Secondary structure effects on DNA hybridization kinetics: a solution versus surface comparison

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Supporting Information

Comparison of solution-phase probe and target mixing via hand pipetting and syringe injection

![Graph](image)

**Figure S1.** Solution-phase hybridization of P₀T₀ monitored by UV absorbance (260 nm). P₀ and T₀ mixed by hand pipetting (gray squares) and syringe injection (black squares). See text for details of concentration and time zero (t = 0) calculations. Both hybridizations carried out in 1M NaCl/TE, [P₀] = [T₀] = 1μM, T=20°C. The solid lines are fits to both sets of data, indicating the mixing methods have no influence on the kinetic data obtained (k_on average = (1.52 ± 0.06) × 10⁶ M⁻¹s⁻¹).

**Thermal melting of dsDNA monitored by UV absorbance**

As described in the text, each probe-target pair was melted rapidly by ramping to 88 °C. The sequences were then slowly cooled to 20 °C at 0.25 °C/minute and monitored by UV absorbance spectroscopy (Varian Cary 100 with Peltier thermostatable multicell holder). Absorbance values were corrected by subtracting a buffer melting profile and then converting the data to ssDNA fraction (1). Exact melting temperatures (T_m’s) were determined at 0.5 ssDNA fraction and found to be: P₀T₀ (76.2 °C), P₄T₃ (76.5 °C), P₃T₃ (77.4 °C), and P₄T₄ (78 °C). Based on thermodynamic calculations (2-4), a difference in T_m of less than 2 °C for the entire range of sequences studied corresponds to a difference in ΔG°₂⁰ of 0.5 kcal/mol.
Figure S2. Thermal melting of all duplex DNA pairs studied as monitored by UV absorbance spectroscopy (260 nm). $P_0T_0$ (black line), $P_3T_3$ (green line), $P_4T_3$ (red line), and $P_4T_4$ (blue line). All sequences melted under conditions of [Probe] = [Target] = [dsDNA] = 1 μM in 1 M NaCl/TE. Only an expanded view of the cooling cycle is shown and all data is plotted as ssDNA fraction for comparison. Actual absorbance changes ($\Delta A$) over the entire melting range for these sequences are 0.095, 0.1, 0.11, 0.092, respectively.
Concentration dependent ssDNA $P_4$ and $T_4$ thermal melting by UV absorbance spectroscopy

**Figure S3.** Thermal melting of ssDNA $P_4$ (A) and $T_4$ (B) monitored by UV absorbance spectroscopy (at 260 nm) at different strand concentrations: 0.3$\mu$M (black), 1$\mu$M (red) and 10$\mu$M (green). Insets show the derivative of each melting curve. Data were collected during the cooling cycle from 90 $^\circ$C - 10 $^\circ$C at a ramp rate of 0.25 $^\circ$C/min in 1 M NaCl/TE. The y-axis represents the fraction of total absorbance change during thermal melting; the concentration-dependent data has been scaled in this way for comparison and clarity.
Absorbance values were corrected by subtracting a buffer melting profile. For both strands P₄ and T₄, the ratio ΔA_{melt}/[strand] is identical for 0.3 μM and 1 μM strand concentrations. However, the ratio value is smaller for 10 μM concentration, indicating dimer contribution (less hypochromicity change during melting due to pre-formed base pairs). The dimerization at very high strand concentration is more clearly shown by the derivative inset plots in Figure S3; a second peak appears in the derivative plots for both P₄ and T₄ at 10 μM (green curves). For P₄, the dimer peak can also be observed at 1 μM but has a lower intensity, reflecting a smaller population. The contributions of hairpin (hp) and dimer (di) are estimated based on their free energies (ΔG’s). Enthalpy, ΔH, and entropy, ΔS, changes associated with ssDNA thermal melting are determined by van’t Hoff analysis:

\[
\Delta H_{hp} = 4RT_m^2 \left( \frac{\partial \alpha}{\partial T} \right)_{T=T_m} \quad \text{Equation 1} \\
\Delta H_{di} = 6RT_m^2 \left( \frac{\partial \alpha}{\partial T} \right)_{T=T_m} \quad \text{Equation 2}
\]

\[
\Delta S = \Delta H / T_m \quad \text{Equation 3} \\
\Delta G = \Delta H - T \cdot \Delta S \quad \text{Equation 4}
\]

where α is the fraction of hairpin or dimer formed. The population (P) of each structure is then calculated using:

\[
P_{hp} = \Delta G_{hp} / (\Delta G_{hp} + \Delta G_{di}) \quad \text{Equation 5} \\
P_{di} = \Delta G_{di} / (\Delta G_{hp} + \Delta G_{di}) \quad \text{Equation 6}
\]

Using this analysis, we find a population of 30% dimer at 10 μM and 10% dimer at 1 μM for P₄ under our experimental conditions (20°C, 1 M NaCl/TE). For sequence T₄, dimers clearly form at high strand concentrations, however, they have very low melting temperatures (< 20 °C), and a population could not be accurately determined. In the concentration range 0.3 – 1 μM, T₄ has no dimer population.
Solution-phase kinetics: Ionic strength dependence

Figure S4. Solution-phase hybridization kinetics of all sequences studied measured in 1M NaCl/TE (A) and 0.1 M NaCl/TE (B). For all studies, [Probe] = [Target] = 1μM and T = 20°C. Kinetic rate constants at all ionic strengths follow the same trend found in 0.5 M NaCl/TE (see text), \( P_0 T_0 > P_3 T_3 > P_4 T_3 > P_4 T_4 \), and \( k_{on} \) values are obtained from linear fits (solid lines) to 1/C vs. time (shown at right). See table below for calculated values.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>( k_{on} ) ( (10^5 \text{ M}^{-1} \text{s}^{-1}) ) in solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 M NaCl/TE</td>
</tr>
<tr>
<td>( P_0 T_0 )</td>
<td>15.6 ± 1.3</td>
</tr>
<tr>
<td>( P_3 T_3 )</td>
<td>8.6 ± 0.2</td>
</tr>
<tr>
<td>( P_4 T_3 )</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>( P_4 T_4 )</td>
<td>4.30 ± 0.16 (fast)</td>
</tr>
<tr>
<td></td>
<td>1.60 ± 0.02 (slow)</td>
</tr>
</tbody>
</table>
Surface kinetics and fitting: Ionic strength dependence

Figure S5. Surface hybridization kinetics of all sequences studied measured in 1 M NaCl/TE (A), 0.5 M NaCl/TE (B), and 0.1 M NaCl/TE (C). For all studies, [Target] = 1μM and T ~ 20°C. Surface probe densities are 4.5 x 10^{12} molecules/cm^2, 6.8 x 10^{12} molecules/cm^2, and 5.7 x 10^{12} molecules/cm^2 for P_0, P_3, and P_4 surfaces, respectively. Lines are fits to the data using a traditional Langmuir model, Equation 4 in text. Insets A and B show fitting to one hour P_0 T_0 and P_4 T_4 data, while inset C shows fitting to 2.5 hours. See table below for k_{off} values obtained from fitting to the first 30 minutes of hybridization except where indicated.
<table>
<thead>
<tr>
<th>Sequence</th>
<th>1M NaCl/TE</th>
<th>0.5 M NaCl/TE</th>
<th>0.1 M NaCl/TE</th>
</tr>
</thead>
<tbody>
<tr>
<td>P_0T_0</td>
<td>0.55 ± 0.05 (0.5 hour)</td>
<td>0.57 ± 0.04 (0.5 hour)</td>
<td>0.100 ± 0.005 (0.5 hour)</td>
</tr>
<tr>
<td></td>
<td>0.51 ± 0.06 (1 hour)</td>
<td>0.57 ± 0.08 (1 hour)</td>
<td>0.110 ± 0.005 (2.5 hours)</td>
</tr>
<tr>
<td>P_1T_3</td>
<td>0.35 ± 0.03</td>
<td>0.24 ± 0.01</td>
<td>0.033 ± 0.001</td>
</tr>
<tr>
<td>P_2T_4</td>
<td>0.099 ± 0.004</td>
<td>0.083 ± 0.003</td>
<td>0.028 ± 0.001</td>
</tr>
<tr>
<td>P_3T_4</td>
<td>0.035 ± 0.001</td>
<td>0.032 ± 0.001</td>
<td>0.021 ± 0.001</td>
</tr>
</tbody>
</table>

References

2. HYTHER™ version 1.0. Peyret, N. and SantaLucia, J. Wayne State University.