Intracellular Concentrations

Impact on Pharmacokinetic Models

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Acknowledgements

- TUSP
  - **Swati Nagar**
  - Priyanka Kulkarni
  - Jaydeep Yadav
  - Kim Holt
  - Min Ye
- WSU
  - **Jeff Jones**
  - Jim Brozik
  - Sarah Humphries
- NIH Grants
  - 5R01GM104178
  - 1R01GM114369

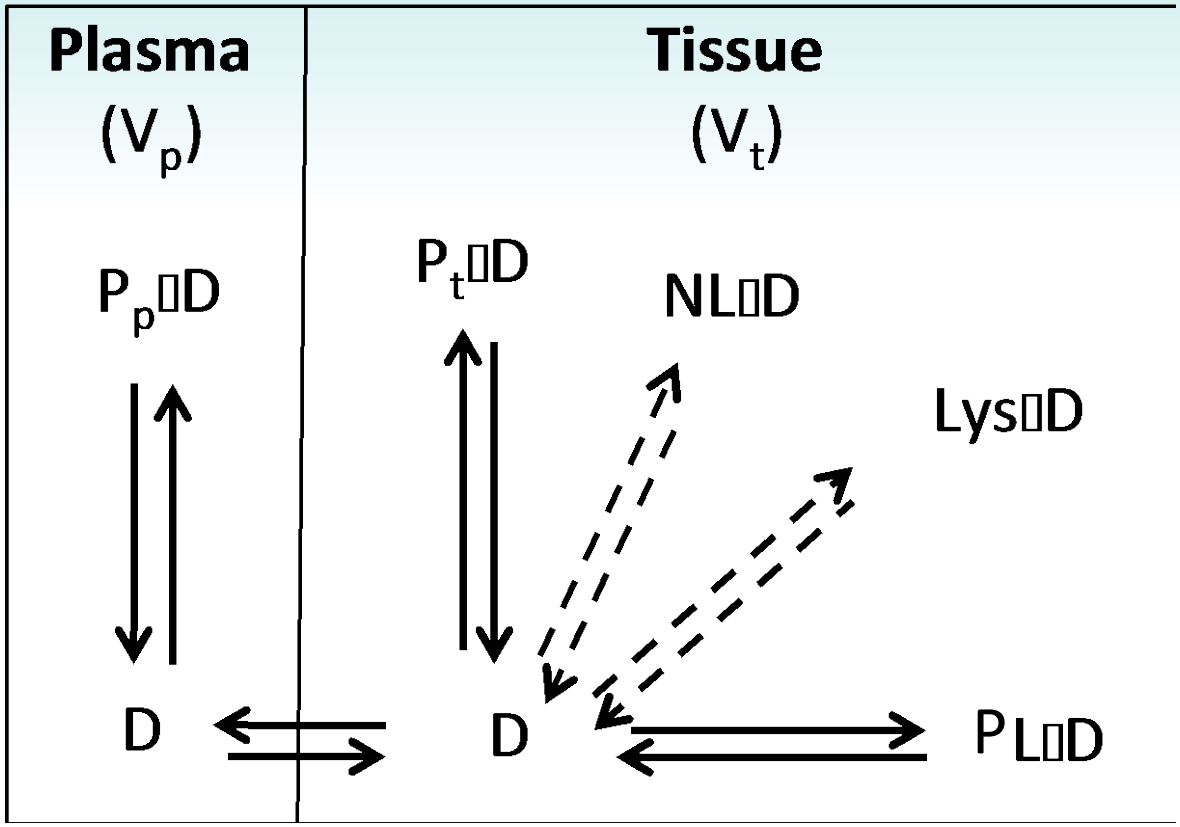
Intracellular Concentrations

- Unbound intracellular concentrations interact with intracellular targets
  - Therapeutic targets
  - Non-therapeutic targets
    - Drug interactions
    - Other toxicities
- Total intracellular concentrations determine tissue partitioning
  - Tissue distribution
  - Volume of distribution

Intracellular Distribution

- Unbound intracellular drug concentrations can partition into different organelles
  - Phospholipid membranes
  - Other lipids (e.g. adipose)
  - Lysosomes
    - pH partitioning
  - Mitochondria
  - Intracellular protein binding is usually not important

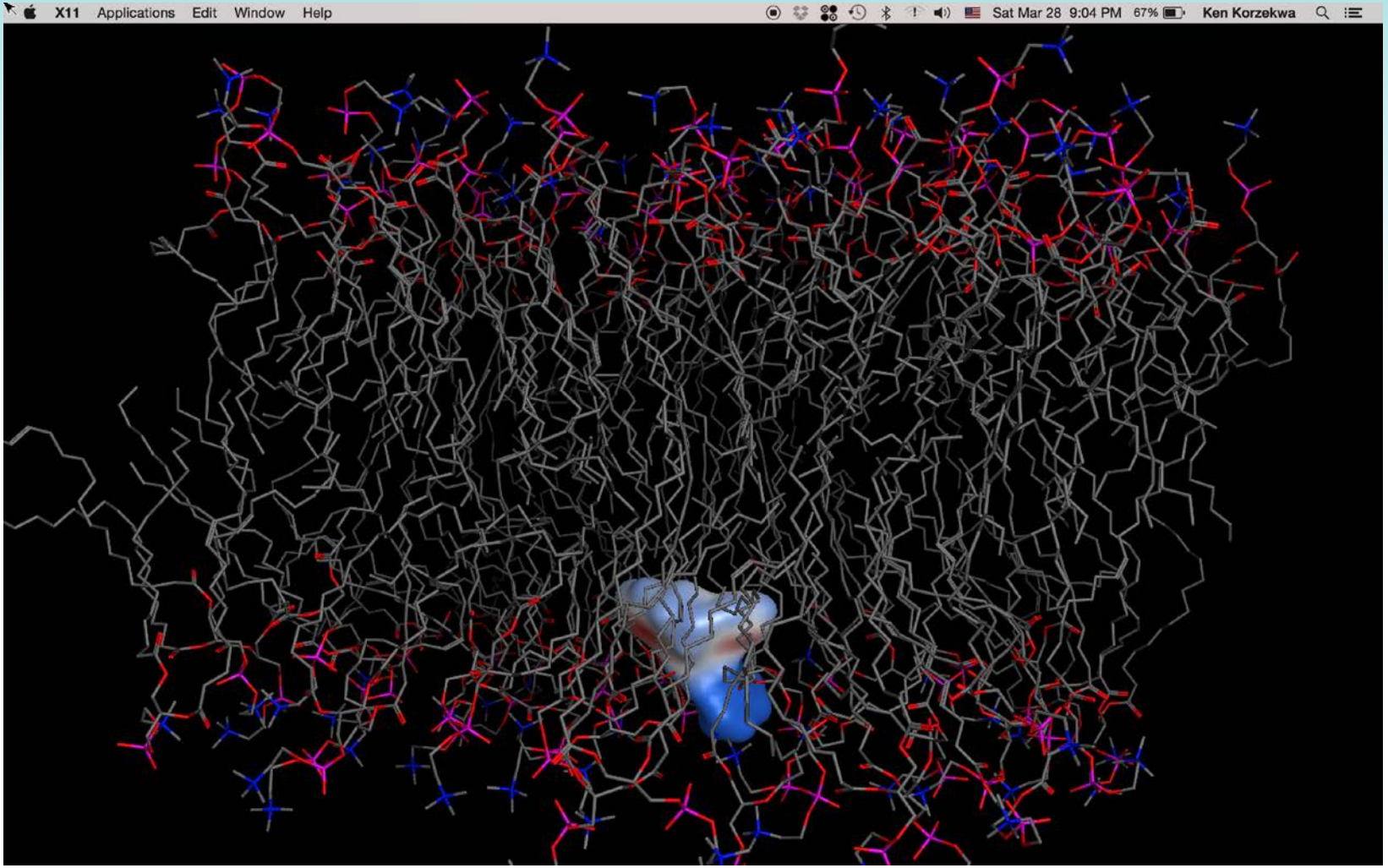
A General Model Drug Partitioning



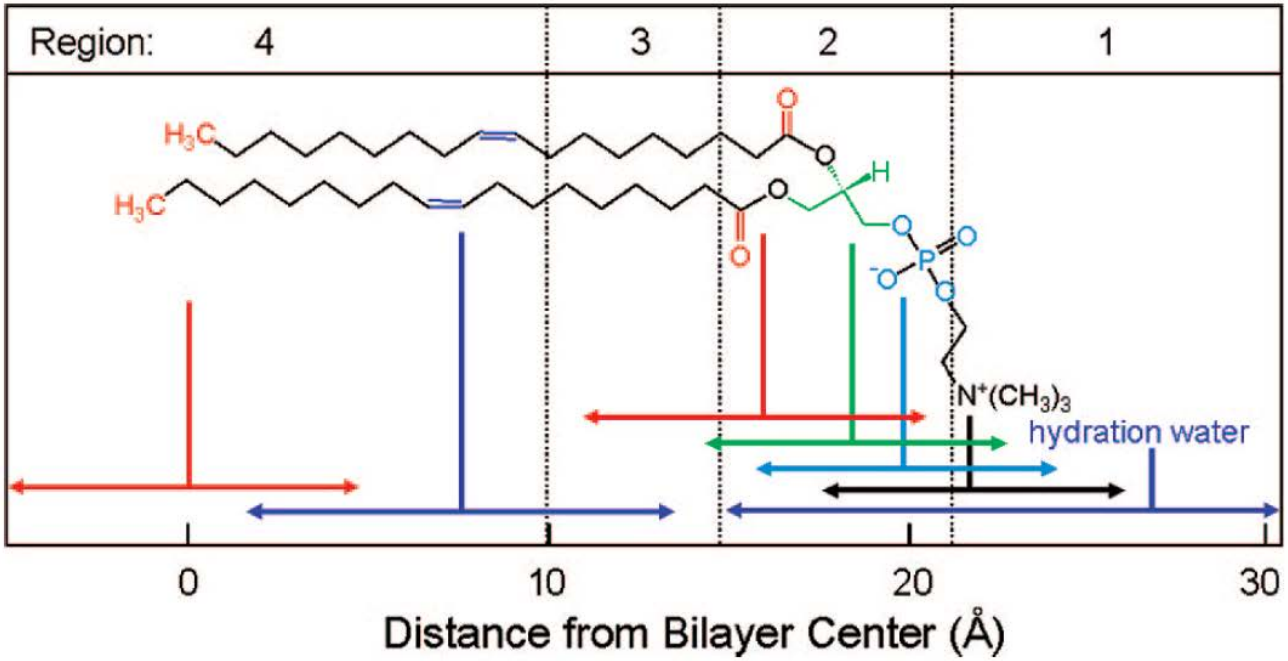
Membrane Partitioning

- "Non-specific binding" to tissues or cells is usually dominated by membrane partitioning.
  - Passive process.
  - Driven to a large extent by the affinity of a drug to an ordered phospholipid environment.
  - Neutral, acidic, and basic compounds partition very differently into phospholipids.
    - The extent that neutral compounds partition into membranes is determined by hydrophobicity, acceptors, donors, etc.
    - The ionized form of acids partition minimally into membranes.
    - Ionized bases readily partition into phospholipids.
- We use microsomal partitioning to parameterize drug-membrane interactions.

Hydrophobic Amine in the Lipid Bilayer



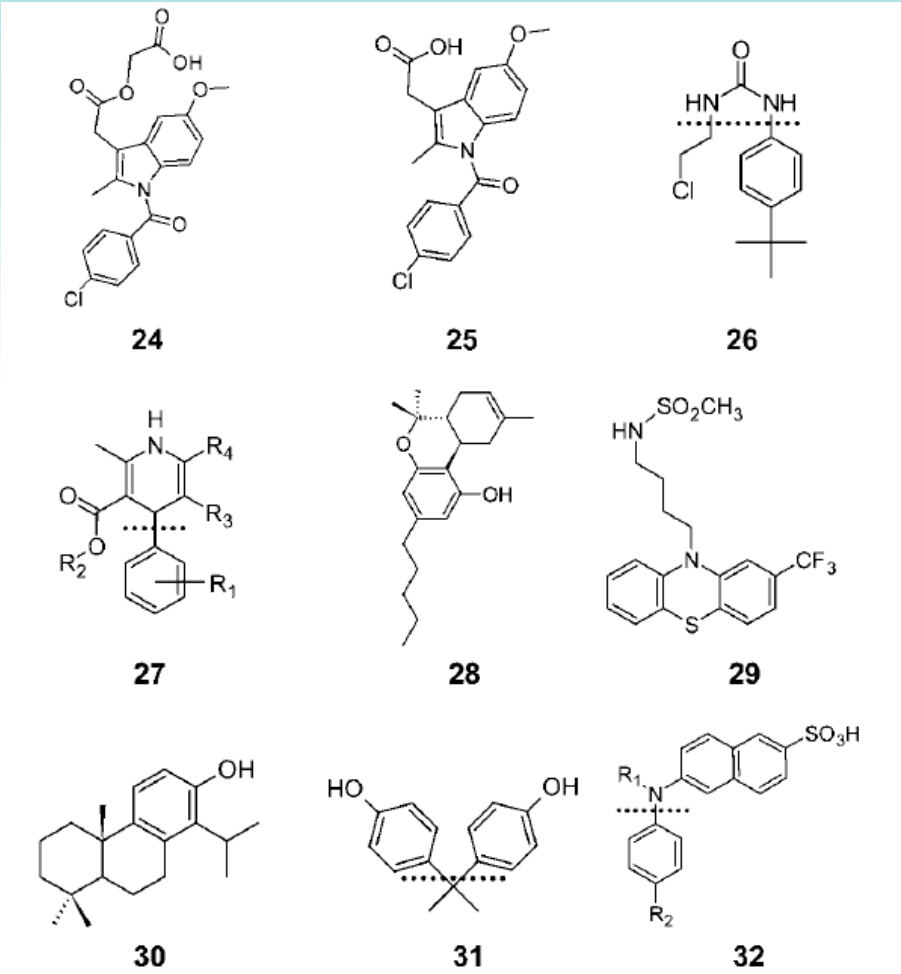
Regions of Phospholipid Membranes



Balaz, S. Modeling kinetics of subcellular disposition of chemicals. *Chem Rev* **109,** 1793-1899 (2009).

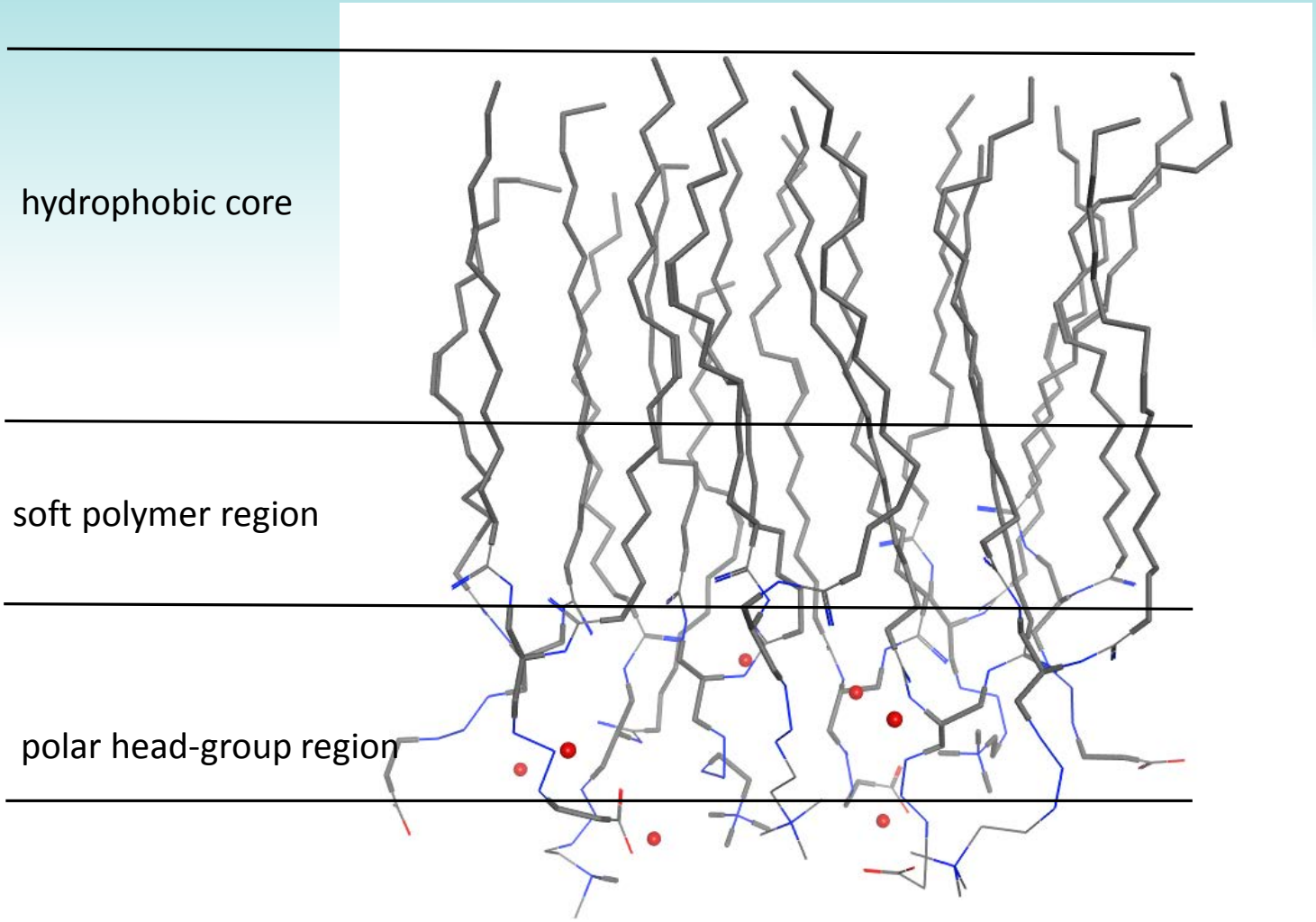
Orientation in Membranes

Molecules Found at the Hydrophobic/Polar Interface

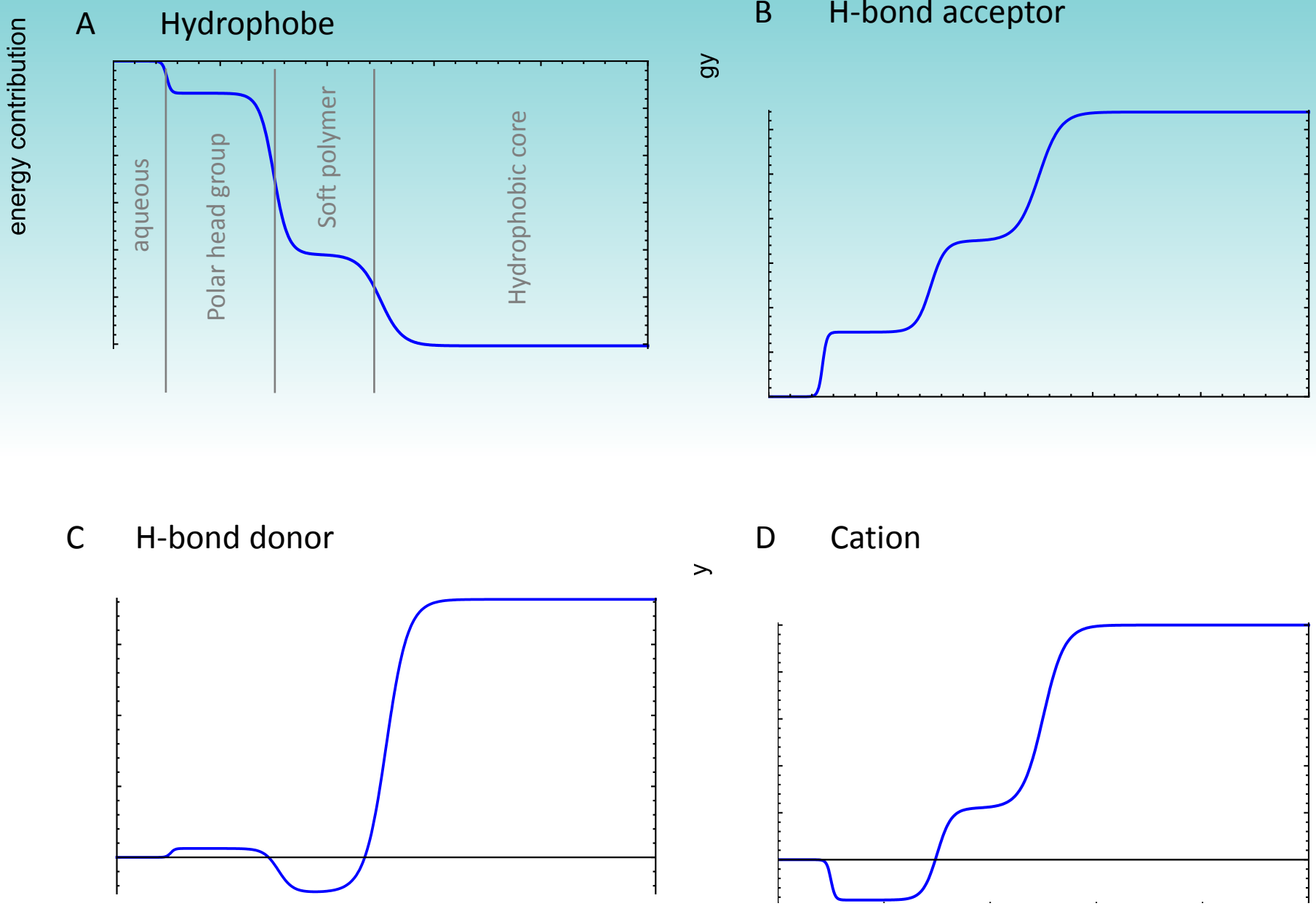


Balaz, S. Modeling kinetics of subcellular disposition of chemicals. *Chem Rev* **109,** 1793-1899 (2009).

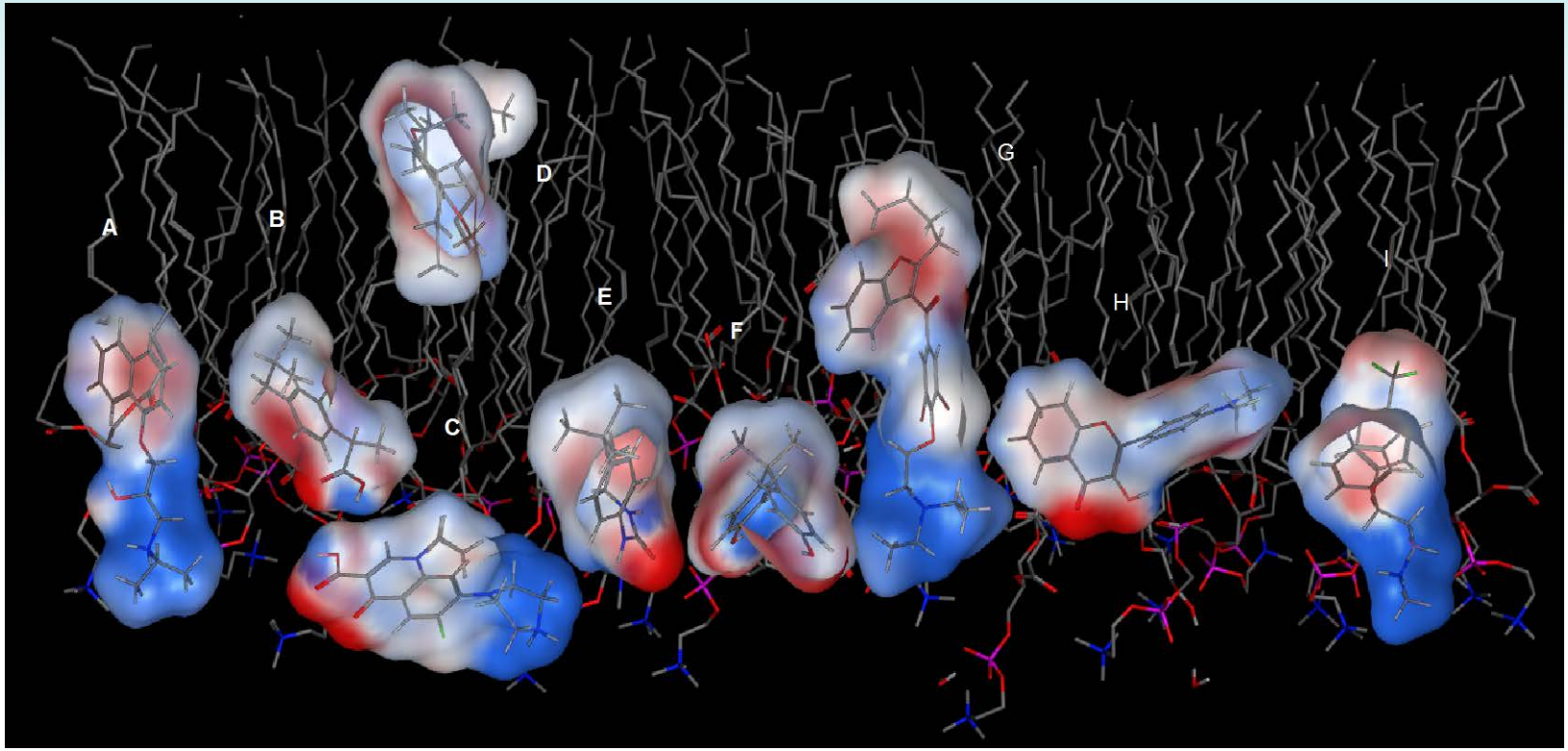
Template for a Membrane-Orientation-Based Model



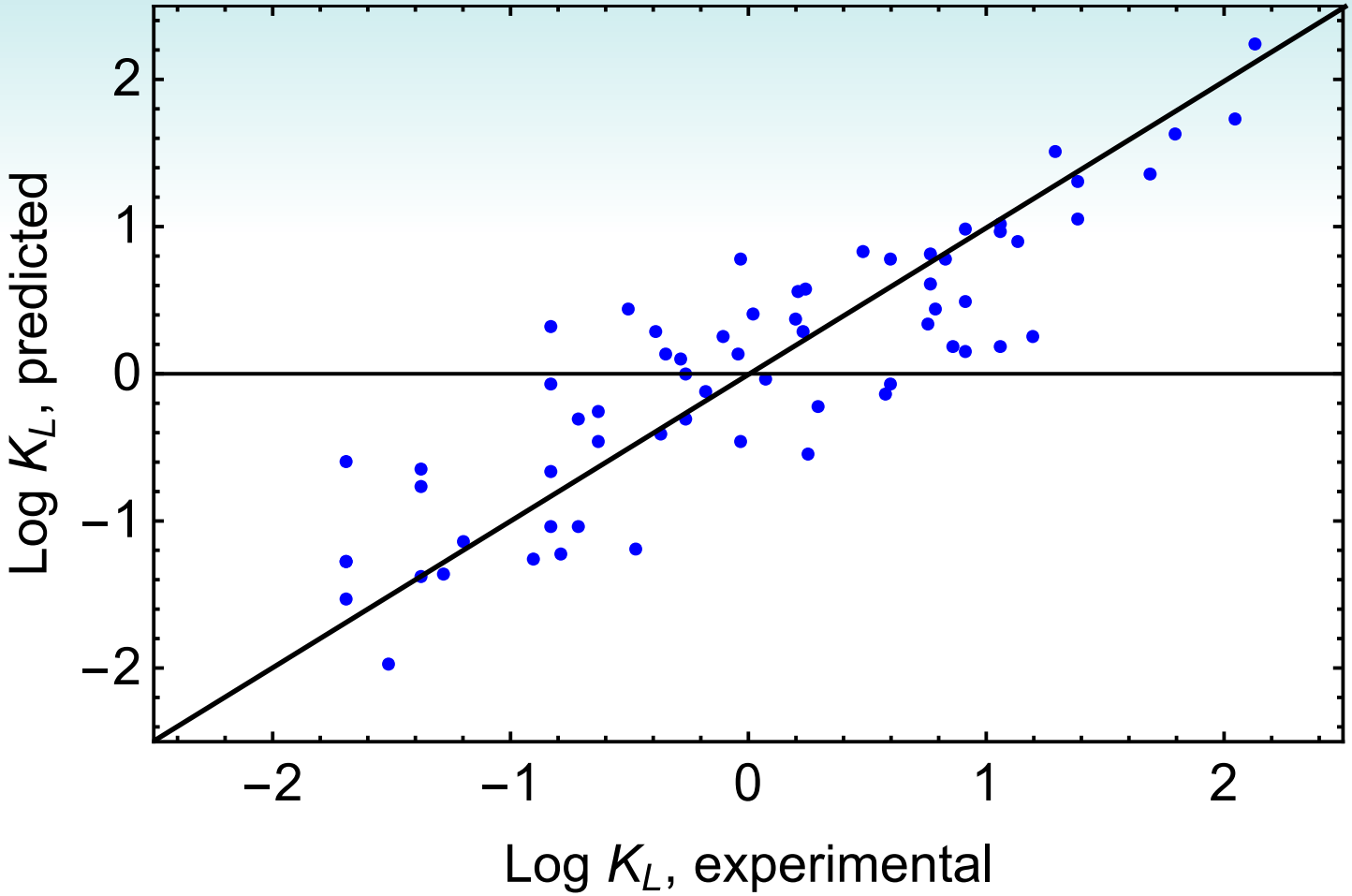
Nagar, S. & Korzekwa, K. Drug Distribution. *Pharm Res* **34,** 535-543 (2017).


energy contribution
A
Hydrophobe
aqueous
Polar head group
Soft polymer
Hydrophobic core
B
H-bond acceptor
gy
C
H-bond donor
y
D
Cation

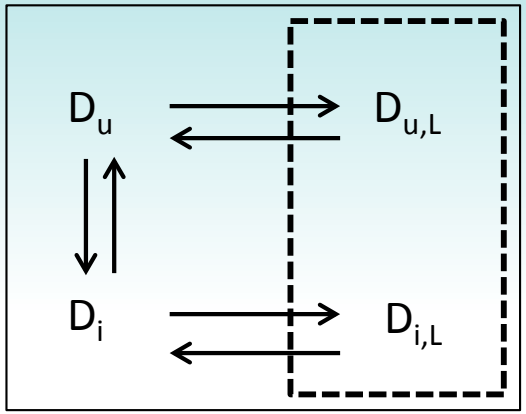
Predicted Membrane Orientation



Predicting f_{um} with an Orientation Model



Descriptor-Based Model for f_{um}

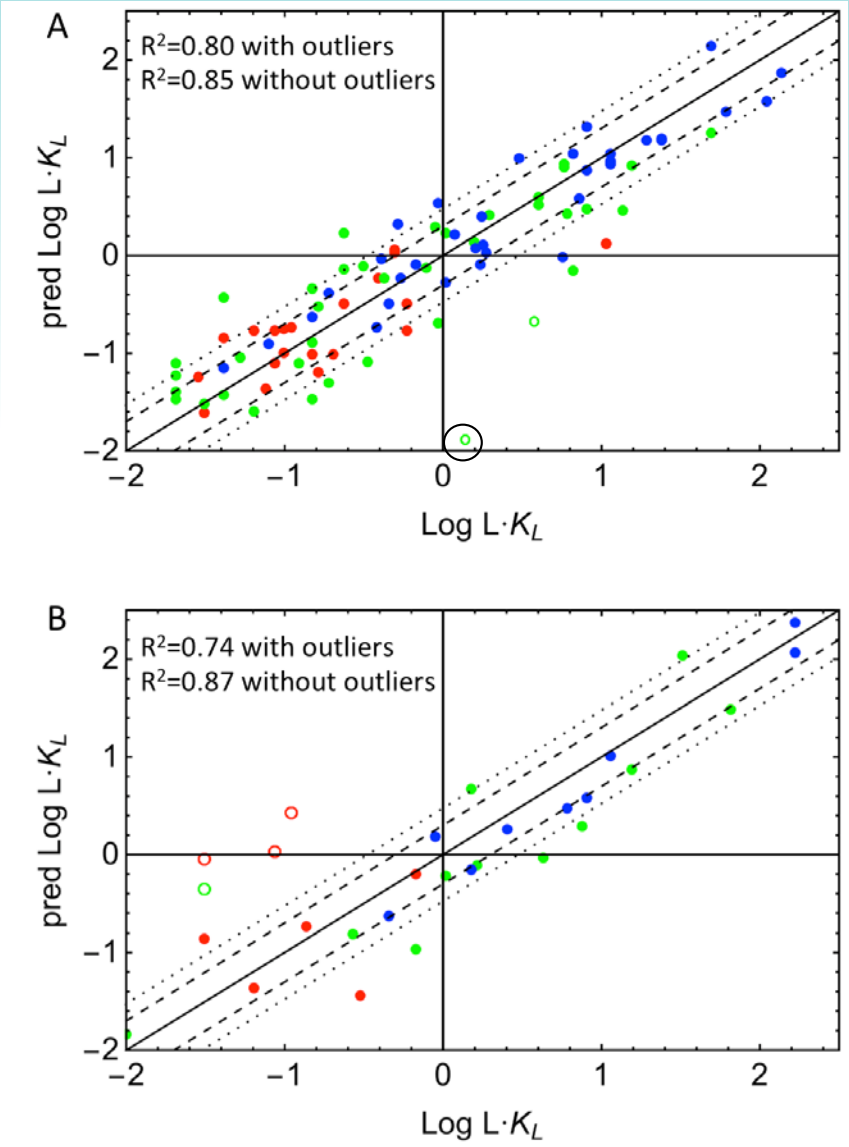


Conformation with the highest hydrophobic moment is selected.

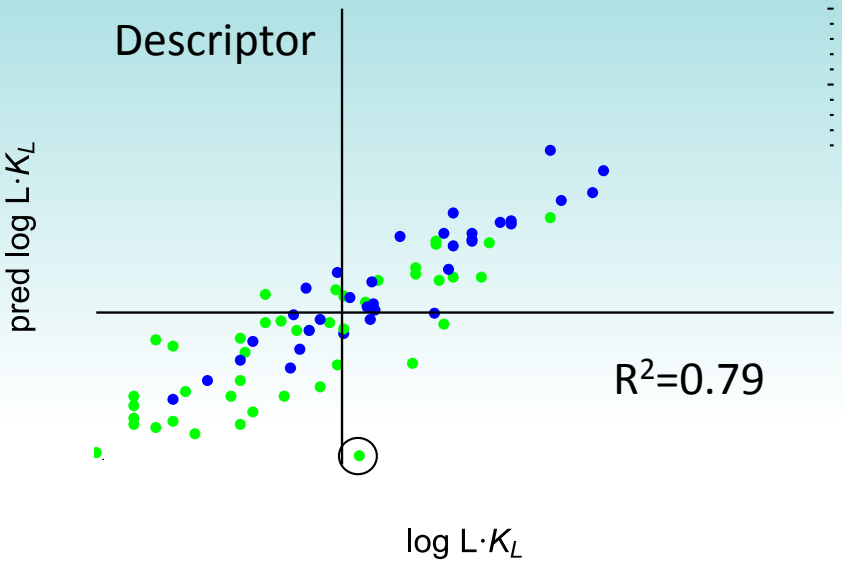
$$Log\ (LK_L) = Log\left(\begin{matrix}10^{const1+a1\ LogP+d1\ dipole+e\ SO+f\ NO2} + \\ 10^{const2+a2\ LogP+b2\ acc+c2\ don+e\ SO+f\ NO2+pKa,b-7.4} + \\ +\ 10^{const3+b3\ acc+e\ SO+f\ NO2-pKa,a+7.4}\end{matrix}\right) -$$

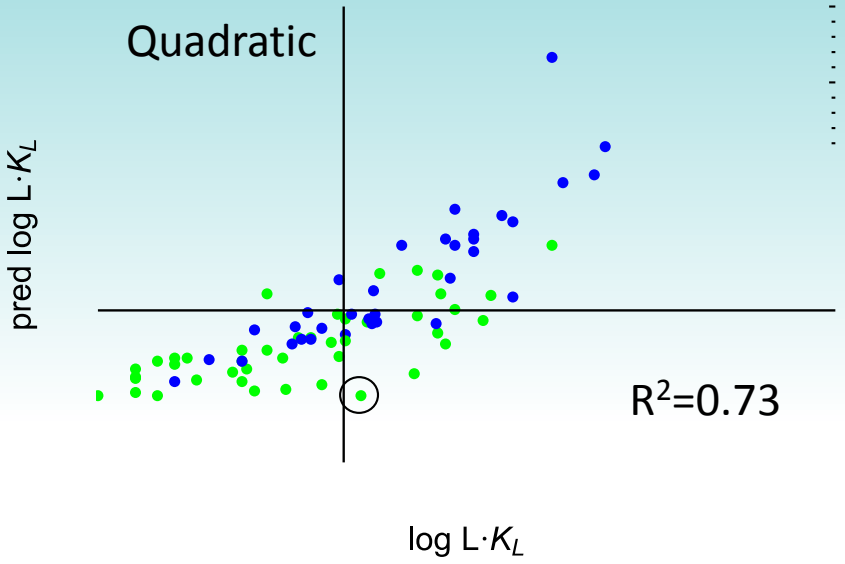
$$Log\left(1+10^{pKa,b-7.4}\right) - Log\left(1+10^{7.4-pKa,a}\right) - Log\left(1+10^{pKa,b-7.4}\right) - Log\left(1+10^{7.4-pKa,a}\right)$$

Descriptor-Based Model for f_{um}

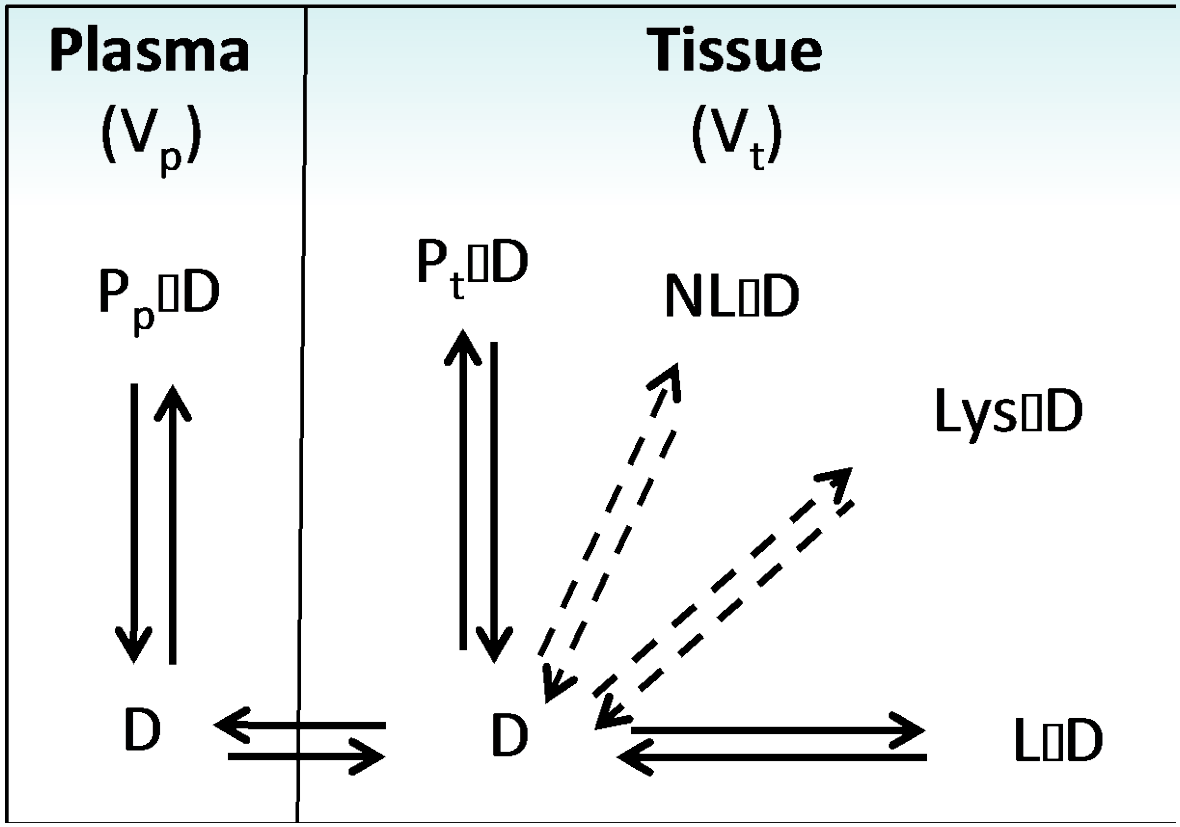


Descriptor-Based and Quadratic Models for fum





A General Model for Vss



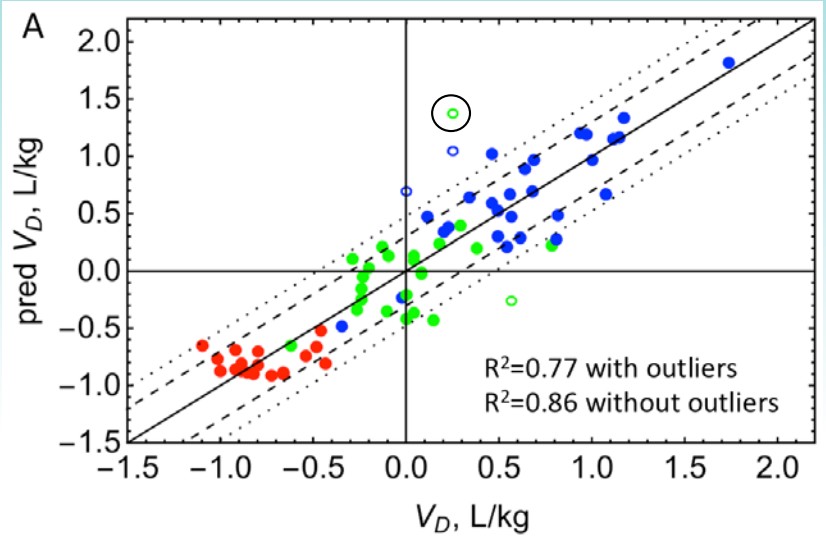
Volume of Distribution Models

Model	AICc[b]	AICc	R^2, AAFE	Parameter Estimate ± error			
	n=63	n=60		a	b	c	e
Linear LK_L $V_D = V_p + V_t R_1 (1 - f_{up}) + V_t f_{up} + f_{up}\left(a\left(\frac{1 - f_{um}}{f_{um}}\right) + b\right)$	39.6	15.4	0.84, 1.6	20.0 ±0.2	0.76 ±0.43	--	--
Linear LK_L + neutral lipids $V_D = V_p + V_t R_1 (1 - f_{up}) + V_t f_{up} + f_{up}\left(a\left(\frac{1 - f_{um}}{f_{um}}\right) + b + c\, P_{OW}\right)$	39.3	15.3	0.84, 1.6	19.9 ±2.5	0.76 ±0.43	0	--
Linear LK_L + lysosomes (bases[d]) $V_{ss} = V_p + V_t f_{up} + V_t R_1 (1 - f_{up}) + f_{up}\left(a\left(\frac{1 - f_{um}}{f_{um}}\right) + b + e\left(\frac{10^{pKa,b-4.8} + 1}{10^{pka,b-7.2} + 1}\right)\right)$	38.1	14.7	0.84, 1.6	18.1 ±2.6	0.62 ±0.40	--	0.003 ±0.002

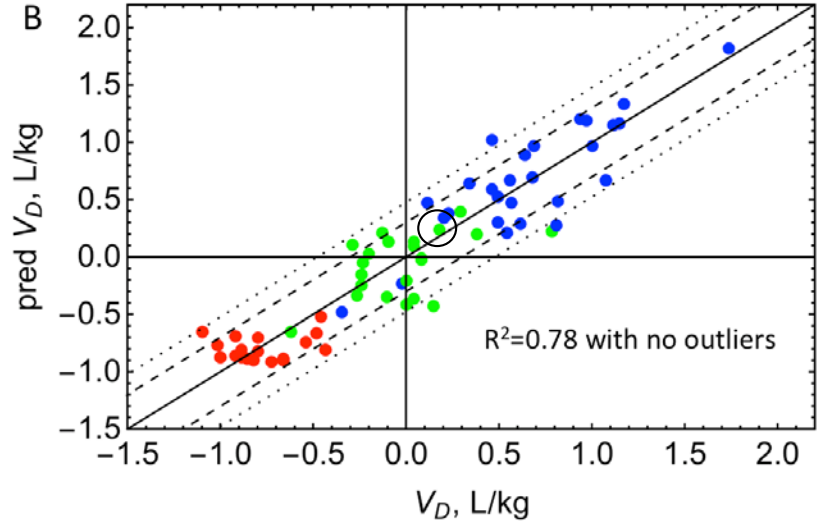
Compounds limited to those reported by Obach, R. S., Lombardo, F. & Waters, N. J. *Drug Metab Dispos* **36,** 1385-1405 (2008).

Korzekwa, K. & Nagar, S. *Pharm Res* **34,** 544-551 (2017).

Model for V_{ss}

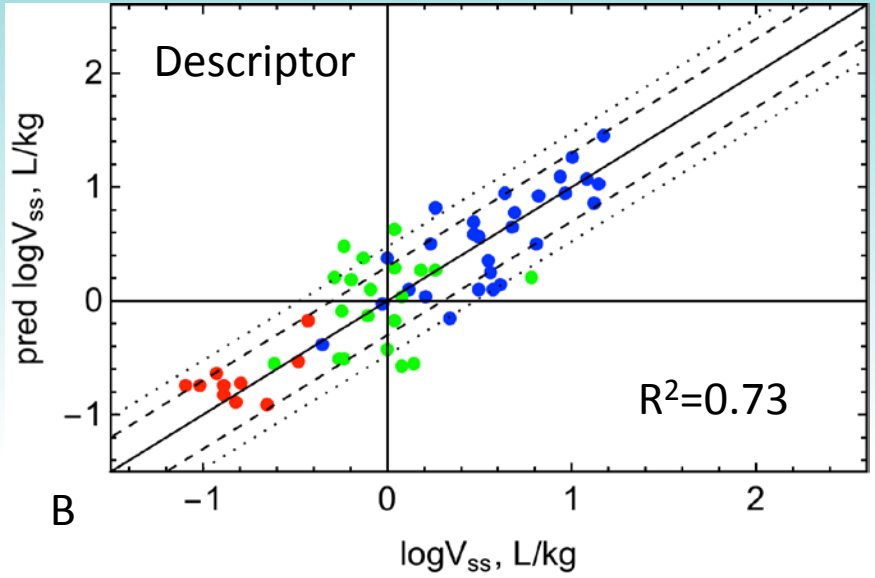


Experimental fum

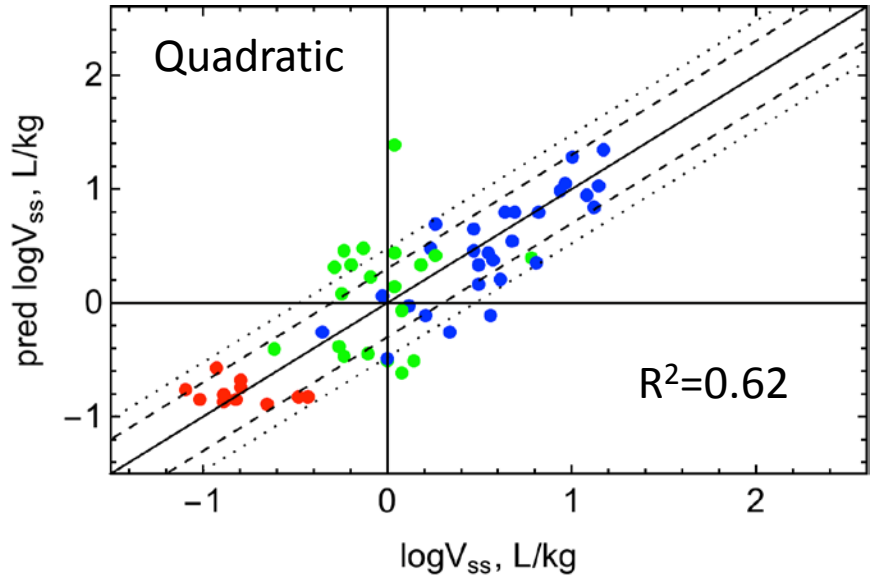


Predicted fum

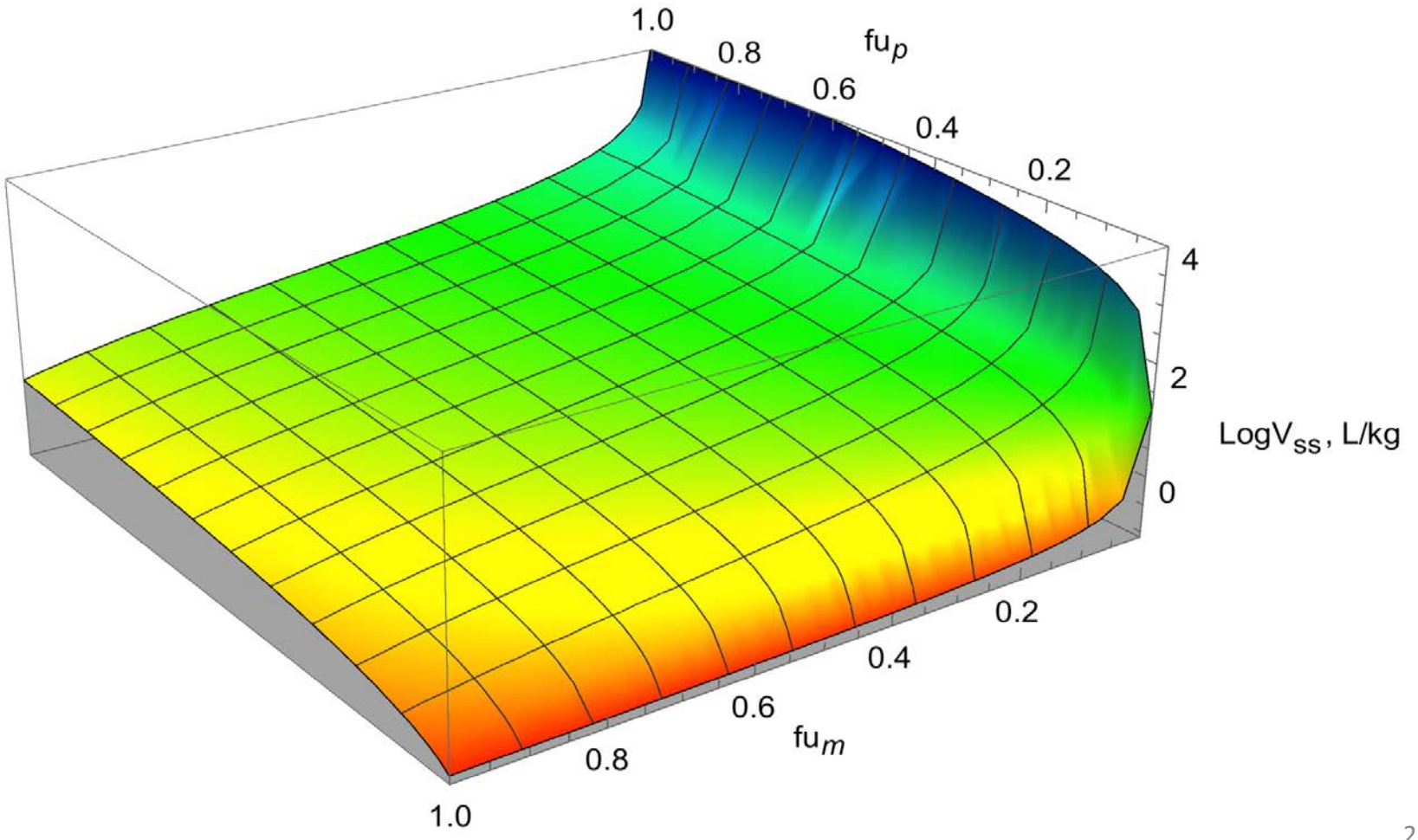
Descriptor and Quadratic fum to Predict Vss



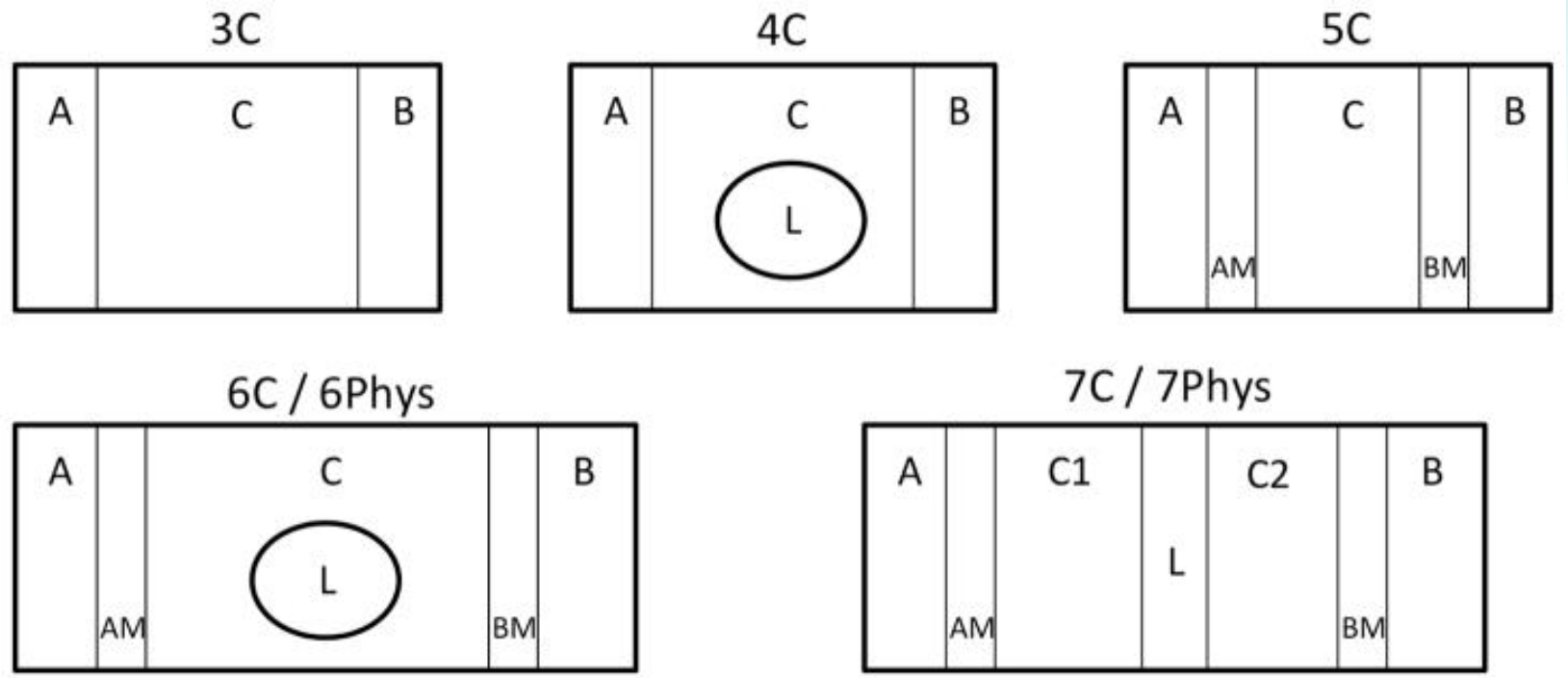
B



Compounds with Low fum or fup Values can have Large Errors in Predicted V_{ss}

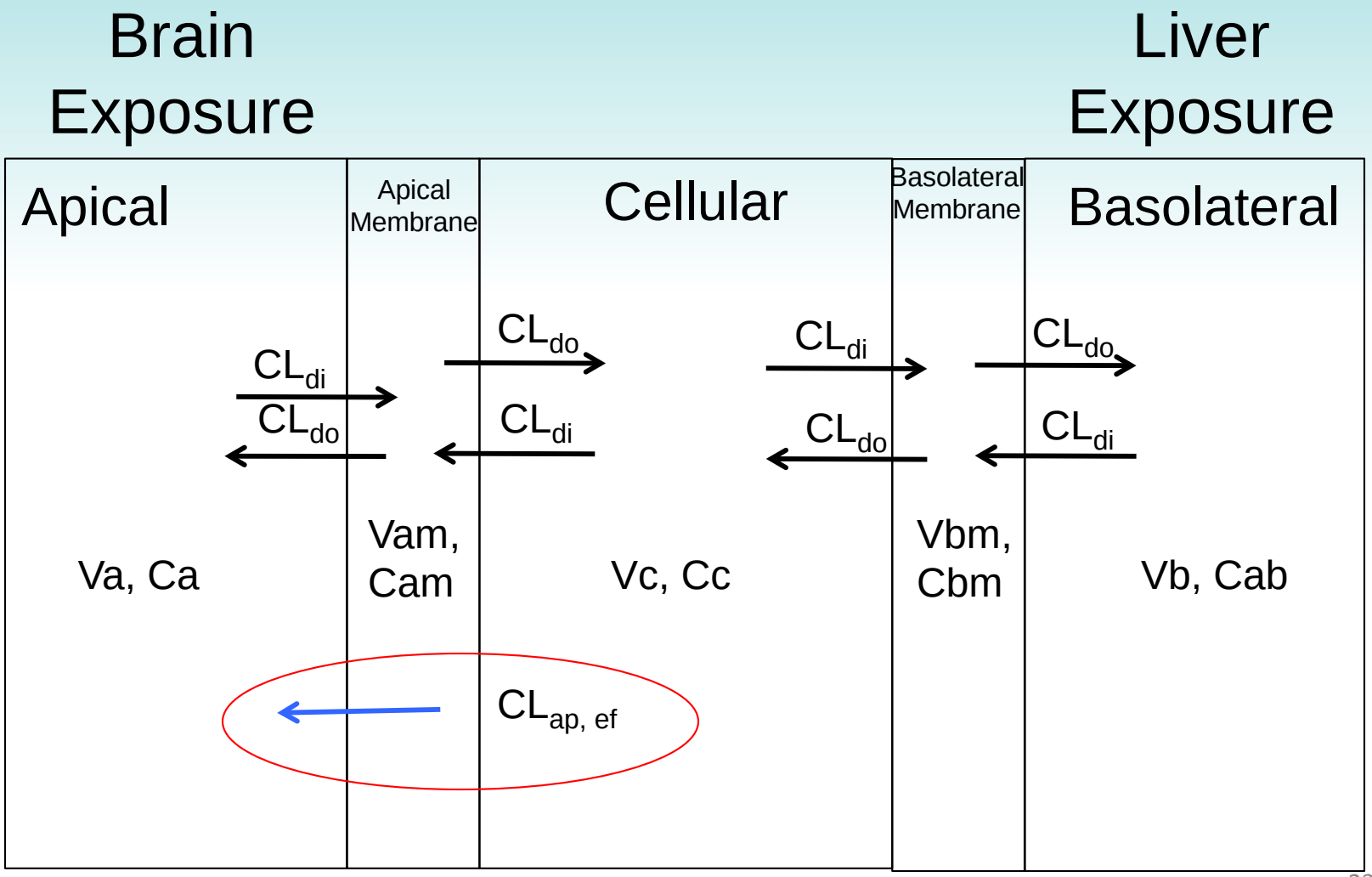


Compartmental Models for Permeability-Limited Distribution



Nagar, S., Tucker, J., Weiskircher, E. A., Bhoopathy, S., *et al*. *Pharm Res* **31,** 347-359 (2014).

5-Compartment Model

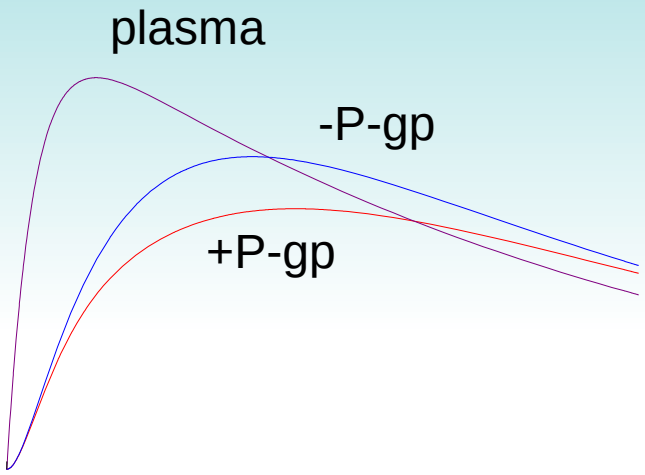


Tissue Concentrations -/+ Efflux Activity

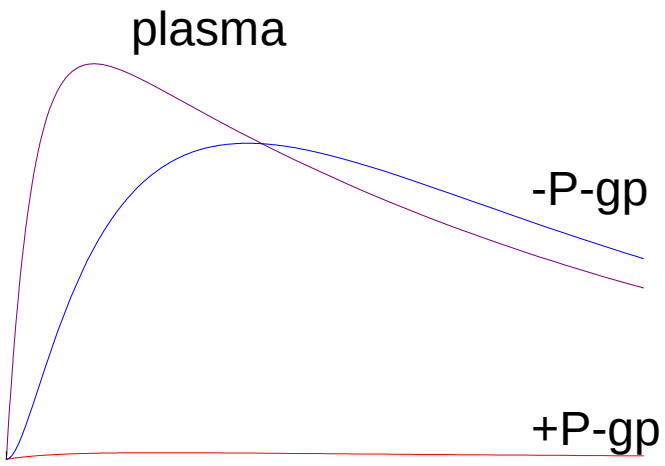
Drug	Brain Conc. Ratio mdr1(-/-)/ mdr1(+/+)	Predicted $C_{cell,AB}$ ratio	Liver Conc. Ratio mdr1(-/-)/ mdr1(+/+)	Predicted $C_{cell,BA}$ ratio
Verapamil	9.5	6.4	1.1	1.9
Verapamil	7.7	6.4	n.d.	1.9
Loperamide	65	43	n.d.	1.9
Loperamide	31	43	n.d.	1.9
Loperamide	13.5	43	3.1	1.9
Pitavastatin	1.3	10	0.88	1.1
Digoxin	35.3	31	2.0	1.5
Morphine	1.7	n.d.	1.1	n.d.
Dex.	2.5	n.d.	1.1	n.d.
CsA	17	n.d.	1.2	n.d.
Ondansetron	4.0	n.d.	0.9	n.d.
Vinblastine	22.4	n.d.	1.8	n.d.
Asimadoline	9.1	n.d.	1.1	n.d.
Nelfinavir	16.1	n.d.	3.0	n.d.
Selamectin	4.9	n.d.	0.5	n.d.
Ivermectin	59	n.d.	3.7	n.d.
Grepafloxacin	2.35	n.d.	0.88	n.d.
Tacrolimus	6.0	n.d.	1.7	n.d.
Apafant	73.6	n.d.	4.5	n.d.
SDZ PSC 833	2.1	n.d.	0.9	n.d.

Schinkel, J Clin Invest 1995; 96: 1698-1705; Kalvass, Pharm Res 2004 21:1867-1870; Hsiao, JPET 2006 317:704-710; Korzekwa, DMD 2012; 40, 865-876

Effect of P-gp on Brain and Liver concentrations

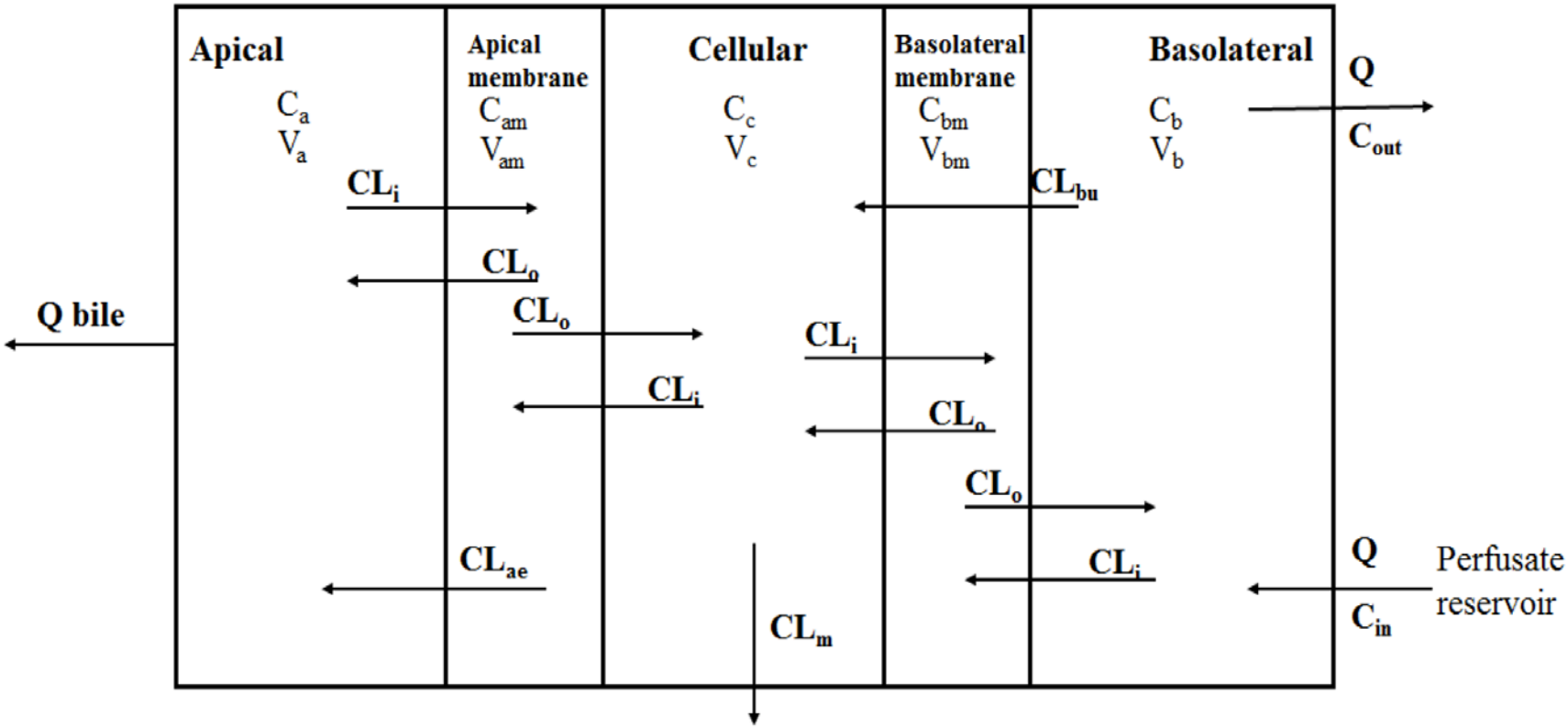


Oral Verapamil
Basolateral Exposure
(Liver)



Oral Verapamil
Apical Exposure
(Brain)

Model for Liver Perfusion Studies



Kulkarni, P., Korzekwa, K. & Nagar, S. *J Pharmacol Exp Ther* **359,** 26-36 (2016).

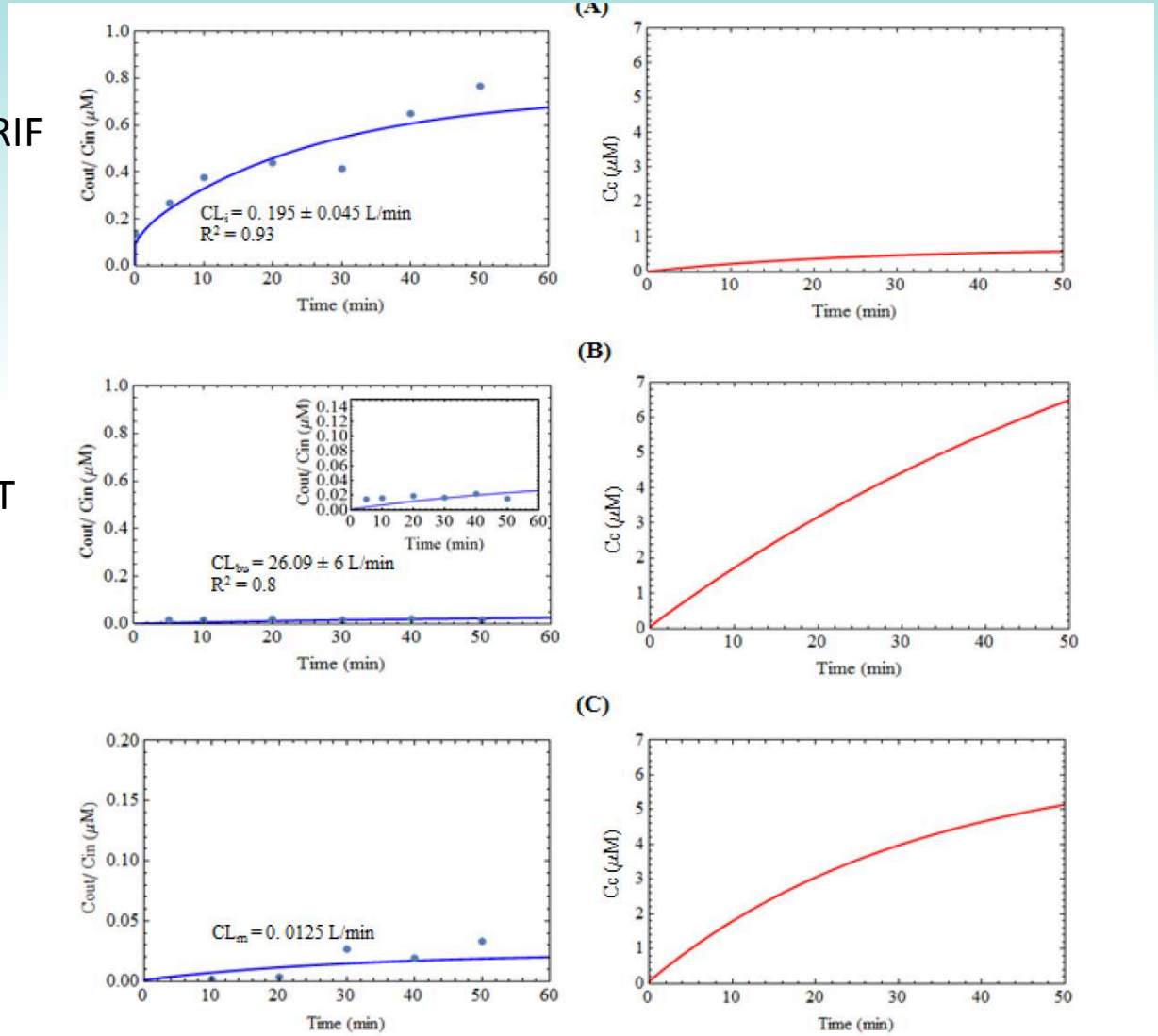
Atorvastatin – Liver Perfusion Studies

C_{in} - C_{out} Intracellular Conc.

ATV+ABT+RIF

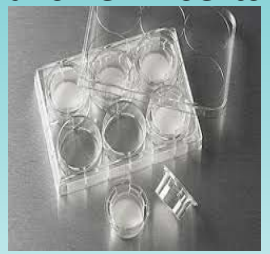
ATV+ABT

ATV



Experimental APPROACH: Confocal Microscopy

transwell inserts

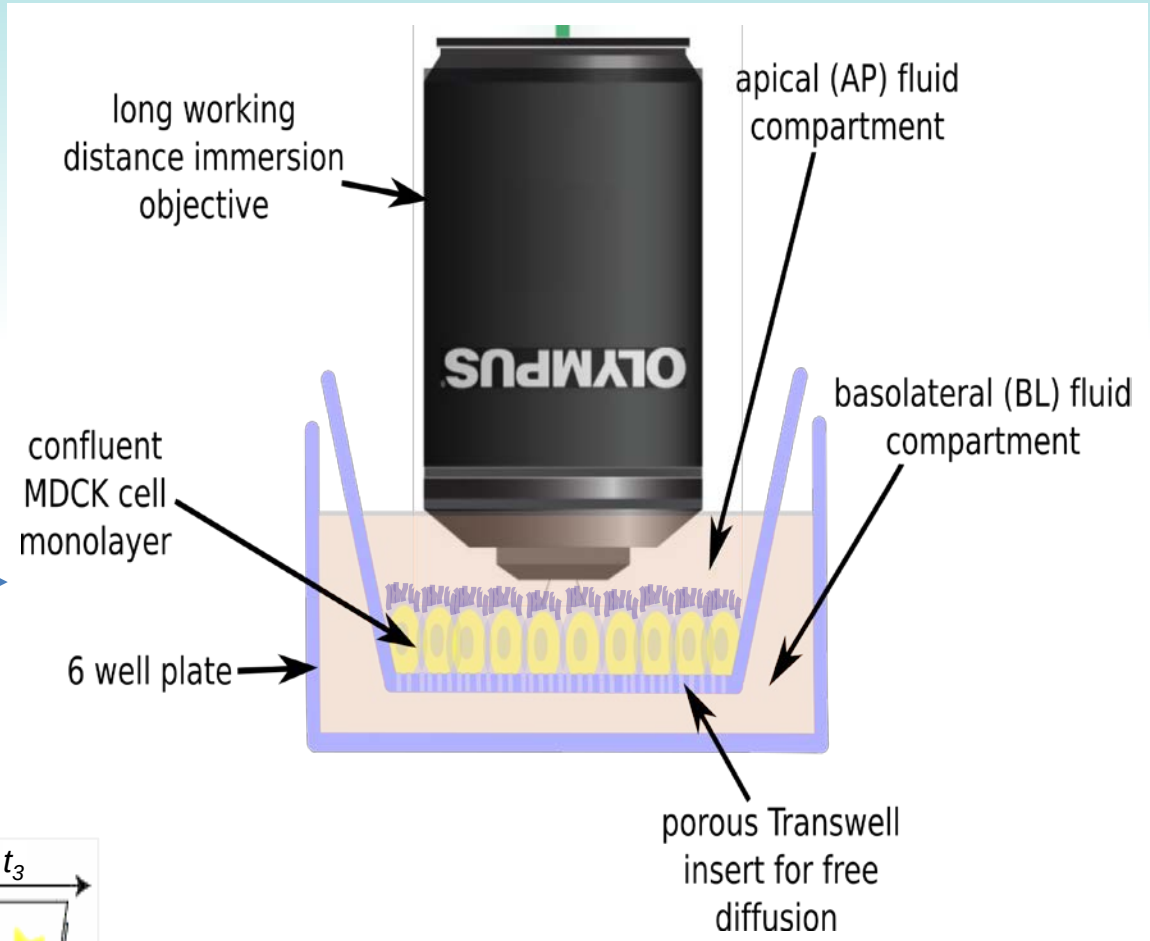


STEP 1: Grow MDCK cells to confluence

STEP 2: Inhibit endogenous transporters

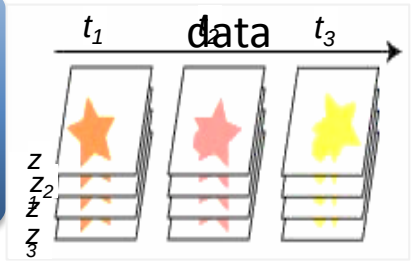
cyclosporine A
rifampicin

STEP 3: Set up on microscope stage



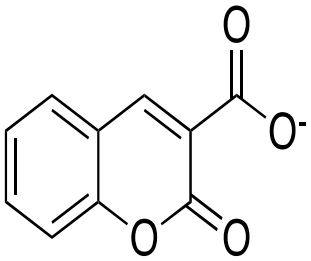
STEP 4: Add fluorescent drug-like compound to AP or BL chamber and collect *xyzt* fluorescence data

xyzt data



TEST compounds

Increasing lipophilicity



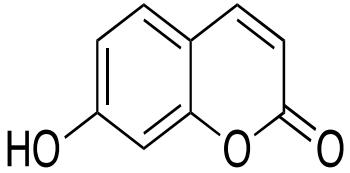
coumarin 3-COOH

pKa = 3.28

anion

cLogD -2.8

hydrophilic

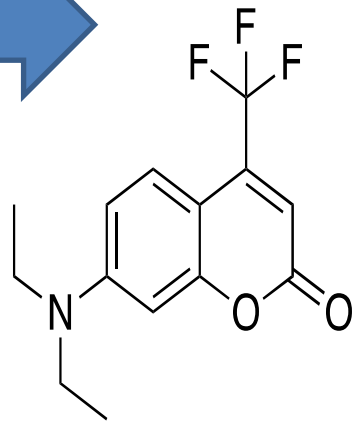


7-OH coumarin

pKa = 7.7

neutral

cLogD
1.6



coumarin 152A

pKa = ~6.57

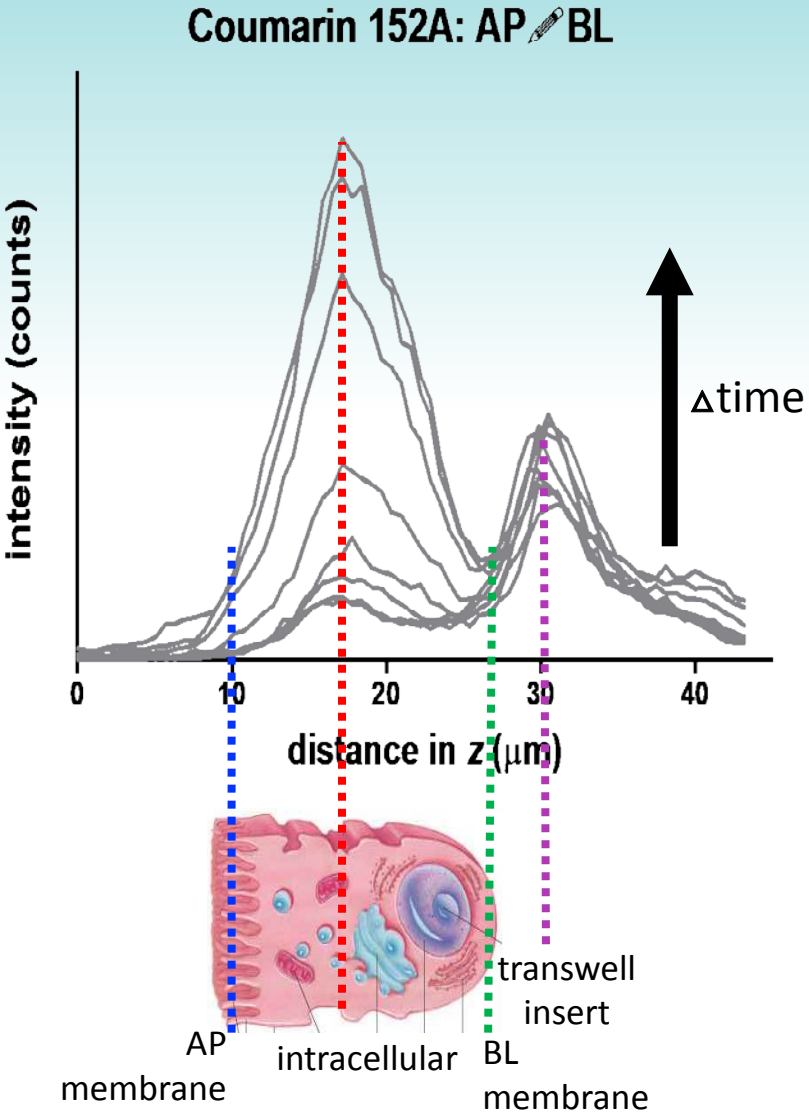
neutral/cation

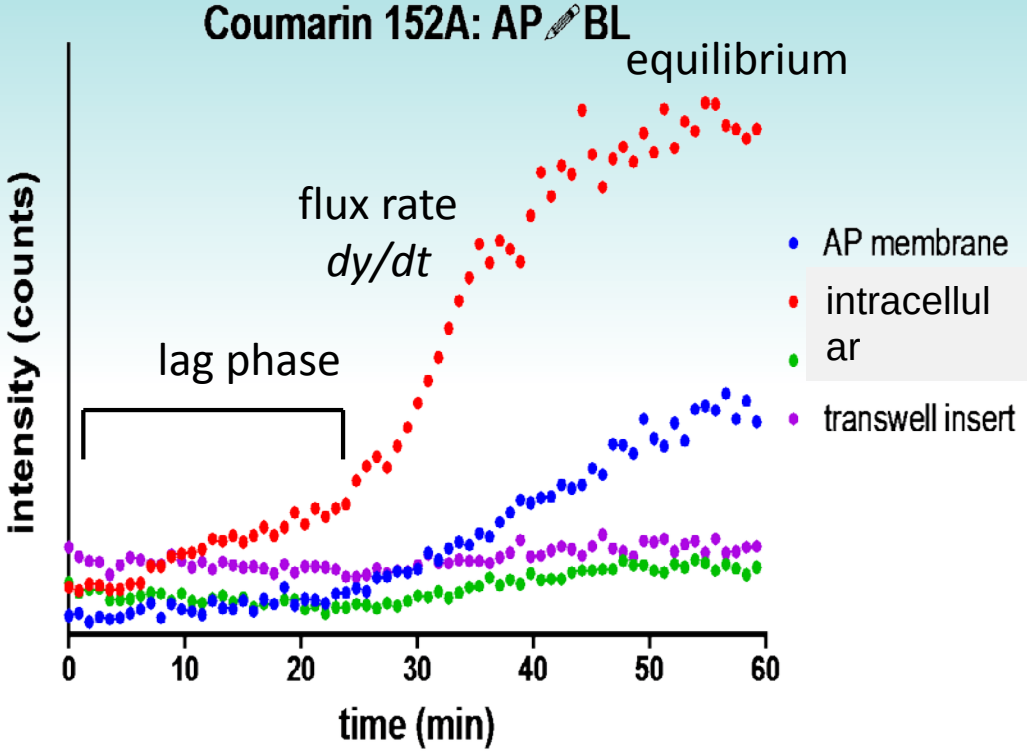
3.5 < cLogD < 4.1

lipophillic

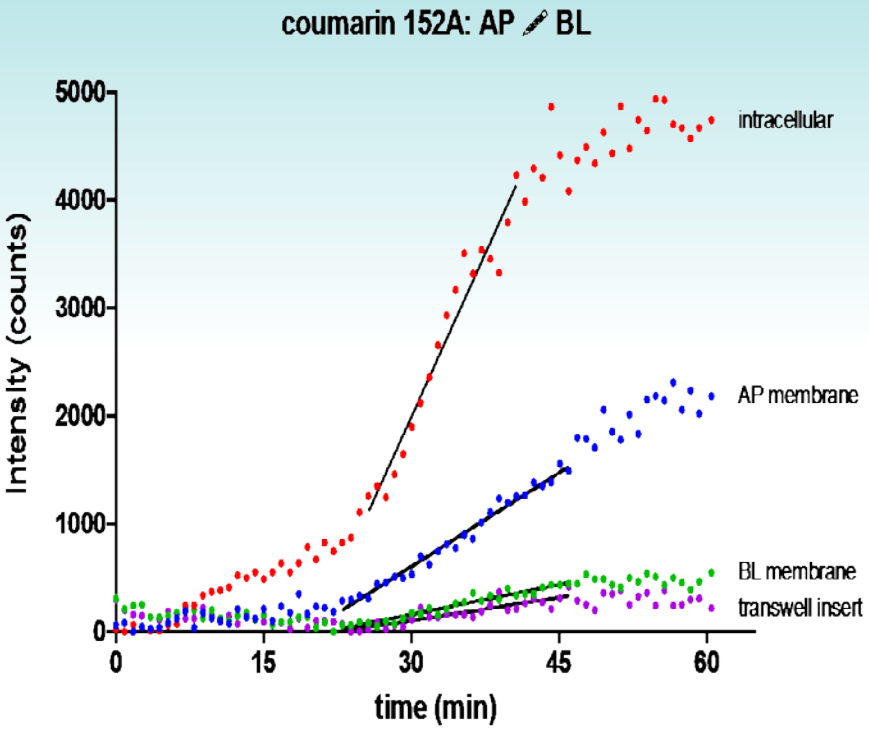
Testa, B. and P. Jenner. Drug Metabolism: Chemical & Biochemical Aspects. New York: Marcel Dekker, Inc., 1976., p. 88

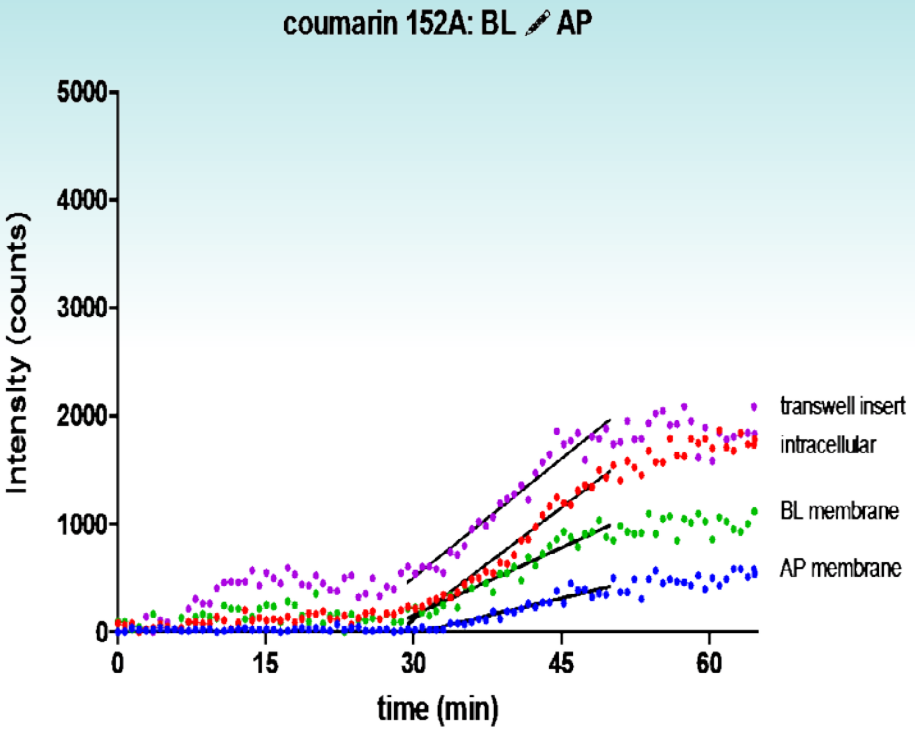
EXAMPLE RAW DATA





Determination of Quasi-apparent Permeability (~P_{app})





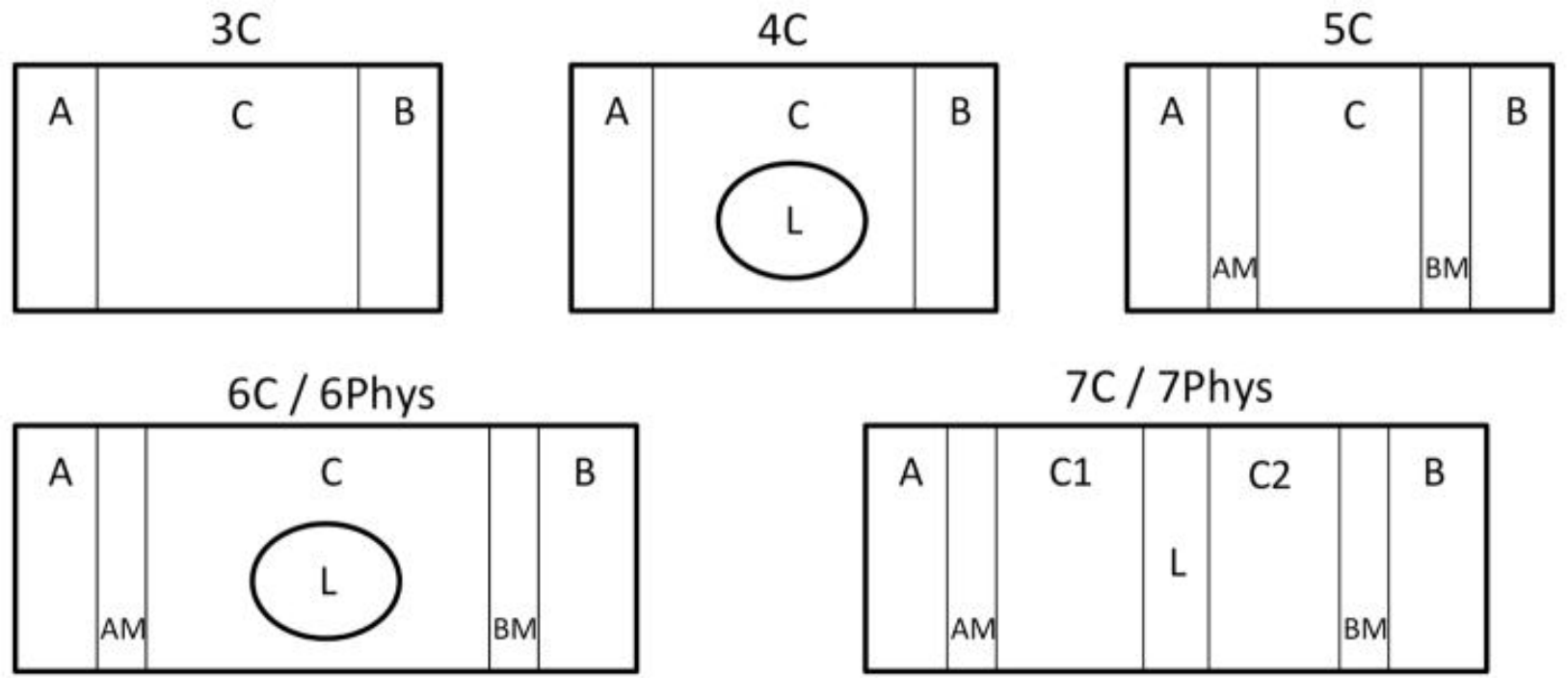
$$\sim P_{app} = dR/dt \times 1/(A\ C_{donor})$$

dR/dT = rate of appearance

A = area

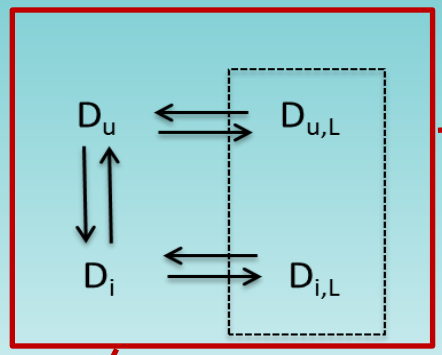
C_{donor} = concentration of donor

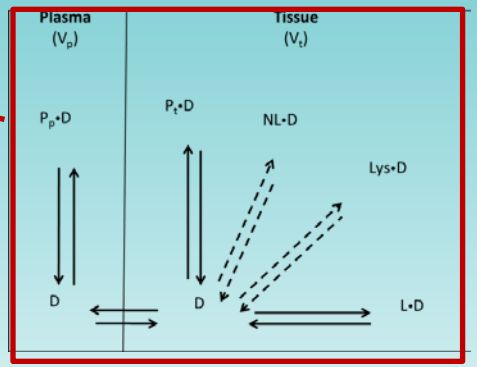
Compartmental Models for Permeability-Limited Distribution



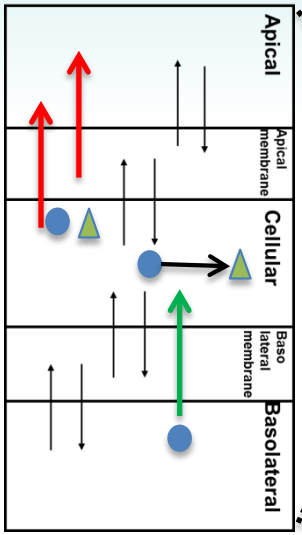
Summary

- Orientation-based and descriptor-based membrane partitioning models have been constructed.
- Membrane partitioning and plasma protein binding can be used to model Vss.
- Hybrid PBPK-compartmental models have been constructed to predict intracellular concentrations.
- Confocal microscopy is being used to watch molecules traverse a cell monolayer.





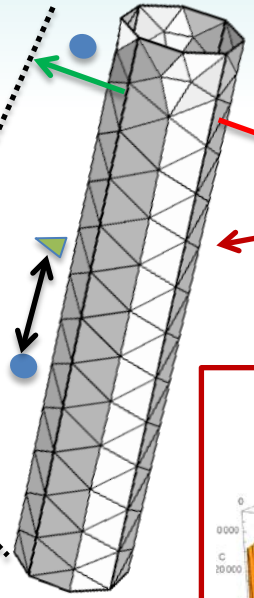
Tissue Distribution

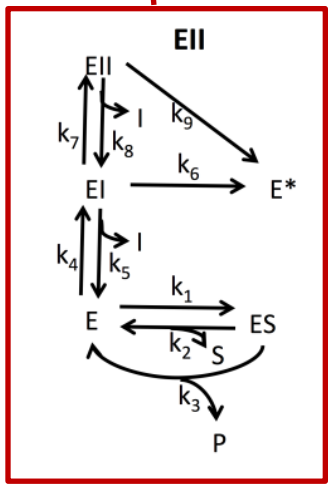


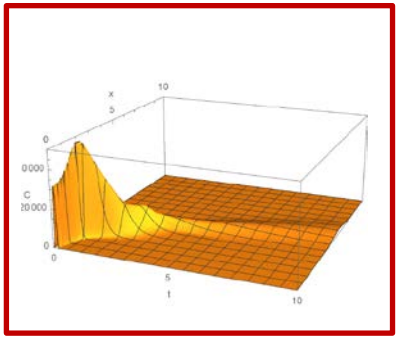
Liver

EHR

Intestine







Drug
Metabolite
Active uptake
Active efflux
Elimination