Supporting Information

Labeling Monomeric Insulin with Renal Clearable Luminescent Gold Nanoparticles

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Experimental Section

Materials and Equipment

Human insulin (Recombinant), $HAuCl_4(3H_2O)$, and L-glutathione (reduced) were purchased from Sigma-Aldrich, Insulin ELISA kit from Life Technologies, Amicon Ultra 0.5ml 50 kDa centrifugal filters from EMD Millipore, MES buffered saline, 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), and N-Hydroxysuccinimide (NHS) from Thermo Scientific, and NAP25 columns from GE Healthcare Life Sciences. The hydrodynamic diameter (HD) and (zeta potential) of the nanoparticles in aqueous solution were analyzed using a Malvern particle size and zeta potential analyzer. Transmission electron microscopy (TEM) images were obtained with a 200 kV JEOL 2100 transmission electron microscope. Fluorescence measurements were conducted using a PTI QuantaMaster™ 30 Fluorescence Spectrofluorometer. Absorption spectra were collected using a Varian 50 Bio UV-Vis spectrophotometer. Fourier Transform Infrared (FTIR) spectra were obtained using Nicolet 380 ThermoScientific. The elemental analysis for Au was conducted using an Agilent 7700x ICP Mass Spectrometer. OneTouch Ultra2 test strips and glucose meter were used to measure the blood glucose level of the mice. The purity of the samples was determined using Shimadzu Prominence High Performance Liquid Chromatography (HPLC), equipped with BioSuite™ 125, 10 μm SEC (7.5 x 300 mm) column. The column was flushed with phosphate buffered saline (PBS) for 25 minutes prior to the sample measurements, which was also used as the solvent. The in vivo near-infrared fluorescence imaging was performed using a Carestream Molecular imaging system In-Vivo FX PRO (U.S.).

Supplementary Figures

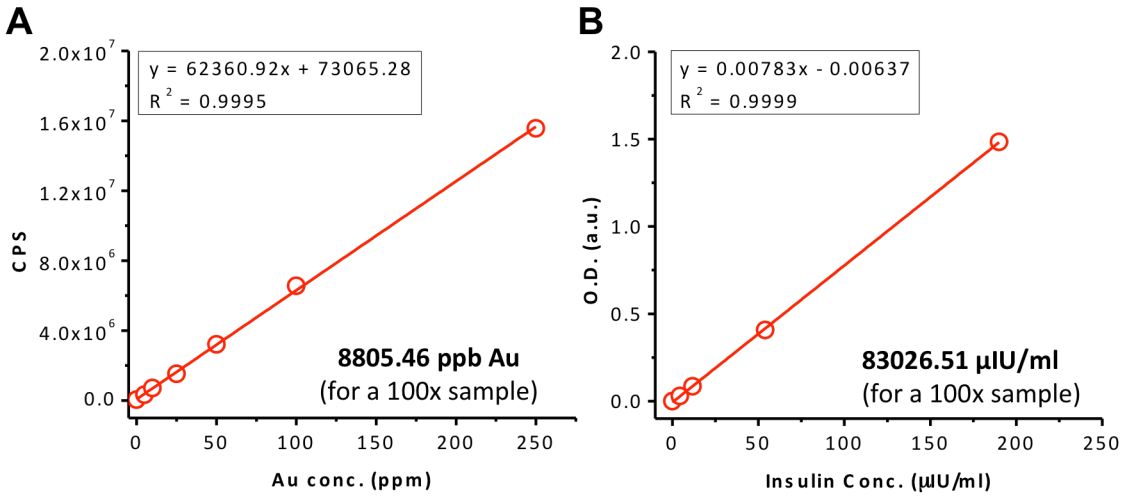

Figure S1. The ICP-MS (A) and insulin ELISA (B) standard curves.

The insulin-GS-AuNPs in Figure S2 show a mean core size of 1.57 nm, which contains roughly 102 Au atoms.[1] Hence, on average, the NPs have a molecular weight of about 20 kDa.

8805.46 ppb Au as shown in Figure S1A is equivalent to 2.69×10^{19} Au atoms/L or 2.64×10^{17} particles/L.

83026.51 µIU insulin/ml as shown in Figure S1B is equivalent to 0.083027 IU insulin/ml insulin or 3.02 µg insulin/ml (1 IU = 36.36 µg insulin), which equates to 3.13×10^{17} insulin molecules/L.

Therefore, the ratio between insulin and GS-AuNP is 1.2 insulin molecule/AuNP.

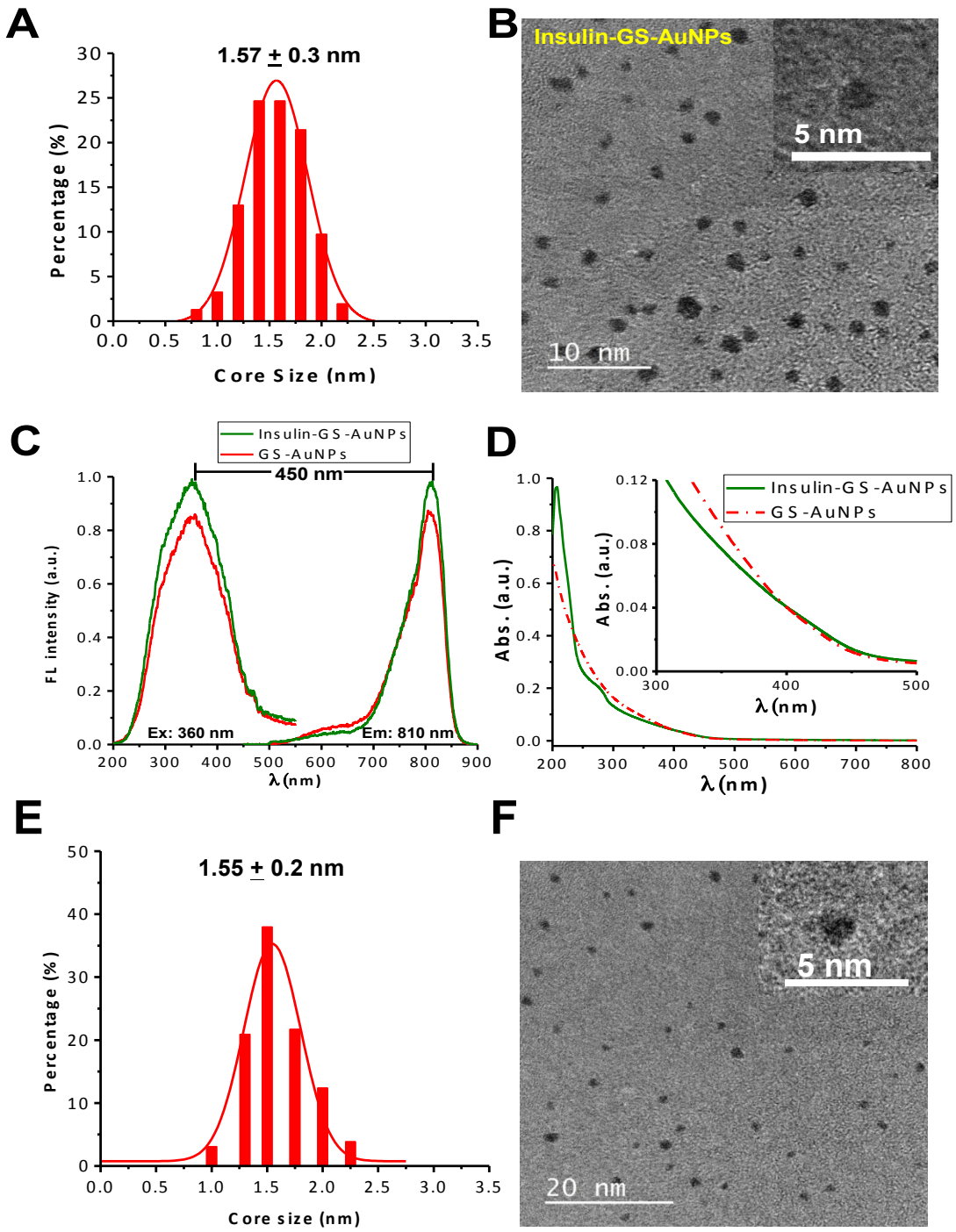

Figure S2. The average core diameter of Insulin-GS-AuNPs (A) based on HR-TEM images (B). The fluorescence spectra of insulin-GS-AuNPs and GS-AuNPs (C) at similar concentration as shown in the UV-Vis spectrum (D). Core size analysis of GS-AuNPs (E) and TEM images (F).

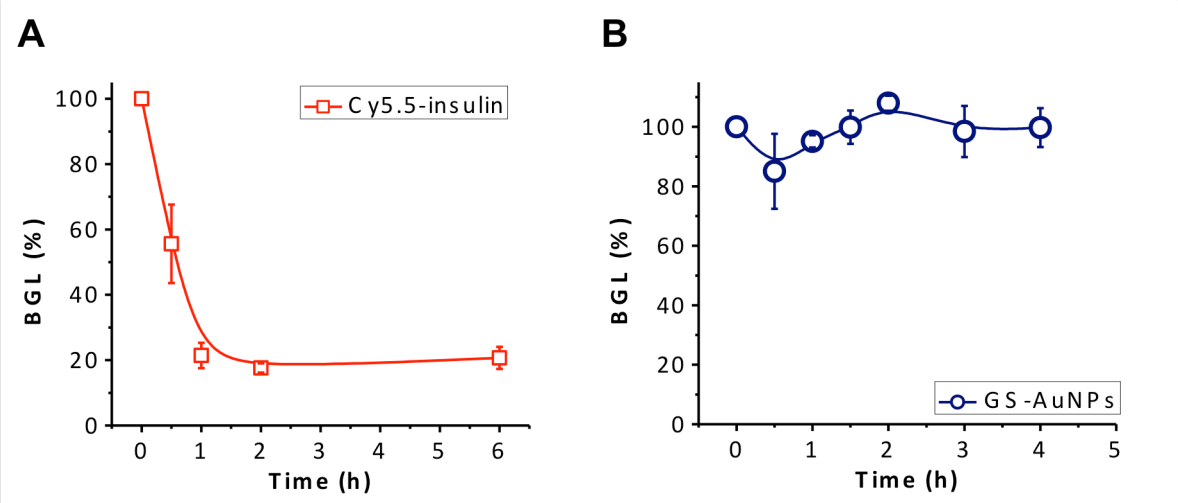

Figure S3. BGL of i.v. injected Cy5.5-insulin in normal balb/c mice (A). A comparison in the BGL of NOD/ShiLtJ mice after i.v. injection with GS-AuNPs (negative control) (B).

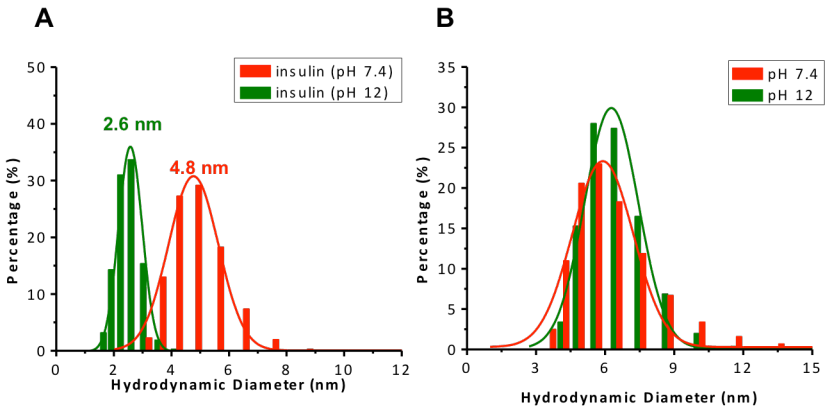

Figure S4. The hydrodynamic diameter (HD) of insulin at different pHs corresponding to the monomeric (2.6 nm) and hexameric (4.8 nm) form (A). The HD of the insulin-GS-AuNPs at pH 7.4 (5.9 ± 1.3 nm) and pH 12 (6.2 ± 1.2 nm) (B).

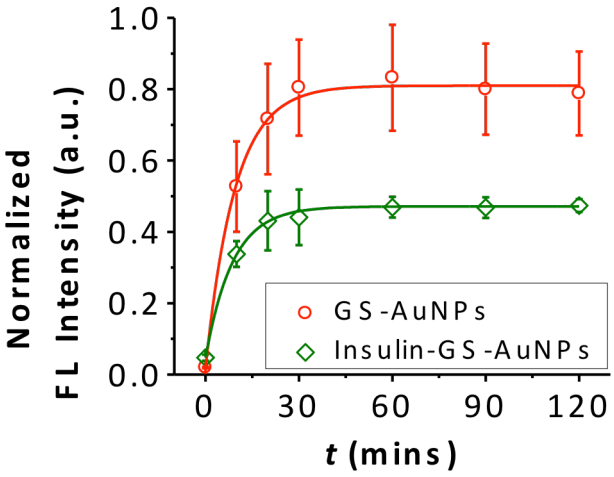

Figure S5. The renal clearance kinetics of pure GS-AuNPs and insulin-GS-AuNPs with $t_{1/2}$ of 9.4 mins and 8.7 mins, respectively.

References

[1] Azubel, M., Koivisto, J., Malola, S., Bushnell, D., Hura, G. L., Koh, A. L., Tsunoyama, H., Tsukuda, T., Pettersson, M., Häkkinen, H. et al. (2014) Electron microscopy of gold nanoparticles at atomic resolution. *Science*, *345*, 909-12.