Supporting Information

Self-Assembly of Glutathione S-transferases into Nanowires

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Protein Expression and Purification. The genetically modified *Sj*GST-6His by adding the His-tag to *Sj*GST was purified using anion exchange chromatography on a DEAE Sepharose CL-6B column, rather than metal-affinity chromatography which was typically used to purify histidine-tagged protein but stripped a small amount of divalent metal ion from the column when using imidazole to elute the bound protein which could interfere with metal-directed protein self-assembly. The recombinant *SjGST-6His* with *SjGST* gene cloned into expression vector pET-6His was transformed into the *Escherichia coli* strain BL21 (DE3) (Novagen). The transformed cells were grown at 37 °C with shaking in 1 L LB medium containing 100 μg/ml Amp to an OD600 in the range of 0.8~1.0, whereupon isopropyl-β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM to induce the expression of the exogenous protein *Sj*GST-6His in cells. After 3 hours of induction, the bacteria were harvested by centrifugation and the pellet was suspended and sonicated in an ice bath after adding phenylmethylsulfonyl fluoride (PMSF) to a final concentration of 0.1 mM to allow the cell lysate to cool down. The lysate was centrifuged at a high speed of 1,8000 rpm for 40 minutes and the supernatant was collected and diluted with the equilibration buffer (Na_2HPO_4-NaH_2PO_4, 2 mM, pH 7.6, EDTA 1 mM) before loading to a DEAE Sepharose CL-6B column (2.6×40 cm) equilibrated with the same buffer. After the sample was loaded onto the column, the genetically modified protein *Sj*GST-6His was eluted from the

column with the same buffer containing 0.05, 0.1, 0.15, 0.2, and 0.5 M NaCl and then analyzed by SDS-PAGE respectively. The eluted fraction mainly containing *Sj*GST-6His was concentrated and applied to a glutathione (GSH) affinity column, and then eluted with GSH. The eluted object protein was concentratede and then loaded on a 2.6×80 cm column of Sephadex G-75 at a flow rate of 0.5 ml/min with 0.05 M sodium phosphate buffer, pH 7.0 containing 0.15 M NaCl. The salt content was removed using a desalting column Sephadex G-25 column (1.8×40 cm). The purity of the sample was verified by SDS-PAGE.

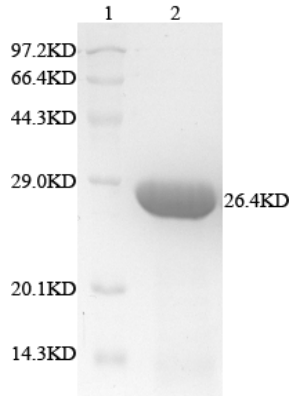


Figure S1. SDS-PAGE analysis of *Sj*GST-6His. Lane1: marker proteins; lane 2: the monomer of *Sj*GST-6His.

AFM Measurement of the Metal-Directed Self-Assembly and EDTA-Driven Disassembly. Approximately 50 μl of solution containing metal-directed *Sj*GST-6His-Ni^{2+} self-assemblies (ca. 10^{-6} M in 10 mM Tris-HCl buffer, pH 7.4), which was just diluted from the concentrated prepared *Sj*GST-6His-Ni^{2+} assemblies solution (ca. 10^{-5} M in 10 mM Tris-HCl buffer at pH 7.4) to minimize the dissociation from the metal-directed supramolecular self-assembly to individual building block by equilibration in diluted state, was dropped on a freshly trimethylsilyl-modified silicon wafer with 3-aminopropyl-dimethyl methoxy silane and left for 5 minutes at room temperature to allow the sample with negative charge to bind onto the wafer surface with positive charge through electrostatic interaction. The residue solution was then removed away from the wafer surface and the wafer was washed 3 times

with Milli-Q grade water to remove the residue buffer salts. The silicon wafer sample was then left in air for a few hours until completely dry at room temperature before it was transferred to NanoScope MultiMode-AFM (Veeco, USA). All the measurements were performed using the tapping mode AFM with a SiN_4 tip having a radius of ca. 10~20 nm. The line scan rate was 0.8 Hz with 512 pixels perline.

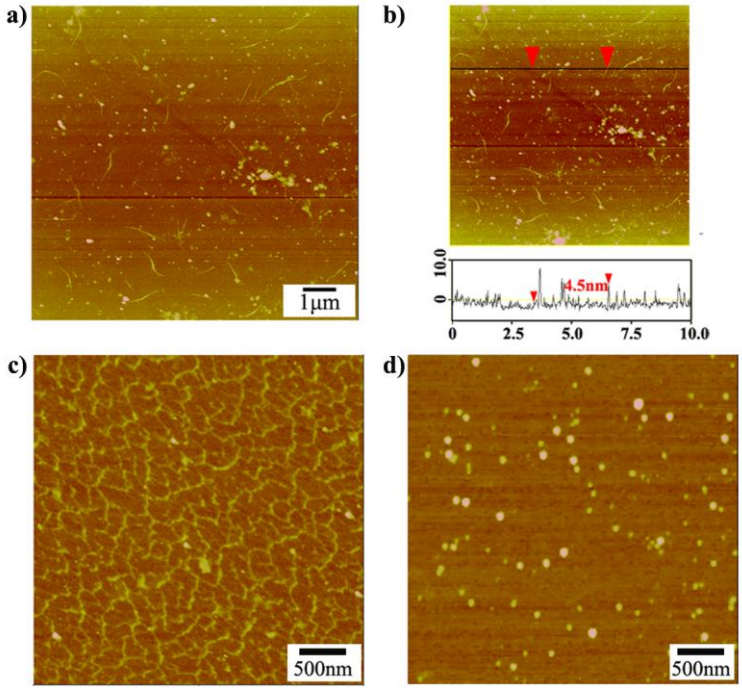


Figure S2. EDTA induced disassembly of the metal-directed of the supramolecular self-assembly. a) The linear assembly of *Sj*GST-6His-Ni^{2+} and its associated height profile along the black line b). c) A planar network of the metal-directed *Sj*GST-6His-Ni^{2+} assemblies through metal coordination. d) EDTA-driven disassembly of the supramolecular polymer.

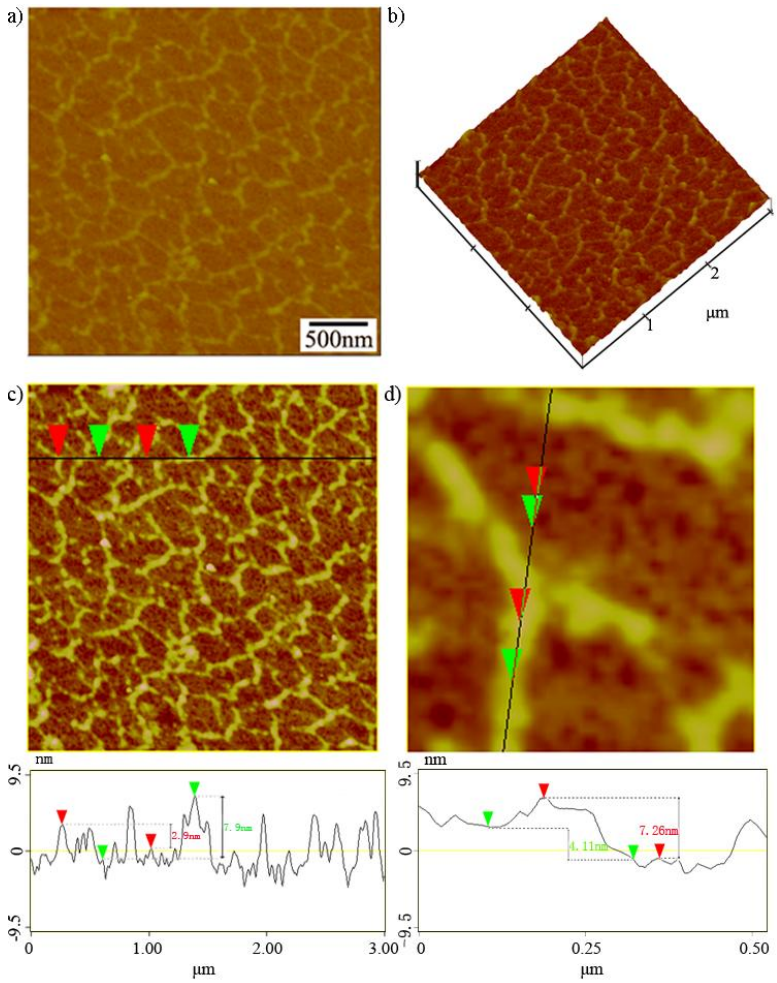


Figure S3. a) AFM topographical images of a planar network of the metal-directed *Sj*GST-6His-Ni^{2+} assemblies through metal coordination when the protein concentration of the molecular building block was up to 20 μM on the trimethylsilyl-modified silicon wafer. b) The three-dimensional image of panel (a). c) The associated height profiles along the black line for panel (a). d) The enlarged image of panel (c) and the associated height profiles along the black line.

SEC Analysis. For the size exclusion chromatography (SEC) analysis, metal-directed *Sj*GST-6His supramolecular self-assemblies were analyzed using a Zorbax GF-250 Gel Filtration Column (9.6 × 250 mm, Agilent, Germany) with a mobile phase of 20 mM sodium phosphate buffer, pH 7.4. The analyses were performed at room temperature at a flow rate of 1.0 ml/min monitored at 280 nm. The Zorbax GF-250 column had a molecular mass range of 400 kDa to 4 kDa for native protein and was calibrated using the following protein: BSA 67 kDa, Ovalbumin 45 kDa, and RNase A 13.7 kDa.

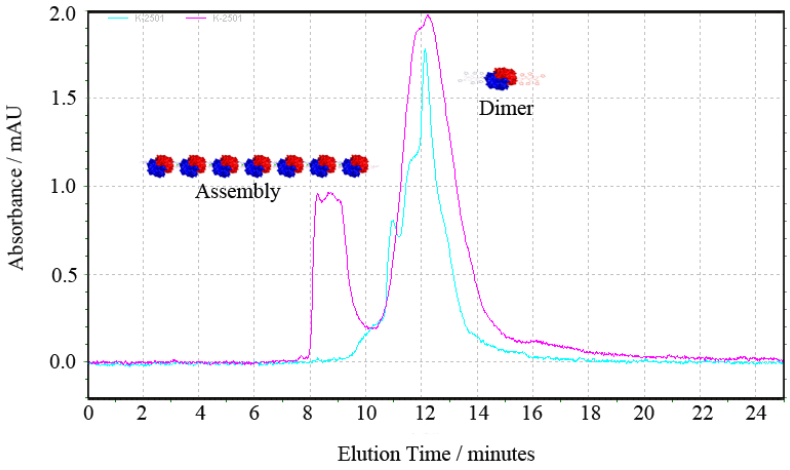


Figure S4. Size exclusion chromatography (SEC) analysis of the nickel ion directed self-assembly of *Sj*GST-6His-Ni^{2+} (red trace, supramolecular enzyme polymer) and its single building block *Sj*GST-6His (green trace, homodimer, 52.8 KD) without adding nickel sulphate.

Dynamic Light Scattering (DLS) Analysis. The particle size of the metal-directed self-assemblies of *Sj*GST-6His-Ni^{2+} were analyzed by dynamic light scattering (DLS) using a Malvern Nano_S instrument (Malvern, U.K.). However, DLS hardly renders dimensional aspects of 1D or rod-shape particles in principle, but DLS is an additional analytical method for demonstrating the formation of the Ni^{2+}-directed self-assembly compared to AFM and SEC, although the hydrodynamic diameters is a little greater than the actual diameters of the nanoparticle. All measurements with the protein concentration of 0.16 mg/ml were repeated for three times at 25 °C. Sample buffer typically contained 20 mM Tris-HCl, pH 7.4, 50 mM NaCl.

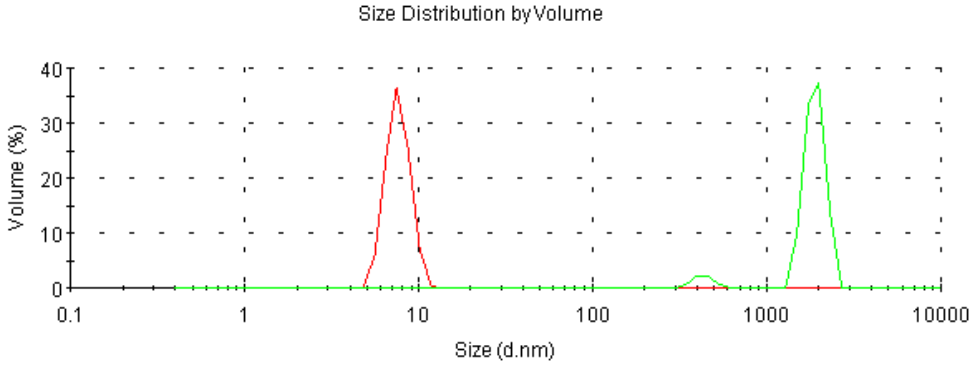


Figure S5. The dynamic light scattering (DSC) analysis of the hydrodynamic diameters of the nickel ion directed self-assembly of *Sj*GST-6His-Ni^{2+} (green trace, supramolecular polymer, mainly 1,886 nm) and its single building block *Sj*GST-6His without adding nickel sulphate (red trace, homodimer, 6.5 nm).

The Self-assembly and Disassembly Switch by Adjusting Ni^{2+} and EDTA Concentration. While preparing the samples for DLS in the presence of excess Ni^{2+}, it was observed that at a high *Sj*GST-6His concentration of 0.1 mM, the metal-directed self-assembly of *Sj*GST-6His-Ni^{2+} resulted in an opalescence of protein assembly solution (Figure S2, b). After adding an excess of EDTA, it was found that the EDTA restored a transparent solution owing to the disassembly of the protein supramolecular polymer of *Sj*GST-6His-Ni^{2+} (Figure S2, c).

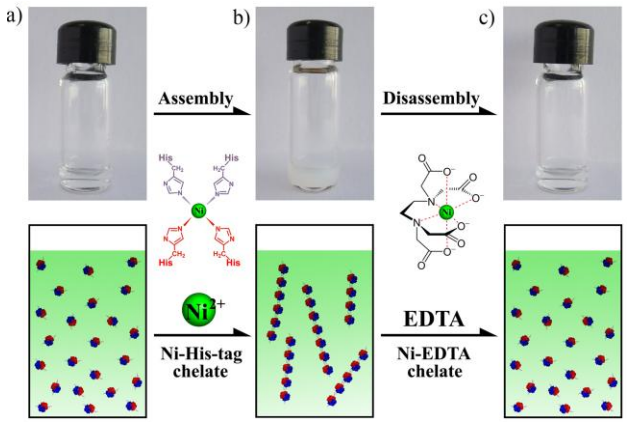


Figure S6. The self-assembly and disassembly switch by Ni^{2+} and EDTA. Ni^{2+} induced self-assembly of *Sj*GST-6His with a high protein concentration up to 0.1 mM through metal coordination and the disassembly of the supramolecular polymer of *Sj*GST-6His-Ni^{2+} after the addition of EDTA. a) Molecular building block of *Sj*GST-6His, 0.1 mM, dissolved in 20 mM Tris-HCl, pH 7.4, containing 50 mM NaCl. b) Ni^{2+} (10 μM)induced self-assembly of *Sj*GST-6His-Ni^{2+}. c) After adding an excess of EDTA (0.1 mM), the protein assembly of *Sj*GST-6His-Ni^{2+} disassembled.

Circular Dichroism Spectroscopy. CD spectra were obtained using a MOS-450/AF-CD spectropolarimeter (Bio-Logic, France) equipped with a thermostatted cell holder, using a 0.1 cm quartz

cell. Spectra were collected by averaging three repetitive scans with protein concentration 0.5 μM for molecular building block *Sj*GST-6His and its metal-directed self-assemblies *Sj*GST-6His-Ni^{2+}. Total sample volume in CD measurements was 300 μl. For Ni^{2+}-dependent CD studies, spectra were acquired at 25 °C using 1 nm intervals, 1 nm bandwidth, and 60 nm min^{-1}. Spectra were baseline corrected before converting to mean residue ellipticities.

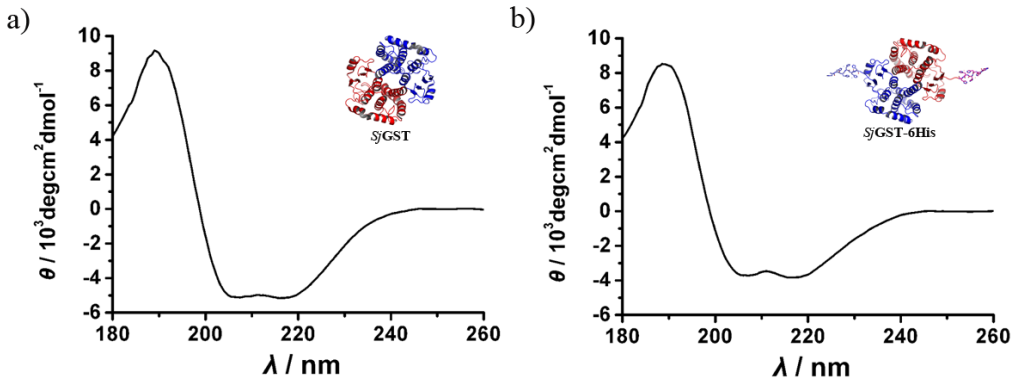
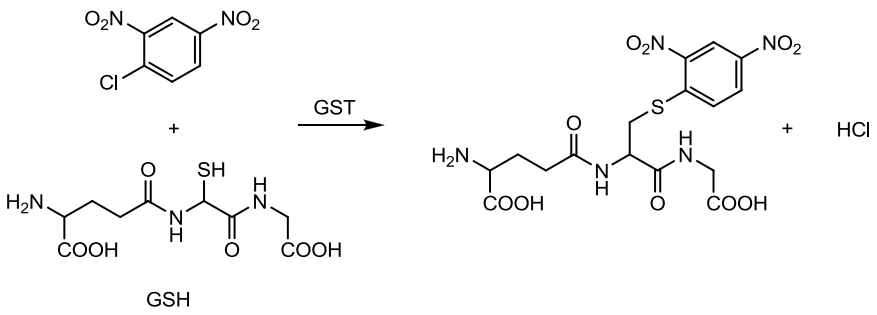


Figure S7. Far-UV circular dichroism spectra for proteins in Milli Q water, measured at 25 °C. a) Molar ellipticity vs. wavelength for *Sj*GST a) and *Sj*GST-6His with genetically introduced N-terminal His-tag b).

Enzymatic Assay. GST catalyzes the conjugation of reduced glutathione-via a sulfhydryl group-to electrophilic center on a wide variety of substrates, including the model substrate 1-chloro-2, 4-dinitrobenzene (CDNB) (Scheme S1). The glutathione conjugate generated in this reaction absorbs maximally at 340 nm, well separated from the wavelength of maximum absorbance of the two starting substrates. The Michaelis-Menten constant (K_m), the turn over number (k_{cat}), and catalytic efficiency (k_{cat}/K_m) for supramolecular building block *Sj*GST-6His and its metal-directed self-assembly *Sj*GST-6His-Ni^{2+} were measured to assess the effect of the metal-directed protein assembly upon catalytic activity. The enzymatic activity was assessed at 25 °C by monitoring the reaction of CDNB with GSH spectrophotometrically at 340 nm. Enzyme was added to 0.1 M potassium phosphate buffer pH 6.5 containing CDNB (0.5 mM) and reduced glutathione at six different substrate concentrations. The

uncatalyzed reaction without enzyme was set up as a control and had a measurable rate. The reaction was carried out in duplicate for the individual *Sj*GST-6His and its metal-directed self-assemblies.



Scheme S1. The reaction of glutathione (GSH) conjugating to 1-chloro-2, 4-dinitrobenzene (CDNB) catalyzed by glutathione S-transferase (GST).

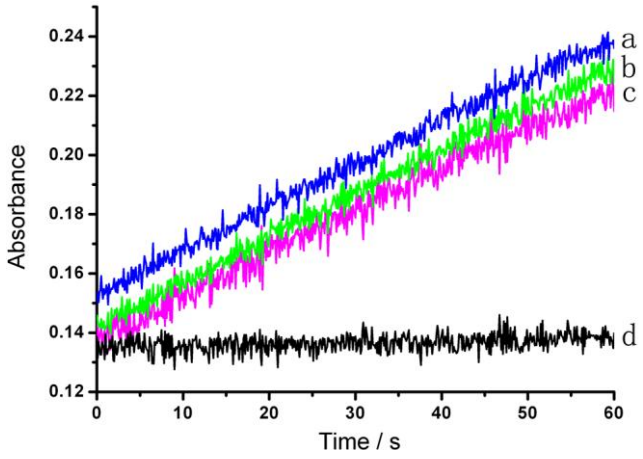


Figure S8. The absorption at 340 nm of the reaction product catalyzed by widetype *Sj*GST a), its genetically modified *Sj*GST-6His b) and its metal-directed self-assembly c). The black trace was the absorption of the reaction product without enzyme d). Reactions were carried out in 0.1 M potassium phosphate buffer,, pH 6.5, at 25 °C, containing CDNB 0.5 mM, GSH 1 mM, and enzyme sample 9 nM.

Steady-State Kinetics of *Sj*GST-6His and Its Ni^{2+}-Directed Self-Assembly. All kinetic experiments were performed in the same way as GST activity assay. The initial catalytic reaction rates were measured by observing the increase of product absorption at 340 nm at a wide range of concentration of one substrate while the concentration of the other substrate was fixed. The glutathione (GSH) concentration

varied from 0.05 to 20 mM. The reaction mixture (0.5 ml) contained 0.1 M potassium phosphate buffer pH 6.5, 0.5 mM CDNB, and varying concentration of GSH. The catalytic reaction was initiated by addition of CDNB. The uncatalytic reaction affecting the measurement of the initial rate was taken into account and subtracted to obtain exact kinetic value. Lineweaver–Burk plots were used to determine apparent K_m and k_{cat} values.