OFFGel Prefractionation Reveals Remarkable Protein Post-Translational Modifications

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Introduction and objectives
Performing a comprehensive nonbiased proteome analysis is an extraordinary challenge due to sample complexity and wide dynamic range, especially in eukaryotic tissues. Thus, prefractionation steps conducted prior to mass spectrometric analysis are critically important to reduce complex biological matrices and allow in-depth analysis.

Methods
Here we demonstrated the use of OFFGel prefractionation to identify low abundant and hydrophobic proteins than in a nonfractionated sample. We examined the capability of OFFGEL prefractionation for detecting PTMs when coupled with targeted enrichment strategy such as TiO2 phospho-enrichment.

Results and Discussion
OFFGel prefractionation of a kidney protein sample was able to unveil protein functional relevance by detecting PTMs, especially when prefractionation was augmented with a targeted enrichment strategy such as TiO2 phospho-enrichment. The OFFGel- TiO2 combination used in this study was comparable to other global phosphoproteomics approaches (SCX-TiO2 , ERLIC-TiO2 , or HILIC-TiO2 ). In addition, OFFGel prefractionation showed improvement in detecting low abundance proteins for deep proteome analysis.

Conclusion
The detailed mouse kidney proteome with the phosphopeptide enrichment presented here serves as a useful platform for a better understanding of how the renal protein modification machinery works and, ultimately, will contribute to our understanding of pathological processes as well as normal physiological renal functions.

Keywords: OFFGel prefractionation, PTM, Proteomics