NMR strategy of interaction analyses for larger protein complexes

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The identification of protein complex interface provides deep insights into the various biological systems, such as signal transduction processes, immune systems, and cellular recognition. Moreover, the recent expansion of structural genomics, in which the three-dimensional structures of proteins from a variety of organisms, including human, are determined in a genome-wide fashion, increases the significance of investigations of protein-protein interactions.
To address the issue, we proposed the NMR method, cross-saturation measurement, which utilizes the TROSY detection and deuteration to a high degree for proteins, for a more rigorous determination of the contact residues of large protein complexes than the conventional approaches, involving chemical shift perturbation and hydrogen-deuterium exchange experiments [Takahashi, H., et al. *Nature Struct. Biol.* (2000) 7, 220-223].
However, within the determination of the contact residues, there are limitations that the cross-saturation method is difficult to apply to protein complexes with a molecular weight over 150 K and/or with a weak binding affinity since the resonances originating from the complexes should be directly observed in the method. To overcome these limitations, we report here another version of the cross-saturation measurement, termed transferred cross-saturation measurement, in which the NMR resonances originating from a free protein under the fast exchange process between the free and bound states on the NMR time scale are utilized [Nakanishi T., et al., *J. Mol. Biol.*, (2002) 318, 245-249.] and also show some examples of the application of the transferred cross saturation method to larger systems [Nishida, N., et al., *Nature Struct. Biol.* (2003) 10, 53-57, Takeuchi, K., et al., *Structure* (2003) 11, 1381-1392, Ichikawa,O. *EMBO J.*(2007) 26, 4168-4178].