TROSY solution-state NMR with a large α-helical membrane protein

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Introduction

Structure-determination with large α-helical membrane proteins by solution-state NMR spectroscopy is very challenging. The main challenges that have to be overcome before applying NMR experiments for structural measurements are as follows: **(i) Expression** - a suitable expression system that will produce milligram quantities of protein. **(ii) Purification and reconstitution** - a purification protocol that achieves milligram quantities of highly-pure protein reconstituted in a membrane mimetic that retains the native structure and function of the protein and that is compatible with NMR experiments. **(iii) Achieving high-resolution NMR spectra** - a prerequisite to applying experiments for assignment of signals and for solving structure. **(iv) Isotope labelling** - NMR-observable isotopes (usually ^{13}C and/or ^{15}N) have to be incorporated into the protein, uniformly or into specific amino acids, often combined with deuteration. **(v) Stability** - the NMR experiments are usually performed with the sample at a temperature of ≥ 20 ºC; the protein has to be stable at this temperature over days to weeks of NMR acquisition time.

For several years we have been developing labelling and sample preparation strategies to achieve high-resolution solution-state NMR spectra of the 12-helix (464 residues, 52 kDa) *E. coli* sugar transporter GalP, which is homologous with the human glucose transporter GLUT1, and is an ideal system to make significant progress on large systems. We have now shown that this is possible by combining selective amino acid labelling with TROSY-type NMR experiments.

Results

In its unlabelled form, we can routinely express GalP up to levels of 50% of total protein in *E. coli* inner membrane preparations, from which it can be purified with the aid of a genetically-engineered C-terminal His-tag to give protein yields of up to 10 mg/litre. This is a good starting point for applying isotope-labelling strategies, which can have a significant effect on lowering protein yield. We have now developed labelling and sample preparation methods with GalP that have allowed successful acquisition of both [^{15}N-^{1}H]-TROSY and [^{13}C-^{1}H]-methyl-TROSY spectra of the protein reconstituted in DDM detergent micelles. Examples are shown below.

[^{15}N-^{1}H]-TROSY spectra of [$^{15}N_2$]tryptophan-labelled GalP. The twelve tryptophan residues in GalP were selectively labelled with ^{15}N combined with uniform deuteration by expression using a tryptophan-auxotrophic host strain of *E. coli* combined with a modified minimal medium that contained [U-^{2}H,$^{15}N_2$]-*L*-tryptophan and [U-^{2}H]-*D*-glucose in D_2O. The protein was purified in the presence of [U-^{2}H]glycerol and reconstituted into [^{2}H]DDM micelles. [^{15}N-^{1}H]-TROSY spectra of the [U-^{2}H,$^{15}N_2$-Trp]GalP sample acquired on a 900 MHz NMR magnet at 25 ºC produced signals from both the side-chain and backbone positions of tryptophan residues in the protein (Figure 1).

[^{13}C-^{1}H]-methyl-TROSY spectra of [^{13}C-ILV]-labelled GalP. The methyl groups of isoleucine, leucine and valine residues of GalP were selectively labelled with ^{1}H,^{13}C combined with uniform deuteration and ^{15}N-labelling (specifically [U-^{2}H, U-^{15}N, Ile-$^{13}CH_3$, Leu/Val-$^{13}CH_3$,$^{12}CD_3$]GalP) by expression in *E. coli* using a modified minimal medium that contained ^{1}H,^{13}C-labelled precursors to Ile/Leu/Val residues, [U-^{2}H]-*D*-glucose and [^{15}N]ammonium chloride in D_2O. The protein was purified in the presence of [U-^{2}H]glycerol and reconstituted into DDM micelles. [^{13}C-^{1}H]-methyl-TROSY spectra of the [^{13}C-ILV]-

labelled GalP sample acquired on a 750 MHz NMR magnet at 20 ºC showed that we can detect and resolve signals that represent ~ 25-30 out of 36 Ile residues and ~ 60 out of 60 + 34 Leu and Val residues (Figure 2).

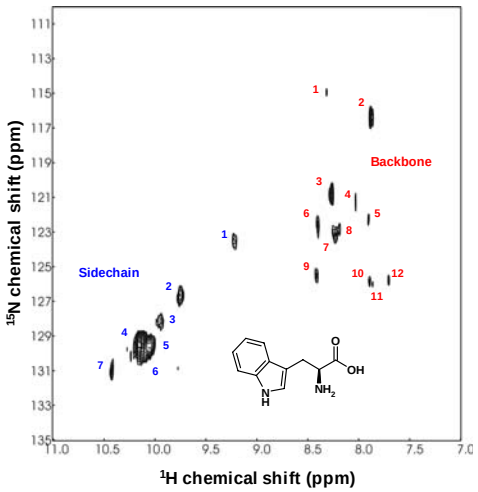

Figure 1: [^{15}N-^{1}H]-TROSY spectrum of [U-^{2}H,$^{15}N_2$-Trp]GalP in [^{2}H]DDM micelles at 25°C. Acquired at 900 MHz at HWB-NMR.

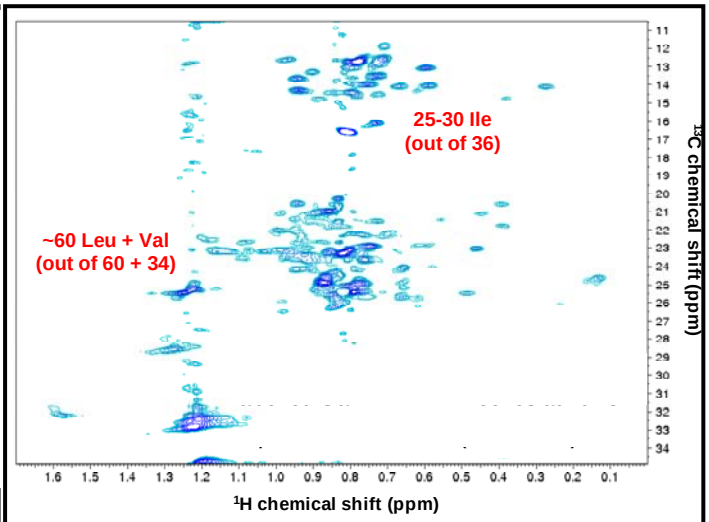

Figure 2: [^{13}C-^{1}H]-methyl-TROSY spectrum of ILV-labelled GalP in DDM micelles at 20°C. Acquired at 750 MHz at Leeds over 17 hours.

We are currently investigating the effects of inhibitors of GalP (forskolin and cytochalasin B) on the spectra and acquiring [^{13}C-^{1}H]-methyl-TROSY spectra on a higher field magnet (900 MHz).

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