

# Parkinson's Disease biomarker discovery via LC-MS/MS characterization and quantification of alpha synuclein PTMs in erythrocytes



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## Introduction

### Parkinson's disease (PD) quick facts

- Neurodegenerative disorder characterized by accumulation of Lewy body protein aggregates in brain
- PD global burden expected to grow significantly over the next 50 years

**Want:** Robust clinical measure (qualitative and quantitative) to

1. Properly recruit clinical trials
2. Assess effectiveness of therapeutic interventions at different stages of disease progression

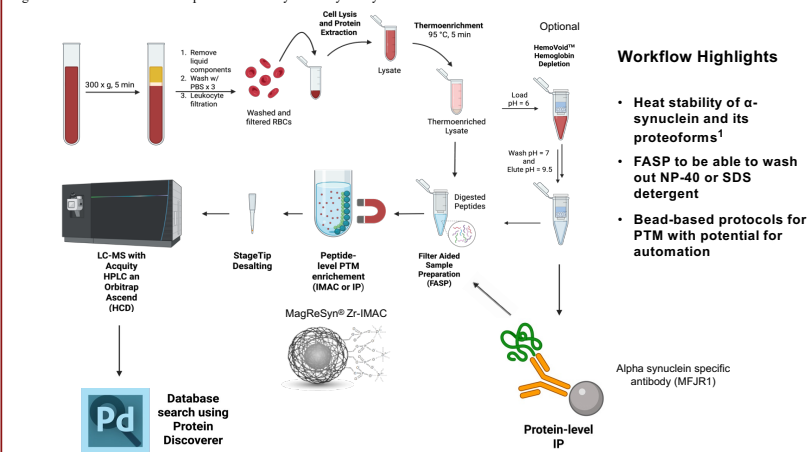
**Need:** Predictive and prognostic biomarkers from readily accessible biofluids

**This work:** Erythrocytic Alpha synuclein protein ( $\alpha$ -syn) as a source of potential PD markers

- Measurements of total  $\alpha$ -syn in CSF, plasma, and RBCs via both antibody and MS approaches have yielded inconsistent or low-powered results
- Miranda et al. demonstrated for the first time with dot blot that combined PTM signature could distinguish PD from HC but work done with antibodies
- In vitro evidence tPTMs directly affect the seeding and fibrillation of  $\alpha$ -syn which underpins the onset and progression of the disease

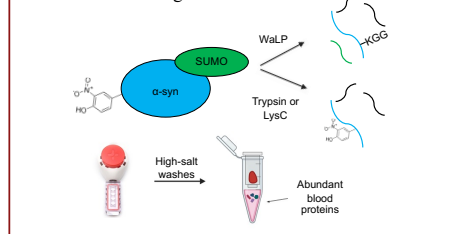
## Materials and Methods

Figure 2. General workflow for proteomics analysis of erythrocytes



## Conclusions and Future Directions

- Alpha synuclein is highly abundant in erythrocytes but its proteoforms are still analytically challenging to characterize by Mass Spec
- With enrichment it is possible to detect phospho S87 even in healthy patients
- From qualitative to quantitative
  - The IS problem
    - Generate in vitro with kinases?
    - is LFQ DIA the solution?
  - For case-control maybe can use relative intensities only but what about clinic?
    - Translation to dried blood spot microsamples
- Changing the workflow
  - Complementarity of search engines (evidence that PSM overlap is <100%)
- Other PTMs
  - OGlcNAc, SUMOylation, nitration
    - Biologically interesting but workflows PTM-specific
    - Harmonizing across methods?



## Results

Figure 2. Some known post-translational modification of alpha synuclein. Reproduced from Magalhães and Lashuel.<sup>2</sup>

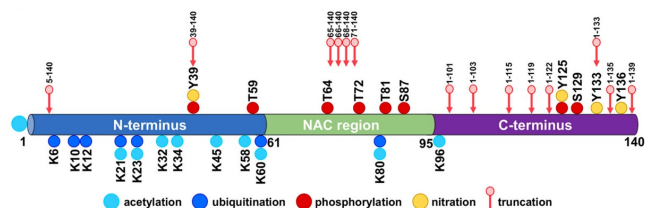


Figure 3. Fragmentation spectra and y ion series for confirmed phosphopeptide

TVEGAGSIAAATGFVK, S7-Phospho  
 Ascend\_MAAP\_2\_25\_08a/raw\_#1781 RT: 47.988 min  
 PTM: 300.3353 (m/z 90.2), z=2; Mono m/z: 779.88036 Da; Mono+1559.75344 Da; Match Tol: +0.02 Da

