The Structure of a Drug-Protein Complex Detected and Analysed by EVV 2DIR Spectroscopy

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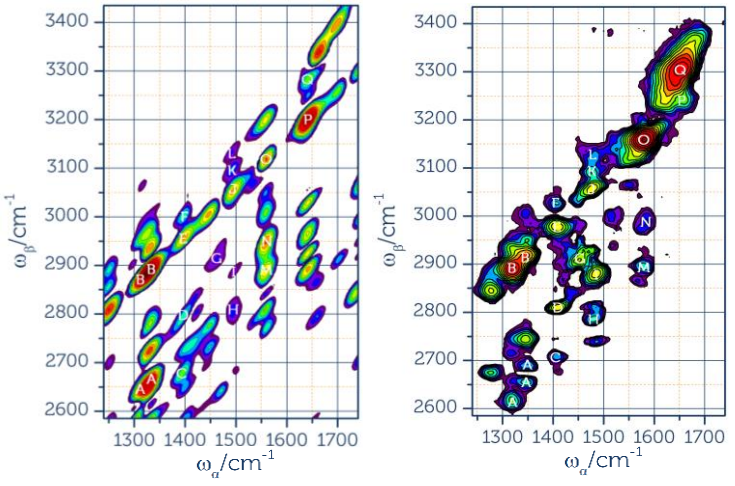
Abstract: The geometry of a drug-bound to its protein target is measured with EVV 2DIR. Of the >200 resolvable peaks at least 7 are due to specific binding. By comparing the spectrum calculated from the crystal structure with that measured by EVV 2DIR we can determine whether the structure as observed by EVV 2DIR is the same as that of the complex in the crystal structure or not.

We have previously shown that it is possible to determine the geometry of molecular complexes using EVV 2DIR spectroscopy [Guo et al, PCCP 2009] by comparing the ratios of crosspeaks taken with all beams parallel in polarisation (PPP) and with the visible beam at 90^0 (PPS) With accurate quantum mechanical calculations for structural refinement it was possible to obtain the distance between molecular species with an accuracy of 0.1Å and the angle between them to 1^0 [Guo et al, PCCP 2012]. In this work we take the same approach to the study of the binding between a kinase inhibitor and its kinase drug target. The first objective is to compare the structure determined by means of EVV 2DIR spectroscopy with that of the crystal structure. The second objective is to determine whether this methodology can be used to determine the structures of unknown complexes and/or validate the predictions of docking models for drug-protein interactions.The data for the drug-protein complex is shown below after having had the protein spectrum subtracted. It is also compared with the spectrum calculated for the drug in the protein binding site and peaks assigned are labelled. The experimental data for the drug-protein complex has a dynamic range of 450 and contains ~100 resolvable and quantifiable crosspeaks 3σ above the noise floor. Of these 100 peaks, 7 are only present when the drug is specifically bound indicating that they are couplings formed only when the protein-drug complex is formed.



By comparing the polarisation ratios in the calculated and measured spectra, it is possible to determine whether there is structural consistency between the EVV 2DIR data and the spectrum predicted from the crystal structure. In this presentation I discuss this comparison and the extent to which the structure determined by EVV 2DIR spectroscopy agrees with the x-ray crystal structure. I also discuss the accuracy that can be obtained by making approximations and suggest how a cycle of refinement can be used to determine drug-protein binding geometries *de novo* and to test the predictions of drug-protein docking models.