Technologies for transporter drug discovery

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Transporters represent attractive targets for drug discovery and are implicated in the pathophysiology of disorders across several therapeutic areas, including asthma, cardiovascular disease, diabetes and neuroscience. However, the intrinsic mechanistic properties of transporters present significant challenges to the development of high-throughput screening methodologies. This review provides an update on potential transporter targets and evaluates the impact of available technologies to enable transporter screening, lead optimization and assessment of pharmacokinetics.

Introduction

The uptake and efflux of essential nutrients and drugs, such as sugars, amino acids, inorganic ions and therapeutically useful agents, into and out of cells and organelles is controlled by transporters and channels that lower the free energy required for molecules to traverse membranes. Ion channels allow the selective movement of ions down their electrochemical gradients through a defined mechanism and when opened they allow permeation at rates close to diffusion control (reviewed in ref. 1). In contrast, transporters have a fixed stoichiometry of ion/solute movement per translocation cycle. Energetically, facilitated transporters allow the passage of solutes down their electrochemical gradients (e.g., glucose through GLUT4 [SLC2A4], Fig. 1, Table 1), while active transporters create ion/solute gradients across membranes utilizing diverse coupling mechanisms. Primary-active ATP-dependent transporters bind or hydrolyze ATP (e.g., P-glycoprotein/MDR1 [ABCB1]) to control the movement of various substances out of cells or into cellular organelles, while ion pumps hydrolyze ATP to generate and maintain electrochemical ion gradients that can be coupled to secondary-active transporters driving the concentrative uptake of substrates across biological membranes (e.g., Na⁺/glucose through SGLT2 [SLC5A2]; reviewed in ref. 2). Taken together, there are currently 46 different solute carrier (SLC)-transporter families and over 360 transport genes reported (www.bioparadigms.org). In addition, there are four distinct types of non-SLC transport ATPases (P-, F-, V- and ABC) (reviewed in ref. 3), of which the ABC superfamily contains 48 human genes in seven families.4

Consistent with the observation that ~60% of drug targets are located at the cell surface,3 transporters have been explicitly identified as the primary molecular target in the action of several important drugs used for the treatment of hypertension, heart failure, gastrointestinal disorders, atherosclerosis and various psychiatric disorders, including anxiety and depression (Table 2). Indeed, a recent estimate places the sodium:neurotransmitter symporter family as seventh in the top ten gene families targeted by currently available drugs.5 Furthermore, a number of transporters are being pursued for an array of indications suggesting that this class of proteins continues to represent attractive targets for drug development (Table 3).

It is important to note that none of the currently available drugs targeting transporters were developed using gene-targeted, high-throughput screening strategies. Chlorothiazide,6,7 furosemide,8 neurotransmitter uptake inhibitors9 and ezetimibe10 were identified using animal pharmacology to guide medicinal chemistry, while anti-cancer agents transported by nucleoside transporters are nucleoside analogues.11 Although clearly successful, such approaches present significant challenges in today’s drug discovery environment. For instance, drugs targeting identified molecular entities are preferred by regulatory agencies because it is easier to understand their pharmacological profile. In addition, the use of substrate analogues tends to generate a congested chemical space leading to obvious intellectual property issues. For these reasons among many others, transporter-targeted drug discovery is moving towards using high throughput platforms that will allow the identification of novel chemical entities.

Unlike the field of ion channels that boasts robust high throughput ligand binding assays, functional fluorescent screening platforms (VIPR™, FLIPR™) and radiotracer fluxes, as well as medium throughput automated electrophysiological technologies (IonWorks Quattro™ (Molecular Devices); PatchExpress (Axon/Molecular Devices); QPatch (Sophion Bioscience); Patchliner (Nanion Technologies); Flyion (GmbH)),12 the implementation of similar platforms for studying transporters has been lagging due to fundamental conceptual and practical distinctions between ion channels and transporters. First, while channels allow ions to move down their electrochemical gradients at rates of 10⁷–10⁸ ions per second, transporters build up and maintain gradients through substantial conformational changes in order to ensure minimal substrate ‘leak’.13 This type of operation translates into a relatively slow movement of substrate(s), typically at approximately 10–10⁵ molecules per second.14-16 Second, while ion channels by definition
move net charge across the membrane, transporters can be either electrogenic (e.g., NCX1 [SLC8A1], SGLT1 [SLC5A1]) or electroneutral (e.g., NCC [SLC12A3], GLUT4) (Fig. 1). Thus, many transporters are not amenable to the standard electrophysiological approaches exploited to study ion channels. Third, many potential transporter targets are expressed in intracellular compartments (e.g., DIC [SLC25A10, mitochondrial dicarboxylate exchanger], ZnT8 [SLC30A8, β-cell secretory granule zinc transporter]), which require novel assay development approaches that are often not cell-based (Fig. 1). This review summarizes the current technologies and approaches available to support the discovery of modulators that target the large transporter family, and speculates on the challenges that must be overcome to improve the delivery of new therapeutics altering the activity of these proteins.

**Technologies for Transporter Drug Discovery**

The prioritization of targets for drug development is a multi-factorial process, taking into consideration many sources of information including, but not restricted to, human and mouse genetics, tissue distribution and pharmacological proof of concept. Rarely is all of this information available when initially considering a target and, therefore, in addition to the often-unknown impact on pathophysiology, tractability represents a significant factor in determining whether to move forward a given target (i.e., the ability to establish high-throughput primary and secondary screens). Assuming that the substrate(s) is known, screening a large compound library (>10⁶ compounds) on a transporter target requires a robust, high-capacity assay operating in a high-density plate format (384- or 1536-well). Furthermore, effective confirmation and optimization of hit compounds requires the development of secondary assays, ideally making use of complementary technologies in order to identify positive hits and support lead optimization efforts. In the following sections, we attempt to illustrate how the nature of the transport mechanism (i.e., electrogenic versus electroneutral) and substrate(s) dictates choice of functional assays and the nature of the read-out.

**Fluorescence**

Voltage-sensing dye(s). Electrogenic transporter and ion channel function can lead to changes in membrane potential, and this property of ion channels has allowed their activity to be indirectly evaluated using voltage sensing dye(s).

Invitrogen provide FRET-based, voltage-sensing dyes, originally developed in Roger Tsien’s laboratory. The FRET donor (dye 1) is a coumarin dye linked to a phospholipid that inserts into the outer leaflet of the cell membrane, and the FRET acceptor (dye 2) is an oxonol derivative (Fig. 2A), a lipophilic anion that quickly partitions within the membrane according to the membrane potential. In a hyperpolarized cell, excitation of the coumarin produces FRET and excites oxonol, which then emits a fluorescent signal at 580 nm. Depolarization of the cell membrane causes the voltage-sensitive oxonol to move to the inner

![Figure 1. Solute-carrier (SLC) and non-SLC transporters are present in the plasma membrane or intracellular compartments of a prototypic cell.](image-url)
dyes was recently introduced (FLIPR T etra, Molecular Devices) with improvements in throughput related to its simultaneous measurement of a whole plate. Finally, Hamamatsu Corp., has just released a fluorescent plate reader (FDS7000) with improved liquid handling capabilities and versatility (www.hamamatsu.com).

Molecular Devices offer a proprietary membrane-potential dye kit used for the measurement of changes in membrane potential. It utilizes a voltage-sensing dye mixed with proprietary fluorescent quenchers that maximize throughput by enabling a homogenous assay format. The quencher(s) absorb the emission of the voltage-sensitive dye when it is positioned in the outer layer of the cell membrane. When the cell membrane is depolarized, the dye moves to the inner layer of the cell membrane and upon excitation emits a detectable signal. The temporal resolution of this dye is in the layer of the cell membrane, increasing the physical distance (>100 nm) between the two dyes and disrupting FRET. Under these conditions, the emission from dye 1 (460 nm) is enhanced while the emission from dye 2 is reduced (Fig. 2B, blue and red traces, respectively). These events are quantified as a ratio of emission detected from the FRET donor and FRET acceptor (Fig. 2B, green trace). Thus, FRET-based voltage dyes provide relatively rapid temporal resolution (approximately seconds) and the ratiometric measurement of change in membrane potential helps to reduce assay artifacts. Originally, the Voltage Ion Probe Reader instrument (VIPRTM, Aurora Discovery, San Diego, CA), specifically designed for FRET-based assays, was used to support a high throughput screening campaign in 384-well format. More recently, a multi-wavelength fluorescence indicator probe reader instrument that can collect data using ratiometric

| Compound (Marketed drug) | Disease | Molecular target(s) |
|--------------------------|---------|---------------------|
| Fluoxetine HCl (SSRI, ProzacTM) | Depression, anxiety, autism | SERT (SLC6A4)9 |
| Reboxetine (NRI, ProliftTM) | Depression, panic, ADD/ADHD | NET (SLC62A2)81 |
| Bupropion (WellbutrinTM) | Depression, smoking sensation | NET (SLC62A2) and DAT (SLC62A3)81 |
| Duloxetine (SNRI, CymbaltaTM) | Depression, diabetic neuropathic pain | NET (SLC62A2) and SERT (SLC6A4)81 |
| Triptane (SGRI, CymbaltaTM) | Anxiety/panic disorder (SGRI) | GAT-1 (SLC6A1)82 |
| Hydrochlorothiazide (OreticTM) | Heart Failure, Hypertension, | NCC (SLC12A3)6,7 |
| Furosemide (LasixTM) | Heart Failure, edema | NKCC2 (SLC12A1)83 |
| Digoxin (LanoxinTM) | Heart Failure, atrial Fibrillation & flutter | Na+/K+-ATPase84,85 |
| Omeprazole (PrilosecTM) | G.I. reflux | H+/K+-ATPase86,87 |
| Ezetimibe (ZetiaTM & VytorinTM) | Atherosclerosis | Niemann-Pick C1-like 1 (NPC1L1)10,88 |

| Table 2 | Transporters are the target of marketed drugs for diverse therapeutic indications |
|---------|----------------------------------|
| Table 3 | A selection of transporter targets across therapeutic areas |

Layer of the cell membrane, increasing the physical distance (>100 nm) between the two dyes and disrupting FRET. Under these conditions, the emission from dye 1 (460 nm) is enhanced while the emission from dye 2 is reduced (Fig. 2B, blue and red traces, respectively). These events are quantified as a ratio of emission detected from the FRET donor and FRET acceptor (Fig. 2B, green trace). Thus, FRET-based voltage dyes provide relatively rapid temporal resolution (approximately seconds) and the ratiometric measurement of change in membrane potential helps to reduce assay artifacts. Originally, the Voltage Ion Probe Reader instrument (VIPRTM, Aurora Discovery, San Diego, CA), specifically designed for FRET-based assays, was used to support a high throughput screening campaign in 384-well format. More recently, a multi-wavelength fluorescence indicator probe reader instrument that can collect data using ratiometric
range of minutes, slower than the FRET-based voltage-sensing dye combination.

High-throughput functional assays for SGLT1,26 GAT-3 (SLC6A15)27 and GlyT2 (SLC6A5)28 have been developed using the FRET-based voltage-sensing dyes and membrane potential dye kit (Fig. 2C). A similar assay has also been shown to provide a read-out for the Na+/K+ ATPase.29 Since these assays take advantage of net charge movement during transporter function, the low turnover rate of transporters, resulting in relatively limited charge movement under steady-state conditions, has presented a challenge in assay development. Critical to the optimization of functional assays monitoring change in membrane potential are the levels of transporter expression in cell lines, which should be able to maximize measurable substrate-dependent changes in membrane potential. In the case of human SGLT1 expressed in HEK293 cells, the electrophysiological demonstration that substrate addition caused cells to depolarize by ~25 mV provided the foundation for the development of a membrane potential, FRET-based assay (Fig. 2B).26 Utilizing this cell line, VIPR™ and FLIPR™ assays were developed which display appropriate substrate specificity, ionic dependence and phlorizin sensitivity.26 Thus, the combination of molecular cell biology and voltage-sensing dyes has the potential for supporting primary screening of electroneutral transporters, such as SGLT2 (SLC5A2), a major re-uptake mechanism of glucose in the proximal tubule of the kidney. Inhibitors of SGLT2 are being evaluated in clinical trials for the treatment of type II diabetes mellitus.30,31

Fluorescently engineered substrate(s). The availability of fluorescent substrate analogues of either electrogenic or electroneutral transporters provides a powerful means for developing high-throughput functional assays using fluorescent plate readers (FlexStation, FLIPR™). For example, 4-((4-diethylaminostyryl)-N-methylpyridinium iodide (ASP+), used to study organic cation transport in LLC-PK1 cells,32 is being exploited to establish high-throughput assays for dopamine (DA), noradrenaline (NE) and serotonin (5-HT) transporters.35,36 the molecular targets for anti-depressants and drugs of abuse. Like [3H]DA and [3H]NE, ASP+ is transported in a Na+-, Cl- - and temperature-dependent manner by the monoamine transporters DAT (SLC6A3), NET (SLC6A2) and SERT (SLC6A4). Critically, by including a quencher of extracellular ASP+ fluorescence, and monitoring intracellular fluorescence (Fig. 3A), it has been possible to develop homogenous assays using either a Flexstation™ or FLIPR™ for these co-transporters (Fig. 3B). Such monoamine transporter assays are currently driving the development of triple re-uptake inhibitors for therapeutic indications including attention deficit hyperactivity disorder, depression, pain, anxiety, obsessive compulsive disorder and panic attacks.39

Ion-specific fluorescent probes. In many cases, it is not possible to engineer a fluorescent version of a transporter substrate while retaining the properties of the unlabeled substrate (e.g., Na+ or Cl- for thiazide-sensitive NCC). Therefore, to enable the development of fluorescence-based functional assays, the interaction of substrate(s) with intracellular dyes leading to a change of fluorescence has been explored.

Extensive engineering of enhanced yellow fluorescent protein (EYFP) has identified a variant, EYFP-H148Q/I152L, that binds halides such as Cl- and I- at physiological concentrations, resulting in fluorescent quenching.40,41 By stably expressing EYFP-H148Q/I152L in mammalian cells together with an appropriate transporter/ion channel target, it is possible to monitor changes in the concentration of intracellular anions with standard fluorescent plate readers (Flexstation™, FLIPR™) to identify CFTR agonists42 and anion channel modulators (http://www.moleculardevices.com/pages/event_presentations_2007_FLIPR_users.html). Recently, the electroneutral anion exchanger PDS (SLC26A4) has been implicated in the pathophysiology of asthma and chronic obstructive pulmonary disease.43,44 Interestingly, PDS-mediated, electroneutral exchange of extracellular iodide for intracellular chloride can be monitored using quench of EYFP fluorescence (Fig. 4),43 demonstrating the feasibility of the approach for the development of high-throughput functional assays that can drive drug development of challenging targets.

Several non-protein, ion-specific fluorescent probes, such as calcium-indicator dyes, are also available for measuring intracellular ion concentrations. Calcium binding to fluorophores alters the fluorescent signal, which can be detected with an instrument, such as

![Figure 2. Exploiting voltage-sensing dyes to monitor the operation of electrogenic transporters. (A) Principle of voltage-sensing dyes. The excitation and emission frequencies of the FRET donor are 400 and 460 nm, respectively, and emission from the FRET acceptor at 580 nm. During depolarization, the FRET acceptor translocates to the inner membrane leaflet, resulting in a decrease in FRET emission at 580 nm and an increase in emission from the FRET acceptor at 460 nm. The relative intensity at 460 nm and 580 nm provides a read-out of changes in the membrane potential of the cell. (B) Substrate-induced depolarization in hSGLT1/HEK293 cells. HEK293 or hSGLT1/HEK293 cells in a 96-well plate were pre-incubated with the membrane potential fluorescence-sensitive dyes described in ref. 26 and placed in a VIPR™ instrument. Upon recording the emission of both CC2-DMPE (blue) and DiSBAC2 (red) for 3 s at 1 Hz, 50 mM α-MeGlc was added, and fluorescence from both dyes was monitored for an additional 47 s. The fluorescence emission ratio is also illustrated as a green line. Where indicated, 100 µM phlorizin was also present. (C) Stoichiometry of electrogenic transporters analyzed with voltage-sensing dyes. Schematic does not reflect oligomeric assembly of transporters.](https://www.landesbioscience.com/channels/315 FIG2.png)
displacement of a bound ligand can provide a means for establishing high throughput assays. Typically, these assays use a radioactive ligand, however, if the compound can be modified with a fluorescent tag while retaining potency, FP can provide an alternative technology for screening.46

FP measurements provide information on molecular probe mobility and the processes that modify it, including the interaction of the transporter with a modulator. In FP assays, free modulator rotates rapidly and the random mobility results in low FP. Upon binding of the low molecular weight tracer to a large, slowly rotating molecule, such as a transporter, bound modulators have restricted mobility resulting in high FP. FP therefore provides a direct insight into the extent of tracer binding. Advantageously, because polarization is a general property of fluorescent molecules, polarization-based readouts are somewhat less dye-dependent and less susceptible to environmental interferences, such as pH changes, than assays based on fluorescent intensity measurements. Furthermore, FP enables homogenous binding assays in a high throughput format since there is not a requirement to separate bound from free ligand. Although the application of FP to ion channels47 and transporters is in its infancy, tools enabling FP screening of therapeutically interesting transporters are beginning to emerge.48

Radioactivity

Traditional flux. Radiotracer is a powerful in vitro way of allowing functional detection of transport-modulating activities of potential pharmaceuticals, and has long been used to monitor transporter-mediated ion fluxes.49,50 Unlike fluorescent approaches, radiotracer substrates can be made available for every class of transporter and thus, although concerns over excessive radioactive waste and safety exist, radiotracers represent the only universal approach to transporter assay development.

Radiolabeled flux assays measure transporter activity by monitoring the uni-directional movement of substrate(s) in or out of cells, subcellular organelles or proteoliposomes. In the case of cells, after an appropriate incubation time, cells are separated from their medium and lysed. Radioactivity in both the lysate and supernatant can be measured by scintillation counting. Inhibition or activation of the transporter by a modulator will affect the concentration of radiolabeled material.51 Critically, radiotracer flux assays are reliable, direct, linear and flexible because they are easily performed with miniaturization and automation.52 Most recently, the substrate analogue of glucose, [14C]alpha-methyl-glucopyranoside (αMeGlc) has been widely used in cell based flux assays for the identification of compounds that inhibit kidney SGLT2 with high affinity while also displaying marked selectivity over the closely-related intestinal SGLT1 transporter.31,53,54

Binding assays. Radiolabel ligand-binding assays have been extensively used in drug screening, especially prior to the availability of cell
that will enable direct functional measurements of electrically active symporters.55-57

be performed with detergent-solubilized neurotransmitter:sodium to transfer energy. Recent studies indicate that SPA assays can also into 384- and 1536-well formats. Radiolabeled ligands bind to these plate to separate free from ligand-transporter complex, and therefore, require a plate wash step, which limits the screening throughput. In contrast, scintillation proximity assays (SPA) use solid scintillant-containing beads to capture cell membranes and can be miniaturized into 384- and 1536-well formats. Radiolabeled ligands bind to these membrane-coated beads, enabling homogenous detection since only the bound labeled ligand is close enough to the SPA beads to transfer energy. Recent studies indicate that SPA assays can also be performed with detergent-solubilized neurotransmitter:sodium symporters.55-57

**Electrophysiology**

**Patch-clamp.** The direct measurement of currents generated by ions flowing through transporters expressed at the cell surface provides a gold standard for measuring compound activity on electrogenic transporters in vitro. However, despite the high-quality functional data generated by this technique, in its traditional format, patch clamping has limited use in transporter drug screening because of the very low throughput. Rather it is utilized either to gain detailed mechanistic information regarding the interaction of modulators with a transporter,58 cell line validation or for determining whether cell lines expressing transporters undergo changes in membrane potential that are of sufficient magnitude to pursue the use of voltage sensitive dyes for establishing high capacity functional assays.26

Recently, several automated patch-clamp instruments have been developed and are now commercially available [IonWorks Quattro™ (Molecular Devices); PatchExpress (Axon/Molecular Devices); QPatch (Sophion Bioscience); Patchliner (Nanion Technologies); Flyion (GmbH)]. Although these automated instruments greatly enable secondary screening of ion channels,12 the current amplitudes required to obtain high quality data and reliable compound titrations is in the order of ~200 pA. Thus, for most transporters the signal to noise of these instruments is not sufficient for the measurement of the small currents typically associated with their function.26,59,60 However, transporters such as EAAC1 (SLC1A1), that display a chloride conductance coupled to substrate (glutamate) transport may be amenable to these automated technologies.59,60

**Surface electrogenic event reader (SURFE2R).** Recognizing the need for a highly sensitive, automated electrophysiological approach that will enable direct functional measurements of electrically active transporters, IonGate have developed and commercialized the SURFE2R instrument (www.iongate.de). The foundation of this technology relies on the ability to observe the charge displacements of electrically active transporters immobilized on solid supported membranes (SSM),61 by taking advantage of the high specific capacitance and the ruggedness of a planar lipid bilayer prepared on a solid support. Cell fragments, membranes or proteoliposomes containing transporter are adsorbed to the SSM, and the capacitively coupled currents generated by the transporter activity are recorded. The compound membrane formed by the adsorbed proteoliposomes and the SSM can withstand high flow velocities, allowing for fast solution exchange at the surface (Fig. 5A). By rapidly exchanging between solutions without and with substrate, a given transporter can be activated, and a transient current can be recorded that contains information about the size and the temporal development of the transport process. The latest generation of this technology, SURFE2R 500, provides the ability to test approximately 500–1000 compounds/day (Fig. 5B). In a typical experiment on rabbit SGLT1, after membranes have been equilibrated with a non-activating solution containing 50 mM mannitol, rapid exchange into an activating solution containing 50 mM αMeGlc leads to a capacitative Na+ current (Fig. 5C). Upon exchange into a non-activating solution, the transported Na+ re-equilibrates with the non-activating bulk solution leading to a slow Na+ current in the opposite direction. Upon total re-equilibration, the cycle, that typically takes ~10 s, can be repeated.

The SURFE2R has been utilized to monitor native or recombinantly expressed bacterial and mammalian transporters in membranes or purified and reconstituted systems (www.iongate.de). Notably, a comparison between manual patch clamp and SURFE2R measurements of NCX1 illustrates that IC50 values for inhibitors are comparable, and sensitivity is similar to patch voltage clamp methodologies.62

An interesting feature of the SURFE2R technology is the ability to measure electrogenic components of electroneutral transporters. For example, the omeprazole-sensitive H+/K+ ATPase utilizes ATP hydrolysis to catalyze the exchange of H+ and K+ ions (Fig. 5D). Using a K+-free non-activating solution to equilibrate membranes on the sensor, followed by the same solution containing 20 μM Na-ATP, the ATP-driven and omeprazole-sensitive H+-capacitative current can be monitored without the movement of K+ in the opposite direction.61 In a similar manner, electroneutral exchangers such as DIC,21 PDS63 and OATP8,64 may be amenable to this technology with the use of appropriate experimental regimes.

A unique feature of the SURFE2R technology is its operation in a cell-free format. Since the primary criteria for operation is the adsorption of membrane vesicles onto a solid support, the SURFE2R should be able to provide automated measurements on transporters and ligand gated ion channels that are expressed in intracellular compartments (e.g., mitochondria, synaptic vesicles). For example, IonGate have demonstrated that it is possible to adsorb synaptic vesicles from serotonergic neurons onto sensors and monitor Na+/Cl-/glutamate transport through the excitatory amino acid transporter EAAC1,65 and its inhibition by compounds.60 Thus, rather than attempting to recombinantly re-route intracellular transporters to the plasma membrane or purify and reconstitute the transporter of interest in planar bilayers for electrophysiological measurements, SURFE2R provides the possibility of performing experiments in a more native
substrate concentrations. Given that drug transporters significantly impact the pharmacokinetics of drug candidates, understanding whether a candidate compound is a substrate for a drug transporter can influence its drug development. Typically, this evaluation is carried out in vitro using monolayers of polarized cells (e.g., MDCK, LLC-PK1) expressing the transporter of interest (e.g., MDR1 to predict brain penetration, OATP1B1/1B3 to predict liver uptake) and presenting the candidate to either the apical or basolateral cell membrane compartment. After an appropriate incubation period, media from either compartment is collected and prepared for quantitation by liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS). Additional throughput can be obtained by exploiting the capability of LC/MS/MS to de-convolute multiple drug mixtures allowing sample pooling (i.e., multiple samples to be pooled before analysis). Biotrove have recently launched an LC-MS methodology allowing the analysis of a sample every 6 s providing a throughput of ~10,000 samples/day.

Conclusions and Perspectives

Physiology and human genetics have implicated some mechanistically well defined, as well as orphan, transporters in the pathophysiology of human disease. Given the diversity of substrates context. The utility of this approach for the mechanistic understanding and tractability of mitochondrial, synaptic vesicle and perhaps other intracellular transporters remains to be thoroughly explored.

Other Technologies for Measuring Transporter Activity

Atomic absorption spectrophotometry (AAS). Commercially available instrumentation for AAS has enabled the development of high-throughput, non-radioactive, ion flux assays for a variety of voltage-gated and ligand-gated channels. In these assays, the flux of non-radioactive tracer ions into or out of cells expressing ion channel/transporter of interest is determined by measuring the concentration of tracer ion in the supernatant and/or within the cells by AAS. Single-channel and multi-channel instruments are currently available for 96-well and 384-well assays providing moderate to high throughput (Aurora Biomed, Vancouver). The application of this technology has been primarily focused on K-channel assays where Rb⁺ is used as the tracer ion. However, AAS has also been used to monitor the activity of zinc transporters, including ZnT8, which transports zinc into secretory granules of β-cells, indicating that an agonist may benefit glucose-dependent insulin secretion.

Mass spectrometry. Mass spectrometry is an extremely sensitive, selective and rapid, label-free technology enabling measurement of substrate concentrations. Given that drug transporters significantly impact the pharmacokinetics of drug candidates, understanding whether a candidate compound is a substrate for a drug transporter can influence its drug development. Typically, this evaluation is carried out in vitro using monolayers of polarized cells (e.g., MDCK, LLC-PK1) expressing the transporter of interest (e.g., MDR1 to predict brain penetration, OATP1B1/1B3 to predict liver uptake) and presenting the candidate to either the apical or basolateral cell membrane compartment. After an appropriate incubation period, media from either compartment is collected and prepared for quantitation by liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS). Additional throughput can be obtained by exploiting the capability of LC/MS/MS to de-convolute multiple drug mixtures allowing sample pooling (i.e., multiple samples to be pooled before analysis). Biotrove have recently launched an LC-MS methodology allowing the analysis of a sample every 6 s providing a throughput of ~10,000 samples/day.
and mechanisms of transport, improving the tractability of transporters for drug discovery will require the integration of several complementary technologies, as summarized in Figure 6. In such an approach, radiotracer and fluorescent assays will provide the foundation for high throughput screening, while the confirmation of hits and understanding their mechanism of action will also make use of automated and/or conventional electrophysiology. In addition, other technologies, such as MS and AAS, may be leveraged to move transporter targets forward.

A fundamental question plaguing the pursuit of certain transporter targets is the feasibility of developing small molecule agonists that directly increase turnover rate. Mechanistically, high resolution, substrate-bound, crystal structures suggest that transporters tend to operate by two basic mechanisms, gated pore or rocker switch. Hypothetically, a direct agonist would likely increase the rate of transition between inward and outward facing conformational states, although it is conceivable that altering the equilibrium distribution between these states may be beneficial. Interestingly, structure-guided functional studies on the bacterial H+/Cl- antiporter (ClC-ec1) have illustrated that the rate of chloride flux through ClC-ec1 can be dramatically increased by point mutations in the gate that prevents the movement of chloride ions and protons, resulting in an uncoupled channel-like electrodiffusion of anions rather than a coupled alternative-exposure conformational cycle. While, ClC-ec1 belongs to the unique mixed ion channel/transporter CLC family, it is tempting to speculate that there may be direct agonists de-stabilizing transporter gates/increasing ‘rocking’, while retaining coupled transport, within the large compound libraries now amenable to screening with available technologies. In this context, Mellitech recently announced the identification of an agonist increasing ZnT8-mediated transport of zinc into secretory granules (www.mellitech.com) and Chorion announced the development of a positive allosteric modulator of KCC2 (SLC12A4) for the treatment of neuropathic pain (www.chorion.com), although the underlying mechanism(s) of increased transport turnover have not been disclosed.

Finally, improving the availability and throughput of transporter functional assays may provide a means to incorporate transporter-driven tissue targeting into drug discovery. For example, GlaxoSmithKline and Xenoporo have developed SolziraTM, a new chemical entity that is designed to improve upon the pharmacokinetics of gabapentin by developing a gabapentin-prodrug that takes advantage of high-capacity transport mechanisms in the gastrointestinal tract to improve drug absorption. SolziraTM (gabapentin enacarbil) extended release was recently submitted to the U.S. Food and Drug Administration as a new drug application for the treatment of moderate-to-severe primary Restless Legs Syndrome (www.xenoporo.com). Similarly, understanding the precise expression profile of drug transporters should enable the development of transport assays that would prioritize hits from high throughput screens based on their delivery or exclusion from target organs via defined transporters. However, despite the clear conceptual advantages of exploiting transporters to accomplish tissue-specific delivery of drugs, a recent genome-wide association study has illustrated that non-synonymous loss-of-function mutations in SLC01B1, the transporter responsible for statin uptake into the liver, lead to higher blood statin levels and increased risk of statin-induced myopathy. Whether potentially remodeling the physiology of drug transporter substrates, by hijacking the transporter for tissue-specific drug delivery, is acceptable will likely be evaluated on a case-by-case basis.

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Conflicts of interest

The authors declare they are employees of Merck and Co., Inc., and potentially own stock and/or hold stock options in the company.

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