Electronic Supplementary Information (ESI)

A fluorescent double-network-structured hybrid nanogel as embeddable nanoglucometer for intracellular glucometry†

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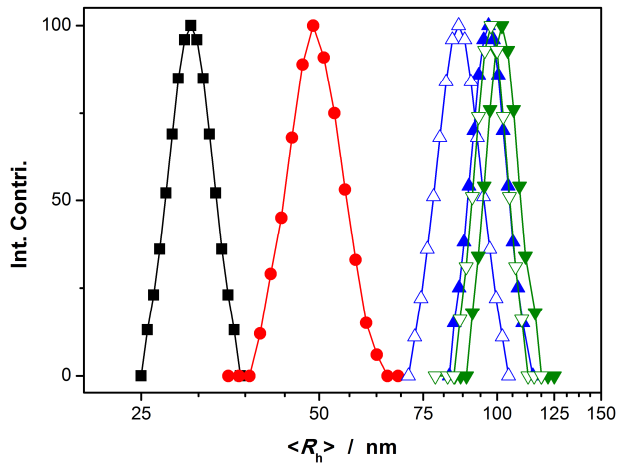
SI-1. Synthesis and DLS sizing of [poly(NIPAM–*co*–FPBA)]$_{core}$–[ZnO@poly(AAm)]$_{shell}$ nanogels

The core-shell structured [poly(NIPAM–*co*–FPBA)]$_{core}$–[ZnO@poly(AAm)]$_{shell}$ nanogels were prepared by a two-step procedures. The poly(NIPAM–*co*–FPBA) template nanogels were preared first following the method described in the maintext. Those poly(NIPAM–*co*–FPBA) template nanogels were used as core particles for the synthesis of the core-shell structured nanogels. The poly(NIPAM–*co*–FPBA) template nanogel dispersion (200 mL) was put into a reactor and heated up to 80 ^{o}C under nitrogen environment for 30 min. Then the shell solution, containing Zn(MAA)$_2$ (0.100 g), AAm (0.040 g), and MBAAm (0.001 g), was dripped into the reactor. It took about 30 min to finish this process. And then AAPH (1 mL, 0.103 M) was added. After refluxing for 2 min, NaOH (0.25 mL, 10 M) aqueous solution was added into the reaction system and continuously refluxed for another 1 h. After cooling to room temperature, the product was purified by centrifugation (20000 rpm, 30 min, 35 ^{o}C), redispersion in water, and 3 days' dialysis against water.

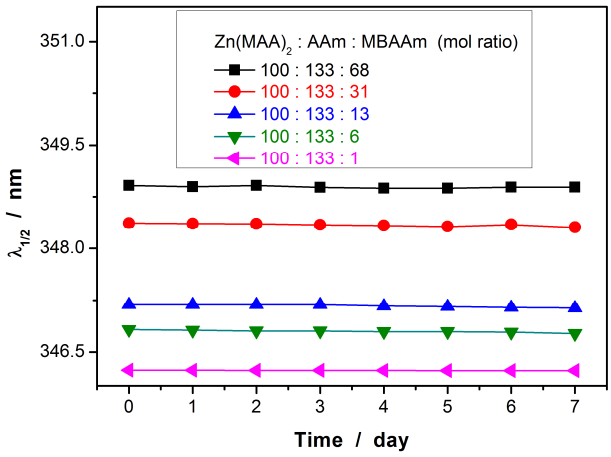


DLS was used to measured the <R_h> of the [poly(NIPAM–*co*–FPBA)]$_{core}$–[ZnO@poly(AAm)]$_{shell}$ nanogels in the presence of glucose (▲, Δ: 0 mg/dL; ▼, ∇ : 540 mg/dL), and shown above. The re-1

sults of the poly(NIPAM–*co*–FPBA) template nanogels (■: 0 mg/dL) and the double-network-structured FNG (●: 0 mg/dL) were also presented for comparison. Closed and open symbols denote the size distribution before and after thirty cycles of additing/removing glucose, respectively.

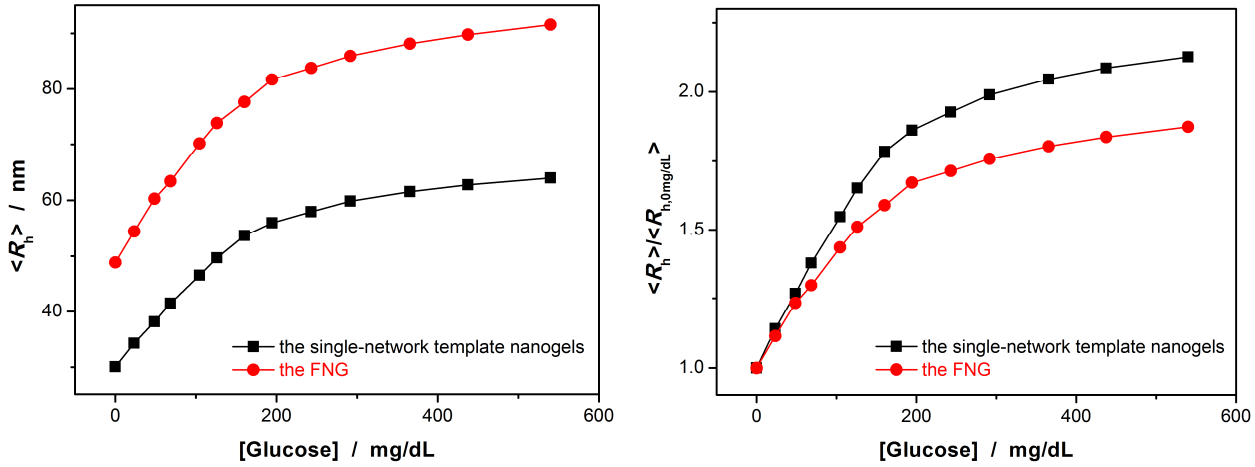
It is possible that the binding of glucose increases the degree of ionization on poly(NIPAM–*co*–FPBA) nanogel core and builds up a Donnan potential for the core to push ZnO@poly(AAm) gel shell to swell. However, because the responsive template gel core is well confined inside the non-responsive SN gel shell, the swelling degree of the core-shell nanogels is quit limited with the $\langle R_{h,540mg/dL}\rangle/\langle R_{h,0mg/dL}\rangle$ being only 1.05. Furthermore, a much small decrease in $\langle R_h\rangle$ (ca. 89% of the original value) and slight broadening in the size distribution were observed on the core-shell $[\text{poly(NIPAM–}co\text{–FPBA)}]_{core}$–$[\text{ZnO@poly(AAm)}]_{shell}$ nanogels.

SI-2. Time-dependent UV-vis absorption showing stability of the FNG



All obtained FNG show good stability. No sediment was observed even after 3 months' storage at room temperature. More importantly, only a marginal change can be detected in the typical $\lambda_{1/2}$ of ZnO QDs, the wavelength at which the absorption is 50% of that at the excitonic peak (or shoulder; see SI3 below), implying that the size of the immobilized ZnO QDs remained unchanged during storage.

SI-3. Comparison of the phase behaviour of the template nanogels and the FNG



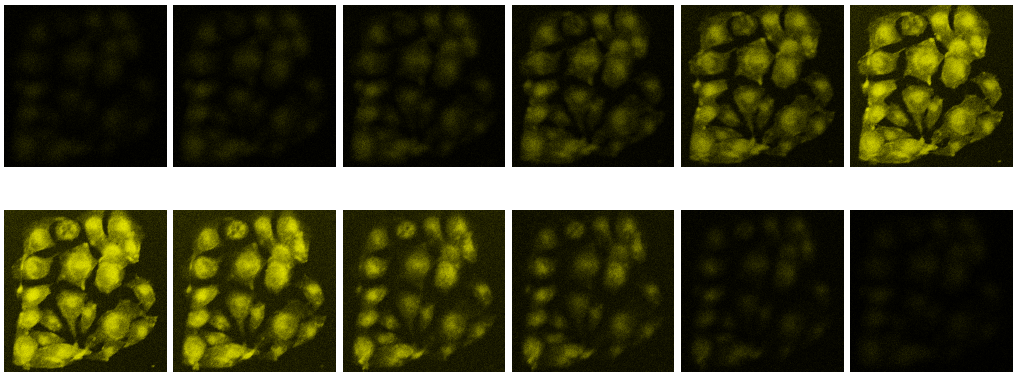
In the glucose concentration range of 0–540 mg/dL, the FNG have a larger average hydrodynamic radius $<R_h>$ than poly(NIPAM–*co*–FPBA) template nanogels (left figure). At a glucose concentration in the range studied, the $<R_h>/<R_{h,0mg/dL}>$ value of the FNG is generally smaller than that of the template nanogels (right figure), which can be attribute to the contribution of the second network to the substantial improvment in mechanical strength.

SI-4. Interference tests on glucose level reading by using the FNG.

Constituents	Concentration	Relative error [a]	Constituents	Concentration	Relative error [a]
human serum albumin	44.0 g/L	+3.6%	Co^{2+}	5.0×10^{-3} mM	–0.8%
L-lactate	20.0 mM	+5.6%	Fe^{3+}	2.0×10^{-3} mM	–0.8%
D(-)-fructose	0.1 mM	+1.3%	urea	5.0×10^{-2} mM	–1.2%
D(+)-galactose	0.1 mM	+0.7%	citric acid	5.0×10^{-2} mM	+0.9%
D(+)-mannose	0.1 mM	+0.6%	vitamin C	5.0×10^{-2} mM	+1.3%
pyruvic acid	0.1 mM	+0.2%	γ-globulins	5.0×10^{-2} mM	+1.1%
K^+	2.0 mM	–0.1%	lysozyme	5.0×10^{-2} mM	+0.8%
Na^+	2.0 mM	–0.3%	glycine	0.1 mM	–0.9%
Ca^{2+}	20.0 mM	–0.2%	arginine	0.1 mM	–1.2%
Mg^{2+}	0.5 mM	–0.5%	L-phenylalanine	0.1 mM	+2.2%
Ba^{2+}	2.0 mM	–1.0%	lysine	0.1 mM	+1.8%
Al^{3+}	5.0×10^{-2} mM	–0.3%	L-cystenine	0.5 mM	+3.6%
Cu^{2+}	2.0×10^{-3} mM	–1.0%	tyrosine	0.5 mM	–1.8%
Zn^{2+}	5.0×10^{-2} mM	–0.2%	cholesterol	1.0×10^{-2} mM	+2.5%

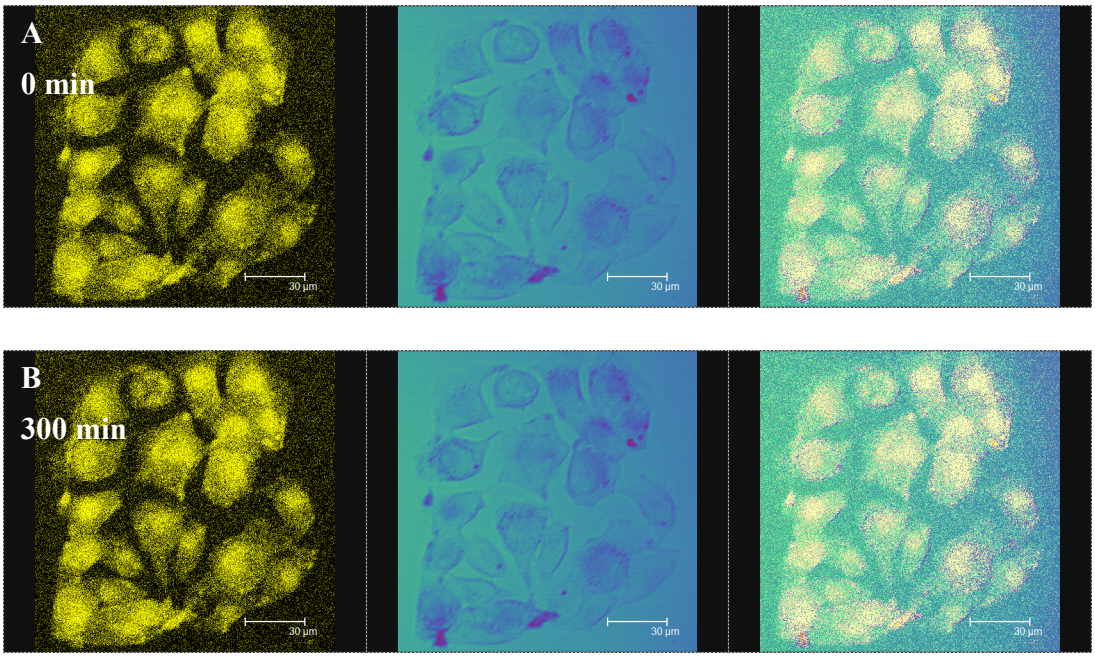
[a] "+" and "–" indicate an increase and decrease in glucose-induced PL quenching of the ca. 549 nm emission intensity, respectively.

SI-5. Permeability of the FNG in B16F10 Cells.



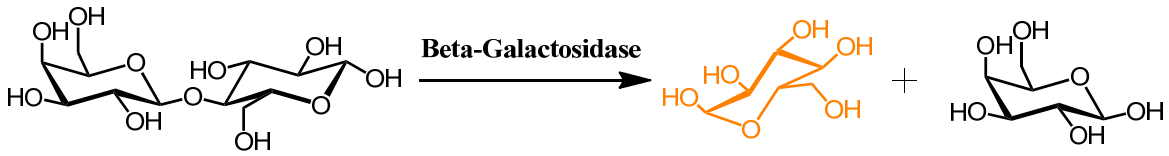
Top-to-bottom Z-scanning confocal fluorescence images of mouse melanoma cells B16F10 incubated with the FNG (10.0 μg/ml) without glucose in the growth media were examined. The FNG was brightly illuminated, which can be noticeably distinguishable from the background. Obviously, on the top and bottom sections of the cells, no bright spots are observed, indicating that no FNG was adsorbed at the surface of the cells.

SI-6. Photostability of the FNG in B16F10 Cells.

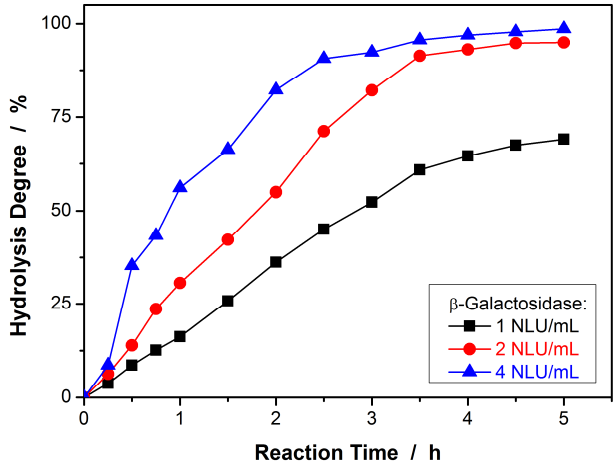


The B16F10 cells loaded with FNG were also continuously illuminated for 30 min. The confocal images indicate that the FNG within the cells still produced a bright color and retained the same PL intensity even after 30 min continuous irradiation, confirming the excellent photostability of the FNG.

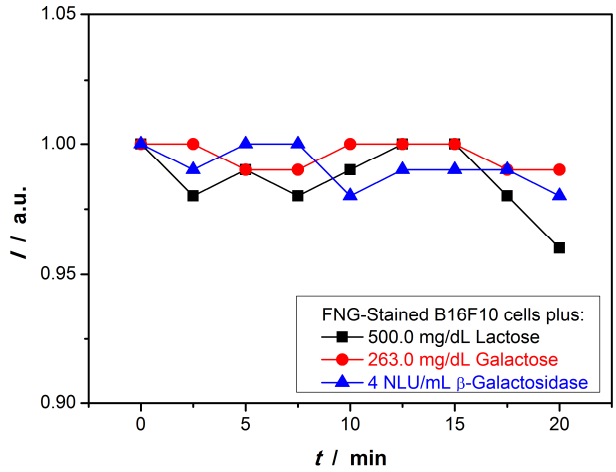
SI-7. The Biocatalytic Reaction of Lactose by β-Galactosidase.



β-Galactosidase catalyzes the breakdown of the substrate lactose into two monosaccharide sugars, glucose and galactose. The oxygen bridge connecting the two sides of the lactose molecule is cleaved through the addition of a water molecule. The addition of the water molecule is known as hydrolysis.



SI-8. Control Experiments on the FNG-Stained B16F10 Cells Fed with Lactose, β-Galactosidase, or Galactose.



To further examine the potential interference of non-glucose constituents, we conducted control experiments to assess the performance of the FNG in B16F10 cells, plus 500.0 mg/dL lactose, 4 NLU/mL β-

galactosidase, or 263.0 mg/dL galactose (an equated amount of that may be produced by comletely hydrolysis of lactose). The FNG clearly shows excellent selectivity for glucose over those non-glucose constituents.