

## Electronic Supplementary Information

### A protein kinase assay based on FRET between quantum dots and fluorescently-labeled peptides

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## Experimental details

### 1. Materials

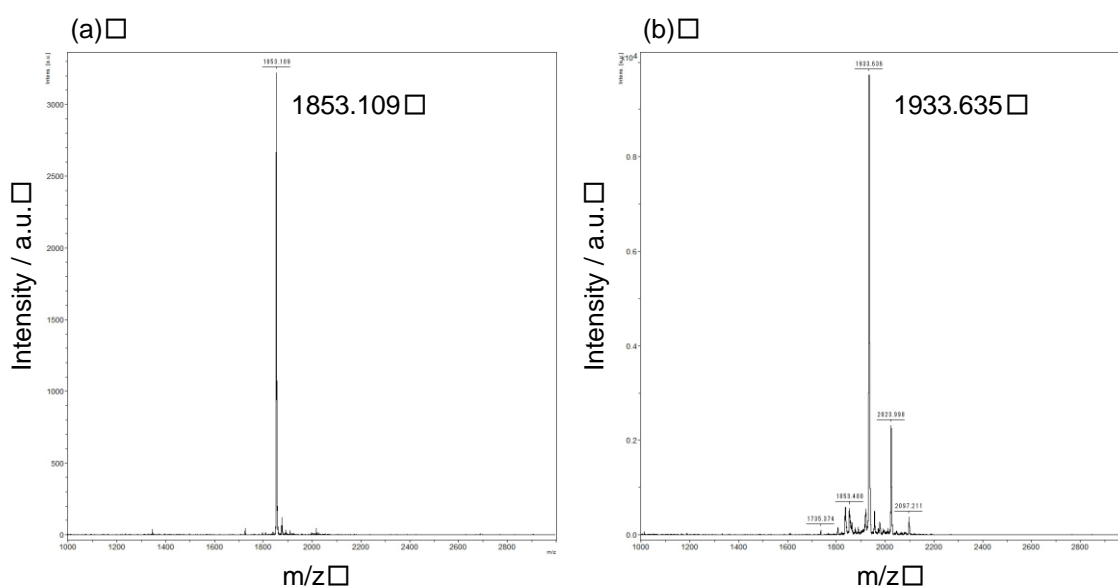
NovaPEG<sup>®</sup> Rink Amide resin and Fmoc protected amino acids were purchased from Novabiochem (Darmstadt, Germany). Fmoc-8-amino-3,6-dioxaoctanoic acid (Fmoc-mini-PEG<sup>TM</sup>) was purchased from Peptide International (Louisville, KY). 5/6-carboxy-tetramethyl-rhodamine succinimidyl ester (5/6-TAMRA) was purchased from Thermo Scientific (Rockford, IL). *N,N*-diisopropylethylamine (DIPEA), dichloromethane (DCM), 1-methyl-2-pyrrolidone (NMP), piperidine, trifluoro acetic acid (TFA) and *N*-[1-(Cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylamino(morpholino)]uranium

hexafluorophosphate (COMU) were purchased from Watanabe Chemical Industries (Hiroshima, Japan). *N,N*-dimethylformamide (DMF) and diethylether were purchased from Kanto Chemical (Tokyo, Japan). Triisopropylsilane (TIS) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Methanol, dimethyl sulfoxide (DMSO), Gö 6983, magnesium chloride hexahydrate and calcium chloride were purchased from Wako Pure Chemical Industries (Osaka, Japan).  $\alpha$ -Cyano-4-hydroxycinnamic Acid (CHCA), adenosine 5'-triphosphate disodium salt hydrate (ATP), protein kinase C alpha isozyme, human, recombinant (PKC $\alpha$ ), diacylglycerol and phosphatidylserine were purchased from Sigma Aldrich (St. Louis, MO). Acetonitrile was purchased from nacalai tesque (Kyoto, Japan). Carboxyl coated quantum dots (Qdot<sup>®</sup> 545 ITK<sup>™</sup> Carboxyl Quantum Dots) was purchased from Invitrogen (Carlsbad, CA). Rottlerin was purchased from Calbiochem (Darmstadt, Germany). 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) was purchased from Dojindo Laboratories (Kumamoto, Japan).

## 2. Synthesis of fluorescent-labeled peptide

Peptides were synthesized by Fmoc chemistry using NovaPEG<sup>®</sup> Rink Amide resin and the corresponding Fmoc amino acids.<sup>1</sup> Coupling reactions were performed with 3 eq. mol (relative to amine group on the resin) of a Fmoc amino acid, 3 eq. mol of COMU in DMF as a coupling agent, 3 eq. mol of DIEPA in NMP as a base for 10 min. Completion of the coupling reaction was confirmed by checking remaining of free amine by Kaiser test. Fmoc protection group was removed by treatment with 20% solution of PPD in DMF for 10 min. For modification of TAMRA at the N-terminal of the peptide, 1.2 eq. mol of 5/6-TAMRA dissolved in DMF and 1.2 eq. mol of DIEA were stirred with the resin overnight in the dark at room temperature. After the dye modification, the resin was washed with DMF, DCM and methanol each 5 times and dried under reduced pressure. Deprotection of the peptide side chain and cleavage from the resin were accomplished by treatment with a mixture of cold TFA/TIS/H<sub>2</sub>O (95:2.5:2.5) for 1 h on ice. The peptide solution was delivered by drops into cold diethyl ether to precipitate the peptide. The obtained peptide was purified by reversed-phase liquid chromatography (LaChrome Elite, Hitachi High-Technologies, Tokyo, Japan) using Atlantis dC18 OBD Prep Column (19 x 100 mm, Waters, Milford, MA) under a linear gradient, at flow-rate of 4 mL min<sup>-1</sup> with CH<sub>3</sub>CN/H<sub>2</sub>O (containing 0.1% TFA)

mobile phase. The purified peptide was lyophilized and was obtained as red powder. The synthesis of peptides were identified by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) (autoflex III, Bruker Daltonics). The representative MALDI-TOF-MS results of peptide **S** and **pS** were shown in Fig. S1. The CHCA prepared in 50% CH<sub>3</sub>CN/H<sub>2</sub>O containing 0.1% TFA to be 10 mg mL<sup>-1</sup> was used as a matrix by mixing in 1:1 ratio.



**Fig. S1** Identification of the peptide **S** (a) and **pS** (b). Calculated exact mass of peptide **S** and **pS** were 1852.00 and 1931.97, respectively.

### 3. Measurement of emission spectrum

QD in 10 mM HEPES buffer (pH 7.4) was mixed with peptide to be 0, 10, 20, 40, 60, 100 in a molar ratio of peptide/QD. Subsequently, the final concentration of the components were adjusted to be 10 mM HEPES (pH 7.4), 10 nM QD, 100  $\mu$ M ATP, 2 mM MgCl<sub>2</sub>, 0.5 mM CaCl<sub>2</sub>, 2.0  $\mu$ g/mL diacylglycerol, 2.5  $\mu$ g/mL phosphatidylserine. The solution was left to stand for 15 min at room temperature. The emission spectrum was measured by using a spectrofluorophotometer (FP6600, JASCO, Tokyo, Japan) equipped with an ETC-273 temperature controller (JASCO). The excitation wavelength and temperature were 400 nm and 30 °C, respectively. Emission ratio (553 nm/580 nm) was simply calculated by using following formula: [fluorescence intensity at 553 nm / fluorescence intensity at 580 nm].

#### 4. Phosphorylation reaction with protein kinases

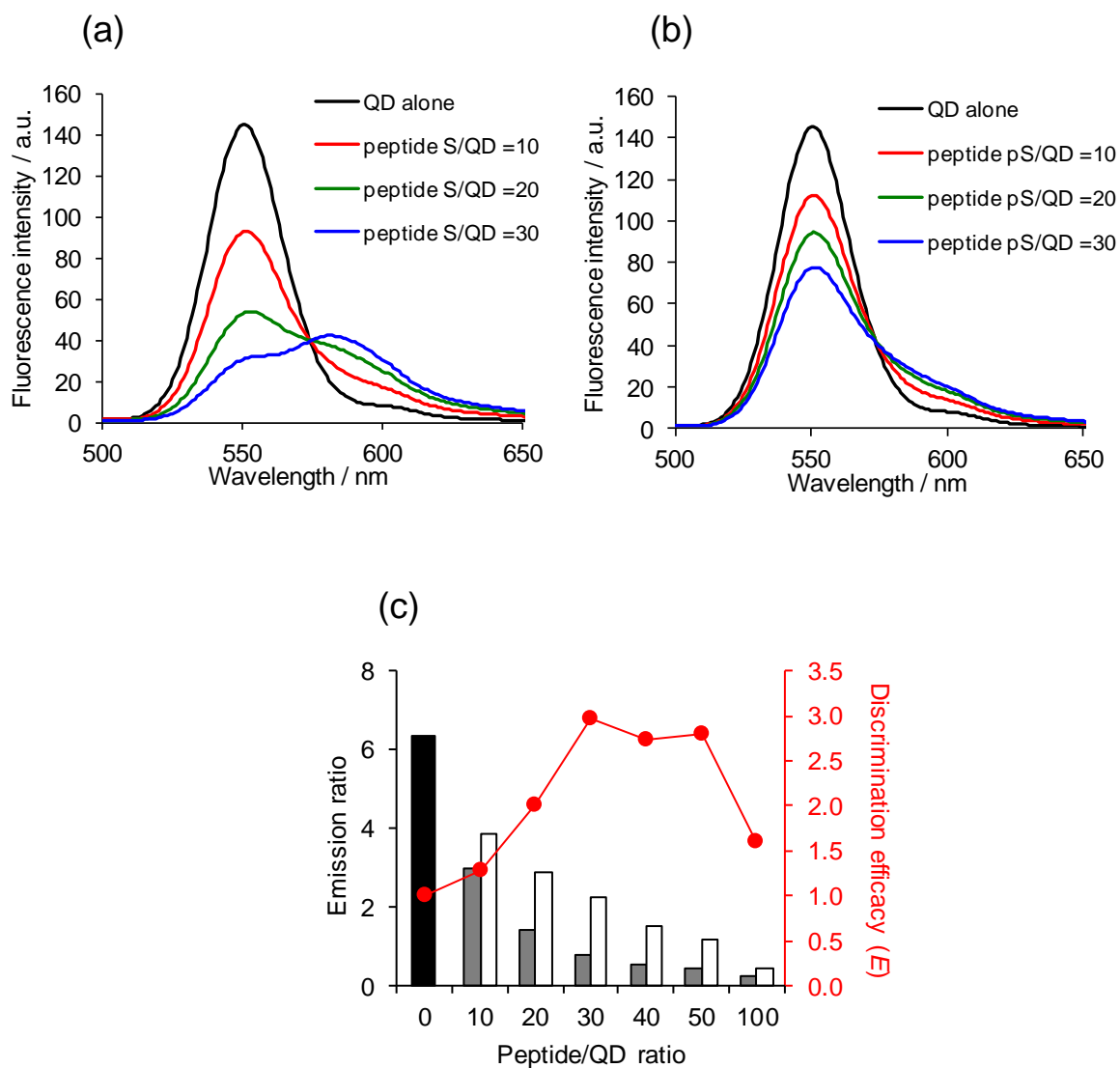
As for PKC $\alpha$ , the phosphorylation of 4  $\mu$ M peptide **S** was performed in PKC $\alpha$  reaction buffer (10 mM HEPES buffer, pH 7.4, 100  $\mu$ M ATP, 2 mM MgCl<sub>2</sub>, 0.5 mM CaCl<sub>2</sub>, 2.0  $\mu$ g/mL diacylglycerol, 2.5  $\mu$ g/mL phosphatidylserine) with each concentration of PKC $\alpha$ . In a negative control experiment, PKC $\alpha$  reaction buffer without ATP was used. As for PKA, the phosphorylation of 3  $\mu$ M peptide **S-2** was performed in PKA reaction buffer (10 mM HEPES buffer, pH 7.4, 100  $\mu$ M ATP, 1 mM MgCl<sub>2</sub>). After incubation for 60 min at 30 °C, 5  $\mu$ L of the reaction solution, equal volume of 100 nM QD and 40  $\mu$ L of the reaction buffer were mixed and the mixture was allowed to remain for 15 min. After that, the emission spectrum was measured and the emission ratio was calculated, as described above. The EC<sub>50</sub> value and limit of detection were calculated by using GraphPad Prism software.

#### 5. Quantification of phosphorylation ratio by MALDI-TOF-MS

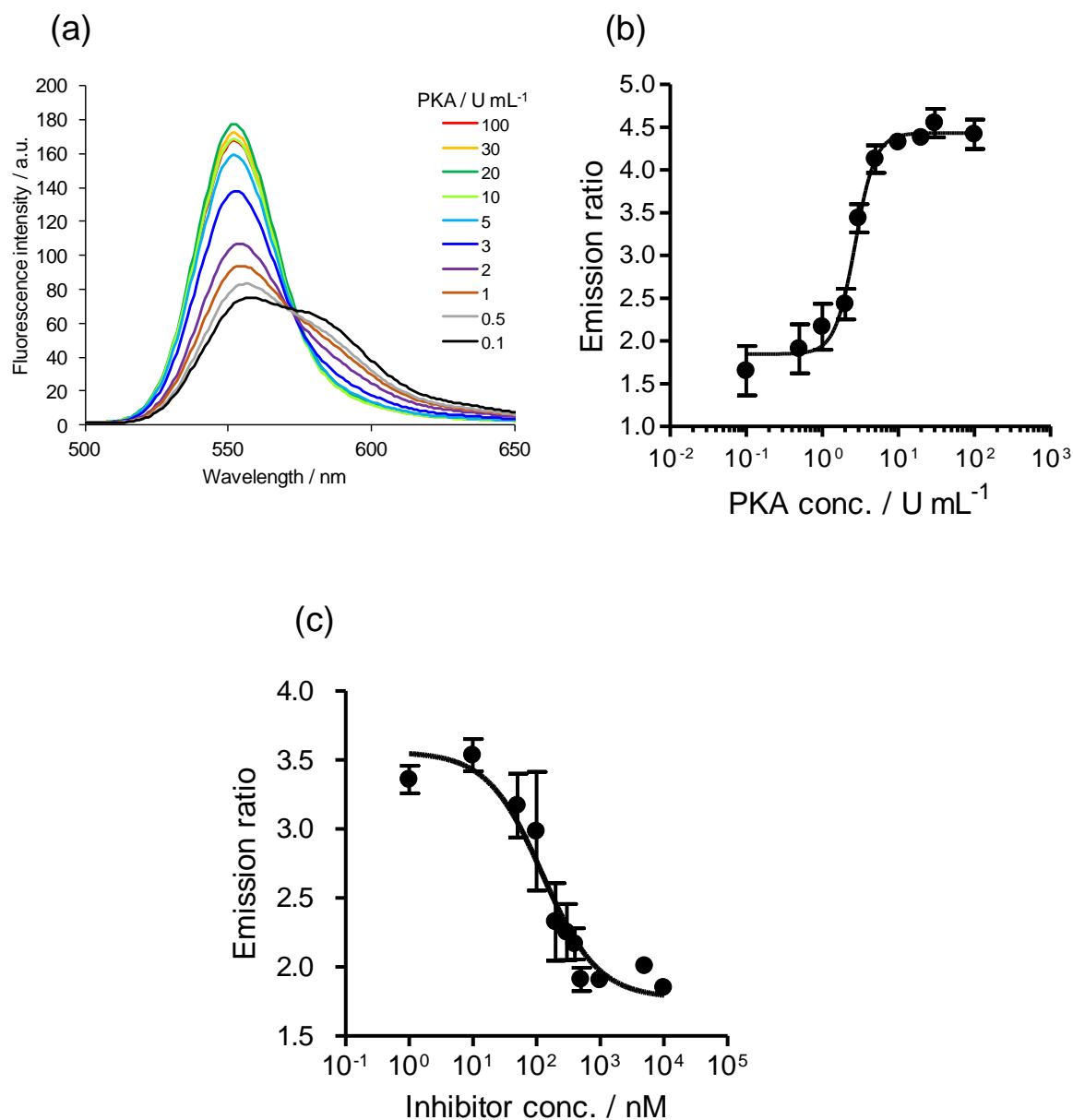
The phosphorylation ratio of the peptide was analyzed by MALDI-TOF-MS. The phosphorylation reaction was performed as described above and was finished by mixing with CHCA solution in 1:1 ratio. The MALDI-TOF-MS measurement was conducted using a positive linear mode. The total intensity of 200 laser shots was analyzed using flexAnalysis software (Bruker Daltonics). The phosphorylation ratio was calculated by following formula: [phosphorylated peptide intensity / (phosphorylated peptide intensity + unphosphorylated peptide intensity) x 100].<sup>2</sup>

#### 6. Determination of IC<sub>50</sub> value

As for PKC $\alpha$ , inhibitors (Gö 6983 and rottlerin) dissolved in DMSO were diluted to be each concentration with PKC $\alpha$  reaction buffer. The various concentrations of inhibitors and 0.2 U mL<sup>-1</sup> of PKC $\alpha$  were pre-incubated before adding to the reaction cocktail. As for PKA, H-89 dissolved in DMSO was diluted to be each concentration with PKA reaction buffer. The phosphorylation reaction and measurement of emission ratio were carried out as described. The IC<sub>50</sub> value was also determined by using GraphPad Prism software.



**Fig. S2** Application of our detection principle to PKA substrate peptide. Emission spectra of QDs and peptide **S-2** (a) and **pS-2** (b) at various peptide/QD molar ratios. (c) The QD/TAMRA emission ratio (553 nm/580 nm) and discrimination efficacy ( $E$ ) at each peptide/QD molar ratio. The emission ratio of peptide **S-2** is in gray and **pS-2** in white.



**Fig. S3** (a) PKA concentration dependent emission spectra of peptide S-2/QD mixtures after the phosphorylation reaction with ATP. (b) Dependence of the emission ratio on the PKA concentration. (c) Inhibition of PKA activity by H-89. The data are the means  $\pm$  SEM of three independent experiments.

## References

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- 2 J. H. Kang, D. Asai, R. Toita, H. Kitazaki, and Y. Katayama, *Carcinogenesis*, 2009, **30**, 1927.