Electronic supplementary information (ESI)

Kinetic Study of DNA Hybridization on DNA-modified Gold Nanoparticles with Engineered Nano-Interfaces

*Akari Takashima and Motoi Oishi**

Division of Materials Science, Faculty of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8573, Japan.

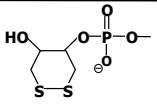
*To whom correspondence should be addressed: Motoi Oishi (E-mail: oishi@ims.tsukuba.ac.jp)

Chemicals and Instruments

Citrate-coated 15 nm gold nanoparticle solution (GNP: BBI solution, USA), sodium chloride (NaCl: Wako, Japan), phosphate buffer powder (pH 7.0; PB: Wako, Japan), 2-mercaptoethanol (Sigma-Aldrich, USA), Tween 20 (Wako, Japan), DEPC-treated water (Wako, Japan), bovine serum albumin (BSA: Jackson ImmunoResearch, USA), and mono-thiolated PEGs with different molecular weights (SH-mPEG$_{2k}$, SH-mPEG$_{6k}$, and SH-mPEG$_{10k}$: Sigma-Aldrich, USA) were used without further purification. Water was purified using the Milli-Q system (Millipore, USA). Oligonucleotides were purchased from Tsukuba Oligo Service, Co., Japan, and the DNA sequences employed are shown in Table S1. Centrifugation of GNP solutions was carried out for 15 min at 25 °C and 20,000 *g* using Micro Refrigerated Centrifuge Model 3740 (Kubota, Japan). UV/vis, and fluorescence spectra were recorded using a UV-2550 spectrometer (Shimadzu, Japan) and an F-7000 spectrometer (Hitachi, Japan), respectively. Dynamic light scattering (DLS) measurement was carried out in PB (10 mM; pH 7.0 containing 0.15 M NaCl) without BSA at 20 °C using Zetasizer Nano-ZSP (Malvern, UK) equipped with a 4.0 mW He-Ne laser (λ = 633 nm).

Table S1. DNA sequences used in this study.

Name	Sequence
probe DNA	**5′–dithiol–AAA AAA GAA AGA GGA GTT AA–3′ (20 mer)**
Q-probe DNA	**5′–BHQ-1–AAA AAA GAA AGA GGA GTT AA–3′ (20 mer)**
diluent DNA (A_{20})	**5′–dithiol–AAA AAA AAA AAA AAA AAA AA–3′ (20 mer)**
target DNA	**5′–TTA GAG TTG CAT GGA TTA ACT CCT CTT TCT–FAM–3′ (30 mer)**

Complementary sequences (15 mer) are represented in underlined portions. **5′–dithiol:** 

Preparation of probe-GNP and probe/diluent-GNP

Both probe-GNP and probe/diluent-GNP were prepared according to methods reported in the literature,[1] with minor modifications. For the preparation of probe-GNPs, a citrate-stabilized 15 nm GNP solution (250 μL, 2.3 nM, 1.4×10^{12} particles/mL) was added to probe DNA (18 μL, 25 μM) in water for the preparation of probe-GNPs (final concentrations were as follows: [GNP] = 2.15 nM; [probe DNA] = 1.7 μM). For the preparation of probe/diluent-GNP, a citrate-stabilized 15 nm GNP solution (250 μL, 2.3 nM, 1.4×10^{12} particles/mL) was added to a mixture of diluent DNA (16 μL, 25 μM) and probe DNA (2 μL, 25 μM) in water (final concentrations were as follows: [GNP] = 2.15 nM; [diluent DNA] = 1.5 μM; [probe DNA] = 0.19 μM). The resulting mixtures were incubated at room temperature for 3 h. An appropriate amount of PB (100 mM; pH 7.0 containing 1.5 M NaCl) was then added stepwise to bring the final NaCl concentration to 300 mM. After incubation for 38 h, the final mixtures were centrifuged three times at 20,000 *g* for 15 min to remove free DNA. After the removal of the supernatants, 250 μL of PB (10 mM; pH 7.0 containing 0.15 M NaCl) was added to the red oily precipitate to re-disperse probe-GNP and probe/diluent-GNP. The prepared probe-GNP and probe/diluent-GNP solutions were stored at 4 °C. Approximately 40 ~ 50 % of the original concentration of GNPs was lost during preparation.

Preparation of probe/PEG_x-GNPs (x = 2k, 6k, and 10k)

The preparation of probe/PEG_x-GNPs (x = 2k, 6k, and 10k) was carried out by means of methods reported in the literature,[2] with minor modifications. The concentration and amount of the additive of each PEG and probe DNA for the preparation of probe/PEGx-GNPs are shown in Table S2. As a typical example, the preparation of probe/PEG_{2k}-GNP is described as follows: To

a mixture of citrate-stabilized 15 nm GNP (1.0 mL, 2.3 nM) and Tween 20 (10 μL, 1 wt% in water), probe DNA (61 μL, 20.4 μM) in water was added. The resulting mixture was incubated at room temperature for 1 h and then SH-mPEG$_{2k}$ (63 μL, 20 μM) in water was added. After incubation for 1 min, 126 μL of PB (100 mM; pH 7.0 containing 1.5 M NaCl) was added (final concentrations were as follows: [probe DNA] = 0.98 μM; [SH-mPEG$_{2k}$] = 1.0 μM; [NaCl] = 150 mM). The resulting mixture was further incubated at room temperature for 1 h. The final mixtures were centrifuged three times at 20,000 *g* for 15 min to remove Tween 20, free DNA, and PEGs. The supernatant was removed, and then 250 μL of PB (10 mM; pH 7.0 containing 0.15 M NaCl and 1 wt% of BSA) was added to the red oily precipitate to re-disperse probe/PEG$_{2k}$-GNP. The prepared probe/PEG$_{2k}$-GNP solution was stored at 4 °C. Approximately 10 % of the original concentration of GNPs was lost during preparation. The preparation of PEG$_x$-GNPs (x = 2k, 6k, and 10k) without the probe DNA was carried also out using the above procedure, and their sizes are listed in Table S3.

Table S2. Concentration and additive amount of each PEG and probe DNA for the preparation of probe/PEG$_x$-GNPs (x = 2k, 6k, and 10k).

	PEG solution	probe DNA solution
	concentration / additive amount	concentration / additive amount
probe/PEG$_{2k}$-GNP	20 μM / 63 μL	20.4 μM / 61 μL
probe/PEG$_{6k}$-GNP	20 μM / 15 μL	20.4 μM / 27 μL
probe/PEG$_{10k}$-GNP	20 μM / 12 μL	20.4 μM / 27 μL

Table S3. Size of PEG$_x$-GNPs (x = 2k, 6k, and, 10k).

	size [a] (nm)
PEG$_{2k}$-GNP	25.3
PEG$_{6k}$-GNP	28.4
PEG$_{10k}$-GNP	34.6

[a]determined by DLS measurement.

Quantitation of Immobilized Probe DNA Strands on GNPs

Quantification of immobilized probe DNA strands on GNPs was carried out according to methods reported in the literature.[1] FAM-labeled DNA (FAM-DNA) with 20 nucleotides (A_{20}) was used in place of probe DNA and diluent DNA to quantify the amount of immobilized DNA strands on the GNPs. Procedures for the preparation of FAM-DNA-modified GNPs and FAM-DNA/PEG-co-modified GNPs were the same as those described above. First, 143 mM 2-mercaptoethanol in PB (10 mM; pH 7.0 containing 0.15 M NaCl) was added to the FAM-DNA immobilized GNP solution to bring the final concentration of 2-mercaptoethanol to 35.75 mM. After incubation for 24 h at room temperature, the solutions were centrifuged at 20,000 *g* for 15 min to separate the displaced DNA from the GNP aggregates. Aliquots of the supernatant were analyzed by fluorescence spectroscopy. Fluorescence maxima (measured at 520 nm) were converted to molar concentrations of FAM-DNA by interpolation from a standard linear calibration curve prepared with known concentrations of FAM-DNA using identical buffer pH, salt, and 2-mercaptoethanol concentrations. The average number of probe DNA strands per particle was obtained by dividing the measured DNA molar concentration by the GNP concentration.

Measurement of DNA Hybridization on GNPs and in Solution

The instrument settings were chosen as follows: λ_{ex} = 492 nm (slit 5 nm), emission spectra = 500–600 nm (slit 5 nm), and PMT detector voltage = 1000 V. The fluorescence intensity at 520 nm was used to evaluate the DNA hybridization kinetics. To a solution of GNPs or Q-probe in PB (10 mM; pH 7.0 containing 0.15 M NaCl and 1 wt% BSA) in a quartz cell, a solution of FAM-labeled target DNA (14 μL, 150 nM) in PB (10 mM; pH 7.0 containing 0.15 M NaCl and 1

wt% BSA) was added. The total volume and final concentrations of target DNA and probe DNA were adjusted to 300 μL and 7 nM ([target DNA] = [probe DNA] = [Q-probe]), respectively. The resulting mixture was incubated at 20 °C for 5400 sec (equilibrium). During the incubation, fluorescence spectra were measured at appropriate time intervals.

Estimation of Hybridization Efficiency (HE)

Hybridization efficiency (HE) (%) at hybridization time = t (sec) for Q-probe and GNPs was calculated from equation S(1),

$$\text{HE (\%)} = 100(1-F_t / F_0) \qquad \text{eq. S(1)}$$

where F_0 and F_t, are fluorescence intensities at $t = 0$ (sec) and t (sec), respectively.

Second-Order Kinetic Model for DNA Hybridization in Solution (Q-probe)

The apparent hybridization rate constant ($_{app}k_h$) value for Q-probe (DNA hybridization in solution) was estimated from the change in fluorescence intensity based on the second-order kinetic model (equation S(2)),

$$d[\text{duplex}]/dt = {}_{app}k_h\,[\text{target DNA}]_t^2 - {}_{app}k_d([\text{target DNA}]_0 - [\text{target DNA}]_t) \qquad \text{eq. S(2)}$$

where $_{app}k_d$ is the apparent dissociation rate constant, [duplex] is the concentration of the duplex, $[\text{target DNA}]_t$ is the concentration of target DNA at time = t, and $[\text{target DNA}]_0$ is the initial concentration of target DNA (7 nM). At low temperatures ($T << T_m$), $_{app}k_d$ can be ignored, and thus, equation S(2) is given by

$$1 / [\text{target DNA}]_t = {}_{app}k_h t + 1 / [\text{target DNA}]_0 \qquad \text{eq. S(2)}'$$

Fig. S1 shows the plot of $1/[\text{target DNA}]_t$ versus t based on equation S(2)′. Thus, the slope in Fig. S1 is the $_{app}k_h$ for Q-probe.

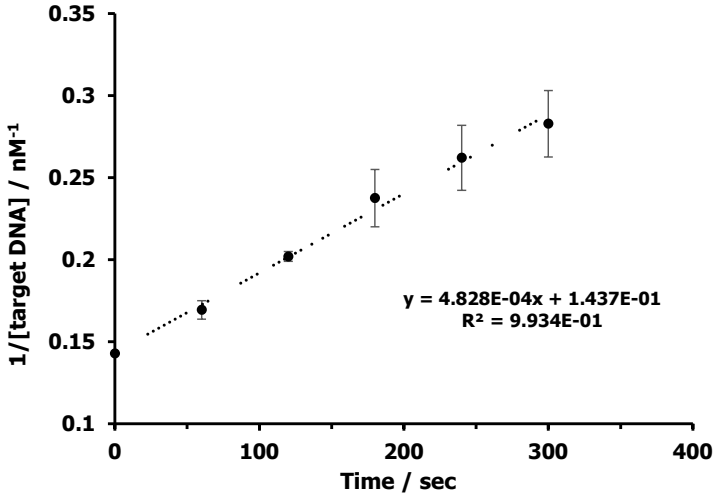


Fig. S1. Plots of 1/[target DNA]$_t$ versus time for the DNA hybridization of Q-probe. Mean values and standard deviations were obtained from three independent experiments.

Since d[duplex]/dt is 0 at equilibrium, equation S(2) is given by

$$0 = {}_{app}k_h\,[\text{target DNA}]_{eq}^2 - {}_{app}k_d([\text{target DNA}]_0 - [\text{target DNA}]_{eq}) \qquad \text{eq. S(3)}$$

where $[\text{target DNA}]_{eq}$ is the concentration of target DNA at equilibrium. Additionally, $[\text{target DNA}]_{eq}$ can be expressed by $[\text{target DNA}]_0(100 - \text{HE}_{eq})/100$, and the apparent equilibrium (binding) constant ($_{app}K_{eq}$) can be expressed as $_{app}k_h/_{app}k_d$, and thus, equation S(3) can be rewritten

$${}_{app}K_{eq} = {}_{app}k_h/{}_{app}k_d = 100 \bullet \text{HE}_{eq} / [\text{target DNA}]_0\,(100 - \text{HE}_{eq})^2 \qquad \text{eq. S(4)}$$

where HE_{eq} is the HE (%) at equilibrium (equal to HE at 5400 sec).

Langmuir Kinetic Model for DNA Hybridization on GNPs

The $_{app}k_h$ values for probe-GNP, probe/diluent-GNP, probe/PEG_{2k}-GNP, probe/PEG_{6k}-GNP, and probe/PEG_{10k}-GNP (DNA hybridization at solid-liquid interfaces) were also estimated from the change in fluorescence intensity based on the Langmuir kinetic model (equation S(5)),

$$\text{d}C_{duplex}/\text{d}t = {}_{app}k_h[\text{target DNA}]_0\,(A - C_{duplex}) - {}_{app}k_d C_{duplex} \qquad \text{eq. S(5)}$$

where C_{duplex} (strands/nm^2) is the surface concentration of duplexes (equal to hybridized target DNA) and A (strands/nm^2) is the surface concentration of duplexes at full coverage (equal to the initial surface concentration of probe DNA). Integration of equation S(5) gives

$$C_{duplex}(t) = C_{duplex}(eq)\{1 - \exp -({}_{app}k_h[\text{target DNA}]_0 + {}_{app}k_d)t\} \quad \text{eq. S(6)}$$

where $C_{duplex}(t)$ is the surface concentration of duplexes (equal to hybridized target DNA) at t, and $C_{duplex}(eq)$ is the surface concentration of duplexes (equal to hybridized target DNA) at equilibrium. With $({}_{app}k_h[\text{target DNA}]_0 + {}_{app}k_d)$ represented by a, equation S(6) is given by

$$C_{duplex}(t) = C_{duplex}(eq)(1 - e^{-at}) \quad \text{eq. S(7)}$$

Since $C_{duplex}(t)$ can be described by $(HE_t \cdot A)/100$ and $C_{duplex}(eq)$ can be described by $(HE_{eq} \cdot A\})/100$, equation S(7) is given by

$$HE = HE_{eq}(1 - e^{-at}) \quad \text{eq. S(8)}$$

Thus, data for time-dependent HE in Figure 2 can be fitted using equation S(8). The determined a values are listed in Table S4.

Table S4. The determined a values for probe-GNP, probe/diluent-GNP, and probe/PEG-GNPs.

	probe-GNP	probe/diluent-GNP	probe/PEG_{2k}-GNP	probe/PEG_{6k}-GNP	probe/PEG_{10}-GNP
$a \times 10^{-3}$	1.02 ± 0.15	1.53 ± 0.31	4.16 ± 0.81	3.16 ± 0.55	1.18 ± 0.18

Since dC_{duplex}/dt is 0 at equilibrium, equation S(5) and $C_{duplex}(eq)$ is given by

$$0 = {}_{app}k_h[\text{target DNA}]_0 (A - C_{duplex}(eq)) - {}_{app}k_d C_{duplex}(eq)$$

$$C_{duplex}(eq) = ({}_{app}k_h[\text{target DNA}]_0 A)/({}_{app}k_h[\text{target DNA}]_0 + {}_{app}k_d)$$

$$= ({}_{app}k_h[\text{target DNA}]_0 A)/a \quad \text{eq. S(9)}$$

Thus, ${}_{app}k_h$ values can be estimated from equation S(10) by fitting the experimental data.

$${}_{app}k_h = a \cdot HE_{eq}/\, 100[\text{target DNA}]_0 \quad \text{eq. S(10)}$$

Additionally, ${}_{app}K_{eq}$ (= ${}_{app}k_h$ /${}_{app}k_d$) values can be estimated from equation S(11).

$${}_{app}K_{eq} = {}_{app}k_h / (a - {}_{app}k_h[\text{target DNA}]_0) \qquad \text{eq. S(11)}$$

References

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(2) J. Li, B. Zhu, X. Yao, Y. Zhang, Z. Zhu, S. Tu, S. Jia, R. Liu, H. Kang and C. J. Yang, *ACS Appl. Mater. Interfaces* 2014, **6**, 16800.