GS02-2 **Improvement of labeled protein analysis with photocross-linker bearing acylsulfonamide linkage**

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Target identification of bioactive molecule has been a very important subject in the fields of life sciences and drug discovery. Recently, we have developed a multifunctional photo-crosslinker having a cinnamate moiety, which largely improved the efficiency on photoaffinity labeling (PAL)-based identification of the labeled site. In this study, we developed one-step synthesis of a PAL probe by sulfo-click reaction, one of bioorthogonal reactions, and utilized the scissile function of acylsulfonamide linkage to improve the efficiency of analysis.

Protein kinase PKCα and its inhibitory peptide were used for the PAL studies. Two PAL probes were prepared by introducing the photocross-linker into the peptide, which was previously incorporated of an unnatural amino acid derivative bearing a sulfonyl azide group, by sulfo-click reaction without protection. The PAL probes labeled PKCα in time-dependent manner under irradiation. *N*-Acylsulfonamide group formed by sulfo-click is extremely stable, and can be easily hydrolyzed upon *N*-alkylation in the same condition as reductive alkylation of cysteine, which is a chemical treatment of protein prior to MS analysis. This cleavage property is a useful function for purification of labeled protein. Photoproducts were analyzed by LC-MS/MS after digestion, which indicated the binding direction of the inhibitory peptide in proximity to the ATP-binding domain of PKCα. Furthermore, the cinnamic acid tag generated by the cleavage is converted to the corresponding coumarin tag by UV irradiation, which can be used as a MS-based varidation of labeled peptide.