



# Prefractionation of Intact Proteins for Mass Spectrometry

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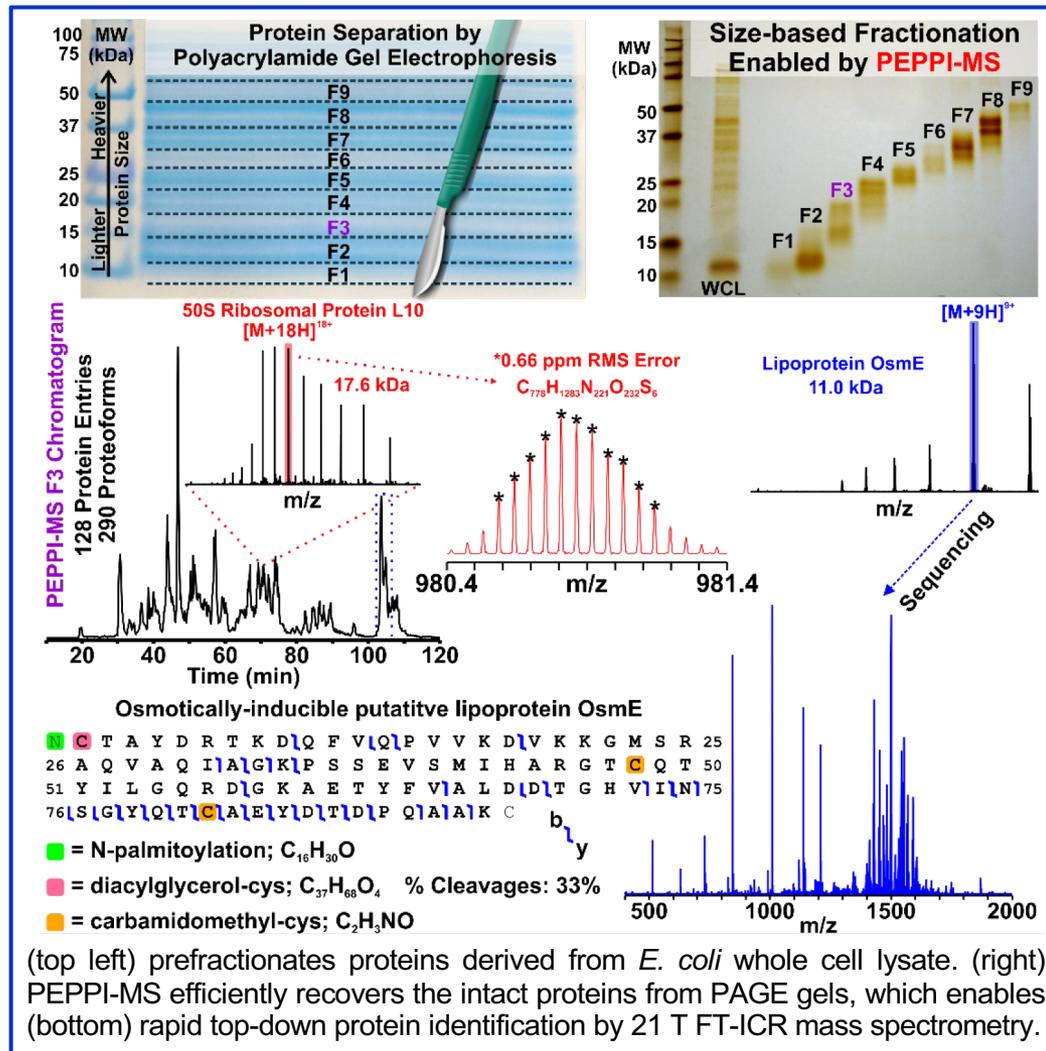
Intact protein mass analysis, or “top-down” proteomics (TDP), provides molecular understanding of phenotype that is otherwise unobtainable. Polyacrylamide gel electrophoresis (PAGE) is a widespread analytical tool that provides approximate molecular weight-based prefractionation of intact proteins. Until now there has been no means to efficiently recover intact proteins from PAGE gels, which has *long* been a barrier for coupling PAGE with high resolution, accurate mass TDP.

A new technique, **PEPPI-MS** (Passively Eluting Proteins from Polyacrylamide gels as Intact species for Mass Spectrometry), prefractionates protein mixtures into discrete molecular weight (MW) ranges with widely available PAGE materials and equipment. These TDP experiments identified more than 1000 proteoforms from PEPPI-MS samples; performance equivalent to other far more complex and expensive prefractionation strategies.

PEPPI-MS will empower many laboratories to prepare samples for intact protein mass analysis. PEPPI-MS does not require expensive or specialized equipment or consumables, and provides several advantages over other methods. PAGE parameters such as gel percentage, buffer system, and the MW range of the fractions can be easily tailored to the proteins of interest in the sample to optimize performance.

**Instrumentation used:** 21T FT-ICR Mass Spectrometer

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(top left) prefractionates proteins derived from *E. coli* whole cell lysate. (right) PEPPI-MS efficiently recovers the intact proteins from PAGE gels, which enables (bottom) rapid top-down protein identification by 21 T FT-ICR mass spectrometry.