Prediction and modelling of hOCT3

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1 Introduction

Organic cation transporters belong to the solute carrier 22 (SLC22) family and mediate the absorption, distribution, and elimination of a broad variety of endogenous and exogenous organic compounds. OCT3 is an organic cation transporter originally cloned from rat placenta [1]. It handles a variety of cationic drugs in addition to the endogenous organic cations such as dopamine, norepinephrine, and histamine. Recent studies by Grundemann et al. [2] with human OCT3 have confirmed the identity of OCT3 as the EMT extraneuronal transporter for monoamine transmitters). EMT consists of 556 amino acids with 12 putative transmembrane segments [2].

To date, seven different OCTs have been identified and characterized in humans, OCT1 (SLC22A1), OCT2 (SLC22A2), OCT3 (SLC22A3), OCTN1 (SLC22A4), OCTN2 (SLC22A5), Fly-like putative transporter 1 (FLIPT1) (SLC22A15) and carnitine transporter 2 (CT2/OCT6) (SLC22A16).

It is widely accepted that human SLC membrane proteins are potential therapeutics target for understating mechanism of drug transport and drug-resistance, and therefore, structural information could help design better drugs. Of 33,137 human protein structures in the RCSB Protein Data Bank, only 326 human membrane proteins are represented (25-Nov-2015). In the absence of known crystal structure, the prediction of 3D structure could be accomplished by comparative modeling, threading and ab-initio modeling because experimental methods are slow and expensive. In addition, some structures were failed to be solved, and usually a representative family structure can suffice to deduce structures of the entire family sequences. However, no member of the SLC22 family has been successfully crystallized. Thus there is a need to generate a 3-D homology model of hOCT3 with a suitable template.

In this study, 3-D homology models for hOCT3 (human OCT3) have been generated using eukaryotic inorganic phosphate transporter (PiPT) as the template.

2 Materials and Methods

The three-dimensional homology model of hOCT3 was based on the crystal structure of a eukaryotic inorganic phosphate transporter (PiPT) [3]. PiPT belongs to the phosphate: H+ symporter family of MFS, similar to the SLC22 family, PiPT also contains 12 TMDs and

intracellular N and C termini, and a large intracellular loop between TMDs 6 and 7. More importantly, the structure includes residues 30518 except for 67 residues in the flexible linker between the N and C domains, predicted from sequence to be disordered [3]. The crystal structure of PiPT (PDB ID: 4J05) was solved in complex with its substrate, inorganic phosphate, by Pedersen et al. at a resolution of 2.9 in an inward-facing occluded state conformation [3]. The structure of PiPT is currently recommended as the best template for OCTs and OATs because it is a crystallized transporter sharing the most sequence similarity with the human solute carrier group at this time, it possesses similar structure compared with OCTs (another OCT template like GLUT3 does not contain the large intracellular loop), it was crystallized in an occluded state, and PiPT is more evolutionarily close to OCTs and OATs. Based on these factors, PiPT has been chosen as the first template in order to generate a 3-D homology model for hOCT3 [4].

2.1 Sequences retrieval and multiple sequences alignment (MSA)

The sequences of the seven human OCTs, OCT1 (SLC22A1, Uniprot ID: O15245, 50%), OCT2 (SLC22A2, Uniprot ID: O15244, 51%), OCT3 (SLC22A3, Uniprot ID: O75751), OCTN1 (SLC22A4, Uniprot ID: Q9H015, 31%), OCTN2 (SLC22A5, Uniprot ID: O76082, 33%), hCT2/OCT6 (SLC22A16, Uniprot ID: Q86VW1, 28%) and FLIPT1 (SLC22A15, Uniprot ID: Q8IZD6, 31%) were retrieved from the Uniprot Knowledgebase database by running BLAST (% identity are indicated).

The sequence of PiPT was downloaded from PDB in a fasta file (4J05).

2.2 Secondary structure analysis

The secondary structure prediction of hOCT3 protein sequence was done using PSIPRED. The transmembrane prediction program TMHMM ver. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) was used to determine the putative topology of the hOCT3.

2.3 Alignment of template

The template PiPT and target (hOCT3) sequence were aligned using ClustalX and T-COFFEE followed by manual adjustments (AliView) based on the TMDs observed in PiPT structure and predicted for hOCT3 using PSIPRED and TMHMM, the result of alignment is shown in Fig.**??** .

2.4 Computational structural modeling

Next, 100 homology models of hOCT3 were generated for each alignment result and the crystal structure of PiPT (downloaded from PDB) using the MODELLER software. A DOPE (discrete optimized protein energy) score was automatically calculated for each model, and it was used to evaluate the quality of a structure model as a whole. Only the first top ten ranked were chosen for further analysis.

2.5 Model validation

The refined and energy minimized models were validated for the stereochemical validation by using the Structural Analysis on SwissModel Server (http://swissmodel.expasy.org/) which has various programs such as Ramachandran plot, PROCHECK, was used. Stereochemical quality

of a 3-D protein model was checked using the PROCHECK function, which produces a Ramanchandran plot that helps visualize energetically allowed regions, and analyzes the residue-by-residue geometry of a model. A model with more than 90% of amino acids located in the favorable regions of a Ramanchandran plot is generally considered as an acceptable model.

3 Results

The first step towards computing 3D structure of hOCT3 was to predict their putative secondary structure and the two-dimensional topology. For accomplishing this, three tools were used: PSIPRED, TMHMM and the target sequence as input. PSIPRED was used for predicting secondary structure of the hOCT3. TMHMM is employed for predicting transmembrane helices in hOCT3. TMHMM predicted 12 transmembrane helices in each hOCTs. TMHMM also mapped a large extracellular and a large intracellular loop in the target sequence.

Also a MSA was done on the different sequences of SCL22 family with the template. The MSA shows that residues involved in the active site of PiPT are relatively conserved [3] (highlighted residues in Fig.**??**). The phosphate is coordinated by Tyr 150(M4), Gln 177(M5), Trp 320(M7), Asp 324(M7) and Tyr 328(M7) which appear to be fully conserved in the family of phosphate:H+ symporters.

One template was used in this study.

3.1 Template PiPT

There can be seen in Fig.**??** the result of a first alignment by using T-coffee? The extracellular loop of PiPT was not part of the crystallization, thus it was not contained in the sequence of the PDB file. Consequently, gaps were added manually at the beginning of the sequence. Similarly, the intracellular loops between putative TMDs 6, 7 of hOCT3 were filled with gaps because no corresponding residues were modeled in the crystal structure of PiPT. These latter residues refer to the 67 missing residues in the flexible linker as mentioned above. After using MODELLER, DOPE scores of the top ten ranked model of hOCT3 are summarized in the table 1. However, the top ranked models lack N-terminal helix region in all seven hOCT3. This could be due to the limited sequence homology of the N-terminal and the C- terminal domains of the hOCTs with available PDB structures. For refining the conformation and orientation of the modeled N-terminal and the C- terminal domain of hOCT3, Python script for refining loop was used with MODELLER. It generates three conformations for each model kept previously. Output models were assessed for the quality of the loop refinement using the DOPE score.

The coloured annotations corroborate secondary structure prediction on TMHMM (Fig.4): best model's regions which are mainly helix-structured match with predicted strucutres. Conversely, on PSIPRED, secondary structure prediction output revealed that the N-terminal of hOCT3 is expected to contain a small helix region that ranges from amino acid Phe4 to Val11. In addition, the supposedly flexible linker region between TMD 6 and 7 does not appear to be fully disordered (Fig.5).

With PROCHECK, the best model was assessed after the refinement. For the model 47, 89.0% of amino acids were in the most favored region, 7.6% in the additional allowed region, and only a total of 3.5% in the generously and disallowed region (disallowed residues highlighted in Figure 6). These observations imply that the structure of model 47 for hOCT3 is plausible.

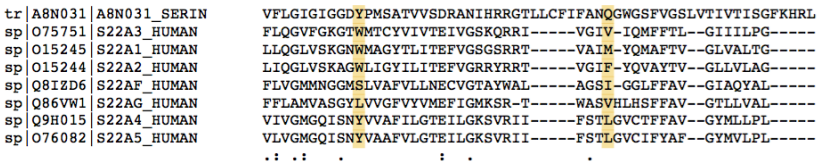

Figure 1: Multiple alignment of PiPT, hOCT1, hOCT2, hOCT3, OCTN1, OCTN2, hCT2/OCT6 and FLIPT1.The figure is focused on the alignment of residues Tyr 150(M4 helix), Gln 177(M5 helix) which may have a role in the phosphate transport

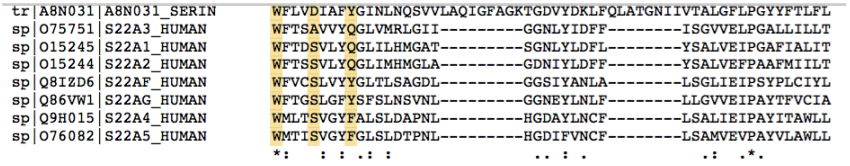

Figure 2: Multiple alignment of PiPT, hOCT1, hOCT2, hOCT3, OCTN1, OCTN2, hCT2/OCT6 and FLIPT1.The figure is focused on the alignment of residues Trp 320(M7 helix), Asp 324(M7 helix) and Tyr 328(M7 helix) which may have a role in the phosphate transport

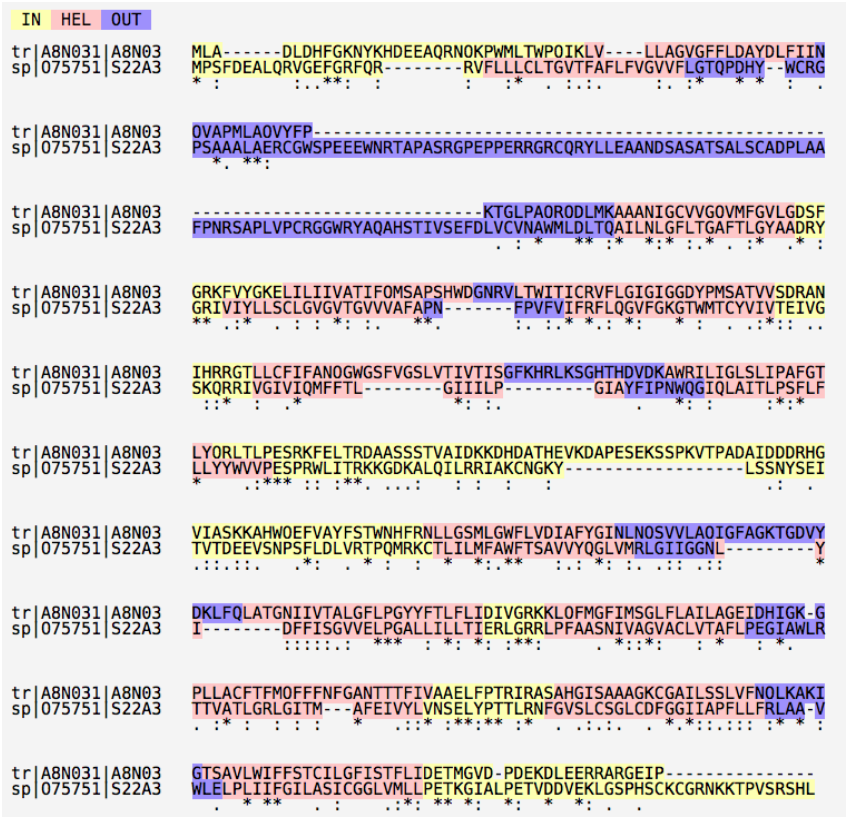

Figure 3: Sequence alignment between PiPT and hOCT3: Asterisks (*) indicate residues that are the same between the template and the target; colons (:) represent residues that are highly conserved between the sequences; periods (.) represent residues that are weakly conserved between the sequences; and blanks indicate that the residues are different.

Model #	DOPE
86	-55969.19
47	-55833.12
46	-55637.74
84	-55593.69
3	-55575.87
71	-55508.38
41	-55460.83
21	-55422.80
02	-55408.29
52	-55388.92

Table 1: DOPE scores for hOCT3.

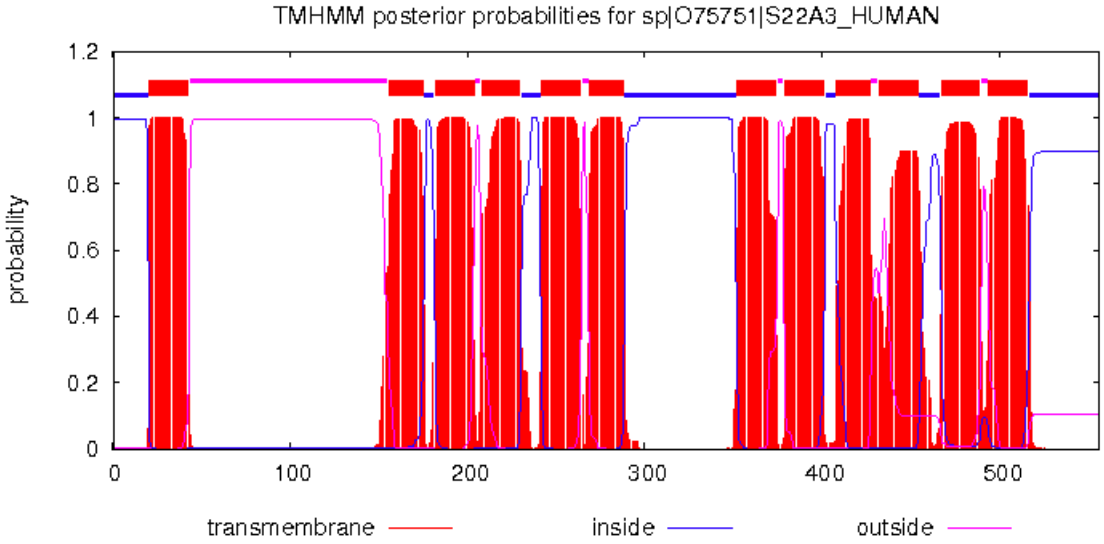

Figure 4: TMHMM secondary structure prediction..

4 References

[1] : Kekuda R, Prasad PD, Wu X, Wang H, Fei YJ, Leibach FH, and Ganapathy V. Cloning and Functional characterization of a potential sensitive, polyspecific organic cation transporter (OCT3) most abundantly expressed in placenta. J Biol Chem 273: 1597115979 , 1998.

[2] : Dirk Grundemann1, Birgit Schechinger, Gudrun A. Rappold and Edgar Schomig. Molecular identification of the corticosterone- sensitive extraneuronal catecholamine transporter. Nat Neurosci. 1998 Sep;1(5):349-51.

[3] : Bjrn P. Pedersen, Hemant Kumar, Andrew B. Waight, Aaron J. Risenmay, Zygy Roe-Zurz, Bryant H. Chau, Avner Schlessinger, Massimiliano Bonomi, William Harries, Andrej Sali, Atul K. Johri Robert M. Stroud. Crystal structure of a eukaryotic phosphate transporter. Nature 496, 533536 (25 April 2013)

[4] :

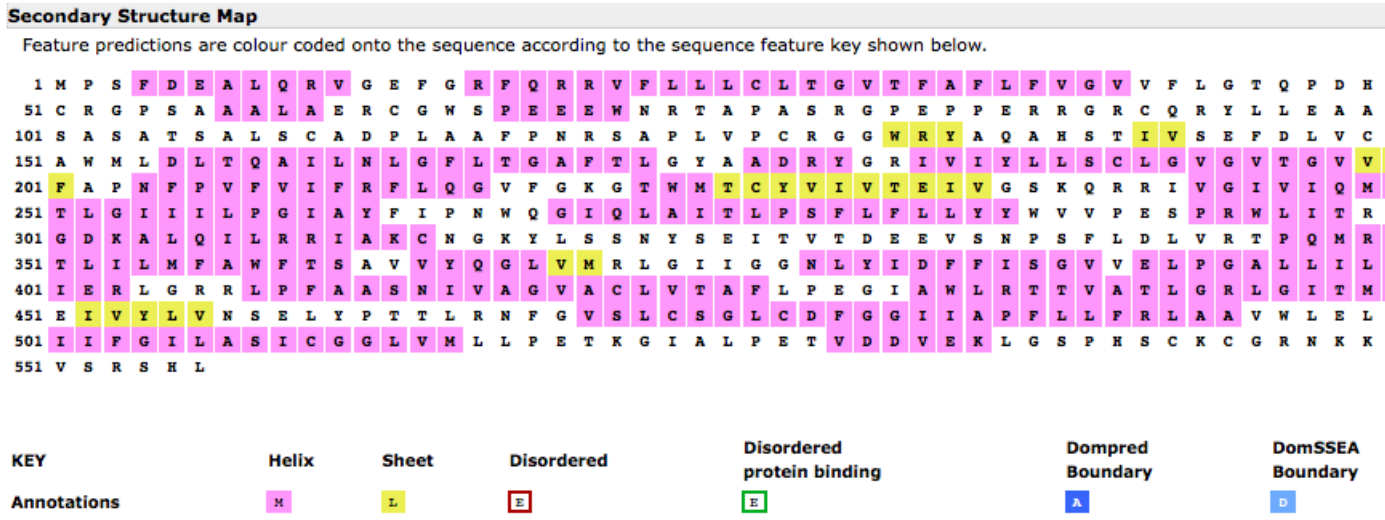

Figure 5: PSIPRED secondary structure prediction.

Schlessinger, A., et al., SLC Classification: An Update. Clinical Pharmacology amp; Therapeutics, 2013. 94(1): p. 19.

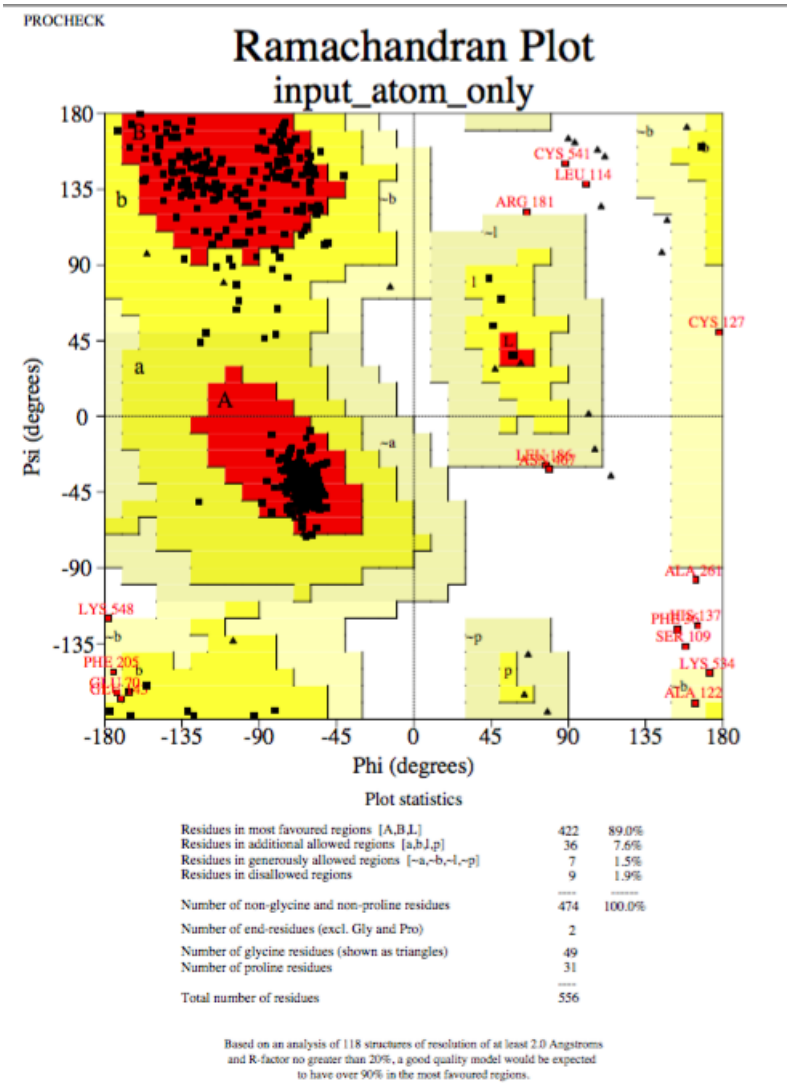

Figure 6: Ramachandran plot for model 47

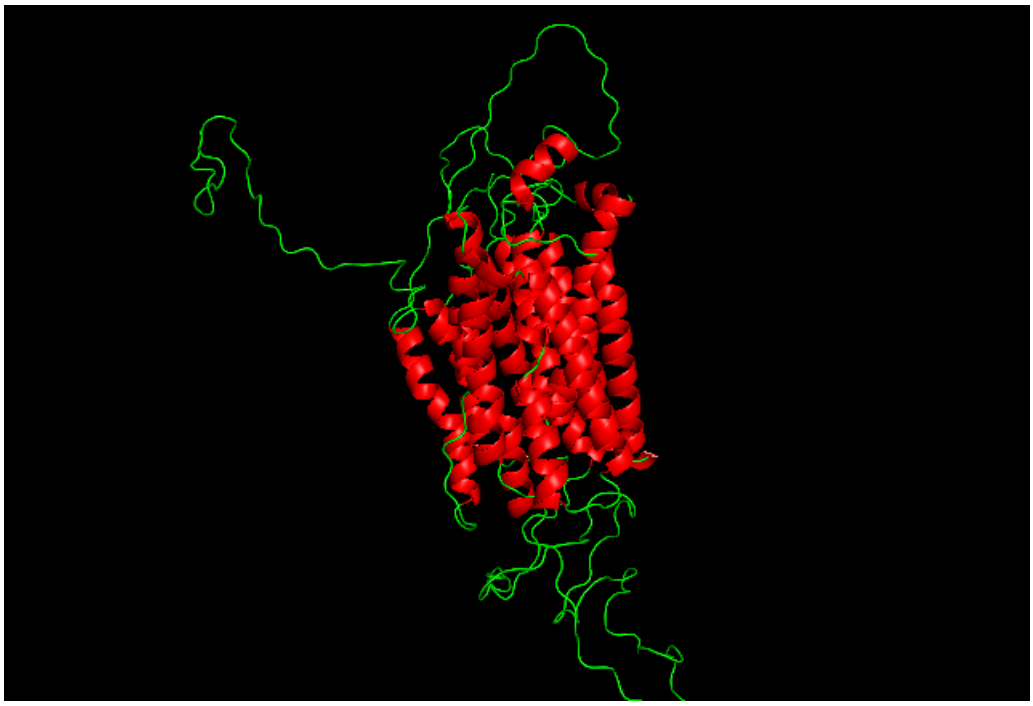

Figure 7: Cartoon representation of model 47