

## Structural studies of UbcH5b~ubiquitin intermediate

Eri SAKATA<sup>1,2</sup>, Tadashi SATOH<sup>3,4</sup>, Shunsuke YAMAMOTO<sup>1</sup>,  
Yoshiki YAMAGUCHI<sup>1,4</sup>, Maho YAGI-UTSUMI<sup>1,5</sup>, Eiji KURIMOTO<sup>1</sup>,  
Keiji TANAKA<sup>2</sup>, Soichi WAKATSUKI<sup>3</sup>, Koichi KATO<sup>1,5\*</sup>

<sup>1</sup>Nagoya City University, Nagoya, Aichi 467-8603, Japan,

<sup>2</sup>Tokyo Metropolitan Institute of Medical Science, Setagaya, Tokyo 156-8506, Japan,

<sup>3</sup>Photon Factory, KEK, Tsukuba, Ibaraki 305-0801, Japan,

<sup>4</sup>RIKEN Advanced Science Institute, Wako, Saitama, 351-0198, Japan,

<sup>5</sup>National Institutes of Natural Sciences, Okazaki, Aichi, 444-8787, Japan

### Introduction

Covalent attachment of ubiquitin (Ub) to target proteins regulates a wide variety of cellular events. Formation and elongation of Ub chains are performed by E2/E3 enzyme complexes. Active E2~Ub thioester intermediates can conjugate Ub to the lysine  $\epsilon$ -amino groups of target proteins in cooperation with E3s. A key question in ubiquitination is how E2/E3 complexes can deal with various acceptor sites distributed on the substrate to perform polyubiquitin elongation and/or multiple ubiquitination.

Here we performed a crystallographic study of an UbcH5b~Ub conjugate in combination with NMR and biochemical analyses and provided mechanical insights for this question [1].

### Experimental Procedure

The thioester-linked UbcH5b~Ub conjugate is a catalytically activated intermediate, and thus unstable and not suitable for crystallization. In order to trap a less activated conjugate, the catalytic residue Cys85 of UbcH5b was mutated to a serine residue. Ub was enzymatically conjugated to UbcH5b (C85S) using E1. Crystals of the UbcH5b~Ub conjugate were obtained in hexagonal  $P6_122$  form by the hanging drop vapor diffusion method. Diffraction data sets were collected at Photon Factory BL5A. The crystal structure was solved by molecular replacement. The refined model of the UbcH5b~Ub has an  $R$ -factor of 23.1% and  $R_{\text{free}}$  is 28.0% for data between 20.0 and 2.2 Å resolution (Fig. 1).

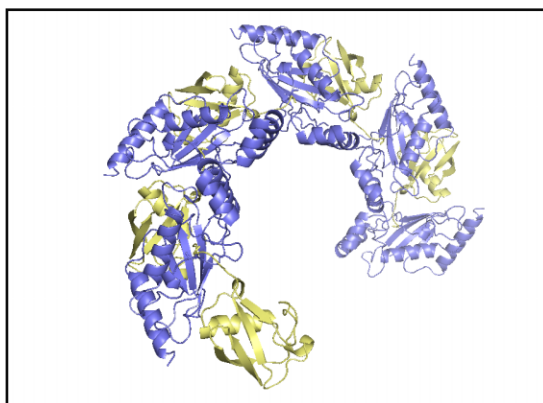


Fig. 1 Crystal structure of UbcH5b~Ub conjugate. The conjugate is assembled into an infinite spiral.

### Results and Discussion

The UbcH5b~Ub structure shows an intermediate state for Ub transfer. In the active site of UbcH5b, Asn77, involved in formation of an oxyanion intermediate during the nucleophilic attack on the thioester bond, interacts with the GG motif at the C-terminus of the conjugated Ub. Moreover, the E2~Ub conjugate is assembled into an infinite spiral through backside interaction with a canonical Ile44 surface of Ub. This active complex may provide multiple E2 active sites, enabling efficient ubiquitination of substrates. Indeed, our biochemical data supports a “riding-on” model, in which self-assembled E2~Ub conjugates act as a bridge to promote E2-substrate contact.

### Reference

- [1] E. Sakata et al., *Structure* **18**, 138 (2010).  
\*kkatonmr@ims.ac.jp