

# Improved Protein Analysis with New Mass Spectrometry-Compatible Surfactant

Sergei Saveliev<sup>1</sup>; Daniel Simpson<sup>1</sup>; Becky Godat<sup>1</sup>; Ethan Strauss<sup>1</sup>; William Daily<sup>2</sup>; Carolyn Woodroffe<sup>2</sup>; Dieter Klaubert<sup>2</sup>; Grzegorz Sabat<sup>3</sup>; Robert Bulleit<sup>1</sup>; Keith Wood<sup>1</sup>

<sup>1</sup>Promega Corp., Madison, WI; <sup>2</sup>Promega Biosciences Inc., San Luis Obispo, CA; <sup>3</sup>Mass spec facility, Biotechnology Center, University of Wisconsin, Madison, WI



## 1. Abstract

Typical protocols for in-gel protein digestion require overnight incubation followed by additional 2 – 3 hours for peptide extraction. We present here a protocol and reagent to streamline this process and in many cases improve protein coverage.

The reagent, ProteaseMAX™ surfactant, enhances peptide cleavage by providing a denaturing environment prior to protease addition. It is also designed to degrade during the proteolytic reaction, generating species that do not interfere with mass spectrometry.

For in-gel applications, complete digestions can be performed in 1 hour and the surfactant provides concurrent peptide extraction. This obviates the requirement for separate extraction processing, greatly simplifying the overall work-flow. Recovery is particularly enhanced for longer and more hydrophobic peptides (2,500-4,000 Da), thereby improving coverage.

Enhanced solubilization by ProteaseMAX™ surfactant improves in-solution digestion of complex proteomic samples. Hydrophobic membrane proteins can be solubilized at room temperature in under 1 hr. Proteome coverage of a mouse membrane extract, analyzed by 2D LC-MS/MS, was increased 70% by incorporating the surfactant into the sample preparation protocol.

## 2. Rapid digestion of Myoglobin

Myoglobin, a relatively protease resistant protein, is fully digested in under 30 min with ProteaseMAX™ surfactant. Without denaturant, digestion of myoglobin required more than 18 hrs to reach completion.

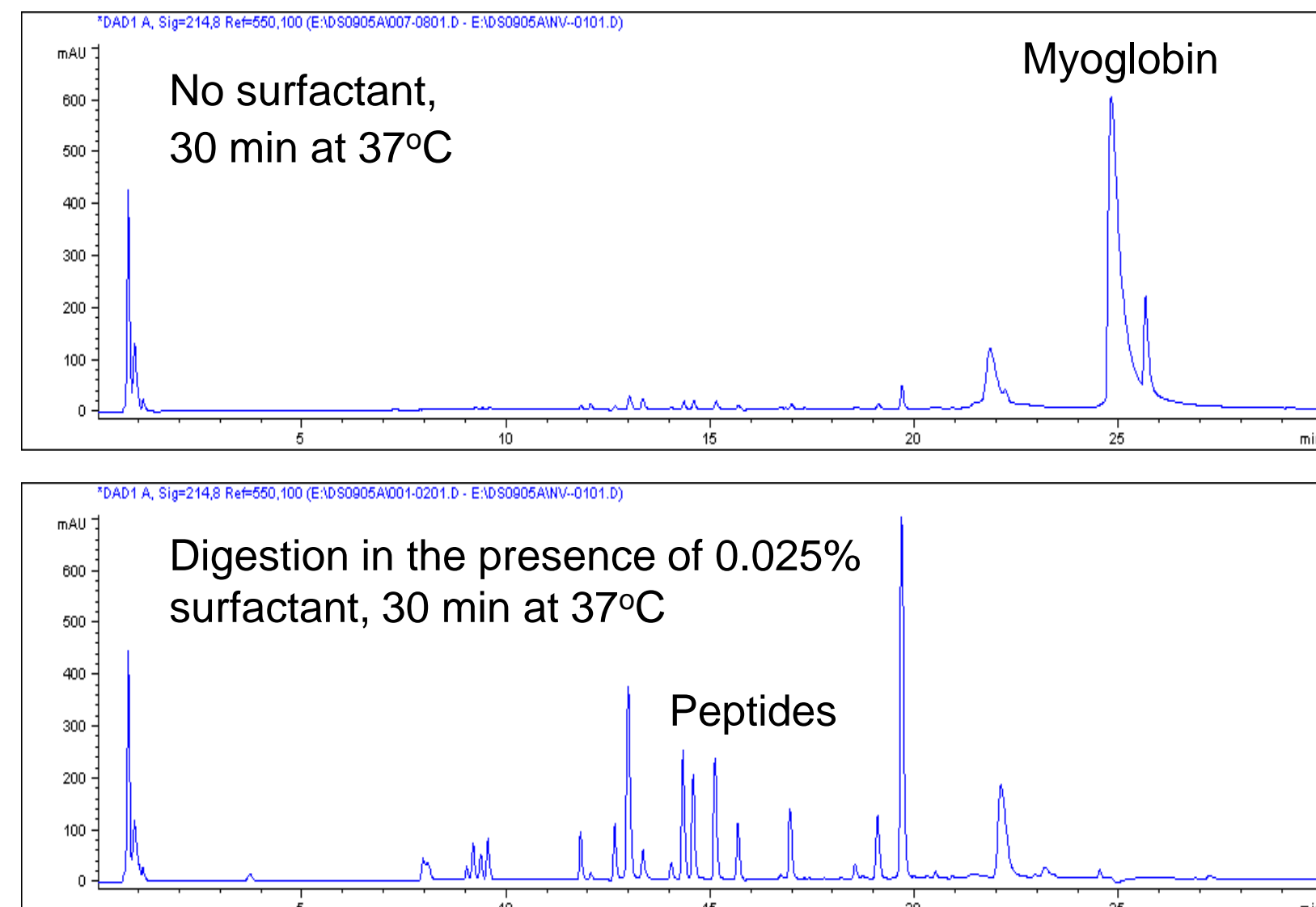
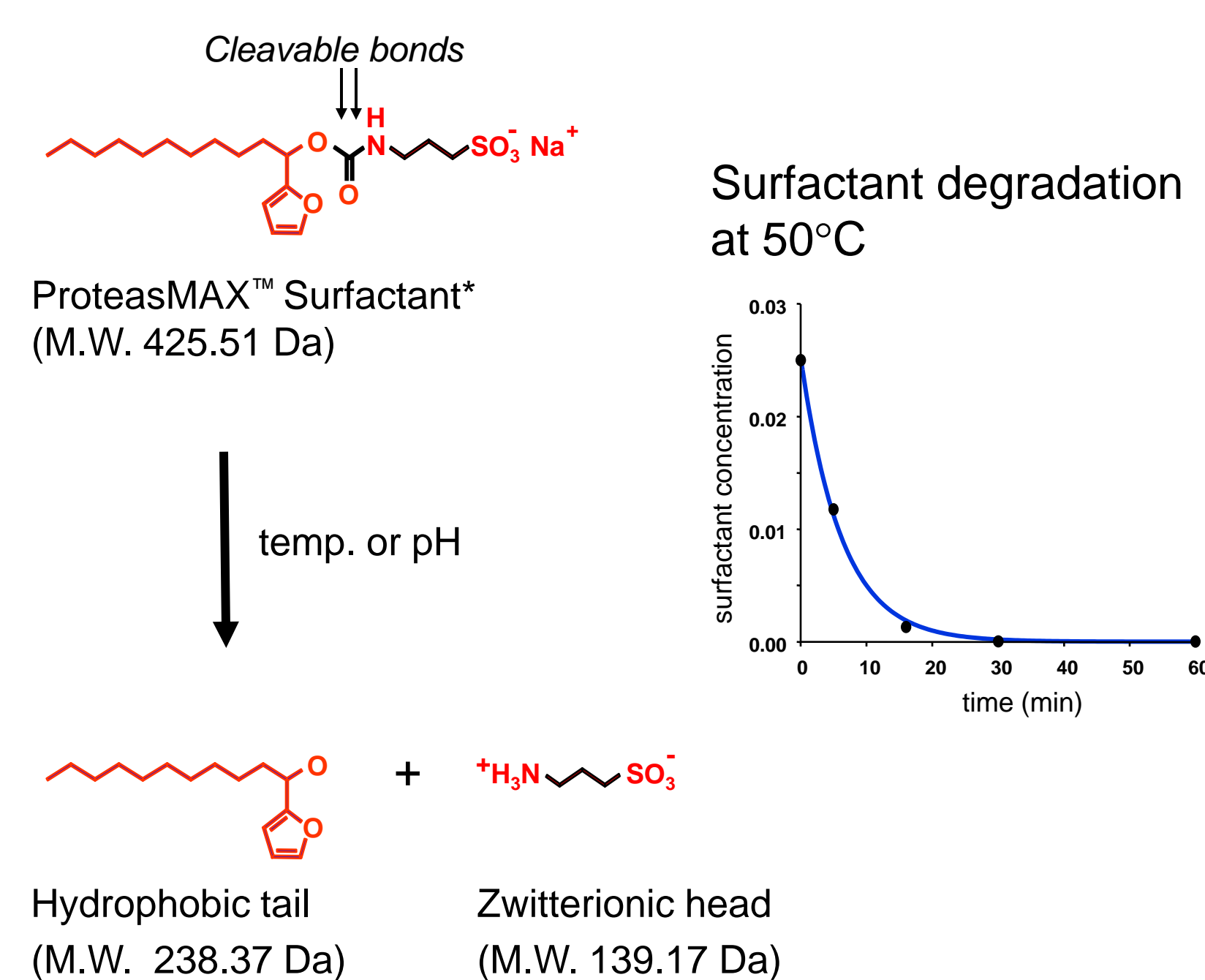


Figure: Myoglobin from horse heart was digested with trypsin (50:1 ratio, 37°C) with or without the surfactant and the tryptic peptides were resolved with RP HPLC (det. @ 214nm).

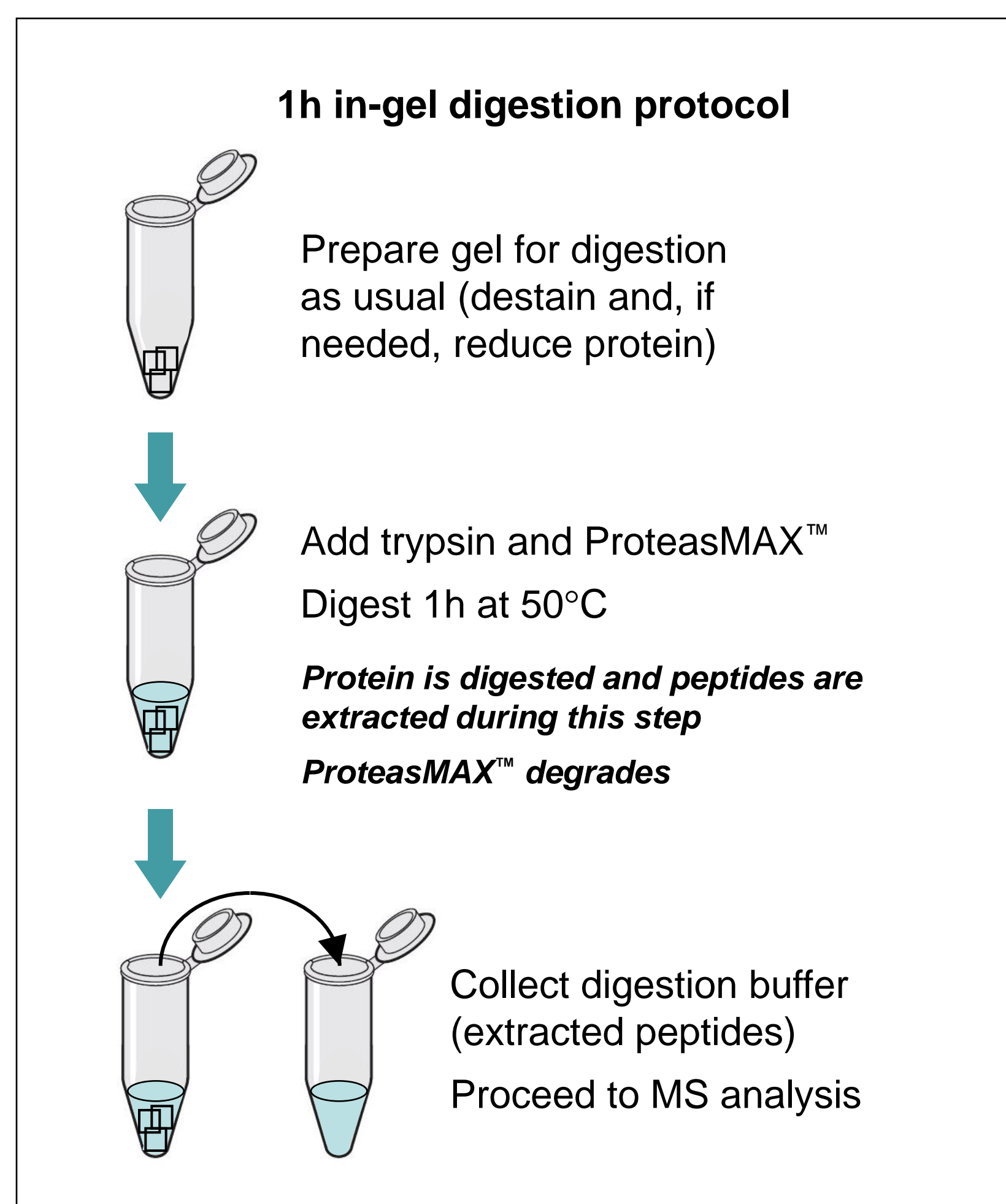
## 3. ProteaseMAX™ surfactant physical properties

\*ProteaseMAX™ is an SDS-like anionic surfactant that is stable as a 1% stock solution at -20°C. However, under digestion conditions (e.g., 1h at 50°C, 0.025% surfactant) it degrades to products that do not interfere with mass spectrometry



\*Patent pending

## 4. In-gel protein digestion and peptide extraction in a single one-hour step



### Mechanism of the protocol

ProteaseMAX™ surfactant assists the in-gel digestion process in two ways:

- Solubilizes and unfolds proteins, thus facilitating access to protease cleavage sites
- Extracts peptides from gel

Note: ProteaseMAX™ surfactant improves recovery of long peptides >2,500 Da to the levels suitable for MS/MS analysis

### In-gel digestion of a model protein

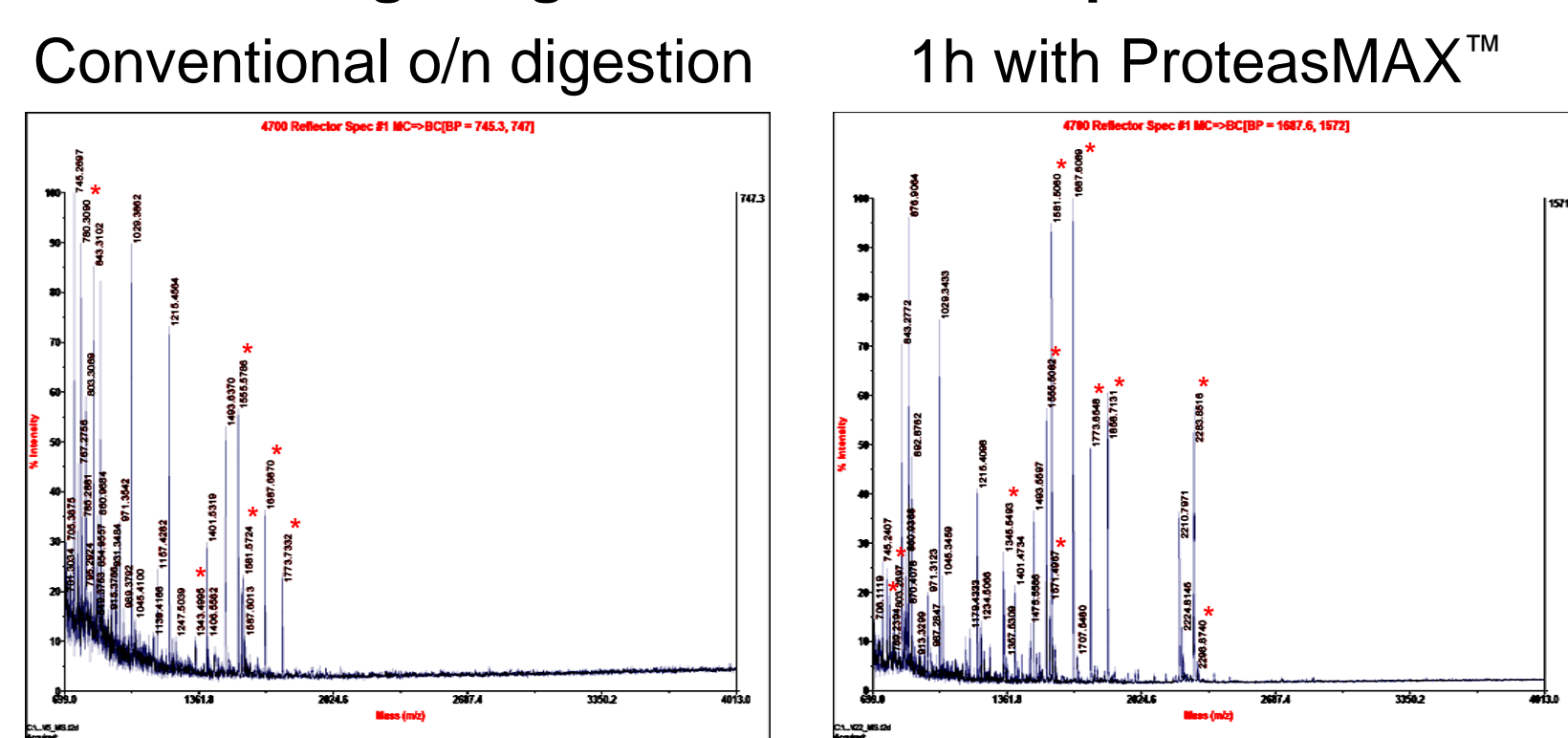


Figure: Ovalbumin (50 ng) was in-gel digested with trypsin overnight or 1h in the presence of ProteaseMAX™. A 1/40 aliquot from each digest was analyzed on an AB 4800 MALDI-TOF/TOF. Asterisks indicate identified peptides.

The data show efficient recovery of peptides after 1h digestion with ProteaseMAX™ surfactant. Ovalbumin protein coverage increased from 20% in a regular protocol to 36% in 1h digestion protocol with ProteaseMAX™.

## 5. Comparison of conventional overnight and one-hour protocols for in-gel digestion of various proteins

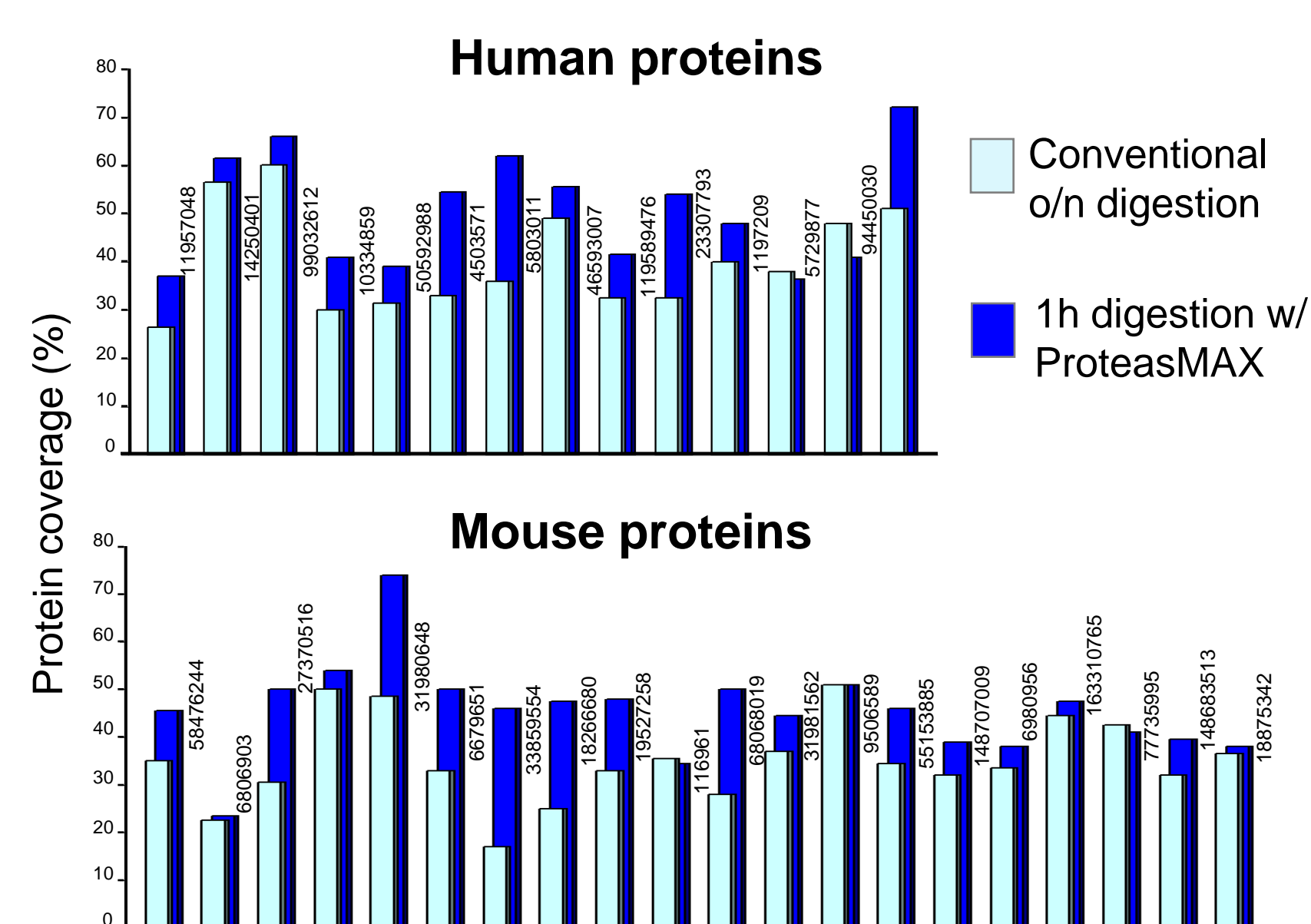


Figure: Human or mouse proteins from cytoplasmic and membrane extracts were resolved with SDS-PAGE, stained with Coomassie Blue, in-gel digested and analyzed with an AB 4800 MALDI-TOF/TOF. Proteins are identified by gi # at the right

Higher protein coverage was achieved with ProteaseMAX™ surfactant in most cases. This was due in part to improved recovery of longer peptides

## 6. LC-MS/MS analysis of a band from gel-fractionated protein extract

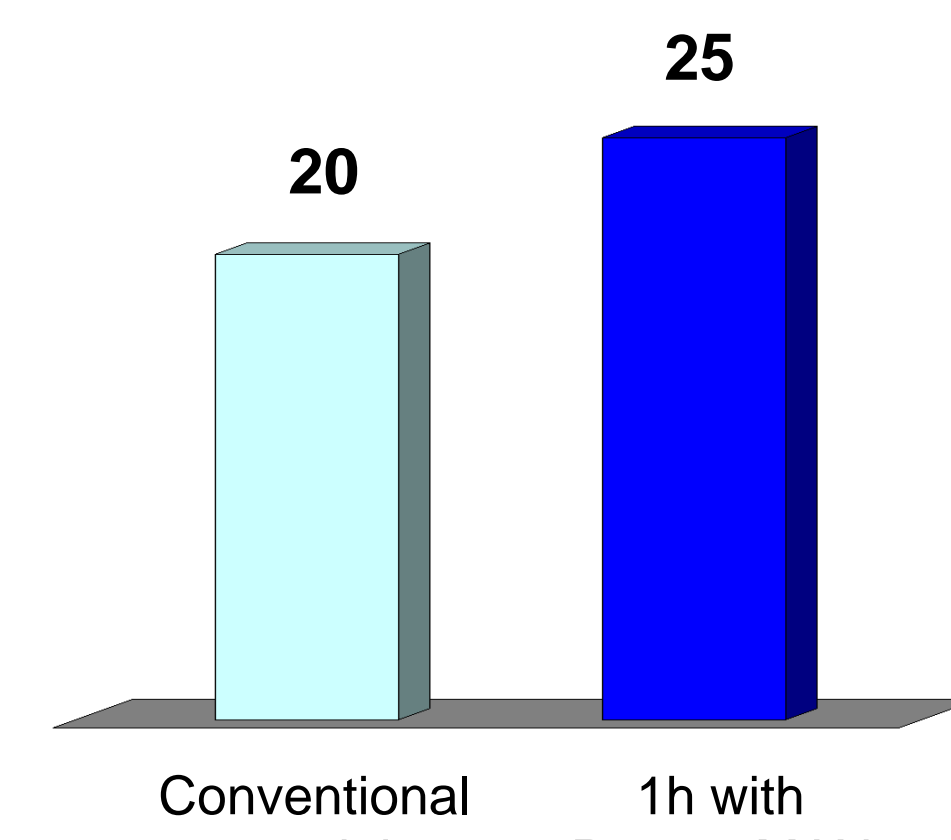


Figure: Membrane protein extract from mouse heart was resolved with 1D SDS-PAGE. Gel was stained with Coomassie Blue. The fraction with an apparent M.W. of 54-56 kD was excised and digested with a conventional overnight or 1h ProteaseMAX-assisted digestion protocol. Digests were analyzed with a Thermo Scientific LTQ/Eksigent Nano-LC. Generated MS/MS data was searched with Sequest against the Swiss-prot *Mus musculus* (House mouse) database.

The data show the number of proteins identified in 54-56 kD fraction of mouse protein extract for both protocols as further indication that a 1h surfactant-assisted protocol is at least as efficient as a conventional protocol for identification of proteins in gel. One third of the proteins recovered with 1h protocol had higher coverage than identical proteins recovered with a conventional protocol (data not shown)

## 6. 2D LC-MS/MS analysis of mouse membrane proteome

Proteins identified in membrane protein extract from mouse heart

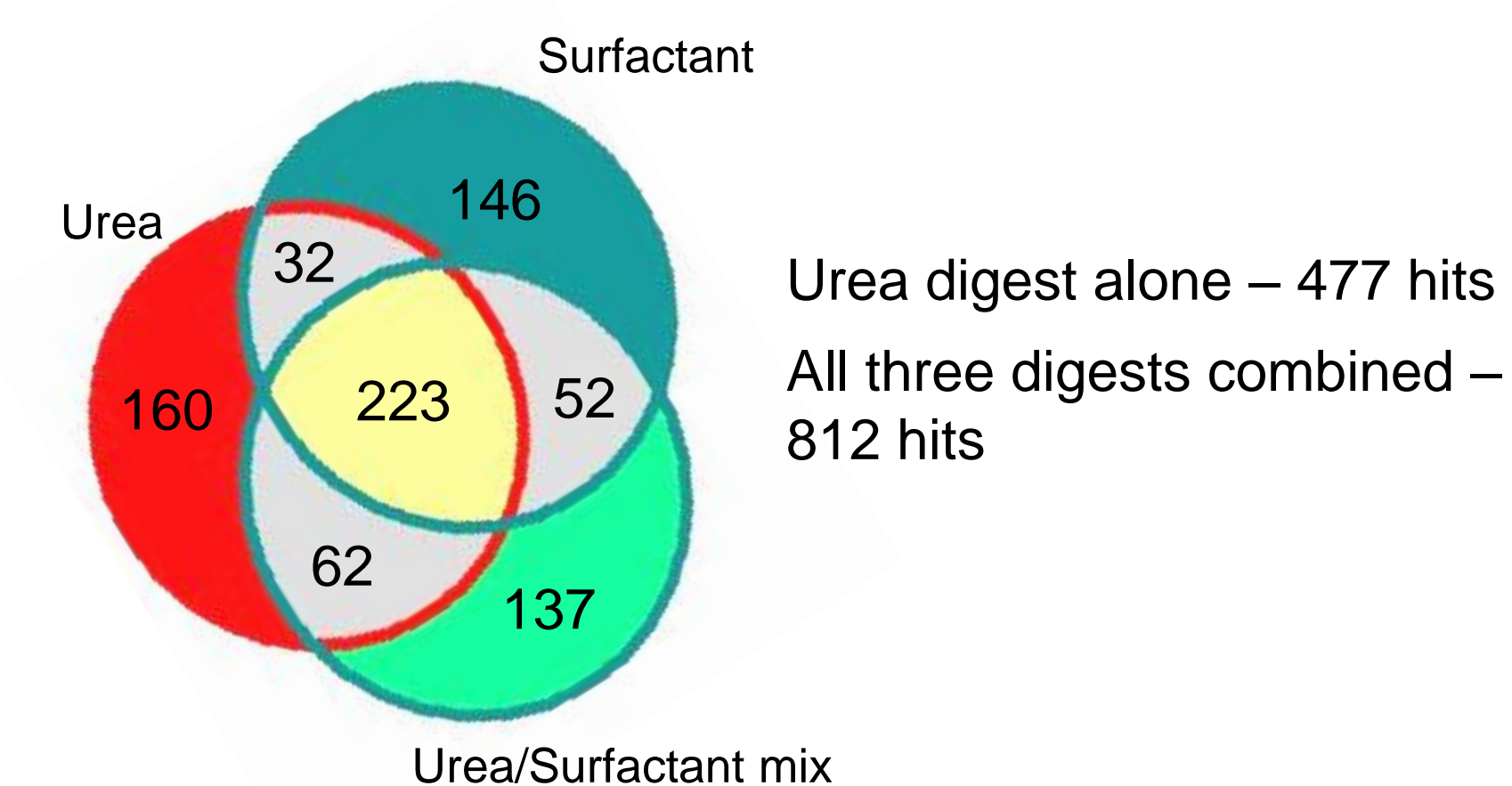


Figure: Membrane protein extract from mouse heart was solubilized under three different conditions: Urea, surfactant or Urea/surfactant mix. Each condition was digested with trypsin and analyzed with off-line 2D LC-MS/MS on an Agilent 1100 series LC/MSD Trap SL spectrometer. MS/MS data was searched against *Mus musculus* (House mouse) non-redundant NCBI database using Mascot.

The data indicate a 70% increase in proteome coverage when ProteaseMAX™ is used in combination with Urea over the use of Urea alone.

## 8. Summary

Improved sample preparation for mass spectrometry with the new ProteaseMAX™ surfactant

- In-gel protein digestion and peptide extraction in a single one-hour step
- Recovery of longer and more hydrophobic peptides from polyacrylamide gels (2,500-4,000 Da), providing improved protein coverage
- Improved coverage of membrane proteome owing to enhanced protein solubilization and synergy with Urea
- Degradation over the course of a protein digestion allowing sample analysis without further clean-up or extraction