

A Calcium-Modulated Plasmonic Switch

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Cellular activities are mediated by proteins, which often undergo conformational changes to regulate binding and enzymatic activities that direct signaling pathways. Hence, it is important to develop and apply tools to characterize dynamic changes in protein conformation. A variety of approaches are available, including those based on NMR,¹ FRET,² and plasmonics.^{3,4} Plasmonic approaches are particularly useful because they provide an intense signal that does not bleach and allow nondestructive measurement over long periods of time. In addition to acting as probes of molecular interactions, plasmonic devices have significant potential as nanoscale optical switches, waveguides, light sources, microscopes, and lithographic tools.^{3,4} Herein, we demonstrate a plasmonic switching device based on the calcium-induced conformational changes of calmodulin. The extinction maximum (λ_{max}) of a localized surface plasmon resonance (LSPR) sensor functionalized with calmodulin reversibly shifts by 2–3 nm in response to changes in Ca^{2+} concentration, creating a unique on/off switch and providing information about the dynamics and structure of the protein. A high-resolution (HR) LSPR spectrometer with a wavelength resolution ($\Delta\lambda$) of 1.5×10^{-2} nm was used to detect the calcium-modulated wavelength shifts (Supporting Information).

The LSPR is a unique nanoscale phenomenon that gives rise to an intense extinction and scattering spectrum in noble metal nanoparticles that is highly dependent on the local refractive index at the nanoparticle surface. Biomolecule adsorption to a nanoparticle alters the local refractive index, causing shifts in the extinction maximum (λ_{max}). The magnitude and direction of these shifts provide detailed information about the packing density of the adsorbed species. LSPR sensors can be used to characterize and detect a wide variety of biological events, including DNA hybridization,⁵ carbohydrate protein binding,⁷ and antigen antibody interactions.^{8,9}

A commercially established technology based on propagating SPR in thin Au films can sense changes in refractive index up to 1 μm away from the sensor surface. Such SPR sensors have been used to detect protein conformational changes due to denaturation.^{10,11} However, the relatively large sensing region of propagating SPR sensors gives rise to interference from bulk refractive index changes. In contrast, the LSPR sensing region in Ag nanoparticles is confined to a thin (2530 nm) shell around the nanoparticle.² As a result, LSPR sensors possess 40-fold greater spatial resolution normal to the sensor surface, allowing improved detection of low molecular weight binding events. Monitoring LSPR changes in real-time provides information about the dynamics of binding events and protein folding, with much less interference from bulk refractive index changes.

Despite the superior spatial resolution of LSPR sensors, low S/N ratios have presented a challenge. However, developments in instrumentation and data analysis have dramatically improved the sensitivity of LSPR sensors, enabling real-time detection in solution. For example, Höök et al. developed a sensor capable of measuring LSPR shifts of less than 5×10^{-4} nm to detect binding to nanohole films.¹³ This level of sensitivity is comparable to propagating SPR sensors in terms of shift/molecule/area. Using single nanoparticles, LSPR spectroscopy has the potential to surpass the sensitivity of SPR.¹⁴

In this work, we use an HR LSPR spectrometer to detect binding and conformational changes in real-time with a standard deviation in LSPR wavelength of 5×10^{-3} nm for nanosphere lithography (NSL)-fabricated Ag nanopillar arrays incubated in solution. While this noise level is 10-fold higher than that reported by Höök, in part because nanoholes absorb more light than NSL arrays, the shift per molecule is higher for NSL arrays due to their shorter electromagnetic field decay lengths, resulting in a similar overall sensitivity per molecule. This noise level corresponds to a S/N ratio of 800 for typical (MW = 60 kDa) protein binding events, facilitating measurement of binding rates.

To demonstrate the capabilities of our real-time, HR LSPR spectrometer, we characterized the calcium-dependent conformational change in calmodulin (CaM), a protein that mediates many cellular responses to the second messenger calcium ion. CaM binds Ca^{2+} with dissociation constants of order 1 μM .¹⁵ Crystal structures show differences in the conformation and shape of the protein in its Ca^{2+} -bound and unbound states, and previous work has demonstrated CaM gels that change shape, making it an ideal protein to test both the real-time and wavelength resolution of our LSPR spectrometer in response to small, reversible refractive index changes.

To immobilize calmodulin, we created a protein construct consisting of a CaM domain sandwiched between two cutinase domains, with the N-terminal cutinase rendered inactive through a mutation. The active C-terminal cutinase reacts with a phosphate ligand on the monolayer to give a covalent, site-specific attachment to the nanoparticle. The design of this construct confers two benefits: (1) CaM is uniformly oriented at the surface, with the cutinase acting as a spacer molecule to reduce substrate interference and more closely approximate a solution-phase protein, and (2) the N-terminal cutinase domain provides an additional mass of 22 kDa, creating a more significant change in the overall protein packing density when treated with calcium. The λ_{max} of the LSPR sensor was monitored in real-time as CutCaMCut bound to the SAM, demonstrating a shift of 3.63 nm in aqueous buffer and a noise level of 4×10^{-3} nm (Figure 1B). Spectra measured in a N_2 environment before and after this immobilization step show an increase in the extinction intensity and a $\Delta\lambda_{\text{max}}$ of +26 nm,

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