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Targeting SLC Transporters: Small Molecules as Modulators and Therapeutic Opportunities

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Abstract

Solute carrier (SLCs) transporters mediate the transport of a broad range of solutes across biological membranes. Dysregulation of SLCs has been associated with various pathologies, including metabolic and neurological disorders, as well as cancer and rare diseases. SLCs are therefore emerging as key targets for therapeutic intervention with several recently approved drugs targeting these proteins. Unlocking this large and complex group of proteins is essential to identifying unknown SLC targets and develop next generation SLC therapeutics. Recent progress in experimental and computational techniques has significantly advanced SLC research including drug discovery. Here, we review emerging topics in therapeutic discovery of SLCs, focusing on state-of-the-art approaches in structural, chemical, and computational biology, and discuss current challenges in transporter drug discovery.

Keywords

Membrane protein; drug design; protein structure prediction; ligand discovery

Biological importance of Solute Carrier (SLC) transporters

Membrane transporters play a critical role in communication between the cell and the environment. The largest membrane transport group in humans is the Solute Carrier (SLC) transporters that consists of 455 members classified into 66 families [1]. The SLCs transport a broad range of **substrates** (see Glossary), including nutrients, neurotransmitters, ions, and drugs, and take part in numerous biological processes, such as regulation of cell signaling and organization of the cellular organelles [2]. A large fraction of the SLCs are secondary

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active transporters that couple electrochemical gradient of ions and/or other molecules to transport substrates (*eg* **symport**, **antiport**) [1, 3]. Genetic variations in many SLC members have been linked to various diseases and disorders, such as neurological disorders, metabolic disease, and cancer [4, 5].

Notably, despite their biological importance, SLCs are still considered to be among the most understudied proteins relative to their family size, and many aspects of their biology remain unknown. Analyses by us and others highlight the gap in the current knowledge of human SLCs [2, 5-8]: many **orphan transporters** have no known substrates [5, 9] and established SLC transporters have no known chemical modulators [10], and a variety of disease-related mutations in SLCs have not been characterized [5]. Further, over ten protein families have been reclassified as SLCs in the past decade, such as the pyrophosphate exporter, progressive ankylosis protein homolog (ANKH; SLC62A1) [11], and the lysosomal cholesterol transporter Niemann-Pick disease type C1 (NPC1; SLC65A1) [12]. Notably, newly developed computational and experimental approaches discussed herein have addressed important questions in SLC biology, including long sought-after questions related to disease mechanisms and substrate specificity, as well as fast-growing fields in SLC research such as **allosteric modulation** and drug development (Key Figure, Figure 1).

Due to their broad physiological roles, the SLCs are proteins of the utmost pharmacological importance. Uptake and efflux SLC transporters are often localized in organs and tissues such as the kidney, liver, and blood-brain-barrier (BBB), where they control the absorption, distribution, and excretion of therapeutic drugs [13, 14]. For example, the peptide transporter PepT1 (SLC15A1) regulates the intestinal absorption of peptide-like drugs, such as β-lactam antibiotics (*eg* cefadroxil) and antiviral drugs (*eg* valacyclovir) across the cell membrane [15, 16]. Therefore, genetic variations in these transporters are often associated with differential drug response among patient populations (*ie* **pharmacogenomics**) [13]. In fact, genetic polymorphisms of the organic cation transporter OCT1 (SLC22A1), which is mainly expressed in the liver, impact the disposition, distribution, and toxicity of prescription drugs such as metformin, among different ethnic groups [17].

Moreover, multiple SLCs have been validated as important targets for therapeutic intervention [18]. Over the past 50 years, the monoamine transporters, including the norepinephrine transporter NET (SLC6A2), dopamine transporter DAT (SLC6A3), and serotonin transporter SERT (SLC6A4) have been targeted by drugs for treating of neurological and psychiatric disorders [19]. For instance, selective serotonin reuptake inhibitors (SSRIs, *eg* Escitalopram) and serotonin–norepinephrine reuptake inhibitors (SNRIs, *eg* Duloxetine), which increase neurotransmitter concentrations in synapses and control neurotransmission, are often prescribed to treat depression and anxiety disorders [20]. More recently, the SLC target space has been expanded to a variety of other indications such as metabolic disorders, cancer, and other pathologies [18]. For example, sodium-glucose cotransporter-2 SGLT2 (SLC5A2) inhibitors (*eg* canagliflozin) are used for the treatment of type 2 diabetes by lowering blood sugar levels [21]. Other novel SLC targets with drug candidates in clinical investigation include the creatine transporter CRTR (SLC6A8; gastrointestinal cancer, clinical trial number NCT03597581) and glycine

transporter 1 GlyT1 (SLC6A9; schizophrenia) [22]. Finally, drugs targeting established SLC targets have been recently repurposed to address new indications. For example, the SGLT2 inhibitor Licogliflozin [23] and the apical sodium bile acid transporter ASBT (SLC10A2) inhibitor Elobixibat (clinical trial number NCT04006145 [24]) are currently in clinical trials for treating liver disease including nonalcoholic steatohepatitis (NASH).

In this review, we discuss emerging topics in SLC biology, including SLC structure and mechanism, the chemical space of SLC ligands, strategies to modulate SLC function, and how the most recent advances in computational modeling can be applied to characterize the SLCs. Finally, we discuss current challenges in SLC drug discovery.

SLC structures in rational drug design

The three-dimensional structures of SLC transporters can help address fundamental questions related to their biology, including description of mechanisms of membrane transport and substrate specificity, as well as development of predictive models for the effect of disease-related mutations on SLC function. Over the past decade, there has been a surge in the number of experimentally determined structures of SLCs, primarily due to progress in cryo-electron micrography (cryo-EM) technologies for structure determination of membrane proteins [25]. These structures have shown that, unlike other functionally defined "Superfamilies" (*eg* ABC transporters, GPCRs), the SLCs are highly diverse in structure, consisting of several evolutionary unrelated, distinct structural classes or folds (Figure 2A) [6, 7, 26], where the Major Facilitator Superfamily (MFS; *eg* glucose transporters GLUTs (SLC2)) and the leucine transporter LeuT-like fold (*eg* neurotransmitters transporters of the SLC6 family) are the most common structural classes in the human SLCs [6] (Figure 2B). It was observed that conserved functionally important elements among members of distinct families (*ie* SLC6 and SLC7) within a structural class (*ie* LeuT-fold), allow for efficient functional annotation among SLC members [27]. Notably, it has been shown that transmembrane helices 1 and 6 (TM1 and TM6) are anchoring the ligands through interactions with backbone atoms of conserved residues (*eg* the GXG motif in TM1), while TM3, TM8, and TM10 consist of variable residues conferring the selectivity of the ligand for the transporter (Figure 2B).

Interestingly, despite their dissimilarity in structure, SLCs use a conserved **'alternating access' transport mechanism**, in which the transporter interchangeably exposes its binding site at either side of the membrane [28]. The alternating access model is facilitated through the internal symmetry within the transporter structure, regardless of its structural class [29]. Notably, multiple structures were determined in different conformation of the transport cycle for only a small number human SLCs, where the majority of SLCs with known structures have only been solved in one or two conformations [18]. For example, proteins belonging to MFS such as the glucose transporters GLUTs (SLC2) use a **rocker-switch mechanism**, while members of the LeuT-like (*eg* neurotransmitters transporters of the SLC6 family) and Glt_{Ph}-like (*eg* the amino acid transporters SLC1) structural classes use **gated-pore** and **elevator transport mechanisms**, respectively (reviewed in [8, 30]).

In addition to describing transport mechanisms, atomic resolution structures of SLC drug targets can be used for rational drug design. For example, structures of the cancer-related transporters of monocarboxylates (MCT1; SLC16A1) [31], glutamine (ASCT2; SLC1A5) [32], cystine (xCT, SLC7A11) [33], leucine (LAT1, SLC7A5) [34], and glucose (GLUT1, SLC2A1) [35] have been used to develop compounds targeting reprogrammed metabolic networks in cancer. Furthermore, the structures of SLCs related to drug transport and dynamics, such as the intestinal transporter PepT1 [36] and the liver and brain transporter OCT3 [37], have revealed previously unknown mechanisms of drug absorption, drug-drug interactions, and pharmacogenomics.

Notably, distinct conformations can be used to develop conformation-specific binders with unique scaffolds and specificity profiles [38]. Indeed, up until recently, it was thought that the most optimal transporter conformation for rational design would be outward-facing conformations, as observed in the SERT-escitalopram complex [39]. However, it was shown that small molecule inhibitors can also target inward conformations (Figure 2B) [34, 40-44]. In fact, SERT–ibogaine complexes were solved in multiple SERT states, including outward-facing, occluded, and inward-facing conformations [44]. Interestingly, one mechanism has been proposed in which an inhibitor targeting inward-facing conformation first diffuses across the membrane and subsequently binds the transporter within the cell [45]. This putative mechanism can potentially allow the compound to avoid competing with endogenous substrates found in the rich extracellular media. Taken together, these studies open new avenues for the design of novel small molecule inhibitors targeting particular conformations of the transport cycle.

Pharmacological space of SLCs

A **tool compound** can be defined as a chemical that selectively controls the function of a protein, allowing researchers to address fundamental and mechanistic questions about the target protein by using a range of experimental approaches, such as biochemical and cellular assays or *in vivo* methodologies [46]. Tool compounds can also potentially provide a starting point for the development of lead compounds for future therapeutics. Over the past decade, newly developed tool compounds targeting SLCs have advanced their structural and functional characterization. For example, a small molecule inhibitor of the cancer-related amino acid transporter ASCT2 (SLC1A5) called *Lc*-PBE was designed using a homology model based on the EAAT1 (SLC1A3) X-ray structure in a ligand bound outward-facing state; the compound facilitated the experimental structure determination of ASCT2 in a unique conformation, which allowed the further development of potent and selective ASCT2 inhibitors [45].

Notably, the current known space of SLC ligands is limited, where major barriers for effective discovery of useful chemical probes for SLCs include the limited availability of assays for this class of proteins [10] and the lack of SLC structures in distinct conformations available for rational drug design [18]. Analysis of the ChEMBL database [47] shows that small molecule ligands of SLCs, as measured by IC_{50} values of 1 mM or lower, exist for only 97 SLCs . Interestingly, there is a significant bias for well-established drug targets such as the neurotransmitter transporters, NET and DAT, and sugar transporters, SGLT1 and

GLUT1, where each protein has more than 1,000 inhibitors (Figure 3), whereas hundreds of SLCs do not have any reported inhibitors. This highlights the need for potent and selective chemical tools for SLC transporters. In addition, analysis of relationships between proteins based on the chemical similarity of their small molecule ligands can reveal functional associations, as well as guide the deorphanization of proteins [48, 49] (Box 1).

Chemical modulation of SLCs

Small molecule ligands of SLCs can control their function *via* different mechanisms. An SLC inhibitor can selectively inhibit the transport of substrates *via* the transporter across the cellular membrane. An inhibitor can bind the substrate binding site (‘**orthosteric inhibitor**’), competing with the substrate and blocking its binding and/or the conformational changes that are associated with transport. Over the past decade, many orthosteric inhibitors have been developed for a range of biomedically important transporters including the Na+/Citrate Cotransporter NaCT (SLC13A5) [50], glycine transporter-1 GlyT1 (SLC6A9) [42], and the GABA transporter 1 GAT1 (SLC6A1) [40]. For example, orthosteric inhibitors of SLCs that are associated with reprogrammed metabolic networks in cancer can deprive the tumor cell of nutrients. A recent study analyzed structure-activity relationship (SAR) around the xCT (SLC7A11) inhibitor sulfasalazine, allowing for the investigation of the toxicity of this drug when administered to patients. As a result, several potent xCT inhibitors were designed and validated in various cancer cell lines and presented minimal toxicity profiles in normal human astrocytes [51]. Interestingly, another recent study showed that inhibition of GLUT1 (SLC2A1) and/or GLUT3 (SLC2A3) resulted in **disulfidptosis,** a unique mechanism of cell death in SLC7A11^{high} cancer cells. This work provides a novel strategy for treating numerous cancers with high xCT expression [52]. Moreover, it was shown that drugs targeting the **orthosteric binding site** in distinct SERT [53] and DAT [38] conformations can lead to differential pharmacological effect. This suggests that SLC transporters can modulate biased signaling potentially allowing for fine tuning pharmacological effects of drugs.

Alternatively, an inhibitor can bind a site distant from the substrate binding site or the substrate transport pathway (‘**allosteric inhibitor**’). Allosteric binding sites are often less conserved within a protein family and can thus potentially be targeted with more selective inhibitors than the substrate binding site inhibitors [54]. Moreover, the allosteric sites are more likely to be targeted by molecules chemically different from the endogenous substrates that bind the substrate binding site, and thus avoid off-target binding to proteins with similar substrate specificity. Thus, one significant advantage of allosteric inhibitors is that they can potentially improve drug-like properties and selectivity, which are often not seen in transporters’ substrate-like compounds such as amino acid-like or sugar-like ligands. Allosteric inhibitors in transporters have so far been described in members of the SLC6 family [55] (*eg* SERT/SLC6A4 [56], DAT/SLC6A2 [57], and GlyT2/SLC6A5 [58]), and the SLC1 family (*eg* in EAAT1/SLC1A3 [59]). For example, one mechanism of allosteric inhibition is binding the interface between the scaffold and mobile domains of the SLC1 family of elevator transporters, hence hindering the conformational change that is needed for transport, as observed for the allosteric EAAT1 inhibitor UCPH$_{101}$ [60] (Figure 4A). Interestingly, the UCPH$_{101}$ binding site overlaps with equivalent lipid binding sites in its

homologs EAAT3 (Figure 4A) and ASCT2 (SLC1A5), where cholesterol was proposed to be important for ASCT2 function [32]. Interestingly, a recent study revealed that this allosteric mechanism is conserved among SLC1 members and that subtle differences in the allosteric binding site allow the identification of selective allosteric inhibitors, thereby providing an avenue for future drug development for members of this highly important SLC family [61].

Additionally, allosteric binding sites can be targeted by small molecule activators – compounds enhancing the transport efficiency of natural or synthetic substrates across biological membranes. To the best of our knowledge, only one allosteric activator has been rationally designed for an SLC, the glutamate transporter EAAT2 (SLC1A2) [62]. This activator is localized at the interface between the trimerization and transport domain, another region proposed to be important for enabling the conformational change required for transport. Binding sites of sterols and other lipids in other transporters have also been shown to be amenable for allosteric modulation, further demonstrating the importance of these molecules for function, similarly to other membrane protein families such as ion channels [63, 64], where cholesterol can interact with its target, triggering conformational changes that are associated with activation or inhibition. For example, recent structural, biochemical, and pharmacological data suggest that the transport of substrates by the LeuT-fold transporter LAT1 (SLC7A5) depends on its interaction with SLC3A2 and is mediated by two lipid molecules, as well as by cholesterol which serves as a LAT1 activator [34] (Figure 4B). Further, a variety of related SLCs with a LeuT-fold are modulated by lipids, such as the dopamine transporter DAT (SLC6A3) [65, 66], serotonin transporter SERT (SLC6A4) [67], and glycine transporter GlyT2 (SLC6A5) [58].

Another type of activators are **pharmacochaperones**, compounds that rescue folding and/or trafficking of misfolded proteins. Pharmacochaperones improve the activity of proteins carrying disease-causing mutations that affect their folding, stability, or localization to the membrane [68]. For example, pharmacochaperones have been recently developed for the treatment of cystic fibrosis, caused by a specific mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) [69, 70]. These drugs represent a novel strategy of rescuing the function of a malfunctioned transporter through small molecule binding. Particularly, the CFTR modulators can be classified into two groups: i) ‘potentiators’ (i.e., Ivacaftor) are compounds that increase the channel conductance; and ii) ‘correctors’ (i.e., Elexacaftor and Tezacaftor) are molecules that rescue CFTR folding and trafficking to the plasma membrane [70]. The necessity of combining both types of modulators for trafficking and functional rescue shows the complexity of pharmacochaperones development. Pharmacocharperoning development has recently been explored for the monoamine transporters of the SLC6 family [71, 72]. For example, derivatives of the psychedelic drug ibogaine were able to correct folding deficient DAT (SLC1A3), thus paving the way for rational design of pharmacochaperones targeting SLC transporters.

Importantly, SLCs have different regions that control other aspects of their function, including post-translational modifications and protein-protein interactions [73]. For example, DAT physically and functionally interacts with the voltage-gated K+ channel

Kv2.1 to modulate dopamine neurotransmission [74]. Moreover, the C-terminus of DAT binds Ca^{2+}/calmodulin-dependent protein kinase II α (CaMKIIα) to facilitate phosphorylation of the N-terminus of DAT, thereby modulating amphetamine-induced dopamine efflux [75]. Interestingly, the termini of the SLC6 members are divergent in sequence and size and are thought to play a critical role in functional differences among these proteins, including their interactions with substrates and lipids [73, 76]. Some of these interacting regions can be highly dynamic, or even involve unstructured regions; thus, developing small molecule compounds targeting these regions is highly challenging.

Finally, over the past decade, new approaches have emerged to control proteins through their selective degradation [77]. For instance, **PROteolysis-TArgeting ChimeraS (PROTACS)** are bifunctional molecules that consist of a small molecule protein ligand that is connected via a linker to an E3 ubiquitin ligase (E3) ligand that recruits the protein degradation machinery [78]. PROTACs as well as other degraders have shown promise on a variety of targets, including tau [79] and KRAS [80], as well as multiple compounds currently tested in clinical trials. Designing a PROTAC for SLCs is particularly challenging. It requires the formation of a tertiary complex from a large variety of warheads, linkers, and E3 ligase ligand. Moreover, it needs to bind the target from the intracellular domain, which might not include an accessible, druggable site. It was recently shown that selected SLCs representing distinct SLC families are amenable for degradation by PROTACS, providing encouraging data for using this approach to modulate transporters [81]. For example, a degrader with broad specificity for the SLC9 family that showed promising data on cancer cell line was developed [81].

Computational modeling approaches

The SLCs adopt distinct conformational states to allow for alternating access transport of substrates. Despite advancements in structure determination of membrane proteins with experimental techniques, using these approaches to characterize different conformations of membrane can be costly and time consuming. Computational protein structure prediction aims to bridge the gap between the sequence space of the SLCs and their structural coverage (Figure 5). Traditional modeling approaches include homology or template-based modeling (TBM), in which the target protein is modeled based on its sequence alignment to one or more known experimentally determined structure(s) of homolog protein(s) that serve as modeling templates. Conversely, in *de novo* or *ab initio* modeling, the target is modeled directly from its amino acid sequence [82]. The accuracy of these methods has significantly improved over the years, partly due to the integration of spatial restraints derived from the analysis of sequence co-evolution [83] or experimental data [84].

Molecular Dynamics (MD) simulations have played a central role in describing dynamic properties of membrane proteins [85], and are particularly useful when combined with experimental methods [86]. For example, MD simulations were used to describe different aspects of SERT mechanism, including domain movement [87], ion binding [88], substrate specificity [89], oligomerization [90], mechanism of inhibitor binding and the resulting conformational changes [44], as well as the effect of mutations on its structure/function [91]. Additionally, advances made in atomistic and coarse-grained (CG) force-fields have

allowed for improved modelling of protein-lipid interactions and the role lipids play on protein dynamics and function (reviewed in [92, 93]). MD has also been used to gain insights on lipid interactions with transporters affect conformation stability and transitions. For example, in DAT, PIP2 is involved in regulating the transition to the inward facing state by interacting with DAT's N-term and intracellular loop 4 [94]. Additionally, simulations aided in identifying a conserved cholesterol binding site in SERT and cholesterol binding stabilizes SERT in an outward facing conformation [67].

Notably, atomic-level simulations were employed to characterize the full transport cycle of the bacterial glucose transporter SemiSWEET, including transitions from the transporter's outward-facing to its inward-facing conformation [95]. This analysis was performed on one of the smallest known transporters with a simple geometry and energy coupling mechanism; however, MD simulations' ability to model the translocation cycle in human SLCs is more challenging due to the long timescales required to overcome the high free-energy barriers that separate distinct states, as well as the inaccuracies of the force fields used.

One way to address some of the challenges in unguided all-atom simulations is by using biased or guided simulation techniques (reviewed in [96]), which often provide novel mechanistic insights on SLC transporters, especially when combined with experimental testing. For example, accelerated MD (aMD) applied to the human DAT revealed unknown insights into the sequential gating and transport events of this protein [97].

Alternatively, artificial intelligence (AI) and machine learning (ML) based methods have recently emerged as powerful and accurate approaches for structure prediction. Specifically, the recent release of the two open-source structure prediction methods AlphaFold2 (AF2) [98] and RosettaFold [99] launched a new era in protein structure prediction, providing tremendous support for addressing fundamental scientific questions using structural biology insights. For example, AF2 has been applied to a variety of challenging problems, such as structure modeling of protein complexes [100] and large assemblies [101], as well as experimental structure determination of particularly challenging targets [102] and identification of protein disordered regions [103].

A current limitation of modern AI based modeling is that there is no straightforward one-size-fits-all procedure that accurately captures the conformational diversity of proteins [104-106]. Moreover, it is unclear whether these methods can accurately model the protein's amino acid sidechains [107], binding pockets or point mutations effect on structure [108], which are critical for rational drug design. Therefore, model generation should be performed and evaluated judiciously. One way to evaluate the relevance of the model for rational design is its ability to enrich for known ligands as compared to a data set of the ligands and likely non-binders or decoys, using docking [109] (Figure 5). By iteratively generating models and evaluating their binding site with enrichment the model's binding site is optimized for protein-small molecule ligand complementarity and structure-based ligand discovery [110, 111]. Sampling biologically relevant conformations to be evaluated with enrichment can be done with a range of modeling approaches such as sidechain modeling on a fixed backbone, MD simulations, as well as other approaches [111]. In addition, using integrated approaches that include data derived from low resolution experimental data can guide rational drug

design. For example, metainference MD simulations generate an ensemble of conformations that are consistent with available cryo-EM data, accounting for the concurrent presence of data ensemble-averaging, structural heterogeneity, and noise level variability in different regions of the experimental map [112]. In a recent study, metainference has advanced the discovery of a unique binding site and inhibitor conformation that was useful for the design of potent inhibitors [45].

Overall, computer guided ligand discovery campaigns have been applied to characterize a range of SLC transporters, representing different families and mechanisms, including PepT1 (SLC15A1) [113], NaCT [114] LAT1 (SLC7A5) [115], BGT1 (SLC6A12) [116], and GLUT3 (SLC2A3) [117]. Recently, a virtual screen of an ultra-large compound library (200 million compounds) on a membrane transporter was conducted using the inward-open SERT structure, leading to the discovery of selective SERT inhibitors with potencies up to 200 times better than the SSRI fluoxetine as well as improved efficacy in various mouse behavioral models. This study also demonstrated that even for a highly studied membrane transporter target, new structural information and improved computational approaches can inform the development novel lead compounds [53].

Concluding remarks

Recent advancements in chemical and structural biology methodologies, as well as in our conceptual understanding of the importance of the transport process, have allowed emergence of the SLCs as a major drug target family. The wealth of structural information and newly established computational methods can also allow us to rationalize how polymorphisms cause disease or differential drug response among individuals. Can we predict which mutation would be neutral, loss-of-function, or gain-of-function [118]? Describing mutation's effect on structure / function is expected to improve the understanding of disease mechanisms, identify novel drug targets, and advance precision medicine.

Furthermore, structures of biomedically important transporters revealed previously unknown mechanisms of transport modulation that can be harnessed for the development of next generation of transporter drugs. For instance, transporters utilizing elevator-like transport mechanisms can be targeted with small molecules physically blocking the movement of the mobile domain (*eg* in EAAT1; Figure 4). Moreover, substrate binding site inhibitors targeting specific conformations, such as those of SERT lead to compounds with improved *in vivo* efficacy, further refining our understanding of pharmacological control of transporter function. Fine-tuning transporter function with chemical tools can lead to new areas in transporter pharmacology and drug design, such as pharmacology of biased signaling in GPCRs [119].

In addition, the CFTR drugs showed that small molecule activators can correct disease phenotype caused by transporter malfunctions. There are hundreds of diseases associated mutations causing defective transporters, which in principle, could potentially be targeted with approaches similar to those taken in the development of the CFTR drugs. However, key questions remain: are there general rules that determine which compound will be a substrate that goes through the transporter, an inhibitor that binds the transporter and blocks

transport, or an activator that improves the transport of a defective transporter? Generalized strategies to develop each compound type are expected to allow the development of chemical tools that facilitate the characterization of SLCs as well as future SLC drugs. Finally, transporters often contain other regions that mediate other aspects of their function, including protein-protein interactions and post-translational modification. Description of the structure / function of these regions is expected to reveal unknown modulatory surfaces that are amenable for drug design.

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Glossary

Allosteric inhibitor
an inhibitor that binds to a site different from the orthosteric site of the transporter

Allosteric modulation
mechanism of regulation in which a molecule that binds at a different site than the substrate binding site and enhances (allosteric activators) or inhibits (allosteric inhibitor) SLC transport

Alternating access
a process in which membrane transporters undergo conformational changes to alternate between different states such as outward-facing, occluded, and inward-facing, enabling selective movement of substrates across the membrane

Antiport
two or more different substrates, such as molecules or ions, are concurrently transported across the cell membrane in opposite directions

Disulfidptosis
regulated cell death arising from disulfide stress induced by elevated SLC7A11 expression combined with glucose starvation

Elevator
a type of alternating access transport where the substrate binds the transport domain and is then moved across the membrane via a significant rigid-body movement of the transport domain against the scaffold domain, which is typically involved in oligomerization

Gated-pore or rocking-bundle
a type of alternating access transport mechanism in which a static scaffold domain and a mobile bundle domain that alternatively opens and close during the transport mechanism

Molecular dynamics (MD) simulation

predicting the positions of atoms in a biomolecular system over time by applying Newton's equations using a force field to specify the system's parameters

Orphan transporter
a transporter whose endogenous substrate or physiological function has not yet been characterized

Orthosteric inhibitor
an inhibitor that binds to the substrate or orthosteric site of the transporter

Orthosteric binding site
the binding site of the transporter's substrate or other competitive ligands

Pharmacochaperone
a small molecule that aids in the correct folding, stabilization, and/or trafficking of misfolded or unstable proteins, thereby rescuing their functional activity

Pharmacogenomics
the study of how individual's response to drugs is influenced by their genetic makeup

PROteolysis-TArgeting Chimera (PROTAC)
bifunctional molecule consisting of a small molecule protein ligand that is connected via a linker to an E3 ubiquitin ligase (E3) ligand that recruits the protein degradation machinery

Rocker-switch
a type of alternating access transport mechanism in which two symmetrically related domains shift around a central substrate-binding site, with the protein essentially moving around the substrate to alternately expose the binding site to either of the membrane

Substrate
a molecule or an ion that gets transported across the membrane by a transporter

Symport
two or more different substrates, such as molecules or ions, are concurrently transported across the cell membrane in the same direction

Transport mechanism
the process of mediating substrate movement across the membrane with three commonly described states, including outward-facing, occluded, and inward-facing conformations

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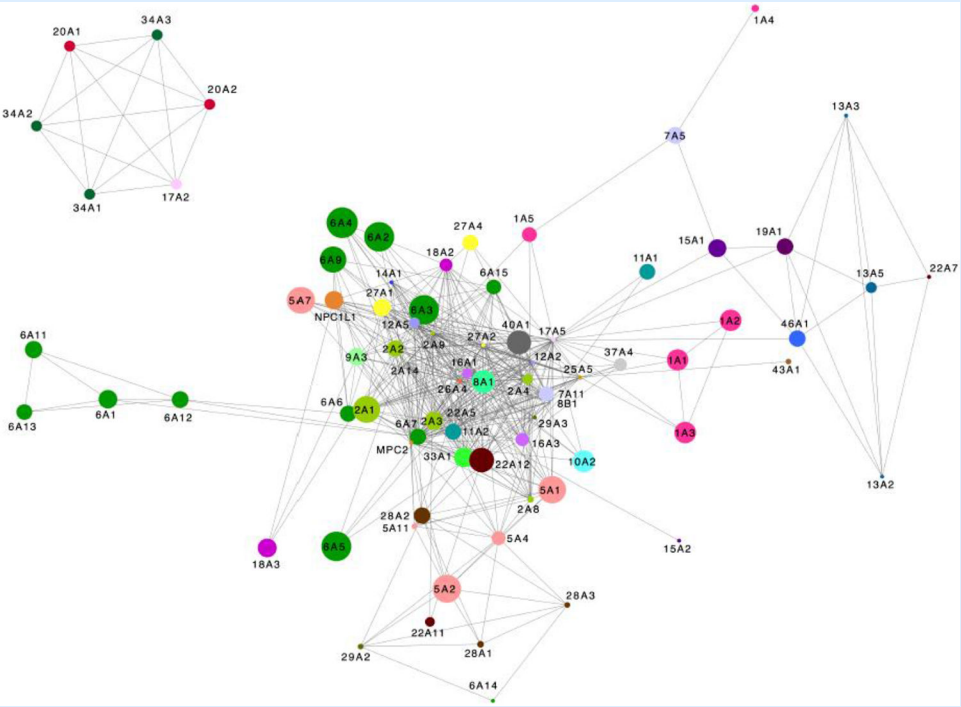
Box 1.

Pharmacological space of SLCs.

To demonstrate the relationships between proteins based on the chemical similarity of their small molecule ligands, we generated, as an example, a network in which the SLCs are connected to each other if they have chemically similar small molecule ligands in ChEMBL (Figure I). We observe that SLCs belonging to different, and often evolutionarily unrelated families, can be highly connected in the pharmacological space (Figure I). Some relationships among SLCs are expected from the overlap in their natural substrates. For example, members the evolutionarily unrelated sugar transporter families, including the SLC2 family of glucose transporters (GLUTs; light green; MFS fold) and the SLC5 family of sodium-dependent glucose transporters (SGLTs; salmon; LeuT-like fold) are highly interconnected based on the chemical similarity of their ligands. Similarly, phosphate transporters belonging to the distinct SLC families SLC34, SLC20, and SLC17 are also connected, forming a unique cluster. Interestingly, other connections among SLCs are not entirely obvious from their natural substrates, such as the subcluster formed by the sodium-dependent citric acid cycle (CAC) metabolites transporters of the SLC13 family (SLC13A2,3,5) and the unrelated folate transporters SLC19A1 and SLC46A1. Moreover, some members of the same SLC family are more closely associated with members of other SLC families than those of their own family. For example, SLC1A5, a neutral amino acid transporter, is linked only indirectly to SLC1 family members (SLC1A1,2,3) that transport acidic amino acids (*eg* glutamate), through SLC17A5, which transports sialic acids. Overall, filling the gap in the knowledge of the SLC ligands will allow us to connect more SLCs in the pharmacological space thereby guiding functional annotation and deorphanization of the SLCs.



Box 1, Figure I: Pharmacological space of SLCs.

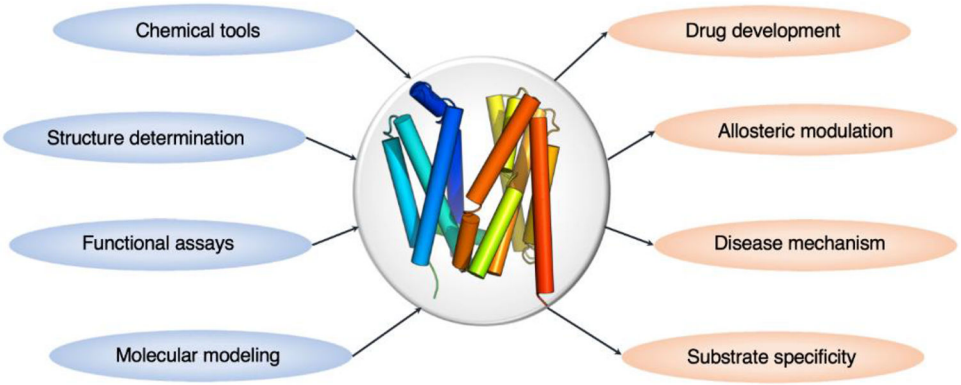
The size of the circle in the map corresponds to the number of known small molecule inhibitors of the SLC according to the criteria described above. The distance between two SLCs is the inverse of the sum of the dice similarities based on the RDkit morgan fingerprint [123], with radius two, of every combination of pair of inhibitors from the two SLC inhibitor sets normalized by the number of comparisons. The arrangement of the SLCs is dictated by the Cytoscape edge weighted, force directed biolayout [124].

Outstanding Questions

- Are there general structural determinants of SLC modulators that discriminate between substrates, inhibitors, and activators?
- Can we accurately predict mutational effect on SLC structure / function (neutral, gain-of-function, loss-of-function), to improve the understanding of disease mechanisms and identify novel SLC drug targets?
- Can we harness emerging AI technologies and superior computational power to improve our understanding of transport mechanisms and guide the development of future SLC drugs?

Highlights

- Solute carrier (SLC) transporters are a highly understudied class of proteins that transport ions, nutrients, and drugs, across biological membranes, and are often mutated in disease.
- Unlocking this large and complex group of proteins is essential to identify unknown SLC targets and develop next generation SLC drugs.
- Recent advances in chemical, structural, and computational biology have allowed to develop innovative strategies to modulate their function as well as unique tool compounds and future drugs.



Key Figure, Figure 1:

Overview of the emerging topics in SLC research. Approaches advancing the characterization of SLCs are shown on the left-hand side of the figure, while the biological questions currently addressed by the community are shown on the right-hand side.

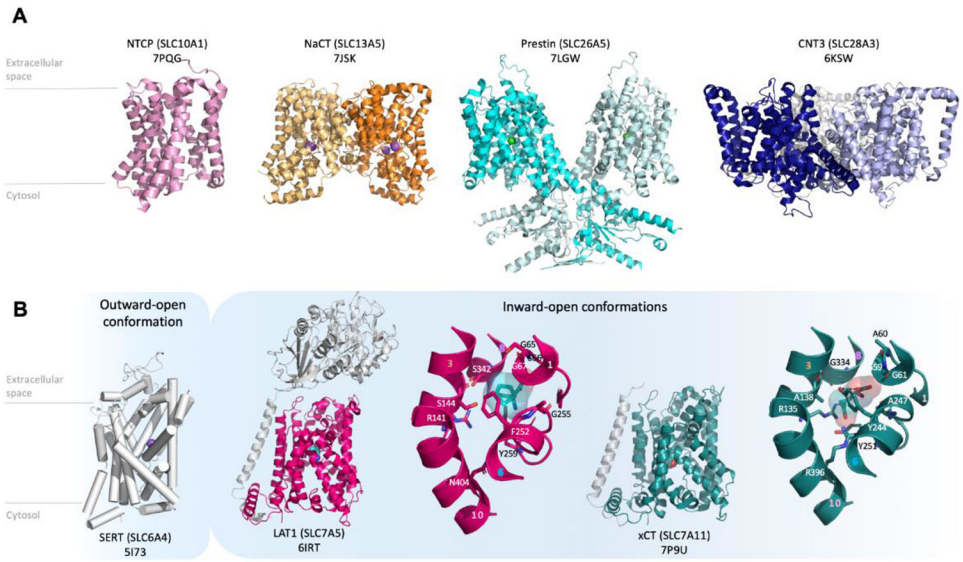


Figure 2: SLC structure and dynamics.

(**A**) Recent SLC structures revealed previously unknown structural classes or folds that operate *via* the alternating access mechanism, including, from left to right NTCP (SLC10A1) (PDB id 7PQG [120]), NaCT (SLC13A5) (PDB id 7JSK [50]), Prestin (SLC26A5) (PDB id 7LGW [121]) and CNT3 (SLC28A3) (PDB id 6KSW [122]). (**B**) Outward open structure of SERT (SLC6A4) (PDB id 5I73 [39]). Inhibitors-bound structures in inward-open conformations of the LeuT fold transporters LAT1 (SLC7A5) (PDB id 6IRT [34]), and, xCT (SLC7A11) (PDB id 7P9U [41]) as well as a close up view of their respective binding sites. Residues defining the binding site are labeled, with TM1 and TM6 labeled in white and blue, and TM3, TM8, and TM10 in orange, purple and pink, respectively.

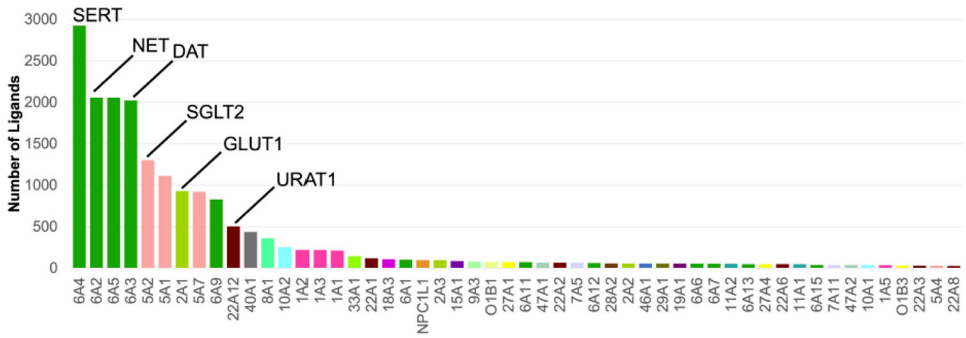


Figure 3: Small molecule ligands of SLCs.

Each inhibitor is defined as having IC_{50} value of 1mM or lower. Analysis of ChEMBL [47] identified 97 SLCs with at least one inhibitor using this criterion (Supplementary Table 1). 52 SLCs had 25 or more ligands and are shown here. The colors correspond to the SLC family of each protein.

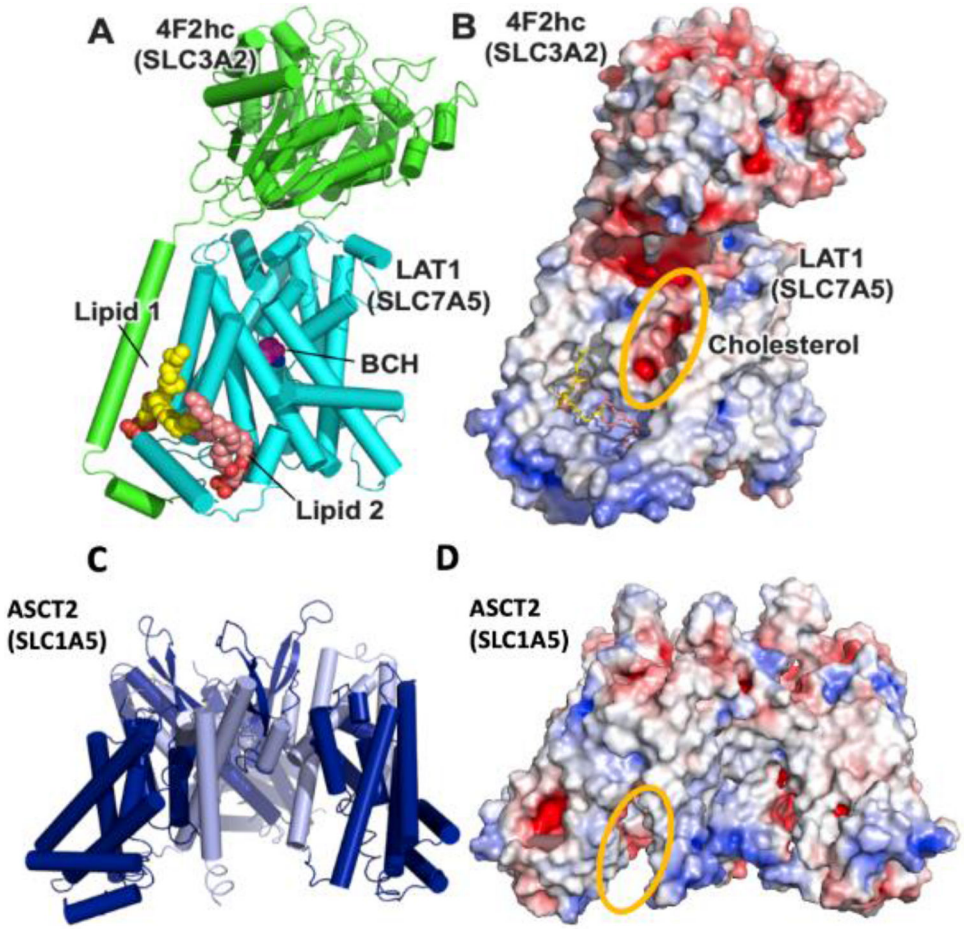


Figure 4: Allosteric modulatory surfaces in amino acid transporters.

(**A**) Trimeric structure of Excitatory amino acid transporter 3 EAAT3 (SLC1A1) shown as cylinder (left; PDB id 6S3Q) where each protomer is shown in a different color. Phospholipids in the interface between protomer 1 and 2 are shown as purple and yellow spheres. Also shown is surface representation of protomer 1 of EAAT1 (SLC1A3) (right; PDB id 5MJU) with the allosteric inhibitor $UCPH_{101}$ (orange sticks), whose location overlaps with the location of the phospholipids colored in purple in EAAT3. (**B**) Heterodimeric structure of LAT1 (SLC7A5; cyan) and 4F2hc (SLC3A2; green) (PDB id 6IRT) in complex with two lipid molecules (yellow and pink), and the substrate binding site inhibitor BCH (magenta; left). Also shown is surface representation of LAT1 structure colored based on electrostatic potential (right). The putative cholesterol binding site is highlighted in orange.

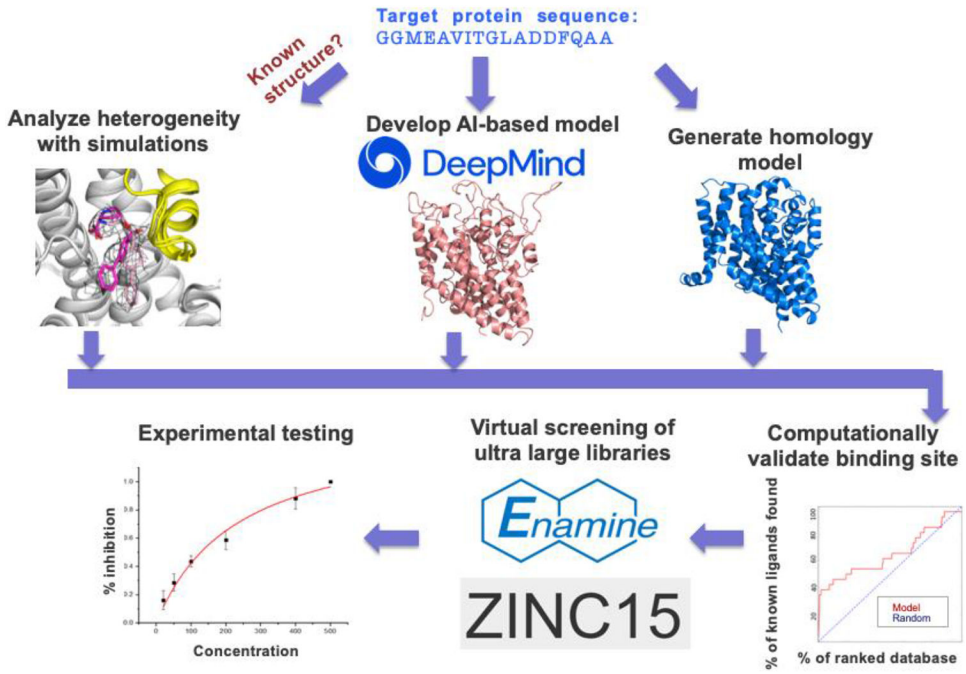


Figure 5: Workflow for structure-based virtual screening of SLC transporters.

For transporters with known structures, MD simulations can be applied to sample different druggable conformations. For SLCs with unknown structure, or for those SLCs that lack structure in a desirable conformation, structural models can be generated using the AI-based method AlphaFold2 or through homology modeling. All models can then be evaluated on the basis of their ability to predict known ligands (when applicable) using enrichment, and subsequently refined based on these results. Virtual screening of large purchasable compound libraries is then performed where top scoring compounds are tested experimentally.