JOHNS HOPKINS
MEDICINE

Discovery and validation of urinary glycoproteins associated with aggressive prostate cancer

Hui Zhang

Professor of Pathology

Johns Hopkins University

The needs for aggressive prostate cancer biomarkers

The intended uses and clinical utilities of glycoprotein biomarkers for the early detection of aggressive prostate cancer

- Replace invasive biopsy procedure by non-invasive urinary analysis
- Select patients with non-aggressive initial biopsy for secondary biopsy
- Monitor prostate cancer progression in active surveillance patients

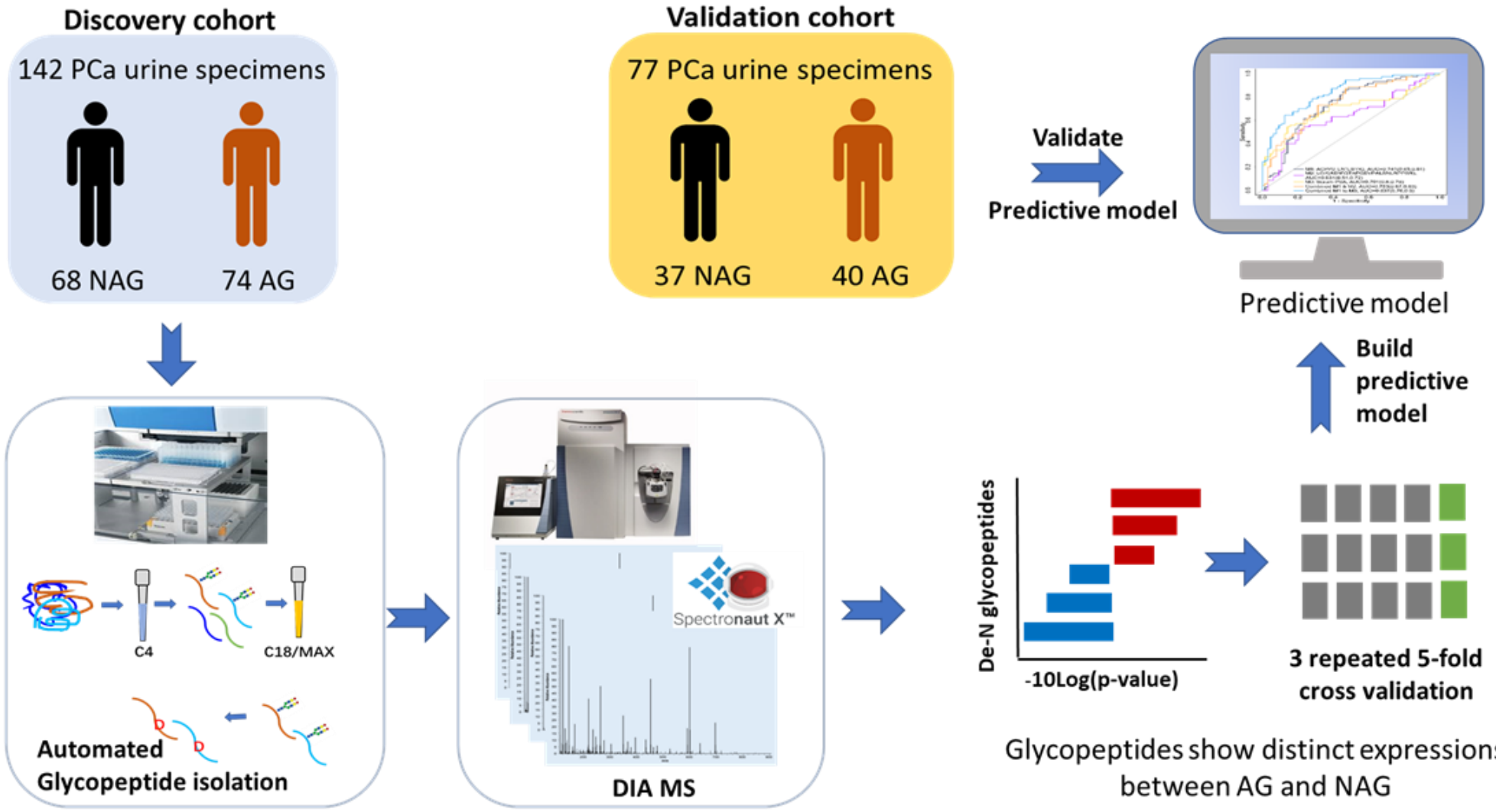
Why Urinary Glycoprotein Biomarkers?

Most FDA-approved biomarkers for cancer diagnosis and prognosis are glycoproteins.

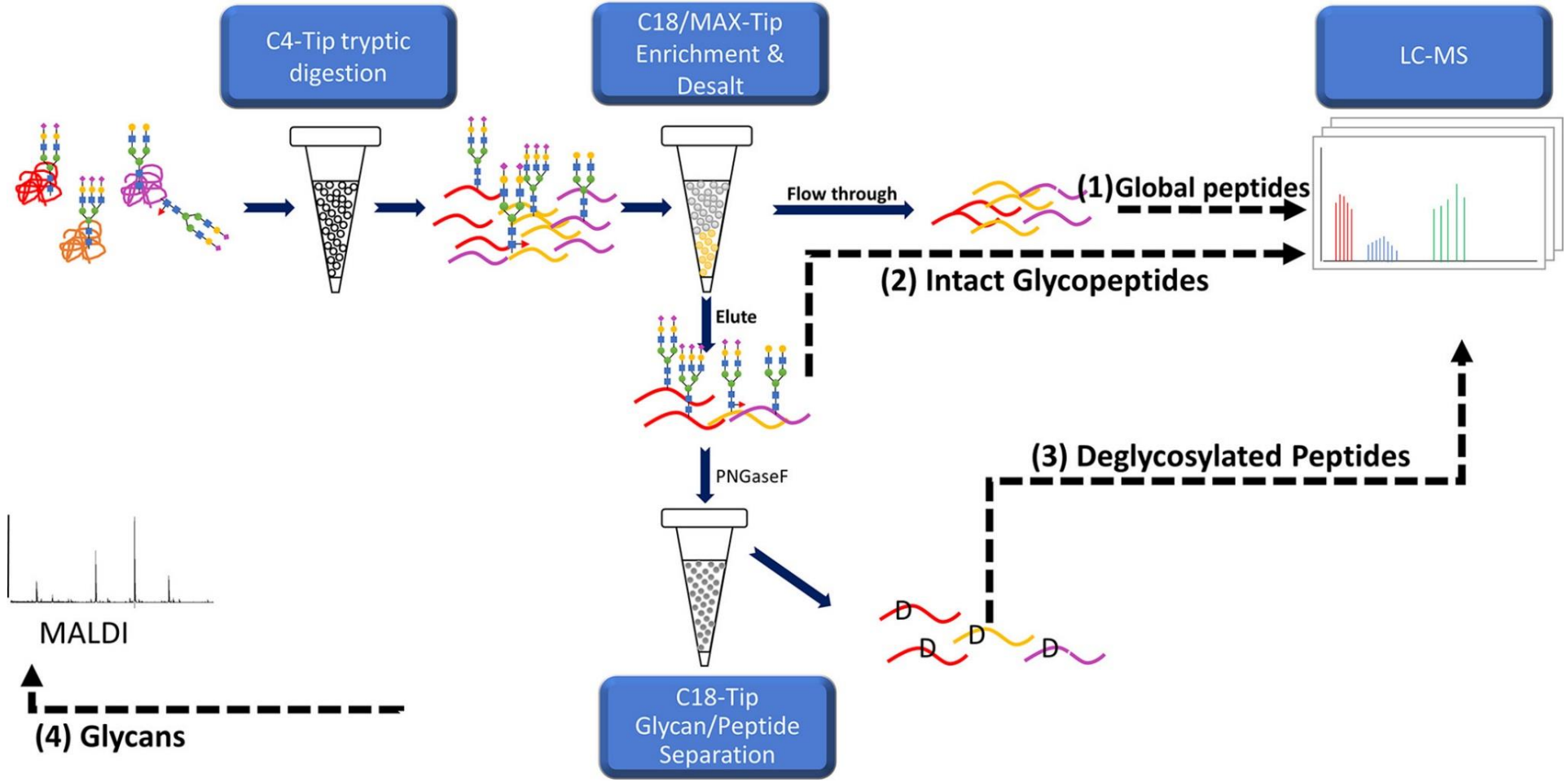
Glycoproteins can be easily secreted into urine, and feasible to be detected as non-invasive biomarkers.

Tumor cells display markedly changed glycosylation states.

Workflow of an integrated urine glycoproteomic analysis



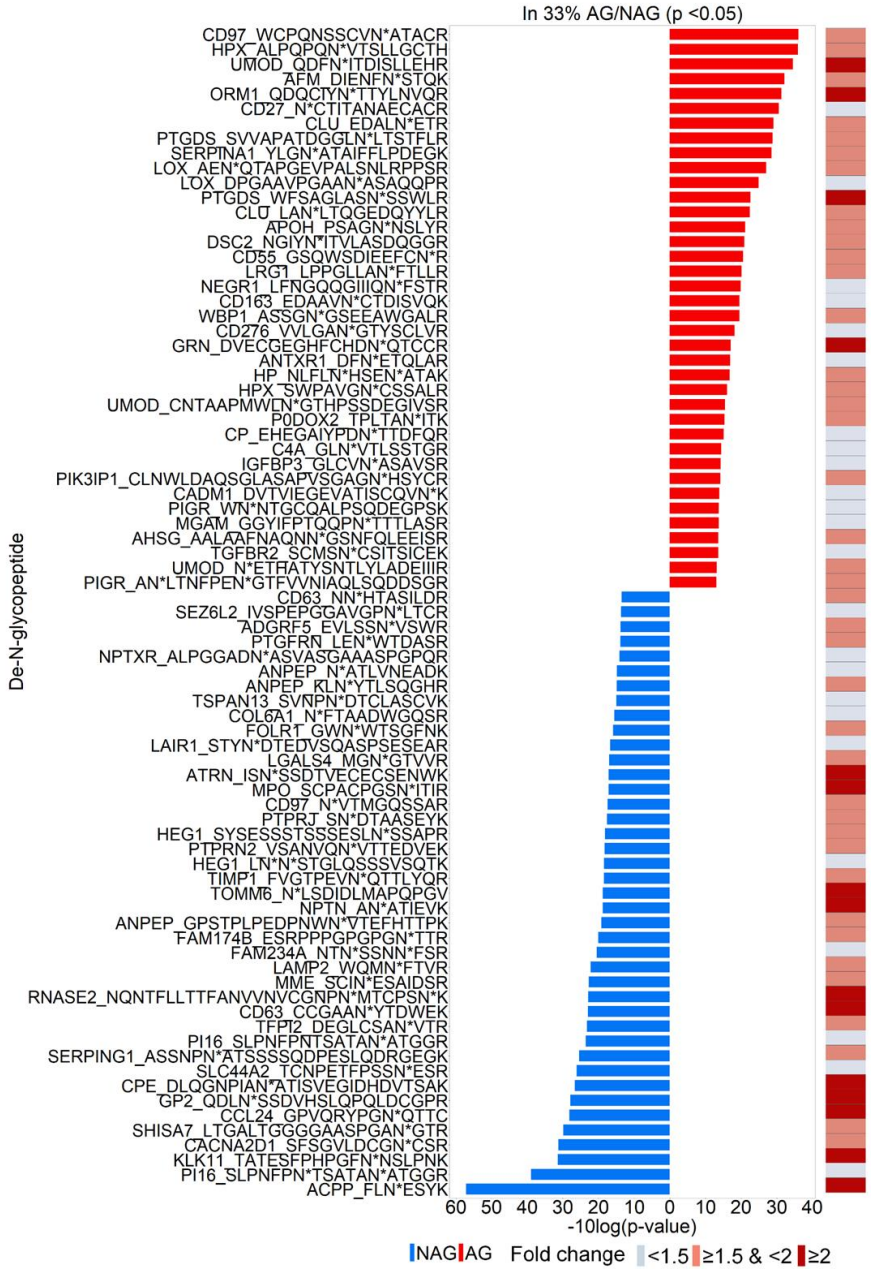
Automated glycans, glycosite, and intact glycopeptide analysis



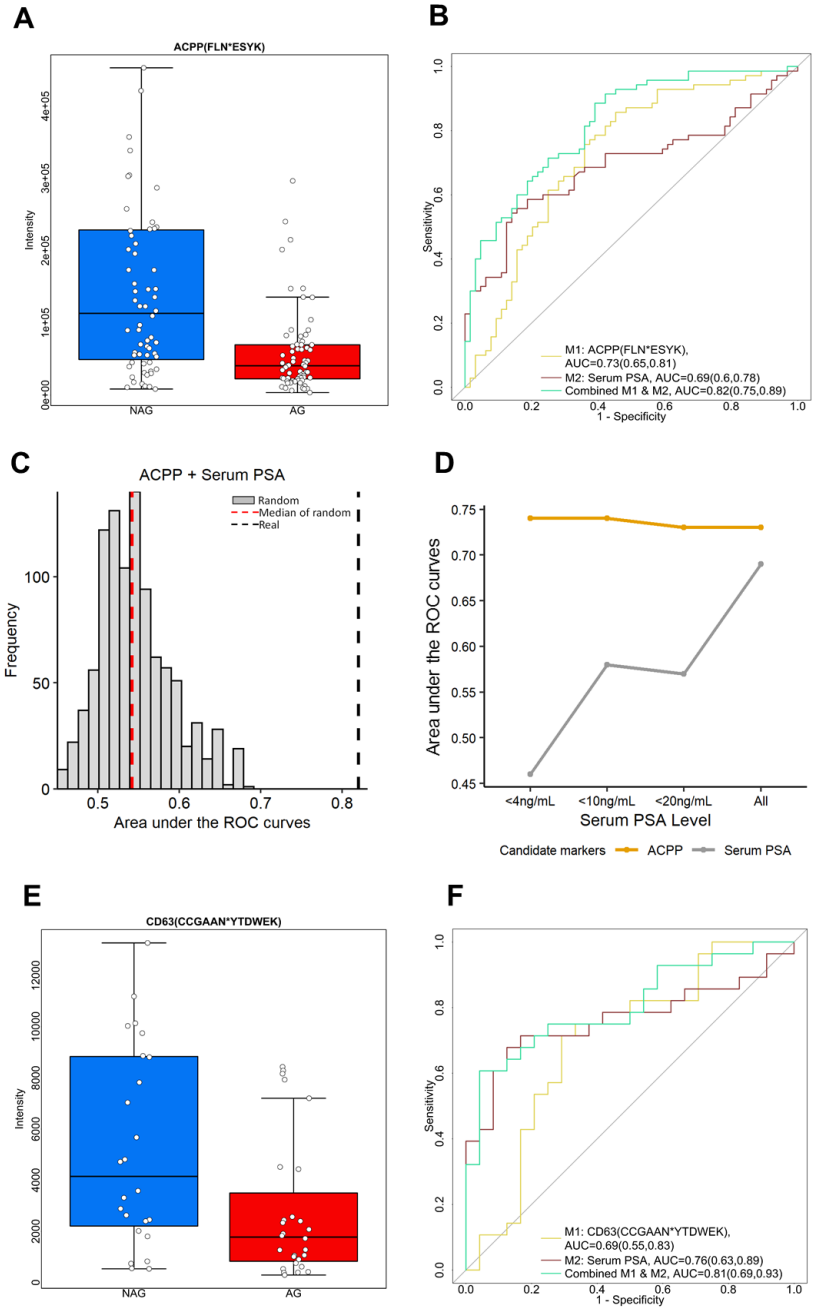
Simple tip-based sample processing method for urinary proteomic analysis. David J Clark, Yingwei Hu, Michael Schnaubelt, Yi Fu, Sean Ponce, Shao-Yung Chen, Y Zhou, P Shah, Hui Zhang; *Anal. Chem.* 2019; 91 (9), 5517-5522.

Glycans, Glycosite, and Intact Glycopeptide Analysis of N-Linked Glycoproteins Using Liquid Handling Systems. Shao-Yung Chen; Mingming Dong; Ganglong Yang; Yangying Zhou; David J. Clark; T. Mamie Lih; Michael Schnaubelt; Zichen Liu; Hui Zhang; *Anal. Chem.* 2019; 92, 1680-1686.

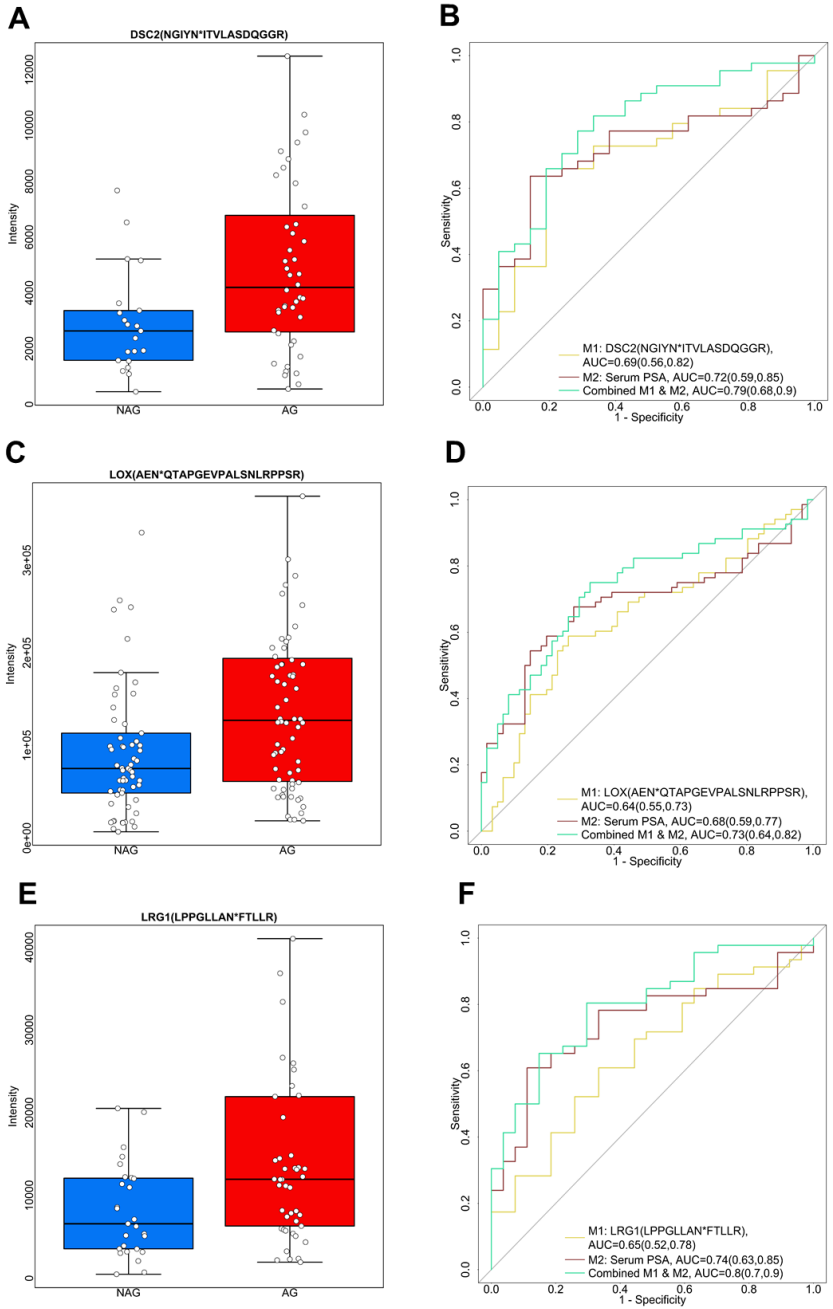
Glycopeptides associated with AG



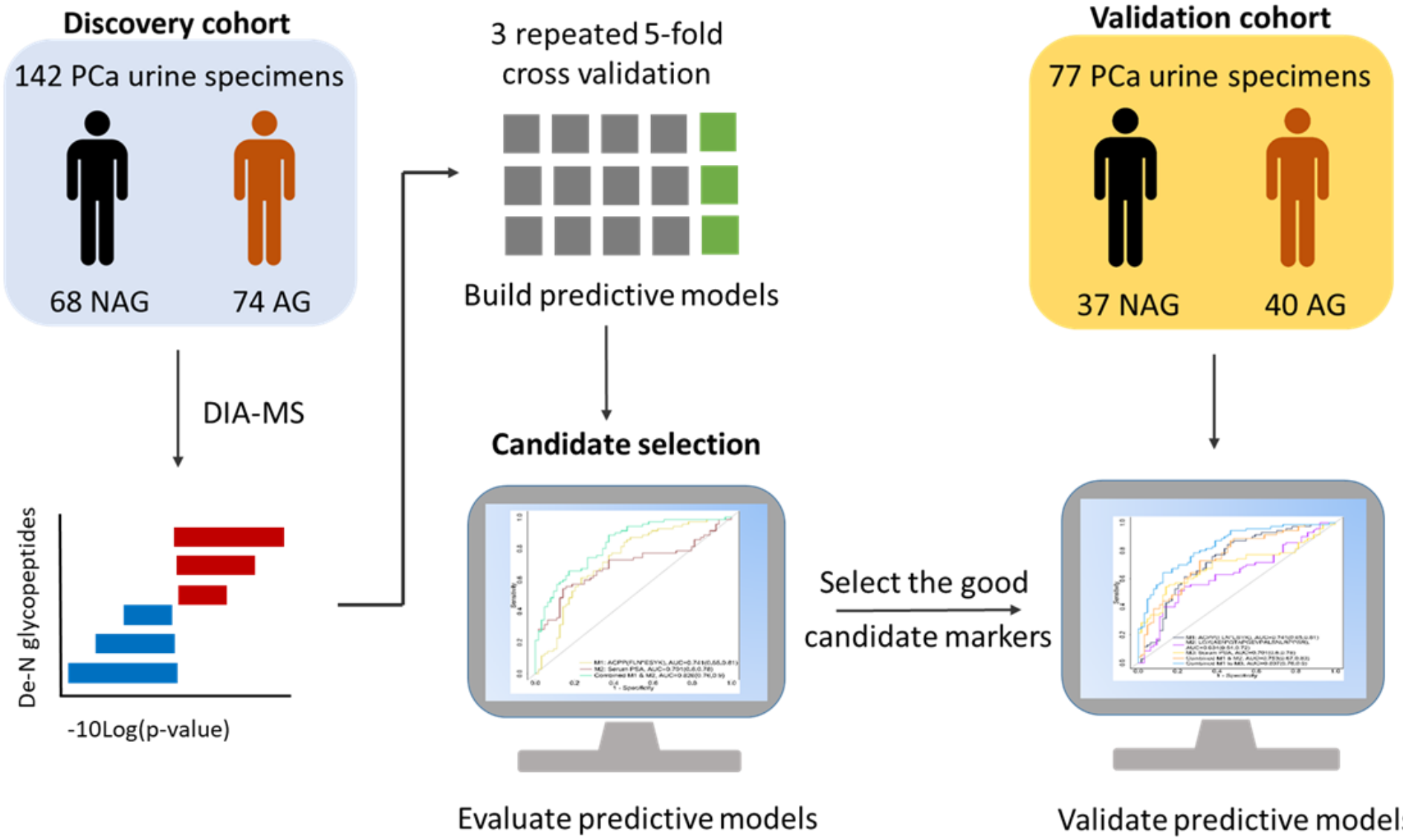
Down-regulated glycopeptides in AG PCa



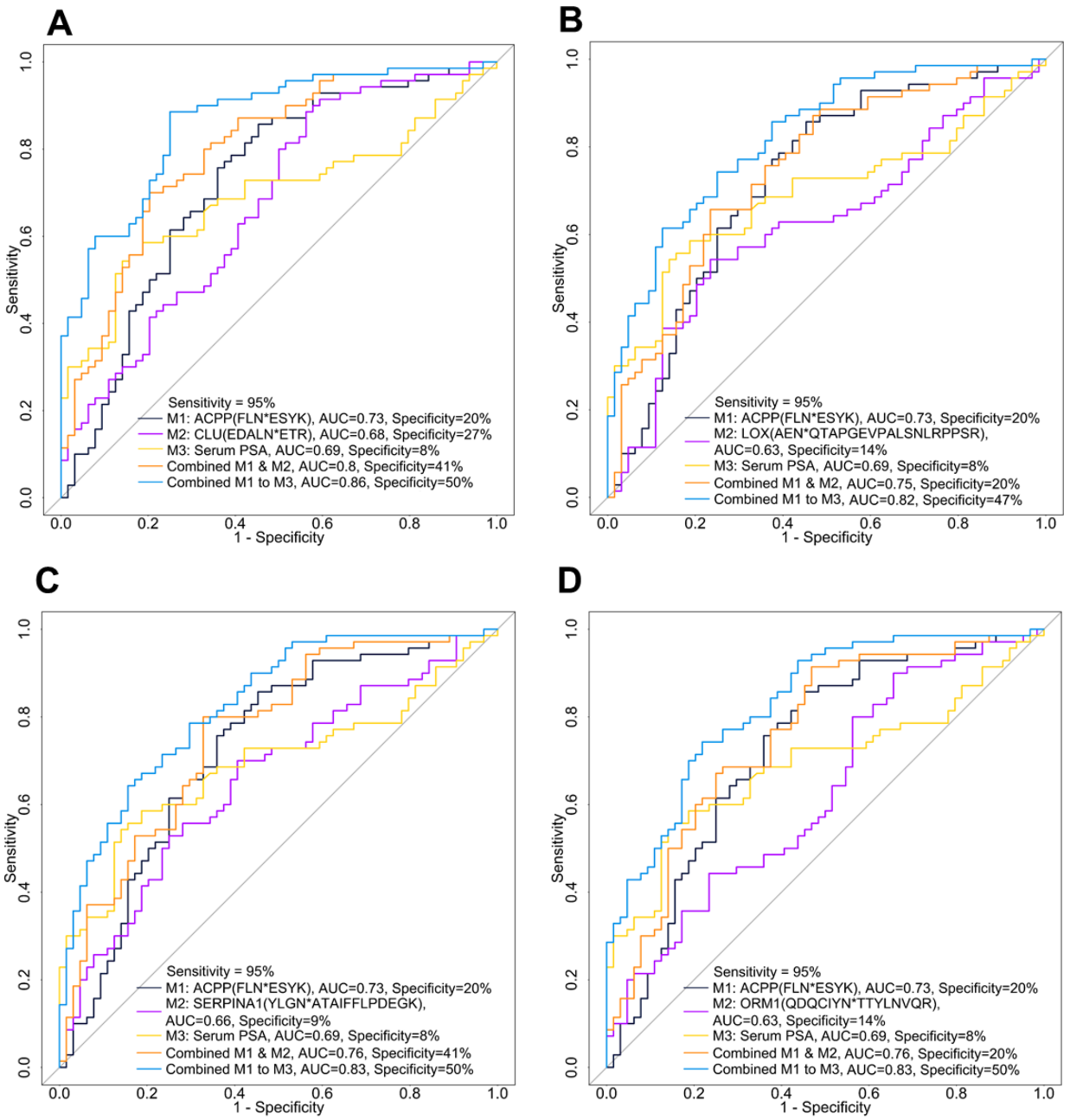
Up-regulated glycopeptides in AG PCa



Schematic overview of candidate glycopeptide discovery and validation



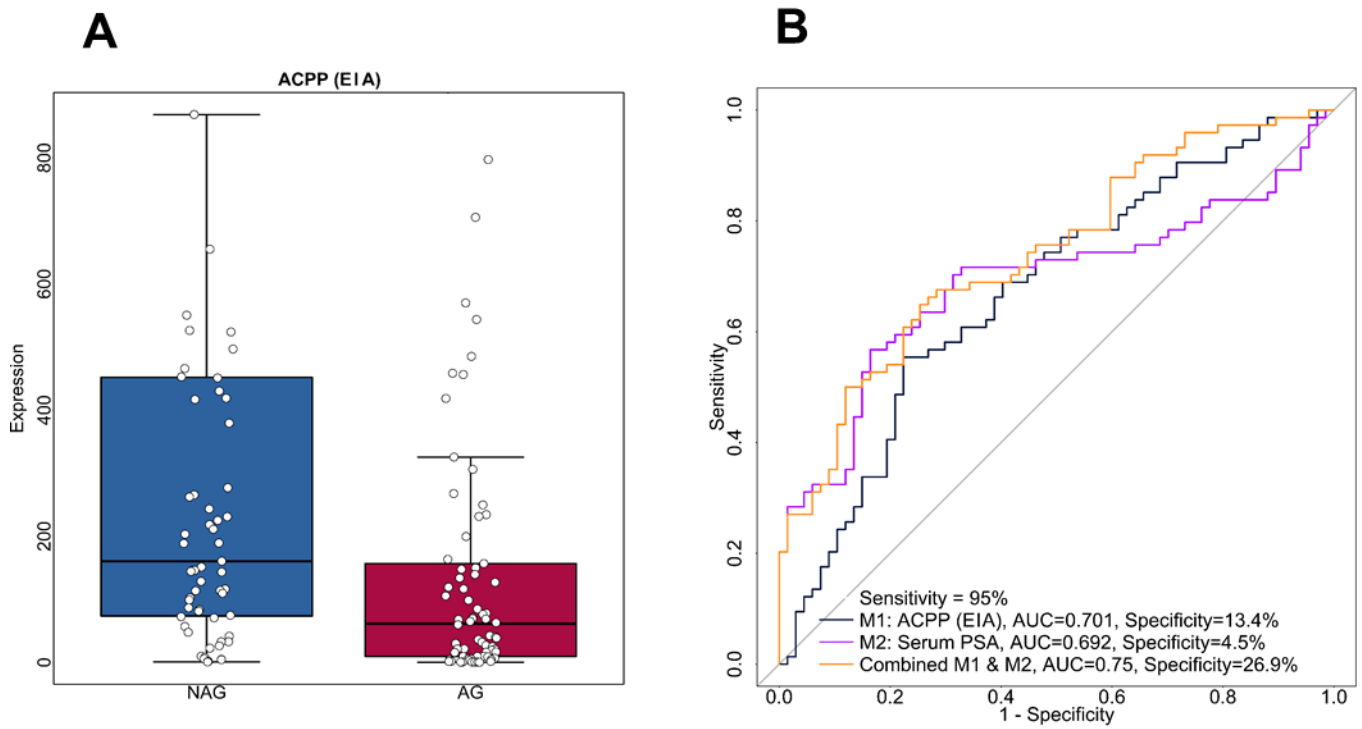
ROC analysis of combined panels



Performance of different panel of candidate biomarkers in discovery cohort (74 AG and 68 NAG), validation cohort set 1 (40 AG and 37 NAG) and validation cohort set 2 (40 AG and 13 NAG)

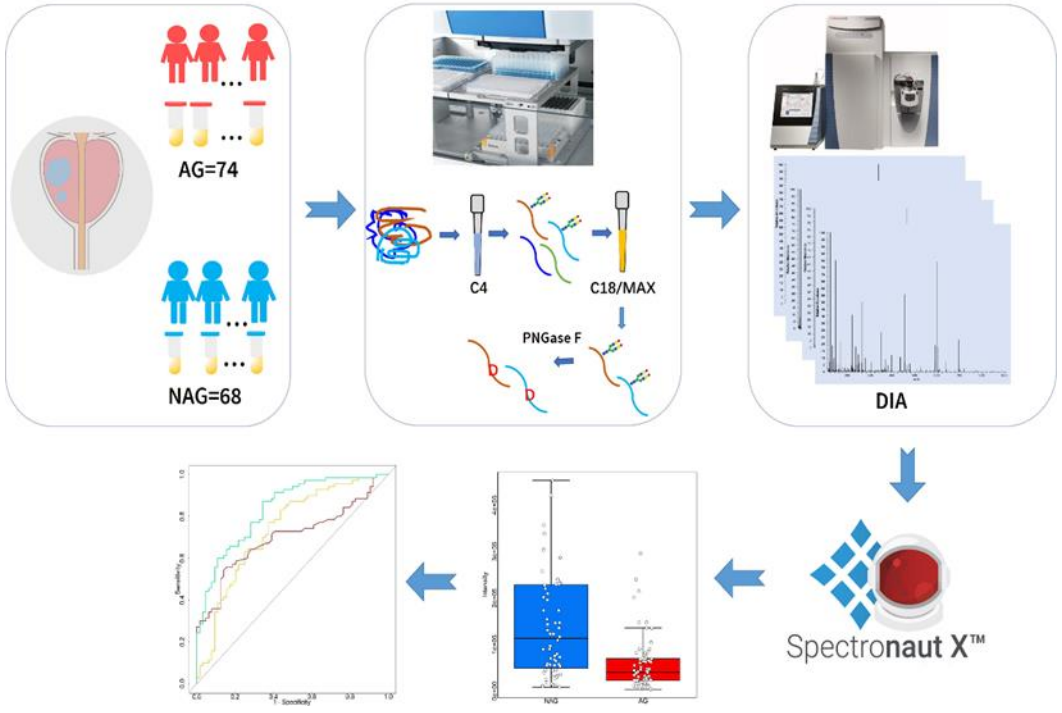
Panels of candidate biomarkers	Area under the ROC curves (95% confidence interval)		
	discovery cohort	validation set 1	validation set 2
ACPP & Serum PSA	0.82 (0.75,0.89)	0.83 (0.74,0.92)	0.8 (0.67,0.93)
ACPP & CLU & Serum PSA	0.86 (0.8,0.92)	0.85 (0.76,0.94)	0.76 (0.6,0.92)
ACPP & LOX & Serum PSA	0.82 (0.75,0.89)	0.85 (0.76,0.93)	0.81(0.69,0.93)
ACPP & SERPINA1 & Serum PSA	0.83 (0.76,0.9)	0.84 (0.75,0.93)	0.82 (0.7,0.94)
ACPP & ORM1 & Serum PSA	0.83 (0.76,0.9)	0.82 (0.72,0.91)	0.82 (0.71,0.94)

Immunoassay and quantitative analysis of urinary ACPP



Accomplishments during this cycle of EDRN

1. Established high throughput glycoproteomic platform for urinary glycoprotein biomarker development



2. Collaborated with EDRN teams to analyze urine cohorts
3. Identified candidate glycoprotein biomarkers for aggressive prostate cancer

The state of glycoprotein markers

Candidate glycoprotein biomarkers							
Candidate Biomarker	Discovery	Pre-validation	Validation	1. My team project	2. Collaborative/Team Projects and Set-aside Projects	3. CLIA/LDT Assay	4. New seminal Discoveries/developments related to biomarkers
Fucosylated serum PSA	---------->			Yes	Yes		Improved performance when combined with phi
Urinary ACPP	---------->			Yes			Improved performance when combined with urinary LOX
Urinary CD63	---------->			Yes			Improved performance when combined with serum PSA
Urinary LOX	---------->			Yes			Improved performance when combined with urinary ACPP
Urinary CLU	---------->			Yes			Improved performance when combined with urinary ACPP and serum PSA
Urinary PSA	---------->			Yes	Yes		Improved performance when combined with urinary ACPP and serum PSA
Urinary ORM1	---------->			Yes			
Urinary DSC2	---------->			Yes			
Urinary PTGD	---------->			Yes			
Urinary SERPINA1	---------->			Yes			
Urinary LRG1	---------->			Yes			
Urinary KLK11	---------->			Yes			
Urinary SCGB1A1	---------->			Yes			
Urinary AZGP1	---------->			Yes			
Urinary CSTA	---------->			Yes			

Summary and the Next Steps

- Use MS- or antibody-based assays to quantify glycopeptides or glycoproteins: fuc-PSA, ACPP, CLU
- Validate the candidate biomarkers using post-DRE urine from PCA3 evaluation trial
- Determine the performance of biomarkers using pre-DRE urine from CPDR

The progress and the current state of trans-network collaborative projects

434 Post-DRD urine collected using EDRN protocol in JHU

AG=289

NAG=145

CVC
JHU

859 Post-DRE urine from the NCI-EDRN's Urinary PCA3 Evaluation Trial (from 11 sites). Urine samples from 562 initial biopsies

AG=138

NAG=126

Non-cancer=298

297 repeated biopsies

AG=26

NAG=41

Non-cancer=230

DMCC
CVCs

178 Pre-DRE urine from Center for Prostate Disease Research (CPDR)

AG=78

NAG=50

Non-cancer=50

BRL
CPDR

EDRN Structure

The unique EDRN Network structure enables the large scale study design and investigation for required biomarker development and validation

Specimen collection, data generation, and data analysis sites are independent and samples are blinded during data generation for reduced bias.

Integrated molecular (genomics, methylation, mRNA, miRNA, exosome, proteins, glycoproteins), pathological, and imaging analyses for improved clinical performance for cancer detection

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Clinical evaluation: Daniel W Chan, Lori Sokoll, Zhen Zhang, Alan Partin

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BRLs: CPDR (Gyorgy Petrovics), JHU (Daniel Chan)
CVCs: (Emory, UM, JHU, UT, Cornel, etc)
DMCC: (Yingye Zheng)

NCI/EDRN: Glycoprotein Biomarkers for the Early Detection of Prostate Cancer, U01CA152813