Reversible Aptamer-Au Plasmon Rulers for Secreted Single Molecules

Somin Eunice Lee,*†,§,∥ Ana Luisa Correia,*† Shayne Petkieiewicz,*§ Jessica M. Smith,*§ Vivian E. Ferry,*§ A. Paul Alivisatos,*§‡ and Mina J. Bissell*†

†Life Sciences Division, §Material Science Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States
‡Department of Chemistry and Materials Science & Engineering, §Kavli Energy NanoScience Institute, University of California, Berkeley, California 94720, United States
∥Department of Electrical & Computer Engineering, §Biointerfaces Institute, University of Michigan, Ann Arbor, Michigan 48109, United States
*Department of Molecular Reproduction Development and Genetics, Indian Institute of Science, Bangalore, 560012, India

ABSTRACT: Plasmon rulers, consisting of pairs of gold nanoparticles, allow single-molecule analysis without photobleaching or blinking; however, current plasmon rulers are irreversible, restricting detection to only single events. Here, we present a reversible plasmon ruler, comprised of coupled gold nanoparticles linked by a single aptamer, capable of binding individual secreted molecules with high specificity. We show that the binding of target secreted molecules to the reversible plasmon ruler is characterized by single-molecule sensitivity, high specificity, and reversibility. Such reversible plasmon rulers should enable dynamic and adaptive live-cell measurement of secreted single molecules in their local microenvironment.

KEYWORDS: plasmon, single-molecule, secreted molecule, imaging, gold nanoparticle

Precise measurement of secreted molecules such as growth factors, proteases, and morphogens can provide insight into how their concentration gradients form between the cell and the cellular microenvironment and how cells respond to these concentration gradients in development and in cancer. Investigations of secreted molecules have relied primarily on fluorescence-based ensemble methods that are inherently limited in sensitivity as well as spatial and temporal resolution, making it difficult to detect the diffusible molecules in their native microenvironment. Micro- and nanopatterned structures have been utilized to physically confine diffusible molecules. But detection of molecules secreted in low abundance remains challenging using currently available methods.

The ability to detect single molecules provides a promising approach for measuring secreted molecules within their local microenvironment. Available fluorescence-based single-molecule methods are prone to photobleaching, blinking, and low signal-to-noise. Recently, plasmon rulers consisting of pairs of gold nanoparticles, have enabled dynamic detection of single-molecule activity without photobleaching or blinking. Two gold nanoparticles can be coupled together using a single biomolecule substrate, such as a cleavable DNA or peptide substrate, where the substrate length determines the distance between the two nanoparticles. When the two gold nanoparticles are brought into close proximity, the surface plasmon resonances of the individual nanoparticles will couple, generating a light scattering spectrum that is dependent on their interparticle distance. High spatial resolution of single molecules is achievable because of the large scattering cross-section and therefore high intensity of the scattered spectrum. The light scattering spectrum also is time invariant and can be continuously acquired in real time, resulting in high (ms-min) temporal resolution over the course of long imaging periods. Intracellular endonuclease cleaving dynamics and intracellular protease activity have been visualized with high spatial and temporal resolution using plasmon rulers that otherwise would be difficult to observe using conventional bulk methods.

Beyond cleavage, visualizing binding can provide a complete picture of molecules secreted into the cellular microenvironment. Current plasmon rulers that rely on cleavage of their biomolecule substrate to generate a change in their interparticle distance are limited to a subset of active enzymatic molecules. Moreover, cleaving is irreversible, restricting detection to single events. Plasmon rulers that utilize binding have been nonspecific so far for the protein-of-interest or have been limited to complementary nucleic acids. Here, we present a reversible plasmon ruler, comprised of coupled gold nanoparticles linked by a single aptamer, capable of binding single protein molecules with high specificity (Figure 1a). A conformational change in the aptamer, upon binding with the protein-of-interest, generates a change in the interparticle distance. This change in interparticle distance is coupled to a change in light scattering spectrum, which is then analyzed (Figure 1b). By analyzing the change in the scattering spectrum, we can determine the binding of secreted molecules to the plasmon ruler.

Supporting Information

Received: March 24, 2015
Revised: May 25, 2015
Published: June 3, 2015

DOI: 10.1021/acs.nanolett.5b01161
Nano Lett. 2015, 15, 4564–4570
distance of the plasmon ruler that is observable in the light scattering spectrum. In principle, a reversible plasmon ruler can be designed to detect any target molecule including growth factors, proteases, and morphogens with high specificity through selection of their cognate aptamer. We show that this class of reversible plasmon rulers is characterized by single-molecule sensitivity, high specificity, and reversibility needed for live-cell detection of secreted single protein molecules in the cellular microenvironment.

Results and Discussion. In order to validate the reversible plasmon ruler strategy to measure secreted single molecules within the cellular microenvironment, we employed a reversible aptamer-Au plasmon ruler, comprised of aptamer-linked gold nanoparticles, to specifically bind individual secreted matrix metalloproteinase molecules in the cellular microenvironment. The binding of the target molecule to the plasmon ruler generates a change in the interparticle distance of the latter, resulting in a shift in the plasmon resonance wavelength that is colorimetrically and spectroscopically observed using transmission-mode dark-field microscopy.

Sample homogeneity was confirmed by transmission electron microscopy (Figure 1b and Supporting Information Figure S1). Having established that we could assemble plasmon rulers consisting of a single aptamer, we investigated whether binding of MMP3 molecules could be measured at the single-molecule level. Plasmon rulers were immobilized such that one particle was bound to the glass surface through biotin-avidin chemistry and the other particle was free to move in solution. Scattering images and spectra of individual plasmon rulers were acquired using a transmission-mode dark-field microscope outfitted with a true-color imaging charge-coupled device (CCD) camera and spectrometer (Supporting Information Figure S2 and Figure S1). To investigate binding, we selected the protein MMP3, which is secreted by the mammary gland to mediate invasion and branching morphogenesis in puberty and is responsible for apoptosis and remodeling in involution, as well as regulation of noncanonical paracrine signaling pathways. DNA aptamers targeting MMP3 were modified with a biotin group on one end and a thiol group on the other end and folded into a tertiary structure. Aptamers can be generally functionalized to Au nanoparticles; however, to achieve single-molecule sensitivity, Au nanoparticles comprised of exactly one aptamer are needed. We controlled the average number of aptamers per Au nanoparticle by adjusting the aptamer/Au nanoparticle ratios. After the addition of aptamers with Au nanoparticles, the heterogeneous mixture was purified using high-performance liquid chromatography (HPLC) to obtain monomeric aptamer-Au nanoparticles (Figure 1b). Two Au nanoparticles were assembled through avidin-biotin and Au-thiol chemistries and purified using gel electrophoresis to form the final plasmon ruler structure. Sample homogeneity was confirmed by transmission electron microscopy (Figure 1b and Supporting Information Figure S1).

Having established that we could assemble plasmon rulers consisting of a single aptamer, we investigated whether binding of MMP3 molecules could be measured at the single-molecule level. Plasmon rulers were immobilized such that one particle was bound to the glass surface through biotin-avidin chemistry and the other particle was free to move in solution. Scattering images and spectra of individual plasmon rulers were acquired using a transmission-mode dark-field microscope outfitted with a true-color imaging charge-coupled device (CCD) camera and spectrometer (Supporting Information Figure S2 and Figure S1). To investigate binding, we selected the protein concentration to be on the order of the dissociation constant $K_d$. Upon addition of recombinant MMP3 (9 nM), single plasmon rulers exhibited a spectral redshift of 12 nm (Figure S1). No spectral shift was observed in a control experiment with plasmon rulers consisting of a mismatched sequence. We surmised that the observed spectral redshift was due to a decrease in the interparticle distance upon binding of the MMP3 molecule to the aptamer between the two Au nanoparticles. The utility of plasmon rulers as nanoscale distance sensors is based on their spectroscopic signatures, which redshift with decreasing distance between nanoparticles. With calibration, the spectroscopic position therefore can be used to determine the interparticle spacing before and after binding of recombinant MMP3. Supporting Information Figure S4 shows the result of a calculation of the peak position with changing interparticle distance based on electromagnetic simulation. The results of this simulation are intended to give qualitative guidance, as they are modeled as dimers in solution.
with a fixed polarization and do not account for the refractive index of the substrate or cellular environment, and the polarization of the incident light is assumed to excite only the longitudinal mode. Spectral shifts of 12 nm are readily achievable with small changes in the interparticle distance. Another way to assess the change in distance is to characterize the plasmon rulers in the absence and presence of recombinant MMP3 protein using transmission electron microscopy.40 The interparticle distance in Figure 2b shows a mean inter-particle distance of 2.5 nm in the presence of recombinant MMP3. Distribution histogram of inter-particle distance in the presence of recombinant MMP3, where P is the number of plasmon rulers with inter-particle distance d / total number of plasmon rulers in the observed population (top). Mean inter-particle distance is 9.5 nm in the absence of recombinant MMP3. Distribution histogram of inter-particle distance in the presence of recombinant MMP3 (9 nM concentration), where P is the number of plasmon rulers with inter-particle distance d / total number of plasmon rulers in the observed population (bottom). Mean inter-particle distance is 2.5 nm in the presence of recombinant MMP3.

To address this question of whether the binding between single MMP3 molecules and the aptamer-Au plasmon ruler were reversible, plasmon rulers were immobilized with one particle bound to the glass surface. Buffer containing 9 and 0 nM recombinant MMP3 was then alternately exchanged, and the peak wavelength of the plasmon resonance was monitored during this buffer exchange. We observed that the peak wavelength alternately redshifted and blueshifted by approximately 12 nm in response to the buffer exchange, indicating that the binding of MMP3 to the aptamer-Au plasmon ruler was reversible (Figure 3a). Notably, the response time of the

![Figure 2](https://example.com/f2.png)

**Figure 2.** Aptamer-Au plasmon rulers bind to single MMP3 molecules. (a) Spectral analysis: Representative scattering spectrum of single aptamer-Au plasmon rulers in the absence of recombinant MMP3 observed using transmission-mode darkfield microscopy (top). Representative scattering spectrum of single aptamer-Au plasmon rulers in the presence of recombinant MMP3 (9 nM concentration), showing a spectral shift of 12 nm due to binding of MMP3 molecule to the aptamer-Au plasmon ruler (bottom). (b) Distance analysis: Distribution histogram of inter-particle distance in the absence of recombinant MMP3 measured by transmission electron microscopy, where P is the number of plasmon rulers with inter-particle distance d / total number of plasmon rulers in the observed population (top). Mean inter-particle distance is 9.5 nm in the absence of recombinant MMP3. Distribution histogram of inter-particle distance in the presence of recombinant MMP3 (9 nM concentration), where P is the number of plasmon rulers with inter-particle distance d / total number of plasmon rulers in the observed population (bottom). Mean inter-particle distance is 2.5 nm in the presence of recombinant MMP3.

![Figure 3](https://example.com/f3.png)

**Figure 3.** Aptamer-Au plasmon ruler binding to MMP3 molecules is reversible and specific. (a) Aptamer-Au plasmon ruler is immobilized with one nanoparticle bound to the glass surface using biotin-avidin chemistry. Peak wavelength of the plasmon resonance of an aptamer-Au plasmon ruler when alternately flushed with buffer containing 9 and 0 nM recombinant MMP3. The peak wavelength of the plasmon resonance increases (redshift) with addition of recombinant MMP3, indicating a decrease in the interparticle distance due to binding of recombinant MMP3 molecule to the aptamer-Au plasmon ruler. The peak wavelength of the plasmon resonance then decreases (blueshift) in the absence of recombinant MMP3, indicating an increase in the interparticle distance when the aptamer-Au plasmon ruler is unbound. Alternately flushing with buffer containing 9 and 0 nM recombinant MMP3 results in the peak wavelength that alternately redshifts and blueshifts, strongly suggesting that binding of MMP3 to the aptamer-Au plasmon ruler is reversible. (b) As a control, the peak wavelength of the plasmon resonance of an aptamer-Au plasmon ruler did not shift when alternately flushed with buffer containing 9 and 0 nM recombinant MMP9.
condition, the yield of the aptamer-protein binding reaction $Y_b$ depends solely on the initial protein concentration $[P]_0$

$$Y_b = \frac{1}{\frac{k_{on}}{[P]_0} + 1}$$

(1)

The detection sensitivity for protein molecules then becomes equal to the dissociation constant (Supporting Information). Thus, 12.5 nM MMP3 protein molecules are needed to bind with 50% of plasmon rulers and to initiate a response. Meanwhile, because of this low plasmon ruler concentration, the yield of the unbinding reaction $Y_u$ is close to 1

$$Y_u = \frac{1}{\frac{k_{off}}{k_{on}} + 1}$$

(2)

The unbinding reaction therefore progresses almost completely once the protein concentration goes to zero (Supporting Information), making the sensing of the plasmon ruler reversible. The $k_{on}$ and $k_{off}$ rates of the aptamer-protein reaction can be estimated from Figure 3: the unbinding event completes on a time scale of less than 1 min, which indicates a $k_{off}$ higher than 0.1 s$^{-1}$ and a $k_{on}$ lower than 8 x 10$^6$ s$^{-1}$ M$^{-1}$.

In order to verify the specificity of the aptamer, we used MMP9 as a control protein because of the sequence and structural similarities between matrix metalloproteinase family members.41 Buffer containing 9 and 0 nM recombinant MMP9 was alternately exchanged. We observed that the peak wavelength did not shift during this buffer exchange (Figure 3b), indicating that reversible aptamer-Au plasmon rulers were indeed specific to MMP3 molecules.

It was observed that a difference in the spectral peak position occurred for plasmon rulers characterized in buffer solution and in cell culture medium. The peak position of the scattering spectrum redshifted in cell culture medium as compared to buffer solution. The plasmon rulers then further redshift in the presence of MMP3, indicating that MMP3 could be detected in cell culture medium. We surmise that the slight difference in peak position may be due to compounds, such as salts, carbohydrates, vitamins, amino acids, metabolic precursors, and growth factors, in the cell culture medium that may decrease the electrostatic repulsion and therefore decrease the separation between the two nanoparticles of the plasmon ruler, resulting in a redshifted spectrum in the cell culture medium.

Finally, we investigated whether aptamer-Au plasmon rulers could be used to measure the level of secreted MMP3 molecules in the mammary epithelial cell microenvironment in culture. Aptamer-Au plasmon rulers were immobilized with one particle bound to the glass surface using avidin chemistry. Non-malignant murine SCp2 mammary epithelial cells secreting MMP3 (control) or with their endogenous MMP3 knocked down using shRNA were cultured on glass surfaces (Figure 4c,d). Using immunofluorescence, we verified that control SCp2 mammary epithelial cells stained for MMP3, whereas cells with MMP3 knockdown did not stain for the protein (Figure 4e,f). We detected a distinct shift in the scattering peak wavelength and decrease in intensity of aptamer-Au plasmon rulers when in the presence of MMP3 knockdown cells, relative to their control counterparts (Figure 4c,d). The peak wavelength distributions show that the mean peak wavelength redshifted in the presence of MMP3 and blueshifted in the absence of MMP3. Beyond merely sensing the presence of secreted MMP3 molecules using plasmon...
rulers, the concentration of secreted MMP3 molecules in the local microenvironment can be quantitatively determined using plasmon rulers. From Figure 4c,d, 75% of plasmon rulers were bound with secreted MMP3. On the basis of this plasmon ruler response, the MMP3 concentration was calculated to be approximately 37.5 nM in the local microenvironment of control Scp2 cells.

In conclusion, we have demonstrated a reversible aptamer-Au plasmon ruler capable of binding single secreted molecules with high specificity. In the future, we anticipate that plasmon rulers can be used to precisely quantify secreted molecules to understand how their concentration gradients form and evolve within their cellular microenvironments. Studies are underway to characterize spatial and temporal resolution in live cells. To move toward in vivo studies, the stability and degradation of aptamer-Au plasmon rulers should be addressed. Phosphorothioate backbone could be incorporated into aptamers to reduce potential degradation by nucleases in the intracellular and in vivo environment. Aptamer-Au plasmon rulers are currently being developed for use in three-dimensional culture models and in vivo, where plasmon rulers could disperse within the extracellular matrix (i.e., collagen). We envision that quantification of secreted single molecules, including growth factors, proteases, and morphogens, within three-dimensional cellular microenvironments can provide insight into how tissues form and get patterned during development and may suggest therapeutic strategies to repair diseased tissues in the future.

**Methods.** Au Nanoparticle Preparation. Citrate-stabilized Au nanoparticles (30 nm, Ted-Pella) at a concentration of 2E11 particles/mL were ligand-exchanged overnight with bis(p-sulfanatophenyl)phenylphosphine (BSPP, Strem Chemicals) at a concentration of 1 mg/mL. To remove excess BSPP, BSPP-stabilized Au nanoparticles were then centrifuged at 5000 rpm for 50 min. The supernatant was removed and the BSPP-stabilized Au nanoparticles were suspended 10 mg BSPP/1 mL deionized (DI) water. The nanoparticle concentration was determined by UV−vis absorption spectroscopy based on the particle size of 30 nm and extinction coefficient of 3.585 × 10⁴ M⁻¹ cm⁻¹ (obtained from Ted-Pella). The stock solution was concentrated to achieve a final concentration of 2 × 10¹³ particles/mL and stored at ambient temperature.

Aptamer Preparation. DNA aptamer targeting MMP3 (ATW0062, Base Pair Biotechnologies) was conjugated with a biotin group on one end and a thiol group on the other end. The stock solution was prepared by suspending the lyophilized aptamer in 10 mM Tris bu solution of Au nanoparticles and 10 mg/mL BSA-biotin (Roche) at concentration of 1 mg/mL. To remove excess BSA, BSA-stabilized Au nanoparticles were then centrifuged at 5000 rpm for 50 min. The supernatant was removed and the BSA-stabilized Au nanoparticles were suspended 10 mg BSA/1 mL deionized (DI) water. The nanoparticle concentration was determined by UV−vis absorption spectroscopy. Puriﬁcation of aptamer-Au nanoparticles was selected for aptamer-Au plasmon ruler assembly. Sample elution was detected by monitoring UV−vis absorption at the plasmon resonance wavelength of 520 nm against a reference wavelength of 850 nm at which Au nanoparticles do not absorb. Automatic fraction collection was triggered by monitoring these wavelengths. The nanoparticle concentration was then determined by UV−vis absorption spectroscopy. Purified monomeric aptamer-Au nanoparticles were stored at 4 °C until use.

Avidin-Au Nanoparticle Preparation. First, 50 μL of stock solution of Au nanoparticles and 10 μL of 25:1 HS-C11-EG6-OCH2-COOH (HS-PEG-COOH, Prochimia)/HS-C11-(EG)6 Biotin (HS-PEG-Biotin, Prochimia) was mixed and reacted for 2 h at room temperature. After 2 h, Au nanoparticles were separated from excess PEG using puriﬁcation columns (NAP-5, GE Healthcare), added with 1 μL of 10 mg/mL avidin (A2666, Invitrogen), and reacted overnight at 4 °C. To remove excess avidin, avidin-Au nanoparticles were washed three times with 10 mM Tris, 30 mM NaCl pH 8.0 solution by centrifugation at 5000 rpm for 10 min. The nanoparticle concentration was determined by UV−vis absorption spectroscopy. Purified avidin-Au nanoparticles were stored in 10 mM Tris, 30 mM NaCl pH 8.0 solution at 4 °C until use.

Aptamer-Au Plasmon Ruler Assembly. Monomeric aptamer-Au nanoparticles were stochiometrically added to avidin-coated Au nanoparticles (1:1) and reacted overnight at room temperature. Gels were prepared with 0.7% agarose by weight in 0.5X tris-borate-EDTA buffer (TBE, Sigma). To obtain aptamer-Au plasmon rulers, the reacted solution was separated by gel electrophoresis for 45 min at 120 V in 0.5X TBE buffer to achieve distinct visible bands. Band corresponding to aptamer-Au plasmon rulers was extracted from the gel, enclosed in dialysis membranes (Spectra/Per 7, Spectrum Laboratories) and separated from the gel by electrophoresis for 20 min at 120 V in 0.5X TBE buffer. Purified aptamer-Au plasmon rulers were stored at 4 °C until use.

Single-Molecule Binding Characterization. Glass flow chamber was modiﬁed with 1 mg/mL BSA-biotin (Roche) and washed with 10 mM Tris, 30 mM NaCl pH 8.0 solution. Purified aptamer-Au plasmon ruler solution was then introduced and incubated overnight at 4 °C. The aptamer-Au plasmon ruler binds such that only the avidin-Au nanoparticle binds to the biotinylated surface while the aptamer-Au nanoparticle is free to move in solution. Then, Superblock (Pierce) was introduced and incubated for 30 min at 4 °C.

For single molecule binding experiments, 9 nM recombinant mouse matrix metalloproteinase (MMP3, Leinco) was introduced into the chamber. Scattering images and spectra were obtained on a transmission mode dark-ﬁeld microscope (Eclipse Ti−S, Nikon) equipped with a dark-ﬁeld condenser (NA 1.2−1.4), 40X air objective (NA 0.60), color camera (Qimaging), spectrometer (2300i, Acton) with a grating of 300 groves per millimeter (500 nm blaze wavelength), and liquid nitrogen cooled CCD (Spec-10:100B, Princeton Instruments). A slit width of 2 μm was used to ensure a single plasmon ruler was in the region-of-interest. Illumination was provided using a 100 W halogen source. A 1 s exposure time was used acquire a spectrum of a single plasmon ruler. For reversibility experiments, the chamber was alternately flushed with buffer containing 9 and 0 nM recombinant MMP3 in 5 min intervals, and spectra were collected at one spectrum per minute.
Transmission Electron Microscopy Characterization. Transmission electron microscopy (LaB6 Tecnai G2 S-TWIN) was used to characterize interparticle distance in the absence and presence of MMP3. Calculated and observed distances by TEM analysis has been shown to be in good agreement when the particle size is 5–10 nm. Thus, 5 nm particles were used here to form plasmon rulers. Purified aptamer-Au plasmon ruler solution was mixed with 0 and 9 nM recombinant mouse matrix metalloproteinase (MMP3, Leinco) and then immobilized on TEM grids for imaging.

Cell Culture and shRNA-Mediated Knockdown. The nonmalignant SCp2 murine mammary epithelial cell line was maintained in DMEM/F12 supplemented with 5% fetal bovine serum (FBS, HyClone), 5 μg/mL insulin (Sigma-Aldrich), and 50 μg/mL gentamycin (83–5072-1M, UCSF) as previously described. Cell medium was changed every other day. For shRNA-mediated knockdown, cells were transduced with lentivirus carrying shRNA construct targeting MMP3 (MISSION shRNA library, Sigma-Aldrich) and selected with 2 μg/mL puromycin as previously described.

Single-Molecule Analysis of Secreted MMP3. Aptamer-Au plasmon rulers were immobilized with one nanoparticle bound to the glass surface of the chamber using biotin–avidin chemistry as described above. SCp2 mammary epithelial cells with MMP3 knock down (MMP3 negative) and control (MMP3 positive) were then cultured in the chambers overnight at 37 °C with 5% CO2 humidified air to achieve a seeding density of 25%. Aptamer-Au plasmon rulers, synthesized from the same synthesis batch, were used for both the MMP3 positive and MMP3 negative mammary epithelial cell culture experiments. Chambers were then washed with fresh media. Scattering images and spectra of aptamer-Au plasmon rulers in the local microenvironment of MMP3 negative cells and MMP3 positive cells were obtained using transmission mode dark-field microscopy.

Immunofluorescence. SCp2 mammary epithelial cells with MMP3 knock down and control were cultured for 72 h on glass coverslips, fixed with 4% paraformaldehyde (15710, EMS) in 1x PBS for 10 min, washed with 1x PBS, and permeabilized in 0.25% Triton X-100 (8532, Sigma-Aldrich) in 1x PBS for 10 min. Cells were then blocked with 2% bovine serum albumin (A7888, Sigma-Aldrich) for 1 h, followed by incubation with a goat polyclonal antibody targeting MMP3 (ab18899, Abcam) in blocking buffer overnight at 4 °C and donkey α-goat secondary antibody (A11058, Life Technologies) for 1 h at room temperature. DNA DAPI (D9542, Sigma-Aldrich) was used to stain cell nuclei. Fluorescence images were acquired with an upright confocal microscope (LSM710, Zeiss) using a 1.4 NA 63× oil immersion.

Electromagnetic Simulation. Simulations were performed using Lumerical FDTD solutions, version 8.7.4. The gold nanoparticles were modeled using refractive index data from Johnson and Christy in a surrounding medium of water. Peak positions of the calculated spectra were extracted by fitting the spectral data to a Lorentzian line shape.

ASSOCIATED CONTENT

Supporting Information Additional figures and discussion on detection limit of plasmon rulers, reversibility of plasmon rulers, kinetics of the binding reaction, and estimation of the protein concentration. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nanolett.5b01161.

Author Contributions Q.C. and R.B. contributed equally.

Notes The authors declare no competing financial interest.

REFERENCES


