SUPPLEMENTARY INFORMATION

Nanoglassified, Optically-Active Monolayer Films of Gold Nanoparticles for *in situ* Orthogonal Detection by LSPR and SALDI-MS

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CONTENTS

METHODS

Synthesis of gold nanoparticle. AuNPs (13nm in diameter) were prepared using previously established methods[1]. In short, a round bottom flask containing 500 mL of 1 mM gold (III) chloride trihydrate (Sigma-Aldrich, St. Louis, MO) is heated until boiling, after which 50mL of 38.8 mM trisodium citrate dihydrate (Sigma-Aldrich, St. Louis, MO) is added. The mixed solution was continuously boiled and stirred until a deep red color was obtained. Nanoparticle formation and size distribution was verified using UV-Vis absorption and TEM.

Fabrication of calcinated AuNP films. Standard glass slides (Fisher Scientific) were first cleaned in a piranha solution (3:1 H_2SO_4/30% H_2O_2) for 30 minutes to ensure hydroxyl group coverage across the surface, and then rinsed with Millipore water, followed by drying with nitrogen. The cleaned glass slides were immersed for two hours in 1.0 mg/mL, pH 8.0, 60,000 MW poly(allylamine hydrochloride) (Sigma-Aldrich, St. Louis, MO). The PAH coated slides were incubated with as-prepared AuNP solution overnight with gentle agitation. After being immersed in another PAH solution with the same conditions described above, the modified glass slides were dipped into a 22 mg/mL, pH 9.5 sodium silicate solution (Fisher Scientific) for one minute. Finally, the slides were dried with nitrogen and heated in a furnace to 450°C for four hours at a rate of 17°C/minute.

Microscopic characterizations. Atomic force microscopy (AFM) images were obtained using a Veeco Dimension 5000 atomic force microscope (Santa Barbara, CA) with manufacturer-provided hardware. All images were obtained in tapping mode. To measure the film thickness, a mask was applied and distance between the top of the calcinated film and the bare glass surface was determined from the AFM height profile. The film morphology was also characterized using a Philips FEI XL30 scanning electron microscope (SEM). Synthesized nanoparticle sizes were

verified using a Philips Tecnai 12 transmission electron microscope (TEM). Both SEM and TEM characterizations were performed in UCR CFAMM.

Preparation of lipid vesicles. An appropriate amount of lipid stock solution containing L-α-phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL) in chloroform was dried with nitrogen to form a thin lipid film. Following this, the vial containing lipids was placed in a vacuum desiccator for 4 hours in order to completely remove all residual solvent. The lipid was then resuspended in water to a lipid concentration of 1.0 mg/mL. After vigorous vortexing to remove all lipid remnants from the vial wall, the solution was probe sonicated for 20 minutes. The resuspended lipids were then centrifuged at 8000 rpm for 15 minutes to remove any titanium particles from the probe tip during sonication. The supernatant was extruded through a polycarbonate filter (100 nm) to produce vesicles of uniform size and stored at 4^{o}C before use. For FRAP analysis, vesicle preparation followed the same procedure with 2% (w/w) 1-palmitoyl-2-{6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl}-*sn*-glycero-3-phosphocholine (NBD-PC, Avanti Polar Lipids, Alabaster, AL).

LSPR Measurements. All extinction spectra were recorded by a Cary 50 UV-Vis-NIR spectrophotometer. To obtain the differential extinction spectra, each spectrum was normalized by dividing by the maximum absorbance intensity, then an absorbance spectrum of the film in pure aqueous solution was subtracted as a reference.

FRAP Measurements. Using previously established methods[2], FRAP measurements were performed on a Meridian Insight confocal laser scanning microscope (CLSM) with 488 nm Argon laser excitation, SPOT Pursuit CCD, and a fluorescein emission filter used in conjunction with a 40x/0.75na Achroplan dipping objective. Laser scanning was stopped for 1 second to obtain a bleach profile, and fluorescence microscopy images were captured each second after.

Sample preparation for MS analysis. Two peptides, [Sar^{1}, Thr^{8}]-angiotensin II (MW = 956.1) and neurotensin (MW = 1672) (Sigma Aldrich, St. Louis, MO) were prepared in 50% acetonitrile containing 0.1% TFA and 10 mM citric acid to a final concentration of 2 μM, respectively. Cytochrome c was prepared with the same condition with the final concentration of 0.1 mg/mL. Cytochrome c digest was prepared to a final concentration of 1 mg/mL, with the addition of trypsin from bovine pancreas (Sigma Aldrich, St. Louis, MO) to make the protein:enzyme ratio 50:1 by weight. The protein enzyme mixture was kept in water bath at 37 ℃ for 16 hours, followed by the addition of 2 μL 100% formic acid after digestion.

Matrix-assisted samples were prepared by spotting 0.5 μL aliquots onto a stainless steel MALDI plate. A CHCA (Sigma Aldrich, St. Louis, MO) stock solution was prepared at 10 mg/mL in a solvent of 50% acetonitrile, 49.95% water, and 0.05% trifluoracetic acid. When used as a matrix, samples were prepared in a 1:10 ratio of peptide solution to CHCA. Matrix-free samples on the calcinated film were dissolved in 50% acetonitrile, 49.95% water, and 0.05% trifluoracetic acid, containing 10 mM citric acid, then spotted in 0.5 μL aliquots onto the calcinated AuNP film. The film was attached onto a stainless steel MALDI plate by adhesive polyimide tape before sample deposition.

Surface-assisted laser desorption/ionization mass spectra were collected by Voyager-DE STR MALDI-TOF mass spectrometer (Applied Biosystems, Framingham, MA). Experiment condition was set in positive reflector mode at accelerating voltage of 20 kV. The spectrometer is equipped with a pulsed nitrogen laser operated at 337 nm with 3 ns duration pulses. MS spectra were acquired as an average of 60 laser shots.

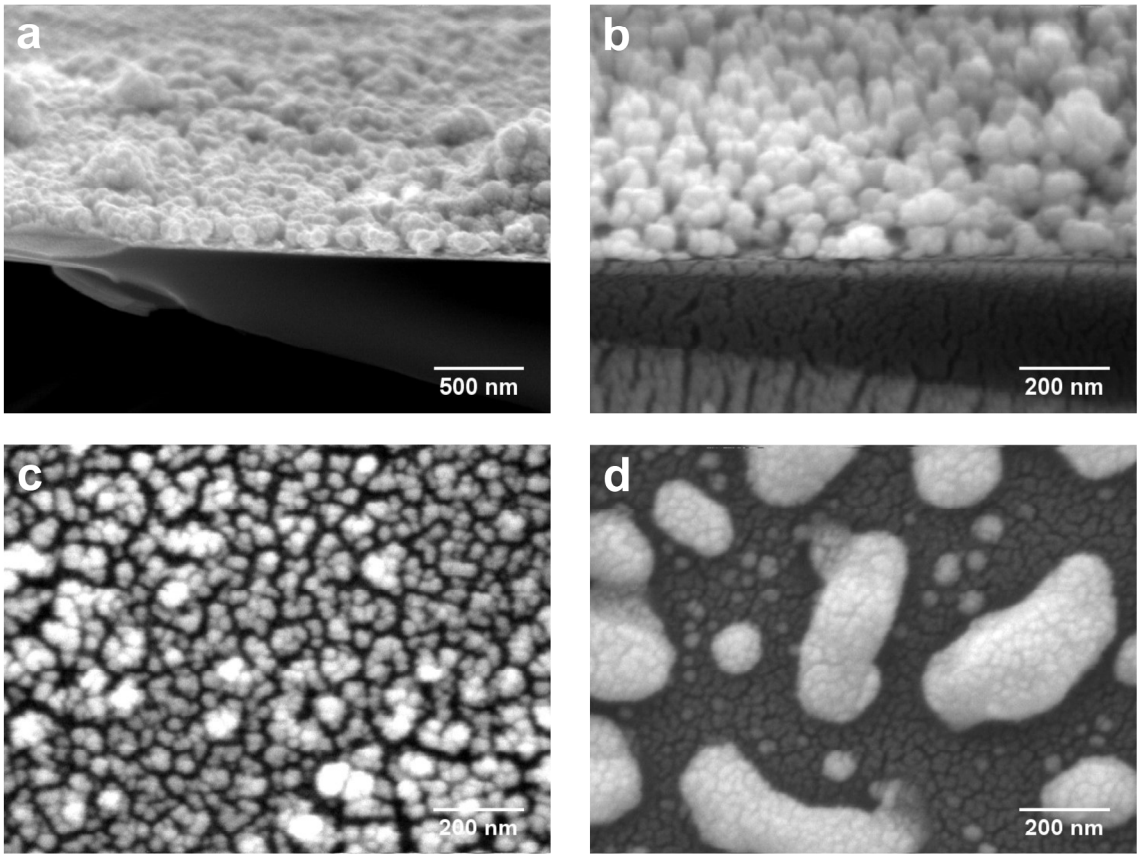

Figure S1. SEM characterization of the nanofilm substrates. (a,b) Images of calcinated AuNP film and the edge on the substrate, collected using a tilt angle. **(c)** Top-down image of calcinated AuNP film on the glass substrate. **(d)** Image of a calcinated AuNP film without deposition of the sodium silicate layer leading to amorphous structure and loss of plasmonic activity.

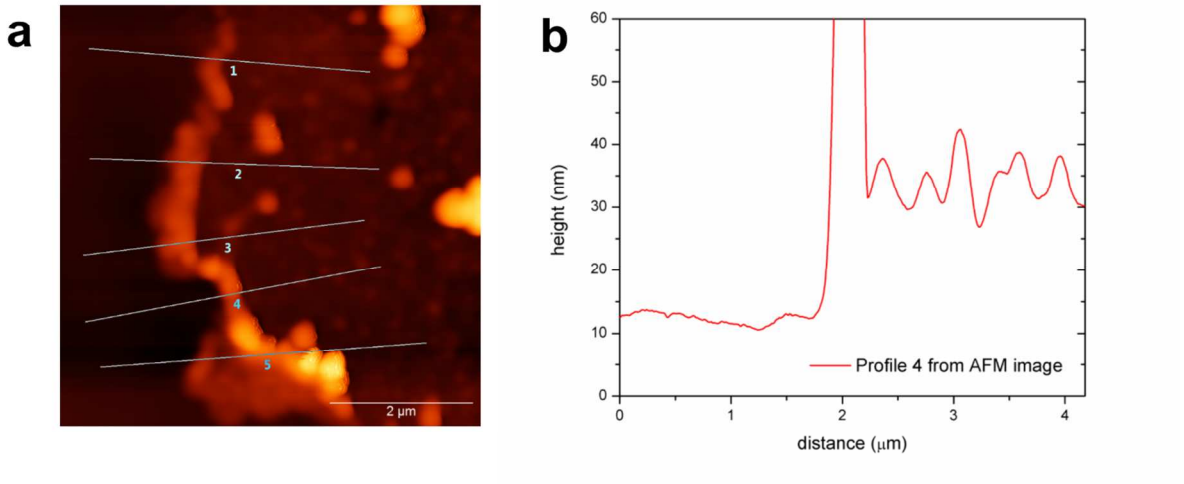

Figure S2. Derivation of film thickness from atomic force microscopy (AFM). (a) AFM image of calcinated gold nanoparticle film, with bare glass exposed to the left. Five height profiles were drawn and the film thickness of 26.1±5.7 nm was derived by subtracting the height of the film from the height of the glass within each of these profiles. **(b)** Representative line profile from AFM image. Left side represents glass, while right side represents the calcinated AuNP film. The spike in the middle is due to the accumulation of material by the mask used to initially cover the glass surface.

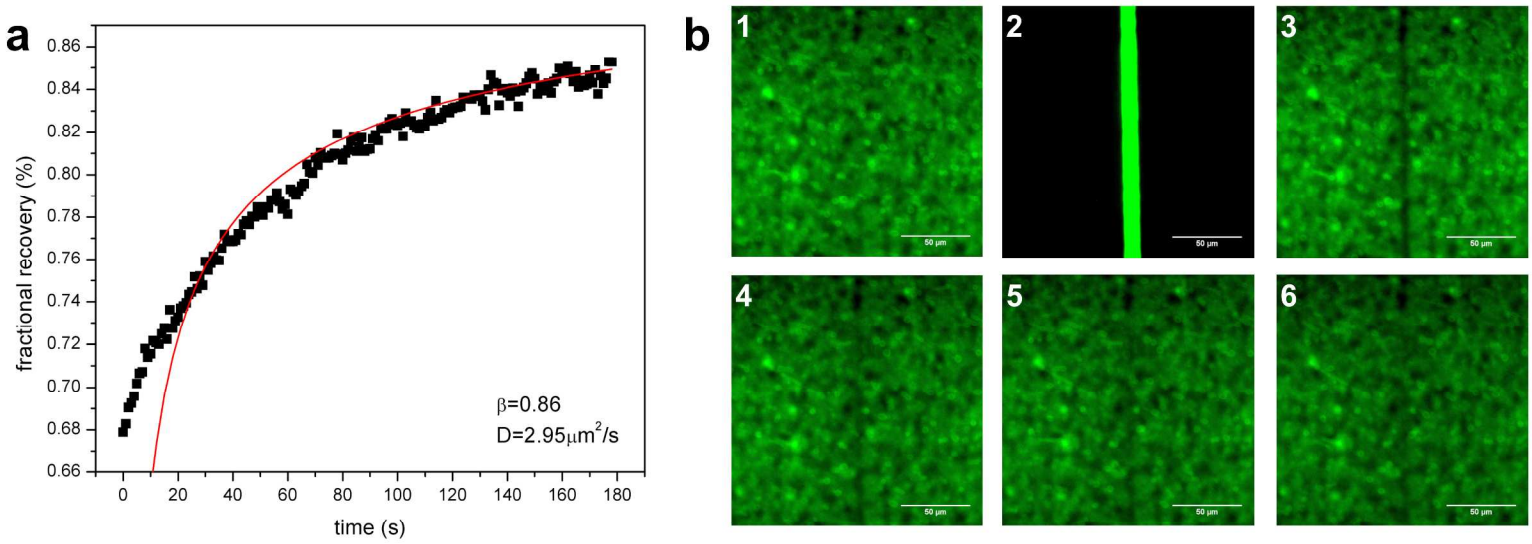

Figure S3. Fluorescence recovery after photobleaching (FRAP) of suspended lipid bilayer. **(a)** FRAP recovery curve for chicken egg-derived phosphatidylcholine on calcinated gold nanoparticle surface, where β is the mobile fraction and D is the diffusion coefficient. **(b)** Fluorescence microscopy images before, during, and after bleaching of lipids. 1 shows the surface before bleaching, 2 shows the bleaching line, and 3-6 show the fluorescence recovery at 0, 60, 120, and 180 seconds after bleaching, respectively.

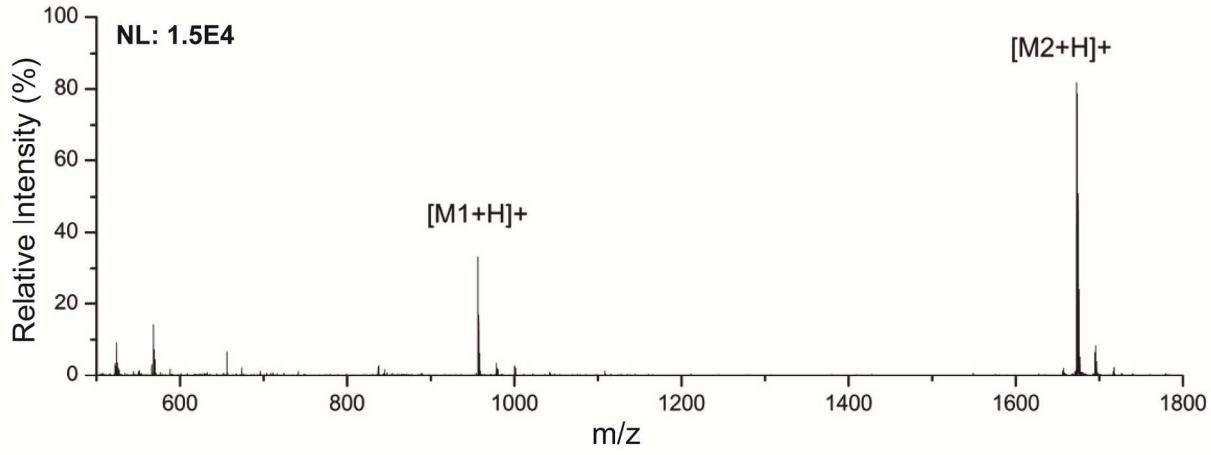

Figure S4. MALDI spectrum of of [Sar1, Thr8]-angiotensin II (M1=956.1) and neurotensin (M2=1672) using CHCA as a matrix.

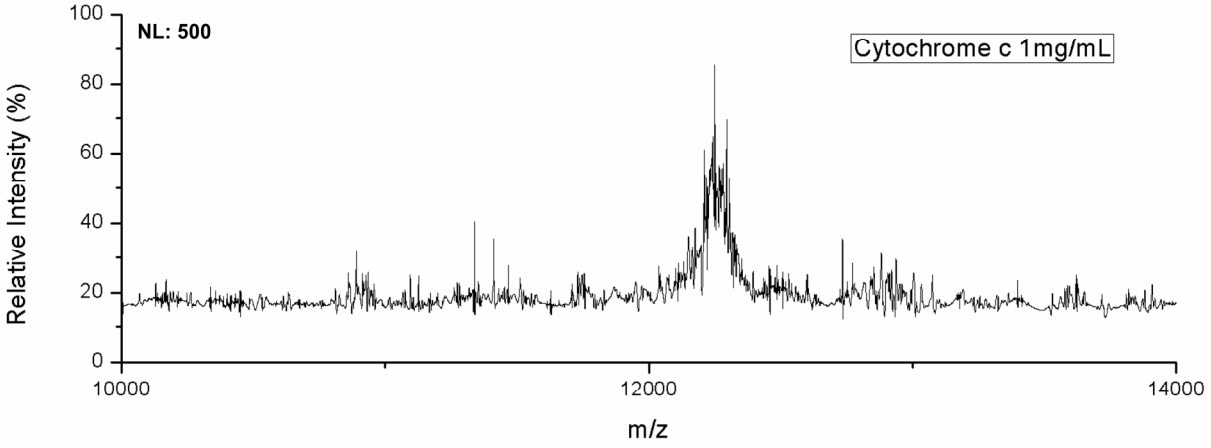

Figure S5. Identification of intact protein by surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS). SALDI mass spectrum of cytochrome c at 1 mg/mL on the calcinated gold nanoparticle film.

Table 1. Identified peptide fragments from cytochrome c digest

Peaks	Theoretical Mass (Da)	Amino acid sequence	Position	Experimental Mass (Da) – MALDI[a]	Experimental Mass (Da) – SALDI[b]
C1[*]	617.7302	KKGER	88-92	617.3	617.3
C2	634.8012	IFVQK	10-14	635.3	---
C3[*]	648.4079	IFAGIK	82-87	649.3	649.3
C4	779.4484	MIFAGIK	81-87	779.6	779.7
C5	908.2012	MIFAGIKK	81-88	907.7	907.8
C6	965.1412	EDLIAYLK	93-100	964.8	964.8
C7	1169.3366	TGPNLHGLFGR	29-39	1168.9	1168.9
C8[*]	1435.6735	KGEREDLIAYLK	90-101	1435.2	1435.3
C9	1562.8290	HKTGPNLHGLFGRK	27-40	1563.3	1563.4
C10	1634.9720	IFVQKCAQCHTVEK	10-23	1634.1	1634.1
C11	2139.4470	GITWGEETLMEYLENPKK	57-74	2138.6	2139.7

[*]Refer to partially digested cytochrome c peptides by trypsin, not recognized by MASCOT mass fingerprint tool.

[a]MASCOT score: 77, Sequence coverage: 55% (MASCOT, SwissProt), 74% (includes partially digested peptides)

[b]MASCOT score: 73, Sequence coverage: 43% (MASCOT, SwissProt), 74% (includes partially digested peptides)

SUPPLEMENTARY REFERENCES

(1) Frens, G. *Nature (London), Phys. Sci.* **1973**, 241, 20-22.

(2) Phillips, K.S.; Cheng, Q. *Anal. Chem.* **2005**, 77, 327-334.