

**P044** Observation of internal cleavage and ligation reactions of a ribozyme

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Hairpin ribozyme is a self-cleaving and ligating RNA enzyme and in its natural form contains an RNA four-way junction as an important structural element. We have used single molecule fluorescence spectroscopy to untangle the structural dynamics (folding and unfolding) and chemistry (cleavage and ligation) of the hairpin ribozyme. The active site of the ribozyme is stably formed by docking two internal loops, but upon cleavage undocking is accelerated by two orders of magnitude. The markedly different kinetic properties allow us to differentiate cleaved and ligated forms, and thereby observe multiple cycles of internal cleavage and ligation of a ribozyme in a uniquely direct way. The position of the internal equilibrium is biased toward ligation, but the cleaved ribozyme undergoes several undocking events prior to ligation, during which products may dissociate. Formation of the stably-docked active site, rapid undocking after cleavage, and a strong bias toward ligation should combine to generate a stable circular template for the synthesis of the viral (+) strand and thus ensure a productive replication cycle.