## **Colouring Crystals with Inorganic Nanoparticles**

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### **Experimental Methods**

Calcite single crystal/ gold nanoparticle (GNP) composites were prepared by co-precipitation of calcium carbonate with GNPs functionalised with PSPMA<sub>30</sub>-PDPA<sub>47</sub> block copolymer, where the PSPMA<sub>30</sub>-PDPA<sub>47</sub> block copolymer was synthesised and characterised as described previously.<sup>1</sup>

### 1. Synthesis of PSPMA<sub>30</sub>-PDPA<sub>47</sub> Block Copolymer

#### Synthesis of 2-(4-Morpholino)ethyl 2-Bromoisobutyrate (ME-Br) Initiator

4-(2-Hydroxyethyl)morpholine (10.0 g, 75.5 mmol) and triethylamine (11.5 g, 113.3 mmol) were dissolved in dichloromethane (250 mL) in a 500 mL round-bottomed flask. The solution was cooled using an ice bath for 20 minutes before dropwise addition of 2-bromoisobutyryl bromide (19.5 g, 83.1 mmol) under dry nitrogen. This reaction mixture was stirred at 20 °C for 48 h, prior to being washed with 0.10 M aqueous sodium carbonate until all the triethylammonium bromide salt was removed. The organic layer was separated and then washed five times with 200 ml portions of deionised water. The combined organic phases were dried over MgSO<sub>4</sub>, and the solvent was removed under reduced pressure at 20 °C. The purified ME-Br initiator (15.34 g, 73 % yield) was stored in a freezer at -20 °C in the absence of light prior to use.

<sup>1</sup>H NMR (CDCl<sub>3</sub>, residual peak 7.27 ppm, 400 MHz): δ = 4.25 (t, 2H), 3.63(t, 4H), 2.62(t, 2H), 2.47(t, 4H), 1.87 (s, 6H).

### ATRP synthesis of the PHPMA<sub>30</sub>-PDPA<sub>47</sub> diblock copolymer precursor

In a 100 mL two-neck round-bottomed flask, ME-Br initiator (99.0 mg, 1.00 mmol) 2-hydroxypropyl methacrylate monomer (HPMA, 4.32 g, 30 mmol, target degree of polymerisation, DP = 30) were dissolved in 2-propanol/water (95:5 % w/w) to obtain a 50 % w/w aqueous solution of HPMA. This mixture was degassed using a stream of dry nitrogen gas for 1 h. Cu(I)Cl catalyst and 2,2'-bipyridine (relative molar ratios of ME-Br: Cu(I)Cl: bpy = 1:1:2) were then added quickly under a nitrogen blanket to start the polymerisation. The reaction mixture turned dark-brown and became viscous as the reaction progressed. After approximately 2.5 h, the polymerisation was essentially complete (more than 99 % conversion) as judged by <sup>1</sup>H NMR (in d<sub>4</sub>-methanol). Then a separately degassed 50 % w/w solution of 2-(diisopropylamino) ethyl methacrylate (DPA) in 2-propanol/water was added to this reaction solution via syringe. The polymerisation continued for 24 h and then was quenched by exposing the reaction mixture to air, followed by dilution with THF (80 ml). The spent ATRP catalyst

was removed by passing the green solution through a silica gel (0.063-0.2 nm particle diameter; Merck, Germany) column. The THF was partially removed under vacuum, and the crude block copolymer was precipitated into deionised water (500 ml). The recovered off-white copolymer was redissolved in THF and precipitated into water to removed traces of unreacted DPA monomer. A white solid was obtained after drying under vacuum (11.68 g, 78 % yield). Gel permeation chromatography (GPC) analysis was performed using a refractive index detector with HPLC grade THF eluent containing 2.0 % v/v triethylamine at a flow rate of 1.0 ml min<sup>-1</sup>, and the GPC column temperature was set at 30 °C. A series of near-monodisperse poly(methyl methacrylate) standards were used for calibration, giving an  $M_n = 17,400$  and an  $M_w/M_n = 1.21$  for the PHPMA-PDPA diblock copolymer precursor. Assuming that the target DP of 30 was achieved for the first PHPMA block, comparison of the integrated NMR signal assigned to the protons of the DPA residues at 2.75-3.25 ppm with that of the oxyethylene protons due to the HPMA and DPA residues at 4.0-4.5 ppm indicated a mean diblock copolymer composition of PHPMA<sub>30</sub>-PDPA<sub>47</sub> (<sup>1</sup>H NMR run in CDCl<sub>3</sub>).

### Full Esterification of PHPMA<sub>30</sub>-PDPA<sub>47</sub> diblock copolymer using succinic anhydride

In a 100 ml round-bottomed flask, 2.0 g of PHPMA<sub>30</sub>- PDPA<sub>47</sub> copolymer was dissolved in anhydrous THF (25 ml). Two molar equivalents of succinic anhydride and triethylamine relative to the hydroxyl groups of the copolymer were added under nitrogen. Esterification was allowed to proceed at 20 °C for 48 h. THF was then removed under vacuum, and the crude zwitterionic diblock copolymer was dissolved in saturated aqueous NaCl solution. The copolymer was purified by dialysis against aqueous NaHCO<sub>3</sub> for at least two days to ensure complete removal of impurities, followed by three days of dialysis against pure water and then freeze-drying overnight. <sup>1</sup>H NMR spectroscopy (in d<sub>5</sub>-pyridine) was used to assess the degree of esterification by comparing the four protons due to the succinic acid groups at 3.05 ppm to the twelve methyl protons due to the DPA residues at 1.05 ppm. The final fully esterified diblock copolymer was obtained as a white solid and was denoted 'PSPMA<sub>30</sub>-PDPA<sub>47</sub>'.

#### 2. Synthesis of Gold Nanoparticles (GNPs)

30 mg of PSPMA<sub>n</sub>-PDPA<sub>47</sub> (where n = 16, 30, 50 and 70) was dissolved in 10 ml deionised water (Milli-Q Standard, resistivity = 18.2 M $\Omega$ cm) and 30 mg of NaAuCl<sub>4</sub> (Sigma-Aldrich) was then added to give a pale yellow solution. GNPs were then formed by addition of 0.5 ml of 25 wt% hydrazine hydrate solution, under vigorous stirring, and the reaction mixture was stirred overnight. Finally, the solution of GNPs was dialysed against deionised water for 4 days to remove residual ions and copolymer not bound to the nanoparticles.

#### **3.** Precipitation of CaCO<sub>3</sub> in the Presence of Gold Nanoparticles (GNPs)

Calcium carbonate was precipitated in the presence of the copolymer-stabilised GNPs using the ammonium carbonate diffusion method.<sup>2</sup> A solution of 3.0 mM CaCl<sub>2</sub>.2H<sub>2</sub>O (Sigma-Aldrich) was

mixed with ~ 0.30 g dm<sup>-3</sup> GNPs, and these reaction mixtures were placed in Petri dishes. Glass slides which had been pre-cleaned with piranha solution were placed at the base of the dishes, which were then transferred to a sealed desiccator containing solid ammonium carbonate (Sigma-Aldrich). Crystallisation was then allowed to proceed for 24 hours, after which time the glass slides were removed from solution, and were washed with water and ethanol, before being allowed to dry in air at 20 °C. Control experiments were also performed using an identical method, but excluding the GNPs.

### 4. Characterisation of diblock copolymers, GNPs and CaCO<sub>3</sub> Crystals

<sup>1</sup>*H NMR Spectroscopy:* All <sup>1</sup>*H* NMR spectra were recorded in  $D_2O$ , CDCl<sub>3</sub>, d<sub>4</sub>-methanol or d<sub>5</sub>pyridine using a 400 MHz Bruker Advance 400 spectrometer. Micellisation of zwitterionic diblock copolymers was studied in  $D_2O$  using either NaOD or DCl to adjust the solution pH. Typically 64 scans were averaged per spectrum.

*TEM Analysis of GNPs:* The gold nanoparticles were characterised using Transmission Electron Microscopy (TEM), Energy-dispersive X-ray Analysis (EDXA) and selected area electron diffraction techniques. Samples were mounted on carbon-coated, formvar-covered Cu TEM grids by placing a drop of the GNP solution on the grid, drawing off excess liquid, and allowing the sample to dry in air at 20°C. TEM and EDXA were carried out using a FEI Tecnai TF20 FEG-TEM / STEM operating at 200 kV which was fitted with an HAADF detector, an Oxford Instruments INCA 350 EDX system/80mm X-Max SDD detector and a Gatan Orius SC600A CCD camera.

*Dynamic Light Scattering:* Hydrodynamic diameters were measured at 25 °C using a Malvern Zetasizer NanoZS Instrument equipped with a 4 mW He-Ne solid-state laser operating at 633 nm. Back-scattered light was detected at  $173^{\circ}$  and the mean particle diameter was calculated from the quadratic fitting of the correlation function over thirty runs of ten seconds duration. All measurements were performed in triplicate on 0.1 w/v % aqueous GNPs dispersions and determined as a function of pH, which was adjusted using NaOH or HCl as required.

*Aqueous Electrophoresis of GNPs:* Zeta potentials were calculated from the electrophoretic mobilities determined using the same Malvern Instruments Zetasizer NanoZS instrument. Measurements were obtained as a function of pH on 0.1 w/v % aqueous GNPs dispersions by gradually adding HCl, starting from an initial pH of around 10. Zeta potentials were averaged over twenty runs at the desired pH, and the variance was typically within the size of the data points shown.

*Optical Microscopy of CaCO<sub>3</sub> Crystals:* The morphologies and colours of the CaCO<sub>3</sub> crystals were studied using a Nikon Eclipse LV 100 polarisation microscope, operating in the reflected light mode.

*SEM Analysis of CaCO<sub>3</sub> Crystals:* The morphologies of the CaCO<sub>3</sub> crystals was determined using Scanning electron microscopy (SEM) by mounting the glass slides supporting the CaCO<sub>3</sub> crystals on SEM stubs using adhesive conducting pads and coating with Pt/Pd. Imaging was performed using a LEO 1530 Gemini FEG-SEM operating at 3 kV using an in-lens detector mode. The cross-sections of particles were also imaged using SEM to investigate particle occlusion. Here, samples were prepared by placing a clean glass slide on top of the glass slide supporting the calcite crystals, and pressing down, resulting in fracture of the crystals.

*TEM Analysis of CaCO<sub>3</sub> Crystals*: The distribution of GNPs throughout the calcite single crystal lattice was investigated using TEM. Cross-sections (lamellae) through the crystals were prepared by sectioning the crystals using Focussed Ion Beam Milling (FIB), where this was carried out with a FEI FIB-201, operating at 30 keV with Ga ions. A lamella of size  $10 \times 5$  micron was mounted on to a bar in the copper FIB lift-out grid, and was held in place during the FIB by depositing platinum over the end of the lamella resting on the grid bar, thereby welding it in place. TEM analysis was then performed as described above.

*Characterisation of Phase of CaCO<sub>3</sub> Particles*: Infra-red spectroscopy and powder XRD were used to characterise the polymorphs present in the CaCO<sub>3</sub> sample, while Raman microscopy enabled analysis of individual particles. IR analysis of powders was performed using a Perkin Elmer AT-IR spectrometer, while Raman was carried out using a Renishaw 2000 Raman microscope operating with a 785 nm diode laser. PXRD was performed using a Bruker D8 Advanced diffractometer with X-ray source emitting Cu K $\alpha_1$  radiation. Samples were gently ground and placed on a piece of silicon wafer, and XRD data were collected between 10° and 60° in intervals of 0.02° and a scan rate of 1°/ minute.

*Characterisation of Amount of GNPs in CaCO<sub>3</sub> Particles:* Atomic Absorption Spectroscopy (AAS) was used to determine the amount of gold within the CaCO<sub>3</sub> crystals, which were dissolved in aqua regia and analysed using a Perkin-Elmer Atomic Absorption Spectrometer AAnalyst 400, operating with an air-acetylene flame. Calibration standards of gold and calcium were obtained from Alfa Aesar (1000  $\mu$ g/ml) and were diluted in 5% HCl prior to use.

## References

- 1. Y. Y. Kim, K. Ganesan, P. Yang, A. N. Kulak, S. Borukhin, S. Pechook, L. Ribeiro, R. Kröger, S. J. Eichhorn, S. P. Armes, B. Pokroy and F. C. Meldrum, *Nature Mater.*, 2011, **10**, 890-896.
- 2. J. Ihli, P. Bots, A. Kulak, L. G. Benning and F. C. Meldrum, *Adv. Func. Mater.*, 2013, 23, 1965-1973.
- 3. C. Gabrielli, R. Jaouhari, S. Joiret and G. Maurin, *Journal of Raman Spectroscopy*, 2000, **31**, 497-501.

Electronic Supplementary Material (ESI) for Chemical Communications This journal is C The Royal Society of Chemistry 2013

# **Supplementary Information**

Figure S1. TEM image of PSPMA<sub>30</sub>-PDPA<sub>47</sub>-stabilised gold nanoparticles.



**Figure S2.** <sup>1</sup>H NMR spectra recorded for PSPMA<sub>30</sub>-PDPA<sub>47</sub> diblock copolymer in 0.10 M NaCl in D<sub>2</sub>O as a function of pH (adjusted using DCl or NaOD). At pH 6.5, the PSPMA and PDPA blocks are both weakly charged and hence can dissolve molecularly in aqueous solution in the presence of NaCl, since this salt screens the electrostatics. Thus all signals due to these two solvated blocks are visible in the <sup>1</sup>H NMR spectrum at pH 6.5. Increasing the solution pH to pH 10 using NaOD led to the disappearance of the PDPA signals at  $\delta$  1.4,  $\delta$  3.8 and  $\delta$  4.4, since the deprotonated PDPA block (pK<sub>a</sub> 6.3) becomes hydrophobic at this pH. On the other hand, addition of DCl to the same PSPMA<sub>30</sub>-PDPA<sub>47</sub> solution led to the disappearance of the PSPMA signals at  $\delta$  2.2-2.6,  $\delta$  4.2 and  $\delta$  5.1.These spectra confirm that this zwitterionic diblock copolymer exhibits so-called 'schizophrenic' behaviour, i.e. it can form PDPA-core micelles in alkaline solution and PSPMA-core micelles in acidic solution. In the context of the gold sol used in this work, the cationic PDPA block behaves as the steric stabiliser at low pH, whereas the anionic PSPMA block acts as the steric stabiliser at high pH (with the latter pH being used for the formation of the CaCO<sub>3</sub> crystals).



**Figure S3**. Optical micrographs of calcite crystals precipitated (a) in the absence of additives and (b) in the presence of PSPMA<sub>30</sub>-PDPA<sub>47</sub>-stabilised gold nanoparticles.

D 0 0 20 µm b 20 µm

**Figure S4.** (a) Powder XRD of CaCO<sub>3</sub> crystals precipitated in the presence of PSPMA<sub>30</sub>-PDPA<sub>47</sub>stabilised gold nanoparticles, showing that only calcite is present. (b) and (c) Raman spectrum of (b) PSPMA<sub>30</sub>-PDPA<sub>47</sub>-stabilised gold nanoparticles and (c) CaCO<sub>3</sub> crystals precipitated in the presence of the same GNPs, showing that they are calcite. The peaks at 1085 cm<sup>-1</sup> ( $\nu_1$ ), 711cm<sup>-1</sup> ( $\nu_4$ ), 281 cm<sup>-1</sup> and 154 cm<sup>-1</sup> (lattice modes) are characteristic of calcite,<sup>3</sup> while all other peaks arise from the copolymerstabilised gold particles.



**Figure S5.** SEM images of fracture surfaces through (a) a calcite crystal containing  $PSPMA_{30}$ -PDPA<sub>47</sub>-stabilised gold nanoparticles and (b) a pure calcite crystal. The fracture surface of the calcite/gold hybrid crystals is significantly roughened, providing strong evidence for particle incorporation.



**Figure S6.** High resolution TEM (HRTEM) micrograph of a  $PSPMA_{30}$ -PDPA<sub>47</sub>-stabilised gold nanoparticle embedded within a single crystal of calcite. The continuous lattice finges in the calcite demonstrate that the GNP does not distrupt its single crystal character.

