Supporting Information

Structure of the stapled p53 peptide bound to Mdm2

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Peptide synthesis

SAH-p53-8 was synthesized as previously described.[1]

Protein expression and purification

The recombinant human Mdm2 (residues 25-111) was cloned into the pET-20 vector (Novagen) without an affinity tag and expressed in *E. coli* BL21-CodonPlus(DE3)-RIL expression cells (Stratagene). Cells were grown at 37 °C and induced at OD_{600nm} of 0.8 with 1 mM IPTG. After 5 h induction at 37 °C the cells were harvested by centrifugation. The harvested cells from 5 liters of *E. coli* cell culture were re-suspended in PBS at pH 7.4 and ruptured by sonication. After centrifugation the inclusion bodies were washed with PBS containing 0.05% Triton X-100 with subsequent low-speed centrifugation (12000G). The procedure was repeated three times. The inclusion bodies were solubilized with 6 M GuHCl in 100 mM Tris-HCl, pH 8.0 including 1 mM EDTA and 10 mM DTT. The protein was dialyzed at 4 °C, against 4 M GuHCl, pH 3.5 including 10 mM DTT. For renaturation, the protein was diluted 1:100 in 10 mM Tris- HCl, pH 7.0, containing 1 mM EDTA and 10 mM DTT by adding the denatured protein drop-wise into the refolding buffer. Refolding was carried out

for 10 h at 4 °C. Ammonium sulfate was added to a final concentration of 1.5M and the protein was applied to the Butyl Sepharose 4 Fast Flow (GE Healthcare) and subsequently eluted with 100 mM Tris-HCl, pH 7.2, including 5 mM DTT. The protein was further purified by gel filtration on HiLoad 16/60 Superdex200 (Pharmacia). The Mdm2 containing fractions are mixed with the 2-fold excess of the peptide and concentrated to about 10 mg/ml before the crystallization trial. The purity and folding of the protein was measured by SDS-PAGE and 1D NMR.

X-ray crystallography

Crystallization of the Mdm2 with the peptide was achieved at room temperature by sitting drop vapor diffusion and the protein to crystallization solution ratio of 1:1, with the crystallization solution containing 100 mM Sodium acetate trihydrate, pH 4.75 and 2.5 M NaCl. The crystals appeared in several days and grew to a final size of 0.1 mm. They were transferred to cryoprotectant solution containing the crystallization solution supplemented with 25% (v/v) glycerol, then directly plunged frozen in liquid nitrogen. Native dataset to 2.0 Å were collected from a single crystal at 100 K on the SLS PXII beam line at Paul Scherrer Institute, Villigen, Switzerland and processed using XDS and XSCALE[2] program. The structure of Mdm2 from PDB entry 1YCR was used as a search model after the p53 peptide was removed. The initial Rfactor of the solution was 0.45. The model was subsequently rebuilt using Xfit[3] and refined using Refmac5 from CCP4 suite[4]. Data collection and refinement statistics are given in table S1. Each asymmetric unit contains two stapled peptide – Mdm2 complexes. Both are virtually identical (RMSD of the main chain atoms 0.7 A) except region between Asp84-Val88 in chain A that is shifted by about 1.2 A due to crystal contacts. The residues outside 18-27 range in the peptide are generally not visible in electron density (one chain shows additionally Gln28-Asn30 stabilized due

to crystal contacts) indicating that this part is flexible and do not take direct part in binding to Mdm2. Several solvent-exposed sidechains had no interpretable electron density and were removed from the model.

Table S1. Data collection and refinement statistics

Data collection	
Space group	$P2_1$
Cell constants (Å)	a = 45.4
	b = 42.41
	c= 50.5
	β = 90.86
Resolution range (Å)	50 – 2.0
Wavelength (Å)	0.999
Observed reflections	48 879
Unique reflections	13 254
Whole range	
Completeness (%)	99.5
R_{merge}	13.6
$I/\sigma(I)$	12.45
Last shell	
Resolution range (Å)	2.0 – 2.1
Completeness (%)	99.8
R_{merge}	60.6
$I/\sigma(I)$	3.14
Refinement	
No. of reflections	11 550
Resolution (Å)	20 – 2.0
R-factor (%)	16.8
R_{free} (%)	21.6
Average B ($Å^2$)	13.42
R.m.s bond lenght (Å)	0.009
R.m.s. angles (°)	1.05
Content of asymmetric unit	
No. of protein complexes	2
No. of protein residues/atoms	197/1660
No. of solvent atoms	146

References

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(4) Collaborative Computational Project, Number 4. *Acta Crystallogr. D. Biol. Crystallogr.* **1994,** 50, 760-3.