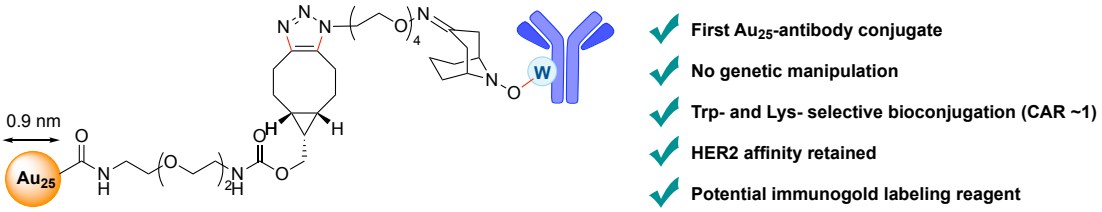
Bioconjugation of Au_{25} nanocluster to trastuzumab

([1]*Graduate School of Pharmaceutical Sciences, The University of Tokyo*, [2]*Graduate School of Science, The University of Tokyo,* [3]*Interdisciplinary Research Center for Catalytic Chemistry (IRC3) National Institute of Advanced Industrial Science and Technology (AIST),* [4]*Graduate School of Medicine, The University of Tokyo*) ○Katarzyna Joanna Malawska,[1] Shinjiro Takano,[2] Kounosuke Oisaki,[3] Haruaki Yanagisawa,[4] Masahide Kikkawa,[4] Tatsuya Tsukuda[2], Motomu Kanai[1]

Keywords: Bioconjugation; Tryptophan; Gold nanocluster; Immunogold labelling; Antibody conjugate

Cryogenic Electron Microscopy (Cryo-EM) is a dynamic field of structural biology, but despite many advances, the images obtained by cryo microscopes remain extremely noisy. One way to tackle this problem is to apply labelling with heavy metal particles such as colloidal gold. Recent years saw a surge in reports on special types of particles called gold nanoclusters. Compared to colloidal gold, their synthesis requires special skills, but they are more stable, their core is smaller, and they can be covalently attached to proteins which makes them excellent candidates for high-resolution electron probing of proteins. Bioconjugation of larger gold nanoclusters such as Au_{71}, Au_{102}, and Au_{144} with the aim to create an antibody-based label for Cryo-EM has already been described in the literature. Reported methods mostly relied on time-consuming genetic manipulation on protein to install additional cysteine residue, whose free thiol would serve as an actual bioconjugation handle.

In this presentation, we report the first example of bioconjugation of an Au_{25} gold nanocluster to protein, without genetic manipulation. The novelty of the method is the application of mild tryptophan-selective bioconjugation protocol which was fine-tuned since the first report to make it compatible with gold nanoclusters. Currently, easier-to-handle keto-ABNOH derivatives can be applied in the reaction instead of keto-ABNO radicals, under aqueous neutral buffered conditions and strain-promoted alkyne-azide cycloaddition is employed to secure cluster on protein. The utility of the method is demonstrated on whole monoclonal antibody trastuzumab. We present successful separation of the Au_{25} conjugate and compare it with the conjugate synthesized by conventional lysine-selective bioconjugation under Cryo-EM.



1) Y. Seki, T. Ishiyama, D. Sasaki, J. Abe, Y. Sohma, K. Oisaki, M. Kanai, *J. Am. Chem. Soc.* **2016**, *138*, 10798.