Evaluation of Ultra A for the detection and analysis of drug-target structural changes using EVV 2DIR spectroscopy

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Introduction

When two molecules come together to form a complex, part of the interaction is the coupling of molecular vibrations that originated on the unbound molecular species. My group has shown in the past that the new couplings formed by the formation of molecular complexes can be detected by EVV 2DIR spectroscopy[1]. Moreover we were also able to show how this data can used to determine the geometry of the molecular complex with high accuracy and precision[2]. Following this we successfully demonstrated that despite the complexity of a protein system, it is possible to detect the binding of a drug to a protein target using EVV 2DIR spectroscopy[3]. As part of this study we also demonstrated that it was possible to assign some of the features using quantum mechanical calculations to predict the spectra expected from the crystal structure. This is potentially important as despite the power of existing structural biology methods, there are still many limitations that mean that structural data is not available in sufficient quantity or quality, to drive particular drug-discovery/development strategies.

The limiting factor in our studies of drug-target binding, is the signal to noise and reproducibility achievable by the spectroscopic apparatus. The age of the laser systems for my group's apparatus at Imperial (15 years old and no longer supported by the laser manufacturers) and its low repetition rate meant that this was now a limiting factor in pushing forwards with the scientific programme. With this in mind, my group undertook an EPSRC-funded knowledge transfer activity through the impact acceleration scheme. In collaboration with the CLF, we ported our EVV experiment onto CLF apparatus. This was successfully completed and showed that EVV 2DIR experiments could be successfully performed on both Ultra A and Lifetime.

Following this knowledge transfer activity, we applied for laser time to explore the utility of the new apparatus. I report on this project below for which there were two main foci. Firstly, we explored the utility of the new apparatus against drug-kinase binding. Secondly, we explored the ability of EVV 2DIR spectroscopy to be used to assign structural features to G-quadruplex DNA. The reason for this second strand is that G4 DNA is a drug target and the ability to interpret EVV 2DIR would lead to a drug-target study.

Drug Binding to FGFR1

The system of study is FGFR1, which was the subject of our original drug-binding study run at Imperial College in collaboration with AstraZeneca and this collaboration is ongoing. There were two main objectives in this part of the work. Firstly repeating the published results to show that the same drug-binding features can be detected and secondly searching for a change in the spectral features associated with phenylalanine on drug binding. Kinases have a short regulatory loop called the DFG sequence, which can move from internal to external to the protein. This changes the solvation state of the phenylalanine molecule, which should be resolvable with EVV 2DIR spectroscopy.

A summary of this part of the study is that unfortunately neither the reproducibility nor the signal to noise was good enough to repeat the Imperial College work. This was true for both identifying the original binding peaks and for monitoring the DFG transition.

Without going into the technical details of the experimental arrangement, extensive analysis of the data identified two main problems. 1) The noise on one spectral axis was very high, apparently due to the instability of one of the pulse trains. The CLF team identified this as being largely due to the long path length between the experimental area and laser sources, which led to substantial variation in beam overlap, leading to high noise along one spectral axis. 2) The spectra of the IR beams used in the experiment were not sufficiently reproducible on a day to day basis, meaning that data between different days could not be averaged or compared meaningfully.

As a result, we concluded with the CLF team that technical changes needed to be made to the experiment for the next experimental run. These were 1) Relocation of the experiment to be significantly closer to the laser sources 2) The addition of a reference arm to the spectrometer in order to normalize variations in beam properties both during the day and day to day.

The anticipation is that a further experimental run on apparatus in which both the signal to noise and reproducibility of the data has been improved, would yield successful results, equal to or surpassing the quality of the original Imperial College data.

Detecting Structural Elements in G4 DNA

There are many DNA sequences that can form the G4 structure. Myc2345 is a well-studied and particularly stable sequence containing 21 bases. The Myc G4 structure can be induced by the addition of ions such as potassium. As such this gave an external variable that could be altered in order to identify spectral changes associated with the transition from a disordered to a G4 structure.

The relative simplicity of the G4 sequence means that we anticipated that neither the quantitative levels of reproducibility, nor the signal to noise of the data would need to be as high as required for the study of protein-drug binding. This proved to indeed be the case.

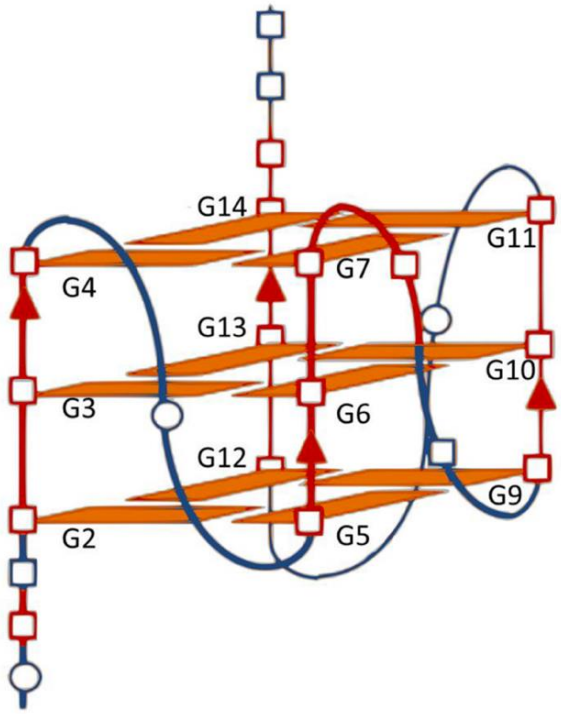

Figure 1 The structure of Myc2345 G-quadruplex. The blue circles represent thymine, red squares represent guanine and blue squares represent adenine. The orange rectangles represent guanine bases in the tetrads, and they are all in the anti conformation. The G-tracts involved in forming the tetrads are in the same direction(i.e. it is a parallel G-quadruplex).

Despite this, the complexity of the data turns out to be significant. Two-dimensional fitting has shown that over 170 peaks are present in the data for the Myc Sequence in the absence of metal ions.

Within these peaks we were able to further identify three further classes of peak which vary with the introduction of metal ions.

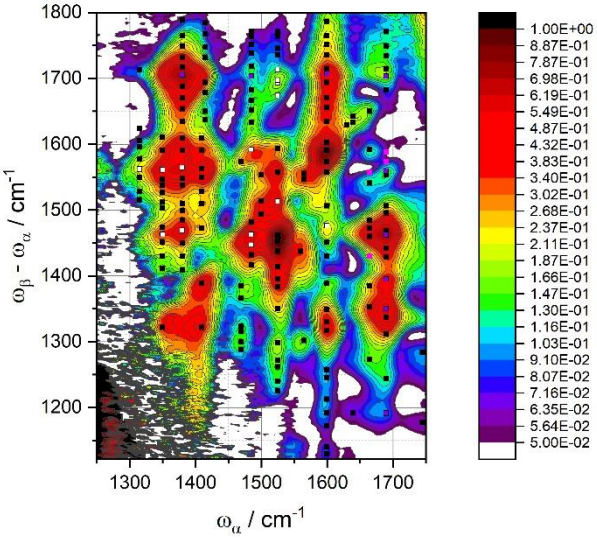

Figure 2 EVV 2DIR spectrum of Myc2345 in the absence of potassium ions taken using Ultra A. Over 170 individual peaks are identifiable.

The first of these are hypochromic peaks. Hypochromism is known to be caused by base stacking and we have identified many such peaks in our data.

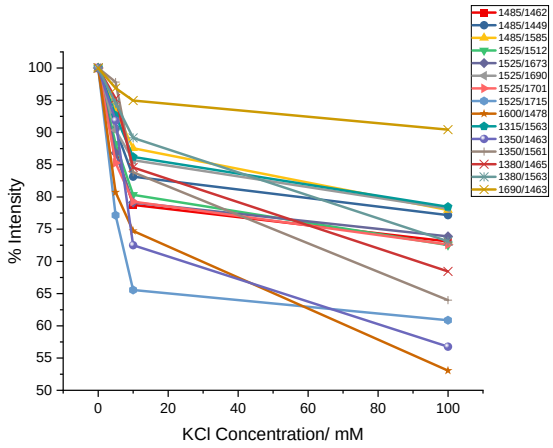

Figure 3 A summary of hypochromic intensity changes as a function of potassium ion concentration. Hypochromism is associated with base stacking and this seems to be largely complete by the time 10mM of ions have been added

An example of a cut through of the data to highlight the complexity is below.

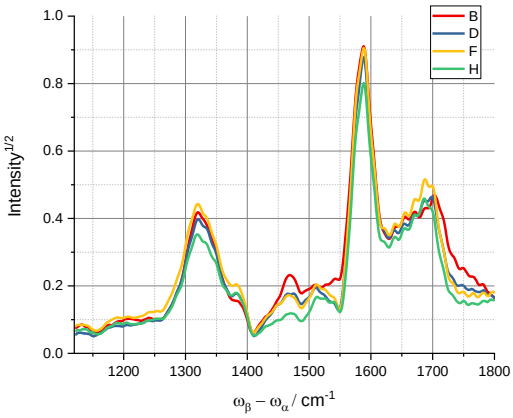

Figure 4 Cut through at ω_α = 1600cm^{-1}. Spectra are shown at 4 ion concentrations. 0, 5,10, 100mM.

In addition to hypochromism there are peak shifts, where a coupling peak at one frequency is replaced by another shifted by 20-30cm^{-1}. While the frequencies that contribute to the peaks can be assigned, it is not yet clear what specific structural changes cause these shifts in frequency.

Finally there is the appearance of entirely new peaks due to the formation of the G4 structure. These are tentatively assigned to the formation of new intramolecular hydrogen bonds as the G4 structure forms. These are largely associated with carbonyl frequencies, but there are also possibly new crosspeaks due to additional inter-ring coupling as the bases stack.

The conclusions from this study are both self-consistent and with previous studies of G4 structures by other methods. The details and the interpretations are in the process of being written up as a paper to be submitted in early 2020.

Conclusions

EVV 2DIR experiments performed on Ultra A, have allowed the identification of three classes of spectral change which are associated with the formation of the Myc2345 G4 structure from disordered DNA. These changes can be assigned to structural features in a self-consistent way and demonstrate that

Ultra A EVV-2DIR provides sufficient capability to be used in structural and drug binding studies in small nucleic acid systems.

Conversely, the experiments on FGFR1 were not successful as neither the reproducibility of the data nor the signal to noise were sufficient for resolving the proportionately smaller changes that occur upon drug binding to a protein. This reflects the larger size and complexity of a protein where the binding-peaks make up a smaller proportion of the total signal/spectrum.

Modifications to the Ultra A-EVV 2DIR setup have been identified which have the potential to abrogate these problems and make the system comparable or superior to the performance of the obsolete Imperial College system.

Acknowledgements

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