

Supporting Information

Sensitive Detection of Antibiotics using Aptamer Conformation Cooperated Enzyme-assisted SERS Technology

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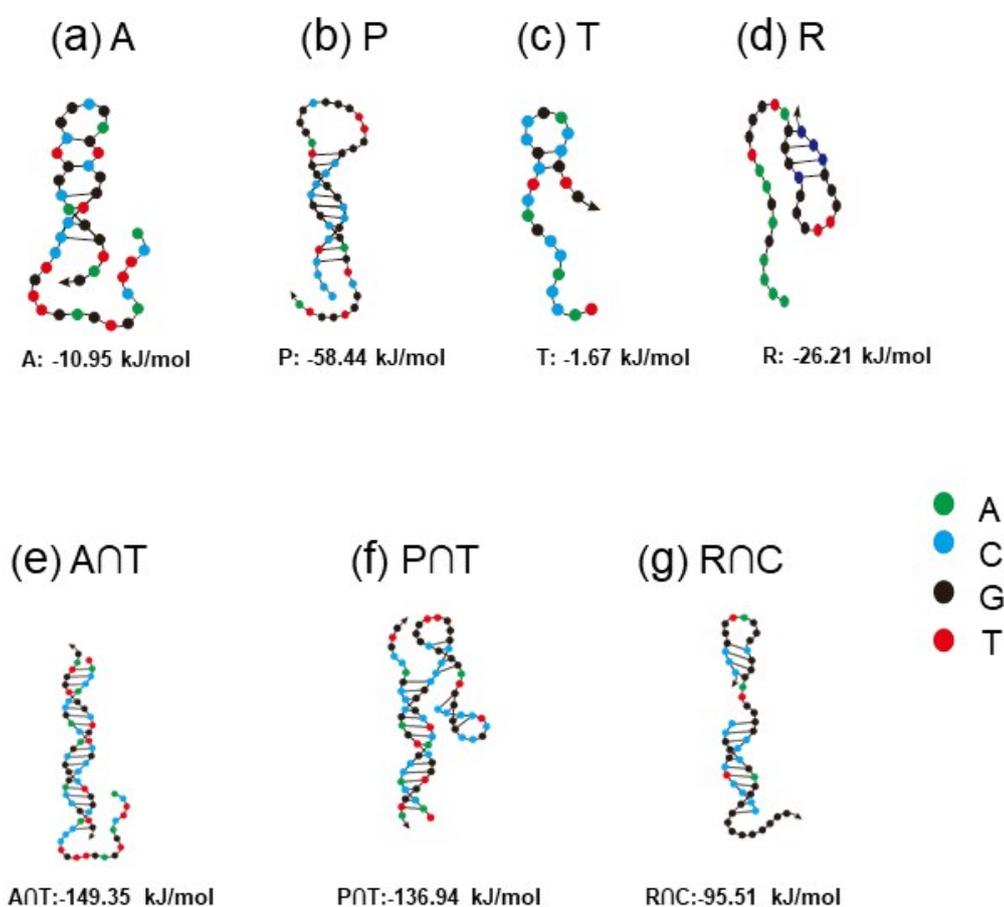


Figure S1. Minimum free energy (MFE) structures of different oligos and oligo hybridization with annotation of free energy calculated by NUPACK.

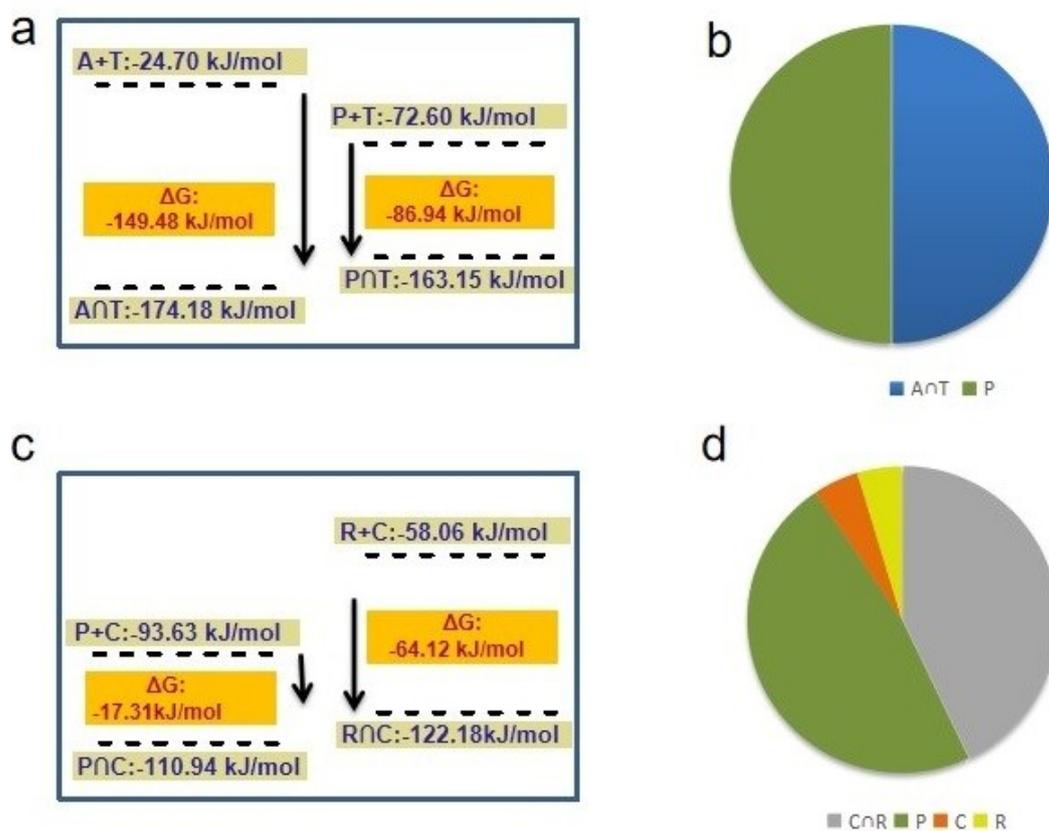


Figure S2. Theoretical calculations of the energy changes for the competitive hybridizations between DNA oligos. (a-b) Gibbs free energy changes and the calculated efficiencies for hybridization in silica: A∩T or P∩T. (c-d) Gibbs free energy changes and the calculated efficiencies for hybridization in silica: P∩C or R∩C. ∩ symbols hybridization of the oligos. (The temperature at 25 °C)

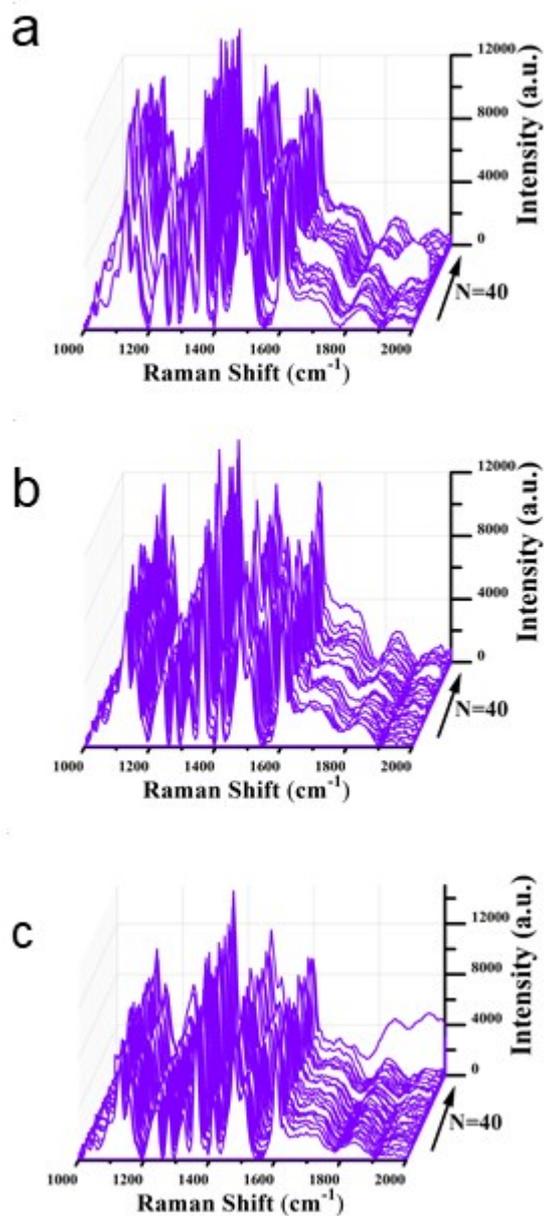


Figure S3. SERS spectra of Cy5 (1 μM) collected from 40 random spots of additional three separate Au NPs@Si substrates. Baseline correction was made for individual spectrum primarily using the software built-in function of automatic adjustment. The occasional issue of baseline over-processing may require manual adjustment for correction.

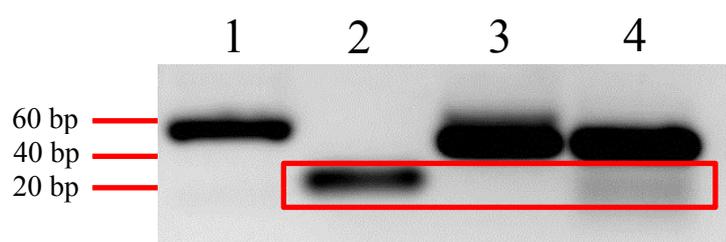


Figure S4 Gel electrophoresis validation of the ACCESS assay including both the first step of aptamer-CAP recognition-induced releasing and the second step of enzymatic cleavage in a single-tube experiment. 1: P+T; 2: R; 3: A+T+P+Exo III; 4: A+T+CAP+P+Exo III. GelRed nucleic acid stain used in the experiment. The red rectangle drawn as a visual guide for comparison of the band position.

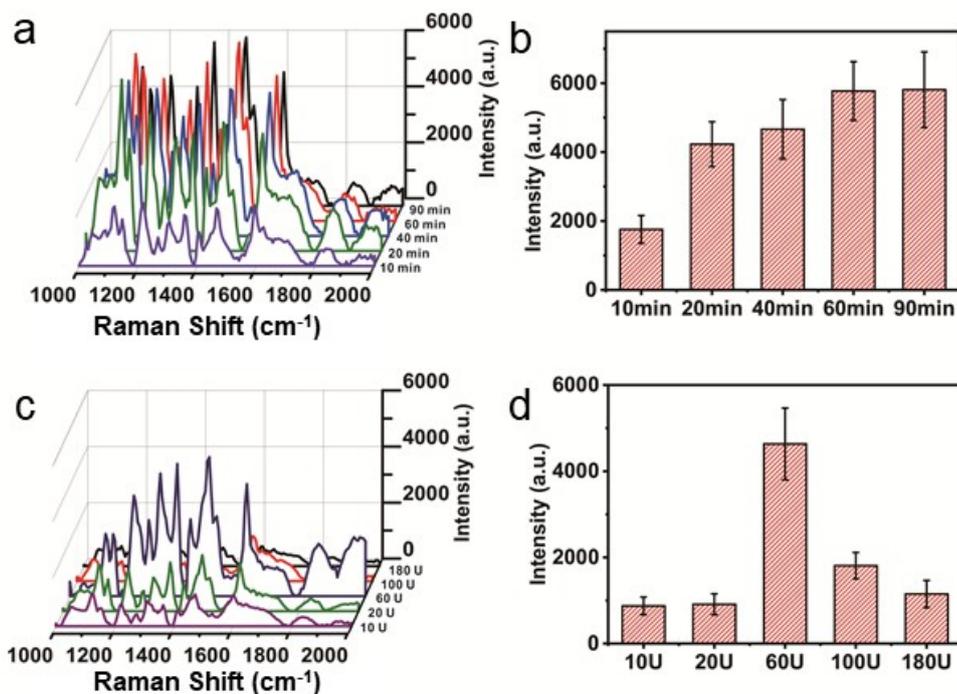


Figure S5. Optimization of the incubation time and Exo III enzyme concentration in the cleavage reaction. (a-b) SERS spectra and intensity quantification at 1366 cm⁻¹ peak in the titration experiments of the enzyme incubation time. (c-d) SERS spectra and intensity quantification at 1366 cm⁻¹ peak in the titration experiments of the enzyme concentration. Error bar: standard deviation (n = 3). Baseline correction was made for individual spectrum primarily using the software built-in function of automatic adjustment.

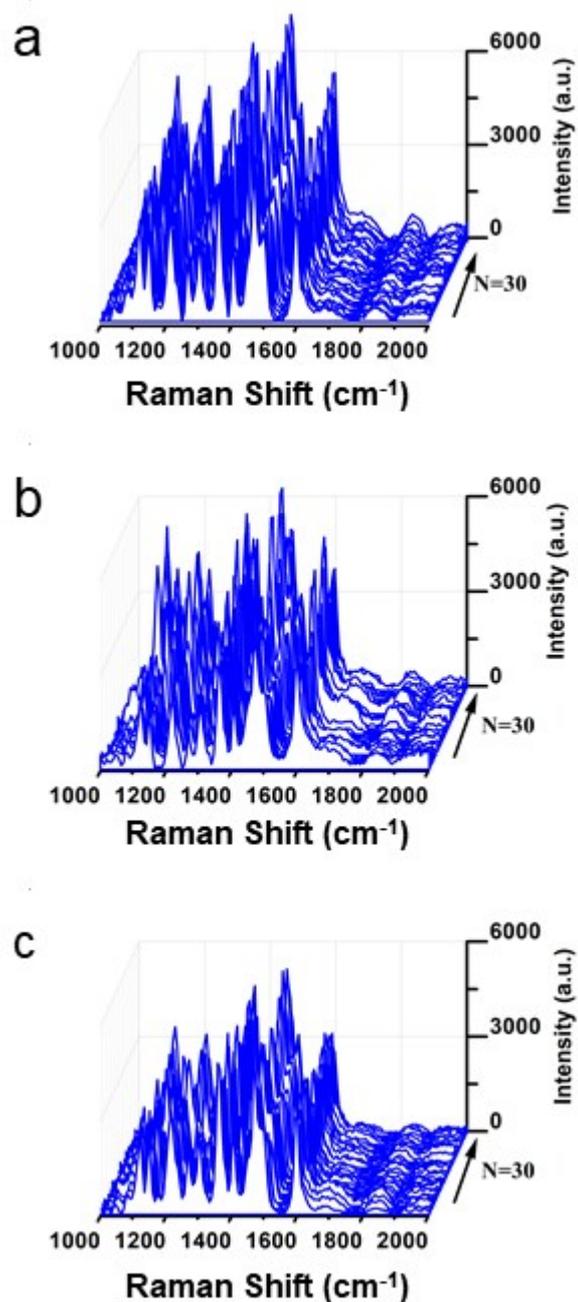


Figure S6. SERS spectra of CAP (150 pM) collected from 30 random spots of three identical Au NPs@Si substrates. Baseline correction was made for individual spectrum primarily using the software built-in function of automatic adjustment. The occasional issue of baseline over-processing may require manual adjustment for correction.

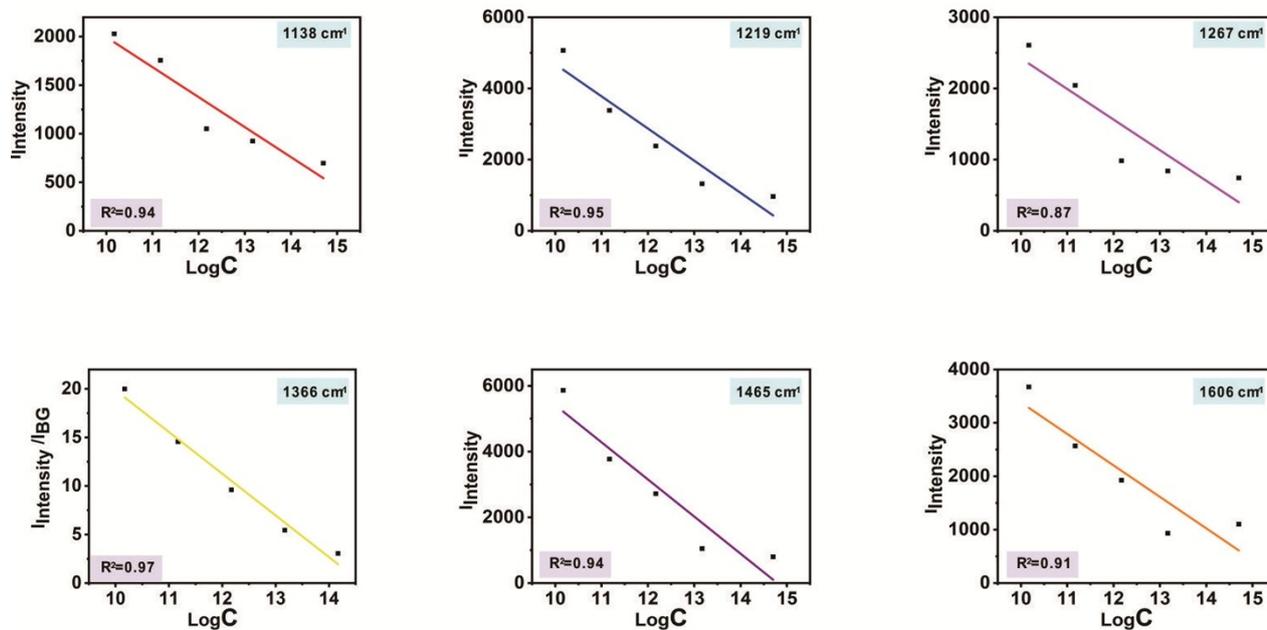
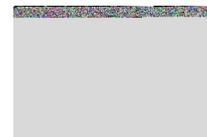


Figure S7. Raman intensities of the characteristic SERS peaks at 1138, 1219, 1267, 1366, 1465 and 1606 cm⁻¹ with logarithmic CAP concentrations in water.

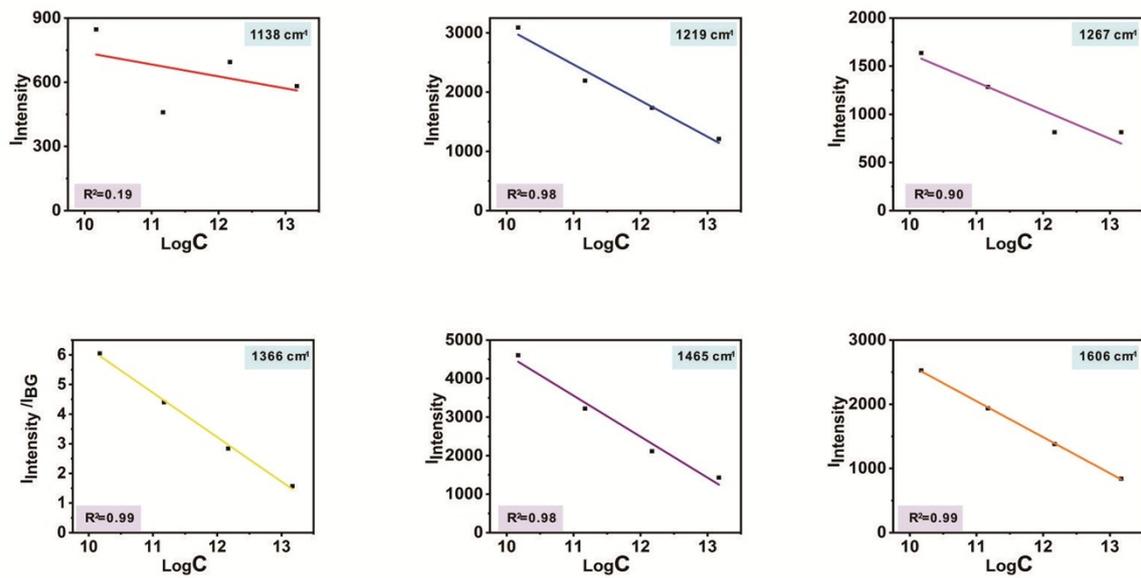
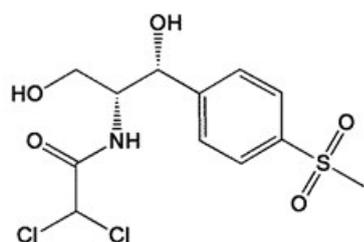
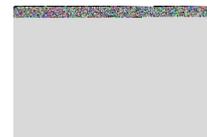
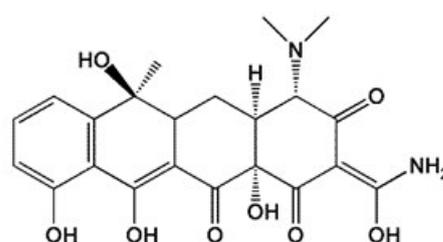


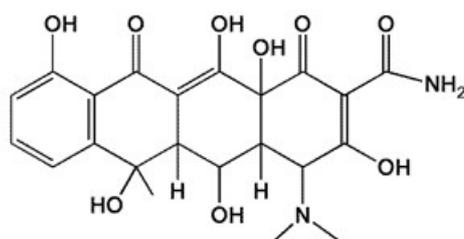
Figure S8. Raman intensities of the characteristic SERS peaks at 1138, 1219, 1267, 1366, 1465 and 1606 cm^{-1} with logarithmic CAP concentrations in milk.



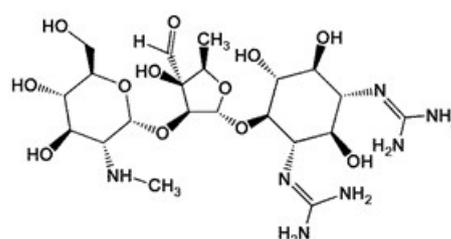
thiamphenicol



tetracycline



oxytetracycline



streptomycin

Figure S9. The chemical structures of different antibiotics, including thiamphenicol (TAP); tetracycline (TE); OT:oxytetracycline (OT); S:streptomycin (S).

**Table 1. The sequences of DNA oligos**

DNA	Sequences (5' → 3')
Probe	CCCCTCGCCGGGGTAGGGCGGGTTGGGCCCCGGCGAGTCGGTGGTA
Capture	CCGGCGAGGGGCGGGCGGG
Target	TACCACCGACTCGCCGACCGTG
Residue	CCCCTCGCCGGGGTAGGGCGGGTTGGGCC
Aptamer	ACTTCAGTGAGTTGTCCACGGTCGGCGAGTCGGTGGTAG

Table 2. Performance comparison of different assays for CAP detection

Assay principle	LOD	Linear Range	Reference
SERS	310 nM	310 nM-15.5 pM	46
Aptamer + Realtime q-PCR	3.1 nM	3.1 nM-62 nM	10
Surface plasmon resonance	164 nM	N/A	47
Chemiluminescent Immunoassay	0.25 nM	3.1 pM-31 pM	48
Electrochemical sensor	2.9 nM	10 nM-1000nM	19
Photoresponsive colorimetric immunoassay	0.03 nM	0.03 nM-12.5 nM	15
Our work	15 fM	15 fM - 150 pM	