Electronic Supplementary Information

Merely Two Mutations Switch a DNA-Hydrolyzing Deoxyribozyme from Heterobimetallic (Zn^{2+}/Mn^{2+}) to Monometallic (Zn^{2+}-only) Behavior

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Reselection of 10MD5 for tolerance of wider Zn^{2+} concentration

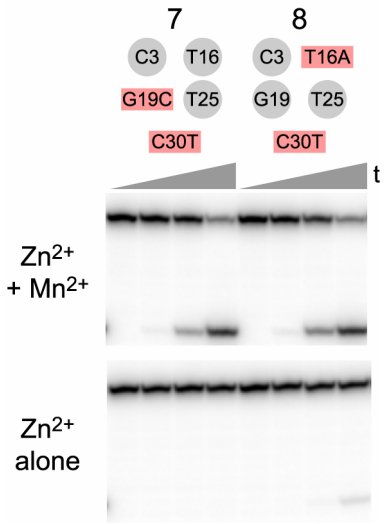

Fig. S1. Assays of the G19C/C30T and T16A/C30T double mutants, analogous to the assays shown in Fig. 2. The G19C/C30T double mutant was essentially inactive (<0.5% cleavage yield at 22 h, assay 7), revealing that the C30T mutation cannot serve functionally in place of T16A. The T16A/C30T double mutant showed only trace activity (0.2% cleavage yield at 2 h and 2.1% cleavage yield at 22 h, assay 8), indicating that C30T can substitute only very ineffectively for G19C. The 2.1% yield at 22 h for the T16A/C30T double mutant with Zn^{2+} alone corresponds to k_{obs} of only 10^{-3} h^{-1}, which is three orders of magnitude lower than the ~2 h^{-1} observed for the T16A/G19C double mutant (i.e., 10MD5-AC) with Zn^{2+} alone.

Reselection of 10MD5 for tolerance of wider Zn^{2+} concentration

A deoxyribozyme pool was generated with 25% randomization relative to the parent 10MD5 sequence, as described previously.[1] In vitro selection rounds were performed as described previously[2] at varying Zn^{2+} concentrations of either 0.3, 1.0, or 3.0 mM (70 mM HEPES, pH 7.5, as measured for the 1 M HEPES buffer stock solution, 20 mM $MnCl_2$, and 150 mM NaCl at 37 °C). The first round of the reselection experiment was performed with 1 mM Zn^{2+} and 14 h incubation time. Starting at round 2, the Zn^{2+} concentration was then alternated between 0.3 and 3 mM. Pool cleavage was first observed (2.4%) in round 4 with 0.3 mM Zn^{2+}. Individual deoxyribozymes were cloned from round 8 (50% pool yield in 18 h with 0.3 mM Zn^{2+}). Most of the new deoxyribozymes showed tolerance of broader Zn^{2+} concentration than the parent 10MD5 deoxyribozyme, similar to 10MD5-AC as shown in Fig. 4. Sequence alignment of the new deoxyribozymes (Fig. S1) revealed that all of them shared T16R and G19Y mutations.

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          1         10        20        30        40
          |         |         |         |         |
10MD5     CGCTAGATAA GTGGGTGCGT TTGCTATAGC TGTCCCTCAA
8PZ6      ..AC......  ....AGATT.  ....C....T  .....TCT..
8PZ7      ..AC......  ....AGA.C.  ....C....T  .....TCT..
8PZ22     GC..C.CAT.  .....G.TC.  ....C....T  ....T.C.C.
8PZ23     ..A.......  .....A..C.  ..........  ..........
8PZ26     ..A.......  .....A.TC.  ......G..T  .........C.
8PZ29     ..AC....G.  ....AGA.T.  ....C....T  .....TCT..
8PZ31     ...A......  .....A.AC.  .........T  ..........
8PZ33     ..A.......  ....AGA.T.  ....C....T  .....TCT..
8PZ35     ..AC......  ....AGA.T.  ....C....T  .....TCT..
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Fig. S2. Artificial phylogeny of the 10MD5 variants reselected for tolerance of broader Zn^{2+} concentration. Shown is the 40 nt enzyme-region sequence for each deoxyribozyme. The gray boxes denote the three variable regions as identified in our previous report.[1] Colored red are the T16 and G19 positions, which were uniformly mutated (T16R and G19Y) relative to the parent 10MD5 sequence. Colored green is the C30 position, which was almost always mutated (C30T) relative to the parent 10MD5 sequence.

Assay of site specificity of 10MD5-AC with expanded recognition site

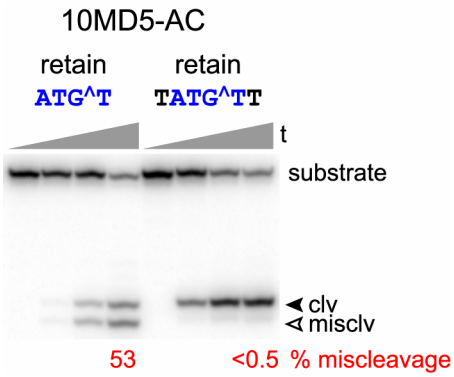

Fig. S3. Expansion of the recognition site beyond ATG^T restores the site specificity of 10MD5-AC. In the substrate sequence, either ATG^T (left) or TATG^TT (right) was retained, and all other nucleotides were changed (A↔T, G↔C). As in our previous report,[1] "% miscleavage" refers to the percentage of the total product that corresponds to miscleavage, i.e., cleavage not at the original ATG^T position but instead one nucleotide to the 5′-side at AT^GT. t = 0, 15 min, 2 h, and 22 h (70 mM HEPES, pH 7.5, 1 mM $ZnCl_2$, 20 mM $MnCl_2$, 150 mM NaCl, 37 °C).

References for Supplementary Information

1. Y. Xiao, M. Chandra and S. K. Silverman, *Biochemistry*, 2010, **49**, 9630-9637.
2. M. Chandra, A. Sachdeva and S. K. Silverman, *Nat. Chem. Biol.*, 2009, **5**, 718-720.