Electronic Supplementary Material (ESI) for Materials Horizons.

Supporting Information

Monodisperse Functional Microspheres from Step-growth "Click" Polymerizations: Preparation, Functionalization and Implementation

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Experimental Section

Materials: Pentaerythritol tetra(3-mercaptopropionate) (PETMP) and trimethylolpropane tri(3-mercaptopropionate) (TMPTMP) was donated by Bruno Bock. Ethylene glycol bis(3-mercaptopropionate) (EGBMP) was purchased from Wako Chemicals USA Inc. Divinyl sulfone (DVS) was purchased from Oakwood Products. Hexylamine, triethylamine (TEA), polyvinylpyrrolidone (K-value 29-32) (PVP), propargyl acrylate, trimethylolpropane triacrylate (TMPTA), di(trimethylolpropane) tetraacrylate (DTPTA), rhodamine B and rhodamine 110-azide (azide-fluor 488) were purchased from Sigma-Aldrich and used as received. Acryloxyethyl thiocarbamoyl rhodamine B was purchased from Polysciences, Inc.

General procedure for preparing microspheres: The general dispersion polymerization procedure for thiol-Michael microspheres involves an *in situ* transition from a homogeneous solution to a heterogeneous colloid. The mixture begins as a clear solution, prepared by dissolving 3.66 g (7.5 mmol) PETMP, 3.0 g TMPTA (10 mmol) and 1.5 g PVP in 150 mL methanol. The catalyst (hexylamine, 60 mg) was added under 400 RPM mechanical stirring and the mixture turned milky immediately. After 2h, the reaction was stopped, and the product was harvested by centrifugation. The product was a white powder after washing with methanol for three times and dried under vacuum, and the yield is measured by weight. Various polymerization conditions were used for the different monomers and catalyst loadings. For thiol-excess microspheres, 2.0 g TMPTA (6.7 mmol) was used so that the thiol group is present in 33 mol% excess. For the

acrylate-excess microspheres, 3.6 g TMPTA (12 mmol) was used so that acrylate it is present as a 20 mol% excess. The other polymerization conditions remain the same. Alkyne functionalized microspheres were made as follows: 2.44 g PETMP (1 eq), 1.8 g TMPTA (0.9 eq), 0.33 g propargyl acrylate (0.15 eq) and 1.5 g PVP were dissolved in 150 mL methanol. The polymerization was triggered by 0.1 g TEA. The mixture was allowed to react for 2h under 400 RPM stirring. The product was a white powder after washing with methanol for three times that was subsequently dried under vacuum. For azide functionalized microspheres, 0.38 g 6-azidohexyl acrylate (0.1 eq) was used instead of propargyl acrylate, and the other conditions were unchanged.

Procedure for preparing polymeric composite: Microspheres made from the PETMP-DVS formulation (after washed with methanol for three times, 0.5 g) were put in a vial then a stoichiometric amount of TMPTMP (0.4 g) and TMPTA (0.3 g) was added. The mixture was well mixed and a drop of TEA was added. Then, the mixture was quickly sandwiched into a glass cell with a sample thickness of 1 mm and cured overnight at 70°C.

*Procedure for preparing fluorescently labeled microspheres:*1.0 g thiol-excess (33 mol%) microspheres were dispersed in 50 mL methanol. 10 mg of acryloxyethyl thiocarbamoyl rhodamine B was then added to the mixture, following by 10 drops of TEA. The mixture was stirring for 1h and then washed with methanol for 5 times.

The functionalization of such microspheres via the CuAAC "click" reaction was begun by treating 1.0 g of microspheres with 0.1 g maleic anhydride in methanol in the presence of TEA to consume the unreacted thiol. Then, 1 mg of azide functionalized Cy-5 dye was added along with 10 mg of a copper (II) chloride-PMDTA complex and 100 mg ascorbic acid. The mixture was stirred overnight then washed with methanol 5 times.

Degradation of fluorescently-labeled microspheres: 0.1 g of rhodamine B labeled thiol-excess microspheres were dispersed in 10 mL of 1 mol/L NaOH solution. Specific timepoint samples were prepared by neutralizing the mixture with ammonia chloride at the desired time, then the mixture was centrifuged, and the upper clear solution was analyzed by UV-vis spectroscopy.

Characterization

Fourier Transform Infrared Spectroscopy (FTIR): FT-IR spectra of microspheres were obtained by a diffuse reflectance FT-IR accessory on a Nicolet 6700. The microspheres were mixed well with KBr powder and mid-IR was utilized to monitor the functional groups in the polymer microspheres.

Scanning Electron Microscope (SEM): SEM images were taken on a JEOL JSM 7401F located in the Colorado Nanofabrication Lab at CU-Boulder. Dilute suspensions of microspheres in methanol were dropped onto clean glass slides. After the solvent evaporated, samples were coated with approximately 4 nm of gold and then evaluated.

The size and size distribution were measured by analyzing SEM images where at least 50 individual microspheres were measured for each sample.

Fluorescence Microscope: Fluorescence images were pictured on a Nikon TE-2000-E microscope. Samples consisting of microspheres dispersed in solution were prepared by sealing the solution between a 1" x 3" glass slide and a cover slide with nail polish. The TRITC channel was used for samples labeled with acrylate functionalized rhodamine B, and the YFP channel was used for samples labeled with azide-fluor 488.

Differential Scanning Calorimetry (DSC): Differential scanning calorimetry measurements were performed on a Diamond DSC (Perkin Elmer), calibrated with an indium standard. Two heating cycles of the samples were carried out between -50 °C and 50 °C at 10 °C/min, in hermetically sealed aluminum pans. The glass transition temperatures were determined by Pyris software.

Dynamic mechanical analysis (DMA): Tests were conducted on a TA Instruments Q800 dynamic mechanical analyzer. Samples with thickness of 1 mm were prepared following the method described above. The temperature was ramped at 3 °C/min and the frequency used was 1 Hz. The glass transition temperature was determined as the temperature of the maximum value in the tan δ curve.

UV-Vis spectroscopy: A fiber optic UV-Vis spectrometer was used to measure the fluorescence response (USB2000-UV-VIS Miniture Fiber Optic Spectrometer, Ocean

Optics, Dunedin, FL). Irradiation of samples containing dyes released from the microspheres was achieved by a white light source (ORIEL, fiber optic illuminator model 77501) with a filter that blocks irradiation below 480nm.

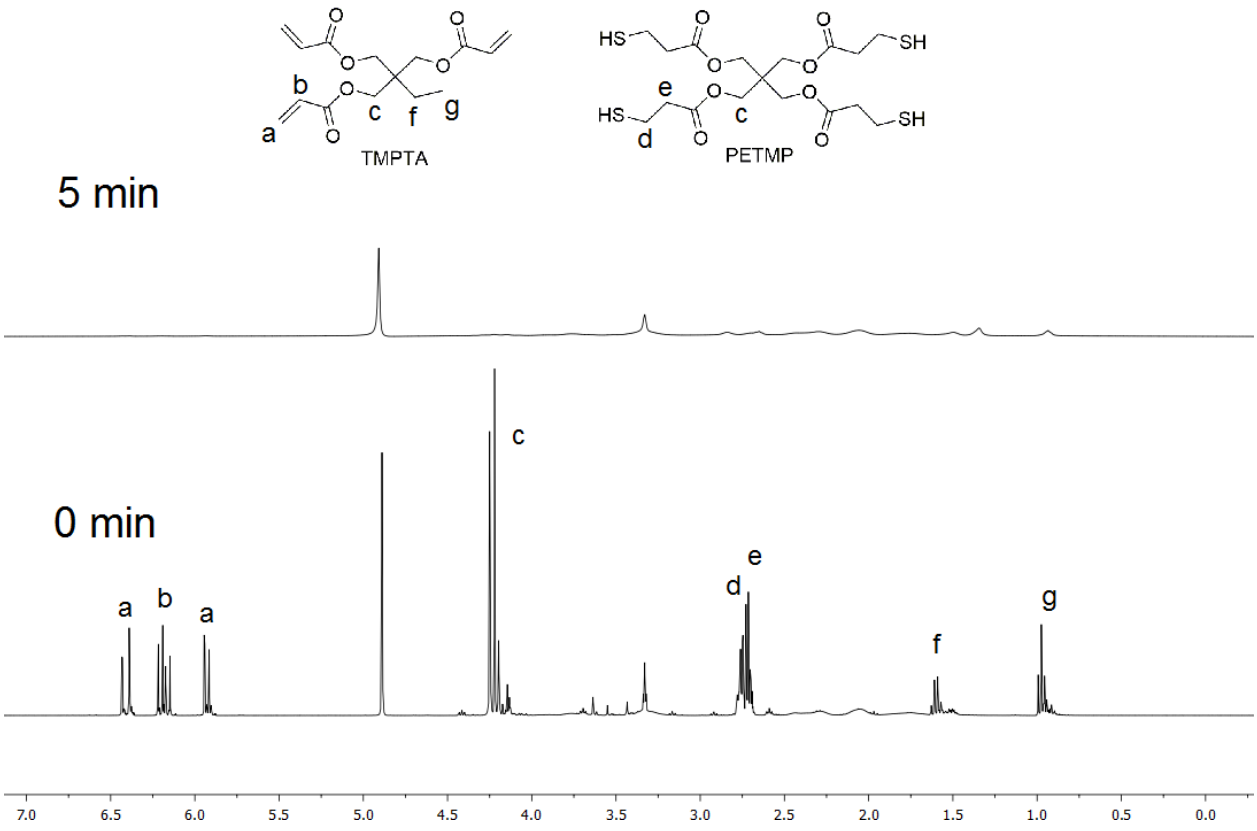

Figure S1. ^{1}H NMR of thiol-Michael addition polymerization in CD_3OD: 0.122 g PETMP (0.25 mmol), 0.10 g TMPTA (0.33 mmol), 0.05 g PVP, 5 mL CD_3OD. A drop of hexylamine was added (12 mg) and the dispersion was taken NMR spectrum immediately.

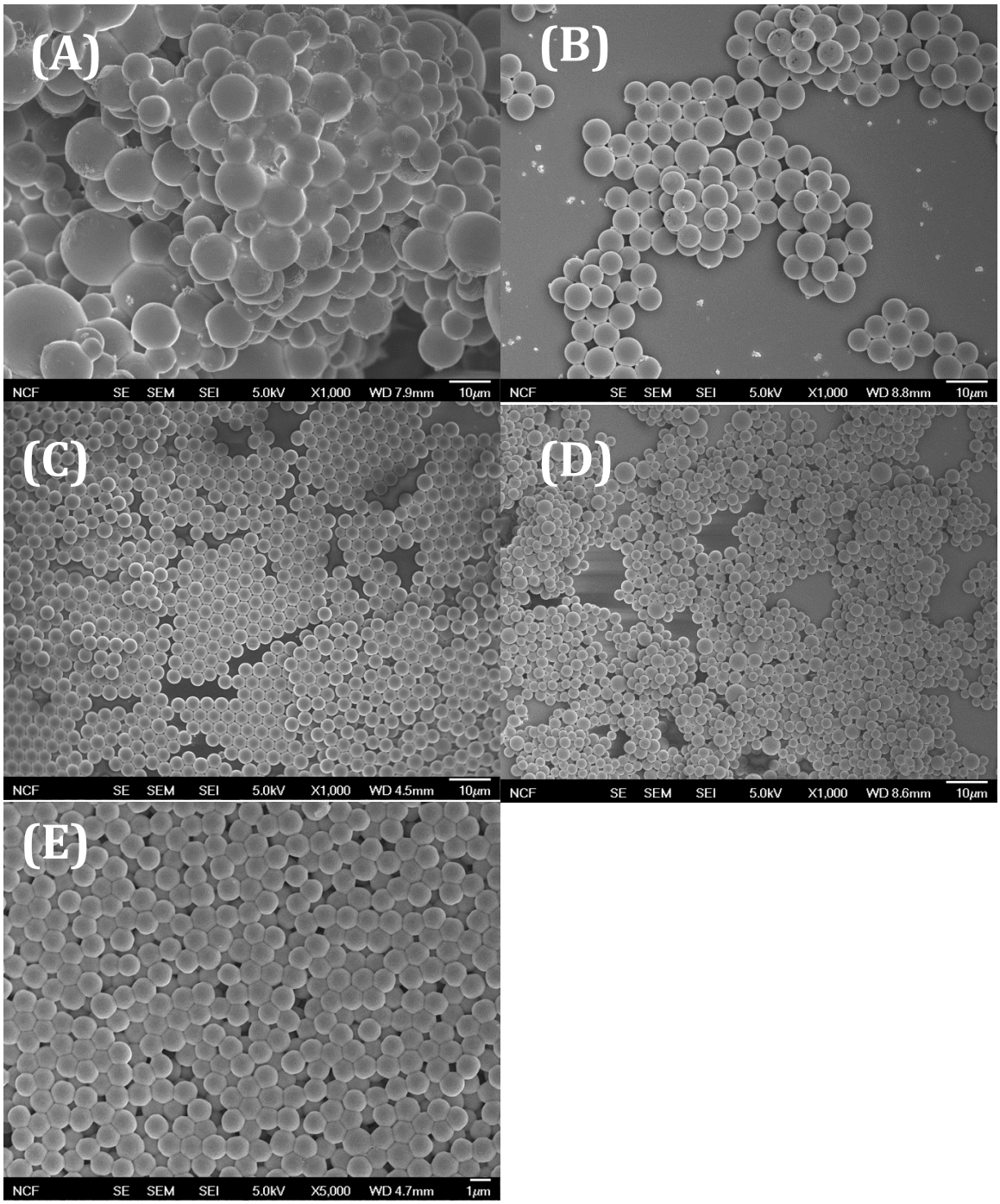

Figure S2. SEM images of thiol-Michael dispersion polymerization with various monomers: (a) EGBMP and TMPTA; (b) TMPTMP and TMPTA; (c) PETMP and TMPTA; (d) PETMP and DTPTA; (e) PETMP and DVS. Polymerization condition for all experiments: 30 mmol for both thiol and acrylate (vinyl sulfone) functional groups, 1.5 g PVP, 150 mL MeOH and 60 mg of hexylamine added under 400 rpm stirring.

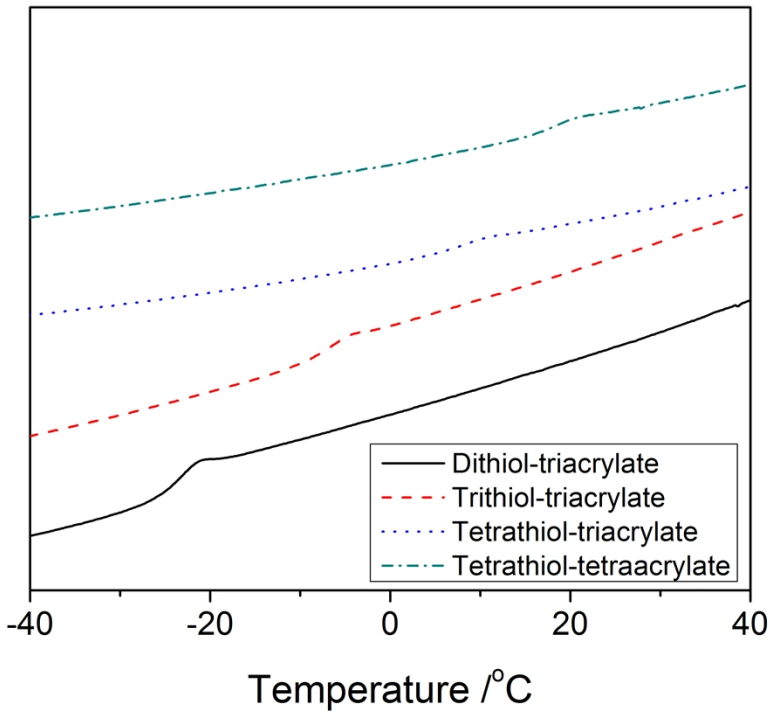

Figure S3. DSC curves of thiol-Michael microspheres made from various monomers: (a) Dithiol-triacrylate (EGBMP and TMPTA); (b) Trithiol-triacrylate (TMPTMP and TMPTA); (c) Tetrathiol-triacrylate (PETMP and TMPTA); (d) Tetrathiol-tetraacrylate (PETMP and DTPTA).

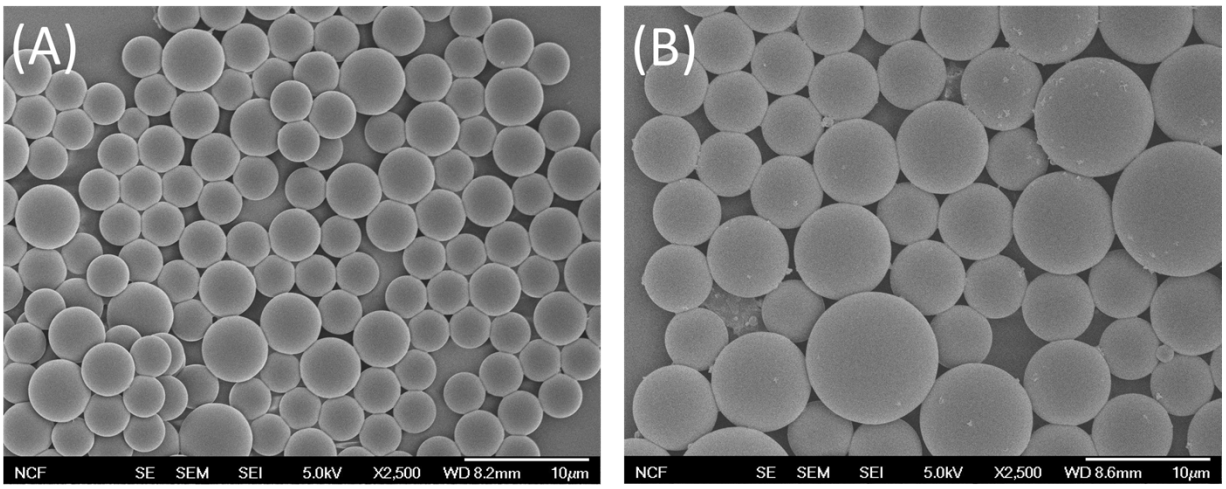

Figure S4. SEM images of thiol-Michael dispersion polymerization with alkyne and azide functionality: (a) alkyne-functionalized; (b) azide-functionalized. Polymerization condition for all experiments: PETMP (2.44 g, 5 mmol) and TMPTA (2.0 g, 6.7 mmol), propargyl acrylate (0.33 g, 3 mmol, for alkyne-functionalized microspheres), propargyl acrylate (0.33 g, 3 mmol, for alkyne-functionalized microspheres, 6-azidohexyl acrylate (0.38 g, 2 mmol, for azide-functionalized microspheres), 1.5 g PVP, 150 mL MeOH and 0.1g of TEA added under 400 rpm stirring for 2h.