

Carrier-Mediated Cellular Uptake Of Pharmaceutical Drugs: An Exception Or The Rule?

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Abstract. It is generally thought that many drug molecules are transported across biological membranes via passive diffusion at a rate related to their lipophilicity. However, the types of biophysical forces involved in the interaction of drugs with lipid membranes are no different from those involved in their interaction with proteins, and so arguments based on lipophilicity could also be applied to drug uptake by membrane transporters or carriers. In this article, we discuss the evidence to support the idea that rather than being an exception, carrier-mediated and active uptake of drugs may be more common than is usually assumed — including a summary of specific cases in which drugs are known to be taken up into cells via defined carriers — and consider the implications for drug discovery and development.

Introduction

Obtaining a better understanding of the factors that affect drug pharmacokinetics and pharmacodynamics is a key factor in improving the effectiveness of the drug discovery process. Critical to this understanding is the question of how a drug (or a metabolite thereof), when applied to an organism or tissue, gains access to its target. Putting aside anatomical details, paracellular transport and endocytosis (see Box 1 for the rationale for these simplifications), and assuming oral administration and a reasonable aqueous solubility, the major issue is then whether drugs normally cross cell membranes by diffusion through a lipid bilayer portion of the membrane, or by ‘hitchhiking’ on carriers or transporters that act on natural endogenous substrates (albeit that these substrates or carriers are often unknown) (FIG. 1).

In studies of drug absorption and distribution, the first of these two possibilities is often considered to be dominant. For example, Lipinski’s influential ‘rule-of-5’ (Ro5)¹ for predicting the likelihood of poor absorption or permeation of orally administered drugs implicitly assumes the pre-eminence of the first method, and explicitly classes the latter as an exception to which the rule does not apply. The rule-

of-5 predicts that compounds are more likely to have poor absorption or permeation when two or more of the following parameters are exceeded: molecular weight (MW) >500, calculated octanol/water partition coefficient CLogP >5, number of H-bond donors >5, and number of H-bond acceptors >10. These empirical guidelines, which concentrate on lipophilicity and on H-bond formation, have been of immense importance in our understanding at a phenomenological level of the transfer of drugs across membranes and their disposition within multicellular organisms, as have a variety of related biophysical measures.

Lipinski also noted that “...orally active therapeutic classes outside the Ro5 are antibiotics, antifungals, vitamins and cardiac glycosides. We suggest that these few therapeutic classes contain orally active drugs that violate the Ro5 because members of these classes have structural features that allow the drugs to act as substrates for naturally occurring transporters.” It is also worth noting that most of these compounds are natural products or derivatives thereof (see below).

The types of biophysical forces that determine the interaction of drugs with lipids (especially hydrophobic and hydrogen-bonding interactions) are no different from those involved in their interaction with proteins, especially hydrophobic transport proteins, and biophysical arguments cannot alone make a mechanistic distinction between the two modes of transport in Fig 1. Indeed, four lines of reasoning together suggest that carrier-mediated cellular uptake of drugs could be more widespread than is typically assumed at present, which we will discuss in this article.

The first, and most direct line, of evidence, is that there are many specific cases in which drugs are known to be taken up into cells via defined carriers. Related to this is the demonstration of the necessity for carriers even for certain lipophilic cations that had been assumed to cross membranes solely via diffusion. Furthermore, it is often the case that drugs accumulate in particular tissues (they do not simply ‘leak out’ of cells down a concentration gradient), and their accumulation is often far greater than any possible number of intracellular binding sites. Finally, we note the ability to enhance cellular uptake substantially with the “prodrug” approach using moieties that are known to be substrates for carriers. Whilst we recognise that all scientific evidence may be open to more than one interpretation, we believe that, when taken together over the wide range of systems that we discuss, the argument for a more prominent role of carrier-mediated uptake is compelling. This view has considerable ramifications for future drug discovery, which are summarized in Box 1.

The transfer of molecules across artificial and biological membranes

A classical means with which to study the transport of molecules across the lipid portion of bilayer membranes is to use membranes made just of those lipids (often including, importantly, considerable amounts of organic solvent)². Typically, the BLM is formed across a small (ca 1 mm) orifice in a teflon cup separating two aqueous phases. Such BLMs ('bilayer' or 'black' lipid membranes)^{3, 4} have been of immense utility in the study of biophysical phenomena, including transmembrane transport across them, since it is possible to measure directly the rate of passage of molecules from one aqueous compartment to the other that the BLM separates. The dissolution of drugs in aqueous media, and their extraction from them into membranes, are necessarily governed by (i) the making and breaking of hydrogen bonds and (ii) a general measure of lipophilicity or hydrophobicity (which tends to increase with molecular weight)^{5, 6}. Leaving aside the question of whether a membrane is truly a solvent, solute partitioning between phases can usually be reasonably well-described by the Abraham model^{5, 7, 8} that includes as its elements terms for hydrogen bond donor and acceptor potential (basicity/acidity), for polarisability/dipolarity, for molecular volume, and for the excess molar refraction.

Models such as this are multiple regression models that weight each of the terms differently for different sets of conditions (e.g. the pH, the tissue of interest, etc.), and are thus capable of wide applicability. Given the biophysical basis of these elements, we see that the biophysical forces involved in the transfer of molecules across membranes are no different in principle from those involved in ligand binding to protein targets (including carriers)⁹, and thus biophysical measures of this type (as described in examples in¹⁰⁻¹³) that predict ADME assuming it is a diffusion-mediated event could also be applied to transport using a protein or proteins.

It is easy to assume that what is true for a BLM is true for a biological membrane, but this is by no means logical. First, significant transport across the comparatively unstable BLMs is known to occur via pore defects^{3, 14}, a mode of transport that is probably much less significant in biological membranes. Secondly, the weight, and sometimes area, ratio of protein:lipid in many biological membranes (1:1 to 3:1)¹⁵ is such that it is inevitable that proteins affect the transport properties of lipids or pores that may be used for small molecule transport in their absence, notwithstanding the effects of specific lipids on the permeability of molecules through BLM or natural membranes. Finally, the interactions between lipids and proteins have profound effects on the behaviour and properties of both of them, e.g. in modulating enzyme activity¹⁶ or in effecting aggregation¹⁷.

Although they have been shown to cross BLMs, the very small neutral molecules urea¹⁸ and glycerol¹⁹, which have been widely assumed to permeate phospholipids membranes rapidly by diffusion, have been shown to use carriers to penetrate biological membranes. Indeed, glycerol is actually an osmolyte in yeasts²⁰, and therefore has to be effectively impermeant across phospholipid bilayers. Even water itself, that as judged by osmotic swelling experiments ostensibly crosses biomembranes extremely rapidly^{21,22}, can be transported via aquaporin carriers²³, while in liposomes the rate of transfer of non-electrolytes depends strongly on molecular weight rather than $\log P$ ²⁴.

Consequently, the extent to which molecules that can be seen to cross BLM actually do so via true dissolution in a 'bulk' membrane phase could be small. Finally, models relating diffusion rates across membranes to specific biophysical properties, such as $\log P$, should be based on large sample numbers and properly validated with examples not used in the construction of the model. To date, we are not aware of any studies that have succeeded in providing the requisite data.

High-throughput analogues of BLM, in particular the parallel artificial membrane permeation assay (PAMPA), have been developed and exploited in the analysis of drug transport. The PAMPA assay²⁵ involves transfer across phospholipid-impregnated filters. However, the flux across them can be very poor for drugs even when their absorption in humans is good (see e.g. cephalexin, tiacrilast and others in Fig 3 of²⁵). The correlations of uptake even of established drugs with both $\log P$ and with transport across Caco-2 cells (a widely used cell model of intestinal transport^{26,27}) can also be rather weak (see e.g.^{28-30, 31} (our Fig 2) and in addition e.g. Fig 4 of³²). Figs 2 and 3 also shows some data replotted from Table 1 of a recent comparison³³ using PAMPA. Despite the fact that these are a selected set of marketed drugs that are both considered to cross membranes by diffusion and also mainly have adequate absorption, it is apparent that a number of molecules with poor PAMPA apparent permeability are in fact absorbed well by cells. Since we are aware of very few serious comparisons of the type required (i.e. between the uptake of drug molecules into biological cells versus that across artificial phospholipid or other hydrophobic membranes lacking carriers), and they will clearly be required in the future, we would recommend that comparisons of artificial membrane apparent permeability, Caco-2 cell apparent permeability and $\log P$ that seek to claim 'good' correlations should give the data in graphical as well as tabular form, and give both the slopes and the correlation coefficients obtained. In addition, they should involve a wide range of chemistries, since a model that describes the behaviour of a homologous series (often via $\log P$) when viewed alone may be quite

inadequate when applied to other moieties, e.g. data based on alcohols were poor at predicting the effects of phthalates³⁴. Many factors including solubility, formulation, pH and intestinal enzymology can affect drug uptake³⁵. The ‘rule of 5’ favours intermediate values of CLogP³⁶, reflecting in part the need for drugs to exist in both the aqueous phases and in hydrophobic milieux such as membranes (or integral membrane proteins). There are many kinds of structural and biophysical cheminformatic descriptors that can be used to account for the relationships between particular molecular properties and a biological activity such as uptake³⁷, and because optima are often at intermediate values it is not necessarily easy to identify the optimal descriptors.

Unstirred water layer effects describe the fact that the transport of molecules to a surface assumes free diffusion at diffusion-controlled rates, but layers of water adjacent to membranes can lower this rate^{32, 38}. Leaving this aside, the rate of uptake of small molecules across BLMs decreases with increasing molecular volume^{39, 40} but otherwise favours molecules with low polarity or high values of log P^{3, 33, 41, 42} (although the number of detailed studies of this matter is surprisingly small). Again, much of this flux in BLMs is likely to be due to pore defects⁴³ (or to dissolution in solvents in the membrane-forming mixture) rather than to true dissolution in a biomembrane-mimicking bilayer type of membrane.

Indeed, for the one case in pharmacology in which it is considered that diffusion almost certainly does affect transport (and maybe efficacy) – the case of general anaesthetics – it was long assumed that the almost non-existent relationship between structure and activity, but the high correlation over many orders of magnitude between activity and log P, meant that both diffusion and their mode of action were controlled simply by the ability of anaesthetic molecules to partition into biological membranes^{44, 45}. However, many facts such as the equivalent interactions of these molecules with a variety of proteins^{46, 47}, including direct structural evidence⁴⁶, and the correlation between specific receptor binding⁴⁸ and potency in specific mutant mice⁴⁹, mean that this view is no longer considered tenable (reviewed in^{50, 51}). Indeed, even such a small molecule as ethanol is now recognised as having relatively specific receptors⁵². As stated above, this merely reflects the fact that the binding sites of certain carriers for solutes, and the biophysical interactions involved, may be very similar to those thought to effect their diffusion across membranes via partitioning.

We shall therefore now review the evidence that drugs can be normally transported across biological cell membranes into cells via carriers – often of previously unknown specificity – from the main sources highlighted in the introduction. We begin by recognising that while this does not *per se* say anything about uptake, the existence and importance of many proteins involved in drug efflux – which are of huge significance for example in anti-infective^{53, 54} and anti-tumour activities⁵⁵ – is well established⁵⁶. Not only does this illustrate the widespread existence of the ability of natural proteins to transport xenobiotic drugs but leads one to recognise that if carriers cause their efflux the same or other carriers might cause their influx too. There could then a balance between influx and efflux (as well as any ‘passive’ carrier-independent permeability), and the issue then is how to determine which carriers these are and to assess what might be their natural substrates. We begin by reviewing knowledge of the uptake carriers known to exist in humans.

What influx carriers are known to exist in humans?

Until recently, the number of identified carriers was modest, but a combination of genomics and post-genomics is rapidly altering this, and a variety of internet resources act as portals to some of this information (Table 1). The approved human gene names for carriers include those that begin SLC (for SoLute Carrier)⁵⁷ and ABC (ATP-binding cassette)⁵⁸ and can be found at <http://www.gene.ucl.ac.uk/nomenclature/index.html> (and see also Tables 1, 2, 3 and 4 and S1). Based on homology/motif searching and semi-automated curation, the <http://membranetransport.org/> website (in June 2007) lists 758 transporters of all kinds for *H. sapiens* and 347 for *S. cerevisiae* (a number reasonably similar to the 285 manually curated proteins at the Yeast Transport Protein Database <http://rsat.scmbb.ulb.ac.be/~sylvain/ytpdb/>). This number for humans exceeds substantially the numbers that appear or are described in most reviews based on ‘wet’ biological experiments, and suggests (as indeed stated explicitly in the recent paper on the reconstruction of the human metabolic network⁵⁹) that we are really only scratching the surface of what is there, leave alone what might be their specificities for natural molecules and xenobiotic drugs. Similar comments may be made about mitochondria, where genomic and post-genomic studies now show many hundreds of proteins to be present in these organelles, many of unknown or novel function⁶⁰, with mitochondrial carrier proteins prominent among them^{61, 62} (many with still-unknown substrates⁶³). Given, as noted above, that water, glycerol and urea can use carriers, to assume that a molecule is not a substrate for one of these carriers seems risky. In particular, all carriers could potentially contribute to the background

permeability of xenobiotics into cells or organelles that are not known to express high levels of any particular carrier of interest.

Proteins of the solute carrier (SLC) family are involved in the transport of a broad range of substrates. SLC transporters can be passive (uniporters), coupled (symporters) or exchangers (antiporters). Currently, there are over 40 families containing around 340-360 transporters. The state of research into SLCs was reviewed in 2004⁵⁷. Of the 43 families summarised, an extensive literature search found evidence of non-endogenous uptake activity by 19 solute carrier families. Amongst these, SLCO/SLC21, the organic anion transporting superfamily (OATPs) (reviewed in ⁶⁴), and SLC22 (reviewed in ⁶⁵), the organic cation/anion/zwitterion transporter family, are heavily involved in the uptake of many diverse substrates. Both exhibit a wide tissue distribution and form part of the major facilitator superfamily.

Amongst the families for which there is as yet no evidence of non-endogenous substrate transport are many (but not all) of the transporters involved in the transport of metal ions, including: $\text{Na}^+/\text{Ca}^{2+}$ (SLC8) and Na^+/H^+ (SLC9), transition metal ions (SLC11), Na^+/K^+ and Cl^- (SLC12), Na^+ and inorganic phosphate (SLC20), $\text{Na}^+/\text{Ca}^{2+}/\text{K}^+$ (SLC24), zinc (SLC30), ferrous iron (SLC40), and divalent metal ions (SLC41). Similarly, the transporters of small ionic species such as bicarbonate (SLC4), and sulphate, oxalate, formate and similar (SLC26) do not seem so far to exhibit evidence of non-endogenous substrate transport. These small endogenous substrates are markedly dissimilar to most xenobiotics, so it might be expected that they are not so readily involved in xenobiotic transport.

Carrier-mediated uptake: the evidence

We now turn to the four lines of evidence highlighted in the introduction supporting a more prominent role for carrier-mediated uptake.

(i) There is abundant evidence for carrier-mediated drug uptake in specific cases where it has been studied.

While some of the later evidence and reasoning we describe may be seen as being more circumstantial, albeit entirely consistent with our thesis, we start by drawing attention to the increasing evidence from

specific cases that particular drugs do in fact enter cells via identified carrier molecules for which they are not the ‘natural’ ligand. A comprehensive and annotated list of human SLCs and some of their known natural and xenobiotic substrates is given in Supplementary Table S1. Listed there are 393 substrate-transporter relationships, covering transporters from 17 solute carrier families and 203 unique substrates. Evidence of transport is mainly on the basis of uptake assays in transfected cells. Some of these SLCs are illustrated diagrammatically in Fig 3.

The data can be interrogated in terms of which transporters act on a given drug or conversely, which drugs are the substrates for a given transporter. In this latter vein, Table 2 lists the main superfamilies of transporters, while Tables 3 and 4 provide details of three of the SLC families — *SLC15*, *SLC22*, and *SLCO* — that are considered especially to have a role in xenobiotic drug uptake.

Members of the oligopeptide transporter family *SLC15*⁶⁶ mediate proton-coupled cotransport of many diverse peptide and peptidomimetic substrates. Well characterised family members are PEPT1 (*SLC15A1*) and PEPT2 (*SLC15A2*). PEPT1 is highly expressed in the intestine and PEPT2 in the kidney, though expression has also been observed in the bile duct epithelia, choroid plexus, lung and mammary gland. All 400 dipeptides and 8000 tripeptides derived from the common protein-forming amino acids are substrates for both, despite large differences in molecular size, net charge and solubility. A detailed characterisation of PEPT1 substrates has been performed⁶⁷, where there is a particular affinity for molecules possessing amino and carboxylic acid groups separated by about 6Å, even in non-peptidic substrates. Such information permits a rational approach to prodrug design, as in the coupling of valine to acyclovir and ganciclovir to enhance substrate-likeness for PEPT1⁶⁶. Uptake of prodrug across the apical membrane and rapid hydrolysis by intracellular dipeptidases leads to increased drug availability. Drug substrates of PEPT transporters include many important classes, including antivirals (valacyclovir), antibiotics (β-lactams), and angiotensin-converting enzyme inhibitors⁶⁶.

Organic cation/anion/zwitterion transporters (*SLC22*⁶⁵) are widely distributed, with various family members being expressed in the liver, kidney, skeletal muscle, placenta, heart, lung, spleen and brain (see also⁶⁸ for details of expression). Substrates include the endogenous prostaglandins, serotonin, carnitine, adrenaline, 2-oxoglutarate, and the drugs acyclovir, ganciclovir, metformin, memantine, verapamil, and zidovudine. There is considerable substrate overlap between group members. We

identified 72 substrates for the family, 24 of which are transported by more than one family member. No substrate is identified as a substrate of every SLC22 transporter.

Organic anion transporting polypeptides (*SLCO*, formerly *SLC21*⁶⁴) mediate bidirectional, sodium-independent, pH-dependent substrate-anion exchange. We identified 59 substrates, 34 of which are transported by more than one family member. Known substrates cover a wide range of substrates, including bile salts, steroid hormones and conjugates, thyroid hormones, organic cations, and various drugs such as atorvastatin, benzylpenicillin, enalapril and pravastatin. Generally, substrates are anionic amphipathic molecules with a molecular weight greater than 450 Daltons. QSAR studies defined a pharmacophore with two hydrogen bond acceptors, one hydrogen bond donor and two hydrophobic regions⁶⁴. Polyspecific family members tend to have a wide tissue distribution, covering the BBB, choroid plexus, lung, heart, intestine, kidney, placenta and testis.

Considering the characteristics of those drugs that have been identified as a substrate for an uptake transporter, there is a rather blurred distinction between which are natural products (e.g. erythromycin), semisynthetic molecules that are typically modified natural products (e.g. benzylpenicillin), completely synthetic products that are nevertheless an analogue of a natural metabolite (e.g. propranolol, nominally an analogue of histamine) or completely synthetic products that are not considered to be an analogue of any human metabolite (e.g. atorvastatin). Scrutiny of Table 3 indicates that almost all compounds do in fact fall into the first 3 categories, and indeed, perhaps they could be seen to be analogues of natural metabolites for which one could reasonably imagine the existence in evolution of transporter molecules, which have been selected implicitly via the experience of medicinal chemists or simply for reasons of efficacy.

Indeed, it is well known that ‘natural products’, i.e. bioactive ‘secondary’ metabolites^{69, 70} do not obey the ‘rule of 5’ (for example, most antibacterials⁷¹), and it is certainly known in some cases that they are the substrates of active transporters in the producing organisms⁷². Given that bioactive microbial products are necessarily secreted, evolution must have produced carriers capable of binding the relevant chemical structures⁷³. The fact that these bioactive ‘secondary’ metabolites are often active on other cells of the producer organism⁷⁴ as well as the higher organism reinforces the view that suitable protein binding motifs must exist widely throughout evolution^{75, 76}. This suggests that it is to be expected that there are likely to be transporters for these kinds of bioactive ‘secondary’ metabolites in higher organisms, as is indeed found to be the case^{58, 67, 77-79}. Note too (Table 3) that a very high

proportion of drugs that we have noted as having transporters are in some sense analogues of natural products. A standard principle in cheminformatics and in medicinal chemistry is the idea that molecules that are ‘like’ each other structurally will tend to have similar activities. It is consequently reasonable that such activities will include the ability to act as substrates for transporters if the molecules are ‘like’ natural molecules endogenous to the target organism. Such a quantitative survey has yet to be done.

An interesting example related to this issue from Table 3 is provided by the statins, a family of drugs that all inhibit HMG-CoA reductase, which includes both agents that could be considered as natural products or derivatives (e.g. lovastatin, simvastatin), and also what would appear to be totally synthetic agents (e.g. atorvastatin). A variety of studies have demonstrated that a major route of transport is via the various organic anion transport proteins⁷⁹⁻⁹¹, many of which naturally transport bile acids. For instance⁷⁹, OATP1B1 and OATP1B3, are both highly expressed in human liver and are able to transport atorvastatin, cerivastatin, fluvastatin, pitavastatin, pravastatin and rosuvastatin. Multiple versions of these transporters are present, and even individual variants can account for 35%⁹¹ to 90%⁹² of the uptake. Statins such as simvastatin, lovastatin acid and pravastatin are also substrates for monocarboxylate transporters⁹³. OATP2 transports pravastatin, lovastatin, simvastatin, and atorvastatin⁸⁴. They can also be transported for instance by the bile acid transporter SLC10A1⁹¹ and the monocarboxylate transporter *SLC16A1*⁹⁴ (see also Table S1).

(ii) Even lipophilic cations need carriers to transfer them across cell membranes

The assumption that highly lipophilic molecules can partition straightforwardly into membranes and thereby transfer across them is both common and implicit in the view of the importance of log P in determining uptake. These considerations are taken to apply to neutral rather than to charged molecules, and it is well recognised that charged molecules effectively cannot cross the interior of black lipid membranes because of the enormously unfavourable Born charging energy required to transfer them across a low dielectric^{43, 95, 96}. However, it is reasonable that the addition of sufficient lipophilic groups to an ion, delocalising the ionic charge, would decrease the Born charging energy and thereby confer membrane-permeating ability to such ions. In this vein, an early series of studies, motivated by questions of bioenergetics following the chemiosmotic proposals of Mitchell⁹⁷, showed that even ionically charged lipophilic molecules could indeed cross both black lipid and cellular membranes, albeit that this activity could be strongly promoted by the presence of ‘catalytic’ amounts

of lipophilic ions of opposite charge such that the membrane-permeating species was then probably neutral⁹⁸. This then led to the widespread assumption (see e.g.^{99, 100}) that such molecules could penetrate biological membranes in the absence of any carriers being necessary. However, there is clear evidence of a requirement of proteinaceous carriers for at least some of these lipophilic cationic molecules, that had been assumed on such biophysical grounds to cross biological membranes without them (for example, the requirement of a functional thiamine carrier to effect transfer of the dibenzyl,dimethyl-ammonium cation¹⁰¹) (and see also^{102, 103}). Other experiments by these authors showed that dibenzyl dimethyl ammonium uptake is inhibited completely by thiamine disulfide, a competitive inhibitor of thiamine transport. These findings of carrier-mediated uptake of such molecules (as in the case of thallos ion transport¹⁰⁴) also possibly calls into question the use of such lipophilic cations in the estimation of transmembrane potentials in such systems.

(iii) Drugs do concentrate in specific tissues

The steady-state concentration of a drug in a particular cell, cellular compartment or tissue is evidently determined in large measure by the activity of the relative rates of influx and efflux and their binding to targets (whether functional and specific or gratuitous and nonspecific). Binding is probably not the major issue since intracellular concentrations can be significantly larger than any plausible stoichiometric concentration of binding sites. Thus, the fact that some drugs can concentrate in specific tissues¹⁰⁵⁻¹¹² suggests that these drugs do not 'leak out' so as to equilibrate with extracellular concentrations as they would if transmembrane diffusion on the basis of log P alone was the whole (or even most of) the story, and the fact that they are concentrated then necessarily (on thermodynamic grounds) suggests some kind of active uptake. Some of these examples are based on specific tissues (e.g.^{105, 106}), while others concentrate of specific organisms (e.g. the mouse), on drug discovery^{107, 110}, on PK/PD^{108, 111, 112} and on drug-drug interactions¹¹³. While we have largely avoided focussing above on specific tissues (cf.^{68, 105, 112, 113}) (but see Box 3 for a discussion of the blood-brain barrier), there are clear cases in which rational modifications can beneficially affect efflux¹¹⁴ as well as influx (see pro-drugs, below), though we note of course in particular that selective tissue concentrating mechanisms may also be a cause of toxicity¹¹⁵, and that there are other problems, such as drug-drug interactions¹¹⁶⁻¹¹⁹, both in general^{116, 118} and in specific tissues such as the liver¹¹⁷ and the kidney¹²⁰, that are not our primary focus. Similarly, if drugs compete with nutrients or intermediary metabolites for carrier sites, one may suppose that this could be a significant mechanism for drug-nutrient interactions¹²¹.

(iv) Impermeable drugs can be made permeable by creating prodrugs that hitchhike on carriers

Many nominally drug-like compounds are recognised as being membrane-impermeable. However, it has been shown in many cases that it is possible to enhance permeability substantially by modifying the drug chemically to form a prodrug that can act as a substrate for known drug carriers and thereby enter cells¹²²⁻¹³⁰. The case of peptide transporters is particularly clear^{67, 129, 131-133} and hitchhiking on peptide transporters can demonstrably improve the activity of certain antibacterials¹³⁴. Coupling of drugs such as chlorambucil¹³⁵, cis-platin¹³⁶ and acyclovir¹³⁷ to bile acid derivatives or of carindacillin to monocarboxylates¹³⁸ can also be highly effective. Such couplings often lower the lipophilicity of the drugs while enhancing their uptake, a phenomenon hard to explain in terms of log P. However, in other cases, permeability is enhanced by making drugs more lipophilic, e.g. by esterifying carboxylic acids. The assumption then is that these can diffuse in, although whether such influx is by diffusion, by carrier mediation (given that any change in the structure of a substrate can often have large effects on the activity of an enzyme for which it is a substrate) or even by endocytosis or even by endocytosis is not in fact known, given that we have little knowledge of the extent to which existing carriers are responsible for the baseline uptake of molecules that is observed.

Implications

The above analysis has implications for both drug design and for present cheminformatic concepts of lead-likeness^{139, 140} and drug-likeness¹⁴¹⁻¹⁴³ (and even CNS-likeness¹⁴⁴) in drug design and discovery, since many of the recent trends in molecular drug design and development have been towards increased lipophilicity, leading to a greater likelihood of both a lack of selectivity and of attrition¹⁴⁵. There is then the clear need to bring together the (moderately limited) bioinformatic knowledge of transporter specificity with the more common and largely biophysical cheminformatics descriptors. If drugs are mainly transported by carriers, this gives a ready explanation of why general descriptors are not normally going to be very effective in individual cases, and promotes the view that we need to understand much better than we do now at a mechanistic level the specificities for existing and candidate drugs of known drug transporters.

If carriers are heavily involved in drug uptake, they will have natural substrates and we may expect not only to find them (in the same way that opioid and other receptors, and their endogenous substrates,

were found by pharmacological means) but to use this knowledge to exploit them via the design of pro-drugs or the re-design of drugs to allow their transport by such carriers. There will also be cases in which simply affecting the carriers themselves will have profound pharmacological effects. Thus¹⁴⁶, glycocholic acid and polyamine conjugates are able to inhibit transporters involved in hepatic and intestinal bile acid uptake, and since secretion and re-uptake are common in chemical neurotransmission it is reasonable that such molecules may prove useful targets. Indeed this is the known mode of action of some important kinds of CNS-active substances, including those targeting the uptake of glutamate and dopamine¹⁴⁷ and serotonin¹⁴⁸.

The way forward – towards a systems biology that includes human drug carriers

In a post-genomic era we can begin to move towards and beyond a knowledge of what transporters exist, and useful starting-points are the web-accessible databases (Table 1). Armed with the knowledge of the existence of these carriers, we can seek to study them as targeted entities using the methods of molecular biology, and this is already providing important new knowledge on their distribution, activities and specificities^{54, 105, 149-153}. Such methods based on expression cloning are likely to be far more powerful and persuasive than the more traditional methods for implicating carriers based on criteria such as saturability, which is a very poor criterion since non-saturability can be caused by multiple carriers of which some may have very weak affinity constants. As web-accessible data on tissue-selective expression profiles become available at both the transcriptomic^{154, 155} (<http://expression.gnf.org/>) and proteomic¹⁵⁶⁻¹⁵⁸ (<http://www.proteinatlas.org/>) levels, this will begin to allow us to understand which transporters are likely to be expressed and thus functionally active in which tissues (an example is given in Fig 4), and thereby provide the wherewithal with which to integrate the available knowledge¹⁵⁹. All else being equal, one may expect straightforward correlations between the extent of accumulation of drugs in a tissue and the tissue expression of the carriers responsible for their import, thereby allowing one to infer the relevant carriers by rank-comparing the tissue distributions of drugs and of the various carriers. Expression cloning studies will then easily establish the specificities of the proposed carriers for existing and candidate drugs, just as is now done routinely for cytochromes P450¹⁶⁰. Cassette dosing and mass spectrometric assays will be especially useful here. Specifically, it is stressed that if a chief determinant of drug uptake into cells is represented by the amount and activities of individual carriers for which these drugs are the substrates, then tissues that express active drug carrier proteins in high concentrations are likely to take such molecules up in greater amounts, with concomitant risks of toxicity.

As more studies on cloned transporters are performed, we may also expect significant improvements in our knowledge of the molecular enzymology of these processes, including details of binding and structure-activity relationships, as per the bottom-up systems biology agenda¹⁶¹. There is also a significant role for model organism studies here^{162, 163}, since many of the carriers known to be active in humans have homologues in experimentally more tractable organisms (see above). For example, existing data regarding the interaction of yeast cells with drugs have pointed up a number of cases in which changes in the activity of specific carriers increase or decrease the sensitivity of cells to xenobiotics¹⁶⁴⁻¹⁶⁷, with the clear implication that such carriers effect the entry of these drugs into cells or their exit from them. Evidently, similar studies in genetically tractable higher organisms will be of immense value. In addition, chemical genetics strategies for determining the mode of action of small-molecule inhibitors on their cellular targets^{168, 169} apply equally to their interactions with the drug transporters that may be required to get them there. As mentioned above, the issue is that we do not know which carriers these are, although a reasonable starting strategy in some cases is to use the methods of cheminformatics and molecular similarity analysis to assess which natural metabolites they most clearly resemble according to appropriate criteria. While the type of such transport (uniport, antiport, symport, group transfer) is not part of the focus of this review, we recognise that once a particular influx is seen to be going via a specific kind of transporter then it will be of considerable interest to determine the mode of transport and role of any cosubstrates.

Systems biology involves an iterative interplay between wet experiments, modelling and technology development, and to take forward the role of carriers in human drug transport a systems biology strategy is apposite. An essentially bottom-up strategy (as illustrated in Fig 5) seems appropriate, since we are at such an early stage, and reflects the primary necessity for establishing which carriers transport which molecules. At the moment the quantitative pharmacological evidence for drug uptake by carriers is comparatively sparse, since this has simply not been a focus of most studies. This will lead to what we essentially desire the eventual availability of a digital human, in which we can simulate far more effectively than we can now the entire metabolism and control in human biochemical networks, including the spatially differentiated metabolism of drugs. This can and should be done as a community effort, preferably in a loosely coupled or distributed way. The availability of the first major versions of the 'entire' human metabolic network^{59, 170} in a machine readable form (as SBML¹⁷¹ and see www.sbml.org) provides an outstanding starting point for this endeavour^{172, 173}

Concluding remarks

What we have sought to do here is to bring together a rather scattered but, we believe, ultimately persuasive literature on the role of membrane transporters in cellular drug uptake. What we hope we have therefore achieved is a more coherent view that leads one to focus on the mechanistic significance of membrane transporters in all aspects of drug absorption, distribution, metabolism, excretion and toxicity, including the effects of polymorphisms, ADRs and drug-drug and drug-nutrient interactions. If one accepts that most of this transport may indeed occur via carriers, the next stage is to begin to understand their specificity and energy coupling mechanisms and put together the relevant transporters into the rest of the metabolic network, using the standard ‘bottom-up’ methods of systems biology¹⁶¹. Only when this is done may we hope to have a predictive biology of human drug disposition.

Acknowledgments

Our interest in pursuing these issues has been helped considerably by grant BB/D007747/1 from the BBSRC, together with attendant funding from GSK. We thank Scott Summerfield and Phil Jeffrey of GSK for their support and interest, and Karin Lanthaler and Steve Oliver for useful discussions. DBK also thanks the EPSRC and RSC for financial support, and the Royal Society/Wolfson Foundation for a Research Merit Award. We apologise to the many authors whose work was not cited due to limitations on references. This is a contribution from the BBSRC- and EPSRC-funded Manchester Centre for Integrative Systems Biology (www.mcisb.org/).

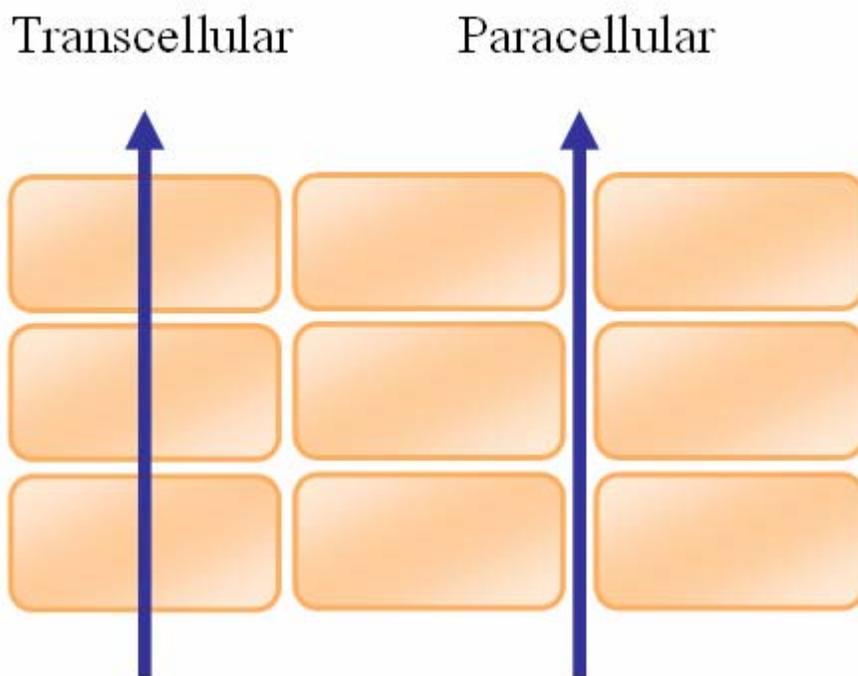
Box 1. Implications of a more prominent role for carrier-mediated drug uptake

- A combination of substrate specificity and carrier distribution, additional to target distribution, can account for much of the different tissue distributions of drugs (and hence warn of toxicity issues)
- Drugs or prodrugs may be designed to be targeted to specific tissues that express highly the carriers for which they are the substrates
- Drugs may be designed to avoid specific tissues that lack carriers for them
- It becomes much easier to understand in principle the tissue distributions of xenobiotics
- Cross-species sequence homologies may allow better interpretation of tissue distributions in different organisms
- Uptake carriers may provide novel and rational drug targets
- Molecular cloning will allow the specificities of individual carriers for target drugs to be measured directly
- Drug-drug interactions may be mediated by competition with or inhibition of influx transporters

Box 2. Molecular transmembrane transport as a focus of this review

As shown in Fig 1, the focus of this review begins with the widespread view of biological membranes as some kind of ‘fluid mosaic’ in which polytopic proteins are embedded in an effectively 2-dimensional ‘sea’ of phospholipid bilayer. Such cells are typical embedded in tissues, whereby arrays of cells form the actual barrier that must be penetrated. We illustrate such a tissue in Box Figure. This shows two other issues regarding the transfer of molecules across the tissue: the first is that there is the potential for a paracellular route, in which molecules ‘sneak past’ the cell membrane barriers via the extracellular spaces. This of course does not provide for intracellular access of the drugs of interest. The second is that the ease of passing a tissue from one side to the other might also depend on anatomical factors such as the number of cell membranes that must be traversed. Very little is known about the latter, and such effects are in a sense additional to transcellular and paracellular transport. There is also the possibility of intracellular vesiculation (endocytosis), allowing molecules in an external aqueous phase to enter an intracellular ‘aqueous’ phase without actually crossing a phospholipid bilayer. This, however, cannot serve to effect transfer across a whole tissue.

Finally, we largely do not consider other second order effects such as membrane curvature, lipid rafts and the like, and simply ask the question ‘do molecules traverse the barrier that the membrane represents largely by diffusion through the lipid portion of the membrane, in a manner governed essentially via $\log P$, or via interactions with proteins that mediate their transmembrane transport?’. This is a very general question that just treats a cell membrane as a closed vesicle separating the inside of a cell from its outside.



Box 2 Figure. Transcellular and Paracellular transport {will need redrawing by NRDD}.

Box 3. The blood brain barrier

The blood brain barrier is of especial interest since CNS-active drugs necessarily have to permeate it, and in many ways (but given experimental difficulties perhaps unsurprisingly) it is still little understood. Certainly, a major feature is the limited possibility for paracellular transport^{174, 175} (see discussion in Box 2). While there are clearly influx carriers^{93, 176-183} there is also considerable evidence that the activity of efflux carriers is very effective in removing xenobiotics from the CNS¹⁸⁴⁻¹⁸⁶ such that both influx and efflux activities as well as binding need to be understood if selective blood brain barrier penetration is to be achieved¹⁸⁷⁻¹⁸⁹. Known influx carriers include those for large neutral amino acids (LAT1), glucose (GLUT1), monocarboxylates (MCT1), choline (CHT) and nucleobases such as adenosine (CNT2), but most remain unknown (e.g.^{130, 190}). Thus System L transports large neutral amino acids, L-glutamine, L-asparagine, D-amino acids, and the drug melphalan⁹³. High expression of hLAT1 mRNA is detected in brain tissue by Northern blot analysis¹⁹¹, while system y⁺ transports cationic amino acids. Its CAT1 RNA is enriched 38-fold in rat cerebral microvessels and choroid plexus compared with whole brain¹⁹². Certain organic cation transporters, such as rOCT3¹⁹³, are

known to be expressed in the brain. rOCT3 mediates the uptake of the neurotoxin 1-methyl-4-phenylpyridinium (MPP⁺) and the neurotransmitter dopamine when expressed in mammalian cells. Organic anion transporters are also reported in the brain, and OATP-A is present at the human BBB¹⁹⁴. Brain expression of the peptide/histidine transporter (PHT1) was confirmed by *in situ* hybridisation. PHT1 substrates include histidine and carnosine, with many di- and tri-peptides inhibiting histidine uptake¹⁹⁵. Lee¹⁷⁷ has reviewed drug transporters in the central nervous system. Evidence for the importance of efflux carriers come from the large increases in brain concentration of a variety of drugs such as amprenavir¹⁹⁶ and SB-487946¹⁹⁷ when for instance the P-glycoprotein carrier is inhibited pharmacologically or knocked out at the genetic level. There is a clear role for *in silico* studies here, as well as ‘wet’ experimental approaches.

Table 1. Some searchable databases for transporter molecules. Note that many of these contain large numbers of acronyms, which may be resolved using Acromine (<http://www.nactem.ac.uk/software/acromine/>)¹⁹⁸. The behaviour of individual proteins determined by literature analysis can be studied at <http://www.ihop-net.org/UniPub/iHOP/>.

Name	URL	Focus	Reference
Human Membrane Transporter Database	http://lab.digibench.net/transporter/ and for drugs http://lab.digibench.net/transporter/drug.html	Human	¹⁹⁹
IUBMB and HUGO Membrane Transport Proteins Nomenclature	http://www.chem.qmul.ac.uk/iubmb/mtp/ http://www.gene.ucl.ac.uk/cgi-bin/nomenclature/searchgenes.pl	Human	²⁰⁰
Transport Classification Database using the above names	http://www.tcdb.org/	Various	²⁰¹
Yeast Transport Protein Database	http://rsat.scmdbb.ulb.ac.be/~sylvain/ytpdb/	Yeast	²⁰²
SoLute Carrier (SLC) Tables	http://www.bioparadigms.org/slc/	Various	⁵⁷
TP-search	http://www.tp-search.jp/	Mammalian	²⁰³
TransportDB	http://www.membranetransport.org/	Multiple and Comparative	^{78, 204}

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Table 2. Overview of human transporter superfamilies and families with possible/known roles in drug uptake. This is largely based on material at <http://www.tcdb.org/> (see also ²⁰¹). (TM = transmembrane)

Superfamily	Family	TCDB	General Topology	Transporter	Substrates
Amino acid/Polyamine/Organocation (APC) Superfamily	Amino Acid/Auxin Permease (AAAP) <i>SLC36</i>	2.A.18	10-11 TM α -helices	Symport / Antiport	Amino acids, auxin (indole-3-acetic acid)
	Amino acid/Polyamine/Organocation (APC) <i>SLC7</i>	2.A.3	14 TM α -helices	Symport / Antiport	Amino acids, choline, polyamines
Anion Transporter (AT) Superfamily	Bile Acid:Na ⁺ Symporter (BASS) <i>SLC10</i>	2.A.28	7-10 TM spanners	Symport	Bile acids and other organic acids
Major Facilitator Superfamily (MFS)	Major Facilitator (MF) <i>SLC2, 16-18, 22, 33, 37, 43</i>	2.A.1	Mostly 12, 14 or 24 α -helical TM α -helices	Uniport/Symport / Antiport	Sugars, drugs, neurotransmitters, metabolites, amino acids, peptides, nucleosides, organic and inorganic anions
	Proton-dependent Oligopeptide Transporter (POT) <i>SLC15</i>	2.A.17	12 TM α -helices	Symport	Peptides, histidine, antibiotics
	Organo Anion Transporter (OAT). <i>SLCO/21</i>	2.A.60	12 TM α -helices	Uniport/Antiport	Broad range: Organic anions, organic cations (bromosulphophthalein, prostaglandins, bile acids, steroid conjugates, oligopeptides, drugs, toxins, others)
Resistance-Nodulation-Cell Division (RND) Superfamily	Eukaryotic (Putative) Sterol Transporter (EST)	2.A.6.6	N-TM-Extracytoplasmic domain-5TM-Extracytoplasmic domain-6TM-C	Antiport	Sterols, lipids
Drug/Metabolite Transporter Superfamily	Nucleotide-Sugar Transporters	2.A.17.10 2.A.17.11 2.A.17.12	8-12 TM α -helices	Antiport	Exchange nucleotides for nucleotide-sugars
ATP-gated Cation Channel (ACC)		1.A.7	2 TM spans + extracellular receptor domains	Facilitated Diffusion	Prolonged exposure of certain forms to ATP leads to pore dilation. Pore permeable to solutes up to 1kDa.
Solute:Sodium Symporter (SSS)		2.A.21 <i>SLC5</i>	13-15 TM α -helices	Symport	Sugars, amino acids, organocations, nucleosides, inositols, vitamins, urea, anions
Neurotransmitter:Sodium Symporter (NSS)		2.A.22 <i>SLC6</i>	12 TM α -helices	Symport	Neurotransmitters, amino acids
Dicarboxylate/Amino Acid:Cation (Na ⁺ or H ⁺) Symporter (DAACS)		2.A.23 <i>SLC1</i>	8 TM spanners and 1 or 2 pore loop structures (putative)	Symport	Malate/succinate/fumarate, glutamate/aspartate, Ala/Ser/Cys/Thr, neutral and acidic amino acids, zwitterionic and dibasic amino acids

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Mitochondrial Carrier (MC). <i>See SLC25</i>	2.A.29 <i>SLC25</i>	6 TM α -helices	Antiport	Citrate, malate, PEP, lysine, arginine, aspartate, glutamate, others
Nucleobase:Cation Symporter-2 (NCS2)	2.A.40 <i>SLC23</i>	12 TM α -helices	Symport	Nucleobases, ascorbate
Concentrative Nucleoside Transporter (CNT)	2.A.41 <i>SLC28</i>	10-14 TM α -helices	Symport	Nucleosides
Reduced Folate Carrier (RFC)	2.A.48 <i>SLC19</i>	12 TM α -helices	Symport / Antiport	Folate, reduced folate and derivatives, methotrexate, thiamine
Equilibrative Nucleoside Transporter (ENT)	2.A.57 <i>SLC29</i>	11 TM α -helices	Symport	Nucleosides and analogs
Bilirubin Transporter (BRT)	2.A.65	Uncertain	Symport	Bilirubin, organic anions, rifamycin, nicotinic acid
Organic Solute Transporter (OST)	2.A.82	Chain α : 7 TM spanners Chain β : 1 TM spanner	Facilitated Diffusion	Bile acids, prostaglandin E1, digoxin, steroids

Table 3. Some common drug substrates of the most prolific SLC transporter families as judged by the number of substrates referenced in Tables 4 and S1.

Substrate	SLC15A1	SLC15A2	SLC22A1	SLC22A2	SLC22A3	SLC22A4	SLC22A5	SLC22A6	SLC22A7	SLC22A8	SLC22A11	SLCO1A2	SLCO1B1	SLCO1B3	SLCO1C1	SLCO2A1	SLCO2B1	SLCO3A1	SLCO4A1	SLCO4C1
acyclovir			•					•												
amoxicillin	•	•																		
atorvastatin													•				•			
benzylpenicillin													•				•	•	•	
bestatin	•	•																		
caspofungin													•							
cefaclor	•	•																		
cefalexin	•																			
ceftibuten	•																			
cephaloridine							•	•		•	•									
cidofovir								•												
cimetidine			•	•	•			•		•										
didanosine								•												
enalapril	•											•								
erythromycin									•											
fexofenadine												•	•	•						
fluvastatin													•	•			•			
ganciclovir			•					•												
glibenclamide																	•			
ibuprofen								•												
lamivudine								•												
metformin			•	•																
methotrexate								•	•	•			•	•						•
midodrine	•																			
pitavastatin													•	•						
pravastatin													•				•			
propranolol				•																
pyrilamine						•	•													
quinidine						•	•													
rifampicin													•	•						
rosuvastatin												•		•			•			
salicylate									•	•										
stavudine								•												
temocaprilat	•											•								
tetracycline								•	•	•	•									
trifluridine								•												
valacyclovir	•									•										
valganciclovir	•	•																		
valproate							•													
verapamil						•	•													
zalcitabine								•												
zidovudine				•				•	•	•	•									

Table 4. Examples of drug uptake by three of the most significant families of transporter (SLC15, SLC22 and SLCO)

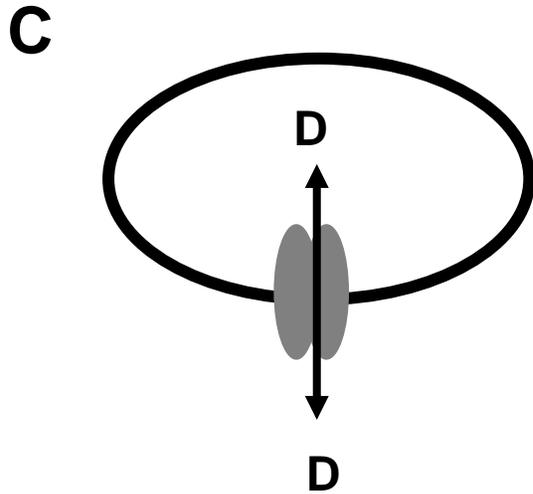
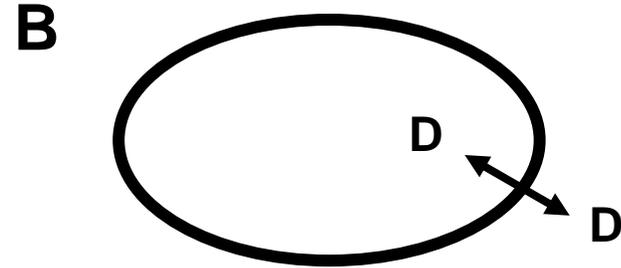
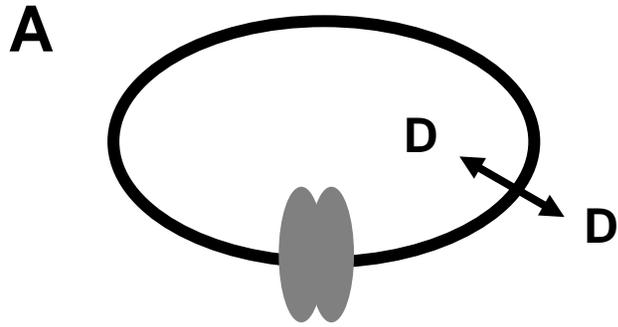
SLC Family	HUGO Symbol + Synonyms	Description	Substrate	Refs	Code
15 66	SLC15A1; PEPT1	Oligopeptide transporter	cefalexin	205	DATC
			bestatin	206	DATC
				207	DATC
			amoxicillin	206	DATC
				208	DADC
			ampicillin	206	DATC
			cefaclor	208	DADC
			cefadroxil	209	DATC
				206	DATC
			cefixime	209	DATC
			ceftibuten	210	DCA
			temocapril	116	DATC
			temocaprilate	116	DATC
			enalapril	116	DATC
	midodrine	211	DATC		
valganciclovir	212	DATC			
valganciclovir	213	DATC			
SLC15A2; PEPT2	H+/peptide transporter	amoxicillin	208	DADC	
		cefaclor	208	DADC	
		cefadroxil	214	DADT	
		bestatin	207	DATC	
		valganciclovir	213	DATC	
22 65	SLC22A1; OCT1	Organic cation transporter	zidovudine	215	DATC
			acyclovir	216	DATC
			ganciclovir	216	DATC
			metformin	217	DATC
			cimetidine	217	DATC
	SLC22A2; OCT2	Organic cation transporter	memantine	218	DATC
			metformin	219	DATC
			propranolol	220	DATC
			cimetidine	221	DATC
			zidovudine	222	DATC
			pancuronium	223	TOAT C
			cyanine863	223	TOAT C
	quinine	223	TOAT C		
	SLC22A3; OCT3; EMT	Extraneuronal monoamine transporter	cimetidine	221	DATC
			tyramine	224	DATC
	SLC22A4; OCTN1	Organic cation transporter	quinidine	225	DATC
			pyrilamine	225	DATC
			verapamil	225	DATC
	SLC22A5; OCTN2	Organic cation transporter	quinidine	226	DATC
			pyrilamine	226	DATC
			verapamil	226	DATC

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			valproate	226	DATC	
			cephaloridine	227	DATC	
	SLC22A6; OAT1	Organic anion transporter	adefovir	228	DATC	
			cidofovir	228	DATC	
			acyclovir	229	DATC	
			zalcitabine	229	DATC	
			didanosine	229	DATC	
			stavudine	229	DATC	
			trifluridine	229	DATC	
			ganciclovir	216	DATC	
			lamivudine	229	DATC	
			zidovudine	229	DATC	
			methotrexate	230	DATC	
			ketoprofen (low uptake)	231	DATC	
			ibuprofen (low uptake)	231	DATC	
			cimetidine	232	DATC	
			tetracycline	233	DATC	
			cephaloridine	234	IATC	
	SLC22A7; OAT2	Organic anion transporter	zidovudine	216	DATC	
			tetracycline	233	DATC	
			salicylate	235	DATC	
			methotrexate	236	DATC	
			erythromycin	237	DATC	
			theophylline	237	DATC	
	SLC22A8; OAT3	Organic anion transporter	valacyclovir	216	DATC	
			zidovudine	216	DATC	
			methotrexate	238	DATC	
			salicylate	238	DATC	
			cimetidine	238	DATC	
			cephaloridine	234	IATC	
	SLC22A11; OAT4	Organic anion/cation transporter	zidovudine	216	DATC	
			cephaloridine	234	IATC	
	SLCO 64	SLCO1A2; OATP; OATP-A; OATP1A2	Organic anion transporter	fexofenadine	239	TOAT C
				rocuronium	240	DATC
enalapril				241	DATC	
temocaprilat				242	DATC	
rosuvastatin				91	DATC	
SLCO1B1; OATP-C; LST1; OATP1B1; OATP2		Organic anion transporter	benzylpenicillin	243	DATC	
			pravastatin	84	DATC	
			rifampicin	244	DATC	
			atorvastatin	81	DATC	
			capsosungin	245	DATC	
			cerivastatin	81	DATC	
			fexofenadine	239	DATC	
			methotrexate	246	IATC	
			fluvastatin	247	DATC	
pitavastatin		92	DATC			
SLCO1B3; LST-2;		Organic anion transporter	digoxin	248	DATC	
			methotrexate	246	IATC	

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	<i>OATP1B3;</i> <i>OATP8</i>		rifampicin	²⁴⁴	DATC
			fexofenadine	²⁴⁹	DATC
			fluvastatin	²⁴⁷	DATC
			pitavastatin	⁹²	DATC
			rosuvastatin	⁹¹	DATC
	<i>SLCO2B1;</i> <i>OATP2B1;</i> <i>OATP-B</i>	Organic anion transporter	pravastatin	⁸³	DATC
			glibenclamide	²⁵⁰	DATC
			atorvastatin	⁸⁷	DATC
			benzylpenicillin	²⁴³	DATC
			fluvastatin	²⁴⁷	DATC
	<i>SLCO4C1;</i> <i>OATP4C1</i>	Organic anion transporter	rosuvastatin	⁹¹	DATC
			methotrexate	²⁵¹	DATC
			digoxin	²⁵¹	DATC



FIGURES FOR DOBSON AND KELL NRDD 7, 205-220 (2008). NOTE THAT THESE DIFFER SLIGHTLY FROM THOSE IN THE PUBLISHED VERSION

CortiAM vs PAMPA

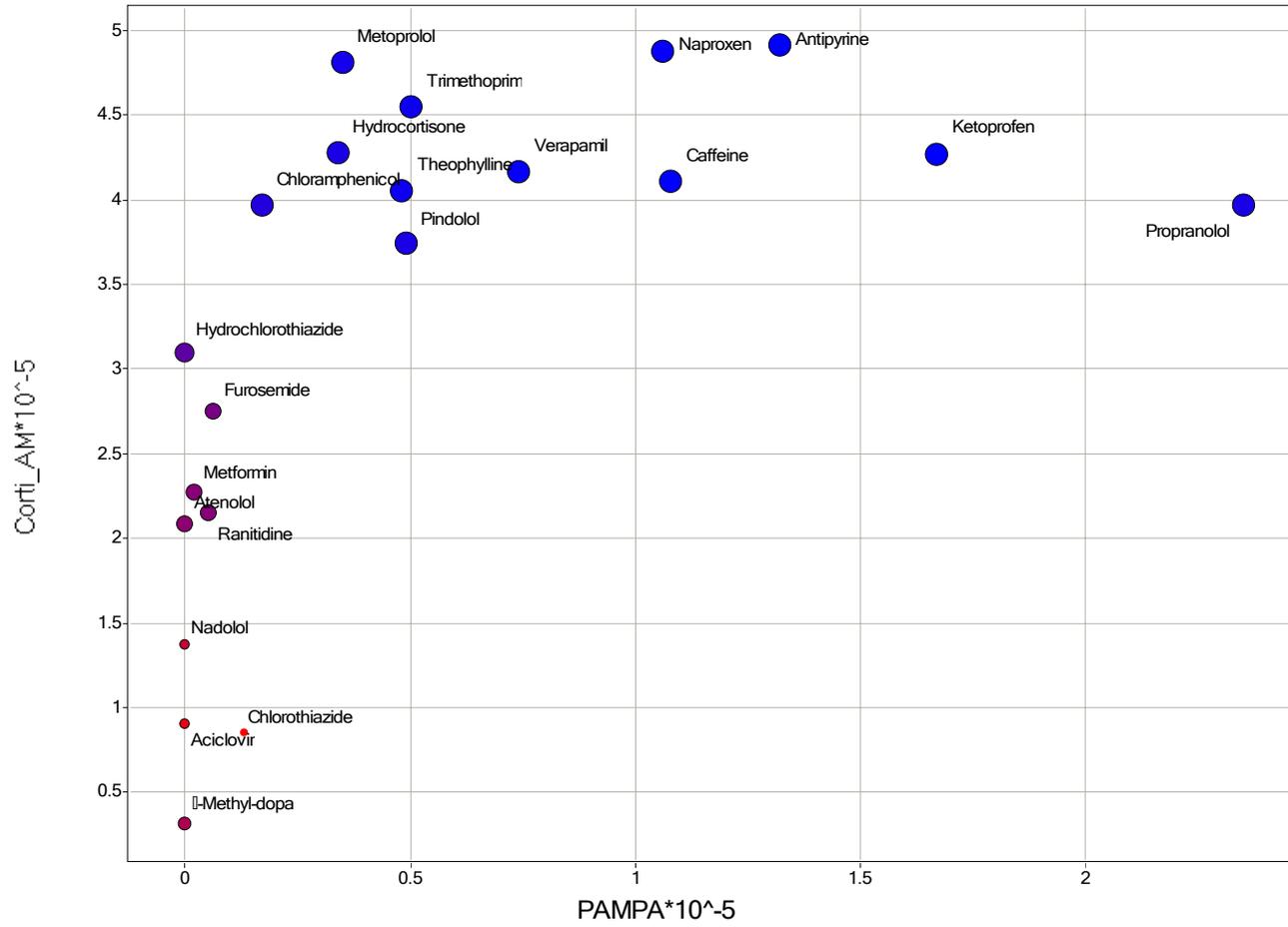


Fig 2A

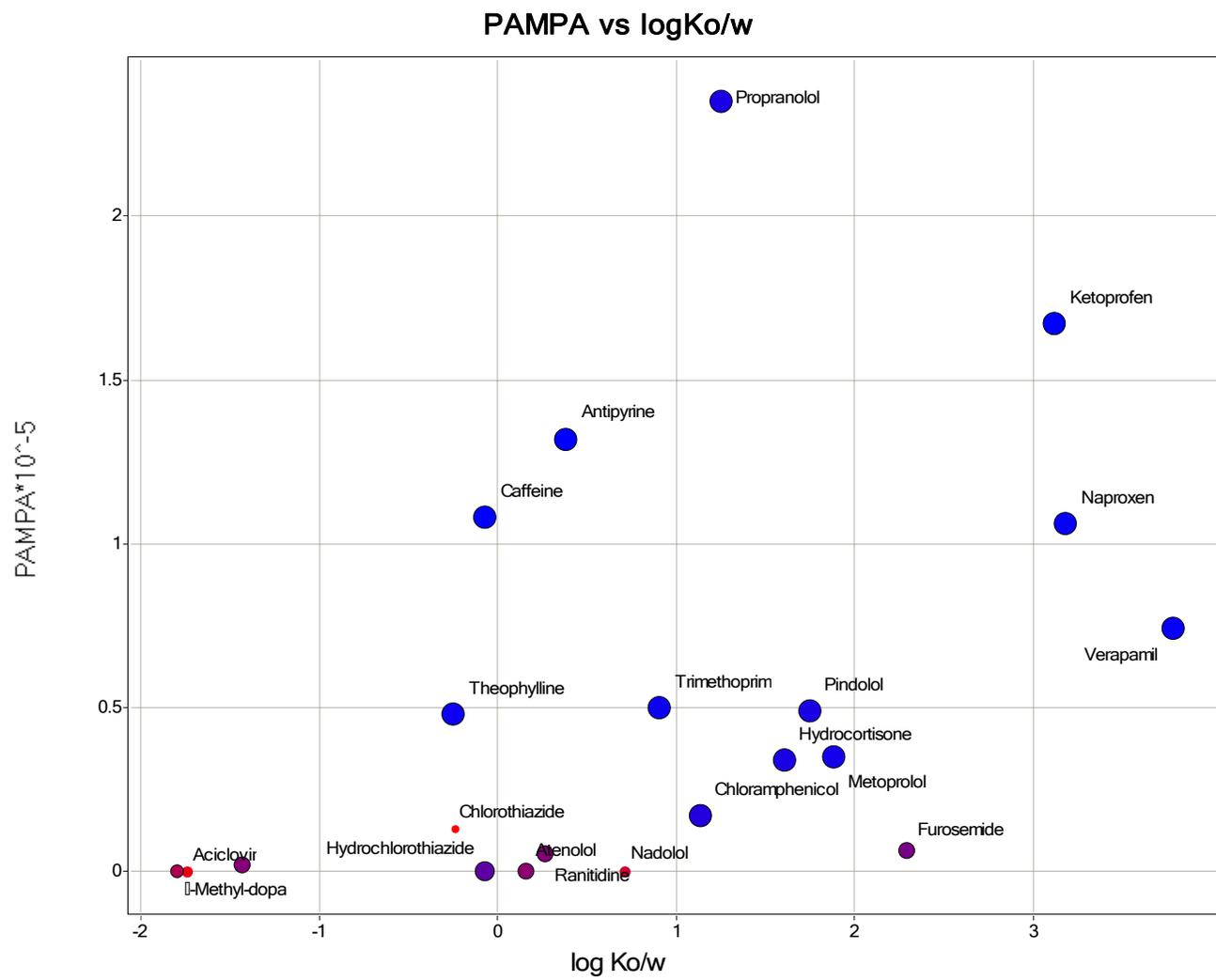


Fig 2B

CaCo vs logKo/w

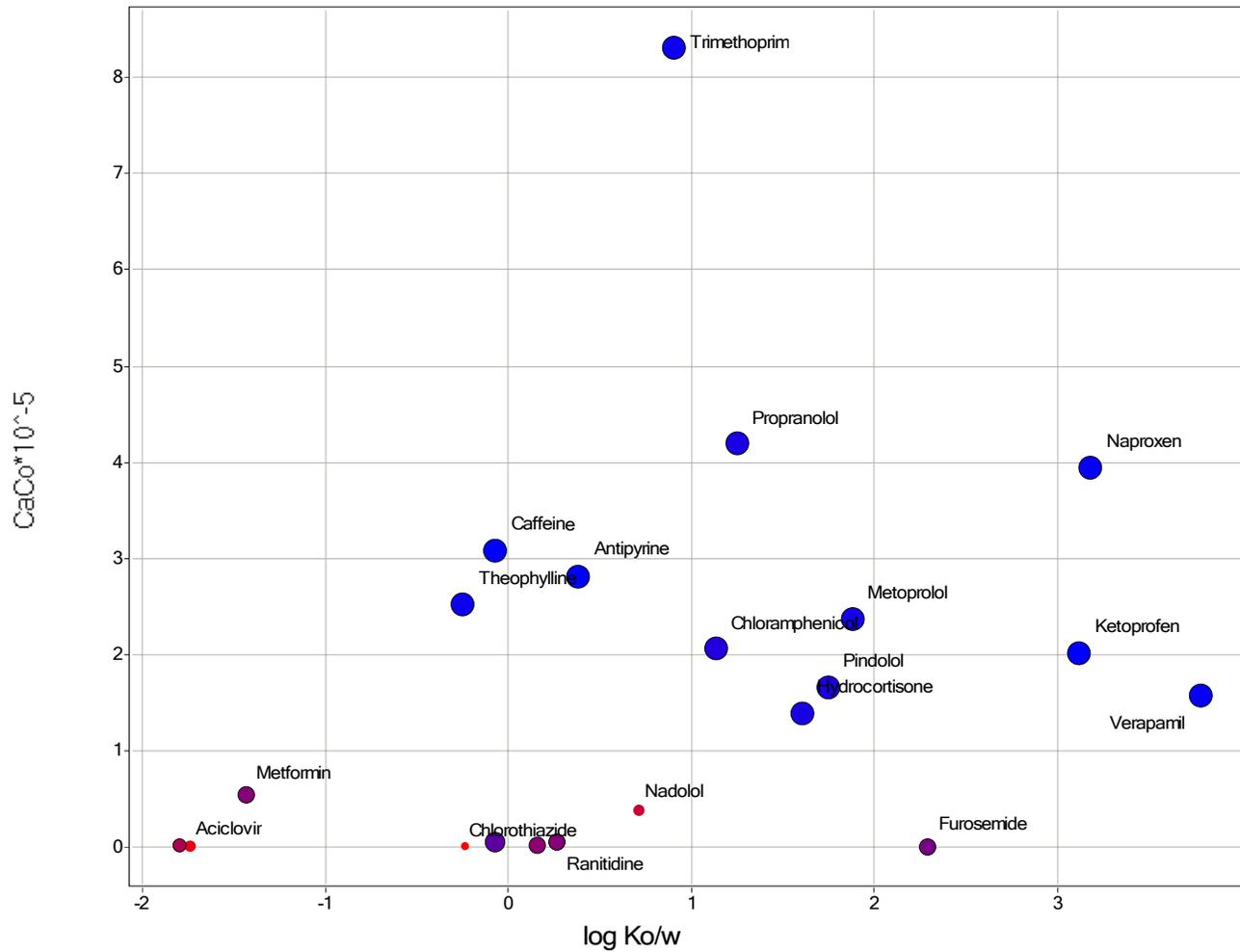
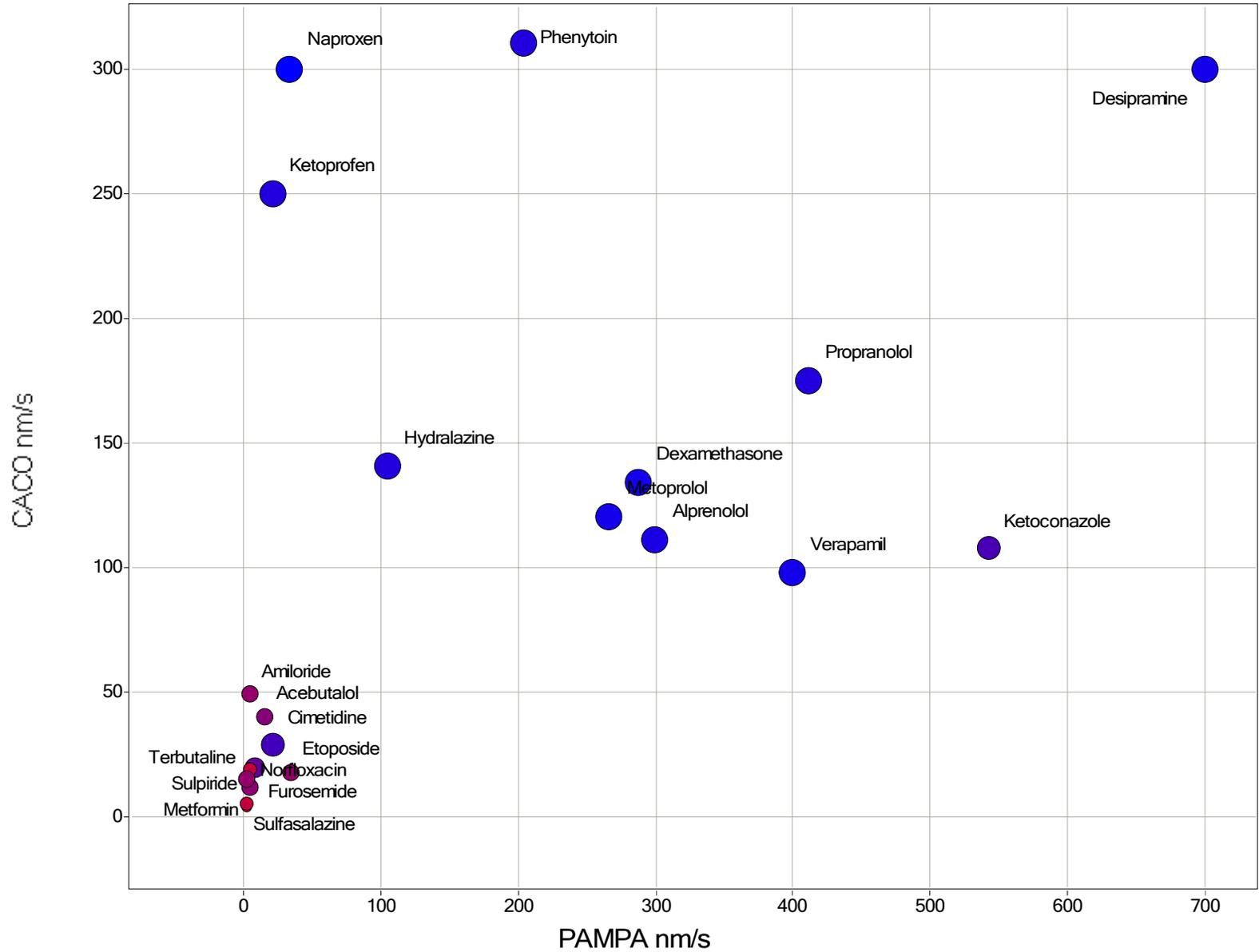
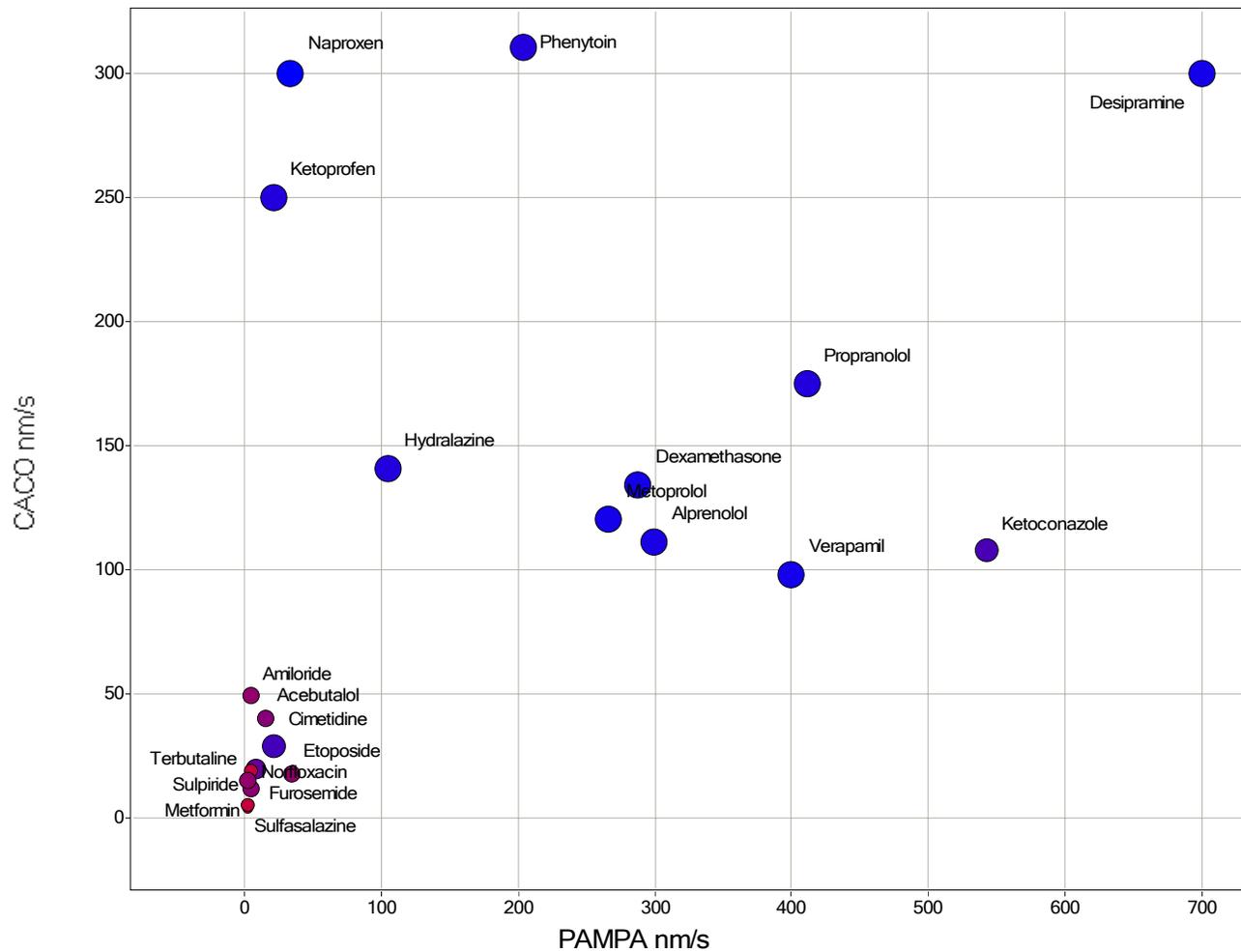


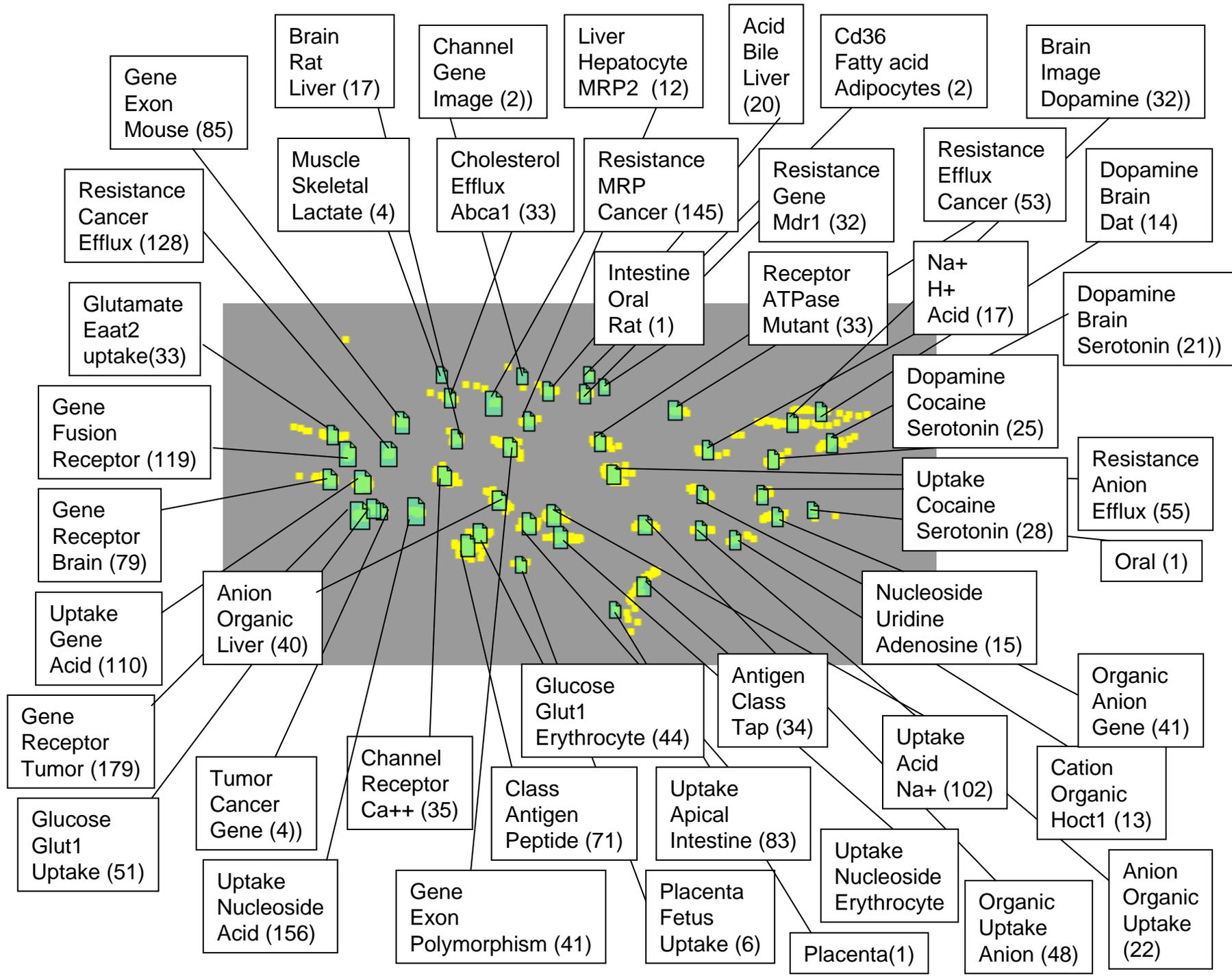
Fig 2C

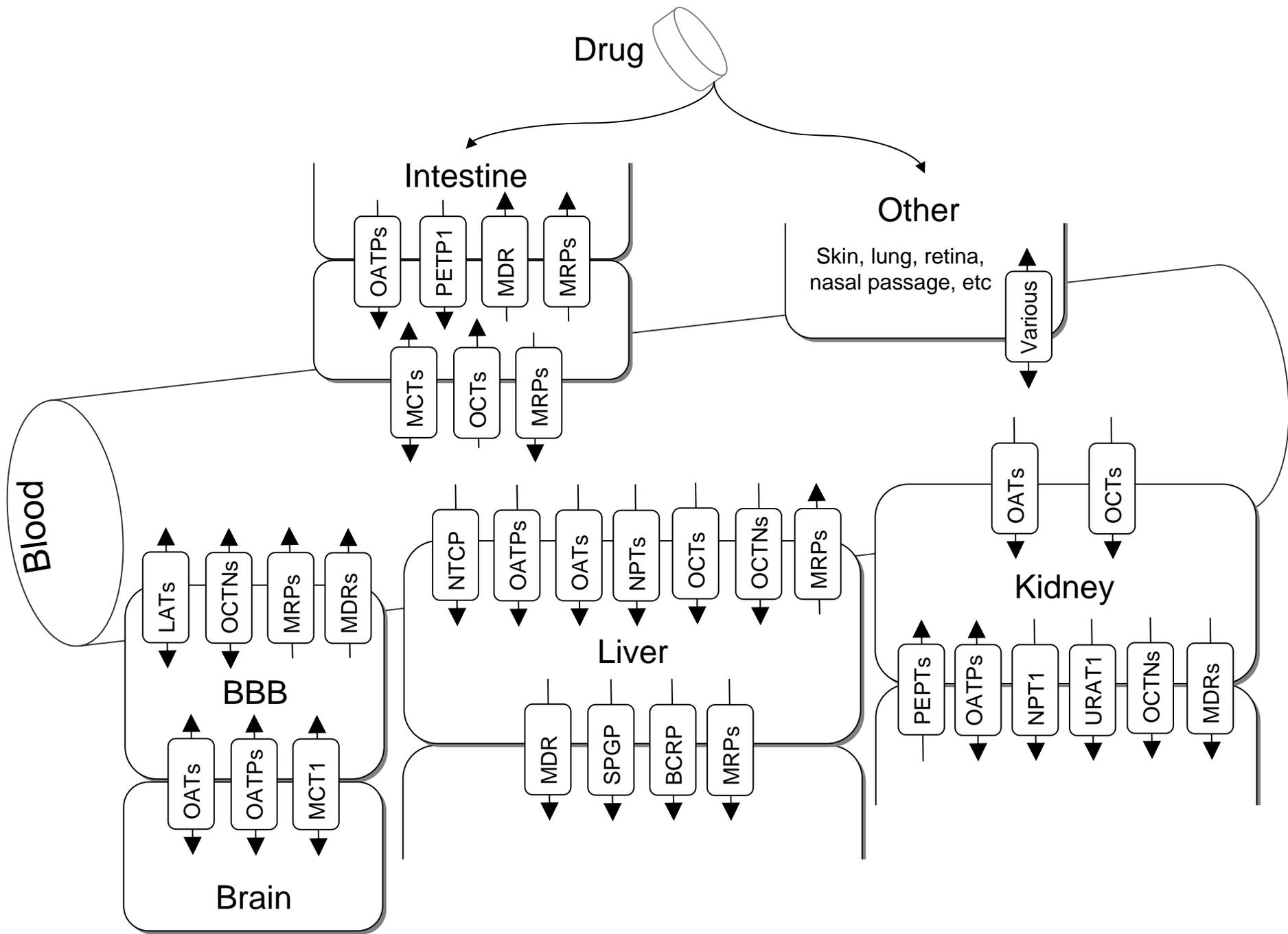
CaCo vs PAMPA



CaCo vs PAMPA

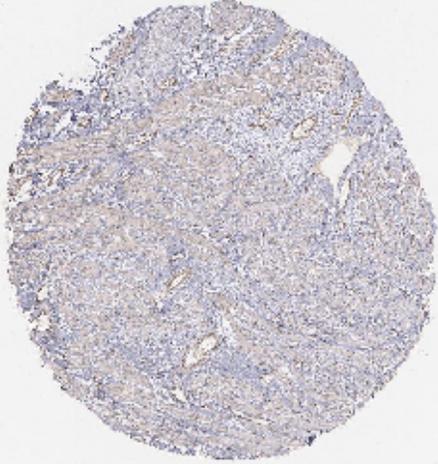
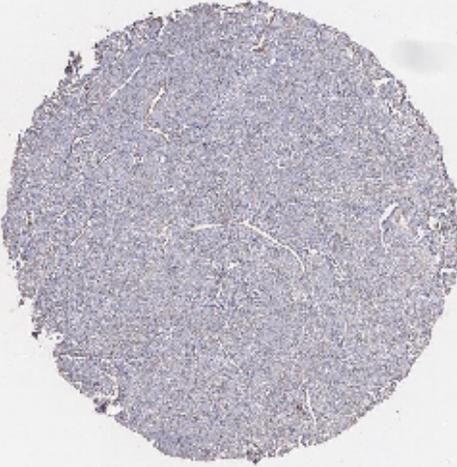
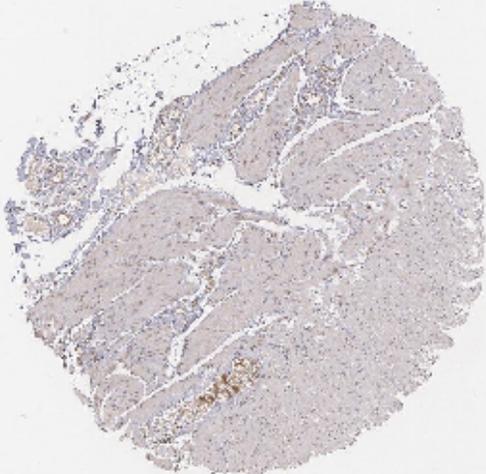






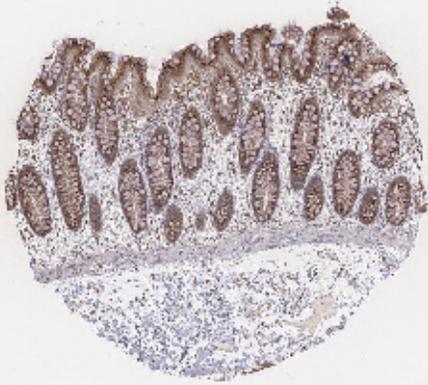
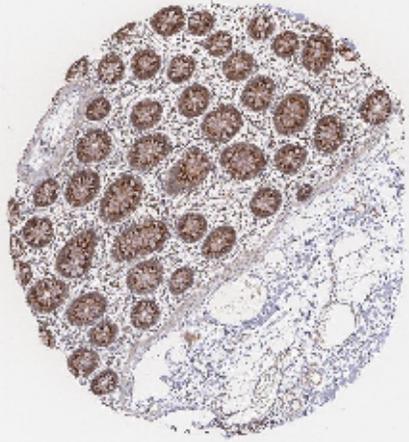
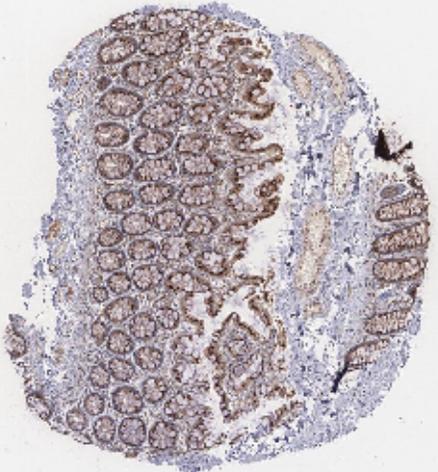
Smooth muscle [SLC1A4]

A

Cell Type	Intensity	Quantity	Localization
Smooth muscle cells	weak	75%-25%	cytoplasmic and/or membranous
			
Female, age 39	Female, age 65	Male, age 67	

Brown color indicates presence of protein, blue color shows cell nuclei. [Image Usage Policy](#)

B

Cell Type	Intensity	Quantity	Localization
Glandular cells	strong	>75%	cytoplasmic and/or membranous
			
Male, age 67	Male, age 14	Female, age 61	

Brown color indicates presence of protein, blue color shows cell nuclei. [Image Usage Policy](#)

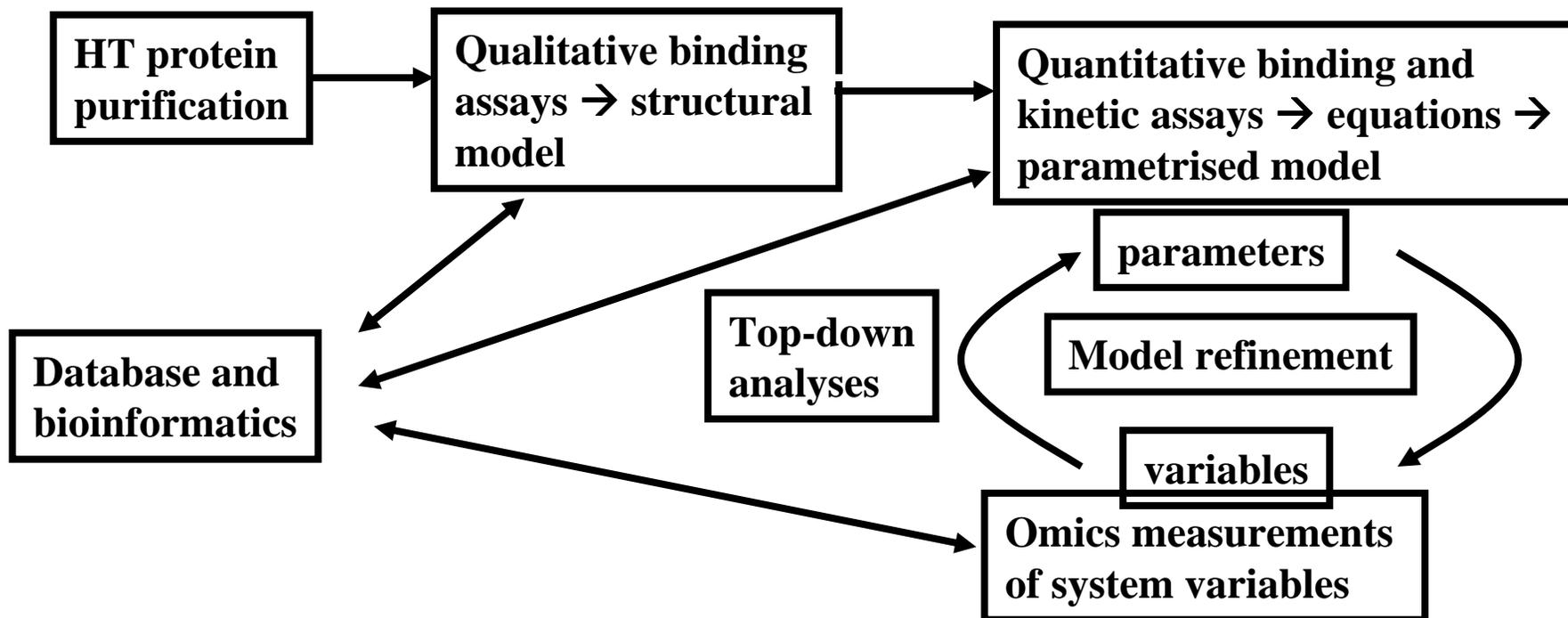


Figure legends

Fig 1. Two means of transmembrane transport of drugs. The membrane-bounded compartment is taken to consist of a lipid bilayer in which are included proteinaceous carriers. **A.** The drug (D) partitions into the lipid bilayer portion of the membrane, roughly according to $\log P$ (the octanol:water partition coefficient) and redissolves in the intracellular fluid. **B.** In this view the drug transport occurs via transfer across the bilayer membrane exactly as it might do in a phospholipid membrane lacking any proteins (although we note that these may more readily admit passage via aqueous pore defects that do not occur nearly so readily in a protein-containing natural biomembrane). **C.** In an alternative view, that is the focus of this review, most or all of the drug transport in fact occurs via proteinaceous carriers that exist in the membrane and that normally transport 'natural' cellular and extracellular metabolites (i.e. those biosynthetically produced by the organism) but which also show activity in transporting the xenobiotic. B and C are not mutually exclusive and could in principle occur together in the same membrane. Overall, the steady-state, free intracellular concentration of a drug will reflect an interplay between passive uptake and the activities of influx and efflux transporters.

Fig 2. Comparisons between drug permeability in natural membranes and artificial systems, and their comparison with oil-water partition coefficients. First, we show the relationship between the apparent permeability of marketed drugs across artificial membranes, across CaCo cells, and the fraction absorbed in humans.

A. Apparent permeability in Corti's artificial membrane vs that in the PAMPA system (note also the numerical differences in the permeability in $\text{cm}\cdot\text{s}^{-1}$), the size encodes the apparent permeability (small = less absorbed) and colour encodes the fraction absorbed in humans (red = low, blue = high). Data plotted from Table 2 of ³¹. The ostensibly hyperbolic shape of the graph is best interpreted in terms of two classes of compounds, one of which has a low PAMPA permeability ($< 0.4 \cdot 10^{-5} \text{ cm}\cdot\text{s}^{-1}$) but considerable variation in the permeability across Corti's membrane system, while the other set has a high permeability ($> 3.5 \cdot 10^{-5} \text{ cm}\cdot\text{s}^{-1}$) in Corti's system and a very variable one in PAMPA.

B. CaCo vs PAMPA (the fraction absorbed in humans in an *in vivo* assay is encoded by both size and colour, blue high). In addition we show the absence of any clear linear relationship between permeability and (logarithm of the) oil-water partition coefficient. Data for **B** from Table 1 of ³³.

Overall, it is clear that even when the assays are tuned by varying the type of lipid and solvent, the uptake into human cells cannot be predicted well by the uptake across artificial membranes since there is no overall correlation between the two.

Figure 3

Comparisons between drug permeability in natural membranes and artificial systems, and their comparison with oil-water partition coefficients.

A. Lack of correlation between apparent permeability in an artificial membrane (PAMPA assay) and $\log K(\text{octanol-water})$.

B. Lack of correlation ($r^2 = 0.097$) between apparent permeability in CaCo-2 cells and $\log K(\text{octanol-water})$. In C and D the data are plotted from those in Table 2 of Corti ³¹, with size encoding the apparent permeability and colour the fraction absorbed in humans (red = low, blue = high).

Overall, it is clear that even when the assays are tuned via the choice of lipid and solvent, the uptake into human cells cannot be predicted well either by the ‘hydrophobicity’ encoded in logK.

Fig 4. Multiple drug carriers in different tissues, all of which may need to be permeated (after ²⁵²).

Fig 5. Tissue-selective expression of solute carrier molecules, where brown colouration indicates presence of protein. Expression levels of SLC7A3 (cationic amino acid transporter, γ^+ system) are high in oesophagus epithelial cells (A) and liver bile duct cells (B), moderate in glandular cells of the small intestine (C), and low in glandular cells of the duodenum (D). Antibody-based histochemical staining pictures taken with its permission from the Human Protein Atlas http://www.proteinatlas.org/tissue_profile.php?antibody_id=3629.

Fig 6. The ‘bottom-up’ systems biology agenda begins with the purification (or at least concentration) of (usually recombinant) proteins, then assays which molecules act as substrates or effectors of these proteins, then uses titrations of these to acquire kinetic parameters and the equations that describe the activity of the individual steps. Such assays may be done *in vitro* (i.e. in liposomes), but will more likely be done in cells (lacking an excessive background) *in situ* by expression cloning, noting that the exact membrane composition can of course affect the kinetic parameters that are estimated. These kinetic data may be used to populate metabolic models, typically described using Ordinary Differential Equations. The models make predictions of the system variables such as metabolic fluxes, and these can be compared with experiment.

References

1. Lipinski, C.A., Lombardo, F., Dominy, B.W. & Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* **23**, 3-25 (1997).
2. Mueller, P., Rudin, D.O., Tien, H.T. & Wescott, W.C. Reconstitution of cell membrane structure *in vitro* and its transformation into an excitable system. *Nature* **194**, 979-& (1962).
3. Jain, M.K. The bimolecular lipid membrane. (Van Nostrand Reinhold, New York; 1972).
4. Tien, H.T. Bilayer lipid membranes (BLM): theory and practice. (Marcel Dekker, New York; 1974).
5. Huque, F.T.T., Box, K., Platts, J.A. & Comer, J. Permeability through DOPC/dodecane membranes: measurement and LFER modelling. *Eur J Pharm Sci* **23**, 223-232 (2004).
6. Camenisch, G., Folkers, G. & van de Waterbeemd, H. Review of theoretical passive drug absorption models: Historical background, recent developments and limitations. *Helv Pharm Acta* **71**, 309-327 (1996).
7. Abraham, M.H. Scales of solute hydrogen bonding - their construction and application to physicochemical and biochemical processes. *Chem. Soc. Rev.* **22**, 73-83 (1993).
8. Abraham, M.H., Chadha, H.S. & Mitchell, R.C. Hydrogen bonding. 33. Factors that influence the distribution of solutes between blood and brain. *J Pharm Sci* **83**, 1257-1268 (1994).
9. Babine, R.E. & Bender, S.L. Molecular recognition of protein-ligand complexes: Applications to drug design. *Chem Rev* **97**, 1359-1472 (1997).
10. Arendt, R.M., Greenblatt, D.J., Liebisch, D.C., Luu, M.D. & Paul, S.M. Determinants of benzodiazepine brain uptake: lipophilicity versus binding affinity. *Psychopharmacology (Berl)* **93**, 72-76 (1987).
11. Abraham, M.H. et al. On the mechanism of human intestinal absorption. *Eur J Med Chem* **37**, 595-605 (2002).
12. Zhao, Y.H. et al. Evaluation of human intestinal absorption data and subsequent derivation of a quantitative structure-activity relationship (QSAR) with the Abraham descriptors. *J Pharm Sci* **90**, 749-784 (2001).
13. Fujikawa, M., Ano, R., Nakao, K., Shimizu, R. & Akamatsu, M. Relationships between structure and high-throughput screening permeability of diverse drugs with artificial membranes: application to prediction of Caco-2 cell permeability. *Bioorg Med Chem* **13**, 4721-4732 (2005).
14. Tien, H.T. & Ottova-Leitmannova, A. (eds.) Planar lipid bilayers (BLMs) and their applications. (Elsevier, New York; 2003).
15. Westerhoff, H.V., Kell, D.B., Kamp, F. & van Dam, K. in Microcompartmentation. (ed. D.P. Jones) 115-154 (CRC Press, Boca Raton, Fl.; 1988).
16. Lee, A.G. How lipids affect the activities of integral membrane proteins. *Biochim Biophys Acta* **1666**, 62-87 (2004).
17. Reynwar, B.J. et al. Aggregation and vesiculation of membrane proteins by curvature-mediated interactions. *Nature* **447**, 461-464 (2007).
18. McDonald, M.D., Wood, C.M., Wang, Y. & Walsh, P.J. Differential branchial and renal handling of urea, acetamide and thiourea in the gulf toadfish *Opsanus beta*: evidence for two transporters. *J Exp Biol* **203**, 1027-1037 (2000).
19. Fujimoto, N., Inoue, K., Hayashi, Y. & Yuasa, H. Glycerol uptake in HCT-15 human colon cancer cell line by Na(+)-dependent carrier-mediated transport. *Biol Pharm Bull* **29**, 150-154 (2006).

20. Hohmann, S. Osmotic stress signaling and osmoadaptation in yeasts. *Microbiol Mol Biol Rev* **66**, 300-372 (2002).
21. Chappell, J.B. & Crofts, A.R. in Regulation of metabolic processes in mitochondria (eds. J.M. Tager, S. Papa, E. Quagliariello & E.C. Slater) 293-321 (Elsevier, Amsterdam; 1966).
22. Kell, D.B., Peck, M.W., Rodger, G. & Morris, J.G. On the permeability to weak acids and bases of the cytoplasmic membrane of *Clostridium pasteurianum*. *Biochem Biophys Res Commun* **99**, 81-88 (1981).
23. Agre, P. et al. Aquaporin water channels--from atomic structure to clinical medicine. *J Physiol* **542**, 3-16 (2002).
24. Cohen, B.E. & Bangham, A.D. Diffusion of small non-electrolytes across liposome membranes. *Nature* **236**, 173-174 (1972).
25. Kansy, M., Senner, F. & Gubernator, K. Physicochemical high throughput screening: Parallel artificial membrane permeation assay in the description of passive absorption processes. *J Med Chem* **41**, 1007-1010 (1998).
26. Calcagno, A.M., Ludwig, J.A., Fostel, J.M., Gottesman, M.M. & Ambudkar, S.V. Comparison of drug transporter levels in normal colon, colon cancer, and Caco-2 cells: impact on drug disposition and discovery. *Mol Pharm* **3**, 87-93 (2006).
27. Hubatsch, I., Ragnarsson, E.G.E. & Artursson, P. Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers. *Nat Protocols* **2**, 2111-2119 (2007).
28. Camenisch, G., Folkers, G. & van de Waterbeemd, H. Comparison of passive drug transport through Caco-2 cells and artificial membranes. *Int J Pharmaceut* **147**, 61-70 (1997).
29. Malkia, A., Murtomaki, L., Urtti, A. & Kontturi, K. Drug permeation in biomembranes: in vitro and in silico prediction and influence of physicochemical properties. *Eur J Pharm Sci* **23**, 13-47 (2004).
30. Subramanian, G. & Kitchen, D.B. Computational approaches for modeling human intestinal absorption and permeability. *J Mol Model (Online)* **12**, 577-589 (2006).
31. Corti, G., Maestrelli, F., Cirri, M., Zerrouk, N. & Mura, P. Development and evaluation of an in vitro method for prediction of human drug absorption - II. Demonstration of the method suitability. *Eur J Pharm Sci* **27**, 354-362 (2006).
32. Flaten, G.E., Dhanikula, A.B., Luthman, K. & Brandl, M. Drug permeability across a phospholipid vesicle based barrier: a novel approach for studying passive diffusion. *Eur J Pharm Sci* **27**, 80-90 (2006).
33. Balimane, P.V., Han, Y.H. & Chong, S.H. Current industrial practices of assessing permeability and P-glycoprotein interaction. *AAPS Journal* **8**, E1-E13 (2006).
34. Salter, G.J. & Kell, D.B. Solvent selection for whole cell biotransformations in organic media. *CRC Crit Rev. Biotechnol.* **15**, 139-177. (1995).
35. Burton, P.S., Goodwin, J.T., Vidmar, T.J. & Amore, B.M. Predicting drug absorption: how nature made it a difficult problem. *J Pharmacol Exp Ther* **303**, 889-895 (2002).
36. Lipinski, C.A., Lombardo, F., Dominy, B.W. & Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* **46**, 3-26 (2001).
37. Gasteiger, J. (ed.) Handbook of Chemoinformatics: From Data to Knowledge. (Wiley/VCH, Weinheim; 2003).
38. Gutknecht, J. & Walter, A. Histamine, theophylline and tryptamine transport through lipid bilayer membranes. *Biochim Biophys Acta* **649**, 149-154 (1981).
39. Lieb, W.R. & Stein, W.D. Biological membranes behave as non-porous polymeric sheets with respect to the diffusion of non-electrolytes. *Nature* **224**, 240-243 (1969).
40. Xiang, T.X. & Anderson, B.D. The relationship between permeant size and permeability in lipid bilayer membranes. *J Membr Biol* **140**, 111-122 (1994).

41. Bean, R.C., Shepherd, W.C. & Chan, H. Permeability of lipid bilayer membranes to organic solutes. *J Gen Physiol* **52**, 495-508 (1968).
42. Walter, A. & Gutknecht, J. Permeability of small nonelectrolytes through lipid bilayer membranes. *J Membr Biol* **90**, 207-217 (1986).
43. Bordi, F., Cametti, C. & Naglieri, A. Ion transport in lipid bilayer membranes through aqueous pores. *Coll Surf A* **159**, 231-237 (1999).
44. Bowman, W.C., Rand, M.J. & West, G.B. Textbook of Pharmacology. (Blackwell, Oxford; 1967).
45. Seeman, P. The membrane actions of anesthetics and tranquilizers. *Pharmacol Rev* **24**, 583-655 (1972).
46. Franks, N.P., Jenkins, A., Conti, E., Lieb, W.R. & Brick, P. Structural basis for the inhibition of firefly luciferase by a general anesthetic. *Biophys J* **75**, 2205-2211 (1998).
47. Miller, K.W. The nature of sites of general anaesthetic action. *Br J Anaesth* **89**, 17-31 (2002).
48. Mihic, S.J. et al. Sites of alcohol and volatile anaesthetic action on GABA_A and glycine receptors. *Nature* **389**, 385-389 (1997).
49. Jurd, R. et al. General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA_A receptor $\beta 3$ subunit. *FASEB J* **17**, 250-252 (2003).
50. Grasshoff, C., Drexler, B., Rudolph, U. & Antkowiak, B. Anaesthetic drugs: linking molecular actions to clinical effects. *Curr Pharm Des* **12**, 3665-3679 (2006).
51. Franks, N.P. Molecular targets underlying general anaesthesia. *Br J Pharmacol* **147 Suppl 1**, S72-81 (2006).
52. Wallner, M., Hancher, H.J. & Olsen, R.W. Low-dose alcohol actions on alpha 4 beta 3 delta GABA_A receptors are reversed by the behavioral alcohol antagonist Ro15-4513. *Proc Natl Acad Sci* **103**, 8540-8545 (2006).
53. Lewis, K. Multidrug resistance pumps in bacteria: variations on a theme. *Trends Biochem. Sci.* **19**, 119-123 (1994).
54. Sipos, G. & Kuchler, K. Fungal ATP-binding cassette (ABC) transporters in drug resistance & detoxification. *Curr Drug Targets* **7**, 471-481 (2006).
55. Borst, P. & Oude Elferink, R. Mammalian ABC transporters in health and disease. *Annu Rev Biochem* **71**, 537-592 (2002).
56. Higgins, C.F. Multiple molecular mechanisms for multidrug resistance transporters. *Nature* **446**, 749-757 (2007).
57. Hediger, M.A. et al. The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins. Introduction. *Pflügers Arch* **447**, 465-468 (2004).
58. Dean, M. & Annilo, T. Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. *Annu Rev Genomics Hum Genet* **6**, 123-142 (2005).
59. Duarte, N.C. et al. Global reconstruction of the human metabolic network based on genomic and bibliomic data. *Proc Natl Acad Sci* **104**, 1777-1782 (2007).
60. Calvo, S. et al. Systematic identification of human mitochondrial disease genes through integrative genomics. *Nat Genet* **38**, 576-582 (2006).
61. Kunji, E.R. The role and structure of mitochondrial carriers. *FEBS Lett* **564**, 239-244 (2004).
62. Palmieri, F. The mitochondrial transporter family (SLC25): physiological and pathological implications. *Pflugers Arch* **447**, 689-709 (2004).
63. Robinson, A.J. & Kunji, E.R. Mitochondrial carriers in the cytoplasmic state have a common substrate binding site. *Proc Natl Acad Sci U S A* **103**, 2617-2622 (2006).
64. Hagenbuch, B. & Meier, P.J. Organic anion transporting polypeptides of the OATP/ SLC21 family: phylogenetic classification as OATP/ SLCO superfamily, new nomenclature and molecular/functional properties. *Pflugers Arch* **447**, 653-665 (2004).

65. Koepsell, H. & Endou, H. The SLC22 drug transporter family. *Pflugers Arch* **447**, 666-676 (2004).
66. Daniel, H. & Kottra, G. The proton oligopeptide cotransporter family SLC15 in physiology and pharmacology. *Pflugers Arch* **447**, 610-618 (2004).
67. Bailey, P.D. et al. How to make drugs orally active: a substrate template for peptide transporter PepT1. *Angew Chem Int Ed Engl* **39**, 505-508 (2000).
68. Hilgendorf, C. et al. Expression of thirty-six drug transporter genes in human intestine, liver, kidney, and organotypic cell lines. *Drug Metab Dispos* **35**, 1333-1340 (2007).
69. Bu'Lock, J.D., Nisbet, L.J. & Winstanley, D.J. Bioactive microbial products: search and discovery. (Academic, New York; 1982).
70. Devlin, J.P. (ed.) High throughput screening: the discovery of bioactive substances. (Marcel Dekker, New York; 1997).
71. Payne, D.J., Gwynn, M.N., Holmes, D.J. & Pompliano, D.L. Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nat Rev Drug Discov* **6**, 29-40 (2007).
72. Kutchan, T.M. A role for intra- and intercellular translocation in natural product biosynthesis. *Curr Opin Plant Biol* **8**, 292-300 (2005).
73. Piggott, A.M. & Karuso, P. Quality, not quantity: the role of natural products and chemical proteomics in modern drug discovery. *Comb Chem High Throughput Screen* **7**, 607-630 (2004).
74. Kell, D.B., Kaprelyants, A.S. & Grafen, A. On pheromones, social behaviour and the functions of secondary metabolism in bacteria. *Trends Ecol. Evolution* **10**, 126-129 (1995).
75. Greene, L.H. et al. The CATH domain structure database: new protocols and classification levels give a more comprehensive resource for exploring evolution. *Nucleic Acids Res* **35**, D291-297 (2007).
76. Marsden, R.L. et al. Exploiting protein structure data to explore the evolution of protein function and biological complexity. *Philos Trans R Soc Lond B Biol Sci* **361**, 425-440 (2006).
77. Tamai, I. et al. The predominant contribution of oligopeptide transporter PepT1 to intestinal absorption of beta-lactam antibiotics in the rat small intestine. *J Pharm Pharmacol* **49**, 796-801 (1997).
78. Ren, Q. & Paulsen, I.T. Comparative analyses of fundamental differences in membrane transport capabilities in prokaryotes and eukaryotes. *PLoS Comput Biol* **1**, e27 (2005).
79. König, J., Seithel, A., Gradhand, U. & Fromm, M.F. Pharmacogenomics of human OATP transporters. *Naunyn Schmiedebergs Arch Pharmacol* **372**, 432-443 (2006).
80. Nakai, D. et al. Human liver-specific organic anion transporter, LST-1, mediates uptake of pravastatin by human hepatocytes. *J Pharmacol Exp Ther* **297**, 861-867 (2001).
81. Kameyama, Y., Yamashita, K., Kobayashi, K., Hosokawa, M. & Chiba, K. Functional characterization of SLCO1B1 (OATP-C) variants, SLCO1B1*5, SLCO1B1*15 and SLCO1B1*15+C1007G, by using transient expression systems of HeLa and HEK293 cells. *Pharmacogenet Genomics* **15**, 513-522 (2005).
82. Nozawa, T., Imai, K., Nezu, J., Tsuji, A. & Tamai, I. Functional characterization of pH-sensitive organic anion transporting polypeptide OATP-B in human. *J Pharmacol Exp Ther* **308**, 438-445 (2004).
83. Kobayashi, D. et al. Involvement of human organic anion transporting polypeptide OATP-B (SLC21A9) in pH-dependent transport across intestinal apical membrane. *J Pharmacol Exp Ther* **306**, 703-708 (2003).
84. Hsiang, B. et al. A novel human hepatic organic anion transporting polypeptide (OATP2). Identification of a liver-specific human organic anion transporting polypeptide and identification of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. *J Biol Chem* **274**, 37161-37168 (1999).

85. Lau, Y.Y., Huang, Y., Frassetto, L. & Benet, L.Z. Effect of OATP1B transporter inhibition on the pharmacokinetics of atorvastatin in healthy volunteers. *Clinical Pharmacology & Therapeutics* **81**, 194-204 (2007).
86. Kivistö, K.T. & Niemi, M. Influence of drug transporter polymorphisms on pravastatin pharmacokinetics in humans. *Pharmaceut Res* **24**, 239-247 (2007).
87. Grube, M. et al. Organic anion transporting polypeptide 2B1 is a high-affinity transporter for atorvastatin and is expressed in the human heart. *Clin Pharmacol Therapeut* **80**, 607-620 (2006).
88. Fujino, H., Saito, T., Ogawa, S. & Kojima, J. Transporter-mediated influx and efflux mechanisms of pitavastatin, a new inhibitor of HMG-CoA reductase. *J Pharm Pharmacol* **57**, 1305-1311 (2005).
89. Kim, R.B. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins) and genetic variability (single nucleotide polymorphisms) in a hepatic drug uptake transporter: what's it all about? *Clin Pharmacol Ther* **75**, 381-385 (2004).
90. Shitara, Y., Itoh, T., Sato, H., Li, A.P. & Sugiyama, Y. Inhibition of transporter-mediated hepatic uptake as a mechanism for drug-drug interaction between cerivastatin and cyclosporin A. *J Pharmacol Exp Ther* **304**, 610-616 (2003).
91. Ho, R.H. et al. Drug and bile acid transporters in rosuvastatin hepatic uptake: function, expression, and pharmacogenetics. *Gastroenterology* **130**, 1793-1806 (2006).
92. Hirano, M., Maeda, K., Shitara, Y. & Sugiyama, Y. Contribution of OATP2 (OATP1B1) and OATP8 (OATP1B3) to the hepatic uptake of pitavastatin in humans. *J Pharmacol Exp Ther* **311**, 139-146 (2004).
93. Tamai, I. & Tsuji, A. Transporter-mediated permeation of drugs across the blood-brain barrier. *J Pharm Sci* **89**, 1371-1388 (2000).
94. Tamai, I. et al. Participation of a proton-cotransporter, MCT1, in the intestinal transport of monocarboxylic acids. *Biochem Biophys Res Commun* **214**, 482-489 (1995).
95. Parsegian, A. Energy of an ion crossing a low dielectric membrane: solutions to four relevant electrostatic problems. *Nature* **221**, 844-846 (1969).
96. Dilger, J.P., McLaughlin, S.G.A., McIntosh, T.J. & Simon, S.A. Dielectric constant of phospholipid bilayers and the permeability of membranes to ions. *Science* **206**, 1196-1198 (1979).
97. Mitchell, P. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biol. Rev.* **41**, 445-502 (1966).
98. Grinius, L.L. et al. Conversion of biomembrane-produced energy into electric form. I. Submitochondrial particles. *Biochim Biophys Acta* **216**, 1-12 (1970).
99. Azzone, G.F., Pietrobon, D. & Zoratti, M. Determination of the proton electrochemical gradient across biological membranes. *Curr. Top. Bioenerg.* **13**, 1-77 (1984).
100. Nicholls, D.G. & Ferguson, S.J. Bioenergetics 2. (Academic Press, London; 1992).
101. Barts, P.W.J.A., Hoerberichts, J.A., Klaassen, A. & Borst-Pauwels, G.W.F.H. Uptake of the lipophilic cation dibenzylidimethylammonium into *Saccharomyces cerevisiae*. Interaction with the thiamine transport system. *Biochim Biophys Acta* **597**, 125-136 (1980).
102. Theuvenet, A.P.R., van de Wijngaard, W.M.H. & Borst-Pauwels, G.W.F.H. 9-Aminoacridine, a fluorescent probe of the thiamine carrier in yeast cells. *Biochim Biophys Acta* **730**, 255-262 (1983).
103. Yoshioka, K., Nishimura, H. & Hasegawa, T. Effect of a phenyl group in quaternary ammonium compounds on thiamine uptake in isolated rat hepatocytes. *Biochim Biophys Acta* **819**, 263-266 (1985).
104. Damper, P.D., Epstein, W., Rosen, B.P. & Sorensen, E.N. Thallous ion is accumulated by potassium transport systems in *Escherichia coli*. *Biochemistry* **18**, 4165-4169 (1979).

105. Kusuvara, H. & Sugiyama, Y. Role of transporters in the tissue-selective distribution and elimination of drugs: transporters in the liver, small intestine, brain and kidney. *J Control Release* **78**, 43-54 (2002).
106. Faber, K.N., Muller, M. & Jansen, P.L. Drug transport proteins in the liver. *Adv Drug Deliv Rev* **55**, 107-124 (2003).
107. Mizuno, N., Niwa, T., Yotsumoto, Y. & Sugiyama, Y. Impact of drug transporter studies on drug discovery and development. *Pharmacol Rev* **55**, 425-461 (2003).
108. Sai, Y. Biochemical and molecular pharmacological aspects of transporters as determinants of drug disposition. *Drug Metab Pharmacokinet* **20**, 91-99 (2005).
109. Alnouti, Y., Petrick, J.S. & Klaassen, C.D. Tissue distribution and ontogeny of organic cation transporters in mice. *Drug Metab Dispos* **34**, 477-482 (2006).
110. Kim, R.B. Transporters and drug discovery: why, when, and how. *Mol Pharmaceut* **3**, 26-32 (2006).
111. Lin, J.H. Tissue distribution and pharmacodynamics: A complicated relationship. *Current Drug Metabolism* **7**, 39-65 (2006).
112. Shitara, Y., Horie, T. & Sugiyama, Y. Transporters as a determinant of drug clearance and tissue distribution. *Eur J Pharm Sci* **27**, 425-446 (2006).
113. Zhang, L., Strong, J.M., Qiu, W., Lesko, L.J. & Huang, S.M. Scientific perspectives on drug transporters and their role in drug interactions. *Mol Pharmaceut* **3**, 62-69 (2006).
114. Raub, T.J. P-glycoprotein recognition of substrates and circumvention through rational drug design. *Mol Pharm* **3**, 3-25 (2006).
115. Sweet, D.H. Organic anion transporter (Slc22a) family members as mediators of toxicity. *Toxicol Appl Pharmacol* **204**, 198-215 (2005).
116. Tsuji, A. Transporter-mediated drug interactions. *Drug Metab Pharmacokinet* **17**, 253-274 (2002).
117. Shitara, Y., Sato, H. & Sugiyama, Y. Evaluation of drug-drug interaction in the hepatobiliary and renal transport of drugs. *Annu Rev Pharmacol Toxicol* **45**, 689-723 (2005).
118. Endres, C.J., Hsiao, P., Chung, F.S. & Unadkat, J.D. The role of transporters in drug interactions. *Eur J Pharm Sci* **27**, 501-517 (2006).
119. Li, M., Anderson, G.D. & Wang, J. Drug-drug interactions involving membrane transporters in the human kidney. *Expert Opinion on Drug Metabolism & Toxicology* **2**, 505-532 (2006).
120. Li, M., Anderson, G.D. & Wang, J. Drug-drug interactions involving membrane transporters in the human kidney. *Expert Opin Drug Metab Toxicol* **2**, 505-532 (2006).
121. Dresser, G.K. et al. Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clin Pharmacol Ther* **71**, 11-20 (2002).
122. Amidon, G.L. & Lee, H.J. Absorption of peptide and peptidomimetic drugs. *Annu Rev Pharmacol Toxicol* **34**, 321-341 (1994).
123. Tsuji, A. Tissue selective drug delivery utilizing carrier-mediated transport systems. *J Controlled Release* **62**, 239-244 (1999).
124. Michalska, D., Morzyk, B., Bienko, D.C. & Wojciechowski, W. Glutarimide: a carrier transporting drug through cell membranes. *Med Hypotheses* **54**, 472-474 (2000).
125. Rubio-Aliaga, I. & Daniel, H. Mammalian peptide transporters as targets for drug delivery. *Trends Pharmacol Sci* **23**, 434-440 (2002).
126. Beaumont, K., Webster, R., Gardner, I. & Dack, K. Design of ester prodrugs to enhance oral absorption of poorly permeable compounds: challenges to the discovery scientist. *Curr Drug Metab* **4**, 461-485 (2003).
127. Ettmayer, P., Amidon, G.L., Clement, B. & Testa, B. Lessons learned from marketed and investigational prodrugs. *J Med Chem* **47**, 2393-2404 (2004).

128. Majumdar, S., Duvvuri, S. & Mitra, A.K. Membrane transporter/receptor-targeted prodrug design: strategies for human and veterinary drug development. *Adv Drug Deliv Rev* **56**, 1437-1452 (2004).
129. Terada, T. & Inui, K. Peptide transporters: structure, function, regulation and application for drug delivery. *Curr Drug Metab* **5**, 85-94 (2004).
130. Pardridge, W.M. Molecular Trojan horses for blood-brain barrier drug delivery. *Curr Opin Pharmacol*, doi:10.1016/j.coph.2006.1006.1001 (2006).
131. Catnach, S.M., Fairclough, P.D. & Hammond, S.M. Intestinal absorption of peptide drugs: advances in our understanding and clinical implications. *Gut* **35**, 441-444 (1994).
132. Tamai, I. et al. Improvement of L-dopa absorption by dipeptidyl derivation, utilizing peptide transporter PepT1. *J Pharm Sci* **87**, 1542-1546 (1998).
133. Bailey, P.D. et al. Conformational and spacial preferences for substrates of PepT1. *Chem Commun (Camb)*, 5352-5354 (2005).
134. Hammond, S.M. et al. A new class of synthetic antibacterials acting on lipopolysaccharide biosynthesis. *Nature* **327**, 730-732 (1987).
135. Kullak-Ublick, G.A. et al. Chlorambucil-taurocholate is transported by bile acid carriers expressed in human hepatocellular carcinomas. *Gastroenterology* **113**, 1295-1305 (1997).
136. Briz, O. et al. Carriers involved in targeting the cytostatic bile acid-cisplatin derivatives cis-diammine-chloro-cholylglycinate-platinum(II) and cis-diammine-bisursodeoxycholate-platinum(II) toward liver cells. *Mol Pharmacol* **61**, 853-860 (2002).
137. Tolle-Sander, S., Lentz, K.A., Maeda, D.Y., Coop, A. & Polli, J.E. Increased acyclovir oral bioavailability via a bile acid conjugate. *Mol Pharm* **1**, 40-48 (2004).
138. Li, Y.H., Tanno, M., Itoh, T. & Yamada, H. Role of the monocarboxylic acid transport system in the intestinal absorption of an orally active beta-lactam prodrug: carindacillin as a model. *Int J Pharm* **191**, 151-159 (1999).
139. Rishton, G.M. Nonleadlikeness and leadlikeness in biochemical screening. *Drug Discovery Today* **8**, 86-96 (2003).
140. Hann, M.M. & Oprea, T.I. Pursuing the leadlikeness concept in pharmaceutical research. *Curr Op Chem Biol* **8**, 255-263 (2004).
141. Walters, W.P. & Murcko, M.A. Prediction of 'drug-likeness'. *Adv Drug Deliv Rev* **54**, 255-271 (2002).
142. Muegge, I. Selection criteria for drug-like compounds. *Med Res Rev* **23**, 302-321 (2003).
143. Wunberg, T. et al. Improving the hit-to-lead process: data-driven assessment of drug-like and lead-like screening hits. *Drug Discov Today* **11**, 175-180 (2006).
144. Reichel, A. The role of blood-brain barrier studies in the pharmaceutical industry. *Curr Drug Metab* **7**, 183-203 (2006).
145. Leeson, P.D. & Springthorpe, B. The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat Rev Drug Discov* **6**, 881-890 (2007).
146. Vicens, M. et al. Novel cationic and neutral glycocholic acid and polyamine conjugates able to inhibit transporters involved in hepatic and intestinal bile acid uptake. *Bioorg Med Chem* **15**, 2359-2367 (2007).
147. Gether, U., Andersen, P.H., Larsson, O.M. & Schousboe, A. Neurotransmitter transporters: molecular function of important drug targets. *Trends Pharmacol Sci* **27**, 375-383 (2006).
148. Hyttel, J. Pharmacological characterization of selective serotonin reuptake inhibitors (SSRIs). *Int Clin Psychopharmacol* **9**, 19-26 (1994).
149. Pacholczyk, T., Blakely, R.D. & Amara, S.G. Expression cloning of a cocaine-sensitive and antidepressant-sensitive human noradrenaline transporter. *Nature* **350**, 350-354 (1991).
150. Zhang, L. et al. Cloning and functional expression of a human liver organic cation transporter. *Mol Pharmacol* **51**, 913-921 (1997).

151. Tian, X., Zhang, P., Zamek-Gliszczynski, M.J. & Brouwer, K.L. Knocking down transport: applications of RNA interference in the study of drug transport proteins. *Drug Metab Rev* **37**, 705-723 (2005).
152. Anzai, N., Kanai, Y. & Endou, H. Organic anion transporter family: current knowledge. *J Pharmacol Sci* **100**, 411-426 (2006).
153. Kim, M.K. & Shim, C.K. The transport of organic cations in the small intestine: current knowledge and emerging concepts. *Arch Pharm Res* **29**, 605-616 (2006).
154. Su, A.I. et al. A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc Natl Acad Sci U S A* **101**, 6062-6067 (2004).
155. Jongeneel, C.V. et al. An atlas of human gene expression from massively parallel signature sequencing (MPSS). *Genome Res* **15**, 1007-1014 (2005).
156. Uhlen, M. et al. A human protein atlas for normal and cancer tissues based on antibody proteomics. *Mol Cell Proteomics* **4**, 1920-1932 (2005).
157. Nilsson, P. et al. Towards a human proteome atlas: high-throughput generation of mono-specific antibodies for tissue profiling. *Proteomics* **5**, 4327-4337 (2005).
158. Persson, A., Hober, S. & Uhlen, M. A human protein atlas based on antibody proteomics. *Curr Opin Mol Ther* **8**, 185-190 (2006).
159. Ekins, S. Systems-ADME/Tox: resources and network approaches. *J Pharmacol Toxicol Methods* **53**, 38-66 (2006).
160. Ekins, S., Stresser, D.M. & Williams, J.A. In vitro and pharmacophore insights into CYP3A enzymes. *Trends Pharmacol Sci* **24**, 161-166 (2003).
161. Kell, D.B. Metabolomics, modelling and machine learning in systems biology: towards an understanding of the languages of cells. The 2005 Theodor Bücher lecture. *FEBS J* **273**, 873-894 (2006).
162. Joyce, A.R. & Palsson, B.O. The model organism as a system: integrating 'omics' data sets. *Nat Rev Mol Cell Biol* **7**, 198-210 (2006).
163. Kaletta, T. & Hengartner, M.O. Finding function in novel targets: *C. elegans* as a model organism. *Nat Rev Drug Discov* **5**, 387-399 (2006).
164. Giaever, G. A chemical genomics approach to understanding drug action. *Trends Pharmacol Sci* **24**, 444-446 (2003).
165. Lum, P.Y. et al. Discovering modes of action for therapeutic compounds using a genome-wide screen of yeast heterozygotes. *Cell* **116**, 121-137 (2004).
166. Parsons, A.B. et al. Exploring the mode-of-action of bioactive compounds by chemical-genetic profiling in yeast. *Cell* **126**, 611-625 (2006).
167. Suter, B., Auerbach, D. & Stagljar, I. Yeast-based functional genomics and proteomics technologies: the first 15 years and beyond. *Biotechniques* **40**, 625-644 (2006).
168. Tochtrop, G.P. & King, R.W. Target identification strategies in chemical genetics. *Comb Chem High Throughput Screen* **7**, 677-688 (2004).
169. Zheng, X.S., Chan, T.F. & Zhou, H.H. Genetic and genomic approaches to identify and study the targets of bioactive small molecules. *Chem Biol* **11**, 609-618 (2004).
170. Ma, H. et al. The Edinburgh human metabolic network reconstruction and its functional analysis. *Mol Syst Biol* **3**, 135 (2007).
171. Hucka, M. et al. The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. *Bioinformatics* **19**, 524-531 (2003).
172. Kell, D.B. Systems biology, metabolic modelling and metabolomics in drug discovery and development. *Drug Disc Today* **11**, 1085-1092 (2006).
173. Kell, D.B. The virtual human: towards a global systems biology of multiscale, distributed biochemical network models. *IUBMB Life* **59**, 689-695 (2007).

174. Rubin, L.L. & Staddon, J.M. The cell biology of the blood-brain barrier. *Annu Rev Neurosci* **22**, 11-28 (1999).
175. Di, L., Kerns, E.H., Fan, K., McConnell, O.J. & Carter, G.T. High throughput artificial membrane permeability assay for blood-brain barrier. *Eur J Med Chem* **38**, 223-232 (2003).
176. Smith, Q.R. Transport of glutamate and other amino acids at the blood-brain barrier. *J Nutr* **130**, 1016S-1022S (2000).
177. Lee, G., Dallas, S., Hong, M. & Bendayan, R. Drug transporters in the central nervous system: brain barriers and brain parenchyma considerations. *Pharmacol Rev* **53**, 569-596 (2001).
178. Shimizu, K. et al. Carrier-mediated processes in blood--brain barrier penetration and neural uptake of paraquat. *Brain Res* **906**, 135-142 (2001).
179. McCormack, A.L. & Di Monte, D.A. Effects of L-dopa and other amino acids against paraquat-induced nigrostriatal degeneration. *J Neurochem* **85**, 82-86 (2003).
180. Pardridge, W.M. Blood-brain barrier genomics and the use of endogenous transporters to cause drug penetration into the brain. *Curr Opin Drug Discov Devel* **6**, 683-691 (2003).
181. Terasaki, T. et al. New approaches to in vitro models of blood-brain barrier drug transport. *Drug Discov Today* **8**, 944-954 (2003).
182. Lee, W. et al. Polymorphisms in human organic anion-transporting polypeptide 1A2 (OATP1A2): implications for altered drug disposition and central nervous system drug entry. *J Biol Chem* **280**, 9610-9617 (2005).
183. Allen, D.D. & Geldenhuys, W.J. Molecular modeling of blood-brain barrier nutrient transporters: In silico basis for evaluation of potential drug delivery to the central nervous system. *Life Sciences* **78**, 1029-1033 (2006).
184. Sun, H.Y., Dai, H.Q., Shaik, N. & Elmquist, W.F. Drug efflux transporters in the CNS. *Adv Drug Delivery Rev* **55**, 83-105 (2003).
185. Begley, D.J. ABC transporters and the blood-brain barrier. *Curr Pharm Design* **10**, 1295-1312 (2004).
186. Bachmeier, C.J., Trickler, W.J. & Miller, D.W. Comparison of drug efflux transport kinetics in various blood-brain barrier models. *Drug Metab Disposition* **34**, 998-1003 (2006).
187. Summerfield, S.G. et al. Improving the in vitro prediction of in vivo central nervous system penetration: integrating permeability, P-glycoprotein efflux, and free fractions in blood and brain. *J Pharmacol Exp Ther* **316**, 1282-1290 (2006).
188. Summerfield, S.G. & Jeffrey, P. In vitro prediction of brain penetration - a case for free thinking? *Expert Opin. Drug Discov.* **1**, 595-607 (2006).
189. Summerfield, S.G. et al. Central nervous system drug disposition: the relationship between in situ brain permeability and brain free fraction. *J Pharmacol Exp Ther* **322**, 205-213 (2007).
190. Pardridge, W.M. Blood-brain barrier delivery. *Drug Discov Today* **12**, 54-61 (2007).
191. Yanagida, O. et al. Human L-type amino acid transporter 1 (LAT1): characterization of function and expression in tumor cell lines. *Biochim Biophys Acta* **1514**, 291-302 (2001).
192. Stoll, J., Wadhvani, K.C. & Smith, Q.R. Identification of the cationic amino acid transporter (System y⁺) of the rat blood-brain barrier. *J Neurochem* **60**, 1956-1959 (1993).
193. Wu, X. et al. Identity of the organic cation transporter OCT3 as the extraneuronal monoamine transporter (uptake₂) and evidence for the expression of the transporter in the brain. *J Biol Chem* **273**, 32776-32786 (1998).
194. Gao, B. et al. Organic anion-transporting polypeptides mediate transport of opioid peptides across blood-brain barrier. *J Pharmacol Exp Ther* **294**, 73-79 (2000).
195. Yamashita, T. et al. Cloning and functional expression of a brain peptide/histidine transporter. *J Biol Chem* **272**, 10205-10211 (1997).
196. Polli, J.W. et al. Role of P-glycoprotein on the CNS disposition of amprenavir (141W94), an HIV protease inhibitor. *Pharm Res* **16**, 1206-1212 (1999).

197. Cutler, L., Howes, C., Deeks, N.J., Buck, T.L. & Jeffrey, P. Development of a P-glycoprotein knockout model in rodents to define species differences in its functional effect at the blood-brain barrier. *J Pharm Sci* **95**, 1944-1953 (2006).
198. Okazaki, N. & Ananiadou, S. Building an abbreviation dictionary using a term recognition approach. *Bioinformatics*, in press (2006).
199. Yan, Q. & Sadée, W. Human membrane transporter database: a Web-accessible relational database for drug transport studies and pharmacogenomics. *AAPS PharmSci* **2**, E20 (2000).
200. Wain, H.M., Lush, M.J., Ducluzeau, F., Khodiyar, V.K. & Povey, S. Genew: the Human Gene Nomenclature Database, 2004 updates. *Nucleic Acids Res* **32**, D255-257 (2004).
201. Saier, M.H., Jr., Tran, C.V. & Barabote, R.D. TCDB: the Transporter Classification Database for membrane transport protein analyses and information. *Nucleic Acids Res* **34**, D181-186 (2006).
202. Van Belle, D. & André, B. A genomic view of yeast membrane transporters. *Curr Opin Cell Biol* **13**, 389-398 (2001).
203. Ozawa, N. et al. Transporter database, TP-Search: a web-accessible comprehensive database for research in pharmacokinetics of drugs. *Pharm Res* **21**, 2133-2134 (2004).
204. Ren, Q., Kang, K.H. & Paulsen, I.T. TransportDB: a relational database of cellular membrane transport systems. *Nucleic Acids Res* **32**, D284-288 (2004).
205. Mitsuoka, K., Kato, Y., Kubo, Y. & Tsuji, A. Functional expression of stereoselective metabolism of cephalexin by exogenous transfection of oligopeptide transporter PEPT1. *Drug Metab Dispos* **35**, 356-362 (2007).
206. Sala-Rabanal, M., Loo, D.D., Hirayama, B.A., Turk, E. & Wright, E.M. Molecular interactions between dipeptides, drugs and the human intestinal H⁺-oligopeptide cotransporter hPEPT1. *J Physiol* **574**, 149-166 (2006).
207. Saito, H., Terada, T., Okuda, M., Sasaki, S. & Inui, K. Molecular cloning and tissue distribution of rat peptide transporter PEPT2. *Biochim Biophys Acta* **1280**, 173-177 (1996).
208. Li, M. et al. Interactions of amoxicillin and cefaclor with human renal organic anion and peptide transporters. *Drug Metab Dispos* **34**, 547-555 (2006).
209. Wenzel, U. et al. Transport characteristics of differently charged cephalosporin antibiotics in oocytes expressing the cloned intestinal peptide transporter PepT1 and in human intestinal Caco-2 cells. *J Pharmacol Exp Ther* **277**, 831-839 (1996).
210. Menon, R.M. & Barr, W.H. Transporters involved in apical and basolateral uptake of ceftibuten into Caco-2 cells. *Biopharm Drug Dispos* **23**, 317-326 (2002).
211. Tsuda, M. et al. Transport characteristics of a novel peptide transporter 1 substrate, antihypertensive drug midodrine, and its amino acid derivatives. *J Pharmacol Exp Ther* **318**, 455-460 (2006).
212. Balimane, P.V. et al. Direct evidence for peptide transporter (PepT1)-mediated uptake of a nonpeptide prodrug, valacyclovir. *Biochem Biophys Res Commun* **250**, 246-251 (1998).
213. Sugawara, M. et al. Transport of valganciclovir, a ganciclovir prodrug, via peptide transporters PEPT1 and PEPT2. *J Pharm Sci* **89**, 781-789 (2000).
214. Ocheltree, S.M. et al. Mechanisms of cefadroxil uptake in the choroid plexus: studies in wild-type and PEPT2 knockout mice. *J Pharmacol Exp Ther* **308**, 462-467 (2004).
215. van Montfoort, J.E. et al. Comparison of "type I" and "type II" organic cation transport by organic cation transporters and organic anion-transporting polypeptides. *J Pharmacol Exp Ther* **298**, 110-115 (2001).
216. Takeda, M. et al. Human organic anion transporters and human organic cation transporters mediate renal antiviral transport. *J Pharmacol Exp Ther* **300**, 918-924 (2002).
217. Wang, D.S. et al. Involvement of organic cation transporter 1 in hepatic and intestinal distribution of metformin. *J Pharmacol Exp Ther* **302**, 510-515 (2002).

218. Busch, A.E. et al. Human neurons express the polyspecific cation transporter hOCT2, which translocates monoamine neurotransmitters, amantadine, and memantine. *Mol Pharmacol* **54**, 342-352 (1998).
219. Kimura, N. et al. Metformin is a superior substrate for renal organic cation transporter OCT2 rather than hepatic OCT1. *Drug Metab Pharmacokinet* **20**, 379-386 (2005).
220. Dudley, A.J., Bleasby, K. & Brown, C.D. The organic cation transporter OCT2 mediates the uptake of beta-adrenoceptor antagonists across the apical membrane of renal LLC-PK(1) cell monolayers. *Br J Pharmacol* **131**, 71-79 (2000).
221. Grundemann, D., Liebich, G., Kiefer, N., Koster, S. & Schomig, E. Selective substrates for non-neuronal monoamine transporters. *Mol Pharmacol* **56**, 1-10 (1999).
222. Urakami, Y., Akazawa, M., Saito, H., Okuda, M. & Inui, K. cDNA cloning, functional characterization, and tissue distribution of an alternatively spliced variant of organic cation transporter hOCT2 predominantly expressed in the human kidney. *J Am Soc Nephrol* **13**, 1703-1710 (2002).
223. Gorboulev, V. et al. Cloning and characterization of two human polyspecific organic cation transporters. *DNA Cell Biol* **16**, 871-881 (1997).
224. Grundemann, D., Schechinger, B., Rappold, G.A. & Schomig, E. Molecular identification of the corticosterone-sensitive extraneuronal catecholamine transporter. *Nat Neurosci* **1**, 349-351 (1998).
225. Yabuuchi, H. et al. Novel membrane transporter OCTN1 mediates multispecific, bidirectional, and pH-dependent transport of organic cations. *J Pharmacol Exp Ther* **289**, 768-773 (1999).
226. Ohashi, R. et al. Na⁺-dependent carnitine transport by organic cation transporter (OCTN2): its pharmacological and toxicological relevance. *J Pharmacol Exp Ther* **291**, 778-784 (1999).
227. Ganapathy, M.E. et al. beta-lactam antibiotics as substrates for OCTN2, an organic cation/carnitine transporter. *J Biol Chem* **275**, 1699-1707 (2000).
228. Cihlar, T. & Ho, E.S. Fluorescence-based assay for the interaction of small molecules with the human renal organic anion transporter 1. *Anal Biochem* **283**, 49-55 (2000).
229. Wada, S. et al. Rat multispecific organic anion transporter 1 (rOAT1) transports zidovudine, acyclovir, and other antiviral nucleoside analogs. *J Pharmacol Exp Ther* **294**, 844-849 (2000).
230. Sekine, T., Watanabe, N., Hosoyamada, M., Kanai, Y. & Endou, H. Expression cloning and characterization of a novel multispecific organic anion transporter. *J Biol Chem* **272**, 18526-18529 (1997).
231. Mulato, A.S., Ho, E.S. & Cihlar, T. Nonsteroidal anti-inflammatory drugs efficiently reduce the transport and cytotoxicity of adefovir mediated by the human renal organic anion transporter 1. *J Pharmacol Exp Ther* **295**, 10-15 (2000).
232. Burckhardt, B.C. et al. Transport of cimetidine by flounder and human renal organic anion transporter 1. *Am J Physiol Renal Physiol* **284**, F503-509 (2003).
233. Babu, E. et al. Human organic anion transporters mediate the transport of tetracycline. *Jpn J Pharmacol* **88**, 69-76 (2002).
234. Khamdang, S. et al. Interaction of human and rat organic anion transporter 2 with various cephalosporin antibiotics. *Eur J Pharmacol* **465**, 1-7 (2003).
235. Sekine, T. et al. Identification of multispecific organic anion transporter 2 expressed predominantly in the liver. *FEBS Lett* **429**, 179-182 (1998).
236. Sun, W., Wu, R.R., van Poelje, P.D. & Erion, M.D. Isolation of a family of organic anion transporters from human liver and kidney. *Biochem Biophys Res Commun* **283**, 417-422 (2001).
237. Kobayashi, Y. et al. Possible involvement of organic anion transporter 2 on the interaction of theophylline with erythromycin in the human liver. *Drug Metab Dispos* **33**, 619-622 (2005).
238. Cha, S.H. et al. Identification and characterization of human organic anion transporter 3 expressing predominantly in the kidney. *Mol Pharmacol* **59**, 1277-1286 (2001).

239. Cvetkovic, M., Leake, B., Fromm, M.F., Wilkinson, G.R. & Kim, R.B. OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. *Drug Metab Dispos* **27**, 866-871 (1999).
240. van Montfoort, J.E. et al. Polyspecific organic anion transporting polypeptides mediate hepatic uptake of amphipathic type II organic cations. *J Pharmacol Exp Ther* **291**, 147-152 (1999).
241. Pang, K.S., Wang, P.J., Chung, A.Y. & Wolkoff, A.W. The modified dipeptide, enalapril, an angiotensin-converting enzyme inhibitor, is transported by the rat liver organic anion transport protein. *Hepatology* **28**, 1341-1346 (1998).
242. Ishizuka, H. et al. Transport of temocaprilat into rat hepatocytes: role of organic anion transporting polypeptide. *J Pharmacol Exp Ther* **287**, 37-42 (1998).
243. Tamai, I. et al. Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family. *Biochem Biophys Res Commun* **273**, 251-260 (2000).
244. Vavricka, S.R., Van Montfoort, J., Ha, H.R., Meier, P.J. & Fattinger, K. Interactions of rifamycin SV and rifampicin with organic anion uptake systems of human liver. *Hepatology* **36**, 164-172 (2002).
245. Sandhu, P. et al. Hepatic uptake of the novel antifungal agent caspofungin. *Drug Metab Dispos* **33**, 676-682 (2005).
246. Abe, T. et al. LST-2, a human liver-specific organic anion transporter, determines methotrexate sensitivity in gastrointestinal cancers. *Gastroenterology* **120**, 1689-1699 (2001).
247. Kopplow, K., Letschert, K., Konig, J., Walter, B. & Keppler, D. Human hepatobiliary transport of organic anions analyzed by quadruple-transfected cells. *Mol Pharmacol* **68**, 1031-1038 (2005).
248. Kullak-Ublick, G.A. et al. Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology* **120**, 525-533 (2001).
249. Shimizu, M. et al. Contribution of OATP (organic anion-transporting polypeptide) family transporters to the hepatic uptake of fexofenadine in humans. *Drug Metab Dispos* **33**, 1477-1481 (2005).
250. Satoh, H. et al. Citrus juices inhibit the function of human organic anion-transporting polypeptide OATP-B. *Drug Metab Dispos* **33**, 518-523 (2005).
251. Mikkaichi, T. et al. Isolation and characterization of a digoxin transporter and its rat homologue expressed in the kidney. *Proc Natl Acad Sci U S A* **101**, 3569-3574 (2004).
252. Sai, Y. & Tsuji, A. Transporter-mediated drug delivery: recent progress and experimental approaches. *Drug Discov Today* **9**, 712-720 (2004).