Contribution ID: **47** Type: **Oral Presentation**

A Top-down Hybrid MS Approach Captures Extent and Dynamics of Simultaneous Phosphorylation Events in AMP- activated kinase Complex

Introduction:
AMP-activated kinase (AMPK) complex is the regulatory hub of cell energy metabolism, linked to heart disease and longevity, making it an attractive drug target. AMPK activity involves sophisticated subunit isoforms, conformational changes, and post-translational modifications. Phosphorylation controls AMPK through upstream kinases, generating diverse proteoforms whose extent and dynamics remain unexplored. We studied AMPK phosphorylation using a novel hybrid top-down and bottom-up MS approach to reveal simultaneous phosphorylation events and capture biochemical reaction dynamics.
Methods:
Full-length heterotrimeric AMPK complexes (α1β1γ1/α2β2γ2) were expressed in E.coli and modified in vitro with CaMKK, GSK3-beta, and PP2A in presence of nucleotides, inhibitors, and activator Pfizer-739. Total subunit phosphorylation was determined using intact mass RPLC-MS on C4 columns with Bruker Impact II Q-ToF. Site-specific phosphorylation kinetics used DDA/PRM on timsToFpro. Top-down MS employed TriVersa Nanomate coupled with solariX XR6 12-Tesla FTICR-MS. Data analysis used FragPipe v.22.0, Skyline, MASH Native v.1.1, and Bruker OmniScape 2025.
Results:
Biochemical assays with CaMKK, PP2A, and Pfizer-739 revealed extensive AMPK phosphorylation dynamics. Top-down LC-MS showed unique plateauing growth curves for three AMPK subunits, revealing distinct proteoform pool dynamics. Bottom-up peptide quantification followed site-specific kinetics using a phosphorylated/unphosphorylated peptide database, quantifying >10 phosphopeptide pairs at MS1 level. Mapping these timsToF kinetic curves onto structural models provides novel insights into AMPK's activation cycle. Top-down MS characterized simultaneous phosphorylation sites missed by bottom-up approaches. Current work focuses on GSK3-β phosphorylation in cardiac isoform AMPK α2 C-terminus and high-resolution top-down MS on ScimaX 7T MRMS. Our complementary results facilitate dysregulation studies and pharmacological interventions.
Novel Aspect:
Hybrid MS approach reveals simultaneous AMPK phosphorylation events and captures biochemical reaction dynamics with unprecedented precision.

User consent

yes

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Session Classification: Biomedical Applications