



TRANSIL Brain Absorption:

A novel in vitro model for predicting CNS penetration

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High Throughput Screening for Biological Systems

March 16th, 2009

Outline

- Significance of the Blood-Brain Barrier
- Key criteria for compound optimization
- What can we measure with existing models?
- Advantage of estimating both f_u brain and logBB

TRANSIL Brain Absorption

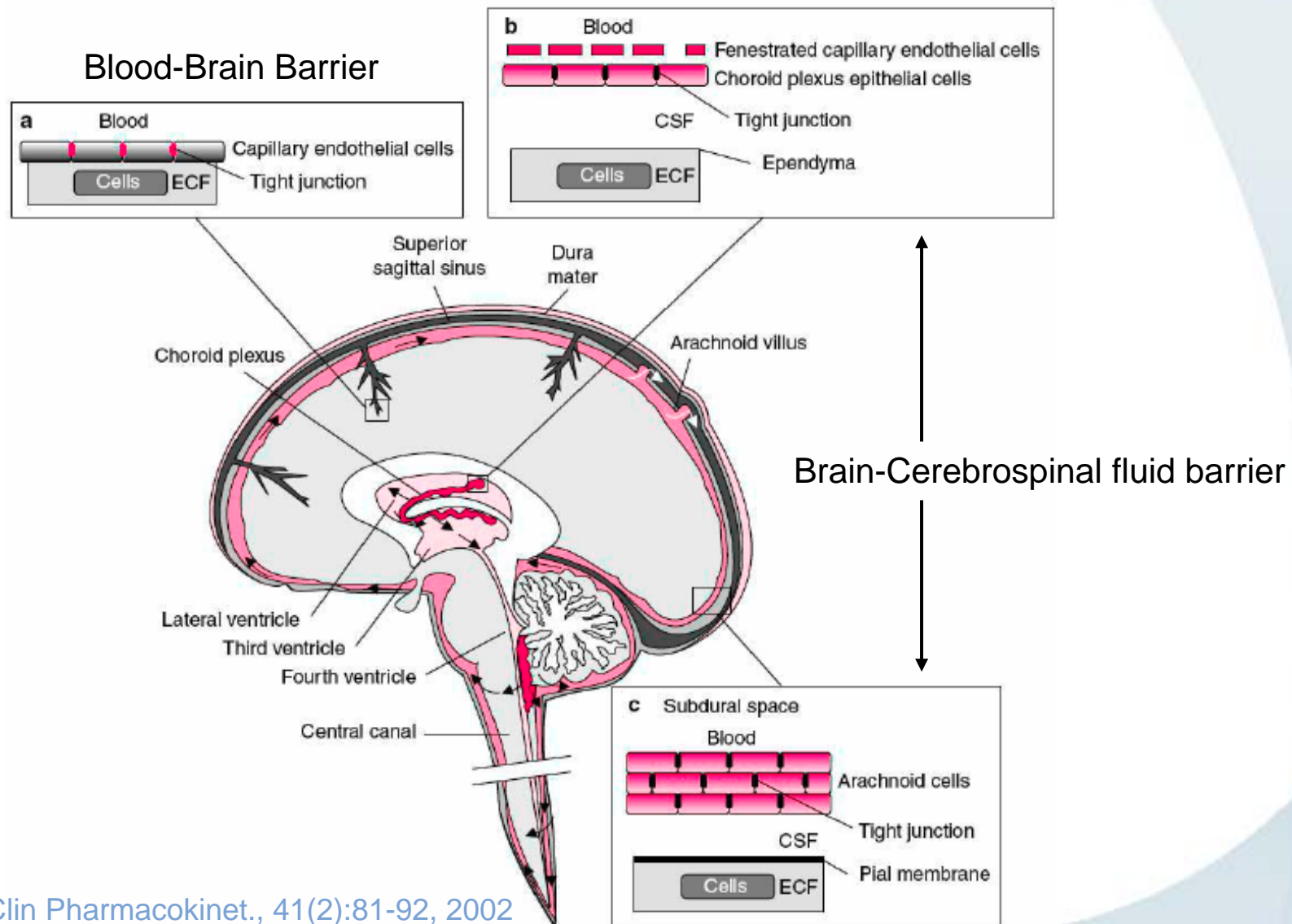
Key Features

- Prediction of brain availability ($\log BB$ and f_u brain)
- Prediction of concentration at receptor site
- Classification of CNS+ /CNS- compounds
- Fast: 2 min incubation time
- Easy: First ready-to-use assay kit

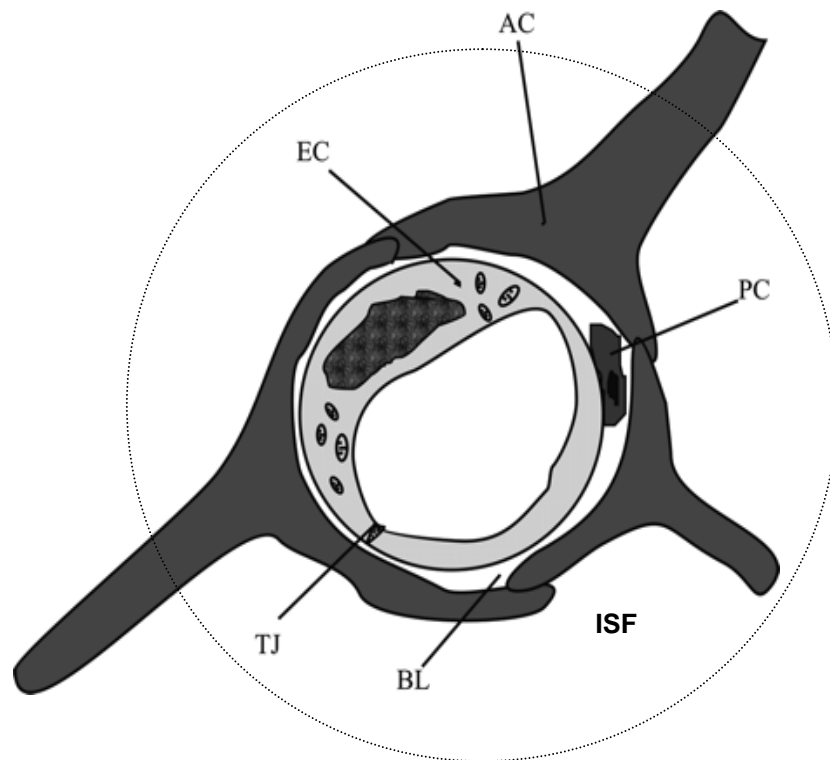
Significance of Blood-To-Brain Absorption

- Blood Brain Barrier protects the brain from xenobiotics while maintaining metabolic functions
- Significant hurdle for CNS targeted drugs
- Important protection from drug side-effects
- CNS-accessible chemical space much smaller in comparison to other organs

Brain Barriers

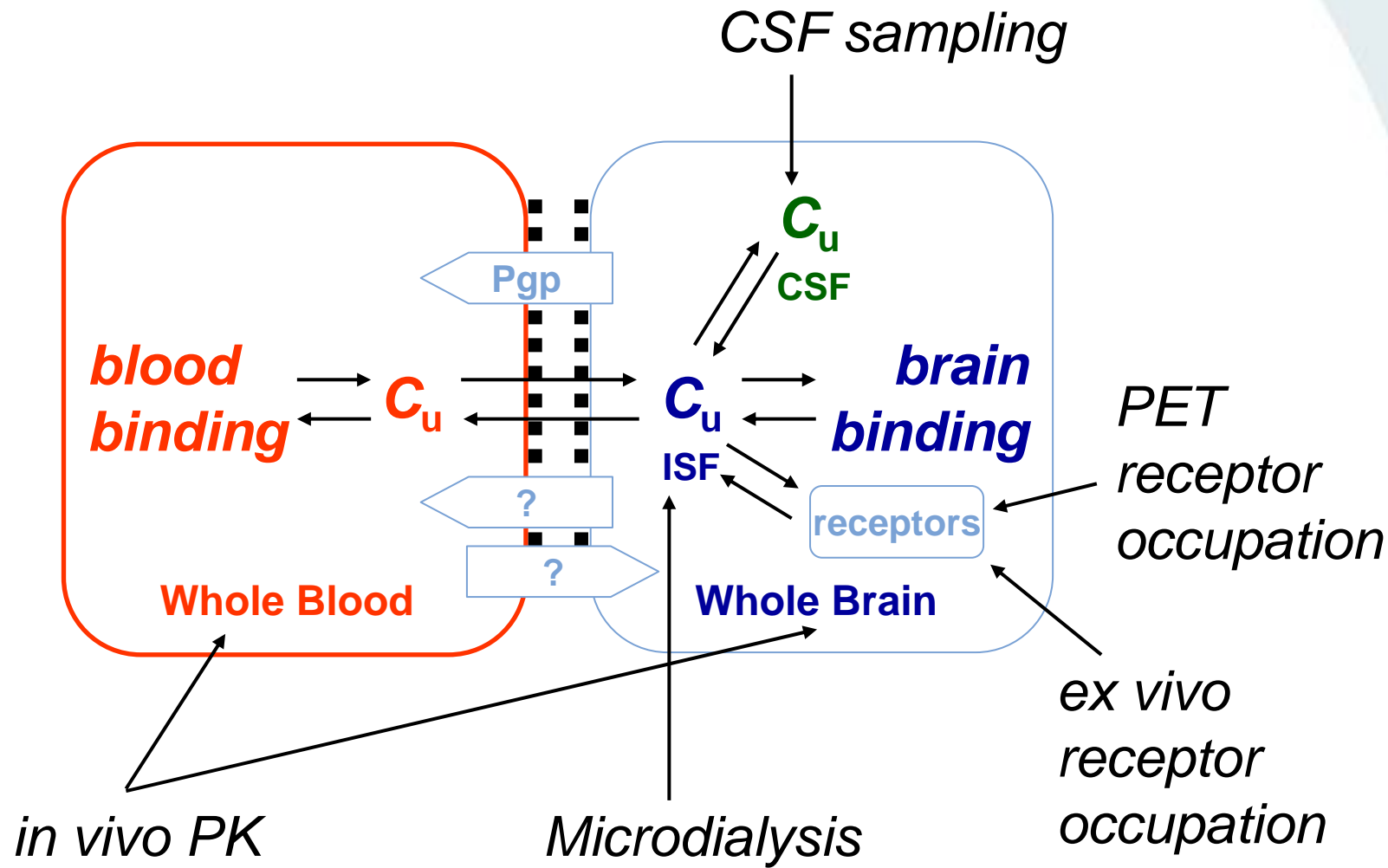


Neurovascular Unit



- Endothelial Cells
 - Interface btw blood and brain
 - Single cell encloses lumen
 - Tight junction holds cell together
- Tight junctions
 - Maintain structure
 - Signal transduction
 - Regulate paracellular diffusion
- Pericyte Cells
 - Flat contractile cells
 - Regulate endothelial cell proliferation
 - Induces BBB tightness
- Extracellular Matrix
 - 20% of brain
 - Organized mesh of secreted proteins
- Astrocytes
 - Surround 99% of BBB
 - Influence endothelial structure
 - Induce BBB tightness
- Interstitial Fluid
 - Receptor biophase

Brain Compartments & Methods



Key Issues

- Key question: will sufficient drug reach the site of action?
- Key issue: ISF if the receptor biophase

$$\text{ISF} \approx C_u\text{brain} = C_t\text{brain} \times f_u\text{brain}$$

Key Criteria for Brain Absorption

- CNS targets
 - What is the concentration at the receptor site?
- CNS side effects
 - How much compound gets into brain?
 - How much compound is freely available to cause havoc in brain?

What Can We Measure?

	Conc.	f_u
• Brain/Plasma distribution (logBB)		
– <i>in vivo</i>	+	-
– IAM	+	-
• Uptake rates		
– PAMPA	0	-
– MDCK, caco-2, BCEC, etc	0	-
• PS-product		
– <i>in situ</i> perfusion	0	-
• Brain free fraction		
– <i>in situ</i> dialysis	-	+
– Microdialysis against brain tissue	-	+

Model Limitations

- logBB confounded by
 - Non-specific tissue binding
 - Efflux and influx transport
 - Brain metabolism
- Rate measurements (logPS, PAMPA, MDCK, etc) yield little information on brain conc.
- Plasma/CSF and Brain/SCF barrier different from Plasma/Brain barrier
- *in vivo* f_u determination expensive and laborious

Classification Schemes

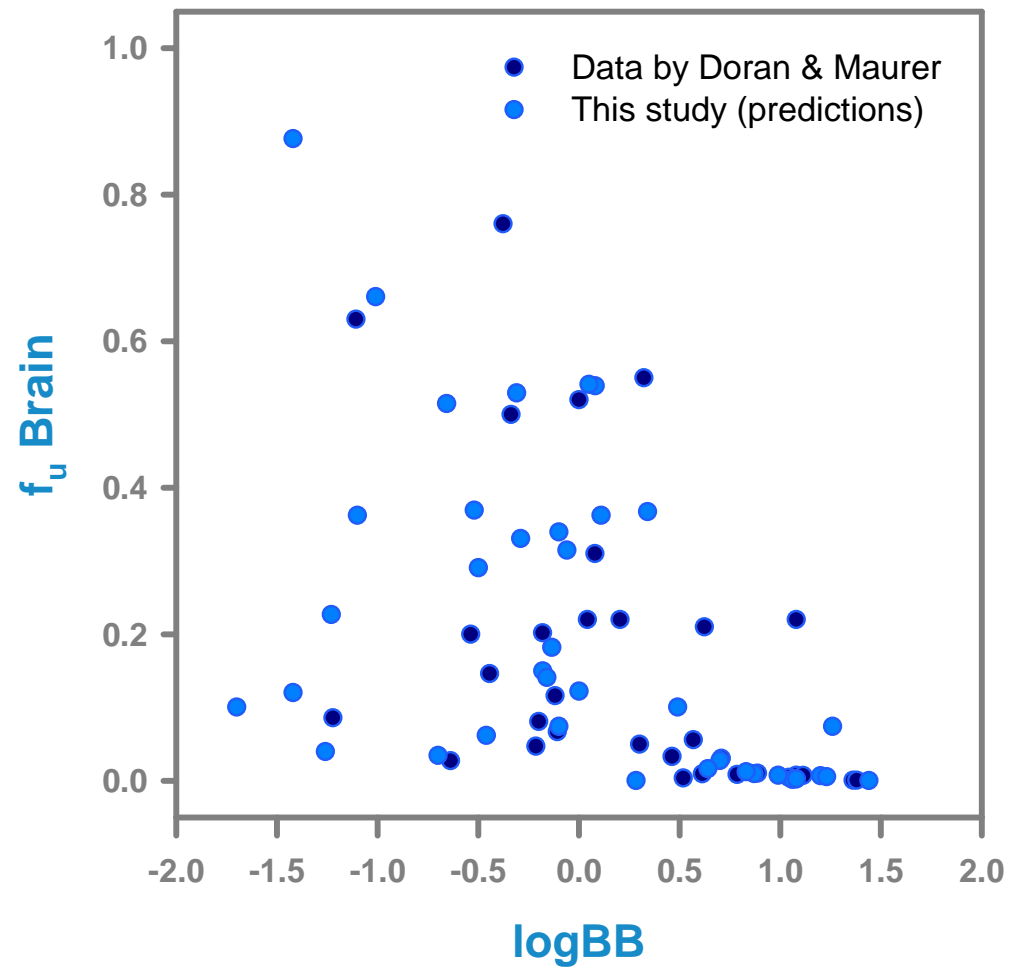
Assay	CNS+
MDR1-MDCKII	$P_{app} > 14 \cdot 10^{-6} \text{ cm/s}$
PAMPA-BBB	$P_e > 4 \cdot 10^{-6} \text{ cm/s}$
<i>in vivo</i> B/P ratio	$B/P > 0.3$
<i>in vivo</i> perfusion	$\text{Log PS} > -2.5$
CNS rules	$\text{PSA} < 60 - 70 \text{ \AA}^2$ $1 < \log D_{7.4} < 3$ $\text{MW} < 450$

Free Drug Hypothesis and logBB

	A	B	C	D
C_t blood	100	100	100	100
Blood f_u [%]	1	10	1	10
Brain f_u [%]	1	1	10	10

C_u brain [ng/ml]	1	10	1	10
C_t brain [ng/g]	100	1000	10	100
B/P ratio	1	10	0.1	1

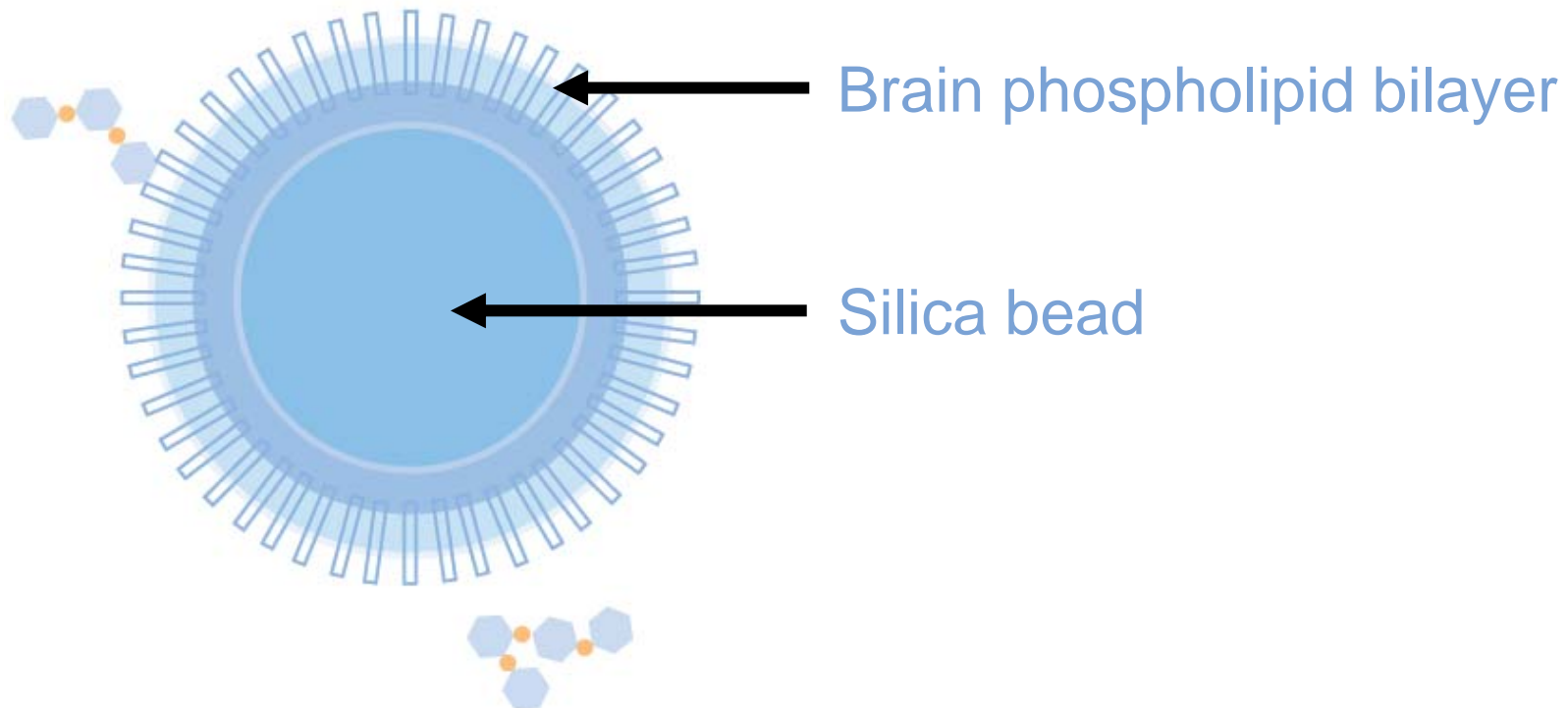
Brain Free Fraction and logBB



BBB Models

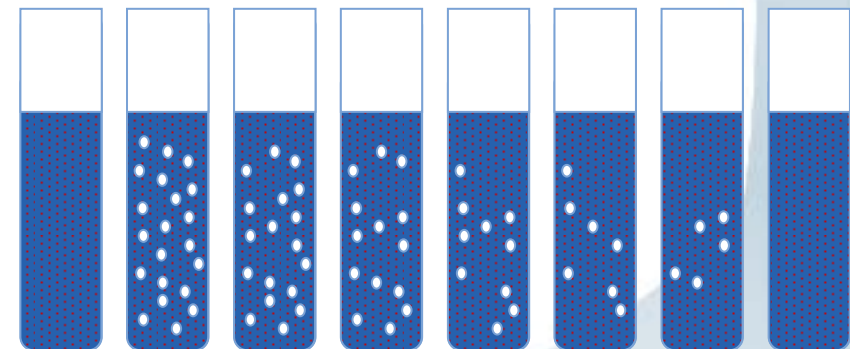
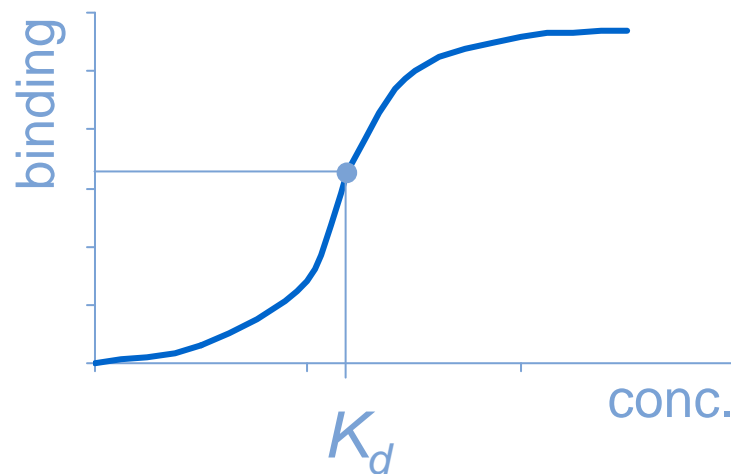
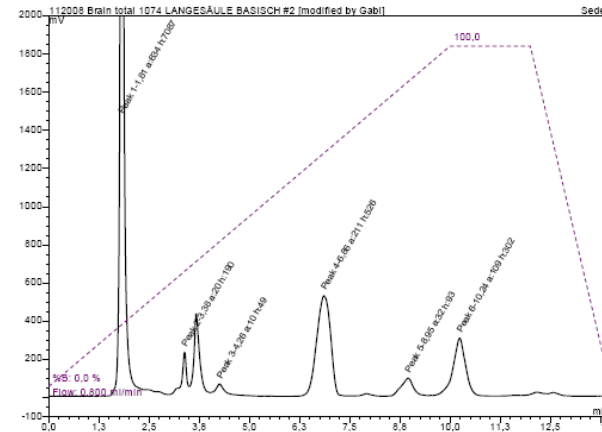
Assay	Permeability		Equil. Dist.	f_u
	passive	active		
• In silico	☹️	-	☺️	-
• MDCK cells	☹️	☺️	-	-
• BCEC/astrocyte co-culture	☺️	☺️	-	-
• PAMPA	☺️	-	-	-
• TRANSIL Brain Absorption	☺️	-	☺️	☺️

TRANSIL Brain Absorption I

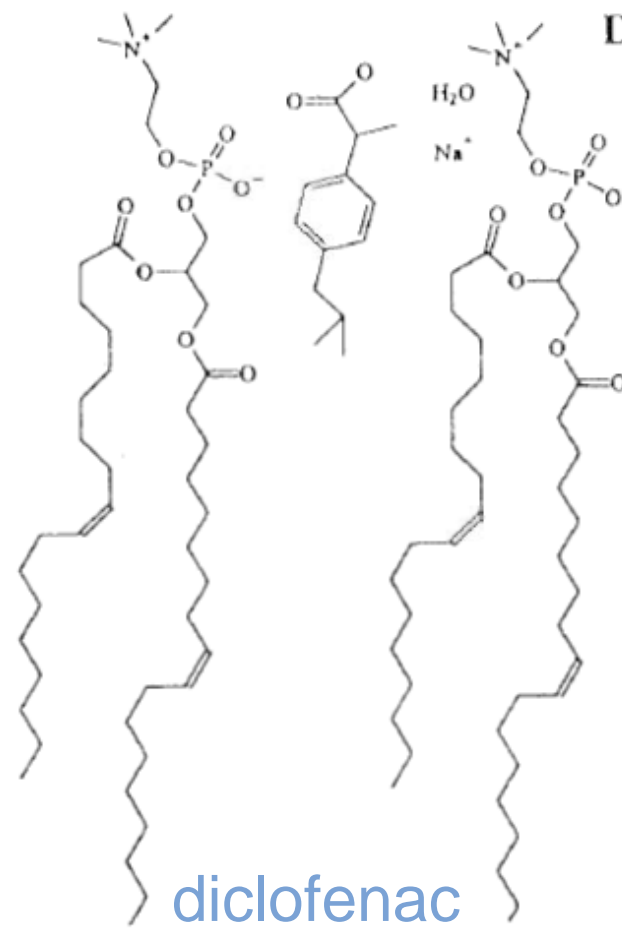
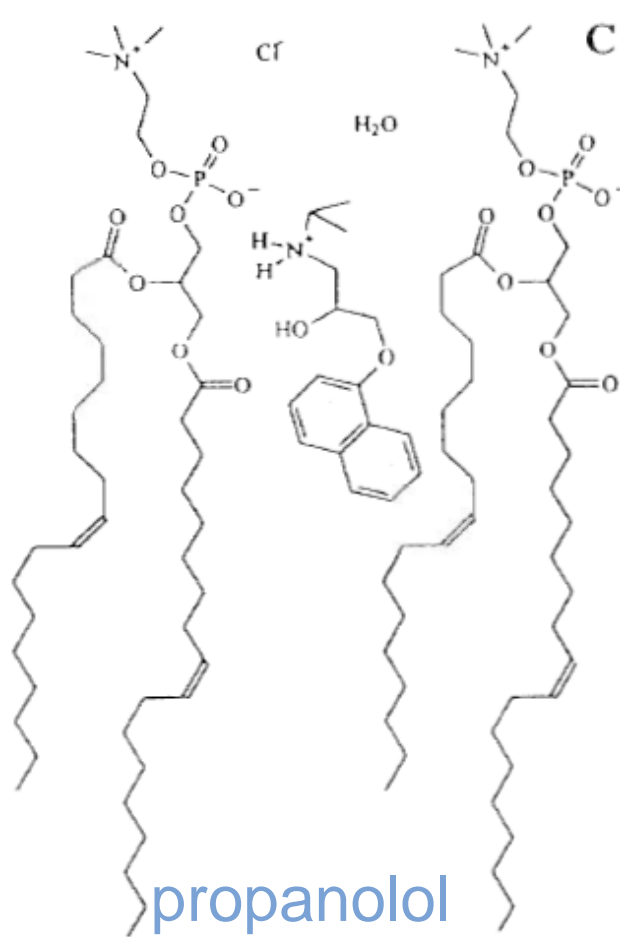


TRANSIL Brain Absorption II

- Porcine brain lipids
- Reconstituted membrane vesicles on 10 μm beads
- Titration of membrane affinity

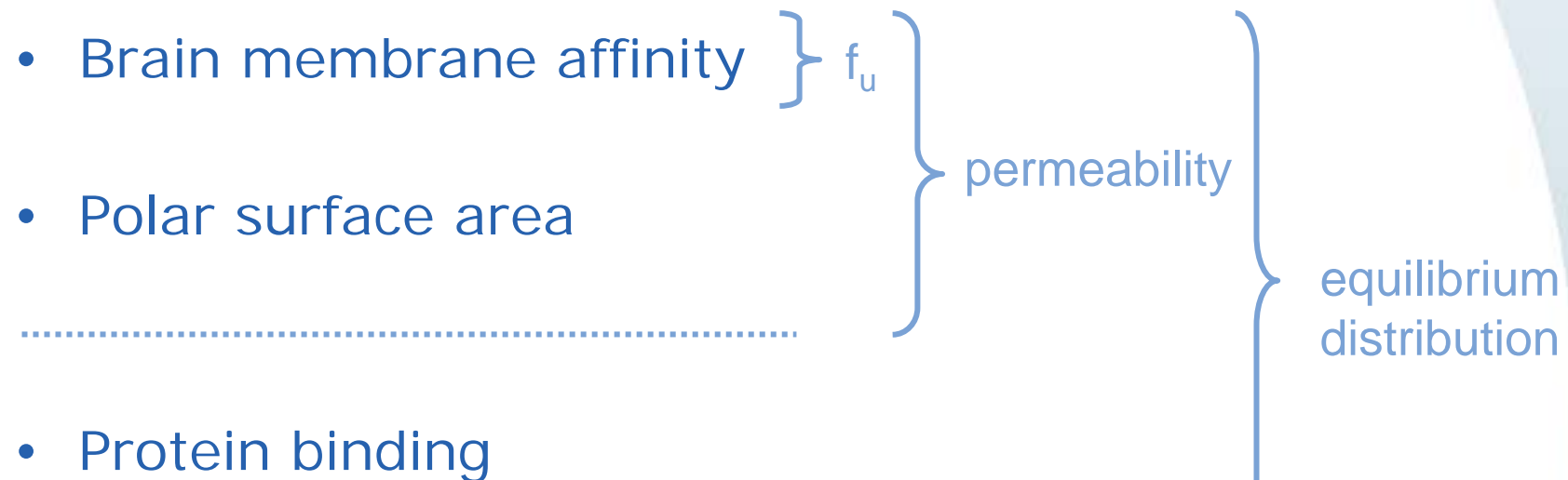


Membrane – Small Molecule Interaction



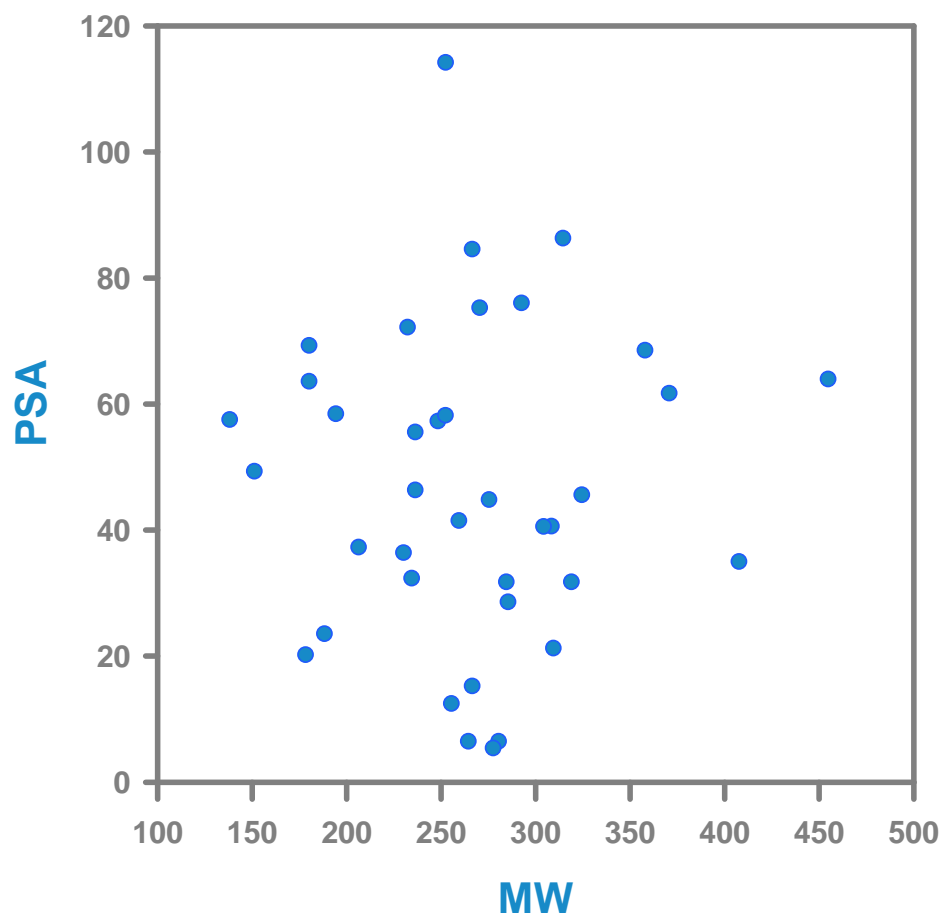
TRANSIL Brain Absorption A Hybrid Model

Prediction based on:

- Brain membrane affinity } f_u
 - Polar surface area
 -
 - Protein binding
- permeability
- equilibrium distribution
- 
- A diagram illustrating the components of a hybrid model for brain absorption. It features a list of three input factors: 'Brain membrane affinity', 'Polar surface area', and 'Protein binding'. A horizontal dotted line is placed between 'Polar surface area' and 'Protein binding'. A right-facing curly bracket groups 'Brain membrane affinity' and 'Polar surface area', with the label
- f_u
- to its right. A larger right-facing curly bracket groups the entire list of three factors. To the right of this larger bracket is the label 'permeability'. A final, even larger right-facing curly bracket groups the entire list and the 'permeability' label, with the label 'equilibrium distribution' to its right.

Validation Data

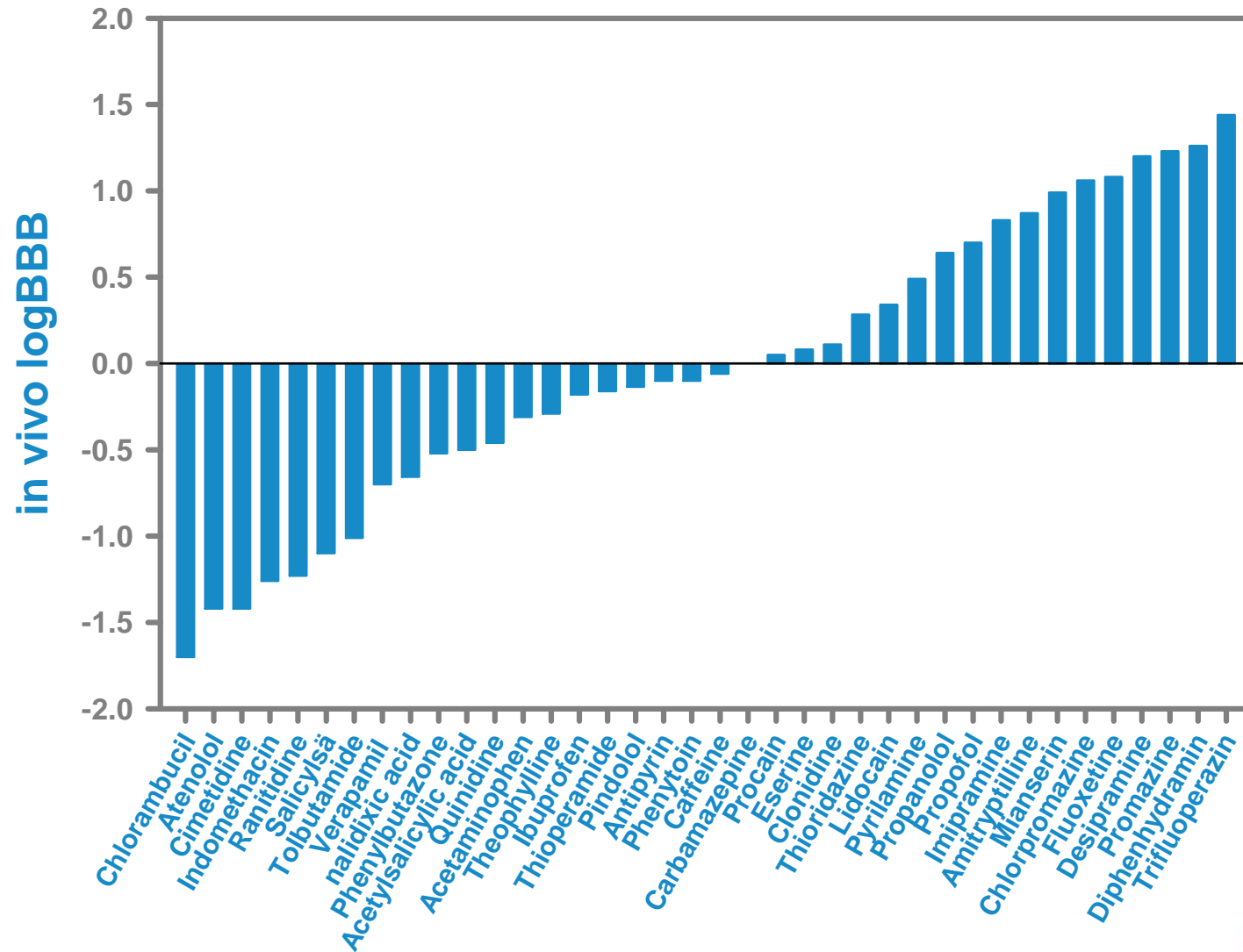
Compound Composition



Atenolol
 Chlorambucil
 Cimetidine
 Clonidine
 Ibuprofen
 Indomethacin
 Lidocain
 Nalidixic acid
 Phenylbutazone
 Pindolol
 Procaine
 Propranolol
 Pyrilamine
 Quinidine
 Ranitidine
 Salicylic acid
 Theophylline
 Tolbutamide

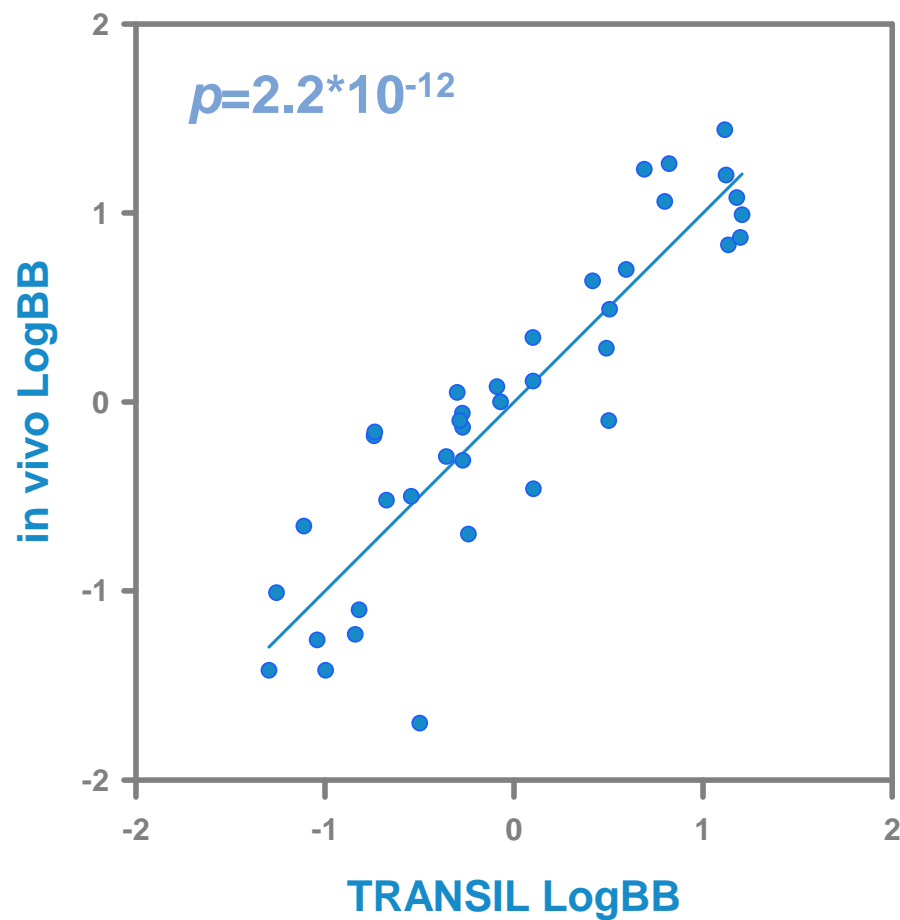
Acetaminophen
 Acetylsalic acid
 Amitryptilline
 Caffein
 Carbamazepine
 Chlorpromazine
 Desipramine
 Diphenhydramie
 Eserine
 Fluoxetine
 Imipramine
 Mianserin
 Phenazone
 Phenytoin
 Promazine
 Propofol
 Thioperamide
 Thioridazine
 Trifluoperazine
 Verapamil

Validation Data: in vivo logBB



Linear Model of Validation Data

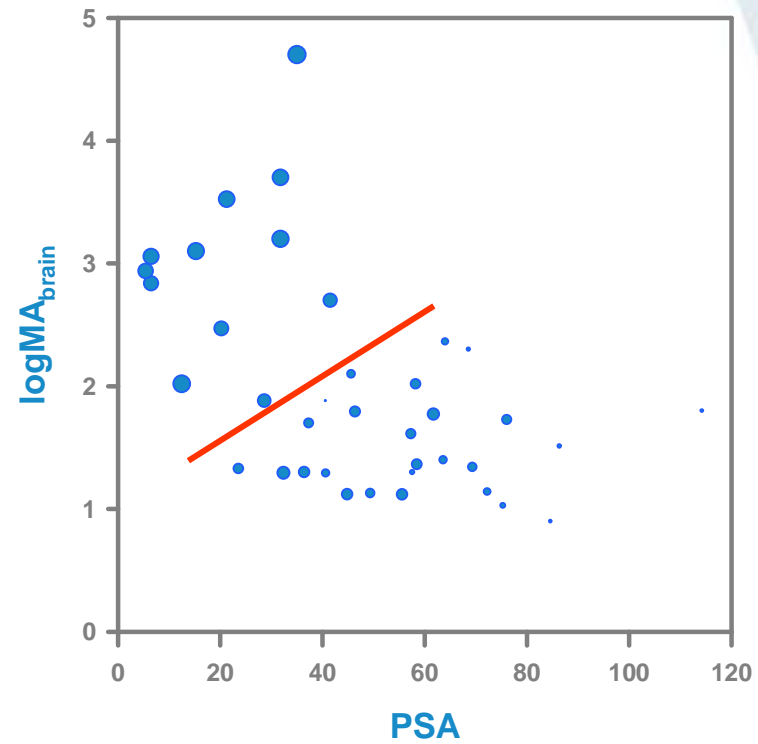
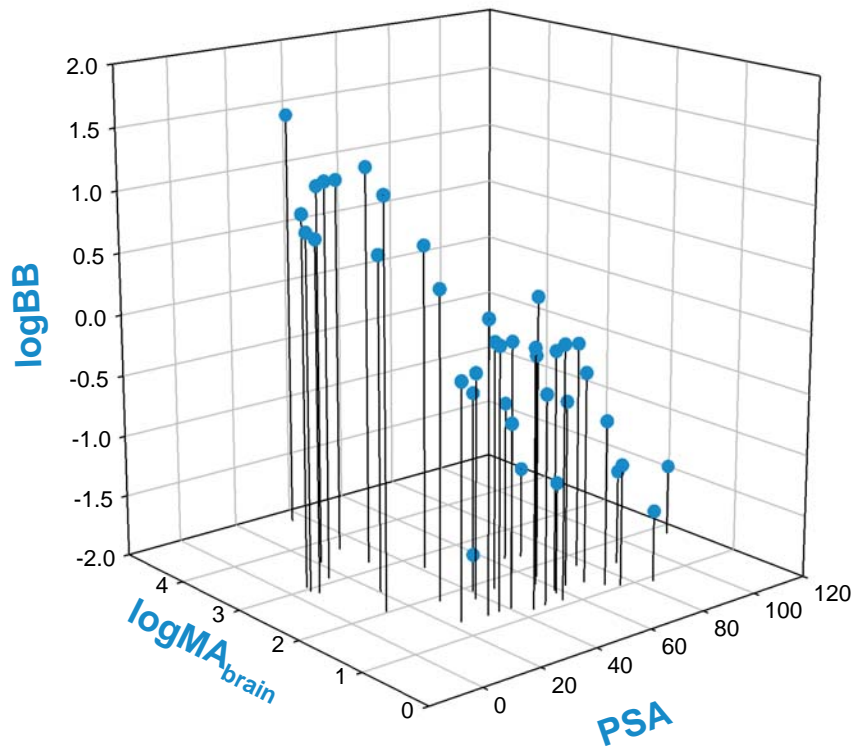
Validation Data



Variable	<i>p</i> -value
$\log MA_{\text{brain}}$	6.9×10^{-7}
$\log K_{B/F}$	2.1×10^{-4}
PSA	1.2×10^{-8}

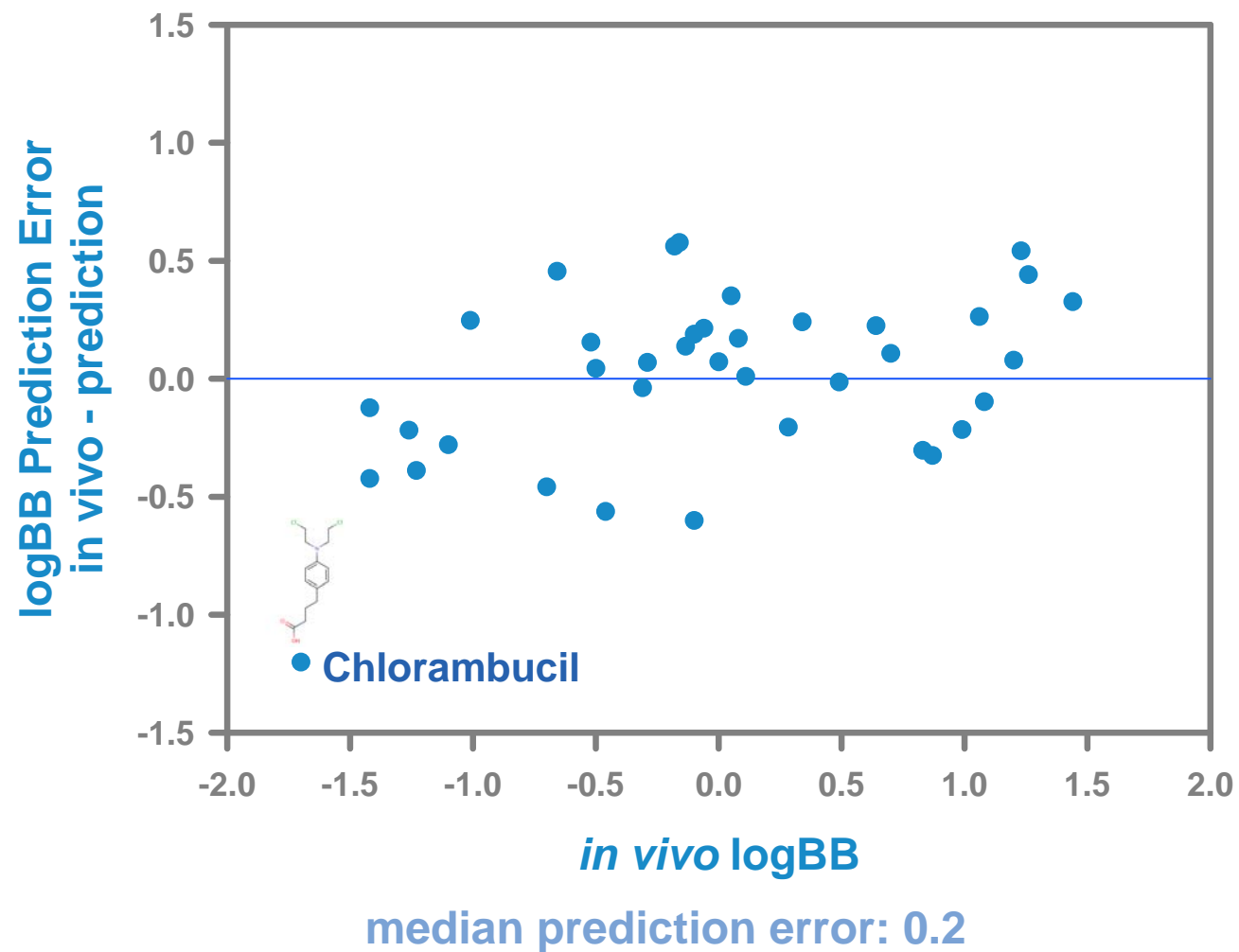
$$\log BB = a \cdot \log MA_{\text{brain}} - b \cdot \text{PSA} - d \cdot \log K^{B/F} + \text{const}$$

Key logBB Predictors: $\log\text{MA}_{\text{brain}}$ and PSA

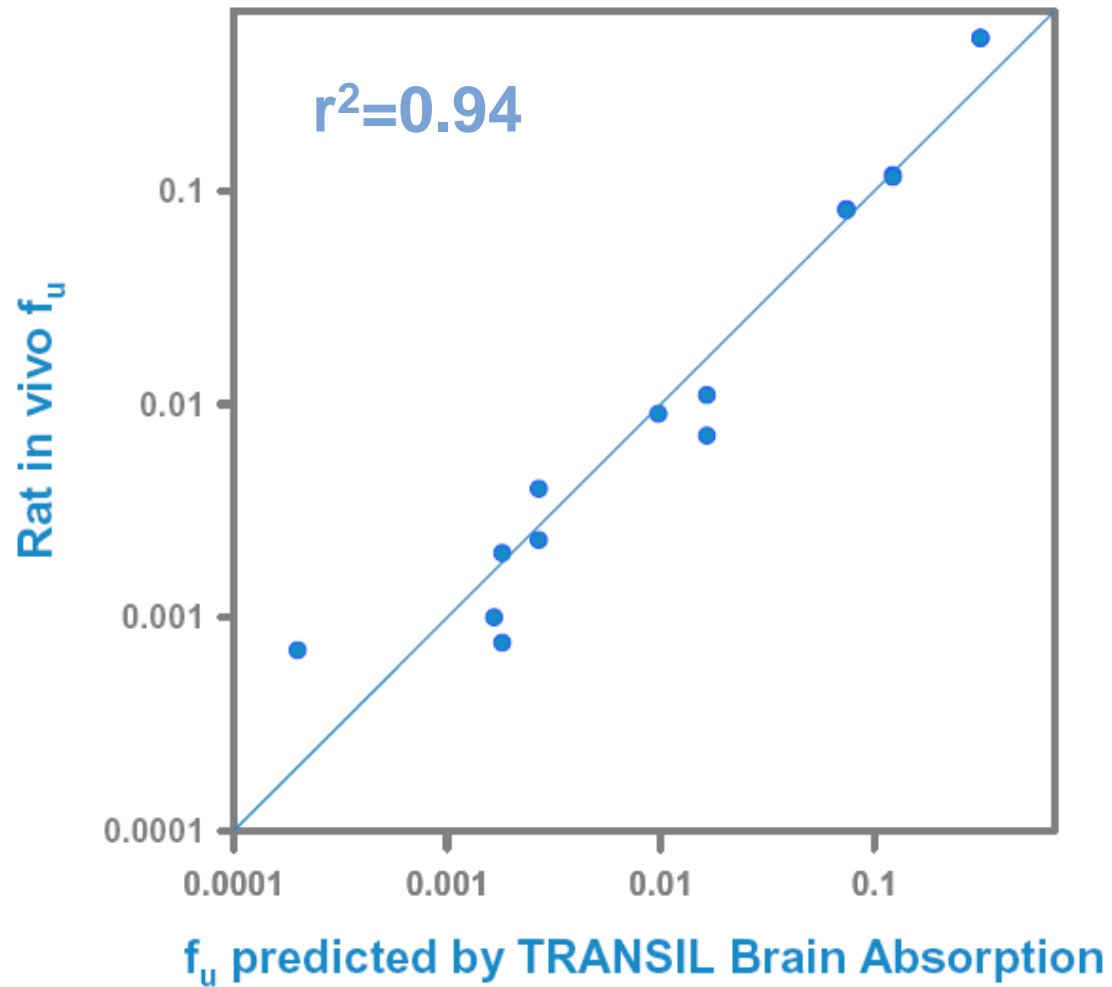


Leave One Out Cross Validation

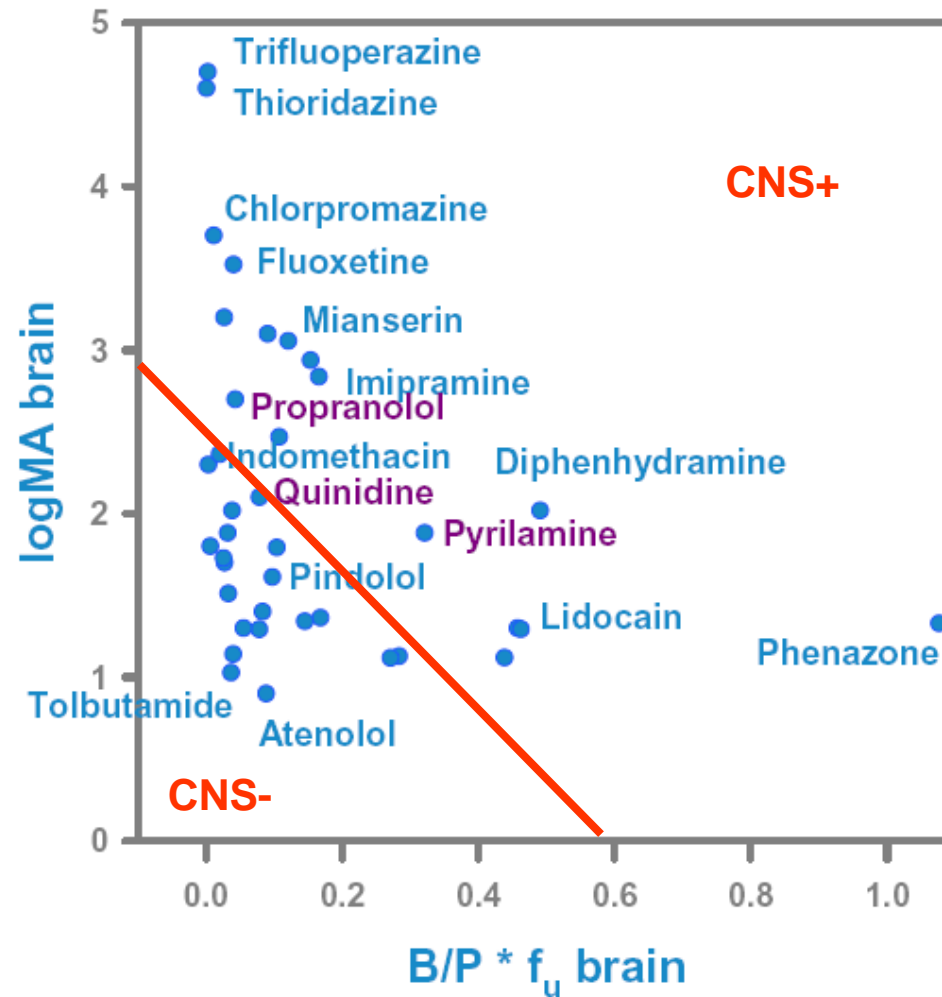
Bland-Altman Plot of Prediction Error



Prediction of Brain Free Fraction



CNS Classification



Applications

- Primary screening in CNS projects
 - Filter: reduce library size by 30 to 60%
 - Build: support CNS specific library design
 - Decrease false negative rate
- Lead optimization in CNS projects
 - Optimize brain penetration
 - Cut cost by more than 50%
- Lead optimization in non-CNS projects
 - Optimize toxicology profile



TRANSIL Brain Absorption Key Benefits

- Prediction of brain availability (\log_{BB} and f_u brain)
- Classification of CNS+ /CNS- compounds
- Screening cost reduction by at least 50%
- Fast: 2 min incubation time
- Easy: First ready-to-use assay kit

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