**Supplementary Data**

# A highly specific sodium aptamer probed by 2-aminopurine for robust $Na^+$ sensing

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**Table S1**. DNA oligonucleotides used in this study. [2AP] = 2-aminopurine; r[2AP] = ribo-2-aminopurine; rA = ribo-adenine; FAM = carboxyfluorescein.

| DNA name | Sequences and modifications (from 5′-terminius) |
|---|---|
| Ce13d | TTTCGCCATAGGTCAAAGGTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC |
| NaA43 | TTTCGCCATCCAGGTCAAAGGTGGGTGAGGGGACGCCAAGAGTCCCCGCGGTTAGTGACTCGTGAC |
| 17E | TTTCGCCATCTTCTCCGAGCCGGTCGAAATAGTGACTCGTGAC |
| Ce13d-$A_{20}$ | TTTCGCCATAGGTCAAAGGTGGGTGCGAGTTTTTACTCGTAATAGTGACTCGTGAC |
| Ce13d-$C_{20}$ | TTTCGCCATAGGTCAAAGGTGGGTGCGAGTTTTTACTCGTCATAGTGACTCGTGAC |
| Ce13d-$G_{20}$ | TTTCGCCATAGGTCAAAGGTGGGTGCGAGTTTTTACTCGTGATAGTGACTCGTGAC |
| Ce13d-$A_{10}$C | TTTCGCCATAGGTCAACGGTGGGTGCGAGTTTTTACTCGTTATAGT GAC TCG TGA C |
| Ce13d-$G_{14}$T | TTTCGCCATAGGTCAAAGGTTGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC |
| Ce13d-$A_3$G | TTTCGCCATGGGTCAAAGGTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC |
| Ce13d-$A_8$G | TTTCGCCATAGGTCGAAGGTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC |
| Ce13d-$G_{14}$A | TTTCGCCATAGGTCAAAGGTAGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC |
| Ce13d-$T_{17}$C | TTTCGCCATAGGTCAAAGGTGGGCG CGA GTT TTT ACT CGT TAT AGT GAC TCG TGAC |
| Ce13d-$A_{10}$C+ $T_{20}$A | TTTCGCCATAGGTCAACGGTGGGTGCGAGTTTTTACTCGTAATAGTGACTCGTGAC |
| Ce13d-$G_{14}$T + $T_{20}$A | TTTCGCCATAGGTCAAAGGTTGGTGCGAGTTTTTACTCGTAATAGTGACTCGTGAC |
| Ce13d-$A_8$2AP | TTTCGCCATAGGTC[2AP]AAGGTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC |
| Ce13d-$A_9$2AP | TTTCGCCATAGGTCA[2AP]AGGTGGGTGCGAGTTTTTACTCGTTATAGTGACTCGTGAC |
| Sub-FAM | GTCACGAGTCACTATrAGGAAGATGGCGAAA-FAM |
| Sub-dA | GTCACGAGTCACTATAGGAAGATGGCGAAA |
| Sub-ribo-2AP | GTCACGAGTCACTATr[2AP]GGAAGATGGCGAAA |
| Sub-deoxyribo-2AP | GTCACGAGTCACTAT[2AP]GGAAGATGGCGAAA |

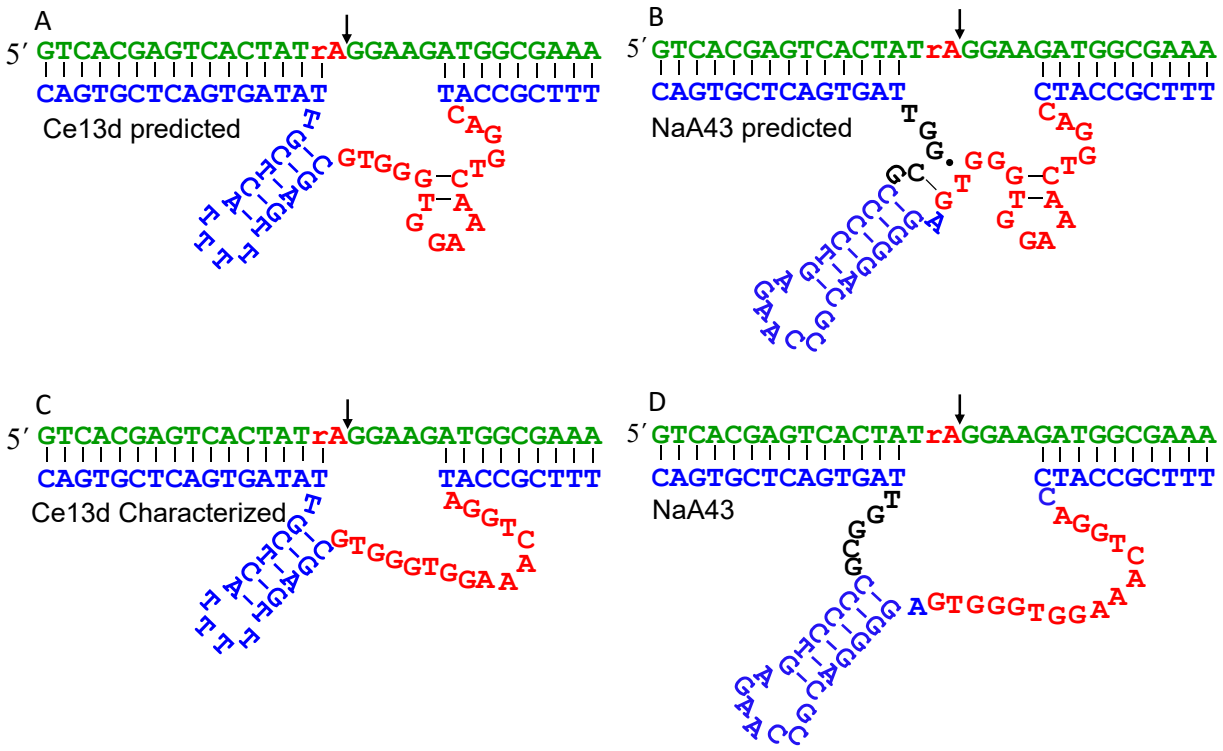


**Figure S1.** The secondary structures of the (A) Ce13d and (B) NaA43 predicted by the Mfold program (1). Based on the Mfold prediction, both structures contain a small hairpin in their identical loop in red. However, our biochemical characterizations (2) do not support such a small hairpin. Therefore, we draw the secondary structure of the Ce13d as in (C) and that of the NaA43 in (D) in the main paper.

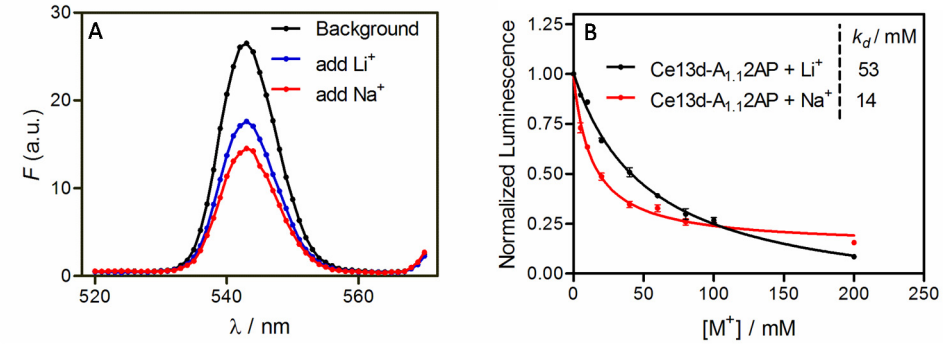


**Figure S2.** (A) To further confirm that 2AP modification does not disrupt $Na^+$ binding, we characterized the $Na^+$-induced Ce13d global folding using $Tb^{3+}$-sensitized luminescence by following the method we previously introduced (3,4). Sensitized $Tb^{3+}$ luminescence spectra with the 2AP-labeled Ce13d in buffer (25 mM $Li^+$, 50 mM Tris, pH 7.5) and after further adding 25 mM $Li^+$ or $Na^+$. The DNAzymes were prepared with Sub-ribo-2AP substrate and the enzyme (each 1 μM, see Table S1 for sequences). After annealing, various concentrations of $Li^+$/$Na^+$ were added. Then, 5 μM $Tb^{3+}$ was added, followed by monitoring the spectra from 520 to 570 nm by exciting at 290 nm. The peak intensity at 543 nm was quantified. The addition of $Na^+$ cause stronger fluorescence decrease which is attributable to the specific $Na^+$ binding, otherwise $Li^+$ would decrease more due to its higher charge density. (B) To have quantitative understanding, a careful titration was performed. Normalized $Tb^{3+}$ luminescence of the 2AP-modified Ce13d as a function of $Li^+$ and $Na^+$ concentration, from which the binding affinity of $Na^+$ ($k_d$ = 14 mM) was evaluated to be lower than that of $Li^+$ ($k_d$ = 53 mM), confirming the $Na^+$ binding by the 2AP-modified Ce13d. Besides confirming $Na^+$ binding, this result also highlights the advantage of 2AP probe compared with previous used $Tb^{3+}$-sensitized luminescence, in which 2AP signal is insensitive to non-specific interaction between metal ions and DNA (e.g., $Li^+$ causes little fluorescence change), while the latter is strongly influenced by competing ions (e.g., $Li^+$ also induces significant fluorescence decrease with higher concentrations).

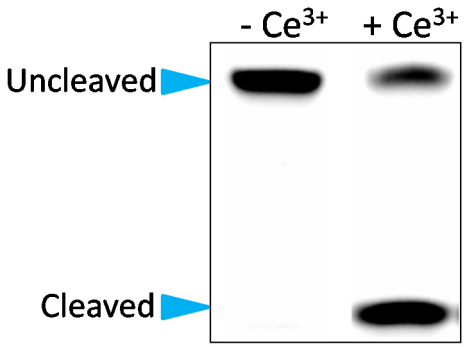


**Figure S3.** Gel image showing the activity of the Ce13d in absence and presence of 10 μM $Ce^{3+}$ after 1 h incubation. The assay was performed in 25 mM NaCl, 50 mM MES, pH 6.0. The result showed that without $Ce^{3+}$, the Ce13d is inactive in presence of $Na^{+}$ alone.

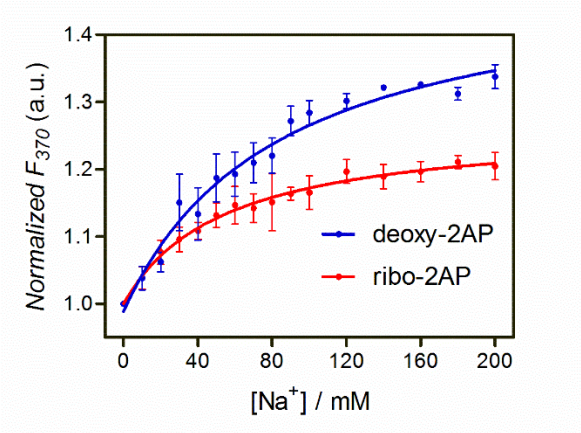


**Figure S4**. Normalized fluorescence enhancement at 370 nm of the NaA43 with ribo-2AP or deoxyribo-2AP substrate as a function of $Na^+$ concentration. While the deoxy-2AP substrate yielded a slightly higher fluorescence increase, it is still less than 40% with 200 mM $Na^+$.

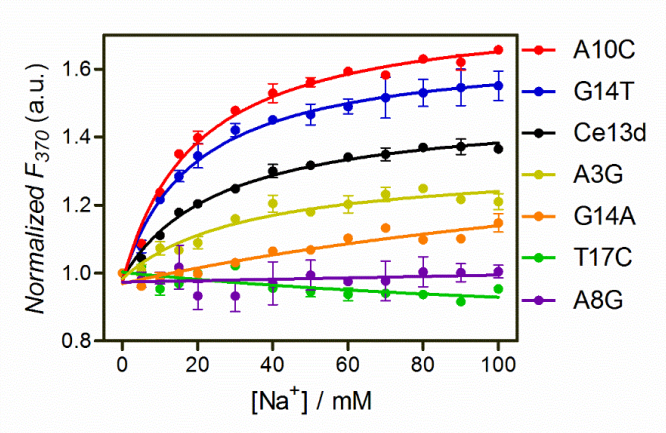


**Figure S5**. Normalized 2AP fluorescence enhancement of various Ce13d mutants as a function of $Na^+$ concentration. All these mutants were demonstrated to bind $Na^+$ based on our previous $Tb^{3+}$ sensitized luminescence experiment (4). Among them, the $A_{10}C$ mutant displayed the highest fluorescence response, and therefore it is the best candidate for further studies.

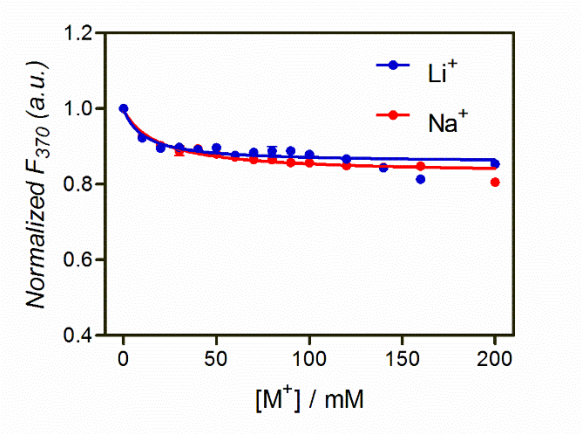


**Figure S6**. Normalized fluorescence intensity at 370 nm of Ce13d-A9 2AP (e.g. 2AP was introduced at $A_9$ position) as a function of salt concentration. In this case, no significant fluorescence decrease was observed with increasing concentration of both $Na^+$ and $Li^+$. On the other hand, labeling the 2AP at the $A_8$ position had a significant $Na^+$-dependent change of fluorescence, indicating that the fluorescence change observed in this work is mainly due to the $Na^+$ binding, instead of non-specific interactions.

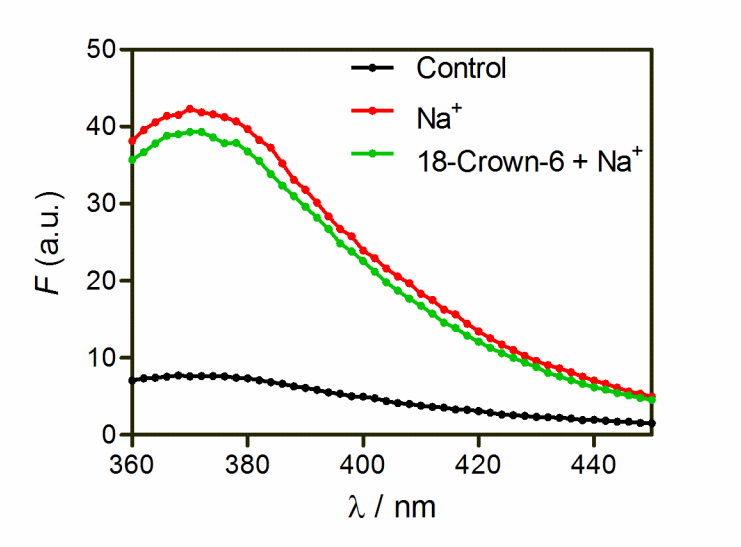


**Figure S7**. The fluorescence spectra of our optimized $Na^+$ sensor (with deoxyribo-2AP substrate and the double mutant enzyme) in absence or presence of 50 mM $Na^+$. The addition of 100 mM 18-crown-6 has little effect on the sensor response.

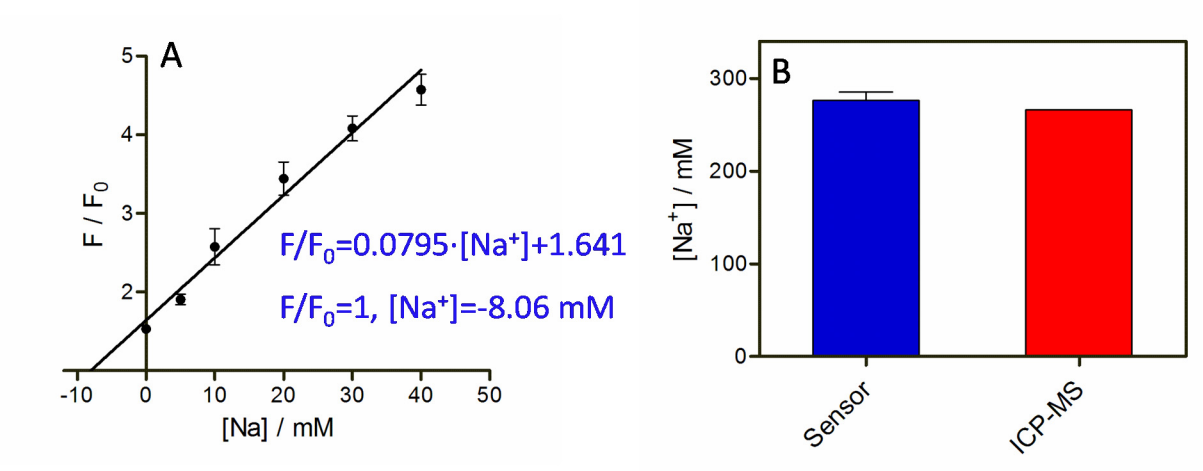


**Figure S8**. (A) The standard addition method for $Na^+$ detection in Atlantic Ocean water. The sensor (200 μL) was mixed with the ocean water (6 μL), and then further titrated with $Na^+$ stock solutions (1 μL of each addition). With the linear increasing curve, the $Na^+$ concentration was calculated to be 8.06 mM in the cuvette. Taking the dilution effect into consideration, the $Na^+$ concentration was estimated to be 276 mM in the ocean water. (B) A comparison of $Na^+$ concentration measured by $Na^+$ sensor and standard ICP quantification, and each with the results of 276 mM and 266 mM $Na^+$, respectively. With the error range, the results obtained by both methods are identical.

**Additional references.**

1. Zuker, M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.*, **31**, 3406-3415.

2. Huang, P.-J.J., Lin, J., Cao, J., Vazin, M. and Liu, J. (2014) Ultrasensitive DNAzyme beacon for lanthanides and metal speciation. *Anal. Chem.*, **86**, 1816-1821.

3. Zhou, W., Zhang, Y., Huang, P.-J.J., Ding, J. and Liu, J. (2016) A DNAzyme requiring two different metal ions at two distinct sites. *Nucleic Acids Res.*, **44**, 354-363.

4. Zhou, W., Ding, J. and Liu, J. (2016) A selective $Na^+$ aptamer dissected by sensitized $Tb^{3+}$ luminescence. *ChemBioChem*.