Supporting Information: Reversible Aptamer-Au Plasmon Rulers for Secreted Single Molecules

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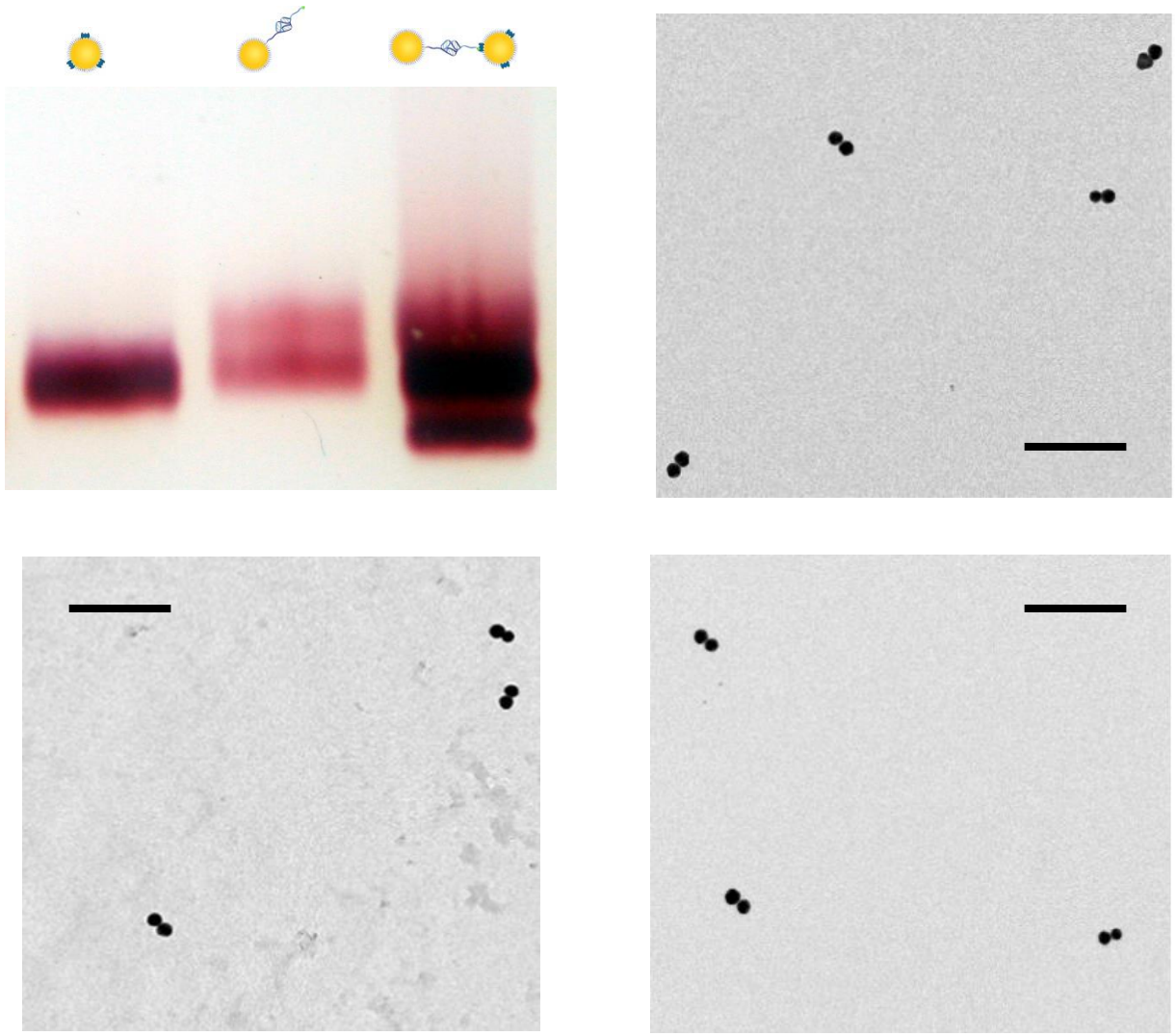
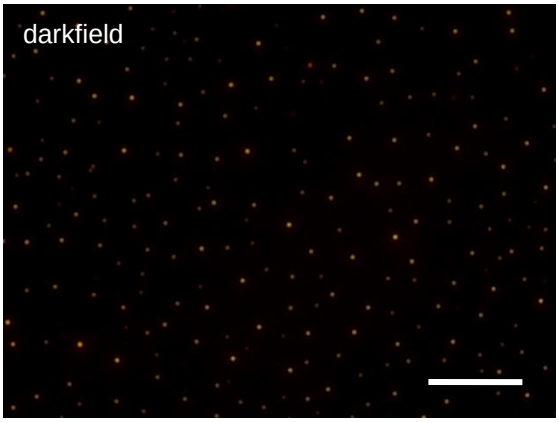


Fig. S1


darkfield

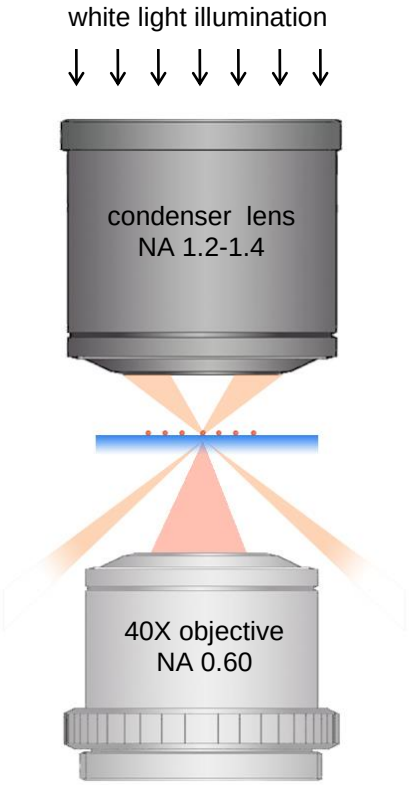

white light illumination
condenser lens
NA 1.2-1.4
40X objective
NA 0.60

Fig. S2

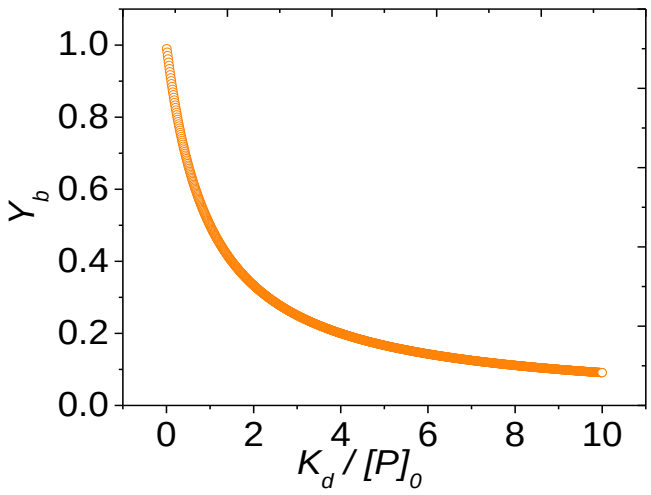


Fig. S3

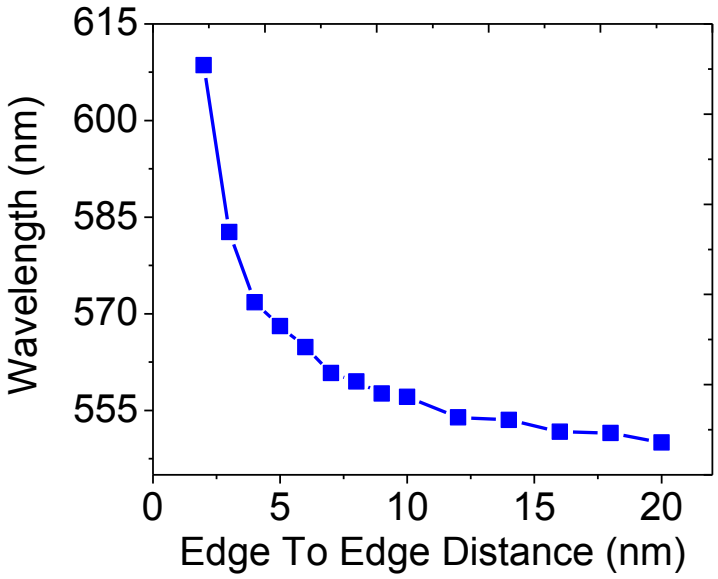

615
600
585
570
555
Wavelength (nm)
0
5
10
15
20
Edge To Edge Distance (nm)

Fig. S4

Figure S1. Assembly of reversible plasmon rulers. Electrophoretic analysis of plasmon ruler assembly. The first lane corresponds to Au nanoparticles (30 nm diameter) functionalized with avidin. The second lane corresponds to HPLC separated monomeric aptamer-Au nanoparticles (30 nm diameter). Monomeric aptamer-Au nanoparticles are then stochiometrically added to avidin-coated Au nanoparticles (1:1). The bottom band of the third lane corresponds to purified aptamer-Au plasmon rulers. A 40% yield of aptamer-Au plasmon rulers is estimated based on the intensity analysis of the gel (Image J). Representative transmission electron microscopy images of aptamer-Au plasmon rulers. Scale bar of the population image is 200 nm.

Figure S2. Single-particle imaging of plasmon rulers. Plasmon rulers were immobilized with one particle bound to the glass surface. Transmission-mode darkfield microscopy was used to visualize single plasmon rulers. Scale bar of the population image is 20 μm.

Figure S2. Percentage of protein molecules detected by plasmon rulers versus protein concentration. Y_b is the yield of the binding reaction, i.e., percentage of protein molecules that can conjugated with plasmon rulers at an initial concentration of $[P]_o$. In our experiment, the initial protein concentration is much higher than the initial plasmon ruler concentration. As a result, we have $Y_b = \frac{1}{\frac{K_d}{[P]_o}+1}$, as shown in the above plot. The percentage of detected protein molecules increases as the initial protein concentration increases.

Figure S4. Calculation of peak position of plasmon rulers. Electromagnetic simulation of peak position of coupled 30 nm Au nanoparticles. The distances are reported as edge-to-edge distances between the nanoparticles. As the distance between nanoparticles is decreased, the spectrum red-shifts. Simulations were performed in a homogeneous refractive index environment corresponding to water (n = 1.333) and consider only the shift in the longitudinal mode.

Supplementary Discussions

Detection limit of plasmon rulers

The reversible plasmon rulers work through the following ligand binding reaction:

MMP-3 Protein + Aptamer ⇌ Complex

The dissociation constant $\boldsymbol{K_d}$ of the formed complex is defined as below:

$$\frac{[P][A]}{[C]} = K_d$$

Where $[P], [A], [C]$ are the concentrations of protein, aptamer, and complex in equilibrium. For the detection of MMP-3 protein molecules, $[A]_o$ and $[P]_o$ are the initial concentrations of aptamer (plasmon rulers) and MMP3 proteins, while the initial concentration of complex is zero. If we assume a concentration of x proteins binded with aptamers after the reaction reaches equilibrium, we have:

$$\frac{[P][A]}{[C]} = \boldsymbol{K_d} = \frac{([P]_o - x)([A]_o - x)}{x} = ([P]_o - x)(\frac{[A]_o}{x} - 1)$$

So the percentage of protein molecules detected is:

$$Y_b = \frac{x}{[A]_o} = \frac{1}{\frac{K_d}{[P]_o - x} + 1}$$

In our experiments: $[P]_o \gg [A]_o > x$, so $Y_b = \frac{\boldsymbol{1}}{\frac{\boldsymbol{K_d}}{\boldsymbol{[P]_o}}+\boldsymbol{1}}$, independent of $[A]_o$;

The physical meaning of this relation is: our plasmon rulers could detect a portion of $\frac{\boldsymbol{1}}{\frac{\boldsymbol{K_d}}{\boldsymbol{[P]_o}}+\boldsymbol{1}}$ MMP-3 protein molecules at an initial protein concentration of $[P]_o$. When $Y_b = 1/2$, the required initial protein concentration $[P]_o = K_d$. In other words, half of the MMP-3 molecules can be detected using the plasmon rulers when the initial protein

concentration equals to the dissociation constant, which defines the detection limit of the plasmon rulers.

Reversibility of plasmon rulers

For the reversibility test of plasmon rulers in Figure 3, plasmon rulers were first reacted with 9 nM MMP3 protein molecules, and then exposed to 0 nM MMP3 protein molecules. A new set of equilibrium concentrations of free protein molecules, aptamers, and complex was established. If we assume y of complex is dissociated after the exposure to 0 nM MMP3 protein molecules, we have:

$$\frac{([P]_o + y)([A]_o + y)}{[C]_o - y} = \boldsymbol{K_d}$$

Where $[P]_o$, $[A]_o$, $[C]_o$ are the initial concentrations of protein, aptamer, and complex.

$$\frac{([P]_o + y)([A]_o + y)}{[C]_o - y} = \boldsymbol{K_d} = \frac{y([A]_o + y)}{[C]_o - y} = \frac{[A]_o + y}{\frac{[C]_o}{y} - 1}$$

So the yield of the dissociation reaction

$$Y_d = \frac{y}{[C]_o} = \frac{1}{\frac{[A]_o + y}{K_d} + 1}$$

In our experimental condition, y <$[C]_o$<< K_d and $[A]_o$ << K_d, the dissociation percentage is 100%, which means ~100% of the complex will be dissociated from the plasmon rulers, and the plasmon rulers could be retrieved for the next round of detection.

Kinetics of the binding reaction

By definition:

$$\boldsymbol{K_d} = \frac{\boldsymbol{k_{off}}}{\boldsymbol{k_{on}}}$$

Where $\boldsymbol{k_{off}}$ is the reaction constant for the dissociation reaction, $\boldsymbol{k_{on}}$ is the reaction constant for the binding reaction.

From Figure 3, the unbinding event completes on a timescale of less than one minute, which means $\boldsymbol{k_{on}}$>0.1 s^{-1}. In this reaction, $\boldsymbol{K_d}$=12.5 nM, so $\boldsymbol{k_{off}}$<8X10^5 $s^{-1}M^{-1}$.

Estimation of the protein concentration in Figure 4

From Figure 4, we can estimate around ¾=0.75 of the central peak positions have been shifted after the secretion of MMP3 in living cells. Then from the relation described in Figure S2, we have

$$Y_b = \frac{\mathbf{1}}{\frac{\boldsymbol{K_d}}{[\boldsymbol{P}]_o} + \mathbf{1}} = \mathbf{0.75}$$

So $[\boldsymbol{P}]_o$=37.5 nM