

Fragment-based drug discovery by NMR – The advantages of isotope-labelling

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Introduction

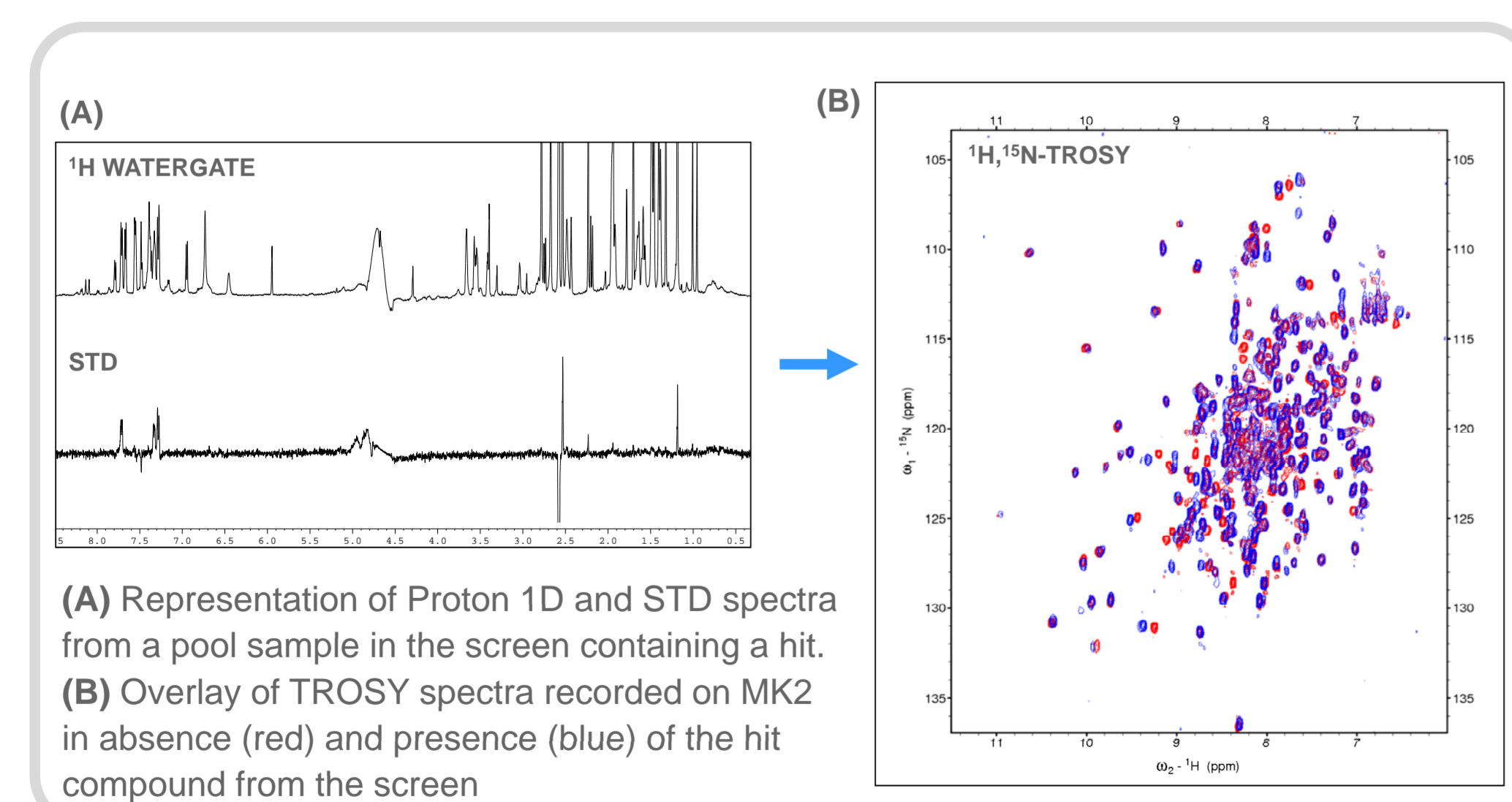
In Fragment-based Drug Design (FBDD) the use of biophysical screening techniques like NMR, Crystallography, Fluorescence Correlation Spectroscopy (FCS) or Surface Plasmon Resonance (SPR) for fragment screening and hit qualification is key to the discovery of a good number of promising starting points for subsequent development and optimisation.

Particularly NMR spectroscopy can be a powerful tool in the quest for fragment-hits suitable as efficient starting points in hit-to-lead programmes. Therefore, the technology is an important asset in the integrated FBDD technology platform EVOlution™ at Evotec. The information content of NMR spectroscopy can be exceptionally high when observing resonances of the protein directly. For that purpose, isotope labelling is almost always required.

Kinases are one of the most prominent classes of proteins for target-based drug discovery. In order to identify novel ligands for specific subsites and kinase conformations we have applied different isotope-labelling approaches to several kinase targets: Ubiquitously isotope-labelled protein is highly useful for screening and in combination with resonance assignments allows focusing on the interaction site of interest. The NMR data obtained on selectively isotope-labelled kinase protein expressed from insect cells can allow easy access to information about binding modes of ligands and produce valuable clues for the development of leads from fragment-based drug discovery.

Screening for novel MK2 ligands with unlabelled protein followed by hit validation with isotope-labelled protein

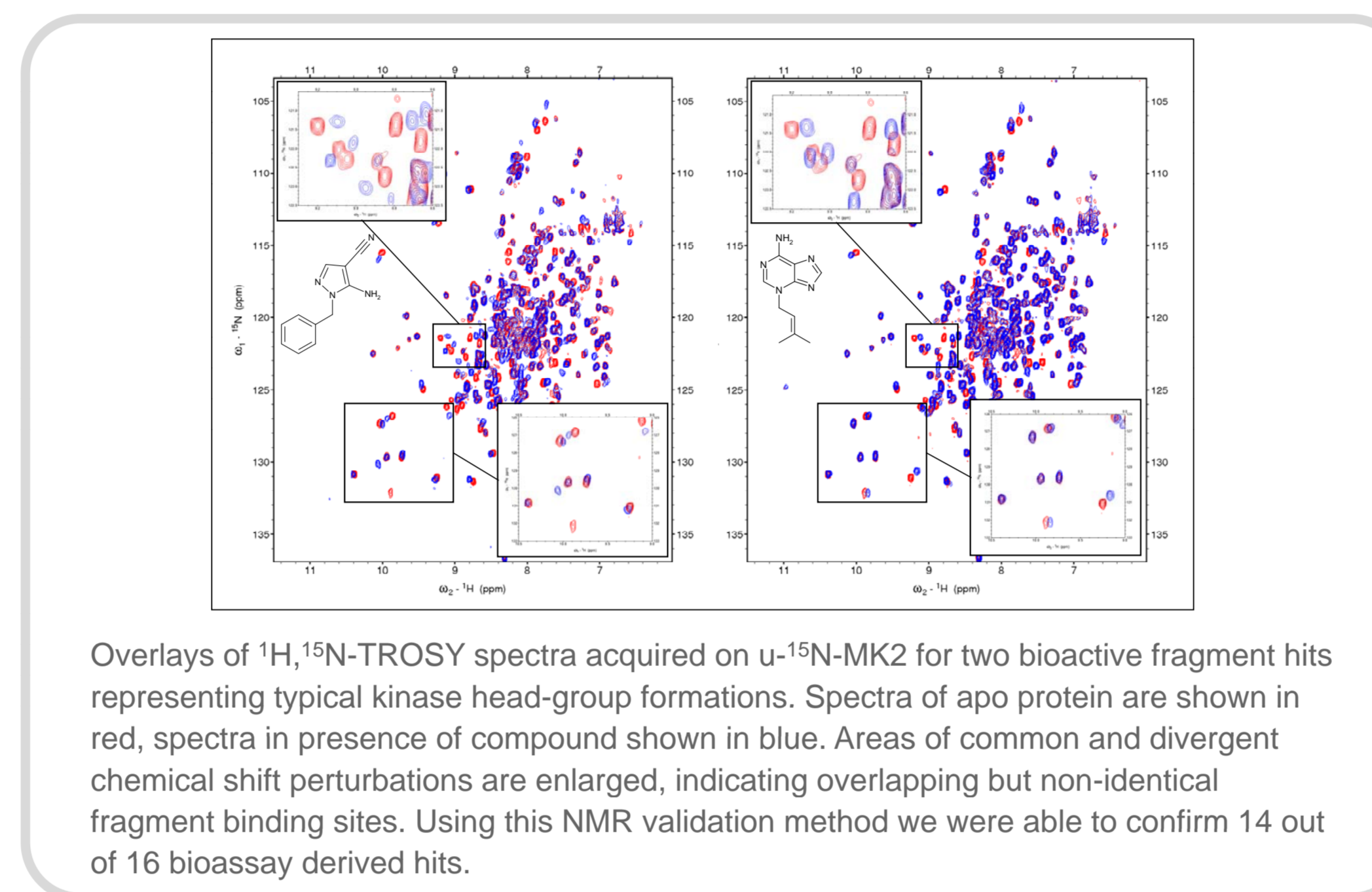
In cases where the availability of isotope-labelled protein is a restricting factor, it is often beneficial to screen using ligand-observed NMR methods, followed by a hit validation applying protein-observed NMR experiments like HSQC or TROSY. This sequence of NMR assays is very cost efficient. Only compounds that have shown initial potency in the screen are subjected to the information-rich protein observed NMR assay, reducing the amount of isotope-labelled protein required to a minimum.



In the quest for novel fragment hits binding to MAPKAP kinase2 (MK2) a ligand-observed screen of 2,500 compounds was performed on the inactive conformation of MK2 using STD NMR as the assay format. Ubiquitously ²H,¹⁵N-labelled protein was obtained from an *E.coli* expression system. With this material the most interesting fragment hits from the screen were subsequently validated for binding by testing their effect on the ¹H,¹⁵N-TROSY spectra of labelled MK2.

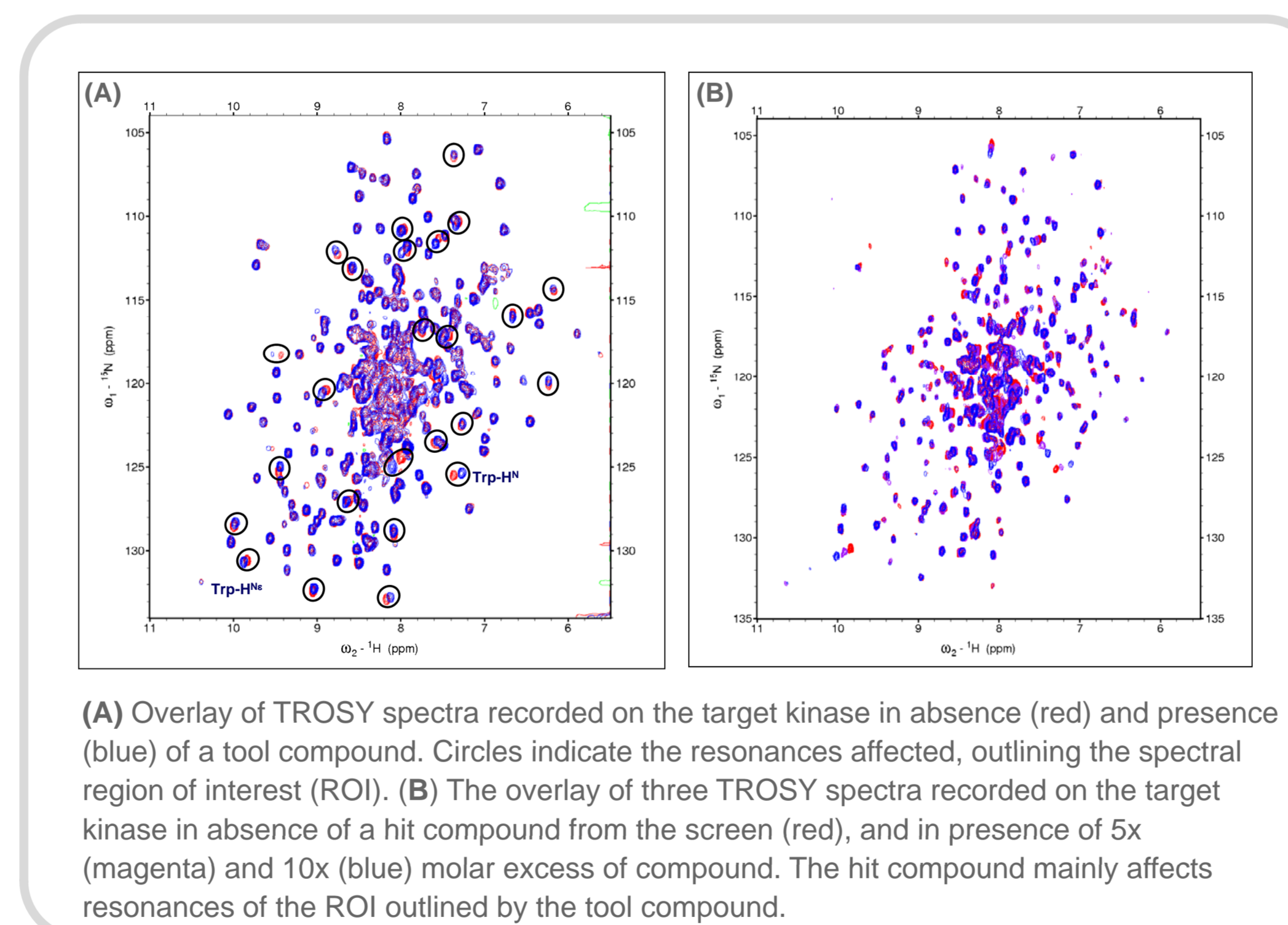
Patterns of chemical shift perturbations induced by ligands allow comparison of binding modes

Hit fragments from an HTFS by bioassay on MK2 were subjected to an NMR validation process by TROSY NMR. Here only functionally active fragments are examined for direct binding to MK2. Compounds binding to the protein induce a “fingerprint” pattern of chemical shift perturbations (CSPs). By comparing the CSP fingerprints of fragments, differences and similarities in binding modes can be deduced quickly.



Screening of a kinase for compounds binding to an allosteric binding site

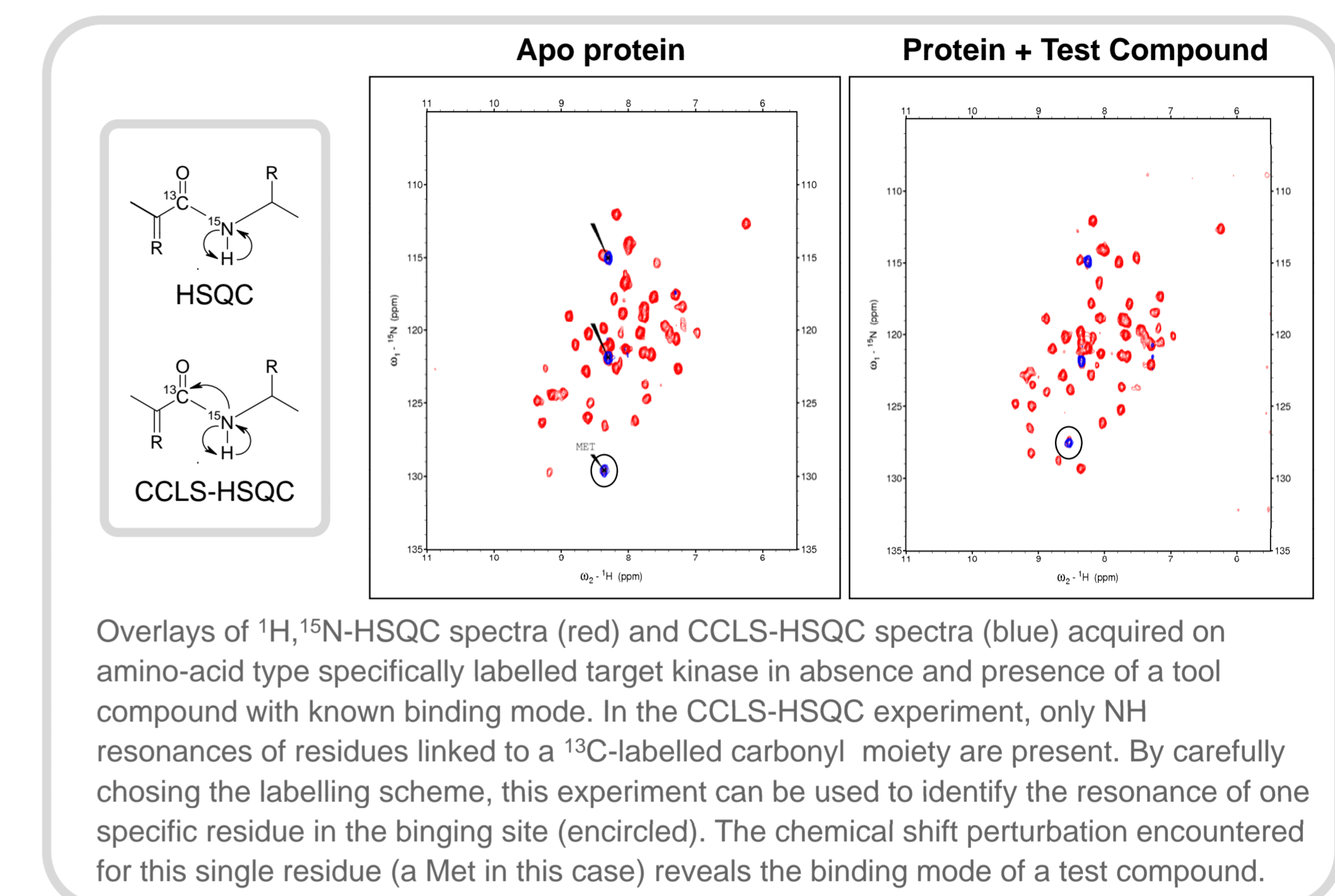
In cases where kinase proteins can readily be obtained in isotope-labelled form and backbone resonance assignments can be achieved, fragment hits binding to a specific interaction site can be identified immediately in the screening campaign.



Uniformly ¹⁵N-labelled protein was produced from an *E. coli* expression system. We performed a protein observed (¹H,¹⁵N-TROSY) NMR screen on the catalytic domain of a cell type specific kinase isoform. A known binder to the allosteric site was used as a tool compound to yield a “fingerprint”. Using backbone resonance assignments, a spectral “region of interest” was established and compounds binding to the allosteric binding site were readily identified in the screen. The most interesting hit compounds from the screen showed concentration dependent chemical shift perturbations restricted to the defined region of interest. This can ultimately be used to determine *K_d* values for the interaction with the protein in an NMR-monitored titration series.

Targeting specific binding sites / binding modes by selective isotope labelling in insect cells

Amino acid-type selective isotope-labelling of proteins can be a very powerful tool to focus on binding sites and sometimes even deduce compound binding modes from a limited number of NMR resonances. Selective isotope-labelling is most efficiently achieved in insect cell expression systems due to the tighter regulation of metabolic pathways avoiding scrambling of isotope labels. Implementing a well designed labelling scheme is key to obtaining maximum information from the NMR data.



For the catalytic domain of a tyrosine kinase, hits from a biophysical screen using SPR were characterised with respect to their binding modes.

A labelling scheme was devised in which several amino acid types were selectively ¹⁵N-isotope labelled and one amino acid type carried a ¹³C-label. Selectively labelled protein was expressed from a baculovirus expression system using insect cells.

Applying a special NMR experiment (carbonyl carbon label sensitive HSQC, CCLS-HSQC^[1]) allowed the identification of the backbone amide resonance of a single methionine residue in the binding site of interest. Compounds that bind into this binding site affect this resonance very differently, depending on their binding mode. So the binding modes of test compounds can be deduced simply by monitoring a single resonance in the ¹H,¹⁵N-HSQC spectra of the labelled catalytic kinase domain.

Summary

Many benefits can be gained from applying an isotope-label to a target protein. As illustrated for three different kinase targets isotope-labelling can open up very useful opportunities to apply NMR spectroscopy to obtain maximum information content:

- Monitor the target protein and binding to it directly
- Put a focus on (allosteric) binding sites
- Deduce information on binding modes
- Correlate NMR and available structure data

If technical hurdles can be overcome, isotope-labelling can therefore be the key to moving fragment hits from a screening campaign into the initial stages of hit-to-lead development.

References

[1] Tonelli *et al.* J. Biomol. NMR 39, 177-185 (2007)