Enzymatic branched ubiquitin chain formation on K63 homotypic chain with branched points defined by photo-induced stepwise poly-ubiquitin synthesis

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Protein ubiquitination is an essential post-translation modification that regulates numerous cellular processes. Ubiquitin modification in any of its seven lysine residues or N-terminal methionine of proximal ubiquitin results in polymeric chains. The structure complexity ranging from monoubiquitin, homotypic chain, and heterotypic chains with more than one linkage-type, gives rise to ubiquitin code and signal diversity[1]. Even though recent studies have stated the importance of heterotypic chains, reports on its biological role or the mechanism involved in the synthesis are limited[2,3]. Thus, the method enabling efficient synthesis of structure-defined ubiquitin chains is highly demanded for systematic studies on their functions.

To achieve the synthesis of structure defined polyubiquitin chains, a semi-synthetic method of step wise ubiquitin chain assembly was established. The proximally or distally modified/blocked monoubiquitins are used for the synthesis of structure-defined polyubiquitin chains by linkage defined ubiquitin ligase. The Ubiquitin with Nvoc-protected lysine at the 63rd position (K63) was synthesized by Solid phase peptide synthesis followed by native chemical ligation. Photo-induced deprotection of Nvoc-protected lysine residue on successive round of enzymatic reaction enables the synthesis of length-controlled K63 ubiquitin chains. Furthermore, blocking the acceptor site K48 by arginine substitution in ubiquitin moiety would limit the ubiquitination site with respect to branch point.

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