Electronic Supplementary Material (ESI) for ChemComm.

Hairpin ribozyme mediated RNA recombination

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Supplementary Information

Supplementary Figures

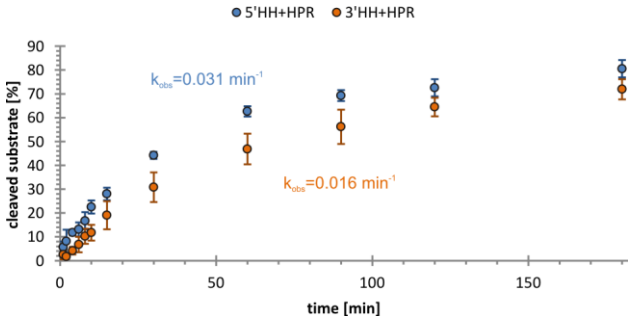

Figure S1: Time course of HPR supported cleavage of recombination substrates 3'HH and 5'HH at equimolar concentration (2 nM).

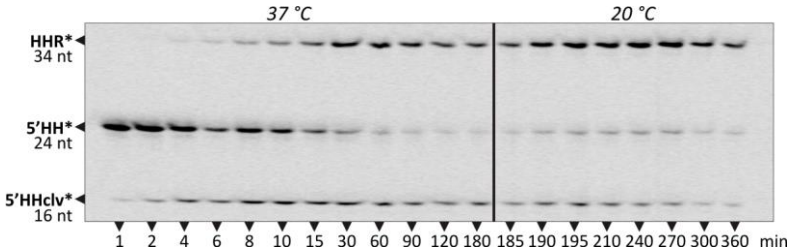

Figure S2: Exemplary denaturing 10% polyacrylamide gel showing recombination reactions over time (compare with figure 2d in the main text) at optimised conditions (50 mM Tris/HCl (pH 7.5), 50 mM $MgCl_2$, 20 nM 5'HH*, 160 nM 3'HH, 80 nM HPR, 3 h 37 °C → 3 h 20 °C). Fluorescence detection was executed with LI-COR 4300 DNA sequencer.

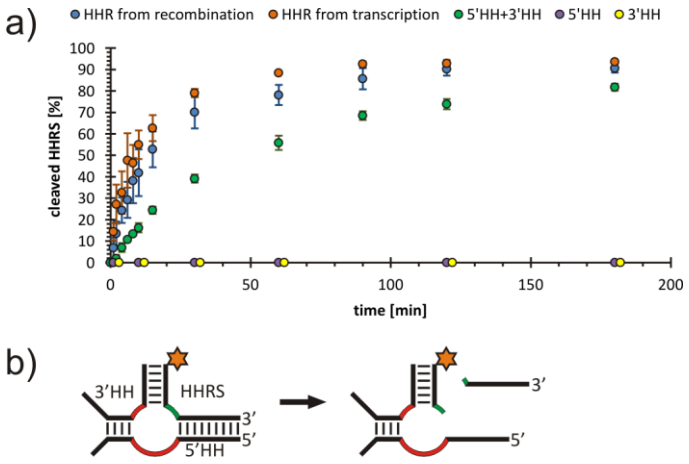

Figure S3: Cleavage activity of the hammerhead ribozyme variants. a) Time courses of reactions. b) Assembly of recombination substrates 5'-HH and 3'HH into a hammerhead ribozyme that is capable of binding and cleaving the substrate HHRS.

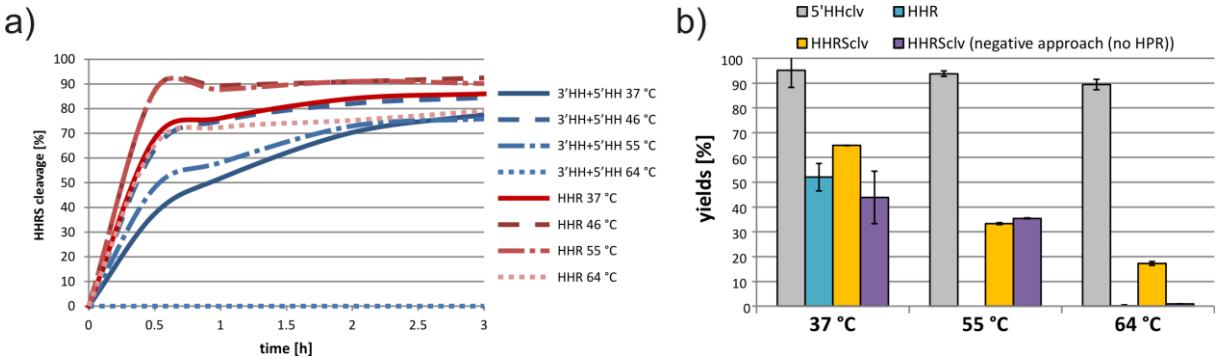

Figure S4: Temperature dependence of HHR mediated cleavage (a) and recombination (b). **a:** Time courses of cleavage reactions of the two-stranded HHR (blue) in comparison to the ligated HHR resulting from recombination (red). Cleavage conditions: 20 nM ribozyme, 20 nM substrate in 50 mM Tris/HCl (pH 7.5), 50 mM $MgCl_2$. **b**: Recombination and HHR cleavage yields in dependence on temperature. Recombination conditions: 50 mM Tris/HCl (pH 7.5), 50 mM $MgCl_2$, 20 nM 5'HH*, 80 nM 3'HH, 40 nM HPR, 2h, followed by addition of the HHR substrate (HHRS*), denaturation and re-incubation for 1h. Fractions of the cleaved recombination substrate 5'HH (grey), recombination product HHR (cyan), cleaved HHR substrate (orange) and cleaved HHR substrate in the absence of HPR (control, purple) were measured.

Experimental Procedures

Materials

The hairpin ribozyme and the hammerhead ribozyme for positive control were obtained from *in vitro* transcription using DNA oligonucleotides (HPR template: 5'-TGATGAAAAT AGCTGGTAAT GTACCGAATC CGAAGATTCG TGTTTCTCAG CTGTGGTTCT CCACGATCTC CCTATAGTGA GTCGTATTA-3', HHR template: 5'-GGTACCGTTT CGTGGGACTC CACTCATCAG GCCTTCTCCC TATAGTGAGT CGTATTA-3', T7 promotor: 5'-TAATACGACTCACTATAGGGAGA-3', purchased from Biomers) and T7 RNA polymerase (produced in-house). The HPR substrates 3'HH (5'-GUGGAGUCCC ACGAAACGGU ACC-3'), 5'HH (5'-$NH_2(CH_2)_6PO_4$-AGGCCUGAUG AGUGGAGUCC CACG-3') and the HHR substrate (5'-$NH_2(CH_2)_6PO_4$-GGUACCGUCG CCUGAGA-3') were generated in-house by phosphoramidite solid-phase synthesis. RNA was purified by denaturing PAGE (10% or 15%, urea and acrylamide (acrylamide/bis-acrylamide 19:1) were purchased from Roth), desalted via precipitation with ethanol and quantified by UV spectrometry.

RNA fluorescence labelling

5'HH, HHRS and 3'HH were labelled with an ATTO680 fluorescence dye. Therefore 5 nmol of the amino linker modified oligonucleotides (see above) were dried and solved in 50 µl of fresh $NaHCO_3$ buffer (200 mM, pH 8.6). This solution was mixed with 50 µg ATT680-NHS (purchased from ATTO-TEC), solved in 50 µl DMF. The reaction was incubated at room temperature for 3 h. The labelled RNA was extracted via ethanol precipitation and purified by HPLC (elution buffer: 0.1 M triethylammonium acetate; gradient: 5 → 26 % acetonitrile; flow rate 0.5 ml/min) on a reversed phase column (Nucleodur 100-5 C18 ec 250/4).

HPR cleavage assay

TRIS/HCl (pH 7.5)	50 mM
$MgCl_2$	10 mM
HPR	2 nM
3'HH/5'HH (labelled with ATTO680)	2 nM
Final volume	50 µl

HPR, the respective ATTO labelled substrate (3'HH or 5'HH), buffer and water were mixed. The solution was heated up to 90 °C for 2 min and then incubated at 37 °C for 15 min. Reaction was started by addition of $MgCl_2$ and proceeded for 3 h at 37 °C. At specific times (1, 2, 4, 6, 8, 10, 15, 30, 60, 90, 120 and 180 min) aliquots of 1 µl were taken from the solution and diluted in 19 µl stop mix (7 M urea, 50 mM EDTA). Samples were analysed on a LI-COR plate sequencer.

HPR recombination assay

TRIS/HCl (pH 7.5)	50 mM
$MgCl_2$	50 mM
HPR	4 nM
3'HH	8 nM
5'HH (labelled with ATTO680)	2 nM
Final volume	100 µl

For recombination, RNA was mixed in buffer, denatured at 90 °C for 2 min and incubated for further 15 min at 37 °C. Reaction was started by injection of $MgCl_2$ and proceeded at 37 °C for 3 h, followed by a temperature shift to 20 °C and incubation for another three hours. Samples of 1 µl volume were taken at the end of the first and the second three hour incubation period, or were collected over time (1, 2, 4, 6, 8, 10, 15, 30, 60, 90, 120, 180, 185, 190, 195, 210, 240, 270, 300 and 360 min), and diluted in 19 µl stop mix (7 M urea, 50 mM EDTA) to stop reaction. Samples were analysed on a LI-COR plate sequencer.

Preparative recombination

TRIS/HCl (pH 7.5)	50 mM
$MgCl_2$	50 mM
HPR	40 µM
3'HH	40 µM
5'HH	10 µM
(labelled with ATTO680)	
Final volume	50 µl

The mixture was denatured (2 min 90 °C) and incubated at 37 °C for 3 h, followed by incubation at20 °C for another 3 h. Reaction was stopped and subjected onto a 12% denaturing polyacrylamide gel. The product containing band was cut off, RNA was eluted with 0.3 M sodium acetate buffer (pH 5.4) and precipitated from ethanol.

Hammerhead ribozyme cleavage assay

TRIS/HCl (pH 7.5)	50 mM
$MgCl_2$	50 mM
HHR	2 nM
HHRS	2 nM
(labelled with ATTO680)	
Final volume	50 µl

Hammerhead ribozymes and the ATTO680 labelled substrate were mixed with buffer and water. Reaction mixtures were heated to 90 °C for 2 min and cooled down to 37 °C over 15 min. Reaction was started by $MgCl_2$ addition and proceeded at 37 °C for 3 h. After suitable time intervals (1, 2, 4, 6, 8, 10, 15, 30, 60, 90, 120 and 180 min), aliquots of 1 µl were taken, diluted in 19 µl stop mix (7 M urea, 50 mM EDTA) and analysed on a 10% denaturing PAGE.