Probing the enthalpies of individual hydrogen bonds in DNA duplexes with isotope edited infrared spectroscopy

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Measuring the strength of the hydrogen bonds between DNA base pairs is of vital importance for understanding how our genetic code is physically accessed and recognized in cells, particularly during replication and transcription. Therefore, it is important to develop probes for these key hydrogen bonds (H-bonds) that dictate events critical to cellular function, such as localized melting of DNA. The vibrations of carbonyl bonds are well-known probes of their H-bonding environment, and their signals can be observed with infrared (IR) spectroscopy. Yet, pinpointing a single bond of interest in the complex IR spectrum of DNA is challenging due to the large number of carbonyl signals that overlap with each other. Here, we use a method we developed that exploits isotope editing and infrared (IR) spectroscopy to isolate IR signals from thymine (T) C=O carbonyls. We use temperature-dependent experiments and van't Hoff plots on model compounds that mimic the T base to determine the change in the enthalpy of H-bond formation between the T base and proton donors of varied strengths. We then construct a correlation plot relating changes in the T C2=O and T C4=O band position to the change in enthalpy when the T base is bound to various H-bond donors. Next, we use isotope editing to extract the band positions of T C=Os in large DNA duplexes and use our correlation plot (change in frequency vs change in enthalpy) to determine the enthalpy of individual H-bonds in DNA base pairs.