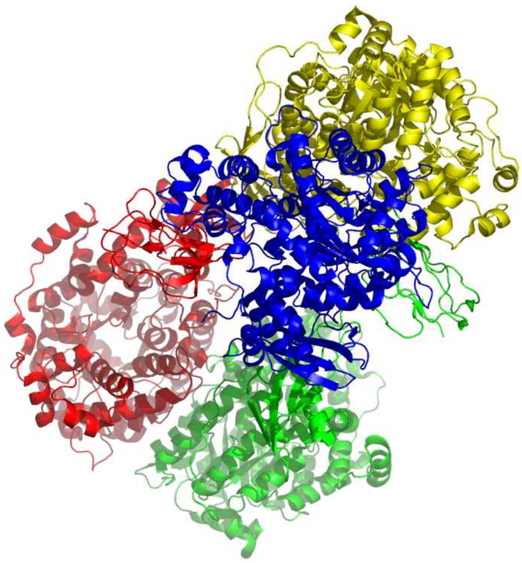
3bg3

Evolutionary trace report by **report_maker**

March 25, 2010



CONTENTS

1 INTRODUCTION

From the original Protein Data Bank entry (PDB id 3bg3):
Title: Crystal structure of human pyruvate carboxylase (missing the biotin carboxylase domain at the n-terminus)
Compound: Mol id: 1; molecule: pyruvate carboxylase, mitochondrial; chain: a, b, c, d; fragment: ct+pt+bccp domain; synonym: pyruvic carboxylase, pcb; ec: 6.4.1.1; engineered: yes
Organism, scientific name: Homo Sapiens;

3bg3 contains a single unique chain 3bg3A (680 residues long) and its homologues 3bg3D, 3bg3C, and 3bg3B.

2 CHAIN 3BG3A

2.1 P11498 overview

From SwissProt, id P11498, 92% identical to 3bg3A:
Description: Pyruvate carboxylase, mitochondrial precursor (EC 6.4.1.1) (Pyruvic carboxylase) (PCB).
Organism, scientific name: Homo sapiens (Human).
Taxonomy: Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Catarrhini; Hominidae; Homo.
Function: Pyruvate carboxylase catalyzes a 2-step reaction, involving the ATP-dependent carboxylation of the covalently attached biotin in the first step and the transfer of the carboxyl group to pyruvate in the second. Catalyzes in a tissue specific manner, the initial reactions of glucose (liver, kidney) and lipid (adipose tissue, liver, brain) synthesis from pyruvate.
Catalytic activity: ATP + pyruvate + HCO(3)(-) = ADP + phosphate + oxaloacetate.
Cofactor: Biotin and manganese.
Pathway: Gluconeogenesis and lipogenesis.
Subunit: Homotetramer.
Subcellular location: Mitochondrial matrix.
Disease: Defects in PC are the cause of pyruvate carboxylase deficiency (PC deficiency) [MIM:266150]. PC deficiency leads to lactic acidosis, mental retardation and death. It occurs in three forms: mild or type A, severe neonatal or type B, and a very mild lacticacidemia.
Similarity: Contains 1 ATP-grasp domain.
Similarity: Contains 1 biotin carboxylation domain.

Similarity: Contains 1 biotinyl-binding domain.
Similarity: Contains 1 carboxyltransferase domain.
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 3bg3A

For the chain 3bg3A, the alignment 3bg3A.msf (attached) with 412 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 to this report, under the name of 3bg3A.msf. Its statistics, from the *alistat* program are the following:

```
Format:                MSF
Number of sequences: 412
Total number of residues:    259021
Smallest:              510
Largest:               680
Average length:        628.7
Alignment length:      680
Average identity:      42%
Most related pair:     99%
Most unrelated pair: 21%
Most distant seq:      38%
```

Furthermore, 3% of residues show as conserved in this alignment.

The alignment consists of 6% eukaryotic (1% vertebrata, <1% arthropoda, 4% fungi), 13% 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 3bg3A.descr.

2.3 Residue ranking in 3bg3A

The 3bg3A sequence is shown in Figs. 1–2, with each residue colored according to its estimated importance. The full listing of residues in 3bg3A can be found in the file called 3bg3A.ranks_sorted in the attachment.

2.4 Top ranking residues in 3bg3A 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 3bg3A 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.

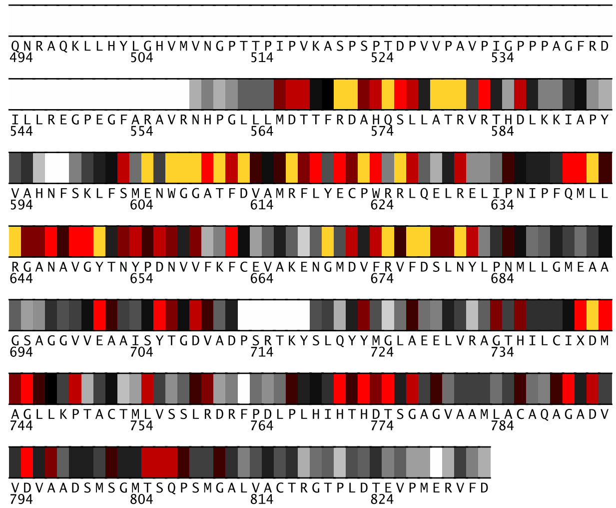


Fig. 1. Residues 494-833 in 3bg3A colored by their relative importance. (See Appendix, Fig.11, for the coloring scheme.)

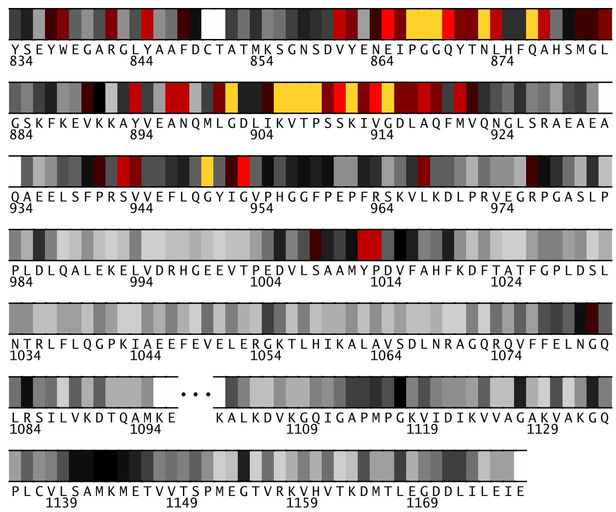


Fig. 2. Residues 834-1178 in 3bg3A colored by their relative importance. (See Appendix, Fig.11, for the coloring scheme.)

Table 1.		
cluster color	**size**	**member residues**
red	160	566,567,568,570,571,572,573 574,575,576,577,579,580,581 583,586,603,605,607,608,609 610,611,612,613,614,616,617 618,619,621,622,623,624,626 627,631,635,640,641,642,643 644,645,646,647,648,649,650 651,653,654,655,656,657,659 662,670,672,674,675,676,677

continued in next column

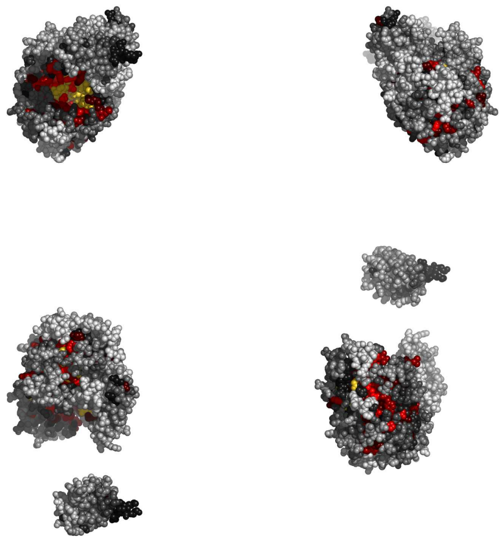


Fig. 3. Residues in 3bg3A, colored by their relative importance. Clockwise: front, back, top and bottom views.

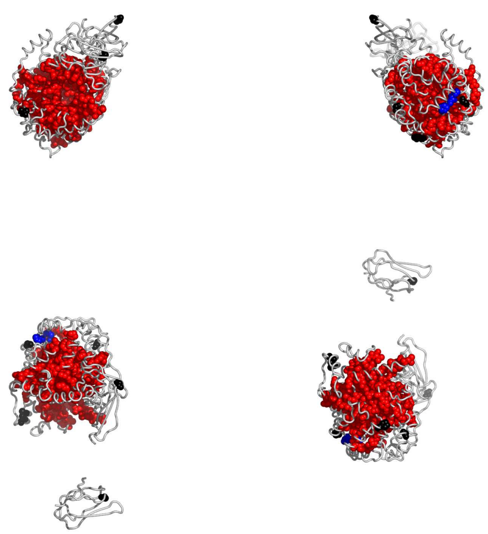


Fig. 4. Residues in 3bg3A, colored according to the cluster they belong to: red, followed by blue and yellow are the largest clusters (see Appendix for the coloring scheme). Clockwise: front, back, top and bottom views. The corresponding Pymol script is attached.

Table 1. *continued*		
cluster color	**size**	**member residues**
		678,679,680,681,682,685,701
		702,706,707,709,710,723,727
		741,742,743,744,745,746,755
		continued in next column

Table 1. *continued*		
cluster color	**size**	**member residues**
		759,760,771,772,773,774,775
		777,779,786,789,790,792,795
		797,802,805,806,807,808,837
		838,842,845,848,861,862,864
		865,866,867,868,869,870,871
		872,873,874,877,878,881,882
		883,890,894,897,898,901,902
		905,906,907,908,909,910,911
		912,913,914,915,916,917,918
		919,921,922,941,943,944,950
		952,953,977,1009,1013,1014
blue	2	736,767

Table 1. Clusters of top ranking residues in 3bg3A.

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.

Manganese (ii) ion binding site. Table 2 lists the top 25% of residues at the interface with 3bg3AMN2001 (manganese (ii) ion). 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)**	**antn**
571	R	R(100)	0.03	1/0	4.47	site
572	D	D(100)	0.03	4/0	2.10	site
741	X	K(99)X	0.06	5/0	1.96	
773	H	H(98) Q(1)	0.06	5/0	2.19	site
743	M	M(98)A Q(1)	0.07	2/1	4.76	
771	H	H(94) Q(5)	0.09	5/0	2.29	site
807	Q	H(29) Q(69)LN	0.10	3/0	4.30	

Table 2. The top 25% of residues in 3bg3A at the interface with manganese (ii) 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 3.		
res	**type**	**disruptive mutations**
571	R	(TD)(SYEVCLAPIG)(FMW)(N)
572	D	(R)(FWH)(KYVCAG)(TQM)
741	X	(Y)(FTEW)(HDR)(SKVMCAG)
773	H	(TE)(D)(SVMCAG)(QLPI)
		continued in next column

Table 3. *continued*		
res	**type**	**disruptive mutations**
743	M	(Y)(H)(T)(R)
771	H	(TE)(D)(SVMCAG)(QLPI)
807	Q	(Y)(T)(FWH)(SCG)

Table 3. List of disruptive mutations for the top 25% of residues in 3bg3A, that are at the interface with manganese (ii) ion.

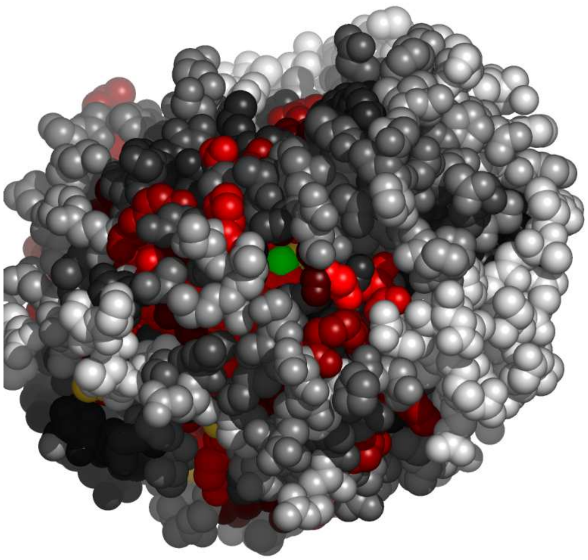


Fig. 5. Residues in 3bg3A, at the interface with manganese (ii) ion, colored by their relative importance. The ligand (manganese (ii) ion) is colored green. Atoms further than 30$\AA$ away from the geometric center of the ligand, as well as on the line of sight to the ligand were removed. (See Appendix for the coloring scheme for the protein chain 3bg3A.)

Figure 5 shows residues in 3bg3A colored by their importance, at the interface with 3bg3AMN2001.

Interface with 3bg3D. By analogy with 3bg3C – 3bg3D interface. Table 4 lists the top 25% of residues at the interface with 3bg3D. 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 ($\AA$)**	**antn**
877	Q	Q(100)	0.03	42/9	2.65	
882	G	G(82) N(13) D(1) S(1) K(1)H	0.20	8/8	3.54	
881	M	Q(34) L(41) V(8) M(9) F(3)ANI	0.23	100/27	1.18	
1143	M	.(16) M(83)	0.25	3/0	4.54	
1144	K	.(16) K(83)	0.25	23/0	2.75	site

Table 4. The top 25% of residues in 3bg3A at the interface with 3bg3D. (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**
877	Q	(Y)(FTWH)(SVCAG)(D)
882	G	(R)(E)(FKW)(M)
881	M	(Y)(TH)(R)(SCG)
1143	M	(Y)(TH)(SCG)(R)
1144	K	(Y)(FTW)(SVCAG)(HD)

Table 5. List of disruptive mutations for the top 25% of residues in 3bg3A, that are at the interface with 3bg3D.

Figure 6 shows residues in 3bg3A colored by their importance, at the interface with 3bg3D.

Pyruvic acid binding site. Table 6 lists the top 25% of residues at the interface with 3bg3APYR2000 (pyruvic acid). 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 ($\AA$)**	**antn**
571	R	R(100)	0.03	10/0	3.03	site
572	D	D(100)	0.03	3/0	4.29	site
575	Q	Q(100)	0.03	10/0	2.73	site
609	G	G(100)	0.03	5/5	3.36	site
908	T	T(100)	0.03	12/3	3.12	site
642	L	L(99)R	0.04	9/0	3.37	
644	R	R(99)V	0.04	15/0	2.62	site
677	F	F(99)I	0.04	8/0	4.15	
907	V	V(99)L	0.05	4/2	3.65	
741	X	K(99)X	0.06	14/0	3.54	
610	A	A(99)T	0.07	3/3	3.82	

Table 6. The top 25% of residues in 3bg3A at the interface with pyruvic acid.(Field names: res: residue number in the PDB entry; type: amino acid type; substs: substitutions seen in the alignment; with the percentage of each

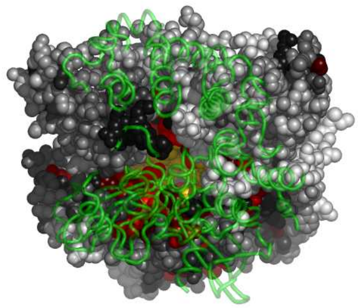


Fig. 6. Residues in 3bg3A, at the interface with 3bg3D, colored by their relative importance. 3bg3D is shown in backbone representation (See Appendix for the coloring scheme for the protein chain 3bg3A.)

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 7.		
res	**type**	**disruptive mutations**
571	R	(TD)(SYEVCLAPIG)(FMW)(N)
572	D	(R)(FWH)(KYVCAG)(TQM)
575	Q	(Y)(FTWH)(SVCAG)(D)
609	G	(KER)(FQMWHD)(NYLPI)(SVA)
908	T	(KR)(FQMWH)(NELPI)(D)
642	L	(Y)(T)(SECHRG)(D)
644	R	(D)(TYE)(SCLPIG)(FVMAW)
677	F	(KE)(T)(QDR)(SCG)
907	V	(YR)(KE)(H)(QD)
741	X	(Y)(FTEW)(HDR)(SKVMCAG)
610	A	(KR)(E)(YQH)(D)

Table 7. List of disruptive mutations for the top 25% of residues in 3bg3A, that are at the interface with pyruvic acid.

Figure 7 shows residues in 3bg3A colored by their importance, at the interface with 3bg3APYR2000.

BTI binding site. By analogy with 3bg3B – 3bg3ABTI2100 interface. Table 8 lists the top 25% of residues at the interface with 3bg3ABTI2100 (bti). The following table (Table 9) suggests possible disruptive replacements for these residues (see Section 3.6).

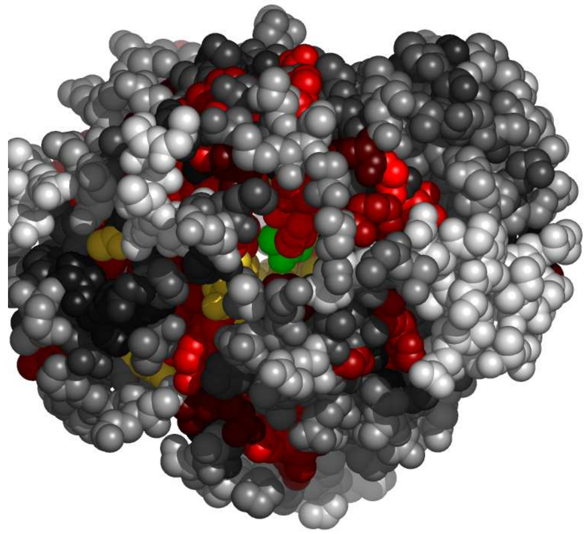


Fig. 7. Residues in 3bg3A, at the interface with pyruvic acid, colored by their relative importance. The ligand (pyruvic acid) is colored green. Atoms further than 30Å away from the geometric center of the ligand, as well as on the line of sight to the ligand were removed. (See Appendix for the coloring scheme for the protein chain 3bg3A.)

Table 8.						
res	**type**	**subst's (%)**	**cvg**	**noc/ bb**	**dist (Å)**	**antn**
575	Q	Q(100)	0.03	5/0	3.41	site
613	D	D(100)	0.03	6/0	3.98	
869	G	G(100)	0.03	1/1	4.73	
908	T	T(100)	0.03	28/6	2.09	site
909	P	P(100)	0.03	2/2	4.76	
644	R	R(99)V	0.04	4/0	3.53	site
651	Y	Y(99)S	0.04	10/0	3.29	
617	R	R(98)NS	0.05	6/0	4.15	
907	V	V(99)L	0.05	2/2	4.81	
912	K	Q(36) K(63)	0.05	18/3	3.51	
911	S	S(99)A	0.06	7/2	2.95	
610	A	A(99)T	0.07	7/1	4.22	
870	Q	M(36) Q(63)T	0.07	8/5	4.43	
618	F	Y(17) F(82)	0.17	4/0	3.39	
614	V	A(14) V(61) S(15) T(8)	0.22	3/0	4.14	

Table 8. The top 25% of residues in 3bg3A at the interface with BTI.(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**
575	Q	(Y)(FTWH)(SVCAG)(D)
613	D	(R)(FWH)(KYVCAG)(TQM)
869	G	(KER)(FQMWHD)(NYLPI)(SVA)
908	T	(KR)(FQMWH)(NELPI)(D)
909	P	(YR)(TH)(SKECG)(FQWD)
644	R	(D)(TYE)(SCLPIG)(FVMAW)
651	Y	(K)(QM)(R)(NELPI)
617	R	(TYD)(E)(FVCLAWPIG)(SM)
907	V	(YR)(KE)(H)(QD)
912	K	(Y)(FTW)(SVCAG)(H)
911	S	(KR)(QH)(FYEMW)(N)
610	A	(KR)(E)(YQH)(D)
870	Q	(Y)(H)(FW)(T)
618	F	(K)(E)(Q)(D)
614	V	(KR)(E)(Y)(QH)

Table 9. List of disruptive mutations for the top 25% of residues in 3bg3A, that are at the interface with BTI.

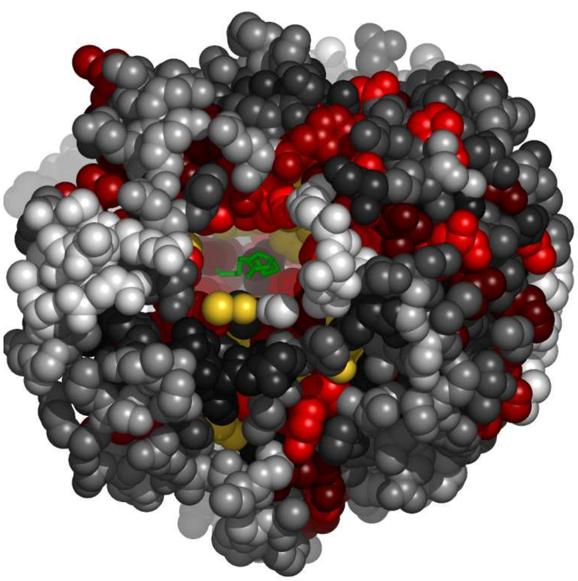


Fig. 8. Residues in 3bg3A, at the interface with BTI, colored by their relative importance. The ligand (BTI) is colored green. Atoms further than 30$\AA$ away from the geometric center of the ligand, as well as on the line of sight to the ligand were removed. (See Appendix for the coloring scheme for the protein chain 3bg3A.)

Figure 8 shows residues in 3bg3A colored by their importance, at the interface with 3bg3ABTI2100.

Interface with 3bg3B. By analogy with 3bg3C – 3bg3B interface. Table 10 lists the top 25% of residues at the interface with 3bg3B. 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 ($\AA$)**
749	P	P(96) A(3)RV	0.11	8/1	3.70
777	G	G(93) S(4)EDA	0.14	8/8	3.40
862	Y	L(30) Y(60) Q(3) F(3) I(1)HW	0.15	14/0	3.32
842	R	R(95)I K(2)VLE SNM	0.16	20/0	3.76
883	L	A(35) L(58) I(2)S M(1)CV	0.17	11/3	3.95
802	S	S(74) A(25)	0.21	5/5	3.98
811	G	E(35) N(20) S(14) G(25). T(1) Q(2)D	0.22	9/9	3.56

Table 10. The top 25% of residues in 3bg3A at the interface with 3bg3B. (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**
749	P	(Y)(R)(TEH)(K)
777	G	(R)(K)(H)(FW)
862	Y	(K)(EQ)(R)(M)
842	R	(Y)(T)(D)(CG)
883	L	(R)(Y)(H)(K)
802	S	(KR)(QH)(FYEMW)(N)
811	G	(R)(FWH)(K)(E)

Table 11. List of disruptive mutations for the top 25% of residues in 3bg3A, that are at the interface with 3bg3B.

Figure 9 shows residues in 3bg3A colored by their importance, at the interface with 3bg3B.

Interface with 3bg3C. By analogy with 3bg3D – 3bg3C interface. Table 12 lists the top 25% of residues at the interface with 3bg3C. The

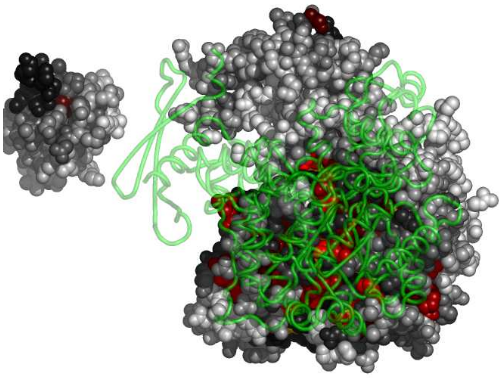


Fig. 9. Residues in 3bg3A, at the interface with 3bg3B, colored by their relative importance. 3bg3B is shown in backbone representation (See Appendix for the coloring scheme for the protein chain 3bg3A.)

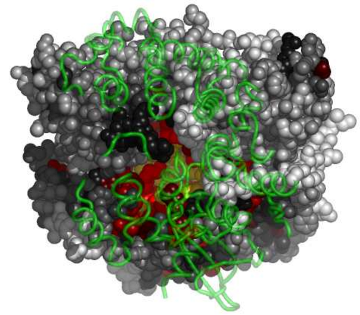


Fig. 10. Residues in 3bg3A, at the interface with 3bg3C, colored by their relative importance. 3bg3C is shown in backbone representation (See Appendix for the coloring scheme for the protein chain 3bg3A.)

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)	antn
1143	M	.(16) M(83)	0.25	9/0	3.25	
1144	K	.(16) K(83)	0.25	32/2	3.28	site

Table 12. The top 25% of residues in 3bg3A at the interface with 3bg3C. (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
1143	M	(Y)(TH)(SCG)(R)
1144	K	(Y)(FTW)(SVCAG)(HD)

Table 13. List of disruptive mutations for the top 25% of residues in 3bg3A, that are at the interface with 3bg3C.

Figure 10 shows residues in 3bg3A colored by their importance, at the interface with 3bg3C.

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 $10\AA^2$, 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 $5\AA$ 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 $5\AA$.

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.

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

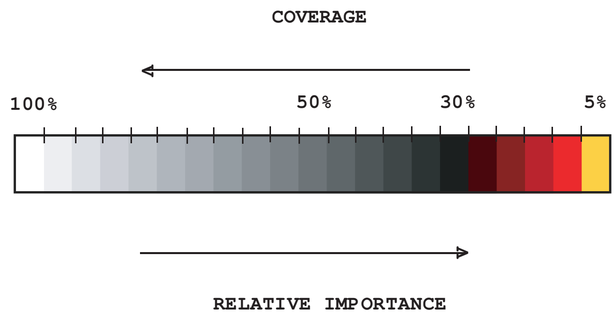


Fig. 11. Coloring scheme used to color residues by their relative importance.

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. 11.

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:

- 3bg3A.complex.pdb - coordinates of 3bg3A with all of its interacting partners
- 3bg3A.etvx - ET viewer input file for 3bg3A
- 3bg3A.cluster_report.summary - Cluster report summary for 3bg3A
- 3bg3A.ranks - Ranks file in sequence order for 3bg3A
- 3bg3A.clusters - Cluster descriptions for 3bg3A
- 3bg3A.msf - the multiple sequence alignment used for the chain 3bg3A
- 3bg3A.descr - description of sequences used in 3bg3A msf
- 3bg3A.ranks_sorted - full listing of residues and their ranking for 3bg3A
- 3bg3A.3bg3AMN2001.if.pml - Pymol script for Figure 5
- 3bg3A.cbcvg - used by other 3bg3A – related pymol scripts
- 3bg3A.3bg3D.if.pml - Pymol script for Figure 6
- 3bg3A.3bg3APYR2000.if.pml - Pymol script for Figure 7
- 3bg3A.3bg3ABTI2100.if.pml - Pymol script for Figure 8
- 3bg3A.3bg3B.if.pml - Pymol script for Figure 9
- 3bg3A.3bg3C.if.pml - Pymol script for Figure 10