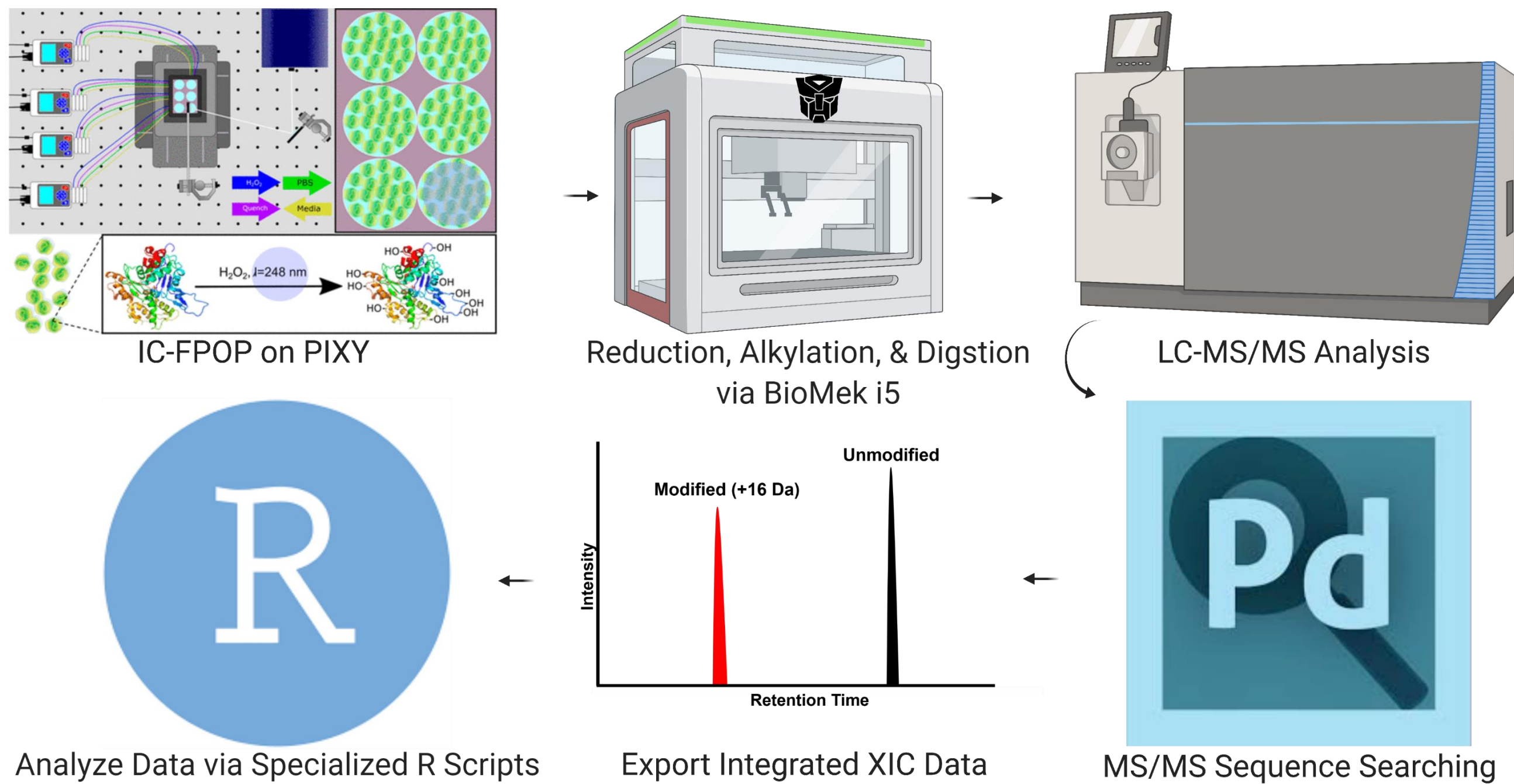


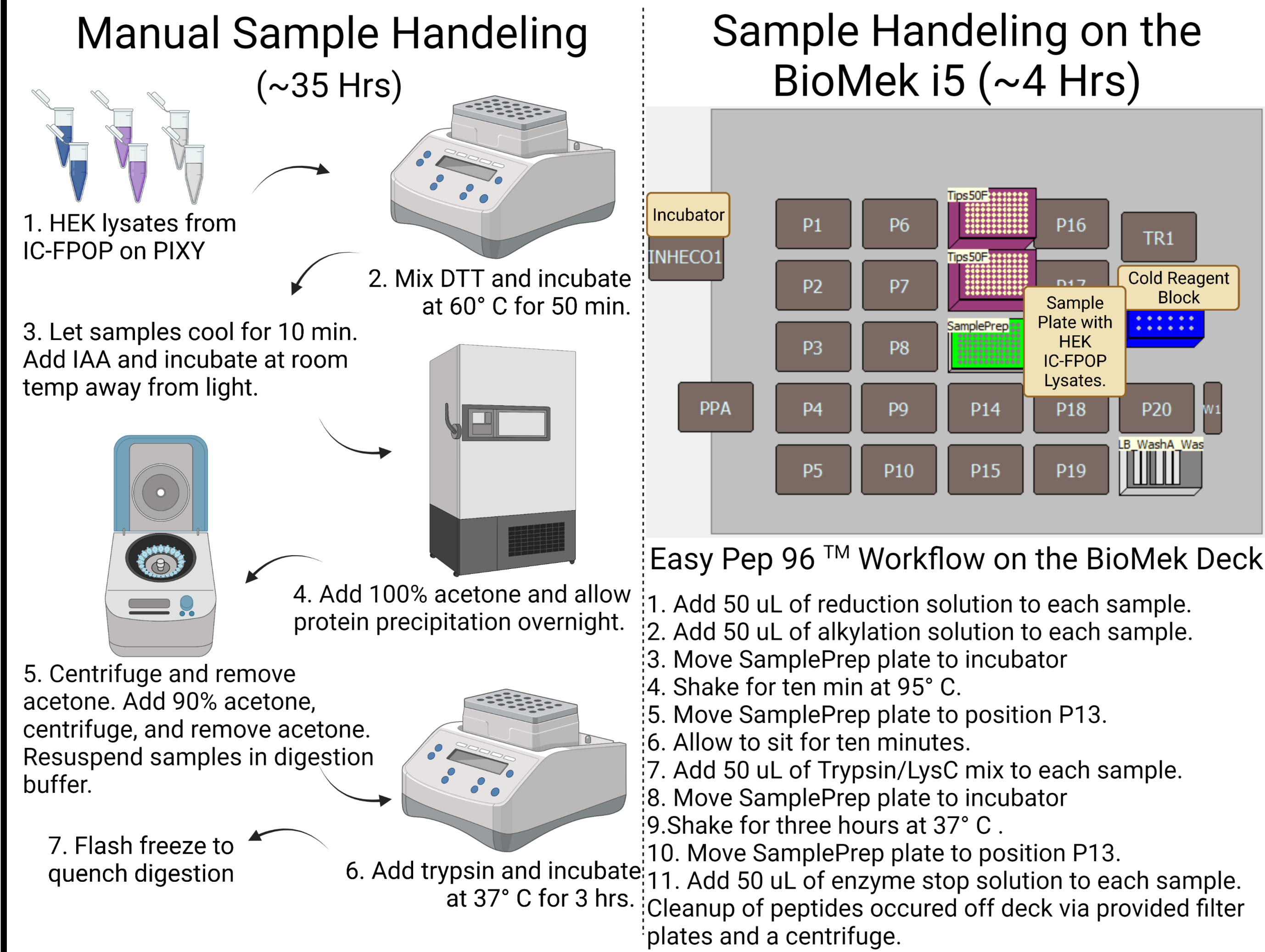


1. Introduction

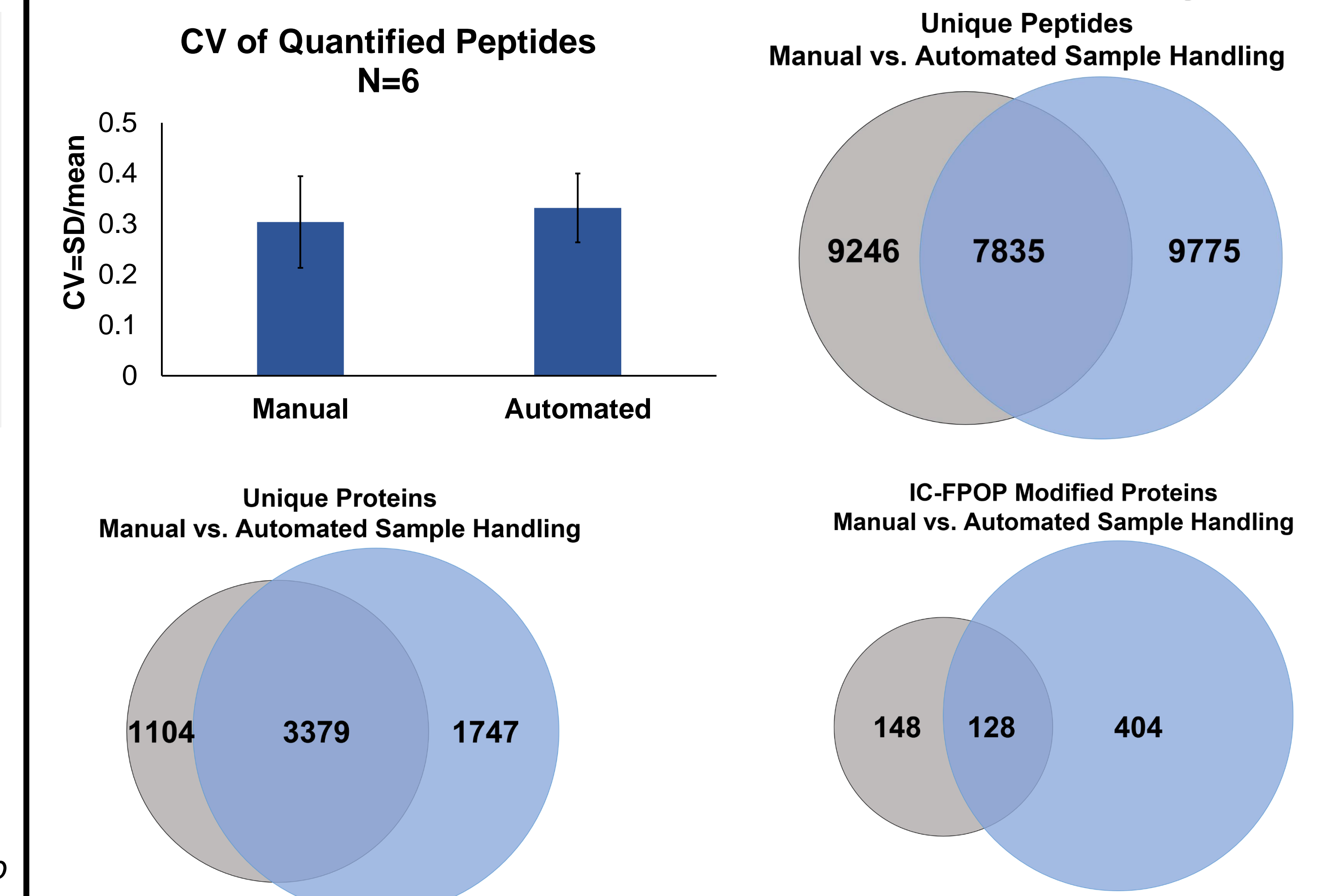
In cell-fast photochemical oxidation of proteins (IC-FPOP) is a valuable, mass spectrometry (MS)-based tool to probe protein structures and interactions¹. It was recently applied on a platform incubator with an XY movable stage (PIXY), where thousands of proteins were modified in cells, in a fraction of time compared to the flow system². The advent of this technology decreases the duration of IC-FPOP but, the workflow still contains a multi-day MS sample preparation procedure followed by a tedious data analysis scheme. In response to these limitations, we implemented the BioMek i5 liquid handling robot to expedite sample processing and developed specialized scripts in R to accelerate data analysis in the IC-FPOP workflow.



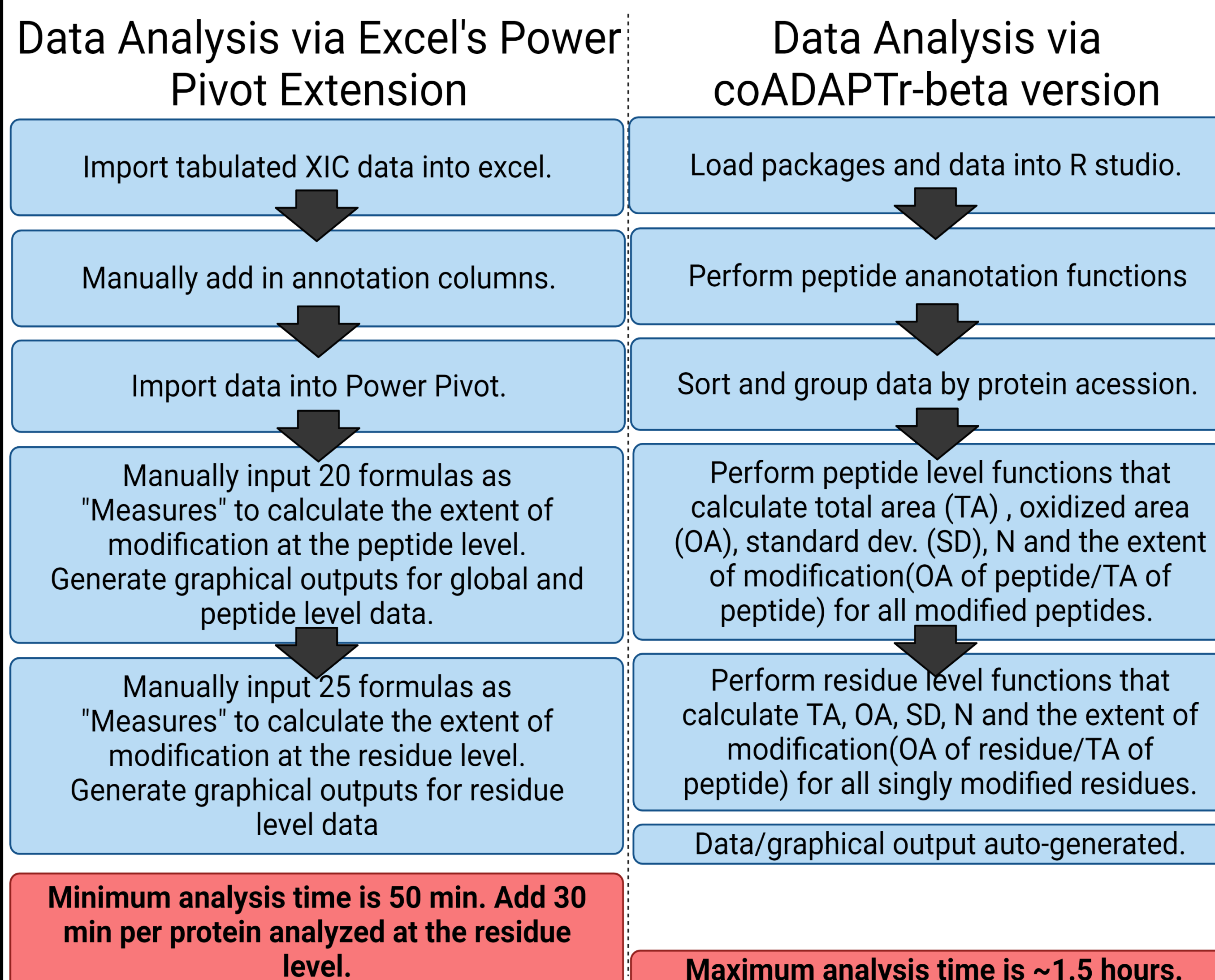
2. Manual vs. Automated Sample Handling



3. Manual vs. Automated Sample Handling

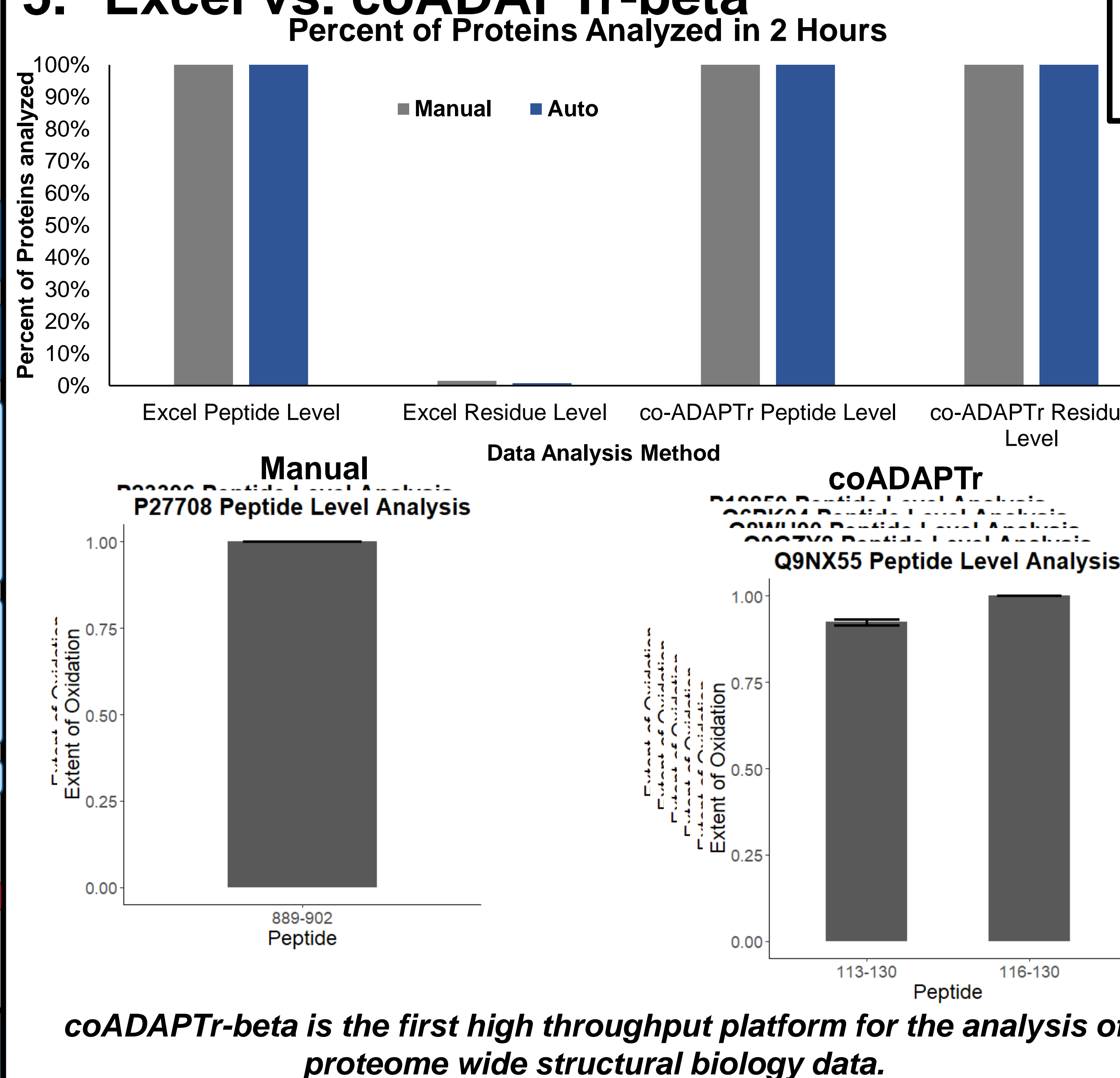


4. Power Pivot in Excel vs R (coADAPTr-beta)



~1 ug of peptides from each method were separated on an EvoSep LC and detected on an Orbitrap Fusion Lumos MS in DDA mode.

5. Excel vs. coADAPTr-beta



The BioMek provides a higher number of identified proteins and peptides in less time than the manual sample handling method.

6. Conclusions

- Manual sample handling resulted in lower CVs for the quantitation of peptides and higher rates of peptide recovery, compared to the automated workflow.
 - Likely due to the peptide clean up step in the OP workflow which we are optimizing.
- Automated sample handling produced more unique proteins, protein groups, and peptide groups detected.
- Automated sample handling resulted in more modified proteins and peptides compared to manual sample handling.
- Although automated sample handling resulted in more sample loss and variability, it produced more modification information in less processing time.
- coADAPTr-beta performed all peptide and residue level functions in a fraction of steps and time compared to previous methods.
- coADAPTr-beta is the first high throughput platform for proteome wide structural biology.**