

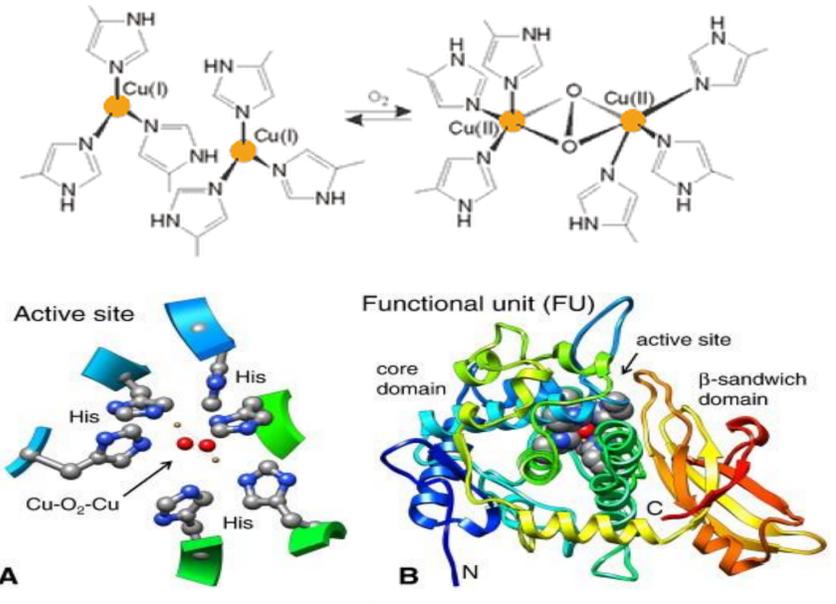


# Hemocyanin; Don't Get in the Way of Blue Bloods

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## Abstract:

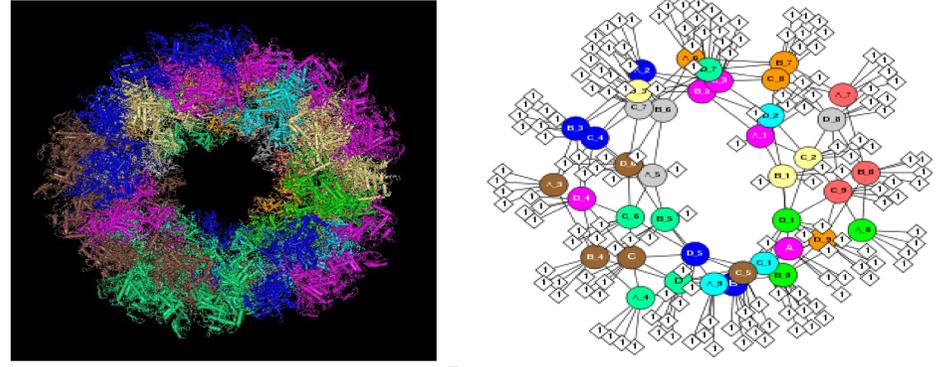
- Hemocyanin is a large oxygen carrying protein in the blood of numerous invertebrates such as horseshoe crabs.
- It is immunogenic, meaning it stimulates the immune system and is a perfect carrier for proteins we want the body to fight.
- Research has shown that hemocyanin is a possible treatment for a number of cancers.
- It is an important precursor to the enzyme phenoloxidase, which is needed by the animals that have it to fight off disease and to facilitate healing.



**Figure 1**  
The top image shows the functional unit of hemocyanin unbound to O<sub>2</sub> on the left and binding to O<sub>2</sub> on the right. Each copper is associated with 3 histidine residues, which help maintain its conformation. The image A shows a close up of the active site where binding of Cu and O<sub>2</sub> takes place, as oriented by the histidine residues. Image B is showing an example of how the active site is situated into the entire functional unit of hemocyanin and phenoloxidase.

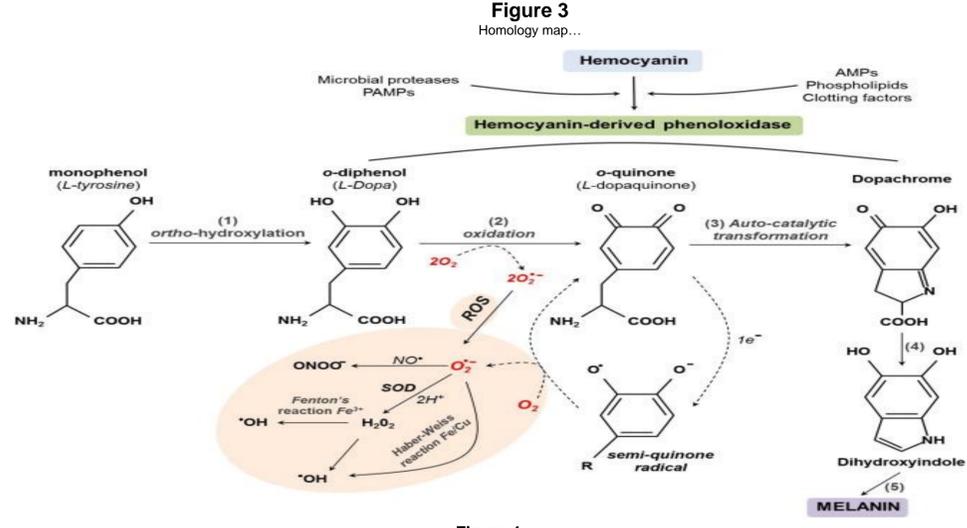
## Introduction:

- Hemocyanins are large free-floating proteins in the hemolymph of arthropods, mollusks, cephalopods, crustaceans and some insects.
- They are functionally homologous to the mammalian protein hemoglobin as an oxygen transport molecule, but it uses copper instead of iron for this function.
- The protein turns blue when bound to oxygen.
- Hemocyanins are very large proteins, with each subunit of the protein weighing about 75kDa. Each functional unit of hemocyanin contains 2 Cu atoms supported by 3 histidine molecules each. O<sub>2</sub> is bound between the two copper atoms (Figure 2).
- Its large size makes it immunogenic, making it useful as an adjuvant for vaccines and for creating anti-tumor or cancer immune responses in mammals[6].
- Hemocyanin is a precursor to the enzyme phenoloxidase[3] (Figure 4) which has a nearly identical active site as the oxygen-binding site of hemocyanin. Phenoloxidase is needed to respond to pathogens by clotting around the pathogen and killing it.
- Phenoloxidase also responds to injury by forming clots when the exoskeleton is injured and stimulating the production of melanin in the healing process.
- It is possible that a mutation in the hemocyanin of an arthropod or other such creature could lead to a dysfunctional phenoloxidase, causing some degree of albinism or melanoma.



**Figure 2**  
Complete keyhole limpet hemocyanin showing 160 functional units.

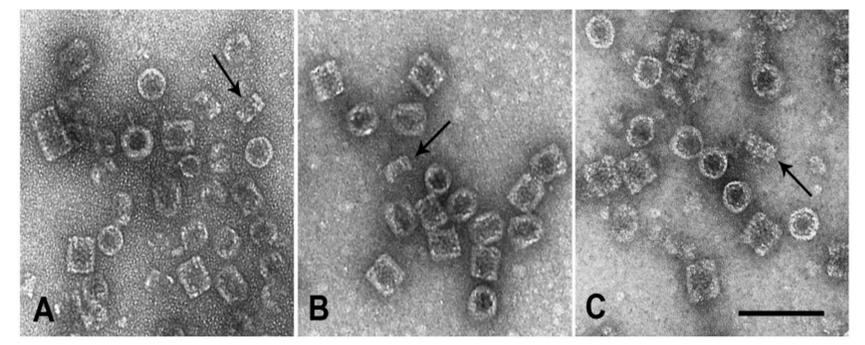
Nasonia vitripennis	1	MALSNasK[Q]SNLILLDFDRPEHYVVPKGERKVFQVDPVPPNLPKQYQNMARILNRFNGESLSSIPKQIAPDLSPL 63
Drosophila melanogaster	1	MAD—KKNLILLDFDHPTEPFMDKQKRVYFDVDFDPSFLTDYRVPISNEVQSRVQKVEQRPVREISPLRIPM 73
Drosophila melanogaster	1	MAD—KKNLILLDFDHPTEPFMDKQKRVYFDVDFDPSFLTDYRVPISNEVQSRVQKVEQRPVREISPLRIPM 73
Nasonia vitripennis	1	ME—KNLILLDFDRPEHYVVPKGERKVFQVDPVPPNLPKQYQNMARILNRFNGESLSSIPKQIAPDLSPL 71
Trichogramma pretiosum	1	MAQME—KSLILLDFDRPEHYVVPKGERKVFQVDPVPPNLPKQYQNMARILNRFNGESLSSIPKQIAPDLSPL 74
Creatosolen sulms marchali	1	MLELAkmKRNLILLDFDRPEHYVVPKGERKVFQVDPVPPNLPKQYQNMARILNRFNGESLSSIPKQIAPDLSPL 77
Nasonia vitripennis	84	SLSRDNFSLFSPSHRKMAGHLITLFGMRTIEDLLSAYACDRVNAQMFVYLSVAILHRPDKHLVPQTEVFPDK 163
Drosophila melanogaster	74	SLGRDEQSLFLPKHRIAGRIDIFMNRSDVDDQSVAYVARDRVNPLFNALSVALLHRPDKGLDLPFSQTFPDR 153
Drosophila melanogaster	74	RLGRSEQSLFLPKHRIAGRIDIFMNRSDVDDQSVAYVARDRVNPLFNALSVALLHRPDKGLDLPFSQTFPDR 153
Nasonia vitripennis	72	SLSRDNFSLFSPSHRKMAGHLITLFGMRTIEDLLSAYACDRVNAQMFVYLSVAILHRPDKHLVPQTEVFPDK 151
Trichogramma pretiosum	75	SLSRRETFSLFPAHRKMATRLTELFMGMRTLEDLVSAAVCRDRVNAQMFVYLSVAILHRPDKHLVPQTEVFPDK 154
Creatosolen sulms marchali	78	SLSRDNFSLFSPSHRKMAGHLITLFGMRTIEDLLSAYACDRVNAQMFVYLSVAILHRPDKHLVPQTEVFPDK 157
Nasonia vitripennis	164	YMDSSVFRHRAKEANVVP-PGSRNAIEPLDWTATDADPEHRVAYVREDVGNLHWHWHVLYPFEGPVA-VQKDRRG 241
Drosophila melanogaster	154	FIDQVIRKMRSEFVYVQ-PGSRMPTIPRDTYASDLDEPHRLVYFRDELGINLHWHWHVLYPFEGPVA-VQKDRRG 232
Drosophila melanogaster	154	FIDQVIRKMRSEFVYVQ-PGSRMPTIPRDTYASDLDEPHRLVYFRDELGINLHWHWHVLYPFEGPVA-VQKDRRG 232
Nasonia vitripennis	152	YMDSSVFRHRAKEANVVP-PGSRNAIEPLDWTATDADPEHRVAYVREDVGNLHWHWHVLYPFEGPVA-VQKDRRG 229
Trichogramma pretiosum	155	YMESSIFHRAKEANVVP-AGSRTPIEPLDWTATDADPEHRVAYVREDVGNLHWHWHVLYPFEGPVA-VQKDRRG 232
Creatosolen sulms marchali	158	YMDGSIHRAKEANVVP-IGSRTPIEPLDWTATDADPEHRVAYVREDVGNLHWHWHVLYPFEGPVA-VQKDRRG 235
Nasonia vitripennis	242	LFYYMHQIARYNAERFNSNLRARVLPFNLRDPIAEGYFPKMSLVASRAVPRPFESTRLSDLNEDQLNVEIGDLR 312
Drosophila melanogaster	233	LFYYMHQIARYNAERFNSNLRARVLPFNLRDPIAEGYFPKMSLVASRAVPRPFESTRLSDLNEDQLNVEIGDLR 312
Drosophila melanogaster	234	LFYYMHQIARYNAERFNSNLRARVLPFNLRDPIAEGYFPKMSLVASRAVPRPFESTRLSDLNEDQLNVEIGDLR 313
Nasonia vitripennis	233	LFYYMHQIARYNAERFNSNLRARVLPFNLRDPIAEGYFPKMSLVASRAVPRPFESTRLSDLNEDQLNVEIGDLR 309
Trichogramma pretiosum	233	LFYYMHQIARYNAERFNSNLRARVLPFNLRDPIAEGYFPKMSLVASRAVPRPFESTRLSDLNEDQLNVEIGDLR 312
Creatosolen sulms marchali	236	LFYYMHQIARYNAERFNSNLRARVLPFNLRDPIAEGYFPKMSLVASRAVPRPFESTRLSDLNEDQLNVEIGDLR 315
Nasonia vitripennis	322	YRDRIMEAVHTQVRNGKGGVQLDEVTDGIDLGNMMEASISDPDYYGVDVHNMGHVAISFAHPDPHRYLEPTIMGDA 401
Drosophila melanogaster	313	WRDRIVEAHDGQVYVDRKRPVDEATGIDLGNMMEASISDPDYYGVDVHNMGHVAISFAHPDPHRYLEPTIMGDA 392
Drosophila melanogaster	314	WRDRIVEAHDGQVYVDRKRPVDEATGIDLGNMMEASISDPDYYGVDVHNMGHVAISFAHPDPHRYLEPTIMGDA 393
Nasonia vitripennis	310	YRDRIMEAVHTQVRNGKGGVQLDEVTDGIDLGNMMEASISDPDYYGVDVHNMGHVAISFAHPDPHRYLEPTIMGDA 389
Trichogramma pretiosum	313	YRDRILEAQISQARLASGGVQLDEVTDGIDLGNMMEASISDPDYYGVDVHNMGHVAISFAHPDPHRYLEPTIMGDA 392
Creatosolen sulms marchali	316	YRDRILEAVHTQVRNGKGGVQLDEVTDGIDLGNMMEASISDPDYYGVDVHNMGHVAISFAHPDPHRYLEPTIMGDA 395
Nasonia vitripennis	402	TTAMRDPVYFRWHAAYVDHVQVFKDLSPPYTVLQVLFVPGINVDIRINTPGANPNTLNHWKSDVLSRGLDFTPRGSI 481
Drosophila melanogaster	393	STAMRDPVYFRWHAAYVDHVQVFKDLSPPYTVLQVLFVPGINVDIRINTPGANPNTLNHWKSDVLSRGLDFTPRGSI 472
Drosophila melanogaster	394	STAMRDPVYFRWHAAYVDHVQVFKDLSPPYTVLQVLFVPGINVDIRINTPGANPNTLNHWKSDVLSRGLDFTPRGSI 472
Nasonia vitripennis	390	TTAMRDPVYFRWHAAYVDHVQVFKDLSPPYTVLQVLFVPGINVDIRINTPGANPNTLNHWKSDVLSRGLDFTPRGSI 469
Trichogramma pretiosum	393	TTAMRDPVYFRWHAAYVDHVQVFKDLSPPYTVLQVLFVPGINVDIRINTPGANPNTLNHWKSDVLSRGLDFTPRGSI 472
Creatosolen sulms marchali	396	TTAMRDPVYFRWHAAYVDHVQVFKDLSPPYTVLQVLFVPGINVDIRINTPGANPNTLNHWKSDVLSRGLDFTPRGSI 475
Nasonia vitripennis	482	MARLTLNHDQFYSNITVNSNKKELIGTVRIFAPRFDETRGQFTFDHQRLMIEMDKFTTQLKRGQNVISRSVDSAL 561
Drosophila melanogaster	473	FARFTLQHLPTTYTISLNNDSCADRFQYVRFMAPKQDROPNLMDQDSMMELDKFTTQLKRGQNVISRSVDSAL 552
Drosophila melanogaster	473	LARFTLQHLQHEFSYTKVENSSEATRYGVYRFLAPKLDROPNLMDQDSMMELDKFTTQLKRGQNVISRSVDSAL 552
Nasonia vitripennis	470	MARLTLNHDQFYSNITVNSNKKELIGTVRIFAPRFDETRGQFTFDHQRLMIEMDKFTTQLKRGQNVISRSVDSAL 549
Trichogramma pretiosum	473	LARLQHLNHDQFYSNITVNSNKKELIGTVRIFAPRFDETRGQFTFDHQRLMIEMDKFTTQLKRGQNVISRSVDSAL 552
Creatosolen sulms marchali	476	MARIQHLHDQFYSNITVNSNNDGDVYGNVRIFMAPKQDFTGHHFNENORLMIEMDKFTTQLKRGQNVISRSVDSAL 555
Nasonia vitripennis	562	TIPFEATFRNLDNRPSDDQIAADAFNFCGCGWPHMLVPGKQSGYPMDFMVTNYELDRNQDPT-GCREGVSF 640
Drosophila melanogaster	553	TIPFEATFRNLDNRPSDDQIAADAFNFCGCGWPHMLVPGKQSGYPMDFMVTNYELDRNQDPT-GCREGVSF 630
Drosophila melanogaster	553	TIPFEATFRNLDNRPSDDQIAADAFNFCGCGWPHMLVPGKQSGYPMDFMVTNYELDRNQDPT-GCREGVSF 630
Nasonia vitripennis	550	TIPFEATFRNLDNRPSDDQIAADAFNFCGCGWPHMLVPGKQSGYPMDFMVTNYELDRNQDPT-GCREGVSF 628
Trichogramma pretiosum	556	TIPFEATFRNLDNRPSDDQIAADAFNFCGCGWPHMLVPGKQSGYPMDFMVTNYELDRNQDPT-GCREGVSF 631
Creatosolen sulms marchali	556	TIPFEATFRNLDNRPSDDQIAADAFNFCGCGWPHMLVPGKQSGYPMDFMVTNYELDRNQDPT-GCREGVSF 634
Nasonia vitripennis	641	GLRDLKYPDLRPNMFFDRLGRGTVGSLNDFLTPNMKVPQITVRFSDVIVKPPRSGQTVIRL 703
Drosophila melanogaster	631	GVDRDLVYDQSGMGGFFDRLRPSGVDRLVNLTPNMKSDVIVKPPRSGQTVIRL 684
Drosophila melanogaster	625	GLRDLKYPDLRPNMFFDRLGRGTVGSLNDFLTPNMKVPQITVRFSDVIVKPPRSGQTVIRL 683
Nasonia vitripennis	629	GLRDLKYPDLRPNMFFDRLGRGTVGSLNDFLTPNMKVPQITVRFSDVIVKPPRSGQTVIRL 691
Nasonia vitripennis	635	GLRDLKYPDLRPNMFFDRLGRGTVGSLNDFLTPNMKVPQITVRFSDVIVKPPRSGQTVIRL 685



**Figure 4**  
Shows the pathway that hemocyanin follows to become phenoloxidase and produce melanin

## Summary:

- Hemocyanin is 1/4 as effective as hemoglobin at oxygen binding at standard temperature, however it is less affected by low oxygen concentrations and cold temperatures, making it a more effective oxygen carrier than our hemoglobin in environments such as the ocean floor [10].
- Hemocyanin is very large, allowing it to easily interact with and activate macrophages, neutrophils and T cells in the body, generating both humoral and cellular immune responses [4], [1].
- The protein itself does not have any adverse side effects when injected into the mammalian body. For this reason, hemocyanin has been used as an adjuvant for a number of vaccines and it is being used as a presenter molecule to activate the immune system against a number of different cancers [1], [2], [5], [6].
- The O<sub>2</sub>-binding site of hemocyanin shares the same conformation as that of phenoloxidase, which is derived from hemocyanin in arthropods.



**Figure 5**  
A. Hemocyanin from the Keyhole limpet (KLH). B. Hemocyanin from Concholepas concholepas, (CCH). C. Hemocyanin from Fissurella latimarginata, (FLH). The images show the top (circles) and lateral (rectangles) views of the molecules. The scale bar represents 100 nm.

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