Moving beyond Molecular Obesity and Potency as addictions in Drug Discovery

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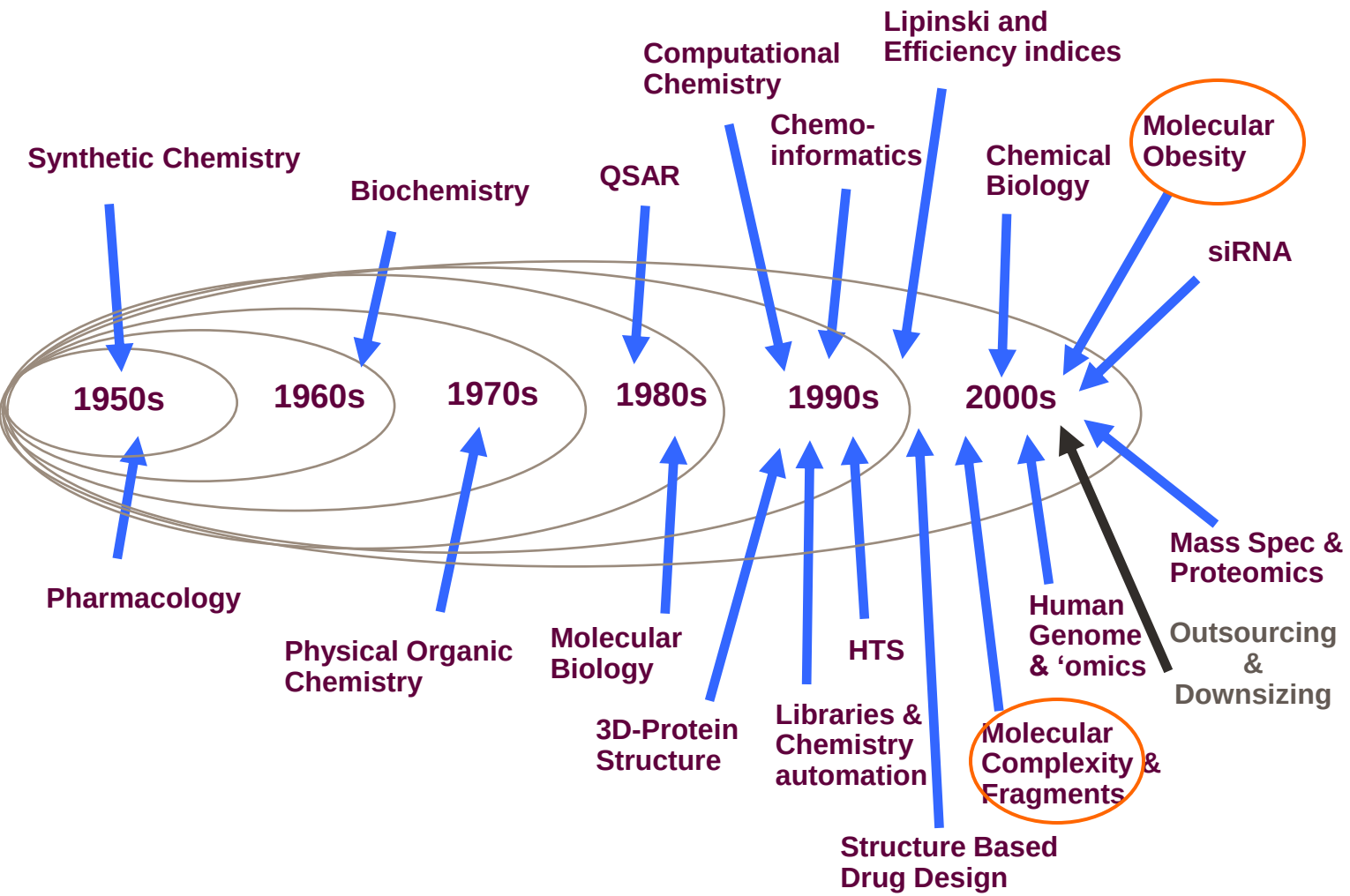
Outline

- Brief recap on what we have (re)learnt and embedded
  - Physchem props PFI, solubility, dose
- What's new in our thinking and experiments and what might just be useful in the future!
  - Cellular concentration and disposition
  - Imaging

Where have we come from & what sort of works for us?



The expanding "sciences" of Medicinal Chemistry and drug discovery

The curse of Molecular Obesity

- The tendency for drug discovery molecules to become too large and too lipophilic for their own good during lead optimisation through the quest for potency and specificity.
  - It presents a high risk to the future “health” of the compound as a drug candidate.
- As with medical obesity, which is measured by Body Mass Index BMI, we now make use of indices such as Ligand Efficiency Index LE and Lipophilic Ligand Efficiency Index LLE to help identify and control the problem.
- But why do we make obese molecules?

Molecular obesity, potency and other addictions in drug discovery, Hann, M. M.. MedChemComm (2011), 2(5), 349-355.

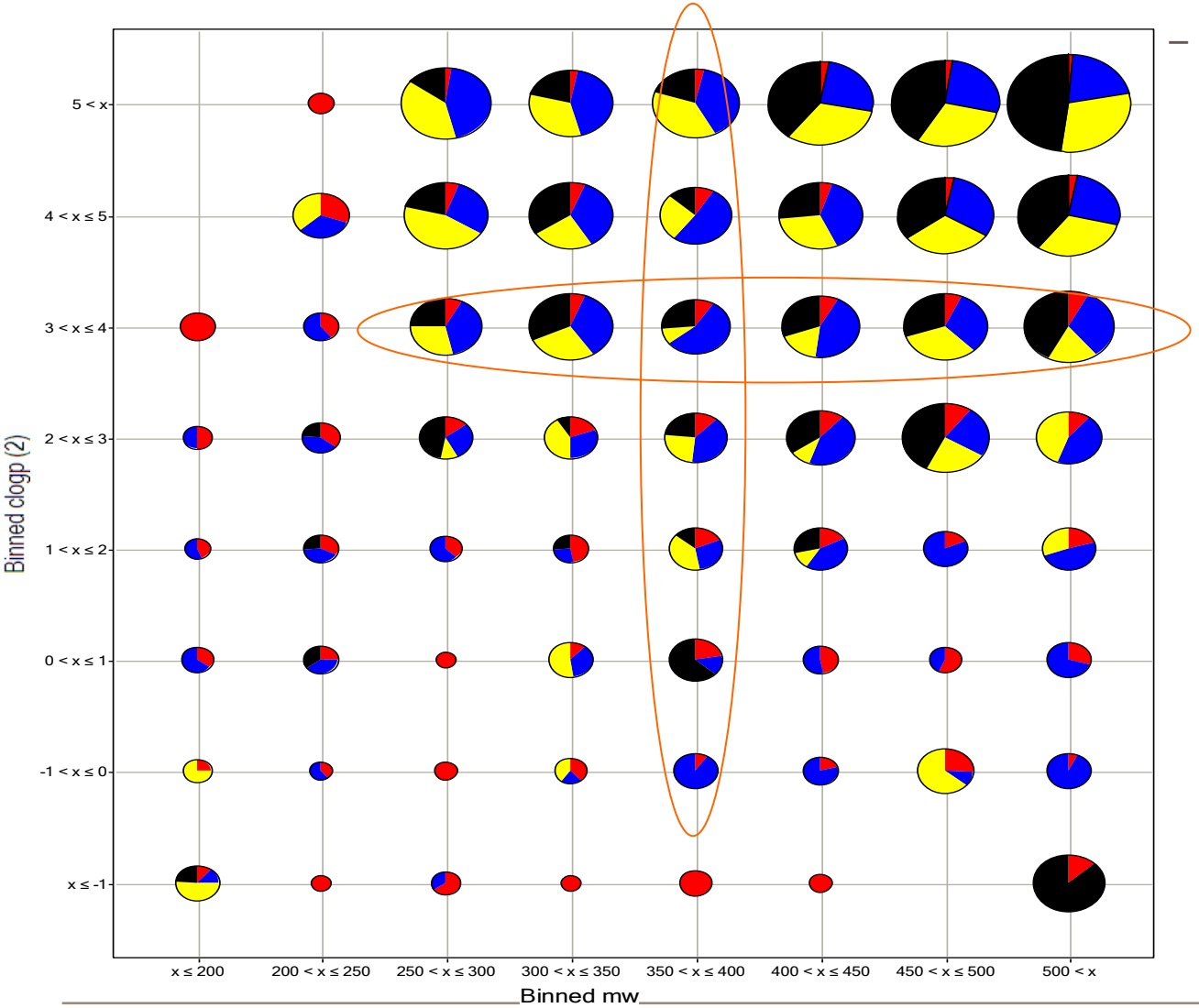
The link of potency and molecular obesity - cont'd?

Typical order of events in a drug discovery screening cascade

1. Biochemical assay
2. Cellular assay
3. *in vivo* assay

- We often start with isolated protein in a biochemical assay with none of the environment of more phenotypic assays to help balance the physicochemical properties.
- We look for early signs of cellular potency in our screening cascades - this needs both some intrinsic potency and cellular penetration
  - Both of these are very easily driven by increasing logP.
  - Once we get cellular activity the damage may already be done if we do not revisit our molecules to look at how we got there.

Is MW or logP the source of promiscuity?



- Graph showing series of pie charts in different cLogP and MW bins for a set of approximately 2500 compounds tested in more than 490 assays. The size of each pie chart represents the average number of hits for compounds in that pie, where a hit is defined as a pXC50 value of 5 or higher. The colours indicate the proportion of compounds within each pie having particular numbers of hits (red: <5; blue: 5-15; yellow: 15-25; black: >25), where a hit is defined as activity greater than 10mM in any of the ~490 assays

Leach, A.R and Hann, M.M.. **Molecular complexity and fragment-based drug discovery: ten years on.** Current Opinion in Chemical Biology 2011, 15:489–496

Property Forecast Index PFI – a useful overall guide to where to look for developable compounds

Drug Discovery Today • Volume 16, Numbers 17/18 • September 2011 REVIEWS

PFI = mChromLogD7.4 + number of Aromatic rings

TABLE 2

Percentages of compounds achieving defined target values in the various developability assays categorised by PFI or iPFI bins[a]

	PFI = mChrom log $D_{pH7.4}$ + #Ar								
Assay / target value	<3	3-4	4-5	5-6	6-7	7- 8	8-9	9-10	>10
Solubility >200 μM	89	83	72	58	33	13	5	3	2
%HSA <95%	88	80	74	64	50	30	17	8	4
2C9 pIC_{50} <5	97	90	83	68	48	32	23	22	38
2C19 pIC_{50} <5	97	95	91	82	67	52	42	42	56
3A4 pIC_{50} <5	92	83	80	75	67	60	58	61	66
Cl_{int} <3 ml/min/kg	79	76	68	61	54	42	41	39	52
Papp >200 nm/s	20	30	46	65	74	77	65	50	33
	iPFI = mChrom log P + #Ar								
hERG pIC_{50} <5 (+1 charge)	86	93	88	70	54	36	29		
Promiscuity <5 hits with pIC_{50} >5	85	78	74	65	49	30	20		

[a] Colouring refers to the % chance of achieving benchmark value in that PFI bin: green, ≥67%; yellow, 34–67%; and red, <33%.

Sweet spot for permeability is in conflict with other desirable properties!

Ask yourself where your hits and lead optimisation compounds fit on this plot?!!

POTENCY

Getting physical in drug discovery II: the impact of chromatographic hydrophobicity measurements and aromaticity, RJ Young, DVS. Green, CN. Luscombe, AP Hill, Drug Discovery Today, 16 (17/18), 2011, 822-830

Cross company analysis of current status of physchem props

NATURE REVIEWS | DRUG DISCOVERY VOLUME 14 | JULY 2015 | 475

An analysis of the attrition of drug candidates from four major pharmaceutical companies

Michael J. Waring[1], John Arrowsmith[2], Andrew R. Leach[3], Paul D. Leeson[3,4], Sam Mandrell[2], Robert M. Owen[5], Garry Pairaudeau[1], William D. Pennie[6,7], Stephen D. Pickett[3], Jibo Wang[8], Owen Wallace[8,9] and Alex Weir[2]

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[5]Pfizer, Cambridge, Cambridgeshire CB21 6GS, UK.
[6]Pfizer, Groton, Connecticut 06340, USA.
[7]Present address: Takeda Pharmaceuticals, Cambridge, Massachusetts 02139, USA.
[8]Eli Lilly, Indianapolis, Indiana 46285, USA.
[9]Present address: Novartis Institutes for Biomedical Research, Cambridge, Massachusetts 02139, USA.
Correspondence to M.J.W.
e-mail: mike.j.waring@astrazeneca.com

Abstract | The pharmaceutical industry remains under huge pressure to address the high attrition rates in drug development. Attempts to reduce the number of efficacy- and safety-related failures by analysing possible links to the physicochemical properties of small-molecule drug candidates have been inconclusive because of the limited size of data sets from individual companies. Here, we describe the compilation and analysis of combined data on the attrition of drug candidates from AstraZeneca, Eli Lilly and Company, GlaxoSmithKline and Pfizer. The analysis reaffirms that control of physicochemical properties during compound optimization is beneficial in identifying compounds of candidate drug quality and indicates for the first time a link between the physicochemical properties of compounds and clinical failure due to safety issues. The results also suggest that further control of physicochemical properties is unlikely to have a significant effect on attrition rates and that additional work is required to address safety-related failures. Further cross-company collaborations will be crucial to future progress in this area.

Reducing the high attrition rates in drug development on promiscuity[5,8] and *in vivo* toxicological outcomes[7,10].

Cross company analysis of current status of physchem props

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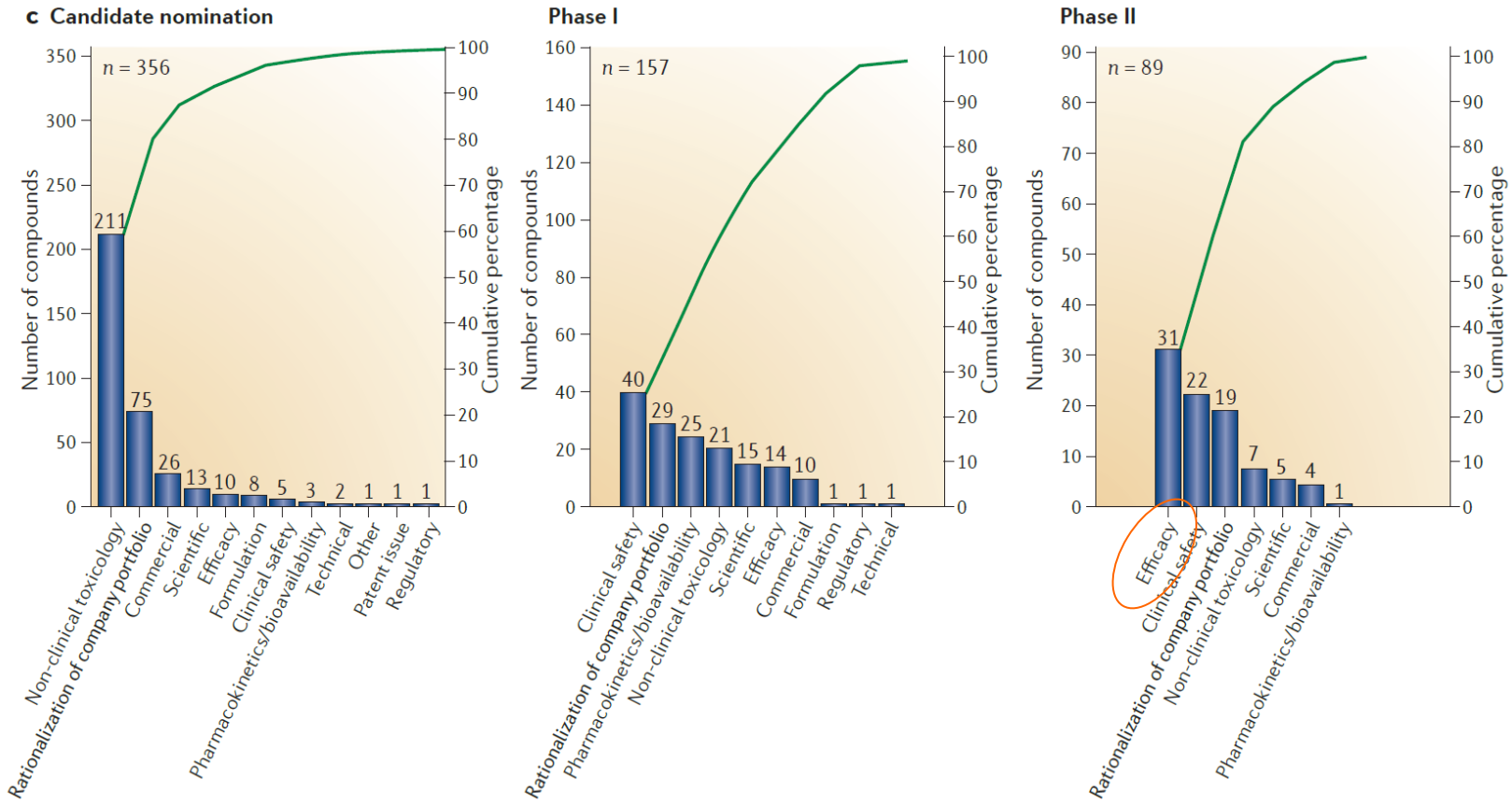

Figure 2 | **Reasons for failure.** **a** | Primary cause of failure for terminated compounds. **b** | Differences in the cause of failure for the first half (2000–2005) and second half (2006–2010) of the decade. **c** | Differences in the cause of failure in preclinical, Phase I and Phase II development.

Cross company analysis of current status of physchem props

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candidates (mean calculated logP = 3.2) towards drugs (mean calculated logP = 2.8), it is questionable whether this would significantly reduce development attrition, based on our findings.

Conclusions

The data set collected and analysed in this study is the largest compiled so far for the purposes of linking attrition to simple molecular properties. We continue to believe that control of physicochemical properties during compound optimization is highly beneficial in identifying compounds of candidate drug quality. This study extends the analysis to their subsequent performance in development and assesses, for the first time, a link to various reasons for failure in development. The most notable finding from this data set is the difference in lipophilicity between compounds failing owing to clinical safety in Phase I and those successfully progressing to Phase II.

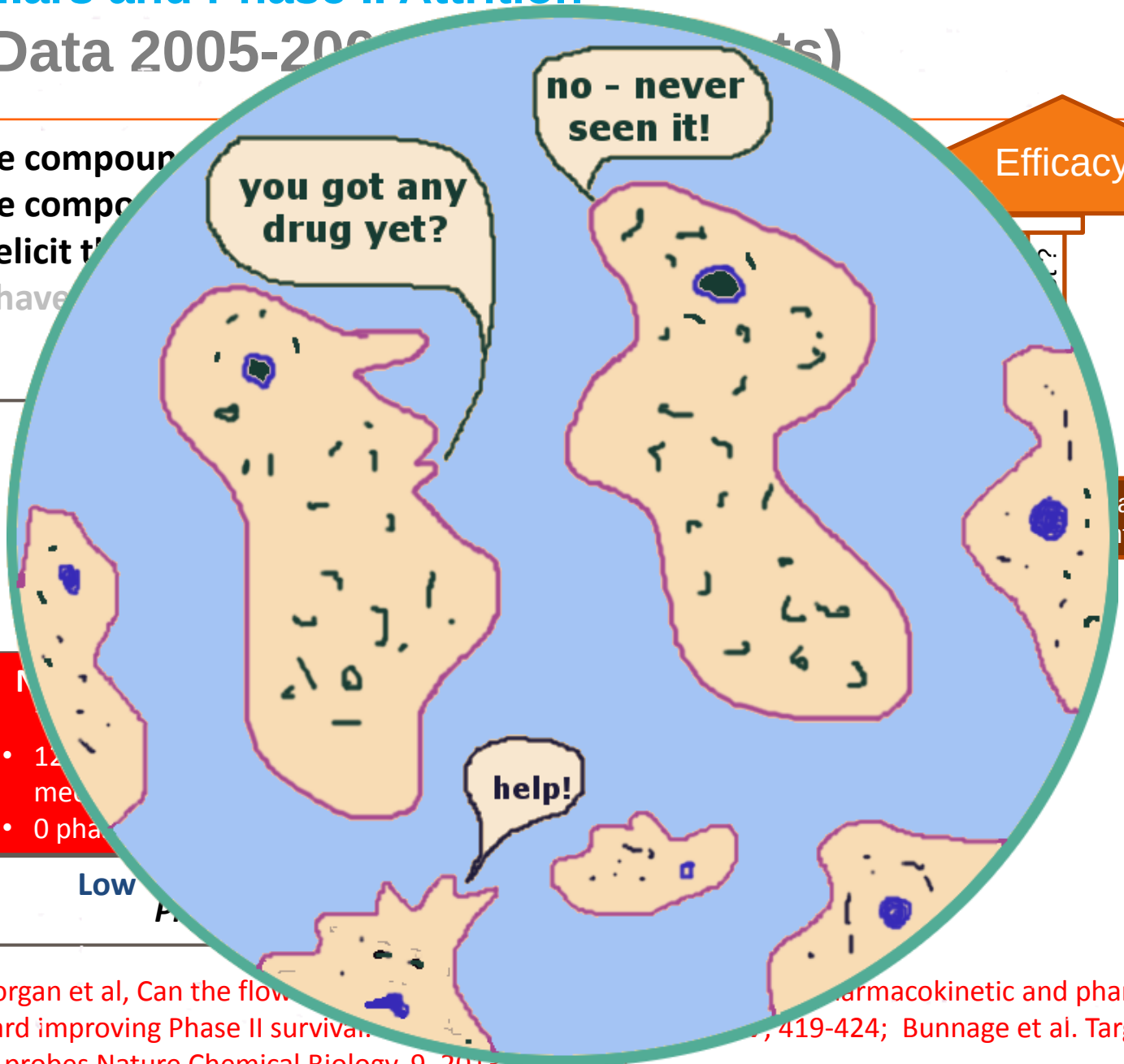
It is clear, however, that fully understanding and addressing the multiple causes of attrition in clinical development will require new thinking and fresh insight. This will be a significant challenge owing to the large number of potential causes of attrition and the complexities of fully understanding these causes at a mechanistic level. We can only hope to address such issues by concerted efforts across the pharmaceutical industry and beyond. That four of the largest pharmaceutical companies were able to work together to address this problem is perhaps the most significant outcome of this study, demonstrating that with an appropriate framework it is possible to tackle the key challenge of attrition by working together while still protecting the intellectual property and commercial interests of the individual organizations. We hope that this study will act as a springboard for future collaborations of a similar nature.

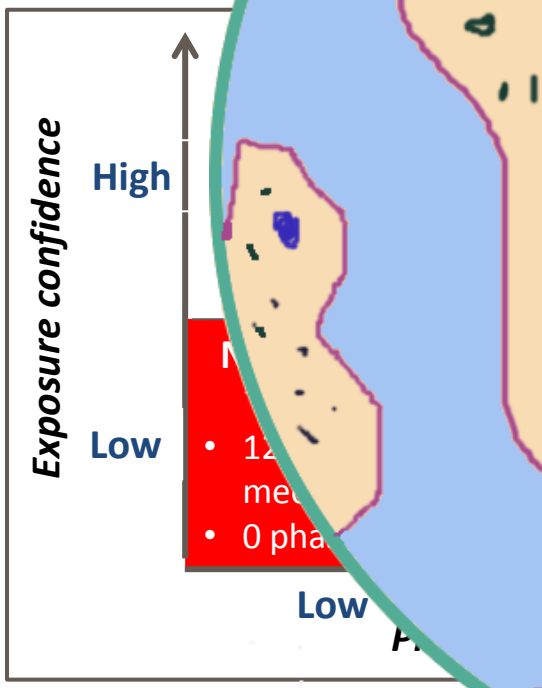
Figure 2 | **Reasons for failure.** **a** | Primary cause of failure for terminated compounds. **b** | Differences in the cause of failure for the first half (2000–2005) and second half (2006–2010) of the decade. **c** | Differences in the cause of failure in preclinical, Phase I and Phase II development.

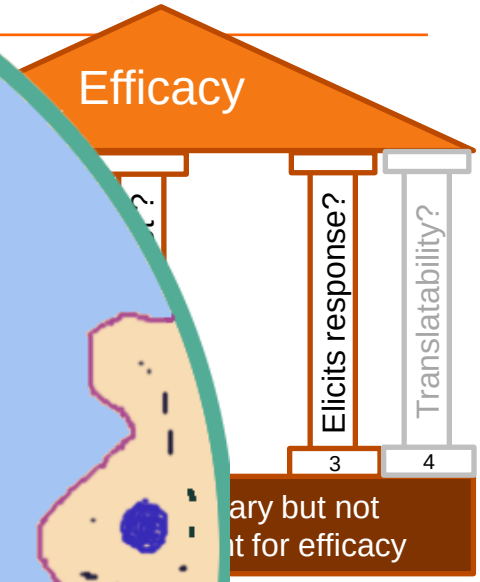
Three pillars and Phase II Attrition

Pfizer Data 2005-20

1) Does the compoun
2) Does the compo
3) Does it elicit t
4) Does it have



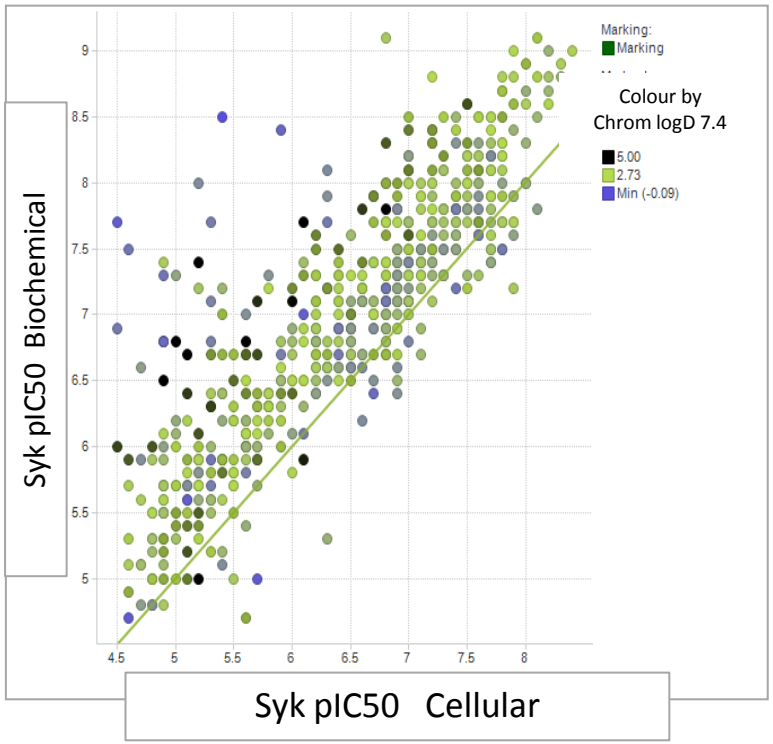




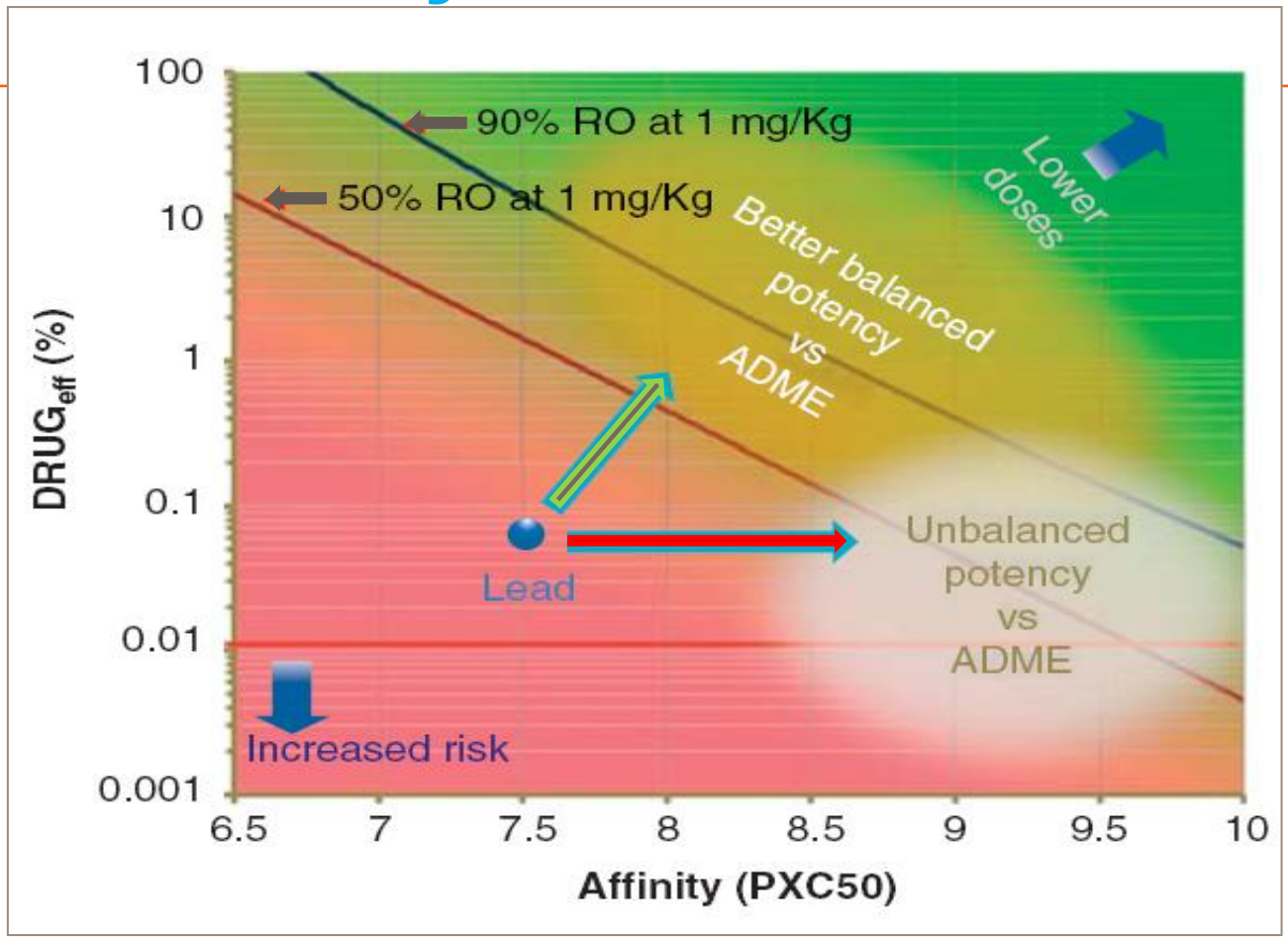
3/4 Pillars: Morgan et al, Can the flow … armacokinetic and pharmacological principles toward improving Phase II survival … 419-424; Bunnage et al. Target validation using chemical probes Nature Chemical Biology, 9, 2013, 195-199

The challenge of Pillar 1. *Does it get there?*

- Differences between compound activities in biochem and cellular assays often challenging to understand
  - leads to much hand waving and distress, particularly by medchemists!
- In lead optimisation indirect estimates are made based on lipophilicity and artificial membrane permeability measurements.
- An increasing percentage of targets are intracellular
- We seek medicines with high **drug efficiency** => low dosage.
  - This requires a balance of binding affinity (ie potency) and access!!



Drug Efficiency – it's the dose stupid!



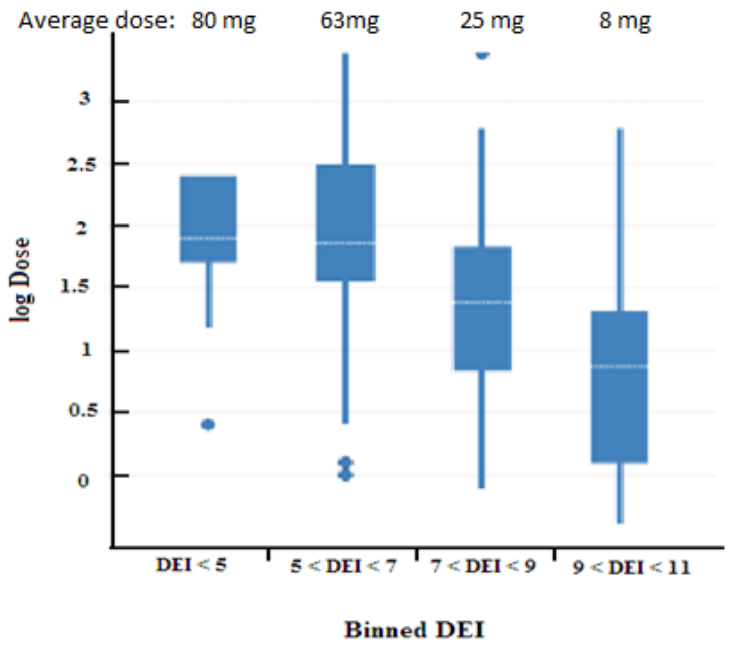
Drug Efficiency Index - a strategy towards low therapeutic dose

Drug Efficiency Index = Log[Biophase Concentration X 100/Dose]+ pXC50

- *Drug efficiency: a new concept to guide lead optimization programs towards the selection of better clinical candidates; S Braggio, D Montanari, T Rossi & E. RattI. Expert Opinion on Drug Discovery 2010, 5(7), 609-618*
- *Application of drug efficiency index in drug discovery: a strategy towards low therapeutic dose. Montanari, Dino; Chiarparin, Elisabetta; Gleeson, Matthew Paul; Braggio, Simone; Longhi, Raffaele; Valko, Klara; Rossi, Tino. Expert Opinion on Drug Discovery, Volume 6, Number 9, September 2011 , pp. 913-920(8)*

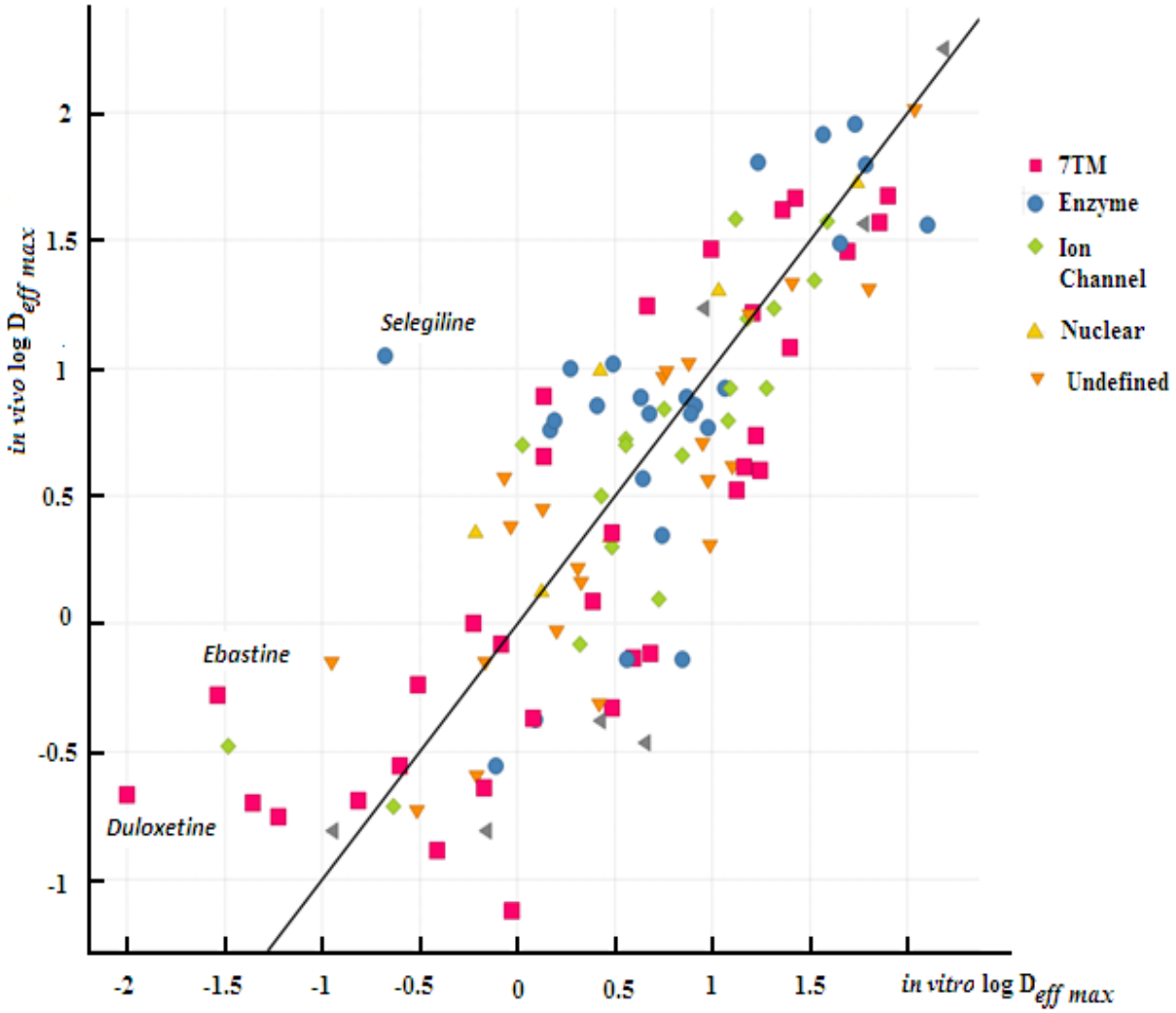
Clinical dose (mg) of marketed drugs is related to Drug efficiency and potency (DEI)

$$Dose = \frac{Potency}{Drug_{eff}} * 100$$



K. Valko, E. Chiarparin, S. Nunhuck, D. Montanari, In vitro measurement of drug efficiency index to aid lead optimization, J. Pharm. Sci. 101(2012) 4155-4169

In vivo drug efficiency shows good correlation with in vitro HPLC Drug eff max



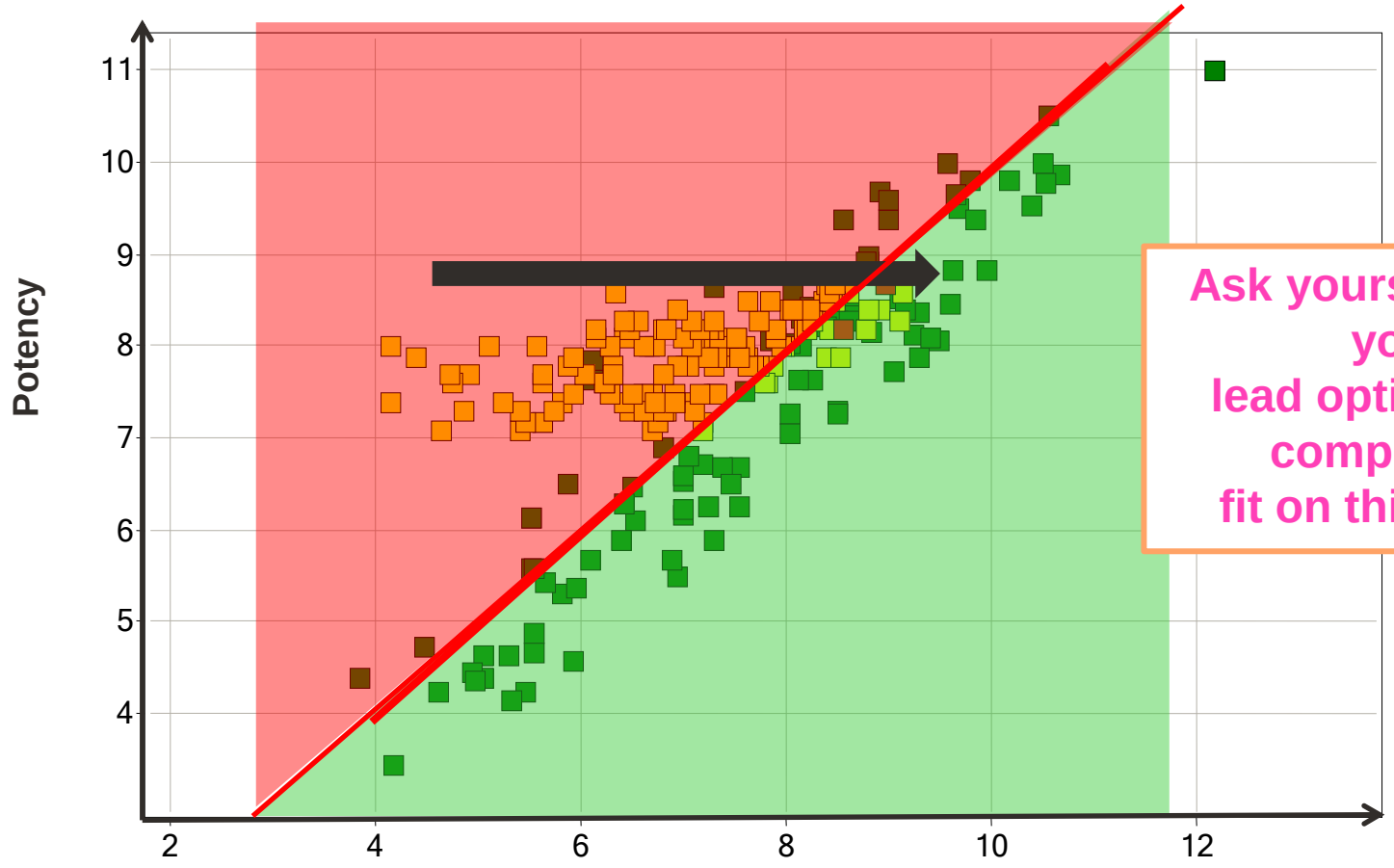
Valko, K. L., Nunhuck, S. B., Hill, A. P., Estimating unbound volume of distribution by I n vitro HPLC-based human serum albumin and immobilized artificial membrane-binding measurements. J. Pharm. Sci., 100 (2011) 849.

Valko, K. L.,, Chiarparin, E.., Nunhuck, S., Montanari, D., In vitro measurements of Drug Efficiency Index to aid lead optimization. J. Pharm. Sci., 101 (2012) 4155.

In vitro HPLC log Deff max = 2 – (0.23xlogk**HSA**+0.43*logk**IAM**-0.72)

Application of DEI within Lead Optimization

(green: known drugs, yellow: project compounds, red: candidates)



Ask yourself where your lead optimisation compounds fit on this plot?!!

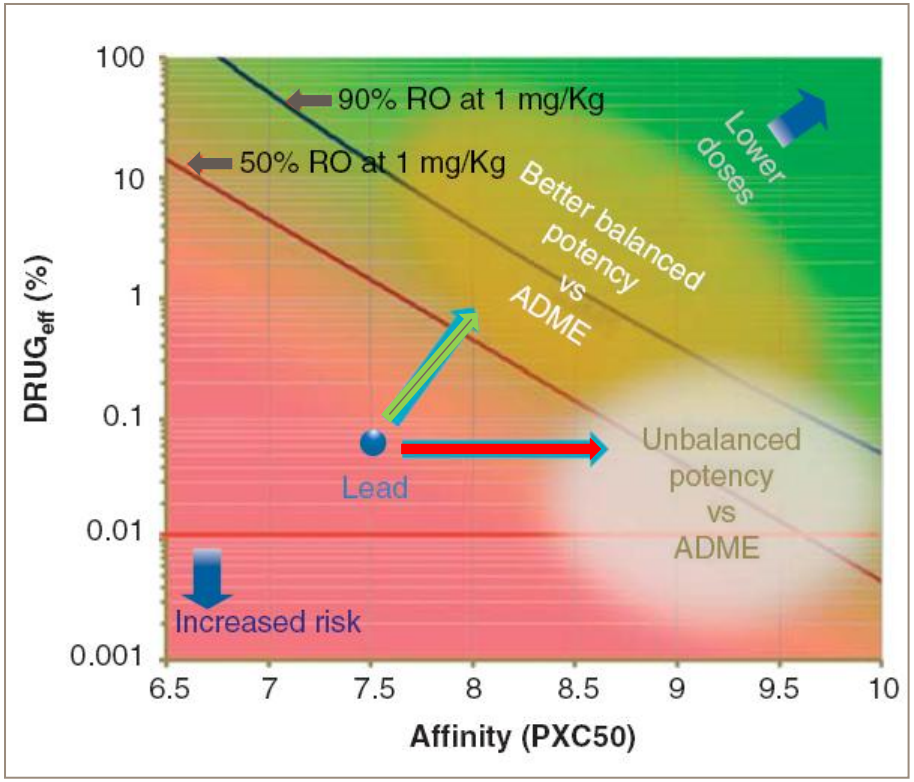
Red line shows when HPLC Drug eff max = 1%

Drug Efficiency – but what does this mean at a cellular level rather than in plasma

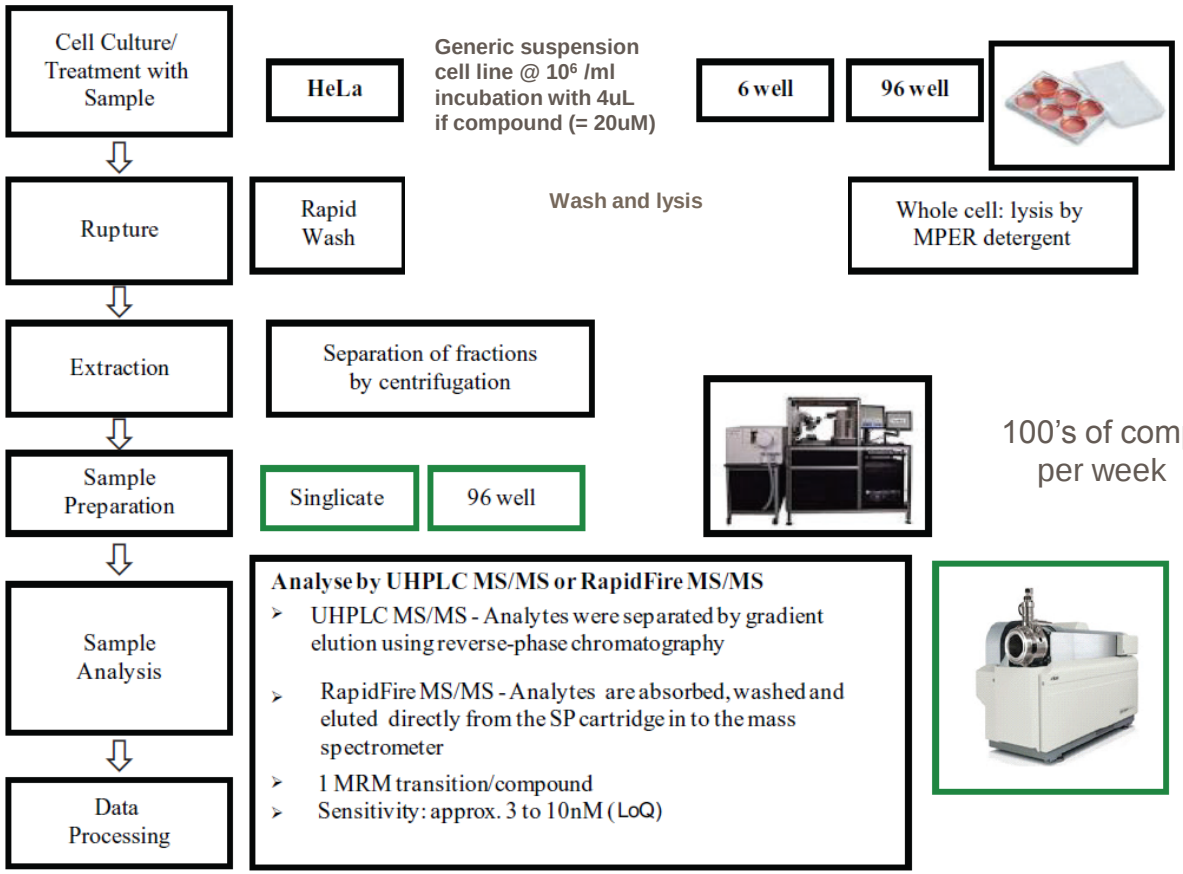
Increasing % of our targets are intracellular

Biophase concentration is traditionally equated to plasma concentration and relies on the free drug hypothesis

Can we measure cell concentration to give an equivalent ***cellular drug efficiency?***



Towards measuring the actual cellular concentration of compounds



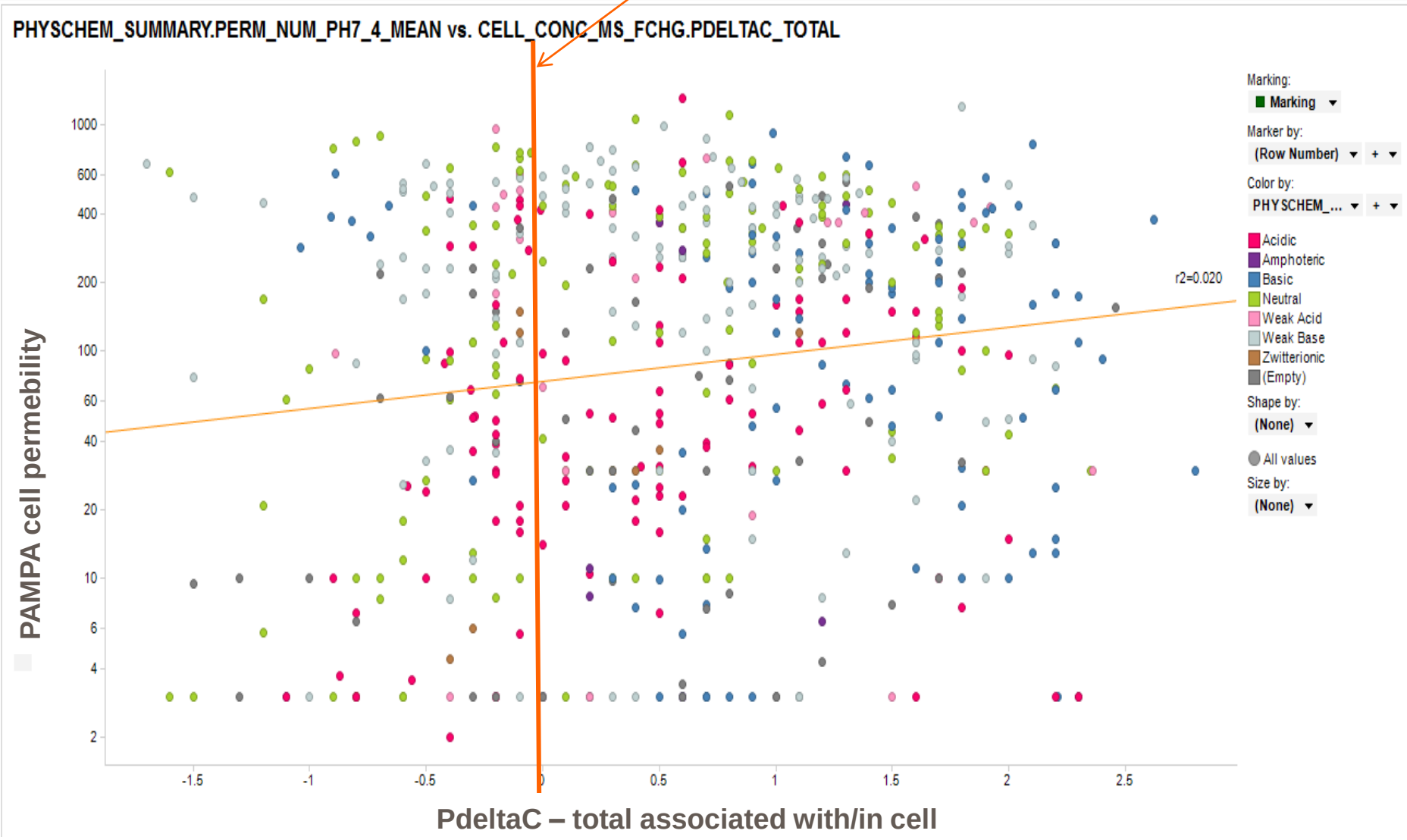
Enables the concept of Cellular Drug Efficiency in early lead optimisation

Data reported as $$p\Delta C = \mathrm{Log}\left[\frac{[C]_{intracellular}}{[C]_{extracellular}}\right]$$

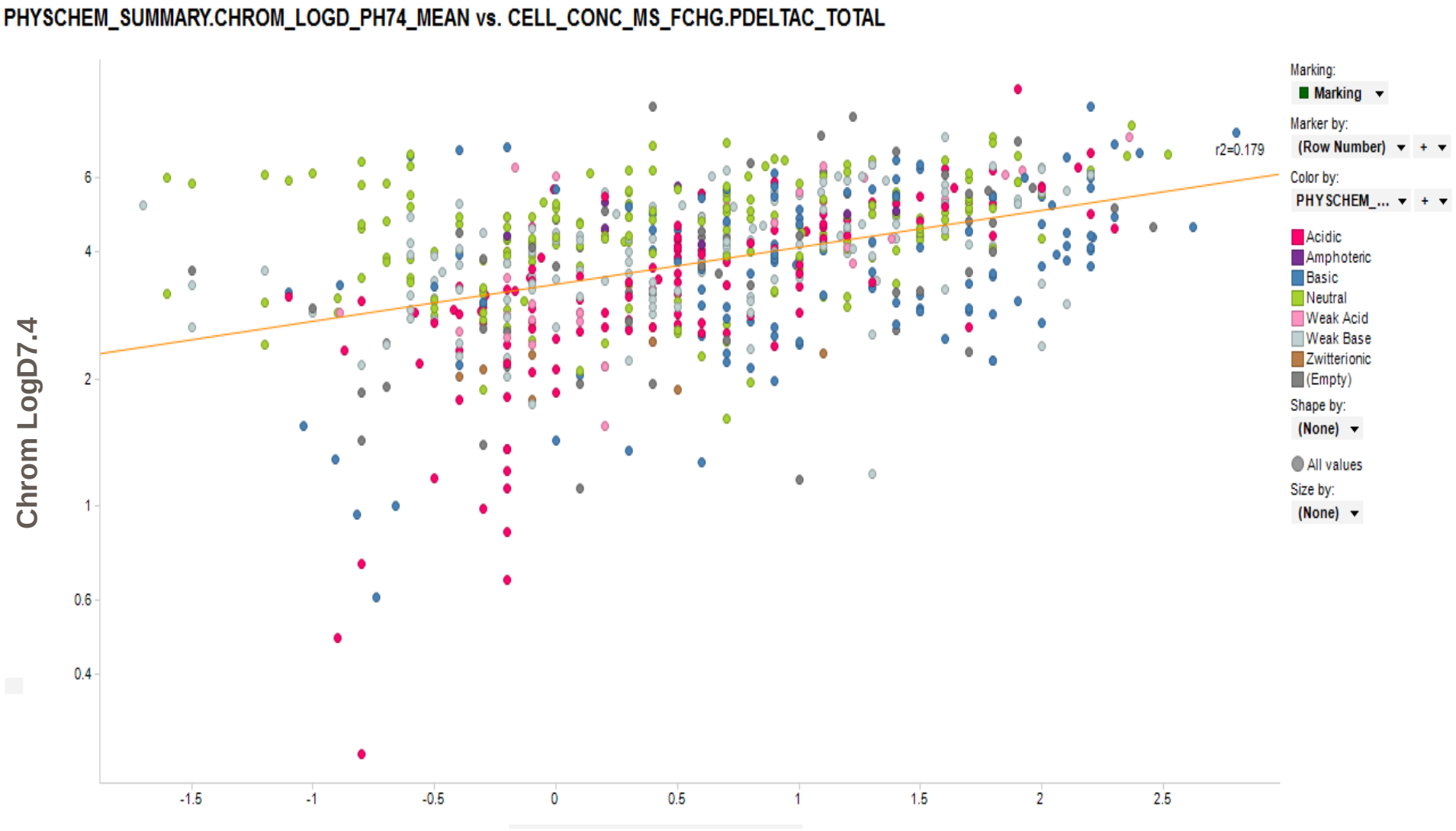
Direct Measurement of Intracellular Compound Concentration by RapidFire Mass Spectrometry Offers Insights into Cell Permeability. J Biomol Screen. 2015 Sep 3. pii: 1087057115604141. Gordon LJ, Allen M, Artursson P, Hann MM, Leavens BJ, Mateus A, Readshaw S, Valko K, Wayne GJ, West A.

Artificial Membrane Permeability vs pDeltaC

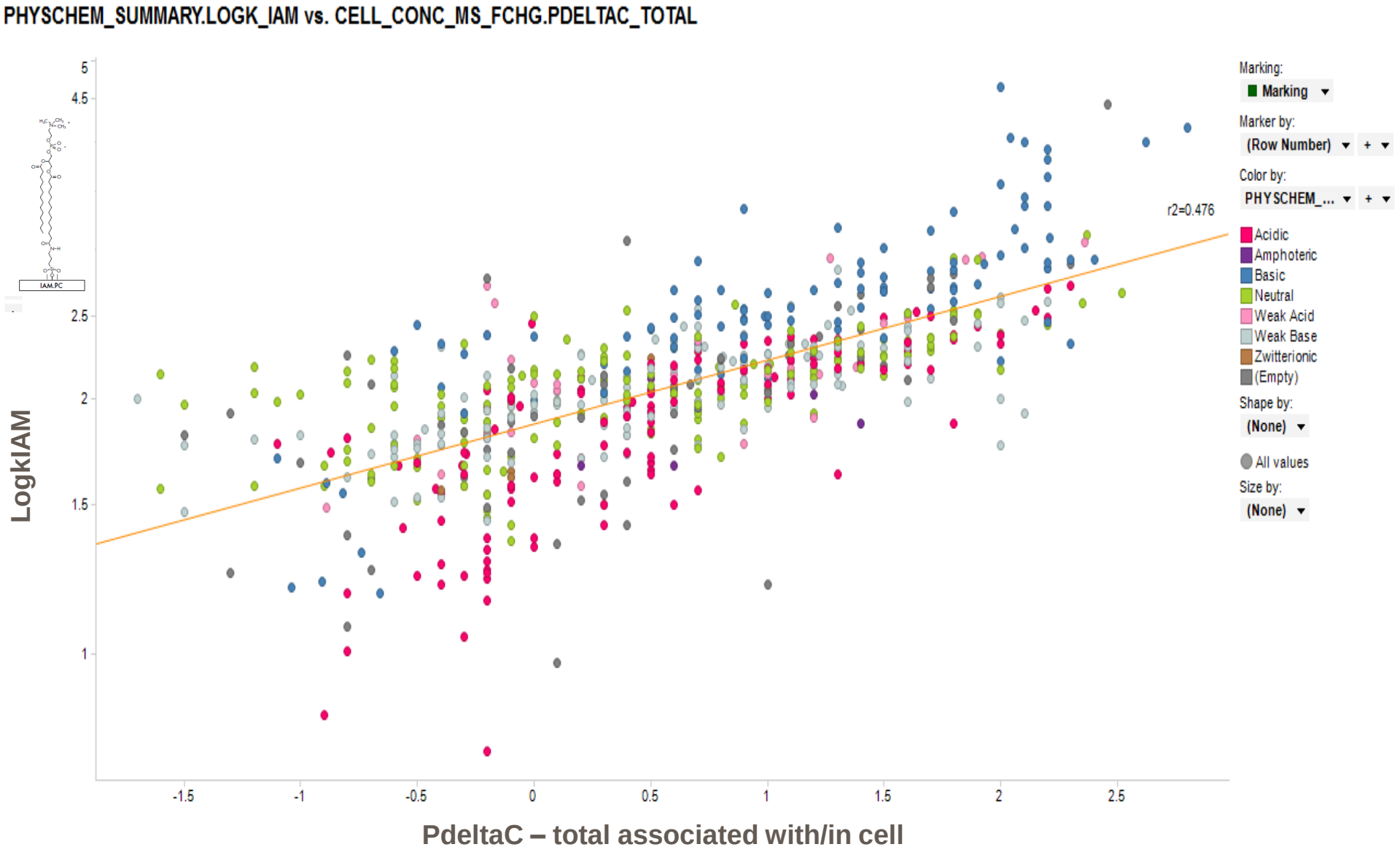
pDeltaC of zero = equal conc inside and outside cell



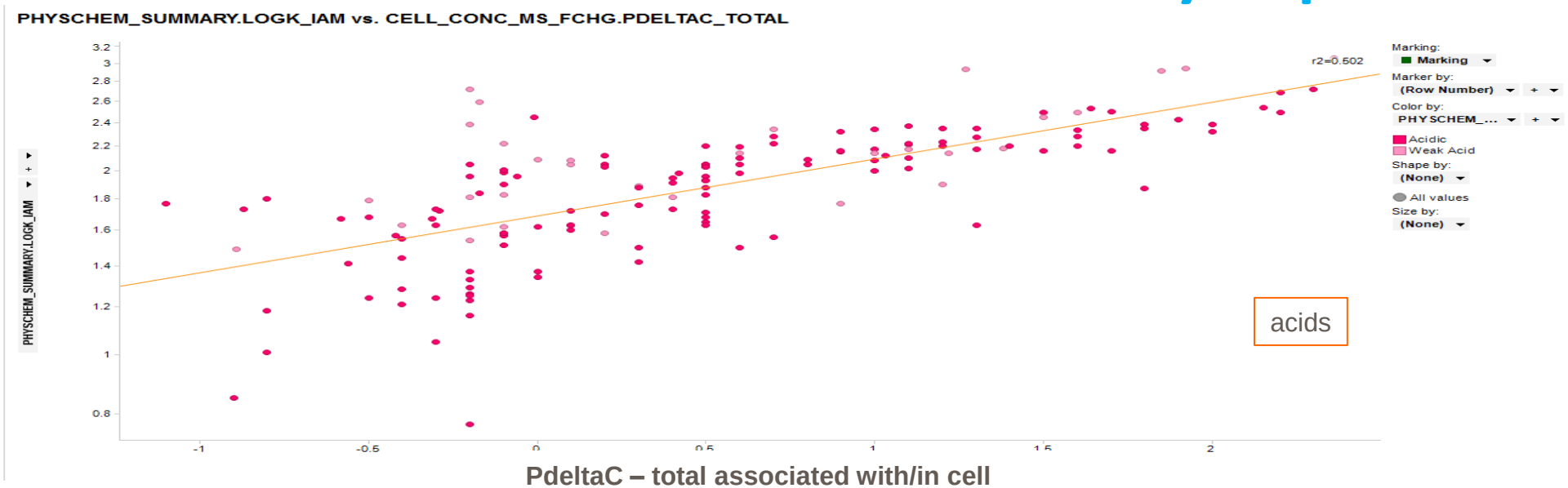
logD7.4 vs pDeltaC

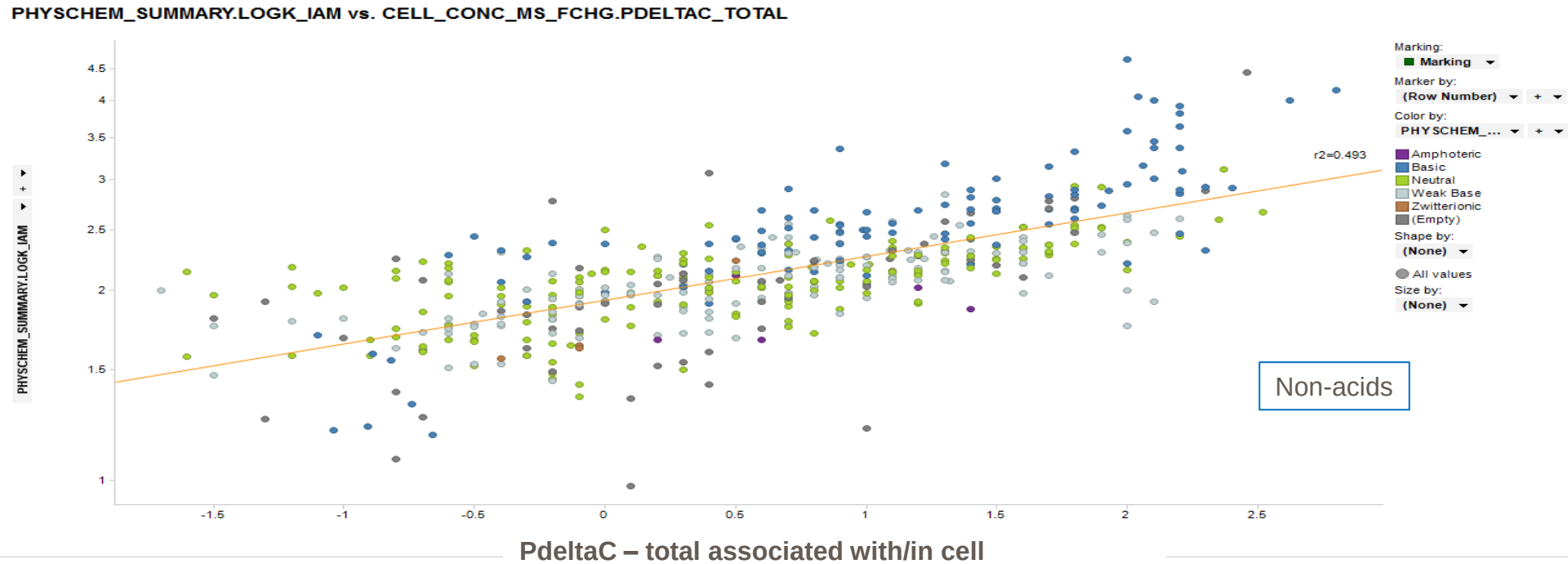


Immobilised artificial membrane affinity vs pDeltaC

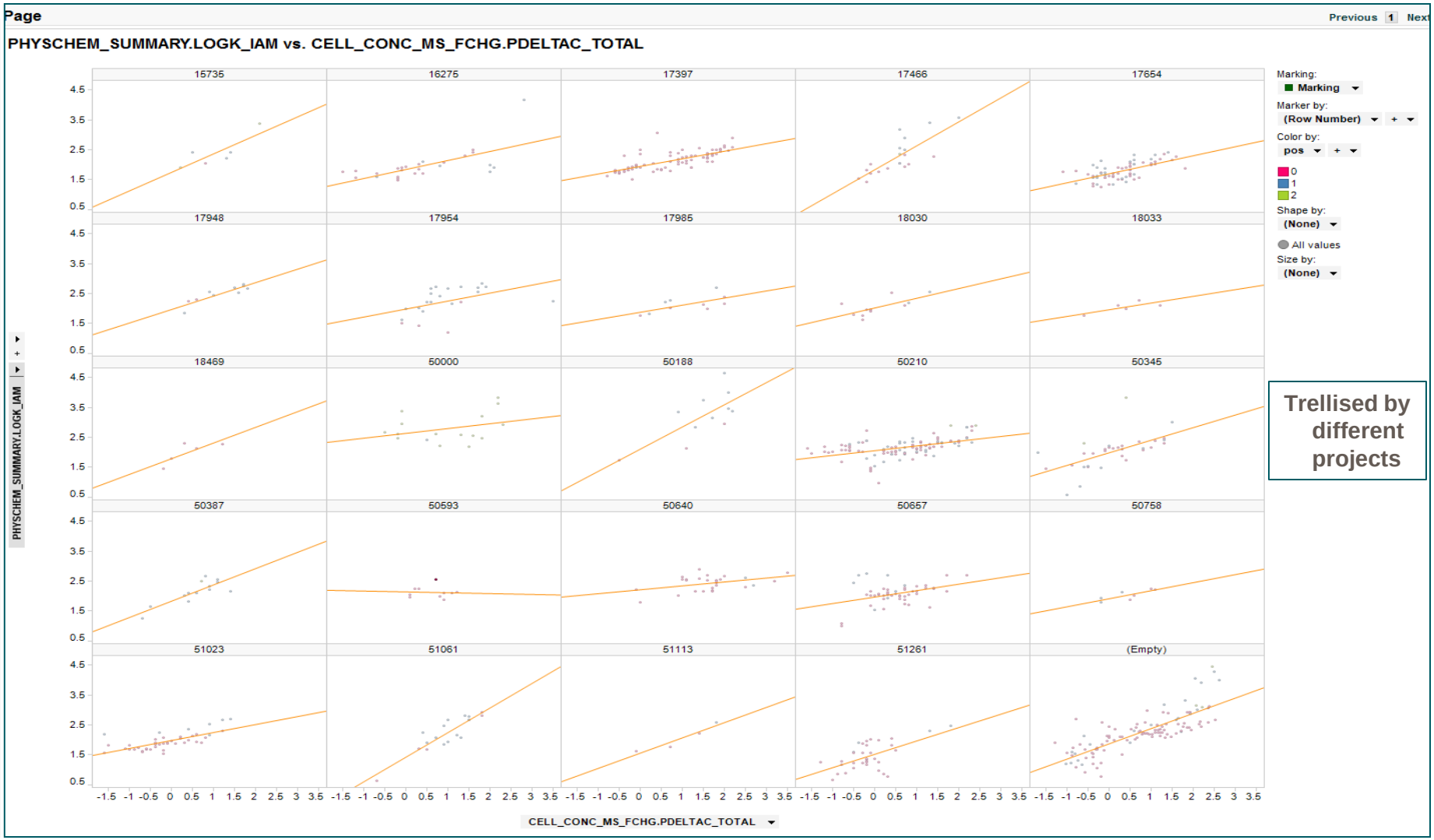


Immobilised artificial membrane affinity vs pDeltaC

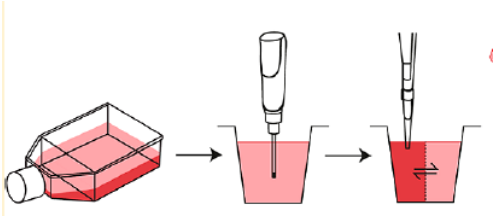




Immobilised artificial membrane affinity vs pDeltaC



Towards understanding intracellular free conc



A High-Throughput Cell-Based Method to Predict the Unbound Drug Fraction in the Brain. Mateus, A., Matsson, P., Artursson, P. (2014). Journal of Medicinal Chemistry, 57(7): 3005-3010

– Binding of compounds to cellular material measured by dialysis using a Rapid Equilibrium Dialysis RED device (Thermo Fisher Scientific Inc.)

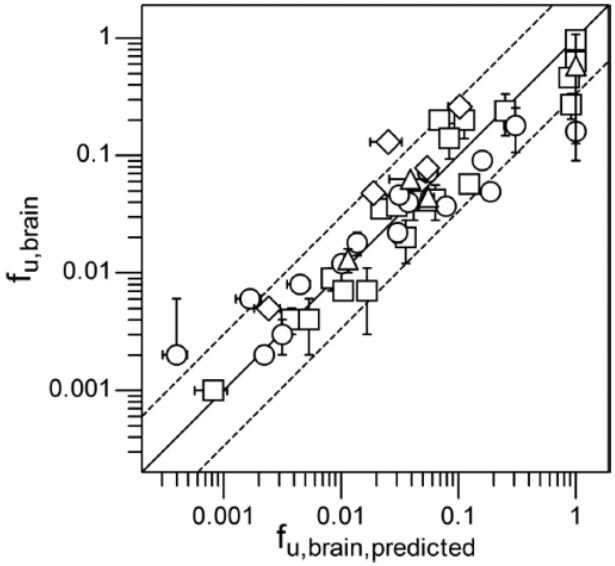
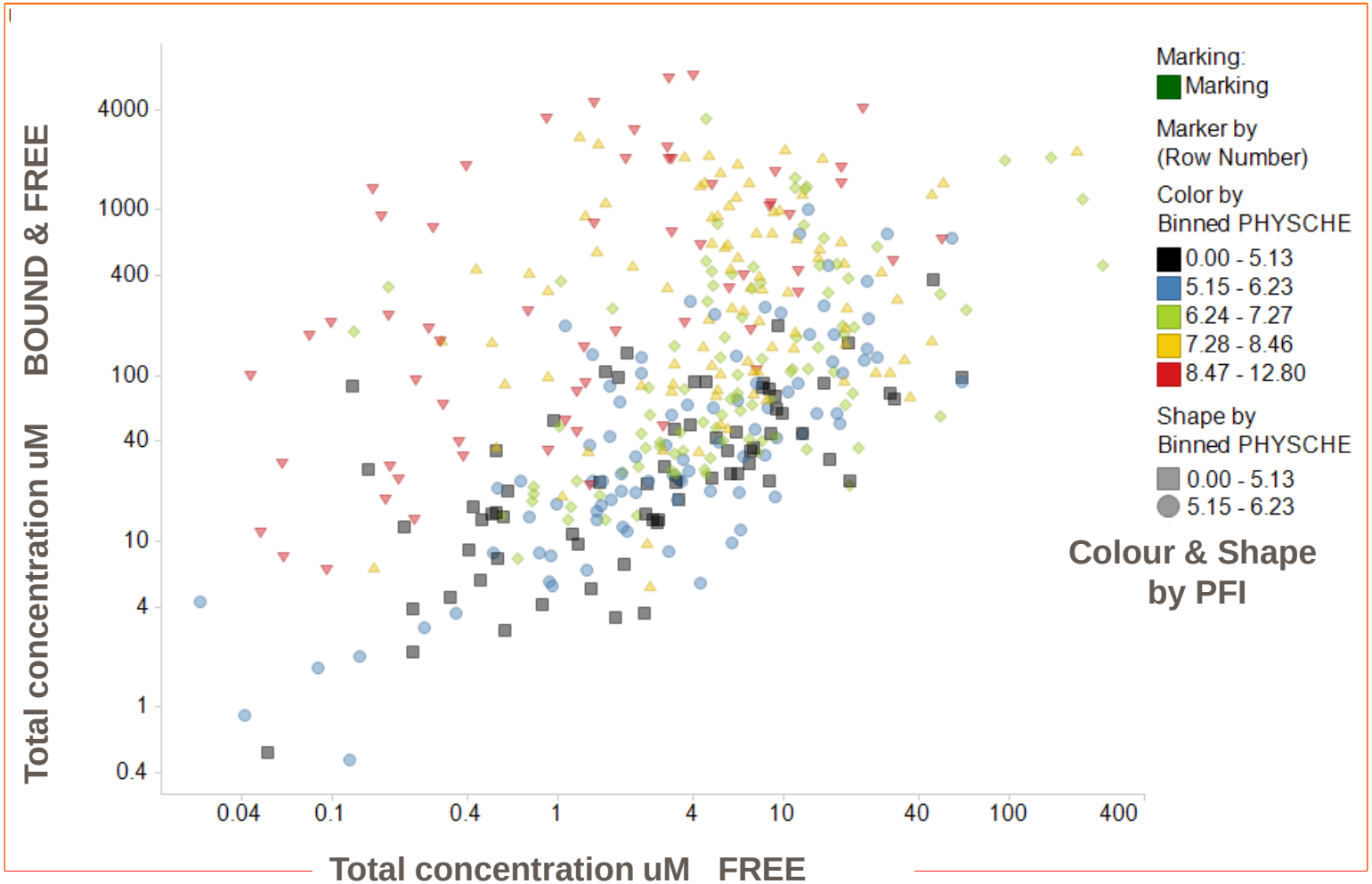


Figure 3. Relationship between $f_{u,brain,predicted}$ (from HEK293 cell homogenates) and experimental $f_{u,brain}$ (from brain homogenates) (RMSE = 0.31). Negatively charged compounds at pH 7.4 are represented by triangles, neutral compounds by circles, positively charged compounds by squares, and zwitterionic compounds by diamonds. The solid line represents a perfect prediction, and the dashed lines represent a 3-fold error interval.

Towards understanding intracellular free conc

Total conc vs Free conc

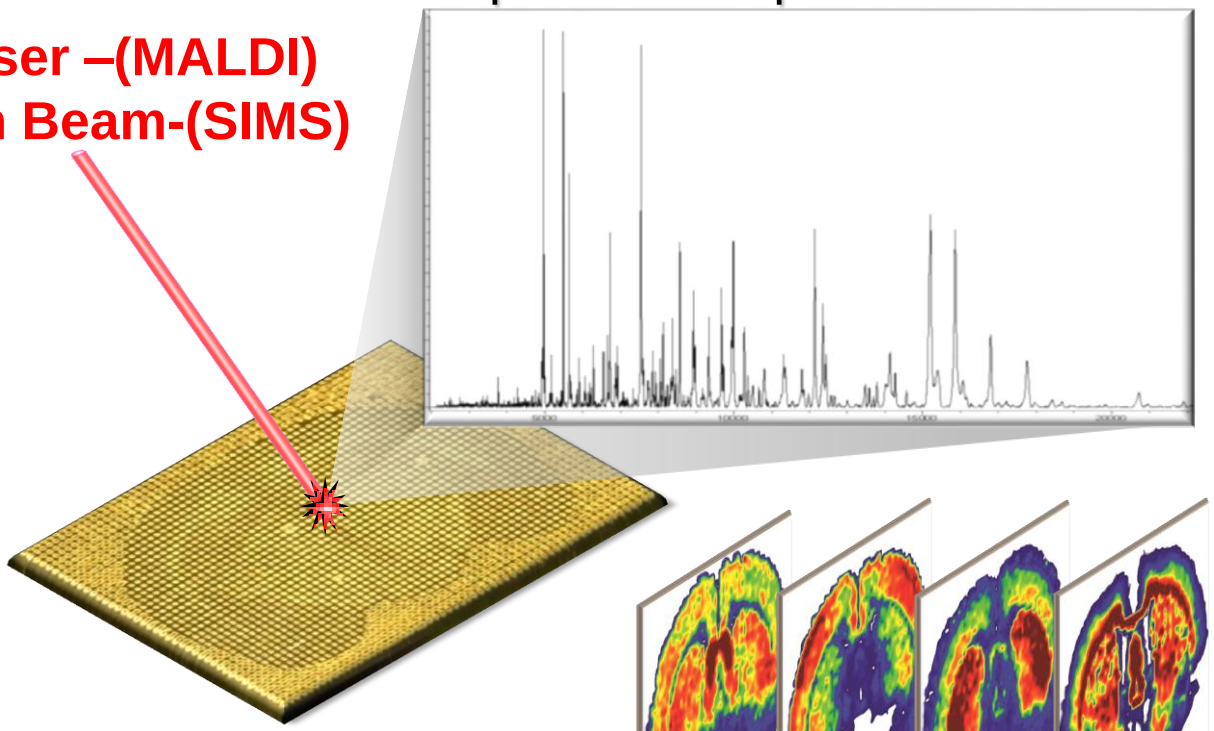


- Ca. 700 compounds measured in consistent assay using HeLa cells
- Opportunity for project specific cell types – highlighting some differences

Towards a better spatial understanding of drug in early discovery

Mass Spectrum Acquired at each Position

- **Laser –(MALDI)**
- **Ion Beam-(SIMS)**

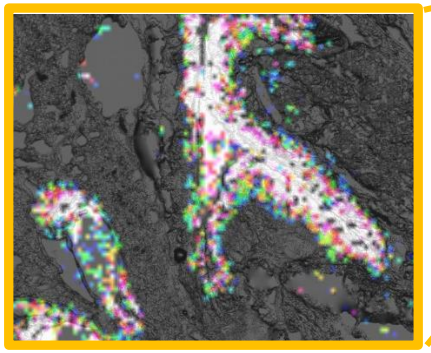


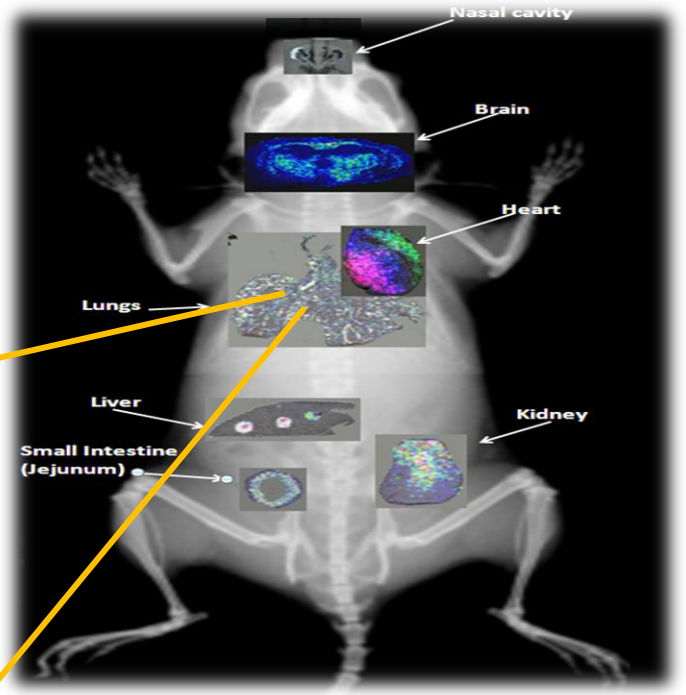
Ion Density Maps

Low *High*

Intensity

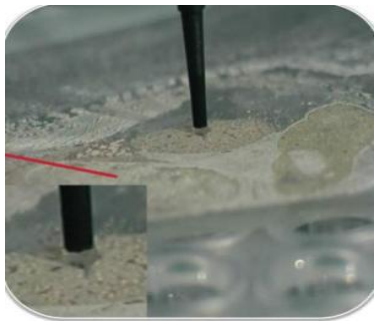
Towards a better spatial understanding of drug in early discovery

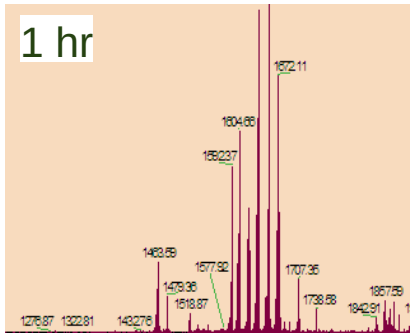


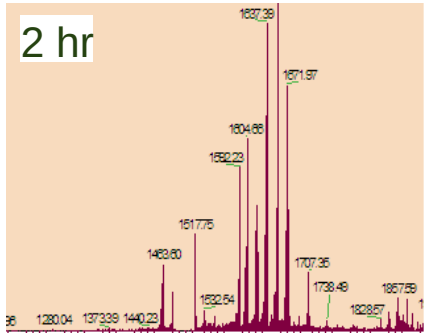


MALDI MSI & LESA well established at GSK

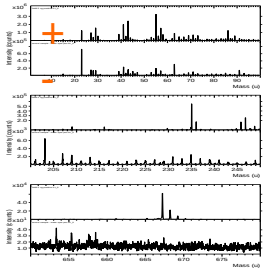
LESA (Liquid Extraction Surface Analysis) MS/MS N-term sequence



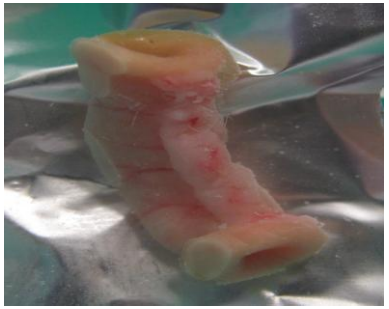




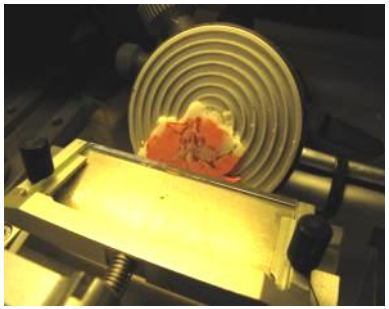
Towards subcellular resolution with Secondary Ion MS = SIMS



Compound Assessment and Feasibility Study



Layout and freeze tissue

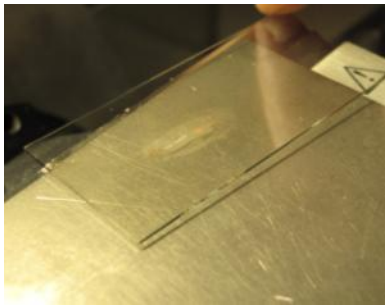


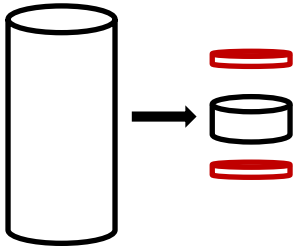
Section on Cryostat (12-16µm thickness)

Photograph tissue

Scan Optical Image

Ship samples to NPL*





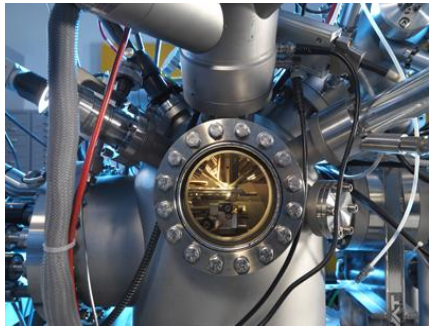
Jejunum section

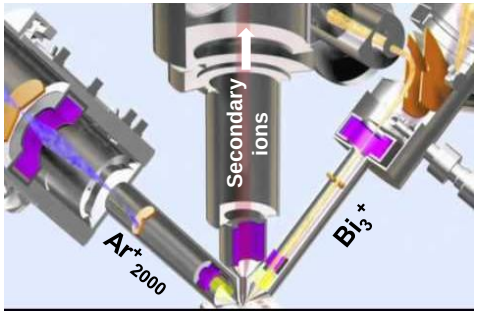
IHC section ~6-10um thick

SIMS section ~ 12-14um thick

IHC section ~6-10um thick

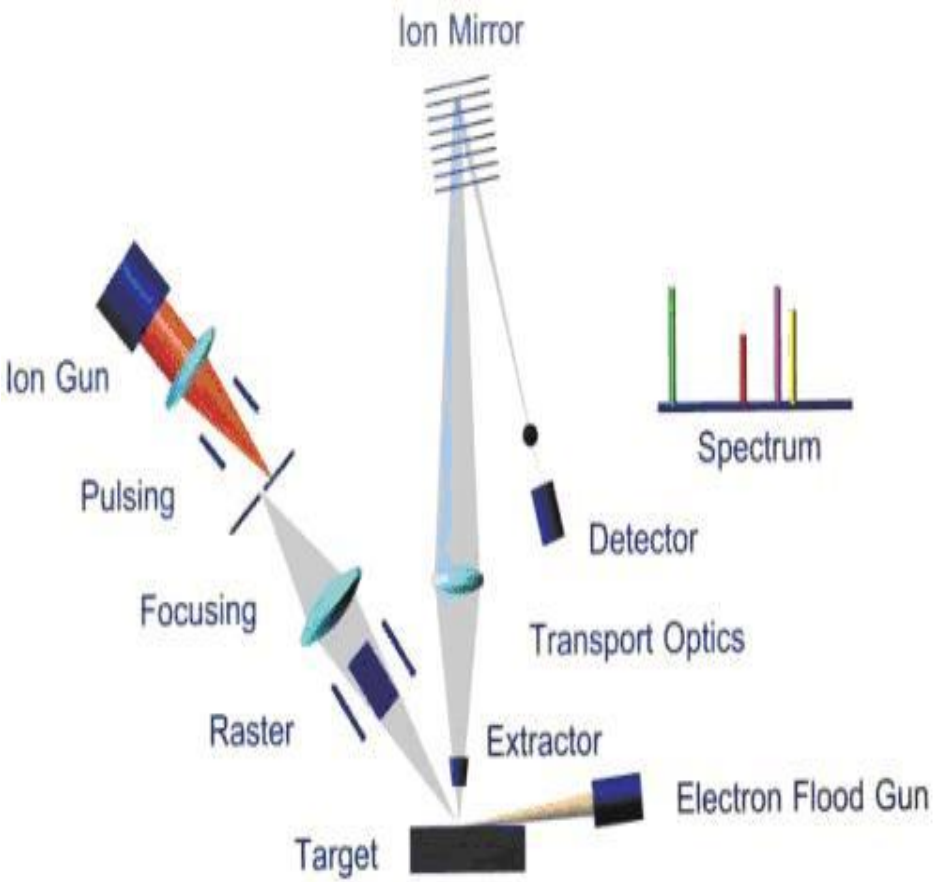
Thaw mount tissue onto MALDI slide for SIMS and adjacent section on histology slide for IHC

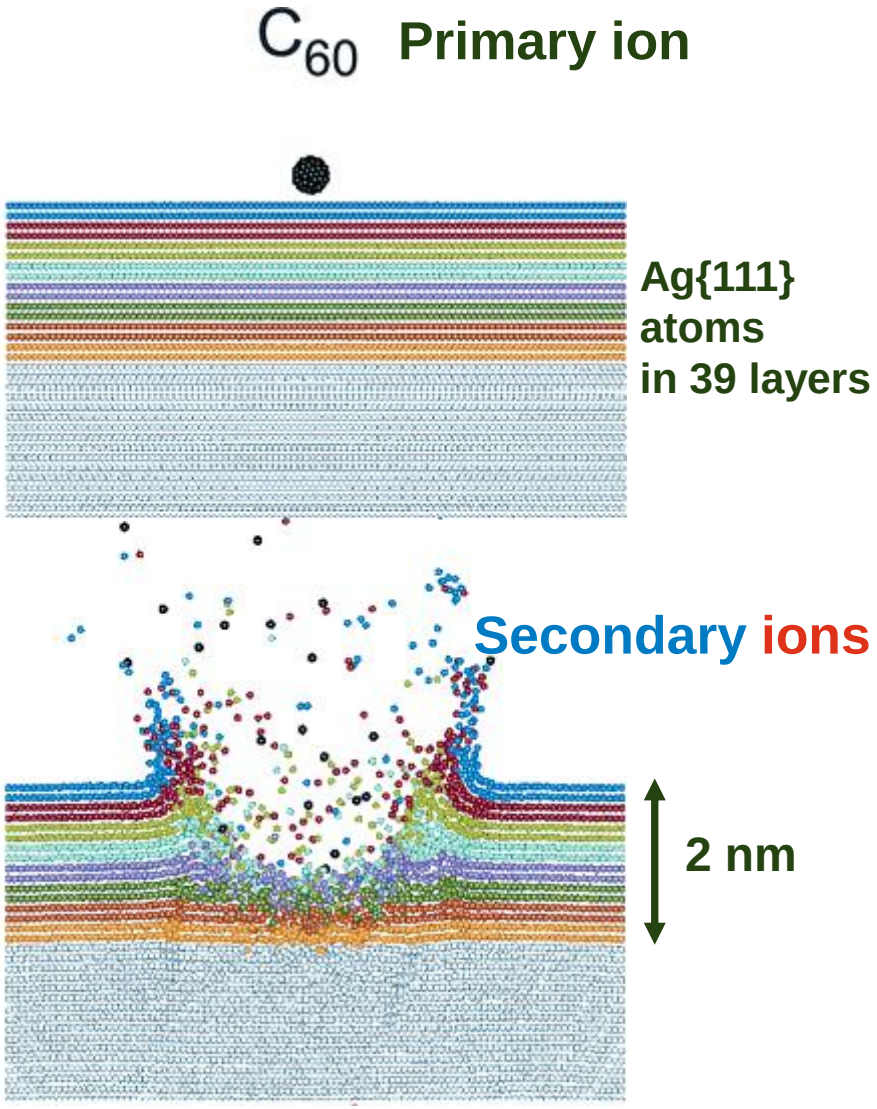




* In collaboration with Prof Ian Gilmore et al at the National Physical Laboratory, Teddington, UK

Secondary ion mass spectrometry, SIMS



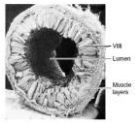


Z Postawa et al., *J. Phys. Chem. B* (2004)

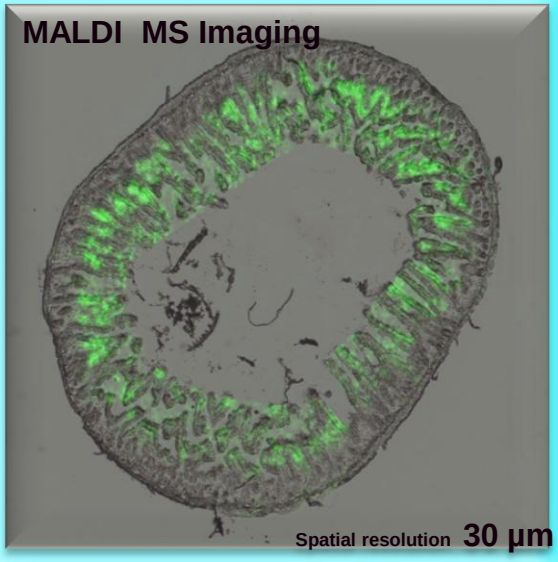
In collaboration with the National Physical Laboratory, Teddington, UK

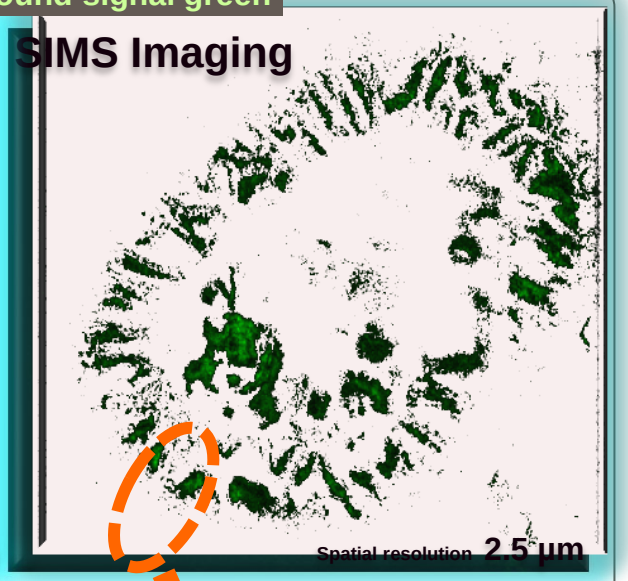
Compound Disposition from Tissue to Cells - Developing Mass Spectrometry Imaging

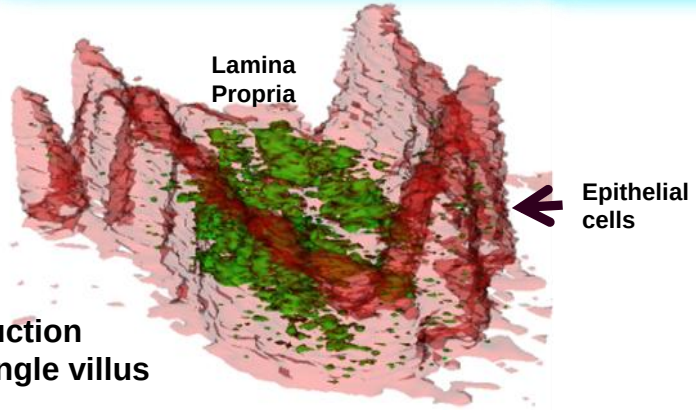
Dime : rat jejunum (~4.5 mm)



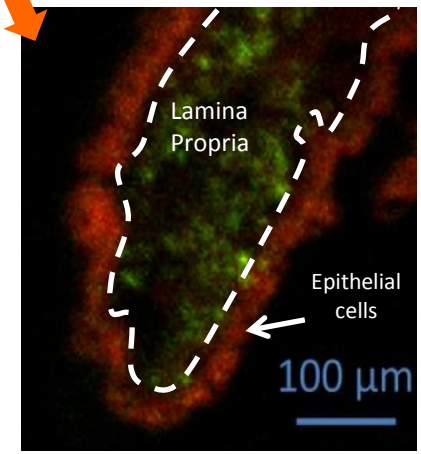
Dosed Compound signal green





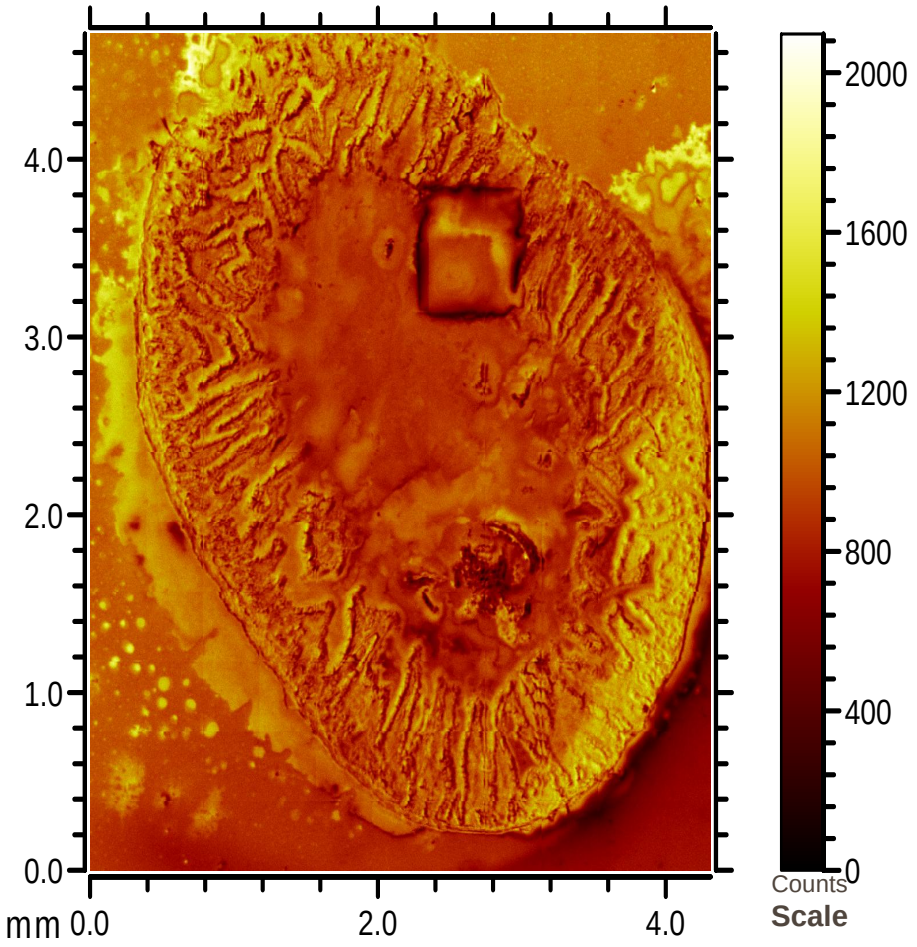


SIMS - 3D reconstruction
depth profile through single villus

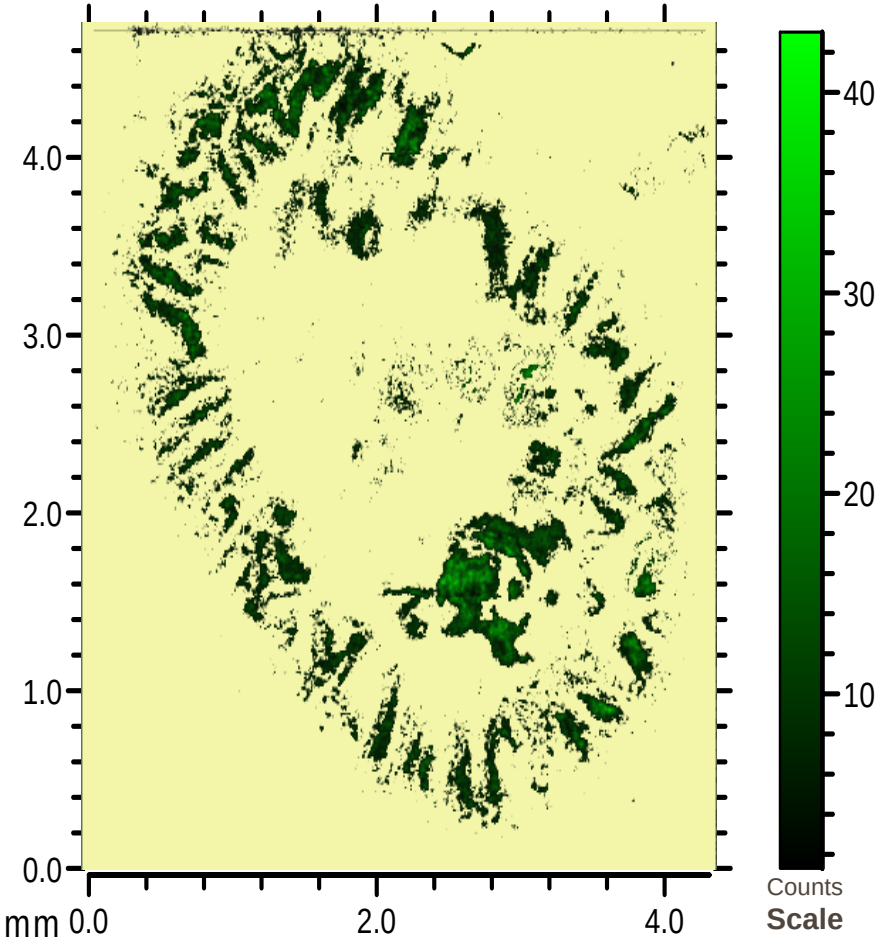


Single-Cell Analysis: Visualizing Pharmaceutical and Metabolite Uptake in Cells with Label-Free 3D Mass Spectrometry Imaging. Melissa K. Passarelli, Carla F. Newman, Peter S. Marshall, Andrew West, I Gilmore, Josephine Bunch, Morgan R. Alexander and Colin T. Dollery. Anal. Chem. 2015, 87, 6696−6702

SIMS: Dosed animal



Sum of all ions in MS (TIC)



Compound specific fragment

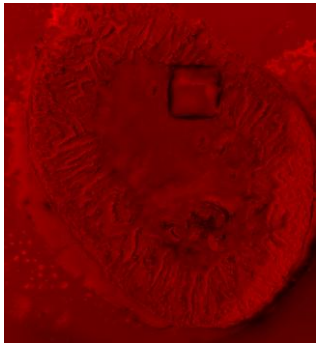
2.5 μm pixel size

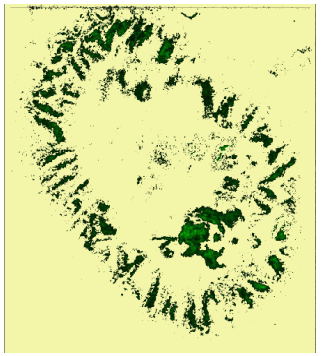
SIMS surface Image

Rat jejunum - 5 day oral dose @ 100 mg/kg, 4 Hr post dose

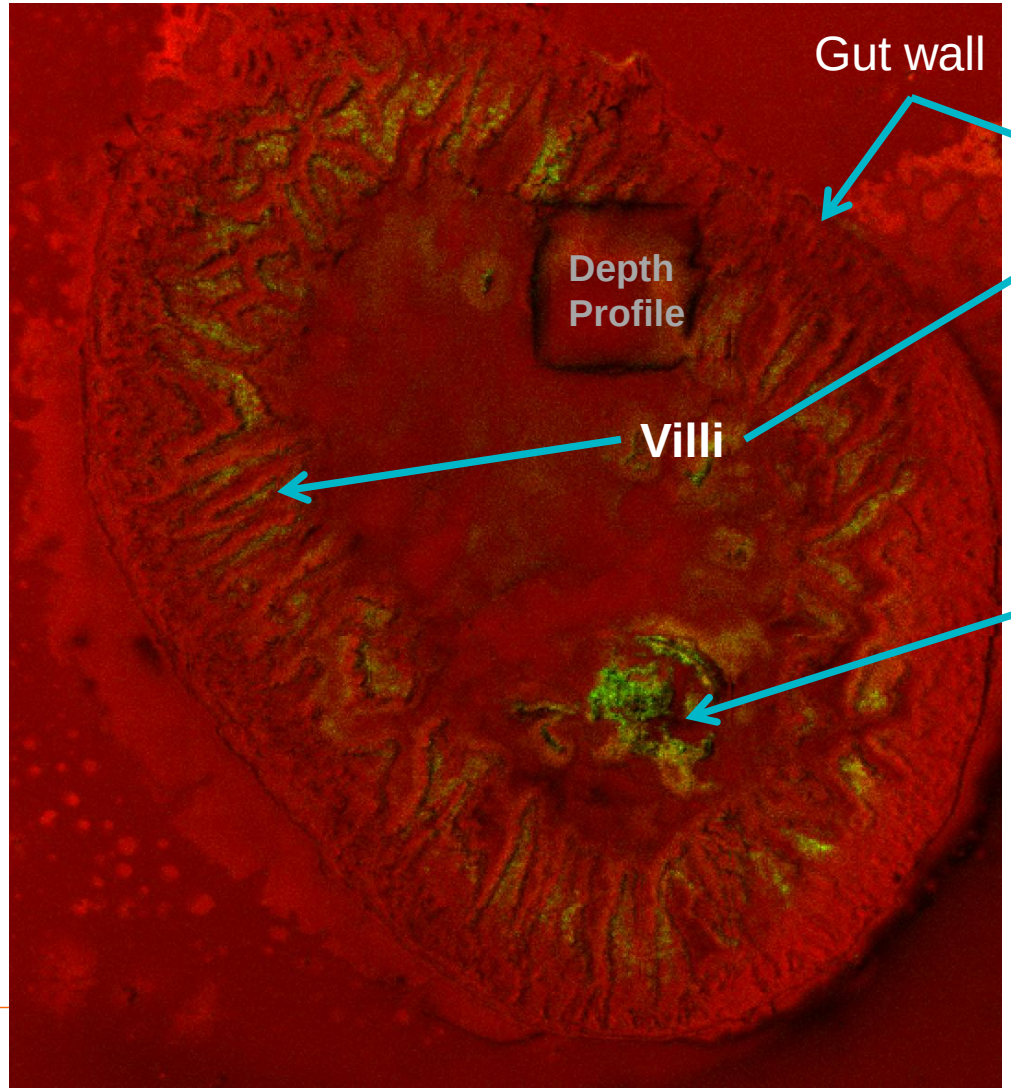
4.3 x 4.7 mm²

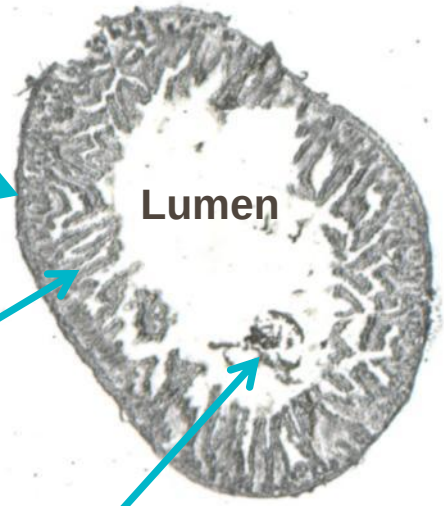
All ions





Compound





optical

"Feed" particle

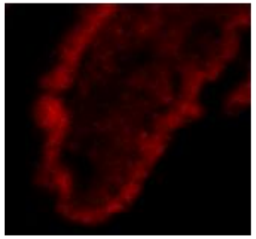
2.5 μm pixel size

Depth profile through individual villus

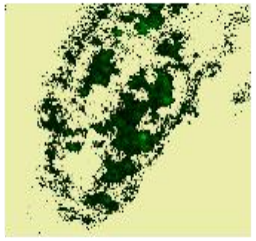
total integrated SIMS signal from the 3D image

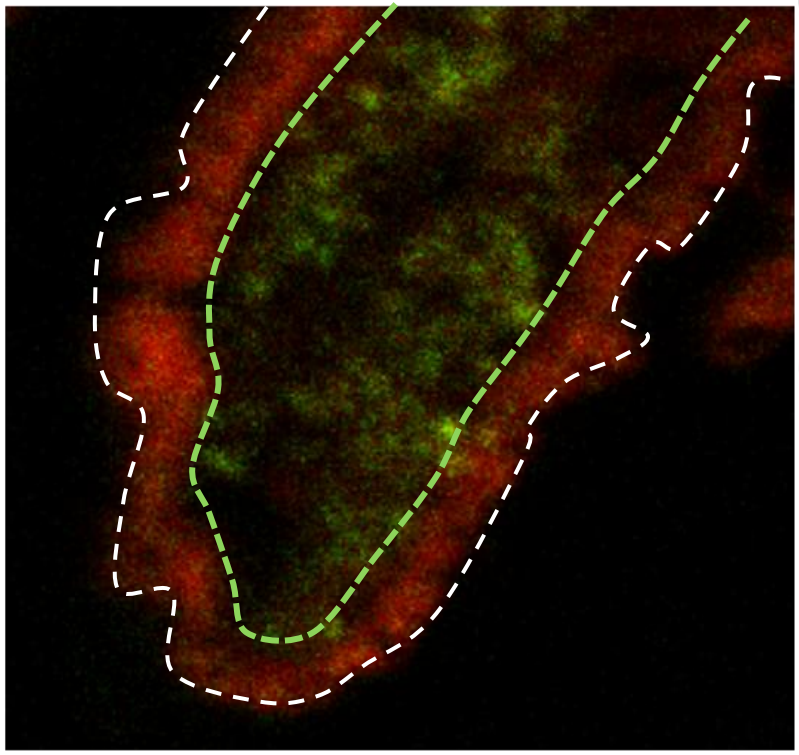
400 x 400 µm²

Lipid fragment
184 m/z
Phosphocholine

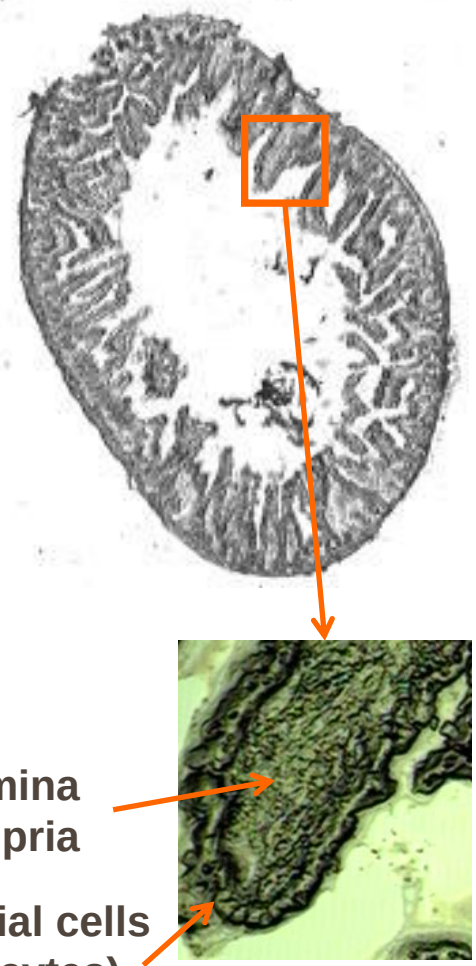


Compound
specific
fragment





optical



Lamina
Propria

Epithelial cells
(enterocytes)

1.5 µm pixel size

SIMS MS imaging of single cells

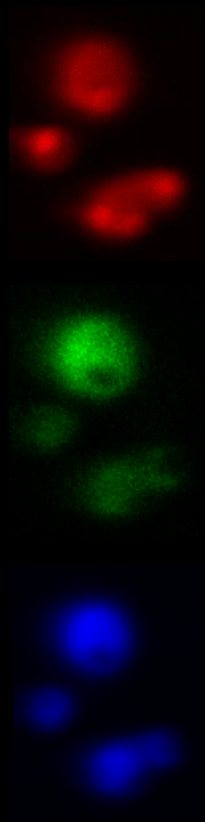
total integrated signal from 3D image

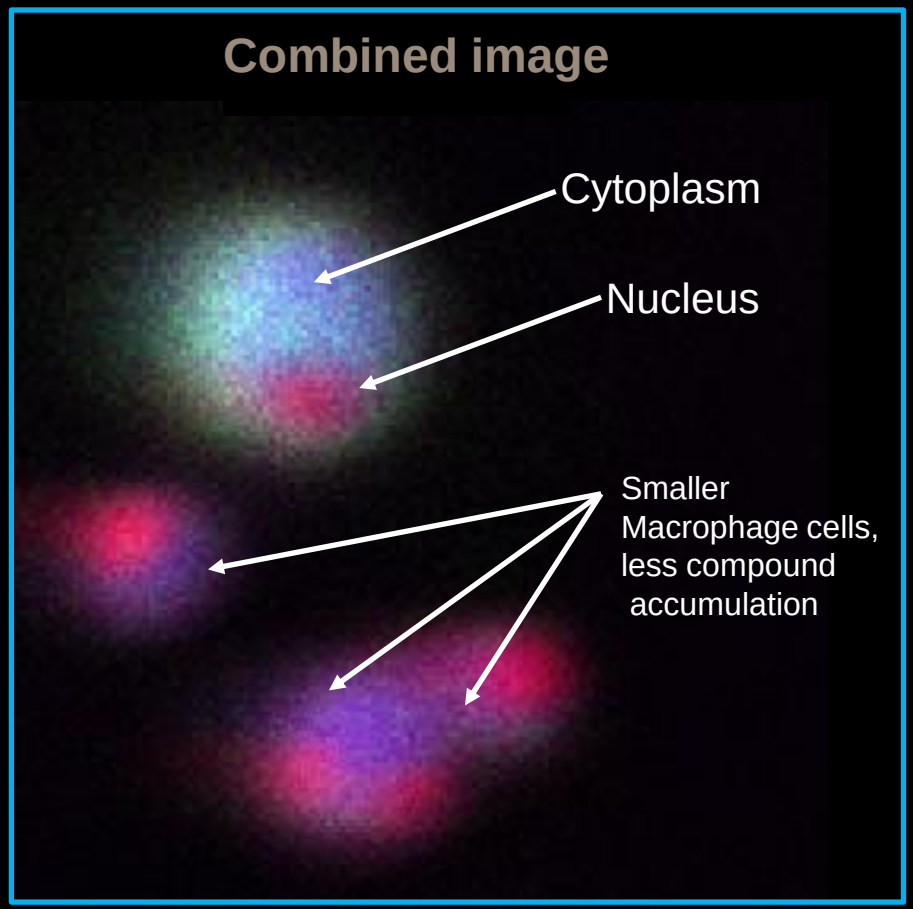
Rat Alveolar macrophage cell line incubated with Amiodarone

Ribose
Marker nucleus

Amiodarone

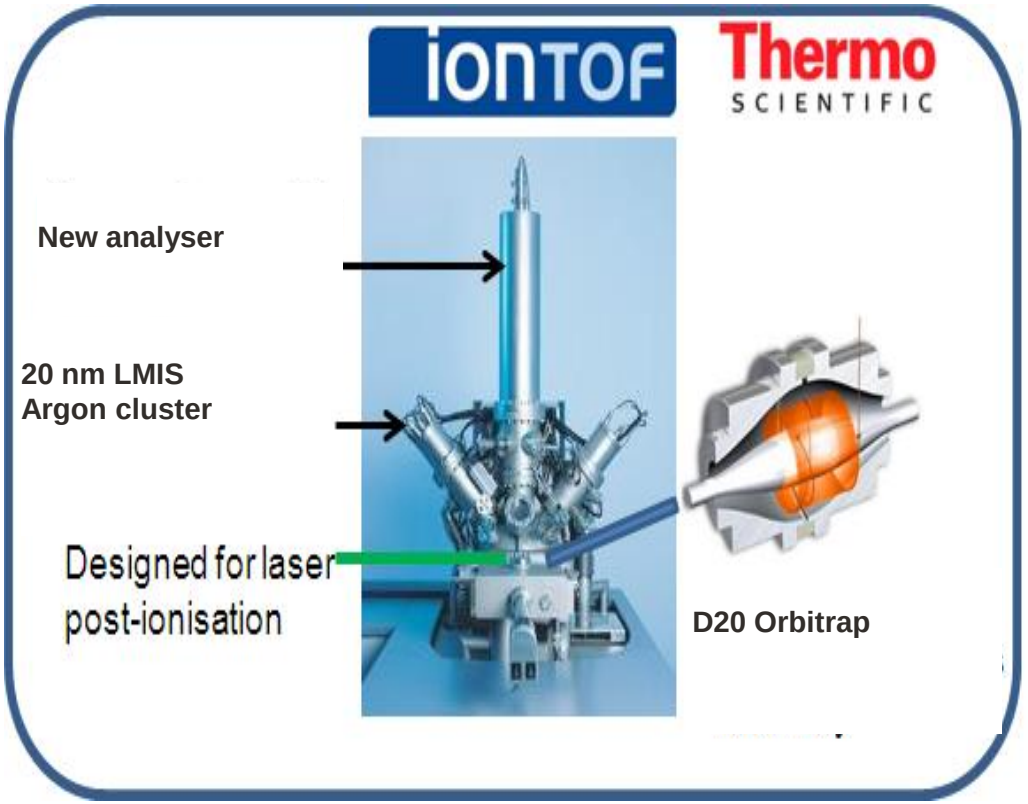
Lipid fragment
Marker cell





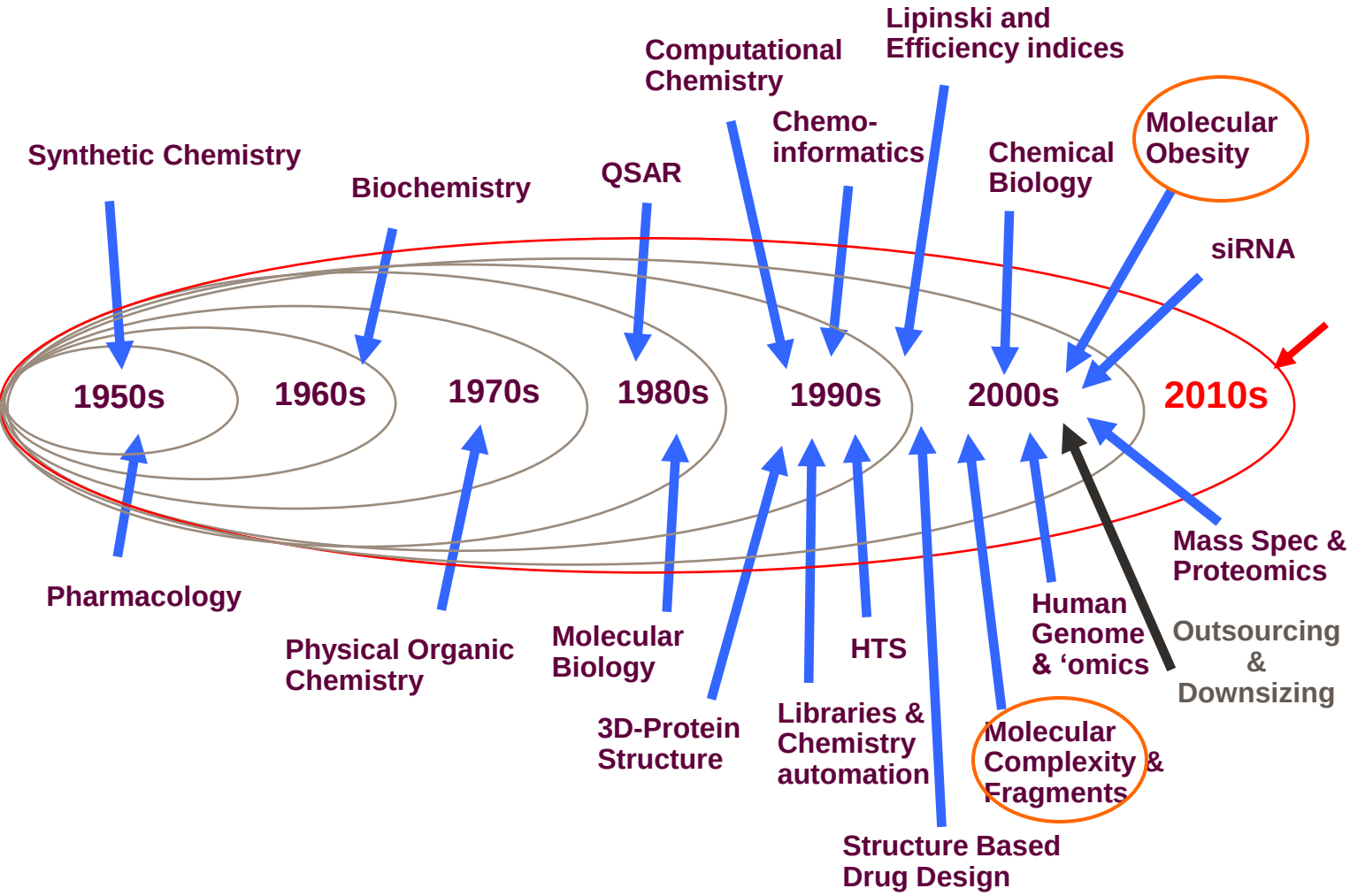
Passarelli et al, *Anal. Chem.*, 2015, 87 (13), pp 6696–6702

Next generation 3D nano-SIMS



What sort of works for us?

What might work for us in the future?



3/4 pillars
Drug Disposition
Drug Accessibility
Drug Efficiency
Target Engagement
Target confidence
Chemical tractability
Phenotypic screens
Quality of Leads
Stem cells
Green Chemistry
Synthetic Biology
Gene editing
Chemical genetics
Chemical Biology
Big Data
Binding Kinetics
CETSA
CryoEM/X-FEL
Free energy
Design tools
Open Innovation
.....

The expanding "sciences" of Medicinal Chemistry and drug discovery

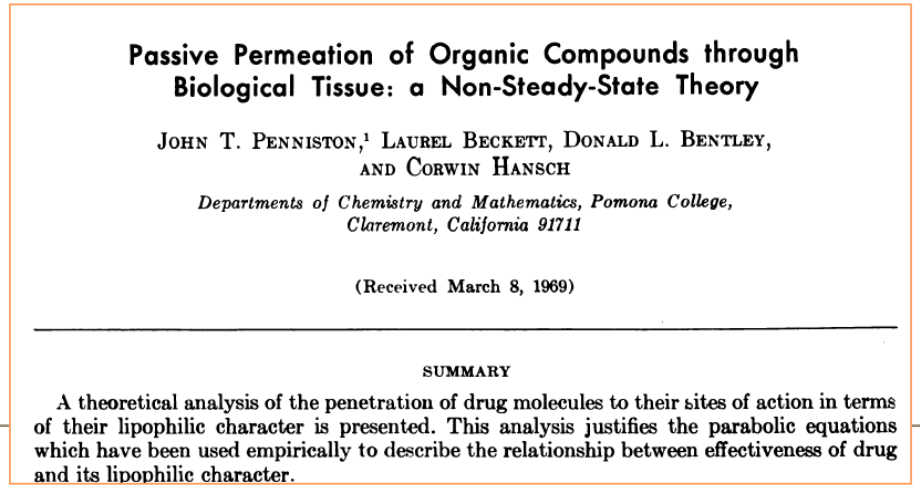
Known knowns, known unknowns, unknown unknowns and ……..

The part that Donald Rumsfeld forgot

Unknown knowns

- Those things that are known but we don't know ourselves
- Those things that are known but we have forgotten
- Those things that are known but we choose to ignore

- Lets try not to ignore the medchem knowledge that has been gained at very considerable expense over many years!



Passive Permeation of Organic Compounds through Biological Tissue: a Non-Steady-State Theory

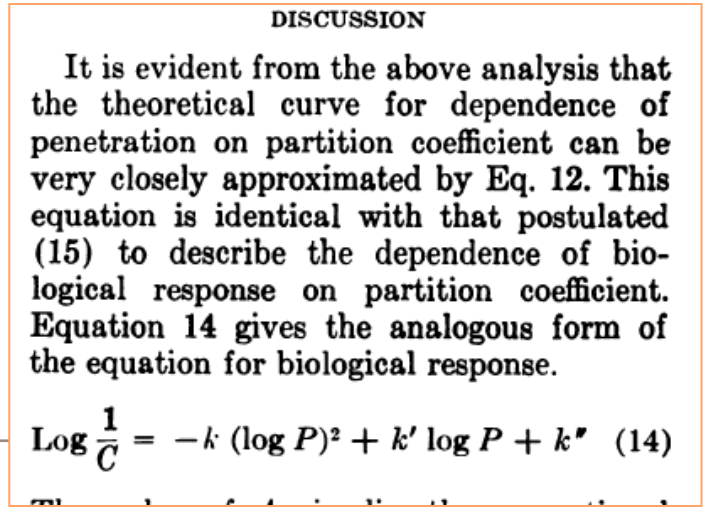
JOHN T. PENNISTON,[1] LAUREL BECKETT, DONALD L. BENTLEY, AND CORWIN HANSCH

Departments of Chemistry and Mathematics, Pomona College, Claremont, California 91711

(Received March 8, 1969)

SUMMARY

A theoretical analysis of the penetration of drug molecules to their sites of action in terms of their lipophilic character is presented. This analysis justifies the parabolic equations which have been used empirically to describe the relationship between effectiveness of drug and its lipophilic character.



DISCUSSION

It is evident from the above analysis that the theoretical curve for dependence of penetration on partition coefficient can be very closely approximated by Eq. 12. This equation is identical with that postulated (15) to describe the dependence of biological response on partition coefficient. Equation 14 gives the analogous form of the equation for biological response.

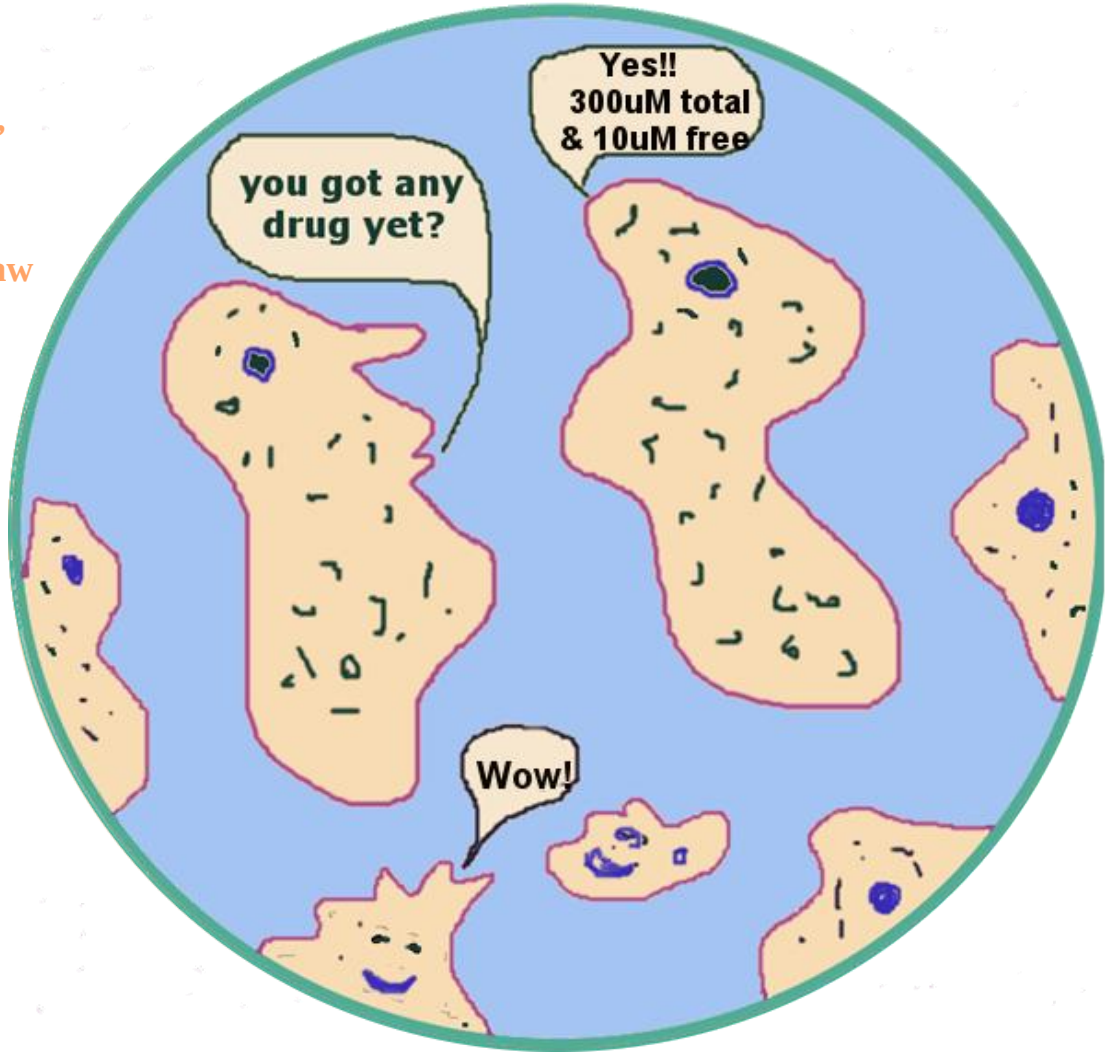
$$\text{Log}\,\frac{1}{C} = -k\,(\log P)^2 + k'\log P + k'' \quad (14)$$

Acknowledgements

Many at GSK and beyond:

- Andrew Leach, Darren Green, Ian Churcher, James Butler, Rob Young, Martin Bayliss;
- Gareth Wayne, Bill Leavens, Morven Evans, Laurie Gordon, Klara Valko, Simon Readshaw et al
- Andy West, Peter Marshall, Carla Newman, Sir Colin Dollery, et al
- Gitte Neubauer, Gerard Drewes, Friedrich Reinhard et al

- Paul Leeson
- Simone Braggio, Dino Montanari et al

- George Keseru – Budapest
- Andre Mateus, Per Artursson – Uppsala.
- Par Nordlund – Karolinska Inst
- Ian Gilmore et al. NPL

Summary