

## Facile fabrication of dextran-based fluorescent nanogels as potential glucose sensors

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### Supporting Information

## Experimental Section

### Materials and apparatus

Dextran (with  $M_w = 40,000$  or  $10,000$  Da), ceric ammonium nitrate (CAN), and 3-aminophenylboronic acid monohydrate (APBA) were purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd. *N,N'*-Methylene bisacrylamide (MBA, Fluka) was recrystallized from methanol. Ceric ammonium nitrate (CAN) was recrystallized from a 3.7 M nitric acid solution containing 11.4 M of ammonium nitrate. Acryloyl chloride was purchased from Aladdin Reagent Co., Ltd. and used as received. Alpha-D-glucose and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich. Dulbecco's modified eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Hyclone, while penicillin and streptomycin were purchased from Gibco. 3HF-AM was a gift kindly provided by Professor Chao-Tsen Chen and doctorate student Chun-Yen Chen. All other chemical reagents were purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd. Dialysis membrane bags with a cut-off molecular weight of 14,000 Da were purchased from Shanghai Green Bird Co., Ltd..

Hydrodynamic diameter and Zeta potential measurements were performed using a Zetasizer Nano ZS90 instrument (Malvern, U.K.) at a concentration of 1 mg/mL and at room temperature. TEM images were recorded using a JEM-2100 (JEOL, Japan) instrument with an acceleration voltage of 200 kV. Infrared spectra (FT-IR) were measured using a Spectrum 100 (Perkin Elmer, Inc., USA) infrared spectrometer. Proton nuclear magnetic resonance ( $^1\text{H}$  NMR) spectra were recorded using an Avance III 400 MHz spectrometer (Bruker, Switzerland). TGA measurements were recorded using a TG 209 F3 Tarsus (Netzsch, Germany) system at a heating rate of 10 °C/min under a nitrogen atmosphere. UV-visible absorption spectra were recorded using a UV-2550 UV-vis spectrophotometer (Shimadzu, Japan). Fluorescence spectra were measured using an RF-5301PC spectrofluorophotometer (Shimadzu, Japan). Optical density (OD) values were measured with a Varioskan Flash Microplate Reader (Thermo Scientific, U.S.A.).

### Synthesis of the 3-acrylamidophenylboronic acid (AAPBA) monomer

The synthesis of the AAPBA monomer was performed according to procedures described in the literature.<sup>1-3</sup> 3-Aminophenylboronic acid monohydrate (5.0 g, 36.5 mmol) was dissolved in 73 mL of 2 M aqueous sodium hydroxide solution, which was then efficiently cooled with ice water. Acryloyl chloride (5.9 mL, 73 mmol) was cooled in an ice bath and added dropwise into the above solution over a period of 15 min under intense stirring, and the reaction was allowed to proceed for 30 min in ice water and subsequently for another 3 h at room temperature. The reaction mixture was slowly adjusted to pH = 1 using dilute hydrochloric acid (0.1 M). The beige precipitate was filtered using a Buchner funnel, washed 5 times with 25 mL of cold water (5 \* 25 mL), and dried in the air overnight, thus yielding a fine powder. This powder was then dissolved in a 20% (v/v) aqueous ethanol (50 mL) solution and gravity filtered using filter paper. AAPBA monomer was crystallized from the filtrate, filtered and dried in a desiccator. The yield of the

AAPBA monomer was 30%, and the purity of the AAPBA was characterized by  $^1\text{H}$  NMR using  $\text{DMSO-}d_6$  as the solvent.

### **Fabrication of the DABA NGs**

5 DABA NGs were fabricated by following a previously reported procedure,<sup>4</sup> with minor modifications. In particular, 0.1 g of dextran was fully dissolved in 10 mL of water and kept under  $\text{N}_2$  protection and gently stirred for 30 min at 30 °C. At this point the designated initiator CAN (15.5 mg or 46.6 mg, dissolved in 0.25 mL of 0.1 N  $\text{HNO}_3$ ) was added, and 5 min later the monomer AAPBA (28.4 mg or 37.9 mg, dissolved in 0.5 mL of DMSO) was added. The  
10 crosslinker MBA (0 - 3.0 mg dissolved in 0.5 mL of  $\text{H}_2\text{O}$ ) was added into the system 30 min after the addition of the monomer. The entire process was allowed to proceed for 4 hours and then the solution was adjusted to  $\text{pH} = 7$  with 1 N NaOH and dialyzed first against an alkaline aqueous solution ( $\text{pH} = 10$ , adjusted with 1 N NaOH) for 2 days and then against deionized water for another day.

15 To investigate the influence of the reagents on the resultant nanogels, several nanogels were synthesized under different reagent ratios.

### **pH-related glucose responsiveness of DABA NGs**

To evaluate their stimuli-responsiveness to changes in the pH and the presence of glucose, the  
20 DABA NGs were dispersed in water, which was adjusted to various pH values by either 0.1 N NaOH or 0.1 N HCl. Subsequently, glucose was added to provide final glucose concentrations of 0, 1, 2, 3 and 5 mg/mL. The hydrodynamic diameters ( $\langle D_h \rangle$ ), polydispersity indices (PDIs) and Zeta potentials of the nanogels were recorded at room temperature using a Zetasizer Nano ZS90 system. All DLS and Zeta measurements were carried out after co-vibrating NG samples  
25 and glucose aqueous solutions for at least 30 minutes to ensure that they reached the dynamic equilibrium.

### **Synthesis of the FDABA NG**

The fluorescent nanogel was denoted as FDABA NG. In order to fabricate FDABA NG, 0.1 g  
30 of dextran was initially dissolved in 10 mL water and kept under  $\text{N}_2$  protection and gently stirred for 30 min at 30 °C. Subsequently, 15.5 mg of CAN (dissolved in 0.25 mL of 0.1 N  $\text{HNO}_3$ ) was added, and 5 min later 37.9 mg of AAPBA (dissolved in 0.5 mL of DMSO) was added. This was followed 30 min later by the addition of 4.5 mg of 3HF-AM (dissolved in 5 mL of DMSO) and subsequently by the addition of 3 mg of the crosslinker MBA. Thereafter, the whole reaction  
35 was allowed to proceed for 4 hours, and then the solution was adjusted to a neutral pH with 1 N NaOH and dialyzed first against NaOH aqueous solution ( $\text{pH} = 10$ ) for 48 h and then against pure water for another 24 h.

### **pH-related glucose responsiveness of FDABA NG**

The responsiveness of the FDABA NG to pH and glucose was tested by dispersing the FDABA NG into aqueous solutions that had been adjusted to various pH values with either 0.1 N NaOH or 0.1 N HCl. Subsequently, glucose was added to provide final glucose concentrations of 0, 1, 2, 3 and 5 mg/mL. The hydrodynamic diameters ( $\langle D_h \rangle$ ), polydispersity indices (PDIs) and Zeta potentials of the nanogels were recorded at room temperature using a Zetasizer Nano ZS90 system. Fluorescence spectra were also recorded using a RF-5301PC spectrofluorophotometer (Shimadzu, Japan) and photos of the fluorescence emission from the samples were taken with a digital camera. All DLS and Zeta measurements were carried out after co-vibrating NG samples and glucose aqueous solutions for at least 30 minutes to ensure that they reached the dynamic equilibrium.

### Cell viability tests

Cytotoxicity tests of the DABA NG3 and FDABA NG were carried out on HeLa cells and mesenchymal stem cells (MSC cells) with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assays. Both cells were purchased from the Shanghai Institute of Cell Biology, the Chinese Academy of Sciences (Shanghai, China). HeLa cells were plated at a density of 6000 cells/well while MSC cells were plated at a density of 8000 cells/well in 96-well culture plates and incubated for 24 h. The supernatant was then removed and 0.15 mL solutions of the DABA NG3 and the FDABA NG in DMEM culture media (supplemented with 10% FBS, 1% penicillin and streptomycin) at various concentrations (0, 25, 50, 100, 200, 400  $\mu\text{g/mL}$ ) were added into each well. At least 5 parallel experiments were performed for each sample. The co-incubation process was performed in an incubator (5%  $\text{CO}_2$ , 37  $^\circ\text{C}$  and saturation humidity) for 24 h. The supernatant was subsequently removed and cells were further washed once with 150  $\mu\text{L}$  of PBS buffer. MTT (0.15 mL of culture medium containing 0.5 mg/mL of MTT) was then added into each well and incubated for another 4 h at 37  $^\circ\text{C}$ . The supernatant was removed and 0.15 mL of DMSO was added into each well to dissolve the formazan crystals that were formed by the living cells. Optical density (OD) values were measured at 570 nm with a microplate reader. The relative cell viability rate was calculated according to Equation S1:

$$\text{Cell Viability (\%)} = \frac{\text{OD}_{\text{experiment}}}{\text{OD}_{\text{control}}} * 100\% \quad (\text{Equation S1})$$

## Results and Discussion

### Characterization of the AAPBA monomer

The successful synthesis of the AAPBA monomer was confirmed by  $^1\text{H}$  NMR spectroscopy (Fig. S1). This monomer exhibited signals at 10.06 (1H, NH), 8.03 (2H,  $\text{B}(\text{OH})_2$ ), 7–8 (1H each, ArH), 6.42 (1H, vinyl CH), 6.21 and 5.69 (1H each, vinyl  $\text{CH}_2$ ) ppm, which was consistent with previous reports.<sup>1-3</sup> The purity of the product was determined to be > 99%, as calculated by  $^1\text{H}$  NMR integration, and the yield was determined to be ~30%.

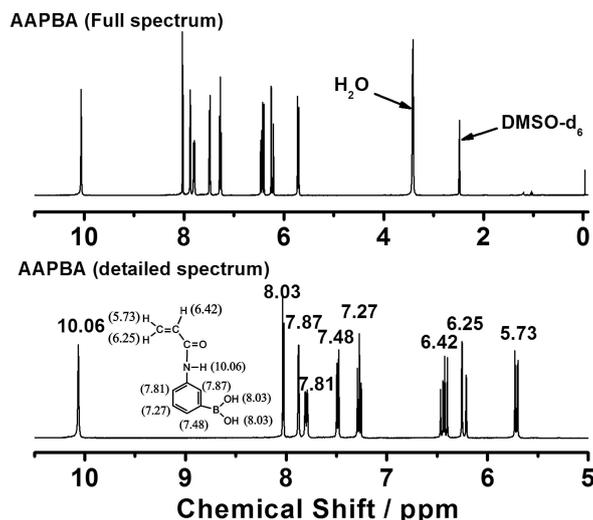
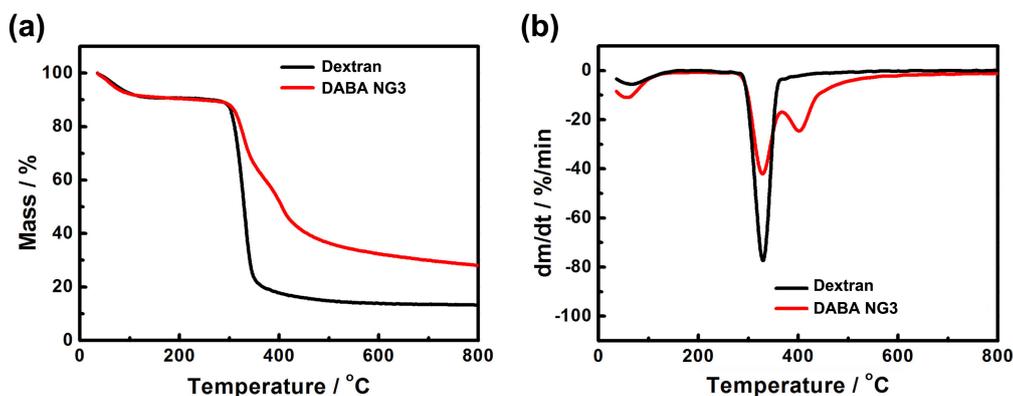


Fig. S1.  $^1\text{H}$  NMR spectrum of the AAPBA monomer.

### Fabrication and characterization of the DABA NGs

The polymerization mechanism is illustrated in Fig. 1 in the main text. Ceric ammonium nitrate (CAN) is a widely used initiator for inducing free radical on polysaccharide backbones, such as dextran, cellulose, chitosan, and their derivatives. In these cases the free radical is typically formed between the 2- and 3- carbons of the carbohydrate ring,<sup>5, 6</sup> and the AAPBA monomer undergoes polymerization at the activated site. Homopolymerization was avoided by keeping CAN at a low concentration.<sup>7</sup>

To evaluate the effect of reactants on the formation of the DABA NGs, we fabricated a series of nanogels with various feed ratios within the targeted size range of 100-300 nm, and the results of this test are listed in Table S1. As we can see, with less monomer input, the size became smaller because of the limited availability of monomers at each polymerization site. Meanwhile, with higher CAN input, the diameters became smaller as more CAN could initiate more free radicals, which thus scattered the monomers at each free radical polymerization site. When no crosslinking agent was used, the diameters of the resultant nanogels were somewhat larger than those of their cross-linked counterparts, which was attributed to the bonding effect of the crosslinker. When the molecular weight of dextran was changed from 10,000 to 40,000 Da, smaller nanogels were formed. This difference arose because longer dextran chains might offer greater steric hindrance and thus keep the reaction within a more confined environment. Similar confinement-regulated behaviour was observed during the fabrication of nanoparticles based on a cationic polysaccharide-chitosan matrix.<sup>8</sup> When all of these results are taken together, it is apparent that DABA NGs with diverse hydrodynamic diameters ranging from 100 to 300 nm and Zeta potentials ranging from -7 to -20 mV were successfully fabricated by controlling the reagent ratios during their synthesis. The DABA NG3 listed in Table S1 were used for further characterization and glucose responsiveness experiments due to their relatively small size and narrow size distribution.



**Figure S2.** Characterization of the DABA NGs: TGA (a) and Differential TG (DTG) (b) curves of dextran and DABA NG3.

**Table S1.** Properties of DABA NGs synthesized using various feed ratios.

Sample No.	$M_w(\text{Dex}^a)$ [Da]	$n(\text{Glu}^b):$ $n(\text{CAN})$	$n(\text{Glu}^b):$ $n(\text{AAPBA})$	$n(\text{AAPBA}):$ $n(\text{MBA})$	$\langle D_h \rangle$ [nm]	PDI	Zeta Potential [mV]
DABA NG1	40,000	21:1	4:1	10:1	$153 \pm 2$	0.144	$-18.9 \pm 0.7$
DABA NG2	40,000	21:1	3:1	10:1	$198 \pm 4$	0.144	$-20.8 \pm 1.8$
DABA NG3	40,000	7:1	3:1	10:1	$119 \pm 1$	0.086	$-7.6 \pm 0.4$
DABA NG4	40,000	7:1	3:1	No MBA	$142 \pm 2$	0.099	$-11.2 \pm 0.9$
DABA NG5	10,000	7:1	3:1	10:1	$299 \pm 11$	0.127	$-7.7 \pm 0.2$

<sup>a</sup>: dextran, <sup>b</sup>: glucose unit.

### Characterization of the FDABA NG

We monitored the pH-related glucose responsiveness of the FDABA NG via DLS, and the results of this experiment are listed in Table S2. The diameters of the FDABA NG became larger in response to increases in the pH and in response to glucose addition.

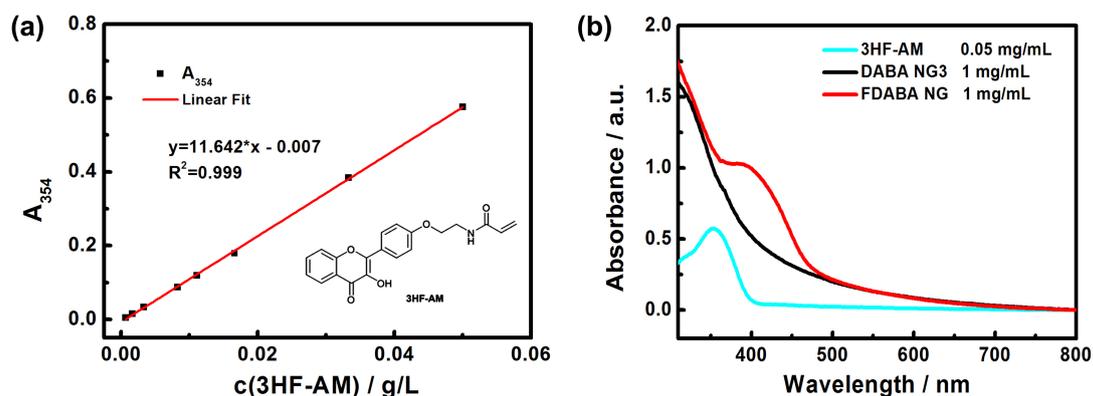
**Table S2.** Size and PDI of FDABA NG at various pH values and glucose concentrations.

Sample conditions	$\langle D_h \rangle$ / [nm]	PDI
pH = 7, c(Glu) <sup>a</sup> = 0 mg/mL	$239 \pm 1$	0.163
pH = 10, c(Glu) = 0 mg/mL	$246 \pm 1$	0.175
pH = 10, c(Glu) = 1 mg/mL	$252 \pm 11$	0.175
pH = 10, c(Glu) = 3 mg/mL	$252 \pm 6$	0.160
pH = 10, c(Glu) = 5 mg/mL	$257 \pm 1$	0.159

<sup>a</sup> c(Glu) denotes the concentration of the glucose

## UV-Vis spectra of FDABA NG

The UV-Vis spectra of the DABA NG3, FDABA NG and the 3HF-AM monomer were measured using a UV-2550 Shimadzu spectrophotometer at concentrations of 1, 1, and 0.05 mg/mL, respectively. A new absorption signal peak appeared at ~ 400 nm in the spectra of the FDABA NG, which indicated that the 3HF-AM probe had been successfully incorporated into the particles. The incorporated 3HF-AM probe exhibited an absorbance signal at 406 nm, while the monomer exhibited an absorbance at 354 nm. This shift in wavelength may be due to the polymerization of 3HF-AM and the shielding effect of the nanogels. The 3HF-AM content in the FDABA NG was determined to be 4.2% through UV-Vis spectroscopy measurements using a calibration curve that had been prepared based on the 3HF-AM monomer (Fig. S3a).



**Fig. S3.** UV-Vis absorption calibration curve for the 3HF-AM monomer (a). This calibration was plotted as the absorbance at 354 nm versus the concentration of the 3HF-AM (the chemical structure of this monomer is also shown in the inset). Also shown are UV-Vis spectra of DABA NG3, FDABA NG and 3HF-AM monomers at concentrations of 1, 1, and 0.05 mg/mL, respectively (b).

## Biocompatibility of DABA NG3 and FDABA NG

A problem encountered with PBA-containing materials is that they may exhibit poor biocompatibility.<sup>9</sup> Various biocompatible polymers<sup>10, 11</sup> have been introduced to address this issue. Dextran is a natural carbohydrate-based polymer with good biocompatibility and hydrophilicity, and it was selected for this study as a means to reduce the cytotoxicity of the PBA-containing polymers and stabilize the formed nanogels in aqueous environments. The cytotoxicities of DABA NG3 and FDABA NG toward both HeLa cells (human cervical cancer cell line) and mesenchymal stem cells (MSC cells) were measured, and the results of this experiment are listed in Fig. S4. The cell viability rates of both cells that were treated with DABA NG3 were above 80% with respect to the untreated control cells, even at DABA NG3 concentrations as high as 400  $\mu$ g/mL. The cytotoxicities of the FDABA NG were slightly greater than those of the DABA NG3 at higher concentrations, indicating that 3HF-AM may have cast some unfavourable effect. In general, however, the results were still acceptable.

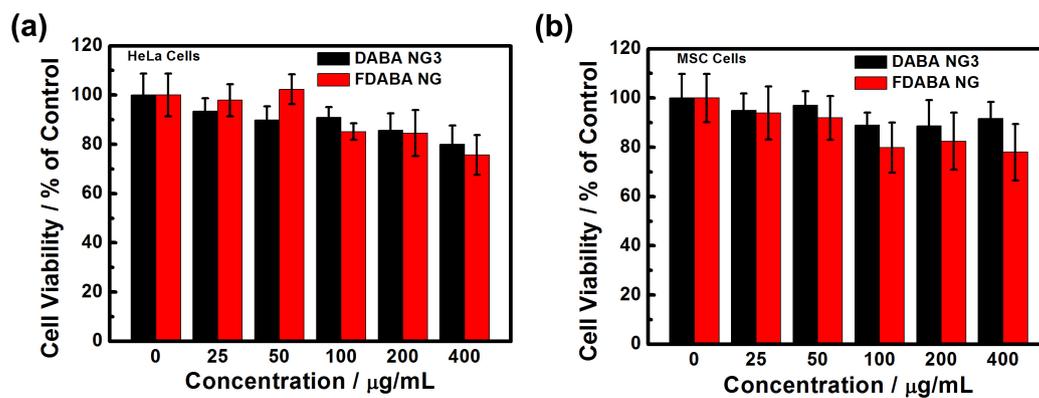


Fig. S4. Cell viability of DABA NG3 and FDABA NG on HeLa (a) and MSC cells (b).

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