ENDscript 2.0

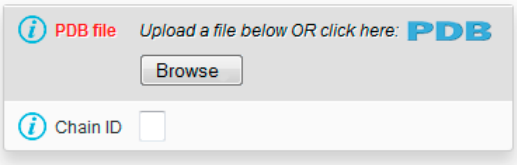
Commented Example

Catalases are tetrameric haeminic enzymes that decompose hydrogen peroxide into water and oxygen. They serve to protect cells from the toxic effects of hydrogen peroxide.

We will use ENDscript to decipher features in the crystal structure of *Proteus mirabilis* catalase (PDB entry **2CAH**).

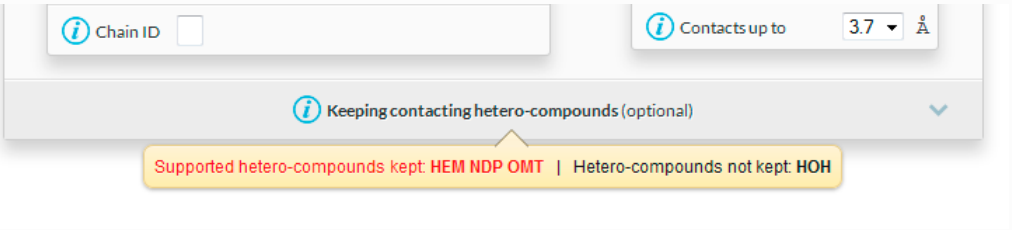
The two fundamental residues of the haem-iron catalytic site are a distal histidine and a proximal tyrosine. A unique methionine sulfone is observed in the distal site of *Proteus mirabilis* catalase. Another peculiarity of this bacterial catalase is its ability to bind nicotinamide-adenine-dinucleotide phosphate (NADPH) for the prevention of inactivation by hydrogen peroxide.

1 The user clicks on the PDB icon of the ENDscript form, then types 2CAH and presses the Retrieve data button



2 The query 2CAH is pre-analyzed by ENDscript

- The following tooltip appears under the panel Keeping contacting hetero-compounds :



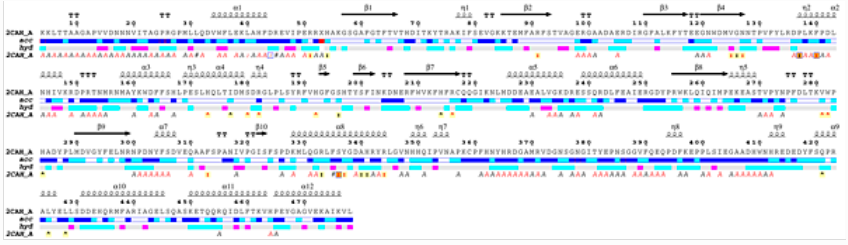
  - HEM is the bound haem, it will be considered as a ligand in ENDscript.
  - NDP is the bound NADPH, it will be considered as a ligand in ENDscript.
  - OMT is the methionine sulphone residue of the polypeptide chain, it will be considered as a protein residue.
  - HOH are water molecules, they will be removed in ENDscript unless the name HOH is written in the tabular form Keeping contacting hetero-compounds (click on the panel to unroll it).

3 The user decides to keep all default settings and press the SUBMIT button

- The sequence of 2CAH is extracted by SPDB.
- A BLAST search is performed against the PDBAA (sequence database derived from PDB protein structures) with a threshold E-value of 1e-6.
- Sequences of homologous proteins found by the BLAST search are aligned with Clustal Omega.
- Secondary structure elements are extracted with DSSP and accessibility is calculated.
- Cα chains of homologous proteins are superimposed to that of 2CAH with ProFit.
- ESPript and PyMOL are used to render 2D and 3D information.

4 The ENDscript "RESULTS" pop-up window appears

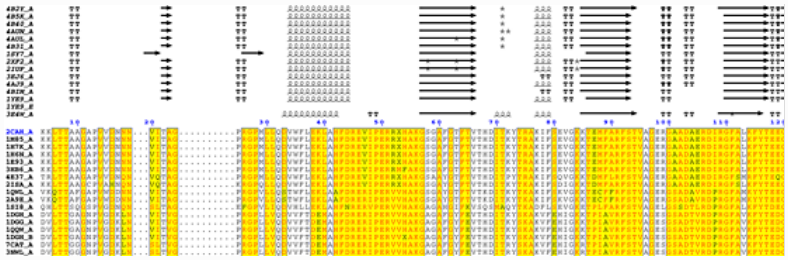
- The user can click on the 2CAH hyperlink to be directed to its PDB summary page.
- The user can open the ESPript - Phase 1 PDF file (download it [9 Kb]) generated by ESPript and notice that:



  - 2CAH crystallizes with one monomer in the asymmetric unit, *e.g.* chain A (labeled 2CAH_A on the figure).
  - The catalase structure has an α + β topology.
  - Residues 1 to 3 are not observed and the 2CAH sequence starts at Lys4.
  - The N-terminal region is involved in extensive protein:protein crystallographic contacts as shown by italic A letters below the sequence block. A red letter identifies a contact < 3.2 Å while a black letter identifies a contact between 3.2 and 5 Å.
  - Leu31 is positioned along a 2-fold crystallographic axis as shown by an italic #.
  - Leu40 is in contact with the haem group of a symmetric monomer as shown by an italic colon :.

- Asp44 is also in contact with the haem group of a symmetric monomer as shown by an italic colon : It is also involved in a protein:protein crystallographic contact as shown by a blue frame.
- Arg51 binds the haem group of the monomer as shown by a normal colon : on a light yellow background.
- The non-standard residue in position 53 (labeled X) is the methionine sulphone of the distal site.
- Phe140 may be a critical residue: it has close contacts with the haem group as shown by the red colon : on the yellow background. It is also involved in protein:protein contacts as shown by the blue frame. Finally, it is involved both in crystallographic and non-crystallographic contacts as shown by the orange background. The detailed list of contacts is accessible *via* the CNS hyperlink of the Tracing files tab in the "RESULTS" pop-up window. With this CNS file, you can check that Phe140 is involved both in a crystallographic contact with Phe43 and in a non-crystallographic contact with the haem group.
- His173 binds tightly NADPH as shown by a red caret ^.

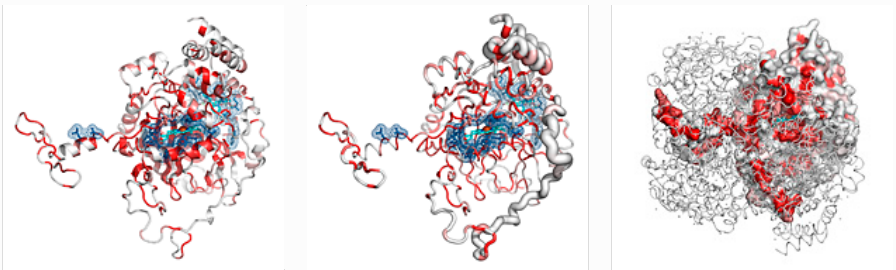
- The user can open the ESPript - Phase 2 PDF file (**download it** [33 Kb]) generated by ESPript and notice that:



Excerpt from the ESPript phase 2 figure

- Secondary structure elements are well conserved in catalases.
- The essential distal His54 is substituted in some sequences to obtain an inactive protein.

- The user can open the PyMOL Sausage representation (**download it** [8.2 Mb]) to interact with the 3D structure.
- He can press on the CONTACTING_RES and SITES buttons in the right-hand PyMOL control panel to observe the haem and the NADPH binding sites.
- He can observe that the Cα trace of the C-terminal region varies between catalases (the tube radius is proportional to the mean r.m.s. deviation between 2CAH and homologous catalases). In agreement, the C-terminal region is poorly conserved in sequence and colored in white (color ramping from white, low conservation, to red, identity).
- By contrast the N-terminal region, which protrudes out of the protein core, is surprisingly well conserved. In fact, this region is deeply buried in the biological tetramer. The user can click on the four BIOUNIT1 buttons in the right-hand PyMOL control panel to generate the tetramer. He can also observe that the four haem groups are well buried in the tetrameric protein.
- The user can cross-check that the N-terminal Leu40 and Asp44 are in contact with the haem of a symmetry-related monomer.
- Finally, the user can click on the SOLV_SURFACE button in the right-hand PyMOL control panel to observe the small channel, which drives the hydrogen peroxide substrate from the protein surface to the haem distal site.



PyMOL representations generated by ENDscript for 2CAH (click on the thumbnails to access full-size images)