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SUPPLEMENTARY ONLINE DATA

Structural changes in the BH3 domain of SOUL protein upon interaction with the anti-apoptotic protein Bcl-xL

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SUPPLEMENTARY INFORMATION

X-ray structure of human SOUL

For the sake of simplicity, only the structure of the orthorhombic crystal form which diffracts to 1.6 Å resolution was described (in the main paper). At the end of this section, we will discuss a difference that was found in the hexagonal crystal form solved to 2.85 Å resolution. The final model of the orthorhombic crystal form of SOUL comprises 180 amino acid residues of both chains A and B, the two monomers present in the asymmetric unit of this crystal form. The maps do not show electron density for the first 18 residues and the last seven residues of both chains. The model contains 2974 non-hydrogen protein atoms, one phosphate and 395 water molecules. The conventional *R* factor is 17.5 % and the free *R* factor is 19.7 % (see Table 1 in the main paper). The *R* factors and rmsd values of Table 1 in the main paper were calculated with the program Phenix.refine [1]. The stereochemical quality of the protein model was assessed with the program PROCHECK [2]; 93.5 % of the residues are in the most favourable region of the Ramachandran plot and the remaining 6.5 % in the additionally allowed region. The two molecules in the asymmetric unit are not related by a non-crystallographic dyad and there are some minor differences between the two chains that can be explained by the packing of the molecules in the crystal (see below). The rmsd between the two main chains is 0.72 Å calculated over 180 Cα pairs of equivalent residues.

The resolution of the orthorhombic crystal form is quite adequate for the analysis of the structure of the solvent molecules within the β-barrel cavity of the protein (see Table 1 of the main paper), and we have identified the water molecules that bind in the interior of barrel in the same position in the two SOUL monomers of the asymmetric unit. Trp^{48} binds a water molecule at its NE1 atom, and another water molecule, in the proximity of the first, binds the NH1 atom of Arg^{132}. Two other water molecules, in the proximity of the first two, bind the carbonyls of Ala^{133} and Gly^{44}, the latter is external to the cavity. The four water molecules are close enough so that the group should be described as a cluster organized in a net of hydrogen-bonded solvent molecules. There is another cluster that involves three conserved water molecules positioned in the interior of the barrel (bound to the OG1 of Thr^{176} and the carbonyl of Val^{89}), at the interface (bound to the carbonyl of Ser^{181}) and outside (bound to the water molecule at the interface). Two other internal water molecules bridge the OG1 of Thr^{186} with the carbonyl of Lys^{84} and the N of Tyr^{110}, and another pair is bound to the carboxy moiety of Asp^{130}. The solvent molecules in the interior of the cavity are in contact with others at the interface which in turn are in the proximity of water molecules in conserved positions on the external surface of SOUL.

An interesting structural difference was observed in the hexagonal crystal form that diffracts to 2.85 Å and contains four SOUL monomers in the asymmetric unit. Two of these monomers (A and B) swap the first portion of their polypeptide chains, the portion running from amino acids 19 to 32, i.e. before the first strand of β-sheet. This structure swapping is not confirmed in the other two monomers, since the portion where it is expected to take place is disordered in the maps. This disorder is a clear indication of high flexibility of that loop and we have not attributed any particular significance to our observation that we think is a consequence of the molecular packing in the crystals.

Comparison of SOUL with HEBP1

The p22HBPs have been studied more extensively than the SOUL family. They are ubiquitously expressed but are extremely abundant in liver, have a cytoplasmic location and there is solid evidence that they bind haem and several porphyrins with micromolar K_d values [3]. The p22HBPs are highly homologous, monomeric and soluble, and bind metalloporphyrins, free porphyrins and *N*-methylprotoporphyrin with similar affinities. Two NMR structures of the same protein, murine p22HBP, have been published [4,5]. In both models, the 22-kDa monomer is described to fold as a distorted β-barrel flanked by two long α-helices arranged on one face. Using a ^{15}N-^{1}H HSQC titration experiment, the porphyrin-binding site of murine p22HBP was mapped and found to comprise a number of loops and one of the two α-helices with all of the residues participating in ligand binding located on a single face of the molecule [5].

Murine p22HBP has approximately 28 % sequence identity with murine SOUL, which was reported to be a dimer in its apo form and to hexamerize upon haem binding with a dissociation constant in the nanomolar range [6]. It was reported further that His^{42}, the only histidine residue present in the sequence, plays a crucial role and that its mutation abolishes haem binding. Comparison of ligand binding of murine p22HBP and murine SOUL reveals that binding has to take place in very different sites and therefore one has to confront the intriguing situation of two highly similar proteins binding the same ligand in a very different way.

The percentage of sequence identity between human SOUL and murine p22HBP is approximately 29 %, which supports the prediction that the two folds should be very similar to one another. In spite of this, all our attempts to solve the crystal structure of SOUL by molecular replacement, using the two available NMR structures as search probes, failed and the SOUL structure had to be solved using the alternative single isomorphous replacement method.

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The co-ordinates of the models, and the structure factors of SOUL and of the complex of human Bcl-xL with the peptide have been deposited in the PDB under accession codes 3R85, 3R8J and 3R8K.

After the final co-ordinates of SOUL became available, the model of chain A of SOUL was superimposed to that of chain B and the two lowest energy NMR structures available of murine p22HBP (PDB codes 2GOV and 2HVA; [4,5]), and the distances between equivalent α carbons were calculated. The results are represented in Supplementary Figure S1(A) as a function of the amino acid number. In the Figure the blue trace represents the differences between the two SOUL molecule models in the orthorhombic crystal asymmetric unit (A and B) and the black trace indicates the difference between the two murine p22HBP NMR structures. The area where the two SOUL molecules in the asymmetric unit differ more are the region before the first strand of β-sheet and the loop connecting strands C and D. The chains before the first strand are totally exposed to the solvent, whereas in the case of the connection of strands C and D the loop in chain A is in close contact with a symmetry related molecule and the equivalent area is in contact with the solvent in molecule B. We thus believe that these differences are simply a consequence of molecular packing in the crystal. Although there is more variability in the two NMR structures of murine p22HBP, the two structures are very similar in the region connecting strands C and D, which is also the region where both are most different from SOUL. This particular region of the SOUL molecule thus appears to be more variable than the rest of the molecule

Supplementary Figure S1(B) is a stereo pair in which the model of SOUL A is superimposed to the two NMR models of murine p22HBP. In the Figure, SOUL is represented in blue, whereas the two models of murine p22HBP are in red and green. As the Figure shows there are areas of larger variability in the conformation of the proteins. The N-terminal region of the two NMR structures is very different and both differ substantially from the X-ray structure, which is due to the fact that this part of the chain is very flexible and becomes more structured in the crystals. A more significant difference is observed in the loop connecting strands C and D between the A chain of SOUL and the two NMR structures. This area of the molecule is, however, in different conformations in the two SOUL molecules of the asymmetric unit and so the differences observed with murine p22HBP probably simply reflect the high degree of flexibility of this area.

Overall the three structures are quite similar, although, as expected, the differences between the two NMR structures are somewhat smaller than those observed between them and the crystal structure of SOUL.

Table S1 Main contacts between the SOUL BH3 domain and human Bcl-xL

Selected distances between the closest human Bcl-xL residues of molecule A and the SOUL BH3 domain in contact with it (labelled molecule E). The hydrophilic contacts are highlighted in bold

SOUL residue	Atom	Bcl-xL residue	Atom	Distance (Å)
Glu^{153}	**OE1**	**Gln^{111}**	**O**	**4.45**
Gln^{154}	**NE2**	**Gln^{111}**	**OE1**	**2.66**
Gln^{154}	CD	Gln^{111}	CD	4.18
Leu^{155}	CB	Gln^{111}	CB	3.67
Leu^{155}	CG	Leu^{112}	CD2	4.00
Leu^{155}	CD1	Val^{126}	CG2	4.13
Leu^{156}	CD1	Glu^{129}	CG	3.76
Leu^{158}	CD1	Gln^{111}	CG	3.51
Leu^{158}	CD1	Leu^{108}	CD1	4.78
Leu^{158}	CD1	Asp^{107}	CB	4.13
Ala^{159}	CB	Val^{126}	CB	4.26
Ser^{160}	**OG**	**Glu^{129}**	**OE2**	**3.81**
Leu^{162}	CB	Leu^{130}	CD1	3.76
Leu^{162}	CD1	Ala^{142}	CB	4.18
Leu^{162}	CD1	Phe^{97}	CZ	3.67
Leu^{162}	CG	Phe^{105}	CZ	4.88
Leu^{162}	CD2	Leu^{108}	CD2	4.30
Arg^{163}	**NH2**	**Asp^{133}**	**OD2**	**4.02**
Arg^{163}	**NH1**	**Glu^{129}**	**OE2**	**3.13**
Arg^{163}	**O**	**Arg^{139}**	**NH2**	**3.26**
Arg^{163}	CG	Arg^{139}	CZ	3.63
Arg^{163}	CG	Arg^{139}	CD	3.75
Arg^{163}	CB	Leu^{130}	CD1	4.16
Glu^{164}	**N**	**Arg^{139}**	**NE**	**4.74**
Asp^{165}	**OD1**	**Tyr^{101}**	**OH**	**3.96**
Asp^{165}	CB	Phe^{97}	CZ	4.11
Asp^{165}	CB	Tyr^{101}	CD1	3.85
Gly^{166}	**O**	**Asn^{136}**	**OD1**	**4.05**
Gly^{166}	C	Gly^{138}	C	4.07
Gly^{166}	CA	Arg^{139}	CG	3.73
Gly^{166}	C	Gly^{138}	C	4.07
Lys^{167}	CB	Arg^{139}	CZ	3.85
Lys^{167}	CB	Asn^{136}	CG	4.90
Lys^{167}	**O**	**Asn^{136}**	**ND2**	**4.59**
Val^{168}	CB	Tyr^{101}	CZ	4.97
Phe^{169}	CD1	Phe^{97}	CD2	3.54
Phe^{169}	CZ	Tyr^{195}	CE2	4.07
Phe^{169}	CE2	Val^{141}	CG2	3.87
Phe^{169}	CE2	Ala^{93}	CB	4.53
Asp^{170}	**OD1**	**Tyr^{195}**	**OH**	**2.44**
Asp^{170}	**OD2**	**Asn^{136}**	**ND2**	**3.25**
Asp^{170}	**OD1**	**Gly^{138}**	**N**	**3.06**
Asp^{170}	**OD2**	**Trp^{137}**	**N**	**4.09**
Asp^{170}	CG	Trp^{137}	C	4.88
Asp^{170}	CG	Asn^{136}	CB	4.50
Lys^{172}	CD	Tyr^{195}	CE2	4.19

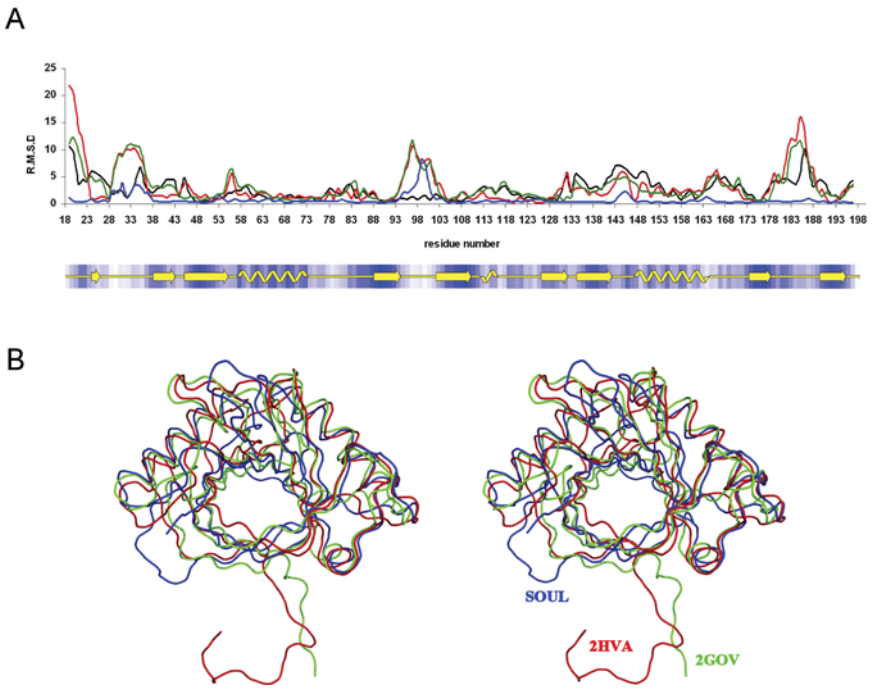

Figure S1 Comparison of the models of human SOUL and murine p22HBP

(**A**) rsmd between α-carbon atoms of the SOUL models and the models of murine p22HBP: A chain compared with B chain of the orthorhombic form (blue); lowest energy NMR murine structure (PDB code 2GOV) compared with the other equivalent structure available of the same protein (PDB code 2HVA) (black). The green and red traces compare chain A of SOUL with the two NMR structures of murine p22HBP [PDB codes 2GOV (green) and 2HVA (red)]. The strip at the bottom of the Figure represents the elements of secondary structure of SOUL. The blue colour identifies the buried residues, whereas white is used to indicate the exposed amino acids. (**B**) Stereoimage with the superposition of the three models. SOUL is in blue and the two NMR models of murine p22HBP are in red and green. The Figures of the models were prepared using the program PyMOL (http://www.pymol.org).

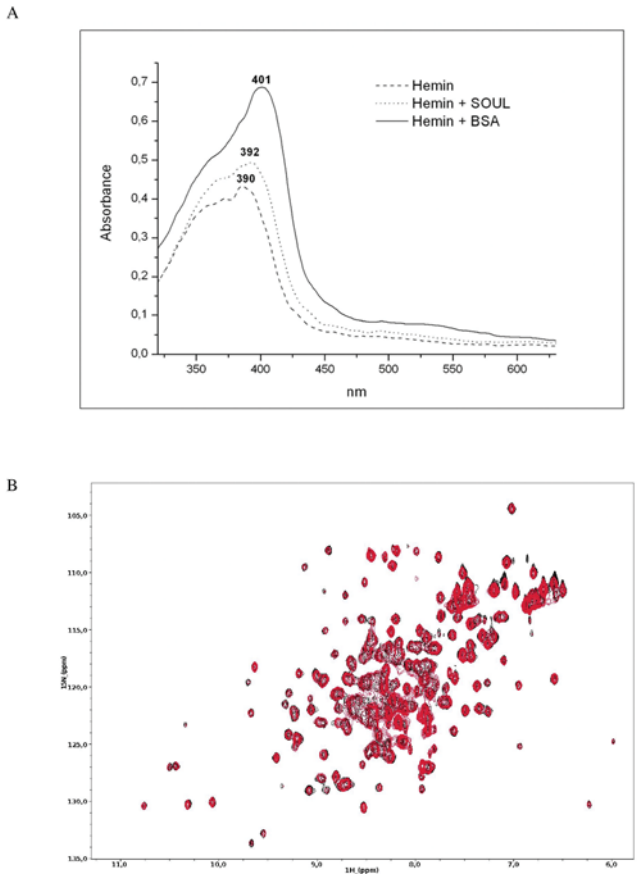

Figure S2 Interaction of SOUL with haemin

(**A**) UV–visible spectra of haemin alone (broken line) and with ten times a molar excess of SOUL (dotted line) and BSA (solid line). (**B**) HSQC titration of ^{15}N-labelled SOUL with haemin. The black spectrum corresponds to protein SOUL before the titration, whereas the red spectrum was recorded after the addition of four equivalents of haemin. No differences were detected between the two spectra.

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