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

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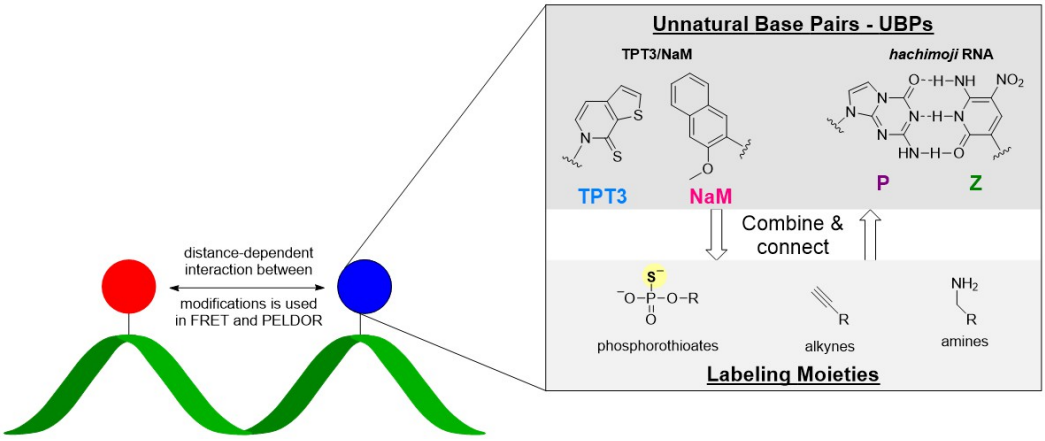
The increasing recognition of RNA in scientific research has prompted the need for advanced methodologies.[1] However, studying RNA proves challenging, as traditional protein research techniques offer limited insights.[2] To understand the functional dynamics of RNA, molecular rulers such as PELDOR (Pulsed Electron Electron Double Resonance) or FRET (Förster Resonance Energy Transfer) are employed on µs-s time scales.[3] 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[3] and *hachimoji* RNA[4], previously employed in RNA synthesis, serve as our unnatural base pair candidates. In addition, we incorporate alkynes[3] and phosphorothioates[5] 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.

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