Stabilization and Functionalization of Polymer Multilayers and Capsules via Thiol–ene Click Chemistry

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SUPPORTING INFOMATION

Experimental

Materials. 3μm–diameter SiO_2 particles were purchased from MicroParticles GmbH as a 5 wt % suspension and were used as received. Poly(methacrylic acid, sodium salt) (PMA), M_w = 15 kDa, was purchased from Polysciences, and poly(vinylpyrrolidone) (PVP), M_w = 55 kDa, was from Sigma-Aldrich. Cysteamine hydrochloride (98%), dithiothreitol (DTT, 99%), *N*-hydroxysuccinimide (NHS, 98%), 2-aminoethyl methacrylate hydrochloride (90%), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide (EDC, 98%), fluorescein isothiocyanate (FITC, 98%), albumin from bovine serum (BSA, 98%), poly(ethylene glycol) monoacrylate (PEG 1, M_w = 556), and fibrinogen (Fg, fraction 1, type I) from human plasma were purchased from Sigma-Aldrich and used as received. Alexa Fluor 488 (AF488) maleimide and hydrazide were obtained from Invitrogen. Succimidyl ester–poly(ethylene glycol)–maleimide (PEG 2, M_w = 5000) was obtained from JenChem. FITC-labeled sheep anti-mouse immunoglobulin G (IgG) was purchased from Silenus Laboratories (Melbourne, Australia). High-purity water with a resistivity greater than 18 MΩ cm was obtained from an in-line Millipore RiOs/Origin

system. The preparation methods for PMA_{THIOL}, fluorescently labeled PMA_{THIOL}, and fluorescently labeled PVP are as described previously.[1]

Protein Labeling

BSA (1.0 g) was dissolved in carbonate buffer (40 mL, 0.1 M, pH 9). To this solution, FITC (2 mg) dissolved in DMSO (2 mL) was added and left to mix gently at room temperature for 8 h. NH_4Cl was added to a final concentration of 50 mM and the solution was left at 4 °C for 4 h. The protein solution was then purified with a G-25 Sephadex™ column (GE Healthcare Life Sciences). A mole ratio of FITC to protein (F:P) of 0.3 was calculated from absorption measurements using an Agilent Technologies 8453 UV-vis spectrophotometer. The protein was freeze-dried and redissolved in PBS (pH 7.2) to a concentration of 2.3 mg mL^{-1}. Fg (50 mg) was labeled in the same manner using 0.7 mL of 1 mg mL^{-1} FITC in DMSO. The conjugated protein was dissolved in PBS and was used without further purification. An F:P ratio of approximately 4 and a protein concentration of 4 mg mL^{-1} was calculated from UV-vis measurements.

Methods. Flow cytometry was performed on a Partec CyFlow Space instrument using an excitation wavelength of 488 and 633 nm. Fluorescence spectroscopy was performed using a Fluorolog Horiba fluorescence spectrophotometer. Particles were imaged on an Olympus IX71 Digital wide field fluorescence microscope equipped with a FITC filter cube. The pH of the buffer solutions was measured with a Mettler-Toledo MP220 pH meter. TEM grids were plasma treated for 10 s prior to use. 5 μL of sample was adsorbed onto a carbon-coated formvar film mounted on 300 mesh copper grids (ProSciTech,

Queensland, Australia) for 5 min. Measurements were taken using an FEI Tecnai TF30 (FEI-Company, Eindhoven, The Netherlands) operated at 200 kV and fitted with a Gatan US1000 2k x 2k CCD Camera (Pleasanton, CA, USA). Scanning electron microscopy measurements were performed on a FEI Quanta 200 SEM equipped with a field emission gun (FEG) and an Everhardt–Thornley detector.

PMA Functionalization. PMA (300 mg, 24 mmol of acid groups), EDC (132 mg, 0.7 mmol), NHS (10 mg, 0.1 mmol), and 2-aminoethyl methacrylate hydrochloride (110 mg, 0.6 mmol) were dissolved in 0.1 M phosphate buffer (3 mL). The reaction was stirred at room temperature for 18 h, followed by purification via column chromatography using a NAP-5 column (GE Healthcare), and recovered by freeze-drying to afford a white powder. The degree of functionalization of PMA was estimated using NMR integrations of the vinyl protons, which was calculated as 13 mol %. ^{1}H NMR (D_2O, 400MHz): δ 0.7-0.9 (br, CH_3), 1.50-1.80 (br, CH_2), 3.40 (m, CH_2), 3.90 (m, CH_2), 5.60 (s, CH_2), 6.0 (s, CH_2).

Assembly of Multilayers. A suspension of the SiO_2 particles (0.25 wt%) was washed with pH 4 buffer via several water centrifugation/redispersion cycles. The resulting suspension was combined with an equal volume of a 2 mg mL^{-1} solution of PVP in 10 mM sodium acetate buffer, pH 4, and adsorption of the PVP was allowed to proceed for 15 min with constant shaking. After this, the particles were washed with fresh pH 4 buffer (three times), redispersed, and combined with a solution of reduced PMA_{THIOL} in 10 mM sodium acetate buffer, pH 4, to a final concentration of PMA_{THIOL} of 1 mg mL^{-1}.

PMA_{THIOL} adsorption was allowed to proceed for 15 min, after which the particles were washed with a solution of fresh pH 4, 10 mM acetate buffer. This procedure describes the assembly of two layers. The same process was repeated with PVP and PMA_{ENE}. An additional five layers (PVP/PMA_{THIOL}/PVP/PME_{ENE}/PVP) were assembled using the above-described procedure.

Stabilization of Capsules Using Click Chemistry. The multilayer-coated particles were dispersed in 10 mM sodium acetate buffer (pH 4) and irradiated with a hand–held UV lamp (256 nm) for 2 h, under constant stirring.

PEGylation of Multilayer-Coated Particles. To a suspension (~0.1 wt %) of the multilayer-coated particles (nine layers) in 10 mM sodium acetate buffer (pH 4), a solution of PEG (PEG 1 or PEG 2) was added to a final concentration of 3 mg mL^{-1}. The suspension was then irradiated with a hand-held UV lamp (366 nm) for 2 h, under constant stirring. Finally, the coated particles were isolated by multiple centrifugation and washing cycles (pH 4).

Preparation of Capsules. Capsules were formed via dissolution of the template silica core by treatment with 2 M HF/8 M NH_4F solution (pH 5) at 20 °C for 5 min, followed by multiple centrifugation (4500 *g* for 5 min)/buffer washing cycles.[2]

Protein Fouling of Multilayer-Coated Particles. Concentrations of the various coated particles described earlier were obtained by counting with a Becton Dickson FACS Calibur flow cytometer using an excitation wavelength of 488 nm. Approximately 3×10^4

coated particles were then added to 100 μL of a FITC-labeled protein (BSA, Fg, IgG)-PBS buffer (pH 7.4) solution, and incubated at room temperature for approximately 14 h. The dispersion was then washed three times with PBS. The relative amounts of protein adsorbed onto the particles were then determined by measuring the fluorescence intensity of the particles by flow cytometry.

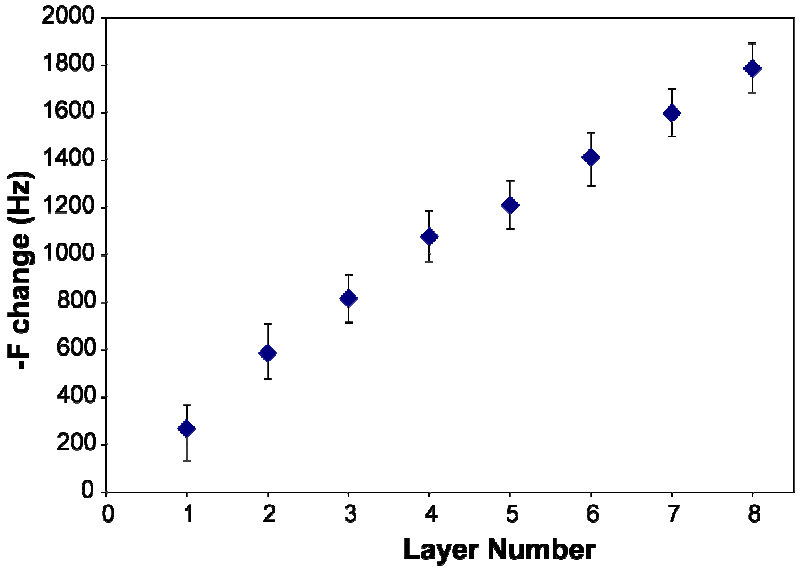

Figure S1. Frequency change of a QCM gold electrode coated with ($PVP/PMA_{THIOL}/PVP/PMA_{ENE}$) multilayers as a function of layer number. The multilayers were deposited from 1 mg mL^{-1} polymer solutions at pH 4 (10 mM sodium acetate) and measurements were taken after each layer was washed three times with pH 4 buffer. PVP was the first layer adsorbed.

1 Zelikin, A. N.; Li, Q.; Caruso, F. *Chem. Mater.* **2008,** *20*, 2655–2661.

2 Wang, Y. J.; Caruso, F. *Chem. Mater.* **2006,** *18*, 4089.