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Profiling RNA-protein interactome using proximity labeling and nanopore sequencing

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RNA-binding proteins (RBPs) regulate every stage of the RNA life cycle. Understanding RNA regulation requires high-resolution mapping of RNA-protein interactions at the single-molecule level. While conventional approaches like CLIP-seq variants can identify binding sites, they lose molecular context through fragmentation during library preparation and only capture population averages. We present **NanoSURF**, a single-molecule technology that combines proximity-based chemical footprinting with nanopore direct RNA sequencing to map protein footprints along full-length RNA molecules. Chemical adducts formed near RNA-protein interaction sites alter nanopore sequencing signals, which NanoSURF identifies by comparing labeled and unlabeled control molecules. We applied NanoSURF to HEK293T cells to track protein interactions throughout the nuclear mRNA life cycle. Our analysis confirms signals coming from exon-junction complexes that enriched ~24 nucleotides upstream of splice junctions specifically in RNAs with poly(A) tails exceeding 120 nucleotides. Additionally, the footprints identify stable multi-site conformations involving intramolecular interactions and protein binding in late-stage RNAs with poly(A) tails shorter than 80 nucleotides. NanoSURF enables single-molecule tracking of RNA-protein interactions throughout the RNA life cycle, preserving information about in situ RNA conformations and long-range interaction patterns that conventional methods cannot capture.