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Production, purification and characterisation of monovalent cation/proton antiporter

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Sodium/proton exchangers (NHX) are essential for cell survival and hence are found in all living cells of every kingdom of life. The activity of these polytopic transmembrane proteins regulates the cellular and vesicular pH-value, cellular ion composition and cell volume and shape. In eukaryotes, they also serve as cytoskeleton anchor. The regulation of these proteins is highly complex reflected by the large number of factors which modulate NHX activity in humans. In this study, a yeast expression system was set up as a first step towards elucidation of the functional specificity of NHX isoforms at the molecular level. Based on structural genomics strategies, conditions were established for production and purification of the recombinant transporters. Functional production of the recombinant targets was probed by a microplate-based growth complementation assay. Detergent solution stability of the purified proteins was determined by static light scattering coupled to size exclusion chromatography (SEC-MALS) and by fluorescence dye based thermal denaturation assays. Liquid handling robotics coupled to automated plate inspection was used to fast and efficiently screen for suitable crystallisation conditions. In order to enhance crystallisation probability and crystal quality surface entropy reduction and antibody fragment mediated co-crystallisation were applied. The x-ray diffraction properties of the obtained crystals were analysed at the SLS synchrotron in Villigen/Switzerland and diffraction data to 6 Å resolutions could be collected.