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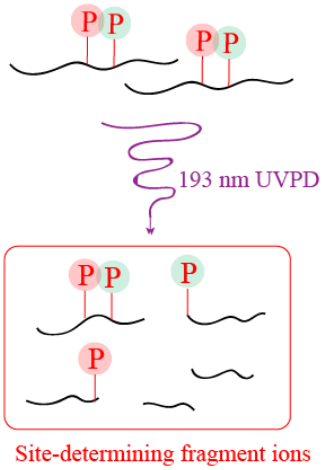
Communication

Elucidating the various multi-phosphorylation statuses of protein functional regions by 193-nm ultraviolet photodissociation

Zheyi Liu [a, #], You Jiang [b, #], Chunlei Xiao [c, *], Xingchuang Xiong [b], Tao Wang [c], Jinan Li [a], Fangjun Wang [a,*], Xiang Fang [b, *], Xueming Yang [c]

[a] *CAS Key Laboratory of Separation Sciences for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China*
[b] *National Institute of Metrology, Beijing 100013, China*
c State Key Laboratory of Molecular Reaction Dynamics, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China

Graphical Abstract



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ABSTRACT

Ultraviolet photodissociation is a high-energy fast excitation method in mass spectrometry and has been successfully applied for the elucidation of sequences and structures of biomolecules. However, its ability to distinguish the phosphorylation sites isomers of multi-phosphopeptides has been not systematically investigated until now. A 193-nm ultraviolet laser dissociation mass spectrometry system was established in this study and applied to elucidate the complex multi-phosphorylation statuses mimicking the functional regions of Sic1, Gli3 and Tau. The numbers of matched fragment ions and phosphorylation site-determining ions were improved on average 123% and 104%, respectively, by utilizing the ultraviolet photodissociation strategy, comparing to the typically utilized collision induced dissociation strategy. Finally, 94% phosphorylation sites within various statuses were unambiguously elucidated.

Phosphorylation is a crucial protein post-translational modification (PTM) and plays key regulatory roles in many vital biological processes, such as signal transduction, mitosis and cell differentiation. Usually, these biological processes are spatiotemporally regulated by sequential and multiple phosphorylation statuses with different phosphorylation sites [1-4]. The involvement of multi-phosphorylation sites could offer much more versatile and precise regulation of the protein functions and has gained more and more

* Corresponding authors.
E-mail addresses: chunleixiao@dicp.ac.cn (C. Xiao), wangfj@dicp.ac.cn (F. Wang), fangxiang@nim.ac.cn (X. Fang)
# These two authors contributed equally to this work.