Supporting Information for

Phosphate-mediated molecular memory driven by two different protein kinases as information input elements

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Contents

(1) General.

Poly(L-aspartic acid) sodium salt (Mw 15,000–50,000) was purchased from Sigma. Poly(L-lysine) hydrobromide (Mw 70,000–150,000) was purchased from Nacalai Tesque (Kyoto, Japan). All solvents and reagents (except amino acid derivatives) were purchased from Wako Pure Chemical Industries (Osaka, Japan) and used as received. Fmoc-amino acid derivatives and reagents for peptide syntheses were purchased from Watanabe Chemical Industries (Hiroshima, Japan). Acetonitrile (HPLC grade) was used for HPLC analysis and purification. Water was purified with the MilliQ production system. Fluorescence signals were recorded on an ARVO MX1420 multilabel counter (PerkinElmer) equipped with a combination of an excitation filter F485 and an emission filter F580 ± 10. Absorbance signals were also acquired on the same plate reader equipped with a 490 nm filter.

(2) Synthesis of spiropyran-containing peptides.

All new spiropyran-containing peptides were synthesized according to the literatures.[S1,2] Briefly, the known spiropyran derivative, 1-(2-hydroxyethyl)-3,3-dimethylindolino-6'-nitrobenzopyrylospiran (**1**)[S3] 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**).[S2] 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.[S4] Side chains of the following amino acids were protected with *t*-butyloxycarbonyl (Boc) for Lys, 2,2,4,6,7-pentamethyldihydrobenzofurane-5-sulfonyl (Pbf) for Arg, *t*-butyl ester (O^{t}Bu) for Glu, *t*-butyl ether (^{t}Bu) for Tyr and Ser, and mono-benzylester (OBzl) for Tyr[P(O)(OH)$_2$] and Ser[P(O)(OH)$_2$]. Initially, Fmoc-[various sequences]-resins were prepared and treated with 20% piperidine in 1-methyl-2-pyrrolidone (NMP) to remove Fmoc protecting group at the N-terminus. The peptide-bound resin was treated with **2** (3 eq) and *N*,*N*-

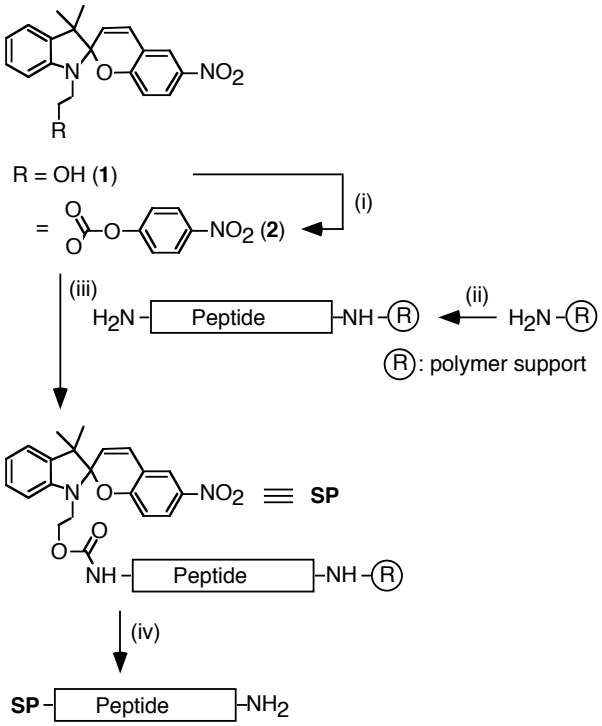

Figure S1. Synthetic scheme of spiropyran-containing peptides. (i) *p*-Nitrophenyl chloroformate, DIEA, CH_2Cl_2. (ii) Solid phase peptide synthesis with Fmoc chemistry. (iii) Peptide–bound resin, DIEA, NMP. (iv) *a*, TFA/*m*-cresol/ethanedithiol/thioanisole (40/1/3/3, v/v/v/v); *b*, HPLC purification,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

diisopropylethylamine (6 eq) in NMP overnight at room temperature in the dark. The reaction mixture was filtered off and the resulting peptide-bound resin was washed with NMP then $CHCl_3$, and dried in *vacuo*. All of the protecting groups 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: SP-YS (13.8 mg, 22%): obsd, 2372.2 [$(M + H)^+$]; calcd 2370.8, SP-pYS (7.4 mg, 12%): obsd, 2450.7 [$(M + H)^+$]; calcd, 2449.8, SP-YpS (19.7 mg, 39%): obsd, 2452.2 [$(M + H)^+$]; calcd 2449.8, SP-pYpS (11.6 mg, 9.1%): obsd, 2531.5 [$(M + H)^+$]; calcd 2529.8.

(3) SP-to-MC thermocoloration for SP-YS in the absence and presence of ionic polymers.

A peptide solution (5.0 μM, 500 μL) in 20 mM Tris HCl buffer (pH 7.4) containing poly(L-aspartate) (10 μM) or poly(L-lysine) (10 μM) was irradiated with indoor lightning for 5 min at room temperature to prepare a colorless SP-dominant solution [we assumed concentrations of poly(L-aspartate) (10 mg/448 μL) and poly(L-lysine) (25 mg/453 μL) to be 0.5 mM in 20 mM Tris HCl buffer (pH 7.4)][S1]. Four aliquots (100 μL each) were transferred from each solution to a 96-well plate (Costar® 3915, black polystylene, Corning Inc.) and the initial fluorescence intensity (F_0) was recorded on a fluorescence platereader. The 96-well plate was sealed with a plate seal to avoid contamination and evaporation, wrapped with an aluminum sheet, and incubated at 25 ˚C in the dark. At the time points requested, the 96-well plate was taken out from the incubator and the aluminum sheet and plate seal were peeled off, subsequently fluorescence intensity (F) was recorded.

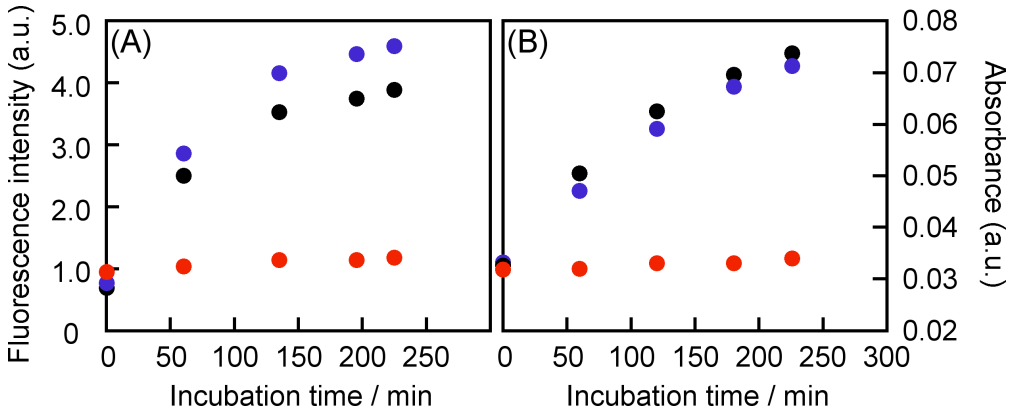

Figure S2. Time courses of the SP-to-MC thermocoloration for SP-YS in the absence (black) and presence of poly(L-aspartate) (red) and poly(L-lysine) (blue) monitored by changes in (A) fluorescence intensity (excitation filter F485, emission filter = F580 ± 10) and (B) absorbance (490 nm-filter). [Peptide] = 5.0 and 25 μM (for panels A and B, respectively) and [ionic polymer] = 10 μM in 20 mM Tris HCl (pH 7.4) at 25 °C. (n = 4, errors within ± 7%).

The SP-to-MC thermocoloration was also monitored by changes in absorbance on the same plate reader. Four aliquots (100 μL each, [peptide] = 25 μM and [ionic polymer] = 10 μM in 20 mM Tris HCl buffer, pH 7.4) after photo-irradiation were transferred to a 96-well plate (Iwaki 3881-096, assay plate, clear, flat bottom). Absorbance data were acquired according to the procedure described above.

(4) Comparison of the changes in fluorescence intensity for the memory cycles 1 and 2.

Two samples (100 μL each) of peptide (50 μM), cAMP (1.0 mM), and ATP (1.0 mM) was reacted with kinase(s) ([SrcN1]/PKA) = 110 nM/60 nM) in 100 mM HEPES buffer (pH 7.0) containing 5.0 mM $MgCl_2$ at 30 ˚C for 60 min in the dark. One was stored on ice as the sample for the memory cycle 1. Another one was further incubated at 75 ˚C for 5 min, 30 ˚C for 2 h, and 75 ˚C for 5 min, then stored on ice as the sample for the memory cycle 2. Two aliquots (45 μL each) were transferred from each reaction mixture to separated eppendorf tubes and mixed with 20 mM Tris HCl buffer (pH 7.4) (405 μL) for a control solution, and 20 mM Tris HCl buffer (pH 7.4) (396 μL) and 0.5 mM poly(L-aspartate) (9.0 μL) for a sample solution. Peptide-containing solutions (four sets of control and sample solutions, 100 μL each) were transferred to a 96-well plate (Costar® 3915, black polystylene, Corning Inc.) and the initial fluorescence intensity (F_0) was recorded on a fluorescence platereader. The 96-well plate was sealed with a plate seal to avoid contamination and evaporation, wrapped with an aluminum sheet, and incubated at 25 ˚C in the dark. After 60 min, the 96-well plate was taken out from the incubator and the aluminum sheet and plate seal were peeled off, subsequently fluorescence intensity (F_{60}) was recorded. Changes in fluorescence intensity (ΔF) were calculated with the following equation, $\Delta F = F_{60} - F_0$.

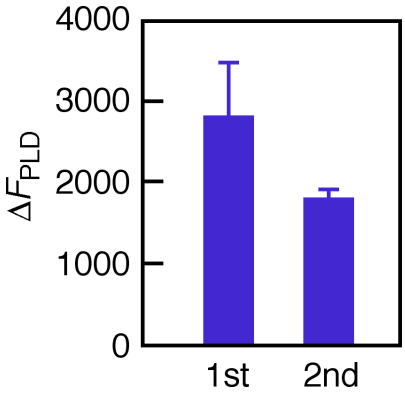

Figure S3. Comparison of the changes in fluorescence intensity for the memory cycles 1 and 2. The colored isomer decomposed gradually by incubation at the elevated temperature.

(5) References

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