



Functional glycan arrays at CFG

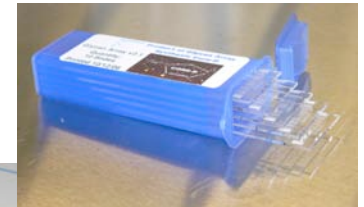
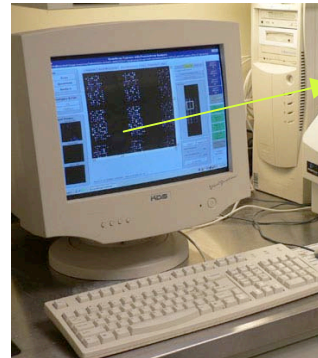
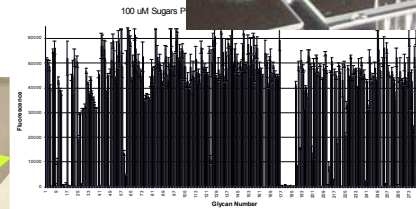
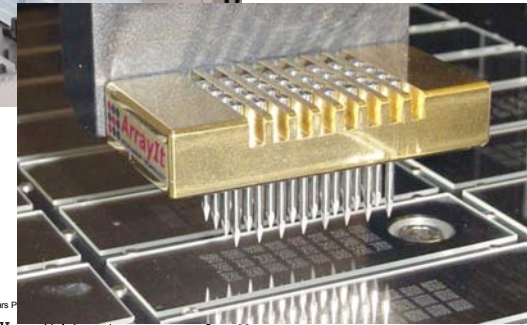
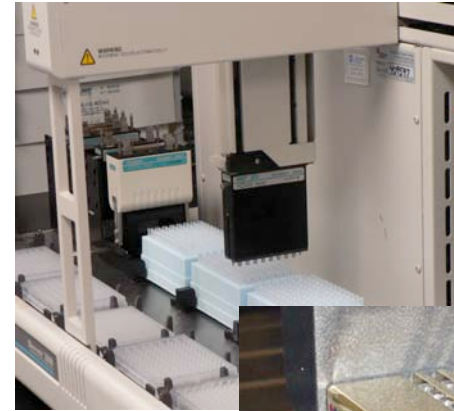
Glycan Array Synthesis Core-D

- I.
 - **Developments of glycan array compound libraries**
 - **Manufacture and expansion of the printed glycan array**
- II.
 - **Development of a pathogen polysaccharide array**
 - **Explore conjugation options for PS conjugates**
 - **In collaboration with Investigators assemble relevant PS**



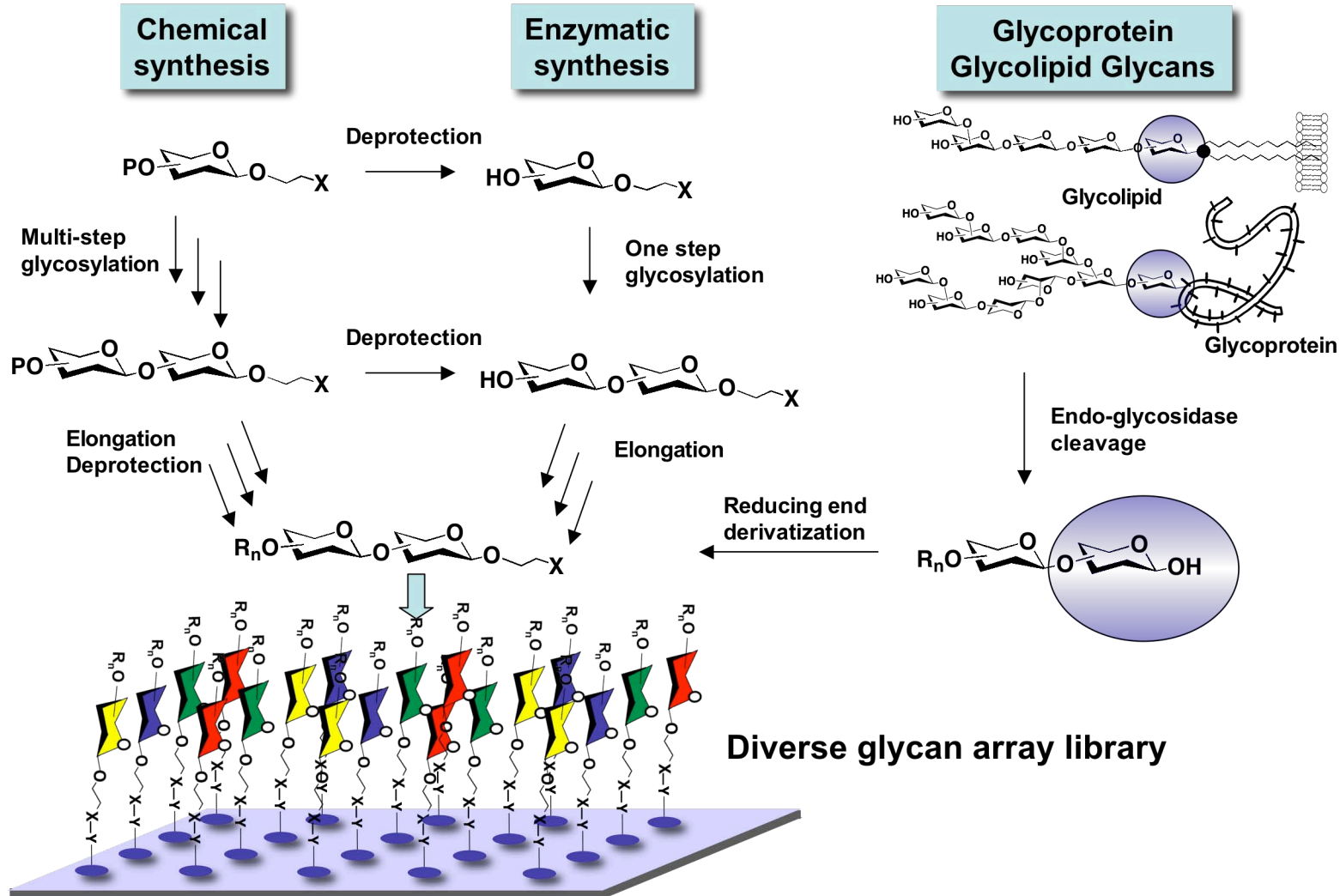
I. The Printed Glycan Array

- Most terminal glycans (>325, v3.0)
- Defined glycans
- Good print capacity
 - Contact printer (800 / month)
 - Pieso-electric (>>>)
- Lectin QC (95% binding)
- Packaging, storage



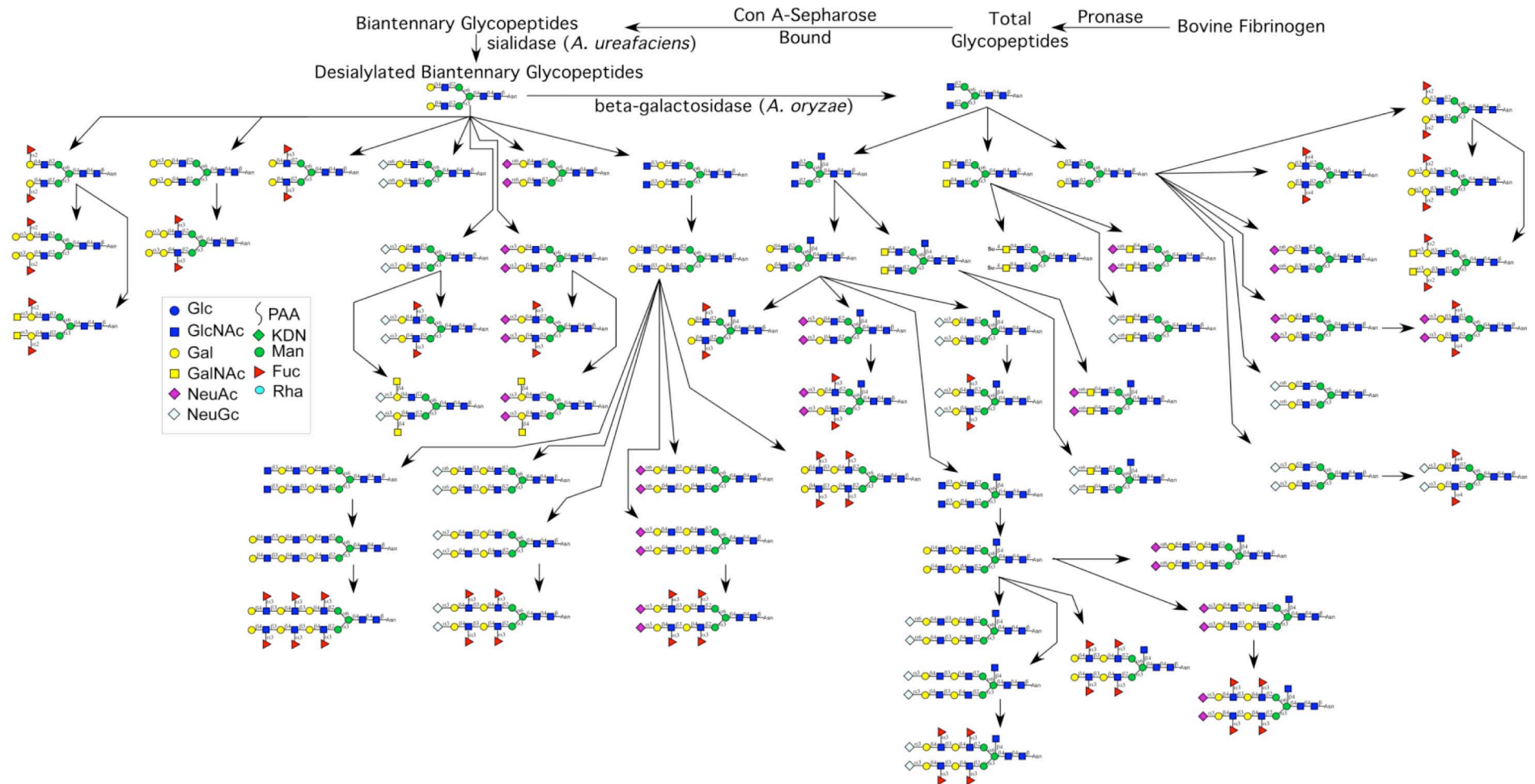


Expansion of array library





Enzymatic modifications of natural N-glycans

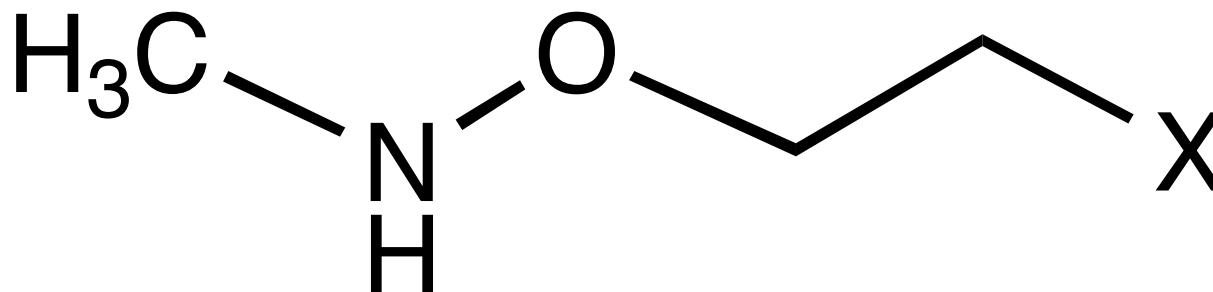


Scheme: a courtesy of R. Cummings



Tagging μg quantities of free glycans

N, O-di-substituted hydroxylamines



Selective anomeric reactivity

Stable (irreversible at neutral conditions)

Ring-closed product

X = NH₂

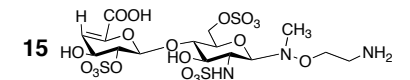
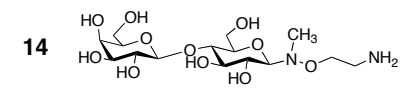
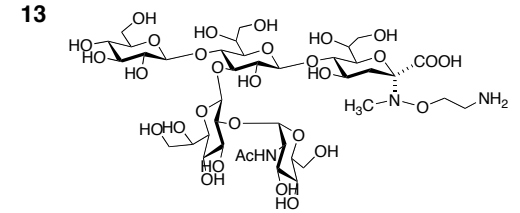
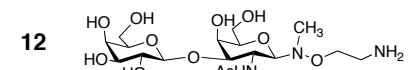
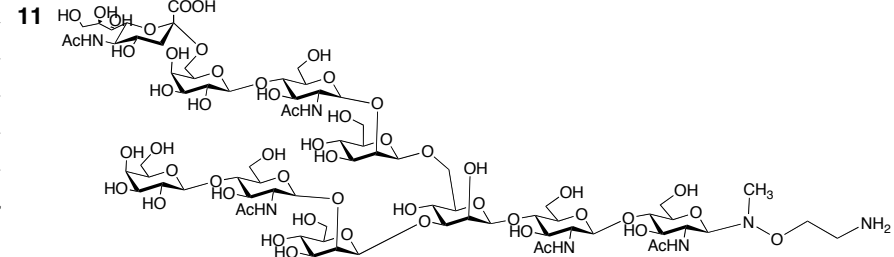
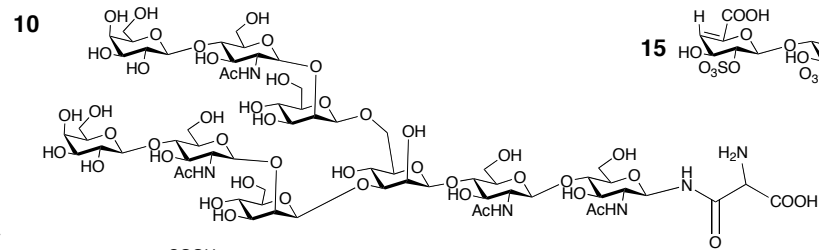
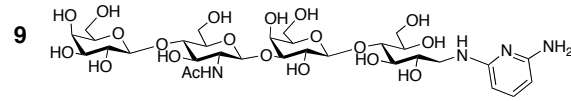
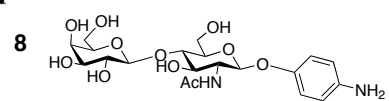
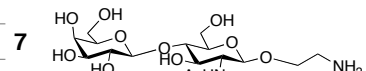
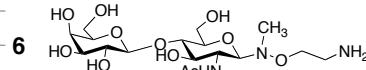
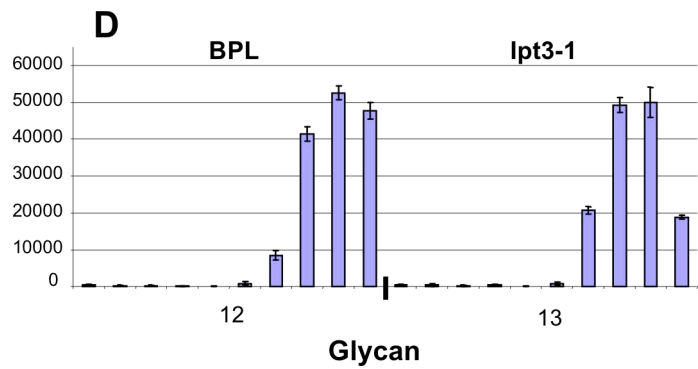
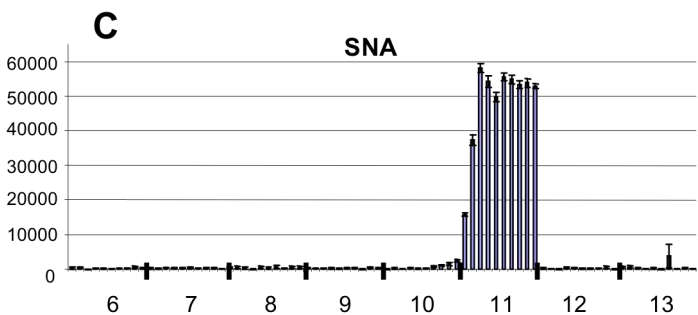
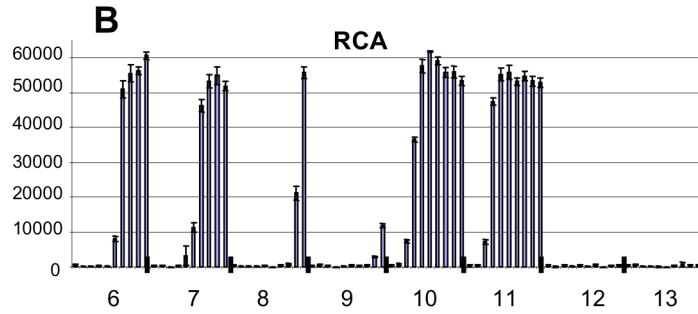
= SH

= Lipid etc...



Test-print of amino-spacers

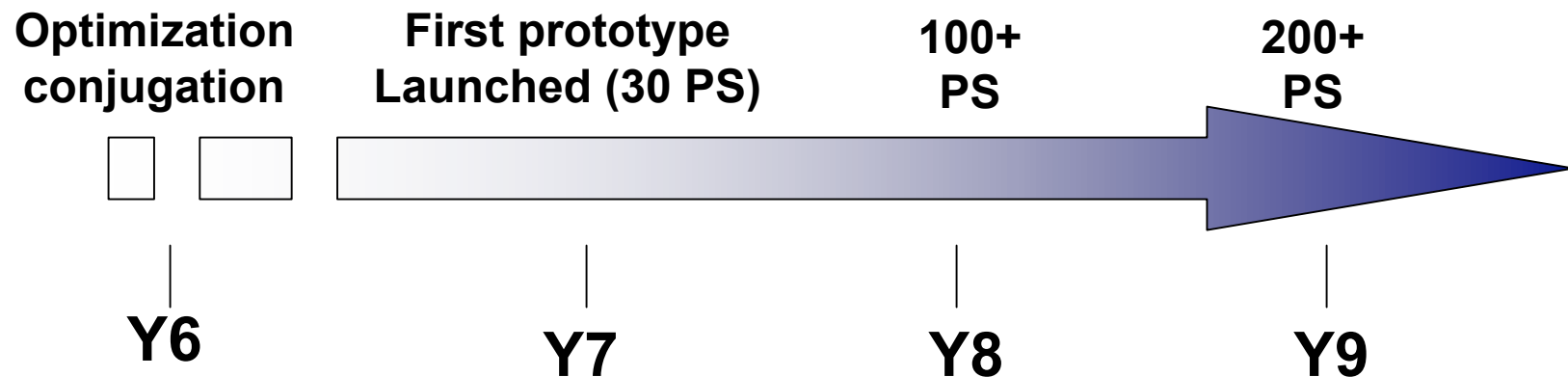
Relative Fluorescence





II. Towards pathogen PS Arrays

- Explore pathogens role in innate immunity
- Assess immune response to natural infections and vaccines





Current selection of PS

- **Opportunistic pathogens**
 - ✓ *Legionella pneumophila* – the cause of the Legionaire’s disease
 - ✓ *Pseudomonas aeruginosa*
- **Enteric bacteria**
 - ✓ *Proteus*
 - ✓ *Providencia*
 - ✓ *Citrobacter*
 - ✓ *Hafnia alvei*
 - ✓ *Escherichia coli* (Category B, CDC)
 - ✓ *Shigella sonnei and flexneri* (Category B, CDC)
 - ✓ *Yokenella regensburgei*
 - ✓ *Salmonella* (Category B, CDC)
- **Vibrios, Campylobacters, Helicobacters**
 - ✓ *Plesiomonas shigelloides* - O54, O74
 - ✓ *C. jejuni*
- **Haemophilus, Bordetella, Brucell, Francisella**
 - ✓ *Bordetella pertussis* - strain 186
 - ✓ *Francisella tularensis* (Category A, CDC)
- **Yersinia, Pasturella**
 - ✓ *Yersinia pestis* (Category A, CDC)
- **Neisseriae**
 - ✓ *N. meningitidis*

Supporting Investigators and Experts:

Dr. Y. Knirel (CFG Bridging project)
Drs. Niedziela and Lugowski (Poland)
Drs Brisson and Richards (Canada)
Dr. T Norberg (Sweden)

Commercial sources



Structural complexity

Oligosaccharide **4** (subgroup O4a,4b)

→ 3)- α -L-Rhap^I-(1 →
 → 3)- β -D-QuipN-(1 →
 → 3)- α -L-FucpN^I-(1 →
 → 3)- α -L-FucpN^{II}-(1 →
 α -L-Rhap^{II}-(1 →

Oligosaccharide **5** (subgroup O11a,11b)

→ 3)- α -L-Rhap^I-(1 →
 → 3)- β -D-FucpN-(1 →
 → 3)- α -L-FucpN-(1 →
 β -D-Glcp-(1 →

Oligosaccharide **6** (subgroup O7a,7b,7d)

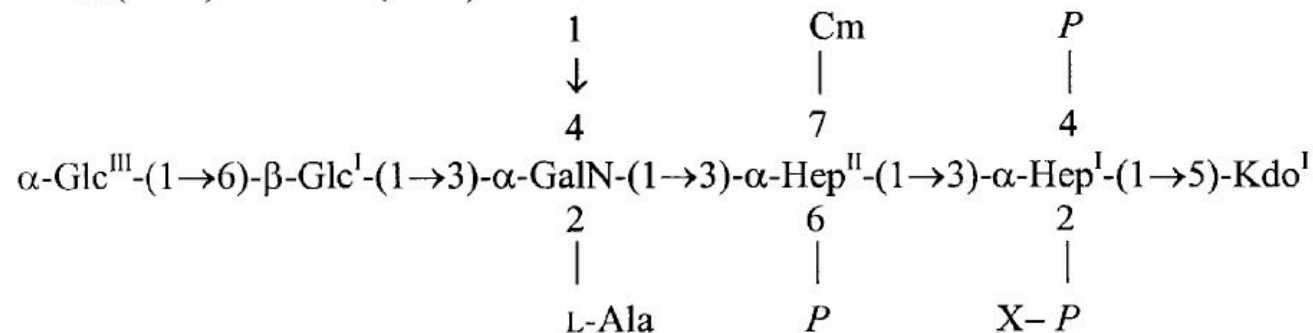
→ 3)- α -L-Rhap^I-(1 →
 → 3)- β -D-FucpN-(1 →
 → 4)- β -D-Xylp-(1 →
 α -Psep-(2 →

Oligosaccharide **7** (subgroup O9a,9d)*

→ 3)- α -L-Rhap^I-(1 →
 → 3)- β -D-QuipN-(1 →
 → 3)- α -D-FucpN-(1 →
 β -Psep-(1 →

Oligosaccharide **13** [subgroup O(2a),2c]

→ 3)- α -L-Rhap^I-(1 →
 β -D-FucpN-(1 →



X = H (**14a** and **15a**) or EtNP (**14b** and **15b**)

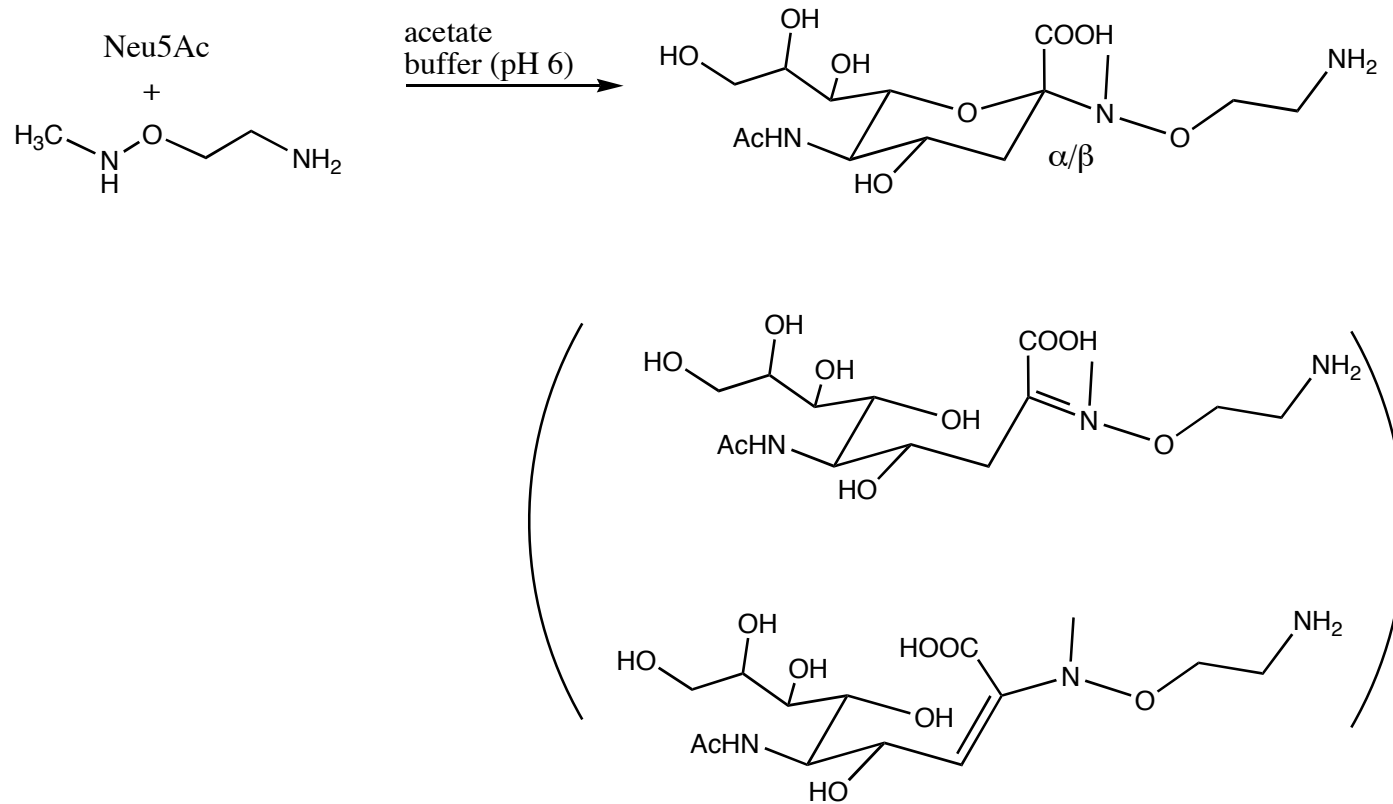
R = H (**14**) or $\alpha\text{-Glc}^{\text{IV}}$ (**15**)

Bystrova OV, Knirel YA, *et al.*

FEMS Immunol Med Microbiol. 2006, **46**, 85-99.



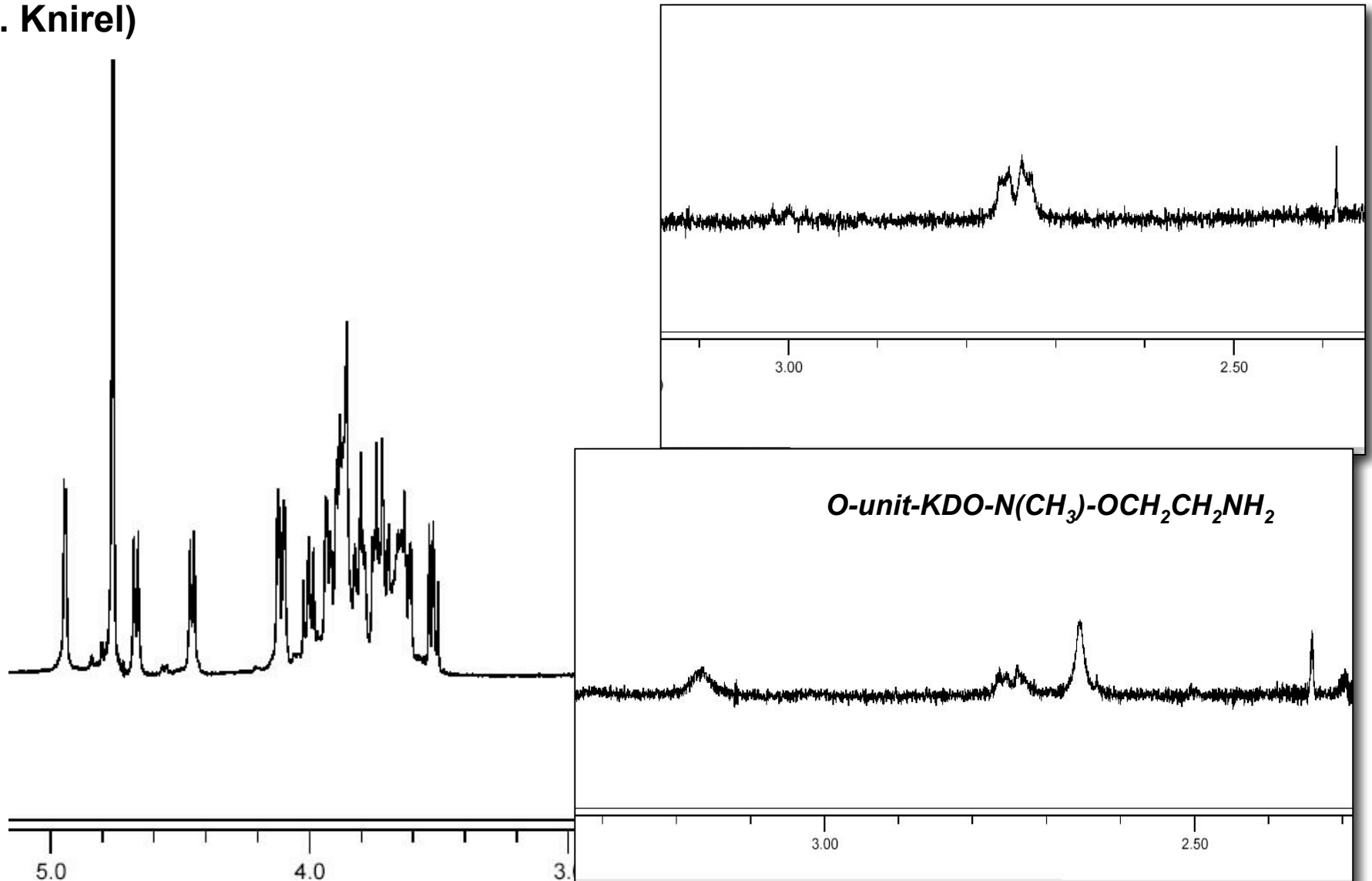
Neu5Ac-conjugate: a model for PS-KDO





PS conjugation

O-antigen *Providencia stuartii* O49 [-6 β -Gal β (1-3)GalNAc β (1-4)Gal α]₂₅-outer Core
(Y. Knirel)





PS conjugates test printed

P. Aerguinosa 09
Un-conjugated

P. Aerguinosa 09
Conjugated

P. Stuartii 049
Un-conjugated

P. Stuartii 049
Conjugated

P. Aerguinosa 04
Un-conjugated

P. Aerguinosa 04
Conjugated

P. Aerguinosa 010
Un-conjugated

P. Aerguinosa 010
Conjugated

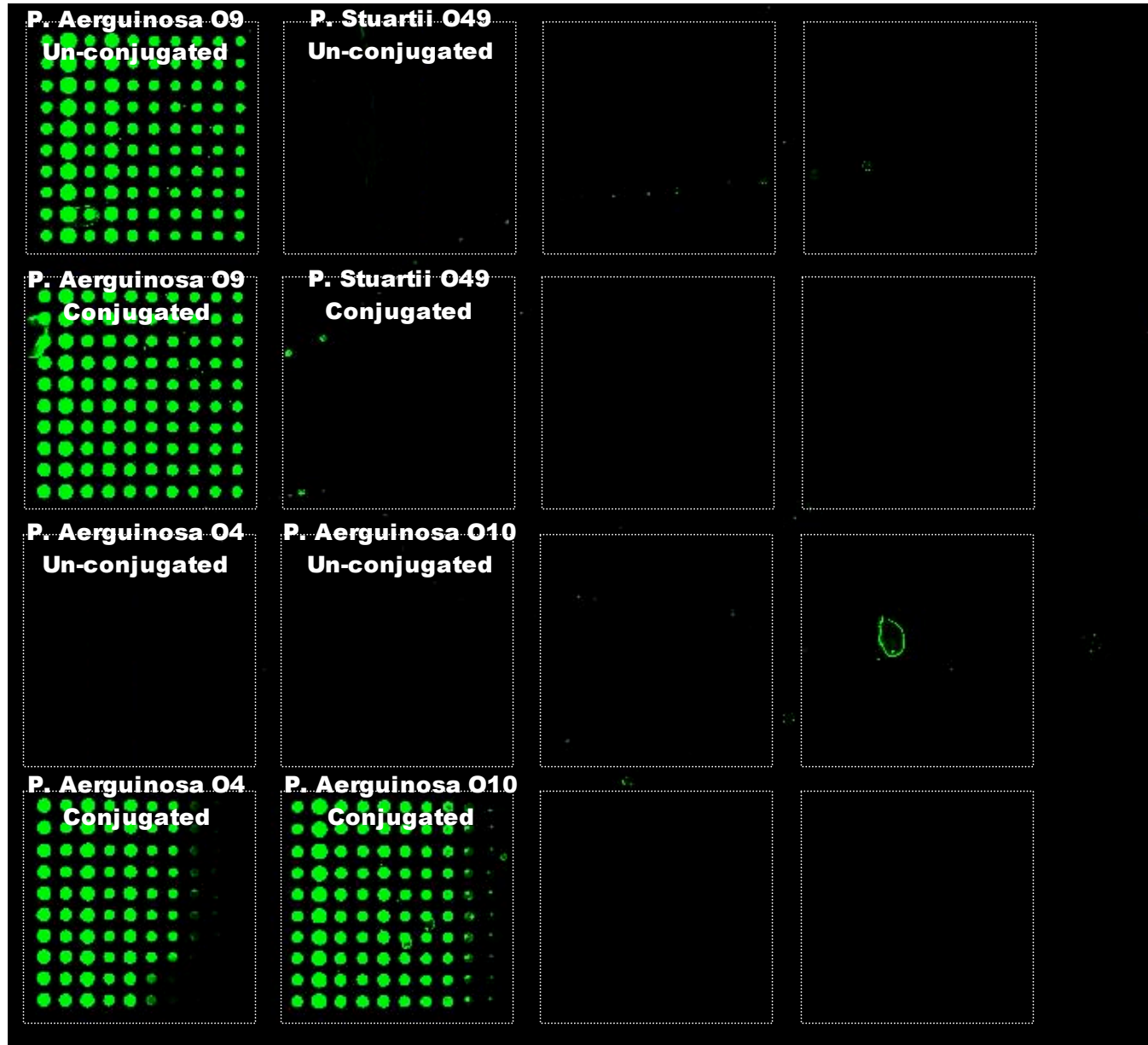
Lectin and antibody detection

Anti P. Aerguinosa 09 mAb

Anti P. Aerguinosa 010 mAb

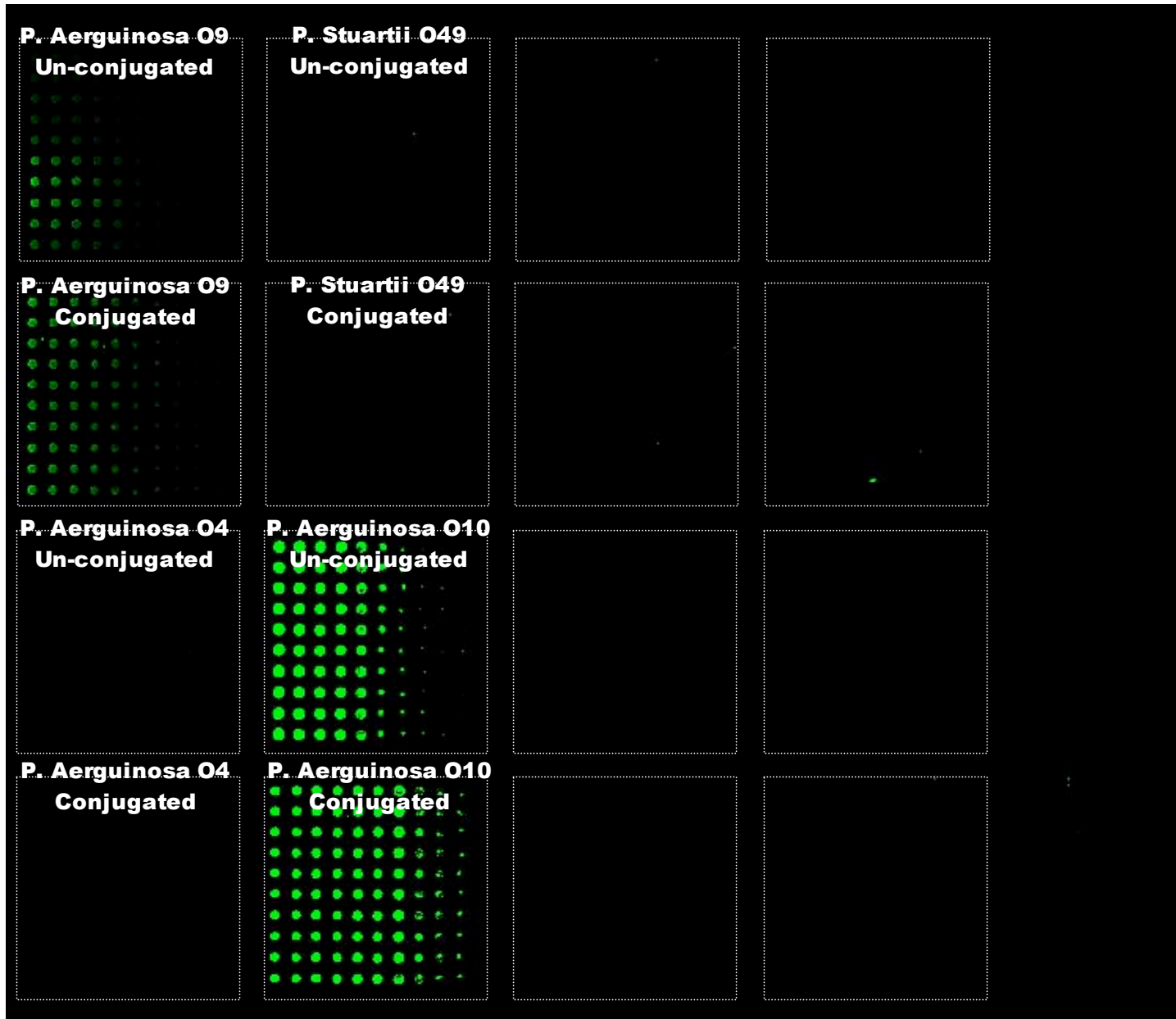


Anti P. Aerguinosa O9 mAb





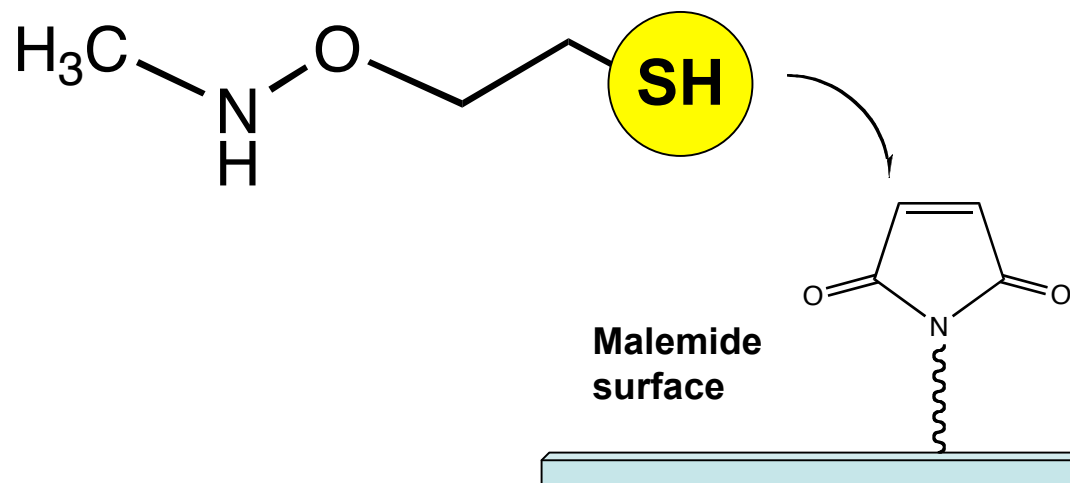
Anti P. Aerguinosa O10 mAb





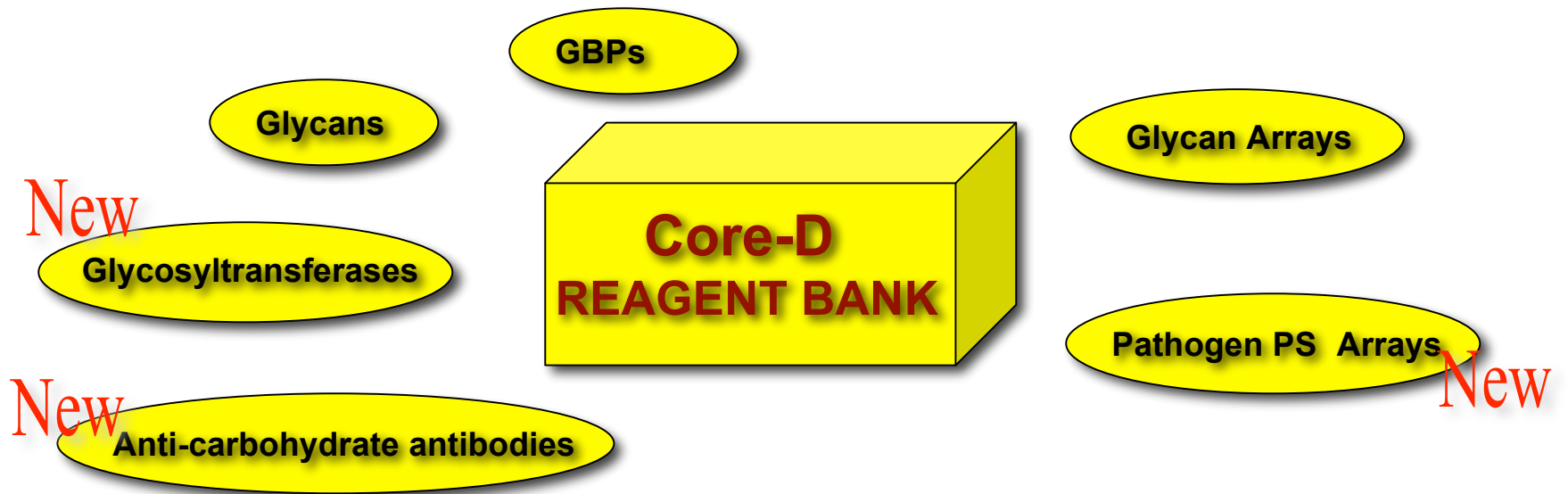
Further developments Y6:

Expand the selection of PS
Continue to evaluate additional PS conjugates
Develop alternative printing chemistries





The Reagent Bank





Acknowledgements

Current Core-D lab members

Dr. N. Razi
Dr. O. Bohorov
J. Hoffmann
K. Allin
O. Berger
H. Andersson-Sand
X. Liu

Dr. J. Paulson
Dr. N. Bovin
Dr. CH. Wong

CFG/TSRI Cores:

Core-H (D. Smith, R. Cummings)
TSRI DNA Array (S. Head)

Participating Investigators and Sponsors:

Enzymes, sugar nucleotides and GBPs

Dr. W. Wakarchuk (NRC, Canada)
Dr. M. Palcic (Carlsberg, Denmark)
Dr. J. Paulson (TSRI, USA)
Dr. P. Qasba (NCI/NIH, USA)
Dr. R. Cummings (Emory, USA)
Dr. G.P. Wang (Ohio State Univ., USA)
Drs. M&M. Fukuda (The Burnham Inst., USA)
Dr. S. Rosen (UCSF, USA)
Dr. A. Varki, (UCSD, USA)
Dr. H. Clausen (Univ. Copenhagen, Denmark)

Kyowa Hakko

Neose technologies Inc.

Bacterial Polysaccharides:

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Drs J.R. Brisson and J. Richards (NRC, Canada)
Drs. T. Niedziela and C. Lugowski (Hirszfield Institute, Poland)
Dr. T. Norberg, (Uppsala University, Sweden)
Dr. S. B. Svenson, (BMC, Sweden)
Dr. A. Bäumler, (UC Davis, USA)