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Communication

# Biochemical properties of $K_{11,48}$-branched ubiquitin chains

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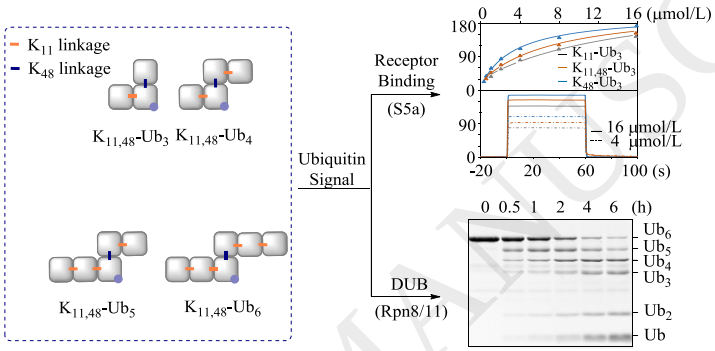


The affinities of chemically synthetic linkage- and length-defined $K_{11,48}$-branched ubiquitin chains binding to ubiquitin receptor S5a were quantitatively measured. Proteasome-associated deubiquitinase Rpn11 showed a higher activity towards $K_{11,48}$-branched ubiquitin chains.

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## ABSTRACT

As one of the most widely existing post-translational modification models, ubiquitination regulates diverse cellular activities. In eukaryotes, $K_{11,48}$-branched ubiquitin chains play key roles in cell cycle and protein quality control. However, the structural and biochemical properties of $K_{11,48}$-branched ubiquitin chains have not been well examined. Here we employed the synthetic linkage- and length-defined $K_{11,48}$-branched ubiquitin chains to examine their binding and hydrolysis properties in vitro. Quantitatively affinity determination of ubiquitin chains to the proteasome ubiquitin receptor S5a indicated that the S5a exhibited preference binding to $K_{11,48}$-branched chains over $K_{11}$-linked chains, but not $K_{48}$-conjugated chains. In addition, deubiquitination experiments were carried out and the results showed that $K_{11,48}$-branched chains were preferably hydrolyzed by proteasome-associated deubiquitinase Rpn11 than homotypic $K_{11}$ or $K_{48}$-linked chains.

Protein ubiquitination plays an essential role in a variety of cellular signaling pathways [1,2]. Ubiquitin (Ub) can form eight types of ubiquitin chains through its own seven lysine residues and an N-terminal amino group. Different ubiquitin chains adopt different conformations, and therefore encode distinct information [3,4]. For instance, $K_{48}$-linked and $K_{63}$-linked ubiquitin chains acting as degradation and non-degradation signal, respectively [5,6], while other atypical ubiquitin chains play important roles in the processes of anti-virus [7-10], protein trafficking [11]. Recent studies have shown that in addition to homogeneously linked ubiquitin chains, mixed or branched ubiquitin chains also control various cellular activities [12-16]. For example, $K_{29,48}$-branched ubiquitin chains