Supplementary Material (ESI) for Molecular BioSystems

Supporting Information for

Rational design of homogenous protein kinase assay platforms that allow both fluorometric and colorimetric signal readouts

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Contents

SI-1. Synthesis of the spiropyran-containing peptides.

All new spiropyran-containing peptides shown in **Main Text (Table 1)** were synthesized according to the literature (**Fig. S1**).[S1] Briefly, the known spiropyran derivative, 1-(2-hydroxyethyl)-3,3-dimethylindolino-6'-nitrobenzopyrylospiran (**1**)[S2] which was prepared from commercially available 2,3,3-trimethyl-3*H*-indole via three steps was treated with *p*-nitrophenyl chloroformate, affording an activated spiropyran (**2**).[S1] Peptides were synthesized by means of Fmoc chemistry on Rink amide MBHA resin with 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole monohydrate (HOBt) as coupling reagents.[S3] Side chains of the following amino acids were protected with acetamidomethyl (Acm) for Cys, *t*-butyloxycarbonyl (Boc) or 4-methyltrityl (Mtt) for Lys, 2,2,4,6,7-pentamethyldihydrobenzofurane-5-sulfonyl (Pbf) for Arg, *t*-butyl ester (O^{t}Bu) for Glu, and *t*-butyl ether (^{t}Bu) for Tyr, Thr, and Ser. Initially, Ac-Cys(Acm)-Gly-Lys(Mtt)-Gly-[various sequences]-Gly-resins were prepared. The peptide-bound resin was dried in *vacuo* and divided in portions required. The obtained peptide-bound resin was treated with trifluoroacetic acid (TFA)/triisopropylsilane (TIS)/CH_2Cl_2 (1/5/94, v/v/v) at room temperature to remove the Mtt protecting group of Lys residue. The resulting peptide-bound resin was washed with 1% diisopropylethylamine (DIEA)/*N*-methyl-2-pyrrolidone (NMP) then NMP and coupled with Fmoc-Glu(O^{t}Bu)-OH on the Lys side chain. Incorporation of Glu residue was repeated if required. The peptide-bound resin was treated with **2** (3 eq) and DIEA (6 eq) in NMP overnight at room temperature in the dark. The reaction mixture was filtered off and washed with NMP then $CHCl_3$, and dried in *vacuo*. All of the protecting groups except Acm on the peptide-bound resin were removed by the treatment of TFA/*m*-cresol/ethanedithiol/thioanisole (40/1/3/3, v/v/v/v) for 60 min at room temperature. The crude peptide obtained was purified by HPLC and characterized by MALDI-TOFMS, affording a fluffy yellow powder: S-PKA-E1 (17.0 mg): obsd, 1737.6 [(M + H)$^+$]; calcd 1736.9. S-PKA-E2 (20.8 mg): obsd, 1868.1 [(M + H)$^+$]; calcd, 1866.1. S-PKA-E3

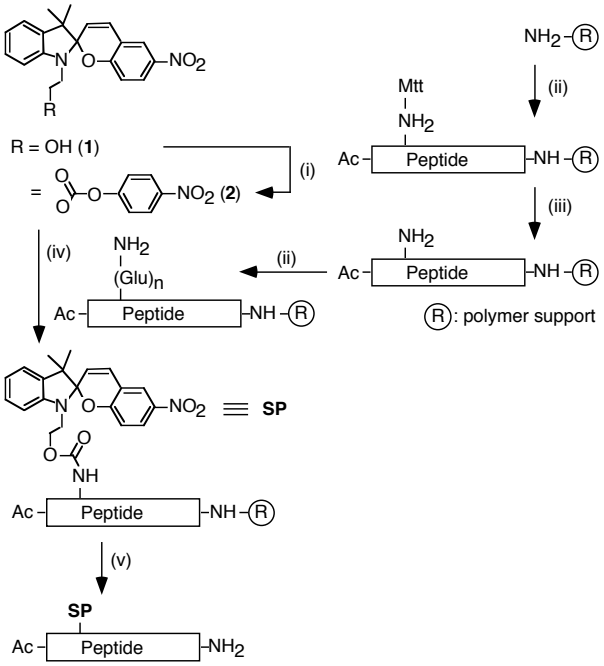

Figure S1. Synthesis of spiropyran-containing peptides. (i) *p*-Nitrophenyl chloroformate, DIEA, CH_2Cl_2. (ii) Solid phase peptide synthesis with Fmoc chemistry. (iii) TFA/TIS/CH_2Cl_2 (1/5/94, v/v/v). (iv) Peptide–bound resin, DIEA, NMP. (v) *a*, TFA/*m*-cresol/ethanedithiol/thioanisole (40/1/3/3, v/v/v/v); *b*, HPLC purification, and *c*, lyophilization.

(24.1 mg): obsd, 1998.1 [(M + H)$^+$]; calcd 1995.2. S-PKA-E4 (19.5 mg): obsd, 2125.5 [(M + H)$^+$]; calcd 2124.3. S-Abl-E1 (12.6 mg): obsd, 1942.4 [(M + H)$^+$]; calcd, 1940.2. S-PKC-E1 (2.2 mg): obsd, 1853.3 [(M + H)$^+$]; calcd 1851.1. S-PKC-E2 (2.9 mg): obsd, 1982.5 [(M + H)$^+$]; calcd 1980.2.

SI-2. Measurements of UV-vis and fluorescence spectra of the spiropyran-containing peptide

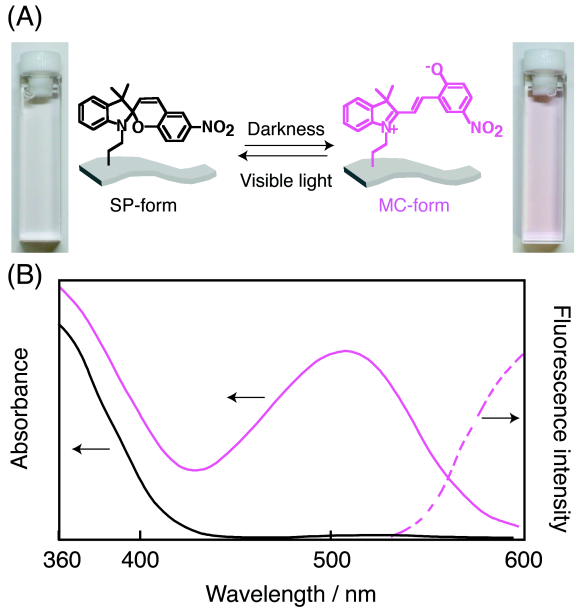

Figure S2. Photochromic properties of the spiropyran-containing peptide in neutral aqueous solution. (A) The SP-to-MC thermocoloration and MC-to-SP photobleaching proceed by incubation in the dark and a visible light irradiation, respectively. These processes are reversible. (B) UV-vis (solid line) and fluorescence (λ_{ex} = 510 nm, dashed line) spectra of S-PKA in the SP-dominant solution (black) and in the SP/MC mixture (magenta) in 20 mM Tris HCl buffer, 100 mM NaCl (pH 7.4) at 4 ˚C. Only the MC-form has an intense absorption band in the visible region. Fluorescence emission from the SP-dominant solution is scarcely observed due to completion of the MC-to-SP photobleaching.

UV-vis and fluorescence (λ_{ex} = 510 nm) spectra of spiropyran-containing peptide, S-PKA were recorded in 20 mM Tris HCl buffer, 150 mM NaCl (pH 7.4) at 4 ˚C. The SP-dominant solution was prepared by continuous irradiation with a 510 nm-light in a fluorescence spectrophotometer at 4 ˚C for 30 min. Photographs were taken at room temperature using more concentrated spiropyran-containing peptide solution than that used in spectroscopic measurements. The SP-dominant solution for photographs was prepared by exposure with inside lightning at room temperature for 10 min.

SI-3. Measurements of the SP-to-MC thermocoloration rates of spiropyran-containing peptides

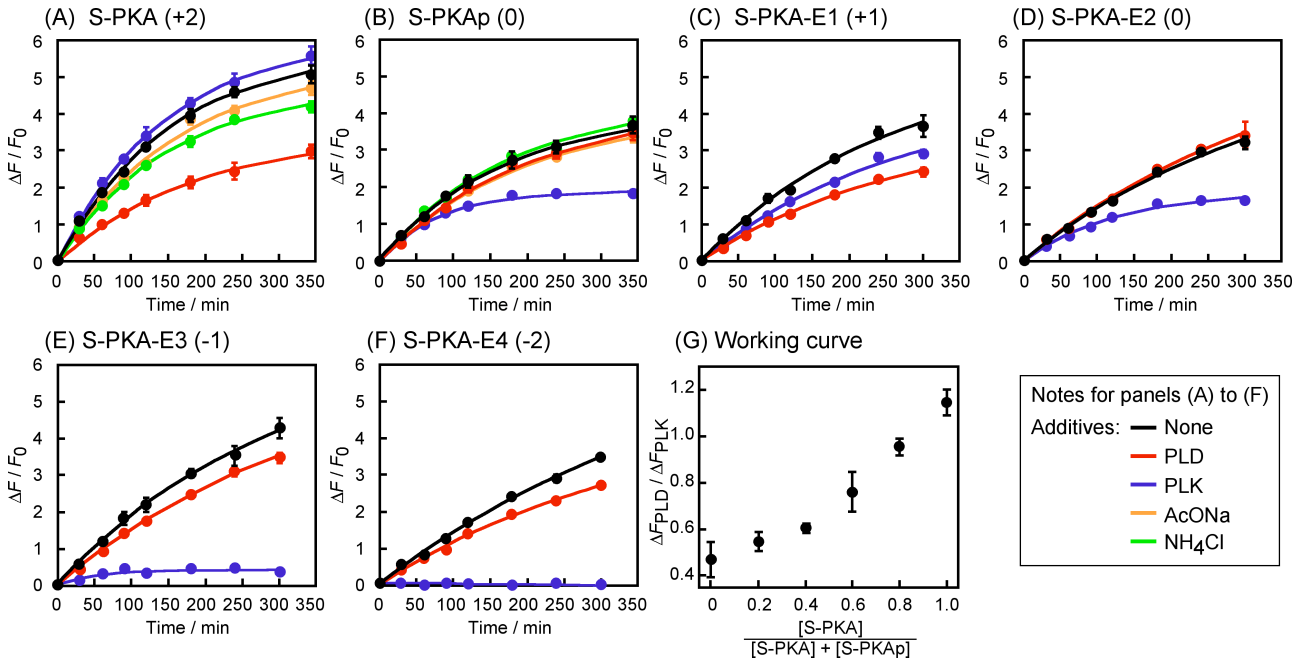

Figure S3. The SP-to-MC thermocoloration of the spiropyran-containing peptides. (A–F) Relaxation processes of spiropyran-containing peptides in the absence and presence of additives. (A) S-PKA (net charge = +2), (B) S-PKAp (0), (C) S-PKA-E1 (+1), (D) S-PKA-E2 (0), (E) S-PKA-E3 (–1), and (F) S-PKA-E4 (–2). [Peptide] = 3.4 □M, [PLD] = [PLK] = 10 □M, [AcONa] = [NH_4Cl] = 10 mM in 20 mM Tris HCl buffer (pH 7.4) at 25 ˚C in the dark. (G) Relationship between fractions phosphorylated and the □F_{PLD}/□F_{PLK} values. Peptides ([total peptide] = [S-PKA] + [S-PKAp] = 3.4 □M) were incubated in 20 mM Tris HCl buffer (pH 7.4) at 25 ˚C in the dark. Plotted values indicate the mean ± s.e.m. for four to eight measurements. Abbreviations: PLD = poly(L-aspartic acid) sodium salt; PLK = poly(L-lysine) hydrobromide; AcONa = sodium acetate; and NH_4Cl = ammonium chloride.

SI-4. HPLC analysis of kinase-catalyzed phosphorylation

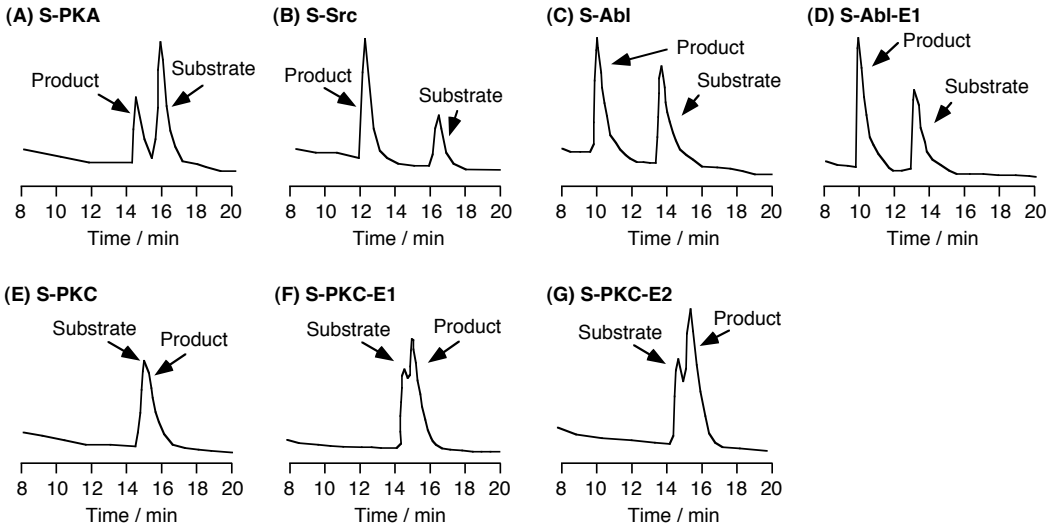

Figure S4. Representative HPLC profiles of kinase-catalyzed phosphorylation of (A) S-PKA (elution condition: 40% acetonitrile/0.1% TFA, [PKA] = 6 nM), (B) S-Src (39% acetonitrile/0.1% TFA, [SrcN1] = 54 nM), (C) S-Abl (39% acetonitrile/0.1% TFA, [v-Abl] = 90 nM), (D) S-Abl-E1 (37% acetonitrilee/0.1% TFA, [v-Abl] = 90 nM), (E) S-PKC (36% acetonitrile/0.1% TFA, [PKC□] = 1.3 nM), (F) S-PKC-E1 (35% acetonitrile/0.1% TFA, [PKC□] = 1.3 nM), and (G) S-PKC-E2 (35% acetonitrilee/0.1% TFA, [PKC] = 1.3 nM).

SI-5. Rational design of chromism-based assay formats for diverse protein kinase activities

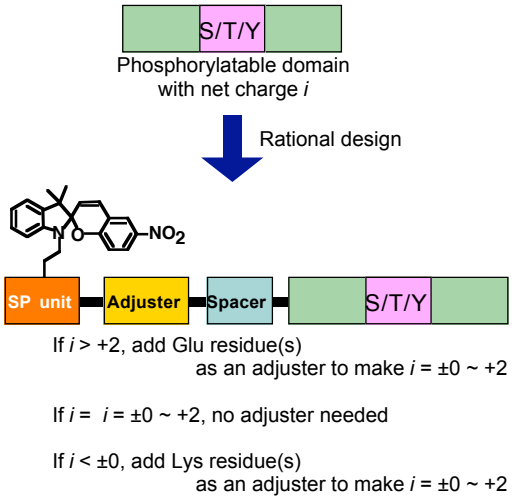

Figure S5. A strategy for rational design of protein kinase substrate peptides suitable for use in CHROBA is proposed. Net charges in the substrate peptides should be adjusted to be between +2 and 0 by incorporating Lys or Glu residue(s) into the peptide substrates for sensitive CHROBA measurements.

SI-6. References

S1. K.-Y. Tomizaki, H. Mihara, *J. Mater. Chem*. **2005**, *15*, 2732–2740.

S2. F. M. Raymo, S. Giordani, *J. Am. Chem. Soc*. **2001**, *123*, 4651–4652.

S3. W. C. Chen, P. D. White, *Fmoc solid phase peptide synthesis: A practical approach*, Oxford University Press, New York, **2000**.