Heterogeneous Protein-Ligand Binding Determined by 2D IR Spectroscopy with the Unnatural Amino Acid Azidohomoalanine

Philip J. M. Johnson, Klemens L. Koziol, and Peter Hamm*

Department of Chemistry, University of Zurich, CH-8057, Switzerland
*peter.hamm@chem.uzh.ch

Using azidohomoalanine as a vibrational probe of local protein structure, we observe multiple ligand binding conformations for C-terminal Aha mutated ligands when bound to a PDZ2 domain, manifest as multiple distinct frequency shifts from the unbound ligand bleach response as observed by 2D IR spectroscopy.

The unnatural amino acid azidohomoalanine (Aha) has been shown to be a sensitive infrared probe of local structure, where frequency shifts of up to ~12 cm^{-1} between folded and unfolded protein conformations [1] or between bound and unbound protein-ligand pairs [2] have been observed. 2D IR experiments of a C-terminal Aha mutant of the ligand which binds to the PDZ2 domain showed what appeared to be significant unbound bleach response even at concentrations which would normally be expected to show near saturated binding [2].

Through a series of 2D IR experiments studying ligand binding as a function of molar fraction with the substrate protein, we resolve two separate frequency shifts associated with two distinct conformations of the C-terminal azido side chain, one which remains solvent-exposed with a frequency shift of ~5 cm^{-1}, and a larger ~12 cm^{-1} frequency shift which implicates the side chain to be buried in the PDZ2 binding pocket (see figure below). The dissociation constants of both conformations are determined by population analysis of the 2D IR spectra, and the results are compared to thermodynamic measures of binding affinity.

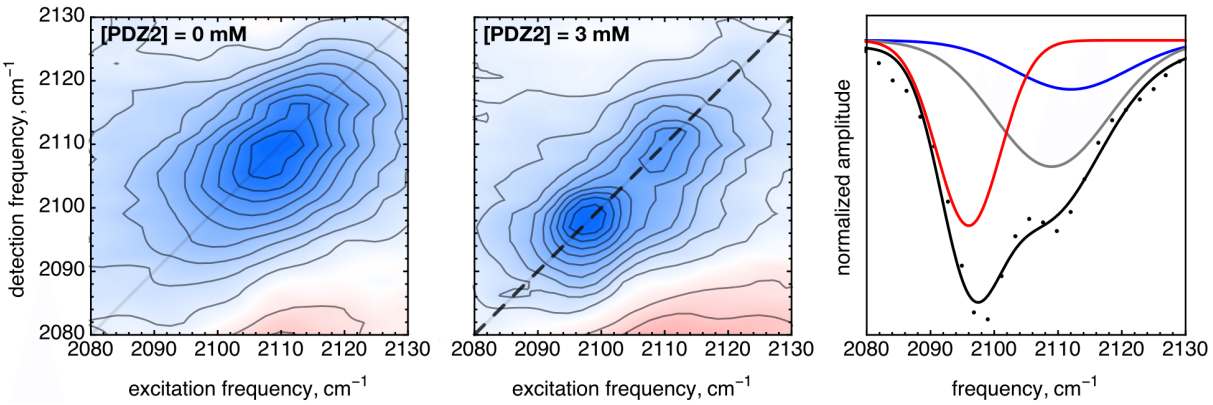

Fig. 1 *(Left)* The 2D IR response from free ligand (0.5mM), unbound to protein. *(Center)* Frequency shifts are observed with the presence of the substrate protein, narrowing and redshifting the bleach response for bound ligand. *(Right)* The diagonal cut of the [PDZ2] = 3mM data is adequately fit by three gaussians, showing that the majority of the population has shifted to a bound state (red and grey curves) with minimal unbound ligand remaining (blue curve).

[1] Taskent-Sezgin *et al.*, Azidohomoalanine: a conformationally sensitive IR probe of protein folding, protein structure, and electrostatics, *Angewandte Chemie, Int. Ed.* **49**, 7473 (2010).
[2] Bloem *et al.*, Ligand Binding Studied by 2D IR Spectroscopy Using the Azidohomoalanine Label, *Journal of Physical Chemistry B* **116**, 13705 (2012).