

Supporting Information

Comprehensive prostate fluid-based spectral libraries for enhanced protein detection in urine.

Annie Ha^{1,2,‡}, Amanda Khoo^{1,2,‡}, Vladimir Ignatchenko², Shahbaz Khan², Matthew Waas², Danny Vesprini^{3,4}, Stanley K Liu^{1,3,4}, Julius O Nyalwidhe^{5,6}, Oliver John Semmes^{5,6}, Paul C Boutros^{7,8,9,10,11,12,13}, Thomas Kislinger^{1,2}.*

‡These authors contributed equally.

¹Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada, M5G 1L7.

²Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, M5G 2C1, Canada.

³Department of Radiation Oncology, University of Toronto, Toronto, Ontario, M5T 1P5, Canada.

⁴Odette Cancer Research Program, Sunnybrook Research Institute, Toronto, Ontario, M4N 3M5, Canada.

⁵Leroy T. Canoles Jr. Cancer Research Center, Eastern Virginia Medical School, Norfolk, Virginia 23501, USA.

⁶ Department of Microbiology and Molecular Cell Biology, Eastern Virginia Medical School, Norfolk, Virginia 23501, USA.

⁷ Department of Human Genetics, University of California, Los Angeles, Los Angeles, California 90095, USA.

⁸ Department of Urology, University of California, Los Angeles, Los Angeles, California 90095, USA.

⁹ Institute for Precision Health, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California 90095, USA.

¹⁰ Eli and Edythe Broad Stem Cell Research Center, University of California, Los Angeles, CA 90095, USA.

¹¹ Broad Stem Cell Research Center, University of California, Los Angeles, California, 90095, USA.

¹² Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California 90024, USA.

¹³ Department of Human Genetics, University of California, Los Angeles, Los Angeles, California 90095, USA.

Corresponding Author

*Thomas Kislinger

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Figure S1

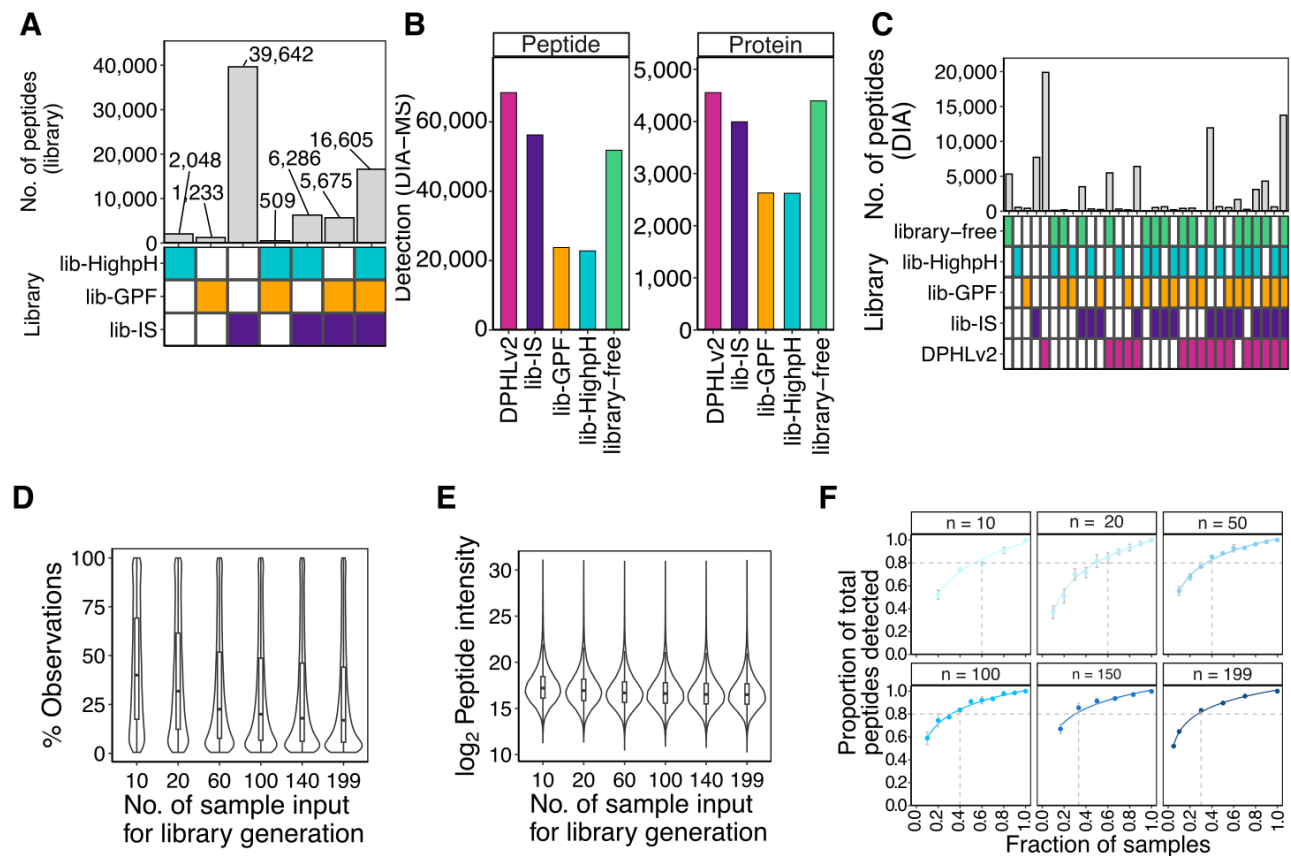


Figure S1. Comparisons between DIA-MS data analysis approaches. **(A)** Intersection of peptides in the sample-specific generated spectral libraries. **(B)** Detection rates **(C)** Intersection of peptides

of the EPS-urine DIA-MS data searched against each sample-specific spectral libraries, DPHLv2, and library-free approach. **(D)** Percent observations of peptides detected in the EPS-urine DIA-MS data when searched against each subsampled library. **(E)** Boxplot of the \log_2 peptide intensities of peptides detected. **(F)** Proportion of total peptides detected in DIA-MS data of each subset cohort using subsampled spectral libraries from DDA-MS of the corresponding subset cohort.

Figure S2

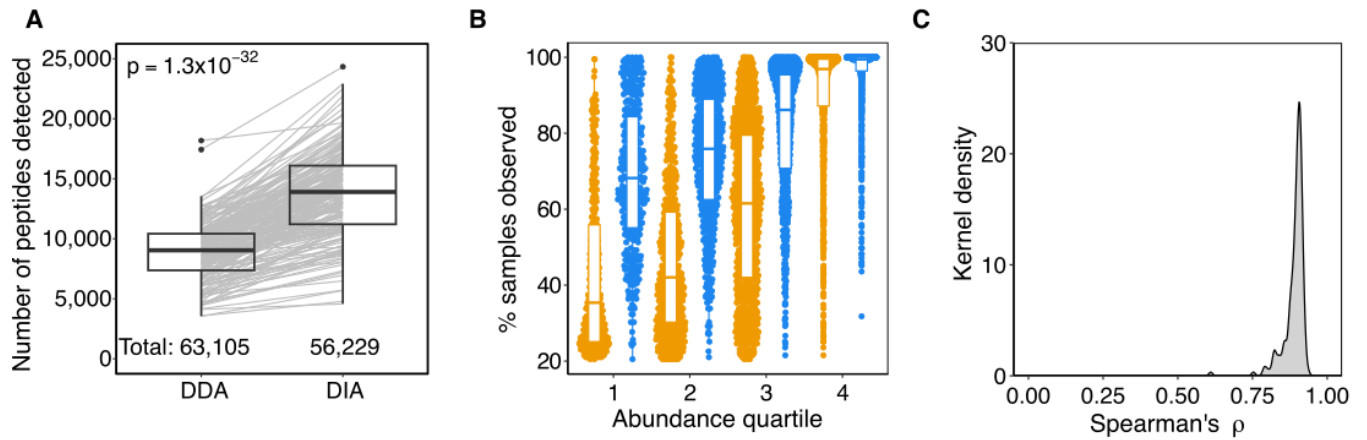
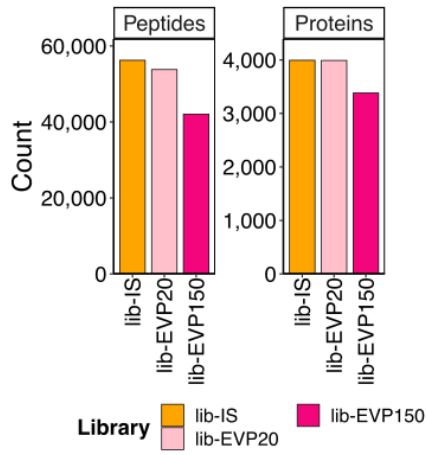


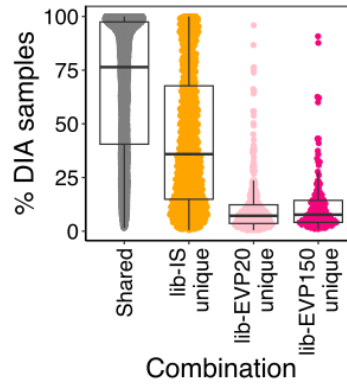
Figure S2. Comparisons between DIA- and DDA-MS on matched EPS-urine samples. **(A)** Number of peptides detected per sample with P-value calculated by Wilcoxon's signed-rank test. **(B)** Percent samples observed for detected proteins in both DIA- and DDA-MS, stratified by abundance quartile quantified by DDA-MS. **(C)** Spearman's correlation of run-to-run protein intensity correlation.

Figure S3

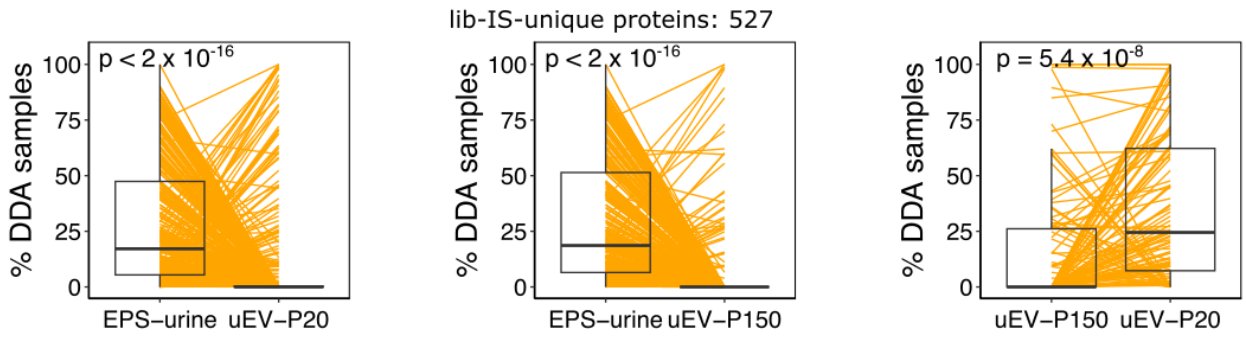
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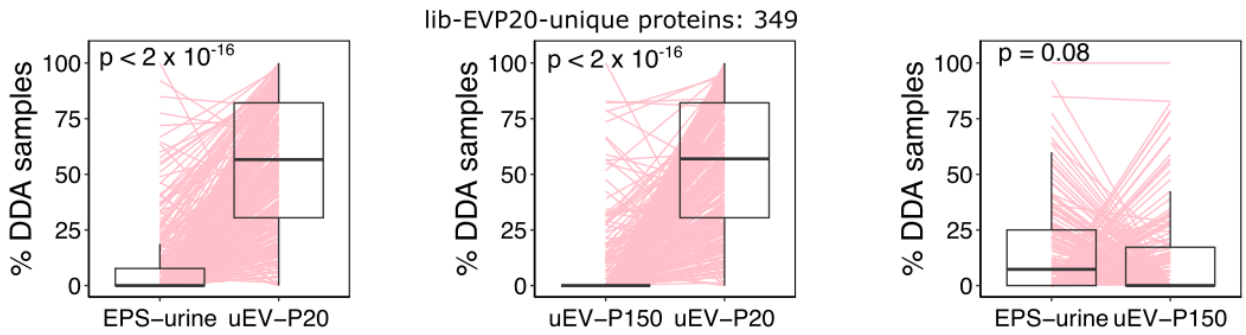
B



C



D



E

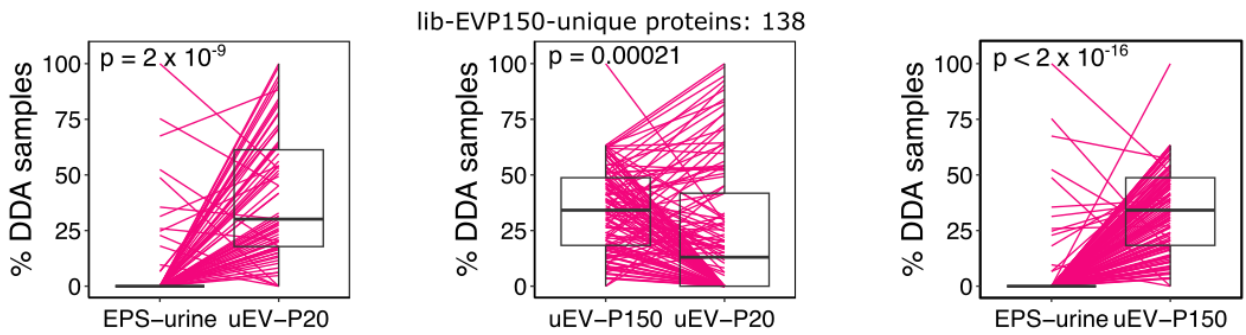


Figure S3. Evaluation of the sample-relevant spectral libraries. **(A)** Library size of the generated spectral libraries. **(B)** Percent observation of proteins detected by all libraries, or uniquely by each of the spectral libraries. **(C-E)** Percent observations of uniquely detected proteins by each spectral library in the corresponding DDA-MS samples of EPS-urine fractions.

Figure S4

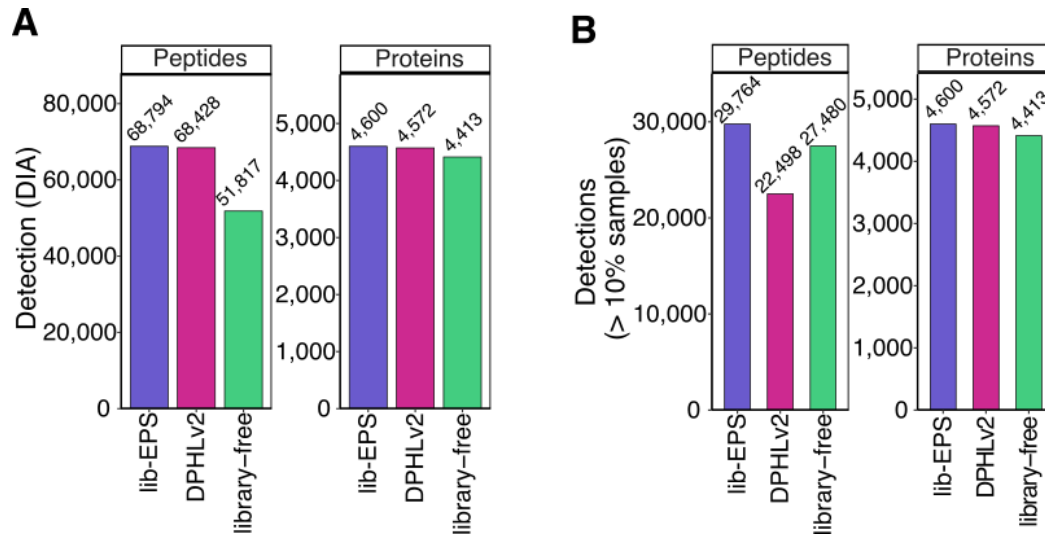


Figure S4. Detection results of the lib-EPS library against DPHLv2 and library free. **(A)** Total number of peptide and proteins detected with each approach. **(B)** Number of peptides and proteins detected in more than 10% of samples with each approach.