Electronic Supplementary Material (ESI) for Molecular BioSystems

Supplementary Information for

Characterization of Non-8-17 Sequences Uncovers Structurally Diverse and Complex RNA-cleaving Deoxyribozymes

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Figure S1

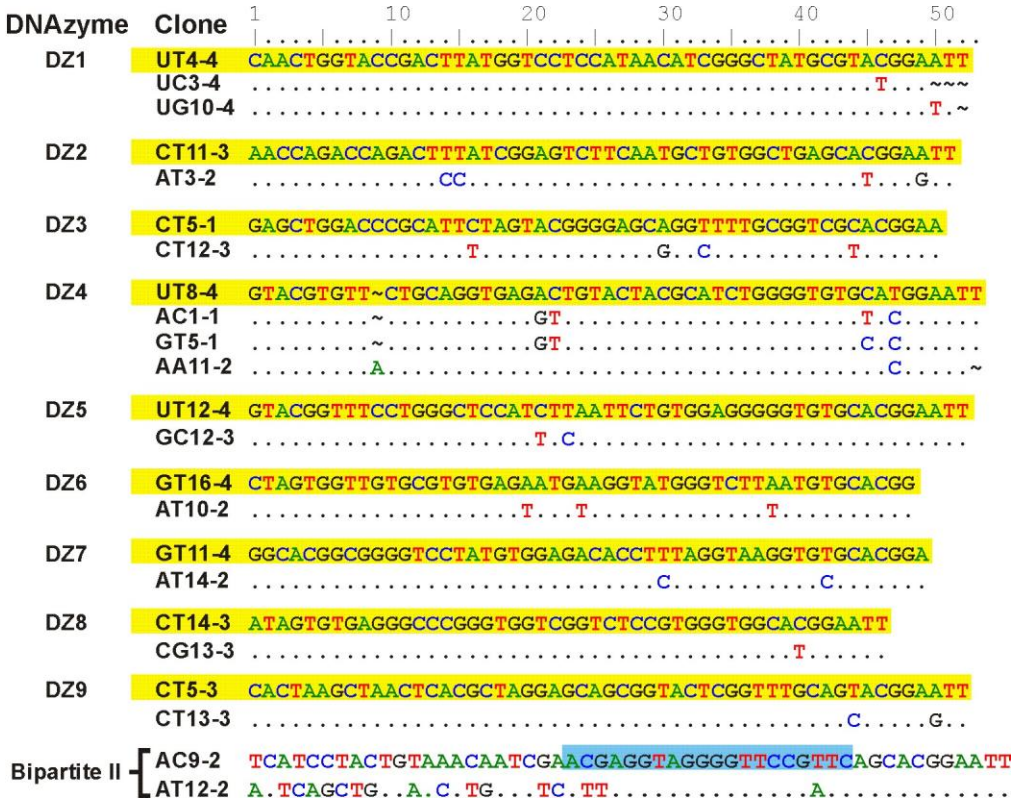

Figure S1. Sequence alignments of 23 non-8-17 RNA-cleaving DNAzymes obtained from a previous *in vitro* selection experiment by our group (1). These sequences are compiled into 10 classes. The clones examined in this study (denoted as DZ1 to DZ9) are highlighted in yellow. The substrate and two primer-binding sites are omitted for clarity (their sequences can be found in Figure 1 of the main text). A, C, G and T are labelled with green, blue, black and red, respectively, for easy viewing. A dot means that the clone has a nucleotide identical to the yellow-highlighted sequence above at the same location and a letter means that the nucleotide at this location has mutated from the highlighted sequence. Two clones, AC9-2 and AT12-2, were removed from the study due to a high sequence homology with the Bipartite II DNAzyme (motif highlighted in blue).

Figure S2

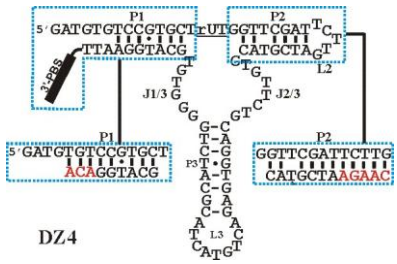

Figure S2. Conversion of DZ4 from unimolecular (*cis*-acting) to bimolecular format (*trans*-acting). DZ4 is shown as a representative example of converting DZ1 to DZ9 from unimolecular (*cis*-acting) to bimolecular (*trans*-acting) format. The secondary structure of DZ4 in *cis* is depicted in the centre. Modifications made to convert the *cis* construct to *trans* format are shown in blue boxes: The 3′ primer binding site (3′-PBS) was removed; the DNAzyme and the substrate strands are separated at loop L2; the substrate binding arms (P1 and P2) were extended to increase stem stability (red nucleotides).

Figure S3

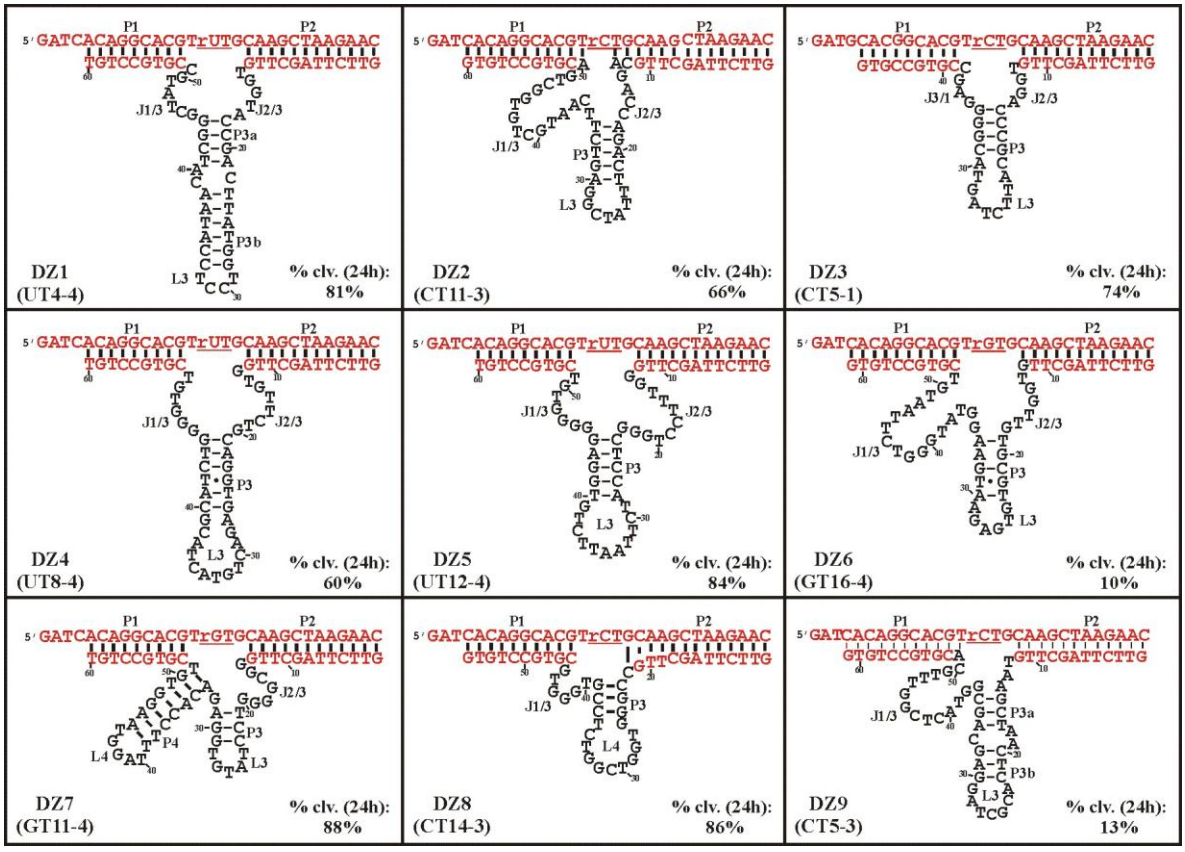

Figure S3. Alteration of binding-arm sequences of each DNAzyme. The two substrate binding arms of each DNAzyme were completely altered into new duplex forming sequences. The percentage of substrate cleavage in 24-h assay is shown for each altered DNAzyme.

Figure S4

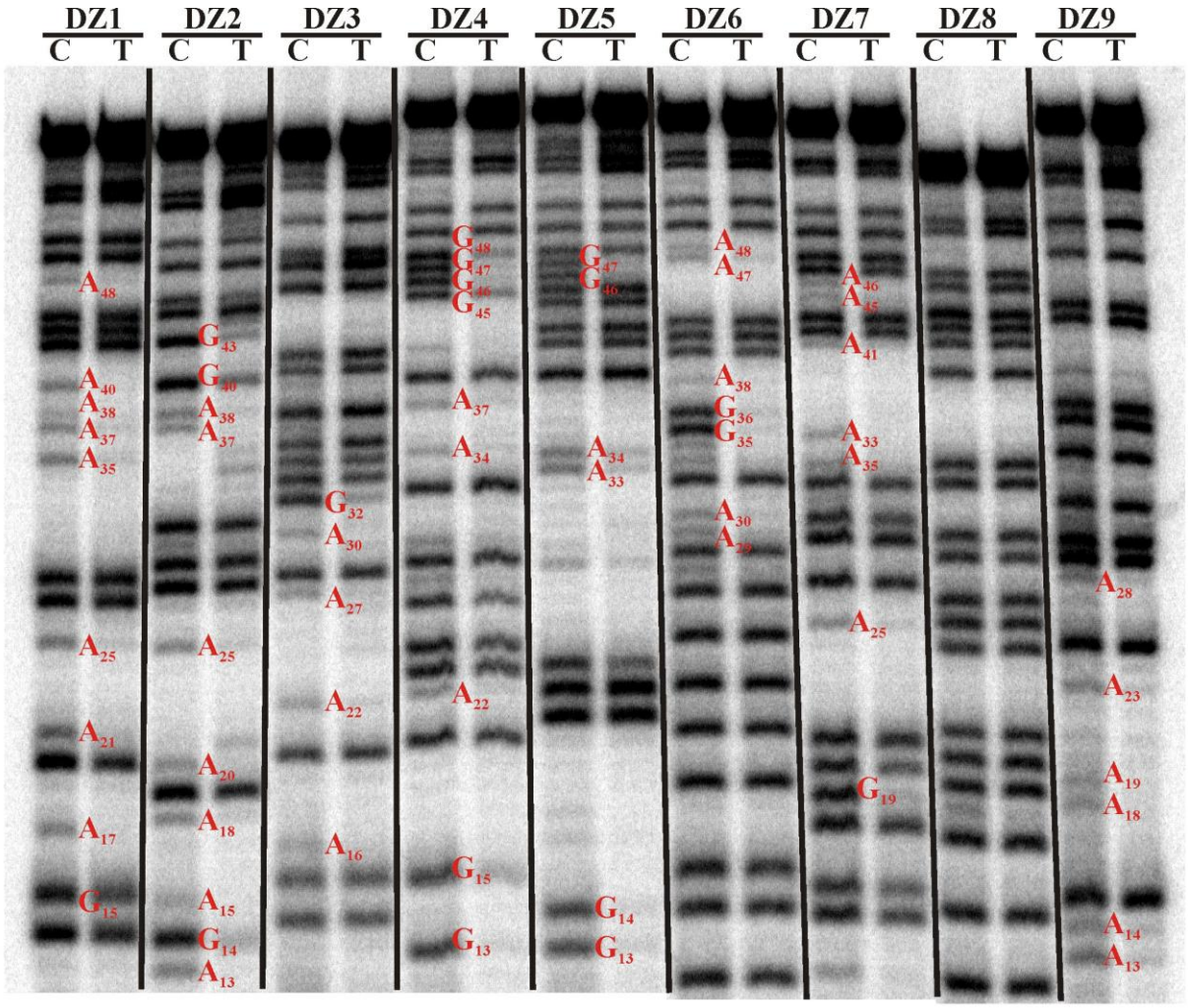

Figure S4. Autoradiograms from dimethyl sulphate (DMS) methylation interference assay of DZ1 to DZ9 are shown. The DNA bands are read from top to bottom in the 3'→5' direction. Lane 'C' represents 'control' (methylation after self-cleavage) and lane 'T' denotes 'test' (methylation before self-cleavage). Reduced intensity in certain bands in lane T are labelled with 'G' or 'A' for guanine or adenine and nucleotide position number corresponding to their respective structural models in Figure 3 of the main text (e.g. 'A_{48}' in lane T of DZ1 designates the adenine at position 48 of DZ1 in Figure 3). These notations are to indicate that the methylation at N7 of guanine or N3 of adenine interfered with tertiary folding or catalytic function of the DNAzyme (thus these guanines' N7 position or adenines' N3 position was protected against methylation). Deoxyribozyme strands that are intolerable to methylation at these nucleotide sites would not be cleaved during piperidine treatment; accordingly, the intensity of relevant bands appears lighter.

Figure S5

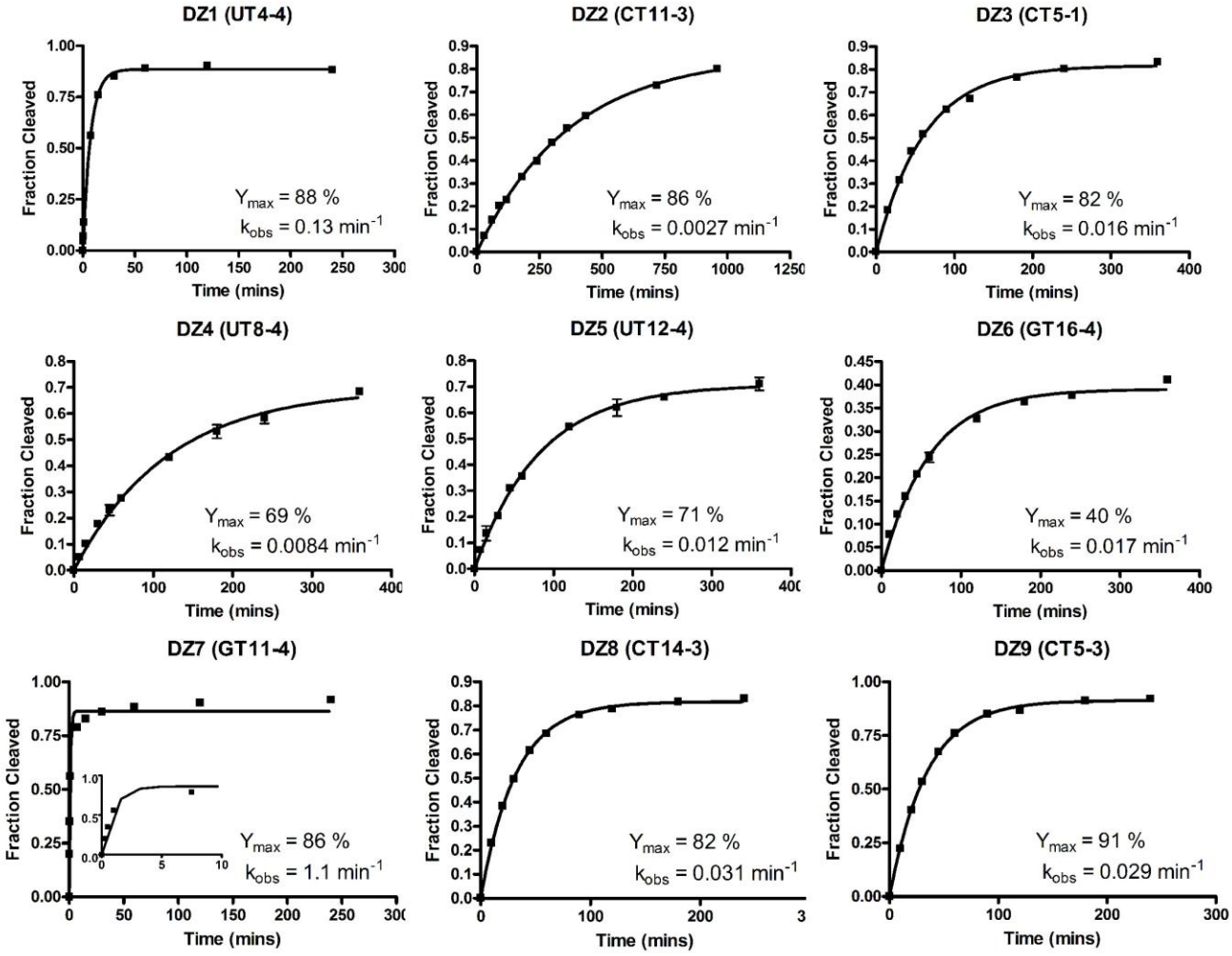

Figure S5. Kinetic plots of DZ1 to DZ9. Original clone names used in *Cruz* et al. are also given in brackets. Time course experiments were performed in *trans* under single-turnover conditions (E/S = 500/1). The total fraction of substrates cleaved were plotted over time and curve fitted under single-turnover kinetics using GraphPad software Prism 4.03. A minimum of two trials were performed for each deoxyribozyme. Rate constants (k_{obs}) and maximum cleavage yields (Y_{max}) are reported on each curve

Reference

1. Cruz, R.P., Withers, J.B. and Li, Y. (2004) Dinucleotide junction cleavage versatility of 8-17 deoxyribozyme. *Chem Biol*, **11**, 57-67.