



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

Lin Rietveld¹, Wei Wu¹, Filip Zawisza¹, Danny Incarnato², Zeshi Li¹ ¹Division of Chemical Biology & Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, Netherlands, ²Department of Molecular Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, Netherlands

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 (SOLiD-MaP), 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.