Supporting Information

Plasmonic Nanosensors for Simultaneous Quantification of Multiple Protein-Protein Binding Affinities

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Methods

Materials. Reagents, metallic salts, buffers, guanosine 5´-diphosphate (GDP) and other analytical grade chemicals were acquired from Sigma-Aldrich or Merck. Deionized water from a Millipore system (> 18 MΩ, Milli Q) was used in all experiments. Isothiocyanobenzyl-NTA was purchased from Dojindo EU GmbH. T-linker-DNA (5'-SH-TTTTTTTTTTT-3') and Thiolated-DNA (5'-SH-TTTTTTTTTTT-NH_2-3') were purchased from Biomers.net GmbH. The proteins were equilibrated against the working buffer (50 mM Tris-HCl pH 7.5, 100 mM KCl, 5 mM $MgCl_2$). For FtsZ we additionally incorporate to the buffer 0.05 mM of GDP.

Protein purification. *Escherichia coli* wild-type FtsZ was expressed and purified by the calcium-induced precipitation method.[1] Mutagenesis of the ZipA gene, overexpression, and purification were carried out by removing the part ranging of the N-terminal domain of wild type ZipA (amino acids 1-25 for s1ZipA, and amino acids 1-188 in the case of s2ZipA).[2, 3] For MinC, the plasmid pWM2688, encoding His-tagged MinC, was generated by amplifying the *minC* gene with primers 890 (GCGAGCTCTCAAACACGCCAATCGAGC) and 803 (CGGGATCCTCAATTTAACGGTTGAACGG) and cloning it as a *Sac*I-*Bam*HI restriction digested fragment into the equally restriction digested plasmid pWM2619 to render pWM2622 and then into *Nco*I-*Bam*HI-cleaved pET28a.[4, 5] After purification, FtsZ, MinC, s1ZipA and s2ZipA were dialyzed against the working buffer, frozen and stored at -80 °C in 20 μL aliquots.

Synthesis of gold nanorods. A stock of gold nanorods (AuNRs) was synthesized as follows. Seeds were prepared by rapid addition of ice cold 0.01 M sodium borohydride to a solution of 0.1 mM $HAuCl_4$ (tetrachloroauric acid) and 25 mM CTAB (hexadecyltrimethyl ammonium bromide) under vigorous stirring. 1 mL of this seed solution was stirred into 1000 mL of growth solution containing 0.1 M CTAB, 0.5 mM $HAuCl_4$, 0.6 mM ascorbic acid and 70 mM $AgNO_3$. The reaction was allowed to proceed for one hour at 30°C. The nanorods were separated from the excess of surfactant by centrifugation at 2,800 *g* for 20 minutes. Centrifugation was repeated twice, the supernatant was discarded each time and replaced with 0.05 M CTAB. The resulting gold nanorods have a width of 31.1 nm ± 5.6 nm and a length of 67.8 nm ± 9.2 nm (values are mean and standard deviation determined from TEM images of 486 particles, Figure S1).

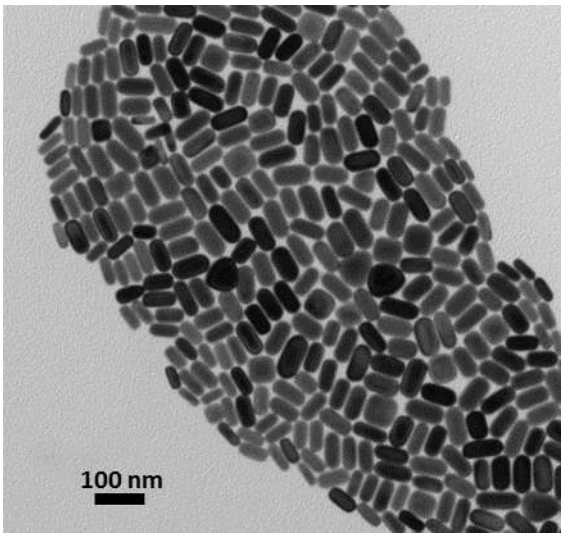

Figure S1. Transmission electron microscopy image of the gold nanorods used in our sensor. These rods have a width of 31.1 nm ± 5.6 nm and a length of 67.8 nm ± 9.2 nm (values are mean and standard deviation determined from 486 particles).

Particle functionalization:

DNA Stabilization. 500 µL of CTAB-stabilized gold nanorods were centrifuged at 6,300 *g* for 5 minutes. After carefully removing the supernatant, the nanorods were resuspended for 10 minutes in a solution containing 5 µL of 500 µM T-Linker DNA-SH (5'-SH-TTTTTTTTTT-3') with 5 µL of 100 mM TCEP and 20 µL of MilliQ water. Then 40 µl of 1% SDS solution were added to resuspend nanorod aggregates. After an additional centrifugation step, a pellet of nanorods was incubated for 1 hour in a solution containing 10 µL of 500 µM NH_2-DNA-SH (5'-SH-TTTTTTTTTT-NH_2-3'), 10 µL of 100 mM TCEP, 30 µL of 1% SDS and 40 µL of MilliQ water. After one hour, 2 µL of 4 M NaCl were added to this mixture with gentle shaking and this step was repeated seven times. The resulting mixture was incubated overnight at room temperature.

Ni^{2+}-NTA-functionalization. The DNA functionalized nanorods were cleaned from unreacted molecules by four centrifugation cycles (at 6,300*g* for 5 minutes), resuspended in PBS buffer, and in the last cycle reacted with 2µL of 1 mg/mL of isothiocyanobenzyl-NTA in 100 µL of PBS buffer (150 mM NaCl) for 3 hours. After that, 10 µL of the NTA-functionalized nanorods were washed twice with 100 µL of MilliQ water and incubated for 10 minutes with 50 µL of 1 mM $NiCl_2$. The resulting Ni^{2+}-NTA functionalized nanorod stock was stable for more than one month.

His-tagged protein immobilization. For the protein immobilization, we took 10µl of the Ni^{2+}-NTA functionalized nanorod stock, centrifuged once at 6,300 *g* for 5 min, and incubated the pellet in 100 µL of 50 nM His-tag protein (s1ZipA, s2ZipA or MinC) solution for 10 minutes. The excess of unbound protein was left in the solution.

Optical characterization. UV-Vis-NIR spectra of the gold nanorods before and after each of the three functionalization steps described above are shown in Figure S2. The two plasmon resonances of the short and long nanorod axis are clearly visible (at 518 nm and 639 nm). The plasmon shifts from the original CTAB rods at 639 nm, to 641 nm after DNA stabilization, to 643 nm after NTA coating and to 647 nm after protein immobilization. The slight redshift at each step was expected due to the increasingly larger molecular layer around the particle. In

the UV region, the presence of the DNA, NTA and s1ZipA was visible by their characteristic resonances: DNA at 260nm, NTA at around 270 nm, and protein at around 280 nm.

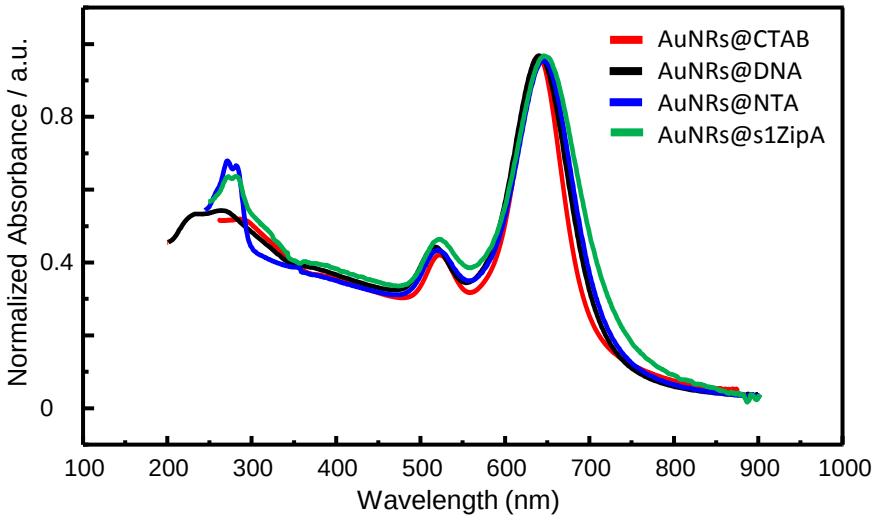

Figure S2. UV-vis-NIR spectra of gold nanorods covered with CTAB (red), DNA (black), NTA (blue), and s1ZipA (green).

Electroporetic characterization. Another check for stable particle functionalization is gel-electrophoresis, where an external electric field is used to drive particles in a gel (here, 0.5% w/v agarose). The migration speed depends on particle size and surface charge.[6] Figure S3 shows an unstained gel for nanoparticles with the following surface coverage: lane 1 with CTAB, lane 2 with DNA, lane 3 with NTA, lane 4 with Ni^{2+}-NTA, and lane 5 with s2ZipA. The particles in lane 6 were the same as those in lane 5 but after treatment with imidazole. Lane 7 shows vitamin B_{12}, a commonly used indicator to correct for electroosmotic effects. The CTAB coated nanorods aggregated, indicating that CTAB did not stabilize the particles under the conditions of electrophoresis. All other particles migrated, demonstrating their successful stabilization. Their differences in mobility were too small for us to claim further insights.

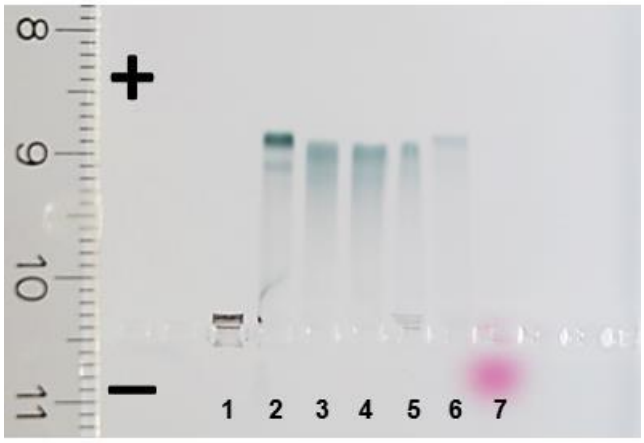

Figure S3. Electrophoretic mobility of nanorods.

Preparation of the sensor substrate. We used a flow cell, consisting of two cover slips held together by a piece of Nescofilm with a void area. One cover slip had two small holes for the connection of thin tubings as in- and outlet. The flow cell was cleaned by rinsing with 100 µL of Hellmanex II special cleaning concentrate and then with 1 mL of MilliQ water. Then, His-tagged protein-functionalized AuNRs were injected and incubated until an adequate surface density of attached particles was reached. Particles were electrostatically immobilized by flushing the previously filtered working buffer in the flow cell and, at the same time, unbound nanoparticles and unbound protein were removed with this procedure. After rinsing with working buffer, we recorded the position of each deposited nanoparticle by taking a photograph of the entire field of view under dark-field illumination. Software identified bright spots and assigned them unique identification numbers. By flowing in one nanoparticle batch at a time, we knew the specific functionalization of each particle in the field of view and grouped their responses accordingly.

Setup Improvements

We equipped a Zeiss Axio Observer Z1 inverted microscope with a PI542 XY-piezo stage (200 µm x 200 µm, closed-loop) and a PI721 Z-piezo (100 µm, closed-loop). Furthermore, for VIS-NIR-spectroscopy, we added an Imspector V10E transmissive imaging spectrograph with an Andor Luca R EM-CCD. The true color images are taken with a Canon EOS 5D Mark II (IR-filter removed). For the automated acquisition and analysis of many single particle spectra, we used MATLAB based control software. Details are described by Rosman et al. 2013.[7]

Experiments

Titration experiments. To carry out the titration experiments of the three interacting partners (FtsZ-s1ZipA, FtsZ-s2ZipA and FtsZ-MinC), solutions with concentration from 100 nM to 300 µM of FtsZ were flushed through the flow cell until the sensor response reached an equilibrium value after about 20 minutes. We used a flow rate of 30 µL/min to add the protein solutions, which should be high enough to avoid mass transport (diffusion) problems.

Sensor regeneration. The sensor was regenerated after s1ZipA binding by treatment with a solution containing imidazole (800 mM) in buffer for 10 minutes. This treatment removes the His-tag protein from the interaction with Ni^{2+}-NTA without deactivating the AuNRs@Ni^{2+}-NTA. A 15 minutes rinse with working buffer removed the imidazole.

SPR experiments. SPR measurements were conducted on a Reichert SPR Instrument to check the K_D values obtained by NanoSPR. The NTA-functionalized sensor chips (NIHCx) were purchased from Xantec Bioanalytics GmhB. Only one channel of the dual channel instrument was functionalized with His-tagged proteins after the incorporation of Ni^{2+} ions, the second channel served as reference. The titration experiments were carried out following the method described by Schuck[8] in the working buffer described in the materials section. In three separate experiments, we tested the interaction of FtsZ with a) s1ZipA, b) s2ZipA and c) MinC.

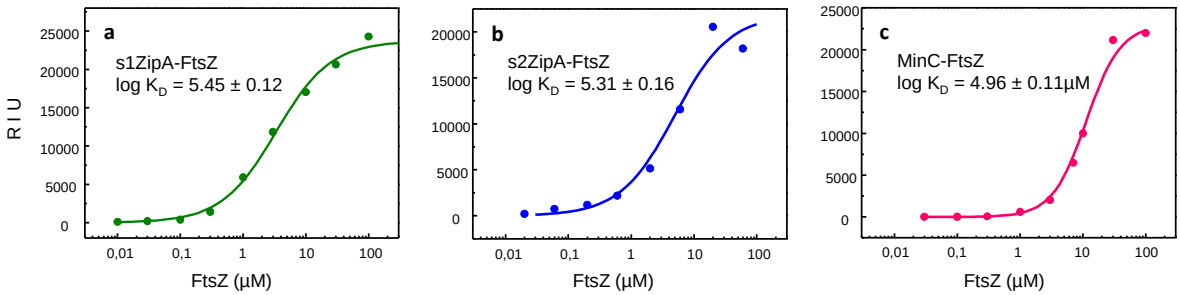

Figure S4. SPR titration curves. The values of RIU (corrected against the reference channel) in equilibrium are shown as function of FtsZ concentration as dots. The line shows Langmuir isotherms fitted to the experimental data. The fits can be used to extract K_D values as displayed.

Additional experiments. To demonstrate a wider range of K_D values obtained in one experiment and, at the same time, learn something about the form of interaction between ZipA and FtsZ, we performed an experiment with three groups of gold nanorods functionalized with: 1. Cysteamine, 2. NTA-Ni^{2+}-s1ZipA, 3. Cysteamine-s1ZipA. Cysteamine functionalization was carried out as described before[9] and confers a positive surface charge to the gold nanoparticles. It is known that this positive charge leads to unspecific binding of most water-soluble proteins.

The result of our experiment is shown in Figure S5 (group 1 in black, group 2 in blue, group 3 in red). The unspecific interaction of FtsZ with cysteamine nanorods shows a K_D value of 0.7 µM. The K_D value for FtsZ-s1ZipA interaction is 5.8 µM (consistent with our previous measurements). The third group of particles consisted of Cysteamine-functionalized nanorods first exposed to s1ZipA. Some s1ZipA proteins unspecifically attached to the gold nanorods, the rest was carefully removed. The interaction of FtsZ with the these non-specifically bound s1ZipA showed a K_D value of K_D = 25.2 µM. This value is much higher (indicating weaker interaction) than the 'native' conditions most likely due to partial denaturation of s1ZipA.

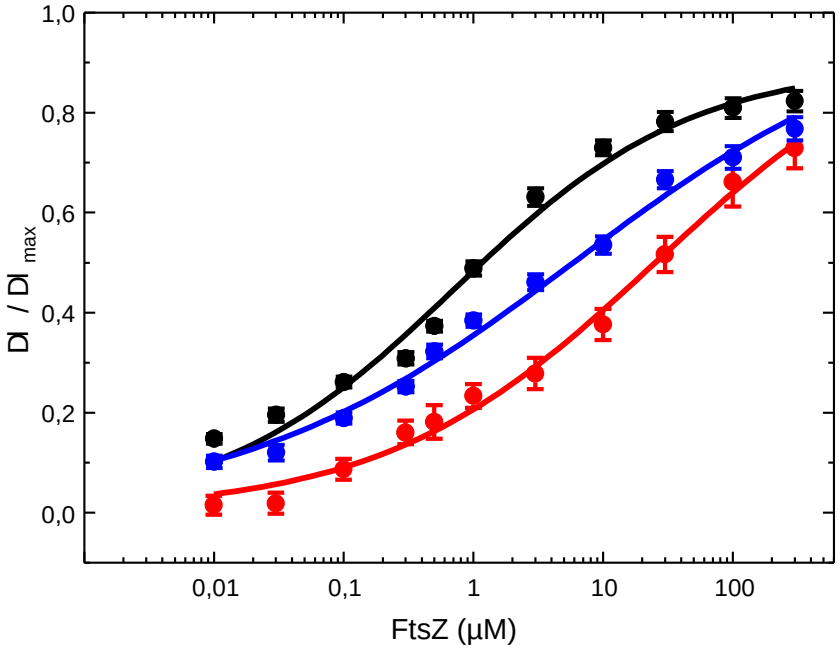

Figure S5. Additional experiment. The data points show the mean plasmon shift (and the corresponding error) observed on three groups of nanoparticles, obtained in a single experiment, for increasing target concentrations. As before, the target protein was FtsZ. The first group of particles was functionalized with positively charged Cysteamine (black), the second with Ni-NTA and s1ZipA (blue), the third with Cysteamine and unspecifically adsorbed s1ZipA (red). The curves show a fit to the Langmuir adsorption isotherm with K_D values of 0.7 µM (black), 5.8 µM (blue) and 25.2 µM (red).

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