Exploring DNA with 2D-IR – spectroscopy, dynamics and ligand binding

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Introduction

DNA is the most fundamental biological molecule. The sequence of four nucleotides of the bases adenine, guanine, thymine and cytosine (A, G, T and C) that comprises the structure of DNA provides a template for the storage of genetic information in most organisms found in Nature. The DNA macromolecule predominantly exists as an anti-parallel duplex, where Watson-Crick base-pairing between A-T and G-C pairs leads to the classic double helix structure. Double stranded (ds) DNA is functionally silent when it is in its duplex form however, and duplex unwinding to reveal the Watson-Crick pairing faces of the nucleobases is a fundamental step in completing the templating function of DNA in replication and transcription.

The link between molecular structure and function is a central tenet of biology and an important basis for understanding biological mechanisms. Rather less well-studied however is the role played by molecular motion, and in particular structural fluctuations, in determining the functional behaviour of biomolecules. This gap in our understanding is particularly acute for DNA where the fundamental process of duplex unwinding invokes a level of dynamism not necessarily found in proteins.

Ultrafast 2D-IR spectroscopy offers the potential to provide bond-level structural insight into complex molecules, combined with sub-100 fs time-resolution enabling measurement of the molecular dynamics that underpin function. Here we present an overview of two recent studies exploiting 2D-IR to investigate the vibrational relaxation of DNA and the structural impact of ligand binding to double-stranded DNA, both in the solution phase. [1,2]

Results – Vibrational Relaxation of DNA

Ultrafast two-dimensional infrared (2D-IR) spectroscopy using the LifeTIME instrument was used to measure the spectroscopy and vibrational relaxation of an all-AT 15-mer DNA duplex in solution.[1] Temperature-dependent 2D-IR experiments exciting the base stretching vibrational modes near 1650 cm^{-1} and probing the base modes (1650 cm^{-1}) and the region of the spectrum near 1050 cm^{-1}, where vibrational modes of the phosphodiester (L_1, L_2) and PO_2 unit of the backbone (P_2) are located, were used to study the vibrational coupling and relaxation dynamics of both single stranded and double stranded DNA.

The experiments revealed vibrational coupling patterns linking bases with the sugar-phosphate backbone for the first time. Melting of the DNA duplex caused significant changes in the positions of off-diagonal peaks in the 2D-IR spectra linking carbonyl and ring-stretching vibrational modes of the adenine and thymine bases with vibrations of the phosphate group and phosphodiester linkage. Figure 1 compares 1- and 2-colour 2D-IR spectra obtained at 20 °C (dsDNA) and 80 °C (above the melting temperature of the duplex). These show that Watson-Crick hydrogen bonding and helix formation leads to a vibrational coupling arrangement of base vibrational modes with those of the backbone that is a unique feature of the double helix.

Measuring 2D-IR spectra as a function of waiting time enabled observation of rapid energy transfer between modes due to the base and backbone of the AT 15-mer. These were mediated by additional modes located on the deoxyribose moiety within the same nucleotide. It was determined that the vibrational relaxation dynamics were insensitive to duplex melting, showing that efficient intramolecular energy relaxation to the solvent via the phosphate groups is the key to excess energy dissipation in both single and double-stranded DNA. [1]

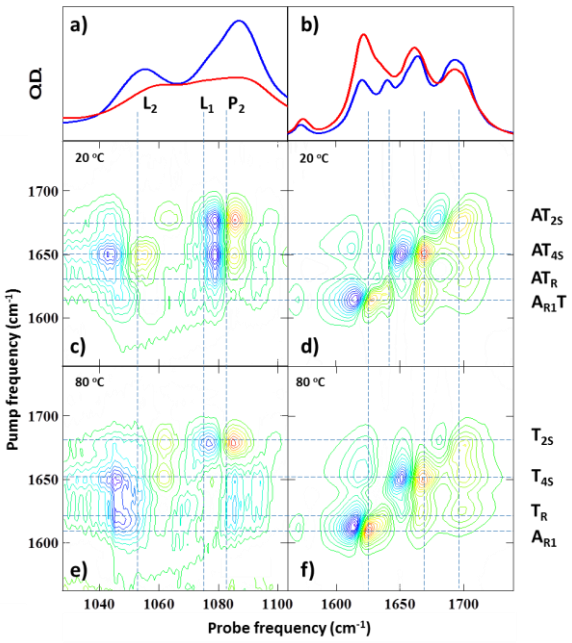

Figure.1: *a) IR absorption spectra of AT 15-mer in the backbone stretching region of the spectrum at 20 ˚C (blue) and 80 ˚C (red). b) IR absorption spectra showing vibrational modes of the bases at 20 ˚C (blue) and 80 ˚C (red). c-f) 2D-IR spectra showing results of one color (d,f) and two color (c,e) experiments at 20 ˚C (c,d) and 80 ˚C (e,f). Vibrational mode assignments are given in black.* [1]

Results – DNA-Ligand Binding

As mentioned briefly above, recognition of dsDNA sequences by transcription factors is an essential aspect of biological function. This offers the opportunity to exploit DNA-binding small molecules, such as minor groove binders (MGBs), to perturb processes associated with gene expression,[3] making them excellent candidates for the design of sequence-selective probes of DNA function or as novel therapeutics. A clear set of molecular rules for the rational design of MGBs to target DNA in a sequence-selective manner is not yet available however, as a consequence of the complex combination of competing enthalpic and entropic contributions from H-bonding, van der Waals forces and changes in hydration of the DNA and ligand.

In order to shed some light on this problem, we applied 2D-IR spectroscopy on ULTRA to investigate the molecular determinants of binding the archetypal MGB Hoechst33258 to 10 base pair sequences of dsDNA. The experiments compared results for an A-tract (AAATTT) sequence, to which Hoechst 33258 binds strongly, with those for an alternating A-T (ATATAT) sequence, to which Hoechst33258 binding is sub-optimal. [2]

Exploiting the fact that 2D-IR is sensitive to H-bonding and molecular structure changes and employing difference spectroscopy to highlight binding-induced spectral changes (Fig.2) it was demonstrated that Hoechst33258 binding results in loss of the minor groove spine of hydration in both the optimal and sub-optimal binding cases. An additional perturbation of the base propeller twists was observed in the A-tract binding region indicating an additional structural factor contributing to the optimal binding case. This change in the DNA structure to accommodate the ligand is reminiscent of induced fit binding and we propose that it maximizes favourable ligand-DNA enthalpic contributions for optimal binding. Induced fit is well-established for small molecule-protein interactions, but its relevance to DNA binding is less clear. Our results demonstrate that controlling the molecular details that induce subtle changes in DNA structure may hold the key to designing novel DNA-binding molecules. [2]

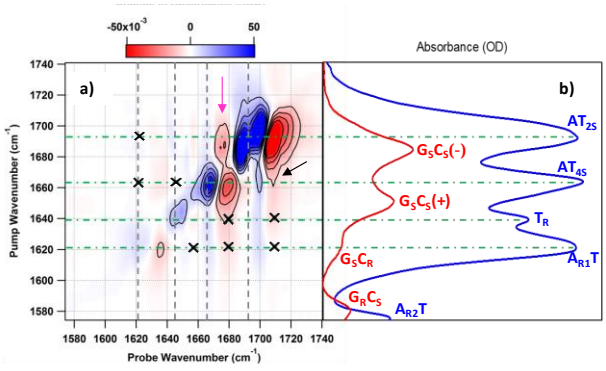

Figure 2. *a) 2D-IR difference spectrum arising from the binding of Hoechst33258 to a 10-mer dsDNA oligomer featuring the AAATTT motif. Crosses show locations of small off-diagonal features. b) FTIR spectra of GC and AT-only DNA sequences show that the perturbation of the DNA spectrum occurs exclusively within the AT region of the dsDNA molecule.* [2]

Conclusions

The results presented show that 2D-IR spectroscopy experiments on LifeTIME and ULTRA offer the potential to probe both the molecular dynamics and intermolecular interactions of dsDNA in the solution phase with base-pair-specific insight, even in complex systems featuring multiple base pairs and additional ligand molecules.

Materials and Methods

2D-IR Spectroscopy: In both studies described above 2D-IR experiments were performed using the Fourier-transform 2D-IR method in which the three input pulses were arranged in a pseudo pump-probe geometry. [4,5]

In the case of the LifeTIME spectrometer, used for vibrational relaxation experiments,[1] the mid-infrared laser pulses were produced by three optical parametric amplifiers (OPA1-3) pumped by two 100 kHz Yb:KGW amplified laser systems, (6W, 180 fs, and 15W, 300 fs). OPA1 generated 2D-IR pulses centered at 1650 cm^{-1}, to excite the base carbonyl/ring stretching modes of the AT-15mer dsDNA sample. OPA2 provided probe pulses centred at 1650 cm^{-1} for single color 2D-IR experiments. OPA3 produced pulses centred at 1050 cm^{-1} to enable two-color 2D-IR experiments probing the phosphate backbone region of the spectrum. The two co-linear pump pulses, separated by a variable time delay, τ, were created using a mid-IR pulse shaper to modulate the OPA1 output. The waiting time was controlled by an optical delay line sited after the pulse shaper. The pump-pulse duration was ~300 fs. The probe beams were dispersed and detected via grating spectrometers equipped with a 128 element MCT array detectors. 2D-IR signals were measured by scanning τ for a fixed waiting time using phase cycling and both ZZZZ and ZZYY polarization geometries.[6]

2D-IR spectra for ligand binding studies[2] were collected using the ULTRA FT-2D-IR spectrometer as described elsewhere.[7,8] The IR pulses had a temporal duration of ~ 100fs; a centre frequency of 1650 cm^{-1} and a bandwidth of ~300 cm^{-1}, at a repetition rate of 10 kHz.

Samples: For all IR measurements, the sample was held between two CaF_2 windows separated by a 25-50 μm thickness poly-tetrafluoroethylene spacer and housed in a thermostatically-controlled mount, allowing the temperature to be controlled, accurate to ± 1°C. For vibrational relaxation measurements, the sample consisted of a 15 base pair oligomer 5'–ATTATTATTATATTA-3' and its complementary sequence dissolved to a concentration of 10 mM in deuterated tris-buffer (100 mM, pD7.4) containing 100 mM NaCl.[1] For ligand binding studies, lyophilised, salt-free DNA oligonucleotides with sequences $d(GGAAATTTGC)_2$ and $d(GGATATATGC)_2$, $(AT)_3$ were used. All samples were prepared using pD7 phosphate buffer solution to a final duplex:Hoechst33258 molecular ratio of 1:1 and annealed at 90 °C for 10 minutes.[2]

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