The cellular transport of pharmaceutical drugs: a problem not of biophysics but of systems biology

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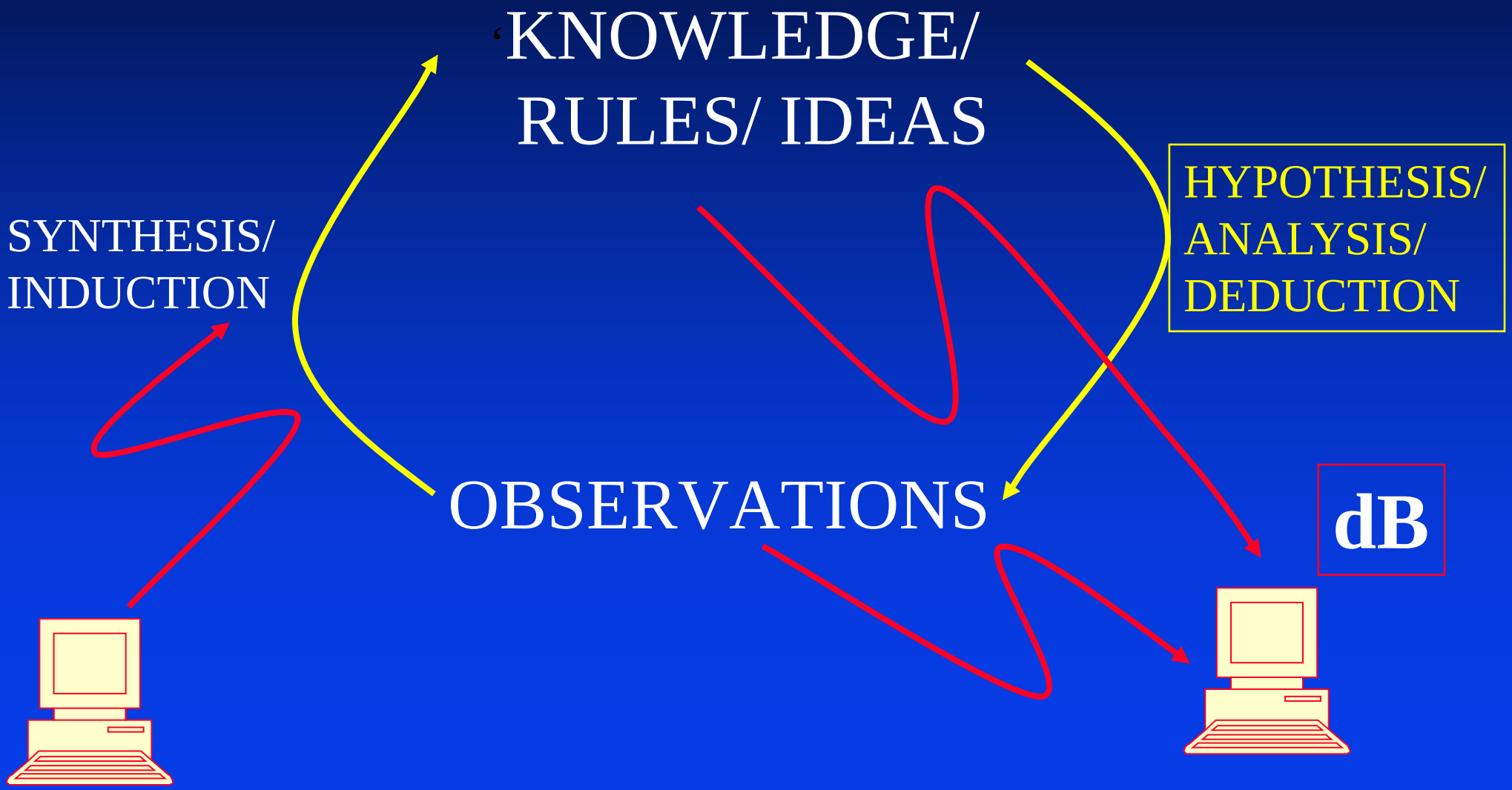
Synopsis of talk

- Philosophy, Systems Biology, 'Omics, and Pharma/AgChem industry issues
- The human metabolic network and its principled informatics/modelling
- The cellular uptake of pharmaceutical drugs: a problem not of biophysics but of systems biology
- An experimental system in yeast
- Implications, e.g. drug-metabolite similarities
- Conclusions
- Afterword – iron behaving badly

The cycle of knowledge



Here is the evidence, now what is the hypothesis? The complementary roles of inductive and hypothesis-driven science in the post-genomic era

Douglas B. Kell[1]* and Stephen G. Oliver[2]

BioEssays 26:99–105, © 2003 Wiley Periodicals, Inc.

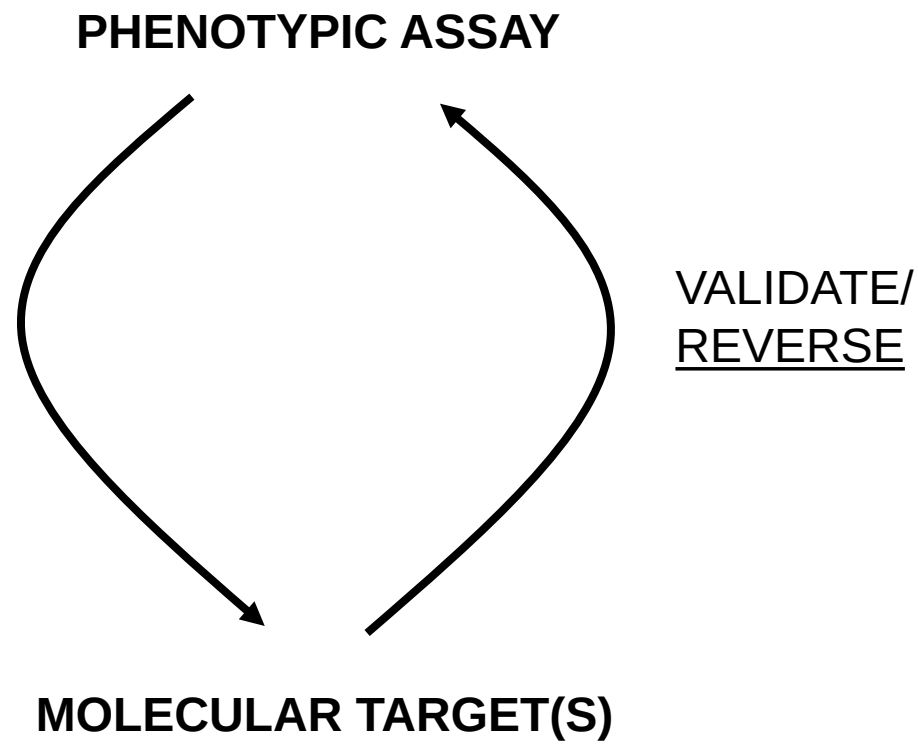
Forward and reverse (chemical) genomics

Classical

Function-first

Screen Organism

Find mechanisms (plural) later



Modern

Gene/target /mechanism -first

Screen target

Test in organism later

Position statement (≡ hypothesis)

- There is in fact no actual evidence (evidence = data plus correct theory and interpretation) that any significant 'passive' permeability goes through lipid bilayers in real (and undamaged) biological membranes, and in the presence of potentially 100s of carriers that might serve to transport drugs it is very hard to obtain it
- Think if your own data are consistent with this

3 counterfactuals that lipid-only theories need (and fail) to explain

1. why most drugs do not diffuse across the blood-brain barrier (and others) where the lipids are not significantly different
2. the substantially varying tissue distributions in cellular drug uptake
3. the very large species differences in cellular drug uptake

Does CO_2 cross intact cellular membranes by using a transporter?

Biochimica et Biophysica Acta xxx (2013) xxx–xxx

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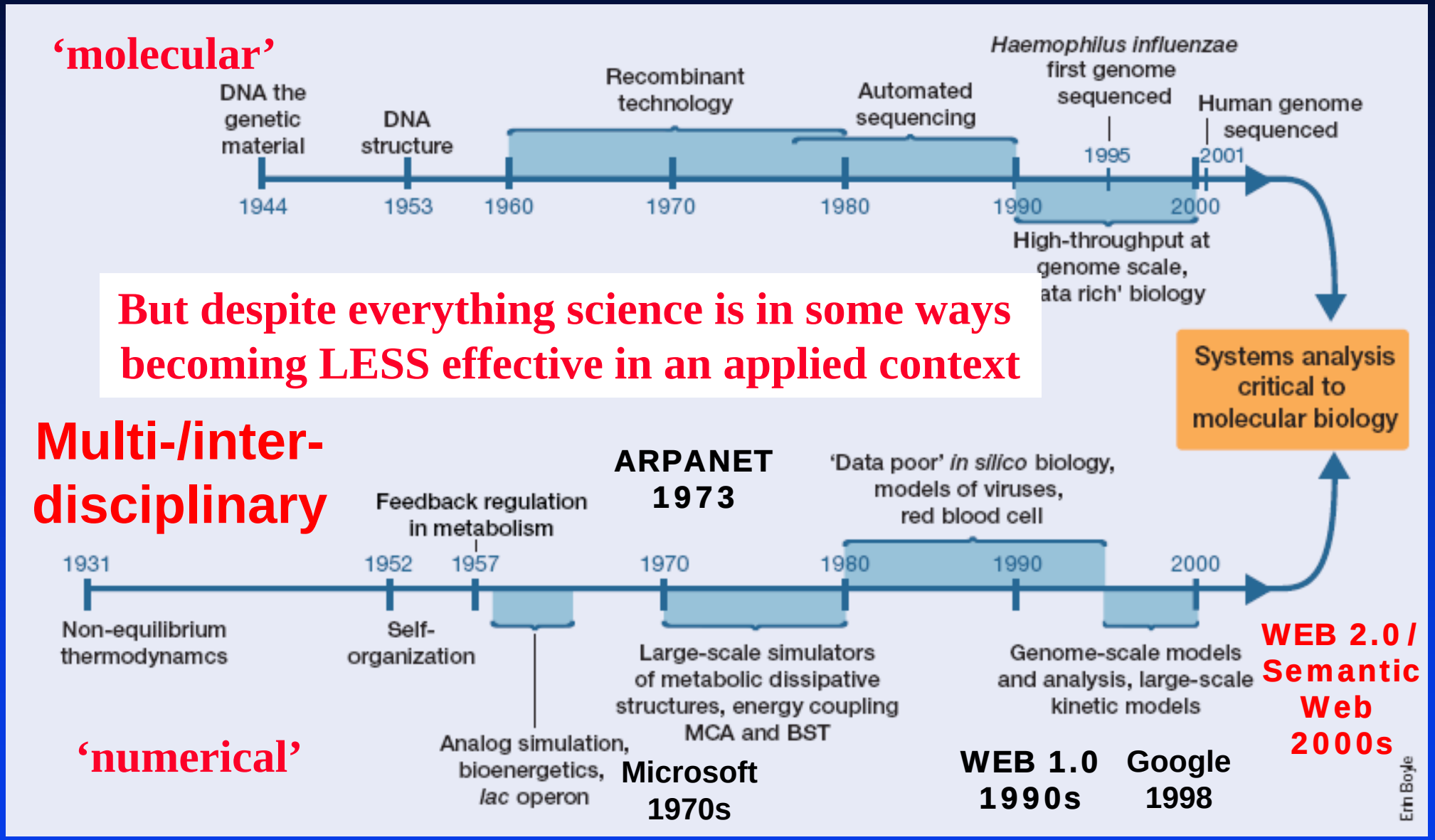
journal homepage: www.elsevier.com/locate/bbagen

Review

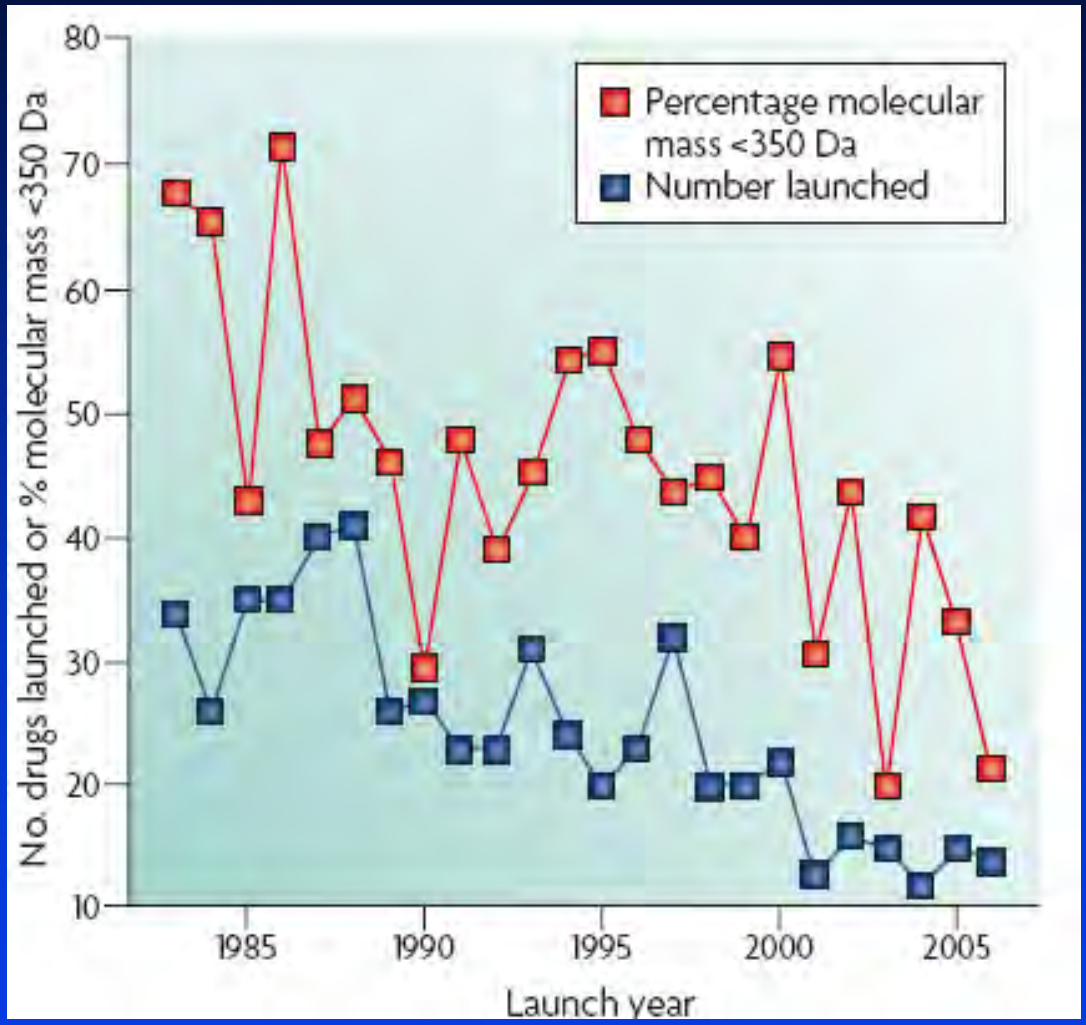
Aquaporins and membrane diffusion of CO_2 in living organisms

Ralf Kaldenhoff *, Lei Kai, Norbert Uehlein

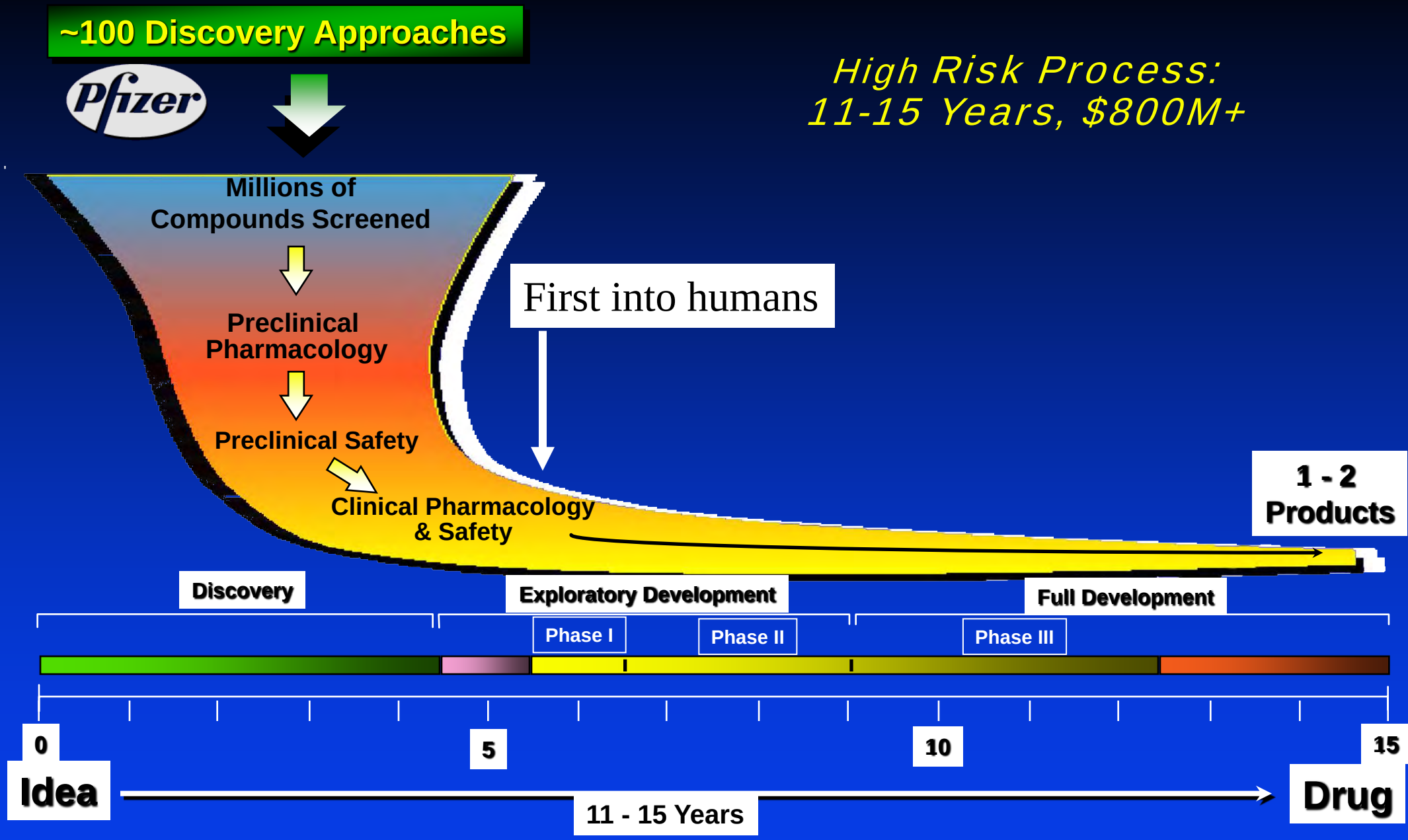
Westerhoff & Palsson NBT 22, 1249-52 (2004)



Declining numbers of drug launches



Leeson & Springthorpe, NRDD 6, 881-890 (2007)



From Lamattina, Pfizer www.wpi.edu/News/Conf/Molecular/Presentations/lamattina.ppt

Attrition

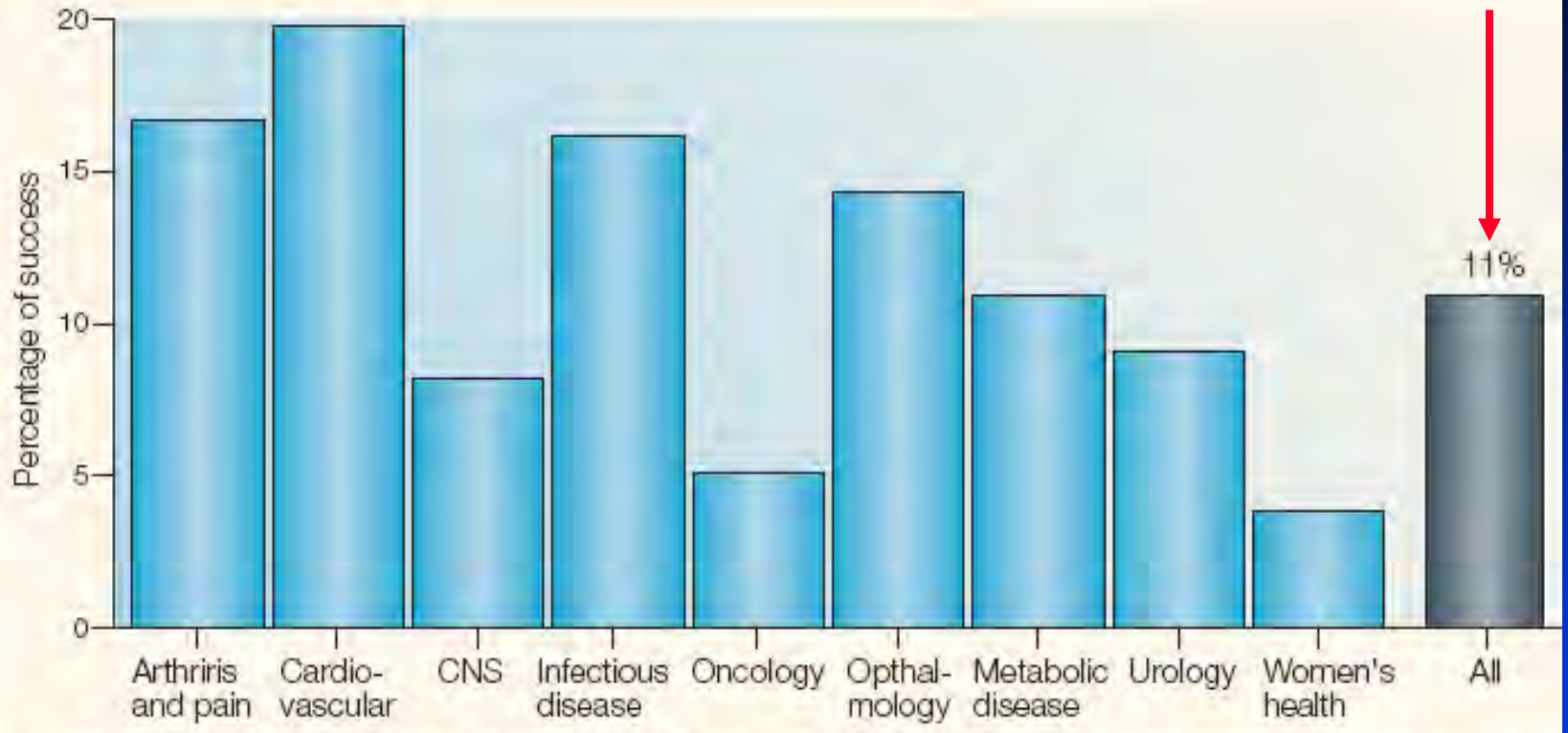


Figure 1 | **Success rates from first-in-man to registration.** The overall clinical success rate is 11%. However, if the analysis is carried out by therapeutic areas, big differences emerge. The data are from the ten biggest drug companies during 1991–2000. (The companies are AstraZeneca, Bristol-Myers Squibb, Eli Lilly, F. Hoffman-LaRoche, GlaxoWellcome, Johnson & Johnson, Novartis, Pfizer, Pharmacia, Schering-Plough and SmithKline Beecham; data were obtained by Datamonitor in the Pharmaceutical Benchmarking Study). CNS, central nervous system.

Kola & Landis, NRDD 3, 711-5 (2004)

Issues of attrition

- Gross PK/PD less of an issue in last decade
- Now mostly due to (i) lack of efficacy, (ii) toxicity
- Both problems are underpinned by the fact that drugs are typically first developed on the basis of isolated molecular assays before being tested in the intact system
- These failures turn drug discovery – if it was not already – into a problem of systems biology

A recent manifesto

FEBS J 280, 5957-5980 (2013)

http://dbkgroup.org/publications

Open Access

the FEBS Journal

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

Need for an Open Access human metabolic network model

IUBMB *Life*, 59(11): 689–695, November 2007

informa
healthcare

A 'grand challenge'….

Feature Article

The Virtual Human: Towards a Global Systems Biology of Multiscale, Distributed Biochemical Network Models

Douglas B. Kell
School of Chemistry and The Manchester Centre for Integrative Systems Biology, The Manchester Interdisciplinary Biocentre, The University of Manchester, Manchester, UK

The human metabolic network (1)

Global reconstruction of the human metabolic network based on genomic and bibliomic data

Natalie C. Duarte, Scott A. Becker, Neema Jamshidi, Ines Thiele, Monica L. Mo, Thuy D. Vo, Rohith Srivas, and Bernhard Ø. Palsson*

- 8 cellular compartments
- 2,712 compartment-specific metabolites
- ~ 1,500 different chemical entities
- 1,496 genes
- 2,233 metabolic reactions (1,795 unique)
- 1,078 transport reactions (32.6%)

PNAS 104, 1777-1782 (2007)

The human metabolic network (2)

Molecular Systems Biology 3; Article number 135; doi:10.1038/msb4100177
Citation: *Molecular Systems Biology* 3:135

www.molecularsystemsbiology.com

molecular systems biology

REPORT

The Edinburgh human metabolic network reconstruction and its functional analysis

Hongwu Ma[1], Anatoly Sorokin[1], Alexander Mazein[1], Alex Selkov[2], Evgeni Selkov[2], Oleg Demin[3] and Igor Goryanin[1,*]

- **Not yet compartmentalised**
- **2,823 reactions (incl 300 'orphans'), of which 2,215 have disease assiociations, plus 1189 transport reactions and 457 exchange reactions**
- **2,322 genes (1069 common with UCSD model)**

Molecular Systems Biology 3, 135 (2007)

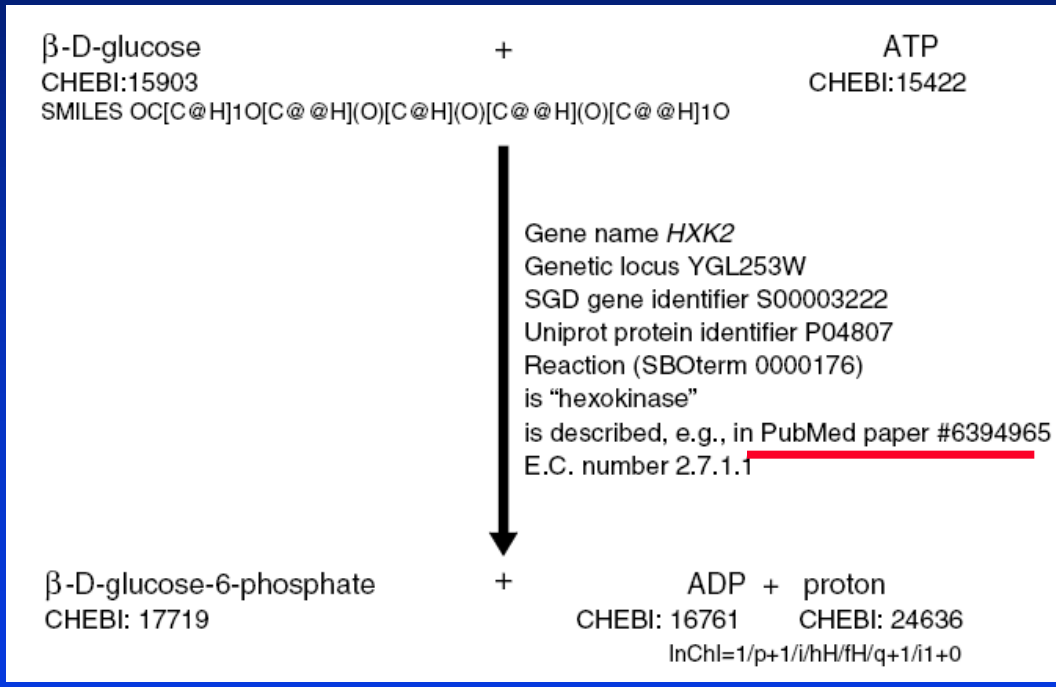
Herrgård *et al.*, Nature Biotechnology 26, 1155-60 (2008)

A consensus yeast metabolic network reconstruction obtained from a community approach to systems biology

Markus J Herrgård[1,19,20], Neil Swainston[2,3,20], Paul Dobson[3,4], Warwick B Dunn[3,4], K Yalçin Arga[5], Mikko Arvas[6], Nils Blüthgen[3,7], Simon Borger[8], Roeland Costenoble[9], Matthias Heinemann[9], Michael Hucka[10], Nicolas Le Novère[11], Peter Li[2,3], Wolfram Liebermeister[8], Monica L Mo[1], Ana Paula Oliveira[12], Dina Petranovic[12,19], Stephen Pettifer[2,3], Evangelos Simeonidis[3,7], Kieran Smallbone[3,13], Irena Spasić[2,3], Dieter Weichart[3,4], Roger Brent[14], David S Broomhead[3,13], Hans V Westerhoff[3,7,15], Betül Kırdar[5], Merja Penttilä[6], Edda Klipp[8], Bernhard Ø Palsson[1], Uwe Sauer[9], Stephen G Oliver[3,16], Pedro Mendes[2,3,17], Jens Nielsen[12,18] & Douglas B Kell[*3,4]

Some key features of yeast consensus reconstruction

- Precise and semantically aware (via InChI, SMILES, dB links and SBO)
- Available online, also as accurate SBML
- Live
- Directly linked to B-net database



http://www.comp-sys-bio.org/yeastnet/

Predicting metabolic fluxes for Industrial Biotechnology

Improving metabolic flux predictions using absolute gene expression data

Open Access

Dave Lee[1†], Kieran Smallbone[1†], Warwick B Dunn[1], Ettore Murabito[1], Catherine L Winder[1], Douglas B Kell[1,2], Pedro Mendes[1,3] and Neil Swainston[1*]

Lee *et al. BMC Systems Biology* 2012, **6**:73

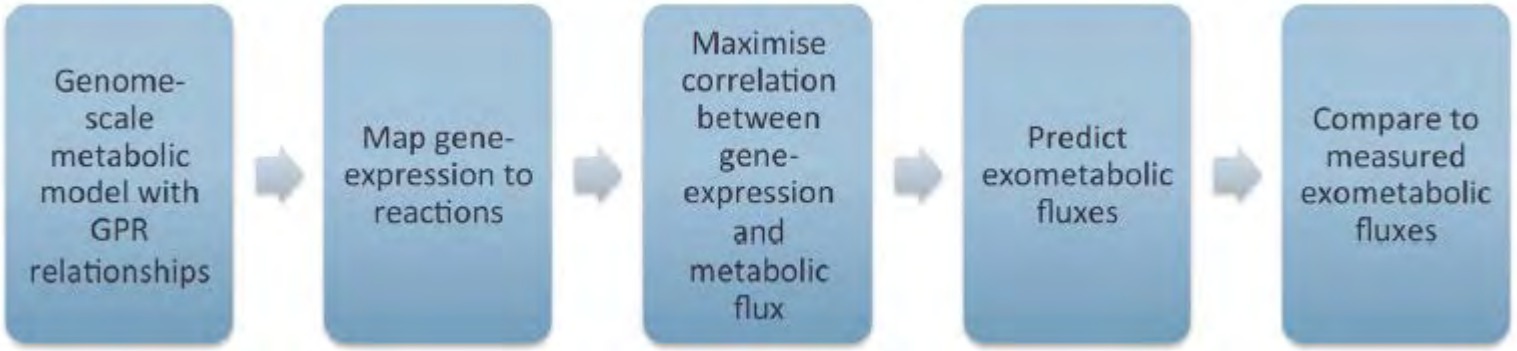


Table 2 Comparison of experimental with predicted exometabolome fluxes, at 85% maximal biomass level

	Experiment	Predicted: Gene expression	Predicted: Standard FBA	Predicted: Fitted FBA	Predicted: GIMME	Predicted: iMAT
Ethanol	13.0	16.2	0	0	0	0
CO_2	21.0	20.1	25.0	21.0	16.0	32.2
Glycerol	2.17	0.126	0	0	0	0
Acetate	0.239	0.00911	0	0	0	0
Trehalose	0.0215	0.0220	0	0	0	0
Lactate	0.00609	0.0176	0	0	0	0
R^2		0.96	0.54	0.58	0.52	0.28

Human network, NBT 31, 419-425 (2013)

A community-driven global reconstruction of human metabolism

Ines Thiele[1,2,36], Neil Swainston[3,4,36], Ronan M T Fleming[1,5], Andreas Hoppe[6], Swagatika Sahoo[1], Maike K Aurich[1], Hulda Haraldsdottir[1], Monica L Mo[7], Ottar Rolfsson[1], Miranda D Stobbe[8,9], Stefan G Thorleifsson[1], Rasmus Agren[10], Christian Bölling[6], Sergio Bordel[10], Arvind K Chavali[11], Paul Dobson[12], Warwick B Dunn[2,13], Lukas Endler[14], David Hala[15], Michael Hucka[16], Duncan Hull[4], Daniel Jameson[3,4], Neema Jamshidi[7], Jon J Jonsson[5], Nick Juty[17], Sarah Keating[17], Intawat Nookaew[10], Nicolas Le Novère[17,18], Naglis Malys[3,19,20], Alexander Mazein[21], Jason A Papin[11], Nathan D Price[22], Evgeni Selkov, Sr[23], Martin I Sigurdsson[1], Evangelos Simeonidis[22,24], Nikolaus Sonnenschein[25], Kieran Smallbone[3,26], Anatoly Sorokin[21,27], Johannes H G M van Beek[28–30], Dieter Weichart[3,31], Igor Goryanin[21,32], Jens Nielsen[10], Hans V Westerhoff[3,28,33], Douglas B Kell[3,34], Pedro Mendes[3,4,35] & Bernhard Ø Palsson[1,7]

7440 reactions (~1/3 transport), 5,063 metabolites, 2,626 unique metabolites

Freely available at http://humanmetabolism.org/

Predicts e.g. Inborn errors of metabolism, exometabolites, drug actions, cellular differences

OPINION

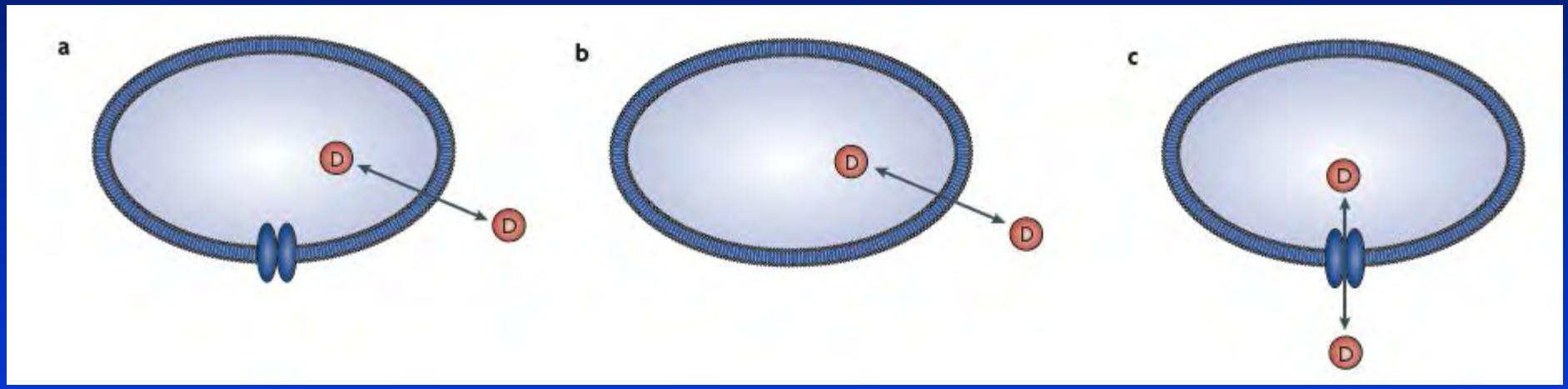
Carrier-mediated cellular uptake of pharmaceutical drugs: an exception or the rule?

Paul D. Dobson and Douglas B. Kell

The types of biophysical forces that determine the interaction of drugs with lipids (especially hydrophobic and hydrogen-bonding interactions) are no different from those involved in their interaction with proteins, especially hydrophobic transport proteins. Therefore, biophysical arguments alone cannot make a mechanistic distinction between the two modes of transport that are outlined in FIG. 1. Indeed, four lines of reasoning together suggest that carrier-mediated

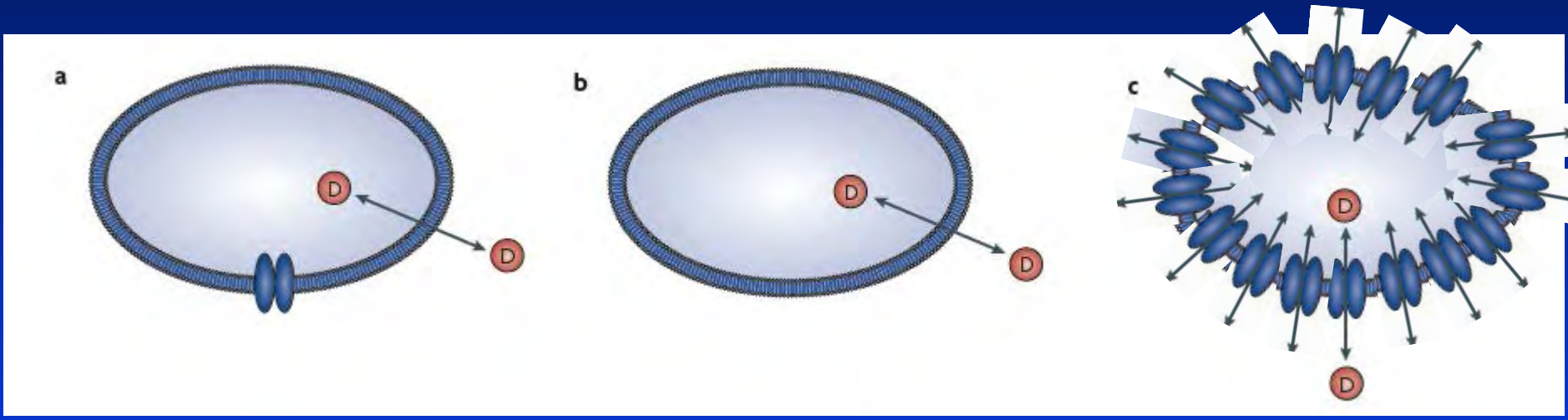
Nature Rev Drug Disc 7, 205-220 (March 2008)

How drugs can cross cellular membranes



By free diffusion or carrier-mediated?

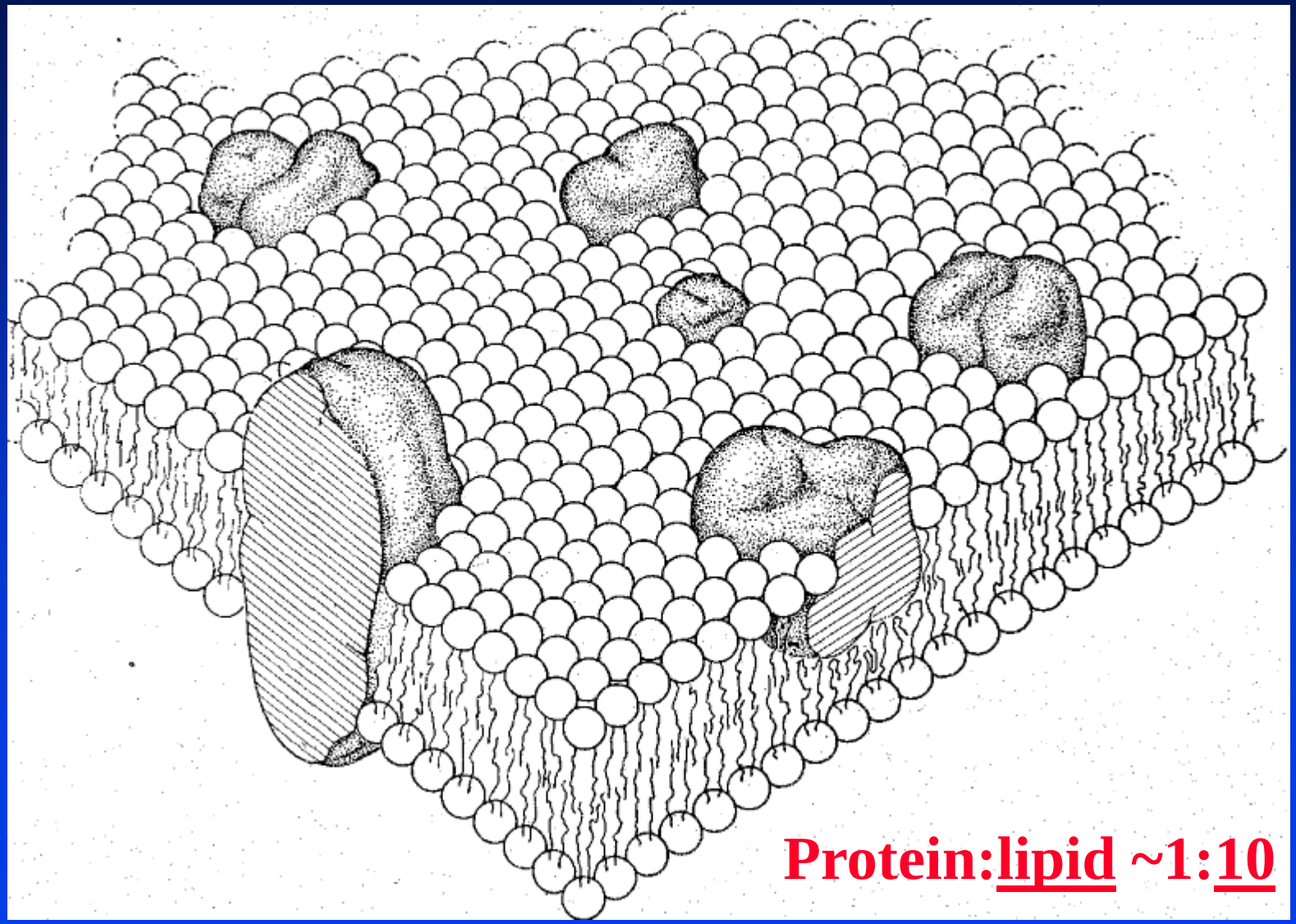
How drugs can cross cellular membranes



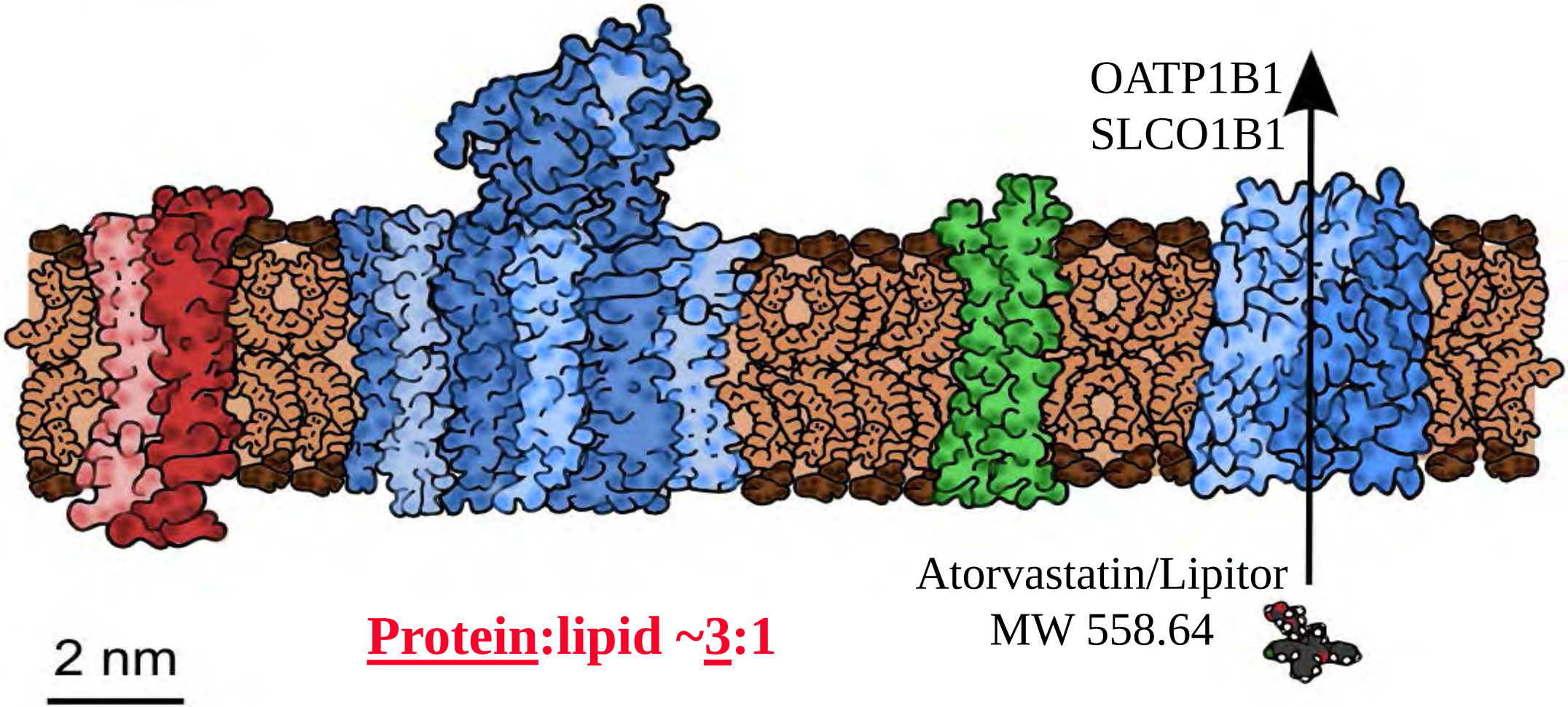
By free diffusion or carrier-mediated?

Note that in real biological membranes there is little or no unperturbed bilayer: Dupuy AD, Engelman DM: Protein area occupancy at the center of the red blood cell membrane. Proc Natl Acad Sci U S A 2008; 105:2848-2852.

Singer & Nicolson, Science 1972



A typical biomembrane drawn to scale



Protein:lipid ~3:1

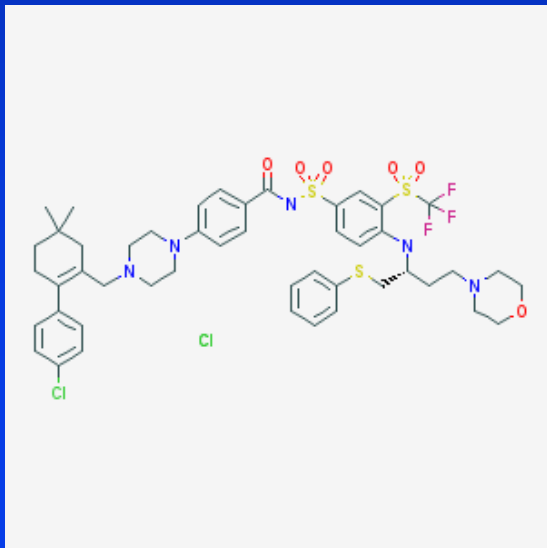
Drug transport – Lipinski's 'Rule of 5'

- **Poor absorption or permeation is more likely when**
- **H-bond donors > 5**
- **H-bond acceptors > 10**
- **MW > 500, and**
- **calculated Log P (CLogP) > 5**

Lipinski *et al.*, Adv Drug Delivery Rev 46, 3-26 (2001) (reprint of 1997 version)

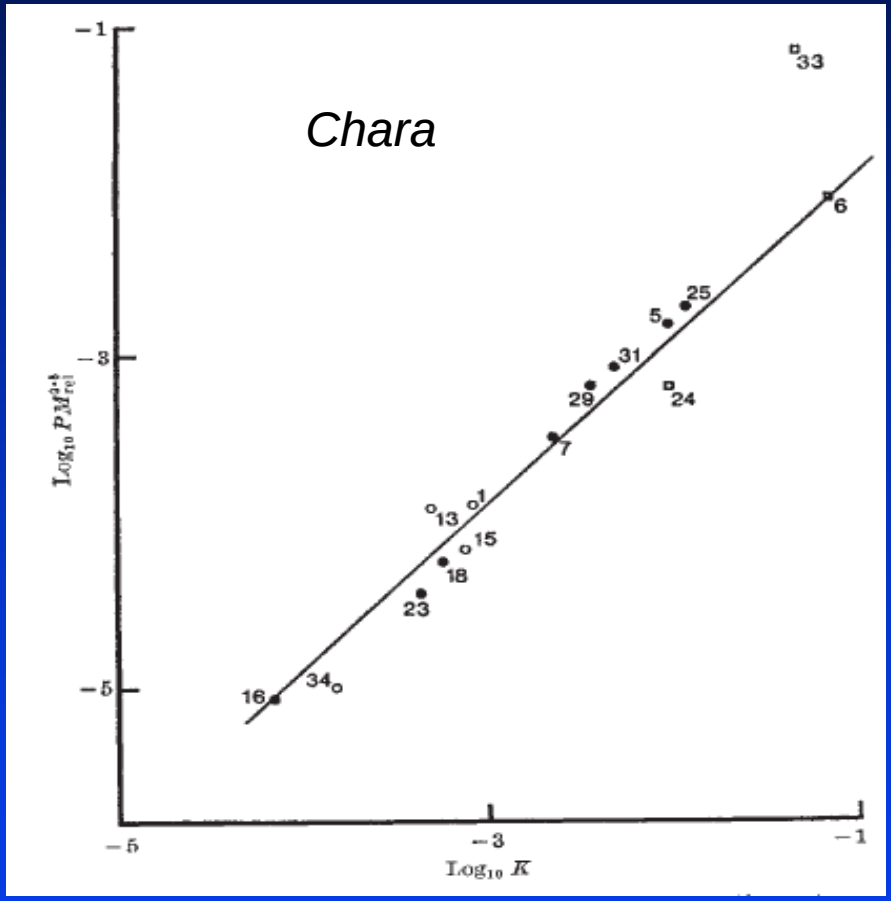
Lipinski Ro5

- While empirical, it effectively imputes a trade-off between lipophilicity to cross membranes and hydrophilicity to ensure aqueous solubility
- Lipinski's Rule-of-5 for determining the likely bioavailability of a compound does <u>implicitly</u> assume the pre-eminence of the lipid diffusion route as it <u>explicitly</u> classes carrier-mediated transport as an exception
- Navitoclax.2HCl – $C_{47}H_{57}Cl_3F_3N_5O_6S_3$, MW1047.53, 7 ring systems!



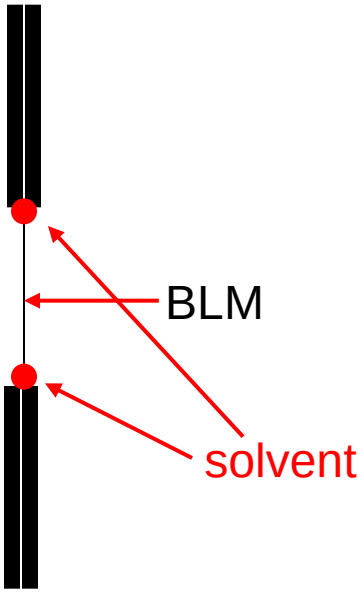
"Overton's Rule" (1899)

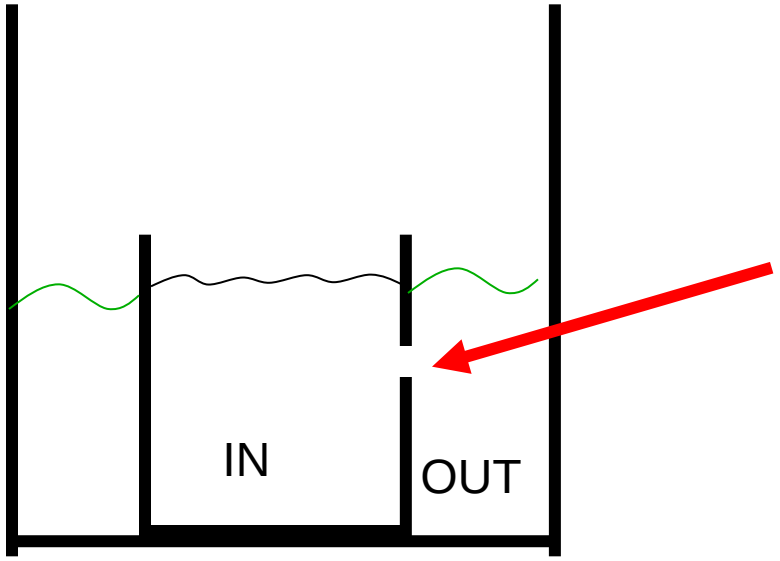
- **Permeability coefficients of nonelectrolytes across biomembranes correlate well with olive oil/water partition coefficients.**
- **(nowadays octanol is more typically used)**
- **Log $K_{o/w}$ = log D or sometimes log P**



Lieb & Stein, Nature 224, 220-3 (1969)

Black (Bilayer) Lipid Membranes (BLMs) – Mueller, Rudin, Tien, Westcott 1962





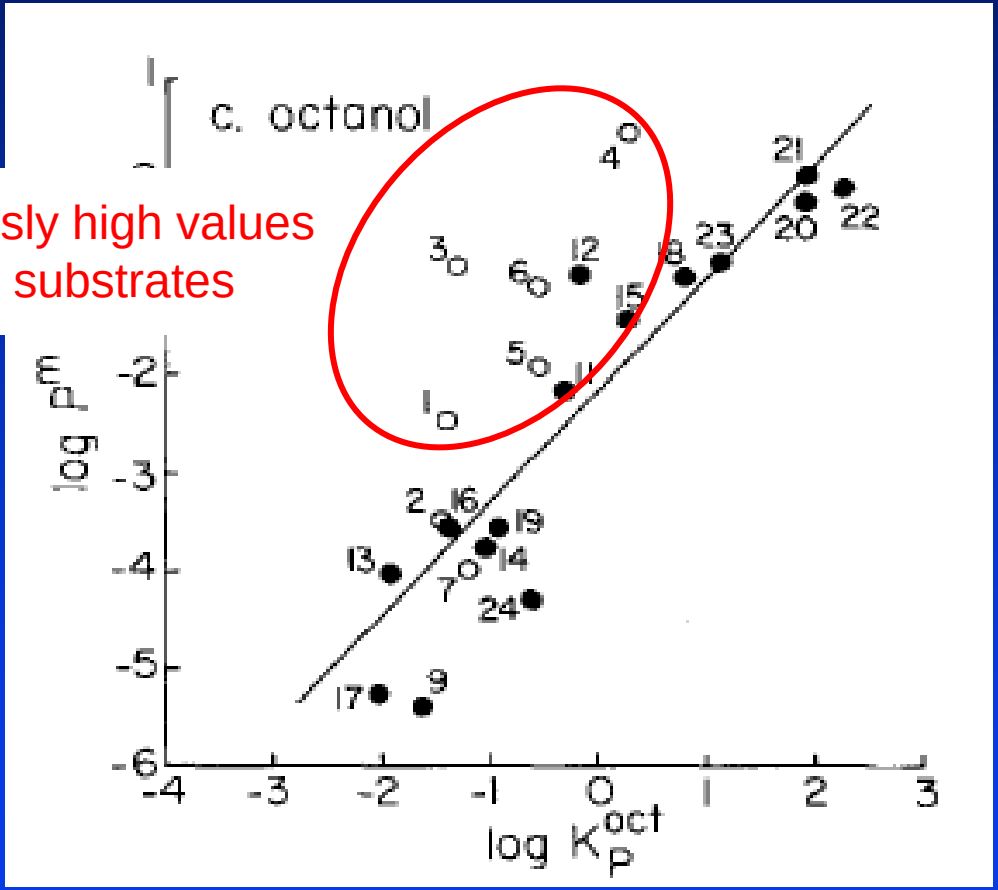
Pore over which is painted a solution of phospholipids and (maybe) ‘oxidised cholesterol’ in an organic solvent

Transmembrane transport can be assessed by adding a substance ‘outside’ and observing the kinetics of its appearance ‘inside’ – unfortunately most is through transient aqueous pores

Black (Bilayer) Lipid Membranes (BLMs)

- Many early studies (70s/80s) were conducted using BLMs
- Some (surprisingly few were performed) showed that uncharged molecules and **even metal ions** could cross them

Note anomalously high values for smallest substrates

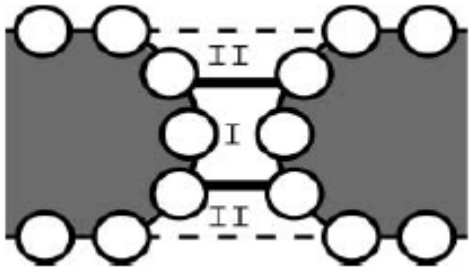


Walter & Gutknecht, J Membr Biol 90, 207-217 (1986)

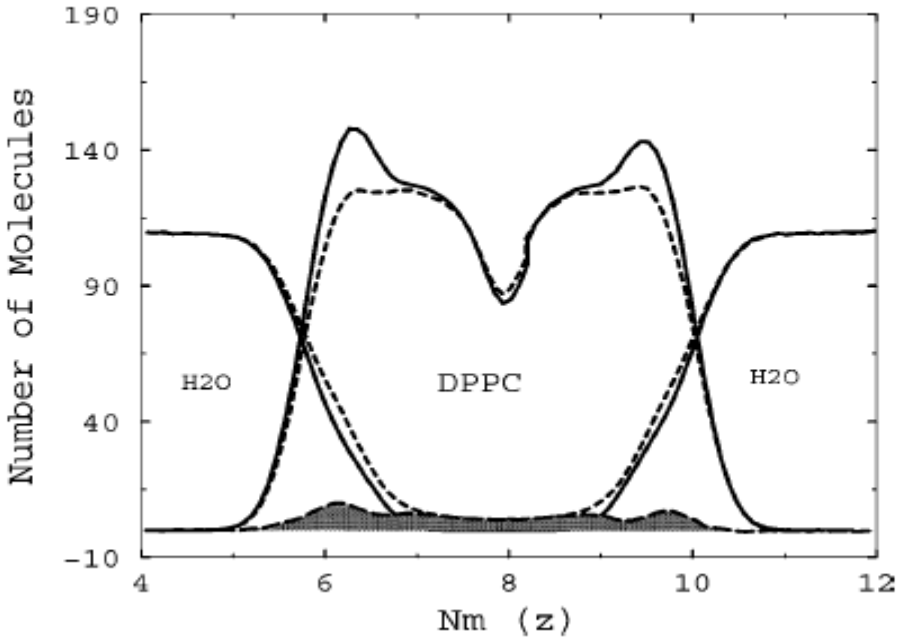
BLMs vs biomembranes

- **It is assumed therefore than they (can) similarly permeate biomembranes, but this is not a logical corollary since (i) biomembranes are NOT *per se* like BLMs as they contain high concentrations of protein, (ii) unlike biomembranes BLMs often contain solvents and always exhibit pore defects (that can be exacerbated by additives such as the molecule under test) such that most passage through BLMs is probably via pore defects**

Pore defects



REAL BIOMEMBRANES DO NOT DO THIS



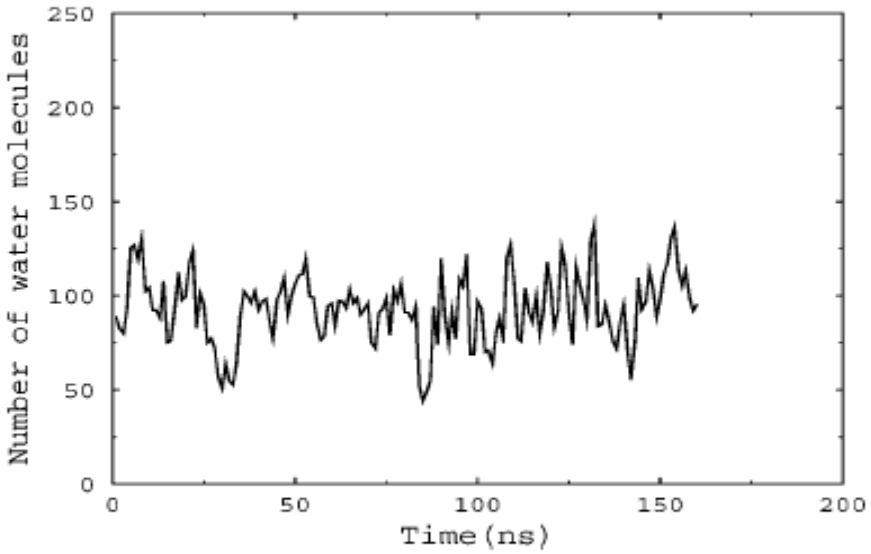


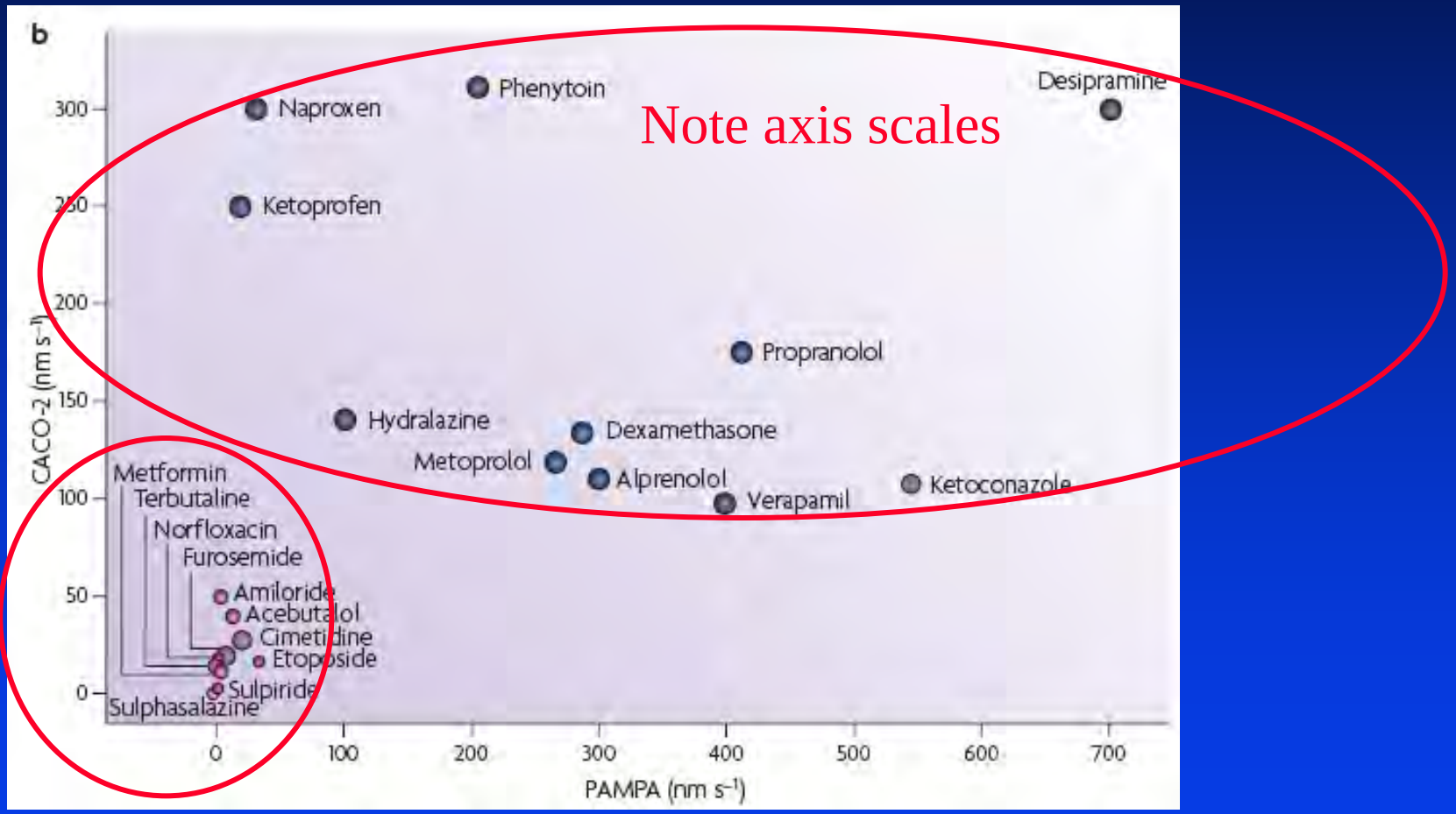
FIGURE 4 The number of water molecules in the central region of the pore as a function of time for the simulation with a lateral pressure of −30 bar (~25 mN/m).

2156 Biophysical Journal Volume 86 April 2004 2156–2164

Molecular Dynamics Simulations of Hydrophilic Pores in Lipid Bilayers

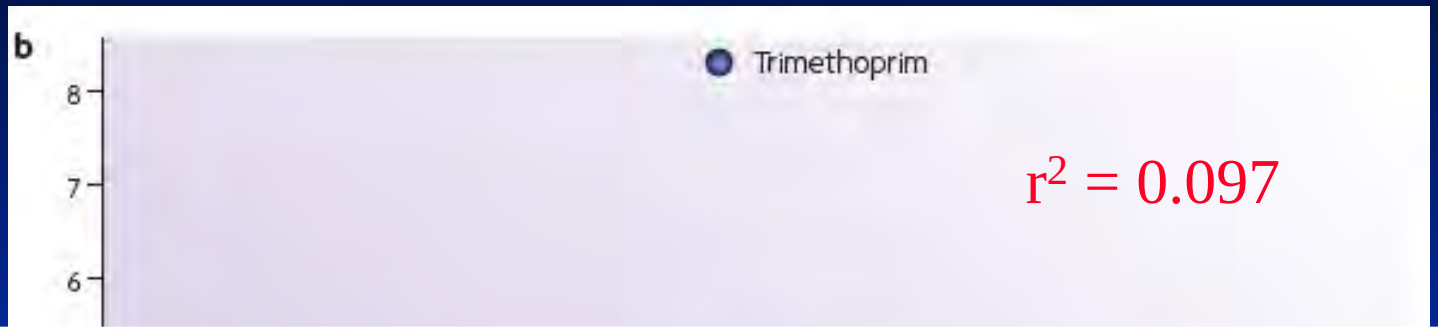
Hari Leontiadou, Alan E. Mark, and Siewert J. Marrink
Department of Biophysical Chemistry, University of Groningen, Nijenborgh, Groningen, The Netherlands

Poor correlation between Caco-2 cells and artificial membrane (PAMPA) assays

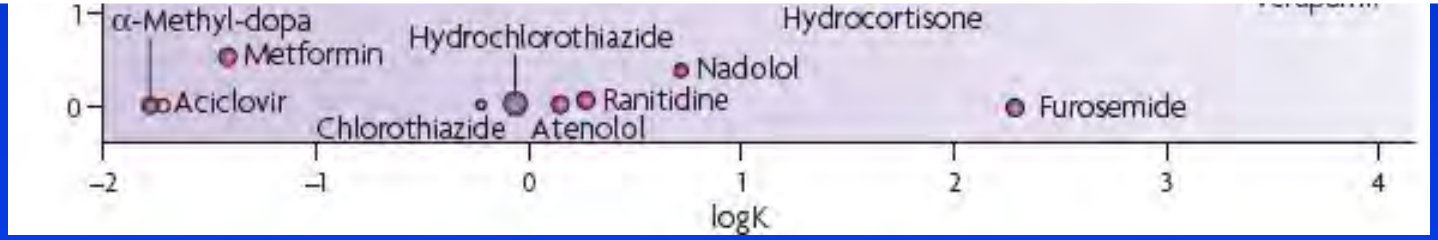


Balimane *et al.*, AAPS J 8, e1-e13 (2006)

Poor relationship between Caco-2 permeability and log $K_{o/w}$ (log P)



THESE log P THEORIES OF DRUG UPTAKE ARE <u>BIOPHYSICAL</u>, 'LIPID-ONLY' THEORIES

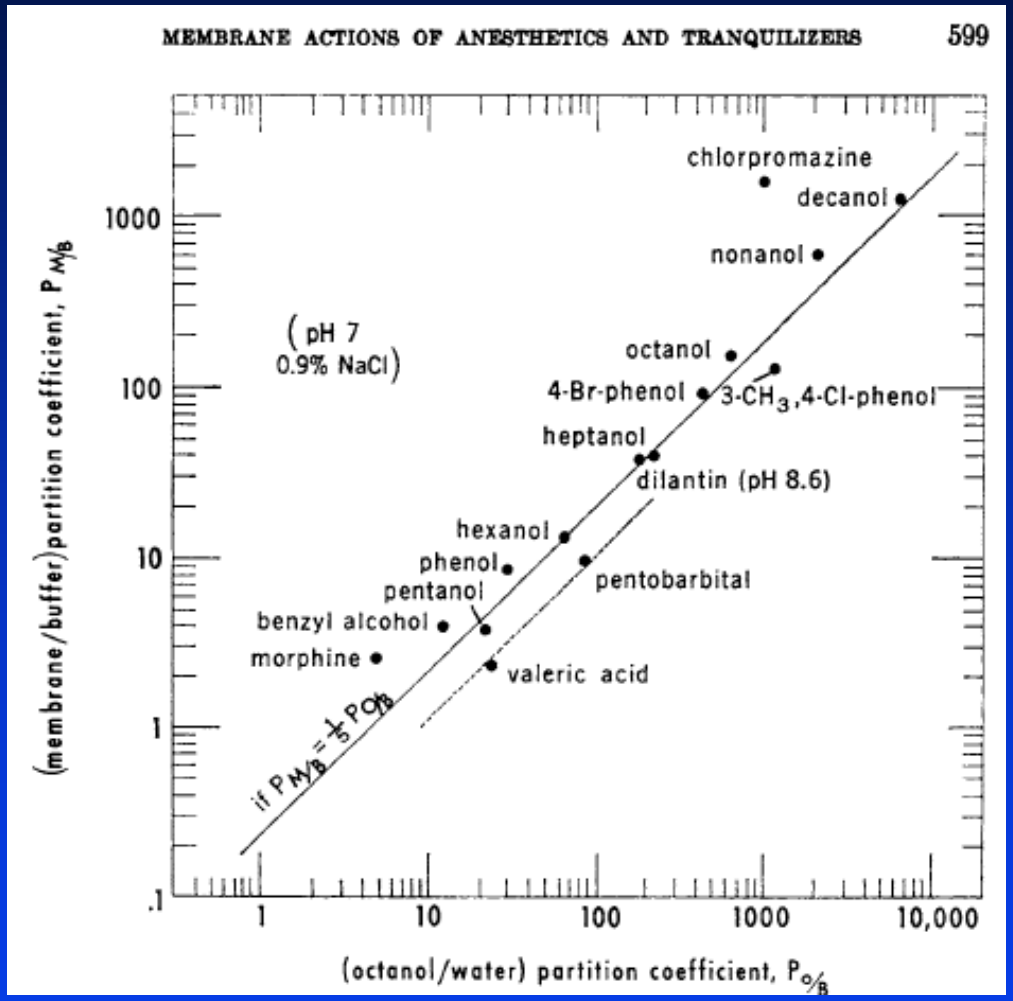


Corti *et al.* Eur J Pharm Sci 7, 354-362 (2006)

Narcotics (‘general anaesthetics’)

- Potency also correlates with log P (up to a cut-off) (Meyer & Overton)
- Negligible structure-activity relationships
- Was assumed that they also act by a ‘biophysical’ mechanism by partitioning ‘nonspecifically’ into membrane and e.g. ‘squeezing’ nerve channels
- This too was a ‘lipid-only’ theory
- None of this now withstands scrutiny of the facts

Membrane-buffer partition correlates with $K_{o/w}$ (though is about 1/5 as great)



P. Seeman, Pharmacol Rev 24, 583-655 (1972) (>2700 citations!)

Anaesthetic potency does largely correlate with partitioning into membrane

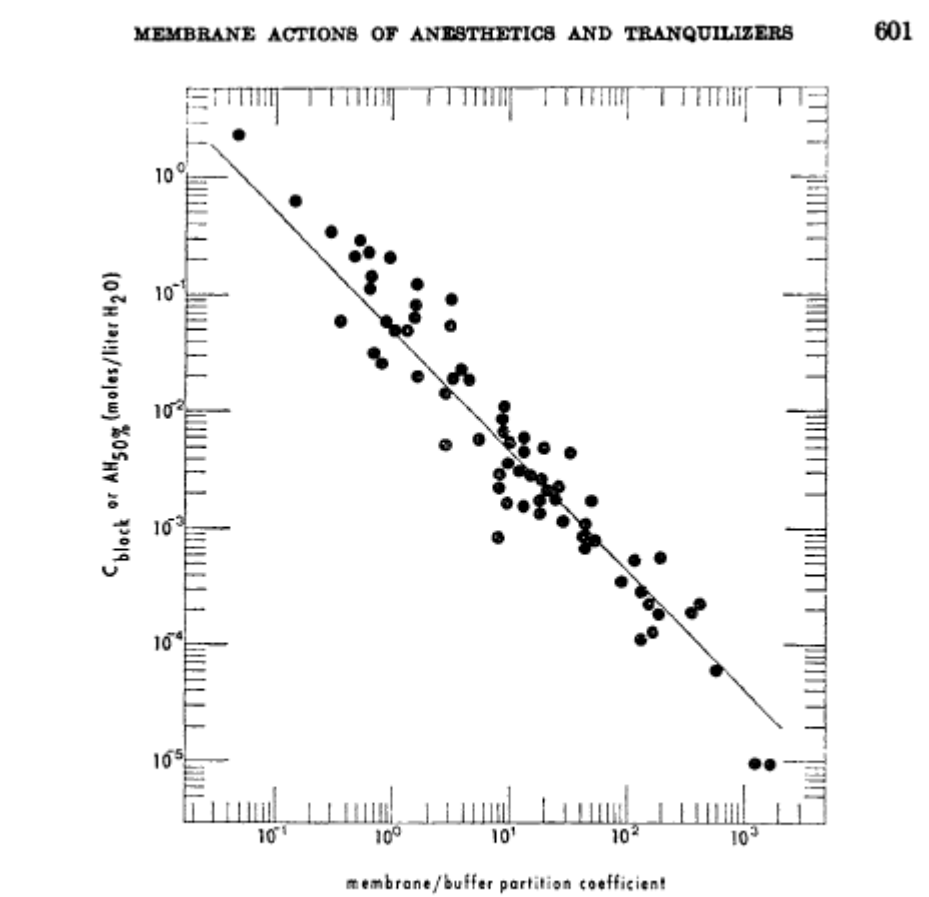
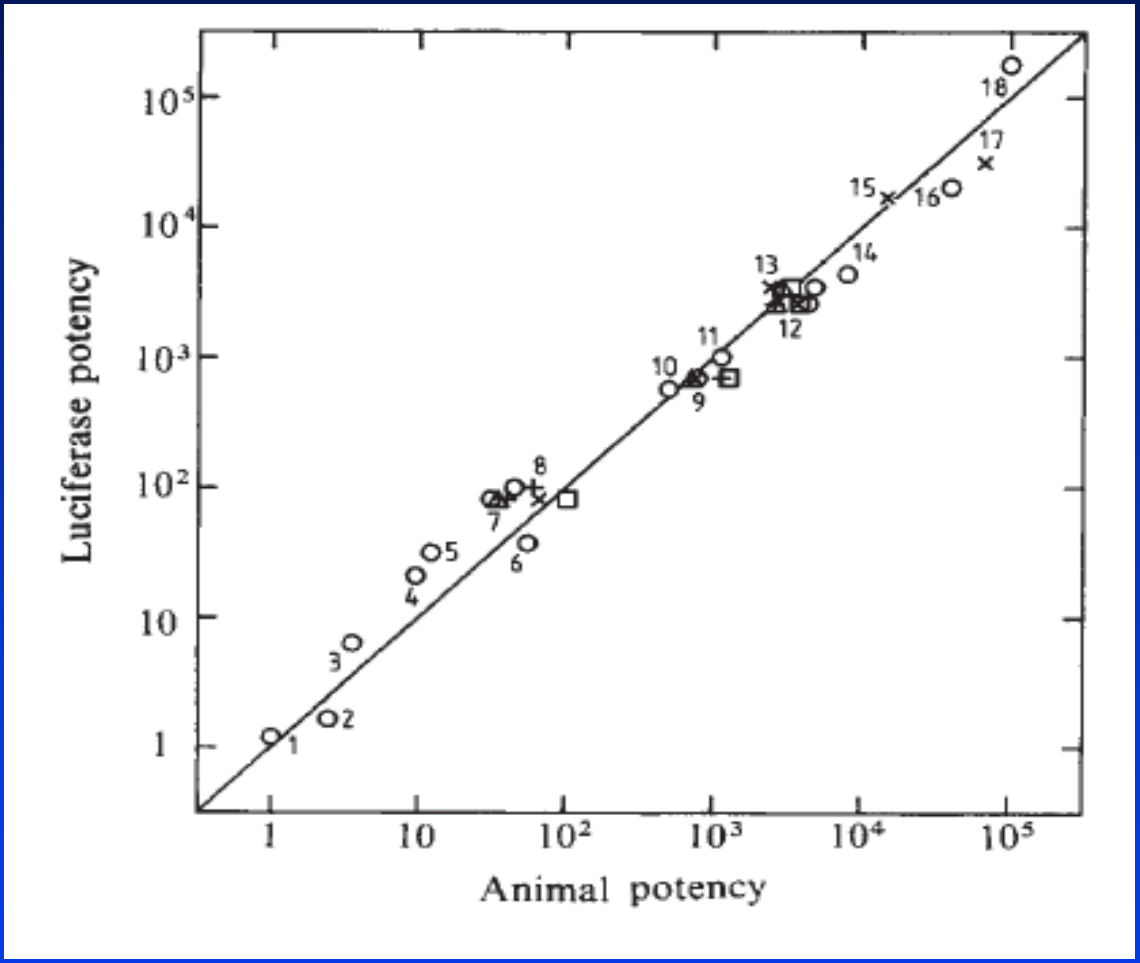


FIG. 9. Showing that the nerve-blocking (or antihemolytic) concentration of an anesthetic directly correlates with the membrane/buffer partition coefficient. See table 5 for code to anesthetics.

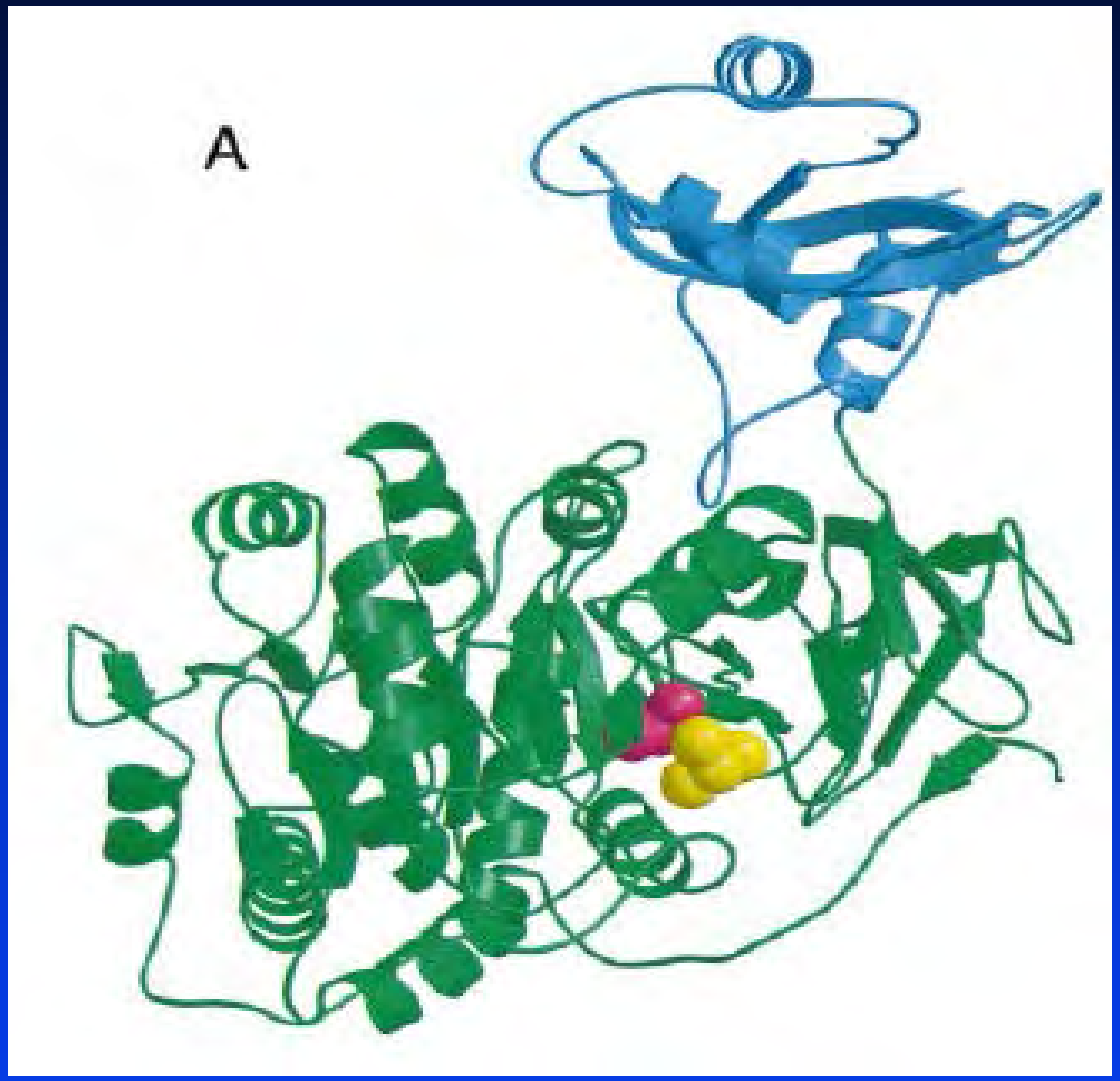
P. Seeman, Pharmacol Rev 24, 583-655 (1972) (>2700 citations!)

Narcotics inhibit luciferase, a soluble protein, with the same potency with which they anaesthetise animals, over 5 logs!



Franks & Lieb, Nature 310, 599-601 (1984)

The structural basis is known



Binding of bromoform to luciferase

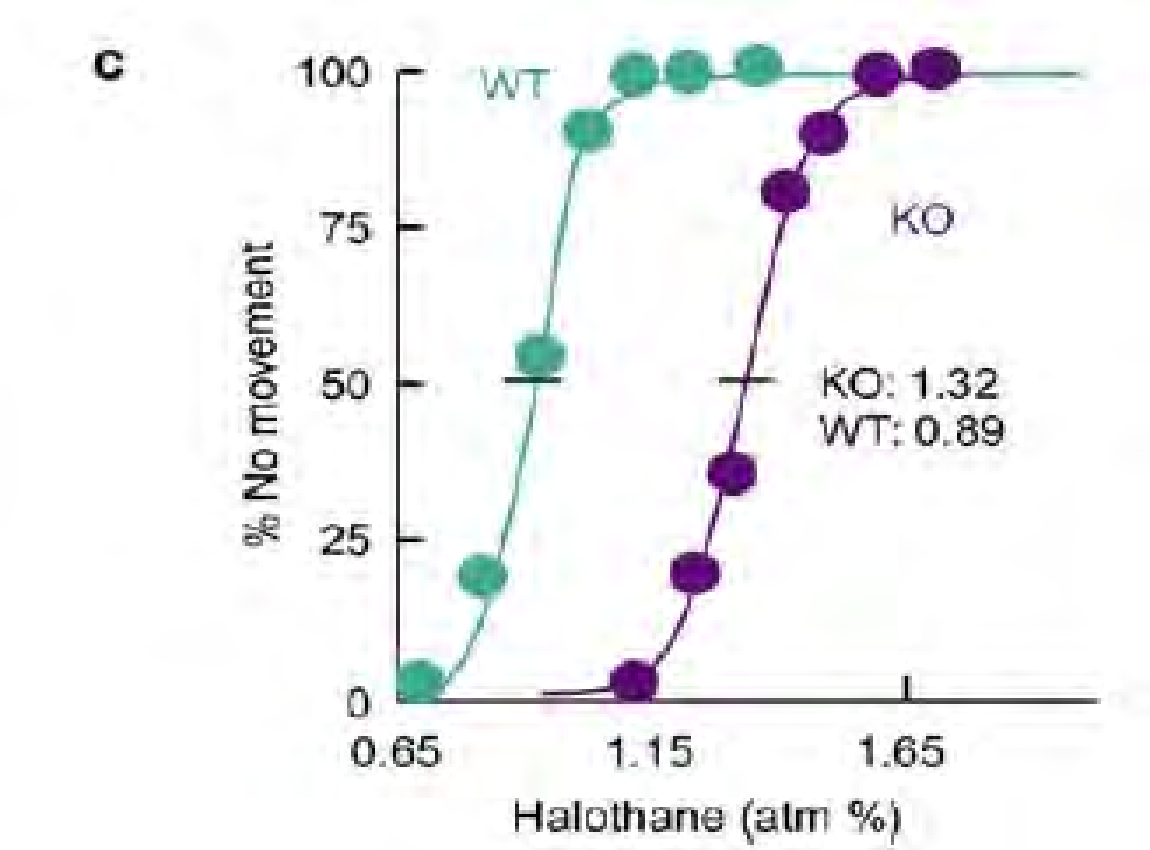
Franks *et al.*, Biophys J 75, 2205-11 (1998)

The biophysical forces required to enter (and cross) a low dielectric are no different from those required to bind to a hydrophobic pocket of a protein

- **Making and breaking H-bonds**
- **‘hydrophobic’ interactions**
- **Polarisation/ polarisability**

Biophysical arguments alone cannot realistically discriminate mechanisms

Halothane affects narcosis in part via a TREK-1 K^+ channel



Heurteaux et al. EMBO J 23, 2684-95 (2004)

In Support of the Pre-eminence of Transporter-mediated Uptake

There are five main sources of evidence that are considered to support the transporter(-only) view:

1) The existence of many proteins involved in drug efflux, which illustrates the widespread existence of the ability of natural proteins to transport xenobiotic drugs.

2) The demonstration of the requirement of carriers for molecules (such as lipophilic cations) that had been assumed on biophysical grounds to cross biological membranes without them.

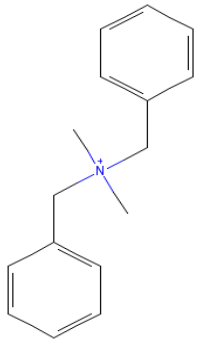
3) The fact that drugs do concentrate in specific tissues and do not, in fact, leak out as they would if transmembrane diffusion on the basis of log P alone was the whole (or even most of) the story (of course this can also in part reflect intracellular binding sites).

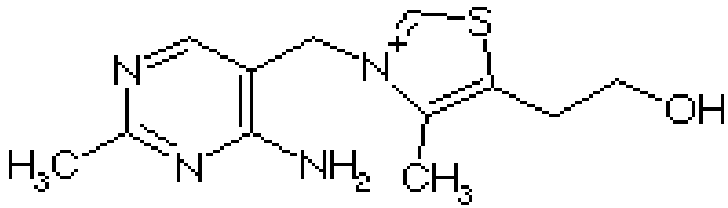
4) The increasing and by now abundant evidence from specific cases that particular drugs do in fact enter cells via identified carrier molecules for which they are not the 'natural' ligand.

5) The ability to enhance permeability substantially by modifying the drug chemically to form a prodrug that can act as a substrate for drug carriers and thereby enter cells.

2) The demonstration of the requirement of carriers for molecules (such as lipophilic cations) that had been assumed on biophysical grounds to cross biological membranes without them.

Uptake of the lipophilic cation dibenzyldimethyl ammonium (DDA^+) is mediated via the transport system.





C00378

Thiamine (clog P ~ 2)

It is not *a priori* easy to guess from structures which xenobiotics use which carriers, though these can be rationalised post hoc via QSAR!

Barts, P. W. J. A., Hoeberichts, J. A., Klaassen, A. & Borst-Pauwels, G. W. F. H. (1980). Uptake of the lipophilic cation dibenzyldimethylammonium into *Saccharomyces cerevisiae*. Interaction with the thiamine transport system. *Biochim Biophys Acta* **597,** 125-36.

3) The fact that drugs do concentrate in specific tissues and do not, in fact, leak out as they would if transmembrane diffusion on the basis of log P alone was the whole (or even most of) the story (of course this can also in part reflect intracellular binding sites).

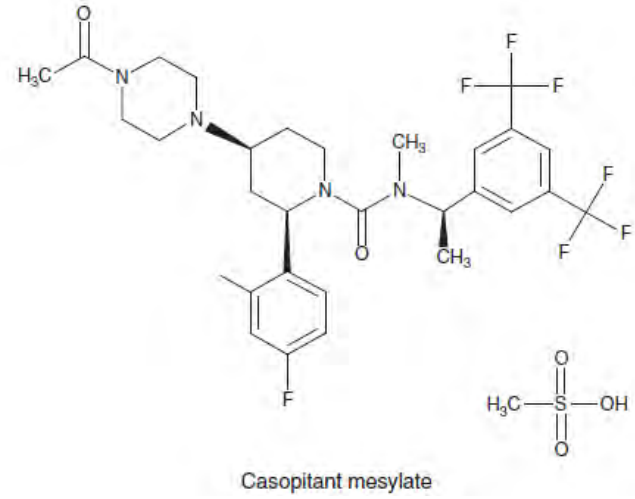
- **In many cases it is far from clear why drugs should accumulate to high levels without any evidence for equivalently (stoichiometrically) increased levels of targets**
- **By contrast, active transport can easily account for this, and there is abundant evidence for major differences in the tissue distributions of carriers**

Exp Op Drug Metab. Toxicol. (2011) 7(2):137-1

Expert Opinion

Drug and metabolite concentrations in tissues in relationship to tissue adverse findings: a review

Mario Pellegatti† & Sabrina Pagliarusco
†*GlaxoSmithKline, Via Fleming 2, Verona, Italy*



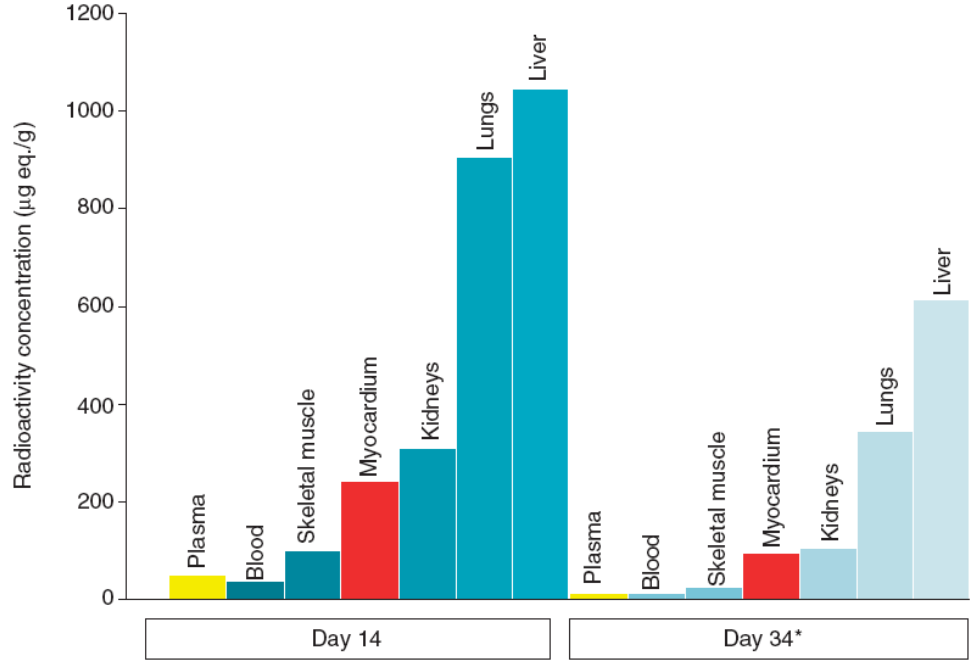
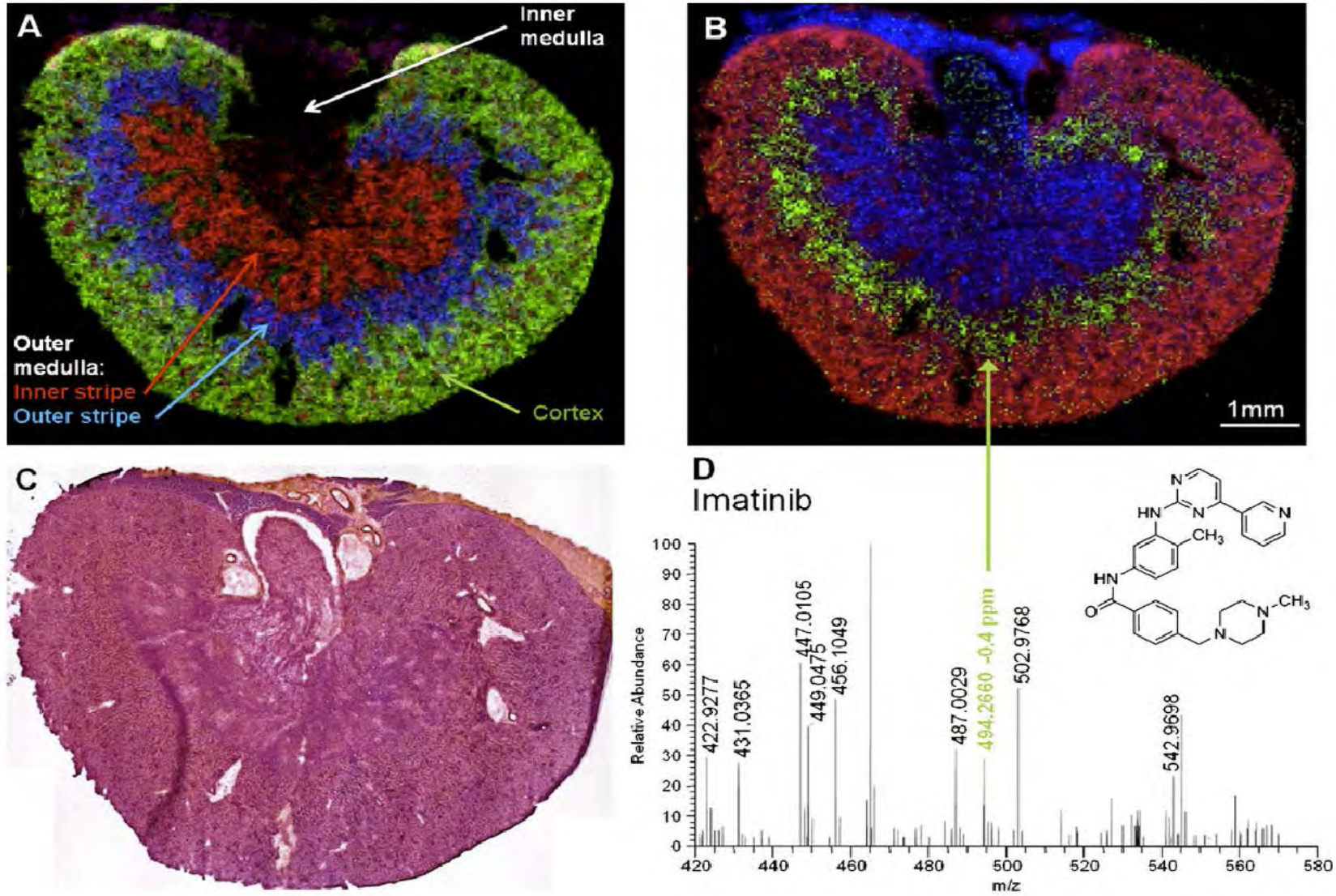


Figure 3. Concentration of drug related material in dog tissues after repeated oral doses of casopitant at 40 mg/kg.
*After a recovery period of 20 days.

Imatinib distribution in mouse kidney

Römpp, A. *et al*. (2011). Anal Bioanal Chem 401, 65-73.



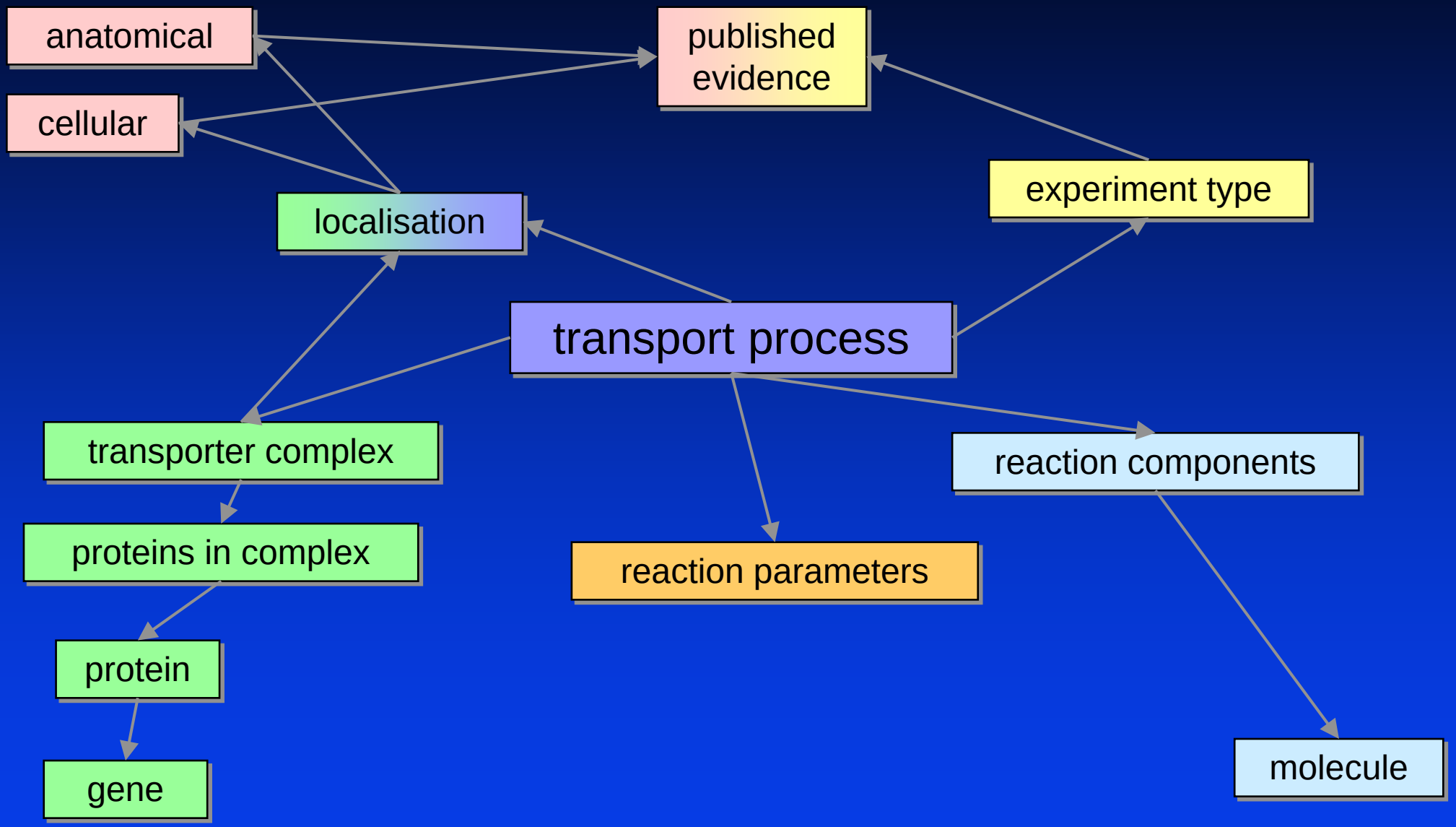
Tissue-selective expression of solute carrier molecules. Expression levels of SLC7A3 (cationic amino acid transporter, y+ system) are high in oesophagus epithelial cells (A), low in liver bile duct cells (B), and moderate in glandular cells of the small intestine (C) and in glandular cells of the duodenum (D). Antibody-based histochemical staining pictures taken with its permission from the Human Protein Atlas http://www.proteinatlas.org/tissue_profile.php?antibody_id=3629

Literature review

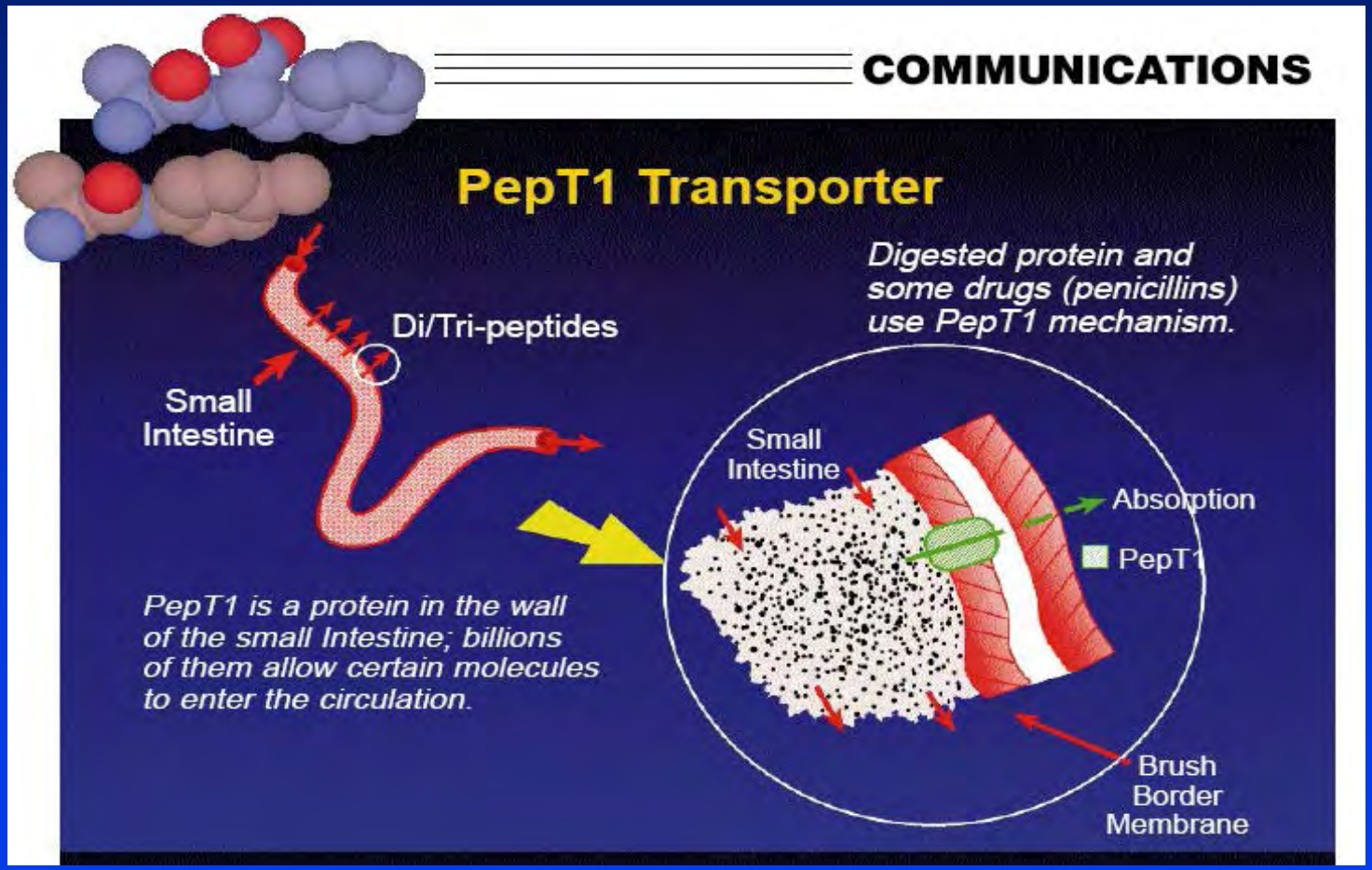
- Our 2008 review at Nature Reviews in Drug Discovery had over 300 references
- No doubt we have missed plenty of others!
- Carriers now recognised to be required even for tiny molecules like water, urea, ammonium and glycerol
- Many/most major drugs are known to interact with carriers, e.g. statins via the liver bile OATP

Substrate	SLC15A1	SLC15A2	SLC22A1	SLC22A2	SLC22A3	SLC22A4	SLC22A5	SLC22A6	SLC22A7	SLC22A8	SLC22A11	SLCO1A2	SLCO1B1	SLCO1B3	SLCO1C1	SLCO2A1	SLCO2B1	SLCO3A1	SLCO4A1	SLCO4C1
acyclovir			•					•												
amoxicillin	•	•																		
atorvastatin													•				•			
benzylpenicillin													•				•	•	•	
bestatin	•	•																		
caspofungin													•							
cefaclor	•	•																		
cefalexin	•																			
ceftibuten	•																			
cephaloridine							•	•		•	•									
cidofovir								•												
cimetidine			•	•	•			•		•										
didanosine								•												
enalapril	•											•								
erythromycin									•											
fexofenadine												•	•	•						
fluvastatin													•	•			•			
ganciclovir			•					•												
glibenclamide																	•			
ibuprofen								•												
lamivudine								•												
metformin			•	•																
methotrexate								•	•	•			•	•						•
midodrine	•																			
pitavastatin													•	•						
pravastatin													•				•			
propranolol				•																
pyrilamine						•	•													
quinidine						•	•													
rifampicin													•	•						
rosuvastatin												•		•			•			
salicylate									•	•										
stavudine								•												
temocaprilat	•											•								
tetracycline									•	•	•									
trifluridine								•												
valacyclovir	•									•										
valganciclovir	•	•																		
valproate							•													
verapamil						•	•													
zalcitabine								•												
zidovudine			•	•				•	•	•	•									

Transport Process Database: Simplifed Schema



5) The ability to enhance permeability substantially by modifying the drug chemically to form a prodrug that <u>can</u> act as a substrate for drug carriers and thereby enter cells.



Ramifications of the view that drug transport is largely carrier-mediated

• A combination of substrate specificity and carrier distribution, additional to target distribution, can account for much of the different tissue distributions of drugs (and hence warn of efficacy/toxicity issues).

• Drugs may be designed to avoid specific tissues that lack carriers for them.

• It becomes much easier to understand in principle the tissue distributions of xenobiotics.

• Cross-species sequence homologies may allow better interpretation of tissue distributions in different organisms.

• Uptake carriers may provide novel and rational drug targets.

• Molecular cloning will allow the specificities of individual carries for target drugs to be measured directly.

•Drug-drug and drug-nutrient interactions may be mediated via interactions with transporters

• **Successful (marketed) drugs will be more like endogenous (intermediary) metabolites**

• **But how do we tell which transporters are used by particular drugs?**

Lanthaler *et al. BMC Biology* 2011, **9**:70
http://www.biomedcentral.com/1741-7007/9/70

BMC Biology
incorporating Journal of Biology

RESEARCH ARTICLE Open Access

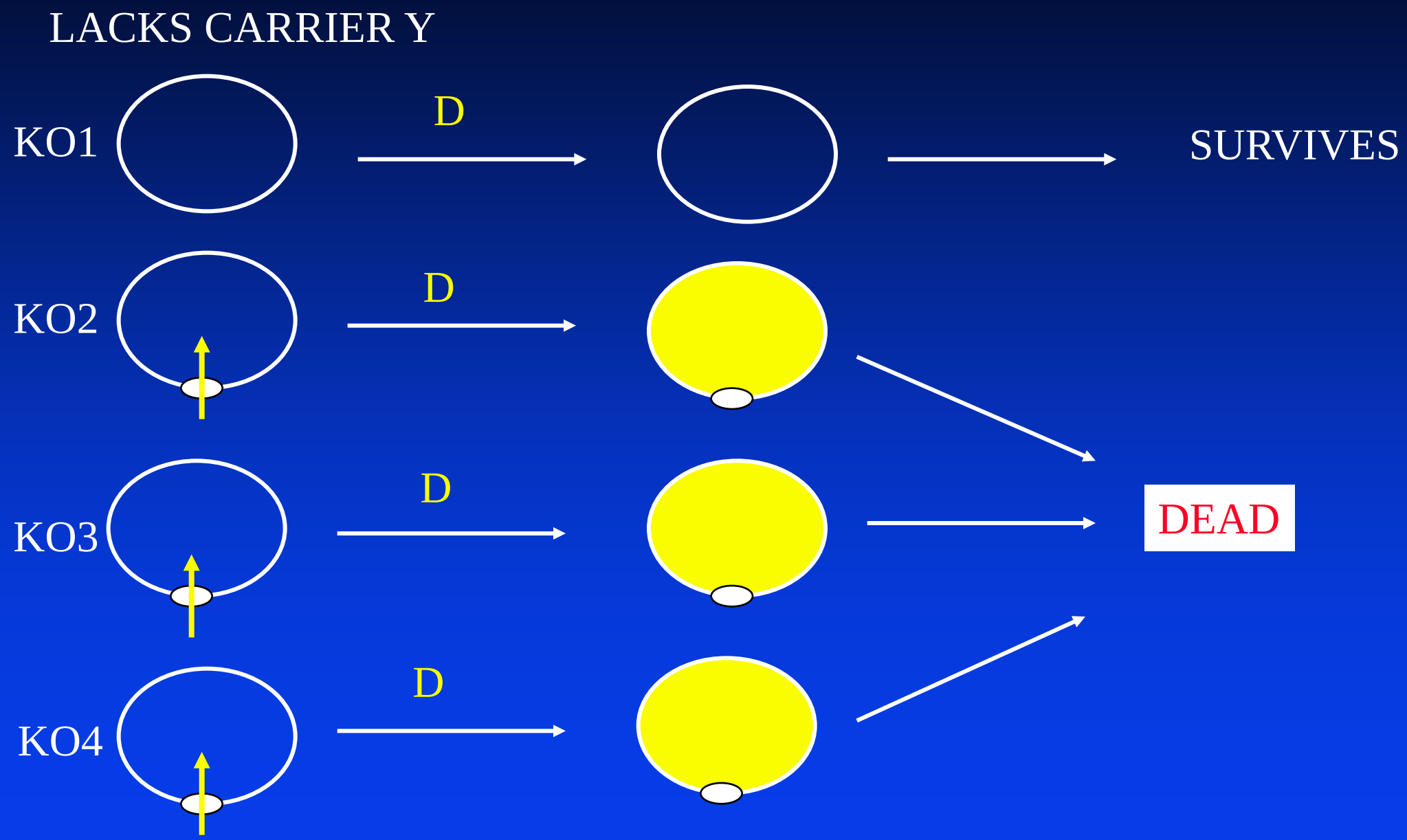
Genome-wide assessment of the carriers involved in the cellular uptake of drugs: a model system in yeast

Karin Lanthaler[1,2,3†], Elizabeth Bilsland[4†], Paul D Dobson[1,2], Harry J Moss[4], Pınar Pir[3,4], Douglas B Kell[1,2] and Stephen G Oliver[3,4*]

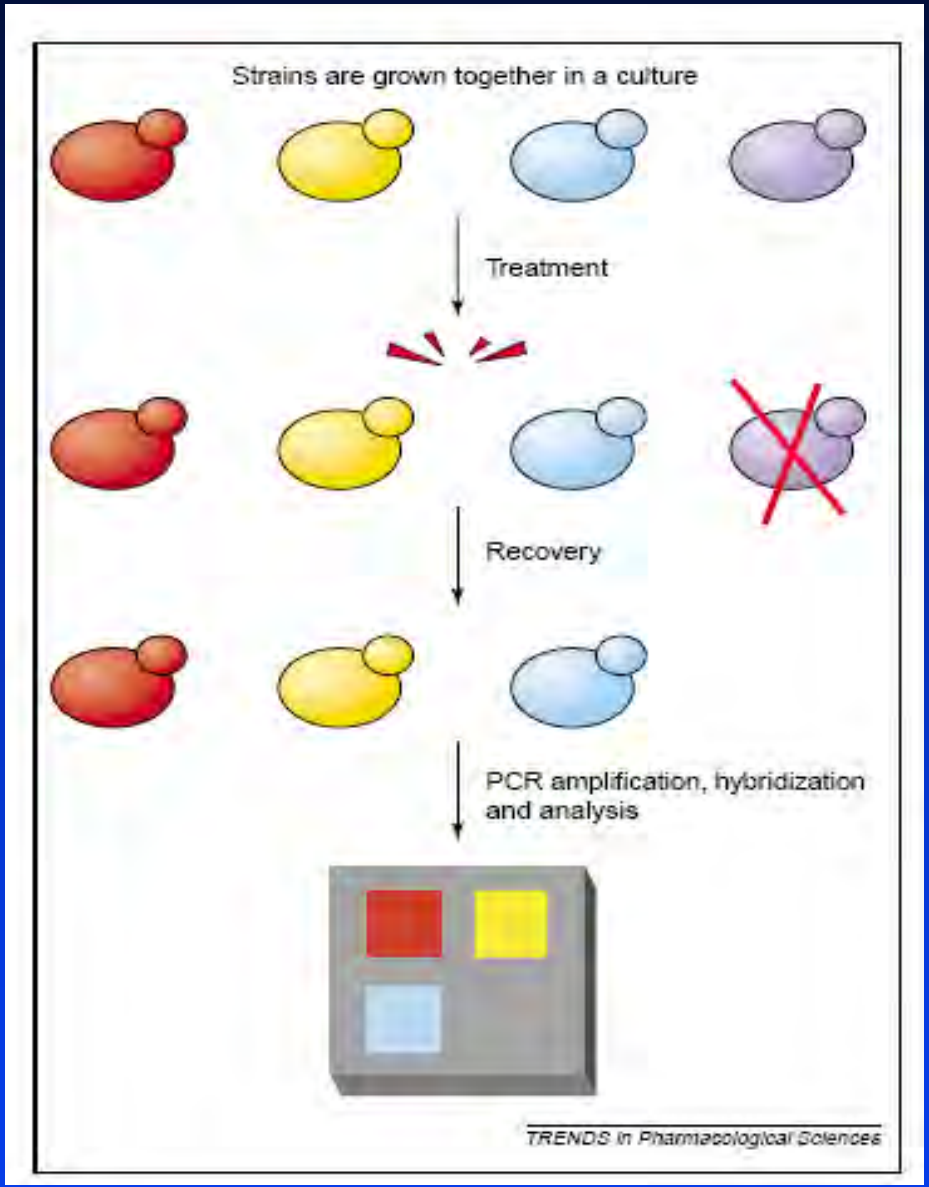
Bar-coded yeast knockout collection

- Every yeast gene KO'd in an individual strain using a neutral replacement marker plus specific 20nt 'uptag' and 'downtag' sequences + a 'universal' PCR primer sequence
- Strains can be competed, DNA extracted, PCR'd and relative growth rates assessed via the tags (on a special Affymetrix chip HT, or by 'deep' sequencing)
- If molecule X is toxic and transported via carrier Y then cells lacking carrier Y will grow faster than the WT in treated cells, thus identifying mode of uptake.

Principle of carrier identification



Cartoon of barcode competitions



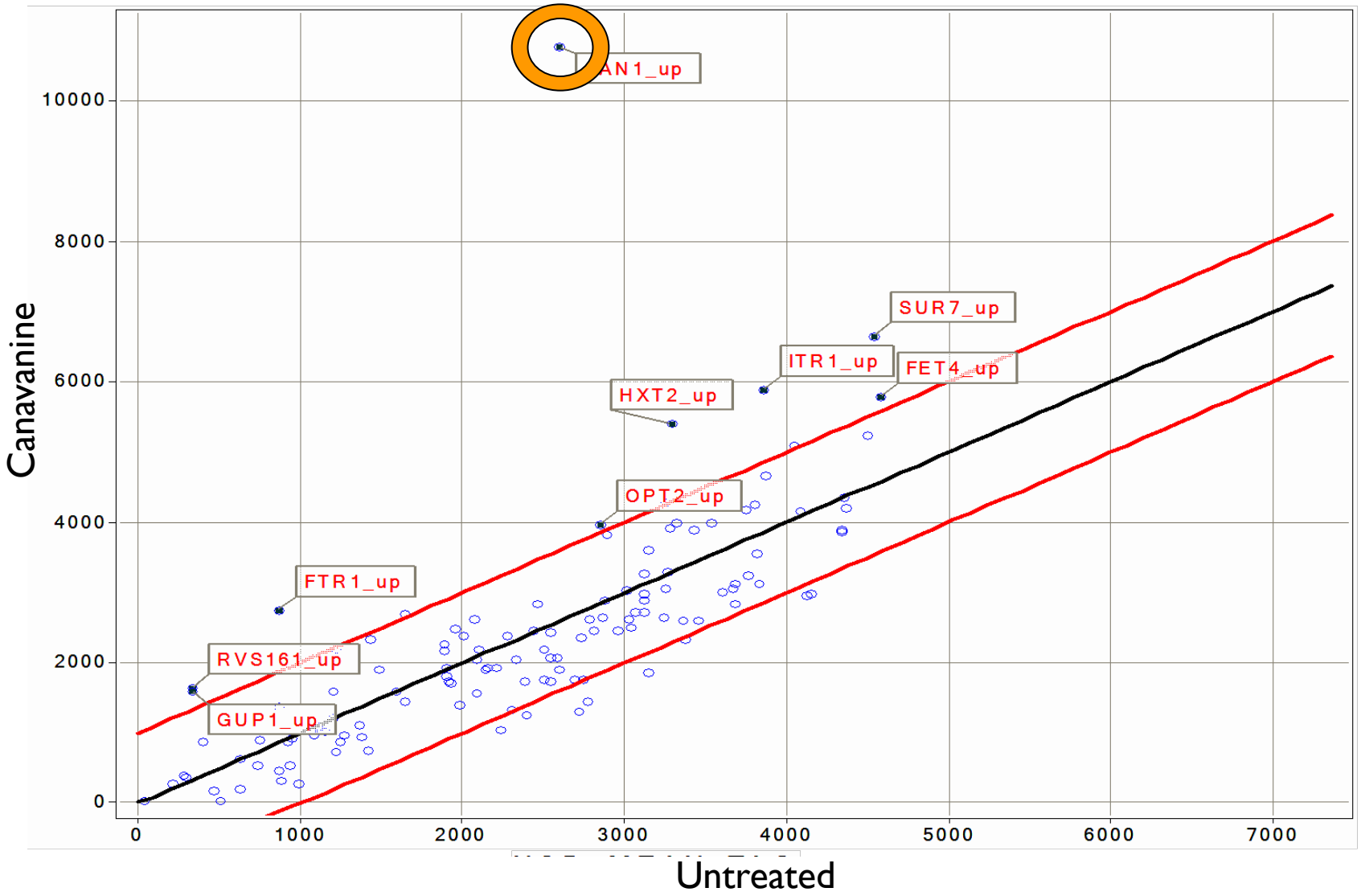
…or nowadays by ‘deep’ sequencing

Canavanine Resistance

Canavanine

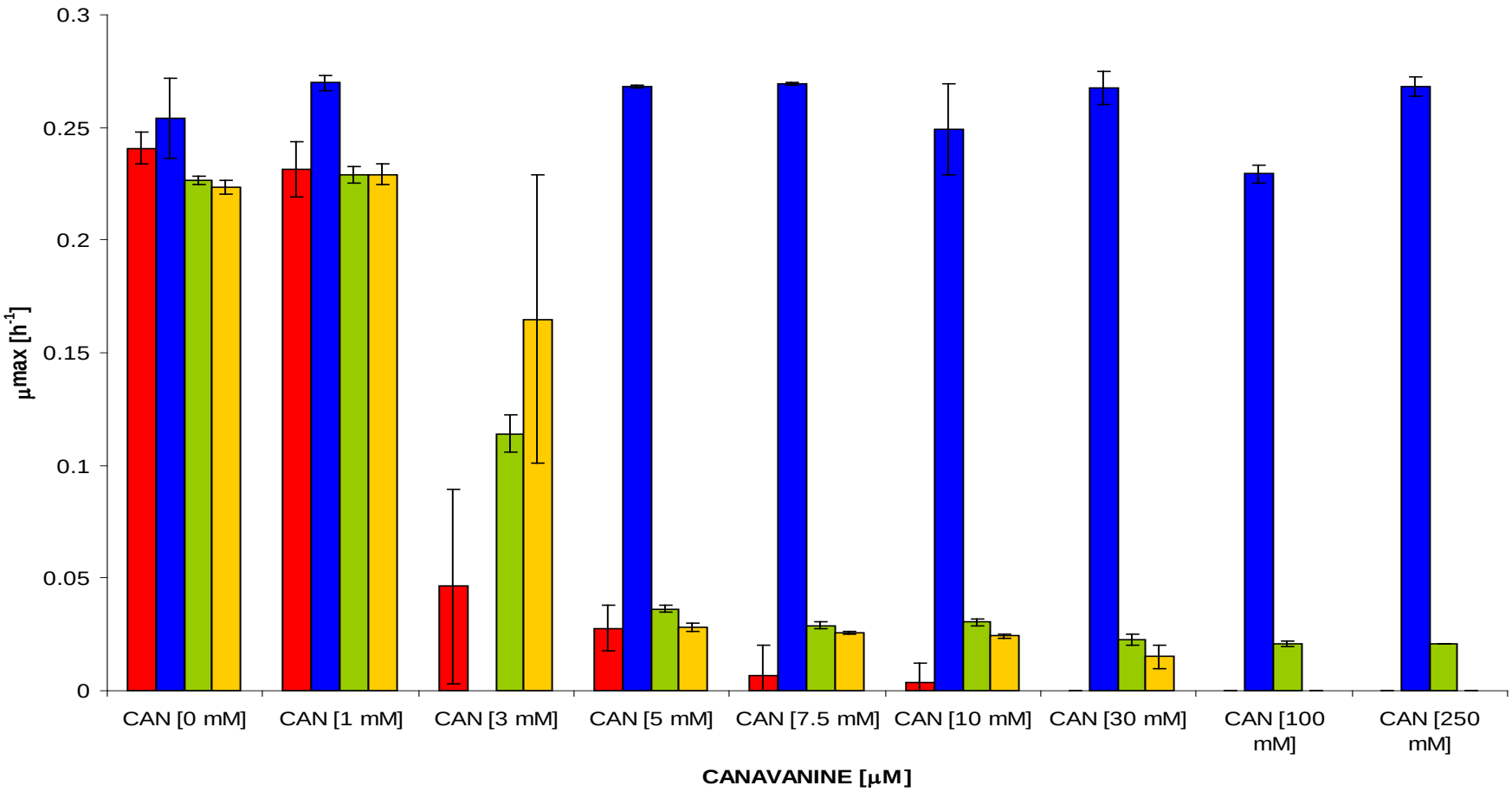
Arginine

Canavanine Resistance

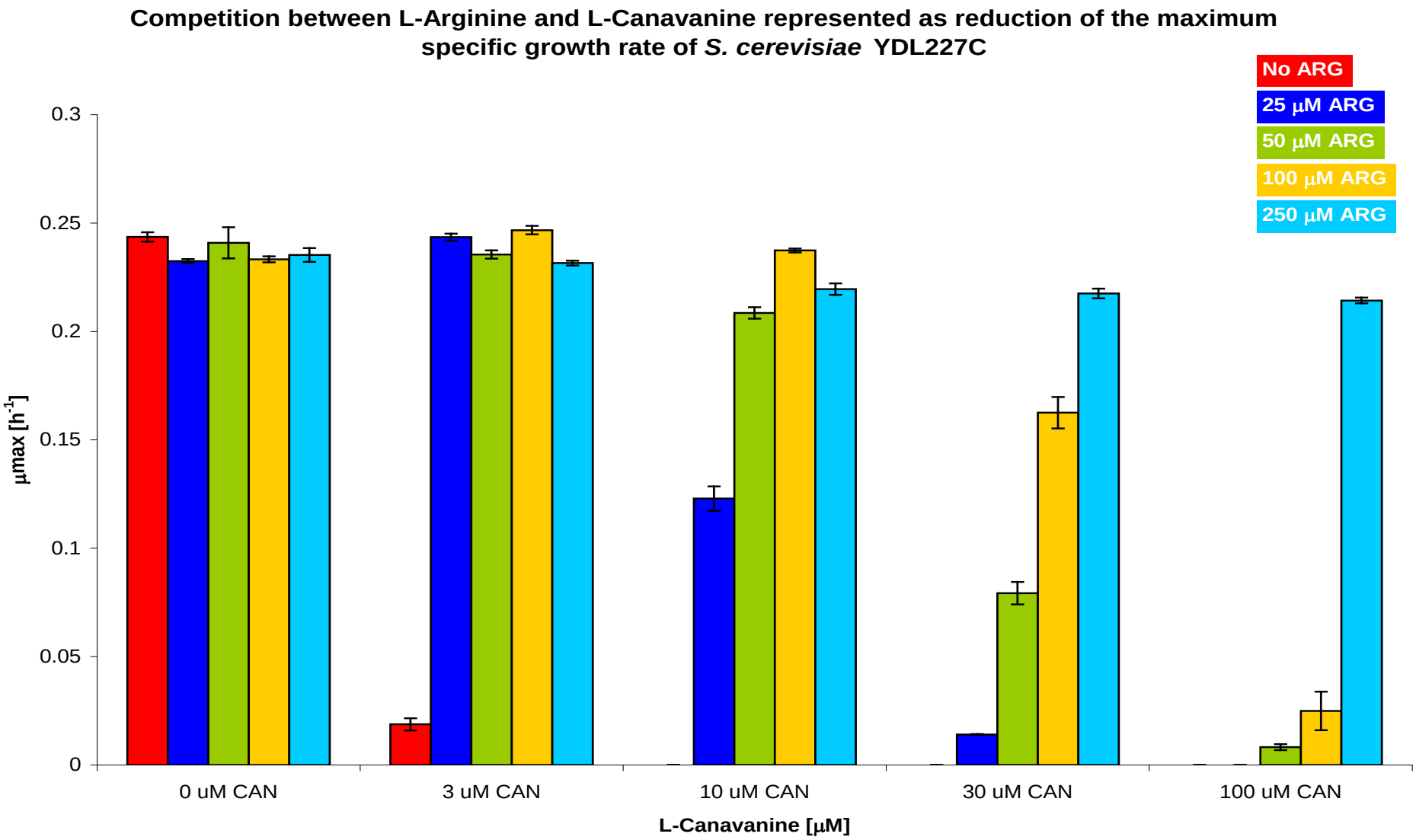


Canavanine Resistance

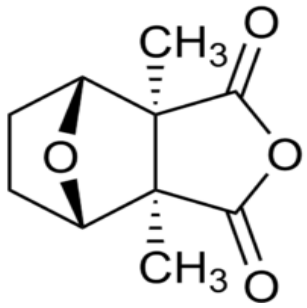
The effect of L-canavanine on the maximum specific growth rate of *S. cerevisiae*
YDL227C (WT), ΔCAN1, ΔFTR1 and ΔFET3
[IC_{90}]=5mM



Competition with the Natural Substrate

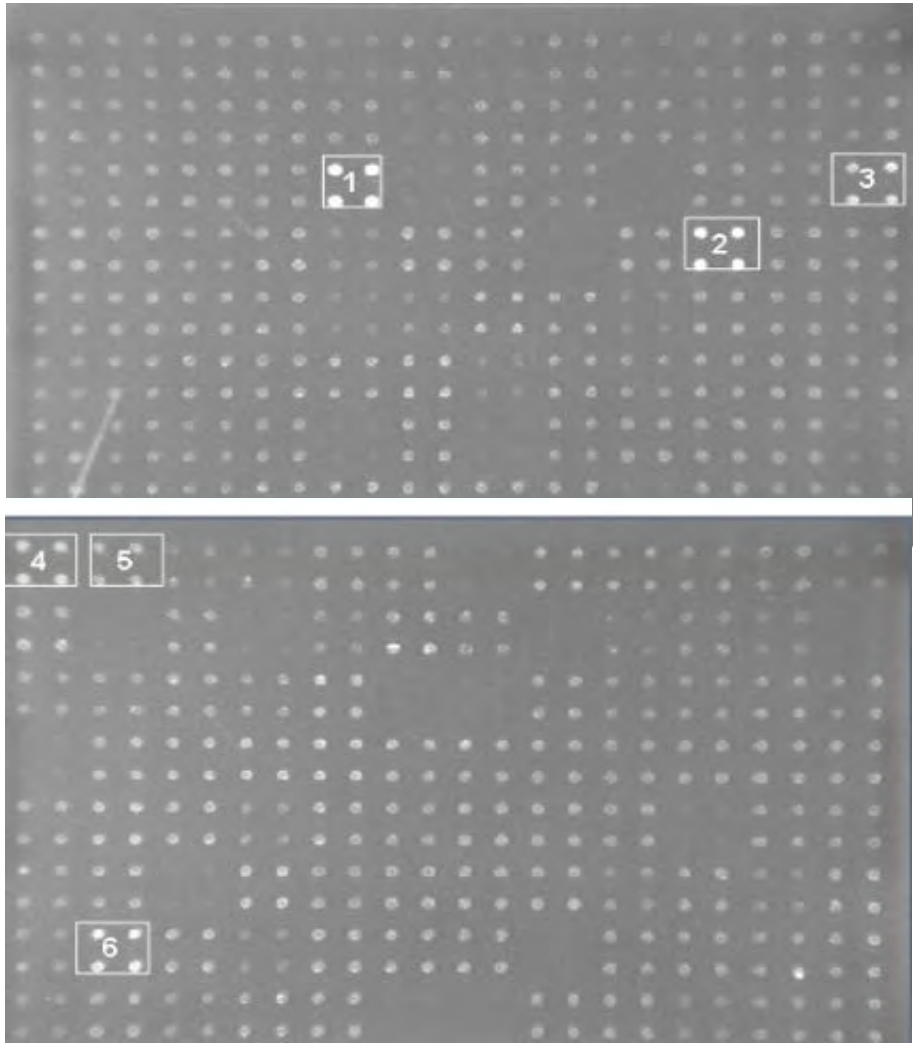


Yeast Robotics



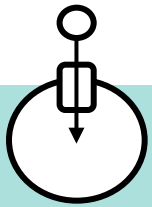
Cantharidin

At least 6 transporters…



1. fen2Δ
2. mal31Δ
3. pca11Δ

4. pho89Δ
5. qdr3Δ
6. snq2Δ



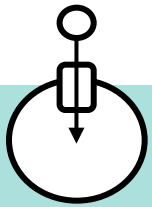
Screening Results

The platforms work (robot is cleanest, fermenter is most parallel)

Broad agreement between solid and liquid robots, but pool effects complicate fermenter

Transporters found for 18/26 drugs

NB – even this approach will miss cases in which there are very many transporters – as is probably often true for marketed drugs

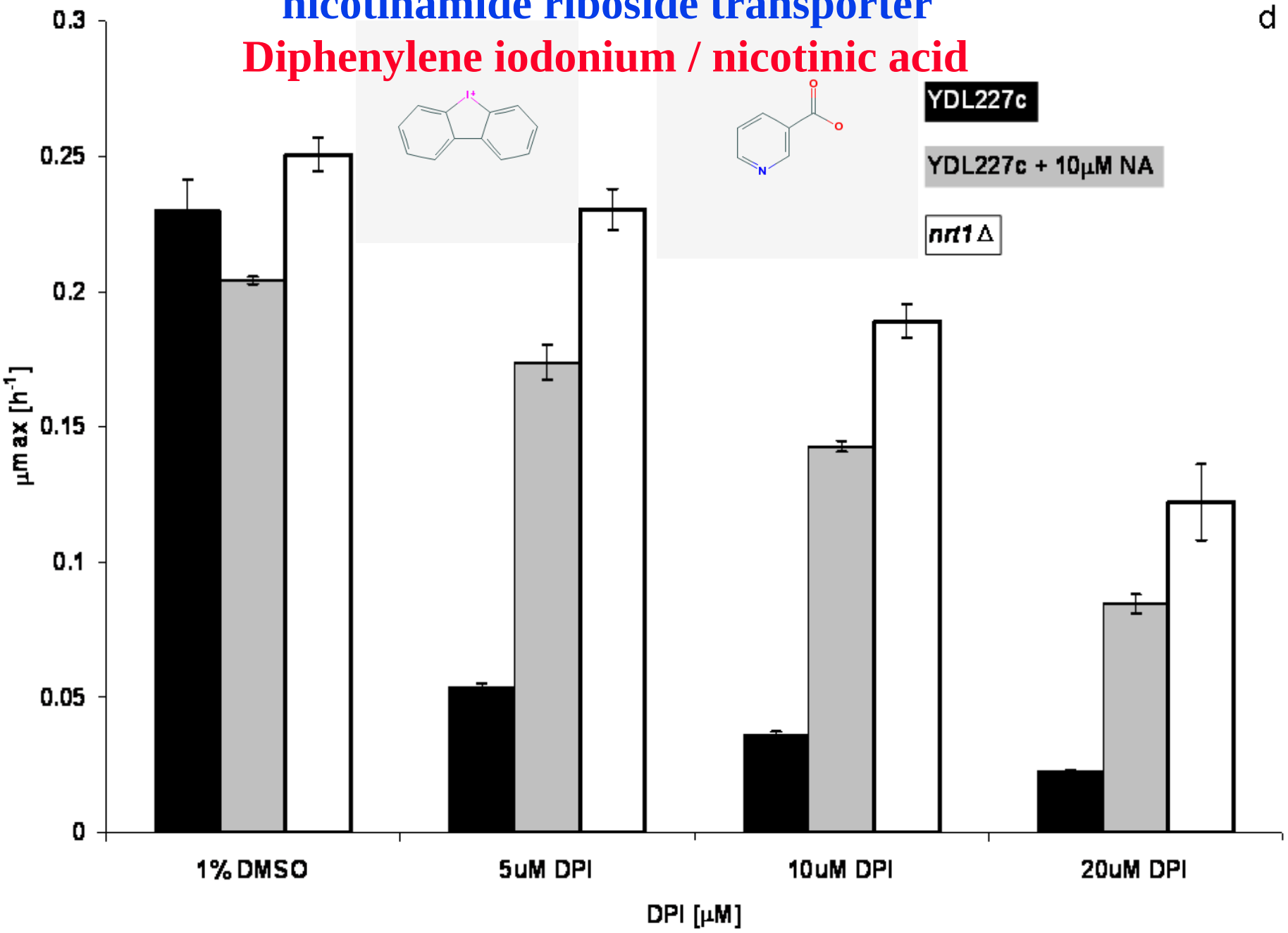


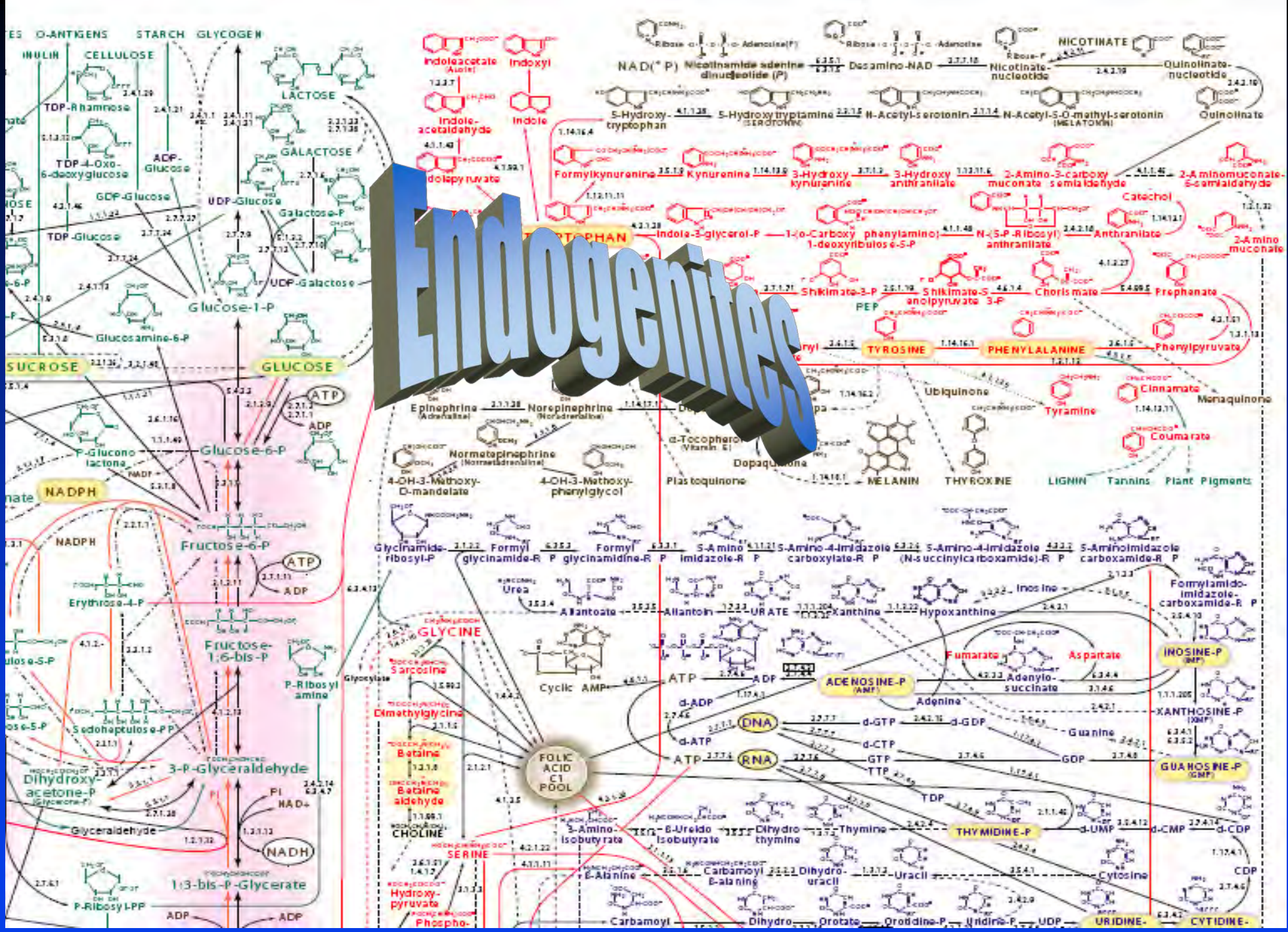
If drugs use transporters that metabolites normally use there are 2 other ramifications

- Drugs and metabolites should compete with each other
- Molecular similarity – successful (marketed) drugs should be more like metabolites than are typical library compounds (that albeit obey the Ro5)

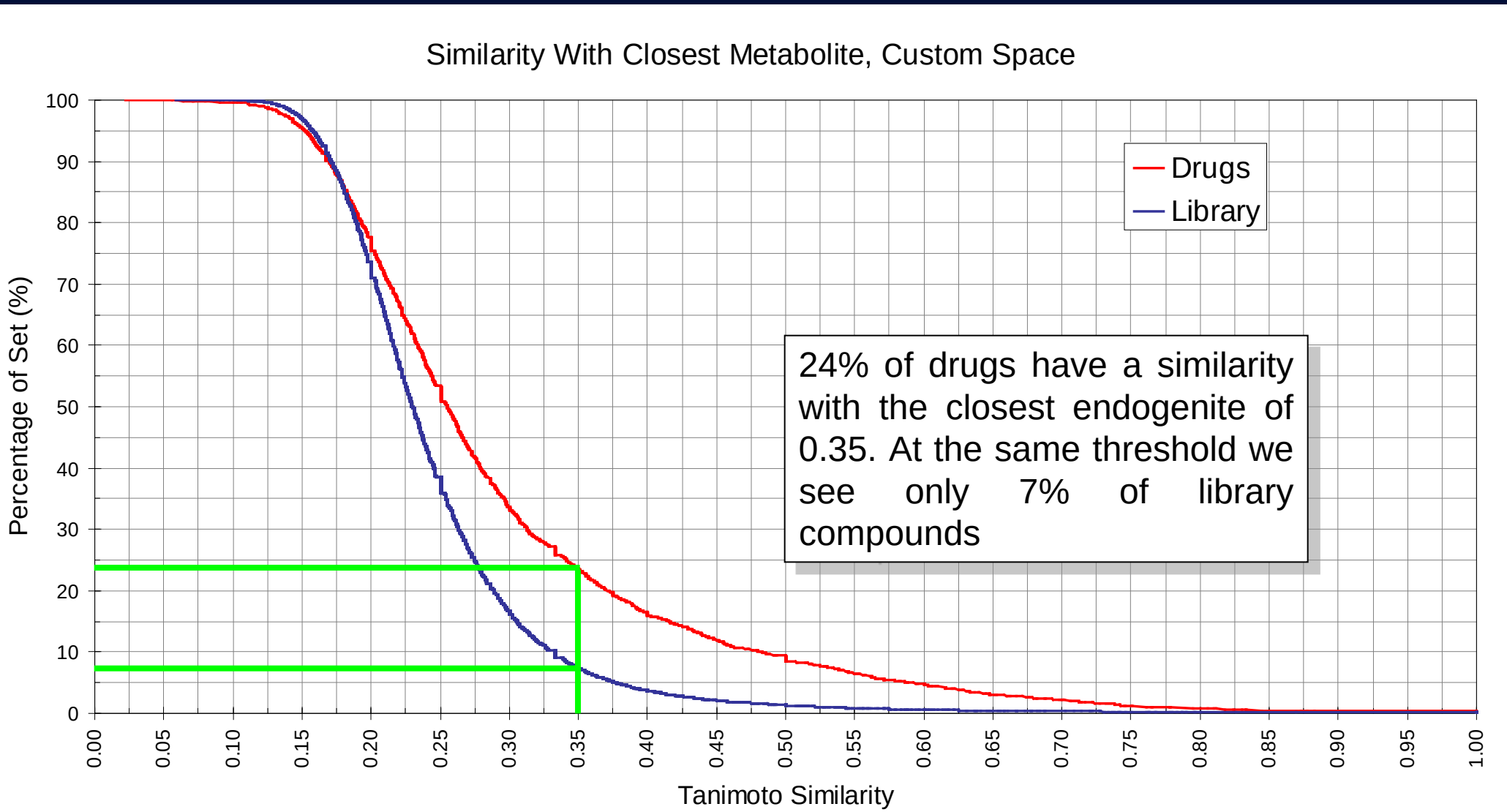
nicotinamide riboside transporter

Diphenylene iodonium / nicotinic acid

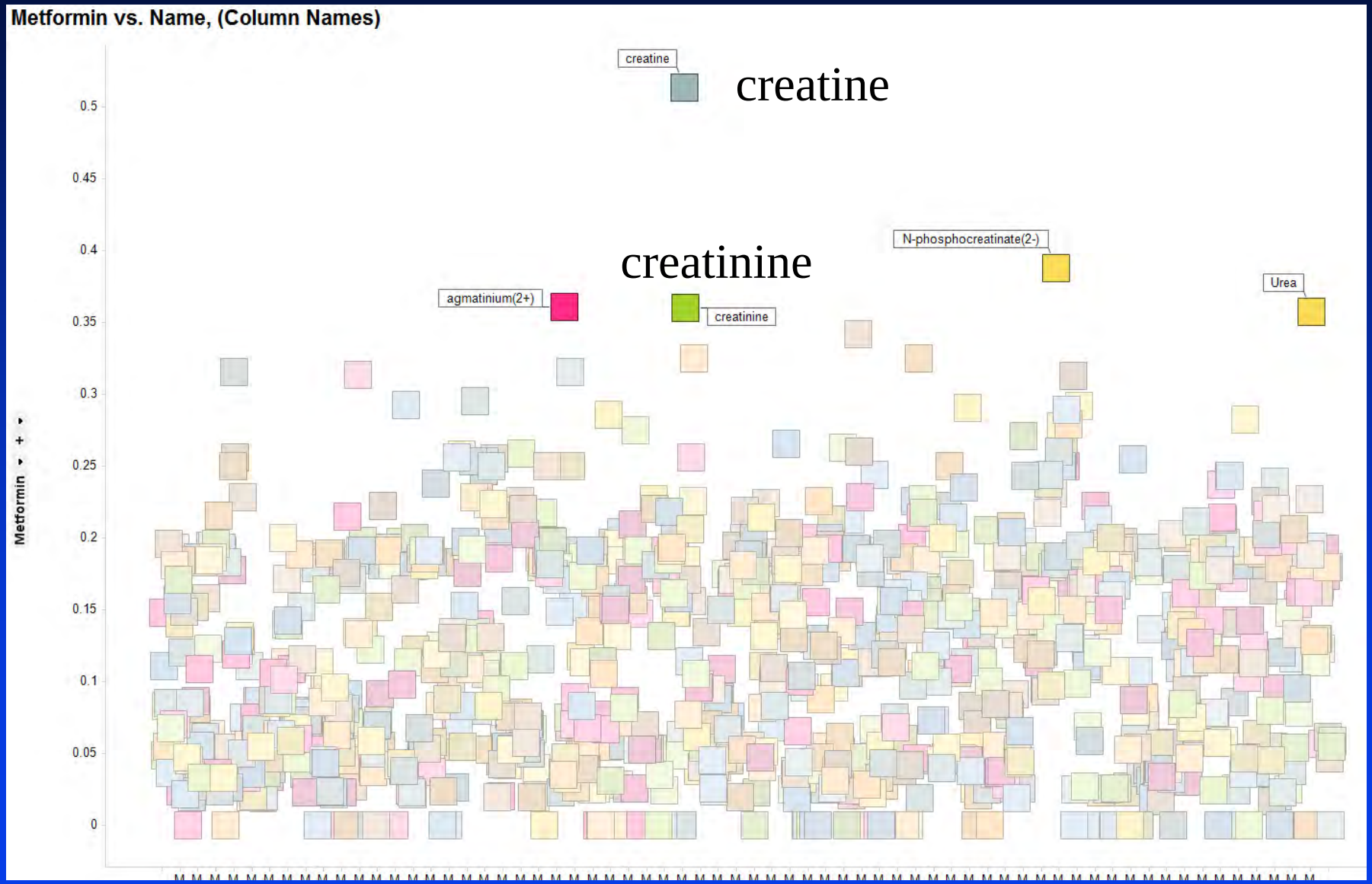



Endogenites
O-ANTIGENS
STARCH
GLYCOGEN
INULIN
CELLULOSE
LACTOSE
GALACTOSE
TDP-Rhamnose
TDP-4-Oxo-6-deoxyglucose
ADP-Glucose
GDP-Glucose
UDP-Glucose
Galactose-P
TDP-Glucose
UDP-Galactose
Glucose-1-P
Glucosamine-6-P
SUCROSE
GLUCOSE
ATP
ADP
P-Gluconolactone
Glucose-6-P
NADP+
NADPH
Fructose-6-P
Erythrose-4-P
Fructose-1;6-bis-P
P-Ribosylamine
Sedoheptulose-P
3-P-Glyceraldehyde
Dihydroxyacetone-P
Glyceraldehyde
PI
NAD+
NADH
1;3-bis-P-Glycerate
P-Ribosyl-PP
Indoleacetate (Auxin)
Indoxyl
Indoleacetaldehyde
Indole
Indolepyruvate
TRYPTOPHAN
Indole-3-glycerol-P
Epinephrine (Adrenaline)
Norepinephrine (Noradrenaline)
Normetapinephrine (Normetadrenaline)
4-OH-3-Methoxy-D-mandelate
4-OH-3-Methoxy-phenylglycol
α-Tocopherol (Vitamin E)
Plastoquinone
Dopaquinone
MELANIN
THYROXINE
LIGNIN
Tannins
Plant Pigments
Ubiquinone
Menaquinone
Tyramine
Cinnamate
Coumarate
NAD(P) Nicotinamide adenine dinucleotide (P)
Deamino-NAD
Nicotinate-nucleotide
NICOTINATE
Quinolinate-nucleotide
Quinolinate
5-Hydroxy-tryptophan
5-Hydroxytryptamine (SEROTONIN)
N-Acetyl-serotonin
N-Acetyl-5-O-methyl-serotonin (MELATONIN)
Formylkynurenine
Kynurenine
3-Hydroxy-kynurenine
3-Hydroxy anthranilate
2-Amino-3-carboxy muconate semialdehyde
2-Aminomuconate-6-semialdehyde
Catechol
Anthranilate
2-Amino muconate
1-(o-Carboxy phenylamino)-1-deoxyribulose-5-P
N-(5-P-Ribosyl) anthranilate
Shikimate-3-P
Shikimate-5-P
Chorismate
Prephanate
PEP
TYROSINE
PHENYLALANINE
Phenylpyruvate
Glycinamide-ribosyl-P
Formyl glycinamide-R P
Formyl glycinamidine-R P
5-Amino imidazole-R P
5-Amino-4-imidazole carboxylate-R P
5-Amino-4-imidazole (N-succinylcarboxamide)-R P
5-Aminoimidazole carboxamide-R P
Formylamido-imidazole-carboxamide-R P
Urea
Allantoin
Allantoate
URATE
Xanthine
Hypoxanthine
Inosine
GLYCINE
Sarcosine
Dimethylglycine
Betaine
Betaine aldehyde
CHOLINE
SERINE
Hydroxy-pyruvate
Glycolate
Cyclic AMP
FOLIC ACID C1 POOL
ATP
ADP
d-ADP
d-ATP
DNA
RNA
ADENOSINE-P (AMP)
Fumarate
Aspartate
Adenylo-succinate
Adenine
INOSINE-P (IMP)
XANTHOSINE-P (XMP)
GUANOSINE-P (GMP)
Guanine
d-GTP
d-GDP
GTP
GDP
TTP
TDP
THYMIDINE-P
d-UMP
d-CMP
d-CDP
CDP
Cytosine
Uracil
Thymine
Dihydro-thymine
Dihydro-uracil
5-Amino-isobutyrate
5-Ureido-isobutyrate
β-Alanine
Carbamoyl β-alanine
Carbamoyl
Dihydro-orotate
Orotidine-P
Uridine-P
UDP
URIDINE-
CYTIDINE-

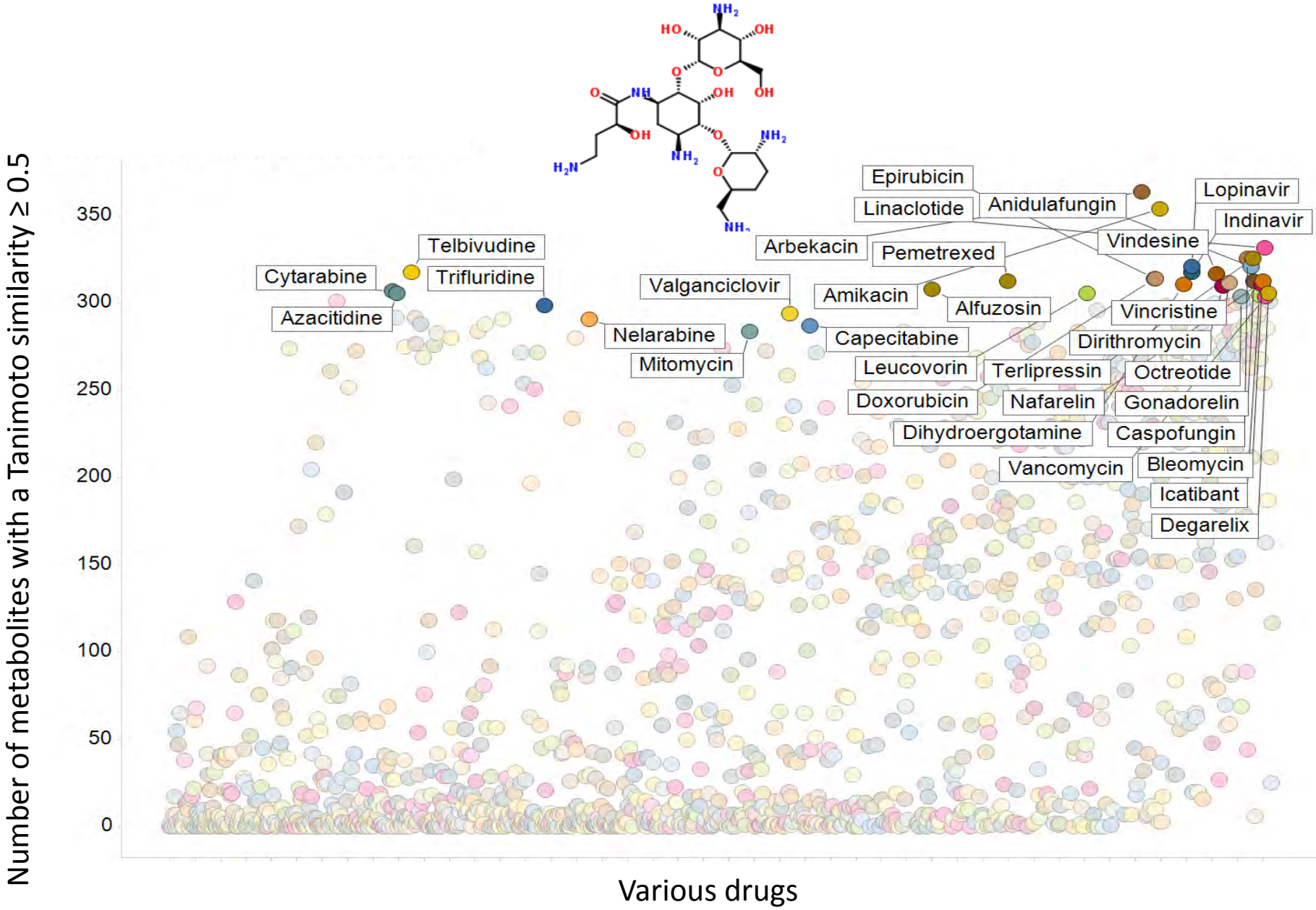
Custom, Tanimoto 0.3



Metformin vs metabolites



D


Number of metabolites with a Tanimoto similarity ≥ 0.5
350
300
250
200
150
100
50
0
Various drugs
Epirubicin
Linaclotide
Anidulafungin
Lopinavir
Indinavir
Arbekacin
Pemetrexed
Vindesine
Telbivudine
Cytarabine
Trifluridine
Valganciclovir
Amikacin
Alfuzosin
Vincristine
Azacitidine
Nelarabine
Capecitabine
Dirithromycin
Mitomycin
Leucovorin
Terlipressin
Octreotide
Doxorubicin
Nafarelin
Gonadorelin
Dihydroergotamine
Caspofungin
Vancomycin
Bleomycin
Icatibant
Degarelix

Drug-Endogenite Likeness

These results hold in further descriptor spaces and at different redundancy thresholds

Drugs are more similar to endogenites than are non-drugs

The concept of endogenite-likeness will contribute to screening library design

Early results comparing drugs and non-drugs to transporter substrates indicate that a similar result holds. This suggests that interactions with transporters are a major component driving the endogenite-likeness of drugs

'Metabolite-likeness' as a criterion in the design and selection of pharmaceutical drug libraries **Drug Disc Today 14, 31-40 (2009)**

Paul D. Dobson, Yogendra Patel and Douglas B. Kell

School of Chemistry and The Manchester Interdisciplinary Biocentre, The University of Manchester, 131 Princess St, Manchester M1 7DN, UK

Latest transporter review

REVIEWS Drug Discovery Today • Volume 18, Numbers 5/6 • March 2013

ELSEVIER

DDT 18, 218-239 (2013)

Reviews • FOUNDATION REVIEW

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

Douglas B. Kell[1,2], Paul D. Dobson[1,2,3], Elizabeth Bilsland[4,5] and Stephen G. Oliver[4,5]

[1] School of Chemistry, The University of Manchester, 131 Princess St, Manchester M1 7DN, UK
[2] Manchester Institute of Biotechnology, The University of Manchester, 131 Princess St, Manchester M1 7DN, UK
[3] ChELSI Institute, Department of Chemical and Biological Engineering, University of Sheffield, Mappin Street, Sheffield S1 3JD, UK
[4] Department of Biochemistry, University of Cambridge, Sanger Building, 80 Tennis Court Road, Cambridge CB2 1GA, UK
[5] Cambridge Systems Biology Centre, University of Cambridge, Sanger Building, 80 Tennis Court Road, Cambridge CB2 1GA, UK

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

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

Conclusions

- The human metabolic network contains many transporters of unknown specficity
- Much evidence shows that specific drugs do enter cells by known carriers, and probably do so only via this route
- Carrier-mediated uptake is thus almost certainly the rule and not the exception, and makes drug and xenobiotic transport a problem of systems biology
- This has considerable implications for the design of safe and efficacious drugs that behave at a SYSTEMS level
- We thus need to develop systems pharmacology

Thanks to….MCISB Management Team

- **Dave Broomhead**
- **Simon Gaskell**
- **John McCarthy**
- **Pedro Mendes**
- **Steve Oliver**
- **Norman Paton**
- **Hans Westerhoff**

Dieter Weichart – Project manager Carole Goble – Taverna

Peter Li – workflows Steve Pettifer – Utopia Rick Dunn, Dave Broadhurst, Ludwig Neyses - HF

Paul Dobson, Karin Lanthaler, Pınar Pir & Elizabeth Bilsland– carrier-mediated drug uptake

Phil Baker – PE and lots of medical insights

and the MCISB team……

BMC Medical Genomics

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Review

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Iron behaving badly: inappropriate iron chelation as a major contributor to the aetiology of vascular and other progressive inflammatory and degenerative diseases

Douglas B Kell*

http://www.biomedcentral.com/1755-8794/2/2/

http://dbkgroup.org/dbkPubs.htm

Arch Toxicol (2010) 84:825–889
DOI 10.1007/s00204-010-0577-x

REVIEW ARTICLE

1,716 refs

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

Douglas B. Kell

The cellular transport of pharmaceutical drugs: a problem not of biophysics but of systems biology

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