

Adsorption of tetracycline antibiotics to gold nanoparticles and feasibility of aptamer-based label-free colorimetric detection

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Abstract

Tetracyclines are a group of very important antibiotics that are still in use to date. To extract, detect and remove tetracyclines from the environment, various nanomaterials have been employed. Although gold nanoparticles (AuNPs) are a commonly cited material for these purposes, fundamental understanding of these tetracycline-AuNP systems are still limited. In this work, the adsorption of tetracycline, oxytetracycline and doxycycline to AuNPs was studied. The effect on the colloidal stability of AuNPs, adsorption kinetics, and the resulting adsorption isotherms were measured. While millimolar concentrations of the tetracyclines can cause aggregation of AuNPs, saturated monolayer adsorption was achieved with low micromolar concentrations of the tetracyclines. Adsorption was instantaneous, and adsorption to AuNPs enhanced their intrinsic fluorescence instead of quenching. With the assumption of aptamer/target complexes cannot be easily adsorbed by AuNPs compared to free aptamers, a label-free colorimetric detection method was tested. While the label-free sensor showed target-dependent aggregation of AuNPs, a non-binding mutant aptamer showed the same trend, suggesting that the color change did not reflect aptamer adsorption but other events such as target adsorption. This study indicates the importance of fundamental understanding of target/AuNP interactions to correctly design aptamer and AuNP-based label-free biosensors.

Keywords: oxytetracycline, doxycycline, aptamers, gold nanoparticles, biosensors

1. Introduction

Tetracycline antibiotics share a similar structure containing four linearly fused rings. Many of the first discovered tetracycline antibiotics such as tetracycline and oxytetracycline were later found to have strong adverse effects in human beings and are now reserved primarily for treatment of animals.¹ Widespread use of tetracyclines in animals has resulted in environmental contamination making efficient and cost-effective detection and removal methods an attractive proposition.^{2,3}

Nanomaterials play important roles in the detection of tetracyclines, including their extraction,⁴ fluorescence spectroscopy,^{5,6} surface enhanced Raman spectroscopy,⁷ and electrochemistry.^{8,9} In addition, studies have been performed gauging the adsorption of tetracyclines to nanomaterials for use in remediation and degradation.¹⁰⁻¹³ Previous studies on the adsorption of tetracyclines to nanomaterials include magnetite nanoparticle-gold nanostar hybrids,⁷ silver nanoparticles,¹⁴ and zirconia nanoparticles.¹⁵ Gold nanoparticles (AuNPs) are particularly important for developing biosensors due to their excellent optical and surface properties.¹⁶⁻²¹ However, current understanding on the adsorption of tetracyclines by AuNPs is still quite limited.

Aptamers are single-stranded oligonucleotides that can selectively bind target molecules.^{22,23} A few aptamers for tetracyclines were previously selected using immobilized target molecules.^{24,25} Using an immobilized DNA library, we recently selected a DNA aptamer with around 100 nM K_d to tetracycline, oxytetracycline and doxycycline.²⁶ Interfacing DNA aptamers with AuNPs is a popular method to develop label-free biosensors,²⁷⁻²⁹ although its feasibility is strongly affected by potential target/AuNP interactions.³⁰⁻³² The main goals of this work are two-fold. First, we aimed to perform fundamental adsorption studies of the three common tetracyclines to AuNPs. For this, we paid particular attention to the effect of pH, which alters the net charge of the tetracyclines. In addition, we studied the effect of the tetracyclines on DNA and aptamer adsorption by AuNPs and the feasibility of using the label-free colorimetric sensing method in the detection of tetracyclines.³⁰

2. Materials and Methods

Chemicals

All the single stranded DNA samples were ordered from Integrated DNA Technologies (Coralville, IA, see Table 1 for DNA sequences). Citrate-deposited gold nanoparticles (13 nm and 100 nm) were synthesized using citrate reduction.³³ The tetracyclines and other chemicals were from Sigma-Aldrich.

Destabilization of AuNPs by the Tetracyclines

To understand the effect of the tetracyclines on the colloidal stability of AuNPs, specimens with varying concentrations of targets (doxycycline: 0.01, 0.02, 0.1, 0.2, 0.3, 0.4, 0.7, 1, 1.2, 1.5, 2 mM, tetracycline: 0.4, 0.7, 1, 1.5, 2 mM, and oxytetracycline: 0.1, 0.2, 0.3, 0.4, 0.5 mM) and 3 nM of AuNP (13 nm size) were buffer in 5 mM of pH 5 acetate buffer or pH 8.2 phosphate buffer. After 15 min incubation, the corresponding UV-Vis spectra were collected (Agilent 8435A).

Surface Enhanced Raman Spectroscopy

Samples including 0.1 nM AuNP (~100 nm size), 1 mM MgSO₄, and 0.3 mM tetracycline were prepared, followed by sitting for 5 min until aggregation was detected through a change in color to blue. Similar samples with 1 mM of tetracycline, but without AuNP was also prepared. Raman spectra were recorded using a Delta Nu Advantage 785 Raman spectrometer.

Adsorption Isotherms

To measure the adsorption isotherms of tetracyclines, a mixture of 2 μ M OTC5, 50 mM pH 6 MES buffer, and 0.5 mM MgCl₂ was gradually titrated with oxytetracycline (0, 100, 300, 500, 700, 1000 nM) to construct a calibration curve. Oxytetracycline of different concentrations (1, 2, 3, 5, 7, 10, 12, and 15 μ M) and 3 nM AuNP (13 nm size) were buffered in 5 mM sodium phosphate buffer (pH 8.2) or acetate buffer (pH 5) and incubated for 15 min. Samples were centrifuged at 15,000 rpm for 10 min, followed by removal of the supernatant and washing of the pellet three times with the buffers. The precipitates were dissolved using 10 mM KCN. Dissolved gold was buffered in a matrix containing 50 mM of pH 6 MES buffer, 2 μ M OTC5, and 0.5 mM MgCl₂, and was analyzed for fluorescence intensity using a Tecan Spark microplate reader (λ_{ex} = 370 nm, λ_{em} = 530 nm). Aside from the proposed incubation conditions, samples containing 0.5 mM MgCl₂ or 30 mM NaCl, and with both 0.5 mM MgCl₂ and 30 mM NaCl were made incubating with 7 μ M oxytetracycline for 15 min at the room temperature. Similar process was carried out for the tetracycline and doxycycline samples.

Adsorption Kinetics

100 nM oxytetracycline was dissolved in 5 mM pH 5 acetate buffer or pH 8.2 sodium phosphate buffer and had fluorescence intensity monitored for 5 mins using an Agilent Eclipse fluorometer ($\lambda_{\text{ex}} = 370$ nm, $\lambda_{\text{em}} = 530$ nm). Fluorescence signals were monitored for another 10 min after adding AuNP (13 nm size) to a final concentration of 0.1 nM. A Similar procedure was carried out to analyze quinine samples ($\lambda_{\text{ex}} = 244$ nm, $\lambda_{\text{em}} = 450$ nm). After recording fluorescence intensity measurements, the oxytetracycline solutions were collected and centrifuged to remove the AuNPs. The supernatants' fluorescence intensity was then collected.

DNA Adsorption onto AuNPs

A 15 min incubation was given to solutions of 0.5 mM MgCl_2 , 500 nM or 10 μM oxytetracycline, and 3 nM AuNP (13 nm) buffered in 5 mM pH 8.2 sodium phosphate buffer. 0.1 μM FAM-labeled DNA and 100 mM NaCl were then added followed by 5 min incubation. Excess FAM-DNA was removed using centrifugation (10 min, 15,000 rpm) and washed, and the precipitates were dissolved in 10 mM of KCN. 5 μL of the supernatant solution recovered after each centrifugation and the dissolved gold solution were buffered to pH 6 in 50 mM MES buffer prior to recording fluorescence intensity using a Tecan Spark microplate reader ($\lambda_{\text{ex}} = 485$ nm, $\lambda_{\text{em}} = 530$ nm). The percentage of DNA adsorption was calculated by dividing the corresponding fluorescence intensity of DNA released from AuNP by the sum of that from the supernatant and the dissolved AuNPs.

Oxytetracycline Binding Assay Using Fluorescence Spectroscopy

To measure the binding of oxytetracycline by aptamers, OTC5 (functional aptamer) and a nonbinding OTC5 mutant were gradually titrated into a mixture of 100 nM oxytetracycline, 0.5 mM MgCl_2 , and 50 mM of pH 6 MES buffer to a final DNA concentration of 300, 900, 1500, 2100, and 2700 nM respectively. The fluorescence intensity was measured using a Tecan Spark microplate reader ($\lambda_{\text{ex}} = 370$ nm, $\lambda_{\text{em}} = 530$ nm).

Biosensor Sensitivity Optimization

Solutions consisting of 10 mM pH 8.2 sodium phosphate buffer, 0.5 mM MgCl_2 , and 3 nM AuNP (13 nm size) were titrated with varying concentrations of OTC5 DNA aptamers (0, 50, 70, 100, 120, 150, 170, 200 nM), followed by adapting AuNP and NaCl concentrations to 3 nM and 50 mM respectively. The prepared samples were incubated for 15 min, and their UV-

Vis spectra were collected (Agilent 8435A).

Label-free biosensor assay

Similar procedures to the biosensor sensitivity optimization were carried out, except the final DNA concentration was fixed at 50 nM, and different DNA sequences (OTC5 aptamer, mutant, or A15 DNA) were used. The UV-Vis spectra of the samples were collected using a spectrometer (Agilent 8435A).

Table 1. DNA sequence and modifications used in this work

Names	Sequences and Modifications (from 5' to 3')
OTC5	ACGACATTCCGTTGATCTCTCCCTTTTGGGTTGGTGTCGT
OTC5 Mutant	ACGACATTCCTGGTATCTCTCCCTTTTGGGTTGGTGTCGT
FAM-OTC5	FAM-CTCTCGACGACATTCCGTTGATCTCTCCCTTTTGGGTTGGT GTCGT
FAM-Random	FAM-ACGCATCTGTGAAGAGAACCTGGG
A15	AAAAAAAAAAAAAAAAA

3. Results and Discussion

3.1. pH-dependent adsorption of the tetracyclines:

tetracycline antibiotics such as doxycycline, tetracycline, and oxytetracycline have multiple ionizable groups with a wide range of pK_a values¹² (doxycycline: $pK_{a1}=3.5$, $pK_{a2}=7.7$, $pK_{a3}=9.5$, tetracycline: $pK_{a1}=3.3$, $pK_{a2}=7.7$, $pK_{a3}=9.7$, oxytetracycline: $pK_{a1}=3.3$, $pK_{a2}=7.3$, $pK_{a3}=9.1$) enabling fine tuning of their charges, which could be important for their interaction with AuNPs. Figures 1a-c show the charge is net neutral in an acidic condition (pH 5) but net negative in a basic solution (pH 8.2). AuNPs are negatively charged due to their surface citrate,³⁴ and they may interact more favorably with the tetracyclines at acidic pH.

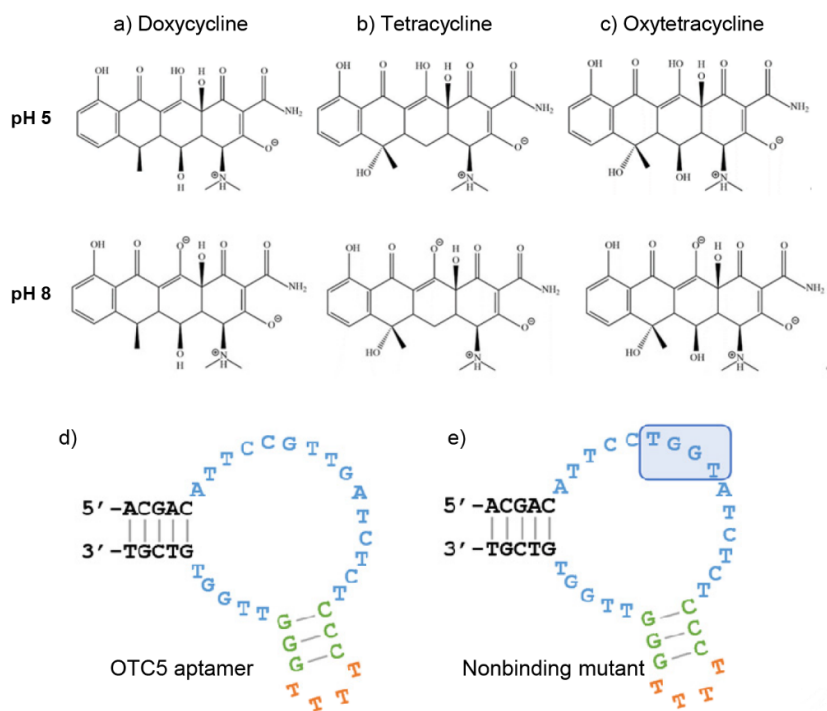


Figure 1: The ionizable groups at pH 5 and pH 8 for the tetracyclines studied in this work: a) doxycycline, b) tetracycline, and c) oxytetracycline. DNA aptamers used in this work and their secondary structures: d) OTC5 (wildtype aptamer), e) OTC5 mutant (mutant).

In this experiment, pH 5 was chosen to keep the tetracyclines charge neutral since it is about 2 units away from pK_{a1} and pK_{a2} . A higher pH of 8.2 was also studied whereby the majority of the tetracycline populations are negatively charged. Due to the buffer range of the

phosphate buffer, we cannot exceed pH values much higher than 8. Other common buffers like Tris that can buffer at a higher pH strongly adsorb to AuNPs and can interfere with adsorption studies.³⁵

Upon adsorption of small molecules to AuNPs, the colloidal stability of AuNPs could be increased or decreased.^{31,36} To investigate the interaction between the tetracyclines and AuNPs, a simple mixing experiment was conducted first to observe the change in color that occurs when AuNPs are destabilized and aggregate. For example, when increasing concentrations of doxycycline were added to the 13 nm diameter AuNPs at pH 5, the AuNPs started to change color with as low as 0.01 mM doxycycline. In contrast, at pH 8, doxycycline did not trigger AuNP aggregation until 0.7 mM (Figure 2a).

The aggregation of the AuNPs were then quantitatively characterized using UV-vis spectroscopy. Based on the spectra, an extinction ratio can be derived by dividing absorbance at wavelength 620 nm by that at 520 nm. These two wavelengths correspond to the aggregated and free AuNPs, respectively (Figure 2b). Figure 2c demonstrates that doxycycline samples buffered at pH 5 triggered full AuNP aggregation starting at 0.1 mM, while samples buffered at pH 8.2 did not cause full aggregation until 2.0 mM. In the case of tetracycline, a similar phenomenon was observed, except that the pH 8.2 sample did not exhibit full aggregation even at 2.0 mM (Figure 2d). Due to limited solubility of oxytetracycline, studies were only conducted up to a maximum concentration of 0.5 mM. In this range, the effect of oxytetracycline was similar in both pH values and it only raised the extinction ratio slightly (Figure 2e).

The stronger destabilization of AuNPs at pH 5 can be explained by the adsorption of a charge neutral molecule, which displaces the surface bound citrate molecules. Such displacement resulted in a loss in repulsion between AuNPs permitting them to reach at closer distances until they are near enough to where van der Waals forces dominate the system and the AuNP visibly aggregate. The proposed mechanism is presented in Figure 2f. Similar effects were also observed with adenosine and ATP, where the former destabilized AuNPs while the latter stabilized them.³⁶

It needs to be noted that the concentration range of the tetracyclines to induce AuNP aggregation is quite high at pH 8.2, most in the 0.1 to 1 mM range. For comparison, molecules

like adenosine,³⁶ kanamycin,³⁷ and dopamine³¹ can aggregate AuNPs in low micromolar regions. This suggests that the interaction between the tetracyclines and AuNPs was quite weak at pH 8.2.

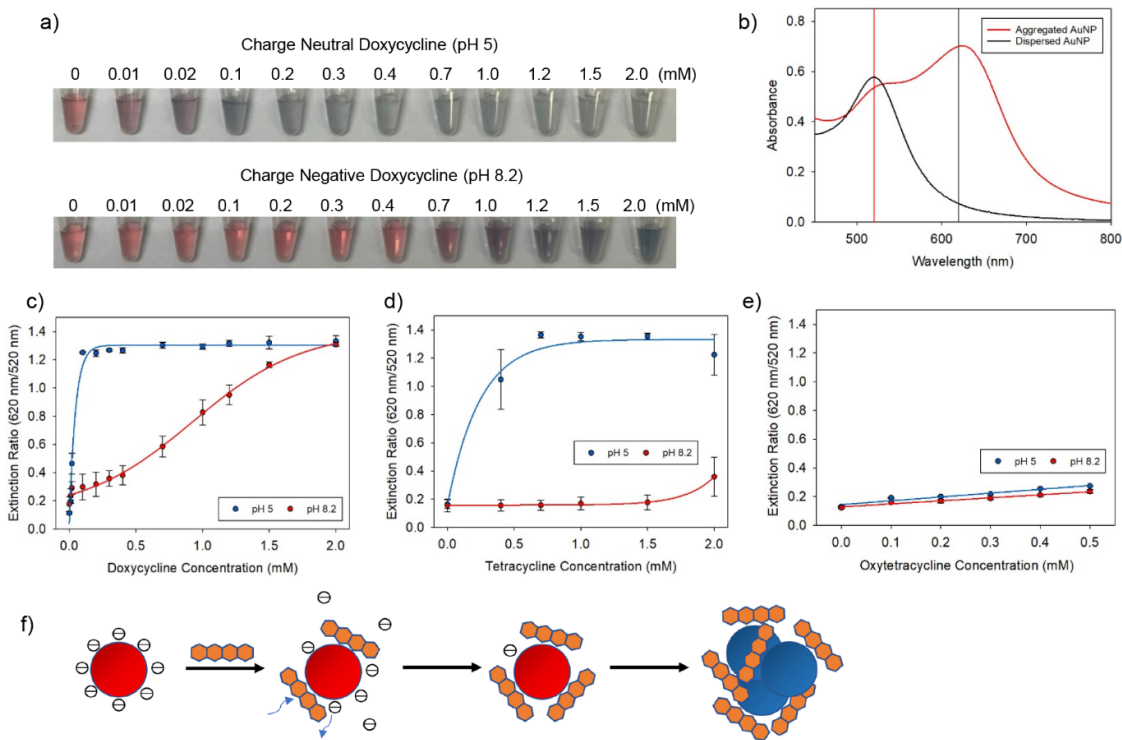


Figure 2: a) Photographs of doxycycline-AuNP samples buffered at pH 5 and pH 8.2. b) UV-Vis absorption spectra of dispersed and aggregated AuNP in an aqueous solution. Extinction ratios of AuNPs incubated with various concentration of (c) doxycycline, (d) tetracycline, and (e) oxytetracycline at pH 5 and pH 8.2. f) A proposed mechanism of AuNP destabilization in the presence of neutral tetracyclines in solution.⁴

3.2 Surface enhanced Raman Spectroscopy (SERS) of Tetracycline-AuNP samples

To confirm the adsorption of the tetracyclines to AuNPs, SERS was performed taking advantage of the Raman enhancement effect of AuNPs.³⁸ SERS increases the sensitivity of the Raman spectrum of a molecule by first adsorbing that molecule to a metal surface.³⁹ The SERS spectrum of 0.3 μ M tetracycline in solution is visible (Figure 3a), while without the AuNPs, even 1 mM tetracycline was too low to produce a Raman spectrum with identifiable characteristic the tetracycline peaks. For the SERS peaks we observed, the dominant SERS characteristic peaks are located at 798, 1014, 1249, 1287, 1342, and 1636 cm^{-1} . Different from

the reported SERS characteristic peaks, there is no prominent peak around 520 cm^{-1} .⁴⁰

The SERS spectrum validated the hypothesis that the destabilization effect seen during the tetracycline adsorption study was due to their adsorption to the AuNPs. However, SERS functions only as a qualitative method. To form more quantitative understandings about the role of target-AuNP interactions, adsorption kinetics and isotherm were also measured.

3.3 Tetracycline adsorption kinetics

Tetracyclines have intrinsic fluorescence,⁴¹ which can be used to sensitively quantify their adsorption. We first monitored the fluorescence of 100 nM free oxytetracycline for 5 min to achieve a stable background. Then, AuNPs amounting to a final concentration of 3 nM were added and the fluorescence was continuously monitored (Figure 3b and 3c, blue traces). At both pH 5 and 8.2, a fluorescence enhancement was observed upon the addition of the AuNPs. Typically, one would expect fluorescence quenching upon adsorption of fluorophores to AuNPs. To confirm the results, both samples were centrifuged, the supernatant collected and analyzed. The resulting fluorescence was very similar to that of free oxytetracycline, indicating the fluorescence enhancement was associated with the presence of the AuNPs. Surface-enhanced fluorescence has been observed in a few fluorophores,^{42,43} and this is the first report of this pertaining to tetracyclines. As a control, the kinetics of adsorption of quinine were also monitored. The fluorescence intensity from quinine dropped upon the addition of AuNPs providing further confirmation as to the tetracyclines yielding a unique fluorescent enhancement (Figure 3d).

From the collected data, the rate of adsorption was very rapid, and it was completed within 10 sec (the time interval of our fluorescence readings). Therefore, for subsequent experiments, a short incubation time of 1 min was sufficient to ensure equilibrium.

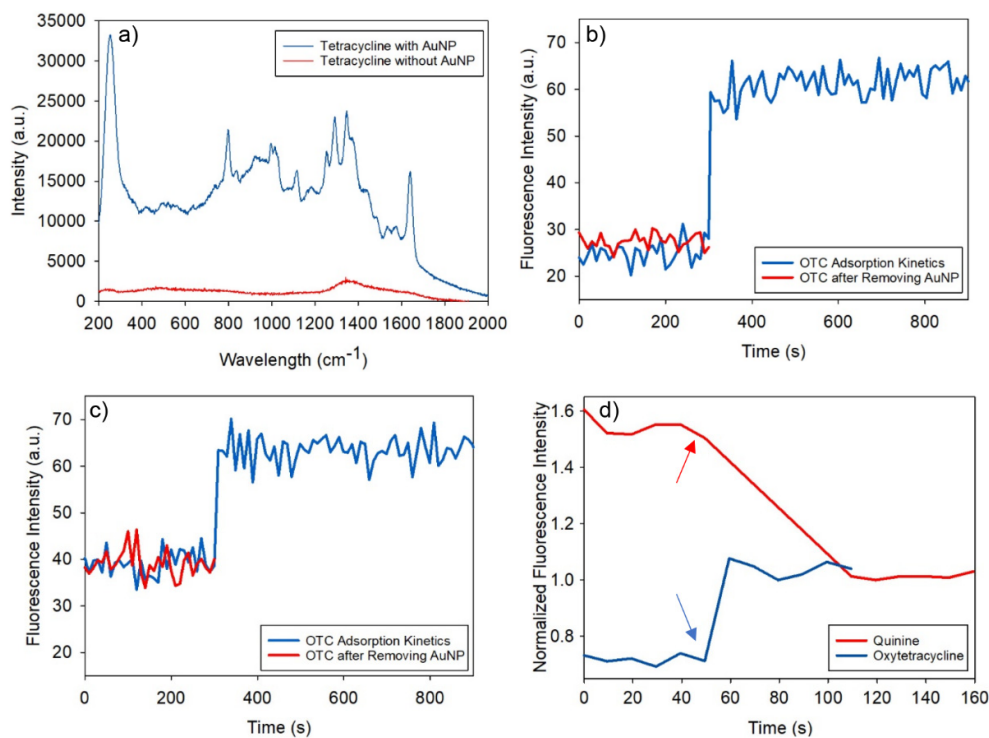


Figure 3: a) SERS spectra of tetracycline without and with AuNPs with characteristic peaks indicated. Oxytetracycline adsorption kinetics at b) pH 5 and c) pH 8.2. AuNPs were added at 300 s (the blue trace), and the sample's fluorescence signal after removing AuNPs is also shown (the red trace). d) The fluorescence of quinine and oxytetracycline before and after adding AuNPs at 50 sec. Fluorescence intensities are normalized based on readings after adding AuNPs.

3.4 Adsorption Isotherms

To measure adsorption isotherms, various concentrations of the tetracyclines were incubated with the AuNPs. After incubation, the excess tetracyclines were washed off, and the AuNPs were dissolved using 10 mM KCN. Although the tetracyclines exhibit fluorescence, it is relatively weak at low concentrations. The OTC5 aptamer was used to amplify its fluorescence for quantification.²⁶ The fluorescence intensities were converted to concentrations using a fluorescence calibration curve.

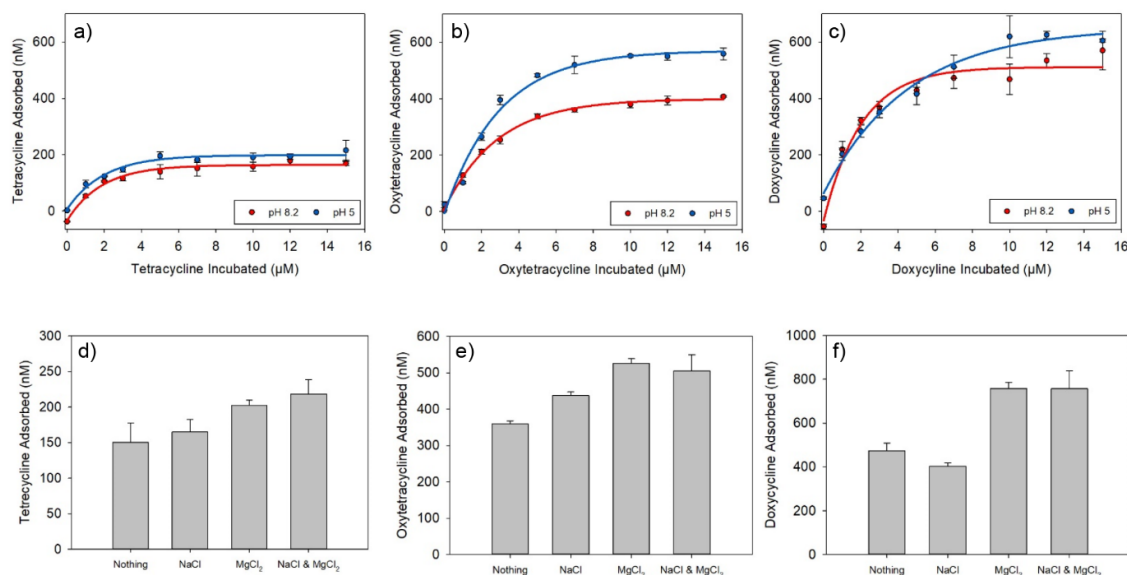


Figure 4: Adsorption isotherms of a) tetracycline, b) oxytetracycline, and c) doxycycline at pH 5 and pH 8.2. d) Tetracycline, e) oxytetracycline, and f) doxycycline adsorbed under various salt conditions at pH 8.2.

All the three tetracyclines followed the Langmuir adsorption isotherm, suggesting monolayer adsorption at both pH 8.2 and pH 5. From the adsorption isotherms, the adsorption reached saturation when incubating the AuNPs (3 nM) with 7 μM or higher concentrations of the tetracyclines. At saturated adsorption, each AuNP adsorbed on average 50 (pH 8.2) or 70 (pH 5) tetracyclines, 130 (pH 8.2) or 190 (pH 5) oxytetracycline, and 150 (pH 8.2) or 200 (pH 5) doxycycline. Such a capacity is consistent with that one layer of tetracyclines can stably adsorb onto AuNP surface based on the thiolated DNA adsorption capacity of around 100 strands on each 13 nm AuNP.⁴⁴ For all the three tetracyclines, their saturated adsorption capacity was higher at pH 5. This may be explained by a lower lateral repulsion when the molecule is overall charge neutral. At pH 8.2, the intermolecular repulsion may lead to a lower density.

In the aggregation data displayed in Figure 2, the range of the tetracyclines tested reached up to a few mM, and all AuNPs were stable with 10 μM tetracyclines (except for doxycycline at pH 5). Therefore, adsorption of a monolayer of the tetracyclines would not aggregate the AuNP. The aggregation by mM level of the tetracyclines was likely due to multilayer

adsorption. For most biosensor applications, a low concentration of tetracycline is likely to be encountered and thus we focused on solutions with lower than 10 μM tetracyclines.

After constructing the adsorption isotherms and understanding the effect of pH, the effect of salt on aggregation was studied (Figure 4d-f). pH 8.2 was employed for this experiment as the tetracyclines would be more sensitive to salt when they are negatively charged. We tested various incubation conditions with MgCl_2 , NaCl and a combination of the two. While 30 mM NaCl had very limited impact on the tetracycline adsorption onto AuNPs, 0.5 mM MgCl_2 and the combination of magnesium chloride and sodium chloride increased the tetracycline adsorption more notably (up to 60% increase). Overall, the effect of salt was quite insignificant.

3.5 Feasibility of label-free biosensors

So far, we know that the tetracyclines can be adsorbed to AuNPs, but within 10 μM , they do not cause aggregation of the AuNPs at pH 8.2. Our group recently reported a DNA aptamer named OCT5 for binding to these tetracyclines.²⁶ A commonly used method to detect aptamer binding is to use AuNPs as shown in Figure 5a.³⁰ Without the target, the aptamer would be adsorbed to the AuNPs and protect its colloidal stability against salt-induced aggregation. In the presence of the target, the aptamer would bind the target to form a rigid structure, and thus the adsorption of the aptamer complex to the AuNPs would be inhibited. This method was originally used to assay DNA hybridization,^{27,28} and later it was adapted for the detection of aptamer binding^{45,46}. We recently found that it is critical to study target/AuNP interactions, which determine the feasibility of this method.³⁰ We now know that the tetracyclines can adsorb to the AuNPs, and thus whether this method can be applied to the detection of the tetracyclines needs to be carefully studied.

To conduct biosensor testing, its working conditions needed to be optimized to achieve high sensitivity. AuNP/aptamer biosensors with higher sensitivity should have a lower concentration of aptamers to protect AuNPs. The conditions were set with 50 mM NaCl , 0.5 mM MgCl_2 , 3 nM AuNPs (13 nm) buffered using 10 mM phosphate (pH 8.2). Mg^{2+} is known to be important for aptamer binding.^{26,47} A series of OCT5 aptamer concentrations were tested to determine their corresponding protection effects over the AuNPs. As shown in Figure 5b, when no aptamer was added, the AuNPs turned blue, and the color retained red with increasing

aptamer concentration confirming the protection effect. We also quantified the color using UV-vis spectrometry (Figure 5c).

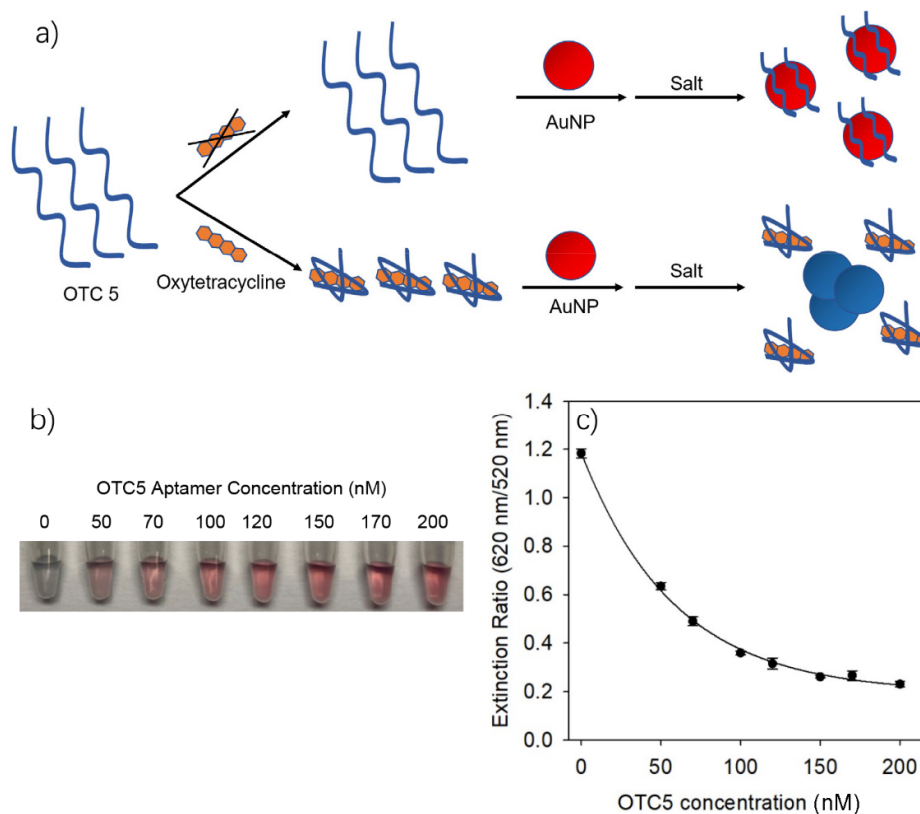


Figure 5: a) A scheme of label-free colorimetric detection of oxytetracycline using OTC5 and AuNPs. Note that this method did not consider the adsorption of oxytetracycline to AuNPs and may not be valid in practice. b) A visual observation of AuNPs protected with different concentrations of OTC5 and NaCl was added to induce AuNP aggregation. c) Extinction ratios of samples protected by different concentrations of OTC5.

Supported by both visual and UV-Vis spectrophotometer observations, an OTC5 concentration of 50 nM was sufficient to protect the AuNPs from total aggregation. Thus, 50 nM was chosen as the optimized concentration used in the later oxytetracycline biosensor testing, since it brings the biosensor the highest sensitivity (e.g. the color difference between 0 and 50 nM is greater than the difference between 100 nM and 50 nM in Figure 5b). This color difference was also quantitatively reflected from the difference in the extinction ratio in Figure 5c.

To determine the feasibility of using AuNPs as indicator in the oxytetracycline/aptamer biosensor system, the OTC5 aptamer and a non-binding mutant control were used (see Figure 1d-e for structures). Samples were prepared by mixing oxytetracycline solutions of varying concentrations, phosphate buffer, DNA aptamers or the control mutant strands followed by adding AuNPs and NaCl. The mutant aptamer cannot bind to oxytetracycline, and would be expected to adsorb onto AuNPs regardless of the presence of oxytetracycline resulting in protection of the AuNPs from aggregation. As shown in Figure 6a, the OTC5 aptamer showed oxytetracycline-dependent changes in the extinction ratio as predicted by the sensor design. The initial ratio was around 0.6, which matched the data in Figure 5c. The ratio increased with increase of oxytetracycline concentration, indicating aggregation of the AuNPs. However, the mutant showed the same trend, given that the mutant was unable to bind oxytetracycline.

To further investigate the specificity, solutions with low nanomolar of oxytetracycline were analyzed (Figure 6b). Again, the difference between controls and samples was negligible (the slope of the mutant was even slightly higher), meaning an absence of feasibility for the oxytetracycline biosensor. To confirm that the OCT5 aptamer possessed specificity for oxytetracycline while the mutant did not, a control experiment was conducted by titrating the aptamers into oxytetracycline solutions and monitoring the fluorescence increase (Figure 6c). Indeed, we only observed the fluorescence change from the aptamer.²⁶ Therefore, we concluded that the AuNPs are not a suitable choice of indicator in the OTC5 aptamer system. The adsorption of oxytetracycline to the AuNPs is a possible root cause.

A15 DNA was also tested as a control sequence for the sensor experiment (Figure 6b). A15 DNA has a very strong stabilizing effect on AuNP and is not known to bind oxytetracycline. Indeed, its initial ratio was the lowest, confirming the strong protection effect. Even with A15, the extinction ratio also increased with the oxytetracycline concentration. It appears that oxytetracycline has an effect in decreasing the protection from DNA. This is another example that target/AuNP interactions disallowed the label-free sensing.³⁰ Given the relative weak interaction between AuNPs and the tetracyclines, successful biosensors might be produced by engineering aptamer structures such as using split aptamers.⁴⁸

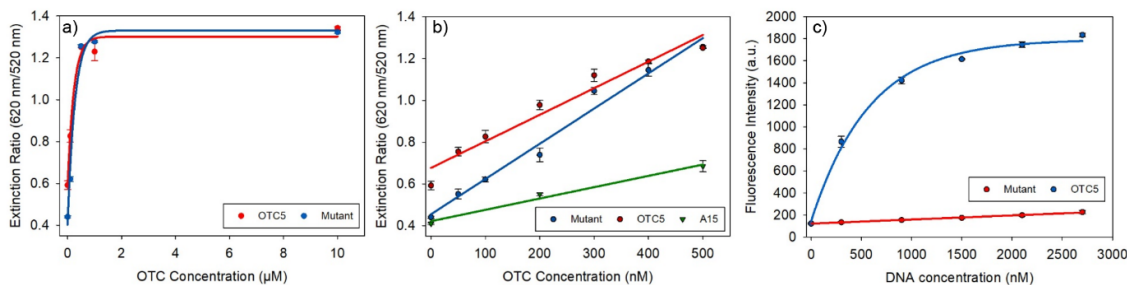


Figure 6. A comparison between OTC5 aptamer, the nonbinding mutant, and A15 DNA through extinction ratios a) at low micromole oxytetracycline concentrations range, b) at nanomolar oxytetracycline concentrations range in pH 8.2 buffer. c) Fluorescence intensity of oxytetracycline solution titrated using the OTC5 aptamer and the nonbinding mutant DNA.

One possibility is that the adsorbed tetracyclines would hinder the further adsorption of single-stranded DNA onto AuNP surface. To test this hypothesis, 0.1 μM fluorophore-labeled DNA (random sequence and OTC5) was incubated with AuNPs in the sensor buffer in the absence or presence of 500 nM and 10 μM tetracyclines. After incubation, excess DNA was washed off, leaving only the AuNPs with adsorbed DNA. We then dissolved the AuNPs using 10 mM KCN to release bound DNA and the fluorescence of the released DNA was quantified. As shown in Figure 7, the amount of adsorbed DNA decreased by the presence of oxytetracycline, which was observed for two different DNA sequences. This decreased DNA adsorption may decrease the stability of AuNPs. We also noticed a difference in the adsorption capacity of the two DNA strands, which was likely due to their sequence difference (e.g. adenine binds to AuNPs more strongly than thymine).¹⁸

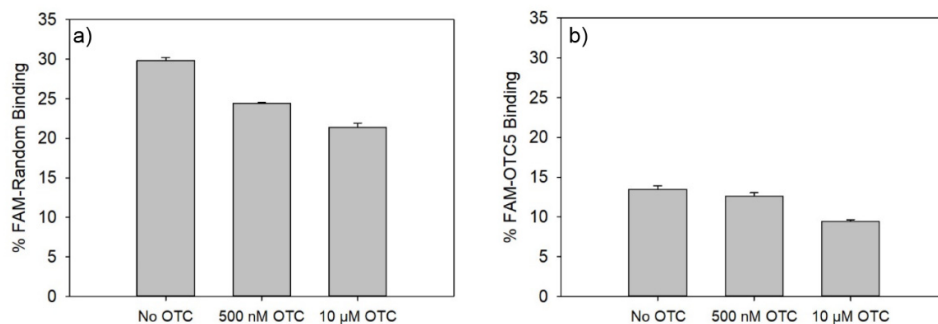


Figure 7: Percentage binding of a) FAM-Random DNA, and b) FAM-OTC5 DNA onto AuNPs,

without oxytetracycline (OTC), and with the presence of 500 nM and 10 μ M of oxytetracycline.

4. Conclusions

In this study, we explored the adsorption of a few common tetracyclines onto AuNPs and its impact over further DNA adsorption onto AuNPs, and the feasibility of a DNA aptamer-AuNP biosensor system. pH-dependent adsorption studies were carried out under pH 5 and pH 8.2, where the tetracyclines were net neutral and net negative, respectively. At pH 5, the adsorbed tetracyclines induced a greater destabilization effect. Kinetics studies elucidated that as small molecules, the tetracyclines underwent a rapid adsorption process. At low μ M concentrations, the tetracyclines followed a Langmuir adsorption isotherm. Adsorption induced fluorescence enhancement was observed with oxytetracycline. The OTC5-AuNP biosensor was optimized to a DNA aptamer concentration of 50 nM. Under such conditions, the biosensor feasibility was poor due to the indistinguishable difference between samples made with OTC5 aptamer and the nonbinding mutant. Among the published aptamers, the OTC5 aptamer is a high affinity one with K_a around 100 nM.^{26,47} Nevertheless, the tested tetracyclines range is broader in the micromolar range, leading to excessed free tetracyclines that can interact with AuNPs and affect the adsorption of aptamers. OTC5 is a short aptamer of round 40-nt and it can fold to a closed hairpin structure. Compared to the adsorption of tetracyclines, the adsorption of the aptamer is likely slower based on its much larger molecular weight and charge repulsion to the AuNPs. The difference of the adsorption of the target bound and free aptamers appeared too small to influence the stability of AuNPs differently. Since this label-free method is not directly applicable for the detection of the tetracyclines, it indicated the dominating interactions between small molecules and AuNPs.

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Data Availability Statement

Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

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