Poster Presentation

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Improving the diffraction of glypican-1 crystals by controlled dehydration

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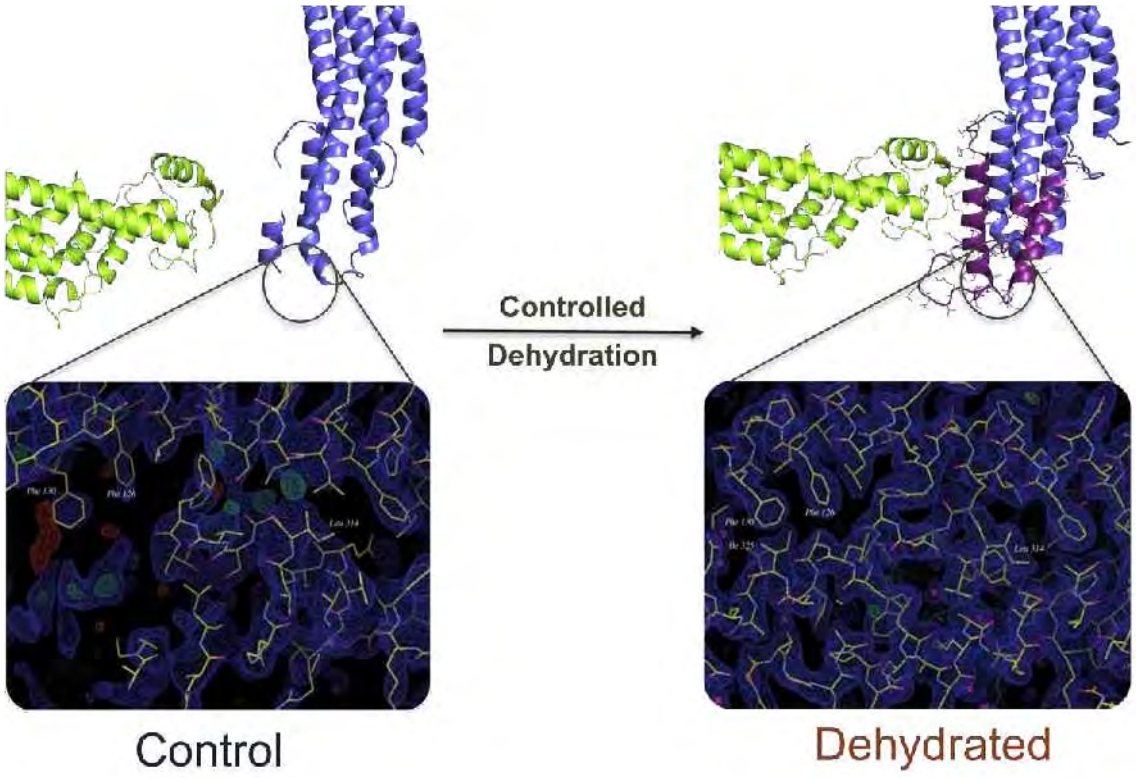
Glypicans are heparan sulfate proteoglycans that are attached to the cell membrane surface by glycosylphosphatidylinositol anchorage. Glypican-1 (Gpc-1) is the predominant heparan sulphate proteoglycan in the developing and adult human brain and is involved in developmental morphogenesis, growth factor and cytokine signalling. We determined the crystal structure of N-glycosylated human glypican-1 core protein at 2.55 Å resolution, which revealed a cylindrical, all α-helical fold (dimensions 120 x 30 x 30 Å), decorated with three major loops and containing the 14 cysteine residues conserved in all members of the glypican family (1). The Gpc-1 crystals were delicate, highly fragile plates, which displayed poor isomorphism, with cell dimensions varying between different crystals. These crystals also diffracted anisotropically, reflected in a Wilson B factor that was twice as large in the c* direction as in the a* and b* directions, which limited the effective resolution to 2.9 Å in the c* direction. Recently we have shown Gpc-1 crystals to be a successful case for improvement in diffraction properties by controlled crystal dehydration using the humidity control device (HC1b), which delivers a humidified air stream of a precise relative humidity that can be used to alter the solvent content inside the crystals (2). The optimal dehydration protocol was developed by investigation of the parameters: final relative humidity RHf, dehydration rate and total incubation time Tinc. Of these, the most important was shown to be Tinc. After dehydration using the optimal protocol, the diffraction quality of the Gpc-1 crystals was clearly improved, with significant reduction in the anisotropy. This generated better, less noisy electron density maps, which allowed the building of previously disordered parts of the model and displayed well-defined side chains.

[1] *Svensson G, Awad W, Hakansson M, et al., (2012). J. Biol. Chem. 287, 14040-14051.,* ***[2]*** *Awad W, Svensson G, Thunnissen M, et al., (2013) Acta Cryst. D69, 2524-33*



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