The promiscuous binding of pharmaceutical drugs and their transporter-mediated uptake into cells: what we (need to) know and how we can do so

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A recent paper in this journal sought to counter evidence for the role of transport proteins in effecting drug uptake into cells, and questions that transporters can recognize drug molecules in addition to their endogenous substrates. However, there is abundant evidence that both drugs and proteins are highly promiscuous. Most proteins bind to many drugs and most drugs bind to multiple proteins (on average more than six), including transporters (mutations in these can determine resistance); most drugs are known to recognise at least one transporter. In this response, we alert readers to the relevant evidence that exists or is required. This needs to be acquired in cells that contain the relevant proteins, and we highlight an experimental system for simultaneous genome-wide assessment of carrier-mediated uptake in a eukaryotic cell (yeast).

Introduction

As part of a continuing discussion [1–6], Di and colleagues [7] recently published a paper in this journal in which they sought to counter the rather voluminous (and increasing) evidence for the proteinaceous carrier-mediated cellular uptake of pharmaceutical and other drugs (by genetically identified carriers) being the norm in favour of passive diffusion through the putative protein-free bilayer portions of biological membranes.

Di *et al.* [7] sought to dismiss a set of 38 articles that we mentioned [5] in favour of transporter-mediated drug uptake and referred to them as 'opinion pieces and not research articles'. These 38 were of course chosen on the basis that they represented review articles that summarised many hundreds of research articles. Moreover, our own first survey [1] had more than 300 references alone (a restricted subset [8–10]). There is burgeoning evidence for the carrier-mediated view of drug uptake, and such reviews continue to appear [11–102].

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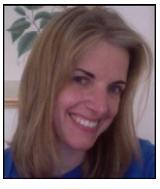
Douglas Kell took an MA (biochemistry) and DPhil (Oxon) in 1978. After several personal fellowships and other posts in what is now the University of Aberystwyth, he was awarded a Personal Chair (1992). He was a Founding Director of Aber Instruments Ltd (Queen's Award for Export Achievement, 1998). He moved to Manchester in 2002 and from 2005 to 2008 was Director, BBSRC Manchester Centre for Integrative Systems Biology (www.mcisb.org/). Awards include the Fleming Award of the Society for General Microbiology (1986), RSC Interdisciplinary Science Award (2004), the FEBS-IUBMB Theodor Bücher prize, Royal Society/Wolfson Merit Award RSC Award in Chemical Biology (all 2005), and the 2006 Royal Society of Chemistry/Society of Analytical Chemistry Gold Medal. Since 2008 he has been serving on secondment as Chief Executive, UK Biotechnology and Biological Science Research Council.

Paul Dobson holds a degree in biochemistry and a PhD (2005) in structural biology with machine learning from UMIST. Following short postdoctoral positions in text mining and Raman spectroscopy, in 2006 he joined the group of Professor Douglas Kell at The University of Manchester, where he led cheminformatics research on mechanisms of drug uptake into cells, and yeast systems biology. He moved to Sheffield in 2010 as a ChELSI postdoctoral researcher with Dr Stephen Wilkinson, and in 2012 was appointed to a lectureship in biomanufacturing. His current research applies computer modelling to improve cell factories for the production of high-value chemicals and biotherapeutics.



Elizabeth (Bessie) Bilsland was born and brought up in Brazil where she graduated in agronomic engineering (ESALQ – USP), mastering in animal science and biotechnology. She obtained her PhD in Prof. Sunnerhagen's laboratory (Goteborg University – Sweden) working on yeast stress responses. She has over a decade of laboratory experience with the yeast *Saccharomyces cerevisiae* and is particularly interested in synthetic biology and assay development for yeast-based drug screens. She has supervised highly successful undergraduate and postgraduate students during both her PhD and post-doctoral work (Cambridge, UK). Recently, she established contacts with FAPESP and the British Consulate in Sao Paulo, which led to the organization of the Workshop on Synthetic Biology and Robotics, and to collaborations with laboratories from the University of Sao Paulo (USP) and Unicamp. She successfully combines a scientific career with raising three children.

Stephen Oliver is Professor of Systems Biology & Biochemistry and Director of the Centre for Systems Biology at Cambridge. He led the team that sequenced the first chromosome, from any organism, yeast chromosome III. His current work employs comprehensive, high-throughput analytical techniques – transcriptomics, proteomics, metabolomics, and rapid phenotyping. He is a member of EMBO, and a Fellow of the: American Association for the Advancement of Science, American Academy of Microbiology, and Academy of Medical Sciences. Prof. Oliver was Kathleen Barton-Wright Memorial Lecturer of the Society for General Microbiology in 1996, and won the Biochemical Society's AstraZeneca Award in 2001.

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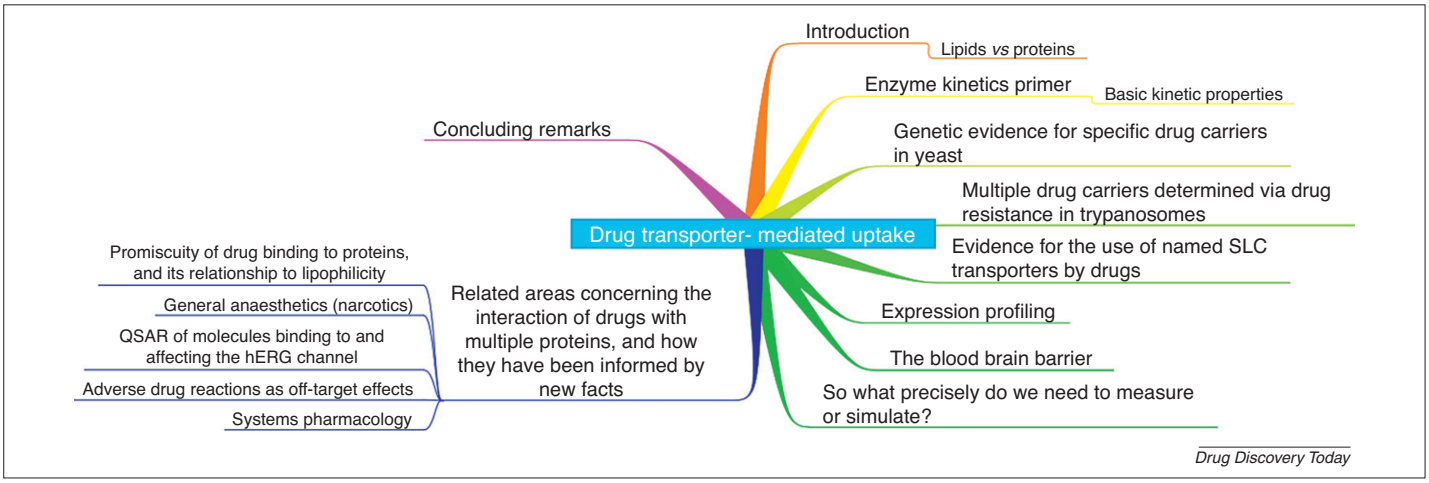

FIGURE 1

A 'mind map' [519] of the contents of this article.

Here, we seek to set down the kinds of experiments that might usefully be done (or indeed have already been done) and that would provide evidence for the overwhelming importance of drug and xenobiotic carriers in real biological membranes. Specifically, in studying transport into and out of cells it is sensible to study living cells rather than artificial membranes. The study of black lipid membranes or any other artificial constructs that are not themselves biological membranes (and thus lack carriers or other proteins) tells us nothing significant about the properties of real biological membranes that possess such carriers, and that is where our *in vivo* interest lies. We lay particular stress on the evidence that proteins and drugs are rather promiscuous with regard to their interactions with each other, because this lies at the heart of the interactions of drugs with multiple carriers. Moreover, we would remind readers of our previous stricture [5], epistemologically based [103], that absence of evidence is not evidence of absence. A 'mind map' summarising this article is shown in Fig. 1.

Lipids versus proteins

As rehearsed previously [5], there is little evidence that specific lipid moieties of the kinds typically found in eukaryotic membranes have substantially different biophysical properties from each other, and thus we assume that any transfer of xenobiotics across biomembranes that is claimed to go via lipid bilayers is similarly constrained. A factor of at most two in the variation of any flux for this seems reasonable. However, because carrier-mediated uptake requires the presence of genetically encoded proteins (any of which may be subject to post-translational modification) our focus is going to be on the evidence that named proteins with identified genetic loci have marked, reasonable and testable (or, indeed, tested) influences on the rate of transport of xenobiotics (and intermediary metabolites) across biological membranes. We shall also seek to avoid making claims not based simply on these facts. Many molecules have negligible permeability in artificial membrane assays, but much greater ones in biological cells; one of many examples is from a recent study [104] of cyclic peptides whose artificial membrane permeability, despite substantial lipophilicity, is both largely negligible and very poorly correlated with lipophilicity.

We also ignore discussions of artificial membranes lacking proteins. Whether biological membranes have protein:lipid ratios of 3:1, 1:1 or 2:3 is not of itself the issue, because one thing is certain [105]: the value is not 0:1. Also it is effectively the area ratio that governs the appearance of a membrane to a substrate as seen from the outside; the molar ratio of proteins to lipids [7] is a poor guide because lipids are so much smaller than proteins, although we certainly recognise the role of lipids in the barrier function of membranes. In addition, we note the rather elastic analysis by which a hexadecane layer either helps or hinders the passage of drugs through aqueous pores (cf. Figs 1 and 2 of Di *et al.* [7]). We note further that a membrane arrangement containing a hexadecane layer of unstated thickness is not really an adequate model for a phospholipid bilayer, if only because hexadecane (unlike pure phospholipid bilayer membranes, and even erythrocyte ghosts [106]) almost certainly does not admit transient aqueous pores. Equally, Di and colleagues [7] cite a remarkable paper [107] in which the correlation between rat brain permeability and the octanol–water partition coefficient is made reasonable solely by excluding the least convenient five of the 27 compounds measured. Finally, in contrast to the view of Di and colleagues [7], cellular membranes and lipid bilayers retain a high capacitance at frequencies low relative to their inverse charging time even when their conductance is quite substantial [108–112]. However, it is worth pointing to evidence that well-made bilayers have a background permeability to ions that is negligible, a fact exploited in nanopore-based methods of nucleic acid sequencing [113,114].

It is also worth stressing that if biological membranes were permeable to all kinds of solutes (whether via the bilayer portion of membranes or otherwise) they would not display osmotic properties at all. Because it is well known that they do so, it is clear that the non-carrier-mediated permeability of biological membranes to most solutes is, in fact, negligible. Recent evidence indicates that even the passage of extremely small molecules, such as water [115], glycerol [116–121], urea [122–125], hydroxyurea [126], ammonia/ammonium [127–132], bicarbonate [133–135], and CO_2 [136–138] across real biomembrane requires (or at least uses) protein transporters.