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Supplemental Data

Tertiary Contacts Distant from the Active Site Prime a Ribozyme for Catalysis

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Additional materials are available at the authors' website:

http://www.chemistry.ucsc.edu/~wgscott/hh

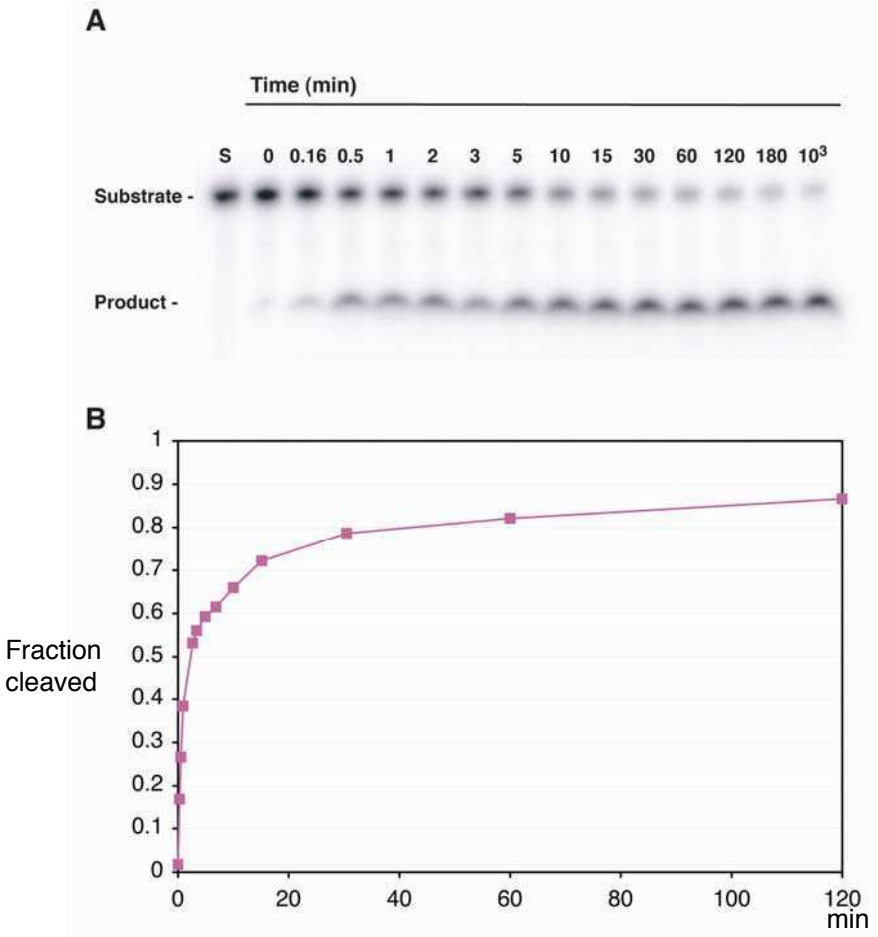


Figure S1. Activity of the Full-Length Hammerhead Ribozyme Construct

(A) 14% polyacrylamide gel electrophoretic assay of hammerhead ribozyme substrate cleavage as a function of time. The data shown are for the hammerhead RNA sequence used in the crystallographic experiments (Figure 1C in the main text). Capital S denotes substrate alone, and the zero time point was obtained before addition of magnesium.

(B) Plot of the fraction of substrate cleaved over time. The cleavage assays was performed under conditions similar to those published previously for the *Schistosoma* ribozyme (Canny et al., 2004). At pH 5.6 and in 10mM $MgCl_2$, the construct used for crystallization cleaves at a rate of about 0.65 min^{-1}, which is comparable to that of the wild type sequence (Canny et al., 2004, Supplementary Information). Furthermore, this construct cleaves to about 90 percent completion, or over 1.3 times more completely than what has been observed with the wild-type *Schistosoma* ribozyme sequence (Osborne et al., 2005, Canny et al., 2004).

The single turnover assay conditions were as follows: Cleavage assays were performed in the similar conditions as assayed previously (Canny et al., 2004), except that the all-ribose enzyme was >50 fold in excess of the all-ribose 5'-labeled substrate. The enzyme concentration was 16.5µM. The crystallographic construct assay conditions were 50mM MES pH5.6, 100mM NaCl, 0.1mM EDTA, 10mM $MgCl_2$. Prior to adding $MgCl_2$ the enzyme and substrate (in the assay solution) were heated at 95° for 2 minutes, then 65° for 2 minutes, then allowed to equilibrate at 27° for 5 minutes. At this point a sample was taken out and designated as the zero time point. The reaction was initiated by adding the $MgCl_2$ and the subsequent time point samples were collected.

Table S1. Br MAD Data Processing Statistics for Doubly Brominated Full-Length Hammerhead Ribozyme Crystals

Spacegroup: C2 Cell: a = 49.6Å b = 69.1Å c = 60.1Å $\alpha = 90^\circ$ $\beta = 112^\circ$ $\gamma = 90^\circ$

MAD Data Set:	**Peak λ: 0.919377 Å**		**Inflection λ: 0.920070 Å**		**Remote λ: 0.926608 Å**	
	Overall	**Outer Shell**	**Overall**	**Outer Shell**	**Overall**	**Outer Shell**
Low resolution limit (Å)	55.73	2.32	55.81	2.32	55.73	2.32
High resolution limit (Å)	2.20	2.20	2.20	2.20	2.20	2.20
R_{merge}	0.041	0.217	0.045	0.241	0.048	0.267
R_{meas} (within I+/I-)	0.055	0.295	0.060	0.325	0.065	0.360
R_{meas} (all I+ & I-)	0.060	0.293	0.061	0.330	0.062	0.355
R_{pim} (within I+/I-)	0.036	0.197	0.040	0.217	0.043	0.240
R_{pim} (all I+ & I-)	0.029	0.144	0.030	0.162	0.030	0.175
Fractional partial bias	-0.029	-0.123	-0.031	-0.147	-0.042	-0.165
Total number of observations	38654	5732	38974	5742	38131	5677
Total number unique reflections	9625	1410	9692	1418	9611	1415
Mean((I)/σ(I))	16.9	4.9	16.6	4.5	15.2	4.0
Completeness	99.2	100.0	99.3	99.8	99.2	99.7
Multiplicity	4.0	4.1	4.0	4.0	4.0	4.0
Anomalous completeness	98.4	98.9	98.3	97.9	97.9	98.1
Anomalous multiplicity	2.0	2.1	2.1	2.1	2.0	2.0
Data rejection cutoff	0.0 σ	0.0 σ	0.0 σ	0.0 σ	0.0 σ	0.0 σ
DelAnom correlation between half-sets	0.193	-0.009	0.082	0.024	-0.135	-0.074
Mid-Slope of Anom Normal Probability	1.186	na	1.105	na	0.934	na

Table S2. MAD Phasing and Refinement Statistics

MAD Phasing: CNS (max. likelihood)

Resolution limits: 55 - 2.2 Å	centrics	acentrics	all
generalized Cullis R-value	N/A	0.7655	0.7655
Kraut R-value	0.0079	0.0357	0.0349
std of lack-of-closure	5.5495	5.5785	5.5748
std of lack-of-isomorphism	0.1133	1.2237	1.1973
Figure of Merit	0.4197	0.4029	0.4031
Phasing Power	1.5020	1.2586	1.2647

Refinement: Refmac (max. likelihood)

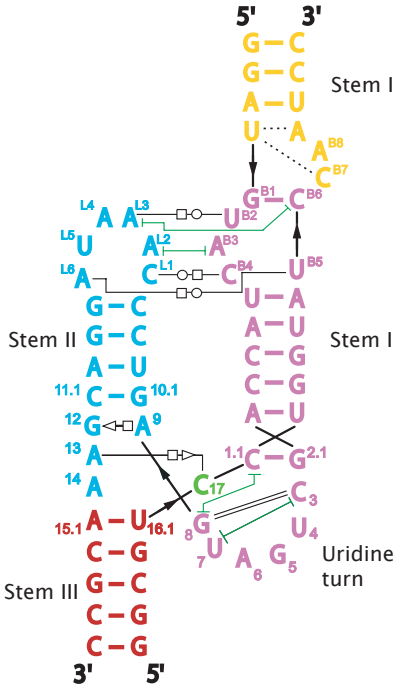
Model error:	
Resolution limits (Å)	55.7 - 2.2
Number of reflections (working set)	8688
Number of RNA atoms (no hydrogens)	1347
Number of water molecules	72
Number of Mg^{2+}	0
Percentage observed	99.08
Percentage of free reflections	9.716
Overall R factor	0.191
Free R factor	0.241
Overall correlation coefficient	0.957
Free correlation coefficient	0.935
Coordinate error based on free R factor (Å)	0.222
Overall figure of merit	0.810
Mean Temperature Factor ($Å^2$)	45.49

Restraint type	**restraints**	**rmsd**	**weight(σ)**
rmsd bond lengths (Å)	1503	0.009	0.021
rmsd bond angles (^{o})	2331	1.676	3.000
chiral centers ($Å^3$)	312	0.076	0.200
rmsd planar angles (Å)	649	0.007	0.020
van der Waals repulsions (Å)	572	0.165	0.200
van der Waals torsions (Å)	913	0.277	0.200
H-bond restraints (Å)	100	0.161	0.200
crystal packing van der Waals (Å)	44	0.127	0.200
crystal packing H-bond restraints (Å)	9	0.164	0.200
σ chain bond β ($Å^2$)	2111	1.204	3.000
σ chain angle β ($Å^2$)	2331	1.628	4.500

Data were processed using MOSFLM and CCP4. The crystal structure was phased to 2.2 Å resolution using Br peak and inflection point data within the crystallographic software suite CNS. Solvent flattening and simulated annealing molecular dynamics were also performed within CNS. Model building was performed in O. The final refinement was carried out using REFMAC and COOT.

Table S3. Sequential and Canonical Numbering Schemes for the Enzyme and Substrate Strands

Substrate strand		Enzyme strand	
Sequential numbering	Canonical numbering	Sequential numbering	Canonical numbering
5'	5'	5'	5'
G-1	G-16.5	GDP-1	GDP-2.10
G-2	G-16.4	G-2	G-2.9
C-3	C-16.3	A-3	A-2.8
G-4	G-16.2	U-4	U-2.7
U-5	U-16.1	G-5	G-B1.1
C-6	C-17	U-6	U-B1.2
C-7	C-1.1	A-7	A-B1.3
U-8	U-1.2	C-8	C-B1.4
G-9	G-1.3	U-9	U-2.6
G-10	G-1.4	A-10	U-2.5
U-11	U-1.5	C-11	C-2.4
A-12	A-1.6	C-12	C-2.3
U-13	U-B1.5	A-13	G-2.2
C-14	C-B1.6	G-14	G-2.1
C-15	C-B1.7	C-15	C-3
A-16	A-B1.8	U-16	U-4
A-17	A-1.7	G-17	G-5
U-18	U-1.8	A-18	A-6
C-19	C-1.9	U-19	U-7
dC-20	dC-1.10	G-20	G-8
3'	3'	A-21	A-9
		G-22	G-10.1
		U-23	U-10.2
		C-24	C-10.3
		C-25	C-10.4
		C-26	C-L2.1
		A-27	A-L2.2
		A-28	A-L2.3
		A-29	A-L2.4
		U-30	U-L2.5
		A-31	A-L2.6
		G-32	G-11.4
		G-33	G-11.3
		A-34	A-11.2
		C-35	C-11.1
		G-36	G-12
		A-37	A-13
		A-38	A-14
		A-39	A-15.1
		C-40	C-15.2
		G-41	G-15.3
		C-42	C-15.4
		C-43	C-15.5
		3'	3'



The sequential and canonical numbering schemes for the enzyme and substrate strands are provided for convenient translation.