New approach in NMR structure determination of weak complexes using RDCs: The complex between the third SH3 domain of CD2AP and Ubiquitin.

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NMR has been known for years as one of the most sensitive tools for examining weak protein–target interactions at physiological conditions. However, the use of this technique in unraveling weak protein-protein interactions and determining their three-dimensional structures has been elusive. Classic NMR strategies have been related with the use of half-filtered NOESY experiments, which require full assignment of both components in the complete bound form, and a very high concentration to detect the intermolecular NOEs for complexes with big binding constants (Kd).

Residual Diploar Couplings (RDCs) can provide information on the orientation of certain dipoles within macromolecules (i.e. HN–N, C–H) relative to the magnetic field, which help to define the relative orientation (longitudinal) of A and B subunits in an A–B complex, although it is not straightforward to extract the RDCs of the fully bound form, and thus to determine the structure.

Here we present a new method for fast structure determination of weak complexes using RDCs by linear extrapolation of the complex couplings following an alternative experimental scheme in combination with ambiguous interaction restraints derived from two classical 3D experiments (HNCACB and HNCO) and a 2D experiment that correlates the CB with the HD and HE chemical shifts in aromatic residues.

We used this RDC-based method to determine the high resolution structure of the complex between the third SH3 domain of CD2AP and Ubiquitin. CD2AP, an adaptor protein involved in cytosolic transport along microtubules, contains three N terminal SH3 domains that mediate most of the intermolecular interactions. We recently solved the structure of the third SH3 domain of CD2AP by NMR [1]. Ubiquitin is known to regulate a wide variety of cellular activities ranging from transcriptional regulation to cell signalling and membrane trafficking. We show that the structure of the complex differs importantly from that between the third SH3 domain of Sla-1 and Ubiquitin.

[1] Ortega-Roldan, J.L., Romero-Romero, M.L., Ora, A., AB, E., López-Mayorga, O., Azuaga, A.I. & van Nuland, N.A.J. *J. Biomol. NMR* **2007**, *39*, 331-336.

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