Supporting Information

Development of an in vitro autocatalytic self-replication system for biosensing application

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1. Optimization of reaction time of hOGG1-catalyzed base excision repair.

The hOGG1-catalyzed 8-oxoG excision repair is a crucial step in the whole reaction, and thus the excision reaction time should be optimized. The hOGG1 (0.1 U/μL) was added to 20 μL of excision reaction system (0.5 U/μL APE 1, 500 nM hairpin substrate, 0.2 μL of 10 mg/mL BSA, 2 μL of 10× NEBuffer 2, and 2 μL of 10× NEBuffer 4), and incubated at 37 °C for certain time. As shown in Figure S1, the fluorescence intensity enhances with the excision reaction time from 0 to 40 min and reaches the plateau at 40 min. Therefore, 40 min of excision reaction time is used in the subsequent research.

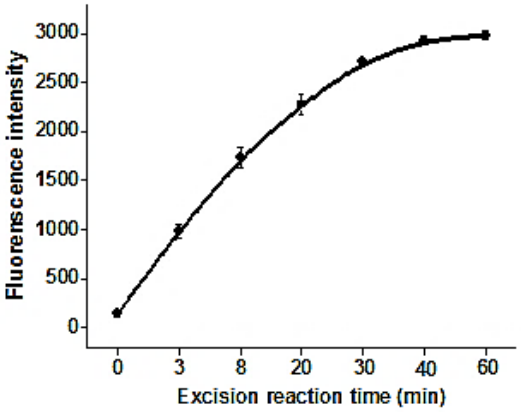

Figure S1. Variance of fluorescence intensity with different excision reaction time. The 0.1 U/μL hOGG1, 0.5 U/μL APE 1, and 500 nM hairpin substrate were used in the experiments. Error bars show the standard deviation of three independent experiments.

2. Optimization of reaction time of autocatalytic self-replicating-directed cascaded recycling amplification.

The reaction time of autocatalytic self-replicating-directed cascaded recycling amplification affects the performance of Fok I-catalyzed recycling cleavage of molecular beacons (MB1 and

MB2), and thus it should be optimized. After the hOGG1-catalyzed 8-oxoG excision, 5 μL of excision products were added to 30 μL of amplification reaction system (9 U of Fok I, 700 nM MB1, 650 nM MB2, and 3 μL of 10× CutSmart Buffer), and incubated at 37 °C in the dark for different time. As shown in Figure S2, the fluorescence intensity increases with the reaction time from 0 to 60 min and reaches the plateau at 60 min. Therefore, the appropriate amplification time is determined to be 60 min.

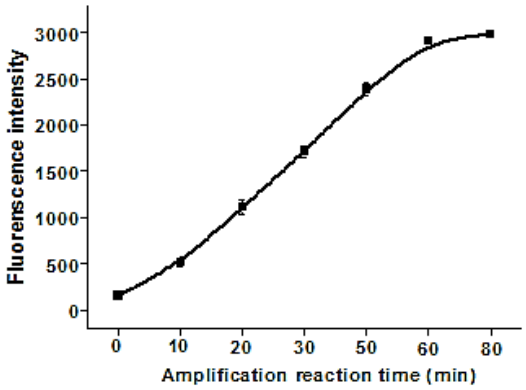

Figure S2. Variance of fluorescence intensity with different amplification reaction time. The 9 U of Fok I, 700 nM MB1, and 650 nM MB2 were used in the experiments. Error bars show the standard deviation of three independent experiments.

3. Optimization of MB1 concentration.

MB1 can adjacently hybridize with the excision product of hOGG1-catalyzed 8-oxoG excision reaction to initiate the autocatalytic self-replicating-directed recycling cleavage of MB1 for the generation of an enhanced fluorescence signal. Thus, the MB1 concentration should be optimized. As shown in Figure S3, the fluorescence intensity improves with the increasing concentration of MB1 from 50 to 700 nM and reaches the plateau at the concentration of 700 nM. Thus, 700 nM is selected as the optimal concentration of MB1.

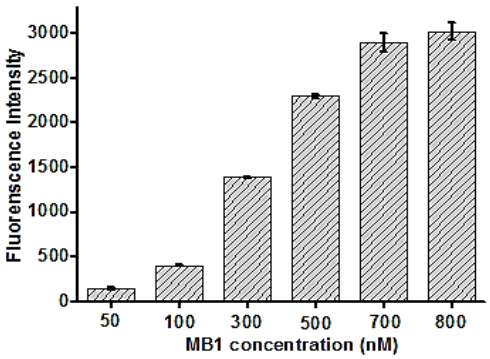

Figure S3. Variance of fluorescence intensity with different-concentration MB1. The 0.1 U/μL hOGG1, 0.5 U/μL APE 1, 500 nM hairpin substrate, 9 U of Fok I, and 650 nM MB2 were used in the experiments. Error bars show the standard deviation of three independent experiments.

4. Optimization of MB2 concentration.

MB2 can partially hybridize with the cleavage product of MB1 to initiate the autocatalytic self-replicating-directed recycling cleavage of MB2, and thus the MB2 concentration should be optimized. As shown in Figure S4, the fluorescence intensity enhances with the increasing concentration of MB2 from 50 to 650 nM and reaches the plateau at the concentration of 650 nM. Therefore, the optimal concentration of MB2 is determined to be 650 nM.

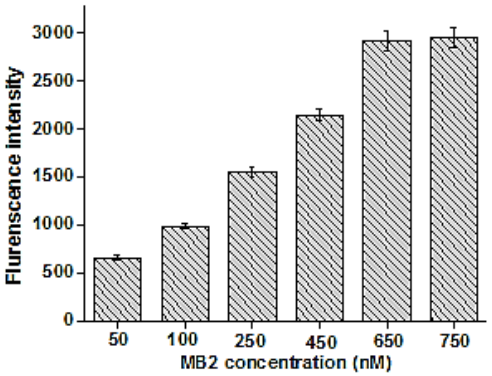

Figure S4. Variance of fluorescence intensity with different-concentration MB2. The 0.1 U/μL hOGG1, 0.5 U/μL APE 1, 500 nM hairpin substrate, 9 U of Fok I, and 700 nM MB1 were used in

the experiments. Error bars show the standard deviation of three independent experiments.

5. Optimization of the amount of Fok I.

Fok I is the key of the whole reaction and thus its amount should be optimized. As shown in Figure S5, the fluorescence intensity improves with the increasing amount of Fok I from 3 to 9 U, followed by the decrease beyond the amount of 9 U. Therefore, 9 U of Fok I is used in the subsequent researches.

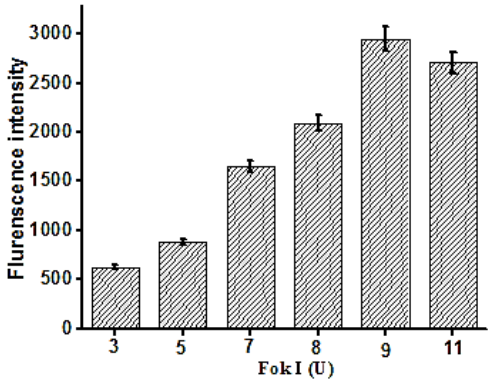

Figure S5. Variance of fluorescence intensity with different-amount Fok I. The 0.1 U/μL hOGG1, 0.5 U/μL APE 1, 500 nM hairpin substrate, 700 nM MB1 and 650 nM MB2 were used in the experiments. Error bars show the standard deviation of three independent experiments.

6. Detection Specificity.

DNA glycosylases are a superfamily of enzymes involved in BER pathway, and it is great challenging to specifically discriminate hOGG1 from other DNA glycosylase members.[1] To investigate the specificity of the proposed method, we used the irrelevant proteins (i.e, immunoglobulin G (IgG) and bovine serum albumin (BSA)) and the nonspecific DNA glycosylase (i.e. uracil-DNA glycosylase (UDG)) as the negative controls. The IgG and BSA do not belong to

DNA glycosylase enzymes, and they cannot excise the damaged 8-oxoG. The UDG is a kind of DNA glycosylase, but it can only specifically recognize and excise the uracil from the U/A mismatch.[2] As shown in Figure S6, under the identical conditions, no significant fluorescence signals are detected in response to the control with only reaction buffer (Figure S6, black column), IgG (Figure S6, blue column), BSA (Figure S6, pink column), and UDG (Figure S6, green column), respectively. In contrast, an extremely high fluorescence signal is observed in response to hOGG1 (Figure S6, red column), which is 12.0-fold higher than that in response to IgG, 10.4-fold higher than that in response to BSA, and 11.4-fold higher than that in response to UDG, suggesting that only hOGG1 can excise the 8-oxoG from 8-oxoG:C base pairs for activating the autocatalytic self-replicating-directed cascaded recycling cleavage of molecular beacons to generate an enhanced fluorescence signal. These results demonstrate that the proposed strategy can discriminate hOGG1 from the irrelevant proteins and other DNA glycosylase members with high specificity.

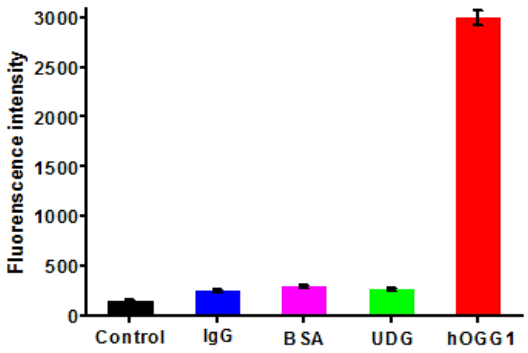

Figure S6. Variance of fluorescence intensity in response to the control with only reaction buffer (black column), 0.1 g/L IgG (blue column), 0.1 g/L BSA (pink column), 0.1 U/μL UDG (green column), and 0.1 U/μL hOGG1 (red column), respectively. Error bars show the standard deviation of three independent experiments.

Table S1. Sequences of the Oligonucleotides [α]

note	sequences (5'-3')
hairpin substrate	CTA **GGA TG**C OGT ATT TAC CG**C ATC C**TA G
molecular beacon 1	GGT AAA T(BHQ1)AC GGG ACT TTG TG**C ATC C**CA CAA AGT CCC(FAM)
molecular beacon 2	G**GG ATG** CAC AAA GT(BHQ1)C CCG ATT GTT CGA TCT CTC GAA CA A T(FAM)

[α]In hairpin substrate, the boldface regions indicate the recognition sites of Fok I and the underlined base "O" indicates the damaged guanine (8-oxoG). In molecular beacon 1 (MB1), the boldface region indicates the recognition site of Fok I, and the underlined bases "T" and "C" indicate the modifications of a black hole quencher 1 (BHQ1) and a fluorescein (FAM), respectively. In molecular beacon 2 (MB2), the boldface region indicates the recognition site of Fok I, and the underlined bases "T" indicate the modifications of a BHQ1 and a FAM, respectively.

References

(1) David, S. S.; O'Shea, V. L.; Kundu, S. Base-Excision Repair of Oxidative DNA Damage. *Nature* **2007**, *447*, 941-950.

(2) Wang, L.-j.; Ren, M.; Zhang, Q.; Tang, B.; Zhang, C.-y. Excision Repair-Initiated Enzyme-Assisted Bicyclic Cascade Signal Amplification for Ultrasensitive Detection of Uracil-DNA Glycosylase. *Anal. Chem.* **2017**, *89*, 4488-4494.