Overview

- Develop a top-down based intact accurate mass and time (AMT) tag approach
- Quantitatively analyze the proteins secreted from human parotid (PS) and submandibular/sublingual gland (SMSL)
- Elucidate the localization of salivary proteins as well as their post translational modifications

Methods

**Adult saliva donors of various ethnic and racial backgrounds, ranging in age from 22 to 30 y, were recruited from the general population, and samples were collected at the UCLA Medical Center with full donor consent, using procedures in accord with the Medical Institution Review Board and the Office of Protection for Research Subjects, as previously described.**

**Constructing a comprehensive catalogue of human salivary gland-derived intact proteome using top-down MS**

**Figure 1.** Schematic representative of quantitative protein identification using the intact AMT tag approach

**Figure 2.** Overall comparison between SMSL and PS intact proteins. Highly abundant proteins in SMSL include histatin, cystatin, SM3B, SM3A, and their isoforms. Low abundant (or not detected) proteins in SMSL are mainly basic proline-rich proteins, such as PRB2 and PRB4. These results indicated secretion of salivary proteins in different glands follows a well-defined pathway.

**Salivary acidic proline-rich proteins (aPRPs)**

- Secreted by both PS and SMSL glands
- Phosphorylation level is higher in PS than in SMSL (kinase involved in PS is more active)
- Glycosylation and phosphorylation may be co-regulated
- Protease cleavage after PTM occurs
- Protease cleavage is more extensive in SMSL

**Figure 3.** Two major groups of aPRP protein isoforms were observed (illustrated in left panel): one group with mass around 15 kDa, and another group with mass around 11 kDa. Proteins with mass around 15 kDa were identified as intact aPRP proteins (PRP1 and its isoforms), while proteins with mass around 11 kDa were found as partially cleaved N-terminus isoforms of PRP1 proteins at residue 106 (also known as PRP3 proteins). The C-terminal fragment was also observed. In addition, our intact AMT tag approach provides relative quantitation information for different aPRP protein isoforms in different glands (in right panel, A,E).

**Results**

**Conclusions**

We demonstrated an intact protein accurate mass and time (AMT) tag approach for confidently identifying and quantifying salivary proteins in different glands.

- High sensitivity and high resolution 12T FTICR-MS allowed us to use a10 ppm filter to more confidently identify and quantify proteins.
- Our in-house developed intact AMT tag pipeline demonstrated high reproducibility.
- For the first time, we observed the new O-ser glycosylations on aPRP proteins at the intact protein level, and correlated this PTM with phosphorylation.
- Our results provide by far the most comprehensive view of intact aPRP proteins and their converase cleavage products.
- In summary, we provide an accurate method to monitor up to 70 intact salivary proteins for biomarker discovery in the context of human disease, using less than 2 µl of an individual’s saliva sample.

**Acknowledgements**

Funding for this work was supported by the NIH-National Center for Research Resources (RR01982), and the U.S. Department of Energy (DOE) Office of Biological and Environmental Research (BER). Work was performed in the Environmental Molecular Science Laboratory (EMSL), a DOE/BER national scientific user facility located on the campus of Pacific Northwest National Laboratory at Richland, Washington.

**CONTACT:** Si Wu, Ph.D., Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, P.O. Box 999, Richland, WA 99352

E-mail: si.wu@pnnl.gov