Electronic Supplementary Information

Natural Lignin Nanoparticles: A Promising Nano-crosslinker for Constructing Fluorescent Photoswitchable Supramolecular Hydrogels

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**Scheme S2.** Synthetic route of α-CD@CEL-NPs and Azo-Gu.

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**Materials and instruments**

1-Amidinopyrazole hydrochloride (C$_4$H$_7$ClN$_4$, CAS No. 4023-02-3), 4-(2-aminoethyl) aniline (C$_8$H$_{12}$N$_2$, CAS No. 13472-00-9), nitroso benzene (C$_6$H$_5$NO, CAS No. 586-96-9), di-tert-butyl dicarbonate (Boc$_2$O) (C$_{10}$H$_{18}$O$_5$, CAS No. 24424-99-5), N,N-diisopropylethylamine (C$_8$H$_{19}$N, CAS No. 7087-68-5) and α-cyclodextrin (α-CD) (C$_{36}$H$_{60}$O$_{30}$, CAS No. 10016-20-3) were purchased from Chengdu Best Reagent Co., Ltd. (Chengdu). N,N-dimethylformamide (DMF) was purchased from Saan Chemical Technology (Shanghai) Co., Ltd. (Shanghai). Hydrochloric acid (HCl, CAS No. 7647-01-0), sodium hydroxide (NaOH, CAS No. 1310-73-2), potassium hydroxide (KOH, CAS No. 1310-58-3), diethyl ether (C$_4$H$_{10}$O, CAS No. 60-29-7), triethylamine (C$_6$H$_{15}$N, CAS No. 121-44-8) and sodium sulfate anhydrous (Na$_2$SO$_4$, CAS No. 7757-82-6) were purchased from Chengdu Cologne Chemical Co., Ltd. (Chengdu). Magnesium sulfate (MgSO$_4$, CAS No. 7487-88-9), sodium carbonate (Na$_2$CO$_3$, CAS No. 497-19-8), acetic acid (C$_2$H$_4$O$_2$, CAS No. 64-19-7), ethanol (C$_2$H$_6$O, CAS No. 64-17-5), ethyl acetate (C$_4$H$_8$O$_2$, CAS No. 141-78-6), triphenylphosphine (C$_{18}$H$_{15}$P, CAS No. 603-35-0), sodium methanolate (CH$_3$NaO, CAS No. 124-41-4), hexane (C$_6$H$_{14}$, CAS No. 110-54-3), dichloromethane (DCM) (CH$_2$Cl$_2$, CAS No. 75-09-2), tetrahydrofuran (THF) (C$_4$H$_8$O, CAS No. 109-99-9), potassium bisulfate (KHSO$_4$, CAS No. 7646-93-7), iodine (I$_2$, CAS No. 7553-56-2) and methanol (CH$_4$O, CAS No. 67-56-1) were purchased from Shanghai Titan Scientific Co., Ltd. (Shanghai). Thiourea (CH$_4$N$_2$S, CAS No. 62-56-6) was purchased from Tianjin Jinbei Fine Chemical Co., Ltd. (Tianjin). Poly(acrylic acid) (PAA) ((C$_3$H$_4$O$_2$)$_n$, Mw=4000000,
CAS No. 9003-01-14) and 2,2-dimethoxy-2-phenylacetophenone (DMAP) (C_{16}H_{16}O_{3},
CAS No. 24650-42-8) were purchased from Aladdin Reagent Co., Ltd (Shanghai).
Trifluoroacetic acid (TFA) (C_{2}H_{2}O_{3}F_{3}, CAS No. 76-05-1) was purchases from Tiexiai
Chemical Industry Development Co., Ltd (Shanghai). All the organic solvents were
used as purchased without further purification. Milli-Q water (resistivity: 18.2
MΩ×cm) was used throughout the project.

\(^1\)H nuclear magnetic resonance (NMR) and nuclear overhauser effect
spectroscopy (NOESY) spectra were measured on a Bruker-400 (Bruker, USA).
Scanning electron microscopic (SEM) images were recorded using a JSM-7500F
(JEOL, JPN). Transmission electron microscopic (TEM) images were measured using
a Tecnai G2 F20 S-TWIN (FEI, USA). Fourier transform infrared spectra (FTIR)
were measured by a Nicolet 6700 (Thermo Fisher Scientific, USA). X-ray
photoelectron spectra (XPS) spectra were recorded on a XSAM 800 (Kratos, UK).
Fluorescence spectra were recorded with a XRF-1800 (Shimadzu, JPN). UV/vis
spectra were recorded by an UV-2600 (Shimadzu, JPN). Photoisomerization of Azo-
Gu was induced by LED with the wavelength of 365 and 470 nm (Zhongjiao Jinyan
Systems). The output intensity of LED was controlled by a LED controller (Zhongjiao
Jinyuan Systems).

Synthesis
Scheme S1. Schematic illustration of the self-assembled process from cellulolytic enzyme lignin (CEL) to cellulolytic enzyme lignin nanoparticles (CEL-NPs).

Scheme S2. The synthetic route of α-CD@CEL-NPs and Azo-Gu.

Synthesis of CEL-NPs. **[1]** CEL (3.41g) was dissolved into ethanol (~25 mg/ml). After stirring for 6 h under room temperature, the suspension was kept for 30 min to precipitate insoluble substances. The supernatant was collected and kept for 6 h to form self-assembled nanoparticles. Then the solvent was evaporated using a rotary evaporator. The product was dried under 40 °C for the next step.

Synthesis of α-CD-SH. **[2]** α-CD (2.01 g, 2.12 mmol) was added to a stirred solution
of triphenylphosphine (10 g, 38 mmol) and iodine (9.68 g, 38 mmol) in DMF (80 mL), the solution was stirred at 80 °C for 15 h. It was then concentrated under vacuum to half the volume, followed by adjusting the pH to 9-10 by the addition of sodium methanolate (3 M, 30 mL). The solution was kept at room temperature for 30 min to remove the formed esters in the reaction, after which it was poured into ice water (1.5 L). The precipitate was collected by filtration to obtain 3.3 g α-CD-I, the yield is 65%.

1H NMR: (400 MHz, DMSO-<sub>d6</sub>, 298K) δ (ppm): 3.24-3.47 (m, 18 H), 3.54-3.68 (m, 12 H), 3.80 (d, J = 9 Hz, 6 H), 4.99 (d, J = 3 Hz, 6 H), 5.94 (d, J = 2 Hz, 6 H), 6.05 (d, J = 6.5 Hz, 6 H).

3.63g α-CD-I (2.22 mmol) and 1.18 g thiourea (15.54 mmol) were dissolved into 30 ml DMF. The mixture was heated to 80 °C for 24 h under nitrogen atmosphere. After the reaction was completed, most DMF was evaporated under reduced pressure. 0.89 g NaOH (22.25 mmol) was added into the solution and the mixture was stirred and refluxed for 1 hour. After cooling to room temperature, 3.03 g KHSO<sub>4</sub> (22.25 mmol) was added, followed by filtration, washing with water, and drying. The crude product was added into 50 ml distilled water, and a little amount of KOH was added to make the solution clear. Adding acidifying with KHSO<sub>4</sub> 1.71 g white powder was obtained, the yield is 72%. 1H NMR: (400 MHz, DMSO-<sub>d6</sub>) δ (ppm): 2.16 (t, J = 6 Hz, 6 H, SH), 2.79 (m, 6 H), 3.21 (d, J = 14 Hz, 6 H), 3.36-3.40 (m, 12 H), 3.60 (t, J = 9 Hz, 6 H), 3.68 (t, J = 8 Hz, 6 H), 4.95 (d, J = 2.5 Hz, 6 H), 5.83 (s, 6 H), 5.97 (d, J = 6 Hz, 6 H)

**Synthesis of α-CD@CEL-NPs.** To a mixture of 2 g CEL-NPs and 2 g (1.87 mmol) α-
CD-SH in 100 ml THF, 20 mg DMPA (0.08 mmol) was added as a photoinitiator. After irradiating for 2 h under a UV lamp of 365 nm, the reaction was completed, and the solvent was then evaporated by rotated evaporation to obtain α-CD@CEL-NPs.

**Synthesis of compound 4.** [3-4] 3 g 2-(4-aminophenyl) ethylamine (21.9 mmol) was dissolved into 100 mL THF and a solution of 4.97 g Boc₂O (22.5 mmol) in 100 mL THF was added dropwise. After stirring for 2 h at room temperature, the mixture was diluted with 400 mL ethyl acetate, and washed sequentially with 100 mL HCl (1 M), 150 mL NaOH (1 M), sat. 150 mL NaHCO₃ and 150 mL brine and dried over Na₂SO₄. The solvent was removed by rotary evaporation and the crude product was purified using silica gel chromatography (30 % ethyl acetate in hexanes) yielding 4.91 g compound (20.81 mmol). The yield is 95%. ¹H NMR: (400 MHz, Chloroform-δ) δ 6.98 (d, J = 8.2 Hz, 2H), 6.64 (d, J = 8.4 Hz, 2H), 3.35-3.28 (m, 2H), 2.68 (t, J = 7.0 Hz, 2H), 1.43 (s, 9H).

**Synthesis of compound 5.** [3-4] 4.91 g compound 3 (20.81 mmol) was dissolved into 30 mL DCM, 2.34 g nitrosobenzene (21.85 mmol) and 12.4 mL acetic acid (217 mmol) were then added. After stirring over night at room temperature, the mixture was diluted with 300 mL ethyl acetate and the organic phase was washed sequentially with 50 mL HCl (1 M), 50 mL NaOH (1 M), sat. 50 mL NaHCO₃ and 50 mL brine. After drying over Na₂SO₄, the solvent was removed in vacuo. The resulting orange oil was dissolved in 10 mL DCM, 16 mL TFA (217 mmol) was then added for Boc-deprotection. After stirring for 1 h, the mixture was basified with 60 mL NaOH (5 M) and extracted with 150 mL ethyl acetate. The organic phase was washed with sat. 50
mL NaHCO$_3$ and 50 mL brine sequentially. The ethyl acetate layer was filtered and concentrated in vacuo, followed by purification using flash column chromatography (SiO$_2$; 100% DCM), yielding primary 2.94 g compound 5 (13.1 mmol). The yield is 63%. $^1$H NMR: (400 MHz, Chloroform-$d$) δ 7.89 (dd, $J = 13.0, 7.8$ Hz, 4H), 7.50 (dt, $J = 13.8, 6.7$ Hz, 3H), 7.36 (d, $J = 8.3$ Hz, 2H), 3.08-3.00 (m, 2H), 2.85 (t, $J = 6.6$ Hz, 2H).

**Synthesis of Azo-Gu.** [3-4] 2.94 g compound 5 (13.1 mmol) was suspended in 15 mL DMF, followed by adding 2.16 g 1-amidinopyrazole hydrochloride (14 mmol) and 5.1 mL N,N-diisopropylethylamine (30.6 mmol), the mixture was then stirred over night at room temperature. The crude product was precipitated from the clear, red solution by addition of diethyl ether, yielding red oil. The oil and supernatant were separated by decantation and the product was dried under vacuum to yielding the corresponding Azo-Gu as an orange solid. $^1$H NMR: (400 MHz, Chloroform-$d$) δ 7.96-7.81 (m, 4H), 7.55-7.45 (m, 3H), 7.35 (d, $J = 8.2$ Hz, 2H), 3.47-3.40 (m, 2H), 2.89 (t, $J = 6.8$ Hz, 2H).

**Calculation of the grafting ratio of α-CD on CEL-NPs.**
**Calculation method 1** (Using the measured data from thiolated β-CD on flat Au films \(^5\))

The surface concentration of thiolated β-CD on flat Au films is \(0.7 \times 10^{-10}\) mol/cm\(^2\). The lower rim outer diameter is 15.3 Å and 13.7 Å for β-CD and α-CD. The surface concentration of thiolated α-CD on flat CEL films is therefore calculated to be \(0.78 \times 10^{-10}\) mol/cm\(^2\).

The areas on CEL-NPs is \(1.13 \times 10^4\) nm\(^2\)

So, the CEL-NPs contains about \(5.31 \times 10^3\) α-CD.

**Calculation method 2** (Using the measured data from thiolated β-CD on Au nanoparticles \(^6\))

Every Au nanoparticle (diameter ~8 nm) can graft 50 β-CD.

From lower rim outer cavity diameter ratio, every CEL-NPs (diameter ~8 nm) can graft 55.84 α-CD.

On CEL-NPs (diameter 8 nm), every α-CD occupies about 3.58 nm\(^2\).

The areas on CEL-NPs is \(1.13 \times 10^4\) nm\(^2\)

So, the CEL-NPs contains about \(3.16 \times 10^3\) α-CD.

The calculated results from the above two methods are similar. So, we believe the calculated results are reliable.
Figure S1. FTIR spectra of α-CD-SH (black), CEL-NPs (red) and α-CD@CEL-NPs (blue).

FTIR spectra indicated successful synthesis of α-CD@CEL-NPs by thiol-ene click reaction between α-CD-SH and CEL-NPs. For α-CD-SH, the absorption band at ∼3374, ∼2566 and ∼1043 cm⁻¹ are attributed to the stretch vibration of O-H, S-H and C-O, respectively. The absorption peak at ∼1603 cm⁻¹ for CEL-NPs is attributed to the stretching vibration of C=Ç on nanoparticles surface, which can be further clicked by thiol of α-CD-SH. After clicking, the absorption at ∼1043 cm⁻¹ assigned to the stretching vibration of C-O was observed for α-CD@CEL-NPs, indicating the successful grafting of α-CD on CEL-NPs surface.
Figure S2. Fluorescence spectra of CEL-NPs (black) and α-CD@CEL-NPs (red).

The fluorescence of CEL-NPs was investigated by applying 365 nm UV light as excitation. A strong blue fluorescence with the wavelength of 460 nm was shown for CEL-NPs. Grafting of α-CD induced a slight red shift of the fluorescence (~30 nm), which is attributed to the introduced electron withdrawing groups and increased rigidity on CEL-NPs surface (Figure S2).
Figure S3. $^1$H NMR spectra (400 MHz at 298 K) of Azo-Gu before and after 365 nm UV light irradiation (40 mW/cm$^2$, 20 min) ([Azo-Gu]=7 mM in D$_2$O).

The $\text{trans}$-$\text{cis}$ photoisomerization of Azo-Gu in D$_2$O was determined by $^1$H NMR. Approximately 75% $\text{trans}$ Azo-Gu switched to $\text{cis}$ Azo-Gu after UV light irradiation.
Figure S4. NOESY spectrum (400 MHz in D$_2$O at 298 K) of the trans Azo-Gu/α-CD ([Azo-Gu]=10 mM, [Azo-Gu]:[α-CD]=1:1 in molar ratio).

Figure S5. NOESY spectrum (400 MHz in D$_2$O at 298 K) of the cis Azo-Gu/α-CD ([Azo-Gu]=10 mM, [Azo-Gu]:[α-CD]=1:1 in molar ratio), cis Azo-Gu was obtained by irradiating trans Azo-Gu for 40 min with 365 nm UV light (40 mW/cm$^2$).
Figure S6. $^1$H NMR spectra (400 MHz at 298 K) of $trans$ Azo-Gu with different concentrations of $\alpha$-CD (0, 35, 70, 105 and 140 mM) in D$_2$O ([$trans$ Azo-Gu]=7 mM in D$_2$O).

Figure S7. $^1$H NMR spectra (400 MHz at 298 K) of $cis$ Azo-Gu with different concentrations of $\alpha$-CD (0, 35, 70, 105 and 140 mM) in D$_2$O ([$cis$ Azo-Gu]=7 mM in D$_2$O). $cis$ Azo-Gu was obtained by irradiating $trans$ Azo-Gu for 20 min with 365 nm UV light (40 mW/cm$^2$).
Figure S8. Association constants (Ka) between Azo-Gu and α-CD. The data for calculating the association constants were obtained from Figure S6 and S7.

A modified Benesi-Hildebrand equation was used for the calculation of the association constants between Azo-Gu and α-CD:

\[
\frac{1}{\Delta\delta_{\text{obs}}} = \frac{1}{\Delta\delta} \times \frac{1}{K_a} \times \frac{1}{C_{\text{CD}}} + \frac{1}{\Delta\delta}
\]

where \( \Delta\delta_{\text{obs}} \) is the observed shifts of the peaks; \( K_a \) is the association constant; \( \Delta\delta \) is a constant correlated to the concentration of Azo-Gu; \( C_{\text{CD}} \) is the concentration of the α-CD.

The concentration of Azo-Gu (in trans or cis) was kept at 7 mM in D₂O, while α-CD was added with the concentrations of 35, 70, 105 and 140 mM, respectively. The cis Azo-Gu sample was obtained by irradiating the trans Azo-Gu with UV light (40 mW/cm²) for 20 min.
The Ka between \textit{trans} Azo-Gu and \(\alpha\)-CD was calculated to be \(\sim2372\ \text{M}^{-1}\), which is in good accordance with previous reports (\textbf{Figure S6 and S8}) \cite{7}. The Ka between \textit{cis} Azo-Gu and \(\alpha\)-CD is much weaker, which is \(\sim7.6\ \text{M}^{-1}\) (\textbf{Figure S7 and S8}).

\textbf{Figure S9.} Photographic images of supramolecular hydrogels formed by PAA with the concentrations of 1, 2, 3, 4 and 5 wt.\%. (a) just dissolved; (b) after keeping for 20 minutes.

PAA was dissolved into water with the concentrations of 1, 2, 3, 4, and 5 wt.\%, respectively. Hydrogels formed quickly when the concentration is beyond 3 wt.\%, and
no hydrogels were formed for the samples of 1 and 2 wt.%. After keeping for 20 minutes, it was found that hydrogels could be formed for the sample with the concentration of 2 wt.%.

**Figure S10.** Strain sweep of ternary supramolecular hydrogels (at an angular frequency of 6.28 rad/s): (a) pristine hydrogels; (b) after 1st UV light irradiation; (c) after 1st blue light irradiation; (d) after 2nd UV light irradiation; (e) after 2nd blue light irradiation; (f) after 3rd UV light irradiation.

The rheology of supramolecular hydrogels was investigated after sequential UV and blue light irradiation. The same supramolecular hydrogels were used without change during the test. When the supramolecular hydrogels were in the gel state, the three-dimensional networks were stable with the shear strain lower than ~3.2%, which were destroyed under the shear strain higher than ~3.2%. When the supramolecular hydrogels were in the sol state, the storage modulus ($G'$) was always lower than the loss modulus ($G''$) (**Figure S10b, S10d and S10f**).
Figure S11. Frequency sweep of ternary supramolecular hydrogels (at a strain of 1%): (a) pristine hydrogels; (b) after 1st UV light irradiation; (c) after 1st blue light irradiation; (d) after 2nd UV light irradiation; (e) after 2nd blue light irradiation; (f) after 3rd UV light irradiation.

For the frequency sweep of the ternary supramolecular hydrogels. An intersected point between $G'$ and $G''$ with increased shearing frequency was observed for the supramolecular hydrogels after UV light irradiation, indicating the hydrogels were deformed after shearing at high frequency (Figure S11b, S11d and S11f). For the hydrogels under gel state, no intersected point was observed, indicating the hydrogels were much stronger (Figure S11a, S11c and S11e). This is attributed to the formed host-guest complexes between Azo and $\alpha$-CD.
References


