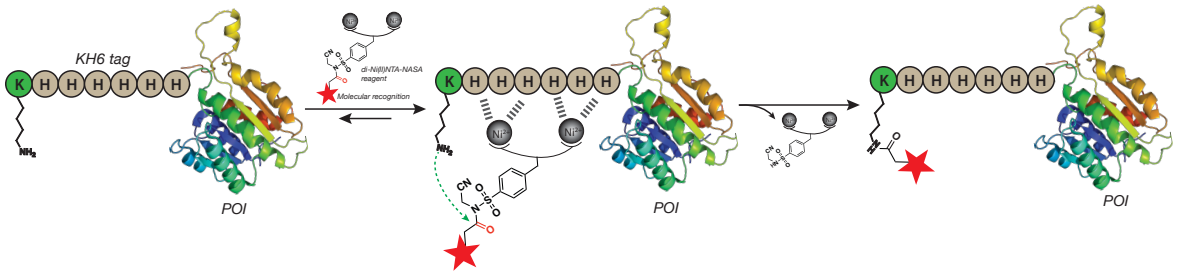
A new reactive peptide tag-probe pair for the site-specific incorporation of designer chemical probes into proteins

([1]*Graduate School of Engineering, Kyoto University*, [2]*JST, ERATO*, [3]*University of Oxford, UK*, [4]*MRC LMB, Cambridge, UK*, [5]*Keio University School of Medicine, Tokyo*) ○Vikram Thimaradka,[1] Jae Hoon Oh,[2] Christina Heroven,[3,4] Radu Aricescu,[3,4] Michisuke Yuzaki,[5] Tomonori Tamura,[1] Itaru Hamachi[1,2]

Keywords: Bioconjugation; Protein modification; Peptide tag.

Installation of desired chemical functionality on protein of interest (POI) provides numerous downstream applications in biology and medicine. Albeit several powerful enzymatic methods, such as SNAP-tag and Halo tag, has been established, the large protein tag domain could perturb natural function and/or behavior of POI. In this context, short peptide tag-probe pairs are promising alternatives for site-specific covalent modification of proteins with minimum perturbation to the inherent protein functions. Our lab had previously developed reactive peptide tag-probe pair system involving a di-Ni(II)NTA–His_6 tag interaction for recruiting a cysteine-reactive alpha chloroacetamide towards tag fused POI.[1] However, oxidation of Cys residues to form disulfide linkages often hinders the labeling in true biological contexts.

Herein, we report a new reactive peptide tag-probe pair employing Lys containing His_6 tag (KH_6/H_6K) with di-Ni(II)NTA tethered *N*-alkyl-*N*-acyl sulfonamide (NASA) reagent, a cleavable electrophile for Lys side chain modification.[2,3] The proximity induced by di-Ni(II)NTA–His_6 interaction facilitates a reaction between a Lys residue of the tag and the NASA group to covalently and site-specifically incorporate a desired reporter into the tag with concomitant removal of di-Ni(II)NTA part. We characterized the labeling kinetics and site specificity using model peptides and several proteins containing the tag sequence *in vitro*. Further, we succeeded in the labeling of the tag-fused EGFR and Neuroligin-1 expressed in live cells.



1) S. Uchinomiya *et al. Chem. Commun.,* 49, 5022 (2013) 2) V. Thimaradka *et al. Bioorg. Med. Chem.,* 30, 115947 (2020) 3) T. Tamura *et al. Nat. Commun.,* 9, 1870 (2018).