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Measuring in situ primary and competitive hybridization events on microspheres VALERIA MILAM, JAMES HARDIN, Georgia Institute of Technology — Understanding hybridization events at surfaces is crucial for optimizing nucleic acid detection platforms as well as DNA-mediated colloidal assembly. We used flow cytometry to measure time-dependent primary and competitive hybridization events of perfectly matched and mismatched targets on microsphere surfaces. In addition to more conventional sample preparation involving multiple wash and resuspension steps prior to measurement, we sampled the reaction volume directly for in situ measurements to minimize potential dissociation events between weaker partner strands during wash steps. Similar to prior reports for oligonucletide solutions, the nearly identical rates for primary hybridization events on microsphere surfaces were independent of target sequence and reached an equilibrium value within 30 min. The extent of in situ primary hybridization events for immobilized probes, however, deviated from solution model predictions. In situ competitive hybridization events were at least 100-fold slower than primary hybridization events and did not appear to reach equilibrium. The kinetics of competitive hybridization events on microspheres are consistent with predicted effects stemming from toehold effects or base length differences between primary and secondary targets.

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