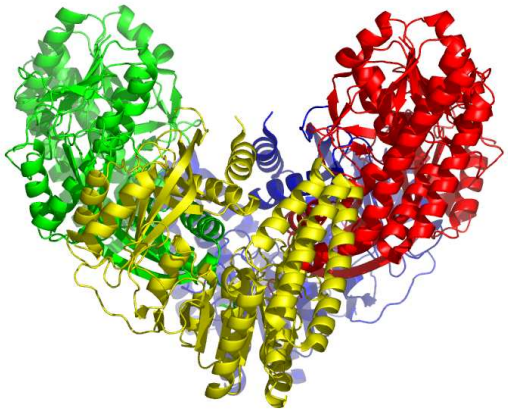
2hmf

Evolutionary trace report by **report_maker**

May 8, 2010



CONTENTS

1 INTRODUCTION

From the original Protein Data Bank entry (PDB id 2hmf):
Title: Structure of a threonine sensitive aspartokinase from methanococcus jannaschii complexed with mg-adp and aspartate
Compound: Mol id: 1; molecule: probable aspartokinase; chain: a, b, c, d; synonym: aspartate kinase; ec: 2.7.2.4; engineered: yes
Organism, scientific name: Methanococcus Jannaschii;

2hmf contains a single unique chain 2hmfA (464 residues long) and its homologues 2hmfD, 2hmfC, and 2hmfB.

2 CHAIN 2HMFA

2.1 Q57991 overview

From SwissProt, id Q57991, 92% identical to 2hmfA:
Description: Probable aspartokinase (EC 2.7.2.4) (Aspartate kinase).
Organism, scientific name: Methanococcus jannaschii.
Taxonomy: Archaea; Euryarchaeota; Methanococci; Methanococcales; Methanocaldococcaceae; Methanocaldococcus.
Catalytic activity: ATP + L-aspartate = ADP + 4-phospho-L-aspartate.
Pathway: Amino-acid biosynthesis; L-lysine biosynthesis via DAP pathway; tetrahydrodipicolinate from L-aspartate: step 1.
Pathway: Amino-acid biosynthesis; L-methionine biosynthesis; L-homoserine from L-aspartate: step 1.
Pathway: Amino-acid biosynthesis; L-threonine biosynthesis; L-threonine from L-aspartate: step 1.
Similarity: Belongs to the aspartokinase family.
About: This Swiss-Prot entry is copyright. It is produced through a collaboration between the Swiss Institute of Bioinformatics and the EMBL outstation - the European Bioinformatics Institute. There are no restrictions on its use as long as its content is in no way modified and this statement is not removed.

2.2 Multiple sequence alignment for 2hmfA

For the chain 2hmfA, the alignment 2hmfA.msf (attached) with 889 sequences was used. The alignment was downloaded from the HSSP database, and fragments shorter than 75% of the query as well as duplicate sequences were removed. It can be found in the attachment

Lichtarge lab 2006

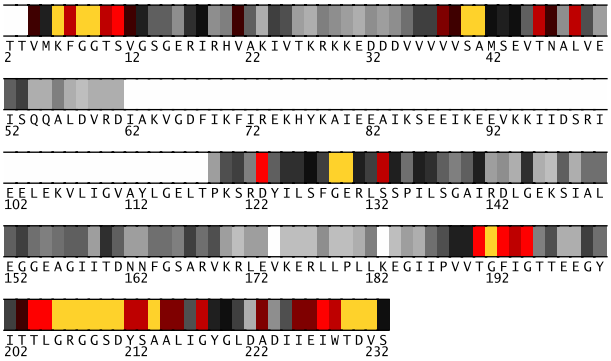


Fig. 1. Residues 2-233 in 2hmfA colored by their relative importance. (See Appendix, Fig.16, for the coloring scheme.)

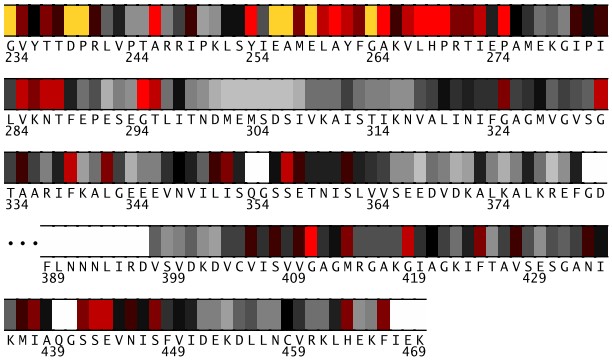


Fig. 2. Residues 234-470 in 2hmfA colored by their relative importance. (See Appendix, Fig.16, for the coloring scheme.)

to this report, under the name of 2hmfA.msf. Its statistics, from the *alistat* program are the following:

```
Format:              MSF
Number of sequences: 889
Total number of residues:    360359
Smallest:            316
Largest:             464
Average length:      405.4
Alignment length:    464
Average identity:    34%
Most related pair:   99%
Most unrelated pair: 15%
Most distant seq:    36%
```

Furthermore, <1% of residues show as conserved in this alignment.

The alignment consists of 2% eukaryotic (1% fungi, 1% plantae), 16% prokaryotic, and 1% archaean sequences. (Descriptions of some sequences were not readily available.) The file containing the sequence descriptions can be found in the attachment, under the name 2hmfA.descr.

2.3 Residue ranking in 2hmfA

The 2hmfA sequence is shown in Figs. 1–2, with each residue colored according to its estimated importance. The full listing of residues in 2hmfA can be found in the file called 2hmfA.ranks_sorted in the attachment.

2.4 Top ranking residues in 2hmfA and their position on the structure

In the following we consider residues ranking among top 25% of residues in the protein . Figure 3 shows residues in 2hmfA colored by their importance: bright red and yellow indicate more conserved/important residues (see Appendix for the coloring scheme). A Pymol script for producing this figure can be found in the attachment.

2.4.1 Clustering of residues at 25% coverage. Fig. 4 shows the top 25% of all residues, this time colored according to clusters they belong to. The clusters in Fig.4 are composed of the residues listed in Table 1.

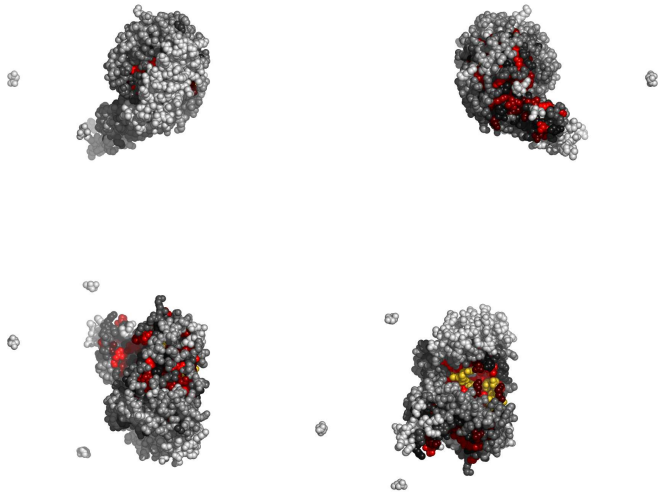


Fig. 3. Residues in 2hmfA, colored by their relative importance. Clockwise: front, back, top and bottom views.

Table 1.

cluster color	size	member residues
red	111	4,6,7,8,9,10,11,12,21,38,39 40,41,46,47,49,123,129,130 133,191,192,193,194,195,203 204,205,206,207,208,209,210 211,212,213,214,215,216,218 223,226,227,228,229,230,231 232,234,235,236,237,238,239 240,241,246,249,254,256,257 258,259,260,261,262,263,264

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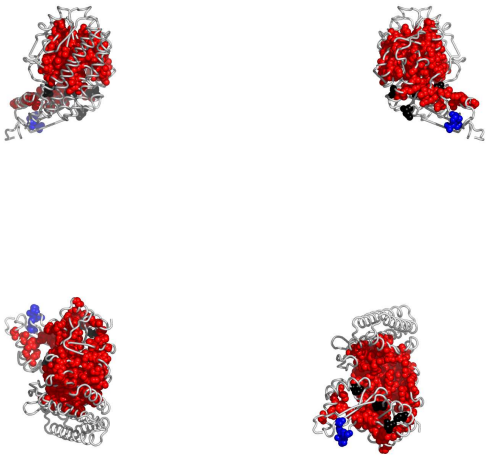

Fig. 4. Residues in 2hmfA, colored according to the cluster they belong to: red, followed by blue and yellow are the largest clusters (see Appendix for the coloring scheme). Clockwise: front, back, top and bottom views. The corresponding Pymol script is attached.

Table 1. *continued*		
cluster color	**size**	**member residues**
		265,266,267,268,269,270,271 272,273,275,276,281,283,285 286,287,288,295,296,333,335 339,342,351,352,362,408,410 411,414,419,421,425,428,437 438,442,443,444,446,448,464 467
blue	3	325,357,358

Table 1. Clusters of top ranking residues in 2hmfA.

2.4.2 Overlap with known functional surfaces at 25% coverage. The name of the ligand is composed of the source PDB identifier and the heteroatom name used in that file.

ADP binding site. Table 2 lists the top 25% of residues at the interface with 2hmfADP403 (adp). The following table (Table 3) suggests possible disruptive replacements for these residues (see Section 3.6).

Table 2.					
res	**type**	**subst's (%)**	**cvg**	**noc/ bb**	**dist (Å)**
503				2/0	4.82

Table 2. The top 25% of residues in 2hmfA at the interface with ADP.(Field names: res: residue number in the PDB entry; type: amino acid type; substs: substitutions seen in the alignment; with the percentage of each type in the bracket; noc/bb: number of contacts with the ligand, with the number of contacts realized through backbone atoms given in the bracket; dist: distance of closest apporach to the ligand.)

Table 3.		
res	**type**	**disruptive mutations**
503		

Table 3. List of disruptive mutations for the top 25% of residues in 2hmfA, that are at the interface with ADP.

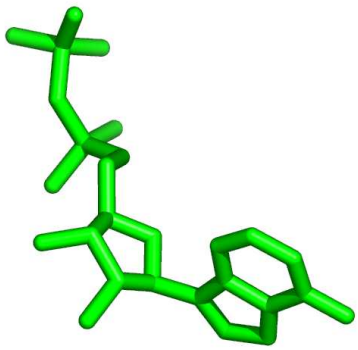

Fig. 5. Residues in 2hmfA, at the interface with ADP, colored by their relative importance. The ligand (ADP) is colored green. Atoms further than 30Å away from the geometric center of the ligand, as well as on the line of sight to the ligand were removed. (See Appendix for the coloring scheme for the protein chain 2hmfA.)

Figure 5 shows residues in 2hmfA colored by their importance, at the interface with 2hmfADP403.

Interface with 2hmfD. Table 4 lists the top 25% of residues at the interface with 2hmfD. The following table (Table 5) suggests possible disruptive replacements for these residues (see Section 3.6).

Table 4.					
res	**type**	**subst's (%)**	**cvg**	**noc/ bb**	**dist (Å)**
501				81/36	0.00
502				77/35	0.00
503				79/35	0.00
504				223/94	0.00
130	E	E(99)VS	0.01	6/0	2.84
continued in next column					

Table 4. *continued*

res	type	subst's (%)	cvg	noc/ bb	dist (Å)
		D			
207	R	R(99)LV K	0.01	25/7	2.66
211	D	D(98)NE	0.01	1/1	4.74
209	G	G(94) Y(3)CA	0.02	6/6	3.36
210	S	S(94)G T(5)	0.02	17/7	3.10
41	A	A(99)Y. SGR	0.03	2/1	4.74
208	G	G(84) N(11) S(1)E D(1)	0.03	21/21	2.82
40	S	S(98)T. KAGL	0.04	10/1	2.67
206	G	G(88)D S(6)P K(2) Q(1)TER	0.04	1/1	4.83
6	K	K(98) .(1)	0.05	4/0	3.83
8	G	G(98) .(1)	0.05	1/1	4.63
9	G	G(98) .(1)AS	0.05	1/1	4.43
193	F	F(94) Y(5)D	0.06	21/0	3.42
46	T	T(91) F(1) S(1)DN G(2)K H(1)P.A I	0.12	15/1	3.19

Table 4. The top 25% of residues in 2hmfA at the interface with 2hmfD. (Field names: res: residue number in the PDB entry; type: amino acid type; substs: substitutions seen in the alignment; with the percentage of each type in the bracket; noc/bb: number of contacts with the ligand, with the number of contacts realized through backbone atoms given in the bracket; dist: distance of closest apporach to the ligand.)

Table 5.

res	type	disruptive mutations
501		
502		
503		
504		
130	E	(H)(FWR)(Y)(CG)
207	R	(TY)(D)(E)(SCG)
211	D	(R)(FWH)(Y)(VCAG)

continued in next column

Table 5. *continued*

res	type	disruptive mutations
209	G	(K)(ER)(Q)(MD)
210	S	(KR)(FQMWH)(E)(NLPI)
41	A	(KE)(R)(YD)(Q)
208	G	(R)(K)(FWH)(YM)
40	S	(R)(K)(H)(FW)
206	G	(R)(FW)(H)(K)
6	K	(Y)(FTW)(SVCAG)(HD)
8	G	(KER)(FQMWHD)(NLPI)(Y)
9	G	(KR)(E)(QH)(FMWD)
193	F	(K)(E)(Q)(R)
46	T	(R)(K)(H)(FQW)

Table 5. List of disruptive mutations for the top 25% of residues in 2hmfA, that are at the interface with 2hmfD.

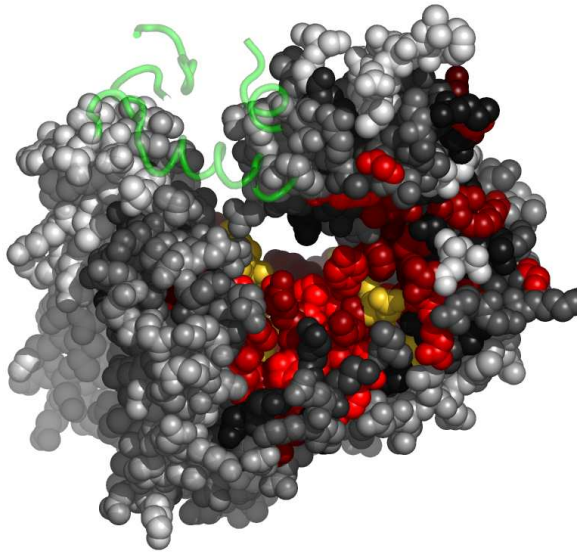

Fig. 6. Residues in 2hmfA, at the interface with 2hmfD, colored by their relative importance. 2hmfD is shown in backbone representation (See Appendix for the coloring scheme for the protein chain 2hmfA.)

Figure 6 shows residues in 2hmfA colored by their importance, at the interface with 2hmfD.

Magnesium ion binding site. Table 6 lists the top 25% of residues at the interface with 2hmfMG301 (magnesium ion). The following table (Table 7) suggests possible disruptive replacements for these residues (see Section 3.6).

Table 6.

res	type	subst's (%)	cvg	noc/ bb	dist (Å)
502				4/0	3.38

continued in next column

Table 6. *continued*					
res	**type**	**subst's (%)**	**cvg**	**noc/ bb**	**dist (Å)**
211	D	D(98)NE	0.01	3/0	4.35
208	G	G(84) N(11) S(1)E D(1)	0.03	4/4	3.85
6	K	K(98) .(1)	0.05	1/0	4.57
266	K	K(68) A(2) Q(4) R(1) S(9) G(4) N(3)V E(4) T(1)CH	0.20	2/1	4.48

Table 6. The top 25% of residues in 2hmfA at the interface with magnesium ion.(Field names: res: residue number in the PDB entry; type: amino acid type; substs: substitutions seen in the alignment; with the percentage of each type in the bracket; noc/bb: number of contacts with the ligand, with the number of contacts realized through backbone atoms given in the bracket; dist: distance of closest apporach to the ligand.)

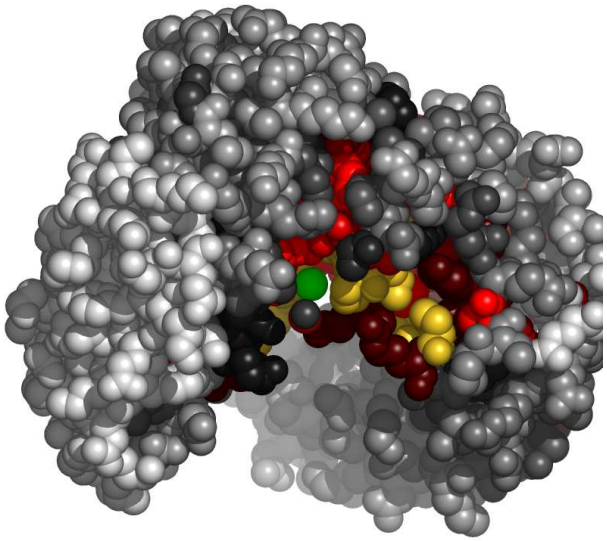

Fig. 7. Residues in 2hmfA, at the interface with magnesium ion, colored by their relative importance. The ligand (magnesium ion) is colored green. Atoms further than 30 Å away from the geometric center of the ligand, as well as on the line of sight to the ligand were removed. (See Appendix for the coloring scheme for the protein chain 2hmfA.)

Table 7.		
res	**type**	**disruptive mutations**
502		
211	D	(R)(FWH)(Y)(VCAG)
208	G	(R)(K)(FWH)(YM)
6	K	(Y)(FTW)(SVCAG)(HD)
266	K	(Y)(FW)(T)(H)

Table 7. List of disruptive mutations for the top 25% of residues in 2hmfA, that are at the interface with magnesium ion.

Figure 7 shows residues in 2hmfA colored by their importance, at the interface with 2hmfMG301.

ADP binding site. Table 8 lists the top 25% of residues at the interface with 2hmfADP402 (adp). The following table (Table 9) suggests possible disruptive replacements for these residues (see Section 3.6).

Table 8.					
res	**type**	**subst's (%)**	**cvg**	**noc/ bb**	**dist (Å)**
501				2/0	4.39

Table 8. The top 25% of residues in 2hmfA at the interface with ADP.(Field names: res: residue number in the PDB entry; type: amino acid type; substs: substitutions seen in the alignment; with the percentage of each type in the bracket; noc/bb: number of contacts with the ligand, with the number of contacts realized through backbone atoms given in the bracket; dist: distance of closest apporach to the ligand.)

Table 9.		
res	**type**	**disruptive mutations**
501		

Table 9. List of disruptive mutations for the top 25% of residues in 2hmfA, that are at the interface with ADP.

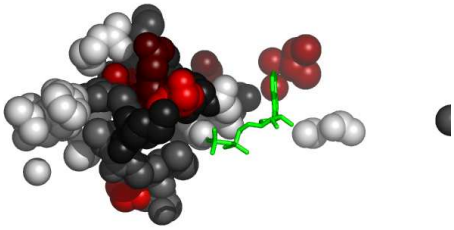

Fig. 8. Residues in 2hmfA, at the interface with ADP, colored by their relative importance. The ligand (ADP) is colored green. Atoms further than 30$\AA$ away from the geometric center of the ligand, as well as on the line of sight to the ligand were removed. (See Appendix for the coloring scheme for the protein chain 2hmfA.)

Figure 8 shows residues in 2hmfA colored by their importance, at the interface with 2hmfADP402.

Interface with 2hmfC. Table 10 lists the top 25% of residues at the interface with 2hmfC. The following table (Table 11) suggests possible disruptive replacements for these residues (see Section 3.6).

Table 10.					
res	**type**	**subst's (%)**	**cvg**	**noc/ bb**	**dist ($\AA$)**
501				81/36	0.00
502				77/35	0.00
503				218/92	0.00
504				79/35	0.00
130	E	E(99)VSD	0.01	6/0	2.84
207	R	R(99)LVK	0.01	25/7	2.66
211	D	D(98)NE	0.01	1/1	4.74

continued in next column

Table 10. *continued*					
res	**type**	**subst's (%)**	**cvg**	**noc/ bb**	**dist ($\AA$)**
209	G	G(94) Y(3)CA	0.02	6/6	3.36
210	S	S(94)G T(5)	0.02	17/7	3.10
41	A	A(99)Y. SGR	0.03	2/1	4.74
208	G	G(84) N(11) S(1)E D(1)	0.03	21/21	2.82
40	S	S(98)T. KAGL	0.04	10/1	2.67
206	G	G(88)D S(6)P K(2) Q(1)TER	0.04	1/1	4.83
6	K	K(98) .(1)	0.05	4/0	3.83
8	G	G(98) .(1)	0.05	1/1	4.63
9	G	G(98) .(1)AS	0.05	1/1	4.43
193	F	F(94) Y(5)D	0.06	21/0	3.42
46	T	T(91) F(1) S(1)DN G(2)K H(1)P.A I	0.12	15/1	3.19

Table 10. The top 25% of residues in 2hmfA at the interface with 2hmfC. (Field names: res: residue number in the PDB entry; type: amino acid type; substs: substitutions seen in the alignment; with the percentage of each type in the bracket; noc/bb: number of contacts with the ligand, with the number of contacts realized through backbone atoms given in the bracket; dist: distance of closest apporach to the ligand.)

Table 11.		
res	**type**	**disruptive mutations**
501		
502		
503		
504		
130	E	(H)(FWR)(Y)(CG)
207	R	(TY)(D)(E)(SCG)
211	D	(R)(FWH)(Y)(VCAG)
209	G	(K)(ER)(Q)(MD)
210	S	(KR)(FQMWH)(E)(NLPI)
41	A	(KE)(R)(YD)(Q)
208	G	(R)(K)(FWH)(YM)

continued in next column

Table 11. *continued*		
res	**type**	**disruptive mutations**
40	S	(R)(K)(H)(FW)
206	G	(R)(FW)(H)(K)
6	K	(Y)(FTW)(SVCAG)(HD)
8	G	(KER)(FQMWHD)(NLPI)(Y)
9	G	(KR)(E)(QH)(FMWD)
193	F	(K)(E)(Q)(R)
46	T	(R)(K)(H)(FQW)

Table 11. List of disruptive mutations for the top 25% of residues in 2hmfA, that are at the interface with 2hmfC.

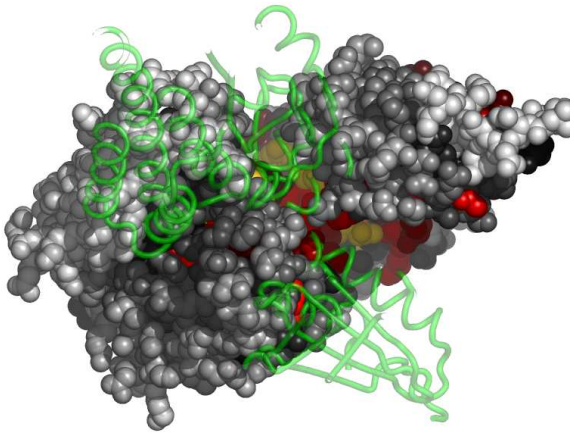

Fig. 9. Residues in 2hmfA, at the interface with 2hmfC, colored by their relative importance. 2hmfC is shown in backbone representation (See Appendix for the coloring scheme for the protein chain 2hmfA.)

Figure 9 shows residues in 2hmfA colored by their importance, at the interface with 2hmfC.

Magnesium ion binding site. Table 12 lists the top 25% of residues at the interface with 2hmfMG304 (magnesium ion). The following table (Table 13) suggests possible disruptive replacements for these residues (see Section 3.6).

Table 12.					
res	**type**	**subst's (%)**	**cvg**	**noc/ bb**	**dist (Å)**
504				3/0	4.29

Table 12. The top 25% of residues in 2hmfA at the interface with magnesium ion.(Field names: res: residue number in the PDB entry; type: amino acid type; substs: substitutions seen in the alignment; with the percentage of each type in the bracket; noc/bb: number of contacts with the ligand, with the number of contacts realized through backbone atoms given in the bracket; dist: distance of closest apporach to the ligand.)

Table 13.		
res	**type**	**disruptive mutations**
504		

Table 13. List of disruptive mutations for the top 25% of residues in 2hmfA, that are at the interface with magnesium ion.

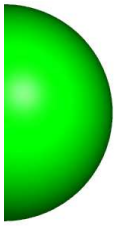

Fig. 10. Residues in 2hmfA, at the interface with magnesium ion, colored by their relative importance. The ligand (magnesium ion) is colored green. Atoms further than 30Å away from the geometric center of the ligand, as well as on the line of sight to the ligand were removed. (See Appendix for the coloring scheme for the protein chain 2hmfA.)

Figure 10 shows residues in 2hmfA colored by their importance, at the interface with 2hmfMG304.

ADP binding site. Table 14 lists the top 25% of residues at the interface with 2hmfADP404 (adp). The following table (Table 15) suggests possible disruptive replacements for these residues (see Section 3.6).

Table 14.					
res	**type**	**subst's (%)**	**cvg**	**noc/ bb**	**dist (Å)**
504				1/0	4.83

Table 14. The top 25% of residues in 2hmfA at the interface with ADP.(Field names: res: residue number in the PDB entry; type: amino acid type; substs: substitutions seen in the alignment; with the percentage of each type in the bracket; noc/bb: number of contacts with the ligand, with the number of contacts realized through backbone atoms given in the bracket; dist: distance of closest apporach to the ligand.)

Table 15.		
res	**type**	**disruptive mutations**
504		

Table 15. List of disruptive mutations for the top 25% of residues in 2hmfA, that are at the interface with ADP.

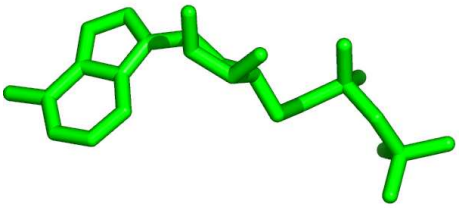

Fig. 11. Residues in 2hmfA, at the interface with ADP, colored by their relative importance. The ligand (ADP) is colored green. Atoms further than 30Å away from the geometric center of the ligand, as well as on the line of sight to the ligand were removed. (See Appendix for the coloring scheme for the protein chain 2hmfA.)

Figure 11 shows residues in 2hmfA colored by their importance, at the interface with 2hmfADP404.

ADP binding site. Table 16 lists the top 25% of residues at the interface with 2hmfADP401 (adp). The following table (Table 17) suggests possible disruptive replacements for these residues (see Section 3.6).

Table 16.					
res	**type**	**subst's (%)**	**cvg**	**noc/ bb**	**dist (Å)**
502				6/0	3.62
211	D	D(98)NE	0.01	3/0	4.38
240	P	P(99)T	0.01	18/2	3.72
264	G	G(99)C	0.01	3/3	4.32
232	V	V(95)MT I(2)FS	0.02	16/0	3.62
231	D	D(93) E(5)	0.03	18/2	2.77
continued in next column					

Table 16. *continued*					
res	**type**	**subst's (%)**	**cvg**	**noc/ bb**	**dist (Å)**
		H(1)N			
234	G	G(97) A(1) S(1)C	0.03	5/5	3.55
40	S	S(98)T. KAGL	0.04	1/0	4.81
230	T	T(87) K(8) S(3)G	0.04	20/3	2.65
239	D	D(91) N(7) H(1)	0.04	49/17	2.54
6	K	K(98) .(1)	0.05	4/0	3.59
8	G	G(98) .(1)	0.05	11/11	3.20
9	G	G(98) .(1)AS	0.05	5/5	2.90
265	A	S(16) A(79) F(3)TLN G	0.06	16/11	3.51
229	W	Y(45) W(43) F(8)MCV NIHL	0.10	2/2	4.20
267	V	V(87) I(11)QR KMG	0.10	60/24	3.29
10	T	T(71) A(1) S(24) .(1)VK	0.14	1/1	4.50
237	T	T(71) N(6) S(12) A(2) V(1)QCG FM(2)KI DR	0.16	1/1	4.79
235	V	V(60) I(24) F(6) M(6) L(1)C	0.17	15/10	3.75
238	T	T(44) C(9) S(1) A(38) V(2)M N(2)GI	0.20	17/17	3.52
266	K	K(68)	0.20	47/23	3.50
continued in next column					

Table 16. *continued*

res	type	subst's (%)	cvg	noc/ bb	dist (Å)
		A(2)			
		Q(4)			
		R(1)			
		S(9)			
		G(4)			
		N(3)V			
		E(4)			
		T(1)CH			
241	R	R(80)	0.21	20/1	2.72
		N(6)			
		K(5)			
		S(1)			
		G(1)QHD			
		ELAT(1)			
236	Y	Y(55)	0.25	26/16	2.80
		M(10)			
		L(13)			
		F(16)			
		H(3)CKA			

Table 16. The top 25% of residues in 2hmfA at the interface with ADP.(Field names: res: residue number in the PDB entry; type: amino acid type; substs: substitutions seen in the alignment; with the percentage of each type in the bracket; noc/bb: number of contacts with the ligand, with the number of contacts realized through backbone atoms given in the bracket; dist: distance of closest apporach to the ligand.)

Table 17.

res	type	disruptive mutations
502		
211	D	(R)(FWH)(Y)(VCAG)
240	P	(R)(YH)(K)(E)
264	G	(KER)(FQMWHD)(NYLPI)(SVA)
232	V	(R)(K)(E)(Y)
231	D	(R)(FW)(H)(YVCAG)
234	G	(KR)(E)(QH)(FMWD)
40	S	(R)(K)(H)(FW)
230	T	(R)(FKW)(H)(M)
239	D	(R)(FW)(VCAHG)(Y)
6	K	(Y)(FTW)(SVCAG)(HD)
8	G	(KER)(FQMWHD)(NLPI)(Y)
9	G	(KR)(E)(QH)(FMWD)
265	A	(R)(K)(E)(Y)
229	W	(E)(K)(TD)(Q)
267	V	(Y)(E)(HR)(D)
10	T	(R)(K)(H)(FW)
237	T	(R)(K)(H)(FW)
235	V	(R)(Y)(KE)(H)
238	T	(R)(K)(H)(FW)
266	K	(Y)(FW)(T)(H)

continued in next column

Table 17. *continued*

res	type	disruptive mutations
241	R	(Y)(T)(D)(E)
236	Y	(K)(EQ)(R)(M)

Table 17. List of disruptive mutations for the top 25% of residues in 2hmfA, that are at the interface with ADP.

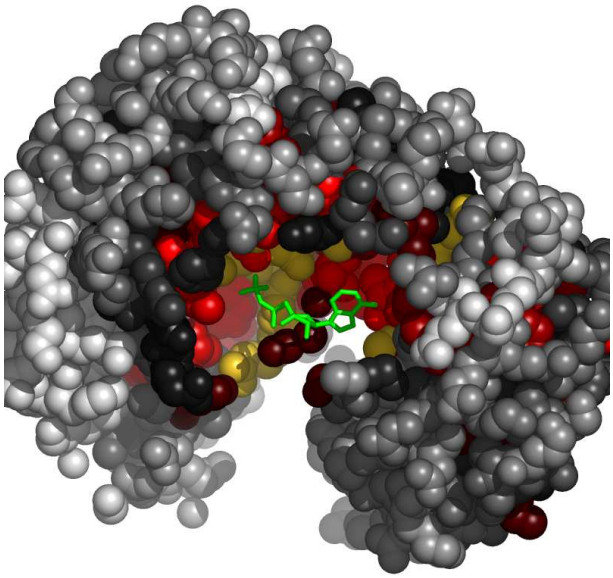

Fig. 12. Residues in 2hmfA, at the interface with ADP, colored by their relative importance. The ligand (ADP) is colored green. Atoms further than 30Å away from the geometric center of the ligand, as well as on the line of sight to the ligand were removed. (See Appendix for the coloring scheme for the protein chain 2hmfA.)

Figure 12 shows residues in 2hmfA colored by their importance, at the interface with 2hmfADP401.

Magnesium ion binding site. Table 18 lists the top 25% of residues at the interface with 2hmfMG303 (magnesium ion). The following table (Table 19) suggests possible disruptive replacements for these residues (see Section 3.6).

Table 18.

res	type	subst's (%)	cvg	noc/ bb	dist (Å)
503				3/0	4.45

Table 18. The top 25% of residues in 2hmfA at the interface with magnesium ion.(Field names: res: residue number in the PDB entry; type: amino acid type; substs: substitutions seen in the alignment; with the percentage of each type in the bracket; noc/bb: number of contacts with the ligand, with the number of contacts realized through backbone atoms given in the bracket; dist: distance of closest apporach to the ligand.)

Table 19.		
res	**type**	**disruptive mutations**
503		

Table 19. List of disruptive mutations for the top 25% of residues in 2hmfA, that are at the interface with magnesium ion.

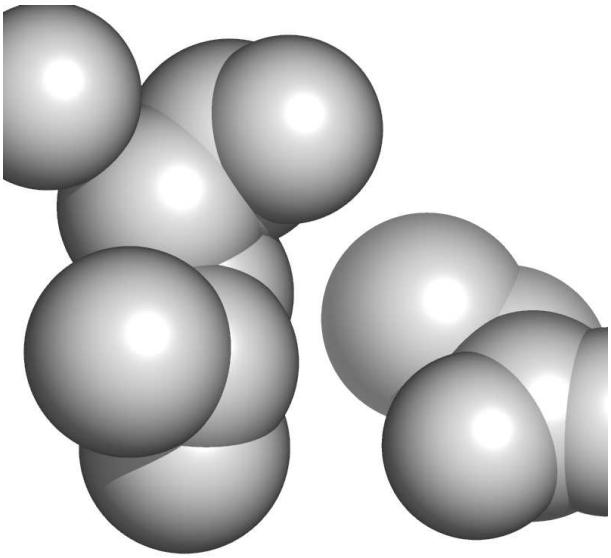

Fig. 13. Residues in 2hmfA, at the interface with magnesium ion, colored by their relative importance. The ligand (magnesium ion) is colored green. Atoms further than 30Å away from the geometric center of the ligand, as well as on the line of sight to the ligand were removed. (See Appendix for the coloring scheme for the protein chain 2hmfA.)

Figure 13 shows residues in 2hmfA colored by their importance, at the interface with 2hmfMG303.

Interface with 2hmfB. Table 20 lists the top 25% of residues at the interface with 2hmfB. The following table (Table 21) suggests possible disruptive replacements for these residues (see Section 3.6).

Table 20.					
res	**type**	**subst's (%)**	**cvg**	**noc/ bb**	**dist (Å)**
501				209/101	0.00
502				77/35	0.00
503				79/35	0.00
504				79/35	0.00
130	E	E(99)VSD	0.01	6/0	2.84
207	R	R(99)LVK	0.01	25/7	2.66
211	D	D(98)NE	0.01	1/1	4.74
continued in next column					

Table 20. *continued*					
res	**type**	**subst's (%)**	**cvg**	**noc/ bb**	**dist (Å)**
209	G	G(94)Y(3)CA	0.02	6/6	3.36
210	S	S(94)GT(5)	0.02	17/7	3.10
41	A	A(99)Y.SGR	0.03	2/1	4.74
208	G	G(84)N(11)S(1)ED(1)	0.03	21/21	2.82
259	E	E(95)DN(2)QMKA	0.03	34/4	2.52
40	S	S(98)T.KAGL	0.04	10/1	2.67
206	G	G(88)DS(6)PK(2)Q(1)TER	0.04	1/1	4.83
6	K	K(98).(1)	0.05	4/0	3.83
8	G	G(98).(1)	0.05	1/1	4.63
9	G	G(98).(1)AS	0.05	1/1	4.43
193	F	F(94)Y(5)D	0.06	21/0	3.42
275	P	F(15)P(47)I(8)L(15)M(2)Y(5)AQ(3)VCDTS	0.07	18/6	3.85
270	P	I(13)P(54)E(3)T(6)N(3)MD(3)L(4)H(1)S(1)V(4)A(1)Q	0.08	10/7	3.32
339	F	F(87)L(3)AC(1)ST(2)V(3)MI	0.10	61/21	3.37
212	Y	T(45)	0.11	5/0	3.88
continued in next column					

Table 20. *continued*

res	type	subst's (%)	cvg	noc/ bb	dist (Å)
		F(4) Y(30) I(4) V(2) L(10)AH W			
357	S	S(89)C T(1)D G(2).F N(2)VQA IHRE	0.11	72/34	2.67
46	T	T(91) F(1) S(1)DN G(2)K H(1)P.A I	0.12	15/1	3.19
333	G	G(94) E(1)S N(1)MLY DKAQVT	0.12	4/4	4.28
443	S	S(79) .(15)T N(3)DK	0.12	11/5	3.48
419	G	G(80) .(15) L(1)YNA EFDH	0.13	9/9	3.25
262	Y	S(35) Y(33) H(4) T(8)M A(6) N(6)WKL F(2)GQR E	0.14	48/0	3.54
216	L	A(48) L(20) V(7) I(19) Y(1) T(1) C(1)ESM	0.15	2/0	3.89
258	M	L(43) A(15) M(16) I(2) Q(2) R(7)V S(3) W(1)K C(3)N	0.15	31/6	3.33

continued in next column

Table 20. *continued*

res	type	subst's (%)	cvg	noc/ bb	dist (Å)
		T(1)GEF D			
263	F	L(34) F(32) S(3) A(5) T(1) M(6)R C(1)N V(3) Y(5) Q(4)EHK	0.16	14/0	3.80
442	S	T(43) A(12) S(22) .(15)C D(2) G(1)VPL I	0.16	76/32	2.77
425	F	F(74) .(15)A C(1)Y T(1) V(2) L(1)MSI W	0.17	27/6	3.59
438	I	I(73) .(15) V(3) Y(1)T L(3)M C(1)FHG	0.17	50/27	2.99
271	R	R(58) D(3) K(7) L(2) E(5) S(2) A(6) F(3) P(3) Q(3)N T(1)YIH	0.18	21/4	3.15
352	I	I(85) V(7)YF L(3)T S(1).M	0.18	51/33	3.02
342	L	L(78) I(3) F(5) M(7) V(3)A	0.19	6/4	4.14

continued in next column

Table 20. *continued*

res	type	subst's (%)	cvg	noc/ bb	dist (Å)
446	N	K(33) N(22) R(9) S(13) .(15)Q A(1) T(2)G	0.20	7/1	3.19
335	A	A(62)F L(14) S(8) T(6) V(1) D(1) I(1)G N(1)MEC Q	0.21	17/9	3.68
358	E	E(81)A N(2) T(1) D(1) K(4)L S(2)MR H(2)YVG QP	0.22	14/3	3.48
351	L	M(53) L(28) F(2) I(3) V(4)PS Q(1) A(3)T.C GRNE	0.23	19/10	3.34
434	N	N(74) .(15)T S(3) P(1)E H(1)KDA Q(1)GRM	0.23	24/0	2.89
437	M	M(46) A(20) .(16) L(5) F(1) V(4) I(3)TQ	0.23	27/20	3.00
362	S	S(62) G(3) T(10) I(3)F M(3) A(4)D C(6)L	0.24	17/3	3.16

continued in next column

Table 20. *continued*

res	type	subst's (%)	cvg	noc/ bb	dist (Å)
421	A	W(2)VYN HEQ A(60)G .(15) S(10) T(6)VF L(2)DEI MCP	0.25	11/6	4.14

Table 20. The top 25% of residues in 2hmfA at the interface with 2hmfB. (Field names: res: residue number in the PDB entry; type: amino acid type; substs: substitutions seen in the alignment; with the percentage of each type in the bracket; noc/bb: number of contacts with the ligand, with the number of contacts realized through backbone atoms given in the bracket; dist: distance of closest apporach to the ligand.)

Table 21.

res	type	disruptive mutations
501		
502		
503		
504		
130	E	(H)(FWR)(Y)(CG)
207	R	(TY)(D)(E)(SCG)
211	D	(R)(FWH)(Y)(VCAG)
209	G	(K)(ER)(Q)(MD)
210	S	(KR)(FQMWH)(E)(NLPI)
41	A	(KE)(R)(YD)(Q)
208	G	(R)(K)(FWH)(YM)
259	E	(H)(FYW)(CRG)(VA)
40	S	(R)(K)(H)(FW)
206	G	(R)(FW)(H)(K)
6	K	(Y)(FTW)(SVCAG)(HD)
8	G	(KER)(FQMWHD)(NLPI)(Y)
9	G	(KR)(E)(QH)(FMWD)
193	F	(K)(E)(Q)(R)
275	P	(R)(Y)(H)(K)
270	P	(R)(Y)(H)(T)
339	F	(K)(E)(R)(Q)
212	Y	(K)(Q)(E)(R)
357	S	(R)(K)(H)(FW)
46	T	(R)(K)(H)(FQW)
333	G	(R)(K)(H)(E)
443	S	(R)(FW)(H)(K)
419	G	(R)(K)(E)(Q)
262	Y	(K)(Q)(EM)(R)
216	L	(R)(Y)(H)(K)
258	M	(Y)(H)(T)(R)
263	F	(E)(K)(D)(T)

continued in next column

Table 21. *continued*		
res	**type**	**disruptive mutations**
442	S	(R)(K)(H)(Q)
425	F	(K)(E)(Q)(R)
438	I	(R)(Y)(K)(H)
271	R	(T)(Y)(D)(E)
352	I	(R)(Y)(H)(K)
342	L	(YR)(T)(H)(KE)
446	N	(Y)(FW)(H)(T)
335	A	(R)(Y)(K)(H)
358	E	(H)(FW)(Y)(R)
351	L	(Y)(R)(H)(T)
434	N	(Y)(FWH)(T)(R)
437	M	(Y)(H)(TR)(S)
362	S	(R)(K)(H)(Q)
421	A	(R)(K)(Y)(E)

Table 21. List of disruptive mutations for the top 25% of residues in 2hmfA, that are at the interface with 2hmfB.

Table 22.					
res	**type**	**subst's (%)**	**cvg**	**noc/ bb**	**dist (Å)**
501				3/0	3.53

Table 22. The top 25% of residues in 2hmfA at the interface with magnesium ion.(Field names: res: residue number in the PDB entry; type: amino acid type; substs: substitutions seen in the alignment; with the percentage of each type in the bracket; noc/bb: number of contacts with the ligand, with the number of contacts realized through backbone atoms given in the bracket; dist: distance of closest apporach to the ligand.)

Table 23.		
res	**type**	**disruptive mutations**
501		

Table 23. List of disruptive mutations for the top 25% of residues in 2hmfA, that are at the interface with magnesium ion.

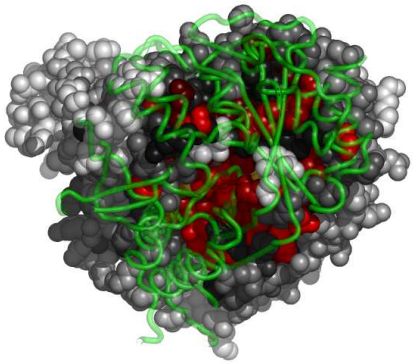

Fig. 14. Residues in 2hmfA, at the interface with 2hmfB, colored by their relative importance. 2hmfB is shown in backbone representation (See Appendix for the coloring scheme for the protein chain 2hmfA.)

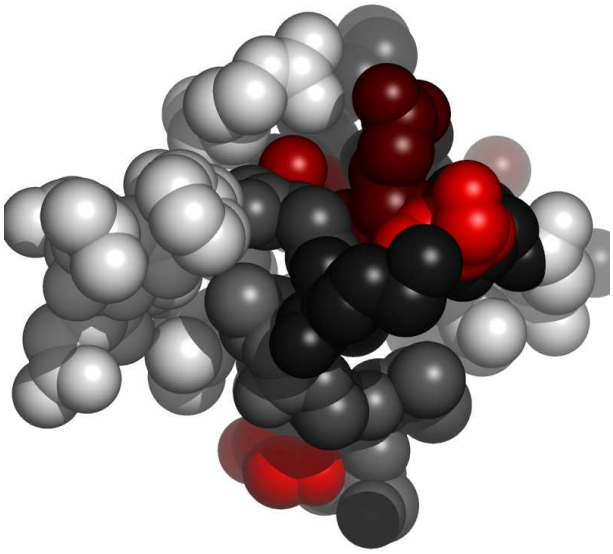

Fig. 15. Residues in 2hmfA, at the interface with magnesium ion, colored by their relative importance. The ligand (magnesium ion) is colored green. Atoms further than 30 Å away from the geometric center of the ligand, as well as on the line of sight to the ligand were removed. (See Appendix for the coloring scheme for the protein chain 2hmfA.)

Figure 14 shows residues in 2hmfA colored by their importance, at the interface with 2hmfB.

Magnesium ion binding site. Table 22 lists the top 25% of residues at the interface with 2hmfMG302 (magnesium ion). The following table (Table 23) suggests possible disruptive replacements for these residues (see Section 3.6).

Figure 15 shows residues in 2hmfA colored by their importance, at the interface with 2hmfMG302.

3 NOTES ON USING TRACE RESULTS

3.1 Coverage

Trace results are commonly expressed in terms of coverage: the residue is important if its "coverage" is small - that is if it belongs to

some small top percentage of residues [100% is all of the residues in a chain], according to trace. The ET results are presented in the form of a table, usually limited to top 25% percent of residues (or to some nearby percentage), sorted by the strength of the presumed evolutionary pressure. (I.e., the smaller the coverage, the stronger the pressure on the residue.) Starting from the top of that list, mutating a couple of residues should affect the protein somehow, with the exact effects to be determined experimentally.

3.2 Known substitutions

One of the table columns is "substitutions" - other amino acid types seen at the same position in the alignment. These amino acid types may be interchangeable at that position in the protein, so if one wants to affect the protein by a point mutation, they should be avoided. For example if the substitutions are "RVK" and the original protein has an R at that position, it is advisable to try anything, but RVK. Conversely, when looking for substitutions which will *n*ot affect the protein, one may try replacing, R with K, or (perhaps more surprisingly), with V. The percentage of times the substitution appears in the alignment is given in the immediately following bracket. No percentage is given in the cases when it is smaller than 1%. This is meant to be a rough guide - due to rounding errors these percentages often do not add up to 100%.

3.3 Surface

To detect candidates for novel functional interfaces, first we look for residues that are solvent accessible (according to DSSP program) by at least $10\mathring{A}^2$, which is roughly the area needed for one water molecule to come in the contact with the residue. Furthermore, we require that these residues form a "cluster" of residues which have neighbor within $5\mathring{A}$ from any of their heavy atoms.

Note, however, that, if our picture of protein evolution is correct, the neighboring residues which *are not* surface accessible might be equally important in maintaining the interaction specificity - they should not be automatically dropped from consideration when choosing the set for mutagenesis. (Especially if they form a cluster with the surface residues.)

3.4 Number of contacts

Another column worth noting is denoted "noc/bb"; it tells the number of contacts heavy atoms of the residue in question make across the interface, as well as how many of them are realized through the backbone atoms (if all or most contacts are through the backbone, mutation presumably won't have strong impact). Two heavy atoms are considered to be "in contact" if their centers are closer than $5\mathring{A}$.

3.5 Annotation

If the residue annotation is available (either from the pdb file or from other sources), another column, with the header "annotation" appears. Annotations carried over from PDB are the following: site (indicating existence of related site record in PDB), S-S (disulfide bond forming residue), hb (hydrogen bond forming residue, jb (james bond forming residue), and sb (for salt bridge forming residue).

3.6 Mutation suggestions

Mutation suggestions are completely heuristic and based on complementarity with the substitutions found in the alignment. Note that they are meant to be **disruptive** to the interaction of the protein with its ligand. The attempt is made to complement the following properties: small $[AVGSTC]$, medium $[LPNQDEMIK]$, large $[WFYHR]$, hydrophobic $[LPVAMWFI]$, polar $[GTCY]$; positively $[KHR]$, or negatively $[DE]$ charged, aromatic $[WFYH]$, long aliphatic chain $[EKRQM]$, OH-group possession $[SDETY]$, and NH2 group possession $[NQRK]$. The suggestions are listed according to how different they appear to be from the original amino acid, and they are grouped in round brackets if they appear equally disruptive. From left to right, each bracketed group of amino acid types resembles more strongly the original (i.e. is, presumably, less disruptive) These suggestions are tentative - they might prove disruptive to the fold rather than to the interaction. Many researcher will choose, however, the straightforward alanine mutations, especially in the beginning stages of their investigation.

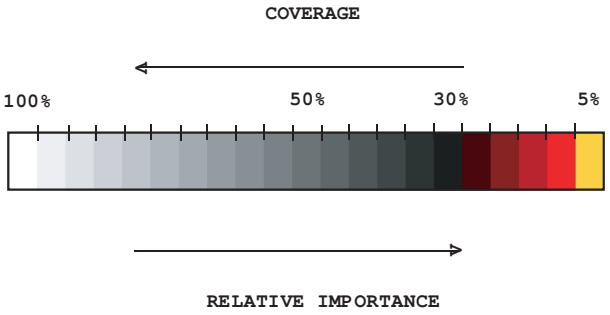

Fig. 16. Coloring scheme used to color residues by their relative importance.

4 APPENDIX

4.1 File formats

Files with extension "ranks_sorted" are the actual trace results. The fields in the table in this file:

- `alignment#` number of the position in the alignment
- `residue#` residue number in the PDB file
- `type` amino acid type
- `rank` rank of the position according to older version of ET
- `variability` has two subfields:
  1. number of different amino acids appearing in in this column of the alignment
  2. their type
- `rho` ET score - the smaller this value, the lesser variability of this position across the branches of the tree (and, presumably, the greater the importance for the protein)
- `cvg` coverage - percentage of the residues on the structure which have this rho or smaller
- `gaps` percentage of gaps in this column

4.2 Color schemes used

The following color scheme is used in figures with residues colored by cluster size: black is a single-residue cluster; clusters composed of more than one residue colored according to this hierarchy (ordered by descending size): red, blue, yellow, green, purple, azure, turquoise, brown, coral, magenta, LightSalmon, SkyBlue, violet, gold, bisque, LightSlateBlue, orchid, RosyBrown, MediumAquamarine, DarkOliveGreen, CornflowerBlue, grey55, burlywood, LimeGreen, tan, DarkOrange, DeepPink, maroon, BlanchedAlmond.

The colors used to distinguish the residues by the estimated evolutionary pressure they experience can be seen in Fig. 16.

4.3 Credits

4.3.1 **Alistat** *alistat* reads a multiple sequence alignment from the file and shows a number of simple statistics about it. These statistics include the format, the number of sequences, the total number of residues, the average and range of the sequence lengths, and the alignment length (e.g. including gap characters). Also shown are some percent identities. A percent pairwise alignment identity is defined as (idents / MIN(len1, len2)) where idents is the number of exact identities and len1, len2 are the unaligned lengths of the two sequences. The "average percent identity", "most related pair", and "most unrelated pair" of the alignment are the average, maximum, and minimum of all (N)(N-1)/2 pairs, respectively. The "most distant seq" is calculated by finding the maximum pairwise identity (best relative) for all N sequences, then finding the minimum of these N numbers (hence, the most outlying sequence). *alistat* is copyrighted by HHMI/Washington University School of Medicine, 1992-2001, and freely distributed under the GNU General Public License.

4.3.2 **CE** To map ligand binding sites from different source structures, report_maker uses the CE program: `http://cl.sdsc.edu/`. Shindyalov IN, Bourne PE (1998) *"Protein structure alignment by incremental combinatorial extension (CE) of the optimal path* . Protein Engineering 11(9) 739-747.

4.3.3 **DSSP** In this work a residue is considered solvent accessible if the DSSP program finds it exposed to water by at least 10Å^2, which is roughly the area needed for one water molecule to come in the contact with the residue. DSSP is copyrighted by W. Kabsch, C. Sander and MPI-MF, 1983, 1985, 1988, 1994 1995, CMBI version by Elmar.Krieger`@cmbi.kun.nl` November 18,2002,

`http://www.cmbi.kun.nl/gv/dssp/descrip.html.`

4.3.4 **HSSP** Whenever available, report_maker uses HSSP alignment as a starting point for the analysis (sequences shorter than 75% of the query are taken out, however); R. Schneider, A. de Daruvar, and C. Sander. *"The HSSP database of protein structure-sequence alignments."* Nucleic Acids Res., 25:226–230, 1997.

`http://swift.cmbi.kun.nl/swift/hssp/`

4.3.5 **LaTex** The text for this report was processed using LaTeX; Leslie Lamport, "LaTeX: A Document Preparation System Addison-Wesley," Reading, Mass. (1986).

4.3.6 **Muscle** When making alignments "from scratch", report maker uses Muscle alignment program: Edgar, Robert C. (2004), *"MUSCLE: multiple sequence alignment with high accuracy and high throughput."* Nucleic Acids Research 32(5), 1792-97.

`http://www.drive5.com/muscle/`

4.3.7 **Pymol** The figures in this report were produced using Pymol. The scripts can be found in the attachment. Pymol is an open-source application copyrighted by DeLano Scientific LLC (2005). For more information about Pymol see `http://pymol.sourceforge.net/`. (Note for Windows users: the attached package needs to be unzipped for Pymol to read the scripts and launch the viewer.)

4.4 Note about ET Viewer

Dan Morgan from the Lichtarge lab has developed a visualization tool specifically for viewing trace results. If you are interested, please visit:

`http://mammoth.bcm.tmc.edu/traceview/`

The viewer is self-unpacking and self-installing. Input files to be used with ETV (extension .etvx) can be found in the attachment to the main report.

4.5 Citing this work

The method used to rank residues and make predictions in this report can be found in Mihalek, I., I. Reš, O. Lichtarge. (2004). *"A Family of Evolution-Entropy Hybrid Methods for Ranking of Protein Residues by Importance"* J. Mol. Bio. **336**: 1265-82. For the original version of ET see O. Lichtarge, H.Bourne and F. Cohen (1996). *"An Evolutionary Trace Method Defines Binding Surfaces Common to Protein Families"* J. Mol. Bio. **257**: 342-358.

report_maker itself is described in Mihalek I., I. Res and O. Lichtarge (2006). *"Evolutionary Trace Report Maker: a new type of service for comparative analysis of proteins."* Bioinformatics **22**:1656-7.

4.6 About report_maker

report_maker was written in 2006 by Ivana Mihalek. The 1D ranking visualization program was written by Ivica Reš. **report_maker** is copyrighted by Lichtarge Lab, Baylor College of Medicine, Houston.

4.7 Attachments

The following files should accompany this report:

- 2hmfA.complex.pdb - coordinates of 2hmfA with all of its interacting partners
- 2hmfA.etvx - ET viewer input file for 2hmfA
- 2hmfA.cluster_report.summary - Cluster report summary for 2hmfA
- 2hmfA.ranks - Ranks file in sequence order for 2hmfA
- 2hmfA.clusters - Cluster descriptions for 2hmfA
- 2hmfA.msf - the multiple sequence alignment used for the chain 2hmfA
- 2hmfA.descr - description of sequences used in 2hmfA msf
- 2hmfA.ranks_sorted - full listing of residues and their ranking for 2hmfA
- 2hmfA.2hmfADP403.if.pml - Pymol script for Figure 5
- 2hmfA.cbcvg - used by other 2hmfA – related pymol scripts
- 2hmfA.2hmfD.if.pml - Pymol script for Figure 6

- 2hmfA.2hmfMG301.if.pml - Pymol script for Figure 7
- 2hmfA.2hmfADP402.if.pml - Pymol script for Figure 8
- 2hmfA.2hmfC.if.pml - Pymol script for Figure 9
- 2hmfA.2hmfMG304.if.pml - Pymol script for Figure 10
- 2hmfA.2hmfADP404.if.pml - Pymol script for Figure 11
- 2hmfA.2hmfADP401.if.pml - Pymol script for Figure 12
- 2hmfA.2hmfMG303.if.pml - Pymol script for Figure 13
- 2hmfA.2hmfB.if.pml - Pymol script for Figure 14
- 2hmfA.2hmfMG302.if.pml - Pymol script for Figure 15