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In silico design of a peptide receptor for triglyceride recognition using a structure-based approach

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SUMMARY

Background: Elevated triglyceride levels constitute a major risk factor for cardiovascular disease, accounting for millions of deaths annually. Current methods for triglyceride quantification rely on enzymatic assays or complex lipid panels, which present limitations in stability, cost, and turnaround time. Peptide-based biosensors offer a promising alternative due to their chemical stability, synthetic accessibility, and amenability to rational design. **Methods:** A structure-based computational approach was employed to design a selective peptide receptor for glycerol —the hydrolysis product of triglycerides— using molecular docking with AutoDock Vina. Twelve peptide receptors were initially derived from the active sites of glycerol-binding enzymes retrieved from the Protein Data Bank. The most promising receptor was subjected to structural modifications, including the addition of styrene units and methylene bridges to increase molecular volume, and the introduction of hydroxyl functional groups on aromatic rings. Selectivity was evaluated *in silico* against eight common fatty acids. A 4×4 Latin square experimental design was applied to optimize the combination of volume modifications and functionalization. **Results:** The receptor based on the TRR sequence (Thr-Arg-Arg), functionalized with styrene units and methylene bridges, and modified with hydroxyl groups, exhibited the highest affinity for glycerol (-4.9 kcal/mol) with interaction distances between 1.744 and 1.925 Å. Selectivity assays demonstrated that the TRR receptor discriminates glycerol preferentially over all tested fatty acids, with affinity differences of at least 1.3 kcal/mol. Statistical analysis confirmed that the receptor's affinity is significantly dependent on the specific arrangement of amino acids in the sequence. **Conclusions:** The TRR receptor represents a promising starting point for the experimental development of a peptide-based biosensor for indirect triglyceride quantification. The computational strategy employed demonstrates the feasibility of designing selective bioreceptors through a structure-based approach, although experimental validation through peptide synthesis and testing in biological matrices remains a necessary next step.

Keywords: Peptide bioreceptor; glycerol recognition; molecular docking; triglyceride biosensor; structure-based design; *in silico* screening.

RESUMEN

Diseño *in silico* de un receptor peptídico para el reconocimiento de triglicéridos mediante un enfoque basado en la estructura

Antecedentes: Los niveles elevados de triglicéridos constituyen un importante factor de riesgo para las enfermedades cardiovasculares, responsables de millones de muertes anuales. Los métodos actuales para la cuantificación de triglicéridos se basan en ensayos enzimáticos o paneles lipídicos complejos, que presentan limitaciones en cuanto a estabilidad, coste y tiempo de procesamiento. Los biosensores peptídicos ofrecen una alternativa prometedora debido a su estabilidad química, accesibilidad sintética y facilidad de diseño racional. **Métodos:** Se empleó un enfoque computacional basado en la estructura para diseñar un receptor peptídico selectivo para el glicerol —producto de la hidrólisis de los triglicéridos— mediante acoplamiento molecular con AutoDock Vina. Inicialmente, se derivaron doce receptores peptídicos de los sitios activos de enzimas que se unen al glicerol, obtenidos del Protein Data Bank. El receptor más prometedor se sometió a modificaciones estructurales, incluyendo la adición de unidades de estireno y puentes de metileno para aumentar el volumen molecular, y la introducción de grupos funcionales hidroxilo en los anillos aromáticos. Se evaluó la selectividad *in silico* frente a ocho ácidos grasos comunes. Se aplicó un diseño experimental de cuadrado latino 4×4 para optimizar la combinación de modificaciones de volumen y funcionalización. **Resultados:** El receptor basado en la secuencia TRR (Thr-Arg-Arg), funcionalizado con unidades de estireno y puentes de metileno, y modificado con grupos hidroxilo, exhibió la mayor afinidad por el glicerol (-4,9 kcal/mol) con distancias de interacción entre 1,744 y 1,925 Å. Los ensayos de selectividad demostraron que el receptor TRR discrimina preferentemente el glicerol sobre todos los ácidos grasos probados, con diferencias de afinidad de al menos 1,3 kcal/mol. El análisis estadístico confirmó que la afinidad del receptor depende significativamente de la disposición específica de los aminoácidos en la secuencia. **Conclusiones:** El receptor TRR representa un punto de partida prometedor para el desarrollo experimental de un biosensor basado en péptidos para la cuantificación indirecta de triglicéridos. La estrategia computacional empleada demuestra la viabilidad del diseño de biorreceptores selectivos mediante un enfoque basado en la estructura, si bien la validación experimental a través de la síntesis de péptidos y las pruebas en matrices biológicas sigue siendo un paso necesario.

Palabras clave: Biorreceptor peptídico; reconocimiento de glicerol; acoplamiento molecular; biosensor de triglicéridos; diseño basado en la estructura; cribado *in silico.*

RESUMO

Projeto *in silico* de um receptor peptídico para reconhecimento de triglicerídeos utilizando uma abordagem baseada em estrutura

Contexto: Níveis elevados de triglicerídeos constituem um importante fator de risco para doenças cardiovasculares, sendo responsáveis por milhões de mortes anualmente. Os métodos atuais para quantificação de triglicerídeos dependem de ensaios enzimáticos ou painéis lipídicos complexos, que apresentam limitações em termos de estabilidade, custo e tempo de resposta. Biossensores baseados em peptídeos oferecem uma alternativa promissora devido à sua estabilidade química, acessibilidade sintética e facilidade de projeto racional. **Métodos:** Uma abordagem computacional baseada em estrutura foi empregada para projetar um receptor peptídico seletivo para glicerol — o produto da hidrólise de triglicerídeos — utilizando acoplamento molecular com o AutoDock Vina. Doze receptores peptídicos foram inicialmente derivados dos sítios ativos de enzimas ligadoras de glicerol obtidas do Protein Data Bank. O receptor mais promissor foi submetido a modificações estruturais, incluindo a adição de unidades de estireno e pontes de metileno para aumentar o volume molecular, e a introdução de grupos funcionais hidroxila em anéis aromáticos. A seletividade foi avaliada *in silico* contra oito ácidos graxos comuns. Um planejamento experimental em quadrado latino 4×4 foi aplicado para otimizar a combinação de modificações de volume e funcionalização. **Resultados:** O receptor baseado na sequência TRR (Thr-Arg-Arg), funcionalizado com unidades de estireno e pontes de metileno, e modificado com grupos hidroxila, apresentou a maior afinidade por glicerol (-4,9 kcal/mol), com distâncias de interação entre 1,744 e 1,925 Å. Ensaios de seletividade demonstraram que o receptor TRR discrimina o glicerol preferencialmente em relação a todos os ácidos graxos testados, com diferenças de afinidade de pelo menos 1,3

kcal/mol. A análise estatística confirmou que a afinidade do receptor depende significativamente do arranjo específico dos aminoácidos na sequência. **Conclusões:** O receptor TRR representa um ponto de partida promissor para o desenvolvimento experimental de um biossensor peptídico para a quantificação indireta de triglicerídeos. A estratégia computacional empregada demonstra a viabilidade de projetar biorreceptores seletivos por meio de uma abordagem baseada na estrutura, embora a validação experimental através da síntese de peptídeos e testes em matrizes biológicas permaneça como uma etapa necessária.

Palavras-chave: Biorreceptor peptídico; reconhecimento de glicerol; ancoragem molecular; biossensor de triglicerídeos; projeto baseado em estrutura; triagem *in silico.*

1. INTRODUCTION

According to the World Health Organization (WHO), approximately 9 million deaths annually are attributed to ischemic heart disease linked to dyslipidemias such as hypertriglyceridemia and hypercholesterolemia. These conditions usually originate from a metabolic imbalance that leads to elevated blood triglyceride levels, either due to endogenous synthesis or dietary intake, and they constitute an important energy source for the body [1-3]. There are three established ranges of triglyceride concentration in which a person can be classified [4]. When triglyceride levels are elevated, they can affect the patient's health without presenting obvious symptoms. Therefore, a blood test is the most reliable method for diagnosis. Typically, triglycerides are calculated from the results of the standard lipid panel, which includes total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TGs). However, the process of measuring triglycerides has limitations that could be improved [5]. Additionally, the extensive analysis process can cause delays in delivering results, making it difficult to continuously monitor triglyceride levels and potentially postponing necessary therapies for the patient's health [6-9].

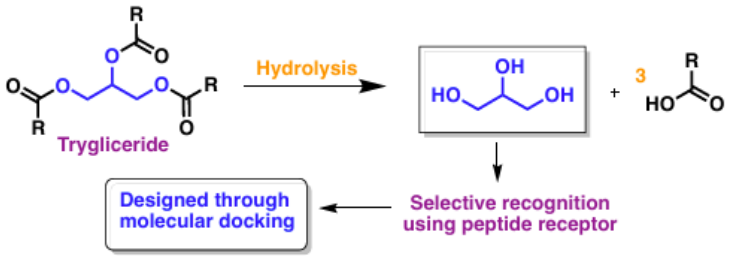
Biosensors represent a promising alternative for measuring specific molecules in blood samples [10]. They offer real-time data, high precision, and excellent selectivity. However, quantifying triglycerides using biosensors faces two significant challenges. The first concerns the receptors used, which can denature under certain environmental conditions, compromising their ability to detect triglycerides. The second relates to the heterogeneity of triglycerides: the various combinations of fatty acids in their structure make quantification in complex biological samples difficult. Essentially, a biosensor must be able to specifically recognize triglycerides and convert the chemical signal into a measurable electrical signal [11].

Currently, the molecular recognition of triglycerides is primarily mediated by three types of biological receptors. Lipase enzymes are the most widely used method: they hydrolyze triglycerides into glycerol and free fatty acids, and the released glycerol is quantified using coupled enzymatic or electrochemical methods [12]. However, lipases are proteins that can denature with pH or temperature changes; their activity varies between batches, and their immobilization on sensor surfaces can compromise long-term stability. Antibodies offer high specificity but are costly to produce, require controlled storage conditions, and generating them against small molecules such as triglycerides is technically challenging [13]. Molecularly imprinted polymers (MIPs) and aptamers have emerged as more stable synthetic alternatives, although the former can degrade due to nucleases in biological samples, and the latter have limited selectivity for complex lipid mixtures [14].

In light of these limitations, short synthetic peptides have emerged as an attractive alternative for designing bioreceptors [15]. Unlike enzymes and antibodies, peptides are chemically

stable, can be synthesized with high purity via solid-phase synthesis, can be chemically modified for immobilization on sensor surfaces, and their sequences can be rationally designed to complement the structure of the analyte of interest. The structure-based design strategy employs computational tools such as molecular docking and molecular dynamics to predict the affinity and binding mode of candidate peptides to their target ligand, enabling virtual screening of hundreds of sequences before synthesizing the most promising ones [16].

This study aims to develop a peptide receptor through computational simulations that mimics the molecular recognition processes observed in natural lipases, while offering the structural stability and chemical-modification versatility of synthetic peptides (Scheme 1) [17, 18].



Scheme 1. Approach to detect glycerol linked to triglycerides.

2. MATERIALS AND METHODS

2.1. Identifying enzymes and analyzing their affinity for glycerol

The enzymes that recognize glycerol at the biological level are selected for the *in silico* receptor design. These enzymes are classified into two families, as defined by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB): oxidoreductases (EC 1) and transferases (EC 2). The selected enzymes include EC 1.14.19.1, EC 1.1.1.35, EC 1.14.99.1, EC 1.13.11.34, EC 1.1.1.6, EC 2.7.1.30, EC 2.7.7.39, and EC 1.1.1.8.

This study focuses on enzymes that process samples and measure triglycerides by determining glycerol concentrations. Furthermore, several identified enzymes play roles in lipid metabolism. Enzymes from the Protein Data Bank (PDB) featuring crystallized structures that bind glycerol were also assessed.

The amino acid residues at each enzyme's active site that recognize glycerol were retrieved from the PDB database. The molecules were obtained in .pdb format for molecular docking, and the binding affinities with glycerol were assessed. Selections were made based on those demonstrating more negative energy, containing fewer than ten amino acids in each active site, and having a resolution below -3 Å (Diagram 1).

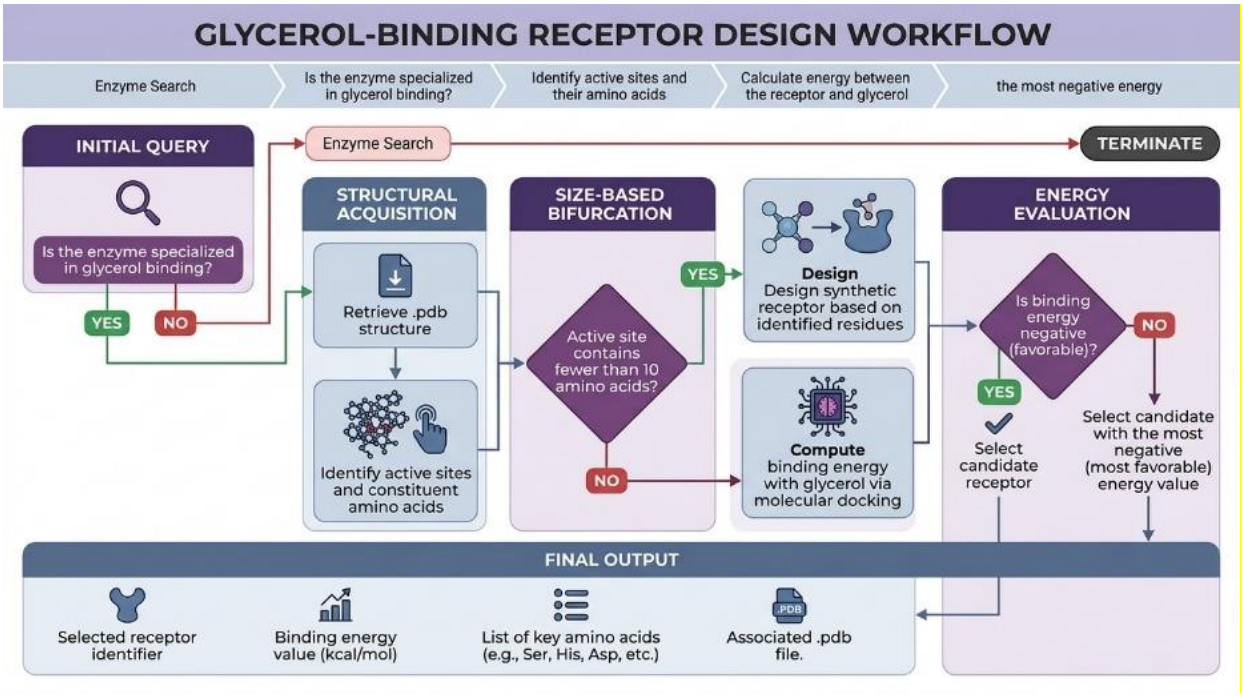


Diagram 1: Enzyme selection workflow

2.2. Molecular Docking

This procedure aimed to calculate the affinity energy (kcal/mol) of enzymes and receptors for glycerol. The initial step involved preprocessing the molecules according to the established protocols in AutoDock Tools. This included removing water molecules from files downloaded from the Protein Data Bank, adding polar hydrogens to the enzymes, and applying Gasteiger charges [19]. After completing these steps, the Grid Box tool in AutoDock Tools was used to define the coordinates for ligand coupling on the macromolecules. The grid box coordinates and the size of the box plot are described in the supporting information (SI). Next, affinity between the analyzed molecules was generated using AutoDock Vina. Each calculation was performed in triplicate with two replicates to ensure data reliability and repeatability. (Table 1SI to Table 14SI) As is well known, the program conducts multiple couplings with randomized ligand positioning on the macromolecule, identifying those that exhibit the highest affinity [20].

2.3. Designed peptide receptors

After selecting the active sites of the enzymes with the highest affinity for glycerol, Avogadro® software was used to design the receptors [21]. Initially, the designs focused solely on amino acids linked by peptide bonds. The use of the MMFF94s force field (Merck Molecular Force Field Variation) for the initial geometric optimization of the designed peptides is based on three considerations. First, the studied peptides are short sequences, whose conformational behavior approximates that of small organic molecules for which MMFF94s was parameterized. Second, optimization with MMFF94s constitutes a pre-optimization step aimed at generating reasonable starting conformations for subsequent molecular docking with AutoDock Vina, which employs its own force field based on AMBER during docking. Third, the gradient value of 0.0000 reported by Avogadro indicates that the geometry has reached an appropriate local energy minimum as a starting point. This strategy reduces computational cost without compromising the quality of the final poses evaluated in the molecular recognition stage [21].

2.4. Determine the interaction distance between the enzyme-glycerol complex and the designed receptor-glycerol complex

Initially, the Autodock software processed the macromolecules, as explained in the earlier molecular docking section. With the coordinates and grid box dimensions available, the enzyme and receptor were defined as rigid molecules within the software. Autodock Vina then calculated the affinity of these molecules with glycerol. The docking results were imported into the software, and using Autodock Tools, the distances between the molecules were measured in angstroms. The Autodock platform visually highlighted the amino acids interacting with glycerol using green spheres. To determine the distances between the evaluated molecules, the average affinity for each molecule was calculated, considering all amino acids that interact with it. This entire procedure was repeated twice across three replicates, mirroring the process described earlier in the molecular docking section.

3. RESULTS AND DISCUSSION

The selection and analysis of enzymes were based on their affinity for glycerol. A literature review identified proteins, primarily enzymes, associated with triglyceride synthesis or the recognition of glycerol. The details of these proteins are outlined in Table 1, which includes PDB nomenclature, standard molecule names, organism information, and resolution data from the Protein Data Bank.

Table 1. Suggested compounds for peptide receptor design

PDB	Proteins	Resolution Å	Organism
4ZYO [22]	Stearoyl-CoA Desaturase	3.25	*Homo sapiens*
3RQS	Hydroxyacyl-CoA dehydrogenase	2.00	*Homo sapiens*
3KK6 [23]	Cyclooxygenase-1	2.75	*Ovis aries*
6N2W [24]	5-Lipoxygenase	2.71	*Homo sapiens*
1KQ3 [25]	Glycerol dehydrogenase	1.50	*Thermotoga maritima*
2W40 [26]	Glycerol kinase	1.49	*Plasmodium falciparum*
3WXL [27]	Glycerol kinase	1.90	*Trypanosoma brucei gambiense*
4FAY [28]	Glycerol-binding protein	1.56	*Limos Lactobacillus reuteri*
6ZQ4 [29]	Glycerol kinase	2.02	*Chaetomium thermophilum*
1COZ [30]	Glycerol-3-phosphate cytidylyltransferase	2.00	*Bacillus subtilis*
1EVY [31]	Glycerol-3-phosphate dehydrogenase	1.75	*Leishmania mexicana*
6UDE	Glycerol kinase	1.90	*Elizabethkingia anophelis* NUHP1

The enzymes contain various sites that interact with glycerol. The affinity between the molecules was evaluated (Table 1 SI). Research indicates that a more negative energy value signifies a greater affinity. Based on these findings, further parameters were analyzed to identify the sites with the highest affinity. These parameters included fewer than 10 amino acids per active site, a resolution of under -3 Å, and a predicted binding affinity of -4.5 kcal/mol for the peptide-glycerol complex, which should be interpreted in the context of the size and chemical nature of the ligand. Glycerol, with a molecular weight of 92.09 Da and only three hydroxyl groups as interaction functionalities, cannot establish the number of non-covalent contacts necessary to achieve affinities comparable to those of drug-sized ligands. Natural enzymes that recognize glycerol as a substrate (glycerol kinases) have Km values in the micromolar to millimolar range, corresponding to binding energies of approximately -4 to -6 kcal/mol. Therefore, a predicted affinity of -4.5 kcal/mol is consistent with the physiological molecular recognition of

this metabolite and represents a favorable peptide-ligand interaction [32]. Twelve sites were selected, as detailed in Table 2, which lists the enzyme names, the corresponding amino acid sequences using single-letter codes, the calculated average affinity, and the standard deviation based on six data points. The sites are numbered in the table according to their respective receptor shapes.

Table 2. Chosen enzymes for creating peptide receptors

Receptor	Enzyme PDB	Amino acid sequence	Average affinity (kcal/mol)	Standard deviation
1.0	6UDE	REWYDQF	-4.5	0.3
2.0		RGTGAISGA	-4.6	0.3
3.0	2W40	F	-4.6	0.0
4.0		TFN	-4.5	0.0
5.0	4FAY	AGHEEC	-4.9	0.1
6.0		GAPGHEE	-4.8	0.1
7.0		TGVNH	-4.8	0.1
8.0		RDGG	-4.8	0.1
9.0		RD	-4.7	0.0
10.0		R	-4.7	0.0
11.0		GAGPTGHEE	-4.8	0.0
12.0	3KK6	HY	-4.7	0.0

A peptide receptor (4.0 Table 2) comprised of three amino acids was constructed and evaluated to examine how the amino acid sequence affects a receptor's affinity for glycerol. The receptor's sequence was modified in three distinct ways, as detailed in Table 3. A statistical analysis, conducted at the 95% confidence level and an alpha level of 0.05 using the Fisher LSD test, revealed a significant difference among the three sequence variations. This finding suggests that the receptor's affinity is closely linked to the specific arrangement of its amino acids.

Table 3. Energy affinities between receptors featuring modified amino acid sequences.

Amino acid combination	Average affinity energy (kcal/mol)	Standard deviation
Thr-Phe-Asn	-2.0 a	0.0
Phe-Asn-Thr	-2.1 b	0.0
Asn-Thr-Phe	-2.2 c	0.0

* Different letters in each column indicate significant differences arising from the altered order of amino acids, with a confidence level of 95% and an alpha of 0.05.

3.1. Creating peptide receptors for glycerol recognition as a substrate

The designed receptors utilized peptide bonds for binding, based on the amino acid sequences of selected active sites, as shown in Table 2. Table 4 presents the molecular docking results between the 12 peptide receptors and glycerol. However, these findings are less negative than those observed in the enzyme-ligand interactions (Table 2). This indicates that glycerol, a smaller molecule, interacts in a manner that diminishes stability and interaction strength. Consequently, modifications were made to the receptors to improve their affinity for the glycerol substrate.

Table 4. Affinity energies between designed receptors and glycerol

Receptors	Average affinity (kcal/mol)	Standard deviation
1.0	-2.5	0.0
2.0	-2.5	0.0
3.0	-1.3	0.0
4.0	-2.1	0.0
5.0	-2.3	0.0
6.0	-2.1	0.1
7.0	-2.0	0.0
8.0	-2.1	0.0
9.0	-1.6	0.0
10.0	-1.3	0.0
11.0	-2.4	0.0
12.0	-1.9	0.0

3.2 Structural modifications

Although the affinity between the molecules increased, the energy level remained insufficient. To enhance it, an initial structural modification was introduced by incorporating methylene bridges between peptide receptor amino acids [17, 18]. This is illustrated in Figure 1, where the bridges are indicated in blue.

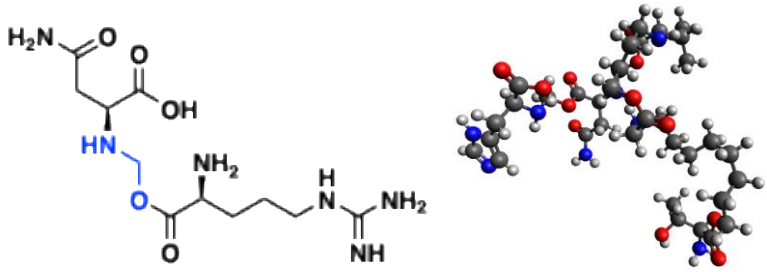


Figure 1. Increase in molecular volume through methylene bridges.

Ten selected receptors containing multiple amino acids in their sequences were modified. However, the number of methylene groups varied among the molecules. A gap of 100 amino acids between the amino acids in the enzyme's sequence would necessitate the addition of 10 methylenes. Consequently, Table 5 presents the receptor structure and the average affinity (3D Representation SI).

Table 5. Affinity of receptors modified by methylene bridges.

Recep-tor	Receptor sequence	Average affinity (kcal/mol)	Standard deviation
1.0	R(CH_2)E$(CH_2)_2$W$(CH_2)_3$Y$(CH_2)_{11}$D(CH_2)Q$(CH_2)_2$F	-2.9	0.0
2.0	R$(CH_2)_{28}$G(CH_2)T$(CH_2)_4$G(CH_2)A(CH_2)I$(CH_2)_2$S$(CH_2)_8$G(CH_2)A	-2.0	0.0
4.0	T(CH_2)F(CH_2)N	-2.4	0.0
5.0	A(CH_2)G$(CH_2)_3$H$(CH_2)_4$E$(CH_2)_6$E(CH_2)C	-2.1	0.0
6.0	G(CH_2)A(CH_2)P$(CH_2)_3$G(CH_2)H$(CH_2)_4$E$(CH_2)_6$E	-2.5	0.0
7.0	T$(CH_2)_8$G$(CH_2)_4$V(CH_2)N(CH_2)H	-2.5	0.0
8.0	R(CH_2)D(CH_2)G(CH_2)G	-2.3	0.0
9.0	R(CH_2)D	-1.9	0.0
11.0	G(CH_2)A(CH_2)G(CH_2)P$(CH_2)_3$T(CH_2)G(CH_2)H$(CH_2)_4$E$(CH_2)_6$E	-3.0	0.1
12.0	H$(CH_2)_{18}$Y	-2.1	0.0

This section reports affinity ranging from -2.0 to -3.0 kcal/mol. Compared to the energies from the interaction between glycerol and receptor compounds made solely of amino acids, the released energy was -0.5 kcal/mol higher. This indicates that increased volume and steric effects enhance glycerol's affinity; however, achieving this affinity still relies on the enzyme-ligand interaction.

Earlier research, including contributions from other authors, has focused on creating small-molecule receptors for dopamine, serotonin, and cortisol [17, 18, 33]. Modifying amino acid trimers by varying their amino acid composition and steric volume improved energy affinity and selectivity. This prompted us to design peptides that included these features. We designed new molecules by adding 30 styrene units to the amino and carboxyl termini of the amino acid trimers, thereby enhancing their affinity for glycerol. Moreover, we combined amino acids with higher affinity. Table 6 details the affinity observed.

Table 6. Glycerol's affinity with the newly designed receptors.

Aminoacid secuence	Average affinity (kcal/mol)	Standard deviation
PPP	-2.3	0.0
AAA	-2.4	0.0
GGG	-2.3	0.0
HHH	-2.8	0.0
EEE	-2.9	0.0
TTT	-2.6	0.1
RRR	-2.9	0.1
DDD	-3.5	0.0
WWW	-2.6	0.0
YYY	-2.5	0.0
QQQ	-3.2	0.1
FFF	-2.5	0.0
III	-3.1	0.2
SSS	-2.2	0.0
TRR	-3.5	0.0
RTT	-3.7	0.0
RHH	-3.6	0.0

*3D structures in SI

After selecting the highest- affinity receptors, the molecules were further structurally modified by altering their volume or introducing organic functional groups. To assess the impact of these modifications on affinity, a 4x4 Latin square experimental design was implemented, categorizing the changes into two treatments: volume variation and molecular functionalization. The following section outlines the modifications required to determine the optimal combination to achieve the highest receptor affinity.

3.3. Volume modifications:

- Inclusion of styrene
- Inclusion of methylene bridges
- Amino acid chain without any volume modification
- Addition of styrene and methylene bridges

3.4. Added functional groups:

- OH
- NH_2
- CH_2
- OH, around the styrene ring

Figure 2. This depicts the four changes applied to the receptor volumes. These modifications guided the functionalization method for the molecules. Specifically, functionalization occurred directly on each amino group of different amino acids, with no volume change observed in the receptors.

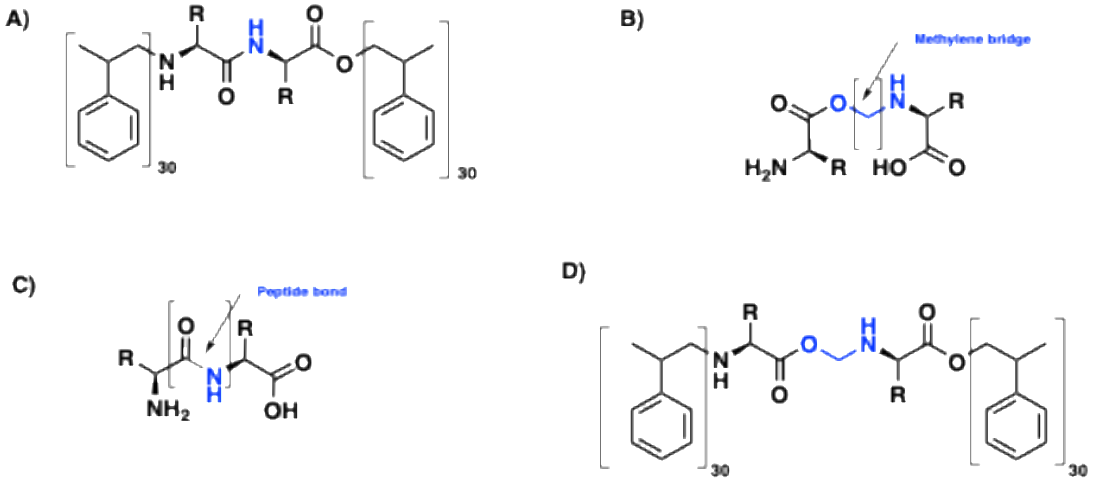


Figure 2. Structural changes resulting from differences in receptor volume: (a) Addition of 30 styrene to the ends of each receptor's amino acid chain; (b) Incorporation of methylene bridges among amino acids; (c) Use of the amino acid chain without alterations in volume; (d) Addition of 30 styrene at the ends of the amino acid chain, along with methylene bridges connecting them.

In molecules containing styrene in the chain, whether alone or connected by methylene bridges, functional groups were directly introduced to the first five rings linked to the receptor's ends, as shown in Figure 3.

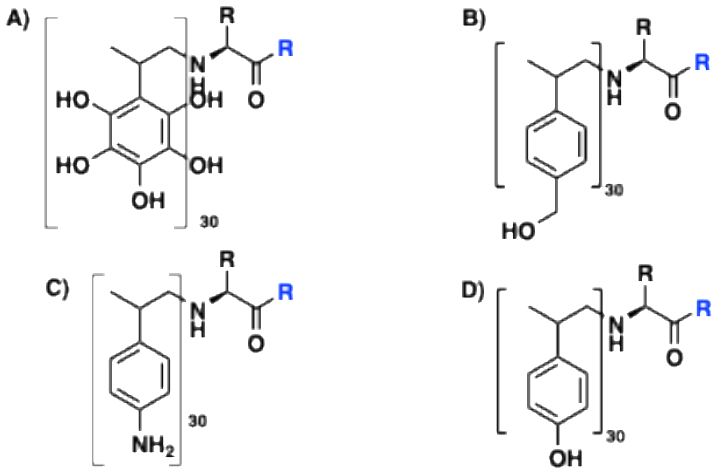


Figure 3. Functional groups modify the volume and properties of styrene-containing molecules. (a) Introducing hydroxyl groups to styrene rings; (b) Placing a methyl hydroxyl at the p-position; (c) Adding an amino group at the p-position; (d) Including a hydroxyl group at the p-position.

On the other hand, functional groups were attached to the amino groups by bonding in molecules composed entirely of amino acids or those with solely methylene bridges. A phenyl group was added to the same amino group while functionalizing styrene with hydroxyl groups. These modifications are depicted in Figures 4 and 5.

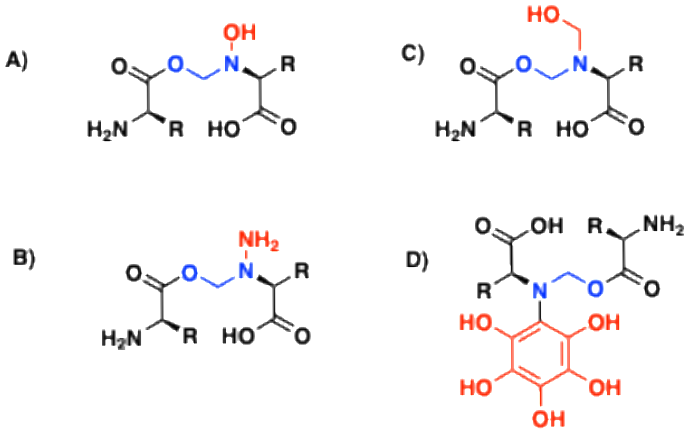


Figure 4. Functionalization of receptors with volume modifications due to methylene bridges. (a) Hydroxyl group; (b) Hydroxyl group linked to a methyl group; (c) Amino group; (d) Functionalization of the ring involving hydroxyl groups.

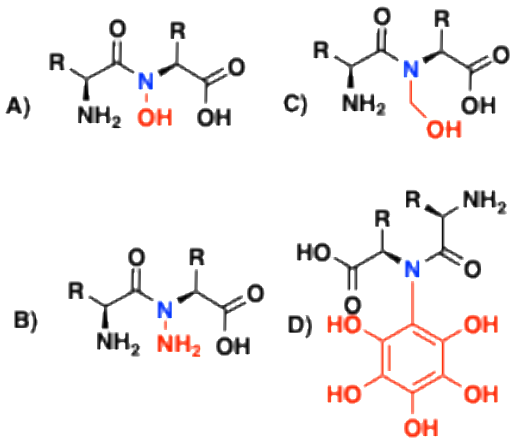
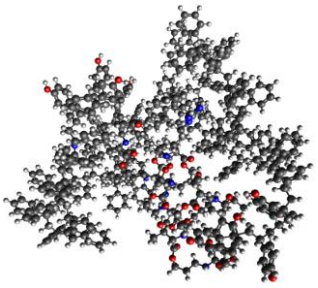
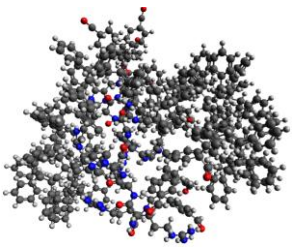
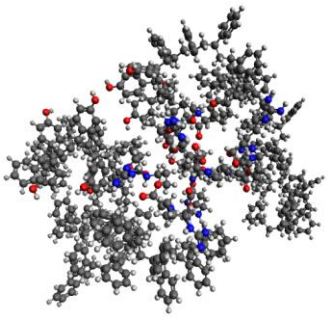
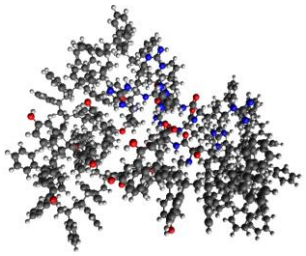


Figure 5. Functionalizing receptors made entirely of amino acids. (a) Hydroxyl group; (b) Hydroxyl group attached to a methyl group; (c) Amino group; (d) Ring functionalization via hydroxyl group.

Ultimately, molecular docking was performed using the constructed receptors to evaluate affinity for glycerol after implementing various modifications in the treatments. A Latin square experimental design was applied to analyze the gathered data. This analysis showed that four configured receptors exhibited the highest affinity, as shown in Table 7. The table illustrates that combining methylene and polystyrene bridges increases molecular volume. At the same time, the inclusion of hydroxyl groups around the polymer rings significantly enhances the molecule's affinity.

Table 7. Methylene bridges and styrene are functionalized in the amino acid sequence to enhance receptor affinity.

Entry	Modifications	Functional groups	Amino acids sequence	Average affinity (kcal/mol)	Standard deviation	3D Visualization
1	30 styrenes	OH-Methyl	RTT	-3.6	0.1	
2	No modification	OH Ring	RHH	-3.7	0.0	
3	30 styrenes + 3 Methylenes	OH Ring	TRR	-4.9	0.0	
4	30 styrenes	OH Ring	TRR	-3.6	0.1	

Based on these findings, the TRR sequence receptor (Table 7 entry 3) exhibited the highest affinity for glycerol. It was enhanced with styrene and methylene bridges for increased volume and functionalized with hydroxyl groups on the styrene rings.

While affinity is crucial in choosing a peptide receptor, the distance and number of bonds formed during receptor-ligand interactions are also significant. The distances from the inter-

action between glycerol and the TRR receptor and those from the superior enzyme were assessed using AutoDock **4FAY**. Figure 6 illustrates the binding interactions of this enzyme with glycerol, represented by green spheres. The ligand (glycerol) is depicted as solid spheres, while the interactions appear as dashed spheres.

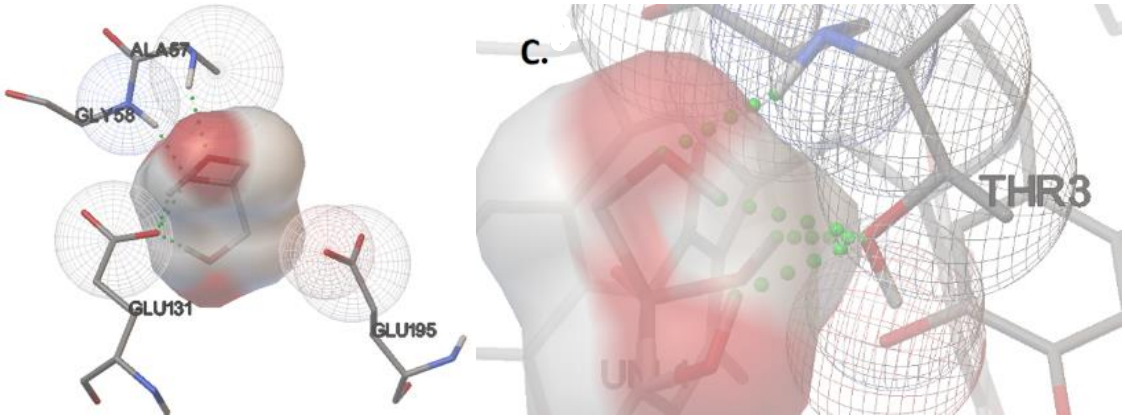


Figure 6. A) The interaction of the 4FAY enzyme with glycerol. B) The interaction of TRR with glycerol.

Five interactions with the enzyme were identified, ranging from 1.780 to 2.157 Å. Additionally, Figure 6B shows the interaction between glycerol and the TRR receptor, which reveals four non-covalent interactions with the threonine amino acid, with distance measurements from 1.744 to 1.925 Å. These measurements indicate a strong interaction between the ligand and the receptor. After determining the two required parameters—affinity for glycerol and interaction distances—we assessed the receptor's selectivity for other biomolecules. Triglyceride esters must be hydrolyzed to identify them as three fatty acid molecules and one glycerol molecule. Thus, we examined the affinity between the most prevalent fatty acids in body metabolism and the TRR receptor, considering these competing molecules during the receptor's recognition phase. The results are summarized in Table 8.

Table 8. The TRR receptor's affinity for various common fatty acids found in the body.

Fatty acids	Average affinity (kcal/mol)	Standard deviation
Docosahexaenoic	-3.6	0.1
Stearic	-2.3	0.1
Oleic	-1.7	0.1
Butyric	-3.0	0.0
Eicosapentaenoic	-2.8	0.0
Caproic	-3.4	0.0
Erucic	-2.1	0.0
Arachidonic	-3.7	0.1

All fatty acids show an average affinity of below -4.9 kcal/mol. This suggests that the TRR receptor interacts more selectively with glycerol than fatty acids.

4. CONCLUSIONS

This study demonstrates that a peptide receptor designed through a structure-based computational approach can recognize glycerol with an affinity of -4.9 kcal/mol and interaction distances of 1.744-1.925 Å, while maintaining selectivity for the most common fatty acids present

in biological samples. The TRR receptor, functionalized with styrene and methylene bridges, serves as a starting point for developing peptide-based biosensors for indirect quantification of triglycerides. However, several limitations must be acknowledged. First, the affinity achieved, although consistent with the molecular recognition of a ligand as small as glycerol, remains below the values reported for enzyme-substrate complexes, indicating that the mimetic capacity of short peptides is still limited. Second, the computational predictions obtained through molecular docking require experimental validation through peptide synthesis, immobilization on sensor surfaces, and evaluation in real biological matrices. Third, the selectivity demonstrated *in silico* against fatty acids must be confirmed in the presence of the complex mixture of biomolecules present in a blood sample.

The future of triglyceride biosensing will depend on the convergence of three areas of innovation. New computational tools with greater predictive capacity, including machine learning algorithms for peptide sequence optimization and enhanced force fields for biomolecular simulations, will accelerate the design of bioreceptors with affinities approaching those of natural enzymes. Advances in surface chemistry and nanomaterials will enable the immobilization of these peptides on electrochemical or optical transducers without compromising their conformational freedom or recognition capacity. Finally, integrating these elements into portable, low-cost microfluidic devices will bring real-time triglyceride monitoring closer to patients, reducing the diagnostic delays that currently limit the management of dyslipidemias. The experimental validation of the TRR receptor and its evolution toward a functional prototype constitute the necessary next steps in this direction.

AUTHOR CONTRIBUTIONS

Conceptualization, J.G-P., D.A-C., and J.V-P; methodology, J.G-P; software, D.A-C., and J.V-P; validation, J.G-P., D.A-C., and J.V-P.; formal analysis, J.G-P., D.A-C., and J.V-P.; investigation, J.G-P., D.A-C., and J.V-P.; resources, J.G-P.; data curation, J.G-P., D.A-C., and J.V-P.; writing—original draft preparation, J.G-P., D.A-C., and J.V-P.; writing—review and editing, J.G-P.; visualization, J.G-P.; supervision, J.G-P.; project administration, J.G-P.; funding acquisition, J.G-P., All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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