

Computer aided study of the oxytocin - receptor complex binding sites

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Research

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**Elisaveta Miladinova^{a, b*}, Peicho Petkov^a, Nevena Ilieva^c and Leandar Litov^a**^a Faculty of Physics, Sofia University "St. Kliment Ohridski", 5 James Bourchier Blvd., 1164 Sofia, Bulgaria^b Section of Biosimulation and Bioinformatics, Center for Medical Statistics, Informatics and Intelligent Systems, Medical University of Vienna, Spitalgasse 23, 1090 Vienna, Austria^c Institute of Information and Communication Technologies, Bulgarian Academy of Sciences, Acad. G. Bonchev St., Block 25A, 1113 Sofia, Bulgaria**CORRESPONDENCE AUTHOR**

Elisaveta Miladinova

Email: e_miladinova@mail.bg

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ABSTRACT

Oxytocin (OT) is a neurohypophysial hormone, which acts both on the peripheral tissues and as a neurotransmitter in the brain. It plays an important role in the control of uterine contractions during labor, secretion of milk and regulates many social and behavioural functions. OT accomplishes its functions via interaction with specific oxytocin receptors (OTRs), which belong to the rhodopsin type (family A) group of G-protein coupled receptors (GPCRs). Increased oxytocin secretion associated with hormonal abnormalities in endocrine glands (thyroid, pituitary) during pregnancy may cause premature birth. Oxytocin receptor antagonists are widely employed for prevention in such cases. Design of these antagonists requires a proper model of the hormone – receptor interaction. However, the 3D structure of the OTR is not known.

The aim of this investigation is to construct a three - dimensional model of the oxytocin receptor, to be used to define the binding sites between oxytocin and its receptor, as a prerequisite for further search for oxytocin antagonists. We employ homology modeling as an efficient technique to generate a family of oxytocin GPCRs structures. The best structure is investigated for its interactions with the hormone oxytocin through molecular dynamics (MD) simulations. The potential oxytocin - receptor binding sites are determined. The structural changes in the receptor induced by oxytocin binding are investigated.

Keywords: homology modelling; molecular dynamics (MD); oxytocin (OT); oxytocin binding sites; oxytocin receptor (OTR); 3D models.

1. INTRODUCTION

Oxytocin is a hormone with a peptide structure, which acts both on the peripheral tissues (hormonal), and as a neurotransmitter in the brain. It is synthesized from neurons located in the hypothalamus [1]. Oxytocin accomplishes its functions via interaction with specific receptor, which belongs to the rhodopsin-type (class I) group of G-protein coupled receptors, by far the largest and most diverse GPCR subfamily [2, 3]. The hormone binds to the transmembrane part of the receptor and thus activates it, causing the start of various intracellular signaling pathways. This leads to an increase in the concentration of Ca^{2+} ions in the cell, actin filaments bind to myosin resulting in contraction of the smooth muscle of the uterus. Increased oxytocin secretion associated with some hormonal abnormalities in endocrine glands during pregnancy may cause uterus contractions and premature birth. In such cases oxytocin receptor antagonists are widely employed for prevention [4, 5], however their efficiency is limited.

As target for many drugs the G protein - coupled receptors are important class of transmembrane (TM) proteins [6, 7]. All members of the GPCRs share structural and functional similarities. They are built from single polypeptide chain, which is composed of seven membrane-spanning α -helices (TM1-7) connected by alternating extracellular (EL1, EL2 and EL3) and intracellular (IL1, IL2 and IL3) loop domains. At all GPCRs the N-terminal part is exposed to the extracellular space and C-terminal tail is located in the intracellular fluid (cytosol) of the cell. The C-terminal portion of the receptor participate in interaction with cytosolic G proteins. An essential disulfide bond, that is formed between two cysteine residues, situated in the TM3/EL1 interface and EL2, plays an important role in the packing and stabilization of the seven TM domains of GPCRs. These cysteine amino acid residues are conserved in almost all receptor structures (Figure 1). The members of the GPCRs differ mainly in the length of the amino acid sequence, their intracellular loops, as well as in length and function of their N- and C-terminal domains. These terminal parts provide specific interactions with the various receptor proteins in extracellular and

intracellular environment. Despite these differences in several subfamilies is found significant sequence homology.

The GPCRs transfer extracellular signals to the intracellular targets by binding the agonistic ligands to their extracellular part. This elicits conformational changes of the receptor and activates the G protein [8, 9].

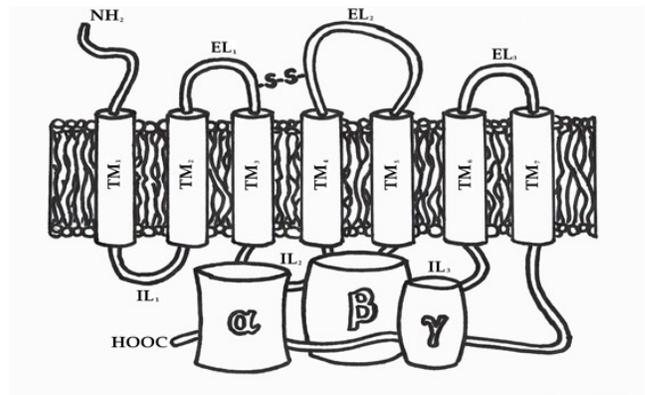


Figure 1. GPCR structure linked to a trimeric guanine-nucleotide binding protein.

There are over 800 unique GPCRs known by now. Based on the degree of sequence homology and functional similarity, these receptors are grouped into five different subfamilies: rhodopsin (family A), secretin (family B), glutamate (family C), adhesion and Frizzled/Taste2. The rhodopsin family is by far the largest and its members are characterized by conserved sequence motifs that imply shared structural features and activation mechanisms [2, 3]. Until now, there is very little structural information available about this subfamily of proteins as they are membrane proteins and thus difficult to crystallize. In order to design rationally an effective drug, which to act on any given target protein, knowledge of the three-dimensional (3D) structure of the target receptor and the binding sites with its ligand is highly desirable [10]. Unfortunately, the 3D structure of the oxytocin receptor is unknown.

The aim of the current investigation is to construct a three-dimensional model of the oxytocin receptor and to define the binding sites by studying complex for-

mation with its natural ligand – oxytocin. The new model will allow the design and the development of more efficient and selective oxytocin-receptor antagonists.

2. MATERIALS AND METHODS

2.1. Homology modelling

Homology or comparative modelling of proteins is a technique that allows the construction of a target protein model based on its amino acid sequence and some known 3D structure of a homologous protein – template [11, 12]. The template structure must be of a reasonable quality – with resolution better than 2.5 Å, and a sequence identity with the target protein of at least 25%. Also the biological function of the two proteins must be similar or at least related [13].

In this study the input sequence of human oxytocin receptor (FASTA format) was retrieved from the NCBI database [14]. The NCBI-Protein BLAST server [15] was used for the search of closest homologue of this target sequence, which contains 348 amino acid residues. Top-ranked template sequences, determined by BLAST, on the basis of optimized E-value of the target sequence, were subjected to multiple sequence alignment. The reciprocal position of the conserved amino acid residues and the alignment score both were used to select the best alignment among all possible ones.

In the present study, the 3D structure of bovine rhodopsin (PDB ID 1JFP [16, 17]) was used as a template. The MODELLER software suit for comparative protein structure modelling [18] was used in the model-building process. More specifically, the sequence alignment was performed with align2d function. The homology models of the human oxytocin receptor were generated with the help of automodel class. The final selection was guided by MODELLER objective function molpdf and the energy profile analysis in terms of DOPE (Discrete Optimized Protein Energy) score. The DOPE potential [19] characterizes the interactions between pairs of atoms and can be decomposed into a score per residue. The energy profile so obtained is useful to detect local regions of high pseudo-energy that usually correspond to errors in the

model.

2.2. Membrane bilayer model

Oxytocin receptor is a transmembrane protein, thus for studying its dynamics it should be considered in complex with the membrane. A membrane bilayer model (consisting of 5 different types of phospholipids) was built for this purpose with the aid of the Membrane Builder tool within the CHARMM GUI web-based graphical user interface [20-22].

2.3. Molecular dynamics simulations

The energy minimization (EM) of the constructed oxytocin receptor structure was performed aiming at structure refinement and stability. The receptor-membrane complex was studied via MD simulations. The simulations were performed with GROMACS 5.0.7 package [23], with CHARMM 36 force field [24]. In all MD simulations a leap-frog integrator was employed. All bonds were constrained by LINCS algorithm. The cutoff radius of Coulomb interactions was set at 12 Å. The protocol comprised initial energy minimization (steepest descent algorithm), followed by equilibration for 375 ps and a production run of 300 ns, with a time-step of 2 fs. The TIP3P water model of solvent was employed. The pressure and temperature were fixed at 1 bar and 310 K by means of Parrinello-Rahman barostat and v-rescale thermostat, respectively. Periodic boundary conditions were applied in all directions. The van der Waals interactions were smoothly switched off at 10-12 Å by a force-switching function [25]. The long-range electrostatic interactions were calculated using the particle-mesh Ewald [26] method. The receptor-membrane system was then subjected to MD simulation (300 ns) with the same settings. The aim of this first simulation was to relax the membrane and solvent residues that form the interface between the receptor model and the rest of the bilayer-solvent environment. Finally, MD simulations of the whole complex membrane-oxytocin receptor-oxytocin were performed. For the energy minimization, the steepest descent method in combination with conjugate gradient method was used. The equilibration simulations in NVT and NPT ensembles with duration 0.5 ns were performed, followed by a production run of 100 ns.

During the NPT equilibration, the structure of the oxytocin - receptor complex was relaxed. The other parameters were the same as for the receptor-membrane simulations.

3. RESULTS AND DISCUSSION

3.1. Homology modelling

The 3D structure of the human oxytocin receptor is not known. We employed homology modeling for its determination. In order to identify the amino acid residues that form the seven transmembrane α -helices of the query protein the sequence of oxytocin receptor was aligned to those of the best template (bovine rhodopsin PDB ID 1JFP). The degree of sequence similarity between the both structures (target protein and template) is important for the accuracy of the predictions through comparative modelling. In our case, the alignment score was 74.3 bits, query coverage was 83% and sequence identity - 22% (obtained by using the BLAST program) [15]. Sequential alignment between the primary amino acid sequences of the target protein (oxytocin receptor) and the template (bovine rhodopsin) was also performed with the MODELLER software package (see Figure 2). The sequence identity was 27%. The members of GPCRs family shared common motifs, which are the extra-cellular disulfide bridge between TM3 and EL2, Asn1.50, Asp2.50, the E/DRY motif (Asp/Glu3.49, Arg3.50, and Tyr3.51), Trp4.50, the two proline residues Pro5.50 and Pro6.50, and the NPXXY motif (Asn7.49, Pro7.50, and Tyr7.53) in TM7. The highly conserved amino acid residues in these important motifs guided the alignment, because they are related to structurally or functionally defining parts of the target protein [2]. The MODELLER align2d function, which takes into account structural information from the template, was employed to perform the sequence alignment. It uses a variable gap penalty function that places gaps in solvent- exposed and curved regions, outside secondary structure segments [27]. Thus, the alignment errors are reduced by about one third compared to those that occur with standard sequence alignment techniques. Based on the alignment with sequence identity of 27%, five homology models were generated. The structure with the lowest val-

ues of the MODELLER objective function molpdf (5399) and DOPE score (-33055), as well as the smallest number of restraints violations was accepted as a prospective 3D model and employed in the further studies (Figure 3).



Figure 2. Alignment of the human oxytocin receptor (query) and bovine rhodopsin receptor (PDB 1JFP) amino acid sequences.

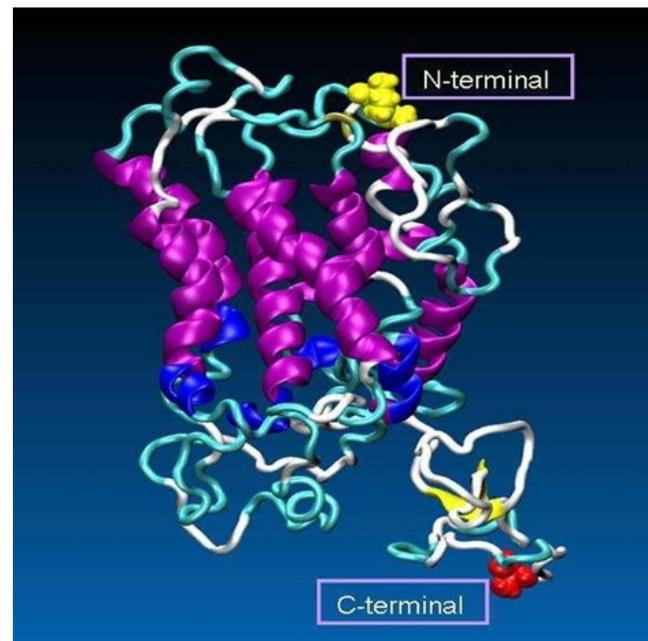


Figure 3. The 3D model of human oxytocin receptor, generated by MODELLER. The secondary structures of the receptor are colored as follows: α -helix (purple), 3-10-helix (blue), extended- β (yellow), turn (cyan), coil (white). Additionally, as VDW spheres are shown N- (in yellow) and C-terminal part (in red) of the receptor.

The geometrical and structural consistency of the constructed model were first evaluated with DOPE assessment function, as implemented in MODELLER, by comparing the energy profiles of the model and the template (Figure 4). The large peak in the template profile in the region of position 70 is most probably related to an active center. Therefore, a possible error indicated by the evaluation function, is not necessarily real.

The degree of sequence identity between target and template, however, is not the only criterion for quality assessment of the model. The choice of template with enough good resolution during homologous modeling also affects the quality of the model. A quantitative measure of the alignment is the root mean square deviation (RMSD) between the two structures,

Eq. 1

$$\text{RMSD} = \sqrt{\frac{1}{N} \sum_{i=1}^N \delta_i^2}$$

where δ_i is the distance between the corresponding superimposed atoms. The alignment is good if $\text{RMSD} < 2 \text{ \AA}$. The template structure (bovine rhodopsin, PDB ID 1JFP) used in our study had a resolution of 1.0 \AA . The model of human oxytocin receptor revealed an excellent agreement with the experimentally determined 3D structure of bovine rhodopsin, with a Ca RMSD of only 1.05 \AA (Figure 5).

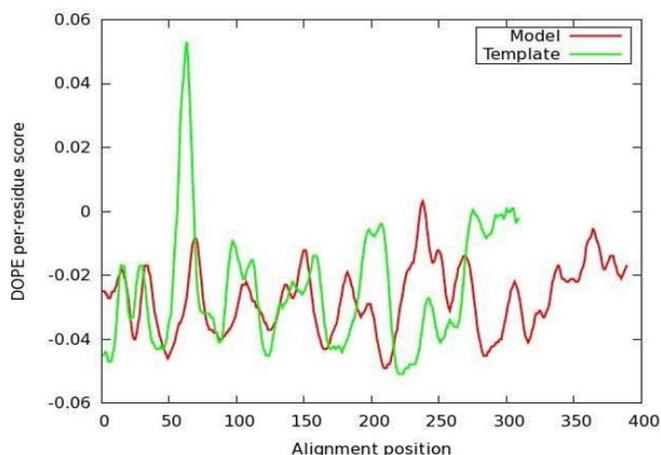


Figure 4. Evaluation of the model of oxytocin receptor. DOPE (Discrete Optimized Protein Energy) score profiles for the model and template.

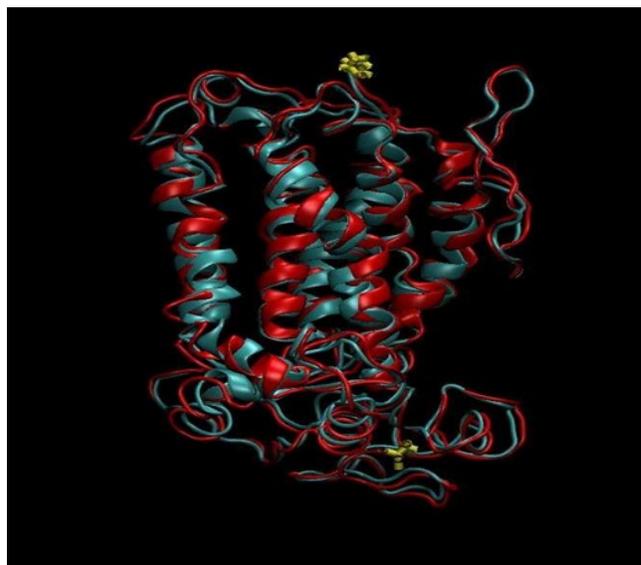


Figure 5. Superimposed structure of the template bovine rhodopsin (red) and the homology model of human oxytocin receptor (cyan). N- and C-terminal parts of both receptor structures are colored in yellow.

3.2. Membrane bilayer model

The conformational stability of GPCRs as membrane proteins strongly depends on their natural environment - the lipid bilayer [28]. For investigation of the binding mechanism of oxytocin to its receptor, a membrane model (smooth uterine muscle) was constructed, consisting of phosphatidyl choline 50.3%, phosphatidyl ethanolamine 24.8%, sphingomyelin 13.8%, phosphatidyl serine 6.4% and cholesterol 2% [29] (Figure 6).

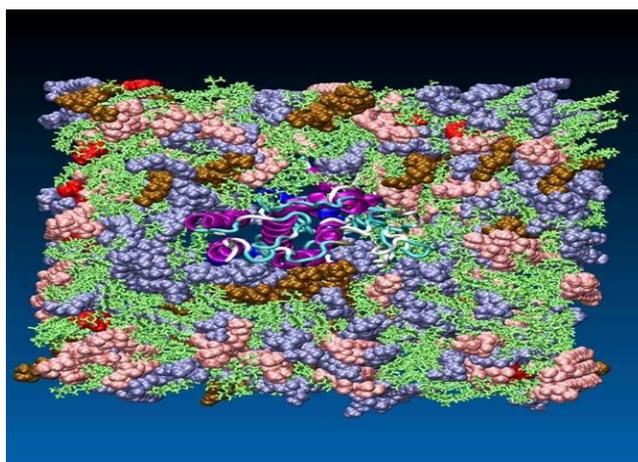


Figure 6. Model of myometrial membrane with a phospholipid composition as follows: phosphatidyl choline 50.3% (green), phosphatidyl ethanolamine 24.8% (blue), sphingomyelin 13.8% (rose), phosphatidyl serine 6.4% (ochre) and cholesterol 2% (red).

3.3. Oxytocin - receptor complex: construction, stability and dynamics studies

The presence of doubly-charged cations, such as Zn^{2+} or Cu^{2+} play an essential role in the specific binding of OT to its receptor. However, it is unclear, whether the metal ions primarily interact with the receptor, OT, or both participants in the process [30]. There are both experimental and theoretical evidence indicating that oxytocin undergoes substantial conformational changes due to interaction with Zn^{2+} [31 - 33]. In the presence of Zn^{2+} , the side chains of Ile3, Gln4, and Asn5 of OT line up to form a cohesive, near-planar surface amenable for coordination with the receptor. These changes permit binding of specific residues in OT with specific residues in its receptor. However, there is no crystallographic information available for oxytocin in complex with metal ion. Thus in order to clarify the role of the divalent ions in the oxytocin – receptor interaction we built a model of such a complex. Stability of the complex was confirmed by a series of molecular dynamics simulations [34]. In our structure the Zn^{2+} ion forms a near-perfect octahedral complex with six of the backbone carbonyl oxygens (associated with Tyr2, Ile3, Gln4, Cys6, Leu8 and Gly9) in the OT (Figure 7), in an agreement with the findings in [35].

Experimental data showed, that residues located in the transmembrane domains, as well as residues in extracellular domains of the receptor are involved in the binding of the peptides like OT and arginine vasopressin (AVP). The amino acid Val115 (helix 3) plays a crucial role in ligand selectivity. The highly conserved Gln residues in the transmembrane domains 3 and 6 create a common agonist-binding pocket to all different subtypes of the rhodopsin-type (class I) receptor family [1].

The oxytocin - metal ion complex is positioned by docking with VMD [36] (VsLab plug-in [37]) within the transmembrane part of the receptor taking into account its experimentally known binding sites. The OT-OTR complex is shown in Figure 8.

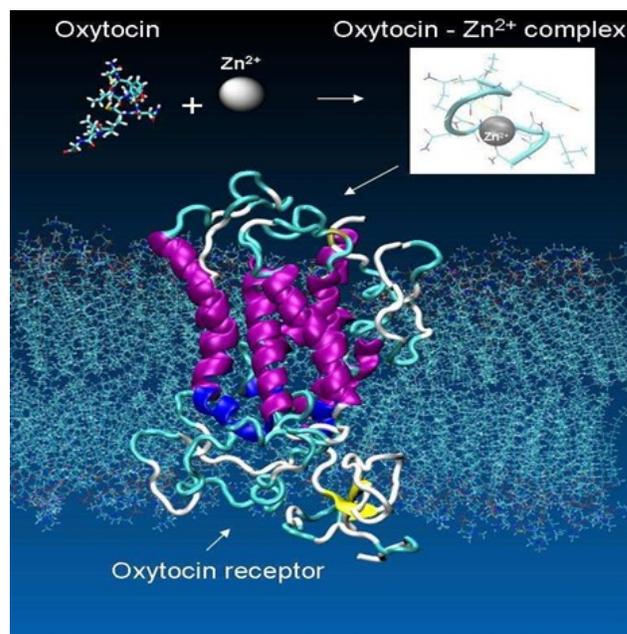


Figure 7. The metal ions as mediator for the specific binding of OT to its receptor.

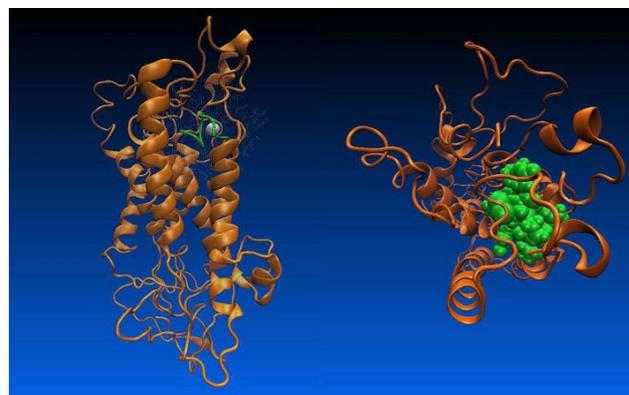


Figure 8. Oxytocin at the binding site of the oxytocin receptor : left - side view; right - top view.

3.4. Determination of the oxytocin binding sites

Both the hydrophobic contacts and hydrogen bonds contribute to the final score of a particular protein-ligand interaction. The tool *g_mmpbsa* [38], implemented in Gromacs was used to calculate binding energies of the oxytocin - OTR complex from MD trajectory. The nonpolar contacts between the receptor and the ligand were determined by decomposing the total calculated binding energy into contributions per residue by the *g_mmpbsa* tool. The hydrogen-bonds analysis of the complex was performed by using the computer algorithm *HBonanza* [39]. The information about the type of each interaction and the amino acids involved in formation of binding pocket

between OTR and its ligand oxytocin is summarised in Table 1.

Table 1 Description of the oxytocin binding sites in the proposed OTR model.

No.	OT	OTR	Intermolecular interaction
1	Cys1	Glu307 (el3)	IONIC HB
2	Cys1	Val115 (helix 3)	VDW ^b
3	Cys1	Tyr200 (el2)	HB ^a
4	Cys1	Gln119 (helix 3)	HB
5	Tyr2	Met123 (helix 3)	VDW
6	Tyr2	Gln119 (helix 3)	VDW
7	Tyr2	Val115 (helix 3)	VDW
8	Tyr2	Tyr200 (el2)	VDW
9	Tyr2	Ile201(el2)	VDW
10	Tyr2	Gln171 (helix 4)	HB
11	Tyr2	Gln171 (helix 4)	VDW
12	Ile3	Phe291 (helix 6)	VDW
13	Ile3	Leu317 (helix 7)	VDW
14	Ile3	Val294 (helix 6)	VDW
15	Ile3	Met123 (helix 3)	VDW
16	Ile3	Gln295 (helix 6)	HB
17	Ile3	Gln119 (helix 3)	HB
18	Gln4	Ile313 (helix 7)	VDW
19	Gln4	Val314 (helix 7)	VDW
20	Gln4	Leu317 (helix 7)	VDW
21	Asn5	Trp300 (el3)	VDW
22	Asn5	Ile312 (helix 7)	VDW
23	Asn5	Ile313 (helix 7)	VDW
24	Asn5	Phe311 (helix 7)	VDW
25	Asn5	Ile313 (helix 7)	HB
26	Asn5	Phe311 (helix 7)	HB
27	Cys6	Tyr200 (el2)	VDW
28	Pro7	Thr202 (el2)	VDW
29	Pro7	Tyr200 (el2)	VDW
30	Leu8	Ile201(el2)	VDW
31	Leu8	Ile204 (helix 5)	VDW
32	Leu8	Trp203 (el2)	VDW
33	Leu8	Thr202 (el2)	VDW
34	Gly9	Gln295 (helix 6)	VDW
35	Gly9	Ser298 (el3)	VDW
36	Gly9	Trp297 (el3)	HB
37	Gly9	Val294 (helix 6)	HB

^a HB, hydrogen bonding interaction.

^b VDW, van der Waals attractive interaction.

In total, 21 binding site residues were defined as potential interaction points in our model of OTR: Glu307 (el3), Val115 (helix 3), Tyr200 (el2), Gln119 (helix 3), Met123 (helix 3), Ile201(el2), Gln171 (helix 4), Phe291 (helix 6), Leu317 (helix 7), Val294 (helix 6), Gln295 (helix 6), Ile313 (helix 7), Val314 (helix 7), Trp300 (el3), Ile312 (helix 7), Phe311 (helix 7), Ile204 (helix 5), Trp203 (el2), Thr202 (el2), Ser298 (el3) and Trp297 (el3). The conserved residues participating in the binding of OT to its receptor are: Val115 (helix 3), Gln119 (helix 3), Met123 (helix 3), Gln171 (helix 4), Phe291 (helix 6), Leu317 (helix 7), Gln295 (helix 6). The experimental data suggests, the important role of the amino acid isoleucine in the OT for the specific binding of the oxytocin molecule to its receptor [40]. Correspondently, in our model the OTR residues binding to the Ile3 were considered crucial for the OT recognition. These residues are: Phe291 (helix 6), Leu317 (helix 7), Val294 (helix 6), Met123 (helix 3), Gln295 (helix 6) and Gln119 (helix 3). The conserved Gln residues Gln119, Gln171 and Gln295 were determined as important residues for the ligand binding and stabilization of the receptor - ligand complex, which is in agreement with both theoretical and experimental results of other research groups [41, 42, 43, 44].

Molecular modeling of other groups in combination with mutagenesis investigations reveals a large number of potential binding sites in OTR that are essential for ligand binding [1]. The comparison with the binding sites in our receptor model shows the following coincidences: Val115, Gln119, Met123, Gln171, Thr202, Val294, Gln295, Trp300, Glu307, Ile313, Val314, four of them - Gln119, Gln171, Gln295 and Glu301 – are in agreement with the experimental data as well.

3.5. Agonist binding induced conformational changes in the OTR

The receptor molecule can exist in two states (inactive and active), that are in conformational equilibrium. The intracellular portion of the receptor participate in interactions with the G protein. The conformational equilibrium is shifted to active receptor state after binding of the ligand in transmembrane pocket

near to extracellular part. Binding of the agonist (ligand) to the receptor results in conformational changes in its extracellular and intracellular loops, as well as in its transmembrane portion [45 - 48]. A common mechanism of activation, in which participate highly conservative amino acid residues, is characteristic of the GPCRs members. These amino acid residues are located in the intracellular parts of helices TM3 (Arg137, which is part of the E(D)RY motif) and TM6 and form "ionic lock", which hold TM3 and TM6 in their inactive form. The disruption of intermolecular interactions between these residues is a key moment in activation process of the receptor. The observed conformational changes are: screening of the electrostatic interaction between the helices, motion of helices TM6, TM5 and divided of cytoplasmic parts of TM3 and TM6 after breakage of the "ionic lock" between them [49]. According to the polar pockets hypothesis the arginine of the motif E(D)RY is constrained in a pocket, in the inactive receptor conformation.

Conserved polar residues located on TM1, TM2 and TM7 form this pocket. In contrast, in the active receptor state triggered by the agonist, the arginine side chain is shifted outside the polar pocket. The negatively charged aspartic acid in the E(D)RY motif is also important, because from its two states, protonated-(neutral) and deprotonated - (anionic), depends the equilibrium between active and inactive receptor forms. These observations are confirmed by the results of mutagenesis studies and as well as by molecular dynamic simulations [50].

In order to investigate the GPCR activation mechanism two MD simulations were carried out for an unbound OTR (300 ns) and for an agonist (oxytocin) bound receptor (100 ns). From the oxytocin bound trajectory it follows that transmembrane helices TM1, TM2, TM6 (after slight initial rotation in the process of oxytocin binding) and TM7 remain stable (Figure 9). Unfolding of the helical structure of the TM3 (Arg113 – Met123) triggered by oxytocin binding at positions Val115, Glu119 and Met123 is ob-

served. The helical structures in TM4 (Val166-Leu177) and TM5 (Trp203-Leu206) are affected significantly in result of hormone interactions with EL2 (Tyr200, Ile201, Thr202 and Trp203), TM4 (Gln171) and TM5 (Ile204).

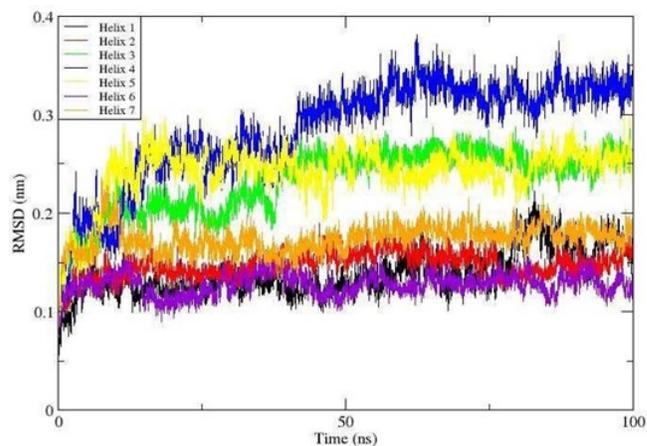


Figure 9. RMSD plot of the 7 transmembrane helices for the trajectory of the agonist-OTR complex. The colors indicate helix 1 (black), helix 2 (red), helix 3 (green), helix 4 (blue), helix 5 (yellow), helix 6 (violet) and helix 7 (orange).

The unbound OTR is characterized by a persistent intra-helix charge-reinforced H-bond between Arg137 (helix 3) of the E/DRY motif and the adjacent aspartate, Asp136. The structural changes in helices TM3 and TM4 result in breakage of this H-bond (Figure 10). At the same time the helical structure of the E/DRY motif is stabilized (Figure 11) and is shifted outside the polar pocket formed by the stable helices TM1, TM2 and TM7 (Figure 12). We do not observe any other changes in the receptor structure in the intracellular region.

Based on these observations we come to hypothesis that the shift of the E/DRY motif influences the G-protein and starts the process of its disintegration from the receptor and the corresponding intracellular process.

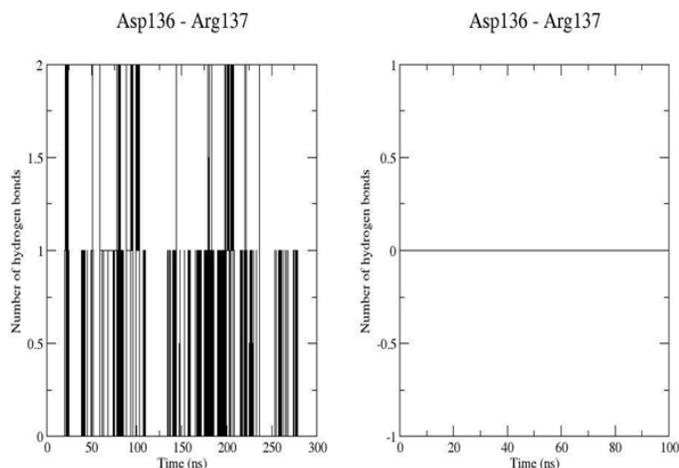


Figure 10. Number of hydrogen bonds between residues Asp136 and Arg137 for the trajectory of the unbound OTR (left) and the trajectory of the agonist-OTR complex (right).

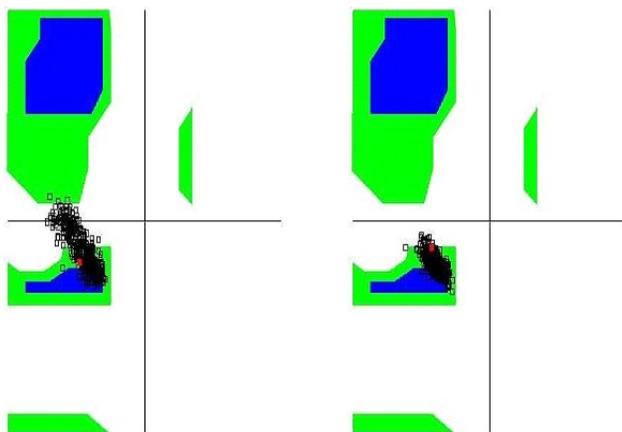


Figure 11. Ramachandran diagram of the Arg137 of the unbound OTR (left) and of the agonist-OTR complex (right).

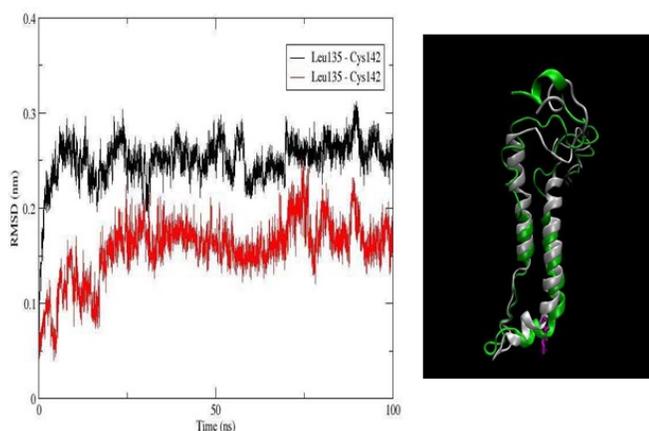


Figure 12. RMSD plot of the amino acid sequence Leu135-Cys142, which includes the E/DRY motif

(Leu135-Asp136-Arg137-Cys138) in the OTR (left). The colors indicate the unbound OTR (black) and the agonist-OTR complex (red). In right panel is shown a superimposed structure of the helix 3 (right) and 4 (left) for the unbound OTR (white) and the agonist-OTR complex (green) - RMSD 3.5 Å. In violet is shown Arg137, which is part of transmembrane helix 3 and E/DRY motif.

4. CONCLUSION

In cases, where there are no crystal structures available of the target proteins, the homology models of GPCRs are highly valuable in drug-designing process [51]. In our study a 3D model of the human oxytocin G-protein coupled receptor was built based on homology modeling. The obtained 3D structure exhibited minor deviations ($C\alpha$ RMSD = 1.05 Å) from the experimentally determined 3D structure of bovine rhodopsin that was used as a template. The additional MD energy minimization of the receptor did not lead to significant deviation from the initial structure. The receptor was embedded in a new improved model of the myometrial membrane (containing five different types of phospholipids). This biomolecular complex was used for investigation of the mechanism of oxytocin binding to its receptor.

A new rigid structure, a complex between oxytocin and a metal ion was built in order to take into account the influence of the Zn^{2+} ions on the hormone binding process. Stability of the complex was confirmed by series of MD simulations. The metal ion changes the 3D structure of the oxytocin and makes possible its binding with the receptor [34].

The complex of oxytocin bound to its receptor embedded in the uterine membrane was used to define the agonist receptor interaction sites and to investigate the mechanism of receptor activation. We found 21 potential binding sites, out of them 11 (essential for the binding) coincide with results of similar investigations by other groups and four of them Gln119, Gln171, Gln295 and Glu301 are in agreement with the experimental data as well. In nine cases was observed formation of H-bonds. The other sites are due to van der Waals interactions between

amino acid residues of oxytocin molecule and the receptor.

The investigation of the structural changes in the receptor induced by the oxytocin binding showed that the transmembrane helices TM1, TM2, TM6 and TM7 are stable when the helices TM3, TM4 and TM5 are partially unfold. The oxytocin – receptor interaction stabilizes the helical structure of the E/DRY motif and shifts it outside the polar pocket formed by the stable helices TM1, TM2 and TM7. This result supports the hypothesis for the critical role of the E/DRY motif in the receptor activation and is consistent with the experimental results for the A GPCRs family [47, 49].

Thus, the validated oxytocin receptor model is suitable for dynamical investigations of the ligand-receptor binding process and therefore, for the design of new highly selective antagonists with possible application for prevention of the premature births.

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Competing interests

The authors declare that they have no competing interests.

List of Abbreviations

AVP	Arginine Vasopressin
DOPE	Discrete Optimized Protein Energy
EL	Extracellular Loop
EM	Energy Minimization
GPCR	G - Protein Coupled Receptor
IL	Intracellular Loop
MD	Molecular Dynamics
OT	Oxytocin
OTR	Oxytocin Receptor
RMSD	Root Mean Square Deviation
TM	Transmembrane

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