

Supporting Information

Photothermal Release of an Encapsulated Therapeutic Agent from Polymer-Wrapped Gold Nanoparticles

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Materials and Methods

General. Chemicals, unless otherwise stated, were used as purchased. For all nanoparticle experiments, CHCl_3 was purchased from ACP Chemicals Inc. For spectroscopic measurements, Spectrograde CHCl_3 was used and for synthesis reagent grade CHCl_3 was used. Ultrapure water was Milli-Q grade (18.2 $\text{M}\Omega\text{cm}$), and benzene was reagent grade. Poly(styrene-*co*-maleic anhydride) (PSMA) was obtained from MilliporeSigma and had an average M_n of $\sim 1,700$ as reported by the manufacture. Jeffamine® M-2070 was procured from Huntsman International LLC. Tyrphostin A8 (4-hydroxybenzylidenemalononitrile, **T8**) (98% purity) was purchased from Alfa Aesar (Thermo Fisher Scientific) and 1-methoxy-1,3-cyclohexadiene was purchased from MilliporeSigma as technical grade (65%). Column chromatography was performed using silica gel 60 (230–400 mesh) from Silicycle Inc. $\text{DMSO-}d_6$ used for NMR spectroscopic analysis was purchased from Cambridge Isotope Laboratories and used as received.

NMR Spectroscopy. ^1H NMR and ^{13}C NMR characterizations of compound **1** was performed on a Bruker QNP 600 cryoprobe working at 600.13 MHz for ^1H NMR spectroscopy and 150.92 MHz for ^{13}C NMR spectroscopy. Chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane using the residual solvent peak as a reference. Coupling constants (J) are reported in hertz. Multiplicities are reported as: s = singlet, d = double, t = triplet, q = quartet, and m = multiplet.

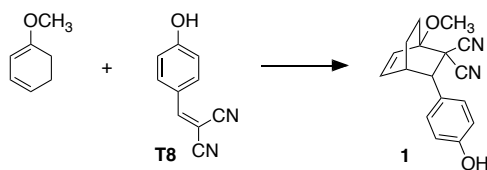
Optical Spectroscopy. UV-vis absorption spectroscopy was performed using a Varian Cary 300 Bio spectrophotometer. Measurements were run in a dual beam setting with a scan rate of 600 nm/min and a 1.00 nm data sampling rate from 800 nm to 200 nm. All measurements were taken in a narrow channel quartz cuvette with a path length of 10 mm. The thermolysis measurements were recorded on a Shimadzu UV-3600 Plus spectrophotometer with similar parameters. Infrared spectroscopy measurements were conducted on a PerkinElmer Spectrum Two™ IR spectrometer equipped with a universal ATR accessory with a 9-bounce diamond top-plate.

Mass Spectrometry (MS). Mass Spectroscopy (HRMS) measurements were performed using an Agilent 6210 TOF LC/MS in ESI-(+) mode.

Melting point measurements. Melting points were measured using a Gallenkamp melting point apparatus and are uncorrected.

Transmission Electron Microscopy (TEM). TEM images were obtained using a Hitachi 8100 Scanning Transmission Electron Microscope operating at 200 keV. Dilute colloids of the nanoparticles (approximately 0.1 wt-%) dispersed in water (10 μ L) were placed on thin, carbon formvar-coated copper grids held by anti-capillary tweezers (Ted Pella, Part # 501-4). The grids were then slowly dried under reduced pressure in a vacuum desiccator. The shape and size of the gold nanoparticles and nanoassemblies were evaluated from the collected TEM images. The size of the nanoparticles was calculated from over 100 particles located at different areas of the TEM grid.

Dynamic Light Scattering (DLS). DLS measurements were carried out using a Malvern Zetasizer Nano-ZS. The colloidal samples were held in a 1 cm path length square glass cuvette (Starna Cells, Part 76 # 23/G/10). A nanoparticle concentration of \sim 0.6 mg/mL was employed for the measurements. All DLS measurements were conducted at 25 $^{\circ}$ C.



Synthesis of bicyclic compound 1. A suspension of Tyrphostin A8 (4-hydroxybenzylidene)malononitrile, (**T8**) (250 mg, 1.47 mmol) in dioxane (0.75 mL) was treated with 1-methoxy-1,3-cyclohexadiene (696 mg, 2.67 mmol¹), and heated at 80 $^{\circ}$ C for 16 h, at which time the solvent was removed under vacuum. Purification by column chromatography on silica gel (25-30% EtOAc:hexanes gradient) yielded the product as a yellow solid. Recrystallization from warm CHCl_3 afforded 133 mg (32% yield) of compound **1** as a white solid.

m.p: 153-157.5 $^{\circ}$ C (decomposed).

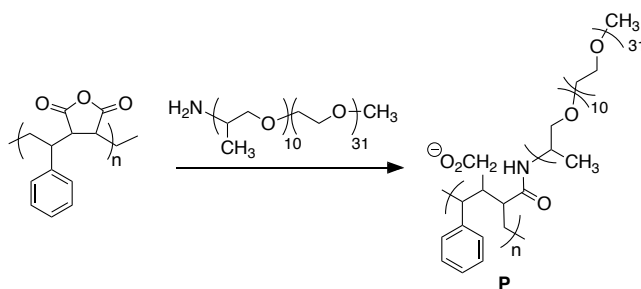
FTIR (neat) $\tilde{\nu}_{max}$ (cm^{-1}): 3420, 2968, 2267, 1616, 1517, 1102, 693.

^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 9.53 (s, 1H), 7.05-7.02 (m, 2H), 6.90 (dd, J = 8.8, 6.5 Hz, 1H), 6.77-6.73 (m, 2H), 6.50 (d, J = 8.8 Hz, 1H), 3.81 (s, 1H), 3.46 (s, 3H), 2.84 (dt, J = 6.4, 2.7 Hz, 1H), 2.01 (ddd, J = 12.6, 9.8, 2.9 Hz, 1H), 1.98-1.92 (m, 1H), 1.79-1.73 (m, 1H), 1.36 (tdd, J = 12.3, 5.9, 2.5 Hz, 1H).

^{13}C NMR (151 MHz, $\text{DMSO}-d_6$) δ 157.53, 137.00, 130.18, 129.66, 129.10, 117.01, 115.92, 114.67, 81.69, 53.61, 52.71, 51.01, 33.98, 26.34, 21.80.

HRMS (ESI⁺): m/z 303.1112 ($\text{M}+\text{Na}$)⁺ Calc. $\text{C}_{17}\text{H}_{16}\text{N}_2\text{ONa}$ 303.1104.

¹ This value corresponds to 65% of the active compound in the reagent grade bottle.



Synthesis of amphiphilic polymer P. A stock solution of the polymer was prepared by stirring Jeffamine® (1.300 g, 0.65 mmol) and poly(styrene-*co*-maleic anhydride) (193.71 mg, 0.114 mM) in CHCl₃ (20 mL) overnight, and then stored at room temperature in a cool, dark location until needed. This stock solution of the polymer was used for further experiments.

FT-IR (neat) $\bar{\nu}$ (cm⁻¹): 3000, 2865, 1723, 1090, 747. The IR supports the formation of the amphiphilic polymer as seen by the amide stretches (N-H) and a shift in the C=O stretches from 1774 cm⁻¹ (PSMA) to 1723 cm⁻¹ (polymer P). See Figure S6 for details.

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.31 (br s), 3.51 (br s), 3.24 (br s), 1.04 (br s).

Synthesis of citrate-coated gold nanoparticles.² A solution of HAuCl₄•(H₂O)₃ (1 mM in 100 mL ultrapure water) was heated to reflux in a 250 mL round-bottom flask. At this temperature, the solution was treated with a pre-warmed (80 °C) solution of sodium citrate (39 mM in 10 mL ultrapure water). The resulting mixture was heated at reflux for 10 min to obtain a deep red coloured solution. This solution was allowed to cool to room temperature. This dispersion was stored and used without any further purification. The UV-visible absorption spectrum of citrate-coated gold nanoparticles showed an intense absorption band centered at 519 nm. The TEM images of these nanoparticles (Figure S1) showed that they have an average size of 11.2 ± 1.2 nm. The concentration of citrate-coated gold nanoparticles was estimated by drying 1 mL of the nanoparticle solution under a high vacuum and weighing the residue. This afforded a value of 2.55 mg/mL.

Preparation of octadecylamine-coated gold nanoparticles.³ The aqueous solution of citrate-coated gold nanoparticles (2 mL) was cautiously layered over a solution of 0.75 mM octadecylamine solution in chloroform (4 mL) in a centrifuge tube, followed by the addition of ultrapure water (2 mL). The two-phase mixture was mixed vigorously for 3 min using a vortex. The organic layer was carefully removed, and its volume reduced to half using a rotary evaporator. This solution of ODA-coated gold nanoparticles was used without any further purification. The UV-vis spectrum of this chloroform solution showed an intense absorption band centered at 525 nm. The TEM images of these nanoparticles showed that they have an average size of 14.6 ± 0.9 nm. A concentration of 0.39 mg/mL of the ODA-coated gold nanoparticles was estimated by drying 1 mL of the nanoparticle solution under a high vacuum and weighing the residue.

Preparation of nano-assembly NP-P-1. This solution above was treated with 2 mL of the stock solution of polymer (P) over the course of 1 min, followed by the bicyclic compound **1** (1 mg, 3.5

² Turkevich, J.; Stevenson, P.C.; Hiller, J. *Discuss. Faraday Soc.*, **1951**, *11*, 55–75.

³ Polavarapu, L.; Xu, Q. H *Nanotechnology* **2009**, *20*, 185606.

μmol) in CHCl_3 (1 mL). The resulting mixture was stirred for 18 h at ambient temperature (22 °C), at which time the solvent was removed using a rotary vacuum until the red mixture became highly viscous. This viscous mixture was treated with alkaline water (1 mL, pH 13, adjusted with 0.1 M aqueous NaOH), and the resulting mixture was sonicated for approximately 30 s. The volume of the red emulsion was reduced using a rotary vacuum until a clear colloid formed. The colloid was centrifuged at 21000 G for 10 min and the flow-through was discarded. The remaining pellet was re-dispersed in 3.0 mL of Milli-Q water and stored in the dark at 4 °C until needed.

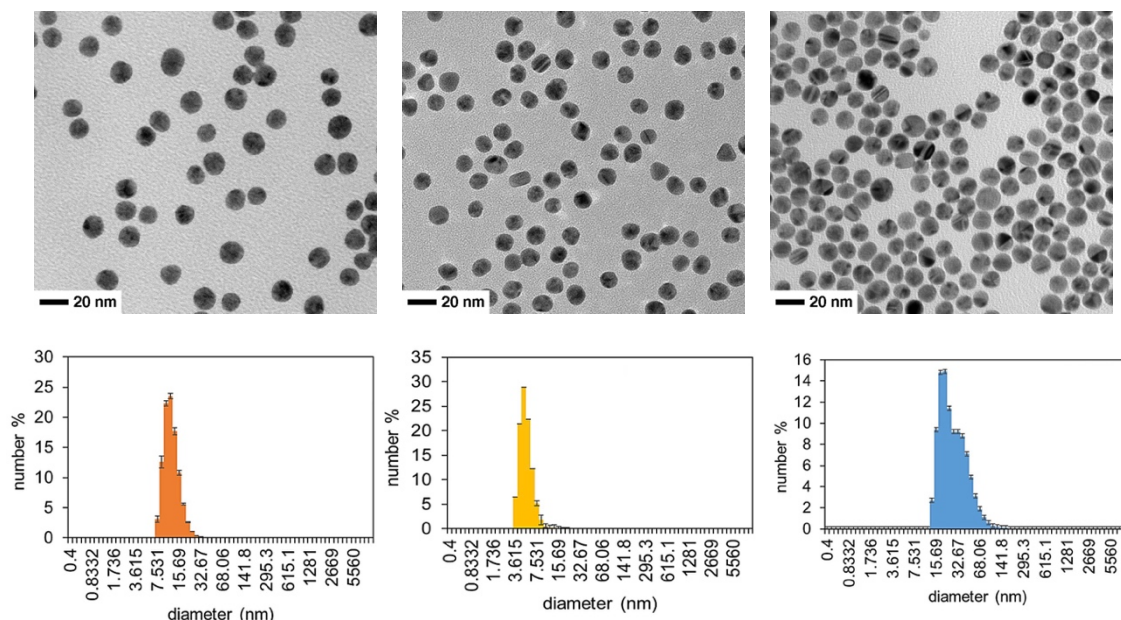


Figure S1. Top row: TEM images of citrate-coated gold nanoparticles (left), octadecylamine-coated gold nanoparticles (middle), and nano-assembly NP-P-1 (right). Bottom row: DLS measurements for citrate-coated gold nanoparticles (left), octadecylamine-coated gold nanoparticles (middle), and nano-assembly NP-P-1 (right).

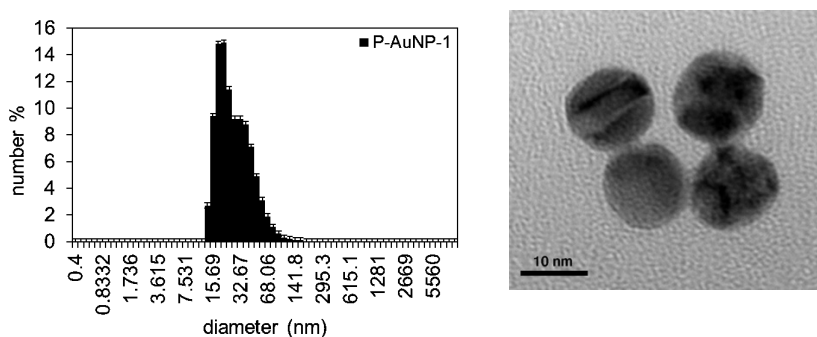


Figure S2. DLS measurements of nano-assembly NP-P-1 (left) and TEM image of nano-assembly NP-P-1 (right) showing faint 'halo' around each nanoparticle.

Estimating the loading of bicyclic compound 1 in NP-P-1. Quantification of the bicyclic compound **1** encapsulated inside the amphiphilic nanoassembly **NP-P-1** was achieved by determining concentration of released Tyrphostin A8 in the flow-through filtrate after the photothermolysis and spin-filtering using UV-vis absorption spectroscopy. The assumption is that all Tyrphostin A8 is released from the nano-assembly, and the retro Diels-Alder reaction have 100% conversion. The mass of a single gold nanoparticle was calculated using the size determined by TEM and assuming that the nanoparticles are perfectly spherical (see the table below for details).

Calculated number of nanoparticles in 1 mL of NP-P-1		
average radius of Au nanoparticle (cm)		$= 5.6 \times 10^{-7}$
volume of Au nanoparticle (cm ³) using $v = 4/3\pi r^3$	$= 4/3 \times 3.14 \times (5.6 \times 10^{-7})^3$	$= 7.4 \times 10^{-19}$
density of gold (g/cm ³)		$= 19.3$
mass of one single Au particle (g)	$= 19.3 \times 7.4 \times 10^{-19}$	$= 1.4 \times 10^{-17}$
weight of residue in 1 mL of NP-P-1 (g)		$= 8.4 \times 10^{-5}$
number of nanoparticles in 1 mL of NP-P-1	$= 8.4 \times 10^{-5} / 1.4 \times 10^{-17}$	$= 5.9 \times 10^{12}$
calculated number of molecules of 1 in 1 mL of NP-P-1 by quantifying released T8		
ϵ at 415 nm for T8 (M ⁻¹ cm ⁻¹)		$= 3.5 \times 10^4$
absorbance at 415 nm of T8 released from nanoparticle in water		$= 3.2 \times 10^{-2}$
concentration of T8 in flow-through filtrate (M)	$= 3.1 \times 10^{-2} / 3.5 \times 10^4$	$= 9.1 \times 10^{-7}$
volume of sample (L)		$= 1.0 \times 10^{-3}$
moles of T8 in flow-through filtrate	$= 9.1 \times 10^{-7} \times 1.0 \times 10^{-3}$	$= 9.1 \times 10^{-10}$
number of molecules of T8 in flow-through filtrate	$= 9.1 \times 10^{-10} \times 6.022 \times 10^{23}$	$= 5.5 \times 10^{14}$
number of molecules of T8 per nanoparticle	$= 5.5 \times 10^{14} / 5.9 \times 10^{12}$	$= 92$

Photothermal experiments. All photolysis experiments were carried out using a nanosecond pulse laser (10 ns, 532 nm) with a tuneable power. A power setting of 800 mW/cm² was used for the photothermal release experiments. Aqueous suspensions of nanoassembly (**NP-P-1**) were irradiated in a quartz cuvette with a pathlength of 1 cm. Nanoparticles were irradiated in one-minute intervals for the duration of time specified.

Verification of T8 in supernatant liquid. To verify that compound **T8** had been released and liberated from the nanoparticles it was essential to separate the nanoparticles from the solution. The sample after photolysis experiments was centrifuged using Amicon® Ultra-0.5 centrifugal filter devices (10 kDa) as per the recommendations of the manufacturer. The resulting supernatant was extracted and analyzed via UV-vis spectroscopy and mass spectrometry. The resulting pellet from the centrifugation was also analyzed by UV-vis dissolving the generated pellet in 500 μ L of Milli-Q water prior to analysis.

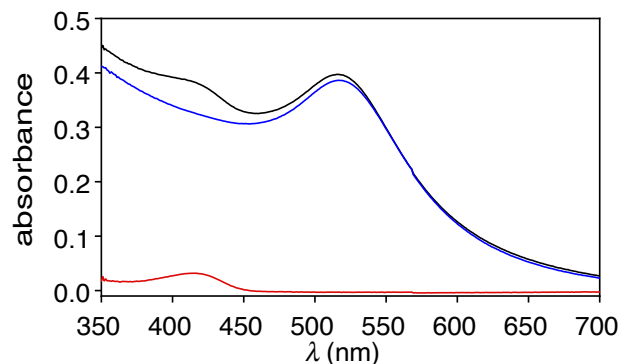


Figure S3. UV-vis absorption spectra when an aqueous solution of **NP-P-1** (2.66 μ g/ μ L) is irradiated with a nanosecond pulse laser (10 ns, 532 nm) at 800 mW/cm² for 5 minutes (black line), the flow-through filtrate after spin-filtering (red line) and the resuspended pellet after spin-filtering (blue line).

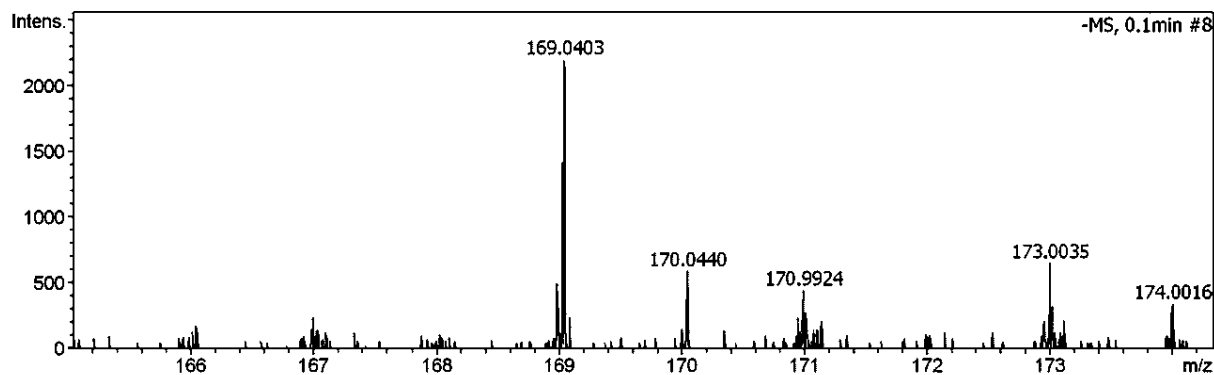


Figure S4. High-resolution mass spectrum analysis of the flow-through confirms the presence of **T8** after the photothermal release from **NP-P-1**. ESI-MS: m/z 169.0403 (M-H) Calc. C₁₀H₅N₂O₂⁻ 169.0407.

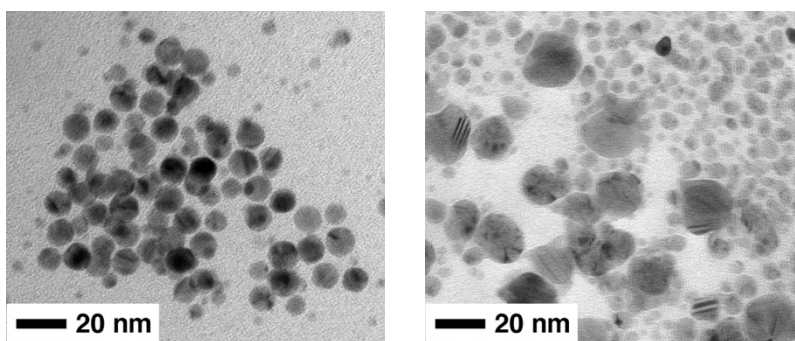


Figure S5. TEM images of nano-assembly **NP-P-1** (left) and citrate-coated nanoparticles (right) after irradiation with a nanosecond pulse laser (10 ns, 532 nm) at 800 mW/cm² for 5 minutes. Irradiation results in minimal changes to **NP-P-1** after irradiation. Citrate-coated nanoparticles undergo restructuring resulting in larger and amorphous shapes. Additionally, numerous fractured nanoparticles can be observed in the upper right corner of the image.

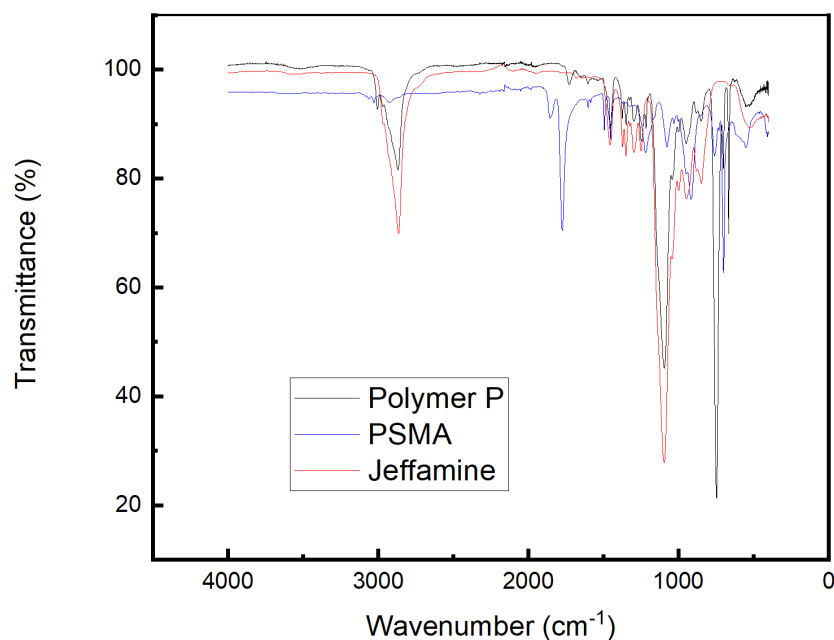


Figure S6. Overlaid IR spectrum of starting materials PSMA and Jeffamine with amphiphilic polymer **P**. The shift of the C=O stretch from 1774 cm⁻¹ in PSMA to 1723 cm⁻¹ in polymer **P** is evidence the ring-opening of the anhydride rings and the formation of the desired polymer.