Insight into the mechanism of lipids binding and uptake by CD36 receptor

Zineb Tarhda*, Azeddine Ibrahimi

Biotechnology lab (MedBiotech), Faculté de Médecine et de Pharmacie de Rabat, Université Mohammed V, Rabat, Morocco ; Zineb Tarhda –Email : zineb.tarhda@um5s.net.ma; *Corresponding author

Abstract:
The membrane protein CD36 is a member of the class B scavenger receptor family. It plays a crucial role in some cardiovascular pathologies and metabolic diseases. Studying the mechanism of action of CD36 receptor is limited due to the absence of its tridimensional crystallized structure. The molecular docking method has allowed us to perform various simulation of the CD36 receptor interaction with their ligands involved in the development of some diseases. In this work, we predicted a tridimensional structure model of CD36 extracellular domain. In addition, we have achieved several tests of rigid and flexible docking by acting on residues proposed in previous experimental researches as essential in fixing of LFCAs. Furthermore, we have acted on regions that appear a key binding site of LFCAs. The physicoc hemical evaluation indicated the reliability of the proposed CD36 structure used for different molecular docking tests. Based on the docking outcome, we were able to propose the different steps of the mechanism allowing the interaction of fatty acids on CD36 receptor and their penetration into the cell cytoplasm. The obtained results and taking in consideration CD36 receptor as a therapeutic target will help us to suggest the mechanism by which an antagonist may inhibit this receptor by acting on its extracellular domain.

Keywords: CD36-LCFAs interaction, rigid docking, flexible docking.

Background:
CD36 is a transmembrane glycoprotein which consists of a single peptide chain of 472 amino acids and has a molecular weight of 88 kDa. CD36 belongs to the class B scavenger receptor family, which includes the receptor for selective cholesteryl ester uptake, scavenger receptor class B type I (SR-BI), and lysosomal integral membrane protein 2 (LIMP-2). The CD36 receptor has a significant role in different cardiovascular diseases **[1]**. This implication is due to its power on lipids fixation. Fatty acid transport regulated by CD36 scavenger receptor occurs in variety of cell types, including monocytes/macrophages **[2, 3]**, microvascular endothelial cells **[4]**, cardiomyocytes **[5, 6]**, adipocytes and skeletal muscle cells **[7, 8]**.

The role of CD36 protein is described in the binding and uptake of long chain fatty acids, and thereby in the control of fatty acid metabolism **[9, 10, 11]**. However, CD36 binding site for long chain fatty acids is poorly mapped to the 127-279

region of the extracellular loop **[12]**. The CD36 also binds to oxidized lipoproteins, especially oxLDL and promote their internalization via the endocytosis pathway. The residues from 155 to 183 are the important functional domain of the CD36 involved in the fixing of the oxLDL, and Lys-164/166 is involved in this interaction. A study showed that PLox bind to CD36 in domain between amino acids 120-155. While, other data reveals that the binding site of PLox on the CD36 is located in the sequence at amino acids 157-171, and the two Lys 164/166 are indispensable for this interaction **[13-16]**. The elucidation of the three-dimensional (3D) structure of the CD36 receptor is indispensable to understand the mode of its interaction with lipids and the mechanism by which this receptor is involved in lipid transport. 3D structure of CD36 has not been experimentally solved via nuclear magnetic resonance (NMR) and/or X-ray crystallography due to the high size of this protein and their transmembrane position. Hence, the structure-function relationship of this receptor is unclear.

Figure 1 : Sequence alignment between CD36 and LIMP-2 extracellular domain

The purpose of our study is to know and understand the fixing mechanism and absorption of lipids by the CD36 receptor scavenger. Thus we were predicted for the first time the CD36 extracellular domain 3D structure by homology modeling method using the lysosomal membrane protein integral 2 (LIMP-2) as a template. Thereafter, we studied the interaction between this protein and some long-chain fatty acids (LCFAs) using rigid and flexible docking approaches.
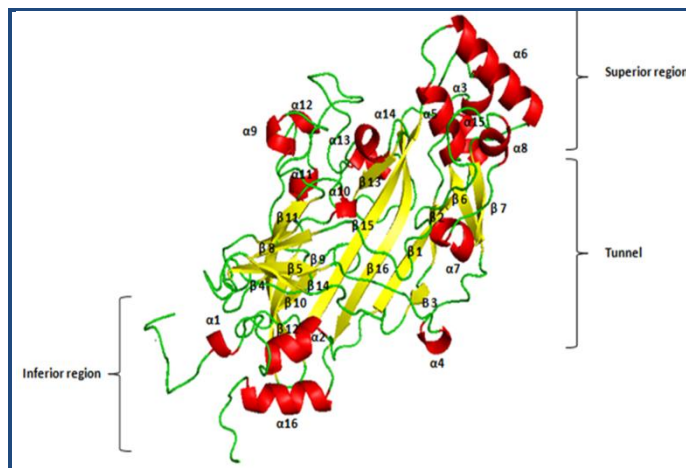

Figure 2: Predicted 3D structure of CD36 extracellular domain

Methodology:
Homology modeling of CD36 receptor
In this study, we have used the crystallized structure of LIMP-2 extacellular domain **[17]** (PDB code: 4F7B) as a template to predict the 3D structure of the CD36 extracellular domain (CD36-ED) using the homology modeling method. We used the Blastp algorithm (protein-protein BLAST) **[18]** to establish the sequence alignment between the extracellular domains of CD36 [29-440 aa] and LIMP-2 [34-429 aa]. The algorithm parameters exploited are those existing by default and this step was performed to extract the locations where the residues are identical or very similar. The MODELLER (version 9v11) program based on homology modeling method was used for

generate the 3D structure of CD36-ED **[19].** To evaluate and validate the CD36-ED structure, three programs were selected, Verify3D **[20],** Procheck **[21]** and Errat **[22].**
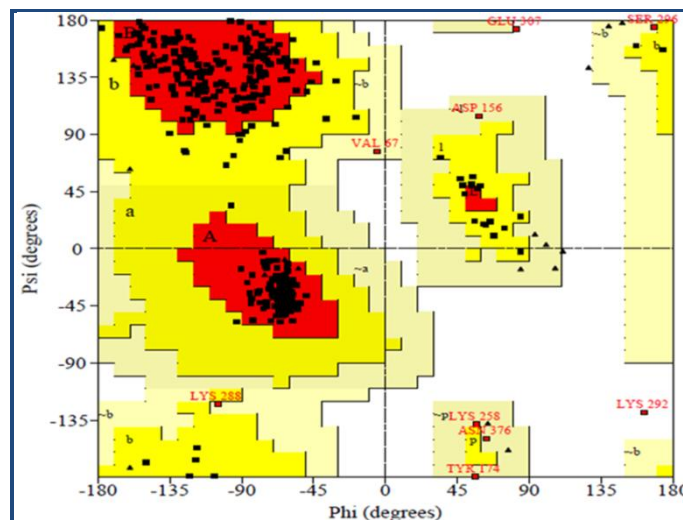

Figure 3: Ramachandran plot graph of CD36 extracellular domain

LCFAs-CD36 interactions studies
The LCFAs used were palmitic acid saturated with 16 C, oleic acid monounsaturated with 18 C and arachidonic acid polyunsaturated with 20 C. The PDB files of ligands were downloaded from the database PubChem structure Search **[23]**. Discovery Studio program chemical modification **[24]** and Chimira **[25]** were used, to examine the role of charged Lys-164 residue on the surface cover of CD36-ED and selective mutagenesis. We have obtained three essential structures of CD36-ED. The CD36-I with a Lys-166 neutralized by acetylating and the CD36-II contains Lys-164 and Lys-166 acetylated. The third one was the CD36-III in which one mutation was generated by changing the Lys-164 positively charged by a negatively charged residue Asp, Lys-164/Asp-164 the Lys-166 is still acetylated. Two types of Docking, rigid and flexible using Autodock Vina were used **[26].** For the Rigid docking between LCFAs and CD36-ED, a docking box which surrounds the entire of the protein was used. Equally, we have performed a second rigid docking with a small box which occupies just the cover loop of CD36-ED. The flexible docking was used to confirm the role of Lys-164 in LCFAs binding by putting its side chain in flexible mode. Side chains of all the residues of the CD36 receptor portal were made Flexible to check the mechanism by which CD36 receptor cover proceeds during the interaction with LCFAs.

Results:
Homology modeling of CD36 receptor
The CD36 superfamily of scavenger receptor proteins consists of SR-BI, LIMP-2 and CD36 and known for its specificity for the lipids binding and uptake **[27].** It was established that the extracellular domain of LIMP-2 is identical (36%) to that of CD36 **[28]**.

Figure 1 elucidates the result of sequences alignment of CD36 and LIMP-2 which have nearly the same lengths with a query cover of 96%. According to BLAST results, the extracellular

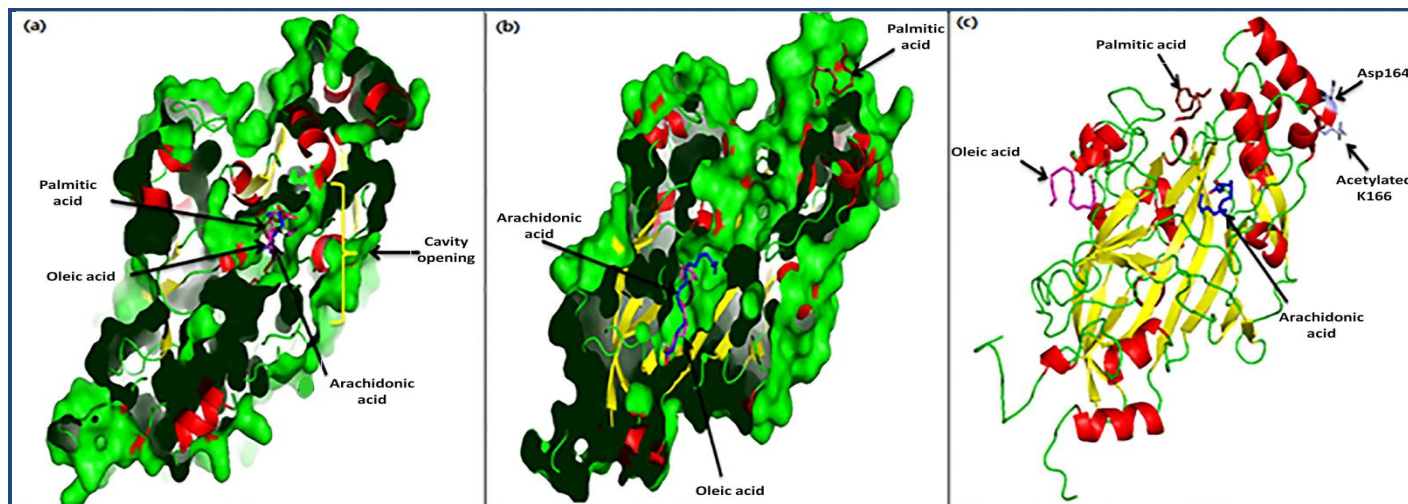domain of CD36 [29-440 aa] is identical by 36% with 2-LIMP [34-429 aa].

Figure 4: Interactions between CD36 and LCFAs: **a)** Location of the LCFAs in the cavity opening of CD36-I; **b)** Affinity of LCFAs to the cavity opening and the cover of the CD36-II; **c)** Interaction between LCFAs and CD36-III on their outer surface.

Figure 2 shows the Pymol visualization of the predicted CD36-ED structure which revealed the presence of 16 α-helix and 16 antiparallel β-strands fragments. The predicted structure can be divided into three regions: The superior region constituted by five α-helices including the two longest helices (α5 and α6) separated by a turn. The helix-turn-helix forming a loop was named the cover; The tunnel comprises the majority of β-strands in the CD36-ED and occupied a large volume; The inferior region which is the domain of the CD36-ED adjacent to the cytoplasmic membrane is composed of three α-helices (α1, α2 and α16) representing the N- and C-terminal of the CD36-ED.
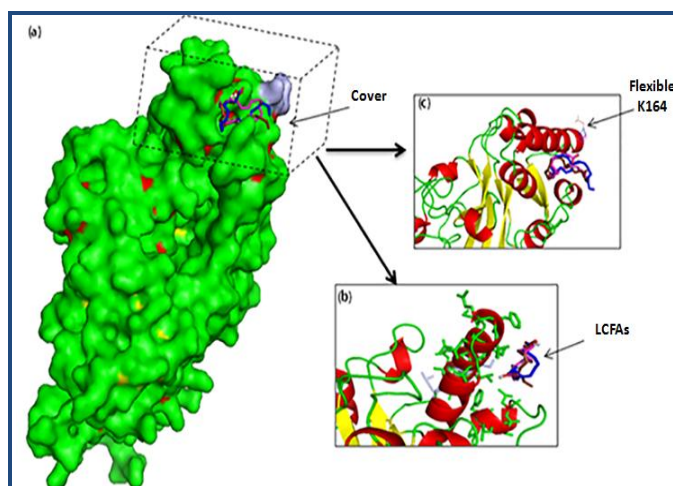

Figure 5: Binding affinity between the cover of CD36 and LCFAs using rigid and flexible docking. **(a)** Location of LCFAs on CD36 using a small box. **(b)** Hydrophobic affinity of LCFAs to the CD36 cover. **(c)** Simulation of CD36-LCFAs interactions by acting on the side chain of K-164.

Verify3D analysis showed that 82.81% of CD36-ED residues have an average score of 3D-1D> 0.2 confirming the compatibility of the 3D structure and the primary sequence of CD36-ED. **Figure 3** of the Ramachandran plot graph shows that 97.5% of CD36-ED residues were distributed in the most favored regions and additional allowed regions and only two residues were on the disallowed regions. Eventhough the overall quality factor obtained by the program Errat of the CD36-ED 3D structure is good by 62.531. To determine the mechanism of CD36 receptor interaction with LCFAs, this validated structure was used to realize molecular docking

The Binding Mechanism between CD36 and LCFAs studies by rigid docking
Two molecular docking studies done to elucidate the interaction between CD36-ED and LCFAs. The rigid docking based on the concept that the ligand is flexible but the receptor remains rigid allowed visualization of different interactions between LCFAs and CD36-ED. Results showed that CD36-I (K-166 neutralized by acetylating) and CD36-II (contains Lys-164 and Lys-166 acetylated) have successively a total charge of -0.0336 and -1.0336. **Figure 4a & 4b** shows that for the LCFAs interactions with CD36-I and CD36-II using the box which surrounds the entire receptor, the ligands are localized in the inner space of the tunnel or they bind to the receptor cavity entry. The LCFAs remain on the outer surface of CD36-III (contains Asp-164 and the Lys-166 is still acetylated) receptor which has a total charge of -2.0335 **(Figure 4c).**

Figure 5a & 5b show the results obtained after visualization of different interactions between LCFAs and the three types of CD36-ED using a small docking box which just surrounds the cover and demonstrating the LCFAs affinity to the outer surface of the cover helices. The flexible docking by influencing the Lys-164 and using a docking box that occupies the cover has shown that the LCFAs are positioned near to the helices α6 and α8 rich in hydrophobic residues **(Figure 5c).** Hence, the hydrophobic interaction between the CD36 and LCFAs appears to be based on the hydrophobicity of fatty acid carboxylic chain and the hydrophobic residues of the cover. It is worth mentioning that in all the shown docking tests, the energy of affinity between LCFAs and CD36 remain very low and not exceeding -5.7 kcal / mol.
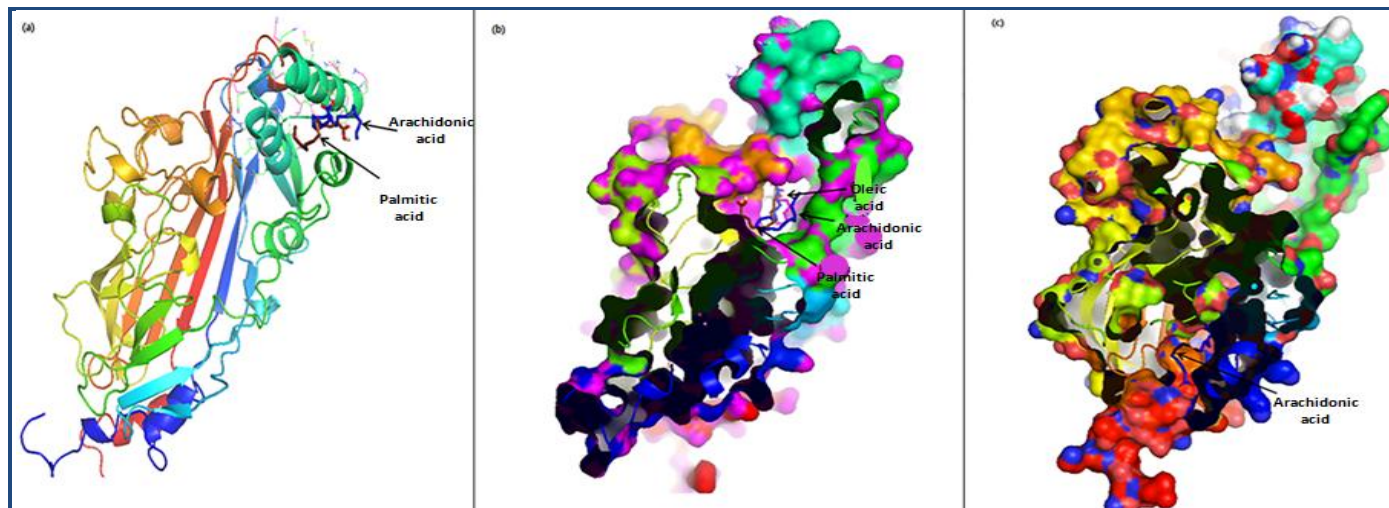
Figure 6: The CD36-LCFAs interaction results of flexible docking by acting on the side chains residues of the CD36 receptor portal. **(a)** LCFAs position in the first contact with CD36; **(b)** Penetration of LCFAs into the CD36 tunnel; **(c)** Localization of LCFAs in the CD36 inferior region adjacent to the cytoplasmic membrane.

The Binding Mechanism between CD36 and LCFAs studies by flexible docking

Results of flexible docking shown in **Figure 6** allowed us to propose the mechanism of the LCFAs binding and uptake by the scavenger receptor CD36. Indeed, setting the side chains of all cover residues in flexible mode showed that the ligand penetrates inside the inner space of the CD36 tunnel during the docking between the receptor and LCFAs. It is worth noting that the energy of affinity in this case of flexible docking is slightly increasing to reach -6.8 Kcal / mol.

Discussion:

In this paper, we exploited the availability of the crystal structure of LIMP-2 extracellular domain recently determined to predict 3D structure of CD36 extracellular domain. We were able to show that the generated model of CD36 receptor has a tunnel formed by interconnected cavities. We also demonstrated that the large part of the sequence from 127 to 279 suggested as the binding site of lipids (LCFAs and modified LDL) **[29]** was included in the constitution of the tunnel and the cover helices. The last Tyr and Arg in the sequence of 127- 279 suggested as a key residues of the LCFAs binding are oriented to the outside surface **[9]**. The cavity opening of CD36-ED has an abundance of hydrophobic amino acids, Leu-200, Phe-201, Pro-203, and a residue positively charged Arg-337. We propose that the aliphatic chain of LCFAs interacts with the receptor by a hydrophobic interaction and its carboxyl group (COOH) interacts with Arg-337 by electrostatic interaction.

Molecular docking studies of the CD36-ED and LCFAs interaction gave important information about the mechanism of CD36 in response to lipids. Indeed, the position of ligands on the external surface of the receptor can be explained by the hydrophobicity and the electropositivity of the residues on the binding site such as the Lys-223 and Lys-316 of the region interacting with LCFAs. The determined cover characterized by seven hydrophobic residues (Phe-153, Val-154, Met-156, Ile-157, Leu-158, Leu-161, Ile-162) was oriented towards the external surface. Since, we determined near the cover, a sequence rich in hydrophobic residues (Pro-185, Phe-186, Leu-187, Leu-189, Val-190, Pro-191 and Pro-193), we propose that LCFAs binds between the sequence mentioned above and the helix α5 of the cover confirming that carboxylic part of LCFAs regulate the recognition step with the CD36 receptor. Given that the results of rigid docking were insufficient to explain accurately the mechanism of interaction between the lipids and the CD36 receptor, we realized the flexible docking by acting in a first step on the Lys-164 and then on the sequence of the CD36 cover. The LCFAs binds to the cover by the hydrophobicity of the majority of these residues and the electropositivity of Lys-164 with which LCFAs interact reversibly. The flexible docking between CD36 and LCFAs in which we put the side chains of the cover residues in a flexible mode allowed a ligand penetration within the receptor tunnel

When we have used the Lys-164 in a flexible mode during docking between CD36 and LCFAs we have got a position of LCFAs just near the helix (α5) rich in hydrophobic residues. Then, we deduce that the interaction between CD36 and LCFAs is mainly hydrophobic. This state depends certainly to the hydrophobicity of the carboxylic chain of fatty acids and the residues of the cover. The pocket of CD36-ED plays a crucial role in the absorption as a tunnel terminated by the two helices of the N-terminus and C-terminus. Hence, the LCFAs will pass through the tunnel and get in the plasma membrane in order to penetrate into the cytoplasm.

The general mechanism by which the CD36 receptor fixes and directs LCFAs into the cytoplasm of cells could be as follow. The first interaction between ligand and the receptor cover cause a movement of this cover and subsequently the opening of the CD36 receptor which facilitates the ligand entry into the hydrophobic pocket. The α5 and α6 helices are the CD36 receptor portal and their conformational change plays a crucial role in the LCFAs fixation. Targeting CD36 receptor scavenger especially its portal which preventing the conformational change of the receptor and the uptake of LCFAs. Eventually, the Sulfo-N-succinimidyl oleate (SSO) molecule may act as an antagonist of CD36 making it as a promising avenue for the

treatment of same disorders. The SSO molecule has an affinity to the Lys-164 and an inhibitory effect on CD36.But it is unable to enter into the cavity of the CD36 receptor in order to reach cytoplasmic membrane, it satisfies by the recognition step with CD36 in which the sulfonate group of SSO bound irreversibly with the primary amine group of Lys-164. This interaction prevents the binding of LCFAs and oxLDL with CD36. The chemical interpretation of the reaction between SSO and Lys-164 was already mentioned **[30].** A covalent bond created between the amine group of Lys-164 that is positively charged and sulfonate group which is negatively charged. The N-amide oleayl protein is the result of this reaction. The covalent bond may be described by quantum mechanics and molecular mechanics. However, no reliable program is available for predict the covalent interaction between SSO and Lys-164.

Conclusion:
Several studies made CD36 receptor as a therapeutic target for pathologies such us athero-inflammatory disorders. Hence, the importance to generate a three-dimensional structure of this transmembrane receptor and study the simulations of its interaction with its ligands.

In this study, we generated and validated CD36 extracellular domain followed by the structure analysis. The CD36 molecular docking allows us to propose a mechanism for the binding of LCFAs of the scavenger receptor CD36 and the lipids orientation towards the cytoplasmic membrane. The elucidation of the mechanism by which CD36 interacts with LCFAs will lead to propose an antagonist molecule for therapeutic purposes

Competing interests:
I'm Zineb TARHDA, corresponding author of the manuscript, I'm enclosing herewith a manuscript entitled "**Insight into the mechanism of lipids binding and uptake by CD36 receptor**" for publication in 'Bioinformation Journal'. With the submission of this manuscript I would like to undertake that: 1) All authors of this research paper have directly participated in the planning, execution, and analysis of this study; 2) All authors of this paper have read and approved the final version submitted; 3) The contents of this manuscript have not been copyrighted or published previously; 4) The contents of this manuscript are not now under consideration for publication elsewhere; 5) The contents of this manuscript will not be copyrighted, submitted, or published elsewhere, while acceptance by the journal is under consideration; 6) There are no directly related manuscripts or abstracts, published or unpublished by any authors of this paper; 7) My Institute's, Faculty of Medicine and Pharmacy, University Mohammed V, Morocco, representative is fully aware of this submission.

Authors' contribution:
ZT carried out the homology modeling and molecular docking studies and drafted the manuscript. AI corrected the manuscript and has given final approval of the version to be published.

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