

## Characterizing Protein Isoforms and PTMs in Microbial Systems by Top-Down Measurements with Capillary HPLC Interfaced to FTICRMS\*

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Novel Aspect: Demonstration of Capillary HPLC-FTICRMS for the High Resolution Characterization of PTMs and Isoforms of Intact Microbial Proteins

### Introduction

Intact protein or “top-down” mass spectrometry provides information on the natural state of the protein, including details about post-translational modifications (PTM’s), truncations, mutations, signal peptides, and isoforms. This information is often difficult to obtain by the more common “bottom-up” MS methods. Because intact proteins vary considerably more than peptides in terms of molecular mass, hydrophobicity, stability, etc., the interfacing of HPLC on-line with MS provides a significant advantage for the top-down MS measurements. The dynamic range, sensitivity, and mass accuracy offered by high performance FTICRMS affords not only unambiguous protein identification in many cases but also detailed information about protein modifications. This approach has been developed and evaluated for characterizing native and modified proteins from the microbe *Rhodospseudomonas palustris*.

### Methods

Capillary HPLC-MS was accomplished with a C4 reverse-phase column interfaced directly to an FTICR instrument (IonSpec electrospray FTICR-MS, 9.4 T magnet). Samples consisting of 10-50 ug of total protein were injected onto the column and eluted at 4 ul/min into the electrospray ion source of the FTICRMS. Mass accuracies of less than 5 ppm and mass resolutions of 50,000-100,000 were achievable for the entire HPLC run. A mixture of 6 standard proteins was used to optimize HPLC-FTICRMS techniques, and evaluate detection limits, dynamic range, and molecular mass range of the experiment. LC-FTICR methods were then applied to *R. palustris* intact proteins to obtain high resolution and accurate mass measurement to determine protein truncations and post translational modifications.

### Preliminary results

The goal of this work was to examine proteins isolated from *R. palustris* by either biochemical or pull-down methodologies. The main objective was to examine intact protein modifications. Particular emphasis was given to characterization of protein truncation, acetylation, and methylation. This LC-FTMS approach provided detailed information about both protein PTM and isoforms. For example, the ribosomal L24 and S8 proteins are not only methylated, but in both cases are present in both the unmodified and modified isoforms; this information would be difficult to obtain from only bottom-up measurements. Various tandem affinity pulldown methods were used to isolate protein complexes for characterization. Initial work involved the GroEL-GroES complex. For *R. palustris*, there are two versions of GroEL. GroEL-2 was tagged with a histag, and used for the pulldown experiment. Top-down measurements revealed the presence of both the tagged GroEL-2 and the untagged GroEL-1 in the purified sample, indicating that the GroEL-GroES complex is likely a mixture of the different proteins. We have isolated the nitrogenase complex from *R. palustris* with a TAP-Tag approach. The function of this nitrogen fixing complex is determined by an ADP-ribosylation (+541 Da) on arginine 101 of the NifH gene when cells are grown under nitrogen fixing conditions. This large PTM is a good candidate for top-down MS measurements to look at a very specific PTM that controls protein function. The bottom-up proteomic analysis failed to identify this modification, most likely due to the missed tryptic cleavage of the modified arginine. This missed cleavage creates a large peptide of 4210 Da (as compared to 1460 Da if arginine is unmodified) since the next tryptic cleavage site is another 24 amino acids away. Although work remains to extend the top-down MS methodology and associated bioinformatics, we have already found this technique to be invaluable for proteome characterization.

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