Electronic Supplementary Information

Construction of a gold nanoparticle-based single-molecule biosensor for simple and sensitive detection of Argonaute 2 activity

Su Jiang,[‡a] Xinyi Zhang,[‡b] Dong-ling Li,[a] Ting-ting Wang,[a] Fei Ma,[*c] and Chun-yang Zhang[*a]

[a] College of Chemistry, Chemical Engineering and Materials Science, Shandong Normal University, Jinan 250014, China.

[b] School of Chemistry and Chemical Engineering, Guangdong Pharmaceutical University, Zhongshan 528458, China.

[c] School of Chemistry and Chemical Engineering, Southeast University, Nanjing, 211189, China.

[†]These authors contributed equally.

* Correspondence authors. E-mail: cyzhang@sdnu.edu.cn, fei@seu.edu.cn.

SUPPLEMENTARY RESULTS

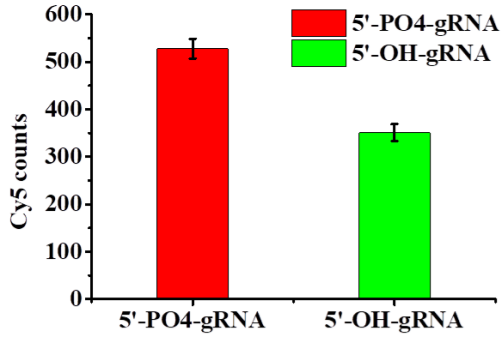

Fig. S1 Measurement of Cy5 signal in response to 5'-PO_4-gRNA (red column) and 5'-OH-gRNA (green column). Error bars represent standard deviations of three experiments.

We measured the Cy5 signal generated by Ago2 using gRNA with 5' hydroxyl end (5'-OH-gRNA) and 5' phosphate end (5'-OH-gRNA) to investigate the influence of 5′ phosphate of gRNA upon the Ago2 cleavage activity. As shown in Fig. S1, both 5'-PO_4-gRNA and 5'-OH-gRNA generate distinct Cy5 signal, and the signal generated by 5'-PO_4-gRNA is 1.5-fold higher than that generated by 5'-OH-gRNA. This can be explained by the factor that the 5′ phosphate of gRNA can better stabilize the RISC structure to increase Ago2 activity.[1] Thus, the 5'-PO_4-gRNA instead of 5'-OH-gRNA is used in this biosensor.

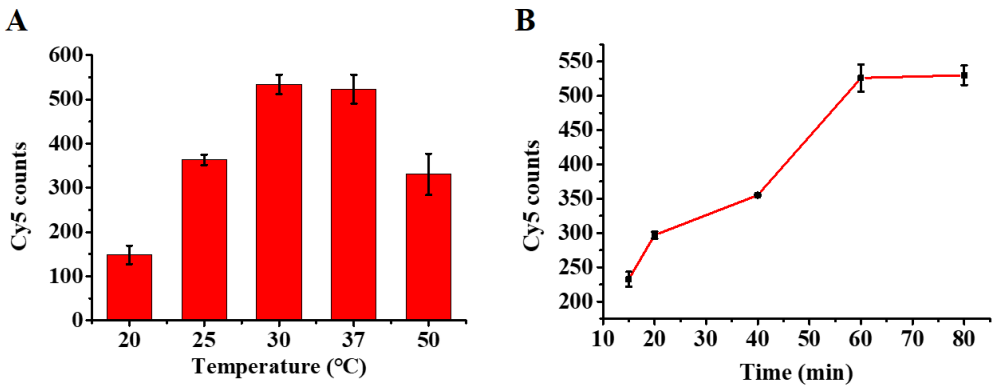

Fig. S2 Variance of Cy5 counts with different reaction temperatures (A) and cleavage reaction time (B), respectively. Error bars represent standard deviations of three experiments.

We investigated the influence of reaction temperature upon the assay performance. As shown in Fig. S2A, a distinct Cy5 signal is observed in the tested reaction temperature from 20 to 30 °C, indicating that the proposed biosensor can work under different temperatures. However, the Cy5 signal decreases beyond the temperature of 30 °C due to either the inhibition of enzyme activity or the dissociation of gRNA/signal probe duplex. To obtain the best assay performance, 30 °C is set as the optimal reaction temperature. We further optimized Ago2 reaction time. As shown in Fig. S2B, the Cy5 counts enhance with the reaction time from 15 to 60 min, and reach a plateau within 60 min due to either the complete consumption of signal probes or the loss of Ago2 activity. Thus, 60 min is set as the optimal Ago2 reaction time.

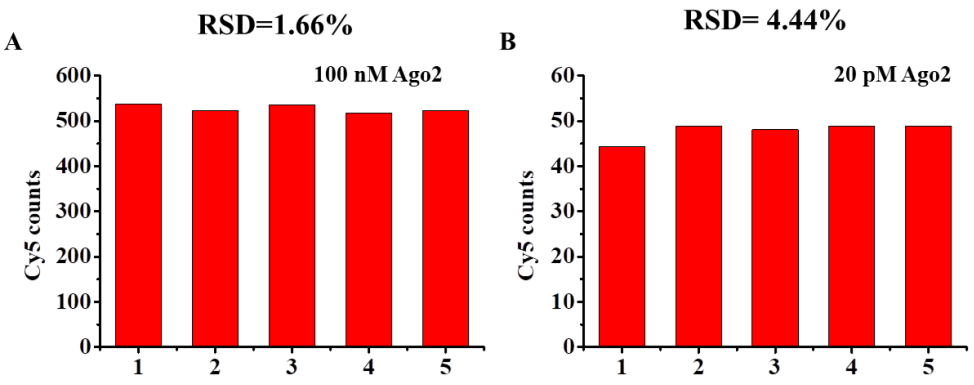

Fig. S3 Reproducibility assessment of the proposed biosensor by measuring 100 nM (A) and 20 pM (B) Ago2, respectively.

We further evaluated the reproducibility of the proposed biosensor. The relative standard deviation (RSD) of the proposed biosensor in response to 100 nM Ago2 (Fig. S3A) and 20 pM Ago2 (Fig. S3B) is measured to be 1.66% and 4.44%, respectively, suggesting the good reproducibility of the proposed biosens

REFERENCES

1. F. V. Rivas, N. H. Tolia, J.-J. Song, J. P. Aragon, J. Liu, G. J. Hannon and L. Joshua-Tor, Nat. Struct. Mol. Biol., 2005, **12**, 340-349.