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1 INTRODUCTION

From the original Protein Data Bank entry (PDB id 1a8s):
Title: Chloroperoxidase f/propionate complex
Compound: Mol id: 1; molecule: chloroperoxidase f; chain: a;
synonym: haloperoxidase f; ec: 1.11.1.10; engineered: yes
Organism, scientific name: Pseudomonas Fluorescens;
1a8s contains a single unique chain 1a8sA (273 residues long).

2 CHAIN 1A8SA

2.1 O31158 overview

From SwissProt, id O31158, 100% identical to 1a8sA:
Description: Non-heme chloroperoxidase (EC 1.11.1.10) (Chloride peroxidase) (CPO-F) (Chloroperoxidase F).
Organism, scientific name: Pseudomonas fluorescens.
Taxonomy: Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas.
Catalytic activity: 2 RH + 2 Cl(-) + H(2)O(2) = 2 RCl + 2 H(2)O.
Subunit: Homodimer.
Similarity: Belongs to the bacterial non-heme bromo- and chloroperoxidases 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 1a8sA

For the chain 1a8sA, the alignment 1a8sA.msf (attached) with 332 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 1a8sA.msf. Its statistics, from the alstat program are the following:
Fig. 1. Residues 1-136 in 1a8sA colored by their relative importance. (See Appendix, Fig.9, for the coloring scheme.)

Fig. 2. Residues 137-273 in 1a8sA colored by their relative importance. (See Appendix, Fig.9, for the coloring scheme.)

Format: MSF
Number of sequences: 332
Total number of residues: 88014
Smallest: 218
Largest: 273
Average length: 265.1
Alignment length: 273
Average identity: 44%
Most related pair: 99%
Most unrelated pair: 15%
Most distant seq: 30%

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

The alignment consists of <1% eukaryotic (<1% fungi), 18% 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 1a8sA.descr.

2.3 Residue ranking in 1a8sA

The 1a8sA sequence is shown in Figs. 1–2, with each residue colored according to its estimated importance. The full listing of residues in 1a8sA can be found in the file called 1a8sA.ranks.sorted in the attachment.

2.4 Top ranking residues in 1a8sA 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 1a8sA 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 1a8sA, colored by their relative importance. Clockwise: front, back, top and bottom views.

Fig. 4. Residues in 1a8sA, 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.

<table>
<thead>
<tr>
<th>Cluster</th>
<th>Size</th>
<th>Red Member Residues</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>66</td>
<td>17, 21, 24, 26, 27, 28, 29, 30, 35, 38, 42, 46, 48, 49, 50, 51, 53, 54, 55, 56, 57, 58, 60, 66, 67, 69, 72, 73, 75, 83, 90, 92, 93, 94, 95, 96, 97, 98, 99, 100, 103, 117, 120, 123, 151, 163, 185, 200, 203, 204, 208, 209, 215, 217, 220, 221, 224, 227, 228, 252, 253, 254, 258, 264, 267, 270</td>
</tr>
</tbody>
</table>

Table 1. Clusters of top ranking residues in 1a8sA.

#### 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.

**Sulfate ion binding site.** Table 2 lists the top 25% of residues at the interface with 1a8sSO44 (sulfate ion). The following table (Table 3) suggests possible disruptive replacements for these residues (see Section 3.6).

### Table 2.

<table>
<thead>
<tr>
<th>Res</th>
<th>Type</th>
<th>Substs (%)</th>
<th>Cvg</th>
<th>NoC/BB</th>
<th>Dist (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>G</td>
<td>G(88)CK</td>
<td>0.15</td>
<td>16/16</td>
<td>2.89</td>
</tr>
</tbody>
</table>

Table 2. The top 25% of residues in 1a8sA at the interface with sulfate 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 approach to the ligand.)

### Table 3.

<table>
<thead>
<tr>
<th>Res</th>
<th>Type</th>
<th>Disruptive Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>G</td>
<td>(FW) (R) (H) (E)</td>
</tr>
</tbody>
</table>

Table 3. List of disruptive mutations for the top 25% of residues in 1a8sA, that are at the interface with sulfate ion.

Figure 5 shows residues in 1a8sA colored by their importance, at the interface with 1a8sSO44.

**Propanoic acid binding site.** Table 4 lists the top 25% of residues at the interface with 1a8sPP1 (propanoic acid). The following table (Table 5) suggests possible disruptive replacements for these residues (see Section 3.6).

### Table 4.

<table>
<thead>
<tr>
<th>Res</th>
<th>Type</th>
<th>Substs (%)</th>
<th>Cvg</th>
<th>NoC/BB</th>
<th>Dist (Å)</th>
<th>Atn</th>
</tr>
</thead>
<tbody>
<tr>
<td>94</td>
<td>S</td>
<td>S(99)AG</td>
<td>0.03</td>
<td>15/5</td>
<td>2.77</td>
<td>site</td>
</tr>
<tr>
<td>253</td>
<td>H</td>
<td>H(97)TV</td>
<td>0.04</td>
<td>10/0</td>
<td>2.88</td>
<td>site</td>
</tr>
<tr>
<td>27</td>
<td>G</td>
<td>G(96)P</td>
<td>0.07</td>
<td>6/6</td>
<td>3.78</td>
<td></td>
</tr>
<tr>
<td>93</td>
<td>F</td>
<td>H(61)</td>
<td>0.09</td>
<td>3/1</td>
<td>4.35</td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>T</td>
<td>M(31)</td>
<td>0.10</td>
<td>16/8</td>
<td>2.75</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>W</td>
<td>Y(3)</td>
<td>0.11</td>
<td>27/16</td>
<td>2.96</td>
<td></td>
</tr>
<tr>
<td>227</td>
<td>V</td>
<td></td>
<td>0.13</td>
<td>2/0</td>
<td>4.03</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. The top 25% of residues in 1a8sA at the interface with propanoic 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 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.)

Fig. 5. Residues in 1a8sA, at the interface with sulfate ion, colored by their relative importance. The ligand (sulfate 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 1a8sA.)

continued in next column
Table 4. The top 25% of residues in 1a8sA at the interface with propanoic acid. (Field names: res: residue number in the PDB entry; type: amino acid type; subs: 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>
<thead>
<tr>
<th>res</th>
<th>type</th>
<th>subst's (%)</th>
<th>cvg</th>
<th>noc/bb</th>
<th>dist (Å)</th>
<th>antn</th>
</tr>
</thead>
<tbody>
<tr>
<td>163</td>
<td>F</td>
<td>V(75)N, I(4)A(3)C(6)L(5)RYMP</td>
<td>0.17</td>
<td>4/0</td>
<td>3.68</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>F</td>
<td>F(71)I(2)Y(4)VSMG</td>
<td>0.19</td>
<td>14/0</td>
<td>3.67</td>
<td></td>
</tr>
</tbody>
</table>

Figure 6. Residues in 1a8sA, at the interface with propanoic acid, colored by their relative importance. The ligand (propanoic acid) 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 1a8sA.)

Table 5. List of disruptive mutations for the top 25% of residues in 1a8sA, that are at the interface with propanoic acid.

<table>
<thead>
<tr>
<th>res</th>
<th>type</th>
<th>disruptive mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>94</td>
<td>S</td>
<td>(KR) (QH) (FMW) (E)</td>
</tr>
<tr>
<td>253</td>
<td>H</td>
<td>(E) (Q) (K) (M)</td>
</tr>
<tr>
<td>27</td>
<td>G</td>
<td>(R) (K) (E) (H)</td>
</tr>
<tr>
<td>93</td>
<td>F</td>
<td>(R) (T) (QD) (R)</td>
</tr>
<tr>
<td>95</td>
<td>T</td>
<td>(R) (K) (H) (Q)</td>
</tr>
<tr>
<td>28</td>
<td>W</td>
<td>(K) (E) (Q) (DR)</td>
</tr>
<tr>
<td>227</td>
<td>V</td>
<td>(R) (Y) (E) (K)</td>
</tr>
<tr>
<td>163</td>
<td>F</td>
<td>(K) (E) (QR) (D)</td>
</tr>
<tr>
<td>200</td>
<td>F</td>
<td>(K) (E) (Q) (DR)</td>
</tr>
</tbody>
</table>

Figure 7. A possible active surface on the chain 1a8sA. The larger cluster it belongs to is shown in blue.

Table 6. Residues forming surface "patch" in 1a8sA.

<table>
<thead>
<tr>
<th>res</th>
<th>type</th>
<th>substitutions (%)</th>
<th>cvg</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>R</td>
<td>R(97)TQ(1)N</td>
<td>0.05</td>
</tr>
<tr>
<td>83</td>
<td>L</td>
<td>FL(96)IEWRKASTM</td>
<td>0.06</td>
</tr>
<tr>
<td>46</td>
<td>G</td>
<td>G(91)R(2)E(1)DQ</td>
<td>0.10</td>
</tr>
<tr>
<td>17</td>
<td>G</td>
<td>G(88)CK(2)N(5)</td>
<td>0.15</td>
</tr>
<tr>
<td>21</td>
<td>P</td>
<td>P(76)T(8)A(3)</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.(1)V(9)ISL</td>
<td></td>
</tr>
</tbody>
</table>
Table 7. Disruptive mutations for the surface patch in 1a8sA.

Another group of surface residues is shown in Fig. 8. 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 8, while Table 9 suggests possible disruptive replacements for these residues (see Section 3.6).

Table 8. Residues forming surface "patch" in 1a8sA.

Table 9. Disruptive mutations for the surface patch in 1a8sA.
Table 9. Disruptive mutations for the surface patch in 1a8sA.

<table>
<thead>
<tr>
<th>res</th>
<th>type</th>
<th>disruptive mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>228</td>
<td>P</td>
<td>(Y) (R) (H) (T)</td>
</tr>
<tr>
<td>93</td>
<td>F</td>
<td>(KE) (T) (QQ) (R)</td>
</tr>
<tr>
<td>95</td>
<td>T</td>
<td>(R) (K) (H) (Q)</td>
</tr>
<tr>
<td>123</td>
<td>P</td>
<td>(Y) (R) (H) (T)</td>
</tr>
<tr>
<td>28</td>
<td>W</td>
<td>(K) (E) (Q) (DR)</td>
</tr>
<tr>
<td>227</td>
<td>V</td>
<td>(R) (Y) (E) (K)</td>
</tr>
<tr>
<td>54</td>
<td>R</td>
<td>(TD) (E) (Y) (CG)</td>
</tr>
<tr>
<td>30</td>
<td>L</td>
<td>(R) (Y) (H) (K)</td>
</tr>
<tr>
<td>163</td>
<td>F</td>
<td>(K) (E) (QR) (D)</td>
</tr>
<tr>
<td>204</td>
<td>D</td>
<td>(R) (H) (Y) (FW)</td>
</tr>
<tr>
<td>69</td>
<td>M</td>
<td>(Y) (T) (H) (R)</td>
</tr>
<tr>
<td>200</td>
<td>F</td>
<td>(K) (E) (Q) (DR)</td>
</tr>
<tr>
<td>66</td>
<td>G</td>
<td>(R) (KE) (FWH) (M)</td>
</tr>
<tr>
<td>208</td>
<td>D</td>
<td>(R) (H) (FW) (Y)</td>
</tr>
<tr>
<td>120</td>
<td>A</td>
<td>(Y) (R) (E) (KH)</td>
</tr>
<tr>
<td>252</td>
<td>P</td>
<td>(R) (Y) (H) (T)</td>
</tr>
<tr>
<td>258</td>
<td>T</td>
<td>(R) (K) (H) (FW)</td>
</tr>
<tr>
<td>67</td>
<td>N</td>
<td>(Y) (TH) (R) (FW)</td>
</tr>
<tr>
<td>203</td>
<td>T</td>
<td>(R) (K) (H) (FQW)</td>
</tr>
<tr>
<td>52</td>
<td>H</td>
<td>(E) (T) (Q) (D)</td>
</tr>
</tbody>
</table>

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 Å², 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 Å 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 Å.

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 (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 [W FY H R], hydrophobic [LPV AM W FT], polar [G TC Y]; positively [K HR], or negatively [D E] charged, aromatic [W FY H], long aliphatic chain [EKRQM], OH-group possession [S D E T Y], and NH2 group possession [N Q R K]. 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 this column of the alignment
4.2 Color schemes used

The following color scheme is used in figures with residues colored by the estimated evolutionary pressure they experience can be seen in Fig. 9.

2. their type
   - ρ ET score - the smaller this value, the lesser variabilty 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 ρh or smaller
   - gaps percentage of gaps in this column

4.2.1 Alistat

alstat 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). alstat is copyrighted by HHMI/Washington University School of Medicine, 1992-2001, and freely distributed under the GNU General Public License.

4.2.2 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.

4.2.3 DSSP

In this work a residue is considered solvent accessible if the DSSP program finds it exposed to water by at least 10Å², 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@cmbH.kun.nl November 18, 2002, http://www.cmbi.kun.nl/gv/dssp/descrip.html.

4.2.4 HSSP


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

4.2.5 LaTeX

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

http://www.latex.org/

4.2.6 Muscle


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

4.2.7 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


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:

- 1a8sA.complex.pdb - coordinates of 1a8sA with all of its interacting partners
- 1a8sA.etvx - ET viewer input file for 1a8sA
- 1a8sA.cluster_report.summary - Cluster report summary for 1a8sA
- 1a8sA.ranks - Ranks file in sequence order for 1a8sA
- 1a8sA.clusters - Cluster descriptions for 1a8sA
- 1a8sA.msf - the multiple sequence alignment used for the chain 1a8sA
- 1a8sA.descr - description of sequences used in 1a8sA msf
- 1a8sA.ranks_sorted - full listing of residues and their ranking for 1a8sA
- 1a8sA.1a8sSO44.if.pml - Pymol script for Figure 5
- 1a8sA.cbcvg - used by other 1a8sA – related pymol scripts
- 1a8sA.1a8sPPI.if.pml - Pymol script for Figure 6