s1.m5.o2 **Crystal Structure of a SUMOylated Target**. Puck Knipscheer,[a] Pim van Dijk,[a] Andrea Pichler,[b] Frauke Melchior[b] and Titia Sixmaa*, [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.

s1.m5.o3 **Mutants at the 4th and 5th Positions of the Sequence d(gcGXYAgc) Suggest a Variety of the DNA Octaplex.** Wataru Adachi, Jiro Kondo, Kenta Mitomi, Tanashaya Ciengshin Tomoko Sunami and Akio Takénaka, *Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama, 226-8501, Japan. E-mail: wadachi@bio.titech.ac.jp*

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It is found that DNA octamer with the sequence d(gcGAGAgc) adopts the base-intercalated duplex, and further associated to form an octaplex in the condition containing potassium ion [1]. This octaplex is the largest multiplex found in nucleic acid structures so far. The eight G_5 residues form two G-quartets through the direct N1-H…O6 and N2-H…N7 hydrogen bonds. Between the two G-quartets, a potassium ion occupies the center to bind the eight O6 atoms of the G_5 residues. In addition, above and below the double G-quartets, two other potassium ions are bound to the four O6 atoms, respectively. To investigate the effects of point mutations at the 4th and 5th residues on the structure, crystal structures of two mutants with the sequence d(gcGGGAgc) and d(gcGAAAgc) have been determined by X-ray crystallography, and compared with that of d(gcGAGAgc). In the d(gcGAAAgc) crystal, eight DNA strands form a right-handed octaplex-assembly. This structure is similar to that of the octaplex found in the d(gcGAGAgc) crystal. In the central part of the d(gcGAAAgc) octa-assembly, the eight A5 residues are bundled through water-mediated hydrogen bonds. This octa-assembly is slightly swollen at the central parts, when compared with that of d(gcGAGAgc). At the fourth residue of both assemblies, water molecules are bridged to the four A_4 residues through hydrogen bonds between the Watson-Crick sites. In the two peripheral parts, many water molecules are found around the helical axis of the octa-assembly. The third G3 residues also seem to be assembled by mediation of water molecules in the major groove of the guanine bases. In their minor groove, the A6 residues of the adjacent anti-parallel strands are bound to form sheared pairs through two hydrogen bonds. At the second and the first residues, the C_2 and G_1 bases are pointed to the outsides of the assembly, and form the Watson-Crick G:C pairs with the complementary bases of the adjacent anti-parallel strands. On the other side of G:C base pairs, water molecules occupy in the center of the assembly. On the surface of the d(gcGAAAgc) octa-assembly, hexa-hydrated magnesium ions are bound to the Watson-Crick site of the G_3 residues and major groove site of G_7 residues. These hydrogen-bonding networks are indispensable to stabilize both assemblies. Crystals of d(gcGGGAgc) were obtained from a solution containing calcium ion. The two DNA strands form a base-intercalated duplex, and the three duplexes are further associated to form a hexa-assembly similar to that of d(gcGAAAgc) containing hexaminecobalt [2]. Crystals are also obtained from a potassium containing solution.

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[2] Sunami T., Kondo J., Hirao I, Watanabe K., Miura K. and Takénaka A. (2004) *Acta. Crystallogr.*, **D60**, 90-96