Supporting Information

Syntheses of the zinc complexes

All zinc complexes (4,4'-Zn(Dpa)-Bpy, 5,5'-Zn(Dpa)-Bpy, 6,6'-Zn(Dpa)-Bpy, 4-Zn(Dpa)-Bpy, and 5-Zn(Dpa)-Bpy) were synthesized from the corresponding halomethyl-2,2'-bipyridine by the nucleophilic substitution with 2,2'-dipicolylamine and the subsequent zinc complexation. Characterizations of these compounds were performed by ^{1}H-NMR, mass spectroscopy, and elemental analysis. Typical synthetic procedure is as follows:

5,5'-bis[(2,2'-dipicolylamino)methyl]-2,2'-bipyridine

A solution of 5,5'-bis(bromomethyl)-2,2'-bipyridine[1] (800 mg, 2.34 mmol), 2,2'-dipicolylamine (979 mg, 4.91 mmol) and potassium carbonate (1.29 g, 9.36 mmol) in anhydrous DMF (6 mL) was stirred for 1.5 h at rt. After dilution with 1 N HCl, the resulting mixture was washed with AcOEt. The aqueous phase was alkalized with 2N NaOH and then extracted with AcOEt (x2). The combined organic layers were washed with water and brine followed by drying over $MgSO_4$. After removal of the solvent in vacuo, the residue was filtered and washed with hexane/AcOEt (1 : 1) to give 5,5'-bis[(2,2'-dipicolylamino)methyl]-2,2'-bipyridine (996 mg, 74%) as a light brown powder: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.78 (4H, s), 3.86 (8H, s), 7.17 (4H, dd, *J*=5.8, 7.0 Hz), 7.57 (4H, d, *J*=7.6 Hz), 7.66-7.71 (4H, m), 7.86 (2H, dd, *J*=2.2, 8.2 Hz), 8.32 (2H, d, *J*=8.0 Hz), 8.54-8.55 (4H, m), 8.71 (2H, d, *J*=1.6 Hz).

5,5'-Zn(Dpa)-Bpy

To a solution of 5,5'-bis[(2,2'-dipicolylamino)methyl]-2,2'-bipyridine (900 mg, 1.56 mmol) in MeOH (20 mL) was added dropwise aqueous solution of 0.596 M of $Zn(NO_3)_2$ (5.22 mL, 3.11 mmol), and the mixture was stirred for 10 min at rt. After removal of the solvent in vacuo, the residue was filtered and washed with CH_3CN followed by vaccum dryness to give 5,5'-Zn(Dpa)-Bpy (1.30 g, 87%) as a pale yellow powder: ^{1}H-NMR(600 MHz, D_2O) δ 3.97 (4H, s), 4.08 (4H, d, *J*=16.1Hz), 4.41 (4H, d, *J*=16.1Hz), 7.64-7.69 (8H, m), 8.01 (2H, d, *J*=7.9 Hz), 8.13-8.18 (6H, m), 8.59 (2H, s), 8.74 (4H, d, *J*=4.9 Hz). FAB-MS *m/e* 892 $[M\text{-}NO_3]^+$. Anal Calcd for $C_{36}H_{34}N_8 \cdot 2Zn(NO_3)_2 \cdot H_2O$: C, 44.32; H, 3.72; N, 17.23. Found: C, 44.50; H, 3.86; N, 16.83.

Solis Phase Peptide Synthesis (SPPS)

All peptides were synthesized by an automated peptide synthesizer (ABI 433A, Applied Biosystems) using the standard Fmoc-based FastMoc coupling chemistry (0.1 mmol scale) on Fmoc-Amide Resin (Applied Biosystems). Fmoc-Ser[PO(Obzl)OH]-OH, Fmoc-Tyr[PO(Obzl)OH]-OH (Watanabe Chemical Industries, Ltd.) were used as the phosphorylated amino acid unit for the peptide

coupling. After the automated SPPS, *N*-terminal amino group was acetylated with 20% Ac_2O in CH_2Cl_2 over 2 hr at rt. Peptide cleavage and side-chain deprotection were carried out by the treatment with TFA-*m*-cresol-thioanisole (86 : 2 : 12) over 1 h at rt . Crude peptide was precipitated in *tert*-butyl-methyl ether, and purified by reverse-phase HPLC (column; YMC-pack ODS-A, 250 x 10 mm). The purification conditions were as follows: mobile phase; CH_3CN (containing 0.1% TFA) / H_2O (containing 0.1% TFA) = 0 / 100 → 40 / 60 (linear gradient over 60 min), flow rate; 3 ml/min, detection; UV (220 nm). Molecular weight of the peptide was confirmed by mass spectrometer (MALDI-TOF): **pS-5,16**; calcd for $C_{65}H_{113}N_{20}O_{32}P_2$ $[M + H]^+$ 1747.7, found 1748.5; **pS-9,16**: calcd for $C_{65}H_{113}N_{20}O_{32}P_2$ $[M + H]^+$ 1747.7, found 1748.8, **pS-12,16**; calcd for $C_{63}H_{111}N_{20}O_{30}P_2$ $[M + H]^+$ 1689.6, found 1690.3, **pS-16**; calcd for $C_{65}H_{112}N_{20}O_{28}P$ $[M + H]^+$ 1651.8, found 1652.8, **IRK-2P**; calcd for $C_{74}H_{113}N_{20}O_{30}P_2$ $[M + H]^+$ 1823.7, found 1823.8, **IRK-1P**; calcd for $C_{74}H_{112}N_{20}O_{27}P$ $[M + H]^+$ 1743.8, found 1745.1, **IRK-0P**; calcd for $C_{74}H_{111}N_{20}O_{24}$ $[M + H]^+$ 1663.8, found 1664.7.

CD Studies

CD spectra were recorded using JASCO J-720W using water-jacketed quartz cell (1 mm path length) at 10 °C. The screening study for the binding of the receptors with the phosphorylated peptides was carried out at a constant concentration of peptide (20 μM) in 10 mM borate buffer (pH 8.0), and the changes of CD signal at 222 nm were measured by addition of 2 eq. of the receptors (the final concentration is 40 μM). Titration experiment was conducted with 20 μM of pS-9,16 in 10 mM borate buffer (pH 8.0) upon addition of 5,5'-Zn(Dpa)-Bpy from 0 to 60 μM. CD titration curve (222 nm) was analyzed with the nonlinear least-square curve-fitting method to evaluate apparent binding constant (K_a, M^{-1}).

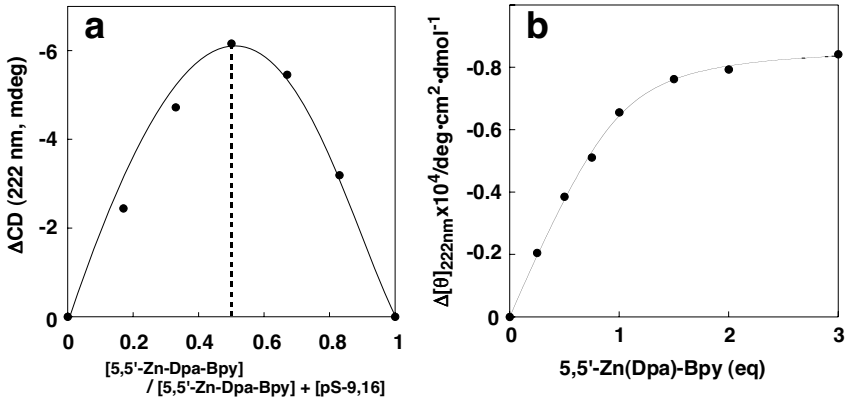

Figure S1. (a) Job plot of pS-9,16 and 5,5'-Zn(Dpa)-Bpy examined by CD measurement (total concentration is 100 μM). (b) CD titration curve (222 nm) of pS-9,16 (20 μM) with 5,5'-Zn(Dpa)-Bpy.

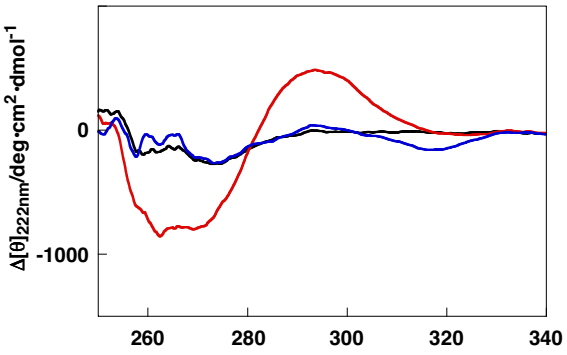

Figure S2. The induced CD due to the bipyridyl moiety of 5,5'-Zn(Dpa)-Bpy (20 μM) upon the addition of 1 eq. of IRK-2P: IRK-2P (—), with 5,5'-Zn(Dpa)-Bpy (—), and 5-Zn(Dpa)-Bpy (—).

Fluorescent Titration

Fluorescent spectra were recorded on a Perkin-Elmer LS55 spectrometer. The titration experiments of 5,5'-Zn(Dpa)-Bpy (5 μM, λ_{ex} = 297 nm) with a series of pS-type peptides were conducted in 10 mM borate buffer (pH 8.0) at 20±1 °C by measuring the changes in fluorescence emission that occurred upon addition of the peptide from 0 to 15 μM. The titration with the phosphorylated peptides **IRK-2P**, **-1P**, and **-0P** were conducted in 50 mM NaCl, 10 mM borate buffer (pH 8.0). Fluorescent titration curves (λ_{em} = 389 nm) were analyzed with the nonlinear least-square curve-fitting method to evaluate apparent binding constant (K_a, M^{-1}).

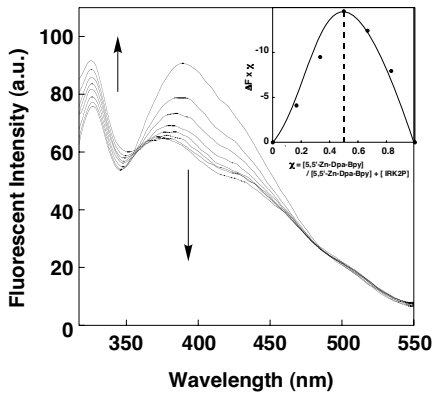

Figure S3. Fluorescence spectral change of 5,5'-Zn(Dpa)-Bpy (5 μM) upon the addition of IRK-2P (0~3 eq). Inset: Job plot of 5,5'-Zn(Dpa)-Bpy and IRK-2P examined by fluorescent measurement (total concentration is 10 μM).

^{31}P NMR studies

^{31}P NMR spectra were recorded on a Brucker DRX-600 (243 MHz) at 25 ±1 °C. Samples were prepared using a concentration of 0.5 mM of pS-9,16 in 50 mM pH 8.0 borate buffer / D_2O (90 : 10) in the absence and presence (1 eq.) of 5,5'-Zn(Dpa)-Bpy. 0.1 mM of H_3PO_4 in H_2O was used as an external reference for ^{31}P NMR.

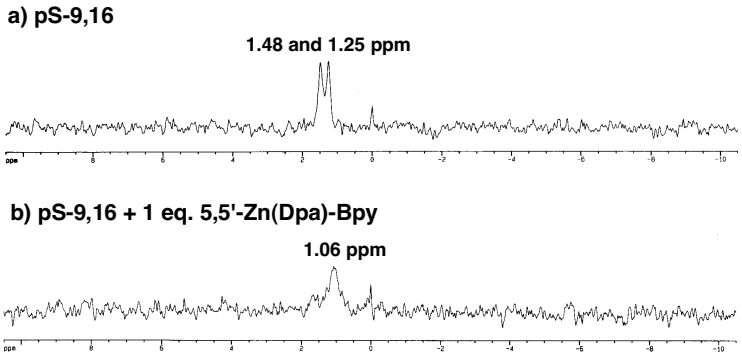

Figure S4. ^{31}P-NMR spectra of pS-9,16 in the absence and in the presence (1 eq.) of 5,5'-Zn(Dpa)-Bpy.

Reference

(1) Greenwald, M.; Wessely, D.; Katz, E.; Willner, I.; Cohen, Y. *J. Org. Chem.*, **2000**, *65*, 1050.