Multistate DNA oligonucleotide dissociation revealed through FTIR, 2D IR, and t-HDVE spectroscopy

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The dehybridization of DNA oligonucleotides is studied with a combination of FTIR, 2D IR, and temperature jump t-HDVE spectroscopies. Nucleobase sequence is found to dictate the dissociation mechanism, with timescales of ~10 μs assigned to strand dissociation and ~70 ns assigned to premelting events such as duplex fraying.

Recently developed coarse-grained models that represent DNA with a reduced number of interaction sites per nucleotide have simulated the hybridization mechanism of DNA in unmatched detail. These models predict rich hybridization dynamics including initial nucleation of a few key contacts followed by zippering of the remaining base pairs, shifted register slithering of one strand along another, and various internal displacement schemes depending on the nucleobase sequence.[1-2] At this time additional experimental insight is needed to directly investigate these or other potential mechanisms, but the required ps-μs temporal resolution along with the need for structural sensitivity poses a challenge.

We have developed an IR spectroscopy based strategy to investigate the dehybridization of DNA oligonucleotides. Through a combination of FTIR, 2D IR, and the aid of a lattice model extension of the nearest-neighbor model[3] we characterize the equilibrium ensemble of intact base-pairs across the melting transition for a set of model oligonucleotides. We find base sequence directs the dehybridization. Some sequences, such as 5'-G(AT)$_4$C-3', dissociate in an essentially two-state manner while others, such as 5'-(AT)$_2$GC(AT)$_2$-3' demonstrate stable intermediates in which the terminal AT bases fray around a stable GC center. Temperature jump transient heterodyne dispersed vibrational echo (t-HDVE) experiments allow the visualization of DNA dehybridization in real-time. We observe 10-20 μs dissociation timescales as well as 10's of ns pre-melting events such as fraying in those sequences demonstrating multistate dissociation (Fig 1).

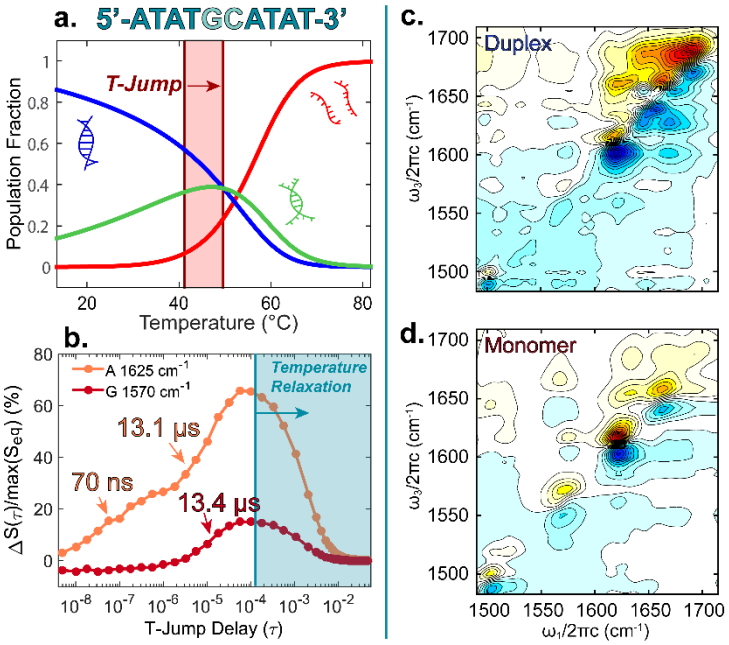

Fig 1. (a) Population profile demonstrating multistate dissociation (b) t-HDVE time traces showing initial fraying of the AT ends of the duplex. 2D IR spectra of DNA (c) duplex and (d) monomer.

[1] Ouldridge, T. E. *et al. Nucleic Acids Res.* **2013**, *41*, 8886-8895.
[2] Hinckley, D. M. *et al. J. Chem. Phys.* **2014**, *141*, 035102.
[3] SantaLucia, J. A. *Proc. Natl. Acad. Sci. USA.* **1998**, *95*, 1460-1465.