SUPPORTING INFORMATION

Semi-Quantification of the Binding Constant Based on Bond Breaking in a Combined Acoustic–Gravitational Field

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Detail procedures of molecular modification to the particle or glass plate.

The carboxylate groups on PS particle (100 μL, 1.4×10^6 mL^{-1}) was activated by adding 100 μL EDC/NHS mixture (30/36 mg mL^{-1}). The surface-activated PS particles were washed using milli-Q water to remove the unreacted EDC/NHS solution. Then, 10 μL of the PS particle suspension (1.4×10^5 mL^{-1}) was added to 2 nmol DNA with various base pairs, Ibu, Man, Gly, Cip or Nap solutions to covalently bind on the particle surface though the condensation reaction. The resultant suspension was washed using 100 mM PBS with 0.5% Tween 20 and milli-Q water.

The glass plate was immersed in 3-glycidylozypropyltrimethoxysilane solution for 1 h to modify the epoxy group on the glass surface through the silane coupling. The resultant glass plate was washed using milli-Q water. Then, the epoxy-modified glass plate was immersed in BSA or ConA solutions including 100 mM boric acid buffer at pH 9.18 with stirring for 4 h to induce the ring-opening reaction of epoxy group. The molecular-modified glass plate was washed using milli-Q water.

For the α determination, the capture and reporter DNA with 20 bases as shown in Table S1 were modified onto the PS microparticle and AuNP using EDC/NHS reaction, respectively. The protocol was the same as above.

Principles of microparticle dissociation in the CAG field.

When the ultrasound standing wave is vertically generated in the channel, acoustic radiation forces (F_{ac}) and sedimentation forces (F_{sed}) are exerted on an microparticle that is immobilized on the glass plate, as shown in Figure 1. F_{ac} and F_{sed} are expressed by the following equations[18]:

$$F_{ac} = \frac{8\pi^2}{3\lambda} r^3 \alpha V^2 \left(\frac{5\rho' - 2\rho}{2\rho' + \rho} - \frac{\gamma'}{\gamma} \right) sin\left(\frac{4\pi z}{\lambda} \right) \quad \text{(S1)}$$

$$F_{sed} = \frac{4}{3}\pi r^3 (\rho' - \rho) g \quad \text{(S2)}$$

where λ is the ultrasound wavelength (1.5 mm in the present study), r is the radius of the particle, α is a device-dependent parameter, V is the voltage applied to the transducer, z is the distance of the particle from the node of the standing wave (the node is defined as z = 0), g is the gravitational acceleration, ρ and γ are the density and compressibility, respectively, of the medium (in the present study, water), and the prime ($'$) identifies the corresponding properties of the particle.

As V increases, F_{ac} increases. Because the interaction force between microparticle and glass plate (F_{bind}) and F_{sed} is constant, the microparticle is dissociated from glass plate when the total force of F_{ac} and F_{sed} exceeds the F_{bind} by increasing V. Thus, we can obtain the following equation from the fact that $F_{bind} = F_{ac} + F_{sed}$ whenever the microparticle is dissociated.

$$V = \sqrt{\frac{F_{bind} - A}{B}}, \quad \text{(S3)}$$

$$A = \frac{4}{3}\pi r^3 g(\rho' - \rho), \quad \text{(S4)}$$

$$B = \frac{8\pi^2}{3\lambda} r^3 \left(\frac{5\rho' - 2\rho}{2\rho' + \rho} - \frac{\gamma'}{\gamma} \right) \alpha sin\left(\frac{4\pi z}{\lambda} \right), \quad \text{(S5)}$$

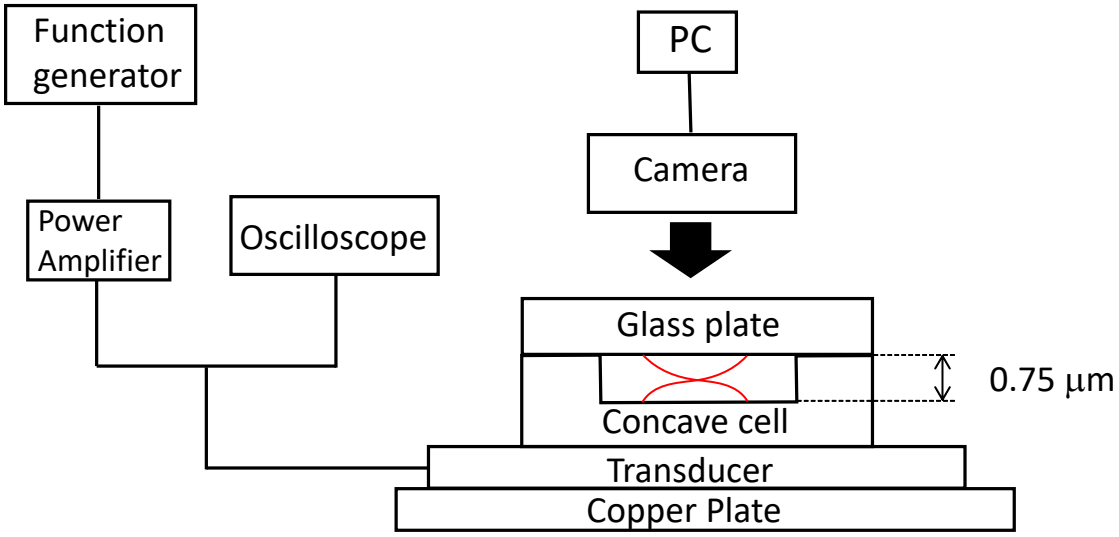

Figure S1 Schematic of the setup for the instrument.

Table S1 Sequences of nucleotides used in this study.

DNA	Sequences (5′→3′)	Functional group
Anchor DNA (3)	AAA	Amino(5′)
Anchor DNA (4)	AAAA	Amino(5′)
Anchor DNA (5)	AAAAA	Amino(5′)
Anchor DNA (6)	AAAAAA	Amino(5′)
Anchor DNA (7)	AAAAAAA	Amino(5′)
Capture DNA (3)	TTT	Amino(5′)
Capture DNA (4)	TTTT	Amino(5′)
Capture DNA (5)	TTTTT	Amino(5′)
Capture DNA (6)	TTTTTT	Amino(5′)
Capture DNA (7)	TTTTTTT	Amino(5′)
Capture DNA (20)	TTTTCCCCCCCCCCCCCCC	Amino(5’)
Reporter DNA (20)	GGGGGGGGGGGGGGGGAAAA	Amino(5’)

The numbers in parenthesis after the DNA name represent the number of Adenine or Thymine.

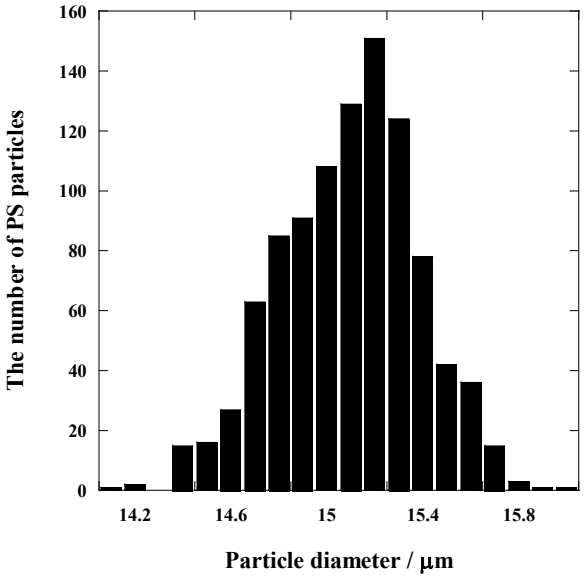

Figure S2. Size distribution of PS particles determined using a microscope. The average size of PS particle was 15.1 ± 0.4 μm.

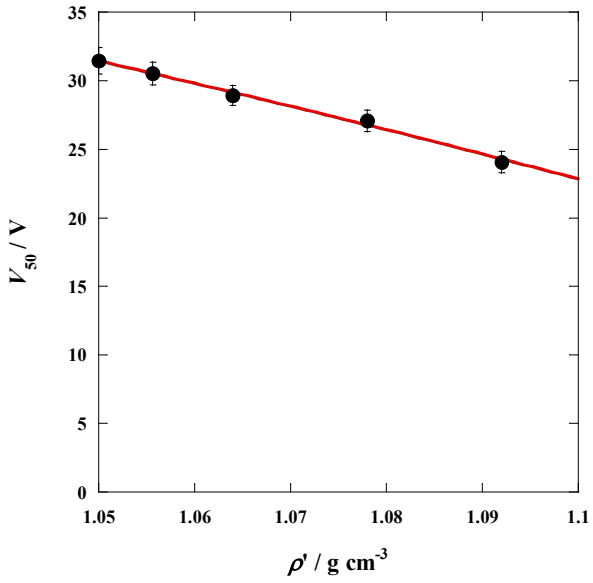

Figure S3. Relationships between ρ' and V_{50}. The ρ' values were changed by binding of AuNPs to PS particles. The red curve was the result of curve-fitting using a = 0.031 as a fitting parameter based on Eqs. (1)–(3).

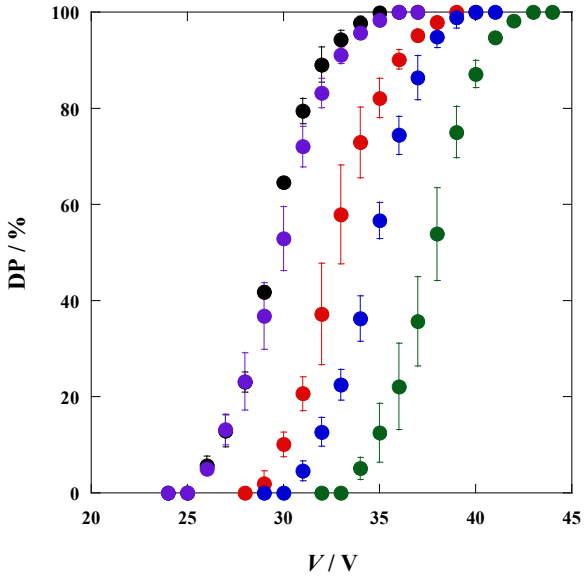

Figure S4. Relationships between *V* and the DP for the PS microparticles. Black: BSA–Ibu, purple: BSA-Cip, red: ConA–Man, blue: ConA–Gly, and green: BSA-Nap.