Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Group trend of lanthanides binding to DNA and DNAzymes with a complex but symmetric pattern

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Materials and Methods

All the DNA sequences were purchased from Eurofin Genomics. The stock DNA solutions were prepared in 5 mM HEPES, pH7.6. Metal chlorides were purchased from Sigma-Aldrich and their solutions were prepared using Milli-Q H_2O. Denaturing gel loading dye was from New England BioLabs Inc. All the gel running reagents were from Bio Basics Inc.

DNAzyme complex preparation. The DNAzyme complex (with non-cleavable Sub-dA substrate) was prepared by dissolving the substrate and enzyme strands (1 μM each) in buffer A (25 mM NaCl, 50 mM MES, pH6.0). The complex was then annealed by heating to 80 °C and cooling to ambient room temperature. The samples were then frozen overnight.

Luminescence. 500 μL DNAzyme complex solution (1 μM) was combined with 5 μL Tb^{3+} or competing ions (10 mM). The samples were incubated overnight at ambient room temperature to allow for competitive binding to reach equilibrium. The sensitized Tb^{3+} emission was measured using a fluorometer (FluoroMax-4, Horiba Scientific) by exciting at 290 nm. Peak high at 543 nm was quantified.

Tb^{3+} luminescence lifetime. The DNAzyme complex solution was dried using centrifugal evaporator (Vacufuge Plus, Eppendorf). The residual DNAzyme and selection buffer salt was dissolved in D_2O to the same concentration (1 μM DNAzyme complex, 25 mM NaCl 50 mM MES, pH 6.0). $TbCl_3$ solution was added to both H_2O and D_2O samples to a final concentration of 100 μM. Samples were incubated overnight. For lifetime measurement, the samples were maintained at 23 °C. Luminescence decay was measured by exciting sample at 290 nm (slit width 5nm) on a Varian Eclipse instrument for 20 ms (emission 543 nm, gate time 0.0200 ms, 50 iterations). The traces were fitted to first-order decay exponential and the decay constant was used to determine H_2O coordination number. The number of coordinated water q is calculated using $q = A(1/\tau_{H2O} - 1/\tau_{D2O})$, where A is 4.2 for Tb^{3+} and τ is the lifetime.[S1]

Terbium^{3+} binding curve. To measure K_d, 1 μM DNAzyme complex was prepared in buffer B (25 mM NaCl, 10 mM $MgCl_2$ 50 mM MES, pH6.0). 200 uL of the DNAzyme *complex* was placed in a quartz cuvette. Its luminescence intensity was measured as a function of Tb^{3+} concentration with an incubation time of 2 min after each Tb^{3+} addition.

Activity assay. Ce13d activity in the presence of 10 μM different lanthanides were carried out in buffer A with 1 μM DNAzyme (using the cleavable Sub-rA substrate). After 1 h of reaction, the samples were analyzed by polyacrylamide gel electrophoresis. The gel was imaged using on a ChemiDoc™ MP imaging system (Bio-Rad)

Additional references

S1. A. L. Feig, M. Panek, W. D. Horrocks, Jr and O. C. Uhlenbeck, *Chem. Biol.*, 1999, **6**, 801-810.