

## Supplementary Information for “Single Molecule Kinetics Reveal Cation Promoted DNA Duplex Formation Through Ordering of Single Stranded Helices”

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A detailed discussion of TST ideas in the context of single molecule kinetic data for nucleic acid conformational change has been described previously.<sup>(1, 2)</sup> Simply summarized, however, this treatment considers one-way crossings over a transition state barrier, with reactants in equilibrium with the barrier and a barrier attempt frequency  $\nu$ . Within such a TST framework, a plot of  $\ln(k/\nu)$  vs.  $1/T$

$$\ln\left(\frac{k}{\nu}\right) = \frac{\Delta S^\ddagger}{R} - \frac{\Delta H^\ddagger}{R} \cdot \frac{1}{T} \quad (6)$$

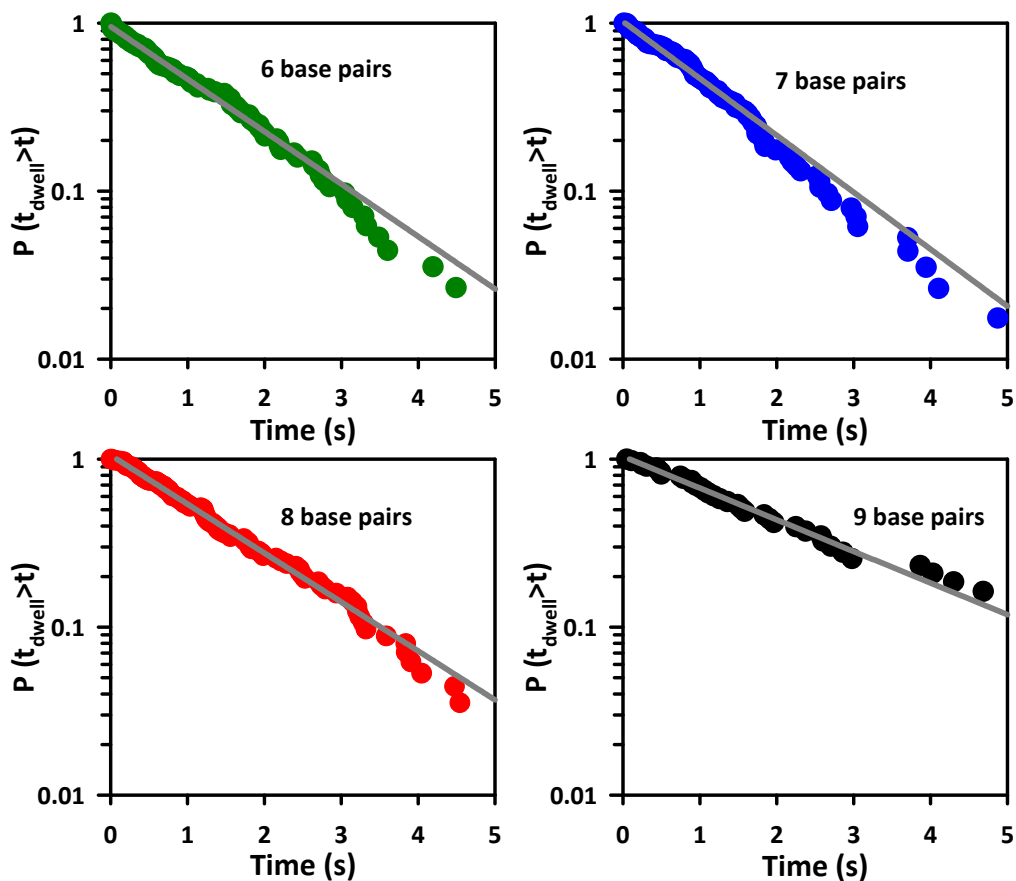
yields a slope and intercept that represent the transition state barrier enthalpy ( $-\Delta H^\ddagger/R$ ) and entropy ( $\Delta S^\ddagger/R$ ), respectively. However, in order to determine absolute transition state entropy, Eq. (6) requires additional information on the attempt frequency,  $\nu$ . From the discussion of diffusion limited duplex formation, it follows that the fastest possible rate constant is the frequency at which the strands can interact. Thus, the diffusion limited rate constant ( $k_{\text{diff}} \approx 2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ) should be a sufficient descriptor of this maximum interaction frequency. For a fixed 200 nM strand concentration, this yields a bimolecular attempt frequency of  $\nu = 400 \text{ s}^{-1}$ . However, as discussed in the main manuscript,  $k_{\text{diff}}$ , through the diffusion coefficient,  $D_{\text{S2-Cy5}}$ , has a linear dependence on temperature and an inverse relationship with the temperature dependent viscosity,  $\eta(T)$ . Thus, the room temperature attempt frequency is multiplied by a factor of  $T/295 \text{ K}$  and  $1 \text{ cP}/\eta(T)$  to correctly describe the temperature dependence of the attempt frequency.<sup>(3)</sup> It is worth noting, of course, that any error in this estimate i) has no impact on  $\Delta H^\ddagger$  and ii) introduces only a *constant offset* in  $\Delta S^\ddagger$ , and indeed one that scales only *logarithmically* (i.e. very slowly) with  $\nu$ . As a result, such errors will have no effect on any predicted *changes* in  $\Delta S^\ddagger$  (i.e.  $\Delta(\Delta S^\ddagger)$ ) as a function of solution parameters.

By way of explicit example, a plot of  $\ln(k_{\text{off}}/\nu)$  vs.  $1/T$  is shown in Fig. S2A, with the corresponding transition state thermodynamic parameters ( $\Delta H^\ddagger$  and  $\Delta S^\ddagger$ ) listed in Table 2. The data clearly show that increasing  $[\text{NaCl}]$  from 25 mM to 1M results in a *less steep* slope, i.e., the transition state enthalpy for duplex dissociation clearly *decreases* with added NaCl. Specifically, at 25 mM  $[\text{NaCl}]$ ,  $\Delta H^\ddagger = 51$  (3) kcal/mol, which decreases significantly to 39 (4) kcal/mol at 1M  $[\text{NaCl}]$ . This  $\Delta(\Delta H^\ddagger) = 12$  kcal/mol *decrease* in slope is also coupled with a compensating decrease in the transition state entropy, with  $\Delta S^\ddagger$  decreasing from 153 (11) cal/mol/K to 117 (12) cal/mol/K, from 25mM to 1M  $[\text{NaCl}]$  respectively.

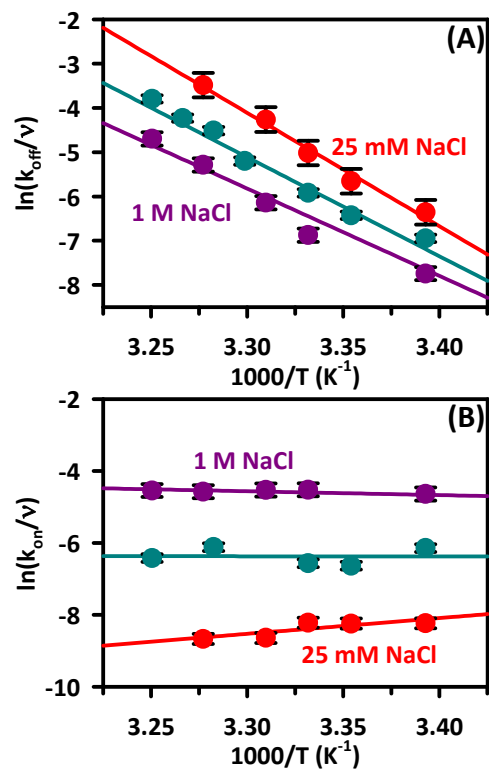
Fig. S2B shows a similar analysis for the association process, i.e.,  $\ln(k_{\text{on}}/\nu)$  vs.  $1/T$ , also as a function of  $[\text{NaCl}]$ . At low  $[\text{NaCl}]$ , (25 mM) the slope is slightly positive ( $k_{\text{on}}$  decreases with increasing temperature), which describes a case with weakly *negative* activation enthalpy ( $\Delta H^\ddagger = -9$ (3) kcal/mol). However, as  $[\text{NaCl}]$  is increased, the slope of the Eyring plot decreases until it is even slightly negative at 1M  $[\text{NaCl}]$ , corresponding to a *positive* activation enthalpy of  $\Delta H^\ddagger = 2$ (1) kcal/mol. Similarly, over the same range of increase in  $[\text{NaCl}]$ , the transition state entropy also becomes considerably more favorable, from  $\Delta S^\ddagger = -46$ (10) cal/mol/K to  $-2$ (3) cal/mol/K. The complete set of thermodynamic parameters is summarized in Table II.

### SUPPORTING REFERENCES

1. Fiore, J. L., Holmstrom, E. D., and Nesbitt, D. J. (2012) Entropic origin of Mg<sup>2+</sup>-facilitated RNA folding, *Proc. Natl. Acad. Sci. U. S. A.* 109: 2902-2907.
2. Holmstrom, E. D., Fiore, J. L., and Nesbitt, D. J. (2012) Thermodynamic Origins of Monovalent Facilitated RNA Folding, *Biochemistry* 51: 3732-3743.
3. Kestin, J., Sokolov, M., and Wakeham, W. A. (1978) Viscosity of Liquid Water in Range - 8-Degrees-C to 150-Degrees-C, *J Phys Chem Ref Data* 7: 941-948.



**Fig. S1** The CDFs of the 6, 7, 8 and 9 base pair constructs for the duplex association process are all straight lines, indicating single exponential processes. The slopes of the plots are largely insensitive to the length of the duplex, which represent the insensitivity of  $k_{\text{on}}$  to the number of base pairs.



**Fig. S2.** Transition state theory (TST) analysis of duplex dissociation (A) and hybridization (B) for the same  $[\text{NaCl}]$  in Fig. 8: 25 mM (red), 125 mM (green) and 1 M (purple).