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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 in [10-13]) 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 activity [16] or in effecting aggregation [17].

Although they have been shown to cross BLMs, the very small neutral molecules urea [18] and glycerol [19], 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 yeasts [20], 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 carriers [23], 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 assay [25] 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 of [25]. 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 of [32]). Figs 2 and 3 also shows some data replotted from Table 1 of a recent comparison [33] 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 phthalates [34]. Many factors including solubility, formulation, pH and intestinal enzymology can affect drug uptake [35]. The 'rule of 5' favours intermediate values of CLogP [36], 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 uptake [37], 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 defects [43] (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 evidence[46], and the correlation between specific receptor binding[48] and potency in specific mutant mice [49], 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 receptors [52]. 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 activities [55] – is well established [56]. 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 network [59]) 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 function[60], with mitochondrial carrier proteins prominent among them [61, 62] (many with still-unknown substrates [63]). 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 in2004[57]. 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: Na^+/Ca^{2+} (SLC8) and Na^+/H^+ (SLC9), transition metal ions (SLC11), Na^+/K^+ and Cl^- (SLC12), Na^+ and inorganic phosphate (SLC20), Na^+-Ca^{2+}/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 performed [67], 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 PEPT1 [66]. 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 inhibitors [66].

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 also [68] 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, bencylpenicillin, 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 regions [64]. 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 antibacterials[71]), and it is certainly known in some cases that they are the substrates of active transporters in the producing organisms [72]. Given that bioactive microbial products are necessarily secreted, evolution must have produced carriers capable of binding the relevant chemical structures [73]. The fact that these bioactive ‘secondary’ metabolites are often active on other cells of the producer organism [74] 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 proteins [79-91], many of which naturally transport bile acids. For instance [79], 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 transporters [93]. OATP2 transports pravastatin, lovastatin, simvastatin, and atorvastatin [84]. They can also be transported for instance by the bile acid transporter SLC10A1 [91] 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 Mitchell[97], 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 neutral [98]. 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 cation [101]) (and see also [102, 103]). Other experiments by these authors showed that dibenzyldimethylammonium 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 thallous ion transport [104]) 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 stoicheiometric concentration of binding sites. Thus, the fact that some drugs can concentrate in specific tissues [105-112] 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 interactions [113]. 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 efflux [114] 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 toxicity[115], and that there are other problems, such as drug-drug interactions [116-119], both in general [116, 118] and in specific tissues such as the liver [117] and the kidney [120], 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 interactions [121].

(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 cells [122-130]. The case of peptide transporters is particularly clear [67, 129, 131-133] and hitchhiking on peptide transporters can demonstrably improve the activity of certain antibacterials [134]. Coupling of drugs such as chlorambucil [135], cis-platin [136] and acyclovir [137] to bile acid derivatives or of carindacillin to monocarboxylates [138] can also be highly effective. Such couplings often lower the lipohilicity 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-likeness[141-143] (and even CNS-likeness[144]) 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 attrition [145]. 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. Thus [146], 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 dopamine [147] and serotonin [148].

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 proteomic [156-158] (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 knowledge [159]. 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 P450 [160]. 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 agenda [161]. 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 xenobiotics [164-167], 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 SBML [171] 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 biology [161]. 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/).

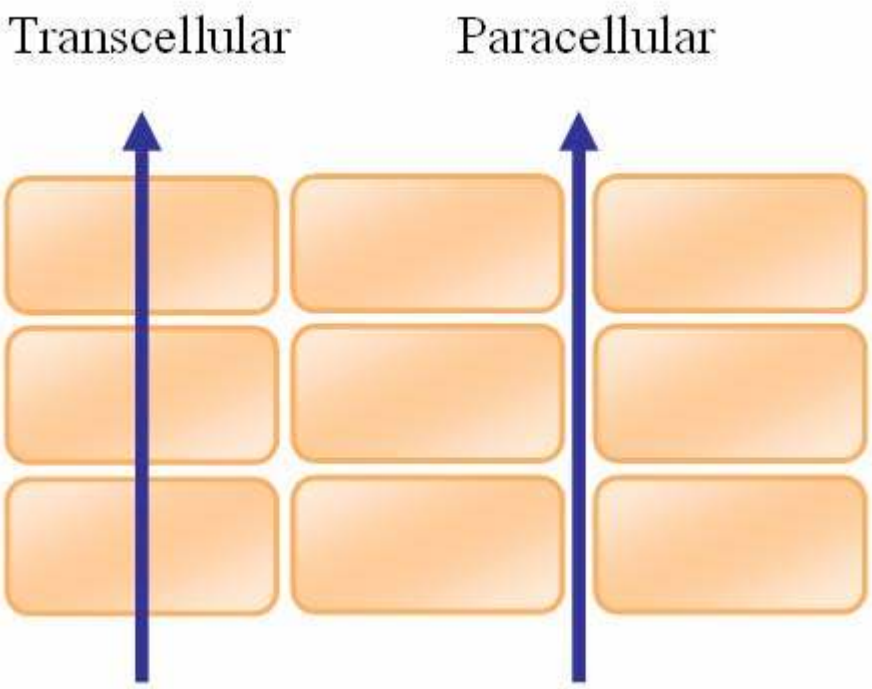
Box 1. Implications of a more prominent role for carrier-mediated drug uptake

- 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 carries 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-dimenional '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 inetrest. 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 CNS[184-186] such that both influx and efflux activities as well as binding need to be understood if selective blood brain barrier penetration is to be achieved [187-189]. 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 melphalan [93]**.** High expression of hLAT1 mRNA is detected in brain tissue by Northern blot analysis [191], while system y+ transports cationic amino acids. Its CAT1 RNA is enriched 38-fold in rat cerebral microvessels and choroid plexus compared with whole brain [192]. Certain organic cation transporters, such as rOCT3 [193], 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 BBB [194]. 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 uptake [195]. Lee [177] 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 amprenavir [196] and SB-487946 [197] 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.scmbb.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]

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**	**Transport**	**Substrates**
Amino acid/Polyamine/ Organocation (APC) Superfamily	Amino Acid/Auxin Permease (AAAP) *SLC36*	2.A.18	10-11 TM α-helices	Symport / Antiport	Amino acids, auxin (indole-3-acetic acid)
	Amino acid/Polyamine/Organocation (APC) *SLC7*	2.A.3	14 TM α-helices	Symport / Antiport	Amino acids, choline, polyamines
Anion Transporter (AT) Superfamily	Bile Acid:Na+ Symporter (BASS) *SLC10*	2.A.28	7-10 TM spanners	Symport	Bile acids and other organic acids
Major Facilitator Superfamily (MFS)	Major Facilitator (MF) *SLC2, 16-18, 22, 33, 37, 43*	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) *SLC15*	2.A.17	12 TM α-helices	Symport	Peptides, histidine, antiobiotics
	Organo Anion Transporter (OAT). *SLCO/21*	2.A.60	12 TM α-helices	Uniport/ Antiport	Broad range: Organic anions, organic cations (bromosulfophthalein, 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 *SLC5*	13-15 TM α-helices	Symport	Sugars, amino acids, organocations, nucleosides, inositols, vitamins, urea, anions
Neurotransmitter:Sodium Symporter (NSS)		2.A.22 *SLC6*	12 TM α-helices	Symport	Neurotransmitters, amino acids
Dicarboxylate/Amino Acid:Cation (Na+ or H+) Symporter (DAACS)		2.A.23 *SLC1*	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

Mitochondrial Carrier (MC). *See SLC25*	2.A.29 *SLC25*	6 TM α-helices	Antiport	Citrate, malate, PEP, lysine, arginine, aspartate, glutamate, others
Nucleobase:Cation Symporter-2 (NCS2)	2.A.40 *SLC23*	12 TM α-helices	Symport	Nucleobases, ascorbate
Concentrative Nucleoside Transporter (CNT)	2.A.41 *SLC28*	10-14 TM α-helices	Symport	Nucleosides
Reduced Folate Carrier (RFC)	2.A.48 *SLC19*	12 TM α-helices	Symport / Antiport	Folate, reduced folate and derivatives, methotrexate, thiamine
Equilibrative Nucleoside Transporter (ENT)	2.A.57 *SLC29*	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.

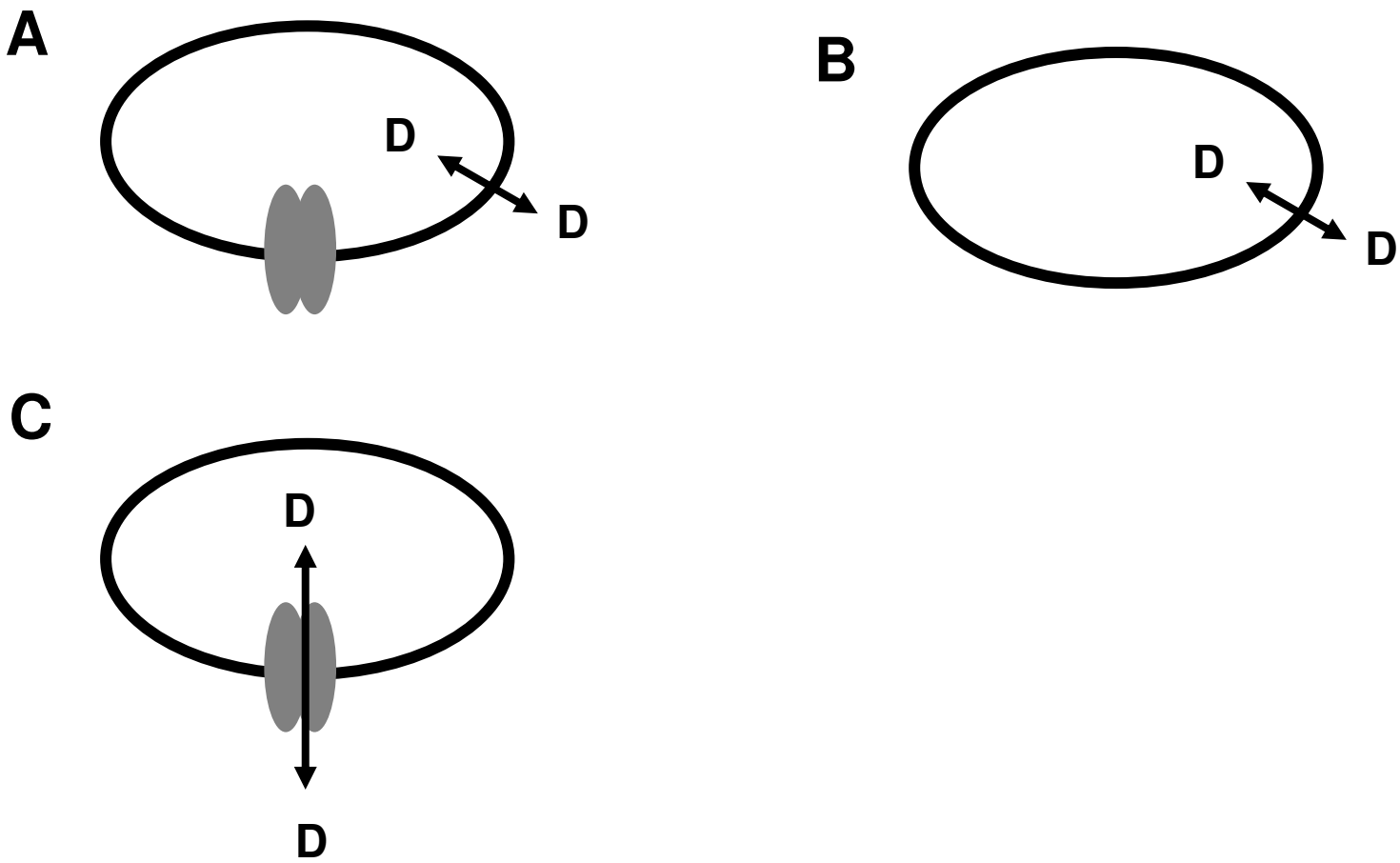
Substrate	*SLC15A1*	*SLC15A2*	*SLC22A1*	*SLC22A2*	*SLC22A3*	*SLC22A4*	*SLC22A5*	*SLC22A6*	*SLC22A7*	*SLC22A8*	*SLC22A11*	*SLCO1A2*	*SLCO1B1*	*SLCO1B3*	*SLCO1C1*	*SLCO2A1*	*SLCO2B1*	*SLCO3A1*	*SLCO4A1*	*SLCO4C1*
acyclovir			●					●												
amoxicillin	●	●																		
atorvastatin													●				●			
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)

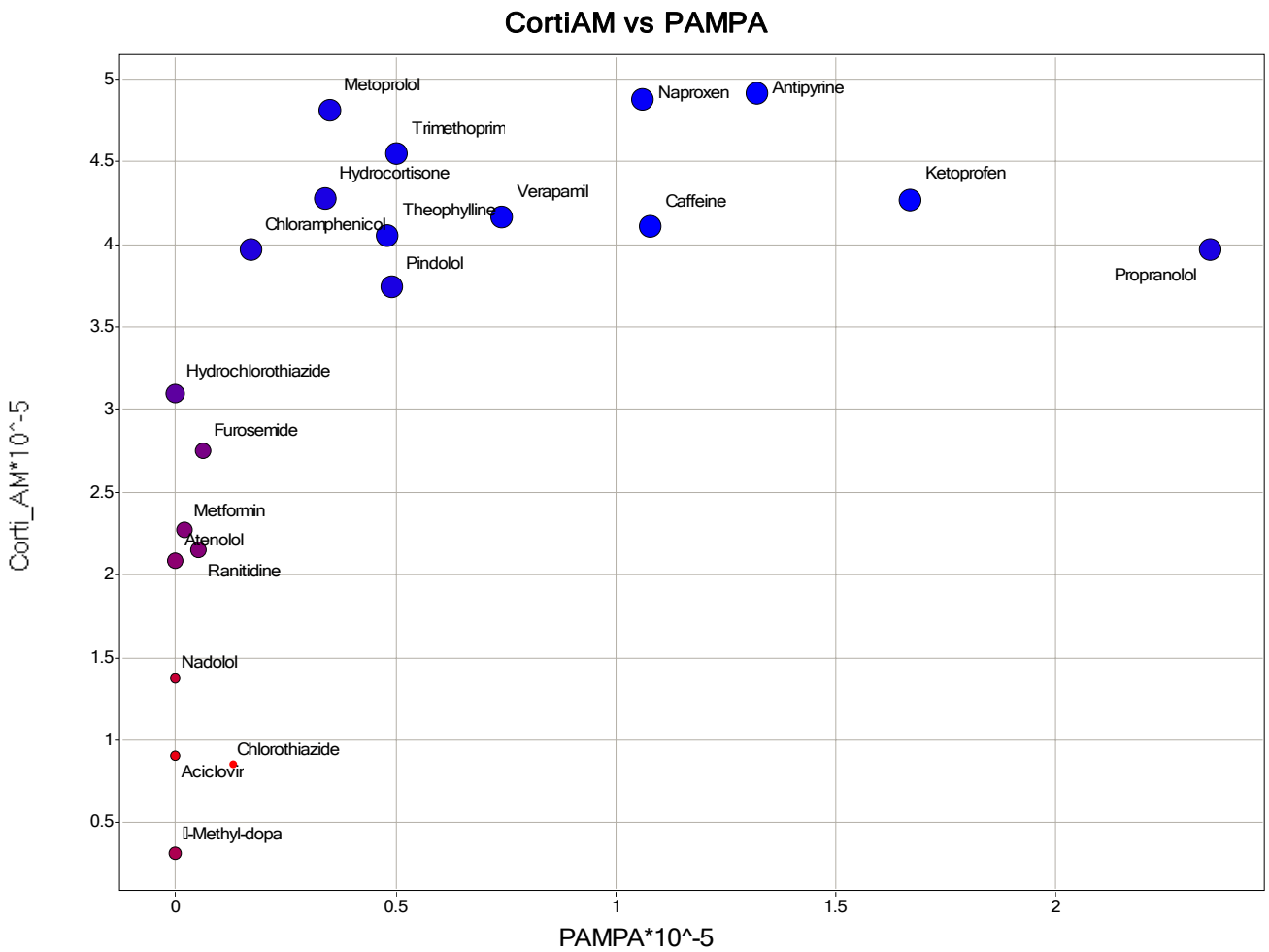
SLC Family	HUGO Symbol + Synonyms	Description	Substrate	Refs	Code
15 [66]	*SLC15A1; PEPT1*	Oligopeptide transporter	cefalexin	[205]	DATC
			bestatin	[206]	DATC
				[207]	DATC
			amoxicillin	[206]	DATC
				[208]	DADC
			ampicillin	[206]	DATC
			cefaclor	[208]	DADC
			cefadroxil	[209]	DATC
				[206]	DATC
			cefixime	[209]	DATC
			ceftibuten	[210]	DCA
			temocapril	[116]	DATC
			temocaprilate	[116]	DATC
			enalapril	[116]	DATC
			midodrine	[211]	DATC
			valacyclovir	[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]	TOATC
			cyanine863	[223]	TOATC
			quinine	[223]	TOATC
	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

			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
			theophyline	[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]	TOATC
			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
			capsofungin	[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

	OATP1B3; OATP8		rifampicin	[244]	DATC
			fexofenadine	[249]	DATC
			fluvastatin	[247]	DATC
			pitavastatin	[92]	DATC
			rosuvastatin	[91]	DATC
	SLCO2B1; OATP2B1; OATP-B	Organic anion transporter	pravastatin	[83]	DATC
			glibenclamide	[250]	DATC
			atorvastatin	[87]	DATC
			benzylpenicillin	[243]	DATC
			fluvastatin	[247]	DATC
			rosuvastatin	[91]	DATC
	SLCO4C1; OATP4C1	Organic anion transporter	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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PAMPA*10^-5
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Antipyrine
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Verapamil
Caffeine
Theophylline
Chloramphenicol
Pindolol
Propranolol
Hydrochlorothiazide
Furosemide
Metformin
Atenolol
Ranitidine
Nadolol
Chlorothiazide
Aciclovir
-Methyl-dopa
5
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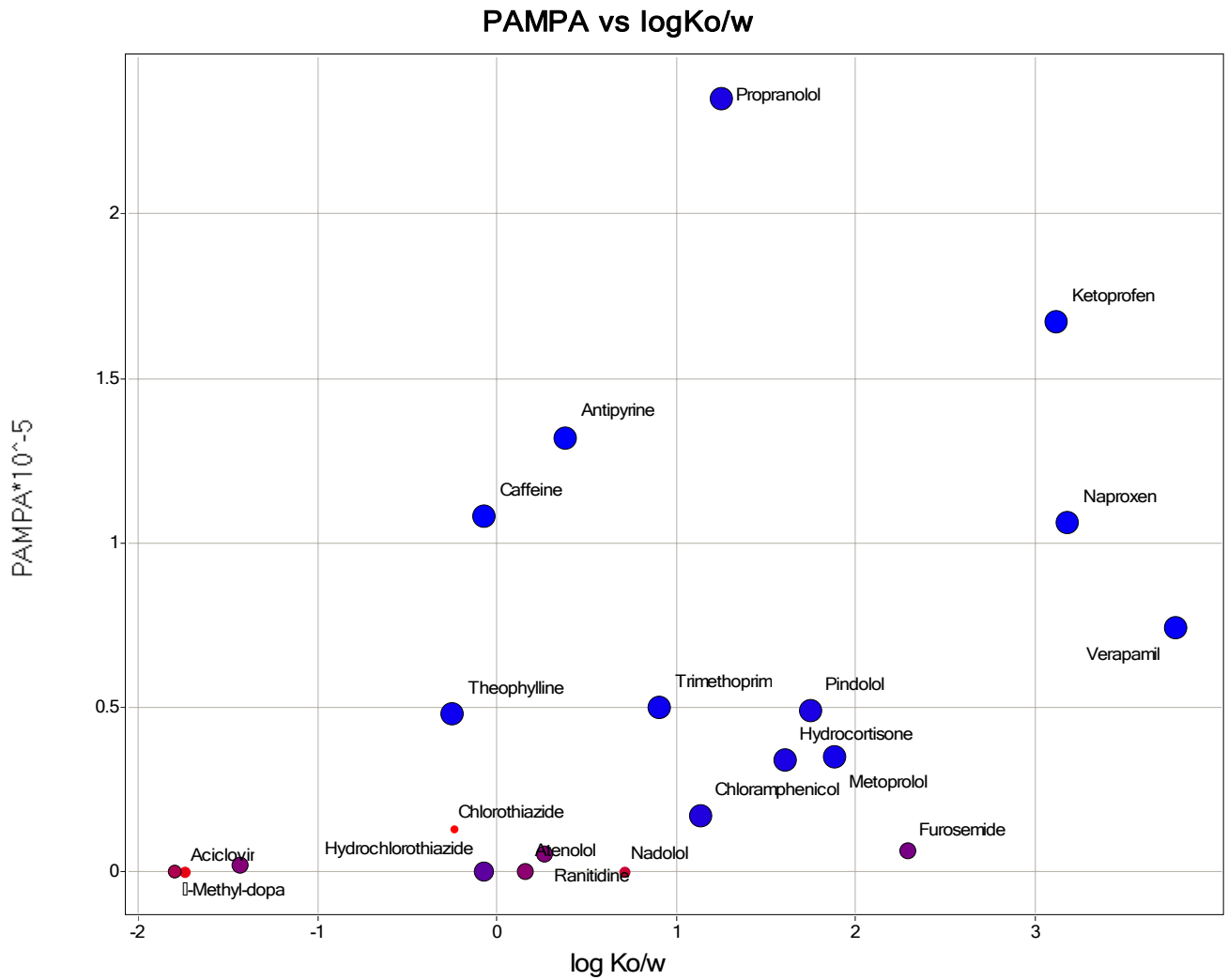
2

Fig 2A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log Ko/w
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Furosemide
Hydrochlorothiazide
Atenolol
Nadolol
Aciclovir
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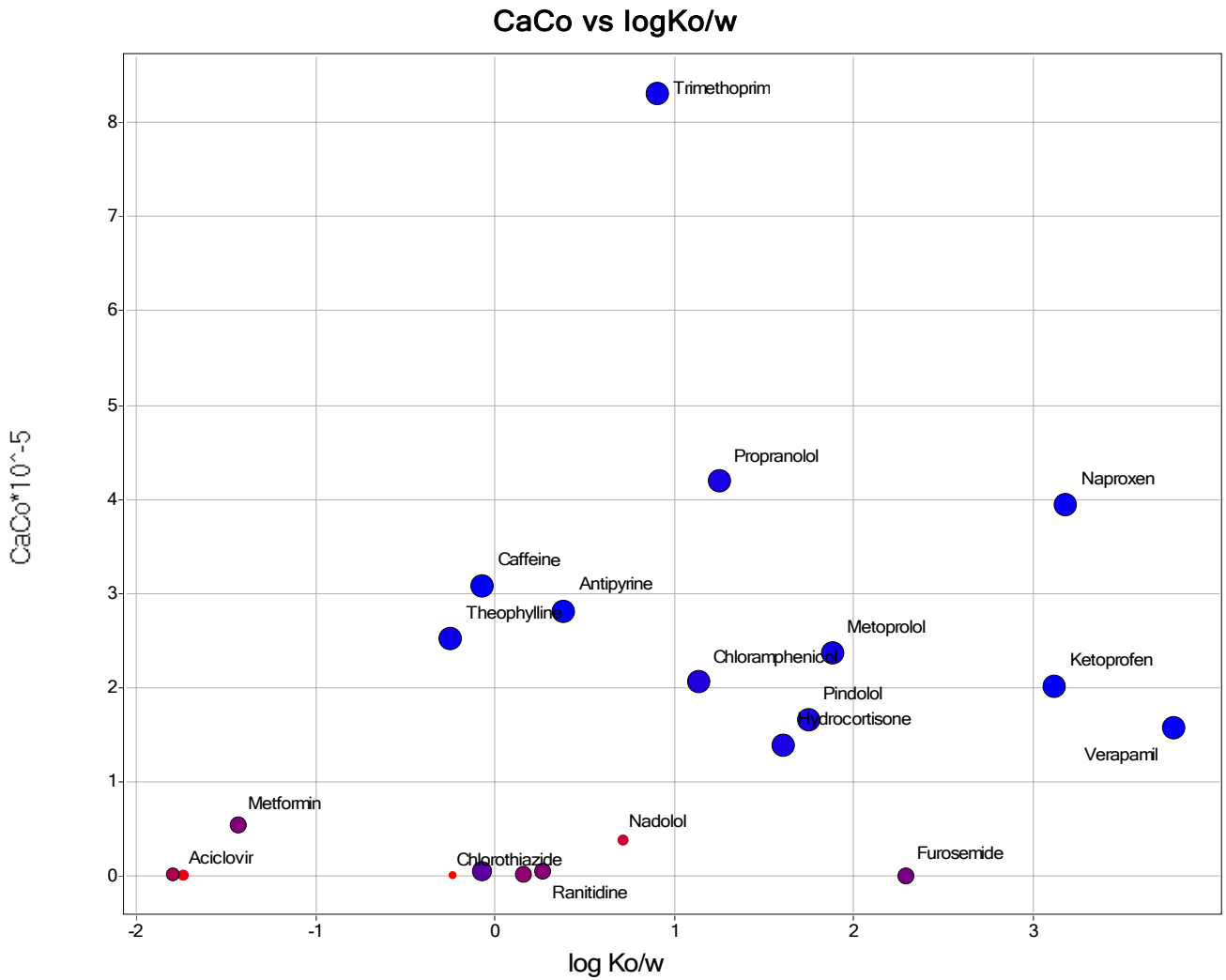
2
3

Fig 2B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Ketoprofen
Pindolol
Hydrocortisone
Verapamil
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Aciclovir
Chlorothiazide
Furosemide
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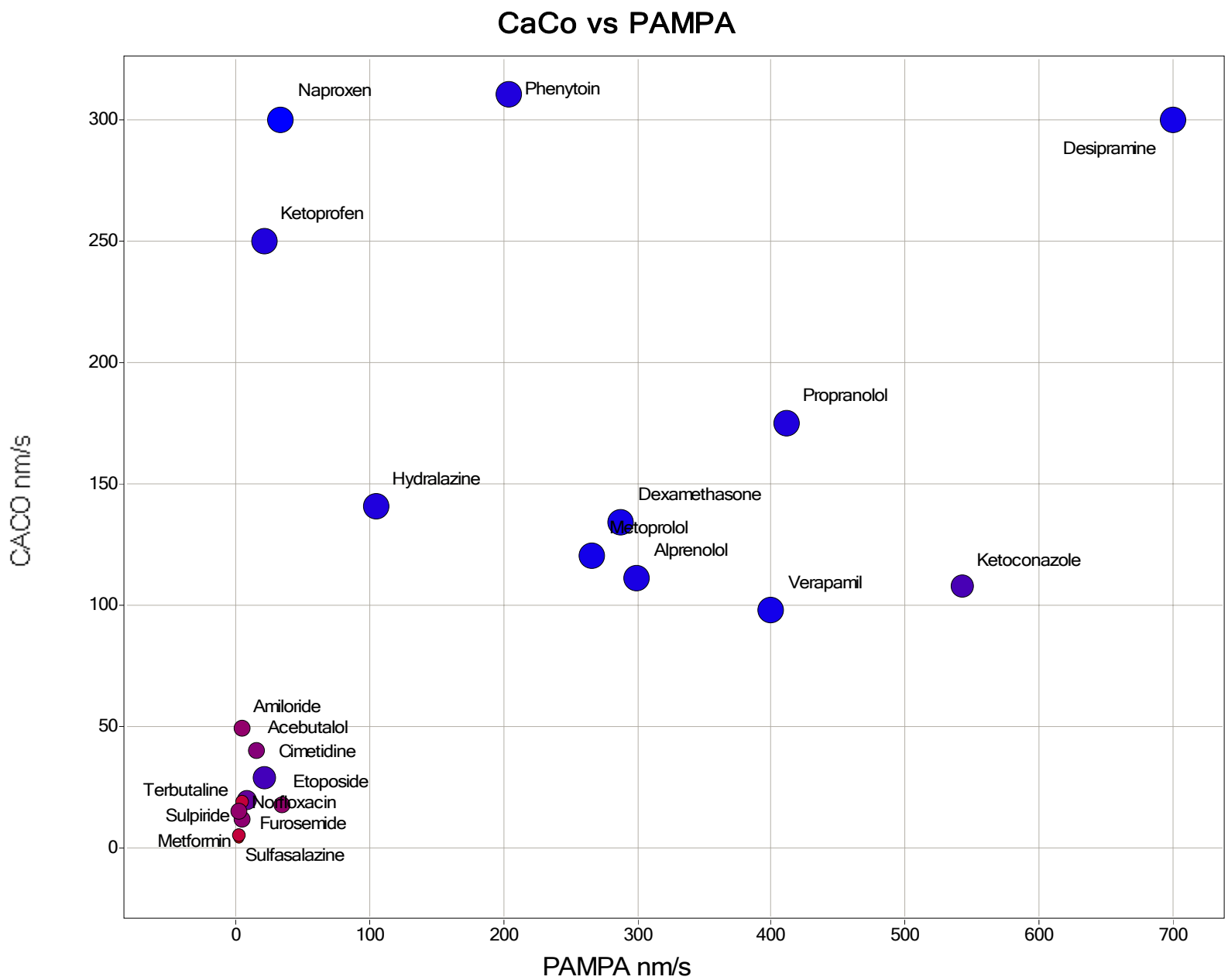
Fig 2C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Alprenolol
Ketoconazole
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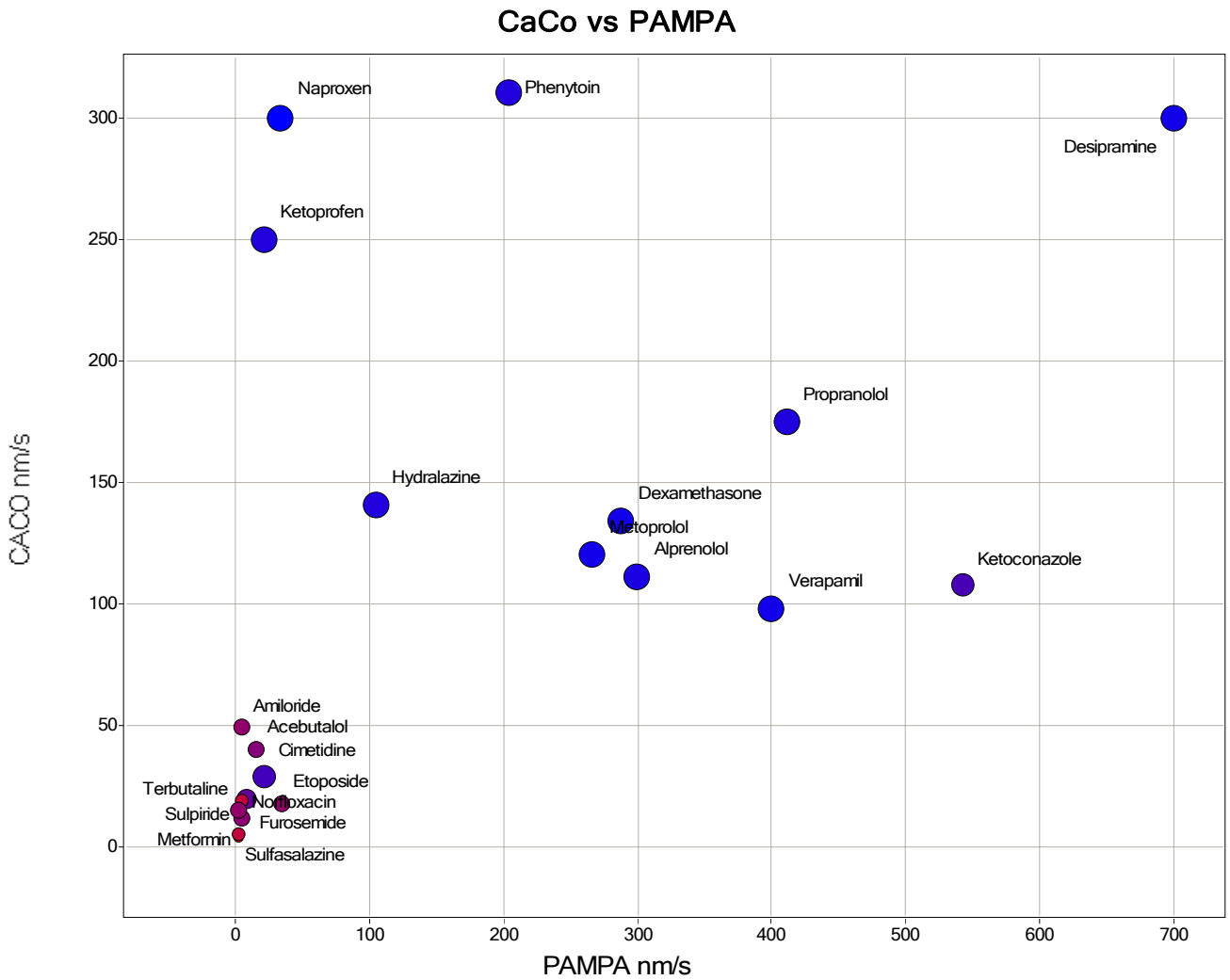
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Propranolol
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Dexamethasone
Metoprolol
Alprenolol
Ketoconazole
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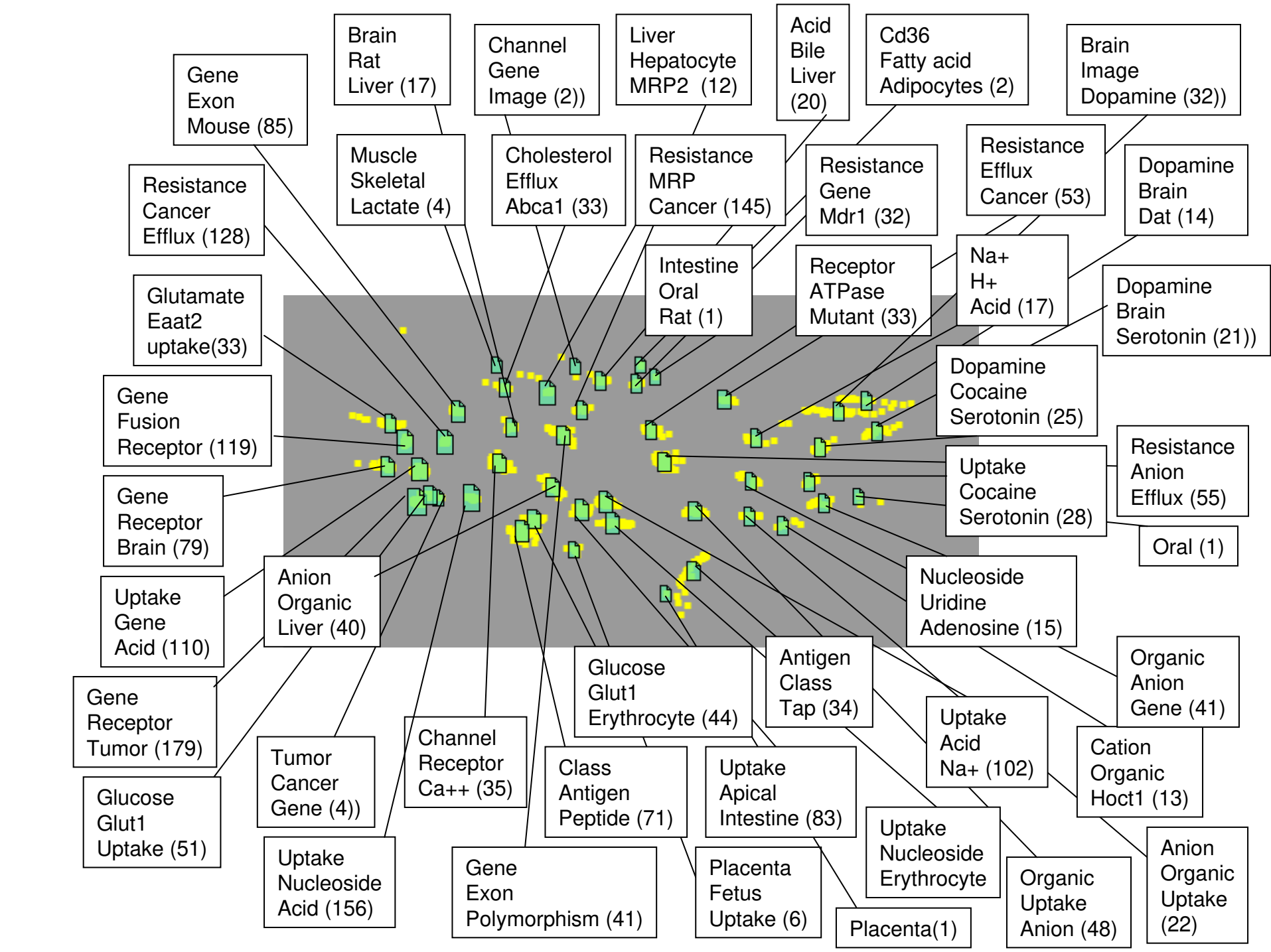
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Acid Bile Liver (20)
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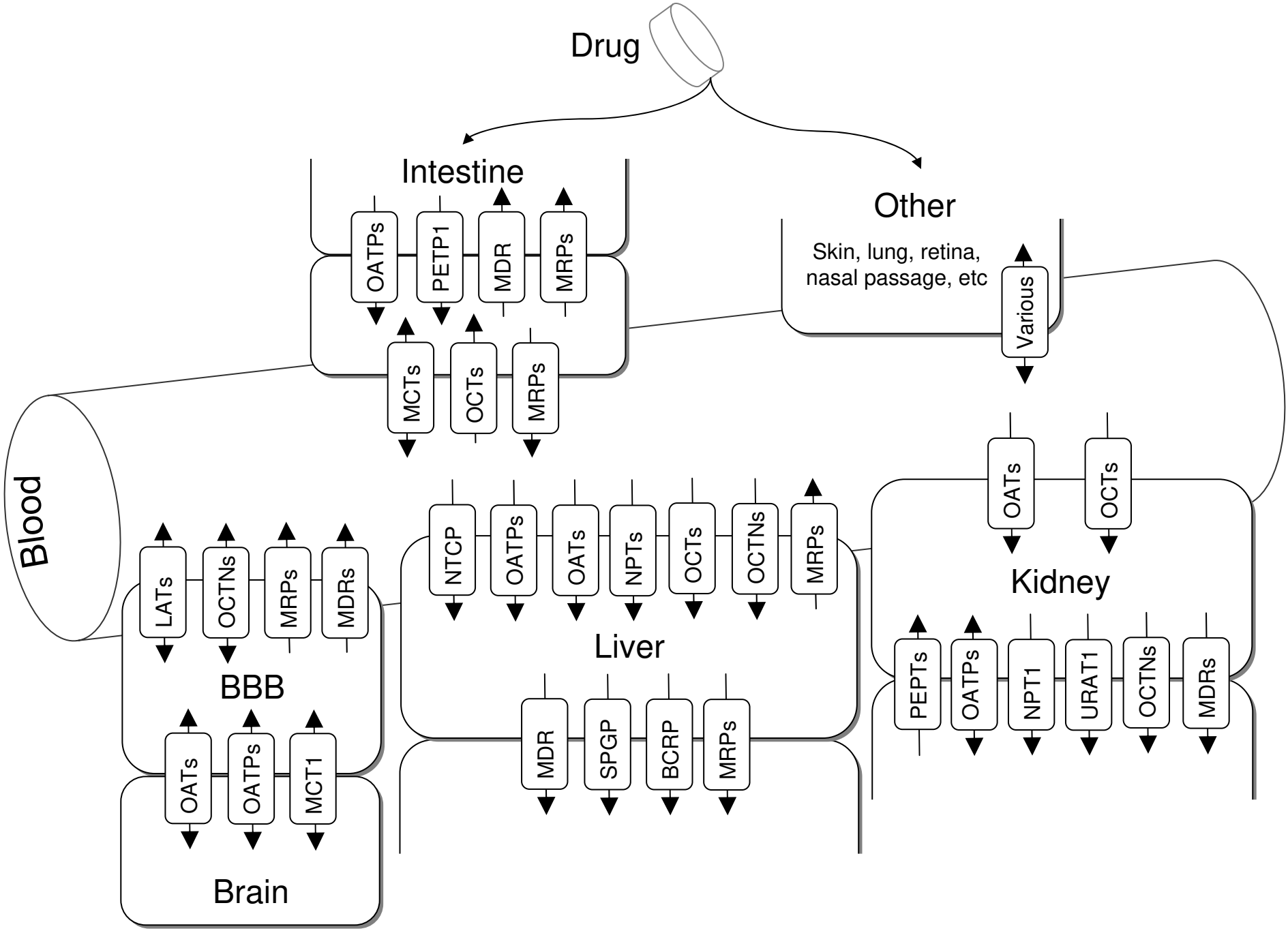
Brain Image Dopamine (32))
Gene Exon Mouse (85)
Muscle Skeletal Lactate (4)
Cholesterol Efflux Abca1 (33)
Resistance MRP Cancer (145)
Resistance Gene Mdr1 (32)
Resistance Efflux Cancer (53)
Dopamine Brain Dat (14)
Resistance Cancer Efflux (128)
Intestine Oral Rat (1)
Receptor ATPase Mutant (33)
Na+ H+ Acid (17)
Dopamine Brain Serotonin (21))
Glutamate Eaat2 uptake(33)
Dopamine Cocaine Serotonin (25)
Gene Fusion Receptor (119)
Uptake Cocaine Serotonin (28)
Resistance Anion Efflux (55)
Gene Receptor Brain (79)
Oral (1)
Anion Organic Liver (40)
Nucleoside Uridine Adenosine (15)
Uptake Gene Acid (110)
Organic Anion Gene (41)
Antigen Class Tap (34)
Glucose Glut1 Erythrocyte (44)
Gene Receptor Tumor (179)
Uptake Acid Na+ (102)
Cation Organic Hoct1 (13)
Channel Receptor Ca++ (35)
Tumor Cancer Gene (4))
Class Antigen Peptide (71)
Uptake Apical Intestine (83)
Glucose Glut1 Uptake (51)
Uptake Nucleoside Erythrocyte
Anion Organic Uptake (22)
Uptake Nucleoside Acid (156)
Gene Exon Polymorphism (41)
Placenta Fetus Uptake (6)
Organic Uptake Anion (48)
Placenta(1)


Drug
Intestine
OATPs
PETP1
MDR
MRPs
MCTs
OCTs
MRPs
Other
Skin, lung, retina,
nasal passage, etc
Various
Blood
LATs
OCTNs
MRPs
MDRs
BBB
OATs
OATPs
MCT1
Brain
NTCP
OATPs
OATs
NPTs
OCTs
OCTNs
MRPs
Liver
MDR
SPGP
BCRP
MRPs
OATs
OCTs
Kidney
PEPTs
OATPs
NPT1
URAT1
OCTNs
MDRs

A

Smooth muscle [SLC1A4]

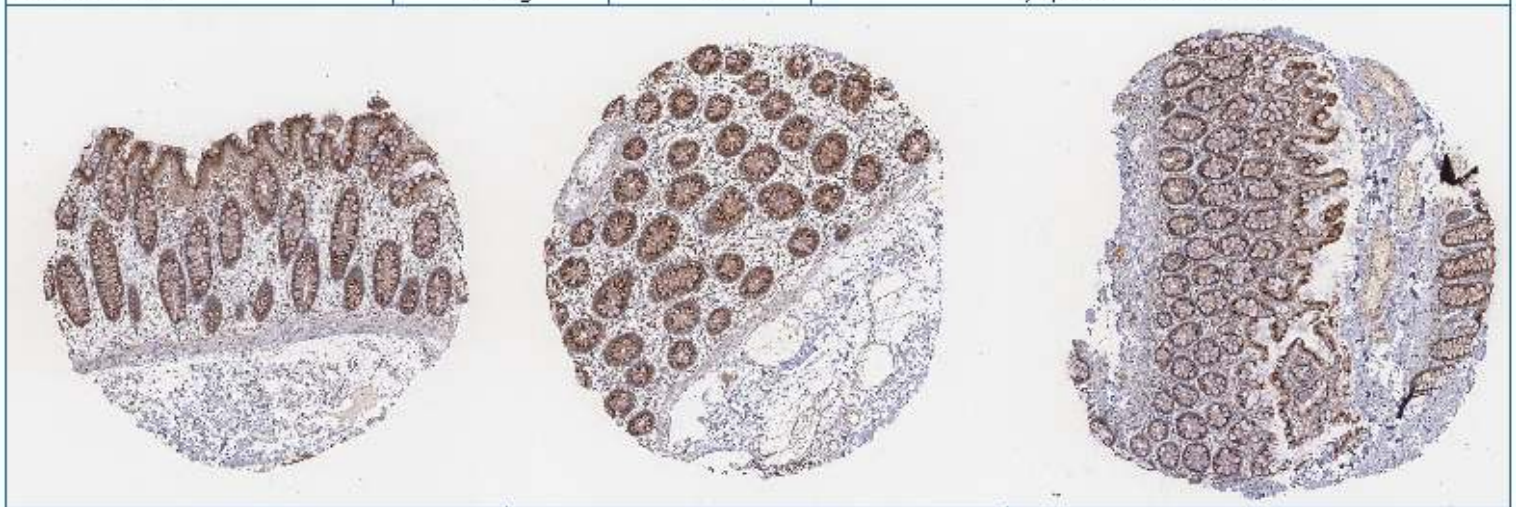
Cell Type	Intensity	Quantity	Localization
Smooth muscle cells	weak	75%-25%	cytoplasmic and/or membranous

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

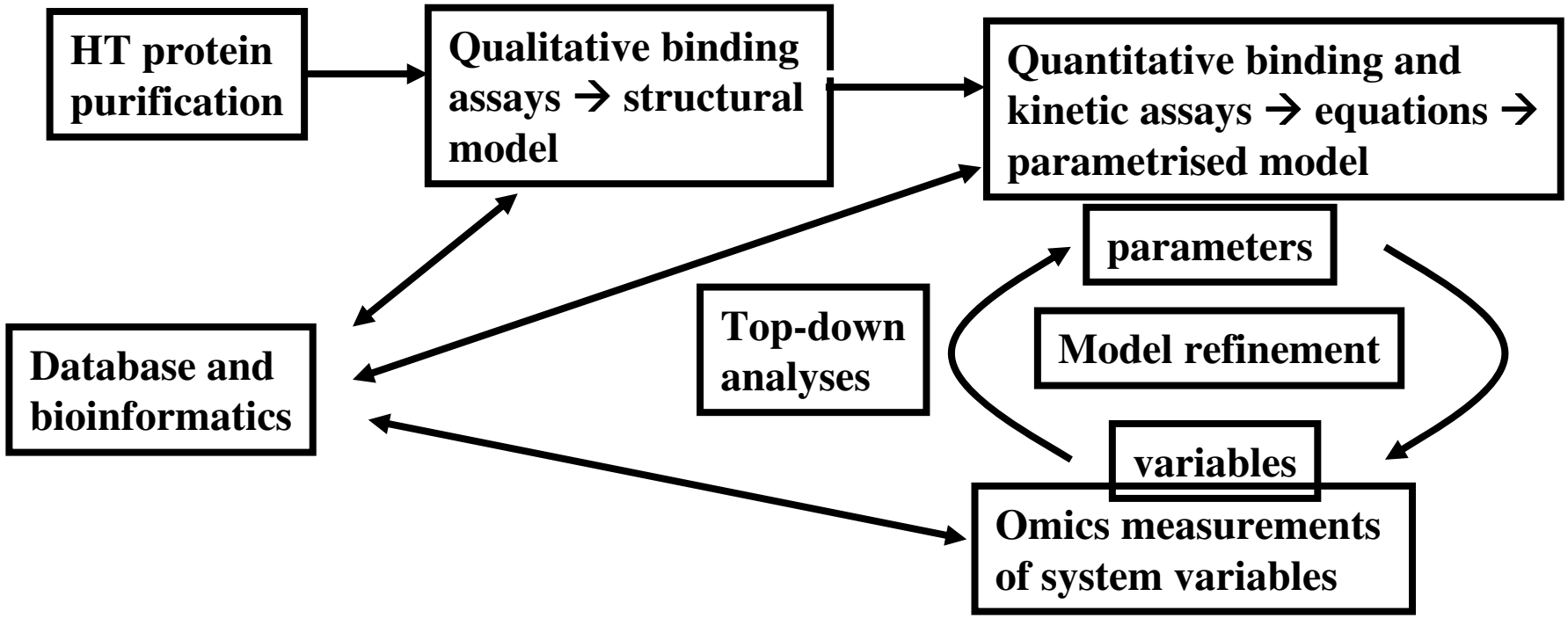

HT protein purification
Qualitative binding assays → structural model
Quantitative binding and kinetic assays → equations → parametrised model
parameters
Top-down analyses
Model refinement
Database and bioinformatics
variables
Omics measurements of system variables

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 organsim) 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 $cm.s^{-1}$), 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 permearbility ($< 0.4. 10^{-5}$ $cm.s^{-1}$) but considerable variation in the permeability across Corti's membrane system, while the other set has a high permeability ($> 3.5 . 10^{-5}$ $cm\ s^{-1}$) 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(octanol-water).
B. Lack of correlation ($r^2 = 0.097$) between apparent permeability in CaCo-2 cells and log K(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, y+ 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.

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