SOLiD-MaP: A Photoproximity Labeling Platform for Small Molecule Binding Site Mapping on RNA

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Background: RNA-targeting small molecules are emerging as promising therapeutics for disease-associated non-coding RNAs, but their development requires methods that define binding sites and evaluate RNA target selectivity. Existing approaches for detecting ligand-RNA interactions have provided powerful foundations, yet many rely on direct crosslinking or covalent-capture chemistries whose performance depends on ligand-specific probe design, warhead compatibility, and local reaction geometry. Method: We report Singlet Oxygen footprinting on RNA in a Ligand-Directed manner for Mutational Profiling (SOLiDMaP), a photoproximity labeling platform for small molecule-RNA interaction analysis. Using the Mango-II aptamer and thiazole orange ligand as a model system, we evaluated the efficiency and selectivity of singlet oxygen-mediated RNA labeling and developed a pairwise reverse transcription stop assay and performed mutational profiling using next-generation sequencing to identify ligand binding sites. Results: We established aniline as an efficient nucleophile for singlet oxygen-mediated RNA labeling and demonstrated target-selective labeling driven by ligand-localized photosensitization. Labeling selectivity was further tuned by chemically constraining the diffusion of singlet oxygen with a quencher. The RT-stop and mutational profiling assays accurately identified ligand-proximal regions and mapped ligand binding sites on RNA. Conclusion: SOLiD-MaP provides a new, orthogonal strategy for analyzing small molecule-RNA recognition and expands the toolkit for studying non-coding RNA-targeting therapeutics.