Functional, composite polythioether nanoparticles *via* thiol-alkyne photopolymerization in miniemulsion

D. N. Amato,[a] D. V. Amato,[a] J. Narayanan,[a] B. R. Donovan,[a] J. R. Douglas,[a] S. E. Walley,[a] A. S. Flynt,[b] and D. L. Patton*[a]

[a]School of Polymers and High Performance Materials, University of Southern Mississippi

[b]Department of Biology, University of Southern Mississippi

1. Experimental

1.1 Materials

Hexadecane, 4-p-methoxy phenol, sodium dodecyl sulfate (SDS), 1,7-octadyne, 1-hexyne, 2,2-dimethoxy-2-phenylacetophenone (DMPA), tetrahydrofuran (THF), 7-mercapto-4-methylcoumarin, sulforhodamine B, silver nitrate ($AgNO_3$), sodium borohydride (NaBH4, ≥96% purity), dodecanethiol (DDT, ≥98% purity), ethanol (EtOH, ACS reagent grade), toluene and butyl acetate (Sigma-Aldrich), pentaerythritol tetra(3-mercaptopropionate) (PETMP, BrunoBock), 1-hydroxycyclohexyl phenyl ketone (Irgacure 184, CIBA), 7-methoxy-4-methylcoumarin (TCI, Tokyo, Japan) and Texas Red® C2 maleimide (Invitrogen) Click-iT® EdU Imaging Kit with Alexa Fluor® 488 Azide (Life Technologies™) were obtained at the highest purity available and used without further purification unless otherwise specified. Trimethylolpropane Tripropargyl Ether (TMPTPE) was prepared according to a previously reported method.[1]

1.2 General Sample Preparation

Each sample was prepared in a 20 mL scintillation vial with a total volume 10 mL. The organic stock solution shown in Table 1 was added into the vial containing a stock solution of SDS and deionized water. The samples were then placed into an ice bath and sonicated using a Q-700A-110 probe ultrasonicator at 20 % amplitude for 20 minutes.

Table 1. General formulation of organic stock solution for thiol-yne photopolymerization in miniemulsion.

Organic Fraction	Mass (g)	Wt. %
Hexadecane	0.5 (2.2 mmol)	7.32
1,7 Octadiyne	0.5 (4.7 mmol)	7.32
PETMP	2.3 (4.7 mmol)	33.67
Irgacure 184	0.1 (0.49 mmol)	1.46
4-p-methoxy phenol	0.03 (0.24 mmol)	0.44
n-butyl acetate	3.4 (29 mmol)	49.78

The miniemulsions were then cured using an Omnicure S1000-1B with a 100W mercury lamp (λ_{max}=365 nm, 320-500 nm filter) and an intensity of 185 mW/cm^2 for 10 minutes unless noted otherwise. All samples were made in triplicate to ensure reproducible data. To optimize the formulation for small nanoparticles, the organic fraction was varied with a constant SDS concentration of 20 mM.

1.3 Preparation of nanoparticles with excess thiol and excess alkyne

Nanoparticles with excess thiol were prepared using a 3.2:1 thiol to alkyne stoichiometry, for example, PETMP (3.68 g, 7.531 mmol) and 1,7-octadiyne (0.50 g, 4.7 mmol). The remaining constituents in the organic formulation from Table 1 were held constant. 250 µL of organic solution was pipetted into 9.75 mL of 20 mM SDS in DI water. The sample was then ultrasonicated for 20 minutes at 10% amplitude and cured under UV light for 10 minutes. Nanoparticles containing excess alkyne were synthesized similarly using a 1.51:1 alkyne to thiol ratio, i.e. 1,7-octadiyne (0.758 g, 7.14 mmol) and PETMP (2.30 g, 4.71 mmol).

1.4 Fluorescent tagging of excess thiol nanoparticles

From the nanoparticle suspension (10 mL) with excess thiol prepared in 1.3, 2 mL were removed and placed into a 20 mL scintillation vial wrapped in aluminum foil with a stir bar. A stock solution of Texas Red® C2 maleimide was made by the addition of 10 µL of Texas Red® C2 maleimide to 100 µL of DMSO. 50 µL of the Texas Red® C2 maleimide stock solution was added to the nanoparticles and stirred overnight. The nanoparticle were purified by centrifugation (5 minutes at 13,300 rpm, Fisher Scientific™ accuSpin™ Micro 17 centrifuge) to remove unreacted Texas Red® C2 maleimide. The supernatant was removed and the nanoparticle pellet was re-suspended in 1 mL DI water. The nanoparticle suspension was then suspended in glycerol on a glass slide and a coverslip was affixed and imaged immediately. The resulting slide was analyzed using a Zeiss LSM 510 confocal laser scanning microscope.

1.5 Fluorescent tagging of excess alkyne nanoparticles *via* 7-mercapto-4-methylcoumarin

From the nanoparticle suspension (10 mL) with excess yne prepared in 1.3, 1 mL was removed and centrifuged for 18 min at 13,300 rpm. The supernatant was removed and the nanoparticle pellet was re-suspended in 1 mL of THF. A solution was prepared containing 21 mg of 7-mercapto-4-methylcoumarin, 30 mg of DMPA and 1 mL of THF was added. The solutions were combined and exposed to UV light for 5 minutes to induce the radical thiol-ene reaction. The solution was centrifuged for 10 minutes, supernatant removed, the pellet re-suspended in THF. The coumarin-functionalized nanoparticle were then cast onto a glass slide with a drop of glycerol and a coverslip for analysis by confocal laser scanning microscopy.

1.6 Fluorescent tagging of excess alkyne nanoparticles *via* Alexa Fluor® 488 Azide

From the nanoparticle suspension (10 mL) with excess yne prepared in 1.3, 2 mL were removed and placed into a 20 mL scintillation vial wrapped in aluminum foil with a stir bar. The 1X Click-iT® EdU buffer was prepared with 1.0 mL of dH_2O to the Click-iT® EdU vial. To the Alexa Fluor® 488 vial, 100 µL of Click-iT® reaction buffer, 800 µL of $CuSO_4$ solution, and 100 µL of the prepared 1X Click-iT® reaction buffer was added. From this, 500 µL of the reaction cocktail was added to the nanoparticle suspension and allowed to react overnight. The solution was then centrifuged (10 minutes at 13,300 rpm, Fisher Scientific™ accuSpin™ Micro 17 centrifuge), supernatant removed, nanoparticles resuspended in 1 mL of H_2O and then added to a slide with glycerol. A coverslip was placed on top and was immediately imaged using a Zeiss LSM 510 confocal laser scanning microscope.

1.7 Preparation of hydrophobically modified silver nanoparticle

Dodecanthiol capped AgNPs were prepared using a modified procedure.[2] Briefly, a 2.25 mM stock solution of $AgNO_3$ was prepared with 19.11 mg $AgNO_3$ in 50 mL of EtOH. A second 69.4 mM solution of $NaBH_4$ was prepared with 0.2625 g in 100 mL of EtOH. Into a 20 mL scintillation vial, 14 mL of $NaBH_4$ stock with 25.7 µL of DDT with a magnetic stirbar. The solution was stirred and in one continuous addition, 4 mL of $AgNO_3$ stock was added, upon which the solution immediately turned yellow and gradually turned dark brown. The solution was centrifuged at 8500 rpm for 12 minutes, supernatant removed, 20 mL EtOH added three times. The final solution was resuspended in varying amounts of either toluene or butyl acetate to adjust the concentration of AgNPs.

1.8 Preparation of composite Ag nanoparticles

Each sample was prepared by mixing 250 µL of the 2:1 SH:yne octadiyne formulation with 50 µL of AgNPs (3% wt AgNP solution in butyl acetate). The solution was pipetted up and down to ensure mixing, upon which 250 µL was dispersed in a solution of SDS and deionized water. The samples were placed into an ice bath and were sonicated using a Q-700A-110 probe ultrasonicator at 20 % amplitude for 20 minutes. The miniemulsions were then cured using an Omnicure S1000-1B with a 100W mercury lamp (λ_{max}=365 nm, 320-500 nm filter) and an intensity of 185 mW/cm^2 for 10 minutes.

1.9. Isolation of the polymer nanoparticles

The polythioether nanoparticles can be recovered in near quantitative yield (based on weight of monomer fraction used in the synthesis) either via centrifugation or freeze-drying directly from the aqueous nanoparticle dispersion.

2.0 Characterization

The size and distribution of the nanoparticles were measured by dynamic light scattering (DLS) using a Microtrac Nanotrac Ultra NPA150. Transmission electron micrographs (Digital Imaging with Gatan Model 785 ES1000W Erlangshen CCD Camera) were taken with a Zeiss 900 TEM operating at 50keV and a JEOL JEM-2100 TEM operating at 200 keV. Samples were applied to 200 mesh copper grids (3.05 mm, 200 lines/inch square mesh, EMS Cat. #G200-Cu) coated with Formvar (5% polyvinyl formal resin). UV-vis measurements were performed on a Perkin-Elmer Lambda 6 UV/Vis spectrophotometer with quartz cuvettes. Atomic force microscopy was performed using a Bruker Icon in tapping mode. The samples were imaged with T300R-25 probes (Bruker AFM Probes) with a spring constant of 40 Nm-1. The samples then stained using OsO4. Samples for DSC analysis were prepared by placing 5–8 mg of freeze-dried cured nanoparticles into an aluminum hermetic DSC pan. Thermal history was erased before running DSC traces as samples were cooled to -50 ºC and heated to 100 ºC at 5 ºC/min. Samples were then cooled to -50 ºC at 10 ºC/min and then heated at a rate of 5 ºC/min to 200 ºC. All DSC tests were run on a TA Instruments Q200 differential scanning calorimeter. High resolution field-emission SEM (FE-SEM) image was obtained with a Zeiss Sigma VP FEG-SEM at 2 kV in high vacuum mode.

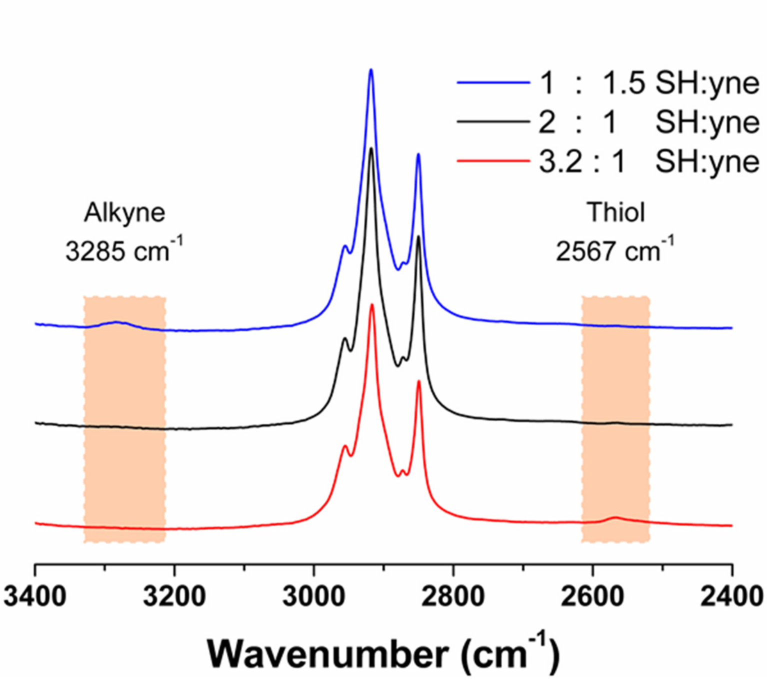

Figure S1. FT-IR spectra of polymer nanoparticles prepared at off stoichiometric ratios of thiol (PETMP) and alkyne (1,7-octadiyne).

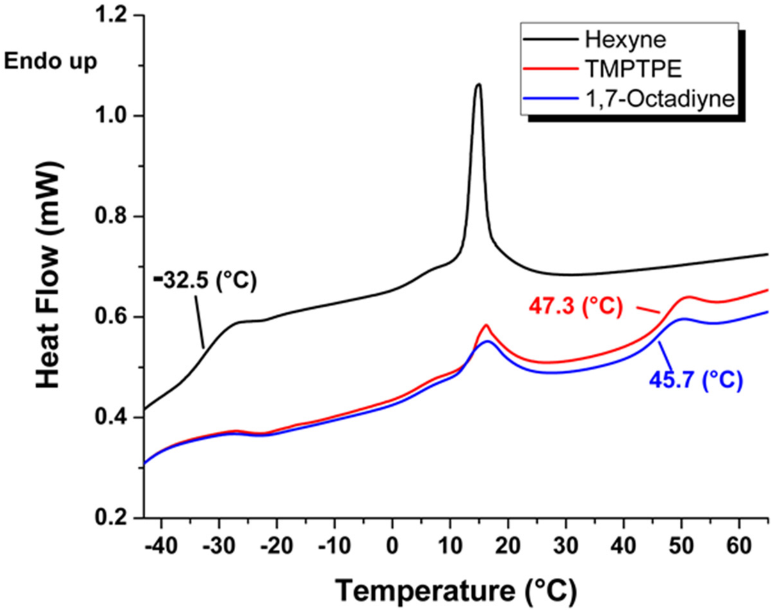

Figure S2. DSC thermograms of all three alkyne formulations prepared in the absence of hexadecane to eliminate additional peaks. Note: The melting peak from 17-18 °C is attributed to SDS crystallization – an assignment supported by Szelaf and Szumala.[3] Other samples prepared with hexadecane (not shown) also showed a melting peak at 17 °C.

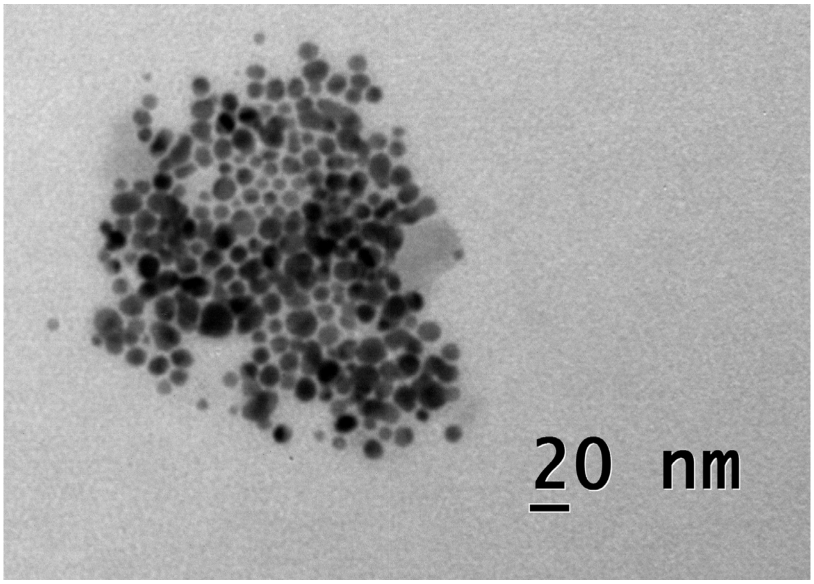

Figure S3. TEM image of synthesized dodecanethiol capped AgNPs.

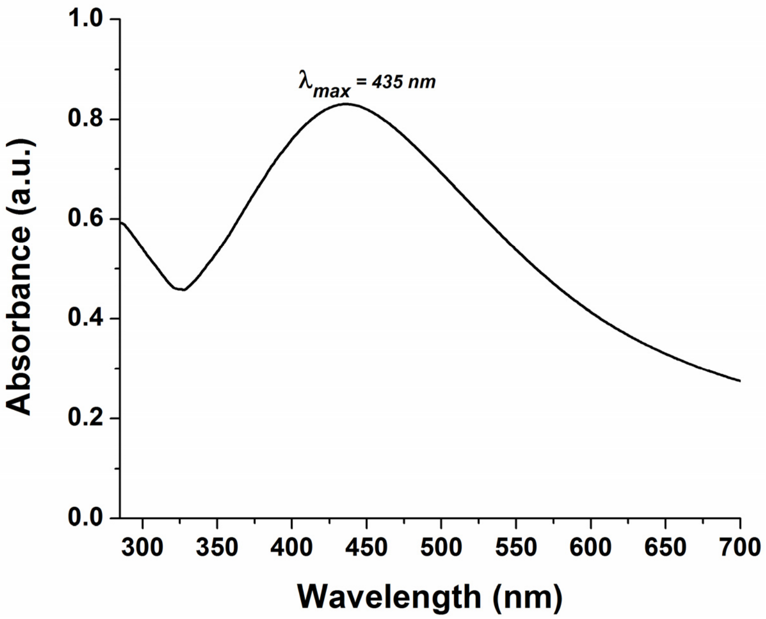

Figure S4. UV-vis spectra of AgNPs dispersed in toluene.

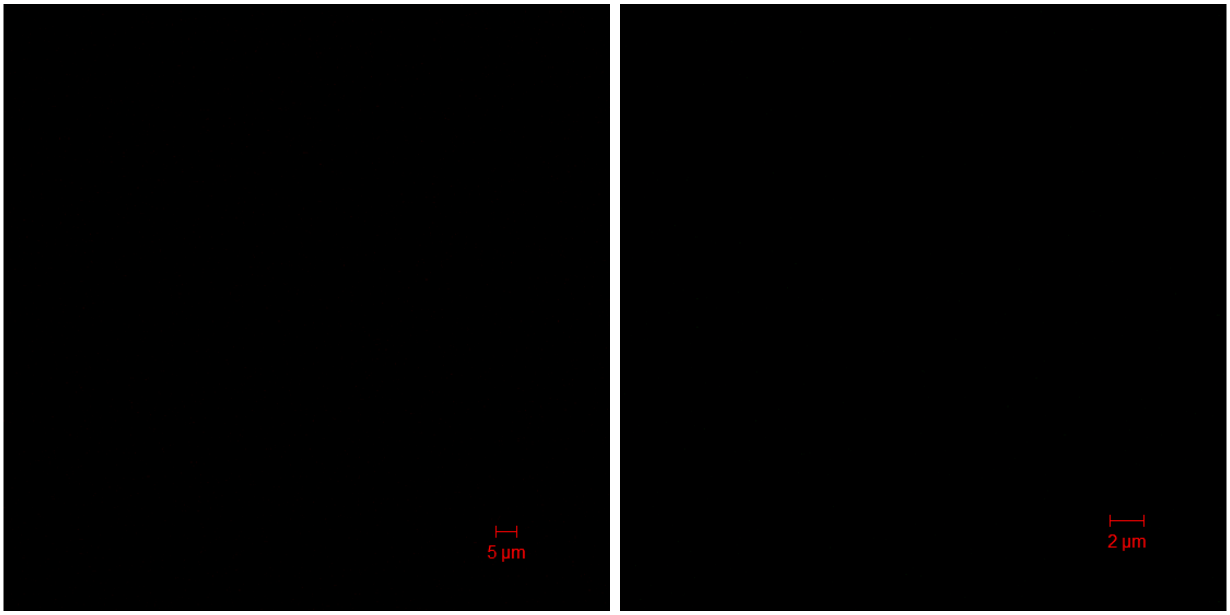

Figure S5. Control experiments for nanoparticle postpolymerization functionalization: (a) Thiol-functionalized nanoparticles (synthesized with excess 3.2:1 excess thiol:yne) were exposed to sulphorhodamine B (without maleimide) using the same reaction conditions as for Texas Red C2 maleimide. (b) Alkyne-functionalized nanoparticles (synthesized with excess 1.51:1 excess yne:thiol) were exposed to 7-methoxy-4-methylcoumarin (without thiol) using the same reaction conditions as for 7-mercapto-4-methylcoumarin. After washing, the absence of nanoparticles in the fluorescence images in (a) and (b) shows that covalent attachment, rather than physisorption is responsible for nanoparticle fluorescence when employing reactive fluorescent tags.

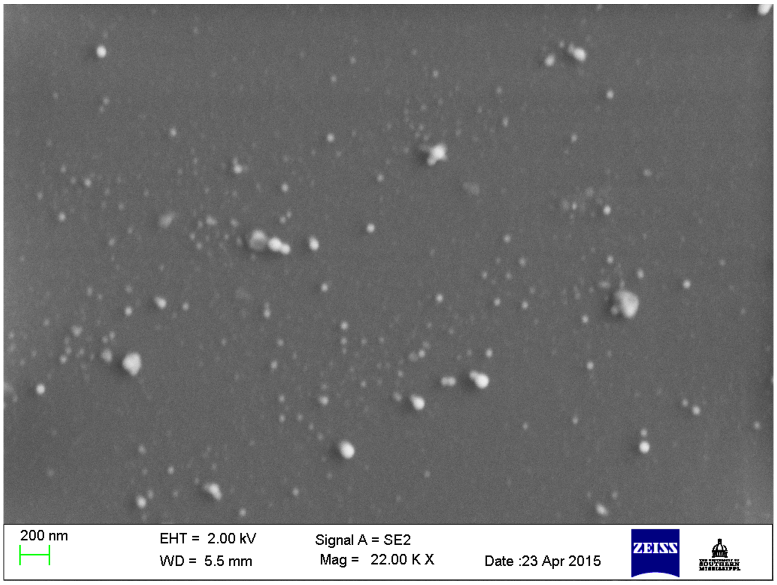

Figure S6. High-resolution FE-SEM micrograph of composite particles. The incorporation of silver nanoparticles did not alter the spherical morphology of the composite particles.

References:

1. Gorman, I. E.; Willer, R. L.; Kemp, L. K.; Storey, R. F., Development of a triazole-cure resin system for composites: Evaluation of alkyne curatives. *Polymer* **2012,** *53* (13), 2548-2558.
2. Farrell, Z.; Shelton, C.; Dunn, C.; Green, D., Straightforward, One-Step Synthesis of Alkanethiol-capped Silver Nanoparticles from an Aggregative Model of Growth. *Langmuir* **2013,** *29* (30), 9291-9300.
3. Szeląg, H.; Szumała, P., Effect of alkyl sulfate on the phase behavior of microemulsions stabilized with monoacylglycerols. *Journal of Surfactants and Detergents* **2011,** *14* (2), 245-255.