Supporting Information

The hammerhead self-cleaving motif as a precursor to complex endonucleolytic ribozymes

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Supplemental figures S1-S7

Supplemental table 1

Supplemental references

SUPPLEMENTAL FIGURES

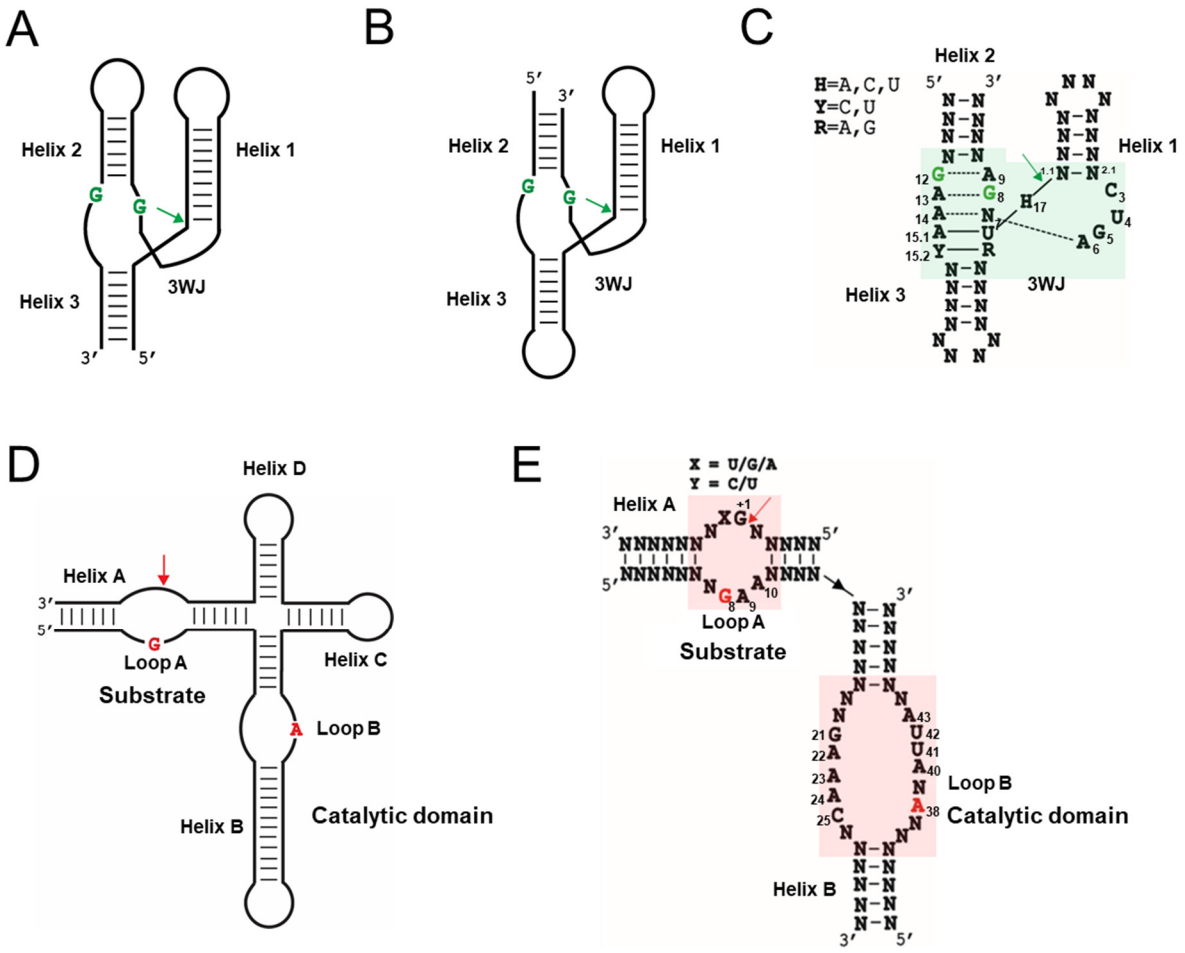

Figure S1. The hammerhead (HH) and hairpin (HP) ribozymes. *(A)* Secondary structure of the wild-type HH ribozyme (type III). Catalytic nucleotides are shown in green, and the cleavage site is indicated by a green arrow. *(B)* Secondary structure of the wild-type HH ribozyme (type II-circular permutation of type III). *(C)* Consensus sequence for a type II HH ribozyme. Conserved/catalytically important nucleotides are highlighted in a green box and residues are numbered according to convention (Hertel, Pardi et al. 1992). *(D)* Secondary structure of wild-type HP ribozyme. Catalytic nucleotides are shown in red, and the cleavage site is indicated by a red arrow. *(E)* Consensus sequence for a minimal HP ribozyme consisting of the substrate and catalytic domains. Conserved/catalytically important nucleotides are highlighted in a red box and residues are numbered according to convention (Fedor 2000).

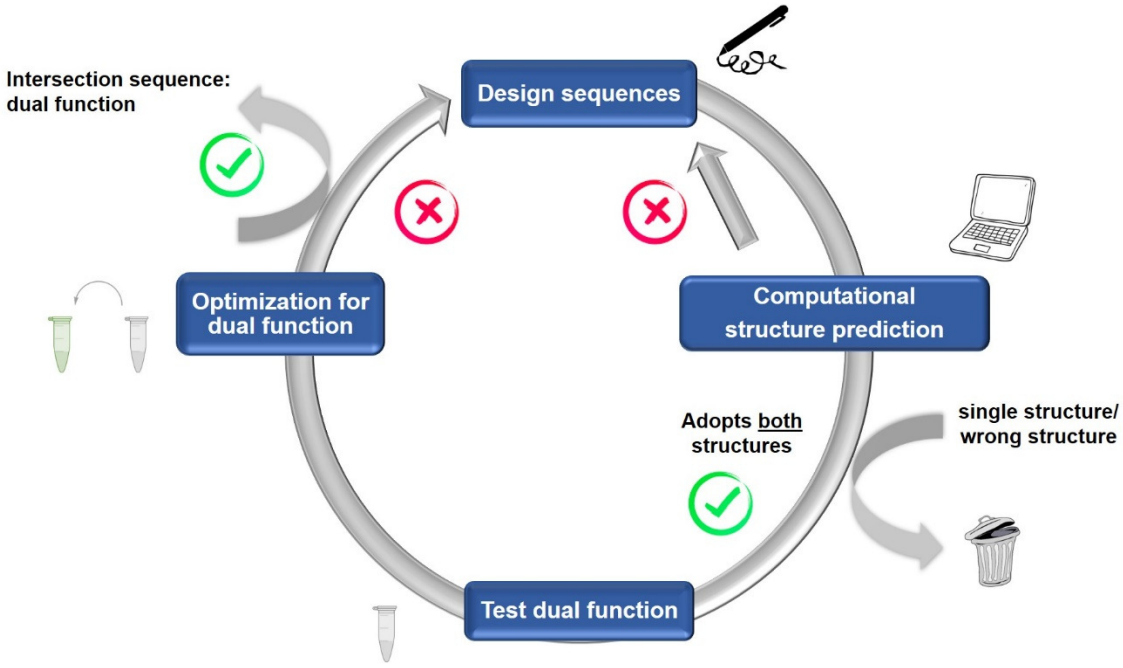

Figure S2. Rational design of intersection sequence, INT, with dual HH-HP activity. The process begins with identifying sequences that can potentially be threaded through the secondary structures corresponding to the HH ribozyme and the catalytic domain of the HP ribozyme (see Fig. S1). These sequences were screened by *in silico* folding algorithms. Sequences that adopted the desired structures were selected and sequences that misfolded or exhibited a single structure were discarded. Selected sequences were tested for *in vitro* cleavage. INT self-cleavage was tested for HH activity and linked to a HP substrate to test HP cleavage. In most cases sequences that exhibited both HH and HP secondary structures *in silico* did not exhibit dual cleavage, therefore sequence optimization (by introducing mutations) was performed to achieve dual function. If the desired bifunctional activity was not obtained, new sequences were designed.

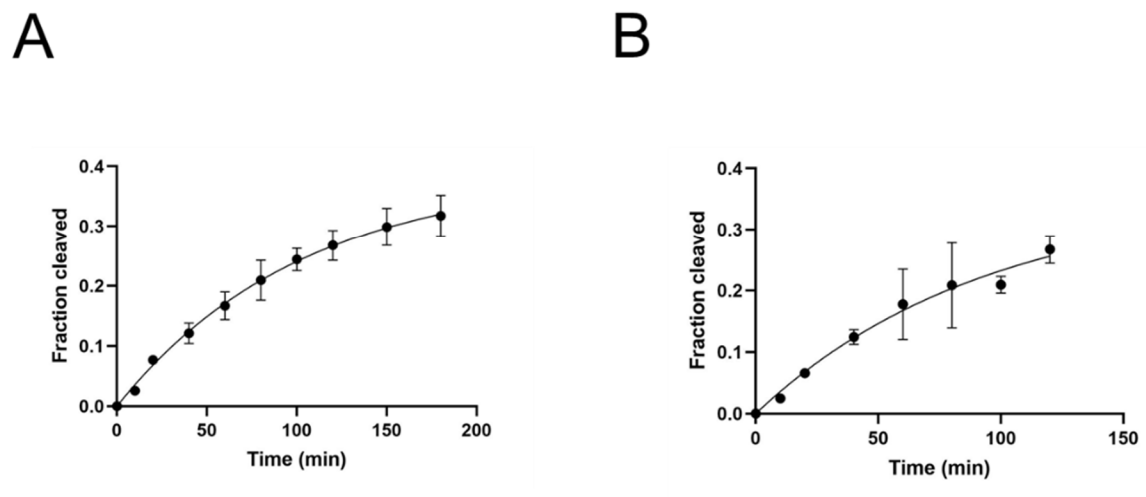

Figure S3. Kinetics of *in vitro* cleavage catalyzed by intersection sequence, INT. *(A)* Self cleavage corresponding to HH activity. *(B)* Self cleavage of INT-HP corresponding to HP activity. Cleavage rates for both ribozymes are ~0.01 min^{-1} (~1000-fold rate acceleration compared to background cleavage).

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5′ GCCUCAGCGAAACAGCUGAUCGUUAUUAGCUGUAUAGUUUGCGAGUCCGUAAAUUAACUGAGGAGCUGAG 3′  HH4
5′ GCCUCAGCGAAACAGCUGAUCGUUAUUAGCUGUAUAGUUUGCGAGUGCGUAAAUUAACUGAGGAGCUGAG 3′  HH3
5′ GCCUCAGCGAAACAGCUGAUCGUUAUUAGCUGUAUAGUUUGCGAGAGCGUAAAUUAACUGAGGAGCUGAG 3′  HH2
5′ GCCUCAGCGAAACAGCUGAUCGUUAUUAGCUGUAUAGUUUGCGACAGCGUAAAUUAACUGAGGAGCUGAG 3′  HH1
5′ GCCUCAGCGAAACAGCUGAUCGUUAUUAGCUGUAUAGUUUGCGUCAGCGUAAAUUAACUGAGGAGCUGAG 3′  INT
5′ GCCUCAGCGAAACAGCUGAUGGUUAUUAGCUGUAUAGUUUGCGUCAGCGUAAAUUAACUGAGGAGCUGAG 3′  HP1
5′ GCCUCAGCGAAACAGCUGAUGCUUAUUAGCUGUAUAGUUUGCGUCAGCGUAAAUUAACUGAGGAGCUGAG 3′  HP2
5′ GCCUCAGCGAAACAGCUGAUGCAUAUUAGCUGUAUAGUUUGCGUCAGCGUAAAUUAACUGAGGAGCUGAG 3′  HP3
5′ GCCUCAGCGAAACAGCUGAUGCAAAUUAGCUGUAUAGUUUGCGUCAGCGUAAAUUAACUGAGGAGCUGAG 3′  HP4
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Figure S4. Single point mutations connect exclusive HH (HH4) and HP (HP4) ribozymes via the intersection sequence (INT). Point mutations to INT involved in the divergence to the HH ribozyme and the catalytic domain of the HP ribozyme are shown in green and red, respectively.

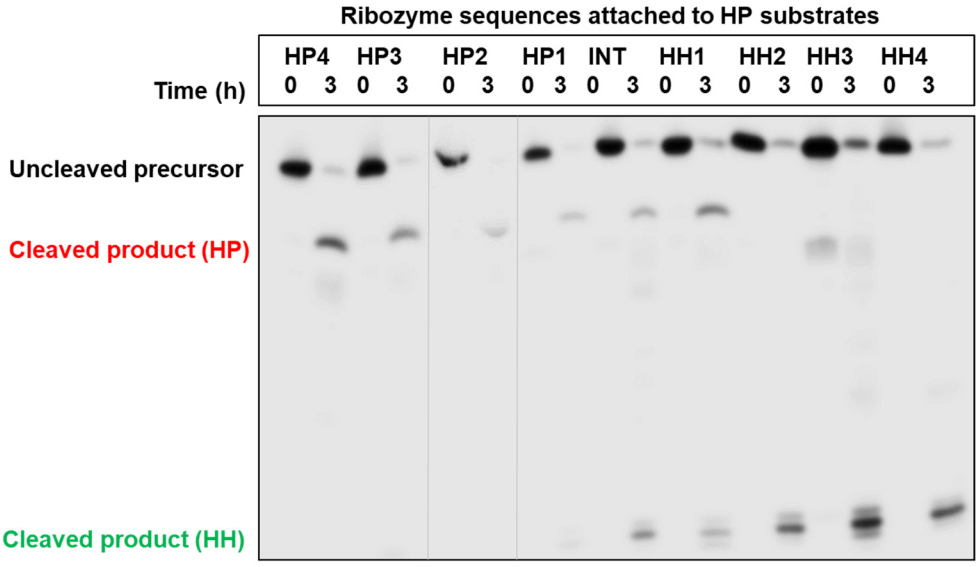

Figure S5. Sequence variants of bifunctional sequence, INT, connected by single point mutations generate either HP or HH ribozymes. INT and HH1 connected to a HP substrate exhibits both HH and HP cleavage generating two product bands (top: HP cleavage, bottom: HH cleavage). Constructs created with HH2, HH3, and HH4 sequences fused to a HP substrate generated one product band corresponding to HH cleavage (bottom band), while constructs created with HP1, HP2, HP3, and HP4 sequences fused to a HP substrate generated one product band corresponding to HP cleavage (top band) (See Figure S4, Table S1 for sequence details).

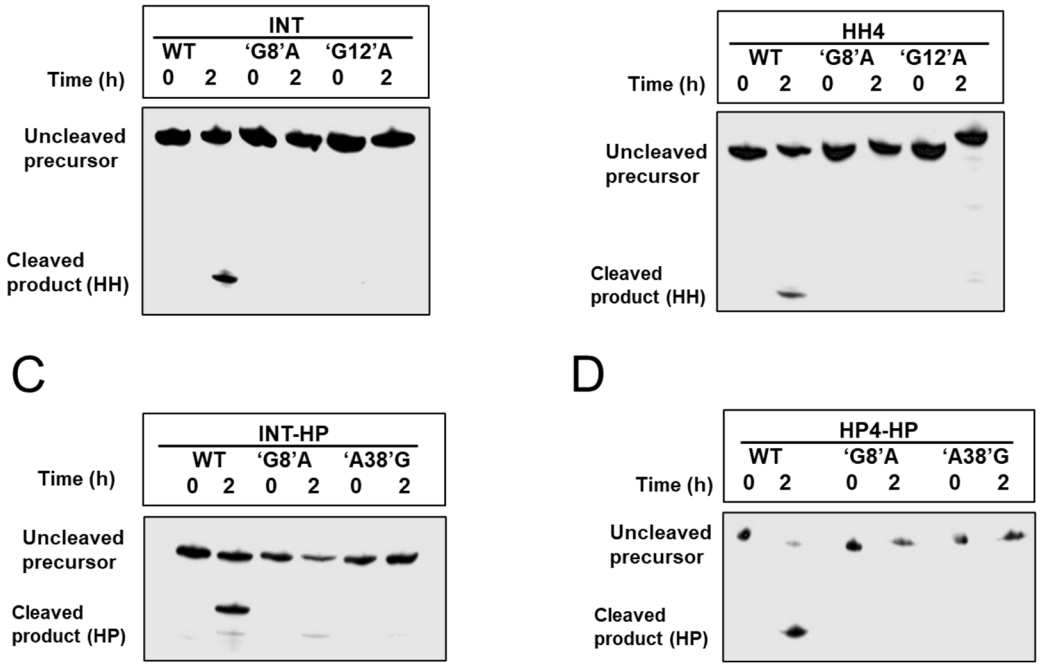

Figure S6. Mutations to catalytic residues of the HH and HP folds abolish activity. 'G8'A and 'G12'A mutations to *(A)* bifunctional sequence, INT and *(B)* exclusive hammerhead sequence, HH4 abolish hammerhead cleavage. **'**G8'A and 'A38'G mutations to *(C)* bifunctional sequence, INT-HP (HH cleavage bands cropped) and *(D)* exclusive hairpin sequence, HP4 abolish hairpin cleavage.

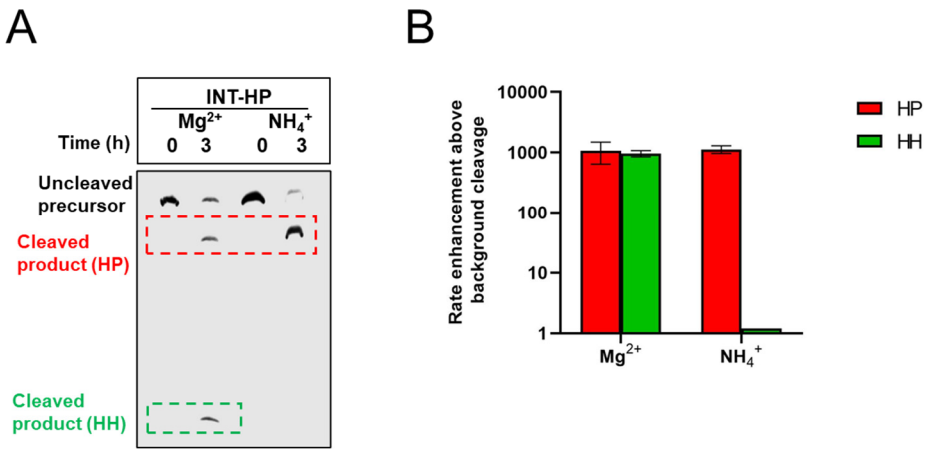

Figure S7. INT-HP loses its HH cleavage activity in 4 M NH_4^+ but retains HP function. Replacing Mg^{2+} with NH_4^+ converts bifunctional sequence, INT-HP, into an exclusive HP ribozyme by eliminating HH cleavage.

SUPPLEMENTAL TABLE 1. RNA sequences used in this work

Construct	Sequence
HH4	GCCUCAGCGAAACAGCUGAUCGUUAUUAGCUGUAUAGUUUGCGAGUCCGUA AAUUAACUGAGGAGCUGAG
HH4-HP	GCCUCAGCGAAACAGCUGAUCGUUAUUAGCUGUAUAGUUUGCGAGUCCGUA AAUUAACUGAGGAGCUGAGauuuuacuaacaagaucGGCAGUCGGCCGUCGUCGCGC CAGAAGCCauu
HH3	GCCUCAGCGAAACAGCUGAUCGUUAUUAGCUGUAUAGUUUGCGAGUGCGUA AAUUAACUGAGGAGCUGAG
HH3-HP	GCCUCAGCGAAACAGCUGAUCGUUAUUAGCUGUAUAGUUUGCGAGUGCGUA AAUUAACUGAGGAGCUGAGauuuuacuaacaagaucGGCAGUCGGCCGUCGUCGCGC CAGAAGCCauu
HH2	GCCUCAGCGAAACAGCUGAUCGUUAUUAGCUGUAUAGUUUGCGAGAGCGUA AAUUAACUGAGGAGCUGAG
HH2-HP	GCCUCAGCGAAACAGCUGAUCGUUAUUAGCUGUAUAGUUUGCGAGAGCGUA AAUUAACUGAGGAGCUGAGauuuuacuaacaagaucGGCAGUCGGCCGUCGUCGCGC CAGAAGCCauu
HH1	GCCUCAGCGAAACAGCUGAUCGUUAUUAGCUGUAUAGUUUGCGACAGCGUA AAUUAACUGAGGAGCUGAG
HH1-HP	GCCUCAGCGAAACAGCUGAUCGUUAUUAGCUGUAUAGUUUGCGACAGCGUA AAUUAACUGAGGAGCUGAGauuuuacuaacaagaucGGCAGUCGGCCGUCGUCGCGC CAGAAGCCauu
INT	GCCUCAGCGAAACAGCUGAUCGUAAUUAGCUGUAUAGUUUGCGUCAGCGUA AAUUAACUGAGGAGCUGAG\
INT-HP	GCCUCAGCGAAACAGCUGAUCGUUAUUAGCUGUAUAGUUUGCGUCAGCGUA AAUUAACUGAGGAGCUGAGauuuuacuaacaagaucGGCAGUCGGCCGUCGUCGCGC CAGAAGCCauu
HP1	GCCUCAGCGAAACAGCUGAUCGUAAUUAGCUGUAUAGUUUGCGUCAGCGUA AAUUAACUGAGGAGCUGAG
HP1-HP	GCCUCAGCGAAACAGCUGAUCGUAAUUAGCUGUAUAGUUUGCGUCAGCGUA AAUUAACUGAGGAGCUGAGauuuuacuaacaagaucGGCAGUCGGCCGUCGUCGCGC CAGAAGCCauu
HP2	GCCUCAGCGAAACAGCUGAUCGAAAUUAGCUGUAUAGUUUGCGUCAGCGUA AAUUAACUGAGGAGCUGAG
HP2-HP	GCCUCAGCGAAACAGCUGAUCGAAAUUAGCUGUAUAGUUUGCGUCAGCGUA AAUUAACUGAGGAGCUGAGauuuuacuaacaagaucGGCAGUCGGCCGUCGUCGCGC CAGAAGCCauu
HP3	GCCUCAGCGAAACAGCUGAUCCAAAUUAGCUGUAUAGUUUGCGUCAGCGUA AAUUAACUGAGGAGCUGAG
HP3-HP	GCCUCAGCGAAACAGCUGAUCCAAAUUAGCUGUAUAGUUUGCGUCAGCGUA AAUUAACUGAGGAGCUGAGauuuuacuaacaagaucGGCAGUCGGCCGUCGUCGCGC CAGAAGCCauu
HP4	GCCUCAGCGAAACAGCUGAUGCAAAUUAGCUGUAUAGUUUGCGUCAGCGUA AAUUAACUGAGGAGCUGAG

HP4-HP	GCCUCAGC**G**AAACAGCUGAUGCAAAUUAGCUGU***AU***AGUUUGCGUCAGCGU**A** AAUUAACUGAG**G**AGCUGAGauuuuacuaacaagaucGGC***AG***UCGGCCGUCGUCGCGC CA**G**AAGCCauu

Hairpin catalytic residues: red, Hammerhead catalytic residues: green; bold italics: cleavage site nucleotides – red for hairpin and green for hammerhead; lowercase: linker connecting INT and its variants to the HP substrate, and 3' substrate overhang

SUPPLEMENTAL REFERENCES

1. Fedor, M. J. (2000). "Structure and function of the hairpin ribozyme." *J. Mol. Biol.* **297**: 269-291.
2. Hertel, K. J., A. Pardi, O. C. Uhlenbeck, M. Koizumi, E. Ohtsuka, S. Uesugi, R. Cedergren, F. Eckstein, W. L. Gerlach, R. Hodgson and et al. (1992). "Numbering system for the hammerhead." *Nucleic Acids Res.* **20**: 3252.