Mechanisms of Surface-Mediated DNA Hybridization

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SUPPORTING INFORMATION

Single-molecule total internal reflection fluorescence microscopy (TIRFM) in conjunction with resonance energy transfer (RET) was used to observe the dynamic behavior of ssDNA at the interface between aqueous solution and solid surface covalently modified with complementary ssDNA via epoxide-amine chemistries. The use of RET provides the ability to probe the spatial distance between proximal complementary strands and was used as an indicator of hybridization. This approach permitted the extraction of state time distributions for the “hybridized” state and “searching” state over a range of temperatures. Control experiments were also performed using a poly-A oligonucleotide instead of the complementary poly-AC strand. Due to the large volume of data obtained in these experiments, some details were omitted from the main text of the manuscript. Therefore, additional information is presented here, including the relative distance between donor and acceptor fluorophores, d, for the negative control (Figure S1), sensitivity analysis of the hybridization threshold (Figure S2), complete temperature data for the fractional analysis of transitions between states (Figure S3), the comparison of poly-AC and poly-A behavior in state time distributions (Figure S4), all the characteristic state times and their respective population fractions for this study (Table S1), Arrhenius analysis of the characteristic surface melting times (Figure S5), the structures of both the poly-TG probe and poly-AC target oligonucleotides (Figure S6), and the data used to determine the optimum epoxysilane (GPTMS) deposition conditions (Figure S7).
Relative distances between donor and acceptor fluorophore distribution for poly-A control

As described in the main text, criteria to distinguish between hybridized oligonucleotides and oligonucleotides in the “searching” state were established using the relative distance between donor and acceptor fluorophores, $d$. The relative distance was calculated for all observed trajectories in both the control and complement target molecule trials. In Figure S1, step-functions were used to describe extreme $d$-values. Values of $d$ below 0.7 were considered to show complete-RET and make up an extremely small fraction of total observations. Zero-RET observations are shown as the step function for $d>2.0$, indicating that a large fraction of poly-A strands engage in negligible RET interactions. Examination of the distribution indicates the presence of two populations, one centered at $d=1.5$, indicating weak RET, and a second showing zero RET, $d>2.0$. This suggests that the $d=1.5$ populations are a result of incidental intermolecular interactions between the control donor-labeled target and the non-complementary immobilized acceptor-labeled probe. This observation, along with analogous data for the complementary donor-labeled target (Figure 2), suggested that $d=1.6$ was an appropriate criterion for assigning hybridization events.

Figure S1. Relative distances between donor and acceptor fluorophore distribution for all poly-A control species observed to undergo RET in experiments at 293K.
**Sensitivity analysis of hybridization threshold**

In the main text of the article the relative distance between donor and acceptor fluorophores was used to identify when hybridization events occurred. The selection criteria involved a cut-off where steps of a trajectory with \( d \) values less than the threshold were considered to be hybridized and values greater than the threshold were searching. It was important when selecting these criteria to choose a hybridization threshold that was low enough to avoid selecting noise or incidental RET events as hybridization events, while not being low enough to identify partial melting events or other fluctuations in RET signal as true dehybridization events. To insure that a sufficiently high hybridization threshold was selected, data was mined and a hybridization threshold sensitivity analysis was performed. This analysis extracted the number of observations of apparent “fluctuations” where a molecule rapidly crossed over the threshold and returned to the hybridized state. The threshold was systematically increased from \( d = 0-100 \) \((E = 1 - 10^{-12})\) and the number of fluctuations calculated. The results of this sensitivity analysis are plotted in Figure S2. The fluctuations that exist at large threshold values are due

![Figure S2](image-url)

**Figure S2.** Distribution of the number of fluctuations as a function of hybridization threshold (•, left axis). Distribution of the number of hybridization events as a function of hybridization threshold (□, right axis)
to the tracking of species that exhibit little RET, or $d$ values approaching infinity, alternating with weak RET signals attributed to noise. In the range of $d = 1.6 - 2.0$ ($E = 0.06 - 0.02$) fluctuations vary by less than 10% from the number of fluctuations at $d \geq 10$ ($E \geq 10-6$). This information combined with the results stated in the main text indicated that a hybridization threshold of $d = 1.6$ would reduce identification of false melting events, incidental RET, and RET signals due to noise.

**Temperature dependence of fractional pathways to hybridization**

Molecular trajectories were examined and then partitioned into “searching” and “hybridized” states based on RET efficiency. Transitions between states could then be counted and normalized by the destination or source state. These fractions were then assembled to provide a picture of the pathway to surface hybridization. This analysis was repeated for the temperature range of 293K – 313K at 10 degree intervals; results are shown in Figure S3. The fractions were insensitive to temperature in this range. This observation was statically confirmed as described in the manuscript.

![Figure S3. Fractional pathway of DNA surface hybridization. Temperatures are read from top to bottom as decreasing temperatures 313K, 303K, and 293K.](image)
**Poly-AC and Poly-A State Time Distributions**

Cumulative state time distributions were prepared by monitoring the dynamic behavior of single-molecule RET trajectories. Using the relative distance between fluorophores “hybridized”- and “searching”-state time intervals were accumulated and used to create cumulative state time distributions. This process was repeated for both the poly-A and poly-AC oligonucleotides. The resulting distributions are compared in Figure S4.

![Figure S4](image_url)

**Figure S4.** Cumulative state time distributions for complementary poly-AC targets (●) and poly-A control (△) targets. (A) transition from searching surface state to hybridized state, (B) desorption from hybridized state, (C) Transition from hybridized to searching state.
**Characteristic State Times as a Function of Temperature**

State time distributions were prepared for all transitions at each measured temperature. These distributions were fit with a number of exponentials selected via the maximum entropy method as described in the text. Hybridization from the searching state had only a single characteristic search time, $\tau_{s,\text{on}}$. While there were multiple characteristic state times for desorption from searching state, $\tau$, desorption from hybridization, $\tau_{\text{des,off}}$, and melting to the surface, $\tau_{s,\text{off}}$. These characteristic residence times and their respective population fractions, $f$, are given in Table S1. As discussed in the text the majority of characteristic state times show little temperature dependence. However, the melting state times exhibit systematic variation. The longer hybridization state has a strong temperature dependence indicated by the decreasing characteristic state times from 15s to 8s over the range of 293K-313K. On the other hand, the shorter hybridization state demonstrates little temperature dependence. This difference is elaborated upon in the primary text.

Table S1. Characteristic state times, $\tau$, and their associated population fractions, $f$, for the exponential fits of all state time distributions over the temperature range 293K – 313K. The numbers in parentheses represent the uncertainty in the least significant digit.

| Mode   | 293 K   | 303 K   | 313 K   |
|--------|---------|---------|---------|
|        | $\tau$  | $f$     | $\tau$  | $f$     |
| $\tau_{s,\text{on}}$ | 0.110(6) | 1       | 0.106(2)| 1       |
| $\tau_{s,\text{off}}$ | 1.47(3)  | 0.55(2) | 1.44(3) | 0.30(5) |
| $\tau_{\text{des,off}}$ | 15.1(8)  | 0.50(1) | 10.8(6)| 0.48(2)|
| $\tau_{1}$       | 2.2(1)   | 0.94(4) | 2.0(4)  | 0.88(6) |
| $\tau_{2}$       | 0.20(1)  | 0.06(2) | 0.17(2) | 0.12(2)|
*Arrhenius Analysis*

In the primary manuscript the temperature was systematically varied from 293K – 313K at intervals of 10°K. For each temperature, state time distributions were extracted and fit with a multi-exponential fit. The characteristic state times (τ) and their associated population fractions were extracted from these fits. Hybridized state times correspond to the time spent hybridized before melting. The hybridization state times demonstrated strong temperature dependence. The characteristic times were inverted to represent an apparent rate of melting and then plotted on an Arrhenius plot (Figure S5). The trend was fit using standard regression analysis; the results of these fits are reported in Figure S5 in the form of the natural log of the Arrhenius equation. The Apparent activation energies were $E_a = 23$ kJ/mol and $E_a = 22$ kJ/mol for melting to the surface and into solution, respectively. These values are somewhat smaller than the analogous activation energies expected for DNA melting behavior in solution and are discussed in more detail in the main body of the text.

![Figure S5. Arrhenius plots for duplex DNA melting to the surface and into solution.](image)
**Structure of Target and Probe Oligonucleotides**

The structures of both the poly-TG probe and poly-AC target oligonucleotides are shown in Figure S6. The linker/fluorophore combinations are drawn explicitly and the DNA sequences are given by their letter code. The two sequences are arranged to resemble the structure of the molecules during hybridization. As discussed in the text the poly-AC target strand is labeled with a C6-Amino/Alexa Fluor 488, linker/fluorophore combination on the 5’ end. The 3’ end of the poly-AC molecule is unmodified. The 5’ end of the poly-TG complementary probe molecule was modified with a C6-Amino linker. A C7-Amino/Quasar 670, linker/fluorophore combination was attached to the 3’ end.

![Figure S6. Structure of labeled probe and target ssDNA.](image-url)
Optimization of GPTMS Monolayer Deposition Conditions

Vacuum assisted chemical vapor deposition was performed on silicon wafers (WRS). The deposition solution was a 1:2:20 solution by volume, of n-butylamine:3-glycidoxypropyltrimethoxysilane (GPTMS):toluene. The solution was placed in the bottom of a desiccator; silicon wafers were mounted in the desiccator via Teflon holder. The desiccator was sealed and evacuated via house vacuum and left to incubate for times ranging from 0-24 hours. Samples were extracted from the deposition chamber and immediately characterized using sessile drop experiments on a contact angle goniometer and variable angle spectroscopic ellipsometry. The results of these analyses are shown in Figure S7. Ellipsometry was used to measure film thickness. The measured film thickness was compared to the length of a fully extended GPTMS molecule film (dotted line, Figure S7); thicknesses equal to this value indicate the presence of a complete monolayer. Contact angle goniometry was used as a complementary technique. The contact angle quantifies the wettability of the surface and is sensitive to different surface chemistries and can be used to characterize a complete monolayer. The combination of these two techniques allowed for the optimization of the deposition of high quality GPTMS monolayers.

Figure S7. Film thickness (Å), left axis, was measured using variable angle spectroscopic ellipsometry (◊). The dotted line indicates the length of a fully extended GPTMS molecule film. Contact angle (degrees, ■), right axis, quantifies the wettability of the surface.