Supporting Information

A Calcium-Modulated Plasmonic Switch

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1. **Preparation of Substrate and SAMs**: Nanosphere lithography (NSL) was used to create monodisperse, surface-confined Ag nanotriangles.[1,2] Glass coverslips (Fisher no.2, 18 mm) were cleaned in a piranha solution (1:3 30% H2O2/H2SO4) 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 H2O/NH4OH/30% H2O2. 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 triangular nanoparticles on the glass substrate.

   The Ag nanoparticle substrates were then functionalized with a self-assembled monolayer according to procedures described in reference 17 of the main text. Substrates were incubated in a 1 mM ethanolic solution of 96% EG3-OH and 4% EG3-maleimide for 24-48 hours, then rinsed with ethanol and dried in N_2. The substrate was then immediately drop-coated with a 1 mM solution of a phosphonate capture ligand in DMSO for 30 minutes at 35° C. Finally, CutCaMCut or CutCut (control) were immobilized by exposing the phosphonate-terminated SAM surface to a 500 nM solution of the protein construct in 20 mM, pH 7.5 Tris buffer at room temperature. The reaction was followed in real-time until it reached completion, generally ~ 10 minutes. The reaction was considered complete when $\Delta\lambda_{max}/dt$ reached zero.

2. **CutCaMCut Construct:** The recombinant CutCamCut construct has an overall mass of 63 kDa, with the calmodulin domain contributing 19 kDa and each cutinase domain contributing 22 kDa. The N-terminal cutinase is rendered inactive by mutagenesis of an active site serine residue to alanine, ensuring that each construct is anchored to the nanoparticle surface by the C-terminal cutinase only.

3. **High-Resolution LSPR Spectrometer:** Figure S1 depicts the HR-LSPR spectrometer used to collect macroscale UV-vis extinction measurements in this study. All measurements were performed in standard transmission geometry with

unpolarized light coupled into a photodiode array spectrometer (BWTek, Newark, DE) using lenses. The probe diameter was approximately 1 mm. A home-built flow cell[3,4] was used to control the external environment of the Ag nanoparticle substrates. A program written in Labview (National Instruments, Austin, TX) acquired spectra at 100 ms intervals, calculated the maximum wavelength by fitting a 100 nm spectral region around the peak to a fourth order polynomial, and displayed λ_{max} in real-time.

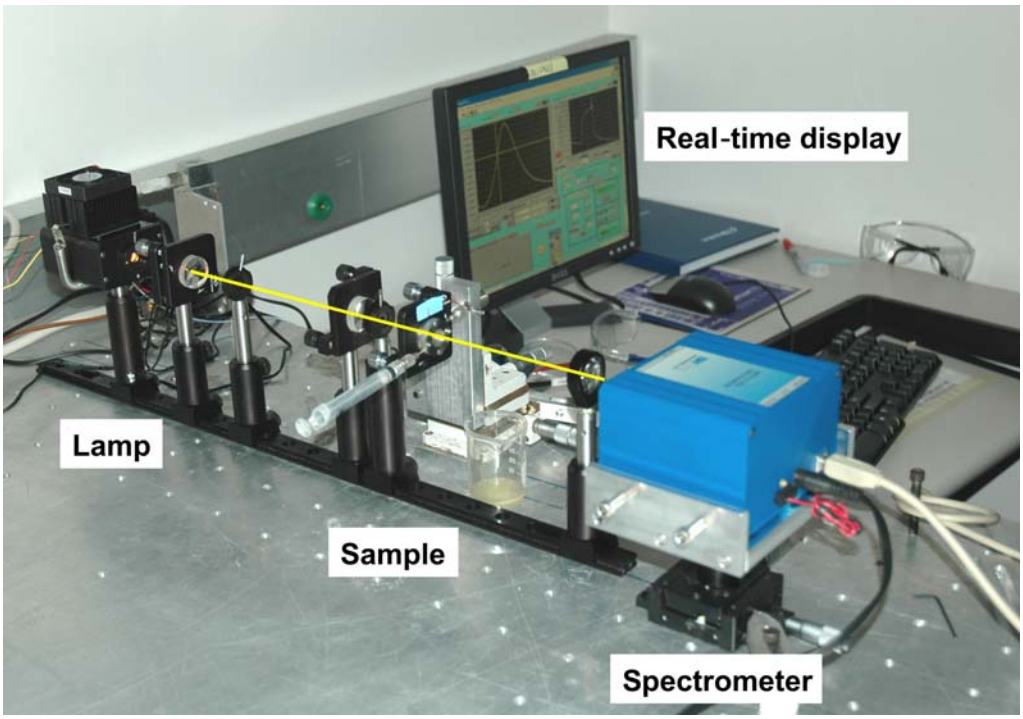

Figure S1. Photograph of the HR-LSPR spectrometer. Light is focused on the sample in a ~ 1 mm beam spot and coupled into a photodiode array spectrometer using lenses. The sample is secured in a home-built flow cell that allows control over the environment of the Ag nanoparticle substrate.

3. Supplementary Data: Figures S2-S5, described in the text, are shown below.

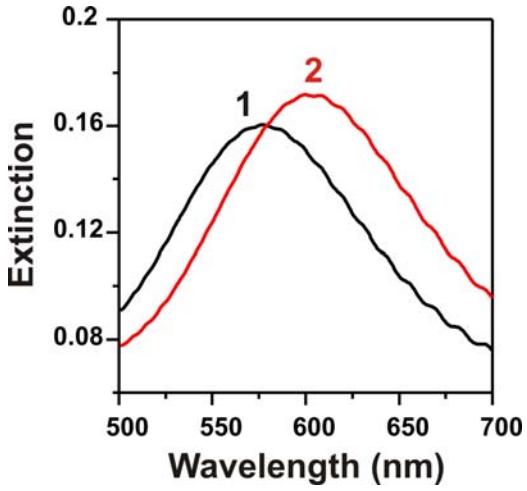

Figure S2. Spectra of a SAM-functionalized Ag nanoparticle substrate taken in a N_2 environment before (1, λ_{max} = 577 nm) and after (2, λ_{max} = 603 nm) immobilization of CutCaMCut.

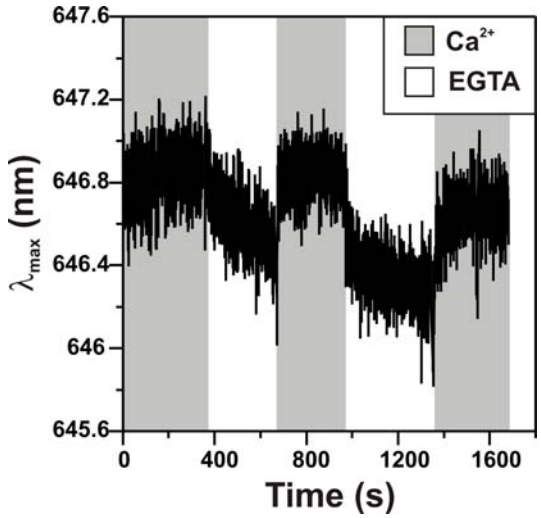

Figure S3. Experimental control demonstrating small, ~ 0.3 nm shifts in λ_{max} for Ag nanoparticles with a phosphonate-terminated SAM and no bound CutCaMCut in response to changing calcium concentration. The SAM preparation is followed through the phosphonate functionalization, but the final step in which the sensor is incubated in CutCaMCut is omitted and replaced with a 10 minute incubation in Tris buffer.

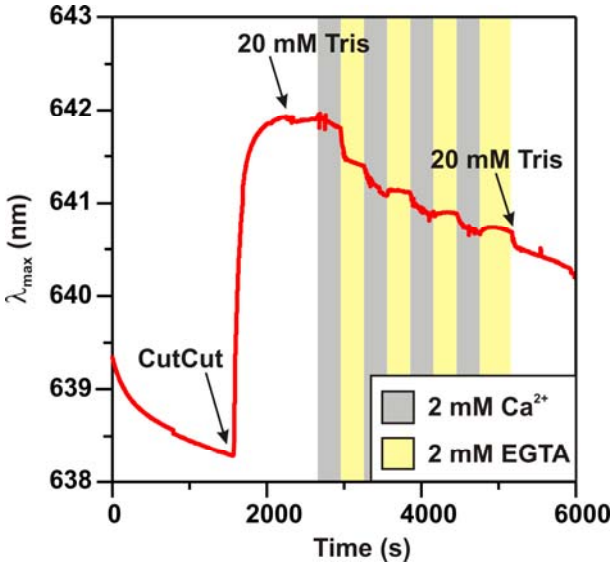

Figure S4. Experimental control in which a sensor with 80 nm deposited Ag is incubated in the non-calcium sensitive cutinase-cutinase (CutCut) construct, followed by a buffer rinse, and finally consecutive 5 minute cycles of 2 mM $CaCl_2$ and EGTA. The constant blue shift in λ_{max} with time is due to annealing of the Ag nanoparticles and is independent of ionic concentration. All solutions are prepared in 20 mM Tris buffer pH 7.5.

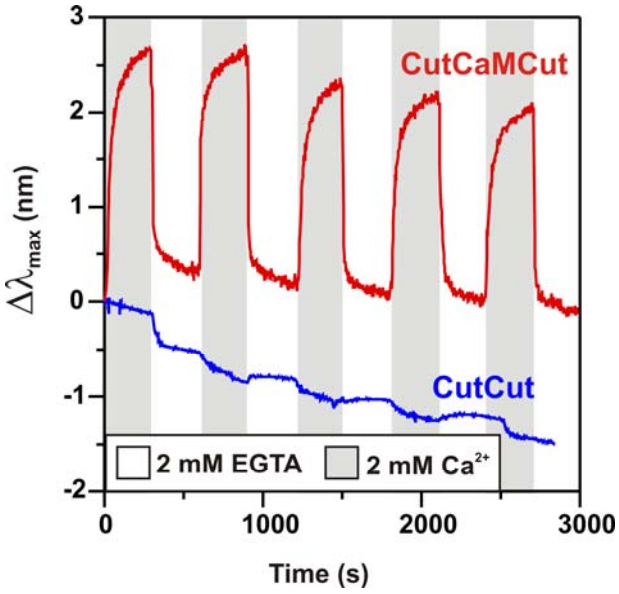

Figure S5. Comparison of the LSPR shifts in response to $CaCl_2$ and EGTA for CutCaMCut (red) and CutCut (blue). For the non-calcium sensitive CutCut, λ_{max} blue shifts an average of 0.247 ± 0.065 nm when exposed to $CaCl_2$. For the calcium sensitive CutCaMCut, λ_{max} red shifts an average of 2.197 ± 0.176 nm when exposed to $CaCl_2$.

References:

[1] Haes, A. J.; Van Duyne, R. P. *J. Am. Chem. Soc.* **2002**, *12*4, 10596.
[2] Haes, A. J.; Zou, S.; Schatz, G. C.; Van Duyne, R. P. *J. Phys. Chem. B* **2004**, *10*8, 109.
[3] Malinsky, M. D.; Kelly, K. L.; Schatz, G. C.; Van Duyne, R. P. *J.Am. Chem. Soc.* **2001**, *12*3, 1471.
[4] Van Duyne, R. P.; Haes, A. J.; McFarland, A. D. *SPIE* **2003**, *5223*, 197.