Defining the substrate scope of DNAzyme catalysis for reductive amination with aliphatic amines

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Mass spectrometry of peptides and oligonucleotide conjugates

For peptides, data were acquired on Waters Q-TOF Ultima ESI mass spectrometer in positive ion mode at the UIUC School of Chemical Sciences Mass Spectrometry Laboratory. All *m*/*z* values are for $M^{+\bullet}$.

For oligonucleotide conjugates, data were acquired on a Bruker UltrafleXtreme MALDI-TOF mass spectrometer with matrix 3-hydroxypicolinic acid in positive ion mode at the UIUC School of Chemical Sciences Mass Spectrometry Laboratory. All *m*/*z* values are for $[M+H]^+$. Samples were desalted by Millipore C_{18} ZipTip before analysis.

Data for peptides and oligonucleotide conjugates were as follows. For those oligonucleotide conjugates that bind to the left-hand DNAzyme binding arm, the DNA sequence was 5′-GGATAATACGACTCACTAT-3′. For those oligonucleotide conjugates that bind to the right-hand DNA binding arm, the DNA sequence was 5′-GAAGAGATGGCGACTTCG-3′.

Peptides (each K protected as Tfa)

AAAKAA	*m*/*z* calcd. 597.6, found 597.5, Δ = –0.02%
ASKKKS	*m*/*z* calcd. 935.8, found 935.5, Δ = –0.03%
ASEKES	*m*/*z* calcd. 745.7, found 745.3, Δ = –0.05%
ASFKFS	*m*/*z* calcd. 781.8, found 781.4, Δ = –0.05%

Oligonucleotide-peptide conjugates that bind to the left-hand DNAzyme binding arm

DNA-AAAKAA	*m*/*z* calcd. 6591.7, found 6595.4, Δ = +0.06%
DNA-ASKKKS	*m*/*z* calcd. 6737.9, found 6743.8, Δ = +0.09%
DNA-ASEKES	*m*/*z* calcd. 6739.8, found 6745.4, Δ = +0.08%
DNA-ASFKFS	*m*/*z* calcd. 6775.9, found 6781.7, Δ = +0.09%
DNA-HEG-AAAKAA	*m*/*z* calcd. 6934.6, found 6941.5, Δ = +0.10%
DNA-HEG-ASKKKS	*m*/*z* calcd. 7080.9, found 7081.6, Δ = +0.01%
DNA-HEG-ASEKES	*m*/*z* calcd. 7082.8, found 7092.0, Δ = +0.13%
DNA-HEG-ASFKFS	*m*/*z* calcd. 7118.9, found 7123.6, Δ = +0.07%

Oligonucleotide conjugate that binds to the right-hand DNAzyme binding arm

5′-benzaldehyde-DNA	*m*/*z* calcd. 5817.7, found 5819.3, Δ = +0.03%

Additional assay data for individual DNAzymes

DNAzyme	yield at 2 h, %	yield at 20 h, %
4HK216	4.2	42.6
4HK218	1.3	11.2
4HK220	3.5	33.2
4HK232	4.3	42.3
4HK236	2.6	25.6
6HR201	1.8	20.9
6HR202	4.1	30.3
6HR204	8.1	62.7
6HR206	3.1	29.2
6HR216	8.4	66.9
6HR222	9.7	74.0
6HR225	8.4	60.8
6HR227	7.0	58.4
6HR228	5.0	48.9
6HR229	1.5	13.2
6HR230	9.4	71.7

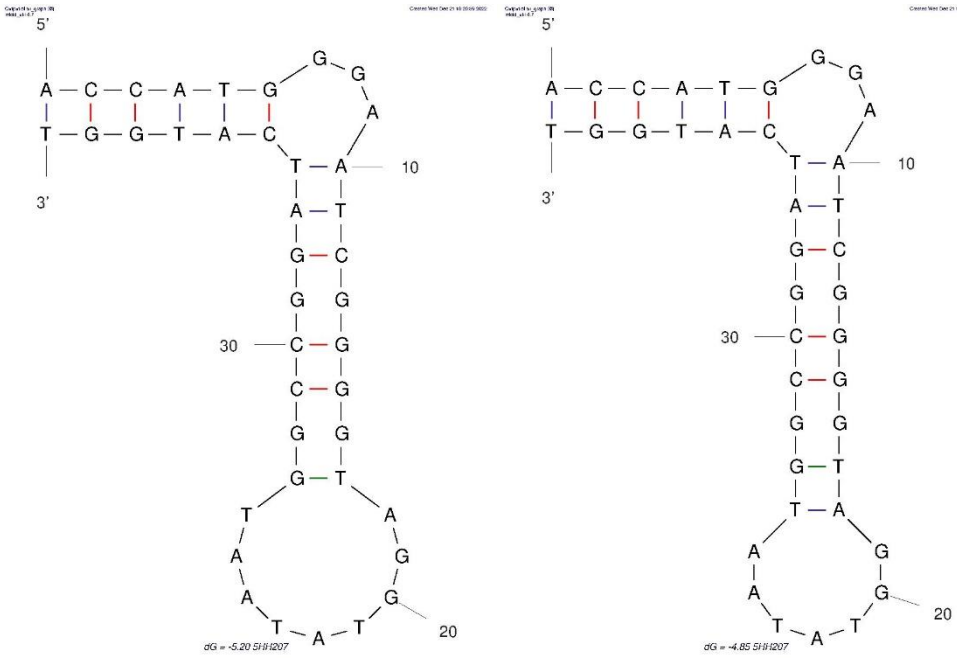
Table S1. Additional data for DNAzymes not shown in Figure 4.

DNAzyme secondary structure predictions using mfold

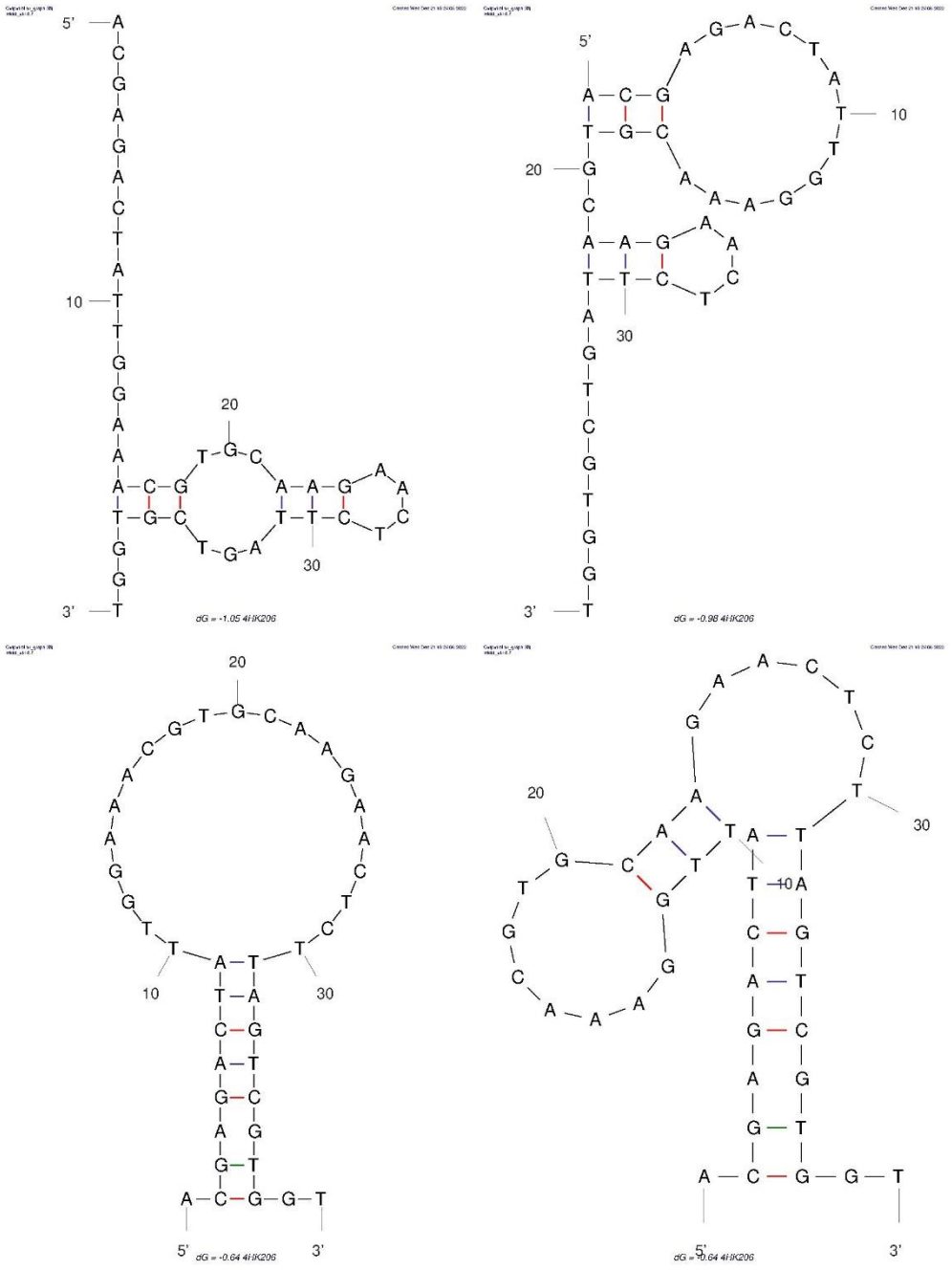
DNAzyme	number of structures	lowest ΔG, kcal/mol
5HH207	2	–5.2
4HK206	6	–1.1
3HP227	4	–2.5
4HQ227	2	–3.7
6HR205	1	–2.3

Table S2. Summary of mfold-predicted[1] secondary structures of the five representative DNAzymes whose characterizations are shown in Fig. 4. The default settings were used for the sequences of the initially random N_{40} or N_{20} regions with the DNA Folding Form at http://www.unafold.org/mfold/applications/dna-folding-form.php, adjusted to 150 mM Na^+ and 40 mM Mg^{2+}. The predicted secondary structures are shown in Fig. S1.

(A) 5HH207



(B) 4HK206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3'
10
20
30
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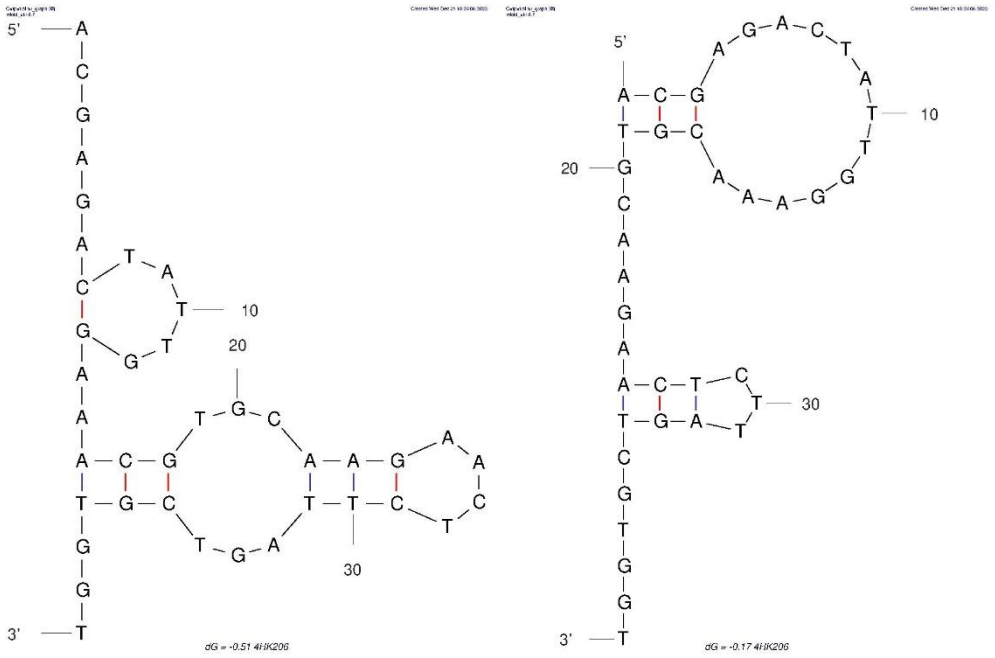
dG = -0.64 4HK206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3'
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3'
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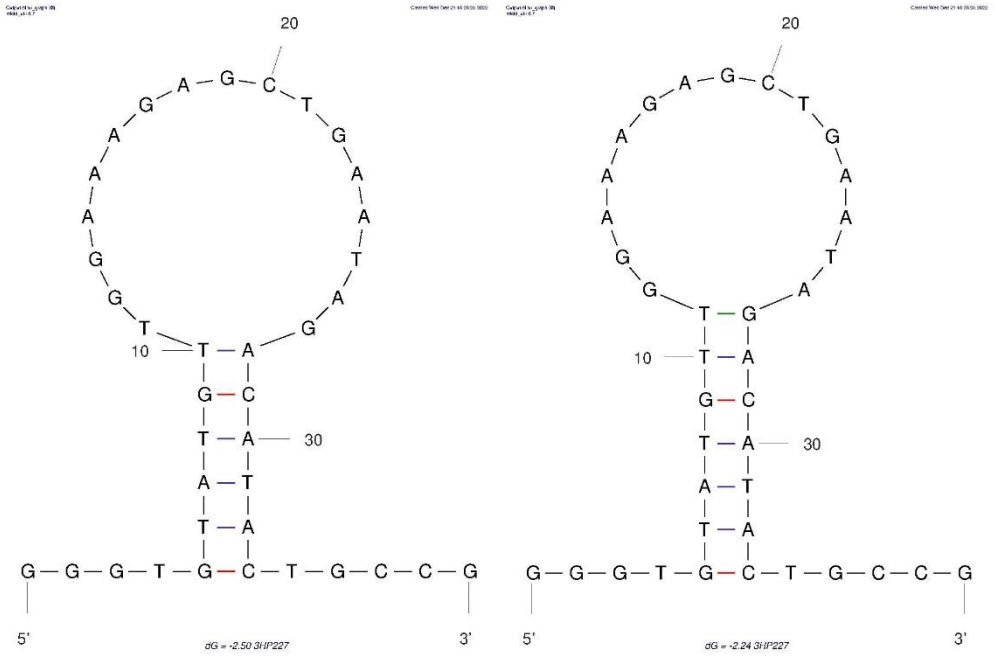
(C) 3HP227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10
30
5'
3'
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10
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5'
3'
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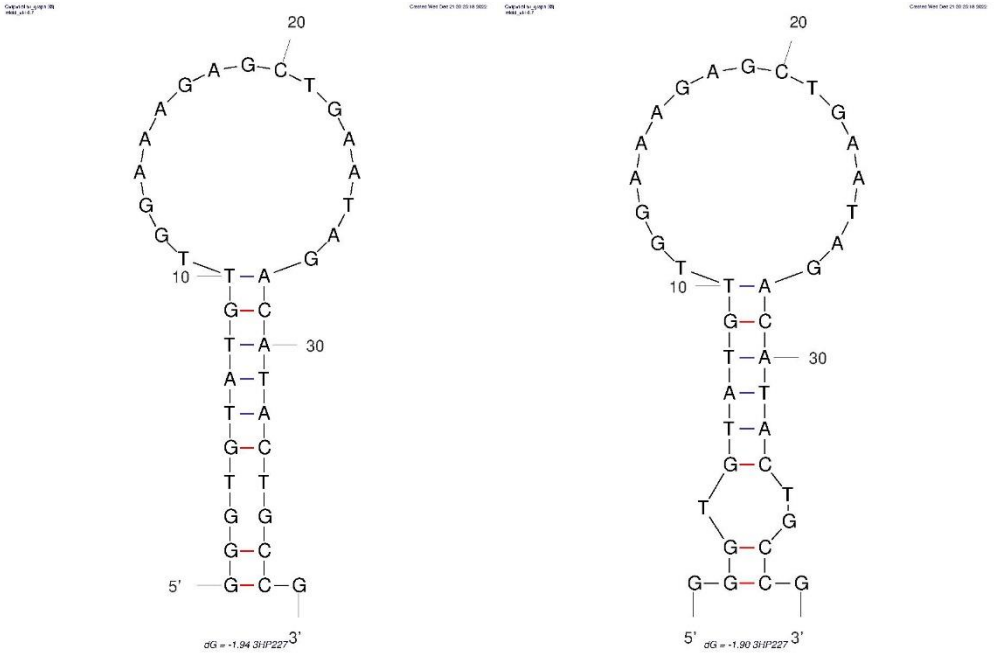
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10
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10
30
5'
3'
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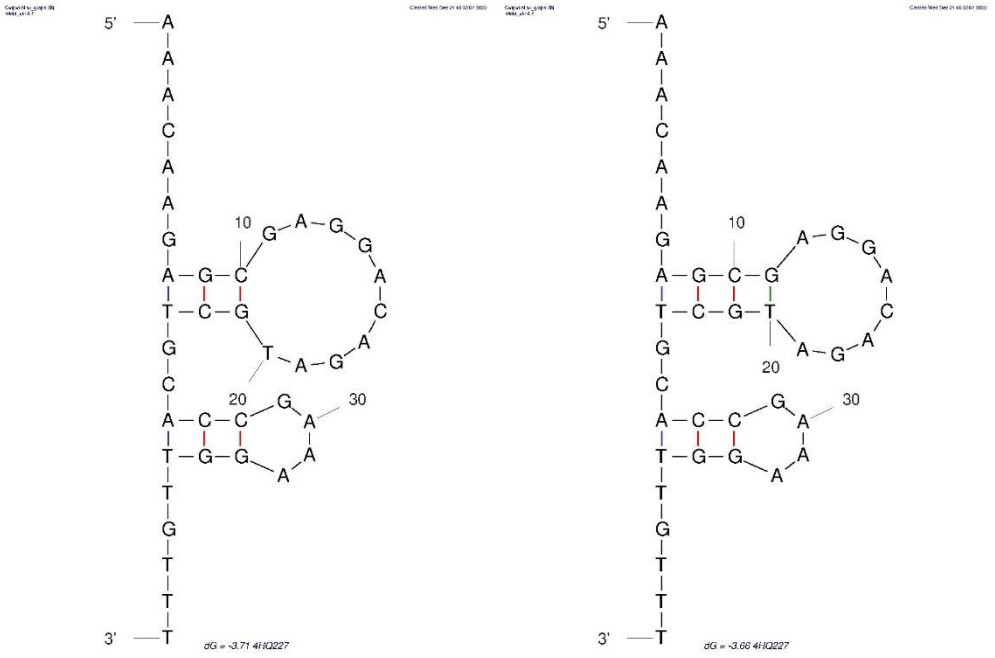
(D) 4HQ227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(E) 6HR205

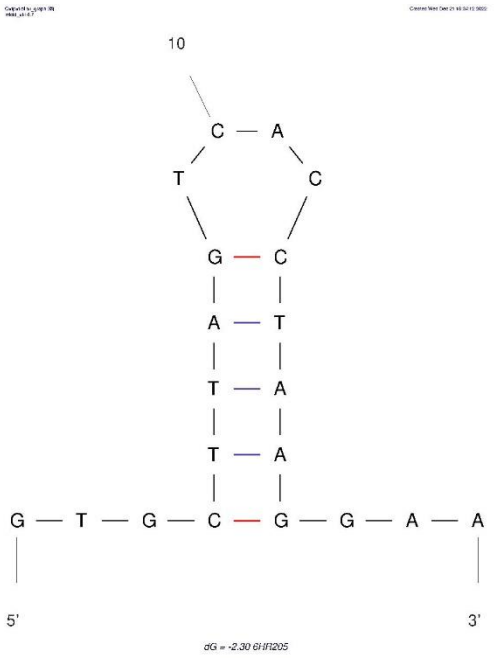

Fig. S1. Representative mfold-predicted[1] secondary structures for the five representative DNAzymes whose characterizations are shown in Fig. 4. The default settings were used for the initially random N_{40} or N_{20} regions with the DNA Folding Form at http://www.unafold.org/mfold/applications/dna-folding-form.php, adjusted to 150 mM Na^+ and 40 mM Mg^{2+}. Where multiple structures are shown for an individual DNAzyme, the lowest-energy structure (with most negative ΔG value) is shown first, followed by the remaining structure(s) in order of increasing energy. See Table S2 for full tabulation of number of structures and lowest ΔG value for each DNAzyme.

References for Electronic Supplementary Information

(1) Zuker, M. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* **2003**, *31*, 3406-3415.