Towards a platform process for the manufacture of glycoconjugate vaccines for pneumococcal disease
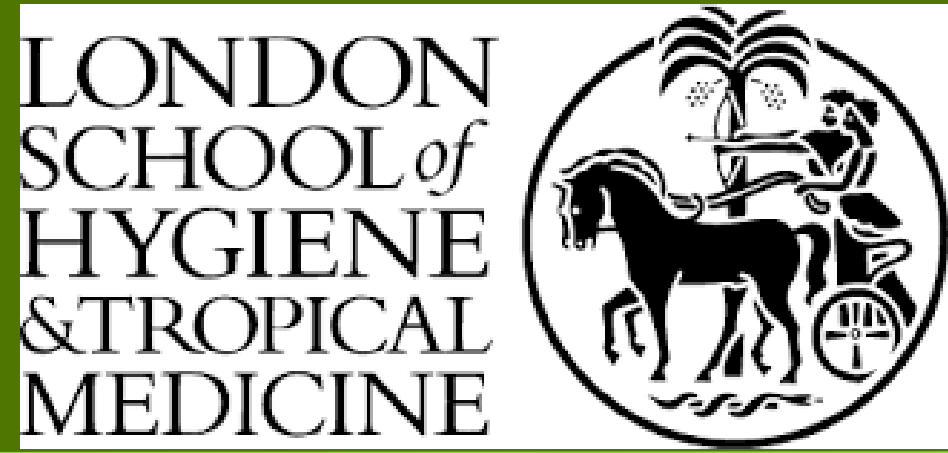
Neha Patel[1], Emily Kay[2], Jon Cuccui[2], Tarit Mukhopadhyay[1,3], Michael Sulu[1], Frank Baganz[1*]

1 Department of Biochemical Engineering University College London, U.K.

2 London School of Hygiene and Tropical Medicine, U.K.

3 Merk Research Laboratories, USA

*f.baganz@ucl.ac.uk

Background

- *Streptococcus pneumoniae* is one of the leading causes of invasive bacterial disease in children resulting in pneumonia and meningitis.
- Introduction of glycoconjugate vaccines led to a significant reduction in invasive bacterial diseases in young children worldwide.
- Current commercial glycoconjugate vaccine for immunisation is Prevnar-13 but cost per dose is high due to complex and long manufacturing process (Fig. 1).
- Protein Glycan Coupling Technology (PGCT) is promising alternative to produce glycoconjugate vaccines intracellularly in *E. coli* [1, 2] (Fig. 2).
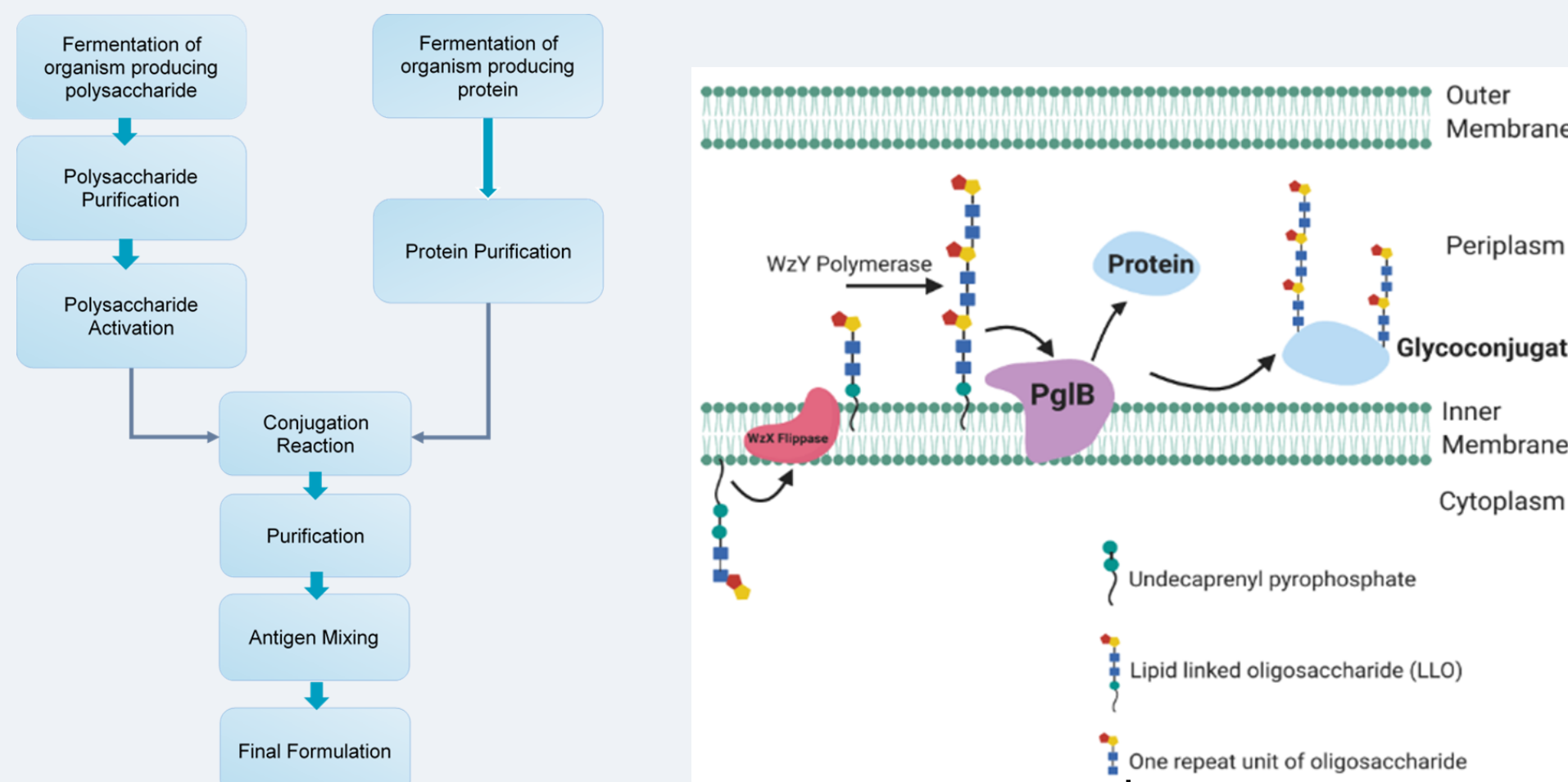

Figure 1: Overview of the chemical conjugation method of glycoconjugate vaccine production.
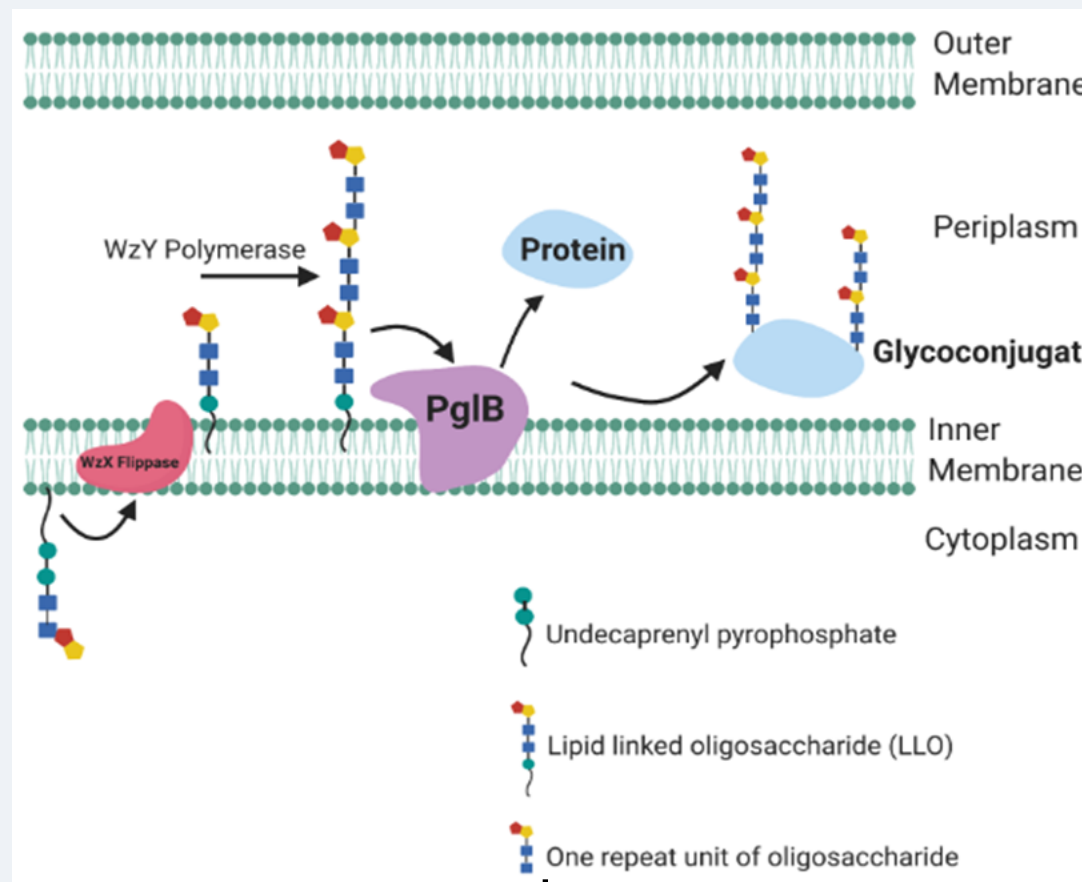
Figure 2: Schematic of PGCT system to produce glycoconjugate recombinantly *in E. coli*. 1) The oligosaccharide is assembled on a lipid core undecaprenyl pyrophosphate (Und-PP) on the cytoplasmic face to form a lipid linked oligosaccharide (LLO), 2) which is flipped into the periplasm by a flippase. 3) A polymerase will then add repeat units of the oligosaccharide to assemble the glycan with multiple repeat units. 4) The oligosaccharyltransferase PglB will recognise the reducing end sugar of the first monosaccharide and transfer the glycan onto the glycotag on the acceptor protein to form the final glycoconjugate product in the periplasm.

Materials and Methods

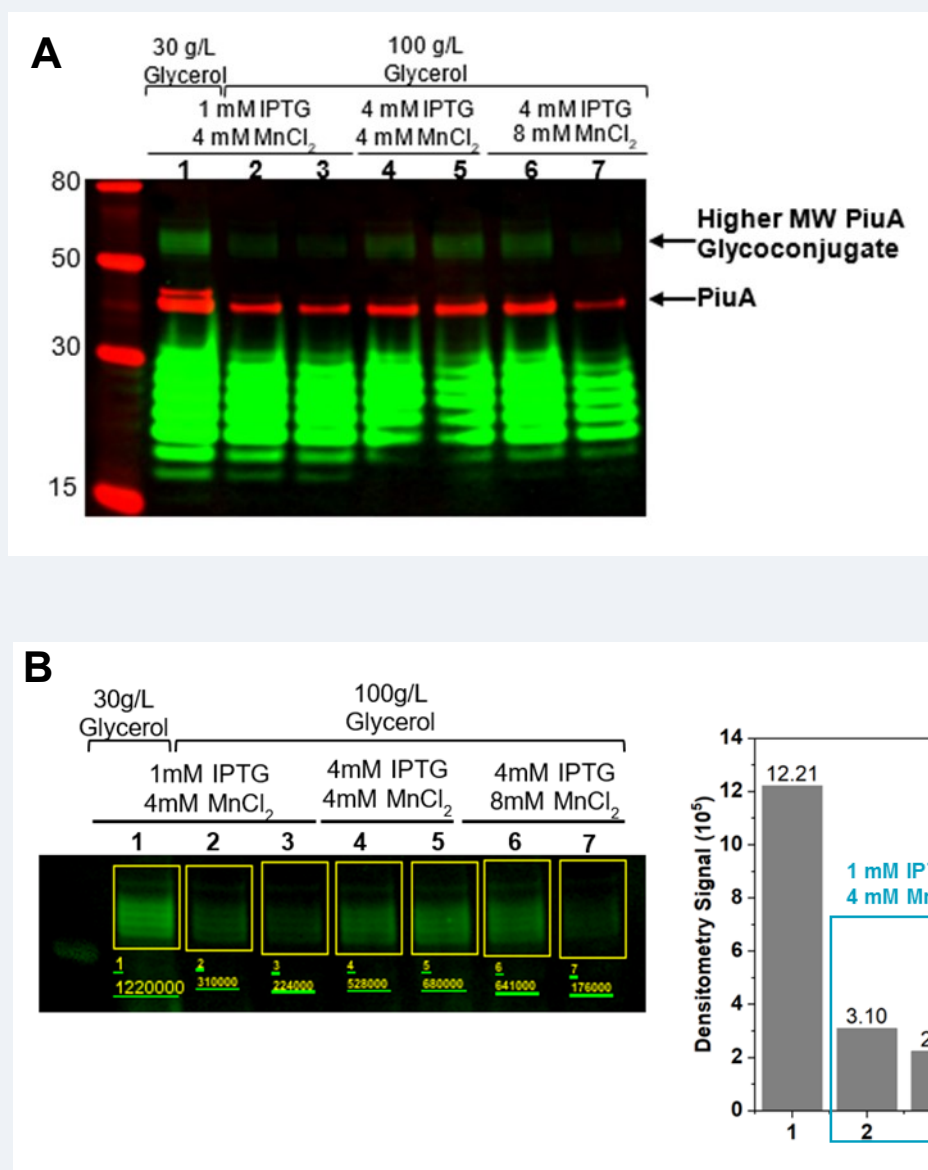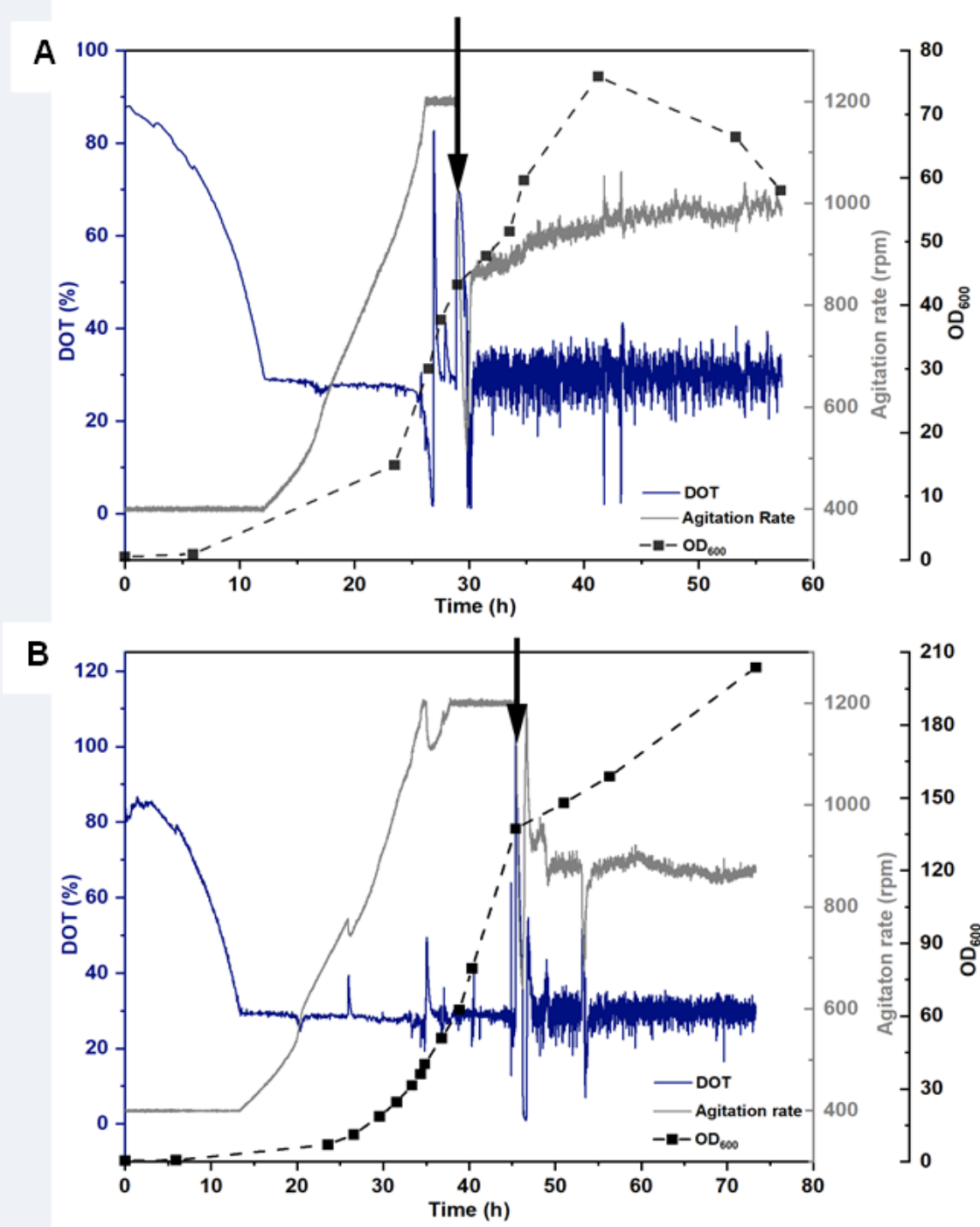**Table 1**: *E. coli* strains used for glycoconjugate production.

Strain Number	Strain and plasmids components	Description	*pglB* Location	Acceptor Protein	Source
1	W311B pb4 pEXT20 (galE) pEXT21 (piuA)	Production of ST4- PiuA Glycoconjugate	Chromosome	PiuA	Reglinski *et al.* (2018)
2	W3110 pb4 pEXT200 (galE+pglB) pEXT21 (piuA)	Production of ST4- PiuA Glycoconjugate	Plasmid	PiuA	This Study
3	W311B pb4 pEXT20 (galE+pglB) pEXT21 (exoA)	Production of ST4- ExoA Glycoconjugate	Chromosome	ExoA	This Study
4	W3110 pb4 pEXT200 (galE+pglB) pEXT21 (exoA)	Production of ST4- ExoA Glycoconjugate	Plasmid	ExoA	This Study

Table 2: Summary of fermentation protocols used in bioreactors.

Fermentation Mode	Media	Inoculation condition	Starting Working Volume	Pre-Induction Feed Condition	Specific Growth Rate value for Exponential Feed	Post-Induction Feed Condition	Induction Conditions
Batch	SSOB	Cells inoculated into bioreactor to an inoculation OD$_{600}$ between 0.3- 0.5	200 mL	Not applicable	Not applicable	Not applicable	1 mM IPTG, 4 mM MnCl$_2$ induction at inoculation
Fed-Batch	SM6Gc 30 g/L or 100 g/L Glycerol in batch phase	Cells inoculated into bioreactor to an inoculation OD$_{600}$ between 0.3- 0.5	200 mL	Not applicable	Not applicable	80% (w/v) Glycerol fed at a rate of 1.6 mL/L/h*	1 mM IPTG, 4 mM MnCl$_2$ induction at DOT Spike; 4 mM IPTG, 4 mM MnCl$_2$ induction at DOT Spike; 4 mM IPTG, 8mM MnCl$_2$ induction at DOT Spike
Fed-Batch with exponential feed	SM6Gc 30 g/L Glycerol in batch phase	Cells inoculated into bioreactor to an inoculation OD$_{600}$ of approximately 0.5	140 mL	Exponential feed initiated at DOT spike maintaining specific growth rate of cells	0.06 h^{-1} or 0.12 h^{-1} in preliminary experiments. 0.07 h^{-1}, 0.085 h^{-1} or 0.1 h^{-1} for fermentations required for DoE study.	80% (w/v) Glycerol fed at a rate of 1.6 mL/L/h . Feed initiated at OD$_{600}$ 138 +/- 9.6	1 mM IPTG 4 mM MnCl$_2$ induction at OD$_{600}$ 138 +/- 9.6

Fed-batch fermentation in defined medium

- Develop a fed-batch fermentation protocol in a defined medium.
- Investigate the impact of IPTG concentration and MnCl$_2$ supplementation on glycoconjugate production in fed-batch fermentations using Strain 1 (Table 1, 2).

Figure 3: Fermentation traces for fed-batch fermentations. (A) fermentation trace for a condition with 30 g/L glycerol in the batch phase and (B) a fermentation trace for 100 g/L glycerol in the batch phase. Arrow indicates point of DOT spike, start of feed of 800 g/L glycerol at a rate of 1.6 mL/L/h and point of induction with 1mM IPTG and supplementation with 4mM MnCl$_2$.

- Glycoconjugate production in normalised samples highest in fermentation with 30g/L glycerol.
- Increasing IPTG led to slightly higher glycoconjugate production using 100 g/L glycerol.

Figure 4: Immunoblot (A) and densitometry analysis (B) of endpoint samples taken from various fed-batch protocols 28 h post-induction. All samples were OD600 matched. Densitometry analysis was performed on glycoconjugate bands. The boxes indicate the bands which were included in this analysis.

Impact of *pglB* location and a different acceptor protein on glycoconjugate production

- Construct three new strains which either express PglB on a plasmid or have a different acceptor protein present (Figure 5).
- Compare the performance of the strains in terms of biomass and glycoconjugate production in fed-batch fermentations with defined medium.
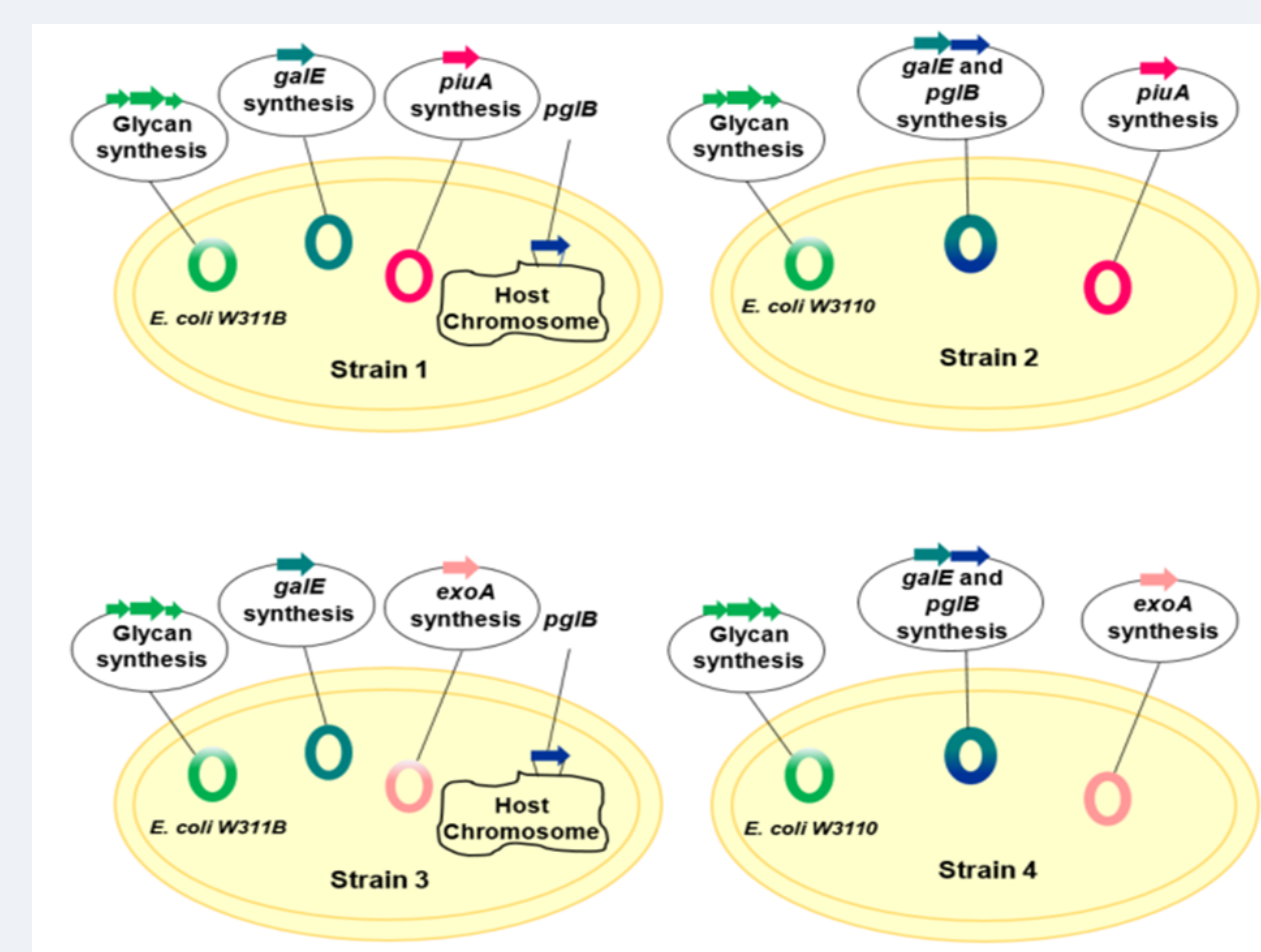

Figure 5: Schematic of glycoconjugate producing strains tested (Table 1).

- Strains with ExoA as an acceptor protein (Strains 3 and 4) have higher glycoconjugate production.
- The glycoconjugate production of a chromosomal versus a plasmidic strain is similar with ExoA as the acceptor protein.
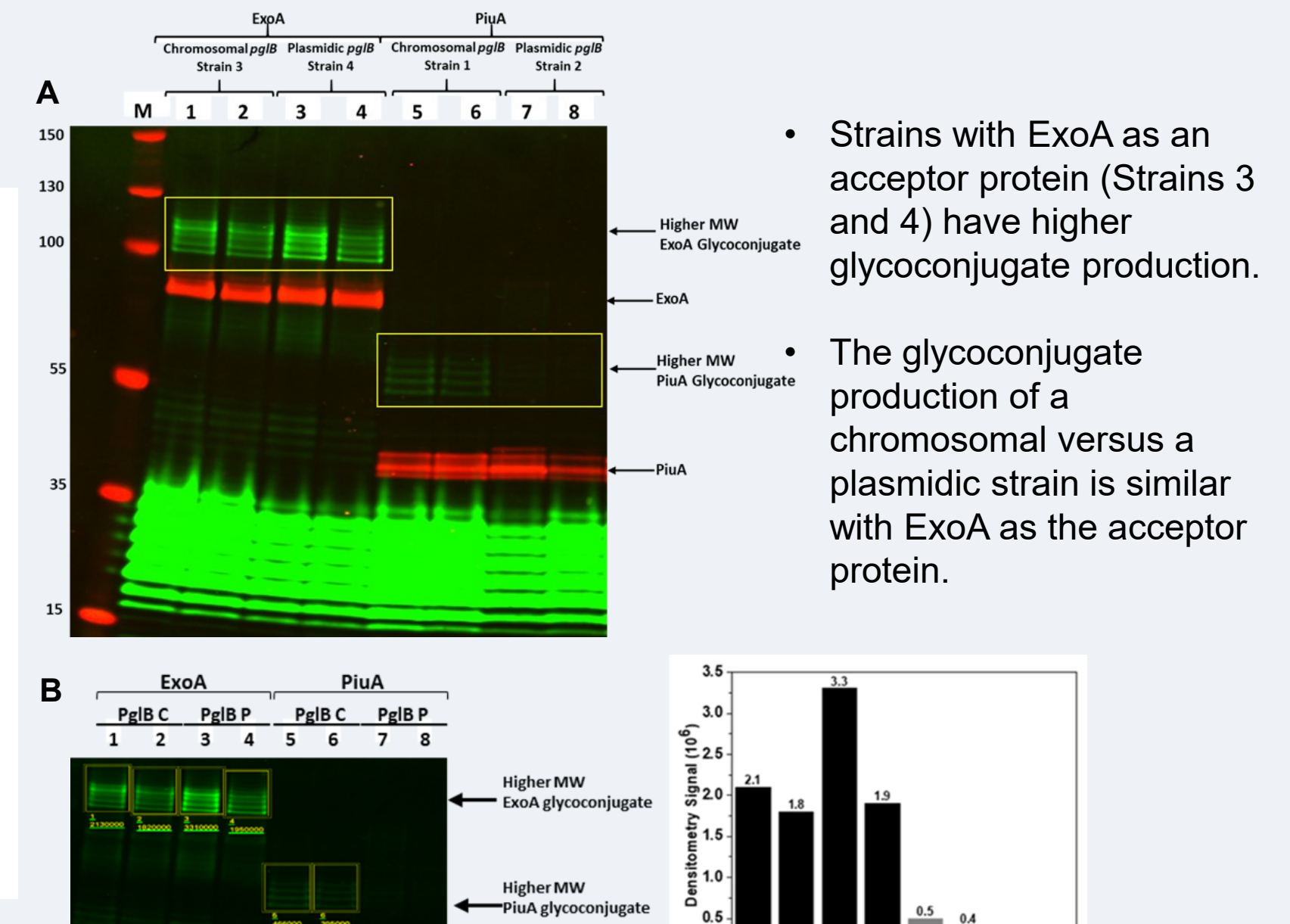
Figure 6: Immunoblot (A) and densitometry analysis (B) of endpoint samples taken from fed-batch fermentations 28 h post-induction. All samples were OD600 matched. Densitometry analysis was performed on glycoconjugate bands.

Optimisation of fermentation conditions using DoE

- Investigate the effect of pre-induction growth rate, post-induction temperature and post-induction feed rate on cell biomass and glycoconjugate production using Strain 3.
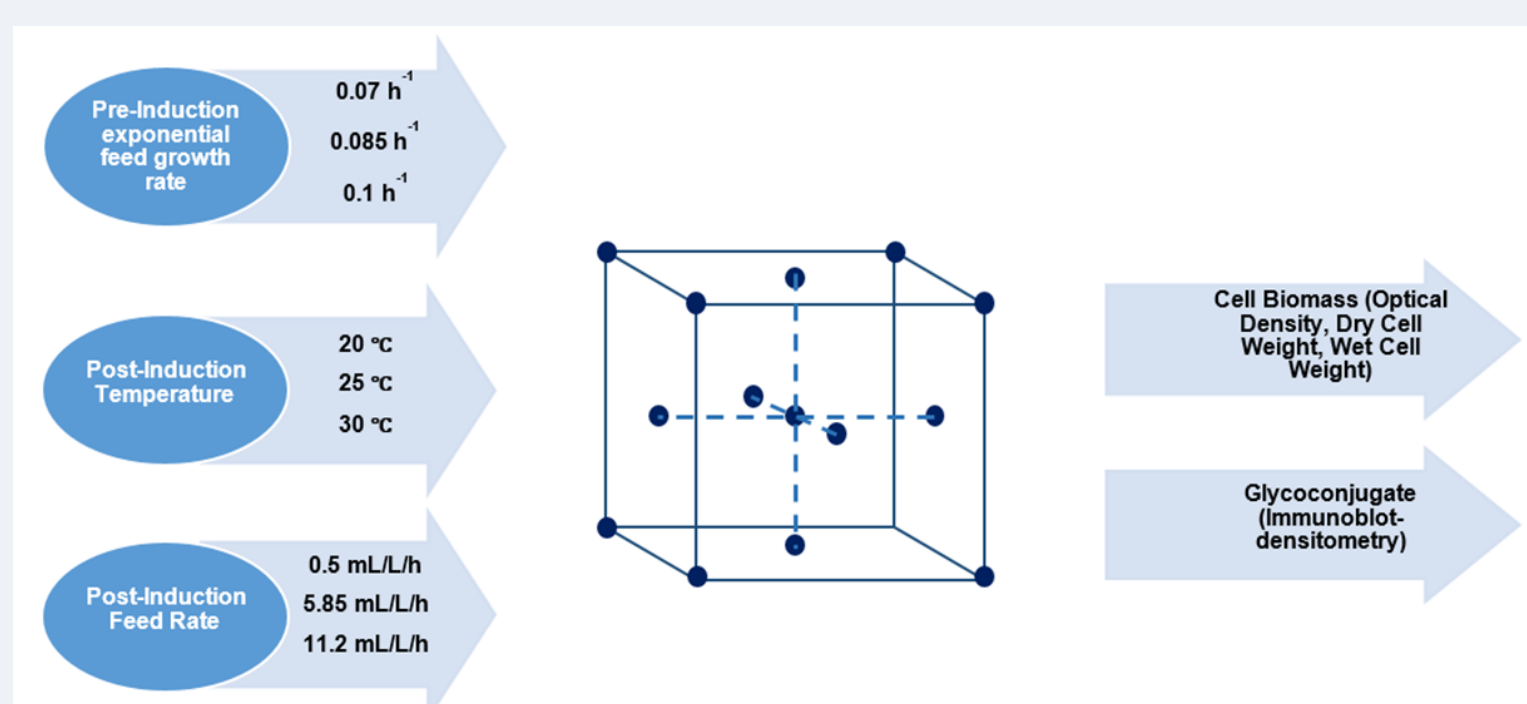

Figure 7: Schematic of central composite design outlining the factors and levels chosen and the responses that were measured. In total 18 different conditions including 4 centre points were run (Table 2).
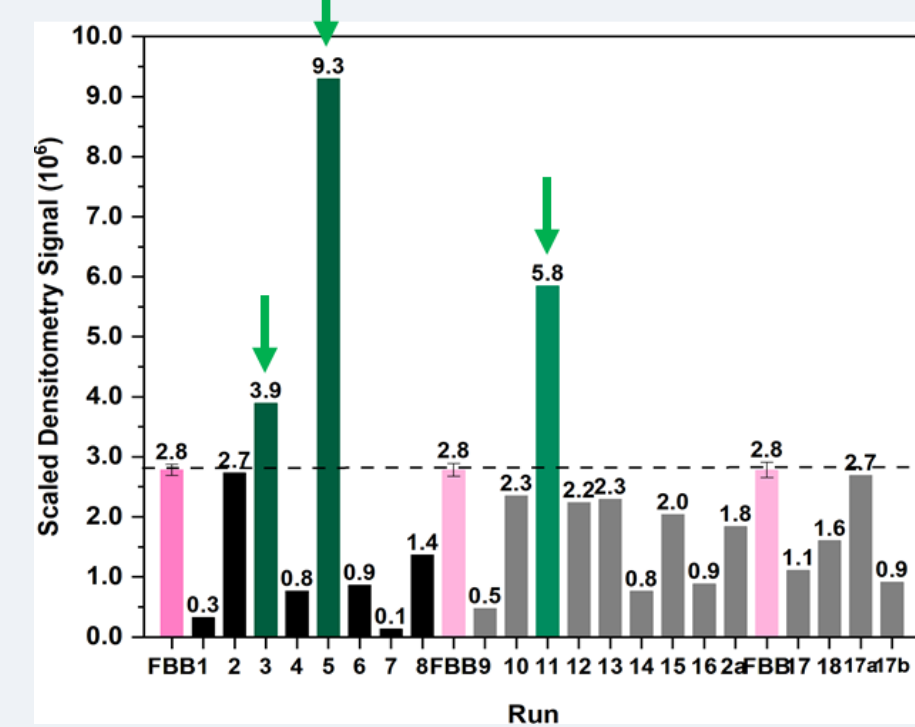

Figure 8: Scaled densitometry signal for glycoconjugate. Bars in green are runs which had a signal higher than the fed-batch benchmark (FFB) sample.
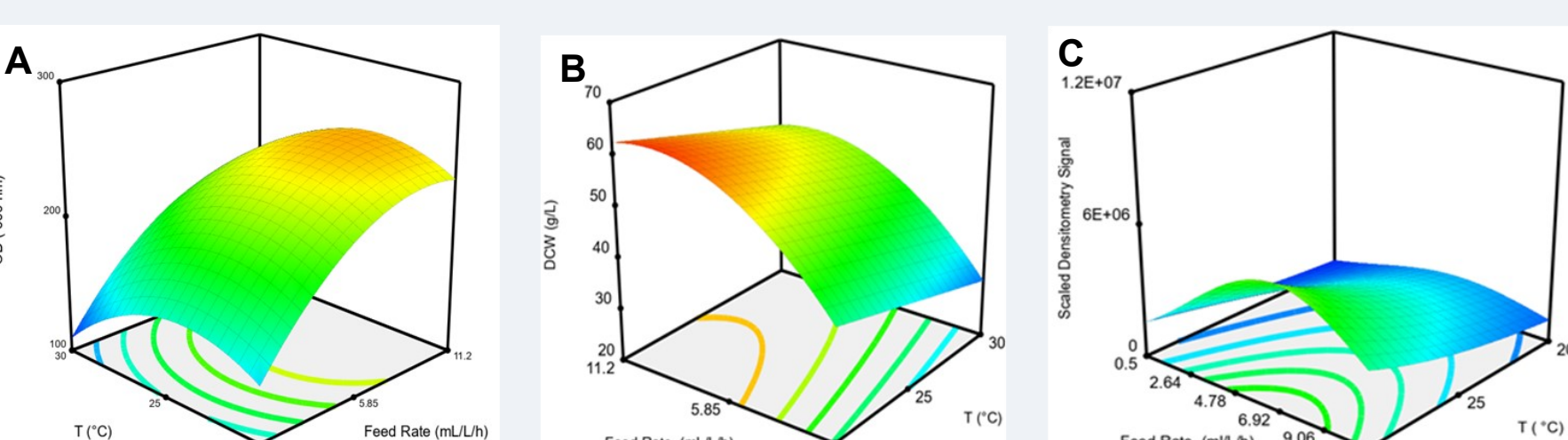

Figure 9: Response surface model plots for OD600 (A), Dry cell weight (B) and scaled densitometry signal (C).

- DOE study has improved cell biomass yield at harvest between 3-7.3 fold compared to a fed-batch benchmark condition.
- Densitometry signal compared to a benchmark sample improved in only three conditions when running normalised samples on immunoblots (Fig. 8)
- Post-induction feed rate and temperature have a statistically significant impact on biomass at harvest and scaled densitometry signal.

Conclusions

- First study to demonstrate production of a serotype 4 pneumococcal glycoconjugate using PGCT in fed-batch fermentation with defined medium.
- The choice of acceptor protein has a considerable impact on glycoconjugate production.
- Optimisation of fermentation process variables can further increase cell biomass yield and glycoconjugate production.

References

1. Cuccui J, et al. (2013) Exploitation of bacterial N-linked glycosylation to develop a novel recombinant glycoconjugate vaccine against Francisella tularensis. Open Biol.; 3(5):130002.

2. Reglinski M, et al. (2018) A recombinant conjugated pneumococcal vaccine that protects against murine infections with a similar efficacy to Prevnar-13. npj Vaccines 3.

Acknowledgments