Supplementary Information

Ratiometric Detection of Oligonucleotide Stoichiometry on Multifunctional Gold Nanoparticles by Whispering Gallery Mode Biosensing

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Experimental setup

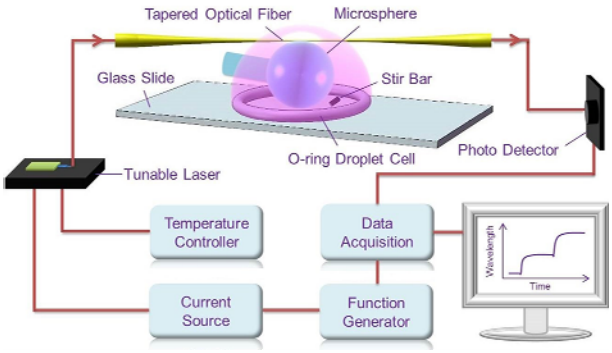


Figure S1. Experimental setup for WGM biosensor.

The experimental setup for the ratiometric detection of GNP oligonucleotide stoichiometry is shown in figure 2. Light from a wavelength-tunable distributed feedback diode tunable laser (~1310 nm, mounted on LDM 4980, ILX Lightwave, Bozeman, Montana, USA) was coupled into the microsphere through a tapered optical fiber (SMF-28e, Corning Inc., Corning, New York, USA). The WGM wavelength was determined by swept wavelength scanning, using a function generator (33120A, Hewlett Packard, Englewood, Colorado, USA) to modulate the laser diode current source (LDX-3207B, ILX Lightwave, Bozeman, Montana, USA). The temperature of the laser diode was thereby kept constant with a thermoelectric temperature controller (LDT-5525, ILX Lightwave, Bozeman, Montana, USA). The output light was detected by a photo detector (PDA 10CS-EC, Thorlabs Inc, Newton, New Jersey,

USA). The transmission spectrum was then acquired by a data acquisition card (NI PCI-6036E, National instruments, Austin, Texas, USA) and LabVIEW software.

Materials and methods

Oligonucleotide strands were synthesized by Eurofins Genomics. Streptavidin was ordered from New England Biolabs (NEB). Biotin-dextran (70,000 MW) was purchased from Marker Gene Technologies, Inc. Gold nanoparticles (15 nm) were bought from Nanopartz TM Inc. Tris-EDTA buffer solution (pH 8.0), MgCl2, Tris-Borate-EDTA buffer (10×concentrate), Glycerol, Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and Agarose - TBE Blend 1.0% were obtained from Sigma Aldrich. All the reagents were used without further purification. Ultra-filter (MW 3,000) was bought from Merck Millipore. Delicate Task Wipers were ordered from Kimtech Science*.

Thiol-modifided oligonucleotide strands treatment:

1.2 μL of 500 mM fresh prepared Tris (2-carboxyethy) phosphine (TCEP) and 60 μL of 100 μM of DNA solution were incubated for 2 hours at room temperature to remove any disulfide bonds. The excessive TCEP was removed by passing through a 3 K ultra-filter. The reduced thiolated-oligonucleotide was then collected.

GNP modification:

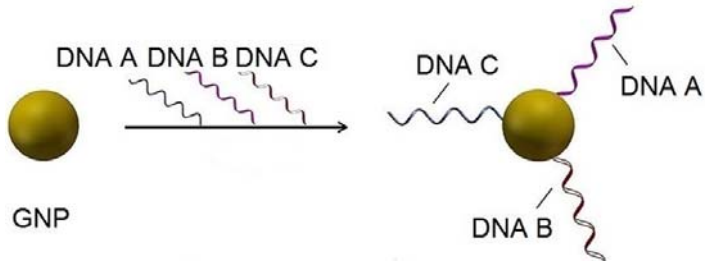


Figure S2. GNP functionalized by DNA A, DNA B and DNA C

Small GNPs with a diameter of 15 nm were mixed with fresh cleaved thiolated-oligonucleotide strands in pH 8.0 buffer (0.5×TBE and 50 mM NaCl) to obtain final concentration of 10 nM GNPs and 2 μM oligonucleotides. After incubating for 8 hours at room temperature, 50 mM NaCl was added to the solution.

This step was repeated 5 times until the final concentration of NaCl was 300 mM. To get rid of excess DNA, the mixture was centrifuged at 5,000 rcf for 10 min. Following removal of supernatant, the red precipitates were washed 3 times with pH 8.0 buffer (0.5×TBE and 50 mM NaCl) to remove excessive DNA and then redispersed in the same buffer.

Oligonucleotide concentration determination:

Oligonucleotide concentration was estimated by measuring the absorbance at 260 nm on an UV-3600 ultraviolet-visible spectrophotometer (Shimadzu Corporation). The concentration can be calculated by Lambert-Beer Law using the extinction coefficients: $\varepsilon_{DNA\,A} = 2.41\times10^5\ M^{-1}cm^{-1}$, $\varepsilon_{DNA\,B} = 2.56\times10^5\ M^{-1}cm^{-1}$, $\varepsilon_{DNA\,C} = 2.67\times10^5\ M^{-1}cm^{-1}$, $\varepsilon_{DNA\ mixture} \approx (\varepsilon_{DNA\,A}+\varepsilon_{DNA\,B}+\varepsilon_{DNA\,C}) / 3 = 2.55\times10^5\ M^{-1}cm^{-1}$.

$C = A / (\varepsilon \times I)$

Where

C = Nucleic acid concentration (M).

A = Absorbance (OD_{260}).

I = Width of the cuvette, 1 cm here.

ε = Extinction coefficient ($M^{-1}cm^{-1}$), calculated from the equation[1] $\varepsilon = N_A\times15.2 + N_G\times12.1 + N_C\times7.05 + N_T\times8.4$, where N_A, N_G, N_C, and N_T are the number of times each nucleotide represented in the sequence of nucleotide.

GNP concentration determination:

The concentration of GNPs was estimated by Lambert-Beer Law mentioned above, where ε equals to $2.4\times10^8\ M^{-1}\,cm^{-1}$ according to the results reported by Sarah J. Hurst[2] *et. al,* with A being the mean absorbance (OD_{524}).

Agarose gel electrophoresis:

Agarose gel electrophoresis was carried out on a Mini-sub cell GT system from Bio-Rad. 10 μL of concentrated oligonucleotide-GNP sample was mixed with 5 μL of

glycerol. The mixture was then loaded on 1% agarose gel and run for 15 min at 90 V in 1×TBE buffer. As a reference, pure GNP mixing with glycerol was loaded on the same gel.

Microsphere fabrication and modification:

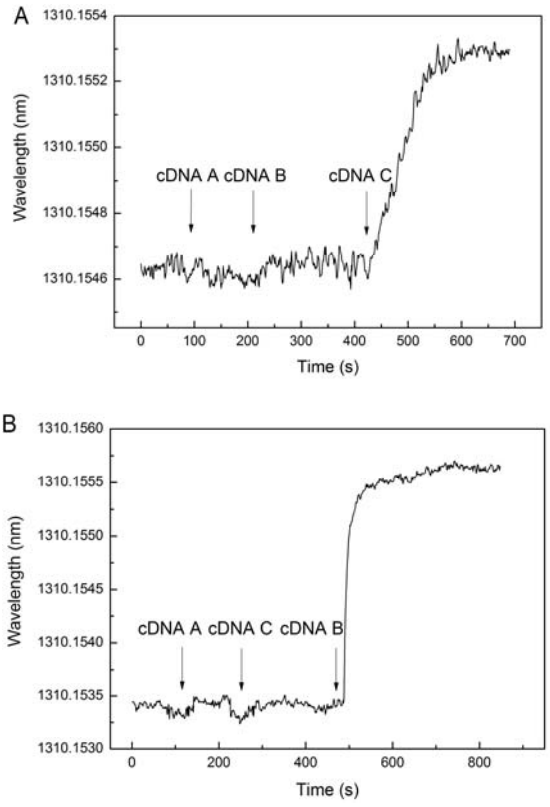
A short (~ 5 cm) length of SMF-28e optical fiber was cut followed by stripping off its polymeric coating at one end using a fiber stripper. The stripped fiber was then cleaned by an acetone-soaked wiper. After melting the tip of striped fiber with a butane-oxygen microtorch, the microsphere was formed by surface tension. Freshly prepared microsphere was cleaned in an air plasma cleaner (PDC-32G, Harrick, Ossining, New York, USA) for 5 min. The sphere was then incubated in a 2 μL hanging drop of dextran-biotin solution (10 mg/ml) until dry, then rinsed in water for 5 min. 24.6 μL of 1 mg/ml streptavidin was mixed with 10 μL of 100 μM biotin-oligonucleotide (cDNA A*, cDNA B*, or cDNA C*) solution to form biotin-oligonucleotide-streptavidin complex. After that, the sphere was incubated in a 2 μL hanging drop of biotin-oligonucleotide-streptavidin complex solution for 10 min, and then immersed in a chamber filled with diluted biotin-oligonucleotide-streptavidin solution for 1 h. Afterwards, the microsphere was immersed in 2 μL hanging drop of 10 nM oligonucleotide-GNP solution until dry followed by rinsing in water for 1h.

Ratiometric detection:

The modified microsphere was fixed on a 3 dimension differential translation stage then coupled to a tapered fiber for WGM excitation. Afterwards, the coupled sphere and fiber were immersed in a droplet cell of pH 8.0 Tris-EDTA-MgCl2 buffer (10 nM pH 8.0 Tris-HCl, 1 mM disodium EDTA, and 12.5 mM MgCl2)[15]. After waiting for the WGM baseline shift signal to become stable, the first cDNA strand was added to the chamber under slow stirring with a micro stir bar to homogenize the hybridization reaction. The second cDNA strand was injected after the WGM shift signal equilibrated.

Test of nonspecific binding:

To evaluate cross-affinity between nucleotides and nonspecific binding on the microsphere, monofunctional GNP modified only with DNA C was conjugated to a microsphere's surface through the DNA C and cDNA C* hybridization. Nonspecific binding was assessed by the subsequent addition of non-complementary strands cDNA A, cDNA B and complementary strand cDNA C. As shown in figure S3A, after the injection of non-complementary strands cDNA A and cDNA B, the WGM wavelength signal did not show any significant shift. However, with the addition of the complementary strand cDNA C, a clear read shift due to the binding of cDNA C with DNA C on the GNP surface is observed. Similar results were observed in the case of monofunctional GNPs modified with DNA B or DNA C (figure S3B, S3C).



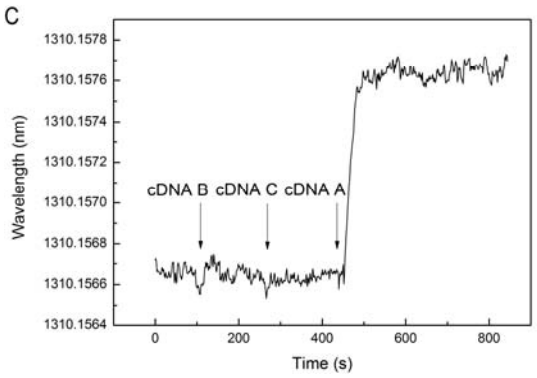


Figure S3. Nonspecific binding test using GNPs-DNA C (A), GNPs-DNA B (B) and GNPs-DNA A (C). Arrows indicate the injection time points.

Table S1. Oligonucleotide sequences

Name	Sequence
DNA A	5’ - Thiol - AAAAA AACCT GGGTT AGTAT - 3’
DNA B	5’ - Thiol - AAAAA AAATG ACCTA CAGAG - 3’
DNA C	5’ - Thiol - AAAAA AAGGA AGGAG GCGTA - 3’
cDNA A	5’ - ATACT AACCC AGG - 3’
cDNA B	5’ - CTCTG TAGGT CAT - 3’
cDNA C	5’ - TACGC CTCCT TCC - 3’
cDNA A*	5’ - Biotin - ATACT AACCC AGG - 3’
cDNA B*	5’ - Biotin - CTCTG TAGGT CAT - 3’
cDNA C*	5’ - Biotin - TACGC CTCCT TCC - 3’

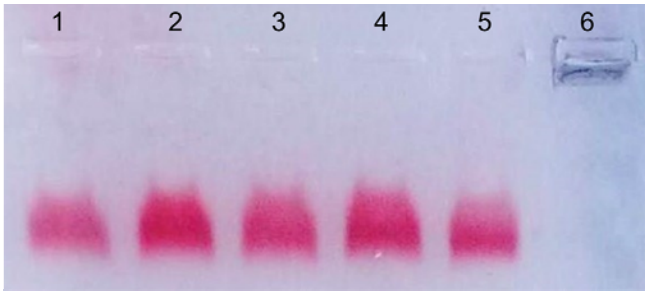


Figure S4. 1% agarose gel electrophoresis analysis of oligonucleotide-GNP

conjugates. The ratio of DNA A to DNA B to DNA C during thiol reaction were 1:1:1 (lane 1), 1:2:1.5 (lane 2), 1:4:2.5 (lane 3), 1:6:3.5 (lane 4), and 1:8:4.5 (lane 5). Bare 15 nm gold nanoparticle control is shown in lane 6.

[1] J. Sambrook, D. W. Russell. 2001. Molecular cloning: a laboratory manual, 3rd ed., vol. 2, Cold Spring Harbor Laboratory Press, New York, U.S.A. PP. 10.13-10.14

[2] S.J. Hurst, A. K. R. Lytton-Jean, C. A. Mirkin, Anal.chem. 2006, 78, 8313.