

# Novel methodology for the Characterization of Ribosome Heterogeneity by Native Mass Spectrometry and Top-Down Proteomics

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## Abstract:

Ribosomes are heterogeneous molecular machines essential for protein synthesis and have been studied for decades. However, there is a significant gap in understanding how structural and compositional heterogeneity modulates ribosomal function under varying biological conditions. Native mass spectrometry in combination with top-down is uniquely capable of addressing this question with high specificity. Here, we compare ribosomal protein proteoform IDs and relative abundances determined by complex-up with top-down analysis data of *E. coli* ribosomal proteins. Complex-up falling under the wings of native mass spectrometry, was performed using a Q Exactive UHMR Orbitrap (Thermo Scientific) modified with a 30W CO<sub>2</sub> laser to enable Infra-red multiphoton dissociation (IRMPD). The 10.6 μm IR laser photons enable the characterization of ribosomal proteins via selective dissociation of rRNA in the ribosomes. This method provides invaluable intact mass information related to proteoforms, yet top-down gives a more in-depth analysis as to the pinpoint locations of post translational modifications (PTMs), isobars and truncations, which are otherwise harder to identify through intact mass data alone. The preliminary results obtained show us harmony between the two approaches, hinting at the agreement between the synergetic of proteomics with native mass spectrometry. So far, all our studies have been performed using commercially available Ribosomes, thereby, the next step will be to grow *E. coli* in the lab, extract ribosomes to reproduce the above key findings, which can reassure the capacity of complex-up approach.

## Key words:

Heterogeneity, Complex-up, Top-down, Native mass spectrometry, IRMPD, PTMs