# Electronic Supplementary Information (ESI)

# A fluorogenic molecular nanoprobe with engineered internal environment for sensitive and selective detection of biological hydrogen sulfide

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# Experimental

## Materials and Instrumentation

All chemical reagents were purchased from Aldrich and TCI and used as received. DSPE-PEG-2000 (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)]) was purchased from Avanti Polar Lipids, Inc. 4-Azidobenzyl bromide was synthesized by following a literature procedure.[ref.1] $^{1}$H NMR and $^{13}$C NMR spectra of ABR was recorded on a Bruker AVANCE 400 spectrometer. Elemental analysis was done using a FLASH 2000 (Thermo SCIENTIFIC, England) CHNS Analyzer. Absorption and photoluminescence spectra were recorded on a UV-visible spectrometer (Agilent 8453) and the F-7000 fluorescence spectrophotometer (Hitachi, wavelength calibrated for excitation and emission), respectively. The nanoparticle size distribution was determined by dynamic light scattering (DLS) method using particle sizer (90Plus, Brookhaven Instruments Corporation) at 25 $^{o}$C.

**Synthesis of ABR:** Resorufin (50 mg, 0.145 mmol) and $K_2CO_3$ (40 mg, 0.29 mmol) were dissolved in DMF (4 mL) and stirred at room temperature under argon atmosphere. After the solution color was changed to dark purple, 4-azidobenzyl bromide (61.5 mg, 0.29 mmol) dissolved in DMF (1 mL) was added dropwise to the stirred solution. The reaction mixture was stirred at 50°C for 3 h. After cooling to room temperature, the reaction mixture was poured into brine and extracted with ethyl acetate two times. The organic layer was separated and dried over anhydrous $MgSO_4$. The solvent was evaporated at reduced pressure, and the residue was purified by column chromatography on a silica gel with ethyl acetate/n-hexane 1:1 (v/v). ABR (35 mg) was obtained as orange solid in 70.3 % yield. $^{1}$H NMR (400 MHz, $CDCl_3$, δ): 7.72-7.70 (d, $J$ = 8.8 Hz, 1H), 7.42-7.39 (m, $J$ = 8.4 Hz and 10.0 Hz, 3H), 7.07-7.05 (d, $J$ = 8.4 Hz, 2H), 6.99-6.97 (dd, $J$ = 8.8 Hz and 2.8 Hz, 1H), 6.86-6.85 (d, $J$ = 2.4 Hz, 1H), 6.84-6.81 (dd, $J$ = 10.0 Hz and 2.0 Hz, 1H), 6.30 (d, $J$ = 2.0 Hz, 1H), 5.12 (s, 2H). $^{13}$C

NMR (150 MHz, $CDCl_3$, δ): 186.34, 162.47, 149.82, 145.76, 145.64, 140.47, 134.74, 134.34, 132.03, 131.70, 129.19, 128.59, 119.44, 114.24, 106.80, 101.07, 70.32. Anal. calcd for $C_{19}H_{12}N_4O_3$: C 66.28, H 3.51, N 16.27 ; found: C 66.10, H 4.13, N 15.47.

**Preparation of nanoABR:** ABR (0.014 mg) was homogeneously mixed with 1-iodooctadecane (0.005 mg) in THF (0.2 mL). After the solvent was evaporated by air flow, the dried mixture was homogeneously dissolved in DMSO (10 μL) and mixed with Milli-Q water (990 μL) containing DSPE-PEG (0.1 mg) with or without SKC (0.4 mg) under vigorous shaking to afford an aqueous dispersion of self-assembled nanoABR. The other nanoABR probes co-assembled with differently charged co-surfactant molecules were prepared by following the same procedure with F127 (5 mg) or sodium dodecyl sulfate (0.5 mg) instead of SKC.

**Selectivity Evaulation:** For selectivity studies, nanoABR was prepared for the titration of biological reactive species in PBS buffer at pH 7.4. The stock solution (100 μM) of RSS were also prepared in PBS buffer. The stock solution (100 μM) of ROS like $H_2O_2$, TBHP, and $OCl^-$ were delivered from 30 wt%, 70 wt%, and 5 wt% aqueous solution, respectively. $NO^{\cdot}$ were generated by adding stock solutions of 3-(aminopropyl)-1-hydroxy-3-isopropyl-2-oxo-1-triazene (NOC-5) in DMSO, respectively. $O_2^{\cdot}$- was obtained from $KO_2$ in DMSO containing 0.2M 18-crown-6 ether for increasing solubility of $KO_2$. $^{\cdot}OH$ and $^{\cdot}OtBu$ were generated by Fenton reaction of 1 mM $Fe^{2+}$ with 100 μM $H_2O_2$ or 100 μM TBHP, respectively.

**In Vitro Cell Labeling and Imaging:** A human cervical epitheloid carcinoma (HeLa) cell line was maintained in Dulvencco's modified eagle medium with 10 % FBS, L-glutamine ($5 \times 10^{-3}$ M) and gentamicin (5 μg $mL^{-1}$), in a humidified 5 % $CO_2$ incubator at 37 $^oC$. The cells were seeded onto 35 mm culture dishes and allowed to grow until 70 % confluence. Prior to the experiment, cells were washed twice with with PBS buffer (pH 7.4) and then incubated in 1.9 mL serum-free medium containing 100 μL of nanoABR dispersion. For endogenous

sulfide imaging, cells were pretreated for 30 min in a serum-free medium containing sodium nitroprusside (SNP, 100 μM). For inhibition test, cells were pretreated with DL-propargylglycine (PAG, 100 μM) for 30 min. The pretreated cells were washed twice with PBS buffer (pH 7.4) to remove free nanoparticles just before the data acquisition, and subjected to microscopic imaging with a LEICA DMI3000B microscope equipped with a Nuance FX multispectral imaging system (CRI, USA).

**In Vitro Diagnostic Imaging of Diabetes:** The animal studies have been approved by the animal care and use committee of Korea Institute of Science and Technology, and all handling of mice was performed in accordance with the institutional regulations. Type 2 diabetes mouse model was prepared using CD-1 mice [ref.2] (male, 10 weeks of age, Orient Bio Inc. Korea) by anaesthetizing with intraperitoneal injection of 0.5% pentobarbital sodium (0.01 mL/g). Diabetes were induced by intra-peritoneal injection of streptozotocin (STZ, 100 uL, 40 mg/mL in PBS buffer), and the injection was repeated 4 times for 1 month. Mice with blood glucose levels between 250-450 mg/dL were selected for the study. Mice were sacrificed and blood was collected in heparinized capillary tubes. The capillary tubes were centrifuged, and the separated plasma collected in microfuge tubes. Fluorescence imaging of $H_2S$ in plasma was done with an IVIS Spectrum imaging system (Caliper, USA).

[Ref.1] H. Zhang, Y. Xie, P. Wang, G. Chen, R. Liu, Yun-Wah Lam, Y. Hu, Q. Zhu, H. Sun, *Talanta*, 2015, 135, 149–154.

[Ref.2] J. Ventura-Sobrevilla, , V.D Boone-Villa, C.N. Aguilar, R. Román-Ramos, E. Vega-Avila, E. Campos-Sepúlveda, F. Alarcón-Aguilar, *Proc. West. Pharmacol. Soc.,* 2011, **54**, 5

## Supplementary Figures

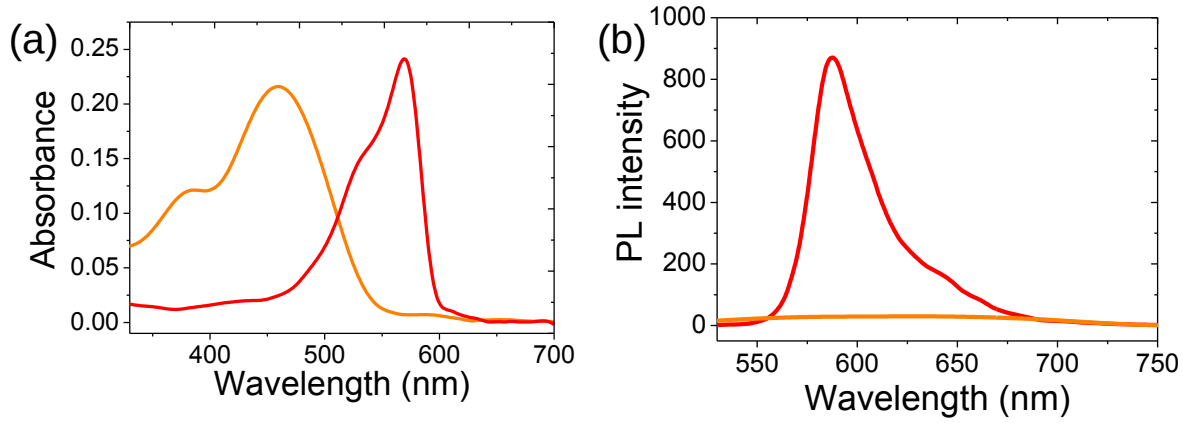


**Fig. S1.** Absorption (a) and fluorescence (b, excited at 480 nm) spectra of resorufin (red curves) and ABR (orange curves) dissolved in DMSO.

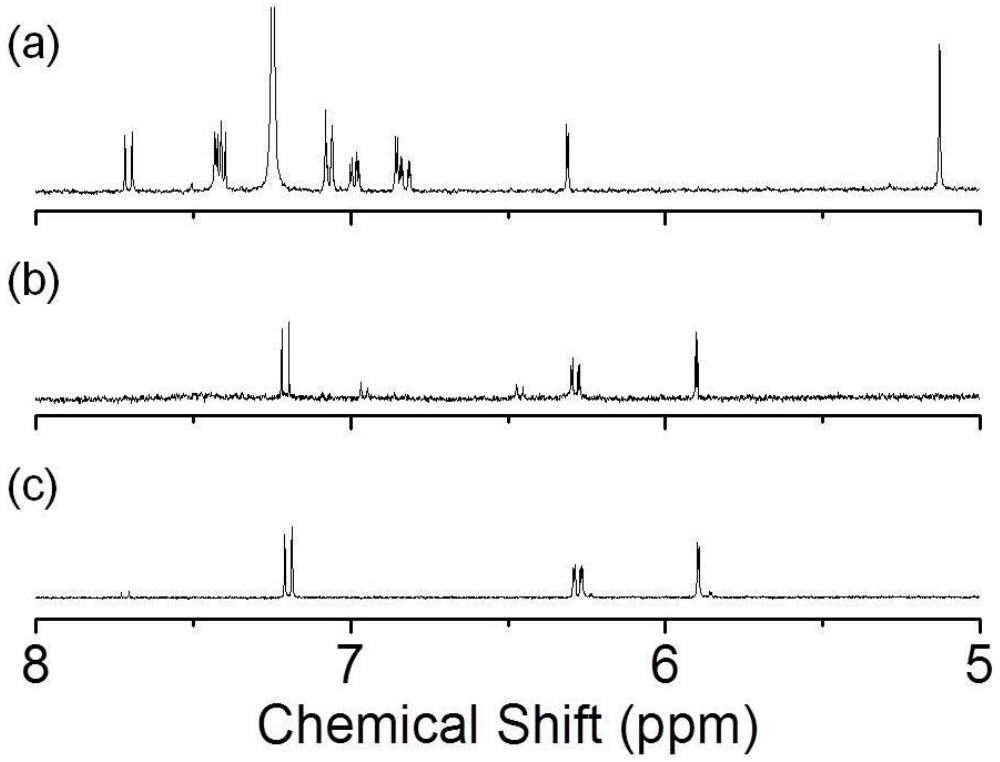


**Fig. S2.** $^{1}$H-NMR spectra of (a) ABR in $CDCl_3$, (b) ABR after reaction with $H_2S$ in DMSO-$d_6$, and (c) resorufin sodium salt in DMSO-$d_6$.

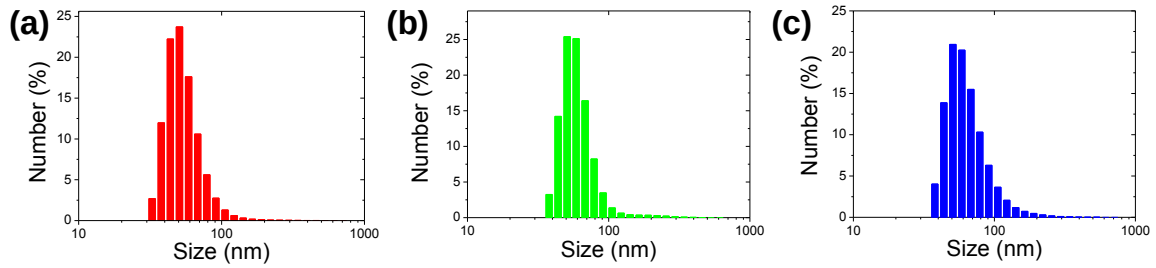


**Fig. S3.** Number-averaged hydrodynamic size distributions of nanoABR co-assembled with (a) SKC, (b) SDS, and (c) F127.

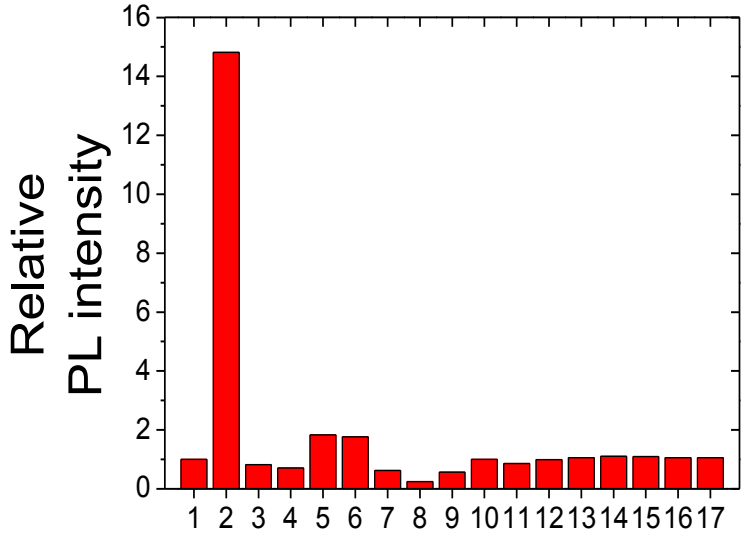


**Fig. S4.** Fluorescence intensity of nanoABR in PBS buffer in the presence of various reactive species (100 μM unless specified otherwise): 1) nanoprobes only; 2) NaHS; 3) $SCN^-$; 4) GSNO; 5) GSH (1 mM); 6) $SO_3^{2-}$; 7) $S_2O_3^{2-}$; 8) L-cysteine; 9) homo-cysteine; 10) $H_2O_2$; 11) TBHP; 12) $O_2^{\cdot-}$; 13) $OCl^-$; 14) $NO^{\cdot}$; 15) $ClO_4^-$; 16) $OH^{\cdot}$; 17) t-$BuO^{\cdot}$.

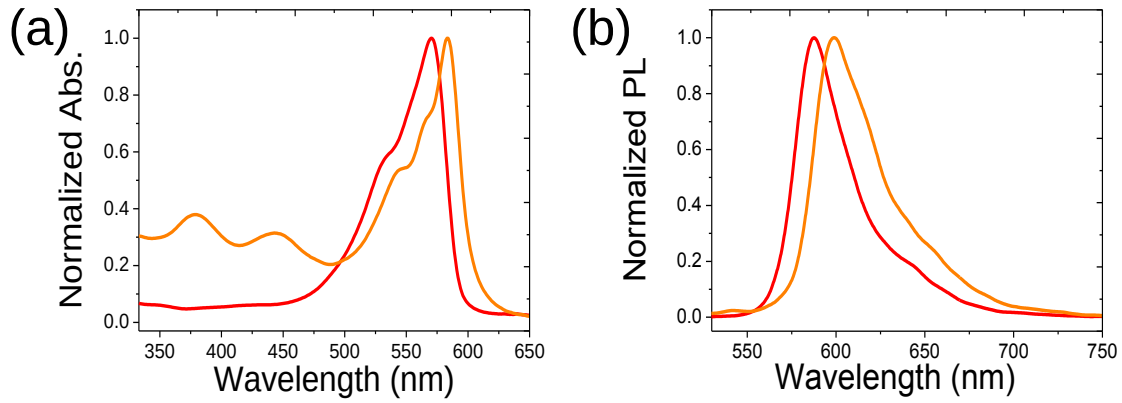


**Fig. S5.** Absorption (a) and fluorescence (b, excited at 480 nm) spectra of resorufin in water (red curves) and nanoABR after reaction with $H_2S$ in PBS buffer (orange curves).

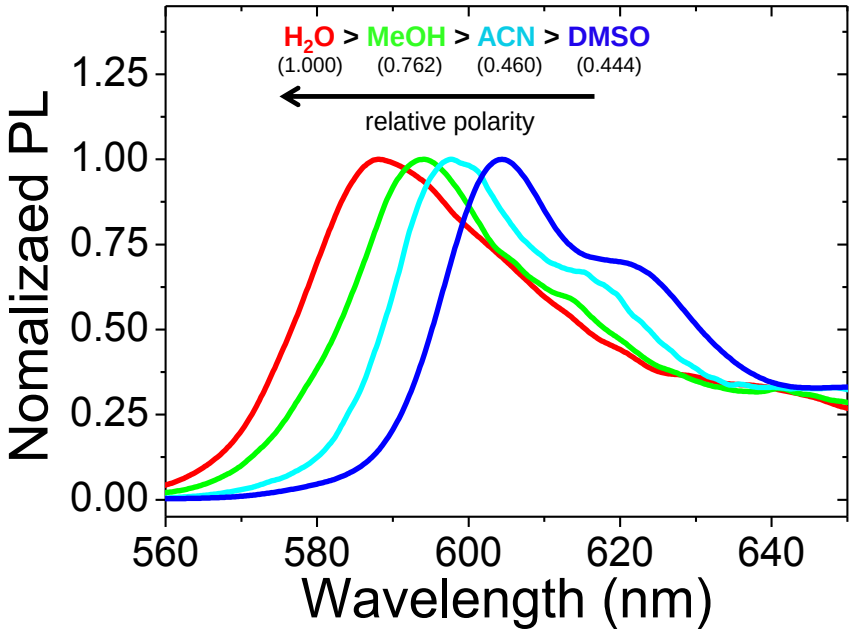


**Fig. S6.** Normalized fluorescence emission spectra of resorufin in different solvent, (red) $H_2O$; (green) methanol; (sky blue) acetonitrile; (blue) dimethylsulfoxide. The observed polarity-dependent peak shift indicates that resorufin has negative solvatochromism.

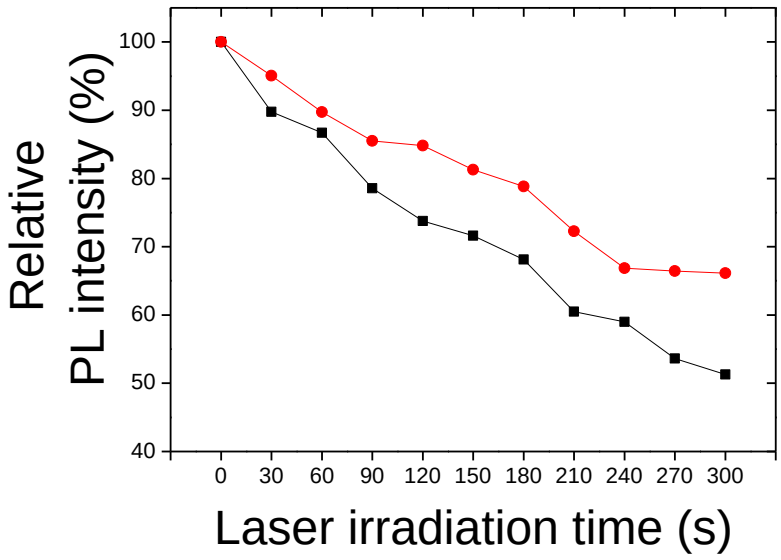


**Fig. S7.** Temporal fluorescence bleaching of free resorufin in water (black dot) and nanoABR after reaction with $H_2S$ in PBS buffer (red dot), under laser irradiation at 532 nm for 5 min.

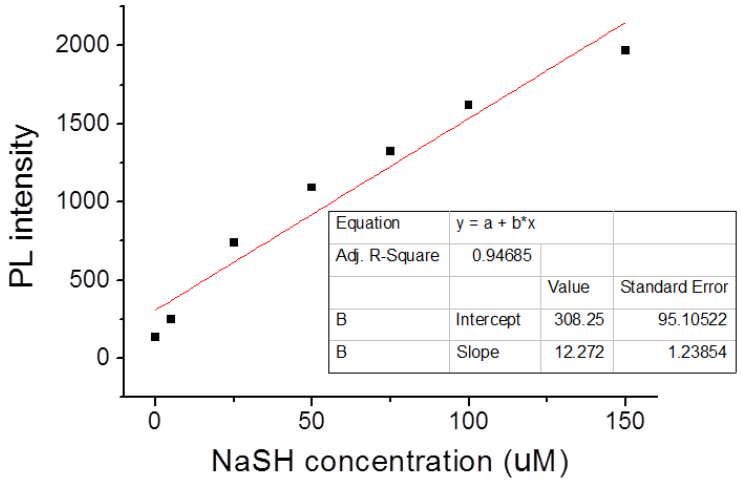


**Fig. S8.** Fluorescence intensity of nanoABR depending on the concentration of NaHS. From the plot, the slope (k) and standard deviation (S.D.) were determined to be 12.272 and 0.0764, respectively. The detection limit is calculated to be 18 nM from an equation (3 × S.D. / k in μM).[ref.3]

[Ref.3] J. Zhang, W. Guo, *Chem. Commun.,* 2014, **50**, 4214.

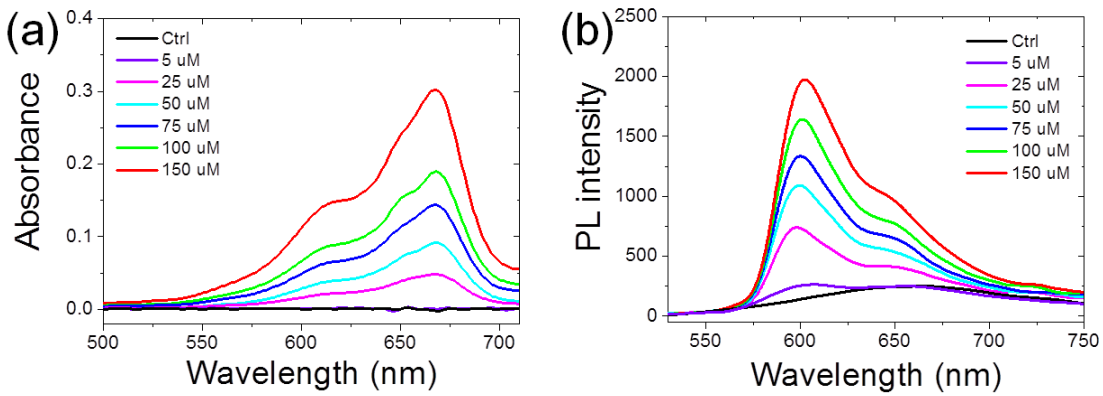


**Fig. S9.** Spectra of (a) methylene blue absorption and (b) nanoABR fluorescence (excited at 480 nm) in the presence of NaHS at various concentrations in PBS.

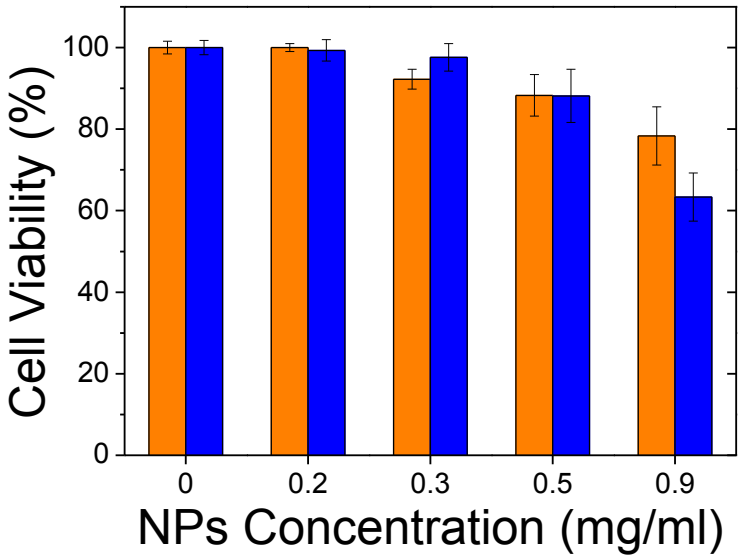


**Fig. S10.** Cytotoxicity of nanoABR against HeLa cells, evaluated by the colorimetric MTT assay. HeLa cells were treated with nanoprobes at different concentrations for 2 h (orange bar) and 4 h (blue bar). The concentration used for imaging corresponds to 0.5 mg/mL, at which cytotoxicity was shown to be minimal.

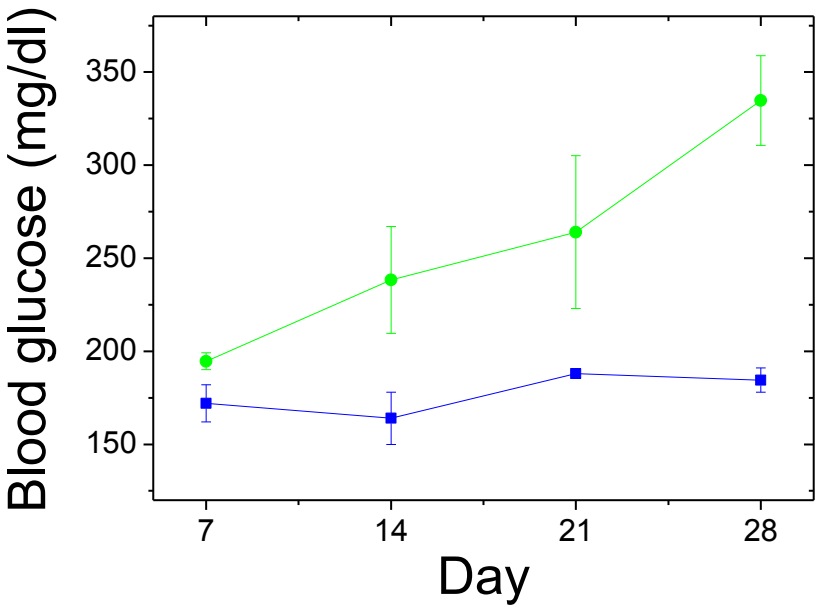


**Fig. S11.** Blood glucose levels of normal (blue) and diabetes (green) mouse models, at selected time points after injection of streptozotocin (STZ) for inducing diabetes.