

# **The structure of the human 4F2hc-LAT1 heteromeric amino acid transporter**

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Amino acids are biologically important molecules that serve as energy source, building blocks of proteins, precursors for metabolites and signaling compounds in all living cells. Transport of proteinogenic amino acids and related substances across biological membranes is mediated by membrane embedded proteins: the amino acid transporters. These membrane proteins belong to different solute carrier (SLC) families (<http://slc.bioparadigms.org>) and transfer amino acids in and out between compartments inside cells, between different cells and between organs. Amino acid transporters have diverse important physiological functions and their absence, overexpression and malfunction can lead to human diseases.

Heteromeric amino acid transporters (HATs) are the unique example of solute transporters composed of two different subunits, a heavy and a light subunit, that are linked by a conserved disulfide bridge (Fotiadis et al., 2013). Heavy subunits belong to the SLC3 family and are required for the correct trafficking of the heterodimer to the plasma membrane. Light subunits are members of the SLC7 family and are responsible for solute transport conferring substrate specificity. Currently, the SLC3 family consists of the two glycoproteins [rBAT (SLC3A1) and 4F2hc (SLC3A2; also known as CD98hc)], and the SLC7 family of 15 amino acid transporters (Fotiadis et al., 2013). Eight of these SLC7 proteins associate with rBAT or 4F2hc to form HATs (Fotiadis et al., 2013).

The L-type amino acid transporter 1 (LAT1; SLC7A5) is a sodium-independent amino acid transporter with specificity for large neutral amino acids, e.g., leucine, tyrosine and tryptophan, and functions as obligatory exchanger with a 1:1 stoichiometry (Fotiadis et al., 2013). The protein is highly hydrophobic, not glycosylated and is predicted to contain 12 transmembrane domains (TMs) with cytoplasmic N- and C-termini. The light subunit LAT1 associates with the heavy subunit 4F2hc, which is a type II membrane glycoprotein that comprises a relatively small, cytoplasmic N-terminal domain, a single TM and a large C-terminal ectodomain. The structure of the 4F2hc-ectodomain was previously determined by X-ray crystallography (Fort et al., 2007).

LAT1 is expressed in different organs and tissues, and is highly overexpressed in immune T cells, the blood-brain barrier and a wide range of tumor cells (Fotiadis et al., 2013; Nii et al., 2001; Scalise et al., 2018). In the central nervous system, LAT1 participates in the transport of L-3,4-dihydroxyphenylalanine (L-DOPA) across the blood-brain barrier. Therefore, the development of prodrugs for transport through LAT1 represents an attractive

strategy for brain drug delivery. Overexpressed LAT1 in various cancer types mediates cellular uptake of leucine and other essential amino acids regulating cell proliferation via activation of the mammalian target of rapamycin (mTOR) (Fotiadis et al., 2013; Scalise et al., 2018). The observation that inhibition of mTOR exerts antitumor effects promoted the idea to develop specific LAT1 inhibitors for therapeutic applications, and PET tracers for tumor imaging and diagnosis (Fotiadis et al., 2013; Scalise et al., 2018). Recently, specific mutations in LAT1 were associated with autism spectrum disorder (Tarlungeanu et al., 2016).

In March 2019, the group of Qiang Zhou published the long-sought structure of the human HAT 4F2hc-LAT1 (Yan et al., 2019). Thus, no experimental structure of LAT1 was available until recently, and most structure-function and pharmacological studies were based on LAT1 homology models built from bacterial amino acid transporter structures (Chien et al., 2018; Geier et al., 2013; Ilgü et al., 2018; Zur et al., 2016). For HATs, structural information was only available at low resolution from negative-stain electron microscopy (EM) (Meury et al., 2014; Rosell et al., 2014) and cryo-EM (Jeckelmann and Fotiadis, 2019) for the human 4F2hc-LAT2 complex. LAT2 is also an SLC7 family member and has an amino acid sequence identity of almost 50% with LAT1. In general, HAT complexes are laborious to produce heterologously and purification is challenging, because of their inherent instability, e.g., breakage of the intersubunit disulfide bridge and complex, and protein aggregation. Because of these features, the 4F2hc glycosylation dependent sample heterogeneity, and that crystallization requires several days for crystal growth thus burdening the protein sample, X-ray crystallography failed in providing a structure of human HATs in the past. The breakthrough came thanks to cryo-EM where crystallization is not required and microgram amounts of protein suffice for three-dimensional (3D) structure reconstruction from single particle projections (Cheng et al., 2015). Because of the unique advantages of cryo-EM biomolecular structure determination, the Nobel Prize for Chemistry 2017 was awarded to this revolutionary technology.

The cryo-EM structure of 4F2hc-LAT1 unveiled the architecture of this HAT at the molecular level (Figure 1A). LAT1 consists of twelve TMs and displays the canonical LeuT fold. The transporter is clamped by three structural elements of 4F2hc, i.e., by the large C-terminal ectodomain (ED), the single transmembrane helix TM1-F and a short N-terminal peripheral helix (H1-F). The large glycosylated ED of 4F2hc is positioned on top of LAT1 and sugar moieties at the four glycosylation sites are exposed to the extracellular space. The

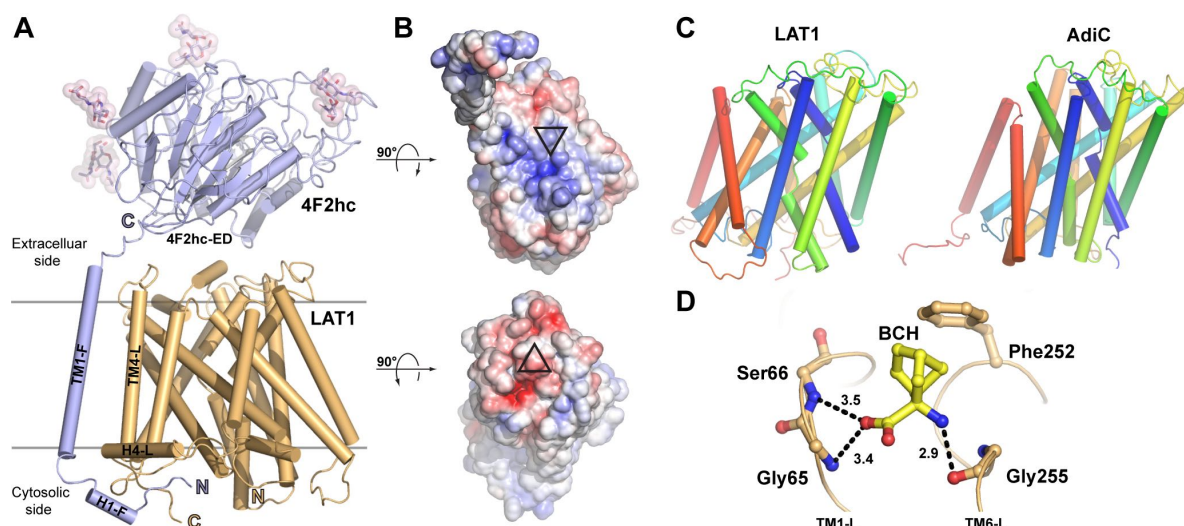
interacting surfaces have opposed surface charges, i.e., LAT1 negatively and 4F2hc positively charged, indicating electrostatic interactions between the two proteins (Figure 1B). Besides the salt bridge between Lys533 (4F2hc) and Glu303 (LAT1), hydrogen bonds between polar residues of 4F2hc and LAT1 further stabilize the interaction between the two subunits on the extracellular side. The single transmembrane helix of 4F2hc (TM1-F) interacts with TM4 of LAT1 (TM4-L) via extensive hydrophobic interactions, which are mediated by highly conserved residues (Figure 1A). A new structural element of LAT1 represents a C-terminal, peripheral amphipathic helix (H4-L) that follows the twelfth and last TM. H4-L interacts with the short cytoplasmic helix H1-F of 4F2hc, thus further stabilizing the complex on the cytoplasmic side. In the intracellular side of the complex, two lipid molecules could be resolved interacting with LAT1 and 4F2hc (Yan et al., 2019).

Comparison of the structures of LAT1 and the L-arginine/agmatine transport AdiC from *Escherichia coli* (Ilgü et al., 2016) shows the high conservation of the LeuT fold from bacteria to human (Figure 1C). The structure of 4F2hc-LAT1 was solved alone and in complex with the non-metabolizable amino acid analog 2-amino-2-norbornanecarboxylic acid (BCH) (Yan et al., 2019). This competitive inhibitor allowed identification of the LAT1 substrate binding site and the molecular binding mechanism of BCH. In the binding pocket, the benzene ring of the Phe252 residue participates in hydrophobic interactions with the norbornane moiety of BCH (Figure 1D). The amino and carboxyl groups of BCH form hydrogen bonds with main-chain atoms of TM6 (Gly255) and TM1 (Gly65 and Ser66) of LAT1 (Figure 1D). This is in line with the previous findings that  $\alpha$ -amino and  $\alpha$ -carboxylate groups of the amino acid substrates Phe and Arg of LAT1 and AdiC are recognized and bound by protein backbone atoms and not residue side chains (Ilgü et al., 2018). Phe252 in LAT1 corresponds to Trp202 in AdiC, which was also shown to be critical for substrate binding (Ilgü et al., 2016). Comparison of the substrate binding sites of human LAT1 and selected bacterial amino acid transporters showed conservation (Yan et al., 2019).

The cryo-EM structures of 4F2hc-LAT1 provide a molecular framework that can be used to analyze disease-related mutations. Two mutations in LAT1 (Ala246Val and Pro375Leu) were recently related to autism spectrum disorders (Tarlungeanu et al., 2016). Both LAT1 mutants are able to form a complex with 4F2hc similar to wild-type (Yan et al., 2019), but have impaired transport functions, i.e., A246V is virtually inactive and P375L has an about five-times higher internal  $K_m$  compared to wild-type (Tarlungeanu et al., 2016). The

4F2hc-LAT1 structure locates Ala246 on TM6. Its substitution to Val was speculated to affect the helix packing between the functionally important TM1 and TM6 of LAT1 (Yan et al., 2019). Pro375 is at the intracellular tip of TM9, which forms a coordination center for surrounding hydrophobic residues of other close structural elements, and the aliphatic tails of the two identified bound lipids (see above) (Yan et al., 2019). Because of this critical position in the structure, it was proposed that the Pro375Leu substitution might alter the local structure and affect LAT1 transport activity (Yan et al., 2019).

In summary, the cryo-EM structure combined with functional studies provide new and important insights into the architecture and working mechanism of the human HAT 4F2hc-LAT1, and the molecular reasons for disease-associated LAT1 mutations.



**Figure 1** Organization and structure of the human 4F2hc-LAT1 complex and comparison of human LAT1 with the bacterial homologue AdIC. **(A)** Side view (i.e., view from within the membrane plane) of the human 4F2hc-LAT1 structure (displayed as ribbon representation). 4F2hc (light blue) is composed of a small N-terminal cytosolic domain (including H1-F), one transmembrane segment (TM1-F) and a large C-terminal ectodomain (4F2hc-ED). The ectodomain structure resolved secondary structure elements and sugar moieties (displayed as sticks and highlighted in pink) at four glycosylation sites. The transporter LAT1 (light orange) is embedded in the lipid bilayer (boundaries marked by horizontal grey lines), and is composed of twelve transmembrane domains (TMs), four  $\alpha$ -helical loop segments, and cytosolic N- and C-termini. **(B)** The extracellular interface between 4F2hc and LAT1 is depicted as electrostatic potential surfaces by splitting apart the individual subunits, and rotating them by 90° and -90°, respectively. The location of residues involved in the salt bridge formation are indicated (4F2hc-Lys533, downwards oriented triangle; LAT1-Glu303, upwards oriented triangle). The colors white, blue and red represent neutral, positively and negatively charged residues, respectively. **(C)** Comparison of the overall topologies of human LAT1 (*left*) and the bacterial homologue AdIC (*right*). In spite of the different conformational states of the two protein structures (LAT1: inward-open and AdIC: outward-open), conservation of the overall topology is evident. The TMs are displayed as ribbon representation and rainbow colored (N-terminus, blue; C-terminus red). **(D)** View into the substrate binding site of LAT1. Amino acids of LAT1 involved in interactions and binding of the competitive inhibitor 2-amino-2-norbornanecarboxylic acid (BCH) (yellow sticks) are displayed. Gly65 and Ser66 are located in TM1-L and interact with the carboxyl-group of BCH. Phe252 and Gly255 are located in TM6-L and interact with the hydrophobic norbornane and amino group of BCH, respectively. Hydrogen bond distances to the main chain atoms of Gly65, Ser66 and Gly255 are indicated.

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