

Supplementary material for article “Noncovalent adsorption of single-stranded and double-stranded DNA on the surface of gold nanoparticles”

Thermal denaturation of DNA duplexes

T_m values were determined in 2×10^{-6} M DNA solutions in the presence of 4 mM $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ (Table S1). Error of measuring the denaturation temperature of DNA duplexes was 0.2 °C.

Table S1. Thermal denaturation temperature of dsDNA.

dsDNA	T, °C
F-R	37.0
FT-R	38.6
FL-R	38.2
FT ₂ -R	37.2
FL ₂ -R	38.1
FT ₄ -R	36.9
FL ₄ -R	38.2

The process of thermal denaturation of dsDNA is described as follows:



where $[\text{AB}]$ is equilibrium concentration of dsDNA, and $[\text{A}]$ and $[\text{B}]$ are equilibrium concentrations of ssDNA constituting the duplex.

At the thermal denaturation temperature of the duplex (T_m), half of the DNA is in solution in the single-stranded state, and the other half in a double-stranded state. The thermal denaturation temperature of dsDNA is related to the initial concentration of duplex DNA according to the formula

$$T_m = - \frac{\Delta G}{R \ln \frac{[\text{AB}]_0}{2}} \quad (6)$$

where $\Delta G = \Delta H - T\Delta S$, ΔG is Gibbs free energy of the reaction, ΔH is reaction enthalpy, ΔS is reaction entropy, and $[\text{AB}]_0$ denotes initial concentration of dsDNA.

The theoretical calculation of T_m is based on the nearest-neighbor model [46 32]. An accurate calculation of T_m requires ΔS and ΔH values of individual complementary

dinucleotide pairs and of all types of noncomplementary dinucleotides, free ends, hairpins, and loops [47-33]. The total ΔG was calculated by summing ΔG_i : standard free-energy changes for Watson–Crick dinucleotides with a step of one nucleotide and with several adjustments:

$$\Delta G_{total} = \sum_i \Delta G_i + \Delta G_{init\ w/term\ G\cdot C} + \Delta G_{init\ w/term\ A\cdot T} + \Delta G_{symmetry} \quad (7)$$

To account for differences between duplexes having terminal A·T and terminal G·C pairs, two initiation parameters are introduced [48, 49 34, 35]: $\Delta G_{init\ w/term\ G\cdot C}$ and $\Delta G_{init\ w/term\ A\cdot T}$. $\Delta G_{symmetry}$ is 0.43 kcal/mol if the duplex is self-complementary and zero if it is non-self-complementary.

We estimated T_m for the F-R pair at various concentrations of dsDNA using the "T_m for Oligos Calculator" [50-36] (Table S2).

Table S2. Thermal denaturation temperatures of the F-R duplex at different DNA concentrations.

[dsDNA], nM	2000	100	50	40	30	20	10	5	2	1	0.5
T _m theoretical	37	33	32	32	32	31	30	30	28	28	27
T _m experimental	37.0	-	-	-	-	-	-	-	-	-	-

*calculated with the help of "RNAstructure - Mathews Lab."

At different temperatures of incubation of dsDNA with GNPs, sets of associates will arise that have different ratios of ssDNA to dsDNA. Nonetheless, it is expected that when DNA is adsorbed on GNPs at all concentrations employed at 25 °C, the proportion of DNA in the double-stranded state will be more than 50%.

Possible secondary structures of ssDNA

It is known that hairpin structures in a DNA molecule can impair its adsorption on GNPs [4]. We built possible secondary structures of ssDNA—R, F, FL, FL2, FL4, FT, FT2, and FT4—under the conditions of the experiments with DNA adsorption on GNPs (4 mM $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$) by means of Web service “RNAstructure - Mathews Lab” [33–38] (Fig. S1). F, FL, FT, FT2, and FT4 do not have stable secondary structure ($\Delta G > 0$).

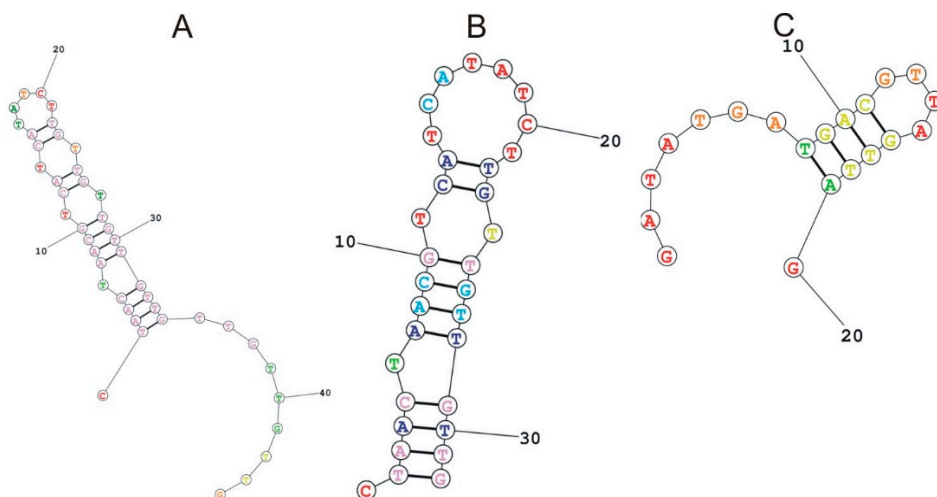


Figure S1. Possible secondary structures of (A) FL4, (B) FL2, and (C) R.

T_m values were determined in DNA solutions having a concentration of 2×10^{-6} M in the presence of 4 mM $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ (Fig. S2).

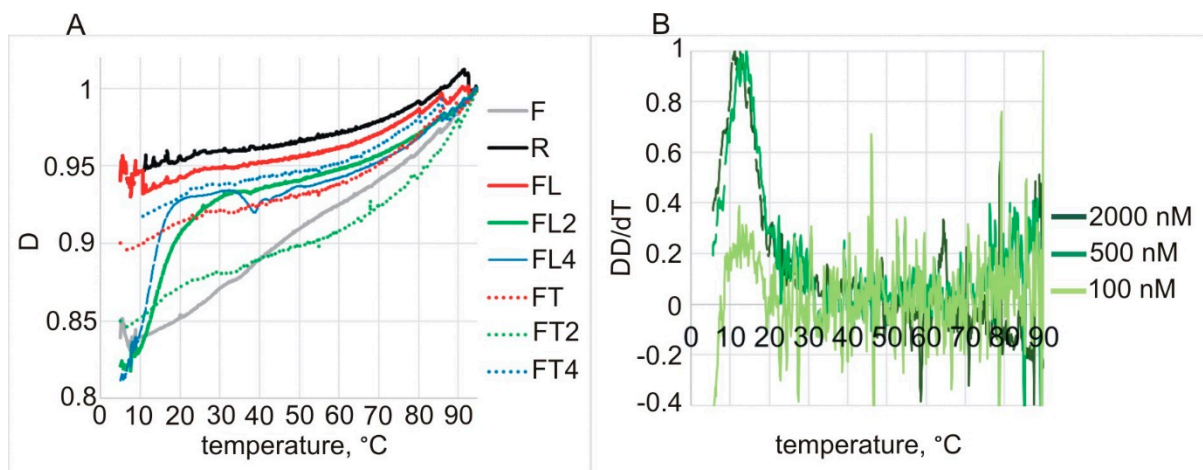


Figure S2. Thermal-denaturation curves of ssDNA in 4 mM $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$. (A) Thermal-denaturation integral curves of ssDNA at 2×10^{-6} M; (B) differential curves of thermal denaturation of FL2 at concentrations of 2×10^{-6} M, 5×10^{-7} M, and 1×10^{-7} M.

Potentially, FL2 and FL4 can form secondary structures at 2×10^{-6} M and 5×10^{-7} M at 11–14 °C in the presence of 4 mM $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ (Fig. S2 A). Nonetheless, when the

concentration of FL2 is lowered to 10^{-7} M, characteristic maximum point of the differential thermal-denaturation curve is absent (Fig. S2 B). Similar results were obtained for FL4. With decreasing concentration, the formation of secondary DNA structures is less efficient. Therefore, the formation of intermolecular secondary structures is more likely for FL2 and FL4. There was no inflection point in the integral thermal-denaturation curve for R, F, FL, FT, FT2, and FT4 ($[DNA] = 2 \times 10^{-6}$ M). These oligonucleotides do not form stable secondary structure.

Additionally, thermal denaturation temperatures of F, R, FL, FL2, FL4, FT, FT2, and FT4 were determined at a DNA concentration of 2×10^{-6} M in a high-ionic-strength solution (10 mM $NaC_2H_6AsO_2$ and 1 M NaCl). The integral thermal-denaturation curves of F, R, FL, FT, FT2, and FT4 had no inflection points under these conditions. The thermal denaturation temperatures of FL2 and FL4 were 43.9 and 47.5 °C, respectively, under these conditions.

ΔG values were assessed for the process of association of dsDNA FL2-R and FL4-R and for the formation of secondary structure of FL2 and FL4 in 4 mM $Na_3C_6H_5O_7$ by means of "Oligo Calc: Oligonucleotide Properties Calculator" [51-42] and "RNAstructure - Mathews Lab" [33-38] (Table S3).

Table S3. ΔG values for processes of association of dsDNA FL2-R and FL4-R and for secondary-structure formation in FL2 and FL4 in 4 mM $Na_3C_6H_5O_7$.

DNA	G, kcal/mol
FL ₂ -R	-21*
FL ₄ -R	-21*
FL ₂	-2.8**
FL ₄	-4.6**

*calculated using "Oligo Calc: Oligonucleotide Properties Calculator";

**calculated with the help of "RNAstructure - Mathews Lab."

The processes of association of duplexes FL2-R and FL4-R are energetically more favorable than the processes of secondary-structure formation in FL2 and FL4. A

competition of these processes during the adsorption of DNA on the surface of GNPs cannot be ruled out.

Magnitude of T20 adsorption on GNPs

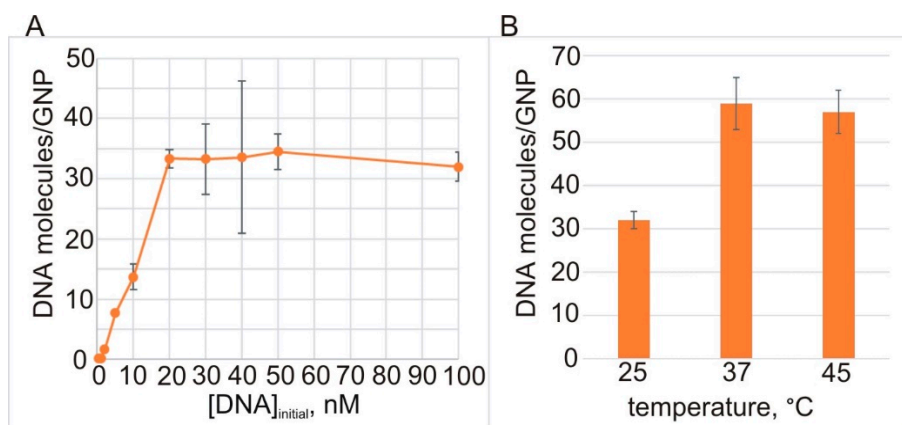


Figure S3. Efficiency of adsorption of T20 on GNPs. (A) The curve of binding of GNPs to the T20 oligonucleotide at 25 °C, (B) magnitude of adsorption of T20 on GNPs at different incubation temperatures.

Evaluation of the mobility of DNA–GNP associates by agarose gel electrophoresis

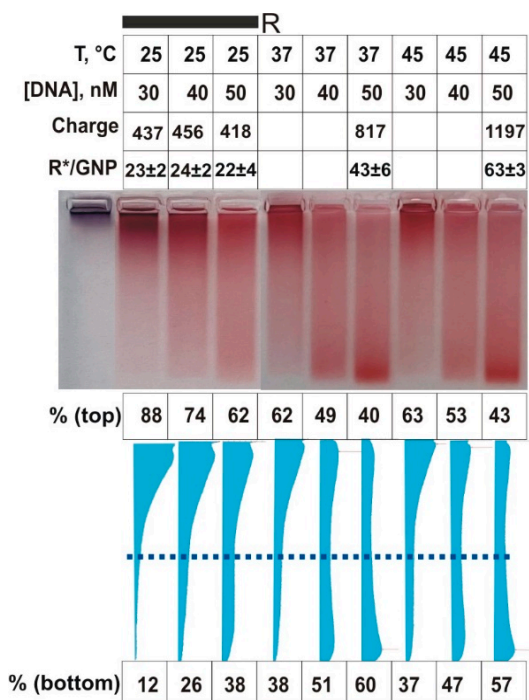


Figure S4. Analyses of the mobility and charge of DNA–GNP associates by agarose gel electrophoresis. An agarose gel image and the analysis of distribution of DNA–GNP associates, as performed by means of GelAnalyzer 19.1 software (www.gelanalyzer.com, created by Istvan Lazar Jr., PhD, and Istvan Lazar Sr., PhD, CSc, March 2022).

For R–GNP associates assembled at 25 °C, the capacity was ~20 R molecules per GNP at all DNA concentrations. The proportion of "fast" associates rose by 25% with an increase in the DNA concentration from 30 to 50 nM. With the elevation of temperature to 37 and 45 °C, the capacity increased by 19 and 20 R molecules, respectively, and the associate's charge increased from 418 to 817 and 1197 phosphate groups, respectively. Growth of the proportion of "fast" associates was noted only when the temperature was raised from 25 to 37 °C (by 22%) and did not change when the temperature was elevated from 37 to 45 °C. That is, when the charge on a GNP corresponding to ~800 internucleotide phosphate groups was reached, the mobility of associates with ssDNA reached a maximum.

Desorption of oligonucleotides from the surface of GNPs

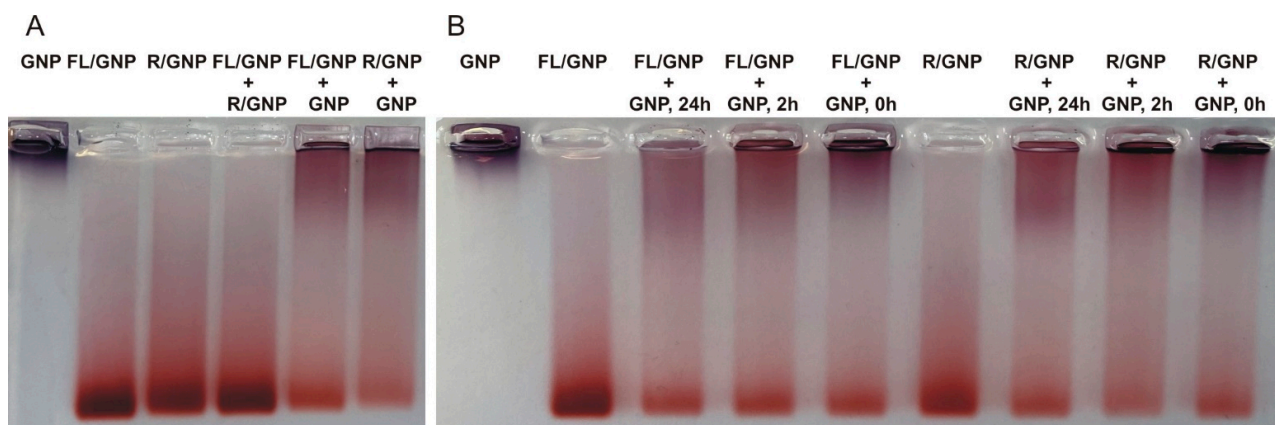


Figure S5. Agarose gel electrophoresis of FL/GNP and R/GNP associates and of their mixtures with each other or with naked GNPs. (A) Incubation for 24 h. (B) Separation of mixtures of FL/GNP and R/GNP associates with naked GNPs immediately after mixing (0 h) and after incubation for 2 or 24 h. Naked GNPs and initial associates FL/GNP and R/GNP were applied to both gels as controls of the mobility and distribution of the associates.

FL and R were adsorbed on GNPs according to the following scheme. Each oligonucleotide was adsorbed on GNPs separately in different test tubes with subsequent mixing of the resultant particles. As a control, each type of associate (FL/GNP or R/GNP) was mixed with naked GNPs. When the nanoparticles were conjugated with two oligonucleotides, desorption of 30–34% of each oligonucleotide was observed (step 1 in Fig. S6), whereas when naked GNPs were added, no more than 6–11% desorption was seen (steps 2 and 2' in Fig. S6), and after washing with sodium citrate: no more than 17–19% (steps 3 and 3' in Fig. S6).

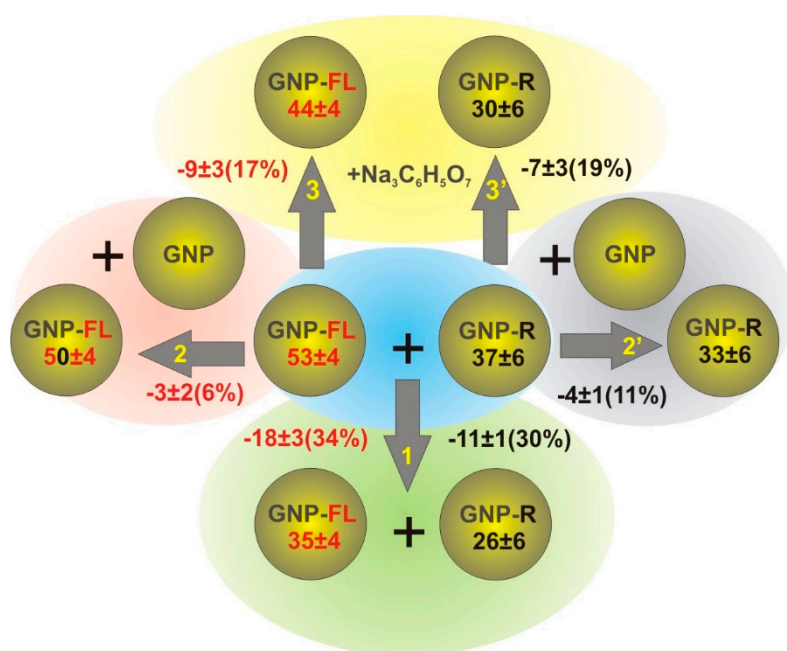


Figure S6. Separate adsorption of complementary oligonucleotides FL and R on GNPs (blue background) followed by combining the two types of DNA-GNP associates with each other (step 1 on a green background) or with naked GNPs [step 2 (FL) on a pink background and step 2' (R) on a gray background]. The background level of desorption after a wash of the associates with a 4 mM solution of sodium citrate is indicated as step 3 (FL) and step 3' (R) on a yellow background. "+" denotes the adsorption of a specified number of DNA molecules, "-" indicates the desorption of a specified number of DNA molecules. Adsorption and desorption of FL are highlighted in red, whereas the adsorption and desorption of R are indicated in black.