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Thyroid hormone transport across L-type amino acid transporters: What can molecular modelling tell us?

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Abstract

Thyroid hormones (THs) and their derivatives require transmembrane transporters (TTs) to mediate their translocation across the cell membrane. Among these TTs, the L-type amino acid transporters (LAT) not only transport amino acids (AAs) but also certain THs and their derivatives.

This review summarizes available knowledge concerning structure function patterns of the TH transport by LAT1 and LAT2. For example, LAT2 imports 3,3'-T_2 and T_3, but not rT_3 and T_4. In contrast to amino acids, THs are not at all exported by LAT2. Homology modelling of LAT1 and LAT2 is based on available crystal structures from the same superfamily the amino acid/polyamine/organocation transporter (APC). Molecular model guided mutagenesis has been used to predict substrate interaction sites. A common recognition feature for amino acid- and TH-derivatives has been suggested in an interior cavity of LAT1 and LAT2. Therein additional distinct molecular determinants that are responsible for the bidirectional AA transport but allowing only unidirectional import of particular THs have been confirmed for LAT2 by mutagenesis. Characterized substrate features that are needed for TH translocation and distinct LAT2 properties will be highlighted to understand the molecular import and export mechanisms of this transporter in more detail.

Introduction

Thyroid hormones (THs) are essential for growth, cellular metabolism and development, especially for the human central nervous system. THs and their derivatives require transmembrane transporters (TTs) to mediate their translocation across the cell membrane (Hennemann et al., 2001).

Among these THTTs only the monocarboxylate transporter (MCT)8 is specific for THs (Friesema et al., 2003), whereas the other TH transporters show broader substrate spectra. For example, the amino acid transporters (LAT)1, (SLC7A5) or 2 (SLC7A8) transport sodium-independently neutral AAs (Hennemann et al., 2001). Uptake studies demonstrated that THs are also imported by LAT1 (Friesema et al., 2001; Kinne et al., 2015) and LAT2 (Friesema et al., 2001; Kinne et al., 2015).

New findings were recently published (Zevenbergen et al., 2015) showing that THs are exported by LAT3 (SLC43A1) and LAT4 (SLC43A2) but that import of TH is impeded. Overall, transport can be competitively inhibited by the LAT inhibitor 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) (Sebastianelli et al., 2008). All TH transporters have a common structure of 12 transmembrane helices (TMHs) (Kinne et al., 2011) but differ in their conformations, since they belong to different transporter families; the APC and the major facilitator superfamily (MFS).

Nevertheless, the roles of LAT transporters as well as their molecular mechanisms in TH transport are not fully understood. Their high potential as a drug delivery system (Tachikawa et al., 2014; Wongthai et al., 2015), especially for THs and their derivatives requires the use of homology models, since crystal structures of these proteins are currently not available.

Diiodothyronines (T_2) are major TH derivatives that are transported by LATs. Interestingly, T_2 which are mostly regarded as mere degradation products also trigger cellular responses, e.g. cell metabolism. For example, 3,3'-T_2 binding sites were discovered in rat mitochondria, suggesting a role in metabolic regulation of the cell (Lanni et al., 1994). While only few possible biological effects of 3,3'-T_2 and 3',5'-T_2 have been reported, there is more information available for 3,5-T_2, which circulates in human serum (Pietzner et al., 2015) and might rapidly and directly act on mitochondrial function. At higher concentrations it also mimics T_3 effects via modulation of TR-dependent transcription in many target cells (Ball, 1997; Jonas et al., 2015; Moreno et al., 2008; Padron et al., 2014; Pietzner et al., 2015). Therefore, the import and the export of T_2 in cell systems seem to play an important role in TH regulation; however the molecular mechanisms are unknown.

Differences between LAT1 and LAT2

In transport studies similar results were observed for the similar transporters LAT1 and LAT2 (Friesema et al., 2001) although there are differences known in substrate specificity.

LAT1 was shown to transport gabapentin (Dickens et al., 2013), but import of gabapentin by LAT2 was not measured (Hinz et al., 2015; Morimoto et al., 2008). A second substrate is alanine which behaves differently with the two transporters. Alanine is not or just less transported by LAT1, depending on the species studies. For LAT2 it was found that alanine is well transported (Khunweeraphong et al., 2012; Morimoto et al., 2008; Pineda et al., 1999; Segawa et al., 1999). Another varying substrate is rT_3, which is transported by LAT1 (Friesema et al., 2001) but not by LAT2 (Kinne et al., 2015). The molecular reasons for the differences were not understood. LAT2 is expressed in several human and mouse tissues such as kidney, placenta, brain, liver, spleen, muscle tissue and small intestine (Braun et al., 2011; Rossier et al., 1999). The substrate specificity of the heterodimer LAT2 and its escort protein CD98 for membrane association was analyzed in co-injected oocytes. A preferential import of 3,3'-T_2 and somewhat less T_3 import was detected, while no transport was observed for rT_3 and T_4 (Kinne et al., 2015). LAT2 import of THs was also studied by competitive inhibition in the presence or absence of AAs, several iodothyronines and the common LAT inhibitor BCH. It has been demonstrated that iodothyronines comprising none (T_0), one (T_1) or two iodine atoms (3,3'-T_2; 3,5-T_2; 3',5'-T_2) clearly compete with the radiolabeled substrate 3,3'-T_2 import indicating that these compounds are very likely also transported by LAT2 (Kinne et al., 2015). However, inhibition of 3,3'-T_2 transport by analogues only indirectly, but not directly, demonstrates that these analogues are transported themselves.

Among others correlated human and mouse LAT1 expression was found for endocrine tissue, immune system, liver, brain, muscle tissue, lung, kidney, male and female tissues and gastrointestinal tract (T Kageyama et al., 2000; Takashi Kageyama et al., 2000; Nakamura et al., 1999; Rossier et al., 1999). When associated with CD98, LAT1 plays a crucial role in AA exchange and is also able to import bulky THs, like T_3 or T_4 (Friesema et al., 2001; Morimoto et al., 2008).

Structure, topology and homology models of transporters

There is a lack of detailed experimental structural information for all THTTs. To gain insight into structure-function relationships crystal structures such as Glycerol-3-phosphate transporter (GlpT, PDB: 1PW4), and xylose transporter (XylE, PDB: 4aj4; PDB: 4gby) have been used as structural templates for homology models of MCT8 which were used for docking of TH derivatives (Braun et al., 2013; Kinne et al., 2010; Protze et al., 2017).

In contrast to MCT8, LAT1/2 belong to APC (Hughes et al., 2004; Schlessinger et al., 2013) in particular to the heteromeric amino acid transporters (HAT). They need an escort protein for efficient cell surface expression (Palacín et al., 2016).

The light chain LAT1/2 and the heavy chain 4F2hc (CD98) form a heterodimer (Rosell et al., 2014; Verrey et al., 2004). LAT1/2 consist of 12 TMHs and have no glycosylation site. Both ends of the light chain are in the cytosol. CD98 is ubiquitously expressed, e.g. in brain, kidney, intestine, placenta, and tumors (Nakamura et al., 1999; Verrey et al., 2004). CD98 consists of only one transmembrane domain (TMD) with four N-linked sites of glycosylation. CD98 almost completely covers the extracellular face of the transporter and increases the stability of the light subunit LAT1/2 by acting as a scaffold protein (Meury et al., 2014; Rosell et al., 2014). A conserved cysteine residue involved in the intersubunit disulfide bridge with CD98 is located between TMH3 and TMH4 of LAT1/2. However, this disulfide bridge is not essential for substrate transport by LAT1/2 (Rosell et al., 2014; Wagner et al., 2000).

To characterize the interactions between the LAT2 and CD98 a LAT2/CD98 interaction model based on the crystal structures of the L-Arginine/Agmatine Antiporter (AdiC; PDB: 3L1L, (Gao et al., 2010); PDB: 3OB6 (Kowalczyk et al., 2011)) and of CD98 (PDB: 2DH2 (Fort et al., 2007)) was generated (Rosell et al., 2014). They found that desolvation is the main contributor to the binding energy of 4F2hc-ED and LAT2. Structure model based ligand discovery for LAT1 is described for T_4 as well as for a range of small molecules (Geier et al., 2013; Zur et al., 2016) and phenyl- and tyrosine analogues (Augustyn et al., 2016).

Single and multiple fragment template approaches generating molecular models of LAT

For LAT transporters structural homology models have recently been suggested based on a single structural template namely AdiC (Geier et al., 2013; Rosell et al., 2014) or ApcT (Geier et al., 2013). Using only a single structural template for homology model building confers a high risk that the single template only matches part of the sequence and thus may provide a wrong structure for the rest of the query protein. Therefore a different strategy of fragmented templates, which was originally developed for G-protein-coupled-receptors (Worth et al., 2011)**,** was also used for LAT2 (Hinz et al., 2015). Initially, a comparative model of LAT2 was generated using AdiC (PDB: 3L1L, (Gao et al., 2010)) and ApcT (PDB: 3GI8, (Shaffer et al., 2009)). Both crystal structures possess 12 TM-helices. The templates share a sequence similarity of about 30 % with LAT2. Both templates are members of the APC superfamily (Saier et al., 2016). The crystal structure of ApcT is solved in the inward-facing apo state and AdiC shows the 12 TMHs in an occluded arginine-bound conformation. It has a structural similarity to the 12 TMHs within the crystal structure of a bacterial homologue for neurotransmitter transporters, the sodium-dependent leucine transporter (LeuT) from *Aquifex aeolicus* (Yamashita et

al., 2005). A common feature of transporters having a LeuT-fold (also named 5+5 inverted repeat fold) is that TMH1 and TMH6 are discontinuous helices interrupted by a highly conserved non-alpha-helical segment (Gao et al., 2010; Yamashita et al., 2005). This structural feature is part of the substrate recognition site and is important for the transport mechanism as reviewed by Palacín et al. (Palacín et al., 2016)

The advantage of using the AdiC crystal structure is the bound substrate arginine. The AA moiety of arginine binds towards the substrate binding site between TMH1 and 6, common for LeuT-like fold transporters. Moreover, like LeuT AdiC contains an aromatic residue that folds like a lid over the AA moiety of the bound substrate after substrate recognition (Nyola et al., 2010).

In the case of LAT2, sequence similarity of fingerprint features in TMH1 and 6 of AdiC and LAT2 suggest the same binding mode for the AA moiety of 3,3'-T_2 in LAT2. AdiC is indeed a sufficient template for LAT2, however, only for particular TMHs (1-4, 6, 7) since for the remaining helices (5, 8-12) some sequence fingerprints of ApcT match better with the LAT2 sequence (figure 1). In contrast to other LAT1 and LAT2 models (Geier et al., 2013; Rosell et al., 2014) a single structural template was not used, but rather both templates were employed for generating a chimeric model (figure1).

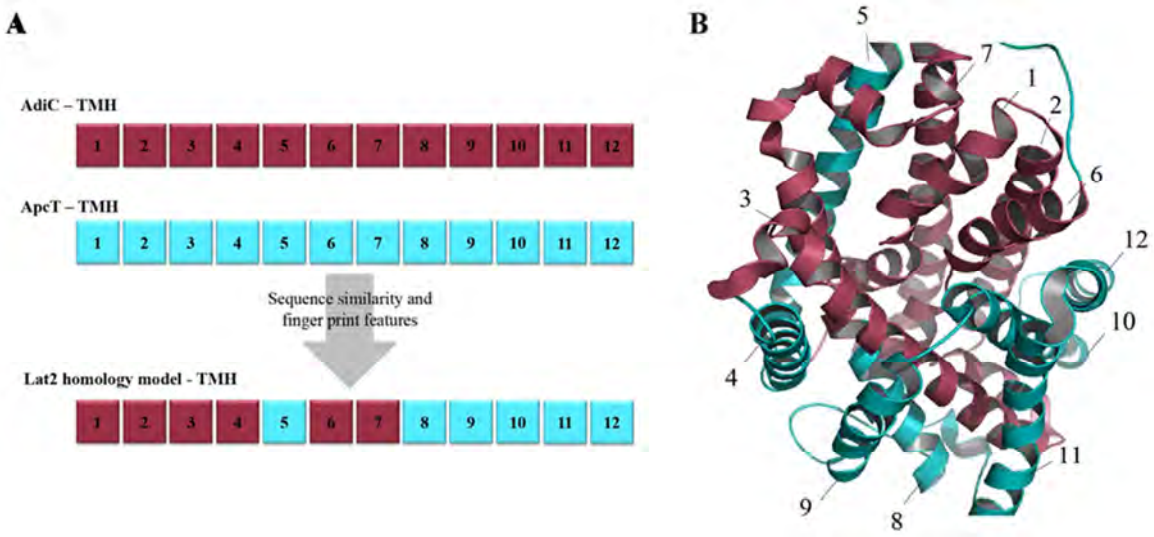

Figure 1: *Multiple fragments from two structural templates AdiC and ApcT are used for the LAT2 mode. A) Helices are selected due to best matching sequence similarity and fingerprint features. B) LAT2 model: Conformation of TMHs 1-4,6,7 are used from AdiC (maroon). Particular sequence dependent conformation of TMHs 5,8-12 from ApcT (cyan) are used as chimeric templates for LAT2 by superimposing the helices to corresponding TMHs of AdiC. The overall outward faced conformation of AdiC is retained for the LAT2 model* (Hinz et al., 2015).

The LAT2 model was mainly built on the outward facing structure of AdiC utilizing the advantage of the bound substrate. TMH5 and 8-12 backbone conformations of ApcT structure are superimposed to the corresponding helices of the overall AdiC structure. This fragment based assembling of best matching TMH sequence similarity was used generating the homology model for LAT2 by retaining the outward facing conformation of the AdiC structure (figure 2) (Hinz et al., 2015).

Concordant effects of mutations of residues in identical positions in both the template structure (N22A (Gao et al., 2010); K158A (Shaffer et al., 2009)) and in LAT2 (N51, K193A (Hinz et al., 2015)), provided evidence of the validity of this chimeric model for LAT2.

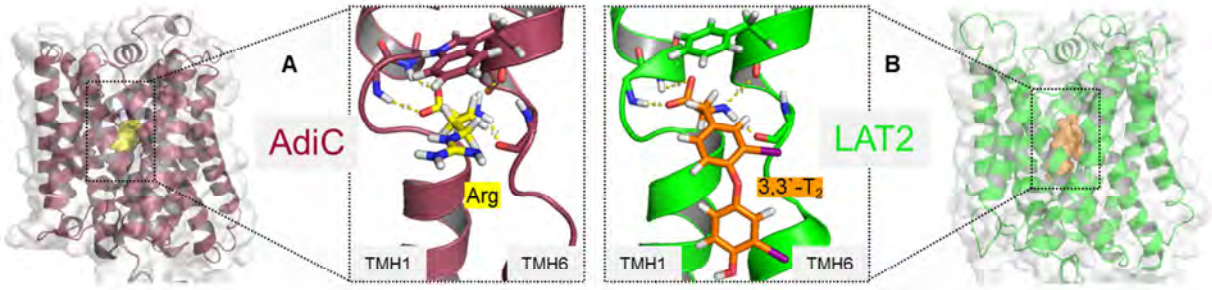

Figure 2: ***Comparison of substrate binding site in template and LAT2 model A)*** *AdiC - 3L1L (maroon) in the occluded state conformation bound to arginine (yellow).* ***B)*** *LAT2 homology model of the 12 TMHs (green) and analogous binding site with 3,3'-T_2 (orange) bound. The AA moieties of arginine and accordingly of 3,3'-T_2 are bound to their recognition site, due to common fingerprint motifs. Substrate is located between TMH1 and 6 and is bound to the backbone via hydrogen bonds. In both cases, the AA moiety of the substrates is covered by an aromatic ring system of side chain Trp^{202} in AdiC and Phe^{242} in LAT2.*

An overview of the LAT subtypes, their classifications, transported THs, crystal structure templates used to generate LAT1/2 models and which substrates have been docked to reveal diverse structure-function studies is provided in table1.

167 ***Table 1:*** *LAT subtypes, classifications, transported THs, crystal structure templates, models and docked substrates for structure function relationships.*

	LAT1	LAT2	LAT3	LAT4
solute carrier members	SLC7A5	SLC7A8	SLC43A1	SLC43A2
transporter classification	APC	APC	MFS	MFS
escort protein	CD98	CD98	-	-
transmembrane helices	12	12	12	12
sequence length	507	507	559	569
mass [kDa]	55,01	55,01	61,48	62,74
glycozilation site	-	-	1	1
exchange	neutral large L-AA	neutral small and large L-AA	neutral large L-AA	neutral large L-AA
import TH	T_4, T_3, rT_3, 3,3'-T_2	T_3, 3,3'-T_2 [7]	-	-
export TH	3,3'-T_2 [1]	-	MIT, DIT, 3,3'-T_2 [12]	MIT, DIT, 3,3'-T_2 [12]
homology model	LAT1 [2]	1. LAT2+CD98 [8] 2. LAT2 [9]	-	-
template (TMH)	AdiC (12) [2], ApcT (12) [2]	1. AdiC (12) + CD98 [8] 2. AdiC (1-4, 6-7) ApcT (5, 8-12)[9]	-	-
PDB	3L1L [3], 3GIA [4]	1. 3L1L, 2DH2 [10] 2. 3L1L, 3GI8 [11]	-	-
docking	AA and derivatives [2], hydroxamic acid [5], meta-substituted phenylalanine and tyrosine analogs [6]	1. no docking 2. TH, BCH, AA and their derivatives	-	-

experiments for elucidation structure function relation	in silico docking and transport assays with screened substrates	1. cross-linking experiments for enlightening the heterodimer interaction [8] 2. in silico docking and transport assays with screened substrates [7,8,9]	transport assays with several substrates [12]	transport assays with several substrates [12]
references	If not otherwise listed data accessed from uniprot.org (1) Friesema et al., 2001 (2) Geier et al., 2014 (3) Gao et al., 2009 (4) Shaffer et al., 2009a (5) Zur et al. 2016 (6) Augustyn et al. 2016	If not otherwise listed data accessed from uniprot.org (7) Kinne et al., 2015 (8) Rosell et al., 2014 (9) Hinz et al., 2015 (10) Fort et al., 2007 (11) Shaffer et al., 2009b	If not otherwise listed data accessed from uniprot.org (12) Zevenbergen et al., 2015	If not otherwise listed data accessed from uniprot.org (12) Zevenbergen et al., 2015

Abbreviations: L-type amino acid transporter (LAT); Solute carrier (SLC); amino acid polyamine organocation superfamily (APC); major facilitator superfamily (MFS); amino acid (AA); thyroid hormone (TH); 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH); monoiodo-thyrosin (MIT), diiodo-thyrosin (DIT); transmembrane helix (TMH); protein data bank identification code (PDB).

LAT2 model guided site directed mutagenesis

Among THTTs least is known about structure-function relationships of LAT2. As molecular and structural details of the interaction between the heavy chain CD98 with the light chain LAT2 has been previously described (Rosell et al., 2014), this review focuses mainly on structure function patterns of LAT2 itself. Amino acids have been selected for site-directed mutagenesis by using the LAT2 homology model and transport studies in *Xenopus laevis* oocytes have been performed (Hinz et al., 2015). All mutations were analyzed initially by western blotting (Hinz et al., 2015) and membrane expression was additionally confirmed by confocal laser scanning microscopy (Hinz et al., 2017). The use of oocytes has the advantage of low endogenous transporter protein expression and thus provide an isolated view of the injected transporter (Van Winkle, 1993).

According to the LAT2 model, amino acids were selected that are located in the proximity to the binding site. Side chain shortening mutations (Y130A, N133S) increased 3,3'-T_2 transport. This indicates a widening of the translocation pathway in the interior of LAT2. The observed import of AAs, like Leu and Phe is not changed by any of the considered mutants (Hinz et al., 2017). Contrariwise side chain enlargements of other residues (I137M, T140F or Y130R) decreased the 3,3'-T_2 import, indicating an obstruction (Hinz et al., 2015).

The pocket in the center of the chimeric LAT2 model exhibits two asymmetrically shaped excavations (figure 3). As docking experiments indicated, they are large enough that two linked aromatic ring structures with bulky substituents such as iodo-substituents in 3,3'- (figure 3A), in 3,5- (figure 3B) or in 3',5'-positions (figure 3C) of T_2-isomers can pass through. The residue Phe^{242} plays a critical role for substrate import. Retaining the hydrophobic but substituting the aromatic property by the non-aromatic valine (F242V), leads to a decreased 3,3'-T_2 import, while mutation to the enlarged aromatic tryptophan (F242W) rescues this effect and even increases the 3,3'-T_2 import compared to WT (Hinz et al., 2015). Side chain shortening or enlargements in Phe^{242} show the opposite of the effects of the other mutations. This indicates a distinct functionality for Phe^{242}. Particularly, Phe^{242} corresponds not only to the identical location of Trp^{202} in the AdiC template structure, but also to the aromatic residue covering the AA moiety in LeuT (Nyola et al., 2010). Thus Phe^{242} strongly supports the hypothesis that an aromatic ring system is in this corresponding position in LAT2. Covering the recognition site seems to play a role at the extracellular side for substrate gating (figure 3C).

TH docking into LAT2-WT model explains T_2 import and T_4 obstruction

From docking experiments it becomes obvious that tyrosine at position 130 narrows the translocation pathway in LAT2 significantly, which makes it impossible for T_4 (grey in figure 3D) to pass through. This explains why LAT2 does not import T_4 at all. In contrast, side chain shortening by Y130A mutation provides an enlarged space for the pocket, which enables the import of T_4 in the expanded translocation pathway (cyan in figure 3D) (Hinz et al., 2017). Other mutations, N133S and F242W do not affect the T_3 and T_4 import. Taking 3,3'-T_2 as an example, studies of the allowed conformational space for the respective TH derivatives indicated that flexibility between both aromatic rings is necessary for the whole molecule to pass through the two asymmetrically shaped excavations of the translocation pathway of LAT2 (Hinz et al., 2015). This is valid also for 3',5'-T_2 with two bulky iodine atoms at the phenolic ring. TH molecules possessing three or four bulky substituents (e.g. iodine)

distributed on both the tyrosyl and phenolic ring are either limiting (T_3) or preventing (rT_3, T_4) their import by steric hindrance of the ring flexibility.

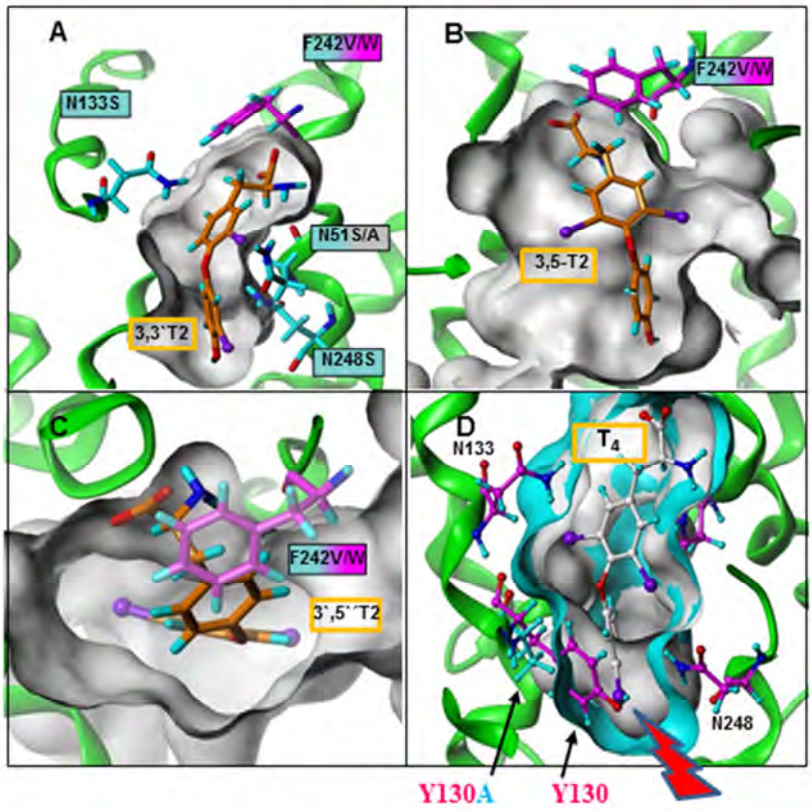

Figure 3: *Possible docking poses for THs on LAT2 homology model (green) into the asymmetrically shaped translocation pathway (sliced grey surfaces) at the center of LAT2 (see Fig. 2B).* ***A)*** *Transport sensitive asparagine residues (cyan) are lining the TH recognition pocket. Their side chain shortening mutations led to pocket enlargement and increases 3,3'-T_2 import. Asymmetric excavations in the translocation pathway allow bulky substituents either* ***B)*** *at the tyrosyl ring of 3,5-T_2 or* ***C)*** *at the* phenolic *ring for 3',5'-T_2. Phe^{242}, here forms a lid covering AA moiety (CO2NH2) of THs in the Arg-bound occluded state (view from extracellular side)* (Hinz et al., 2015)*.* ***D)*** *Side chain Y130 of LAT2-WT model clashes sterically with an iodine atom (lilac) of T_4 (grey sticks) Iodine atom on position 5' exceeds the available space (enclosed grey surface) by protruding the limits of the given translocation pathway of LAT2-WT and clashes with Y130 side chain (red arrow) Y130 is thus obstructing T_4. Mutation Y130 to the shorter alanine side chain (cyan), provides a largely widened translocation pathway (open cyan surface), enabling the import of T_4* (Hinz et al., 2017)*.*

Patterns for bidirectional and unidirectional transport by LAT2

There are distinct molecular determinants revealed, which are responsible for bidirectional AA transport but only for unidirectional import of 3,3'-T_2 and T_3 by LAT2-WT (figure 4). LAT2-WT is unique in just importing TH and not exporting it. LAT2 mutants identified key residues that allow T_4 import (Y130A) and even TH export (N133S) but only for T_2. According to the molecular model (figure 3), N133 is located closer to the central recognition pattern for the AA moiety than the more intracellular facing side where Y130 is located. From these data it can be concluded that an export barrier is localized in the center, while the import barrier is localized towards the intracellular side of LAT2, here

Y130. It was also shown to play a crucial role for several transporters of the APC superfamily (Celik et al., 2008; Kowalczyk et al., 2011).

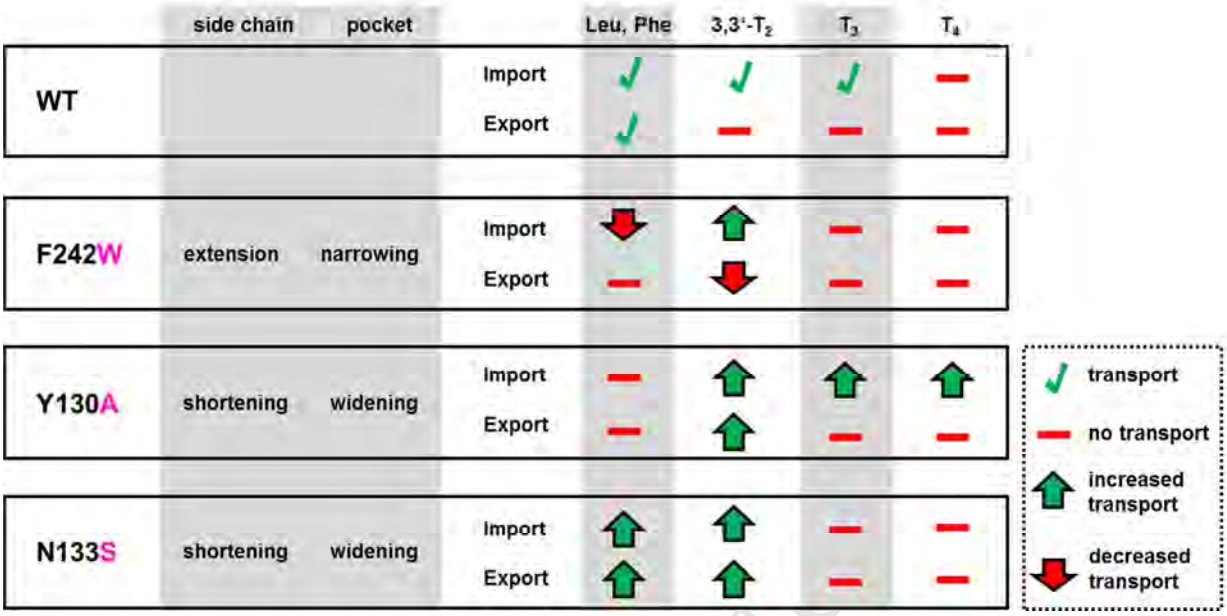

Figure 4: *Summary of import and export results by LAT2-WT and by mutations (leftmost white column), their effects on sizes of side chains and recognition pocket (leftmost gray column) that either selectively enhanced/allowed (green) or blocked (red) the import (upper line) and export (lower line) of AAs (Leu, Phe) and TH (3,3'-T_2, and T_3, T_4) (adapted from* Hinz et al., 2017). *The mutated positions represent molecular features that are responsible for different import and export profiles of* AAs and THs.

Diversity between LAT1/ LAT2 and LAT3/ LAT4

Although LAT3 and LAT4 belong to the LAT family they are members of a subfamily (SLC43) which diverges from that of LAT1 and LAT2 (SLC7A). This is illustrated by the fact that LAT3 and 4 do not need an escort protein (CD98) for membrane localization. LAT3 and LAT4 are new TH transporter candidates. Very recently it was shown by indirect measurements that LAT3 and LAT4 are involved in THs export in transient transfected Cos-1 cells (Zevenbergen et al., 2015). Expression of LAT3 or LAT4 did not affect import of T_4, T_3, rT_3, but slightly affected import of T_2 and clearly affected that of 3-iodo-L-tyrosine (MIT). However, co-transfection of LAT1 or LAT2 with LAT3 or LAT4 strongly diminished cellular accumulation for 3,3'-T_2 and MIT of LAT1 and LAT2. For LAT1 it was shown that 3,3'-T_2 is exported by oocytes co-injected with LAT1 and CD98 (Friesema et al., 2001). However, LAT2 is not involved in TH export, examined in LAT2 overexpressed *Xenopus laevis* oocytes (Hinz et al., 2017). In summary, there are initial hints that LAT1 and LAT2 show distinct preferences for import of particular iodo-compounds, whereas in contrast LAT3 and LAT4 specifically facilitate the export of 3,3'-T_2 and MIT. These findings suggest that different sets of transporters with specific capacities either for import or export may cooperate to regulate cellular thyroid states.

Substrates need distinct features to cross LAT

As well as the transporter mutations, substrate variants, like TH and BCH-similar compounds have also been investigated for LAT2. Six important substrate features are depicted in figure 5. Substrates that are similar to TH, but show different variations at its functional groups have been selected for 3,3'-T_2 inhibition studies of LAT2. It turned out that only if both functional groups (NH2; COOH) of the AA moiety are available, is an inhibition of import of 3,3'-T_2 by LAT2 detectable (Hinz et al, 2017). They probably serve as matching counterparts for the substrate recognition site between TMH1 and 6. In close proximity to the AA functional groups a distinct spatial dimension of this hydrophobic moiety is needed to fit into the translocation pathway of LAT2 otherwise the transport is sterically hindered. Hydrophilic and hydrophobic substrate variants of substituents at the tyrosyl ring in positions 3 and 5 are still transported. All these substrates, even a trifluoromethyl group inhibited the import of 3,3'-T_2 competitively (Hinz et al., 2015). No inhibition was observed for bulky substituents on the phenolic ring, which limits the flexibility of the substrate. It is obvious that diminished ring flexibility of the two aromatic rings hampers and rigid conformation prevents translocation. This explains why import of T_3 by LAT2 is restricted and that of T_4 is impeded. In contrast, TH molecules possessing at least two iodine atoms irrespective of the position are flexible enough to be imported or are able to inhibit the 3,3'-T_2 uptake by LAT2.

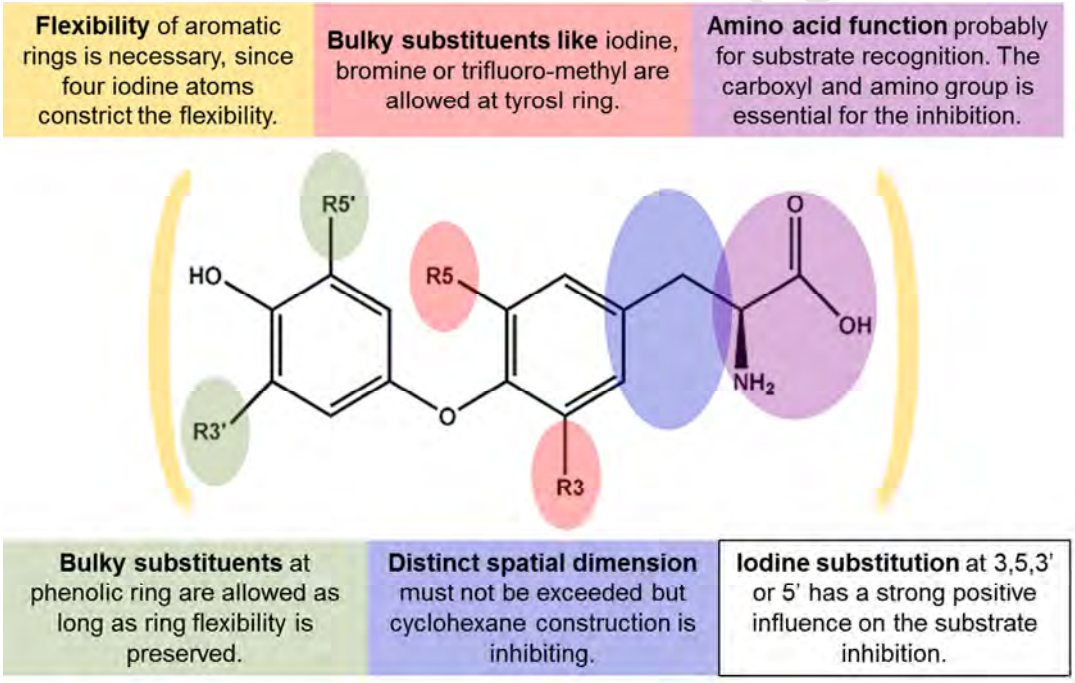

Figure 5: *Result scheme of various TH derivatives indicating pharmacophoric features (colored) that are necessary for experimentally detected import by LAT2* (Hinz et al., 2017, 2015)*. Conformational flexibility of the* phenolic *ring in relation to the tyrosyl ring of TH in dependence of differing iodine substitutions showed that 3,3'-T_2 and 3',5'-T_2 are flexible enough for import, while in T_4 steric hindrance of the four bulky iodine atoms restricts flexibility.*

Several docking and transport studies have been performed for LAT1. As mentioned before, substrate spectrum is not to the same as LAT2. Among AAs, THs, BCH and their derivatives, more features were found for LAT1. The amino acid function is not a stringent requirement. For LAT1 it has recently

been shown that it is possible to replace the ligand's carboxyl group by hydroxamic acid, albeit with a reduced effect on efflux rate and gabapentin inhibition (Zur et al., 2016). Experimental studies with tyrosine or phenylalanine derivatives demonstrated loss of efflux activity, but increased the inhibition of gabapentin. This suggests that large lipophilic meta substitution of tyrosine or phenylalanine would result in inhibitors rather than substrates (Augustyn et al., 2016). A specific LAT1 inhibitor, JPH203, which suppresses L-leucine uptake has been shown by Wempe et al. (Wempe et al., 2012).

Positive influence of iodine atoms

Although small methyl or bulkier isopropyl aliphatic substitutions on positions R3, R5 of the tyrosyl- or on R3' of the phenolic ring (figure 5) are allowed for thyroid hormone derivatives. A clear strong influence of iodine atoms on these positions has been shown by transport or competition studies of 3,3'-T_2 (Hinz et al., 2017).

The results of the experiment greatly indicate that iodine atoms indeed have a critical influence on the transport/import of 3,3'-T_2. A similar behavior was detected for LAT1. 3,5-diiodo-L-tyrosine shows a 25 % higher inhibition than 3-iodo-L-tyrosine on the Gabapentin import (Geier et al., 2013). This can be explained by an established X-bond interaction of polarized halogens. X-bond interaction is most easily understood as primarily an electrostatically driven molecular interaction. According to quantum chemical considerations halogens exhibit an electropositive crown, or σ-hole, that serves as a Lewis acid to attract a variety of electron-rich Lewis bases, in analogous fashion to classical hydrogen bonding (H-bond) interactions. The bulky iodine possesses the largest σ-hole, which preferentially attracts carboxyl atoms, aromatic pi-system and hydrogens of H-bonds (reviewed in (Scholfield et al., 2013)). This also explains why the translocation pathway in LAT2 is lined not only by aromatic residues (Y130, F242), providing the pi-system, but also by hydrophilic residues (N133, N248) providing hydrogens of H-donating bonds respectively.

Conclusions

Taken together molecular modelling is an invaluable tool for modelling proteins that lack crystal structures, such as LATs. Molecular models supported the elucidation of structure-function relationships of TH interaction, especially in LAT2. LAT2 is completely covered extracellularly by CD98 and modelling also provided information about putative interactions between the two proteins. LAT2 models contributed to our understanding of the molecular transport mechanisms of recently characterized determinants that are responsible for different TH import and export profiles. Finally, a LAT1 model helped explain why both carboxylic acid and hydroxamic acids can be recognized, which were found to be transported by LAT1. LAT models are also suitable in predicting novel substrate pharmacophores or specific inhibitors.

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Characterization of different TH import and export profiles for LAT2.

LAT2-features identified that determine bidirectional amino acid transport but only a unidirectional 3,3'-T_2 and T_3 import.

LAT models are also suitable in predicting novel substrate.

Structure-function patterns of the TH transport by LAT.