Supporting Information

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Ligand-Functionalized Core/Shell Microgels with Permselective Shells

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Experimental
Materials
N-isopropylacrylamide (NIPAm, Aldrich) was purified by recrystallization from hexane (J. T. Baker) prior to use. N,N′-methylenebis(acrylamide) (BIS), ammonium persulfate (APS), sodium dodecyl sulfate (SDS), acrylic acid (AAc), N,N′-(1,2-Dihydroxyethylene)- bisacrylamide (DHEA) and sodium periodate were purchased from Aldrich and used as received. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), biotin hydrazide, avidin, avidin-horse radish peroxidase conjugate (avidin-HRP) and 2-(4'-hydroxyazobenzene) benzoic acid (HABA) were purchased from Pierce. Water was purified with Barnstead E-Pure system to a resistance of 18 MΩ and then filtered through a 0.2-µm filter to remove particulate matter.

Methods
Microgel Synthesis
A detailed procedure for preparation of microgels by free radical precipitation polymerization is described elsewhere.\[1\] The total monomer concentration in the pregel solution was kept constant at 70 mM, out of which 10 mol-% was BIS and 10 mol-% was AAc, with the remaining 80 mol-% being NIPAm. All the monomers and 11 mg of SDS were dissolved in 25 mL of water and the resulting solution was filtered through a 0.2-µm-membrane filter (Pall Gelman Metricel) to remove particulate matter. The reaction mixture was heated in a 3-neck round bottom flask equipped with a condenser and inlet for nitrogen. The mixture was heated to 70 °C under a gentle stream of nitrogen for 1 hour, after which 8 mg of APS dissolved in 1 mL of water was added to initiate the reaction. The reaction mixture was kept at 70 °C for 4 hours to complete the reaction. After synthesis, the microgel solution was filtered using fine porosity filter paper (Fisher Scientific) to remove aggregated material, if any. The particles were then purified by centrifugation and resuspension in water at room temperature at least seven times to remove any unreacted monomer and oligomer. 1H NMR measurements of pNIPAm particles prepared from 20 mol-% DHEA pregel solutions reveal ~95% cross-linker incorporation efficiency into the particles.

Conjugation of Biotin
Cleaned particles were freeze-dried and resuspended in 25 mL of phosphate buffer of pH 4.7. 84 mg of EDC was added to the resuspended polymer to activate the acid groups in the microgels, the solution was then agitated for 30 min. 46 mg of biotin hydrazide dissolved in 2 mL of DMSO was added to the EDC-treated polymer solution. The reaction was allowed to
proceed overnight at room temperature. These biotinylated particles were cleaned by dialysis with daily changes of water for 4 weeks.

Addition of Shell

The detailed procedure of the shell addition to the core can be found in previous submissions.[2] In brief, 5 mL of the core (biotinylated or non-biotinylated) microgel solution was taken in a three-necked round bottom flask, to which 8 mg of SDS and 44 mL of deionized water was added. The solution was heated under nitrogen to 70 °C. Separately, 271 mg of NIPAm and 120 mg of DHEA were dissolved in 5 mL of deionized water. This solution was degassed at room temperature for 1 h and then was added to the heated core solution. Finally, 10 mg of APS dissolved in 1 mL of water was added to the solution to initiate the reaction. The reaction was allowed to proceed for 4 h at 70 °C and then was cooled and filtered. The polymer solution was then cleaned by centrifugation and resuspension to remove unreacted monomer and oligomers. The above procedure is for 20% cross-linked shells. For 15% cross-linker, we used 288 mg of NIPAm and 90 mg of DHEA. For 2% cross-linker, we used 330 mg of NIPAm and 12 mg of DHEA.

Degradation of Shell

Since the shell has diol cross-linker we use sodium periodate to degrade it. For each degradation 1 mL of core-shell solution was used for quantitative degradation of the shell. Depending upon the amount of degradation required, an equivalent number of moles of sodium periodate solution was added to the polymer solution and the total volume was made up to 1.5 mL. The amount of DHEA to be degraded was calculated from the amount of DHEA used in pregel solution.

Biotin Assay

To determine the amount of avidin binding to the biotin in the core, we use the avidin-HABA assay.[3] The avidin-HABA solution was prepared by dissolving 100 mg of avidin in 10 mL of 0.1 M PBS followed by addition of 6 mL of 10 mM HABA solution. Finally, the total volume was made up to 200 mL by further addition of PBS. For the avidin-HRP-HABA complex, 5 mg of avidin-HRP was dissolved in 5 mL of PBS and to it 130 µL of 10 mM HABA solution was added. Final volume was made up to 10 mL with PBS.

For the assay 250 µL of appropriately degraded polymer solution is used. To it 750 µL of the avidin-HABA or avidin-HRP-HABA solution is added. The mixture is allowed to equilibrate for 4 h before characterization.

UV-VIS characterization

The absorbances of the polymer-avidin-HABA mixture were obtained using a Shimadzu UV 1601 spectrophotometer. For each measurement, the reference cell had the same volume of polymer solution as used in the sample cell but instead of avidin-HABA mixture it had same volume of PBS.

Photon Correlation Spectroscopy

Particle sizes were determined via photon correlation spectroscopy (PCS, Protein Solutions Inc.) equipped with an integrated Peltier temperature control device (± 0.1 °C), as previously reported.[4] The hydrodynamic radii of the particles were calculated from the diffusion coefficient using the Stokes-Einstein equation. The sample was allowed to equilibrate at the proper temperature for 10 minutes before data collection. Scattered light from the fiber-coupled diode laser (798 nm) was collected at 90° with a fiber-coupled avalanche photodiode detector connected to a 248-channel autocorrelator board. The data was analyzed with Protein Solutions’ Dynamics Software Version 5.25.44.
References