Supporting Information File

Adaptable DNA-Interactive Probe Proficient at Selective Turn-On Sensing for Al^{3+}: Insight from the Crystal Structure, Photophysical Studies, and Molecular Logic Gate

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Table S1. Crystal data and structure refinement for H_{2}NPV.

| Parameters          | H_{2}NPV          |
|---------------------|-------------------|
| Formula             | C_{19}H_{16}N_{2}O_{4} |
| Formula Weight      | 336.34            |
| Crystal System      | Orthorhombic      |
| Space group         | P n a 21          |
| a, b, c [Å]         | 6.3075(3) 19.6917(11) 12.4837(7) |
| α, β, γ [°]         | 90                |
| V [Å^{3}]           | 1550.54(14)       |
| Z                   | 4                 |
| D(calc) [g/cm^{3}]  | 1.441             |
| μ(MoKα) [/mm ]      | 0.103             |
| F(000)              | 704               |
| Crystal Size [mm]   | 0.21 x 0.31 x 0.35 |
| Temperature (K)     | 100               |
| Radiation [λ, Å]    | 0.71073           |
| Theta Min-Max [°]   | 3.4, 32.6         |
| Dataset             | -8: 9 ; -25: 29 ; -18: 15 |
| Tot., Uniq.Data, R(int) | 13435, 5186, 0.040 |
| Observed data       | 4472              |
| | [I > 2σ(I)] |
| N_{o}f, N_{pur}     | 5186, 239         |
| R, wR_2, S          | 0.0552, 0.1335, 1.05 |
Figure S1: The ORTEP view (30% ellipsoid probability) of H₂NPV ligand.

Figure S2: The 2D framework in crystallographic $ac$ plane in H₂NPV ligand.

Table S2. Hydrogen bonds for H₂NPV [Å and °].

| D-H...A            | d(D-H) | d(H...A) | d(D...A) | $\angle$(DHA) |
|--------------------|--------|----------|----------|----------------|
| O(1)-H(1O)...O(2)#1 | 0.82(4) | 1.80(4)  | 2.607(3) | 170(4)         |
| O(3)-H(3O)...N(2)  | 0.78(4) | 1.89(4)  | 2.593(3) | 150(4)         |
| N(1)-H(1N)...O(1)  | 0.83(4) | 1.89(4)  | 2.568(3) | 138(3)         |
| C(19)-H(19B)...O(4)#2 | 0.98    | 2.65     | 3.147(3) | 111.9          |

Symmetry transformations used to generate equivalent atoms:
#1 -x+1,-y+1,z+1/2   #2 x+1/2,-y+1/2,z
Figure S3: $^1$H-NMR spectra of ligand H$_2$NPV.

Figure S4: $^{13}$C-NMR spectra of ligand H$_2$NPV.
Figure S5: ESI-MS spectra of ligand H$_2$NPV.

Figure S6: FTIR spectra of ligand H$_2$NPV.
Figure S7: ESI-MS spectra of complex-1

Figure S8: Infra-red(IR) spectra of complex-1
**Evaluation of binding constant**

Solution of receptor H$_2$NPV (c=20 × 10$^{-6}$ mL$^{-1}$) in MeOH-H$_2$O (9:1, v/v 5 µM aqueous HEPES buffer, pH 7.2) was used for both absorbance and emission titration studies for the Al$^{3+}$. The binding constant $K_a$ of the metal-receptor complex was determined using Benesi-Hildebrand (B-H) equation from emission titration data.

$$\frac{1}{I - I_0} = \frac{1}{I_{\text{max}} - I_0} + \frac{1}{K_a(I_{\text{max}} - I_0)[M^{n+}]^m}$$

where, $I_0$ is the emission intensity of the sensor H$_2$NPV (20 µM) only, at a wave length 524 nm. $I$ is the emission intensity of the sensor H$_2$NPV in the presence of intermediate concentrations of the metal ion, and $I_{\text{max}}$ is the maximum emission intensity of the sensor H$_2$NPV in the presence of excess metal ion.
Figure S10: Benesi–Hildebrand plot from fluorescence titration data of H$_2$NPV(20µM) with Al$^{3+}$.

Calculations of the detection of limit (LOD)

The detection limit was calculated on the basis of the fluorescence titration. The detection limit (LOD) of the probe H$_2$NPV for Al$^{3+}$ were determined using the following equation.

$$\text{Detection limit (LOD)} = 3\sigma/k$$

Where $\sigma$ is the standard deviation of blank measurement, and $k$ is the slope between the fluorescence emission intensity versus metal ion concentration.

Figure S11: Changes of emission intensity of H$_2$NPV($c = 2 \times 10^{-5}$M) as a function of [Al$^{3+}$]($c = 2 \times 10^{-3}$M) at 524 nm.
Figure S12: pH dependence of fluorescence responses of H$_2$NPV and its Al$^{3+}$-complex in 9:1 (v/v) MeOH: HEPES buffer.

**Determination of fluorescence quantum yield:**

Here, the quantum yield $\phi$ was measured by using the following equation,

$$\phi_X = \phi_S \left( \frac{F_X}{F_S} \right) \left( \frac{A_S}{A_X} \right) \left( \frac{n_S^2}{n_X^2} \right)$$

Where, $X$ & $S$ indicate the unknown and standard solution respectively, $\phi = \text{quantum yield}$, $F = \text{area under the emission curve}$, $A = \text{absorbance at the excitation wave length}$, $n = \text{index of refraction of the solvent}$. Here $\phi$ measurements were performed using fluorescein in 0.1M NaOH as standard [$\phi = 0.79$] (error $\sim 10\%$).
Absorbance and emission spectral overlap profile for H$_2$NPV and H$_2$NPV+Al$^{3+}$

Figure S13: Absorption and emission spectral overlap profile for H$_2$NPV and H$_2$NPV+Al$^{3+}$

Figure S14: $^1$H-NMR spectra of complex 1.

Absorbance and fluorescence spectral titration with DNA
The absorption spectral titrations were performed at on a Jasco V660 unit (Jasco International Co. Ltd., Hachioji, Japan) equipped with a thermoelectrically controlled cell holder and temperature controller in matched quartz cuvettes of 10 mm path length, following the methods standardized in our laboratory and reported earlier. The electronic spectra of H$_2$NPV were monitored as a function of the concentration of DNA. In each case a fixed concentration
of the H$_2$NPV (20 μM) was titrated with increasing concentration of DNA over a range of 0–20 μM.

Steady-state fluorescence measurements were performed on a Shimadzu RF-5301 PC unit (Shimadzu Corporation, Kyoto, Japan) in fluorescence free quartz cuvettes of 1 cm path length. The excitation wavelength for H$_2$NPV was 350 nm. All measurements were performed keeping an excitation and emission band pass of 5 nm. The sample temperature was maintained at 298.15 ± 1.0 K using an EyelaUni Cool U55 water bath (Tokyo Rikakikai Co. Ltd., Tokyo, Japan).

**Estimation of the binding parameters**

**Analysis of the binding data**

Binding data obtained from absorbance titration were converted into Scatchard plots (r/C$_f$ vs r) and analyzed using the McGhee–von Hippel methodology described previously. The Scatchard isotherms with positive slope at low r values were analyzed using the following McGhee–von Hippel equation for cooperative binding.

\[
\frac{r}{C_f} = K_i(1-nr)\times \left(\frac{(2\omega+1)(1-nr)+(r-R)}{2(1-nr)(1-nr)}\right)^{(n-1)/2} \left(\frac{1-(n+1)r+R}{2(1-nr)}\right)^{2}
\]

Here, R= \{[1- (n+1)r]^2 + 4ωr(1-nr)}^{1/2},

Scatchard plots with negative slopes at low r values were analysed by the non-cooperative binding model of McGhee and von Hippel as per the following equation:

\[
\frac{r}{C_f} = K_i(1-nr)/(1-(n-1)r)\times [n-1]
\]

Here, $K_i$ is the intrinsic binding constant to an isolated binding site, n is the number of base pairs excluded by the binding of a single H$_2$NPV molecule and $\omega$ is the cooperativity factor. All the binding data were analysed using the Origin 7.0 software that determines the best-fit parameters to $K_i$, n and $\omega$ to Eq. 1 and $K_i$ and n to Eq. 2.

In this case, the intrinsic binding constant($K_i$) and n were estimated to be $(4.02\pm0.18) \times 10^5$ M$^{-1}$ and 1.47 respectively.

The fluorescence spectral titration data were analyzed by employing the Benesi–Hildebrand plot. The binding affinity of DNA for the H$_2$NPV was determined using modified Benesi–Hildebrand equation (3) (Benesi& Hildebrand, 1949).

\[
\frac{1}{\Delta I} = \frac{1}{\Delta I_{max}} + \frac{1}{(\Delta I_{max})K_{BH}} \times \frac{1}{[M]}
\]

Where, $\Delta I$ is the difference in fluorescence and [M] is the DNA concentration. By plotting the reciprocal of the difference in fluorescence intensity against the reciprocal of DNA concentration, the Benesi–Hildebrand association constant ($K_{BH}$) for the H$_2$NPV–DNA
association was calculated from the ratio of the intercept to the slope (Benesi & Hildebrand, 1949). From spectrofluorimetry the value of $K_{BH}$ was estimated to be $(4.72\pm0.03) \times 10^5 \, M^{-1}$.

**Isothermal titration calorimetric study**

Isothermal titration calorimetry experiments were performed on a VP-ITC micro-calorimeter (Malvern Instruments, United Kingdom) to study the binding interaction of $H_2$NPV with DNA. The data were analysed using Origin 7.0 software to obtain the thermodynamic parameters by following the protocols described in details previously. Buffer solutions were degassed extensively to prevent air bubble formation during titration. Titrations were performed by injecting $H_2$NPVsolution (140 $\mu$M) from the rotating syringe into the isothermal chamber containing 1.4235 mL of DNA solutions (60 $\mu$M) at 298.15 K. The titration was performed in 28 sequential injections and each injection released 10 $\mu$L aliquots from the rotating syringe into the calorimeter cell. The corresponding dilution study of each reaction was performed in separate experiment by injecting identical volumes of the same concentration of the $H_2$NPV into the buffer alone. The heat associated with each injection was observed as a heat spike which is actually the measure of the power needed to maintain the sample and reference cells at same temperatures. The area under each peak was integrated to obtain the heat associated with that injection. The heats of dilution were subtracted from the corresponding heat associated with the binding experiment that afforded the actual heat of $H_2$NPV-DNA interaction. The corrected injection heats were thereafter plotted as a function of the molar ratio and this was fitted with a model for one set of binding sites to calculate the equilibrium constant ($K$), the binding stoichiometry ($N$) and the standard molar enthalpy change ($\Delta H^0$) of association. The standard molar Gibbs energy change ($\Delta G^0$) was calculated using the standard relationship

$$\Delta G^0 = -RT \ln K$$  \hspace{1cm} (4)

where $R$ (1.987 cal $\cdot$ K$^{-1} \cdot$ mol$^{-1}$) is the gas constant and $T$ is the temperature in kelvins (K). Analysis of ITC data yielded the values of standard molar Gibbs energy and standard molar enthalpy change that enabled the calculation of $T\Delta S^0$, where $\Delta S^0$ is the calculated standard molar entropy change, using the relationship

$$T\Delta S^0 = \Delta H^0 - \Delta G^0$$  \hspace{1cm} (5)
Table S3: Comparison of sensing of receptor H$_2$NPV to the other reported Al$^{3+}$ sensor

| Probe | Solvent | emission maxima, Stokes shift (nm) | LOD | Sensing in other solvent | DNA binding study | Paper strip kit | Reference |
|-------|---------|-----------------------------------|-----|--------------------------|-------------------|----------------|-----------|
| ![Image](https://example.com/probe1.png) | EtOH-H$_2$O (4:6) | 500, 80 | 7.1×10$^{-8}$ M | NO | NO | NO | 1 |
| ![Image](https://example.com/probe2.png) | MeOH-H$_2$O (2:8) | 436, 19 | 4.57×10$^{-7}$ M | NO | NO | NO | 2 |
| ![Image](https://example.com/probe3.png) | EtOH-H$_2$O (3:1) | 503, 43 | 3.48×10$^{-8}$ M | No | No | Yes | 3 |
| ![Image](https://example.com/probe4.png) | H$_2$O | 490, 84 | 2.09 nM | no | No | yes | 4 |
| ![Image](https://example.com/probe5.png) | EtOH | 496, 82 | ND | ND | NO | NO | 5 |
| ![Image](https://example.com/probe6.png) | MeOH | 505, 100 | 28 nM | NO | NO | NO | 6 |
| ![Image](https://example.com/probe7.png) | MeOH-H$_2$O (4:6) | 487, 93 | 114.54 nM | NO | NO | YES | 7 |
| ![Image](https://example.com/probe8.png) | MeOH-H$_2$O (8:2) | 480, 62 | 1.68 nM | NO | NO | YES | 8 |
| ![Image](https://example.com/probe9.png) | MeOH-H$_2$O (9:1) | 528, 138 | 4.39 µM | NO | NO | NO | 9 |
| ![Image](https://example.com/probe10.png) | MeOH-H$_2$O (1:1) | 517, -- | 10$^{-5}$ M | NO | NO | YES | 10 |
| Compound | pH | pK<sub>a</sub> | Concentration | Result | Result | Result |
|----------|----|-------------|---------------|--------|--------|--------|
| Bis-tris | 535,68 | 18.07 µM | NO | NO | NO |
| MeOH | 433,48 | 0.61×10<sup>-7</sup> M | YES | NO | NO |
| DMSO-H<sub>2</sub>O (2:8) | 450,94 | 1.2 nM | NO | NO | NO |
| H<sub>2</sub>O, HEPES | 468,78 | 0.062 µM | YES | NO | NO |
| DMSO-H<sub>2</sub>O (7:3) | 515,55 | ND | NO | NO | NO |
| Bis-tris buffer | 452,42 | 0.19 µM | NO | NO | NO |
| H<sub>2</sub>O | 442, - | 7.21×10<sup>-8</sup> M | YES | NO | YES |
| MeCN-H<sub>2</sub>O (1:1) | 392, - | 1.98 µM | NO | NO | NO |
| MeOH | 545,138 | 5.08×10<sup>-8</sup> M | YES | NO | NO |
| MeCN-H<sub>2</sub>O (2:8) | 480,590; 40,100 | 0.1 µM, 0.11 ppm | NO | YES | NO |
| MeOH-H<sub>2</sub>O (9:1) | 552,23 | 2.86 nM | NO | NO | NO |
| DMF-H<sub>2</sub>O (1:99) | 568,32 | 1.87×10<sup>-8</sup> M | NO | NO | YES |
| MeOH-H$_2$O (1:9) | EtOH-H$_2$O (4:1) | Tris-HCl buffer | MeOH-HEPES buffer (9:1) |
|-----------------|-----------------|----------------|------------------------|
| **555, 25**     | **582, 28**     | **451, 41**    | **524,124**            |
| 1.4-5.3 nM      | 1.1×10$^{-7}$ M | 1.09 nM        | 1.7 µM                 |
| YES             | NO              | NO             | YES                    |
| NO              | NO              | NO             | YES                    |
| NO              | NO              | NO             | YES                    |

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