How to take full advantage of the CH2-TROSY experiment ?

Rachel Lenoir-Capello[1], Lionel Imbert[2], Soha Abou Ibrahim Alami[3], Ludovic Carlier[3], Nicolas Birlirakis[4], Chiara Zanato[5], Thierry Brigaud[6], Julien Pytkowicz[7], Grégory Chaume[7], Claire Loison[8], Jérôme Boisbouvier[2], and Emeric Miclet*[1]

[1]Laboratoire des Biomolécules – Sorbonne Universités, UPMC, CNRS – 4 place Jussieu 75005 Paris, France

[2]Institut de Biologie Structurale (IBS) – Commissariat à l'Énergie Atomique et aux Énergies Alternatives (CEA) - Grenoble – Grenoble, France

[3]Laboratoire des Biomolécules (LBM) – Sorbonne Universités, UPMC, CNRS – 4 place Jussieu 75005 Paris, France

[4]Laboratoire des Biomolécules (LBM) – Ecole Normale Supérieure de Paris - ENS Paris – 4 place Jussieu 75005 Paris, France

[5]Laboratoire de Chimie Biologie – Université de Cergy Pontoise – Neuville, France

[6]Laboratoire de Chimie Biologie – Universté Cergy-Pontoise – Neuville, France

[7]Laboratoire de Chimie Biologie (LCB) – Université de Cergy Pontoise – Neuville, France

[8]Institut Lumière Matière [Villeurbanne] (ILM) – Université Claude Bernard Lyon 1, Université de Lyon, Centre National de la Recherche Scientifique : UMR5306 – UMR5306 CNRS Université Claude Bernard Lyon 1 Domaine Scientifique de La Doua Bâtiment Kastler, 10 rue Ada Byron 69622 Villeurbanne CEDEX, Franc, France

Résumé

Liquid state NMR greatly benefits from Transverse Relaxation Optimized (TROSY) experiments that increase the sensitivity and the resolution of amide NH or aromatic CH pairs, methyl, trifluoromethyl or methylene groups. Among them, the CH2-TROSY has been largely underexploited so far, despite the large fraction of methylene-type hydrogens in biomolecules. This could be explained by the difficulty of isotopically labelling methylenes in a perdeuterated background whereas this is readily achievable for the popular NH and methyl TROSY. Here, we describe a cell-free protein expression protocol that allows specific glycine 13C-15N labelling of a subunit of the heavy-metal ATPase HMA8, whilst all other amino acids are perdeuterated 2H-15N. This technique enables us to eliminate a great fraction of amino acid scrambling and cross-protonation from the solvent resulting in high labelling selectivity. We show how the sample, recorded with CH2-TROSY experiments, improves the spectral quality of glycine residues in medium sized proteins. A titration experiment of a peptide ligand on the catalytic domain of the Pin1 protein demonstrates the benefit of this approach for ligand binding studies. Precise aliphatic CSP are obtained and can be used for Kd determination.

*Intervenant