Supplementary Information

for

Design of a hyperpolarized ^{15}N NMR probe that induces a large chemical-shift change upon binding of calcium ion

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1. Figure

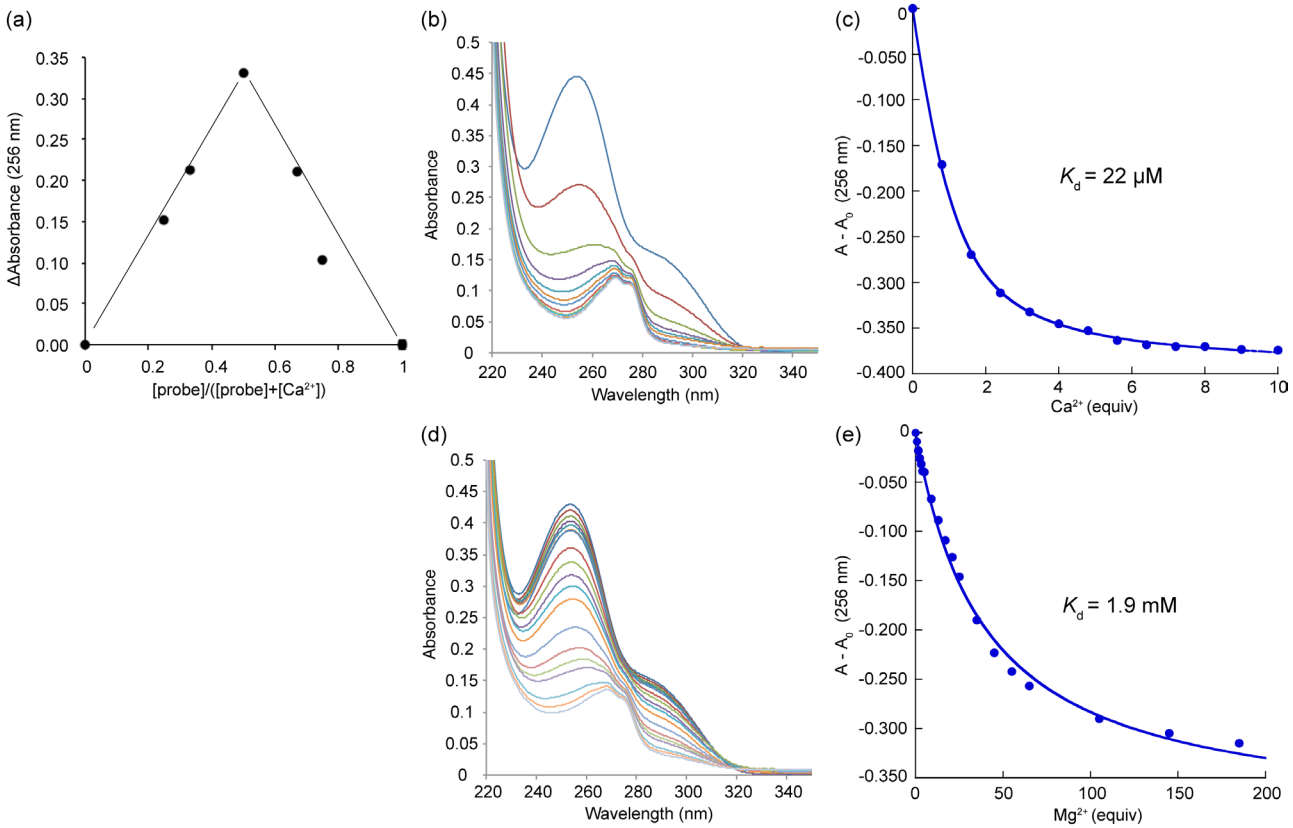

Figure S1. (a) Job plot of the complex formation between ^{15}N labelled *o*-aminophenol-*N,N,O*-triacetic acid (^{15}N APTRA) and Ca^{2+} ([^{15}N APTRA] + [Ca^{2+}] = 10 mM, 256 nm, room temperature). Ultraviolet (UV) absorption spectra of ^{15}N APTRA (50 μM) upon addition of (b) Ca^{2+} (0–10 equiv) or (d) Mg^{2+} (0–200 equiv), measured at 25 °C. UV titration plot (λ = 256 nm) of ^{15}N APTRA (50 μM) with (c) Ca^{2+} (0–10 equiv) or (e) Mg^{2+} (0–200 equiv) and titration curve obtained by the non-linear least-squares curve-fitting method (1:1 binding), giving dissociation constants K_d = 22 μM for Ca^{2+}, 1.9 mM for Mg^{2+}. Figures S1 (a) and (b, c, d, e) were recorded using NanoDrop ND-1000 and a JASCO V-630, respectively. All solutions were prepared using 20 mM HEPES buffer (pH 7.4), 150 mM NaCl.

2. Methods

2-1. Synthesis

General information on synthesis

Reagents and solvents were purchased from standard suppliers and used without further purification. Gel permeation chromatography (GPC) was performed on a JAIGEL GS310 using a JAI Recycling Preparative HPLC LC-9201 (Japan Analytical Industry Co., Ltd.). NMR spectra were measured using a Bruker Avance III spectrometer (400 MHz for ^{1}H). Chloroform-d_1 (7.26 ppm), methanol-d_4 (3.31 ppm) and methanol-d_4 in D_2O (3.31 ppm) were used as the internal standards for ^{1}H NMR. Chloroform-d_1 (77.2 ppm), methanol-d_4 (49.0 ppm) and methanol-d_4 in D_2O (49.0 ppm) were used as the internal standards for ^{13}C NMR. Ammonium chloride-^{15}N (–352.9 ppm) was used as the external standard for ^{15}N NMR. Mass spectra were measured using a JEOL JMS-HX110A (FAB) and a Bruker micrOTOF-QIII (ESI).

Synthesis of 2-nitrophenol-^{15}N

Nitric acid-^{15}N 40% wt/wt (3.6 mL, 32 mmol) was added dropwise to phenol (3.60 g, 38.3 mmol) in acetic acid (10 mL) at –5 °C and the mixture was stirred at room temperature. After 10 h, the mixture was poured into cold water and extracted with chloroform five times. The combined organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The resulting residue was purified using silica gel chromatography (eluent: ethyl acetate:hexane = 1:30 to 4:30) to give 2-nitrophenol-^{15}N as a yellow solid (942 mg, 21%): ^{1}H NMR ($CDCl_3$, 400 MHz), δ = 6.97–7.01 (m, 1H), 7.14–7.16 (m, 1H), 7.56–7.60 (m, 1H), 8.10 (ddd, J = 8.5, 1.8, 1.8 Hz, 1H), 10.57 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ = 120.2, 120.4, 125.3, 133.9 (d, J = 14 Hz), 137.7, 155.3; ^{15}N NMR ($CDCl_3$, 40 MHz), δ = –3.7 ppm.

Synthesis of 2-aminophenol-^{15}N

10 wt% palladium on activated carbon (20 mg) was added to 2-nitrophenol-^{15}N (640 mg, 4.57 mmol) in ethanol (10 mL) and the mixture was stirred under hydrogen at room temperature. After 12 h, the solution

was filtered through Celite, and the filtrate was evaporated to give 2-aminophenol-^{15}N as a brown solid (490 mg, 97%): ^{1}H NMR (CD_3OD, 400 MHz), δ = 6.56–6.66 (m, 2H), 6.68–6.70 (m, 1H), 6.74 (ddd, J = 7.6, 1.8, 1.6 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz), δ =115.6, 117.5, 120.2, 121.0, 136.0 (d, J = 9.5 Hz), 146.6; ^{15}N NMR (CD_3OD, 40 MHz), δ = −332.8 ppm.

Synthesis of Boc-protected ^{15}N APTRA

2-Aminophenol (490 mg, 4.45 mmol) and NaI (341 mg, 2.27 mmol) were dissolved in acetonitrile (10 mL). Diisopropylethylamine (2.4 mL, 14 mmol) and *tert*-butyl bromoacetate (1.3 mL, 8.8 mmol) were added and the mixture was refluxed under nitrogen at 80 °C. After 14 h, diisopropylethylamine (800 μL, 4.6 mmol) and *tert*-butyl bromoacetate (1.3 mL, 8.8 mmol) were added and the mixture was refluxed for an additional 12 h. The mixture was cooled to room temperature. Ethyl acetate was added. The precipitate was removed by filtration. The filtrate was washed with brine three times and water. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The resulting residue was purified using silica gel chromatography (eluent: ethyl acetate:hexane:dichloromethane = 3:100:200) to give Boc-protected ^{15}N APTRA as a yellow oil (825 mg, 41%): ^{1}H NMR ($CDCl_3$, 400 MHz), δ = 1.42 (s, 18H), 1.46 (s, 9H), 4.09 (s, 4H), 4.55 (s, 2H), 6.76–6.79 (m, 1H), 6.84–6.92 (m, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz), δ = 28.1, 28.2, 54.7 (d, J = 10 Hz), 66.7, 81.1, 82.1, 114.4, 119.7, 122.0, 122.2, 139.7 (d, J = 14 Hz), 149.9, 168.4, 170.7; ^{15}N NMR ($CDCl_3$, 40 MHz), δ = –333.4 ppm; HRMS (FAB): m/z calc. for $C_{24}H_{37}{}^{15}NO_7$ $[M]^+$ = 452.2540, found = 452.2544.

Synthesis of ^{15}N APTRA

Boc-protected ^{15}N APTRA (629 mg, 1.39 mmol) was dissolved in 4 M HCl/AcOEt (5 mL). The solution was stirred at room temperature. After 3 h, the solution was evaporated *in vacuo*. The resulting residue was dissolved in 1.1 M NaOH(aq) and purified using gel permeation chromatography (eluent: H_2O) to give ^{15}N APTRA as a white powder (328 mg, 0.80 mmol, 58%). ^{1}H NMR (D_2O, 400 MHz), δ = 3.74 (s, 4H), 4.48 (s, 2H), 6.85–6.87 (m, 1H), 6.93–7.01 (m, 3H); ^{13}C NMR (D_2O, 100 MHz), 58.2 (d, J = 8.0 Hz), 68.0, 113.6,

119.9, 122.4, 123.6, 140.6 (d, J = 12 Hz), 151.1, 177.9, 180.2; ^{15}N NMR (D_2O, 40 MHz), δ = –328.0 ppm; HRMS (ESI): m/z calc. for $C_{12}H_{10}{}^{15}NNa_4O_7$ $[M–3H^+ + 4Na^+]^+$ = 373.0013, found = 373.0021.

2-2. ^{1}H and ^{15}N NMR measurements (Fig. 3)

^{1}H and ^{15}N NMR spectra were measured on a JEOL JNM-ECS 400 spectrometer (400 MHz for ^{1}H NMR and 40 MHz for ^{15}N NMR). Chemical shifts are reported in ppm relative to methanol (δ = 3.31 ppm, external standard) for ^{1}H and $^{15}NH_4Cl$ (δ = –352.9 ppm, external standard) for ^{15}N.

2-3. Hyperpolarization and ^{15}N DNP-NMR measurements

Hyperpolarization was performed according to the method reported previously,[1] with slight modifications (1.5 h and 1 h polarization for Fig. 4a, b and Fig. 4d, respectively). After polarization, samples were dissolved in 20 mM HEPES buffer (pH = 7.4) containing 150 mM NaCl heated to 10 bar. The DNP-NMR measurements were performed using a Japan Redox JXI-400Z spectrometer (9.4 T). $^{15}NH_4Cl$ (–352.9 ppm) was used as an external standard for ^{15}N NMR. The DNP-NMR spectra were obtained using pulse angles of 25° (Fig. 4a, b; *TR* = 4 s), 90° (Fig. 4d).

2-4. Calculation of T_1 value (Fig. 4c)

Tris{8-carboxyl-2,2,6,6-tetra[2-(1-hydroxyethyl)]-benzo(1,2-d:4,5-d′)bis(1,3)dithiole-4-yl}methyl sodium salt (OX63 radical, GE Healthcare) and the ^{15}N APTRA were dissolved in a 1:1 solution of D_2O:glycerol-d_8 (final concentration of OX63 = 15 mM). The sample was hyperpolarized at 1.4 K by irradiation at 94 GHz and 100 mW for 2 h, using a HyperSense (Oxford Instruments). The DNP-NMR measurements were performed using a Japan Redox JXI-400Z spectrometer (9.4 T, 5° pulse angle, *TR* = 2 s). The T_1 values were obtained by fitting the hyperpolarized signal decay to eq. 1, where M_0 is the original magnetization, *TR* is the repetition time and θ is the pulse angle.[2] Conditions: 3.3 mM ^{15}N APTRA, 20 mM HEPES (pH = 7.4), 0 or 6.6 mM $CaCl_2$.

$$M_z(t) = M_0 \sin\theta \, (\cos\theta)^{t/TR} \exp(-t/T_1) \qquad (1)$$

3. References

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