Supplementary Material

Three critical hydrogen bonds determine the catalytic activity of the Diels-Alderase ribozyme

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Supplementary Figures S1 – S8

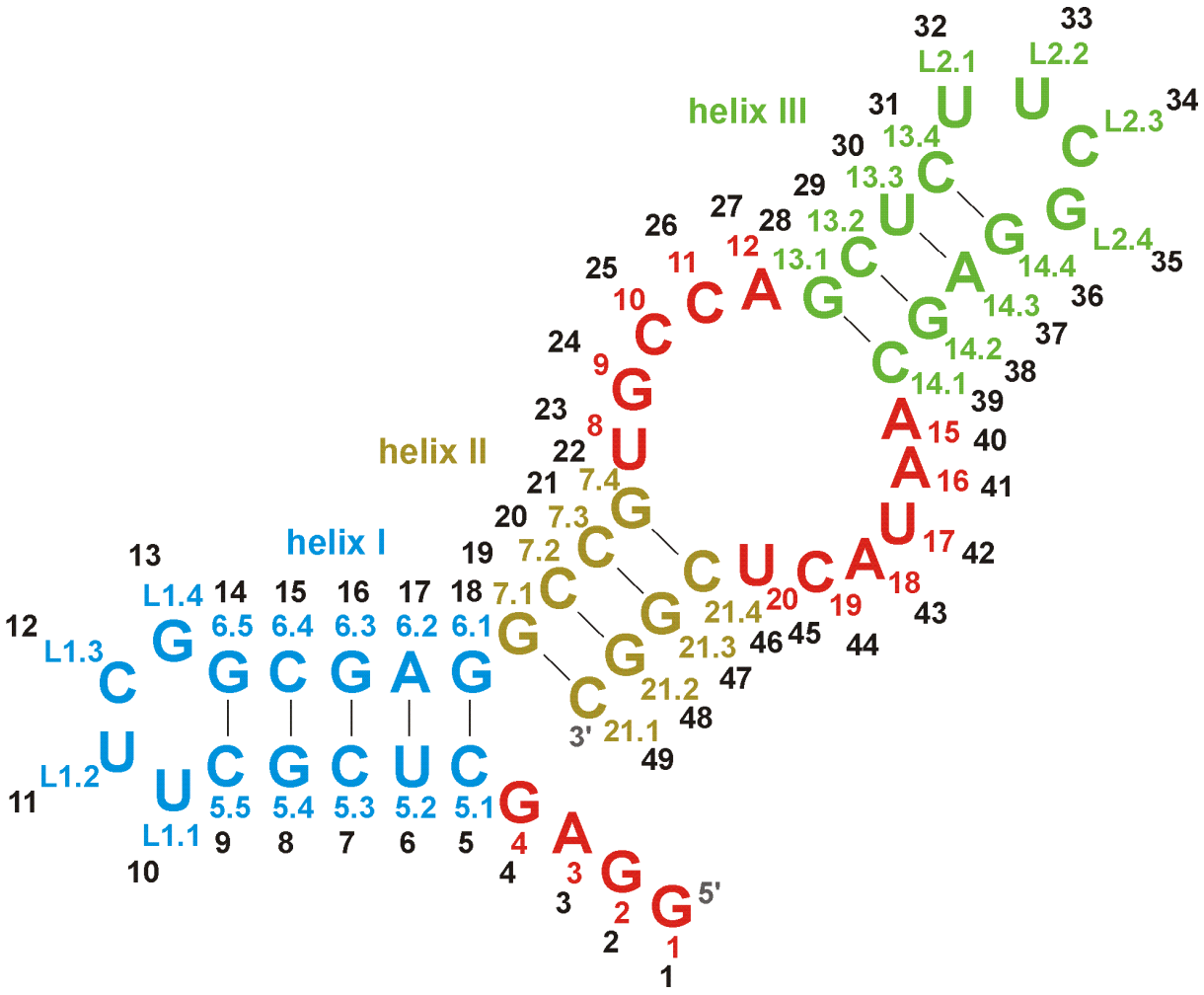

Figure S1. Diels-Alderase ribozyme translation map between the different numbering schemes in use. The colored scheme represents the original numbering (5) used in this manuscript, while Ref. (11) introduced a consecutive numbering scheme shown here in black.

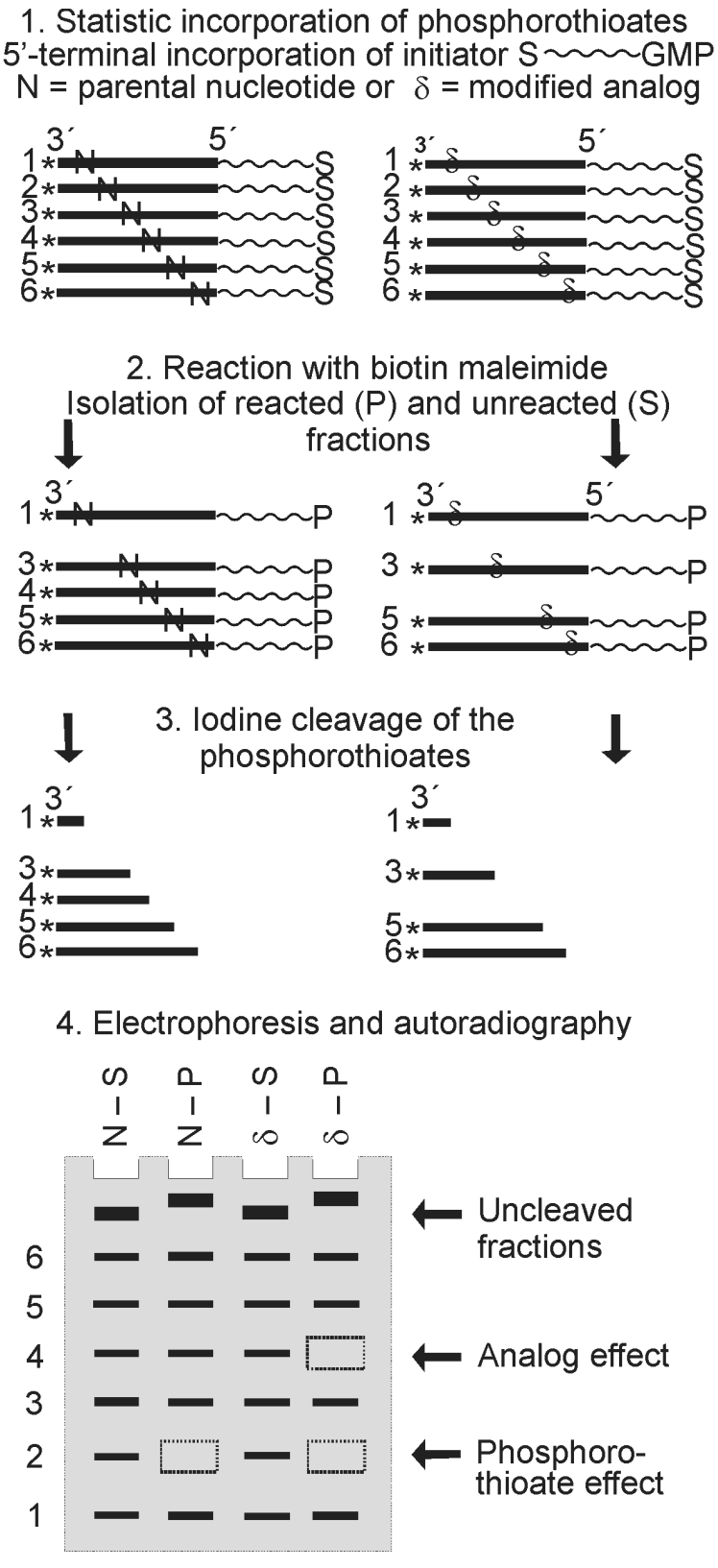

Figure S2. Schematic representation of the NAIM experiments for Diels-Alderase ribozymes.

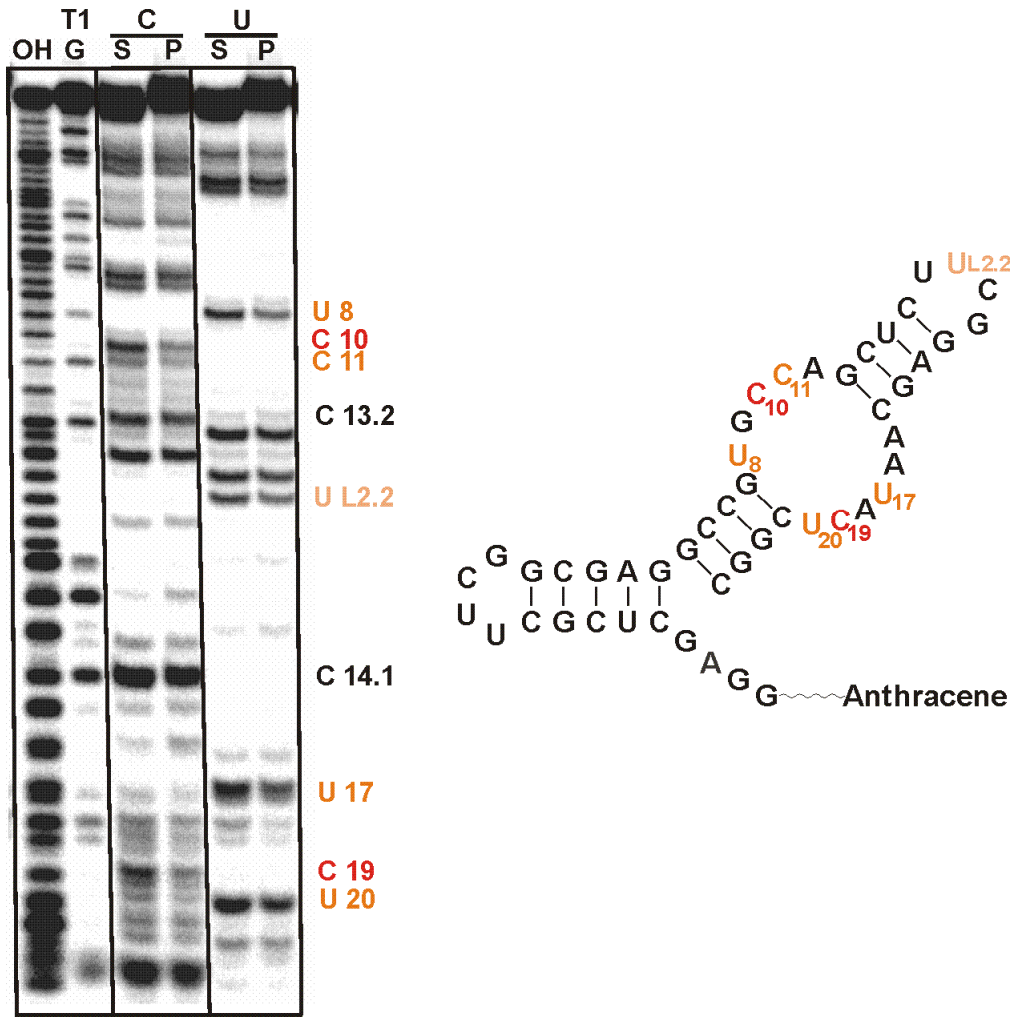

Figure S3. Phosphorothioate interference of Diels-Alderase ribozymes. Left: PAGE gel of the analysis of the parental pyrimidine nucleoside phosphorothioates. Lanes: OH – limited alkaline hydrolysis ladder, T1/G: RNase T1 cleavage ladder for sequence assignment, S – substrate (unreacted) fraction and P – product (reacted) fraction from reaction with biotin maleimide. Right: Summary of the effects. Red – activity 40-45% of wild-type activity, orange – 45-55%, light orange – ~75%, black – full activity.

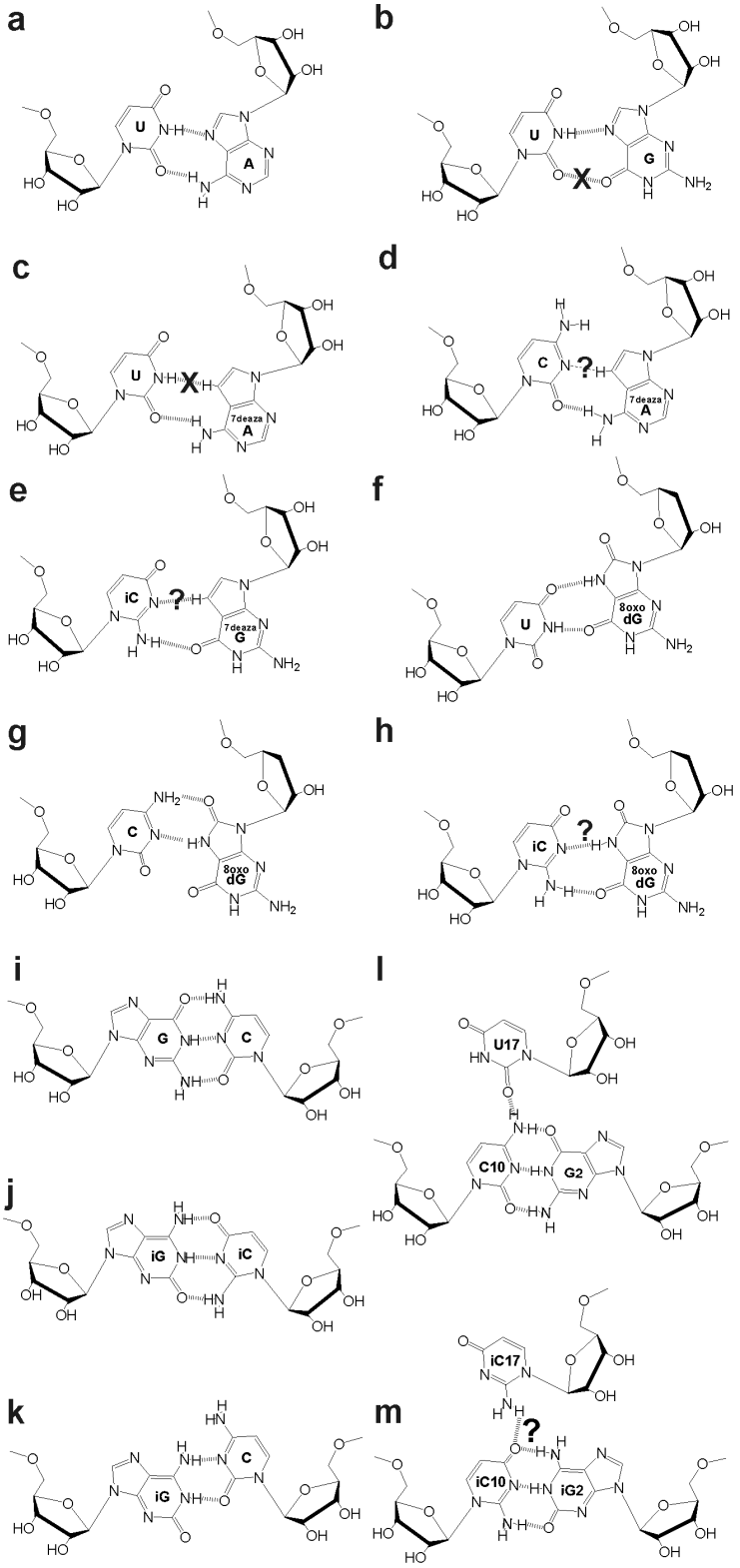

Figure S4. Different base pairing and triple interactions. a-h: combinations designed to support the U8-A18 reverse Hoogsteen base pair. i-k: G-C, isoG-isoC, and isoG-C base pairs. l-m: G2-C10-U17 base triple and the putative isosteric isoG2-isoC10-isoC17 mutant.

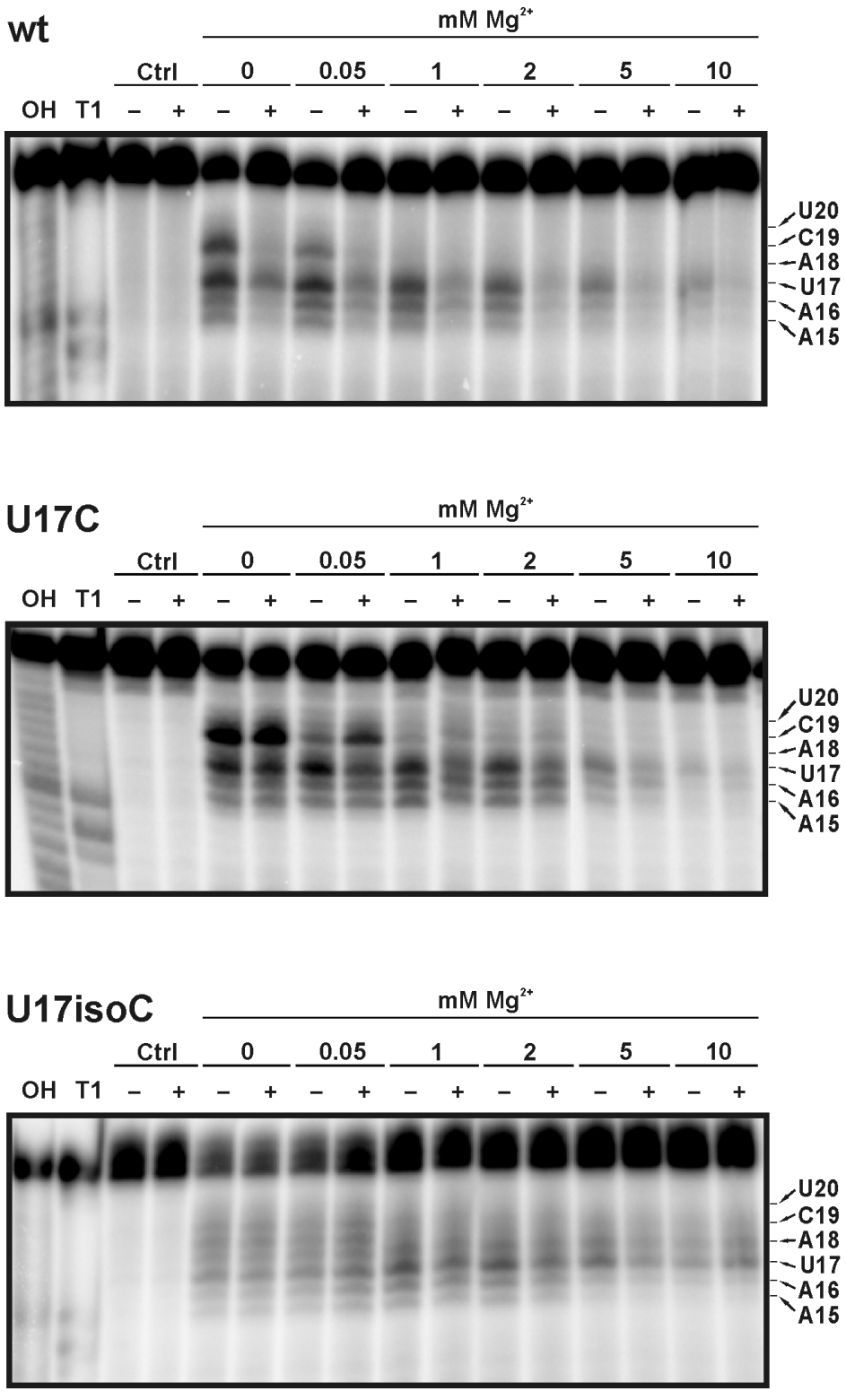

Figure S5. Lead probing PAGE gel showing 5'-^{32}P-labeled wildtype, U17C and U17isoC mutant ribozyme in dependence of Mg^{2+} (0-10 mM) and Diels-Alder product. '+' 1 mM Diels-Alder product, '-', no product. The "+" and "-" lane at one Mg^{2+} ion concentration should be directly compared with each other for changes in the probing pattern. Lane designations as in Fig. 3c.

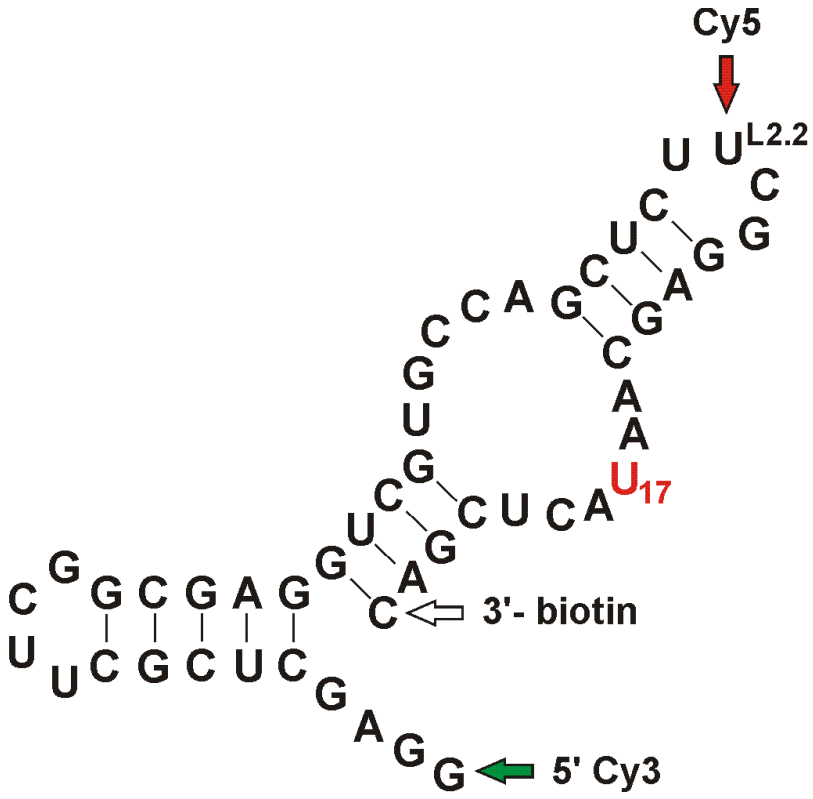

Figure S6. Structure of the dye-labeled ribozyme variants used in the smFRET studies. In position 17, either U or isoC are incorporated.

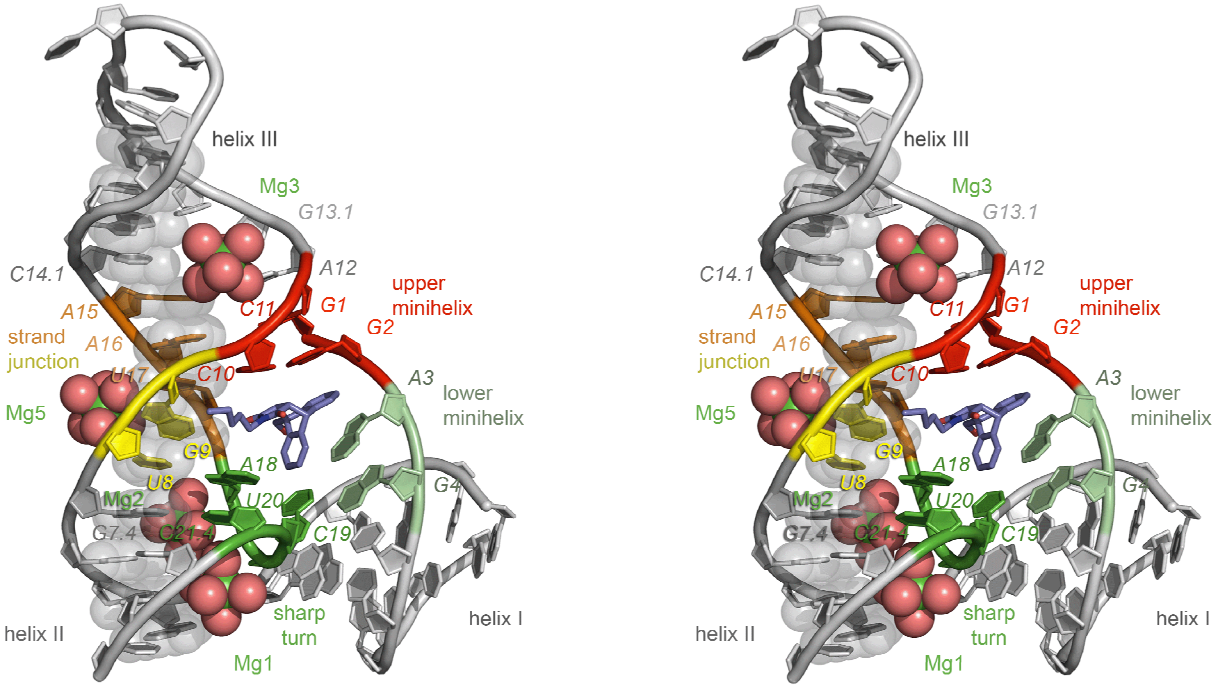

Figure S7. Stereo view (cross-eyed) of the Diels-Alderase ribozyme.

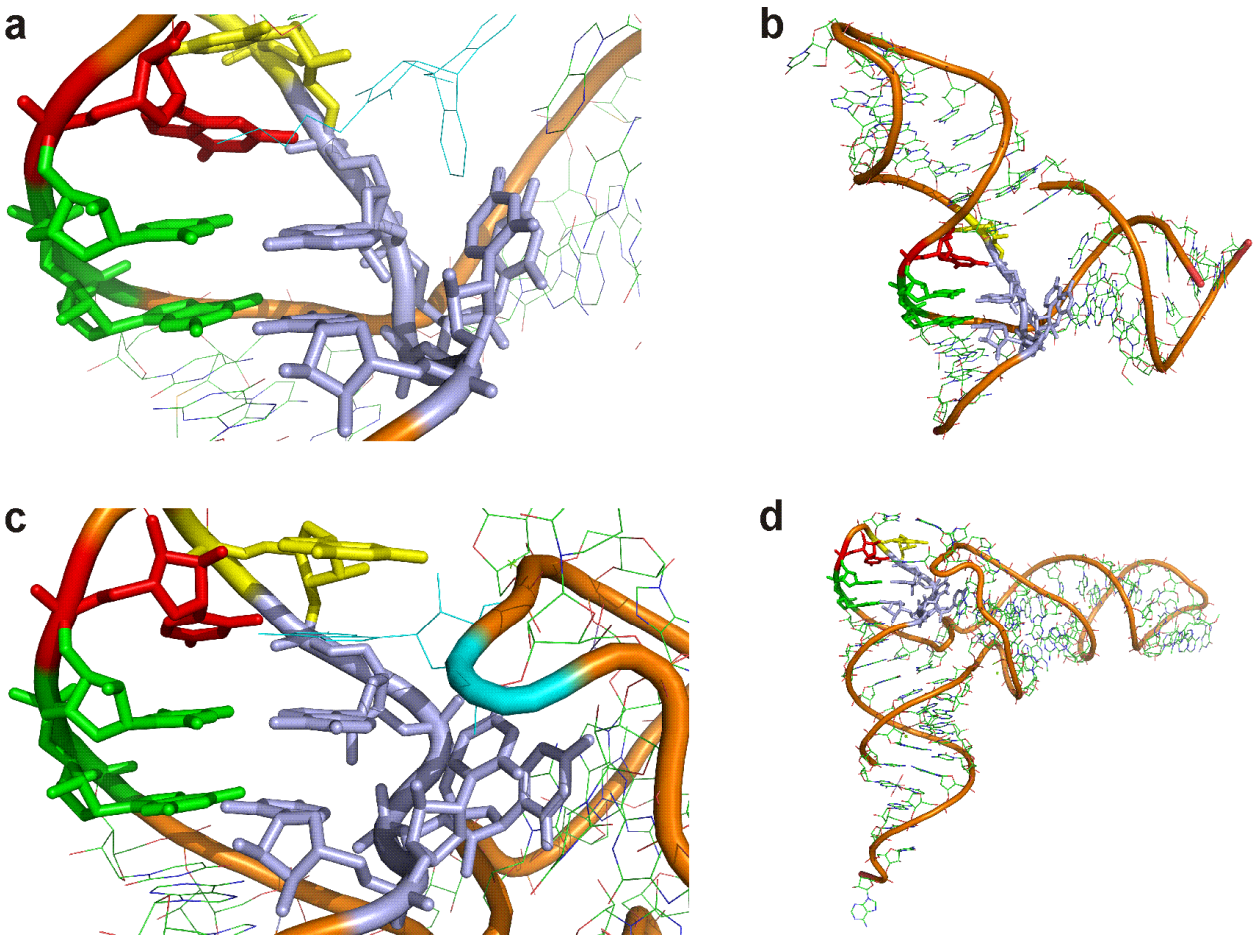

Figure S8. Comparison of the structures of the Diels-Alderase sharp turn (panels **a,b**) and the T-loop of $tRNA^{Phe}$ (**c,d**).