Measuring the interplay between uptake and loss processes of xenobiotics

13th June 2019

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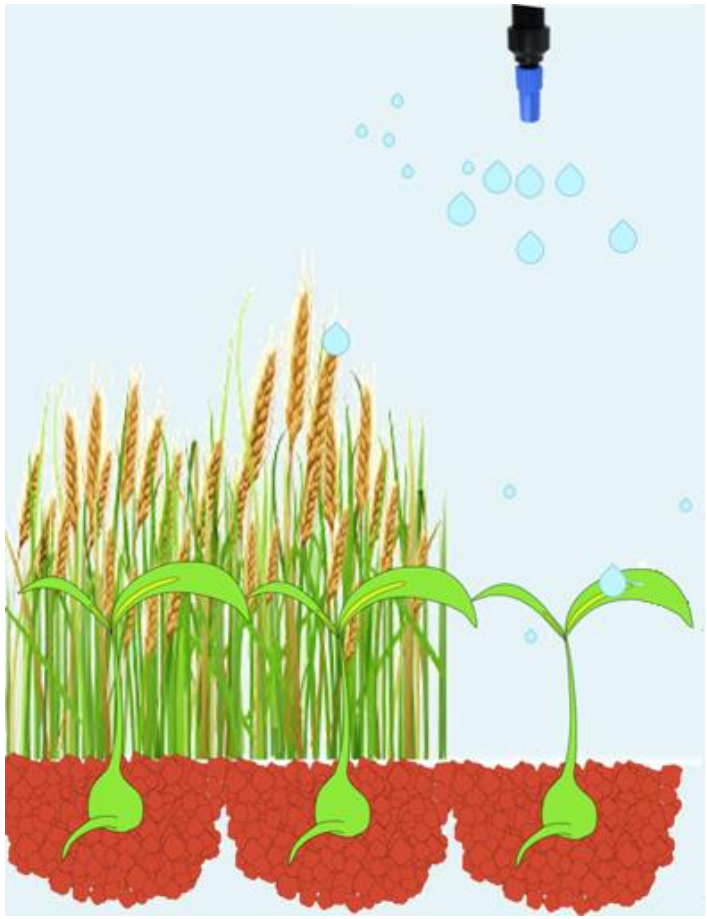
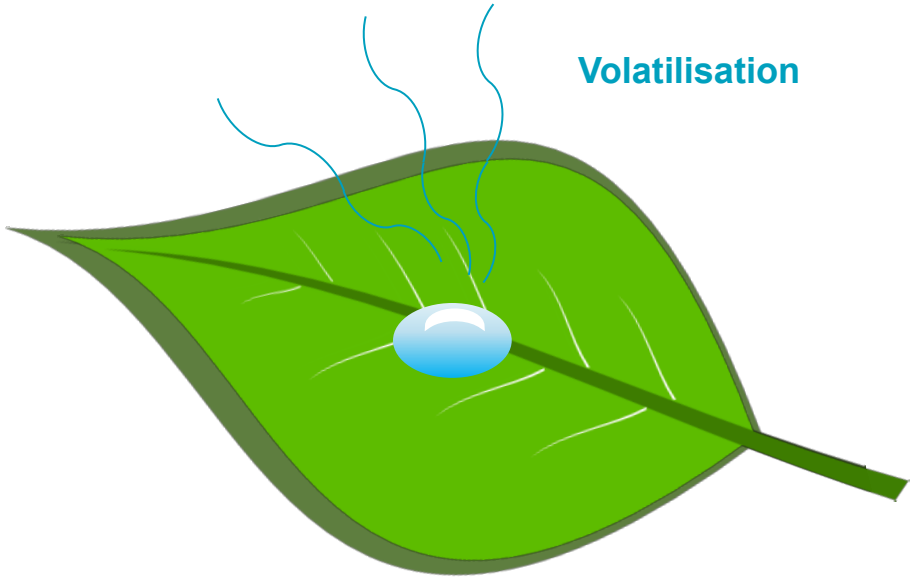
Understanding losses from a foliar surface





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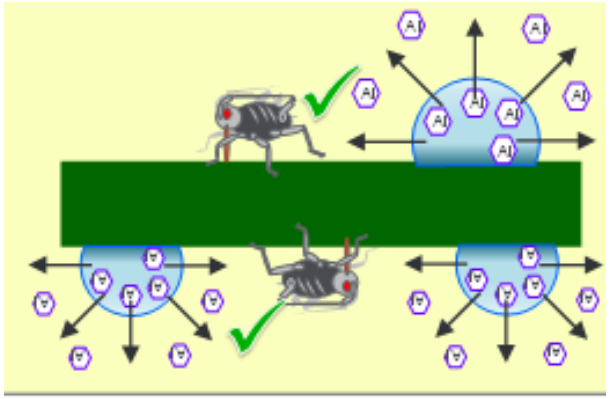
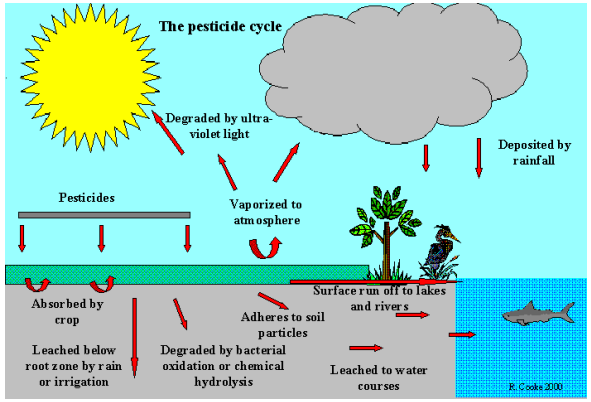
Why is pesticide volatility so important?

- Environmental fate (loss and persistence)
- Registrability
- Activity (vapour activity and volatility as a benefit)



Vapour activity on Aphids

Powdery mildew on Fruit



Environmental fate

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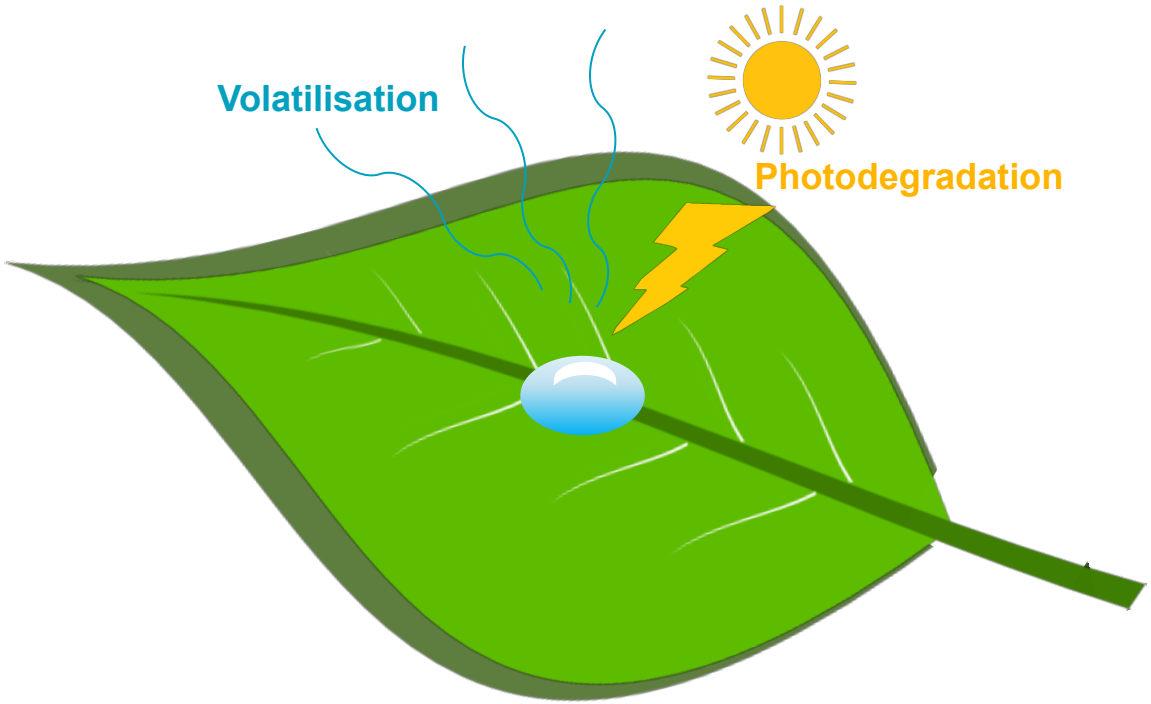
Understanding losses from a foliar surface



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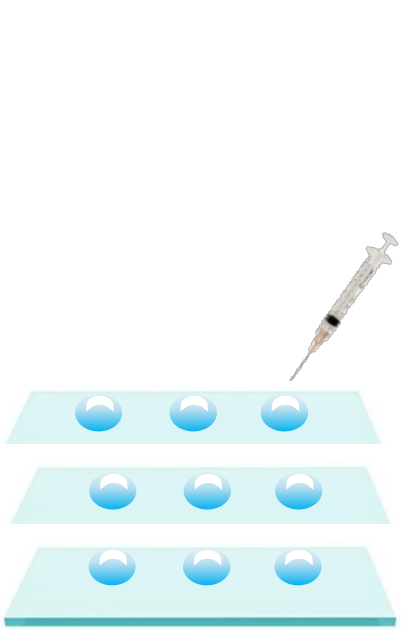
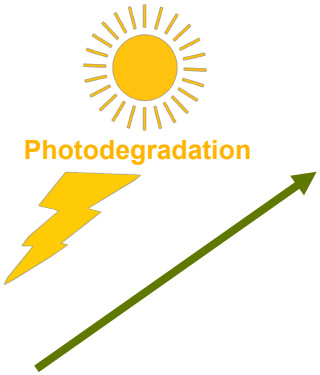
Why is pesticide photodegradation important?

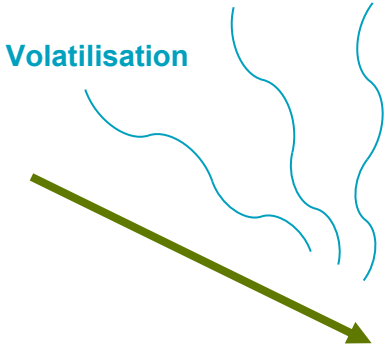
- Activity (photodegradation is one of most destructive post-application pathways)
- Environmental fate (product and persistence)
- Marketability
- Registrability

Pesticide sprayed on leaf surface

In lab test for volatility and photodegradation







Suntest

Wind Tunnel

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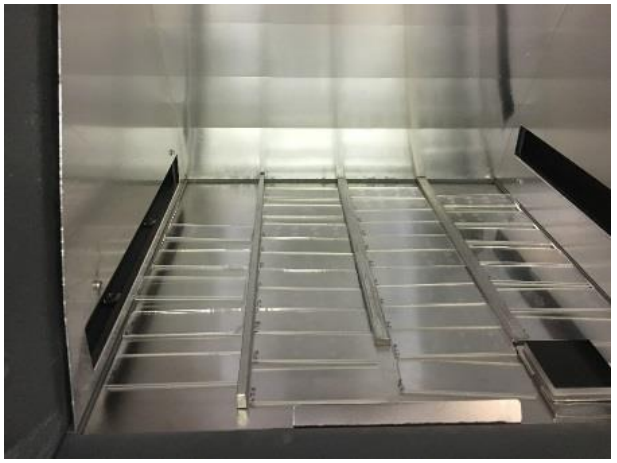
Testing for photodegradation - Suntest



- Atlas XLS+ Suntest
- UV-filtered xenon lamp
- Mimics sunlight intensity & spectrum
- Irradiance set to 750W/m^2
- Thermostated baseplate (circulating water @ 15ºC → baseplate ≈ 20 ± 3ºC)

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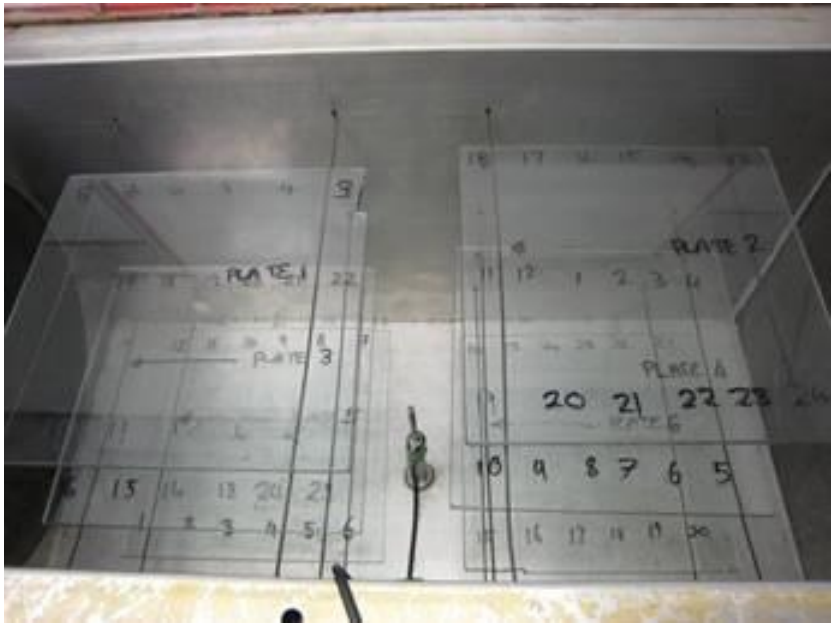
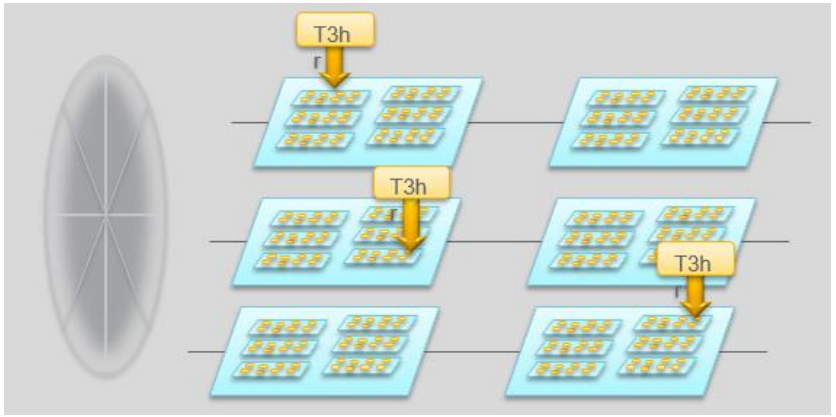
Test for volatility- The Wind Tunnel



- Very sample-efficient means of estimating vapour pressure (typically <100µg)]
- Studies are normally run at 40C with a standard wind speed of 1m/s (no control of the R.H.)



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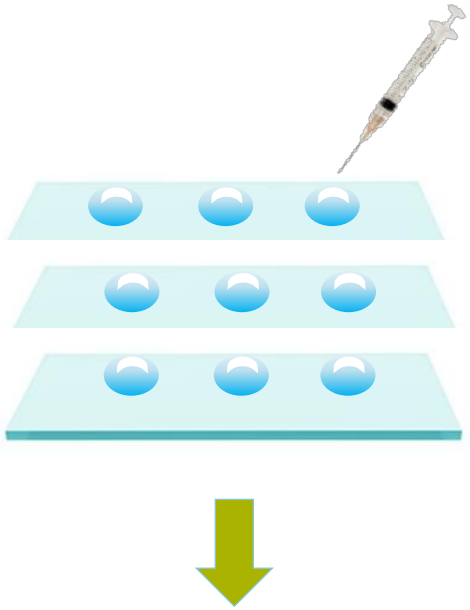
Photodegradation and volatility test – glass as substrate



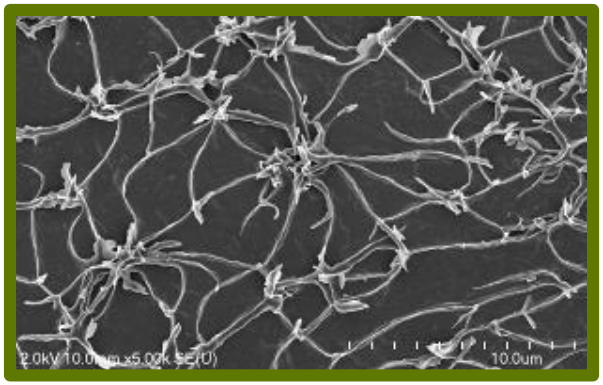
Pros:

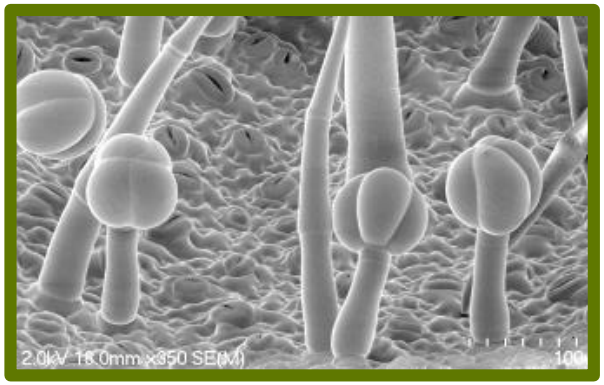
- Good proxy for foliar surface
- Uniform, clean substrate to use for the tests
- High-throughput test
- Worst case scenario
- Indicative but imperfect

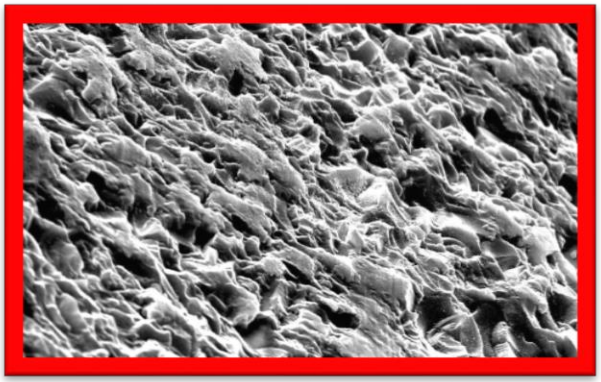
Limitations:

- Different polarity and morphology between glass and leaves–> differences in redistribution mechanism and deposit shape?

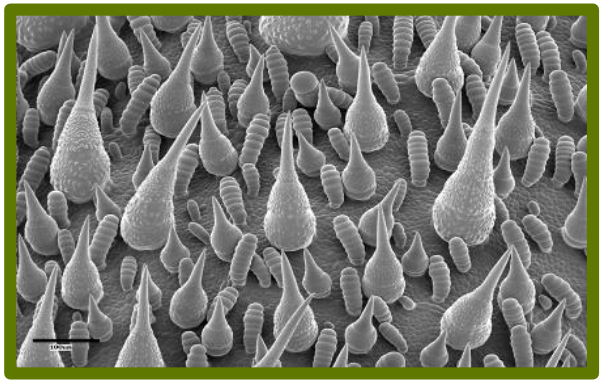
SEM images of leaf and glass surfaces







Glass



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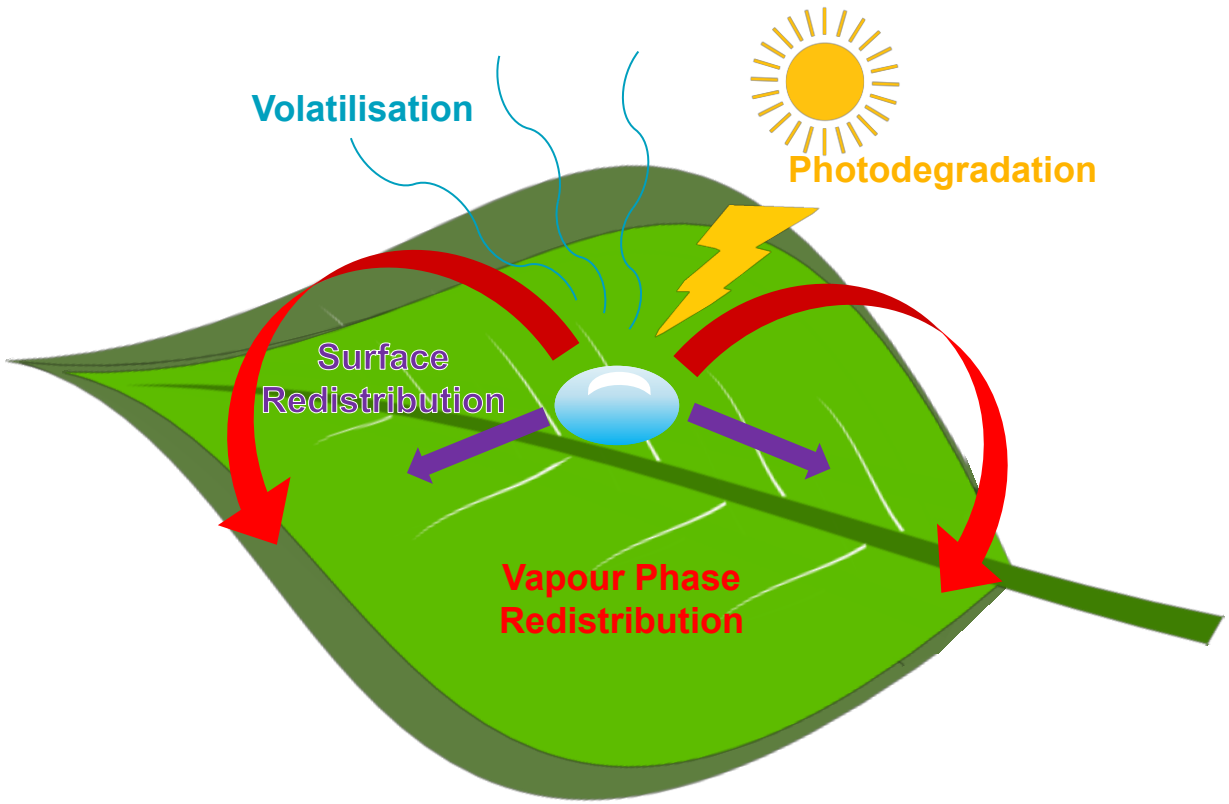
Surface redistribution mechanisms



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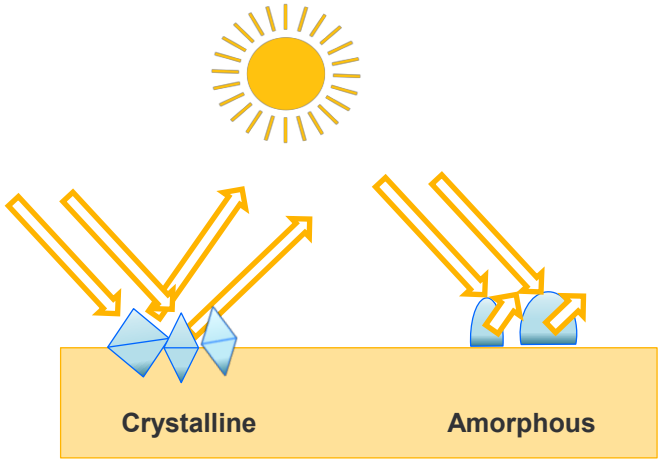
Different deposit – effect on photodegradation and volatility



A highly crystalline deposit

→ increased reflection/refraction/diffraction of incident radiation

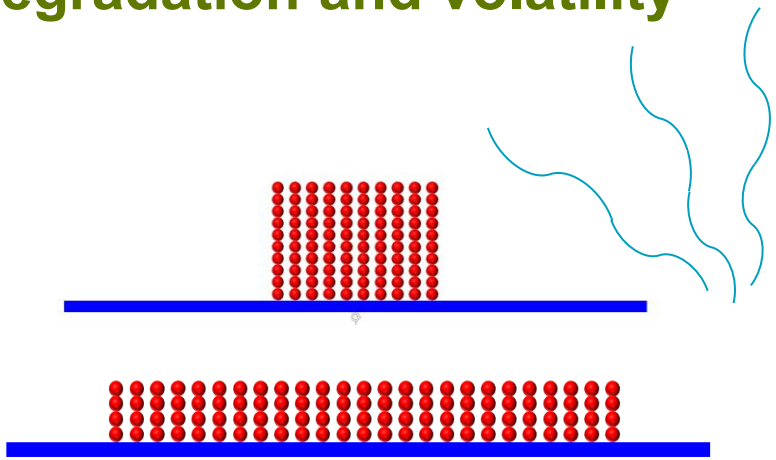
→ less absorption of energy

→ reduced photodegradation

✚

→ strong intermolecular interactions

→ less photo-induced mechanisms available



The larger the deposit area, the faster the compound volatilises.

A highly crystalline deposit

→ strong intermolecular interactions

→ less prone to volatilise (true for small organic molecules)

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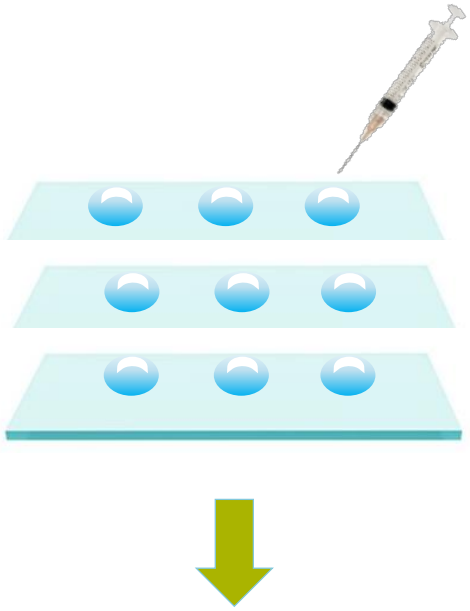
Photodegradation and volatility test – glass as substrate



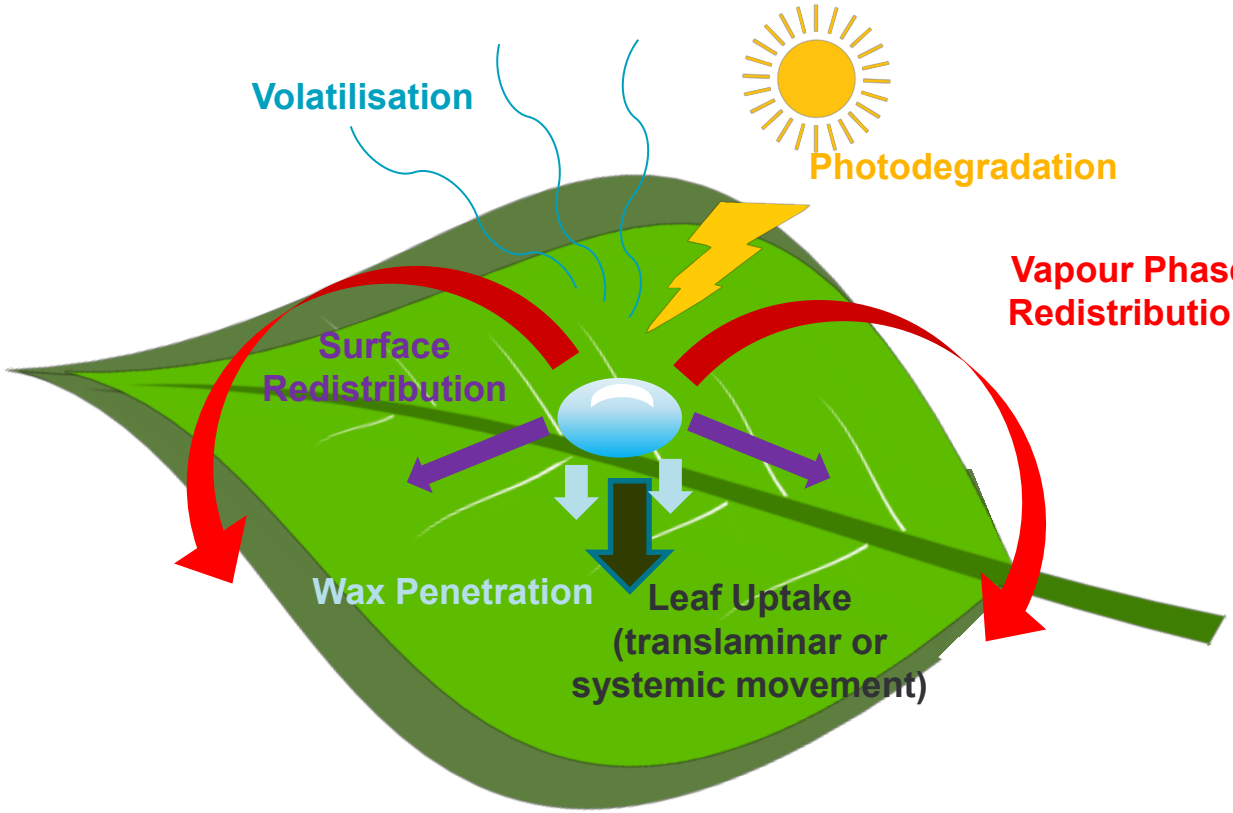
Pros:

- Good proxy for foliar surface;
- Quite smooth and clean substrate to use for the tests
- High-throughput test
- Worst case scenario
- Indication for the photostability or volatility

Limitations:

- Different polarity and morphology between glass and leaves–> differences in deposit? Differences in volatility and photostability?
- Glass is an impermeable substrate: it does not take into account biological interactions that can influence transfer and movement of pesticides

Inside



Lab tests can highlight photochemical instability or a post-deposition volatilization but they do not guarantee this is likely to translate to be a problem in the field

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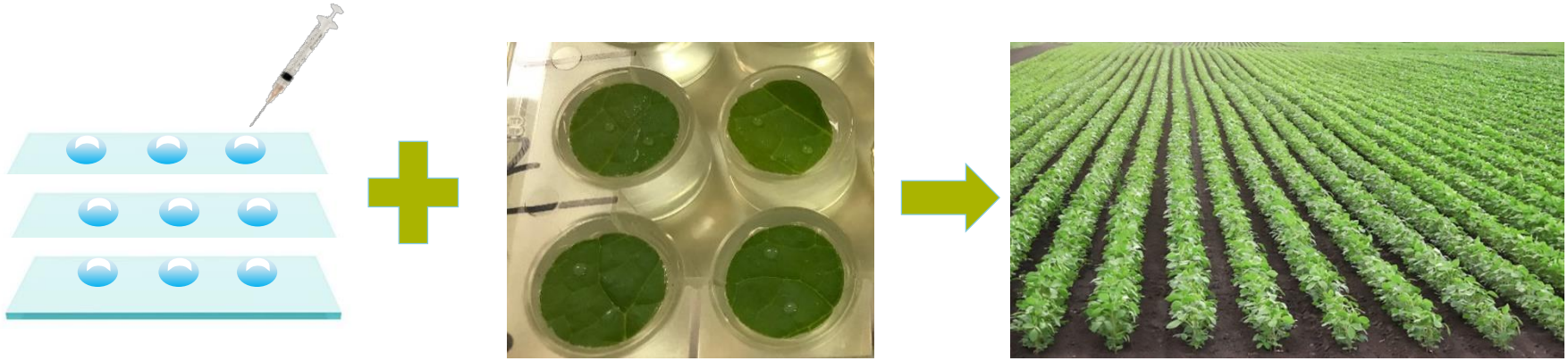
Photodegradation and volatility – alternative substrates



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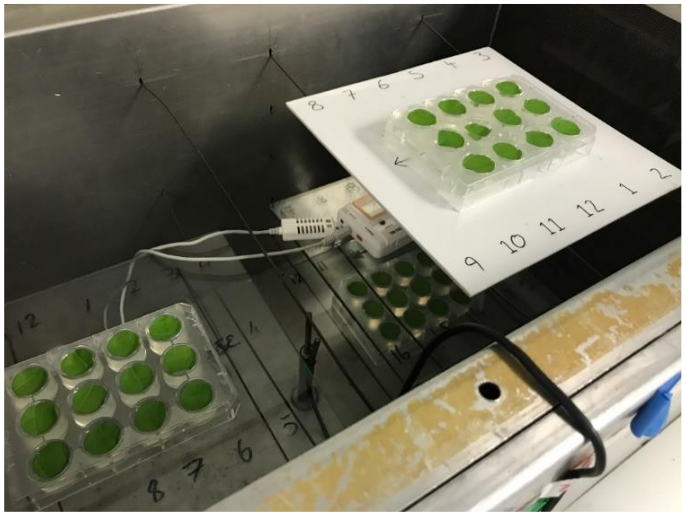
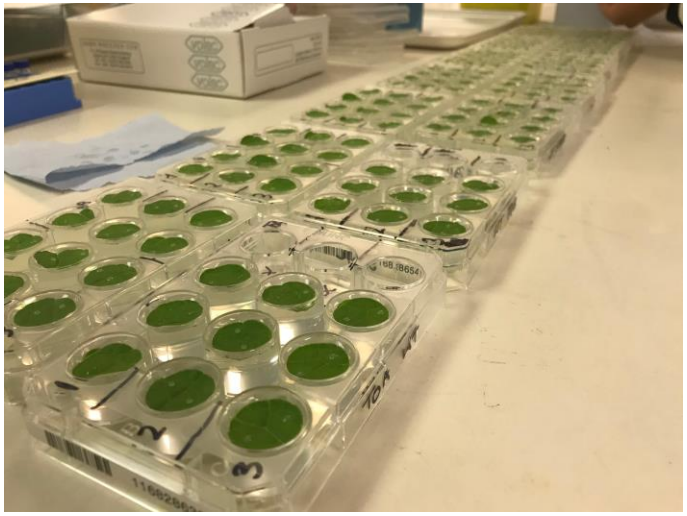
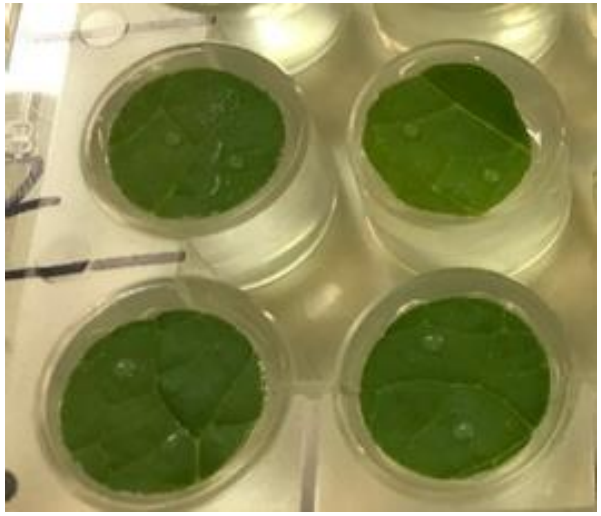
Methodology – leaf as substrate







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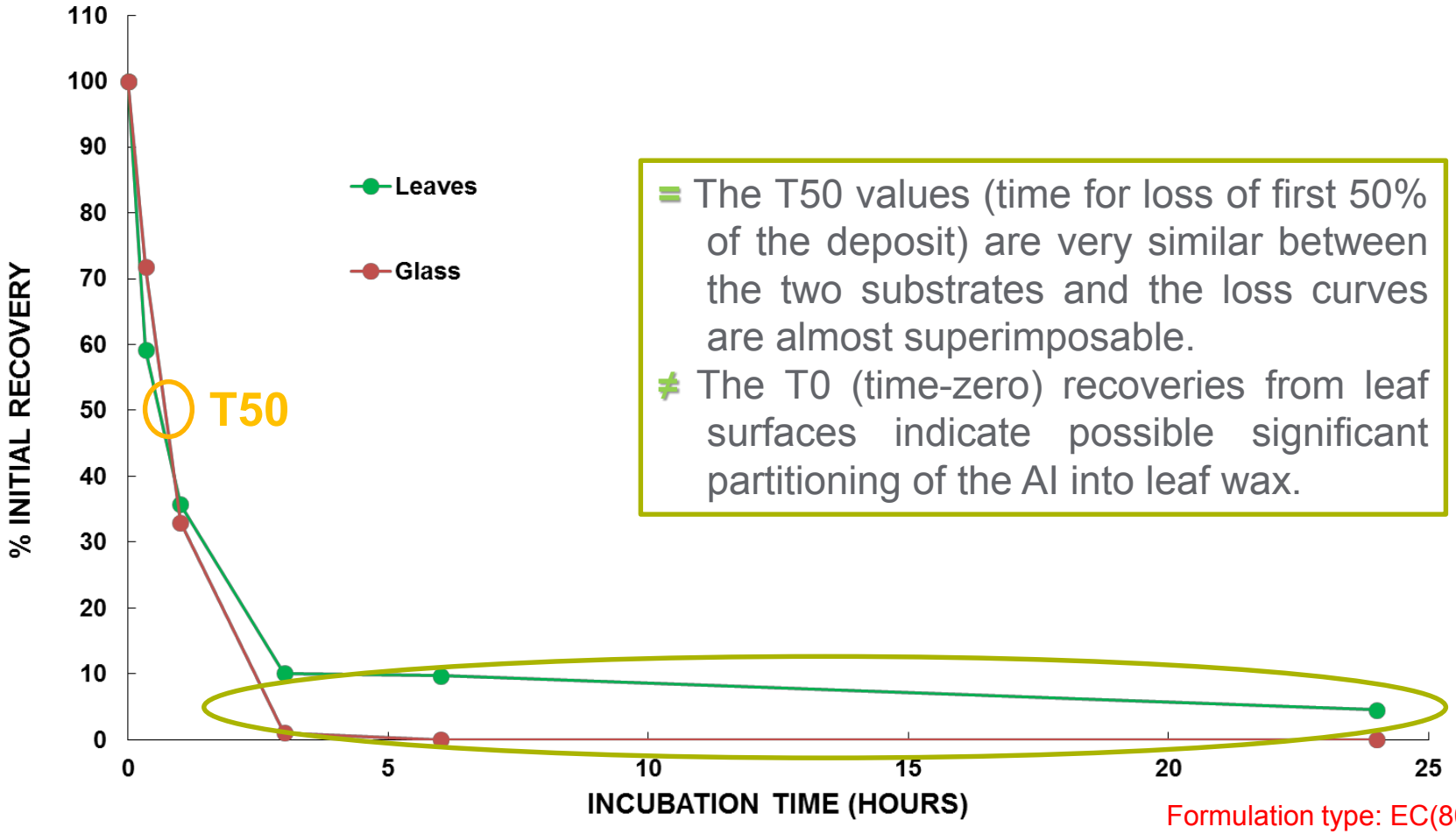
Volatility Study – Glass vs Leaf

LIPOPHILIC VOLATILE HERBICIDE



= The T50 values (time for loss of first 50% of the deposit) are very similar between the two substrates and the loss curves are almost superimposable.

≠ The T0 (time-zero) recoveries from leaf surfaces indicate possible significant partitioning of the AI into leaf wax.

Formulation type: EC(800)

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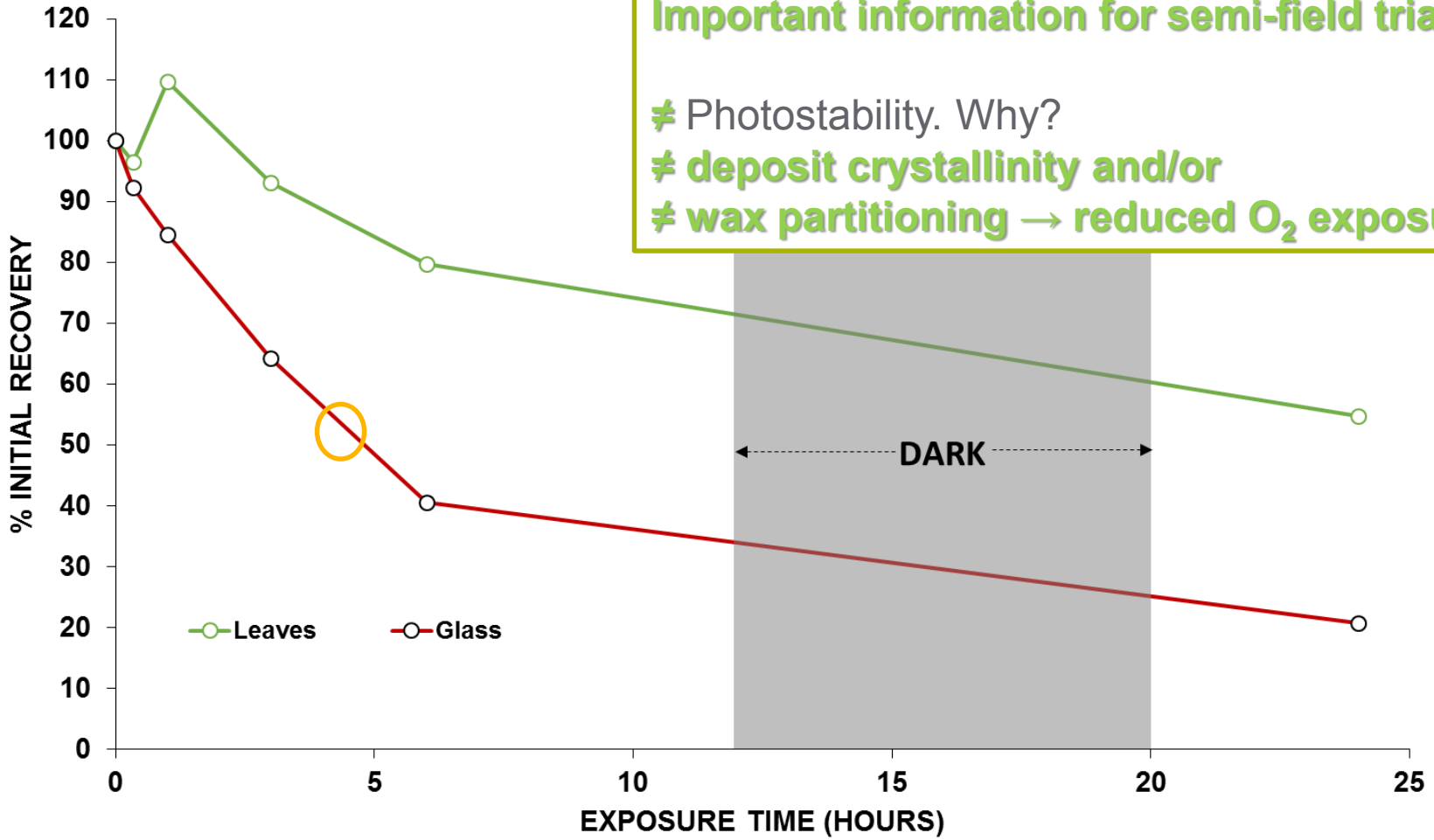
Outdoor Photostability Study – Glass vs Leaf

LIPOPHILIC PHOTOUNSTABLE INSECTICIDE



Formulation type: SC(500)

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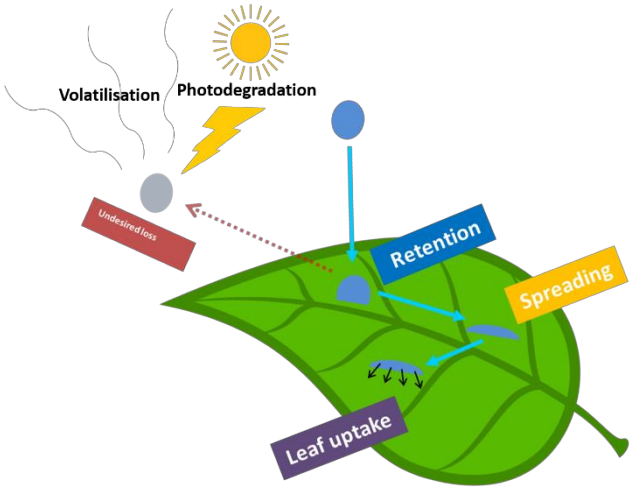
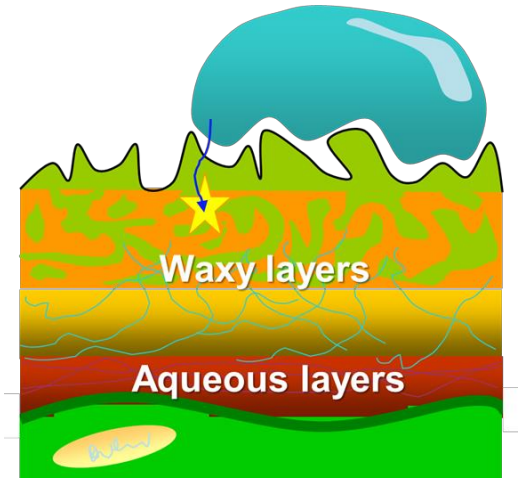
Profiling Uptake and Distribution

- Limitation of the first two studies: only surface wash analysis
- Partial indication of mobility/distribution profile – limited to the surface





In order to have an understanding of the AI partitioning within the leaf

- penetration through the waxy cuticle
- uptake into the leaf and potential translaminar movement

Introduction of the wax wash and extract analysis

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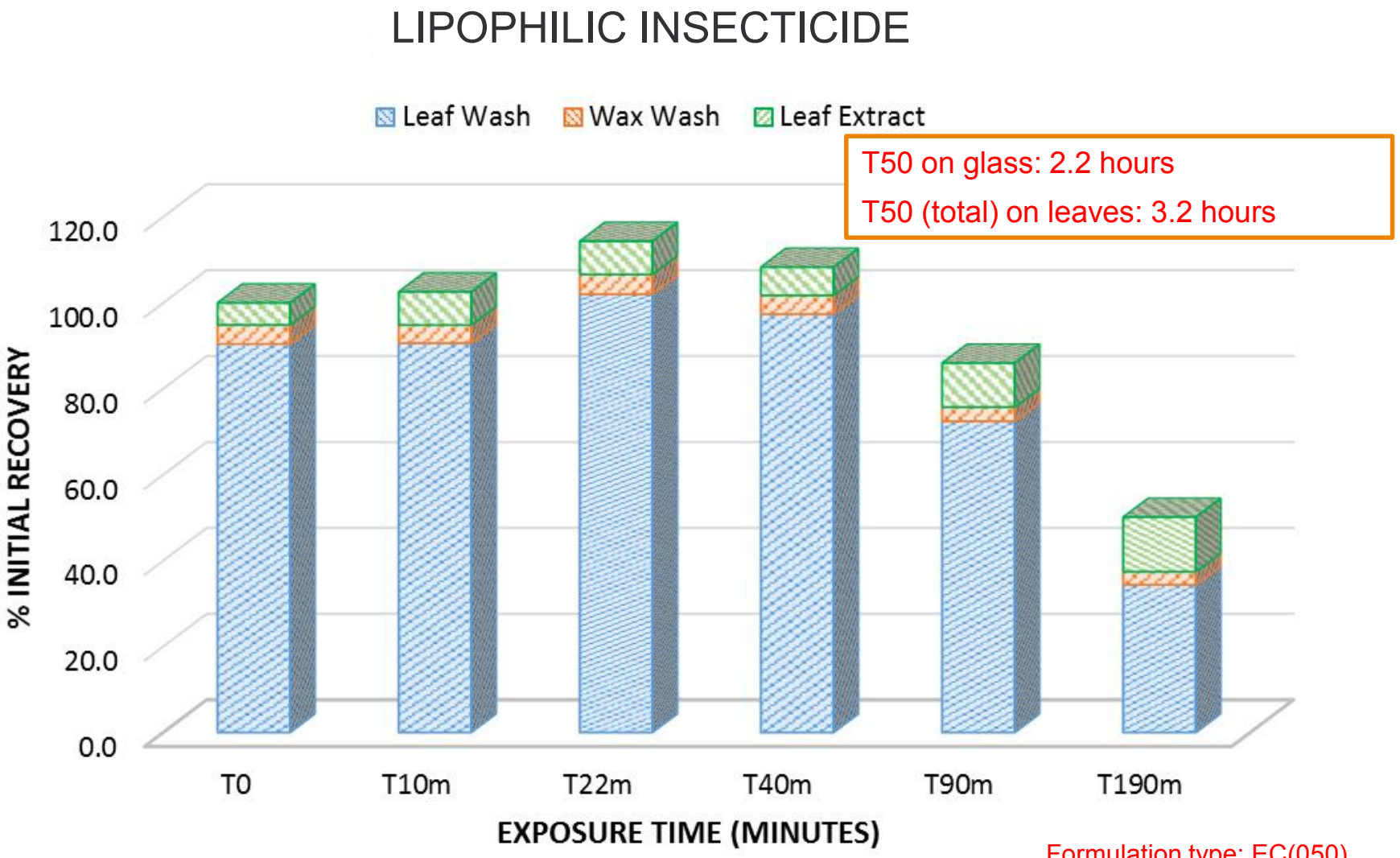
Suntest Photostability Study on leaf substrate



Formulation type: EC(050)

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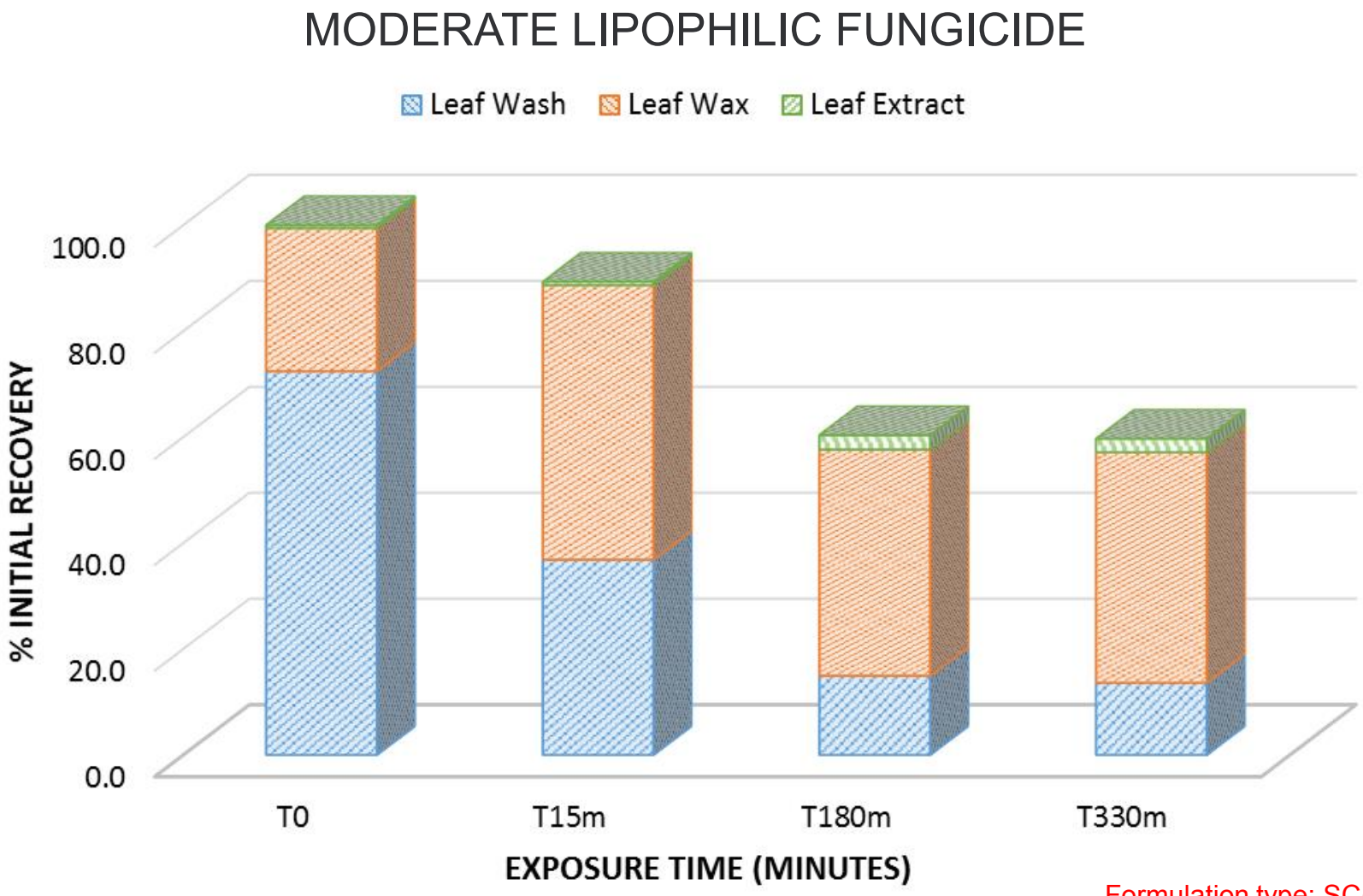
Suntest Photostability Study on Foliar substrate



Formulation type: SC(100)

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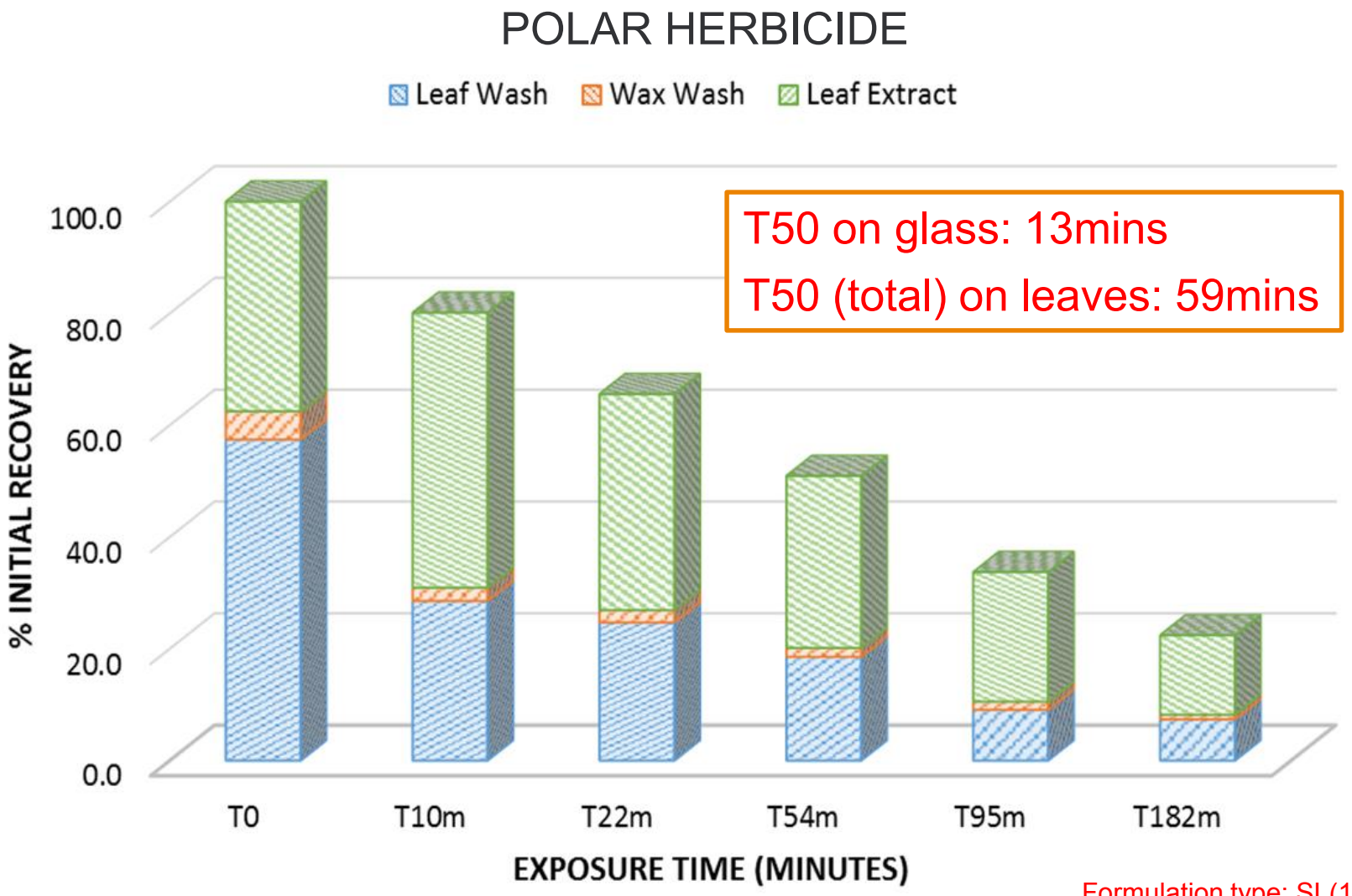
Suntest Photostability Study on Foliar substrate



Formulation type: SL(100)

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Conclusions

- Simple platform that improves our ability to determine interplay between abiotic loss processes and foliar uptake;
- Additional information for interpretation of biological efficacy data;
- Excised cabbage leaves are used as a model system -> extension into different species;
- Help in the design of new AIs.

New developments

- Need to improve the viability of leaf discs in the Suntest
- Use of entire leaves for mobile compounds

Acknowledgements

- Physical Chemistry Group
- Beata Kuzmierczyk-Manole, Discovery Biology
- Reza Nourani, Ins. Biokinetics
- Catherine Waller, Formulation Development
- Phil Lestrange, Plant Production

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No transporters means no transport – and assessment of the 'real' (natural) substrates of xenobiotic transporters

Douglas Kell

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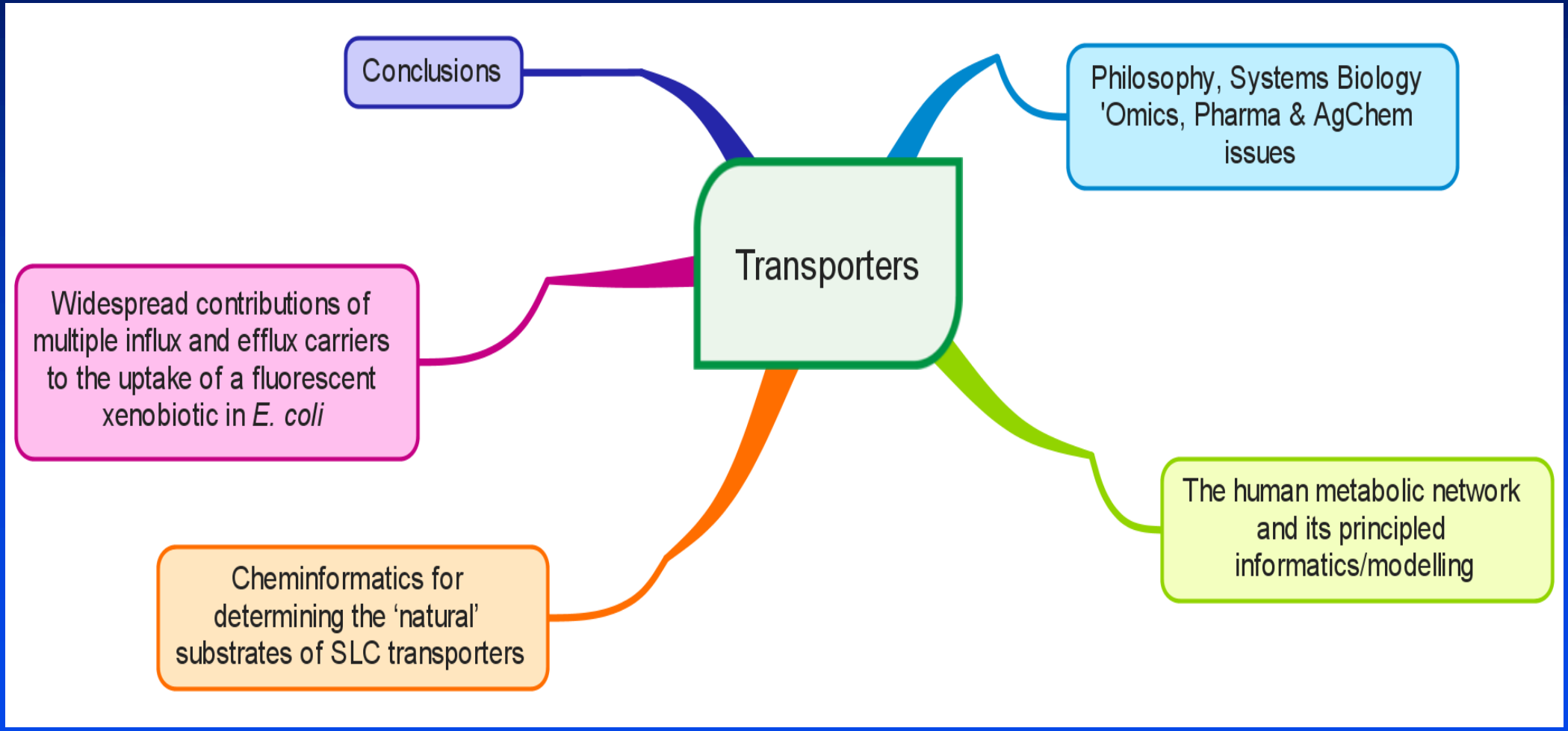
http://dbkgroup.org/publications/

BBSRC bioscience for the future

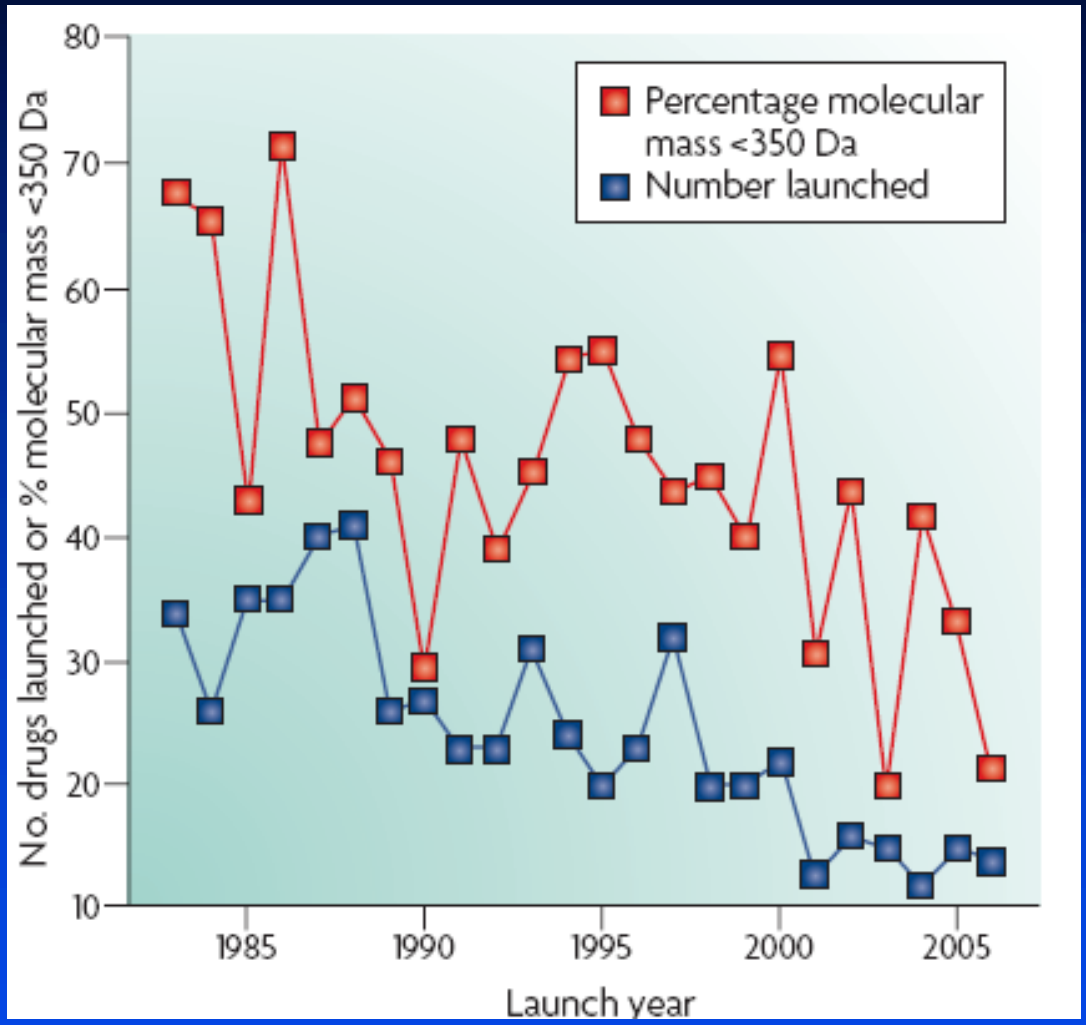
Center for Biosustainability

UNIVERSITY OF LIVERPOOL

Synopsis


Conclusions
Philosophy, Systems Biology 'Omics, Pharma & AgChem issues
Transporters
Widespread contributions of multiple influx and efflux carriers to the uptake of a fluorescent xenobiotic in *E. coli*
The human metabolic network and its principled informatics/modelling
Cheminformatics for determining the 'natural' substrates of SLC transporters

Declining numbers of drug launches



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

~100 Discovery Approaches

Pfizer

Millions of Compounds Screened

Preclinical Pharmacology

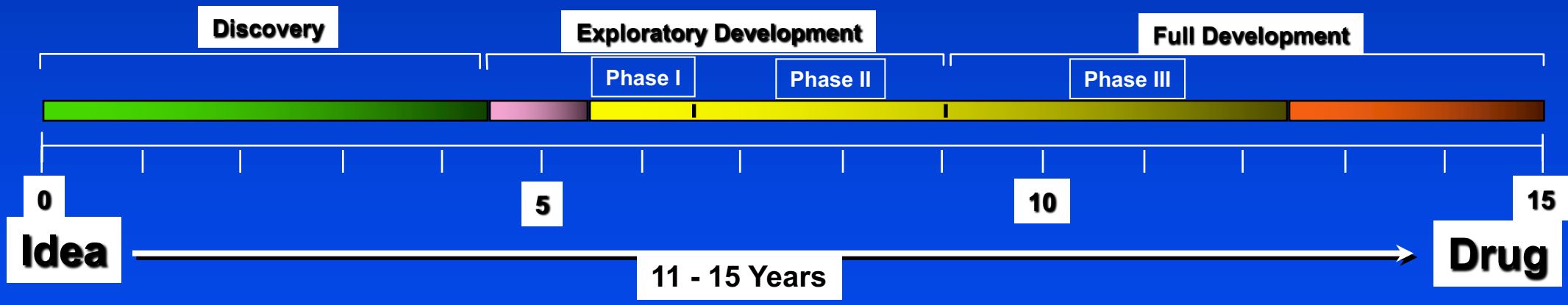
Preclinical Safety

Clinical Pharmacology & Safety

First into humans

High Risk Process:
11-15 Years, $800M+
Now ~ $2500M

1 - 2 Products



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

Attrition

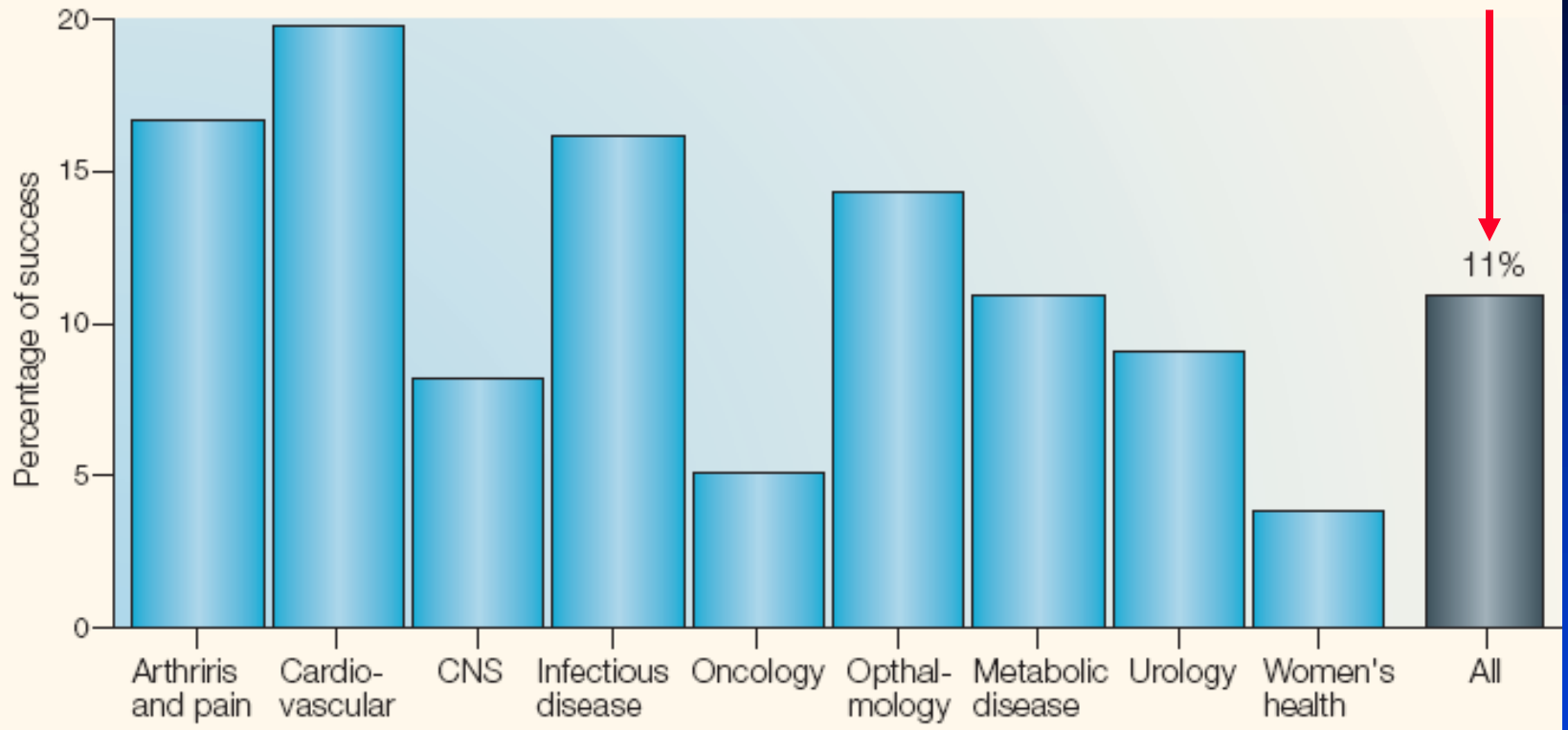


Figure 1 | **Success rates from first-in-man to registration.** The overall clinical success rate is 11%. However, if the analysis is carried out by therapeutic areas, big differences emerge. The data are from the ten biggest drug companies during 1991–2000. (The companies are AstraZeneca, Bristol-Myers 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

OPINION

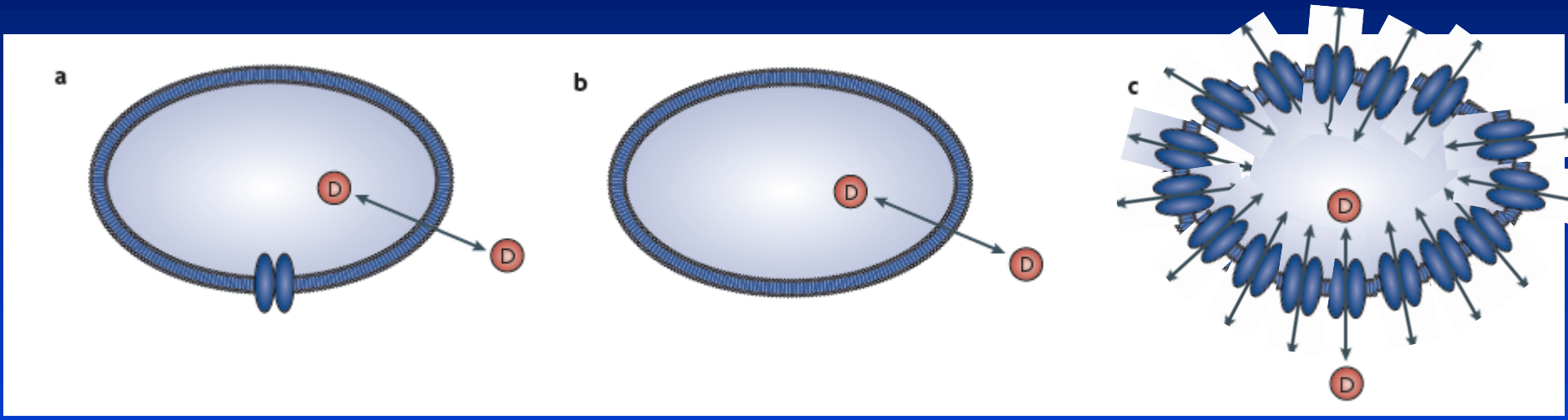
Carrier-mediated cellular uptake of pharmaceutical drugs: an exception or the rule?

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)

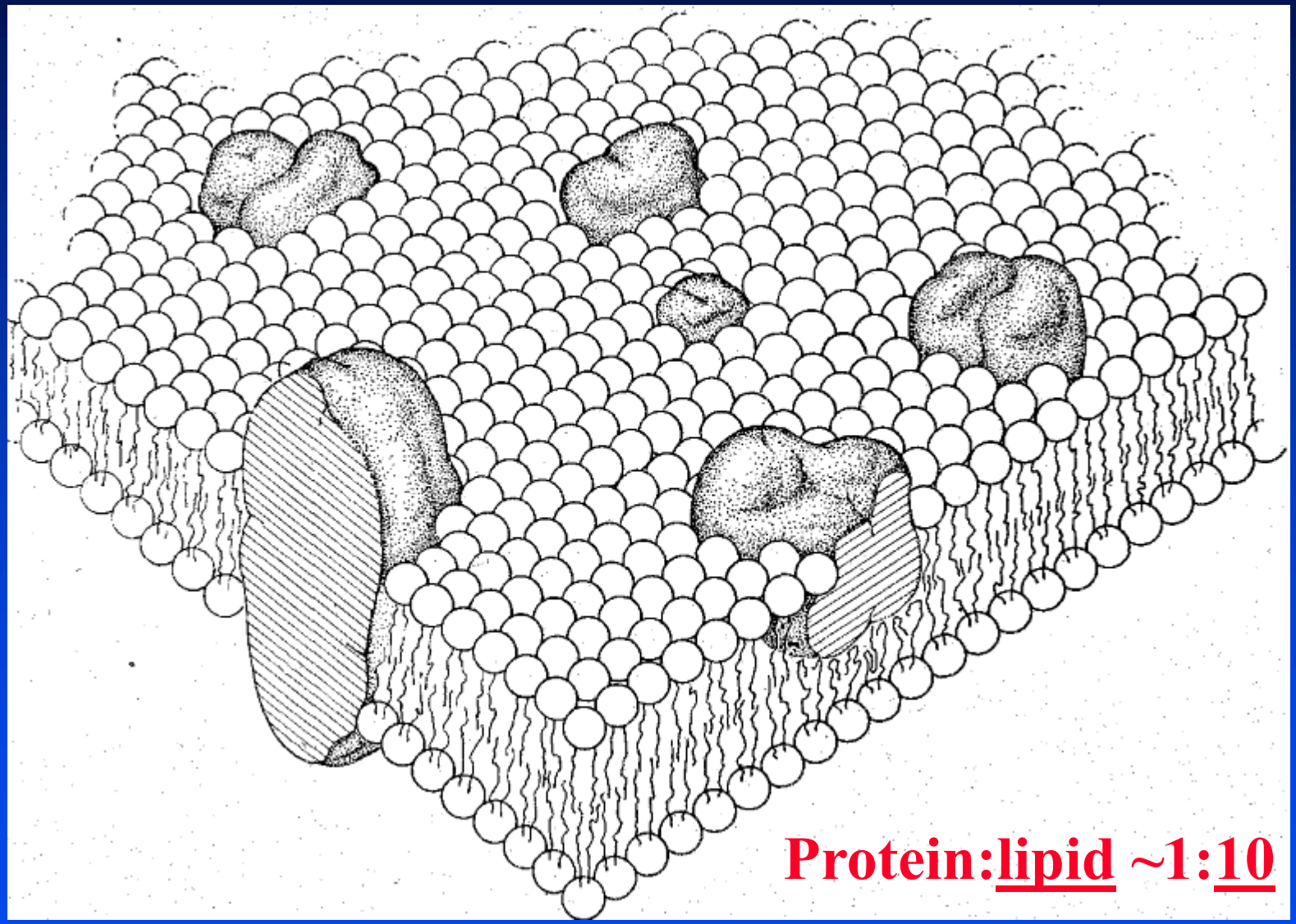
How drugs can cross cellular membranes



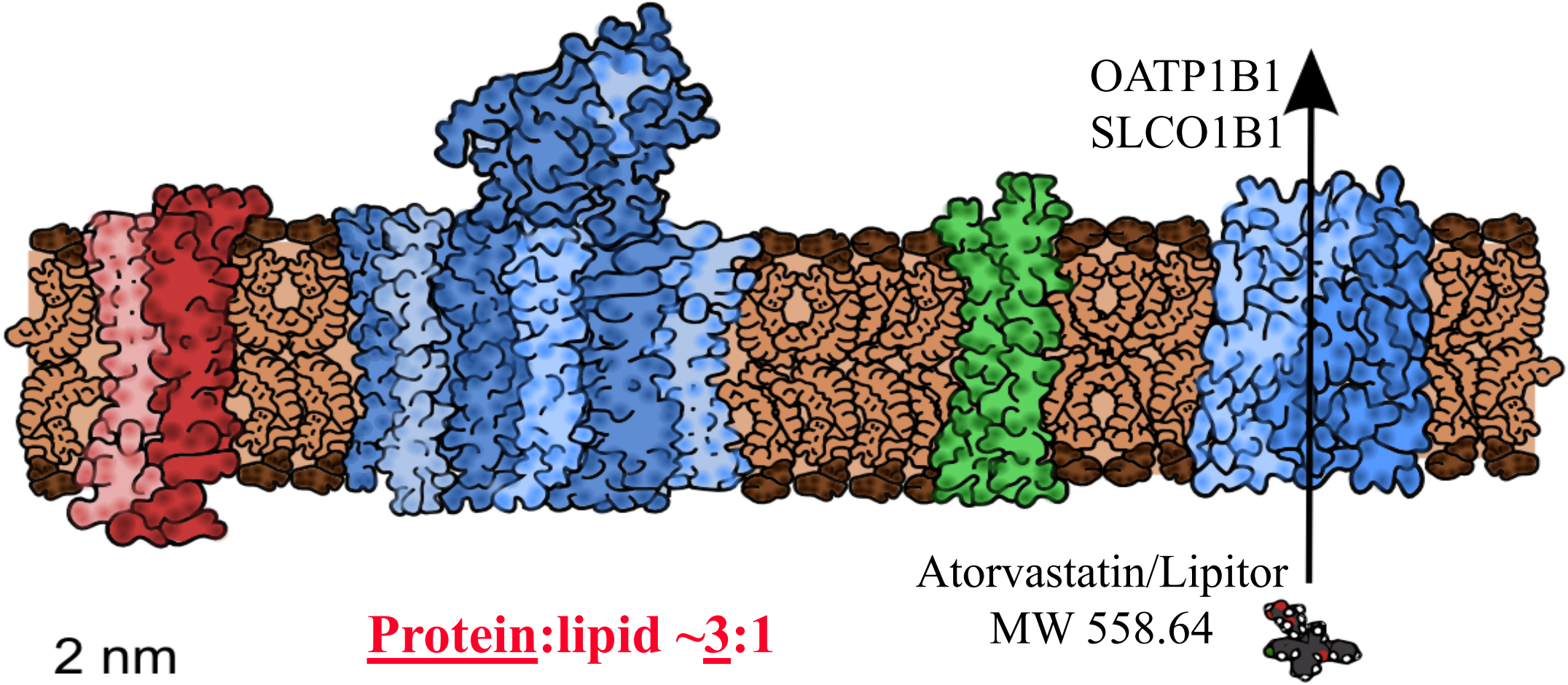
By free diffusion or carrier-mediated?

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

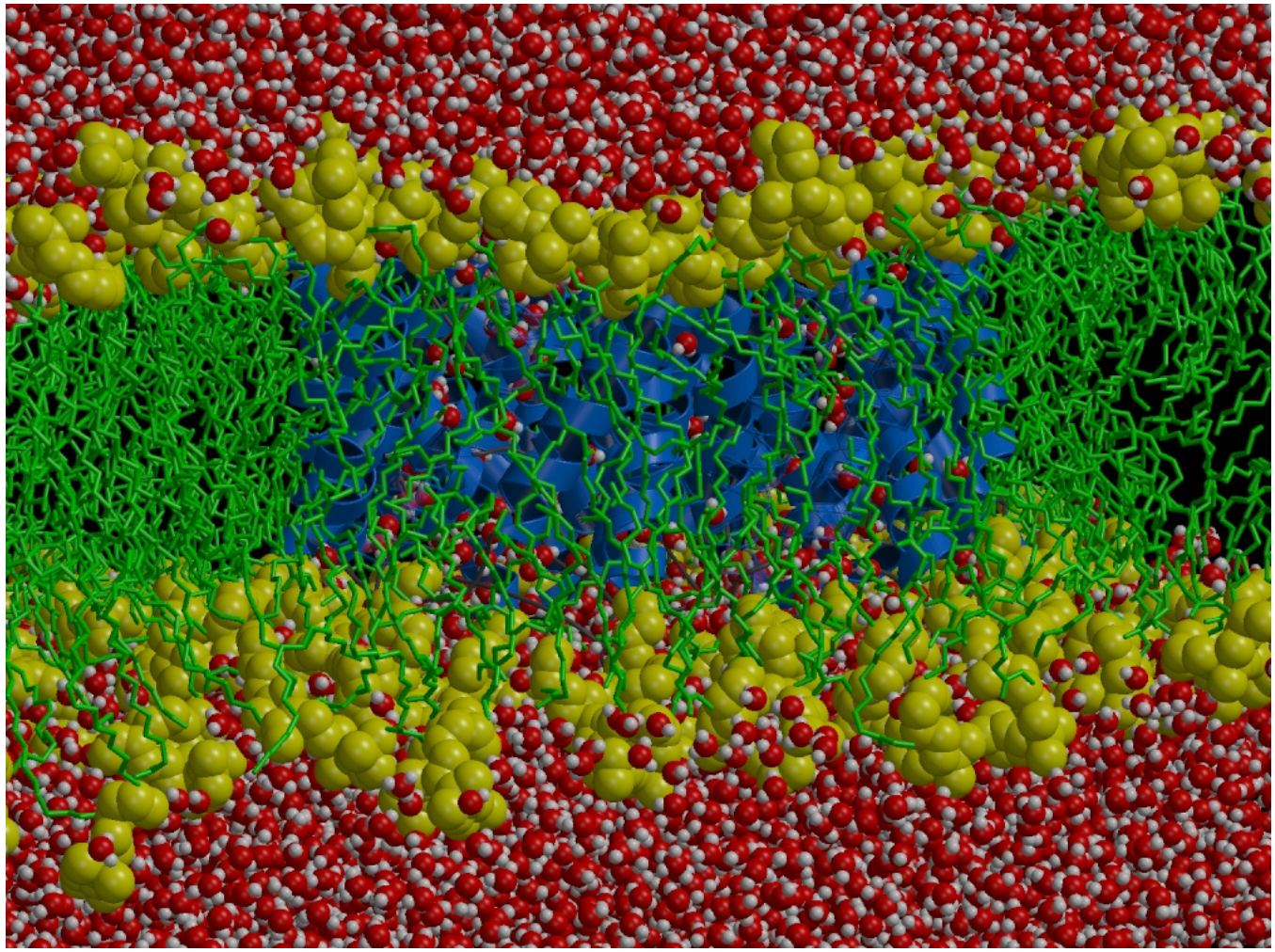


A typical biomembrane drawn to scale



Protein:lipid ~3:1

Membrane with aquaporin



No water crossing via phospho-lipids

http://www3.mpibpc.mpg.de/groups/de_groot/gallery/aqp1_snapshot.jpg

Two views

molecular pharmaceutics

Review

11, 1727-1738 (2014)

pubs.acs.org/molecularpharmaceutics

Passive Lipoidal Diffusion and Carrier-Mediated Cell Uptake Are Both Important Mechanisms of Membrane Permeation in Drug Disposition

Dennis Smith,*,† Per Artursson,‡ Alex Avdeef,§ Li Di,∥ Gerhard F. Ecker,⊥ Bernard Faller,# J. Brian Houston,¶ Manfred Kansy,□ Edward H. Kerns,■,▽ Stefanie D. Krämer,○ Hans Lennernäs,‡ Han van de Waterbeemd,● Kiyohiko Sugano,△ and Bernard Testa▲

frontiers in PHARMACOLOGY

5, 231 (2014) (32pp)

REVIEW ARTICLE
published: 31 October 2014
doi: 10.3389/fphar.2014.00231

How drugs get into cells: tested and testable predictions to help discriminate between transporter-mediated uptake and lipoidal bilayer diffusion

Douglas B. Kell[1,2] and Stephen G. Oliver[3,4]*

Phospholipid Bilayer diffusion Is Negligible (PBIN)

Position statement (≡ hypothesis)

- There is in fact no actual evidence (evidence = data plus correct theory and interpretation) that any significant drug permeability goes via undisturbed 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
- In real, intact biomembranes, for drug transport, "**Phospholipid Bilayer diffusion Is Negligible**" (**PBIN**)
- Think if your own data are consistent with this

Does CO_2 cross intact cellular membranes by using a transporter?

BBA 1840, 1592-5 (2014) Biochimica et Biophysica Acta 1840 (2014) 1592–1595

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Review

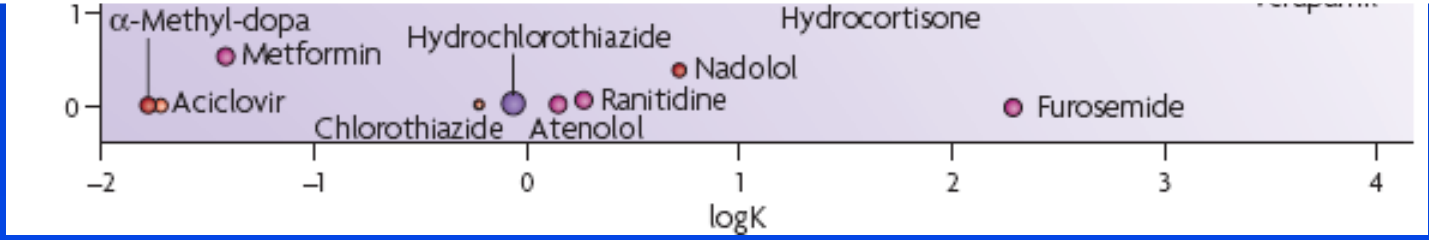
Aquaporins and membrane diffusion of CO_2 in living organisms ☆

Ralf Kaldenhoff *, Lei Kai, Norbert Uehlein

Department of Biology, Applied Plant Sciences, Technische Universität Darmstadt, Schnittspahnstrasse 10, D-64287 Darmstadt, Germany

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

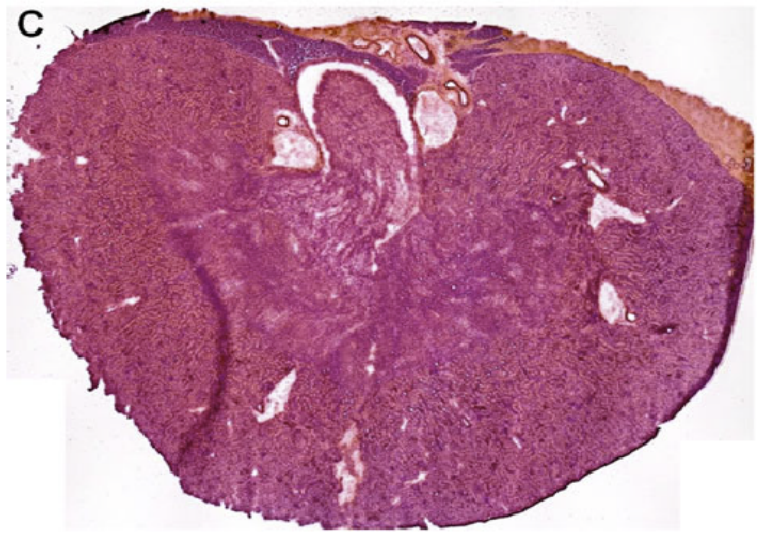
THESE log P THEORIES OF DRUG UPTAKE ARE <u>BIOPHYSICAL</u>, 'LIPID-ONLY' THEORIES

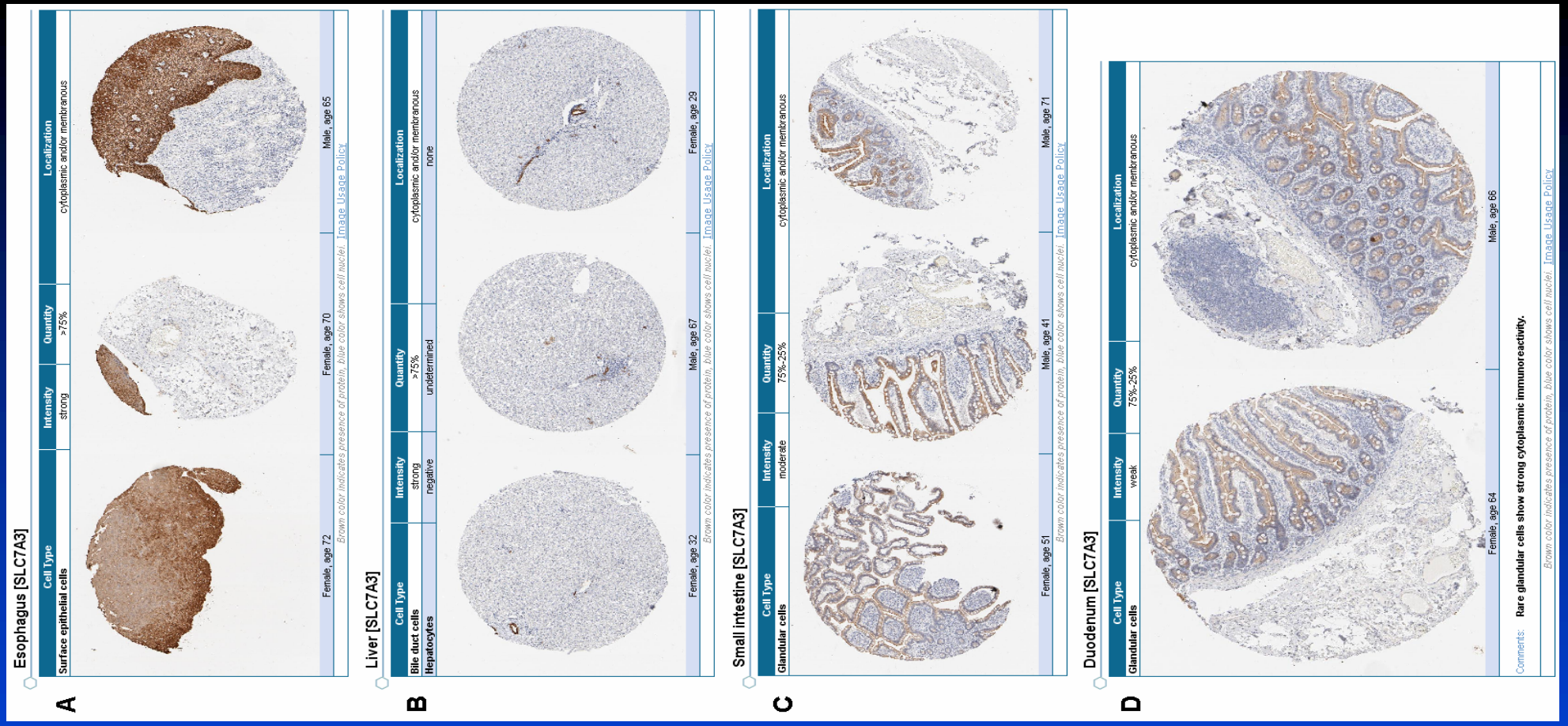


Corti *et al.* Eur J Pharm Sci 7, 354-362 (2006)

Imatinib distribution in mouse kidney

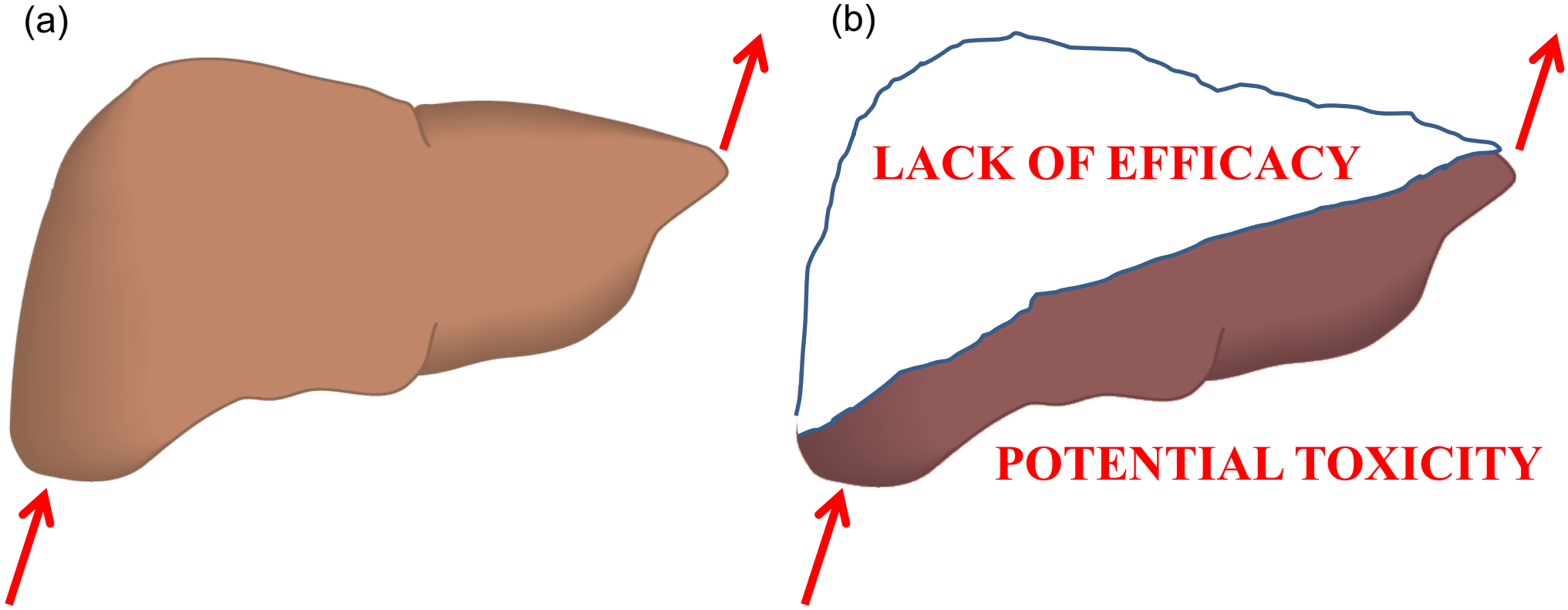
Römpp, A. *et al*. (2011). Anal Bioanal Chem 401, 65-73.





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

Heterogeneous distributions of a drug in an organ can lead to a lack of efficacy while retaining the same gross PK/PD



Total amount of drug in organ is the same, but in (b) it has efficacy in only a fraction of the cells (as it does not enter them all) and may exhibit toxicity (as it is more highly concentrated in some)

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

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]

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

Recon 2.2

Metabolomics _####################_
DOI 10.1007/s11306-016-1051-4

12, 109 (2016)

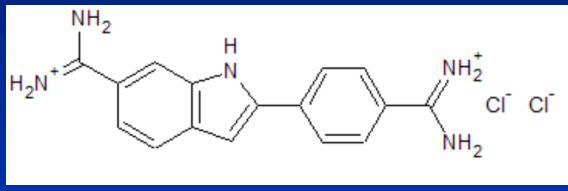
SHORT COMMUNICATION

Recon 2.2: from reconstruction to model of human metabolism

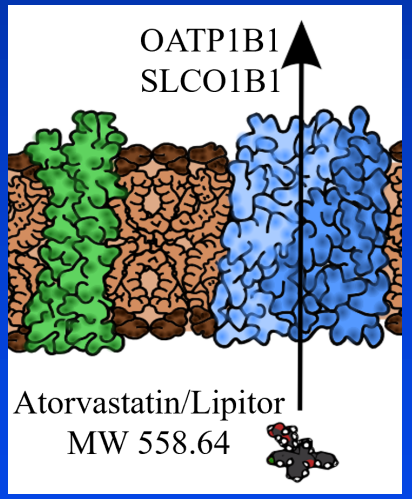
Neil Swainston[1,2,3] · Kieran Smallbone[3] · Hooman Hefzi[4,5] · Paul D. Dobson[3] · Judy Brewer[6,7] · Michael Hanscho[8,9] · Daniel C. Zielinski[4] · Kok Siong Ang[10,11] · Natalie J. Gardiner[2] · Jahir M. Gutierrez[4,5] · Sarantos Kyriakopoulos[11] · Meiyappan Lakshmanan[11] · Shangzhong Li[4,5] · Joanne K. Liu[13] · Veronica S. Martínez[12] · Camila A. Orellana[12] · Lake-Ee Quek[12] · Alex Thomas[5,13] · Juergen Zanghellini[9] · Nicole Borth[8,9] · Dong-Yup Lee[10,11] · Lars K. Nielsen[12] · Douglas B. Kell[1,14] · Nathan E. Lewis[5,15] · Pedro Mendes[1,3,16]

Two (of many) ramifications of a transporters-only view

- 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

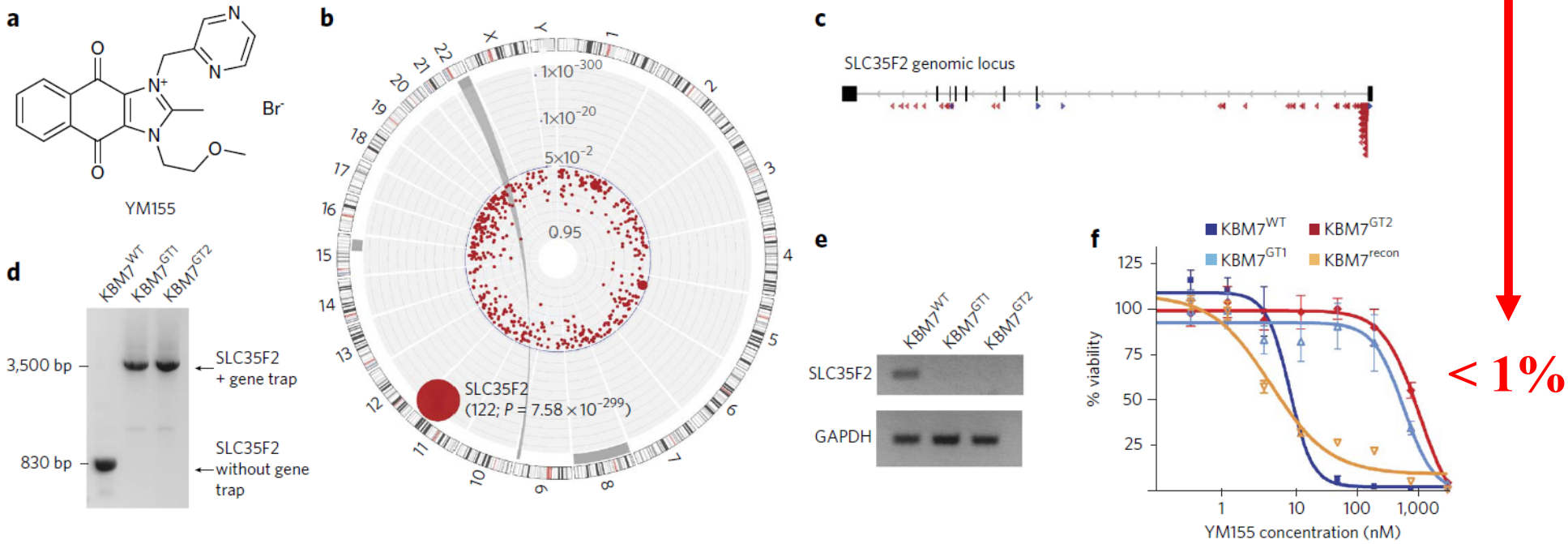
Genome-wide assessment of the carriers involved in the cellular uptake of drugs: a model system in yeast

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*]

ARTICLE
10, 768-773 (2014)
PUBLISHED ONLINE: 27 JULY 2014 | DOI: 10.1038/NCHEMBIO.1590
nature chemical biology

The solute carrier SLC35F2 enables YM155-mediated DNA damage toxicity

Georg E Winter[1], Branka Radic[1,4], Cristina Mayor-Ruiz[2,4], Vincent A Blomen[3], Claudia Trefzer[1], Richard K Kandasamy[1], Kilian V M Huber[1], Manuela Gridling[1], Doris Chen[1], Thorsten Klampfl[1], Robert Kralovics[1], Stefan Kubicek[1], Oscar Fernandez-Capetillo[2], Thijn R Brummelkamp[1,3] & Giulio Superti-Furga[1]*



ARTICLE 10, 768-773 (2014)
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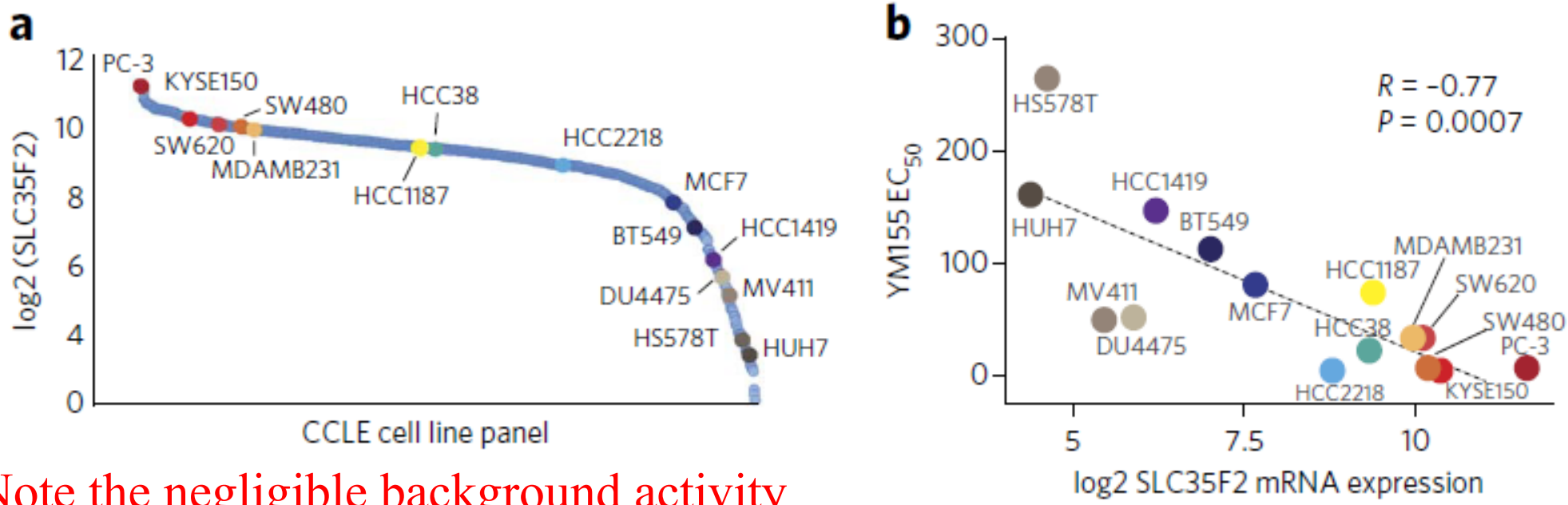
The solute carrier SLC35F2 enables YM155-mediated DNA damage toxicity

Georg E Winter[1], Branka Radic[1,4], Cristina Mayor-Ruiz[2,4], Vincent A Blomen[3], Claudia Trefzer[1], Richard K Kandasamy[1], Kilian V M Huber[1], Manuela Gridling[1], Doris Chen[1], Thorsten Klampfl[1], Robert Kralovics[1], Stefan Kubicek[1], Oscar Fernandez-Capetillo[2], Thijn R Brummelkamp[1,3] & Giulio Superti-Furga[1]*



Note the negligible background activity

Drug-metabolite likenesses

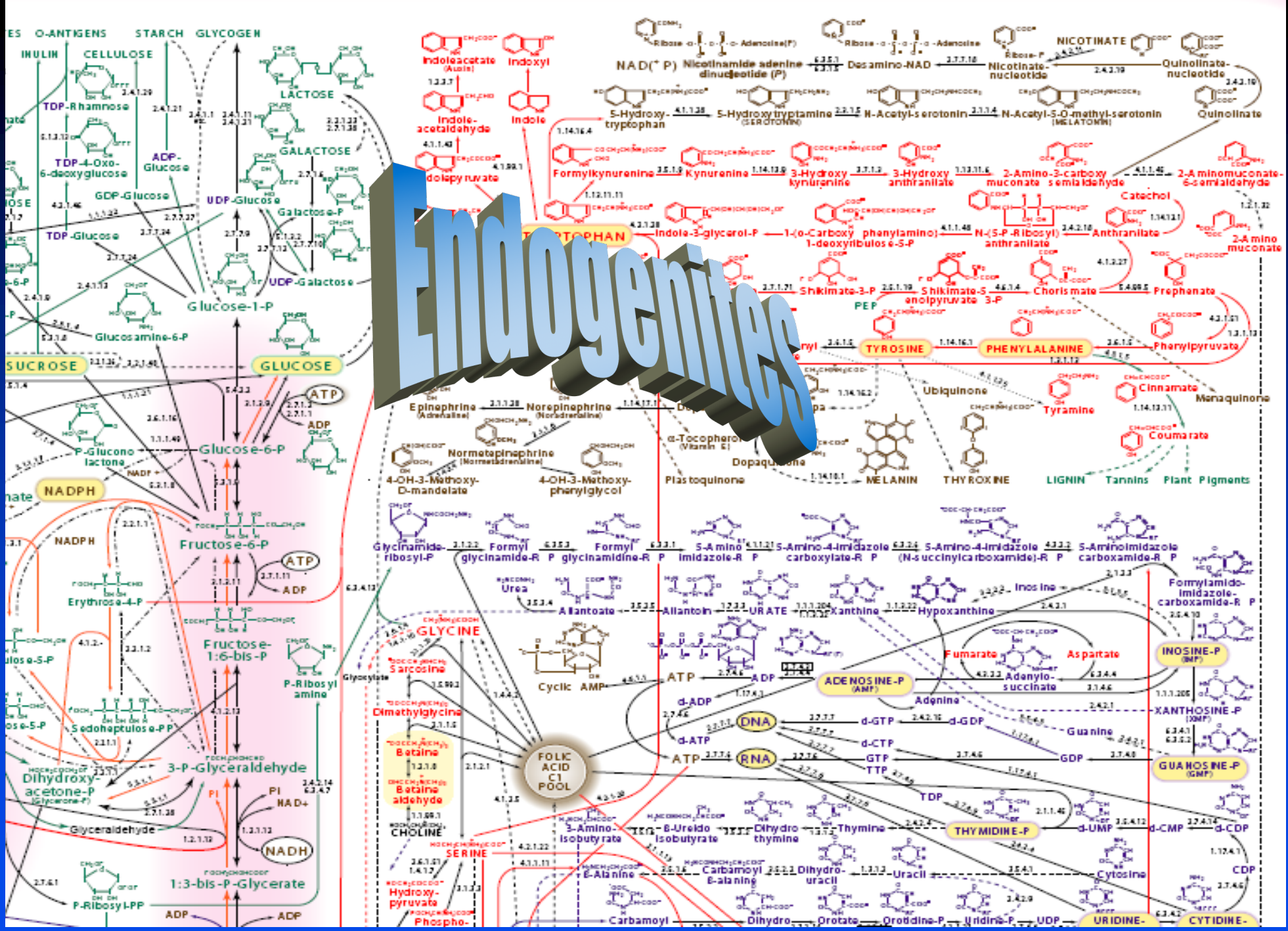
Metabolomics
DOI 10.1007/s11306-014-0733-z

11, 323-339 (2015)

ORIGINAL ARTICLE

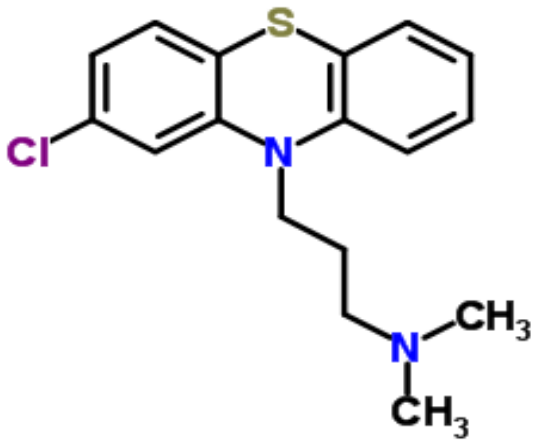
A 'rule of 0.5' for the metabolite-likeness of approved pharmaceutical drugs

Steve O'Hagan · Neil Swainston · Julia Handl · Douglas B. Kell

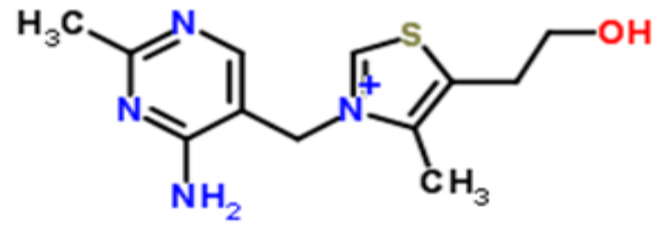

Endogenites
O-ANTIGENS
STARCH
GLYCOGEN
INULIN
CELLULOSE
LACTOSE
GALACTOSE
TDP-Rhamnose
TDP-4-Oxo-6-deoxyglucose
ADP-Glucose
GDP-Glucose
UDP-Glucose
Galactose-P
TDP-Glucose
UDP-Galactose
Glucose-1-P
Glucosamine-6-P
SUCROSE
GLUCOSE
ATP
ADP
Glucose-6-P
P-Gluconolactone
NADP+
NADPH
Fructose-6-P
Erythrose-4-P
Fructose-1:6-bis-P
P-Ribosylamine
Sedoheptulose-P
3-P-Glyceraldehyde
Dihydroxyacetone-P
Glyceraldehyde
NAD+
NADH
1:3-bis-P-Glycerate
P-Ribosyl-PP
Indoleacetate (Auxin)
Indoxyl
Indole-acetaldehyde
Indole
Indolepyruvate
TRYPTOPHAN
Indole-3-glycerol-P
1-(o-Carboxy phenylamino)-1-deoxyribulose-5-P
N-(5-P-Ribosyl) anthranilate
Anthranilate
Catechol
2-Amino muconate
2-Amino-3-carboxy muconate semialdehyde
2-Aminomuconate-6-semialdehyde
Shikimate-3-P
Shikimate-5-P
Chorismate
Prephanate
PEP
TYROSINE
PHENYLALANINE
Phenylpyruvate
Epinephrine (Adrenaline)
Norepinephrine (Noradrenaline)
Normetapinephrine (Normetadrenaline)
4-OH-3-Mathoxy-D-mandelate
4-OH-3-Mathoxy-phenylglycol
α-Tocopherol (Vitamin E)
Plastoquinone
Dopaquinone
MELANIN
THYROXINE
Ubiquinone
Tyramine
Cinnamate
Coumarate
Menaquinone
LIGNIN
Tannins
Plant Pigments
NAD(+P) Nicotinamide adenine dinucleotide (P)
Desamino-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
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
Allantoate
Allantoin
URATE
Xanthine
Hypoxanthine
Inosine
Cyclic AMP
GLYCINE
Sarcosine
Dimethylglycine
Betaine
Betaine aldehyde
CHOLINE
SERINE
Hydroxy-pyruvate
Glycolate
FOLIC ACID C1 POOL
ATP
ADP
ADENOSINE-P (AMP)
Fumarate
Aspartate
Adenylo-succinate
Adenine
INOSINE-P (IMP)
XANTHOSINE-P (XMP)
Guanine
GUANOSINE-P (GMP)
d-ADP
d-ATP
DNA
RNA
d-GTP
d-GDP
GTP
GDP
d-CTP
TTP
TDP
THYMIDINE-P
d-UMP
d-CMP
d-CDP
CDP
3-Amino-Isobutyrate
B-Ureido Isobutyrate
Dihydro thymine
Thymine
B-Alanine
Carbamoyl B-alanine
Dihydro-uracil
Uracil
Cytosine
Carbamoyl
Dihydro
Orotate
Orotidine-P
Uridine-P
UDP
URIDINE-
CYTIDINE-

DRUG-METABOLITE SIMILARITIES

Chlorpromazine



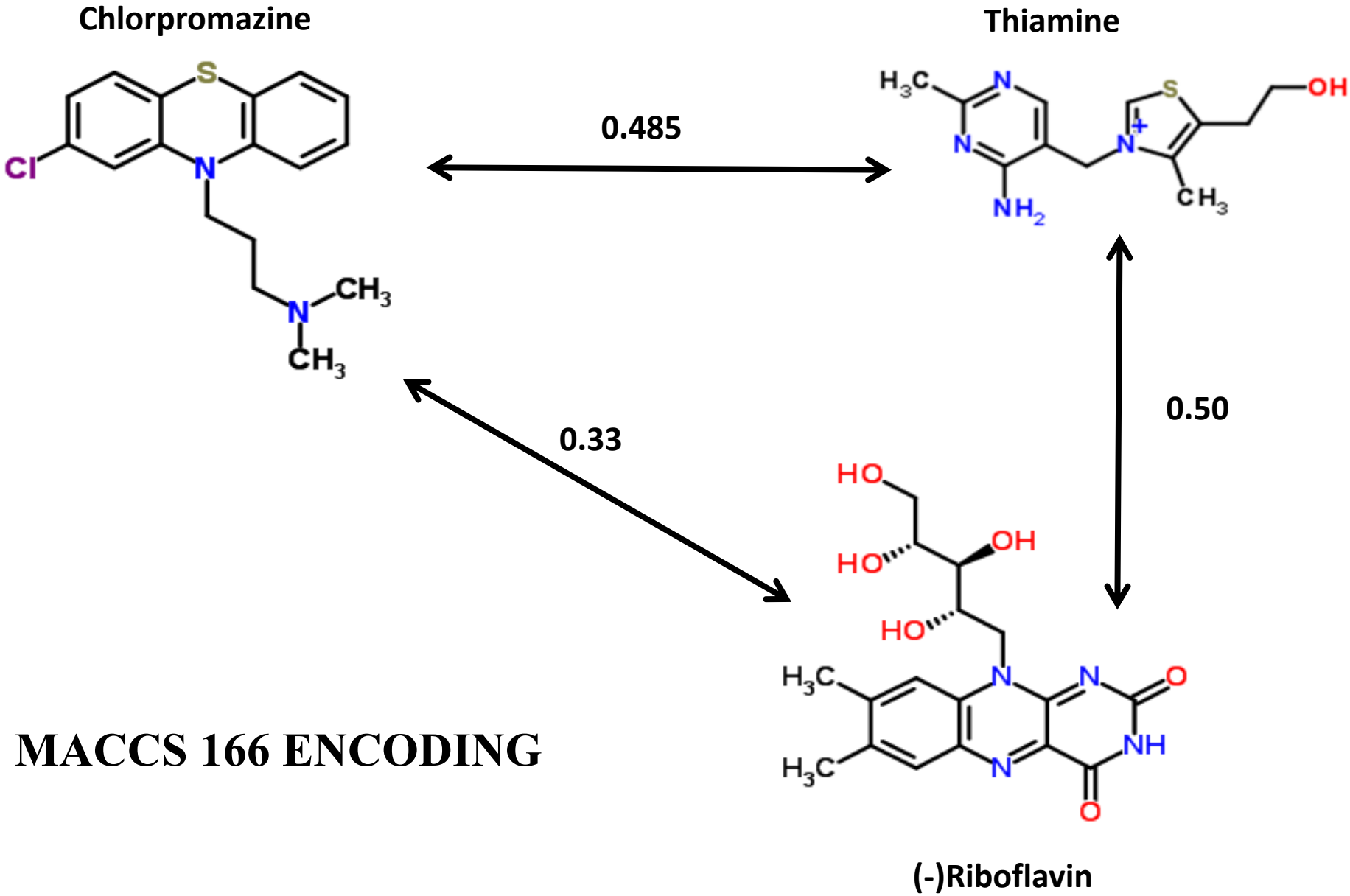
Thiamine



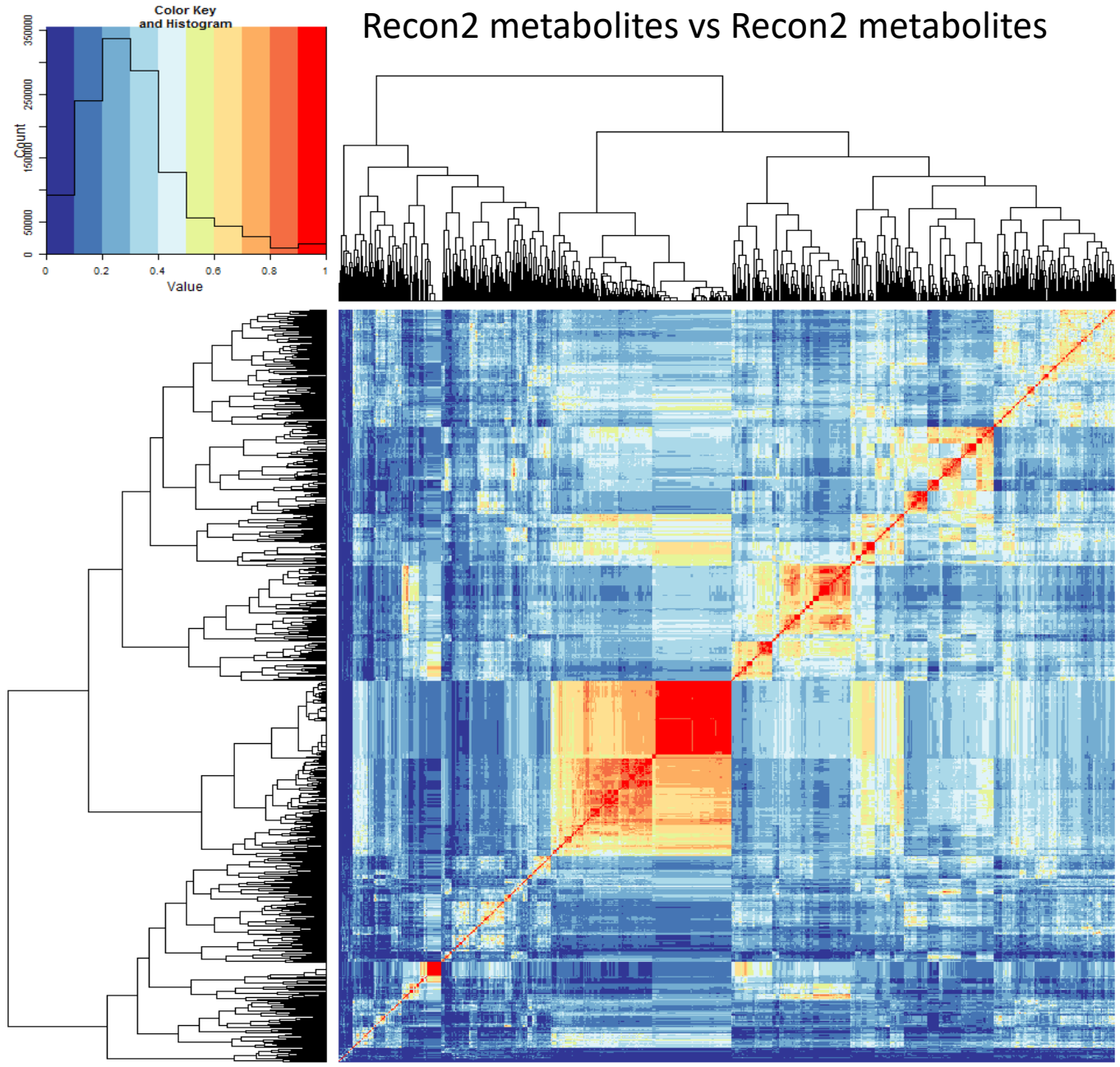
ENCODE AS A STRING OF 1s and 0s (VARIOUS ENCODINGS EXIST)

COMPARE STRINGS, COMMMONLY AS JACCARD/TANIMOTO DISTANCE OF SHARED BITS/ TOTAL BITS

DRUG-METABOLITE SIMILARITIES

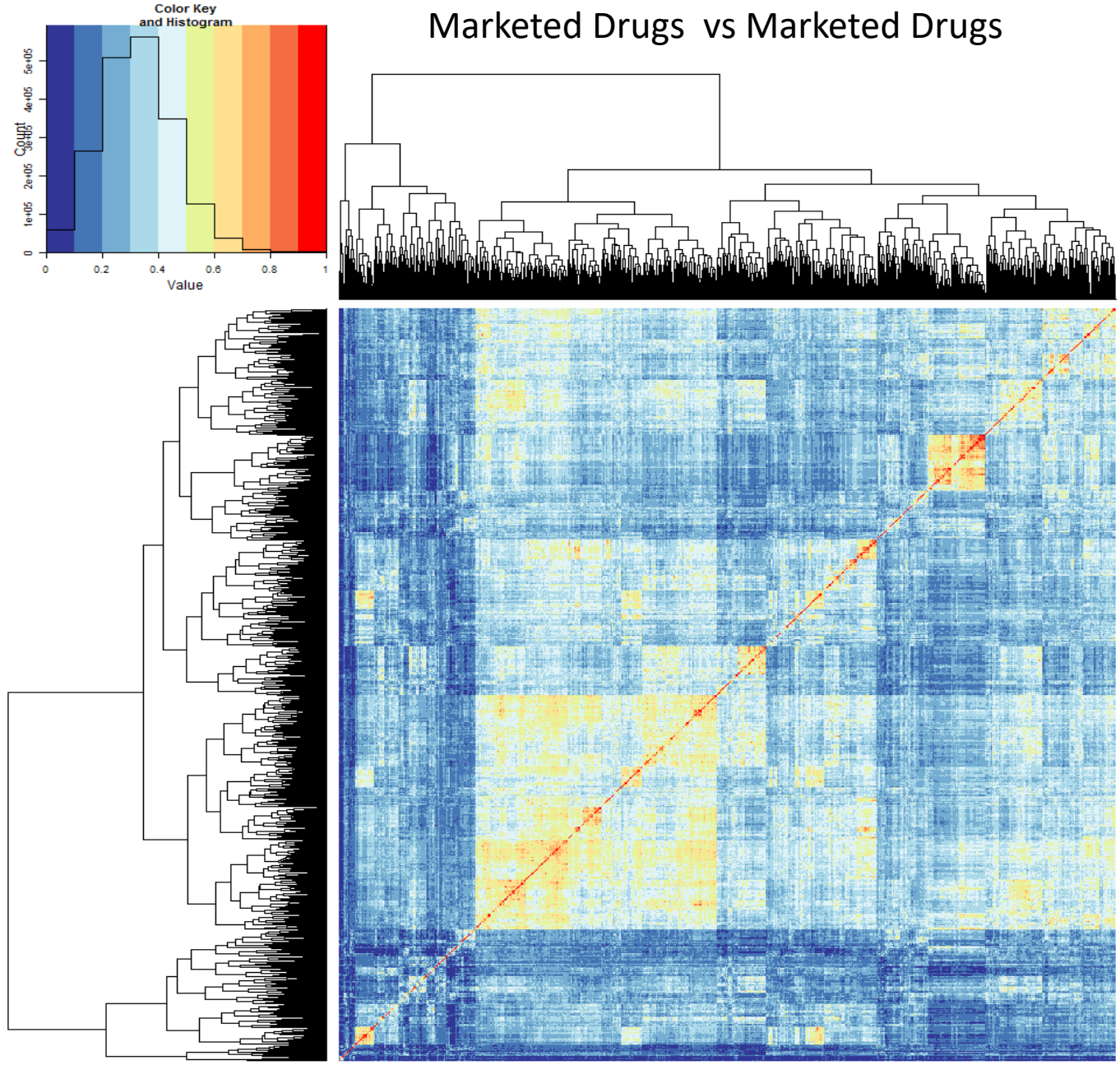


MACCS 166 ENCODING



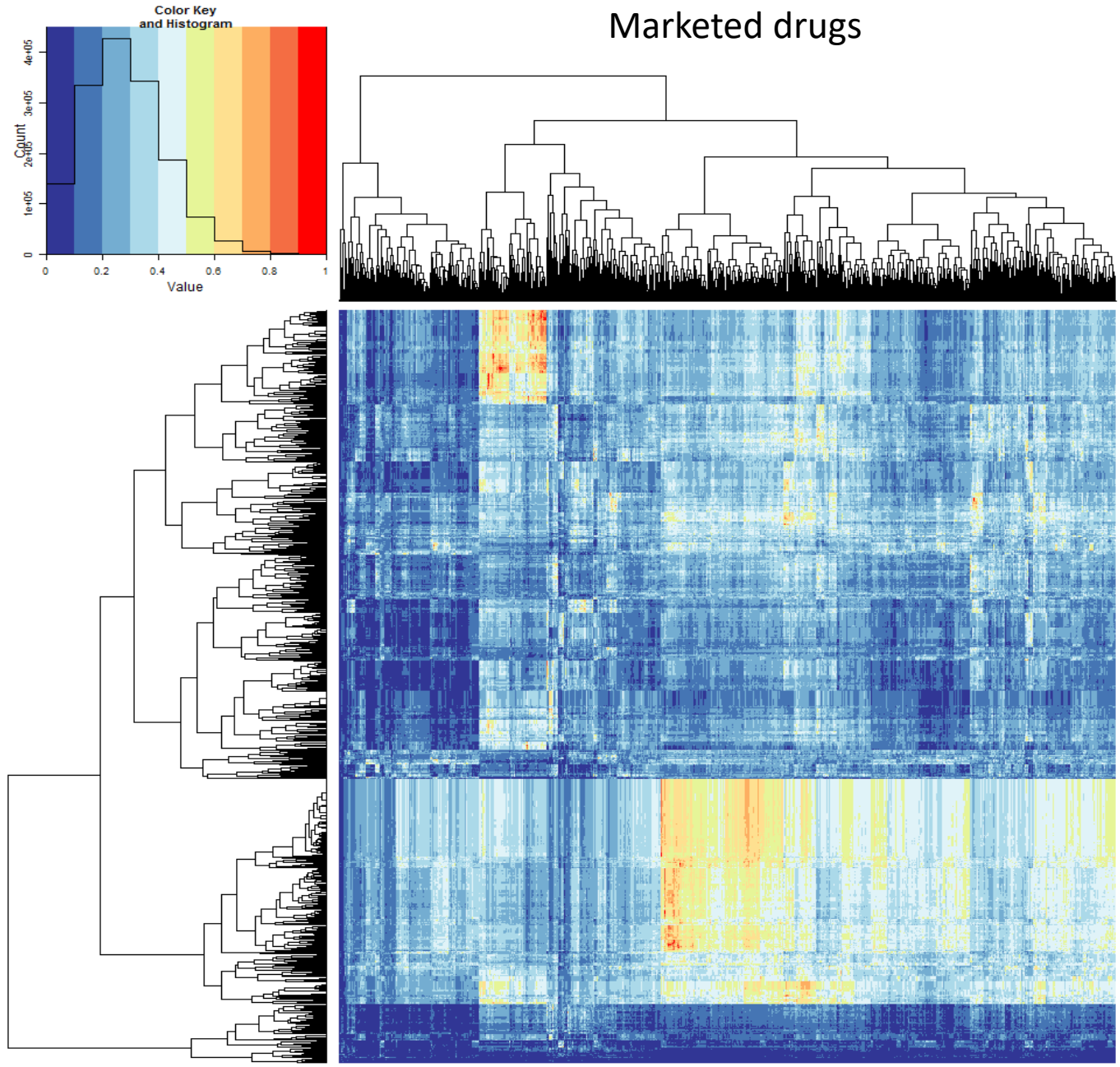
Recon2 metabolites vs Recon2 metabolites

A


Marketed Drugs vs Marketed Drugs
Color Key and Histogram
Count
Value
0
0.2
0.4
0.6
0.8
1
0
1e+05
2e+05
3e+05
4e+05
5e+05

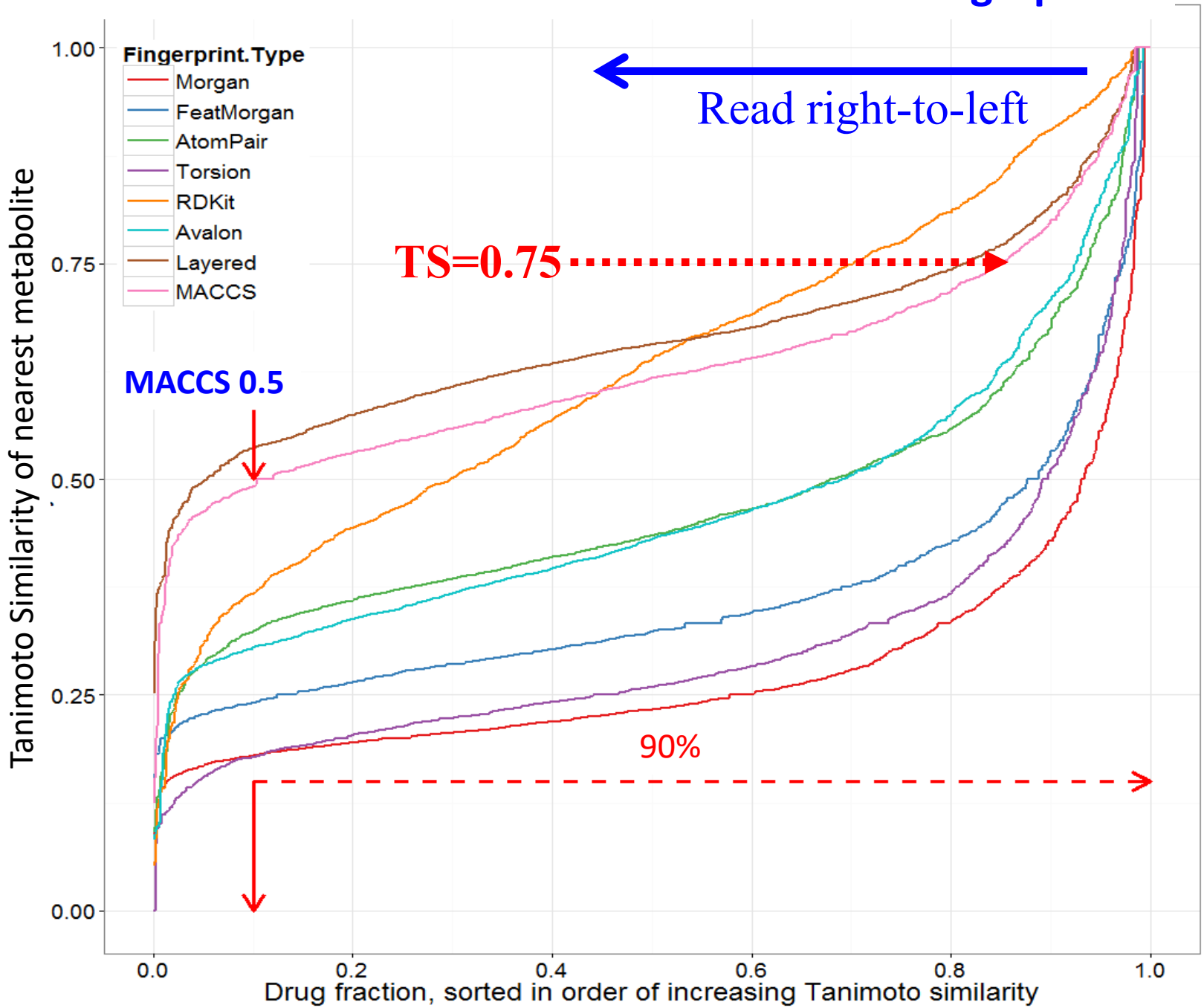
B

C


Color Key
and Histogram
Count
Value
Marketed drugs
Recon2 metabolites

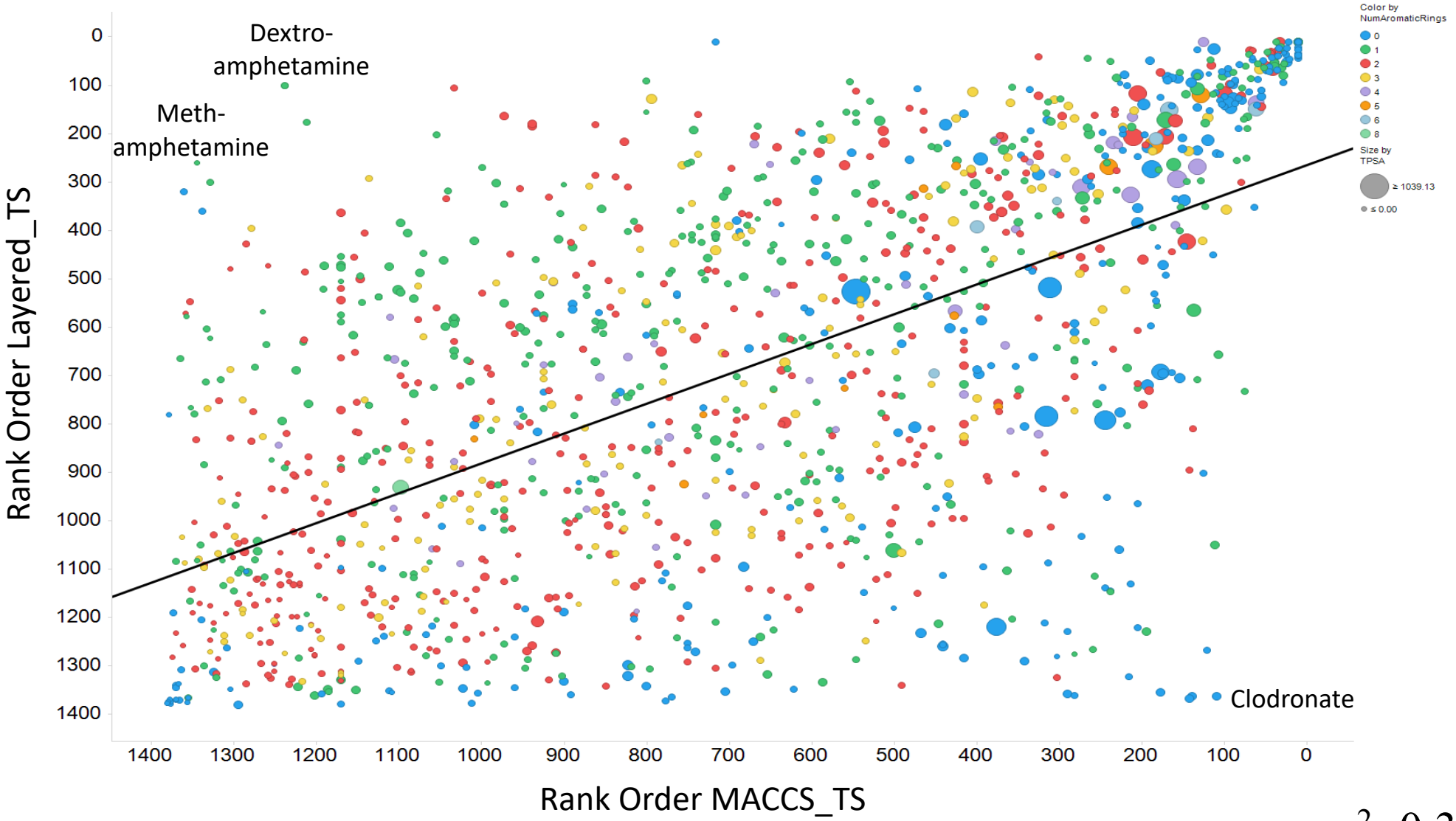
Cumulative Closest Tanimoto distance for different fingerprints

A



Rank Order of Layered_TS vs MACCS_TS

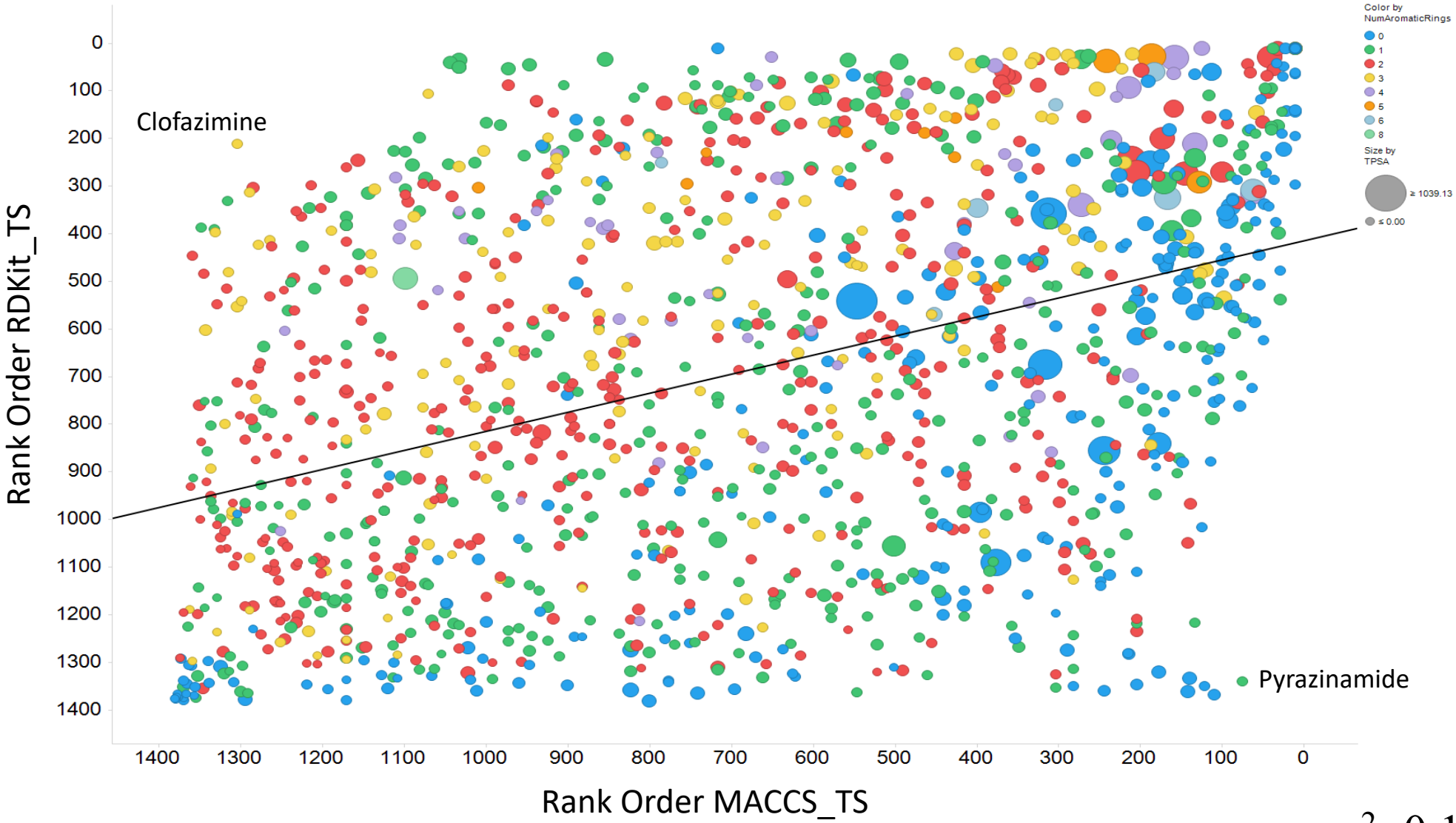
B



r^2=0.38

C

Rank Order of RDKit_TS vs MACCS_TS

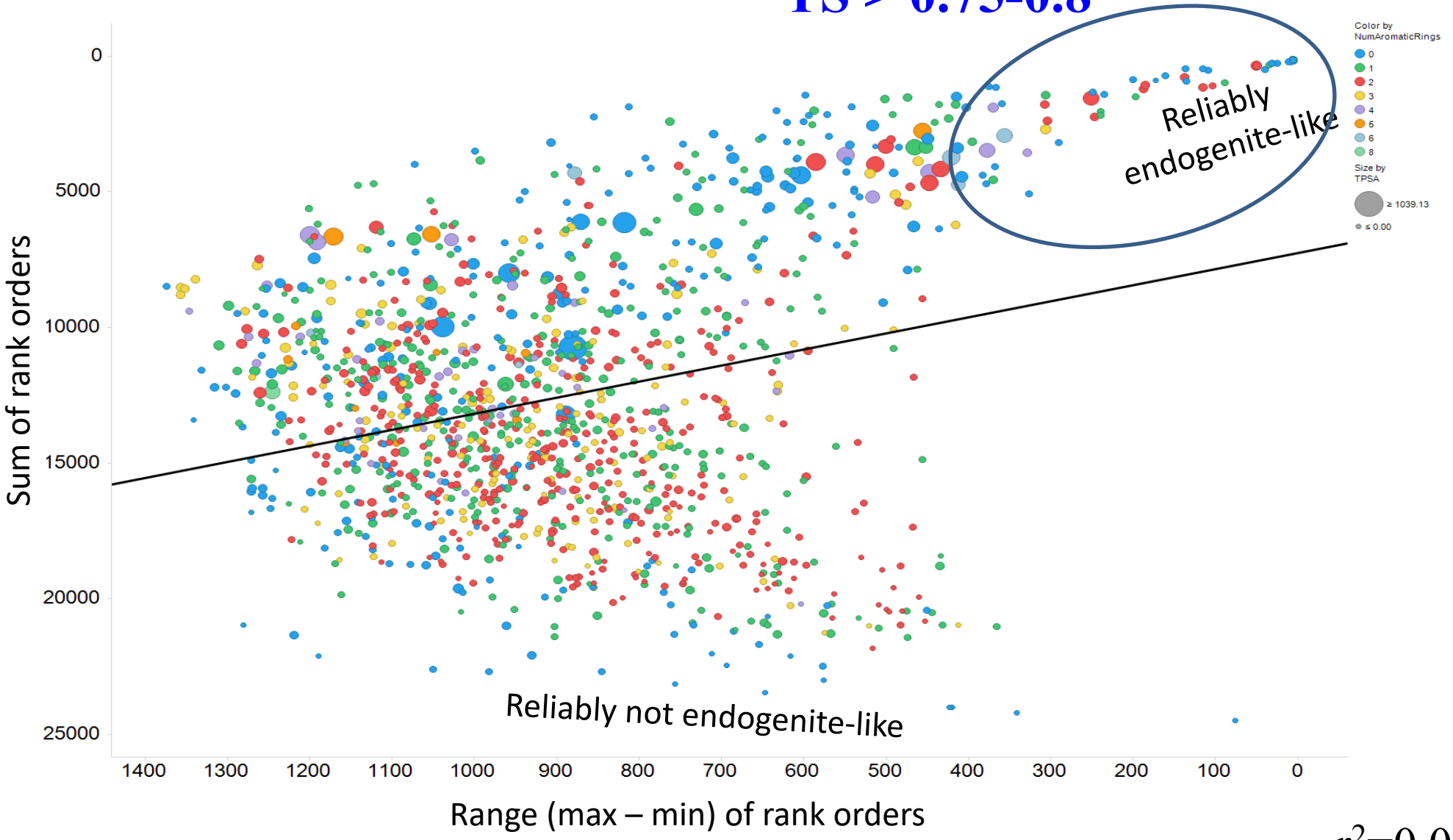


r^2=0.16

A

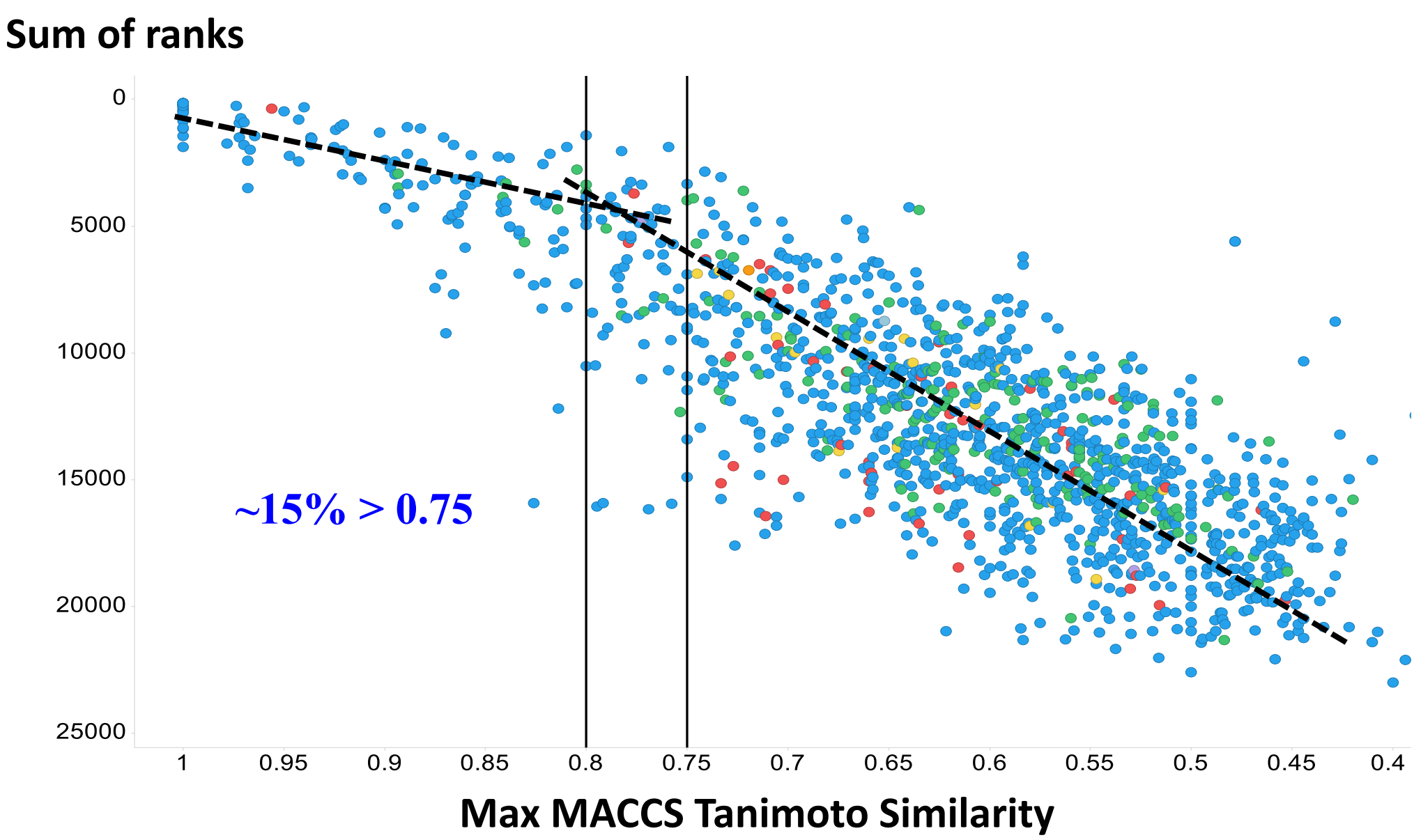
Sum of rank orders vs range

TS > 0.75-0.8



r^2=0.087

Sum of ranks vs MACCS max_TS implies 0.75-0.8 cutoff



PNAS 102, 5256-5261 (2005)

Discovery of the ergothioneine transporter

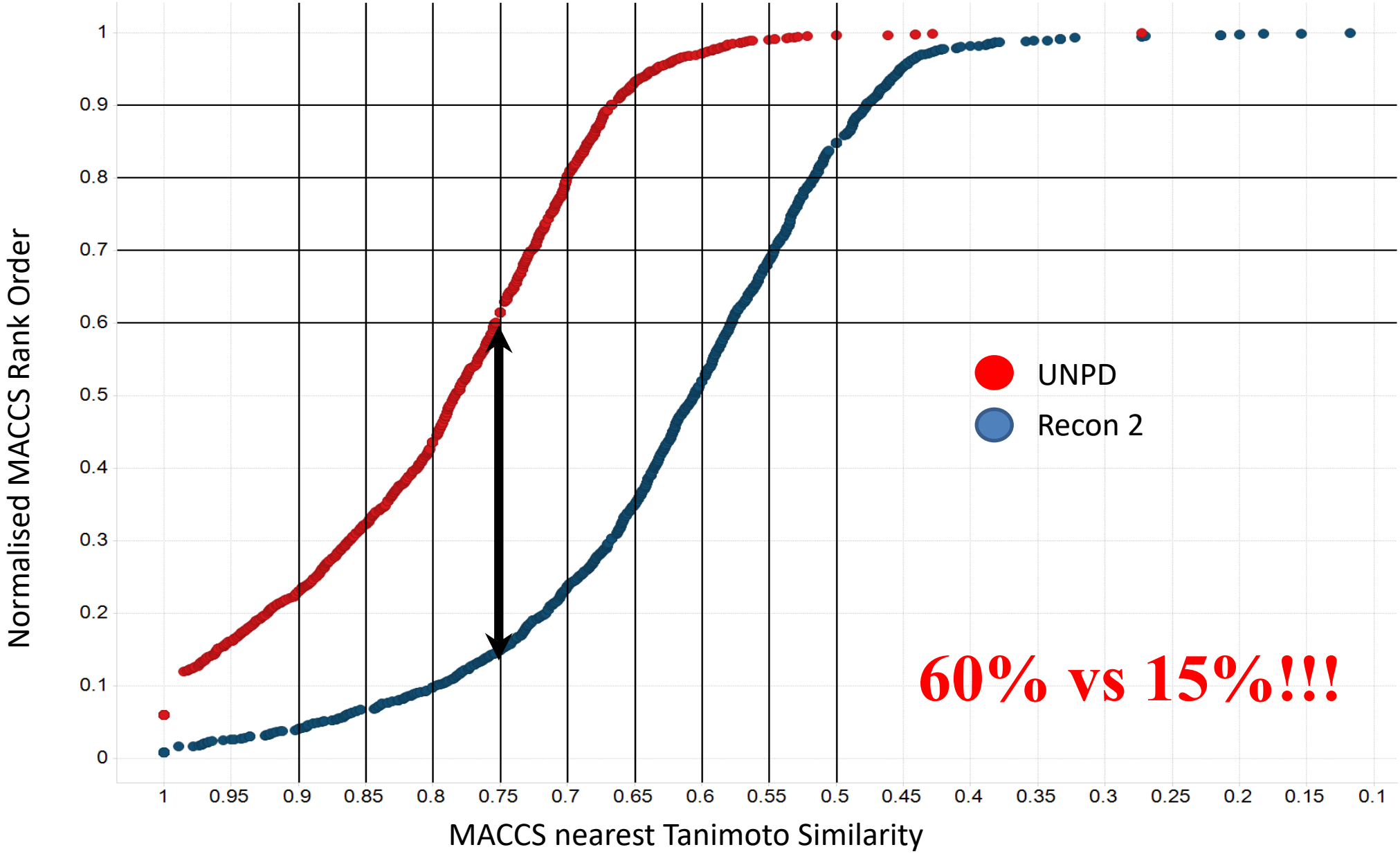
Dirk Gründemann*†‡, Stephanie Harlfinger*, Stefan Golz§, Andreas Geerts§, Andreas Lazar*, Reinhard Berkels*¶, Norma Jung‖, Andrea Rubbert‖, and Edgar Schömig*†

- **OCTN1/ SLC22A4**
- **Supposedly carnitine/ TEA+**
- **Untargeted metabolomics assay**
- **Best substrate ergothioneine ~100x faster**
- **Proline betaine also a good substrate**

- **Implies natural products could be good substrates**

A

Normalised MACCS rank order vs MACCS_TS full



But there is nothing magic about MACCS

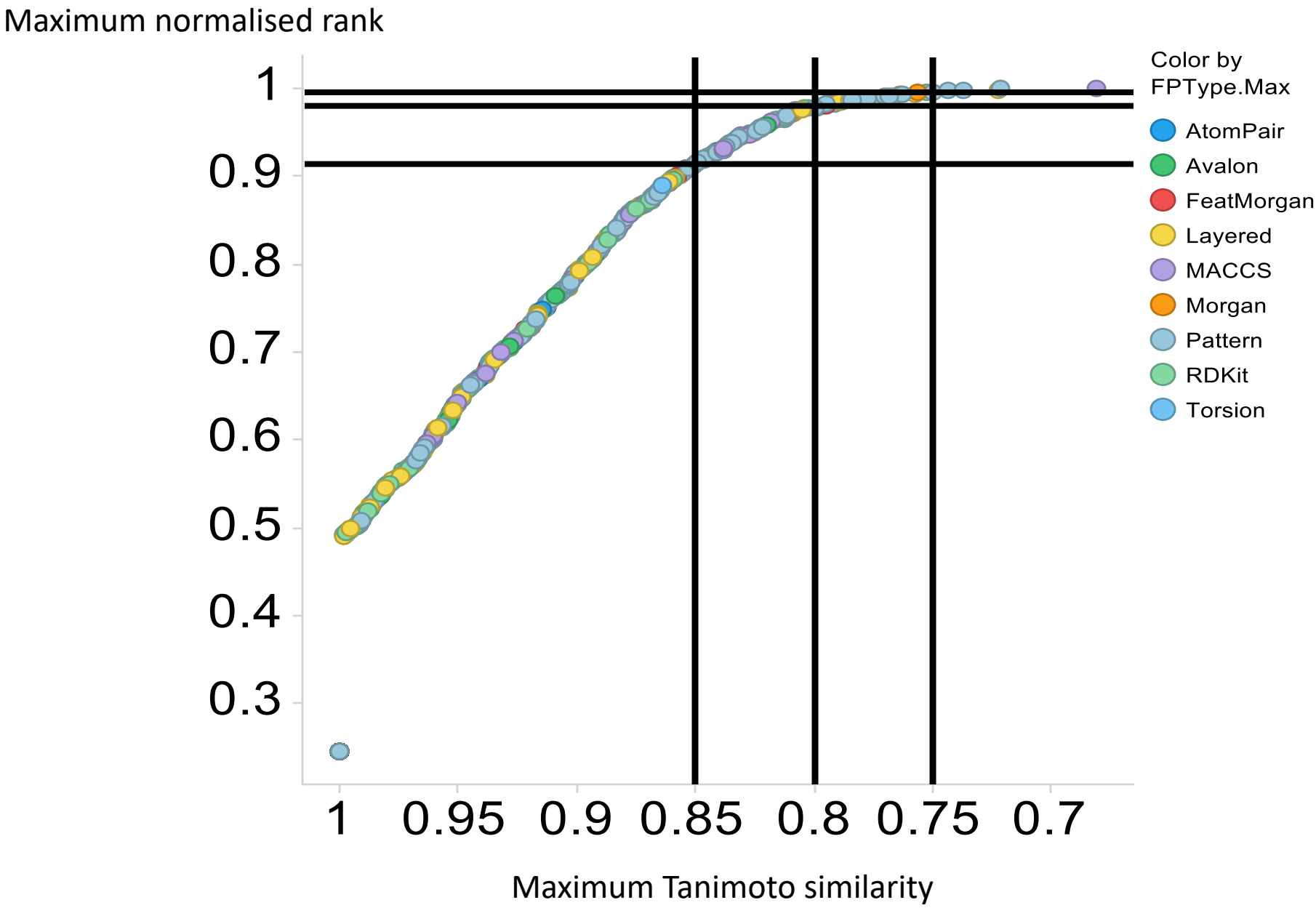
Why not just choose the best one for each molecule?

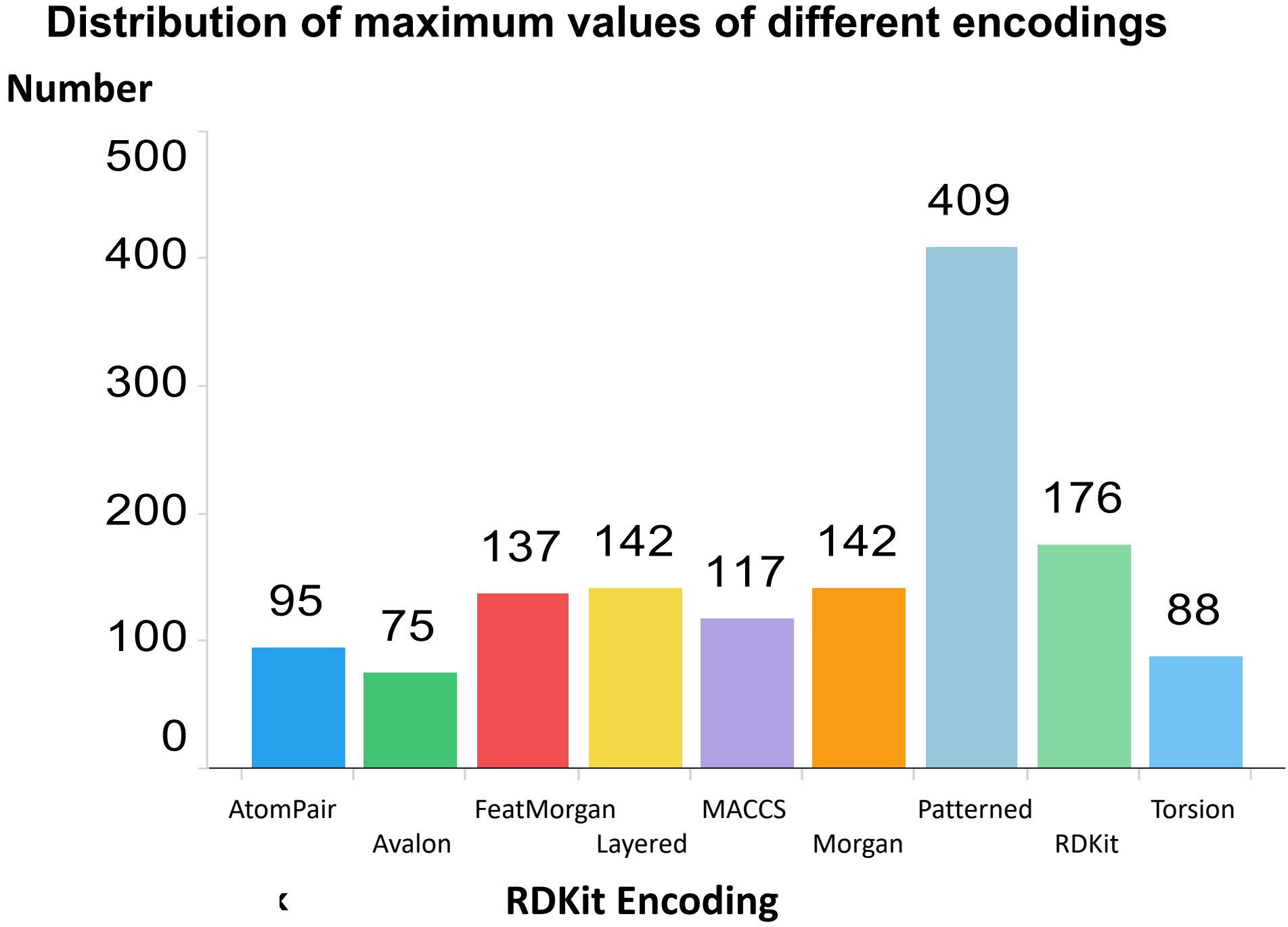
We call this the TYPICAL encoding

Take Your Pick Improved Cheminformatic Analytical Likeness

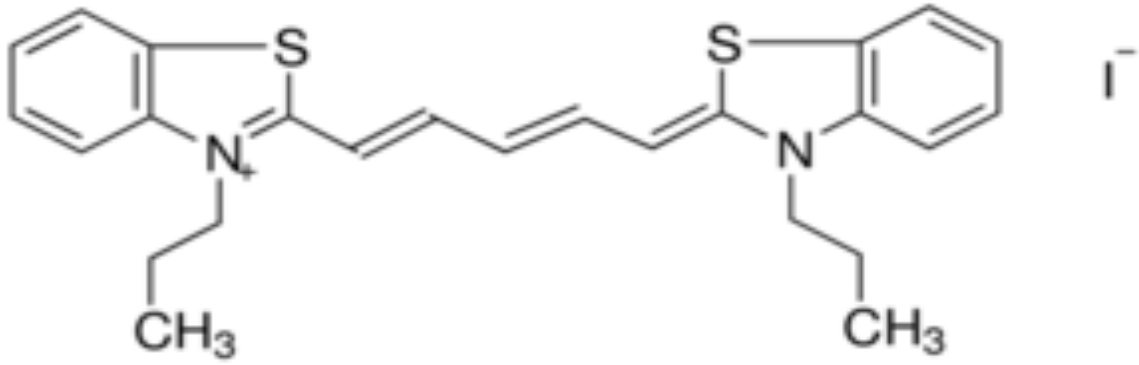
D

Maximum rankwith TYPICAL encoding, 196k NPs

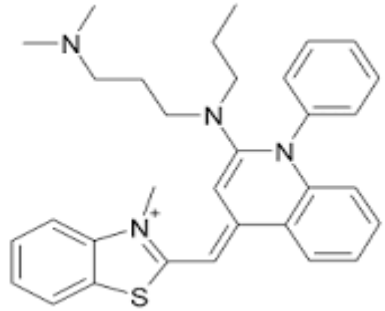



Distribution of maximum values of different encodings
Number
500
400
300
200
100
0
95
75
137
142
117
142
409
176
88
AtomPair
Avalon
FeatMorgan
Layered
MACCS
Morgan
Patterned
RDKit
Torsion
RDKit Encoding

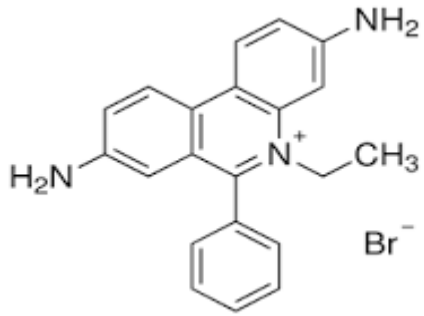
Some cationic dyes used in uptake studies



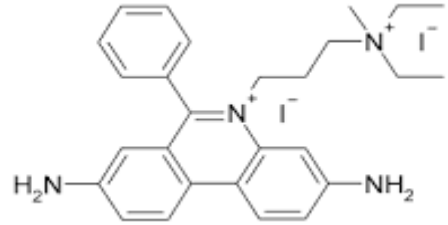
3,3'-Dipropylthiadicarbocyanine iodide ('carbocyanine' or diS-C3(5))
Excitation 640nm, Emission 675 ± 15nm



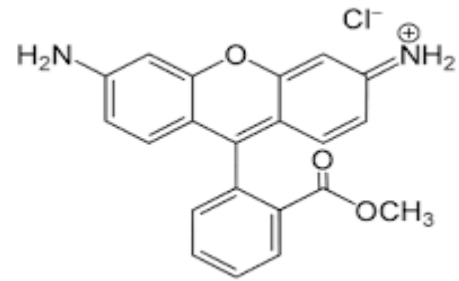
Sybr Green



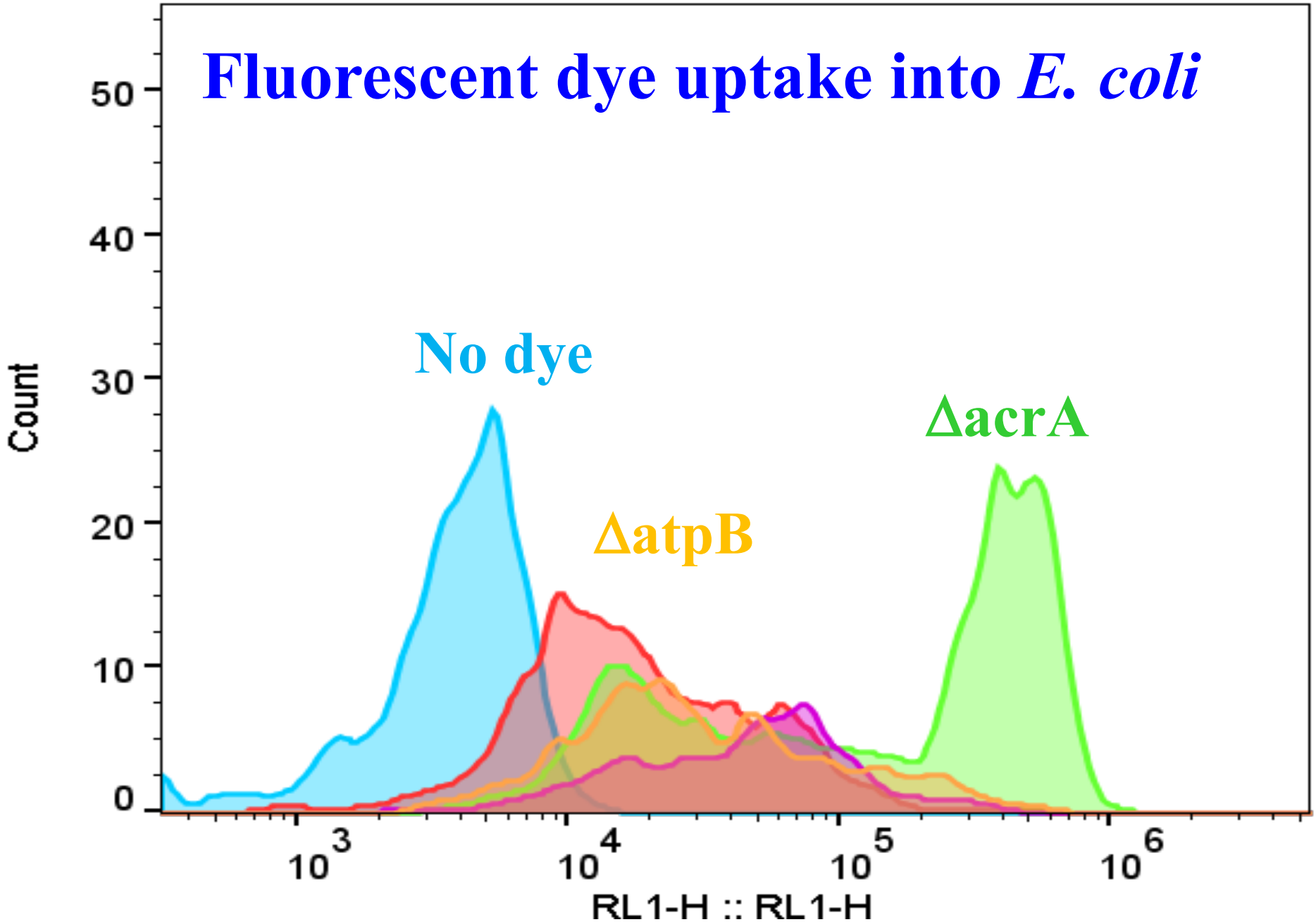
Ethidium bromide



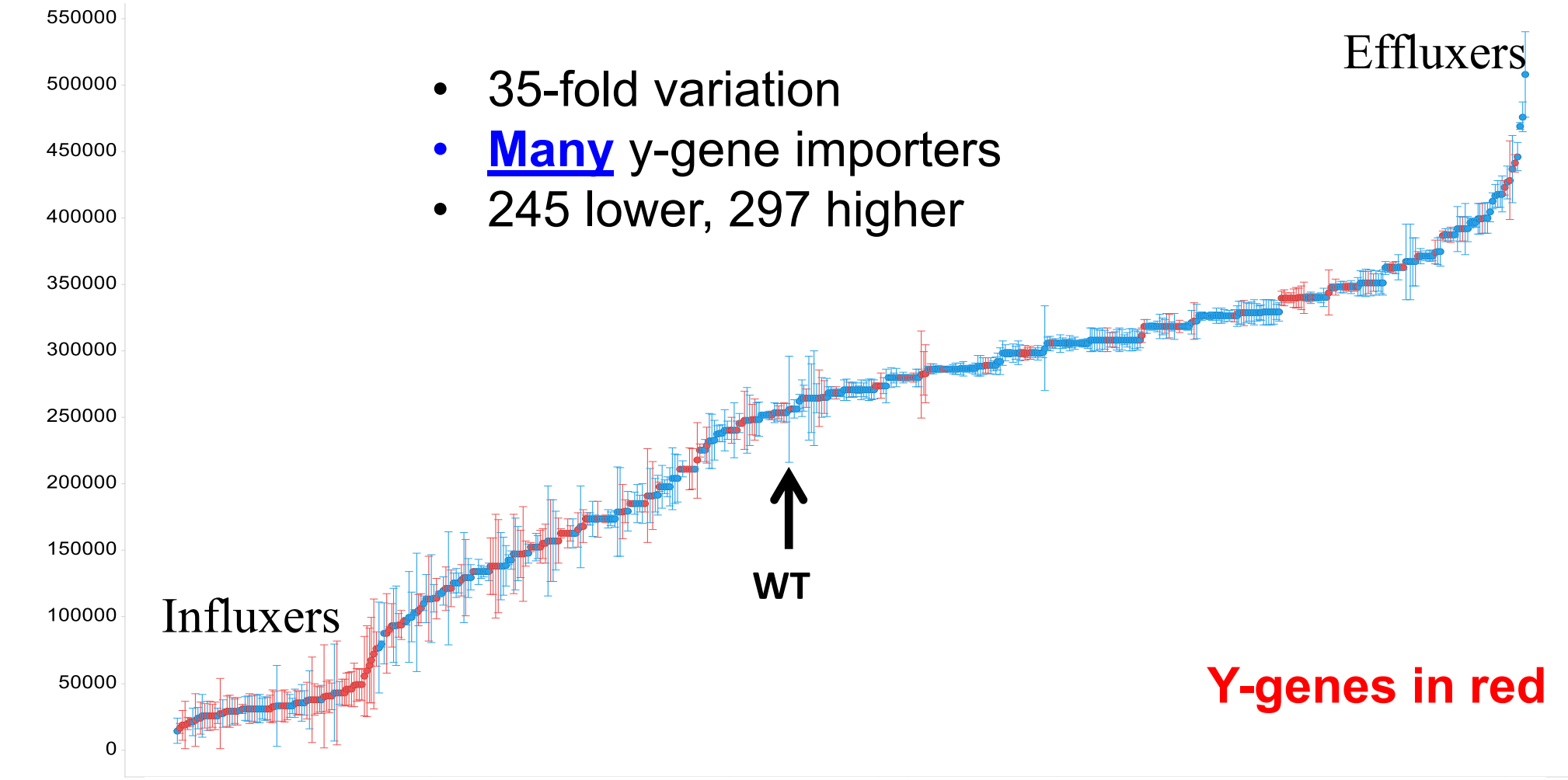
Propidium iodide



Rhodamine 123


Fluorescent dye uptake into E. coli
No dye
ΔatpB
ΔacrA
Count
50
40
30
20
10
0
10^3
10^4
10^5
10^6
RL1-H :: RL1-H

Fluorescent dye uptake in *E. coli* KOs



Knockout list ordered by uptake

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Conclusions

No transporters means no transport – and assessment of the 'real' (natural) substrates of xenobiotic transporters

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