MS5-P36 High-density transfection is superior for production of readily crystallizable glycoproteins in suspension adapted HEK293S GnTI- cells: a case study of human lymphocyte receptor LLT1

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Human embryonic kidney 293 cell line deficient in N-acetylglucosaminyltransferase I (HEK293S GnTI-) is well known tool for recombinant expression of proteins with homogeneous and deglycosylatable N-glycosylation, a feature crucial especially within protein crystallography [1]. So far production protocols using this cell line were based either on lengthy stable cell line generation or transient transfection of adherent cell culture that is costly to scale-up and has reportedly lower expression yields [2].

In this work we have adapted HEK293S GnTI- cell line to growth in suspension and optimized its transient transfection. While transfection at standard cultivation cell density proved very little success we have found out that concentrating the cells to high cell density substantially increases transfection efficiency, greatly enhancing protein yields and creating fast and scalable production process.

We demonstrate this on the production of soluble lectin-like transcript 1 (LLT1, gene *clec2d*) receptor naturally present on natural killer and T-lymphocytes, but upregulated in glioblastoma cells, one of the most lethal tumors, where it acts as a mediator of immune escape. Furthermore, we show that His176Cys mutation is critical for LLT1 stability, leading to reconstruction of disulfide bridge and that LLT1 forms non-covalent homodimer whose dimerization does not depend on presence of its N-glycans [3].

The prepared soluble domain of LLT1 with homogeneous glycosylation was readily crystallized and following optimization of crystal conditions this protein preparation ultimately led to the first structure determination of this receptor described so far [4].

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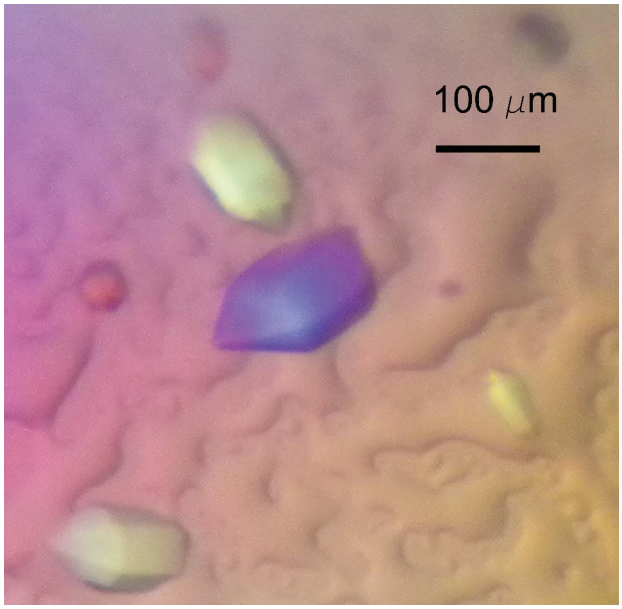

Figure 1. First observed crystals of glycosylated LLT1(H176C) receptor soluble domain produced in HEK293S GnTI- cell line.

Keywords: LLT1; HEK293S GnTI-; C-type lectin-like; NK cell; N-glycosylation; transient transfection; lymphocyte receptor