Automated NMR resonance assignment of large proteins for protein-ligand interaction studies

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Author(s) Gossert, Alvar D.; Hiller, Sebastian; Fernández, César
Author(s) at UniBasel Hiller, Sebastian ;
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The detection and structural characterization of protein-ligand interactions by solution NMR is central to functional biology research as well as to drug discovery. Here we present a robust and highly automated procedure for obtaining the resonance assignments necessary for studies of such interactions. The procedure relies on a combination of three automated projection spectroscopy (APSY) experiments, including the new 4D APSY-HNCACB, and the use of fractionally deuterated protein samples. This labeling pattern increases the experimental sensitivity on the one hand, but it leads to peak multiplets on the other hand. The latter complications are however overcome by the geometric APSY analysis of the projection spectra. The three APSY experiments thus provide high precision chemical shift correlations of the backbone and side chain methyl groups, allowing a reliable and robust assignment of the protein by suitable algorithms. The present approach doubles the molecular size limit of APSY-based assignments to 25 kDa, thus providing the basis for efficient characterization of protein-ligand interactions at atomic resolution by NMR, such as structure-based drug design. We show the application to two human proteins with molecular weights of 15 and 22 kDa, respectively, at concentrations of 0.4 mM and discuss the general applicability to studies of protein-protein and protein-nucleic acid complexes.

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