



# What the HEC?: Functionality of Human Erythrocyte Catalase

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## Abstract

Human Erythrocyte Catalase (HEC) is an enzyme that catalyzes the 2-step decomposition of hydrogen peroxide, H<sub>2</sub>O<sub>2</sub>, to water and oxygen inside the red blood cells of humans. In 1 second, HEC can decompose up to 1 million molecules of H<sub>2</sub>O<sub>2</sub>. H<sub>2</sub>O<sub>2</sub> is a byproduct of aerobic respiration. It can become a free radical and damage the membrane proteins and lipids of red blood cells, resulting in heme degradation. HEC has also been shown to protect somatic cells from the oxidative damage caused by H<sub>2</sub>O<sub>2</sub>. We need H<sub>2</sub>O<sub>2</sub> in our body as a way to help fight off infection. H<sub>2</sub>O<sub>2</sub> is produced by leukocytes to help fight infections because it is an antibacterial agent.

## Structure

Catalase is a tetramer (Figure 1) made up of 4 subunits. The threading arm from one subunit (Figure 2) interlocks through the wrapping loop of another subunit, forming a dimer. The tetramer is formed when 2 arm-exchanged dimers interlock. The interlocking arms cover the barrel channel, which has the heme active site at its bottom. (Figure 3) Restricted access to the active site, the hydrophobic properties and the size of the channel act as a "molecular ruler" to ensure that only H<sub>2</sub>O<sub>2</sub> reaches the active site and also prevents any hydroxyl radical formation.

The metal region of catalase (Figure 5) is a pentacoordinated Fe<sup>3+</sup> that serves to reduce the 1<sup>st</sup> molecule of H<sub>2</sub>O<sub>2</sub>, then oxidize the 2<sup>nd</sup> molecule of H<sub>2</sub>O<sub>2</sub>. Catalase can be found in the peroxisome of almost all plants, animals, and fungi. Red blood cells don't have any organelles, therefore HEC is found freely distributed in its cytoplasm.

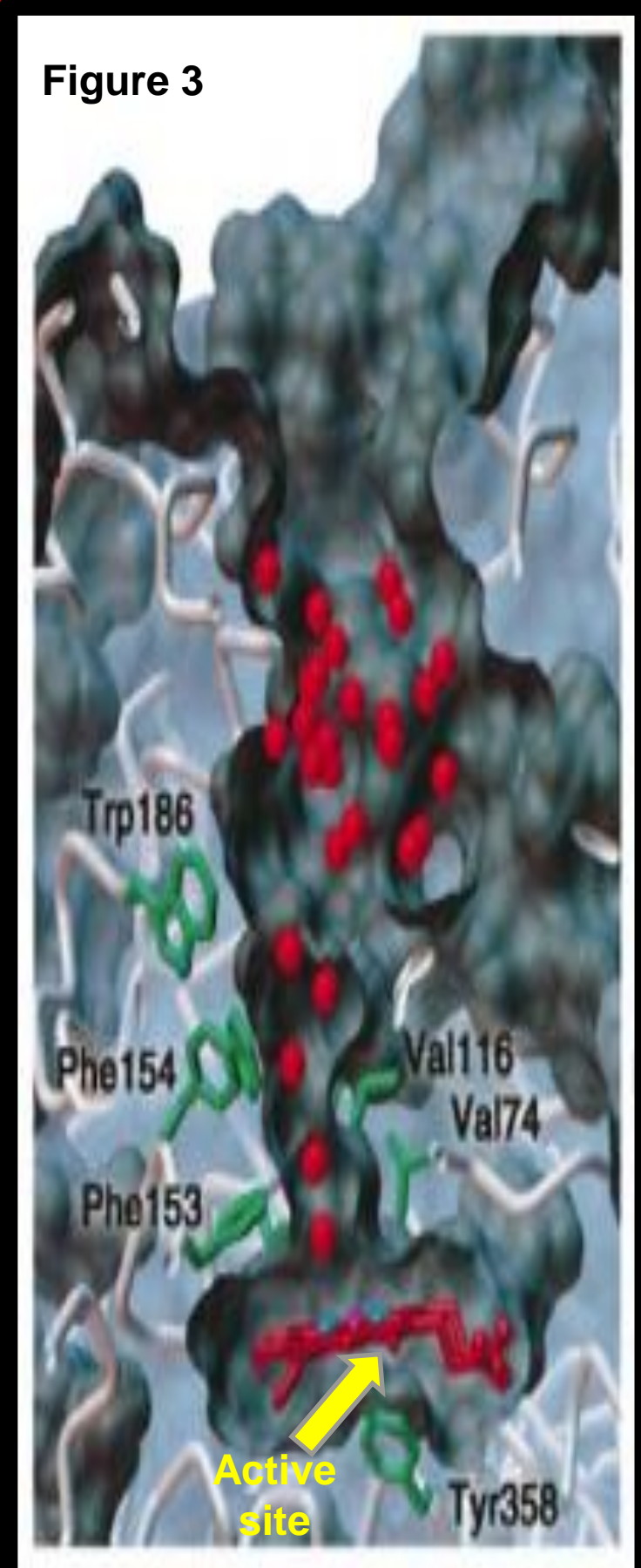


Figure 1

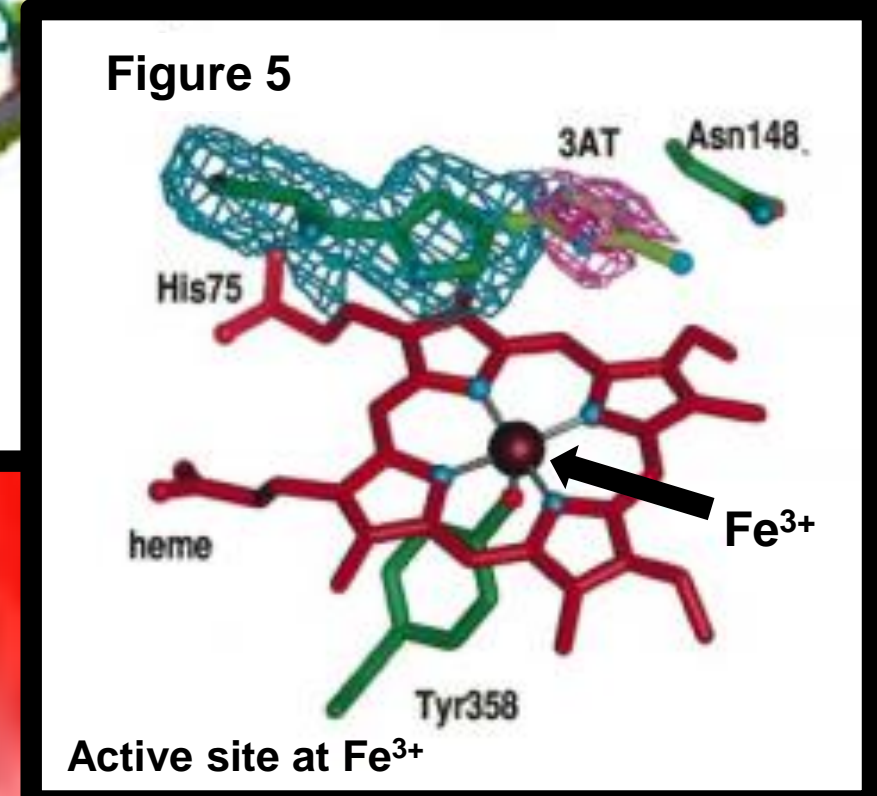
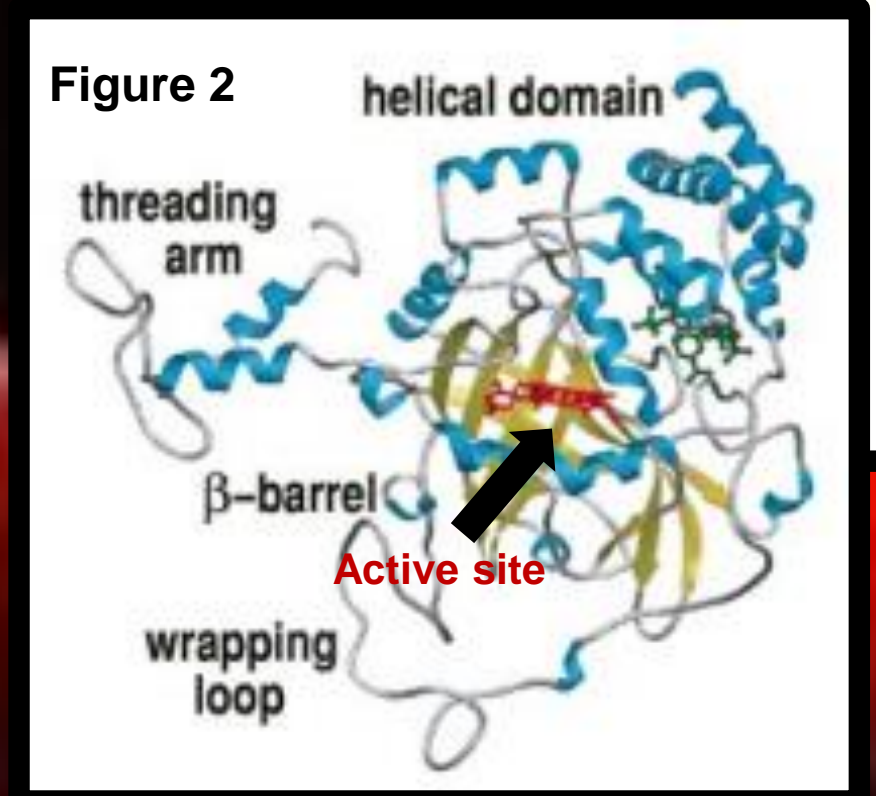
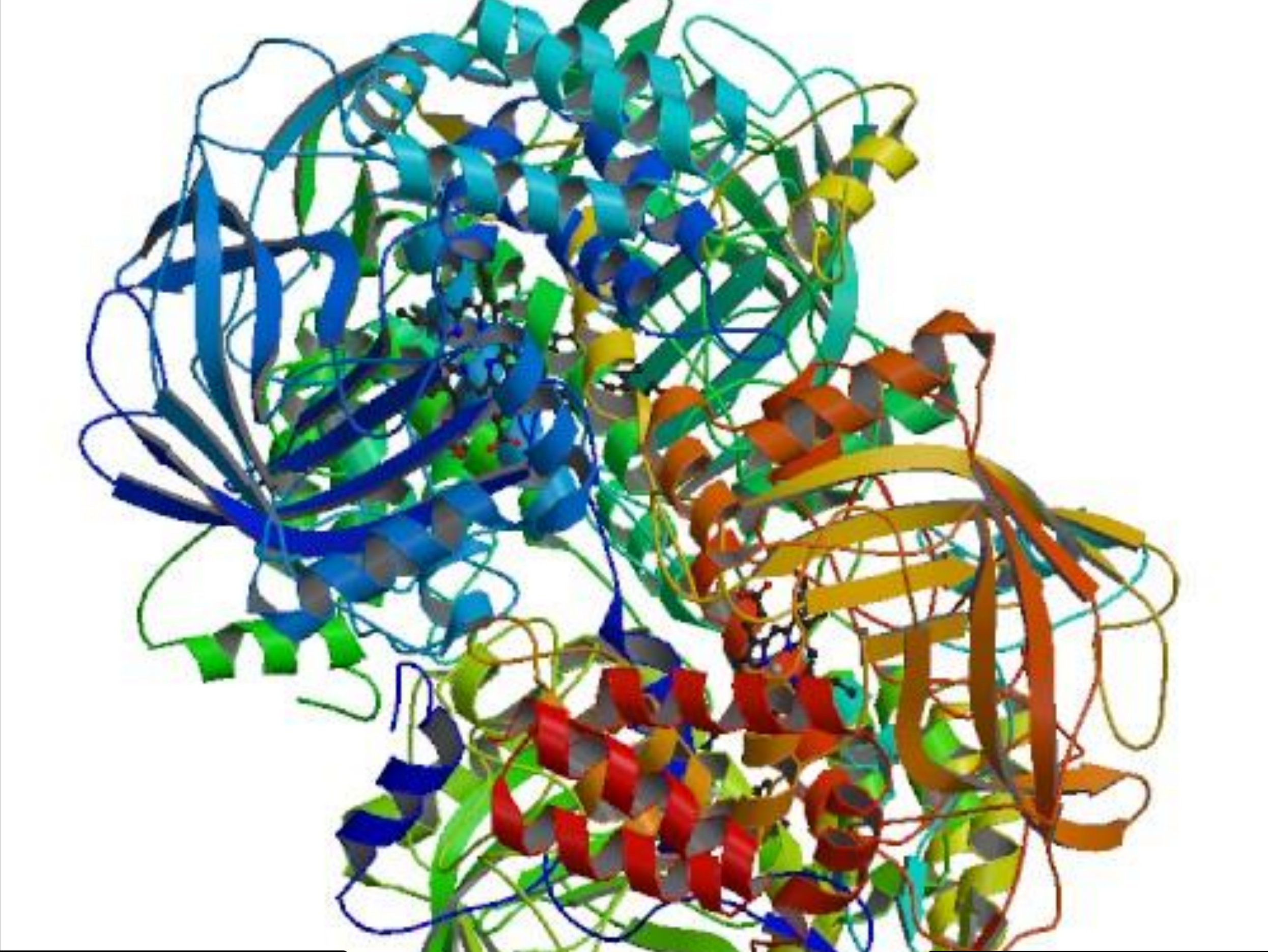


Figure 1 from "1QWQ Crystal structure of human erythrocyte catalase" at rcsb.org

Figures 2, 3, and 5 Putnam et al. (2000)

## Mutations

Homozygous mutation of the CAT gene has been found in Japanese type, a result of splicing of intron 5 or deletion in exon 4, and Hungarian type, a result of insertion in exon 2. It's an autosomal recessive trait that can cause the metabolic disorder acatalasemia. The patient has little to no catalase activity due to increased access to active site. Often asymptomatic, it may result in oral lesions known as Takahara's disease. Build up of H<sub>2</sub>O<sub>2</sub> causes obstructed blood flow to teeth and gums leading to oral gangrene. Low catalase activity has also been linked to diabetes, schizophrenia and greying of the hair. Figure 6 shows a mutation of HEC. These mutations are pointed out by the blue boxes.

Figure 6

|        |     |   |     |
|--------|-----|---|-----|
| Human  | 1   | MADSRDPASDQMHWKEQRAAQKADVLTTGAGNPVGDKLNVTIVGPRGPLLVDVVFTE   | 60  |
| Mutant | 1   | MADSRDPASDQMHWKEQRAAQKADVLTTGAGNPVGDKLNVTIVGPRGPLLVDVVFTE   | 60  |
| Human  | 61  | MAHFRERIPERVVHAKGAGAFGYFEVTHDITKYSKAKVFEHIGKKTPIAVRFSTVAGES | 120 |
| Mutant | 61  | MAHFRERIPERVVHAKGAGAFGYFEVTHDITKYSKAKVFEHIGKKTPIAVRFSTVAGES | 120 |
| Human  | 121 | GSADTVRDRPRGFVAVKFTYEDGNWDLVGNNTPIFFIRDPLFSPFHSQKRNPTQLKDDP | 180 |
| Mutant | 121 | GSADTVRDRPRGFVAVKFTYEDGNWDLVGNNTPIFFIRDPLFSPFHSQKRNPTQLKDDP | 180 |
| Human  | 181 | MVWDFWLSRPESLHQVSLFSDRGIPIGDRHMGYSHTFGLVANGAEVYCKFYKTDQ     | 240 |
| Mutant | 181 | MVWDFWLSRPESLHQVSLFSDRGIPIGDRHMGYSHTFGLVANGAEVYCKFYKTDQ     | 240 |
| Human  | 241 | GIKNLSVEDAARLSQEDDPYGIKLNIAITGKYPSTWTFYIQVMTFNQAEFFPNPDLT   | 300 |
| Mutant | 241 | GIKNLSVEDAARLSQEDDPYGIKLNIAITGKYPSTWTFYIQVMTFNQAEFFPNPDLT   | 300 |
| Human  | 301 | KVWPHKDYPLIPVGLVLRNPNVYFAEVEQIAFDPSNMPGGIEASPKMLQGRFLFAYPD  | 360 |
| Mutant | 301 | KVWPHKDYPLIPVGLVLRNPNVYFAEVEQIAFDPSNMPGGIEASPKMLQGRFLFAYPD  | 360 |
| Human  | 361 | THRHRLGPNYLHVPNCYRVARVANYQRDGPVCMQDQGGAPNYNSFGAPEQQPSALE    | 420 |
| Mutant | 361 | THRHRLGPNYLHVPNCYRVARVANYQRDGPVCMQDQGGAPNYNSFGAPEQQPSALE    | 420 |
| Human  | 421 | HSIQSGEVRRFNTANDDNVTQVRAFVYVNLNEEQKRLCENIAGHLKDAQIFIKKAVK   | 480 |
| Mutant | 421 | HSIQSGEVRRFNTANDDNVTQVRAFVYVNLNEEQKRLCENIAGHLKDAQIFIKKAVK   | 480 |
| Human  | 481 | NFTEVHPDYGSHIQALLDKYNAEKPKNAIHTFVQSGSHLAAREKANL             | 527 |
| Mutant | 481 | NFTEVHPDYGSHIQALLDKYNAEKPKNAIHTFVQSGSHLAAREKANL             | 527 |

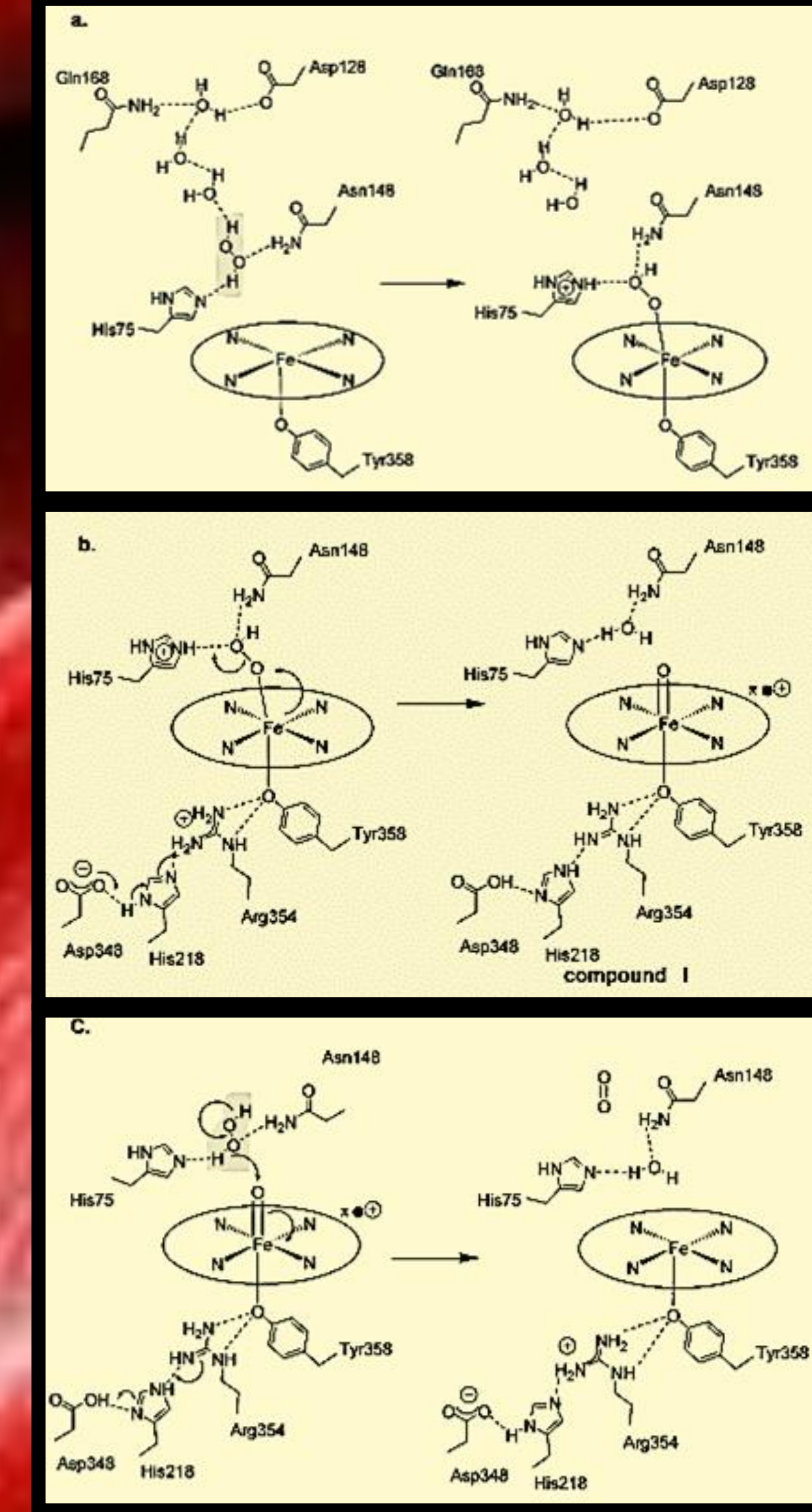
Figure 6 from Altschul et al. (1997)

## Conserved domains throughout evolution:

Figure 4 shows the amino acid sequence of HEC as compared to the amino acid sequence of catalase in other species. Letters in red represent amino acids that are identical. Letters in blue represent amino acids that are different. The n-terminus, as well as the majority of the protein, has been conserved throughout evolution. Conserved domains are the heme binding pocket, the NADPH binding site and the tetramer interface. A binding pocket is a cavity in a protein where a substrate can bind. Domains are conserved because they are important to the function of the protein. The c-terminus of the protein has not been conserved.

## References:

Goyal M, Basak A. Human catalase: looking for complete identity. Protein & Cell [serial online]. October 2010;1(10):888-897. Available from: MEDLINE, Ipswich, MA. Accessed March 26, 2016  
Putnam, Christopher D., et al. "Active and inhibited human catalase structures: ligand and NADPH binding and catalytic mechanism." Journal of molecular biology 296.1 (2000): 295-309.  
Stephen F. Altschul, Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402



**Figure a.** H<sub>2</sub>O<sub>2</sub> travels down the channel and binds to the Fe<sup>3+</sup> active site.

**Figure b.** Heterolytic cleavage of the O-O bond in H<sub>2</sub>O<sub>2</sub>. One oxygen remains bonded to the active site, which is now oxidized to Fe<sup>4+</sup>. The other oxygen leaves as a water molecule.

**Figure c.** A 2<sup>nd</sup> molecule of H<sub>2</sub>O<sub>2</sub> reduces Fe<sup>4+</sup> back to Fe<sup>3+</sup> and leaves as a water molecule and a molecular oxygen.

Figure a, b, c from Putnam et al. (2000)

Figure 4

## Amino acid sequence of HEC and homologs

|            |     |   |     |
|------------|-----|---|-----|
| Human      | 1   | MADSRDPASDQMHWKEQRAAQKADVLTTGAGNPVGDKLNVTIVGPRGPLLVDVVFTE                         | 80  |
| Mouse      | 1   | MADSRDPASDQMHWKEQRAAQKADVLTTGAGNPVGDKLNVTIVGPRGPLLVDVVFTE                         | 80  |
| Fruit fly  | 1   | MA-GRDAASNLIDYKNSQTVS-PGALTTGNGAFIIGKIDASQTVGPRGPIILQDQVNFLEMSHFDRERI             | 78  |
| chimpanzee | 1   | MADSRDPASDQMHWKEQRAAQKADVLTTGAGNPVGDKLNVTIVGPRGPLLVDVVFTE                         | 80  |
| Human      | 81  | AFGYFEVTHDITKYSKAKVFEHIGKKTPIAVRFSTVAGESGSADTVRDRPRGFVAVKFTYEDGNWDLVGNNTPIFFIRDPL | 160 |
| Mouse      | 81  | AFGYFEVTHDITKYSKAKVFEHIGKKTPIAVRFSTVAGESGSADTVRDRPRGFVAVKFTYEDGNWDLVGNNTPIFFIRDPL | 160 |
| Fruit fly  | 79  | AFGYFEVTHDITKYSKAKVFEHIGKKTPIAVRFSTVAGESGSADTVRDRPRGFVAVKFTYEDGNWDLVGNNTPIFFIRDPL | 158 |
| chimpanzee | 81  | AFGYFEVTHDITKYSKAKVFEHIGKKTPIAVRFSTVAGESGSADTVRDRPRGFVAVKFTYEDGNWDLVGNNTPIFFIRDPL | 160 |
| Human      | 161 | FPSFIHSQKRNPTQLKDDPDMVDFWLSRPESLHQVSLFSDRGIPIGDRHMGYSHTFGLVANGAEVYCKFYKTDQ        | 240 |
| Mouse      | 161 | FPSFIHSQKRNPTQLKDDPDMVDFWLSRPESLHQVSLFSDRGIPIGDRHMGYSHTFGLVANGAEVYCKFYKTDQ        | 240 |
| Fruit fly  | 159 | FPSFIHSQKRNPTQLKDDPDMVDFWLSRPESLHQVSLFSDRGIPIGDRHMGYSHTFGLVANGAEVYCKFYKTDQ        | 238 |
| chimpanzee | 161 | FPSFIHSQKRNPTQLKDDPDMVDFWLSRPESLHQVSLFSDRGIPIGDRHMGYSHTFGLVANGAEVYCKFYKTDQ        | 240 |
| Human      | 41  | GIKNLSVEDAARLSQEDDPYGIKLNIAITGKYPSTWTFYIQVMTFNQAEFFPNPDLTKVWPHKDYPLIPVGLVLRN      | 320 |
| Mouse      | 41  | GIKNLSVEDAARLSQEDDPYGIKLNIAITGKYPSTWTFYIQVMTFNQAEFFPNPDLTKVWPHKDYPLIPVGLVLRN      | 320 |
| Fruit fly  | 39  | GIKNLDVKTADQLASTDFDYSIRLDYLRKCKCFPSWTMYIQVMTYEQAKFKYKPNFDVTKVWSQKYEPLIPVGLVLRN    | 318 |
| chimpanzee | 41  | GIKNLSVEDAARLSQEDDPYGIKLNIAITGKYPSTWTFYIQVMTFNQAEFFPNPDLTKVWPHKDYPLIPVGLVLRN      | 320 |
| Human      | 321 | NPVNYFAEVEQIAFDPSNMPGGIEASPKMLQGRFLFAYPDTHRHRLGPNYLHVPNCYRVARVANYQRDGPVCMQDQGG    | 400 |
| Mouse      | 321 | NPVNYFAEVEQIAFDPSNMPGGIEASPKMLQGRFLFAYPDTHRHRLGPNYLHVPNCYRVARVANYQRDGPVCMQDQGG    | 400 |
| Fruit fly  | 319 | NPVNYFAEVEQIAFDPSNMPGGIEASPKMLQGRFLFAYPDTHRHRLGPNYLHVPNCYRVARVANYQRDGPVCMQDQGG    | 398 |
| chimpanzee | 321 | NPVNYFAEVEQIAFDPSNMPGGIEASPKMLQGRFLFAYPDTHRHRLGPNYLHVPNCYRVARVANYQRDGPVCMQDQGG    | 400 |
| Human      | 401 | APNYNPNFSGAPEQQPS-ALEHSIQSGEVRRFNTA-EDNVTQVRAFVYVNLNEEQKRLCENIAGHLKDAQIFIKK       | 477 |
| Mouse      | 401 | APNYNPNFSGAPEQQPS-ALEHSIQSGEVRRFNTA-EDNVTQVRAFVYVNLNEEQKRLCENIAGHLKDAQIFIKK       | 477 |
| Fruit fly  | 399 | APNYNPNFSGAPEQQPS-ALEHSIQSGEVRRFNTA-EDNVTQVRAFVYVNLNEEQKRLCENIAGHLKDAQIFIKK       | 478 |
| chimpanzee | 401 | APNYNPNFSGAPEQQPS-ALEHSIQSGEVRRFNTA-EDNVTQVRAFVYVNLNEEQKRLCENIAGHLKDAQIFIKK       | 477 |
| Human      | 478 | AVKNFTEVHPDYGSHIQALLDKYNAEKPKNAIHTFVQSGSHLAAREKANL                                | 527 |
| Mouse      | 478 | AVKNFTEVHPDYGSHIQALLDKYNAEKPKNAIHTFVQSGSHLAAREKANL                                | 527 |
| Fruit fly  | 479 | AVKNFTEVHPDYGSHIQALLDKYNAEKPKNAIHTFVQSGSHLAAREKANL                                | 506 |
| chimpanzee | 478 | AVKNFTEVHPDYGSHIQALLDKYNAEKPKNAIHTFVQSGSHLAAREKANL                                | 527 |

Figure 4 from Altschul et al. (1997)