Roadmap towards Multimodal Enrichment Strategy for Glycoconjugate Studies

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Introduction

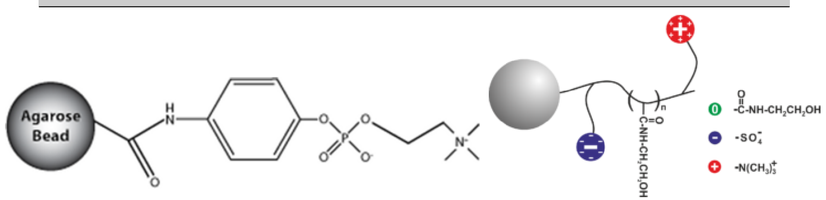
Glycopolymers can be made of glycans attached to proteins, also known as glycoproteins. Alteration of glycoproteome is substantially associated with cancers, autoimmune disorders, and cognitive impairments. Bottom-up glycoproteomics is the workhorse approach for unveiling site-specific glycan profile alterations.[1] To tackle the huge dynamic range and heterogeneity of complex sample proteome which is often masking glycosylation information, enrichment is a convenient replacement for exhaustive protein fractionation in the context of fast screening of at-risk population.[2] Targeting the HILIC retention difference between ion-paired tryptic digested glycopeptides and peptides,[3] ZIC-HILIC remains one of the most popular enrichment approaches.[1-2, 4] However, the potential bias of commercially available ZIC-HILIC materials has been criticized.[5]

Research Question

What are the enrichment biases of phosphorylcholine and iSPE-HILIC materials regarding glycan types and glycoprotein types?

Methods

Materials	• 250 µg 10 kDa MWCO filtered HEK293 cell peptides • 250 µg depleted normal human plasma digest	
	Pierce/G-Biosciences immobilized *p*-aminophenyl phosphorylcholine agarose beads (PCE) slurry 200 µL	HILICON iSPE-HILIC cartridges (iSPE) 100 mg/1 cc
Enrichment	"Drop-HILIC" in microfuge tube • Loading: 100 µL 80% ACN-1% TFA • Binding: end-over-end 20 min • Washing: 250 µL loading buffer × 2 • Elution: 100 µL 1% TFA × 3	Cartridge format • Loading: 1 mL 80% ACN-1% TFA • Binding: passing & reloading × 5 • Washing: 1 mL loading buffer × 5 • Elution: 2.5 mL 0.1% TFA
LC-MS/MS	• Enrichment eluates were reconstituted in 40 µL 0.1% FA, injected 15 µL • Unenriched samples were injected 0.5 µg • Charge-prior stepped HCDpdETD 3 h gradient on Orbitrap Eclipse	
Data Analysis	• Raw data was searched with Byonic v5.0 against 214 built-in human N-glycans and 20377 Swiss-Prot reviewed human proteins with decoys • Results filtered with Score > 150, PEP 2D < 0.01, |ppm Err.| < 3	



PCE Functionalities **iSPE Functionalities**

Results

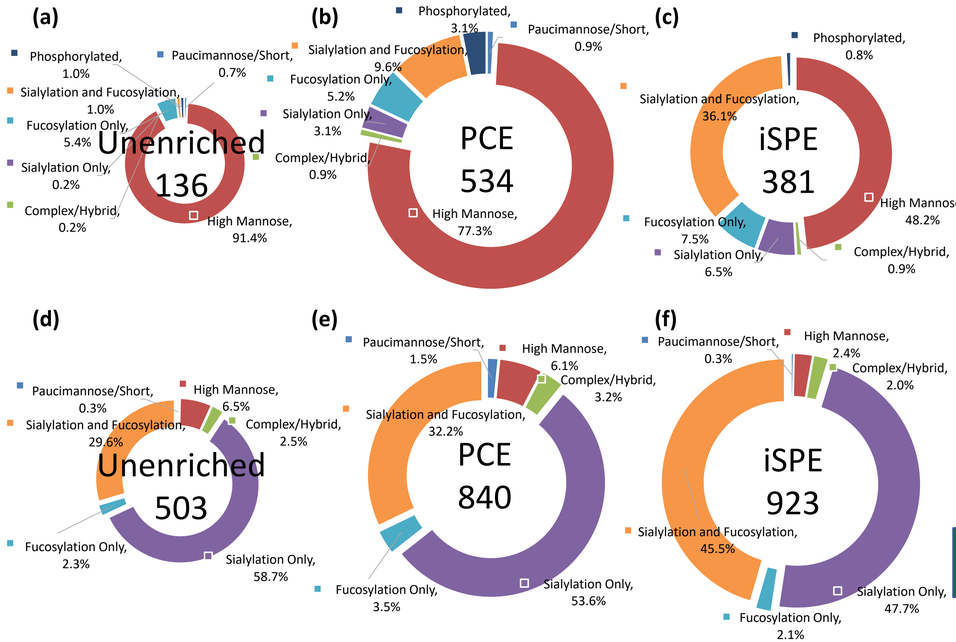


Figure 1 Averaged unique glycopeptide numbers and normalized glycan type ratios detected in **(a-c)** HEK293 cell samples and **(d-f)** depleted normal human plasma samples. Unique glycopeptide numbers are averaged among triplicate LC-MS/MS runs; glycan type ratios are normalized by the sum of detection frequency of triplicates.

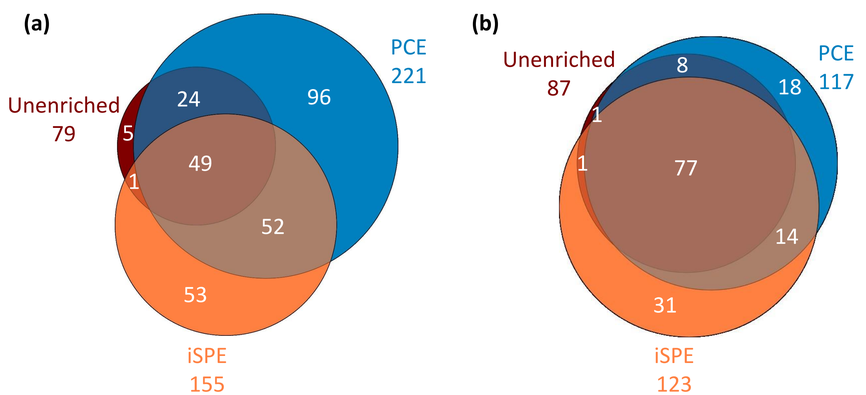


Figure 2 Venn diagrams of total unique glycoproteins detected in **(a)** HEK293 cell samples and **(b)** depleted normal human plasma samples. Glycoprotein coverages are the sums of triplicate LC-MS/MS runs.

Conclusion

- We compared two ZIC-HILIC-based glycopeptide enrichment protocols. PCE (in-house optimized) method has a preference of enriching high mannose type glycans, while iSPE (well-established) method favors sialylated and fucosylated glycans.
- Unique challenges of sample complexity will affect enrichment results. HEK293 cells possess a highly heterogenous glycoproteome which is mostly related to protein processing, while in depleted plasma most proteins are decorated with sialylation and fucosylation.
- PCE outperforms iSPE method in identifying a higher number of glycopeptides and glycoproteins for HEK293 cell samples and has comparable results for plasma samples.
- Further, the unique enrichment properties of both resins can be combined using mixed resin enrichment strategy, as well as complementary and sequential enrichment in different orders.
- Stacking off-line ZIC-HILIC enrichment with on-line FAIMS enrichment might further improve the depth of glycoproteome coverage of current shotgun style rapid analysis.

Acknowledgement

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References

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