Supplementary Data: Hybridization Kinetics between Immobilized Double-Stranded DNA Probes and Targets Containing Embedded Recognition Segments

Bryan A. Baker¹ and Valeria T. Milam¹,²,³*

¹School of Materials Science & Engineering
²Wallace H. Coulter Department of Biomedical Engineering
³Petit Institute for Bioengineering and Bioscience
Georgia Institute of Technology
771 Ferst Dr. NW, Atlanta, GA 30332-0245

Washing Studies in the Absence of Target

In order to determine if washing steps alone affect the fraction of reporter strands released from the dsProbes, a series of washing studies was performed in the absence of target. Figure S1 shows the results of this analysis in which P₁₅:T₁₁, P₁₅:T₁₃, and P₁₅:T₁₅m dsProbes were subjected to the indicated number of washing steps. For all studies, two wash steps are performed immediately after incubation with the reporter strands. For any suspensions then incubated with targets, there are two additional wash steps for a total of four wash steps.
Thus, any changes to dsProbe density values between wash numbers two and four are the most important data points to consider.

Figure S1 shows that only small changes in reporter strand release occur between washing step two and four for **P15:T13** and **P15:T15m** dsProbes. The **P15:T11** dsProbe, however, shows a continual increase in fraction released for each wash step. The increase in fraction released between wash steps two and four is ~17%. This amount of reporter strand release from the **P15:T11** dsProbe due to washing alone is still, however, significantly less than the 40% (total) reporter released over 72 h in the absence of targets or the ~50-80% reporter released over 72 h in the presence of various long targets (see Figure 4). Since the samples at each time point for the competitive hybridization studies were measured after a total of four washes, washing steps alone appear to only modestly promote reporter release for one system studied, namely the low affinity **P15:T11** dsProbe.

![Figure S1](image)

**Figure S1.** Fraction of reporter strands released from **P15:T11**, **P15:T13** and **P15:T15m** dsProbes as a function of the number of washing steps. Each data point corresponds to the average of three separate measurements with error bars representing the standard deviation.
**Reporter Release Studies for P15:T13 and P15:T15m dsProbes**

Figure S2 shows the fraction of reporter strands released from the **P15:T13** dsProbe system in the absence or presence of several targets. The dsProbes incubated with the **NC-100** target (100 thymines only) show little change in duplex density values. In fact, the duplex density values appear to slightly increase as indicated by the small negative fraction released values (~0.05) at long times. We theorize that these negative values may result from some initial imperfect hybridization of the reporter strands to the immobilized strands. For the samples taken at early time points, these weakly associated reporter strands may dissociate during the wash steps. For samples given longer incubation times before final wash steps, however, this additional time may allow the initially imperfectly hybridized reporter strands to form more stable duplexes that are more resistant to final washes. Similar trends in duplex density fluctuations have been attributed to imperfectly hybridized targets in other immobilized DNA probe systems[1]. The **None** sample (in which target strands are absent) shows a small amount of reporter release due to dissociation alone, but only at early time points. While dissociation is not negligible for **P15:T13** dsProbe systems, its stability still surpasses that of the low affinity **P15:T11** (~42% dissociation in Figure 4). This higher stability associated with the **P15:T13** dsProbes, however, also slows competitive hybridization events significantly with all complementary long targets. For the long targets appreciable release is not observed until after 24 h and a plateau value is not reached with any target studied. Though equilibrium is not achieved for this dsProbe system, incubation with targets having the recognition sequence at either the **3’ End** or **5’ End** does result in the greatest release of reporter strands over the time period examined. Though slower and more extensive release occurred, the high affinity **P15:T13** dsProbe does exhibit a more pronounced stratification in the release profiles for the **3’ End**, **5’**
End and Middle targets in comparison to the low affinity P15:T11 dsProbe system (see Figure 4). These differences in behavior are attributed to the greater affinity of the P15:T13 dsProbe in comparison to the P15:T11.

![Graph](image1.png)  ![Graph](image2.png)

**Figure S2.** Fraction of reporter strands released from the P15:T13 dsProbe as a function of time of incubation with various targets after the first (a) hour and (b) 72 h.

Figure S3 shows the fraction of reporter strands released from the P15:T15m dsProbe system in the absence or presence of several targets. While some dissociation is evident in the absence of target for the None sample, the faster and more extensive reporter release achieved for this low affinity mismatched dsProbe indicates that it is more responsive to the long target than the high affinity P15:T13 dsProbe system (see Figure S2). Notably, while more responsive, the release profiles for the low affinity P15:T15m dsProbe system show little, if any, differences for the 3’ End and 5’ End targets, unlike the high affinity P15:T13 dsProbe system.
Figure S3. Fraction of reporter strands released from the P15:T15m dsProbe as a function of incubation time with various targets after the first (a) hour and (b) 72 h.

References

1. Glazer, M., Fidanza, J.A., McGall, G.H., Trulson, M.O., Forman, J.E., Suseno, A. and Frank, C.W. (2006) Kinetics of oligonucleotide hybridization to photolithographically patterned DNA arrays. *Anal. Biochem.*, 358, 225 - 238.