Characterisation of ligand binding to membrane transport proteins from *Escherichia coli* by solid state NMR

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**Transport proteins from *E. coli***

Bacterial membranes contain transport proteins that mediate the uptake of specific nutrients into the organism from the environment, or that expel waste products, antibiotics and toxins. In some cases these proteins are homologous with human transporters that provide a route of entry for drugs into cells and tissues in addition to their natural substrates. The more accessible bacterial proteins can therefore serve as a model for their human counterparts where knowledge about substrate specificities and binding characteristics has implications for therapeutic development. Unlike the human transporters, the bacterial proteins can be overexpressed to provide sufficient quantities of protein that enables the use of techniques such as NMR for analysis. We have been using solid state NMR to characterise ligand binding to a range of transporters present in the inner bacterial membrane of *E. coli*, including a glucuronide transporter (GusB), two nucleoside transporters (NupC and NupG) and the galactose transporter (GalP).

**Observation of ligand binding to membrane proteins by CP-MAS solid state NMR**

The cross-polarisation magic-angle spinning (CP-MAS) solid state NMR experiment allows the direct observation of ligand binding to membrane proteins overexpressed in their native membranes without the need for their purification. The approach requires a substrate for the protein of interest that is labelled with an NMR active isotope, usually $^{13}$C. In our experiments the substrates are equilibrated with non-energised *E. coli* inner membrane preparations in which a specific transporter has been expressed at levels of 20-50% of total protein. The CP experiment only produces a signal for substrate that has bound in the membranes and does not detect substrate that remains in solution; for example, the binding of methyl $[1-^{13}$C$]-\beta$-D-glucuronide to GusB (Fig. 1A).

![Fig. 1.](image)

**Fig. 1.** (A) A $^{13}$C CP-MAS NMR spectrum of *E. coli* inner membranes overexpressing the glucuronide transporter GusB and containing 6 mM methyl $[1-^{13}$C$]-\beta$-D-glucuronide ($[^{13}$C$]$MG). (B) Peak intensities for two different concentrations of $[^{13}$C$]$MG in GusB membranes from spectra recorded with a range of contact times and showing the best fitting simulations of CP profiles for the given values of $K_d$ and $k_{off}$. 

$K_d = 4.17$ mM

$k_{off} = 698$ s$^{-1}$
Methods to quantify substrate affinities for membrane proteins by CP-MAS NMR
When the CP-MAS NMR spectra are recorded over a range of CP contact times, we have shown that the shape of the resultant substrate peak intensity profile is sensitive to changes in binding affinity. Simulations of CP intensity profiles can be fitted to the experimental data to characterise substrate binding in terms of the binding constant ($K_d$) and of the rate constant for dissociation from the binding site ($k_{off}$) (Fig. 1B). In some cases this is the only method that can be used to obtain such information for a particular transporter. We have also developed a competitive displacement approach that allows the determination of the binding constants for unlabelled ligands when at least one suitable labelled substrate has been identified, therefore allowing the screening of an unlimited number of compounds.

Elimination of non-specific signals and reduction of obtrusive backgrounds
When using more hydrophobic substrates in these experiments, e.g. $[1^\prime-^{13}C]$uridine with the nucleoside transporters, non-specific interactions may contribute to the substrate signal. This complicates the measurement of substrate affinities and so we have applied a spectral editing experiment to selectively eliminate the non-specific component of the signal.

The $^{13}$C label introduced into the chosen ligand may produce a signal that overlaps with the natural abundance $^{13}$C background from the membranes in the $^{13}$C NMR spectrum, which interferes with its detection. We have overcome this problem for observing the binding of the inhibitor $[22-^{13}C]$forskolin to the galactose transporter, GalP, by growth of the producing organism in medium containing $^{13}$C-depleted D-glucose as the carbon source (Fig. 2).

Publications


**Collaborators**

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