Biophysical studies on multi drug transporter EmrE

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With multidrug resistance (MDR) being an increasing challenge for modern medicine it is vital to understand its mechanisms. One important aspect is the efflux of drugs via membrane proteins. We are studying the "Small multi drug resistance" protein EmrE (UniProt P23895) by solid-state NMR. EmrE is a secondary transporter that uses the proton gradient across the cell membrane to export drugs from the cell. Typical substrates are aromatic, cationic compounds like TPP^+ or ethidium. It consists of 110 amino acids with a highly conserved glutamate as the only charged residue residing inside the membrane with a proposed pka of 7.5. We have used cell free expression to express a single glutamate mutant with 13C labeling. Cell free expression is useful to minimize scrambling. Solid state NMR analysis of the cell free produced protein precipitate shows a high alpha helical content. Cryo-solid state MAS NMR studies at different pH values indicate a protonation dependent shift of the Cδ signal below a ph of 6.