

THE CELLULAR UPTAKE OF PHARMACEUTICAL DRUGS IS MAINLY CARRIER-MEDIATED AND IS THUS AN ISSUE NOT SO MUCH OF BIOPHYSICS BUT OF SYSTEMS BIOLOGY

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ABSTRACT

It is widely believed that most drug molecules are transported across the phospholipid bilayer portion of biological membranes via passive diffusion at a rate related to their lipophilicity (expressed as log P, a calculated c log P or as log D, the octanol:water partition coefficient). However, studies of this using purely phospholipid bilayer membranes have been very misleading since transfer across these typically occurs via the solvent reservoirs or via aqueous pore defects, neither of which are prevalent in biological cells. Since the types of biophysical forces involved in the interaction of drugs with lipid membranes are no different from those involved in their interaction with proteins, arguments based on lipophilicity also apply to drug uptake by membrane transporters or carriers. A similar story attaches to the history of mechanistic explanations of the mode of action of general anaesthetics (narcotics). Carrier-mediated and active uptake of drugs is far more common than is usually assumed. This has considerable implications for the design of libraries for drug discovery and development, as well as for chemical genetics/genomics and systems chemistry.

INTRODUCTION

As is well known (e.g. [1–4]), attrition rates of drugs in pharmaceutical companies remain extremely high, and nowadays this is mainly due either to lack of efficacy or for reasons of toxicity. Arguably these issues are mainly due to the fact that drug candidates are typically isolated on the basis of their potency in a screen against a molecular target, and only subsequently are they tested in organisms *in vivo*. Since most modern targets have enjoyed some degree of validation using e.g. genetic knockouts, it is likely that the problem of ostensible potency *in vitro* but lack of efficacy *in vivo* is not so much with the target but with the ability of the drug to find the target. In a similar vein, if drugs are accumulated to high levels in particular tissues via the action of active solute transporters [5–7], it is the cellular and tissue distributions of the relevant carriers, rather than any general biophysical properties of the drugs of interest, that largely determine differential tissue distributions. An overview of this article is given in Figure 1. We begin by rehearsing some of the relevant arguments.

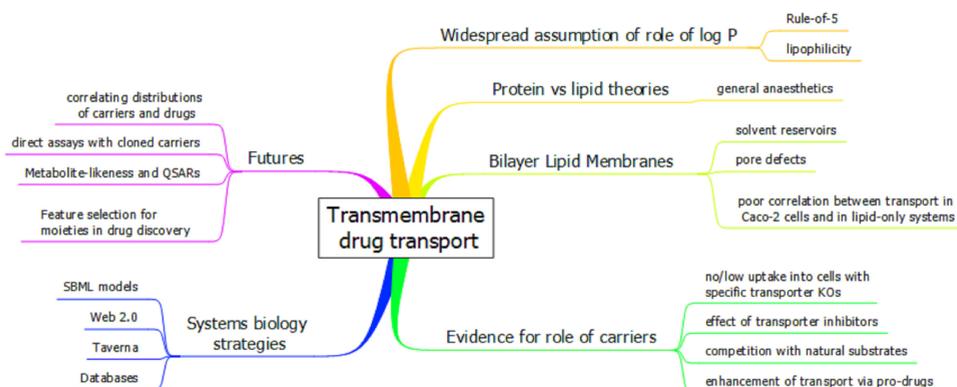


Figure 1. An overview of this article in the form of a ‘mind map’ [8].

HOW DRUGS CROSS CELL MEMBRANES

The prevailing view of cell membranes, popularised in Singer and Nicolson’s celebrated paper of 1972 [9], is that of polytopic proteins floating (and diffusing) in a ‘sea’ of phospholipid bilayer, as illustrated in the cartoon of Figure 2.

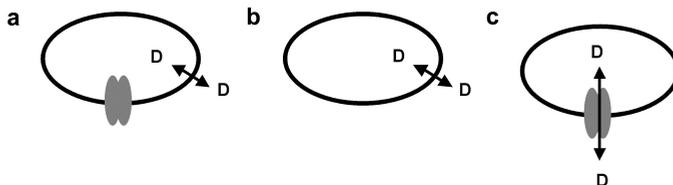


Figure 2. A simple cartoon of two means by which a molecule such as a drug (D) may cross a cellular membrane, either by diffusing through the phospholipid bilayer portion (a, b) or being taken up via a carrier (c) (or both).

While the main elements of this are broadly accepted, two features are of note. First, the protein:lipid ratio in membranes (by mass) is typically 1:1 and may be 3:1 [10], and secondly that most lipids are partially or significantly influenced by the presence of the protein component (and vice versa [11, 12]). However, the cartoon serves to cover the nexus of this article, viz. the question of whether drugs mainly cross cellular membranes via passage through the phospholipid bilayer portion or using carrier-mediated transport. Because it may be active, i. e. coupled to sources of free energy, the latter in particular, *modulo* the existence of any membrane potential differences between compartments, is capable of effecting considerable concentrative uptake. The question then arises as to whether there are molecular or biophysical properties of drugs that can serve to explain their rate of transfer across biological membranes.

‘LIPOPHILICITY’ AS A CANDIDATE DESCRIPTOR FOR RATES OF DRUG TRANSPORT ACROSS BIOMEMBRANES

From the time of Overton [13] it has been recognised that the transmembrane permeability of non-electrolytes correlates well with their olive oil (nowadays octanol): water partition coefficients, typically referred to as $\log D$ or $\log P$ (A more recent example with data can be found in [14]). Thus there has been a tendency to assume that this gives a mechanistic explanation by which such solutes must ‘dissolve’ or partition into the bilayer portion of such biological membranes in order to cross them. Actually it means no such thing, as the biophysical forces and mechanistic acts (e. g. making and breaking of H-bonds) required for ‘partitioning’ into appropriately hydrophobic protein pockets are the same, and so such correlations may also mean that solute transfer is protein-mediated (and see below).

LIPINSKI'S “RULE OF FIVE” FOR DESCRIBING DRUG BIOAVAILABILITY

As indicated above, drugs will only work when they can reach and thereby interact with their ‘targets’, and a first step in understanding this relates to their so-called ‘bioavailability’ [15–17], a term that covers (among others things) solubility, absorption and permeability. Indeed, it was the need to understand bioavailability that led Lipinski to devise his famous ‘rule of five’ (Ro5) [18]. The Ro5 predicts that poor absorption or permeation is more likely

when there are more than 5 H-bond donors, 10 H-bond acceptors, the molecular weight (MW) is greater than 500 and the calculated Log P (CLogP) is greater than 5. While empirical, the Ro5 has been massively important in influencing thinking about the kinds of molecules companies might wish to consider in designing drug screening libraries and the subsequent drugs [4, 19–21]. Clearly it recognises the need to balance the forces that enable a molecule to be at once both sufficiently hydrophilic to dissolve adequately in aqueous media with a requirement to be sufficiently lipophilic to penetrate to or via more hydrophobic environments. It was also explicitly recognised [18] that the Ro5 did not apply to carrier-mediated uptake, and that many/most natural products ‘disobey’ the Ro5 (In the more recently developed fragment-based screening – see e.g. [22, 23] – there is an even more stringent ‘rule of three’ [24]). Log P in its various incarnations is thus seen as a very important property of a candidate drug molecule, although as a macroscopic property it is not entirely obvious how this would be terribly predictive of drug distributions.

BILAYER OR BLACK LIPID MEMBRANES

Notwithstanding this, the long history of the relation between permeability and log P, coupled to the implication that it simply involves dissolving in a hydrophobic environment while crossing from one aqueous phase to another *in vivo*, has meant that many have sought to simplify the understanding of transmembrane molecule transport by studying it in bilayer or ‘black’ lipid membranes (BLMs) [25–28] lacking protein (Fig. 3).

Black (Bilayer) Lipid Membranes (BLMs)

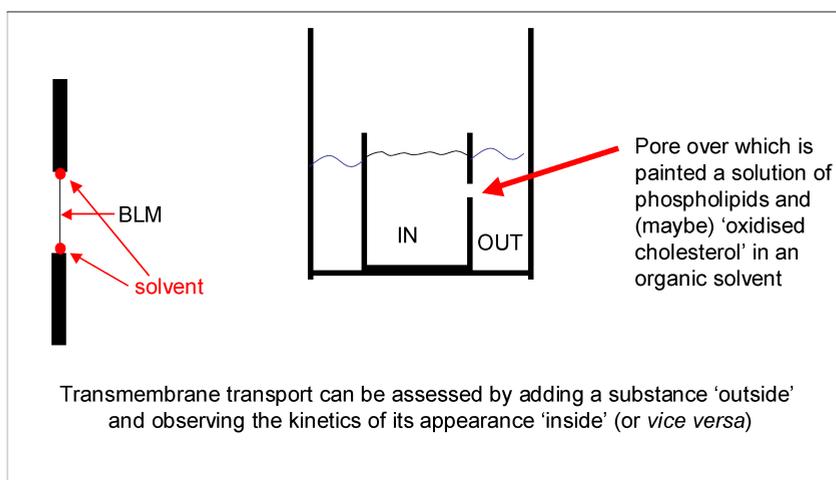


Figure 3. The principle of formation of conventional BLMs

The problems with this kind of system are (i) that most BLMs are formed using organic solvents, and the residual solvent reservoirs (forming an annulus as the edge of the BLM, see Fig. 3) provide a vehicle for transport that does not involve the phospholipid bilayer, and (ii) that many BLMs exhibit aqueous pore defects that biomembranes do not (see also [29, 30]), some potentially induced by solutes themselves, and that these permit transport that does not therefore involve dissolution in any phospholipid (e.g. [31–38]). Indeed, the enormous Born charging energy for transferring electrostatic charge across any low dielectric medium is prohibitive to the trans-lipid transport of ionic charges [39, 40]. Consequently, it is rather doubtful whether such model systems possess the properties necessary for them to act as a useful guide for the mechanisms of transport via natural membranes. The rate of transport of drugs across more recently devised lipid-only membrane systems is also only weakly correlated with the transport of the same molecules across biological membranes (see e.g. [7, 41–44]).

THE MECHANISM OF ACTION OF GENERAL ANAESTHETICS (NARCOTICS)

Correlation is of course a poor guide to mechanism or causality, and another example where there are excellent correlations between bioactivity and lipophilicity, but where these have proved mechanistically highly misleading, is represented by the mode of action of narcotic agents ('general anaesthetics'). Starting with the studies of Meyer [45] and of Overton [46] (see also [47]), a close relationship between lipophilicity (log *D*) and narcotic potency was established. The almost complete lack of a structure-activity relationship over 5 orders of magnitude (but cf. [48, 49]) led many to assume that a simple biophysically based partitioning of anaesthetic molecules into cell membranes (followed, presumably, by some kind of inhibitory pressure-induced effect on membrane ion channels) could account for narcosis [50] (and also tended to imply a unitary mechanism). However, a number of molecules deviate considerably from this picture, and some isomers with similar biophysical properties have very different anaesthetic potencies [51–53]. Now, the biophysical forces underpinning the interaction of such molecules with lipids are no different from those describing interactions with proteins [54], and indeed equivalent interactions of these molecules with fully soluble (non-membranous) proteins (e.g. [55–58], including direct structural evidence for binding [57, 59], and the correlation between specific receptor binding (e.g. [60]) and potency in specific mutant mice [61] (and see [62]), mean that this view is no longer considered tenable (e.g. [54, 63–70]), and it is now recognised that general anaesthetics of different functional classes have a variety of proteinaceous targets [54, 71], in particular GABA_A receptor subtypes [72, 73]. Indeed, even such a small molecule as ethanol is now recognised as having relatively specific receptors [74]! Lipophilicity, and a gross analysis of chemical structure *per se*, then, are poor guides to mechanism.

EVIDENCE THAT DRUGS DO HITCHHIKE ON ‘NATURAL’ CARRIERS

Space does not permit an exhaustive review, and printed papers as such are a poor means to summarise knowledge of this type [75]. However, following early indications that even lipophilic cations require carriers for transmembrane transport [76], a huge number of ‘exceptions’ (or at least instances) have been found. Some are listed in the supplementary information to our recent review [7] while others can be found in other summaries [5, 6]. To this end, we shall shortly be making available a database of human drug transporters (see also [77]), based in part on our data model for metabolite databases [78, 79].

SYSTEMS BIOLOGY, DATABASES AND WEB 2.0 FOR UNDERSTANDING DRUG UPTAKE

There is now a convergence [80–82] between (i) our understanding of those human metabolites that can be determined from genomic reconstructions and the literature [83–85] and (ii) the metabolic network models that alone will allow to effect true systems biology modelling [86–89]. Our main strategy for assisting this involves the use of workflows of loosely coupled elements [80–82, 90], with the models encoded in SBML [91] (www.sbml.org) according to principled markup standards [92, 93]. Bottom-up approaches (Fig. 4) have the merit of starting with molecular mechanism, but do rely on knowledge of the relevant participants.

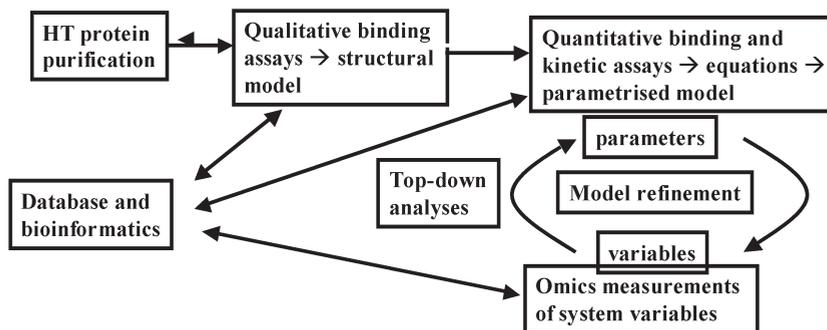


Figure 4. A ‘bottom-up’ systems biology approach (including top-down strategies, and thereby ‘middle-out’ [94]) with which to develop metabolic network models that include drug transporters

WHERE NEXT?

The human genome encodes some 900+ drug transporters [95, 96], and while the main ones involved in cellular drug uptake are a comparatively small subset of these [7], it is clear that we need to understand the specificity and distribution of these, just as is the case with the cytochromes P450 that are so important in drug metabolism. Studies of specificity and

 The Cellular Uptake of Pharmaceutical Drugs is Mainly Carrier-mediated

QSAR measurements will require comparison of drug transport into cells containing or lacking cloned carriers. The tissue and even subcellular distributions of carriers (e.g. in mitochondria [97]) are emerging from studies such as the Human Proteome Atlas (<http://proteomeatlas.org/>) (e.g. [98, 99]). An example, showing the extreme differences in carrier expression between tissues that can be observed, is given in figure 5. Such quantitative proteomic data will be extremely valuable in assisting us in the development of systems biology models, since although it is possible to make substantial progress by ‘guessing’ kinetic parameters from the topology and stoichiometry of metabolic networks alone [100], or better inferring them from measured fluxes and concentrations (e.g. [101 – 107]), experimental measurements of K_m , k_{cat} and protein concentrations is altogether more satisfactory for building and constraining kinetic models.

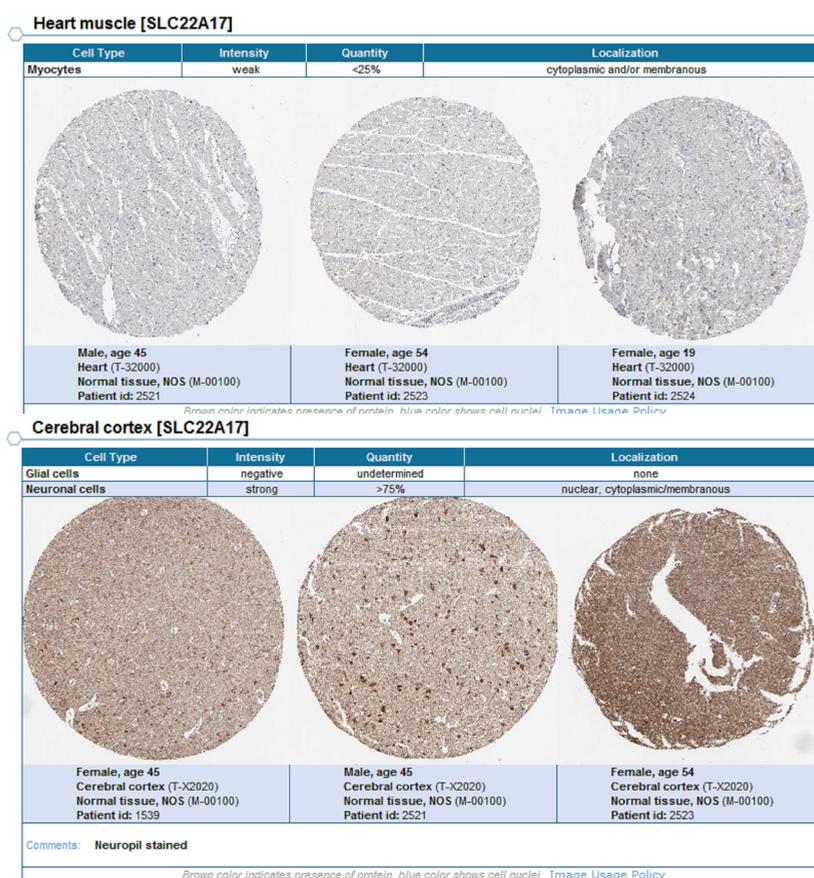


Figure 5. An example from the Human Protein Atlas, taken with permission on its website, of representative tissue distributions of the protein SLC22A17, a so-called brain-specific organic cation transporter. Links are via http://proteomeatlas.org/tissue_profile.php?antibody_id=2728.

We need to understand much better than we do now the biophysical, chemical and molecular descriptors that are important in determining drug dispositions, and this requires the production of suitable models [96]. Evolutionary computing methods (e.g. [108–110]) are extremely powerful but surprisingly underutilised for these purposes. Log D measurements are still of value as a ‘baseline’, but tend to be poor predictors even of gross biological effects when the chemical involved are not in homologous series [111].

FRAGMENT-BASED DRUG DISCOVERY AND DRUG TRANSPORTERS

One interesting approach to drug discovery, rather akin to an evolutionary computing type of approach, involves the evolution of drug structures from smaller fragments (e.g. [23, 24, 112–130]), and the obvious question arises as to which kinds of fragments might best be included in the libraries used. Clearly it will be of interest to compare the similarity of such fragments to natural metabolites [131], since those that are most similar to ‘natural’ metabolites that are known to enter cells are most likely to serve as transporter substrates (the principle of molecular similarity [132–135]).

CONCLUDING REMARKS

“When one admits that nothing is certain one must, I think, also admit that some things are much more nearly certain than others.” [136]

One cannot fail to remark on the huge volume and continuing growth of the scientific literature. Two and a half million peer-reviewed papers are published per year [137], with over 1 million per year in Medline alone (http://www.nlm.nih.gov/bsd/medline_cit_counts_yr_pub.html). The former equates to nearly 5 refereed scientific papers being published per minute – and in a somewhat similar vein presently 10 hours of (albeit largely non-science-related) video material are added at www.youtube.com in the same time! A consequence of this is a kind of ‘balkanisation’ [138] of the literature in which scientists focus solely on more detailed analyses of ever smaller parts of biology. This is clearly going to change [89], and will have to do so, as a result of computerization, the internet and the emergence of systems biology, since only a global overview can lead to general truths (inductive reasoning [139]). Only by looking at many hundreds of papers did we recognize that carrier-mediated uptake is the rule and not the exception [7]. Automation is therefore required.

Given suitably digitised literature and attendant metadata [75], we need to exploit methods such as text mining [140–143], conceptual associations [144–146] and literature-based discovery (e.g. [146–152]) to create new knowledge.

Consequently, we hope we can look forward to the development of many computational tools that will assist chemical biologists in putting together systems biology models that describe accurately the internal biochemical mechanisms of the ‘digital human’ [88].

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REFERENCES

- [1] Ajay (2002). Predicting drug-likeness: why and how? *Curr. Top. Med. Chem.* **2**:1273 – 86.
 - [2] Hann, M.M., Oprea, T.I. (2004). Pursuing the leadlikeness concept in pharmaceutical research. *Curr. Op. Chem. Biol.* **8**:255 – 263.
 - [3] Kola, I., Landis, J. (2004). Can the pharmaceutical industry reduce attrition rates? *Nat. Rev. Drug Discov.* **3**:711 – 5.
 - [4] Leeson, P.D. & Springthorpe, B. (2007). The influence of drug-like concepts on decision-making in medicinal chemistry. *Nat. Rev. Drug Discov.* **6**:881 – 90.
 - [5] Sai, Y., Tsuji, A. (2004). Transporter-mediated drug delivery: recent progress and experimental approaches. *Drug. Discov. Today.* **9**:712 – 20.
 - [6] Sai, Y. (2005). Biochemical and molecular pharmacological aspects of transporters as determinants of drug disposition. *Drug. Metab. Pharmacokinet.* **20**:91 – 9.
 - [7] Dobson, P.D., Kell, D.B. (2008). Carrier-mediated cellular uptake of pharmaceutical drugs: an exception or the rule? *Nat. Rev. Drug Discov.* **7**:205 – 220.
 - [8] Buzan, T. (2002). *How to mind map*. Thorsons, London.
 - [9] Singer, S.J., Nicolson, G.L. (1972). The fluid mosaic model of the structure of cell membranes. *Science* **175**:720 – 31.
 - [10] Westerhoff, H.V., Kell, D.B., Kamp, F., van Dam, K. (1988). The membranes involved in proton-mediated free-energy transduction: thermodynamic implications of their physical structure. In *Microcompartmentation* (ed. D. P. Jones), pp. 115 – 154. CRC Press, Boca Raton, FL.
 - [11] Lee, A.G. (2004). How lipids affect the activities of integral membrane proteins. *Biochim. Biophys. Acta* **1666**:62 – 87.
-

- [12] Reynwar, B.J., Illya, G., Harmandaris, V.A., Muller, M.M., Kremer, K., Deserno, M. (2007). Aggregation and vesiculation of membrane proteins by curvature-mediated interactions. *Nature* **447**:461 – 464.
- [13] Overton, E. (1899). Über die allgemeinen osmotischen Eigenschaften der Zelle, ihre vermutliche Ursachen und ihre Bedeutung für die Physiologie *Vierteljahrsschr. Naturforsch. Ges. Zürich* **44**:88 – 114.
- [14] Lieb, W.R., Stein, W.D. (1969). Biological membranes behave as non-porous polymeric sheets with respect to the diffusion of non-electrolytes. *Nature* **224**:240 – 3.
- [15] Amidon, G.L., Lennernas, H., Shah, V.P., Crison, J.R. (1995). A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharm. Res.* **12**:413 – 20.
- [16] Lennernäs, H., Abrahamsson, B. (2005). The use of biopharmaceutic classification of drugs in drug discovery and development: current status and future extension. *J. Pharm. Pharmacol.* **57**:273 – 85.
- [17] Wu, C.Y., Benet, L.Z. (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.
- [18] Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **23**:3 – 25.
- [19] Lipinski, C.A. (2000). Drug-like properties and the causes of poor solubility and poor permeability. *J. Pharmacol. Toxicol. Methods* **44**:235 – 49.
- [20] van De Waterbeemd, H., Smith, D.A., Beaumont, K., Walker, D.K. (2001). Property-based design: optimization of drug absorption and pharmacokinetics. *J. Med. Chem.* **44**:1313 – 33.
- [21] Owens, J., Lipinski, C. (2003). Chris Lipinski discusses life and chemistry after the Rule of Five. *Drug Discov. Today* **8**:12 – 16.
- [22] Carr, R., Jhoti, H. (2002). Structure-based screening of low-affinity compounds. *Drug Discov Today* **7**:522 – 7.
- [23] Hartshorn, M.J., Murray, C.W., Cleasby, A., Frederickson, M., Tickle, I.J., Jhoti, H. (2005). Fragment-based lead discovery using X-ray crystallography. *J. Med. Chem.* **48**:403 – 13.
- [24] Congreve, M., Carr, R., Murray, C., Jhoti, H. (2003). A rule of three for fragment-based lead discovery? *Drug Discov. Today* **8**:876 – 877.
-

- [25] Mueller, P., Rudin, D.O., Tien, H.T., Wescott, W.C. (1962). Reconstitution of cell membrane structure *in vitro* and its transformation into an excitable system. *Nature* **194**:979–980.
- [26] Jain, M.K. (1972). *The bimolecular lipid membrane*. Van Nostrand Reinhold, New York.
- [27] Tien, H.T. (1974). *Bilayer lipid membranes (BLM): theory and practice*. Marcel Dekker, New York.
- [28] Tien, H.T., Ottova-Leitmannova, A. (2003). *Planar lipid bilayers (BLMs) and their applications*. Elsevier, New York.
- [29] Deamer, D.W. (2008). Origins of life: How leaky were primitive cells? *Nature* **454**:37–8.
- [30] Mansy, S.S., Schrum, J.P., Krishnamurthy, M., Tobe, S., Treco, D.A., Szostak, J.W. (2008). Template-directed synthesis of a genetic polymer in a model protocell. *Nature* **454**:122–5.
- [31] Jansen, M., Blume, A. (1995). A comparative study of diffusive and osmotic water permeation across bilayers composed of phospholipids with different head groups and fatty acyl chains. *Biophys. J.* **68**:997–1008.
- [32] Bordi, F., Cametti, C., Naglieri, A. (1999). Ion transport in lipid bilayer membranes through aqueous pores. *Coll. Surf. A* **159**:231–237.
- [33] Leontiadou, H., Mark, A.E., Marrink, S.J. (2004). Molecular dynamics simulations of hydrophilic pores in lipid bilayers. *Biophys. J.* **86**:2156–64.
- [34] Tieleman, D.P., Marrink, S.J. (2006). Lipids out of equilibrium: energetics of desorption and pore mediated flip-flop. *J. Am. Chem. Soc.* **128**:12462–7.
- [35] Xiang, T.X., Anderson, B.D. (2006). Liposomal drug transport: a molecular perspective from molecular dynamics simulations in lipid bilayers. *Adv. Drug. Deliv. Rev.* **58**:1357–78.
- [36] Gurtovenko, A.A., Vattulainen, I. (2007). Molecular mechanism for lipid flip-flops. *J. Phys. Chem. B* **111**:13554–9.
- [37] Gurtovenko, A.A., Anwar, J. (2007). Ion transport through chemically induced pores in protein-free phospholipid membranes. *J. Phys. Chem. B* **111**:13379–82.
- [38] Leontiadou, H., Mark, A.E., Marrink, S.J. (2007). Ion transport across transmembrane pores. *Biophys. J.* **92**:4209–15.
- [39] Parsegian, A. (1969). Energy of an ion crossing a low dielectric membrane: solutions to four relevant electrostatic problems. *Nature* **221**:844–6.
-

- [40] Weaver, J.C., Chizmadzhev, Y.A. (1996). Theory of electroporation: a review. *Bioelectrochem. Bioenerg.* **41**:135 – 160.
- [41] Balimane, P.V., Han, Y.H., Chong, S.H. (2006). Current industrial practices of assessing permeability and P-glycoprotein interaction. *AAPS Journal* **8**:E1-E13.
- [42] Corti, G., Maestrelli, F., Cirri, M., Zerrouk, N., Mura, P. (2006). Development and evaluation of an in vitro method for prediction of human drug absorption – II. Demonstration of the method suitability. *Eur. J. Pharm. Sci.* **27**:354 – 362.
- [43] Galinis-Luciani, D., Nguyen, L., Yazdanian, M. (2007). Is PAMPA a useful tool for discovery? *J. Pharm. Sci.* **96**:2886 – 92.
- [44] Avdeef, A., Bendels, S., Di, L., Faller, B., Kansy, M., Sugano, K., Yamauchi, Y. (2007). PAMPA – critical factors for better predictions of absorption. *J. Pharm. Sci.* **96**:2893 – 909.
- [45] Meyer, H.H. (1899). Welche Eigenschaft der Anästhetica bedingt ihre narkotische Wirkung? *Arch. Exp. Pathol. Pharmacol.* **42**:109 – 118.
- [46] Overton, C.E. (1901). *Studien über die Narkose zugleich ein Beitrag zur allgemeinen Pharmakologie*. Gustav Fischer, Jena.
- [47] De Weer, P. (2000). A century of thinking about cell membranes. *Annu. Rev. Physiol.* **62**:919 – 26.
- [48] Sewell, J.C., Sear, J.W. (2006). Determinants of volatile general anesthetic potency: a preliminary three-dimensional pharmacophore for halogenated anesthetics. *Anesth. Analg.* **102**:764 – 71.
- [49] Eckenhoff, R., Zheng, W., Kelz, M. (2008). From anesthetic mechanisms research to drug discovery. *Clin. Pharmacol. Ther.* **84**:144 – 8.
- [50] Seeman, P. (1972). The membrane actions of anesthetics and tranquilizers. *Pharmacol. Rev.* **24**:583 – 655.
- [51] Dickinson, R., Franks, N.P., Lieb, W.R. (1994). Can the stereoselective effects of the anesthetic isoflurane be accounted for by lipid solubility? *Biophys. J.* **66**:2019 – 23.
- [52] Dickinson, R., White, I., Lieb, W.R., Franks, N.P. (2000). Stereoselective loss of righting reflex in rats by isoflurane. *Anesthesiology* **93**:837 – 43.
- [53] Bertaccini, E.J., Trudell, J.R., Franks, N.P. (2007). The common chemical motifs within anesthetic binding sites. *Anesth. Analg.* **104**:318 – 24.
- [54] Franks, N.P. (2008). General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. *Nat. Rev. Neurosci.* **9**:370 – 86.
-

- [55] Ueda, I. (1965). Effects of diethyl ether and halothane on firefly luciferin bioluminescence. *Anesthesiology* **26**:603–6.
- [56] Franks, N.P., Lieb, W.R. (1984). Do general anaesthetics act by competitive binding to specific receptors? *Nature* **310**:599–601.
- [57] Franks, N.P., Jenkins, A., Conti, E., Lieb, W.R., Brick, P. (1998). Structural basis for the inhibition of firefly luciferase by a general anesthetic. *Biophys. J.* **75**:2205–11.
- [58] Miller, K.W. (2002). The nature of sites of general anaesthetic action. *Br. J. Anaesth.* **89**:17–31.
- [59] Szarecka, A., Xu, Y., Tang, P. (2007). Dynamics of firefly luciferase inhibition by general anesthetics: Gaussian and anisotropic network analyses. *Biophys. J.* **93**:1895–905.
- [60] Mihic, S.J., Ye, Q., Wick, M.J., Koltchine, V.V., Krasowski, M.D., Finn, S.E., Mascia, M.P., Valenzuela, C.F., Hanson, K.K., Greenblatt, E.P., Harris, R.A., Harrison, N.L. (1997). Sites of alcohol and volatile anaesthetic action on GABA_A and glycine receptors. *Nature* **389**:385–9.
- [61] Jurd, R., Arras, M., Lambert, S., Drexler, B., Siegwart, R., Crestani, F., Zaugg, M., Vogt, K.E., Ledermann, B., Antkowiak, B., Rudolph, U. (2003). General anesthetic actions in vivo strongly attenuated by a point mutation in the GABA_A receptor β 3 subunit. *FASEB J.* **17**:250–2.
- [62] Heurteaux, C., Guy, N., Laigle, C., Blondeau, N., Duprat, F., Mazzuca, M., Lang-Lazdunski, L., Widmann, C., Zanzouri, M., Romey, G., Lazdunski, M. (2004). TREK-1, a K⁺ channel involved in neuroprotection and general anesthesia. *EMBO J.* **23**:2684–95.
- [63] Thompson, S.A., Wafford, K. (2001). Mechanism of action of general anaesthetics: new information from molecular pharmacology. *Curr. Opin. Pharmacol.* **1**:78–83.
- [64] Urban, B.W. (2002). Current assessment of targets and theories of anaesthesia. *Br. J. Anaesth.* **89**:167–83.
- [65] Campagna, J.A., Miller, K.W., Forman, S.A. (2003). Mechanisms of actions of inhaled anesthetics. *New England Journal of Medicine* **348**:2110–2124.
- [66] Franks, N.P., Honoré, E. (2004). The TREK K_{2P} channels and their role in general anaesthesia and neuroprotection. *Trends Pharmacol. Sci.* **25**:601–8.
- [67] Rudolph, U., Antkowiak, B. (2004). Molecular and neuronal substrates for general anaesthetics. *Nat. Rev. Neurosci.* **5**:709–20.
-

- [68] Hemmings, H.C., Jr., Akabas, M.H., Goldstein, P.A., Trudell, J.R., Orser, B.A., Harrison, N.L. (2005). Emerging molecular mechanisms of general anesthetic action. *Trends Pharmacol. Sci.* **26**:503–10.
- [69] Grasshoff, C., Drexler, B., Rudolph, U., Antkowiak, B. (2006). Anaesthetic drugs: linking molecular actions to clinical effects. *Curr. Pharm. Des.* **12**:3665–79.
- [70] Franks, N.P. (2006). Molecular targets underlying general anaesthesia. *Br. J. Pharmacol.* **147 Suppl 1**, S72–81.
- [71] Urban, B.W., Bleckwenn, M., Barann, M. (2006). Interactions of anesthetics with their targets: non-specific, specific or both? *Pharmacol. Ther.* **111**:729–70.
- [72] Solt, K., Forman, S.A. (2007). Correlating the clinical actions and molecular mechanisms of general anesthetics. *Curr. Opin. Anaesthesiol.* **20**:300–6.
- [73] Bonin, R.P., Orser, B.A. (2008). GABA_A receptor subtypes underlying general anesthesia. *Pharmacol. Biochem. Behavior* **90**:105–112.
- [74] Wallner, M., Hanchar, H.J., Olsen, R.W. (2006). Low-dose alcohol actions on alpha 4 beta 3 delta GABA_A receptors are reversed by the behavioral alcohol antagonist Ro15–4513. *Proc. Natl. Acad. Sci. U.S.A.* **103**:8540–8545.
- [75] Hull, D., Pettifer, S.R., Kell, D.B. (2008). Defrosting the digital library: bibliographic tools for the next generation web. *PLoS Comp. Biol.* **4**(10):e1000204.
- [76] Barts, P.W.J.A., Hoeberichts, J.A., Klaassen, A., Borst-Pauwels, G.W.F.H. (1980). Uptake of the lipophilic cation dibenzylidimethylammonium into *Saccharomyces cerevisiae*. Interaction with the thiamine transport system. *Biochim. Biophys. Acta* **597**:125–36.
- [77] Yan, Q., Sadée, W. (2000). Human membrane transporter database: a Web-accessible relational database for drug transport studies and pharmacogenomics. *AAPS Pharm-Sci.* **2**:E20.
- [78] Jenkins, H., Hardy, N., Beckmann, M., Draper, J., Smith, A.R., Taylor, J., Fiehn, O., Goodacre, R., Bino, R., Hall, R., Kopka, J., Lane, G.A., Lange, B.M., Liu, J.R., Mendes, P., Nikolau, B.J., Oliver, S.G., Paton, N.W., Roessner-Tunali, U., Saito, K., Smedsgaard, J., Sumner, L.W., Wang, T., Walsh, S., Wurtele, E.S., Kell, D.B. (2004). A proposed framework for the description of plant metabolomics experiments and their results. *Nat. Biotechnol.* **22**:1601–1606.
- [79] Spasic, I., Dunn, W.B., Velarde, G., Tseng, A., Jenkins, H., Hardy, N.W., Oliver, S.G., Kell, D.B. (2006). MeMo: a hybrid SQL/XML approach to metabolomic data management for functional genomics. *BMC Bioinformatics* **7**:281.
-

- [80] Kell, D.B. (2006). Systems biology, metabolic modelling and metabolomics in drug discovery and development. *Drug Disc. Today* **11**:1085 – 1092.
- [81] Kell, D.B. (2006). Metabolomics, modelling and machine learning in systems biology: towards an understanding of the languages of cells. The 2005 Theodor Bücher lecture. *FEBS J.* **273**:873 – 894.
- [82] Kell, D.B. (2007). Metabolomic biomarkers: search, discovery and validation. *Exp. Rev. Mol. Diagnost.* **7**:329 – 333.
- [83] Duarte, N.C., Becker, S.A., Jamshidi, N., Thiele, I., Mo, M.L., Vo, T.D., Srivivas, R., Palsson, B.Ø. (2007). Global reconstruction of the human metabolic network based on genomic and bibliomic data. *Proc. Natl. Acad. Sci. U.S.A.* **104**:1777 – 1782.
- [84] 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.
- [85] Ma, H., Goryanin, I. (2008). Human metabolic network reconstruction and its impact on drug discovery and development. *Drug Discov. Today* **13**:2 – 8.
- [86] Alon, U. (2006). *An introduction to systems biology: design principles of biological circuits*. Chapman and Hall/CRC, London.
- [87] Palsson, B.Ø. (2006). *Systems biology: properties of reconstructed networks*. Cambridge University Press, Cambridge.
- [88] Kell, D.B. (2007). The virtual human: towards a global systems biology of multi-scale, distributed biochemical network models. *IUBMB Life* **59**:689 – 95.
- [89] Kell, D.B., Mendes, P. (2008). The markup is the model: reasoning about systems biology models in the Semantic Web era. *J. Theoret. Biol.* **252**:538 – 543.
- [90] Li, P., Oinn, T., Stoiland, S., Kell, D.B. (2008). Automated manipulation of systems biology models using libSBML within Taverna workflows. *Bioinformatics* **24**:287 – 289.
- [91] Hucka, M., Finney, A., Sauro, H.M., Bolouri, H., Doyle, J.C., Kitano, H., Arkin, A.P., Bornstein, B.J., Bray, D., Cornish-Bowden, A., Cuellar, A.A., Dronov, S., Gilles, E.D., Ginkel, M., Gor, V., Goryanin, I., Hedley, W.J., Hodgman, T.C., Hofmeyr, J.H., Hunter, P.J., Juty, N.S., Kasberger, J.L., Kremling, A., Kummer, U., Le Novere, N., Loew, L. M., Lucio, D., Mendes, P., Minch, E., Mjolsness, E.D., Nakayama, Y., Nelson, M.R., Nielsen, P.F., Sakurada, T., Schaff, J.C., Shapiro, B.E., Shimizu, T.S., Spence, H.D., Stelling, J., Takahashi, K., Tomita, M., Wagner, J., Wang, J. (2003). The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. *Bioinformatics* **19**:524 – 31.
-

- [92] Le Novère, N., Finney, A., Hucka, M., Bhalla, U.S., Campagne, F., Collado-Vides, J., Crampin, E.J., Halstead, M., Klipp, E., Mendes, P., Nielsen, P., Sauro, H., Shapiro, B., Snoep, J.L., Spence, H.D., Wanner, B.L. (2005). Minimum information requested in the annotation of biochemical models (MIRIAM). *Nat. Biotechnol.* **23**:1509–15.
- [93] Hergård, M.J., Swainston, N., Dobson, P., Dunn, W.B., Arga, K.Y., Arvas, M., Blüthgen, N., Borger, S., Costenoble, R., Heinemann, M., Hucka, M., Le Novère, N., Li, P., Liebermeister, W., Mo, M.L., Oliveira, A.P., Petranovic, D., Pettifer, S., Simeonidis, E., Smallbone, K., Spasiæ, I., Weichart, D., Brent, R., Broomhead, D.S., Westerhoff, H.V., Kýrdar, B., Penttilä, M., Klipp, E., Palsson, B.Ø., Sauer, U., Oliver, S.G., Mendes, P., Nielsen, J., Kell, D.B. (2008). A consensus yeast metabolic network obtained from a community approach to systems biology. *Nat. Biotechnol.* **26**:1155–1160.
- [94] Noble, D. (2006). *The music of life: biology beyond genes*. Oxford University Press, Oxford.
- [95] Anderle, P., Huang, Y., Sadée, W. (2004). Intestinal membrane transport of drugs and nutrients: genomics of membrane transporters using expression microarrays. *Eur. J. Pharm. Sci.* **21**:17–24.
- [96] Ekins, S., Ecker, G.F., Chiba, P., Swaan, P.W. (2007). Future directions for drug transporter modelling. *Xenobiotica* **37**:1152–70.
- [97] Palmieri, F. (2008). Diseases caused by defects of mitochondrial carriers: A review. *Biochim. Biophys. Acta* **1777**:564–78.
- [98] Persson, A., Hober, S., Uhlen, M. (2006). A human protein atlas based on antibody proteomics. *Curr. Opin. Mol. Ther.* **8**:185–90.
- [99] Barbe, L., Lundberg, E., Oksvold, P., Stenius, A., Lewin, E., Björling, E., Asplund, A., Ponten, F., Brismar, H., Uhlén, M., Andersson-Svahn, H. (2008). Toward a confocal subcellular atlas of the human proteome. *Mol. Cell Proteomics* **7**:499–508.
- [100] Smallbone, K., Simeonidis, E., Broomhead, D.S., Kell, D.B. (2007). Something from nothing: bridging the gap between constraint-based and kinetic modelling. *FEBS J.* **274**:5576–5585.
- [101] Mendes, P., Kell, D.B. (1998). Non-linear optimization of biochemical pathways: applications to metabolic engineering and parameter estimation. *Bioinformatics* **14**:869–883.
- [102] Moles, C.G., Mendes, P., Banga, J.R. (2003). Parameter estimation in biochemical pathways: a comparison of global optimization methods. *Genome Res.* **13**:2467–74.
-

- [103] Hoops, S., Sahle, S., Gauges, R., Lee, C., Pahle, J., Simus, N., Singhal, M., Xu, L., Mendes, P., Kummer, U. (2006). COPASI: a COMplex PATHway SIMulator. *Bioinformatics* **22**:3067–74.
- [104] Rodriguez-Fernandez, M., Mendes, P., Banga, J.R. (2006). A hybrid approach for efficient and robust parameter estimation in biochemical pathways. *Biosystems* **83**:248–65.
- [105] Bongard, J., Lipson, H. (2007). Automated reverse engineering of nonlinear dynamical systems. *Proc. Natl. Acad. Sci. U.S.A.* **104**:9943–8.
- [106] Jayawardhana, B., Kell, D.B., Rattray, M. (2008). Bayesian inference of the sites of perturbations in metabolic pathways via Markov Chain Monte Carlo. *Bioinformatics* **24**:1191–1197.
- [107] Wilkinson, S.J., Benson, N., Kell, D.B. (2008). Proximate parameter tuning for biochemical networks with uncertain kinetic parameters. *Mol. Biosyst.* **4**:74–97.
- [108] Bäck, T., Fogel, D.B., Michalewicz, Z. (1997). *Handbook of evolutionary computation*. IOP Publishing/Oxford University Press, Oxford.
- [109] Corne, D., Dorigo, M., & Glover, F. (1999). *New ideas in optimization*. McGraw Hill, London.
- [110] Kell, D.B., Darby, R.M., Draper, J. (2001). Genomic computing: explanatory analysis of plant expression profiling data using machine learning. *Plant Physiol.* **126**:943–951.
- [111] Salter, G.J., Kell, D.B. (1995). Solvent selection for whole cell biotransformations in organic media. *CRC Crit. Rev. Biotechnol.* **15**:139–177.
- [112] Shuker, S.B., Hajduk, P.J., Meadows, R.P., Fesik, S.W. (1996). Discovering high-affinity ligands for proteins: SAR by NMR. *Science* **274**:1531–4.
- [113] Schneider, G., Lee, M.L., 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**:487–94.
- [114] Rees, D.C., Congreve, M., Murray, C.W., Carr, R. (2004). Fragment-based lead discovery. *Nat. Rev. Drug Discov.* **3**:660–72.
- [115] Erlanson, D.A., Hansen, S.K. (2004). Making drugs on proteins: site-directed ligand discovery for fragment-based lead assembly. *Curr. Opin. Chem. Biol.* **8**:399–406.
- [116] Erlanson, D.A., McDowell, R.S., O'Brien, T. (2004). Fragment-based drug discovery. *J. Med. Chem.* **47**:3463–82.
-

- [117] Hajduk, P.J. (2006). Fragment-based drug design: how big is too big? *J. Med. Chem.* **49**:6972–6.
- [118] Leach, A.R., Hann, M.M., Burrows, J.N., Griffen, E.J. (2006). Fragment screening: an introduction. *Mol. Biosyst.* **2**:430–46.
- [119] Albert, J.S., Blomberg, N., Breeze, A.L., Brown, A.J., Burrows, J.N., Edwards, P.D., Folmer, R.H., Geschwindner, S., Griffen, E.J., Kenny, P.W., Nowak, T., Olsson, L.L., Sanganee, H., Shapiro, A.B. (2007). An integrated approach to fragment-based lead generation: philosophy, strategy and case studies from AstraZeneca's drug discovery programmes. *Curr. Top. Med. Chem.* **7**:1600–29.
- [120] Alex, A.A., Flocco, M.M. (2007). Fragment-based drug discovery: what has it achieved so far? *Curr. Top. Med. Chem.* **7**:1544–67.
- [121] Ciulli, A., Abell, C. (2007). Fragment-based approaches to enzyme inhibition. *Curr. Opin. Biotechnol.* **18**:489–96.
- [122] Hajduk, P.J., Greer, J. (2007). A decade of fragment-based drug design: strategic advances and lessons learned. *Nat. Rev. Drug Discov.* **6**:211–9.
- [123] Hubbard, R.E., Davis, B., Chen, I., Drysdale, M.J. (2007). The SeeDs approach: integrating fragments into drug discovery. *Curr. Top. Med. Chem.* **7**:1568–81.
- [124] Hubbard, R.E., Chen, I., Davis, B. (2007). Informatics and modeling challenges in fragment-based drug discovery. *Curr. Opin. Drug Discov. Devel.* **10**:289–97.
- [125] Jhoti, H., Cleasby, A., Verdonk, M., Williams, G. (2007). Fragment-based screening using X-ray crystallography and NMR spectroscopy. *Curr. Opin. Chem. Biol.* **11**:485–93.
- [126] Jhoti, H. (2007). Fragment-based drug discovery using rational design. *Ernst Scheering Found Symp. Proc.* 169–85.
- [127] Morphy, R., Rankovic, Z. (2007). Fragments, network biology and designing multiple ligands. *Drug Discov. Today* **12**:156–60.
- [128] Siegal, G., Ab, E., Schultz, J. (2007). Integration of fragment screening and library design. *Drug Discov. Today* **12**:1032–9.
- [129] Hesterkamp, T., Whittaker, M. (2008). Fragment-based activity space: smaller is better. *Curr. Opin. Chem. Biol.* **12**:260–8.
- [130] Hubbard, R.E. (2008). Fragment approaches in structure-based drug discovery. *J. Synchrotron Res.* **15**:227–230.
-

- [131] Dobson, P.D., Patel, Y., Kell, D.B. (2008). “Metabolite-likeness” as a criterion in the design and selection of pharmaceutical drug libraries. *Drug Disc. Today*, **14**(1–2):31–40.
- [132] Willett, P., Barnard, J.M., Downs, G.M. (1998). Chemical similarity searching. *J. Chem. Inf. Comp. Sci.* **38**:983–996.
- [133] Bajorath, J. (2004). *Cheminformatics: concepts, methods and tools for drug discovery*. Humana Press, Totowa, NJ.
- [134] Maldonado, A.G., Doucet, J.P., Petitjean, M., Fan, B.T. (2006). Molecular similarity and diversity in cheminformatics: from theory to applications. *Mol. Divers.* **10**:39–79.
- [135] Eckert, H., Bajorath, J. (2007). Molecular similarity analysis in virtual screening: foundations, limitations and novel approaches. *Drug Discov. Today* **12**:225–33.
- [136] Russell, B. (1947). *Am I an atheist or an agnostic?*
- [137] Harnad, S., Brody, T., Vallieres, F., Carr, L., Hitchcock, S., Gingras, Y., Oppenheim, C., Hajjem, C., Hilf, E.R. (2008). The access/impact problem and the green and gold roads to open access: An update. *Serials Review* **34**:36–40.
- [138] Kostoff, R.N. (2002). Overcoming specialization. *Bioscience* **52**:937–941.
- [139] Kell, D.B., Oliver, S.G. (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.
- [140] Hoffmann, R., Krallinger, M., Andres, E., Tamames, J., Blaschke, C., Valencia, A. (2005). Text mining for metabolic pathways, signaling cascades, and protein networks. *Sci. STKE* **2005**, pe21.
- [141] Ananiadou, S., McNaught, J. (2006). *Text mining in biology and biomedicine*. Artech House, London.
- [142] Ananiadou, S., Kell, D.B., Tsujii, J.-I. (2006). Text Mining and its potential applications in Systems Biology. *Trends Biotechnol.* **24**:571–579.
- [143] Jensen, L.J., Saric, J., Bork, P. (2006). Literature mining for the biologist: from information retrieval to biological discovery. *Nat. Rev. Genet.* **7**:119–29.
- [144] Torvik, V.I., Smalheiser, N.R. (2007). A quantitative model for linking two disparate sets of articles in MEDLINE. *Bioinformatics* **23**:1658–65.
- [145] Smalheiser, N.R., Swanson, D.R. (1998). Using ARROWSMITH: a computer-assisted approach to formulating and assessing scientific hypotheses. *Comput. Methods Programs Biomed.* **57**:149–53.
-

- [146] Swanson, D.R., Smalheiser, N.R., Torvik, V.I. (2006). Ranking indirect connections in literature-based discovery: The role of medical subject headings. *J. Amer. Soc. Inf. Sci. Technol.* **57**:1427 – 1439.
- [147] Weeber, M., Kors, J.A., Mons, B. (2005). Online tools to support literature-based discovery in the life sciences. *Brief Bioinform.* **6**:277 – 86.
- [148] van der Eijk, C.C., van Mulligen, E.M., Kors, J.A., Mons, B., an den Berg, J. (2004). Constructing an associative concept space for literature-based discovery. *J. Amer. Soc. Information Sci. Technol.* **55**:436 – 444.
- [149] Lindsay, R.K., Gordon, M.D. (1999). Literature-based discovery by lexical statistics. *J. Amer. Soc. Inf. Sci.* **50**:574 – 587.
- [150] Yetisgen-Yildiz, M., Pratt, W. (2006). Using statistical and knowledge-based approaches for literature-based discovery. *J. Biomed. Informatics* **39**:600 – 611.
- [151] Weeber, M., Kors, J.A., Mons, B. (2005). Online tools to support literature-based discovery in the life sciences. *Briefings in Bioinformatics* **6**:277 – 286.
- [152] Kostoff, R.N., Briggs, M.B., Solka, J.L., Rushenberg, R.L. (2008). Literature-related discovery (LRD): Methodology. *Technol. Forecast. Soc. Change*, doi:10.1016/j.techfore.2007.11.010.
-