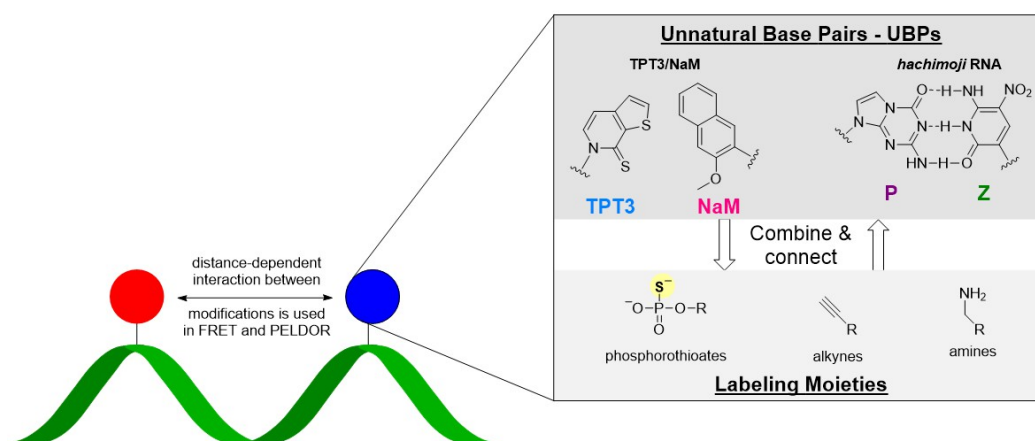


## Advancing RNA Research: A Novel Approach for High-Yield Synthesis and Labeling of Long RNA Strands

N. S. Lindlar<sup>1</sup>, S. Zelger-Paulus<sup>1</sup>, R. K. Sigel<sup>1\*</sup>

<sup>1</sup>University of Zurich, Department of Chemistry

The increasing recognition of RNA in scientific research has prompted the need for advanced methodologies.<sup>[1]</sup> However, studying RNA proves challenging, as traditional protein research techniques offer limited insights.<sup>[2]</sup> To understand the functional dynamics of RNA, molecular rulers such as PELDOR (Pulsed Electron Electron Double Resonance) or FRET (Förster Resonance Energy Transfer) are employed on  $\mu$ s-time scales.<sup>[3]</sup> Yet, these methods require the presence of multiple modifications at specific sites on the RNA molecule, which is a significant hurdle.



In this work, we propose a solution by combining two classes of unnatural base pairs with modifiable moieties. Leveraging enzymatic synthesis for RNA production and well-established chemical reactions for labeling, we anticipate achieving high yields of long RNAs (>100 nt). The synthetic bases NaM/TPT3<sup>[3]</sup> and *hachimoji* RNA<sup>[4]</sup>, previously employed in RNA synthesis, serve as our unnatural base pair candidates. In addition, we incorporate alkynes<sup>[3]</sup> and phosphorothioates<sup>[5]</sup> as modifiable moieties, well-known in bio-orthogonal chemistry. Our proposed method represents a promising approach to overcome the limitations of current techniques and enable efficient synthesis and labeling of long RNA strands.

<sup>[1]</sup> Falese *et al. Chem. Soc. Rev.* **2021**, 50, 2224. <sup>[2]</sup> Reyes *et al. Methods Enzymol.* **2009**, 469, 119; Kappel *et al. Nat. Methods* **2020**, 17, 699. <sup>[3]</sup> Wang *et al. Chem. Sci.* **2020**, 11, 9655; Wang *et al. PNAS* **2020**, 117, 22823. <sup>[4]</sup> Hoshika *et al. Science* **2019**, 363, 884. <sup>[5]</sup> Hu *et al. ACS Chem. Biol.* **2022**, 17, 2248