# The Oxidized Proteome of Peripheral Blood Mononuclear Cells: A Valuable Repository for Clinical Proteomics

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## Overview

**Purpose:** Peripheral blood mononuclear cells (PBMCs) and its specific cell subsets have allowed for a very broad collection of applications such as in vitro cell-based assays to study the immune response to a given stimuli, or to monitor in vivo or ex vivo changes before and after a drug treatment. More importantly, they are an easily accessible cellula part of the blood, and they are the only component of the blood that could have a gene expression activity. Access to complete atlas of the gene expression and posttranslationa modifications in PBMCs will permit more sophisticated studies such as the selection of potential biomarkers that could be used for many purposes ranging from diagnostic, prognosis or even help selecting the appropriate therapy for a patient.

**Results:** This study compiles the most extensive proteome map of PBMCs. We have demonstrated that the combination of the results yielded the identification of over 8000 proteins. In addition, over 5000 proteins were accurately quantified and over 7000 oxidation events were identified. Remarkably, our data also suggest that $H_2O_2$ might play a role modulating signaling pathways by reacting with specific protein targets. Overall, thi study not only adds significant value in the mechanistic understanding of redox signaling but it also creates a valuable protein repository that could lead to the development of nev therapeutic strategies.

## Introduction

Peripheral blood mononuclear cells (PBMCs) are a popular model system to study the physiological and metabolic activity of cells within the body. PBMCs have enabled a very broad collection of biomedical applications. Monitoring gene expression and posttranslational modifications are very promising areas in biomarker discovery and translational research. In this study, we have aimed to have the most extensive proteome map of PBMCs and monitor the in vitro effect of reactive peroxide at low concentration under different exposure times. Over 8000 proteins were mapped, more than 5000 proteins were accurately quantified and over 7000 oxidation events were identified. Thes observations represent the largest proteome profiling dataset for PBMCs to date, and create useful warehouse in the clinical blood proteomics field.

## Methods

**Sample Preparation**

PBMCs from a healthy male individual were purchased from AllCells. 1mill cells aliquots were in vitro treated with 5 mM $H_2O_2$ for 0, 2, 10, 30 and 80 min. Cell lysis, protein precipitation and digestion were performed using the Mass Spec Sample Prep Kit for Cultured Cells (Pierce, Rockford IL).

**Liquid Chromatography and Mass Spectrometry Analsys**

Peptide digests were then analyzed by LC-MS/MS analysis on a Easy nLC1000 coupled to a Thermo Scientific™ Q Exactive™ Plus mass spectrometer over a 2-hour gradient.

**Data Analysis**

Database search and oxidation site localization were performed using SEQUEST and phosphor RS. These tools were used as nodes within Thermo Scientific™ Protein Discoverer 2.0 software. Inferno was then used for further statistical analysis and ProteinCenter was used to extract biological context and set comparisons with publicly available datasets.

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## Study Design

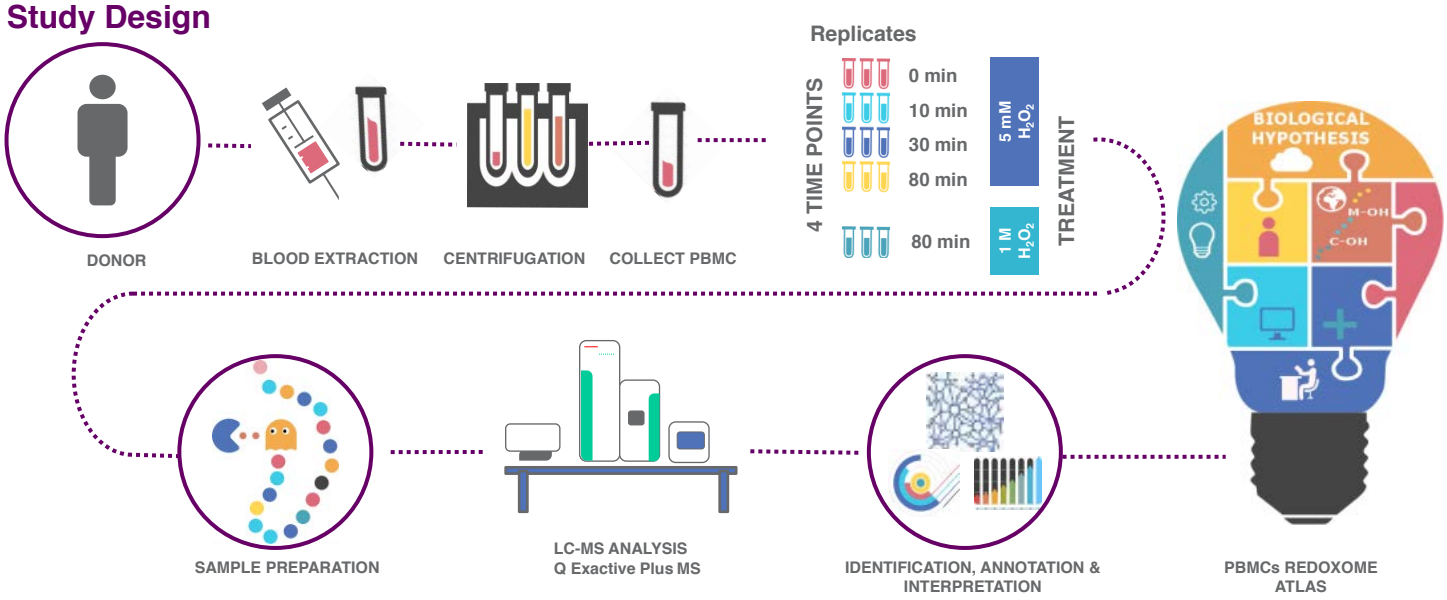


**FIGURE 1. Workflow summarizing the analytical approach.** Samples were obtained from a donor, slow centrifuged to separate plasma and erythrocytes from PBMCs. Lysis was then performed, proteins precipitated and digested. After digestion, the samples were acidified, dried down and analyzed by LC-MS. Finally, peptide and protein identification was performed, as well as, label free quantitation. Subsequently, statistics and data mining including gene ontology enrichment was performed.

## Results

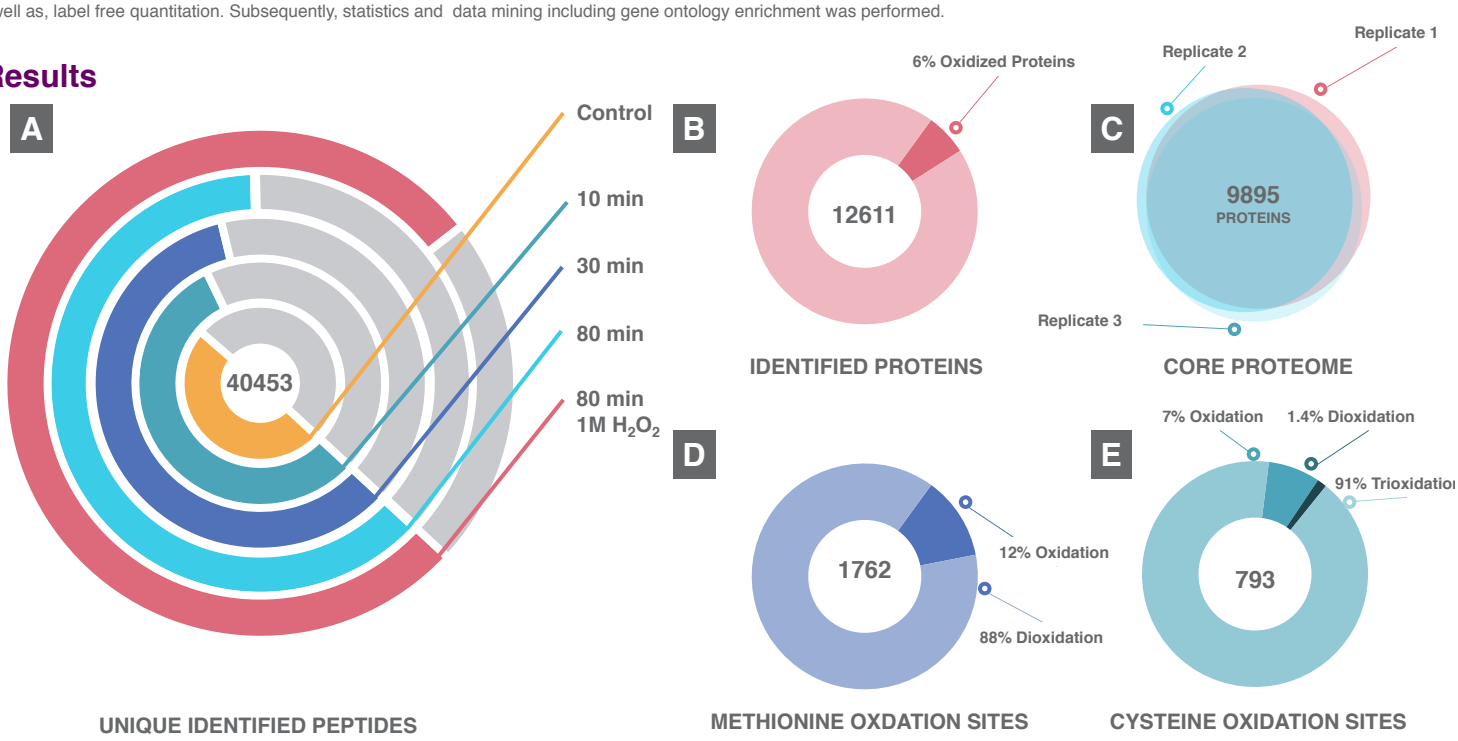


**FIGURE 2. In-depth coverage of the PBMCs proteome**. A. Number of unique identified peptides gained after the run for each condition. B. Number of identified proteins and number of proteins with oxidized cysteines and/or methionine. C. Venn diagram representing the frequency of protein identification within the three replicates. Proteins identified in all 3 replicates were designated as core proteome. D and E. Number of unique oxidation sites for methionine and cytsteine, and percentage of the different oxidation states identified within each amino acid

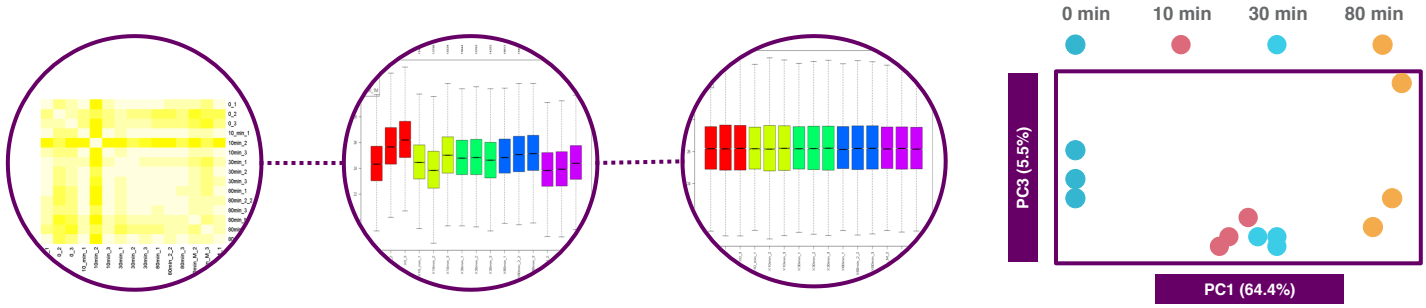


**FIGURE 3. Statistical Analysis Workflow.** Areas from the identified peptides were extracted using the Precursor Ions Area Detector plug-in in Proteome Discoverer software and exported in to a text file for further analysis. Reproducibility was first evaluated using a correlation plot to discard outliers. The average correlation was 0.9. Then raw intensities were $\log_2$ transformed and normalized using LOWES. Peptides were roll-up to protein as follows. A reference peptide with the most presence across all the datasets, was chosen from the group of peptides that belong to a protein. Then the ratios of peptide abundances with respect to the reference were computed, and their median was used as a scaling factor. Protein abundance was obtained as the median of the resulting peptide abundances. Finally, PCA and ANOVA test were performed to classify the samples and discover those protein that changed in abundance.

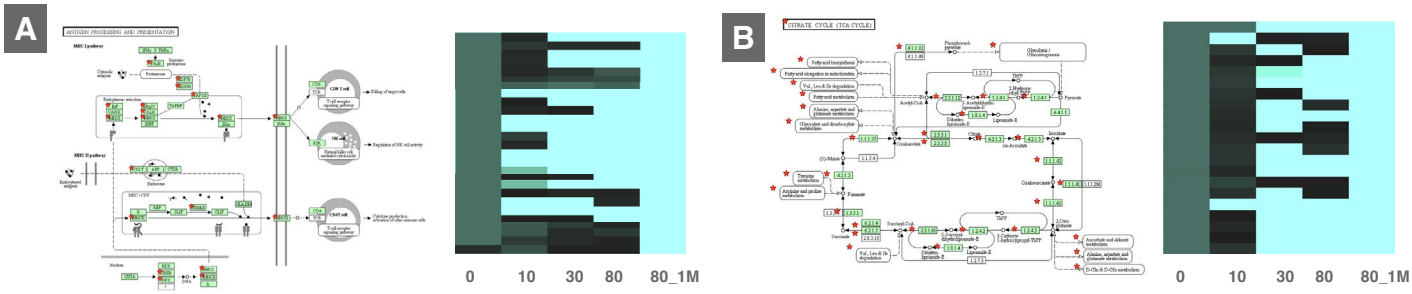


**Figure 6.Differential protein expression upon oxidative stress treatment for the whole proteome.** Differentially, expressed proteins (p-value<0.01) were analyzed by KEGG.db package after the extraction of the corresponding genes. The top functional identifiers mapped to (A) the Antigen and Presentation signaling pathway in T-cells, as well as to (B) the TCA cycle pathway (red stars). Heatmaps represent the normalized abundance for the significant proteins that map to those pathways.

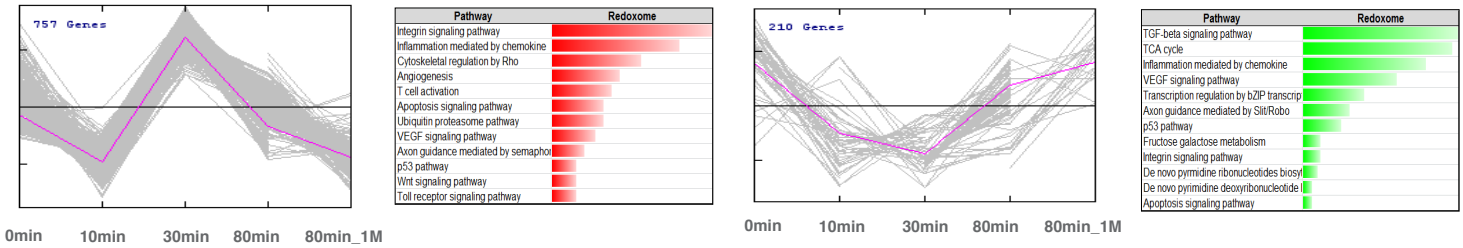


**Figure 7. Time course profiles of the oxidation sites**. Groups of oxidized proteins (query genes) with similar dynamic behaviors were clustered using k-means method. Euclidian distances were calculated for a total of 9 different clusters. Two clusters with opposite profiles are exemplified. In addition, pathway enrichment analyses were performed for each cluster. The analysis demonstrates that both the immunological response and TCA cycle reaction pathways were among the most affected in PBMCs after an oxidative stress treatment.

## Conclusion

- Simple, but yet powerful proteomic workflow, based on label free quantitation, single UHPLC runs on a bench top mass spectrometer and data analysis by Proteome Discoverer software.
- Largest coverage of the PBMC proteome and its dynamics to oxidative stress[1].

1 Saša Končarević, et al, "In-Depth Profiling of the Peripheral Blood Mononuclear Cells Proteome for Clinical Blood Proteomics,"International Journal of Proteomics, vol. 2014, Article ID 129259 doi:10.1155/2014/129259