Base dependent adsorption of single-stranded homo-oligonucleotides to gold nanoparticles

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Abstract: Many bioanalytical devices now feature DNA immobilised on optically or electrically addressed gold surfaces, either via covalent (thiol) tethers, or non-specifically adsorbed via the DNA nucleobases. To guide the development of colorimetric biosensors that depend on the dissociation of adsorbed DNA aptamers, the interaction of homo-30-mers composed of each of the bases with gold nanoparticles was investigated. Through colorimetric measurements of the stability of DNA-coated gold nanoparticle dispersions, stability was found to decrease in the order A > T > C ≥ G, counter to expectations based on intrinsic affinities. These observations were reconciled using electrochemical measurements of DNA surface densities on gold nanoparticle electrodes; while the measured surface densities correlated with the dispersion stabilities, it was apparent that many bases of a long DNA strand were dangling from the surface, rather than directly adsorbed. Thus, even (dT)_{30}, whose bases have the weakest affinity to gold, can cover a gold surface with high total density since many of the bases will simply be tethered, and still contributing to the stability of a nanoparticle dispersion.

Keywords: oligonucleotides; DNA; gold nanoparticles; biosensor; aptasensor; colorimetric.


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1 Introduction

A growing number of biotechnology and nanotechnology devices feature single stranded (ss)DNA probes immobilised on optically or electronically addressed gold surfaces [1–3]. Such interfaces can be exploited for detecting complementary strands of DNA [4,5], or in the case of DNA aptamer sequences, other targets ranging from ions [6], to small molecules [7,8], proteins [9], and even cells [10]. In most cases, thiolated DNA probes are covalently tethered via stable Au-S bonds [9,11–13]. However, DNA is also known to non-specifically adsorb to gold surfaces via its constituent bases [11,12,14–17].

DNA aptamers non-specifically adsorbed to gold nanoparticles (AuNPs) can be used to create simple and versatile colorimetric sensors [16,18–23]. AuNPs coated with negatively charged DNA aptamers are stable in moderate salt concentrations, whereas bare AuNPs aggregate. When the aptamers bind to the target molecule, they have lower affinity to the AuNPs, and their dissociation from the AuNPs leads to aggregation of the AuNPs. The result of these interactions is detectable by naked-eye because the characteristic strong surface plasmonic absorption of monomeric AuNPs is red-shifted in the aggregated state.

Since this sensing method depends on the differential affinities of DNA bases to AuNPs vs. to their target, achieving high sensitivity depends on achieving the right balance of interaction strengths [24,25]. Aptamer sequences that adsorb too strongly to AuNPs may not dissociate in the presence of their targets, yet weakly adsorbed sequences may not sufficiently protect the AuNPs in the first place. It is therefore essential to understand how each of the bases contributes to the affinity of DNA aptamers to AuNPs.

Previous calorimetric measurements of DNA nucleobases and nucleosides binding to Au surfaces have not established a consistent view. Isothermal titration calorimetry studies of bases binding to AuNPs found that affinities decreased in the order $C > G > A > T$ [26]. Thermal desorption measurements, on the other hand, found that both nucleobases and nucleosides had affinities to Au surfaces decreasing in the order $G > C > A > T$ [27]. Competitive adsorption measurements of homo-oligonucleotides on Au surfaces probed by FTIR spectroscopy [15], along with AuNP
aggregation studies [28], agree that oligo(dT) has the weakest affinity. However the competitive adsorption method arrives at yet another order of affinities; A > C ≥ G > T [15].

To understand base adsorption in the context of colorimetric AuNP-based sensors, we used UV-visible absorption spectroscopy to study salt-dependent aggregation of homo-oligonucleotide coated AuNPs as a function of the base identity and concentration of DNA sequences. These measurements are complemented with electrochemical measurements that quantify surface coverage densities as well as indicating binding modes. We find that the surprising order of stability A > T > G > C is explained by a combination of intrinsic affinities along with the ability of weakly adsorbed DNA to pack with high density when only a fraction of the DNA strand is in contact with the AuNP surface.

2 Experimental method

Materials. AuNPs of 10 nm diameter were synthesised by reduction of chloroauric acid with sodium citrate according to the method described previously [29]. The concentration of AuNP dispersions was estimated to be 14 nM according to the extinction coefficient of the surface plasmon peak at 525 nm (2.7 × 10^8 M⁻¹ cm⁻¹) [30]. Homo-oligonucleotide sequences were purchased from Alpha DNA. Chloroauric acid (HAuCl₄) was purchased from Sigma-Aldrich. The ssDNAs were dissolved in deionised water (Milli-Q, 18.2 MΩ cm) and kept at −5°C before use. Deionised water was used in all experiments (unless stated) and all other chemicals were of analytical grade.

Colorimetric titrations. 1 µL aliquots of 0.5 M NaCl were added successively to adjust the ionic strength of 100 µL samples of bare AuNPs and AuNPs with each of the homo-oligonucleotide (previously incubated with AuNPs at the desired concentration). After thorough mixing, UV-Vis absorption was measured after 70 s to monitor the extent of salt induced aggregation.

Chronocoulometry. AuNPs were electrochemically deposited on the surface of a glassy carbon electrode (GCE) as previously reported. Briefly, a three-electrode cell with a volume of 5 mL was used, comprising a polished glassy carbon working electrode (GCE) (eDAQ, 1.0 mm in diameter), Ag/AgCl (3 M NaCl, +0.197 V vs. SHE) reference electrode and Pt wire counter electrode (using a potentiostat Bio-Logic SP-300 instrument). The Au deposition was done by immersing the electrodes in a 5 mL solution containing 1 mM HAuCl₄, 0.01 M Na₂SO₄ and 0.01 M H₂SO₄ at a constant potential of −0.2 V (Ag/AgCl) for 30 s. All solutions were de-gassed for 15 min using N₂ gas. This created a surface with an area of 0.0157 cm² vs. 0.00785 cm² of GCE. 0.1 nmole (16.7 µM in 5 mM trisodium citrate buffer, pH 3) [13] of homo-oligonucleotide (or SH-35-mer aptamer) were incubated with GCE-AuNPs for 20 min. The DNA surface density on GCE-AuNPs was determined using the chronocoulometry (CC) method developed by Steel et al. [31]. The adsorbed ssDNAs on GCE-AuNPs was first immersed in a low ionic strength electrolyte, 20 mM tris–HCl buffer at a pH 7.4, the potential stepped from 200 mV to −500 mV vs. (Ag/AgCl) for 500 ms (using BAS 100A electrochemical analyser), and the resulting charge flow was measured.
The electrode was then immersed for 20 min in a solution of 150 µM Ru(NH₃)₆³⁺ (RuHex) in Tris buffer, and the measurement repeated. By plotting the charge \( Q \) vs. the square root of time \( t^{1/2} \), the excess of RuHex was determined which can be related to the surface density of the DNA [31].

**Electrochemical impedance spectroscopy.** EIS measurements were recorded in a three-electrode cell containing 5 mL PBS solution with 5.0 mM of \([\text{Fe}-(\text{CN})_{6}]^{3–/4–}\) (1:1, mol:mol) at an applied bias of 0.230 V (Ag/AgCl). EIS measurements were recorded on a Bio-Logic instrument (Bio-Logic SP-300). The measurements were carried out with a 10 mV sinusoidal amplitude and collected for harmonic frequencies between 100 mHz and 100 kHz at 12 steps per decade and analysed using Zfit [32].

### 3 Results and discussion

Figure 1(a) shows the UV-Vis absorption spectra of a stable (monomeric) dispersion of AuNPs compared with AuNPs that have been forced to aggregate in the presence of salt. While the negatively charged surface of AuNPs provides interparticle repulsion that stabilizes the dispersion in water, salt screens this repulsion and triggers aggregation. Aggregation is evident from the growth of a broad plasmonic absorption shoulder extending beyond 600 nm, at the expense of the 523 nm peak that is characteristic of the plasmonic absorption of monomeric AuNPs. The loss of absorption at 523 nm can be used to monitor the stability of DNA coated AuNPs to understand interactions between different DNA sequences and AuNPs.

Figure 1(b) shows a series of titrations against salt for AuNPs mixed with 66 nM of 30-mers of each of the deoxynucleosides, along with the response of bare AuNPs for comparison. The plot shows the loss of the monomeric absorption peak at 523 nm as a function of ionic strength. At this DNA concentration, the presence of DNA makes little difference to the stability of AuNPs towards salt, and the different DNA sequences are barely distinguished from each other. This observation suggests that the concentration of DNA was too low for sufficient adsorption to occur.

Figure 1(c) shows a series of titrations against salt for AuNPs mixed with over 100-fold higher concentration of each of the DNA sequences – 1 µM. At this DNA concentration, surface adsorption is more favourable, which is observed via the increased stability of the AuNP dispersion towards higher ionic strengths. Moreover, each of the different DNA sequences affects the AuNP stability to a different degree, even when the same concentrations of DNA are added. Since stability of the AuNP dispersion relates to the density of surface charges that cause particles to repel each other, Figure 1(c) suggests that different amounts of DNA are adsorbed to AuNPs for each sequence. Specifically, the amount of adsorbed DNA is found to follow the order A > T > G > C, which is also reflected in the photographs of AuNP dispersions shown in Figure 1(d).

The lack of correlation between this stability order and the base affinity measurements described above [15,26–28] suggests that factors other than the affinity of DNA bases to Au must play a role. Strikingly, previous studies using other methods all conclude that dT has the lowest affinity to Au of all nucleosides (owing to it having only one contact point with Au), however Figure 1(c) shows that (dT)₁₀ coated AuNPs have the second highest stability under these conditions.
Figure 1 (a) UV-Vis absorption spectra of an AuNP dispersion and AuNPs aggregated in the presence of 23.8 mM ionic strength NaCl. (b) Salt dependent aggregation of AuNPs mixed with 66 nM of different homo-oligonucleotides, probed via the loss of plasmonic absorption at 523 nm. (c) Salt dependent aggregation of AuNPs mixed with 1 µM of different homo-oligonucleotides, probed via the loss of plasmonic absorption at 523 nm. (d) Photographs of AuNPs mixed with 1 µM of different homo-oligonucleotides at 23.8 mM ionic strength as indicated by the arrow in (c) (see online version for colours).

To explore a greater parameter space, binding isotherms were measured via AuNP stability as a function of DNA concentration at a fixed ionic strength of 20 mM. Figure 2 shows that, as expected, higher DNA concentrations lead to greater dispersion stability in all cases, as shown by the increase in relative absorption at 523 nm. However, the amount of DNA required to completely stabilise the dispersions at this salt concentration differed as a function of sequence. The dA sequence was found to confer stabilisation to AuNPs at a lower concentration of DNA compared with the other sequences, noting again that conferred stability does not necessarily reflect intrinsic affinity. The DNA binding isotherm plot in Figure 2 also confirms the observation in Figure 1(a) that different sequences are not expected to be distinguished from each other or from bare AuNPs at low DNA concentrations where little adsorption occurs.

To confirm that the observed AuNP aggregation trends relate to differences in the total coverage of homo-oligonucleotides, we measured DNA coverage densities on AuNP electrodes using the electrochemical method of chronocoulometry. As detailed in the experimental method section, AuNPs were deposited and the desired DNA sequence was
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adsorbed, then the amount of adsorbed RuHex was measured via chronocoulometry in the time domain, which enables surface adsorbed species to be distinguished from the diffusive component of bulk redox probes. The DNA surface densities were then obtained by first subtracting the amount adsorbed to bare AuNPs, then considering the charge per DNA strand, and dividing by the electrode surface area obtained by cyclic voltammetry, as detailed in the experimental method section.

Figure 2  Relative plasmonic absorption at 523 nm as a function of the concentration of different homo-oligonucleotide sequences with a fixed ionic strength of 20 mM (see online version for colours)

Figure 3  Surface densities of homo-oligonucleotides adsorbed on AuNP electrodes, as measured by chronocoulometry. For comparison, the surface density of a 35-mer aptamer tethered via a covalent thiol linkage is also shown

The result of this analysis is presented in Figure 3. For each of the homo-oligonucleotide sequences, surface densities of $2\text{–}4 \times 10^{13}$ molecules/cm$^2$ are measured. The relative surface densities follow the order $A > T > C > G$. The correlation between these surface densities and the AuNP dispersion stabilities measured above confirms that the differences in dispersion stabilities can be explained by the different amounts of adsorbed
DNA in each case. The magnitude of surface densities measured also provides some clues about the nature of DNA binding to AuNPs. Taking a representative surface density of $3 \times 10^{13}$ molecules/cm$^2$, this corresponds to a surface area of 3 nm$^2$ per 30-mer DNA molecule. Given that each base directly bound to the surface and the accompanying backbone segment must occupy an area substantially greater than 0.1 nm$^2$, this analysis makes it clear that some segments of DNA must be dangling away from the surface. To confirm this idea, we also measured the surface density of 35-mer DNA strands, in this case an aptamer sequence that binds 17β-estradiol, covalently tethered away from the surface via thiol linkages. In that case, the tethered configuration with bases extending away from the surface results in a higher surface density of $5 \times 10^{13}$ molecules/cm$^2$ approaches the maximum packing density of tethered DNA [33].

The surface density measurements are complemented by electrochemical impedance spectroscopy measurements of the DNA coated AuNP electrodes, shown in Figure 4. The impedance measurements, presented as Nyquist plots, show the characteristic shape of the Randles cell equivalent circuit that typically describes an electrode/electrolyte interface [34]. The radius of the semicircle indicates the charge-transfer resistance ($R_{CT}$) encountered by a redox probe approaching the electrode surface, which in this case is affected by the charges that DNA brings to the surface. The measured charge-transfer resistances follow the same order as the surface density measurements in Figure 3 above since the resistance encountered by the negatively charged $[\text{Fe}-(\text{CN})_6]^{3-/4-}$ redox probe depends on the density of negative charges encountered while approaching the electrode. Interestingly, all of the homo-oligonucleotides apart from (dA)$_{30}$ have a lower charge-transfer resistance than the bare AuNPs, whereas thiol tethered DNA leads to an increase in charge-transfer resistance [35]. The observed decrease may result from non-specifically adsorbed DNA displacing some of the dense ion layer that exists an electrode surfaces at such high potentials (electrochemical impedance spectroscopy is carried out at 0.23 V vs. Ag/AgCl).

**Figure 4**  Electrochemical impedance spectra of homo-oligonucleotides adsorbed on AuNP electrodes, presented as Nyquist plots. Charge-transfer resistance ($R_{CT}$) values are colour coded and indicated for each case (see online version for colours).
The electrochemical measurements allowed us to reconcile the observed order of stability of homo-oligonucleotide coated AuNPs with previous measurements on intrinsic affinities. The correlation between the electrochemical measurements and colorimetric titrations confirmed that the stability of dispersions relates to the total amount of DNA adsorbed to AuNPs. However, under conditions of high DNA concentration, the surface density of adsorbed DNA may not be correlated with the intrinsic affinity of each of the nucleosides to AuNPs since many of the bases in a sequence are not in contact with the surface, but rather tethered through other bases. The surface density measurements show that this is the case. While dT is known to have the lowest affinity for Au, if a dT homo-oligomer is sufficiently long, some of the bases will adsorb to AuNPs, and the rest will still confer electrostatic stability. In fact, lower affinity may even help to load more DNA onto AuNP surfaces since a greater fraction of the bases will be dangled away from the surface, leaving space for more DNA strands to anchor to the AuNP.

4 Conclusions

To understand the adsorption interactions of DNA on AuNPs for biosensor applications, we evaluated the stability of dispersions of AuNPs coated with 30-mer homo-oligonucleotides comprised of each of the bases. From measurements of AuNP dispersion stability as a function of ionic strength, and also as a function of DNA concentration, we found that stability decreased in the order A > T > C ≥ G. This order of stability did not correlate with measurements of intrinsic nucleoside affinity to Au surfaces, which, in spite of some experiment dependent discrepancies, always place T with the lowest affinity. By undertaking electrochemical measurements of DNA density on AuNP electrodes, we concluded that the order of increased stability correlated with increased surface density. However, nucleotides with lower affinity can still adsorb with high density under these conditions because many of the bases of a long DNA strand are tethered rather than being in contact with the Au surface. These insights will help to guide the engineering of DNA aptamer sequences in sensors where the colorimetric response depends on effective DNA dissociation from AuNPs in the presence of target molecules.

Acknowledgements

Omar A. Alsager acknowledges a fellowship from King Abdulaziz City for Science and Technology, and Bicheng Zhu and Jadranka Travas-Sejdic acknowledge a grant from New Zealand Ministry of Business, Innovation and Employment (UOAX1201).

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