initial stage (about 0~45 min), there was only positive CD effect at the wavelength of 300~400 nm. The CD effect did not changed at this stage. This was due to that $Cu(NH_3)_4^{2+}$ ions formed coordination with surface –OH groups of twisting CNCs and then chirally distributed on the surface of CNCs. Thus, the $Cu(OH)_2$ or CuO formed *in situ* also chirally distributed on the surface of CNCs. At the second stage, the negative CD signal between 450 and 800 nm existed, indicating that the helical CuO structure was preliminarily generated. At the last stage, the intensity of peak-dip CD signal increased and the final chiral nanoarchitecture was formed.

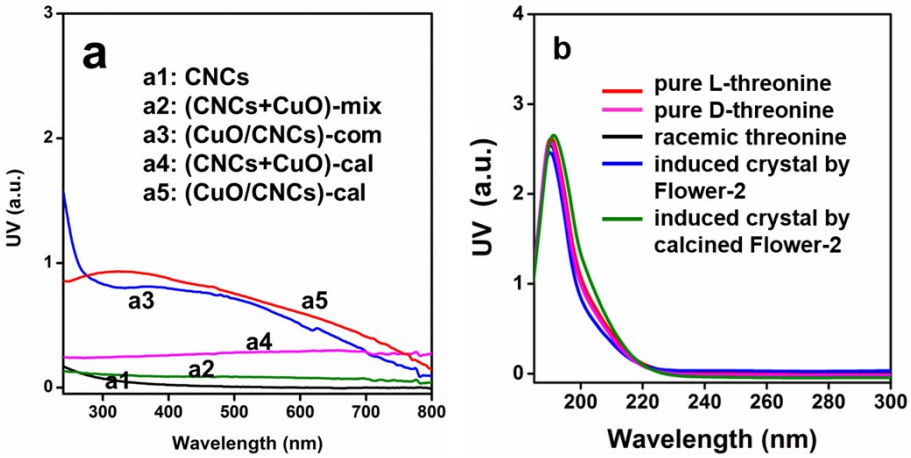

Fig. S13. UV-vis spectra of CNCs, nanoflowers, threonine crystals. CNCs (**a1**), CNCs mixed with pure CuO (CNCs+CuO, **a2**), Flower-2 (**a3**), calcined CuO from (CNCs+CuO) mixtures (**a4**) and calcined CuO from Flower-2 (**a5**). **b**, CD spectra of pure L-threonine (**b1**); Pure D-threonine (**b2**); threonine crystals obtained without any additive (**b3**); threonine crystals induced by Flower-2 (**b4**); threonine crystals induced by calcined Flower-2 (**b5**).

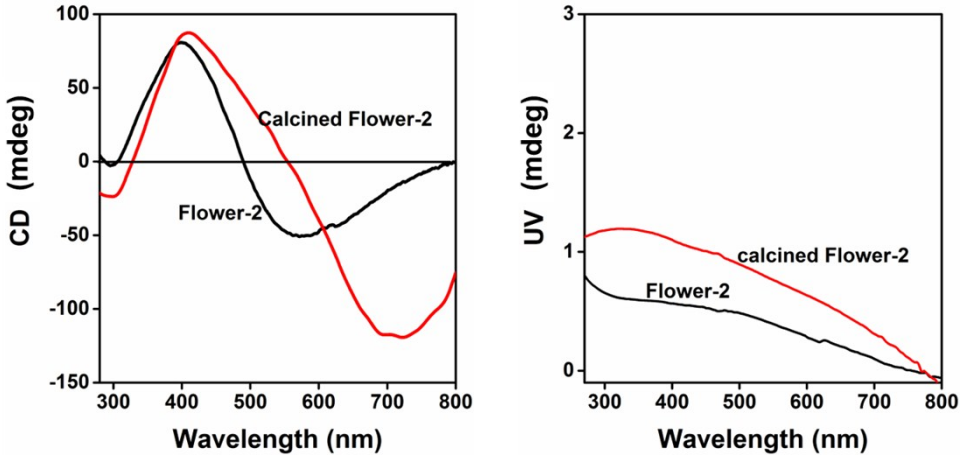

Fig. S14. CD and UV spectra of Flower-2 and calcined Flower-2 measured in the solid state.

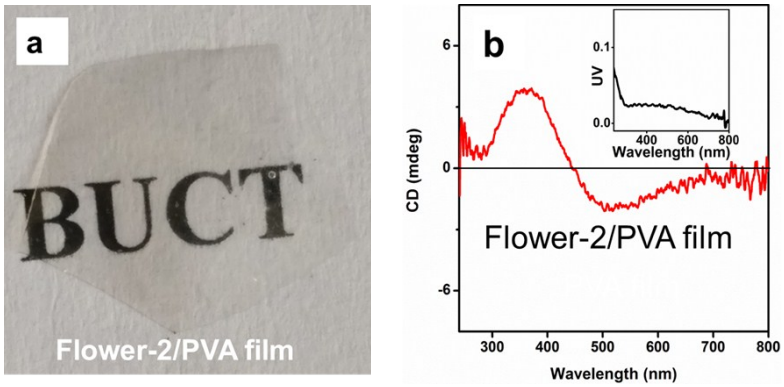

Fig. S15. The chiroptical composite film of Flower-2/poly(vinyl alcohol) (PVA). **a,** the photograph of the composite chiroptical film. b, CD and UV-vis spectrum of the film. Flower-2/PVA water solution (PVA, 5 wt.%)=10 mg/10 ml; The mixture of Flower-2/PVA was treated by ultrasound for about 10 minutes and then cast on glass dishes for 2 days.

The composite film of Flower-2/PVA is transparent and demonstrates a similar CD shape with Flower-2. The UV adsorpion is weak because of the low content of Flower-2 in the composite film. Therefore, the composite film is of optical activity with desirable transparent and chiroptical properties. The film may have important applications in developing chiroptical materials.

References

1. H. Yu and J. P. Deng, *Macromolecules,* 2016, **49**, 7728.
2. M. Hasani, E. D. Cranston, G. Westman and D. G. Gray, *Soft Matter,* 2008, **4**, 2238.
3. E. Kloser and D. G. Gray, *Langmuir,* 2010, **26**, 13450.
4. K. E. Shopsowitz, H. Qi, W. Y. Hamad and M. J. Maclachlan, *Nature,* 2010, **468**, 422.
5. B. Yang, S. Raza, S. Li and J. P. Deng, *Ploymer*, 2017, **127**, 214.

6. W. Feng, J. Y. Kim, X. Wang, H. A. Calcaterra, Z. Qu, L. Meshi and N. A. Kotov, *Sci. Adv.,* 2017, **3**, e1601159.

7. P. McPhie, *Anal. Biochem*., 2001, **293**, 109.

8. F. Jiang, A. R. Esker and M. Roman, *Langmuir*, 2010, **26**, 17919.

9. J. Sugiyama, R. Vuong and H. Chanzy, *Macromolecules*, 1991, **24**, 4168.

10. G. Zou, H. Li, D. Zhang, K. Xiong, C. Dong and Y. Qian, *J. Phys. Chem. B*, 2006, **110**, 1632.

11. Y. Duan, X. Liu, L. Han and S. Asahina, D. Xu, Y. Cao, Y. Yao, S. Che, *J. Am. Chem. Soc*., 2014, **136**, 7193.

12. A. Ben-Moshe, S. G. Wolf, M. B. Sadan, L. Houben, Z. Fan, A. O. Govorov and G. Markovich, *Nat. Commun.,* 2014, **5**, 4302.