GlycosAminoGlycan Project Report

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Scientific Background : The GAG project aims for the structural characterization of enzymes involved in heparan sulfate chain elongation (EXT1/EXT2) and modification (HSulf-2). Heparan sulfates are linear, but highly complex polysaccharide chains, that are attached to serine residues of a core protein, thereby modulating its interaction with growth factors, signaling receptors, cytokines, adhesion molecules and diverse matrix components. Malfunctioning of heparan sulfate biosynthesis has been linked to Alzheimer's disease, acute and chronic inflammation, tumorigenesis and diabetes. Exostosin-1 (EXT1) and Exostosin-2 (EXT2) form a hetero-dimeric complex that carries out the chain elongation by the alternating addition of N-acetylglucosamine and glucuronic acid sugar moieties. The aim of the present project is to obtain a high-resolution cryo-EM structure of the 150 kDa EXT1/EXT2 complex to provide the first insight into the complex architecture and the catalyzed glycosyltransferase reactions. A second aim was to determine a high-resolution structure of HSulf-2, an extracellular sulfatase that regulates heparan sulfate (HS) activity through its ability to catalyze specific 6-O-desulfation of the polysaccharide. So far, no data acquisition time for project part 2 (HSulf-2) has been attributed.

Data collection summary:
A 48hrs data acquisition for project part 1 (EXT1/EXT2 complex) took place on 22/01/20201 – 24/01/2021. The sample was prepared using Quantifoil 1.2/1.3 carbon-coated copper grids which allowed to perform three exposures per foil hole. A total number of 8655 images was collected at a magnification of 215k, equalling a pixel size of 0.65Å. The total accumulated dose was 58.5 $e^-/Å^2$.

Results:
Data processing is currently ongoing. Preliminary results from data processing in cryoSPARC v.3.0, using 4000 automatically pre-processed images suggests, that the image contrast and the particle distribution are good (Figure 1A). The large majority of the micrographs has an estimated CTF resolution between 2.8 and 4.0Å. 2D templates for particle picking were generated from template-free picked particles. These particles were subjected to several rounds of 2D classification, after which a total number of 275549 particles remained. The obtained 2D class averages show the hetero-dimeric EXT1/EXT2 complex in several different orientations and with clear secondary structure features (Figure 1B). First attempts to generate an ab-initio 3D reconstruction were only partially successful, as particle alignment of this rather small proteins seems more challenging than for bigger protein complexes (Figure 1C). Several different strategies are currently investigated in order to generate a more reliable initial 3D map that can then be refined to high resolution. In parallel, we have started to process the full-data set using the raw data of the movies in Relion 3.1.

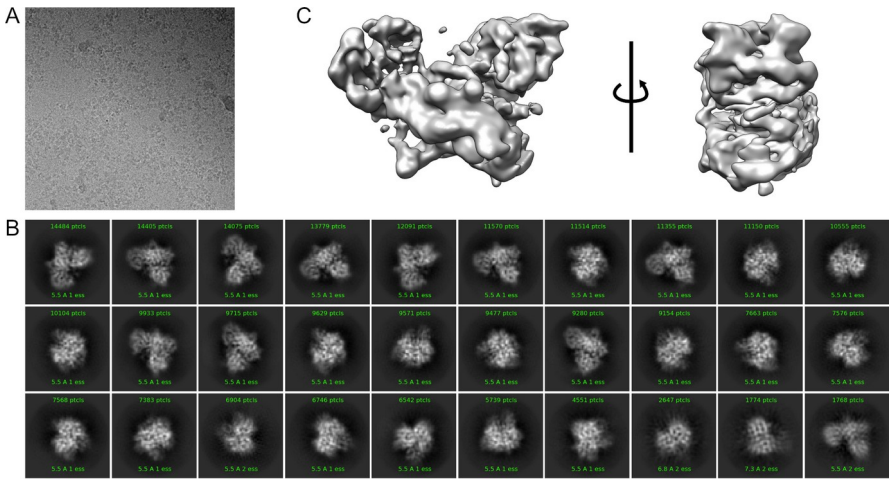

Figure 1: Preliminary data processing results for the EXT1/EXT2 complex.
A) Representative micrograph
B) 2D class averages
C) 3D reconstruction showing the complex from the front and rotated by 90°.