Pharmaceutical drug transport: The issues and the implications that it is essentially carrier-mediated only

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All cells necessarily contain tens, if not hundreds, of carriers for nutrients and intermediary metabolites, and the human genome codes for more than 1000 carriers of various kinds. Here, we illustrate using a typical literature example the widespread but erroneous nature of the assumption that the 'background' or 'passive' permeability to drugs occurs in the absence of carriers. Comparison of the rate of drug transport in natural versus artificial membranes shows discrepancies in absolute magnitudes of 100-fold or more, with the carrier-containing cells showing the greater permeability. Expression profiling data show exactly which carriers are expressed in which tissues. The recognition that drugs necessarily require carriers for uptake into cells provides many opportunities for improving the effectiveness of the drug discovery process.

Introduction

There is considerable and increasing evidence that drugs get into cells more or less solely by hitchhiking on carriers normally used for the transport of nutrients and intermediary metabolites [1–38]. This said, one can find reviews (e.g. [39,40]) that play up the importance of a different cellular route of uptake of pharmaceutical drugs considered to be occurring by simple diffusion through the hydrophobic lipid bilayer portions of biological membranes (and thereby strongly dependent on lipophilicity). Such works also lay stress on studies using artificial membrane systems lacking any proteins, although, as we shall see below, the relevance of such systems (given that the protein:lipid ratio in biological membranes is 1:1–3:1) is at best questionable.

A particular example of a detailed review seeking to provide evidence for the 'mainly bilayer' mode of transport is a recent article by Sugano *et al.* [40], which sought to show that the well-established carrier-mediated transport effecting the cellular uptake of pharmaceutical drugs [1,10,13,14,17] nevertheless coexists with a so-called 'passive' uptake of comparable magnitude, mediated via the bilayer portions of biological membranes. (In the literature, 'passive' normally means non-concentrative, including facilitated diffusion via a carrier; however, where appropriate in this article, we adopt the usage of Sugano *et al.* [40] to imply, as in their Fig. 1 and Box 3, transport through a bilayer portion of a phospholipid cell membrane.) That review [40] served to highlight some of the reasoning used by those who believe in the importance of trans-bilayer transport and the evidence that is purported to underpin it; therefore, the article is helpful in highlighting where the intellectual issues lie. Consequently, we focus several strands of our argument here on those detailed by Sugano *et al.* [40].

At the outset, one should comment that, despite the considerable literature surveyed [40], the title assertion of [40] [a coexistence of such 'passive' and carrier-mediated processes in (cellular) drug transport] fails, because it is simply that (an assertion). Specifically, the authors (and much of the literature they cite) make (and repeat) the same assumption throughout, which is that anything that is not taken up using a carrier that they know about therefore goes through the presumed bilayer portion of the relevant biological membrane, rather than through carriers they either do not know about (in biological membranes) or through

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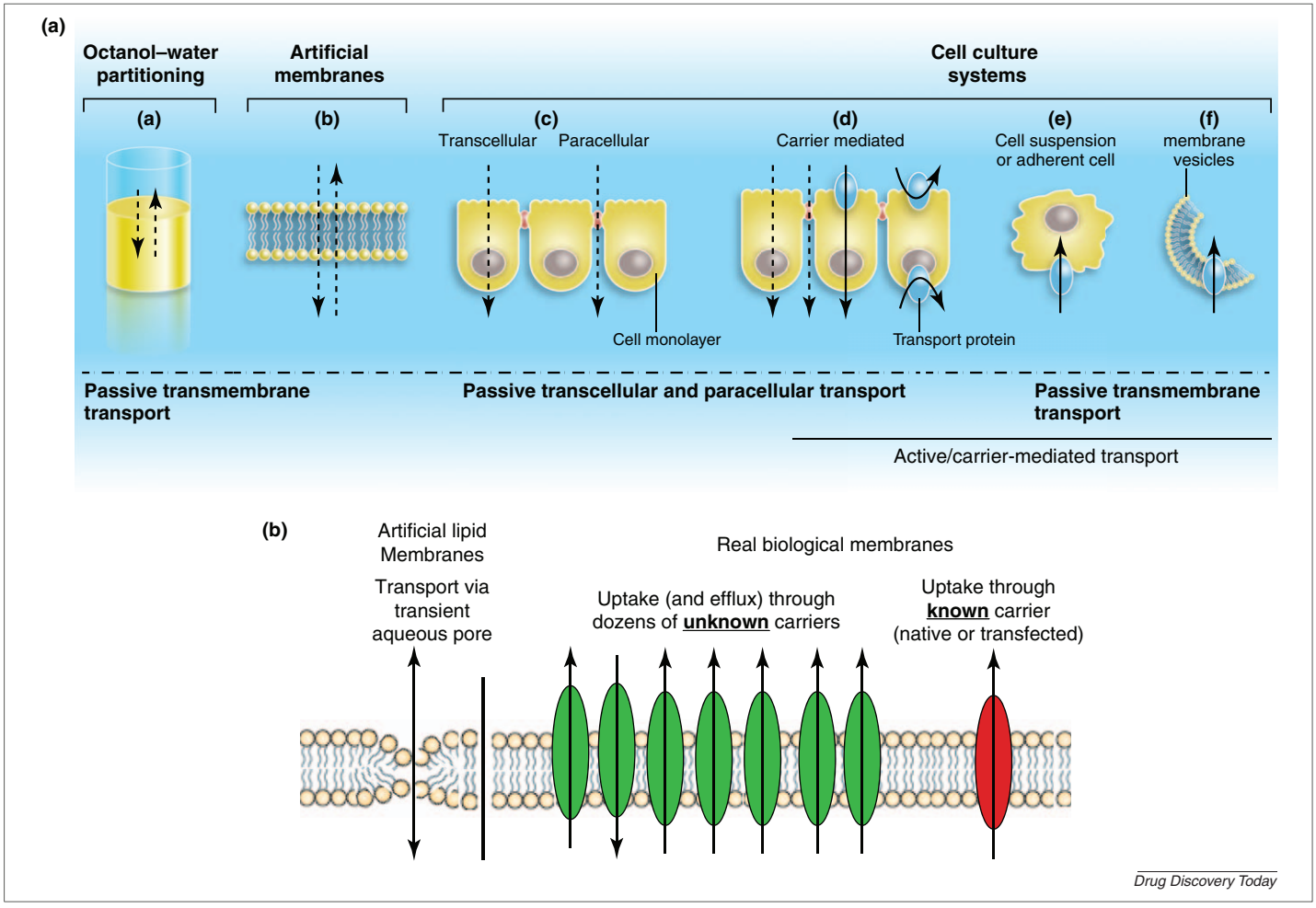

FIGURE 1

Different views of the transport of pharmaceutical drugs across biological and artificial membranes. **(a)** The mechanisms discussed by Sugano *et al.* [40], in which it is assumed that: (i) drugs can transfer directly across the lipid bilayer portion of artificial membranes; (ii) such bilayers are present in natural membranes with properties little or no different from those of the artificial membranes (e.g. that the high protein content of biological membranes does not influence them); and (iii) biological membranes exist with negligible carrier activity. **(b)** A view in which we recognize that: (i) all biological membranes have tens if not hundreds of carriers (even mitochondria have more than 50 carriers [166]); and (ii) most are of as yet unknown specificity and can therefore contribute to the 'background' uptake of any drug, occurring in the absence of any known native or transfected carrier. This view also recognizes that much of the transport, especially of the more hydrophilic molecules, that occurs 'through' artificial membranes in fact occurs via transient aqueous pores that form on a nanosecond timescale [117,118].

transient aqueous pores that occur widely in artificial lipid membranes but not in real biological membranes. When these features are taken into account, the actual requirement, and any evidence, for such 'passive' permeation disappears. Although it is possible that such trans-bilayer 'passive' permeation occurs at meaningful rates, there appears to be no compelling evidence as yet that any pharmaceutical drug crosses real cell membranes by passing through the hydrophobic portion of a phospholipid bilayer. However, as noted above, [40] does provide an excellent basis to help focus on some key issues. We therefore compare the assumptions made by Sugano *et al.* (Fig. 1a; redrawn from Fig. 1 of [40]) with the mechanisms that they did not include (Fig. 1b) and which, in our view, are dominant.

Nature of 'passive' transport in different cell types

Sugano *et al.* [40] assert 'basic passive transcellular transport occurs regardless of cell type (for example between *in vivo* organs and *in vitro* cells). The extent of passive transcellular transport could be dependent on the lipid composition of the membrane, but is usually of comparable magnitude between different cell types'. However, the well-established existence of the blood–brain barrier (BBB) shows that it can vary considerably between different cell types. The BBB is generally impermeable to most drugs (e.g. [41–45]) as tight junctions preclude paracellular transport; however, although its lipid composition is not seen as particularly atypical [46], the BBB does contain many transporters that do allow selective uptake (e.g. [47–59]) as well as catalyzing efflux (e.g. [60–68]). In addition, methods that use chemoinformatic substructural analyses (which would detect determinants of transporter substrates [12]) are at least as effective at predicting BBB uptake as are those based on descriptors such as lipophilicity (e.g. [59,69]). Finally, several studies (e.g. [70–74]) demonstrate the utility of delivering drugs to the central nervous system (CNS) as prodrugs designed to be taken up via known carriers. This shows that,

although there are efflux carriers at the BBB, they alone cannot account for the apparent impermeability of most drugs observed. The BBB also contains many tight junctions, but these would affect only paracellular and not transcellular permeability (Fig. 1a).

The permeability of Caco-2 versus MDCK cells to drugs

One way of assessing the claim that 'background' rates are similar in different cells is to compare them directly. Thus, Sugano *et al.* [40] state 'For instance, Caco-2 cells (derived from the human colon) and Madin–Darby canine kidney (MDCK) cells show similar magnitudes of passive transcellular transport [75]. Therefore, in general, a lipophilic drug that displays a high passive transcellular transport across the intestinal epithelial cell membrane may also display a high passive transcellular transport across, for example, the sinusoidal and canalicular cell membranes of the hepatocyte.' However, the reference cited [75] presented no evidence that the drugs they labeled 'passively permeable' (in any case an ambiguous term; see above) did cross the bilayer 'passively', that is, without a carrier. Moreover, a careful analysis of their data suggests that carrier-free 'passive' permeability is unlikely to be the transport mechanism. First, the relationship between the uptake into the two kinds of cell is obscured by the use of a log–log plot; for 24 of the 55 drugs tested (Table 1 of [75] and the present Fig. 2a), the rates vary more than twofold between the two cell types. Secondly, comparison of the data in Table 1 and Fig. 1 of [75] enables one to identify which (11) of the 55 drugs are claimed to be uptake (seven) or efflux (four) transporter substrates and which (44) are thus supposedly taken up 'passively'. Of these 44, we can find clear literature evidence (see also [10]) for the carrier-mediated transport of 18 of them, namely acebutolol [76], acyclovir [77], atenolol [78], bretylium [79–81], bupropion [82], cefuroxime [83], chlorothiazide [84,85], gabapentin [86,87], hydrocortisone (cortisol) [88,89], ketoprofen [90], mannitol [91,92], methylprednisolone [93,94], phenytoin [95–97], progesterone [98], propranolol [99], testosterone [100] and trimethoprim [101].

The assumption that these molecules cross membranes only 'passively' is, therefore, incorrect. Of course, we cannot say much about the other drugs for which carriers have not (yet) been found. Nevertheless, it would be surprising if the others did not have carriers, because, for instance, we know of many carriers for substances that are closely related structurally to the substrates for which we did not find explicit literature evidence for carrier-mediated uptake. Thus, there are carriers for steroids [102], penicillin G [103] (rather than penicillin V as studied by Irvine *et al.* [75]), as well as for many other β-lactams [104–108], and so on [23]. We thus re-plot the data of Fig. 1 of [75] in Fig. 2b, where it can be seen that the general shape of the plot (as in the original, in fact) is the same whether carriers are known to be used or not. The most obvious interpretation of this plot, then, is that all the drugs tested are using carriers, but that, in some cases, it is not known which ones.

The membranes of any cells, including Caco-2 and MDCK, necessarily contain tens, if not hundreds, of carriers for all kinds of nutrients and intermediary metabolites and it is probably with these 'unknown' carriers that pharmaceutical drugs hitchhike a lift to effect their entry into cells (e.g. [4,9,10,12,13]). More generally, it is becoming increasingly clear from genome-wide association studies that many individual proteins contribute what are individually small amounts to what might be a substantial phenotype [109–111]. Drug transporters are unlikely to be an exception.

What are the proper controls for so-called 'passive' uptake?

Sugano *et al.* [40] assert 'usually when transfected cells are used, non-transfected cells (mock) are simultaneously used as a control experiment to evaluate the contribution of passive transcellular membrane transport.' However, non-transfected cells are emphatically not such a control, because they naturally contain many carriers (scores if not hundreds), and it is not correct to assume that unknown carriers are either absent or irrelevant when assessing 'background' rates of transport in the absence of any known transfected carrier. Although such non-transfected cells might provide a background that enables the determination of the incremental contribution to drug transport of a transfected carrier, they tell one nothing about the mechanisms of any 'background' uptake. The same applies to the assertion [40] (from a survey of carrier-mediated uptake papers) 'Clear indications of the involvement of passive transport were presented in 81 cases. In 46 cases, passive transcellular transport contributed to more than 30% of total uptake and/or permeance.' However, this 'background' uptake could have occurred by any means, most plausibly via the numerous carriers that were present but not identified, as in the case of [75] discussed above.

Known transporters encoded by the human and other genomes

Much is now known about the transporters encoded by the human [112] and other genomes. Thus, the analysis given online at http://www.membranetransport.org/ indicates that the human genome encodes 1022 (uptake and efflux) membrane transporters (0.32 per Mbase genome), with the numbers for some common model eukaryotes being *Arabidopsis thaliana* 1210 (9.68), *Caenorhabditis elegans* 654 (6.74), *Drosophila melanogaster* 603 (5.03), *Mus musculus* 1090 (0.4) and *Saccharomyces cerevisiae* 318 (24.46). This set of eukaryote data is shown in Fig. 3, from which it is clear that all higher organisms contain genes for hundreds or thousands of carrier proteins.

The properties of artificial phospholipid bilayer membranes lacking proteins

One potential way round the issue of transport by unknown carriers is to study it in artificial 'membranes' that do not contain proteins. Thus, Sugano *et al.* [40] state 'A good, but not perfect, choice for such a reference membrane is the black lipid membrane model and unilamellar vesicles (liposomes)'. Unfortunately, such models are entirely inappropriate, precisely because, by lacking proteins, whose ratio to lipids in real membranes is in the range 1:1–3:1, they do not represent biological membranes. Moreover, they are essentially leaky via the formation of transient hydrophilic pores (Fig. 1b). Although these enable molecules to cross such membranes, this is by a mechanism that does not involve their transfer through the hydrophobic portion of the lipid bilayer. This is most obvious when these molecules are charged, because they do appear to cross bilayer membranes (e.g. [113]); however, the enormous Born charging energy required [114–116] means that this cannot be other than via hydrophilic pores, which form

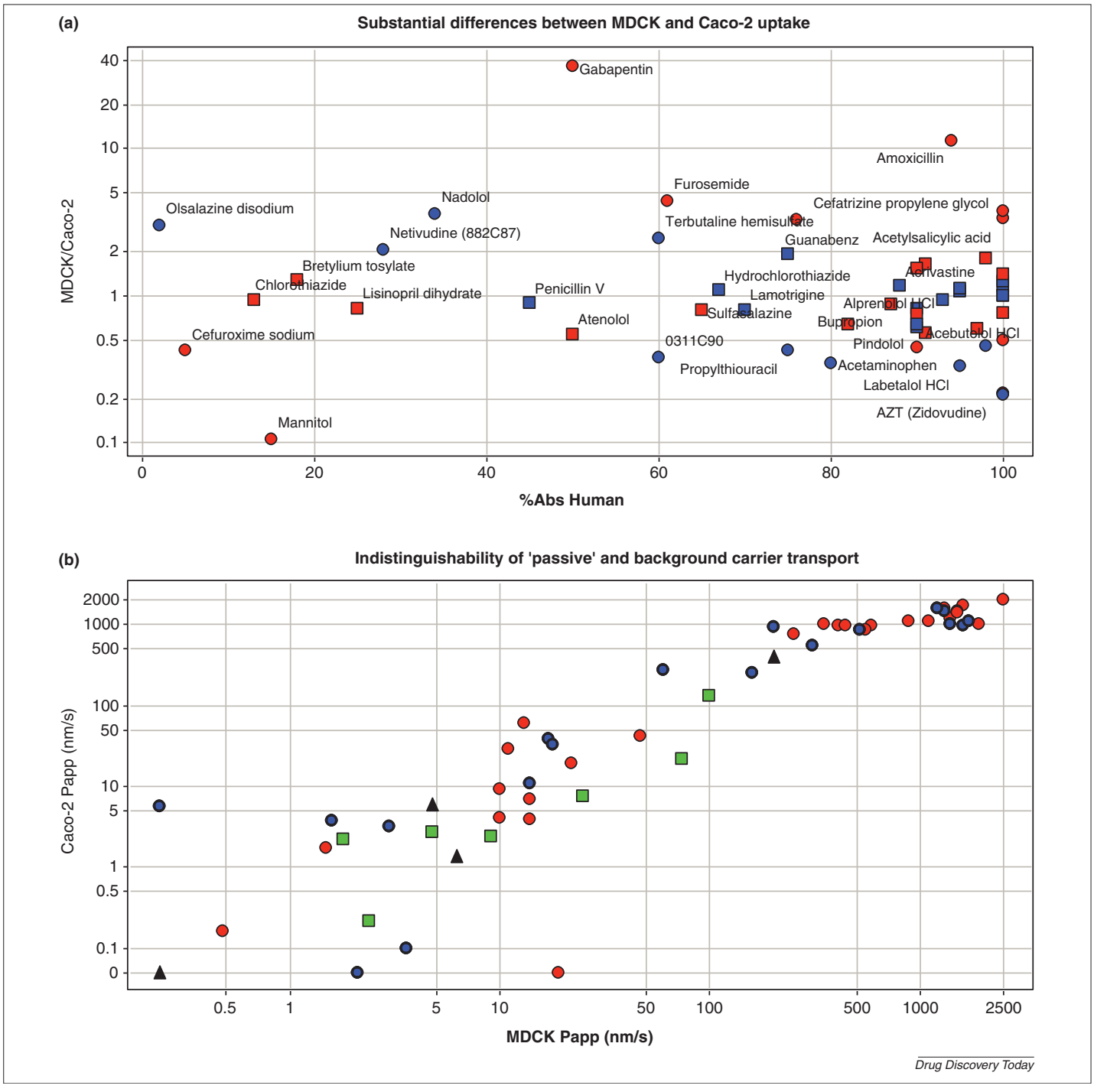

FIGURE 2

Reanalysis of the permeability properties of MDCK and Caco-2 cells as published in [75]. **(a)** Comparison of the rates of drug transfer in the two cells lines, expressed as a ratio, as a function of the percentage absorbed in humans. It is clear that, far from being similar as claimed [40], the rate of uptake of individual drugs across the two cell lines varies considerably [by a factor of at least twofold (circles) in 24 of the 55 drugs tested]. The degree of variation does not appear to depend on whether a carrier is already known (red) or not (blue). The permeability of mannitol in MDCK cells is indistinguishable from zero (unsurprisingly, as it is a well-known osmoticum); in addition, three drugs (acyclovir, ranitidine and sumatriptan succinate) are not shown because their permeability through Caco-2 cells is indistinguishable from zero. The abscissa reflects the percentage of an oral dose that is absorbed by humans *in vivo*. **(b)** Independence, on whether a carrier is known, of the relationship between drug uptake by MDCK and Caco-2 cells. Drugs are classified into whether they were assumed [40,75] to be 'passively' permeable through a membrane bilayer portion (circles), actively taken up (squares), or actively effluxed (triangles). Of the 'passively permeable' drugs, 18 have known carriers (blue), whereas, for others, no carrier is, as yet, known (red).

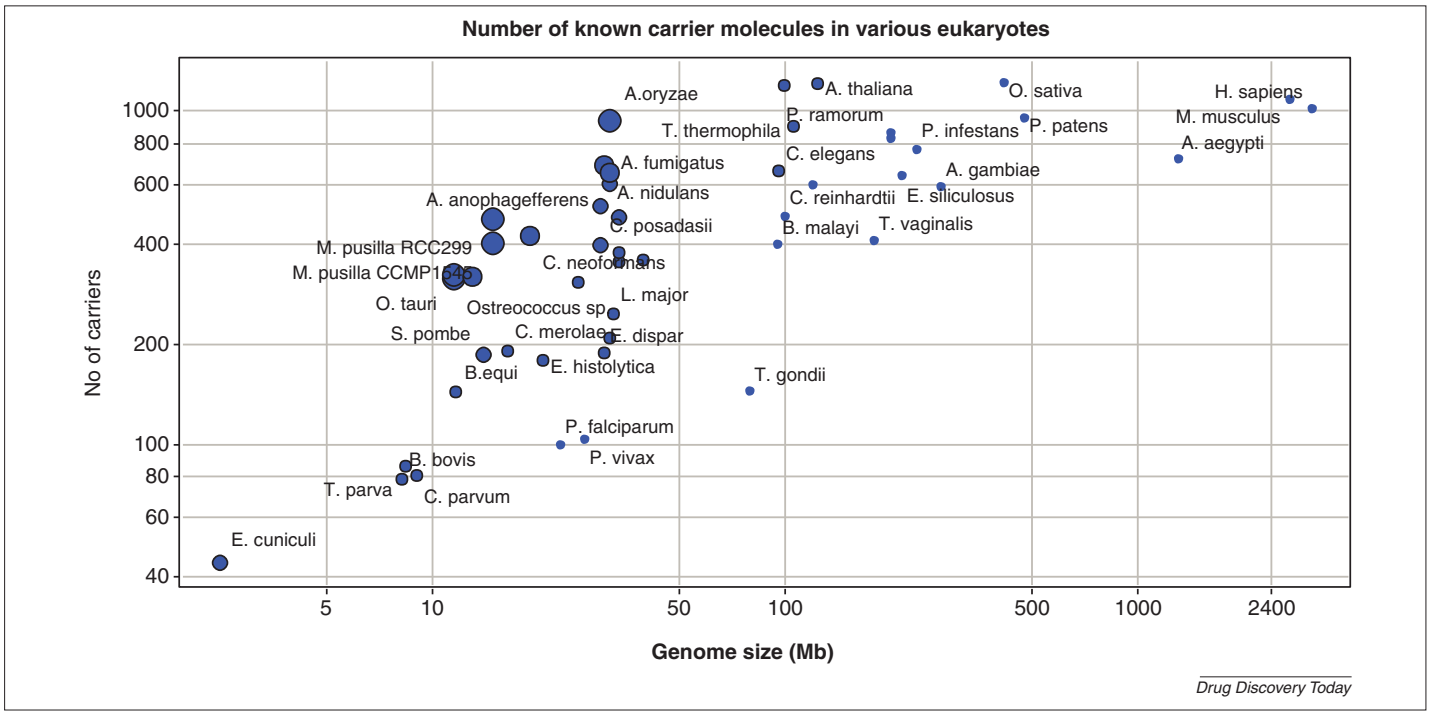

FIGURE 3

The number of carriers known to be encoded within the genomes of various eukaryotes, plotted against their genome size. Data are taken from http://www.membranetransport.org/. In addition, the size of the symbols is proportional to the number of carriers per Mbase of genome. It is evident that, in most cases, hundreds of carriers are already known from each type of organism.

frequently and spontaneously (on a nanosecond timescale) in artificial phospholipid bilayer membranes [117,118]. However, this cannot be occurring in real biological membranes because, if it did, they would exhibit no significant osmotic properties and any osmolytes added externally (such as mannitol or sorbitol, as in typical biochemical incubation media) would be able to cross the membrane freely and would thus not serve as osmotica.

Black lipid membranes and liposomes versus real biological membranes

Sugano *et al*. [40] also state 'In black lipid membranes and liposomal membranes, numerous reports suggest that compounds with mid to high lipophilicity [e.g. a $\log D_{oct}$ {i.e. octanol:water distribution coefficient} > 0) [119] rapidly permeated, whereas compounds with low lipophilicity slowly permeated (for example, glycerol ($\log D_{oct} = -1.76$) and urea ($\log D_{oct} = -1.66$)...). These studies indicate that many drug-like compounds can pass through the lipid bilayer in proportion to their lipophilicity [120]'. Although these studies do indicate this (although there are plenty of outliers, and in fact an entirely different relationship is shown in Fig. 4 of ref. [40]), they indicate neither how this is done, nor what relevance this has to real biological membranes. It is well known that many hydrophobic molecules can disrupt membrane integrity, the effect being greater, within a given structural class, for more hydrophobic molecules [121]. Thus, more hydrophobic molecules are more likely to disrupt the black lipid membrane or liposome structure and thereby induce aqueous pores. A plot is also given (Fig. 5a in [40]) that, although not identifying the specific drugs representing each data point, does imply a 'correlation' (coefficient) of 0.84 between the permeability of drugs into MDCK cells and their permeability in an artificial membrane assay containing hexadecane, 'suggesting that passive transcellular transport is dominant in the permeation of these compounds in MDCK cells' [40]. Although this interpretation would not follow from the data even if the correlation coefficient were unity, the relationship is far from being close. This is because the slope of the log–log plot is not close to 1; instead, it is approximately 0.5. Thus, at the lower end, there is nearly a 100-fold discrepancy in the absolute fluxes; that is, the real biological MDCK cells, which contain carriers, take up the less permeable drugs approximately 100-fold more rapidly than do the artificial membranes. If there were a true correlation (whatever its interpretation), it should manifest itself linearly. We plot the digitized data in linear coordinates in Fig. 4, where it can be seen that the linearity of the relationship is poor; the slope of the 'best' straight line fit is again 0.5, but the correlation coefficient is now only 0.45.

Table 1 of [40] also contains a list of papers that seek to relate various measures of cellular uptake to the behavior of drugs in various artificial membrane assays. Leaving aside the fact that there are many flavors of these assays, such that they are often 'tuned' to optimize the correlations that can be found [10], and there is rarely a separate validation set [122], it is of interest that correlations are poor. Let us take one example ([123], Ref. 100 of [40]), chosen because the artificial membrane is referred to as 'biomimetic'. What this paper (and our Fig. 5) shows (before extra corrections are introduced) is similar to what is described in the previous paragraph: a log–log plot in which the permeability of the less permeable molecules is (in this case) typically greater in the Caco-2 cells, in which the slope is 0.59 and the linear correlation coefficient is again 0.45.

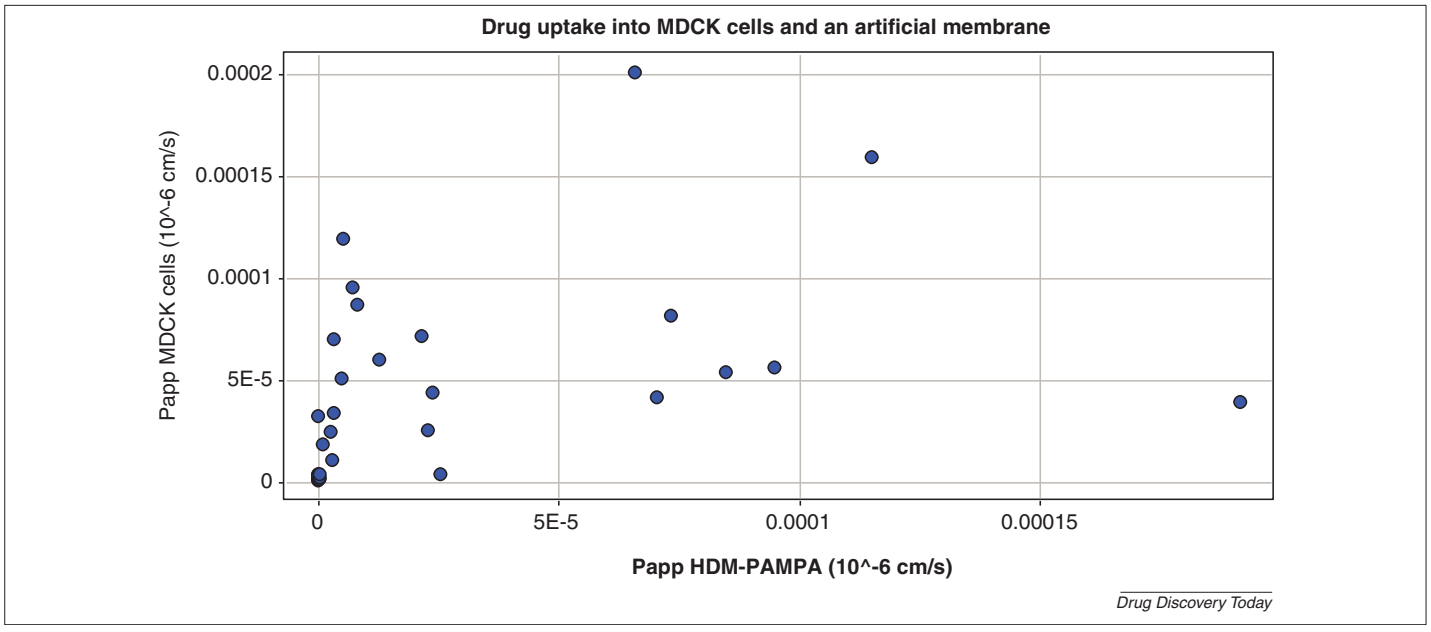

FIGURE 4

Relationship between the rate of drug uptake by MDCK cells and by an artificial membrane system containing hexadecane (HDM-PAMPA). Data are re-plotted (using the Ungraph software from Elsevier Biosoft) from Fig. 5 [40], but on a linear scale. The slope of the 'best fit' line is 0.5, but this is not shown as the correlation coefficient is only 0.45. The low correlation coefficient is not surprising because there are discrepancies of typically two orders of magnitude in the absolute rates (see also [40]), with the MDCK cells (which contain carriers) effecting the faster uptake.

The non-significance of structural specificity to enzymes

Much is made in these kinds of discussion (e.g. [40]) of the supposed difference between the assumed structural specificity towards the substrates of carrier molecules and its essential independence (other than on lipophilicity) from 'passive' transport: 'Correlation between indicators of biological membrane permeation and passive permeation (the whole molecule physicochemical property and artificial membrane permeation) for structurally diverse compounds also suggests that passive transcellular membrane transport of drugs exists' [40]. Balaz [39] also focuses on the importance of specificity in carrier-mediated uptake. However, this ignores the fact that biological membranes are replete with transporters and that many enzymes, such as various cytochromes P450 (e.g. [124]), or transporters, such as the organic anion transporting proteins [125], PEPT1/2 [106–108] and the P-glycoprotein carrier [63–67,126–128], have very broad specificities [129]. Furthermore, narcotics (general anesthetics), which are a structurally diverse set of molecules once thought [130] to depend for their activity solely on lipophilicity, interact rather specifically with their target proteins (e.g. [10,13,14,131–139]). Thus, although a degree of specificity (especially stereospecificity) can be used to imply a role for enzymes and carriers, its absence (especially in the absence of knowledge of the actual specificities of the carriers involved) cannot really tell one anything.

Expression profiling of solute transporters in biological cells

A useful method to find out which carriers are present in real (biological) cell lines and membranes, and, therefore, to study their properties, is to carry out expression profiling analyses. This is starting to be done, for instance via transcriptomics [140–143], and it is known that the plasma membrane of Caco-2 cells contains over 1000 proteins, including several hundred transporters [144–148], of broad (but usually unknown) specificity, whereas the membranes of MDCK cells contain over 800 proteins [149]. Searching the Pharmacogenomic Knowledge Base with the query 'transporter' and limiting the results to genes (http://pharmgkb.org/search/search.action?typeFilter=Gene&exactMatch=false&query=transporter) gives more than 500 separate genes (500 being the maximum that can be displayed in this way). The antibody-based Human Protein Atlas (HPA; http://proteinatlas.org/; [150,151]) now lists expression profiles in multiple cells and tissues of more than 350 solute carriers, and of over 240 of the 461 proteins labeled (http://www.tcdb.org/hgnc_explore.php) as transporters. Here, we highlight two examples from searching the HPA. The first (http://proteinatlas.org/tissue_profile.php?antibody_id=15468&g_no=ENSG00000197208) shows that the organic cation transporter (member 4 of the SLC22 solute carrier family [152]) is expressed in most cells. A second (using the 'advanced search' facility) shows that HeLa cells express 154 different transporters. Although data are not yet available for cells such as MDCK or Caco-2, the expression profiles available in the HPA no longer make it possible to claim that transporters are not expressed in very large numbers in all cells tested. We also highlight here the experimental analyses of transporter proteins in mice [55], the BBB [57] and human intestine [153], all of which illustrate the presence in every cell studied of a considerable number of 'background' transporters. To take just one example, MDCK cells even benefit from a special carrier for urea [154].

Stereospecificity and enzyme kinetics

Although stereospecificity of uptake is unlikely to be observable for any passive diffusion mechanisms, any stereospecificity of uptake

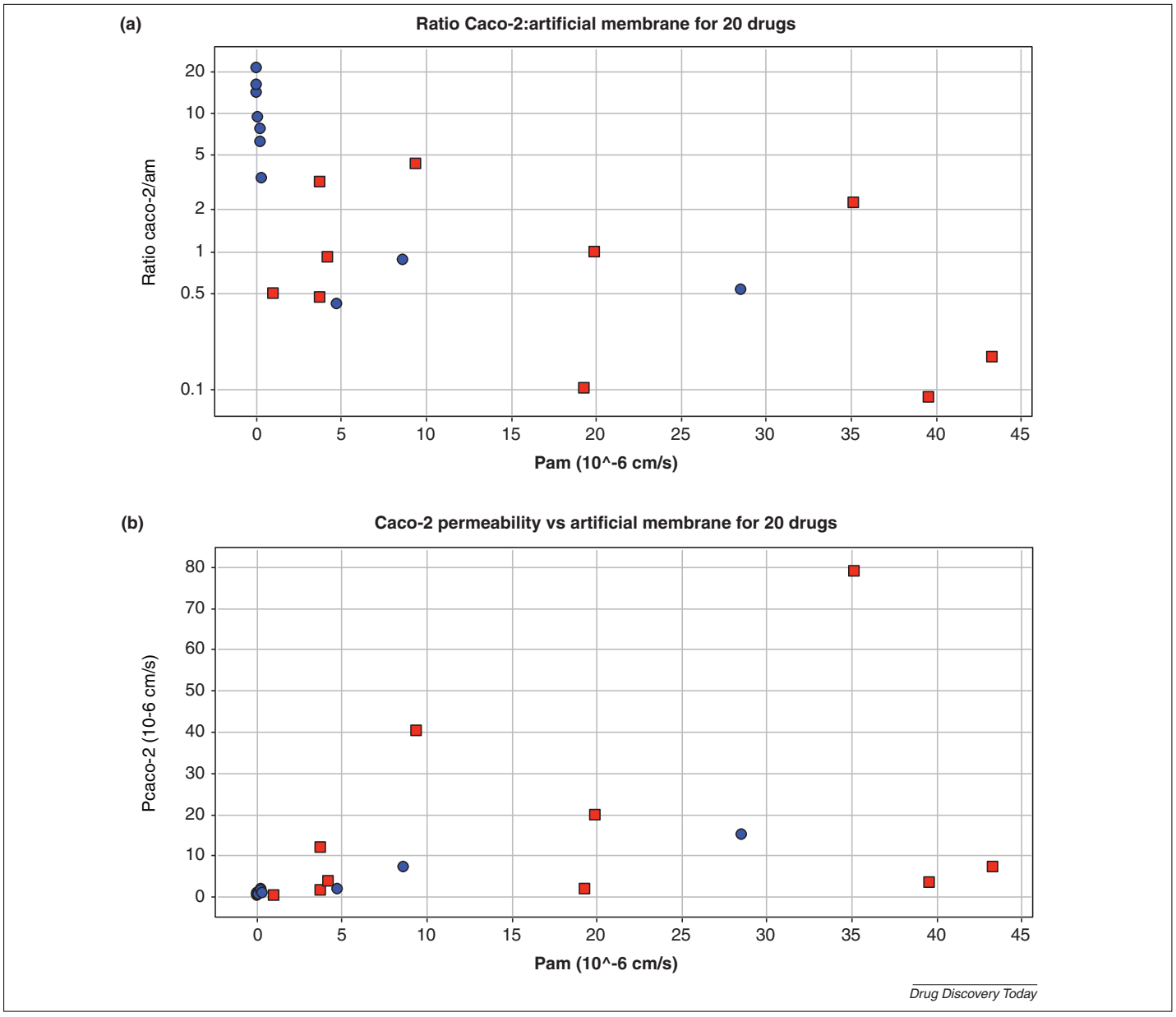

FIGURE 5

Relationship between Caco-2 permeability (P) and permeability through an artificial membrane (am) in a published experiment [123]. Data were digitized from Fig. 6a of [123]. **(a)** Ratio of uptake into Caco-2 cells to that of the artificial membrane versus that of the artificial membrane alone. Only four of the 20 drugs are within a factor 2 of each other. **(b)** Poor linear 'correlation' between Caco-2 cell and artificial membrane permeability. The slope is 0.59 and the correlation coefficient is 0.45. Drugs are classed (by [123]) as hydrophobic (prologD $>$ 0; blue squares) or hydrophilic (prologD $<$ 0; red circles).

observed could be taken as good evidence for carrier mediation (albeit that the converse is not true, as many enzymes are highly promiscuous [129,155,156]). However, Sugano *et al.* [40] also ascribe some unusual properties to carriers. Thus, 'as carrier proteins are made of chiral amino acids, carrier-mediated transport is stereospecific' [40]; however, stereoselectivity comes from the three-dimensional arrangement of atoms in the protein that bind the substrates, and the fact that there are multiple interactions between protein and substrate [157–159]; this would be a sufficient condition whether the building blocks of proteins were inherently chiral or otherwise. Another comment reads 'Analyses of concentration dependency data using kinetic models, such as the Michaelis–Menten equation, are also used to differentiate carrier-mediated transport from passive transcellular membrane transport. According to the Michaelis–Menten equation, the fraction of a permeant that crosses the membrane by passive transcellular membrane transport can become more significant when the concentration of the permeant is higher than the K_m' [40]. This is a curious interpretation of standard enzyme kinetics, because the rate of an enzyme-catalyzed reaction obeying the Michaelis-Menten equation continues to rise monotonically with its substrate concentration, the amount being transported therefore increasing with its substrate concentration, and with the absolute rate being determined more by k_{cat} than by K_m.

Concluding remarks

Given that the kinds of experiment being claimed, repeatedly, to support a significant 'passive' (carrier-independent) permeability of pharmaceutical drugs across the bilayer portion of biological membranes are both commonly performed and commonly misinterpreted, we have found it useful here to highlight our view of some of the issues, which we now do in summary form below:

1 All biological membranes contain tens and possibly hundreds of different kinds of transporter molecule.
2 The presumed absence of evidence for transporter molecules (or the ignoring of it) is not evidence of their absence.
3 Modern expression profiling methods, especially proteomics, can determine which transporters are present in different cell types.
4 Most of these proteins are of unknown specificity and catalytic power (k_{cat}/K_m).
5 Some proteins have wide substrate specificity, and will use a suite of substrates with imperceptible structural relatedness.
6 Consequently, these many native transporters, each possibly contributing only weakly (but with some predominating in specific cases) are most likely, together, to be responsible for the 'background' permeability of most cells to various drugs. This can be tested by determining their expression profiles and studying the properties of the carriers directly.
7 Artificial (lipid or other) membranes lacking these or other proteins cannot be used to assess the role of transporters in pharmaceutical drug uptake in biological cells.
8 Any correlations between transport across an artificial membrane and a real biological membrane cannot have a mechanistic basis because the membranes are different; for example, real biological membranes have protein:lipid ratios of 1:1–3:1, not 0:1.
9 Unlike biological membranes, artificial bilayers are leaky and form transient aqueous pores that enable the transfer of small molecules across the membrane; such molecules do not *per se* cross the hydrophobic core of the lipid bilayer.
10 We now know of carriers for all kinds of molecule [160], from water, urea and glycerol to highly hydrophobic molecules, such as the dibenzyldimethylammonium cation [161], as well as for hundreds, if not thousands, of different drugs [1–4,6–10,17,23].
11 Because of the enormous Born charging energy, small charged molecules cannot diffuse through the hydrophobic portion of a phospholipid bilayer.
12 It is well known that many hydrophobic molecules can disrupt membrane integrity, the effect being greater within a structural class for more hydrophobic molecules [121]; thus, small molecules might themselves induce such hydrophilic pores, possibly as a function of their lipophilicity.
13 Cells lacking a transfected protein are not suitable controls for the same cells containing them.
14 Suitable controls would involve using cells that had a negligible background activity.

Given the continuing huge attrition rates in the pharmaceutical industry [162–165], it is important to find out which carriers are used in which cells. Ignoring their existence serves only to prevent their identification and exploitation in improving the safety and efficacy of new drugs.

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References

1 Sai, Y. and Tsuji, A. (2004) Transporter-mediated drug delivery: recent progress and experimental approaches. *Drug Discov. Today* 9, 712–720
2 Cascorbi, I. (2006) Role of pharmacogenetics of ATP-binding cassette transporters in the pharmacokinetics of drugs. *Pharmacol. Ther.* 112, 457–473
3 Sai, Y. (2005) Biochemical and molecular pharmacological aspects of transporters as determinants of drug disposition. *Drug Metab. Pharmacokinet.* 20, 91–99
4 Yan, Q. (2005) Pharmacogenomics and systems biology of membrane transporters. *Mol. Biotechnol.* 29, 75–88
5 Li, M. *et al.* (2006) Drug–drug interactions involving membrane transporters in the human kidney. *Expert Opin. Drug Metab. Toxicol.* 2, 505–532
6 Shitara, Y. *et al.* (2006) Transporters as a determinant of drug clearance and tissue distribution. *Eur. J. Pharm. Sci.* 27, 425–446
7 Koepsell, H. *et al.* (2007) Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. *Pharm. Res.* 24, 1227–1251
8 Xia, C.Q. *et al.* (2007) Evaluation of drug-transporter interactions using *in vitro* and *in vivo* models. *Curr. Drug Metab.* 8, 341–363
9 You, G. *et al.* eds (2007) *Drug Transporters: Molecular Characterization and Role In Drug Disposition*, John Wiley & Sons
10 Dobson, P.D. and Kell, D.B. (2008) Carrier-mediated cellular uptake of pharmaceutical drugs: an exception or the rule? *Nat. Rev. Drug Discov.* 7, 205–220
11 El-Sheikh, A.A. *et al.* (2008) Mechanisms of renal anionic drug transport. *Eur. J. Pharmacol.* 585, 245–255
12 Dobson, P.D. *et al.* (2009) 'Metabolite-likeness' as a criterion in the design and selection of pharmaceutical drug libraries. *Drug Discov. Today* 14, 31–40
13 Dobson, P. *et al.* (2009) Implications of the dominant role of cellular transporters in drug uptake. *Curr. Top. Med. Chem.* 9, 163–184
14 Kell, D.B. and Dobson, P.D. (2009) The cellular uptake of pharmaceutical drugs is mainly carrier-mediated and is thus an issue not so much of biophysics but of systems biology. In *Proceedings of the International Beilstein Symposium on Systems Chemistry* (Hicks, M.G. and Kettner, C., eds), pp. 149–168, Logos Verlag
15 Lee, E.J.D. *et al.* (2009) Role of membrane transporters in the safety profile of drugs. *Expert Opin. Drug Metab. Toxicol.* 5, 1369–1383
16 Feng, B. *et al.* (2010) Renal clearance in drug discovery and development: molecular descriptors, drug transporters and disease state. *Expert Opin. Drug Metab. Toxicol.* 6, 939–952
17 Giacomini, K.M. *et al.* (2010) Membrane transporters in drug development. *Nat. Rev. Drug Discov.* 9, 215–236
18 Goole, J. *et al.* (2010) The effects of excipients on transporter mediated absorption. *Int. J. Pharm.* 393, 17–31
19 Gumbleton, M. *et al.* (2010) Spatial expression and functionality of drug transporters in the intact lung: objectives for further research. *Adv. Drug Deliv. Rev.* 10.1016/j.addr.2010.1009.1008
20 Hagenbuch, B. (2010) Drug uptake systems in liver and kidney: a historic perspective. *Clin. Pharmacol. Ther.* 87, 39–47
21 Huang, S.M. and Woodcock, J. (2010) Transporters in drug development: advancing on the critical path. *Nat. Rev. Drug Discov.* 9, 175–176
22 Kroetz, D.L. *et al.* (2010) The pharmacogenomics of membrane transporters project: research at the interface of genomics and transporter pharmacology. *Clin. Pharmacol. Ther.* 87, 109–116
23 Lai, Y. *et al.* (2010) Evaluation of drug transporter interactions in drug discovery and development. *Comb. Chem. High Throughput Screen.* 13, 112–134
24 Leeson, P.D. and Empfield, J.R. (2010) Reducing the risk of drug attrition associated with physicochemical properties. *Annu. Rep. Med. Chem.* 45, 393–407

25 Masereeuw, R. and Russel, F.G. (2010) Therapeutic implications of renal anionic drug transporters. *Pharmacol. Ther.* 126, 200–216
26 Sarkadi, B. and Szakács, G. (2010) Understanding transport through pharmacological barriers – are we there yet? *Nat. Rev. Drug Discov.* 9, 897–898
27 Wang, J. and Skolnik, S. (2010) Mitigating permeability-mediated risks in drug discovery. *Expert Opin. Drug Metab. Toxicol.* 6, 171–187
28 Yee, S.W. *et al.* (2010) Pharmacogenomics of membrane transporters: past, present and future. *Pharmacogenomics* 11, 475–479
29 Aw, W. *et al.* (2011) A new trend in personalized medicine: rapid detection of SNPs in drug transporter genes by the SmartAmp method. *Clin. Pharmacol. Ther.* 89, 617–620
30 Ciarimboli, G. (2011) Role of organic cation transporters in drug-induced toxicity. *Expert Opin. Drug Metab. Toxicol.* 7, 159–174
31 Gleeson, M.P. *et al.* (2011) In-silico ADME models: a general assessment of their utility in drug discovery applications. *Curr. Top. Med. Chem.* 11, 358–381
32 Gupta, S. *et al.* (2011) SLC22 transporter family proteins as targets for cytostatic uptake into tumor cells. *Biol. Chem.* 392, 117–124
33 Koepsell, H. (2011) Substrate recognition and translocation by polyspecific organic cation transporters. *Biol. Chem.* 392, 95–101
34 Kramer, W. (2011) Transporters, Trojan horses and therapeutics: suitability of bile acid and peptide transporters for drug delivery. *Biol. Chem.* 392, 77–94
35 Lancaster, C.S. and Sparreboom, A. (2011) Pharmaceutical relevance of drug transporter malfunctions. *Clin. Pharmacol. Ther.* 89, 471–474
36 Tirona, R.G. (2011) Molecular mechanisms of drug transporter regulation. *Handb. Exp. Pharmacol.* 201, 373–402
37 Zhao, P. *et al.* (2011) Applications of physiologically based pharmacokinetic (PBPK) modeling and simulation during regulatory review. *Clin. Pharmacol. Ther.* 89, 259–267
38 Zhang, L. *et al.* (2011) Transporter-mediated drug–drug interactions. *Clin. Pharmacol. Ther.* 89, 481–484
39 Balaz, S. (2009) Modeling kinetics of subcellular disposition of chemicals. *Chem. Rev.* 109, 1793–1899
40 Sugano, K. *et al.* (2010) Coexistence of passive and carrier-mediated processes in drug transport. *Nat. Rev. Drug Discov.* 9, 597–614
41 Pardridge, W.M. (2006) Molecular Trojan horses for blood–brain barrier drug delivery. *Curr. Opin. Pharmacol.* 10.1016/j.coph.2006.1006.1001
42 Nies, A.T. (2007) The role of membrane transporters in drug delivery to brain tumors. *Cancer Lett.* 254, 11–29
43 Eyal, S. *et al.* (2009) Drug interactions at the blood–brain barrier: fact or fantasy? *Pharmacol. Ther.* 123, 80–104
44 Mensch, J. *et al.* (2009) *In vivo*, *in vitro* and *in silico* methods for small molecule transfer across the BBB. *J. Pharm. Sci.* 98, 4429–4468
45 Cheng, Z. *et al.* (2010) Central nervous system penetration for small molecule therapeutic agents does not increase in multiple sclerosis- and Alzheimer's disease-related animal models despite reported blood–brain barrier disruption. *Drug Metab. Dispos.* 38, 1355–1361
46 Krämer, S.D. *et al.* (2002) Lipids in blood–brain barrier models *in vitro* I: thin-layer chromatography and high-performance liquid chromatography for the analysis of lipid classes and long-chain polyunsaturated fatty acids. *In Vitro Cell. Dev. Biol. Anim.* 38, 557–565
47 Tamai, I. and Tsuji, A. (2000) Transporter-mediated permeation of drugs across the blood–brain barrier. *J. Pharm. Sci.* 89, 1371–1388
48 Lee, G. *et al.* (2001) Drug transporters in the central nervous system: brain barriers and brain parenchyma considerations. *Pharmacol. Rev.* 53, 569–596
49 Pardridge, W.M. (2003) Blood–brain barrier genomics and the use of endogenous transporters to cause drug penetration into the brain. *Curr. Opin. Drug Discov. Dev.* 6, 683–691
50 Begley, D.J. (2004) Delivery of therapeutic agents to the central nervous system: the problems and the possibilities. *Pharmacol. Ther.* 104, 29–45
51 Fricker, G. and Miller, D.S. (2004) Modulation of drug transporters at the blood–brain barrier. *Pharmacology* 70, 169–176
52 Calabria, A.R. and Shusta, E.V. (2006) Blood–brain barrier genomics and proteomics: elucidating phenotype, identifying disease targets and enabling brain drug delivery. *Drug Discov. Today* 11, 792–799
53 Ohtsuki, S. and Terasaki, T. (2007) Contribution of carrier-mediated transport systems to the blood–brain barrier as a supporting and protecting interface for the brain; importance for CNS drug discovery and development. *Pharm. Res.* 24, 1745–1758
54 Bernacki, J. *et al.* (2008) Physiology and pharmacological role of the blood–brain barrier. *Pharmacol. Rep.* 60, 600–622
55 Kamiie, J. *et al.* (2008) Quantitative atlas of membrane transporter proteins: development and application of a highly sensitive simultaneous LC/MS/MS method combined with novel *in-silico* peptide selection criteria. *Pharm. Res.* 25, 1469–1483
56 Roberts, L.M. *et al.* (2008) Subcellular localization of transporters along the rat blood–brain barrier and blood–cerebral–spinal fluid barrier by *in vivo* biotinylation. *Neuroscience* 155, 423–438
57 Pottiez, G. *et al.* (2009) Understanding the blood–brain barrier using gene and protein expression profiling technologies. *Brain Res. Rev.* 62, 83–98
58 Abbott, N.J. *et al.* (2010) Structure and function of the blood–brain barrier. *Neurobiol. Dis.* 37, 13–25
59 Fan, Y. *et al.* (2010) Insights for predicting blood–brain barrier penetration of CNS targeted molecules using QSPR approaches. *J. Chem. Inf. Model.* 50, 1123–1133
60 Tsuji, A. and Tamai, I. (1997) Blood–brain barrier function of P-glycoprotein. *Adv. Drug Deliv. Rev.* 25, 287–298
61 Löscher, W. and Potschka, H. (2005) Drug resistance in brain diseases and the role of drug efflux transporters. *Nat. Rev. Neurosci.* 6, 591–602
62 Löscher, W. and Potschka, H. (2005) Role of drug efflux transporters in the brain for drug disposition and treatment of brain diseases. *Prog. Neurobiol.* 76, 22–76
63 Takano, M. *et al.* (2006) Expression and function of efflux drug transporters in the intestine. *Pharmacol. Ther.* 109, 137–161
64 Ha, S.N. *et al.* (2007) Mini review on molecular modeling of P-glycoprotein (Pgp). *Curr. Top. Med. Chem.* 7, 1525–1529
65 Leschziner, G.D. *et al.* (2007) ABCB1 genotype and PGP expression, function and therapeutic drug response: a critical review and recommendations for future research. *Pharmacogenomics J.* 7, 154–179
66 Aller, S.G. *et al.* (2009) Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science* 323, 1718–1722
67 Lagas, J.S. *et al.* (2009) Pharmacokinetic assessment of multiple ATP-binding cassette transporters: the power of combination knockout mice. *Mol. Interv.* 9, 136–145
68 Warren, M.S. *et al.* (2009) Comparative gene expression profiles of ABC transporters in brain microvessel endothelial cells and brain in five species including human. *Pharmacol. Res.* 59, 404–413
69 Clark, D.E. (2003) *In silico* prediction of blood–brain barrier permeation. *Drug Discov. Today* 8, 927–933
70 Yang, C. *et al.* (2001) Prodrug-based optimal drug delivery via membrane transporter/receptor. *Expert Opin. Biol. Ther.* 1, 159–175
71 Majumdar, S. *et al.* (2004) Membrane transporter/receptor-targeted prodrug design: strategies for human and veterinary drug development. *Adv. Drug Deliv. Rev.* 56, 1437–1452
72 Pavan, B. *et al.* (2008) Progress in drug delivery to the central nervous system by the prodrug approach. *Molecules* 13, 1035–1065
73 Gynther, M. *et al.* (2008) Large neutral amino acid transporter enables brain drug delivery via prodrugs. *J. Med. Chem.* 51, 932–936
74 Gynther, M. *et al.* (2009) Glucose promoiety enables glucose transporter mediated brain uptake of ketoprofen and indomethacin prodrugs in rats. *J. Med. Chem.* 52, 3348–3353
75 Irvine, J.D. *et al.* (1999) MDCK (Madin–Darby canine kidney) cells: a tool for membrane permeability screening. *J. Pharm. Sci.* 88, 28–33
76 Kawazu, K. *et al.* (2006) Transport of acebutolol through rabbit corneal epithelium. *Biol. Pharm. Bull.* 29, 846–849
77 Wada, S. *et al.* (2000) Rat multispecific organic anion transporter 1 (rOAT1) transports zidovudine, acyclovir, and other antiviral nucleoside analogs. *J. Pharmacol. Exp. Ther.* 294, 844–849
78 Bagwell, E.E. *et al.* (1989) Stereoselective uptake of atenolol into storage granules isolated from PC12 cells. *J. Pharmacol. Exp. Ther.* 249, 476–482
79 Ross, S.B. and Kelder, D. (1976) Active transport of 3H-bretylium in the rat vas deferens *in vitro*. *Acta Physiol. Scand.* 97, 209–221
80 Batmanglidj Eckhardt, S. *et al.* (1977) Influence of desipramine on the uptake and efflux of radiolabelled bretylium and bethanidine in the adventitial and media-intimal layers of rabbit aortic strips. *Blood Vessels* 14, 303–317
81 Hosotani, T. and Misu, Y. (1977) Sodium sensitive active transport of bretylium into adrenergic neurons for the development of blockade in rabbit ileum. *Arch. Int. Pharmacodyn. Ther.* 226, 235–245
82 Hemauer, S.J. *et al.* (2010) Role of transporter-mediated efflux in the placental biodisposition of bupropion and its metabolite, OH–bupropion. *Biochem. Pharmacol.* 80, 1080–1086
83 Kågedal, M. *et al.* (2007) A study of organic acid transporter mediated pharmacokinetic interaction between NXY-059 and cefuroxime. *J. Clin. Pharmacol.* 47, 1043–1048
84 Hart, L.G. and Schanker, L.S. (1966) Active transport of chlorothiazide into bile. *Am. J. Physiol.* 211, 643–646
85 Lee, L.J. *et al.* (1988) Renal transport kinetics of chlorothiazide in the isolated perfused rat kidney. *J. Pharmacol. Exp. Ther.* 247, 203–208

86 Uchino, H. *et al.* (2002) Transport of amino acid-related compounds mediated by L-type amino acid transporter 1 (LAT1): insights into the mechanisms of substrate recognition. *Mol. Pharmacol.* 61, 729–737
87 Urban, T.J. *et al.* (2008) Effects of genetic variation in the novel organic cation transporter, OCTN1, on the renal clearance of gabapentin. *Clin. Pharmacol. Ther.* 83, 416–421
88 Gross, S.R. *et al.* (1968) The active transport of cortisol by mouse fibroblasts growing *in vitro*. *Biochem. Biophys. Res. Commun.* 32, 66–72
89 Gross, S.R. *et al.* (1970) The outward transport of cortisol by mammalian cells *in vitro*. *J. Cell Biol.* 44, 103–114
90 Morita, N. *et al.* (2005) Functional involvement of rat organic anion transporter 2 (Slc22a7) in the hepatic uptake of the nonsteroidal anti-inflammatory drug ketoprofen. *Drug Metab. Dispos.* 33, 1151–1157
91 Noiraud, N. *et al.* (2001) Identification of a mannitol transporter, AgMaT1, in celery phloem. *Plant Cell* 13, 695–705
92 Opačić, M. *et al.* (2010) Localization of the substrate-binding site in the homodimeric mannitol transporter, EII^{mtl}, of *Escherichia coli*. *J. Biol. Chem.* 285, 25324–25331
93 Chen, T.C. *et al.* (1996) Cellular uptake and transport of methylprednisolone at the blood–brain barrier. *Neurosurgery* 38, 348–354
94 Oka, A. *et al.* (2002) Secretory transport of methylprednisolone possibly mediated by P-glycoprotein in Caco-2 cells. *Biol. Pharm. Bull.* 25, 393–396
95 Potschka, H. and Löscher, W. (2001) *In vivo* evidence for P-glycoprotein-mediated transport of phenytoin at the blood-brain barrier of rats. *Epilepsia* 42, 1231–1240
96 van Vliet, E.A. *et al.* (2006) Inhibition of the multidrug transporter P-glycoprotein improves seizure control in phenytoin-treated chronic epileptic rats. *Epilepsia* 47, 672–680
97 Zhang, C. *et al.* (2010) *In vitro* concentration dependent transport of phenytoin and phenobarbital, but not ethosuximide, by human P-glycoprotein. *Life Sci.* 86, 899–905
98 Ogihara, T. *et al.* (2004) Functional characterization of active transport of progesterone to adrenal cells. *J. Pharm. Pharmacol.* 56, 79–84
99 Wang, Y. *et al.* (2010) Stereoselective transport and uptake of propranolol across human intestinal Caco-2 cell monolayers. *Chirality* 22, 361–368
100 Sharifi, N. *et al.* (2008) A polymorphism in a transporter of testosterone is a determinant of androgen independence in prostate cancer. *BJU Int.* 102, 617–621
101 Cacini, W. and Myre, S.A. (1985) Uptake of trimethoprim by renal cortex. *Biochem. Pharmacol.* 34, 3483–3488
102 Ballatori, N. *et al.* (2005) OSTalpha-OSTbeta: a major basolateral bile acid and steroid transporter in human intestinal, renal, and biliary epithelia. *Hepatology* 42, 1270–1279
103 VanWert, A.L. *et al.* (2007) Organic anion transporter 3 (Oat3/Slc22a8) knockout mice exhibit altered clearance and distribution of penicillin G. *Am. J. Physiol. Renal Physiol.* 293, F1332–1341
104 Tamai, I. *et al.* (1997) 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
105 Tsuji, A. (1999) Tissue selective drug delivery utilizing carrier-mediated transport systems. *J. Control. Release* 62, 239–244
106 Bailey, P.D. *et al.* (2000) How to make drugs orally active: a substrate template for peptide transporter PepT1. *Angew. Chem. Int.* 39, 505–508
107 Bailey, P.D. *et al.* (2006) Affinity prediction for substrates of the peptide transporter PepT1. *Chem. Commun.* 323–325
108 Rubio-Aliaga, I. and Daniel, H. (2008) Peptide transporters and their roles in physiological processes and drug disposition. *Xenobiotica* 38, 1022–1042
109 Frazer, K.A. *et al.* (2009) Human genetic variation and its contribution to complex traits. *Nat. Rev. Genet.* 10, 241–251
110 Moore, J.H. *et al.* (2010) Bioinformatics challenges for genome-wide association studies. *Bioinformatics* 26, 445–455
111 Yang, J. *et al.* (2010) Common SNPs explain a large proportion of the heritability for human height. *Nat. Genet.* 42, 565–569
112 Sakaeda, T. *et al.* (2004) Pharmacogenetics of drug transporters and its impact on the pharmacotherapy. *Curr. Top. Med. Chem.* 4, 1385–1398
113 Tien, H.T. (1974) *Bilayer Lipid Membranes (BLM): Theory and Practice*. Marcel Dekker
114 Parsegian, A. (1969) Energy of an ion crossing a low dielectric membrane: solutions to four relevant electrostatic problems. *Nature* 221, 844–846
115 Weaver, J.C. and Chizmadzhev, Y.A. (1996) Theory of electroporation: a review. *Bioelectrochem. Bioenergy* 41, 135–160
116 Neumann, E. *et al.* (1999) Fundamentals of electroporative delivery of drugs and genes. *Bioelectrochem. Bioenergy* 48, 3–16
117 Leontiadou, H. *et al.* (2004) Molecular dynamics simulations of hydrophilic pores in lipid bilayers. *Biophys. J.* 86, 2156–2164
118 Leontiadou, H. *et al.* (2007) Ion transport across transmembrane pores. *Biophys. J.* 92, 4209–4215
119 Cohen, B.E. (1975) The permeability of liposomes to nonelectrolytes. II. The effect of nystatin and gramicidin A. *J. Membr. Biol.* 20, 235–268
120 Cao, Y. *et al.* (2008) Development of structure–lipid bilayer permeability relationships for peptide-like small organic molecules. *Mol. Pharm.* 5, 371–388
121 Salter, G.J. and Kell, D.B. (1995) Solvent selection for whole cell biotransformations in organic media. *CRC Crit. Rev. Biotechnol.* 15, 139–177
122 Broadhurst, D. and Kell, D.B. (2006) Statistical strategies for avoiding false discoveries in metabolomics and related experiments. *Metabolomics* 2, 171–196
123 Saitoh, R. *et al.* (2004) Correction of permeability with pore radius of tight junctions in Caco-2 monolayers improves the prediction of the dose fraction of hydrophilic drugs absorbed by humans. *Pharm. Res.* 21, 749–755
124 Leong, M.K. *et al.* (2009) Development of a new predictive model for interactions with human cytochrome P450 2A6 using pharmacophore ensemble/support vector machine (PhE/SVM) approach. *Pharm. Res.* 26, 987–1000
125 Hagenbuch, B. and Meier, P.J. (2004) Organic anion transporting polypeptides of the OATP/SLC21 family: phylogenetic classification as OATP/SLCO superfamily, new nomenclature and molecular/functional properties. *Pflügers Arch.* 447, 653–665
126 Didziapetris, R. *et al.* (2003) Classification analysis of P-glycoprotein substrate specificity. *J. Drug Target* 11, 391–406
127 Schinkel, A.H. and Jonker, J.W. (2003) Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. *Adv. Drug Deliv. Rev.* 55, 3–29
128 Cianchetta, G. *et al.* (2005) A pharmacophore hypothesis for P-glycoprotein substrate recognition using GRIND-based 3D-QSAR. *J. Med. Chem.* 48, 2927–2935
129 Khersonsky, O. and Tawfik, D.S. (2010) Enzyme promiscuity: a mechanistic and evolutionary perspective. *Annu. Rev. Biochem.* 79, 471–505
130 Seeman, P. (1972) The membrane actions of anesthetics and tranquilizers. *Pharmacol. Rev.* 24, 583–655
131 Franks, N.P. and Honoré, E. (2004) The TREK K_{2P} channels and their role in general anaesthesia and neuroprotection. *Trends Pharmacol. Sci.* 25, 601–608
132 Heurteaux, C. *et al.* (2004) TREK-1, a K+ channel involved in neuroprotection and general anesthesia. *EMBO J.* 23, 2684–2695
133 Franks, N.P. (2006) Molecular targets underlying general anaesthesia. *Br. J. Pharmacol.* 147 (Suppl. 1), S72–S81
134 Grasshoff, C. *et al.* (2006) Anaesthetic drugs: linking molecular actions to clinical effects. *Curr. Pharm. Des.* 12, 3665–3679
135 Wallner, M. *et al.* (2006) 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. U.S.A.* 103, 8540–8545
136 Eckenhoff, R. *et al.* (2008) From anesthetic mechanisms research to drug discovery. *Clin. Pharmacol. Ther.* 84, 144–148
137 Bonin, R.P. and Orser, B.A. (2008) $GABA_A$ receptor subtypes underlying general anesthesia. *Pharmacol. Biochem. Behav.* 90, 105–112
138 Franks, N.P. (2008) General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. *Nat. Rev. Neurosci.* 9, 370–386
139 Garcia, P.S. *et al.* (2010) General anesthetic actions on GABA(A) receptors. *Curr. Neuropharmacol.* 8, 2–9
140 Bédrine-Ferran, H. *et al.* (2004) Transcriptome variations in human CaCo-2 cells: a model for enterocyte differentiation and its link to iron absorption. *Genomics* 83, 772–789
141 Traka, M. *et al.* (2005) Transcriptome analysis of human colon Caco-2 cells exposed to sulforaphane. *J. Nutr.* 135, 1865–1872
142 Chicault, C. *et al.* (2006) Iron-related transcriptomic variations in CaCo-2 cells, an *in vitro* model of intestinal absorptive cells. *Physiol. Genomics* 26, 55–67
143 Aubry, M. *et al.* (2008) Iron-related transcriptomic variations in Caco-2 cells: *in silico* perspectives. *Biochimie* 90, 669–678
144 Sun, D. *et al.* (2002) Comparison of human duodenum and Caco-2 gene expression profiles for 12,000 gene sequences tags and correlation with permeability of 26 drugs. *Pharm. Res.* 19, 1400–1416
145 Anderle, P. *et al.* (2004) Intestinal membrane transport of drugs and nutrients: genomics of membrane transporters using expression microarrays. *Eur. J. Pharm. Sci.* 21, 17–24
146 Landowski, C.P. *et al.* (2004) Transporter and ion channel gene expression after Caco-2 cell differentiation using 2 different microarray technologies. *AAPS J.* 6, e21
147 Pshezhetsky, A.V. *et al.* (2007) Subcellular proteomics of cell differentiation: quantitative analysis of the plasma membrane proteome of Caco-2 cells. *Proteomics* 7, 2201–2215
148 Ahlin, G. *et al.* (2009) Endogenous gene and protein expression of drug-transporting proteins in cell lines routinely used in drug discovery programs. *Drug Metab. Dispos.* 37, 2275–2283

149 Chen, Y.S. *et al.* (2010) Proteomic profiling of MDCK plasma membranes reveals Wnt-5a involvement during oncogenic H-Ras/TGF-{beta}-mediated epithelial–mesenchymal transition. *Mol. Cell. Proteomics* 10 M110.001131
150 Berglund, L. *et al.* (2008) A genecentric Human Protein Atlas for expression profiles based on antibodies. *Mol. Cell. Proteomics* 7, 2019–2027
151 Pontén, F. *et al.* (2008) The Human Protein Atlas: a tool for pathology. *J. Pathol.* 216, 387–393
152 Koepsell, H. and Endou, H. (2004) The SLC22 drug transporter family. *Pflügers Arch.* 447, 666–676
153 Hilgendorf, C. *et al.* (2007) Expression of thirty-six drug transporter genes in human intestine, liver, kidney, and organotypic cell lines. *Drug Metab. Dispos.* 35, 1333–1340
154 Fröhlich, O. *et al.* (2004) Urea transport in MDCK cells that are stably transfected with UT-A1. *Am. J. Physiol. Cell. Physiol.* 286, C1264–C1270
155 O'Brien, P.J. and Herschlag, D. (1999) Catalytic promiscuity and the evolution of new enzymatic activities. *Chem. Biol.* 6, R91–R105
156 Nobeli, I. *et al.* (2009) Protein promiscuity and its implications for biotechnology. *Nat. Biotechnol.* 27, 157–167
157 Ogston, A.G. (1948) Interpretation of experiments on metabolic processes, using isotopic tracer elements. *Nature* 162, 963
158 Mesecar, A.D. and Koshland, D.E. (2000) A new model for protein stereospecificity. *Nature* 403, 614–615
159 Koshland, D.E. (2002) Case of the hidden assumptions. *Biochem. Mol. Biol. Educ.* 30, 27–29
160 Hediger, M.A. *et al.* (2004) The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins. Introduction. *Pflügers Arch.* 447, 465–468
161 Barts, P.W.J.A. *et al.* (1980) Uptake of the lipophilic cation dibenzyldimethylammonium into *Saccharomyces cerevisiae*. Interaction with the thiamine transport system. *Biochim. Biophys. Acta* 597, 125–136
162 Kola, I. and Landis, J. (2004) Can the pharmaceutical industry reduce attrition rates? *Nat. Rev. Drug Discov.* 3, 711–715
163 Leeson, P.D. and Springthorpe, B. (2007) The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat. Rev. Drug Discov.* 6, 881–890
164 Hopkins, A.L. (2008) Network pharmacology: the next paradigm in drug discovery. *Nat. Chem. Biol.* 4, 682–690
165 Keserü, G.M. and Makara, G.M. (2009) The influence of lead discovery strategies on the properties of drug candidates. *Nat. Rev. Drug Discov.* 8, 203–212
166 Palmieri, F. and Pierri, C.L. (2010) Structure and function of mitochondrial carriers – role of the transmembrane helix P and G residues in the gating and transport mechanism. *FEBS Lett.* 584, 1931–1939