Crystal structure of a SUMOylated target, Puck Knipscheer,[a] Pim van Dijk,[a] Andrea Pichler,[b] Frauke Melchior[b] and Titia Sixma[a*], [a]*The Netherlands Cancer Institute, Amsterdam, The Netherlands, and* [b]*Max Planck Institute for Biochemistry, Martinsried, Germany*. E-mail: t.sixma@nki.nl

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Post-translational modifications play a critical role in many cellular processes due to their ability to rapidly change the behaviour of the modified protein. The transfer and covalent linkage of ubiquitin to lysine residues of proteins has long been known to target the ubiquitinated protein for degradation by the proteasome. It has been recently shown that ubiquitination also has a function in both endocytosis and DNA repair. In addition to ubiquitin, ubiquitin-like proteins relay different signals in the cell but utilize the same overall mechanism of transfer onto target lysines. Of these, SUMO (**s**mall **u**biquitin-related **mo**difier) is one of the best characterized. SUMOylation does not target proteins for degradation but has been implicated in nuclear transport, regulation of transcription and cell division. Among proteins known to get SUMOylated are p53, IκBα, PML and RanGAP1. In contrast to ubiquitin, attachment of SUMO usually involves the four amino acid consensus sequence ψKx(D/E). Detailed information on the transfer and target recognition of ubiquitin and ubiquitin-like proteins is scarce, therefore we set out to study this mechanism using X-ray crystallography. We identified a novel SUMO target that is specifically SUMOylated on a single lysine in vivo and in vitro. Here, we present the 2.3 Å crystal structure of SUMO covalently attached to this target. We have also solved the structure of the target alone at 1.7 Å resolution. This enables us to study changes in the target upon SUMOylation. This first structure of a SUMOylated target reveals that, in addition to the covalent bond between the ε-amino group of the target lysine and the c-terminus of SUMO, several other residues are involved in the interaction across the target-SUMO interface. Understanding Molecular insights into the target specificity are of great importance in understanding the mechanism of SUMOylation and its in vivo function.