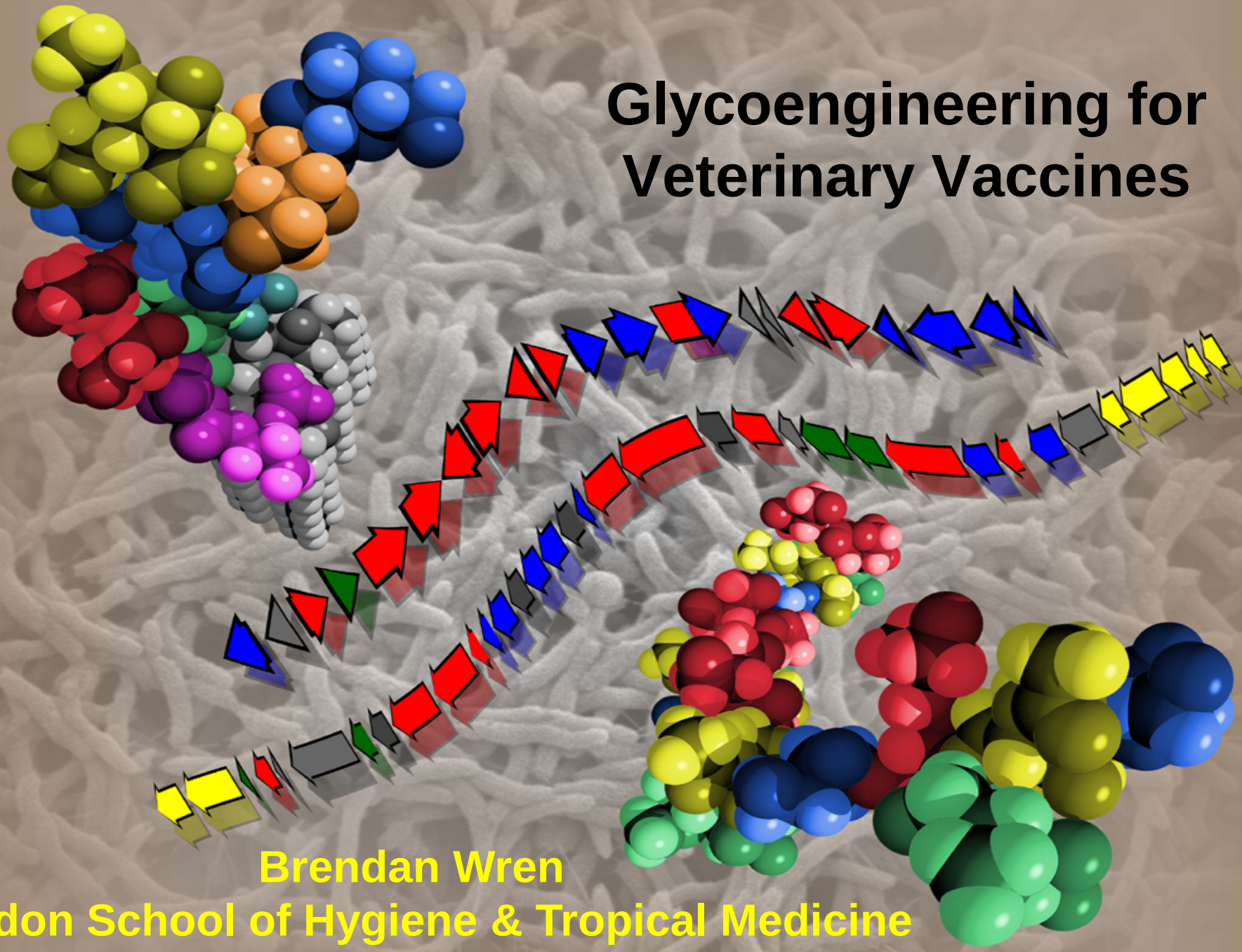
Glycoengineering for Veterinary Vaccines



Brendan Wren
London School of Hygiene & Tropical Medicine

BDS

Protein glycosylation

- More than 80% of human proteins are modified by addition of sugar structures (glycoproteins)
- Glycoproteins are involved in many biological processes ranging from conception to death
- Glycoproteins are present in bacteria –
  The dark side of microbiology
- In contrast to the cloning revolution for DNA and proteins, glycoproteins have escaped biotechnological applications

Glycoconjugate-based vaccines

Polysaccharide-based vaccines produce a T-independent immune response with IgM that opsonises bacteria.

To convert to a more favourable T-dependent response polysaccharides are often conjugated to proteins

Examples of successful human glycoconjugate vaccines

1. ***Haemophilus influenzae***
2. ***Neisseria meningitidis*** **(except type B)**
3. ***Streptococcus pneumoniae*** **(some serotypes)**

Long lasting immunity & suitable for children

The benefits of veterinary vaccines

1. Healthily maintained livestock are essential for economic and societal prosperity

2. Prevention of zoonotic infections reduces human disease

3. Better vaccines may reduce antibiotic usage and reduce the spread of AMR

Why have glycoconjugate vaccines not been used in veterinary medicne?

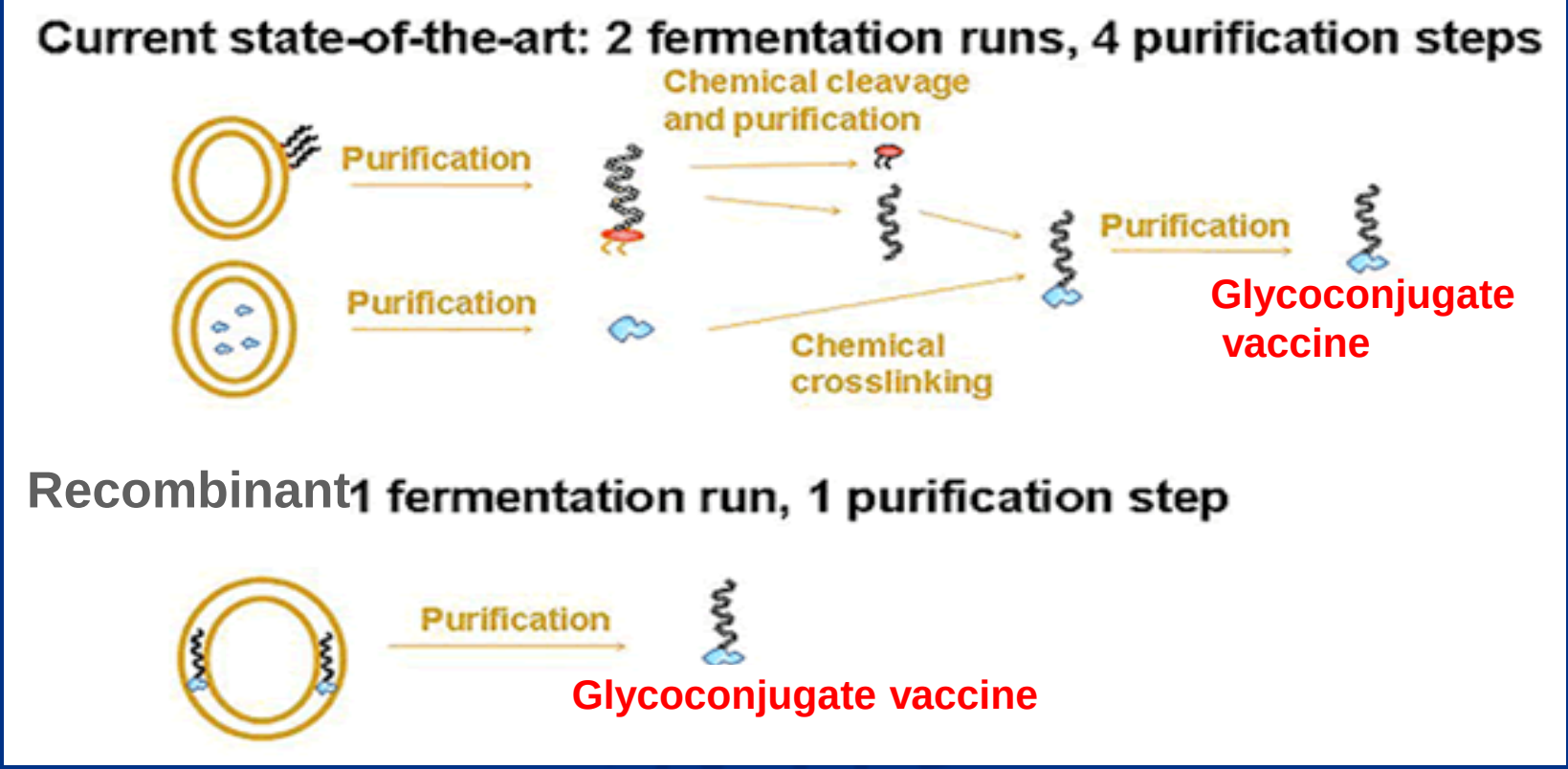
They are effective, but

COST!!

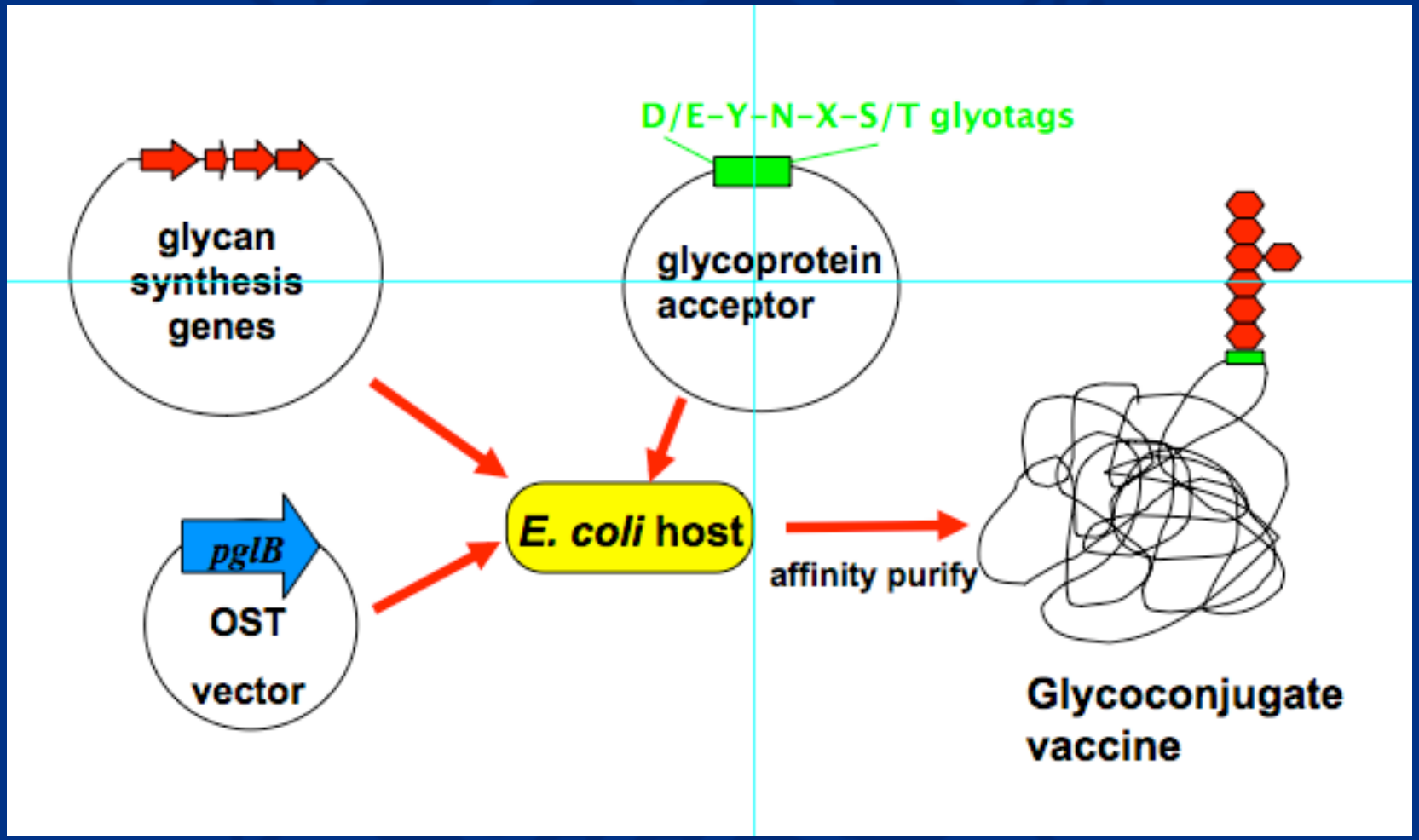
Current glycoconjugate vaccine development

Require purification of polysaccharide from native pathogen and chemical coupling to a protein carrier

Multistep, time consuming and expensive procedure



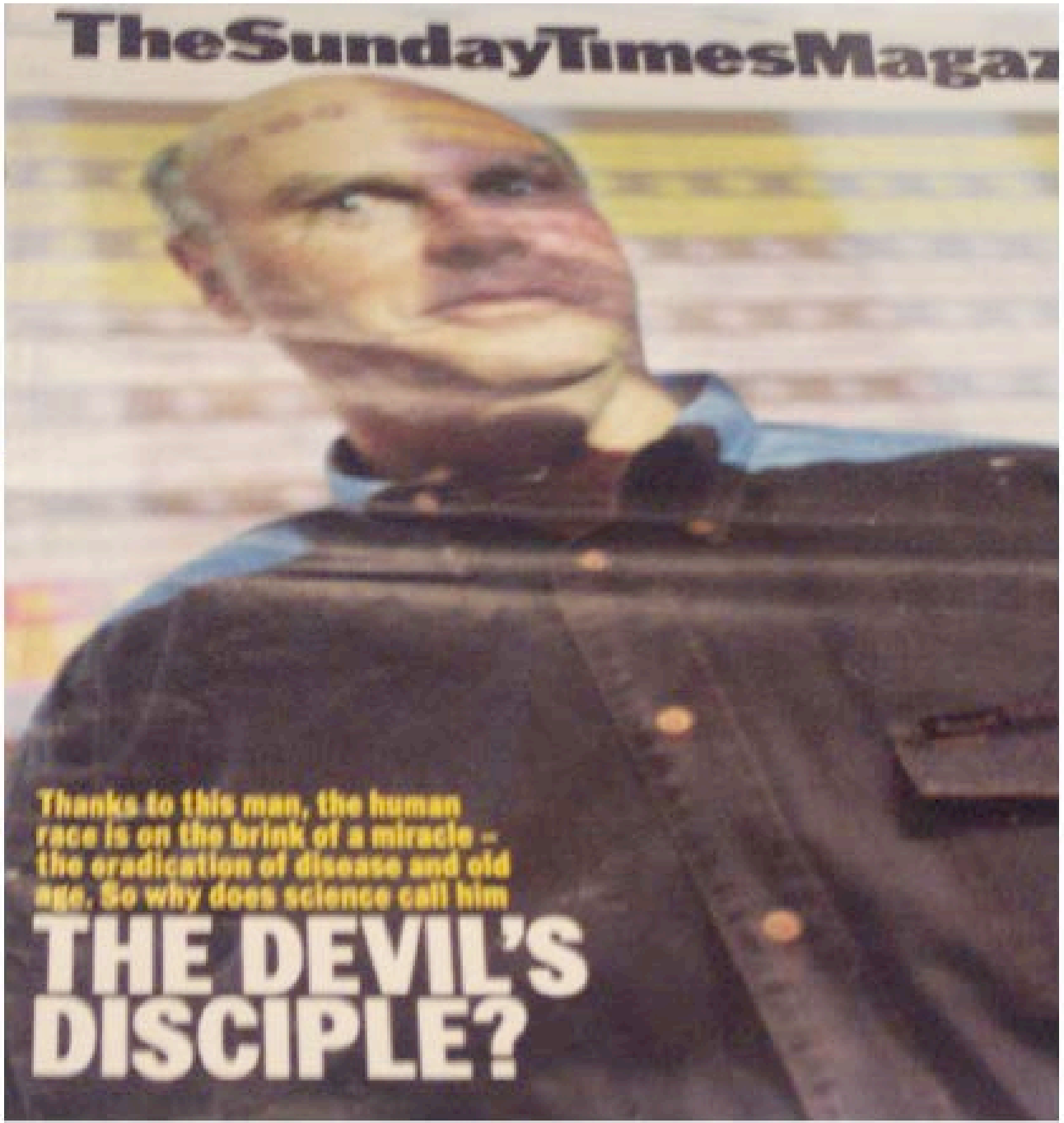
Protein Glycan Coupling Technology – new era for glycoengineering



One step process –
Flexibility of mixing & matching of protein/glycan combinations

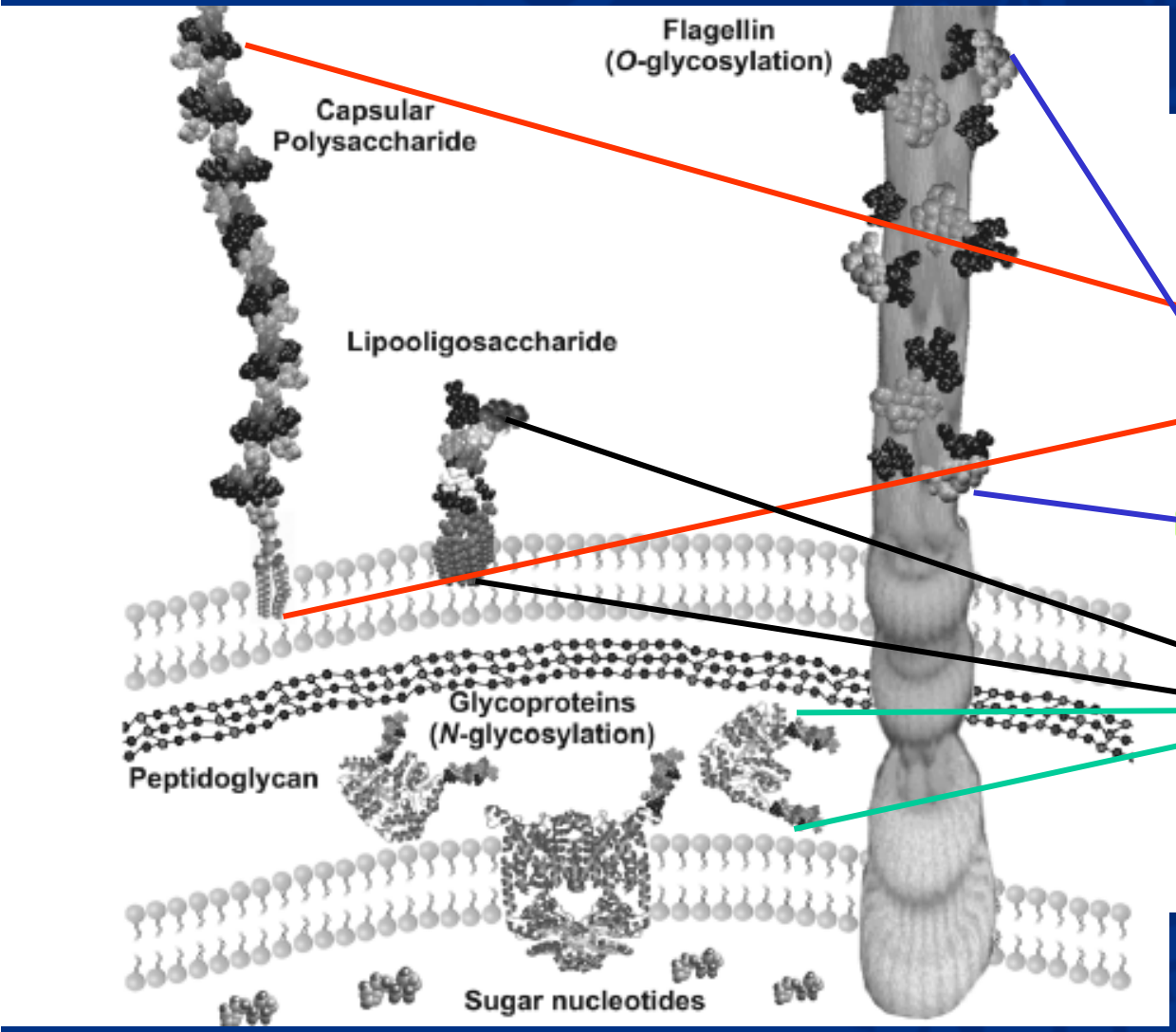
Recombinant glycoconjugate vaccines 20 years in the making

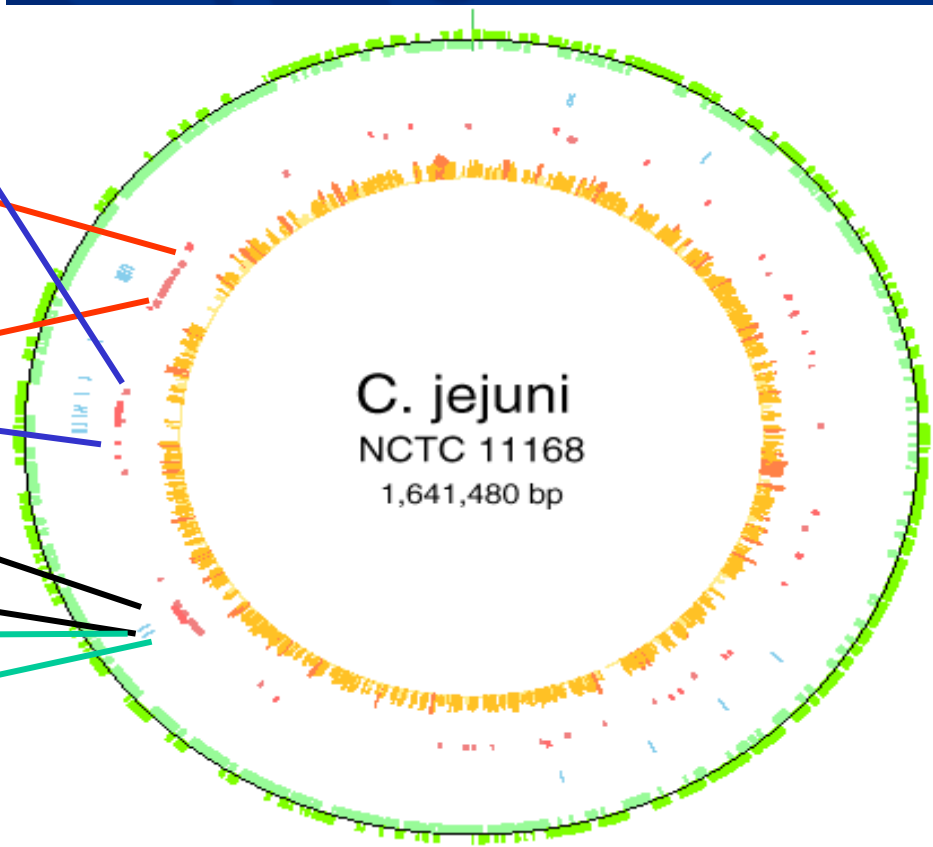
bbsrc



Glycostructures - from genome project to structure & function

Campylobacter jejuni **a hyperglycaemic bug >8% genome encode glycostructures**

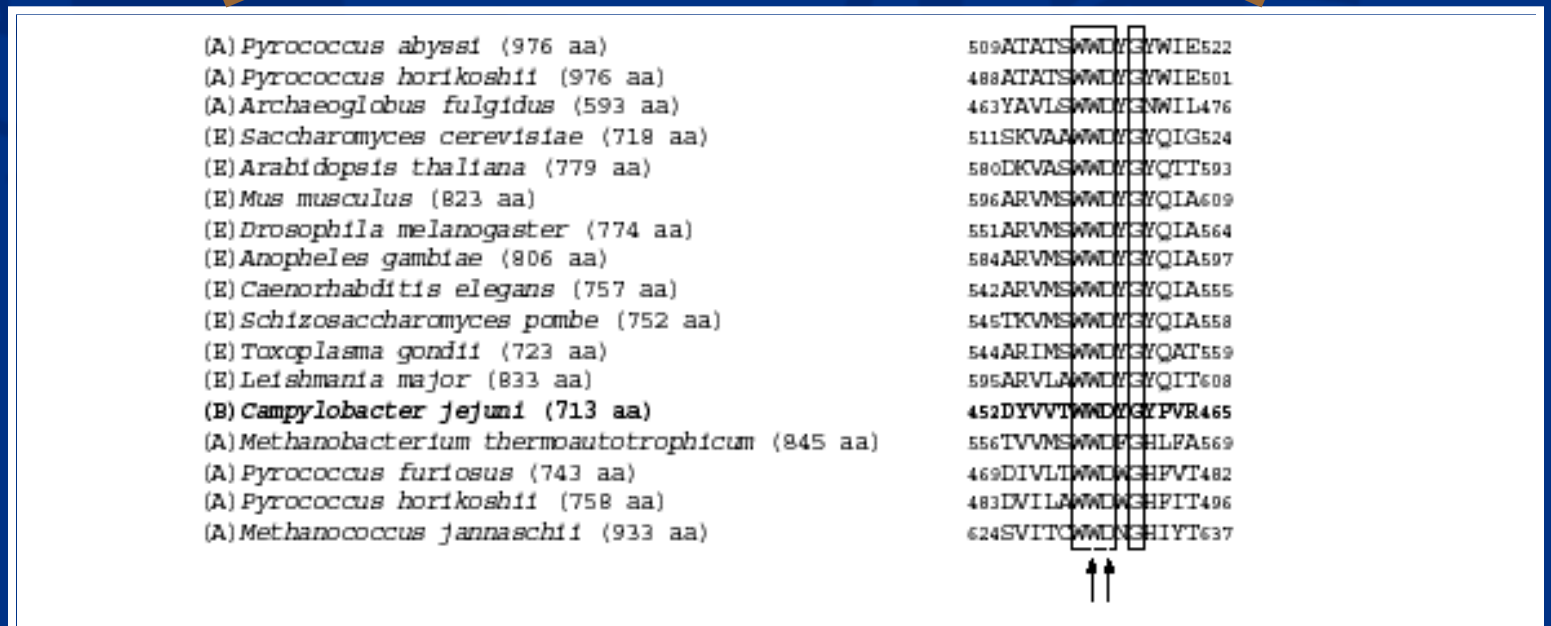




Parkhill *et al* Nature 2001

Campylobacter N-linked general glycosylation system & the importance of PglB

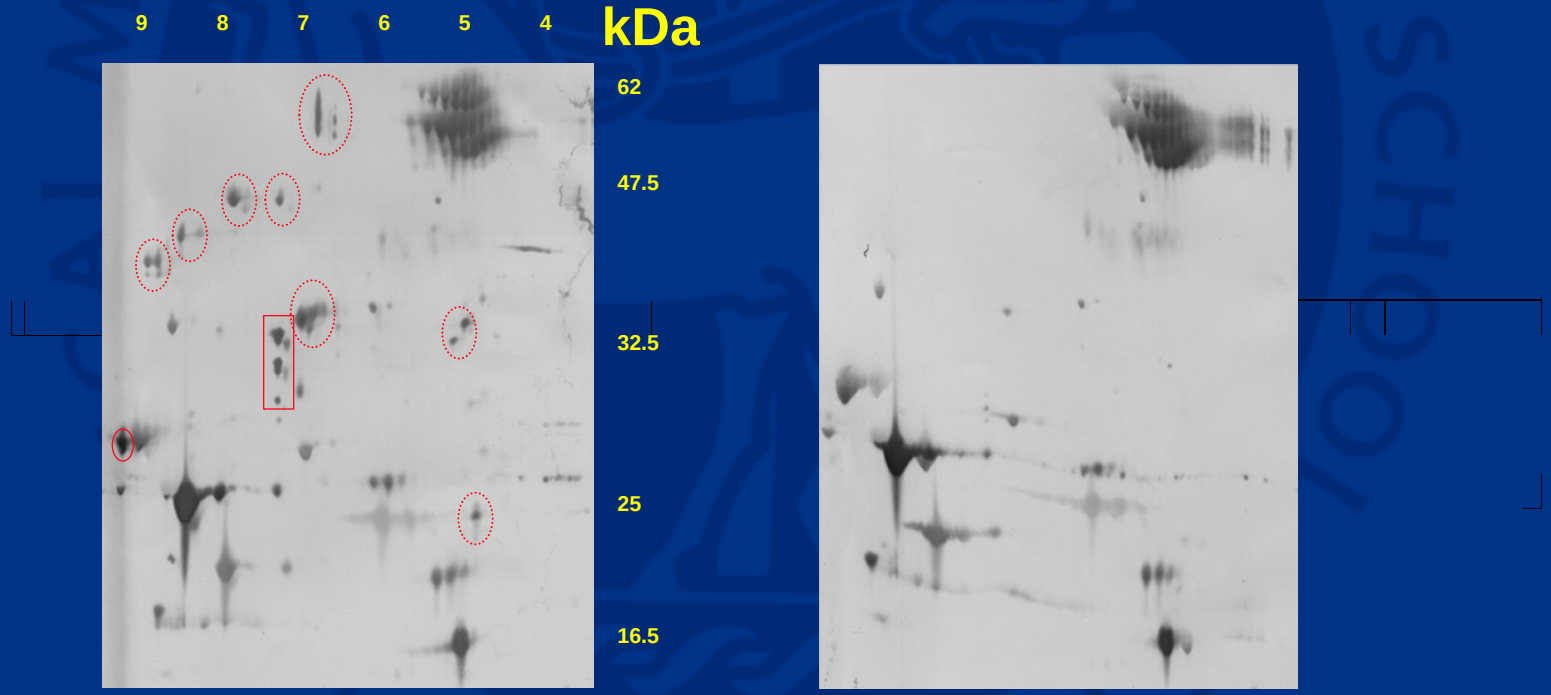
Campylobacter only bacterial Otase (oligosaccharyl transferase)



Multiple *Campylobacter* glycoproteins

PglB mutant abolishes *Campylobacter* general glycosylation pathway resulting in the loss of several lectin-binding proteins

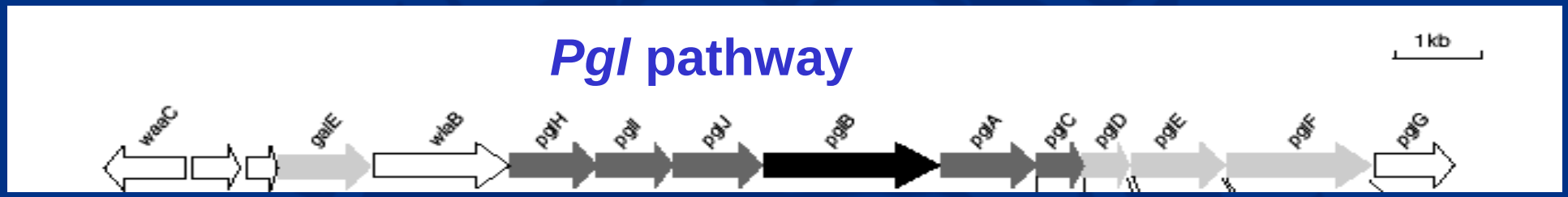
Proteome and mass spec analysis identifies >50 glycoproteins (Linton *et al* Mol Micro 2001, Young *et al* JBC 2002)



Acid extract wildtype

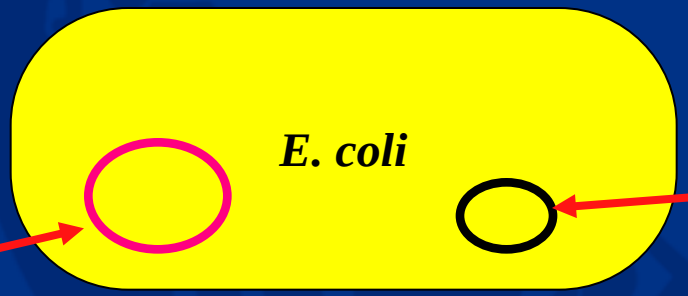
Acid extract PglB mutant

Structural analysis of *Campylobacter N*-glycosylation machinery



To produce sufficient pure yields of the glycan clone into *E. coli?*

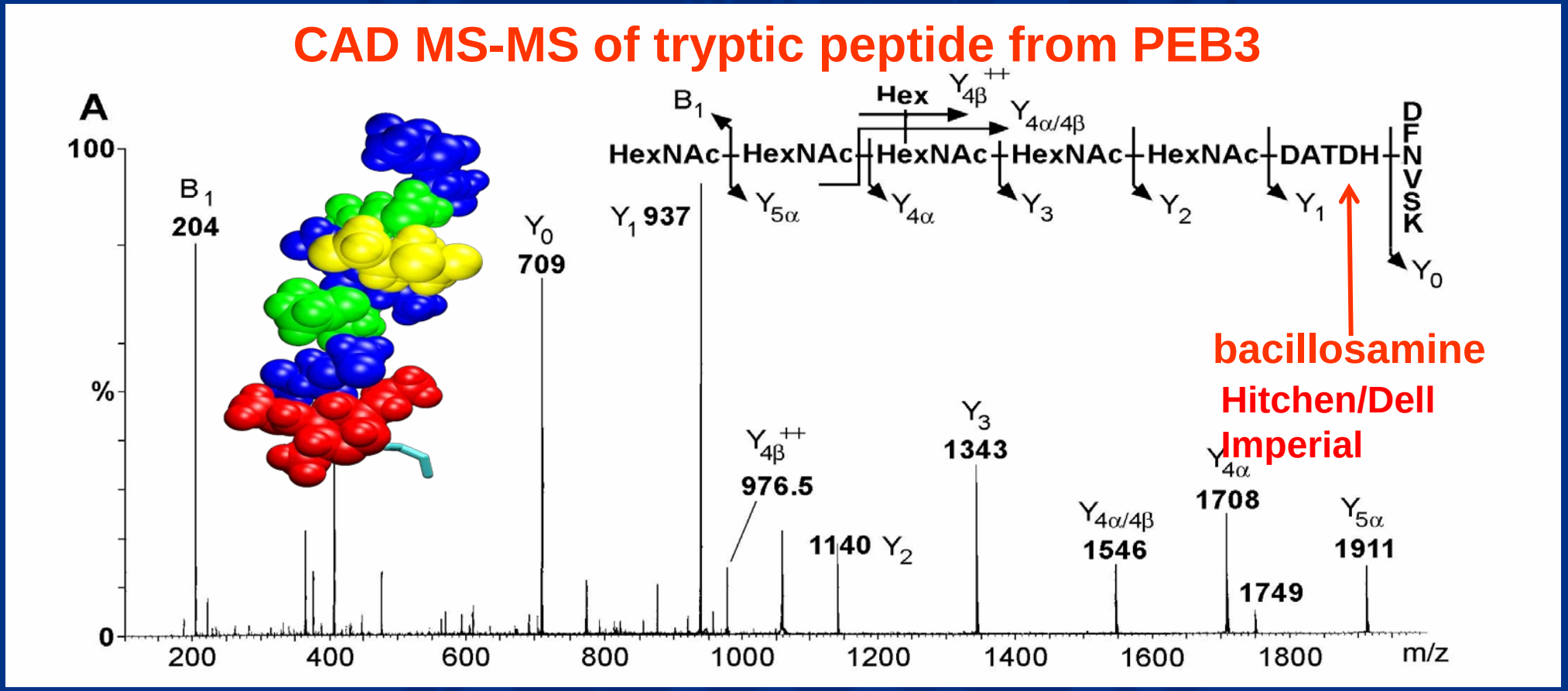
plasmid containing Pgl pathway



PEB3 in plasmid (reporter of glyco-sylation)

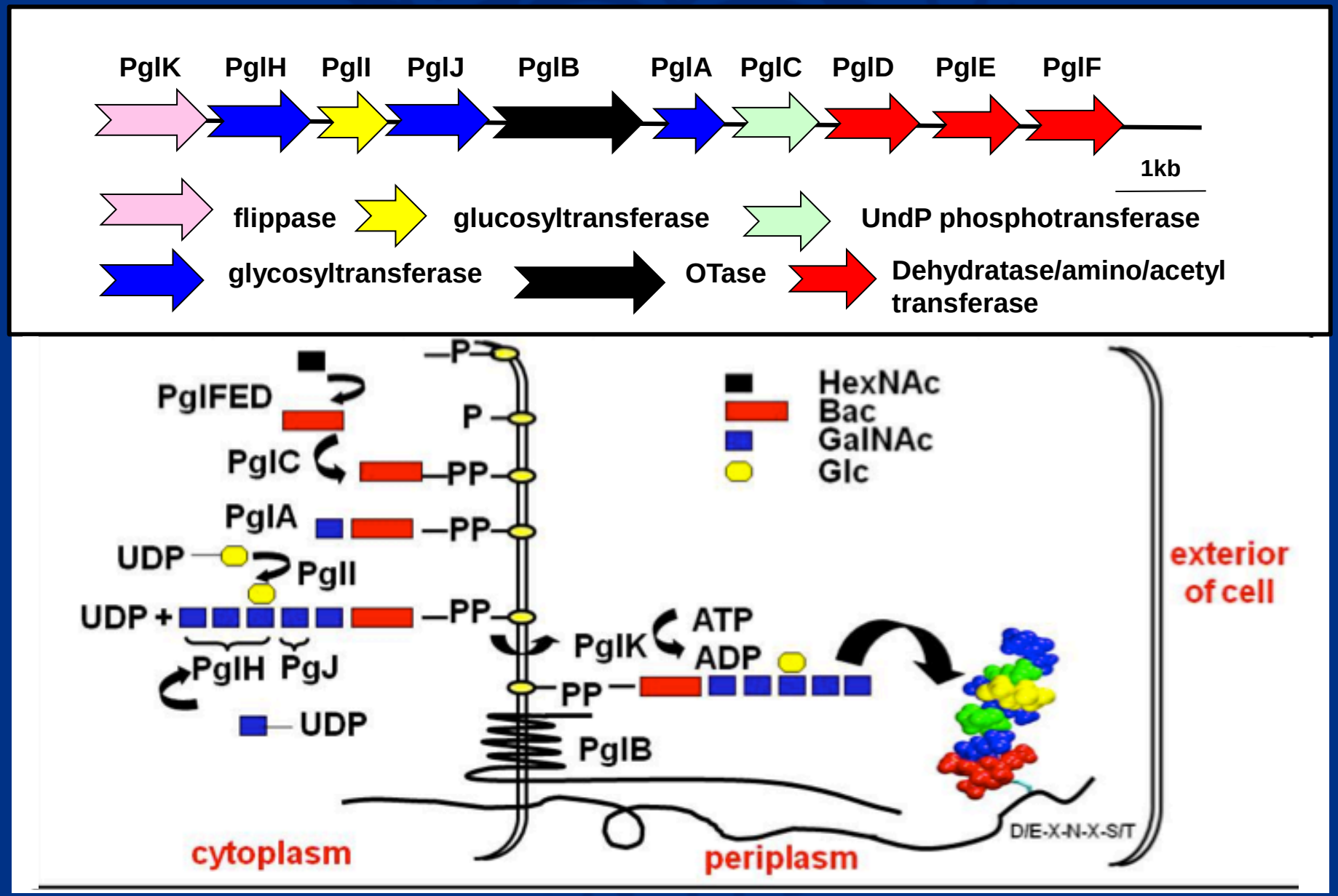
Purify cloned PEB3 and determine glycan structure by mass spec

Structure of PEB3 □*N*-linked glycoprotein heptasaccharide bacillosamine



Wacker *et al* Science 2002

Biosynthesis of *N*-linked glycoproteins in *Campylobacter*



You never know where your genome project will lead?

Can clone *Campylobacter* general glycosylation system in *E. coli* to dissect the role of each gene

But also produce recombinant glycoproteins

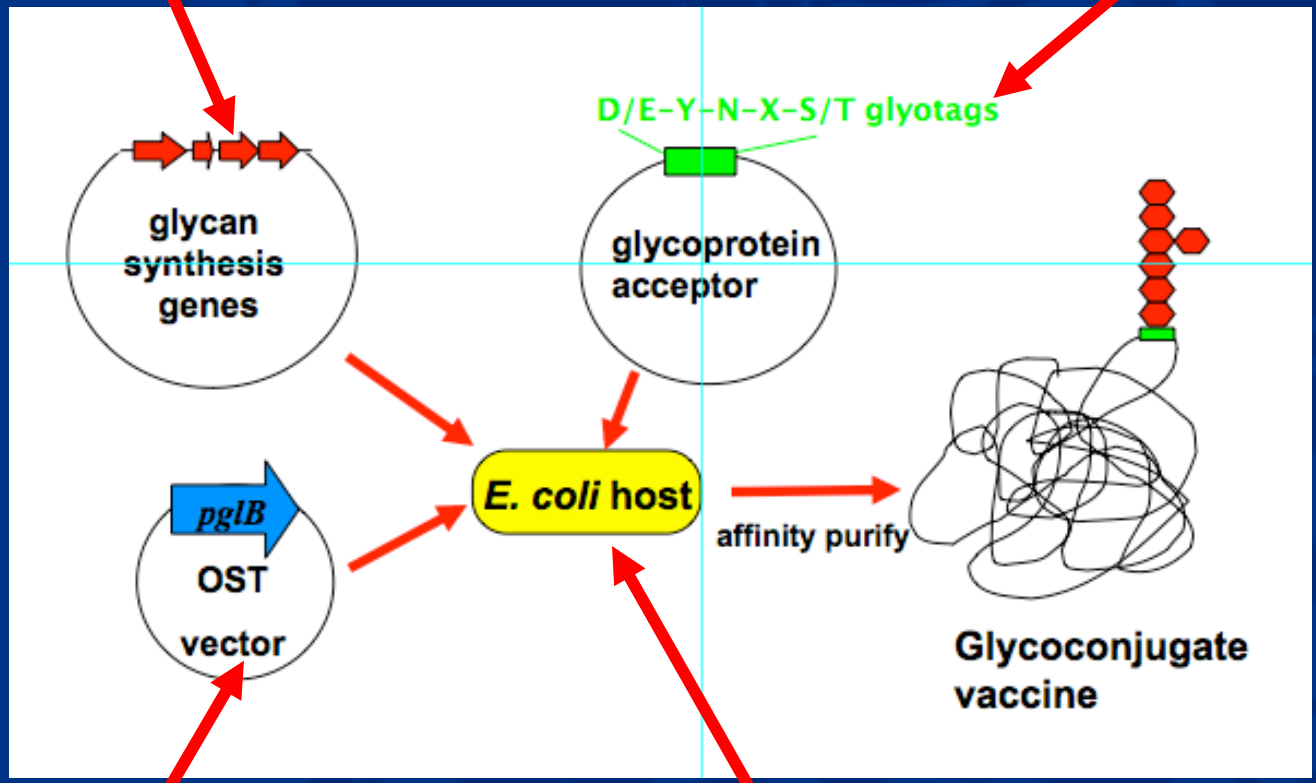
Possibility to produce recombinant glycoproteins and to develop glycoconjugate vaccines

Named process Protein Glycan Coupling Technology (PGCT)

F. tularensis glycoconjugate vaccine design

F. tularensis O-antigen

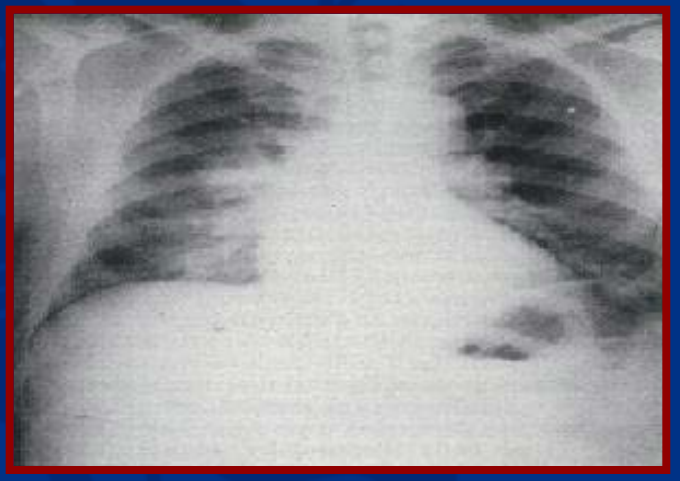
Pseudomonas ExoA

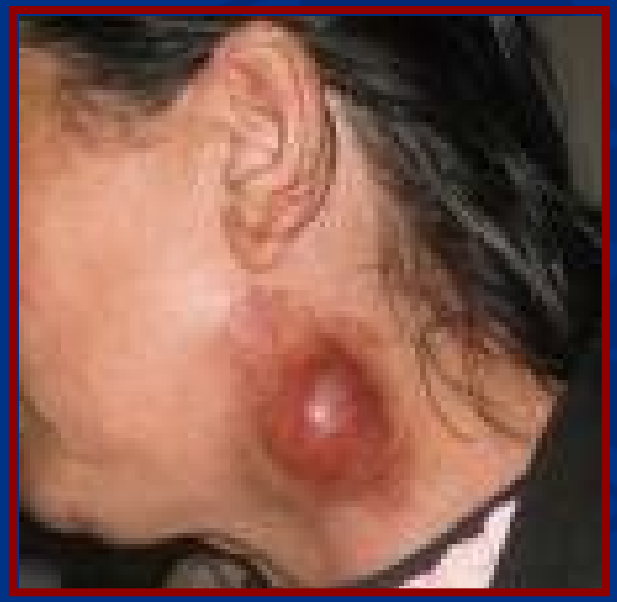


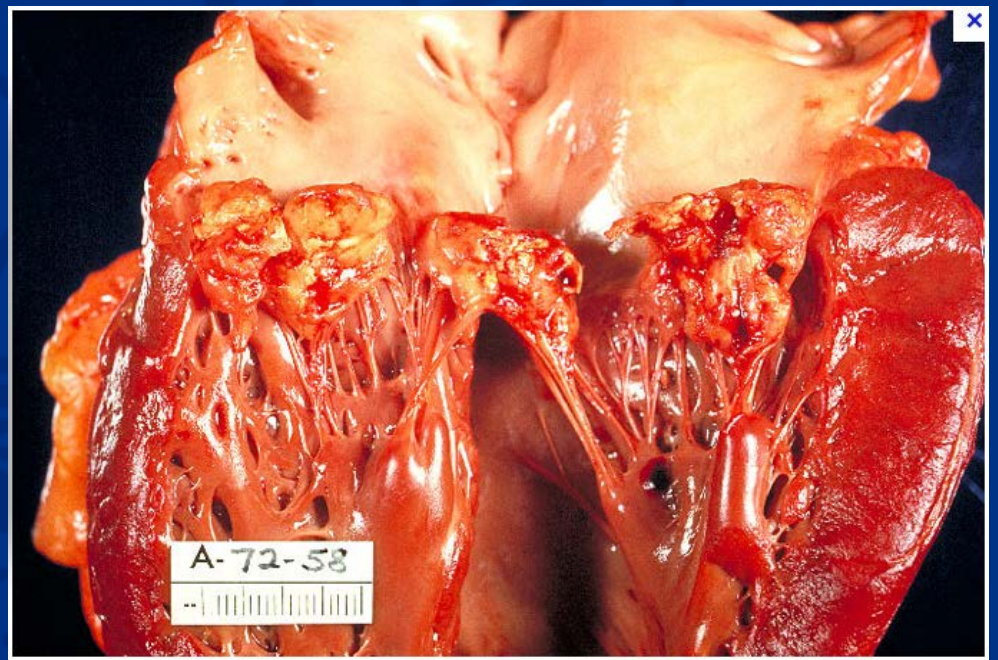
C. jejuni PglB

E. coli CLM24

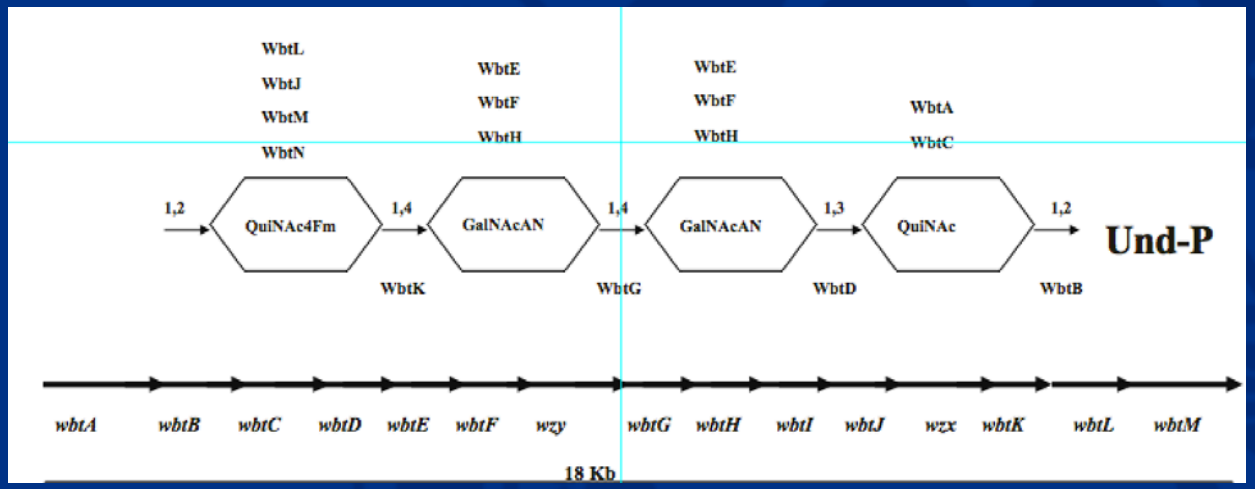
Francisella tularensis
lethal disease – no current vaccine



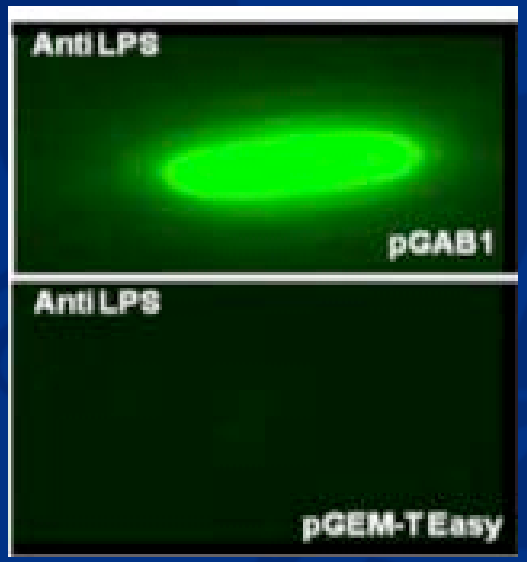




First stage – express glycan locus in *E. coli*



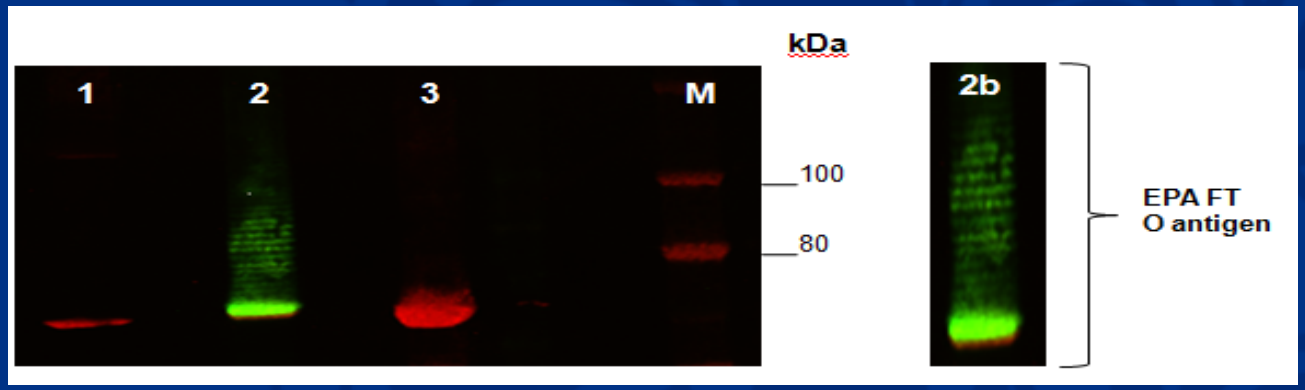
***Francisella* LPS has terminal QuiNAc**



Confirmed LPS expression in *E. coli*

Second stage add protein carrier and CjPglB coupling enzyme

Red ab stain of ExoA
Green ab stain of *Francisella* O-antigen (Mab FB11)

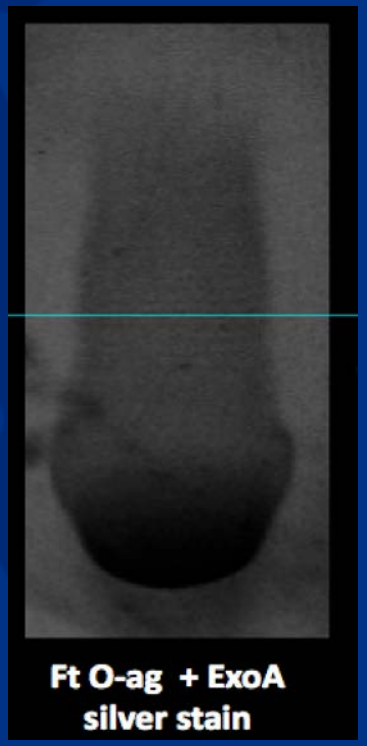


Exo - plasmid

Exo + plasmid

Plasmid alone

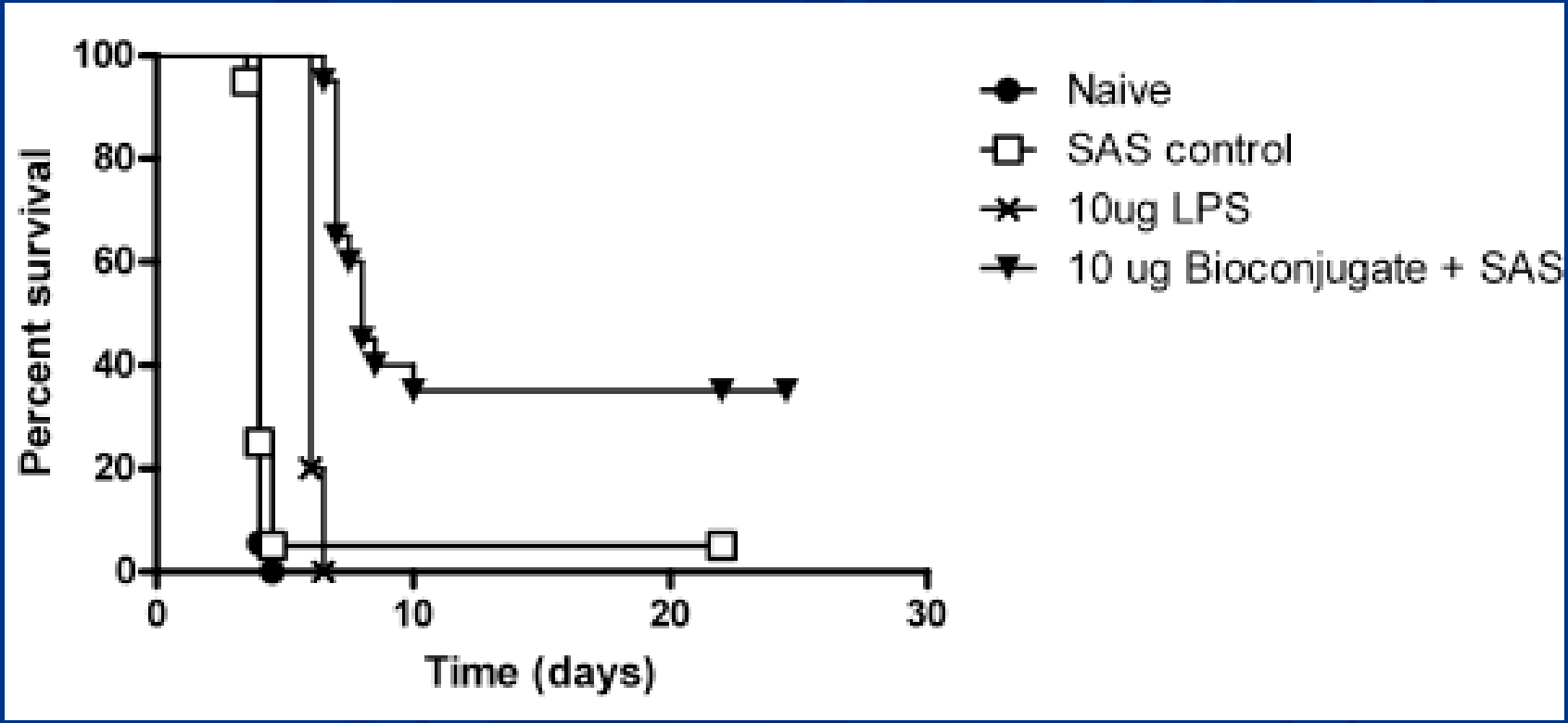
Exo + plasmid



Yield 50 mg per 10 L *E. coli*

Francisella tulerensis LPS coupled to exotoxin A

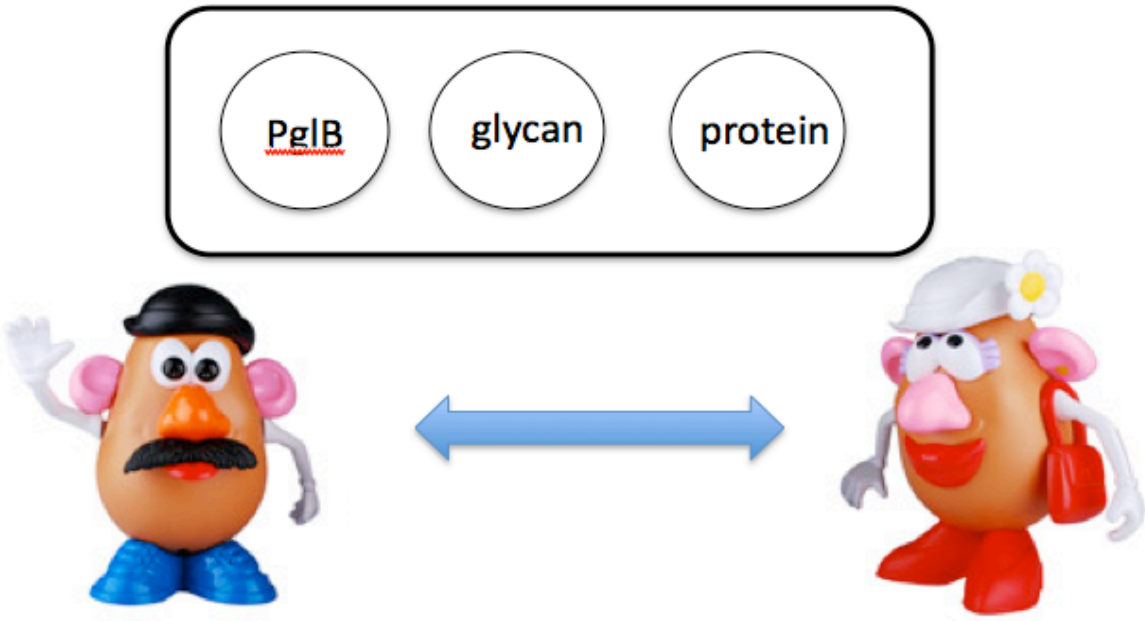
Tested in mice, first attempt - best vaccine to date



Confirmed protection & Th1-dependent response

Cuccui *et al* Open Biol 2013

Second & third generation PGCT glycoconjugate vaccines



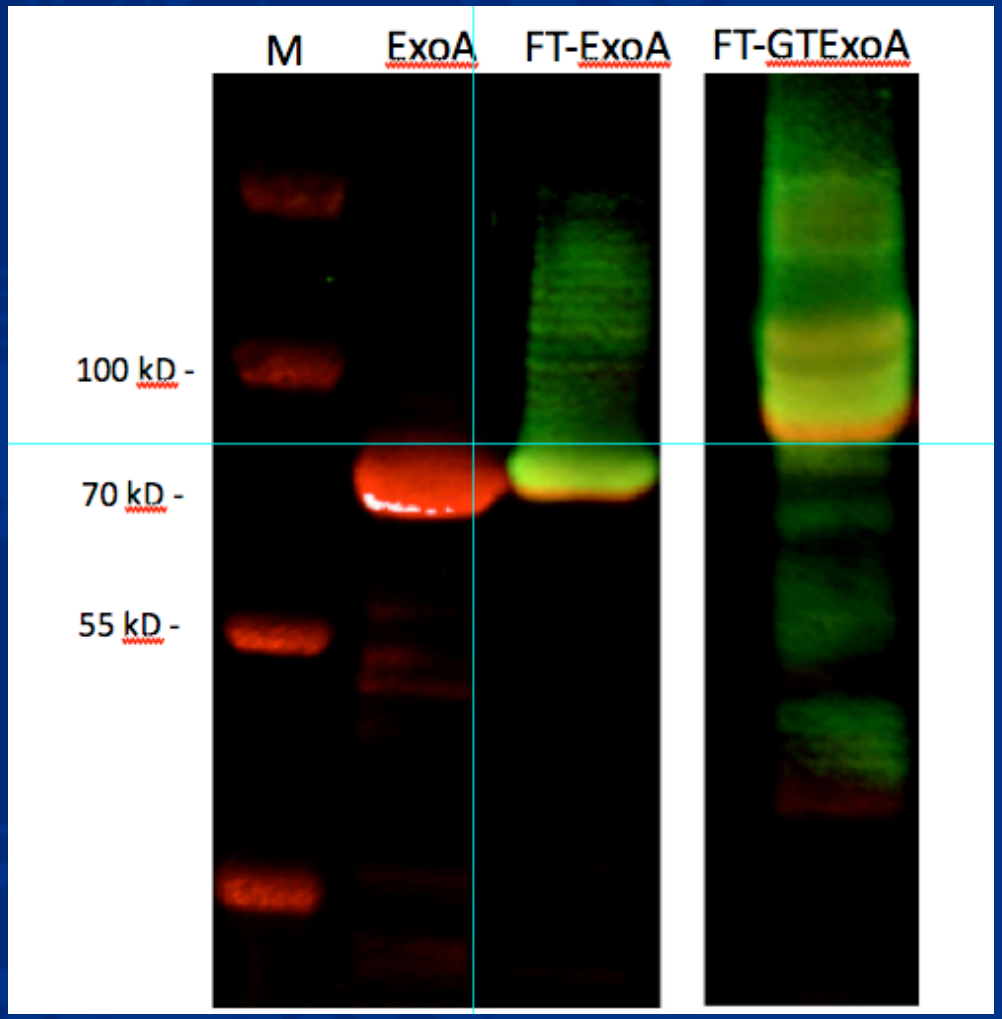
1) Alter Exotoxin A carrier protein to be more heavily glycosylated

2) Swap carrier protein to a native *Francisella* protein to provide dual protection against glycan and protein

Heavily glycosylated ExoA with glycotags

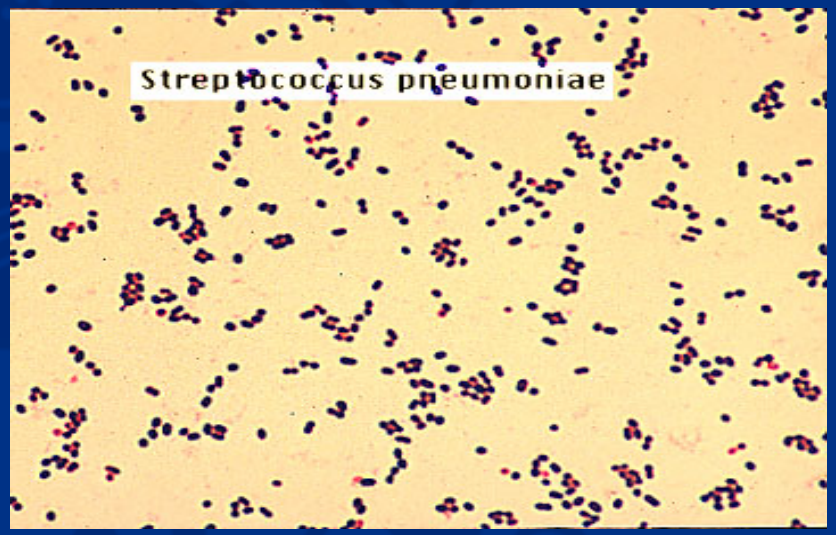
Red ab stain of ExoA

Green ab stain of *Francisella* O-antigen (Mab FB11)

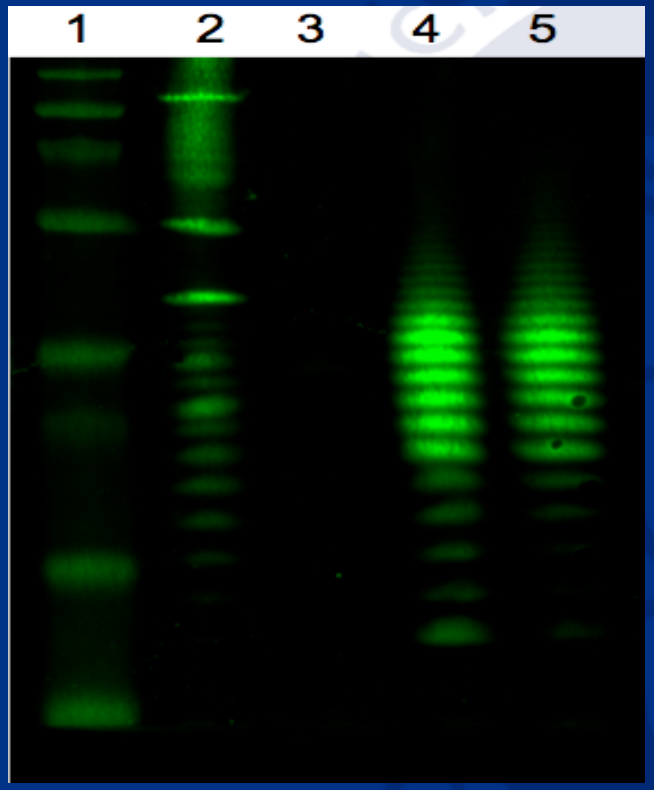


Streptococcus pneumoniae

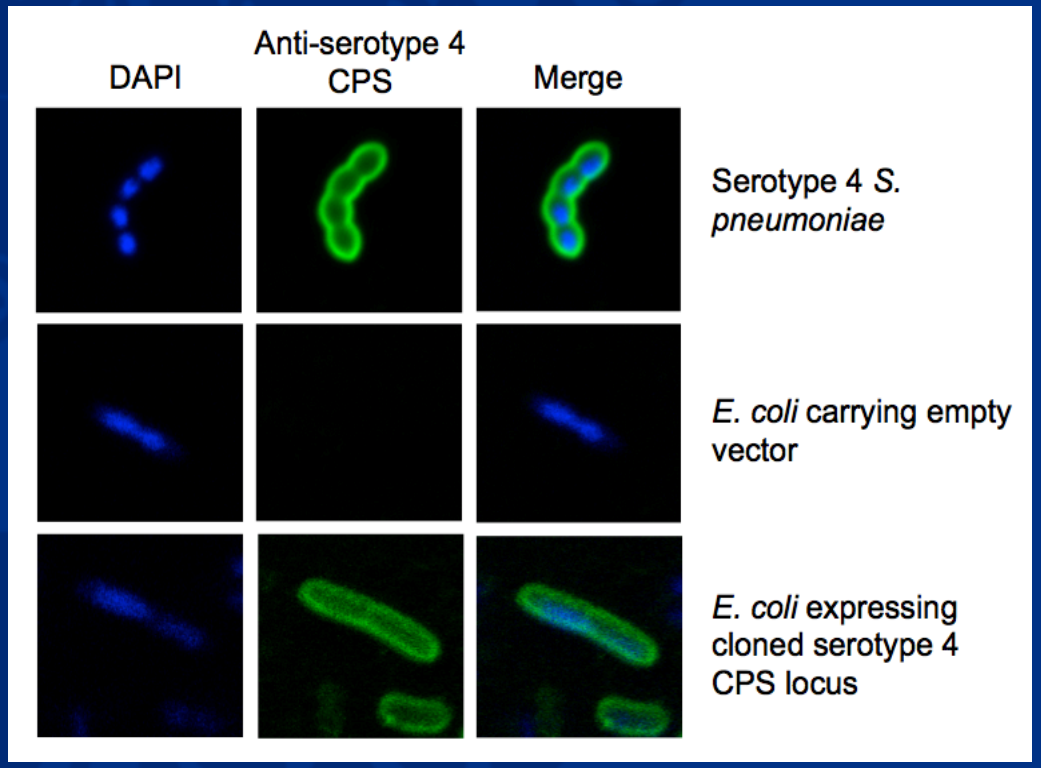
- **Gram positive, alpha-haemolytic diplococcus**
- **Over 90 different serotypes**
- **Causes pneumonia, meningitis, conjunctivitis, bacteraemia and otitis media**
- **Estimated that globally one million children under five die of pneumococcal disease each year**



Strep pneumoniae capsule expressed in *E. coli*

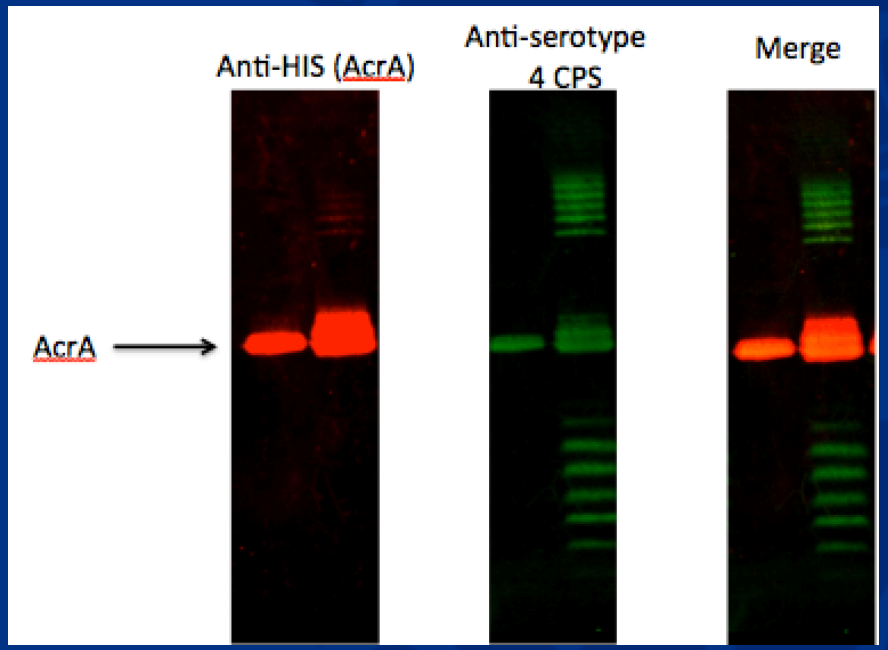


Type 4, 8, 12F, 38 & 46 capsules expressed in *E. coli*

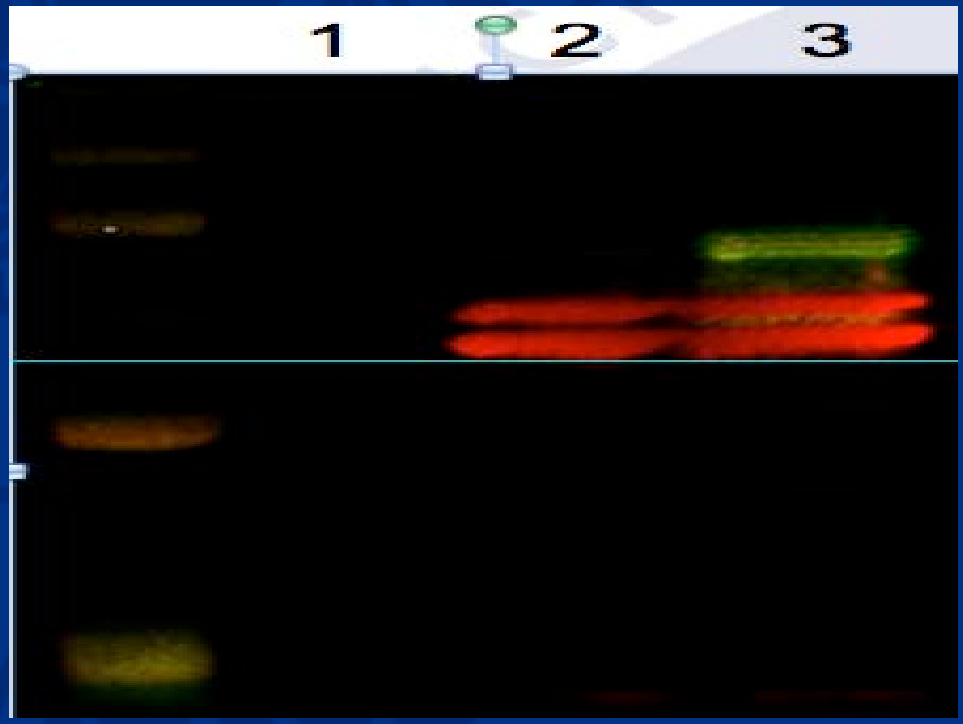


Confirmed cell surface expression

S. pneumoniae capsule coupled to AcrA and pneumolysin

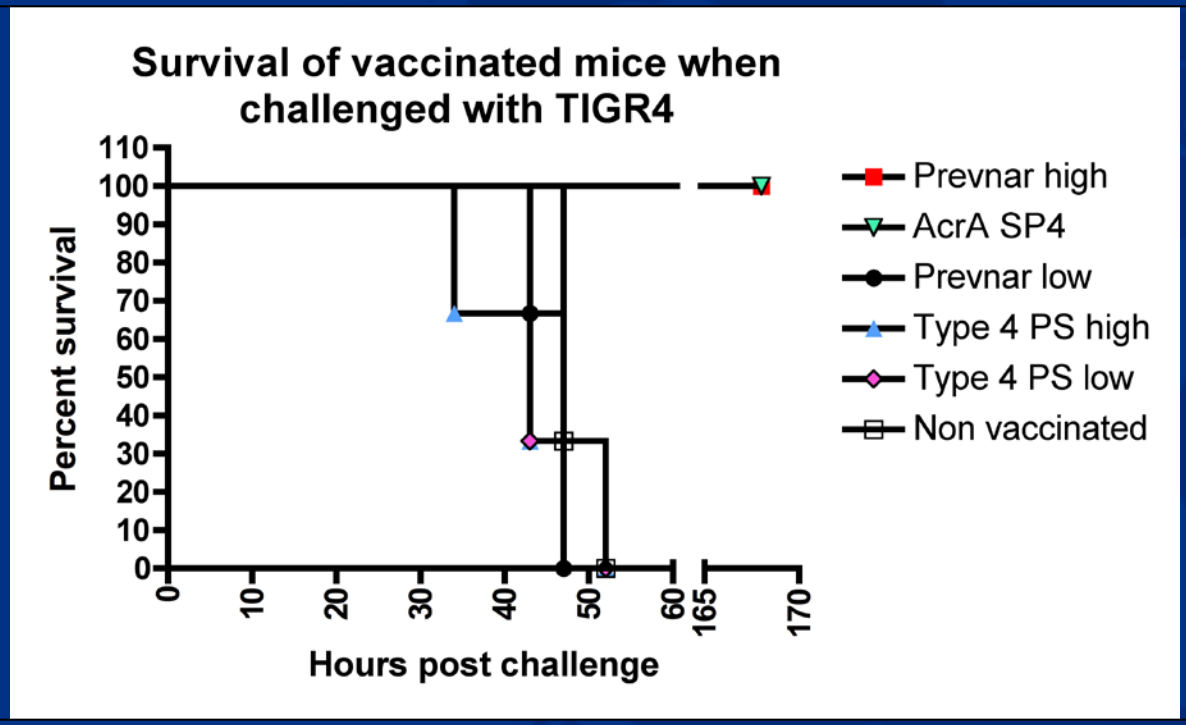


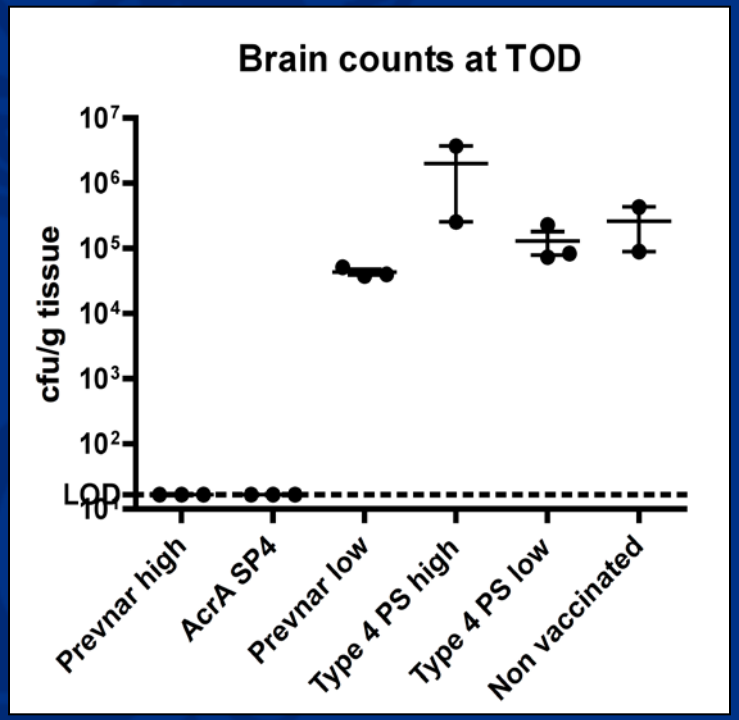
Confirmed PglB modification of AcrA



Confirmed PglB modification of glycotagged pneumolysin

S. pneumoniae capsule coupled to AcrA alone protects and is as good as commercial vaccine



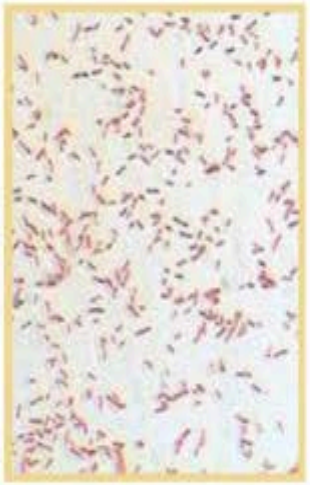


Collaboration with Tim Mitchell

Burkholderia pseudomallei and *mallei* lethal disease – no current vaccines

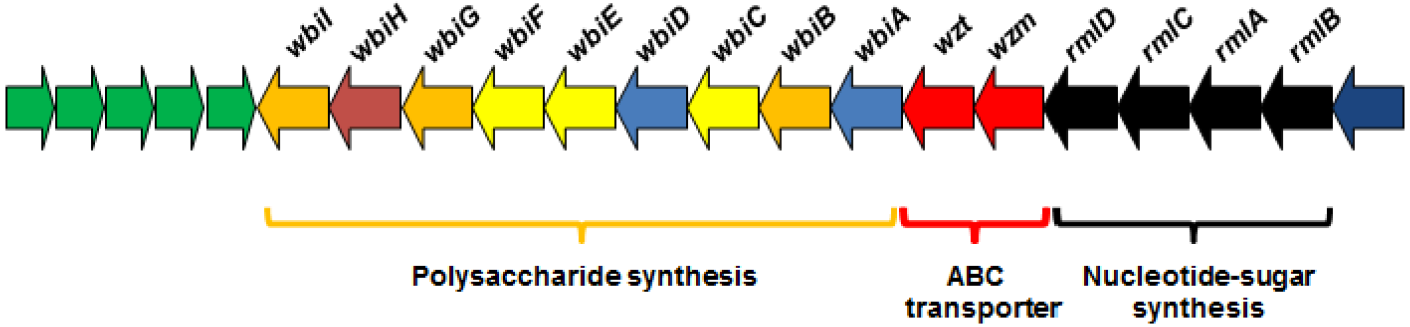
- Gram-negative facultative motile rod bacterium
- Environmental saprophyte endemic in SE Asia and N Australia.
- Intracellular pathogen, causative agent of melioidosis.
- Infection can occur through contamination of wounds or inhalation.
- Acute septicaemia. 40-80 % mortality despite therapy.
- Chronic/Latency up to 62 years reported.
- No vaccine available.
- Infectious dose = 10
- Profoundly antibiotic resistant
- Select Agent Tier 1

Mallei cause of glanders

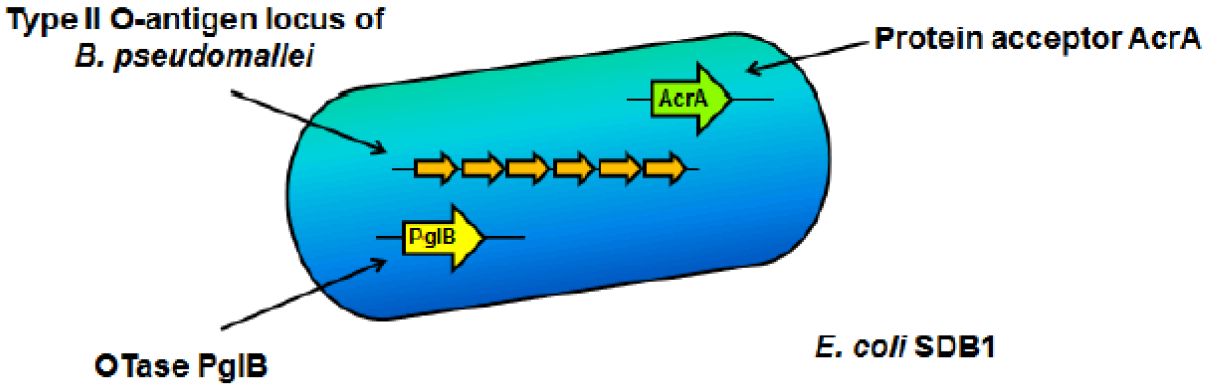


Recombinant *Burkholderia pseudomallei* glycoconjugate vaccine design

Organization of *B. pseudomallei* K96243 O-antigen polysaccharide (II) locus



II O-PS (LPS)	heteropolymer (disaccharide)	-3)- β-D-glucopyranose-(1-3)-6-deoxy-α-L-talopyranose-(1- 33% of L-6dTalp = 4-o-acetylated, 2-o-methylated. 67% = 2-o-acetylated



Other PGCT bacterial vaccines

1. Uropathogenic *E. coli* – human trials (Glycovaxyn, Zurich)

2. *Shigella* LOS/ExoA – human trials (Glycovaxyn, Zurich)

3. Triple combination poultry vaccine (*E. coli, Campylobacter, Salmonella*, *Clostridium perfringens*) – (LSHTM)

4. Coxiella human and veterinary vaccine – (LSHTM)

Triple poultry vaccine 1

Colibacillosis is a severe and recalcitrant avian diseases caused by avian pathogenic *E. coli* (APEC). A successful treatment for colibacillosis is PoulVac an attenuated *E. coli* strain.

Necrotic enteritis growing problem in poultry due to *Clostridium perfringens.* NetB is an outstanding protein candidates in their own right.

Piggy back onto PoulVac (or other attenuated strain) *C. perfingens* NetB protein, coupled to *Campylobacter* heptasaccharide

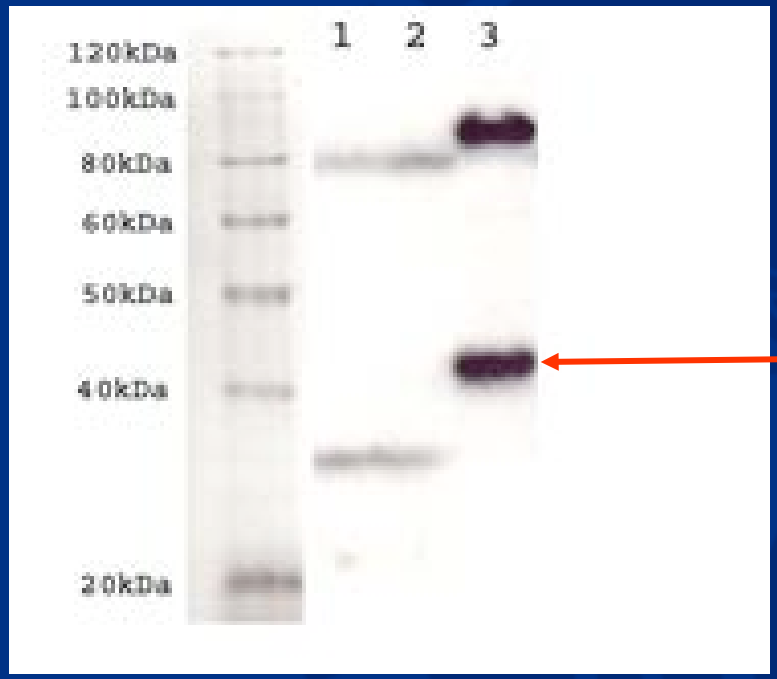
Triple poultry vaccine 2

Exploit *Salmonella* attenuated vaccine strain (*cca-*) extensively used in the poultry industry

Piggy back onto attenuated strain *C. perfingens* NetB protein, coupled to *Campylobacter* heptasaccharide

But will PGCT work in *Salmonella*?

Combination glycoconjugate vaccines



Can use alternative host to *E. coli* and include multiple sites

tetra-glycosylated CjaA in Salmonella

Dual Salmonella/Campylobacter vaccine

Dual ruminant vaccine

Protect ruminants against *Coxiella* (Q fever) and *C. perfringens* infection

Couple the *Coxiella* LPS pathway with NetB or epsilon toxin from *C. perfringens*

Purify from *E. coli* in a single step and produce inexpensive injectable vaccine

Other animal glycoconjugate vaccines under consideration

Exploiting capsules from pathogenic *Strep*

Pig – *Strep suis* (& App)

Equine – *Strep equi*

Mastitis – *Strep uberus* (& *Staph aureus*)

Fish – *Strep innuae* (& *Y. ruckeri*)

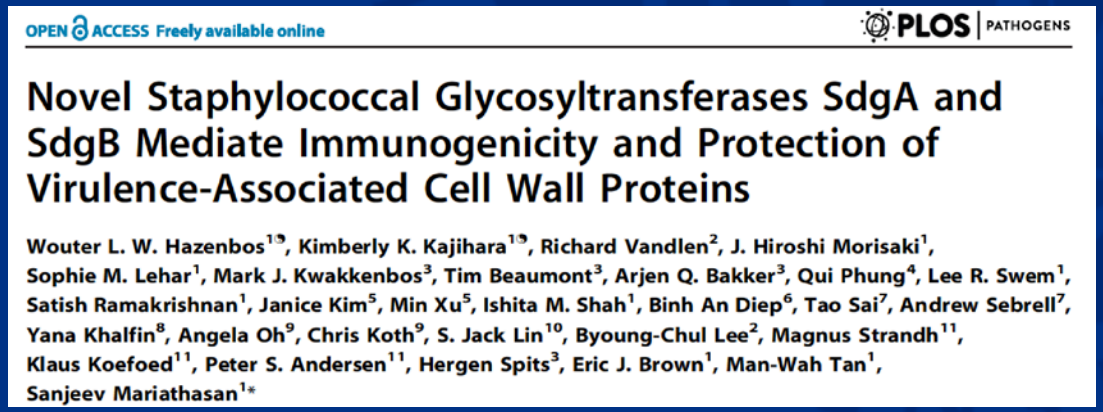
Bacterial glycosylation - the rule rather than the exception

Recently identified bacterial glycosylation systems

Acinetobacter baumannii and Its Role in Virulence and Biofilm Formation. M Feldman PLoS Pathog. 2012 Jun;8

O-linked protein glycosylation in *Vibrio cholerae* and *Burkholderia thailandensis*. Gebhart C Glycobiology. 2012 Jul;22

O-polysaccharide glycosylation in *Aggregatibacter actinomycetemcomitans*. Tang G Infection Immun. 2012 Aug 22



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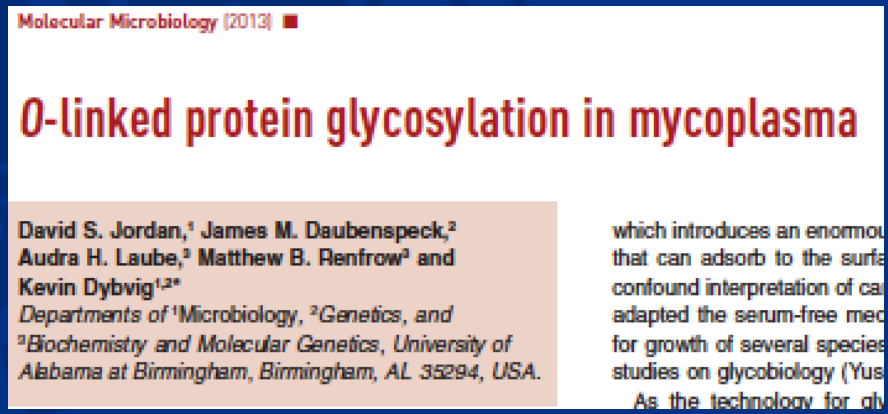
Novel Staphylococcal Glycosyltransferases SdgA and SdgB Mediate Immunogenicity and Protection of Virulence-Associated Cell Wall Proteins

Wouter L. W. Hazenbos[1☯], Kimberly K. Kajihara[1☯], Richard Vandlen[2], J. Hiroshi Morisaki[1], Sophie M. Lehar[1], Mark J. Kwakkenbos[3], Tim Beaumont[3], Arjen Q. Bakker[3], Qui Phung[4], Lee R. Swem[1], Satish Ramakrishnan[1], Janice Kim[5], Min Xu[5], Ishita M. Shah[1], Binh An Diep[6], Tao Sai[7], Andrew Sebrell[7], Yana Khalfin[8], Angela Oh[9], Chris Koth[9], S. Jack Lin[10], Byoung-Chul Lee[2], Magnus Strandh[11], Klaus Koefoed[11], Peter S. Andersen[11], Hergen Spits[3], Eric J. Brown[1], Man-Wah Tan[1], Sanjeev Mariathasan[1*]



Molecular Microbiology (2013) ■

O-linked protein glycosylation in mycoplasma

David S. Jordan,[1] James M. Daubenspeck,[2] Audra H. Laube,[3] Matthew B. Renfrow[3] and Kevin Dybvig[1,2*]

Departments of [1]Microbiology, [2]Genetics, and [3]Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL 35294, USA.

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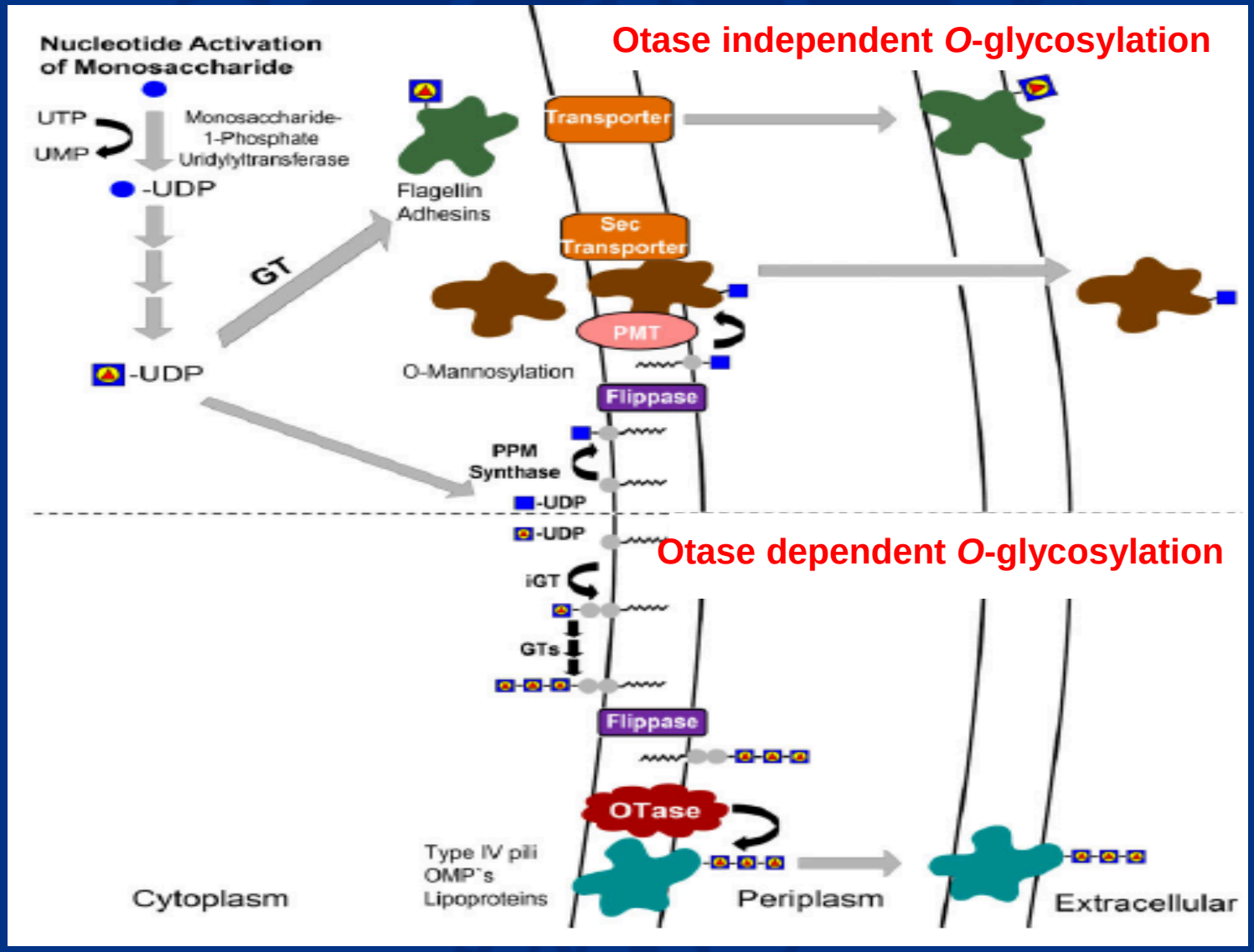
confound interpretation of ca
adapted the serum-free mec
for growth of several species
studies on glycobiology (Yus
As the technology for gly

Towards a classification

O-Otase-dependent and independent glycosylation



Modified from Iwashaki Mol Micro 2014

Current bacterial glycosylation studies on pet pathogens

Clostridium difficile Both flagellin and S-layer glycosylation
Faulds-Pain *et al* Mol Micro 2014

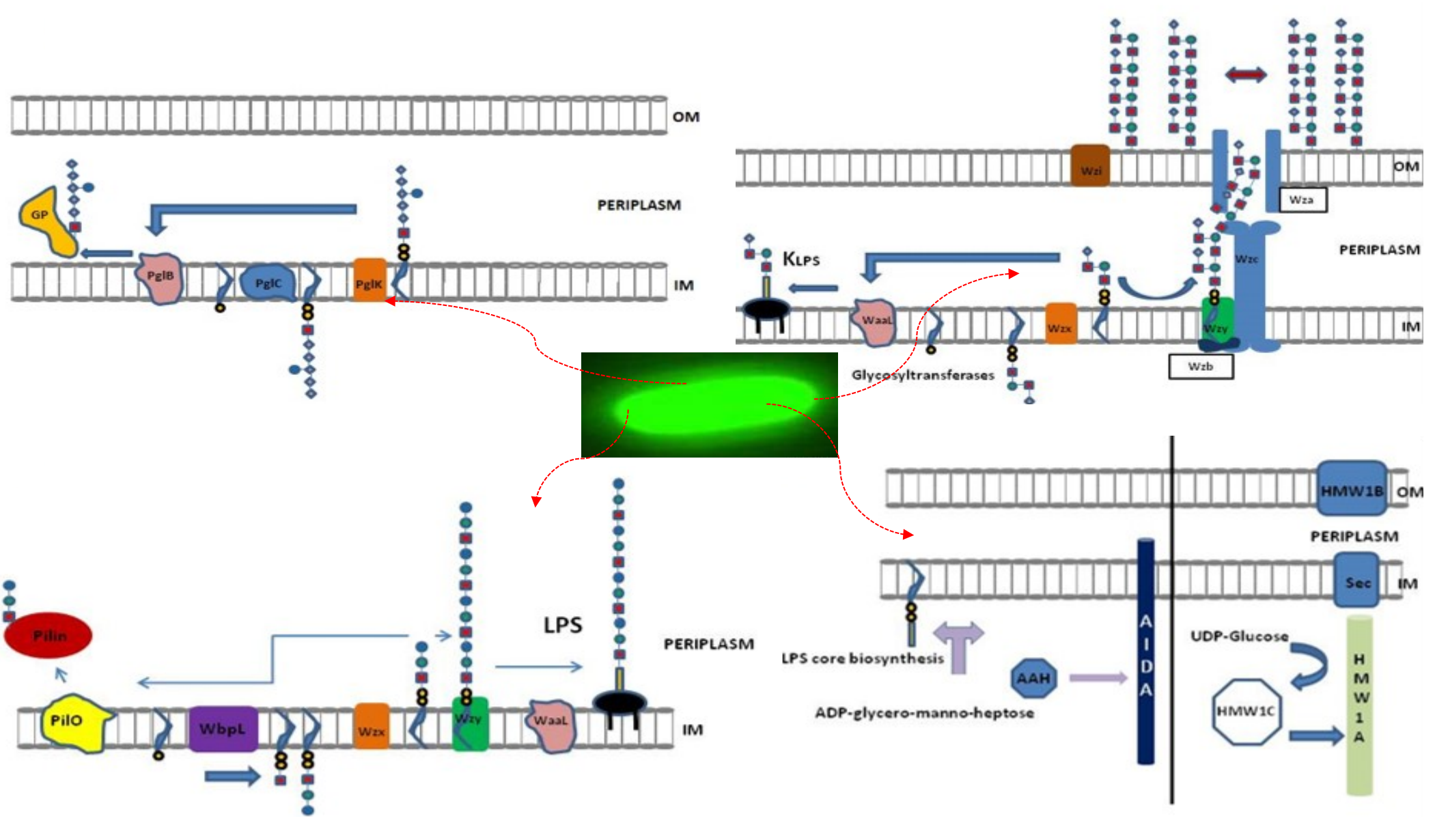
Burkholderia glycosylation x 2

Vibrio cholerae glycosylation

Francisella glycosylation x 2

Actinobacillus glycosylation (new *N*-linked system)
Cuccui *et al* Mol Micro submitted

Bacteria are the best glycoengineers



Conclusions

Basic curiosity driven research can lead to practical applications

- Described the first fully characterised *N*-linked glycosylation system in bacteria
- The development of Protein Glycan Coupling Technology
- Application to novel inexpensive glycoconjugate vaccines
- Potential era for synthetic glycobiology
- Discovery of other bacterial glycosylation systems, role in pathogenesis and potential exploitation

Acknowledgements – LSHTM & PGCT

Current LSHTM

Sherif Aboelhadid
Michelle Cairns
Jon Cuccui
Lisa Dawson
Elizabeth Donahue
Nick Dorrell
Chinelo Ebruke
Abdi Elmi
Alex Faulds-Pain
Alex Shaw
Ozan Gundogdu
Adrian Jervis
Emily Kay
Jiali Lim
Carine Makendi
Melissa Martin
Dominic Mills
Madeleine Moule
Ian Passmore
Richard Stabler
Vanessa Terra
Sam Willcocks
Laura Yates

Past LSHTM

Elaine Allan
Suaad Al-Jaberi
Olivia Champion
Stewart Hinchliffe
Sarah Howard
Andrey Karlyshev
Rebecca Langdon
Dennis Linton
Eric Sampane Donker
Pippa Strong
Gill Thacker
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