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Deciphering the Phosphorylation Barcode of G Protein-Coupled Receptors (GPCRs) using CZE-TDMS

Kevin Jooß[1,2], Ashley N. Ives[3], Rafael D. Melani[4,5], Ryan T. Fellers[5], John Janetzko[6,7], Neil L. Kelleher[3,4,5]

[1]Division of Bioanalytical Chemistry, Amsterdam Institute of Molecular Life Sciences, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands, k.jooss@vu.nl

[2]Centre for Analytical Sciences Amsterdam (CASA), The Netherlands

[3]Northwestern University, Evanston, Illinois, United States

[4]Department of Molecular Biosciences, Northwestern University, Evanston, Illinois, United States

[5]National Resource for Translational and Developmental Proteomics, Northwestern University, Evanston, Illinois, United States

[6]Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, California, United States

Summary

G protein-coupled receptors (GPCRs) are a critical class of biomolecule that are regulated in time and space by post-translational modifications. An area of long-standing interest is mapping modification states (proteoforms) of GPCRs to specific stimuli or disease states, however, characterizing proteoforms of GPCRs is inherently challenging due to their hydrophobicity and low copy number. Even with recent advancements in membrane mimetics and mass spectrometry instrumentation, current workflows for GPCR characterization require large amounts of relatively clean and homogenous protein, which limits their application to endogenous samples. Here, we describe a capillary electrophoresis top-down mass spectrometry (CE-TDMS) workflow to characterize proteoforms of both intact and partially cleaved GPCRs. By this approach, we are able to resolve multiple proteoforms of both beta-2-adrenergic receptor and metabotropic glutamate receptor 2 without the need for prior desalting while using very small volumes of samples (<40 nL injections). In addition, for smaller phosphorylated protein analytes (4.2 kDa), CE resolves phosphorylated isomers, which significantly improves the fragmentation coverage that can be accomplished in comparison to conventional reverse phase-liquid chromatography (RP-LC). Localization of these phosphorylation hot spots was also achieved. Overall, this study demonstrates that CE-TDMS is successful for mass limited samples in complex matrices, making it an ideal candidate to pursue in-depth GPCR proteoform characterization.