# Electronic Supplementary Information

**A General and Versatile Fluorescence Turn-On Assay for Detecting the Activity of Protein Tyrosine Kinases Based on Phosphorylation-Inhibited Tyrosyl Oxidation**

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**1. Materials and reagents**

Tyrosinase, hexokinase (HK, from Saccharomyces cerevisiae), 4-amino-5-(4-chlorophenyl)-7-(t-butyl) pyrazolo [3,4-d] pyrimidine (PP2) and 7-dimethoxy-N-(4-phenoxyphenyl)-4-quinazolinamine (Src inhibitor-1) were purchased from Sigma-Aldrich. c-AMP dependent protein kinase A (PKA) and T4 polynucleotide kinase (T4 PNK) were obtained from New England Biolabs (NEB). Glucose oxidase (GOx) and adenosine triphosphate (ATP) were obtained from Sangon Biotech (Shanghai, China). Protein tyrosine kinase Src (active, 1020 U/mg) was supplied by Millipore. All of the peptides including TAMRA-IYGEFKK/TAMRA-I(pY)GEFKK, TAMRA-RRRRIYGEFKK, TAMRA-DDDDIYGEFKK, TAMRA-KKKAEEEEY and TAMRA-RRRRAEEEEYKKK were custom synthesized by GL Biochem Ltd. (Shanghai, China). All other reagents were of analytical grade and used as received without further purification.

**2. Standard experimental procedures for Src and inhibition assay**

Typically, in a totally 20 μL of Src reaction buffer (25 mM HEPES, 10 mM $MgCl_2$, pH 7.5), the Src-specific peptide TAMRA-RRRRIYGEFKK (2.5 μM) was incubated respectively with series dilutions of Src at 37°C for 1 hour in the presence of 150 μM of ATP. Afterward, the 20 μL Src reaction solution was directly mixed with 180 μL Tris-HCl buffer (50 mM, pH 6.6) containing 30 U/mL of tyrosinase. After incubation at 37°C for 30 min, the fluorescence signal of the final mixture was recorded on an FLS-980 fluorescence spectrometer (Edinburgh Instruments) under the excitation of 554 nm.

For Src inhibition assay, the experiments were carried out with the same procedure as those for Src assay stated above, except for the pre-incubation of a fixed Src concentration of $7\times10^{-4}$ μg/μL with varying concentrations of PP2 (0~5 μM) or Src inhibitor-1 (0~10 μM) before the reaction of Src and the substrate peptide.

**3. Optimization of tyrosinase concentration and the reaction time of tyrosinase**

Efficient fluorescence quenching of the TAMRA-peptide by tyrosinase-mediated Try oxidation is important in this proposed assay, so the effect of tyrosinase concentration on the quenching efficiency of TAMRA-IYGEFKK is firstly investigated. It can be seen from Fig. S1a that the fluorescence of the phosphopeptide (TAMRA-I(p)YGEFKK) will not be obviously affected by tyrosinase, while the fluorescence signal of TAMRA-IYGEFKK decreases gradually with the increase of tyrosinase. However, when the concentration of tyrosinase is higher than 20 U/mL, the fluorescence intensity will be almost leveled off. Therefore, to ensure high oxidation efficiency, 30 U/mL tyrosinase is used in the following work.

Furthermore, the effect of reaction time between TAMRA-IYGEFKK and tyrosinase on the fluorescence quenching efficiency is also investigated. As shown in Fig. S1b, upon incubation with tyrosinase, the fluorescence signal of TAMRA-IYGEFKK decreases sharply at the beginning. But when the reaction time is longer than 20 min, the fluorescence signal would not be further decreased, indicating the peptide may be completely oxidized. Therefore, 30 min of reaction time are chosen for the subsequent Src analysis in this work.

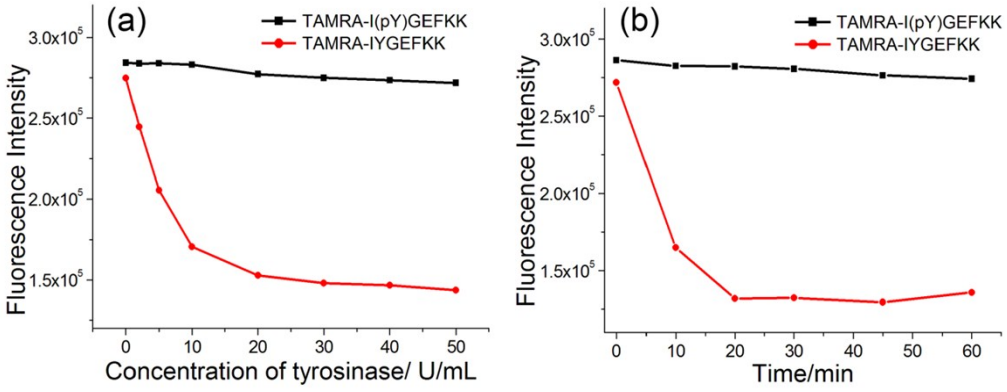


**Fig. S1** (a) Effect of tyrosinase concentration on the fluorescence signal of nonphosphorylated peptide (TAMRA-IYGEFKK, red line) and phosphopeptide (TAMRA-I(p)YGEFKK, black line); (b) Time-dependent fluorescence quenching of the nonphosphorylated peptide (red line) and phosphopeptide (black line) in the presence of 30 U/mL tyrosinase. Other conditions: peptide, 500 nM; excitation, 554 nm; slit widths for fluorescence measurements, 1.5 nm for the excitation while 0.8 nm for the emission. The fluorescence intensities are recorded at 583 nm.

## 4. The influence of peptide sequence on the tyrosinase-mediated fluorescence quenching

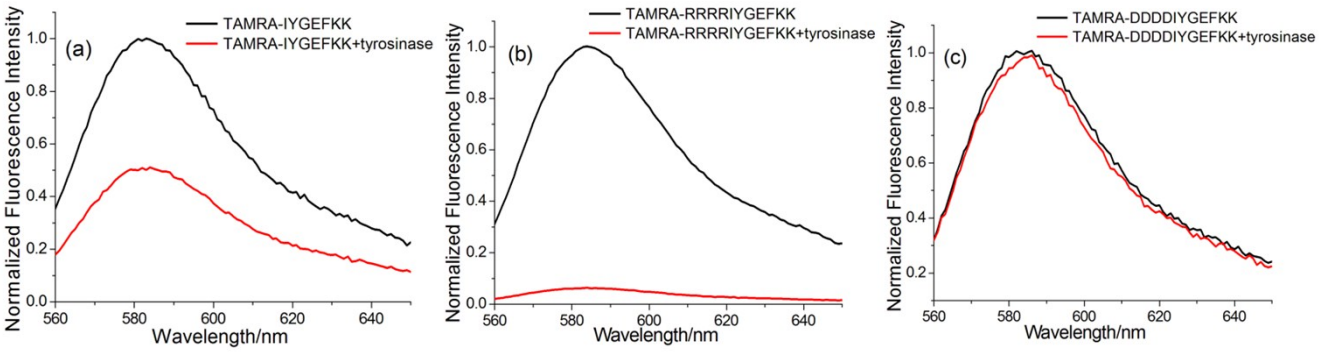


**Fig. S2** Normalized fluorescence spectra of different peptide sequences in the absence or presence of tyrosinase. The sequences of peptides are (a) TAMRA-IYGEFKK, (b) TAMRA-RRRRIYGEFKK and (c) TAMRA-DDDDIYGEFKK, respectively. Experimental conditions: peptide, 250 nM; tyrosinase, 30 U/mL.

We find that the peptide sequence can remarkably affect the tyrosinase-mediated fluorescence quenching efficiency.

As shown in Fig. S2, the tyrosinase-mediated oxidation can quench ~50% of the fluorescence of TAMRA-IYGEFKK (0 net charge in the used buffer) with an S/B ratio of 2, where S/B ratio represents the ratio of fluorescence intensity of the peptide untreated with tyrosinase to that of peptide after incubation with tyrosinase. Meanwhile, the TAMRA-RRRRIYGEFKK (+4 net charges) with four additional positive Arg (R) residues can achieve more than 93% fluorescence quenching with a significantly enhanced S/B ratio of 15.7. In contrast, if several negatively-charged Asp (D) residues are added on the peptide motif (TAMRA-DDDDIYGEFKK, -4 net charges), negligible fluorescence quenching is observed. So it can be concluded that positively-charged peptide sequences are favorable for the tyrosinase-mediated fluorescence quenching in the proposed Src assay, although the mechanism is still unclear to us and needs further investigation. Therefore, the positively-charged TAMRA-RRRRIYGEFKK is chosen as the ideal substrate in subsequent experiment for the detection of Src activity.

Furthermore, the effect of peptide net charges on the tyrosinase-induced fluorescence quenching efficiency is further evaluated by using two randomly synthesized EGFR-specific substrate peptides without detailed sequence optimization. It can be seen from Fig. S3, after incubation with tyrosinase, the fluorescence of TAMRA-KKKAEEEEY (-2 net charges) can be quenched by ~40%, while ~75% fluorescence of the positively-charged TAMRA-RRRRAEEEEYKKK (+2 net charges) will be quenched, which is in accordance with the conclusion stated above. Therefore, it is believed that by rationally design a positively-charged and kinase-specific peptide, the proposed tyrosinase-assisted fluorescent assay can be easily extended to the detection of different PTKs.

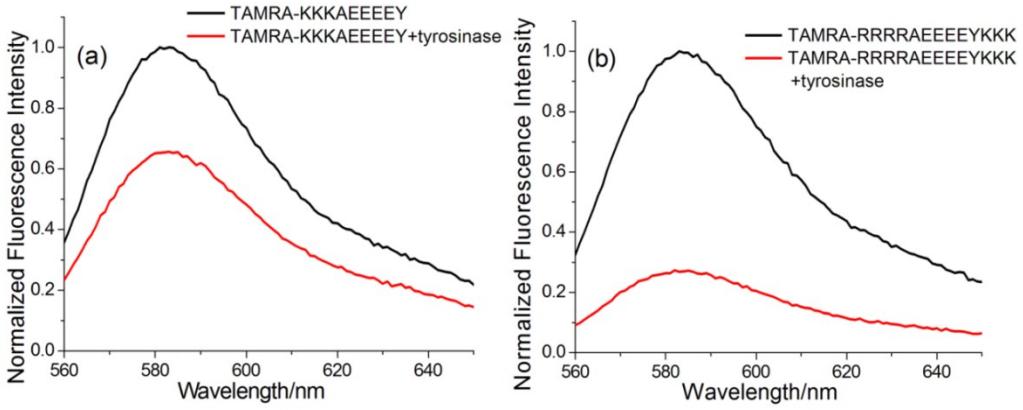


**Fig. S3** Normalized fluorescence spectra of EGFR-specific peptides with different net charges in the absence or presence of tyrosinase. The peptides are (a) TAMRA-KKKAEEEEY and (b) TAMRA-RRRRAEEEEYKKK, respectively. Experimental conditions: peptide, 250 nM; tyrosinase, 30 U/mL.

**5. Optimization of ATP concentration for the detection of Src activity**

In this work, Src-catalyzed phosphorylation reaction is based on the transfer of phosphate group from ATP to Tyr residue in the peptide. So the optimization of ATP concentration is also investigated. Varied concentrations of ATP (from 0 to 250 μM) was respectively incubated with TAMRA-RRRRIYGEFKK (2.5 μM) and $5\times10^{-4}$ μg/μL Src in a 20 μL HEPES buffer at 37°C for 1 hour to perform the phosphorylation reaction. Then the tyrosinase-based oxidation reaction and fluorescence measurement were conducted according to the standard assay procedure stated above. The blank control was performed with the same procedure but without addition of Src. As shown in Fig. S4, the fluorescence intensities of blank control almost keep stable at a low level and will not be influenced by the ATP dosage. But the Src-produced fluorescence intensity increases gradually with the increase of ATP. When ATP concentration is higher than 100 μM, the fluorescence intensity will be no longer obviously changed. Therefore, 150 μM of ATP is chosen as the optimal concentration for further work.

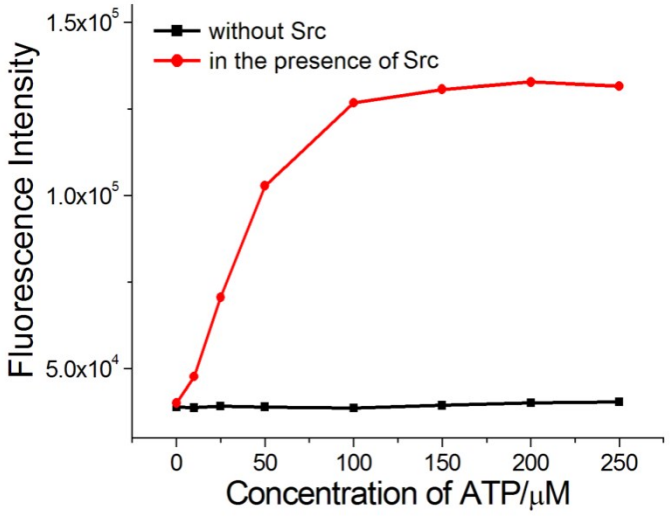


**Fig. S4** Effect of the ATP concentration on the detection of Src. Other conditions: peptide, 250 nM; tyrosinase, 30 U/mL; Src, $5\times10^{-4}$ μg/μL.

**6. Specificity evaluation of the proposed assay for Src**

To estimate the specificity of this proposed strategy for Src, several other enzymes such as GOx, PKA, HK and T4 PNK are detected respectively under the same condition as in the detection of Src. It can be seen from Fig. S5 that only Src can arouse remarkable fluorescence signal while the responses of GOx, PKA, HK and T4 PNK are all negligible, indicating that the proposed method exhibits high specificity for the detection of Src.

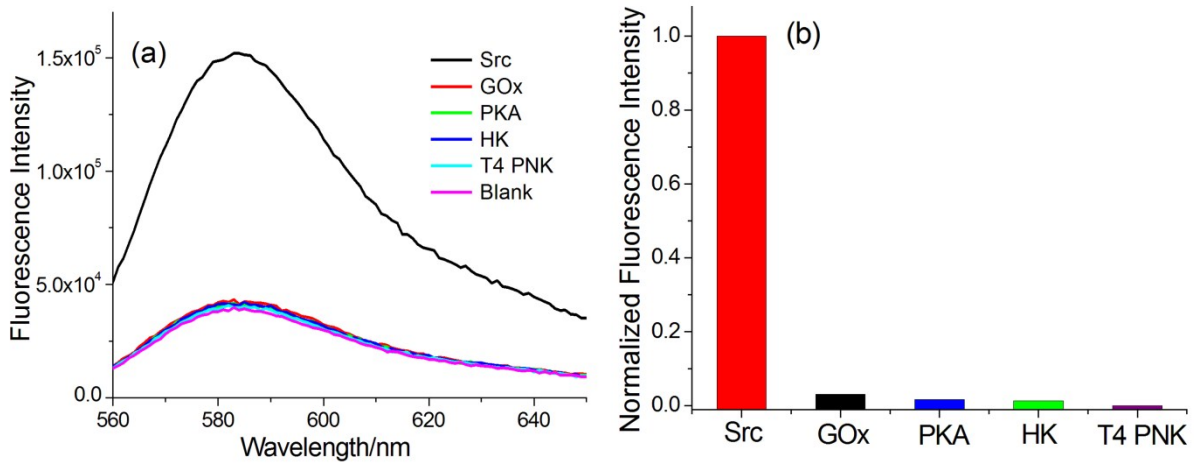


**Fig. S5** Specificity evaluation of the proposed Src assay. (a) Fluorescence spectra produced by Src, GOx, PKA, HK and T4 PNK, respectively. All of these targets are controlled at the same concentration of 0.5 U/mL in a 20 μL reaction solution. It should be noted that 0.5 U/mL Src is equal to $5\times10^{-4}$ μg/μL. Slit widths for fluorescence measurements: 2.0 nm for the excitation while 1.0 nm for the emission. (b) The relative responses of different targets. The fluorescence response of Src (by subtraction of the blank signal) is normalized to be 1.

## 7. Inhibition assay by using Src inhibitor-1

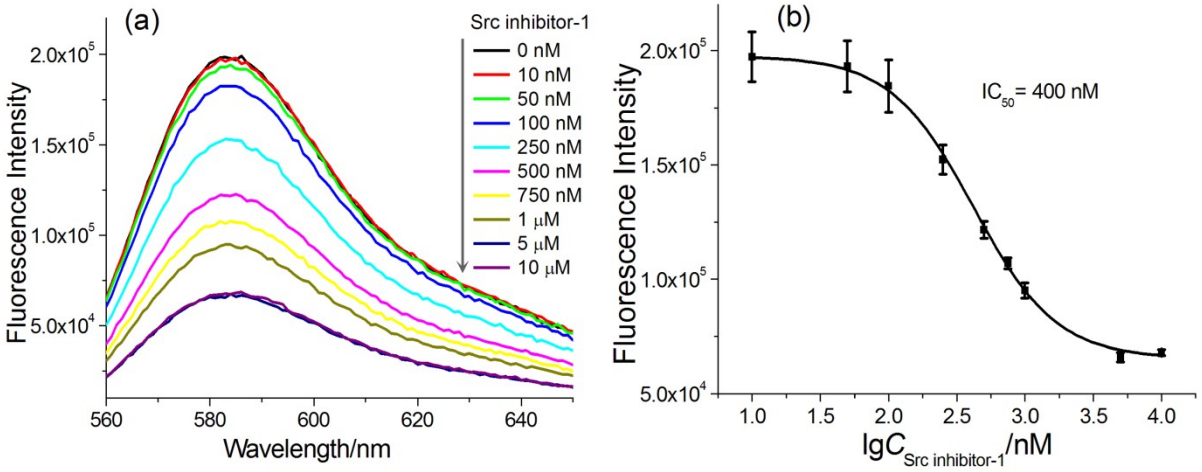


**Fig. S6** (a) Fluorescence spectra of Src assay system in the presence of different concentrations of Src inhibitor-1 by fixing Src concentration at $7\times10^{-4}$ μg/μL; (b) The relationship between fluorescence intensities at 583 nm and Src inhibitor-1 concentrations on the logarithm scale. Slit widths for fluorescence measurements: 2.0 nm for the excitation while 1.0 nm for the emission. Error bars represent standard deviation of three replicates for each data point.