Detection and Assignment of Inhibitor-Protein Interactions from EVV 2DIR Data

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Electron-vibration-vibration (EVV) 2DIR spectroscopy is used to investigate inhibitor binding to mammalian and plant proteins. We demonstrate the ability to detect inhibitor binding, and suggest potential applications in label-free screening and identification of previously unknown binding sites.

EVV 2DIR is a frequency-resolved technique in which three picosecond laser pulses are overlapped in a biological sample and the emitted photons are detected whilst the frequencies of the two mid-IR beams are systematically varied, probing vibrational couplings [1]. EVV 2DIR spectra for the binding domains of FGFR1 & HPPD have been measured, in complex with their respective inhibitors, across a range of different molar ratios of inhibitor to protein, from no inhibitor to excess inhibitor. The spectra of FGFR1 in complex with SU5402 displays >200 peaks (Fig. 1a). Peak intensities as a function of concentration give information about the binding of the inhibitor: certain cross-peaks show an increase in intensity up to 1:1 stoichiometry, after which no further increase in intensity is observed, indicating that the signal originates from bound inhibitor only, with no contribution from the unbound inhibitor or non-specific binding. These peaks are also absent in spectra of SU5402 with non-binding proteins. Other cross-peaks show an increase in intensity with inhibitor concentration which are diagnostic of quantity of inhibitor, arising from vibrations unrelated to binding (Fig. 1b).

This demonstrated that EVV 2DIR has the potential to detect drug protein binding. Potential applications include label-free screening, detection of binding and identification of previously unknown binding sites. Combining appropriate calculations with the experimental results has the potential to provide information on both the geometry of the bound inhibitor and interactions between inhibitor and protein [2].

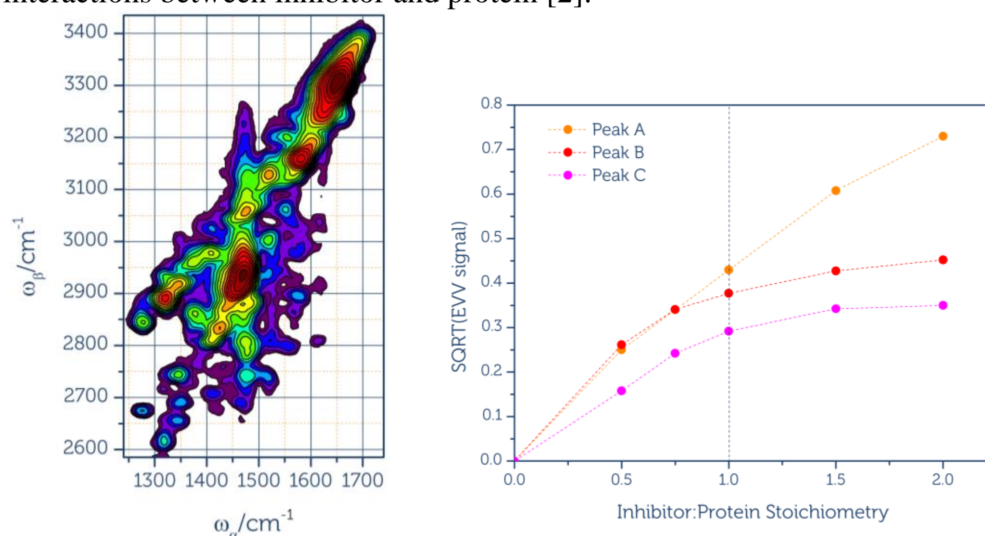

Fig. 1a (left) EVV 2DIR spectra of FGFR1 in complex with inhibitor SU5402. Fig. 1b (right) Peak intensities as a function of inhibitor:protein molar ratio for 2 binding dependant peaks (B+C) and a binding independent peak (A)

[1] F. Fournier *et al.,* Accounts Chem Res, **42,** 1322 (2009)
[2] R. Guo *et al.*, Faraday Discuss, **150,** 160, (2011)