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Screening criteria for qualified antibiotic targets in unmodified gold nanoparticles based aptasensing

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Abstract: In designing unmodified gold nanoparticles-based aptasensing (uGA) assays for antibiotics, we find that some antibiotics can adsorb directly on gold nanoparticles (GNP) regardless of the presence of aptamers, which have been long overlooked in the past. Some adsorptions, however, would strongly disturb the charge distribution on the GNP surface, break up the static colloidal profile, and thus generate false positive colorimetric signals. To identify antibiotics qualified for uGA assays, we established two rational screening criteria for antibiotic targets relying on their oil-water partition coefficients (LogPs) and net physiological charges: logP > 0 and charge $\leqslant$ 0. A good agreement of the GNP color change was obtained between the two criteria-based predictions and the actual tests using six representative antibiotics. The proposed criteria help to shed light on GNP-target interactions, which is significant for developing novel GNP-based colorimetric assays with high reliability.

Main text

Introduction

Gold nanoparticles (GNPs) have emerged as excellent tools in nanotechnology due to their unique physical and chemical properties.[1-2] The interparticle plasmon coupling during GNP aggregation, which leads to clear color change (from red to purple/blue) has attracted considerable attention in the field of colorimetric biosensing.[3-4] GNP aggregation (or redispersion) triggered by targets with predictable color change could be observed by naked eye with sensitivity in the range of nM to μM without additional signal amplification steps.[5-6] The most simplistic mechanism relies on controlled loss of electrostatic stabilization of unmodified GNPs (particles covered merely by a thin capping layer), which has been widely used together with functional DNAs, especially aptamers (*in vitro* selected oligonucleotides with specific target binding abilities[7-9]), to develop colorimetric biosensors. Briefly, GNPs stabilized by small charged moieties (for example, citrate-stabilized GNPs[10]) would aggregate when the surface charges are neutralized by adding salt; Single-stranded DNA (ssDNA) can physically adsorb onto GNPs and stabilize GNPs electrostatically against such salt-induced aggregation (Fig. 1A).[11] Free aptamers (ssDNA) and aptamer/target complexes enable GNPs with different tolerances against salt-induced aggregation, which breeds numerous unmodified GNP-based aptasensing (uGA) assays for rapid label-free colorimetric detection[12-13] or aptamer affinity determination.[14-15] Since capping ligand strongly affects GNP's colloidal property, the discussion here is limited to the most widely used citrate-stabilized GNPs.

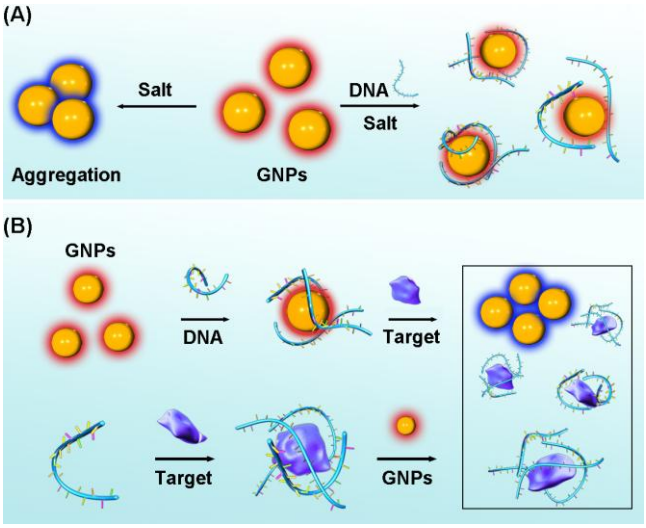

Fig. 1 Unmodified GNP-based aptasensing (uGA) assays. (A) single-stranded DNA (ssDNA) adsorbed GNPs showed enhanced stability against salt-induced aggregation. (B) Two strategies used in uGA assays.

Targets that pose a significant public health threat (such as antibiotics) are of particular concern for developing rapid, low cost, simple-to-use and instrument-free assays with minimum scientific burden on the user. Two general strategies have been discussed during the development of such uGA assays (Fig. 1B): should GNPs be incubated with aptamers prior to (strategy 1)[16-17] or after (strategy 2)[18-19] the addition of target? Regardless of which, one common assumption shared by default is that the target itself has little effect on the color response in uGA assays. However, this assumption has often been overlooked. In developing uGA assays for antibiotics, we found that certain antibiotic targets could adsorb directly onto GNPs and induce aggregation, thereby ruining detection accuracy with false positive signals. This paper describes two rational screening criteria for prediction of qualified antibiotic targets based on *in silico* feasibility analysis. The proposed criteria help to decrease

unnecessary and costly experimental trials, and shed light on GNP-target interactions, which is significant for developing novel GNP-based colorimetric assays with high reliability.

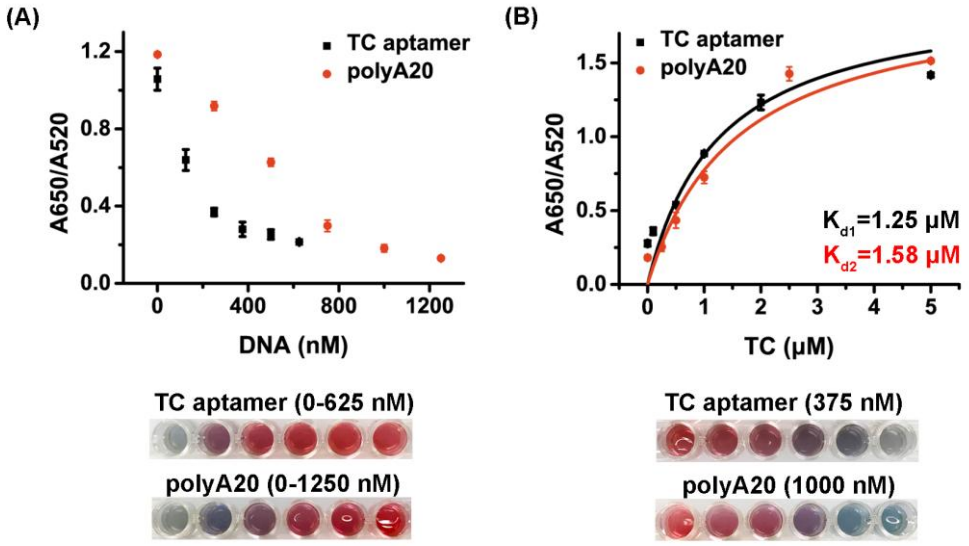

Fig. 2 False positive signals in uGA assays for tetracycline (TC) detection: (A) DNA concentration optimization; (B) TC titration curves fitted by Michaelis-Menten model.

False positivity occured in uGA assays for tetracycline (TC)

Without appropriate control group, uGA signals could be misleading. In the case of tetracycline (TC) detection, TC aptamer (K_d 3.4 nM)[20-21] and polyA20 were applied as experimental and control DNA, respectively. PolyA20 was used as control DNA since no TC-polyA20 interaction could be observed (Fig. S5), thus negative uGA signals could be expected. Firstly, the minimum volume of TC binding buffer that lead to GNP aggregation was optimized to be 40 μL in a 200 μL system (Fig. S3). Then the minimum concentrations of TC aptamer and polyA20 required for GNP stabilization against salt were optimized to equal 375 nM and 1000 nM, respectively (Fig. 2A).

Unexpectedly, uGA assays adopting TC aptamer and polyA20 showed almost no difference in the shape of TC titration curves and even the two K_d values obtained were close to each other (Fig. 2B). Considering that adenine showed the highest GNP stabilizing ability among four DNA nucleotides,[22-23] and no TC-polyA20 interaction could be observed (Fig. S5), polyA20-stabilized GNPs were assumed to remain stable (red suspension) regardless of the presence of TC. Thus, the observed colorimetric responses could be designated to false positive responses. If target is treated as the only variable with fixed aptamers,[17, 24-25] it may be wrongly concluded that colorimetric responses are caused by specific aptamer-target bindings.

As for reasons to explain such false positive signals, the first possibility – TC as additional electrolytes – is ruled out since TC concentration was less than 5 μM in the system (it takes over 30 mM of NaCl to induce aggregation, Fig. S1B). Another possibility is the formation of TC-adsorbed GNPs. If so, in the abovementioned control group (uGA assays adopting polyA20), TC and polyA20 would act as destabilizer and stabilizer for the GNP system, respectively. According to the experimental results, it seemed that the destabilization effect of TC-adsorption was stronger than the stabilization effect of polyA20. Among many explored adsorption profiles,[26-28] the most studied case is the BSA-gold binding, which is favored in the order of "hydrophobic > COO^- >NH^{3+} > OH > ethylene glycol".[29] TC-adsorbed GNPs is likely to exhibit weaker repulsive interparticle forces, resulting in a loss of colloidal stability even in the presence of polyA20. It is important to notice that not only the loss of stabilizer (aptamer), but also the gain of destabilizer (adsorbed antibiotics)

would induce GNP aggregations. In the latter case, it is useful to screen qualified targets for uGA assays.

Proposal for screening criteria of qualified antibiotic targets in uGA assays

We propose to use LogP (log10 of the coefficient for solvent partitioning between 1-octanol and water) and physiological charges of antibiotics as two screening criteria based on the following considerations: (1) LogP is a metric parameter for hydrophobicity, which plays a key role in protein-gold binding and contains substantive thermodynamic free energy information.[30] (2) Electrostatic interaction is important in GNP stabilization,[5] and most aptamers work at neutral pH,[9] therefore the physiological charges of antibiotics matters. Depending on specific buffer used in aptamer SELEX process, the application scenarios of aptamers usually involve solution with near neutral pH,[9] thus the physiological charges of antibiotics are considered to be another important factor. More specifically, positively charged antibiotics are more easily to be adsorbed onto negatively charged citrate-stabilized GNPs than others due to electrostatic attraction. For qualified antibiotic targets, such adsorption should be circumvented. (3) The two parameters are easily accessible based on both experiments and computational methods.[30-31] Therefore it seems that only antibiotics with charge ≤ 0 and logP > 0 would not destabilize GNPs and could be regarded as qualified targets for uGA assays (Fig. 3).

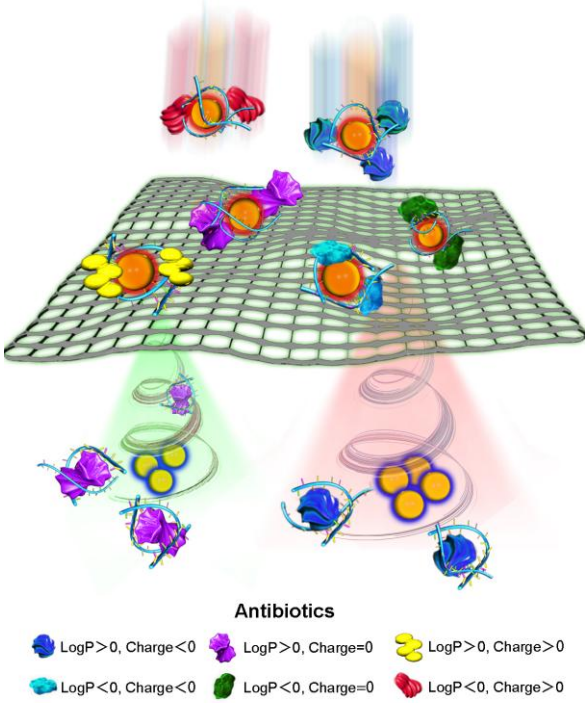

Fig. 3 Screening of qualified antibiotics for uGA assays based on two criteria: Six types of representative antibiotics are being filtered by a mesh filter. Only antibiotics possess logP>0 and charges⩽0 (irregular shapes in blue and magenta) meet the filter criteria and could go further for downstream detection; Whereas the opposite is true for other four types of antibiotics (irregular shapes in yellow, cyan, green and red), which would destabilize GNPs and induce aggregation.

Validation of the screening criteria

In order to test the criteria, antibiotics were divided into six types with representative antibiotics (PNG, CHL, ERY, TC, ENO and KN, Table 1). Their adsorption profiles were shown in Fig. 4. As expected, GNP colloidal suspensions remained stable and redish only with CHL and PNG, which possessed logP>0 and charges≤0. For CHL and PNG, it might be tactless to claim that no adsorption take place. Actually there are two possibilities: (1) Barely no adsorption of antibiotics took place; (2) Antibiotics adsorbed onto GNPs as new stabilizers, forming antibiotic-stabilized GNPs, which

could be further discriminated based on zeta-potential analysis.[29] Originally, GNP system showed a zeta potential at −15.1 mV. After adding 20 μM of PNG or CHL, zeta potentials of the system were measured to equal −11.7 mV and −30.9 mV, respectively. GNP colloids become more negatively charged and stable upon addition of CHL molecules, which suggest adsorption of CHL onto GNP surface as a new stabilizer. In principle, uGA assays can still be designed for targets like CHL, although with some compromised sensitivity.

In the case of PNG, first of all, the variation of zeta potential was relative small when compared with CHL. Second, the negative charges carried by PNG molecules may hinder their adsorption toward electronegative GNPs due to electrostatic repulsion. Third, although there was observed variation in zeta potential, colloidal systems containing GNP and PNG were colorimetrically stable according to experimental results (Fig 4A). Moreover, detection of trace PNG has been achieved using nanogold-based system,[33-34] and no specific interactions between PNG and nanogold were reported in these researches. Taken together, we tentatively tend to attribute the zeta potential variation in PNG-GNP system to slight changes in the chemical environment.

Table 1 Properties of representative antibiotics from six types*

Type (Charge, LogP)	Drug name	Abbreviation	Physiological Charge	LogP
I (−, +)	Penicillin G	PNG	−1	+1.08
II (0, +)	Chloramphenicol	CHL	0	+0.88
III (+, +)	Erythromycin	ERY	+1	+2.6
IV (−, −)	Tetracycline	TC	−1	−3.5
V (0, −)	Enoxacin	ENO	0	−0.98
VI (+, −)	Kanamycin A	KN	+4	−7.1

*All LogP values (source: ALOGPS) and physiological charges were adopted from the DrugBank database (https://www.drugbank.ca).[31] Structures of the six representative antibiotics are plotted in Fig. S6.

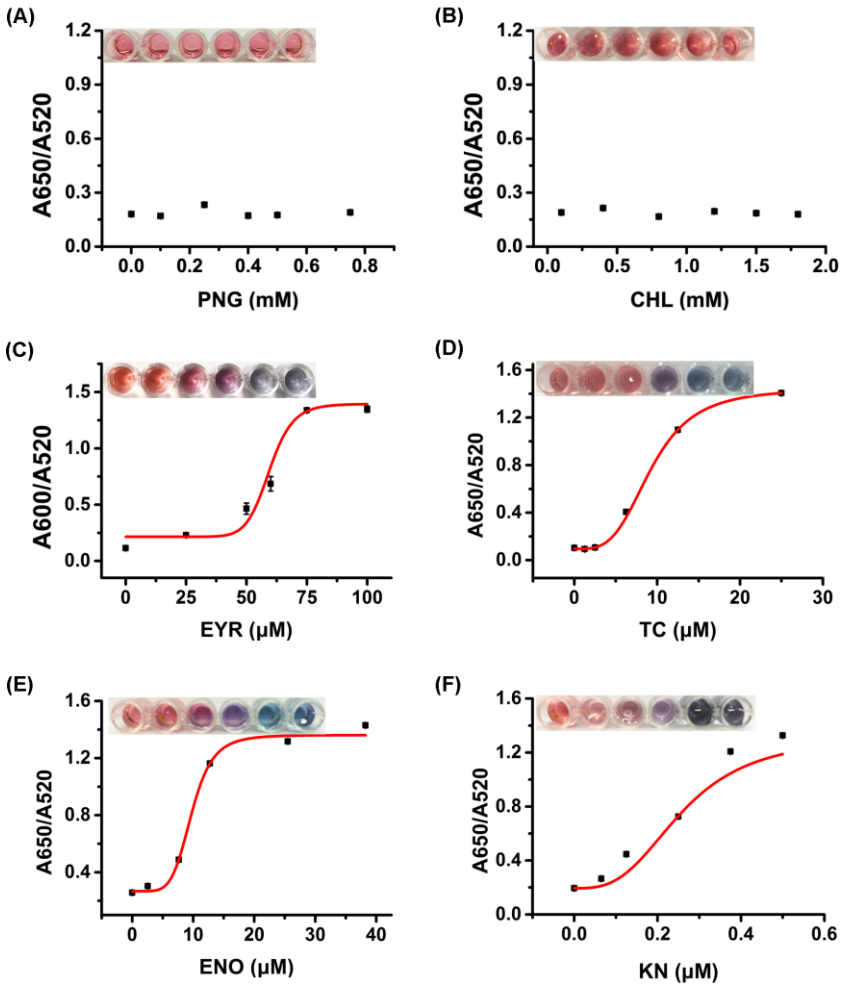

Fig. 4 Adsorption profiles of six representative antibiotics. (A)-(F): PNG, CHL, EYR, TC, ENO and KN from type I-VI, respectively. Data in (C)-(F) were fitted by four-parameter Logistic fitting. Inset: corresponding photographic images.

For four unqualified antibiotics that caused GNP aggregation, GNP colorimetric responses were rapid for ENO and KN, medium speed for EYR and slow for negatively charged TC (Fig. S8). Considering that citrate-stabilized GNPs are originally negatively charged, such differences in response speed may suggest that the adsorption is primarily dominated by electrostatic attraction. Furthermore, it is interesting that the EC_{50} values (fifty percent effect concentrations) in adsorption profiles show roughly linear relation to logP values (Fig. S9). Therefore, we speculate that charges and logP values account for adsorption dynamics and thermodynamic stability, respectively. Besides, even though this work is based on 13 nm-diameter GNPs, similar phenomena were observed in the cases using GNPs of other sizes (Fig. S10). Besides the six antibiotics in Table 1, the criteria were further tested the criteria using five other randomly selected antibiotics whose aptamers have been reported (Table S1). The adsorption of five antibiotics onto GNPs were conducted using the same experimental condition with that mentioned in the manuscript (see details in section 10, supporting information). As expected, unqualified targets would directly lead to colorimetric responses of GNPs under the experimental condition, as shown in Fig. S12. The observed phenomenon also fits the criteria.

Detection of unqualified targets using uGA assays

In principle, we do not recommend to design uGA assays for unqualified targets. However, we found that the differences between experimental and control groups could be used for target quantification. In the case of uGA assays for KN, KN aptamer[24] and its mutant (control group, no KN binding ability, Fig. S11) were applied

using two previously described strategies (Fig. 1B). As shown in Fig. 5C, unqualified target KN (logP<0, charge<0) led to GNP aggregation in control groups as expected. However, due to specific KN-aptamer binding, the Abs. Ratios of uGA assays in experimental group were always higher than that of control group (Fig.5 A, B), which can be used for KN detection (Fig. 5D). The slope obtained for delta Abs. against KN concentration using strategy 2 is 4.4 times larger than that of strategy 1, suggesting that higher sensitivity for uGA assays was achieved using strategy 2. Such advantages in sensitivity is reasonable: It has been reported that the energy of DNA hybridization is much lower than the DNA base coordination energy on GNPs. Thus, addition of DNA cannot detach its complementary DNA from GNPs.[32] Therefore the molecular recognition ability is impaired for DNA adsorbed onto GNPs. Although this is acceptable as a compromised method, it is laborious to use the differences between experimental and control groups for target quantification. In this condition, immunoassays could serve as alternative troubleshooting techniques other than uGA assays for unqualified antibiotics.[35-38] For diagnostic and therapeutic applications, DNA-decorated GNPs could also serve as powerful tools.[39-41] At a more macroscopic level, the effect of antibiotic abuse could be measured by sequence analyses of antibiotics resistance genes instead of direct quantifying antibiotics.[42-44]

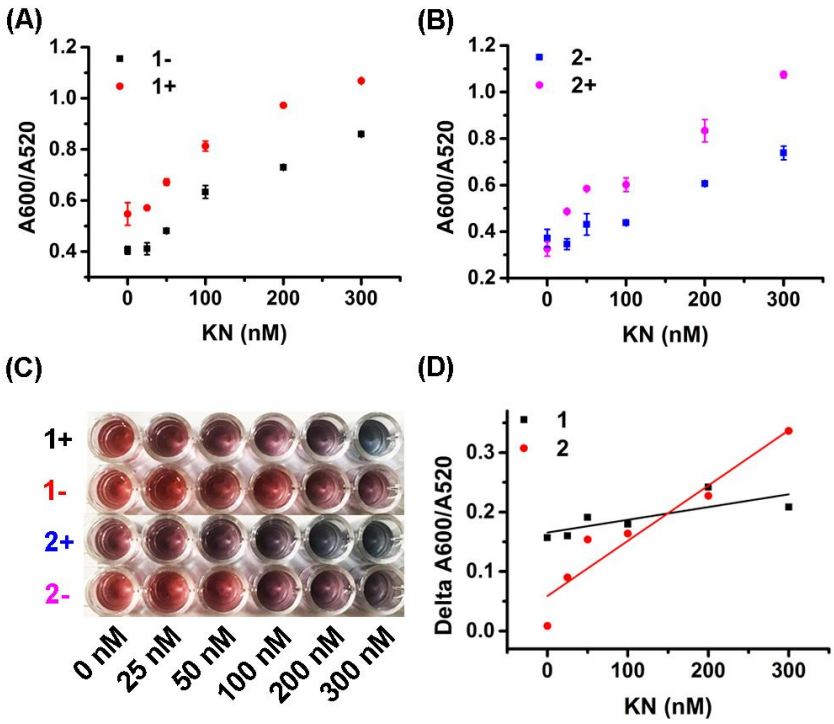

Fig. 5 uGA assay for KN using strategy (A) 1 and (B) 2 (+ and − represent for experimental and control group, respectively.); (C) Corresponding photographic images; (D) Detection of KN using delta Abs. Ratios.

Conclusion

In summary, GNPs are promising tools for developing inexpensive and user-friendly sensors for rapid antibiotics detection, which have important applications in drug industry regulatory, medical research, personal/home care products and environmental monitoring. Two screening criteria for qualified antibiotic targets for uGA assays were established using easily available parameters: (1) logP >0; (2) physiological charge ≤0. Thus *in silico* feasibility of uGA assays could be analyzed prior to real experiments. A good agreement of the GNP color change was obtained between the two criteria-based predictions and the actual tests in laboratory using six representative antibiotics. In general, we do not recommend to use unqualified targets in uGA assays. If uGA

assays must be used for detection of unqualified targets (on the basis of specific high-affinity aptamers), it is strongly recommended to incubate aptamer with antibiotics prior to GNP addition in order to obtain an enhanced sensitivity. More work is needed to go beyond the concern of this article for more rigorous investigation of the relationship between GNPs' colorimetric responses and two parameters (logP and charge) based on smart design, accurate mathematical analysis and computer modelling. Moreover, the universality of this developed criteria could be validated using other organic molecules instead of antibiotics.

Supporting information

The supplementary information (PDF file) includes materials, instruments, experimental procedures and supplementary figures.

Acknowledgments

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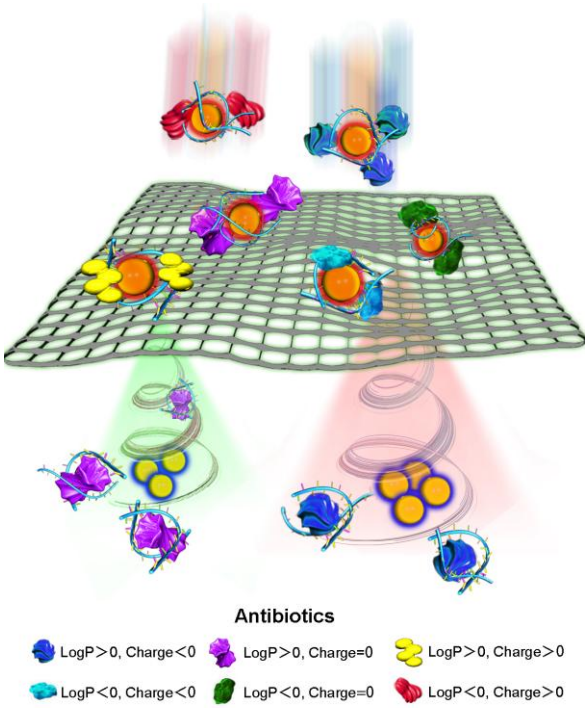
Antibiotics
LogP>0, Charge<0
LogP>0, Charge=0
LogP>0, Charge>0
LogP<0, Charge<0
LogP<0, Charge=0
LogP<0, Charge>0

For TOC only