

Supporting information for

Single molecular multi analyte sensor 'Jewel Pendant Ligand'

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Experimental sections:

Synthesis of JPL-1

General.

All starting materials and reagents were purchased from Tokyo Kasei Kogyo (Tokyo, Japan), Aldrich Chemical (Milwaukee, WI, USA) or Wako Pure Chemical (Tokyo, Japan). THF was distilled over sodium benzophenone ketyl, and CH_2Cl_2 was distilled over CaCl_2 . All other solvents, purchased from Aldrich or Kanto (Tokyo, Japan), were dry grade and used without further purification. ^1H -NMR spectra were recorded on a JEOL JNM-LA300 spectrometer or a Varian MVX-300 in CDCl_3 (Merck). Coupling constants are given in Hz and all chemical shifts are relative to an internal standard of tetramethylsilane. The ESI-MS spectra were recorded on a PerSeptive Biosystems MarinerTM spectrometer with MeOH as the eluent. All air- or moisture-sensitive reactions were carried out in dried glassware under an argon atmosphere with magnetic stirring.

Purification of the final compound was performed by a recycle HPLC LC-918 (Japan Analytical Industry) equipped with two GPC columns JAIGEL-1H, 2H (Japan Analytical Industry) eluted with CHCl_3 .

Synthesis of JPL-1 (Scheme1)

{Phenyl-[2-(tetrahydro-pyran-2-yloxy)-ethyl]-amino}-ethanol (2). 2-[(2-Hydroxy-ethyl)-phenyl-amino]-ethanol (**1**) (13.21 g, 72.9mmol, 1.1eq.) and p-toluenesulfonic acid monohydrate (20.0 g, 116 mmol, 1.7 eq.) were dissolved in 500 mL of CH_2Cl_2 . Then 3,4-dihydro-2H-pyran (5.56 g, 67.7 mmol, 1.0 eq) was added dropwise. The resulting solution was stirred for 12 hours at room temperature. The reaction mixture was washed with saturated aqueous NaHCO_3 solution, water, and brine, dried over Na_2SO_4 , filtered and concentrated by evaporation of the solvent. The crude product was purified by silica gel column chromatography (CHCl_3 : EtOAc 5/1 v/v) to give **2** as colorless oil (9.19g, 34.6 mmol, 51.1%): ^1H NMR (300MHz, CDCl_3 , TMS, r.t.) δ 1.50-1.80 (m, 6H), 3.40-4.00 (m, 10H), 4.58 (m, 1H), 6.65-6.75 (m, 3H), 7.15-7.25 (m, 2H)

Toluene-4-sulfonic acid 2-{phenyl-[2-(tetrahydro-pyran-2-yloxy)-ethyl]-amino}

-ethyl ester (3). Compound **2** (9.19 g, 34.6 mmol, 1 eq.) was dissolved in 100 mL of CH_2Cl_2 , 10mL of pyridine was added and cooled in ice bath. Then toluenesulfonyl chloride (10.94g, 57.4mmol, 1.7eq.) was added dropwise and the resulting mixture was stirred for 12 hours at room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with 0.1M HCl, water and brine, filtered and concentrated by evaporation of the solvent. The crude product was purified by silica gel column chromatography (hexane: EtOAc 5/1 v/v) to give **3** as yellowish oil (6.063 g, 14.5 mmol, 41.8%): ^1H NMR (300MHz, CDCl_3 , TMS, r.t.) δ 1.45-1.80 (m, 6H), 2.42 (s, 3H), 3.40-3.95 (m, 10H), 4.52 (m, 1H), 6.55-6.70 (m, 3H), 7.16 (t, 7.2 Hz, 2H), 7.28 (d, 8.7 Hz, 2H), 7.74 (d, 8.7 Hz, 2H)

[2-(4-Bromo-phenylsulfanyl)-ethyl]-phenyl-[2-(tetrahydro-pyran-2-yloxy)-ethyl]-amine (4).

4-Bromo-benzenethiol (6.05 g, 32.0 mmol, 2.2 eq.) was dissolved in 200 mL of acetone and K_2CO_3 (10.0 g, 72.4 mmol, 5.0 eq) was added. The resulting solution was refluxed for an hour and cooled to room temperature. Then a solution of **3** (6.06 g, 14.4 mmol, 1.0 eq.) in 50 mL of acetone was added dropwise and refluxed for 12 hours. After evaporation of the reaction solvent, the residue was diluted with CHCl_3 , washed with water and brine, filtered and concentrated by evaporation of the solvent. The crude product was purified by silica gel column chromatography (hexane: EtOAc 20/1 v/v) to give **4** as colorless oil (5.78g, 13.2mmol mmol, 91.7%: ^1H NMR (300MHz, CDCl_3 , TMS, r.t.) δ 1.48-1.78 (m, 6H), 3.31(t, 7.8 Hz, 2H), 3.45-3.60 (m, 6H), 3.75-3.95 (m, 2H), 4.55 (m, 1H), 6.60 (d, 8.0 Hz, 2H), 6.69 (m, 1H), 7.19 (m, 2H), 7.27 (d, 8.8 Hz, 2H), 7.42 (d, 8.3 Hz, 2H)

(2-{4-[2-(4-Nitro-phenyl)-vinyl]-phenylsulfanyl}-ethyl)-phenyl-[2-(tetrahydro-

pyran-2-yloxy)-ethyl]-amine (5). Compound **4** (3.91 g, 8.96 mmol, 1 eq.), 1-nitro-4-vinyl-benzene (2.10 g, 14.1 mmol, 1.6 eq.), tri-o-tolylphosphine (113 mg, 0.37mmol, 0.04 eq.), and palladium (II) acetate (90.3 mg, 0.40 mmol, 0.04 eq.) were dissolved in 20 mL of triethylamine in a pressure tube and heated for 2 hours at 120 $^\circ\text{C}$. The reaction mixture was cooled to room temperature and filtered. The solution was diluted with CHCl_3 and washed with 0.1M HCl and water, filtered and concentrated by evaporation of the solvent. The crude product was purified by silica gel column chromatography (CHCl_3 : EtOAc 2/1 v/v) and then purified by recrystallization from hexane to give **5** as an yellow solid (3.04 g, 6.02 mmol, 67.2%): ^1H NMR (300MHz, CDCl_3 , TMS, r.t.) δ 1.45-1.80 (m, 6H), 3.17 (t, 7.8 Hz, 2H), 3.40-3.65 (m, 6H), 3.75-

3.95 (m, 2H), 4.56 (m, 1H), 6.60-6.75 (m, 3H), 7.10-7.30 (m, 4H), 7.37 (d, 8.7 Hz, 2H), 7.48 (d, 8.7 Hz, 2H), 7.63 (d, 8.7 Hz, 2H), 8.22 (d, 8.7 Hz, 2H)

(2-{4-[2-(4-Nitro-phenyl)-vinyl]-phenylsulfanyl}-ethyl)-phenyl-[2-(tetrahydro-pyran-2-yloxy)-ethyl]-amine (6). Compound **5** (3.04 g, 6.02 mmol, 1 eq.) and p-toluenesulfonic acid monohydrate (2.95 g, 7.02 mmol, 1.2 eq) were dissolved in 50 mL of CHCl₃/MeOH (1/1 v/v) and stirred for 68 hours at room temperature. The reaction mixture was diluted with CHCl₃ and washed with water and brine, dried over Na₂SO₄, filtered and concentrated by evaporation of the solvent.

The crude product was purified by crystallization from CH₂Cl₂/hexane to give **6** as a yellow solid (2.22 g, 8.11 mmol, 87.1%). ¹H NMR (300MHz, CDCl₃, TMS, r.t.) δ 3.17 (t, 7.1 Hz, 2H), 3.49 (t, 5.6 Hz, 2H), 3.61 (t, 7.4 Hz, 2H), 3.77 (dt, 5.3 Hz, 2H), 6.70-6.80 (m, 3H), 7.12 (d, 16.2 Hz, 1H), 7.20-7.30 (m, 3H), 7.37 (d, 8.4 Hz, 2H), 7.48 (d, 8.7 Hz, 2H), 7.63 (d, 8.7 Hz, 2H), 8.22 (d, 8.7 Hz, 2H)

(2-Methanesulfonyl-ethyl)-(2-{4-[2-(4-nitro-phenyl)-vinyl]-phenylsulfanyl}-ethyl)-phenyl-amine (7). Compound **6** (702 mg, 1.67 mmol, 1 eq.) was dissolved in 50 mL of pyridine and cooled in an ice-bath. Then methanesulfonyl chloride (428 mg, 3.67 mmol, 2 eq.) was added dropwise to the reaction solution and the mixture was stirred for 2 hours. The reaction solution was diluted with CHCl₃ and washed with concentrated HCl, 0.1M HCl, water and brine, dried over Na₂SO₄, filtered and concentrated by evaporation of the solvent to give **7** as an yellow solid (763mg, 1.53 mmol, 91.6%): ¹H NMR (300MHz, CDCl₃, TMS, r.t.) δ 2.93 (s, 1H), 3.15 (t, 7.4 Hz, 2H), 3.62 (t, 7.2 Hz, 2H), 3.71 (t, 5.9 Hz, 2H), 4.33 (t, 6.0 Hz, 2H), 6.65 (d, 8.7 Hz, 2H), 6.77 (m, 1H), 7.05-7.30 (m, 4H), 7.37 (d, 8.1 Hz, 2H), 7.48 (d, 8.1 Hz, 2H), 7.62 (d, 8.7 Hz), 8.22 (d, 8.4 Hz, 2H)

(2-{4-[2-(2,4-Dinitro-phenyl)-vinyl]-phenoxy}-ethyl)-(2-{4-[2-(4-nitro-phenyl)-vinyl]-phenylsulfanyl}-ethyl)-(4-nitroso-phenyl)-amine (8). 4-Hydroxybenzaldehyde (91 mg, 0.745 mmol, 1.2 eq.) was dissolved in 50 mL of acetone, K₂CO₃ (200 mg, 1.44 mmol, 2.4 eq) was added and refluxed for one hour. The mixture was then cooled to room temperature and a solution of **7** (384 mg, 0.77 mmol, 1 eq.) in 30 mL of acetone was added dropwise before refluxing for 24 hours. The reaction mixture was cooled to room temperature, excess solvent evaporated, and the crude compound was purified by silica gel column chromatography (CHCl₃: hexane 2/1 v/v) to give **8** as colorless oil (263mg, 0.501mmol, 67.2%). ¹H NMR (300MHz, CDCl₃, TMS, r.t.) δ 3.19 (t, 7.8 Hz, 2H), 3.69 (t, 7.1 Hz, 2H), 3.81 (t, 5.6 Hz, 2H), 4.20 (t, 5.6 Hz, 2H), 6.67 (d, 8.0 Hz, 2H), 6.75 (t, 7.1 Hz, 1H), 6.83 (d, 8.5, 2H), 6.94

(d, 8.8 Hz, 2H), 7.12 (d, 16.3 Hz, 1H), 7.20-7.30 (m, 3H), 7.38 (d, 8.3 Hz, 2H), 7.47 (d, 8.3 Hz, 2H), 7.63 (d, 8.8 Hz, 2H), 7.80 (d, 8.8 Hz, 2H), 8.23 (d, 8.8 Hz, 2H), 9.87 (s, 1H)

2-[2-(4-{2-[(2-{4-[2-(4-Nitro-phenyl)-vinyl]-phenylsulfanyl}-ethyl)-phenyl-amino]-ethoxy}-benzylidene)-3-oxo-indan-1-ylidene]-malononitrile (9). Compound **8** (131.2 mg, 0.250 mmol, 1eq.) was dissolved in 5 mL of ethanol/benzene (1/1 v/v) 2-(3-dicyanomethylene-indan-1-ylidene)-malononitrile (146 mg, 0.75 mmol, 3eq.) added, and the reaction mixture stirred for 14 hours at room temperature. The reaction solution was evaporated to dryness and purified by preparative TLC (CHCl₃) to give **9** (110 mg, 0.157 mmol, 62.8%) as a red solid. ¹H NMR (300MHz, CDCl₃, TMS, r.t.) δ 3.19 (t, 6.9 Hz, 2H), 3.70 (t, 6.9 Hz, 2H), 3.81 (t, 5.7 Hz, 2H), 4.23 (t, 5.7 Hz, 2H), 6.70 (d, 7.8 Hz, 2H), 6.76 (t, 7.5 Hz, 1H), 6.91 (d, 8.7 Hz, 2H), 7.09 (d, 16.5 Hz, 1H), 7.15-7.30 (m, 3H), 7.37 (d, 8.1 Hz, 2H), 7.47 (d, 8.7 Hz, 2H), 7.62 (d, 8.7 Hz, 2H), 7.70-7.85 (m, 2H), 7.89 (d, 6.9 Hz, 1H), 8.16 (d, 8.7 Hz, 2H), 8.26 (d, 9.0 Hz, 2H), 8.50 (s, 1H), 8.64 (d, 6.9 Hz, 1H)

2-[2-(4-{2-[[4-(1,3-Bis-dicyanomethylene-indan-2-ylideneamino)-phenyl]-(2-{4-[2-(4-nitro-phenyl)-vinyl]-phenylsulfanyl}-ethyl)-amino]-ethoxy}-benzylidene)-3-oxo-indan-1-ylidene]-malononitrile (JPL-1). Compound **9** (18 mg, 0.0257 mmol, 1eq.) was dissolved in 5 mL of THF and cooled in an ice-bath. Then to the solution, 75 mL of HCl was added and a solution of NaNO₂ (1.8 mg, 4 eq.) dissolved in the smallest amount of water possible was added and stirred for 3 hours at room temperature. The reaction mixture was cooled in an ice-bath and washed with water, dissolved in CHCl₃ and dried over Na₂SO₄, filtered and concentrated by evaporation of the solvent. The crude product was purified by preparative TLC and then using recycle HPLC (GPC, CHCl₃) to give JPL-1 as a green solid (1.9 mg, 1.99 mmol, 7.7 %). ¹H NMR (300MHz, CDCl₃, TMS, r.t.) δ 3.24 (t, 6.6 Hz, 2H), 3.83 (t, 6.8 Hz, 2H), 3.92 (t, 5.1 Hz, 2H), 4.29 (t, 5.1 Hz, 2H), 6.92(d, 9.0 Hz, 2H, Ar-H), 7.13 (d, 16.1 Hz, 1H), 7.19-7.26 (m, 1H), 7.32(d, 8.3 Hz, 2H), 7.40 (d, 9.0 Hz, 2H), 7.50 (d, 8.5 Hz, 2H), 7.61 (d, 8.8 Hz, 2H), 7.75-7.85 (m, 4H), 7.89(d, 6.9 Hz, 1H), 8.18 (d, 8.8 Hz, 2H), 8.25 (d, 9.0 Hz, 2H), 8.51 (s, 1H), 8.57-8.60 (m, 2H), 8.67 (d, 7.1 Hz, 1H). ESI-MS *m/z* 954.2(MH⁺). The ¹H NMR spectrum of JPL-1 is shown as Fig. S2.

UV-Vis Spectrometry

The dyes were dissolved in MeCN (Spectroscopic grade, KANTO, Japan) to the final concentrations of 10 μM. All metal nitrate salts were of highest purity available, purchased from KANTO. Absorption

spectra were recorded on a Hitachi U-2001 double-beam spectrophotometer (Hitachi, Tokyo, Japan) or a JASCO V-5600 UV/Vis Spectrophotometer (JASCO, Tokyo, Japan) using a 1 cm standard quartz cell.

For colored ion solutions, (Cu^{II} , Co^{III}), ion-dependent absorbance was subtracted.

Determination of apparent dissociation constants for metal ions

To determine the association constant (K) for JPL-1 with the metal ions, mathematical fitting to the equilibrium equation was used,

$$K = [\text{Complex}] / [\text{JPL-1}] [\text{M}] \text{ (equation 1)}$$

where, M stands for the individual metal ion. (Fig.S3)

Artificial neural network analysis

The back-propagation neural networks having 60 input layers (absorbance measured at 10 nm wavelength steps between 300 and 900 nm), 5 hidden layers and 2 output layers (concentrations of Fe^{III} and Cu^{II}) were developed¹¹ and used. The applied basis functions are sigmoidal. Learning was continued 1000000 times.

Measured data points were 25 combinations of Fe^{III} and Cu^{II} (3.13×10^{-6} , 6.25×10^{-6} , 1.25×10^{-5} , 2.50×10^{-5} , 5.00×10^{-5} M.) One sample was alternately used as test sample, while the remaining 24 samples were used to train the network (leave-one-out method¹⁴). The differences between experimental and ANN-estimated values were compared.

Result of ANN Analysis.

The detailed data of the simultaneous determination of Fe^{III} and Cu^{II} by BP-ANN is presented in Table S2.

Scheme S1.

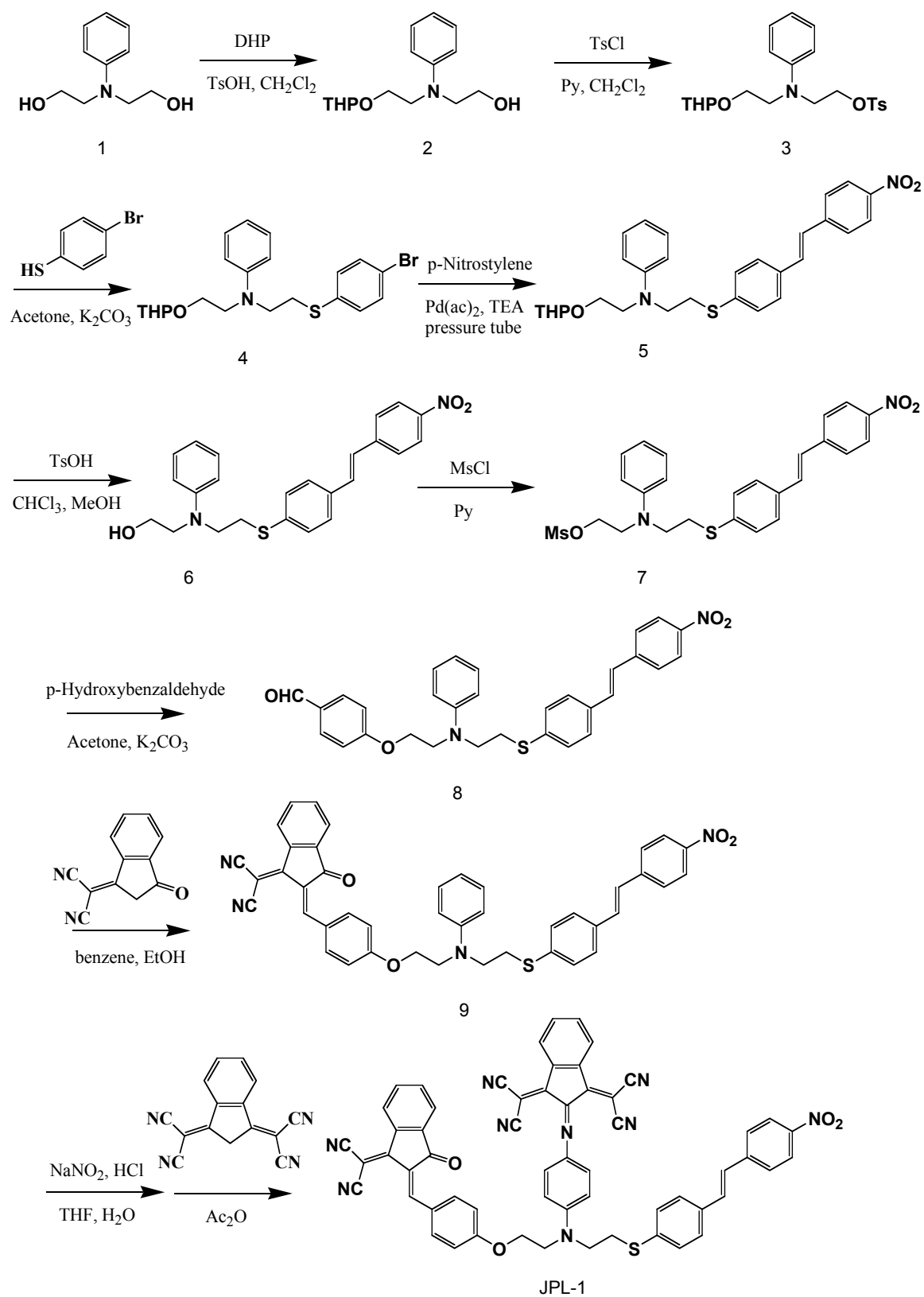


Table S1. Binding constants (M^{-1}) of JPL-1 with the metal cations (Cu^{II} , Fe^{III} , Al^{III} , Cr^{III})

Chemical species	Binding Constant
JPL-1(Cu)	7.2×10^5
JPL-1(Fe)	1.5×10^5
JPL-1(Al)	7.6×10^5
JPL-1(Cr)	1.6×10^6

*binding constant for Pb^{II} was not determined due to the low solubility of $Pb(NO_3)_2$.

Table S2. Simultaneous determination of Fe^{III} and Cu^{II} in MeCN solution by ANNs.

Fe(exp.) [10 ⁻⁵ M]	Cu(exp.) [10 ⁻⁵ M]	Fe(ANN) [10 ⁻⁵ M]	Cu(ANN) [10 ⁻⁵ M]	Rel.error(Fe) (%)	Rel.error(Cu) (%)	Abs.error(Fe) [10 ⁻⁵ M]	Abs.error(Cu) [10 ⁻⁵ M]
5	5	3.69	4.08	26.2	18.3	1.31	0.92
5	2.5	3.24	4.18	35.3	-67.0	1.76	1.68
5	1.25	5.71	1.01	-14.1	19.0	0.71	0.24
5	0.625	4.58	0.62	8.3	0.4	0.42	0.00
5	0.313	4.17	0.63	16.6	-101.3	0.83	0.32
2.5	5	2.22	4.50	11.1	9.9	0.28	0.50
2.5	2.5	4.40	2.80	-76.0	-12.0	1.90	0.30
2.5	1.25	2.00	1.45	19.9	-16.3	0.50	0.20
2.5	0.625	0.67	0.31	73.4	50.9	1.83	0.32
2.5	0.313	3.02	0.33	-20.7	-5.0	0.52	0.02
1.25	5	1.81	4.24	-44.6	15.2	0.56	0.76
1.25	2.5	1.11	3.19	11.0	-27.6	0.14	0.69
1.25	1.25	1.71	0.73	-37.1	41.8	0.46	0.52
1.25	0.625	1.22	0.31	2.1	51.2	0.03	0.32
1.25	0.313	1.44	0.39	-14.9	-24.7	0.19	0.08
0.625	5	0.58	3.50	7.0	30.1	0.04	1.50
0.625	2.5	0.54	3.08	13.1	-23.3	0.08	0.58
0.625	1.25	0.26	1.95	58.1	-55.7	0.36	0.70
0.625	0.625	0.50	0.50	20.2	20.2	0.13	0.13
0.625	0.313	0.45	0.33	27.4	-6.3	0.17	0.02
0.313	5	0.51	4.61	-63.2	7.9	0.20	0.39
0.313	2.5	0.35	2.34	-13.5	6.4	0.04	0.16
0.313	1.25	0.27	0.91	14.7	27.3	0.05	0.34
0.313	0.625	0.28	0.59	12.0	5.4	0.04	0.03
0.313	0.313	0.37	0.32	-17.0	-1.7	0.05	0.01

R (Fe) R (Cu) CV (Fe) (%) CV (Cu)(%) Avr.error (Fe) Avr.error (Cu)

0.90

0.94

34.08

35.60

0.50

0.43

Concentrations are given in units of 10^{-5} M. The correlation coefficient R was found to be 0.90 for Fe^{III} and 0.94 for Cu^{II} . The variation coefficient CV was 34 % for Fe^{III} and 36 % for Cu^{II} with an average error of 5.0 μM for Fe^{III} and 4.3 μM for Cu^{II} .

Table S3. Comparison of the response of JPL-1 at different wavelengths (380, 460, 710nm) to the 10 equivalents of various metals(Pb^{II} , Fe^{III} , Al^{III} , Cu^{II} , Cr^{III}).

	Free	Pb	Fe	Al	Cu	Cr
N-dye(710)	1	0.43	0.15	0.31	0.08	0.48
O-dye(460)	1	0.90	1.05	1.00	0.89	0.99
S-dye(380)	1	0.96	0.90	0.94	0.72	0.96

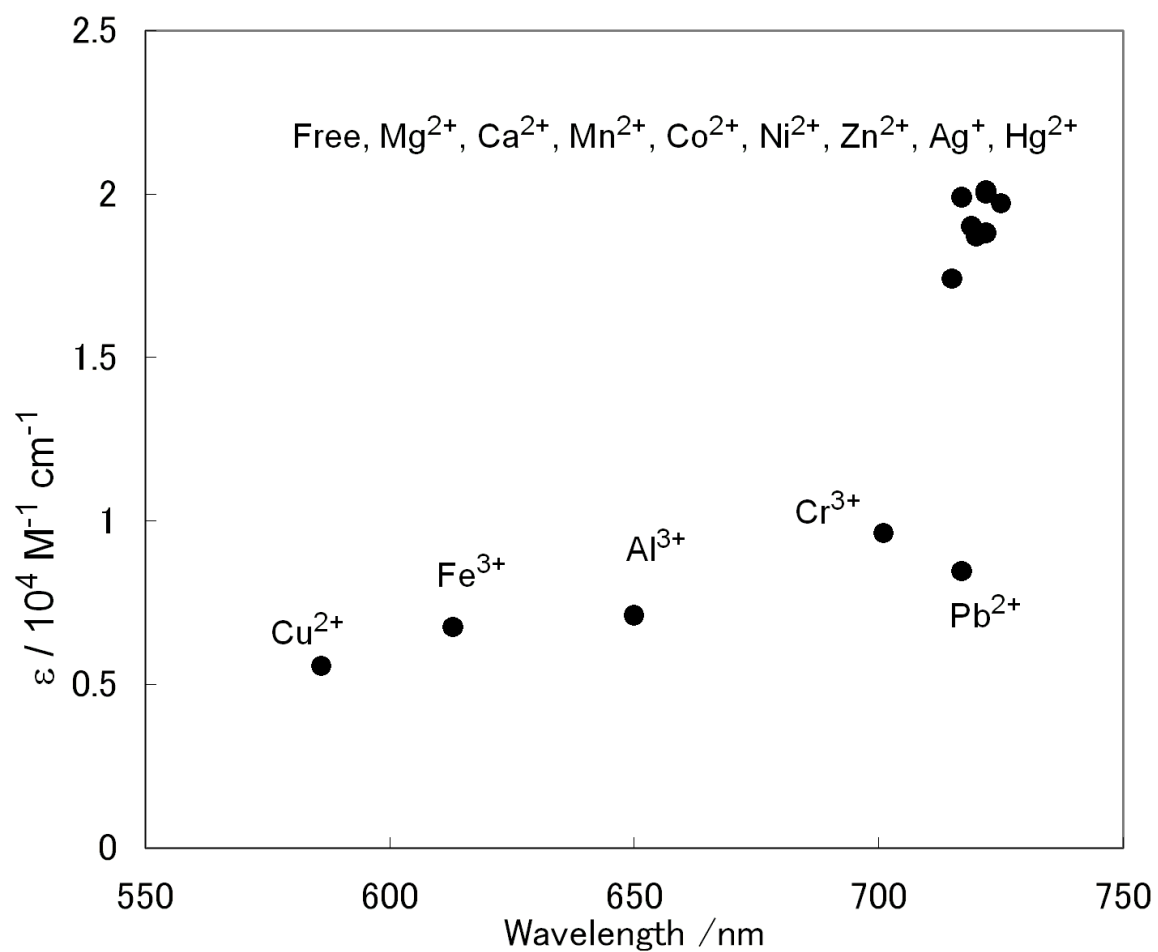


Fig.S1 Long wavelength absorbance (the absorbance peak of the N-dye) of JPL-1 (10 μM) in the presence of 10 μM of various metal cations.

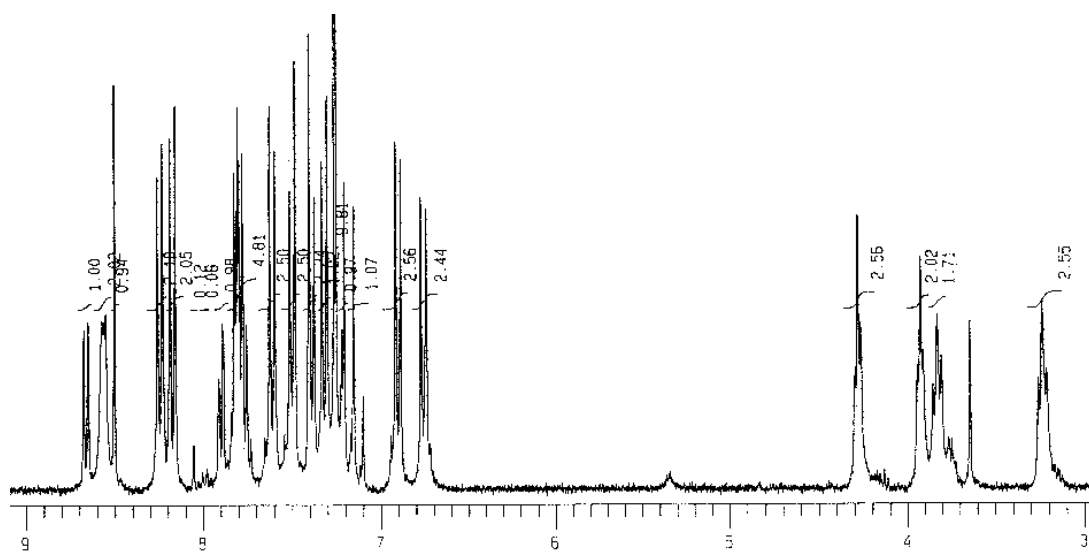


Fig. S2 ^1H NMR-chart of JPL-1 in CDCl_3 .

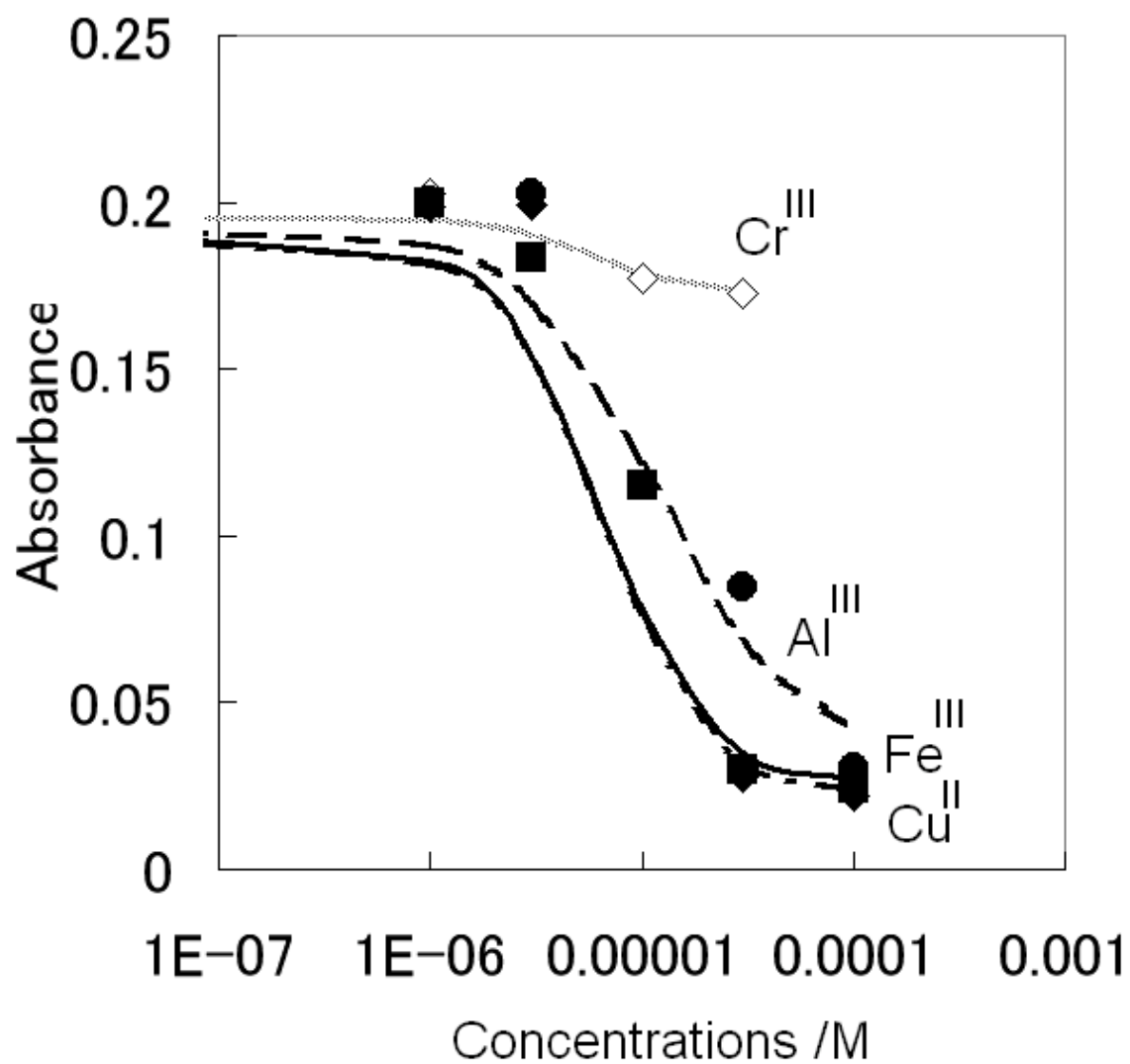


Fig. S3 Absorbance at 710nm of JPL-1 (10 μ M) in the presence of different concentrations of various metal cations (\bullet Fe^{III}, \blacksquare Cu^{II}, \blacklozenge Al^{III}, \diamond Cr^{III}) in MeCN and the theoretical fitting curves.