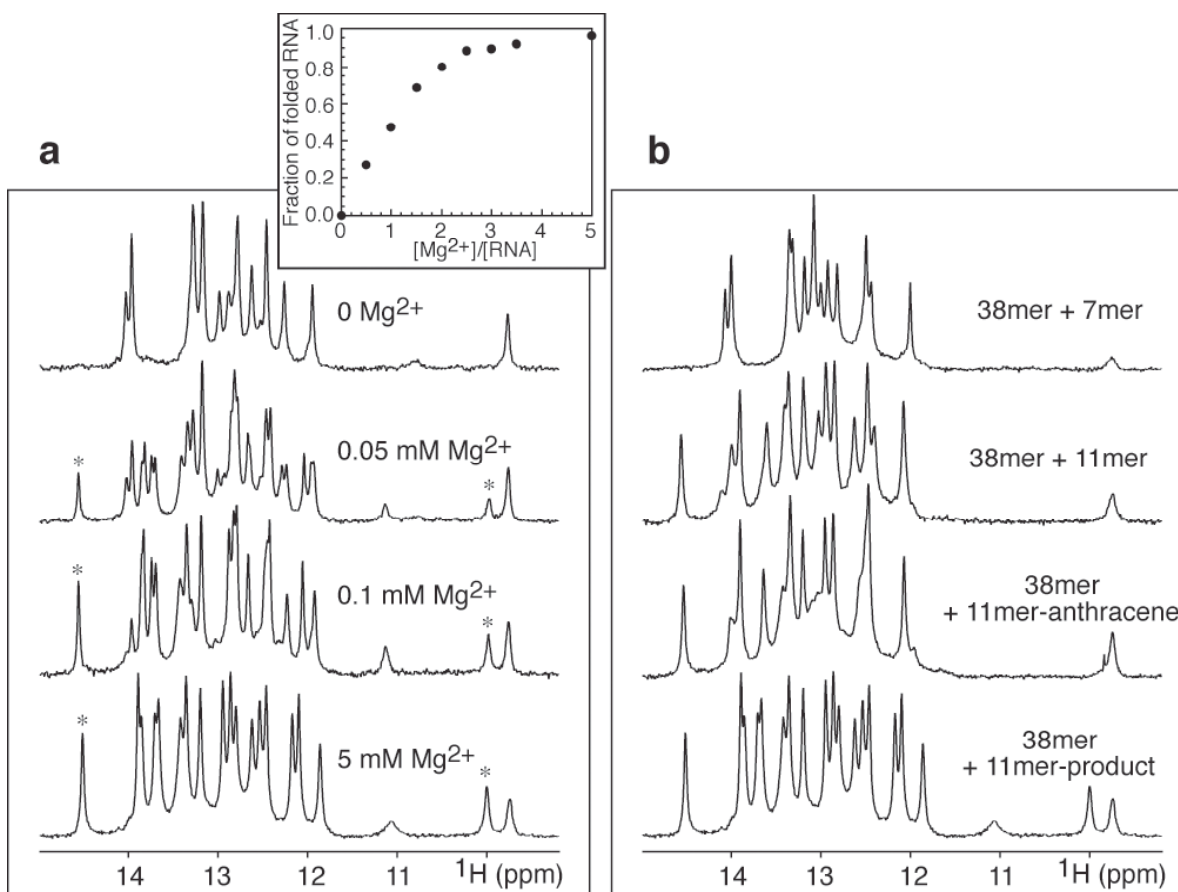
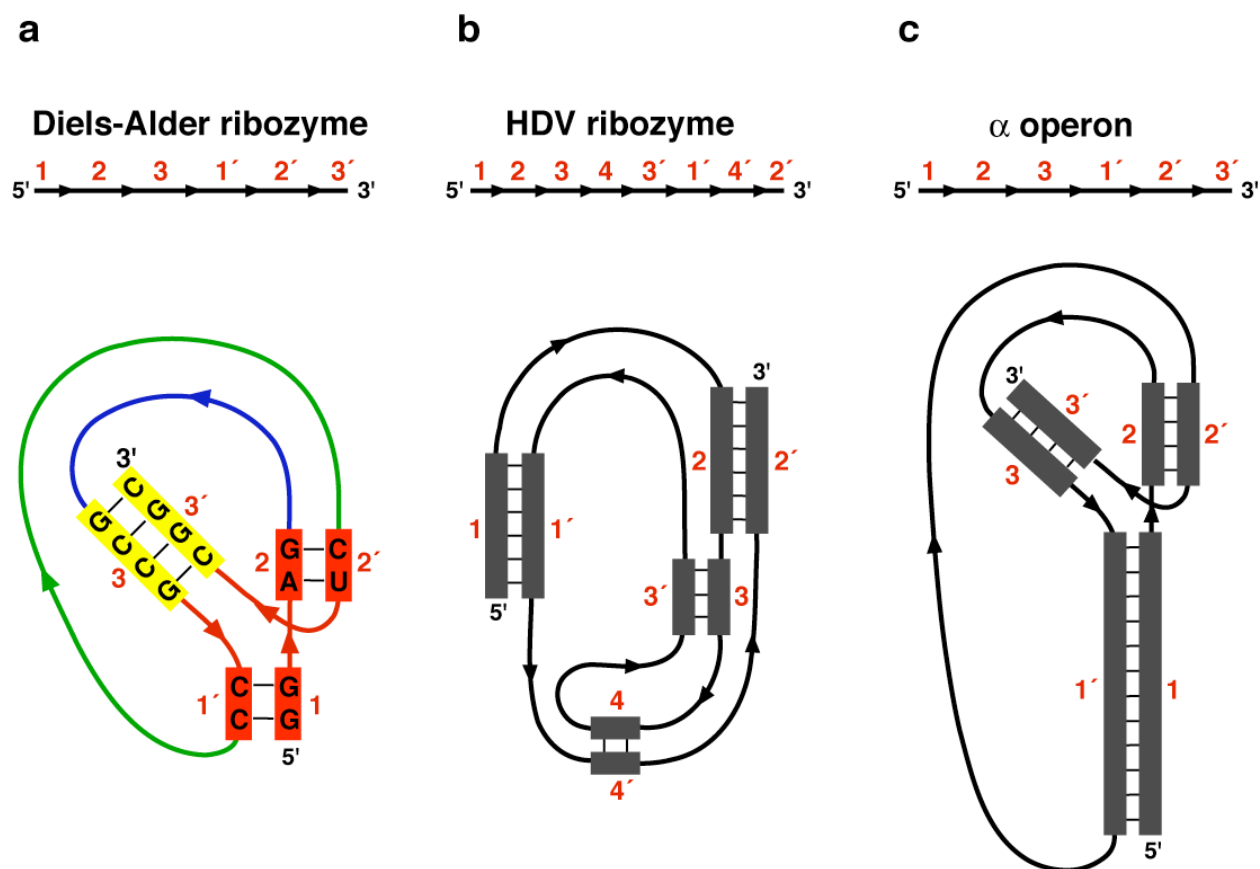


Supplementary Figure 1 Pairing alignments, turns and extensions within the structure of the ribozyme-product complex. (a) The alignment of the G27•A40 non-canonical pair stacked over the A41•(G1-C26) three-base platform. (b) The A41•(G1-C26) platform stacked over the U42•(G2-C25) triple. (c) Intercalation of G24 (in green) between the extended U42-A43 step,

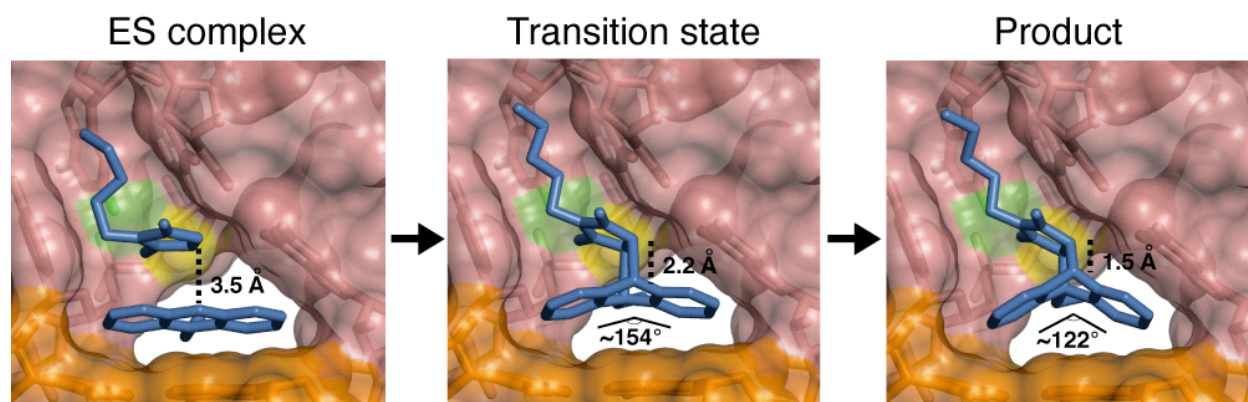
together with hydrogen bond alignments involving the Watson-Crick edge and sugar 2'-OH of G24. **(d)** The A43-C44-U45-C46 turn, stabilized by Mg1 and Mg2, with stacking of A43 and C46. **(e)** The U23-G24-C25-C26 segment, with splaying out of adjacent G24 and C25 bases. **(f)** Surface representation of the packing of the C44-U45 backbone segment of stem I (blue) positioned against the major groove of stem II (gold), mediated by bridging Mg1 and Mg2 cations.



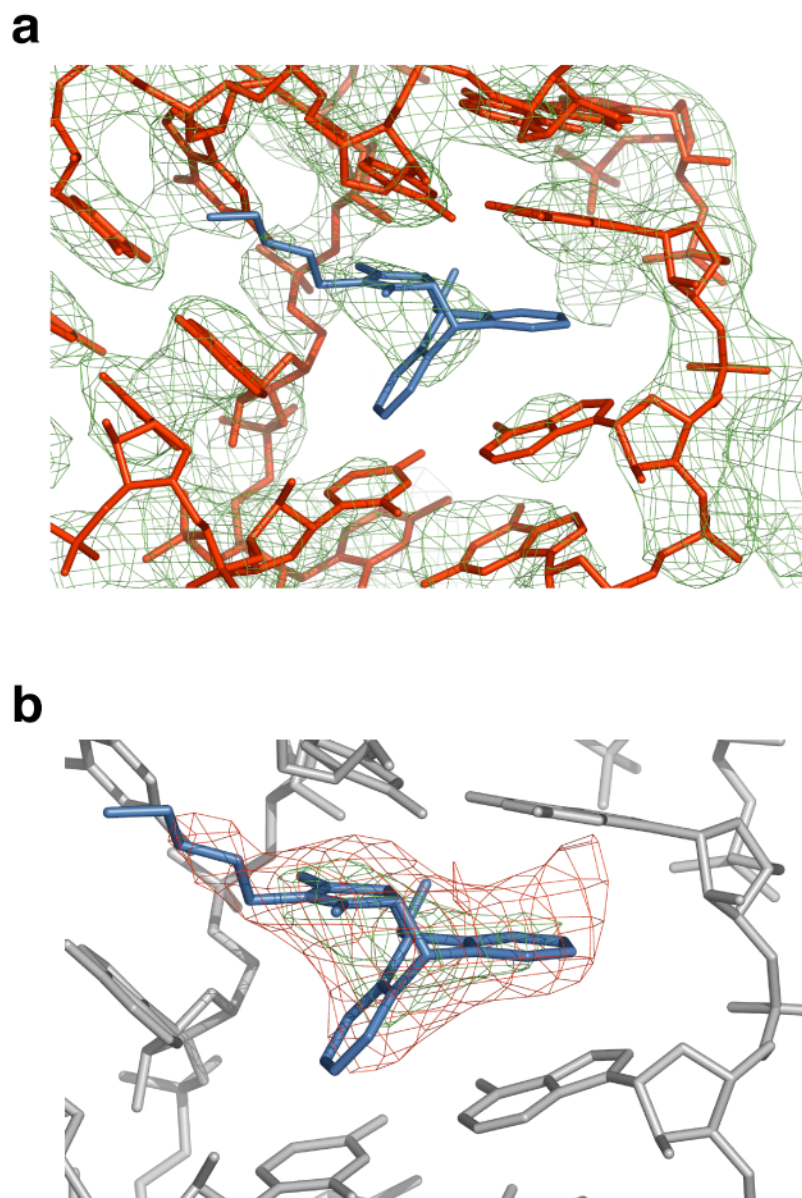
Supplementary Figure 2 Folding of the Diels-Alder ribozyme monitored by imino proton NMR spectra (9 to 15 ppm). **(a)** Mg²⁺-dependence of the spectra of the 38-mer plus 11-mer-HEG-linked-product. Titration data are summarized in the insert, which shows that the majority of the RNA is folded at RNA:Mg²⁺ ratio of 1:2. Peaks used for quantification of Mg-dependent RNA folding are indicated by stars. Note that many more imino protons of the Diels-Alder product are observed on addition of Mg²⁺ to a sample lacking divalent cations. Also note that the exchange between Mg-free and Mg-bound states is slow, resulting in doubling of spectra. NMR spectra are recorded in 30 mM NaCl, 5 mM Tris buffer at pH 7.2. The RNA concentration was 0.05 mM. **(b)** NMR spectra of the ribozyme components. The 7-mer lacks the G1-G2-A3-G4 segment. The 11-mer is either free or HEG-linked with anthracene or product. The pairing of G1-G2-A3-G4 segment is reflected by appearance of additional imino protons. NMR spectra are recorded in 5 mM MgCl₂, 5 mM NaCl, 5 mM Tris buffer, pH 7.2. All NMR spectra were recorded at 25 °C, using jump-and-return water suppression for detection (Plateau & Gueron, (1982), *J. Am. Chem. Soc.* 104, 7310-7311; Phan *et al.*, (2001), *Methods Enzymology*, **338**, 341-371).



Supplementary Figure 3 Schematic drawings of RNA pseudoknot topologies. **(a)** The Diels-Alder ribozyme, which adopts a 1-2-3-1'-2'-3' topology. For comparison, the same coloring scheme as in **Figure 1** was used. Note that the blue and green connectors are partly double-stranded, but not involved in pseudoknot interactions. **(b)** The HDV ribozyme, which adopts a 1-2-3-4-3'-1'-4'-2' topology²⁹. **(c)** The regulatory region of *E. coli* α operon, which adopts a 1-2-3-1'-2'-3' topology³⁰.



Supplementary Figure 4 Proposed model for the catalytic mechanism of the Diels-Alder ribozyme. Enzyme-substrate (ES) complex and transition state models have been obtained by manually docking the molecules into the pocket. The transition state was computed using MOPAC. The product-RNA complex is derived from the crystallographic structure. The surfaces of the A3-U45 base pair, amino group of G24 and 2'-OH of U42 are orange, green and yellow, respectively. Angles showing the buckling of the anthracene ring system and key distances during formation of the carbon-carbon bonds are indicated.



Supplementary Figure 5 Electron density maps of the catalytic pocket of the Diels-Alder ribozyme. **(a)** Solvent-flattened SAD electron density map (green) for the cycloaddition product (blue) and surrounding RNA (red), contoured at 1σ level. **(b)** F_o-F_c map, contoured at 2σ (green) and 1σ (red) levels, calculated for the product region after the RNA refinement and prior to the addition of the product to the model. The product and RNA, superimposed with the density, are from the refined model.

Supplementary Methods

Protocol for preparation and purification of the 11-mer Diels-Alder product

Synthesis of the stereochemically correct 11-mer with the HEG-linked Diels-Alder product was achieved by reaction between N-pentyl maleimide and anthracene-linked 11-mer annealed with 38-mer RNA, and subsequent HPLC purification.

A solution of 0.33 mM 38-mer and 0.2 mM 11-mer anthracene-conjugate in buffer (30 mM Tris-HCl pH 7.4, 300 mM NaCl) was heated to 65 °C for 4 min and allowed to cool to room temperature for 15 min. 80 mM MgCl₂ was added and the reaction was initiated by addition of N-pentylmaleimide (20 mM in ethanol) to a final concentration 10%. After incubation for 6 min at room temperature, the reaction was stopped by addition of β-mercaptoethanol. The product-oligomer was recovered by reversed phase HPLC on a semipreparative C18-column (Luna, Phenomenex, 10 x 250 mm) at 45 °C. Flow rate: 3 ml/min; eluent A: 0.1 M triethylammonium acetate (TEAAc) buffer (pH 7.0); eluent B: 0.1 M TEAAC in 80% aq. MeCN (pH 7.0); detection at 260 nm; 1-50% B in A within 15 min followed by 50-60% B in A within 5 min and then 60-74% B in A within 2 min. Fractions containing the purified RNA were desalted by NAP-5 gel filtration (Amersham) and subsequent ethanol precipitation.

Protocol for preparation and purification of Se-labeled RNA oligomers

The 5'-anthracene-HEG-linked 11-mer with U_{Se} at position 2 and C_{Se} at position 10 was converted into the product as described above. The 38-mer was prepared with C_{Se} at positions 15 and 20 and U_{Se} at positions 30 and 33.

Deprotection of the selenium-labeled RNAs and cleavage from the solid support (40 mg; 11-mers, 20 mg; 38-mers) was achieved in the mixture of MeNH₂ in ethanol (8 M, 0.65 ml) and MeNH₂ in water (40%, 0.65 ml) containing 100 mM DTT for 5 h at 25 °C (11-mers) and for 6.5 h at 30 °C (38-mers), respectively; the solution was then evaporated to dryness. Removal of the 2'-O-TOM groups was achieved by treatment with tetrabutylammonium fluoride in tetrahydrofuran (1 M, 0.95 ml) at 42 °C until a clear solution was obtained. The solution was then kept at room temperature for 12 h (11-mers) and for 15 h (38-mers), respectively. The reaction was quenched by the addition of TEAAc buffer (1 M, pH 7.0, 0.95 ml) and the volume of the solution was reduced to 1 ml by evaporation and directly applied on a Sephadex G10 column (30 x 1.5 cm) controlled by UV-detection at 270 nm. The product was eluted with water

(1 ml/min) and the RNA containing fraction was evaporated to dryness. All RNAs were purified by anion-exchange chromatography on a semipreparative Dionex DNAPac column (9 x 250 mm) at 80 °C. Flow rate: 2 ml/min; eluent A: 25 mM Tris-HCl (pH 8.0), 6 M urea; eluent B: 25 mM Tris-HCl (pH 8.0), 0.5 M NaClO₄, 6 M urea; detection at 265 nm; 3-10% B in A within 20 min. Fractions containing the purified RNA were desalted by loading onto a C18 SepPak cartridge (Waters/Millipore), followed by elution with 0.1 - 0.2 M (Et₃NH)HCO₃, water, and then H₂O/CH₃CN (6/4). Combined fractions containing the RNA were lyophilized to dryness.