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Chemical-computational comparison of organometallic complexes in oxygen carriers and their incidence on blood color

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Abstract. Blood typically carries small amounts of molecular oxygen (O_2) dissolved in the plasma and large amounts chemically combined with hemoglobin. Partial pressure depends only on physically dissolved oxygen, which determines how much oxygen will combine with hemoglobin. The blood needs an O_2 carrier in humans because this gas is not sufficiently soluble in the blood plasma to satisfy the body's requirement. At 37 ºC, a liter of blood only dissolves 2.3 mL of O_2. However, one liter of blood contains 150 g of hemoglobin, and since each gram of hemoglobin dissolves 1.34 mL of O_2, 200 mL of O_2 are transported per liter of blood. This quantity is 87 times more than what plasma could carry by itself. Without an O_2 carrier like hemoglobin, blood would have to circulate 87 times faster, requiring a high-pressure pump, turbulent flow, and a considerable ventilation-perfusion mismatch. This is why other transporter molecules exist in various organisms, giving each of them a representative color. This is how it is in the human being that blood is red; in spiders, crustaceans, mollusks, octopuses, and squids, the blood is blue; in some segmented worms, some leeches, and some marine worms, there is green blood; finally, in marine worms and brachiopods, the blood is violet.

Keywords: Blood Color, Oxygen, Organometallic Complex, Carriers.

INTRODUCTION

Blood is a tissue mainly responsible for oxygen and carbon dioxide transport. However, it has other important functions, such as transporting nutrients and hormones or capturing and dissipation of heat. Reptiles, amphibians, and fish usually transmit heat through their skin, so they are exposed to sunlight to heat up since they do not have their own source. When the body needs to conserve body heat, blood flow is reduced, and conversely, when it needs to dissipate heat, blood flow increases (Arvanitis et al., 2020; Cho et al., 2020; Fuchs & Whelton, 2020).

Blood is produced in specialized tissues such as the red bone marrow (found in flank bones) in mammals and the kidneys in fish, in a process known as hematopoiesis ("blood creation"). Blood comprises a transparent tissue called plasma and cells such as red blood cells, immune system cells, and platelets, responsible for repairing or healing areas where the tissue has been damaged. In most cases, inside each red blood cell, a protein captures oxygen molecules (Brass et al., 2019; Versteeg et al., 2013). This protein is a pigment.

The type of pigment in each group of organisms determines the color of the blood. Some animals have red blood due to hemoglobin; others may have various pigments that will change their blood color (blue, green, orange, yellow, purple); others are usually colorless because they do not have any pigment. Even so, all organisms develop respiration to oxygenate their tissues (Ji et al., 2022; Noris & Galbusera, 2023; Simpson et al., 2022).

Due to the presence of hemoglobin in vertebrates and some invertebrates, red blood is the most common for transporting gases. Hemoglobin is a globular protein comprising four coordinated subunits, differentiated only by their chains: two alpha and the remaining beta (Ahmed et al., 2020; Ali et al., 2022; Giardina, 2022).

Certain invertebrates, instead of hemoglobin, have hemocyanin, an organometallic oxygen-carrying complex. The critical difference is that you have a metallic coordinating agent of copper; it turns blue in the oxygenated form, and after exchanging, it turns colorless in its deoxygenated form. The organometallic complex owns two copper atoms that coordinate with O_2. Certain mollusks and some arthropods have it in their hemolymph. (Li et al., 2019; Zhan et al., 2019; Zhao et al., 2022; Zheng et al., 2021)

One genus of skinks (Prasinohaema) has green blood and hemoglobin in its blood, but they have a higher concentration of biliverdin. The metabolism of precursor hemoglobin generates this pigment. Thus, being a derivative of it, it is a secretion generated in the liver secreted with bile. In the green-blooded skink, the biliverdin in its blood reaches critical levels that would be toxic to other lizards, predators, or organisms such as humans (Cimini et al., 2022; Mancuso, 2021). Segmented worms of the (Annelida) family and leeches have chlorocruorin, the green pigment in their blood. This molecule not always shows their color, since some other annelids have hemoglobin, and this red color masks the green (Bankar et al., 2021; Benbelkhir & Medjekal, 2022; Clauss & Clauss, 2021).

In the case of brachiopods, they are known to have purple blood, the color provided by their oxygen carrier. Hemerythrin is a bulky organometallic complex that conjugates a bidentate ligand and coordinates with the oxygen molecule. They have an open circulatory system, a small heart suspended above the stomach. Some of the cells in the coelomic fluid contain hemerythrin. This suggests that the coelomic vessels constitute the primary transport system for respiratory gases. Some other invertebrate-type marine organisms have hemerythrin as an organometallic complex that transports oxygen in the blood. It is pink-violet in color when oxygenated, and in its deoxygenated state, it lacks color (Kitanishi, 2022; Stenkamp, 1994; Wilkins & Wilkins, 1987).

There are colorless fluids in ice fish that are representatives of the family (Channichthyidae), they are vertebrates, and they are called white or colorless blood. No organometallic complex provides color to their blood; due to this characteristic, they have adapted other physiological functions to live in cold waters (DeVries, 1988; Garofalo et al., 2009; Padang et al., 2020). Oxygen dissolves better in cold water than in warm water; this is not enough to keep fish alive. One of the adaptations is having a large heart that pumps much blood with each beat, with a greater blood volume than fish of comparable size with red blood. Numerous blood vessels in the skin absorb some oxygen, while the gill membranes aid gas exchange (Kim et al., 2019).

Organisms with alternating fluids, such as insects, have their hemolymph pale yellow/green or colorless; hemolymph does not transport O_2, so it does not require an organometallic coordination complex. Thus, tubular networks transport O_2 through a tracheal system that exchanges gas in aerobic respiration (Blow & Douglas, 2019; Theopold et al., 2002; Wyatt, 1961).

MATERIALS AND METHODS

Blood Type Contrast in Different Organisms

The literature search will seek various types of organisms that may present notable variability in their blood fluids. With discrimination of results, it is proposed to observe mainly the color of the oxygenated blood fluid, compared with the standard molecule for the human being. Here, the hemoglobin, with its red color, becomes a pattern to be compared for the multiple species and transporter molecules of oxygen.

It is proposed to compare with the substrate enzyme complexes reported in the RCSB Protein Data Bank (RCSB PDB) platform, and to obtain the crystalline structures, exporting the complex for purification, showing only the three-dimensional structure of each one (Duarte et al., 2022).

Basal Oxygen Carriers and Organometallic Complexes

It is well known that oxygen is transported physically dissolved in the blood and chemically combined with carrier molecules. Under normal circumstances, more oxygen is transported with these molecular carriers than is physically dissolved in the blood. In this way, these molecular targets will be reported as individual structures, that is, it is their basal state, and it will be contrasted with the internal structures in organometallic complexes that function as oxygen carriers.

The basal structures will be compared. Some will present a porphyrin structure in common, looking for the explanation of why they need a metallic agent and how their coordination number makes possible the conformation of the organometallic complex.

Three-Dimensional Biological Complexes

Knowing that each crystalline complex has protein chains with quaternary structures, always associated with a ligand, also called exogenous complement structure. The individual purification of each complex will be carried out using the UCSF Chimera 1.16 Software (Pettersen et al., 2004), eliminating complementary chains and non-compatible amino acid residues at 4 Angstroms, a length universally considered by default to predict potential intermolecular interactions (Núñez-Navarro et al., 2016; Santana-Romo, Duarte, et al., 2020; Santana-Romo, Lagos, et al., 2020).

Each generated image will report the organometallic complex in three dimensions and the transported oxygen molecule. In the same way, it will be possible to visualize the coordination links with the metallic agents and how they, through intermolecular interactions, hold and support the entire complex of oxygen-transporting molecules.

RESULTS AND DISCUSSION

Blood Type in Different Organisms

Red blood. This quantity is 87 times more than what plasma could carry by itself. In the most developed organisms, blood is red, which takes its color mainly from its content of red blood cells, as it is in humans (Figure 1). It contains hemoglobin, a protein responsible for transporting oxygen throughout the body. Hemoglobin is made up of iron, which turns red when it encounters oxygen. The reddish color refers presence of iron in nature, for example, in soils with high iron content (Ali et al., 2022; Giardina, 2022).

Now, at certain times the red color of the blood varies. For example, blood that is more oxygenated and carried from the lungs to the body's organs and muscles through the arteries tends to be pinker. On the other hand, dark blood has already transported oxygen to some destination and returns, through the veins, to the heart and lungs, carrying CO_2 with it to be expelled through respiration to start the cycle again (Ahmed et al., 2020).

Blue blood. Certain organisms such as spiders, crustaceans, some mollusks, octopuses, and squids have their blood flow blue (Figure 1b). It is important to note that they have the disadvantage that their oxygen-carrying capacity is three times less than that of red-blooded organisms. Oxygen is essential to generate energy, and that energy is used to perform bodily movements or other activities such as growing and searching for food or a mate. The reduced oxygen-carrying capacity of blue-blooded organisms has led to cephalopods in the Southern Ocean leading a sedentary lifestyle, marked by little movement and no bodily changes during their life. (Li et al., 2019; Zheng et al., 2021).

The scientific community is currently concerned that these organisms will not be able to respond adequately to global warming and will disappear. The main reason is that the affinity of hemocyanin for oxygen decreases with increasing temperature. What was an adaptation to extreme cold is a drawback in the current global situation, in which the seas are warming (Zhan et al., 2019; Zhao et al., 2022).

Green blood. Species of lizards, leeches, some segmented worms, and marine worms have their blood green (Figure 1c). It is not because they do not have hemoglobin but rather because of an accumulation of biliverdin, a bile pigment produced by the catabolism of hemoglobin, which first becomes bilirubin and then, through oxidation, biliverdin. When it forms its porphyrin conjugation and unites with the iron atom to form the organometallic complex, this molecule takes the name of chlorocruorin. This molecule transports oxygen in organisms that have green blood. Green color predominates on cells and stains blood, bones, muscles, tongue, and mucous membranes (Cimini et al., 2022; Mancuso, 2021).

In all other vertebrates, this excess bile pigment in the blood causes a pathological problem known as jaundice, which can cause severe motor and brain damage. The pigments in certain lizards have a toxic function for their predators; their absorption in the visible spectrum provides protection against ultraviolet rays. Unlike warm-blooded organisms, they do not have thermoregulation, so they are exposed to the sun's rays to get warm (Bankar et al., 2021; Benbelkhir & Medjekal, 2022; Clauss & Clauss, 2021).

Violet blood. Purple blood tends to have this hue due to hemerythrin (Figure 1d); they are usually present in brachiopods and marine worms, including peanut and penis worms (Kitanishi, 2022). This complex is another pigment that contains iron, which binds to oxygen molecules and gives a purple-pink hue. This blood is found in some mollusks, such as brachiopods (lamp shells), and urochordates or sea squirts (Stenkamp, 1994; Wilkins & Wilkins, 1987).

Transparent Blood. There is evidence of a particular group of fish whose blood is transparent. In the Southern Ocean, the so-called "ice fish" lack respiratory pigment in their blood, which is why it is considered colorless or transparent (DeVries, 1988; Garofalo et al., 2009). Although it seems illogical not to have respiratory pigment in the blood, it is likely because the pigment would increase the viscosity of the blood fluid, which in cold places would make it difficult for the heart to pump. Thus, natural selection would have favored the removal of pigment in these organisms. On the other hand, oxygen availability in this habitat is very high, so it is unnecessary to have respiratory pigment (Kim et al., 2019; Padang et al., 2020).

Other types of fluids. As a complement to the deep search for information, we detail that other organisms have marked differences in their blood (Figure 1e).

Insect blood. These organisms do not have blood but instead have a fluid called hemolymph, which is a fluid that carries gases and hormones through the system. Insects do not absorb oxygen from the blood through openings along their backs and sides.

Hemolymph usually has yellowish or bluish-green pigments. These are often due to these animals' diets (Blow & Douglas, 2019; Theopold et al., 2002).

Bloodless animals. In the sea, there is an animal called a flatworm: it looks like a worm; the oxygenation of the tissues depends on the skin since it lacks a circulatory system. Gas exchange provides respiration for the body while nutrients go to the intestine. On the other hand, starfish and sea cucumbers do not have blood, as water is the equivalent, and it transports nutrients and gases through a water-based vascular system (Herhold et al., 2023).

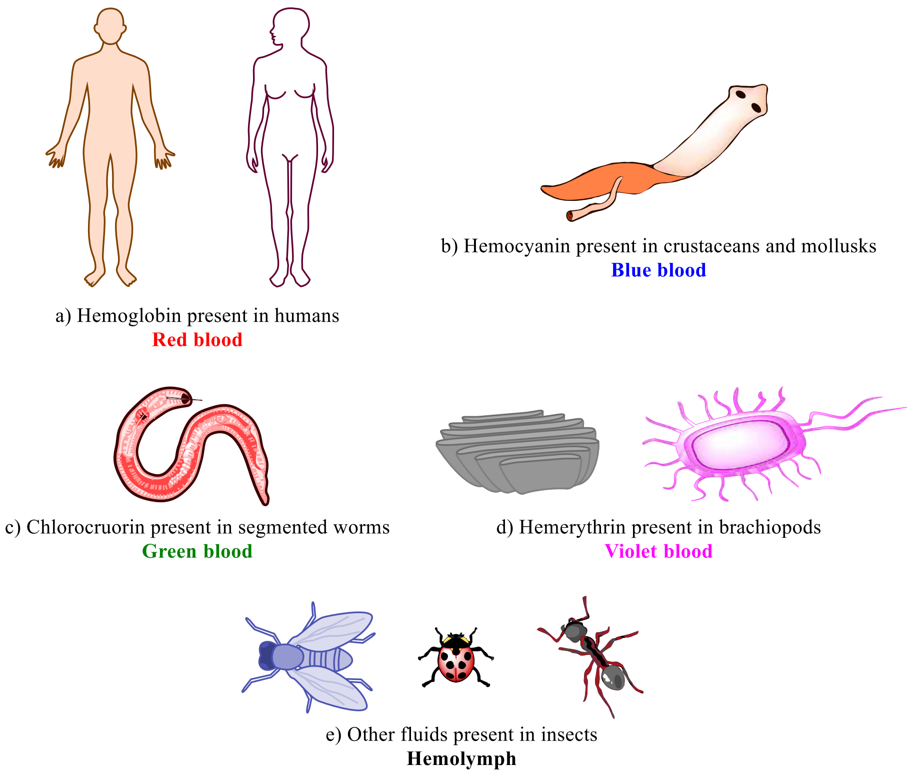
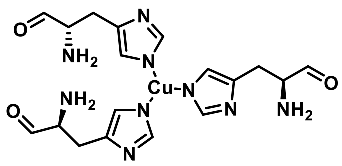


Fig 1. Organisms with various types of blood.

Basal Oxygen Carriers and Organometallic Complexes

The complexes reported in Figure 3 clearly show that they are not associated with oxygen; thus, some are organometallic complexes, such as hemoglobin, chlorocruoryn, and hemerythrin. On the other hand, in Figure 2, hemocyanin shows two individual complexes of tris(4-((S)-2-amino-3-oxopropyl)-1H-imidazol-1-yl)copper.

The metallic agent is significant; only in hemocyanin is the copper atom in the two individual complexes that will form a single organometallic complex by generating their coordination bonds during oxygen transport.



tris(4-((S)-2-amino-3-oxopropyl)-1H-imidazol-1-yl)copper

Fig 2. Hemocyanin organometallic complex subunit

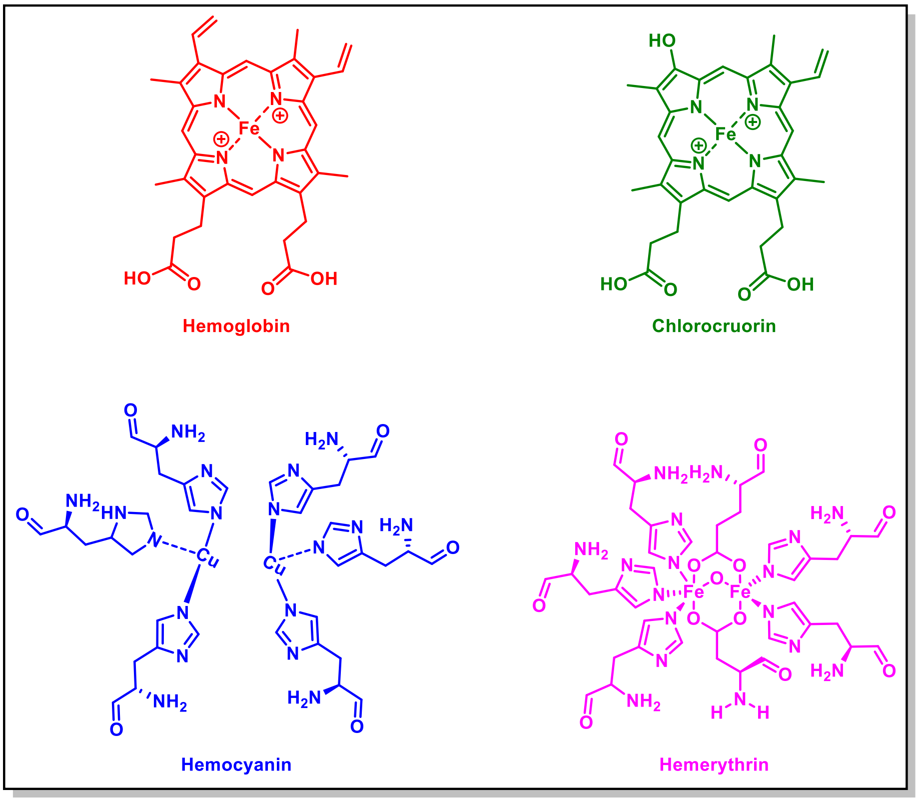


Fig 3. Organometallic no oxygenated complexes in the blood.

Figure 4 shows all the organometallic complexes duly associated with the oxygen molecule, and it should note that oxygen is generally represented as O_2, diatomic, molecular, or gaseous state. Oxygen transporter complexes show both formal bonds, sigma σ type, and attractive intermolecular interactions for coordination bonds.

For hemoglobin and chlorocruorin, it is noted that a pair of the nitrogen atoms of the porphyrin ring has a positive formal charge, indicating that they have a more electrophilic character when generating false bonds from the coordination of the iron atom.

Regarding the hemocyanin and hemerythrin molecules, no positive or negative formal charges are shown; this suggests in terms of reactivity that the carrier molecule remains relatively stable associated with the presence of O_2.

Oxygen is transported and dissolved in the blood in combination with enzymatic factors and inorganic cofactors, which are necessary for hemostasis processes; In this way, several types of organisms present oxygen transporter molecules, different for each one of them, and it is known that according to their complexing metal and structural disposition of the transporter. They can present different colors to the sight of the blood fluids of each specie.

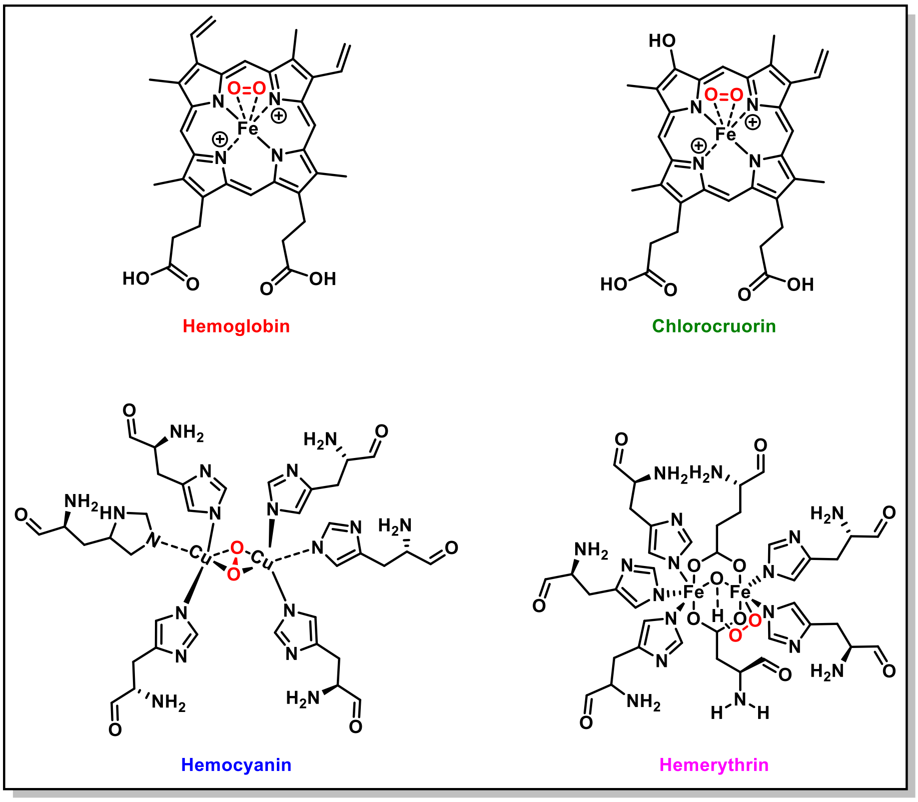


Fig 4. Organometallic oxygenated complexes in the blood.

Three-Dimensional Biological Complexes

Hemoglobin. This molecule has the characteristic conformation of the porphyrin ring; thus, its planarity is the product of sp^2 hybridizations of its constituent heteroatoms. It is striking that the central iron atom associates through its coordination bonds with molecular oxygen but also interacts with a complementary residue of a porphyrin fragment of 2-amino-3-(4H-imidazol-4-yl)propanal. The one that complements and stabilizes the two faces of the linear plane with its evidence in Figure 5. The hemoglobin structure was refined from the crystal reported in PBD ID (5EE4) (Wong et al., 2015); their Root Mean Square Deviation (RMSD) is 2.30 Å, thus obtaining the organometallic complex of Figure 6.

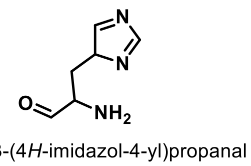


Fig 5. Associated porphyrin residue.

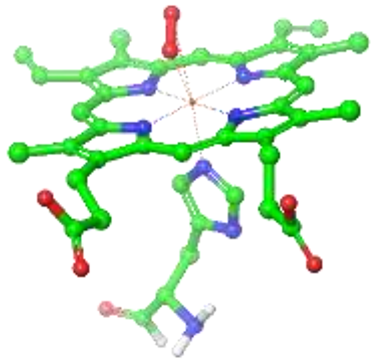


Fig 6. The three-dimensional complex of hemoglobin.

Hemocyanin. This oxygen transporter, having subunits of 2-amino-3-(4H-imidazol-4-yl)propanal, makes the electronic pair available on the cycle nitrogen become donors in the form of a coordinated covalent bond, generating thus the new coordination bonds with the copper heteroatoms. By constituting this, the organometallic complex can receive molecular oxygen, forming bonds in a dipyramid shape, complementing its overall structure. The hemocyanin structure was clean from the crystal reported in PBD ID (1NOL) (Hazes et al., 1993); RMSD 2.40 Å, obtaining the complex shown in Figure 7.

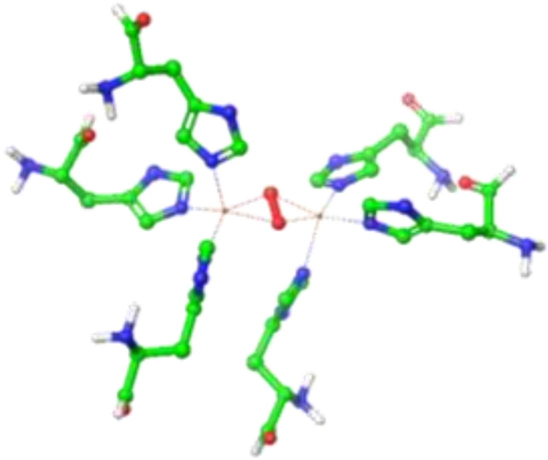


Fig 7. The three-dimensional complex of hemocyanin.

Erythrocruonin. Also called chlorocruorin, and with characteristics very similar to hemoglobin, taking into account the minimal variation of the porphyrin ring with the addition of a peripheral hydroxyl radical, it was detailed via literature that it is a derivative thereof that can vary its concentration in organisms and that due to this concentration will depend on the final tonality of the blood fluid, three-dimensionally it fulfills the structural functions of its predecessor molecule. The erythrocruonin structure was purified from the crystal reported in PBD ID (1ECA) (Steigemann & Weber, 1979); RMSR 1.40 Å, thus obtaining the organometallic complex of Figure 8.

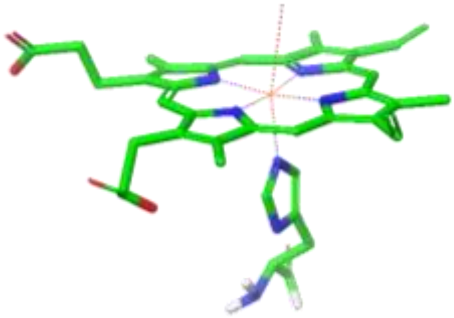


Fig 8. The three-dimensional complex of erythrocruonin.

Emerytrin. This carrier molecule is much more complex than the previous ones. It can be seen in Figure 9 that it can form bridges in bidentate oxygenated molecules, which gives it the necessary three-dimensionality to accommodate molecular oxygen on the outer face, thus being an oxygen carrier with stable characteristics and with the capacity to carry out a gas exchange with ease due to the adequate and external disposition of the links with O_2. One of the possible bonds is saturated with an N_3 to complement the organometallic complex is emphasized. This is demonstrated in the report of the ligand crystallized in the PDB ID (2HMZ) (Holmes & Stenkamp, 1991), RMSD 1.66 Å.

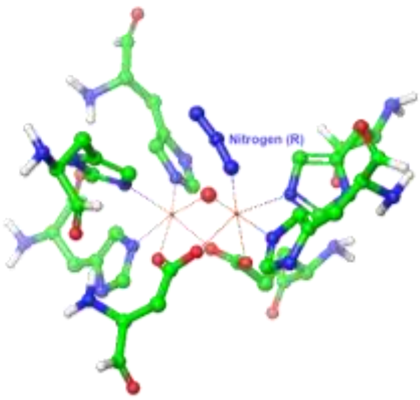


Fig 9. The three-dimensional complex of emerytrin-nitrogen.

Figure 10 shows that the iron atom presents a last coordination bond with a chlorine heteroatom, as it has the protein complex PDB ID (3AGT) (Onoda et al., 2010), 1.40 Å. We can compare this with Figure 9 while for these ligands crystallized on the RCSB PDB platform, a complement ligand was needed; in this way, it was possible to obtain and report the ligands through the X-ray diffraction technique.

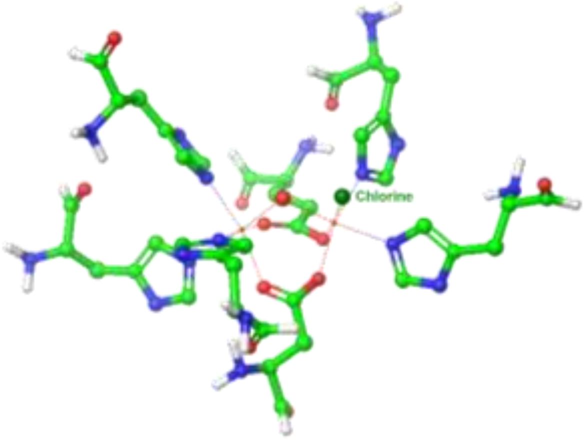


Fig 10. The three-dimensional complex of emerytrin-clorine (Onoda et al., 2010).

CONCLUSION

Using technological tools in computational chemistry helps to understand three-dimensional molecular conformations. The porphyrin fragments derivatives complexes are significantly related to metal atoms and present the most real spatial conformations based on the crystal complex biological origin. The RMSD parameter showing the best quality was 1.40 Å, corresponding to the chlorinated emerytrin and erythrocruonin complexes. Data shows that the organometallic complexes in their oxygenated form, and according to their metal complexation, will provide the color to the blood, which will be visible in the gas exchange in the organism's respiration. A realistic projection of this work is the application of artificial intelligence (AI) for the simulation of new organometallic structures that allow the transport of O_2. This would provide another alternative to biomedical applications such as organ transportation, and *ex vivo* growth of stem cells.

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