1 INTRODUCTION
From the original Protein Data Bank entry (PDB id 1a0o):
Title: Chey-binding domain of chea in complex with chey
Compound: Mol id: 1; molecule: chey; chain: a, c, e, g; engineered: yes; mol id: 2; molecule: chea; chain: b, d, f, h; fragment: chea 124-257; ec: 2.7.3.-; engineered: yes
Organism, scientific name: Escherichia Coli;
1a0o contains a single unique chain 1a0oa (128 residues long) and its homologues 1a0oe, 1a0oc, and 1a0og. Not enough homologous sequences could be found to permit analysis for chain 1a0ob.

2 CHAIN 1A0OA
2.1 P06143 overview
From SwissProt, id P06143, 89% identical to 1a0oa:
Description: Chemotaxis protein cheY.
Organism, scientific name: Escherichia coli, Escherichia coli O157:H7, and Shigella flexneri.
Function: Involved in the transmission of sensory signals from the chemoreceptors to the agellar motors. In its active (phosphorylated or acetylated) form, cheY exhibits enhanced binding to a switch component, iM, at the agellar motor which induces a change from counterclockwise to clockwise flagellar rotation.
Cofactor: Binds 1 magnesium ion per subunit.
Interaction:
Subcellular location: Cytoplasmic.
Ptms: Phosphorylated by cheA or acetylated by acetyl-CoA synthase, depending on which acetate metabolism pathway is available.
Mass spectrometry: MW=13966; METHOD=Electrospray; RANGE=1-128; NOTE=Ref.12.
Mass spectrometry: MW=14008; METHOD=Electrospray; RANGE=1-128; NOTE=With N6-acetylLys-91 (Ref.12).
Similarity: Contains 1 response regulatory 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 1a0oA

For the chain 1a0oA, the alignment 1a0oA.msf (attached) with 852 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 1a0oA.msf. Its statistics, from the alistat program are the following:

Format: MSF
Number of sequences: 852
Total number of residues: 101171
Smallest: 100
Largest: 128
Average length: 118.7
Alignment length: 128
Average identity: 31%
Most related pair: 98%
Most unrelated pair: 12%
Most distant seq: 35%
Furthermore, <1% of residues show as conserved in this alignment.

The alignment consists of 3% eukaryotic (<1% arthropoda, 1% fungi, <1% plantae), 94% prokaryotic, 1% arcaean, and <1% viral sequences. (Descriptions of some sequences were not readily available.) The file containing the sequence descriptions can be found in the attachment, under the name 1a0oA.descr.

2.3 Residue ranking in 1a0oA

The 1a0oA sequence is shown in Fig. 1, with each residue colored according to its estimated importance. The full listing of residues in 1a0oA can be found in the file called 1a0oA.ranks_sorted in the attachment.

2.4 Top ranking residues in 1a0oA and their position on the structure

In the following we consider residues ranking among top 25% of residues in the protein. Figure 2 shows residues in 1a0oA 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. 3 shows the top 25% of all residues, this time colored according to clusters they belong to. The clusters in Fig.3 are composed of the residues listed in Table 1.

Fig. 1. Residues 2-129 in 1a0oA colored by their relative importance. (See Appendix, Fig. 7, for the coloring scheme.)

Fig. 2. Residues in 1a0oA, colored by their relative importance. Clockwise: front, back, top and bottom views.

Fig. 3. Residues in 1a0oA, 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.

in Table 1.
Table 1. Clusters of top ranking residues in 1a0oA.

<table>
<thead>
<tr>
<th>cluster color</th>
<th>size</th>
<th>member residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>red</td>
<td>28</td>
<td>9,11,12,13,14,18,36,39,42,43,56,57,60,61,64,65,73,85,86,87,88,98,102,103,106,109,110,111</td>
</tr>
<tr>
<td>blue</td>
<td>2</td>
<td>25,29</td>
</tr>
</tbody>
</table>

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 1a0oMN4 (manganese (ii) ion). The following table (Table 3) suggests possible disruptive replacements for these residues (see Section 3.6).

Table 2. The top 25% of residues in 1a0oA at the interface with manganese (ii) ion. (Field names: res: residue number in the PDB entry; type: amino acid type; subst’s: 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>
</tr>
</thead>
<tbody>
<tr>
<td>57</td>
<td>D</td>
<td>D(99)E</td>
<td>0.02</td>
<td>4/0</td>
<td>2.43</td>
</tr>
<tr>
<td>109</td>
<td>K</td>
<td>K(99)RH</td>
<td>0.02</td>
<td>1/0</td>
<td>4.57</td>
</tr>
<tr>
<td>13</td>
<td>D</td>
<td>D(99)SN</td>
<td>0.03</td>
<td>5/1</td>
<td>2.58</td>
</tr>
<tr>
<td>60</td>
<td>M</td>
<td>M(75)</td>
<td>0.06</td>
<td>3/2</td>
<td>4.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I(1)KV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>D</td>
<td>D(69)</td>
<td>0.09</td>
<td>2/0</td>
<td>4.08</td>
</tr>
</tbody>
</table>

Table 3. List of disruptive mutations for the top 25% of residues in 1a0oA, that are at the interface with manganese (ii) ion.

<table>
<thead>
<tr>
<th>res</th>
<th>type</th>
<th>disruptive mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>57</td>
<td>D</td>
<td>(R) (FWH) (YVCAG) (K)</td>
</tr>
<tr>
<td>109</td>
<td>K</td>
<td>(T) (Y) (SVCAG) (FW)</td>
</tr>
<tr>
<td>13</td>
<td>D</td>
<td>(R) (FWH) (Y) (K)</td>
</tr>
<tr>
<td>60</td>
<td>M</td>
<td>(Y) (TH) (R) (SCG)</td>
</tr>
<tr>
<td>12</td>
<td>D</td>
<td>(R) (FWH) (YVCAG) (K)</td>
</tr>
</tbody>
</table>

Figure 4 shows residues in 1a0oA colored by their importance, at the interface with 1a0oMN4.

Interface with 1a0oB. Table 4 lists the top 25% of residues at the interface with 1a0oB. The following table (Table 5) suggests possible disruptive replacements for these residues (see Section 3.6).
Fig. 4. Residues in 1a0oA, at the interface with manganese (ii) ion, colored by their relative importance. The ligand (manganese (ii) 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 1a0oA.)

Table 4. The top 25% of residues in 1a0oA at the interface with 1a0oB. (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 5. List of disruptive mutations for the top 25% of residues in 1a0oA, that are at the interface with 1a0oB.

2.4.3 Possible novel functional surfaces at 25% coverage. One group of residues is conserved on the 1a0oA surface, away from (or substantially larger than) other functional sites and interfaces recognizable in PDB entry 1a0o. It is shown in Fig. 6. The right panel of Figure 5 shows residues in 1a0oA colored by their importance, at the interface with 1a0oB.
shows (in blue) the rest of the larger cluster this surface belongs to.

![Image](image)

**Fig. 6.** A possible active surface on the chain 1a0oA. The larger cluster it belongs to is shown in blue.

The residues belonging to this surface "patch" are listed in Table 6, while Table 7 suggests possible disruptive replacements for these residues (see Section 3.6).

<table>
<thead>
<tr>
<th>res</th>
<th>type</th>
<th>substitutions(%)</th>
<th>cvg</th>
</tr>
</thead>
<tbody>
<tr>
<td>102</td>
<td>G</td>
<td>G(96)HLKFRNV</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SPI</td>
<td></td>
</tr>
<tr>
<td>98</td>
<td>A</td>
<td>A(46)A(34)C(8)</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F(1)S(2)NV(1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L(2)YMHQIQT</td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>P</td>
<td>P(80)V(1)K(6)</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E(1)LA(2)NGF</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Y(1)R(1)HDTQSIW</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>A(71)V(9)M(8)</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C(2)T(1)I(1)</td>
<td></td>
</tr>
<tr>
<td>103</td>
<td>A</td>
<td>A(71)R(1)K(16)L</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NH(1)MGTYIACVD</td>
<td></td>
</tr>
</tbody>
</table>

**Table 6.** Residues forming surface "patch" in 1a0oA.

<table>
<thead>
<tr>
<th>res</th>
<th>type</th>
<th>disruptive mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>102</td>
<td>G</td>
<td>(R) (E) (K) (H)</td>
</tr>
<tr>
<td>98</td>
<td>A</td>
<td>(R) (K) (E) (Y)</td>
</tr>
<tr>
<td>82</td>
<td>P</td>
<td>(R) (Y) (H) (T)</td>
</tr>
<tr>
<td>103</td>
<td>A</td>
<td>(R) (K) (Y) (E)</td>
</tr>
<tr>
<td>73</td>
<td>R</td>
<td>(D) (Y) (T) (E)</td>
</tr>
</tbody>
</table>

**Table 7.** Disruptive mutations for the surface patch in 1a0oA.

### 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 10Å², which is roughly the area needed for one water molecule to come in contact with the residue. Furthermore, we require that these residues form a “cluster” of residues which have neighbor within 5Å 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 5Å.

**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 [LPNQDEM1K], large
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, Light SlateBlue, 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. 7.

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 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


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).


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

4.7 Attachments
The following files should accompany this report:

- 1a0oB.complex.pdb - coordinates of 1a0oB with all of its interacting partners
- 1a0oA.complex.pdb - coordinates of 1a0oA with all of its interacting partners
- 1a0oA.etvx - ET viewer input file for 1a0oA
- 1a0oA.cluster_report.summary - Cluster report summary for 1a0oA
- 1a0oA.ranks - Ranks file in sequence order for 1a0oA
- 1a0oA.clusters - Cluster descriptions for 1a0oA
- 1a0oA.msf - the multiple sequence alignment used for the chain 1a0oA
- 1a0oA.descr - description of sequences used in 1a0oA msf
- 1a0oA.ranks_sorted - full listing of residues and their ranking for 1a0oA
- 1a0oA.1a0oMN4.if.pml - Pymol script for Figure 4
- 1a0oA.cbcvg - used by other 1a0oA – related pymol scripts
- 1a0oA.1a0oB.if.pml - Pymol script for Figure 5