Soutenance

THESE

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Salle des séminaires

Institut de biologie structurale - 71 avenue des Martyrs CS 10090 38044 Grenoble Cedex 9 - T.+33 (0)4 57 42 85 00

www.ibs.fr

par **Zsofia Solyom**

Institut de Biologie Structurale

Groupe NMR

NMR methods for intrinsically disordered proteins – Application to studies of NS5A protein of hepatitis C virus

Thèse de Doctorat de l'Université de Grenoble

Intrinsically disordered proteins are characterized by a lack of a stable, 3D structure and are able to fulfill their biological role as such. NMR spectroscopy is the method of choice for their atomic resolution studies, as X-ray crystallography is not amenable to them because of their highly dynamic character. However, NMR spectroscopic studies of these proteins are challenging, because of the high extent of signal overlap in the spectra, resulting from the absence of a hydrogen-bonding network that would lead to structuring and higher signal dispersion. A further problem is experimental sensitivity as often measurement time is limited due to their predisposition for proteolytic degradation. In the first part of this thesis intrinsically disordered proteins are introduced. The second part focuses on NMR spectroscopy of IDPs, BEST-TROSY-type NMR methods are presented and are shown to be well suited for large IDPs, especially for those with high extent of residual structure. 3D BEST-TROSY experiments are presented for assignment, a proline-edited version for aiding amino acid-type identification, and the HET^{ex}-BEST-TROSY experiment that allows rapid measurement of solvent exchange rates. In the third part of this thesis NMR methods are applied for study of the entire intrinsically disordered region (domains 2 and 3) of NS5A protein of hepatitis C virus. The residual secondary structure in this protein fragment is analyzed. Comparison of NMR data on three protein constructs of different lengths together with SAXS data allows identification of transient long range interactions between different regions of this protein. Furthermore, the binding modes of this protein fragment to Bin1 SH3 domain are analyzed. Finally, the preliminary results obtained on investigation of phosphorylation of NS5A of HCV by certain kinases, reported to be biologically relevant, are presented.