

Internship supervisor and host laboratory:

Laboratory: Structural biology of macromolecular complexes

Name, position: FUKAI, Shuya, Associate Professor

E-mail address: fukai@iam.u-tokyo.ac.jp

Lab homepage: <http://www.iam.u-tokyo.ac.jp/srro/SRRORLifeSciDivEn2/Top.html>

Research project:

Ubiquitin (Ub) is a highly conserved 76-residue protein that can be covalently attached to substrate proteins to regulate a wide variety of biological processes. The terminal carboxylate of Ub is typically attached to the side-chain amino group of substrate lysine residues. Ub itself can be linked to another Ub through its own lysine and N-terminal methionine residues to form Ub chains. Ub has seven lysine residues (K6, K11, K27, K29, K33, K48 and K63) and one terminal methionine residue (M1). Therefore, eight different types of Ub chains are produced and transmit different signals to control specific cellular processes. For instance, Ub chains for a signal of the proteasomal degradation are linked through K48, whereas K63- and M1-linked chains play important roles in proteasome-independent processes such as receptor down-regulation, inflammatory signaling and DNA damage response. We have synthesized Ub chains and investigated how Ub-chain signals are recognized by Ub receptors and deubiquitinating enzymes by X-ray crystallography and structure-based mutational analyses *in vitro* and *in vivo*. This internship program will focus on learning techniques for enzymatically and semi-synthetically preparing different types of ubiquitin chains and ubiquitinated proteins. Further, students can try X-ray crystallography, surface plasmon resonance spectroscopy and enzyme assays using the prepared ubiquitin chains and ubiquitinated proteins. We can accept students in either autumn (from September to December, 2014) or spring (from February to May, 2015).

Recent publication:

1. Sato, Y., Yamagata, A., Goto-Ito S., Kubota, K., Miyamoto, R., Nakada, S., Fukai, S. "Molecular basis of K63-linked polyubiquitination inhibition by the interaction between human deubiquitinating enzyme OTUB1 and ubiquitin-conjugating enzyme UBC13" *J. Biol. Chem.*, **287**, 25860-25868, 2012
2. Sato, Y., Fujita, H., Yoshikawa, A., Yamashita, M., Yamagata, A., Kaiser, S. E., Iwai, K., Fukai, S. "Specific recognition of linear ubiquitin chains by the Npl4 zinc finger (NZF) domain of the HOIL-1L subunit of the linear ubiquitin chain assembly complex." *Proc. Natl. Acad. Sci. U. S. A.*, **108**, 20520-20525, 2011
3. Sato, Y., Yoshikawa, A., Yamashita, M., Yamagata, A., Fukai, S. "Structural basis for specific recognition of Lys 63-linked polyubiquitin chains by NZF domains of TAB2 and TAB3", *EMBO J.*, **28**, 3903-3909, 2009
4. Sato, Y., Yoshikawa, A., Mimura, H., Yamashita, M., Yamagata, A., Fukai, S. "Structural basis for specific recognition of Lys 63-linked polyubiquitin chains by tandem UIMs of RAP80.", *EMBO J.*, **28**, 2461-2468, 2009
5. Sato, Y., Yoshikawa, A., Yamagata, A., Mimura, H., Yamashita, M., Ookata, K., Nureki, O., Iwai, K., Komada, M., Fukai, S. "Structural basis for specific cleavage of Lys 63-linked polyubiquitin chains.", *Nature*, **455**, 358-362, 2008