Supporting Information for

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

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Contents

Synthesis of spiropyran-containing peptides .............................................................(S2)
Measurements of UV-vis and fluorescence spectra of spiropyran-containing peptide .......(S3)
Measurements of the SP-to-MC thermocoloration rates of spiropyran-containing peptides .....(S4)
HPLC analysis of kinase-catalyzed phosphorylation .....................................................(S5)
Rational design of photochromic protein kinase substrates for CHROBA measurements ....(S5)
References .......................................................................................................................(S5)

All new spiropyran-containing peptides shown in Main Text (Table 1) were synthesized according to the literature (Fig. S1). Briefly, the known spiropyran derivative, 1-(2-hydroxyethyl)-3,3-dimethylindolino-6’-nitrobenzopyrylospiran (1) which was prepared from commercially available 2,3,3-trimethyl-3H-indole via three steps was treated with p-nitrophenyl chloroformate, affording an activated spiropyran (2). Peptides were synthesized by means of Fmoc chemistry on Rink amide MBHA resin with 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole monohydrate (HOBT) as coupling reagents. Side chains of the following amino acids were protected with acetamidomethyl (Ac) 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’Bu) for Glu, and t-butyl ether (’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$_2$Cl$_2$ (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’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

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 (λex = 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 (λex = 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.

S3
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 mM, [PLD] = [PLK] = 10 μM, [AcONa] = [NH₄Cl] = 10 mM in 20 mM Tris HCl buffer (pH 7.4) at 25 °C in the dark. (G) Relationship between fractions phosphorylated and the $\frac{[F_{P,L,D}]}{[F_{P,L,K}]}$ values. Peptides ([total peptide] = [S-PKA] + [S-PKAp] = 3.4 mM) 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 NH₄Cl = 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% acetonitrile/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% acetonitrile/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