No membrane? No problem: Engineering the ASGPR receptor for soluble assay development

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Introduction

Asialoglycoprotein receptor (ASGPR) is a trimeric transmembrane receptor that can be exploited for the targeted delivery of therapeutic oligonucleotides to the liver via its natural ligand N-acetyl-D-galactosamine (GalNAc). Developing novel ligands for liver-targeted delivery forms part of the ongoing AstraZeneca Oligonucleotide Platform Build.

In vitro studies of ASGPR have been limited to the monomeric carbohydrate binding domain (CBD). The absence of a membrane precludes analysis of the trimeric form of the receptor, which is expected to have a higher affinity for trivalent ligands targeting the CBD.

Here, we designed an artificial trimeric construct in the absence of a membrane and comprising the soluble CBD of ASGPR fused to the NC1 trimerisation domain of collagen.

ASGPR structure and engineering strategy

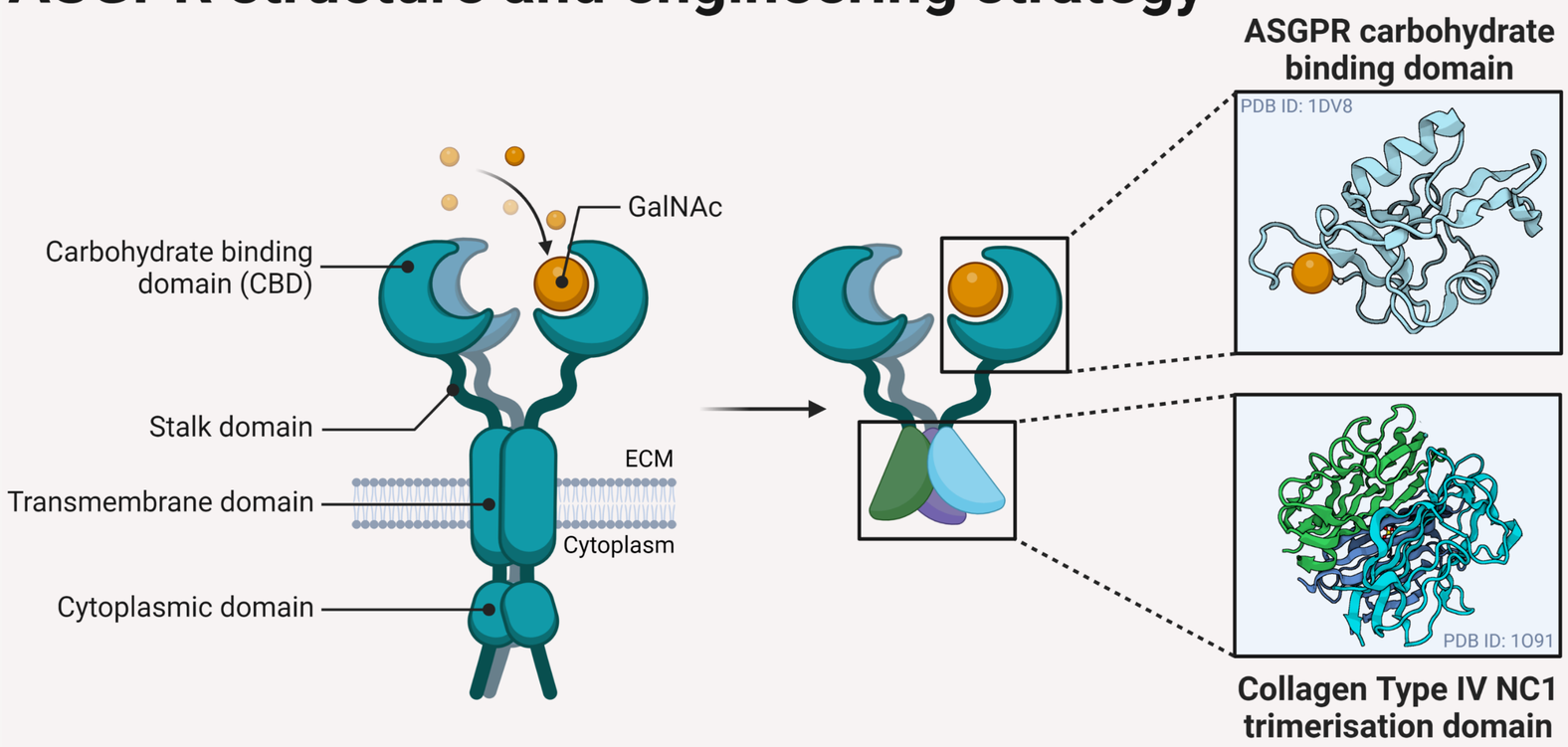

FIGURE 1: ASGPR is a heterotrimeric receptor which is internalised following GalNAc binding. Here, we isolated the CBD and engineered a trimerisation domain from collagen onto its N-terminus to promote oligomerisation in the absence of a membrane

Engineered ASGPR forms trimers

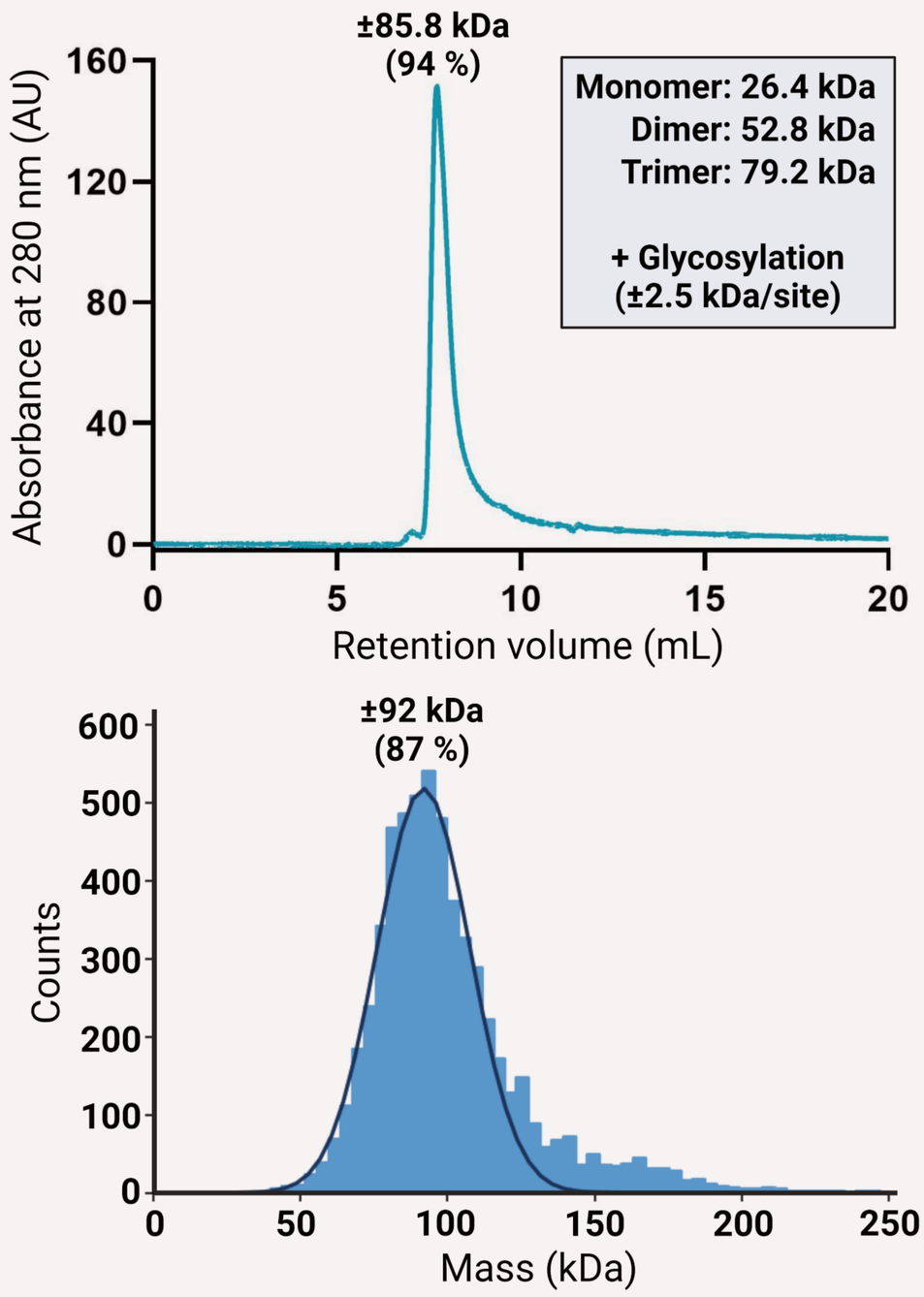

FIGURE 2: Engineered ASGPR forms stable trimers when analysed by SEC-MALS (A) and mass photometry (B). The apparent higher M_w compared to the theoretical trimer is due to the presence of three glycosylation sites (+ 7.5 kDa)

Tri-ASGPR is binding-competent

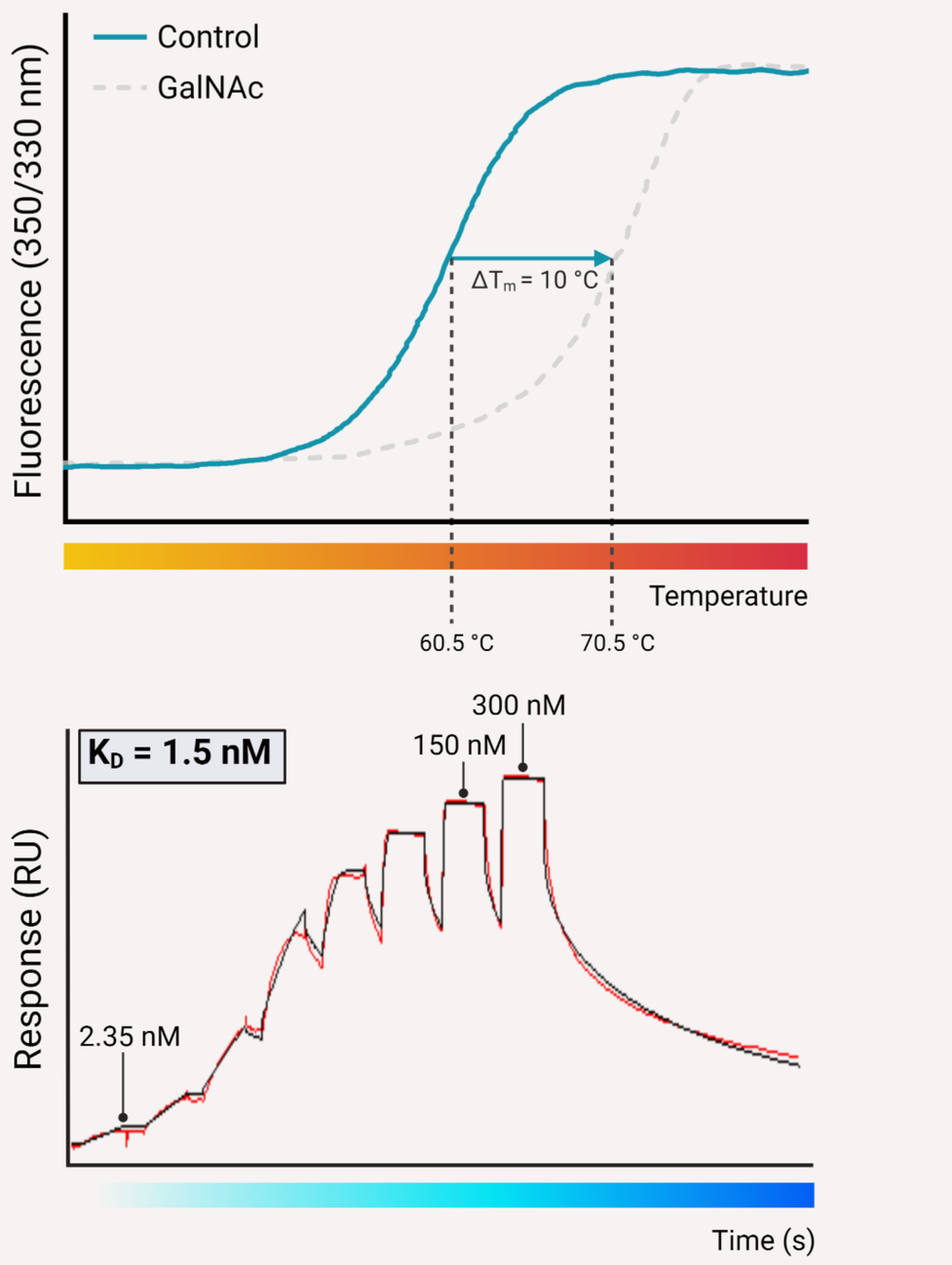

FIGURE 3: Engineered ASGPR is capable of binding the native GalNAc substrate. A large thermal shift was observed upon GalNAc binding (ΔT_m = 10 °C) using nanoDSF (A). Binding was also confirmed using SPR (B), measuring an apparent K_D of 1.5 nM

Conclusions

This approach represents a different way of tackling membrane receptor drug discovery, with the benefits of a native-like soluble oligomer gained whilst avoiding the potential challenges associated with membrane protein purification.

Our next step is to use the Tri-ASGPR construct to generate a high-throughput assay for compound screening.

Acknowledgements

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Figures generated using BioRender.

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