



THE UNIVERSITY
OF ARIZONA

Barnyard Dust Composition and Implications for Asthma in Children

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NEMC 2017
Washington, DC



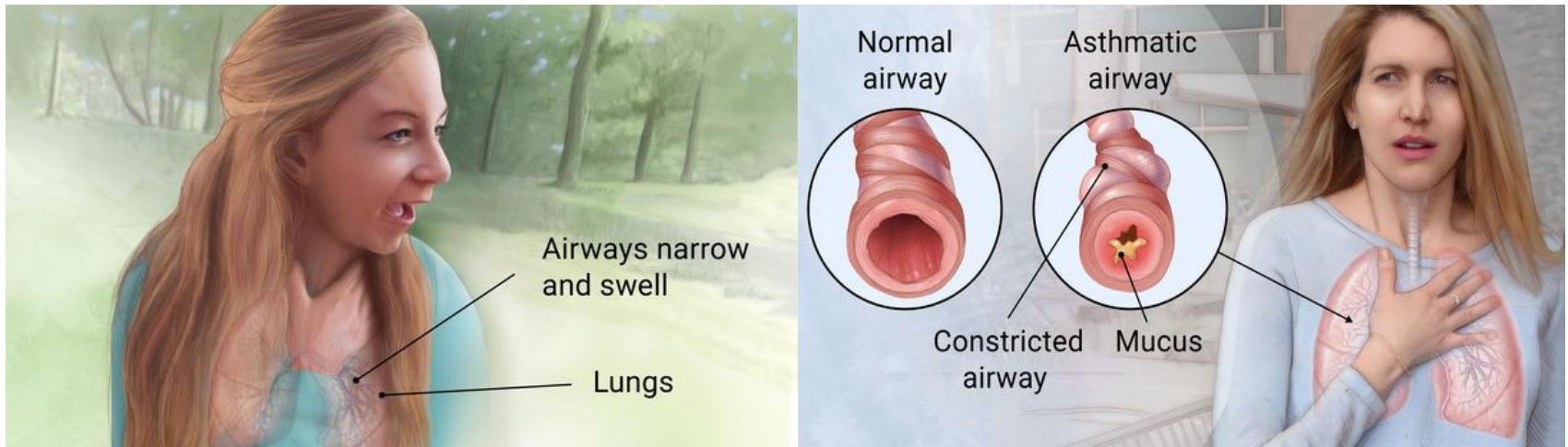
Barnyard Dust Composition and Implications for Asthma in Children

- **Objective:** Identify components of barnyard dust extracts that mediate asthma protection *in vivo*
- **Rationale:** Asthma prevalence in children exposed to farming early in life is reduced over 5-fold compared to non-farming children, Amish dust extracts have been shown to enable Asthma protection in *in vivo* models

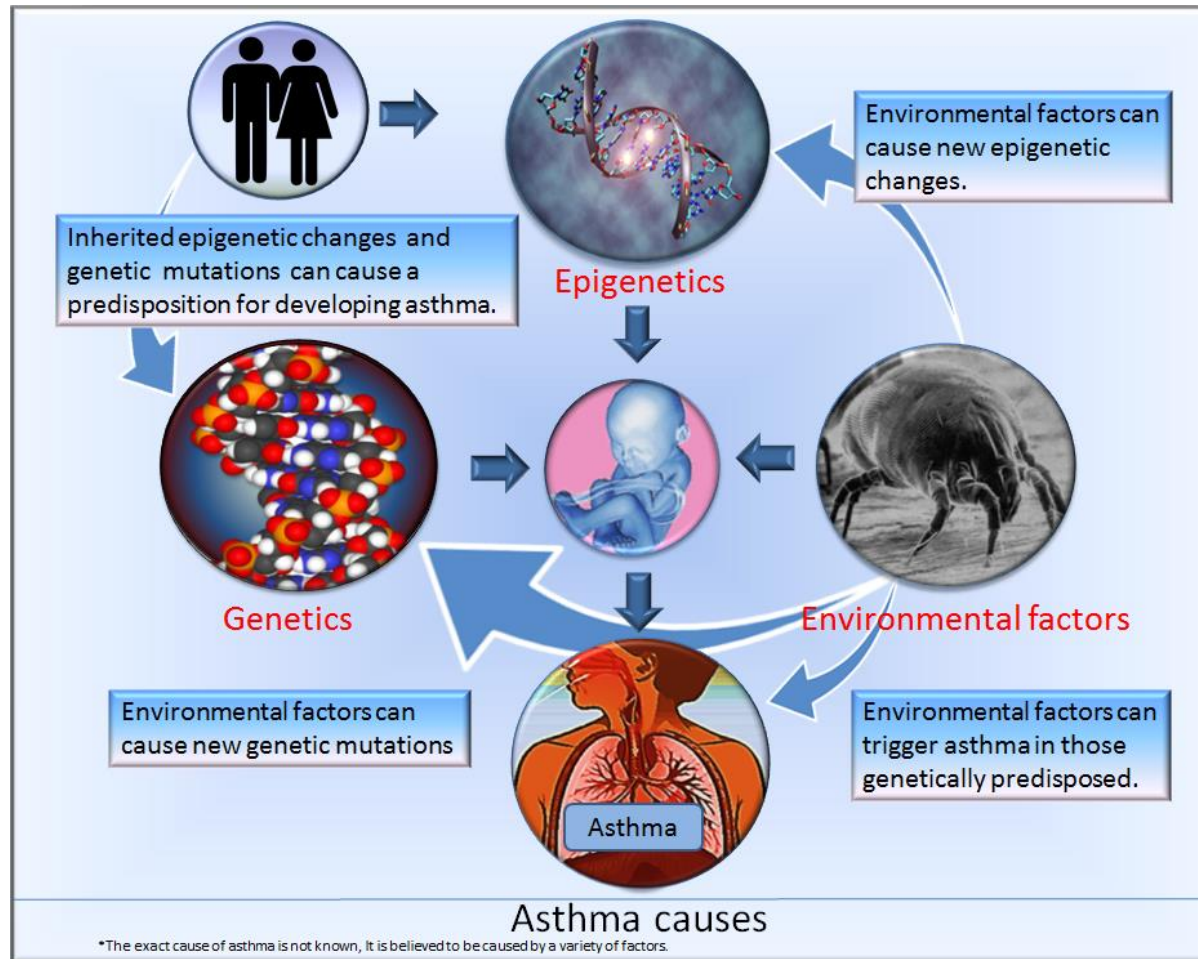


Asthma

- Asthma affects approximately 300 million people worldwide and is the most prevalent chronic disease of childhood**



Asthma



Asthma and the Environment

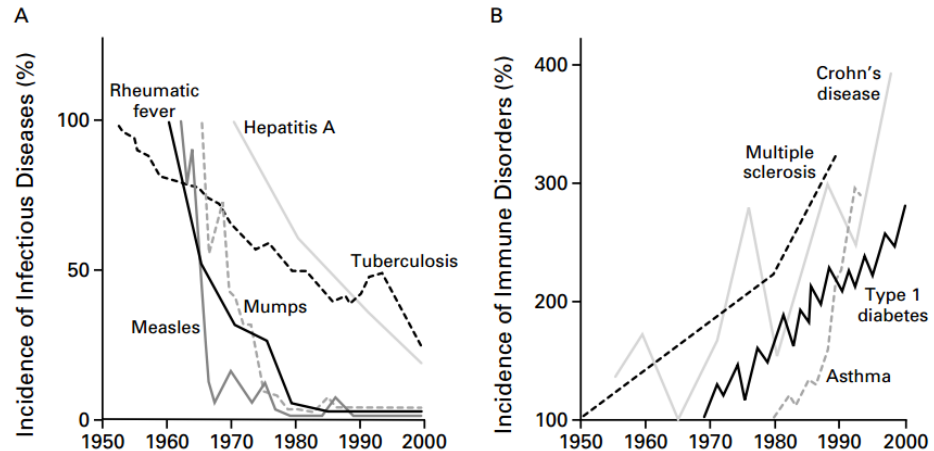


Figure 1. Inverse Relation between the Incidence of Prototypical Infectious Diseases (Panel A) and the Incidence of Immune Disorders (Panel B) from 1950 to 2000.

In Panel A, data concerning infectious diseases are derived from reports of the Centers for Disease Control and Prevention, except for the data on hepatitis A, which are derived from Joussemet et al.¹² In Panel B, data on immune disorders are derived from Swarbrick et al.,¹⁰ Dubois et al.,¹³ Tuomilehto et al.,¹⁴ and Pugliatti et al.¹⁵

N Engl J Med, Vol. 347, No. 12 • September 19, 2002

- **Important role of the environment;**
- **Humans are exposed to a vast numbers of chemicals**

Asthma and the Environment

The New York Times | <https://nyti.ms/2aAILzp>

HEALTH

Barnyard Dust Offers a Clue to Stopping Asthma in Children

By GINA KOLATA AUG. 3, 2016

Scientists say they may have found a sort of magic ingredient to prevent asthma in children: microbes from farm animals, carried into the home in dust.

The results of their research, published on Wednesday in The New England Journal of Medicine, were so convincing that they raised the possibility of developing a spray to do the same thing for children who do not have regular contact with cows and horses.



THE RUNDOWN

A BLOG OF NEWS AND INSIGHT

HEALTH SUPREME COURT

HEALTH

Does modern society worsen allergies and asthma? Ask the Amish

[f](#) 919 BY LINDZI WESSEL, STAT August 4, 2016 at 1:41 PM EDT



Amish people, who live a traditional lifestyle, have low rates of asthma. [apcrn.az](#)

The New York Times | <https://>

SUBSCRIBE

The Opinion Pages | CONTRIBUTING OP-ED WRITER

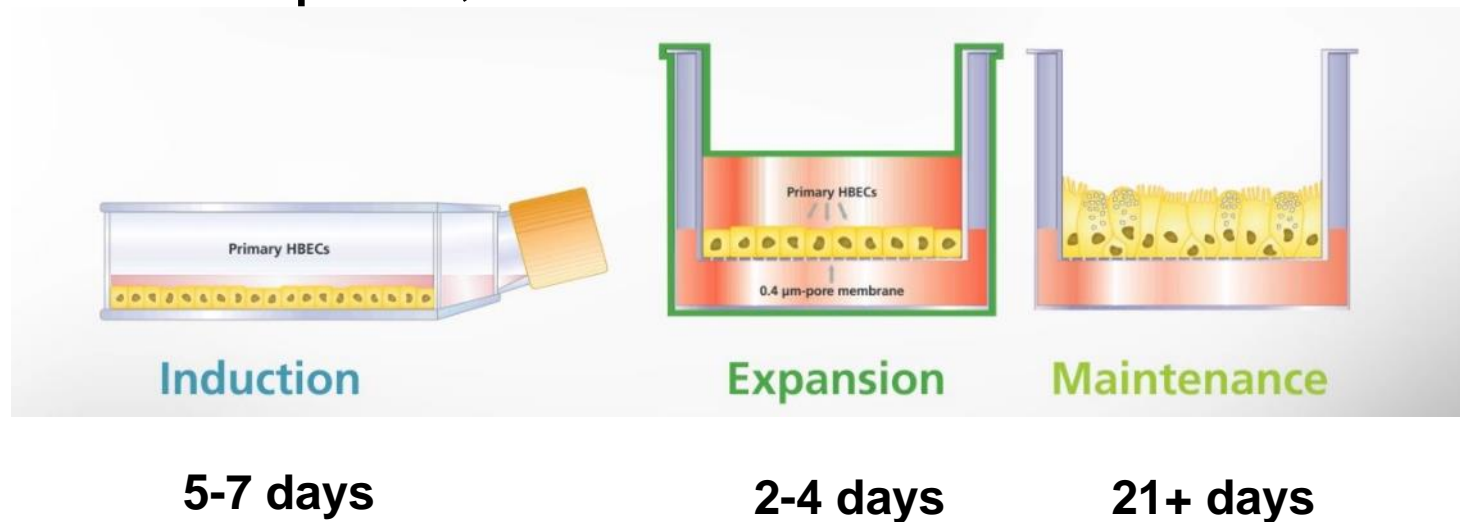
Health Secrets of the Amish

Moises Velasquez-Manoff AUG. 3, 2016

In recent decades, the prevalence of asthma and allergies has increased between two- and threefold in the United States. These days, one in 12 kids has asthma. More are allergic.

Environmental monitoring with an *in vitro* bioassay

- *in vitro* bioassay
 - An air liquid interface (ALI) culture model of immortalized epithelial cells;
 - IL-6 endpoint;

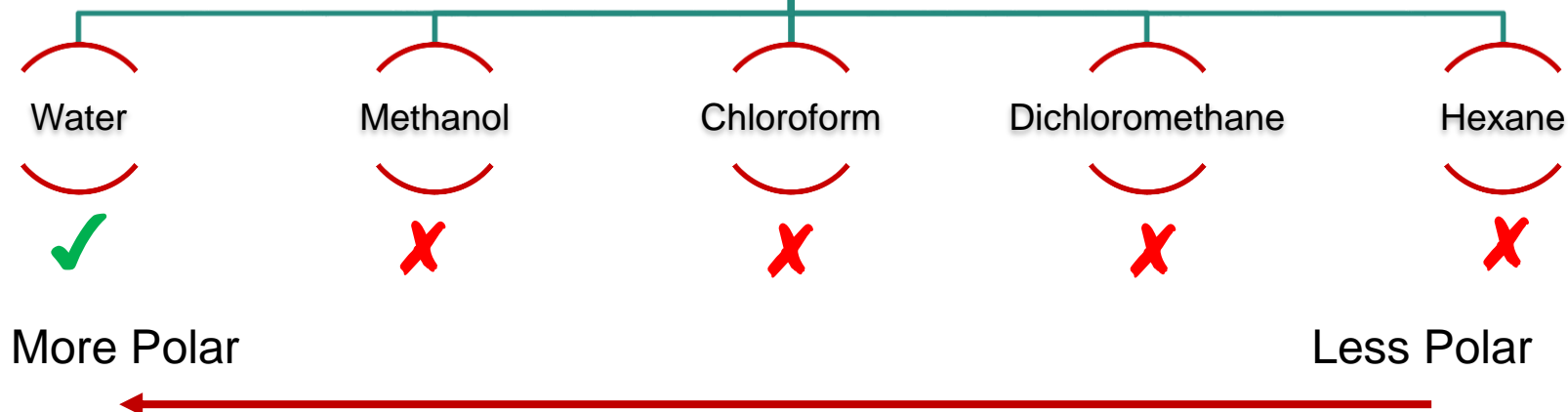


Sample Extraction

Polarity



Barn Dust Extract



Sample Stability

Temperature and Pressure



Barn Dust

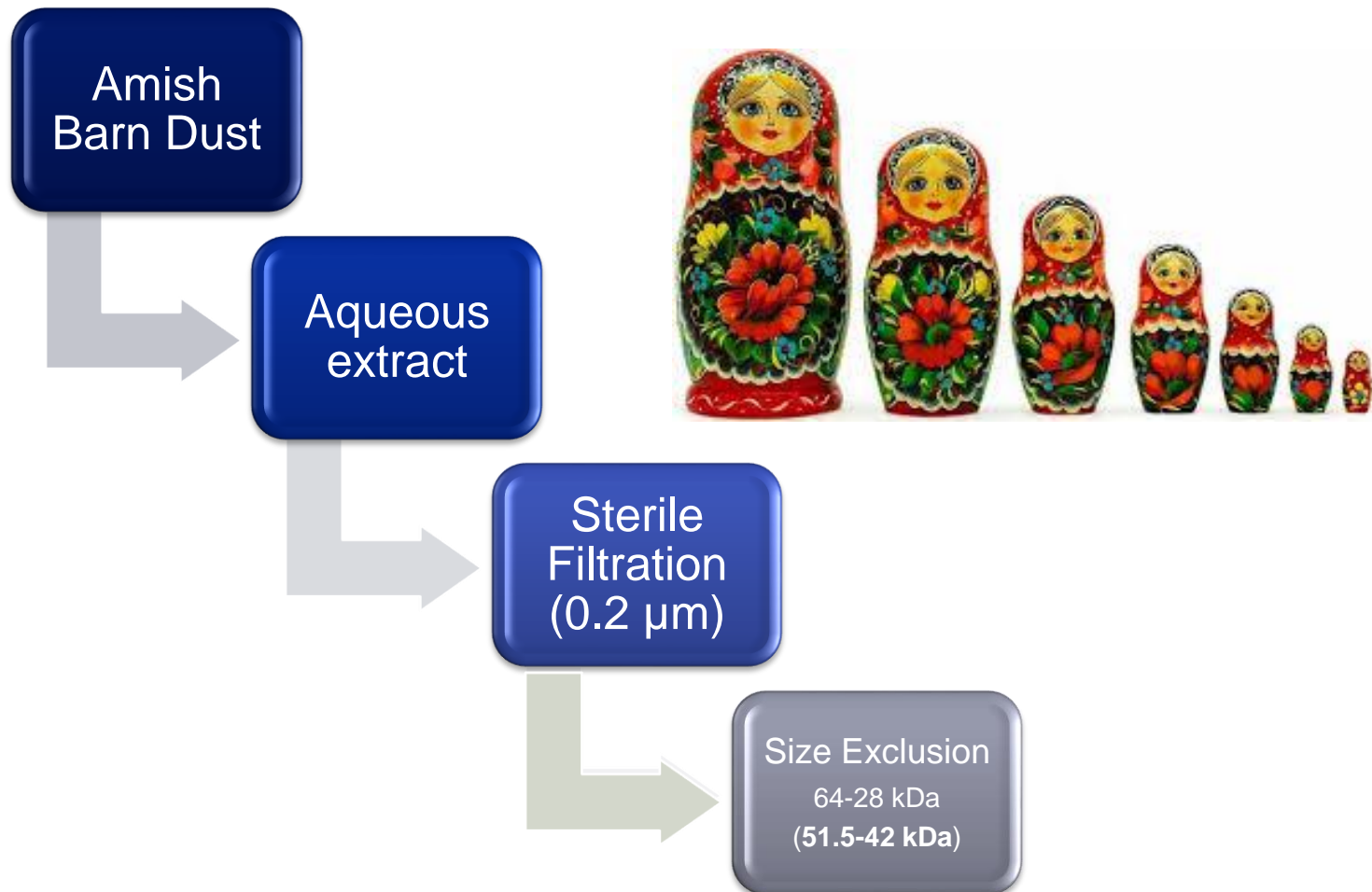
25 °C, 1 atm

80 °C, 103 atm

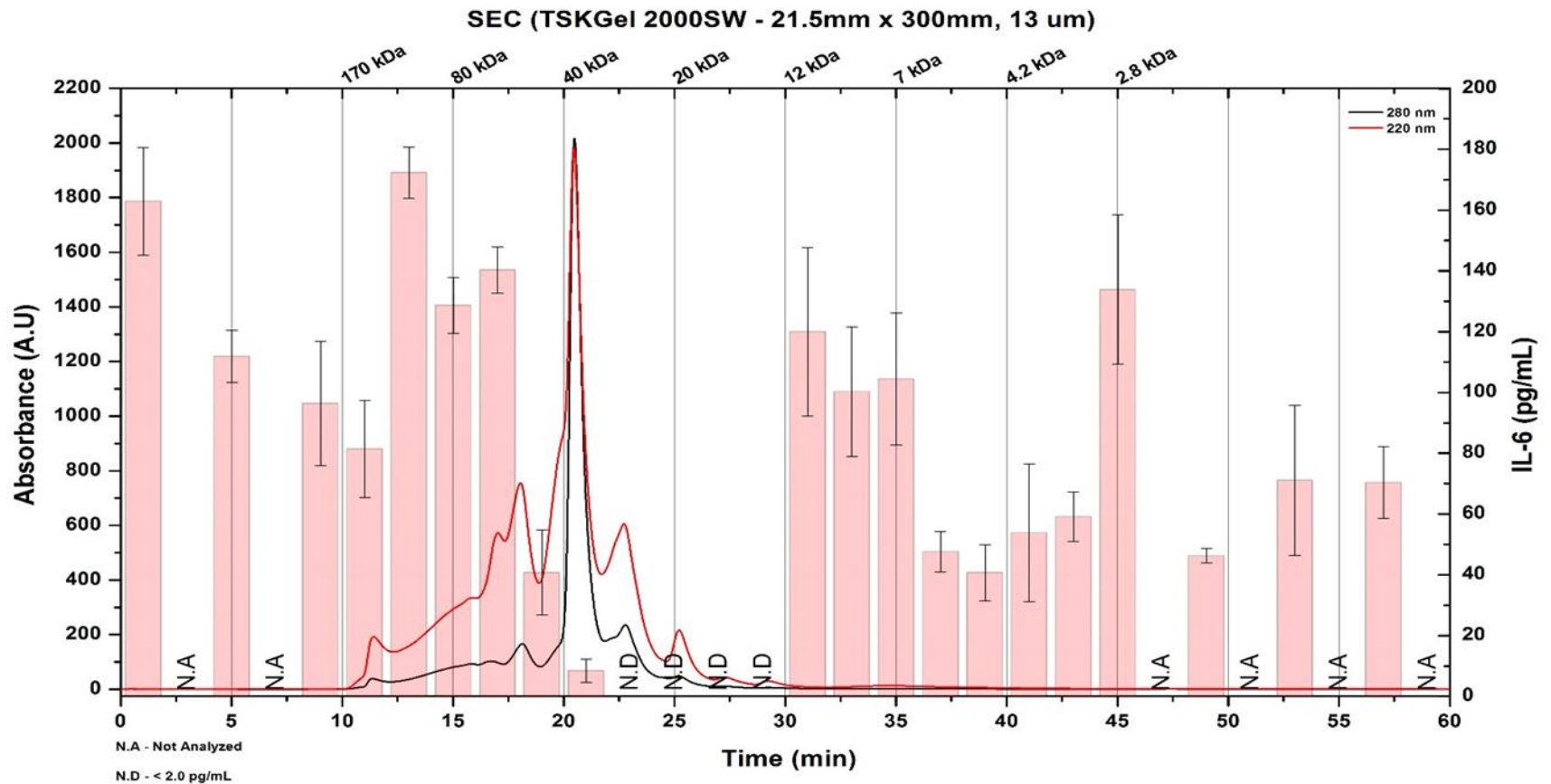
121 °C, 2 atm
autoclave



Sample Preparation



SEC fractions and biological response



Sample Preparation

Affinity Separation - Lectins

SEC
(42-51.5 kDa)

**Affinity
Chromatography**

WGA



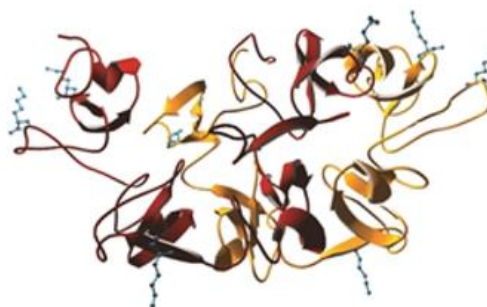
WGA

ConA



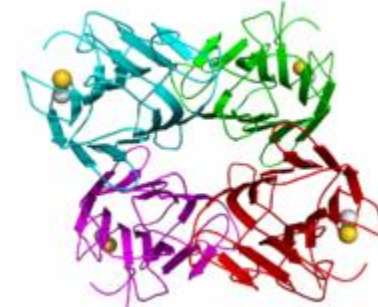
ConA

WGA



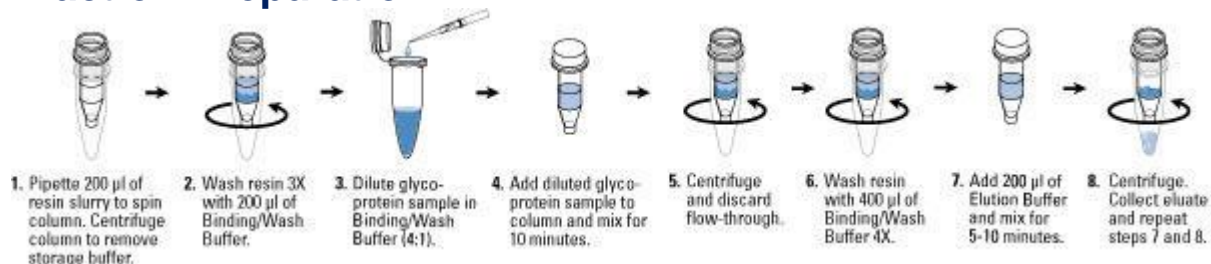
Ligand Motifs:
Sialic Acid
N-acetyl-D-glucosamine

ConA



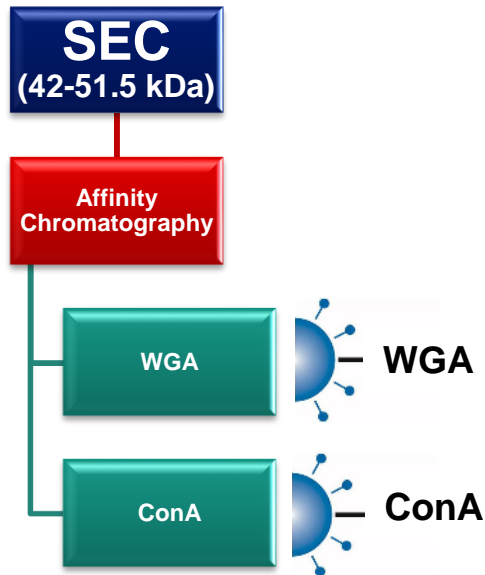
Ligand Motifs:
 α -D-mannosyl residues
 α -D-glucosyl residues

Fraction Preparation:



Sample Preparation

Affinity Separation - Lectins



Lectins:

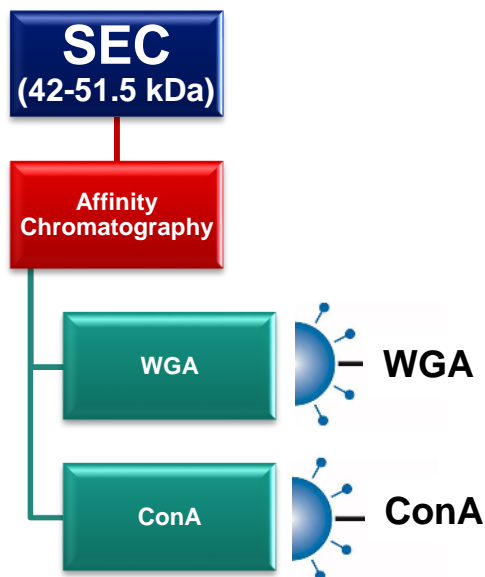
Carbohydrate binding proteins

Targets:

- **Glycoproteins**
- **Glycopeptides**
- **Peptidoglycans**
- **Glycolipids**
- **Glycosides**
- **Lipopolysaccharides**

Sample Preparation

Affinity Separation - Lectins

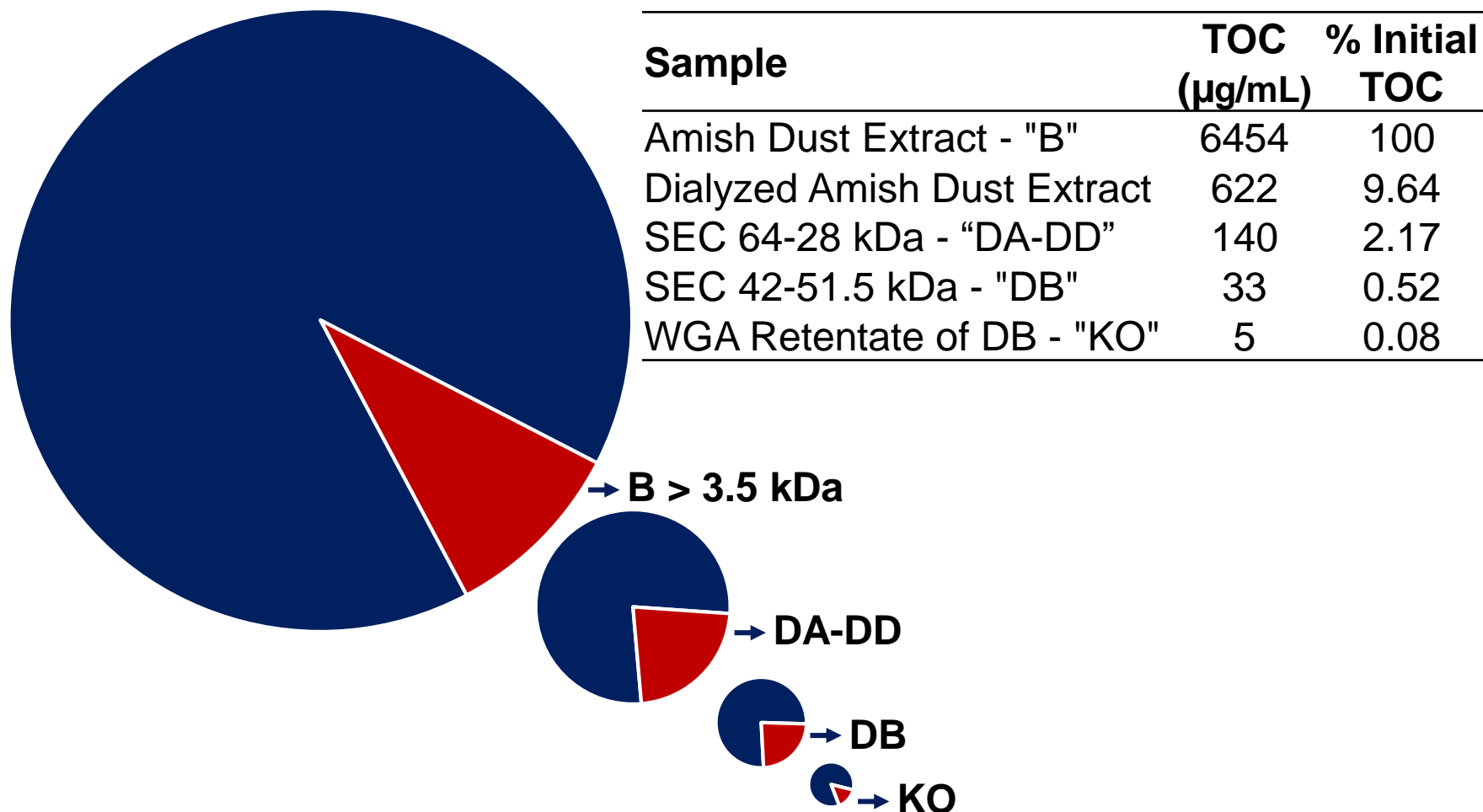


Lectins-Glycoconjugate Interactions

Group of bio-molecules	Range of affinity (Kd)
Lectin-oligosaccharide (Lectin-Glycoconjugate with multiple glycosylation)	$10^{-7} - 10^{-3} \text{ M}$ ($<10^{-7} \text{ M}$)
Complex Salt	$10^{-8} - 10^{-2} \text{ M}$
Protein A-IgG	10^{-6} M
Antigen-antibody (monoclonal)	$10^{-9} - 10^{-7} \text{ M}$
Hormone-receptor	$10^{-12} - 10^{-5} \text{ M}$
Avidin-biotin	10^{-15} M
Covalent bond	10^{-60} M

Deconvolution Progress

Total Organic Carbon (TOC) of fractions



Deconvolution Progress

Estimation of active components concentration

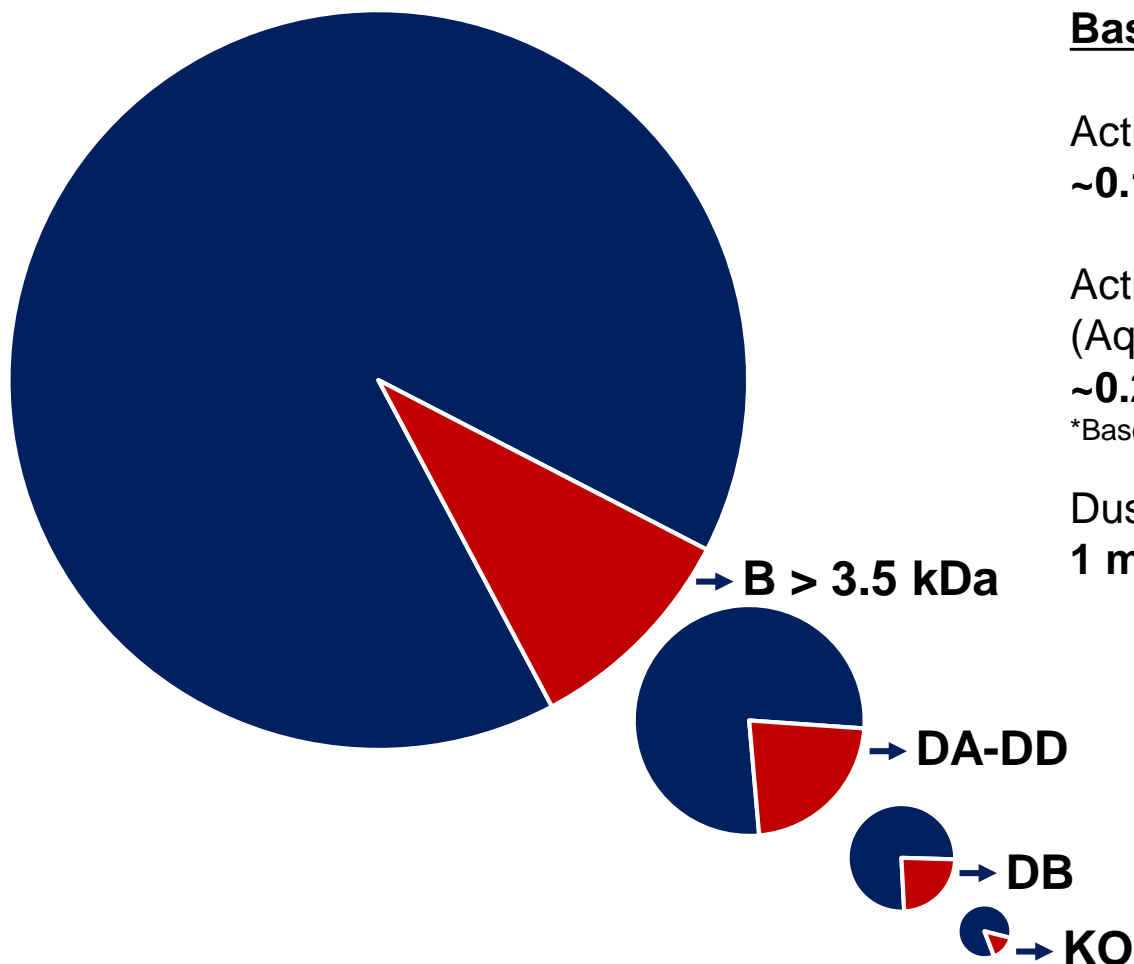
Based on KO TOC concentration:

Active Components in Raw dust:
~0.1 mg/g Dust

Active Components Concentration
(Aqueous extract)*:
~0.2 μM

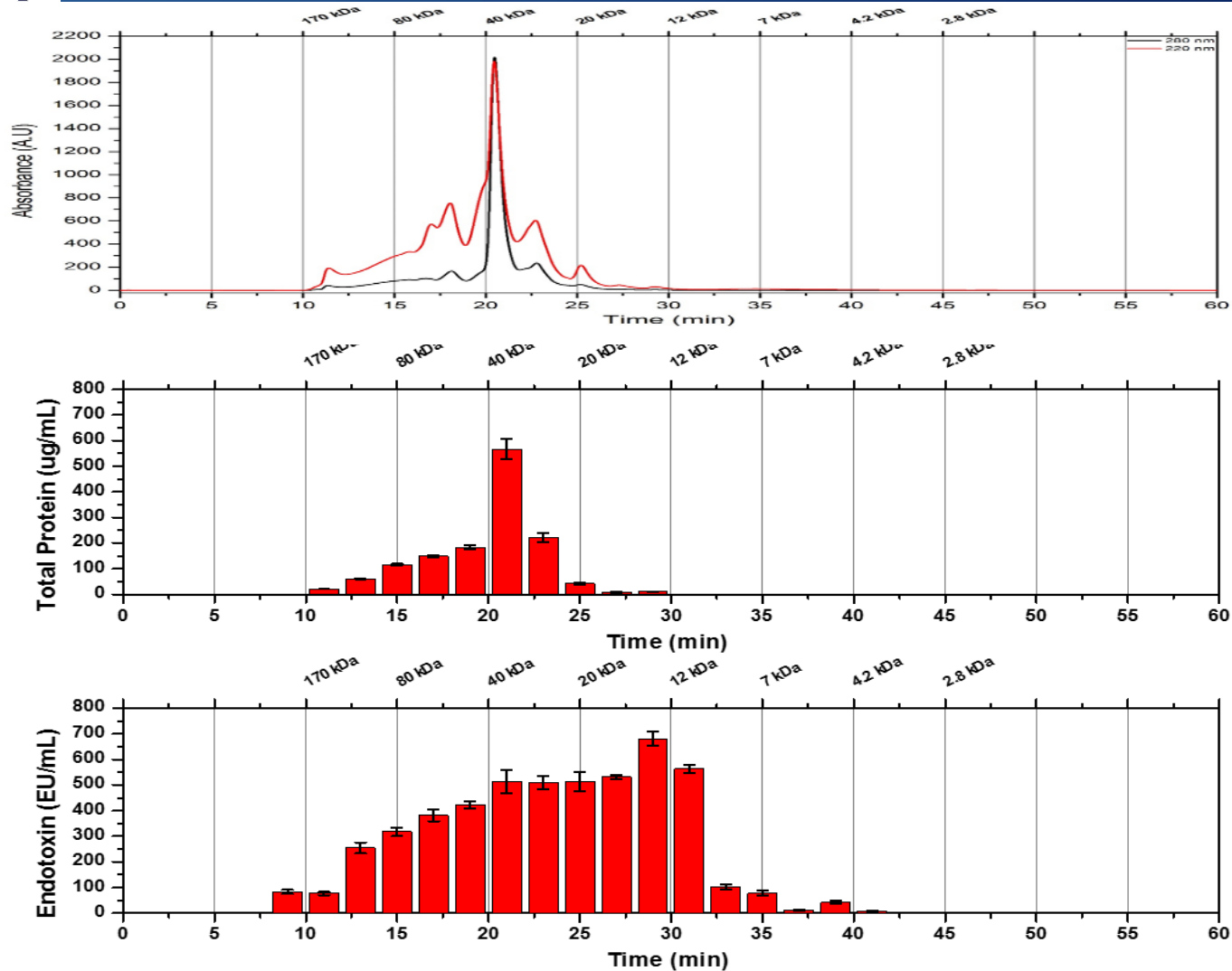
*Based on average MW of DB fraction

Dust Equivalent (DEQ) 100 mg/mL
1 mg DEQ dose = ~ 0.1 μg





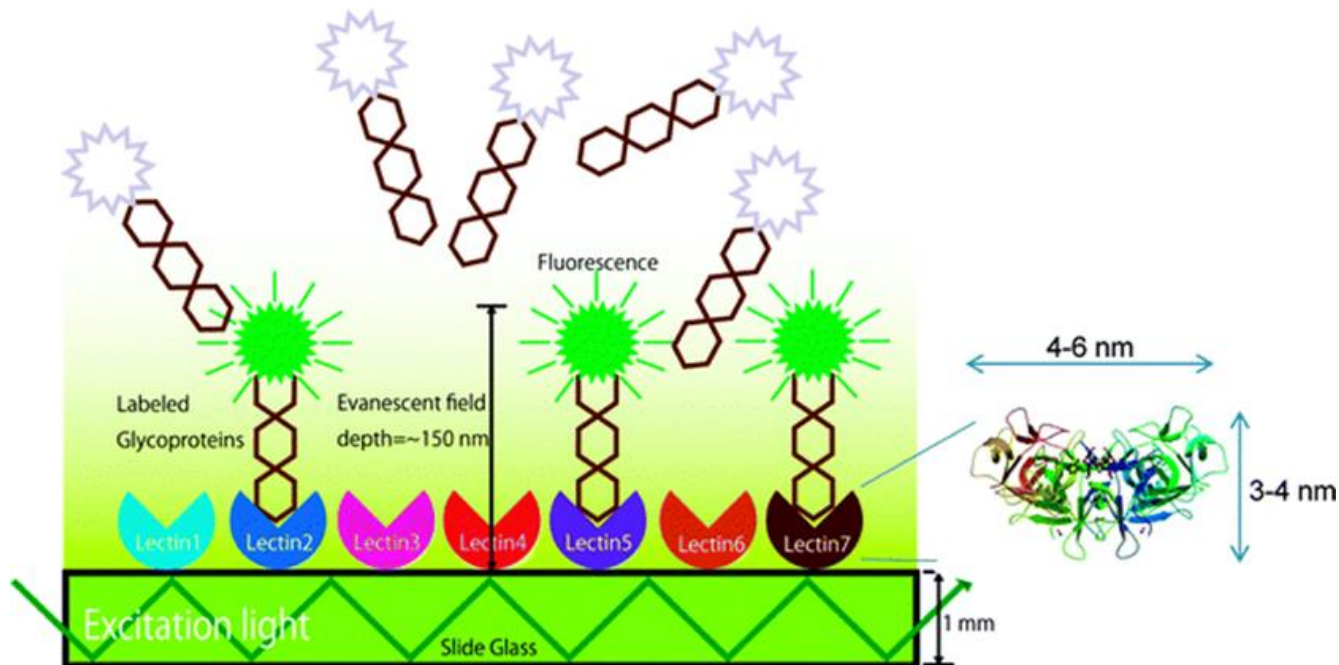
Total Protein and Endotoxin Concentration on SEC fractions



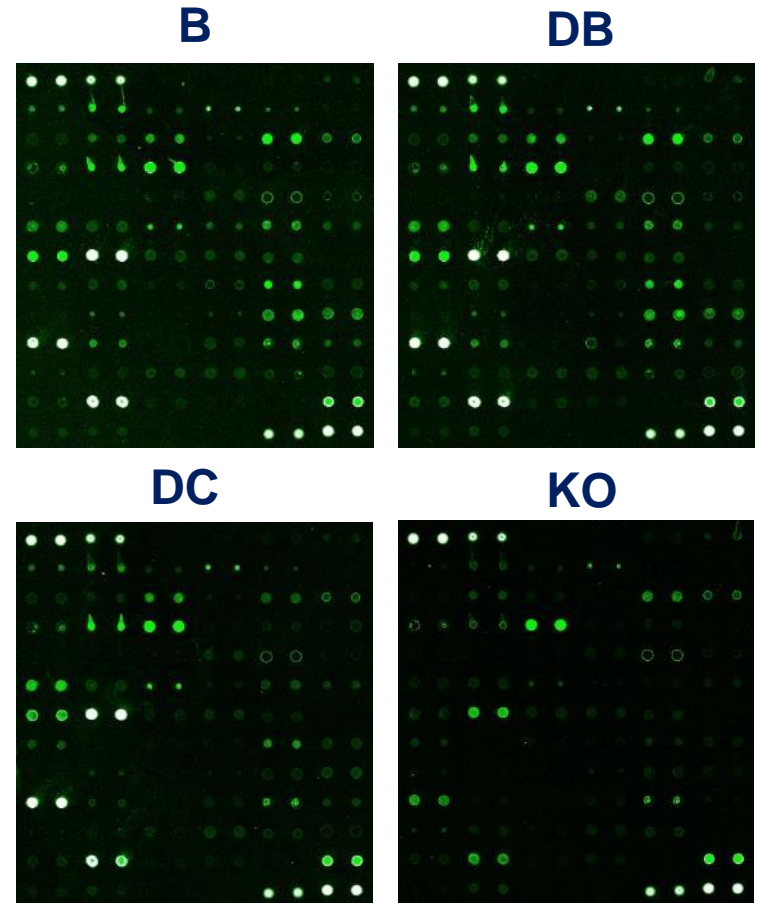
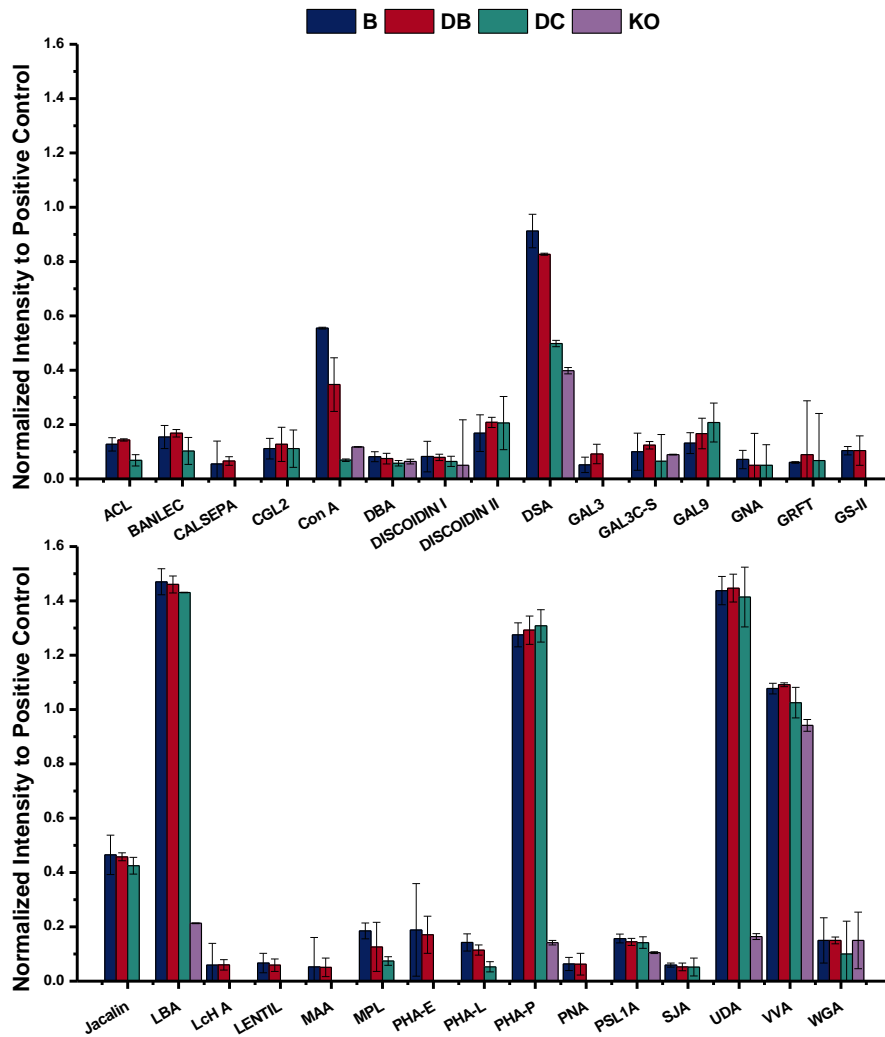
Glycan Profiling

Lectin Array Principle

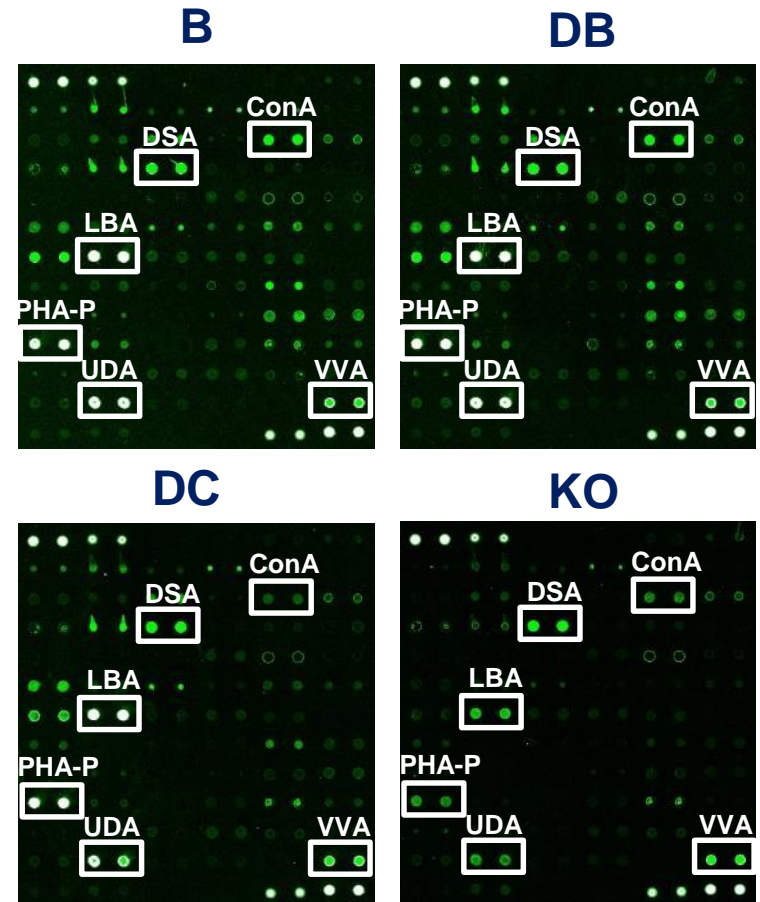
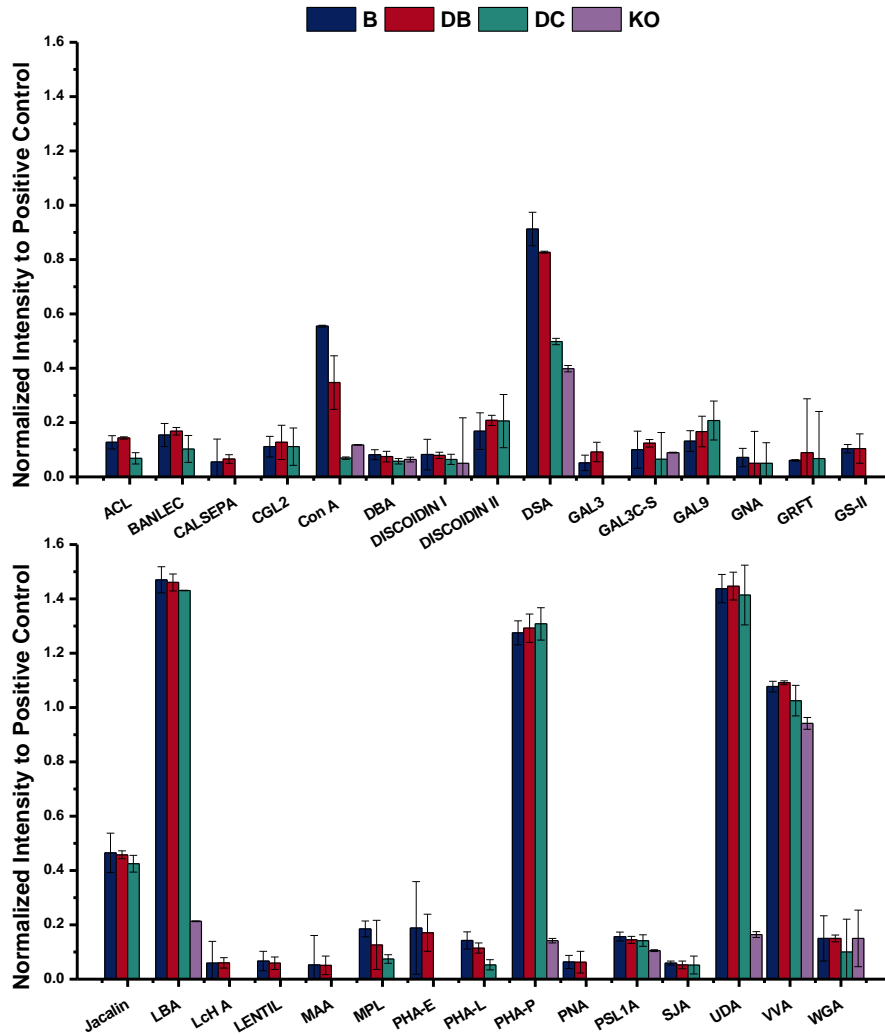
- Rapid and high-sensitivity profiling of complex glycan features without the need for liberation of glycans.



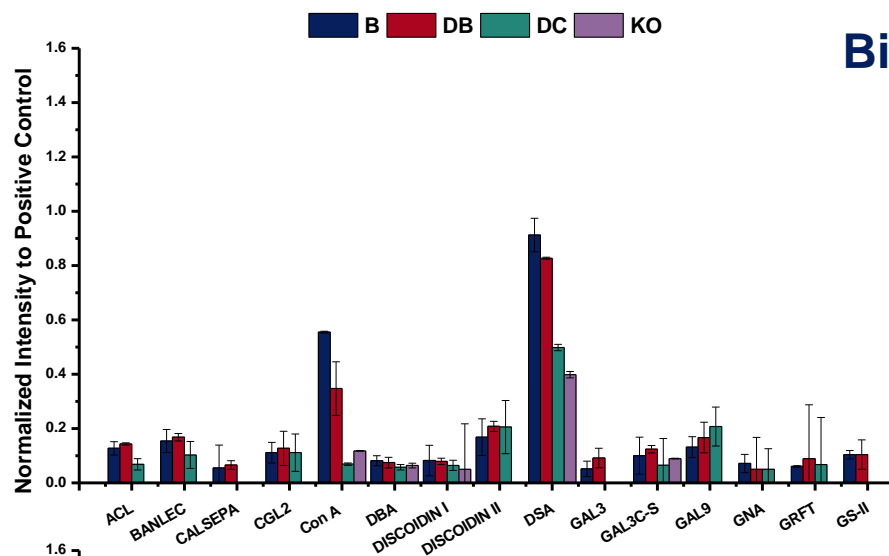
Glycan Profiles



Glycan Profiles

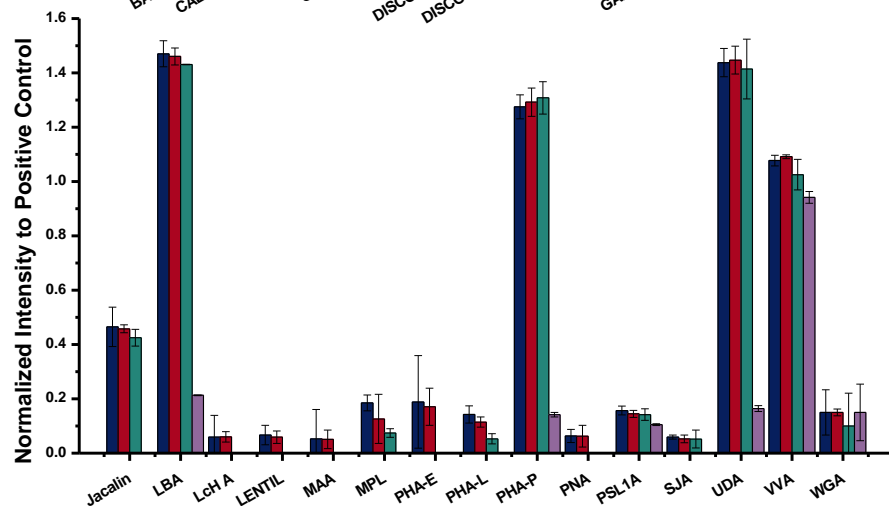


Glycan Profiles



Binding to 30 of lectins present in array

ACL	GAL3C-S	MPL
BANLEC	GAL9	PHA-E
CALSEPA	GNA	PHA-L
CGL2	GRFT	PHA-P
Con A	GS-II	PNA
DBA	Jacalin	PSL1A
DISCOIDIN I	LBA	SJA
DISCOIDIN II	LcH A	UDA
DSA	LENTIL	VVA
GAL3	MAA	WGA



Top 7 interactions and specificities

Lectin	Carbonhydrate specificity
Con A	α Man, α Glc
DSA	(GlcNAc) ₂₋₄
Jacalin	Gal β 3GalNAc
LBA	GalNAc α (1,3)[α Fuc(1,2)Gal
PHA-P	Gal β 4GlcNAc β 2Man α 6(GlcNAc β 4)
UDA	GlcNAc
VVA	GalNAc

Liquid Chromatography – Mass Spectrometry

LC-QTOF Conditions



Agilent 6540 Ultra High Definition (UHD) Accurate-Mass Quadrupole Time-of-Flight (Q-TOF)

Gas Temperature 300 °C

Drying gas flow 13 L/min

Nebulizer pressure 45 psig

Sheath gas Temperature 400 °C

Sheath gas flow 12 L/min

Vcap 5000 V

Fragmentor 175 V

Nozzle Voltage 2000V

Skimmer 65 V

Column ZORBAX 300SB-C18 (2.1mm x 150 mm, 1.8 µm)

Flow 0.5 mL/min

Injection Volume 20 µL

Column Temperature 60 °C

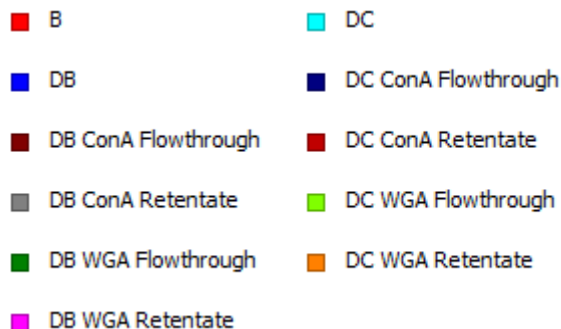
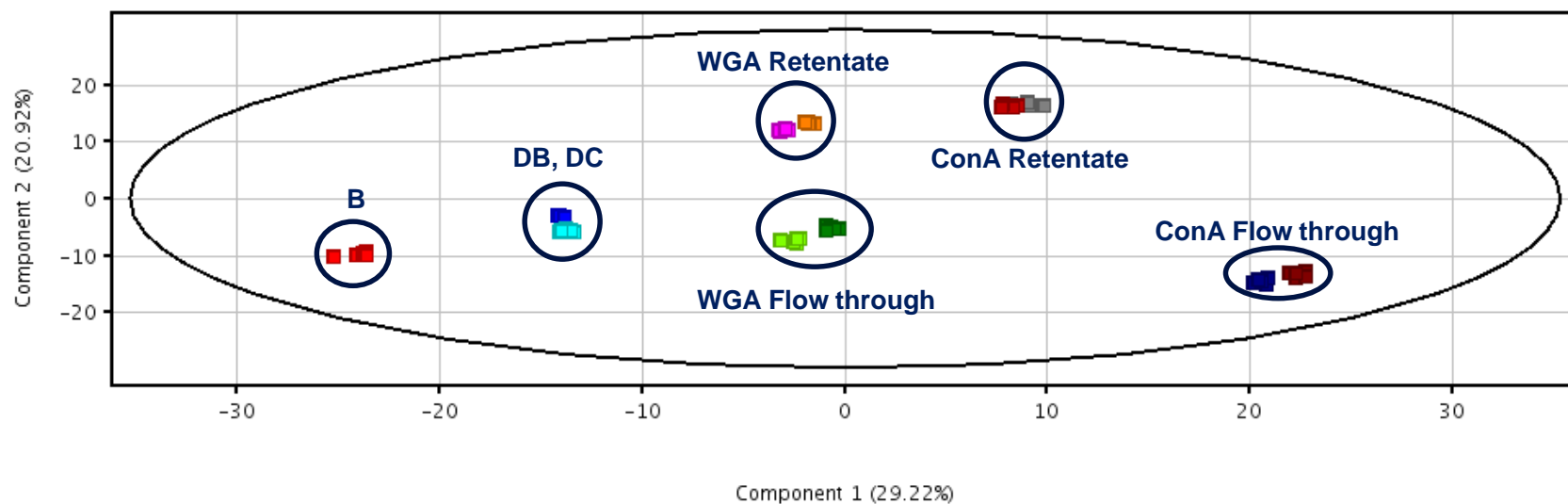
Solvent A Water 0.1% Formic Acid

Solvent B Acetonitrile 0.1% Formic Acid

	Time	A	B
1	2.00 min	95 %	5 %
2	16.00 min	0 %	100 %
3	18.00 min	0 %	100 %
4	18.01 min	95 %	5 %
5	20.00 min	95 %	5 %

LC-QTOF Data Analysis

Principal Components Analysis (PCA)

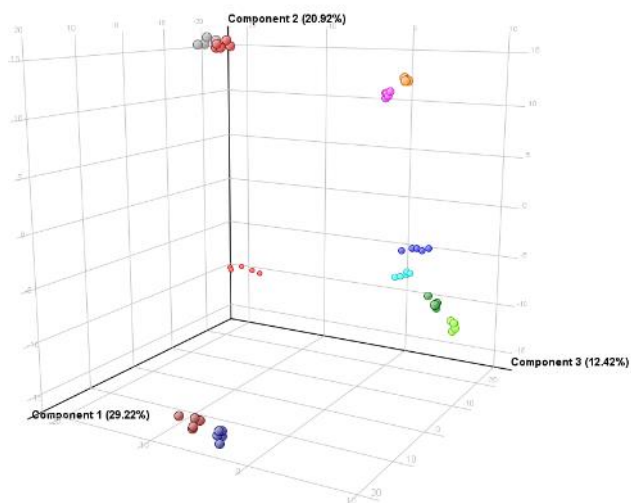


DB and DC pairs of treatments are (SEC fractions 1.5 min apart)

LC-QTOF Data Analysis

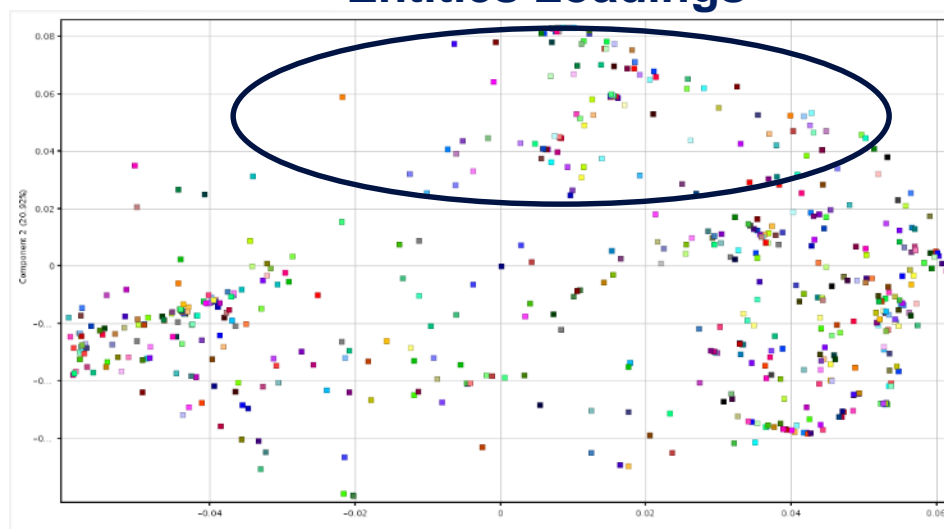
Principal Components Analysis (PCA)

3D PCA Plot



- | | |
|-----------------------|-----------------------|
| ■ B | ■ DC |
| ■ DB | ■ DC ConA Flowthrough |
| ■ DB ConA Flowthrough | ■ DC ConA Retentate |
| ■ DB ConA Retentate | ■ DC WGA Flowthrough |
| ■ DB WGA Flowthrough | ■ DC WGA Retentate |
| ■ DB WGA Retentate | |

Entities Loadings

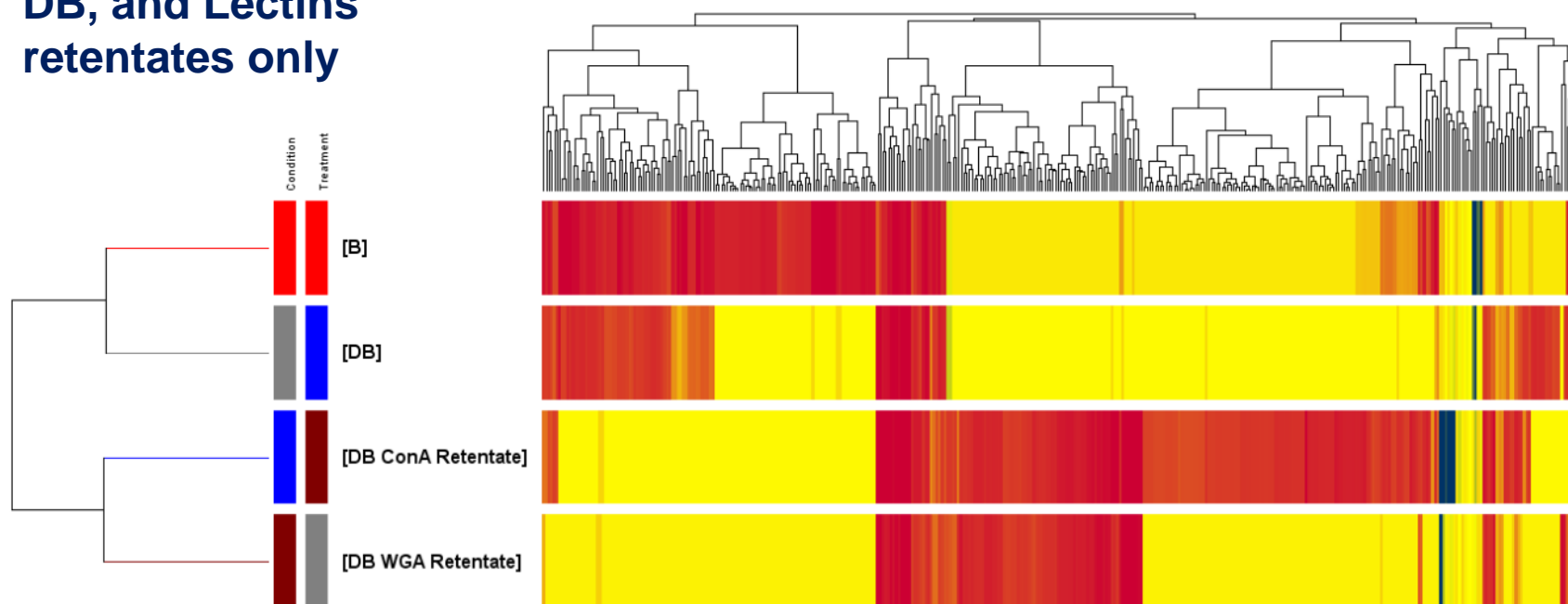


Entities with affinity to Lectins (WGA and ConA motifs)

LC-QTOF Data Analysis

Hierarchical Clustering of Entities and Sample Treatments

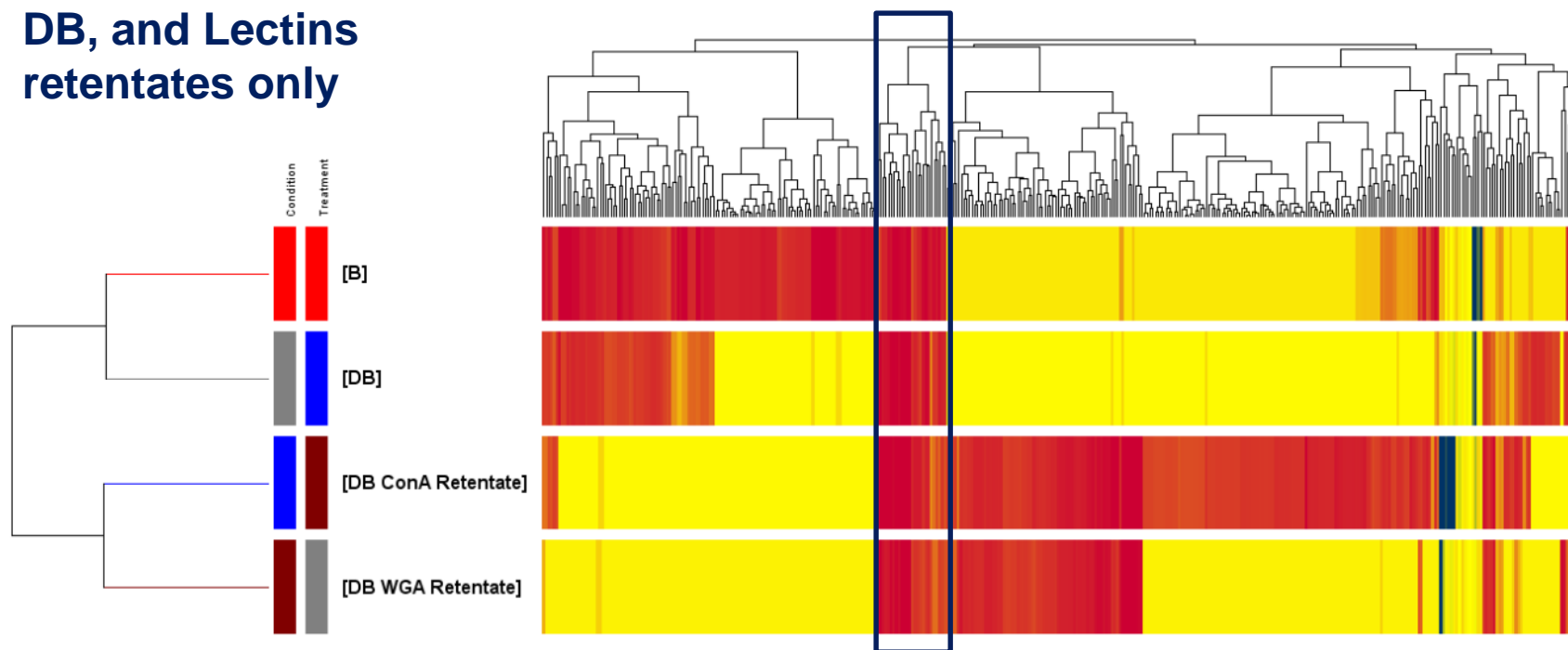
Entities present in B,
DB, and Lectins
retentates only



LC-QTOF Data Analysis

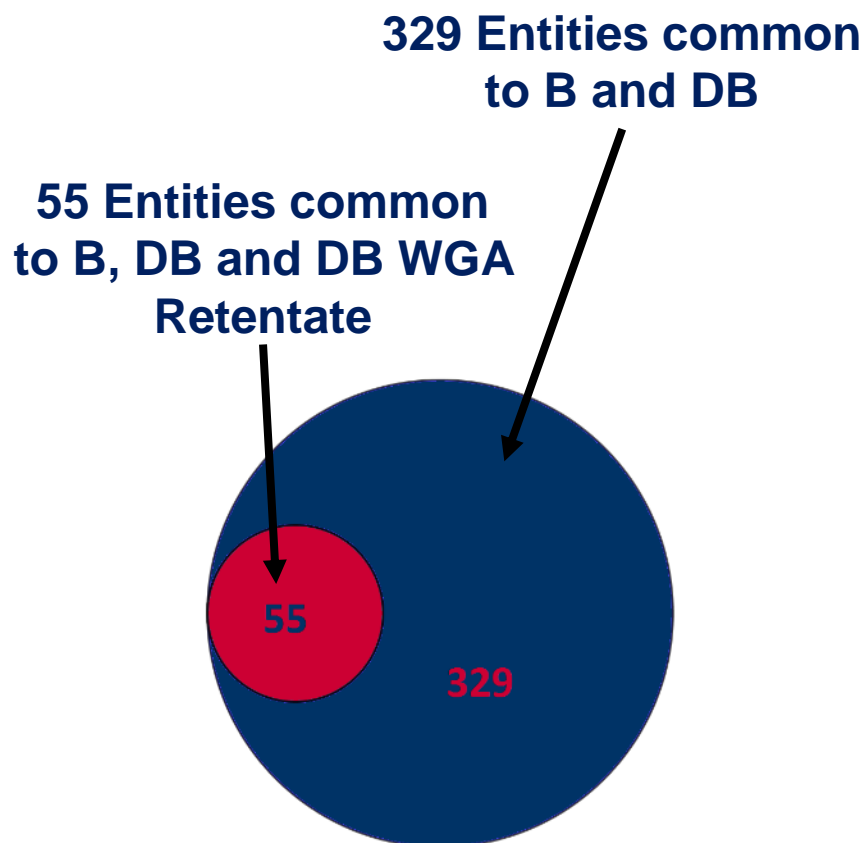
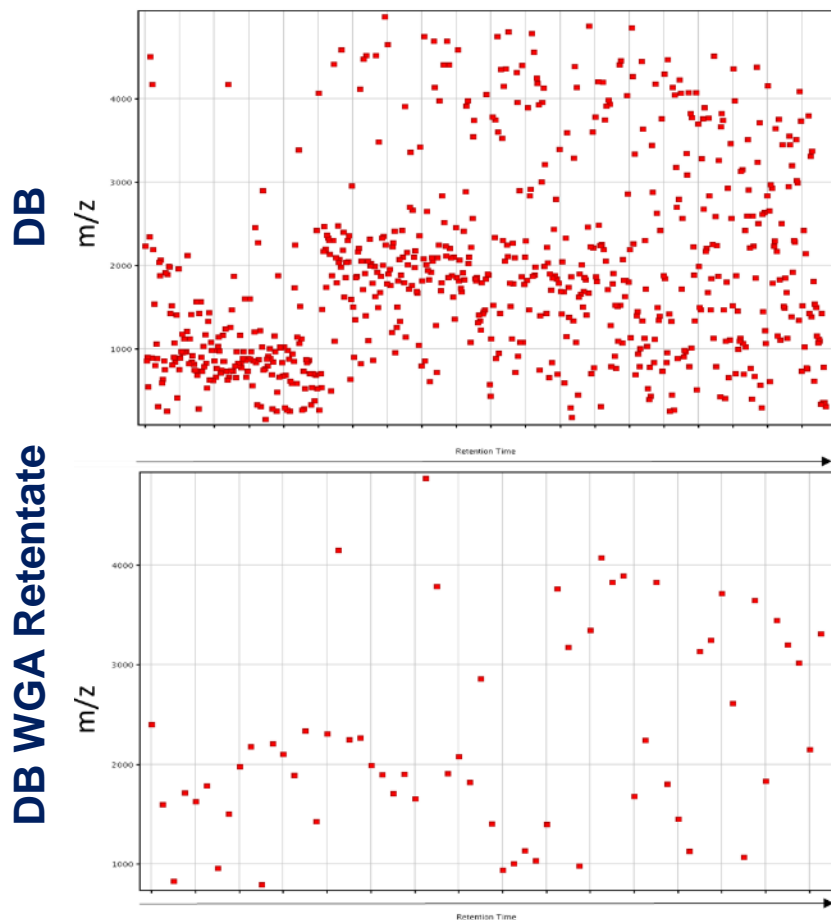
Hierarchical Clustering of Entities and Sample Treatments

Entities present in B,
DB, and Lectins
retentates only



LC-QTOF Data Analysis

Potential Target Entities





Acknowledgements



Ministério da
Educação

