A Conformation- and Ion-Sensitive Plasmonic Biosensor

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Nanosphere Lithography Preparation of LSPR Substrates:

Glass coverslips (Fisher no.2, 18 mm) were cleaned in a piranha solution (1:3 30% H_2O_2/H_2SO_4) at 80 °C for 30 min (CAUTION: piranha solution should be handled with extreme care). Once cooled, the glass substrates were rinsed with copious amounts of mili-Q water and then sonicated for 60 min in 5:1:1 $H_2O/NH_4OH/30\%$ H_2O_2. Next, the glass was rinsed repeatedly with water and was stored in water until use. Polystyrene nanospheres (2 μl, diameter = 390 nm + 19.5 nm, Duke Scientific) were drop-coated onto the cleaned glass coverslips and allowed to dry, forming a monolayer in a close-packed hexagonal formation which served as a deposition mask. The samples were mounted into a Consolidated Vacuum Corporation vapor-deposition chamber. A Leybold Inficon XTM/2 quartz crystal microbalance was used to monitor the thickness of the metal being deposited. For all experiments, 80 nm of Ag (D. F. Goldsmith) was evaporated onto the samples. Following metal deposition, the samples were sonicated for 3-5 min in ethanol (Pharmco) to remove the polystyrene nanosphere mask, creating Ag prismoidal nanoparticles on the glass substrate. SAM molecules were synthesized in-house and comprise two tri ethylene glycol thiols: 3,6,9,34,37,40-hexaoxa-21,22-dithiadotetracontane-1,42-diol and N-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-24,25-dithiado-4,7,10,13,37,40,43-heptaoxatritetracosan-45-ol-1-amide.

Cloning, Expression, and Purification of Cutinase-Calmodulin (CutCaM): Cutinase-calmodulin is a fusion protein construct containing an N-terminal Cutinase fused to a C-terminal calmodulin domain linked through a short, flexible GGGS peptide linker(*PNAS 2002 99 (8) 5048-5052)*. In previous work[21] we designed and cloned a similar construct, cutinase-calmodulin-cutinase, into a pET-22a (Novagen) vector using stepwise ligation of PCR products from either cutinase and calmodulin between appropriate restriction sites to achieve the three domain fusion protein. For this work, we used the truncated cutinase-calmodulin (CutCaM) fragment generated in the aforementioned work for cloning this gene fragment into a pET-21d (Novagen) vector between the N-terminal NcoI and C-terminal XhoI sites. A BamHI site separates the cutinase- and GGGS-calmodulin domains. Briefly, CutCaM PCR products containing NcoI and XhoI sites were digested with the appropriate restricition enzymes and ligated into an empty pET-21d vector using T4 DNA ligase (Promega) so that the gene was in frame with a C-terminal his-tag. Ligation reactions were transformed into chemically competent Origami B (Novagen) cells and plated on agar containing 50 ug/mL kanamycin and 75ug/mL carbenicillin. Positive transformants were determined by sequencing analysis of the isolated colonial DNA.

Expression of CutCam was carried out in 1L Terrific Broth (TB) containing 50ug/mL kanamycin and 75ug/mL carbenicillin that had been inoculated with 15mL of an overnight culture containing the same antibiotics and a stab of Origami B cells harboring the CutCam plasmid. This culture was grown to an OD_{600} of ~0.8 at 37°C with vigorous shaking, cooled to ~20°C in an ice bath and induced with 500mM IPTG. The induced culture was then shaken at 18°C for 16hr. Cells were harvested by centrifugation

and disrupted by lysis with 30mL Cell Lytic B (Sigma-Aldrich) containing EDTA-free Protease Inhibitor Tablets (Roche). Cellular debris was pelleted by centrifugation and the resulting supernatant applied to a 100mL Flex-Column (Kontes) containing 2mL Talon (Clontech) cobalt IMAC resin. The resin and cleared lysate were then rotated together on an orbital shaker at 4°C for 1hr. The lysate was drained from the column and the beads rinsed with 250mL DPBS (Invitrogen). The bound protein was then eluted with two portions of 30mL elution buffer (25mM Tris-HCl, 150mM NaCl, 150mM imidazole, pH 7.8 @ 25°C). The eluate was concentrated to 500uL by ultrafiltration in a 15mL 30kDa cutoff Amicon centrifugal filter unit (Millipore) and purified via an Äkta FPLC (Amerhsam Pharmacia) by size exclusion chromatography on a Superose 6 10/300 GL column and a Superdex 75 10/300 GL (GE Healthcare) column connected in series using 25mM Tris-HCl, 150mM NaCl and 0.05% NaN_3, pH 7.8 @ 25°C as the running buffer. Pure fractions were determined by SDS-PAGE, pooled together, and concentrated to 500uL using a 30kDa cutoff Microcon 500uL centrifugal filter unit (Millipore).

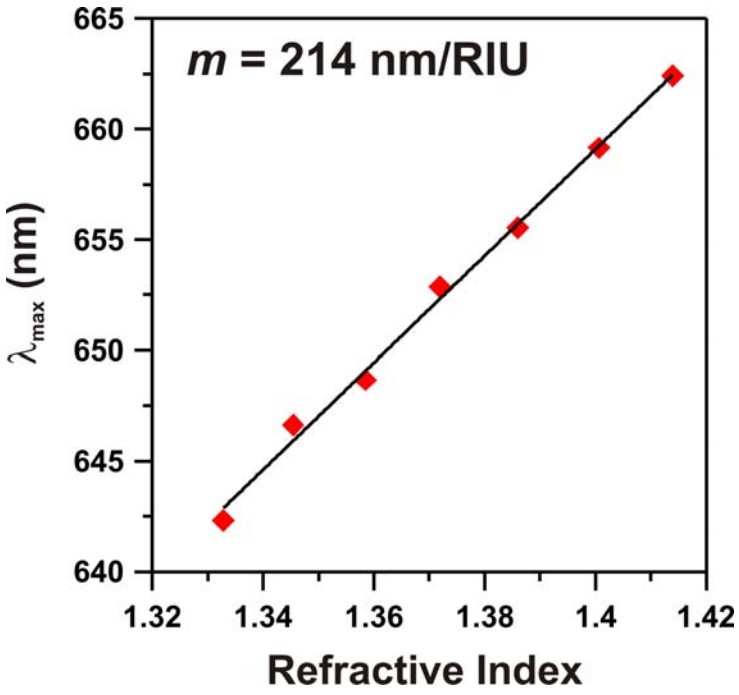

Figure S1. Refractive Index Sensitivity of the LSPR sensor. The LSPR sensor was exposed to aqueous glycerol solutions ranging from 0 – 60% glycerol. The λ_{max} of the LSPR sensor in each of these solutions was plotted versus refractive index. A linear fit to the data, where $\Delta\lambda_{max} = m(\Delta n)$, revealed a slope ($m$) of 214 nm/RIU.

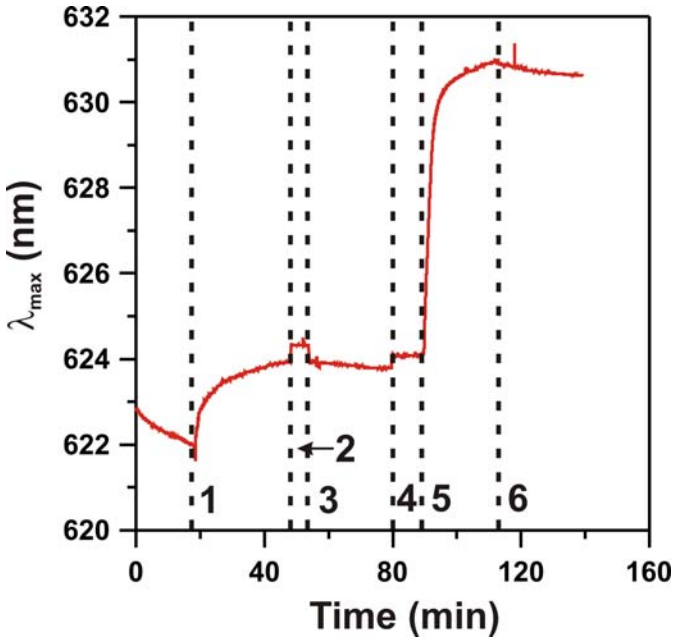

Figure S2. Non-specific binding assay. The affinity of the CutCaMCut functionalized LSPR sensor toward non-specific ligands was assayed by exposing the sensor to 1 μM bovine serum albumin (BSA). No λ_{max} shift was observed, indicating resistance to non-specific binding. A subsequent exposure to anthrax edema factor (EF) resulted in a red shift in λ_{max}, indicating retention of specific binding activity. The time trace follows λ_{max} changes as the sensor is exposed to (1) 500 nM CutCaMCut, (2) 20 mM Tris, (3) 1 μM BSA, (4) 100 μM $CaCl_2$ in 20 mM Tris, (5) 1 μM EF, and (6) 20 mM Tris.