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# Structural modeling of human organic cation transporters

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ABSTRACT

Human organic cation transporters (hOCTs) belong to solute carriers (SLC) 22 family of membrane proteins that play a central role in transportation of chemotherapeutic drugs for several clinical and pathological conditions, including cancer and diabetes. These transporters mediate drug transport; however, the precise mechanism of drug-binding and transport by them is not fully uncovered yet, partly due to unavailability of any crystal structure record. In this work, we performed a multi-phasic approach to compute the 3D structural models of seven human organic cation transporters (hOCTs) starting from primary protein sequence. Our structure modeling approach included 1) I-TASSER based comparative sequence alignment, threading and *ab-initio* protein modeling; 2) models comparison with PSIPRED secondary structure prediction; 3) loop modeling for incongruent secondary structure in Chimera 1.10.1; 4) high resolution structure simulation, refinement, energy minimization using ModRefiner, and 5) validation of the structure models using PROCHECK at SAVEs. From structural point, the computed 3D structures of hOCTs consist of a typical major facilitator superfamily (MFS) fold of twelve α-transmembrane helix domains arranged in a manner rendering hOCTs a barrel shaped structure with a large cleft that opens in cytoplasm. The modeled 3D structure of all hOCTs closely resemble to human SLC2A3 (GLUT3) transporter (PDB ID: 5c65) and displayed an outward-open confirmation and putative cyclic C1 protein symmetry. In addition, hOCTs has a large (>100 amino acids) unique extracellular loop between TMH1 and TMH2 having potential glycosylation sites (Asn-Xaa-Ser/Thr) and cysteine residues, both features indicative of putative role in drug binding and uptake. There is an intracellular three/four-helix loop between TMH6 and TMH7 containing putative phosphorylation sites for precise regulation of hOCTs function as drug transporters. There are nine loops of 4 to 11 amino acids length that protrude from membrane, both intracellularly and extracellularly, and connect adjacent TMHs. The 2D structure prediction showed $N_{in}$-$C_{in}$ topology of all hOCTs. In the unavailability of the crystal structures of hOCTs, the 3D structural models computed *in-silico* and presented herein can be used for studying the mechanism of drug binding and transport by hOCTs.

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## 1. Introduction

Members of the solute carrier (SLC) 22 gene family are the transporters for organic cations, anions, and zwitterions, thus playing a major role in the cellular organic ions homeostasis (Koepsell et al., 2007; Volk, 2014). A large number of endogenous metabolites as well as xenobiotic drugs are organic in nature, and their uptake, distribution, and excretion are strongly depending on the expression of transport systems SLC22 gene family (Koepsell et al., 2007; Volk, 2014). Among the SLC 22 membrane transporter proteins are the organic cation transporters (OCTs) that mainly mediate bi-directional facilitated movement of a variety of lipophilic organic cations based on their electrogenic properties but independent of a $Na^+$ ion or proton gradient. 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) and they share approximately 70% amino acid

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sequence identity among them (data not shown). Northern blot analysis have shown that human OCT1 and OCT3 transcripts are expressed primarily in the sinusoidal and basolateral membrane of the hepatocytes (liver), respectively (Gorboulev et al., 1997; Nies et al., 2008, 2009) while human OCT2 transcripts have been detected in basolateral domain of the renal tubular cells of the kidney (Gorboulev et al., 1997). OCTN1 expresses mainly in kidney (Tamai et al., 1997) while its homologue OCTN2 expressed in kidney as well as in skeletal muscle, heart, and placenta in adult humans (Tamai et al., 1998). FLIPT1 (SLC22A15) expression has been detected in kidney, skeletal muscle, placenta, heart, lung, spleen, liver, and brain; whereas, hCT2 (SLC22A16) expresses in kidney, testis, liver, bone marrow, and leukocytes (Jacobsson et al., 2007).

Membrane proteins participates in almost all cellular activities, therefore, ascertaining their 3D structures could help unraveling how they coordinate their biological responsibilities. 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 (Lin et al., 2015). Approximately 30% of human genes code for membrane proteins. Of 29,779 human protein structures in the RCSB Protein Data Bank, only 308 human membrane proteins are represented (as on 20-Oct-2015). The low number is not a reflection of lack of interest. On the contrary, the low number highlights that membrane proteins are not amenable to purification and crystallization as well as are associated with a number of other drawbacks such as low abundance in a cell; all affect the ultimate success of structural biology endeavors. In the absence of known crystal structure, the prediction of 3D structure could be accomplished by comparative modeling, threading and *ab-initio* modeling that represent a breakthrough approach for a fast and accurate determination of 3D structure of membrane proteins.

Since past few years, structural bioinformatics has made spectacular progress in elucidation and elaboration of the intricate information present in the protein sequence to predict 3D structures of proteins that have no reported crystal structures. A number of state-of-the-arts approaches are currently available that integrate comparative modeling, threading, and *ab initio* modeling to build complete protein model. I-TASSER has proven record in predicting correct folding for proteins and generating high-resolution 3D structural models (Wu et al., 2007; Wu and Zhang, 2007). On the basis of the CASP7 experiment, I-Tasser generated models were found to be with 16.9% improved average TM-score than the best templates (Zhang, 2007). We used I-Tasser server to generate 3D models for seven human organic cation transporters. I-Tasser modeled all the hOCTs using the GLUT3 protein structure (PDB ID: 5c65) as the template with high accuracy and time efficient manner. We found almost congruent folding in all seven hOCTs when the 3D tertiary structure was compared with its secondary structure prediction done using PSIPRED in I-Tasser. Although the I-Tasser based *ab-initio* modeling generated reliable predictions, we faced some challenges such as incongruent secondary structures and orientation of N- and C-terminal, which were dealt in this paper. N- and C-terminal regions that were expected to possess a helix region in I-Tasser models based on PSIPRED prediction; however, were found lacking. In order to address this issue, we employed the 'Model Loop' tool in the Chimera 1.0.1 to predict possible alternate conformations of N- and C-terminal of hOCTs. Since, the modeling of loops (inexact environment) is much more complicated than loop reconstruction in crystal structures (Sellers et al., 2008), we generated five alternate conformation and choose one based on best fit data with PSIPRED prediction and best statistical scores obtained during loop refinement. These selected loop refined models, one model for each hOCT protein, were selected for further complete model refinement and energy minimization using ModRefiner. The energy minimized structures were verified using PROCHECK at SAVES server and steric by Ramachandran plots. The finalized models were submitted to the protein model database. Altogether, the current study has brought in depth understanding related to hOCTs structural features. We envisage that with the availability of 3D structure models of human OCTs, the intricate progress towards understanding mechanism of drug-binding and transport by human OCTs would become comprehensively feasible.

## 2. Methods

### 2.1. Sequence retrieval

The sequences of the seven human OCTs, OCT1 (SLC22A1, Uniprot ID: O15245), OCT2 (SLC22A2, Uniprot ID: O15244), OCT3 (SLC22A3, Uniprot ID: O75751), OCTN1 (SLC22A4, Uniprot ID: Q9H015), OCTN2 (SLC22A5, Uniprot ID: O76082), hCT2/OCT6 (SLC22A16, Uniprot ID: Q86VW1) and FLIPT1 (SLC22A15, Uniprot ID: Q8IZD6) were retrieved from the Uniprot Knowledgebase database (Magrane, 2011). The sequences of the rat OCT1 (Slc22a1, Uniprot ID: Q63089) was also retrieved from the Uniprot Knowledgebase database (Magrane, 2011).

### 2.2. Secondary structure analysis and two-dimension topology prediction

The secondary structure prediction of human OCTs protein sequences was done using PSIPRED in I-Tasser. The transmembrane prediction program TMHMM ver. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) (Möller et al., 2001) was used to determine the putative topology of the hOCTs. The two-dimension topology was generated using PROTTER (http://wlab.ethz.ch/protter/#) (Omasits et al., 2014).

### 2.3. Computational structural modeling

3D structure models for hOCTs and rOCT1 were built using I-Tasser (http://zhanglab.ccmb.med.umich.edu/I-TASSER/), which employs an integrated combinatorial approach comprising all three standard conventional methods for structure modeling that includes comparative modeling, threading, and *ab initio* modeling (Roy et al., 2010), and predicts protein 3D structure with almost no manual intervention.

### 2.4. Loop modeling

The structural editing of the loops present at the N-terminal and C-terminal was done using 'Refine Loops' tool using Chimera 1.10.1 interface to Modeller using DOPE-HR loop modeling protocol. Using 'Refine Loops' tool, we generated five alternate conformations for the N-terminal and C-terminal domain of the top ranked I-Tasser model for all seven hOCTs. Subsequently, the most acceptable models were finalized based on best fit data with PSIPRED secondary structure prediction and best statistical scores obtained during loop refinement.

### 2.5. Global model refinement, energy minimization

Selected loop refined I-TASSER 3D models and rOCT1 model were then subjected to overall structural refinement and energy minimization so as to generate a model close to native form in terms of H-bonding, topology of backbone and positioning of the side chains. Model refinement was done using ModRefiner (http://zhanglab.ccmb.med.umich.edu/ModRefiner/).

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