Supporting Information

SERS-Based Label-Free Insulin Detection at Physiological Concentrations for Analysis of Islet Performance

Hyunjun Cho[1,†], Shailabh Kumar[2,†], Daejong Yang[2], Sagar Vaidyanathan[1], Kelly Woo[1], Ian Garcia[1], Hao J. Shue[1], Youngzoon Yoon[3], Kevin Ferreri[4], Hyuck Choo[,1,2]*

[1]Department of Electrical Engineering, [2]Department of Medical Engineering, California Institute of Technology, Pasadena, CA 91125, United States

[3]Device Lab, Device & System Research Center, Samsung Advanced Institute of Technology (SAIT), Suwon, 16678, Republic of Korea

[4]Department of Translational Research and Cellular Therapeutics, Diabetes and Metabolism Research Institute, Beckman Research Institute of the City of Hope, Duarte, CA

† These authors contributed equally

Corresponding author *E-mail: hchoo@caltech.edu

Experimental Section:

Fabrication and characterization: The SERS substrate was prepared using a hydrothermal method as shown in Figure S1.[1-2] In order to grow ZnO nanowires (NWs), a ZnO-seed solution consisting of 5 mM zinc acetate dihydrate in ethanol was applied to the surface of a 4-inch Si wafer.[3] The substrate was annealed at 350˚C for 0.5 hour and then immersed in a ZnO-NW precursor solution consisting of 25 mM zinc nitrate hexahydrate, 25 mM hexamethylenetetramine (HTMA), and 25 mM polyethylenimine (PEI) at 95˚C in a convection oven for 2.5 hours.[2] The ZnO nanowires were decorated with gold-nanoparticles (Au-NP) by dipping the Si-ZnO nanowire substrate in a Au-NP precursor solution at 90˚C for 1.5 hour.[4] The Au-NP precursor solution was made of DI water containing 1 mM sodium tetrachloroaurate (III) dihydrate and 200 μM sodium citrate dihydrate, and the pH level of the precursor solution was adjusted to 9 to prevent rapid etching of ZnO nanowires by adding 0.1 M NaOH solution.[5] The Au-NP-attachment process was repeated five times to maximize the uniformity of coverage and Raman enhancement by making dense Au-NP stacks in pillar forms, during which the ZnO nanowires eventually dissolved.[1] Scanning electron microscopy (SEM) was performed using FEI Nova 200 NanoLab dualbeam system.

Insulin samples: Purified human recombinant insulin was obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA). Insulin solutions at concentrations ranging from 100 pM to 1 mM were prepared in phosphate-buffered saline (PBS), pH 7.4.

Islet secretions: Human islets were provided for research use by the Southern California Islet Cell Resources Center at the City of Hope (Duarte, CA) with the approval of the COH Institutional Review Board and with the written informed consent. Human islets were isolated by a modified Riccordi method as described previously.[6] Functional assessment of the islets was

performed using a flow culture system as described previously.[6] Briefly, 750 human islets were placed into a flow culture system and perfused at 37 °C in an air/5% CO_2 incubator with low (3 mM) or high glucose (17 mM) Krebs-Ringer-Bicarbonate buffer containing 1% human serum albumin. Samples (375 microliters) were collected at 5 minute intervals and kept at -80 °C until assessed for insulin concentration by ELISA (Human Insulin ELISA kit, Mercodia Inc., Winston Salem, NC, cat# 10-1113-01) or SERS.

Sample preparation for SERS: The silicon wafer with 3D Au-NP clusters was cleaved into 5×5 mm^2 sensing chips and placed inside polydimethylsiloxane (PDMS) chambers with an inner volume of 200 μL. The insulin solutions or islet secretions (200 μL) were added to the chambers without stirring and the PDMS chambers were covered with quartz cover slips. The samples were left undisturbed for 12 hours at room temperature for diffusion-dependent transport and binding of insulin to the highly-dense 3D stacked AuNP pillars. Following this incubation, the samples were rinsed with DI water and dried using an air gun.

SERS and Raman mapping: Raman spectra of insulin were obtained using a Renishaw inVia Raman Microscope. Buffer samples were used as negative control for the experiments. The following measurement conditions were employed for measurement: 785 nm laser wavelength, 0.93 mW laser power at the sample, and 100 second integration time. Two-dimensional (2D) Raman mapping of the substrate was over a 1×1 mm^2 area, with step size of 20 μm, 12 mW laser power at the sample and 10 second integration time at each spot.

Signal Analysis: Raman signal was calculated from five points, at the center of each chip and at four additional points -- top, bottom, left, and right, about 0.5 mm away from the center location -- to characterize the Raman intensity and spatial uniformity. For areal averaging, single point measurements were replaced with 2D Raman scans over increasing areas, as indicated in Figure

2e. Mean intensity from the areal scans was used to calculate the relative standard deviation (RSD) of the signal. Detection limit of the sensors was calculated by using signal-to-noise ratio of 3 as threshold, commonly used for analytical methods.[7] Data was analyzed and plots were prepared using Matlab R2016b (The Mathworks, Inc.) and OriginPro8 (OriginLab Corporation).

Figures:

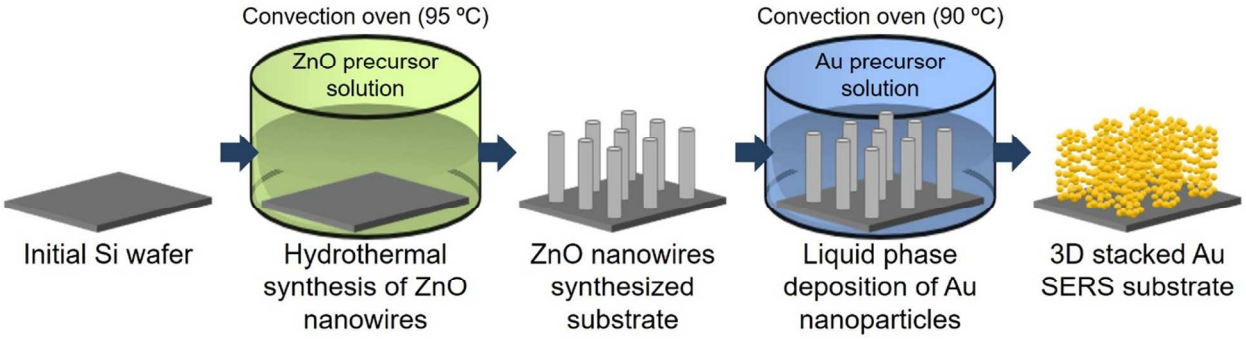

Figure S1: Schematics showing the synthesis of the 3D stacked Au-NP cluster sensors using a hydrothermal method.

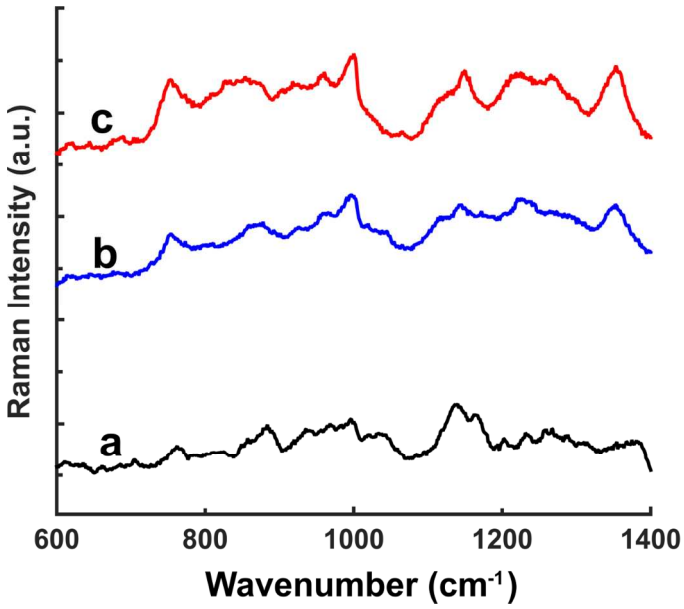

Figure S2: Representational SERS spectra obtained from (a) buffer with no insulin (b) Islet secretions in low glucose buffer with ELISA measured insulin concentration of approximately 138 pM. (c) Islet secretions in high glucose buffer with ELISA measured insulin concentration of approximately 513 pM.

References:

1. Yang, D.; Cho, H.; Koo, S.; Vaidyanathan, S. R.; Woo, K.; Yoon, Y.; Choo, H., Simple, Large-Scale Fabrication of Uniform Raman-Enhancing Substrate with Enhancement Saturation. *ACS Appl. Mater. Interfaces* **2017**, 9, 19092-19101.
2. Law, M.; Greene, L. E.; Johnson, J. C.; Saykally, R.; Yang, P., Nanowire dye-sensitized solar cells. *Nat. Mater.* **2005,** *4*, 455-459.

3. Greene, L. E.; Law, M.; Tan, D. H.; Montano, M.; Goldberger, J.; Somorjai, G.; Yang, P., General route to vertical ZnO nanowire arrays using textured ZnO seeds. *Nano Lett.* **2005,** *5*, 1231-1236.

4. Acosta, B.; Smolentseva, E.; Beloshapkin, S.; Rangel, R.; Estrada, M.; Fuentes, S.; Simakov, A., Gold supported on ceria nanoparticles and nanotubes. *Appl. Catal., A* **2012,** *449*, 96-104.

5. Zang, J.; Li, C. M.; Cui, X.; Wang, J.; Sun, X.; Dong, H.; Sun, C. Q., Tailoring zinc oxide nanowires for high performance amperometric glucose sensor. *Electroanalysis* **2007,** *19*, 1008-1014.

6. Sweet, I.; Gilbert, M.; Scott, S.; Todorov, I.; Jensen, R.; Nair, I.; Al‐Abdullah, I.; Rawson, J.; Kandeel, F.; Ferreri, K., Glucose-stimulated increment in oxygen consumption rate as a standardized test of human islet quality. *Am. J. Transplant.* **2008,** *8*, 183-192.

7. Shrivastava, A.; Gupta, V., Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chron. Young Sci.* **2011,** *2*, 21-25.