## Electronic Supplementary Information

# Fluorescence Turn-On Detection of a Protein through the Displaced Single-Stranded DNA Binding Protein Binding to a Molecular Beacon

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## Experimental section

### Materials

The *E. coli* SSB protein was purchased from Promega Corporation. Lysozyme and hemoglobin were from Worthington Biochemical Corporation. Thrombin was purchased from Beijing Dingguo Biotechnology Co. Ltd. Collagenase was obtained from Invitrogen Corporation. Bovine serum albumin ( BSA ) was purchased from Bio Basic Inc. (BBI). Protein concentration was determined by reading the UV-Vis absorbance at 280 nm. Lysozyme concentration was estimated using an extinction coefficient of 38,940 $M^{-1}cm^{-1}$,[S1] and *E. coli* SSB protein concentration was estimated with an extinction coefficient of 30,250 $M^{-1}cm^{-1}$.[S2]

Two oligonucleotides have been used in our experiments. They are the anti-lysozyme DNA aptamer (5′-ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3′), and the molecular beacon (5′-FAM-CCA TCG CGC TGG TAT CAC TAA ATCT CGA TGG-DABCYL-3′). The fluorophore and the quencher are carboxyfluorescein (FAM) and 4-(4′-dimethylaminophenylazo) benzoic acid (DABCYL), respectively. Both oligonucleotides were synthesized and purified by Shanghai Sangon Biological Engineering Technology & Service Co. Ltd. The aptamer and protein solutions were stored at 4 ºC before use. All other chemicals were of analytical grade. All stock and buffer solutions were prepared using water purified with a Milli-Q A10 filtration system (Millipore, Billerica, MA, USA).

**Measurements**

UV-Vis absorption spectra were obtained using a Cary 50 Bio Spectrophotometer (Varian Inc., CA, USA) equipped with a xenon flash lamp. Emission spectra were recorded using a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., USA) with an excitation wavelength of 490 nm. Excitation and emission slit widths were both of 5 nm. Quartz cuvettes with 10-mm path length were used for UV-vis and emission measurements. All spectra were taken at an ambient temperature of 25 ºC in 10 mM NaCl, 10 mM $MgCl_2$, 20 mM Tris-HCl buffer solution at pH 7.5 unless specified.

**Assay optimization**

We have studied the effects of the solution ionic strength, pH, and temperature. Briefly, increasing solution NaCl concentration resulted significantly decreased SSB protein binding (Fig. S1). And since literature reports show that divalent ions such as $Mg^{2+}$ can increase the stability of MB and reduce its background fluorescence, 10 mM NaCl and 10 mM $MgCl_2$ were used to adjust the assay solution's ionic strength.[S3] MB's background fluorescence was hardly influenced by the solution pH value from 6.8 – 9.0. However, binding of SSB protein was affected by the solution pH, the optimum pH value was determined to be 7.5 (Fig. S2). MB's background fluorescence kept practically unchanged at a temperature range from 10 ºC to 30 ºC, further increase of the solution temperature caused a gradual increase of the background fluorescence. In addition, when the temperature was increased from 10 ºC to 25 ºC,

SSB protein binding to MB was not affected, and the SSB protein showed decreased binding to MB at 30 ºC – 50 ºC (Fig. S3). The optimum solution temperature was therefore set at 25 ºC.

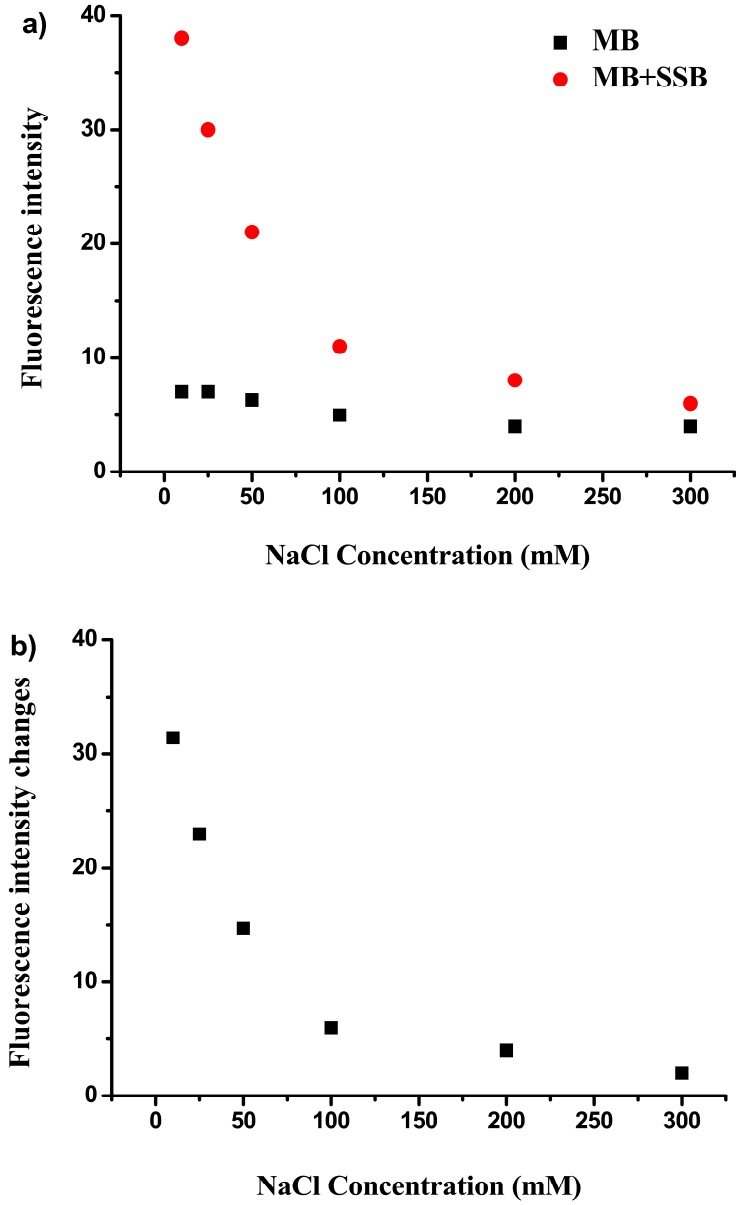


**Figure S1.** a) Fluorescence intensity of molecular beacon (20 nM) at different NaCl concentrations in the presence (•) and absence (▪) of SSB protein (20 nM). b) MB fluorescence intensity changes upon its binding to SSB protein at different NaCl concentrations. Conditions: 20 mM Tris-HCl buffer (pH 7.5), 10 mM $MgCl_2$.

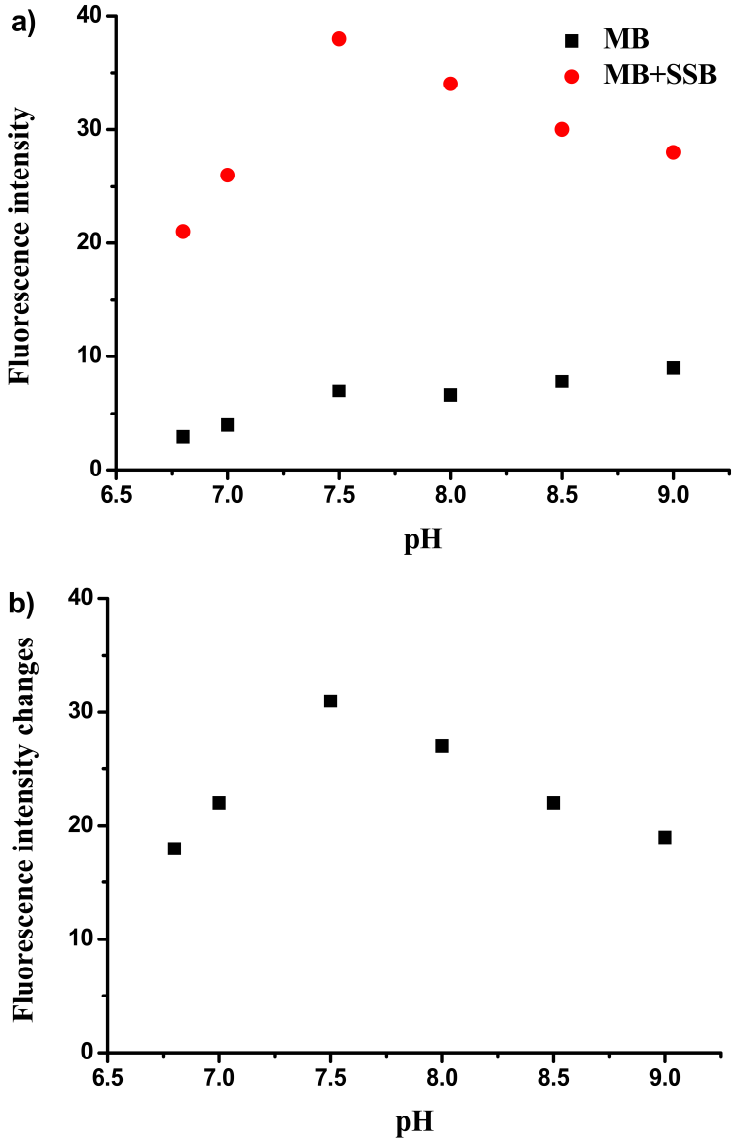


**Figure S2.** a) Fluorescence intensity of molecular beacon (20 nM) at different solution pH values (6.8 – 9.0) in the presence (•) and absence (▪) of SSB protein (20 nM). b) MB fluorescence intensity changes upon its binding to SSB protein at different pH values. Conditions: 20 mM Tris-HCl buffer, 10 mM NaCl and 10 mM $MgCl_2$.

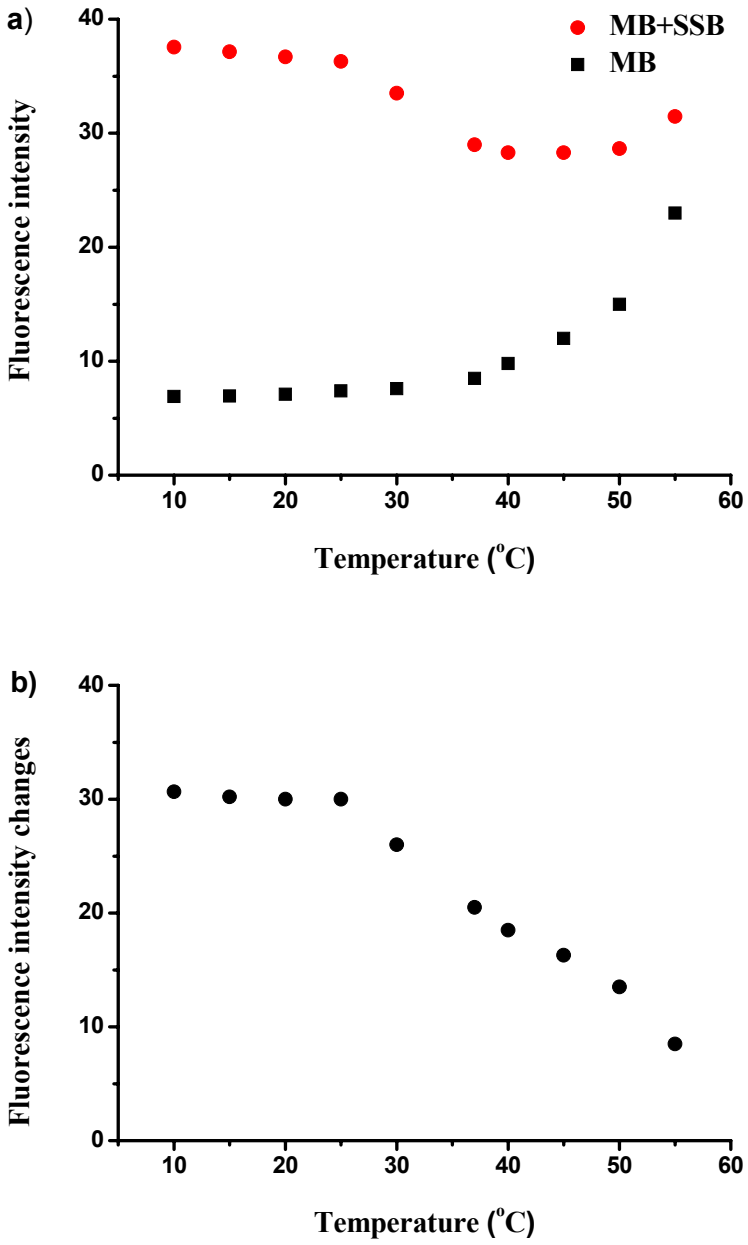


**Figure S3.** a) Fluorescence intensity of molecular beacon (20 nM) at different solution temperatures (10 ºC – 55 ºC) in the presence (•) and absence (▪) of SSB protein (20 nM). b) MB fluorescence intensity changes upon its binding to SSB protein at different temperatures. Conditions: 20 mM Tris-HCl buffer (pH 7.5), 10 mM NaCl and 10 mM $MgCl_2$.

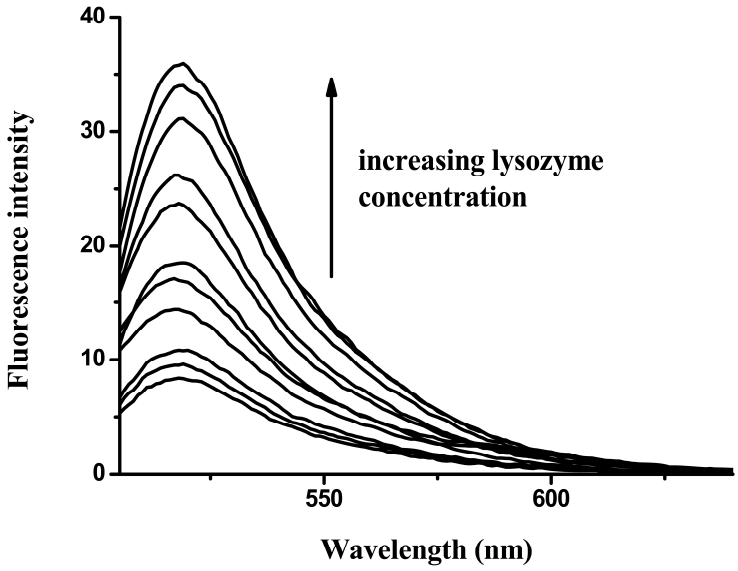


**Figure S4.** Fluorescence intensity changes of MB (20 nM) upon the addition of increasing concentrations of lysozyme. Conditions: 20 nM aptamer, 20 nM SSB protein. Buffer: 20 mM Tris-HCl buffer (pH 7.5), 10 mM NaCl and 10 mM $MgCl_2$.

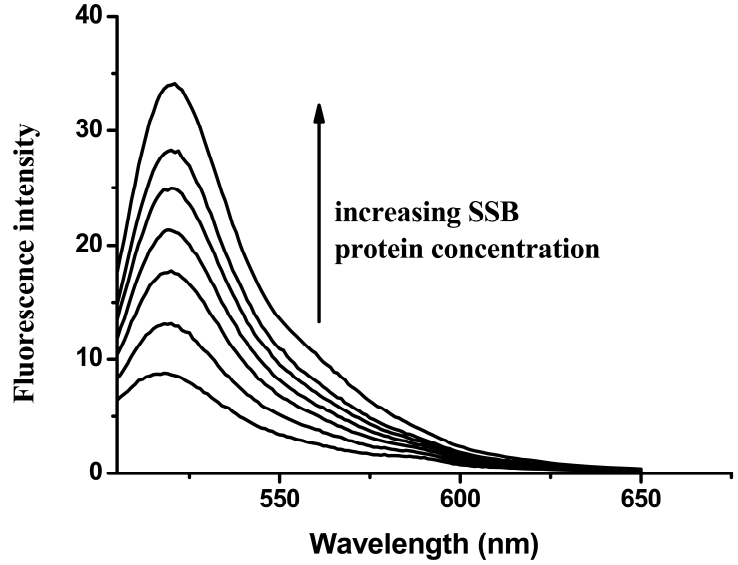


**Figure S5.** Fluorescence intensity changes of MB (20 nM) upon the addition of increasing concentrations of SSB protein. Buffer: 20 mM Tris-HCl buffer (pH 7.5), 10 mM NaCl and 10 mM $MgCl_2$.

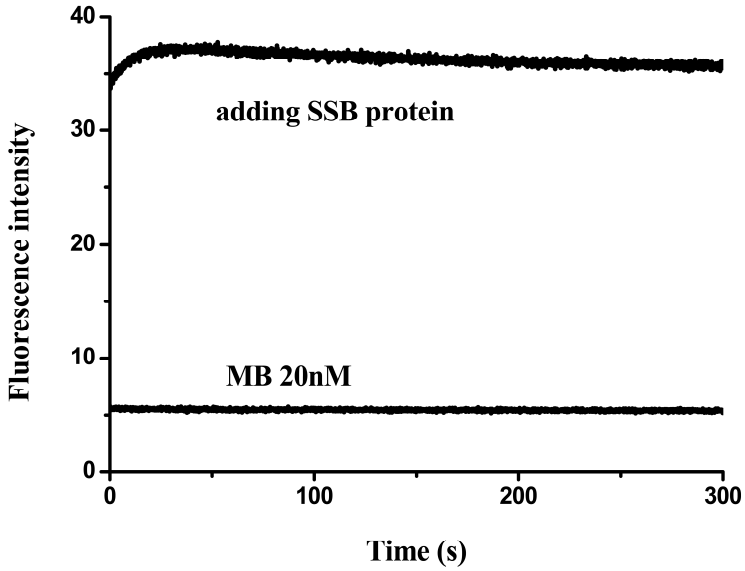


**Figure S6.** Fluorescence intensity changes of 20 nM MB at 518 nm upon the addition of 20 nM SSB protein. Conditions: 20 mM Tris-HCl buffer (pH 7.5), 10 mM NaCl and 10 mM $MgCl_2$.

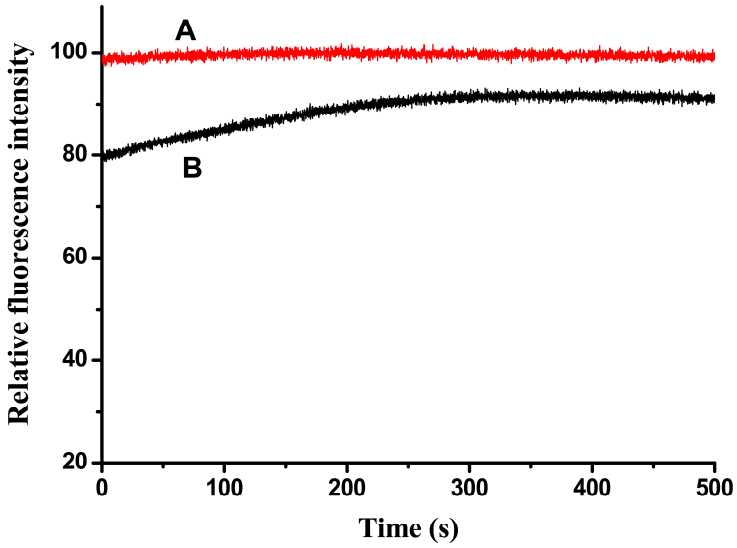


**Figure S7.** Relative fluorescence intensity changes with time. **A**: aptamer was first incubated with lysozyme for 5 min (240 μL buffer solution), then 5 μL of SSB protein was added, the sample was incubated for another 5 min, 5 μL of MB was added and emission intensity (at 518 nm) was immediately measured. The maximum emission intensity value was normalized as 100 for easy comparison. **B**: aptamer was first incubated with SSB protein for 5 min (240 μL), then 5 μL of lysozyme and 5 μL of MB were sequentially added and emission intensity (at 518 nm) was immediately measured. Final solution (250 μL) contained 20 nM aptamer, 20 nM lysozyme, 20 nM SSB protein, 20 nM MB, in 20 mM Tris-HCl buffer (pH 7.5), 10 mM NaCl, 10 mM $MgCl_2$. Experiments were performed at 25 ºC.

**Table S1.** Proteins studied in the current investigation.

| Protein | Molar mass (kDa) | pI |
|---|---|---|
| Lysozyme | 14.3 | 11.0 |
| Thrombin | 37.4 | 6.3-7.6 |
| Hemoglobin | 64.5 | 6.8 |
| Albumin bovine | 69 | 4.7 |
| Collagenase | 68-125 | 5.6 |

**Table S2.** Lysozyme concentration determination in human saliva samples.

| Saliva sample | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Lysozyme concentration (μM) | 1.2 | 2.5 | 1.5 | 1.7 | 1.5 |

**Table S3.** Lysozyme recovery in diluted human saliva samples.

| sample | Addition of standard solution (nM) | Recovery (nM) | Percentage recovery (%) | Relative error (%) | RSD (%) |
|---|---|---|---|---|---|
| 1 | 5.5 | 5.21 | 94.72 | 5.3 | |
| 2 | 5.5 | 5.45 | 99.09 | 0.9 | |
| 3 | 5.5 | 5.37 | 97.63 | 2.4 | 2.73 |
| 4 | 5.5 | 5.59 | 101.63 | -1.6 | |
| 5 | 5.5 | 5.54 | 100.72 | -0.7 | |

Note: Lysozyme samples were diluted, 5.5 nM of standard lysozyme solution was added and lysozyme recovery was determined.

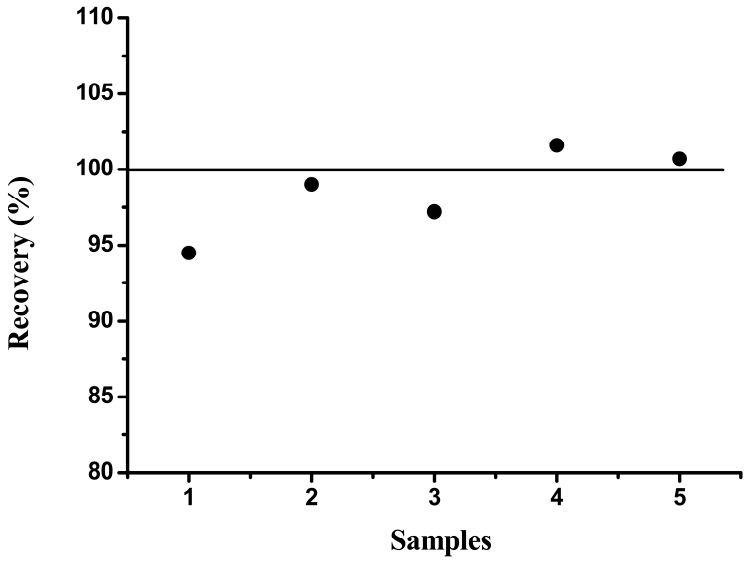


**Figure S8.** Percentage recovery of lysozyme (5.5 nM) from diluted human saliva samples.

## References

(S1) S. C. Gill, P. H. von Hippel, *Anal. Biochem*., 1989, **182**, 319-326.

(S2) K. R. Williams, E. K. Spicer, M. B. LoPresti, R. A. Guggenheimer, J. W. Chase, *J. Biol*. *Chem*., 1983, **258**, 3346-3355.

(S3) X. J. Liu, W. H. Tan, *Anal. Chem.*, 1999, **71**, 5054-5059.