Multi-Stimuli Responsive Nanocomposite Tectons for Pathway Dependent Self-Assembly and Acceleration of Covalent Bond Formation

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Supporting Information

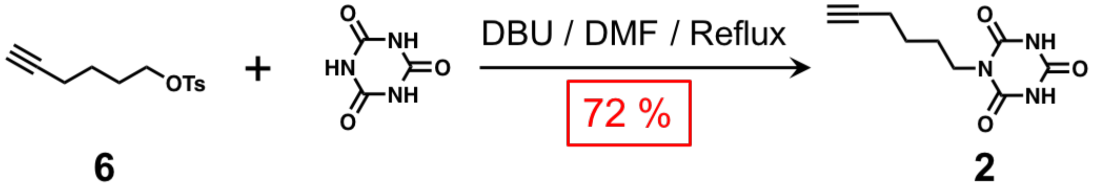
Table of Contents

1. Materials and General Methods

Chemicals were purchased as reagent grade and used without further purification. Commercial grades of anhydrous dichloromethane (DCM), MeCN and *N*,*N*-dimethylformamide (DMF) were used as solvents in all reactions. Compounds **1**,[1] **6**[2] and **7**[3] were prepared according to literature procedures. Thin layer chromatography (TLC) was performed on silica gel 60F254 (E Merck). Column chromatography was carried out on silica gel 60F (Merck 9385, 0.040–0.063 mm). UV/Vis Studies were performed on a Cary-500 UV-Vis Spectrophotometer with a thermal multicell accessory. Nuclear magnetic resonance (NMR) spectra were recorded at 298 K on Bruker Avance 400 spectrometers, with working frequencies of 400 MHz for ^{1}H and 101 for ^{13}C nuclei respectively. Chemical shifts are reported in ppm relative to the signals corresponding to the residual non-deuterated solvents.[4] High-resolution mass spectra (HRMS) were measured on an Agilent 6210 Time-of-Flight (TOF) LC-MS, using an ESI source, coupled with Agilent 1100 HPLC stack, using direct infusion (0.6 mL min^{-1} Measurements at X-band (9.5 GHz) were performed with a Bruker Elexsys E580, equipped with a variable Q dielectric resonator (ER-4118X-MD5-W1). Small angle X-ray scattering (SAXS) experiments were performed at the Massachusetts Institute of Technology (MIT) Center for Materials Science and Engineering (CMSE) X-ray Diffraction Shared Experimental Facility on a SAXSLAB system. The Rigaku 002 microfocus X-ray source produced Cu Kα1 x-rays of wavelength 1.5409 Å; Osmic staggered parabolic multilayer optics focused the beam crossover at the second pinhole. Two sets of JJ X-ray jaw collimation slits set at 0.45 mm and 0.2 mm, respectively, were used to define the beam. The system was calibrated using silver behenate as a standard. Each sample was added to a short section of 1.5 mm diameter Polyimide Tubing (Cole-Parmer) and sealed at both ends with epoxy. The tubes were placed horizontally in the sample chamber and pumped down to 0.08 mbar.

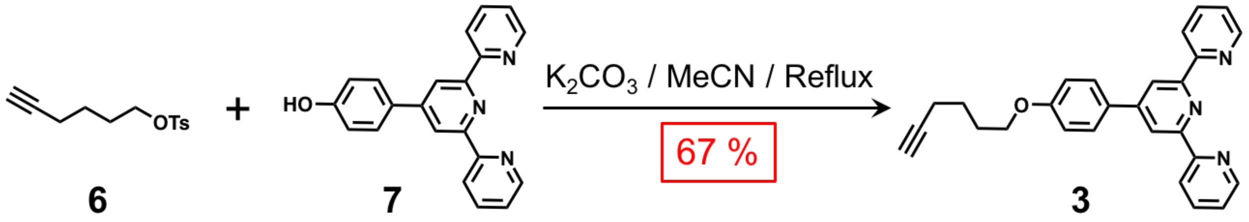
2. Synthetic Protocols

Scheme S1. One-Step Synthesis of Compound 2



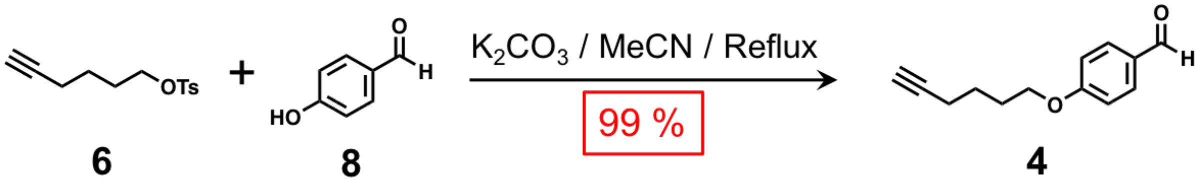
Compound **2**: Cyanuric acid (650 mg, 5 mmol) and Compound **6** (252 mg, 1 mmol) was dissolved in anhydrous DMF (30 mL), and DBU (150 µl, 1 mmol) was added dropwise to the solution. The reaction mixture was then heated under reflux overnight. The solvent was removed under reduced pressure and the crude mixture was re-dissolved in MeOH. The solid was filtered off and the solvent was removed under reduced pressure. The crude mixture was purified by column chromatography (SiO_2) using 4:1 DCM/MeOH as the eluent to afford **2** as an off-white powder (150 mg, 71 %). ^{1}H NMR (400 MHz, d_6-DMSO): δ = 11.34 (br, 2H), 3.64 (t, *J* = 8.0 Hz, 2H), 2.17 (t, *J* = 8.0 Hz, 2H), 1.60 (p, *J* = 4.0 Hz, 2H), 1.44 (p, *J* = 8.0 Hz, 2H). ^{13}C NMR (101 MHz, d_6-DMSO): δ = 150.4, 150.3, 149.1, 84.7, 71.8, 27.1, 25.7, 17.9. HRMS (ESI): m/z calcd for $C_9H_{12}N_3O_3$ $[M + H]^+$ 210.0873, found 210.0877.

Scheme S2. One-step Synthesis of Compound 3



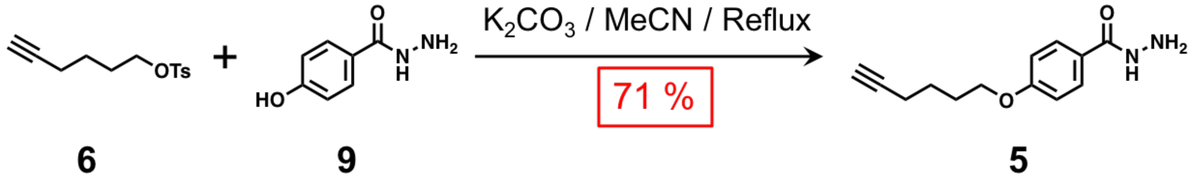
Compound **3**: Compound **6** (252 mg, 1 mmol), compound **7** (325 mg, 1 mmol) and K_2CO_3 (276 mg, 2 mmol) was dissolved in 20 ml MeCN, and the reaction mixture was heated to reflux overnight under stirring. The reaction mixture was then cooled to room temperature and the solvent was evaporated off under vacuum. The crude was washed with H_2O and MeOH and the remaining solid was collected to afford compound 3 as a pale-yellow powder (270 mg, 67 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.73 (d, *J* = 4.0 Hz, 2H), 8.71 (s, 2H), 8.67 (d, *J* = 4.0 Hz, 2H), 7.85–7.91 (m, 4H), 7.35 (dd, *J* = 4.0 Hz, 2H), 7.02 (d, *J* = 4.0 Hz, 2H), 4.07 (t, *J* = 4.0 Hz, 2H), 2.28–2.34 (m, 2H), 1.92–2.00 (m, 3H), 1.77 (p, *J* = 8.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 159.9, 156.4, 155.8, 149.8, 149.1, 136.8, 130.7, 128.5, 123.7, 121.4, 118.2, 114.8, 84.1, 68.7, 67.4, 28.3, 25.0, 18.2. HRMS (ESI): m/z calcd for $C_{27}H_{24}N_3O$ $[M + H]^+$ 406.1914, found 406.1907.

Scheme S3. One-step Synthesis of Compound 4



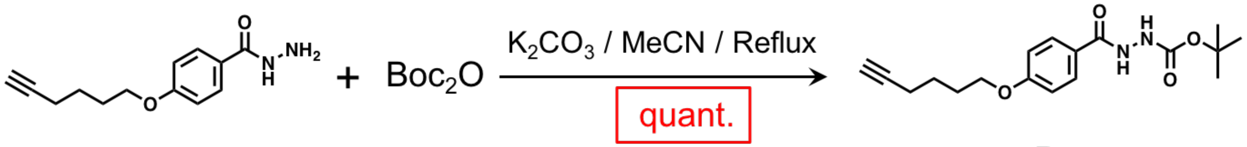
Compound **4**: Compound **6** (1.26 g, 5 mmol), compound **8** (610 mg, 5 mmol) and K_2CO_3 (1.38 g, 10 mmol) was dissolved in 50 ml MeCN, and the reaction mixture was heated to reflux overnight under stirring. The reaction mixture was then cooled to room temperature and the solvent was evaporated off under vacuum. The crude was dissolved in DCM and washed with brine for 3 times. The organic phase was then dried over $MgSO_4$ and the solvent was removed under vaccum to afford compound **4** as a yellow crystalline solid (1.00 g, 99 %). ^{1}H NMR (400 MHz, CD_3OD): δ = 9.82 (s, 1H), 7.85 (d, J = 4.0 Hz, 2H), 7.07 (d, J = 4.0 Hz, 2H), 8.73 (d, J = 4.0 Hz, 2H), 4.10 (t, J = 4.0 Hz, 2H), 2.20–2.29 (m, 3H), 1.93 (p, J = 4.0 Hz, 2H), 1.70 (p, J = 4.0 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 190.8, 164.1, 132.0, 129.9, 114.7, 83.9, 68.8, 67.7, 28.1, 24.9, 18.1. HRMS (ESI): m/z calcd for $C_{13}H_{15}O_2$ $[M + H]^+$ 203.1066, found 203.1067.

Scheme S4. One-step Synthesis of Compound 5



Compound **5**: Compound **6** (1.08 g, 4 mmol), compound **9** (608 mg, 4 mmol) and K_2CO_3 (1.10 g, 8 mmol) was dissolved in 40 ml MeCN, and the reaction mixture was heated to reflux overnight under stirring. The reaction mixture was then cooled to room temperature and the solvent was evaporated off under vacuum. The crude was washed with H_2O and hexane to remove the impurities, and the resulting solid was dissolved in DCM and the product was obtained by recrystallization in a DCM/Hexanes mixture as an off-white powder (660 mg, 71 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.70 (d, J = 4.0 Hz, 2H), 6.92 (d, J = 4.0 Hz, 2H), 4.01–4.11 (m, 4H), 2.25–2.32 (m, 2H), 1.97 (s, 1H), 1.93 (p, $J = 4.0$ Hz, 2H), 1.72 (p, $J = 4.0$ Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 161.9, 132.1, 128.7, 114.5, 114.1, 83.9, 68.8, 67.5, 28.1, 24.9, 18.1. HRMS (ESI): m/z calcd for $C_{13}H_{17}N_2O_2$ $[M + H]^+$ 233.1276, found 233.1284.

Scheme S5. One-step Synthesis of Compound 5-Boc



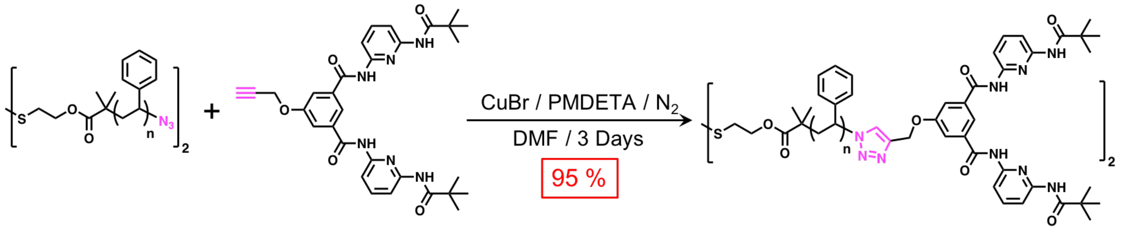
5

5-Boc

Compound **5-Boc**: Compound **5** (230 g, 1 mmol) was dissolved in 5 ml DCM, and in a separate container, Boc_2O (650 mg, 3 mmol) was dissolved in 10 mL DCM, and this solution was added dropwise to the DCM solution of **5**. The reaction mixture was then warmed up to room temperature and stirred overnight. The solvent was then evaporated off under vacuum, and the crude mixture was purified by column chromatography (SiO_2) using 1:1 EtOAC/Hexanes as the eluent to afford **5-Boc** as an off-white powder (330 mg, quant.)^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.96 (br, 1H), 7.76 (d, $J = 4.0$ Hz, 2H), 6.89 (d, $J = 4.0$ Hz, 2H), 6.71 (br, 1H), 4.01 (t, $J = 4.0$ Hz, 2H), 2.29 (td, $J = 4.0$ Hz, 2H), 1.97 (t, $J = 4.0$ Hz, 1H), 1.89–1.95 (m, 2H), 1.72 (p, $J = 4.0$ Hz, 2H), 1.49 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 162.2, 129.2, 114.3, 83.9, 81.9, 68.8, 67.5, 28.2, 28.1, 27.4, 25.0, 18.1. HRMS (ESI): m/z calcd for $C_{18}H_{25}N_2O_4$ $[M + H]^+$ 333.1804, found 333.1809.

We show the synthesis of PS-HW, PS-CA and PS-Tpy using 22 kDa PS-N_3 (11 kDa on each side) as examples. The 52 kDa-PS-HW, PS-CA and PS-Tpy polymers were synthesized using the similar protocol.

Scheme S6. One-Step Synthesis of PS-HW



PS-N$_3$

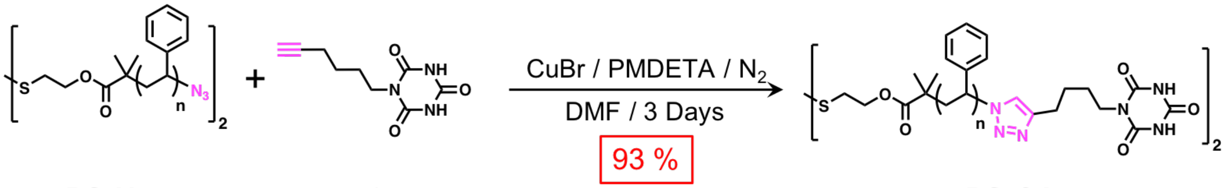
1

PS-HW

PS-HW: **PS-N$_3$** (440 mg, 0.02 mmol) and compound **1** (46 mg, 0.08 mmol) was dissolved in anhydrous DMF (3 mL). After stirring for 30 min under a N_2 atmosphere, PMDETA (12 µl, 0.055

mmol) and CuBr powder (3 mg, 0.02 mmol) was added to the solution. The reaction mixture was stirred for 3 days under a N_2 atmosphere and then was poured into an excess amount of MeOH (50 mL). The precipitate was filtered and re-dissolved in DCM, to which a saturated aqueous solution of EDTA was added. After stirring overnight the organic phase was separated and poured into MeOH (50 mL), and the precipitate was collected by filtration and washed with MeOH to afford PS-HW as a white solid (420 mg, 95 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.40 (br), 8.06 (t, *J* = 8.0 Hz), 7.70–7.88 (m), 6.89–7.26 (m, polystyrene), 6.31–6.79 (m, polystyrene), 4.98–5.10 (br), 3.68–3.81 (br), 2.46–2.57 (br), 1.27–2.32 (m, polystyrene), 0.85–1.06 (m).

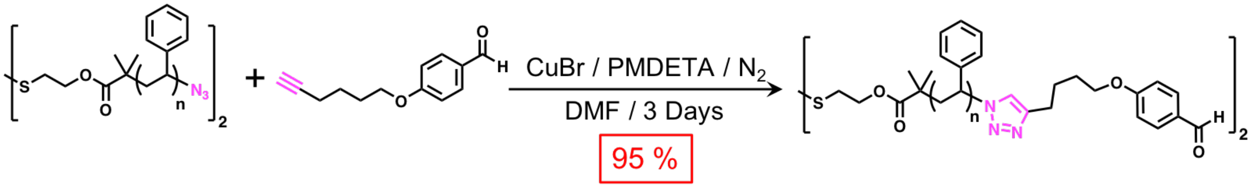
Scheme S7. One-Step Synthesis of PS-CA



PS-N$_3$ **2** **PS-CA**

PS-CA: **PS-N$_3$** (440 mg, 0.02 mmol) and compound **2** (16 mg, 0.08 mmol) was dissolved in anhydrous DMF (3 mL). After stirring for 30 min under a N_2 atmosphere, PMDETA (12 μl, 0.055 mmol) and CuBr powder (3 mg, 0.02 mmol) was added to the solution. The reaction mixture was stirred for 3 days under a N_2 atmosphere and then was poured into an excess amount of MeOH (50 mL). The precipitate was filtered and re-dissolved in DCM, to which a saturated aqueous solution of EDTA was added. After stirring overnight the organic phase was separated and poured into MeOH (50 mL), and the precipitate was collected by filtration and washed with MeOH to afford **PS-CA** as a white solid (410 mg, 93 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.50 (br), 6.89–7.26 (m, polystyrene), 6.31–6.79 (m, polystyrene), 4.98–5.10 (br), 3.76–3.91 (br), 2.61–2.75 (br), 2.45–2.54 (br), 1.27–2.32 (m, polystyrene), 0.85–1.04 (m).

Scheme S8. One-Step Synthesis of PS-Tpy

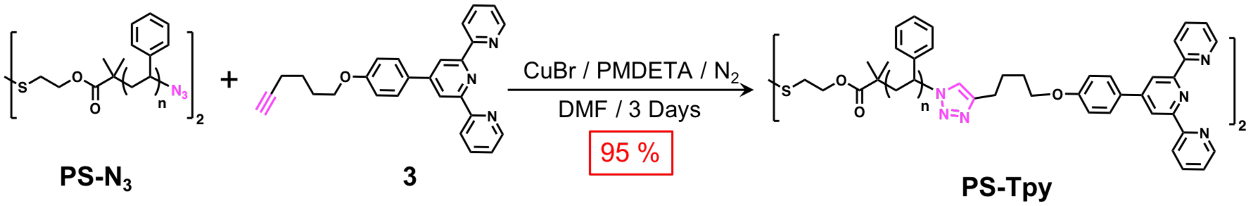


PS-N$_3$ **4** **PS-CHO**

PS-Tpy: **PS-N$_3$** (1.1 g, 0.05 mmol) and compound **3** (81 mg, 0.2 mmol) was dissolved in anhydrous DMF (3 mL). After stirring for 30 min under a N_2 atmosphere, PMDETA (54 μl, 0.25

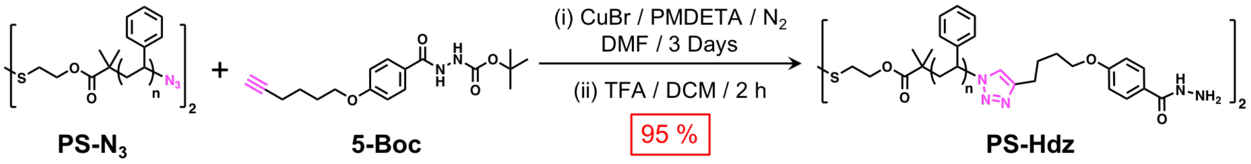
mmol) and CuBr powder (30 mg, 0.23 mmol) was added to the solution. The reaction mixture was stirred for 3 days under a N_2 atmosphere and then was poured into an excess amount of MeOH (50 mL). The precipitate was filtered and re-dissolved in DCM, to which a saturated aqueous solution of EDTA was added. After stirring overnight the organic phase was separated and poured into MeOH (50 mL), and the precipitate was collected by filtration and washed with MeOH to afford **PS-Tpy** as a white solid (1.05 g, 95 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.72 (t, *J* = 8.0 Hz), 8.68 (d, *J* = 8.0 Hz), 7.83–7.90 (m), 7.31–7.37 (m), 6.89–7.26 (m, polystyrene), 6.31–6.79 (m, polystyrene), 4.98–5.10 (br), 3.76–3.91 (br), 2.61–2.75 (br), 2.45–2.54 (br), 1.27–2.32 (m, polystyrene), 0.85–1.04 (m).

Scheme S9. One-Step Synthesis of PS-CHO



PS-CHO: **PS-N$_3$** (440 mg, 0.02 mmol) and compound **4** (16 mg, 0.08 mmol) was dissolved in anhydrous DMF (3 mL). After stirring for 30 min under a N_2 atmosphere, PMDETA (12 µl, 0.055 mmol) and CuBr powder (3 mg, 0.02 mmol) was added to the solution. The reaction mixture was stirred for 3 days under a N_2 atmosphere and then was poured into an excess amount of MeOH (50 mL). The precipitate was filtered and re-dissolved in DCM, to which a saturated aqueous solution of EDTA was added. After stirring overnight the organic phase was separated and poured into MeOH (50 mL), and the precipitate was collected by filtration and washed with MeOH to afford **PS-CHO** as a white solid (420 mg, 95 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.87 (br), 7.81 (br), 6.89–7.26 (m, polystyrene), 6.31–6.79 (m, polystyrene), 4.98–5.10 (br), 3.99–4.08 (br), 2.61–2.75 (br), 2.45–2.54 (br), 1.27–2.27 (m, polystyrene).

Scheme S10. Synthesis of PS-Hdz



PS-Hdz: **PS-N$_3$** (440 mg, 0.02 mmol) and compound **5-Boc** (26 mg, 0.08 mmol) was dissolved in anhydrous DMF (3 mL). After stirring for 30 min under a N_2 atmosphere, PMDETA (12 µl, 0.055

mmol) and CuBr powder (3 mg, 0.02 mmol) was added to the solution. The reaction mixture was stirred for 3 days under a N_2 atmosphere and then was poured into an excess amount of MeOH (50 mL). The precipitate was filtered and re-dissolved in 5 mL 1:1 DCM:TFA (v/v), and the solution was stirred for 2 hours at room temperatrue to remove the Boc group. The solution was then poured into an excess amount of MeOH (50 mL). The precipitate was filtered and re-dissolved in DCM, to which a saturated aqueous solution of EDTA was added. After stirring overnight the organic phase was separated and poured into MeOH (50 mL), and the precipitate was collected by filtration and washed with MeOH to afford **PS-Hdz** as a white solid (420 mg, 95 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.22 (br), 7.72–7.79 (m), 6.89–7.26 (m, polystyrene), 6.31–6.79 (m, polystyrene), 4.90–5.07 (br), 3.97–4.06 (br), 2.81–2.98 (br), 2.45–2.54 (br), 1.22–2.26 (m, polystyrene), 0.84–1.02 (m).

The molecular weight characterization of the a) 22 kDa and b) 52 kDa polystyrene used in this work is shown in Figure S1.

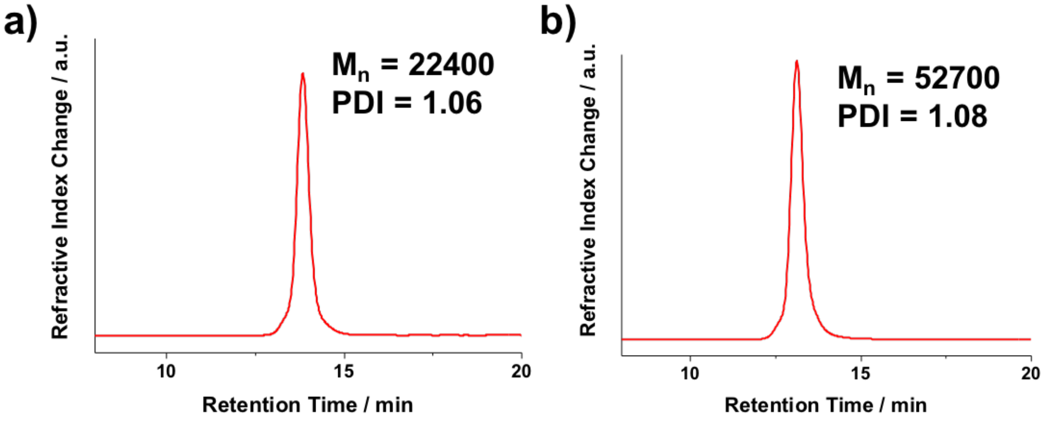


Figure S1. GPC traces of the polystyrene polymers (disulfide bond-contained) used in this work. The molecular weight of the corresponding thiol group functionalized polystyrene is therefore 11 and 26 kDa, respectively. Eluent: THF, 1 mL/min.

3. Experimental Protocols for the Studies of NCT Assemblies

Synthesis of HW- and CA-NCTs: In a typical synthetic process, an appropriate amount of HW-PS or CA-PS polymers was dissolved in acetone (2 mL) in a glass vial to yield a 1.0×10^{-4} M solution. The solution was vigorously stirred while 28 nm AuNP solution (~1 nM, 2 mL)[5] was quickly poured into the vial, and the resulting suspension was allowed to stir for 10 min. After the formation of dark red precipitate, the colorless supernatant was removed by direct decantation or with the aid of light centrifugation. DMF (3 mL) was added to the vial to re-disperse the precipitate, and the resulting dispersion was distributed in centrifuge tubes and purified for 3 centrifuge cycles (5000 rpm, 40 min). For the HW-NCTs, the solvents used for re-dispersing the AuNPs precipitate at the end of each cycle were DMF, toluene, and toluene, respectively. For the CA-NCTs, the solvents were DMF, anisole and anisole, respectively. The final toluene and anisole dispersions were used for the self-assembly studies.

Synthesis of Tpy-NCTs: 0.5 mg 11 kDa-PS-Tpy and 1.5 mg 11 kDa-PS-Br polymers were dissolved in acetone (2 mL) in a glass vial. The solution was vigorously stirred while 28 nm AuNP solution (~1 nM, 2 mL) was quickly poured into the vial, and the resulting suspension was allowed to stir for 10 min. After the formation of dark red precipitate, the colorless supernatant was removed by direct decantation or with the aid of light centrifugation. DMF (3 mL) was added to the vial to re-disperse the precipitate, and the resulting dispersion was distributed in centrifuge tubes and purified for 3 centrifuge cycles (5000 rpm, 40 min). The solvents used for re-dispersing the AuNPs precipitate at the end of each cycle were DMF, toluene, and toluene, respectively, and the final toluene dispersions were used for the self-assembly studies.

Synthesis of HW-Tpy-NCTs: 0.5 mg 11 kDa-PS-Tpy and 4.5 mg 11 kDa-PS-HW polymers were dissolved in acetone (5 mL) in a glass vial. The solution was vigorously stirred while 28 nm AuNP solution (~1 nM, 5 mL) was quickly poured into the vial, and the resulting suspension was allowed to stir for 10 min. After the formation of dark red precipitate, the colorless supernatant was removed by direct decantation or with the aid of light centrifugation. DMF (6 mL) was added to the vial to re-disperse the precipitate, and the resulting dispersion was distributed in centrifuge tubes and purified for 3 centrifuge cycles (5000 rpm, 40 min). The solvents used for re-dispersing the AuNPs precipitate at the end of each cycle were DMF, toluene, and toluene, respectively, and the final toluene dispersions were used for the multi-stimuli responsive self-assembly studies.

Synthesis of HW-Hdz-NCTs: 0.5 mg 11 kDa-PS-Hdz and 1.5 mg 11 kDa-PS-HW polymers were dissolved in acetone (~1 nM, 2 mL) in a glass vial. The solution was vigorously stirred while 16 nm AuNP solution (~2 nM, 2 mL) was quickly poured into the vial, and the resulting suspension was allowed to stir for 10 min. After the formation of dark red precipitate, the colorless supernatant was removed by direct decantation or with the aid of light centrifugation. DMF (3 mL) was added to the vial to re-disperse the precipitate, and the resulting dispersion was distributed in centrifuge tubes and purified for 3 centrifuge cycles (7000 rpm, 50 min). The solvents used for re-dispersing the AuNPs precipitate at the end of each cycle were DMF, toluene, and toluene, respectively, and the final toluene dispersions were used for the covalently-linked NCTs studies.

Synthesis of CA-CHO-NCTs: 0.5 mg 11 kDa-PS-CHO and 1.5 mg 11 kDa-PS-CA polymers were dissolved in acetone (2 mL) in a glass vial. The solution was vigorously stirred while 16 nm AuNP solution (~2 nM, 2 mL) was quickly poured into the vial, and the resulting suspension was allowed to stir for 10 min. After the formation of dark red precipitate, the colorless supernatant was removed by direct decantation or with the aid of light centrifugation. DMF (3 mL) was added to the vial to re-disperse the precipitate, and the resulting dispersion was distributed in centrifuge tubes and purified for 3 centrifuge cycles (7000 rpm, 50 min). The solvents used for re-dispersing the AuNPs precipitate at the end of each cycle were DMF, anisole, and anisole, respectively, and the final anisole dispersions were used for the covalently-linked NCTs studies.

Grafting Density: In this work, the NCTs were prepared using a previously reported protocol,[6] where the grafting density of the polymer chains on the nanoparticle surface was determined to be ~ 0.7 chain/nm^2.

Thermal analysis of NCT assembly: Melt analysis was performed on a Cary-500 UV-Vis Spectrophotometer with a thermal multicell accessory. Before the experiment, a clean quartz cuvette was treated with hexamethyldisilazane (HMDS) by solvent annealing in a 1:1 HMDS:hexanes (v/v) solution in a sealed chamber at room temperature for 20 hours. Then, in a typical experiment, equal volumes of HW NCTs in toluene and CA NCTs in anisole at the same optical density were mixed first in the treated quartz cuvette, followed by the addition of additional toluene and anisole solvents to reach the desired solvent ratio. The cuvette was then placed in the thermal multicell accessory, and heated at a rate of 0.25°C/minute. The extinction was monitored at 520 nm to generate the melt profiles, and normalized to generate Figure 2.

Acid-responsive studies of Tpy-NCT assembly: Three samples of the assembled 11 kDa-PS-Tpy functionalized AuNPs were prepared in the presence of 10 μM $Zn(OTf)_2$, $Mn(ClO_4)_2$ and $Fe(BF_4)_2$, respectively, in toluene. Then TFA was titrated to these three samples and the absorption spectra of three samples were recorded by UV-Vis spectroscopy and the absorption intensity at 520 nm was utilized to reflect (Figure 3b) spectral change.

Acid-base cycles of Tpy NCT assembly: To analyze the reversiblity of the Tpy NCT assemblies, the assembly of the 11 kDa-PS-Tpy functionalized AuNPs were prepared in the presence of 10 μM $Zn(OTf)_2$ in toluene. The sample was subjected to through multiple acid-base cycles by adding TFA and TEA in an alternative manner. After each step, the absorption spectrum of the sample was recorded by UV-Vis spectroscopy and the absorption intensity at 520 nm was utilized to reflect (Figure 3c) spectral change resulting from the assembly/disassembly processes.

Covalent bond formation between functionalized NCTs: Two types of di-ligand-functionalized NCTs were prepared. One type of NCTs was co-functionalized with 11 kDa-PS-HW and 11 kDa-PS-Hdz polymers (3:1 feed ratio), and the other type was co-functionalized with 11 kDa-PS-CA and 11 kDa-PS-CHO (3:1 feeding ratio). The HW-Hdz NCTs and the CA-CHO NCTs were dispersed in toluene and anisole, respectively, and equal volume of the two sets of NCTs with the same optical density were then mixed together. At certain time after mixing the NCTs, an aliquot was taken and a minimum amount of DMF (~1% volume percent) that can interupt the hydrogen bonding was added. The absorption of the aliquot was then measured by UV-Vis spectroscopy for the kinetic study.

4. Solvent Effect on the Thermostability of the HW/CA-based NCT assemblies.

We took 11 kDa-PS-HW and 11 kDa-PS-CA functionalized 28 nm AuNPs as an example to study how the stronger interactions between HW/CA pairs make it possible to assemble the NCTs in more challenging enviorment. Figure S2 shows that the assembly can readily form in the 1:1 mixed solvents of toluene and halogenated solvents including dichloromethane (DCM), *o*-dichlorobenzne (DCB) and chloroform at room temperature. Furthermore, we evaluate how the change of the solvent can tune the thermostability of the NCT assemblies in detail, where the NCTs assembled in mixed solvents of toluene and anisole with different ratios, and the T_m of these aggregates were then measured by UV/Vis spectroscopy. Figure S3 shows that the T_m of the NCT assemblies changes linearly upon changing the percentage of the anisole in the mixed solvent, an observation which suggests that the T_m of the NCT assemblies can be precisely controlled by tuning the solvent ratio.

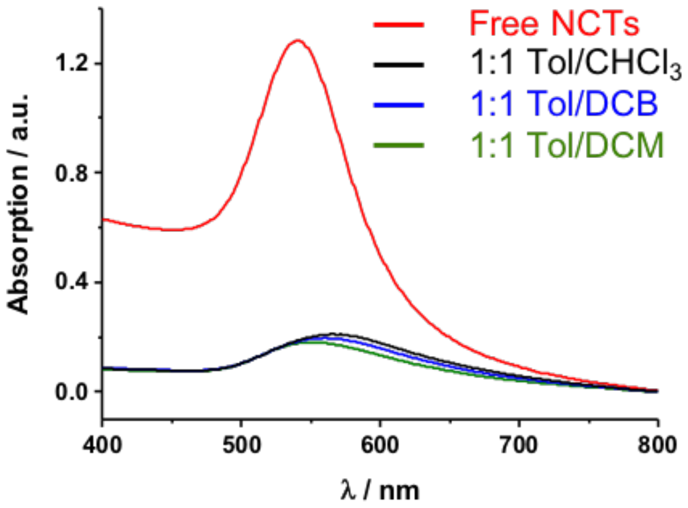


Figure S2. UV/Vis Spectra of the NCTs (11 kDa PS, HW/CA recognition motifs, 28 nm AuNPs) in different solvent mixtures, which demonstrate that the NCTs can assemble in a wide range of solvent environments.

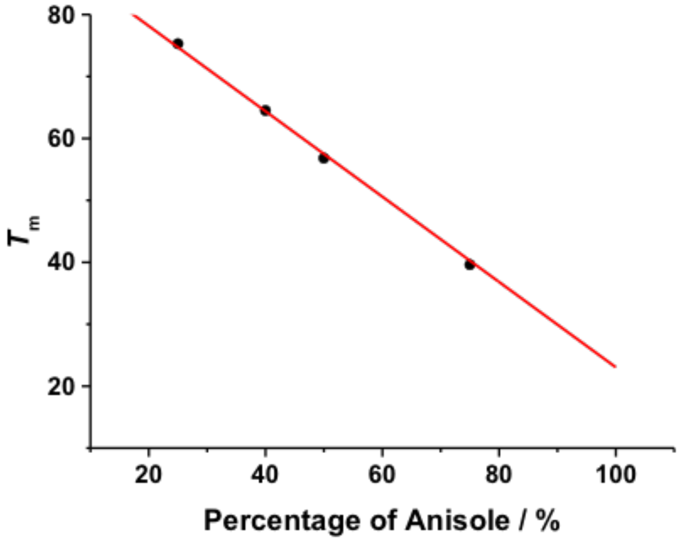


Figure S3. Linear relationship between the T_m of the NCT assemblies (11 kDa PS, HW/CA recognition motifs, 28 nm AuNPs) and the percentage of anisole in the anisole/toluene mixed solvent.

5. Reversiblity Study of the Metal Ion-Tpy NCT Assembly

Figure S4a shows that Fe^{2+}-Tpy-linked NCTs functionalized with 26 kDa PS polymer readily disassembled upon the addition of 260 mM TFA at 22 °C, which is consistent with the fact that alterations to polymer configuration in the NCT system provide a unique design handle to tune the assembly properties of NPs. Figure S4b shows that in addition to acid (TFA, red trace), the assembled Tpy-NCTs (black trace) induced by Zn^{2+} can also disassemble (blue trace) upon the addtion of an excess amount of free Tpy ligand (compound **3**), which offers an alternative approach to tune the assembly process of the Tpy-NCT system.

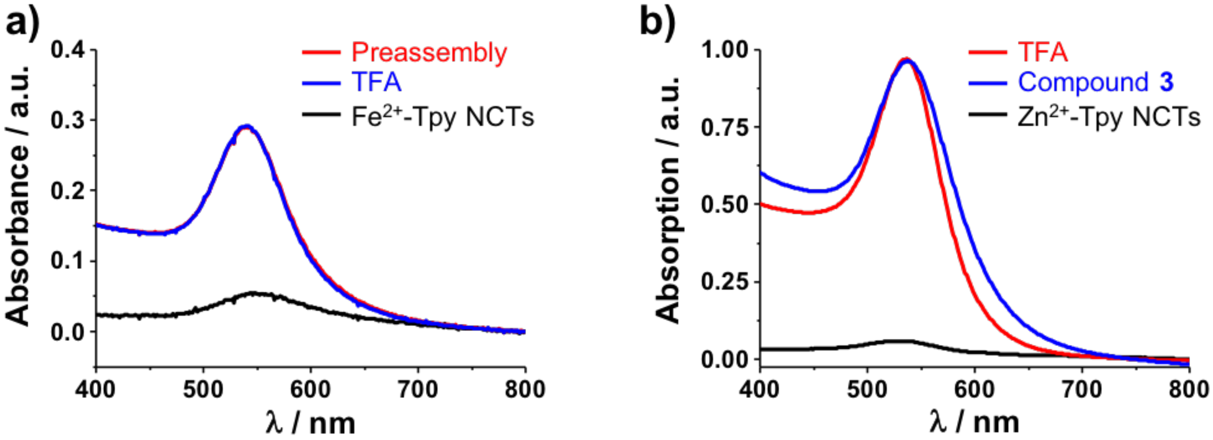


Figure S4. a) UV/Vis Spectra of the assembled NCTs (26 kDa PS, Tpy recognition motifs, 28 nm AuNPs) induced by Fe^{2+} and the recovery of the absorption intensity upon the addition of TFA. b) UV/Vis Spectra of the assembled NCTs (11 kDa PS, Tpy recognition motifs, 28 nm AuNPs) induced by Zn^{2+} and the recovery of the absorption intensity upon the addition of TFA or free Tpy compound **3**, which demonstrate that the disassembly process can be triggered by both types of stimuli.

6. Study of the Photo-responsive AuNPs Systems

Experimental protocol

(1) **HW/CA system**: Equal volumes of 26 kDa-PS-HW and 26 kDa-PS-CA functionalized 16 nm AuNPs with the same optical density (~ 1 nM) were mixed together. Then, SP were added and its concentration was 10 μM in the final solution. This solution was subsequently treated under the irradiation of UV light and visible light in an alternative manner, and the corresponding spectra after each treatment were recorded by UV-Vis spectroscopy.

(2) **Tpy-based system**: To a toluene solution of 26 kDa-PS-Tpy-functionalized 16 nm AuNPs, Zn(OTf)$_2$, TFA and SP were added and their concentrations were 10 μM, 6 and 4 mM in the final solution, respectively. This solution was subsequently treated under the irradiation of visible light and in dark in an alternative manner, and the corresponding spectra after each treatment were recorded by UV-Vis spectroscopy.

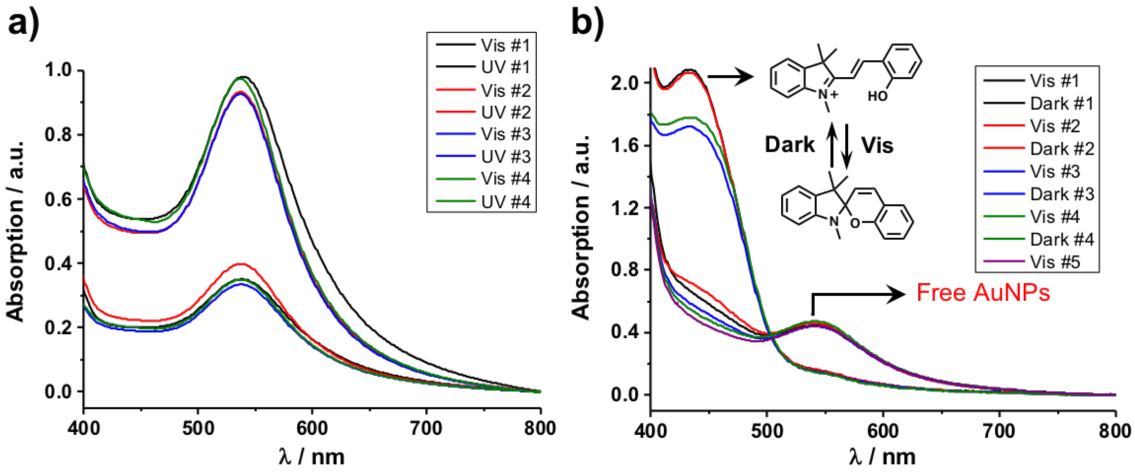


Figure S5. UV/Vis Spectroscopic characterization of the reversible assembly and disassembly behavior of a) the HW/CA NCTs in the presence of SP and b) the Zn^{2+}-Tpy-NCTs in the presence of TFA and SP upon applying different light stimuli.

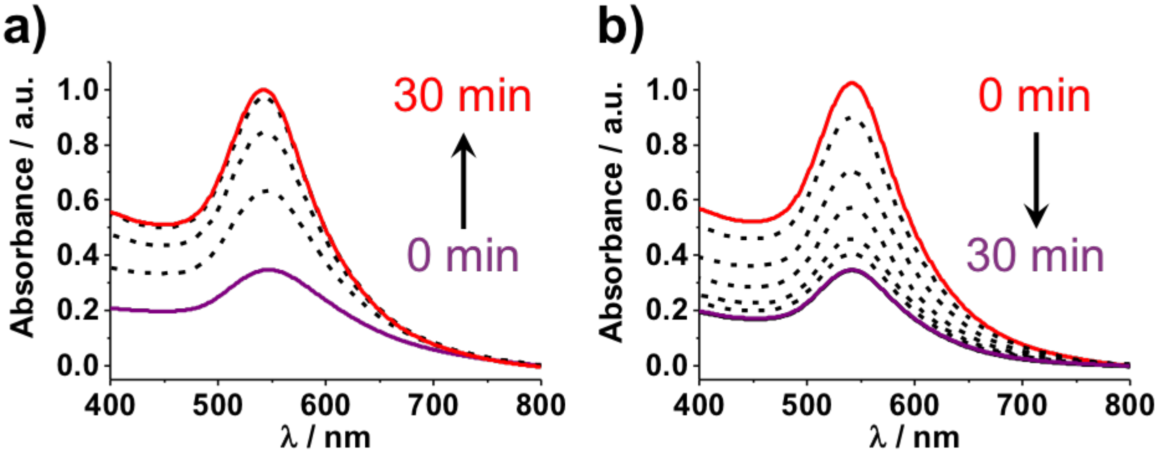


Figure S6. UV/Vis Spectroscopic characterization of a) the disassembly process of the HW/CA NCTs upon exposure to UV light, and b) the re-assembly process of the HW/CA NCTs under visible light, which indicates that in both cases the light-induced assembly/disassembly processes were completed in 30 min.

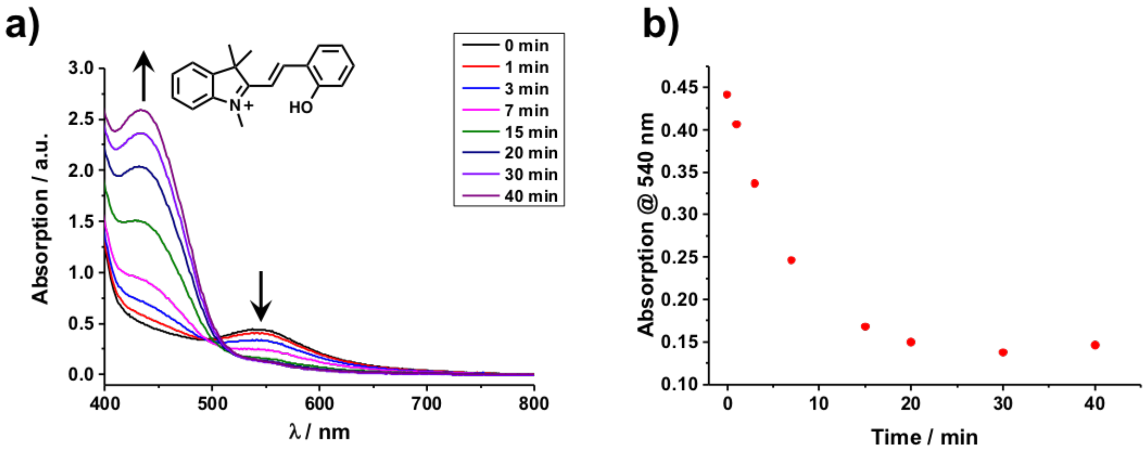


Figure S7. a) UV-Vis Spectroscopic characterization of the kinetic of the assembly process of the Tpy-NCTs upon the removal of the visible light. b) The change of the absorption intensities of the band centered at 540 nm as a function of time, which indicates that the assembly of the AuNPs is completed within 30 min.

The spectra corresponding to the reversible assembly and disassembly of the HW/CA- and Tpy-based system are shown in Figure S5, and the kinetic of these photo-responsive processes were also investigated. For the HW/CA-based NCT system, both the assembly and the disassembly processes were completed in about 30 min after exposure to UV light and removal of the optical stimulus, respectively (Figure S6). For the Tpy-based NCTs, the result (Figure S7a) shows that upon the removal of the visible light, the absorption band centered at 460 nm—characteristic absorption of MCH species—increased its intensity concurrently with the decrement of the absorption band centered at 540 nm—which indicates the assembly of the AuNPs. This observation demonstrates that SP recombined the protons in solution and reformed the MCH species in dark, which facilitates the interactions between the Tpy ligands and Zn^{2+} ions and consequently induces the aggregation of the AuNPs. More importantly, this process is completed (Figure S7b) within 30 mins. Finally, the absorption band at 540 nm which corresponds to the disassembled AuNPs can be restored by irradiating the system with visible light for 5 mins. Taken together, these observations demonstrate that the non-photoresposive group functionalized AuNPs can respond to external light stimluli in a rapid manner through rational design.

7. TEM Characterization of the Multi-stimuli Responsive NCT System

In addition to the UV/Vis spectroscopic measurements, the multi-stimuli responsive behavior of the NCTs was also confirmed by TEM. NCT samples in 3:1 toluene/anisole (~ 1 nM) that had been used to study the orthogonal stimuli-responsive assembly behavior were dropped cast onto a formavar stabilized with carbon 200 mesh copper TEM grid. Specifically, when the assembly behavior is driven exclusively by coordination interactions, only 16 nm NCTs are observed in the supernatant (Figure S8a) and only 28 nm NCTs are observed (Figure S8b) in the aggregate. In comparison, when the assembly is driven by hydrogen bonding or both hydrogen bonding and coordination interactions, the aggregate is comprised of (Figure S8c-e) a mixture of 28 and 16 nm NCTs.

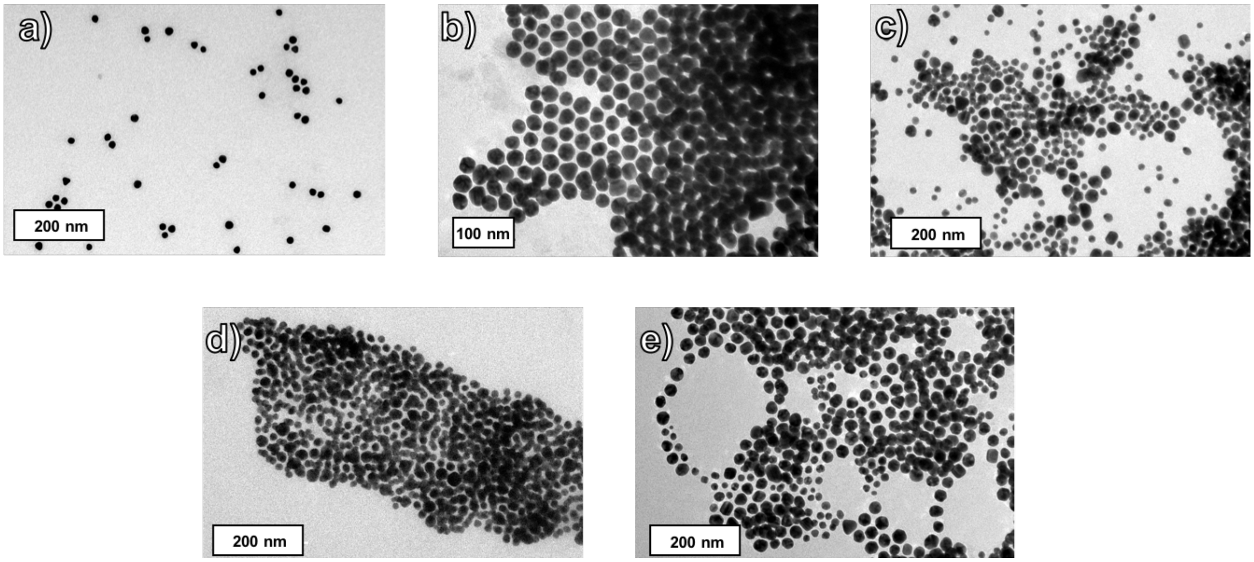


Figure S8. a) TEM images of the dispersed 16 nm NCTs in the supernatant. b-e) TEM images of the NCT assemblies induced by b) metal ion coordination interactions, c) hydrogen bonding interactions, d) hydrogen bonding followed by coordination interactions and e) coordination followed by hydrogen bonding interactions.

Interestingly, when the assembly is governed by both types of interactions, the morphology of the final aggregates exhibits pathway-dependent features. The 28 and 16 nm NCTs are intermixed (Figure S8d) when the aggregates are driven by hydrogen bonding first then coordination chemistry. In comparison, the 28 and 16 nm NCTs are segregated into distinct regions in the aggregates that are driven by coordination chemistry first then hydrogen bonding: the 28 nm NCTs form aggregates which are surrounded (Figure S8e) by 16 nm NCTs.

8. SAXS Characterization of the Pathway-Dependent NCT Assembly Behavior

In addition to TEM, the pathway-dependent assembly of NCTs (28 nm functionalized with HW/Tpy and 16 nm functionalzied with CA) was also confirmed by SAXS characterization. The interparticle spacings of the NCT aggregates obtained through different pathways are summarized in Table S1.

Table S1. Interparticle Spacings of the NCT Assemblies Induced by Different Stimuli.

	Zn^{2+}	H-bonding	Zn^{2+} then H-bonding	H-bonding then Zn^{2+}
q_0 (Å^{-1})	0.0126	0.0171	0.0121 and 0.0171	0.0166
d (nm)	50	37	52 and 37	38

As shown in Table S1, when the 28 and 16 nm NCTs were assembled via steps (i) and (v) as shown in Figure 6a (first hydrogen bonding, then Zn^{2+} complexation), NCTs formed homogeneous aggregates with a single interparticle distance of 38 nm observed in the SAXS data. In comparison, when the two sets of NCTs were assembled in the opposite pathway (steps (iii) and (i) in Figure 6a, Zn^{2+}-Tpy-complex formation followed by hydrogen bonding), two distinct peaks were observed in the low q region, corresponding to the two different interparticle distances that would be expected: a larger distance of 52 nm for 28nm-28nm particle bonds linked with Zn^{2+}-Tpy complexes, and a shorter distance of 37 nm for the 28nm-16nm particle bonds consisting of HW/CA pairs. These observations and TEM images confirm the pathway-dependent assembly process of the NCTs described here.

9. UV/Vis Spectroscopic Study and SAXS Characterization of the NCT Assembly Linked by Covalent Bonds

Figure S9 shows that when DMF (1 % v/v) was added to the 1:1 toluene/anisole solution containing 16 nm HW-Hdz and CA-CHO NCT samples immediately after mixing, the NCTs remain dispersed, as characterized by the strong absorbance around 540 nm, because the hydrogen bonding interactions between HW and CA groups were disrupted by DMF.

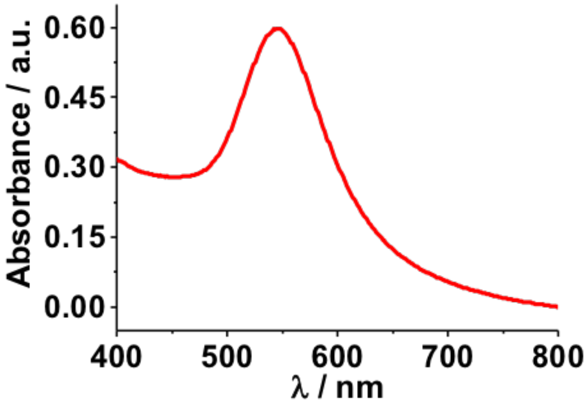


Figure S9. UV/Vis Spectrum of the 1:1 toluene/anisole solution of 16 nm HW-Hdz and CA-CHO NCTs in the presence of 1% v/v DMF.

In comparison, when DMF was added to the mixture after 48 h of mixing the 16 nm HW-Hdz and CA-CHO NCT samples, the NCTs remained assembled (Figure 7c) and these aggregates (1 nM in toluene) were subjected to SAXS measurements to characterize the interparticle spacings. The result shows (Figure S10) that these spacings match well with those for the assembly formed by hydrogen bondings alone, an observation which proves that the assemblies are resulting from the interactions between the termini of the polymer chains, i.e., the hydrazone bond formation.

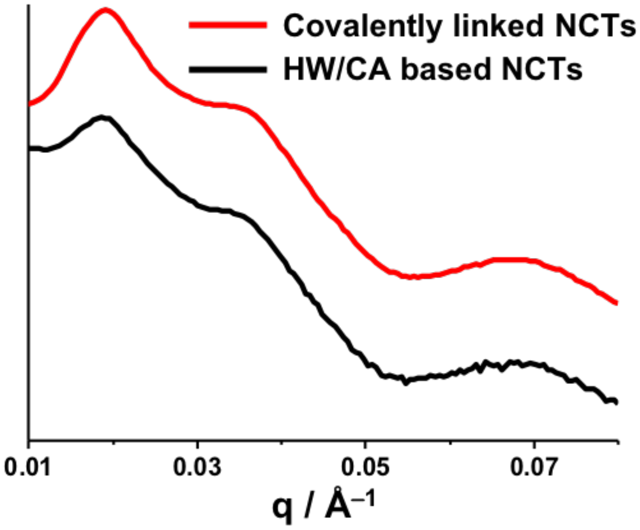


Figure S10. SAXS Measurements of the NCT assemblies induced by covalent bond formation (red trace) and hydrogen bonding interactions (black trace). The well matched interparticle spacings in these two cases demonstrate that the Hdz- and CHO-functionalized NCT assembly are resulting from the interactions between the termini of the polymer chains, which is the formation of the hydrazone bonds.

In addition to HW/CA pairs, covalent hydrazone bonds between NCTs were also achieved using coordination chemistry driven interactions to increase the local concentration of reactive groups. Specifically, two types of 16 nm NCTs were prepared, where one type was co-functionalized with Tpy/CHO groups (11 kDa PS, 3:1 feed ratio), and the other type was co-functionalized with Tpy/Hdz groups (11 kDa PS, 3:1 feed ratio) groups. In a control experiment, simply mixing the toluene solution of these two types of NCTs resulted in no covalent bond formation between the NCTs, as indicated by the lack of change in absorbance of the sample compared with the initial state (Figure S11, blue and red dash traces). In comparison, when Zn^{2+} ions were added to the NCT mixtures, aggregates were formed due to the coordination complexes formed between particles (Figure S11, black trace). After 48 h, the absorption of the NCTs was only partially recovered upon the addition of free tpy ligand, a stimuli which was shown to be able to break any coordination interactions between NCTs. In comparison, the absorption was fully recovered when TFA was added to the aggregates, consistent with hydrozone formation between the different particle types. It is also noteworthy that 75 % of the NCTs were covalently linked after 48 h, which is slower than the HW/Hdz and CA/CHO case. This could occur because the Tpy-linked NCTs are a unary system, meaning that some particles within an aggregate may not actually bind to their neighbors in an arragement that would allow for covalent bond formation (e.g. two Hdz-bearing NCTs linked with Tpy connections would not be able to form a hydrozone bond between them). It is also possible that the acidic cyanuric acid group may also serve as a catalyst to promote the covalent bond formation. Regardless of the specific mechanism, however, the control experiment in the absence of Zn^{2+} indicates that the colocalization of multiple reactive species (and potentially a catalyst) enhances the formation of the covalent bonds.

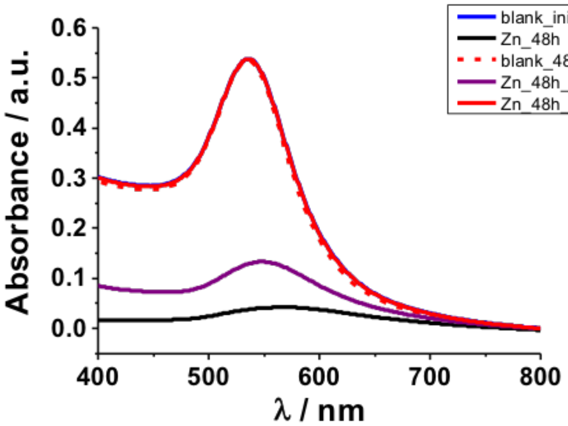


Figure S11. UV/Vis Spectra of the toluene mixture of 16 nm NCTs co-loaded with Tpy/CHO and Tpy/Hdz groups under different conditions. When Zn^{2+} was added to the mixture, the coordination interactions induced the formation of NCT aggregates (black trace), which enhanced the local concentration of the reacting CHO and Hdz groups; 75 % of the NCTs were covalently linked after 48 h (purple trace). The covalent hydrazone bond can be readily cleaved upon the addition of strong acid such as TFA (red trace).

References

(1) Alintas, O.; Gerstel, P.; Dingenouts, N.; Barner-Kowollik, C. *Chem. Commun.*, **2010**, *46*, 6291–6293.
(2) Jackson, P. M.; Moody, C. J.; Shah, P. *J. Chem. Soc., Perkin Trans. 1,* **1990**, *0,* 2909–2918.
(3) Wang, J.; Hanan, G. S. *Synlett* **2005**, *8*, 1251–1254.
(4) Fulmer, G. R.; Miller, A. J.; Sherden, N. H.; Gottlieb, H. E.; Nudelman, A.; Stoltz, B. M.; Bercaw, J. E.; Goldberg, K. I. *Organometallics* **2010**, *29*, 2176–2179.
(5) Liu, X.; Atwater, M.; Wang, J.; Huo, Q. *Colloids Surf., B*. **2007**, *58*, 3–7.
(6) Zhang, J.; Santos, P. J.; Gabrys, P. A.; Lee, S.; Liu, C.; Macfarlane, R. J. *J. Am. Chem. Soc.* **2016,** *138*, 16228–16231.