Cell Surface Glycosylation

-an indicator of the health of the cell

6th Bioanalytical School

INCTBio , Londrina , Brazil

29th November 2018

‘Biologics’ make up 7 out of the 10 best selling drugs in 2018

1. Humira® *autoimmune-RA*	12.05 *billion dollars*
2. Enbrel® *autoimmune-RA*	10.50 *billion dollars*
3. Remicade® *autoimmune-RA*	10.20 *billion dollars*
4. Advair/Seretide® *Beta-2 agonist*	9.25 *billion dollars*
5. Lantus® *insulin analogue*	9.00 *billion dollars*
6. Rituxan® (MabThera) *anti-cancer*	8.80 *billion dollars*
7. Avastin®	8.00 *billion dollars*
8. Herceptin® *anti-cancer*	7.90 *billion dollars*
9. Crestor® *cholesterol lowering statin*	6.80 *billion dollars*
10. Abilify® *anti-psychotic drug*	6.50 *billion dollars*

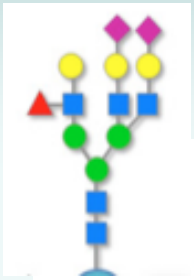
Green – Biopharma biologic **Blue** – Pharma chemical drug

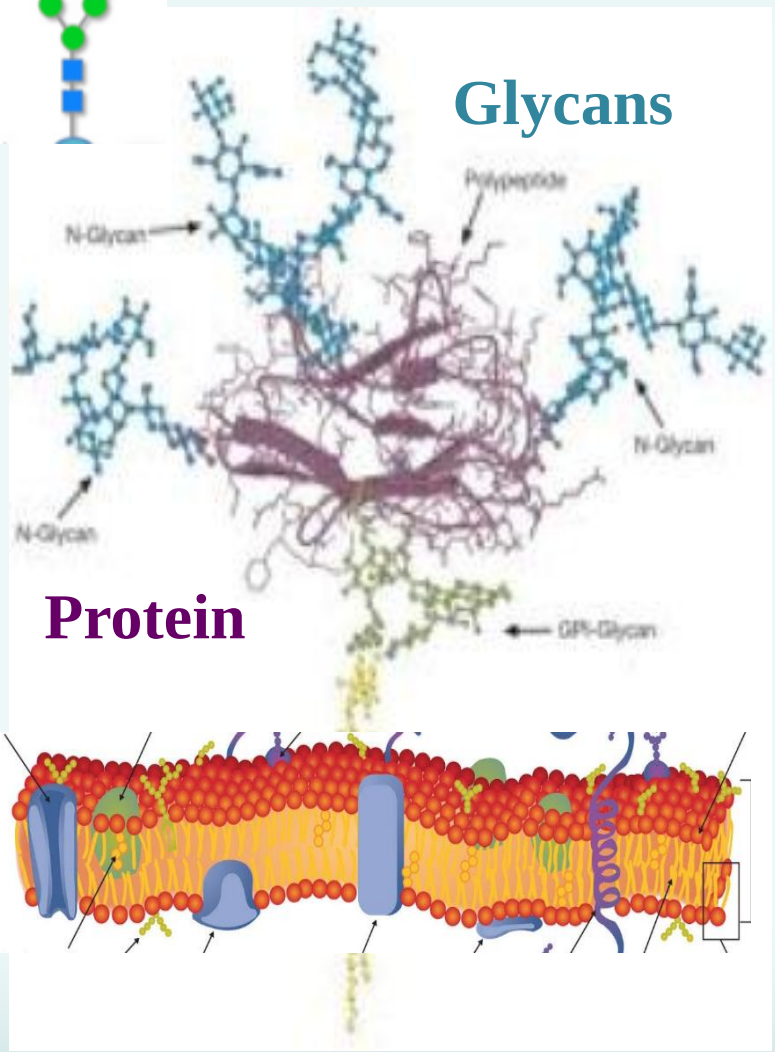
Why measure/monitor cell surface glycosylation of CHO cells ?

Flavio Ferreira

- **The Chinese Hamster Ovary (CHO) cell is the most commonly used cell to produce Biopharmaceuticals (*10,000 litre bio-reactors)***
- **Many of these batches fail – leading to high cost**
- **There is a need for a fast and accurate way to monitor the health of these cells in the bioreactor**
- **Changes in cell surface glycosylation are an early indicator of CHO cell health**
- **If this can be measured quickly then 'remedial' action may be taken in time to save the expensive bioreactor runs**

Cell Surface Glycosylation





The surface of all cells are covered in surface glycans

These surface glycans are nearly always attached to proteins

These surface glycans are involved in cell recognition , adhesion etc

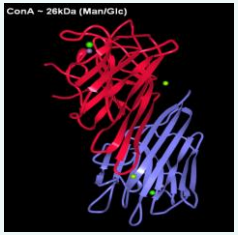
If the cell is stressed the surface Glycoprofile is often the to alter…….

Detecting glycosylation changes at the cell surface using novel 'lectin probes'

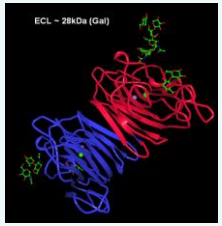
***Lectins** – proteins with high Glycan selectivity*

***Lectin probes** – labelled biotinylated lectins used to probe the glyco-surface of a cell*

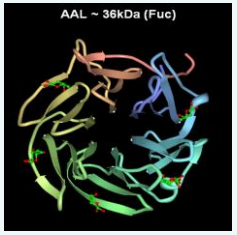
***Novel recombinant lectins** derived from micro-organisms*



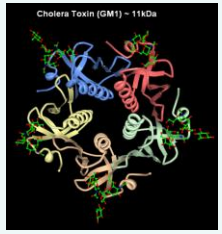
Lec A



Lec B



GafD



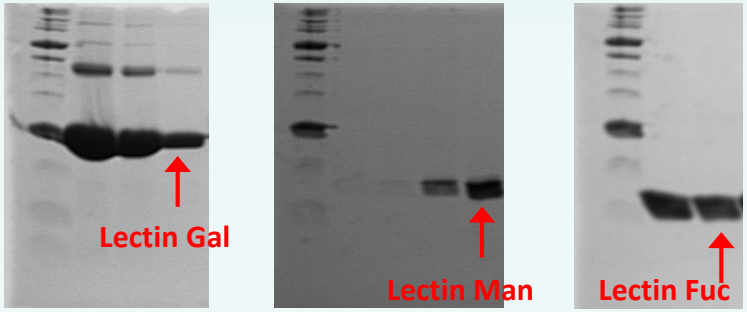
AAL-2

***Target** – the Chinese Hamster Ovary (CHO) cell*

Brendan O'Connor, Donal Monaghan, Jonathan Cawley . (2017) 'Lectin Affinity Chromatography'. ***Methods in Molecular Biology*** **, 1486, 411 - 420.**

Recombinant production of pure lectin proteins

- **Clone , express & purify lectins from bacterial host cells**



- **These lectins have the ability to selectively bind ; Manose, Fucose, GlcNAc ,GalNAc and Sialyic acid**

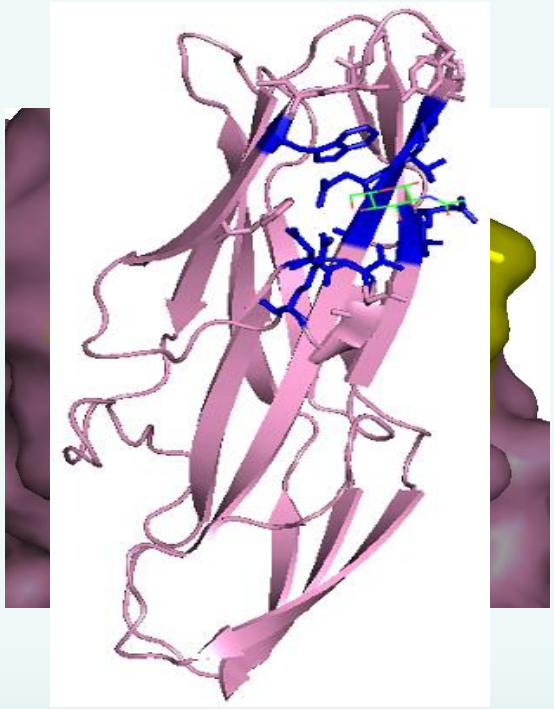
- **Able to extend the 'lectin library' by site directed mutagenesis**

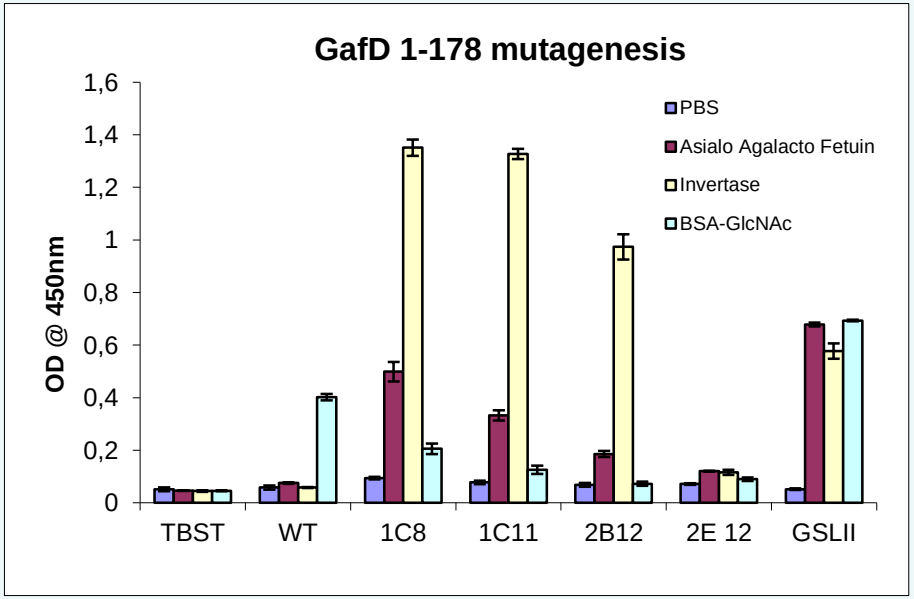
  - able to alter both the lectins affinity and specificity for glycans

Damian Keogh, Ruth Larragy, Brendan O'Connor . 'Exploring the specificity determinants of the *Pseudomonas aeruginosa* PA-IL lectin through site specific mutagenesis'. (2014) *Biochem. Biophys. Acta* , 1840, 6 ,2091-2104.

Expanding the 'Lectin probe' library by site-directed mutagenesis

- **Mutagenesis – *altered affinities as measured by ELLA***

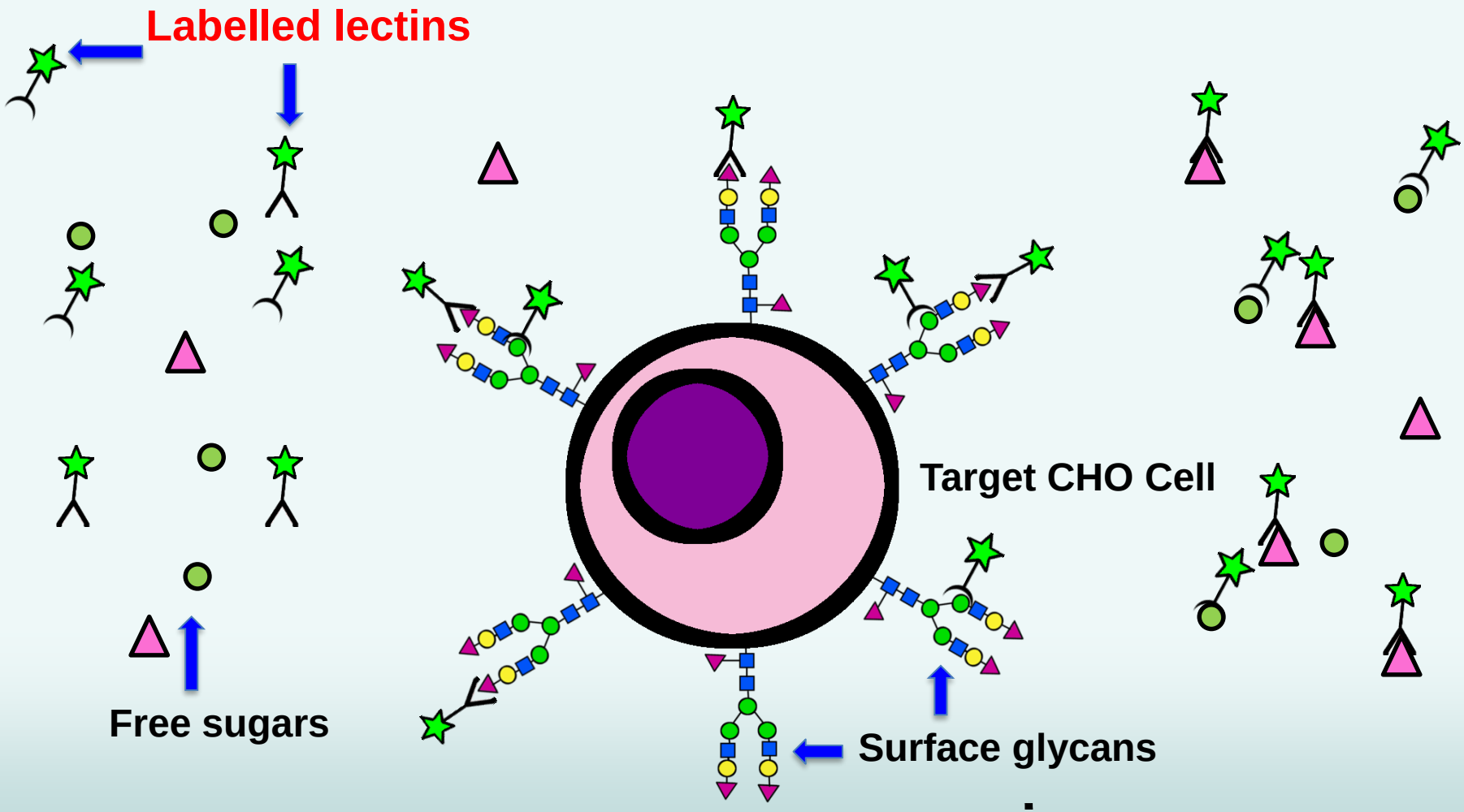




Roisin Thompson, Aileen Creavin, Michael O'Connell, Brendan O'Connor 'Optimisation of the enzyme-linked lectin assay for enhanced glycoprotein and glycoconjugate analysis'. (2011) Analytical Biochemistry , 413 , 2, 114-122.

Lectin probes binding onto surface glycans

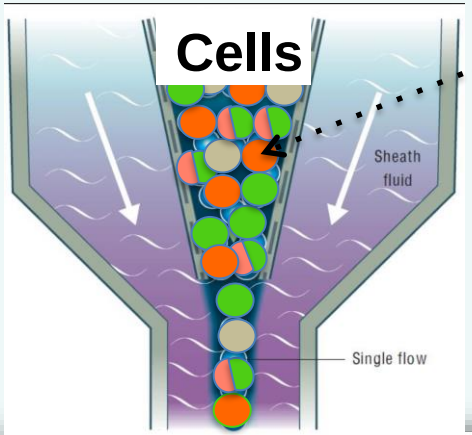
followed by selective elution with free sugar

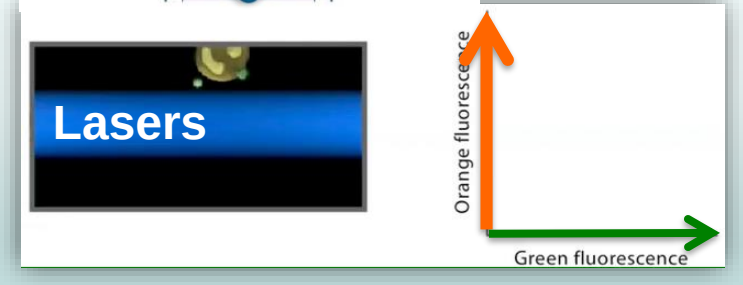


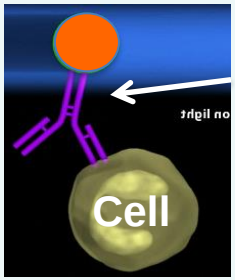
Measuring Lectin binding using Flow Cytometry

- *Flow Cytometry is a multiparametric single cell analysis platform*
- *May be used to measure the fluorescent properties of cells in suspension.*

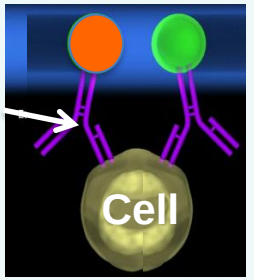
BD FACSAria

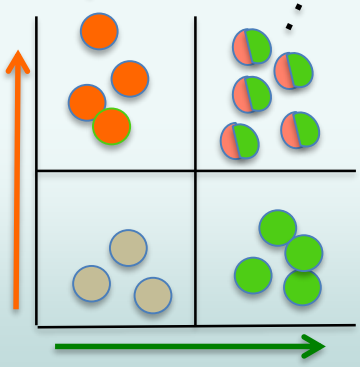




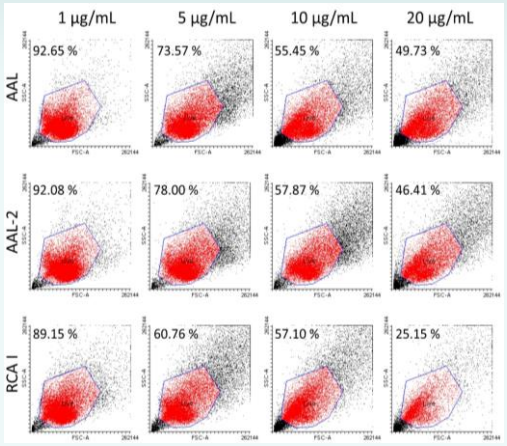


Labelled probes (eg. Antibodies or *lectins*)

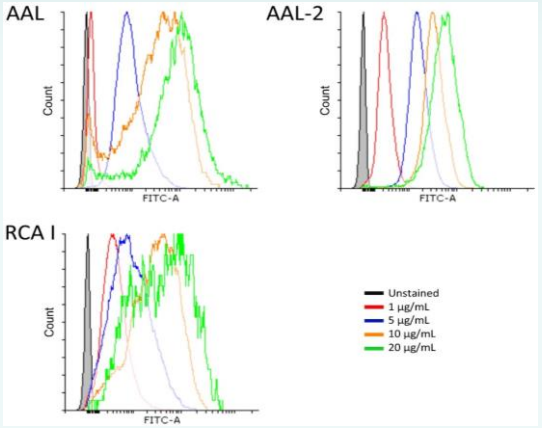




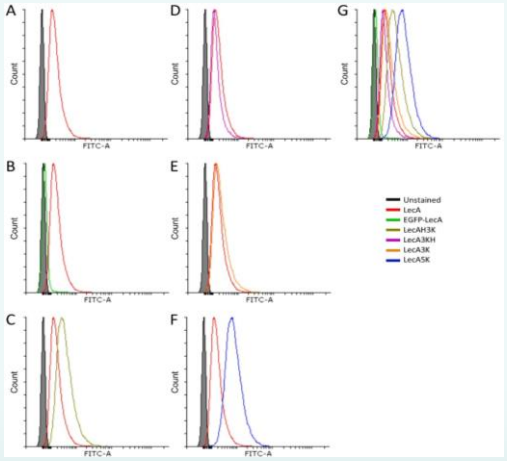
Determining the appropriate lectin probe conc.



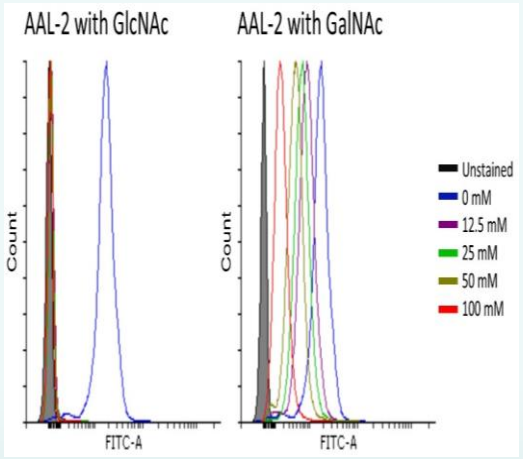
Effect of different lectin conc. binding to CHO cells *(% survival)*



Optimizing % lectin binding to CHO cells

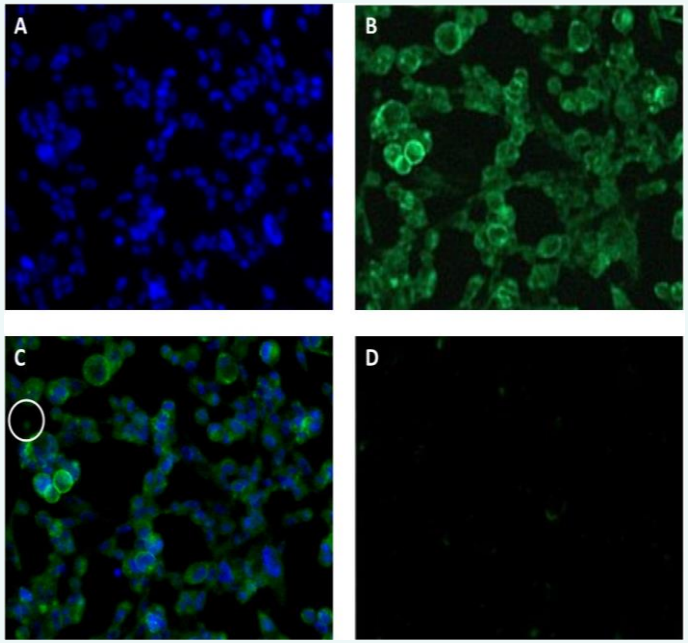


Differential binding of Lec A and Lec A mutants to CHO cells

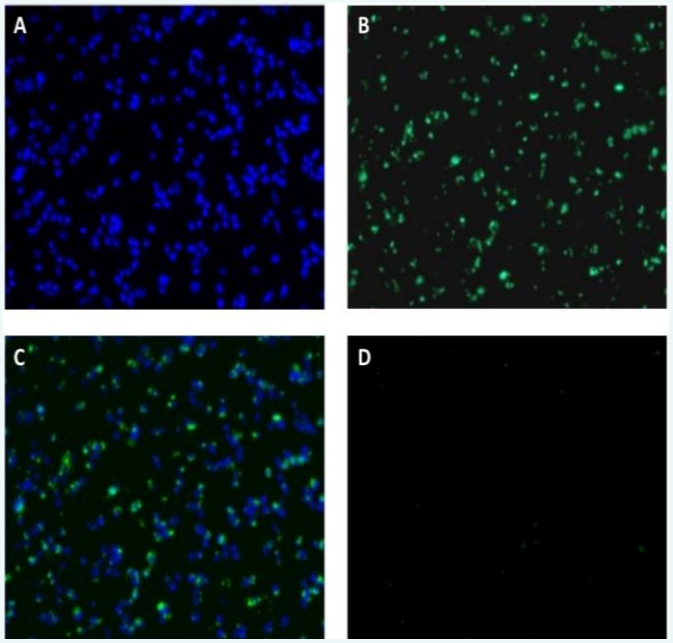


AAL-2 inhibition check with a competitive & non-competitive control free sugar

Visualization of lectin binding to CHO surface

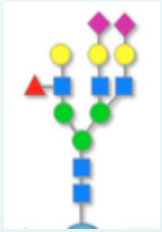


Free sugar



Free sugar

CHO cells probed with AAL lectin probe *(Fucose)*



CHO cells probed with RCA lectin probe *(terminal galactose)*

Testing the effect of variations in culture conditions on CHO cell surface glycosylation using lectin probes.

1. Is it possible to detect **early** cell surface glycosylation changes in response to variations in culture conditions such as media depletion, temperature and CO_2 levels ?

2. Are these **reversible** changes if remedial action is taken ?

i.e. fresh media added or temperature or CO2 level adjustments etc

3. Can we identify latest point of '**no return**' in these changes

i.e. when do the changes become irreversible

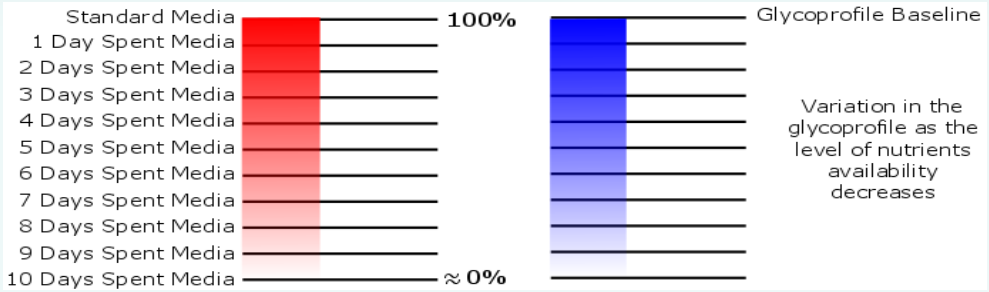
4. When do these changes reflect changes in the **product quality** ?

The CHO culture conditions to be varied include ;

(probed with multiple lectins of different specificities)

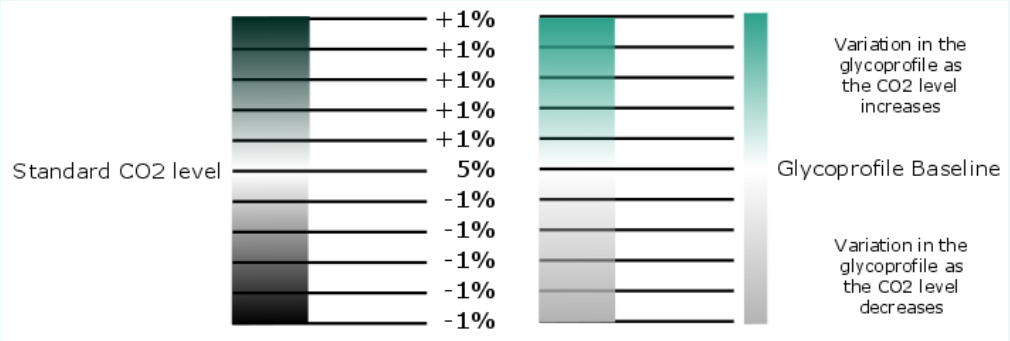
Media Depletion :

(0 to 10.0% day spent media)



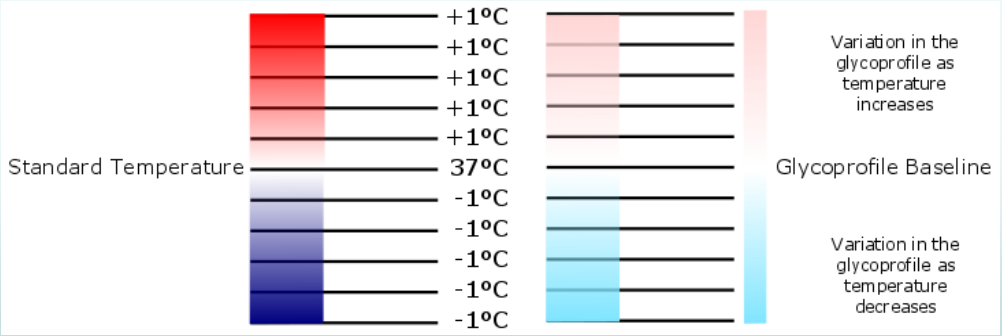
CO_2 levels :

(0% to 5.0 %)



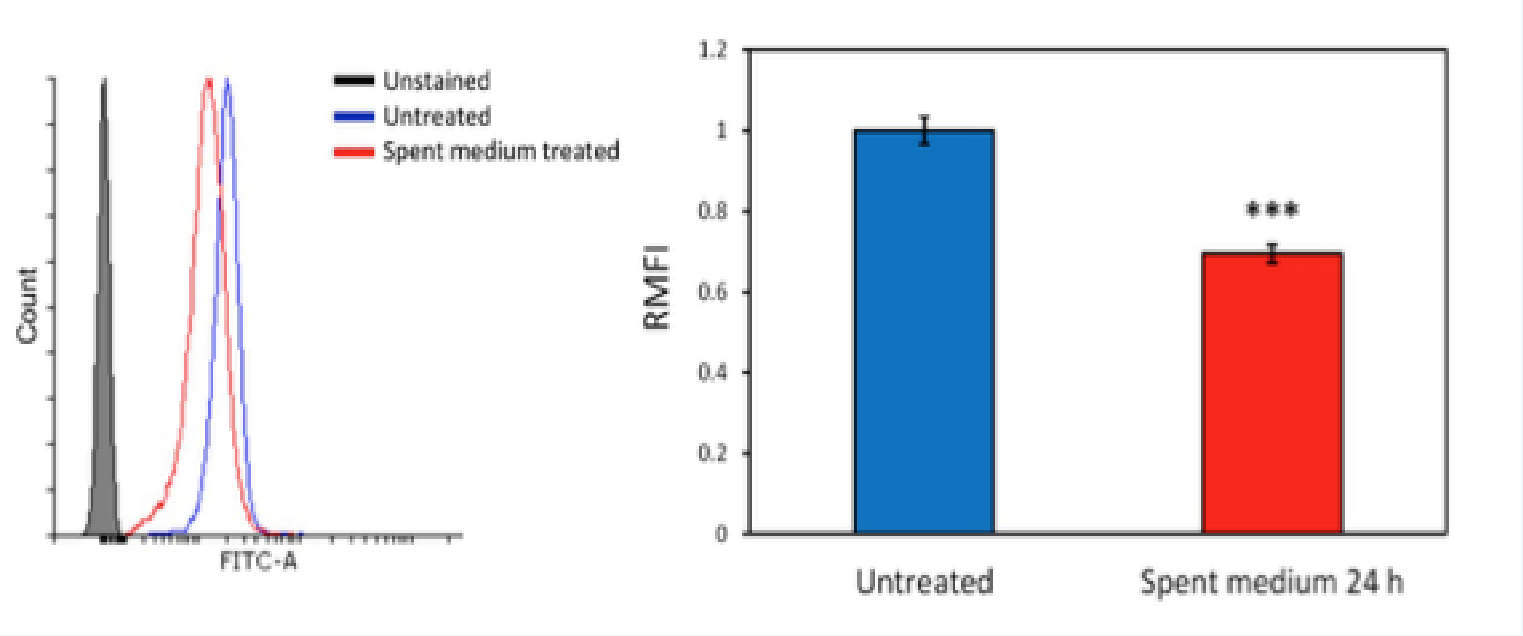
Temperature :

(32^{0}C- 42^{0}C)

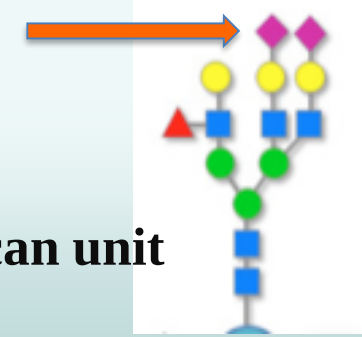


CHO cells probed with MAL-II

following spent medium treatment



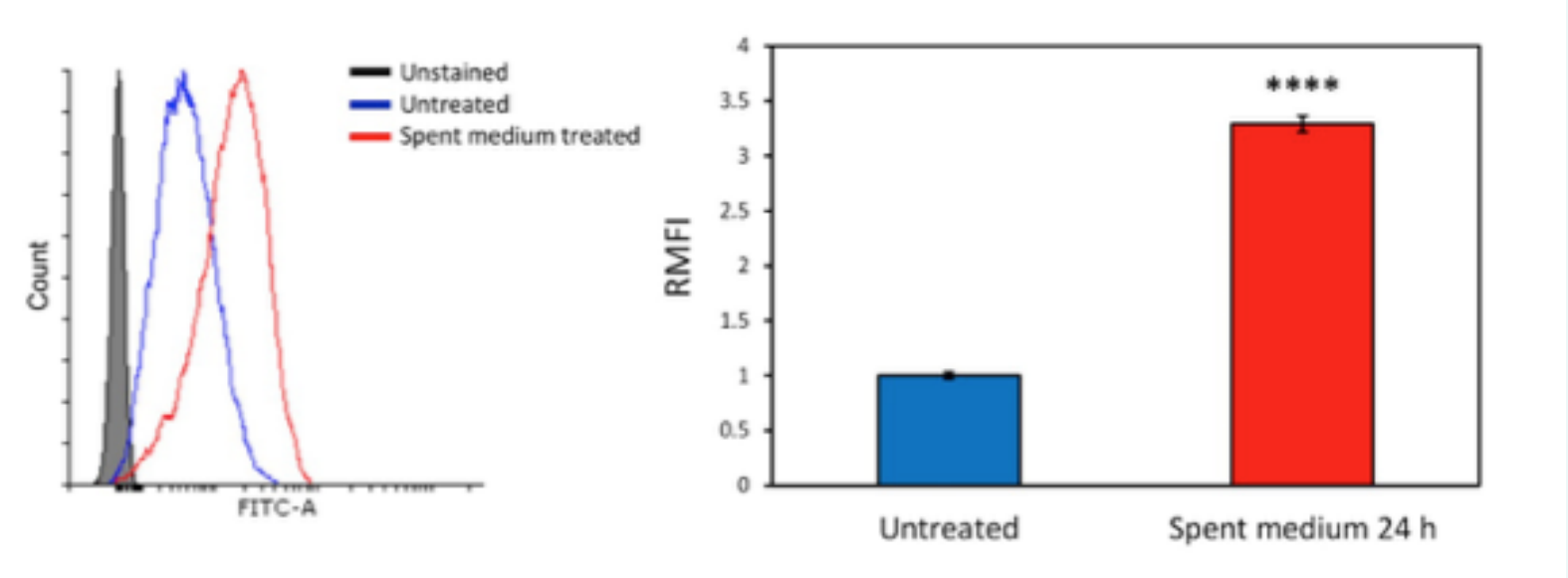
MAL-II has an affinity for 'capping' sialic acid



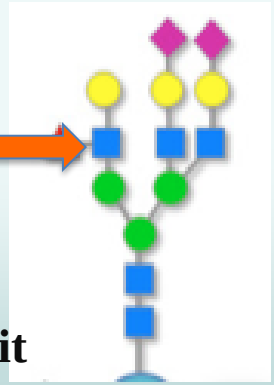
Typical glycan unit

CHO cells probed with Lec A

following spent medium treatment



Lec A has an affinity for the underlying' galactose

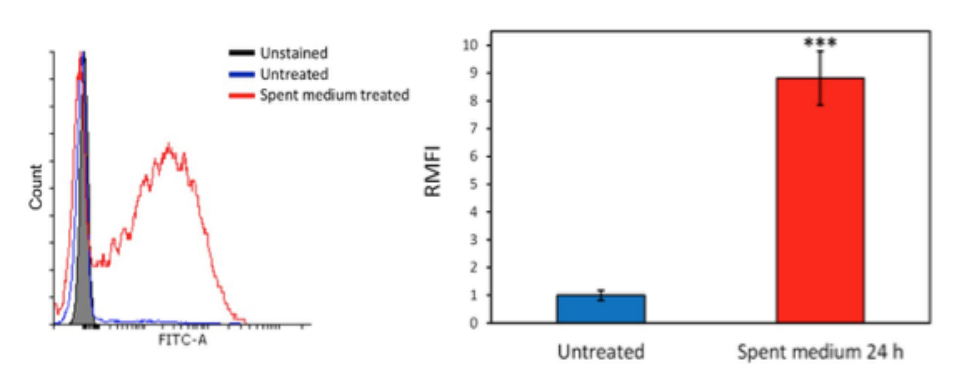


Typical glycan unit

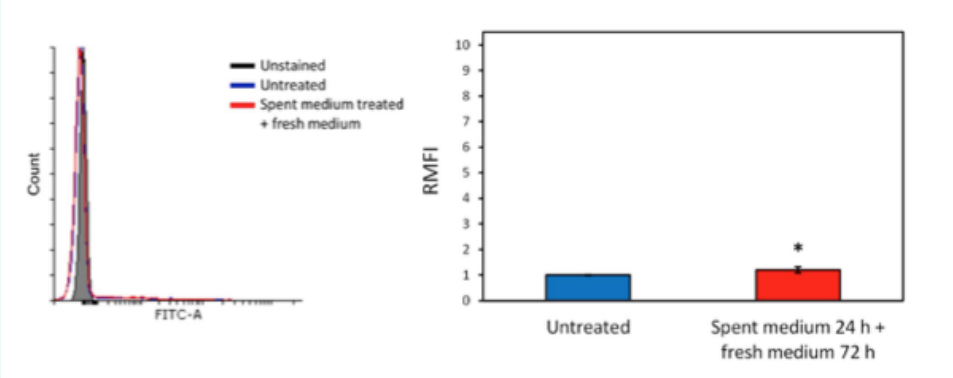
CHO cells probed with PNA

following spent medium & fresh media treatments

Spent media :



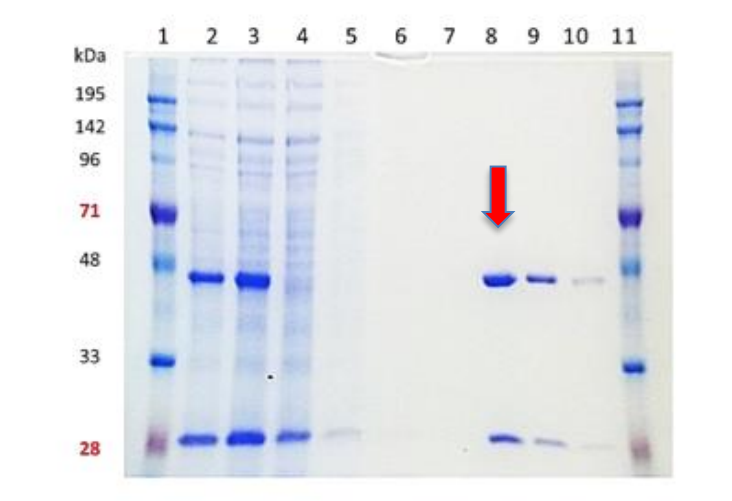
Fresh media :
(recovery)



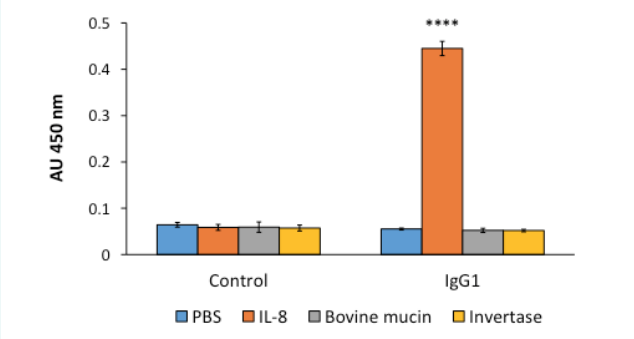
PNA has an affinity for the 'underlying' galactose

Evaluating product quality

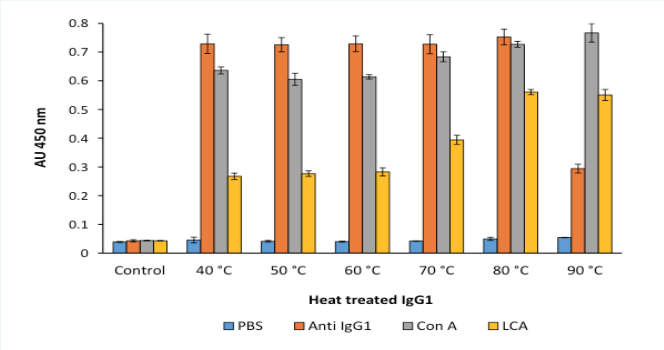
by looking at its glycosylation status



Purification of the antibody product *(by Protein G spin columns)* secreted from the CHO cells



Measuring Product Glycosylation using the Enzyme Linked Lectin Assay (ELLA)



Equating cell surface glycosylation to product quality !!!!

In summary ;

1. **Yes - lectin probes may be used to detect early changes in CHO cell surface glycosylation due to cell culture variations**

2. **Yes - lectin probes may be used to show that early changes in CHO cell surface glycosylation are reversible when remedial action is taken**

3. **Yes - lectin probes may be used to identify the 'point of no return' when changes in CHO cell surface glycosylation become irreversible**

4. **Yes – lectins may be used to correlate changes in CHO cell surface glycosylation to changes in the quality of the anti-body based product**

Detecting glycosylation changes on the surface of colorectal carcinoma cells (SW480 and SW620)

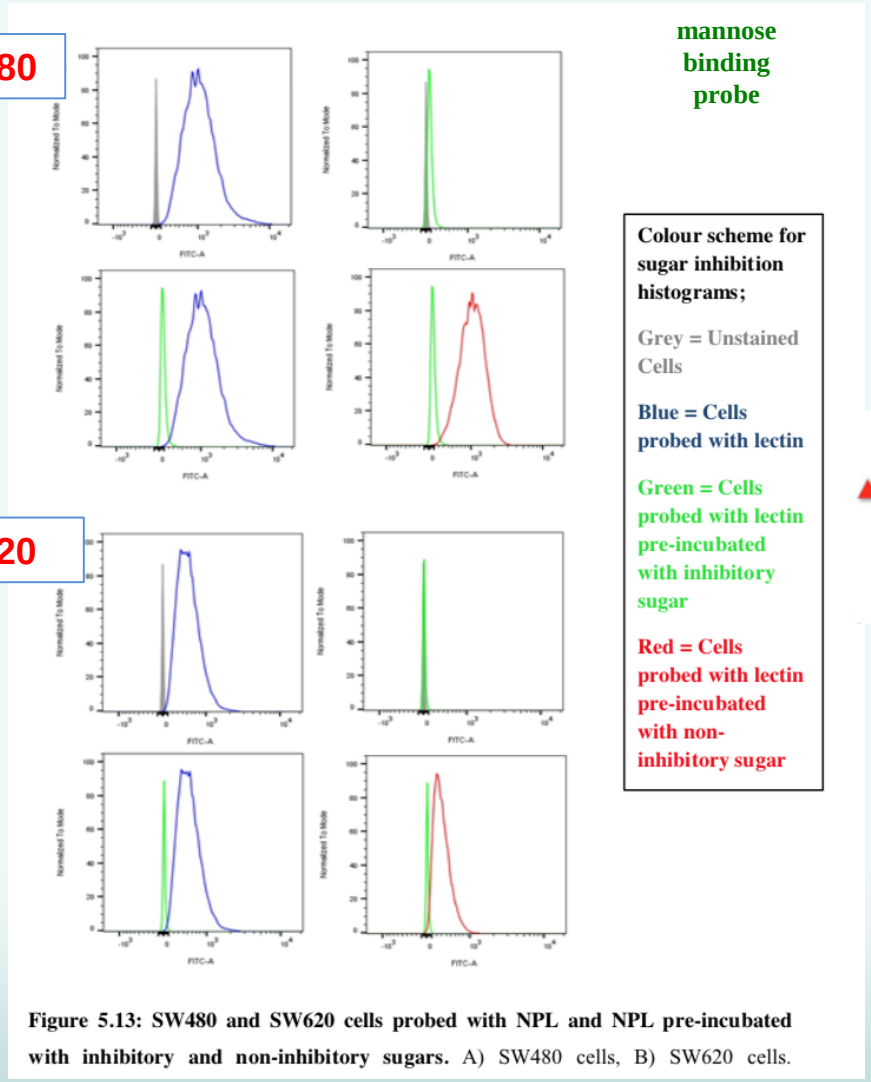
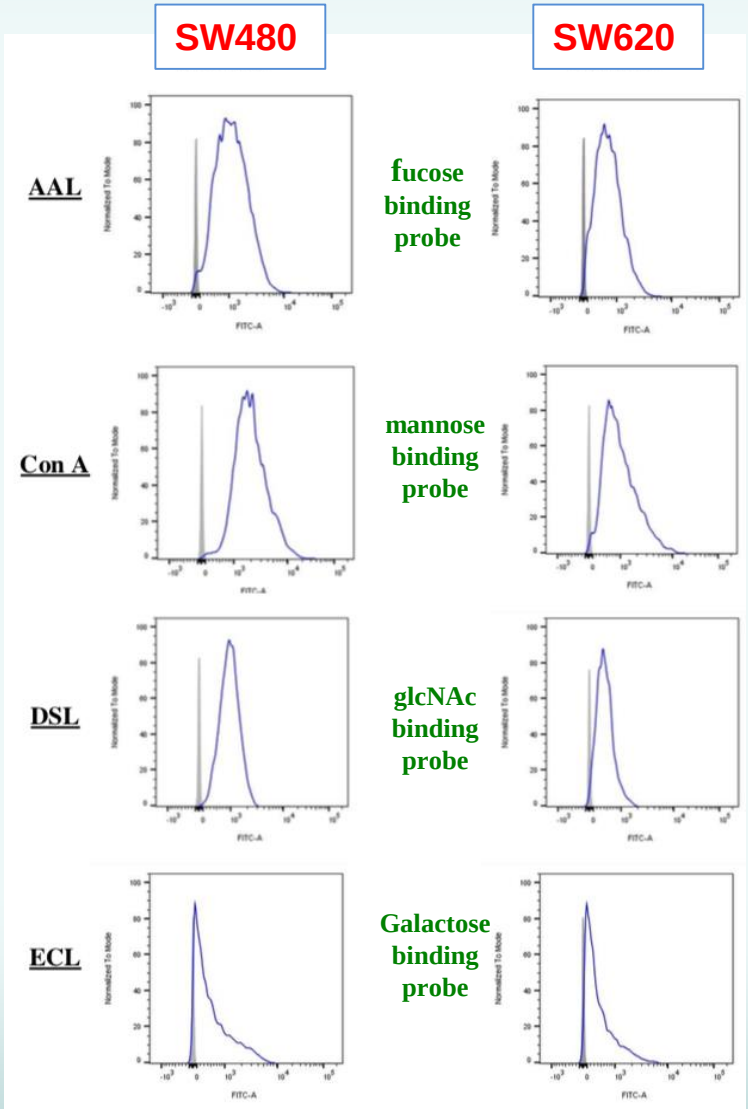
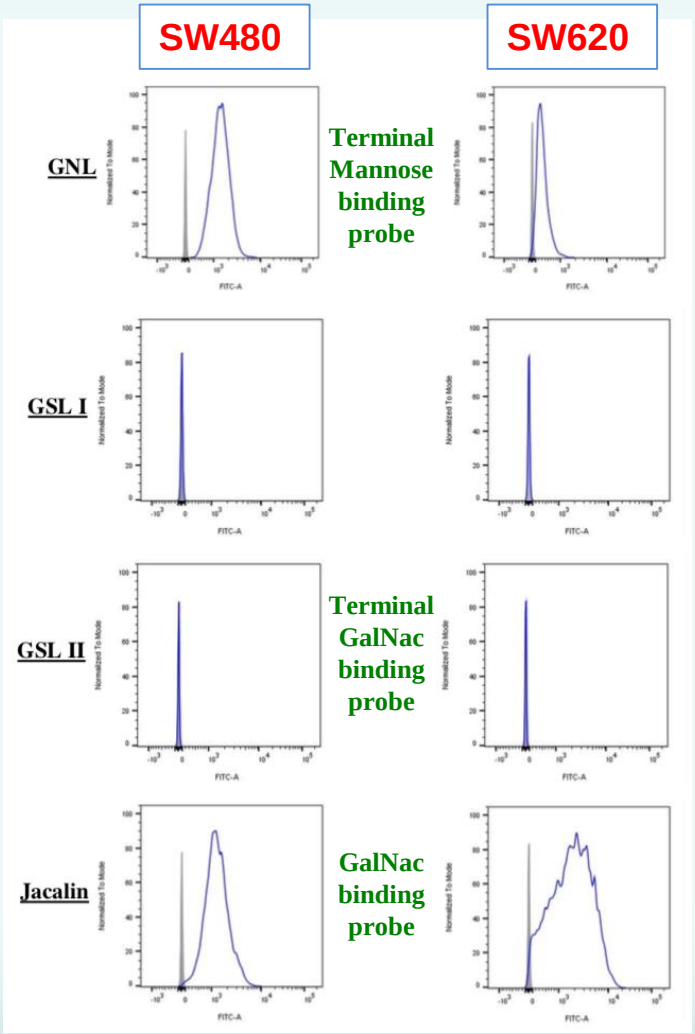
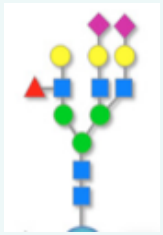


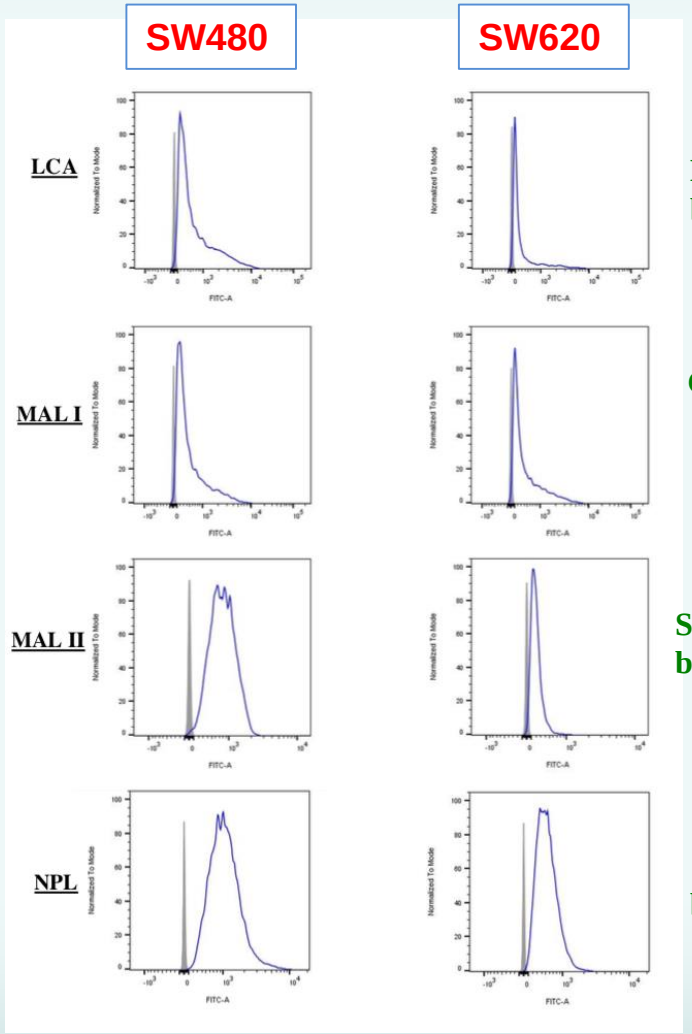
Figure 5.13: SW480 and SW620 cells probed with NPL and NPL pre-incubated with inhibitory and non-inhibitory sugars. A) SW480 cells, B) SW620 cells.



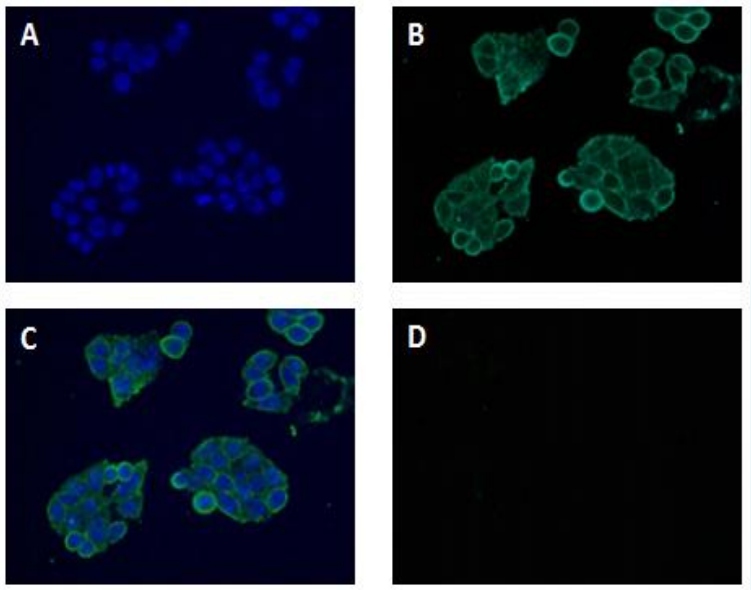
Detecting glycosylation changes on the surface of colorectal carcinoma cells (SW480 and SW620)





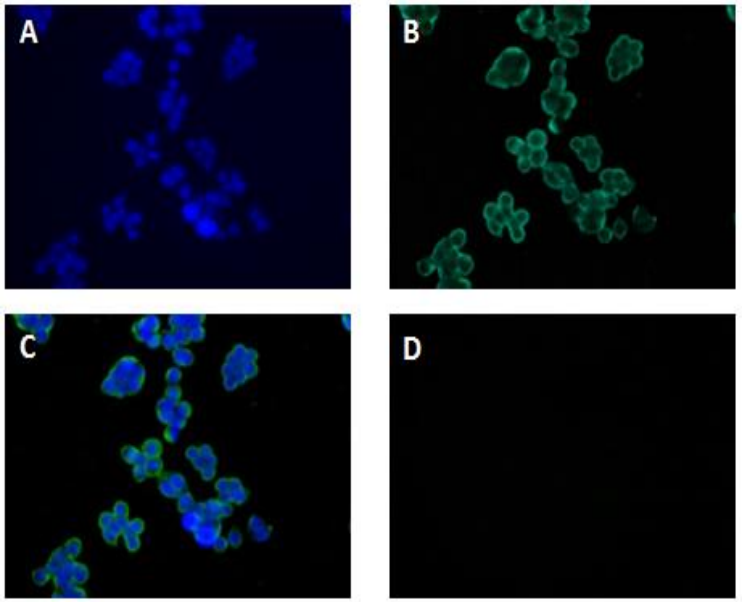


Detecting glycosylation changes on the surface of colorectal carcinoma cells



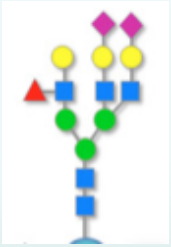
Fucose binding probe

Free sugar



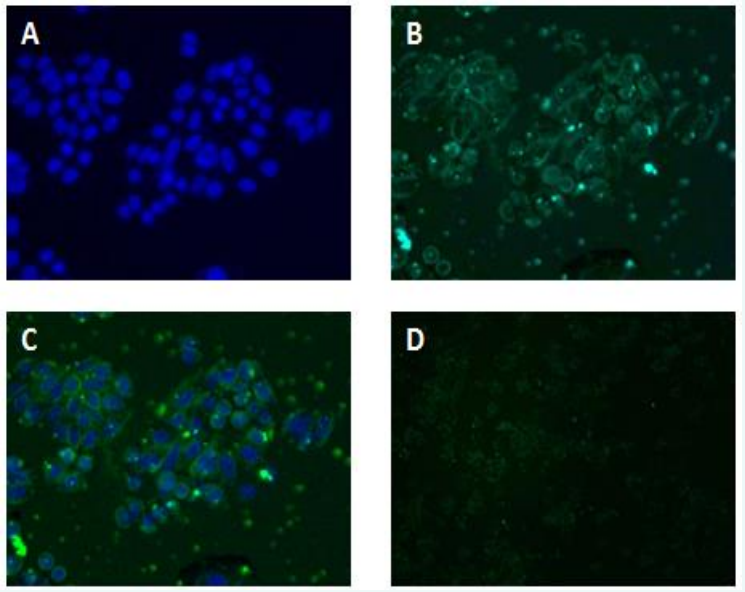
Free sugar

SW480 cells probed with AAL lectin probe



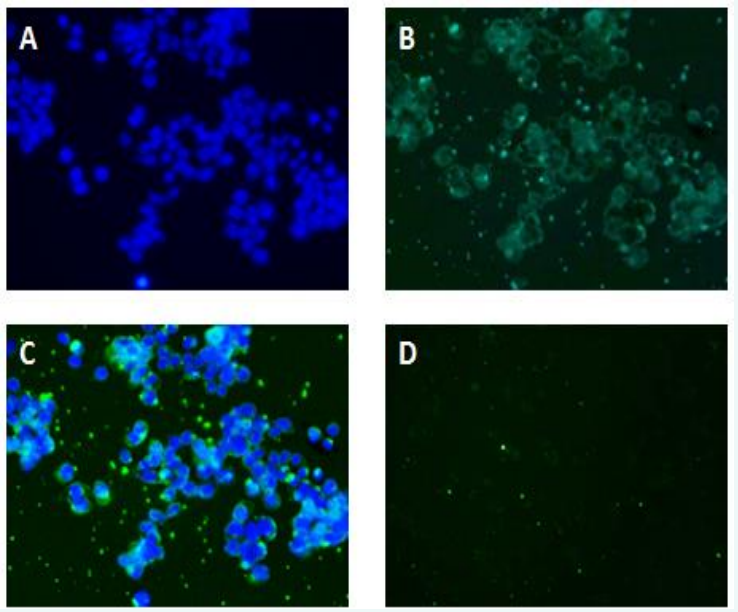
SW620 cells probed with AAL lectin probe

Detecting glycosylation changes on the surface of colorectal carcinoma cells



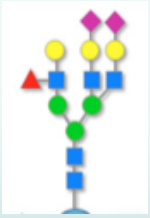
GalNac binding probe

Free sugar



Free sugar

SW480 cells probed with **Jacalin** lectin probe



SW620 cells probed with **Jacalin** lectin probe

Detecting glycosylation changes on the surface of colorectal cancinoma cells

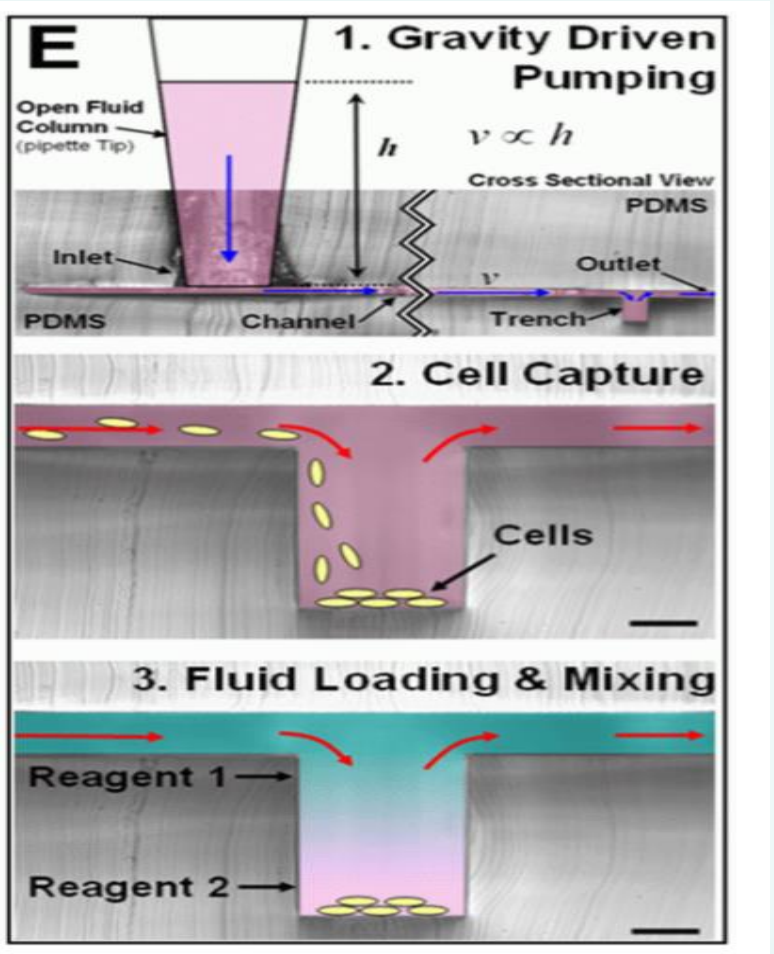
The large decrease (*over 200%*) of sialic acid binding lectins to SW620 cells (*over SW480*) are a direct indication of the loss of the sialic acid capping.

This was found to correspond with a significant increase (*over 150%*) in binding of the galactophilic lectins to the (*now exposed*) underlying Galactose residues of the SW620 cells

Measuring lectin binding to cell surfaces using micofluidic platforms

‘Lab-in-a-trench’ (LiaT)

- Microfluidic platform
- CHO cells captured by gravity
- Gentle laminar flow
- Lectin probes added
- Lectins may be selectively released by use of free sugars

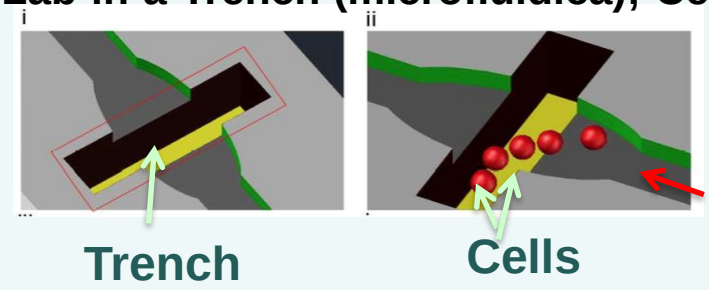


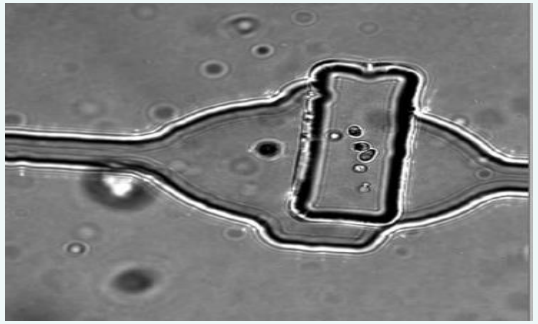
O’Connell T, Walls, D and O’Connor , B. (2014) ‘ Glycan Profiling at Single Cell Level with the Microfluidic Lab-in-a-Trench Platform: A New Era in Experimental Cell Biology’. *Lab on a Chip* , 14 , 3629-3639.

Microfluidic *Lab-in-a-Trench* Platform

-sequential glycan profiling of single cells

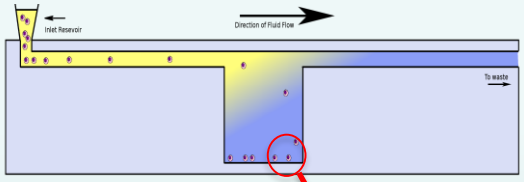
Lab-in-a-Trench (microfluídica); Células capturados pela gravidade

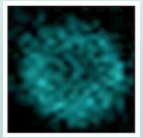




Surface Glycan Profiling using labdelled lectin probes (fluorophores)

Add Lectin #1 → **Lectin binds to cell**





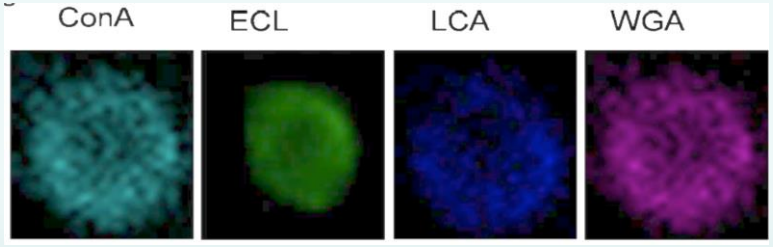
Add free sugar

↓

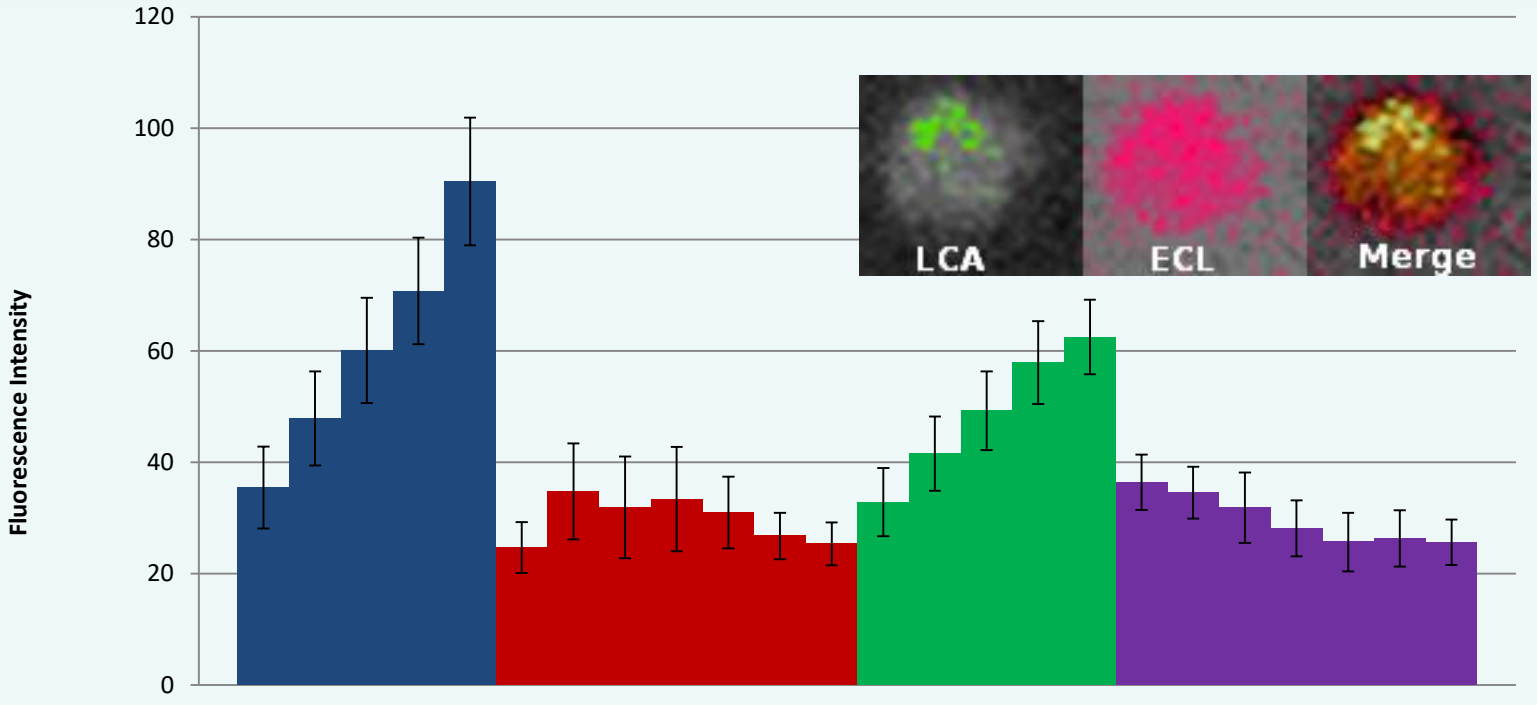
Lectin release

↓

Add Lectin #2 etc



‘Sequential’ probing of the same live cells



Sequential Glycan Profile at 5 min intervals

LCA (Blue - Lectin), Mannose (Red - Elution), ECL (Green - Lectin), Lactose (Purple - Elution)

n=93 cells 6 Trenches

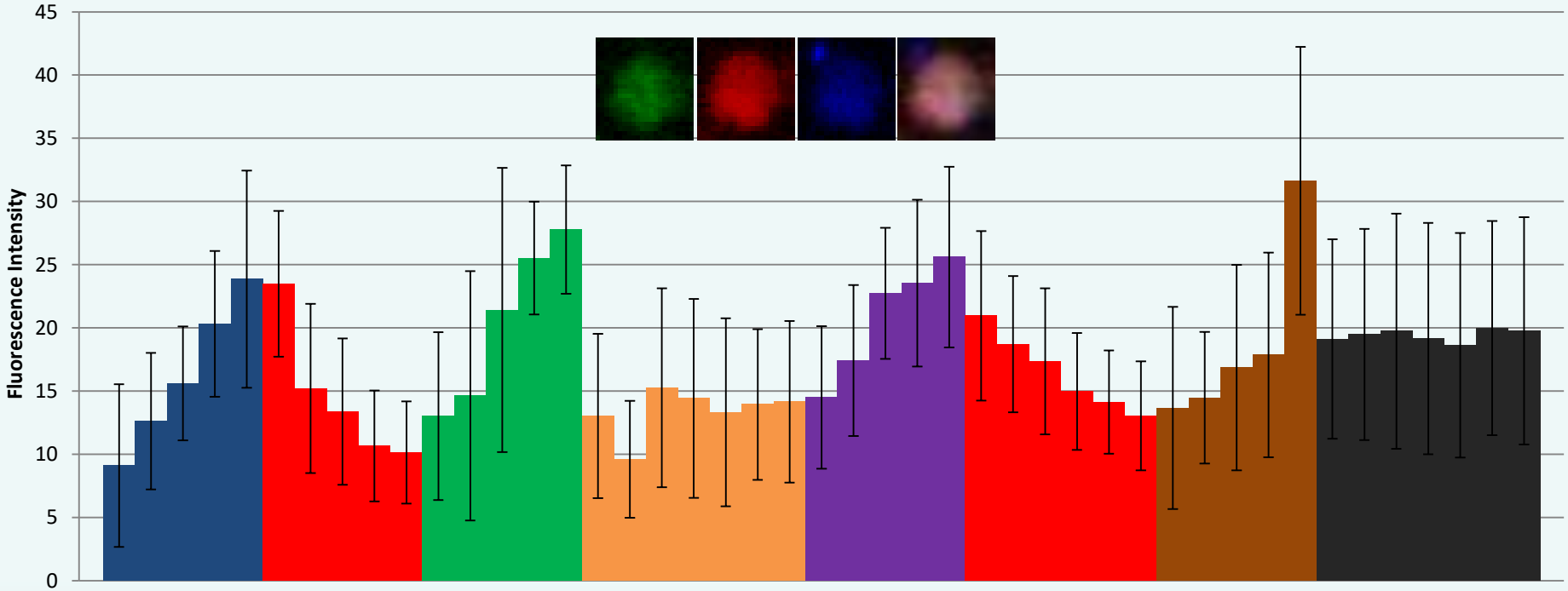
O’Connell T, Walls, D and O’Connor , B. (2014) ‘ Glycan Profiling at Single Cell Level with the Microfluidic Lab-in-a-Trench Platform: A New Era in Experimental Cell Biology’. *Lab on a Chip* , 14 , 3629-3639.

Four lectin sequence

Sequential Glycan Profile 3

LCA (Lectin) Mannose (Elution) ECL (Lectin) Galactose (Elution) ConA (Lectin) Mannose (Elution) WGA (Lectin) GlcNAc (Elution)

n=75 cells ***6 Trenches***



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Final Conclusions

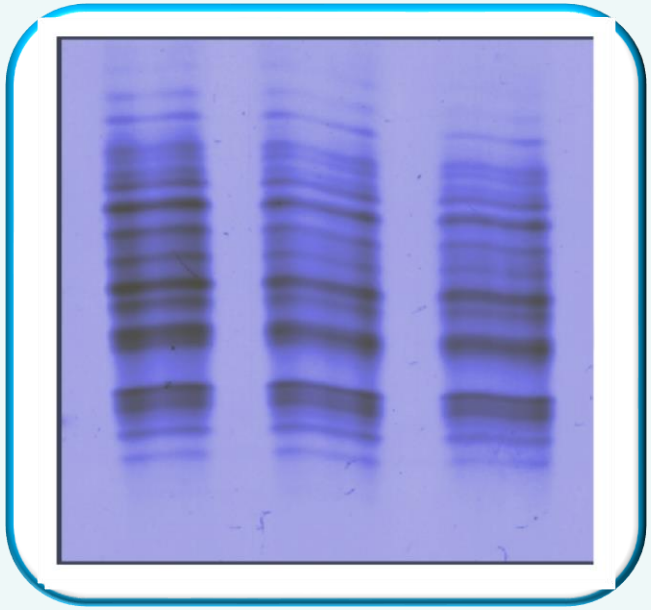
- **Lectin probes are novel method for monitoring cell surface glycosylation**
- **The status of cell surface glycosylation may be linked directly to product quality**
- **Lectin probes may also be used for the sequentially labelling the *same* cell surface**
- **Therefore, they are very useful for imaging**

OBRIGADO !

The clinical significance of Glyco-forms of Biologic drugs

❑ Biological diversity of glyco-forms

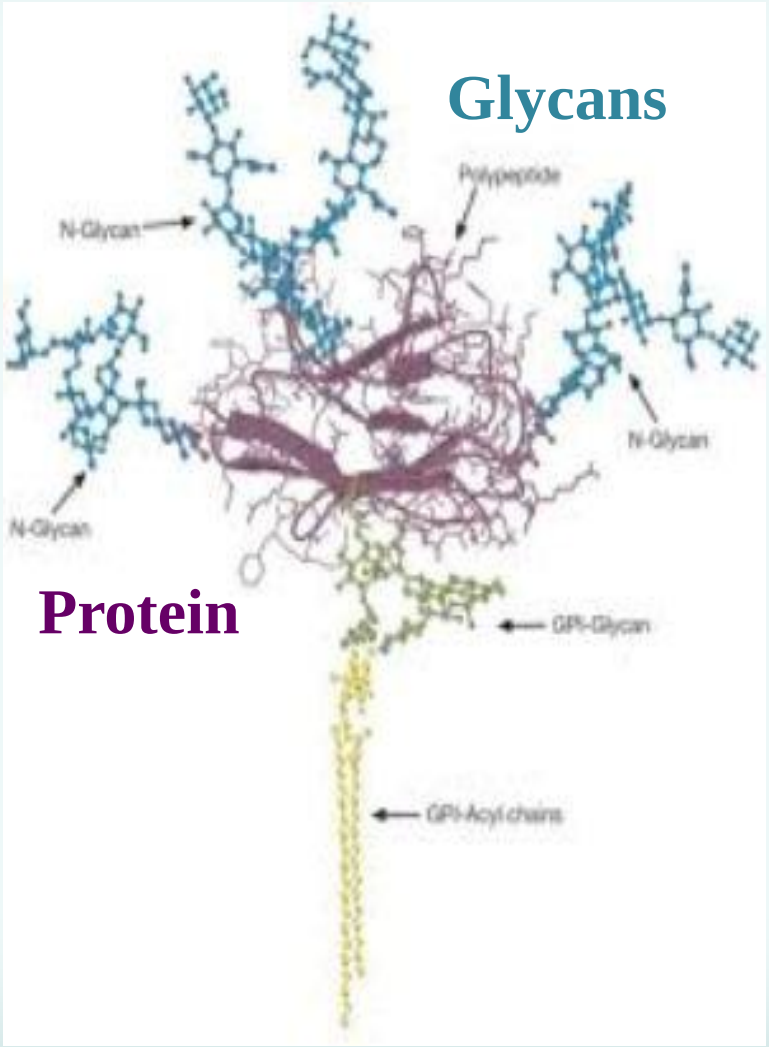
Different Glyco-forms of the same biologic drug will have different ;



- **Stabilities (resistance to breakdown or liver clearance rates) and serum half-lives**
- **Immunogenicity (glycans mask antigenic determinants)**
- **Efficacy i.e. different binding affinities to targets**

Glycosylation has a huge effect on the structural and functional of Biologic protein drugs !

Protein Glycosylation - Glycoforms



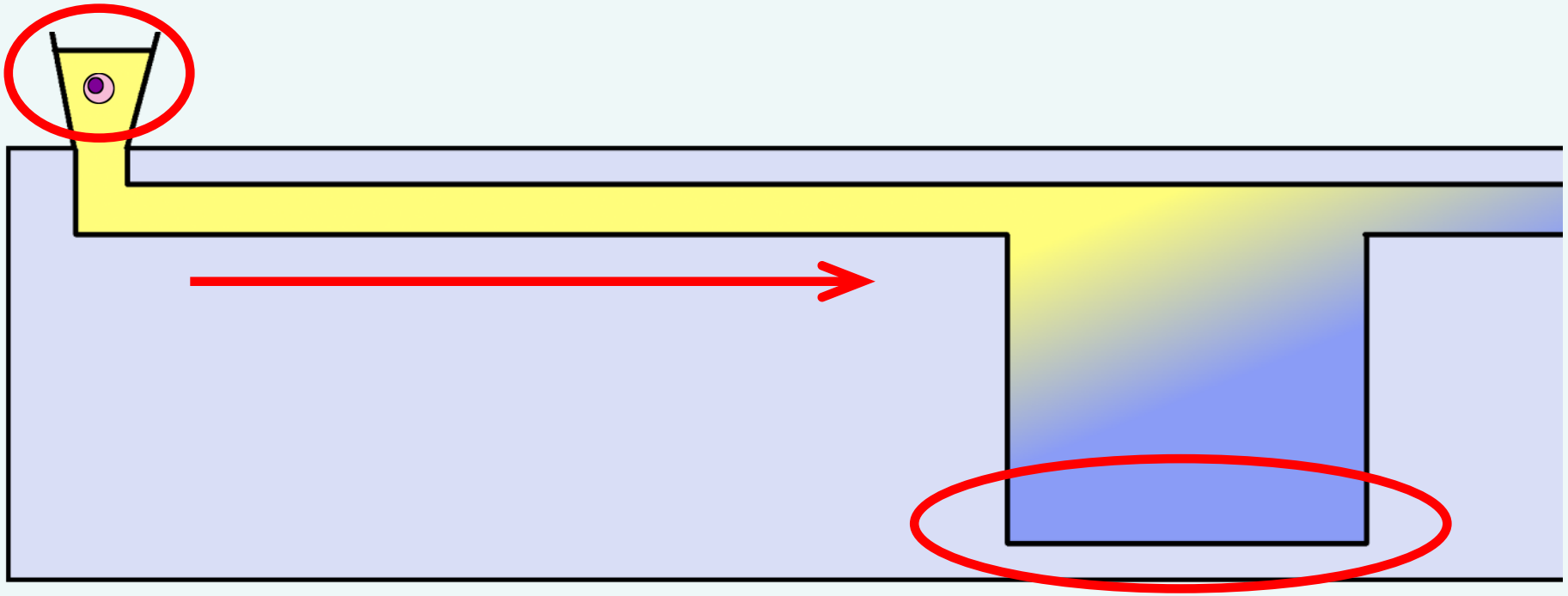
Most of the 'Biologics' are recombinant Glyco-proteins

They may also have *several* Glycosylation sites

They may exist as several 'Glyco-forms' i.e. protein part is identical but the glycans may vary

Each glyco-form of the drug has different stability and efficacy

Lab in a Trench ; *'gentle' cell capture*



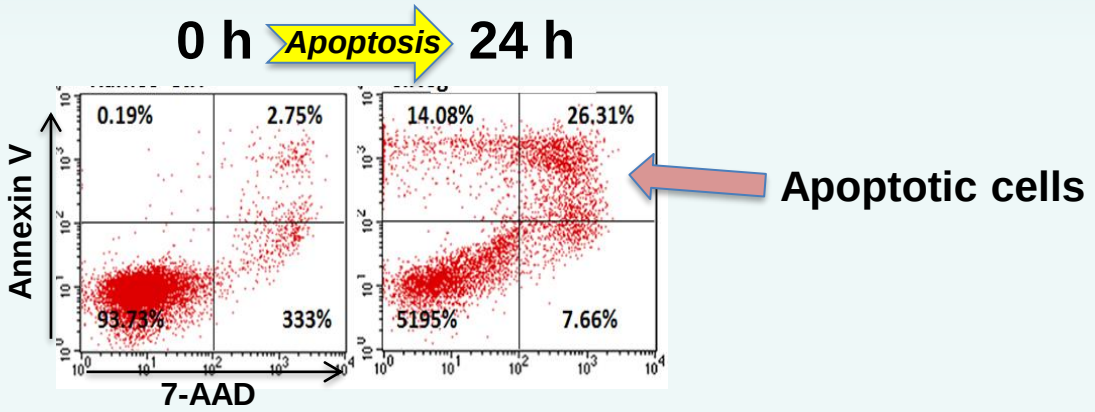
Transfer of lectins or releasing sugars by Laminar flow

Lab-in-a-Trench platform

detection of apoptotic cells using lectin probes

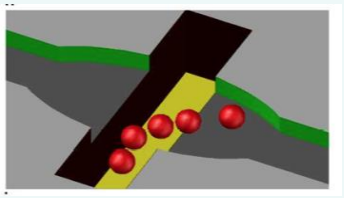
Flow Cytometry

- *CHO Cells*
- *Induce apoptosis*
- *Stained with Annexin V/7-AAD)*



Lab-in-a-Trench

-Add labelled lectin (NPL)



-NPL lectin binds to α-linked mannose and polymannose structures containing (α-1,6) linkages -these are exposed on the surface apoptotic cells

DaSilva, E., Walls, D and O'Connor , B. (2016) ' Glycan Profiling at Single Cell Level with the Microfluidic Lab-in-a-Trench Platform: A New Era in Experimental Cell Biology'. *RSC Soft Matter* , 12 , 9158 - 9169.

Pharmaceutical Industry in Ireland

- **13 of the 15 top International Pharmaceutical producers have facilities in Ireland.**
- **Export 85 billion euros annually (2018)**
- **Represents 55% of total Irish exports (2018)**
- **Number 1 National priority area**
- **Requires highly trained staff/education**