

## Single-molecule studies elucidate the 5'exon binding mechanism for group II intron splicing

B. Fazlji<sup>1</sup>, K. M. Egger<sup>1</sup>, S. Zelger-Paulus<sup>1</sup>, R. K. Sigel<sup>1\*</sup>

<sup>1</sup>University of Zurich

Group II introns are large ribozymes found in bacteria and eukaryotic organelles, capable of self-splicing. However, the precise intron-exon binding mechanism to detect the cleavage site remains unclear.<sup>[1-3]</sup> To elucidate the binding process, we employed single-molecule Förster Resonance Energy Transfer (smFRET).

By gradually increasing  $Mg^{2+}$  concentrations, we investigated the role of metal ions in intron folding. Our smFRET analysis revealed two folding states: a high FRET state consistent with the predicted conformation, indicating a tight intron-exon binding, and a dominant low FRET state.  $Mg^{2+}$  facilitates tertiary interactions and 5'exon binding.<sup>[4,5]</sup> However, even at high  $Mg^{2+}$  concentrations, the low FRET prevailed. These findings validate the existing 3D model and unveil a prevalent low FRET state. We conclude that the group II intron primarily adopts this low FRET state, temporarily binding the 5'exon to achieve the necessary folding state for subsequent cleavage. To elucidate the binding mode, we perturbed specific contacts, demonstrating the connection between structural changes, reduced cleavage activity, and the function of group II introns.

Our study unveils novel insights into the conformational dynamics of group II introns, deepening our understanding of the binding and functional mechanisms in self-splicing ribozymes.

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