Abstract Submitted
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Unexpected phenomena in multiplex hybridization STEVE BLAIR, University of Utah — We discuss the characteristics of multiplex hybridization using experimental (using a custom multi-color real-time evanescent wave platform) and computational methods. The hybridization of multiple nucleic acid sequences to the same probe challenges quantitative microarray interpretation. Multiplex hybridization follows the rules of competitive displacement, in which lower affinity species are displaced by higher affinity species. This phenomenon increases the time to equilibrium and is the underlying mechanism of specificity in molecular capture. A simple three-component kinetic model of hybridization quantitatively describes multiplex hybridization, where cross-hybrids are explicitly accounted for without *a priori* information. Melting analysis is a recognized methodology to study the thermodynamics of DNA interactions and positively identify sequence-specific DNA targets in a multi-component environment. Melting analysis typically assumes that multi-component melts are superpositions of individual melts. We show that under probe-limiting conditions this assumption is not valid. Multi-target interactions with a single probe sequence result in three counter-intuitive phenomena: 1) suppression of all but one apparent melt transition 2) the bound concentration of a lower-affinity species increasing with temperature and 3) the ratio between bound concentrations of high and low affinity species decreasing with temperature.

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