Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information for:

Pyrene Modification Leads to Increased Catalytic Activity in Minimal Hammerhead Ribozymes

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Content:

Experimental details for:

Ribozyme synthesis: Ribozymes containing Pyrene-based hairpin mimics were synthesized using standard solid phase phosphoramidite chemistry with 2´-TOM-protection on an ABI 394 DNA/RNA synthesizer in 0.2 µmol scale. DMT-protected Pyrene-phosphoramidites were synthesized as described previously (Langenegger, S. M.; Häner, R. *ChemBioChem* **2005**, 6, 848-851. Langenegger, S. M.; Häner, R. *Chem. Commun.* **2004**, 2792-2793). The pyrene modifications were incorporated in high yields (judged by trityl monitoring) using a standard RNA cycle protocol. After sequential deprotection with methylamine and TBAF, RNAs were desalted followed by PAGE-purification (For an analytic PAGE of ^{32}P-labelled ribozymes see figure S1 below). In order to determine cleavage rates, ribozymes were 5´-phosphorylated by incubating 1 µM ribozyme, 1 µCi γ-P^{32}-ATP, 10 U T4 polynucleotide kinase in 50 mM Tris-HCl (pH 7,6), 5 mM DTT, 0,1 mM spermidine, 0,1 mM EDTA and 10 mM $MgCl_2$ at 37°C. The reactions included a DNA oligonucleotide that hybridizes to the ribozymes in order to prevent self cleavage during phosphorylation (50 µM concentration, blocking strand sequences for wt-HHR, S2-HMR, S2.2-HMR: ATT CCT GAC TCG TAA CCG (hybridizes to 3´-end of respective ribozymes) and for ribozymes S3-, S3.2-, and S2+S3-HMR: GGC CTC ATC AGA GGA ACC (binding to the 5´-end of respective ribozymes, see also Salehi-Ashtiani K, Szostak JW. In vitro evolution suggests multiple origins for the hammerhead ribozyme. *Nature* **2001**, 414, 6859.). After phosphorylation, ribozymes were again purified by PAGE. Below (figure S1) an analytical PAGE is shown that proves the homogeneity of the individual phosphorylated ribozymes.

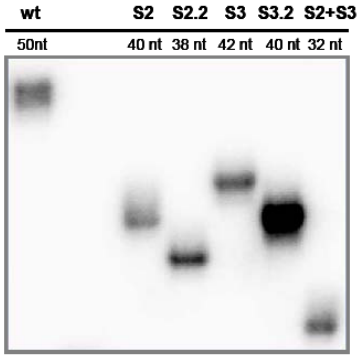

Figure S1: Autoradiography of a 12% denaturing PAGE of the synthesized ribozymes after PAGE purification and prior to cleavage reactions.

In addition to the PAGE analysis, fluorescence spectra were determined that display characteristic emission of the incorporated pyrene modifications. In figure S2, a representative spectrum for S2-HMR is shown.

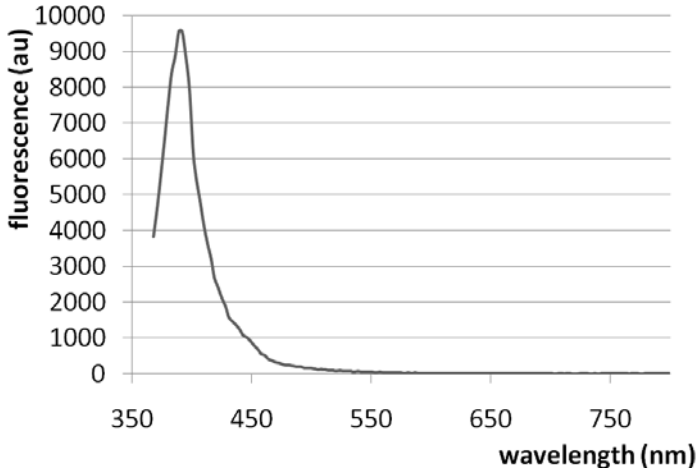

Figure S2: Fluorescence spectrum of a 1 µM solution of ribozyme S2-HMR excited at 335 nm. The observed fluorescence matches the emission spectrum of a pyrene monomer.

Ribozyme reactions: Cleavage rates of ***in cis***-cleaving hammerhead ribozymes were determined in 20 µl volume containing 10 nM of ^{32}P-phosphorylated ribozymes in 50 mM Tris buffer, pH 7.5, 50 mM NaCl. Reactions were started by addition of $MgCl_2$ to a final concentration of 10 mM. At timepoints ranging from 10 seconds to 60 minutes samples were removed and quenched in four volumes of 95 % (v/v) formamid containing 30 mM EDTA. The cleavage products were separated by 10 % denaturing PAGE and quantified by autoradiography. Observed rate constants were obtained from plotting the fraction of cleaved ribozyme versus time by non-linear fitting to the equation $F_t = F_\infty - F_0(1 - e^{kt})$. All reactions were carried out in duplicates.