

Electronic Supplementary Information

**Development of a phos-tag-based fluorescent biosensor for sensitive detection of protein kinase in cancer cells**

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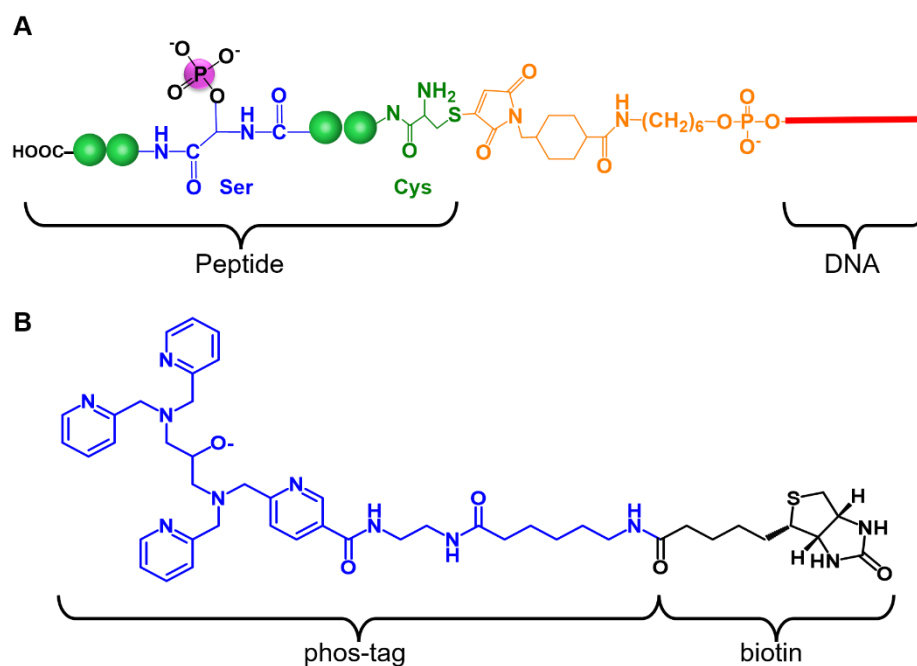
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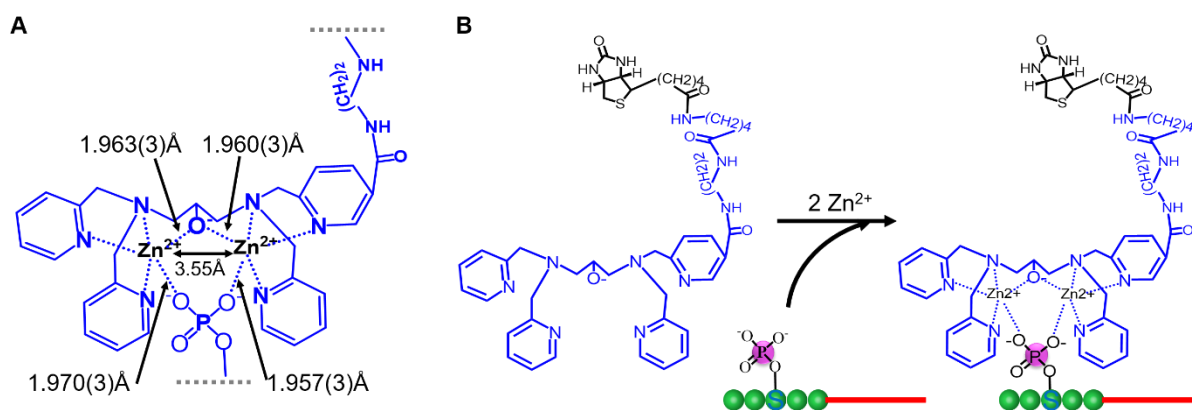
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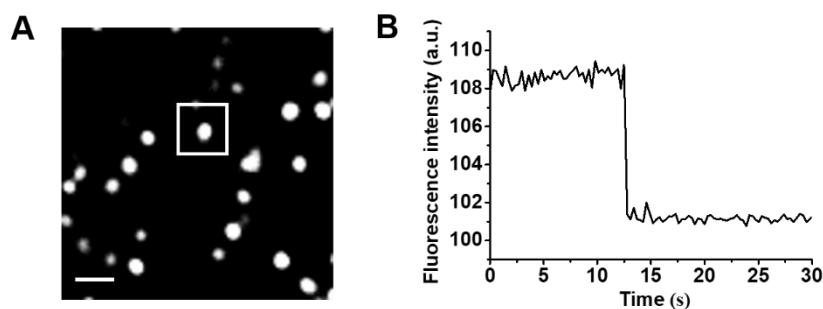
## SUPPLEMENTARY RESULTS



**Fig. S1** (A) Chemical structure of the phosphorylated peptide-DNA conjugates. (B) Structure of the biotinylated phos-tag.



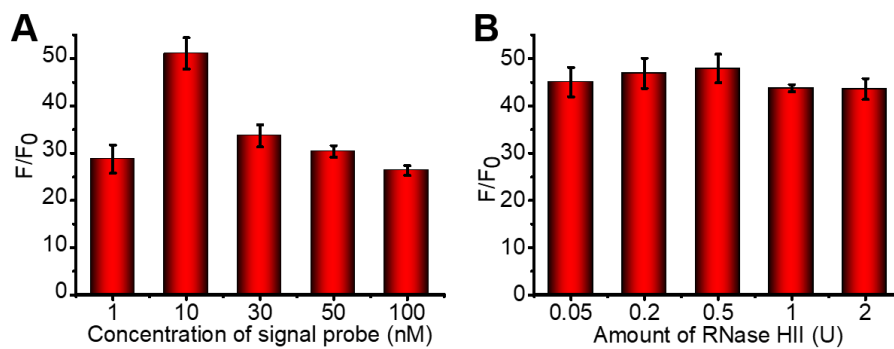
**Fig. S2** (A) Bond distances of phos-tag zinc (II) complex.<sup>1</sup> Red dotted line, coordination bond. (B) Detailed structure of phosphate skeleton of DNA in peptide-DNA.<sup>2</sup>



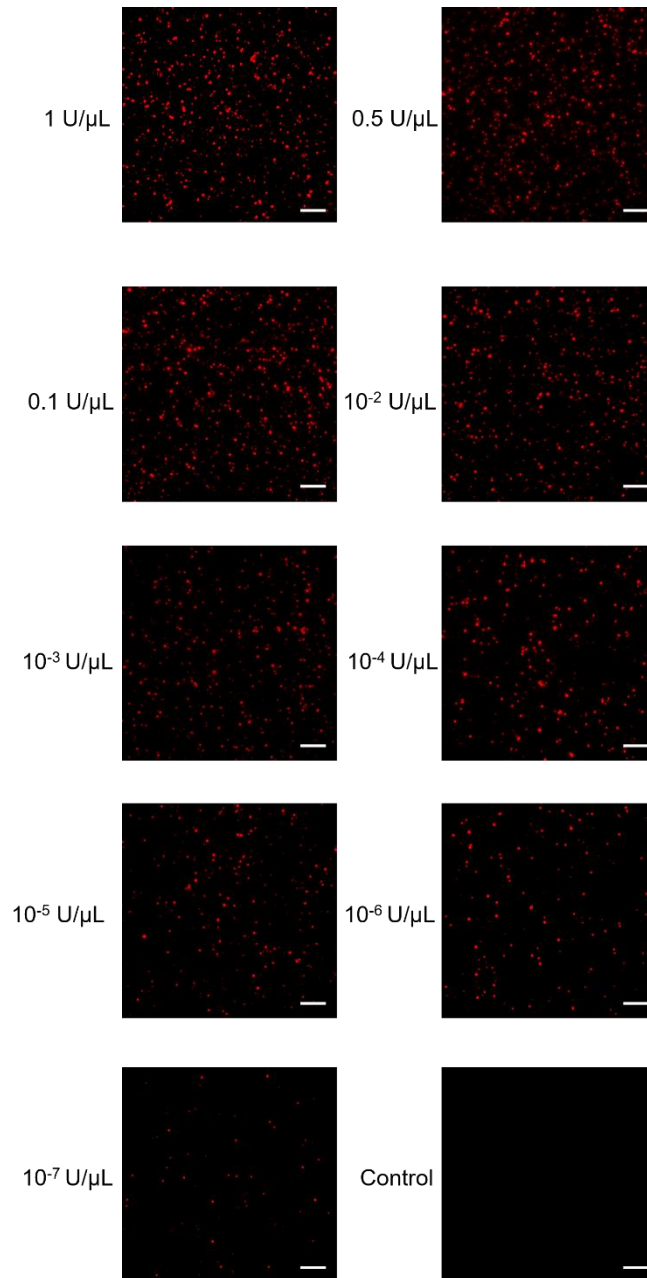
**Fig. S3** (A) Cy5 fluorescence images prior to photobleaching step. Scale bar is 1  $\mu\text{m}$ . (B) Intensity traces of single Cy5 fluorescence spots over time showing one photobleaching process.

### Optimization of experimental conditions

To improve the detection sensitivity, we optimized the experimental conditions of RNase HIII-actuated single-ribonucleotide repairing-mediated cycling signal amplification, including the concentration of signal probes and the amount of RNase HIII. The value of  $F/F_0$  was used to evaluate the experiments, where  $F$  and  $F_0$  are the fluorescence intensity in the presence and absence of peptide-DNA conjugates, respectively. As shown in Fig. S4A, the  $F/F_0$  value enhances with the increasing concentration of signal probe from 1 to 10 nM, and reaches the maximum at 10 nM. Thus, 10 nM signal probe is used in subsequent experiments. We further optimized the amount of RNase HIII (Fig. S4B). The  $F/F_0$  value improves with the increasing amount of RNase HIII from 0.05 to 0.5 U, followed by decrease beyond the amount of 0.5 U. Thus, 0.5 U of RNase HIII is used in subsequent experiments.

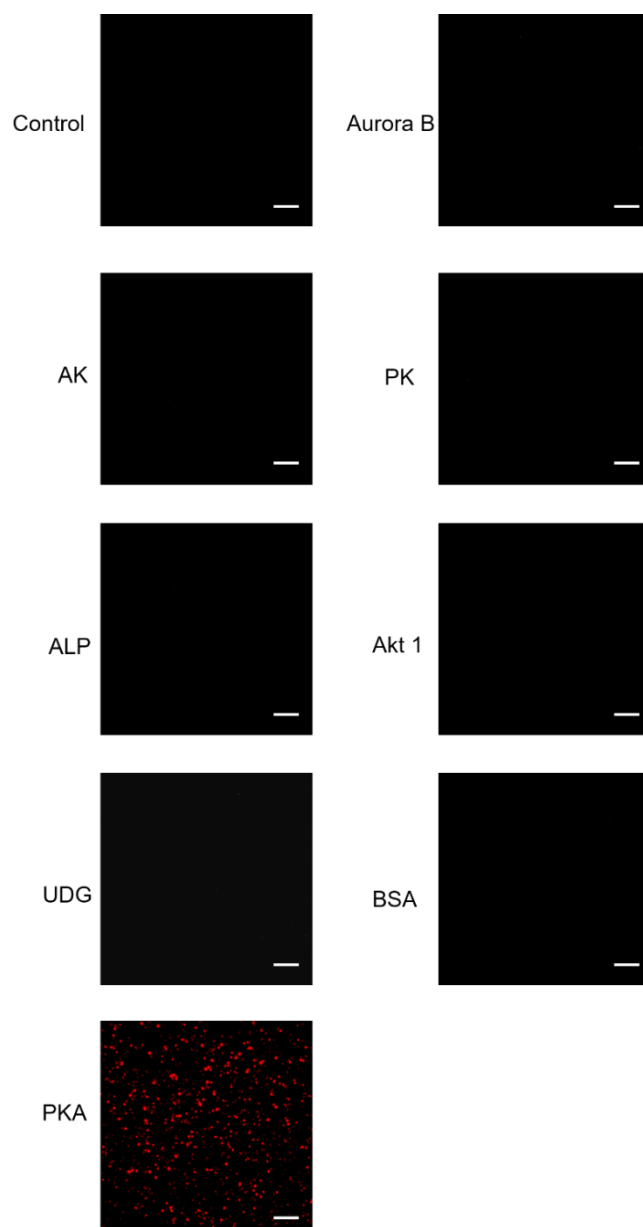


**Fig. S4** (A) Variance of the  $F/F_0$  value with different concentration of signal probes. (B) Variance of the  $F/F_0$  value with different amounts of RNase HII. Error bars show the standard deviation of three experiments.



**Fig. S5** Fluorescence images of phos-tag-based fluorescent biosensor as a function of PKA concentration.

The red color represents the signal of Cy5. The scale bar is 5 μm.



**Fig. S6** Fluorescence images of phos-tag-based fluorescent biosensor in response to 100 nM Aurora B, 0.5 U/ $\mu$ L AK, 0.5 U/ $\mu$ L PK, 0.5 U/ $\mu$ L ALP, 100 nM Akt 1, 0.5 U/ $\mu$ L UDG, 10  $\mu$ g/mL BSA, and 1 U/ $\mu$ L PKA, respectively. The red color represents the signal of Cy5. The scale bar is 5  $\mu$ m.

## Reference

- 1 E. Kinoshita, M. Takahashi, H. Takeda, M. Shiro and T. Koike, *Dalton Trans*, 2004, DOI: 10.1039/b400269e, 1189-1193.
- 2 M. Liu, D. Zhang, X. C. Zhang, Q. F. Xu, F. Ma and C. Y. Zhang, *Chem. Commun.*, 2020, **56**, 5243-5246.