

**Label-free detection of C-reactive protein using highly dispersible gold nanoparticles synthesized by reducible biomimetic block copolymers**

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## EXPERIMENTS

### *Materials*

2-Methacryloyloxyethyl phosphorylcholine (MPC) was kindly provided by NOF Co., Ltd. and used without further purification. 4-Cyanopentanoic acid dithiobenzoate was synthesized according to the method reported by McCormick and co-workers.<sup>1</sup> *N*-Methacryloyl-(*L*)-tyrosine methylester (MAT) was synthesized by a previously described method.<sup>2</sup> CRP from human plasma was purchased from Sigma-Aldrich Co. LLC. Other chemicals were purchased from Wako Pure Chemical Industries, Ltd. or Sigma-Aldrich Co. LLC. and used without further purification. Pre-purified water was further purified using a Millipore Milli-Q system that includes ion exchange, distillation, and filtration steps (18.2 M $\Omega$ ).

### *Preparation of block copolymers*

Poly(MPC)-*b*-poly(MAT) (PMPC-*b*-PMAT) was prepared by RAFT polymerization. In the typical synthesis for PMPC as a macro chain-transfer agent (macro-CTA), MPC (10.0 g, 33.9 mmol), 4,4'-azobis(4-cyanopentanoic acid) (0.019 g, 0.068 mmol), and an appropriate amount of 4-cyanopentanoic acid dithiobenzoate (0.158 g, 0.565 mmol) were dissolved in water, and the concentration of MPC was adjusted to 1 M. Then, to remove oxygen, argon gas was passed through the solution for 30 min. The solution was heated to 70 °C. To remove any unreacted monomer, the solution was dialyzed against methanol and water for 2 days each. The solution of the dialysis tube was collected and freeze-dried to obtain PMPC in powder form.

MAT (0.717 g, 2.56 mmol), 4,4'-azobis(4-cyanopentanoic acid), and

0.126 mmol of macro-CTA were dissolved in methanol and the volume of the solution was adjusted to 38.2 mL. Polymerization was performed at 60 °C for 15 h. Block copolymers were purified by dialysis against methanol for 2 days and pure water for three days and then recovered by freeze-drying. The molar fraction of monomer units and number-average molecular weight ( $M_n$ ) of each block copolymer was calculated from  $^1\text{H}$  NMR data. Their molecular weight distribution ( $M_w/M_n$ ) was determined with a JASCO GPC system with a refractive index detector and size-exclusion columns, using the PEG standard made up in distilled water containing 10 mM LiBr. The molar contents of the MAT units in the block copolymers were estimated by  $^1\text{H}$  NMR.

A block copolymer (2.42 g) was dissolved in methanol and the volume of the solution was adjusted to 18.4 mL. After the polymer was completely dissolved in water, a trace of (tris(2-carboxyethyl)phosphine (TCEP) was added to the solution, and then 32.5 mole equivalent of ethanolamine was added. The solution was stirred at ambient temperature for 3 h or until the polymer solution became colorless. The product was obtained after purification by dialysis against methanol for 2 days and water for 3 days and lyophilized. Then, the polymer was characterized by  $^1\text{H}$  NMR, a JASCO UV-Vis spectrometer, and GPC.

Critical micelle concentration (cmc) is one of the most important parameters for micellization. The cmc value of PMPC-*b*-PMAT in water was determined in water at room temperature. In the process of aggregation, the ratios of the fluorescence peak intensities at 373 and 384 nm of pyrene are lower when the concentration is below the cmc value, whereas the ratios of the peak intensities ( $I_{384}/I_{373}$ ) are enhanced with increased PMPC-*b*-PMAT concentration when pyrene is encapsulated in the micelles.<sup>3</sup>

### ***Preparation of PMPC-*b*-PMAT-protected AuNPs***

In a typical experiment, HAuCl<sub>4</sub>/phosphate buffer saline (PBS) suspension (200 μL, 10 mM) was added to the polymer solution (200 μL, 6 mM) under vigorous stirring. NaOH solution (0.5 mL, 1 M) was introduced 2 min later, and the reaction was allowed to proceed under vigorous stirring at 60 °C for 15 h. The PMPC-*b*-PMAT-protected AuNPs were purified by dialysis in deionized water for three days, freeze-dried, and collected as a powder.

### ***Characterization of AuNPs bearing MPC block copolymers***

**Transmission Electron Microscopy (TEM).** TEM measurements were performed on a JEOL model JEM-1400 instrument operated at an accelerating voltage of 100 kV. A sample was prepared by placing a drop of gold nanoparticle solution on a collodion-coated copper grid followed by air-drying.

**Dynamic Light Scattering (DLS) analysis.** A Malvern Zetasizer Nano-ZS operating at a laser wavelength of 633 nm and a fixed detector angle of 173° was used for DLS measurements on highly dilute aqueous gold dispersions.

**Zeta-potential measurement.** Measurements were performed using a Malvern Zetasizer Nano-ZS. Mobility  $\mu$  is converted into a  $\zeta$ -potential using the Smoluchowski equation,  $\zeta = \mu\eta/\epsilon$ , where  $\eta$  and  $\epsilon$  are the viscosity and dielectric constant of the solvent, respectively. The solution pH was adjusted by the addition of HCl or NaOH.

**Thermogravimetric Analysis (TGA).** TGA was performed using a Rigaku Thermo plus TG-8120 instrument. Samples were heated from 25 to 450 °C at 10 °C/min. The surface density of PMPC-*b*-PMAT (chains/nm<sup>2</sup>) can be estimated by TGA as follows:

$$\text{Surface density of block copolymers (chains/nm}^2\text{)} = \frac{\left(\frac{W_{\text{polymer}}}{100 - W_{\text{polymer}}}\right) \rho V_{\text{particle}} N_A}{M_{\text{polymer}} S_{\text{particle}}} \dots\dots(2)$$

Here,  $W_{\text{polymer}}$  is the percent weight loss corresponding to the decomposition of the block copolymer chains,  $\rho$  is the density of bulk gold (19.32 g/cm<sup>3</sup>),  $V_{\text{particle}}$  is the volume of bare gold nanoparticles determined from the TEM image (8.6 nm),  $N_A$  is Avogadro's number,  $M_{\text{polymer}}$  is the molecular weight of a block polymer chain ( $M_n$ ), and  $S_{\text{particle}}$  is the specific surface area of the bare gold nanoparticles calculated from the TEM image.

**UV-Vis absorption spectroscopy.** UV-vis absorption spectroscopic measurements were performed with a JASCO V-530 spectrophotometer with a 1.0 cm path length quartz cell at various temperatures.

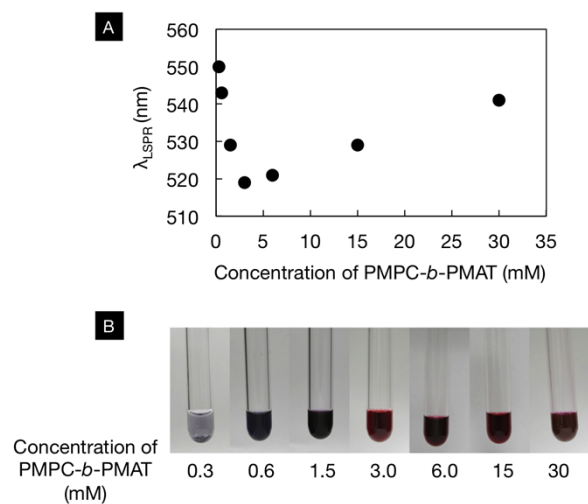
***Detection of C-reactive protein (CRP) with AuNPs bearing MPC block copolymers***

AuNPs were dissolved in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; 0.1 M, pH7.4) and the concentration was adjusted to 20 mg/mL. The AuNPs suspension (20 μL) was mixed with 500 μL of 2 mM CaCl<sub>2</sub> in HEPES (pH7.4), 3-Morpholinopropanesulfonic acid (MOPS, pH 6.5), 2-morpholinoethanesulfonic acid

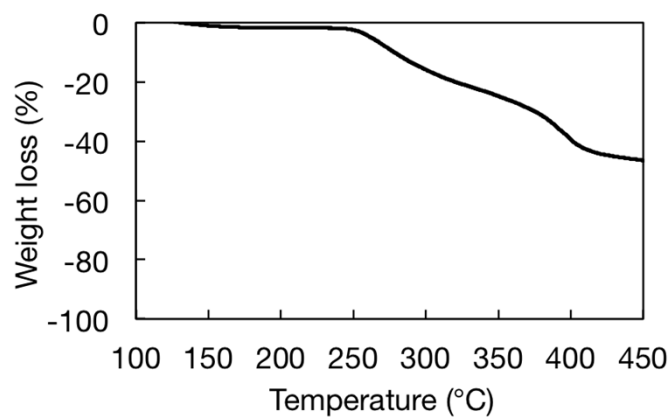
monohydrate (MES, pH 5.5) buffer or only each buffer ( $\text{Ca}^{2+}$  free) in a plastic tube. Appropriate amounts of 2800 nM CRP/HEPES and MES buffer were then added to the tube. The total volume of the test liquids was adjusted to 1.0 mL. After all samples were mixed, the tubes were stored at room temperature for 30 min and optical analyses for AuNPs were performed.

## REFERENCES

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**Figure S1.** (A) Effect of polymer concentration on  $\lambda_{LSPR}$  of AuNPs dispersion; (B) Photographs of AuNPs prepared with PMPC-*b*-PMAT of different concentrations. The AuNPs are suspended in 153 mM NaCl aq.



**Figure S2.** TGA data for PMPC-*b*-PMAT-protected AuNPs.