GlycoNZ
Glycoconjugates for Glycobiology

Frequently Asked Questions – FAQs
Version 23 June 2025

Primary Question INDEX

0	**Contact, frequently asked questions (FAQ), website and catalogue**
	Related questions:
0.1	To contact GlycoNZ product please email us at office@glycoNZ.com
0.2	If you ask a question which is already in our FAQ list we will send you this list and refer you the answer number(s) which relates to your question. If you ask a question which is not in our FAQ list we will add your question to the next FAQ list and answer by email.

<table>
<tr><td>0.3</td><td>The GlycoNZ website can be found at www.glyconz.com

On this website you will find a worksheet (may take a few moments to load) of our entire catalogue http://www.glyconz.com/products/

Catalogue ID - this is the number you should use to order
Oligosaccharide / FSL Structure – basic structural formula
Designation (Label) – what is typically written on the vial label
Trivial Name – alternative abbreviated naming
Unit [mg] – the amount of material in a vial
Price [USD] – price in USD (excluding USA/Canada customers who buy from Merck)
Series - type of construct (BM, BP, PA, FP, FF)
Glycan size – number of glycan residues
Presentation – monomer of polymer
Carrier - if carried on polyacrylamide backbone (PAA)
Label – biotin or FITC
Glycan MW – Mw of the glycan residue (also as in COA)
Glycan-binding protein – listed if known
Other ID – other IDs previously used, e.g GlycoTech

http://www.glyconz.com/documents/

Product specifications and safety data sheets (SDS) are provided for customer use.

You can also download SDS files from Millipore-Sigma (just web-search our catalogue number)
And a link to the latest copy (pdf) of these FAQs</td></tr>
<tr><td>0.4</td><td>Download the Merck "GlycoNZ" Catalogue at
https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/marketing/global/documents/144/274/glyconz-intro-ca6885en-mk.pdf</td></tr>
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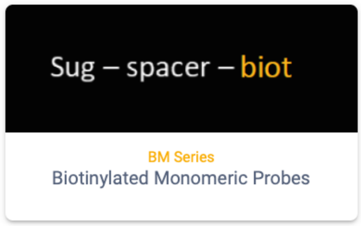
1.	**Who are the suppliers of GlycoNZ glycoconjugates?**
	Related questions: 4.4
1.1	GlycoNZ glycoconjugates are supplied by GlycoNZ Limited (NZBN# 9429042199526), a 100% subsidiary of Kode Biotech Limited (NZBN# 9429038366413), based in Auckland, New Zealand
1.2	GlycoNZ supplies catalogued GlycoNZ products to the the world excluding the US & Canada (and non-catalogued and bespoke items to the US/Canada)
1.3	Merck has an exclusive license to supply catalogue GlycoNZ products in the US & Canada, and non-exclusive to the rest of the world. You can get to their website here https://www.sigmaaldrich.com/ Then search for GlycoNZ 
1.4	 **Innovative Applications of Polyacrylamide Glycan Conjugates: Advancing Glycobiology Research** Available On Demand 59 minutes https://www.sigmaaldrich.com/NZ/en/collections/webinars/innovative-applications-polyacrylamide-glycan-conjugates? **You will need to register to view this webinar** **Detailed description:** Polyacrylamide glycan conjugates (Glyc-PAA; PA series) are powerful and versatile tools in glycobiology, extensively adopted by researchers for microplate coating and as antigens or ligands for viral and bacterial lectins. PAA's hydrophilicity, flexibility, and stability under diverse chemical conditions enable increased affinity for glycan-binding proteins. Biotinylated versions (Glyc-PAA-biot; BP series) with biotin tags are especially valuable for visualization and quantification assays, while fluorophore glycan conjugates (Glyc-fluorescein; FP series) are extensively employed in the study of cell-bound lectins. Join us for an innovative webinar exploring glycan conjugates and their applications in glycobiology. Our expert speakers, Professor Nicolai Bovin and Dr. Karnakar Chitta, will share valuable insights through a live discussion and Q&A. **Key topic include:** • The role of glycoconjugates in key biological processes and their associations. • The diverse types of glycoconjugates available and their specific applications. • How the use of glycoconjugates significantly expands the potential for discovering drug targets related to infectious diseases, inflammation, and cancer.
1.5	**Glycotech vs GlycoNZ?** Prior to 2020 the US based company Glycotech was a distributor for GlycoNZ products (with the distributorship transferring to Merck in 2020). Those products previously supplied by Glycotech are the same products as supplied by GlycoNZ (although the Glycotech catalogue number system is different to GlycoNZ).

<table>
<tr><td>2.</td><td colspan="2">What are the different classes of GlycoNZ glycoconjugates products?</td></tr>
<tr><td></td><td colspan="2">Related questions: A.3, 12</td></tr>
<tr><td>2.1</td><td>BM series: Sugar-spacer-biotin
These probes have an elongated spacer giving them the possibility to bind avidin or streptavidin. The biotin fragment is hydrophobic enough to permit the probes to be adsorbed on SepPack C18 cartridges from aqueous solutions and to be eluted by methanol similarly to glycosides bearing a “Lemieux-type” spacer. The spacer is the normal spacer (usually C3 or C2) of the ligand plus C6 spacer of biotin label.
BM series construcst are soluble in aqueous buffers up to at least 1mg/mL</td><td>
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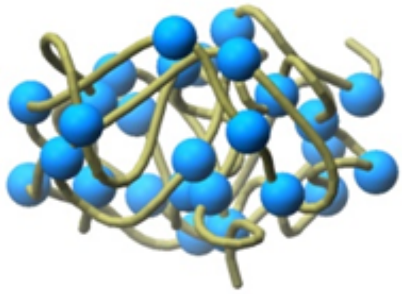
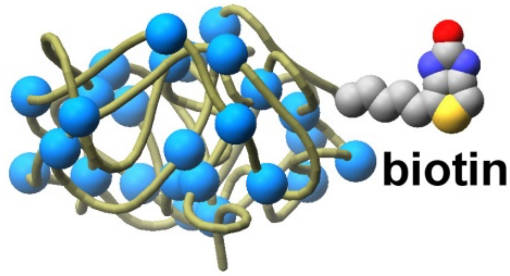
<tr><td>2.2</td><td colspan="2"> 

Polyacrylamide glycoconjugates PA & BP series
Polyacrylamide glycan conjugates (Glyc-PAA; PA series) without label/tag, have been widely used in many research areas of glycobiology, primarily for coating of polystyrene microplates with Glyc-PAA as an antigen or ligand for antibodies and glycan-binding proteins or lectins of viruses and bacteria, in enzyme immunoassays or other types of assays. Note this image shows a single biotin residue in a short segment of PAA, but there is approximately one biotin residue for every 20 PAA monomers (5%)

PAA is used as the carrier polymer because it has following features that are attractive for use in glycobiology:

hydrophilicity and absence of groups adhering to the cell surface and proteins;
PAA is stable under diverse chemical conditions

Stable to proteases
PAA matrix is stable at pH 3.0 – 10.0
Withstands 100 ^{o}C
The aqueous solution can be frozen and thawed many times

Optimal composition: 20 mol% Glyc, 5 mol% biot or 1 mol% fluo
Side groups $–CH_2CONHCH_2CH_2OH$, not $–CH_2CONH_2$
Weak total negative charge ($-COO^-$)
Cells tolerant to Glyc-PAA and Glyc-PAA-fluo probe
PAA, unlike protein matrices, does not have Trp and Tyr, and therefore does not affect fluorescence events
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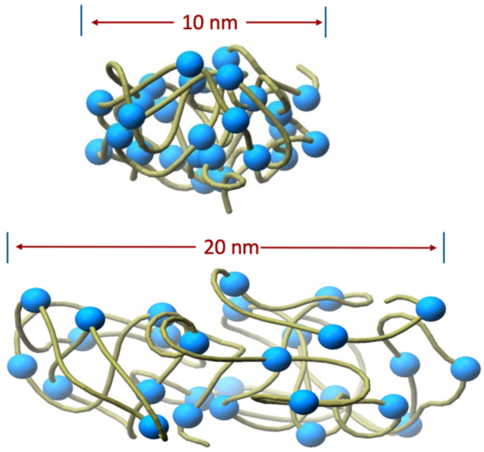
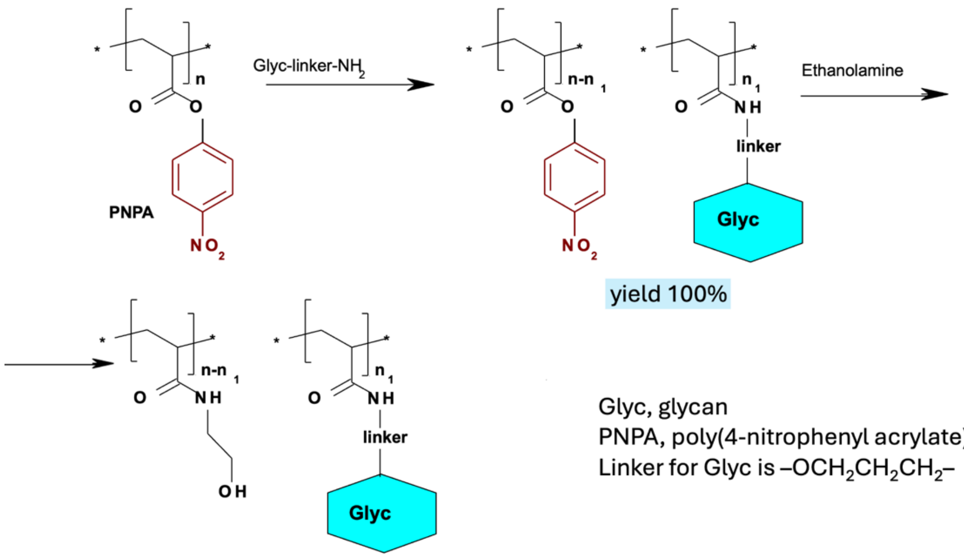
- the flexibility of the polymeric chain (a random coil), which allows practically any distance between glycan residues – this is a fundamental property to increase the affinity towards two (or more) subunit glycan-binding proteins;



- Reliable and standardized chemistry



Glyc, glycan
PNPA, poly(4-nitrophenyl acrylate)
Linker for Glyc is $–OCH_2CH_2CH_2–$

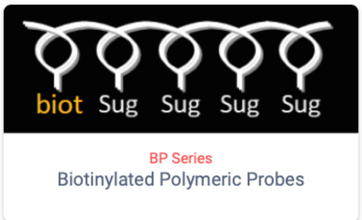
Biotinylated polyacrylamide glycan conjugates (Glyc-PAA-biot; BP series) have found even more applications as the biotin tag allows for quantitative immobilization of Glyc-PAA on any avidinylated surface. Additionally, Glyc-PAA-biot conjugates are used in a variety of assays as a tag, where the first step is the attachment of the glycan to a binding protein (e.g. virus, bacteria or mammalian cell) thereby tagging it with biotin, which can then be followed by visualization/quantification with fluorescent avidin or enzyme-conjugated avidin, or immobilization on an avidinylated surface. Glyc-PAA-biot conjugates are convenient as tags for simple (just by a mixing in aq. media) "glycosylation" of various avidinylated solid materials (chips, affinity adsorbents, beads, immunological plates, etc.).

Why is ethanolamine used to "quench" excess active groups?
To convert an activated polymer into a true actylic acid amide, you need to use a solution of ammonia in water, and this leads to the fact that some of the groups are converted into carboxyls. It is undesirable and poorly controlled. We compared more than 12 other small amines, including oligoethylene glycols, to ethanolamine. It turned out worse in terms of background in ELISA.

See also reference list in appendix for reviews on these constructs.

2.3 **When did polyacrylamide conjugates BP & PA change from 30kDa to 20kDa polymer?**
Earlier versions of the BP & PA products (manufactured pre 2012) used PAA with an average mass of 30 kDa, which was changed in 2012 to have an average mass of 20kDa. We have many dozens of examples where

<table>
<tr><td></td><td colspan="2">30kDa and 20kDa conjugates were used and no differences in their protein-binding properties were observed (including in relation to influenza viruses). However, we do not exclude that there may be a visible discrepancy when the analytical platform is acutely sensitive to a change in conjugate size from 30 to 20 kDa. With due respect to the expiry dates these 30kDa products are not supplied by GlycoNZ but some old stock may exist in individual laboratories.</td></tr>
<tr><td>2.4</td><td colspan="2">What does PAA terminology % mol., mean
Saccharide 20% mol. (or carbohydrate content) means that every 5th PAA monomer has a glycan residue
biotin 5% mol. means that every 20th PAA monomer has a biotin residue
fluorophore 1% means that every 100th PAA monomer has a fluorophore residue

Why was the glycan load chosen to be 20 mol%?
At the very beginning of sales, we offered some customers series with glycan content of 2%, 5%, 10%, 20% and 30%. This allowed both ourselves and our clients to choose the best option. The 20% option almost always turned out to be optimal.</td></tr>
<tr><td>2.5</td><td>BP series: Biotinylated polymeric probes
PAA is poly[N-(2-hydroxyethyl)acrylamide], used as a carrier, has low non-specific sorption and it is stable to chemical and proteolytic action.
The affinity of the probes is $10^2 – 10^5$ times higher than that of the corresponding free sugars.
$HOCH_2CH_2NH_3^+$-salts for acidic sugars,
Spacer-arm is normally C3, spacer arm for biotin is C6. The flexible polymer chain behaves as an additional spacer.
Mr approx. 20kDa, Content: saccharide 20% mol., biotin 5% mol.
Purity: synthetic ω-aminoalkyl glycosides used for coupling with the polymer have a purity >95% (HPLC and ^{1}H-NMR)</td><td>
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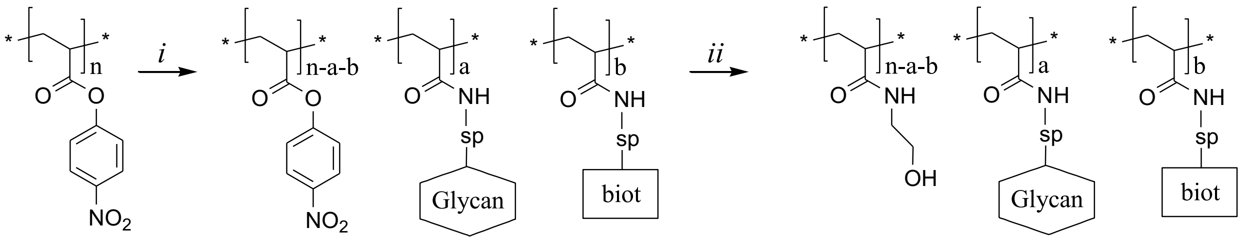
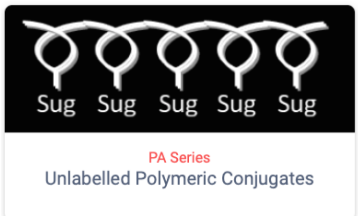
<tr><td></td><td colspan="2"></td></tr>
<tr><td>2.6</td><td>PA series: Unlabelled polyacrylamide conjugates
$HOCH_2CH_2NH_3^+$-salts for carboxylates and sulfates
Spacer-arm for oligosaccharides is normally C3.
PAA is poly[N-(2-hydroxyethyl)acrylamide], a flexible polymer chain serves as an additional spacer.
Mr approx. 20kDa, Content: carbohydrate 20%.</td><td>
</td></tr>
<tr><td>2.7</td><td>FP series: Fluorophore polymeric probes
Probes designed to reveal lectins directly on cell membranes, in glycocalyx, and in the cytoplasm. The low fluorescein content does not affect the probe solubility in water but it still allows a reliable probe detection. The fluorophore used for labelling is fluorescein isothiocyanate.
The affinity of the probes is $10^2 – 10^5$ times higher than that of corresponding free sugars.
PAA is poly[N-(2-hydroxyethyl)acrylamide], a flexible polymer chain serves as an additional spacer.
Mr approx. 20kDa, Content: carbohydrate 20% mol; fluorescein 1% mol.</td><td>
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</table>

2.8 **FF series: Sepharose affinity absorbents**

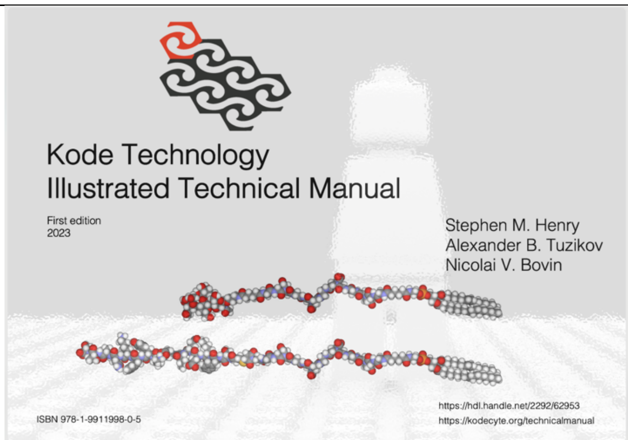
The carbohydrate ligand is attached to aminosepharose via PAA. Content: 0.6 micromol Sug per 1 mL. Affinity adsorbents are obtained by conjugation of Sug-PAA to aminated Sepharose6FF*; density of Sug groups in composition of the polymer corresponds to one of Sug-PAA, i.e. 20% mol. Capacity is 0.6 μmol/mL of adsorbent. Regeneration can be made at pH 11-12 without loss of quality; Store condition is in a 20% ethyl alcohol at 4°C. We recommend to use regenerated adsorbent <10 times. Elution of bound proteins (lectins, antibodies) can be performed by acidic buffers (pH 2.5-3) or by basic buffers (pH 9-10); borate containing buffers are not applicable

2.9 **How to calculate mol% for each construct**

For quantitative calculations, you do not need the molecular weight of the polymer (which has a wide mass distribution) instead use data on micromoles per mg. If required please email us and request the Micromol/milligram data table (22 pages) which looks like the following to look up data related to your construct

Cat. Nr.		(oligo)saccharide and its Spacer (OS+S)	Short name	Short name for Table presentation	Molecular weight of OS+S	μmol OS/mg conjugate (blocked by ethanolamine)		
						PAA	PAA – 5% biot	PAA – 1% flu (Flu-cadaverine)
		Trisaccharides						
42		**Fucα1-2Galβ1-3GlcNAcβ-sp3**	Le^d, H (type 1)	LeD	586.6	0.908	0.853	0.888
89		**Fucα1-2Galβ1-4GlcNAcβ-sp3**	H (type 2)	Htype2	586.6	0.908	0.853	0.888
740		**Fucβ1-2Galβ1-4GlcNAcβ-sp3**	Fucβ2'LN		586.6	0.908	0.853	0.888
59		**Fucα1-2Galβ1-3GalNAcα-sp3**	H (type 3)	Htype3	586.6	0.908	0.853	0.888

2.10



FSL series: Function(glycan)-spacer-lipid – designed for direct labelling of cells and viruses

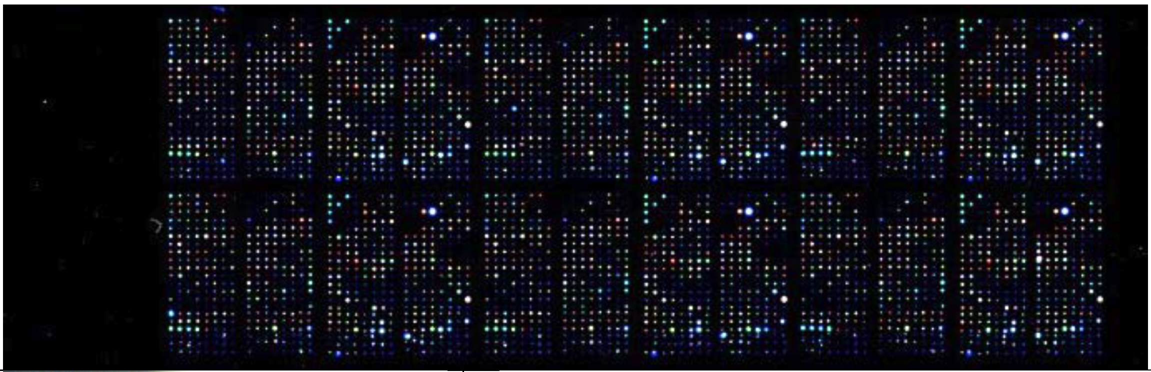
Kode Technology is explained in detail in the **free Kode Technology Illustrated Technical Manual eBook**

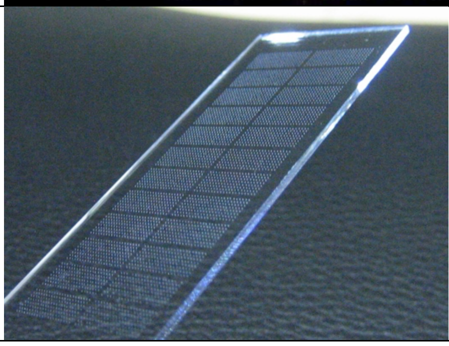
The 56mB eBook can be downloaded from the University of Auckland server **https://hdl.handle.net/2292/62953**

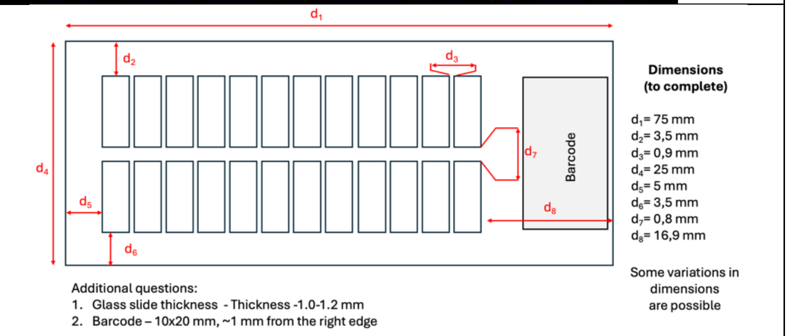
or see www.kodecyte.org to download a copy from our website

FSL (Kode Constructs) are available generally bespoke and made upon request (except FSL-biotin and FSL-FLRO4 which are always in stock)

2.11 **Glycochip/Glycoarrays** (**bespoke product – made to order, minimum quantities**):







Glycochip for detection of natural and adaptive immunoglobulins in blood/plasma, as well as other glycan-recognising proteins.

Ligands are covalently immobilised using epoxide residues on a glass slide and amino group in composition of oligo- or polysaccharide ligand

Characteristics:

- 25×75×1 mm glass or plastic slides
- epoxy-activated surface to print glycans
- ~600 immobilized ligands
- ~400 synthetic mammalian glycans
- ~200 pathogenic bacteria polysaccharides ~200 polysaccharides from pathogenic bacteria *(Shigella flexneri, Shigella dysenteriae, Shigella boydii, Salmonella enterica, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Proteus vulgaris, Proteus pinneri, Cronobacter sacazaki,*etc.)
- Specific IgG, IgM and IgA are measurable.
- Shelf life: 1 year at +4°C under vacuum or dessication
- Identification barcodes
- ~3 hours analysis time
- Chips are packed under vacuum and can be shipped at room temperature. After opening a package, chips are stable for 1 (2 days maximum) days at room temperature at low humidity. After that you should re-pack them under vacuum (or under argon) or place them into the dessicator and store them for a long time (several months), you can re-pack them when you need. If you do not have a vacuum station or dessicator, you can place the box into the zipped packet with desiccant and store them for 1 week at room temperature. The critical point is humidity.

(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6411795/)
Any custom configuration of the chip is possible on request. Specialized thematic arrays, as

- Blood group antigens
- Cancer-associated antigens

- Galectin ligands
- Siglec ligands
- Bacterial polysaccharides
- Contact us for custom design

Glycochip uses:

- Search for glycan-binding proteins and characterization of their specificity; the proteins are, antibodies, lectins, glycosyltransferases, glycosidases;
- Functional revealing of lectins and other carbohydrate-binding and carbohydrate-modifying molecules in composition of bacteria, viruses and cells;
- Development of anti-bacterial and anti-viral compounds acting on microorganism's lectins;
- Search for diagnostic signatures (combination of several antibodies-markers), for cancer, autoimmune and other diseases diagnostics.

Glycochip readers common requirements for reader:

- Suitable for 1" × 3" (one inch by three inches) slide with 1 mm thickness;
- Reading area: 22 mm × 73 mm;
- Able to accurately read Cy3 / Alexa555 and Cy5 / Alexa647 fluorescent molecules;
- Resolution: ≤ 10 μm

See also references in Appendix
Video: Olivera-Ardid, S., Khasbiullina, N., Nokel, A., Formanovsky, A., Popova, I., Tyrtysh, T., Kunetskiy, R., Shilova, N., Bovin, N., Bello-Gil, D., Mañez, R. Printed Glycan Array: A Sensitive Technique for the Analysis of the Repertoire of Circulating Anti-carbohydrate Antibodies in Small Animals. *J. Vis. Exp.* (144), e57662, doi:10.3791/57662 (2019).

https://www.jove.com/video/57662

In addition, there is also a video at https://cloud.mail.ru/public/5AnX/5AYJqGeQn

See also Appendix B : WORKING WITH GLYCOCHIPS

Discuss with us your needs – minimum order size is 25 glycochips, cost is USD350 per chip (excluding bespoke)

3.	**Why does it sometimes take what seems a long time for a construct to be made (back-order) or arrive in our lab?**
	Related questions:
3.1	We actively try to maintain levels of our existing stock products to meet anticipated demand, however we often get an unexpected "run" on certain products, which can rapidly deplete our stock. When we see or can anticipate this, we immediately implement stock replacement manufacturing. Replacement of new stock can be reasonably quick if the oligosaccharide exists as a raw material. In most cases synthesis of the product simply requires conjugation of the amino-spacered oligosaccharide with the polymer, which requires three days, plus another two to three days for analytical procedures for quality control. However, if the parent oligosaccharide is unavailable and requires synthesis, this can be very time consuming. For example, the synthesis of a sialylated tetrasaccharide, from the first to the last stage, takes 6-8 months in experienced hands. Fortunately, in most cases, we already have ready-made oligosaccharides, or at least di- and tri-saccharide building blocks ready for further synthesis, and so typically we only need a few months for synthesis and final purification before conjugation to polymers and/or modification with biotin or fluorophore. However, it must also be appreciated with a catalogue of over 1000 different products, and typically up to 30 different products always simultaneously being synthesised, dispensed and quality controlled (each at a different stage) at the same time, new synthesis requests must be dove-tailed into the synthesis workflow (which can result in a further delay of up to 2 weeks).

3.2	Finally, the products are then imported to GlycoNZ in New Zealand (a process which can take up to 10-20 working days) for shipping, import clearance, final inspection, labelling and packing into orders before exporting to customers where export/import clearances can also take up to 10-15 working days. These delays are quite variable and country specific (especially where there are taxes and tariffs applicable, which are paid by you the importing customer). It should also be noted that on average about 10% of shipments are randomly pulled aside for additional checks. Don't worry our products are stable at RT so will not deteriorate during this time. In these current times there are significant global issues related to shipping and import/export shipping so please expect occasional delays which are outside of control. Typically products shipped from us, if they do not encounter unexpected export issues should arrive in your lab within 1 week, but unfortunately delays of 3 weeks are common.

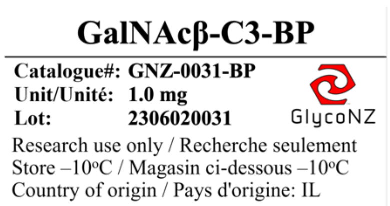
4.	**What are the HS Codes and other import information?**
	Related questions:
4.1	Standard description is: sample of synthetic carbohydrate conjugates for RUO (research use only)
4.2	All vials contain 1mg (0.001 g) or less. Total weight of all combined product (<0.xg) HARMLESS NON-RESTRICTED MATERIALS CLASSED AS SUGARS , Non-infectious, non-hazardous, not of biological origin or derived from animal or plant matter. NOT FOR HUMAN USE, FOR RESEARCH PURPOSES ONLY.
4.3	**HS Codes are** BP series HS code 3906.90.5000 BM series HS code 2940.00.6000 PA Series HS code 3906.90.5000 FSL-series HS code 3822.90.00 FF-series HS code 3914.00.90 Glycochips HS code 3822.90.00
	HS Code 3822.90.00 - Composite diagnostic or laboratory reagents, other than pharmaceutical preparations of heading 3002 or 3006 - HS C ode 3906.90.5000 - Acrylic polymers, in primary forms (excluding poly"methyl methacrylate") HS Code 2940.00.6000 - Sugars, chemically pure, other than sucrose, lactose, maltose, glucose and fructose; sugar ethers and sugar esters, and their salts, others – HS Code 3914.00.90 Ion-exchangers based on polymers of natural or synthetic plastics materials
4.4	GlycoNZ glycoconjugates primarily come from Israel, although a number of different glycan raw materials required for synthesis may been obtained from a variety of sources. "Country of Origin: Israel" or "Made in Israel "
4.5	The origin of GlycoNZ products is assigned to the country where the product's last substantial transformation took place, which is Israel. The glycan ligands used in manufacturing are either synthesized in-house or sourced throughout the world, sometimes as complete saccharides and sometimes as building blocks. All glyco ligands are in-house quality assured and then modified and incorporated into GlycoNZ polymers which is a substantial transformation process and where the products obtains its essential character (and its harmonized code (HTS) number), and hence this becomes the product origin as defined by regulation (e.g. the US regulation § 134.1 and § 177.22 Definitions, which are essentially the same as those used in New Zealand and elsewhere in the world) § 134.1 "(b) Country of origin. 'Country of origin' means the country of manufacture, production, or growth of any article of foreign origin entering the United States. Further work or material added to an article in another country must effect a substantial transformation in order to render such other country the 'country of origin' within the meaning of this part; ..." § 177.22 Definitions "(a) Country of origin. For the purpose of this subpart, an article is a product of a country or instrumentality only if 1. it is wholly the growth, product, or manufacture of that country or instrumentality, or 2. in the case of an article which consists in whole or in part of materials from another country or instrumentality, it has been substantially transformed into a new and different article of

	commerce with a name, character, or use distinct from that of the article or articles from which it was so transformed...." Finished products are then shipped to GlycoNZ in New Zealand where they undergo final quality checks and labelling.
4.6	**Generic import statements regarding product** (compliant with USDA/Customs Source Statement : Declaration with respect to USDA Guideline 1105) 1. Material name: catalogue number 00nn-**BP** (details) a. (detailed name) - xxxxx-sp3-PAA-biot b. (shorthand name) - xxxxx-BP c. (description) Biotinylated polymeric glycan probe. PAA is poly[N-(2-hydroxyethyl)acrylamide], Mr approx. 20kDa, Content: saccharide 20% mol., biotin 5% mol. 2. Material name: catalogue number 00nn-**BM** (details) a. (detailed name) - xxx-sp3-biot b. (shorthand name) - xxxx-C3-biot c. (description) Biotinylated monomeric probe. 3. The material is confirmed as being 100% chemically synthesized 4. The material is not recombinant nor sourced from human or animal 5. The material does not come from a lab that works with exotic viruses affecting avian species or livestock 6. The intended use of the material is for in vitro (lab) use only – research use only 7. The material does not contain any animal, microorganism, or cell culture product. 8. The materials are not derived from and were not exposed to any animal, microorganism, or cell culture product at any step in the production process 9. The origin of the raw material for manufacturing location is Israel. The certification of origin is in the Product Specification and the Certificate of Analysis The statements of how it is known that the material does not contain an infectious biological agent, is based on the manufacturing route which uses 100% non-biological raw materials

5.	**How do I order and pay for GlycoNZ catalogue products?**
	Related questions:
5.1	If in the USA/Canada you need to order GlycoNZ catalogue products from Merck-Millipore (The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada) https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/marketing/global/documents/144/274/glyconz-intro-ca6885en-mk.pdf NOTE: We have no involvement in the prices set by Merck-Millipore, any discounts offered or not, or payment terms.
5.2	Order GlycoNZ product direct from GlycoNZ – email us at office@glycoNZ.com to request a quote, or to submit a purchase order. English is the only language we use.
5.3	GlycoNZ Payment terms for verified buyers are **30 days after shipping of product from NZ**. (unless agreed otherwise in writing)
5.4	If your organisation is unable to pay via an emailed invoice, (i.e. you require registration by us into your accounting or e-invoicing system) or pay by credit card in NZ currency (via a Pay Station (https://paystation.co.nz) invoice in NZ$, see also 5.8) then we are unable supply you. You will need to purchase your products from Merck. If a complex **supplier onboarding process is required for us please discuss with us before placing an order**.
5.5	We cannot accept payment by cheque (the NZ banking system no longer accepts them)
5.6	Payment is always in USD (unless agreed otherwise) or making payment by credit card, then it is in NZ$

5.7	Payment is by USD bank transfer – these instructions are on the invoice, and additional information may be requested by emailing us at office@glyconz.com (note if your accounting system does not allow for the bank account suffix of USD the replace USD with 000)
5.8	By prior arrangment credit card payment can be made via a Pay Station (https://paystation.co.nz) invoice. Note this credit card transcation is in NZ$, and an invoice converted into NZ$ will be issued. Note this form of payment also incurs an additional 5.0% transaction fee.
5.9	Sorry discounts are not available unless you are ordering very large quantities of material (>10 of the same construct) and we have pre-agreed, and then with a maximum of 10% discount.
5.10	There is a minimum order value of $250 (if less then a handling fee of $50 is applied)
5.11	A standard shipping fee of $120 is applied unless the buyer provides their own FedEx or DHL account number, then no charge is made
5.12	Incoterm is DAP – deliver at place. GlycoNZ is responsible for arranging carriage and for delivering the goods, ready for unloading from the arriving means of transport, at the named place. Risk transfers from seller to buyer when the goods are available for unloading; so unloading is at the buyer's risk. The buyer is responsible for import clearance and any applicable local taxes or import duties and tariffs.

6.	**What documentation is available for GlycoNZ products?**
	Related questions: A.3
6.1	Most GlycoNZ product documentation is available online http://www.glyconz.com/documents/
6.2	Specifications and Safety Data Sheets Product specifications and safety data sheets (SDS) are provided for customer use. The information contained in each SDS is based on the data available to GlycoNZ and is believed to be accurate. Each SDS available through this webpage is the latest version available but may not have been updated with information that has recently become available after publications.
6.3	CoA – certificates of Analysis are available upon request – email office@glycoNZ.com
6.4	FAQ (this document)
6.5	Also see documents available from Merck Millipore Sigma (see above)

7.	**What do the GlycoNZ products look like?**
	Related questions:
7.1	Most GlycoNZ products are packaged in plastic cryo vials (Cryo.S PP, Greiner Bio-One GmbH), with a colored cap (the cap colour has no relevance). However, some glycoconjugates may be in a range of different containers (including glass vials) depending on where they were manufactured.
7.2	A typical label looks like the example below and has the following fields Trivial product label name (will be in many different formats – see CoA/Specification) Catalogue#: A catalogue number Unit: the number of mg of product per vial Lot: the manufacturing batch number – variable formats depending on source Labels are printed on Avery Heavy Duty Identification Inkjet Labels J4776. These labels are tough (tear proof) and resist oil, dirt and water and suitable for use between -20 to +80 degrees Celsius. 
7.3	The product in the vial usually appears as a freeze-dried powder but variations exist (see below)
7.4	Acceptable product variations include 1. Small dry spot, grain or flake (often glass-like) – and sometimes in the cap 2. Coloured – coffee coloured is typical for BP-series, while fluorophores will have colour 3. Spotting on bottom of vial 4. Thin film (maybe invisible – you will be notified if your product looks like this, but be assured it is present in the vial)

7.5	We inspect all product vials before shipment to ensure they are OK
7.6	**It is reasonable common for the product to be lodged inside the cap.** During shipment product may move inside the container, so always carefully check no product is stuck in the lid/cap of the vial (and take high precautions when opening vial). Always reconstitute product with the lid on, and thoroughly mix to capture any product on wall in in the lid. Centrifude to recover any reconsituted product remaining in the cap.

8.	**What are shipping and storage temperature requirements for GlycoNZ products?**
	Related questions:
8.1	Product can be shipped at ambient temperature for up to 4 weeks
8.2	**BM series** Monomeric biotinylated glycans in dry form in the dark are stable at -18°C for more than ten years and at room temperature up to three months. Aqueous solutions at -18°C are stable more than one year, at +4°C at least two months, and at room temperature at least 48 h.
8.3	**PA, BP and FP conjugates** Polyacrylamide glycoconjugates as dry compounds are stable in the dark at -18°C for more than ten years.Conjugate #0058 PA (Neu5Acα2-6GalNAcα-sp3, SiaTn) was stored at -18°C for 17 years. Comparison ofthis sample with a freshly prepared one by TLC (silica gel plates, in three different eluents) showed fullidentity. There were no any signs of degradation (e.g. sialic acid cleavage). At ambient temperature in the dark, dry glycoconjugates are stable for at least six months. There was a case when #0065 PA (Lewis-A trisaccharide 3'-sulfate) conjugate was “lost” by UPS and returned six months later without any trace of degradation (storage conditions unknown). We recommend long-term storage of PAA conjugates at -18°C, and short-term storage at +4°C.
8.4	**FF series** (Affinity adsorbents of Sug-PAA conjugated to aminated Sepharose6FF). Ship at room temperature (25°C/77°F). Store resin in a 20% ethyl alcohol buffer at 4°C. **Do not freeze**
8.5	A survey of our biologists The following opinion about stability of aqueous solutions of various Glyc polyacrylamide conjugates (according to reproducible results in ELISA and FACS analysis) under the following storage conditions: PA, BP and FP aqueous solutions (in the dark) are stable at -18°C for more than one year. PA, BP aqueous solutions are stable at +4°C at least six months. FP aqueous solutions (in the dark) are stable at +4°C ~1 week. PA, BP and FP aqueous solutions are stable at room temperature at least 48 h. Repeated freezing/thawing of aqueous solutions does not affect the properties of polyacrylamide glycoconjugates.
8.6	**Summary, we advise**: Conjugates of sialoglycans and sulfated glycans are less stable in the aqueous media (especially in unbuffered solvents and distilled water) than the others, we therefore recommend minimizing their storage time in the aqueous media. In general dry PA, BP and FP glycoconjugates until expiry date (-18°C), 1 year (+4°C), 6 months (RT) PA and BP aqueous solutions 1 year (-18°C) 6 months (+4°C) at least 48h (RT) FP aqueous solutions 1 year (-18°C) ~ 1 week (+4°C) at least 48 h (RT) BM aqueous solutions 1 year (-18°C) 2 months (+4°C) at least 48 h (RT)
8.7	**Expiry dates for products (provided correct storage)** 10-15 years for most glycoconjugates except sulfated and sialylated. 6 years for all sulfated and sialylated glycoconjugates 15 years for FSL series. Note: Some products may have shorter or longer dates

9.	**What are the rehydration/reconstitution and storage requirements for GlycoNZ products?**
	Related questions:
9.1	Without exception, all PAA conjugates are very soluble in water and in aqueous buffers
9.2	All buffers are suitable, except for borate one and strongly acidic solutions.
9.3	**Product can be dissolved in any aqueous buffer** Example buffer: 0.3M Sodium Phosphate Buffer (0.1M Na_2HPO_4, 0.2M NaH_2PO_4)

	1.42g Na_2HPO_4 (Mm = 141.96) and 2.4g NaH_2PO_4 (Mm = 119.98) diluted to 100mL with reagent grade water (adjust pH to 7.4)
9.4	Example 1 Rehydration: Add 0.5mL of Sodium Phosphate Buffer to 0.5mg vial of polymer in vial. Rotate until totally dissolved. Note: cap of vial is lined with Teflon and is relatively unreactive. May be diluted from this stock concentration to a working buffer such as PBS. Storage: This product may be stored for up to 5 days at 4°C. Thereafter, it should be stored frozen. Aliquoting the sample will avoid freeze/thaw cycles. When frozen, the product is stable for at least 1 year.
9.5	Example 2 Rehydration: Add 0.5mL of 50% Sodium Phosphate Buffer and 50% glycerol to 0.5mg vial of polymer in vial. Rotate until totally dissolved. Must be diluted from this stock to working buffers such as PBS. Use this method only with large dilutions to avoid interference with glycerol. Storage: Store at -20 °C. In 50% glycerol product will not freeze at -20 °C. This method avoids freeze/thaw cycles but must be controlled for glycerol content at low working dilutions.
9.6	We recommend keeping the aqueous solution at room temperature for no more than 8 hours, and at +4 degrees - no more than a week.
9.7	Storage of conjugates containing sialic acid or sulfate residues in distilled water should be avoided, as it is often acidified with carbon dioxide. If you have dissolved the conjugate in water, then for subsequent storage it can be kept at -20 °C or lyophilized.
9.8	FF series (Affinity adsorbents of Sug-PAA conjugated to aminated Sepharose6FF). Ship at room temperature (25°C/77°F). Store resin in a 20% ethyl alcohol buffer at 4°C. Do not freeze. Storage at RT for a maximum of one month is probably OK.

10.	**What quality control is done on GlycoNZ products?**
	Related questions:
10.1	All GlycoNZ products undergo rigorous QC checks during synthesis and as a final product
10.2	**BM series** 1) NMR spectrum of the original glycan to be conjugated; 2) NMR spectrum of the final product; 3) Ability to bind to streptavidin-coated plates.
10.3	**PA & BP series** We do not routinely record NMR spectra for PAA conjugates, since the resolution of peaks in polymer molecules is poor, and the meaning of such spectra is lost. The quality of conjugate synthesis (the conjugation completeness) is controlled by the sum of the following data: 1) NMR spectrum of the original glycan to be conjugated; 2) comparison of the mass of the isolated PAA conjugate with the calculated one; 3) the completeness of the disappearance of the starting glycan from the reaction mixture, using a very sensitive TLC ninhydrin method. If we see compliance with expectations on all three points, then we consider that the conjugate corresponds to the expected composition. The PAA polymer used is always the same (one large batch) for the synthesis of all PAA conjugates
10.4	**TLC** Biotinylated PAA conjugates (BP) are prepared by coupling of amino spacered glycans and biotin with poly(4-nitrophenyl-acrylate) ~20 kDa, and subsequent treatment with ethanolamine. TLC is used to control the completeness of binding of glycan and biotin to the polymer prior to its treatment with ethanolamine. However, TLC is not suitable for characterization of the polymer, because of its wide molecular mass distribution it moves on the plate as a strip. The information from TLC is thus to determine the absence of starting (free, unconjugated) saccharide and biotin in the reaction mixture.

11.	**What are the different spacers that are used?**	
	Related questions:	
	For most of the chemically synthesized glycans, the sp3 (C3) spacer is used. In some glycans (usually obtained biosynthetically), the spacer is introduced at the last step, and then it is then sp4.	
sp2	C2	$-O(CH_2)_2NH-$
sp3	C3	$-O(CH_2)_3NH-$
sp4	glycylamino	$-NH(CO)CH_2NH-$

sp5	long	$-O(CH_2)_3NH-CO(CH_2)_5NH-$
sp8	PEG_6	$-(OCH_2CH_2)_6NH-$
sp10	PEG_2	$-(OCH_2CH_2)_2NH-$
Ad	$-OC(CH_2)_4CO-$	For FSL spacers see Kode eBook https://hdl.handle.net/2292/62953
CMG(2)		For FSL spacers see Kode eBook https://hdl.handle.net/2292/62953

12.	**What is the molecular weights of the glyconjugates?**
	Related questions: 2.2, 17
12.1	When we talk about molecular weight, in fact this refers to the relative size of the molecule, as determined by gel permeation HPLC, dynamic light scattering or a zeta sizer, and not the true molecular mass. Indeed, the modification of the bare backbone of PAA with 20 molar percent (mol%, i.e., every fifth monomeric unit is modified) of a disaccharide ligand increases the MM by a factor of two, whereas with a decasaccharide by a factor of six. Nevertheless, the relative MW (actually – molecular size) measured by the mentioned above methods turned out to be similar, because actually measured is the hydrodynamic size of the molecule, which depends on the length of the polymer chain more than on the size of the pendant groups. The true molecular mass of Glyc-PAA is illusory, 20 (or 1000) kDa is a value averaged for the continuum of larger and smaller polymers. This is why we generally avoid using the term “number of Glyc residues in a single molecule”; instead, more accurate and obviously correct value is the content as X mol per mg, which is convenient for calculating concentrations, stoichiometry, etc. The conventional loading of Glyc ligands is 20 mol%, this is enough for a reasonably high density of Glyc residues along the backbone, formally (in an extended conformation) the average distance between adjacent residues is ~ 1 nm. Comparison of the series 5, 10, 20, 30 and 40 mol% demonstrated that the optimal density in relation to the affinity for glycan-binding proteins is case-specific, but 20% loading was optimal in most cases. Similarly, it was found that the optimal content of the biotin tag is 5 mol%; “optimal” in this case means the signal/noise ratio in the assays, where Glyc-PAA-biot (in combination with the streptavidin reagent in the next step) is used as a tracer of immobilized glycan-binding protein. For Glyc-PAA-fluo probes, the 1% content of fluorescein (fluo) residue turned out to be the best in the FASC analysis of cell surface lectins and fluorescent microscopy. Initially found for 30 kDa probes, all the percentages mentioned above turned out to be optimal for 1000 kDa probes. For less common labels/tags, it is easy to find the percentage that is optimal for a specific analysis using microscale synthesis of a series with its different content.

13.	**Where is the biotin residue on BP glyconjugates?**
	Related questions: 2
13.1	The biotinyl residue BP, unlike biotin as a vitamin, is devoid of the ionized carboxylate group and, therefore, is an apolar fragment, especially if it is (as usual) extended by a C6 spacer. There are approx. ten of biot residues distributed randomly in the composition of the glycopolymer 20 kDa with a standard 5 mol% biot, whereas in the variant 1000 kDa – proportionally more. This suggests a risk of non-specific binding, especially in complex media, such as serum; non-specific interaction is enhanced due to the large number of copies of the tag in the polymer chain. Indeed, when biotinylated probes were directly compared with fluorescein analogues

14.	**What can I use as a negative control ?**
	Related questions:
14.1	We do not recommend the use of sugar-free biotinylated PAA as a negative control due to the hydrophobicity of the biotin (in the absence of a hydrophilic sugar ligand). Similarly for Glyc-PAA-fluo assays (FP-series), the hydrophobicity of fluorescein is compensated by the hydrophilicity of the Glyc residue. There is no universal negative control for these BP & FP assays; we always use several probes with different Glyc residues. As a control, it is better to choose a suitable (preferably two) monosaccharide or disaccharide conjugate. As an example: if you're working with sialyl-lactosamine conjugate, a good control is just lactosamine-PAA-biot (no sialic acid), or biotinylated PAA with some kind of monosaccharide. The Glycan should not interact for general reasons, but should be of similar size (i.e. the negative control

	should not be a monosaccharide for a tetrasaccharide) and charge (one of the two controls should have the same charge as the Glyc being studied). Empty wells are not an appropriate negative control.
14.2	0000-PA, or 0000-BP (its ligand is aminoglucitol) can be used as a "PAA" negative control. Alternatively you can use your own PAA polymer without ligand, i.e. N-(2-hydroxyethyl)polyacrylamide.

15.	**What is the nomenclature for the PAA polymers are we using?**
	Related questions:
15.1	The first years of using polyacrylamide glyco-probes, especially in cell glycobiology, shown that the backbone with the $–CONHCH_2CH_2OH$ pendant groups is slightly better than the simplest $–CONH_2$ version in relation of non-specific binding, as a result practically all the following probes were and remain derivatives of 2-ethanolamide. Thus, the abbreviation "PAA" and "polyacrylamide" used for simplicity, actually mean poly[N-(2-hydroxyethyl)acrylamide]; Glyc-PAA according to strict nomenclature is a co-polymer of poly[N-(2-hydroxyethyl)acrylamide], while Glyc-PAA-label is triple co-polymer. Their formulas should be designated as $–[(Glyc\text{-}sp\text{-}CO)CH\text{-}CH_2]_n\ [(HOCH_2CH_2NHCO)CH\text{-}CH_2]_m–$ or $–[(Glyc\text{-}sp\text{-}CO)CH\text{-}CH_2]_n\ [–(label\text{-}sp\text{-}CO)CH\text{-}CH_2]_w\ [(HOCH_2CH_2NHCO)CH\text{-}CH_2]_m$

16.	**What is the methodology for EIA (PAA & FSL)?**
	Related questions: A.3
16.1	**ELISA coating buffer:** 15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH9.6. Many other buffers perform just as well, but traditionally we use this one.
16.2	**EIA with PA series (example method)** MaxiSorp microtiter plates (Nunc, ThermoFisher Scientific, Denmark) are coated with glycan-PAA in Na-carbonate buffer ($Na_2CO_3/NaHCO_3$, 0.05 mol/L pH 9.6) 10 µg/mL, 60 µL per well for 60 minutes at 37°C and washed. The plates are blocked with 1% BSA in PBS, 60 µL/well, for 45 minutes at 37°C. Two-fold serial dilutions of blood sera (initial dilution is x20) or affinity-purified human blood antibodies (initial concentration 1-2 µg/ml) with PBS containing 0.3% BSA are added to the wells (50 µL/well) of plate and incubated for 60 min at 37°C. Then HRP-labeled anti-human IgM,G,A (Southern Biotechnology, USA) or HRP-labeled anti-human IgG (Invitrogen, USA), taking into account the working dilutions 1:4500 and 1:12000, correspondingly, in PBS containing 0.3% BSA are added to the wells (50 µL/well) of plates and incubated for 60 minutes at 37°C. Color is developed by a 20-minute incubation at room temperature in 0.1 mol/L sodium phosphate / 0.1 mol/L citrate buffer containing 0.04% of *o*-phenylenediamine and 0.03% of H_2O_2. The color reaction is stopped by the addition of 1 mol/L H_2SO_4. The absorbance is read at 492 nm with a multitask plate reader. Between each stage the plates are washed four times with PBS containing 0.1% Tween-20. All the tests are performed at least in duplicate; the differences between readings (intra-assay) should not exceed 5%.
16.3	**EIA with BM (example method)** For the polystyrene coating Glyc-sp-biot (BM series), this is possible ONLY if the plate is pre-coated with streptavidin, otherwise sorption does not occur. With this method of immobilization, you will know exactly the amount of immobilized Glyc-sp-biot, since the coating is quantitative (provided the amount of Glyc-sp-biot does not exceed the capacity of the Streptavidin plate, which is known from the manufacturer).
16.4	**EIA with FSL-constructs (example method)** PolySorp microtiter plates (Nunc, ThermoFisher Scientific, Denmark) are coated with peptide-FSL or glycan-FSL in PBS (0.15 mol/L, pH 7.4), not less than 60 pmol/well (60 µL/well) for 120 minutes at 37°C, and washed. The plates are blocked with 1% BSA in PBS, 60 µL/well, for 45 minutes at 37°C. Two-fold serial dilutions of blood sera (initial dilution is x5), or affinity-purified human blood antibodies (initial concentration 2 µg/ml) in PBS containing 0.3% BSA, are added to the wells (50 µL/well) of plate and incubated for 60 min at 37°C. Then HRP-labeled anti-human IgG antibodies (Invitrogen, USA, working dilution 1:12000 in PBS containing 0.3% BSA), 50 µL/well, are added to the plates and incubated for 60 minutes at 37°C. Color is developed by a 20-minute incubation at room temperature in 0.1 mol/L sodium phosphate / 0.1 mol/L citrate buffer containing 0.04% of *o*-phenylenediamine and 0.03% of H_2O_2. The color reaction is stopped by the addition of 1 mol/L H_2SO_4. The absorbance is read at 492 nm with a multitask plate. Between each stage the plates are washed four times with PBS containing 0.1%

	Tween-20. All the tests are performed at least in duplicate; the differences between readings (intra-assay) should not exceed 5%.

17.	**Can we compare quantitatively the data obtained with different biotinylated/fluorescent polymeric probes?**
	Related questions: 12
17.1	Yes. Starting polymer, i.e. 20 kDa poly(4-nitrophenylacrylate), is modified in the first step with cadaverine-fluoresceine (1 mol%) with quantitative yield. Gram quantity of this polymer is stored as stock solution, material for synthesis of all glycopolymers is taken from this batch. At the second stage, Glyc-sp-NH2 is attached to the fluo-labeled polymer, this reaction occurs in ~100% yield due to the large excess of activated COOH groups over the Glyc-sp-NH2 component, the long period of time given for reaction (24 hrs) and elevated temperature (40°C). In all cases, completeness of the reaction is controlled by: (i) thin-layer chromatography monitoring for absence of Glyc-sp-NH2 in the final reaction mixture (ninhydrine probe), and (ii) correspondence of purified product weight to theoretical value. All glycoprobes have identical molecular weight because are based on the same batch of initial polymer.

18.	**Is BSA appropriate to use for an ELISA where I am trying to detect anti-Neu5Gc antibodies? I ask because I thought BSA contained Neu5Gc glycans, and if so, this could interfere with the assay.**
	Related questions:
18.1	As A.Varki wrote, "for example, bovine serum albumin (BSA) is a common blocking reagent in many applications, including ELISAs. However, although BSA is not itself sialylated, even highly-purified preparations are contaminated with other bovine serum glycoproteins that contain Neu5Gc glycans (unpublished observations). These can absorb out anti-Neu5Gc antibodies, resulting in a markedly lower observed signal for anti-Neu5Gc antibodies. Many other commonly used blocking agents (e.g., milk or bovine/porcine gelatin) suffer from the same problem. To overcome this issue, we have been using chicken ovalbumin (15) or fish gelatin (24) as blocking reagents, since they both lack Neu5Gc and thus do not interfere with detection assays. Using such methods we detected a broad and variable spectrum of anti-Neu5Gc antibodies of IgM, IgG and IgA constituting ~0.1% of total Igs, ranging at 0.1–23 μg/ml against several potential targets, some at levels similar to anti-α-Gal antibodies" (see doi: 10.1111/j.1399-3089.2011.00622.x). In fact, we had serious problems with calf serum in our work, but not with BSA, perhaps because we were lucky and worked with well-purified BSA."

19.	**Can I add oligosaccharides as a carbon source to the microbial culture medium and observe whether the bacteria can use this sugar chain for metabolic process?**
	Related questions:
19.1	Yes. An example would be using (0042) Fucα1-2Galβ1-3GlcNAcβ-sp-biot. After incubation with bacteria, it will turn into defucosylation and degalactosylation products, Galβ1-3GlcNAcβ-sp-biot and GlcNAcβ-sp-biot, all deglycosylation products and the starting Fucα1-2Galβ1-3GlcNAcβ-sp-biot can be isolated using a cartridge with a C18 sorbent, and further composition of the mixture can be determined using TLC or MS. We can also supply the standards for this, i.e., Galβ1-3GlcNAcβ-sp-biot and GlcNAcβ-sp-biot if required.

20	**Is BP of FP better for studying living cells?**
	Related questions:
20.1	For the study of living cells, the FP series is the primary and preferred choice for two reasons: 1) biotin residues are quite hydrophobic, and there are five times more of them than fluorescein residues; 2) a two-step process (incubation with a biotin probe - washing - incubation with labeled avidin) is not only an additional time, but also a risk of injuring cells. However, it is sometimes necessary to use a two-step process if the fluorescein label is not suitable due to suboptimal wavelength or because it fades (burns out) quickly; avidin can be taken with any fluorescent label. A similar case is when one needs to incubate a pair of probes with different labels with a cell; then fluorescein and biotin can be used.

	There is an alternative approach in order to avoid a two-step process - this is a custom synthesis of a probe or probes with other, non-fluorescein (for example, red) labels

21	**Can GlycoNZ products be coupled with SA biosensor chip for Surface Plasmon Resonance experiment applications?**
	Related questions:
21.1	Glycoconjugates of the Glyc-PAA-biot (BP series) type can be easily attached to a streptavidin-coated SPR sensor, but we do not recommend doing this, since the distance between the surface and the glycan becomes unfavorably large (streptavidin + PAA), our experience with influenza virus analysis was negative. It is better to use a non-polymeric version (Glyc-biot, BM series), and even better to immobilize the glycan chemically directly, on the activated SPR chip.

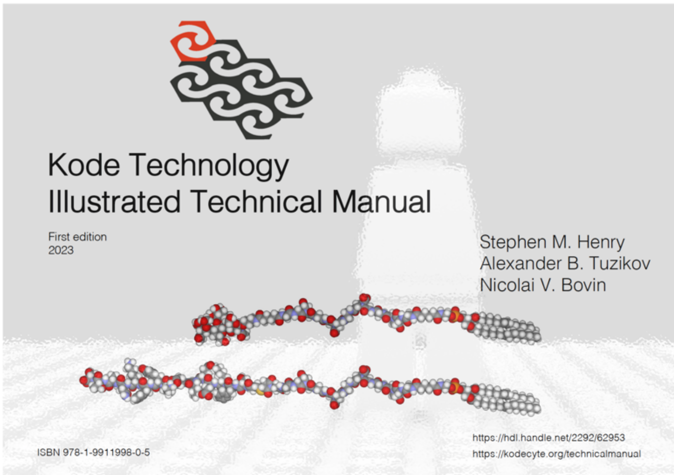
22	**What products in the GlycoNZ catalogue are known or potential influenza probes?**			
	Related questions: 11 (spacers)			
22.1	The following list are known and potential influenza probes available as glycoNZ products . A = avian, H = human, ? = uncertain, less studied.			
22.2	Cat#	Ligand	Short name	Species
	72	Neu5Acα2-8Neu5Acα-sp3	(Neu5Acα)$_2$-C3	?
	83	Neu5Acα2-3Galβ-sp3	Neu5Acα3Galβ-C3	A
	84	Neu5Acα2-6Galβ-sp3	Neu5Acα6Galβ-C3	H
	709	Neu5Gcα2-3Galβ-sp3	Neu5Acα(Gc)3Galβ-C3	A
	983	Neu5Gcα2-6GalNAcα-sp3	Neu5Gcα6GalNAcα-C3	?
	993	Neu5Acα2-3GalNAcα-sp3	Neu5Acα3GalNAcα-C3	A
	36	Neu5Acα2-3Galβ1-4GlcNAcβ-sp3	Neu5Acα3'LN-C3	A
	54	Neu5Acα2-3Galβ1-3GlcNAcβ-sp3	Neu5Acα3'Le^c-C3	A
	60	Neu5Acα2-3Galβ1-4Glcβ-sp4	Neu5Acα3'Lac-Gly	A
	63a	Neu5Acα2-6Galβ1-4Glcβ-sp3	Neu5Acα6'Lac-C3	H
	96	Neu5Acα2-3Galβ1-3GalNAcα-sp3	Neu5Acα3'TF-C3	A
	705	Neu5Acα2-6Galβ1-3GlcNAcβ-sp3	Neu5Acα6'Le^c-C3	H
	706	Neu5Acα2-6Galβ1-3(6-O-Su)GlcNAcβ-sp3	Neu5Acα6'(6-su)Le^c-C3	?
	730	Neu5Gcα2-3Galβ1-3(6-O-Su)GlcNAcβ-sp3	Neu5Gcα3'(6-su)Le^c-C3	A
	731	Neu5Gcα2-3Galβ1-4(6-O-Su)GlcNAcβ-sp3	Neu5Gcα3'(6-su)LN-C3	A
	760	Neu5Acα2-6Galβ1-3GalNAcα-sp3	Neu5Acα6'TF-C3	?
	785	Neu5Acα2-3(6-O-Su)Galβ1-3GalNAcα-sp3	Neu5Acα3'(6'-su)TF-C3	A
	839	Neu5Acα2-3(6-O-Su)Galβ1-4GlcNAcβ-sp3	Neu5Acα3'(6'-su)LN-C3	A
	908	Neu5Acα2-6Galβ1-4(6-O-Su)GlcNAcβ-sp3	Neu5Acα6'(6-su)LN-C3	?
	917	Neu5Acα2-3Galβ1-3(6-O-Su)GalNAcα-sp3	Neu5Acα3'(6-su)TF-C3	A
	949	Neu5Acα2-3Galβ1-3(6-O-Su)GlcNAcβ-sp3	Neu5Acα3'(6-su)Le^c-C3	A
	951	Neu5Acα2-3Galβ1-4(6-O-Su)GlcNAcβ-sp3	Neu5Acα3'(6-su)LN-C3	A
	956	Neu5Gcα2-3Galβ1-4GlcNAcβ-sp3	Neu5Gcα3'LN-C3	A
	957	Neu5Gcα2-6Galβ1-4GlcNAcβ-sp3	Neu5Gcα6'LN-C3	?
	962	Neu5Gcα2-3Galβ1-3GlcNAcβ-sp3	Neu5Gcα3'Le^c-C3	A
	975	Neu5Gcα2-3Galβ1-4Glcβ-sp3	Neu5Gcα3'Lac-C3	A
	984	Neu5Acα2-6(Galβ1-3)GalNAcα-sp3	Neu5Acα6TF-C3	H
	997	Neu5Acα2-6Galβ1-4GlcNAcβ-sp3	6'SLN-C3	H
	17	Neu5Acα2-3(6-O-Su)Galβ1-4(Fucα1-3)GlcNAcβ-sp3	Neu5Acα3'(6'-su)Le^x-C3	A
	20	Neu5Acα2-3Galβ1-4(Fucα1-3)(6-O-Su)-GlcNAcβ-sp3	Neu5Acα3'(6-su)Le^x-C3	A
	61	Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAcβ-sp3	SiaLe^a-C3	A
	62	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ-sp3	SiaLe^x-C3	A
	757	Neu5Acα2-6(Fucα1-2)Galβ1-4GlcNAcβ-sp3	Neu5Acα6'(Fucα2')LN-C3	?
	850	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ-sp3	3'SLN3Galβ-C3	A
	898	Neu5Acα2-8Neu5Acα2-3Gaβl-4Glcβ-sp4	(Neu5Acα)$_2$-3'Lac-Gly (GD3)	?
	925	Neu5Aα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-sp3	6'SLN3'LN-C3	H

	987	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-sp4	11-OS	H

23	**What GlycoNZ products are suitable for binding to Norovirus?**
	Related questions:
23.1	We have experimental data only for GII.4, it binds very well to blood group B(type 3) tetrasaccharide Gala1-3(Fuca1-2)Galb1-3GalNAca. Assuming that Noroviruses prefer to bind to antigens of the ABO system, we recommend testing antibody blocking with all variants of synthetic ABH oligosaccharides in the form of PAA derivatives (PA series in our catalog).
23.2	The catalogue list of available ABH glycans is 0010 Galα1-3Galβ1-4Glcβ-sp2 0042 Fucα1-2Galβ1-3GlcNAcβ-sp3 0059 Fucα1-2Galβ1-3GalNAca-sp3 0070 Galα1-3Galβ1-4GlcNAcβ-sp3 0080 Fucα1-2Galβ1-3GalNAcβ-sp3 0085 GalNAcα1-3(Fucα1-2)Galβ-sp3 0085a GalNAcα1-3(Fucα1-2)Galβ-sp5 0086 Galα1-3(Fucα1-2)Galβ-sp3 0086a Galα1-3(Fucα1-2)Galβ-sp5 0089 Fucα1-2Galβ1-4GlcNAcβ-sp3 0747 GalNAcα1-3Galβ1-4GlcNAcβ-sp3 0006 GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ-sp3 0007 Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ-sp3 0009 Galα1-3Galβ1-4(Fucα1-3)GlcNAcβ-sp3 0721 GalNAcα1-3(Fucα1-2)Galβ1-3GalNAcβ-sp3 0755 GalNAcα1-3Galβ1-4(Fuα1-3)GlcNAcβ-sp3 0816 GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ-sp3 0847 Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ-sp3 0848 Galα1-3(Fucα1-2)Galβ1-3GalNAcα-sp3 0849 Galα1-3(Fucα1-2)Galβ1-3GalNAcβ-sp3 0857 Galα1-3Galβ1-4GlcNAcβ1-3Galβ-sp3 0860 GalNAcα1-3(Fucα1-2)Galβ1-3GalNAcα-sp3 0968 Galα1-3(Fucα1-2)Galβ1-4(Fucα1-3)GlcNAcβ-sp3

24	**What Biotinylated glycopolymers and monomers selectively bind to CD33 and CD22?**
	Related questions:
25.1	CD22 prefers 2-6 sialoglycans, here we recommend #0997 Neu5Acα2-6Galβ1-4GlcNAcβ
25.2	CD33 is “promiscuous”, in early articles they wrote about a preference for 2-3 sialoglycans, in later ones - about an approximate parity between 2-3 and 2-6, but it is clear that their fucosylated versions are inactive. Our colleagues have their own data (unpublished) according to which cells transfected with the Siglec-3 gene bound only benzylsialoside (# 035a). Neu5Acα-sp9- It appears that the specificity of cellular recognition by Siglec-3 is strongly determined by the protein “context” and not just by the sialoligand. Therefore, in the case of CD33, we recommend testing several sialoglycans in your specific test system and choosing the best one for further studies.

APPENDIX

A.	What are the key references and published examples?
A.1	Tuzikov A, et al. 40 years of glyco-polyacrylamide in glycobiology. Glycoconj J. 2021 Feb;38(1):89-100. doi: 10.1007/s10719-020-09965-5. Epub 2021 Jan 14. PMID: 33443721. Glycoconjugate Journal (2021) 38:89–100 https://doi.org/10.1007/s10719-020-09965-5 REVIEW ARTICLE **40 years of glyco-polyacrylamide in glycobiology** Alexander Tuzikov[1] · Alexander Chinarev[1] · Nadezhda Shilova[1] · Elena Gordeeva[1] · Oxana Galanina[1] · Tatyana Ovchinnikova[1] · Marcel Schaefer[2] · Nicolai Bovin[1,2] Received: 21 August 2020 / Revised: 30 October 2020 / Accepted: 17 November 2020 / Published online: 14 January 2021 © Springer Science+Business Media, LLC, part of Springer Nature 2021 **Abstract** Polyacrylamide conjugates of glycans have long been widely used in many research areas of glycobiology, mainly for immobilizing glycans in solid-phase assays and as multivalent inhibitors. Pending biotin tag allows immobilizing Glyc-PAA quantitatively on any surface, and acts as a tracer for detection of carbohydrate-binding proteins. However, the scope of already realized capabilities of these probes is immeasurably richer than those listed above. This review is not so much about routine as about less common, but not less significant applications. Also, the data on the glycopolymers themselves, their molecular weight, size and polymer chain flexibility are presented, as well as the methods of synthesis, clusterisation and entropy factor in their interaction with proteins.
A.2	Bovin NV. Review: Polyacrylamide-Based Glycoconjugates as Tools in Glycobiology June 1998 Glycoconjugate Journal 15(5):431-46 DOI: 10.1023/A:1006963717646 Glycoconjugate Journal 15, 431–446 (1998) © 1998 Kluwer Academic Publishers. Manufactured in the U.K. **REVIEW** Polyacrylamide-based glycoconjugates as tools in glycobiology Nicolai V. Bovin *Shemyakin Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, Moscow GSP-7, 117871 Russia* **This review describes the synthesis, physicochemical characteristics and application for studying carbohydrate-binding proteins of polyacrylamide (PAA) type neoglycoconjugates. An approach to the synthesis of conjugates based on the interaction of activated polyacrylic acid with ω-aminoalkyl glycosides has been developed. Both the molecules of Glyc-PAA and the conjugates bearing various labels and effectors, as well as sorbents, and glycosurfaces can be designed using this method. Examples of the application of the conjugates as tools for the study of lectins, antibodies, and glycosyltransferases in glycobiology, cytochemistry and histochemistry are described along with the prospects of the further development of the presented approach in glycotechnology and medicine.**
A.3	Henry, S.M., Tuzikov, A.B., Bovin, N.V. (2023), Kode Technology Illustrated Technical Manual, First Edition. Kode Biotech. https://hdl.handle.net/2292/62953 

Series	application	references
PA	Immuno- and similar solid-phase assays, for coating of microplates with Glyc-PAA as a “capture” antigen or ligand. In study of glycan-binding proteins, lectins (including whole viruses and bacteria	Lopes, A., et al: Host specific glycans are correlated with susceptibility to infection by lagoviruses, but not with their virulence. *J. Virology* **92**, e01759-17 (2017). Meichenin, A.M., et al.: Tk, a new colon tumor-associated antigen resulting from altered O-glycosylation. *Cancer Res*. **60**, 5499-5507 (2000). Khraltsova, L.S. et al: An enzyme-linked lectin assay for α1,3-galactosyltransferase. *Anal. Biochem*. **280**, 250-257 (2000).
PA	As a multivalent form of the glycan. Works much better as an inhibitor of glycan-protein interactions	Meichenin, A.M., et al.: Tk, a new colon tumor-associated antigen resulting from altered O-glycosylation. *Cancer Res*. **60**, 5499-5507 (2000). Pochechueva, T.V., et al: P-selectin blocking potency of multimeric tyrosine sulfates in vitro and in vivo. *Bioorgan. Med. Chem. Lett*. **13**, 1709-1712 (2003). Obukhova, P., et al: Are there specific antibodies against Neu5Gc epitopes in the blood of healthy individuals? *Glycobiology* **30**, 395-406 (2020).
PA	In solid-phase assays and histochemistry as a “tracer” for revealing glycan-binding proteins	Weitz-Schmidt, G., et al: An E-selectin binding assay based on polyacrylamide-type glycoconjugates. *Anal.Biochem*.**238**, 184-190 (1996). Gabius, H.-J., et al: Reverse lectin histochemistry: Design and application of glycoligands for detection of cell and tissue lectins. *Histol.Histopathol*. **8**, 369-383 (1993). Rapoport, E.M., et al: Glycan-binding profile of DC-like cells. Glycoconj. J. 37, 129-138 (2020).
PA	As a reagent for glycan coating of various solid materials (chips including SPR, affinity adsorbents, beads, immunological plates, etc.)	Chinarev, A.A., et al: Biotinylated multivalent glycoconjugates for surface coating. Methods Mol. Biol. 600, 67-78 (2010). Matrosovich, M.N., Gambaryan, A.S.: Solid-phase assays of receptor-binding specificity. Methods Mol. Biol. 865, 71-94 (2012). Rye, P.D., Bovin, N.V.: Selection of carbohydrate-binding cell phenotypes using oligosaccharide-coated magnetic particles. Glycobiol. 7, 179-182 (1997).
PA	Instant *in situ* production of a soluble complex of the glycan with an enzyme, such as peroxidase	No refs.
FP	Flow cytometry studying cell surface lectins such as selectins, siglecs, galectins, DC-SIGN, etc. Search for unknown yet lectins in composition of viruses, eukaryotic and bacterial cells	Kurmyshkina, O., et al: Glycoprobes as a tool for the study of lectins expressed on tumor cells. *Acta Histochem*.**112**, 118-126 (2010). Rapoport, E.M., et al: Glycan recognition by human blood mononuclear cells with an emphasis on dendritic cells. *Glycoconj. J*. **35**, 191-203 (2018). Galanina, O., et al: Fluorescent carbohydrate probes for cell lectins. *Spectrochimica Acta*, Part A **57**, 2285-2296 (2001). Galanina, O.E., et al: Carbohydrate-based probes for detection of cellular lectins. *Anal. Biochem*. **265**, 282-289 (1998). Dutta, S., et al: Sulfated Lewis A trisaccharide on oviduct membrane glycoproteins binds bovine sperm and lengthens sperm lifespan. *J. Biol. Chem*. **294**, 13445-13463 (2019). Silva,E., et al: Lactadherin is a candidate oviduct Lewis X trisaccharide receptor on porcine spermatozoa. *Andrology* **5**, 589-597 (2017).
FP	Fluorescent microscopy and histochemistry: the study of glycan-	Galanina, O., et al: Fluorescent carbohydrate probes for cell lectins. *Spectrochimica Acta*, Part A **57**, 2285-2296 (2001).

	binding proteins of cells and tissues	
BM/BP	As substrates for glycosyl transferases	Mollicone, R., et al. Activity, splice variants, conserved peptide motif, and phylogeny of two new alpha1,3-fucosyltransferase families (FUT10 and FUT11). *J. Biol. Chem.*, **284**, 4723-4738 (2009). Patil SA, et al. Scaling down the size and increasing the throughput of glycosyltransferase assays: activity changes on stem cell differentiation. *Anal. Biochem.*, **425**(2),135-144 (2012).
BM/BP	Solid phase assays, SPR, glycan array	Pochechueva, T., et al. Multiplex suspension array for human anti-carbohydrate antibody profiling. *Analyst.* , **136**(3), 560-9 (2011). http://www.functionalglycomics.org/static/consortium/resources/resourcecoreh6.shtml
FSL	Cell/virus/solid phase	All FSL references are found in the Kode eBook https://hdl.handle.net/2292/62953 www.kodecyte.com
Glycochip	References **Video:** Olivera-Ardid, S., Khasbiullina, N., Nokel, A., Formanovsky, A., Popova, I., Tyrtysh, T., Kunetskiy, R., Shilova, N., Bovin, N., Bello-Gil, D., Mañez, R. Printed Glycan Array: A Sensitive Technique for the Analysis of the Repertoire of Circulating Anti-carbohydrate Antibodies in Small Animals. *J. Vis. Exp.* (144), e57662, doi:10.3791/57662 (2019). M.E.Huflejt, M.Vuskovic, D.Vasiliu, H.Xu, P.Obukhova, N.Shilova, A.Tuzikov, O.Galanina, B.Arun, K.Lu, N.Bovin. Anti-carbohydrate antibodies of normal sera: findings, surprises and challenges.*Molecular Immunology*, 46, 3037-3049 (2009). PMID: 19608278. T.Pochechueva, F.Jacob, D.R.Goldstein, M.E.Huflejt, A.Chinarev, R.Caduff, D.Fink, N.Hacker, N.V.Bovin, V.Heinzelmann-Schwarz. Comparison of suspension array, ELISA and printed glycan array in the detection of human anti-glycan antibodies. *Glycoconjugate J.*, 8-9, 507-517 (2011). PMID: 21948103. P.Obukhova, V.Piskarev, V.Severov, G.Pazynina, A.Tuzikov, M.Navakouski, V.Shilova, N.Bovin. Profiling of serum antibodies with printed glycan array: room for data misinterpretation. *Glycoconj. J.*,8-9, 501-505 (2011). PMID: 22057658. N.V.Shilova, M.J.Navakouski, N.Khasbiullina, O.Blixt, N.V.Bovin. Printed glycan array: antibodies as probed in undiluted serum and effects of dilution. *Glycoconj. J.*, 29, 87-91 (2012). PMID: 22258790. N.Bovin, P.Obukhova, N.Shilova, E.Rapoport, I.Popova, M.Navakouski, C.Unverzagt, M.Vuskovic, M.Huflejt. Repertoire of human natural anti-glycan immunoglobulins. Do we have auto-antibodies?*Biochim. Biophys. Acta*, *General Subjects*, 1820, 1373-1382 (2012). PMID: 22365885. Bovin N.V. Natural antibodies to glycans. *Biochemistry* (Moscow), 78, 786-797 (2013). Published in Russian in *Biochemistry (Mosc)*, 2013, 78, 1008-1022. PMID: 24010841. (English version available on request) N.Shilova, M.E.Huflejt, M.Vuskovic, P.Obukhova, M.Navakouski, N.Khasbiullina, G.Pazynina, O.Galanina, A.Bazhenov, N.Bovin. Natural antibodies against sialoglycans. *Top Curr Chem.*, Sept 14 (2013). PMID: 24037491.	

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Appendix B : WORKING WITH GLYCOCHIPS

How to work with blood serum (method outline)

- Obtain blood serum using standard protocol
- Block the remaining active groups on the glycochip surface by immersing in a blocking buffer according to activated slides manufacturer protocol. Wash chips by MilliQ (or bidistillated water) and dry with centrifugation or in nitrogen stream
- Pre-wet the microchips for 15 min in 0.1 M isotonic phosphate buffer, containing 0.1% of Tween 20 (0.1% PBS)
- Dilute blood serum at 1:15 in appropriate volume of 1% phosphate buffer saline, pH 7.4 (PBS), containing 3% BSA and incubate for 10 min at 37°C
- Centrifuge a sample for 15 min at 12 000 rpm
- Aspirate an upper part of sample and apply it onto microchip surface (1 ml per chip)
- Keep microchip on a shaker for 1 h at 37°C and 80% of relative humidity
- Wash the chips 0.05% PSB and add a 1 ml of a solution of biotinylated goat antibodies recognizing human immunoglobulins in appropriate dilution in 0.1% PBS or a solution containing fluorescently labeled goat antibodies recognizing human IgG, IgM or IgA (labeled with Alexa555 (or Cy3, or analogous) or Alexa647 (or Cy5, or analogous)) - in appropriate dilution in 0.1% PBS
- Incubate microchip on shaker (30 rpm) for 1 h at 37°C and 80% of relative humidity (in the dark in case of fluorescently labeled antibodies) and wash the chips with 0.05% PBS
- In case of biotinylated secondary antibodies add a solution of fluorescence labeled (Alexa 555 (or Cy3) or Alexa 647 (or Cy5) streptavidin - in appropriate dilution in 0.1% PBS and keep for 0.5 h at room temperature in the dark
- Wash the chips with 0.05% PBS followed by MilliQ (or bidistillated water)
- Dry microchips with centrifugation or in nitrogen stream
- Measure the intensity of fluorescence using a fluorescent reader/scanner
- Convert obtained images to Excel using reader's software and GAL-file (supplied)
- Analyze your data.

List of recommended fluorescence readers:

- Molecular Devices Axon (GenePix 4000/4100/4200/4300/4400 Series);
- Perkin Elmer (ScanArray, ProScanArray);
- TECAN (LS 200/300/400, LS Reloaded, Power Scanner);
- GE (Typhoon FLA 7000/9500);
- Ditabis (MArS);
- Sensovation (Flair, SensoSpot);
- Innopsys (InnoScan 710/910/1100);
- Agilent Technology (DNA Microarray Scanner G2565CA)

Data processing:

- Download ScanArray (the best software for image processing at this moment) from https://cloud.mail.ru/public/3TWA/5eb2u7EbX (only works on PC, not Mac)
- The .GAL file contains information about spot positions and glycan IDs. After applying of a Gal.-file a csv-file is generated. Then this file is converted to the xlsx-file containing the full information about structures and signals (using our Web-application). We will send you the corresponding link and instruction as soon as you receive the chips.
- The tool for the data processing is located in our Website https://semiotik-array.com/. We will send you the detailed instruction how to work with it and create your personal login and password as soon as you will start the work.
- the titer of bound antibodies, i.e. relative content of antibodies in a sample of blood/plasma is determined by the spot fluorescence intensity using a fluorescence reader;
- the protein in test should possess a direct fluorescent label, an alternative is labeled antibody against the protein;

- mathematical tools for the search of diagnostic signatures are described in Marko I. Vuskovic, Hongyu Xu, Nicolai V. Bovin, Harvey I. Pass and Margaret E. Huflejt Processing and analysis of serum antibody binding signals from Printed Glycan Arrays for diagnostic and prognostic applications. November 22, 2011pp 402-426 https://doi.org/10.1504/IJBRA.2011.043771
- clients use their own software for a deep data processing and plotting.

Control spots:

- Composition of the negative control spots: Array contains several negative control spots: 1) print buffer, 2) #629 Trehalose (not binding disaccharide) +ethanolamine, 3) blocking buffer. You can use each of them individually or you can calculate the average value.
- Positive controls: these spots contain streptavidin. Array contains: 1) fluorescently labeled BSA ("BSA-Cy3"), 2) streptavidin labeled with Alexa555 and streptavidin labeled with Alexa647, as a mixture ("FM").

See also references in Appendix

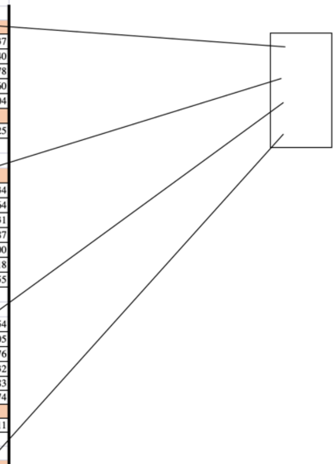
Video: Olivera-Ardid, S., Khasbiullina, N., Nokel, A., Formanovsky, A., Popova, I., Tyrtysh, T., Kunetskiy, R., Shilova, N., Bovin, N., Bello-Gil, D., Mañez, R. Printed Glycan Array: A Sensitive Technique for the Analysis of the Repertoire of Circulating Anti-carbohydrate Antibodies in Small Animals. *J. Vis. Exp.* (144), e57662, doi:10.3791/57662 (2019).

https://www.jove.com/video/57662

In addition, there is also a video at https://cloud.mail.ru/public/5AnX/5AYJqGeQn

Typical slide layout

432	334	Fmix	Fmix	496	230	491	481	421	329	Fmix	Fmix	304	Fmix
159	138	168	305	Fmix	436	410	282	34	825	823	340	435	337
80	15	77	114	66	75	Fmix	232	245	216	215	225	426	140
223	368	219	389	851	345	234	342	366	37	30	21	45	78
121	403	113	388	57	378	22	374	33	377	44	381	365	360
98	483	Fmix	812	535	536	59	6	65	53	52	Fmix	112	404
326	empty	89	88	60	100	73	97	239	101	105	252	217	Fmix
Fmix	505	Fmix	Fmix	504	150	193	203	145	143	print	195	Fmix	25
335	806	419	print	630	629	498	331	390	816	Fmix	392	Fmix	Fmix
395	371	363	309	131	165	130	181	129	198	128	817	338	434
Fmix	Fmix	62	Fmix	Fmix	Fmix	247	246	241	324	323	320	256	264
359	172	346	852	364	850	344	901	373	Fmix	81	Fmix	Fmix	31
362	184	396	189	166	179	277	136	369	199	379	376	361	387
85	321	14	267	828	295	931	268	855	293	854	829	191	200
512	empty	83	281	122	292	123	287	236	288	125	266	633	318
190	315	808	291	537	298	514	161	204	139	178	Fmix	Fmix	955
250	222	431	900	827	826	499	319	423	401	386	383	380	254
327	316	263	384	Fmix	63	20	99	96	107	17	19	227	205
118	303	302	300	322	260	Fmix	Fmix	Fmix	226	243	253	428	276
188	158	144	182	151	171	408	853	47	116	41	64	1	32
137	Fmix	164	Fmix	Fmix	328	275	255	310	Fmix	156	370	146	183
126	104	111	23	13	233	237	240	259	206	258	173	Fmix	174
36	empty	71	110	9	55	29	811	235	10	5	24	35	Fmix
170	809	290	485	297	479	274	142	194	169	Fmix	177	214	211
132	Fmix	441	Fmix	249	Fmix	Fmix	208	425	202	135	350	372	Fmix
242	229	Fmix	Fmix	154	38	162	16	153	18	167	48	133	72
93	117	76	46	800	632	813	627	495	493	508	534	429	231
492	818	820	294	482	278	block	Fmix	87	94	43	92	84	82
501	631	488	819	299	822	489	503	821	Fmix	7	3	86	502
106	197	Fmix	Fmix	180	196	192	58	Fmix	289	103	Fmix	438	437
844	empty	442	Fmix	2	26	61	50	251	102	51	28	49	4
Fmix	279	810	273	815	272	528	187	160	149	block	804	954	805
		Fmix	fluorescent control spots										

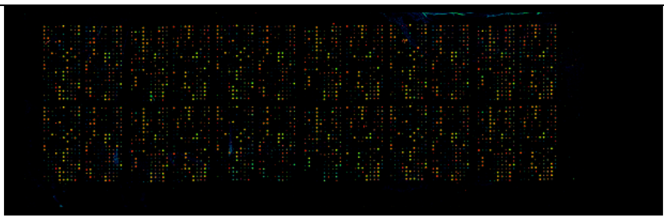


How to process microarray images

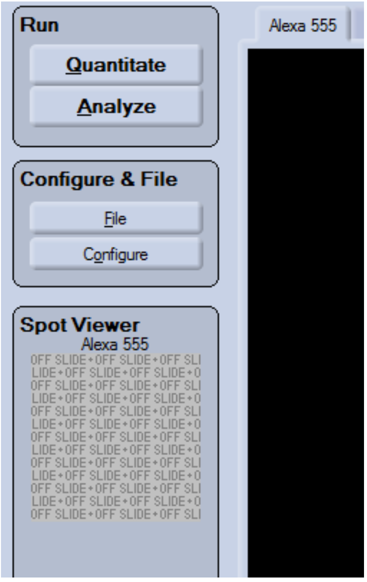
Please download ScanArray (the best software for image processing at this moment) from https://cloud.mail.ru/public/3TWA/5eb2u7EbX
and install it on your computer in mode «without connecting to a scanner»

Instructions for ScanArray

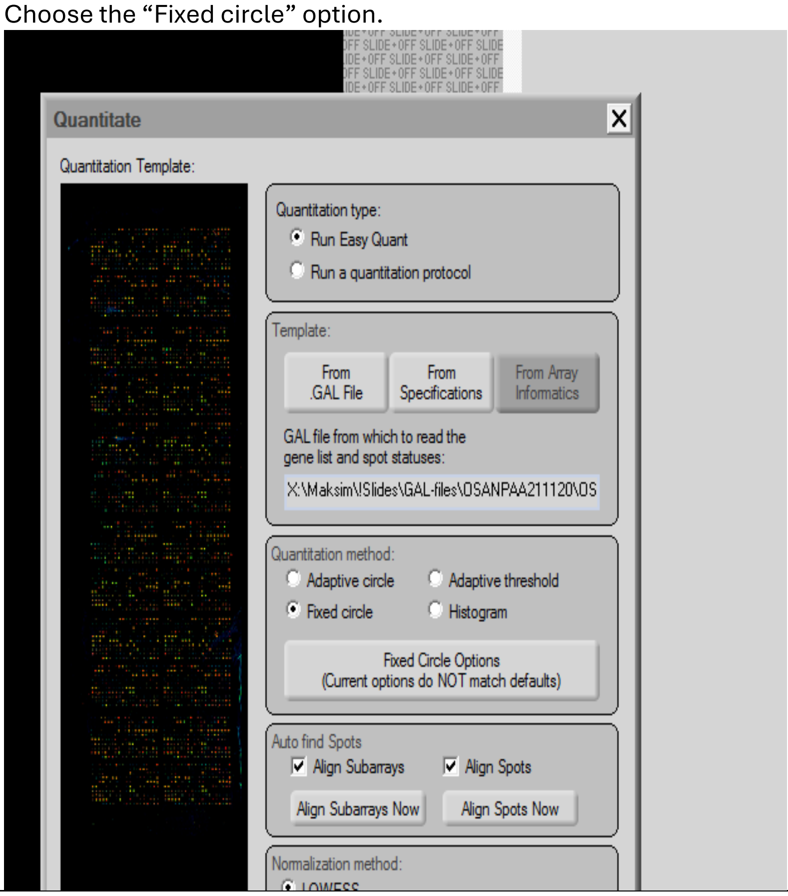
Open your tiff in ScanArray, choose a rainbow palette. See an example of image in rainbow palette below:



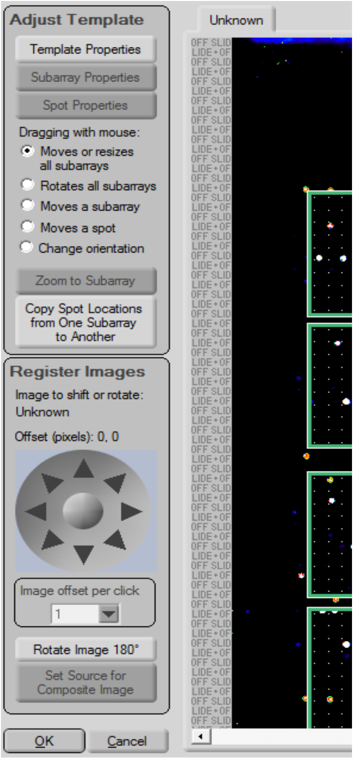
Press the Quantitate button.



Choose a GAL file pressing to “From . GAL File” button.
Delete all marks for Align Subarrays and Align spots.
Press Adjust Template and Register images button.
Choose the “Fixed circle” option.



Please note that the chip number should be located at the bottom. If necessary, press Rotate Image 180° (barcode faced down).



Firstly, adjust size and position of a 1st subarray according to your image. Press Moves a subarray and align all subgrids to right position (re-size the subarrays if necessary). Do not change an order of subgrids. Please use a bright control spots as a control points (see a file with print layout).

Press OK.
Remove the check marks from Align subarrays and Align spots and press Align Spots now. Wait a minute.
Press Adjust Template and Register images button again.
Choose Move a spot and align all non-aligned spots manually.
Press OK.
Press Start.
Save a resulted table as csv-file. Using of the microarray number and scanning parameter as a name of the file are strongly recommended.
Use Web-application Semiotik to convert csv to xls.

Instruction for Web-application Semiotik

- Open Web-site https://semiotik-array.com/.
- Scroll down and press (double click) .
- Fill the Login and Password rows.
  Login – XXXXX, Password – XXXX
  Press Enter.
- Press button Выберите файл (it's a WebApps bug, sorry) and Choose your csv -file obtained after the ScanArray software.
- Copy the name of the processed file.
- Past the file name in opened window and save your xlsx to the desired folder.