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

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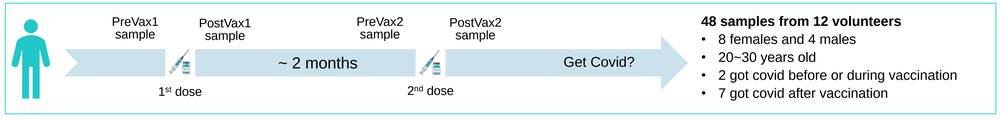
Proteograph™ workflow coupled with TMTpro 18Plex

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 in human and vaccination efficiency. Furthermore, our understanding of the underlying molecular mechanisms behind the adaptive response to COVID-19 vaccine remains 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 the Proteograph workflow enables identification and quantification of more than 3,000 proteins from human plasma samples in this study. Since 7 volunteers still contracted COVID-19 after two-dose vaccination, our analysis gained further insights into the personalized response to COVID-19 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

- 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 TMTpro 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 MS 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.

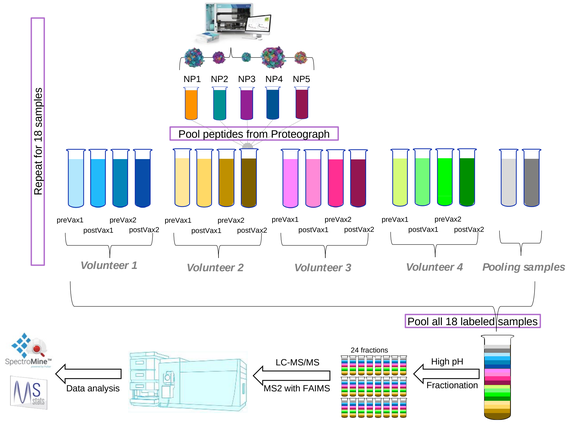


Figure 1. Proteograph workflow with TMT labeling. For each biological sample, five peptide mixtures prepared by five distinct nanoparticles of Proteograph™ Assay were pooled into one single sample. Then, 16 pooled samples from 4 volunteers were labeled with TMTpro 18plex and combined to produce a single TMT mixture.

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

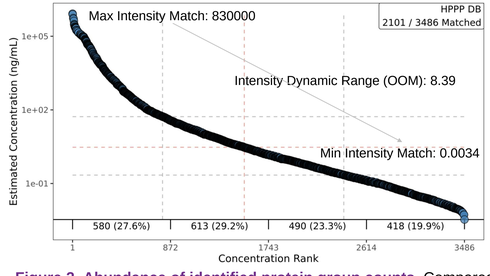


Figure 2. Abundance of identified protein group counts. Compared with the existing plasma proteomics studies on COVID-19 vaccination[2,3], the proposed workflow provides much deeper access to human plasma proteome, i.e., 23,372 peptides and 3,094 proteins. Mapping these proteins to the HPPP protein database[4] shows detection across the entire concentration range of the database, 20% of which fell in the low abundance range.

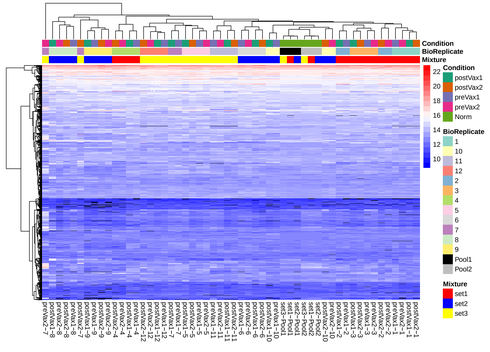


Figure 3. Hierarchical clustering analysis. Data shows that samples from the same volunteer were clustered together and demonstrated a high inter-individual variation.

Results

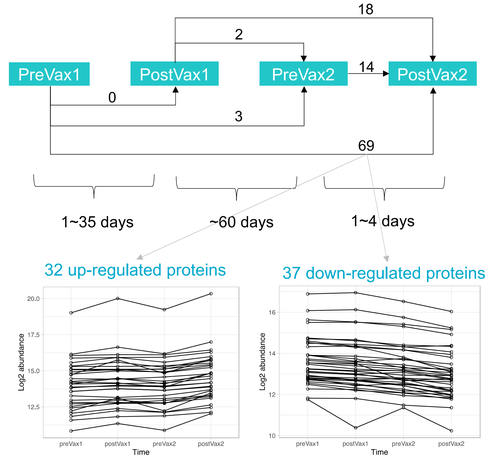


Figure 4. Differential protein analysis across the cohort. None of the proteins were differentially expressed after the first dose but 69 proteins became differential after the second dose.

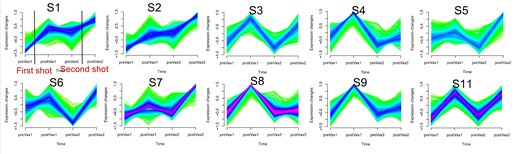


Figure 5. Individual-specific hierarchical clustering analysis. Proteins from each volunteer were clustered into significant discrete groups to illustrate various patterns of protein expression changes after vaccination. Most volunteers shared a common N (up-regulation) pattern, where protein abundances were up-regulated by the first dose, decreased after the first dose, and boosted by the second dose.

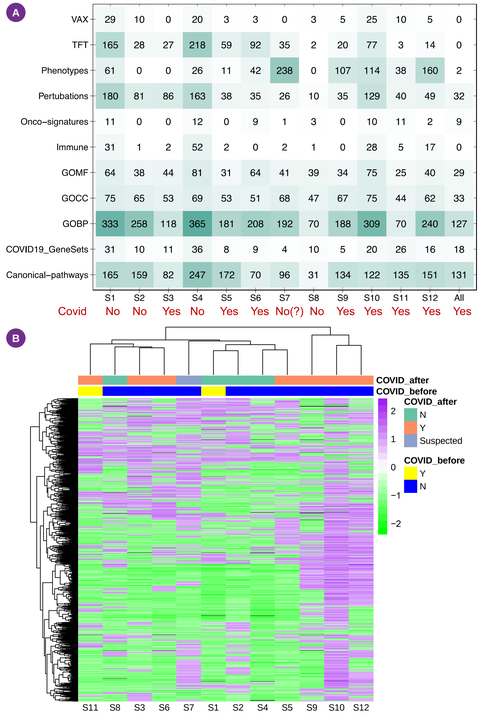


Figure 6. Individual-specific pathway enrichment and clustering analysis. A) Pathway enrichment analysis shows Individuals enriching most gene ontology biological processes (GO-BP) and immune pathways didn't get COVID-19 within 10 months after vaccination. **B)** Clustering on immune pathways-related proteins separates individuals who didn't get Covid-19.-

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)
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[4] Schwenk, et al. Journal of Proteome Research. (2017)