Three-dimensional model of ligand-binding domain from glucagon like peptide-1 receptor, molecular target of antidiabetic treatment

Model tridimensional al domeniului asociator al ligandului din receptorul pentru glucagon like peptide-1, țintă moleculară în tratamentul antidiabetic

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Abstract

Using the applications of Schrödinger library a 3D model of ligand-binding domain of the glucagon like peptide-1 receptor (LBD-GLP-1R) was created. The template structures were related receptors, worked out by X-ray/NMR, like GIPR, CRFR and PACAPR. The procedure starts with the graft of GLP-1R radicals on the GIPR backbone. This stage is justified by the fact that the best sequence homology is between these two receptors. The resulted raw structure, which is a GIPR backbone bearing GLP-1R aligned radicals, has some gaps because the GLP-1R chain is longer than the GIPR. These gaps were completed in the following stage by chain building. The dihedral adjustments were made concomitantly with the chain building, based on the Φ-Ψ-Ω torsion angle values of the aligned template residues from the other receptors (CRFR and PACAPR). The resulted model was statistically compared with some models received from 3D structure prediction servers and with the crystallographic solution model of LBD-GLP-1R from PDB. Secondary structure and disulfide analyses reveal in Model the conserved conformational elements (α-helix, β-sheets and the disulfide pattern). A more detailed analysis showed that the Model atomic topologies (Asp-Arg ion-pairs, Trp-Arg-Trp sandwich contacts) are very similar with those of solution model and of server released models.

Key words: *GLP-1 receptor, N-terminal domain, multiple sequence alignment, structure prediction*

Rezumat

Folosind aplicațiile librăriei Schrödinger a fost creat un model tridimensional al LBD-GLP-1R. Structurile matrice au fost receptori înrudiți, rezolvați prin difracție de raze X sau RMN aşa cum sunt GIPR, CRFR şi PACAPR. Procedeul începe cu grefarea radicalilor GLP-1R pe scheletul GIPR. Această etapă este justificată de faptul că între cei doi receptori există cea mai bună omologie secvențială. Structura brută rezultată, care este un schelet de GIPR încărcat cu radicalii aliniați ai GLP-1R, are câteva goluri datorită faptului că GLP-1R are ca-

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tena mai lungă decât GIPR. Aceste goluri au fost completate în următoarea etapă prin construcție catenară. Concomitent cu construcția catenară au fost făcute ajustările diedrice pe baza valorilor unghiurilor de torsiune Φ-Ψ-Ω ale resturilor aliniate ale aminoacizilor matrice din ceilalți receptori (CRFR şi PACAPR). Modelul rezultat a fost comparat statistic cu câteva modele primite de la servere de predicție a structurilor 3D şi cu modelul rezolvat cristalografic al LBD-GLP-1R din PDB. Analiza structurilor secundare şi a punților disulfurice relevă prezența în Model a structurilor α-helix, foaie β-pliată şi pattern-ul disulfuric. Analiza mai detaliată a arătat că topologiile atomice ale Model-ului (perechea de ioni Arg-Asp, contactele din sandwich-ul Trp-Arg-Trp) sunt foarte asemănătoare cu cele aflate în structura determinată cristalografic şi cu cele din modelele elaborate de servere.

Cuvinte cheie: *receptorul pentru GLP-1, domeniul N-terminal, aliniament secvențial multiplu, predicție structurală*

Introduction

Glucagon-like peptide 1 (GLP-1) is an incretin hormone secreted by the intestinal L cells into the blood stream when greasy food, protein hydrolysate, and/or glucose enter the duodenum. The incretin effect is defined by a significantly higher insulin stimulatory effect evoked after an oral glucose load rather than that from an intravenous glucose infusion when plasma glucose concentrations are matched. Another incretin hormone is the gastric inhibitory polypeptide (GIP), secreted by K-cells from the mucosa of the duodenum/jejunum. GLP-1 itself is not suitable as a therapeutic agent for the antidiabetic treatment, because it is quickly destroyed by the dipeptidyl peptidase IV, but its analogues *(*exenatide, liraglutide*)* extend the duration of action retaining the regulatory activity of GLP-1[5]. Peptidase inhibitors (vildagliptin, sitagliptin) are other agents which target the incretin system[1]. These inhibitors enhance the lifetime of endogenous GLP-1. These antidiabetes medications (analogues and enhancers) address both the insulin secretion deficiency as well as the decline in β-cell mass[4]. GLP-1R is a member of the superfamily of the 7-transmembrane-spanning receptors coupled with G-protein. This superfamily is grouped into 6 classes: A (rhodopsin like), B (secretin like), C (metabotropic glutamate/pheromone), D (fungal pheromone), E (cAMP) and the frizzled/ smoothened family. The class B consists of many receptors, as those of calcitonin, corticotropin releasing factor (CRF), GIP, glucagon, growth hormone-releasing hormone (GHRH), parathyroid hormone (PTH), pituitary adenylate cyclase-activating polypeptide (PACAP), secretin, vasoactive intestinal polypeptide. The receptors of GLP-1, GLP-2, GIP, GHRH, glucagon and secretin constitute the glucagon family[6]. Aside from the seven transmembrane helixes, the members of the above mentioned superfamily possess 3 extracellular loops, 3 intracellular loops, an amino-terminal extracellular domain (consisting in a *signal* segment that serves as a marker for endoplasmic reticulum translocation and the ligand-binding domain, LBD), and an intracellular carboxy-terminal domain which mediates the signal transduction via G-protein to adenylyl cyclase. Another feature of N-domains is a disulfide pattern, highly conserved in this family, consisting of three disulfide bonds essential for the LBD conformation[3,8]. It is an important fact that the isolated recombinant N-domains of the GLP-1R[11], PTHR[12], CRFR[14] are able to bind their respective ligands. This property suggests the possibility to use this N-domain for studying ligand binding properties of the whole receptor. Before solutioning LBD-GLP-1R, the huge interest for diabetes treatment with incretin analogues/ mimetics stimulated modeling of LBD-GLP-1R. The purpose of this study is the *in silico* construction of a large part of the N-domain from GLP-1R. This fragment contains mainly the ligand-binding domain of the receptor. This method is different from the algorithms used by the automatic 3D structure prediction servers

like ESyPRED-3D[•], 3D-JIGSAW[••], CPH[•••]. The main distinctive element refers to the dihedral adjustment of the *Model* backbone which is based on the Φ-Ψ dihedral values of some 'evolutionary relatives' of GLP-1R like CRFR and PACAPR. However, the multiple sequence alignment, using ClustalW program, is a common stage for the method and the algorithms of automatic prediction. The attained three-dimensional structure of *Model* is compared by superposition with the solved structure of LBD-GLP-1R obtained by X-ray crystallography and the models received from some prediction servers.

Methods

The visualization of proteins was made using the graphical user interface *Maestro* of the Schrödinger Suite 2007 Library. The energy minimization was performed with *Impact* application in an *Optimized Potential for Liquid Simulations* force field. Measurement and superposition tools were used to calculate the distances between significant atoms, to reveal the contacts between contiguous atoms and to compare statistically two protein backbones. The multiple sequence alignment was carried out on ClustalW v1.81[••••]

Importing and fixing of molecular systems

The template molecules were imported from the Protein Data Bank (PDB) using the import control of *Protein Preparation Wizard* tool. To convert the raw structures into all-atom molecules and to fix structural defects the *Fix structure setup* tool was used. The template molecules used for LBD-GLP-1R construction were the fragments of GIPR, CRFR and PACAPR solved by crystallography (marked with asterisk). They were imported from PDB with the following codes: 2QKH[13], 1U34[7] and respectively 2JOD[16]. The solved structure of LBD-GLP-1R[15] can be accessed, since February 19th, 2008, on www.rcsb.org at code 3C5T. This structure here is named GLP-1R*.

Energy minimization

In order to calculate the electrostatic component of the energy it was used a *constant dielectric* environment with relative static permittivity ε = 78. Since the protein systems are big, *Non-bonded cutoffs* option with *Update neighbor-list frequency* of 10 steps and *Residue-based cutoff distance* of 12 Å was activated. The *Truncated Newton* and *Energy and gradient* were the minimization algorithm and respectively convergence criterion. The values of *Energy change criterion* and *Gradient criterion* were 10^{-7} kcal/mol and respectively 10^{-2} kcal/mol·Å. The long range forces were updated to each 8th step. *Long range force cutoff > d Å* option was set to 10 Å.

Clustal W2 multiple sequence alignment

The sequences introduced to ClustalW Server were the followings: (*i*) sequence 28-131 of human GLP-1R, named Target in *Figure 1*; (*ii*) sequence 29-122 of human GIPR, named GIPR; (*iii*) sequence 39-133 of mouse CRFR, named CRFR, and (*iv*) three sequences of native PACAPR (22-24, 26-88 and 110-143) plus a Gly residue introduced by the substitution of native Cys25 in order to ameliorate the protein stability. This sequence contains totally 101 residues which constitute the extracellular domain of human splice variant PAC1-R-short, named PACAPR. The fragment 89-109 was deleted by engineering from native receptor. The scores of alignment suggest that the most related sequence pair is Target-GIPR, the other pairs having lower scores (*Figure 1*). Based on this result the 3D construction of LBD-GLP-1R began with the graft of the Target aligned radicals upon the GIPR* backbone. The tool was setted at slow/accurate level of Pairwise Alignment.

Construction of a raw LBD-GLP-1R backbone and gap completion

The loading of GIPR* backbone with

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3C5T   28 ATVSLWETVQKWREYRRQCQRSLTEDPPPATDLFCNRTFDEY-ACWPDGEPGSFV  81
2QKH   29 -GQTAGELYQRWERYRRECQETLAAAEPPS-GLACNGSFDMY-VCWDYAAPNATA  80
1U34   39 ------------TLLEQYCHRTTIGN-FSGPYTYCNTTLDQIGTCWPQSAPGALV  80
2JOD   22 --HSDGIFKKEQAMCLEKIQRANELMGFNDSSPGCPGMWDNI-TCWKPAHVGEMV  73

3C5T   82 NVSCPWYLPWASSVP-QGHVYRFCTAEGLWLQKDNSSLPWRDLSECEESKR---  131
2QKH   81 RASCPWYLPWHHHVA-AGFVLRQCGSDGQWG-------LWRDHTQCENPE----  122
1U34   81 ERPCPEYFNGIKYNT-TRNAYRECLENGTWASRVNYSHCEPILDDKQRKYDLHY  133
2JOD   74 LVSCPELFRIFNPDQDMGVVSRNCTEDG-WSEPFPHYFDACGFDEYESET----  122
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Alignment scores: 3C5T:2QKH = 42; 3C5T:1U34 = 21; 3C5T:2JOD = 12

Figure 1. ClustalW multiple sequence alignment of the Target with template sequences. The template residues for dihedral adjustment are highlighted by green background and the conserved residues by yellow. On magenta background there are the Target residues inserted into $Target_{raw}$ to fill in the gaps and on green background there are the chosen template residues from CRFR and PACAPR.

radicals was performed by substitution using *Mutate mode* tool. In this way a raw chain, named $Target_{raw}$, was obtained. It is a GIPR* backbone bearing radicals from Target. The alignment from *Figure 1* reveals some gaps in the pairing of Target with GIPR. These gaps appear because the Target sequence is longer by 10 residues than the GIPR sequence. Three isolated residues and a 7-aa sequence of Target are not superposable with GIPR: Ala28, Thr58, Arg131 and the Gln112-Lys-Asp-Asn-Ser-Ser-Leu sequence. The gap completion with Target residues was performed by chain building in *grow mode* by adding step by step residues maintaining *Grow Direction*, *Joining Geometry* and *Secondary Structure* parameters on the options *forward* (except Ala28), *trans* (except Thr58) and *user defined* (except Ala28), respectively. Ala28 was added with *backward grow* direction, *trans* joining geometry and *alpha helix* secondary structure. Before filling the inner gaps with threonine and 7-aa fragment, the $Target_{raw}$ chain was cleft by deleting a peptide bond with *Deleter* tool. The joining of the inserted Thr58 C-terminus with Asp59 N-terminus, to stick the chain together, was performed by using *Connect & Fuse* tool.

Dihedral adjustments

In *Figure 1* the highlighted residues were chosen considering the hydrophobic and volume similarities between inserted and aligned residues. The hydrophobic criterion was applied on the hydropathy scale derived by Kyte and Doolittle[10]. There was no doubt in the choosing of residues Glu104 and Arg112 considering the hydrophobic and volume criteria. The residues Asn114 and Ser116 were chosen based on identity. When selecting residues Val113 and His108 first the volume and, in the case of Phe110, the hydrophobicity were taken into consideration.

Using the *Dihedrals* tool the Φ-Ψ values of template residues were recorded. During the gap filling of $Target_{raw}$, the dihedrals of the inserted residues were adjusted with *User defined* option of *Build* tool. The adjustment of Φ-Ψ-Ω dihedrals of Thr58 was performed with the corresponding values of Ser50 from PACAPR*. The χ dihedrals values were not adjusted. The final 3D structure, named *Model*, was compared with GLP-1R* using the *Superposition* tool. The atomic RMSD values were compared with the values obtained by superposition of GLP-1R* with other spatial models of LBD-GLP-1R released by the 3D structure prediction servers mentioned above. The superposition was performed for the whole structure as well as for certain parts of the models. These parts are mentioned in the head of *Table 1*.

Table 1. Atomic RMSD values (in Ångström) resulted from the statistical comparison between GLP-1R* and each of the automatic predicted models. In the first column the compared models are mentioned. The all-atom column contains the values of RMSD for all-atom comparison. In the backbone column the group C_β-C_α-CO-N is the protein backbone which includes C_β and carbonyl oxygen atoms, and C_α-C-N is backbone minus carbonyl oxygen atoms. In the side-chain column the side-chain atoms with and without C_β are taken in consideration.

		Backbone		**Side-chain**	
	All-atom	C_β-C_α-CO-N	C_α-C-N	including C_β	no C_β
ESyPRED-3D	4.3674	2.3392	2.1115	5.2240	5.8488
3D-JIGSAW	4.8160	3.0208	2.7779	5.6627	6.1861
CPH	4.6629	2.9786	2.7760	4.7738	5.9940
Model	4.5060	4.1301	3.6419	4.9298	5.0312

Results and Discussion

In the *Table 1* are presented the RMSD values resulted from the statistical comparison between the atomic coordinates of GLP-1R* on one hand and each automatic predicted model on the other hand. It is noticeable the fact that in all-atom mode the *Model* is better than the models obtained from 3D-JIGSAW and CPH. The precision of side-chain coordinates of *Model* is better than that of the models generated by prediction servers. The RMSD value of 5.0312, resulted in the *side-chain-no* C_β mode, is the lowest value. s

The evolutionary relatedness among GLP-1R, GIPR, CRFR and PACAPR is reflected by some structural similarities. *Figure* 2

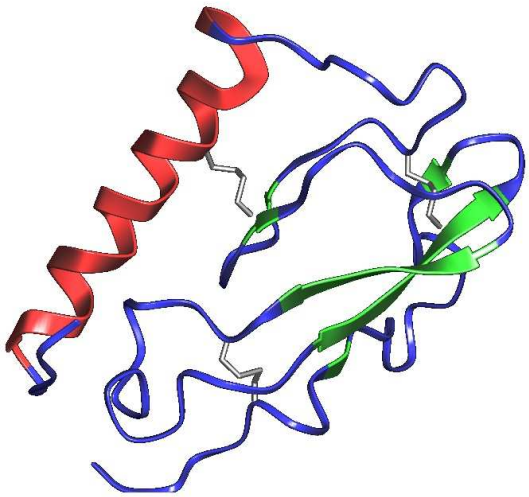

Figure 2. Secondary structures of *Model*. The conformations are α-helix (red) and β-sheets (green). These conformational elements are visualized based on the representation convention of the proteins. The picture suggests the stabilizing role of disulfide bonds presented as white broken sticks.

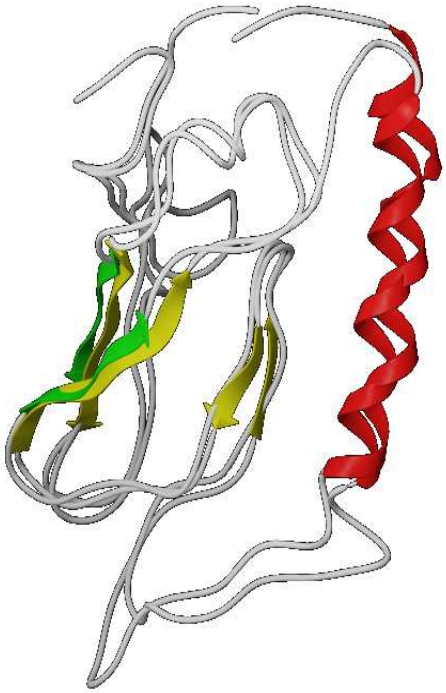

Figure 3. Superposed secondary structures of *Model* and GLP-1R*. The figure reveals that GLP-1R* is richer in β-sheet secondary structures (yellow antiparallel oriented bands) than *Model* (green antiparallel oriented bands).

***Table 2*. Centroid and N-O distances of some PDB structures (1-4), *Model* (5) and the models received from 3D structure prediction servers (6-8).** The bolded data are also represented in *Figure 4*. The defining of the centroid (dummy) atoms was made using the *Centroids* panel in *Edit* menu of *Maestro* graphical interface. The distances between dummy atoms were measured using *Measurements* panel of the same interface.

	Structure	PDB code	Centroid distance (Å)	N-O distance (Å)	Ion-pair type
1	X-ray-GLP-1R	3C5T	4.960	3.632	N-O bridge
2	**GIPR**	2QKH	5.207	**3.465**	N-O bridge
3	**CRFR2 β**	1U34	4.164	**3.206**	N-O bridge
4	**PACAPR**	2JOD	4.526	**3.416**	N-O bridge
5	***Model***	-	6.728	**5.384**	Long-range
6	ESyPRED-3D	-	6.265	5.346	Long-range
7	3D-JIGSAW	-	4.276	3.069	N-O bridge
8	CPH	-	3.907	3.058	Salt bridge

shows the conserved structural elements, α-helix, antiparallel β-sheets and the three disulfide bonds, which are common features not only for the above mentioned receptors but also for other class B members. Absolute conserved residues, on yellow background in *Figure 1*, constitute the hard core of LBD-GLP-1R.

Figure 3 presents the superposed backbones of GLP-1R* and *Model* of which statistical comparison, in *all-atom* mode of superposition, produced an atomic RMSD value of 4.5060 Å (*Table 1*).

Asp67 plays a central role forming intramolecular interactions also observed in GIPR*, CRFR* and PACAPR* for corresponding Asp residues. The residue Asp67 from GLP-1R* interacts indirectly with Arg102 and directly with Trp72 while in GIPR* the residue Asp66 forms a N-O bridge with Arg101, in CRFR* the residue Asp65 contacts Arg101 by a

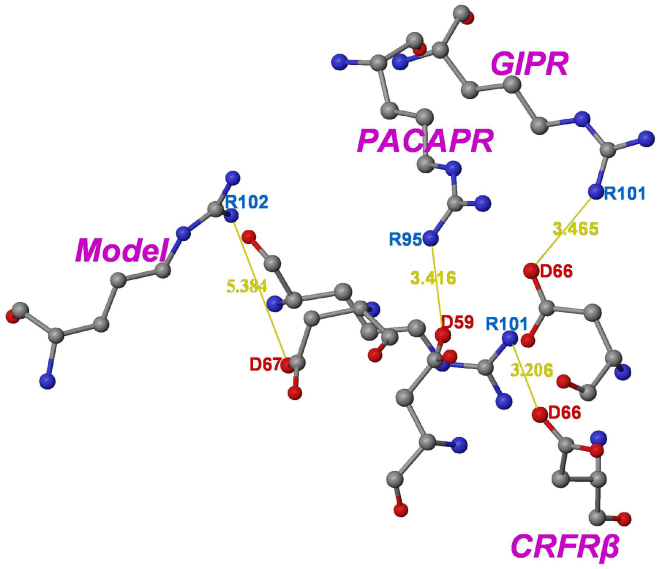

***Figure 4*. The topology of Asp-Arg interactions of template receptors and *Model*.** In figure the Asp-Arg distances are represented by yellow segments and their values are mentioned in Ångströms. The distinctive atoms are noted accordingly the one-letter code.

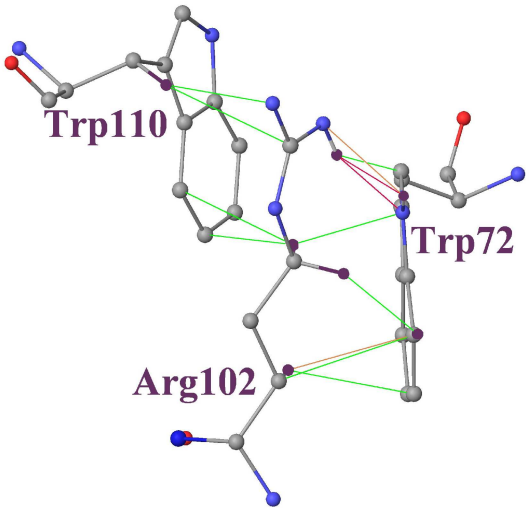

***Figure 5*. Contact topology of the sandwich system Trp72-Arg102-Trp110 in *Model*.** The good, poor and unusable contacts are represented by green, orange and respectively red segments. Good contacts make the atoms separated from each other by distances which range from $0.98(R_1 + R_2)$ to $1.02(R_1 + R_2)$, where R_1 and R_2 are ideal Van der Waals radii of the atoms *1* and *2*. The atom colour convention is C-grey, O-red, N-blue and H-violet.

N-O bridge and in PACAPR* the residue Asp59 forms a N-O bridge with Arg95. In conclusion, the interaction between absolute conserved aspartate and arginine residues is highly conserved in these receptors. *Figure 4* shows the distances between charged atoms of Arg/Asp side-chains from solved LBDs and *Model*.

The N-O distance value for *Model* is very similar with that of the predicted model received from ESyPRED-3D. In the *Table 2* are synopsized the data from *Figure 4* with the centroid topology data of receptors to define the ion-pair interaction types. Ion-pairs are divided into four geometrical categories[2]: salt bridges, N–O bridges, C–C bridges and longer-range ion-pairs.

A ion-pair is classified as a salt bridge when (*i*) the side-chain charged centroids are within a 4.0 Å distance and (*ii*) at least one pair

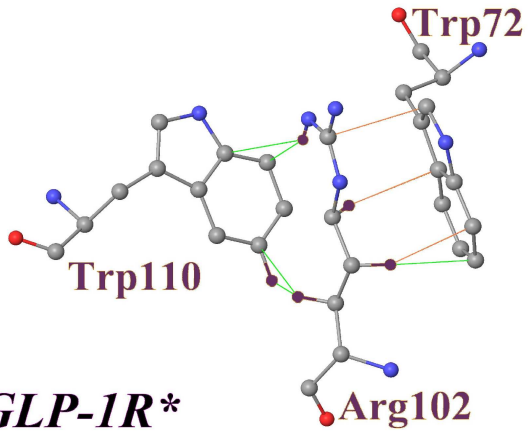

Figure 6. Contact topology of the sandwich system in GLP-1R*. The colour convention and the setting values are the same as those from *Figure 5*.

of Asp side-chain carbonyl oxygen and Arg side-chain nitrogen atoms are within a 4.0 Å distance. A ion-pair is a N-O bridge when it violates the first criterion but it satisfies the second. In a longer-range ion-pair the both salt bridge criteria are violated. The atoms that define the centroids are: (*i*) C^{γ}, $O^{\delta 1}$ and $O^{\delta 2}$ in aspartate side-chain and (*ii*) N^{ε}, C^{ξ}, $N^{\eta 1}$ and $N^{\eta 2}$ in arginine side-chain[9].. Applying the criteria for ion-pair type assessment, in the last column of *Table 2* are mentioned these types. The only model which satisfies the two conditions for the salt-bridge topology is that received from CPH. In the *Model* the N-O and centroid topologies reveal the case of long-range ion-pair (similar to ESyPRED-3D model). However, all template structures and GLP-1R* reveal the case of N-O bridge.

A feature of class B receptors is the sandwiching of a conserved arginine side-chain between the side-chains of two conserved tryptophane residues. The importance of Trp72 and Trp110 residues from GLP-1R* for ligand binding was previously documented by receptor mutagenesis[17]. The same relationship is observed in GIPR* where the aromatic indol ring system constituted by Trp71 and Trp109 residues sandwiches residue Arg101. In CRFR*

the Arg101 side-chain is sandwiched between the aromatic rings of Trp71 and Trp109. These examples support the above mentioned observation and this feature is also revealed in the *Model* structure (*Figure 5*).

To analyse the interatomic contacts in Trp-Arg-Trp system, *Contact cutoff ratios* were set to the following values: 1.02, 0.98 and 0.90 Å. This was performed in order to highlight the *good*, *bad* and respectively *ugly* contacts taking into consideration the classification nomenclature of *Contacts* tabs from *Measurements* panel in *Tools* menu. For comparison the contact topology of the GLP-1R* arginine-tryptophane sandwich is shown in *Figure 6*. *Figure 5* reveals many good contacts in *Model* but also some ugly contacts between Trp72 and Arg102 atoms. The GLP-1R* sandwich does not have ugly contacts suggesting a better stability.

Conclusions

A manual method for the construction of a three-dimensional model of a protein (target) could start with the graft of the target radicals upon the backbone of a protein which is the best sequence homologue of target identified by multiple sequence alignement. On this way it could obtain a raw 3D model of which dihedral adjustments, based on the known values of angular coordinates of other related proteins, could lead to a refined structure of target protein here named *Model*. In all-atom mode the comparison with GLP-1R* reveals that the *Model* is better than the models obtained from 3D-JIGSAW and CPH. Surprisingly, the positional deviations of the *Model* side-chain atoms, beside GLP-1R* side-chain atoms, are smaller than those of the server-released models.

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- ●●●● http://align.genome.jp/

Abbreviations

CRF = corticotropin releasing factor
CRFR = corticotropin releasing factor receptor
GHRH = growth hormone releasing hormone
GIP = gastric inhibitory polypeptide
GIPR = gastric inhibitory polypeptide receptor
GLP-1 = glucagon-like peptide 1
GLP-1R = glucagon-like peptid-1 receptor
GLP-2 = glucagon-like peptide 2
LBD = ligand-binding domain
NMR = nuclear magnetic resonance
PACAP = pituitary adenylate cyclase-activating polypeptide
PACAPR = pituitary adenylate cyclase-activating polypeptide receptor
PDB = protein data bank
PTH = parathyroid horrmone
PTHR = parathyroid horrmone receptor