Sequence-resolved hybridisation kinetics for RNA-guided target recognition

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Background: RNA guided target recognition lies at the heart of both gene regulation and RNA-based medicine: small interfering RNAs, microRNAs, and RNA-editing guide RNAs act by hybridising to a target transcript, and their therapeutic selectivity depends on discriminating the intended site from a background of near-identical sequences. This selectivity is fundamentally kinetics, set by how the target sequence shapes association and dissociation in real time, yet guide and oligonucleotide design still rests largely on equilibrium thermodynamics. This approach captures the affinity of a duplex but not the kinetics through which it forms and dissociates. Method: We use SPARXS (Single-molecule Parallel Analysis for Rapid eXploration of Sequence space), a high-throughput single-molecule platform that couples next generation sequencing with singlemolecule fluorescence to quantify hybridisation across defined libraries of sequence variants. SPARXS allows association and dissociation rates to be resolved for every sequence in parallel. We begin with a controlled library and scale towards the full complexity of short sequence space. Results: For each sequence variant we resolve the rate constants and assemble these into kinetic maps that reveal how sequence composition reshapes hybridisation across the probed sequence space. Conclusion: These kinetic maps provide mechanistic insights into how RNA encodes specificity at the level of binding kinetics, and establish a quantitative, kinetics-resolved framework for the rational design of siRNA, microRNA-based, and RNA-editing therapeutics with predictable, sequence-tuned bidning kinetics, and a foundation for predicting off-target discrimination as the approach scales across sequence space