Supporting information

Quantifying Ligand-Protein Binding Kinetics with Self-Assembled Nano-Oscillators

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Detection limit

The theoretical detection limit of nano-oscillators in distance is 1.5 nm (Figure 2c), which can be converted into charge using Eq. 2. The spring constant of DNA linkers are determined using freely-jointed chain model, given by

$$k = \frac{3k_BT}{nb^2}, \qquad \text{(S1)}$$

where b is the Kuhn length of DNA, and n is the number of segments of Kuhn length in the DNA. For a 245 nm DNA molecule, k is ~1×10^{-6} N/m. We estimated the electric field using the method

in reference 31 in the main text, which is about 2500 V/m. Thus, q is about 7.5 e (electron charge) after considering the charge screening effect. The surface area of 540 nm particle is ~1 μm^2, and the detection limit in charge density is 7.5 e/ μm^2.

To determine the detection limit for the ligand molecules, we calculated the charge of each ligand at pH 7.4 (Table S1). By assuming the charge change induced by the ligand upon binding is the same as the charge of the ligand, we obtain the detection limit for each ligand.

Table S1. Detection limit for different ligands.

Ligand	MW	Charge at pH 7.4	Detection limit
Anti-BSA	150 kDa	-1 e	1.9 pg/mm^2
1	320 Da	+1 e	4.0 fg/mm^2
ShK	4 kDa	+6 e	8.5 fg/mm^2

Equation of motion

The motion of a nano-oscillator driven by electrical force is described by,

$$m\frac{d^2z}{dt^2} + c\frac{dz}{dt} + kz = qE - (F_g - F_b), \quad \text{(S2)}$$

where m, z, c, q, F_g, and F_b are the mass, oscillation amplitude, damping coefficient, effective charge, gravity and buoyancy of the particle, k is the entropic spring constant of the DNA tethers, and $E = E_0 e^{j\omega t}$ is the applied electric field ($\omega = 2\pi f$ is the angular frequency, and f is the frequency of the field).

We estimate the value for each term and simplify the equation. In this work, the 540 nm silica particle (density ~2.0 g/cm^3) is tethered by 245 nm DNA and driven into oscillation by a field with f = 5 Hz. Thus, the inertia term $m\frac{d^2z}{dt^2}$ is ~10^{-8} pN. The damping term is described by,

$$c\frac{dz}{dt} = 3\pi\eta D\frac{dz}{dt}, \quad \text{(S3)}$$

where η and D are viscosity of the solution and diameter of the particle. The damping term is determined to be ~0.02 pN. The entropic term for fully stretched DNA (z = 245 nm) is ~0.2 pN, which is much greater than the inertia term and damping term. The term (F_g - F_b) is also small (~10^{-3} pN) compared to the entropic term. Eq. S2 is simplified as,

$$kz = qE. \quad \text{(S4)}$$

From Eq. S4 we obtained Eq. 2 in the main text.

Detection with 5 µm nano-oscillators using prism based plasmonic imaging setup

We fabricated 5 µm nano-oscillators and imaged the nano-oscillators with a prism based plasmonic imaging setup (SPRM 200, Biosensing Instrument Inc.). About 200 individual nano-oscillators can be imaged simultaneously, indicating potentially high-throughput detection capability (Figure S1a). Using 5 µm nano-oscillators also improves the signal to noise ratio, which is demonstrated with measuring the binding of compound **1** to KcsA-Kv1.3. Compared to the measurement using 540 nm nano-oscillators (Figure 4b), the lowest detectable concentration is 0.5 nM, which is 20-fold smaller. The k_a, k_d, and K_D were determined to be 9.0×10^5 $M^{-1}s^{-1}$, 2.9×10^{-3} s^{-1}, and 3.2 nM, respectively (Figure S1b).

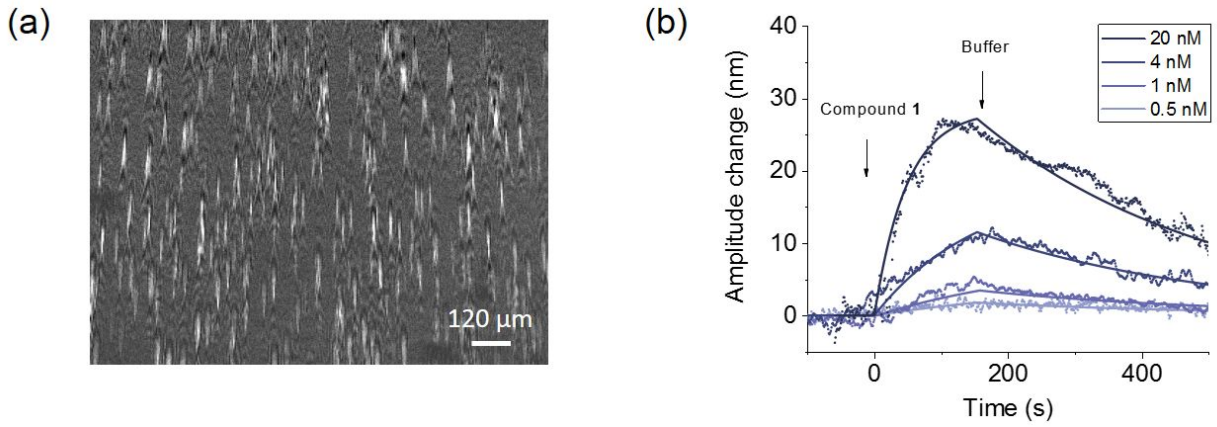

Figure S1. Binding kinetics results measured with 5 µm nano-oscillators. (a) About 200 individual nano-oscillators can be resolved simultaneously, indicating potentially high-throughput detection capability. Particle size, 5 µm; DNA length, 245 nm. (b) Binding kinetics of KcsA-Kv1.3 – **1** interaction measured with 5 µm nano-oscillators. The signal of each concentration was averaged over more than 10 individual nano-oscillators. Applied potential: U_0 = 1.0 V, f = 5 Hz. Buffer: 3 mM nanodisc buffer and pH = 7.4.

Particle-surface interactions

To study the interaction between nano-oscillators and the surface, we fabricated nano-oscillators on two surfaces covered with neutral spacers (MT(PEG)4) and negatively charged spacers (dithiolalkanearomatic PEG6-COOH), respectively, and recorded the oscillation in one minute. The result shows that negatively charged spacers reduce the sudden decrease in the oscillation amplitude due to stronger electrostatic repulsion between the particles and surface. (Figure S2).

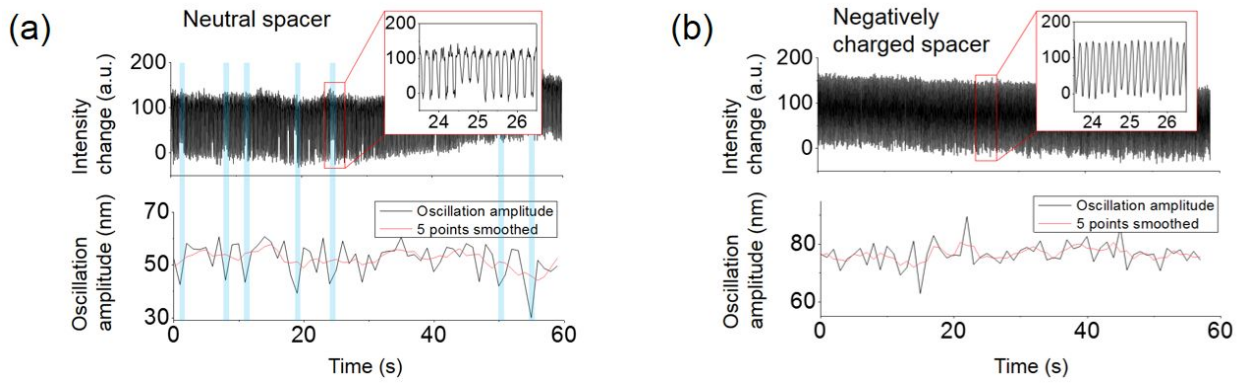

Figure S2. The oscillation of nano-oscillators on surfaces with different charge polarities. (a) Top panel shows oscillation profile of a nano-oscillator fabricated on a gold surface with neutral spacer (no charge) in one minute. The blue shadows mark where the nano-oscillator is trapped to substrate due to particle-substrate interaction. The inset figure shows a zoom-in of a trapping event. Bottom panel shows translation from intensity to oscillation amplitude with FFT. The blue shadows mark the defects induced by corresponding trapping events. The mean value and standard deviation of oscillation amplitude are 52.1 nm and 6.3 nm. (b) Oscillation profile of a nano-oscillator fabricated on a gold surface with negatively charged spacer in one minute. Particle trapping is reduced due to stronger electrostatic repulsion between the particles and substrate. Bottom panel shows translation from intensity to oscillation amplitude with FFT. The mean value and standard deviation of oscillation amplitude are 76.4 nm and 3.8 nm. Applied potential: U_0 = 0.3 V and f = 5 Hz. Buffer: 6 mM PBS and pH = 7.4.

Brownian motion and DNA density

We have optimized the density of DNA linkers on the surface by diluting with MT(PEG)4 spacers at a linker/spacer ratio of 1/6000, and the Brownian noise is significantly reduced. In Figure S3 we

show that the Brownian motion of nanoparticles increases with the decrease of DNA density, because the particle is tethered by less linkers and is more flexible.

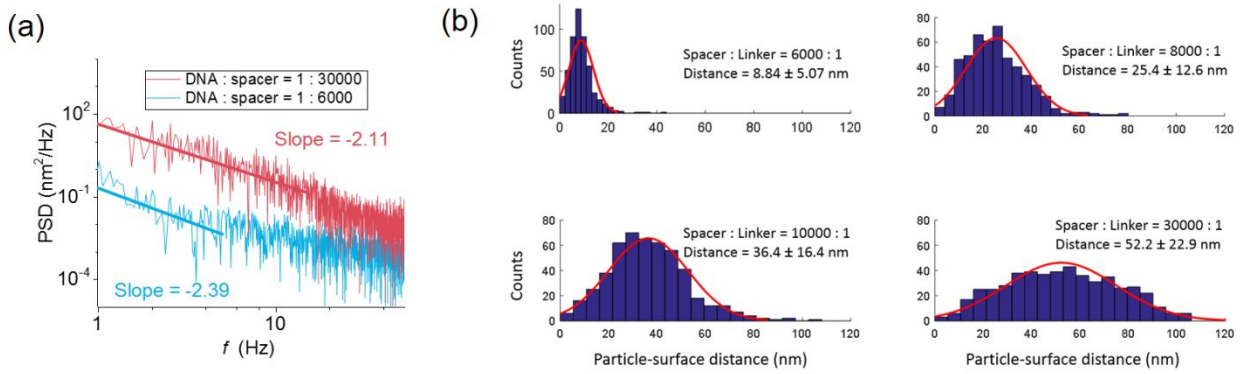

Figure S3. Brownian motion of nano-oscillators fabricated using different linker/spacer ratios. (a) Power density spectrum of the motion of nano-oscillators (without applying voltage) fabricated with different linker/spacer ratios. The solid curves are linear fits in low frequency region. The slopes of the fits are close to -2, indicating Brownian motion. (b) Brownian motion of nano-oscillators in z direction increases with the decrease of DNA density. The histogram of particle-surface distance is obtained by measuring the distance in every 100 ms for a total of 50 seconds. The red curve is fitting of the data to normal distribution.

Effects of DNA length on oscillation amplitude

Besides the 245 nm DNA linkers mentioned in the main text, we have synthesized DNA linkers of two different lengths (102 nm and 500 nm) using polymerase chain reaction (PCR). The 102 nm and 500 nm DNA are segments from λ DNA at positions of 4135-4436 and 2966-4436, respectively. The segments share the same reverse primer 5' Biotin-TAC GCA GCT CTG CTG TCA CTC-3'. The forward primer for 102 nm DNA is 5' Thiol – AGT TTT CAG GAA GCC

CGC AGT-3', and for 500 nm DNA is 5' Thiol-GTG TGG ATG CAG CCC TGT T-3'. After PCR and purification, the DNA linkers were characterized by gel electrophoresis as shown in Figure S4a.

The length of DNA linkers should be long enough to have as broad moving range as possible but should not exceed the depth of evanescent field. To find an appropriate length, we measured the oscillation amplitude of 540 nm particles tethered by 102 nm, 245 nm, and 500 nm DNA linkers respectively (Figure S4b). The amplitude plateau of 500 nm linkers is determined to be ~250 nm, indicating the particles exceed the sensing depth due to overlong linkers. Both 102 nm and 245 nm linkers are within the sensing depth because the maximum amplitude is close to their linker length. This is confirmed by the oscillation profiles of the three DNA linkers, as shown in Figure S4c. Unlike the oscillation of 102 nm and 245 nm, the oscillation of 500 nm DNA has a flat valley, which indicates the particle moves beyond detection range. Both the 102 nm and 245 nm linkers are good for fabricating nano-oscillators, but the 245 nm linkers provide broader moving range for particles thus enable detection for more charges.

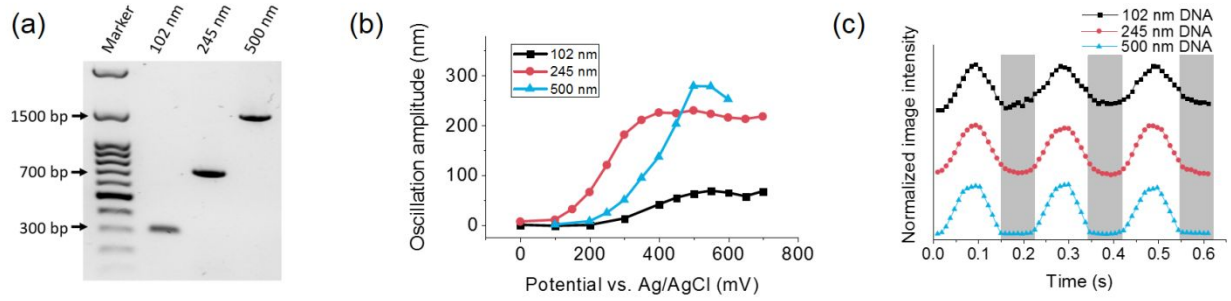

Figure S4. Oscillation of nano-oscillators with DNA linkers of different lengths. (a) Characterization of DNA linkers with gel electrophoresis. From left to right: marker, 102 nm DNA, 245 nm DNA, and 500 nm DNA. (b) The oscillation amplitude of 540 nm silica nanoparticles tethered by 102 nm, 245 nm and 500 nm DNA linkers. The solution is 6 mM PBS and the

frequency of field is 5 Hz. (c) The oscillation profiles of 540 nm nano-oscillators fabricated with 102 nm, 245 nm, and 500 nm DNA linkers. The dark shadows mark the valley of intensity, which shows that the 500 nm DNA linker exceeds the detection range.