ThP 781

A Small-Scale, Flexible , yet Automated Protein Digestion and Peptide Desalting Process

Kojo Abdul-Hadi[1], Martin Technau[2], Qiaozhen (Cheryl) Lu[1], Bekim Bajrami[1], Nathan Swentko[3], Peter Juhasz[1], Ru Wei[1]

[1]Biogen Idec, Cambridge MA, [2]Intavis AG, Cologne, Germany, [3]Intavis, Inc. Chicago, IL

INTRODUCTION

- Rationale
  - Protein digestion and peptide desalting are labor intensive processes that are carried out manually in many proteomics laboratories today. Automating these procedures has presented a challenge because high-end liquid handlers which are capable of performing complex sample preparation procedures, are usually not suited for protocols and studies where low amounts of biological material are available or small numbers of samples need to be processed.
- Objective
  - To modify an Intavis DigestPro MSi, designed for automated in-gel protein digestion and MALDI sample preparation, and expand it's capabilities to perform in-solution digestions and sample desalting.

DigestPro MSi Modifications

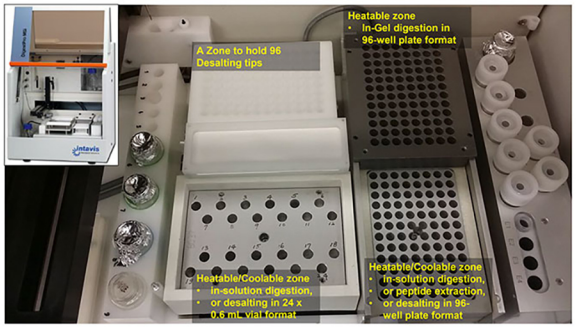
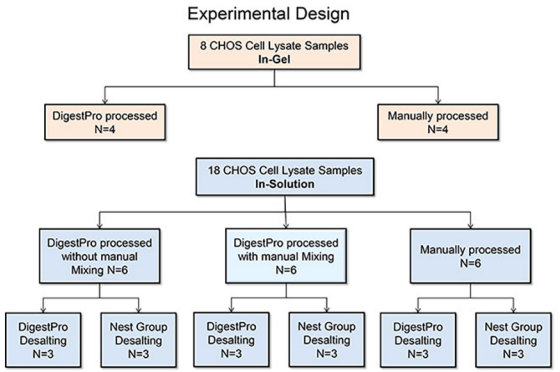


Fig. 1. Image of the DigestPro working station. Modified zones and function capabilities are labeled.

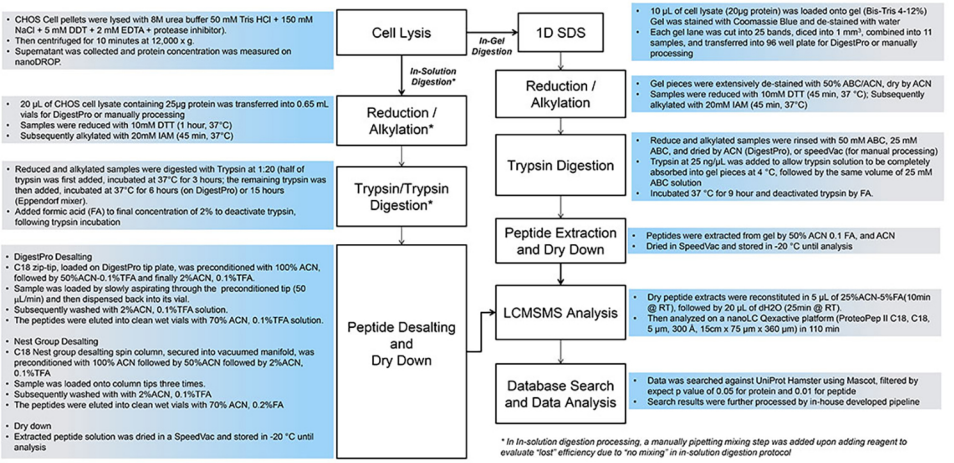
Experimental Design

- An in-gel digestion experiment was performed to compare
  - In-Gel digestion on the DigestPro to our manual in-gel digestion process
- An In-Solution experiment was performed to compare:
  - In-Sol digestion by the DigestPro to manual digestion by lab scientist
  - In-Sol digestion by the DigestPro without mixing upon reagent adding to manually mixing
  - De-salting by DigestPro to NestGroup desalting on a Vacuum device



Methods

Sample Processing Workflow



** In In-solution digestion processing, a manually pipetting mixing step was added upon adding reagent to evaluate "lost" efficiency due to "no mixing" in in-solution digestion protocol*

Results – In-Solution Digestion and Desalting

Summary

- Table 1 lists 18 samples processed through in-solution digestion by either DigestPro or manually by a lab scientist, followed by desalting either by DigestPro or maunally by a lab scientist using NestGroup C18 desalting spin column and vacuum device
- ~2000 proteins were identified from in-solution digested samples, shown in Fig 1.
  - Protein ID is consistent within the replicates (1-2%CV)
  - When adding manually pipette mixing step, DigestPro performs similarly to a lab scientist in terms of protein identification (DPmDP vs. KAvDP)
  - Without mixing, the protein ID from DigestPro processing is slightly lower ((DPnDP vs. DPmDP/KAvDP)
- Comparing protein ID by different desalting methods, Fig.2 shows
  - DigestPro performed consistently better then manual processing (DPnDP vs. DPnNG; DPmDP vs. DPmNG; KAvDP vs. KAvNG)
  - Manually desalting using spin column and vacuum device may generate larger variation
- Protein ID overlapping among replicates (%) is shown in Fig 3.
  - Each process performs similarly in terms of overlapping proteins among replicates
- Quantitative sensitivity comparison by spectral counting method is shown in Fig 4.
  - Similarly to protein ID, when adding mixing step, similar performance is observed on DigestPro to manual processing (Fig 4A, DPmDP vs. KAvDP)
  - Without mixing, a slightly lower sensitivity (DPnDP) is seen (Fig 4A and 4B)
  - Quantitative reproducibility, however, is very similar among 3 processes (Fig 4C)

Table 1 Cell Lysate Samples for In-Sol Sample Processing

Sample_No	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10	S11	S12	S13	S14	S15	S16	S17	S18
Proc.ID	DPnDP			DPnNG			DPmDP			DPmNG			KAvDP			KAvNG		
Replicate	r1	r2	r3	r1	r2	r3	r1	r2	r3	r1	r2	r3	r1	r2	r3	r1	r2	r3
Digestion	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP	DP	KA	KA	KA	KA	KA	KA
mixing	n	n	n	n	n	n	m	m	m	m	m	m	v	v	v	v	v	v
desalting	DP	DP	DP	NG	NG	NG	NG	NG	NG	DP	DP	DP	NG	NG	NG	DP	DP	DP

• DP = DigestPro; KA is one of our scientists
• n=no mixing; m=manually mix after adding reagent; v=vortex in manually digestion process
• NG=Nest Group spin column and desalting in vacuum assisted device

Fig. 2. Protein ID by processing methods.

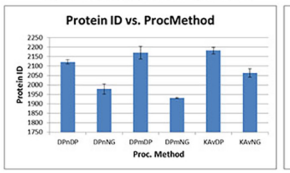


Fig. 3. Protein ID overlapping among replicates (%)

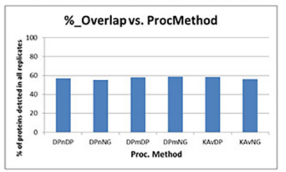
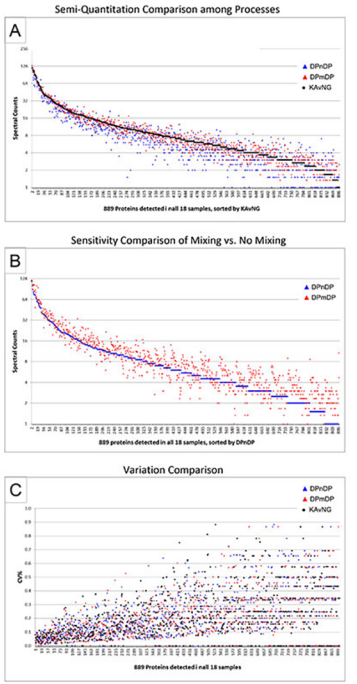


Fig. 4 Sensitivity Comparison



Results – In-Gel Digestion

Summary

- Table 2 lists 8 samples gone through in-gel digestion by either DigestPro or manually by a lab scientist
- ~6000 proteins were identified from each gel lane, shown in Fig 5.
  - While protein ID is very similar among processes(Fig. 5A), DigestPro digested samples show better protein coverage (more peptide and spectra, Fig. 5B and Fig 5C)
- Quantitative sensitivity comparison by spectral counting method is shown in Fig 6.
  - For high abundance proteins, manually processed samples acquired higher spectral counts, it becomes similar for lower abundance proteins (Fig. 6A, towards right and bottom curve)
  - DigestPro, however, seems to have a better quantitative reproducibility, comparing to manually processed samples (Fig. 6B)

Fig. 5 Protein Identification Comparison

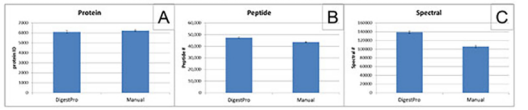
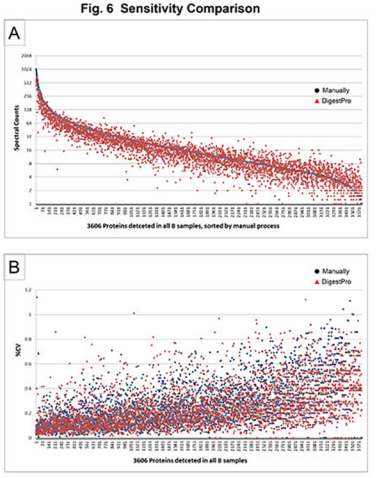


Fig. 6 Sensitivity Comparison



SUMMARY

- The customized instrument allows complete hands-free in-solution digestion and peptide desalting, following commonly used lab protocols. This is in addition to its standard in-gel digestion function. Up to 48 samples in a 96-well plate format or 24 samples in 0.6 mL vial format can be in-solution digested or desalted.
- Task change from in-gel digestion to in-solution digestion is simply done by selecting an in-solution digestion method (no hardware change) and preparing a different set of reagents according to the protocol. A simple needle change and a valve switch enables desalting function.
- Additional customized steps such as deglycosylation can be easily incorporated into in-solution digestion protocols, and multiple conditioning or eluting steps can be added to desalting protocols.
- Reagents like Lys-C and trypsin can be kept as stock solutions at optimum temperature and can be diluted at an appropriate time point to minimize reagent loss and activity.
- Evaluation of the platform included the comparison of automated procedures with manual sample preparation in terms of proteome coverage and reproducibility/sensitivity of protein abundance measured by spectral counts.
- Preliminary results suggest comparable performance between the manual and automated procedures with obvious advantage demonstrated by the automated platform in sample throughput and overall productivity.