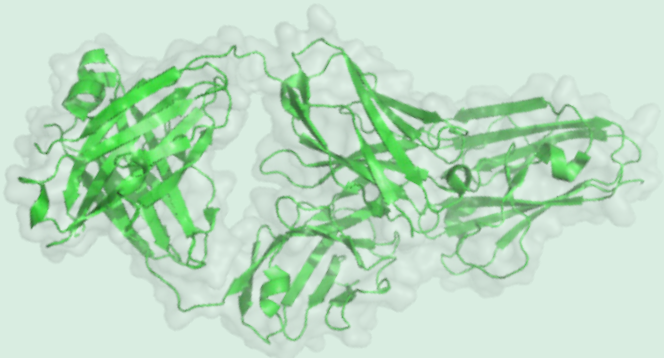
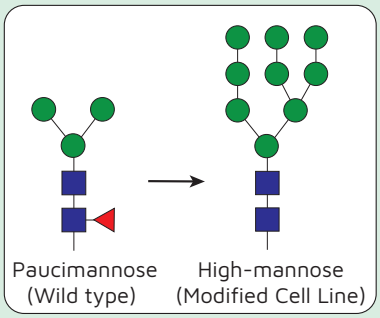
New Glyco-modified Cell Line - HighMan-S2 - for Enhanced Immunogenicity of Your Antigen



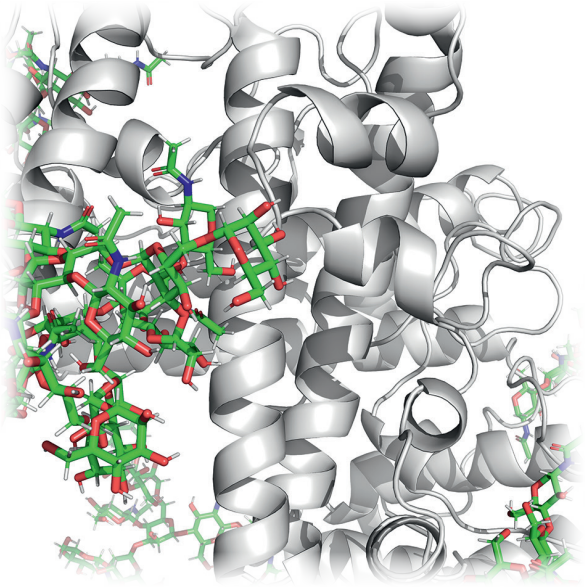
Pfs48/45 (malaria vaccine candidate)



- Increased Immunogenicity
- Humanised Proteins
- Improving Pharmacokinetics

CRISPR/Cas9

A Tool to Engineer the Glycosylation Profile

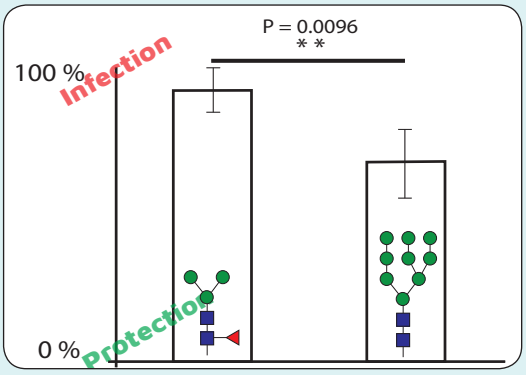


Close-up of selected glycans on VAR2CSA

The ExpreS^2 platfrom has a solid track record of producing ***difficult-to-express*** proteins, where other systems fail, such as VAR2CSA.

Using CRISPR/Cas9, the enzymatic activity can be edited to yield high-mannose N-glycan structures of your favorite protein, such as VAR2CSA, here verified by LC-MS.

PoC: Improving Immune Response by Generating Antibodies with Increased Binding Functionality towards Malaria Parasites



An *in vitro* functional antibody assay can serve as a useful tool to estimate the amount of blocking antibodies in mouse serum.

The new HighMan-S2 cell line produces proteins, which induce immunogenicity and raise more functional antibodies in mice.

100% binding indicates that the antibodies are not able to prevent binding (Infection), while 0% binding indicates the binding of parasites to CSA was prevented (Protection).

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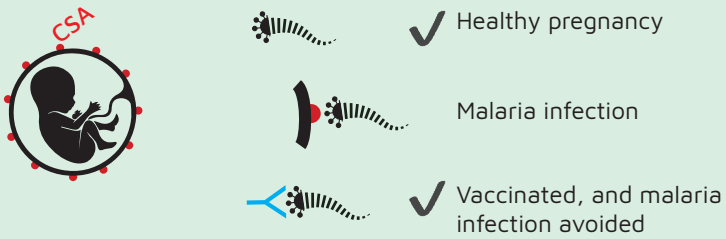
Case: How to Make Your Protein Stand Out

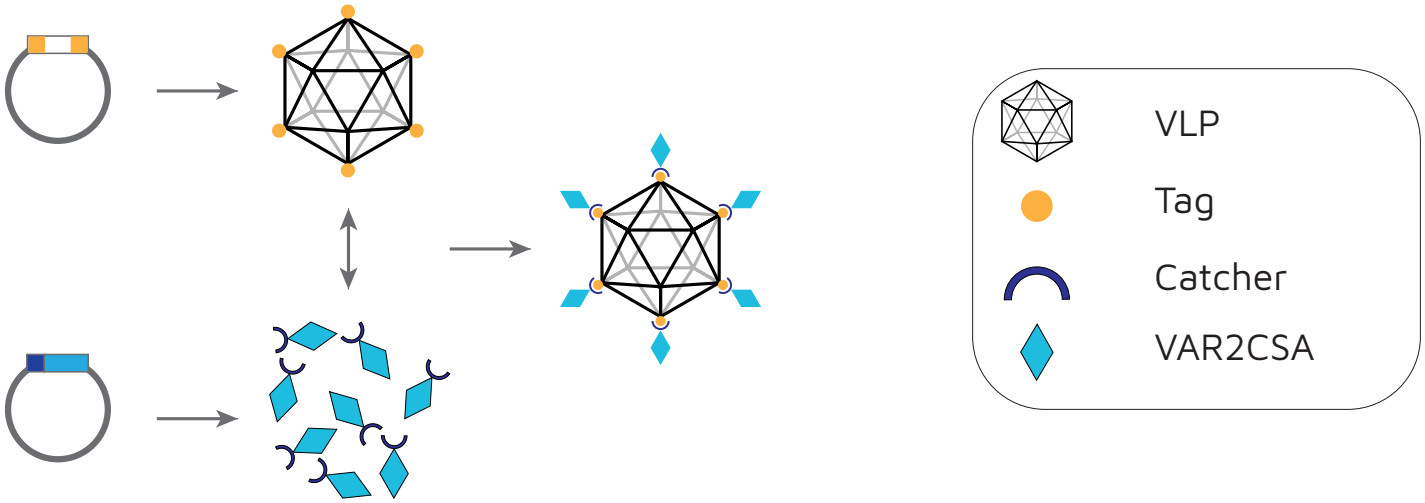
In pregnant women with malaria, the parasites bind via VAR2CSA to CSA on placental cells and prevent nutrient transfer, which causes low birth weight, premature birth or stillbirth.

The ExpreS^2 system has shown to be superior at expressing secreted viral and parasitic proteins, such as the placental malaria vaccine candidate, VAR2CSA, which is now in ***clinical phase Ib***.

VAR2CSA specific antibodies from vaccinated, pregnant women prevent binding of parasite infected erythrocytes to the placental cells, and the fetus develops normally.



Hypothesis: By glyco-engineering of the VAR2CSA antigen to target glycan receptors and circulating lectins, we can enhance the efficacy of the vaccine.



The VAR2CSA antigen was expressed using a CRISPR/Cas9 engineered *Drosophila* S2 cell line, called HighMan-S2, to generate high-mannose N-linked glycan structures for comparison with wild type paucimannose structures. Proteins were purified and formulated with AddaVax™ (A) or covalently linked to a virus-like particle (VLP), prior to mice immunisations.

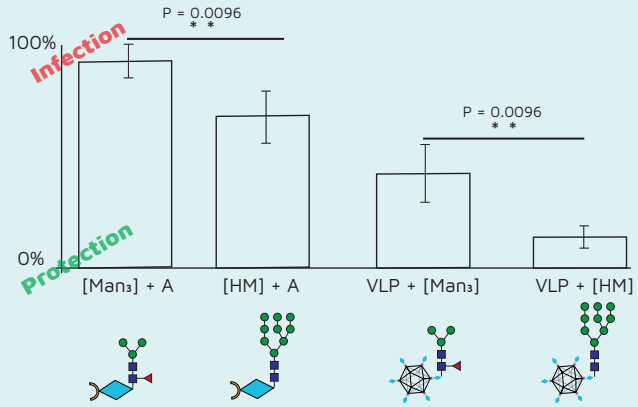
The antibodies' ability to prevent binding of infected erythrocytes to CSA was analysed by an inhibition assay, as shown below where antibodies from mice sera were evaluated for their ability to prevent binding of parasite to CSA.

VLP Technology Facilitates a Synergistic Effect

Functionality of the raised antibodies was evaluated by a parasite inhibition assay, where the inhibition of parasite infected erythrocytes binding to CSA was evaluated.

100% binding indicates that the antibodies are not able to prevent binding (Infection), while 0% binding indicates the binding of parasites to CSA was prevented (Protection).

In conclusion, this demonstrates the ability of engineering the N-linked glycosylation of the placental malaria antigen from native *Drosophila* S2 paucimannose (Man_3) to High-mannose (HM), in order to establish a more functional antibody response in immunised mice.



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