In Silico Design of a Peptide Receptor for Dopamine Recognition

CURRENT STATUS: POSTED

Luna Rodriguez-Salazar
Universidad El Bosque

James Guevara Pulido
Universidad El Bosque

joguevara@unbosque.edu.co

Corresponding Author
ORCiD: https://orcid.org/0000-0001-9134-3719

DOI: 10.21203/rs.2.18104/v1

SUBJECT AREAS
General Cell Biology & Physiology Molecular Biology

KEYWORDS
dopamine, molecular docking, bioreceptor, in silico
Abstract

Background: Dopamine (DA) is an important neurotransmitter with a fundamental role in regulatory functions related to the central, peripheral, renal, and hormonal nervous systems. Dopaminergic neurotransmission dysfunctions are commonly associated with several diseases; thus, in situ quantification of DA is a major challenge. To achieve this goal, enzyme-based biosensors have been employed for substrate recognition in the past. However, owing to their sensitivity to changes in temperature and pH levels.

Results: New peptide bioreceptors have been developed. Based on this, 45 in silico bioreceptors were designed to exhibit a higher affinity than that of the DA transporters (DATs). The design was based on the active sites of crystallized enzyme structures that are physiologically related to DA. These affinities were calculated using AutoDock Vina and by assessing the interaction energy between DA and the active DAT site. The controlled variables in the design were amino acids, bond type, steric volume, stereochemistry, affinity, and interaction distances.

Conclusions: Three bioreceptor candidates presenting promising values in terms of DA affinity and distance were obtained.

1. Background

Dopamine (DA) is a catecholamine that acts as a hormone or a neurotransmitter [1] owing to its functions in the central nervous system and peripheral system as well as in other tissues, such as the gastrointestinal and renal tissues [2]; it also has functions of sodium regulation [3]. Further, its importance in motor control, learning, motivation, memory, and the reward system has also been described [4]. Because DA plays a critical role in the regulation of vital functions, dopaminergic neurotransmission dysfunctions are commonly associated with multiple disorders [5], such as schizophrenia, Parkinson's disease, [6] and Alzheimer's disease [7].

The importance of DA as a neurotransmitter extends beyond its relationship with diseases because it is also a diagnostic tool [8]; for example, catecholamines, detected in urine samples, are tumor markers for neuroblastomas and pheochromocytomas.

Hence, this evidences the need for reliable bioanalytical methods for the extraction, separation, and
quantification of these diagnostic markers [1]. Nevertheless, it must also be considered that catecholamines are unstable, are prone to spontaneous oxidation, easily decompose at high pH values [9], and are also found at low concentrations in biological samples needing rigorous pretreatment for quantification without interference [3]. Therefore, DA quantification methods are being researched, and their mechanisms of action and ligands of interaction with nervous system receptors are also being assessed using in silico models [10]. This study is conducted because DA is considered as one of the most important neurotransmitters in mammals [8]. There are currently new in vitro techniques for the quantification of DA; however, the standard method used is high-performance liquid chromatography with electrochemical detection (ECD) [11]. Considering the above-mentioned difficulties, these techniques do not yield reproducible results in real time or in situ analysis. Therefore, biosensors have emerged as a tool capable of potentially offering real-time detection with high selectivity and sensitivity to the substrate [12].

The DA biosensors proposed to date use the tyrosinase enzyme as a bioreceptor (EC 1.14.18.1) [13]. However, enzymatic bioreceptor biomolecules exhibit their own disadvantages, such as low stability against pH and temperature variations and critical operating conditions, which significantly hinder their usage in real-time analysis [14]. Consequently, new unconventional bioreceptors have been designed for the recognition of substrates, representing a fundamental step in the development of new diagnostic tools [15]. One of these bioreceptors are peptides and are emerging as an alternative for molecular recognition, in addition to being considered versatile molecules because they can be synthesized with a wide variety of structural modifications [16,17]. In silico models of peptide bioreceptors [18] and biosensors using peptide bioreceptors have already been designed for substrate recognition [19].

Therefore, as part of our study of our bioreceptor design group for the recognition of a particular substrate [18], the in silico design of possible bioreceptors that recognize in silico DA is being completed to subsequently perform its synthesis. This way, the in vitro design and testing process can be more efficient and faster owing to the implementation of bioinformatics tools, such as molecular modeling, to assess the bioreceptor-ligand interaction and improve it against the protein-
ligand complex interaction [20], which is usually formed with the DA transporter (DAT) in the biological process of dopaminergic neurotransmission [21].

2. Results And Discussions
The results of this study are presented below in three sections. The first section presents the enzymes chosen owing to their association with DA and the interaction energy resulting from the enzyme-DA complex. The second section presents the variables that were controlled during the designing of the bioreceptor and the affinity results for DA after each structural modification. Finally, the third section includes the description of the parameters that allowed three bioreceptor candidates to be selected for future synthesis as well as the molecular interaction distances between DA and the bioreceptor.

2.1. Selection and analysis of enzymes with DA interaction

2.1.1. Enzyme Selection
According to the methodology proposed and followed, enzymes belonging to the enzyme class 1.14.18 were retrieved from the Protein Data Bank (PDB). However, the results only yielded the following three crystallized enzymes: tyrosinase (1.14.18.1) as 5M8L, methane monooxygenase (particulate) (1.14.18.9) as 1YEW, and methylsterol monooxygenase (1.14.18.9) as 4IIT. Because only three crystallized enzymes were found out of the nine enzymes proposed, the authors decided to seek other enzymes that interact with DA or its analogs in the physiological processes as well as those crystallized using DA as the ligand. Accordingly, the following 15 enzymes were found (Table 1)

Table 1. Enzymes related to dopamine that have been crystallized and registered in the PDB.
| Common name                                                                 | PDB Nomenclature |
|-----------------------------------------------------------------------------|------------------|
| Crystal structure of human tyrosinase related protein 1                     | 5M8L             |
| The Phenylacetyl-CoA monooxygenase PaaABC subcomplex with phenylacetyl-CoA | 4IIT             |
| Structure of Drosophila dopamine transporter bound to neurotransmitter dopamine | 4XP1             |
| Structure of AED7-norepinephrine complex                                     | 3DYE             |
| ABC-transporter choline binding protein in complex with acetylcholine       | 2RIN             |
| Structure of biogenic amine binding protein from Rhodnius prolixus          | 4GET             |
| Structure of human sulfotransferase SULT1A3 in complex with dopamine and 3-phosphoadenosine 5-phosphate | 2A3R             |
| Quinone reductase 2 in complex with dopamine                                | 2QMZ             |
| Structure of the human dopamine D3 receptor in complex with eticlopride    | 3PBL             |
| Structure of the human D4 dopamine receptor in complex with Nemonapride    | 5WIU             |
| Crystal structure of the N-terminal domain of DrrA/SidM from Legionella pneumophila | 3NKU             |
| Structure of human I113T SOD1 mutant complexed with dopamine in the p21 space group | 4A7V             |
| Structure of Norcoclaurine synthase from Thalictrum flavum in complex with dopamine and hydroxybenzaldehyde | 2VQ5             |
| Structure of Drosophila melanogaster E47D dopamine N-acetyltransferase in ternary complex with CoA and Acetyl-dopamine | 5GIG             |
| Structure of human D-amino acid oxidase complexed with imino-DOPA           | 2E82             |

2.1.2. Interaction analysis of the enzyme-DA complex.

Table 2 presents the results obtained for each enzyme, calculated as the average of the three tests performed. Based on these results, the three enzymes selected with the highest affinity for DA were 5M8L, 2A3R, and 4IIT, which are in bold text the Table 2. These enzymes will be used as a starting point for bioreceptor design.

Table 2. Intercation energy (average) result of each enzyme for the calculation of affinity for dopamine.
| Enzyme | Interaction energy (average) (kcal/mol) | Standard deviation |
|--------|----------------------------------------|--------------------|
| 5M8L   | -6.2                                   | 0.1                |
| 4IIT   | -6.1                                   | 0.1                |
| 4XP1   | -5.2                                   | 0.0                |
| 3DYE   | -5.6                                   | 0.1                |
| 2RIN   | -5.0                                   | 0.0                |
| 4GET   | -5.4                                   | 0.0                |
| 2A3R   | -6.3                                   | 0.0                |
| 2QMZ   | -5.6                                   | 0.2                |
| 3PBL   | -5.6                                   | 0.1                |
| 5WIU   | -4.5                                   | 0.1                |
| 3NKU   | -5.5                                   | 0.0                |
| 4A7V   | -5.2                                   | 0.1                |
| 2VQ5   | -5.0                                   | 0.1                |
| 5GIG   | -4.9                                   | 0.1                |
| 2E82   | -5.9                                   | 0.1                |

2.2. Design and analysis of bioreceptors that interact with DA

The bioreceptors were designed to perform a miniaturization allowing competition in affinity and molecular distances with DAT, a protein capable of modulating the DA availability in the synaptic space, because it recaptures extracellular DA and enters the presynaptic neurons [22]. Considering that this protein is activated or deactivated according to the both short- and long-term physiological demands of the neurons [22], it must be mimicked to recognize the DA not captured by postsynaptic neurons within the system when it is coupled to a biosensor system.

It is important to mention that miniaturization is a concept that has been defined as “the process of doing something on a very small scale using modern technology” [23] or as “a version of something on a small scale or small size” [24]. In addition, this miniaturization trend was first applied to electronic devices in the 1960’s and later evolved to their replacement by biological molecules [25] and to drug releasing processes [26].

It is important to keep in mind that human DAT (hDAT) has not been crystallized yet; therefore, there is no report on its crystalline structure determined through experimental methods. Nevertheless, the structure of DATs of other species, such as Drosophila melanogaster, has already been determined [27] (the PDB code for this protein is 4XP1). Therefore, this registered 4XP1 protein was deemed as analogous to hDAT. However, there are computational models that have simulated the structure and interactions of hDAT [28], and this information will also be considered in the following design of
Further, the initial bioreceptors were designed from the amino acids of the active site of the 5M8L, 4IIT, and 2A3R enzymes to which structural changes were sequentially made as per the provisions included in the methodology.

The first group of bioreceptors designed comprised peptides constructed from the amino acids of the 5M8L, 4IIT, and 2A3R enzymes. Table 3 denotes their identifying code, their composing amino acid sequence, the affinity result calculated as the average of the triplicate calculation results along with their standard deviation. In addition, Figure 1 displays the images related to the design of these three bioreceptors.

Table 3. Results of bioreceptors designed exclusively based on the previously selected amino acids.

| Bioreceptor code | Amino acid sequence | Average affinity (kcal/mol) | Standard deviation (kcal/mol) |
|------------------|---------------------|-----------------------------|------------------------------|
| 1.0               | NWR                 | −2.6                        | 0.0                          |
| 2.0               | RQKYSSMMGSPNKNFI    | −3.6                        | 0.0                          |
| 3.0               | FPFDKHEAH           | −3.5                        | 0.1                          |

As expected, the results indicated the affinity of the three bioreceptors’ affinity DA was low in terms of the interaction energy of the DAT, which is −5.2 kcal/mol. These results were expected because the steric hindrance exhibited by a peptide within this design length range is not comparable with that of the enzyme [18]. As previously reported [18], it is important to identify amino acids capable of generating interaction with the substrate and crucial to mimic the steric volume of the target protein with which competition is desired in terms of interaction energy for the substrate of interest because this is a criterion that directly influences affinity.

Thus, two options were considered. The first was to consider the number of residues between the selected amino acids and the second was to increase the steric volume of the bioreceptors to increase their affinity [18].

Accordingly, Table 4 presents the code for each bioreceptor and its sequence when adding methylene bridges among the amino acids to preserve the distances established in the protein sequence. Finally, this Table also presents the affinity results, which are calculated as the average of the triplicate calculation along with their standard deviation. In addition, Figure 2 presents the images
corresponding to each of the bioreceptors designed for this structural modification.

Table 4. Results of the bioreceptors designed maintaining the distances between the selected amino acids.

| Bioreceptor code | Amino acid sequence with methylene bridges | Average affinity (kcal/mol) | Standard deviation |
|------------------|-------------------------------------------|----------------------------|--------------------|
| 1.1              | N(CH₂)₄W(CH₂)₂R                           | −3.0                       | 0.0                |
| 2.1              | R(CH₂)Q(CH₂)₇KYSS(CH₂)₁₀MM(CH₂)GP(CH₂)SPN(CH₂)K(CH₂)N(CH₂)₅F(CH₂)₃I | −3.1                       | 0.1                |
| 3.1              | F(CH₂)P(CH₂)₂F(CH₂)₅K(CH₂)H(CH₂)₂EAH      | −3.6                       | 0.1                |

In this case, if the interaction energies of the bioreceptors composed solely of peptides are compared against the interaction energies from the bioreceptors to which the methylene bridges were added, no conclusive pattern may be observed. In some cases, the energy increased (bioreceptors 1.1 and 3.1, Table 3, second and fourth row), whereas in others, it decreased (bioreceptor 2.1). However, it was considered that because DA is a small molecule and does not have many atoms that may allow it to interact with other molecules, the amino acids are distant. Therefore, for the bioreceptor 2.1, the possible interactions that existed before adding the methylene bridges are lost. This result contradicts that in a previous report [18], but if it is considered that the substrate size in the previous study was much larger in terms of DA, it could be construed that the molecule can only generate a small number of interactions.

Because the previous results did not reflect a specific pattern, wherein one group of bioreceptors denotes better results than the other, it was decided that the following structural modifications were required in all previous designs to determine which bioreceptor and under which variables better interaction with DA occurs.

Therefore, as mentioned previously, the steric volume of the six bioreceptors already designed had to be increased to boost their affinity for DA (Tables 3 and 4). Therefore, each bioreceptor designed so far was polymerized by C and N terminal. The general structure of this process is shown in Figure 3, and the images of the bioreceptors designed are presented in Figures 5 and 6, both for the bioreceptors comprising only the peptide chain as for those with methylene bridges among the amino acids.

The following two different polymers were used for this purpose: polyethylene and polystyrene. Once
designed, the interaction energy for DA was calculated for each one. The results of these dockings with polyethylene and polystyrene are presented in Tables 5 and 6 respectively, with their corresponding code and sequence. Addition of the third level 1 corresponds to the polymerization with polyethylene and of the level 2 corresponds to the polymerization with polystyrene.

Table 5. Results of designed receptors maintaining the distances between the selected amino acids.

| Bioreceptor code | Amino acid sequence                                                                 | Average affinity (kcal/mol) | Standard deviation (kcal/mol) |
|------------------|--------------------------------------------------------------------------------------|----------------------------|-----------------------------|
| 1.0.1            | poly (ethylene)-NWR-poly (ethylene)                                                | -4.0                       | 0.0                         |
| 2.0.1            | poly (ethylene)-RQKYSSMMGPNKNFI-poly (ethylene)                                   | -3.7                       | 0.0                         |
| 3.0.1            | poly (ethylene)-FPFDKHEAH-poly (ethylene)                                         | -3.9                       | 0.0                         |
| 1.1.1            | poly (ethylene)-N(CH\textsubscript{2})\textsubscript{4}W(CH\textsubscript{2})\textsubscript{2}R-poly (ethylene) | -4.0                       | 0.0                         |
| 2.1.1            | poly (etileno)-R(CH\textsubscript{2})Q(CH\textsubscript{2})\textsubscript{2}KYSS(CH\textsubscript{2})\textsubscript{10}MM(CH\textsubscript{2})GP(CH2)SPN(CH\textsubscript{2})K(CH\textsubscript{2})N(CH\textsubscript{2})\textsubscript{5}F(CH\textsubscript{2})\textsubscript{9}-poly (ethylene) | -4.1                       | 0.1                         |
| 3.1.1            | poly (ethylene)-F(CH\textsubscript{2})P(CH\textsubscript{2})\textsubscript{2}F(CH\textsubscript{2})D(CH\textsubscript{2})\textsubscript{3}K(CH\textsubscript{2})H(CH\textsubscript{2})\textsubscript{2}EAH-poly (ethylene) | -4.4                       | 0.0                         |

Table 6. Results of the designed receptors maintaining the distances between the chosen amino acids.

| Bioreceptor code | Amino acid sequence                                                                 | Average affinity (kcal/mol) | Standard deviation (kcal/mol) |
|------------------|--------------------------------------------------------------------------------------|----------------------------|-----------------------------|
| 1.0.2            | poly (styrene)-NWR-poly (styrene)                                                  | -5.1                       | 0.1                         |
| 2.0.2            | poly (styrene)-RQKYSSMMGPNKNFI-poly (styrene)                                     | -4.8                       | 0.0                         |
| 3.0.2            | poly (styrene)-FPFDKHEAH-poly (styrene)                                           | -4.8                       | 0.1                         |
| 1.1.2            | poly (styrene)-N(CH\textsubscript{2})\textsubscript{4}W(CH\textsubscript{2})\textsubscript{2}R-poly (styrene) | -4.4                       | 0.1                         |
| 2.1.2            | poly (styrene)-R(CH\textsubscript{2})Q(CH\textsubscript{2})\textsubscript{2}KYSS(CH\textsubscript{2})\textsubscript{10}MM(CH\textsubscript{2})GP(CH2)SPN(CH\textsubscript{2})K(CH\textsubscript{2})N(CH\textsubscript{2})\textsubscript{5}F(CH\textsubscript{2})\textsubscript{9}-poly (styrene) | -5.2                       | 0.0                         |
| 3.1.2            | poly (styrene)-F(CH\textsubscript{2})P(CH\textsubscript{2})\textsubscript{2}F(CH\textsubscript{2})D(CH\textsubscript{2})\textsubscript{3}K(CH\textsubscript{2})H(CH\textsubscript{2})\textsubscript{2}EAH-poly (styrene) | -5.1                       | 0.1                         |

According to the results presented in Tables 5 and 6 in comparison with those in Tables 3 and 4, it is evident that affinity increases in all cases when the steric volume increases by means of polymerization. For this case particularly, there is a recognizable pattern. In addition, for polymerization with polystyrene, affinity for DA increases with respect to the results of all bioreceptors when polymerization is performed with polyethylene. On the other hand, these results follow the pattern set out previously [18], which it has been stated that recognition interaction improves when polymerization is done with polystyrene.
Considering that the affinity results are not equal or better than those of the DAT up to this point, variables other than the amino acids identified from the PDB enzymes were evaluated while considering that the bioreceptor design is targeted toward a miniaturized DAT. Therefore, variables other than the amino acids identified from the PDB enzymes were evaluated to lower the number of amino acids that make up the bioreceptor even further.

Based on the above, we decided to study which amino acid variables could influence affinity for DA. Thus, the first criterion was stereochemistry. With the exception of glycine, amino acids have a chiral carbon, which exhibits four bonds with different functional groups [29], thus generating an enantiomer pair of spatial isomers defined as non-superimposable mirror images [30]. It is important to keep in mind that its natural stereochemistry in the human body is the L configuration and not the D configuration [29]. However, this section uses the R and S nomenclature, which applies to natural compounds and determines the stereochemistry based on the importance defined by the atomic number of the chiral carbon substituent. Using this information, amino acid stereochemistry was selected as the first variable to be analyzed for identifying its influence on the interaction with bioreceptors.

The next parameter considered was not directly focused on the nature or structure of the amino acids but on the ability of DA to form bonds or to interact with other molecules and how many of these could be formed. For this section, the authors used information taken from publications, such as the computational modeling of hDAT [28] and the models of DA receptor interaction dynamics [10]. Therefore, the second variable analyzed was the relationship between the interaction energy between bioreceptors and DA according to the amount of amino acids in the bioreceptor.

The third criterion was the nature of the amino acids, considering that they are divided into four groups. The first group includes non-polar amino acids, the second includes polar amino acids, the third includes acidic amino acids, and the fourth includes basic amino acids [31]. Thus, the next variable studied was the chemical characteristics of amino acids.

To address the first variation, the NWR bioreceptor polymerized with polystyrene (code: 1.0.2) was selected because this was the polymerization that provided the best results only with these three
amino acids. Furthermore, the interaction could be only with a portion of the bioreceptors with more amino acids, which cannot be identified. This is also supported by the interactions identified between the computational model of hDAT and DA, wherein it is generally determined whether they occur with groups of two to four amino acids [28].

Based on this, all possible combinations of variations between the R and S stereochemistry were performed for the NWR tripeptide by polymerizing with polystyrene. Figure 6 presents the flat structure of this bioreceptor, wherein each amino acid whose stereochemistry will be modified is identified with a different color.

Table 7 denotes the codes for these bioreceptors, the stereochemistry of each amino acid in the respective order, and the average triplicate calculation of the interaction energy in kcal/mol; Figure 7 presents the images related to each one.

| Bioreceptor code | Stereochemistry | Average affinity (kcal/mol) | Standard deviation (kcal/mol) |
|------------------|-----------------|-----------------------------|-----------------------------|
| 1.0.2            | SSS             | −5.1                        | 0.1                         |
| 1.0.2.2          | RRS             | −4.5                        | 0.0                         |
| 1.0.2.3          | RSR             | −4.9                        | 0.0                         |
| 1.0.2.4          | RSS             | −5.0                        | 0.1                         |
| 1.0.2.5          | SRS             | −4.8                        | 0.0                         |
| 1.0.2.6          | SSR             | −4.9                        | 0.1                         |
| 1.0.2.7          | RRR             | −5.0                        | 0.0                         |

As seen in Table 7, there are variations in the affinity results for each bioreceptor according to variations in the stereochemistry of the amino acids that compose them. Hence, it was determined that the bioreceptor that best interacts with DA is the one with SSS stereochemistry, which corresponds to code 1.0.2, is in bold text in Table 7. Although the other results were not considerably distant, this result was obtained because this is the natural stereochemistry of amino acids, and therefore, the other results exhibited decreased affinity. It is worth mentioning that the bioreceptor with SSS stereochemistry is the same one that was designed by polymerization with polystyrene, which is why the code did not change. The standard deviation of the data is generally reduced to one decimal or even becomes null in some cases, which means that the data dispersion is not very variable.
However, once we defined that we wanted to maintain the natural amino acid stereochemistry in the bioreceptor design, we proceeded to determine how many amino acids they should have.

Based on the hDAT model described [28] and the analysis of the number of interactions that DA can form, the influence of the number of amino acids was evaluated only with three styrene-polymerized bioreceptors according to the previous results. The design of these bioreceptors was based on glycines so that only the interaction of DA with the amount of peptide bonds could be assessed with no influence of the functional groups that compose the substituents of the other amino acids. The amount of amino acids varied from two to four glycine molecules, as shown in Figure 8.

Considering this, Table 8 indicates the affinity results for the three glycine bioreceptors, specifying the amount of glycine they contain through their code and the interaction energy in kcal/mol. Figure 9 also presents the images related to each bioreceptor corresponding to this variable.

| Bioreceptor code | Peptide | Average affinity (kcal/mol) | Standard deviation |
|------------------|---------|----------------------------|--------------------|
| 4.0              | GG      | −4.9                       | 0.0                |
| 4.1              | GGG     | −5.1                       | 0.0                |
| 4.2              | GGGG    | −4.8                       | 0.0                |

As shown in Table 8, the best results were obtained with the bioreceptor comprising three glycine molecules (4.1, in bold text in Table 8) with a value of −5.1 kcal/mol, which was the parameter used to build the following bioreceptors to study the relationship of bioreceptor affinity for DA according to the amino acids that compose it.

Based on this, the bioreceptors configured by tripeptides were designed according to their nature. A total of seven bioreceptors were modeled while studying this characteristic, comprising glycine, phenylalanine, alanine, asparagine, serine, cysteine, and histidine. The general structure for this group of bioreceptors is shown in Figure 10.

Phenylalanine and alanine were used as standards for the group of non-polar amino acids to simultaneously compare the influence of the amino acid with an aromatic substituent, which made it possible to analyze the π-π interaction that can occur between the amino acids themselves or with DA. We identified that this interaction can occur with phenylalanine [32]. Therefore, the bioreceptor results with phenylalanine can be compared with those that are formed only by alanine, which are
also non-polar, but with an aliphatic and single-carbon substituent. This allows the result to be related to the type of interaction that can be formed and to the steric volume of the amino acid. Regarding the previous results, as mentioned, the glycine was tested to determine the importance of the presence or absence of the substituent in the amino acids that made up the bioreceptor or if the peptide bonds alone could generate enough affinity for DA. This result is presented in the first row of Table 12 and is the same one presented in Table 8 under code 4.1. The results of the average affinity of the triplicate calculation of the bioreceptor comprising the phenylalanine tripeptide is reported in Table 9 on line two under code 5. The alanine bioreceptor corresponds to code 6 and is reported in the third row of the same Table; these bioreceptors correspond to images B and C in Figure 11.

Regarding the bioreceptors designed from asparagine (code 7), serine (code 8), and cysteine (code 9), the group of polar amino acids has been addressed. However, there are differences in the substituents of these three amino acids, which were considered during their selection. Asparagine is an amino acid that, in addition to being polar, has the amide functional group (RCONH₂) in its substituent and has the capacity to accept three and donate two hydrogen bonds [33]. On the other hand, serine has a hydroxyl group in its substituent and can donate three and accept four hydrogen bonds [34]. Cysteine is a thiol [35]. The results of these three bioreceptors are reported in rows four, five, and six, respectively, of Table 9.

Histidine is a basic amino acid because of the chain in its substituent. It was selected owing to its high reactivity and because it is an amino acid that plays an important role in the catalytic activity of proteins [36]. The bioreceptor designed based on histidine was assigned code 10 and the result of interaction energy with the DA is reported in row seven of Table 9.

Figure 11 presents the images corresponding to each of these seven bioreceptors designed to assess the importance of the nature of their composing amino acid. Each one is identified by its code, as specified in Table 9.

Table 9. Results of the receptors composed of amino acids of different chemical nature.
| Bioreceptor code | Tripeptide | Average affinity (kcal/mol) | Standard deviation (kcal/mol) |
|-----------------|------------|----------------------------|-------------------------------|
| 4.1             | GGG        | −5.1                       | 0.0                           |
| 5               | FFF        | −5.0                       | 0.0                           |
| 6               | AAA        | −4.3                       | 0.0                           |
| 7               | NNN        | −4.5                       | 0.0                           |
| 8               | CCC        | −4.8                       | 0.0                           |
| 9               | SSS        | −4.9                       | 0.0                           |
| 10              | HHH        | −4.8                       | 0.1                           |

As denoted in Table 9, the results for this series of amino acids range from −4.3 to −5.1 kcal/mol, wherein only one of the bioreceptors has a standard deviation other than zero, which means that there was no variability between them and that in the case of bioreceptor 10, data dispersion decreased. Bioreceptor 6, which comprises a tripeptide of alanine, exhibited a more distant result than the others, as shown in row four of Table 9. This may mean that the alanine substituent (CH₃) did not generate a significant affinity with DA, and this result is comparable with that of phenylalanine (row 3 of Table 9), which is also non-polar and provides better results. Therefore, these π–π interactions are stronger than those formed by alanine, as mentioned in the amino acid selection criteria.

The next lowest result found was −4.5 kcal/mol, corresponding to bioreceptor 7, recorded in row five of Table 9. Here, the substituent was an amide did not exhibit any result despite being able to donate two and receive three hydrogen bonds. This may be because these protons possess a very weak acidic character; therefore, in contrast, hydrogen bond interactions may be unlikely, for example, with protons of aspartic acid [37]. These results are comparable with those of serine and cysteine bioreceptors which are also weak despite having acidic protons.

Because with the structural modifications made and the assessment of the three variables above failed to achieve a bioreceptor with better affinity results, it was considered that mixtures between the different amino acids will potentiate the results, specially because it had been observed that the expected results were not obtained for bioreceptors comprising only one amino acid. Then, to combine these amino acids, we decided to use the groups of amino acids reported as exhibiting interaction in the models of hDAT and the group of DA receptors. These groups were taken from the previous studies [10,28].
Before analyzing the results obtained for this group of bioreceptors, it was necessary to highlight that it had already been determined that the amount of amino acids should not exceed four to ensure that interactions with DA were specifically occurring with the amino acids of interest. The existence of aromatic amino acids showed an increase in the interaction, and to add steric volume, the polymerization had to be performed with polystyrene.

Consequently, after testing the variables above, 13 additional bioreceptors were designed which correspond to the amino acid groups identified in the computational models both of the hDAT [28] and the interaction mechanism of DA receptors [10].

Overall, it has been reported that both intra and extracellular DA interactions with DATs and DA receptors involve groups of amino acids, with the number ranging from two to four. In fact, a study argues that aspartic acid is a very important amino acid and essential for the DA reuptake process. In addition, several aromatic interactions were also identified as playing a prominent role in the activity of the protein with DA [28].

In total, 14 groups of amino acids were identified. They were polymerized by the C and N terminal with polystyrene to give them steric volume, a characteristic that had already been proven by increasing bioreceptor interaction. Table 10 displays the code assigned to each bioreceptor, the peptide for which it was composed, and the average affinity result of the triplicate calculation.

| Bioreceptor code | Peptide | Average affinity (kcal/mol) | Standard deviation (kcal/mol) |
|------------------|---------|----------------------------|------------------------------|
| 11               | LS      | −4.5                       | 0.1                          |
| 12               | RD      | −4.7                       | 0.0                          |
| 13               | RDYF    | −5.1                       | 0.0                          |
| 14               | SD      | −5.1                       | 0.0                          |
| 15               | SDW     | −5.3                       | 0.1                          |
| 16               | WFF     | −4.6                       | 0.0                          |
| 17               | WFFH    | −4.6                       | 0.1                          |
| 18               | WFFN    | −5.1                       | 0.0                          |
| 19               | WFT     | −5.4                       | 0.0                          |
| 20               | WH      | −4.5                       | 0.1                          |
| 21               | WHF     | −4.5                       | 0.1                          |
| 22               | YDN     | −5.1                       | 0.0                          |
| 23               | YF      | −5.0                       | 0.0                          |

When observing the results obtained for the 14 bioreceptors presented in Figure 12 the affinity range obtained was determined to be ranging from −4.5 to −5.4 kcal/mol, with null or 0.1 standard
deviations, indicating that there was no significant data dispersion. We were able to obtain bioreceptors, the energy of which exceeded the interaction energy of the DAT at −5.2 kcal/mol. The two bioreceptors that improved interaction with DA were 15 and 19, which are in bold text in rows five and ten of Table 10.

First, bioreceptor 15, denoted in row five of the previous table and image D in Figure 12, will be discussed. This bioreceptor comprises serine, aspartic acid, and tryptophan. As per the above-mentioned findings, serine is a polar amino acid because of its hydroxyl group, which has been described as playing a prominent role in the catalytic activity of enzymes [34]. Conversely, aspartic acid was not addressed or considered in the tests; therefore, it is essential to emphasize its characteristics. This amino acid is acidic and can donate three and accept five hydrogen bonds; therefore, it could be said that it is the amino acid with the highest number of interactions so far [31,38]. The final one is tryptophan, which is part of the group of non-polar and aromatic amino acids. Its substituent has the indole functional group and can donate and accept three hydrogen bonds [39]. This bioreceptor reported an interaction energy of −5.3 kcal/mol. Its corresponding image shows how the amino acids were exposed and the polymer provided steric volume leaving a free pocket for interaction with DA.

Bioreceptor 19 corresponds to image I in Figure 12. With this design, we obtained an affinity of −5.4 kcal/mol, and its composing amino acids are tryptophan, phenylalanine, and tyrosine. Of these amino acids, tyrosine, which is an aromatic and polar amino acid, capable of donating three and accepting four hydrogen bonds, was not analyzed [40]. It is one of the amino acids found at the highest percentage of protein composition and has the phenol functional group in its substituent [41]. As described previously, this bioreceptor contains a tripeptide of aromatic amino acids, which reaffirms the finding that the π−π interaction is essential for DA recognition. However, it is evident that the aromatic group is not strong enough to interact with DA alone, but when supplemented by the hydroxyl group in the tyrosine ring and the benzofused substituent of tryptophan, they interact together to release more energy. The image depicting this bioreceptor also shows that the peptide bonds form a curve that exposes the amino acid substituents so that they can interact with DA.
This applies to the bioreceptors that exceeded the affinity of the DAT. However, it is evident that there are four more bioreceptors that approach this affinity, presenting a value of -5.1 kcal/mol. Therefore, the affinity value while assessing these cases was considered and so were the difference between the first interaction identified by the AutoDock Vina software as well as the difference between the upper and lower quadratic distances of the different configurations tested by the software to yield the results. This was performed because the interactions are more likely to form when the difference between the first and the second values is not greater than 2 Å [42]. Thus, the first two values obtained on performing molecular docking with bioreceptors 13, 14, 18, and 22 are denoted in Figure 13. This is the result for the four bioreceptors being analyzed. As mentioned above, bioreceptor 14 is the only one in which one of the two values, between the upper or lower limit, of the quadratic root of the average atomic distances deviations in the interaction is <2 Å. This bioreceptor corresponds to image (b) in the figure. Therefore, this bioreceptor may exhibit a good interaction with DA beyond the energy value recorded. Thus, there are several bioreceptors that may offer better recognition characteristics for DA than DAT.

2.3. Analysis of candidate bioreceptors

Based on the results, the study continued with two candidates that exceeded the affinity parameter and an additional one with promising characteristics owing to the differences in the upper and lower root-mean-square deviation (RMSD) values. These three bioreceptors were further assessed to determine the distances used for the interaction in the docking process and to compare them against the distance of the DAT. Another criterion used to assess the docking result was through a graphical interface that displays the calculated interaction model [43]. This visualization was conducted using the PyMOL software for both the DAT and the three selected bioreceptors.

Figure 14 presents the possible interactions through hydrogen bonds (yellow dotted lines) that were simulated to calculate the affinity of the DAT for DA. The distances of these hydrogen bonds are shown in the image. Only four possible interactions were evaluated because the hydroxyl groups in
the ring were closer to the protein. The results yielded two of 2.8 Å, one of 2.7 Å, and one of 2.4 Å.

With these criteria, we determined whether the distances of the selected bioreceptors were similar to those of the DAT. The images corresponding to the results of the docking for bioreceptors 14, 15, and 19 are denoted in Figure 15.

As presented in Figure 14, the measured length values of the interactions created between DA and each of the bioreceptors can be observed. These results are further summarized in Table 11, wherein bioreceptors 19 and 15 exhibit distances greater than the distances reported for the hydrogen bonds formed between the DAT and DA, although they have better interaction energy in terms of affinity.

Conversely, for bioreceptor 19, the distance of one of the hydrogen bonds between the bioreceptor and DA is 2.3 Å. Therefore, the distance is decreased by one tenth when compared against the shortest bond that can be formed with the DAT according to the simulations. However, this bioreceptor has no affinities higher than the DA reuptake.

| Bioreceptor code | Hydrogen bond length (Å) |
|------------------|--------------------------|
| 19               | 3.3                      |
| 15               | 3.7                      |
| 14               | 2.3                      |

Based on these results, three bioreceptors designed were candidates to be synthesized for in vitro tests for selectivity, stability to be a functional part of a biosensor, and affinity for DA.

In brief, two of the bioreceptors report better DAT interaction energies, and both comprise three amino acids and are polymerized by the C and N terminal with polystyrene. The third bioreceptor has shorter distances for interaction with DA although the energy interaction is −0.1 kcal/mol weaker than the energy reported for the DAT.

3. Conclusions

An in silico methodology was implemented to design bioreceptors through multiple structural changes; controlling variables, such as amino acid sequences according to their quantity; their linking bond; their stereochemistry; and the classification group and steric volume of the bioreceptor. From this, it was possible to obtain three candidate designs for later synthesis.

In comparison, three bioreceptors were designed, which in structural terms, are simpler than a
protein, and in this case, the DAT; for example, the DAT contains more than six hundred residuals, whereas the bioreceptors have a maximum of three amino acids. Therefore, miniaturization of the DAT was achieved in terms of recognition and molecular interaction with DA. For the simulated interactions, the interaction energy improved by −0.2 kcal/mol compared with the interaction energy presented by the DAT owing to DA. In fact, in one case, it was possible to obtain a distance 0.1–0.5 Å closer.

The first bioreceptor corresponds to code 19 assigned in the paper; it comprises the polystyrene-WFT-polystyrene sequence, with an interaction energy of −5.4 kcal/mol and an interaction distance of 3.3 Å. The second bioreceptor corresponds to code 15 and is formed by the polystyrene-SDW-polystyrene structure. It presented an affinity of −5.3 kcal/mol for DA and an interaction distance of 3.7 Å. Finally, the third one comprises two amino acids and corresponds to bioreceptor code 14, with a polystyrene-SD-polystyrene structure, an interaction energy of −5.1 kcal/mol, and a length of interaction with DA of 2.3 Å.

Bioreceptor candidates were obtained by implementing computational tools that minimize the trial and error stage of the design, synthesis, and in vitro testing processes.

4 Methods

4.1. Enzyme and receptor analysis

As described, tyrosinase has been previously used to design DA biosensors because it is active in vivo. This enzyme catalyzes the hydroxylation and oxidation of diphenols to o-quinones in the presence of oxygen [44], and this reaction can be measured with electrodes that monitor the reduction of catecholamine [13].

Hence, the group to which tyrosinase belongs was identified and is 1.14.18 according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB). This group represents the class of oxidoreductases EC 1, with the subclass that acts by donating electron pairs with the incorporation or reduction of molecular oxygen [14] and with the subclass that uses another compound as a donor and incorporates an oxygen atom [18] [45]. There are eight different types of tyrosinase in this type of enzyme, all of which are denoted in Table 12.

Table 12. Nomenclature of preselected enzymes for analysis.
| Systematic nomenclature | Common name                                                                 |
|-------------------------|-----------------------------------------------------------------------------|
| EC. 1.14.18.1           | tyrosinase                                                                  |
| EC. 1.14.18.2           | CMP-N-acetylneuraminate monooxygenase                                       |
| EC. 1.14.18.3           | methane monooxygenase (particulate)                                        |
| EC. 1.14.18.4           | phosphatidylcholine 12-monooxygenase                                       |
| EC. 1.14.18.5           | sphingolipid C4-monooxygenase                                               |
| EC. 1.14.18.6           | 4-hydroxysphinganine ceramide fatty acyl 2-hydroxyl                         |
| EC. 1.14.18.7           | dihydroceramide fatty acyl 2-hydroxylase                                   |
| EC. 1.14.18.8           | 7α-hydroxycholest-4-en-3-one 12α-hydroxylase                               |
| EC. 1.14.18.9           | methylsterol monooxygenase                                                 |

Conversely, the receptors and enzymes characteristic of DA interactions in biological processes were identified. In the case of DA receptors, the five D1-5 receptors have been described in the literature [10]. These receptors reuptake DA once it has been released from the presynaptic terminals. Many drugs involved in dopaminergic neurotransmission have been designed to block these receptors [5]. We also considered the DAT, the protein that modulates the availability of DA in the synaptic space, because it is responsible for reuptake of extracellular DA and introduces it into the presynaptic neurons [22].

In addition, other proteins that had been crystallized with DA or its analogs in the PDB were evaluated. These proteins were studied in the PDB, along with their sequences and interaction amino acids, and they were also downloaded in the .pdb format.

### 4.2. Molecular docking

Molecular docking was done for performing the simulations for the affinity calculations. As part of the procedure used for performing simulations in AutoDock Tools (version 1.5.6), the water molecules that could have been included in the protein.pdb file downloaded from the PDB were removed, polar hydrogens were added to the proteins, and the Gasteiger charges were added as described previously [25]. Each analysis grid was a variant and was selected according to the protein or bioreceptor analyzed to verify whether all the amino acids involved in the interaction were being evaluated. The results were analyzed by two criteria. The first criterion was the lowest energy released between the protein–ligand or bioreceptor–ligand complex because the stability of a complex can be measured by the negative magnitude of the Gibbs free energy [20]. The second criterion was RMSD. Each calculation was performed in triplicate, and the averages and standard deviation of the lowest
energy value for each protein were obtained.

4.3. Bioreceptor design

Once the triplicate enzyme calculations have been completed, the three enzymes with the best affinity values (kcal/mol) were selected, and the corresponding bioreceptors were designed based on the amino acids that interact with DA. Thus, possible bioreceptors were designed for the three selected enzymes using the ChemDraw® software.

Each designed peptide structure was stored with the .cdmxl extension in a folder named “bioreceptors” and was subsequently optimized using the Universal Forcefield Program from the Avogadro® software tool, with four update steps and using the down-gradient algorithm option [46]. The .pdbqt files were generated after saving each bioreceptor in a folder called “bioreceptors.” The description of this procedure is found in previous studies [46,47].

We selected this software and this molecular energy and geometry optimization algorithm because they allow global minimum to be reached instead of a partial value through convergence toward the minimum, wherein the forces must reach the zero value and the accepted threshold is $4.5 \times 10^{-4}$ [48]. However, it should be considered that important parameters, such as the speed of convergence, stability, and computational cost, can deteriorate when processing some of the bioreceptors because as the number of links increases as well as the link and torsion angles, the difficulty and time for molecular geometry to converge also usually increase [48].

The designed bioreceptors, based on the amino acids of the enzymes involved in the DA recognition process, were evaluated based on an adapted Trott & Olson methodology (2009), which is as follows. Because the bioreceptors designed in silico were constructed by the authors instead of obtaining them from the PDB, the procedure bypasses the pretreatment that adds polar hydrogens to the ligand and conversion of the file to .pdbqt format because this is the original extension with which they were created.

The previous section describes how the bioreceptor affinities were calculated as per the adapted Trott & Olson methodology (2009). Structural modifications were also applied to each bioreceptor designed to achieve interaction energies similar to those reported for the enzymes. These variations are
presented below:

1. Amino acid sequences (i.e., AGA).

2. Amino acid sequence with intermediate methylene bonds (i.e., A-(CH2)n-G-A).

3. Amino acid sequences polymerized by the N and C terminal with both polyethylene and polystyrene (i.e., Poly-AGA-Poly).

4. Amino acid sequence with intermediate methylene bonds, polymerized by the terminal N and C with both polyethylene and polystyrene (i.e., Poly-A-(CH2)n-GA-Poly).

5. Variation of amino acids according to their chemical nature, considering the results of the bioreceptors with the previous modifications.

Abbreviations
Dopamine (DA)
Electrochemical detection (ECD)
DA transporter (DAT)
Protein Data Bank (PDB)
Human DAT (hDAT)
Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB).
Root-mean-square deviation of atomic positions (RMSD)

Declarations
**Ethics approval and consent to participate:** Not applicable

**Consent for publication:** Not applicable

**Availability of data and material:** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing Interest:** The authors declare no conflicts of interest.

**Funding:** This research was funded by UNIVERSIDAD EL BOSQUE, grant number PCI-2017-9646.

**Author Contributions:** Conceptualization, JGP, and LRS.; methodology, JGP.; software, LRS.;

22
validation, JGP, and LRS.; formal analysis, JGP, and LRS.; investigation, JGP, and LRS.; data curation, JGP, and LRS.; writing—original draft preparation, JGP, and LRS.; writing—review and editing, JGP.; supervision, JGP.; project administration, JGP.; funding acquisition, JGP, and LRS.; all authors have read and approved the manuscript JGP, and LRS.

Acknowledgments: Not applicable

References

[1] Rozet, E.; Morello, R.; Lecomte, F.; Martin, G. B.; Chiap, P.; Crommen, J.; Boos, K. S.; Hubert, P. Performances of a Multidimensional On-Line SPE-LC-ECD Method for the Determination of Three Major Catecholamines in Native Human Urine: Validation, Risk and Uncertainty Assessments. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 2006, 844 (2), 251–260. https://doi.org/10.1016/j.jchromb.2006.07.060.

[2] Zeng, C.; Eisner, G. M.; Felder, R. A.; Jose, P. A. Dopamine Receptor and Hypertension. Curr. Med. Chem. Cardiovasc. Hematol. Agents 2005, 3 (1), 69–77.

[3] Bicker, J.; Fortuna, A.; Alves, G.; Falcão, A. Liquid Chromatographic Methods for the Quantification of Catecholamines and Their Metabolites in Several Biological Samples - A Review. Anal. Chim. Acta 2013, 768 (1), 12–34. https://doi.org/10.1016/j.aca.2012.12.030.

[4] Juárez Olguín, H.; Calderón Guzmán, D.; Hernández García, E.; Barragán Mejía, G. The Role of Dopamine and Its Dysfunction as a Consequence of Oxidative Stress. Oxid. Med. Cell. Longev. 2016, 2016, 1–13. https://doi.org/10.1155/2016/9730467.

[5] Beaulieu, J.-M.; Gainetdinov, R. R. The Physiology, Signaling, and Pharmacology of Dopamine Receptors. Pharmacol. Rev. 2011, 63 (1), 182–217. https://doi.org/10.1124/pr.110.002642.

[6] Tsunoda, M.; Aoyama, C.; Nomura, H.; Toyoda, T.; Matsuki, N.; Funatsu, T. Simultaneous Determination of Dopamine and 3,4-Dihydroxyphenylacetic Acid in Mouse Striatum Using Mixed-Mode Reversed-Phase and Cation-Exchange High-Performance Liquid Chromatography. J. Pharm. Biomed. Anal. 2010, 51 (3), 712–715. https://doi.org/10.1016/j.jpba.2009.09.045.

[7] Trillo, L.; Das, D.; Hsieh, W.; Medina, B.; Moghadam, S.; Lin, B.; Dang, V.; Sanchez, M. M.; De Miguel, Z.; Ashford, J. W.; et al. Ascending Monoaminergic Systems Alterations in Alzheimer’s Disease.
Translating Basic Science into Clinical Care. *Neurosci. Biobehav. Rev.* 2013, 37 (8), 1363-1379. https://doi.org/10.1016/j.neubiorev.2013.05.008.

[8] Marsden, C. A. Dopamine: The Rewarding Years. *Br. J. Pharmacol.* 2009, 147 (S1), S136-S144. https://doi.org/10.1038/sj.bjp.0706473.

[9] Rosano, T. G.; Swift, T. A.; Hayes, L. W. Advances in Catecholamine and Metabolite Measurements for Diagnosis of Pheochromocytoma. *Clin. Chem.* 1991, 37 (10 Pt 2), 1854-1867.

[10] Bueschbell, B.; Barreto, C. A. V.; Preto, A. J.; Schiedel, A. C.; Moreira, I. S. A Complete Assessment of Dopamine Receptor-Ligand Interactions through Computational Methods. *Molecules* 2019, 1–26. https://doi.org/10.3390/molecules24071196.

[11] Quaiserová-Mocko, V.; Novotný, M.; Schaefer, L. S.; Fink, G. D.; Swain, G. M. CE Coupled with Amperometric Detection Using a Boron-Doped Diamond Microelectrode: Validation of a Method for Endogenous Norepinephrine Analysis in Tissue. *Electrophoresis* 2008, 29 (2), 441-447. https://doi.org/10.1002/elps.200700398.

[12] Perumal, V.; Hashim, U. Advances in Biosensors: Principle, Architecture and Applications. *J. Appl. Biomed.* 2014, 12 (1), 1-15. https://doi.org/10.1016/j.jab.2013.02.001.

[13] Florescu, M.; David, M. Tyrosinase-Based Biosensors for Selective Dopamine Detection. *Sensors (Switzerland)* 2017, 17 (6). https://doi.org/10.3390/s17061314.

[14] Bazin, I.; Tria, S. A.; Hayat, A.; Marty, J. L. New Biorecognition Molecules in Biosensors for the Detection of Toxins. *Biosens. Bioelectron.* 2017, 87, 285–298. https://doi.org/10.1016/j.bios.2016.06.083.

[15] Rivas, L.; Mayorga-Martinez, C. C.; Quesada-González, D.; Zamora-Gálvez, A.; de la Escosura-Muñiz, A.; Merkoçi, A. Label-Free Impedimetric Aptasensor for Ochratoxin-A Detection Using Iridium Oxide Nanoparticles. *Anal. Chem.* 2015, 87 (10), 5167–5172. https://doi.org/10.1021/acs.analchem.5b00890.

[16] Thyparambil, A. A.; Bazin, I.; Guiseppi-Elie, A. Evaluation of Ochratoxin Recognition by Peptides Using Explicit Solvent Molecular Dynamics. *Toxins (Basel).* 2017, 9 (5), 1–20. https://doi.org/10.3390/toxins9050164.
[17] Anisimov, V. M.; Ziemys, A.; Kizhake, S.; Yuan, Z.; Natarajan, A.; Cavasotto, C. N. Computational and Experimental Studies of the Interaction between Phospho-Peptides and the C-Terminal Domain of BRCA1. *J. Comput. Aided. Mol. Des.* **2011**, *25* (11), 1071-1084. https://doi.org/10.1007/s10822-011-9484-3.

[18] Rodríguez-Salazar, L.; Guevara-Pulido, J.; Morales-Mendoza, E.; Ibla, F. In-Silico Design of Peptide Receptor for Carboxyhemoglobin Recognition. *Informatics Med. Unlocked* **2019**, *14*, 1-5. https://doi.org/10.1016/J.IMU.2019.01.003.

[19] Thyparambil, A.; Bazin, I.; Guiseppi-Elie, A. Molecular Modeling and Simulation Tools in the Development of Peptide-Based Biosensors for Mycotoxin Detection: Example of Ochratoxin. *Toxins (Basel)*. **2017**, *9* (12), 395. https://doi.org/10.3390/toxins9120395.

[20] Du, X.; Li, Y.; Xia, Y.-L.; Ai, S.-M.; Liang, J.; Sang, P.; Ji, X.-L.; Liu, S.-Q. Insights into Protein-Ligand Interactions: Mechanisms, Models, and Methods. *Int. J. Mol. Sci.* **2016**, *17* (2). https://doi.org/10.3390/ijms17020144.

[21] Cheng, M. H.; Bahar, I. Molecular Mechanism of Dopamine Transport by Human Dopamine Transporter. *HHS Public Acces* **2015**, *23* (11), 2171-2181. https://doi.org/10.1016/j.str.2015.09.001.

[22] Vaughan, R. A.; Foster, J. D. Mechanisms of Dopamine Transporter Regulation in Normal and Disease States. *Trends Pharmacol. Sci.* **2013**, *34* (9), 489-496. https://doi.org/10.1016/j.tips.2013.07.005.

[23] Cambridge University Press. MINIATURIZATION | definition in the Cambridge English Dictionary https://dictionary.cambridge.org/us/dictionary/english/miniaturization (accessed Aug 28, 2019).

[24] Collins. Miniaturize definition and meaning | Collins English Dictionary https://www.collinsdictionary.com/dictionary/english/miniaturize (accessed Aug 28, 2019).

[25] Weibel, N.; Grunder, S.; Mayor, M. Functional Molecules in Electronic Circuits. *Org. Biomol. Chem.* **2007**. https://doi.org/10.1039/b703287k.

[26] Delcassian, D.; Patel, A. K.; Cortinas, A. B.; Langer, R. Drug Delivery across Length Scales. *J. Drug Target.* **2019**, *27* (3), 229-243. https://doi.org/10.1080/1061186X.2018.1438440.

[27] Wang, K. H.; Penmatsa, A.; Gouaux, E. Neurotransmitter and Psychostimulant Recognition by
the Dopamine Transporter. *Nature* **2015**, *521*, 322–327. https://doi.org/10.2210/PDB4XP1/PDB.

[28] Cheng, M. H.; Bahar, I. Molecular Mechanism of Dopamine Transport by Human Dopamine Transporter. *Structure* **2015**, *23* (11), 2171–2181. https://doi.org/10.1016/j.str.2015.09.001.

[29] Blaber, M. Stereochemistry of Amino Acids

https://chem.libretexts.org/Bookshelves/Biological_Chemistry/Supplemental_Modules_(Biological_Chemistry)/Stereochemistry_of_Amino_Acids (accessed Aug 12, 2019).

[30] Lexico by Oxford Dictionary. Definition of enantiomer

https://www.lexico.com/en/definition/enantiomer (accessed Aug 12, 2019).

[31] Reddy, M. K. Amino acid - Standard amino acids https://www.britannica.com/science/amino-acid/Standard-amino-acids (accessed Aug 12, 2019).

[32] McGaughey, G. B.; Gagné, M.; Rappé, A. K. Pi-Stacking Interactions. Alive and Well in Proteins. *J. Biol. Chem.* **1998**, *273* (25), 15458–15463. https://doi.org/10.1074/jbc.273.25.15458.

[33] National Center for Biotechnology Information. Asparagine

https://pubchem.ncbi.nlm.nih.gov/compound/Asparagine.

[34] National Center for Biotechnology Information. Serine

https://pubchem.ncbi.nlm.nih.gov/compound/5951 (accessed Aug 14, 2019).

[35] National Center for Biotechnology Information. Cysteine

https://pubchem.ncbi.nlm.nih.gov/compound/Cysteine (accessed Aug 14, 2019).

https://doi.org/10.1107/S0108270196003952.

[36] Liao, S.-M.; Du, Q.-S.; Meng, J.-Z.; Pang, Z.-W.; Huang, R.-B. The Multiple Roles of Histidine in Protein Interactions. *Chem. Cent. J.* **2013**, *7* (1), 44. https://doi.org/10.1186/1752-153X-7-44.

[37] Universidad de Buenos Aires. Tabla de valores de pKa para aminoácidos

http://www.calvo.qb.fcen.uba.ar/Tabla_pKa.html (accessed Aug 28, 2019).

[38] National Center for Biotechnology Information. Aspartic Acid (Compound)

https://pubchem.ncbi.nlm.nih.gov/compound/Aspartic-acid (accessed Aug 19, 2019).

https://doi.org/10.1107/S0108270107021671.

[39] National Center for Biotechnology Information. Tryptophan
https://pubchem.ncbi.nlm.nih.gov/compound/Tryptophan (accessed Aug 19, 2019).

[40] National Center for Biotechnology Information. Tyrosine
https://pubchem.ncbi.nlm.nih.gov/compound/Tyrosine (accessed Aug 19, 2019).

[41] Encyclopaedia Britannica, inc. Tyrosine | chemical compound
https://www.britannica.com/science/tyrosine (accessed Aug 19, 2019).

[42] Morris, G. M.; Lim-Wilby, M. Molecular Docking. In Methods in Molecular Biology; Kukol, A., Ed.; Hummana Press: Totowa, 2015; Vol. 443, pp 1-1. https://doi.org/10.1002/9783527678679.dg07851.

[43] Seeliger, D.; de Groot, B. L. Ligand Docking and Binding Site Analysis with PyMOL and Autodock/Vina. J. Comput. Aided. Mol. Des. 2010, 24 (5), 417–422. https://doi.org/10.1007/s10822-010-9352-6.

[44] Decker, H.; Tuczek, F. Tyrosinase/Catecholoxidase Activity of Hemocyanins: Structural Basis and Molecular Mechanism. Trends Biochem. Sci. 2000, 25 (8), 392–397. https://doi.org/10.1016/S0968-0004(00)01602-9.

[45] Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB). EC 1.14.18 http://www.sbcsc.qmul.ac.uk/iubmb/enzyme/EC1/14/18/ (accessed Sep 19, 2018).

[46] Hanwell, M. D.; Curtis, D. E.; Lonie, D. C.; Vandermeersch, T.; Zurek, E.; Hutchison, G. R. Avogadro: An Advanced Semantic Chemical Editor, Visualization, and Analysis Platform. J. Cheminform. 2012, 4 (1), 17. https://doi.org/10.1186/1758-2946-4-17.

[47] Avogadro Chemistry. Auto Optimize Tool - Avogadro https://avogadro.cc/docs/tools/auto-optimize-tool/ (accessed Oct 3, 2018).

[48] Cuevas, G.; Cortés, F. Optimización de La Geometría Molecular. In Introducción a la Química Computacional; Fondo de Cultura Económica: México D.F., 2003; pp 114–126.

Figures
Figure 1

Peptide bioreceptors. (a) Bioreceptor made up by the NWRD peptide. (b) Bioreceptor consisting of the RQKYSSMMGPSPNKNFI peptide. (c) Bioreceptor made up by the FPFDKHEAH peptide.

Figure 2

Bioreceptors with methylene bridges as spacers among amino acids. (a) Bioreceptor comprising the NWRD peptide. (b) Bioreceptor comprising the RQKYSSMMGPSPNKNFI peptide. (c) Bioreceptor comprising the FPFDKHEAH peptide.
General structure of the polymerization of bioreceptors. (a) For bioreceptors composed solely of peptides. (b) For bioreceptors with methylene bridges among amino acids.

Bioreceptors polymerized with ethylene by C and N terminal. (a) Bioreceptor 1.0.1; (b) 2.0.1; (c) 3.0.1; (d) 1.1.1; (e) 2.1.1; and (f) 3.1.1
Bioreceptors polymerized with styrene by C and N terminal. (a) Bioreceptor 1.0.2; (b) 2.0.2; (c) 3.0.2; (d) 1.1.2; (e) 2.1.2; and (f) 3.1.2.

Polystyrene–NWR–polystyrene bioreceptor whose amino acid stereochemistry was modified.
Polystyrene–NWR–polystyrene bioreceptors with modifications in the stereochemistry of each amino acid. (a) Bioreceptor 1.0.2.1 (SSS); (b) Bioreceptor 1.0.2.2 (RRS); (c) Bioreceptor 1.0.2.3 (RSR); (d) Bioreceptor 1.0.2.4 (RSS); (e) Bioreceptor 1.0.2.5 (SRS); (f) Bioreceptor 1.0.2.6 (SSR); and (g) Bioreceptor 1.0.2.7 (RRR).
Figure 8

General structure of the receptors with variations in the amount of glycine.

Figure 9

Bioreceptors composed of glycine and polymerized with polystyrene. (a) Polystyrene–GG–Polystyrene bioreceptor. (b) Polystyrene–GGG–Polystyrene. (c) Polystyrene–GGGG–Polystyrene.
The general structure of the receptors wherein the nature of amino acids is studied.

Figure 10

\[ R: \text{Alanine, Asparagine, Phenylalanine, Cysteine, Serine, Histidine, glycine} \]
Figure 11

Bioreceptors varying with respect to the nature of the amino acids that compose them and polymerized with polystyrene by the C and N terminal. (a) Bioreceptor 4.1; (b) Bioreceptor 5; (c) Bioreceptor 6; (d) Bioreceptor 7; (e) Bioreceptor 8; (f) Bioreceptor 9; and (g) Bioreceptor 10.
Figure 12

Images of bioreceptors designed from simulated molecular interactions for hDAT and dopamine receptors with DA. a) Bioreceptor 11; b) Bioreceptor 12; c) Bioreceptor 13; d) Bioreceptor 14; e) Bioreceptor 15; f) Bioreceptor 16; g) Bioreceptor 17; h) Bioreceptor 18; i) Bioreceptor 19; j) Bioreceptor 20; k) Bioreceptor 21; l) Bioreceptor 22; and m) Bioreceptor 23.
Figure 13

Second result for bioreceptors 13, 14, 18, and 22. (a) Bioreceptor 13; (b) Bioreceptor 14; (c) Bioreceptor 18; and (d) Bioreceptor 22.

Figure 14

Visualization of the interaction between the DAT and DA. The hydrogen bonds formed (yellow dotted line) and the measurement of their lengths are shown.
Figure 15

Visualization of the interactions of the selected bioreceptors with the measurements of the lengths of the hydrogen bonds formed between dopamine and the amino acids of the bioreceptor. (a) Bioreceptor 19. (b) Bioreceptor 15. (c) Bioreceptor 14.