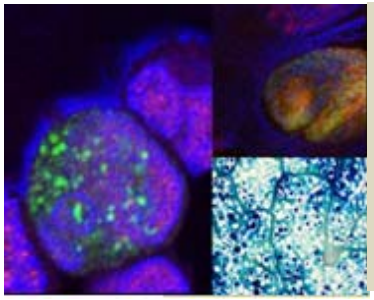

# Comparison between ultra-high pressure and atmospheric tryptic digestion for proteomic analyses

**E. Bonneil[1], R. Biringer[2], C. Bell[1], P. Thibault[1]**

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Université de Montréal

IRIC

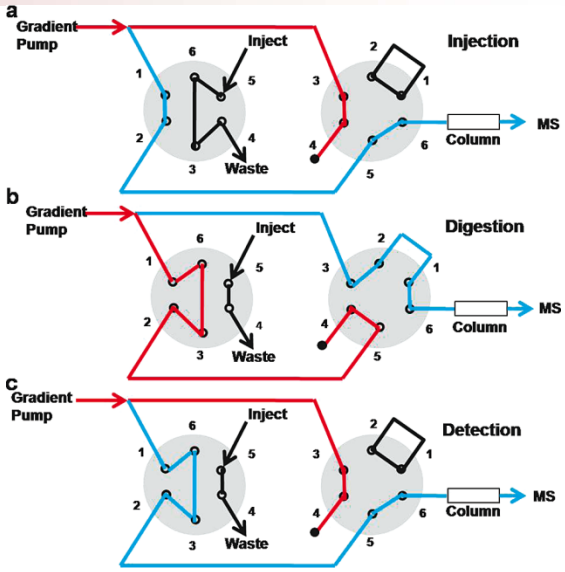
# Pressure cycling technology for proteomics

- Proteomics is the large-scale study of proteins:
    1. Identification of proteins
    2. Identification of protein modifications (phosphorylation, acetylation….)
    3. Monitoring of protein or modification expression changes between control and stimulated samples
- Proteomic analysis is a tedious process involving
    1. protein denaturation (1 hr)
    2. protein digestion (4-18hr)
    3. LC-MS analysis of peptides (1hr)
    4. data processing (1hr)
- Bottleneck for high-throughput analyses is the **protein digestion**
- Strategies to speed-up the digestion:
    - increase enz/prot ratio → more enzyme autolysis peptides, non-specific cleavages
    - heating to increase protein denaturation requires enzyme modification
    - addition of detergents might be incompatible with LC-MS or enzyme activity
    - immobilized enzyme microspin columns or microreactors have a limited capacity
    - ultrasonic energy might degrade the protein or the gel bands → blockage of the LC-MS system

# Pressure cycling technology for proteomics

High pressure digestion inside an HPLC valve

- On-line coupling with the LC-MS sample → reduces sample loss
- No pressure cycling available
- temperature control of the valve could be challenging
- Limited to liquid sample, not applicable to gel samples



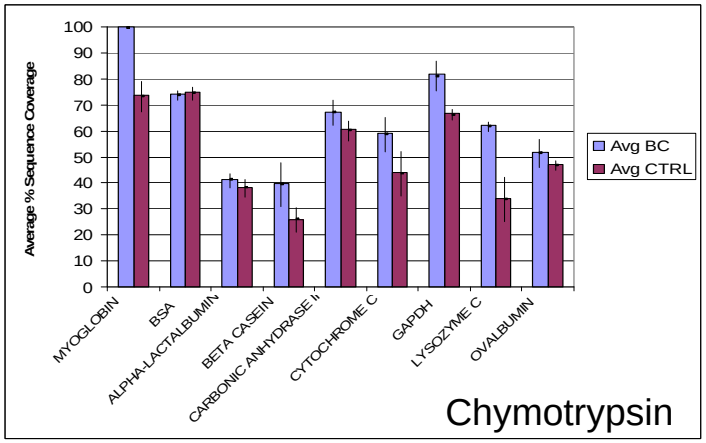

Gross et al. *Anal. Chem.* 82, 1771-1174

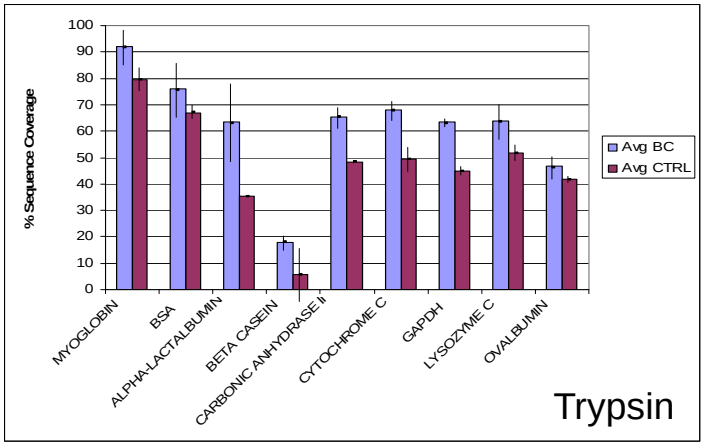
High pressure digestion using the Barocycler

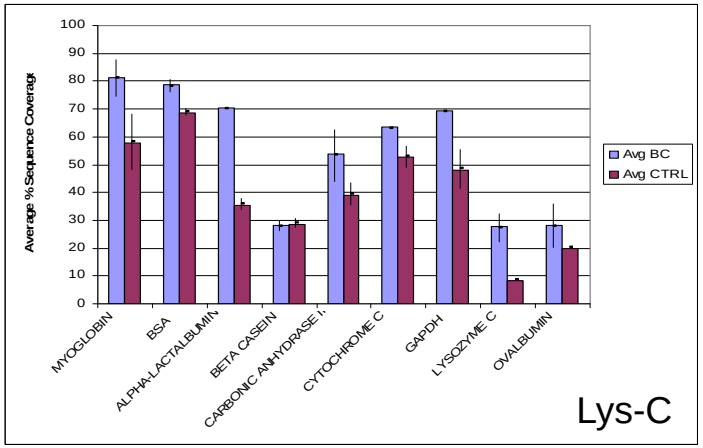
- Off-line digestion system
- Temperature control available
- No restrictions to sample format

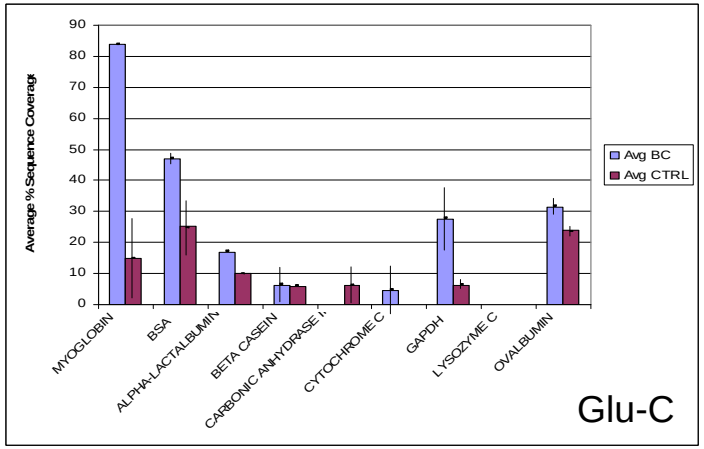
# Digestion efficiency for standard protein mixtures

- Standard proteins were reduced and alkylated
- Control experiments were performed for 16hr without mixing
- PCT digest were performed for 30 min



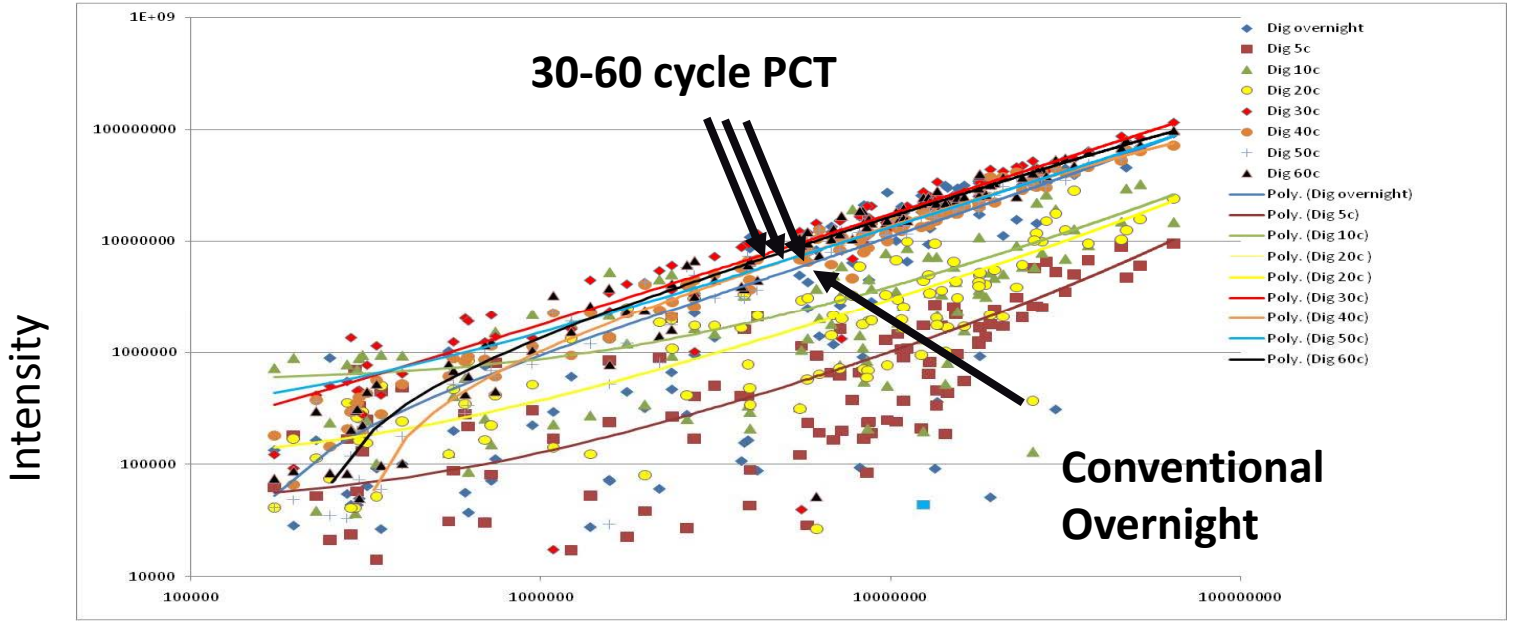




- **PCT produces higher % sequence coverage than the control for almost all proteins substrates and proteases examined**

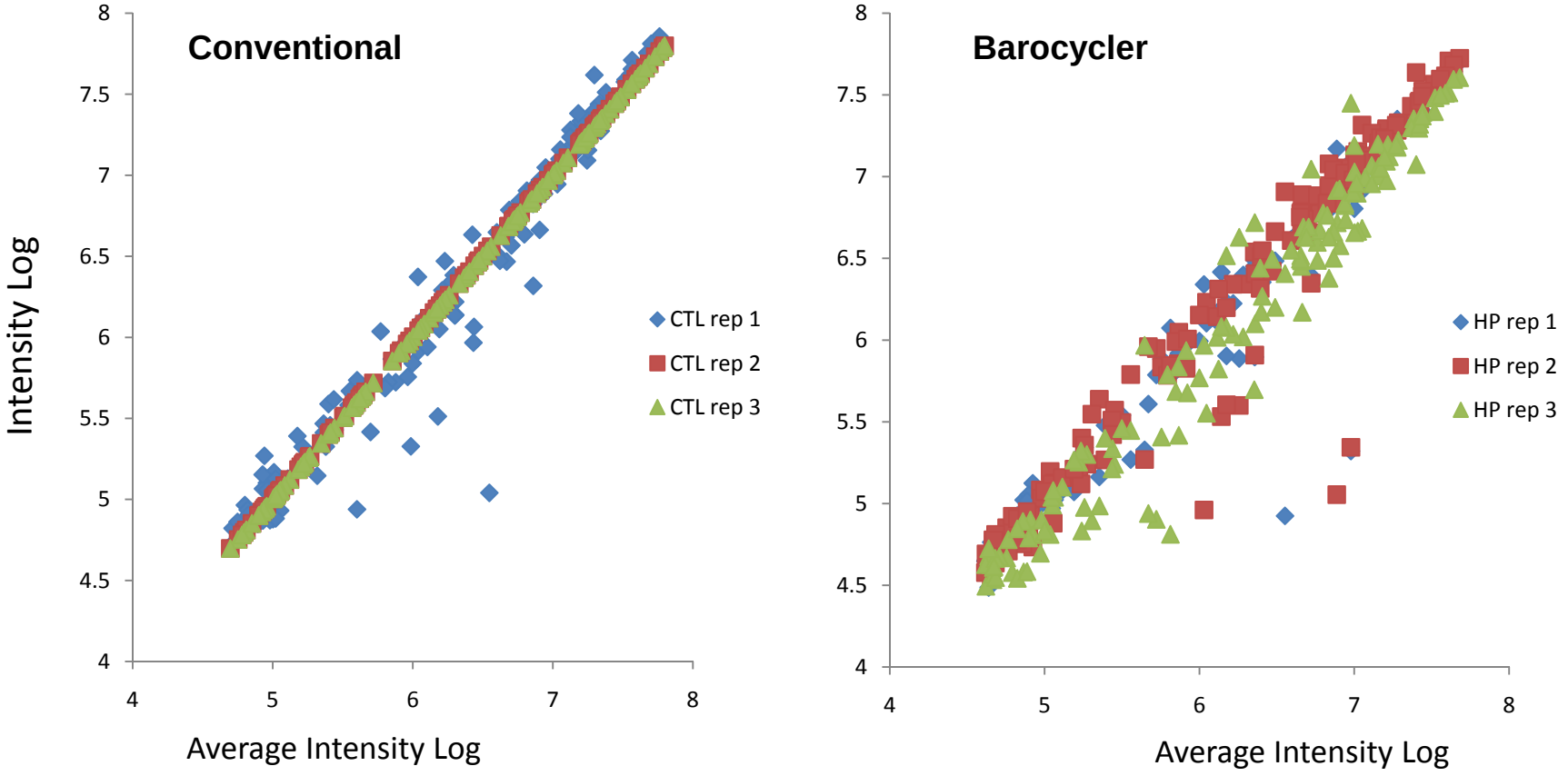
# Digestion efficiency for BSA 1D band

- BSA 1D Band reduced and alkylated with chloroacetamide
- 1ug were digested in solution (**50 ul**) at 1 atm with mixing or with PCT (1c 50s HP 10s LP)
- Search with 4 miscleavages
- 250 ng were injected.




- **Similar peak intensity when using PCT (> 30 min) or conventional digestion**

# Digestion efficiency for BSA 1D band

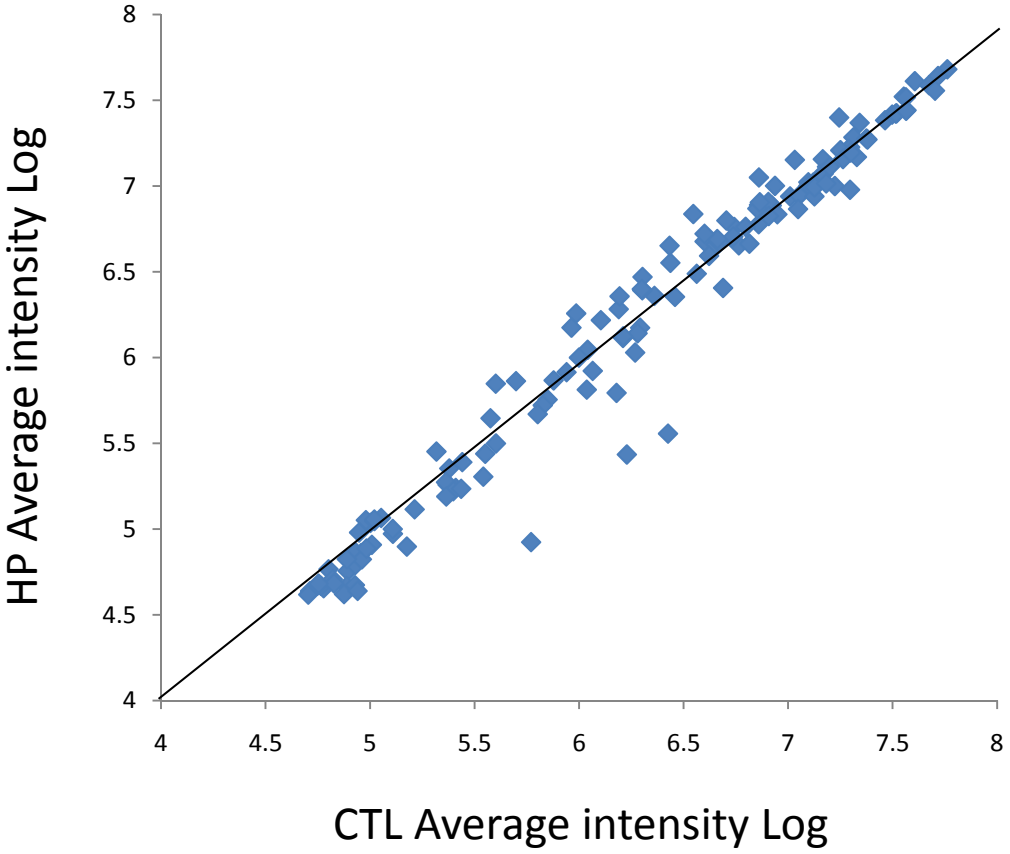


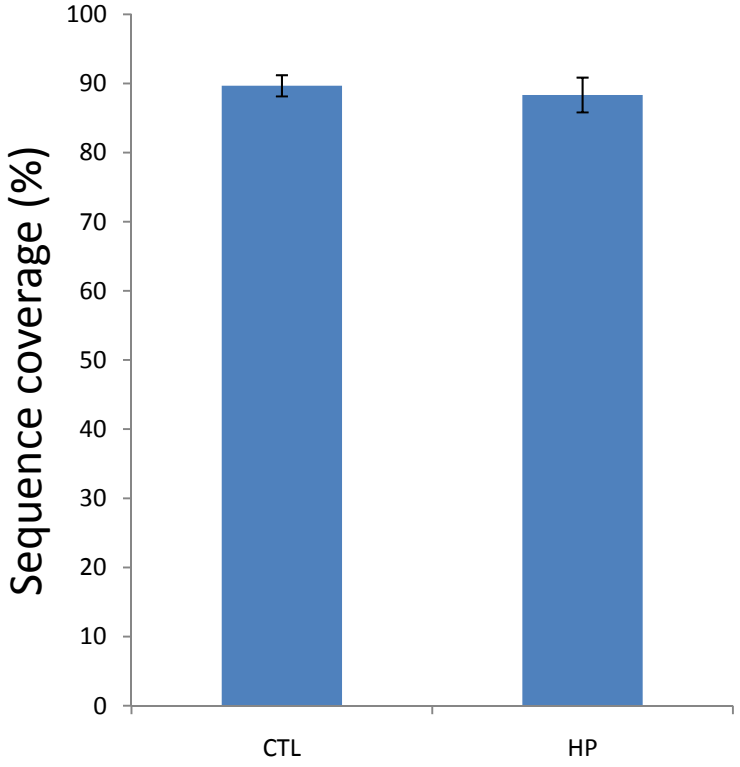




- **Reproducibility of peak intensity slightly better with the conventional method.**
- **The transfer step from the gel pieces to the small tubes might cause some losses.**

# Digestion efficiency for BSA 1D band

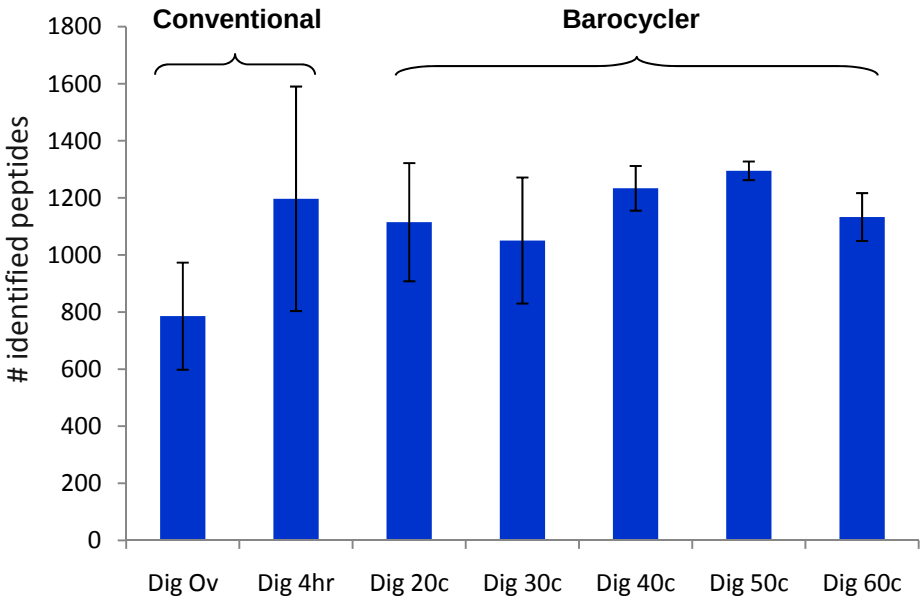


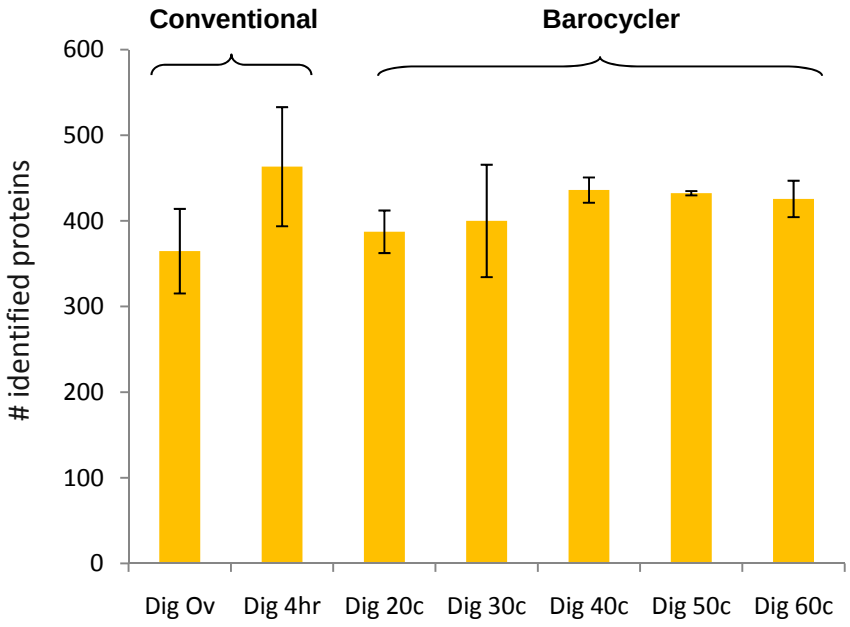




- **No loss of peptide intensity by doing the digestion at HP**
- **Similar sequence coverage**

# Reproducibility comparison for U937 cell lysate digestion

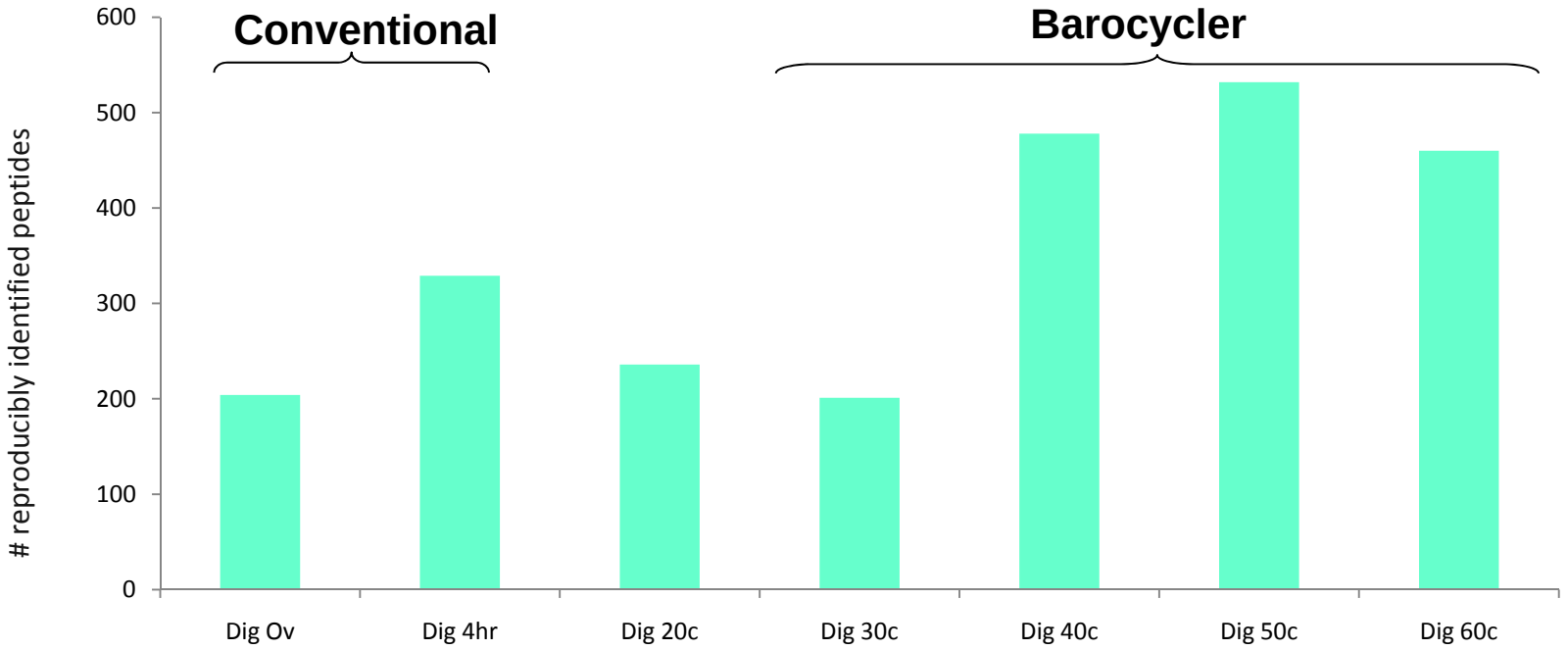
- U937 cell lysate reduced with TCEP 0.5 mM 37  C 30 min
- 5ug were digested in solution at 1 atm or with PCT (enz/prot =1/25) (1c 50 s HP 10s LP)
- Conventional digestions were performed with mixing at 650 rpm
- 1ug were injected on a nano-LC system coupled to an LTQ-Orbitrap.




- **Number of protein and peptides more reproducible with PCT (>40 cycles)**

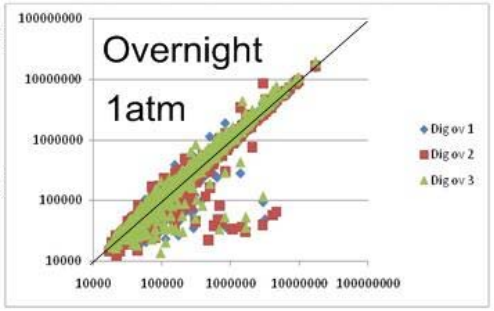
# Reproducibility comparison for U937 cell lysate digestion

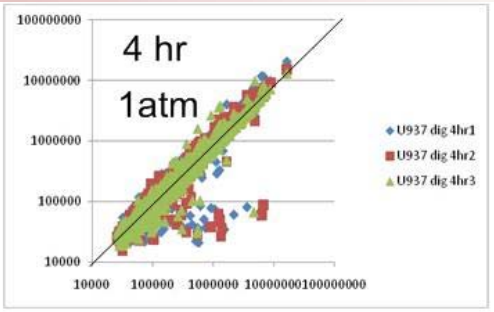



- **Better reproducibility of protein identifications with the Barocycler. Enhances confidence in protein identification. Advantage for MS/MS based quantitation (iTRAQ, TMT…)**

# Reproducibility comparison for U937 cell lysate digestion

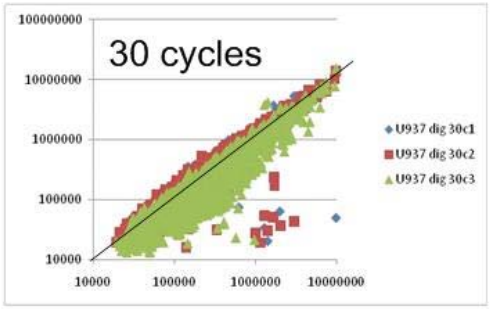
RSD 18%

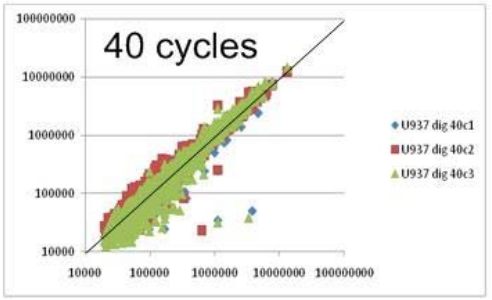



RSD 22%

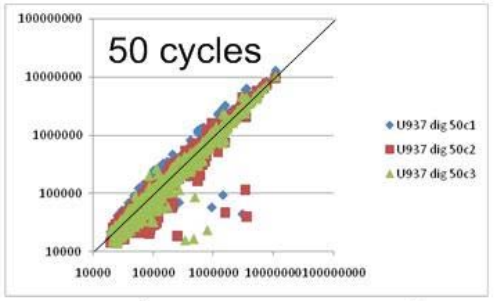
RSD 49%

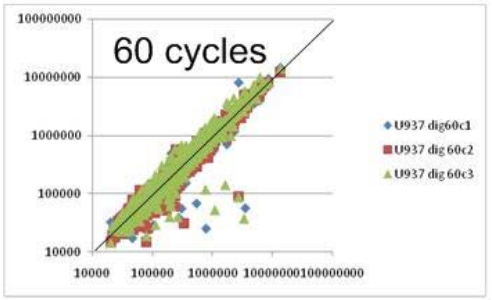



RSD 28%

RSD 17%

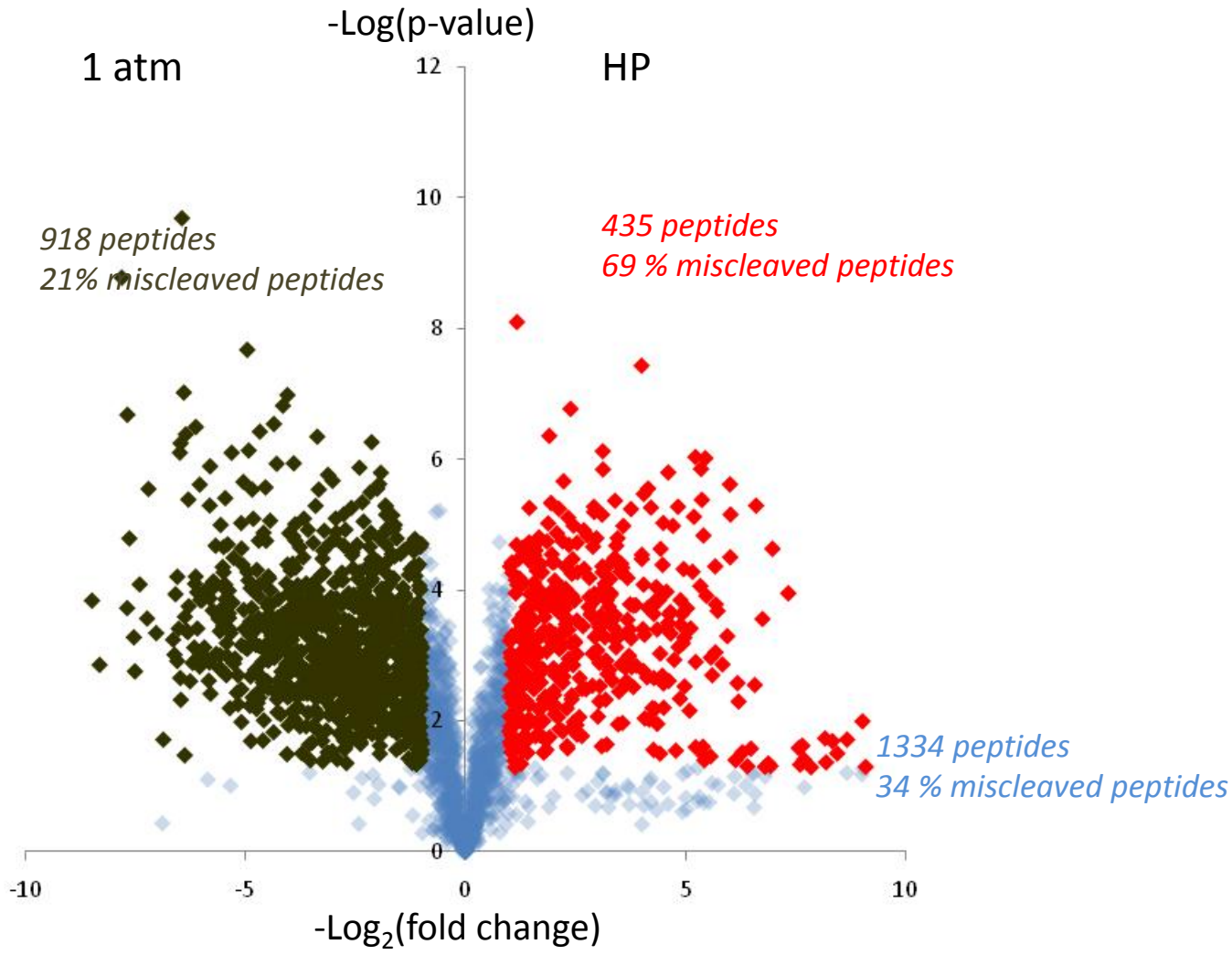



RSD 15%

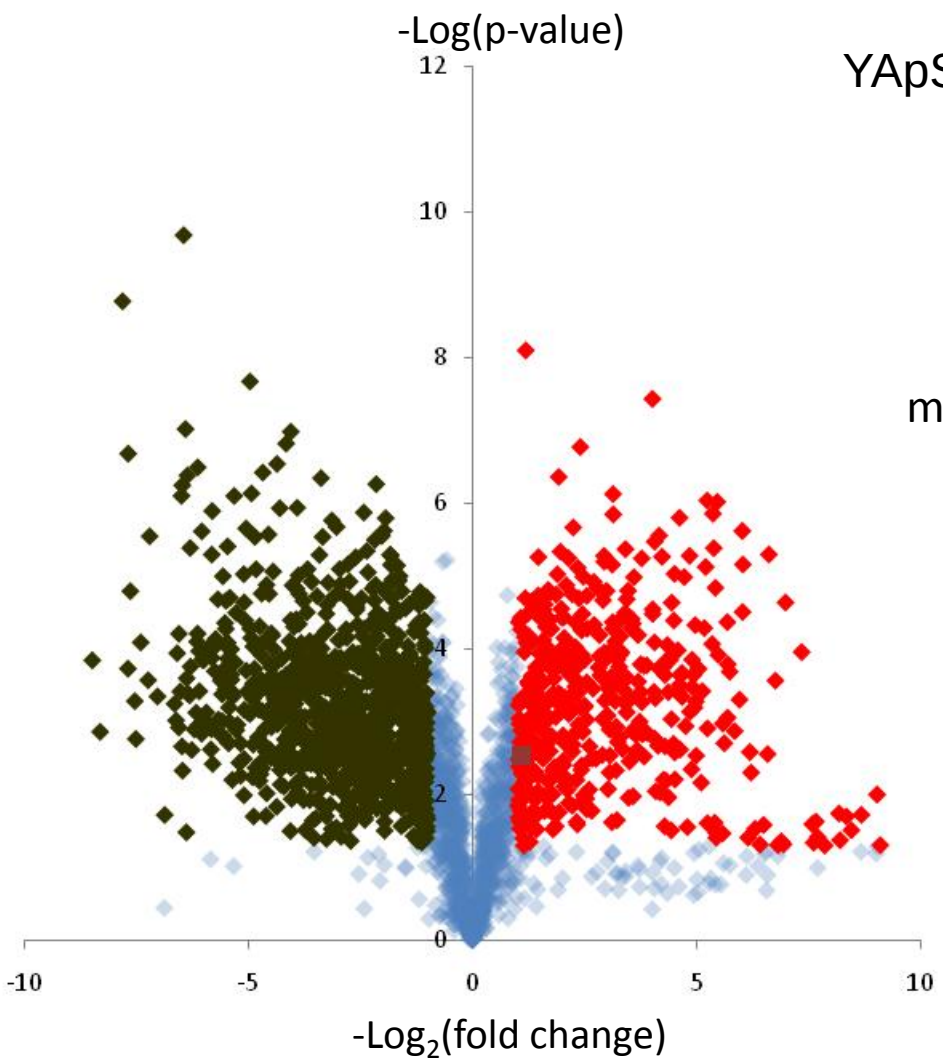
- **Tighter distribution of intensities along the diagonal axis for 50 c and 60 c**
- **Less outliers with 50 or 60 cycles than the conventional digestion method**

# Digestion extent of U937 cell lysate

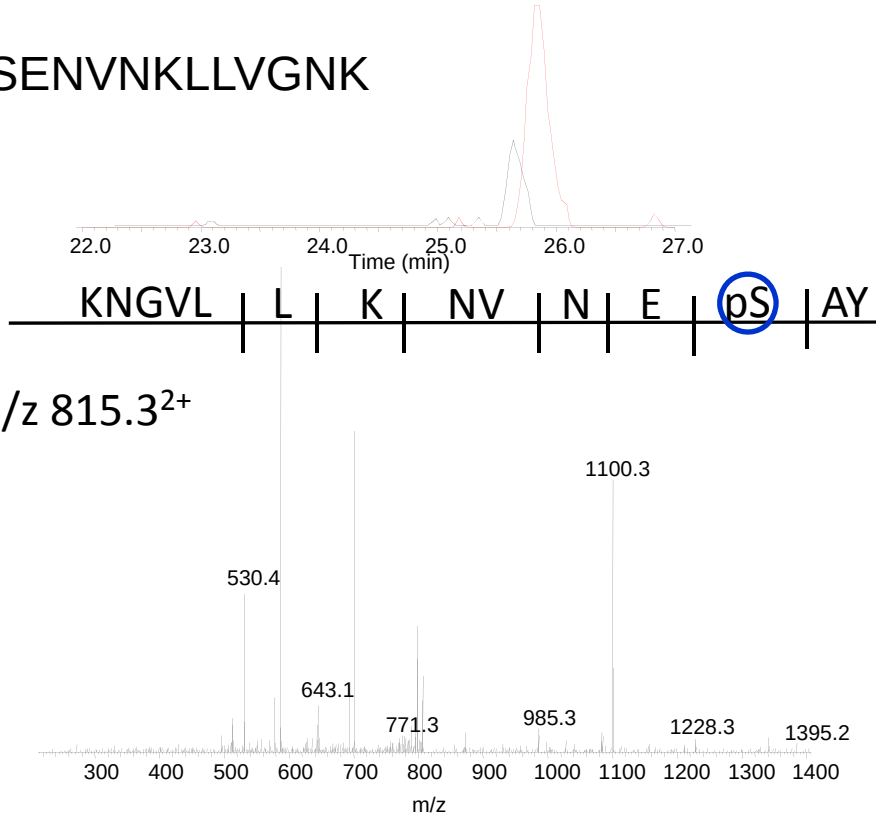



- **Higher intensity miscleaved peptides after digestion at HP for 1 hr than overnight at 1 atm**

# Digestion extent of U937 cell lysate




YApSENVNKLLVGNK

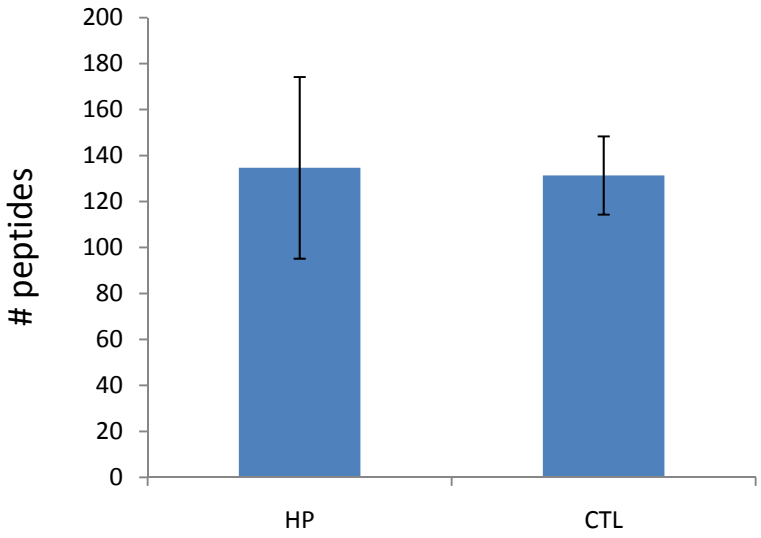


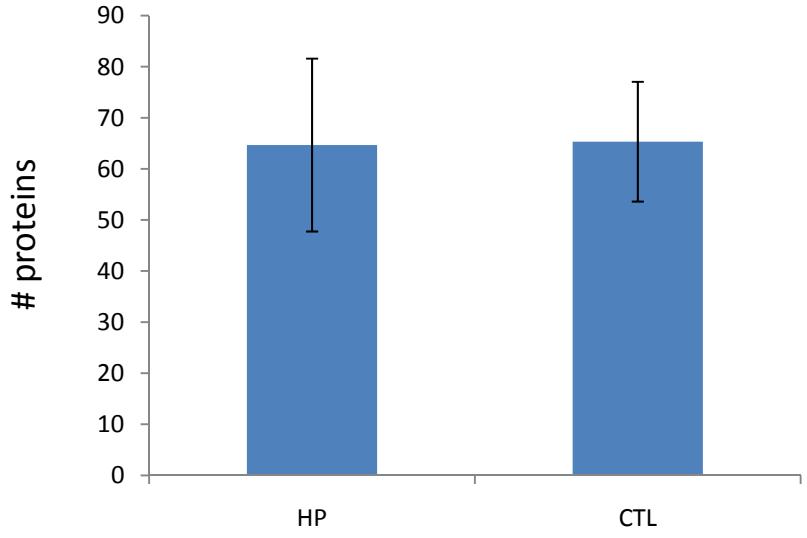

*Rab 1B anchored membrane protein*

**Incomplete digestion can be an edge for phosphopeptide detection**

# Mouse macrophage membrane samples

- Mouse macrophage membrane samples 500 ng reduced with TCEP 0.5 mM 37  C 30 min
- Digestion in solution at 1 atm or with PCT **120 cycles (25 s at 20kpsi 5s at 1 atm)**
- Conventional digestions were performed with mixing at 650 rpm
- 500 ng were injected on a nano-LC system coupled to an LTQ-Orbitrap.

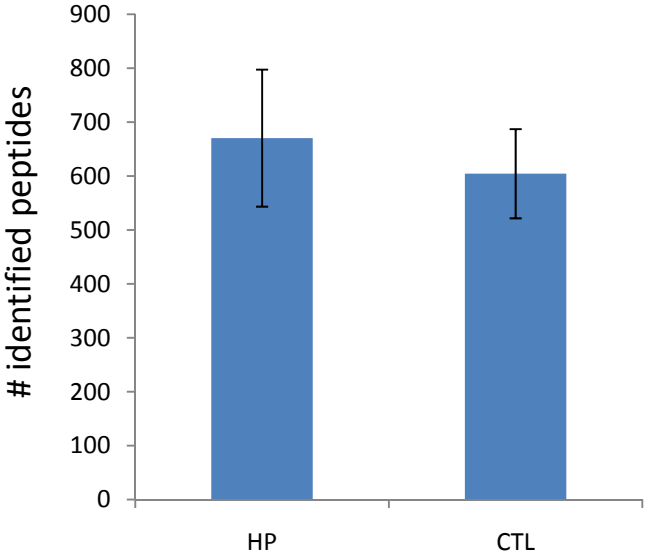



- **Similar performances between HP 1hr and 1 atm 18 hrs**
- **No material losses**

# Mouse macrophage membrane samples

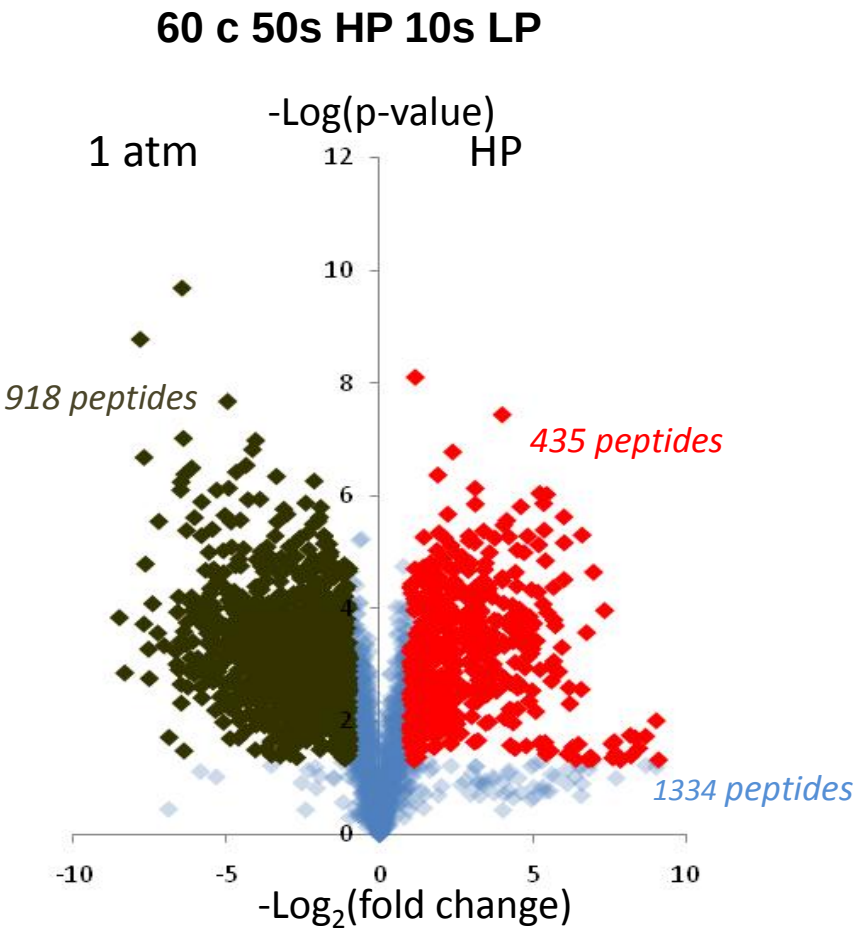
- Mouse macrophage membrane samples 3 ug reduced with TCEP 0.5 mM 37  C 30 min
- Digestion in solution at 1 atm or with PCT **120 cycles (25 s at 20kpsi 5s at 1 atm)**
- Conventional digestions were performed with mixing at 650 rpm
- 500 ng were injected on a nano-LC system coupled to an LTQ-Orbitrap.

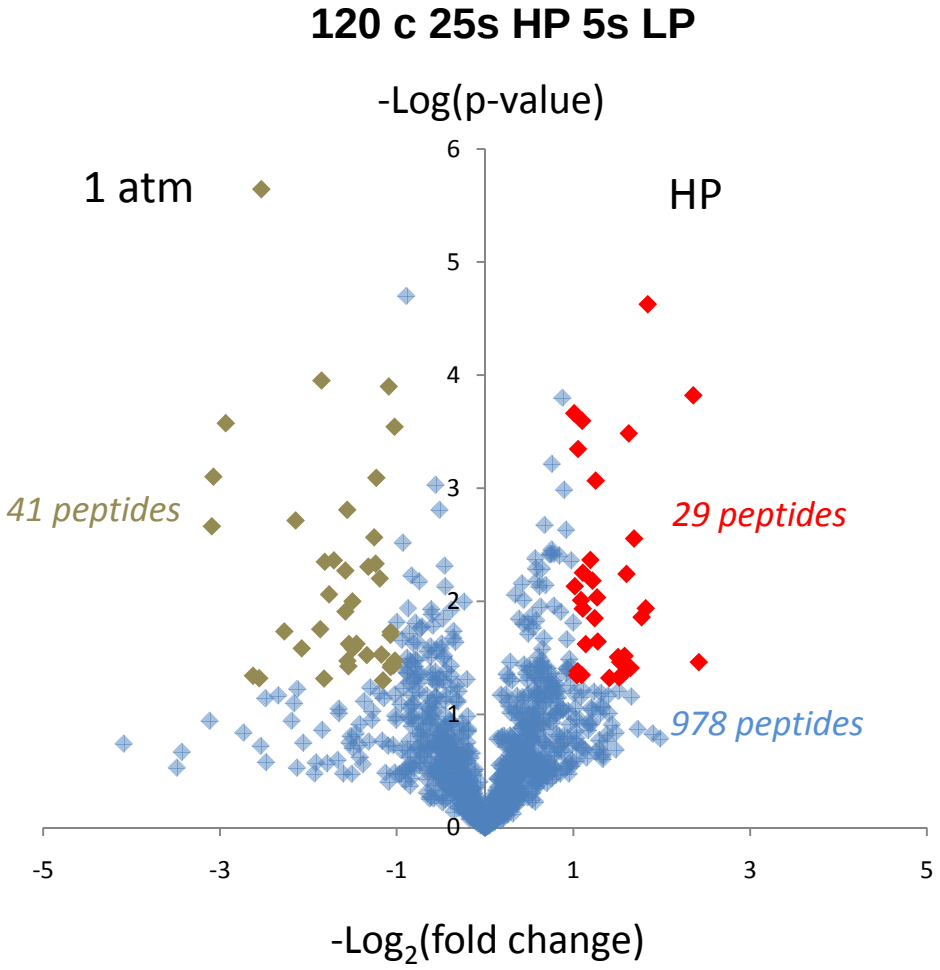



| | CTL | HP |
|---|---|---|
| # peptides | 1167 | 1182 |
| # proteins | 358 | 302 |
| # MPs | 135 | 132 |
| # IMPs | 23 | 23 |

- **Similar performances between HP 1hr and 1 atm 18 hr**

# Mouse macrophage membrane samples




- **Digestion extent similar with both methods**
- **Increasing the number of HP/LP cycles improves the digestion extent**

# Conclusion

- Higher sequence coverage for protein standard with digestion at HP for 1hr

- Similar performances for the digestion of BSA in gel in terms of sequence coverage and peptide intensity
- Reproducibility of peak intensity is better for the digestion at 1 atm overnight

- Numbers of identified peptides and proteins are comparable for cell lysate
- Reproducibility of peak intensity is better at HP for one hour
- Reproducibility of identification is better at HP for one hour

- Performances are again comparable for membrane sample digestion in terms of identified peptides , proteins and peptide intensity when using a higher number of cycles

- Future work: increase the number of cycles to improve digestion efficiency

# Acknowledgements