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# In-silico design of peptide receptor for carboxyhemoglobin recognition

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ABSTRACT

A series of peptide receptors were designed using an in-silico approach for the recognition of the heme prosthetic group in carboxyhemoglobin (COHb). These peptide chains were designed from the amino acids present in the enzyme Cytochrome b561 (Cyt b561) active site. The evaluation of the interaction energy of the heme prosthetic group against the Cyt b561 active site was found to be −7.1 kcal/mol. However, after evaluating the interaction energies of the heme group against the different peptides representing the enzyme's active site, a higher affinity was discovered with one particular peptide chain (−8.2 kcal/mol). For the design, the peptide was built preserving the distance between the amino acids involved in the catalytic process of Cyt b561, using methylenes as spacers. Additionally, the peptide was polymerized with styrene, resulting in the following peptide chain poly (*styrene*)-A$(CH_2)_5$QW$(CH_2)_5$GF$(CH_2)_5$YW$(CH_2)_6$M$(CH_2)_5$HA$(CH_2)_6$G-(*styrene*)*poly*. This result promotes the rational design of peptide receptors in the detection and quantification of COHb from the recognition of its prosthetic group.

## 1. Introduction

Dyshemoglobinemia are a group of disorders characterized by a functional alteration in the hemoglobin (Hb) molecule, which hinders oxygen transportation. Hemoglobin's ability as an oxygen-carrier molecule depends on a non-protein prosthetic unit called the heme group. Each Hb molecule features four heme groups surrounding a globin group, forming a tetrahedral structure. The heme prosthetic group is composed of a porphyrin ring to which an iron (II) atom is attached (Fe-protoporphyrin IX). Upon binding to oxygen or carbon monoxide, the iron atom changes its oxidation state from iron(II) to iron(III) (Scheme 1). The most relevant dyshemoglobinemia disorder is the COHb formation, as a result of carbon monoxide (CO) poisoning.

These kinds of disorders are common but difficult to diagnose, especially in low and middle income countries. The diagnosis requires a high index of clinical suspicion, the knowledge of a carbon monoxide exposure history, and a blood sample for CO-oximetry [1–3]. However, CO-oximeters are usually inaccessible to less privileged populations due to their cost [4].

CO poisoning could be responsible for up to 40,000 deaths and more than 500,000 illnesses per year worldwide [5]. The disease prevalence and the majority of its cases are registered in low and middle income countries [6], where the most common cause is said to be improperly vented combustion sources.

A significant number of patients who survive CO poisoning suffer from long term neurological afflictions and affective sequelae, along with long-term neurocognitive deficits in up to 15–40% of patients. Approximately one third of patients with middle and high levels of CO poisoning present cardiac dysfunctions, such as arrhythmia, left ventricular systolic dysfunction, and myocardial infarction. Nevertheless, neurological deficits are not necessarily related to CO intoxication, as there are a wide variety of factors which could affect mitochondrial respiration, cellular energy utilization, inflammation, and free radical generation, in both brain and heart [5].

COHb levels in blood are commonly measured using either standard CO-oximetry or hemoximetry [7], although alternate techniques also include gas chromatography [8] and selective oxyhemoglobin (OHb) and methemoglobin (MetHb) reduction of the iron (III) heme group in the presence of COHb with sodium dithionite [9,10]. The latter technique is based on the use of spectrophotometry to quantify the percentage of COHb present in a blood sample, observing a maximum difference in the spectra at 540 nm, while at the isosbestic point (579 nm), the same absorbance is perceived [9].

Despite the fact that the techniques mentioned above quantify the

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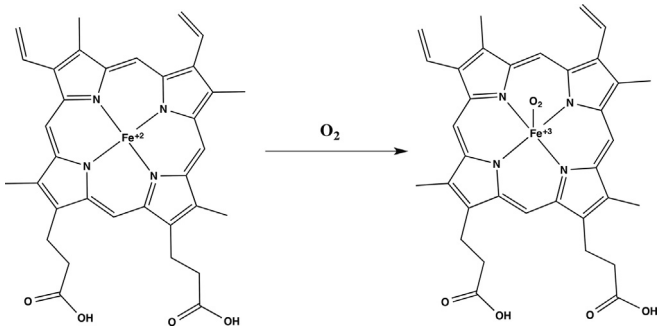


**Scheme 1.** Heme Prosthetic Group, Fe-protoporphyrin IX.

concentration of COHb in a given sample, the development of biosensors during the last decade has brought great advantages into quantitative analysis techniques. Biosensors are highly specific devices that provide real-time measurements and allow the application of additional in-situ methods and analysis [11].

Biosensors are analytical devices primarily comprised of a bioreceptor and a transducer. A bioreceptor can be defined as an immobilized recognition element or bio component, such as antibodies, nucleic acids, enzymes, peptides, etc., able to recognize a specific target analyte [12]. These biocomponents are integrated with a transducer in order to convert the recognition event into an electrical or measurable signal [13]. The purpose of a biosensor is to provide real-time, accurate and reliable information about the analyte. Ideally, this device is capable of responding continuously and reversibly without disturbing the study sample. Therefore, biosensors have been designed to perform analytically in areas such as medicine, agriculture, food safety, bioprocessing and in environmental and industrial monitoring [14].

The most common bioreceptors used are enzymes, although it has been recently described that their stability is affected by changes in temperature and pH conditions, which limits their use in real samples [15]. As a result, many non-enzymatic receptors have emerged which are able to maintain substrate selectivity in complex biological matrices versus interfering with similar reduction equivalences, and offer a much better stability than their enzymatic counterparts.

Some of these non-enzymatic bioreceptors are described in Ref. [16]. Thus, the design of new bioreceptors is an important step in the development of new diagnostic tools. Peptides, for example, have emerged as alternatives for molecular recognition [16]. These compounds can be synthesized offering a wide variety of structural modifications, making them very versatile [17]. In addition, a particular sequence of amino acids can be constructed in accordance with the active site of a particular enzyme using a variety of coupling algorithms, which provide a complex model that quantifies, theoretically, the best suited bioreceptor-analyte interaction. The software AutoDock Vina is a useful example of a particular coupling algorithm [18].

Cyt b561 was taken as reference for this project. This enzyme features two cavities present on each side of the membrane. As described by Ref. [19], each cavity is surrounded by positive charges, with a heme group located underneath. This study strongly suggest that these positively charged cavities may be the substrate-binding sites, which in turn seems to allude that Cyt b561 family members may have electron-donating ferric-chelates [19].

### 1.1. Procedure

Each peptide structure was first built using the Avogadro Software [20]. Next, the self-optimization was performed employing the UFF (Universal Forcefield) with four update steps. Then, a gradient descent algorithm was used to optimize the molecules. Molecular modeling was later carried out with the AutoDock Vina [18] and the enzyme was optimized according to what is described in literature. The dimension of the GRID chosen for Cyt b561 is 0.375 Å. The dimensions X, Y and Z had 22, 24 and 28 number of points respectively, while the Center GRID BOX for these dimensions was 11, 90.5 and 57.5 respectively. Finally, the GRID chosen in the modeled bioreceptors included the amino acid sequence, exclusively. All calculations were made in triplicate to obtain statistically reliable results.

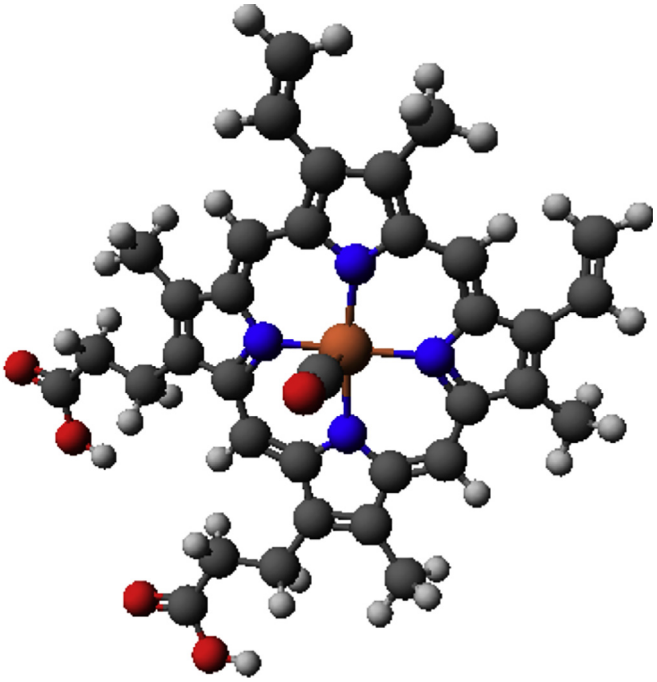

**Fig. 1.** CO binding to iron (III) of heme prosthetic group.

## 2. Results and discussion

Initially, the evaluation of the interaction energy between the active site of Cyt b561 and the heme prosthetic group in COHb (Fig. 1) by means of AutoDock Vina, was determined as an initial step [18]. This calculation showed that the interaction energy is −7.1 kcal/mol (Table 1).

Based on the interaction energy of the iron (III) present in the heme group in COHb against Cyt b561 active site, it was considered as a working hypothesis that building a peptide made out of the same sequence of amino acids directly involved in both, the substrate recognition process and the catalysis of Cyt b561, would yield interaction values similar to those found in the molecular modeling using the whole Cyt b561 structure (Table 1). Based on the working hypothesis, a peptide was built in Avogadro [20] using the amino acid sequence *AQWGFYWMHAG,* reported by Ref. [19] (Fig. 2) to begin to replicate the structure of the enzyme's active site.

First, the interaction energies of the heme prosthetic group in COHb against the amino acid sequence *AQWGFYWMHAG* were evaluated and are shown in Table 2. In comparison with the energies derived against Cyt b561 (Table 1), it can be observed that the COHb-undecapeptide interaction releases less energy. This phenomenon can be explained by

**Table 1**
Interaction energies of COHb against Cyt b561.

| Mode | Affinity (Kcal/mol) | dist from best mode | |
|---|---|---|---|
| | | rmsd l.b. | rmsd u.b |
| 1 | −7.1 | 0.000 | 0.000 |
| 2 | −7.1 | 1.959 | 5.996 |
| 3 | −7.0 | 2.499 | 8.084 |

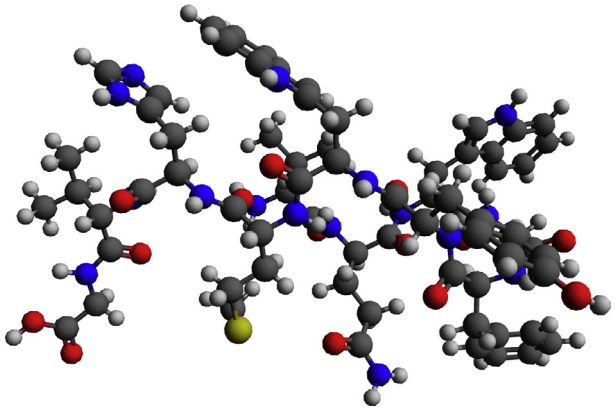

**Fig. 2.** Amino acid sequence AQWGFYWMHAG [19].

**Table 2**
Interaction energies of heme group against AQWGFYWMHAG.

| Mode | Affinity (Kcal/mol) | dist from best mode | |
|---|---|---|---|
| | | rmsd l.b. | rmsd u.b |
| 1 | −5.4 | 0.000 | 0.000 |
| 2 | −5.3 | 3.103 | 7.893 |
| 3 | −5.2 | 3.644 | 6.580 |

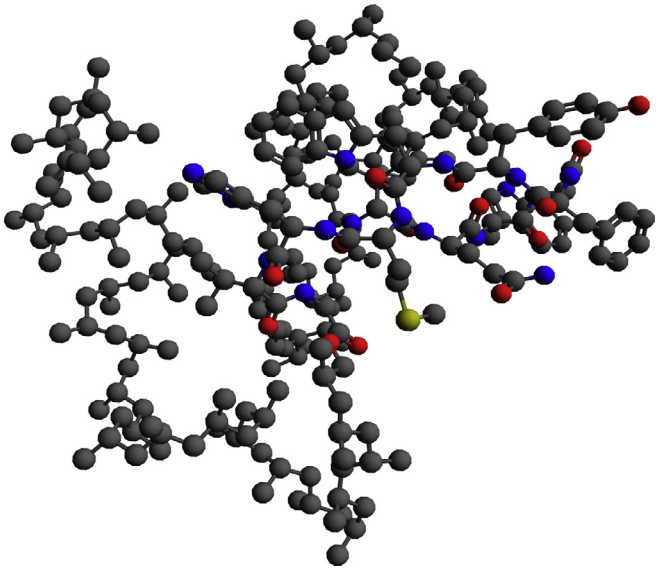

**Fig. 3.** Peptide polymerized with ethylene poly(ethylene)-AQWGFYWMHAG-(ethylene)poly.

the low steric hindrance that results from a peptide sequence of that size. Based on the data obtained, several possibilities can be analyzed. One possibility is to polymerize [21] the amino acid sequence in both the N and C terminals in order to achieve a larger steric volume (Fig. 3).

By increasing the steric volume of the undecapeptide and exposing the amino acids of the peptide chain, an increase in the interaction energies (Table 3) was obtained. However, these energies are still lower than those derived from the interaction between Cyt b561 and the heme prosthetic group in COHb. Hence, the compound used for the peptide polymerization was changed to one with a higher volume, such as styrene (Fig. 4), and its interaction energy was again calculated (Table 4).

The interaction energies calculated were the same as with the ethylene polymer. According to these results, the possibility of preserving the distances between amino acids of the active site was

**Table 3**
Interaction energies of heme group against polyethylene-peptide chain.

| Mode | Affinity (Kcal/mol) | dist from best mode | |
|---|---|---|---|
| | | rmsd l.b. | rmsd u.b |
| 1 | −6.9 | 0.000 | 0.000 |
| 2 | −6.9 | 3.930 | 8.070 |
| 3 | −6.8 | 3.046 | 7.981 |

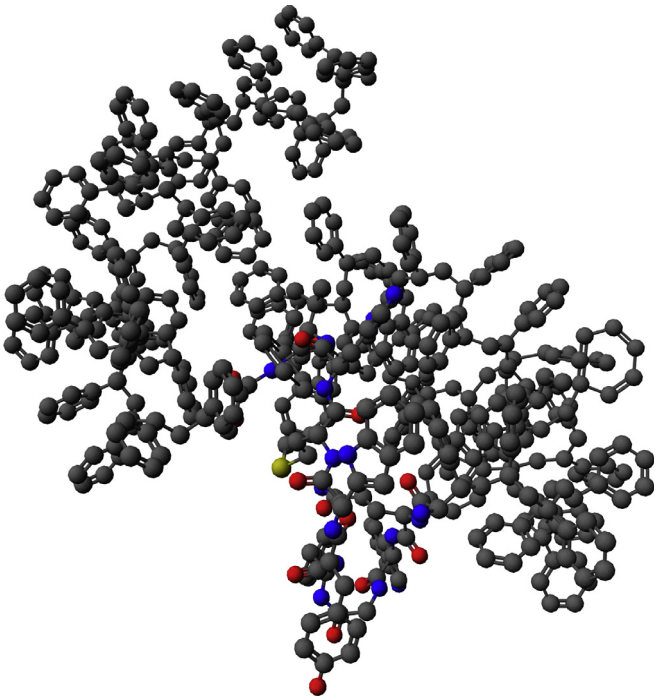

**Fig. 4.** Peptide polymerized with styrene poly(styrene)-AQWGFYWMHAG-(styrene)poly.

**Table 4**
Interaction energies of heme group against polystyrene-peptide chain.

| Mode | Affinity (Kcal/mol) | dist from best mode | |
|---|---|---|---|
| | | rmsd l.b. | rmsd u.b |
| 1 | −6.9 | 0.000 | 0.000 |
| 2 | −6.8 | 3.865 | 7.841 |
| 3 | −6.8 | 3.015 | 8.053 |

considered [19], taking into account that these amino acids are not arranged in a linear sequence but are instead interleaved by other amino acids that do not interact with the heme prosthetic group. Therefore, the sequence of amino acids (*AQWGFYWMHAG)* involved in the catalytic process will be preserved, but those other amino acids not directly involved in substrate recognition will be replaced with methylene chains with the same number of carbons as the intermediate peptide bond found in Cyt b561. Thus, a new peptide chain was built in Avogadro [20] to include the methylene bridges, which resulted in the following sequence $A(CH_2)_5QW(CH_2)_5GF(CH_2)_5YW(CH_2)_6M(CH_2)_5HA(CH_2)_6G$, in order to evaluate the affinity of this peptide against the prosthetic group. Subsequently, the peptide was polymerized with ethylene in both the N and C terminals (Fig. 5).

The following theoretical calculations (Table 5) show an interaction energy equal to the one obtained against Cyt b561 (see Table 1 line 1 vs Table 5 line 1), which highlights the fact that a peptide chain made up of the amino acids present in the active site of the enzyme could be a

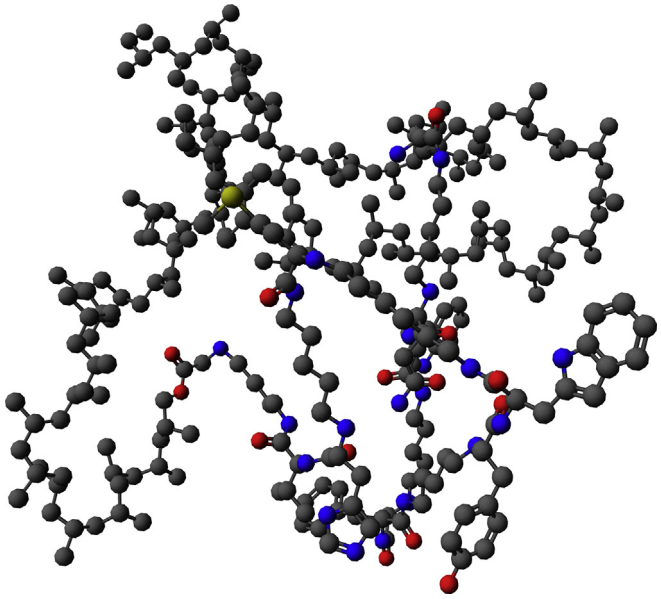

**Fig. 5.** Peptide featuring methylene bridges and ethylene polymerization poly (ethylene)-A$(CH_2)_5$QW$(CH_2)_5$GF$(CH_2)_5$YW$(CH_2)_6$M$(CH_2)_5$HA$(CH_2)_6$G-(ethylene)poly.

**Table 5**
Interaction energies of heme group against polyethylene-peptide chain, featuring methylene bridges.

| Mode | Affinity (Kcal/mol) | dist from best mode | |
|---|---|---|---|
| | | rmsd l.b. | rmsd u.b |
| 1 | −7.1 | 0.000 | 0.000 |
| 2 | −6.8 | 10.942 | 14.402 |
| 3 | −6.8 | 2.527 | 7.629 |

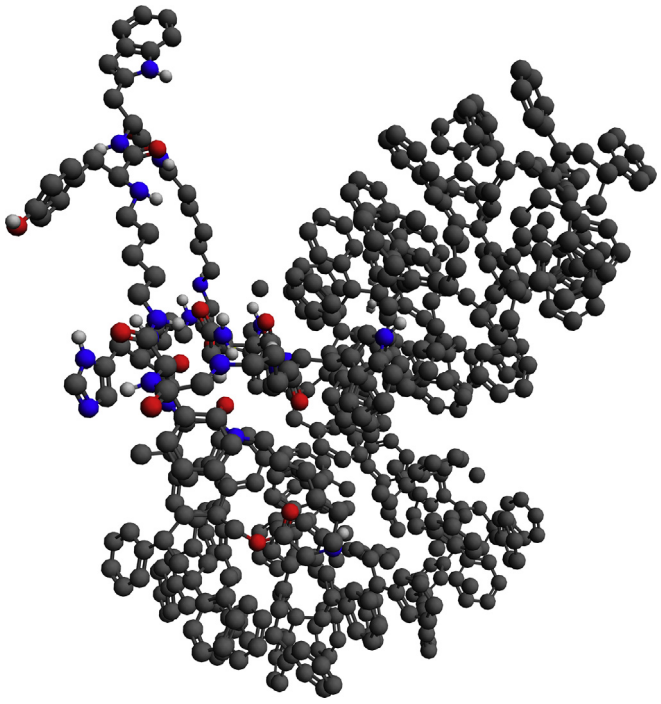

**Fig. 6.** Peptide featuring methylene bridges and styrene polymerization poly (styrene)-A$(CH_2)_5$QW$(CH_2)_5$GF$(CH_2)_5$YW$(CH_2)_6$M$(CH_2)_5$HA$(CH_2)_6$G-(styrene) poly.

**Table 6**
Interaction energies of heme group against polystyrene-peptide chain, featuring methylene bridges.

| Mode | Affinity (Kcal/mol) | dist from best mode | |
|---|---|---|---|
| | | rmsd l.b. | rmsd u.b |
| 1 | −8.2 | 0.000 | 0.000 |
| 2 | −8.0 | 3.300 | 8.416 |
| 3 | −7.9 | 3.399 | 8.269 |

useful bioreceptor for the detection of COHb from the recognition of its heme prosthetic group. The main advantage of using a peptide as a COHb receptor is to avoid the arduous process of enzymatic purification [22]. Moreover, it might be considered that this type of bioreceptor will be able to resist changes in both temperature and pH conditions, as it does not have the extensive amino acid sequence Cyt b561 (251 amino acids) [19]. Therefore, to improve the affinity of the peptide against the heme group, the peptide was polymerized with styrene instead (Fig. 6). The interaction energies were then calculated (Table 6).

Based on the data described in Table 6, it is important to notice that a significant increase in the interaction energy was observed between the heme prosthetic group in COHb with the peptide chain (Fig. 6). This in-silico approach could be the starting point for the rational design of non-enzymatic receptors to be used in the real-time detection of biomolecules of physiological interest.

Considering that the heme group can be found presenting several structural differences at the physiological level, compounds such as Hb, MetHb and OxHb were built in Avogadro. The interaction energies of each of their prosthetic groups, Hb featuring iron (II) and MetHb and OxHb featuring iron (III), against the proposed peptide receptor (Fig. 6), were calculated. As expected, a similar energy was obtained for all three substrates (Table 7) when compared with COHb (Table 6 line 1), due to their structural equivalence.

Theoretically, any molecule with the ability to selectively recognize other molecules can be named a bioreceptor [23]. Therefore, in past years, the literature has reported that the use of peptides as bioreceptors offer a variety of appealing advantages compared to those of other commonly used bioreceptors (i.e., enzymes) [16]. Peptides are the simplest of biological elements used for the recognition of small molecules [24,25], thus being easier to synthesize in a profitable manner.

Yang et al. [25] identified, using a phage display technique, that a heptapeptide sequence (CKSLENSYC) presents a specific affinity for a small molecule, bisphenol A (BPA). Then, using the previously mentioned sequence as the bioreceptor, along with a gold electrode as a transducer, an electrochemical biosensor was developed.

However, as described before, simple peptide sequences lacking N and C terminal polymerization, such as *AQWGFYWMHAG,* exhibit less interaction energy that those sequences exhibiting terminal polymerization (Table 2 vs. Tables 3–6).

On the other hand, several reports found in the literature have shown that biopolymers like chitosan aid peptide chains in the recognition of mycotoxins [26], yielding excellent recognition results, and facilitating peptide chain management.

**Table 7**
Interaction energies of interference against polystyrene-peptide chain, featuring methylene bridges.

| Sustrate | Affinity (Kcal/mol) | dist from best mode | |
|---|---|---|---|
| | | rmsd l.b. | rmsd u.b |
| Hb | −8.3 | 0.000 | 0.000 |
| MetHb | −8.3 | 0.000 | 0.000 |
| OxHb | −8.3 | 0.000 | 0.000 |

In the present article, it was confirmed in-silico that increasing steric volume at both end terminals of the peptide sequences increases the bioreceptor-COHb interaction, compared to the one obtained with unpolymerized peptide chains.

Considering the similar energies obtained between the proposed peptide receptor (Fig. 6) and the three most common forms of hemoglobin, OxHb, Hb and MetHb, it is imperative to consider an additional differentiation step in order to differentiate OxHb and MetHb from COHb. Hence, to avoid the misquantification of COHb in a given sample, sodium dithionite can be used to selectively reduce OxHb and MetHb in the presence of COHb [9,10]. The indirect quantification of COHb via selective reduction of the other two species is currently being evaluated in our lab. as is the synthesis of the proposed peptide receptor (Fig. 6), in order to evaluate its functionality in-vitro.

## 3. Conclusion

The interaction energy (Kcal/mol) of the heme prosthetic group in carboxyhemoglobin (COHb) against Cyt b561 was first evaluated using AutoDock Vina, and later compared against five newly-proposed peptides, whose sequence was based on the amino acids present at the active site of the enzyme. It was discovered that the spaces between said amino acids must be preserved and that the N and C terminals of the peptide must be polymerized to increase the selectivity of recognition of COHb. As a result, the peptide chain *poly(styrene)-A$(CH_2)_5$QW$(CH_2)_5$GF$(CH_2)_5$YW$(CH_2)_6$M$(CH_2)_5$HA$(CH_2)_6$G-(styrene)poly* showed the highest affinity against the heme group in COHb.

## Conflicts of interest

None declared.

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