Supporting Information for

Sensitive and specific detection of proteins based on target-responsive DNA polymerase activity

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Table S1. Oligonucleotide sequences studied in this work.

Name	DNA sequence (5' → 3')[a]
Apt-$_{pol(10)}$[b]	ATT CGT AGA TCA ATG TAC AGT ATT G
Apt-$_{pol(8)}$[b]	TCG TAG ATC AAT GTA CAG TAT TG
Apt-$_{pol(12)}$[b]	GAA TTC GTA GAT CAA TGT ACA GTA TTG
Apt-$_{pol(14)}$[b]	ATG AAT TCG TAG ATC AAT GTA CAG TAT TG
Apt-$_{pol(16)}$[b]	TGA TGAA TTC GTA GAT CAA TGT ACA GTA TTG
Apt-$_{lysozyme}$	ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG TTG ACC AGG A
Control Apt-$_{pol(10)}$[b]	AAA AAA AAA ACA ATG TAC AGT ATT G
Control Apt-$_{lysozyme}$	TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTG ACC AGG A
HP	TCC TGG TC
Template	CAG AAA TCT CAG GGA CTC TAA AGC TCA ACT TGC ATA AAC TTC TGA GGA
Primer	TCC TCA GAA GTT TAT GCA
Complement	TCC TCA GAA GTT TAT GCA AGT TGA GCT TTA GAG TCC CTG AGA TTT CTG
TaqMan probe	FAM-TAG AGT CCC TGA GAT TTC TG-BHQ1

[a] The colors of oligonucleotide sequences correspond to those of the domains depicted in Scheme 1.

[b] The underlined letters in Apt-$_{pol}$ indicate the sequence that is complementary to Apt-$_{lysozyme}$ and the corresponding hybridization length is denoted in the bracket.

Table S2. Comparison of the developed method with other fluorescent methods.

Key materials or methods	Linear range (nM)	LOD (nM)	Reference
Pyrene-modified molecular beacon	-	0.2	[1]
Exo III-assisted amplification	8.74-69.93	5.59	[2]
Assembly of quantum dots (QDs)/G-quadruplex	5.0-500	2.6	[3]
Molecular beacon	0-15	0.2	[4]
Fluorescence resonance energy transfer (FRET)	30-210	2.5	[5]
Target-responsive DNA polymerase activity	0-20	0.8	This work

Fig. S1. Polyacrylamide gel electrophoresis image to confirm the binding of the detection probe to DNA polymerase. (1) Apt-$_{pol}$, (2) Apt-$_{lysozyme}$, (3) Apt-$_{pol}$ + Apt-$_{lysozyme}$, (4) Apt-$_{pol}$ + Apt-$_{lysozyme}$ + lysozyme, (5) Apt-$_{pol}$ + Apt-$_{lysozyme}$ + DNA polymerase, and (6) Apt-$_{pol}$ + Apt-$_{lysozyme}$ + DNA polymerase + lysozyme. The final concentrations of Apt-$_{pol}$, Apt-$_{lysozyme}$, DNA polymerase, and lysozyme are 500 nM, 500 nM, 110 nM, and 1 μM, respectively.

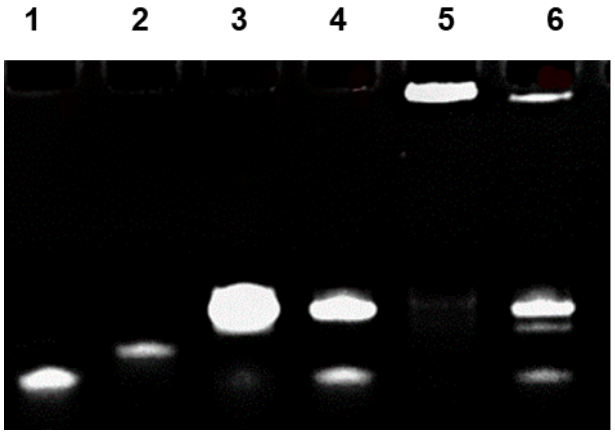


Fig. S2. Fluorescence signal change (F_{90}-F_0) at different concentrations of Apt-$_{lysozyme}$. The concentration of Apt-$_{pol}$, the hybridization length between Apt-$_{pol}$ and Apt-$_{lysozyme}$, and the concentration of HP are 200 nM, 10 mer, and 200 nM, respectively.

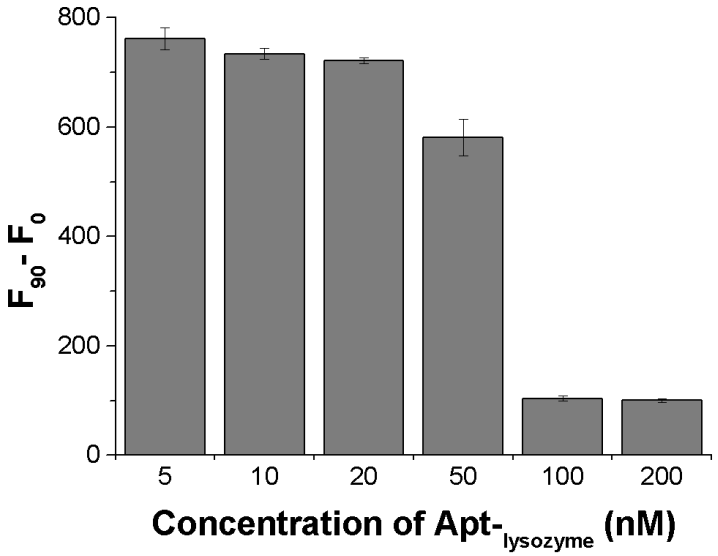


Fig. S3. The linear relationship between fluorescence signal change (F_{90}-F_0) and lysozyme concentration (0-20 nM) spiked in diluted human serum (1%), where F_{90} and F_0 are the fluorescence intensities measured at 90 min and 0 min in the primer extension reaction, respectively. The final concentrations of Apt-$_{pol}$, Apt-$_{lysozyme}$, HP, and DNA polymerase are 200 nM, 100 nM, 200 nM, and 5.5 nM, respectively. Due to the significant interference by serum proteins at higher percentages of serum protein, 1% human serum was employed.

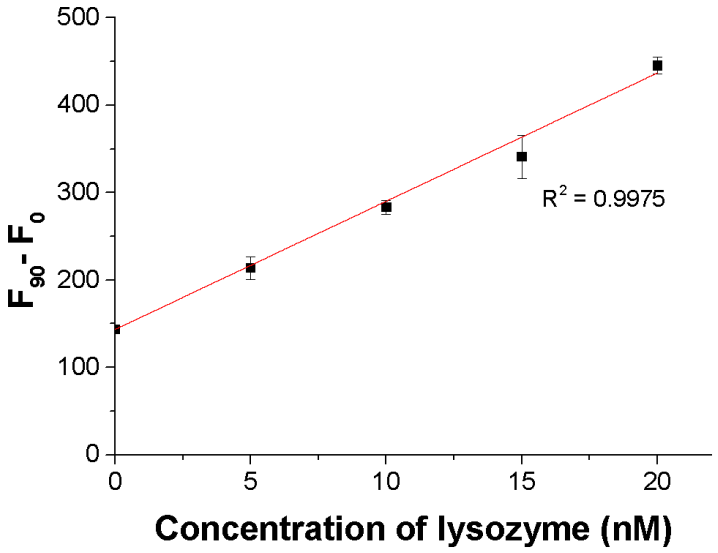
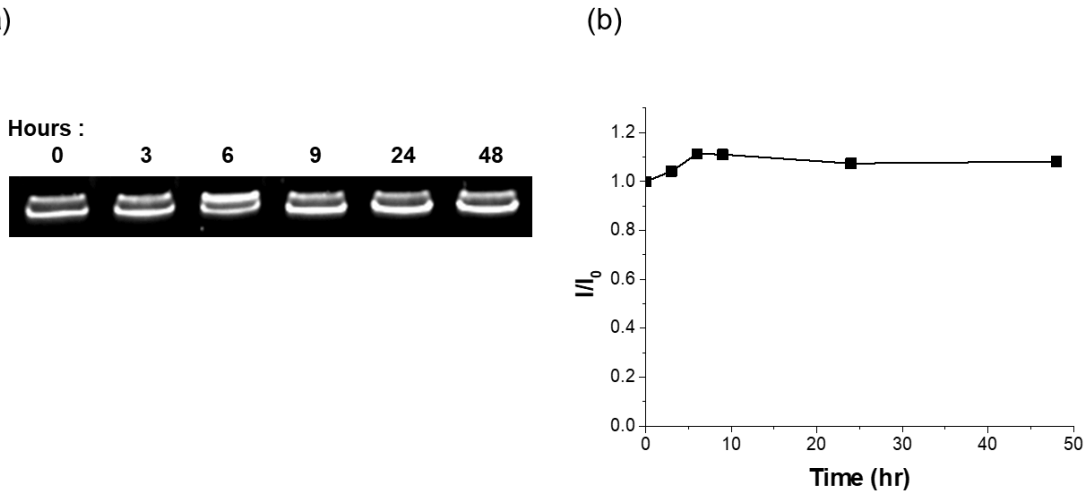


Fig. S4. Stability of the complex of detection probe and DNA polymerase (Apt-$_{pol}$/Apt-$_{lysozyme}$/DNA polymerase) in diluted human serum (1%). (a) Polyacrylamide gel electrophoresis image and (b) band intensities for the complexes (Apt-$_{pol}$/Apt-$_{lysozyme}$/DNA polymerase) incubated for different incubation times (0, 3, 6, 9, 24, and 48 hrs) in diluted human serum, where I and I_0 are the band intensities for the complexes measured at corresponding incubation time and 0 hr, respectively. The final concentrations of Apt-$_{pol}$, Apt-$_{lysozyme}$, and DNA polymerase are 500 nM, 500 nM, and 110 nM, respectively.



The extra bases were introduced at the 3'-end of DNA aptamer, which may cause its binding affinity to be reduced. However, in this study, the extra bases were utilized for the recycling of lysozyme by HP extension reaction. Thus, the deleterious influence of extra bases to the aptamer binding affinity, which may occur, could be compromised by target recycling resulting from HP extension on the aptamer strand. This was evidenced by the sensitive detection of lysozyme with the LOD of 0.80 nM, a value that is comparable or superior to those from previous fluorescent methods.

References

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