the FEBS Journal
MINIREVIEW

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

Douglas B. Kell[1,2]

1 School of Chemistry, The University of Manchester, UK
2 Manchester Institute of Biotechnology, The University of Manchester, UK

Keywords
drug discovery, drug resistance, drug transporters, enzyme kinetics, expression profiling, genomics, polypharmacology, promiscuity, robustness

Correspondence
D. B. Kell, Manchester Institute of Biotechnology, The University of Manchester, 131 Princess Street, Manchester M1 7DN, UK.
Tel: +44 (0)161 306 4492
E-mail: dbk@manchester.ac.uk
Website: http://dbkgroup.org

(Received 3 February 2013, revised 20 March 2013, accepted 26 March 2013)

doi:10.1111/febs.12268

Despite the sequencing of the human genome, the rate of innovative and successful drug discovery in the pharmaceutical industry has continued to decrease. Leaving aside regulatory matters, the fundamental and interlinked intellectual issues proposed to be largely responsible for this are: (a) the move from 'function-first' to 'target-first' methods of screening and drug discovery; (b) the belief that successful drugs should and do interact solely with single, individual targets, despite natural evolution's selection for biochemical networks that are robust to individual parameter changes; (c) an over-reliance on the rule-of-5 to constrain biophysical and chemical properties of drug libraries; (d) the general abandoning of natural products that do not obey the rule-of-5; (e) an incorrect belief that drugs diffuse passively into (and presumably out of) cells across the bilayers portions of membranes, according to their lipophilicity; (f) a widespread failure to recognize the overwhelmingly important role of proteinaceous transporters, as well as their expression profiles, in determining drug distribution in and between different tissues and individual patients; and (g) the general failure to use engineering principles to model biology in parallel with performing 'wet' experiments, such that 'what if?' experiments can be performed *in silico* to assess the likely success of any strategy. These facts/ideas are illustrated with a reasonably extensive literature review. Success in turning round drug discovery consequently requires: (a) decent systems biology models of human biochemical networks; (b) the use of these (iteratively with experiments) to model how drugs need to interact with multiple targets to have substantive effects on the phenotype; (c) the adoption of polypharmacology and/or cocktails of drugs as a desirable goal in itself; (d) the incorporation of drug transporters into systems biology models, en route to full and multiscale systems biology models that incorporate drug absorption, distribution, metabolism and excretion; (e) a return to 'function-first' or phenotypic screening; and (f) novel methods for inferring modes of action by measuring the properties on system variables at all levels of the 'omes. Such a strategy offers the opportunity of achieving a state where we can hope to predict biological processes and the effect of pharmaceutical agents upon them. Consequently, this should both lower attrition rates and raise the rates of discovery of effective drugs substantially.

Abbreviations
NF-κB, nuclear factor-kappa B; Ro5, rule-of-5.

Introduction

As illustrated in Fig. 1, classical drug discovery (or pharmacology or chemical genetics) started with an organism displaying a phenotype where there was a need for change (e.g. a disease) and involved the assay of various drugs *in vivo* to identify one or more that was efficacious (and nontoxic). There was no need to discover (let alone start with) a postulated mechanism of drug action; for a successful drug, this could come later (often much later) [1–3]. This approach is thus 'function first', and is equivalent in terms of (chemical) genetic or genotype–phenotype mapping [4] to 'forward' genetics, and has lead to the discovery of many drugs that are still in use (and mainly still without detailed knowledge of their mechanisms of action). By contrast, particularly as a result of the systematic (human) genome sequencing programmes, drug discovery largely changed to an approach that was based on the ability of chemicals to bind to or inhibit chosen molecular targets at low concentrations *in vitro* [5]. This would then necessarily be followed by tests of efficacy in whole organisms. This approach is thus 'target-first', and is equivalent to 'reverse' genetics, and (despite some spectacular new molecules that work on selected patients, as well as the important rise of biologicals) has been rather ineffectual because the vast majority of small molecule drugs (90–95%) fail to go forward, even from the 'first into humans' phase, to become successful and marketable drugs; a set of phenomena known as 'attrition' [6–11]. This is not unexpected to systems biologists, who would see the distinction as being similar to the distinction between hypothesis-dependent and data-driven science [12,13]. The present review aims to illustrate why this is the case, as well as what we might seek to do to improve matters. Figure 2 provides an overview of the present review, which begins by recognizing the role of robustness in biochemical networks.

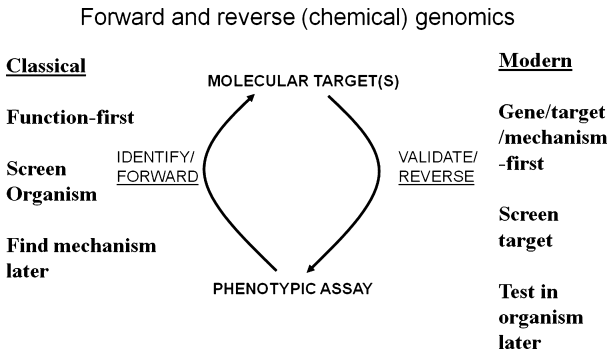

Fig. 1. A contrast between classical ('function first') forward chemical discovery with the more recent target-first or 'reverse' strategy. It is suggested that a reversion to the more classical approach through phenotypic screening is likely to prove beneficial from a systems point of view.

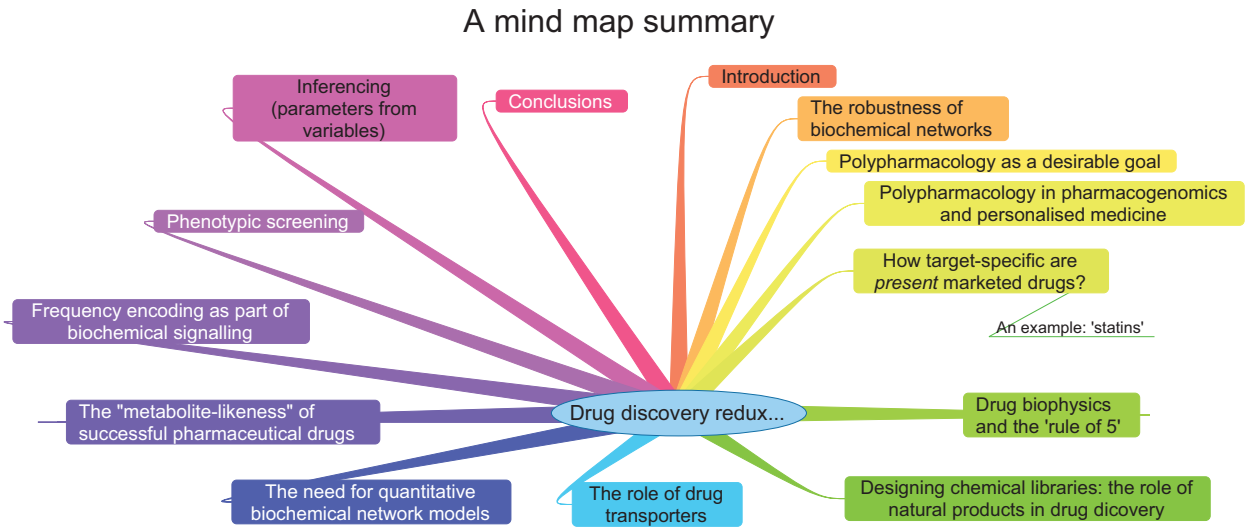

Fig. 2. A 'mind map' [436] summarizing the present review. The map should be read starting at the 12 o'clock position and working clockwise.

The robustness of biochemical networks

Somewhat in contrast to designed and artificial network structures such as roads, railways and process plants [14], natural evolution has selected much less for cheapness and efficiency than for robustness to parameter changes (whether caused by mutation or otherwise) [15–26]. This is straightforwardly understandable in that an organism with a mutation messing up a whole pathway will soon be selected out, and so the selection pressure for robustness is very high. Typically, it is the network topologies and feedback structures themselves, rather than the exact parameter values involved, that are responsible for the robustness to parameter changes [27]. However, another way to think about this is that, by diminishing the sensitivity of individual steps to particular changes in their parameters (or to inhibitors), no individual enzyme or target or inhibitor is likely to have much effect unless it affects many other steps by itself. This is easily achieved by having enzymes obeying Henri–Michaelis–Menten kinetics operating at (or below) their K_m values (Fig. 3), where a certain amount of inhibition of them (other than uncompetitive inhibition) [28] simply raises the concentration of their substrate and restores flux. (If the substrate of an enzyme has a concentration that is maintained essentially constant by regulatory mechanisms, then competitive inhibition of an enzyme that uses it in a minor pathway can be expected to be as effective *in vivo* as it is in the spectrophotometer.)

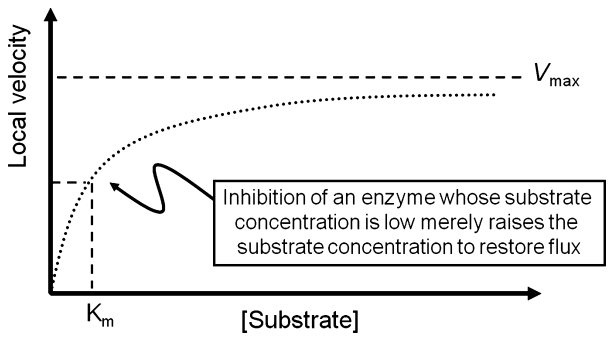

Fig. 3. The kinetics of a typical enzyme obeying Henri–Michaelis–Menten kinetics; if the substrate concentration is near the K_m, initial inhibition of the enzyme increases the substrate concentration that restores the local flux. The enzyme is said to have a high elasticity towards its substrate. This is common in biology. A rare [437] but highly important exception is the inhibition of 5-enolpyruvoylshikimate-3-phosphate synthase (ESPS; EC 2.5.1.19) by the herbicide glyphosate, which is uncompetitive with respect to one of the substrates, shikimate-3-phosphate [438,439], such that the extent of inhibition is effectively increased by the raised substrate concentration.

The corollary is clear: to have a major effect on a typical biochemical network, it is necessary to modulate multiple steps simultaneously (see below), such that any drug that acts solely on a single (molecular) target is unlikely to be successful. The same is true of schemes designed to increase the fluxes in pathways of biotechnological interest [29–35]. This distributed nature of flux control, which contributes to robustness, has long been established, and indeed is proven mathematically for certain kinds of networks via the theorems of metabolic control analysis [36–42]. These show that, by normalizing appropriately, the contributions ('control coefficients', also known as their local sensitivities) [43] to a particular flux of all the steps in a biochemical pathway add up to 1, and thus most individual steps are likely to have only a small contribution.

Polypharmacology as a desirable goal

If we are to design drugs that overcome this robustness, we need either to find individual molecules that hit a useful set of multiple targets [44–46] (for an example from neuropharmacology, see [47–51]) or use cocktails of drugs [24,52–54], each of which hits mainly an individual target. The former is known as polypharmacology [44,45,55–67] or multi-target drug discovery [68,69] and the recognition that we need to attack multiple targets in pharmacology is reflected in names such as 'systems pharmacology' [6,70–88] or 'systems medicine' [89–93]. The use of cocktails is of course commonplace in diseases such as cancer and HIV-AIDS [94].

One issue is that finding a good subset of even a small number of targets from a large number of possible targets is a combinatorial optimization problem [95]. All combinations of n drugs specific for n targets gives 2^n possibilities [54], whereas finding the best combination of even just three or four drugs or targets out of 1000 gives 166 million or 41 billion combinations, respectively, resulting in numbers that are too large for typical experimental analyses (but easily accessible computationally; see below).

Polypharmacology in pharmacogenomics and personalized medicine

An important recognition, if not that recent in origin [96], is that every patient is different and thus their response to drugs will also be different [97–102]. As

neatly phrased by Henney [103], quoting an 18th Century physician (Caleb Parry), 'It is much more important to know what kind of patient has a disease than to know what kind of disease a patient has'. The essential combinatorial argument is straightforward [104]: if we define for any character, such as the fasting low-density lipoprotein-cholesterol level, the 'normal range' to be the middle 95 percentiles, then any individual has a probability of 0.95 of being 'normal' (for that character). (This is conventional but thereby ignores systematic errors or biases [105].) The probability of being normal for two (independent) characters is thus 0.95^2 and, for n independent characters, is 0.95^n. This drops below 1% when $n = 90$, and there are of course thousands of characters. What is probably more unexpected, therefore, is not that individuals are different but that they display any similarity of response at all (in part, this presumably reflects the evolution and selection for robustness described above, and the fact that many characters are not of course entirely independent.)

From the point of view of polypharmacology, a drug that interacts usefully with n targets can more easily afford to 'lose' one of them (e.g. as a result of an inactivating single nucleotide polymorphism or other mutation) if n is large, whereas a drug that has only one target may provide a very strong variation in response between individuals. Assuming that adverse drug reactions are taken into account, a drug with multiple useful targets is thus likely to show significantly less variation in the response across populations. Drugs do of course require transporters to reach to their sites of action (see below) and this concept should also be included as part of the relevant polypharmacological analysis of multiple 'targets' (i.e. preferred macromolecules with which the drug is intended to interact).

How target-specific are the presently available marketed drugs?

The argument that one should seek to hit multiple targets begs the question of which proteins do successful (and thus marketed) drugs actually bind to, given that many of them were in fact isolated on the basis of their ability to bind to a specific and isolated molecular target? What takes place in real cells, tissues and organisms, however, is very different: individual drugs [44,46,55,57,61,63,64,66,67,106–145], and even intermediary metabolites [146–149], are now seen to bind to a great many more entities than just the single 'target' via which they were typically discovered. Drugs on average bind to six targets [150], whereas ligands in some classes typically bind to many more [44,114]. This 'drug promiscuity' [151] can be accounted for in terms of the comparatively limited number of protein motifs used in evolution [152], which are often related to each other [145,153], as well as the fact that only a small number of biophysical forces determine binding; together, these make complete specificity generally implausible in small molecules and, as a consequence, bioactivity in one species is often enriched in other species [154,155]. A typical example of promiscuity is outlined below.

An example: statins

Although low-density lipoprotein-cholesterol is widely regarded as a major determinant of cardiovascular diseases as a result of its appearance in atherosclerotic plaques, its correlation with disease when in its normal range is poor [115,156]. Nonetheless, subsequent to the discovery of a ligand (later marketed as lovastatin) from *Aspergillus terreus* that would inhibit HMG-CoA reductase, and thus lower cholesterol, a great swathe of 'statins' have been marketed, and the epidemiological evidence that they can prolong life is good. It is again widely assumed that this is because they lower cholesterol, whereas this is neither logical, nor (as stated) true. Although there is a highly unfortunate tendency to lump all such molecules as 'statins' (presumably because they were discovered via their ability to inhibit HMG-CoA reductase), expression profiling studies straightforwardly show that they have no such unitary mode of action [157]. The resolution of the paradox [158] is uncomplicated [62]. All statins consist of a substructure that mimics hydroxymethylglutarate (and not, incidentally, its CoA derivative), termed the 'front end', bound to a wide variety of other structures (the 'back ends'). In most cases, it is likely the 'back end' that accounts for most of the biological activity, and mainly because such molecules are anti-inflammatory. In a previous review [62], more than 50 literature citations up to June 2008 were provided. More recent examples are now available [159–165]. A similar tale can be told for 'glitazones' [62].

Drug biophysics and the rule-of-5

Lipophilicity is widely seen as an important concept in drug discovery, albeit that there is no doubt that drug promiscuity tends to increase with lipophilicity [107,119,122,124,126,127,144,150,166–172]. In an extremely influential review [173] and later reprint [174],

Chris Lipinski and colleagues, when seeking to minimize the number of drugs that failed for reasons of pharmacodynamics and pharmacokinetics, proposed four rules (known as the 'rule-of-5' or Ro5 because each rule contains elements that are multiples of 5). They predicted that poor absorption or permeation into cells for a molecule is more likely when the number of hydrogen-bond donors > 5, the number of hydrogen-bond acceptors > 10, the relative molecular mass > 500 and the calculated log *P* (cLog *P*) > 5. This last in particular is a measure of lipophilicity, and those who design chemical libraries will always seek molecules that obey the Ro5, including through experimental measurements of the partition coefficient log *P* [175–177] and/or the distribution coefficient log *D* [178]. Note, however, that natural product-based drugs (still a major source of leads and indeed marketed drugs; see below) very rarely, if ever, obey the Ro5 and, indeed, even some synthetic drugs have very large molecular weights [169]; for example, navitoclax dihydrochloride [179], a Bcl-2 inhibitor, has seven ring systems and a relative molecular mass of 1047.5. Indeed, there is an increasing recognition [154,155,166,180–186] that over-reliance on Ro5 compliance would lose many desirable drugs, including known 'blockbusters'.

Designing chemical libraries: the role of natural products in drug discovery

Originally, of course, all drugs were natural products, and even now natural products (or chemical moieties derived therefrom) continue to contribute to many useful and profit-making drugs. Notwithstanding, many drug companies have abandoned them. This makes little sense [187] because they represent an exceptionally rich resource that occupies a distinct chemical space [188–204], and they continue to provide approximately half of all useful drugs [205–212]. The ability to detect novel and previously cryptic natural products, whether via pheromone activity [213] and co-culture [214,215], pharmacognosy [216], proteomics [217] and metabolomics [218], or via (meta)genomics [219] and genome mining [220], will increase greatly the utility of natural products in drug discovery. Their common role as iron chelators [221–224] makes them of special interest [26,62,225].

One reason given for the otherwise very odd loss of interest in natural products is that their high fraction of stereocentres often makes them difficult to manipulate chemically. Probably a more pertinent reason is that their failure to obey Lipinski's rules has led to the perception that they do not easily permeate cells. The facts of permeation speak otherwise, not least because, if they are active against intracellular targets as most are (and, in humans, are active orally, and thus must cross at least the gut epithelium), they must cross membranes easily enough. There remains a question as to how (Fig. 4).

The role of drug transporters

> … what is certain today is that most molecules of physiological or pharmacological significance are transported into and out of cells by proteins rather than by a 'passive' solubility into the lipid bilayer and diffusion through it … [226]

Notwithstanding the above quotation (dating from 1999), it is widely assumed that drugs cross membranes according to their lipophilicity, via what little [227] phospholipid bilayer sections of biological membranes may be uninfluenced by proteins (Fig. 4A). Actually, the evidence for this mode of transport is essentially non-existent (and, in truth, it is hard to acquire directly). There is an alternative view that we have reviewed extensively [151,228–231], for which there is abundant evidence, as well as a number of recent reviews (e.g. from 2012 alone: [84,232–267]); this is that transbilayer transport *in vivo* is negligible, and drugs cross biomembranes by hitchhiking on genetically encoded solute transporters that are normally involved in the intermediary metabolism of the host. In humans, there are more than 1000 of these [231], and a number of online databases exist [151,250].

The evidence cited above comes in various flavours, although the most pertinent for our purposes are the many clear experimental examples that show precisely which genetically-encoded transporters are used to

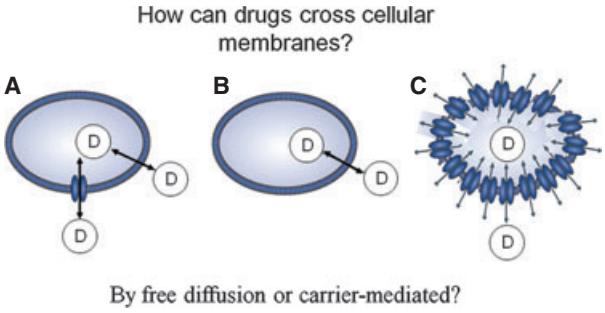

Fig. 4. Two means by which pharmaceutical drugs can cross cellular (and intracellular) membranes, namely via 'free' diffusion or via one or more carriers (A). In a first assumed method (B), they can do so by 'dissolving' in any phospholipid bilayer portions of the cell membrane. Alternatively (C), they can hitchhike on one of the many hundreds of natural (genome-encoded) carrier molecules.

transport specific drugs. This is especially easily achieved, and can be made quantitative, when the drugs themselves are toxic (or can be added at toxic concentrations), as in yeast [268] and trypanosomes [269–272]. It is important that the assays are at least semi-quantitative because binary (qualitative yes/no) assays that look for resistance when carriers are deleted may miss them. To emphasize once more, this is because multiple carriers can often transport each drug, and so the loss of just one is not normally going to confer 'complete' resistance. It probably underpins the widespread belief in 'passive' diffusion across membranes because 'passive' is often used erroneously as a synonym for 'transporters that we do not happen to know about and that are in fact important' [151,231].

A flipside of this is illustrated by examples where there is clear evidence that the expression (profiles) of a subset of transporters substantially determines the efficacy of the drug in question. Gemcitabine, the best drug against pancreatic cancer, provides an excellent example because the drug is only efficacious when a suitable nucleoside transporter is well expressed in the target tissue [233,245,249,252,273–288].

Drug transporters: 'barriers', tissue and interspecies differences

As well as the historical change in an understanding of the mode of action of narcotics ('general anaesthetics'), which went from entirely lipid-only views to one where the protein targets were identified and recognized [151,231], there are at least three contrafactuals that those who believe in lipid-transport-only theories need to explain: (a) the fact that most drugs do not diffuse across the blood–brain barrier (and others) where the lipids are not significantly different [151,231]; (b) the substantially varying tissue distributions [289–296]; and (c) the very large species differences in cellular drug uptake [297–299]. By contrast, the transporter-only view recognizes the possibility of varying degrees of tissue/individual/species enzyme distribution [289,291,293,296,300–305] and specificity [151], and their requirement for effecting transport provides a simple explanation for all these phenomena. In other words, the primacy of the need for transporters to effect drug transport into any cell at meaningful rates means that we need to seek to understand which drugs use which transporters. As noted above, if a drug can hitchhike on half a dozen transporters, a knockout of only one will tend to show little phenotypic effect, and thus careful quantitative methods may be necessary to discriminate which transporters are involved; in such cases, therefore, although the knowledge of the multiple transporters is interesting, it may not be that important to the function of getting drugs to intracellular targets.

Overall, this recognition of the importance of drug transporters shows that the problem of understanding how drugs get into cells is not so much a problem of biophysics, but rather a problem of quantitative systems biology. What is meant by this is outlined below.

The need for quantitative biochemical network models

It is a commonplace in engineering that, if one aims to understand the system being designed, especially if it is complex, then it is necessary to have a parallel mathematical or computational *in silico* model of the artefact of interest. This has long been recognized in a few areas of biology (e.g. neurophysiology) [306,307], although only more recently are we beginning to see human biochemical and physiological (and especially metabolic) network models of the type that we require [92,308–319], both for the entire organism or for elements such as the liver [320], a liver cell [321] or a macrophage [322,323]. The development of these is best performed using crowd-sourcing or community-based methods [319,324–326]. The great utility of such reconstructed networks [327–330] lies in areas such as: testing whether the model is accurate, in the sense that it reflects (or can be made to reflect) known experimental facts; analyzing the model to understand which parts of the system contribute most to some desired properties of interest; hypothesis generation and testing, allowing rapid analysis of the effects of manipulating experimental conditions in the model without having to perform complex and costly experiments (or to restrict the number that are performed); and testing what changes in the model would improve the consistency of its behaviour; along with experimental observations.

They also provide the necessary ground substance for inferencing modes of action of compounds with unknown or off-target effects (see below).

The metabolite-likeness of successful pharmaceutical drugs

Because we know the structures of successful, marketed drugs, it is possible to develop concepts such as drug-likeness [331–333] that capture the properties possessed by successfully marketed drugs. However, armed with the widely available metabolomics data indicating the metabolites that cells, tissues or body

fluids typically possess [334–337], it is possible to investigate whether (because we consider that they must hitchhike on carriers used in intermediary metabolism) successful (i.e. marketed) drugs are more similar to human metabolites than to say the Ro5-compliant molecules typically found in drug discovery libraries. When such studies are performed, the answer is that most synthetic compounds in chemical databases are not metabolite-like [338], whereas successful drugs are indeed commonly metabolite-like [339–341]. This adds weight to the view that those seeking to discover new drugs should consider the metabolite-likeness of their molecules early in the discovery process, along with the question of which transporters they are likely to use. It also leads to the obvious recognition [84] that it is important to incorporate into human metabolic network models the reaction steps that cover the metabolism of candidate and marketed pharmaceuticals (including their absorption, distribution and excretion).

Frequency encoding as part of biochemical signalling

Assays are an important part of the drug discovery process, although a simple binding or inhibition assay of a specific target (whether isolated or even when within a cell) does not clarify whether the inhibition serves any useful function. A particularly clear and interesting example comes from signalling pathways in which the signal is not based on amplitude (i.e. that might reasonably reflect an inhibition) but on frequency (that almost certainly will not, at least not directly). The transcription factor nuclear factor-kappa B (NF-κB) provides a good example.

Because a collection of nominally similar cells or unicellular organisms is not even close to being identical (thermodynamically, an 'ensemble'), for fundamental statistical reasons [104], there is the question of how to correlate macroscopic measurements of metabolic or signalling molecules with phentotypic effects. In cases such as when the phenotype is the ability to replicate or divide, which is necessarily a single-cell property, one simply cannot make such as correlation, even in principle [342–344], and sometimes the variability of the expression profiles between single, axenic microbial cells of even single proteins is huge [345,346].

Another specific case in which we cannot expect to relate the properties of collections of cells to a phenotype of interest is when they are not in a steady-state, and especially when they oscillate. This is exactly what happens in the NF-κB system. What we found, on comparing the behaviour of a mathematical model of the system [347,348] (Fig. 5) with the behaviour of individual cells determined microscopically [349,350], was that there is indeed a substantial oscillation in the distribution of NF-κB between the nucleus and the cytoplasm, and that this dynamic behaviour (rather than say a 'static' concentration of the NF-κB) can be related to changes in gene expression controlled by the transcription factor. More simply, macroscopic snapshots of the NF-κB concentration provide no information on the dynamics (and their heterogeneity) [351], and it is the dynamics that is important: the protein signal is frequency-encoded [352,353]. This phenomenon appears to be widespread, and also applies, for example, to p53-Mdm2 [354–359], ERK [360], Stat/Smad [361] and elsewhere [362,363]. Such studies indicate the need to study their interaction (and effects on biology) at as high a level of organization as possible, and certainly not solely by focussing on individual molecules. Analysis of cells (often called high-content screening) [364–381] is a start, although we need to return to 'phenotypic' screening at the level of the differentiated organism.

Phenotypic screening

Thus, we come full circle to the distinction made in Fig 1. If we wish to discover new drugs that work effectively at the level of the organism, we need to move towards initial analyses that are conducted in differentiated organisms [382–394]. For financial and ethical reasons, this mainly means model organisms, with

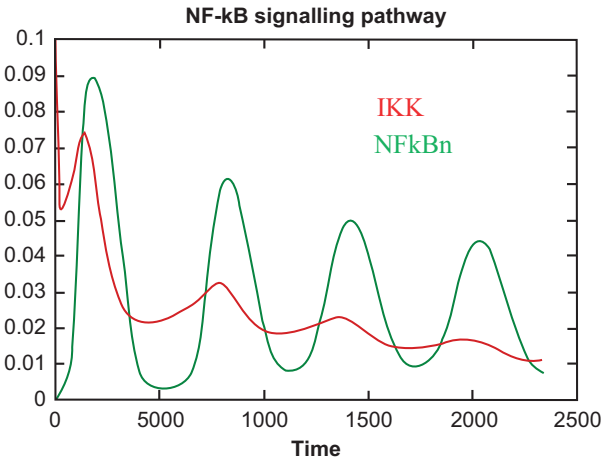

Fig. 5. The behaviour of a model of the NF-κB pathway. At time zero, after a 2000-s period of pre-equilibration *in silico*, NF-κB is 'added' at a concentration of 0.1 μM. For more details, see Ihekwaba *et al.* [347].

candidates including *Saccharomyces cerevisiae* [394,395], *Caenorhabditis elegans* [154,396–402], *Drosophila melanogaster* [403–405] and *Danio rero* (zebrafish) [405–410]. (Because of the numbers of organisms involved, fragment-based discovery methods [172,411–422] are preferable.) This will find us the effects, under circumstances where transporters are not a major issue, and will assess toxicity at once. What this will not necessarily clarify is the modes of action of the drugs; for this, appropriate analyses are needed, many of which can now be performed on a genome-wide scale [268,269,423,424]. An important additional strategy is based on the use of inferencing methods.

Inferencing (parameters from measurement of variables)

In a typical biochemical network, the parameters are the topology of the network, the starting (or fixed) concentrations of enzymes, their kinetic properties (e.g. K_m and V_{max}) and the starting or 'fixed' concentrations of metabolites and effectors. pH and time are also usually treated as honorary parameters. The variables of the system are then the changes in metabolite concentrations or fluxes that occur when one of the parameters is changed (e.g. by adding a substrate or effector to the system). The issue (Fig. 6) is how to identify which parameters have changed by measurement of changes in the variable alone (i.e. what effectors do is modify some of the parameters). The welcome answer is that they can [139,425–435],

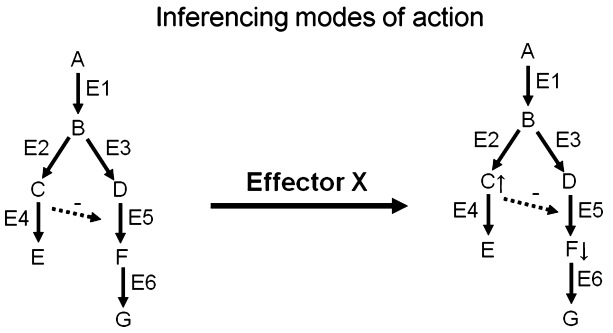

Fig. 6. A typical inferencing problem. Left: a (simple) network for which the properties are understood, with metabolite C being an inhibitor of enzyme E5. We then add an effector with an unknown mode of action, whose observable effects on the variables are to raise C and lower F. Does the effector stimulate E2, inhibit E4, inhibit E5, stimulate E6, or all or none of these? Simple inspection of the qualitative network makes it hard to decide, although quantitative (Bayesian types of) inferencing methods can point to the sites of action of the effectors based on a knowledge of the starting network and the two sets of variables alone [428].

although many of these problems are quite underdetermined, and the numerical methods do not yet scale well. However, what this tells us is that the availability of candidate networks, together with series of 'omics' measurements of variables, does indeed allow the possibility of inferring the modes or molecular sites of action of polypharmacological agents when added to whole cells or organisms.

Concluding remarks

The present review has sought to identify a number of areas where we might beneficially look again at how useful medicines are discovered:

- recognizing that the solution to failed target-first approaches that lead to attrition involves adopting function-first approaches
- recognizing that this follows in part from the fact that very few diseases (and no complex ones) have a unitary cause, and thus poly-pharmacology approaches are required
- recognizing the need for quantitative biochemical models that we can interrogate *in silico* and then validate
- recognizing the major role of drug transporters in getting drugs to their sites of action (and stopping their accumulation at toxic levels)
- recognizing that this involves a radical re-evaluation of the utility of the Ro5 as commonly used
- recognizing that most transporters evolved and were selected to transport natural, endogenous metabolites, and that successful drugs are structurally 'like' metabolites
- recognizing that this invites a major consideration of the benefits of natural products in drug discovery
- recognizing that phenotypic screening is important, although establishing mechanisms and modes of action requires genome-wide analyses coupled with sophisticated inferencing methods.

Taking all these together will once again set us more securely on a path to successful drug discovery.

References

1 Grunberg E & Schnitzer RJ (1952) Studies on the activity of hydrazine derivatives of isonicotinic acid in the experimental tuberculosis of mice. *Q Bull Sea View Hosp* **13**, 3–11.
2 Pletscher A (1991) The discovery of antidepressants: a winding path. *Experientia* **47**, 4–8.
3 Mdluli K, Slayden RA, Zhu Y, Ramaswamy S, Pan X, Mead D, Crane DD, Musser JM & Barry CE III

(1998) Inhibition of a *Mycobacterium tuberculosis* beta-ketoacyl ACP synthase by isoniazid. *Science* **280**, 1607–1610.
4 Kell DB (2002) Genotype:phenotype mapping: genes as computer programs. *Trends Genet* **18**, 555–559.
5 Drews J (2000) Drug discovery: a historical perspective. *Science* **287**, 1960–1964.
6 van der Greef J & McBurney RN (2005) Rescuing drug discovery: *in vivo* systems pathology and systems pharmacology. *Nat Rev Drug Discov* **4**, 961–967.
7 Kola I & Landis J (2004) Can the pharmaceutical industry reduce attrition rates? *Nat Rev Drug Discov* **3**, 711–715.
8 Kola I (2008) The state of innovation in drug development. *Clin Pharmacol Ther* **83**, 227–230.
9 Empfield JR & Leeson PD (2010) Lessons learned from candidate drug attrition. *IDrugs* **13**, 869–873.
10 Leeson PD & Empfield JR (2010) Reducing the risk of drug attrition associated with physicochemical properties. *Annu Rep Med Chem* **45**, 393–407.
11 Kwong E, Higgins J & Templeton AC (2011) Strategies for bringing drug delivery tools into discovery. *Int J Pharm* **412**, 1–7.
12 Kell DB & Oliver SG (2004) Here is the evidence, now what is the hypothesis? The complementary roles of inductive and hypothesis-driven science in the post-genomic era. *BioEssays* **26**, 99–105.
13 Elliott KC (2012) Epistemic and methodological iteration in scientific research. *Stud Hist Philos Sci* **43**, 376–382.
14 Leveson N (2004) A new accident model for engineering safer systems. *Saf Sci* **42**, 237–270.
15 Bornholdt S & Sneppen K (2000) Robustness as an evolutionary principle. *Proc Biol Sci* **267**, 2281–2286.
16 Morohashi M, Winn AE, Borisuk MT, Bolouri H, Doyle J & Kitano H (2002) Robustness as a measure of plausibility in models of biochemical networks. *J Theor Biol* **216**, 19–30.
17 Papin JA, Price ND, Wiback SJ, Fell DA & Palsson BØ (2003) Metabolic pathways in the post-genome era. *Trends Biochem Sci* **28**, 250–258.
18 Stelling J, Sauer U, Szallasi Z, Doyle FJ III & Doyle J (2004) Robustness of cellular functions. *Cell* **118**, 675–685.
19 Wagner A (2005) Robustness, evolvability, and neutrality. *FEBS Lett* **579**, 1772–1778.
20 Becker SA, Feist AM, Mo ML, Hannum G, Palsson BO & Herrgard MJ (2007) Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox. *Nat Protoc* **2**, 727–738.
21 Kim PJ, Lee DY, Kim TY, Lee KH, Jeong H, Lee SY & Park S (2007) Metabolite essentiality elucidates robustness of *Escherichia coli* metabolism. *Proc Natl Acad Sci USA* **104**, 13638–13642.
22 Kitano H (2007) A robustness-based approach to systems-oriented drug design. *Nat Rev Drug Discov* **6**, 202–210.
23 Daniels BC, Chen YJ, Sethna JP, Gutenkunst RN & Myers CR (2008) Sloppiness, robustness, and evolvability in systems biology. *Curr Opin Biotechnol* **19**, 389–395.
24 Lehár J, Krueger A, Zimmermann G & Borisy A (2008) High-order combination effects and biological robustness. *Mol Syst Biol* **4**, 215.
25 Wu Y, Zhang X, Yu J & Ouyang Q (2009) Identification of a topological characteristic responsible for the biological robustness of regulatory networks. *PLoS Comput Biol* **5**, e1000442.
26 Kell DB (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.
27 von Dassow G, Meir E, Munro EM & Odell GM (2000) The segment polarity network is a robust development module. *Nature* **406**, 188–192.
28 Eisenthal R & Cornish-Bowden A (1998) Prospects for antiparasitic drugs. The case of *Trypanosoma brucei*, the causative agent of African sleeping sickness. *J Biol Chem* **273**, 5500–5505.
29 Thomas S & Fell DA (1998) The role of multiple enzyme activation in metabolic flux control. *Adv Enzyme Regul* **38**, 65–85.
30 Park JH, Lee KH, Kim TY & Lee SY (2007) Metabolic engineering of *Escherichia coli* for the production of L-valine based on transcriptome analysis and *in silico* gene knockout simulation. *Proc Natl Acad Sci USA* **104**, 7797–7802.
31 Park JH, Lee SY, Kim TY & Kim HU (2008) Application of systems biology for bioprocess development. *Trends Biotechnol* **26**, 404–412.
32 Becker J, Zelder O, Häfner S, Schröder H & Wittmann C (2011) From zero to hero–design-based systems metabolic engineering of *Corynebacterium glutamicum* for L-lysine production. *Metab Eng* **13**, 159–168.
33 Lee JW, Kim TY, Jang YS, Choi S & Lee SY (2011) Systems metabolic engineering for chemicals and materials. *Trends Biotechnol* **29**, 370–378.
34 Lee D, Smallbone K, Dunn WB, Murabito E, Winder CL, Kell DB, Mendes P & Swainston N (2012) Improving metabolic flux predictions using absolute gene expression data. *BMC Syst Biol* **6**, 73.
35 Lee JW, Na D, Park JM, Lee J, Choi S & Lee SY (2012) Systems metabolic engineering of microorganisms for natural and non-natural chemicals. *Nat Chem Biol* **8**, 536–546.

36 Kacser H & Burns JA (1973) The control of flux. In Rate Control of Biological Processes Symposium of the Society for Experimental Biology, Vol. 27 (Davies DD, ed.), pp. 65–104. Cambridge University Press, Cambridge.
37 Heinrich R & Rapoport TA (1974) A linear steady-state treatment of enzymatic chains. General properties, control and effector strength. *Eur J Biochem* **42**, 89–95.
38 Kell DB & Westerhoff HV (1986) Metabolic control theory: its role in microbiology and biotechnology. *FEMS Microbiol Rev* **39**, 305–320.
39 Giersch C (1988) Control analysis of metabolic networks. 1. Homogeneous functions and the summation theorems for control coefficients. *Eur J Biochem* **174**, 509–513.
40 Fell DA (1996) Understanding the Control of Metabolism. Portland Press, London.
41 Cornish-Bowden A, Hofmeyr J-HS & Cárdenas ML (1995) Strategies for manipulating metabolic fluxes in biotechnology. *Bioorg Chem* **23**, 439–449.
42 Heinrich R & Schuster S (1996) The Regulation of Cellular Systems. Chapman & Hall, New York.
43 Saltelli A, Tarantola S, Campolongo F & Ratto M (2004) Sensitivity Analysis in Practice: A Guide to Assessing Scientific Models. Wiley, New York.
44 Paolini GV, Shapland RH, van Hoorn WP, Mason JS & Hopkins AL (2006) Global mapping of pharmacological space. *Nat Biotechnol* **24**, 805–815.
45 Hopkins AL (2008) Network pharmacology: the next paradigm in drug discovery. *Nat Chem Biol* **4**, 682–690.
46 Metz JT & Hajduk PJ (2010) Rational approaches to targeted polypharmacology: creating and navigating protein-ligand interaction networks. *Curr Opin Chem Biol* **14**, 498–504.
47 Kupershmidt L, Weinreb O, Amit T, Mandel S, Bar-Am O & Youdim MB (2011) Novel molecular targets of the neuroprotective/neurorescue multimodal iron chelating drug M30 in the mouse brain. *Neuroscience* **189**, 345–358.
48 Weinreb O, Amit T, Bar-Am O & Youdim MBH (2011) A novel anti-Alzheimer's disease drug, ladostigil neuroprotective, multimodal brain-selective monoamine oxidase and cholinesterase inhibitor. *Int Rev Neurobiol* **100**, 191–215.
49 Kupershmidt L, Amit T, Bar-Am O, Youdim MBH & Weinreb O (2012) Neuroprotection by the multitarget iron chelator M30 on age-related alterations in mice. *Mech Ageing Dev* **133**, 267–274.
50 Weinreb O, Amit T, Bar-Am O & Youdim MBH (2012) Ladostigil: a novel multimodal neuroprotective drug with cholinesterase and brain-selective monoamine oxidase inhibitory activities for Alzheimer's disease treatment. *Curr Drug Targets* **13**, 483–494.
51 Pollak Y, Mechlovich D, Amit T, Bar-Am O, Manov I, Mandel SA, Weinreb O, Meyron-Holtz EG, Iancu TC & Youdim MB (2013) Effects of novel neuroprotective and neurorestorative multifunctional drugs on iron chelation and glucose metabolism. *J Neural Transm* **120**, 37–48.
52 Zimmermann GR, Lehár J & Keith CT (2007) Multi-target therapeutics: when the whole is greater than the sum of the parts. *Drug Discov Today* **12**, 34–42.
53 Lehár J, Krueger AS, Avery W, Heilbut AM, Johansen LM, Price ER, Rickles RJ, Short GF III, Staunton JE, Jin X *et al*. (2009) Synergistic drug combinations tend to improve therapeutically relevant selectivity. *Nat Biotechnol* **27**, 659–666.
54 Small BG, McColl BW, Allmendinger R, Pahle R, Lopez-Castejon G, Rothwell NJ, Knowles J, Mendes P, Brough D & Kell DB (2011) Efficient discovery of anti-inflammatory small molecule combinations using evolutionary computing. *Nat Chem Biol* **7**, 902–908.
55 Roth BL, Sheffler DJ & Kroeze WK (2004) Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat Rev Drug Discov* **3**, 353–359.
56 Mencher SK & Wang LG (2005) Promiscuous drugs compared to selective drugs (promiscuity can be a virtue). *BMC Clin Pharmacol* **5**, 3.
57 Hopkins AL, Mason JS & Overington JP (2006) Can we rationally design promiscuous drugs? *Curr Opin Struct Biol* **16**, 127–136.
58 Gregori-Puigjané E & Mestres J (2008) A ligand-based approach to mining the chemogenomic space of drugs. *Comb Chem High Throughput Screen* **11**, 669–676.
59 Morphy R & Rankovic Z (2007) Fragments, network biology and designing multiple ligands. *Drug Discov Today* **12**, 156–160.
60 Daws LC (2009) Unfaithful neurotransmitter transporters: focus on serotonin uptake and implications for antidepressant efficacy. *Pharmacol Ther* **121**, 89–99.
61 Hopkins AL (2009) Predicting promiscuity. *Nature* **462**, 167–168.
62 Kell DB (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.
63 Mestres J & Gregori-Puigjané E (2009) Conciliating binding efficiency and polypharmacology. *Trends Pharmacol Sci* **30**, 470–474.
64 Park K, Lee S, Ahn HS & Kim D (2009) Predicting the multi-modal binding propensity of small molecules: towards an understanding of drug promiscuity. *Mol BioSyst* **5**, 844–853.
65 Hu Y & Bajorath J (2010) Polypharmacology directed compound data mining: identification of promiscuous chemotypes with different activity profiles and

comparison to approved drugs. *J Chem Inf Model* **50**, 2112–2118.
66 Yang L, Wang KJ, Wang LS, Jegga AG, Qin SY, He G, Chen J, Xiao Y & He L (2011) Chemical-protein interactome and its application in off-target identification. *Interdiscip Sci* **3**, 22–30.
67 Simon Z, Peragovics Á, Vigh-Smeller M, Csukly G, Tombor L, Yang Z, Zahoránszky-Köhalmi G, Végner L, Jelinek B, Hári P *et al*. (2012) Drug effect prediction by polypharmacology-based interaction profiling. *J Chem Inf Model* **52**, 134–145.
68 Morphy JR & Harris CJ (2012) Designing Multi-Target Drugs. RSC Publishing, London.
69 Csermely P, Korcsmáros T, Kiss HJM, London G & Nussinov R (2013) Structure and dynamics of molecular networks: a novel paradigm of drug discovery. A comprehensive review. *Pharmacol Ther* doi:10.1016/j.pharmthera.2013.01.016
70 van der Greef J (2005) Systems biology, connectivity and the future of medicine. *Syst Biol* **152**, 174–178.
71 Berger SI & Iyengar R (2009) Network analyses in systems pharmacology. *Bioinformatics* **25**, 2466–2472.
72 Wist AD, Berger SI & Iyengar R (2009) Systems pharmacology and genome medicine: a future perspective. *Genome Med* **1**, 11.
73 Allerheiligen SR (2010) Next-generation model-based drug discovery and development: quantitative and systems pharmacology. *Clin Pharmacol Ther* **88**, 135–137.
74 Berger SI & Iyengar R (2010) Role of systems pharmacology in understanding drug adverse events. *Wiley Interdiscip Rev Syst Biol Med* **3**, 129–135.
75 Taboureau O, Nielsen SK, Audouze K, Weinhold N, Edsgärd D, Roque FS, Kouskoumvekaki I, Bora A, Curpan R, Jensen TS *et al*. (2010) ChemProt: a disease chemical biology database. *Nucleic Acids Res* **39**, D367–372.
76 Yang R, Niepel M, Mitchison TK & Sorger PK (2010) Dissecting variability in responses to cancer chemotherapy through systems pharmacology. *Clin Pharmacol Ther* **88**, 34–38.
77 van der Graaf PH & Benson N (2011) Systems pharmacology: bridging systems biology and pharmacokinetics-pharmacodynamics (PKPD) in drug discovery and development. *Pharm Res* **28**, 1460–1464.
78 Agoram BM & Demin O (2012) Integration not isolation: arguing the case for quantitative and systems pharmacology in drug discovery and development. *Drug Discov Today* **16**, 1031–1036.
79 Antman E, Weiss S & Loscalzo J (2012) Systems pharmacology, pharmacogenetics, and clinical trial design in network medicine. *Wiley Interdiscip Rev Syst Biol Med* **4**, 367–383.
80 Benson N, Cucurull-Sanchez L, Demin O, Smirnov S & van der Graaf P (2012) Reducing systems biology to practice in pharmaceutical company research; selected case studies. *Adv Exp Med Biol* **736**, 607–615.
81 Cucurull-Sanchez L, Spink KG & Moschos SA (2012) Relevance of systems pharmacology in drug discovery. *Drug Discov Today* **17**, 665–670.
82 Dar AC, Das TK, Shokat KM & Cagan RL (2012) Chemical genetic discovery of targets and anti-targets for cancer polypharmacology. *Nature* **486**, 80–84.
83 Hansen J, Zhao S & Iyengar R (2012) Systems pharmacology of complex diseases. *Ann NY Acad Sci* **1245**, E1–5.
84 Rostami-Hodjegan A (2012) Physiologically based pharmacokinetics joined with *in vitro-in vivo* extrapolation of ADME: a marriage under the arch of systems pharmacology. *Clin Pharmacol Ther* **92**, 50–61.
85 Winter GE, Rix U, Carlson SM, Gleixner KV, Grebien F, Gridling M, Müller AC, Breitwieser FP, Bilban M, Colinge J *et al*. (2012) Systems-pharmacology dissection of a drug synergy in imatinib-resistant CML. *Nat Chem Biol* **8**, 905–912.
86 Zhao S & Iyengar R (2012) Systems pharmacology: network analysis to identify multiscale mechanisms of drug action. *Annu Rev Pharmacol Toxicol* **52**, 505–521.
87 Bai JP & Abernethy DR (2013) Systems pharmacology to predict drug toxicity: integration across levels of biological organization. *Annu Rev Pharmacol Toxicol* **53**, 451–473.
88 Kell DB & Goodacre R (2013) Metabolomics and systems pharmacology: why and how to model the human metabolic network for drug discovery. *Drug Discov Today*, in press.
89 Auffray C, Chen Z & Hood L (2009) Systems medicine: the future of medical genomics and healthcare. *Genome Med* **1**, 2.
90 Capobianco E (2012) Ten challenges for systems medicine. *Front Genet* **3**, 193.
91 Hood L & Flores M (2012) A personal view on systems medicine and the emergence of proactive P4 medicine: predictive, preventive, personalized and participatory. *New Biotechnol* **29**, 613–624.
92 Mardinoglu A & Nielsen J (2012) Systems medicine and metabolic modelling. *J Intern Med* **271**, 142–154.
93 Wolkenhauer O, Auffray C, Jaster R, Steinhoff G & Dammann O (2013) The road from systems biology to systems medicine. *Pediatr Res* **73**, 502–507.
94 Henney A & Superti-Furga G (2008) A network solution. *Nature* **455**, 730–731.
95 Kell DB (2012) Scientific discovery as a combinatorial optimisation problem: how best to navigate the landscape of possible experiments? *BioEssays* **34**, 236–244.

96 Emperor of China Huang Ti & Maoshing Ni (Translator) (1995) Neijing Suwen (The Yellow Emperor's Classic of Medicine). Shambala Publications, Boston, MA.
97 Sadiq SK, Mazzeo MD, Zasada SJ, Manos S, Stoica I, Gale CV, Watson SJ, Kellam P, Brew S & Coveney PV (2008) Patient-specific simulation as a basis for clinical decision-making. *Philos Transact A Math Phys Eng Sci* **366**, 3199–3219.
98 Holmes MV, Shah T, Vickery C, Smeeth L, Hingorani AD & Casas JP (2009) Fulfilling the promise of personalized medicine? Systematic review and field synopsis of pharmacogenetic studies. *PLoS One* **4**, e7960.
99 Squassina A, Manchia M, Manolopoulos VG, Artac M, Lappa-Manakou C, Karkabouna S, Mitropoulos K, Del Zompo M & Patrinos GP (2010) Realities and expectations of pharmacogenomics and personalized medicine: impact of translating genetic knowledge into clinical practice. *Pharmacogenomics* **11**, 1149–1167.
100 Johnson SK, Heuck CJ, Albino AP, Qu P, Zhang Q, Barlogie B & Shaughnessy JD Jr (2011) The use of molecular-based risk stratification and pharmacogenomics for outcome prediction and personalized therapeutic management of multiple myeloma. *Int J Hematol* **94**, 321–333.
101 Ventola CL (2011) Pharmacogenomics in clinical practice: reality and expectations. *P & T* **36**, 412–450.
102 Wei CY, Lee MTM & Chen YT (2012) Pharmacogenomics of adverse drug reactions: implementing personalized medicine. *Hum Mol Genet* **21**, R58–R65.
103 Henney AM (2012) The promise and challenge of personalized medicine: aging populations, complex diseases, and unmet medical need. *Croat Med J* **53**, 207–210.
104 Williams RJ (1956) Biochemical Individuality. John Wiley, New York.
105 Broadhurst D & Kell DB (2006) Statistical strategies for avoiding false discoveries in metabolomics and related experiments. *Metabolomics* **2**, 171–196.
106 Krejsa CM, Horvath D, Rogalski SL, Penzotti JE, Mao B, Barbosa F & Migeon JC (2003) Predicting ADME properties and side effects: The BioPrint approach. *Curr Opin Drug Discov Devel* **6**, 470–480.
107 Ekins S (2004) Predicting undesirable drug interactions with promiscuous proteins *in silico*. *Drug Discov Today* **9**, 276–285.
108 Fliri AF, Loging WT, Thadeio PF & Volkmann RA (2005) Analysis of drug-induced effect patterns to link structure and side effects of medicines. *Nat Chem Biol* **1**, 389–397.
109 Li H, Gao Z, Kang L, Zhang H, Yang K, Yu K, Luo X, Zhu W, Chen K, Shen J *et al.* (2006) TarFisDock: a web server for identifying drug targets with docking approach. *Nucleic Acids Res* **34**, W219–224.
110 Azzaoui K, Hamon J, Faller B, Whitebread S, Jacoby E, Bender A, Jenkins JL & Urban L (2007) Modeling promiscuity based on *in vitro* safety pharmacology profiling data. *ChemMedChem* **2**, 874–880.
111 Keiser MJ, Roth BL, Armbruster BN, Ernsberger P, Irwin JJ & Shoichet BK (2007) Relating protein pharmacology by ligand chemistry. *Nat Biotechnol* **25**, 197–206.
112 Yıldırım MA, Goh KI, Cusick ME, Barabási AL & Vidal M (2007) Drug-target network. *Nat Biotechnol* **25**, 1119–1126.
113 Campillos M, Kuhn M, Gavin AC, Jensen LJ & Bork P (2008) Drug target identification using side-effect similarity. *Science* **321**, 263–266.
114 Karaman MW, Herrgard S, Treiber DK, Gallant P, Atteridge CE, Campbell BT, Chan KW, Ciceri P, Davis MI, Edeen PT *et al.* (2008) A quantitative analysis of kinase inhibitor selectivity. *Nat Biotechnol* **26**, 127–132.
115 Peterson RT (2008) Chemical biology and the limits of reductionism. *Nat Chem Biol* **4**, 635–638.
116 Keiser MJ, Setola V, Irwin JJ, Laggner C, Abbas AI, Hufeisen SJ, Jensen NH, Kuijer MB, Matos RC, Tran TB *et al.* (2009) Predicting new molecular targets for known drugs. *Nature* **462**, 175–181.
117 Keiser MJ & Hert J (2009) Off-target networks derived from ligand set similarity. *Methods Mol Biol* **575**, 195–205.
118 Bantscheff M, Scholten A & Heck AJR (2009) Revealing promiscuous drug-target interactions by chemical proteomics. *Drug Discov Today* **14**, 1021–1029.
119 Peters JU, Schnider P, Mattei P & Kansy M (2009) Pharmacological promiscuity: dependence on compound properties and target specificity in a set of recent Roche compounds. *ChemMedChem* **4**, 680–686.
120 Garcia-Serna R & Mestres J (2010) Anticipating drug side effects by comparative pharmacology. *Expert Opin Drug Metab Toxicol* **6**, 1253–1263.
121 Keiser MJ, Irwin JJ & Shoichet BK (2010) The chemical basis of pharmacology. *Biochemistry* **49**, 10267–10276.
122 Waring MJ (2010) Lipophilicity in drug discovery. *Expert Opin Drug Discov* **5**, 235–248.
123 Allen JA & Roth BL (2011) Strategies to discover unexpected targets for drugs active at G protein-coupled receptors. *Annu Rev Pharmacol Toxicol* **51**, 117–144.
124 von Eichborn J, Murgueitio MS, Dunkel M, Koerner S, Bourne PE & Preissner R (2011) PROMISCUOUS: a database for network-based drug-repositioning. *Nucleic Acids Res* **39**, D1060–D1066.
125 Garcia-Serna R, Ursu O, Oprea TI & Mestres J (2011) iPHACE: integrative navigation in pharmacological space. *Bioinformatics* **26**, 985–986.

126 Leeson PD & St-Gallay SA (2011) The influence of the 'organizational factor' on compound quality in drug discovery. *Nat Rev Drug Discov* **10**, 749–765.
127 Leeson PD, St-Gallay SA & Wenlock MC (2011) Impact of ion class and time on oral drug molecular properties. *MedChemComm* **2**, 91–105.
128 Meanwell NA (2011) Improving drug candidates by design: a focus on physicochemical properties as a means of improving compound disposition and safety. *Chem Res Toxicol* **24**, 1420–1456.
129 Mestres J, Seifert SA & Oprea TI (2011) Linking pharmacology to clinical reports: cyclobenzaprine and its possible association with serotonin syndrome. *Clin Pharmacol Ther* **90**, 662–665.
130 Metz JT, Johnson EF, Soni NB, Merta PJ, Kifle L & Hajduk PJ (2011) Navigating the kinome. *Nat Chem Biol* **7**, 200–202.
131 Nisius B & Bajorath J (2011) Mapping of pharmacological space. *Expert Opin Drug Discov* **6**, 1–7.
132 Nonell-Canals A & Mestres J (2011) *In silico* target profiling of one billion molecules. *Mol Inform* **30**, 405–409.
133 Oprea TI, Nielsen SK, Ursu O, Yang JJ, Taboureau O, Mathias SL, Kouskoumvekaki L, Sklar LA & Bologa CG (2011) Associating drugs, targets and clinical outcomes into an integrated network affords a new platform for computer-aided drug repurposing. *Mol Inform* **30**, 100–111.
134 Pérez-Nueno VI & Ritchie DW (2011) Using consensus-shape clustering to identify promiscuous ligands and protein targets and to choose the right query for shape-based virtual screening. *J Chem Inf Model* **51**, 1233–1248.
135 Prado-Prado F, García-Mera X, Escobar M, Sobarzo-Sánchez E, Yañez M, Riera-Fernandez P & González-Díaz H (2011) 2D MI-DRAGON: a new predictor for protein-ligands interactions and theoretic-experimental studies of US FDA drug-target network, oxoisoaporphine inhibitors for MAO-A and human parasite proteins. *Eur J Med Chem* **46**, 5838–5851.
136 Xie L, Xie L & Bourne PE (2011) Structure-based systems biology for analyzing off-target binding. *Curr Opin Struct Biol* **21**, 189–199.
137 Zolk O & Fromm MF (2011) Transporter-mediated drug uptake and efflux: important determinants of adverse drug reactions. *Clin Pharmacol Ther* **89**, 798–805.
138 Besnard J, Ruda GF, Setola V, Abecassis K, Rodriguiz RM, Huang XP, Norval S, Sassano MF, Shin AI, Webster LA *et al.* (2012) Automated design of ligands to polypharmacological profiles. *Nature* **492**, 215–220.
139 Colinge J, Rix U, Bennett KL & Superti-Furga G (2012) Systems biology analysis of protein-drug interactions. *Proteomics Clin Appl* **6**, 102–116.
140 Drewes G & Bantscheff M (2012) Chemical Proteomics. Springer, Berlin.
141 Hawley SA, Fullerton MD, Ross FA, Schertzer JD, Chevtzoff C, Walker KJ, Peggie MW, Zibrova D, Green KA, Mustard KJ *et al.* (2012) The ancient drug salicylate directly activates AMP-activated protein kinase. *Science* **336**, 918–922.
142 Lounkine E, Keiser MJ, Whitebread S, Mikhailov D, Hamon J, Jenkins JL, Lavan P, Weber E, Doak AK, Côté S *et al.* (2012) Large-scale prediction and testing of drug activity on side-effect targets. *Nature* **486**, 361–367.
143 Pérez-Nueno VI & Ritchie DW (2012) Identifying and characterizing promiscuous targets: implications for virtual screening. *Expert Opin Drug Discov* **7**, 1–17.
144 Peters JU, Hert J, Bissantz C, Hillebrecht A, Gerebtzoff G, Bendels S, Tillier F, Migeon J, Fischer H, Guba W *et al.* (2012) Can we discover pharmacological promiscuity early in the drug discovery process? *Drug Discov Today* **17**, 325–335.
145 Xie L, Xie L, Kinnings SL & Bourne PE (2012) Novel computational approaches to polypharmacology as a means to define responses to individual drugs. *Annu Rev Pharmacol Toxicol* **52**, 361–379.
146 Wellendorph P, Johansen LD & Brauner-Osborne H (2009) Molecular pharmacology of promiscuous seven transmembrane receptors sensing organic nutrients. *Mol Pharmacol* **76**, 453–465.
147 Li X, Gianoulis TA, Yip KY, Gerstein M & Snyder M (2010) Extensive *in vivo* metabolite-protein interactions revealed by large-scale systematic analyses. *Cell* **143**, 639–650.
148 Li X & Snyder M (2011) Metabolites as global regulators: a new view of protein regulation: systematic investigation of metabolite-protein interactions may help bridge the gap between genome-wide association studies and small molecule screening studies. *BioEssays* **33**, 485–489.
149 Kell DB (2011) Metabolites do social networking. *Nat Chem Biol* **7**, 7–8.
150 Mestres J, Gregori-Puigjané E, Valverde S & Solé RV (2009) The topology of drug-target interaction networks: implicit dependence on drug properties and target families. *Mol BioSyst* **5**, 1051–1057.
151 Kell DB, Dobson PD, Bilsland E & Oliver SG (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.
152 Orengo CA & Thornton JM (2005) Protein families and their evolution-a structural perspective. *Annu Rev Biochem* **74**, 867–900.
153 Khersonsky O & Tawfik DS (2010) Enzyme promiscuity: evolutionary and mechanistic aspects. In Comprehensive Natural Products II Chemistry and

Biology (Mander L & Lui H-W, eds), pp. 48–90. Elsevier, Oxford.
154 Burns AR, Wallace IM, Wildenhain J, Tyers M, Giaever G, Bader GD, Nislow C, Cutler SR & Roy PJ (2010) A predictive model for drug bioaccumulation and bioactivity in *Caenorhabditis elegans*. *Nat Chem Biol* **6**, 549–557.
155 Wallace IM, Urbanus ML, Luciani GM, Burns AR, Han MK, Wang H, Arora K, Heisler LE, Proctor M, St Onge RP *et al*. (2011) Compound prioritization methods increase rates of chemical probe discovery in model organisms. *Chem Biol* **18**, 1273–1283.
156 Couzin J (2008) Cholesterol veers off script. *Science* **322**, 220–223.
157 Wagner BK, Kitami T, Gilbert TJ, Peck D, Ramanathan A, Schreiber SL, Golub TR & Mootha VK (2008) Large-scale chemical dissection of mitochondrial function. *Nat Biotechnol* **26**, 343–351.
158 Libby P & Aikawa M (2002) Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. *Nat Med* **8**, 1257–1262.
159 Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG *et al*. (2008) Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med* **359**, 2195–2207.
160 Mira E & Manes S (2009) Immunomodulatory and anti-inflammatory activities of statins. *Endocr Metab Immune Disord Drug Targets* **9**, 237–247.
161 Montecucco F & Mach F (2009) Update on statin-mediated anti-inflammatory activities in atherosclerosis. *Semin Immunopathol* **31**, 127–142.
162 Bereswill S, Munoz M, Fischer A, Plickert R, Haag LM, Otto B, Kuhl AA, Loddenkemper C, Gobel UB & Heimesaat MM (2010) Anti-inflammatory effects of resveratrol, curcumin and simvastatin in acute small intestinal inflammation. *PLoS One* **5**, e15099.
163 Dinarello CA (2010) Anti-inflammatory agents: present and future. *Cell* **140**, 935–950.
164 Bu DX, Griffin G & Lichtman AH (2011) Mechanisms for the anti-inflammatory effects of statins. *Curr Opin Lipidol* **22**, 165–170.
165 Antonopoulos AS, Margaritis M, Lee R, Channon K & Antoniades C (2012) Statins as anti-inflammatory agents in atherogenesis: molecular mechanisms and lessons from the recent clinical trials. *Curr Pharm Des* **18**, 1519–1530.
166 Leeson PD & Springthorpe B (2007) The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat Rev Drug Discov* **6**, 881–890.
167 Whitlock GA, Fish PV, Fray MJ, Stobie A & Wakenhut F (2008) Pyridyl-phenyl ether monoamine reuptake inhibitors: impact of lipophilicity on dual SNRI pharmacology and off-target promiscuity. *Bioorg Med Chem Lett* **18**, 2896–2899.
168 Price DA, Blagg J, Jones L, Greene N & Wager T (2009) Physicochemical drug properties associated with *in vivo* toxicological outcomes: a review. *Expert Opin Drug Metab Toxicol* **5**, 921–931.
169 Hann MM (2011) Molecular obesity, potency and other addictions in drug discovery. *MedChemComm* **2**, 349–355.
170 Gleeson MP, Hersey A, Montanari D & Overington J (2011) Probing the links between in vitro potency, ADMET and physicochemical parameters. *Nat Rev Drug Discov* **10**, 197–208.
171 Good AC, Liu J, Hirth B, Asmussen G, Xiang Y, Biemann HP, Bishop KA, Fremgen T, Fitzgerald M, Gladysheva T *et al*. (2012) Implications of promiscuous Pim-1 kinase fragment inhibitor hydrophobic interactions for fragment-based drug design. *J Med Chem* **55**, 2641–2648.
172 Hann MM & Keserü GM (2012) Finding the sweet spot: the role of nature and nurture in medicinal chemistry. *Nat Rev Drug Discov* **11**, 355–365.
173 Lipinski CA, Lombardo F, Dominy BW & Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* **23**, 3–25.
174 Lipinski CA, Lombardo F, Dominy BW & Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* **46**, 3–26.
175 Ayouni L, Cazorla G, Chaillou D, Herbreteau B, Rudaz S, Lanteri P & Carrupt PA (2005) Fast determination of lipophilicity by HPLC. *Chromatographia* **62**, 251–255.
176 Gocan S, Cimpan C & Comer J (2006) Lipophilicity measurements by liquid chromatography. *Adv Chromatogr* **44**, 79–176.
177 Demare S, Slater B, Lacombe G, Breuzin D & Dini C (2007) Accurate automated log P-o/w measurement by gradient-flow liquid-liquid partition chromatography Part 1. Neutral compounds. *J Chromatogr A* **1175**, 16–23.
178 Scherrer RA & Howard SM (1977) Use of distribution coefficients in quantitative structure-activity-relationships. *J Med Chem* **20**, 53–58.
179 Wendt MD (2012) The discovery of navitoclax, a Bcl-2 family inhibitor. *Top Med Chem* **8**, 231–258.
180 Vieth M & Sutherland JJ (2006) Dependence of molecular properties on proteomic family for marketed oral drugs. *J Med Chem* **49**, 3451–3453.
181 Abad-Zapatero C (2007) A sorcerer's apprentice and the rule of five: from rule-of-thumb to commandment and beyond. *Drug Discov Today* **12**, 995–997.

182 Oprea TI, Allu TK, Fara DC, Rad RF, Ostopovici L & Bologa CG (2007) Lead-like, drug-like or 'pub-like': how different are they? *J Comput Aided Mol Des* **21**, 113–119.
183 Zhang MQ & Wilkinson B (2007) Drug discovery beyond the 'rule-of-five'. *Curr Opin Biotechnol* **18**, 478–488.
184 Giménez BG, Santos MS, Ferrarini M & Fernandes JPS (2010) Evaluation of blockbuster drugs under the Rule-of-five. *Pharmazie* **65**, 148–152.
185 Ridder L, Wang H, de Vlieg J & Wagener M (2011) Revisiting the rule of five on the basis of pharmacokinetic data from rat. *ChemMedChem* **6**, 1967–1970.
186 Petit J, Meurice N, Kaiser C & Maggiora G (2012) Softening the rule of five-where to draw the line? *Bioorg Med Chem* **20**, 5343–5351.
187 Baker DD, Chu M, Oza U & Rajgarhia V (2007) The value of natural products to future pharmaceutical discovery. *Nat Prod Rep* **24**, 1225–1244.
188 Feher M & Schmidt JM (2003) Property distributions: differences between drugs, natural products, and molecules from combinatorial chemistry. *J Chem Inf Comput Sci* **43**, 218–227.
189 Larsson J, Gottfries J, Bohlin L & Backlund A (2005) Expanding the ChemGPS chemical space with natural products. *J Nat Prod* **68**, 985–991.
190 Sprous DG & Salemme FR (2007) A comparison of the chemical properties of drugs and FEMA/FDA notified GRAS chemical compounds used in the food industry. *Food Chem Toxicol* **45**, 1419–1427.
191 Ertl P, Roggo S & Schuffenhauer A (2008) Natural product-likeness score and its application for prioritization of compound libraries. *J Chem Inf Model* **48**, 68–74.
192 Ertl P & Schuffenhauer A (2008) Cheminformatics analysis of natural products: lessons from nature inspiring the design of new drugs. *Prog Drug Res* **66**, 217, 219–235.
193 Grabowski K, Baringhaus KH & Schneider G (2008) Scaffold diversity of natural products: inspiration for combinatorial library design. *Nat Prod Rep* **25**, 892–904.
194 Khanna V & Ranganathan S (2009) Physicochemical property space distribution among human metabolites, drugs and toxins. *BMC Bioinformatics* **10**, S10.
195 Osada H & Hertweck C (2009) Exploring the chemical space of microbial natural products. *Curr Opin Chem Biol* **13**, 133–134.
196 Renner S, van Otterlo WA, Dominguez Seoane M, Mocklinghoff S, Hofmann B, Wetzel S, Schuffenhauer A, Ertl P, Oprea TI, Steinhilber D *et al.* (2009) Bioactivity-guided mapping and navigation of chemical space. *Nat Chem Biol* **5**, 585–592.
197 Rosén J, Gottfries J, Muresan S, Backlund A & Oprea TI (2009) Novel chemical space exploration via natural products. *J Med Chem* **52**, 1953–1962.
198 Singh N, Guha R, Giulianotti MA, Pinilla C, Houghten RA & Medina-Franco JL (2009) Chemoinformatic analysis of combinatorial libraries, drugs, natural products, and molecular libraries small molecule repository. *J Chem Inf Model* **49**, 1010–1024.
199 Bauer RA, Wurst JM & Tan DS (2010) Expanding the range of 'druggable' targets with natural product-based libraries: an academic perspective. *Curr Opin Chem Biol* **14**, 308–314.
200 Clemons PA, Bodycombe NE, Carrinski HA, Wilson JA, Shamji AF, Wagner BK, Koehler AN & Schreiber SL (2010) Small molecules of different origins have distinct distributions of structural complexity that correlate with protein-binding profiles. *Proc Natl Acad Sci USA* **107**, 18787–18792.
201 Faller B, Ottaviani G, Ertl P, Berellini G & Collis A (2011) Evolution of the physicochemical properties of marketed drugs: can history foretell the future? *Drug Discov Today* **16**, 976–984.
202 Chen HM, Engkvist O, Blomberg N & Li J (2012) A comparative analysis of the molecular topologies for drugs, clinical candidates, natural products, human metabolites and general bioactive compounds. *MedChemComm* **3**, 312–321.
203 Jayaseelan KV, Moreno P, Truszkowski A, Ertl P & Steinbeck C (2012) Natural product-likeness score revisited: an open-source, open-data implementation. *BMC Bioinformatics* **13**, 106.
204 López-Vallejo F, Giulianotti MA, Houghten RA & Medina-Franco JL (2012) Expanding the medicinally relevant chemical space with compound libraries. *Drug Discov Today* **17**, 718–726.
205 Chin YW, Balunas MJ, Chai HB & Kinghorn AD (2006) Drug discovery from natural sources. *AAPS J* **8**, E239–253.
206 Newman DJ & Cragg GM (2007) Natural products as sources of new drugs over the last 25 years. *J Nat Prod* **70**, 461–477.
207 Butler MS (2008) Natural products to drugs: natural product-derived compounds in clinical trials. *Nat Prod Rep* **25**, 475–516.
208 Ganesan A (2008) The impact of natural products upon modern drug discovery. *Curr Opin Chem Biol* **12**, 306–317.
209 Harvey AL (2008) Natural products in drug discovery. *Drug Discov Today* **13**, 894–901.
210 Rishton GM (2008) Natural products as a robust source of new drugs and drug leads: past successes and present day issues. *Am J Cardiol* **101**, 43D–49D.
211 Li JW-H & Vederas JC (2009) Drug discovery and natural products: end of an era or an endless frontier? *Science* **325**, 161–165.

212 Kingston DGI (2011) Plant-derived natural products as anticancer agents. In *Cancer Management in Man: Chemotherapy, Biological Therapy, Hyperthermia and Supporting Measures* (Minev BR, ed) pp, 3–23 Springer, New York.
213 Kell DB, Kaprelyants AS & Grafen A (1995) On pheromones, social behaviour and the functions of secondary metabolism in bacteria. *Trends Ecol Evol* **10**, 126–129.
214 Burgess JG, Jordan EM, Bregu M, Mearns-Spragg A & Boyd KG (1999) Microbial antagonism: a neglected avenue of natural products research. *J Biotechnol* **70**, 27–32.
215 Peiris D, Dunn WB, Brown M, Kell DB, Roy I & Hedger JN (2008) Metabolite profiles of interacting mycelial fronts differ for pairings of the wood decay basidiomycete fungus, *Stereum hirsutum* with its competitors *Coprinus micaceus* and *Coprinus disseminatus*. *Metabolomics* **4**, 52–62.
216 Bohlin L, Goransson U, Alsmark C, Wedén C & Backlund A (2010) Natural products in modern life science. *Phytochem Rev* **9**, 279–301.
217 Bumpus SB, Evans BS, Thomas PM, Ntai I & Kelleher NL (2009) A proteomics approach to discovering natural products and their biosynthetic pathways. *Nat Biotechnol* **27**, 951–956.
218 Rochfort S (2005) Metabolomics reviewed: a new 'omics' platform technology for systems biology and implications for natural products research. *J Nat Prod* **68**, 1813–1820.
219 Li MH, Ung PM, Zajkowski J, Garneau-Tsodikova S & Sherman DH (2009) Automated genome mining for natural products. *BMC Bioinformatics* **10**, 185.
220 Challis GL (2008) Genome mining for novel natural product discovery. *J Med Chem* **51**, 2618–2628.
221 Holinsworth B & Martin JD (2009) Siderophore production by marine-derived fungi. *Biometals* **22**, 625–632.
222 Perron NR & Brumaghim JL (2009) A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. *Cell Biochem Biophys* **53**, 75–100.
223 Perron NR, Wang HC, Deguire SN, Jenkins M, Lawson M & Brumaghim JL (2010) Kinetics of iron oxidation upon polyphenol binding. *Dalton Trans* **39**, 9982–9987.
224 Sharpe PC, Richardson DR, Kalinowski DS & Bernhardt PV (2011) Synthetic and natural products as iron chelators. *Curr Top Med Chem* **11**, 591–607.
225 Funke C, Schneider SA, Berg D & Kell DB (2013) Genetics and iron in the systems biology of Parkinson's disease and some related disorders. *Neurochem Int* **62**, 637–652.
226 Al-Awqati Q (1999) One hundred years of membrane permeability: does Overton still rule? *Nat Cell Biol* **1**, E201–E202.
227 Dupuy AD & Engelman DM (2008) Protein area occupancy at the center of the red blood cell membrane. *Proc Natl Acad Sci USA* **105**, 2848–2852.
228 Dobson PD & Kell DB (2008) Carrier-mediated cellular uptake of pharmaceutical drugs: an exception or the rule? *Nat Rev Drug Discov* **7**, 205–220.
229 Kell DB & Dobson PD (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 Proc Int Beilstein Symposium on Systems Chemistry (Hicks MG & Kettner C, eds), pp. 149–168. http://www.beilstein-institut.de/Bozen2008/Proceedings/Kell/Kell.pdf. Logos Verlag, Berlin.
230 Dobson P, Lanthaler K, Oliver SG & Kell DB (2009) Implications of the dominant role of cellular transporters in drug uptake. *Curr Top Med Chem* **9**, 163–184.
231 Kell DB, Dobson PD & Oliver SG (2011) Pharmaceutical drug transport: the issues and the implications that it is essentially carrier-mediated only. *Drug Discov Today* **16**, 704–714.
232 Bi YA, Kimoto E, Sevidal S, Jones HM, Barton HA, Kempshall S, Whalen KM, Zhang H, Ji C, Fenner KS *et al*. (2012) *In vitro* evaluation of hepatic transporter-mediated clinical drug-drug interactions: hepatocyte model optimization and retrospective investigation. *Drug Metab Dispos* **40**, 1085–1092.
233 Borbath I, Verbrugghe L, Lai R, Gigot JF, Humblet Y, Piessevaux H & Sempoux C (2012) Human equilibrative nucleoside transporter 1 (hENT1) expression is a potential predictive tool for response to gemcitabine in patients with advanced cholangiocarcinoma. *Eur J Cancer* **48**, 990–996.
234 Cheng CY & Mruk DD (2012) The blood-testis barrier and its implications for male contraception. *Pharmacol Rev* **64**, 16–64.
235 Clarke JD & Cherrington NJ (2012) Genetics or environment in drug transport: the case of organic anion transporting polypeptides and adverse drug reactions. *Expert Opin Drug Metab Toxicol* **8**, 349–360.
236 DeGorter MK, Xia CQ, Yang JJ & Kim RB (2012) Drug transporters in drug efficacy and toxicity. *Annu Rev Pharmacol Toxicol* **52**, 249–273.
237 Delespaux V & de Koning HP (2012) Transporters in antiparasitic drug development and resistance. In Antiparasitic and Antibacterial Drug Discovery: Trypanosomatidae (Flohe L, Koch O & Jäger T, eds), in press. Wiley-Blackwell, London.
238 Ekins S, Polli JE, Swaan PW & Wright SH (2012) Computational modeling to accelerate the identification of substrates and inhibitors for transporters that affect drug disposition. *Clin Pharmacol Ther* **92**, 661–665.
239 Fardel O, Kolasa E & Le Vee M (2012) Environmental chemicals as substrates, inhibitors or

inducers of drug transporters: implication for toxicokinetics, toxicity and pharmacokinetics. *Expert Opin Drug Metab Toxicol* **8**, 29–46.
240 Fromm MF (2012) Prediction of transporter-mediated drug-drug interactions using endogenous compounds. *Clin Pharmacol Ther* **92**, 546–548.
241 Gallegos TF, Martovetsky G, Kouznetsova V, Bush KT & Nigam SK (2012) Organic anion and cation SLC22 'drug' transporter (Oat1, Oat3, and Oct1) regulation during development and maturation of the kidney proximal tubule. *PLoS One* **7**, e40796.
242 Harwood MD, Neuhoff S, Carlson GL, Warhurst G & Rostami-Hodjegan A (2013) Absolute abundance and function of intestinal drug transporters: a prerequisite for fully mechanistic in vitro-in vivo extrapolation of oral drug absorption. *Biopharm Drug Dispos* **34**, 2–28.
243 Iusuf D, van de Steeg E & Schinkel AH (2012) Functions of OATP1A and 1B transporters in vivo: insights from mouse models. *Trends Pharmacol Sci* **33**, 100–108.
244 Karlgren M, Vildhede A, Norinder U, Wisniewski JR, Kimoto E, Lai YR, Haglund U & Artursson P (2012) Classification of inhibitors of hepatic organic anion transporting polypeptides (OATPs): influence of protein expression on drug–drug interactions. *J Med Chem* **55**, 4740–4763.
245 Kobayashi H, Murakami Y, Uemura K, Sudo T, Hashimoto Y, Kondo N & Sueda T (2012) Human equilibrative nucleoside transporter 1 expression predicts survival of advanced cholangiocarcinoma patients treated with gemcitabine-based adjuvant chemotherapy after surgical resection. *Ann Surg* **256**, 288–296.
246 Lai Y, Varma M, Feng B, Stephens JC, Kimoto E, El-Kattan A, Ichikawa K, Kikkawa H, Ono C, Suzuki A *et al*. (2012) Impact of drug transporter pharmacogenomics on pharmacokinetic and pharmacodynamic variability – considerations for drug development. *Expert Opin Drug Metab Toxicol* **8**, 723–743.
247 Li RW, Yang C, Sit AS, Lin SY, Ho EY & Leung GP (2012) Physiological and pharmacological roles of vascular nucleoside transporters. *J Cardiovasc Pharmacol* **59**, 10–15.
248 Mandery K, Glaeser H & Fromm MF (2012) Interaction of innovative small molecule drugs used for cancer therapy with drug transporters. *Br J Pharmacol* **165**, 345–362.
249 Morinaga S, Nakamura Y, Watanabe T, Mikayama H, Tamagawa H, Yamamoto N, Shiozawa M, Akaike M, Ohkawa S, Kameda Y *et al*. (2012) Immunohistochemical analysis of human equilibrative nucleoside transporter-1 (hENT1) predicts survival in resected pancreatic cancer patients treated with adjuvant gemcitabine monotherapy. *Ann Surg Oncol* **19** (Suppl 3), 558–564.
250 Morrissey KM, Wen CC, Johns SJ, Zhang L, Huang SM & Giacomini KM (2012) The UCSF-FDA TransPortal: a public drug transporter database. *Clin Pharmacol Ther* **92**, 545–546.
251 Mulgaonkar A, Venitz J & Sweet DH (2012) Fluoroquinolone disposition: identification of the contribution of renal secretory and reabsorptive drug transporters. *Expert Opin Drug Metab Toxicol* **8**, 553–569.
252 Murata Y, Hamada T, Kishiwada M, Ohsawa I, Mizuno S, Usui M, Sakurai H, Tabata M, Ii N, Inoue H *et al*. (2012) Human equilibrative nucleoside transporter 1 expression is a strong independent prognostic factor in UICC T3–T4 pancreatic cancer patients treated with preoperative gemcitabine-based chemoradiotherapy. *J Hepatobiliary Pancreat Sci* **19**, 413–425.
253 Nakanishi T & Tamai I (2012) Genetic polymorphisms of OATP transporters and their impact on intestinal absorption and hepatic disposition of drugs. *Drug Metab Pharmacokinet* **27**, 106–121.
254 Neuvonen PJ (2012) Towards Safer and more predictable drug treatment – reflections from studies of the first BCPT Prize Awardee. *Basic Clin Pharmacol Toxicol* **110**, 207–218.
255 Obaidat A, Roth M & Hagenbuch B (2012) The expression and function of organic anion transporting polypeptides in normal tissues and in cancer. *Annu Rev Pharmacol Toxicol* **52**, 135–151.
256 Roth M, Obaidat A & Hagenbuch B (2012) OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. *Br J Pharmacol* **165**, 1260–1287.
257 Saadatmand AR, Tadjerpisheh S, Brockmoller J & Tzvetkov MV (2012) The prototypic pharmacogenetic drug debrisoquine is a substrate of the genetically polymorphic organic cation transporter OCT1. *Biochem Pharmacol* **83**, 1427–1434.
258 Salomon JJ & Ehrhardt C (2012) Organic cation transporters in the blood-air barrier: expression and implications for pulmonary drug delivery. *Ther Deliv* **3**, 735–747.
259 Sissung TM, Troutman SM, Campbell TJ, Pressler HM, Sung H, Bates SE & Figg WD (2012) Transporter pharmacogenetics: transporter polymorphisms affect normal physiology, diseases, and pharmacotherapy. *Discov Med* **13**, 19–34.
260 Sprowl JA, Mikkelsen TS, Giovinazzo H & Sparreboom A (2012) Contribution of tumoral and host solute carriers to clinical drug response. *Drug Resist Updat* **15**, 5–20.
261 Sprowl JA & Sparreboom A (2012) Drug trafficking: recent advances in therapeutics and disease. *Clin Pharmacol Ther* **92**, 531–534.

262 Tamai I (2012) Oral drug delivery utilizing intestinal OATP transporters. *Adv Drug Deliv Rev* **64**, 508–514.
263 Umans RA & Taylor MR (2012) Zebrafish as a model to study drug transporters at the blood-brain barrier. *Clin Pharmacol Ther* **92**, 567–570.
264 Vadlapudi AD, Vadlapatla RK & Mitra AK (2012) Sodium dependent multivitamin transporter (SMVT): a potential target for drug delivery. *Curr Drug Targets* **13**, 994–1003.
265 Varma MVS, Lai Y, Feng B, Litchfield J, Goosen TC & Bergman A (2012) Physiologically based modeling of pravastatin transporter-mediated hepatobiliary disposition and drug-drug interactions. *Pharm Res* **29**, 2860–2873.
266 Yoshida K, Maeda K & Sugiyama Y (2012) Transporter-mediated drug-drug interactions involving OATP substrates: predictions based on *in vitro* inhibition studies. *Clin Pharmacol Ther* **91**, 1053–1064.
267 Zamek-Gliszczynski MJ, Hoffmaster KA, Tweedie DJ, Giacomini KM & Hillgren KM (2012) Highlights from the international transporter consortium second workshop. *Clin Pharmacol Ther* **92**, 553–556.
268 Lanthaler K, Bilsland E, Dobson P, Moss HJ, Pir P, Kell DB & Oliver SG (2011) Genome-wide assessment of the carriers involved in the cellular uptake of drugs: a model system in yeast. *BMC Biol* **9**, 70.
269 Alsford S, Turner DJ, Obado SO, Sanchez-Flores A, Glover L, Berriman M, Hertz-Fowler C & Horn D (2011) High-throughput phenotyping using parallel sequencing of RNA interference targets in the African trypanosome. *Genome Res* **21**, 915–924.
270 Baker N, Alsford S & Horn D (2011) Genome-wide RNAi screens in African trypanosomes identify the nifurtimox activator NTR and the eflornithine transporter AAT6. *Mol Biochem Parasitol* **176**, 55–57.
271 Schumann Burkard G, Jutzi P & Roditi I (2011) Genome-wide RNAi screens in bloodstream form trypanosomes identify drug transporters. *Mol Biochem Parasitol* **175**, 91–94.
272 Alsford S, Eckert S, Baker N, Glover L, Sanchez-Flores A, Leung KF, Turner DJ, Field MC, Berriman M & Horn D (2012) High-throughput decoding of antitrypanosomal drug efficacy and resistance. *Nature* **482**, 232–236.
273 Spratlin J, Sangha R, Glubrecht D, Dabbagh L, Young JD, Dumontet C, Cass C, Lai R & Mackey JR (2004) The absence of human equilibrative nucleoside transporter 1 is associated with reduced survival in patients with gemcitabine-treated pancreas adenocarcinoma. *Clin Cancer Res* **10**, 6956–6961.
274 Giovannetti E, Del Tacca M, Mey V, Funel N, Nannizzi S, Ricci S, Orlandini C, Boggi U, Campani D, Del Chiaro M *et al.* (2006) Transcription analysis of human equilibrative nucleoside transporter-1 predicts survival in pancreas cancer patients treated with gemcitabine. *Cancer Res* **66**, 3928–3935.
275 Marce S, Molina-Arcas M, Villamor N, Casado FJ, Campo E, Pastor-Anglada M & Colomer D (2006) Expression of human equilibrative nucleoside transporter 1 (hENT1) and its correlation with gemcitabine uptake and cytotoxicity in mantle cell lymphoma. *Haematologica* **91**, 895–902.
276 Mori R, Ishikawa T, Ichikawa Y, Taniguchi K, Matsuyama R, Ueda M, Fujii Y, Endo I, Togo S, Danenberg PV *et al.* (2007) Human equilibrative nucleoside transporter 1 is associated with the chemosensitivity of gemcitabine in human pancreatic adenocarcinoma and biliary tract carcinoma cells. *Oncol Rep* **17**, 1201–1205.
277 Nakano Y, Tanno S, Koizumi K, Nishikawa T, Nakamura K, Minoguchi M, Izawa T, Mizukami Y, Okumura T & Kohgo Y (2007) Gemcitabine chemoresistance and molecular markers associated with gemcitabine transport and metabolism in human pancreatic cancer cells. *Br J Cancer* **96**, 457–463.
278 Oguri T, Achiwa H, Muramatsu H, Ozasa H, Sato S, Shimizu S, Yamazaki H, Eimoto T & Ueda R (2007) The absence of human equilibrative nucleoside transporter 1 expression predicts nonresponse to gemcitabine-containing chemotherapy in non-small cell lung cancer. *Cancer Lett* **256**, 112–119.
279 Pérez-Torras S, García-Manteiga J, Mercadé E, Casado FJ, Carbó N, Pastor-Anglada M & Mazo A (2008) Adenoviral-mediated overexpression of human equilibrative nucleoside transporter 1 (hENT1) enhances gemcitabine response in human pancreatic cancer. *Biochem Pharmacol* **76**, 322–329.
280 Farrell JJ, Elsaleh H, Garcia M, Lai R, Ammar A, Regine WF, Abrams R, Benson AB, Macdonald J, Cass CE *et al.* (2009) Human equilibrative nucleoside transporter 1 levels predict response to gemcitabine in patients with pancreatic cancer. *Gastroenterology* **136**, 187–195.
281 Maréchal R, Mackey JR, Lai R, Demetter P, Peeters M, Polus M, Cass CE, Young J, Salmon I, Devière J *et al.* (2009) Human equilibrative nucleoside transporter 1 and human concentrative nucleoside transporter 3 predict survival after adjuvant gemcitabine therapy in resected pancreatic adenocarcinoma. *Clin Cancer Res* **15**, 2913–2919.
282 Santini D, Schiavon G, Vincenzi B, Cass CE, Vasile E, Manazza AD, Catalano V, Baldi GG, Lai R, Rizzo S *et al.* (2011) Human equilibrative nucleoside transporter 1 (hENT1) levels predict response to gemcitabine in patients with biliary tract cancer (BTC). *Curr Cancer Drug Targets* **11**, 123–129.
283 Hagmann W, Jesnowski R & Lohr JM (2010) Interdependence of gemcitabine treatment, transporter

expression, and resistance in human pancreatic carcinoma cells. *Neoplasia* **12**, 740–747.
284 Matsumura N, Nakamura Y, Kohjimoto Y, Inagaki T, Nanpo Y, Yasuoka H, Ohashi Y & Hara I (2010) The prognostic significance of human equilibrative nucleoside transporter 1 expression in patients with metastatic bladder cancer treated with gemcitabine-cisplatin-based combination chemotherapy. *BJU Int* **108**, E110–116.
285 Tanaka M, Javle M, Dong X, Eng C, Abbruzzese JL & Li D (2010) Gemcitabine metabolic and transporter gene polymorphisms are associated with drug toxicity and efficacy in patients with locally advanced pancreatic cancer. *Cancer* **116**, 5325–5335.
286 Spratlin JL & Mulder KE (2011) Looking to the future: biomarkers in the management of pancreatic adenocarcinoma. *Int J Mol Sci* **12**, 5895–5907.
287 Wang H, Word BR & Lyn-Cook BD (2011) Enhanced efficacy of gemcitabine by indole-3-carbinol in pancreatic cell lines: the role of human equilibrative nucleoside transporter 1. *Anticancer Res* **31**, 3171–3180.
288 Fisher SB, Fisher KE, Patel SH, Lim MG, Kooby DA, El-Rayes BF, Staley CA III, Adsay NV, Farris AB III & Maithel SK (2013) Excision repair cross-complementing gene-1, ribonucleotide reductase subunit M1, ribonucleotide reductase subunit M2, and human equilibrative nucleoside transporter-1 expression and prognostic value in biliary tract malignancy. *Cancer* **119**, 454–462.
289 Sai Y (2005) Biochemical and molecular pharmacological aspects of transporters as determinants of drug disposition. *Drug Metab Pharmacokinet* **20**, 91–99.
290 Wu CY & Benet LZ (2005) Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharm Res* **22**, 11–23.
291 Shitara Y, Horie T & Sugiyama Y (2006) Transporters as a determinant of drug clearance and tissue distribution. *Eur J Pharm Sci* **27**, 425–446.
292 Pacanowski MA, Hopley CW & Aquilante CL (2008) Interindividual variability in oral antidiabetic drug disposition and response: the role of drug transporter polymorphisms. *Expert Opin Drug Metab Toxicol* **4**, 529–544.
293 Watanabe T, Kusuhara H & Sugiyama Y (2010) Application of physiologically based pharmacokinetic modeling and clearance concept to drugs showing transporter-mediated distribution and clearance in humans. *J Pharmacokinet Pharmacodyn* **37**, 575–590.
294 Pagliarusco S, Martinucci S, Bordini E, Miraglia L, Cufari D, Ferrari L & Pellegatti M (2011) Tissue distribution and characterization of drug-related material in rats and dogs after repeated oral administration of casopitant. *Drug Metab Dispos* **39**, 283–293.
295 Pellegatti M & Pagliarusco S (2011) Drug and metabolite concentrations in tissues in relationship to tissue adverse findings: a review. *Expert Opin Drug Metab Toxicol* **7**, 137–146.
296 Sreedharan S, Stephansson O, Schiöth HB & Fredriksson R (2011) Long evolutionary conservation and considerable tissue specificity of several atypical solute carrier transporters. *Gene* **478**, 11–18.
297 Ho RH, Tirona RG, Leake BF, Glaeser H, Lee W, Lemke CJ, Wang Y & Kim RB (2006) Drug and bile acid transporters in rosuvastatin hepatic uptake: function, expression, and pharmacogenetics. *Gastroenterology* **130**, 1793–1806.
298 Grover A & Benet LZ (2009) Effects of drug transporters on volume of distribution. *AAPS J* **11**, 250–261.
299 Lai Y (2009) Identification of interspecies difference in hepatobiliary transporters to improve extrapolation of human biliary secretion. *Expert Opin Drug Metab Toxicol* **5**, 1175–1187.
300 Anderle P, Sengstag T, Mutch DM, Rumbo M, Praz V, Mansourian R, Delorenzi M, Williamson G & Roberts MA (2005) Changes in the transcriptional profile of transporters in the intestine along the anterior-posterior and crypt-villus axes. *BMC Genomics* **6**, 69.
301 Ayrton A & Morgan P (2008) Role of transport proteins in drug discovery and development: a pharmaceutical perspective. *Xenobiotica* **38**, 676–708.
302 Lee EJD, Lean CB & Limenta LMG (2009) Role of membrane transporters in the safety profile of drugs. *Expert Opin Drug Metab Toxicol* **5**, 1369–1383.
303 Ho RH & Kim RB (2010) Drug Transporters. Handbook of Drug-Nutrient Interactions, 2nd edn. pp. 45–84.
304 Burckhardt G & Burckhardt BC (2011) *In vitro* and *in vivo* evidence of the importance of organic anion transporters (OATs) in drug therapy. *Handb Exp Pharmacol* **201**, 29–104.
305 Keogh JP (2012) Membrane transporters in drug development. *Adv Pharmacol* **63**, 1–42.
306 Hodgkin AL & Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol* **117**, 500–544.
307 Noble D (2006) The Music of Life: Biology Beyond Genes. Oxford University Press, Oxford.
308 Kell DB (2006) Systems biology, metabolic modelling and metabolomics in drug discovery and development. *Drug Discov Today* **11**, 1085–1092.
309 Kell DB (2007) The virtual human: towards a global systems biology of multiscale, distributed biochemical network models. *IUBMB Life* **59**, 689–695.

310 Duarte NC, Becker SA, Jamshidi N, Thiele I, Mo ML, Vo TD, Srvivas R & Palsson BØ (2007) Global reconstruction of the human metabolic network based on genomic and bibliomic data. *Proc Natl Acad Sci USA* **104**, 1777–1782.
311 Ma H, Sorokin A, Mazein A, Selkov A, Selkov E, Demin O & Goryanin I (2007) The Edinburgh human metabolic network reconstruction and its functional analysis. *Mol Syst Biol* **3**, 135.
312 Clapworthy G, Viceconti M, Coveney PV & Kohl P (2008) The virtual physiological human: building a framework for computational biomedicine I. Editorial. *Philos Transact A Math Phys Eng Sci* **366**, 2975–2978.
313 Ma H & Goryanin I (2008) Human metabolic network reconstruction and its impact on drug discovery and development. *Drug Discov Today* **13**, 402–408.
314 Hao T, Ma HW, Zhao XM & Goryanin I (2010) Compartmentalization of the Edinburgh Human Metabolic Network. *BMC Bioinformatics* **11**, 393.
315 Hao T, Ma HW, Zhao XM & Goryanin I (2012) The reconstruction and analysis of tissue specific human metabolic networks. *Mol BioSyst* **8**, 663–670.
316 Lee SY, Sohn SB, Kim HU, Park JM, Kim TY, Orth JD & Palsson BØ (2012) Genome-scale network modeling. *Syst Metab Eng* **2012**, 1–23.
317 Resasco DC, Gao F, Morgan F, Novak IL, Schaff JC & Slepchenko BM (2012) Virtual Cell: computational tools for modeling in cell biology. *Wiley Interdiscip Rev Syst Biol Med* **4**, 129–140.
318 Wu M & Chan C (2012) Human metabolic network: reconstruction, simulation, and applications in systems biology. *Metabolites* **2**, 242–253.
319 Thiele I, Swainston N, Fleming RMT, Hoppe A, Sahoo S, Aurich MK, Haraldsdottír H, Mo ML, Rolfsson O, Stobbe MD *et al.* (2013) A community-driven global reconstruction of human metabolism. *Nat Biotechnol*, doi:10.1038/nbt.2488.
320 Holzhütter HG, Drasdo D, Preusser T, Lippert J & Henney AM (2012) The virtual liver: a multidisciplinary, multilevel challenge for systems biology. *Wiley Interdiscip Rev Syst Biol Med* **4**, 221–235.
321 Gille C, Bölling C, Hoppe A, Bulik S, Hoffmann S, Hübner K, Karlstädt A, Ganeshan R, König M, Rother K *et al.* (2010) HepatoNet1: a comprehensive metabolic reconstruction of the human hepatocyte for the analysis of liver physiology. *Mol Syst Biol* **6**, 411.
322 Bordbar A, Lewis NE, Schellenberger J, Palsson BØ & Jamshidi N (2010) Insight into human alveolar macrophage and *M. tuberculosis* interactions via metabolic reconstructions. *Mol Syst Biol* **6**, 422.
323 Bordbar A, Mo ML, Nakayasu ES, Schrimpe-Rutledge AC, Kim YM, Metz TO, Jones MB, Frank BC, Smith RD, Peterson SN *et al.* (2012) Model-driven multi-omic data analysis elucidates metabolic immunomodulators of macrophage activation. *Mol Syst Biol* **8**, 558.
324 Herrgård MJ, Swainston N, Dobson P, Dunn WB, Arga KY, Arvas M, Blüthgen N, Borger S, Costenoble R, Heinemann M *et al.* (2008) A consensus yeast metabolic network obtained from a community approach to systems biology. *Nat Biotechnol* **26**, 1155–1160.
325 Thiele I & Palsson BØ (2010) Reconstruction annotation jamborees: a community approach to systems biology. *Mol Syst Biol* **6**, 361.
326 Thiele I, Hyduke DR, Steeb B, Fankam G, Allen DK, Bazzani S, Charusanti P, Chen FC, Fleming RM, Hsiung CA *et al.* (2011) A community effort towards a knowledge-base and mathematical model of the human pathogen *Salmonella typhimurium* LT2. *BMC Syst Biol* **5**, 8.
327 Kell DB & Knowles JD (2006) The role of modeling in systems biology. In System Modeling in Cellular Biology: From Concepts to Nuts and Bolts (Szallasi Z, Stelling J & Periwal V, eds), pp. 3–18. MIT Press, Cambridge.
328 Oberhardt MA, Palsson BØ & Papin JA (2009) Applications of genome-scale metabolic reconstructions. *Mol Syst Biol* **5**, 320.
329 Bordbar A & Palsson BØ (2012) Using the reconstructed genome-scale human metabolic network to study physiology and pathology. *J Intern Med* **271**, 131–141.
330 Kim HU, Sohn SB & Lee SY (2012) Metabolic network modeling and simulation for drug targeting and discovery. *Biotechnol J* **7**, 330–342.
331 Walters WP & Murcko MA (2002) Prediction of 'drug-likeness'. *Adv Drug Deliv Rev* **54**, 255–271.
332 Ursu O & Oprea TI (2010) Model-free drug-likeness from fragments. *J Chem Inf Model* **50**, 1387–1394.
333 Bickerton GR, Paolini GV, Besnard J, Muresan S & Hopkins AL (2012) Quantifying the chemical beauty of drugs. *Nat Chem* **4**, 90–98.
334 Brown M, Dunn WB, Dobson P, Patel Y, Winder CL, Francis-McIntyre S, Begley P, Carroll K, Broadhurst D, Tseng A *et al.* (2009) Mass spectrometry tools and metabolite-specific databases for molecular identification in metabolomics. *Analyst* **134**, 1322–1332.
335 Wishart DS, Knox C, Guo AC, Eisner R, Young N, Gautam B, Hau DD, Psychogios N, Dong E, Bouatra S *et al.* (2009) HMDB: a knowledgebase for the human metabolome. *Nucleic Acids Res* **37**, D603–610.
336 Nobata C, Dobson P, Iqbal SA, Mendes P, Tsujii J, Kell DB & Ananiadou S (2011) Mining metabolites: extracting the yeast metabolome from the literature. *Metabolomics* **7**, 94–101.
337 de Matos P, Adams N, Hastings J, Moreno P & Steinbeck C (2012) A database for chemical proteomics: ChEBI. *Methods Mol Biol* **803**, 273–296.

338 Gupta S & Aires-de-Sousa J (2007) Comparing the chemical spaces of metabolites and available chemicals: models of metabolite-likeness. *Mol Divers* **11**, 23–36.
339 Adams JC, Keiser MJ, Basuino L, Chambers HF, Lee DS, Wiest OG & Babbitt PC (2009) A mapping of drug space from the viewpoint of small molecule metabolism. *PLoS Comput Biol* **5**, e1000474.
340 Dobson PD, Patel Y & Kell DB (2009) 'Metabolite-likeness' as a criterion in the design and selection of pharmaceutical drug libraries. *Drug Discov Today* **14**, 31–40.
341 Peironcely JE, Reijmers T, Coulier L, Bender A & Hankemeier T (2011) Understanding and classifying metabolite space and metabolite-likeness. *PLoS One* **6**, e28966.
342 Kell DB, Ryder HM, Kaprelyants AS & Westerhoff HV (1991) Quantifying heterogeneity: Flow cytometry of bacterial cultures. *Antonie Van Leeuwenhoek* **60**, 145–158.
343 Davey HM & Kell DB (1996) Flow cytometry and cell sorting of heterogeneous microbial populations: the importance of single-cell analysis. *Microbiol Rev* **60**, 641–696.
344 Kell DB, Kaprelyants AS, Weichart DH, Harwood CL & Barer MR (1998) Viability and activity in readily culturable bacteria: a review and discussion of the practical issues. *Antonie Van Leeuwenhoek* **73**, 169–187.
345 Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O'Shea EK & Weissman JS (2003) Global analysis of protein expression in yeast. *Nature* **425**, 737–741.
346 Newman JR, Ghaemmaghami S, Ihmels J, Breslow DK, Noble M, DeRisi JL & Weissman JS (2006) Single-cell proteomic analysis of *S. cerevisiae* reveals the architecture of biological noise. *Nature* **441**, 840–846.
347 Ihekwaba AEC, Broomhead DS, Grimley R, Benson N & Kell DB (2004) Sensitivity analysis of parameters controlling oscillatory signalling in the NF-κB pathway: the roles of IKK and IκBα. *Syst Biol* **1**, 93–103.
348 Ihekwaba AEC, Broomhead DS, Grimley R, Benson N, White MRH & Kell DB (2005) Synergistic control of oscillations in the NF-κB signalling pathway. *Syst Biol* **152**, 153–160.
349 Nelson DE, Ihekwaba AEC, Elliott M, Gibney CA, Foreman BE, Nelson G, See V, Horton CA, Spiller DG, Edwards SW *et al.* (2004) Oscillations in NF-κB signalling control the dynamics of gene expression. *Science* **306**, 704–708.
350 Ashall L, Horton CA, Nelson DE, Paszek P, Ryan S, Sillitoe K, Harper CV, Spiller DG, Unitt JF, Broomhead DS *et al.* (2009) Pulsatile stimulation determines timing and specificity of NFkappa-B-dependent transcription. *Science* **324**, 242–246.
351 Paszek P, Ryan S, Ashall L, Sillitoe K, Harper CV, Spiller DG, Rand DA & White MRH (2010) Population robustness arising from cellular heterogeneity. *Proc Natl Acad Sci USA* **107**, 11644–11649.
352 Nelson DE, See V, Nelson G & White MR (2004) Oscillations in transcription factor dynamics: a new way to control gene expression. *Biochem Soc Trans* **32**, 1090–1092.
353 Kell DB (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.
354 Lahav G, Rosenfeld N, Sigal A, Geva-Zatorsky N, Levine AJ, Elowitz MB & Alon U (2004) Dynamics of the p53-Mdm2 feedback loop in individual cells. *Nat Genet* **36**, 147–150.
355 Geva-Zatorsky N, Rosenfeld N, Itzkovitz S, Milo R, Sigal A, Dekel E, Yarnitzky T, Liron Y, Polak P, Lahav G *et al.* (2006) Oscillations and variability in the p53 system. *Mol Syst Biol* **2**, 2006.0033.
356 Lahav G (2008) Oscillations by the p53-Mdm2 feedback loop. *Adv Exp Med Biol* **641**, 28–38.
357 Abou-Jaoude W, Ouattara DA & Kaufman M (2009) From structure to dynamics: frequency tuning in the p53-Mdm2 network I. Logical approach. *J Theor Biol* **258**, 561–577.
358 Ouattara DA, Abou-Jaoude W & Kaufman M (2010) From structure to dynamics: frequency tuning in the p53-Mdm2 network. II Differential and stochastic approaches. *J Theor Biol* **264**, 1177–1189.
359 Geva-Zatorsky N, Dekel E, Batchelor E, Lahav G & Alon U (2010) Fourier analysis and systems identification of the p53 feedback loop. *Proc Natl Acad Sci USA* **107**, 13550–13555.
360 Shankaran H, Ippolito DL, Chrisler WB, Resat H, Bollinger N, Opresko LK & Wiley HS (2009) Rapid and sustained nuclear-cytoplasmic ERK oscillations induced by epidermal growth factor. *Mol Syst Biol* **5**, 332.
361 Yoshiura S, Ohtsuka T, Takenaka Y, Nagahara H, Yoshikawa K & Kageyama R (2007) Ultradian oscillations of Stat, Smad, and Hes1 expression in response to serum. *Proc Natl Acad Sci USA* **104**, 11292–11297.
362 Tiana G, Krishna S, Pigolotti S, Jensen MH & Sneppen K (2007) Oscillations and temporal signalling in cells. *Phys Biol* **4**, R1–R17.
363 Cai L, Dalal CK & Elowitz MB (2008) Frequency-modulated nuclear localization bursts coordinate gene regulation. *Nature* **455**, 485–490.
364 Kell DB (2000) Metabolic Footprinting – a novel high throughput, high content screening method for functional genomics. SBS Meeting, Vancouver, http://wwwsbsonlineorg/brochure/PPosters/KellAbstractpdf.

365 Liptrot C (2001) High content screening – from cells to data to knowledge. *Drug Discov Today* **6**, 832–834.
366 Giuliano KA, Haskins JR & Taylor DL (2003) Advances in high content screening for drug discovery. *Assay Drug Dev Technol* **1**, 565–577.
367 Abraham VC, Taylor DL & Haskins JR (2004) High content screening applied to large-scale cell biology. *Trends Biotechnol* **22**, 15–22.
368 Edwards BS, Oprea T, Prossnitz ER & Sklar LA (2004) Flow cytometry for high-throughput, high-content screening. *Curr Opin Chem Biol* **8**, 392–398.
369 Perlman ZE, Mitchison TJ & Mayer TU (2005) High-content screening and profiling of drug activity in an automated centrosome-duplication assay. *ChemBioChem* **6**, 145–151.
370 Erfle H & Pepperkok R (2005) Arrays of transfected mammalian cells for high content screening microscopy. *Methods Enzymol* **404**, 1–8.
371 Giuliano KA, Cheung WS, Curran DP, Day BW, Kassick AJ, Lazo JS, Nelson SG, Shin Y & Taylor DL (2005) Systems cell biology knowledge created from high content screening. *Assay Drug Dev Technol* **3**, 501–514.
372 Grånäs C, Lundholt BK, Heydorn A, Linde V, Pedersen HC, Krog-Jensen C, Rosenkilde MM & Pagliaro L (2005) High content screening for G protein-coupled receptors using cell-based protein translocation assays. *Comb Chem High Throughput Screen* **8**, 301–309.
373 Smellie A, Wilson CJ & Ng SC (2006) Visualization and interpretation of high content screening data. *J Chem Inf Model* **46**, 201–207.
374 Young DW, Bender A, Hoyt J, McWhinnie E, Chirn GW, Tao CY, Tallarico JA, Labow M, Jenkins JL, Mitchison TJ *et al.* (2008) Integrating high-content screening and ligand-target prediction to identify mechanism of action. *Nat Chem Biol* **4**, 59–68.
375 Naumann U & Wand MP (2009) Automation in high-content flow cytometry screening. *Cytometry A* **75**, 789–797.
376 Zock JM (2009) Applications of high content screening in life science research. *Comb Chem High Throughput Screening* **12**, 870–876.
377 Bickle M (2010) The beautiful cell: high-content screening in drug discovery. *Anal Bioanal Chem* **398**, 219–226.
378 Soleilhac E, Nadon R & Lafanechere L (2010) High-content screening for the discovery of pharmacological compounds: advantages, challenges and potential benefits of recent technological developments. *Expert Opin Drug Discov* **5**, 135–144.
379 Thomas N (2010) High-content screening: a decade of evolution. *J Biomol Screen* **15**, 1–9.
380 Kümmel A, Selzer P, Beibel M, Gubler H, Parker CN & Gabriel D (2011) Comparison of multivariate data analysis strategies for high-content screening. *J Biomol Screen* **16**, 338–347.
381 Xia X & Wong ST (2012) Concise review: a high-content screening approach to stem cell research and drug discovery. *Stem Cells* **30**, 1800–1807.
382 Grozinger CM, Chao ED, Blackwell HE, Moazed D & Schreiber SL (2001) Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotypic screening. *J Biol Chem* **276**, 38837–38843.
383 Yarrow JC, Feng Y, Perlman ZE, Kirchhausen T & Mitchison TJ (2003) Phenotypic screening of small molecule libraries by high throughput cell imaging. *Comb Chem High Throughput Screen* **6**, 279–286.
384 Clemons PA (2004) Complex phenotypic assays in high-throughput screening. *Curr Opin Chem Biol* **8**, 334–338.
385 Hart CP (2005) Finding the target after screening the phenotype. *Drug Discov Today* **10**, 513–519.
386 Kaminuma E, Heida N, Yoshizumi T, Nakazawa M, Matsui M & Toyoda T (2005) *In silico* phenotypic screening method of mutants based on statistical modeling of genetically mixed samples. *J Bioinform Comput Biol* **3**, 1281–1293.
387 Klekota J, Brauner E & Schreiber SL (2005) Identifying biologically active compound classes using phenotypic screening data and sampling statistics. *J Chem Inf Model* **45**, 1824–1836.
388 Abdulla MH, Ruelas DS, Wolff B, Snedecor J, Lim KC, Xu F, Renslo AR, Williams J, McKerrow JH & Caffrey CR (2009) Drug discovery for schistosomiasis: hit and lead compounds identified in a library of known drugs by medium-throughput phenotypic screening. *PLoS Negl Trop Dis* **3**, e478.
389 Etzion Y & Muslin AJ (2009) The application of phenotypic high-throughput screening techniques to cardiovascular research. *Trends Cardiovasc Med* **19**, 207–212.
390 Jenkins JL & Urban L (2010) Phenotypic screening: fishing for neuroactive compounds. *Nat Chem Biol* **6**, 172–173.
391 Stine MJ, Wang CJ, Moriarty WF, Ryu B, Cheong R, Westra WH, Levchenko A & Alani RM (2011) Integration of genotypic and phenotypic screening reveals molecular mediators of melanoma-stromal interaction. *Cancer Res* **71**, 2433–2444.
392 Pruss RM (2011) Phenotypic screening strategies for neurodegenerative diseases: a pathway to discover novel drug candidates and potential disease targets or mechanisms. *CNS Neurol Disord Drug Targets* **9**, 693–700.
393 Swinney DC & Anthony J (2011) How were new medicines discovered? *Nat Rev Drug Discov* **10**, 507–519.

394 Trabocchi A, Stefanini I, Morvillo M, Ciofi L, Cavalieri D & Guarna A (2011) Chemical genetics approach to identify new small molecule modulators of cell growth by phenotypic screening of *Saccharomyces cerevisiae* strains with a library of morpholine-derived compounds. *Org Biomol Chem* **8**, 5552–5557.
395 Giaever G, Flaherty P, Kumm J, Proctor M, Nislow C, Jaramillo DF, Chu AM, Jordan MI, Arkin AP & Davis RW (2004) Chemogenomic profiling: identifying the functional interactions of small molecules in yeast. *Proc Natl Acad Sci USA* **101**, 793–798.
396 Kamath RS, Martinez-Campos M, Zipperlen P, Fraser AG & Ahringer J (2001) Effectiveness of specific RNA-mediated interference through ingested double-stranded RNA in *Caenorhabditis elegans*. *Genome Biol* **2**, RESEARCH0002.
397 Artal-Sanz M, de Jong L & Tavernarakis N (2006) *Caenorhabditis elegans*: a versatile platform for drug discovery. *Biotechnol J* **1**, 1405–1418.
398 Burns AR, Kwok TC, Howard A, Houston E, Johanson K, Chan A, Cutler SR, McCourt P & Roy PJ (2006) High-throughput screening of small molecules for bioactivity and target identification in *Caenorhabditis elegans*. *Nat Protoc* **1**, 1906–1914.
399 Collins JJ, Evason K & Kornfeld K (2006) Pharmacology of delayed aging and extended lifespan of *Caenorhabditis elegans*. *Exp Gerontol* **41**, 1032–1039.
400 Olsen A, Vantipalli MC & Lithgow GJ (2006) Using *Caenorhabditis elegans* as a model for aging and age-related diseases. *Ann NY Acad Sci* **1067**, 120–128.
401 Leung MCK, Williams PL, Benedetto A, Au C, Helmcke KJ, Aschner M & Meyer JN (2008) *Caenorhabditis elegans*: an emerging model in biomedical and environmental toxicology. *Toxicol Sci* **106**, 5–28.
402 Mohr SE & Perrimon N (2012) RNAi screening: new approaches, understandings, and organisms. *Wiley Interdiscip Rev RNA* **3**, 145–158.
403 Wheeler DB, Bailey SN, Guertin DA, Carpenter AE, Higgins CO & Sabatini DM (2004) RNAi living-cell microarrays for loss-of-function screens in *Drosophila melanogaster* cells. *Nat Methods* **1**, 127–132.
404 Mehta A, Deshpande A & Missirlis F (2008) Genetic screening for novel *Drosophila* mutants with discrepancies in iron metabolism. *Biochem Soc Trans* **36**, 1313–1316.
405 Geldenhuys WJ, Allen DD & Bloomquist JR (2012) Novel models for assessing blood-brain barrier drug permeation. *Expert Opin Drug Metab Toxicol* **8**, 647–653.
406 Peterson RT, Link BA, Dowling JE & Schreiber SL (2000) Small molecule developmental screens reveal the logic and timing of vertebrate development. *Proc Natl Acad Sci USA* **97**, 12965–12969.
407 Eilertson CD, White A, Doan T & Rubinstein AL (2003) Fluorescent zebrafish lipid assay for compound library screening. *Arterioscler Thromb Vasc Biol* **23**, A74–A74.
408 Kokel D, Bryan J, Laggner C, White R, Cheung CY, Mateus R, Healey D, Kim S, Werdich AA, Haggarty SJ *et al*. (2010) Rapid behavior-based identification of neuroactive small molecules in the zebrafish. *Nat Chem Biol* **6**, 231–237.
409 Peal DS, Peterson RT & Milan D (2010) Small molecule screening in zebrafish. *J Cardiovasc Transl Res* **3**, 454–460.
410 Rihel J, Prober DA, Arvanites A, Lam K, Zimmerman S, Jang S, Haggarty SJ, Kokel D, Rubin LL, Peterson RT *et al*. (2010) Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. *Science* **327**, 348–351.
411 Shuker SB, Hajduk PJ, Meadows RP & Fesik SW (1996) Discovering high-affinity ligands for proteins: SAR by NMR. *Science* **274**, 1531–1534.
412 Schneider G, Lee ML, Stahl M & Schneider P (2000) *De novo* design of molecular architectures by evolutionary assembly of drug-derived building blocks. *J Comput Aided Mol Des* **14**, 487–494.
413 Rees DC, Congreve M, Murray CW & Carr R (2004) Fragment-based lead discovery. *Nat Rev Drug Discov* **3**, 660–672.
414 Leach AR, Hann MM, Burrows JN & Griffen EJ (2006) Fragment screening: an introduction. *Mol BioSyst* **2**, 430–446.
415 Alex AA & Flocco MM (2007) Fragment-based drug discovery: what has it achieved so far? *Curr Top Med Chem* **7**, 1544–1567.
416 Hajduk PJ & Greer J (2007) A decade of fragment-based drug design: strategic advances and lessons learned. *Nat Rev Drug Discov* **6**, 211–219.
417 Hubbard RE, Chen I & Davis B (2007) Informatics and modeling challenges in fragment-based drug discovery. *Curr Opin Drug Discov Devel* **10**, 289–297.
418 Jhoti H (2007) Fragment-based drug discovery using rational design. *Ernst Schering Found Symp Proc*, **3**, 169–185.
419 Fattori D, Squarcia A & Bartoli S (2008) Fragment-based approach to drug lead discovery: overview and advances in various techniques. *Drugs R D* **9**, 217–227.
420 Boettcher A, Ruedisser S, Erbel P, Vinzenz D, Schiering N, Hassiepen U, Rigollier P, Mayr LM & Woelcke J (2010) Fragment-based screening by biochemical assays: Systematic feasibility studies with trypsin and MMP12. *J Biomol Screen* **15**, 1029–1041.
421 Abad-Zapatero C & Blasi D (2011) Ligand Efficiency Indices (LEIs): more than a simple efficiency yardstick. *Mol Inform* **30**, 122–132.

422 Filz OA & Poroikov VV (2012) Fragment-based lead design. *Russ Chem Rev* **81**, 158–174.
423 Lopez A, Parsons AB, Nislow C, Giaever G & Boone C (2008) Chemical-genetic approaches for exploring the mode of action of natural products. *Prog Drug Res* **66**, 237, 239–271.
424 Smith AM, Durbic T, Kittanakom S, Giaever G & Nislow C (2012) Barcode sequencing for understanding drug-gene interactions. *Methods Mol Biol* **910**, 55–69.
425 Mendes P & Kell DB (1998) Non-linear optimization of biochemical pathways: applications to metabolic engineering and parameter estimation. *Bioinformatics* **14**, 869–883.
426 Bongard JC & Lipson H (2005) Nonlinear system identification using coevolution of models and tests. *IEEE Trans Evol Comput* **9**, 361–384.
427 Bongard J & Lipson H (2007) Automated reverse engineering of nonlinear dynamical systems. *Proc Natl Acad Sci USA* **104**, 9943–9948.
428 Jayawardhana B, Kell DB & Rattray M (2008) Bayesian inference of the sites of perturbations in metabolic pathways via Markov Chain Monte Carlo. *Bioinformatics* **24**, 1191–1197.
429 Vyshemirsky V & Girolami MA (2008) Bayesian ranking of biochemical system models. *Bioinformatics* **24**, 833–839.
430 Vyshemirsky V & Girolami M (2008) BioBayes: a software package for Bayesian inference in systems biology. *Bioinformatics* **24**, 1933–1934.
431 Wilkinson SJ, Benson N & Kell DB (2008) Proximate parameter tuning for biochemical networks with uncertain kinetic parameters. *Mol BioSyst* **4**, 74–97.
432 Brown M, He F & Wilkinson SJ (2010) Properties of the proximate parameter tuning regularization algorithm. *Bull Math Biol* **72**, 697–718.
433 Xu TR, Vyshemirsky V, Gormand A, von Kriegsheim A, Girolami M, Baillie GS, Ketley D, Dunlop AJ, Milligan G, Houslay MD *et al*. (2010) Inferring signaling pathway topologies from multiple perturbation measurements of specific biochemical species. *Sci Signal* **3**, ra20.
434 Kleemann R, Bureeva S, Perlina A, Kaput J, Verschuren L, Wielinga PY, Hurt-Camejo E, Nikolsky Y, van Ommen B & Kooistra T (2011) A systems biology strategy for predicting similarities and differences of drug effects: evidence for drug-specific modulation of inflammation in atherosclerosis. *BMC Syst Biol* **5**, 125.
435 Zhan C & Yeung LF (2011) Parameter estimation in systems biology models using spline approximation. *BMC Syst Biol* **5**, 14.
436 Buzan T (2002) How to Mind Map. Thorsons, London.
437 Cornish-Bowden A (1986) Why is uncompetitive inhibition so rare? A possible explanation, with implications for the design of drugs and pesticides. *FEBS Lett* **203**, 3–6.
438 Rubin JL, Gaines CG & Jensen RA (1984) Glyphosate inhibition of 5-enolpyruvylshikimate 3-phosphate synthase from suspension-cultured cells of *Nicotiana silvestris*. *Plant Physiol* **75**, 839–845.
439 Alibhai MF & Stallings WC (2001) Closing down on glyphosate inhibition – with a new structure for drug discovery. *Proc Natl Acad Sci USA* **98**, 2944–2946.