

## A enzyme-free Fluorescence quenching sensor for amplified detection of kanamycin in milk based on Competitive triggering strategies

Table S1 Sequences of oligonucleotides used in this work.

DNA Name	Sequence (5' → 3')
APT	TGGGGGTTGAGGCTAAGCCGA
C-APT	GCCATATCGGCTGCCATACAACCCCA
H1	TCGGCTGCCATAT <u>AGGCT</u> TATGGCAGCCGATATGGC-FAM
H2	BHQ-1-GCCATATCGGCTG <u>ACATA</u> AAGCCGATATGGCAGCCGA
H1'	TCGGCTGCCATATCGGCTTATGGCAGCCGATATGGC-FAM
H2'	BHQ-1-GCCATATCGGCTGCCAT <u>AGCCGATATGGCAGCCGA</u>
H1 <sub>m12</sub>	TCGGCTGCCATAT <u>AGC</u> CTTATGGCAGCCGATATGGC
H2 <sub>m12</sub>	GCCATATCGGCTG <u>ACG</u> TAAGCCGATATGGCAGCCGA
H1 <sub>m13</sub>	TCGGCTGCCATAT <u>AGCC</u> CTATGGCAGCCGATATGGC
H2 <sub>m13</sub>	GCCATATCGGCTG <u>ACGT</u> IAGCCGATATGGCAGCCGA
H1 <sub>m22</sub>	TCGGCTG <u>AC</u> ATAT <u>AGG</u> CTTATGGCAGCCGATATGGC
H2 <sub>m22</sub>	GCCATAT <u>AGG</u> CTG <u>ACG</u> TAAGCCGATATGGCAGCCGA
H2 <sub>m23</sub>	TCG <u>C</u> TG <u>C</u> GATAT <u>AGG</u> CTTATGGCAGCCGATATGGC
H2 <sub>m23</sub>	GCC <u>C</u> TAT <u>C</u> I <u>G</u> CTG <u>ACG</u> TAAGCCGATATGGCAGCCGA

Underscores indicate mismatched bases. Italics are toehold fields.

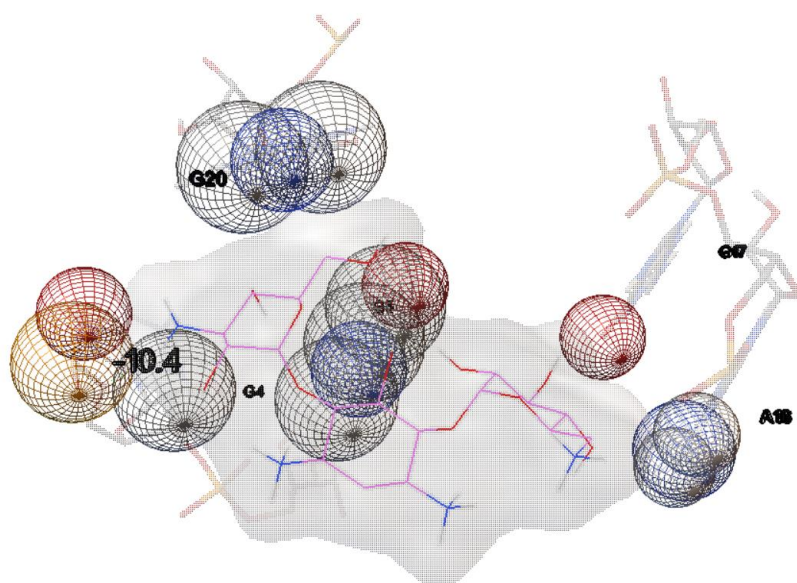


Figure S1 Molecular docking diagram of kanamycin and aptamer

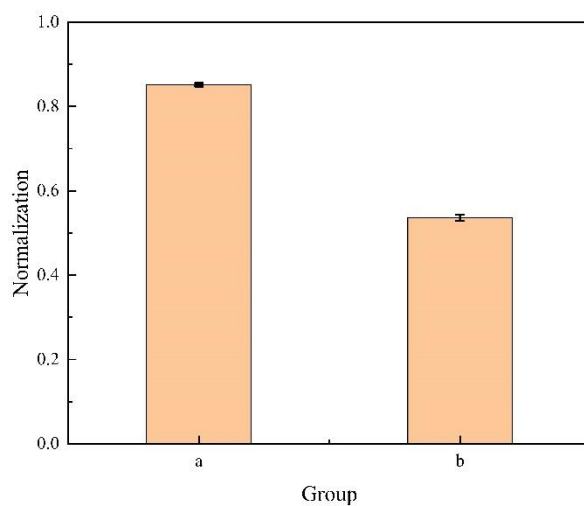


Figure S2 Normalization of fluorescence intensity. (a) Fluorescence ratio with mismatch (b) Fluorescence ratio without mismatch

Normalization =  $F_0/F$ ;  $F_0$  is the fluorescence intensity of H1(H1'), and  $F$  is the fluorescence intensity of H1(H1') mixed with H2(H2')

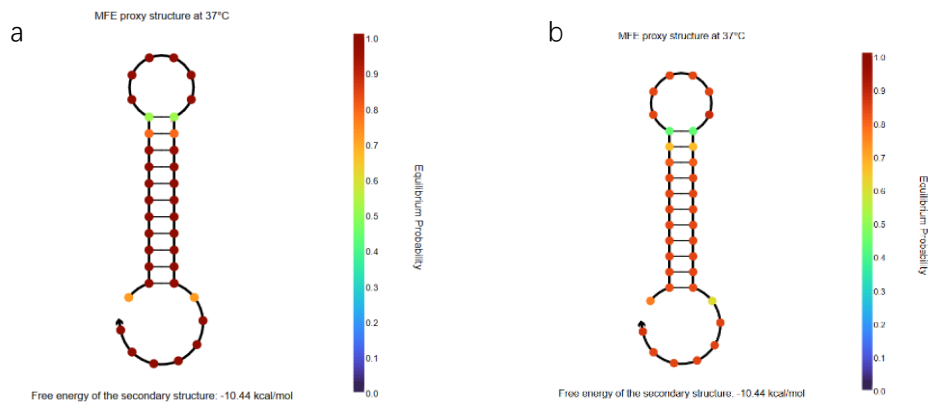


Figure S3 Diagram of NUPACK. (a) H1; (b) H1'

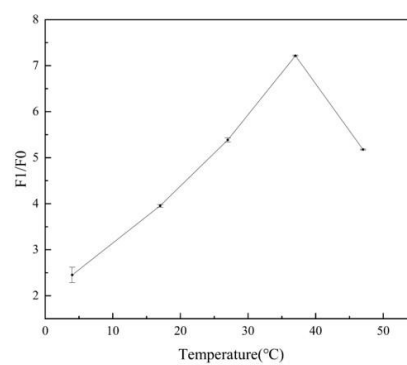
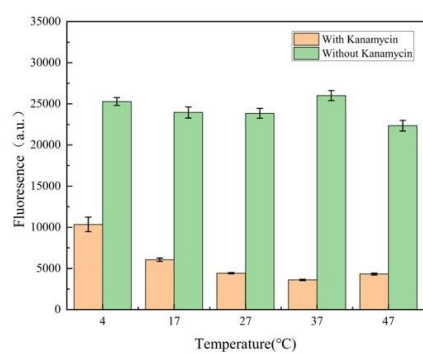


Figure S4 Optimization of reaction temperature

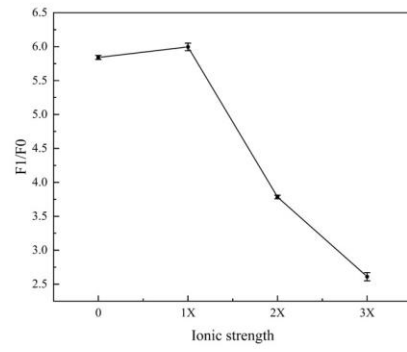
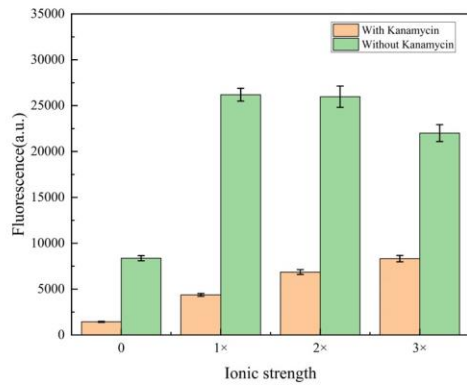


Figure S5 Optimization of ionic strength.

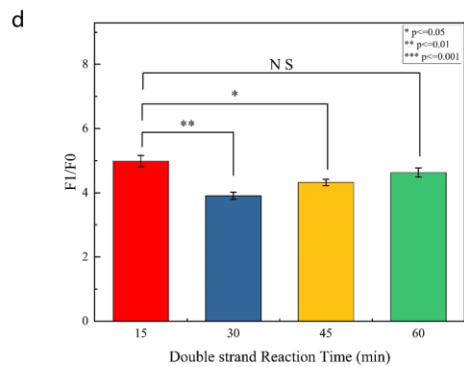
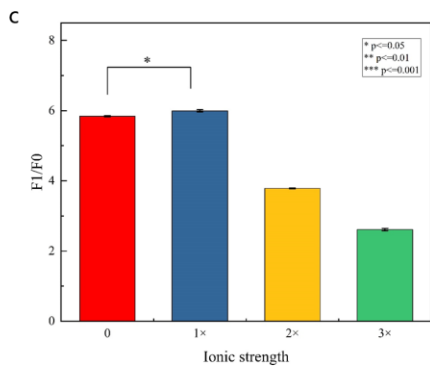
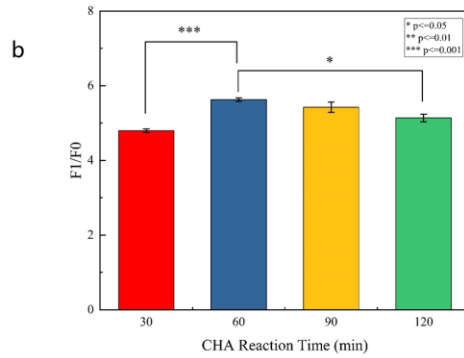
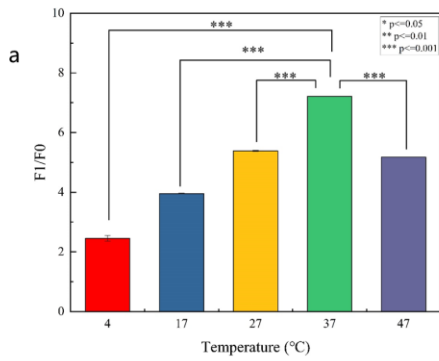


Figure S6 Figure S6 Significant difference analysis. (a)Temperature; (b)CHA Reaction Time; (c)Ionic strength; (d) Double strand Reaction Time

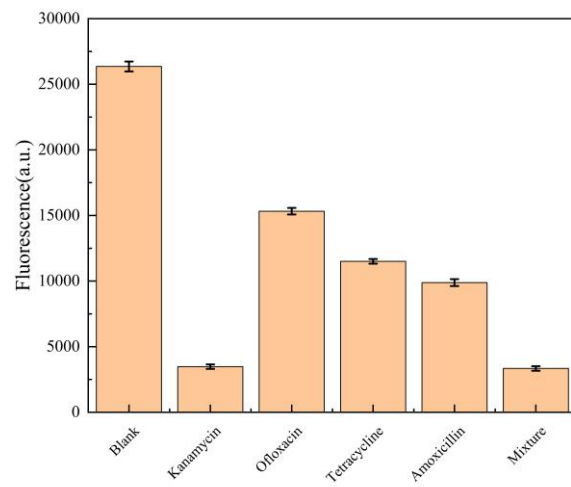


Figure S7 Specific detection of our biosensors. From left to right are blank control, kanamycin, ofloxacin, tetracycline, amoxicillin, and mixture.