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 H₂O. 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 Tb³⁺ or competing ions (10 mM). The samples were incubated overnight at ambient room temperature to allow for competitive binding to reach equilibrium. The sensitized Tb³⁺ emission was measured using a fluorometer (FluoroMax-4, Horiba Scientific) by exciting at 290 nm. Peak high at 543 nm was quantified.

Tb³⁺ luminescence lifetime. The DNAzyme complex solution was dried using centrifugal evaporator (Vacufuge Plus, Eppendorf). The residual DNAzyme and selection buffer salt was dissolved in D₂O to the same concentration (1 µM DNAzyme complex, 25 mM NaCl 50 mM MES, pH 6.0). TbCl₃ solution was added to both H₂O and D₂O 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 H₂O coordination number. The number of coordinated water \( q \) is calculated using \( q = A \left( \frac{1}{\tau_\text{H₂O}} - \frac{1}{\tau_\text{D₂O}} \right) \), where \( A = 4.2 \) for Tb³⁺ and \( \tau \) is the lifetime.\( ^{S1} \)

Terbium³⁺ binding curve. To measure \( K_d \), 1 µM DNAzyme complex was prepared in buffer B (25 mM NaCl, 10 mM MgCl₂ 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 Tb³⁺ concentration with an incubation time of 2 min after each Tb³⁺ 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