Deep, Unbiased and Quantitative mass spectrometry-based plasma proteome analyses of adaptive response to COVID-19 vaccine

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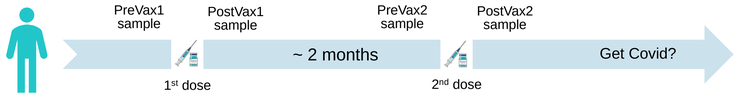
Proteograph™ workflow coupled with TMTpro 18Plex provides deep access to plasma proteome

Proteograph workflow provide valuable insights on personalized human responses to Covid-19 vaccination

Introduction

COVID-19 vaccines have been extensively used to immunize a large worldwide population during COVID-19 pandemic. However, individuals respond to the vaccine differently, leading to distinct proteome changes and vaccination efficiency. At the same time, our understanding of the underlying molecular mechanisms behind the adaptive response to COVID-19 vaccine is still limited. Here, we used the Proteograph workflow[1]coupled with TMT labeling to quantify the proteome changes in a cohort of 12 volunteers immunized with two doses of COVID-19 vaccine. Our data confirmed that Proteograph workflow enables identification of more than 3,000 proteins from human plasma samples in this study. Since 7 volunteers still got COVID-19 after two-dose vaccine, our analysis gained further insights into the personalized response to vaccination.

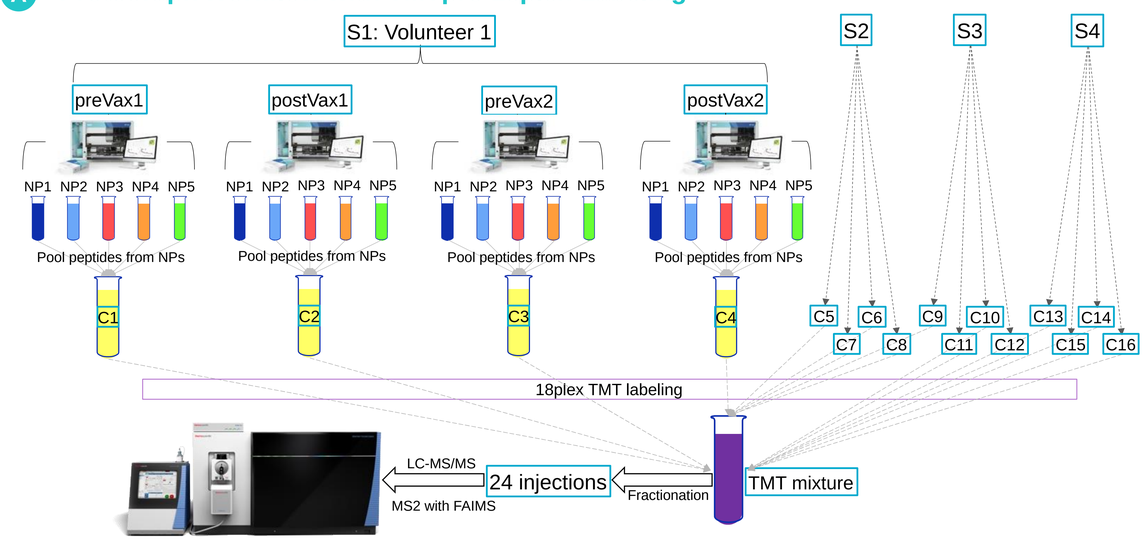


48 samples from 12 volunteers
- 8 females and 4 males
- 20~30 years old
- 2 got covid before or during vaccination
- 7 got covid after vaccination

Methods: Proteograph Workflow and TMT Labeling

Plasma samples were collected before and after first and second vaccination from each volunteer. Samples were processed by Proteograph™ Product Suite (Seer Inc.) using five distinct nanoparticles (NPs). Tryptic peptides were then pooled into one single sample for TMT labeling. A total of 48 samples were allocated into three 18-plex TMT mixtures, followed by peptide fractionation by high pH RP and LC-MS/MS analysis, comprised of an EASY nanoLC system coupled to an Orbitrap Fusion Lumos MS equipped with FAIMS Pro Interface (all from Thermo Fisher Scientific). Peptides were separated using a 25-cm analytical C18 Aurora column (IonOpticks) into 24 fractions. A two-hour gradient with three FAIMS Pro compensation voltages was used in this study. The raw spectra data were processed by SpectraMine (Biognosys) to generate peptide identification and quantification data. We then used R/Bioconductor package MSstatsTMT for protein quantification, normalization, and differential analysis.

A Proteoraph Workflow with TMTpro 18plex Labeling



B Experimental Design

Plex	126	127N	127C	128N	128C	129N	129C	130N	130C	131N	131C	132N	132C	133N	133C	134N	134 C	135 N
set1	S1 preVax1	S1 postVax1	S1 preVax2	S1 postVax2	S2 preVax1	S2 postVax1	S2 preVax2	S2 postVax2	S3 preVax1	S3 postVax1	S3 preVax2	S3 postVax2	S4 preVax1	S4 postVax1	S4 preVax2	S4 postVax2	pool1	pool2
set2	S6 preVax1	S6 postVax1	S6 preVax2	S6 postVax2	S8 preVax1	S8 postVax1	S8 preVax2	S8 postVax2	S9 preVax1	S9 postVax1	S9 preVax2	S9 postVax2	S10 preVax1	S10 postVax1	S10 preVax2	S10 postVax2	pool1	pool2
set3	S5 preVax1	S5 postVax1	S5 preVax2	S5 postVax2	S7 preVax1	S7 postVax1	S7 preVax2	S7 postVax2	S11 preVax1	S11 postVax1	S11 preVax2	S11 postVax2	S12 preVax1	S12 postVax1	S12 preVax2	S12 postVax2	pool1	pool2

C

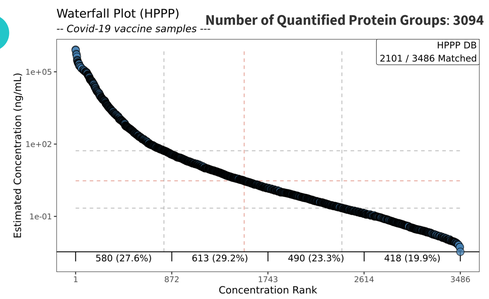
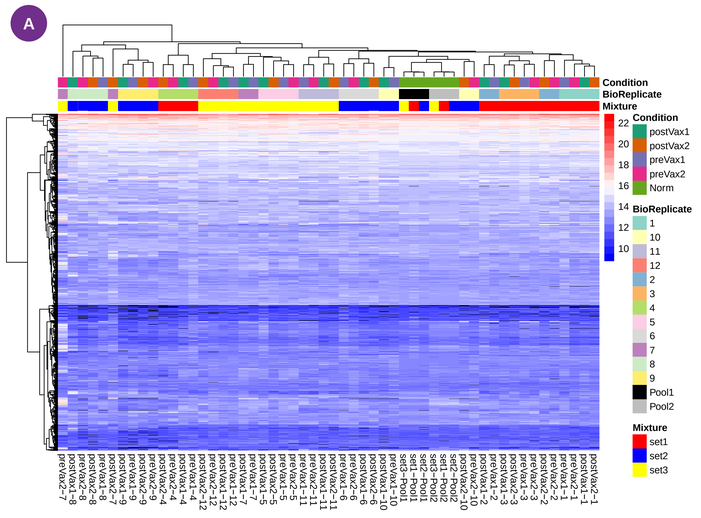


Figure 1. Proteograph workflow with TMT labeling: An overview of protein quantification performance.

A) For each biological sample, five peptide mixtures prepared by five distinct nanoparticles of Proteograph™ Assay were pooled into one single sample. Then, 16 pooled samples from 4 volunteers were labeled with 18plex TMT and combined to produce a single TMT mixture. B) 48 samples from 12 volunteers were allocated into three 18plex TMT mixtures. Each mixture includes two masterpool samples for normalization which was labeled with extra 2 labels in TMTpro 18plex labeling kit . C) Compared with the existing plasma proteomics studies on COVID-19 vaccination[2,3], the combination of Proteograph workflow with TMTpro 18Plex has provided much deeper access to human plasma proteome, i.e., 23,372 peptides and 3,094 proteins. Mapping these 3,094 proteins to the Human Plasma Proteome Project (HPPP) protein database[4] shows detection across the entire concentration range of the database, 20% of which fell in the low abundance range.

A



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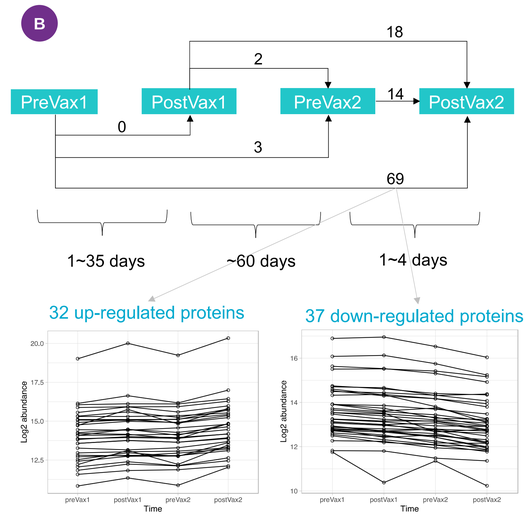
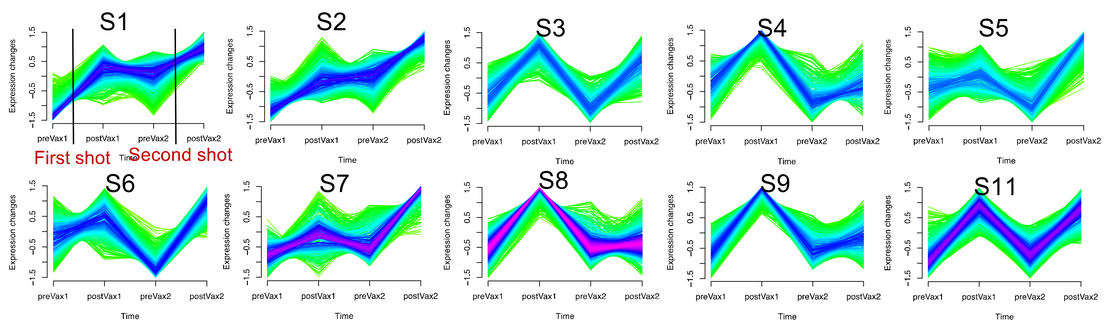


Figure 2. Clustering and differential protein analysis across the Cohort.

A) Hierarchical clustering analysis showed that samples from the same volunteer were clustered together and demonstrated a high inter-individual variation. B) Differential analysis using samples from 12 volunteers before and after two doses of COVID-19 vaccine. None of the proteins were differentially expressed after the first dose but 69 proteins became differential after the second dose.

A



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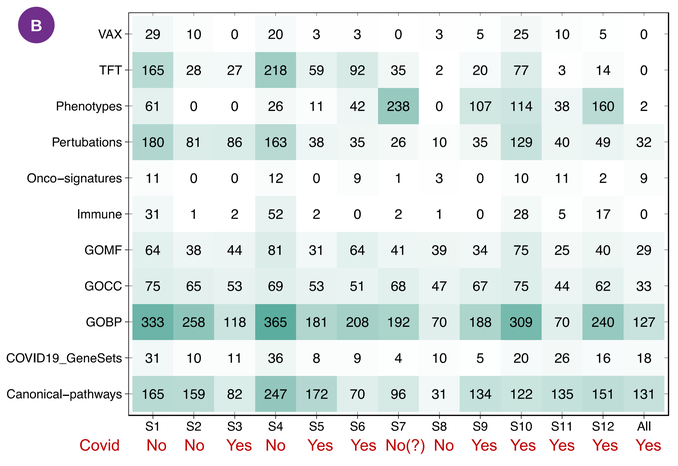

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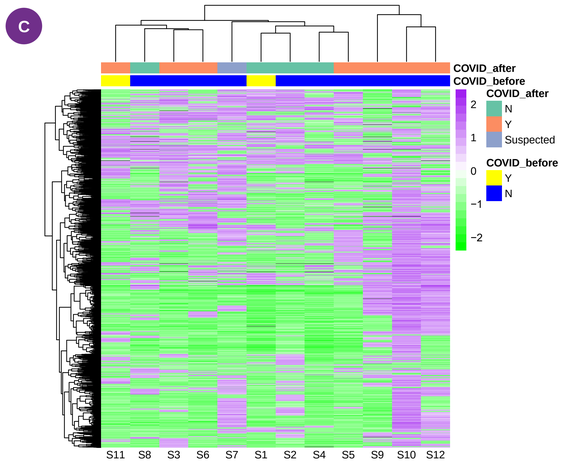

Figure 3. Individual-specific differential proteomic analysis.

A) Proteins from each volunteer were clustered into significant discrete groups using mFuzz package to illustrate various patterns of protein expression changes after vaccination. Most volunteers shared a common N (up-regulation) pattern, where protein abundances were up-regulated by the first dose, decreased after the first dose, and boosted by the second dose. B) Pathway enrichment analyses on individual volunteers showed that individuals who enriched most GO-BP and immune pathways didn't get COVID-19 within 10 months after two-dose vaccination. C) Clustering on immune pathways-related proteins, individuals who didn't get Covid-19 were grouped together.

Conclusion

- Proteograph workflow coupled with TMT labeling quantifies >3,000 protein groups from a cohort of COVID-19 vaccination study with 48 plasma samples
- Covid-19 vaccine related plasma proteomics data show a high between-subject variance
- Individuals with enriched gene oncology and immune pathways didn't get covid within 10 months after vaccination

References

[1] Blume et al. Nat. Comm. (2020)
[2] Wang et al. Front. Immunol. (2022)
[3] Wang et al. medRxiv (2022)
[4] Schwenk, et al. Journal of Proteome Research. (2017)

Publications

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