Electronic Supplementary Information (ESI)

Facile One-pot Synthesis of Organic Dye-Complexed Microgels for Optical Detection of Glucose at Physiological pH

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SI 1. Experimental Procedures

1.1 Chemicals and materials

D(+)-glucose and Bordeaux R dye sample were purchased from ACROS, 2-vinyl-4,6-diamino-1,3,5-triazine (VDT) was obtained from TCI America, and all other chemicals were purchased from Aldrich. *N*-isopropyl acrylamide (NIPAM) was recrystallized with a hexane-acetone mixture (v/v, 1:1) and dried in vacuum. 4-vinylphenylboronic acid (VPBA), *N*,*N*'-methylenebisacrylamide (MBAAm), sodium dodecyl sulfate (SDS), 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH), sodium *L*-lactate, *D*(+)-glucose, human serum albumin (HSA), and Bordeaux R dye sample were used as received without further purification. The water used in all experiments was deionization water.

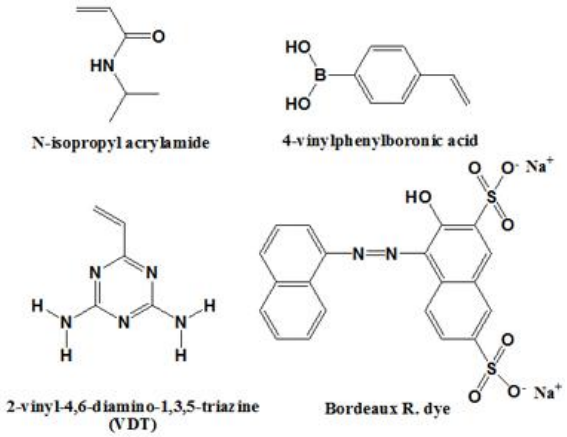

Fig. S1. Chemical structures of the co-monomers and the Bordeaux R dye molecule.

1.2 One-pot synthesis of the poly(NIPAM-VPBA-VDT)-dye composited microgels

The strategy for fabrication of dye composited microgels is *via* free radical precipitation polymerization reaction. Firstly, Bordeaux R dye molecules and VDT were mixed in 120 mL of

water, and stirred for 30 min. VDT with amino functional groups can complex with sulfonic acid groups from Bordeaux R dye molecules, so the solubility of the VDT dramatically increased in an aqueous solution. Then, NIPAM, VPBA, and dye-complexed VDT can be copolymerized in one pot by using AAPH as an initiator. The feeding composition is shown in Table 1. Typically, a mixture of NIPAM, Bordeaux R dye-complexed VDT, VPBA (0.115 g), MBAAm (0.018 g), SDS (0.020 g) was put in a 250 mL three-neck round-bottom flask equipped with a stirrer, a nitrogen gas inlet, and a condenser. The mixture was heated to 70^{o}C under a N_2 purge. After 30 min, 2 mL of 0.089 M AAPH was added to initiate the polymerization. The reaction was allowed to proceed for 4 h. The obtained poly(NIPAM-VPBA-VDT)-dye composite microgels were purified by centrifugation (Thermo Electron Co. SORVALL RC-6 PLUS superspeed centrifuge, 20,000 rpm, 35^{o}C, 30 min), decantation, and then washed with water. The resultant dye-composited microgels were further purified by dialysis for 2 weeks (Spectra/Por molecularporous membrane tubing, cutoff 12,000-14,000) against frequently changed water at room temperature (~ 22^{o}C) until the dialyzed water became colorless. The dye composited microgels with different feeding amount of VDT are coded as PTB-1 (1 mol%), PTB-5 (5 mol%), and PTB-10 (10 mol%), respectively.

Table S1. Feeding composition for synthesis of poly(NIPAM-VPBA-VDT)-dye composite microgels

Sample	NIPAM (g)	VPBA (g)	VDT (g)	Bordeaux (g)	MBAAm (g)	SDS (g)
PTB-1	0.708	0.115	0.011	0.033	0.018	0.020
PTB-5	0.719	0.115	0.053	0.167	0.018	0.020
PTB-10	0.675	0.115	0.106	0.334	0.018	0.020

2. Characterization

The transmission electron microscopy (TEM) images were taken on a FEI TECNAI transmission electron microscope at an accelerating voltage of 120 kV. Approximately 30 μL of the dye composite microgel dispersion was air-dried on a carbon-coated copper grid for the TEM measurements.

Dynamic light scattering (DLS) was performed on a standard laser light scattering spectrometer (BI-200SM) equipped with a BI-9000 AT digital time correlator (Brookhaven Instruments, Inc.). A He-Ne laser (35 mW, 633 nm) was used as the light source. All dye composite microgels were passed through Millipore Millex-HV filters with a pore size of 0.80 μm to remove dust before the measurements. All measurements were made at ~37^{o}C. In DLS, the Laplace inversion of each measured intensity-intensity time correlated function can result in a characteristic line width distribution $G(\Gamma)$. For a purely diffusive relaxation, Γ is related to the

translational diffusion coefficient D by $(\Gamma/q^2)_{c\to 0,q\to 0}=D$, where $q=(4\pi n/\lambda)\sin(\theta/2)$, with n, λ, and θ being the solvent refractive index, the wavelength of the incident light in vacuo, and the scattering angle, respectively. $G(\Gamma)$ can be further converted to a hydrodynamic radius (R_h) distribution by using the Stokes-Einstein equation, $R_h=(K_BT/6\pi\eta)D^{-1}$, where T, K_B, and η are the absolute temperature, the Boltzmann constant, and the solvent viscosity, respectively.

The UV-vis absorption spectra and absorbance values of the dye-composited microgel dispersions were obtained on a Thermo Electron Co. Helios β UV-vis Spectroscopy. The PL spectra of the composite microgel dispersions at various glucose concentrations were obtained on a JOBIN YVON Co. FluoroMax-3 spectrofluorometer equipped with a Hamamatsu R928P photomultiplier tube and a calibrated photodiode for excitation reference correction from 200 to 800 nm, with an integration time of 1 s.

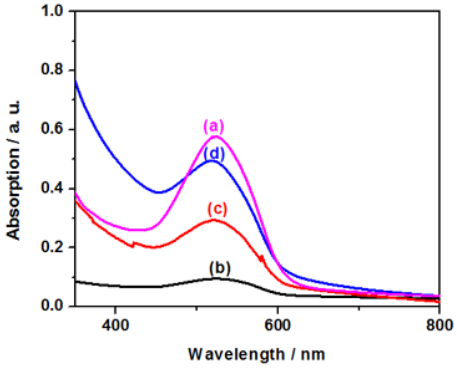

Fig. S2. UV-Vis absorption curves of (a) the Bordeaux R dye solution in 5 mM PBS buffer of pH = 7.47 and (b-d) the poly(NIPAM-VPBA-VDT)-dye composite microgels dispersed in 5 mM PBS buffer of pH = 7.47: PTB-1 (b), PTB-5 (c), and PTB-10 (d).

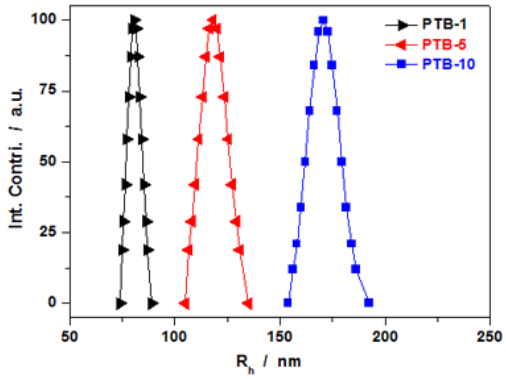

Fig. S3. The size distribution in terms of R_h of the poly(NIPAM-VPBA-VDT)-dye composite microgel particles with a very narrow size distribution: PTB-1 (-▶-), PTB-5 (-◀-), and PTB-10 (-■-) in the presence of 13 mM glucose, measured at 37°C, pH = 7.47, and a scattering angle θ=45°.

3. Stability

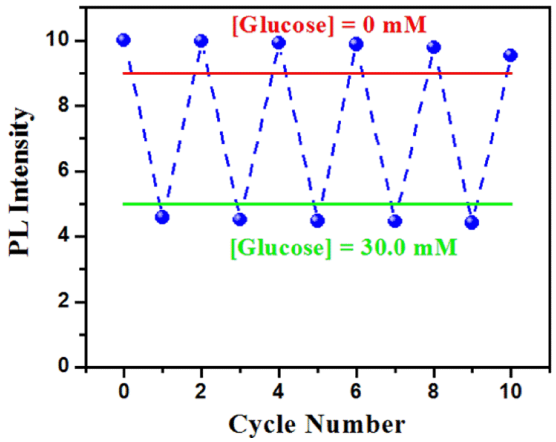

Fig. S4. PL quenching and recovery cycles upon the repeated addition (30.0 mM) and dialysis removal of glucose (0 mM) in the dispersion medium of poly(NIPAM-VPBA-VDT)-dye composite microgel (PTB-10) at pH = 7.47.

4. Interferences

The glucose sensing of the microgels is based on the reversible binding of cis-diols of glucose to the phenyl boronic acids (PBA) on the microgel network chains, thus other cis-diol metabolites presented in blood can potentially interfere the glucose detection of the PBA-based microgels. The typical concentration of pyruvate, galactose and fructose in blood is < 0.1mM,[S1] thus it is unlikely that these compounds will interfere significantly with the glucose sensing of our microgels. The major potential interferents for PBA-based microgel sensor are lactate and human serum albumin (HSA) because of their relatively high concentrations in blood. Considering the typical lactate concentration of 0.36-0.75 mM in a resting healthy adult[S2,S3] and typical HSA concentration of 44 g/L in serum,[S3] we have studied the impact of 1 mM lactate and 44 g/L HSA on the glucose sensing response of the prepared microgels (**Fig. S5**). The results show that our dye-composited microgel glucose sensors should be free from significant interferences of lactate, as there was only about 1 – 5% decrease in glucose-induced PL quenching in the presence of 1.0 mM lactate at glucose concentrations below 25 mM. On the other hand, the presence of 44 g/L HSA increases

the glucose-induced PL quenching, resulting in a plausibly slight increase in the glucose responsive sensitivity. At low glucose concentrations (⩽15 mM), the HSA-induced increase in the glucose-responsive PL quenching is not significant (< 6%). At higher glucose concentrations, however, the glucose-responsive PL quenching does not reach a plateau as it does in the absence of HSA, which can be attributed to the competitive binding of HSA to boronic acids. As the HSA is a relatively large molecule compared to glucose, the binding of HSA to the PBA sites of microgels can cause a larger swelling degree, thus lead to a larger PL quenching. Based on our results, we expect that the sensitivity change of the microgels to glucose will be less than 5% in real matrices at glucose concentrations below 15 mM. At higher glucose concentrations (>15 mM), the sensitivity change of the microgels to glucose should be less than 10% in real matrices

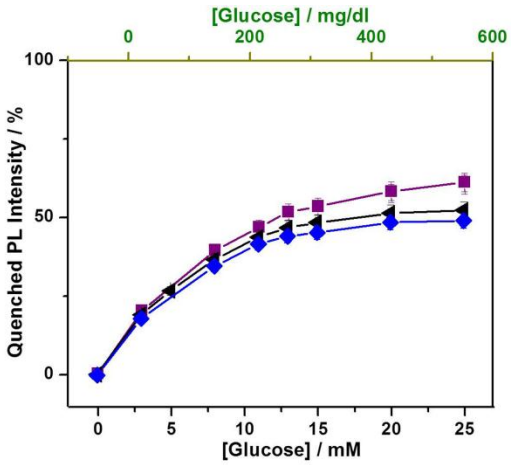

Fig. S5. Glucose response of the p(NIPAM-VPBA-VDT)-dye composite microgels (PTB-10) in the presence of *L*-lactate (-◀-: 0 mM; -◆-: 1 mM) and HSA (-◀-: 0 mM; -■-: 44 g/L) in the PBS solutions at pH = 7.47.

References:

S1. T. D. James, K. Sandanayake, S. Shinkai, *Angew. Chem. Int. Ed. Engl.* 1994, **33**, 2207.

S2. M. M. Muscatello, L. E. Stunja, S. A. Asher, *Anal. Chem.* 2009, **81**, 4978.

S3. C. A. Burtis, E. R Ashwood (Eds), *Tietz Textbook of Clinical Chemistry* 1999, 3rd ed. W. B. Saunders, Philadelphia, PA.