Review

Membrane transporter engineering in industrial biotechnology and whole cell biocatalysis

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Because they mainly do not involve chemical changes, membrane transporters have been a Cinderella subject in the biotechnology of small molecule production, but this is a serious oversight. Influx transporters contribute significantly to the flux towards product, and efflux transporters ensure the accumulation of product in the much greater extracellular space of fermentors. Programmes for improving biotechnological processes might therefore give greater consideration to transporters than may have been commonplace. Strategies for identifying important transporters include expression profiling, genome-wide knockout studies, stress-based selection, and the use of inhibitors. In addition, modern methods of directed evolution and synthetic biology, especially those effecting changes in energy coupling, offer huge opportunities for increasing the flux towards extracellular product formation by transporter engineering.

The control of flux in biochemical networks

In any complex biochemical network, all steps contribute to the control of the flux through a particular pathway or even that catalysed by a specific enzyme, but some steps exert a greater degree of control on the fluxes of interest than do others. Although all steps contribute to flux control, in devising strategies to increase such fluxes [1], it is wise to pay special attention to the particular steps that exert the greatest control over the pathway. In many cases, and especially for those systems involving xenobiotics, these steps include the cellular transporters that catalyse the influx of substrates and the efflux of products or potentially cytotoxic compounds. In other words, these steps are typically significantly rate-controlling. A quantitative measure of the extent of this rate or flux control is encapsulated in the flux-control coefficient (Box 1). With a relative density barely greater than 1, even at 100 g.l^{-1} wet cell concentration most of the volume of a fermentor is extracellular; thus, to maximise volumetric productivity it will be desirable to ensure that cells excrete the products of interest [2].

But doesn't stuff just diffuse into and out of cells unaided?

There is a surprisingly widespread view in the pharmaceutical industry, starting with the relevant textbooks [3], that the main means by which most xenobiotics (e.g., drugs) enter and exit cells is simply by diffusing passively across the lipid bilayer portion of cell membranes down their concentration gradients and according to their lipophilicity (log P or log D; see Glossary). If this were the case we should have little to say in this review, but it is not [4–14]. The main means by which small molecules cross biological cell membranes is through genetically encoded, proteinaceous transporter molecules, and this gives the cells, and the biotechnologist, important means by which to control and influence the process. The first thing to know, then, is qualitative [15,16]: which small molecules use which transporters? While we shall mainly consider microbes and fermentations, the principles we enunciate are general, and we recognise their role in the metabolic engineering of plants where especially vacuolar, peroxisomal, chloroplast, and root transporters can exert significant flux control.

'Unexpected' transporters for the uptake of uncharged nonelectrolyte nutrients and other small molecules

Based on the 19th-century studies of Overton, who showed a close correlation between the logarithm of the rate of

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Glossary

The logarithm of the distribution coefficient (log D): D is the ratio of the sum of the concentrations of all forms of a compound (ionised plus non-ionised) in each of two phases, typically 1-octanol and an equilibrated aqueous buffer, whose pH must be specified.

The logarithm of the partition coefficient (log P): P is a measure of the hydrophobicity of a molecule; log P is the logarithm (base 10) of the ratio of the concentration of a solute molecule in an organic solvent, usually 1-octanol, to that of the non-ionised form of the same molecule in water.

Box 1. Metabolic control and flux balance analysis

Imagine a metabolic network or pathway in which we vary the concentration of an enzyme E by an amount ΔE, with a concomitant change in the flux of interest ΔJ. In the limit of small changes, this becomes dJ/dE. By normalising these changes to the flux and enzyme concentration at the operating point (J,E) we can obtain a dimensionless quantity C^J_E, the flux-control coefficient of enzyme E on the flux of interest J, which describes in quantitative terms the extent to which that enzyme controls the flux. C^J_E is equivalent to a local sensitivity coefficient. If C^J_E is 0 then the enzyme exerts no flux control, while if it is 1 then it is completely flux-controlling. The sum of the flux-control coefficients for all enzymes on a particular flux is 1. This means that most enzymes have small flux-control coefficients, and even a 50% knockdown typically has a limited effect on flux (Figure I). Thus, to have major effects, one should seek to use haploid organisms or homozygous diploid deletants [9,66]. A related concentration-control summation theorem shows that the sum of the concentration control coefficients = 0. Note that the flux-control coefficient is not constant – at a different operating point it would be higher or lower as flux control shifts among different parts of the network. The thesis in this review is that, where the flux-control coefficients of transporters are determined, they will often be found to be larger than those of other enzymes, providing suitable suggestions for transporter engineering.

Flux balance analysis describes a series of techniques for estimating relative metabolic fluxes without the requirement to know any of the kinetics of the participating enzymes. All it requires is knowledge of the stoichiometries of the participating reactions, the molecular identities of the reactants and products themselves, and an objective function that one is trying to optimise. Linear programming techniques can then be used to optimise the latter. The stoichiometries, including mass, charge, and energy balances, provide a very effective series of constraints to determine the possible fluxes; however, the objective function is more problematic. Typically, biomass is used (i.e., the rate of biomass formation), with biomass being encoded as a 'molecule' with a non-integer empirical formula. It is desirable to add further constraints, for example by confining specific fluxes to restricted ranges or finding flux distributions that best correlate with expression profiles [63].
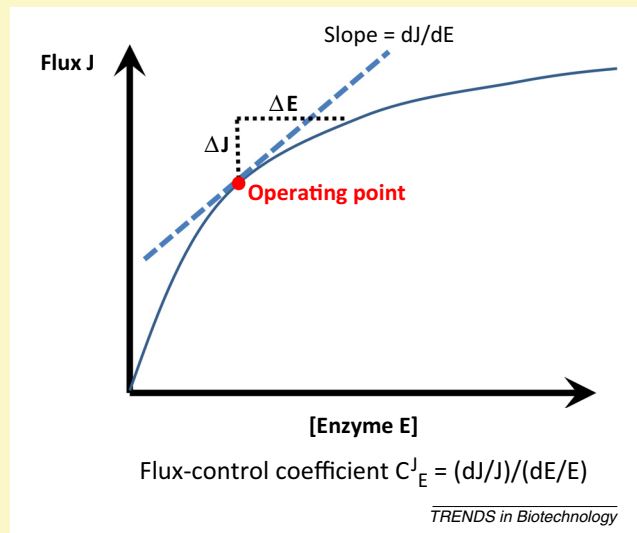

$$C^J_E = (dJ/J)/(dE/E)$$

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Figure I. Flux as a function of enzyme concentration.

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cellular uptake of non-electrolytes and their log P values, it had been widely assumed that small, uncharged molecules could permeate freely across the bilayer portion of biological membranes (even though it is well known that glucose and other sugars do not). However, it is now recognised that this is not at all the case, with transporters having been found (and required) for the uptake of many small, uncharged substances such as alkanes [17], ammonia (NH_3) [18], carbon dioxide (CO_2) [19,20], ethanolamine [21], fatty acids [22], glycerol [23], hydrogen peroxide (H_2O_2) [24], hydroxyurea [25], nitric oxide [26], (di)oxygen [27], urea [28,29], and even water [23,30]. The last was a finding for which Peter Agre received the 2003 Nobel Prize [31,32].

It has long been known that acetate enters cells mainly in its uncharged form, as acetic acid. This may be determined by osmotic swelling experiments [33], but these kinds of experiments do not say anything about the mechanism by which it enters: bilayer diffusion or a transporter. However, it is now known in the important amino acid producer *Corynebacterium glutamicum* that even the uptake of electroneutral acetic acid involves the use of a specific transporter [34].

Ethanol is another small nonelectrolyte of much biotechnological interest, and it is desirable to increase its export from producer cells [35]. It is not yet entirely certain which transporters are responsible for this, but the ATP-binding cassette (ABC) transporter [36] Pdr18 [37] and the glyceroaquaporin Fps1 [38] possess properties that might be consistent with such a role, although other mechanisms may also be involved [39]. While we later discuss in more detail export (efflux) transporters of molecules not normally produced by the host, this section leads naturally to a discussion of those that are known to be involved in the secretion of metabolites that the host naturally produces.

Some useful case histories from classical fermentations

A notable example of the role of transporters in improving the yield of an important fermentation product (more than 2M tonnes per annum) comes from the history of the glutamate fermentation carried out using various coryneform bacteria, notably *C. glutamicum* [40]. Following the initial discovery of the fermentative production of glutamate, various empirical treatments in the 1960s and 1970s were found to enhance the efflux of glutamate from producer strains. It was later established that this was due to a change in membrane tension that activated a mechanosensitive glutamate efflux pump encoded by a gene called *NCgl*1221, a homologue of the *Escherichia coli ygg*B gene, now known as *msc*S, the mechanosensitive channel of small conductance. Similar efflux pumps are now known to be involved in the export of products during a variety of other amino acid fermentations [41], such as those for lysine, isoleucine, threonine, methionine, and others [2].

Why would a cell export its metabolites?

One may wonder (from an evolutionary perspective) why bacteria see fit to excrete important nutrients or metabolites, often at fast rates. The most persuasive general explanation [42] is to the effect that soil bacteria (such as corynebacteria) that have experienced drought and are hit by a raindrop (a common stress), experience truly massive osmotic stresses or turgor pressures that can only

realistically be dealt with by a virtually instantaneous excretion of internal osmolytes. This excretion is catalysed by a mechanically-sensitive, membrane-triggered osmoregulatory process which can also occur in plants [43]. Such a role for the glutamate exporter, and one may suppose for other such exporters, is consistent with the similar role of its *E. coli* homologue [44]. Indeed, since their initial discovery in bacteria, a considerable number of such mechanosensitive, turgor-regulating exporters are now known [45], including seven (in two families) in *E. coli*.

Citric acid production

In a similar vein, the large-scale (well over 1M tonnes per annum) fermentative production of the tricarboxylate citric acid by the fungus *Aspergillus niger* involves active export of the product from the producer strain using a proton-symporting transporter [46]. Dicarboxylate titre is also improved by enhancing dicarboxylate efflux transporters [47].

Biomass production

In some fermentations the biomass itself is the product, and it is of interest to know what role transporters may play in controlling growth rate more generally. By using a pHauxostat to select strains of the fast-growing yeast *Kluyveromyces marxianus* for even faster growth [48], it was possible to evolve one that could grow up to 30% faster than the starting strain. This decrease in doubling time to 52 min, apparently the fastest reported for a eukaryote, was accompanied by an increase in surface area of some 40% at essentially constant volume. These results imply that membrane processes, such as substrate uptake, were most limiting to growth rate. Indeed, 80% of the growth rate increase was ascribed to membrane processes [48]. Continuous selection is also an excellent strategy for selecting strains resistant to toxins such as solvents [49,50], especially in turbidostats [51] in which growth rate can be measured online [52].

In this context is noteworthy that a high-throughput screen [53] of heterozygous deletants of diploid *S. cerevisiae* identified 145 transporter-encoding genes that exerted significant control over growth rate (so-called high-flux-control or HFC genes) in turbidostat culture. Ninety of these genes had a haploinsufficient (HI) phenotype – that is, they reduced the maximum growth rate of yeast when present in only one copy in a diploid – while the remainder had a haploproficient (HP) phenotype, increasing the growth rate when in the heterozygous state. These HFC genes included those encoding plasma membrane transporters, but also genes specifying proteins involved in transporting ions and metabolites into subcellular organelles, especially the mitochondria and the vacuole. Amongst the HI genes were those encoding plasma membrane transporters of metals (particularly iron and zinc), organic acids (including amino acids), ammonium, phosphate, sulphate, vitamins, sugars (including glucose) and sugar alcohols, and also the aquaporin gene, *AQY1*. This group of HI genes also includes four that encode drug efflux pumps. Given the discussion of efflux transporters, above, it would seem sensible for biotechnologists and synthetic biologists to pay attention not only to transporters of important nutrients but also to those responsible for the efflux of potentially toxic products of metabolism, such as ethanol and other biofuels.

Transcriptome-based strategies for determining transporter-mediated activities

Virtually since its inception, it has been clear that genome-wide expression profiling at the level of the transcriptome provides an excellent strategy for identifying which gene products may be pertinent for particular biological processes. This applies equally to the role of transporters in biotechnology. Thus, the availability of the *Penicillium chrysogenum* genome made it possible [54] to compare the expression profiles of low- and high-producing strains, finding a considerable enhancement in transporter expression in the high-producers, again implying strongly that enhanced transporter expression could drive increased fluxes. Although, in general terms, the expression of an individual gene does not necessarily correlate with the productivity of a fermentation, and certainly not over a wide range because of changes in the distribution of flux control (Box 1), genome-wide trawls relating expression to activity can be highly beneficial, especially for metabolic networks. This is because metabolic transformations are subject to strict stoichiometric controls (no 'alchemy' is allowed).

Flux balance analysis

While the counsel of perfection in genome-scale metabolic modelling includes mechanistic details of every enzymatic step, which can then be turned into an ordinary differential equation (ODE) model that may be used to model or predict all the fluxes and concentrations of interest, we very rarely have sufficient of the kinetic parameters to do this [55–58]. However, the stoichiometric constraints alluded to above mean that the methods of flux balance analysis [58–60] (Box 1) may be used to attempt to predict the fluxes of interest. As part of a strategy to minimise the number of possible flux patterns that can explain the observable data [61,62], we have found [63] that absolute transcriptomics provides a valuable surrogate for the flux through each step.

The distribution of expression levels for transporter and non-transporter genes were determined (but not shown) in a recent study [63] of a yeast strain growing at 85% of its maximum growth rate. The transporter nature of the genes was assessed (Figure 1) by the present version of the yeast metabolic network. As judged by their median levels, as well as by 5000 permutations, there is a significantly lower level of expression ($P < 0.0004$) of transporter genes (19.3 transcripts per cell) than of non-transporter genes (31.7). This is not inconsistent with the fact that cellular membranes, as 2D structures, possess a more limited amount of real estate for incorporating transporters and other membrane proteins than do the 3D intracellular spaces. The 'surfaceome', including SLCs, is also the most variable between different and differentiated cells [64].

Detecting relevant uptake transporter genes through genome-wide knockout analyses

Although individual genes were classically and typically discovered individually, it is now possible to extend the
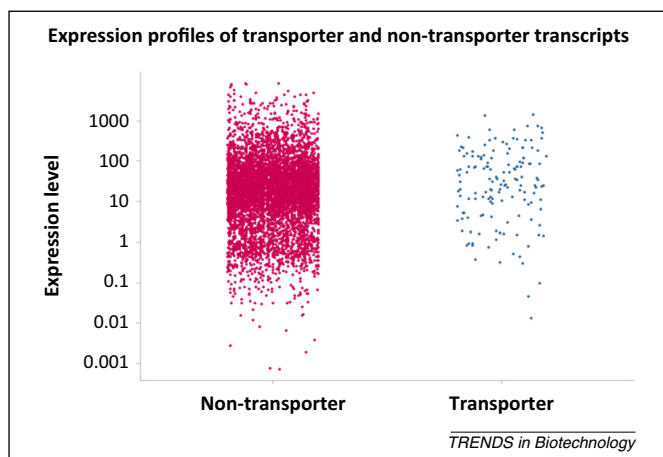
Figure 1. Expression profiles of 151 transporter and 6373 non-transporter transcripts in baker's yeast. Data from [63]. Note that fewer transport reactions in the model (327/1079, 30.3%) have associated genes (hence transcripts) than do all other metabolic reactions (1983/2255, 87.9%).

analysis of transporter roles to the genomic level (Box 2), and there are useful online databases focussing on transporters (Table 1). The sole requirements are for a suitable variation in the extent of expression of different enzymes in different strains, and this is most conveniently achieved using single gene knockouts combined with a means of selecting the phenotype of interest such as growth selection (Figure 2). Thus, in the case of *S. cerevisiae*, we were able to exploit the barcoded yeast deletion mutant collection to identify transporters for 18 out of 26 drugs tested [9]. Most had multiple transporters. For the eight where we could not detect which transporters were used, this is likely because there were simply too many transporters, and removing only one did not provide sufficient selectivity.

That study [9] used haploid strains (Box 1) and a purpose-designed microarray chip, but nowadays it is recognised that deep sequencing is much more effective and reliable [65]. Thus, in an exciting development, a near-haploid human cell line (KBM7) with a retroviral gene-trap was used to demonstrate that only a single transporter (called SLC35F2) is responsible for the uptake of the cytotoxic anticancer drug candidate sepantronium bromide (also known as YM155) into these cells [66]. Clearly, these kind of methods may be applied to any system for which cells that have or have not taken up a particular drug may be discriminated and separated (e.g., by cell sorting [67]), and then identified genetically. It is worth stressing that this kind of experiment would not 'work' – in other words, return any hits – if bilayer diffusion were the dominant mechanism of transmembrane transport. Put another way, it would indeed seem from such experiments that, for drug transport into cells, phospholipid bilayer diffusion is negligible [13,14].

In theory these kinds of knockout strategies could also be used to select strains with knockouts in efflux transporters (if such exist), via their greater sensitivity to a compound, but positive selection methods for resistance are always more reliable (Box 3 and Figure 2). While it has already been noted the deletion of only one of the two copies of a gene can be sufficient to produce a significant reduction in growth rate [53], it was also found that the removal of two genes,

PDR10 and *PDR12*, encoding ABC multidrug transporters, actually enhanced growth rate. Thus, further investigation of the substrate preferences of these apparently promiscuous efflux pumps might pay dividends in both biotechnology and drug design.

Genes for efflux transporters

As well as the genes for efflux transporters described above, there is considerable interest in the recognition that a chief cause of antibiotic resistance, a huge continuing and present problem [68], is the ability of microbes to pump out such molecules using 'multidrug resistance' (MDR) efflux transporters [69,70]. MDR pumps are often of wide specificity, for example, for lipophilic compounds, and increasing numbers of structures are becoming known [71]. Efflux transporters are of wider significance in medicine because, by removing toxins, they lower the intracellular concentrations. This can be good in the case of genuine toxicants [72] but less so when they encode phenotypic resistance, for example, to anticancer agents [73]. However, in biotechnology it is both desirable and possible to select for strains that are particularly resistant to

Table 1. Some databases with a focus on membrane transporters

Name	Focus/organism	URL
Bioparadigms SLC tables	Human	http://www.bioparadigms.org/slc/intro.htm
Caenorhabditis elegans solute transporter database	*C. elegans*	http://www.wormslc.org/
Drugbank	Human/drugs	http://drugbank.ca
Human intestinal transporter database	Human/drugs	Not apparently directly online; data are downloadable from each paper's supplementary information.
Human transporter database	Human	http://htd.cbi.pku.edu.cn
Transportal	Human/drug transport	http://bts.ucsf.edu/fdatransportal/
TransportDB	Comparative genomics of transporters	http://membranetransport.org/
Transporter classification database (TCDB)	IUBMB-approved transporter classifications	http://www.tcdb.org/
Transporter database TP-search	Human/drug uptake	http://www.tp-search.jp
TransportTP	Transporter prediction	http://bioinfo3.noble.org/transporter/
Yeast metabolome database	*S. cerevisiae*	http://www.ymdb.ca/
Yeast transport protein database (YTPdb)	*S. cerevisiae*	http://ytpdb.biopark-it.be/ytpdb/
Yeti: yeast transport information	*S. cerevisiae*	http://genolevures.org/yeti.html

stresses, including stresses from organic solvents [74] and/or from high intra- and/or extracellular product titres.

Indeed, it is precisely this kind of positive selection that can be used to our advantage in biotechnology. Thus, by seeking tolerance to added compounds, efflux transporters have been found for alkanes [17,75–79], arenes [80,81], short-chain alcohols [82,83], terpenoids [78], short-chain fatty acids [84,85], and long-chain fatty acids [86,87], while those for isoprene and isoprenoids are eagerly sought [88]. Unusual efflux transporters produced by microbes for specific purposes include one for FAD in *Shewanella oneidensis* [89], while virtually all free-living aerobes must and do secrete siderophores to permit them to effect iron uptake [90–93].

Transporter-mediated osmotic stress engineering
If cells are to accumulate soluble products to high titres, there will always be the danger of significant osmotic

Figure 2. One approach to genome-wide identification of transporters for a toxic drug. This evaluates the enrichment of survivors when the gene encoding the transporter for an appropriate concentration of a cytotoxic drug is knocked out, relative to other strains in which other genes may or may not be knocked out.

stresses as well as lowered water activities [94]. While these osmotic stresses can be relieved by the synthesis of so-called compatible solutes [95], such as betaine, another strategy includes their intracellular accumulation via uptake transporters [96]. *Corynebacterium glutamicum* provides an excellent example [97,98]. Note that inducing the synthesis of such compatible solutes can also be of value in the production of soluble and functional recombinant proteins [99,100].

Transporter engineering
Having established which transporters are important for the problem of interest, it is possible to improve them, typically by the methods of directed evolution [101–103]. These involve varying the primary sequence of the protein, and selecting those with improved properties, in an iterative manner. The variation in primary sequence is carried out by various forms of mutation and recombination, nowadays including the methods of synthetic biology in which we can control rather precisely which sequences are made by creating them at the DNA level by chemical synthesis [103–109]. The question then arises as to what kind of objective function we might seek (Box 4). We might wish to turn a concentrative uptake transporter into one that merely catalyses equilibration, in other words the efflux of product formed intracellularly. There is ample precedent for this loss of energy coupling, for example, in mutants of the normally concentrative lactose permease of *E. coli* [110] or of the mammalian intestinal di- and tripeptide transporter PepT1 (SLC15a1) [111], and – for influx of substances normally pumped out – of drug uptake via uncoupled variants of the LmrP 'efflux' transporter in lactobacilli [112]. By contrast, it was possible [113] to change a multidrug monovalent 'efflux' antiporter into one that used divalent ions. Thus there seems little doubt that we should be able to change the specificity [114], promiscuity [115], or detailed molecular transport pathways [116] of transporters by directed evolution as easily [117] as we can for other proteins [118]. Indeed, evidence for the selection of efflux transporters during the development of various amino acid fermentations was given
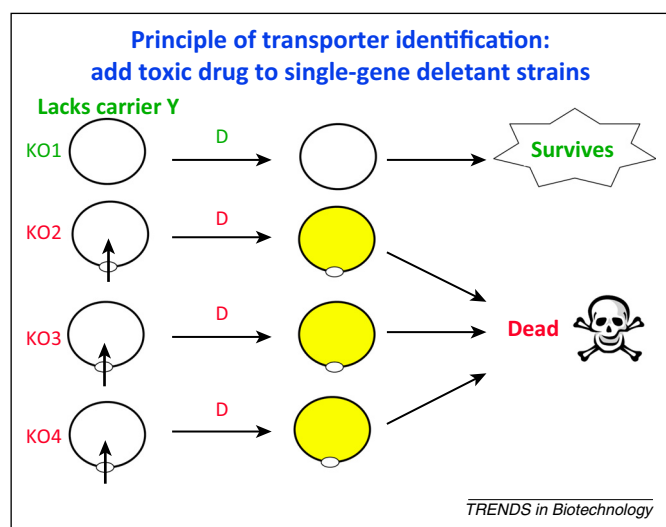
Box 3. Classical strategies for detecting the roles of particular cell membrane transporters

Originally, the determination of which transporters accounted for the uptake of particular nutrients or other compounds used classical genetic techniques, often obtaining mutants in transporter genes by selecting for resistance to cytotoxic structural analogues of those nutrients. For example, canavanine is a structural analogue of arginine that can be taken up by cells, including those of *Saccharomyces cerevisiae* [9] and humans, and is incorporated into proteins where it disrupts their function, thus proving cytotoxic. Such cytotoxic molecules, that bear structural similarities to intermediary metabolites, are known as antimetabolites, and antimetabolite molecules such as analogues of folate, nucleobases, and nucleosides (Figure I) continue to play a major role in cancer chemotherapy [145]. In yeast, the overwhelming bulk of canavanine uptake and, in mutants, resistance to it, is determined by the arginine transporter Can1p encoded by the gene *can1*. Strains lacking this gene function are, depending upon the precise metric, more than 100-fold more resistant to the antimetabolite than is the wild type [9], and of course the gene encoding the arginine transporter is explicitly named after its ability to encode resistance to this antimetabolite.
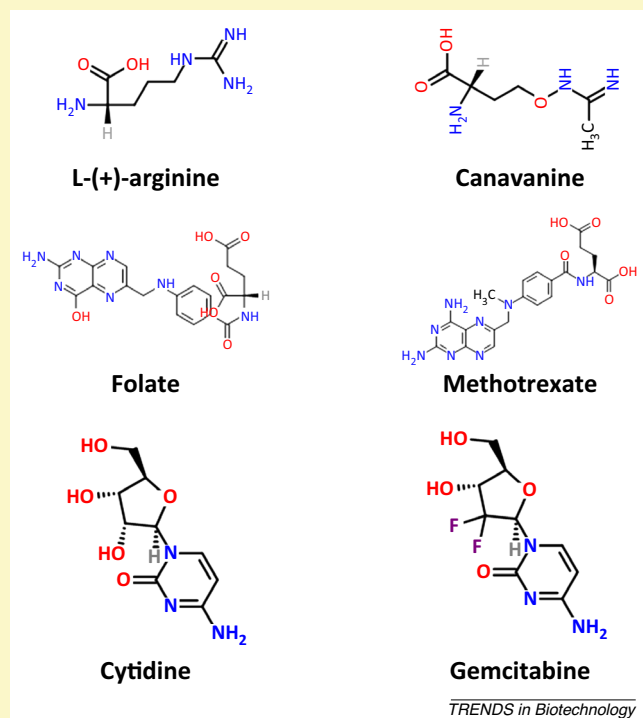

L-(+)-arginine

Canavanine

Folate

Methotrexate

Cytidine

Gemcitabine

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Figure I. Some antimetabolites that bear structural similarities to natural metabolites with which they compete for uptake transport (and intracellular activity).

Box 4. 'Influx' and 'efflux' transporters

Assessing the contributions of membrane proteins to the tolerance of stresses induced by fermentation or incubation conditions is also an important experimental approach to detecting 'efflux' transporters, a comment that leads us to note that thermodynamic principles mean that any transporter is theoretically reversible in its direction of operation, although for kinetic reasons connected with the Haldane relationship this may not appear to be the case. Thus, 'influx' and 'efflux' transporters refer to their normal direction of operation *in vivo*, and this is determined both by the thermodynamics and the mechanistic details of any energy coupling involved (Figure I). While our chief interest here is in identifying cases where transporters exert significant flux control, increasing numbers of 3D protein structures for transporters are becoming available [146], and these are beginning to allow calculation of their molecular mechanisms from first principles, based on molecular dynamics [147]. This will also, in time, assist in their rational redesign.
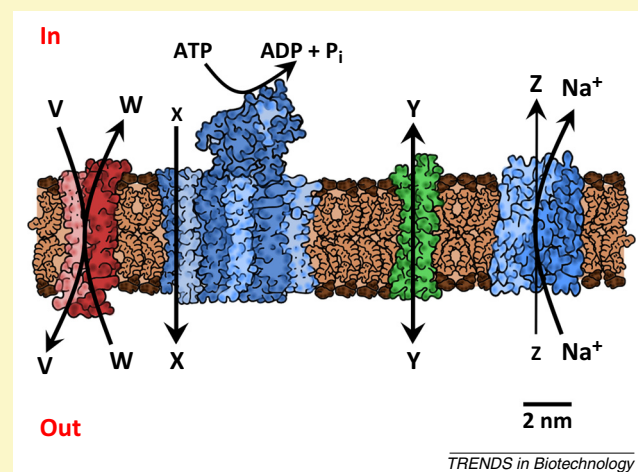

Figure I. An illustration of four types of transporters. V and W are transported in exchange for each other – if there is a concentration gradient of one, it will drive the transport (antiport) of the other. X is transported out of the cell, potentially against its concentration gradient, by a transporter that couples its transport activity to ATP hydrolysis. Y enters and exits the cell by facilitated diffusion (it is a uniporter), while Z is taken up concentratively in symport with a sodium ion (that descends its own concentration gradient). The terms 'active' (concentrative) and 'passive' (equilibrative) are best used solely to describe the thermodynamics, with no mechanism being implied unless stated [13]. The membrane is drawn approximately to scale, with a typical *in vivo* protein:lipid ratio of 3:1 by mass. Note too that there can be a highly-intimate interaction between specific lipids and transporter function such that changing the former may affect the latter.

above. Papers showing a gain-of-function of NCgl1221 to constitutive glutamate excretion [119,120] are of special note, indicating the potential for transporter engineering.

E. coli contains a (possibly) surprising number of efflux pumps, comprising one sixth of all its transporters [117], even for sugars. Indeed, in *E. coli* there are as many as 37 MDR transporters [121], most commonly from the Major Facilitator Superfamily [122]. Arguably, the main efflux transporters are AcrB [123,124], MdfA [125], EmrE [126,127], and MtdM [122,128]. Thus, and while n-alkanes are much less cytotoxic than many other organic solvents [129], a particularly nice example of the directed evolution of a

membrane protein for catalysing product efflux is the study of Foo and Leong [78], who evolved AcrB to drive improved efflux of the hydrocarbons n-octane and α-pinene from *E. coli* using selection against the toxicity of n-octanol (that was also presumably excreted) while Fisher *et al.* did the same for shorter-chain alcohols [82]. Mutations in several other genes, such as *lon*, *pro*V, *sox*S, and *mar*R, also act via AcrB to increase the solvent tolerance of *E. coli* [130,131]. Multidrug resistance transporters have also been used to export dipeptides [41] and arabinose [132] from *E. coli*, while NAD transporter engineering has been exploited to advantage in the whole cell biocatalytic production of dihydroxyacetone [133].

S. cerevisiae contains 28 members of the Major Facilitator Superfamily of multidrug efflux pumps and at least six members of the ABC multidrug transporter family

[134–136]. All these efflux pumps reside in the plasma membrane, while Vmr1p is a vacuolar membrane protein. While the importance of the plasma membrane pumps in drug resistance (notably to azoles) in pathogenic yeasts is well recognised [137], any possible role in the efflux of diesel fuels from engineered yeast seems not to have been considered [138] or, at least, not published.

Concluding remarks and future perspectives

In this short review we have sought to summarise some of the evidence that membrane transporters represent somewhat underutilised yet excellent targets for the purposes of strain improvement in biotechnology. Some of the evidence comes from more classical fermentations where such changes 'emerged' from undirected (mutation and selection) strain improvement programmes, while more recently there are examples of more deterministic strategies based on metabolic engineering. We anticipate many major improvements in the future as the powerful techniques of directed evolution are brought to bear on selected membrane transporters, especially those catalysing concentrative efflux of the desired product. Much as with pharmaceutical drug transporters [13], what we need now are good, predictive, quantitative structure–activity relationship models that will help to determine the activity of any transporter sequence for any drug. Such models will bring us truly closer to the era of 'designer transporters for biotechnology'.

References

1 Kell, D.B. and Westerhoff, H.V. (1986) Metabolic control theory: its role in microbiology and biotechnology. *FEMS Microbiol. Rev.* 39, 305–320

2 Van Dyk, T.K. (2008) Bacterial efflux transport in biotechnology. *Adv. Appl. Microbiol.* 63, 231–247

3 Gabrielsson, J. and Hjorth, S. (2012) *Quantitative Pharmacology: An Introduction to Integrative Pharmacokinetic–Pharmacodynamic Analysis*, Apotekarsocieteten

4 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

5 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

6 Dobson, P. et al. (2009) Implications of the dominant role of cellular transporters in drug uptake. *Curr. Top. Med. Chem.* 9, 163–184

7 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

8 Kell, D.B. et al. (2011) Pharmaceutical drug transport: the issues and the implications that it is essentially carrier-mediated only. *Drug Discov. Today* 16, 704–714

9 Lanthaler, K. et al. (2011) Genome-wide assessment of the carriers involved in the cellular uptake of drugs: a model system in yeast. *BMC Biol.* 9, 70

10 Kell, D.B. et al. (2013) The promiscuous binding of pharmaceutical drugs and their transporter-mediated uptake into cells: what we (need to) know and how we can do so. *Drug Discov. Today* 18, 218–239

11 Kell, D.B. (2013) Finding novel pharmaceuticals in the systems biology era using multiple effective drug targets, phenotypic screening, and knowledge of transporters: where drug discovery went wrong and how to fix it. *FEBS J.* 280, 5957–5980

12 Kell, D.B. and Goodacre, R. (2014) Metabolomics and systems pharmacology: why and how to model the human metabolic network for drug discovery. *Drug Discov. Today* 19, 171–182

13 Kell, D.B. and Oliver, S.G. (2014) How drugs get into cells: tested and testable predictions to help discriminate between transporter-mediated uptake and lipoidal bilayer diffusion. *Front. Pharmacol.* 5, 231

14 Kell, D.B. (2015) What would be the observable consequences if phospholipid bilayer diffusion of drugs into cells is negligible? *Trends Pharmacol. Sci.* 36, 15–21

15 Kell, D.B. and Knowles, J.D. (2006) The role of modeling in systems biology. In *System Modeling in Cellular Biology: From Concepts to Nuts and Bolts* (Szallasi, Z. et al., eds), pp. 3–18, MIT Press

16 Kell, D.B. (2006) Metabolomics, modelling and machine learning in systems biology: towards an understanding of the languages of cells. The 2005 Theodor Bücher lecture. *FEBS J.* 273, 873–894

17 Grant, C. et al. (2014) Identification and use of an alkane transporter plug-in for applications in biocatalysis and whole-cell biosensing of alkanes. *Sci. Rep.* 4, 5844

18 Wang, J. et al. (2013) Ammonium transport proteins with changes in one of the conserved pore histidines have different performance in ammonia and methylamine conduction. *PLoS ONE* 8, e62745

19 Kaldenhoff, R. et al. (2014) Aquaporins and membrane diffusion of CO_2 in living organisms. *Biochim. Biophys. Acta* 1840, 1592–1595

20 Kai, L. and Kaldenhoff, R. (2014) A refined model of water and CO_2 membrane diffusion: effects and contribution of sterols and proteins. *Sci. Rep.* 4, 6665

21 Penrod, J.T. et al. (2004) A pH-sensitive function and phenotype: evidence that EutH facilitates diffusion of uncharged ethanolamine in *Salmonella enterica*. *J. Bacteriol.* 186, 6885–6890

22 van den Berg, B. (2005) The FadL family: unusual transporters for unusual substrates. *Curr. Opin. Struct. Biol.* 15, 401–407

23 Ishibashi, K. et al. (2011) The evolutionary aspects of aquaporin family. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 300, R566–R576

24 Bienert, G.P. and Chaumont, F. (2014) Aquaporin-facilitated transmembrane diffusion of hydrogen peroxide. *Biochim. Biophys. Acta* 1840, 1596–1604

25 Walker, A.L. et al. (2011) Transcellular movement of hydroxyurea is mediated by specific solute carrier transporters. *Exp. Hematol.* 39, 446–456

26 Herrera, M. and Garvin, J.L. (2011) Aquaporins as gas channels. *Pflugers Arch.* 462, 623–630

27 Wang, Y. and Tajkhorshid, E. (2010) Nitric oxide conduction by the brain aquaporin AQP4. *Proteins* 78, 661–670

28 Shayakul, C. et al. (2013) The urea transporter family (SLC14): physiological, pathological and structural aspects. *Mol. Aspects Med.* 34, 313–322

29 Strugatsky, D. et al. (2013) Structure of the proton-gated urea channel from the gastric pathogen *Helicobacter pylori*. *Nature* 493, 255–258

30 Day, R.E. et al. (2013) Human aquaporins: regulators of transcellular water flow. *Biochim. Biophys. Acta* 1840, 1492–1506

31 Agre, P. (2004) Aquaporin water channels (Nobel Lecture). *Angew. Chem. Int. Ed. Engl.* 43, 4278–4290

32 Benga, G. (2012) The first discovered water channel protein, later called aquaporin 1: molecular characteristics, functions and medical implications. *Mol. Aspects Med.* 33, 518–534

33 Kell, D.B. et al. (1981) On the permeability to weak acids and bases of the cytoplasmic membrane of *Clostridium pasteurianum*. *Biochem. Biophys. Res. Commun.* 99, 81–88

34 Jolkver, E. et al. (2009) Identification and characterization of a bacterial transport system for the uptake of pyruvate, propionate, and acetate in *Corynebacterium glutamicum*. *J. Bacteriol.* 191, 940–948

35 Dunlop, M.J. *et al.* (2011) Engineering microbial biofuel tolerance and export using efflux pumps. *Mol. Syst. Biol.* 7, 487

36 Sá-Correia, I. *et al.* (2009) Drug:H$^+$ antiporters in chemical stress response in yeast. *Trends Microbiol.* 17, 22–31

37 Teixeira, M.C. *et al.* (2012) Increased expression of the yeast multidrug resistance ABC transporter Pdr18 leads to increased ethanol tolerance and ethanol production in high gravity alcoholic fermentation. *Microb. Cell Fact.* 11, 98

38 Teixeira, M.C. *et al.* (2009) Genome-wide identification of *Saccharomyces cerevisiae* genes required for maximal tolerance to ethanol. *Appl. Env. Microbiol.* 75, 5761–5772

39 Dikicioglu, D. *et al.* (2014) Yeast cells with impaired drug resistance accumulate glycerol and glucose. *Mol. Biosyst.* 10, 93–102

40 Sano, C. (2009) History of glutamate production. *Am. J. Clin. Nutr.* 90, 728S–732S

41 Mitsuhashi, S. (2014) Current topics in the biotechnological production of essential amino acids, functional amino acids, and dipeptides. *Curr. Opin. Biotechnol.* 26, 38–44

42 Morbach, S. and Krämer, R. (2002) Body shaping under water stress: osmosensing and osmoregulation of solute transport in bacteria. *ChemBioChem* 3, 384–397

43 Kell, A. and Glaser, R.W. (1993) On the mechanical and dynamic properties of plant cell membranes: their role in growth, direct gene transfer and protoplast fusion. *J. Theor. Biol.* 160, 41–62

44 Booth, I.R. and Blount, P. (2012) The MscS and MscL families of mechanosensitive channels act as microbial emergency release Valves. *J. Bacteriol.* 194, 4802–4809

45 Booth, I.R. (2014) Bacterial mechanosensitive channels: progress towards an understanding of their roles in cell physiology. *Curr. Opin. Microbiol.* 18, 16–22

46 García, J. and Torres, N. (2011) Mathematical modelling and assessment of the pH homeostasis mechanisms in *Aspergillus niger* while in citric acid producing conditions. *J. Theor. Biol.* 282, 23–35

47 Chen, J. *et al.* (2014) Activating C$_4$-dicarboxylate transporters DcuB and DcuC for improving succinate production. *Appl. Microbiol. Biotechnol.* 98, 2197–2205

48 Groeneveld, P. *et al.* (2009) Super life: how and why 'cell selection' leads to the fastest-growing eukaryote. *FEBS J.* 276, 254–270

49 Brown, S.W. and Oliver, S.G. (1982) Isolation of ethanol-tolerant mutants of yeast by continuous selection. *Eur. J. Appl. Microbiol. Biotechnol.* 16, 119–122

50 Lane, P.G. *et al.* (1999) Analysis of a continuous-culture technique for the selection of mutants tolerant to extreme environmental stress. *Biotechnol. Bioeng.* 65, 397–406

51 Markx, G.H. *et al.* (1991) The permittistat: a novel type of turbidostat. *J. Gen. Microbiol.* 137, 735–743

52 Davey, H.M. *et al.* (1996) Oscillatory, stochastic and chaotic growth rate fluctuations in permittistatically-controlled yeast cultures. *Biosystems* 39, 43–61

53 Pir, P. *et al.* (2012) The genetic control of growth rate: a systems biology study in yeast. *BMC Syst. Biol.* 6, 4

54 van den Berg, M.A. *et al.* (2008) Genome sequencing and analysis of the filamentous fungus *Penicillium chrysogenum*. *Nat. Biotechnol.* 26, 1161–1168

55 Smallbone, K. *et al.* (2013) A model of yeast glycolysis based on a consistent kinetic characterization of all its enzymes. *FEBS Lett.* 587, 2832–2841

56 Smallbone, K. and Mendes, P. (2013) Large-scale metabolic models: from reconstruction to differential equations. *Ind. Biotechnol.* 9, 179–184

57 Almquist, J. *et al.* (2014) Kinetic models in industrial biotechnology – improving cell factory performance. *Metab. Eng.* 24, 38–60

58 Palsson, B.Ø. (2015) *Systems Biology: Constraint-Based Reconstruction and Analysis*, Cambridge University Press

59 Curran, K.A. *et al.* (2012) Using flux balance analysis to guide microbial metabolic engineering. *Methods Mol. Biol.* 834, 197–216

60 Lakshmanan, M. *et al.* (2014) Software applications for flux balance analysis. *Brief. Bioinform.* 15, 108–122

61 Smallbone, K. *et al.* (2007) Something from nothing: bridging the gap between constraint-based and kinetic modelling. *FEBS J.* 274, 5576–5585

62 Smallbone, K. and Simeonidis, E. (2008) Flux balance analysis: a geometric perspective. *J. Theor. Biol.* 258, 311–315

63 Lee, D. *et al.* (2012) Improving metabolic flux predictions using absolute gene expression data. *BMC Syst. Biol.* 6, 73

64 da Cunha, J.P.C. *et al.* (2009) Bioinformatics construction of the human cell surfaceome. *Proc. Natl. Acad. Sci. U.S.A.* 106, 16752–16757

65 Smith, A.M. *et al.* (2009) Quantitative phenotyping via deep barcode sequencing. *Genome Res.* 19, 1836–1842

66 Winter, G.E. *et al.* (2014) The solute carrier SLC35F2 enables YM155-mediated DNA damage toxicity. *Nat. Chem. Biol.* 10, 768–773

67 Davey, H.M. and Kell, D.B. (1996) Flow cytometry and cell sorting of heterogeneous microbial populations: the importance of single-cell analysis. *Microbiol. Rev.* 60, 641–696

68 Laxminarayan, R. *et al.* (2013) Antibiotic resistance – the need for global solutions. *Lancet Infect. Dis.* 13, 1057–1098

69 Nikaido, H. and Pagès, J.M. (2012) Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria. *FEMS Microbiol. Rev.* 36, 340–363

70 Prasad, R. and Rawal, M.K. (2014) Efflux pump proteins in antifungal resistance. *Front. Pharmacol.* 5, 202

71 Du, D. *et al.* (2014) Structure of the AcrAB–TolC multidrug efflux pump. *Nature* 509, 512–515

72 Wen, X. *et al.* (2014) MDR1 transporter protects against paraquat-induced toxicity in human and mouse proximal tubule cells. *Toxicol. Sci.* 141, 475–483

73 Callaghan, R. *et al.* (2014) Inhibition of the multidrug resistance P-glycoprotein: time for a change of strategy? *Drug Metab. Dispos.* 42, 623–631

74 Segura, A. *et al.* (2012) Solvent tolerance in Gram-negative bacteria. *Curr. Opin. Biotechnol.* 23, 415–421

75 Doshi, R. *et al.* (2013) Transporter-mediated biofuel secretion. *Proc. Natl. Acad. Sci. U.S.A.* 110, 7642–7647

76 Ling, H. *et al.* (2013) Transcriptome response to alkane biofuels in *Saccharomyces cerevisiae*: identification of efflux pumps involved in alkane tolerance. *Biotechnol. Biofuels* 6, 95

77 Chen, B. *et al.* (2013) Transporter engineering for improved tolerance against alkane biofuels in *Saccharomyces cerevisiae*. *Biotechnol. Biofuels* 6, 21

78 Foo, J.L. and Leong, S.S.J. (2013) Directed evolution of an *E. coli* inner membrane transporter for improved efflux of biofuel molecules. *Biotechnol. Biofuels* 6, 81

79 Nishida, N. *et al.* (2013) ABC transporters and cell wall proteins involved in organic solvent tolerance in *Saccharomyces cerevisiae*. *J. Biotechnol.* 165, 145–152

80 Sun, X. *et al.* (2011) An antirepressor, SrpR, is involved in transcriptional regulation of the SrpABC solvent tolerance efflux pump of *Pseudomonas putida* S12. *J. Bacteriol.* 193, 2717–2725

81 Fillet, S. *et al.* (2012) Transcriptional control of the main aromatic hydrocarbon efflux pump in *Pseudomonas*. *Environ. Microbiol. Rep.* 4, 158–167

82 Fisher, M.A. *et al.* (2014) Enhancing tolerance to short-chain alcohols by engineering the *Escherichia coli* AcrB efflux pump to secrete the non-native substrate n-butanol. *ACS Synth. Biol.* 3, 30–40

83 Foo, J.L. *et al.* (2014) Improving microbial biogasoline production in *Escherichia coli* using tolerance engineering. *MBio* 5, 01932–2014

84 Moschen, I. *et al.* (2012) Significance of short chain fatty acid transport by members of the monocarboxylate transporter family (MCT). *Neurochem. Res.* 37, 2562–2568

85 Sá-Pessoa, J. *et al.* (2013) SATP (YaaH), a succinate–acetate transporter protein in *Escherichia coli*. *Biochem. J.* 454, 585–595

86 Lin, M.H. and Khnykin, D. (2014) Fatty acid transporters in skin development, function and disease. *Biochim. Biophys. Acta* 1841, 362–368

87 Villalba, M.S. and Alvarez, H.M. (2014) Identification of a novel ATP-binding cassette transporter involved in long-chain fatty acid import and its role in triacylglycerol accumulation in *Rhodococcus jostii* RHA1. *Microbiology* 160, 1523–1532

88 Lohr, M. *et al.* (2012) Isoprenoid biosynthesis in eukaryotic phototrophs: A spotlight on algae. *Plant Sci.* 185, 9–22

89 Kotloski, N.J. and Gralnick, J.A. (2013) Flavin electron shuttles dominate extracellular electron transfer by *Shewanella oneidensis*. *MBio* 4, e00553-12

90 Kell, D.B. (2009) Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases. *BMC Med. Genomics* 2, 2

91 Hider, R.C. and Kong, X. (2010) Chemistry and biology of siderophores. *Nat. Prod. Rep.* 27, 637–657

92 Kell, D.B. (2010) Towards a unifying, systems biology understanding of large-scale cellular death and destruction caused by poorly liganded iron: Parkinson's, Huntington's, Alzheimer's, prions, bactericides, chemical toxicology and others as examples. *Arch. Toxicol.* 577, 825–889

93 de Carvalho, C.C.C.R. *et al.* (2011) Recent achievements on siderophore production and application. *Recent Pat. Biotechnol.* 5, 183–198

94 Nicolaou, S.A. *et al.* (2010) A comparative view of metabolite and substrate stress and tolerance in microbial bioprocessing: from biofuels and chemicals, to biocatalysis and bioremediation. *Metab. Eng.* 12, 307–331

95 Hohmann, S. *et al.* (2007) Yeast osmoregulation. *Methods Enzymol.* 428, 29–45

96 Kempf, B. and Bremer, E. (1998) Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. *Arch. Microbiol.* 170, 319–330

97 Weinand, M. *et al.* (2007) Characterization of compatible solute transporter multiplicity in *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 76, 701–708

98 Ochrombel, I. *et al.* (2011) Osmotic stress response in *C. glutamicum*: impact of channel- and transporter-mediated potassium accumulation. *Arch. Microbiol.* 193, 787–796

99 Prasad, S. *et al.* (2011) Effect of chemical chaperones in improving the solubility of recombinant proteins in *Escherichia coli*. *Appl. Environ. Microbiol.* 77, 4603–4609

100 Fahnert, B. (2012) Using folding promoting agents in recombinant protein production: a review. *Methods Mol. Biol.* 824, 3–36

101 Turner, N.J. (2009) Directed evolution drives the next generation of biocatalysts. *Nat. Chem. Biol.* 5, 567–573

102 Kell, D.B. (2012) Scientific discovery as a combinatorial optimisation problem: how best to navigate the landscape of possible experiments? *Bioessays* 34, 236–244

103 Currin, A. *et al.* (2015) Synthetic biology for the directed evolution of protein biocatalysts: navigating sequence space intelligently. *Chem. Soc. Rev.* Published online December 15, 2014. http://dx.doi.org/10.1039/c4cs00351a

104 Cameron, D.E. *et al.* (2014) A brief history of synthetic biology. *Nat. Rev. Microbiol.* 12, 381–390

105 Church, G.M. *et al.* (2014) Realizing the potential of synthetic biology. *Nat. Rev. Mol. Cell Biol.* 15, 289–294

106 Currin, A. *et al.* (2014) SpeedyGenes: a novel approach for the efficient production of error-corrected, synthetic gene libraries. *Protein Evol. Des. Sel.* 27, 273–280

107 Nielsen, J. *et al.* (2014) Engineering synergy in biotechnology. *Nat. Chem. Biol.* 10, 319–322

108 Swainston, N. *et al.* (2014) GeneGenie: optimised oligomer design for directed evolution. *Nucleic Acids Res.* 12, W395–W400

109 Way, J.C. *et al.* (2014) Integrating biological redesign: where synthetic biology came from and where it needs to go. *Cell* 157, 151–161

110 Brooker, R.J. *et al.* (1989) Characterization and Sequencing of the LacY54-41 uncoupled mutant of the lactose permease. *J. Biol. Chem.* 264, 8135–8140

111 Meredith, D. (2009) The mammalian proton-coupled peptide cotransporter PepT1: sitting on the transporter-channel fence? *Philos. Trans. R. Soc. Lond. B: Biol. Sci.* 364, 203–207

112 Schaedler, T.A. and van Veen, H.W. (2010) A flexible cation binding site in the multidrug major facilitator superfamily transporter LmrP is associated with variable proton coupling. *FASEB J.* 24, 3653–3661

113 Tirosh, O. *et al.* (2012) Manipulating the drug/proton antiport stoichiometry of the secondary multidrug transporter MdfA. *Proc. Natl. Acad. Sci. U.S.A.* 109, 12473–12478

114 Madej, M.G. *et al.* (2013) Evolutionary mix-and-match with MFS transporters. *Proc. Natl. Acad. Sci. U.S.A.* 110, 5870–5874

115 Khersonsky, O. and Tawfik, D.S. (2010) Enzyme promiscuity: a mechanistic and evolutionary perspective. *Annu. Rev. Biochem.* 79, 471–505

116 Yao, X.Q. *et al.* (2013) Drug uptake pathways of multidrug transporter AcrB studied by molecular simulations and site-directed mutagenesis experiments. *J. Am. Chem. Soc.* 135, 7474–7485

117 Daley, D.O. *et al.* (2005) Global topology analysis of the *Escherichia coli* inner membrane proteome. *Science* 308, 1321–1323

118 Chakraborty, S. *et al.* (2013) Promiscuity-based enzyme selection for rational directed evolution experiments. *Methods Mol. Biol.* 978, 205–216

119 Nakayama, Y. *et al.* (2012) A gain-of-function mutation in gating of *Corynebacterium glutamicum* NCgl1221 causes constitutive glutamate secretion. *Appl. Environ. Microbiol.* 78, 5432–5434

120 Becker, M. *et al.* (2013) Glutamate efflux mediated by *Corynebacterium glutamicum* MscCG, *Escherichia coli* MscS, and their derivatives. *Biochim. Biophys. Acta* 1828, 1230–1240

121 Nishino, K. and Yamaguchi, A. (2001) Analysis of a complete library of putative drug transporter genes in *Escherichia coli*. *J. Bacteriol.* 183, 5803–5812

122 Holdsworth, S.R. and Law, C.J. (2012) Functional and biochemical characterisation of the *Escherichia coli* major facilitator superfamily multidrug transporter MdtM. *Biochimie* 94, 1334–1346

123 Pos, K.M. (2009) Drug transport mechanism of the AcrB efflux pump. *Biochim. Biophys. Acta* 1794, 782–793

124 Eicher, T. *et al.* (2012) Transport of drugs by the multidrug transporter AcrB involves an access and a deep binding pocket that are separated by a switch-loop. *Proc. Natl. Acad. Sci. U.S.A.* 109, 5687–5692

125 Sigal, N. *et al.* (2006) MdfA from *Escherichia coli*, a model protein for studying secondary multidrug transport. *J. Mol. Microbiol. Biotechnol.* 11, 308–317

126 Schuldiner, S. (2009) EmrE, a model for studying evolution and mechanism of ion-coupled transporters. *Biochim. Biophys. Acta* 1794, 748–762

127 Nakashima, R. *et al.* (2011) Structures of the multidrug exporter AcrB reveal a proximal multisite drug-binding pocket. *Nature* 480, 565–569

128 Paul, S. *et al.* (2014) A single-component multidrug transporter of the major facilitator superfamily is part of a network that protects *Escherichia coli* from bile salt stress. *Mol. Microbiol.* 92, 872–884

129 Salter, G.J. and Kell, D.B. (1995) Solvent selection for whole cell biotransformations in organic media. *CRC Crit. Rev. Biotechnol.* 15, 139–177

130 Watanabe, R. and Doukyu, N. (2014) Improvement of organic solvent tolerance by disruption of the *lon* gene in *Escherichia coli*. *J. Biosci. Bioeng.* 118, 139–144

131 Doukyu, N. *et al.* (2012) Improvement in organic solvent tolerance by double disruptions of proV and marR genes in *Escherichia coli*. *J. Appl. Microbiol.* 112, 464–474

132 Koita, K. and Rao, C.V. (2012) Identification and analysis of the putative pentose sugar efflux transporters in *Escherichia coli*. *PLoS ONE* 7, e43700

133 Zhou, Y.J. *et al.* (2013) Engineering NAD$^+$ availability for *Escherichia coli* whole-cell biocatalysis: a case study for dihydroxyacetone production. *Microb. Cell Fact.* 12, 103

134 Goffeau, A. *et al.* (1997) Multidrug-resistant transport proteins in yeast: complete inventory and phylogenetic characterization of yeast open reading frames with the major facilitator superfamily. *Yeast* 13, 43–54

135 Balakrishnan, R. *et al.* (2012) YeastMine – an integrated data warehouse for Saccharomyces cerevisiae data as a multipurpose tool-kit. *Database (Oxford)* 2012, bar062

136 Cherry, J.M. *et al.* (2012) *Saccharomyces* Genome Database: the genomics resource of budding yeast. *Nucleic Acids Res.* 40, D700–D705

137 Noël, T. (2012) The cellular and molecular defense mechanisms of the *Candida* yeasts against azole antifungal drugs. *J. Mycol. Med.* 22, 173–178

138 Westfall, P.J. and Gardner, T.S. (2011) Industrial fermentation of renewable diesel fuels. *Curr. Opin. Biotechnol.* 22, 344–350

139 Sahoo, S. *et al.* (2014) Membrane transporters in a human genome-scale metabolic knowledgebase and their implications for disease. *Front. Physiol.* 5, 91

140 Ananiadou, S. *et al.* (2014) Event-based text mining for biology and functional genomics. *Brief. Funct. Genomics.* Published online June 6, 2014. http://dx.doi.org/10.1093/bfgp/elu015

141 Swainston, N. *et al.* (2011) The SuBliMinaL Toolbox: automating steps in the reconstruction of metabolic networks. *J. Integr. Bioinform.* 8, 186

142 Herrgård, M.J. *et al.* (2008) A consensus yeast metabolic network obtained from a community approach to systems biology. *Nat. Biotechnol.* 26, 1155–1160

143 Thiele, I. *et al.* (2013) A community-driven global reconstruction of human metabolism. *Nat. Biotechnol.* 31, 419–425

144 Swainston, N. *et al.* (2013) An analysis of a 'community-driven' reconstruction of the human metabolic network. *Metabolomics* 9, 757–764

145 Tiwari, M. (2012) Antimetabolites: established cancer therapy. *J. Cancer Res. Ther.* 8, 510–519

146 Forrest, L.R. *et al.* (2011) The structural basis of secondary active transport mechanisms. *Biochim. Biophys. Acta* 1807, 167–188

147 Koldsø, H. *et al.* (2013) Ligand induced conformational changes of the human .serotonin transporter revealed by molecular dynamics simulations. *PLoS ONE* 8, e63635