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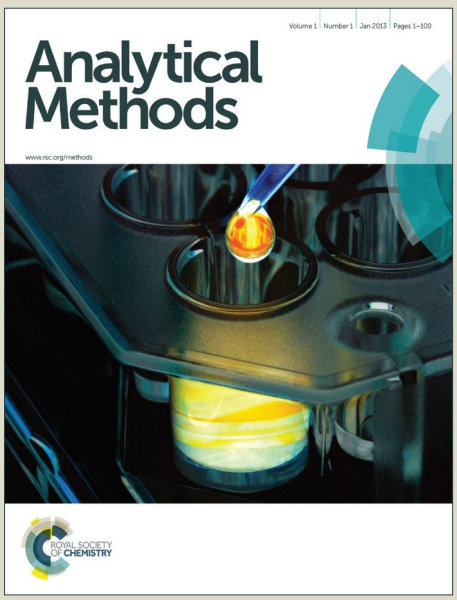

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ARTICLE

Understanding the Competitive Interactions in the Aptamer–Gold Nanoparticle Based Colorimetric Assays using Surface Enhanced Raman Spectroscopy (SERS)

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Aptamer–gold nanoparticles (AuNPs) based colorimetric assays have become increasingly popular as a viable rapid detection method, but the molecular interactions governing the mechanism and successful interpretation of the color changes has not been explored well. The objective of this study was to evaluate the competitive interactions that occur in this detection assay at the molecular level by employing surface enhanced Raman spectroscopy (SERS). The SERS signals of molecules in close proximity to AuNPs was exploited to give information on AuNPs surface coverage as well as ssDNA aptamer conformational changes during target capture. Two antibiotics, ampicillin and kanamycin, and their respective aptamers, were used in this study. Results indicate the reason for the lack of AuNPs aggregation with ampicillin could be due to a stronger binding affinity of AuNPs to the ampicillin than to the aptamer. Kanamycin, on the other hand, induced AuNPs aggregation to produce color change and the SERS data indicate a stronger binding affinity of kanamycin to the aptamer compared to the AuNPs as well as aptamer conformational changes. The use of SERS can be a potential tool to rapidly screen and validate the aptamer and target interaction for application of aptamer–gold nanoparticles (AuNPs) based colorimetric assays.

Introduction

Gold nanoparticles (AuNPs) are popular detection probes because they are able to produce a range of colors, caused by the shift of surface plasmon resonance (SPR) upon aggregation.[1] Target induced AuNPs aggregation has been widely applied in a variety of AuNPs based colorimetric assays for rapid sensing of ions (e.g. Hg^{2+}), small molecules (e.g. cocaine), complex molecules (e.g. proteins) and even cells.[2–5] One of the main advantages of using this kind of assay is the ability to detect changes with the naked eye. In addition, if an accurate and quantitative result is desired, a relatively inexpensive and commonly available spectrophotometer can be used to analyze a range of absorbance wavelength with precise optical density values. On the other hand, a major limitation hindering the application of many modes of AuNPs based colorimetric assays is the tendency for nonspecific interactions that can significantly alter the aggregation status of AuNPs. Indeed, a considerable number of papers have been dedicated to overcoming the nonspecific interactions, or biofouling, of AuNPs in the colorimetric detection assays.[6,7]

In order to reduce the non-specificity of AuNPs based colorimetric assays, aptamers have been employed as target specific bio-capture agents. Aptamers are single stranded oligonucleotides that are artificially synthesized to capture its target. They have many benefits as a target capture agent due to the ease in reproducibility for synthesis, versatility in labelling, immobilizing, signaling and regenerating.[8,9] Furthermore, recent technological advancements have made it faster and cheaper to create new aptamers.[10,11] However, the most compelling reason for choosing aptamers as opposed to other target capture agents (e.g. antibodies) is its ability to capture virtually any target, including small molecules (e.g. toxic chemicals).[4,12–21] One of the common strategies for applying ssDNA in the AuNPs based assay is to adsorb ssDNA onto AuNPs surfaces to stabilize the AuNPs. The flexibility of the ssDNA aptamer to partially uncoil its bases allows the negative charge of the phosphate backbone to be sufficiently distant so that van der Waals forces between the bases and the gold is strong enough to cause the ssDNA to stick to the gold.[22] When a target is later introduced into the mixture, the aptamer will bind to the target, which can cause the aptamer to de-attach from AuNPs due to conformational change in its tertiary structure. Hence, the AuNPs becomes prone to aggregation by neutralization of the surface charge with salts added later.

Despite the numerous benefits of using aptamers in a detection method such as the AuNPs based colorimetric assay, there are several limitations that can hinder the successful implementation of this method. For example, aptamers are often selected in a buffer environment with a defined concentration of salts and pH, and thus, work optimally in those conditions, which happen to be critical factors contributing to the aggregation of AuNPs.[19] Hence,

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premature AuNPs aggregation may occur if sample solution matrix environment can significantly destabilize the AuNPs. Another challenge is the variable affinity of different aptamer sequences to AuNPs. For example, dT was found to have a much lower binding affinity to AuNPs than dG, dC, and dA., hence an aptamer sequence with more dTs may have lower binding affinity to AuNPs.[23] The same study also showed the length of oligonucleotides was able to significantly affect the stability of AuNPs. Lastly, there is still a possibility of other molecular interactions in the colorimetric assay that can significantly alter the results

In this study, we aim to understand the competitive interactions between AuNPs, aptamers, and targets in the aptamer-AuNPs based colorimetric assay described above. Two aptamers for the food-related antibiotics, ampicillin and kanamycin, respectively[14,17] were chosen for this study. Several key factors are considered and investigated. Firstly, a critical step to ensure the reliability and sensitivity of the detection assay is the optimization of the concentration ratio of AuNPs to aptamer. A lower than optimal AuNPs:aptamer concentration ratio will lead to false positives because the AuNPs will not be covered fully whereas a higher than optimal concentration ratio will lead to false positives. Secondly, for reliable application, the aptamer must have a stronger binding affinity to the target than to the AuNPs. Thirdly, it is equally important that the target has a stronger binding affinity to the aptamer than to the AuNPs. If it was the other way around, the method will be unreliable and potentially give false results.

Here, we applied surface enhanced Raman spectroscopy (SERS) to investigate these competitive interactions. SERS is a combination of Raman spectroscopy and nanotechnology. Raman spectroscopy is capable of molecular fingerprint specificity for every distinct molecule that is being analyzed. Noble metallic nanosubstrates are essential in the enhancement of these Raman signals, attributed to the electromagnetic and charge transfer mechanisms. AuNPs are excellent SERS nanosubstrates when aggregated together and the chemical signatures of analytes staying in close proximity get mostly enhanced, and hence, the use of SERS not only requires little sample preparation, but allows for quick analysis of the surface interactions occurring between AuNPs, aptamer and/or target.

To the best of our knowledge, this is the first study that is conducted to understand the competitive interactions of molecules involved in the aptamer-AuNPs based colorimetric assay. The procedure and results from this study not only help us understand the interactions that occur in the molecular level, but also give us an analytical framework for future studies that may require understanding of competitive interactions in a complex system.

Experimental

Chemicals and Reagents

Chloroauric acid ($HAuCl_4$), was purchased from Sigma Aldrich (St. Louis, MO, USA). Tetrasodium citrate, potassium phosphate monobasic, potassium phosphate dibasic, sodium chloride, ampicillin and kanamcycin and were purchased from Fisher Scientific (Pittsburg, PA, USA). ssDNA aptamers were purchased as lyophilized powder (salt-free) through Eurofins MWG Operon (Huntsville, AL, USA). Double distilled water was obtained from MicroPure system UV/UF (Fisher Scientific, Pittsburg, PA, USA) and was used throughout the experiment.

Synthesis of citrate-stabilized AuNPs

13 nm diameter AuNPs, as used in many aptamer-based colorimetric assays[4,12–21], were synthesized using a citrate reduction method. [19,24,25] Prior to synthesis, glassware were soaked in aqua regia (3 parts HCl, 1 part HNO_3), rinsed copiously with double distilled water and dried. Then, in a 250 ml beaker, 100 ml of 1 mM $HAuCl_4$ solution was heated on a hot plate with vigorous stirring using a magnetic stir bar. When the aqueous 1 mM $HAuCl_4$ solution started to boil, 10 ml of 38.8 mM trisodium citrate was quickly added and allowed to continuously boil for 20 min. During the boil, the color of the mixture turned from pale yellow to colorless, then to dark red. After 20 min, the mixture was taken off the hot plate and cooled to room temperature in a clean, closed lid container. TEM was performed to verify the size and consistency of the AuNPs (13 $\pm$ 1.1 nm) (**Fig. S1**). The AuNPs concentration was then determined using Beer's Law with an extinction coefficient of 1.39 X 10^8 M^{-1}, resulting in calculated value of 17 nM.[26]

Aptamer preparation

ssDNA aptamer specific to ampicillin and kanamycin were obtained from literature[14,17]. The ampicillin specific aptamer (AMP17) sequence was 5'- GCGGGCGGTTGTATAGCGG-3'while the kanamycin specific aptamer (Ky2) sequence was 5'-TGGGGGTTGAGGCTAAGCCGA-3'. Prior to use, the aptamers were dissolved in 10 mM potassium phosphate buffer (pH 8.0), heated to 95 °C for 2 min and cooled to room temperature for 1 h in order to ensure the proper, 3-dimensional ssDNA conformation. The aptamer solution was then further diluted with buffer to various concentrations for analysis.

Procedure of aptamer-AuNPs based colorimetric assay

In a 96-well microplate, 40 µL of AuNPs was first mixed and incubated with 60 µL of 600 nM aptamer solution for 5 min. Then, 60 µL of target at various concentrations (final: 0, 30, 300, 600, 1200 nM) were added and mixed with the aptamer-AuNPs solution for 5 min. After incubation, 40 µL of 500 mM NaCl solution was added to induce AuNPs aggregation and the mixture was left for another 5 min to equilibrate. Pictures were taken and absorbance spectra (i.e. wavelength range: 450-750 nm) were measured using a SpectraMax M2e UV-vis spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). Finally, the absorbance ratio (620/520) was calculated to quantify the degree of AuNPs aggregation for each sample. Each sample was conducted in triplicates.

SERS study of molecular interactions in matrix

One of the main advantages of using SERS to study molecular interactions is the ability to analyze samples in situ. Since little or no sample preparation is required, it can be analyzed rapidly without the limitation of time lag and destruction of samples. In this study, 1

µL of each sample mixture obtained from the aptamer-AuNPs-based colorimetric assay were deposited onto a gold-coated slide and left on a counter for a few minutes to dry. Then, the samples were analyzed using a DXR Raman spectro-microscope (Thermo Scientific, Waltham, MA, USA).

OMNIC™ software version 9.1 was used to control the DXR Raman spectro-microscope. The sample surface was first focused using a 50x microscope objective lens, then ten spots were randomly selected within the region of aggregated AuNPs to maximize Raman enhancement effects. Aggregated AuNPs were mainly formed around the sample coffee ring and were characterized by dark, black spots.

Raman instrumentation parameters used include a 780 nm laser excitation wavelength connected to a 50 µm slit width aperture, which produced a 4.7–8.7 cm^{-1} spectral resolution and 3.1 µm spot diameter. The laser power used was set at 5 mW with an integration time of 2 s per spot. The spectral range analyzed was 400 - 3000 cm^{-1}.

After collecting all the spectra, the data was inputted into TQ Analyst software (Thermo Scientific, Waltham, MA, USA) for statistical analysis. First, all the spectra gathered from different spots in each sample were averaged, and then normalized using the full spectral range (i.e. 400-3000 cm^{-1}) in order to reduce peak intensity variations attributed to Raman enhancement differences. Then, second derivative transformation and smoothing were applied so as to reduce spectral noise and to separate any overlapping bands. Finally, the principal component analysis (PCA) function was used to quantitatively compare the differences between several sets of spectra. This function is primarily used to focus the spectral data analysis onto the most dominant features, thereby removing random variations that are of less significance. The extent of variation between different samples is then measured based on how far the sample spots are from each other on the PCA plot. For example, if the spots representing "A" is clustered away from spots representing "B", then we can conclude that these two samples are significantly different from each other ($p<0.05$). On the other hand, if "A" and "B" spots overlap each other, then they are not significantly different from each other. Hence, this type of discriminant analysis is useful to determine the extent of variation between different sample spectra.

Results and Discussion

Target-Aptamer Interactions

To understand the competitive interactions in the aptamer-AuNPs colorimetric assay, it is important to first understand the basic mechanism for target-aptamer interactions. The underlying molecular interaction between aptamers and their target is largely due to hydrogen bonding, electrostatic interactions, stacking of aromatic rings and van der Waals interaction.[11] They typically follow an induced fit model, whereby the aptamer changes its 3-dimensional structure to interact fully with their target. Their secondary structure is also characterized by stems, internal loops, bulges, hairpins, tetra loops, pseudoknots, triplicates, kissing complexes, or G-quadruplex; these specific structures influences the binding sites of the target.

The chemical structure of ampicillin and kanamycin, together with the secondary structure of ssDNA aptamers specific to these two compounds are shown in **Fig. 1**.

Ampicillin is a β-lactam antibiotic, and is characterized by a β-lactam ring in its molecular structure. Its side chain (circled) contains a primary amine functional group and a benzene ring. Interestingly, the ampicillin aptamer was reported to be highly specific to the ampicillin side chain, as other β-lactam antibiotics with similar chemical structures such as amoxicillin (i.e. additional hydroxyl group) and benzylpenicillin (i.e. lacks primary amino group) had significantly lower binding affinity to the aptamer (AMP17).[17] The aptamer sequence responsible for the binding of ampicillin was also reported to be "-GGT(T)-" in the loop region as well as "-GC-" base pairing (circled) at the joint of a loop and stem.[17]

Kanamycin is an aminoglycoside antibiotic, and is characterized by amino-modified glycoside groups in its molecular structure. Similar antibiotic structures such as kanamycin b (i.e. one hydroxyl group replaced by an amine) and tobramycin (i.e. one hydroxyl group replaced by an amine and another hydroxyl group removed) were shown to have similar binding affinity to the kanamycin aptamer (Ky2).[14] Other antibiotics with relatively different chemical structures such as ampicillin (i.e. penicillin), streptomycin (i.e. aminoglycoside) and sulfadimethoxine (i.e. sulfonamide) were reported to have significantly less affinity to the aptamer.[14] This suggests that this aptamer sequence may be broad-specific for kanamycin and its derivatives. The "-TGGA"- motif (circled) was also found in a stem and loop structure in every aptamer candidates, hence it was concluded that this structure was primarily responsible for the capture of kanamycin.[14]

Optimization of Concentration-Ratios

In order to study competitive interactions in the aptamer-AuNP based colorimetric assay, it is important to optimize the detection assay to best represent the implementation of this method. The use of the right concentrations of each component in the aptamer-AuNP based colorimetric assay is crucial to not only improve sensitivity, but to also prevent false colorimetric results. **Fig. 2a** shows a schematic illustration of the aptamer-AuNP based colorimetric assay.

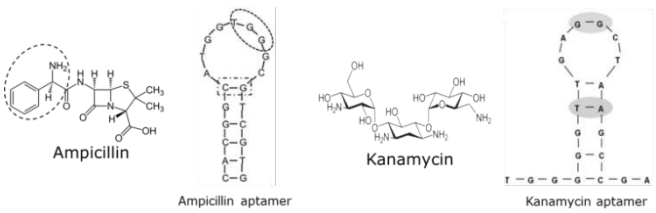

Fig. 1 Chemical structures of target antibiotics and aptamers used (i.e. circled regions on aptamer structures indicate conserved motifs) [aptamers obtained from Song et. al.[14,17] Used with permission from Elsevier]

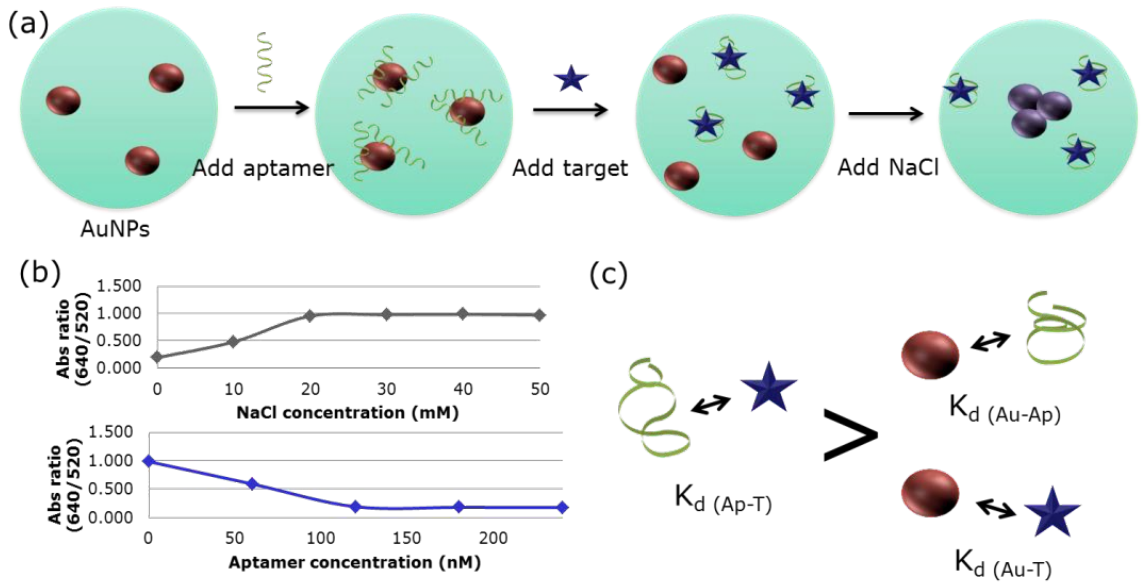

Fig. 2 **(a)** Schematic Illustration of a suitable aptamer-based colorimetric assay; **(b)** Optimization of aptamer and NaCl concentrations using absorbance ratio (i.e. 640/520); **(c)** Relative binding affinity (Kd) requirements for successful implementation of the aptamer-based colorimetric assay (Ap = aptamer; T = target; Au = gold nanoparticle)

The first important optimization step is the concentration of NaCl added to induce the aggregation. NaCl is used in this assay to neutralize the surface charge caused by citrate-capped (i.e. negatively charged) AuNPs. Adding too little NaCl will cause insufficient neutralization of the surface charge of AuNPs, hence it will not induce aggregation (i.e. change color) even if aptamers were not bound to the AuNPs. If too much NaCl is added, it can potentially neutralize the surface charge of ssDNA aptamers, and thus cause AuNPs to get close enough for a color change, even though steric hindrance can prevent full aggregation. Our results show that 30 mM NaCl was optimal for the full aggregation of AuNPs without aptamer (Fig. 2b), and hence this concentration was used for subsequent experiments in this study.

The second optimization step in this assay requires knowledge of how much aptamer to add to AuNPs. If insufficient aptamer is added, the AuNPs will be partially exposed and will be more sensitive to AuNPs aggregation. On the other hand, if there is an oversaturated amount of aptamer, the AuNPs will more likely still be covered by aptamer even after target addition as the target will preferentially bind to the aptamers not bound to AuNPs, hence a false negative result can occur. **Fig. 2b** shows the absorbance ratio (640/520) of the samples decreasing as more aptamer is added to AuNPs until an optimum concentration (i.e. 180 nM), where it begins to remain constant even after more aptamer addition.

Competitive Interactions Influencing the Detection of Ampicillin

Fig. 2c shows the key competitive interactions and their relative affinities to each other in order to have a reliable aptamer-AuNPs colorimetric assay. Briefly, the relative binding affinity between aptamer and target must be stronger than the aptamer-AuNPs and the target-AuNPs interactions. The detection of ampicillin was used as the first example for investigating these competitive interactions. To do this, ampicillin was tested at several concentrations that represented a range of aptamer:target concentration ratios (i.e. 16:1, 2:1, 1:1, and 1:2). The absorption spectrums of these samples are shown in **Fig. 3a**.

Surprisingly, the absorption peak for all target concentration samples was 520 nm, which is representative of a wine-red color (**Fig. 3c**). **Fig. 3b** shows the absorption ratio vs ampicillin concentration graph, where the higher the absorption ratio, the more aggregation of AuNPs has occurred. This result showed no significant difference ($p<0.05$) in the aggregation status of these samples.

SERS was then used to elucidate the surface chemistry of AuNPs. **Fig. S2** shows the full raw SERS spectra of all the samples analyzed. In order to interpret the SERS data, the second derivative SERS

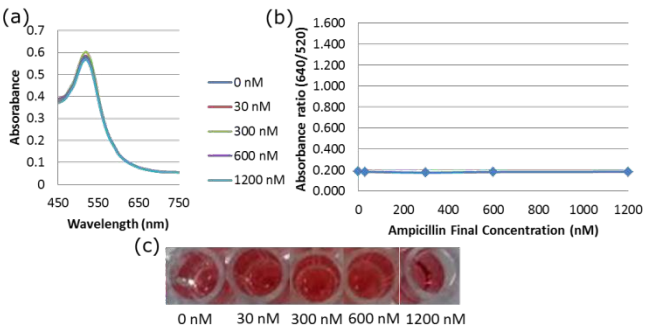

Fig. 3 **(a)** UV-vis absorbance of aptamer-based colorimetric samples with various ampicillin concentrations; **(b)** Absorbance ratio (640/520) based on UV-vis absorbance; **(c)** Optical image of aptamer-based colorimetric samples with increasing concentrations of ampicillin (left to right).

spectra after smoothing and normalization was used to reduce spectral noise and to focus on changes caused by the addition of ampicillin. **Fig. 4** includes the second derivative SERS spectra and principal component analysis (PCA) 3-D graph of samples analyzed at a lower (i.e. 30 nM) and higher (600 nM) ampicillin concentration.

At the lower ampicillin concentration, it is evident that the AuNPs surface coverage is dominated by peaks attributed to the binding of aptamers. The PCA graph shows a statistical similarity between the "Aptamer only" sample (i.e. AuNPs mixed only with aptamer) and the "Aptamer+Ampicillin (30 nM)" sample (i.e. AuNPs mixed with aptamer and 30 nM ampicillin).

The SERS spectra at higher ampicillin concentrations, however, show a different result. A quick observation of the spectra shows a huge difference between the "Aptamer only" and the "Aptamer + Ampicillin (600nM)" samples, but a very close resemblance between the "Aptamer + Ampicillin (600nM)" and "Ampicillin only (600nM)" samples. The PCA graph confirms this observation and thus, we can conclude that the AuNPs surface was almost fully covered with ampicillin and furthermore, displaced the aptamer previously bound to the AuNPs.

From the SERS results (i.e. structure), the colorimetric results (i.e. function) can be better explained. In this case, the SERS results showed that in these sample matrices, the AuNPs-ampicillin interaction was stronger than the AuNPs-aptamer and ampicillin-aptamer interactions, so that the ampicillin can replace the aptamer on the AuNPs. From the molecular structure of ampicillin, we speculate the interaction between ampicillin and AuNPs is primarily through the Au-S bond, which is very strong and commonly used for surface modification of Au.[27,28] After ampicillin has covered the AuNPs surface, the addition of salt was unable to induce aggregation due to the steric repulsion of the ampicillin molecules. It is also possible some aptamers were still attached to the ampicillin even after ampicillin covered the AuNPs, as some little aptamer signals were observed in the SERS spectra of the "Aptamer + Ampicillin (600nM)" sample, and thus provide additional repulsive charge/hindrance to prevent AuNPs aggregation. In this scenario, because of the proximity of the aptamer from the AuNPs was further than the ampicillin, the signals of the aptamer was much weaker than the ampicillin. In either case, however, the competitive interactions and their relative affinity to each other do not meet the assay criteria to obtain desired results in the aptamer-AuNPs based colorimetric assay.

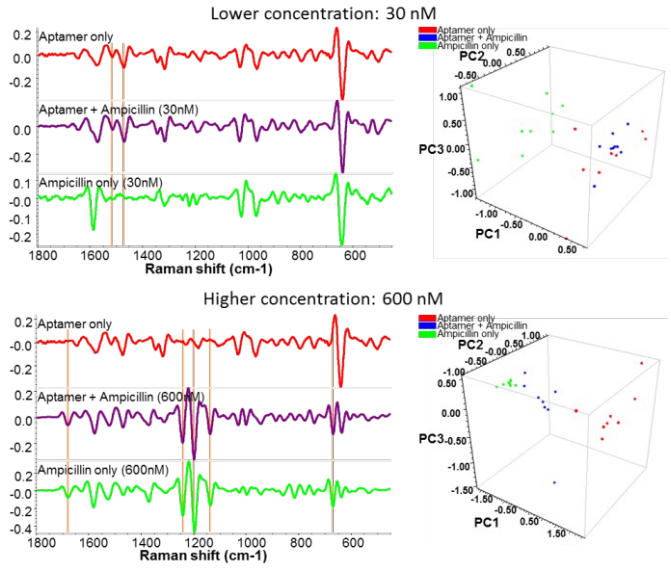

Fig. 4 Second derivative SERS spectra and principal component analysis chart of aptamer-based colorimetric samples at low (i.e. 30 nM) and high (600 nM) ampicillin concentrations. Aptamer and ampicillin peaks are represented by orange lines on the spectra. Other specific peaks can be located by referring to Fig. S2.

Competitive Interactions Influencing the Detection of Kanamcyin

To better understand the competitive interactions influencing the detection of analyte in the aptamer-AuNPs colorimetric assay, kanamycin was used as the second target with its specific aptamer sequence. **Fig. 5a** shows the colorimetric results as kanamycin concentration was added from 0 to 1200 nM.

As the concentration of kanamycin increased, the absorption peak wavelength shifted from 520 nm to approximately 640 nm, which corresponds to the visual color change from red to blue (**Fig. 5c**). The absorption ratio (640/520) increased from 0.2 to 1.4 when the kanamycin concentration was increased from 0 to 600 nM, with a linear, concentration-dependent curve from 30 to 600 nM (R^2 = 0.994) (**Fig. 5b**). These colorimetric results show the successful aggregation of AuNPs due to kanamycin introduction.

The raw SERS spectra of kanamycin at different concentrations in the aptamer-AuNPs based colorimetric assay are found in **Fig. S3**. The spectra in **Fig. 6** use the same data and provide information on the surface coverage of AuNPs in this assay. At lower kanamycin concentrations (i.e. 30 nM), the "Aptamer + Kanamycin (30nM)" sample's spectral profile was not the same as the "Aptamer only" sample's, although there were several peaks that were similar to each other. The "Kanamycin only" sample's spectral profile was also not the same as the "Aptamer + Kanamycin (30nM)" sample's, but had some similar peaks. This suggests that the aptamer and kanamycin were both present on or near the surface of AuNPs,

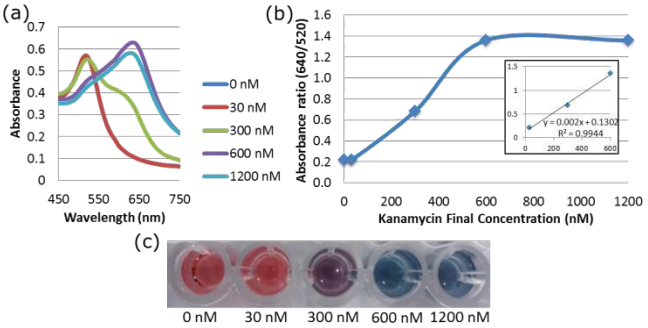

Fig. 5 **(a)** UV-vis absorbance of aptamer-based colorimetric samples with various kanamycin concentrations; **(b)** Absorbance ratio (620/520) based on UV-vis absorbance; **(c)** Optical image of aptamer-based colorimetric samples with increasing concentrations of kanamycin (left to right).

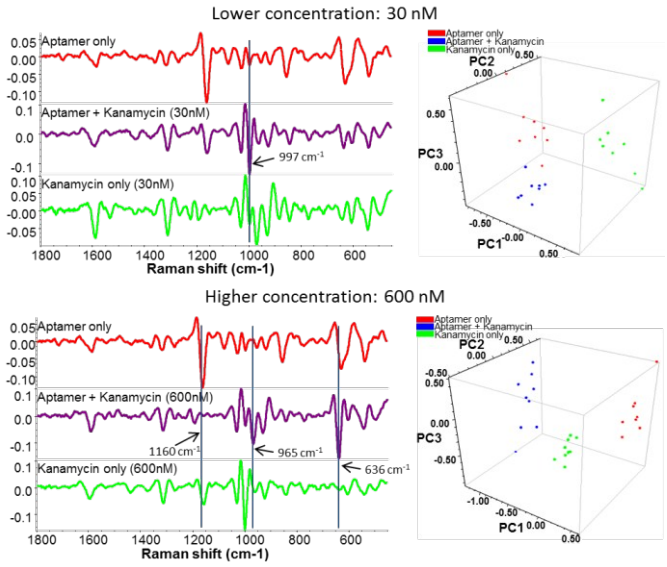

Fig. 6 Second derivative SERS spectra and principal component analysis chart of aptamer-based colorimetric samples at low (i.e. 30 nM) and high (600 nM) kanamycin concentrations. Aptamer and kanamycin peaks are represented by dotted lines on the spectra. Other specific peaks can be located by referring to Fig. S3.

possibly due to the interaction between aptamer and kanamycin. The PCA graph shows the "Aptamer + Kanamycin (30nM)" sample spectra had a PC1 score between the "Aptamer only" sample and the "Kanamycin only (30 nM)" sample, which strengthens this claim. In addition, the enhanced peak at 997 cm^{-1} for "Aptamer + Kanamycin (30nM)" is possibly another indication of an interaction between aptamer and kanamcyin.

At the higher kanamycin concentration (600 nM), the "Aptamer + Kanamycin (600nM)" sample's spectral profile was still not the same as the "Aptamer only" or "Kanamycin only (600nM)" sample's. However, the spectral profile of "Aptamer + Kanamycin (600nM)" was different from "Aptamer + Kanamycin (30nM)". The changes that occurred could be due to the wrapping of kanamycin by the aptamer, thus producing conformational changes that can be detected by SERS. For example, the aptamer peak at 1160 cm^{-1} disappeared on "Aptamer + Kanamycin (600nM)". At 965 cm^{-1}, a new peak was seen on "Aptamer + Kanamycin (600nM)", whereas a shift in the aptamer peak was spotted at 636 cm^{-1} on "Aptamer + Kanamycin (600nM)". These SERS results suggest that the aggregation of AuNPs was due to the stronger affinity of aptamer binding to the kanamycin than the affinity of the kanamycin to the AuNPs, as seen in the case of ampicillin and its aptamer. The lack of resemblance of the "Kanamycin only (600nM)" sample's spectral profile to "Aptamer + Kanamycin (600nM)" also suggests that the affinity of kanamycin binding to AuNPs was weaker than the affinity of aptamer to kanamycin. Hence, the aptamer-AuNPs based colorimetric assay using the kanamycin aptamer and its target meets the criteria to successfully interpret the colorimetric results.

Conclusions

With the increasing popularity of aptamer-AuNPs based colorimetric assay, it is important to understand the underlying factors that contribute to the success of this detection method. This study was able to evaluate the competitive interactions present in the aptamer-AuNPs colorimetric assay using SERS to ensure the correct interpretation of the colorimetric results. The interaction between aptamer and target must be stronger than the interaction between AuNPs and aptamer as well as the interaction between AuNPs and target. In addition, SERS was employed as a simple and rapid (<30 min) method to investigate surface chemistry of AuNPs at the molecular level in order to ensure reliable colorimetric detection. Future studies would involve the investigation of the colorimetric assay in a complex matrix such as food.

Acknowledgements

The acknowledgements come at the end of an article after the conclusions and before the notes and references.

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Graphical Abstract

for

Understanding the Competitive Interactions in the Aptamer–Gold Nanoparticle Based Colorimetric Assays using Surface Enhanced Raman Spectroscopy (SERS)

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