SiteSpecific Conjugation Applied to Glycoconjugate Vaccines

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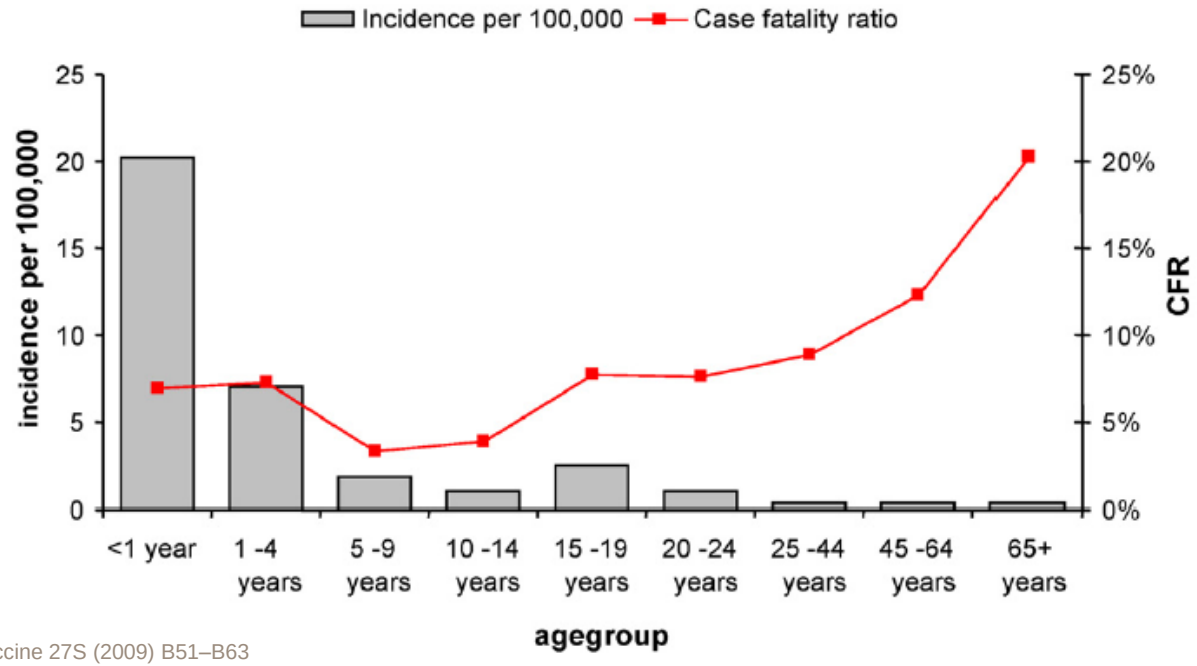
IASOC 28 Sep 2016

Capsular Polysaccharides (CPS) are virulence factors for pathogenic bacteria

- Resistance to non specific host immunity
  - Capsule may confer some resistance to complement mediated killing
  - Sialic acid containing CPS are poor activator of alternative complement pathway
- Resistance to specific host immunity
  - Molecular mimicry: capsules that mimic host structures are poorly immunogenic and provide protection against specific arm of host's immune response
- Adherence
  - Group A streptococcus hyaluronic capsule
- Resistance to dessication
  - CPS are highly hydrated and protect bacteria from dessication during exposure to enviroment

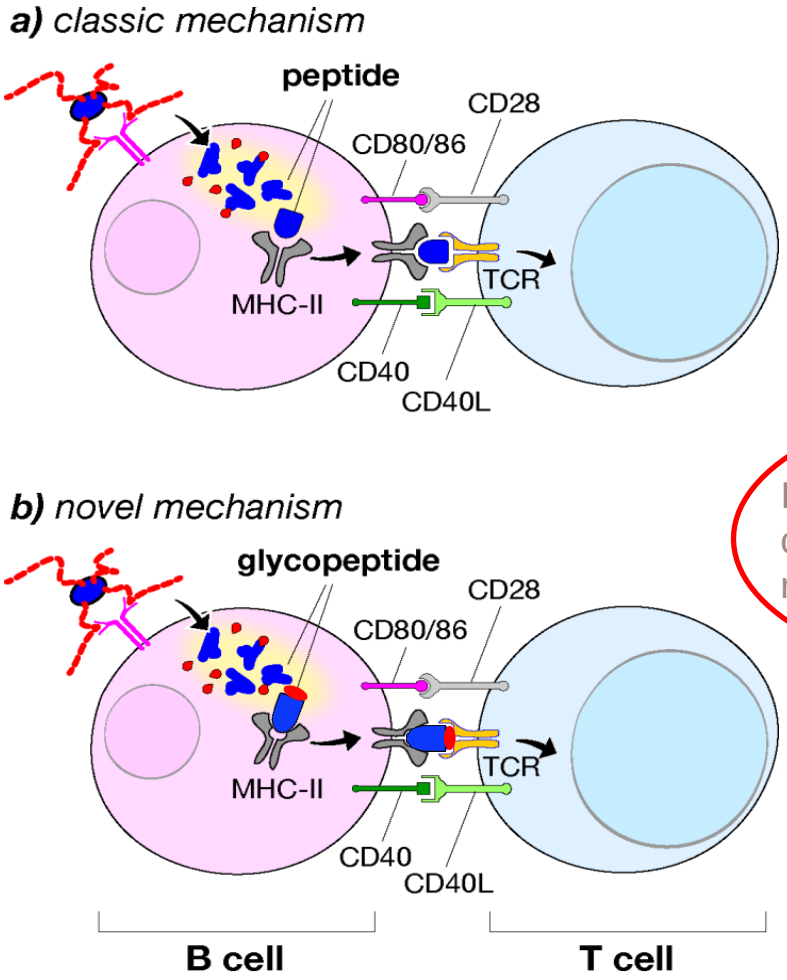
Vaccines based on capsular polysaccharides are protective but suffer of important limitations

- Immunogenic and protective in adults
- T-cell independent immune response
- No immunological memory: no booster effect upon re-vaccination
- No affinity maturation (IgM to IgG)
- No effect in young children (below 18 months)
- Immunological hyporesponsiveness



L.H. Harrison et al . Vaccine 27S (2009) B51–B63

The mechanism of action for glycoconjugate vaccines



Is the amino acid targeted for conjugation to the protein carrier relevant for immunogenicity?

F. Y. Avci, X. Li, M. Tsuji, D. L. Kasper, *Nat. Med.* **2011,** *17* (12), 1602
F. Berti, R. Adamo ACS Chem. Biol. **2013**, *8*, 1653

Glycoconjugate Vaccines Currently Licensed (US or EU or WHO)

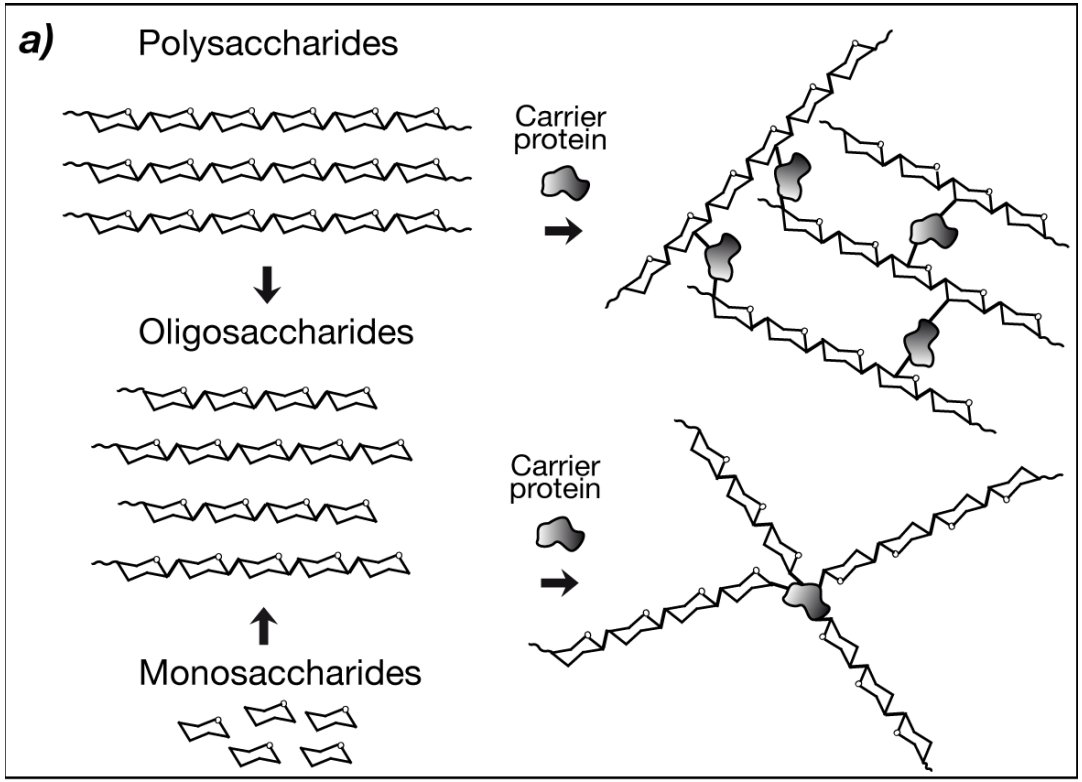
Indication	Type of Conjugate	Manufacturer
Haemophilus influenzae type b	PRP-TT PRP-OMPC PRP-CRM	Sanofi/Pasteur; GSK Merck Pfizer; Novartis/GSK
Neisseria meningitidis group C	MenC-CRM MenC-TT	Pfizer, Novartis/GSK Baxter
Neisseria meningitidis group A	MenA-TT	Serum Institute India
Haemophilus influenzae type b/*Neisseria meningitidis* group C	MenC/Hib-TT	GSK
Neisseria menigitidis A,C,W135,Y	MenACWY-DT MenACWY-CRM MenACWY-TT	Sanofi Pasteur Novartis/GSK GSK
Streptococcus pneumoniae 4,6B,9V,14,18C,19F, 23F	7 valent-CRM	Pfizer
Streptococcus pneumoniae 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F	10 valent-DT/TTProteinD	GSK
Streptococcus pneumoniae 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F.	13 valent-CRM	Pfizer

TT= tetanus toxoid; DT= Diphtheria Toxoid OMPC= MenB Outer membrane protein complex; CRM= non toxic mutant of Diphtheria toxin; Protein D : lipoprotein from Haemophilus influenzae type b

Examples of glycoconjugate vaccines under development

Indication	Type of conjugate	Stage
Streptococcus Group B	Glycoconjugates of type Ia, Ib, II, III and V	Clinical
Streptococcus pneumoniae	Glycoconjugates of type 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F conjugated to CRM197	Clinical
Staphylococcus aureus	Glycoconjugates of type 5 and 8 CPS PNAG glycoconjugates Bio-conjugates	Clinical and preclinical
C. difficile	Glycoconjugates of surface polysaccharide	Preclinical
Salmonella typhi (Vi)	rEPA-Vi conjugate; CRM-Vi conjugate	Clinical
Shigella species	Various O-antigen-protein conjugates	Preclinical and Clinical
Group A Streptococcus(GAS)	GASCHO-TT; GASCHO-CRM	Preclinical
Neisseria meningitidis group B	coreLOS-Protein	Preclinical
Candida albicans	Synthetic Oligomannosyl conjugates β-glucan-CRM197 conjugates	Preclinical
Shigella Dysenteriae	Bio-conjugate	Clinical
Breast cancer	Various synthetic carbohydrate antigens Conjugated to carrier proteins or peptides or organic adjuvants	Preclinical and clinical stages
Epithelial cancer		
Prostate cancer		

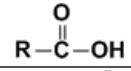
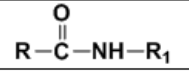
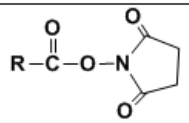
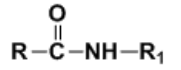
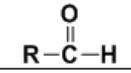
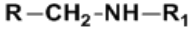
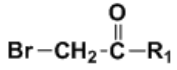
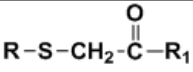
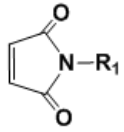
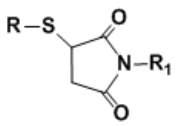
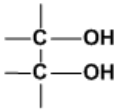
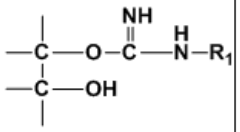
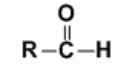
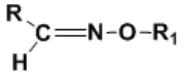
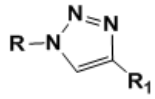
Different ways to make glycoconjugates



Adapted from Costantino et al. Expert Opin. Drug Discov. (2011) 6(10):1045-1066

A variety of chemistries can be used to make conjugate vaccines

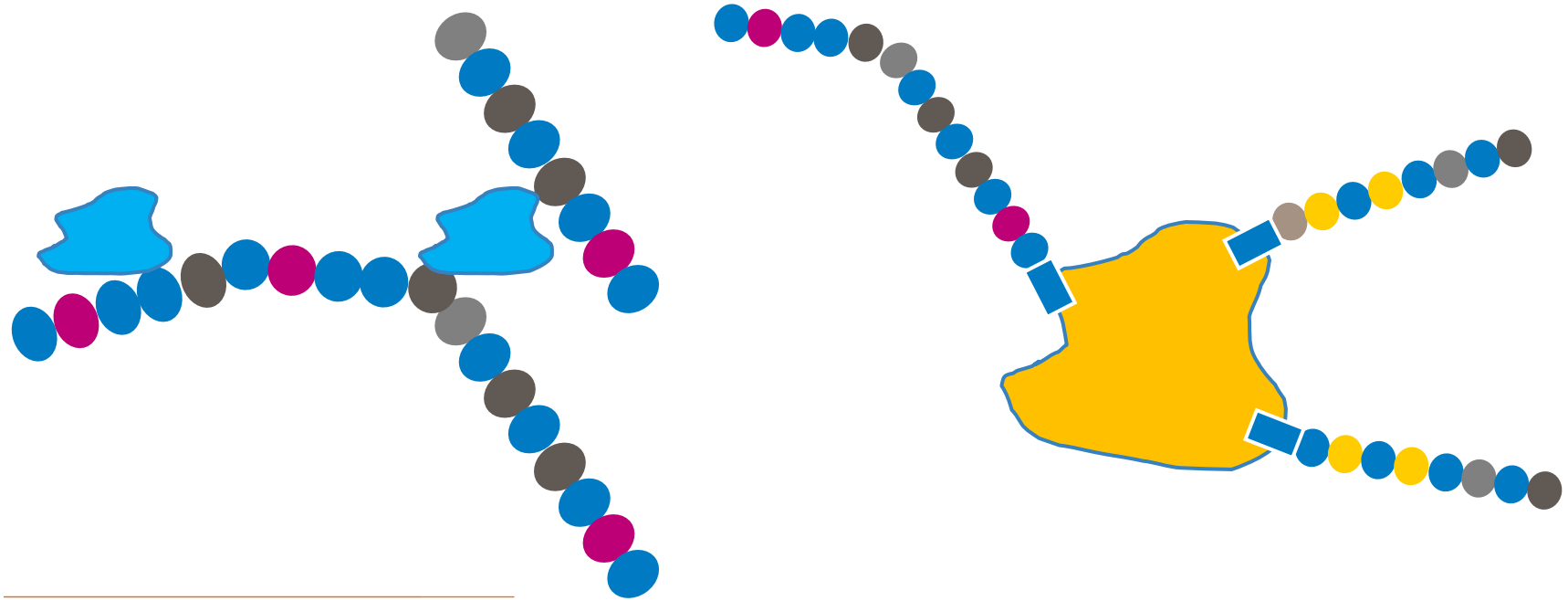
Costantino et al. Expert Opin. Drug Discov. (2011) 6(10):1045-1066

Chemistry	Functional groups involved		Product
Carbodiimide mediated condensation		H_2N-R_1	
Active ester		H_2N-R_1	
Reductive amination		H_2N-R_1	
Thioalkylation			
Thiol addition	$R-SH$		
Disulfide formation		$HS-R_1$	$R-S-S-R_1$
Thiol-ene	$R-SH$	$CH_2=CH-R_1$	$R-S-CH_2-CH_2-R_1$
Cyanogen Bromide or CDAP Activation		H_2N-R_1	
Oxime formation		H_2N-O-R_1	
"Click Chemistry"	$R-N=N^+=N^-$	$HC\equiv C-R_1$	

Many Elements May Impact the Product Profile of a Conjugate Vaccine

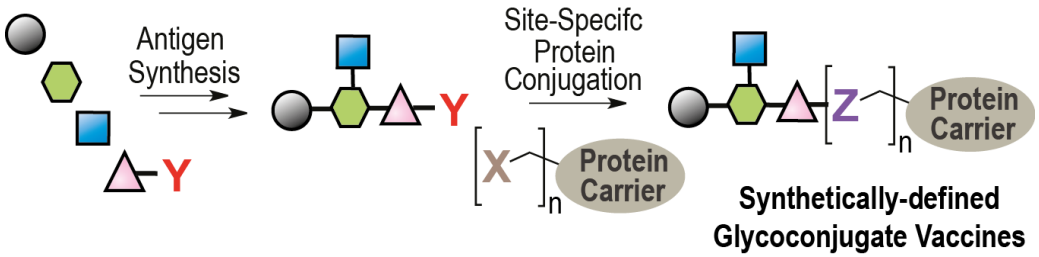
- Conjugation Chemistry
- Length of the saccharide chain
- Ratio carbohydrate/protein
- The nature of the spacer
- The nature of the carrier protein

Different Models of Conjugation Chemistry



A route for well-defined glycoconjugates

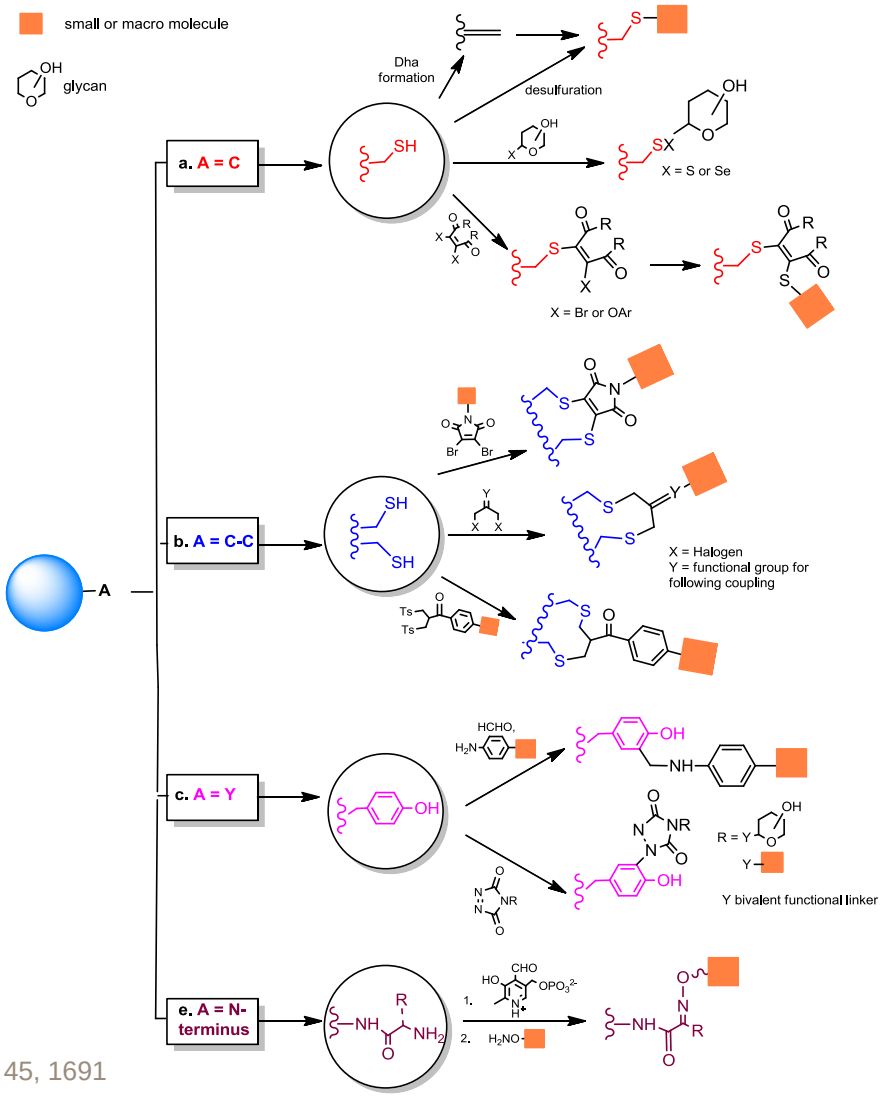
- Protein conjugation has great importance to biotherapies and chemical biology
- Glycoconjugate vaccines allow preventing a number of infectious diseases
- Well-defined glycoconjugates may represent an important tool to investigate the immunological properties due to different epitope density and presentation, and the mechanism of action of this class of vaccines.



R. Adamo, A. Nilo, B. Castagner, O. Boutureira, F. Berti, G. J. L. Bernardes *Chem. Sci*., **2013**, *4*, 2995

Overview of methods enabling site selective modifications

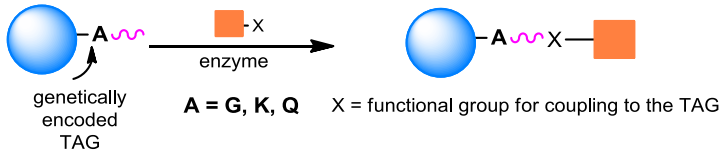
- Chemical modifications



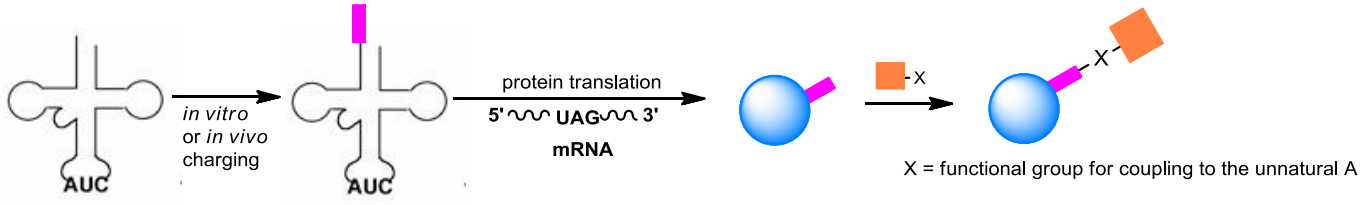
Q.-Y. Hu, F. Berti, R. Adamo *Chem. Soc. Rev.* **2016,** 45, 1691

Overview of methods enabling site selective modifications

- **Enzymatic modifications**

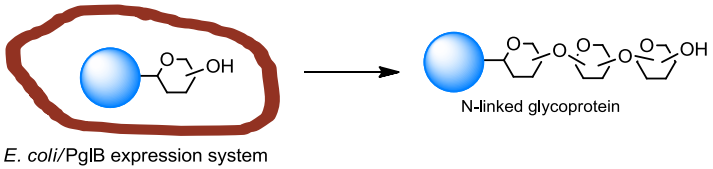


- **Incorporation of unnatural amino acids**

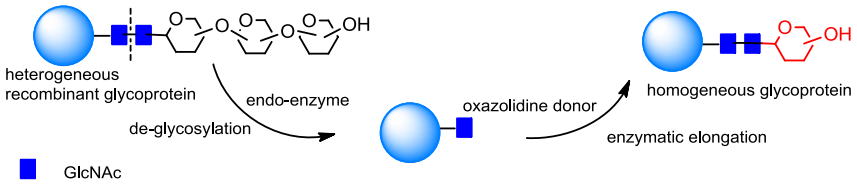


- **Glycoengineering**

a. protein glycan coupling technology



b. glycan remodelling

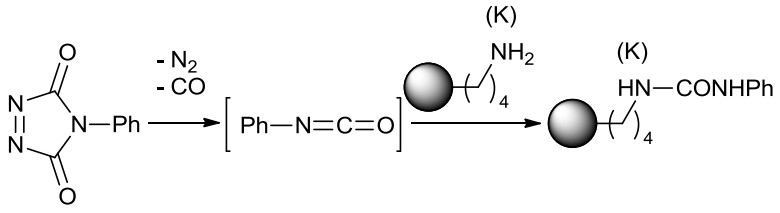


Tyrosine selective conjugation method

- 4-substituted-1,2,4-triazoline-3,5-diones have recently been proposed as highly reactive conjugation reagents for selective tyrosine labeling

S. D. Tilley, M. B. Francis, *J. Am. Chem. Soc.* **2006**, *128*, 1080-1081

- We found that this reagent underwent a rapid spontaneous decomposition, and the reaction gave the compound derived from urea formation at lysines as major product



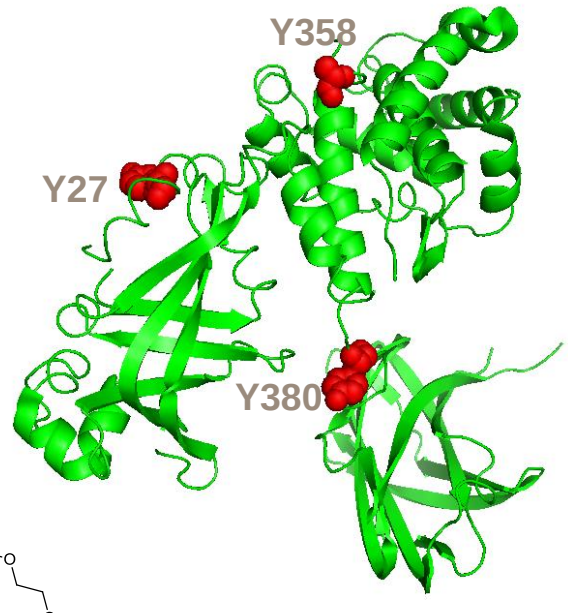
PTAD

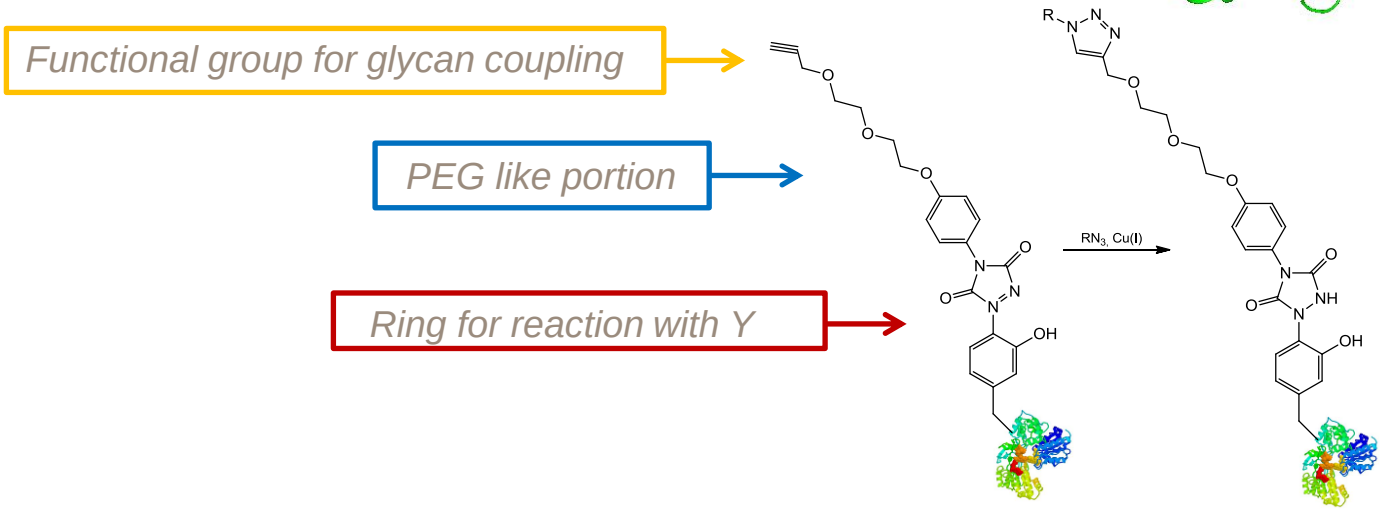
- A protocol was optimized to give the desired product at tyrosine in high yield without detectable urea formation

  - ❑ Increased PTAD stability in acetonitrile
  - ❑ Tris(hydroxymethyl)amino-methane (Tris) was selected to function as both the isocyanate scavenger and buffer

A protocol for selective modification of Y27, 46, 358 and 380 on protein carrier CRM_{197}

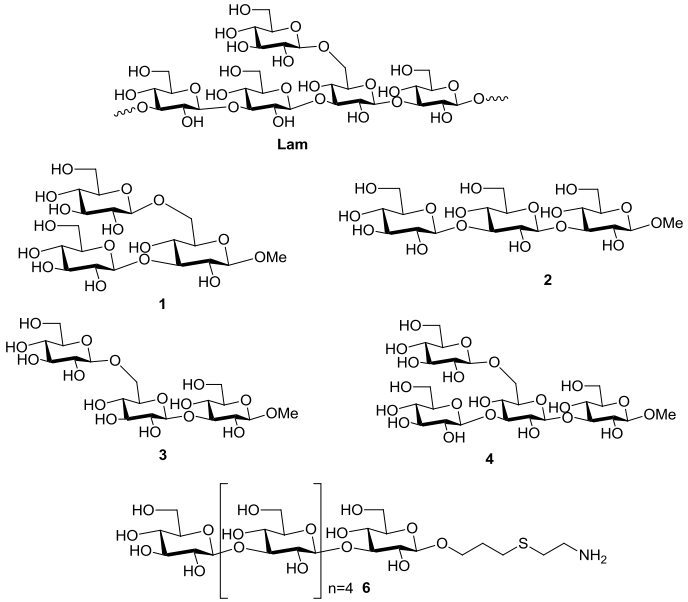
- ✓ Good homogeneity as determined by LC-MS (ESI) with an average labeling ratio of 3.8 alkyne functionalities
- ✓ Four major modification sites at **Y27**, **46**, **358** and **380** out of total 18 tyrosines determined by MS/MS analysis of proteolytic digests
- ✓ Good agreement with solvent accessibility calculations by ICM software package



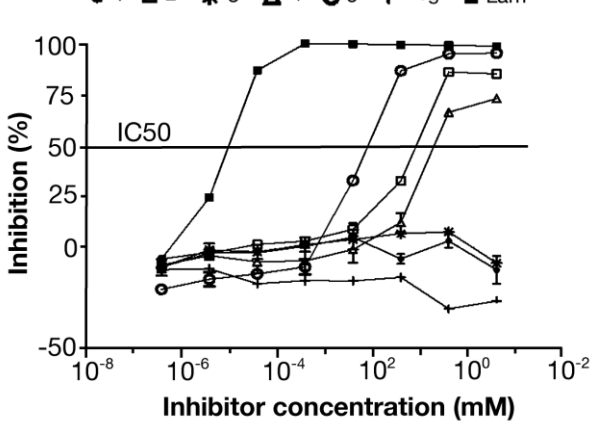


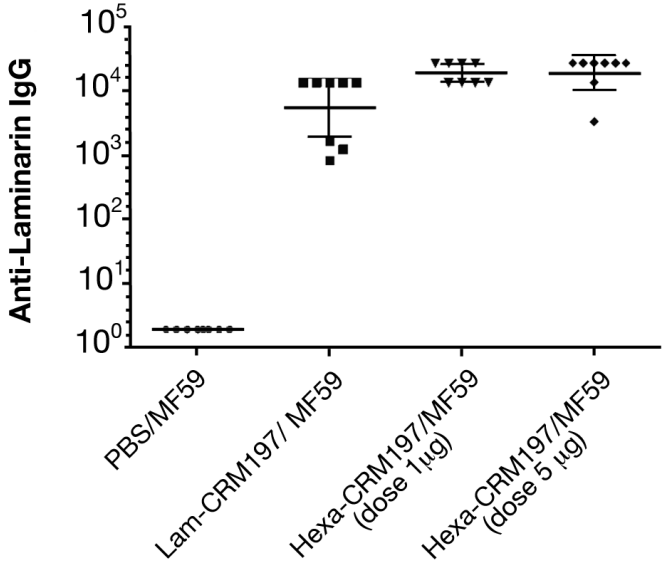
Q.-Y Hu, M. Allan, R. Adamo, D. Quinn, H. Zhai, G. Wu, K. Clark, J. Zhou, S. Ortiz, B. Wang, E. Danieli, S. Crotti, M. Tontini, G. Brogioni, F. Berti. *Chem. Sci.* **2013**, *4*, 3827

A linear β-1,3 glucan hexasaccharide has been identified as anti-Candida vaccine candidate



Inhibitors	2	4	6	Laminarin
IC50	$1x10^{-1}$	$6x10^{-2}$	$5x10^{-3}$	$7x10^{-6}$





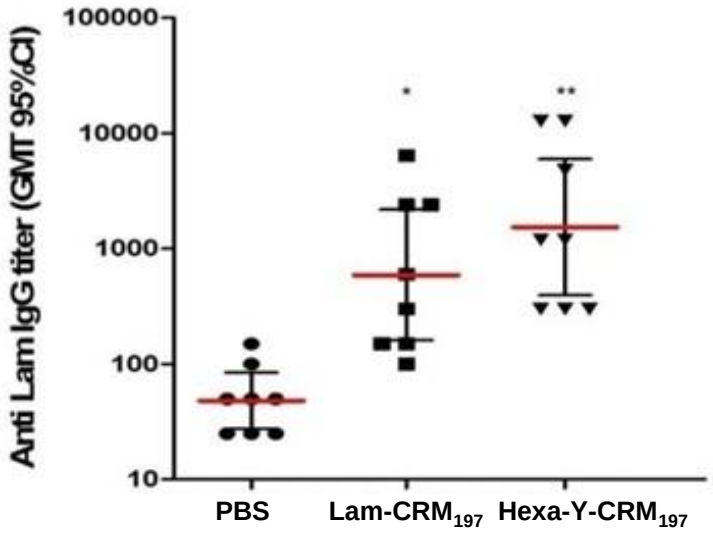
- Laminarin-CRM_{197} is glycoconjugate vaccine against *Candida albicans* ready to move to clinical phase I

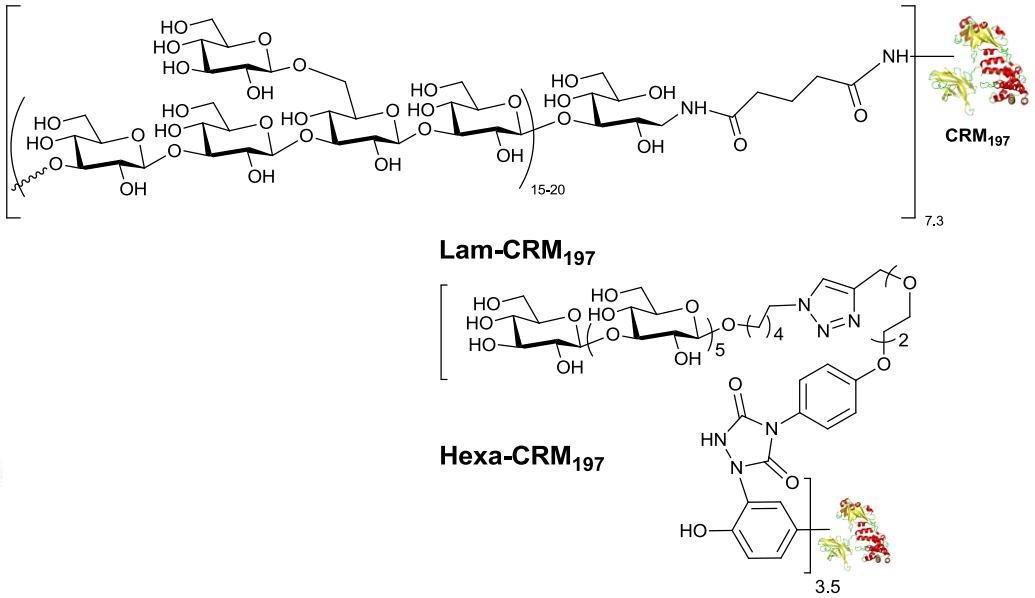
  A. Torosantucci et al. *J. Exp. Med.* **2005**, *202*, 597

- β-1,6 linkages are not necessary to elicit protective antibodies

  C. Bromuro et al. *Vaccine*, **2010**, *28*, 2615
  R. Adamo et al. *J. Carbohydr. Chem.* **2011,** *30,* 249.

Immunological evaluation in comparison to Laminarin-CRM_{197} conjugate

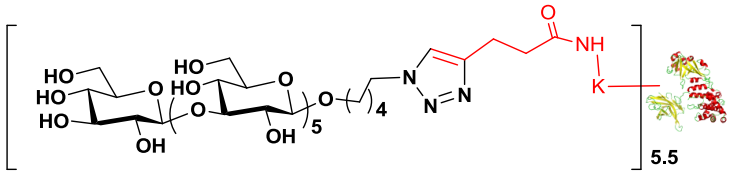




- Immunization of 8 groups of CD1 mice at 2 µg carbohydrate dose
- The anti-Candida Laminarin-CRM_{197} conjugate, with average glycosylation degree of 7.3 oligosaccharides, was used as the positive control
- Antigens formulated with MF59
- Sera were analysed by ELISA for their content of anti-Laminarin IgG
- Statistically comparable IgG titers against Laminarin, but lower glycosylation degree

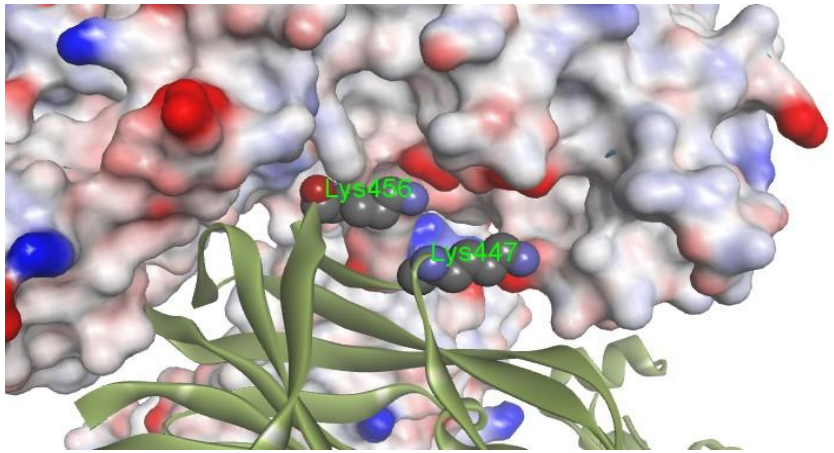
Localization of the more exposed lysines on CRM_{197}

Controlled insertion of 6 NHS linkers



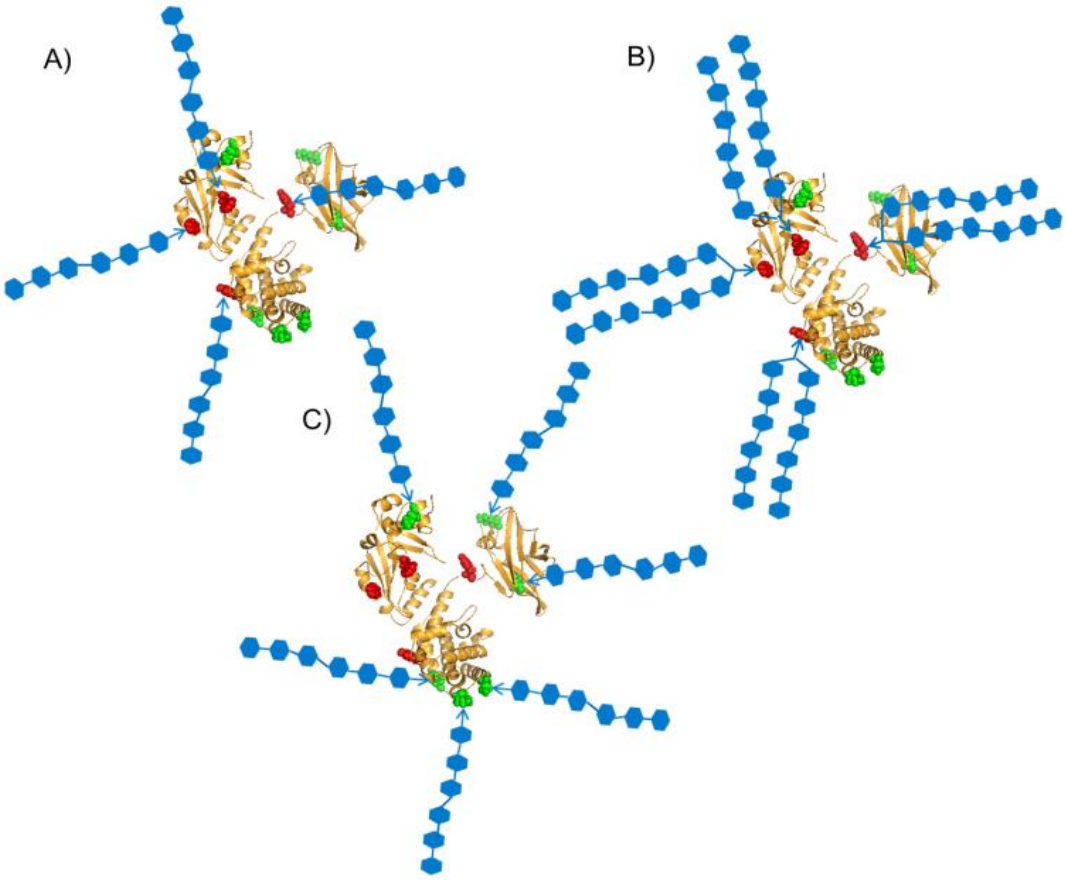
CRM_{197} was modified with 6 linkers onto K

- By Trypsin digestion, GluC digestion and AspN/GluC double digestion 31 out of the 39 available lysine residues were labelled with the linker in addition to the N-terminus glycine
- The more exposed lysines were identified: **K103, K221, K242, K236, K498, K526**
- Water accessibility of lysine residues showed an excellent correlation with the modification level.
- The homodimer interface functions as a non-covalent self-protecting group. For instance, K456 is highly exposed but is buried in the interface of the homodimer explaining why modification of this residue was found to be low.



S. Crotti, H. Zhai, J. Zhou, M. Allan, D. Proietti, Q.-Y. Hu, W. Pansegrau, F. Berti, R. Adamo, *ChemBioChem* **2014**, *15*, 836

Creation of defined patterns of conjugation sites



Patterns of defined sites for conjugation of synthetic glycans.
Y27, **Y46**, **Y358** and **Y380** are red-highlighted;

K103, **K221**, **K236**, **K242**, **K498**, K526 are green-highlighted.

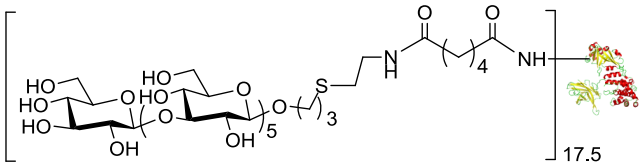
Three different types of constructs were designed:

A. selective attachment of oligomers
B. bivalent clusters of oligomers on tyrosine residues
C. attachment of oligomers on the more surface accessible lysine residues.

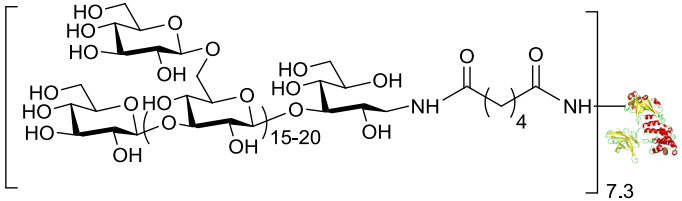
R. Adamo, Q.-Y. Hu, A. Torosantucci, S. Crotti, G. Brogioni, M. Allan, P. Chiani, C. Bromuro, D. Quinn, M. Tontini, F. Berti. *Chem. Sci.* **2014**, *5*, 4302

Set of glycoconjugates to evaluate the effect of carbohydrate density/conjugation site

Random glycoconjugates

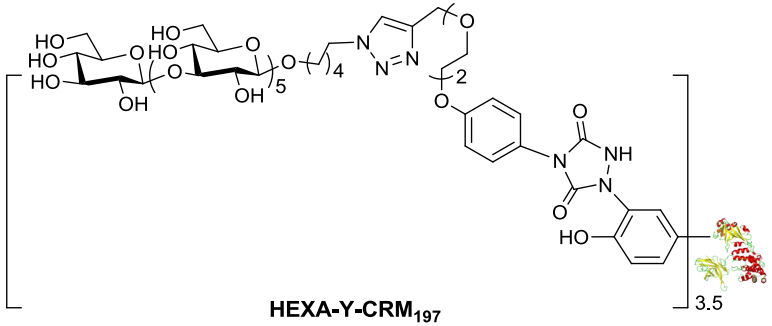


Lam-CRM$_{197}$

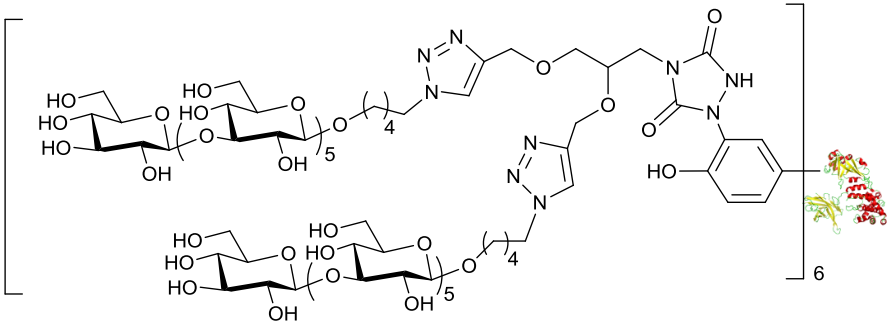


HEXAsidea-CRM$_{197}$

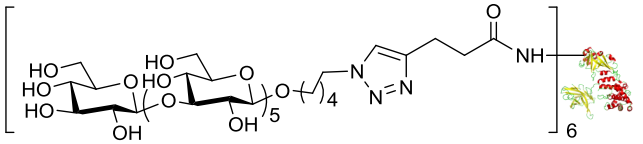
Defined glycoconjugates



HEXA-Y-CRM$_{197}$



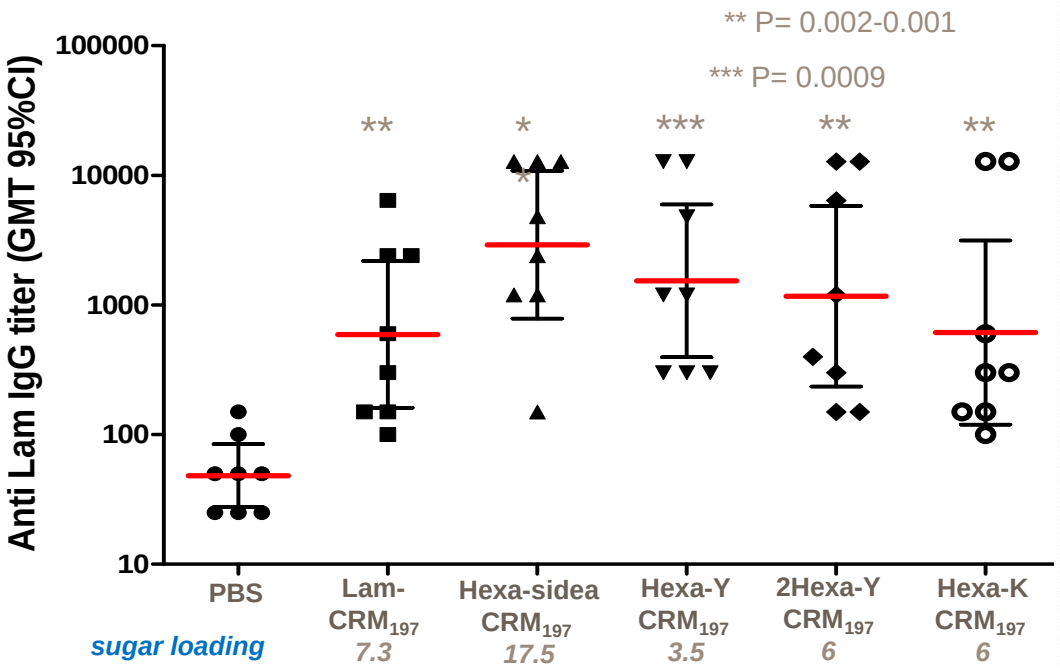
2HEXA-Y-CRM$_{197}$

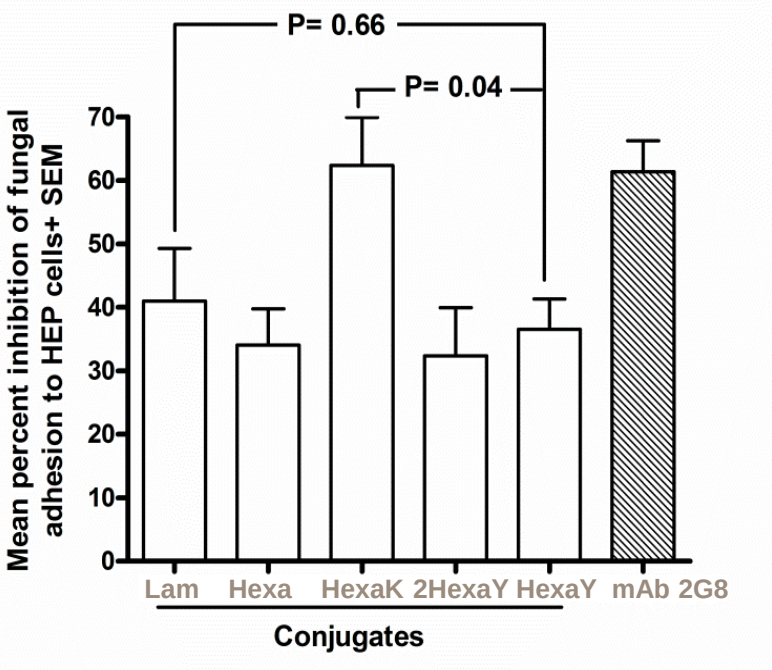


HEXA-K-CRM$_{197}$

Immunological evaluation of synthetic β-glucans conjugates in comparison to Laminarin-CRM_{197} conjugate

Anti Laminarin IgG - Sera post 3rd immunization





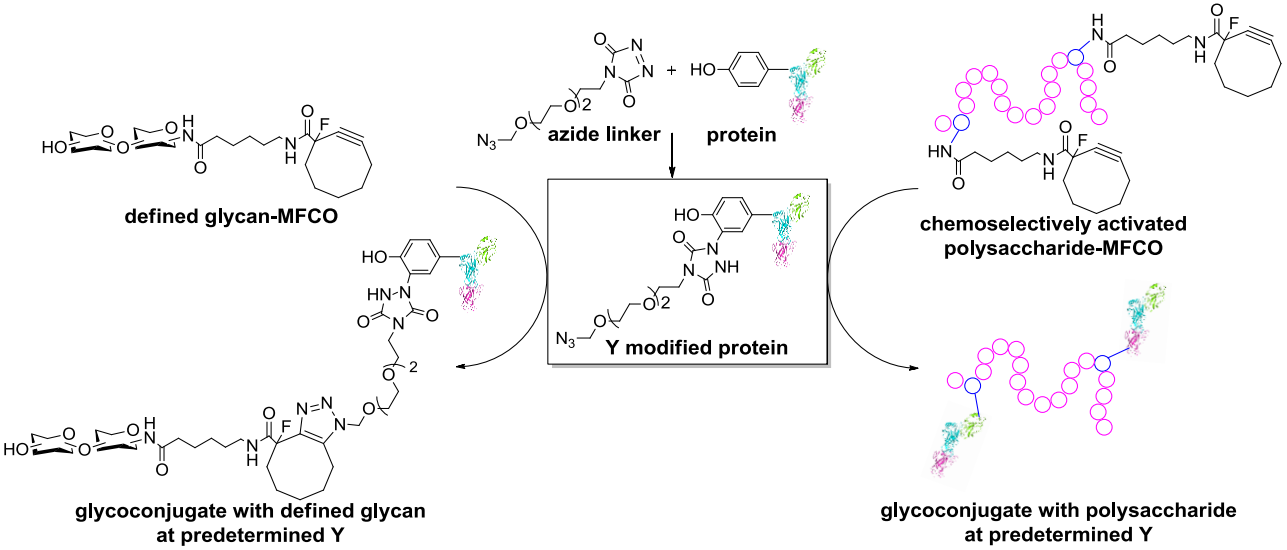
- Immunization of 8 groups of CD1 mice at 2 µg carbohydrate dose, MF59 as adjuvant
- Sera were analysed by ELISA for their content of anti-laminarin IgG
- Statistically comparable IgG titers to laminarin, but lower glycosylation degree and defined attachment point
- Anti linker antibodies, particularly directed to the phenyl ring of spacer used for Y-ligation, were revealed. No effect on the anti-carbohydrate response

Development of GBS vaccines based on capsular polysaccharide and pilus proteins

- Group B *Streptococcus* (GBS) is a leading cause of severe bacterial infections in first 3 months of life
- GBS is also an important cause of morbidity and mortality among non-pregnant adults, particularly the elderly with underlying medical conditions
- Most of GBS strains possess a capsular polysaccharide (CPS) on their surface which is a major virulence factor
- 10 different CPS serotypes have been characterized (Ia, Ib, II, III, IV, V, VI, VII, VIII, and IX), of which 5 (Ia, Ib, II, III, V) are responsible for the vast majority of the disease in North America and Europe
- NVD has a trivalent carbohydrate-based vaccine against serotypes Ia, Ib and III in phase II clinical trials
- A pentavalent conjugate vaccines including additional serotypes II and V might broad vaccine coverage
- A combination of three GBS pilus components encoded by GBS PI-1, PI-2a, and PI-2b can confer protection to immunized mice against lethal challenge with 12 GBS strains.

D. Maione, I. Margarit, C. D. Rinaudo et al. *Science* **2005**, *309*, 148

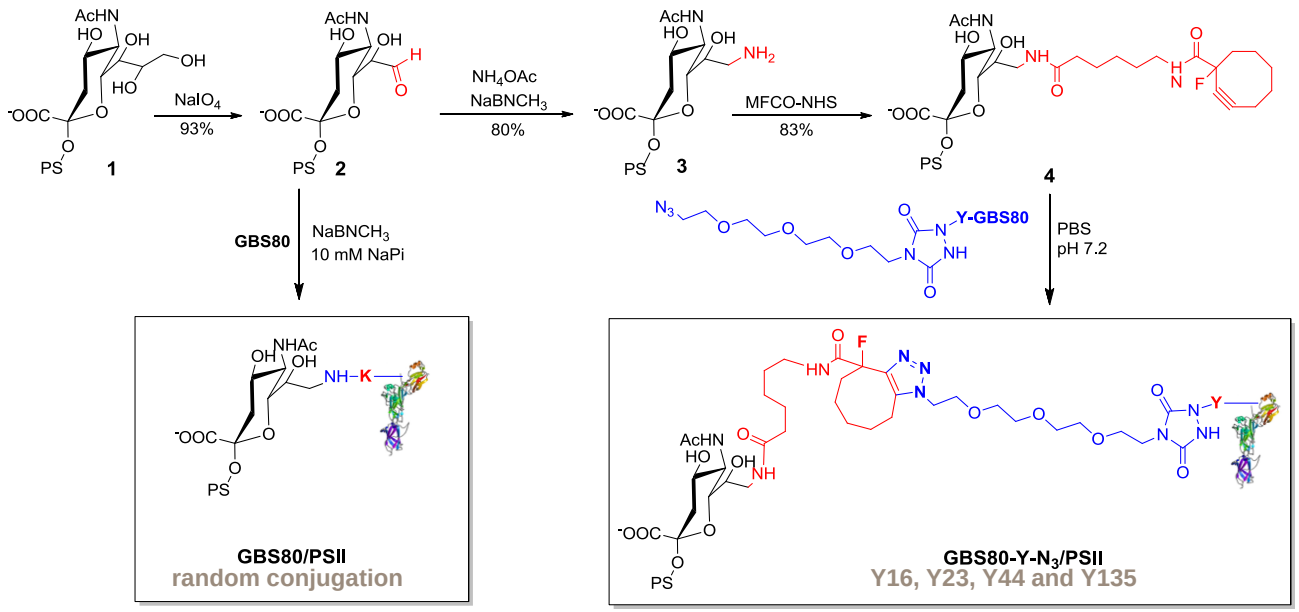
Conjugation of GBS polysaccharide to pilus proteins with the dual role of carrier and antigen



- Tyrosine-ligation is a powerful method for polysaccharide conjugation
  (*i*) enables targeting defined sites of the protein
  (*ii*) ensures higher batch-to-batch consistency
- These are important features when the protein is used with the dual role of antigen and carrier
- Attempts to conjugate high molecular weight negatively charged GBS polysaccharides to the tyrosine residues of CRM_{197} by CuAAC provided the desired glycoconjugates at poor yield
- Use of MFCO linker and SPACC gave very good coupling efficiencies

A. Nilo, M. Allan, B. Brogioni, D. Proietti, V. Cattaneo, S. Crotti, S. Sokup, H. Zhai, I. Margarit, F. Berti, Q.-Y. Hu, R. Adamo. *Bioconj. Chem* **2014**, *25*, 2105

Coupling PSII to GBS80: random vs tyrosine conjugation

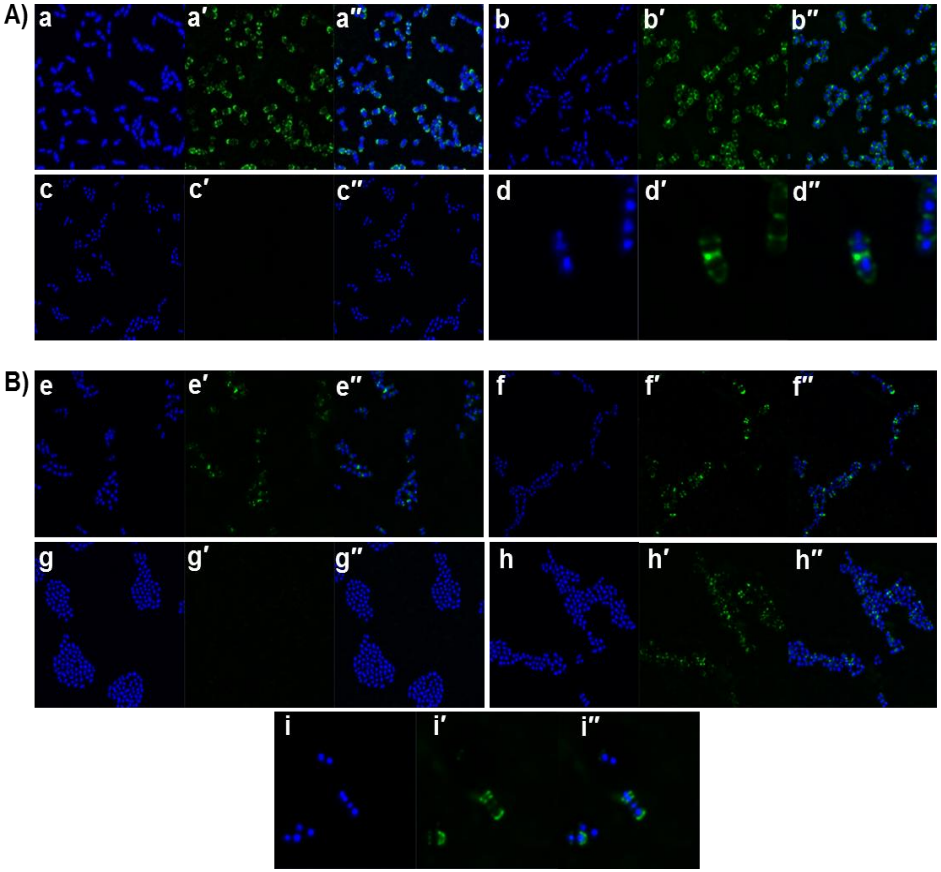


Characteristics of the synthesized glycoconjugates.

Glycoconjugate	Saccharide:protein stoichiometry (w/w)[a]	Saccharide:protein ratio (w/w)[b] in the product	Free saccharide[c]	Conjugation efficiency[d]
CRM_{197}/PSII	1:1	1.1	<3%	>95%
GBS80/PSII	2:1	1.8	14.7%	86%
GBS80-Y-N_3/PSII	1:1	1.1	<4.5%	>95%

•Ratio of reagents used in the conjugation reaction; b. carbohydrate:protein ratio in the purified glycoconjugate; c. conjugated and unconjugated PS in the purified products were estimated by HPAEC-PAD quantification of Gal; d. percentage of carbohydrate attached to protein in respect to the amount used in conjugation.

Anti glycoconjugate sera recognize individually PSII and GBS80



Immunofluorescence staining of PSII and pilus protein GBS80 on the surface of GBS strains 5401 (A) and COH1 (B), respectively, using sera from conjugates GBS80/PSII (a, e) and GBS80-Y-N_3/PSII (b, f). Serum against a conjugate of PSV with pilus protein GBS67 (c, g) and anti-GBS80 serum (h) were used as controls. (d) and (i) are the magnification of (a) and (e), respectively. In panels a-i staining of the bacteria is shown; in panels a′-i′ is the staining in the presence of anti-conjugate serum; panels a″-i″ are superimpositions of the other two previous panels.

GBS80 pilus protein functions as carrier for PSII (A) and antigen (B)

1 μg polysaccharide and protein dose, respectively, after three immunizations using Alum as adjuvant

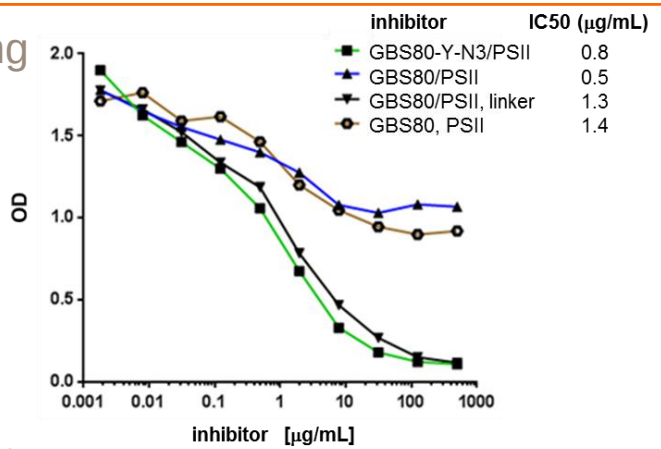
	PBS	CRM_{197}/PSII	GBS80/PSII	GBS80-Y-N_3/PSII	GBS80
A. GBS80 as carrier					
Anti PSII IgG GMT EU/ml (95% CI)	<10	3881 (4691-17632)[a]	3495 (975-12231)[a]	1337 (844-5830)[a]	<10
OPKA titer[c] (strain 5401)	<30	2823±65[a]	1768±337[a]	2235±220[a]	<30
Protected/Treated[d] (%) (strain DK21)	6/59 (10%)	na[b]	36/60 (60%)	68/69 (98%)	na
B. GBS80 as antigen					
Anti PSII IgG GMT EU/ml (95% CI)	<10	<10[e]	775 (83-7240)	3111 (426-22705)	3099 (69-137225)
OPKA titer (strain COH1)	<30	<30[e]	676±104	282±113	270±16
Protected/Treated[e] (%) (strain COH1)	28/80 (35%)	na	30/60 (50%)	49/70 (70%)	35/60 (58%)

.

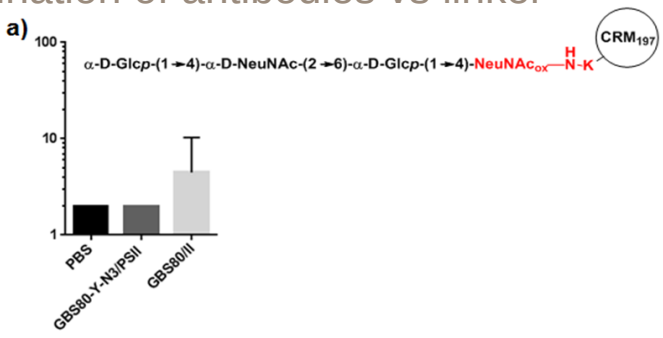
Results from two different immunization schemes. b. na = not available. c. OPKA titers ± standard deviation from duplicates run in the same plate (intra-assay variability); d. GBS80-Y-N_3/PSII vs GBS80/PSII, GBS80-Y-N_3/PSII vs PBS and GBS80/PSII vs PBS $p < 0.01$, according to Fisher's exact test; e. GBS80-Y-N_3/PSII vs GBS80/PSII $p < 0.05$ according to Fisher's exact test; e. titers from mice vaccinated with CRM_{197}/PSII conjugate in the second immunization experiment reported in Panel A.

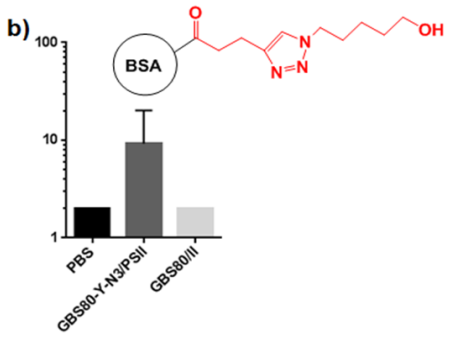
Anti-linker antibodies were directed to the cyclooctene rather than the triazole with no effect on the polysaccharide immunogenicity

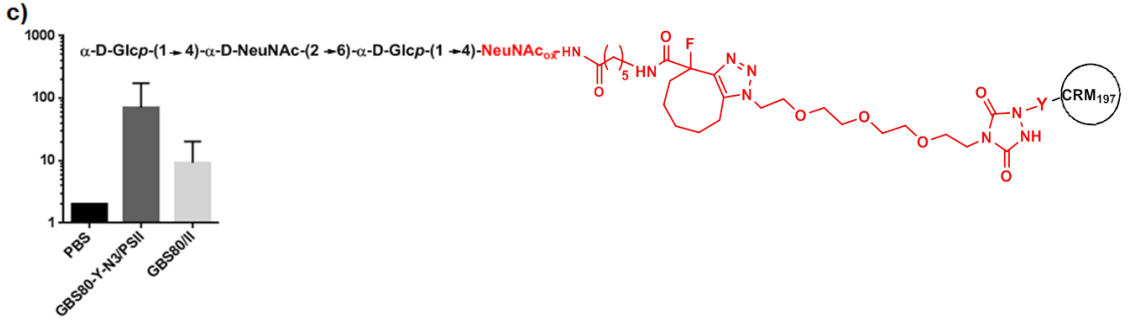
- Analysis of anti glycoconjugate serum using PSII for coating



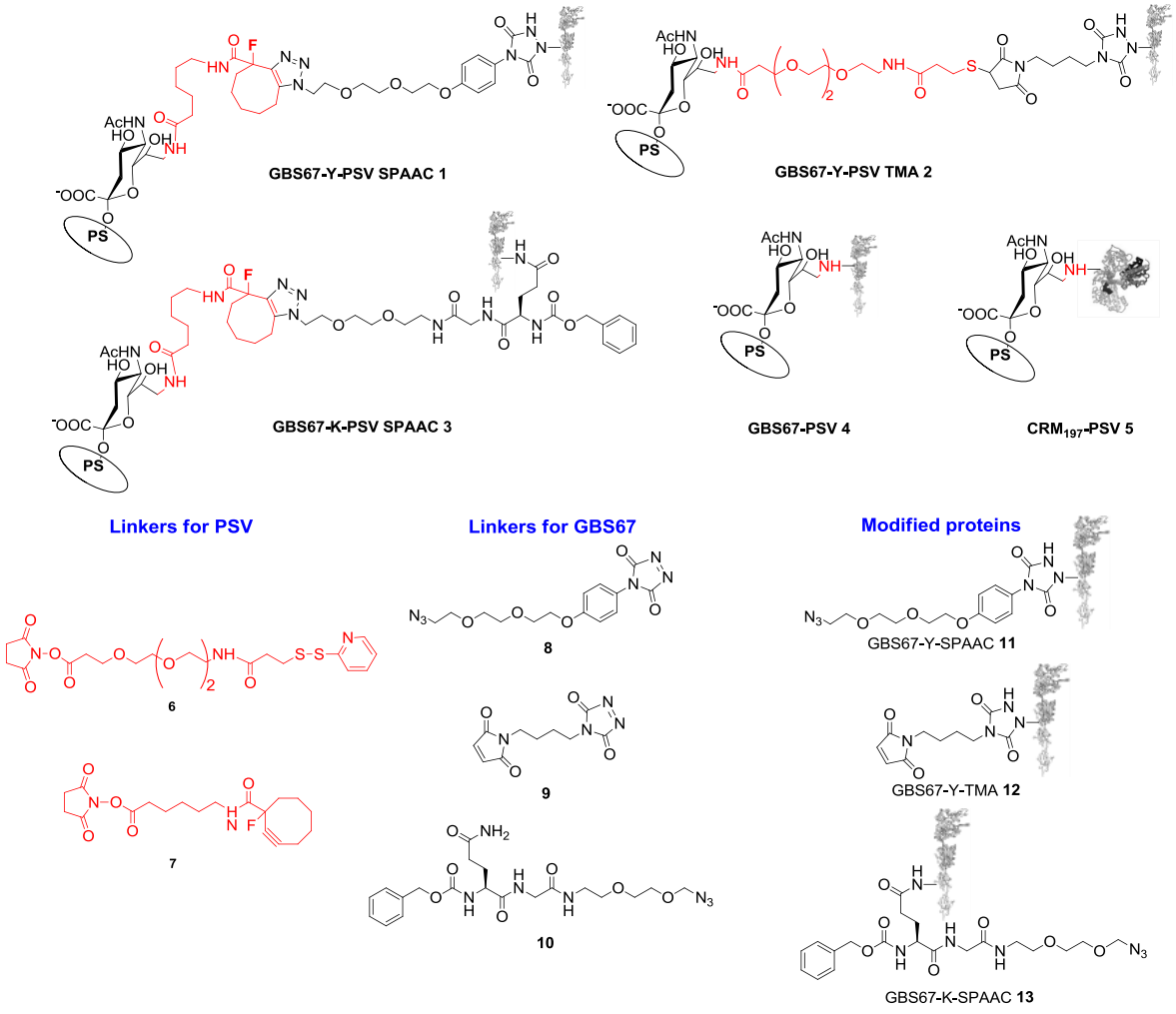
- ELISA determination of antibodies vs linker







Novel set of GBS PSV-GBS67 conjugates using tyrosine ligation/thiol-maleimide addition and microbial transglutaminase



A. Nilo, I. Passalacqua, M. Fabbrini, M. Allan, A. Usera, F. Carboni, B. Brogioni, A. Pezzicoli, J. Cobb, M. R. Romano, I. Margarit, Q.-Y. Hu, F. Berti, R. Adamo. Bioconj. Chem. **2015**, DOI: 10.1021/acs.bioconjchem.5b00365

Characteristics of synthesized glycoconjugates

Modified sites

Y744, Y282/283, Y336/337 and Y403

K320, K340, K558 and K812

Glycoconjugate	Saccharide:protein stoichiometry (w/w)[a]	Saccharide:protein in conjugate (w/w)[b]	Free saccharide (%)[c]	Conjugation efficiency (%)[d]
GBS67-Y-PSV SPAAC 1	3:1	2.5	<4.8	86
GBS67-Y-PSV TMA 2	6:1	3.0	14.7	50
GBS67-K- PSV SPAAC 3	3:1	2.0	<6.1	67
GBS67-PSV 4	2:1	1.8	<6.6	90
CRM_{197}-PSV 5	0.7:1	1.9	<6.3	40

Amount of reagents used in the conjugation reaction; b. carbohydrate:protein ratio in the purified glycoconjugate; c. conjugated and unconjugated PS as estimated by HPAEC-PAD quantification of GlcNAc; d. amount of conjugated polysaccharide vs amount of polysaccharide used for conjugation.

GBS67 is effective as antigen and carrier for PSV

Functional activity of the glycoconjugates 1-4 and corresponding controls

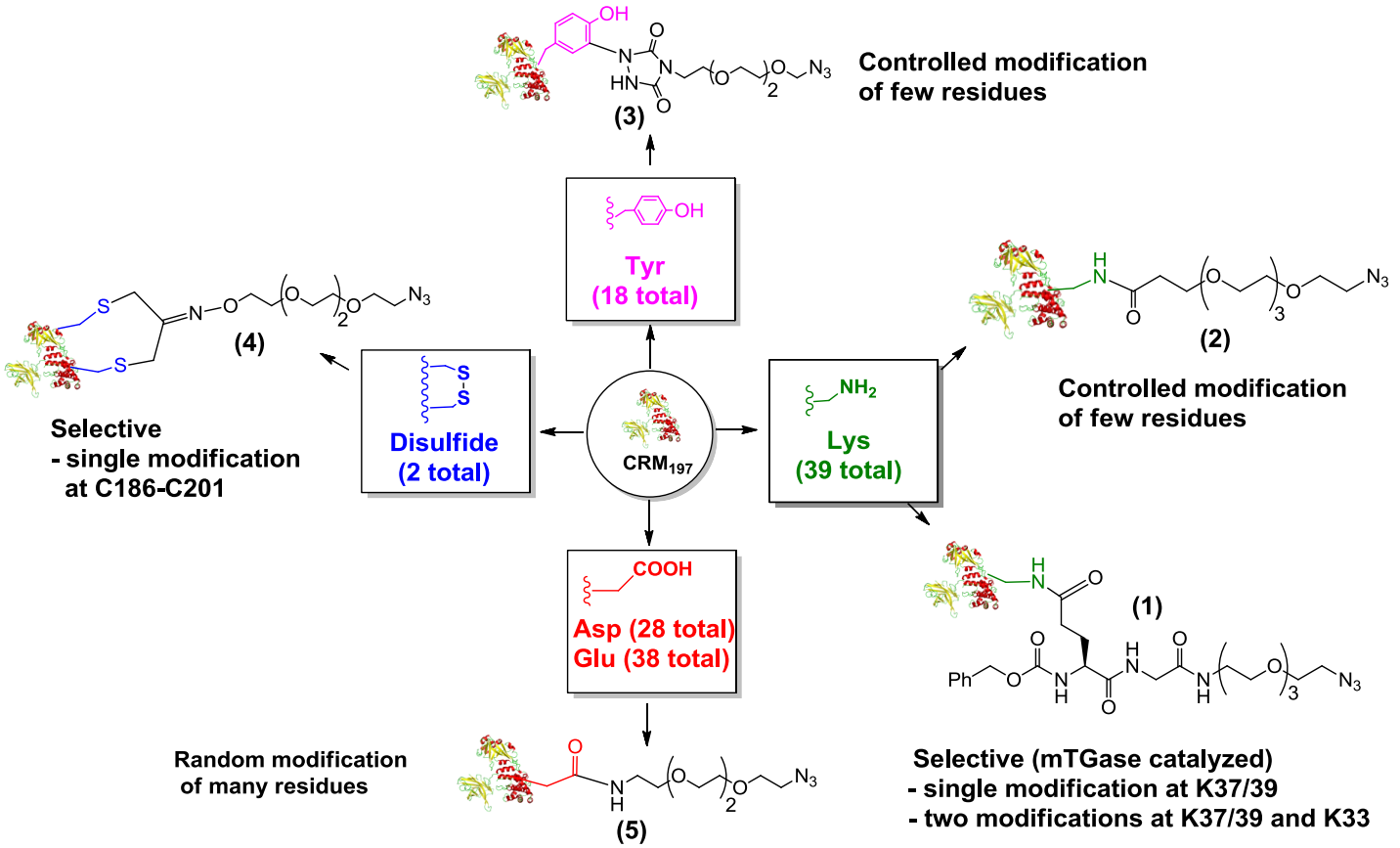
Glycoconjugate	Anti PSV activity		Anti PSV/GBS67 activity	Anti GBS67 activity	
	Anti-PSV IgG Median titers[a]	OPKA titers (2603)	OPKA titers (CJB111)	Anti-GBS67 IgG Median titers[a]	OPKA titers[e] (515)
dose	0.5 mg PSV	0.5 mg PSV	0.5 mg PSV	1 mg GBS67	1 mg GBS67
Alum	<10	<30	<30	<10	<30
GBS67-Y-PSV SPAAC 1	354 (23-4396)	197	493	162621 (122197-183447)	260
GBS67-Y-PSV TMA 2	956 (30-4035)	731	1497	542567 (423899-639251)	323
GBS67-K-PSV SPAAC 3	145 (94-214)	294	628	163709 (119336-324948)	174
GBS67-PSV 4	3596 (811-9430)[b]	2465	4626	381001 (261968-492158)	336
CRM_{197}-PSV 5	2785 (1543-8275)[c]	1307	2372	-	-
GBS67	-	-	-	691079 (442301-1290000)[d]	529

Median titers with 25-75% percentile range; statistical analysis was calculated according to the Mann-Whitney test. b. **4** vs **3** p = 0.0006; c. **5** vs **3** p = 0.0003; d. GBS67 vs **1** p = 0.001; GBS67 vs **3** p = 0.002; GBS67 vs **4** p = 0.03. e. OPKA titers from duplicates.

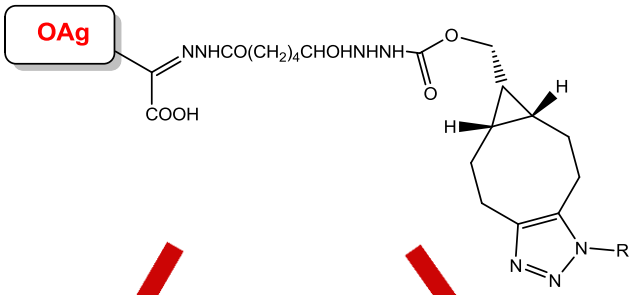
- ✓ Thiol maleimide addition is the best combination of site/chemistry
- ✓ Copper free click chemistry induced anti-linker antibodies while thiol-maleimide addition did not

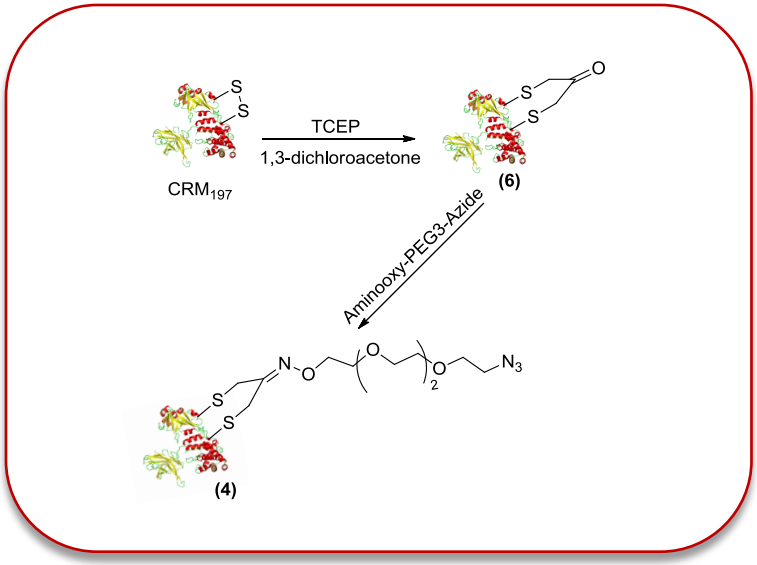
Study of the effect of glycan-protein connectivity in Salmonella O-antigen based vaccines

Collaboration with G. Stefanetti and F. Micoli, GVGH

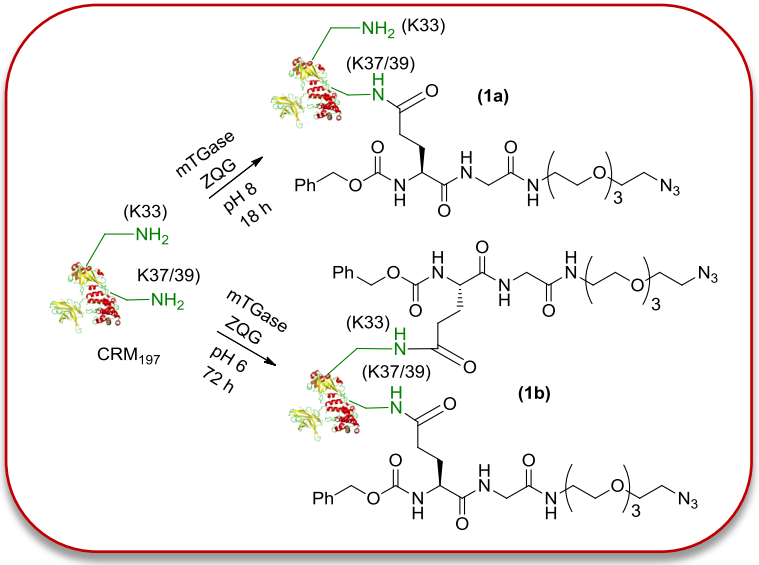


Two novel bioconjugation methods for selective modification of disulfide and lysine were developed





Reaction of dichloroacetone with sulfhydryls

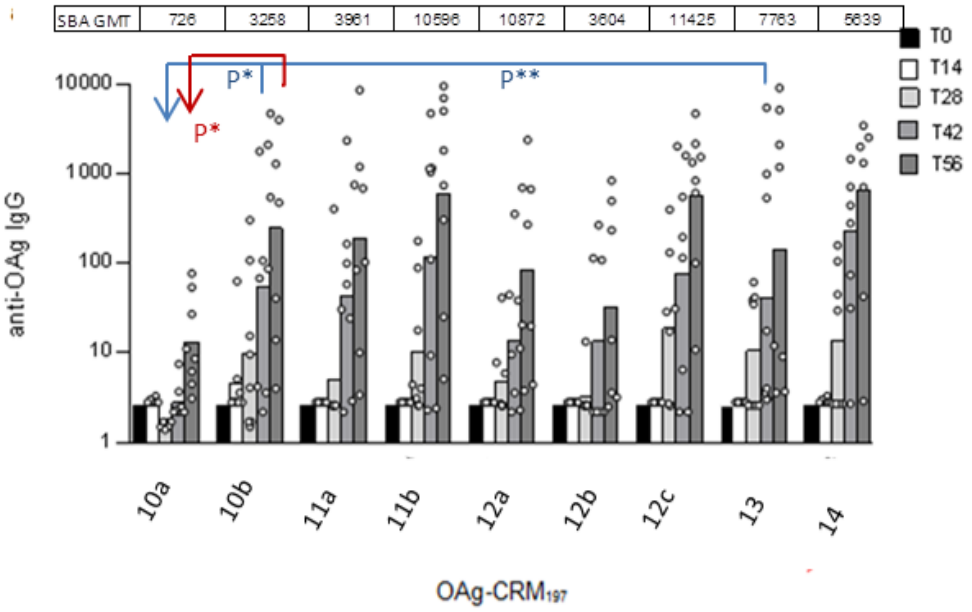


mTGase catalyzed modification of lysine

Glycan-protein impacts the vaccine immunoactivity

Characterization of OAg-CRM_{197} conjugates generated by copper-free click chemistry targeting different amino acids on CRM_{197}

Conjugate	Average CRM_{197} labeling	Modified sites on CRM_{197}	OAg chains per CRM_{197}
OAg-CRM_{197}(K+1) 10a	+1.0	K37/K39	0.7
OAg-CRM_{197}(K+2) 10b	+2.0	K37/K39, K33	0.6
OAg-CRM_{197}(K+3.8) 11a	+3.8	K103, K221, K242	1.5
OAg-CRM_{197}(K+7.1) 11b	+7.1	K103, K221, K236, K242, K498, K526	2.0
OAg-CRM_{197}(Y+1.5) 12a	+1.5	Y27, Y46, Y358, Y380	2.0
OAg-CRM_{197}(Y+2.6) 12b	+2.6	Y27, Y46, Y358, Y380	2.4
OAg-CRM_{197}(Y+4.3) 12c	+4.3	Y27, Y46, Y358, Y380	3.7
OAg-CRM_{197}(C-C+1) 13	+1.0	C186-C201	1.2
OAg-CRM_{197}(E/D+4.8) 14	+4.8	Random E/D	1.4

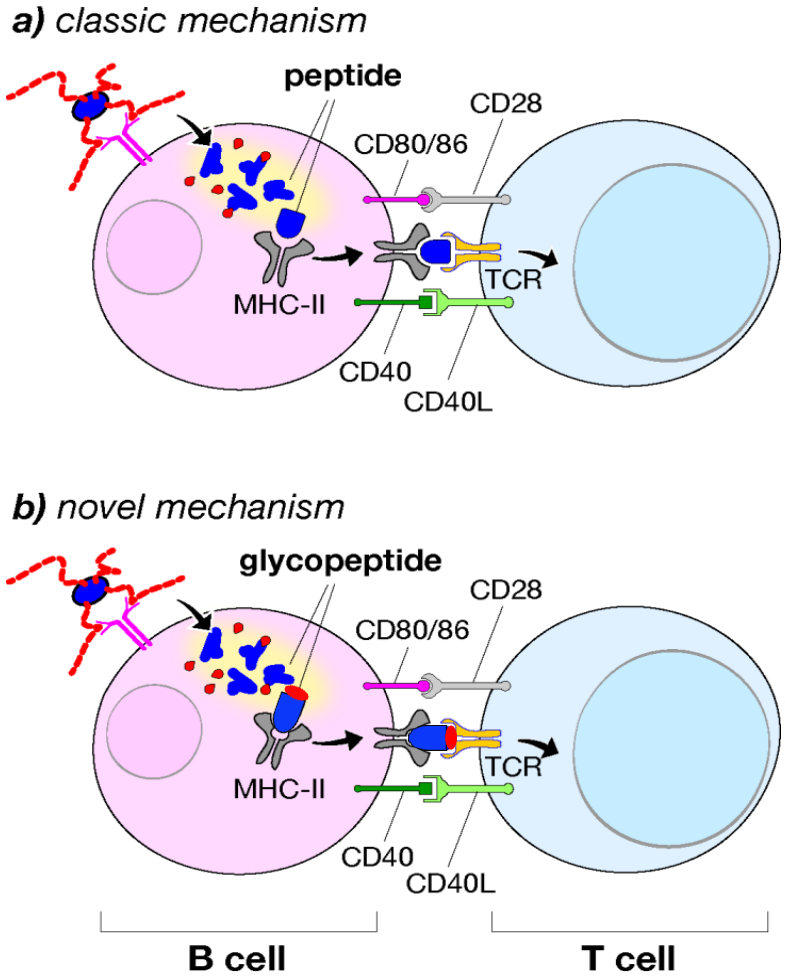


- The conjugate prepared at C186-C201 gave an immune response significantly higher than conjugate at K37/K39

- The anti OAg titer were comparable to conjugate with higher loading

G. Stefanetti, Q.-Y. Hu, A. Usera, Z. Robinson, M. Allan, A. Singh, H. Imase, J. Cobb, H. Zhai, D. Quinn, M. Lei, A. Saul, R. Adamo, C. A. MacLennan, F. Micoli. *Angew. Chem. Intl. Ed*. **2015**, DOI: 10.1002/anie.201506112R1

A comparison between classic and novel mechanism of action for glycoconjugate vaccines



- ✓ Different attachment sites could result in
  1) diverse exposure/presentation of the glycoconjugate to antigen presenting cells
  2) different processing of the vaccine inside B-cells, or
  3) formation of different peptides after processing of the conjugates inside B-cells to be presented to T-cells

- ✓ The importance of the amino acids targeted in the conjugation reaction is not apparent when random chemistries are used, when the immune response averages the contribution of individual “optimal” position

- ✓ Very few attachement sites (even one single) might be sufficient to develop efficacious vaccines

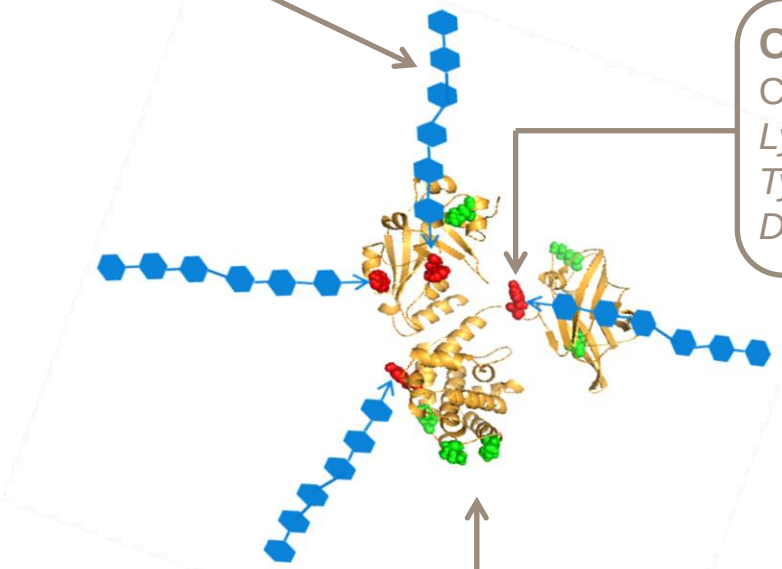
F. Y. Avci, X. Li, M. Tsuji, D. L. Kasper, *Nat. Med.* **2011,** *17* (12), 1602
F. Berti, R. Adamo ACS Chem. Biol. **2013**, *8*, 1653

Conclusions

- We have developed four methods for modification of tyrosine, lysine (chemical and TGase-based) and disulfide bridge respectively
- We have used these methods in the preparation of a variety of novel glycoconjugate vaccine candidates
- Site selective methods ensure high consistency in the preparation of glycan-protein conjugates, definition of the attachment site and correlation of the immune response with the targeted site
- This feature is relevant for the preparation of carbohydrate-based vaccines using protein with the dual role of carrier for the carbohydrate and antigen
- The use of site selective methods has allowed deciphering the impact of conjugation chemistry on the immunological activity of glycoconjugate vaccines

Overwiew of appraches towards well defined glycoconjugate vaccines

Chemical or chemo-enzymatic synthesis of the carbohydrate antigen



Choice of the conjugation site
Chemical or enzymatic modification of
Lysine
Tyrosine
Disulfide

Selection of the carrier protein
Classic carriers (CRM_{197})
Protein with dual role of carrier and antigen (GBS pilus proteins)

Acknowledgements.....
...to my working team

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Carla Bromuro
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... and you for your kind attention!