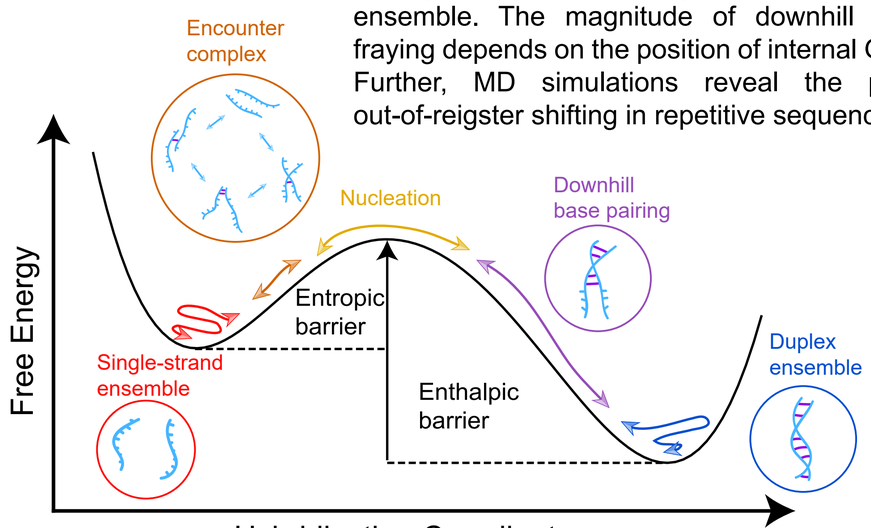
Tokmakoff Group

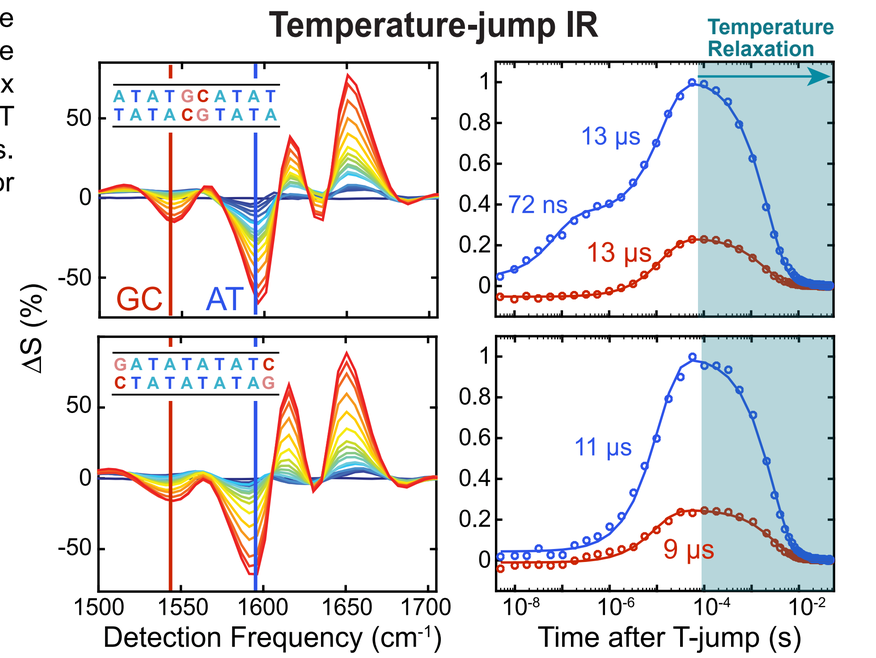
Nucleic Acid Structural Dynamics

DNA Duplex Hybridization

We seek to understand the physical properties that govern the series of molecular events during nucleic acid duplex hybridization. Along these lines we are investigating how nucleobase sequence can alter the melting behavior of duplex DNA. We experimentally interrogate nucleic acid structural changes through steady-state and temperature-jump infrared spectroscopy (T-jump IR) and NMR spectroscopy. We obtain further molecular insight from analysis of molecular dynamics (MD) simulations of hybridization through collaboration with Prof. Andrew Ferguson.

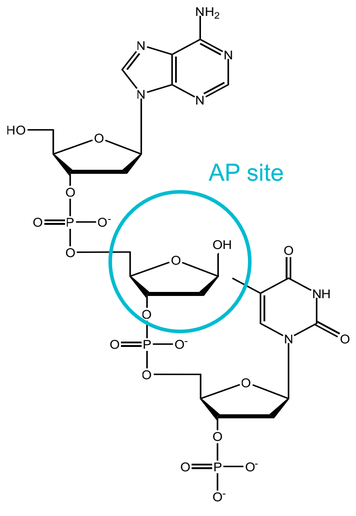


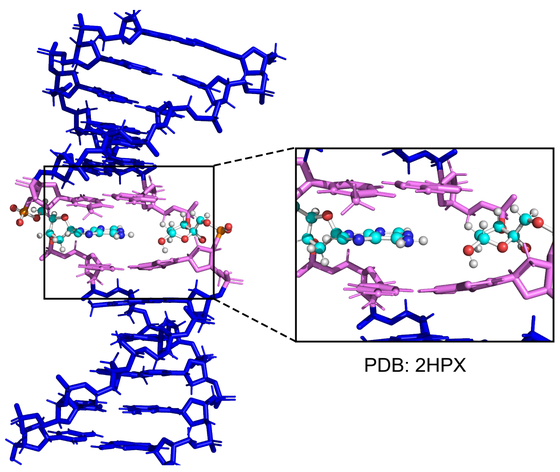
In our initial work, we investigated how the relative position of A:T and G:C base pairs shapes the base pairing energetics and dynamics within the duplex ensemble. The magnitude of downhill terminal A:T fraying depends on the position of internal G:C contacts. Further, MD simulations reveal the potential for out-of-reigster shifting in repetitive sequences.

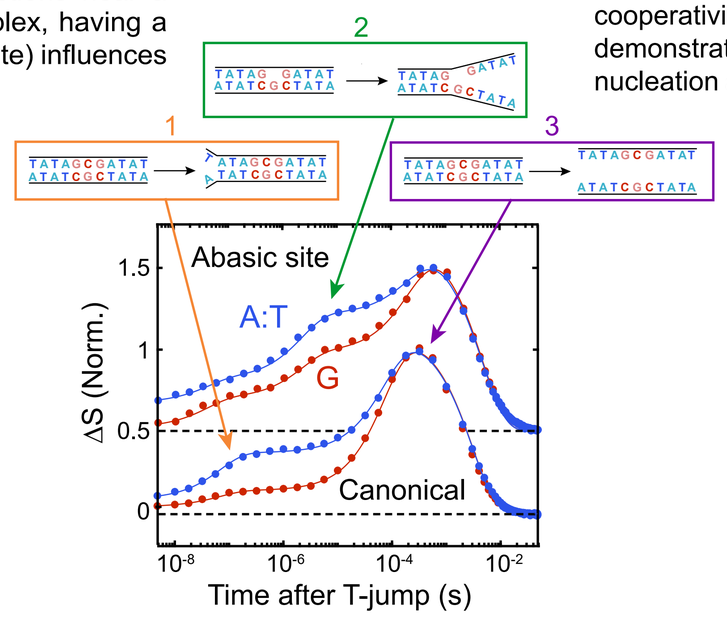


Disruption of Duplex Cooperativity

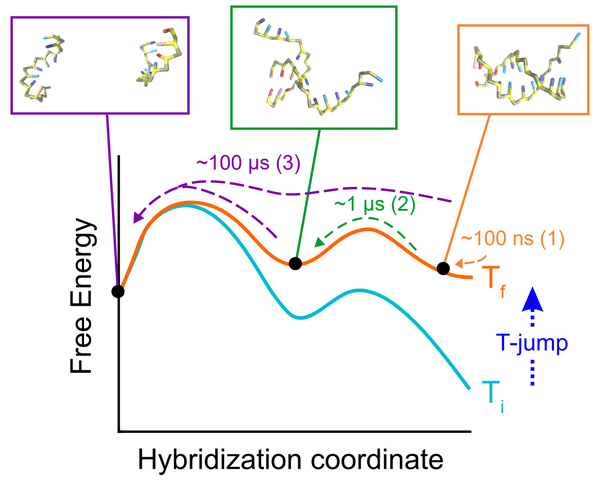
Nucleic acids exhibit several non-canonical base pairs due to chemical modifications, mispairing, and chemical damage. The local reduction of base-pairing and stacking interactions near a modified site may disrupt the cooperativity of base pairing throughout a short duplex, having a drastic impact on stability and dynamics. We are studying how an abasic site (AP site) influences base-pairing cooperativity in short DNA duplexes.





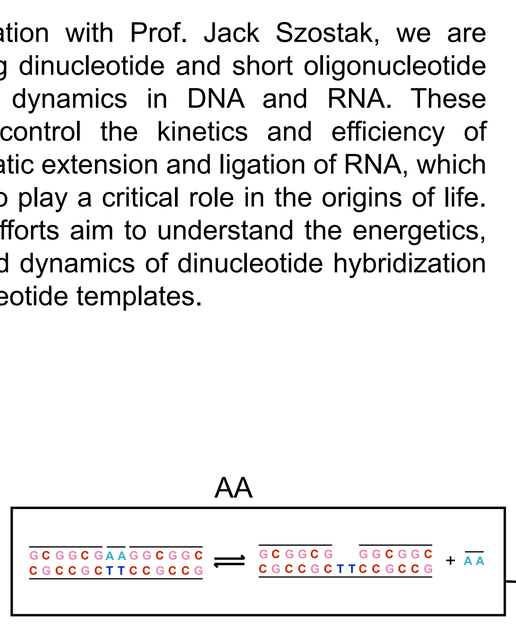


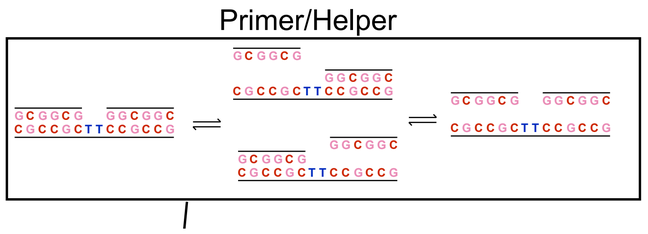
While canonical duplexes show a single dehybridization barrier, T-jump IR directly observes half-dehybridization (~1 µs) and full-strand separation (~100 µs) in duplexes with an AP site and demonstrates that each process is activated. These differences illustrate how an AP site disrupts the cooperativity of base pairing in DNA. Further, simulated trajectories demonstrate that the dynamics of hybridization are broken into steps of nucleation and zippering on one side of the AP site and then the other.



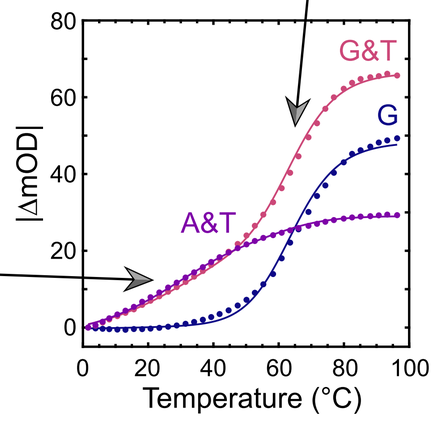
Hybridization of Short Nucleic Acids

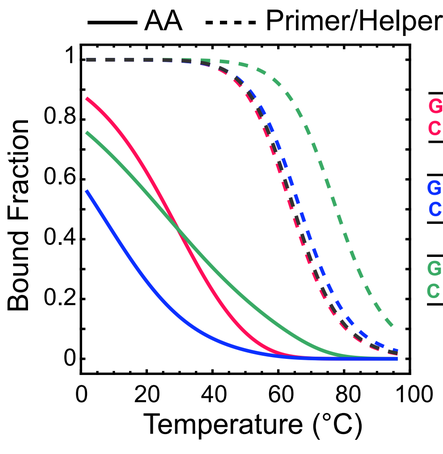
In collaboration with Prof. Jack Szostak, we are investigating dinucleotide and short oligonucleotide (un)binding dynamics in DNA and RNA. These processes control the kinetics and efficiency of non-enzymatic extension and ligation of RNA, which is thought to play a critical role in the origins of life. Our initial efforts aim to understand the energetics, kinetics, and dynamics of dinucleotide hybridization to oligonucleotide templates.





Multi-step thermal dissociation of oligonucleotide complexes





GCGGCGAAGGCGGC / CGCCGCTTCCGCCG (DNA)

GCGGCGAA / CGCCGCTTCCCCAG (DNA)

GCGGCGAAGGCGGC / CGCCGCUUCCGCCG (RNA)

Our initial results demonstrate the ability to measure accurate AA dissociation curves with temperature-dependent IR spectroscopy and dissociation kinetics with T-jump IR spectroscopy. Dinucleotide binding stabily is highly sensitive to the type of template and whether DNA or RNA is used. Future experiments aim to understand energetics and kinetics across a wider sequence space and evaluate the potential role of non-canonical base-pairing interactions.

Temperature-jump of AA dissociation