MOLECULAR BASIS FOR SPECIFICITY OF NOROVIRUS TOWARDS HUMAN GLYCANS

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Noroviruses (NoVs) are a leading cause of viral gastroenteritis worldwide and are responsible for a significant public health and economic burden [1]. These highly infectious pathogens bind to human glycans, such as histo-blood group antigens (HBGAs), to attach to the host cell and initiate infection [2]. NoV strains, with potentially unique glycan specificities, pose a growing challenge, particularly in the absence of an effective vaccine [3]. However, is known in literature that Human milk oligosaccharides (HMOs), which structurally mimic HBGAs, are able to act as natural decoys that inhibit norovirus binding, thereby reducing the risk of infection [4]. This project aims to investigate the molecular determinants of glycan specificity in predominant (GII.4), rare (GII.2) and emerging (GII.17) NoV strains to assist the development of glycomimetics for anti-adhesion therapy (AAT).
To achieve this, a novel and efficient protocol for the expression and purification of the P domain in *Escherichia coli* for these three NoV strains was successfully developed, yielding to 10-15 mg of highly pure protein per litre of culture. The dimerization necessary for carbohydrate binding was confirmed by SEC-MALLS and SEC-SAXS on the purified P-domains. Those were then subjected to crystallisation trials, resulting in the identification of successful crystallisation conditions for the three strains. In particular, new co-crystals were obtained with new HMOs for the GII.4 strain, and a high-resolution structure was obtained for the GII.2 apo protein in an unreported space group. Diffractions tests are underway for GII.17 in complex with HMOs and HGBAs. Comparative structural and biophysical analyses of different NoV genotypes will reveal both conserved and variable binding motifs, providing a deeper understanding of the determinants for NoV specificity.
The knowledge gained from this study will facilitate the rational design of glycan-based inhibitors capable of blocking NoV-host interactions, providing a promising avenue for the development of novel therapeutic strategies against norovirus infections.

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