Enhancing Carbohydrate Metabolite and Glycan Analysis through Porous Graphitic Carbon HPLC Columns

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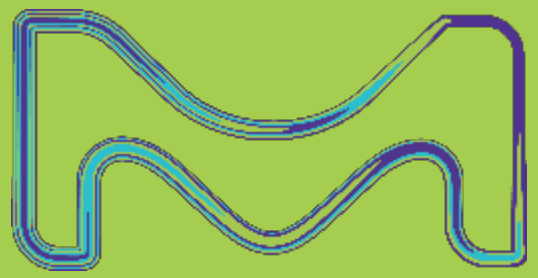

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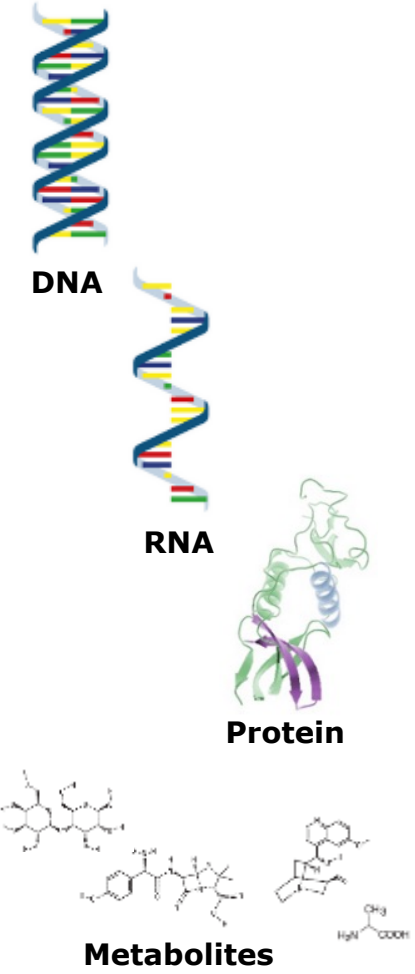
The Genome

- Humans have 23 chromosome pairs with ~ 23.000 genes
- Human races **differ in 0.1% of their genome**
- Chimpanzee and humans differ in **1.5 – 5% of their genome**
- The genome of 2 chimpanzee differs of **about 1.2%.**

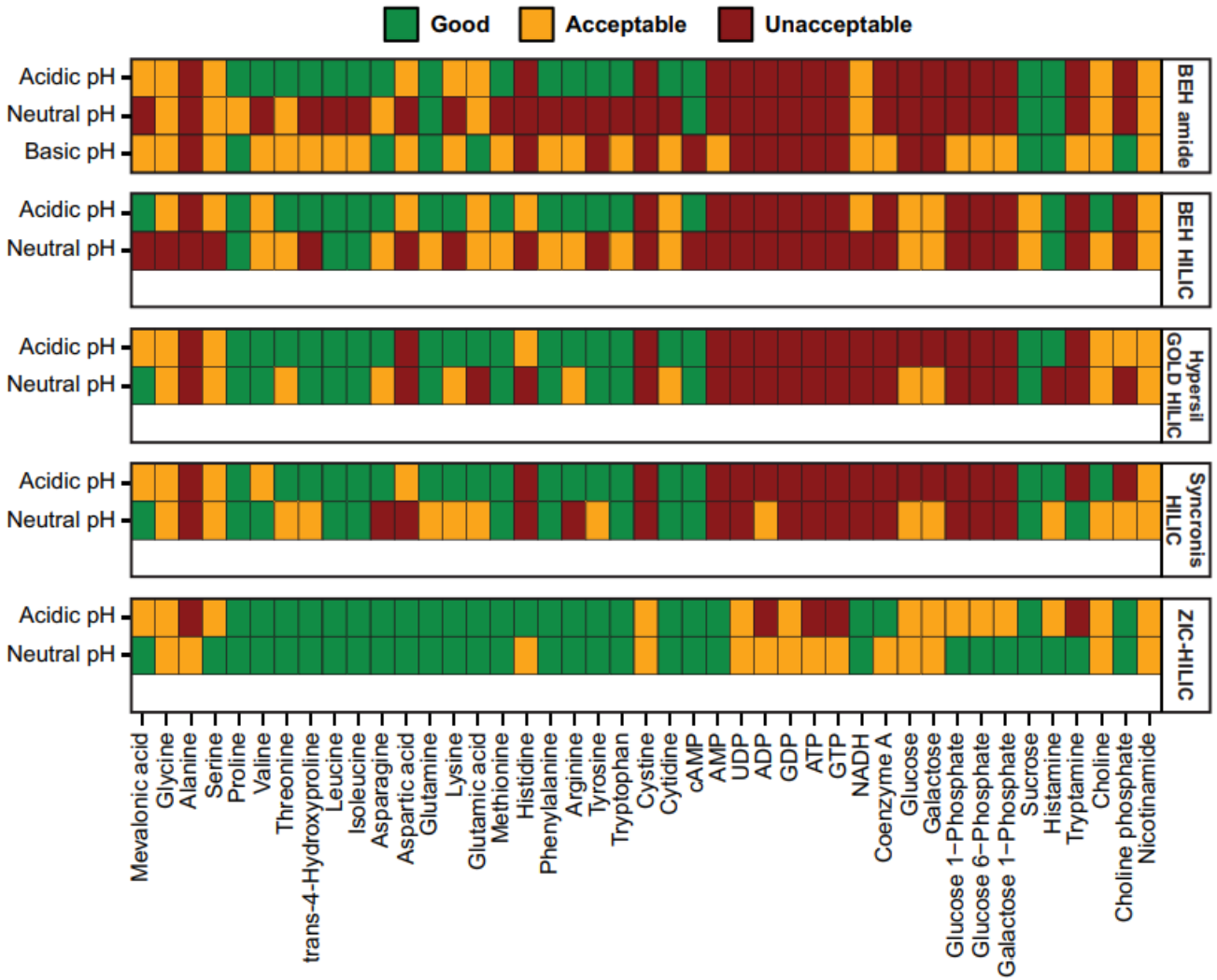
The OMICs Approach



OMICS approach	Read-out	Results	Technology
Genomics	Genes (DNA)	Genetic variants, gene presence or absence, genome structure	Sequencing Exome sequencing
Epigenomics	Modifications of DNA	Location, type, or degree of reversible DNA modifications	Modification-sensitive PCR and qPCR Next-generation sequencing MS
Transcriptomics	RNA and/or cDNA	Gene expression, gene presence or absence, splice sites, RNA editing sites	RT-PCR and RT-qPCR Gene arrays RNA-sequencing
Proteomics	Protein	Abundance of peptides, peptide modifications, and interactions between peptides	**HPLC/LC-MS** Western blotting ELISA
Metabolomics	Metabolites	Abundance of small molecules such as carbohydrates, amino acids and fatty acids	**HPLC/LC-MS** GC-MS NMR
Glycomics	Glycomes	Entire complement of sugars, free or present in more complex molecules, including genetic, physiologic, pathologic and other aspects	**HPLC/LC-MS**

Conventional separation of polar Metabolites

Comparison of HILIC Columns

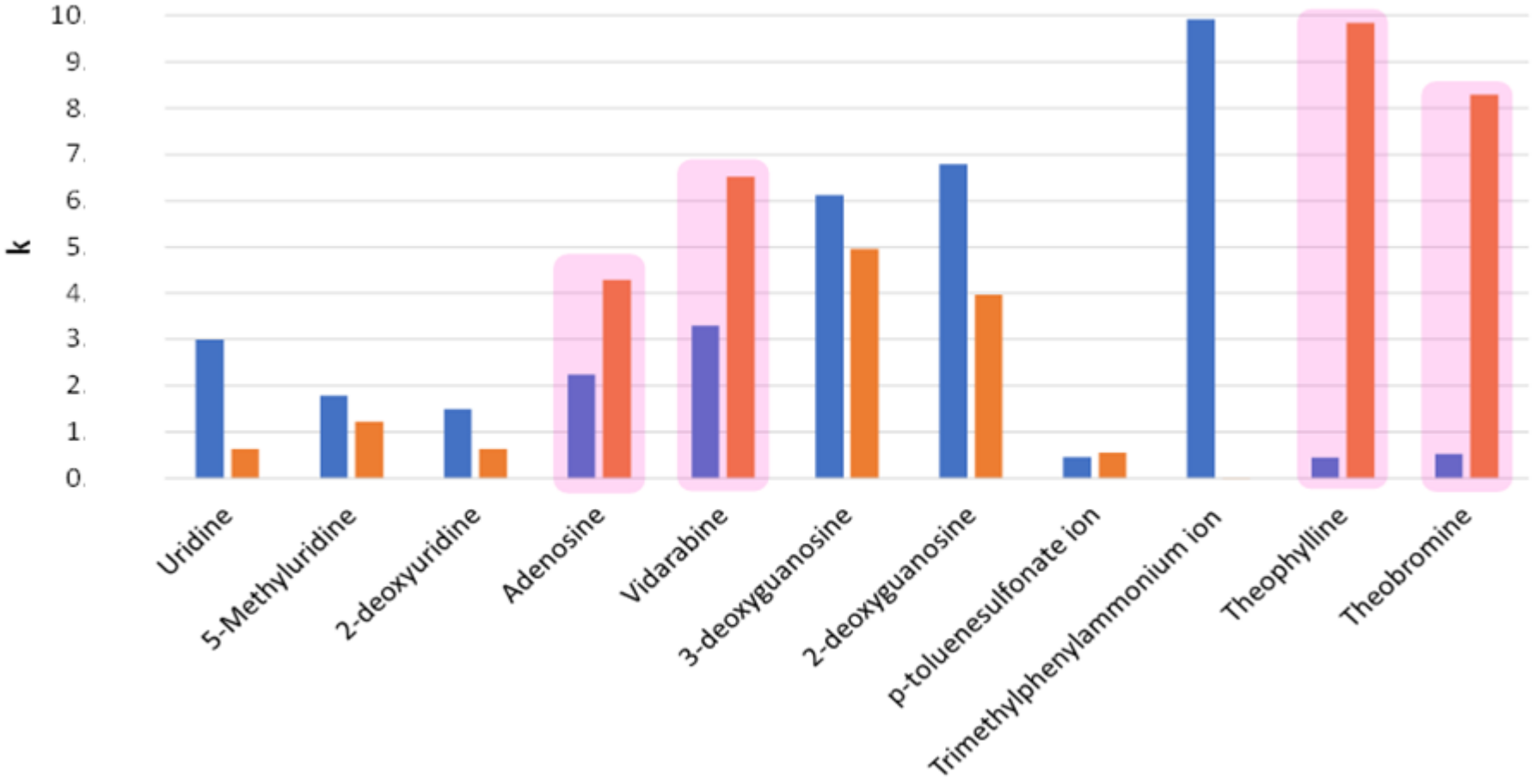


"The zwitterionic Sulfobetaine column operated at neutral pH was **superior for separating urine and plasma metabolites with the best peak shape** in many cases. Even though the gradient condition, the oven temperature, and the flow rate have been shown to be secondary parameters, they were also optimized in HILIC mode."

Optimized Analytical Procedures for the Untargeted Metabolomic Profiling of Human Urine and Plasma by Combining Hydrophilic Interaction (HILIC) and Reverse-Phase Liquid Chromatography (RPLC)–Mass Spectrometry; Michael Snyder et al.; 2015 by The American Society for Biochemistry and Molecular Biology, Inc. ; Open Access: https://doi.org/10.1074/mcp.M114.046508

Zwitterionic Sulfobetaine HILIC vs Porous graphitic carbon (PGC)

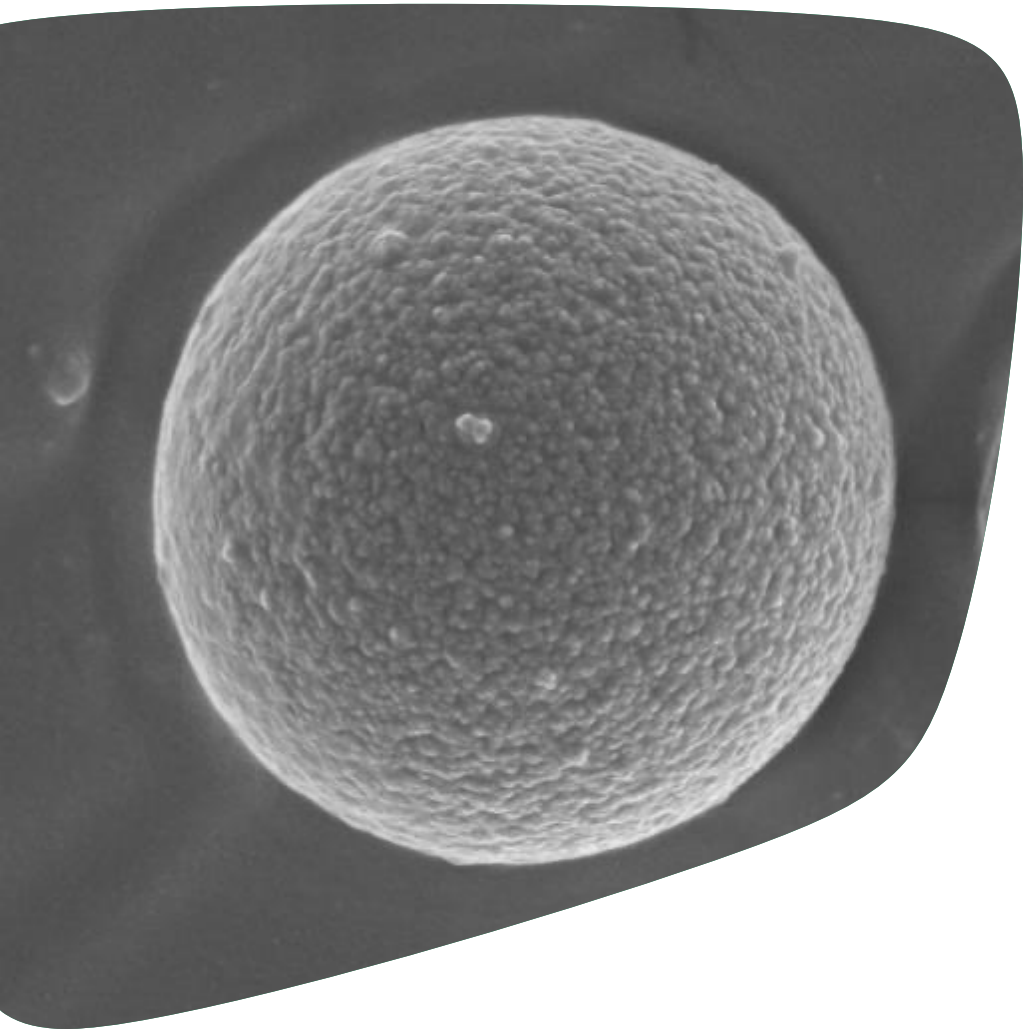
Retention factor k



Comparison of retentions **Sulfobetaine-HILIC** to **PGC**

Theobromine and theophylline interacting on PGC showing the enhanced retention.

Porous graphitic carbon (PGC) particles



- 99.9% of carbon surface with a certain degree of graphitization.
- PGC is unique compared to Graphitized Carbon Blacks (GCB's) in that the morphology of the carbon structure allows it to withstand the back pressure requirements for HPLC and UHPLC. GCB's cannot handle these pressures.

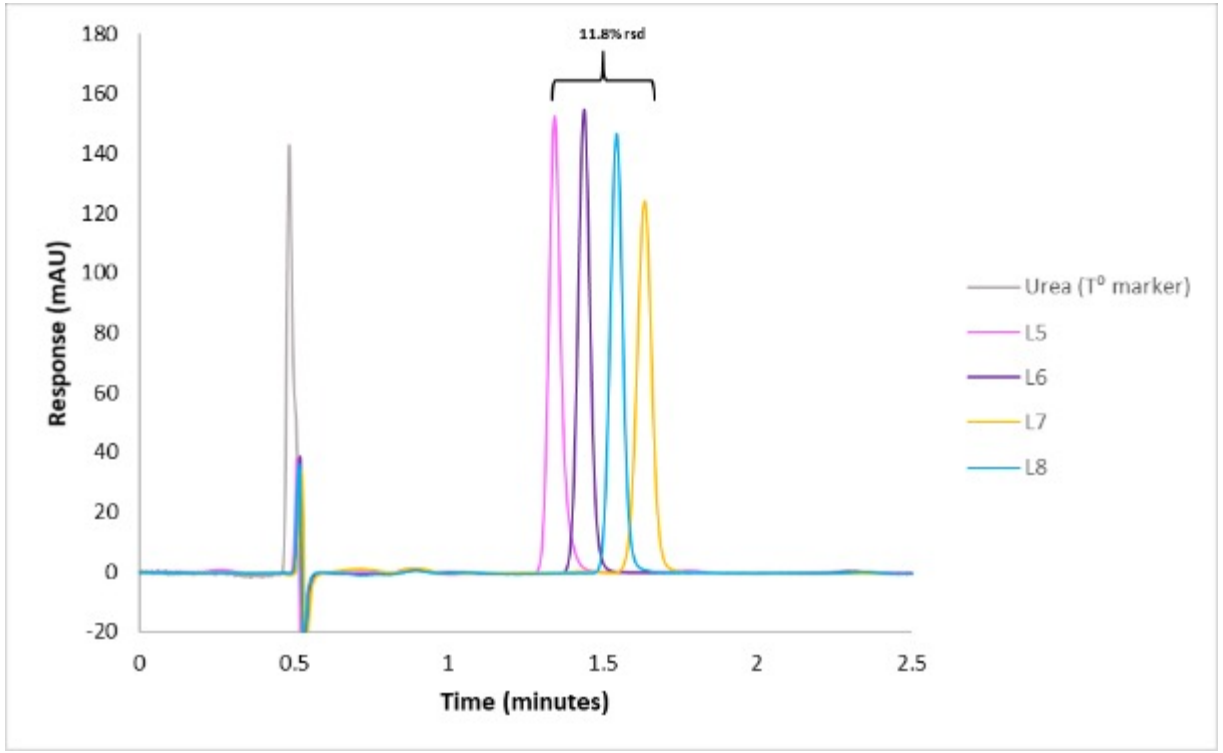
Providing

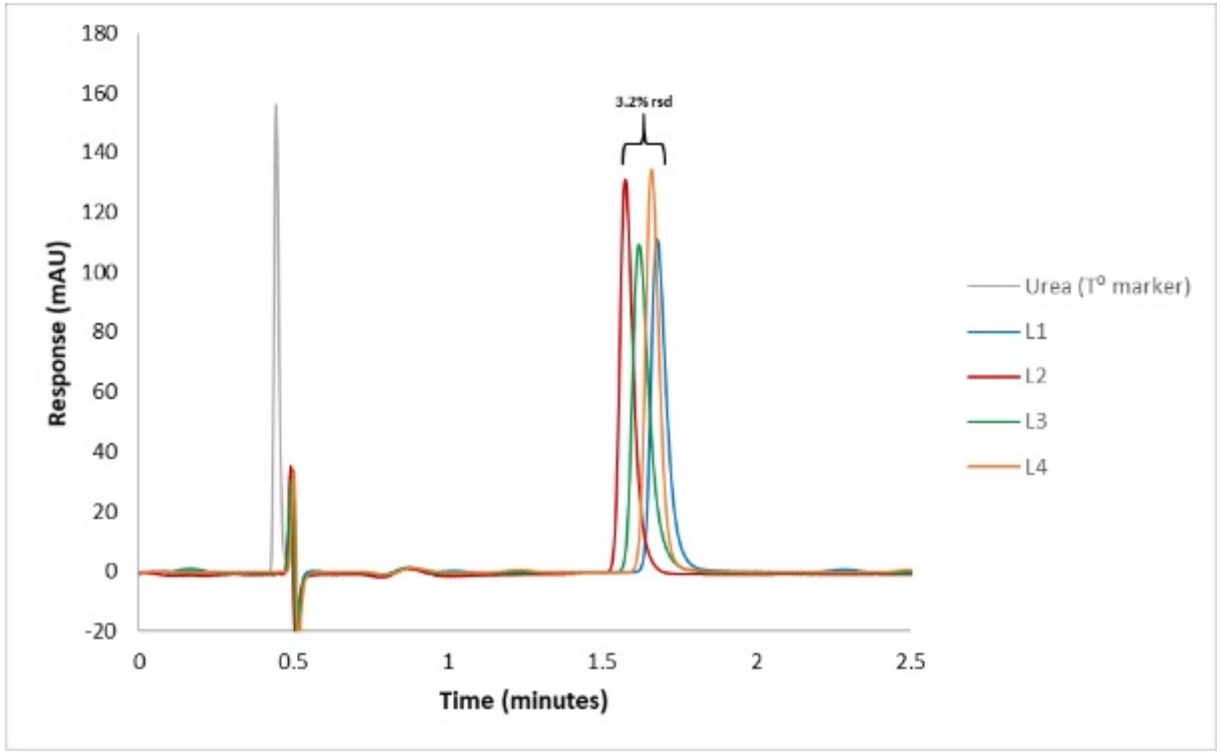
- High temperature Stability (250 °C), good choice for high temperature applications
- Pressure up to 620 Bar
- pH compatible from 0 - 14
- Wide range of mobile phases, not affected by harsh solvents
- Extends the range of polar analytes in reversed-phase mode, beyond polar embedded C18 phases
- Excellent analyte shape selectivity

Porous graphitic carbon (PGC) particles

Improvements to Current Technology – Lot to Lot Variability

PGC A Uracil, 254 nm **PGC B**



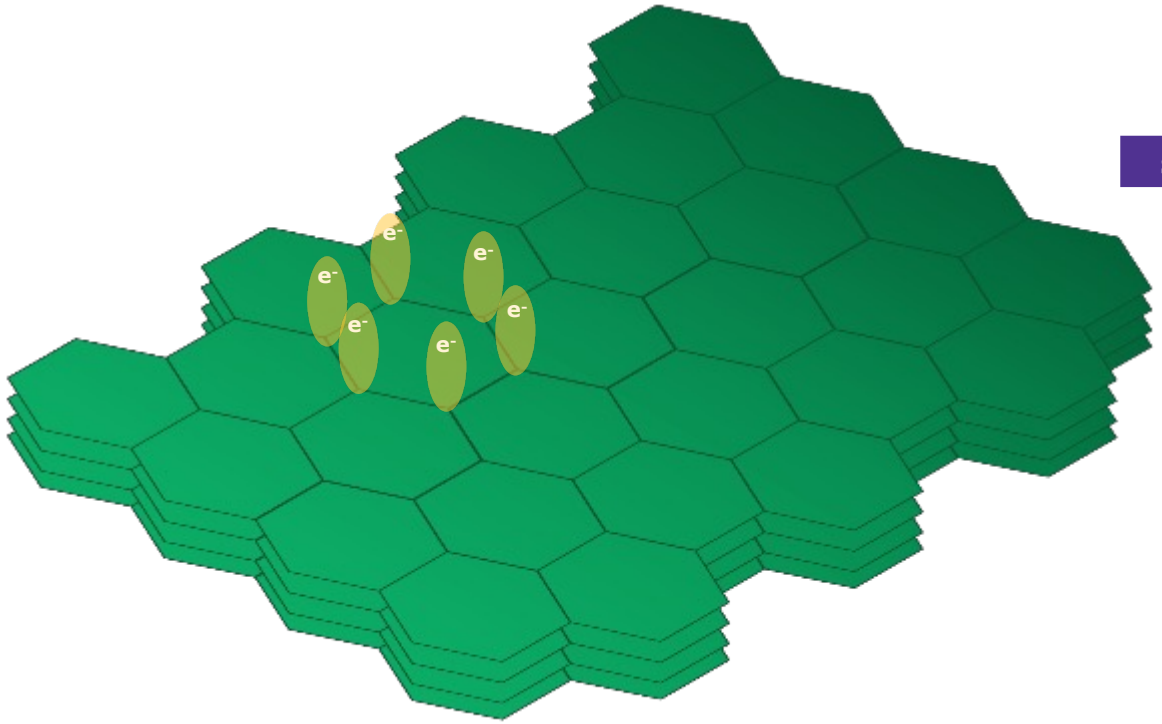


Significantly better lot to lot variance on PGC B

Porous graphitic carbon (PGC) particles

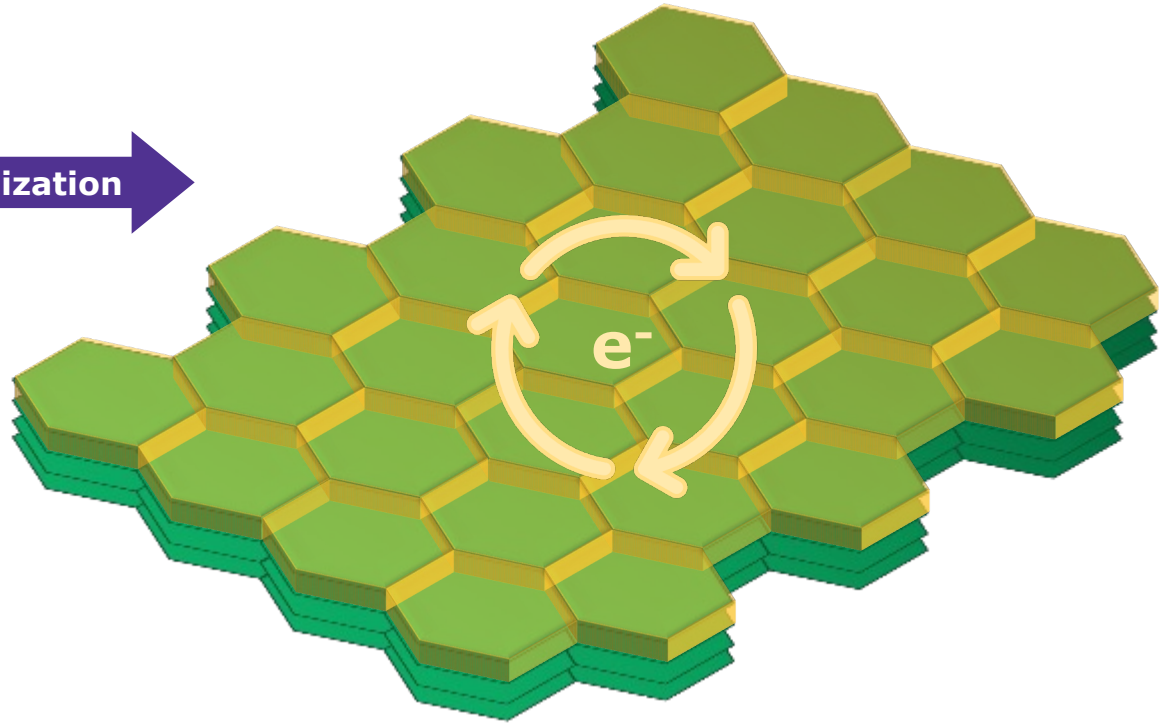
π-Cloud of Graphite

Delocalized e^- on all carbon atoms in p-orbitals



sp^2 Hybridization

Continuous π-cloud system



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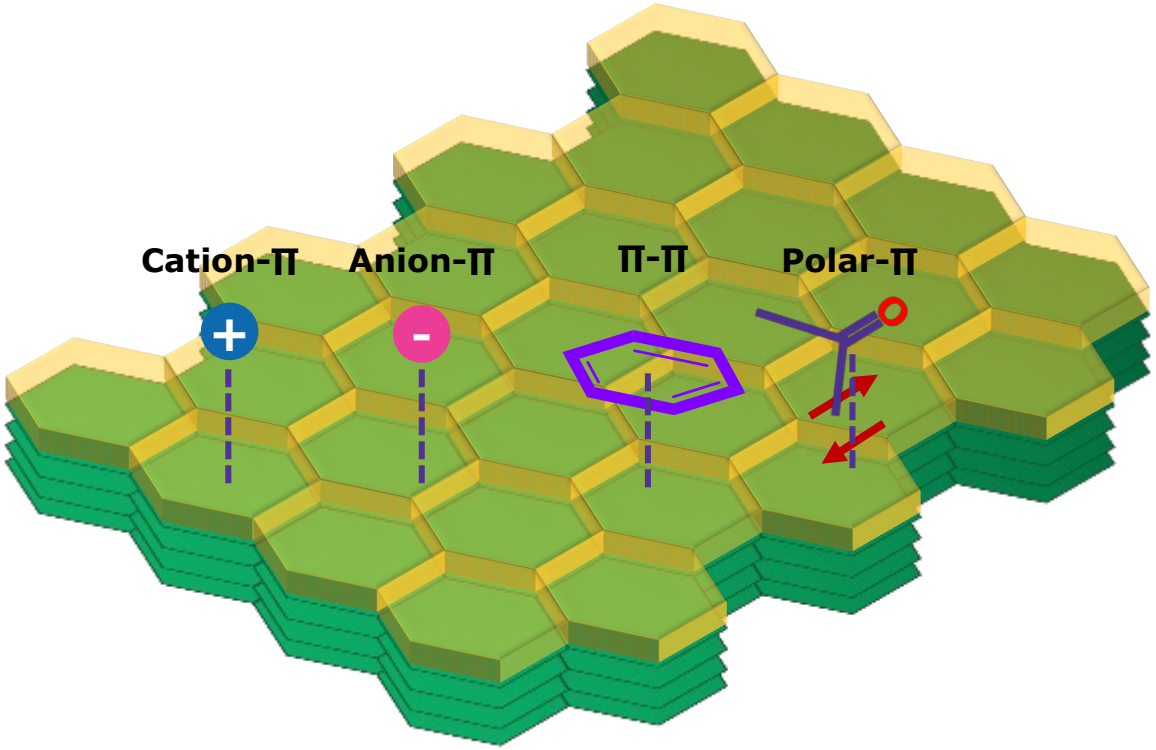
Porous graphitic carbon (PGC) particles

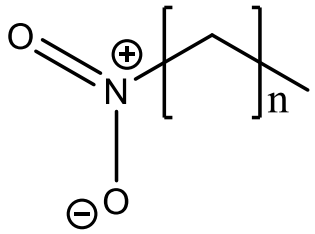
Some Interaction Types with the π Cloud of Graphite

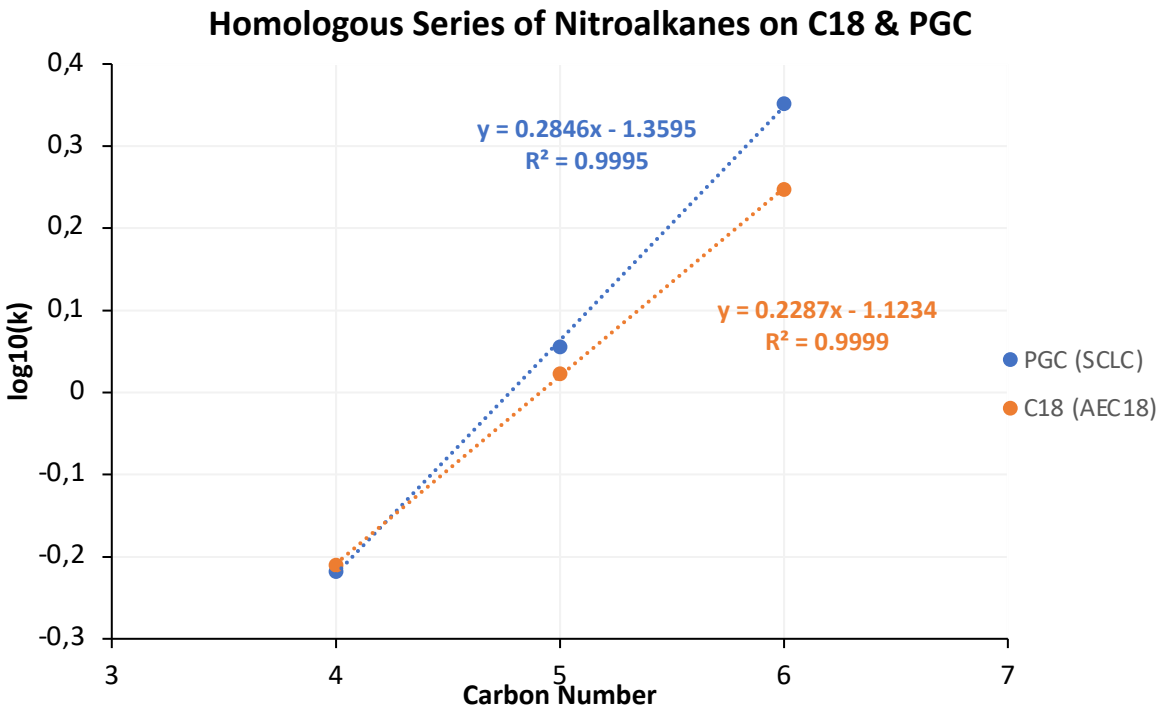


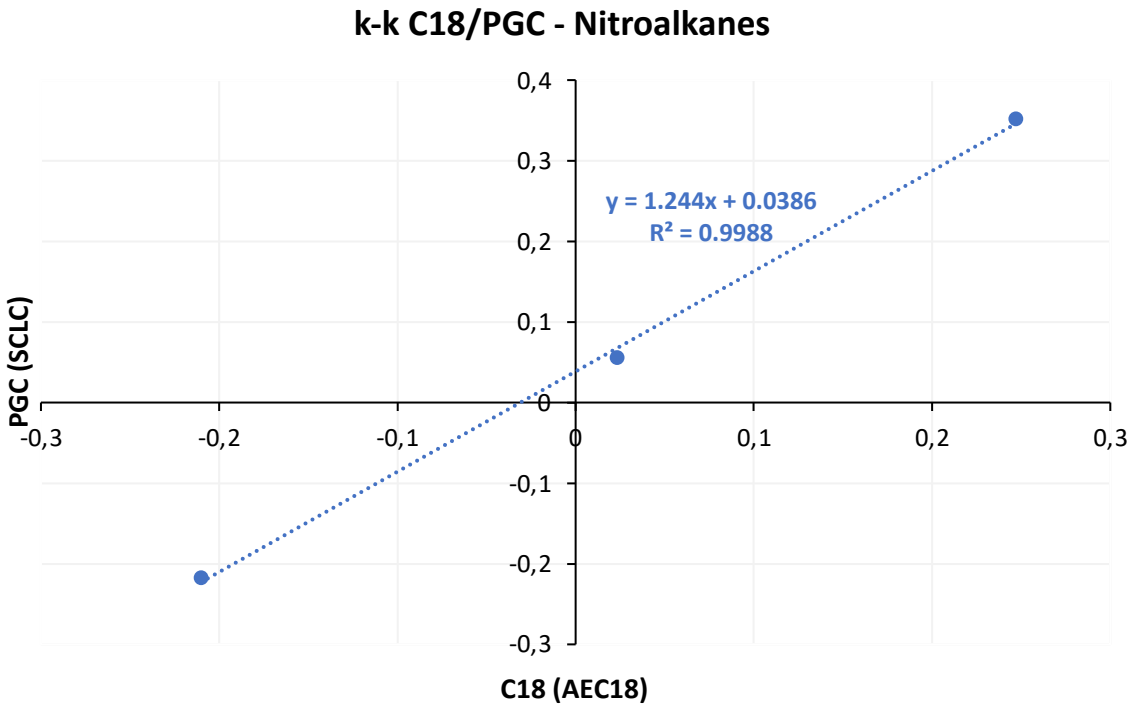
- **Cation-π**, positively charged analyte attracted to electron rich surface
- **Anion-π**, negatively charged analyte interacts with electron rich surface. Repulsion would be expected; however, this is not normally the case on PGC.
- **π-π**, orbital overlap between analyte and surface
- **Polar-π**, molecular dipole/quadrupole induces dipole/quadrupole moment with the surface

Comparison PGC to C18

Nitroalkanes in 60/40 Acetonitrile/Water



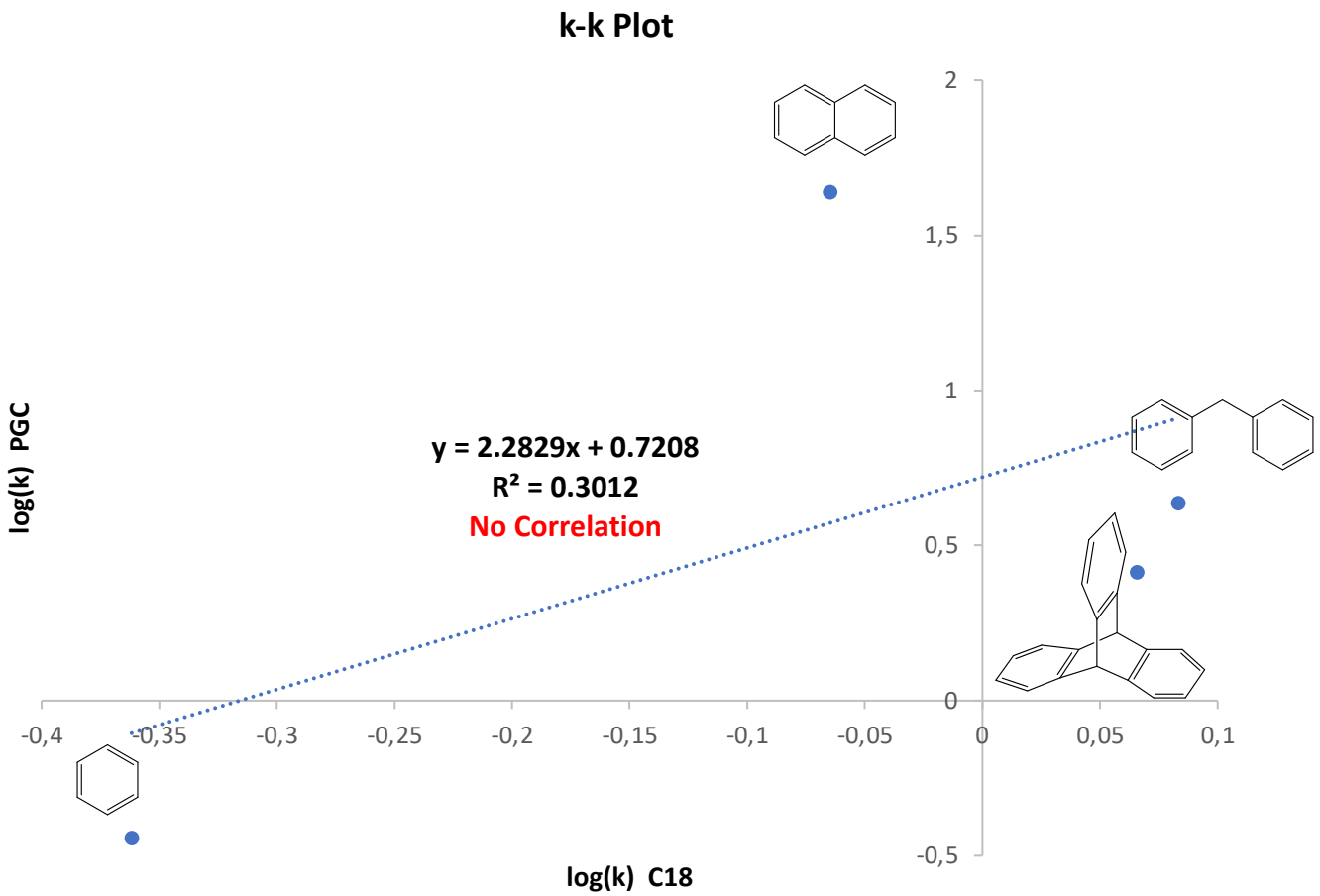


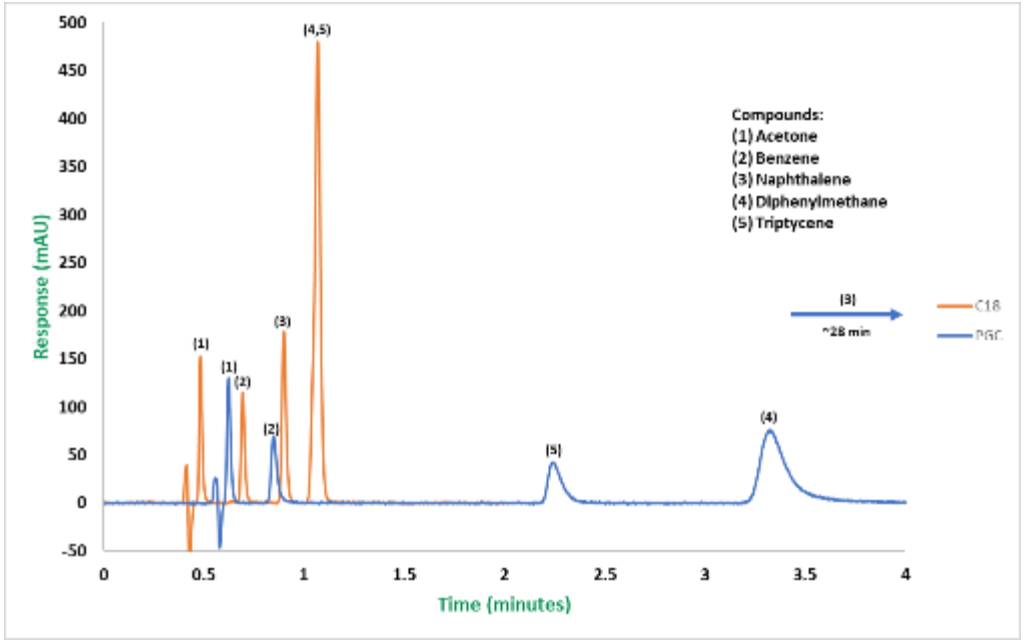


Similar trend PGC and C18

Comparison PGC to C18

Stereochemistry Considerations



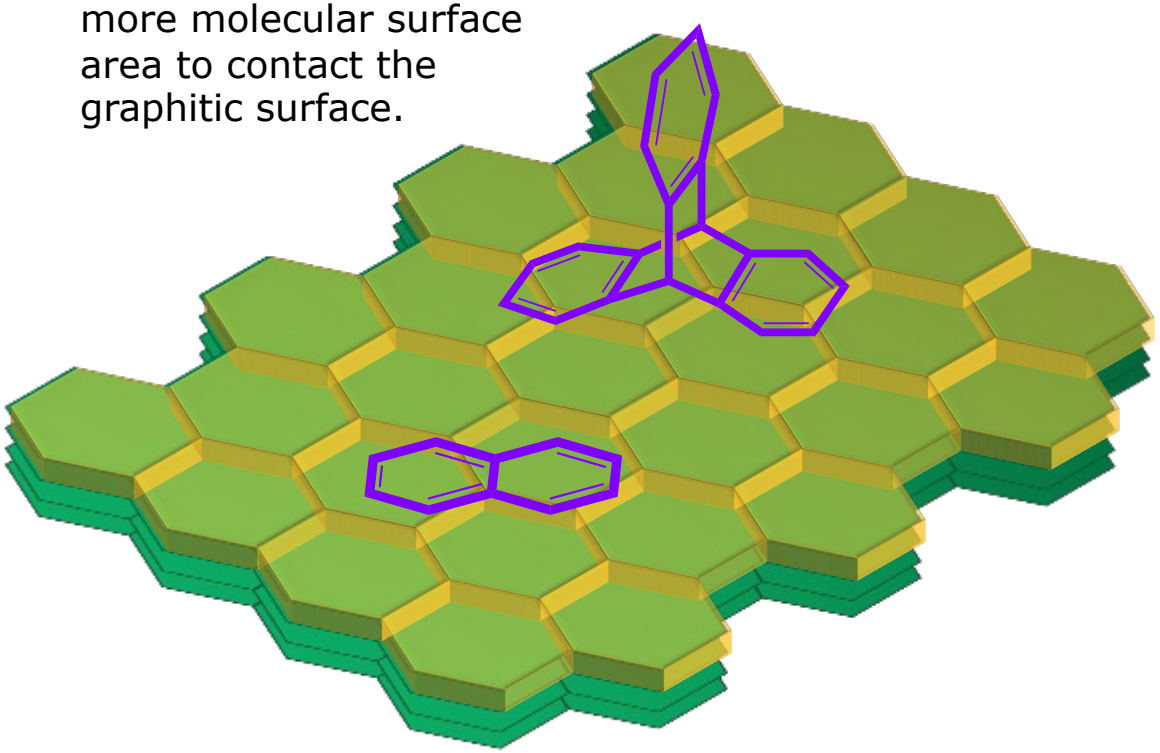


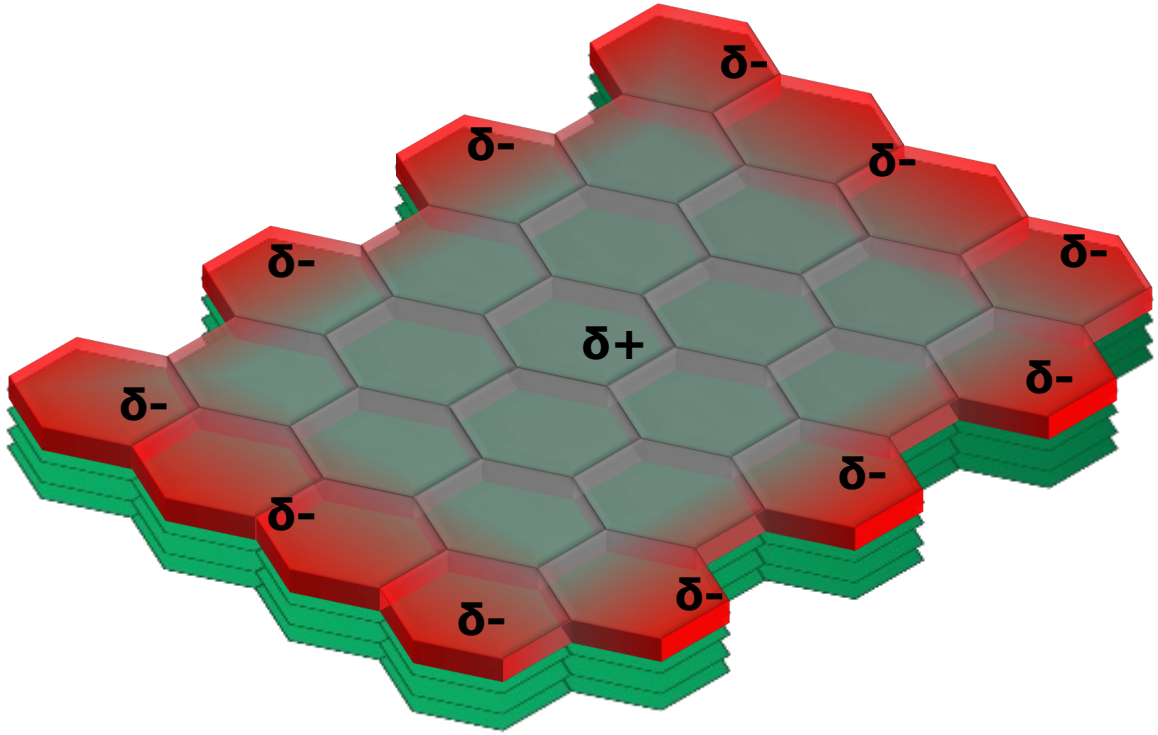
Differences PGC and C18

PGC's Proclivity Towards Aromatics
Stereochemistry Considerations

Key Takeaway: PGC shows strong retention properties towards flat/aromatic analytes

Naphthalene can orient more molecular surface area to contact the graphitic surface.





One theory: Increased electron density towards the extremities resulting in increased negative charge – neutral and anionic analytes interact in the central region, positively charged and H-Bonding interactions at the outermost regions of the basal planes.

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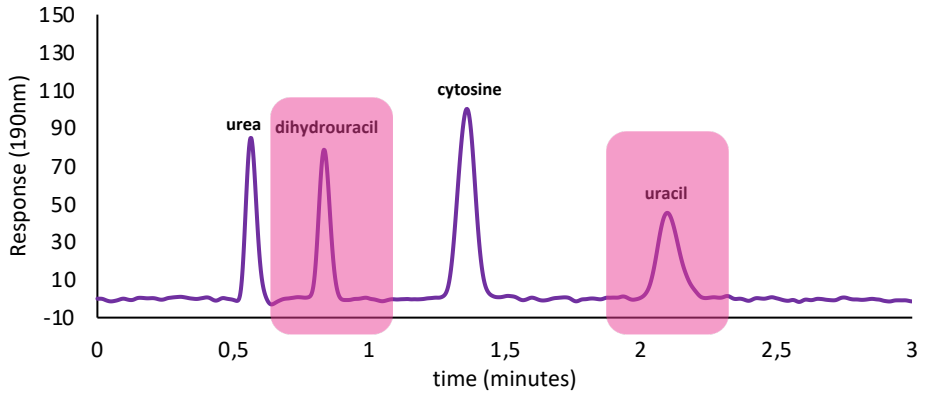
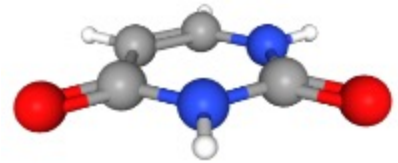
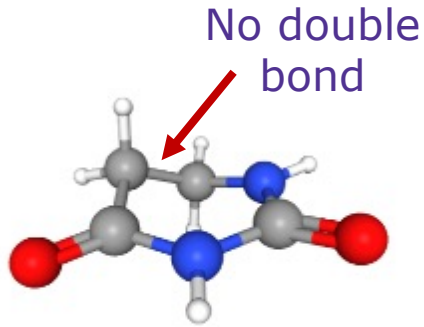
Porous graphitic carbon (PGC) particles
Interaction with Hydrophilic Molecules

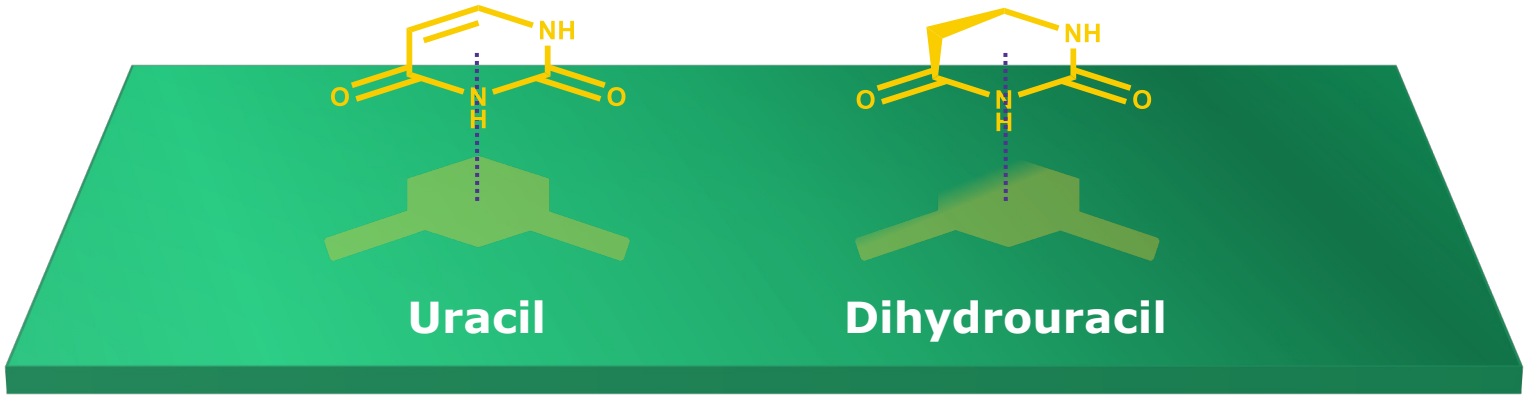




Planar



Non-planar

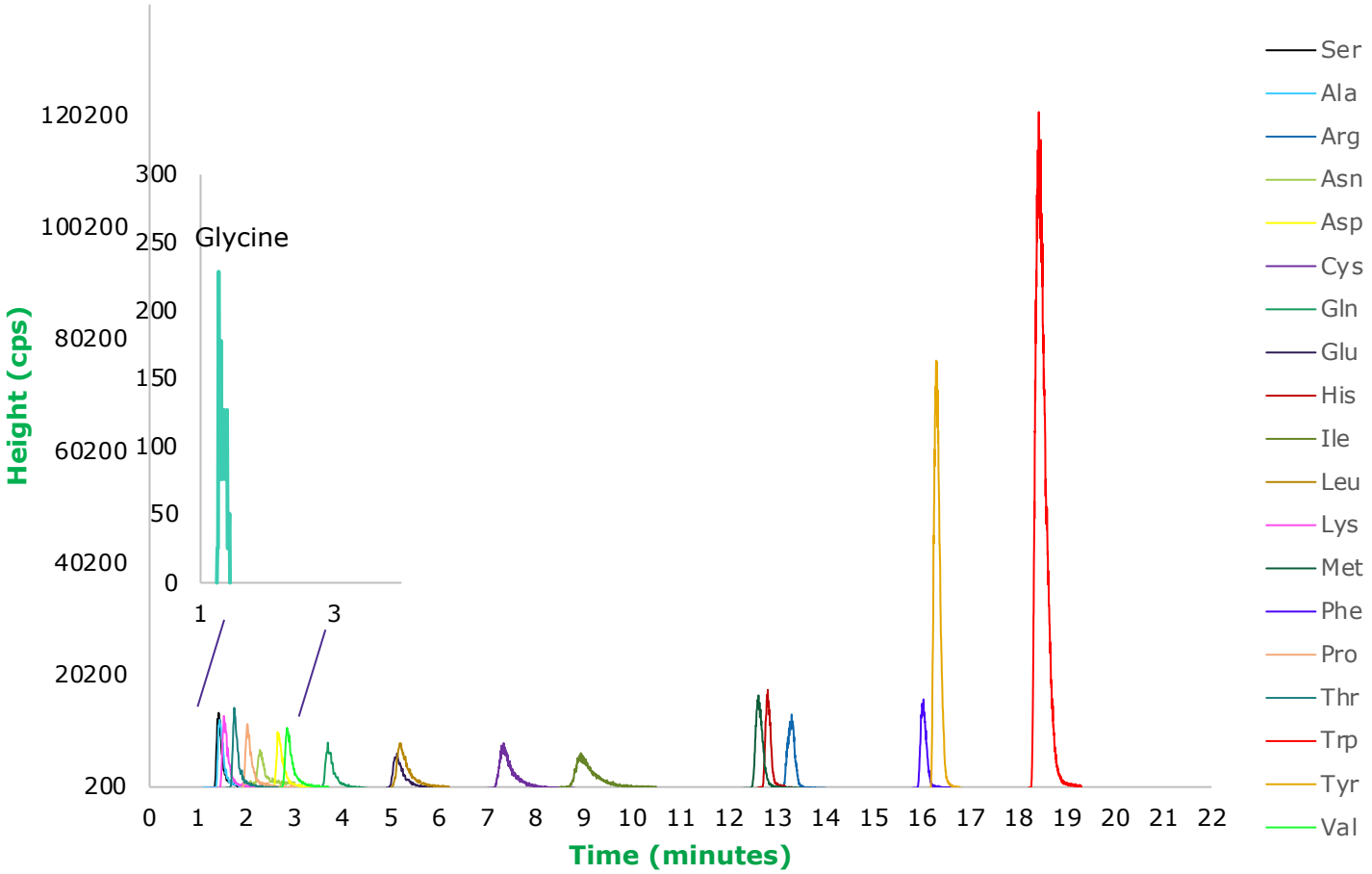


Planar/aromatic molecules interact more favorably with PGC: stronger retention.

Analyte shape very important in explaining retention !

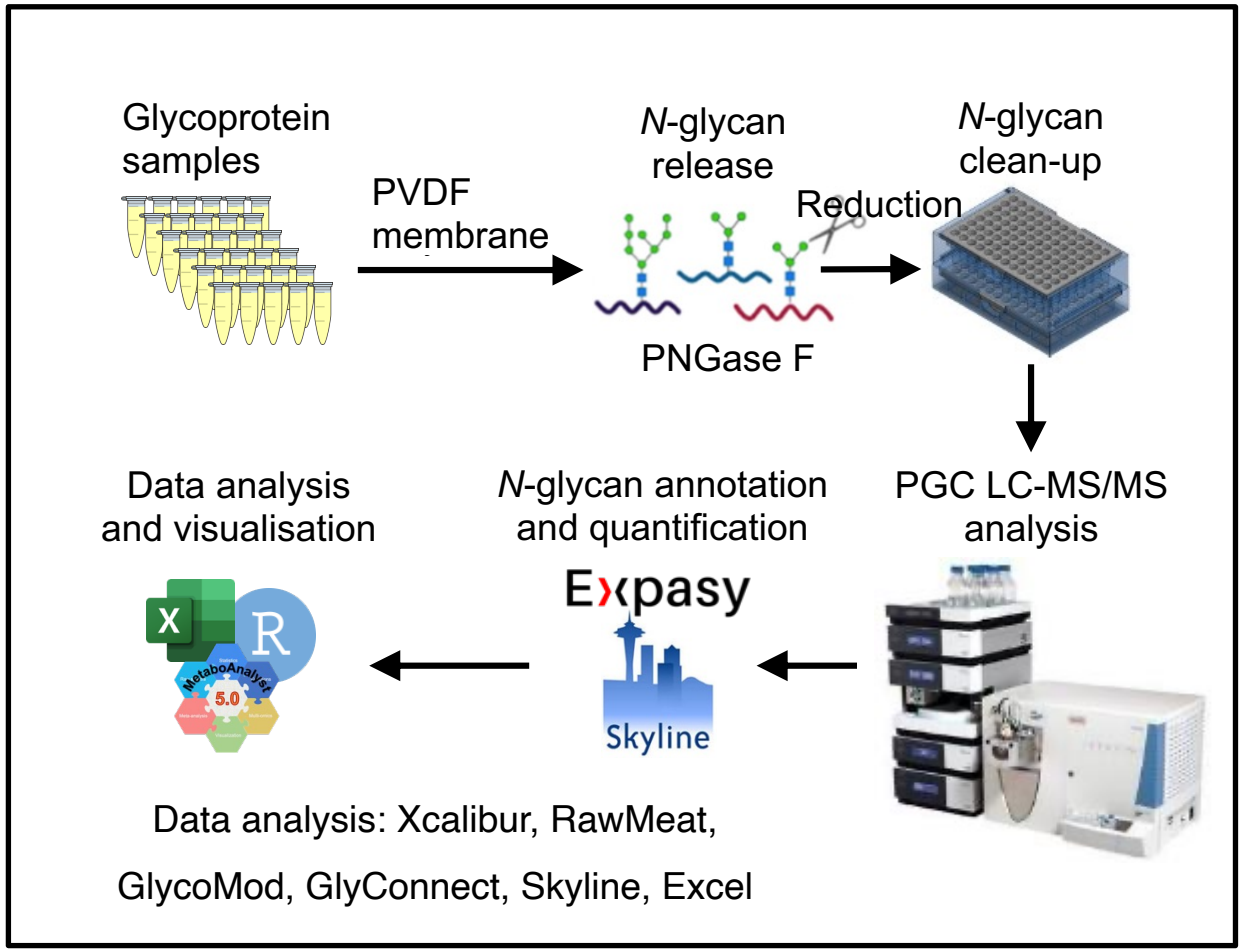
Porous graphitic carbon (PGC) particles

LC-MS/MS Analysis of 20 Underivatized Amino Acids



Column:	PGC 10 cm x 2.1 mm, 2.7 µm
Mobile phase:	[A] Water (0.1% (v/v) DFA); [B] Acetonitrile (0.1% (v/v) DFA)
Gradient:	Hold at 0% B for 7 min; 0% B to 5% B in 5 min; 5% B to 100% B in 10 min
Flow rate:	0.2 mL / min
Column temp.:	12 °C
Detector:	MSD
Injection:	1.0 µL
Sample:	Amino acids mix, varied concentrations water (0.1% v/v DFA)

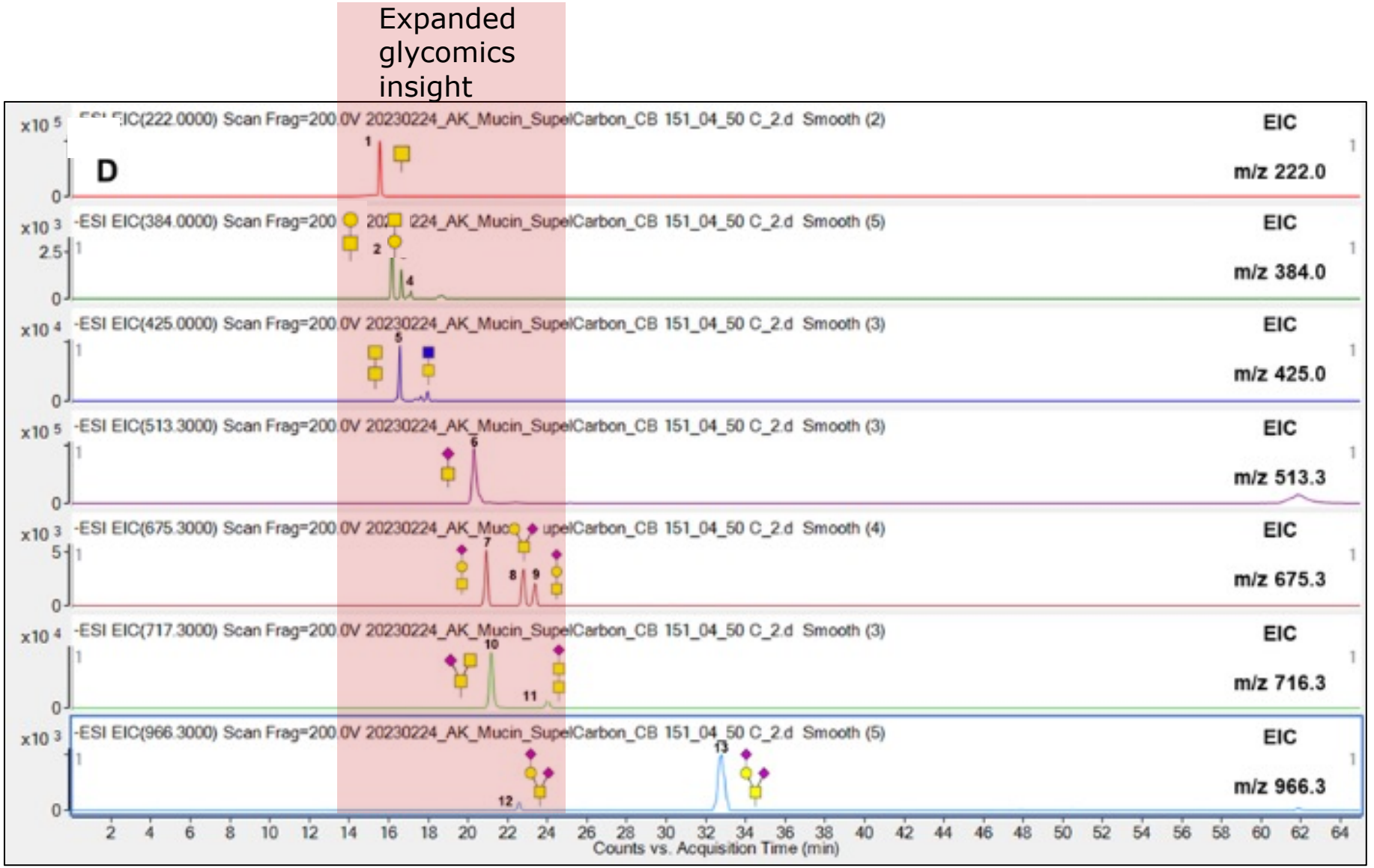
Porous graphitic carbon (PGC) particles
LC-MS/MS-based glycomics

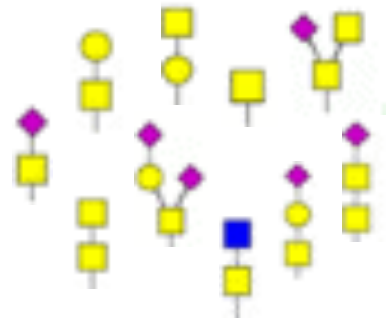


Jensen et al, *Nature Protocol*, 2012
Hinneburg et al., *Anal Chem*, 2019

Porous graphitic carbon (PGC) particles

Separation Mucin O-glycans





Column: PGC
150 x 1 mm, 2.7 µm

Mobile phase: [A] 10 mM ammonium bicarbonate, pH 8
[B] 10 mM ammonium bicarbonate in 70% acetonitrile

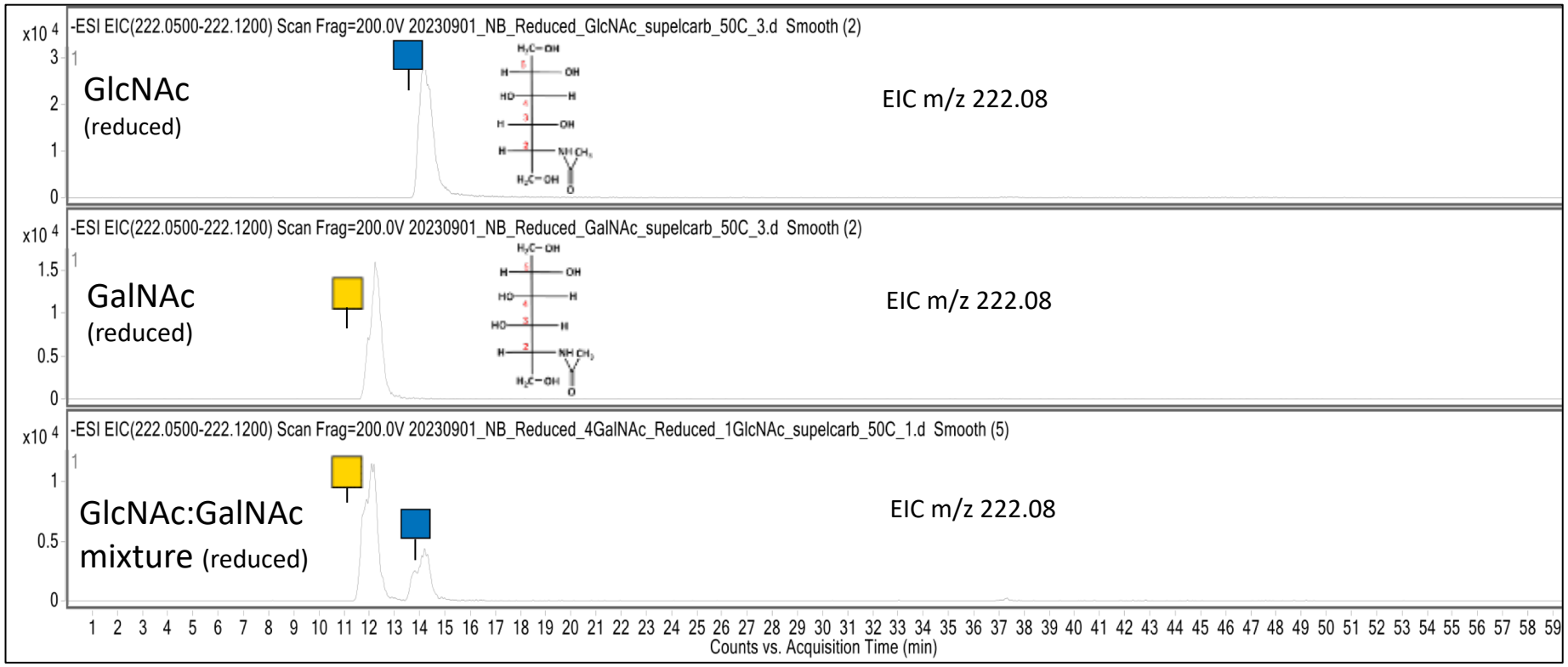
Column temp.: 50 °C

Sample: O-glycan mixture from bovine salivary mucin

Courtesy of Macquarie University Sydney, Australia (unpublished data)

Porous graphitic carbon (PGC) particles

Separation non-derivatized GlcNAc and GalNAc monosaccharides



Column: PGC
150 x 1 mm, 2.7 µm

Mobile phase: [A] 10 mM ammonium bicarbonate, pH 8
[B] 10 mM ammonium bicarbonate in 70% acetonitrile

Column temp.: 50 °C

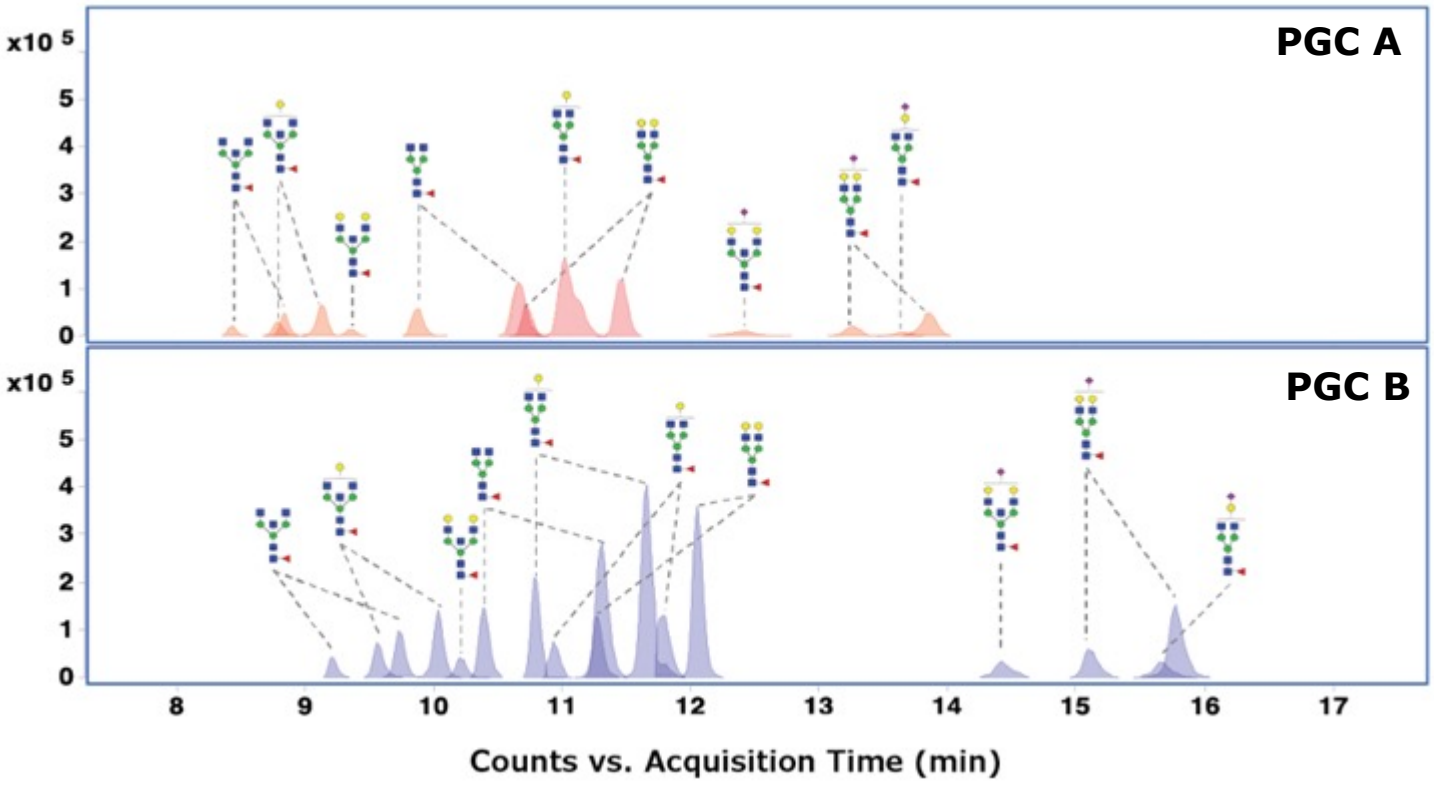
Sample: GlcNAc and GalNAc

Courtesy of Macquarie University
Sydney, Australia
(unpublished data)

PGC expands our view of the human glycome

Porous graphitic carbon (PGC) particles

UHPLC-MS Analysis of Released N-Glycans from Human IgG



Column:	PGC 10 cm x 2.1 mm, 2.7 µm + guard 2 cm x 2.1 mm , 2.7 µm
Mobile phase:	[A] 97:3 Water (0.1% (v/v) Formic acid): Acetonitrile (0.1% (v/v) Formic acid); [B] 10:90 Water (0.1% (v/v) Formic acid): Acetonitrile (0.1% (v/v) Formic acid)
Gradient:	Hold at 3% B for 2 min; 3% B to 16% B in 8 min; 16% B to 40% B in 8 min; 40% B to 60% B in 2 min; 60% B to 100% B in 2 min; hold at 100% B for 8 min
Flow rate:	0.3 mL / min
Column temp.:	40 °C
Detector:	MSD
Injection:	1.0 µL
Sample:	Released N-glycans from human IgG, 3 µg on column, water

Courtesy of Prof. Hyun Joo An, Chungnam National University, South Korea

Excellent resolution was achieved without derivatization, which allows accurate quantitation of glycans by MS.
PGC B showed more than doubled sensitivity and half as wide peaks.
The PGC is a valid alternative to HILIC-based glycan methods

To Summarize

- Porous graphitic carbon (PGC) is a Reversed Phase column with unique properties that allow it to retain some very polar (hydrophilic) molecules without the need for HILIC
- PGC demonstrates strong shape selectivity making it a good choice for separating isomers and structurally similar analytes
- PGC allows flexibility in pH, mobile phases and high temperatures
- Retention mechanism seems to be primarily explained by adsorption, electrostatic interactions and analytes' stereochemistries
- PGC is an excellent option for the HPLC / LCMS analyses of polar metabolites and glycans

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Thank you very much for your attention