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Towards Neutron crystallography of membrane proteins: Insights into production of deuterium-labelled OmpF

Content

Hydrogen bonds play a crucial role for protein function and involved in almost every mechanism from foundation of protein structure to enzyme catalysis. Hydrogen (1H) atoms form the basis of hydrogen bond that is not scattered by X-ray crystallography due to its poor scattering power. Neutron protein crystallography (NPX) is a powerful tool that is capable of locating hydrogens and study the significance of hydrogen bonding interactions in biomacromolecules. However, due to the requirement of large crystals very few neutron structures have been deposited in PDB with no membrane protein structure determined yet. Additionally, 1H has a negative scattering length and large incoherent cross-section giving rise to a significant background noise in neutron data collection. This effect can be minimized by isotopic substitution of 1H with its heavier isotope deuterium (2H or D) leading to less ambiguous data analysis and better structure interpretation. Overall ˜25% H atoms in a protein are solvent exchangeable and can be exchanged by dissolving in heavy water. However, complete deuterium labelling (perdeuteration) is required for the remaining 75% H atoms. In this work, an optimized methodology for large scale production of perdeuterated bacterial outer membrane protein F (OmpF) has been designed. OmpF was produced in deuterated minimal medium with different carbon sources. Mass spectrometry and thermal stability experiments verified the purity and level of deuteration of OmpF protein. Perdeuterated OmpF crystals also diffracted X-rays to 9 Å resolution emphasising the need of fine tuning of perdeuterated crystallisation conditions.

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