

## Olefin Metathesis for Site-Selective Protein Modification

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Progress Report

### Introduction

Site-selective protein modification has become an important tool to study protein functions in chemical biology. The bio-orthogonal chemical reaction of choice was olefin metathesis. Despite its wide applications for C-C bond formation in organic synthesis and the increasing development of aqueous olefin metathesis for green chemistry, its reactivity on biomolecules was unknown at the time this project was started.

### Allylic chalcogens in Aqueous Cross Metathesis

Among the different types of small molecule alkenes screened in model metathesis reactions, allyl sulfides were found to be the most reactive substrates compared to allyl ethers, allyl amines or the longer-chained sulfide derivatives (butenyl-, pentenyl sulfides). Through literature research, the activation effect of allylic chalcogens in olefin metathesis appears to be a general phenomenon. However, the unique reactivity of allyl sulfide in metathesis was overlooked in other studies of olefin metathesis of sulfur-containing compounds. This enhanced reactivity was explained with a sulfur-assisted mechanism for the first time. The superior reactivity of allyl sulfide led to rapid metathesis reaction in aqueous reaction conditions and enabled the first examples of olefin cross-metathesis on protein surface.

### Chemical routes to S-allyl cysteine on Protein Surface

In order to access the allyl sulfide tag on protein surface, facile chemical methods to install S-allyl cysteine on protein surface has been developed. The strategy was based on the direct allylation of a cysteine residue on a protein surface. In particular, a cysteine-specific allylating reagent – allyl selenocyanate was used on protein substrate for the first time.

### Cross-Metathesis on Protein: Investigation of Substrate Scope and Steric Effects

In the first report of cross-metathesis on protein surface, only few examples were reported and also the scope of the reaction on protein was not fully understood. The investigation of different factors that may affect the outcome of cross-metathesis on protein surface was therefore conducted. A range of metathesis partners containing different olefin tether of various lengths were screened. Unhindered alkenyl ethers were found to be the best metathesis partners. Moreover, the influence of steric hindrance in cross-metathesis of proteins was examined. By reducing the steric hindrance around the allyl sulfide tag on protein surface through a chemical spacer, the rate and conversion of metathesis reaction on proteins was greatly enhanced. Allyl selenides were also discovered to be an even more effective substrate than allyl sulfides in olefin metathesis. This superior reactivity of allyl selenide has enabled 9 different examples of cross-metathesis on a protein with full conversion. Through this work, substrate selection guidelines that give the best chance for successful metathesis reaction on proteins were established.

### Genetic Approach to Installing Allyl Sulfide/Selenide into Proteins

In order to make olefin metathesis a more general strategy for bioconjugation, the genetic incorporation of metathesis handles was investigated. S-allyl homocysteine and Se-allyl homoselenocysteine were tested as a methionine surrogate in protein over-expression in methionine auxotrophic cell line. Gratifyingly, it was demonstrated that both amino acids can be incorporated successfully into proteins as a methionine surrogate. This is the first time that allyl sulfide/selenide containing amino acids were shown to be a substrate for Met tRNA synthase, providing further insight into the promiscuity of substrate recognition of this enzyme.

### Plan for Completion of Thesis

Investigate olefin metathesis on cell surface (in collaboration with Hamachi laboratory at Kyoto University). Finish outstanding experiments for genetic approach to install allyl sulfide into protein for publication. Please refer to Section 2(ii) of GSO14 for further details.