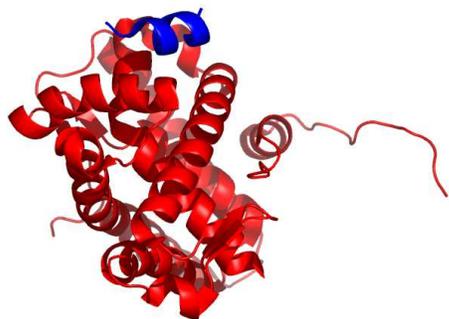


3mne

Evolutionary trace report by **report_maker**

September 23, 2010



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1 INTRODUCTION

From the original Protein Data Bank entry (PDB id 3mne):

Title: Crystal structure of the agonist form of mouse glucocorticoid stabilized by f608s mutation at 1.96a

Compound: Mol id: 1; molecule: glucocorticoid receptor; chain: a; fragment: unpaired residues 527-783; synonym: gr, nuclear receptor subfamily 3 group c member 1; engineered: yes; mutation: yes; mol id: 2; molecule: nuclear receptor coactivator 2 peptide; chain: b; fragment: tif2 coactivator motif, residues 740-752; synonym: ncoa-2, transcriptional intermediary factor 2, glucocorticoid receptor-interacting protein 1, grip-1; engineered: yes

1 **Organism, scientific name:** Mus Musculus;

3mne contains a single unique chain 3mneA (253 residues long).

1 Chain 3mneB is too short (10 residues) to permit statistically significant analysis, and was treated as a peptide ligand.

2 CHAIN 3MNEA

2.1 P06537 overview

From SwissProt, id P06537, 97% identical to 3mneA:

Description: Glucocorticoid receptor (GR).

Organism, scientific name: Mus musculus (Mouse).

Taxonomy: Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Sciurognathi; Muroidea; Muridae; Murinae; Mus.

6 Function: Receptor for glucocorticoids (GC). Has a dual mode of action: as a transcription factor that binds to glucocorticoid response elements (GRE) and as a modulator of other transcription factors. Affects inflammatory responses, cellular proliferation and differentiation in target tissues.

7 Subunit: Heteromultimeric cytoplasmic complex with HSP90, HSP70, and FKBP5 or another immunophilin, or the immunophilin homolog PPP5C. Upon ligand binding FKBP5 dissociates from the complex and FKBP4 takes its place, thereby linking the complex to dynein and mediating transport to the nucleus, where the complex dissociates. Binds to DNA as a homodimer, and as heterodimer with NR3C2 or the retinoid X receptor. Binds STAT5A and STAT5B

homodimers and heterodimers. Interacts with NRIP1, TGFB111, POU2F1, POU2F2 and TRIM28. Interacts with several coactivator complexes, including the SMARCA4 complex, CREBBP/EP300, TADA2L (Ada complex) and p160 coactivators such as NCOA2 and NCOA6. Interaction with BAG1 inhibits transactivation (By similarity).

Interaction:

Subcellular location: Cytoplasmic in the absence of ligand; nuclear after ligand-binding.

Alternative products:

Event=Alternative splicing; Named isoforms=2; Name=1; Synonyms=1-A, GR form A; IsoId=P06537-1; Sequence=Displayed; Note=Isoform 1-B is produced by alternative initiation at Met-28 of isoform 1-A; Name=2; Synonyms=2-A, GR form B; IsoId=P06537-2; Sequence=VSP 003704; Note=Isoform 2-B is produced by alternative initiation at Met-28 of isoform 2-A; Event=Alternative initiation; Comment=4 isoforms, 1-A (shown here), 1-B, 2-A and 2-B, are produced by alternative initiation at Met-1 and Met-28;

Induction: Down-regulated by glucocorticoids.

Domain: Composed of three domains: a modulating N-terminal domain, a DNA-binding domain and a C-terminal steroid-binding domain.

Ptm: Increased proteasome-mediated degradation in response to glucocorticoids.

Ptm: Phosphorylated in the absence of hormone; becomes hyperphosphorylated in the presence of glucocorticoids.

Ptm: Sumoylated; this reduces transcription transactivation (By similarity).

Similarity: Belongs to the nuclear hormone receptor family. NR3 subfamily.

Similarity: Contains 1 nuclear receptor DNA-binding 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 3mneA

For the chain 3mneA, the alignment 3mneA.msf (attached) with 139 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 3mneA.msf. Its statistics, from the *alistat* program are the following:

```

Format:                MSF
Number of sequences:   139
Total number of residues: 32841
Smallest:              193
Largest:               253
Average length:        236.3
Alignment length:      253
Average identity:      58%
Most related pair:     99%
Most unrelated pair:   20%
Most distant seq:     32%

```

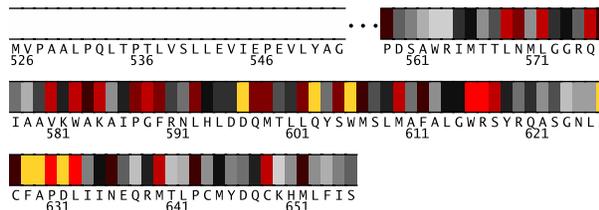


Fig. 1. Residues 526-656 in 3mneA colored by their relative importance. (See Appendix, Fig.10, for the coloring scheme.)

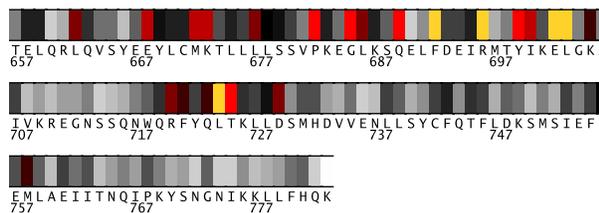


Fig. 2. Residues 657-783 in 3mneA colored by their relative importance. (See Appendix, Fig.10, for the coloring scheme.)

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

The alignment consists of 46% eukaryotic (46% vertebrata) sequences. (Descriptions of some sequences were not readily available.) The file containing the sequence descriptions can be found in the attachment, under the name 3mneA.descr.

2.3 Residue ranking in 3mneA

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

2.4 Top ranking residues in 3mneA 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 3mneA 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.

Table 1.		
cluster color	size	member residues
red	60	569, 570, 572, 576, 577, 581, 583 584, 585, 588, 589, 590, 593, 597 598, 599, 602, 603, 605, 606, 607 610, 612, 616, 617, 618, 627, 628
<i>continued in next column</i>		

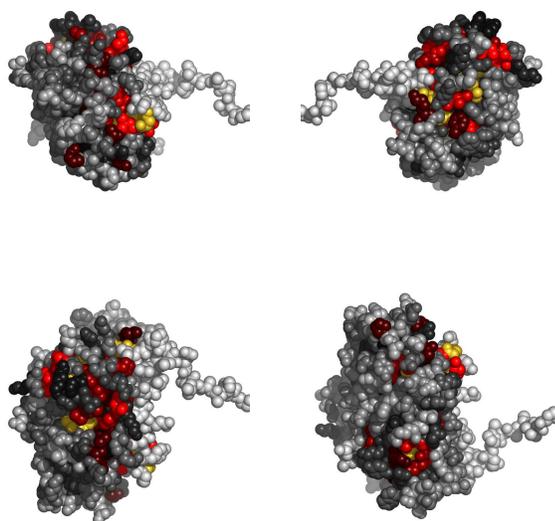


Fig. 3. Residues in 3mneA, colored by their relative importance. Clockwise: front, back, top and bottom views.

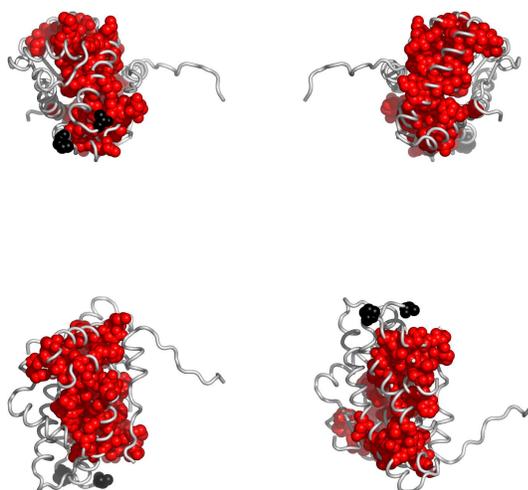


Fig. 4. Residues in 3mneA, colored according to the cluster they belong to: red, followed by blue and yellow are the largest clusters (see Appendix for the coloring scheme). Clockwise: front, back, top and bottom views. The corresponding Pymol script is attached.

Table 1. continued		
cluster color	size	member residues
		629, 630, 631, 632, 633, 636, 640 649, 652, 662, 668, 672, 673, 677 682, 685, 686, 689, 692, 696, 699
<i>continued in next column</i>		

Table 1. continued		
cluster color	size	member residues
		700, 702, 703, 705, 720, 721, 723 724, 725, 729, 758

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

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.

Glycerol binding site. Table 2 lists the top 25% of residues at the interface with 3mneAGOL1 (glycerol). 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
616	W	W(98)FY	0.06	11/0	3.92	
617	R	R(99)K	0.08	13/1	2.76	site
673	K	K(98) E(1)	0.13	3/0	3.69	site

Table 2. The top 25% of residues in 3mneA at the interface with glycerol. (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 approach to the ligand.)

Table 3.		
res	type	disruptive mutations
616	W	(K)(E)(Q)(D)
617	R	(T)(YD)(SVCAG)(FELWPI)
673	K	(Y)(FW)(T)(VCAG)

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

Figure 5 shows residues in 3mneA colored by their importance, at the interface with 3mneAGOL1.

Dexamethasone binding site. Table 4 lists the top 25% of residues at the interface with 3mneADEX784 (dexamethasone). 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)	antn
629	F	F(99)Y	0.03	23/3	3.27	site
606	W	W(99)R	0.04	3/0	3.91	
617	R	R(99)K	0.08	5/0	2.97	site
569	L	L(99).	0.10	30/17	3.37	site

continued in next column

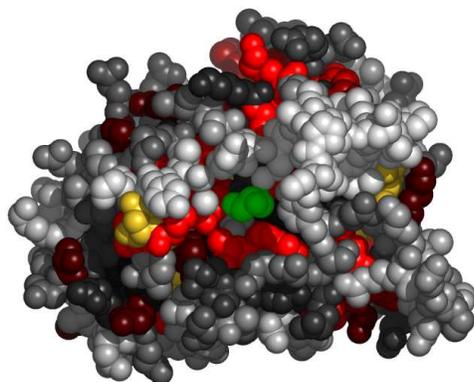


Fig. 5. Residues in 3mneA, at the interface with glycerol, colored by their relative importance. The ligand (glycerol) is colored green. Atoms further than 30Å away from the geometric center of the ligand, as well as on the line of sight to the ligand were removed. (See Appendix for the coloring scheme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

res	type	subst's (%)	cvg	noc/ bb	dist (Å)	antn
576	Q	Q(97) E(2)	0.12	17/0	2.88	site
610	M	M(82) L(2) S(15)	0.12	21/11	3.73	
572	L	L(98)	0.14	8/2	4.28	site
570	N	N(97) ST .C	0.16	34/9	2.82	
607	M	M(82) L(17) A	0.21	14/1	3.76	
652	M	M(94) I(2) C L(1)	0.21	15/0	3.70	

Table 4. The top 25% of residues in 3mneA at the interface with dexamethasone. (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 approach to the ligand.)

res	type	disruptive mutations
<i>continued in next column</i>		

res	type	disruptive mutations
629	F	(K) (E) (Q) (D)
606	W	(E) (TD) (K) (SQCG)
617	R	(T) (YD) (SVCAG) (FELWPI)
569	L	(YR) (TH) (SCG) (KE)
576	Q	(Y) (FWH) (T) (VCAG)
610	M	(Y) (H) (R) (T)
572	L	(Y) (R) (H) (T)
570	N	(Y) (FWH) (R) (E)
607	M	(Y) (H) (T) (R)
652	M	(Y) (H) (R) (T)

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

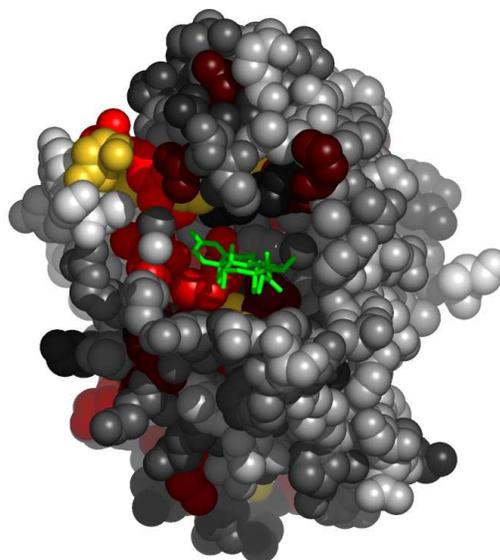


Fig. 6. Residues in 3mneA, at the interface with dexamethasone, colored by their relative importance. The ligand (dexamethasone) is colored green. Atoms further than 30Å away from the geometric center of the ligand, as well as on the line of sight to the ligand were removed. (See Appendix for the coloring scheme for the protein chain 3mneA.)

Figure 6 shows residues in 3mneA colored by their importance, at the interface with 3mneADEX784.

Interface with the peptide 3mneB. Table 6 lists the top 25% of residues at the interface with 3mneB. The following table (Table 7) suggests possible disruptive replacements for these residues (see Section 3.6).

res	type	subst's (%)	cvg	noc/ bb	dist (Å)
603	Q	Q(98)ER	0.03	6/1	3.72
585	K	K(99)	0.10	30/0	3.21
581	V	V(97) I(2)	0.12	19/1	3.73
590	F	F(98) L(1)	0.15	1/0	4.78
598	Q	Q(98)LP	0.17	10/2	3.80
602	L	L(33) I(66)	0.18	6/0	4.06
599	M	I(25) M(74)V	0.19	28/6	3.45
758	M	M(95)SH L.(2)	0.24	12/4	2.98

Table 6. The top 25% of residues in 3mneA at the interface with 3mneB. (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 approach to the ligand.)

res	type	disruptive mutations
603	Q	(Y)(FW)(T)(H)
585	K	(Y)(FTW)(SVCAG)(HD)
581	V	(Y)(R)(KE)(H)
590	F	(KE)(T)(QDR)(SCG)
598	Q	(Y)(H)(T)(FW)
602	L	(YR)(TH)(SKECG)(FQWD)
599	M	(Y)(H)(T)(R)
758	M	(Y)(T)(H)(R)

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

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

Glycerol binding site. Table 8 lists the top 25% of residues at the interface with 3mneAGOL785 (glycerol). The following table (Table 9) suggests possible disruptive replacements for these residues (see Section 3.6).

res	type	subst's (%)	cvg	noc/ bb	dist (Å)	antn
702	E	E(97) S(1)T	0.02	9/0	2.77	site
705	K	K(53) R(43)D H(2)	0.23	14/0	2.71	

Table 8. The top 25% of residues in 3mneA at the interface with glycerol. (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

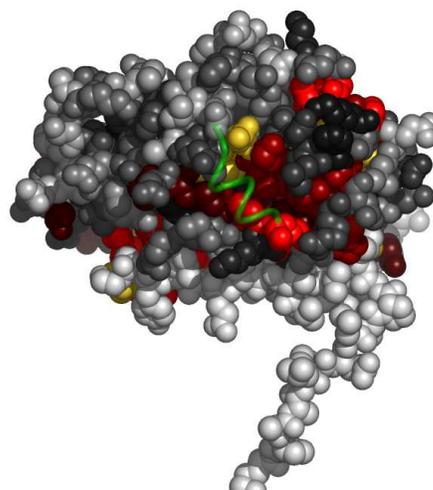


Fig. 7. Residues in 3mneA, at the interface with 3mneB, colored by their relative importance. 3mneB is shown in backbone representation (See Appendix for the coloring scheme for the protein chain 3mneA.)

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 approach to the ligand.)

res	type	disruptive mutations
702	E	(FWH)(R)(Y)(K)
705	K	(Y)(T)(FW)(VCAG)

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

Figure 8 shows residues in 3mneA colored by their importance, at the interface with 3mneAGOL785.

2.4.3 Possible novel functional surfaces at 25% coverage. One group of residues is conserved on the 3mneA surface, away from (or substantially larger than) other functional sites and interfaces recognizable in PDB entry 3mne. It is shown in Fig. 9. The right panel shows (in blue) the rest of the larger cluster this surface belongs to. The residues belonging to this surface "patch" are listed in Table 10, while Table 11 suggests possible disruptive replacements for these residues (see Section 3.6).

res	type	substitutions(%)	cvg	antn
597	D	D(100)	0.01	continued in next column
632	D	D(100)	0.01	
702	E	E(97)S(1)T	0.02	

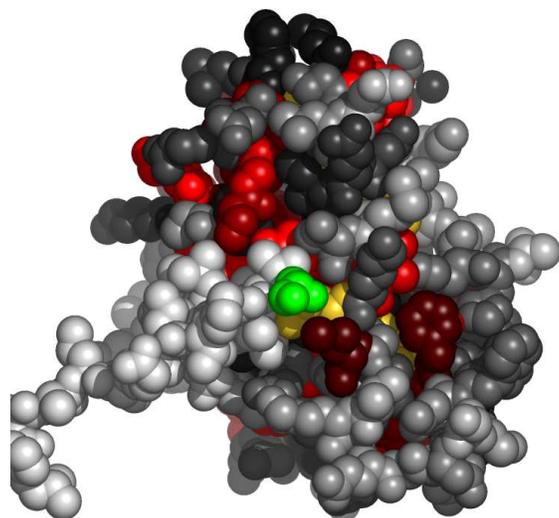


Fig. 8. Residues in 3mneA, at the interface with glycerol, colored by their relative importance. The ligand (glycerol) is colored green. Atoms further than 30Å away from the geometric center of the ligand, as well as on the line of sight to the ligand were removed. (See Appendix for the coloring scheme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

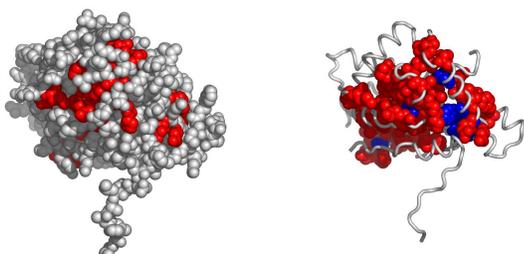


Fig. 9. A possible active surface on the chain 3mneA. The larger cluster it belongs to is shown in blue.

Table 10. continued					
res	type	substitutions(%)	cvg	antn	
603	Q	Q(98)ER	0.03	site	
629	F	F(99)Y	0.03		
606	W	W(99)R	0.04		
696	R	R(98)L(1)	0.04		
616	W	W(98)FY	0.06		
631	P	P(98)TE	0.06		
633	L	L(98)VY	0.06		
689	Q	Q(97)SH(1)	0.06		
682	P	P(97)ILS	0.07		
685	G	G(97)LTV	0.07		
725	T	T(96)L(2)P(1)	0.07		
617	R	R(99)K	0.08		
continued in next column					

Table 10. continued				
res	type	substitutions(%)	cvg	antn
569	L	L(99).	0.10	site
583	W	W(99).	0.10	
585	K	K(99).	0.10	site
589	G	G(98)WD	0.11	
700	I	I(97)LT(1)E	0.11	site
576	Q	Q(97)E(2).	0.12	
581	V	V(97)I(2).	0.12	site
610	M	M(82)L(2)S(15)	0.12	
618	S	S(98)T(1)	0.13	site
673	K	K(98)E(1)	0.13	
572	L	L(98).V	0.14	site
588	P	P(98)GL	0.15	
590	F	F(98)L(1)	0.15	site
662	L	L(97)IM(1)	0.15	
570	N	N(97)ST.C	0.16	site
598	Q	Q(98)LP	0.17	
720	R	R(97)HGSK	0.17	site
729	D	D(95)PXS YGR	0.17	
593	L	M(2)L(97)	0.18	site
602	L	L(33)I(66)	0.18	
599	M	I(25)M(74)V	0.19	site
686	L	L(97)PSQ	0.19	
628	C	Y(69)C(28)FIV	0.20	site
607	M	M(82)L(17)A	0.21	
652	M	M(94)I(2)CL(1)	0.21	site
723	Q	H(7)Q(91)NL	0.22	
577	V	L(53)V(28)M(16)	0.23	site
705	K	K(53)R(43)DH(2)	0.23	
721	F	F(88)Q(1)L(7)S Y(1)	0.24	site
758	M	M(95)SHL.(2)	0.24	
678	L	L(65)F(31)SM(1) A	0.25	site
756	P	P(94)L(2)IH.(2)	0.25	

Table 10. Residues forming surface "patch" in 3mneA.

Table 11.		
res	type	disruptive mutations
597	D	(R)(FWH)(KYVCAG)(TQM)
632	D	(R)(FWH)(KYVCAG)(TQM)
702	E	(FWH)(R)(Y)(K)
603	Q	(Y)(FW)(T)(H)
629	F	(K)(E)(Q)(D)
606	W	(E)(TD)(K)(SQCG)
696	R	(T)(YD)(SECG)(VA)
616	W	(K)(E)(Q)(D)
631	P	(R)(YH)(K)(T)
633	L	(R)(KY)(EH)(T)
689	Q	(Y)(FW)(T)(H)
continued in next column		

Table 11. continued		
res	type	disruptive mutations
682	P	(R)(Y)(H)(T)
685	G	(R)(K)(E)(H)
725	T	(R)(K)(H)(FQW)
617	R	(T)(YD)(SVCAG)(FELWPI)
569	L	(YR)(TH)(SCG)(KE)
583	W	(KE)(TQD)(SNCG)(R)
585	K	(Y)(FTW)(SVCAG)(HD)
589	G	(K)(R)(E)(Q)
700	I	(R)(Y)(H)(TK)
576	Q	(Y)(FWH)(T)(VCAG)
581	V	(Y)(R)(KE)(H)
610	M	(Y)(H)(R)(T)
618	S	(KR)(FQMWH)(NELPI)(Y)
673	K	(Y)(FW)(T)(VCAG)
572	L	(Y)(R)(H)(T)
588	P	(R)(Y)(H)(TKE)
590	F	(KE)(T)(QDR)(SCG)
662	L	(Y)(R)(TH)(CG)
570	N	(Y)(FWH)(R)(E)
598	Q	(Y)(H)(T)(FW)
720	R	(D)(E)(T)(Y)
729	D	(R)(FWH)(K)(Y)
593	L	(Y)(R)(TH)(SCG)
602	L	(YR)(TH)(SKECG)(FQWD)
599	M	(Y)(H)(T)(R)
686	L	(Y)(R)(H)(T)
628	C	(K)(ER)(Q)(D)
607	M	(Y)(H)(T)(R)
652	M	(Y)(H)(R)(T)
723	Q	(Y)(T)(FWH)(SCG)
577	V	(Y)(R)(KEH)(D)
705	K	(Y)(T)(FW)(VCAG)
721	F	(K)(E)(R)(Q)
758	M	(Y)(T)(H)(R)
678	L	(R)(Y)(H)(T)
756	P	(R)(Y)(T)(H)

Table 11. Disruptive mutations for the surface patch in 3mneA.

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 *not* 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 [WIFYHR], hydrophobic [LPVAMWFI], polar [GTCY]; positively [KHR], or negatively [DE] charged, aromatic [WIFYH], long aliphatic chain [EK RQM], 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

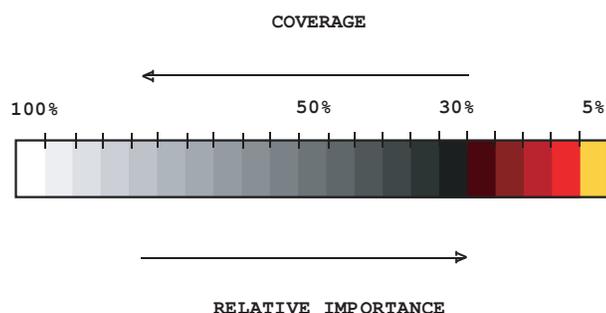


Fig. 10. Coloring scheme used to color residues by their relative importance.

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

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. 10.

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 $(\text{idents} / \text{MIN}(\text{len1}, \text{len2}))$ 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 10\AA^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 L^AT_EX; 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:

- 3mneA.complex.pdb - coordinates of 3mneA with all of its interacting partners
- 3mneA.etvx - ET viewer input file for 3mneA
- 3mneA.cluster_report.summary - Cluster report summary for 3mneA
- 3mneA.ranks - Ranks file in sequence order for 3mneA
- 3mneA.clusters - Cluster descriptions for 3mneA
- 3mneA.msf - the multiple sequence alignment used for the chain 3mneA
- 3mneA.descr - description of sequences used in 3mneA msf
- 3mneA.ranks_sorted - full listing of residues and their ranking for 3mneA
- 3mneA.3mneAGOL1.if.pml - Pymol script for Figure 5
- 3mneA.cbcvg - used by other 3mneA – related pymol scripts
- 3mneA.3mneADEX784.if.pml - Pymol script for Figure 6
- 3mneA.3mneB.if.pml - Pymol script for Figure 7
- 3mneA.3mneAGOL785.if.pml - Pymol script for Figure 8