Supporting Information

MNAzymes are a versatile new class of nucleic acid enzymes which can function as biosensors and molecular switches

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Table S1. Candidate partzyme pairs for the Mz1 MNAzymes

MNAzyme	Partzyme	Name	Sequence 5'-3'*
Mz1a	A	RO5Aa/2	**CAAACGAGTCCTGGCCTTGTCT**GCTACAACGA*GAGGAAACCTT*
	B	RO5Ba/2	*TGCCCAGGGA*GGCTA**GTGGAGACGGATTACACCTTC**
Mz1b	A	RO5Ab/2	**CAAACGAGTCCTGGCCTTGTCT**CTACAACGA*GAGGAAACCTT*
	B	RO5Bb/2	*TGCCCAGGGA*GGCTAG**GTGGAGACGGATTACACCTTC**
Mz1c	A	RO5Ac/2	**CAAACGAGTCCTGGCCTTGTCT**TACAACGA*GAGGAAACCTT*
	B	RO5Bc/2	*TGCCCAGGGA*GGCTAGC**GTGGAGACGGATTACACCTT**
Mz1d	A	RO5Ad/2	**CAAACGAGTCCTGGCCTTGTCT**ACAACGA*GAGGAAACCTT*
	B	RO5Bd/2	*TGCCCAGGGA*GGCTAGCT**GTGGAGACGGATTACACCTTC**
Mz1e	A	RO5Ae/2	**CAAACGAGTCCTGGCCTTGTCT**CAACGA*GAGGAAACCTT*
	B	RO5Be/2	*TGCCCAGGGA*GGCTAGCTA**GTGGAGACGGATTACACCTTC**
Mz1f	A	RO5Af/2	**CAAACGAGTCCTGGCCTTGTCTAA**CGA*GAGGAAACCTT*
	B	RO5Bf/2	*TGCCCAGGGA*GGCTAGCTAC**GTGGAGACGGATTACACCTTC**
Mz1g	A	RO5Ag/2	**CAAACGAGTCCTGGCCTTGTCT**ACGA*GAGGAAACCTT*
	B	RO5Bg/2	*TGCCCAGGGA*GGCTAGCTACA**GTGGAGACGGATTACACCTTC**

* The bases underlined form part of the core of the assembled MNAzyme, bases in **bold** hybridize with the assembly facilitator and bases in *italics* hybridize to the substrate.

Table S1. To demonstrate the engineering of MNAzymes from DNAzymes, the 10:23 catalytic core[1] was split at several locations. A series of candidate partzyme pairs were synthesized with sensor arms designed to hybridise to AF-RO5 and with substrate arms directed against the substrate, Sub2.

Figure S1. Cleavage activity of Dz1 and Mz1 MNAzyme core splits.

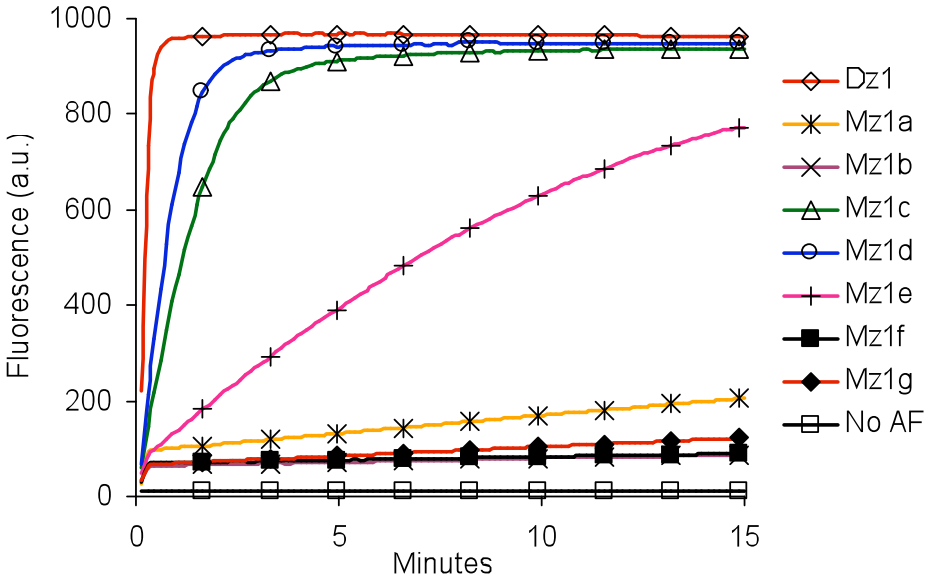

Figure S1. Comparison of the catalytic activity of the 10:23 DNAzyme (Dz1) and the series of Mz1 MNAzymes with different locations of the partial core splits. The splits Mz1c and Mz1d demonstrated cleavage activity that was comparable to Dz1. The other splits showed reduced (Mz1e) or minimal activity (Mz1a, Mz1b, Mz1f and Mz1g). Reactions were run in triplicate and results were averaged before plotting using Excel (Version 11). The k_{obs} was generated by fitting the equation (($Y = Y_{max}*(1-\exp(-k_{obs}*X))+Y_{min}$) where Y_{max} was set at 1000 or Y_{min} was set at 1) to the data .

Method for data shown in Table 1 and Figure S1. Analysis of the catalytic activity of partzyme pairs was performed using a SmartCycler System thermocycler (Cephied). Reactions were initiated by the addition of substrate and the total volume of all reactions was 25 µL. Cleavage of Sub2i-FB was monitored in real time at 9 second intervals for 15 minutes by observing the increase in fluorescence of isothermal reactions performed at 55°C. Each reaction was performed in triplicate and contained 1 x PCR Buffer II (Applied Biosystems), 50 mM $MgCl_2$ and 0.2 µM of Sub2i-FB. Test reactions for the 10:23 DNAzyme also contained 0.2 µM of Dz1. MNAzyme test reactions also contained 0.2 µM of each of the candidate partzyme A and B pairs and either 0.2 µM AF-RO5 or no assembly facilitator (No AF). The candidate partzyme A and B pairs assayed for cleavage activity were RO5Aa/2 and RO5Ba/2 (Mz1a), RO5Ab/2 and RO5Bb/2 (Mz1b), RO5Ac/2 and RO5Bc/2 (Mz1c), RO5Ad/2 and RO5Bd/2 (Mz1d), RO5Ae/2 and RO5Be/2 (Mz1e), RO5Af/2 and RO5Bf/2 (Mz1f) and RO5Ag/2 and RO5Bg/2 (Mz1g).

Table S2. Variant 8:17 DNAzymes and candidate partzyme pairs

DNAzyme	Sequence of the complete catalytic core domain
8:17	$C_1\,C_2\,G_3\,A_4\,G_5\,C_6\,C_7\,G_8\,G_9\,A_{10}\,C_{11}\,G_{12}\,A_{13}$
variant 1	$C_1\,C_2\,G_3\,A_4\,G_5\,C_6\,C_7\,G_8\,G_9\,\mathbf{T_{10}}\,C_{11}\,G_{12}\,A_{13}\,\mathbf{A_{14}}$
variant 2	$\mathbf{G_1}\,C_2\,\mathbf{C_3}\,A_4\,G_5\,C_6\,\mathbf{G_7}\,G_8\,\mathbf{C_9}\,\mathbf{T_{10}}\,C_{11}\,G_{12}\,A_{13}\,\mathbf{A_{14}}$
Variant 3	$C_1\,C_2\,G_3\,A_4\,G_5\,C_6\,C_7\,G_8\,G_9\,A_{10}\,C_{11}\,G_{12}\,A_{13}\,\mathbf{A_{14}}$

Candidate partzyme pair core*	Partial catalytic sequences in candidate partzyme pairs		Based on
	Partzyme B (N_1 to N_Y)[#]	Partzyme A (N_x to A_Y)[#]	
Mz2a	C_1 to C_6	C_7 to A_{14}	variant 1
Mz2b	G_1 to C_6	G_7 to A_{14}	variant 2
Mz2c	G_1 to C_3	A_4 to A_{14}	
Mz2d	G_1 to C_9	T_{10} to A_{14}	
Mz2e	C_1 to C_6	C_7 to A_{13}	8:17
Mz2f	C_1 to C_6	C_7 to A_{14}	variant 3

*Partzyme pair core sequences based on the 8:17 DNAzyme and variants thereof are designated Mz2 with candidate sequences designated a to f.
[#]N represents a base and x and y denote the positions with respect to the complete catalytic core of the DNAzyme which are split when incorporated into candidate partzymes A and B, respectively.

Table S2. MNAzymes engineered from the 8:17 DNAzyme (and variants thereof). Several variants of the partial core sequence for partzyme A were investigated for Mz2 MNAzymes. The partzyme A partial core sequences were based on the 8:17 DNAzyme[1]. Further sequences were tested which were based on the 8:17 DNAzyme that incorporated variant bases within the unpaired region of the core (variant 1 and 3) or the unpaired region and the stem region of the core (variant 2) (indicated in bold) which has been shown to be tolerant to alteration without abolition of catalytic activity[2]. Table S2 shows the complete catalytic core domain of DNAzymes and partial sequence incorporated into candidate partzymes.

Table S3. Candidate partzyme pairs for the Mz2 MNAzymes

MNAzyme	Partzyme	Name	Sequence 5'-3'*
Mz2a	A	RO4Aa/1	**GCTGGTCATCCAGCAG**CGGTCGAA*ATAGTGAGT*
	B	RO4Ba/1	*CATCTCTTCT*CCGAGC**GTGTTCGACAATGGC**
Mz2b	A	RO4Ab/1	**GCTGGTCATCCAGCAG**GGCTCGAA*ATAGTGAGT*
	B	RO4Bb/1	*CATCTCTTCT*GCCAGC**GTGTTCGACAATGGC**
Mz2c	A	RO4Ac/1	**GCTGGTCATCCAGCAG**AGCGGCTCGAA*ATAGTGAGT*
	B	RO4Bc/1	*CATCTCTTCT*GCC**GTGTTCGACAATGGC**
Mz2d	A	RO4Ad/1	**GCTGGTCATCCAGCAG**TCGAA*ATAGTGAGT*
	B	RO4Bd/1	*CATCTCTTCT*GCCAGCGGC**GTGTTCGACAATGGC**
Mz2e	A	RO4Ae/1	**GCTGGTCATCCAGCAG**CGGACGA*ATAGTGAGT*
	B	RO4Ba/1	*CATCTCTTCT*CCGAGC**GTGTTCGACAATGGC**
Mz2f	A	RO4Af/1	**GCTGGTCATCCAGCAG**CGGACGAA*ATAGTGAGT*
	B	RO4Ba/1	*CATCTCTTCT*CCGAGC**GTGTTCGACAATGGC**

* The bases underlined form part of the core of the assembled MNAzyme, bases in **bold** hybridize with the assembly facilitator AF-RO4 and bases in *italics* hybridize to the substrate Sub1i-FB.

Figure S2. Cleavage activity of Mz2 MNAzyme core splits.

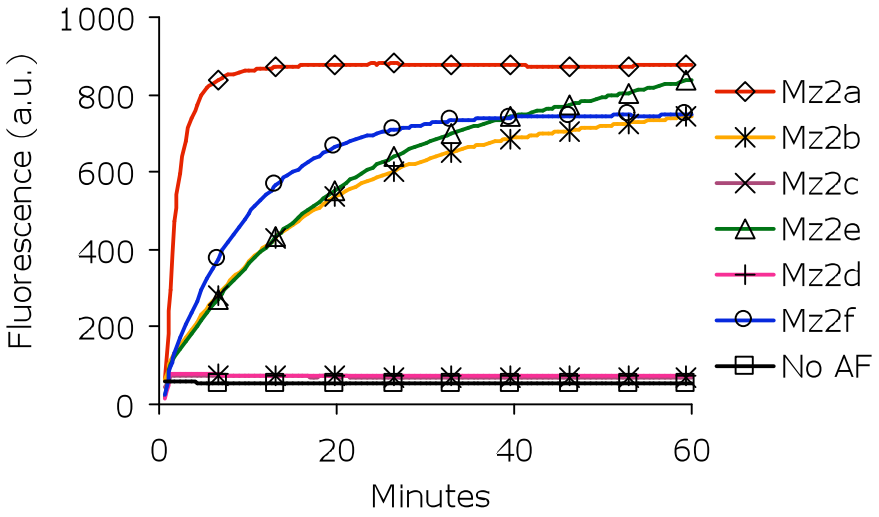

Figure S2. Comparison of the catalytic activity of Mz2 partzyme core pairs based on the DNAzymes variant 1 (Mz2a), variant 2 (Mz2b, Mz2c and Mz2d), 8:17 (Mz2e) and variant 3 (Mz2f). Mz2a, which contained a partzyme A with a core region based on the core of the 8:17 variant 1, cleaved Sub1i-FB at a faster rate than Mz2e which was based on the original 8:17 core and the Mz2f based on the variant 3 core. In addition partzyme pairs based on a variant 2 8:17 DNAzyme split at various locations were shown to be either catalytically active (Mz2b) or inactive (Mz2c and Mz2d).

Each reaction was performed in triplicate and was monitored in real time at 36 seconds intervals for 1 hour at 40^{o}C using the isothermal direct detection method with the modification of 50 mM $MgCl_2$ and 50 mM Tris HCl (pH 9.0 at 25°C). Reactions also contained either 0.2 µM of AF-RO4 or no assembly facilitator (No AF).

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Figure S3. Catalytic activity of the Mz1 MNAzyme at various temperatures.

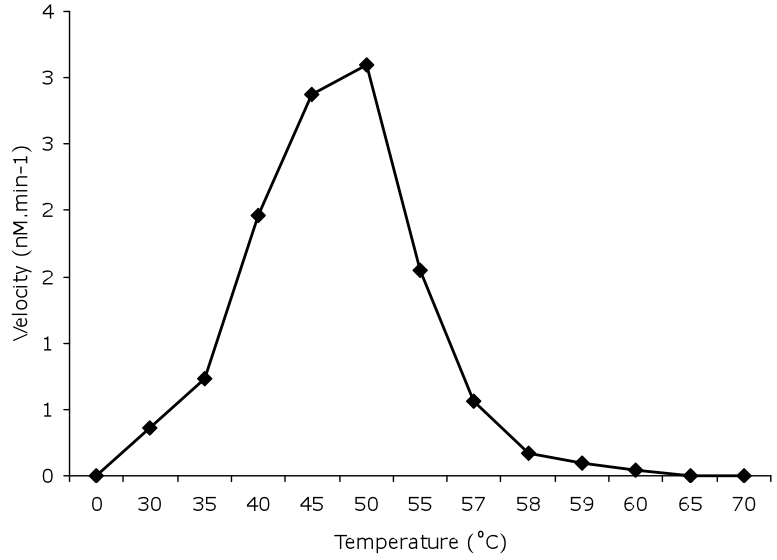

Figure S3. MNAzyme Mz1d was tested at various temperatures to determine turnover. The optimal temperature for cleavage by this MNAzyme, of the 22 base substrate in this buffer, is approximately 50^{o}C.

The partzyme pairs of Mz1d, RO5Ad/2-P and RO5Bd/2-P, had sensor arms that hybridise to AF-RO5 and substrate arms directed against the substrate Sub2i-FB. Each reaction was performed in triplicate and was monitored in real time at 7 second intervals for 10 minutes at 60^{o}C, 59^{o}C, 58^{o}C, 57^{o}C, 55^{o}C, 50^{o}C, 45^{o}C, 40^{o}C, 35^{o}C or 30^{o}C, using the isothermal direct detection method with the modification of 50 mM $MgCl_2$. Reactions also contained 0.1 μM of AF-RO5 or no assembly facilitator (measured at 55^{o}C). The k_{obs} values were obtained by performing a fit of a hyperbolic equation to the data (using Prism Version 4.0, GraphPad Software Inc.)

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