TRANSIL Brain Absorption:

A novel in vitro model for predicting CNS penetration

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High Throughput Screening for Biological Systems

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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

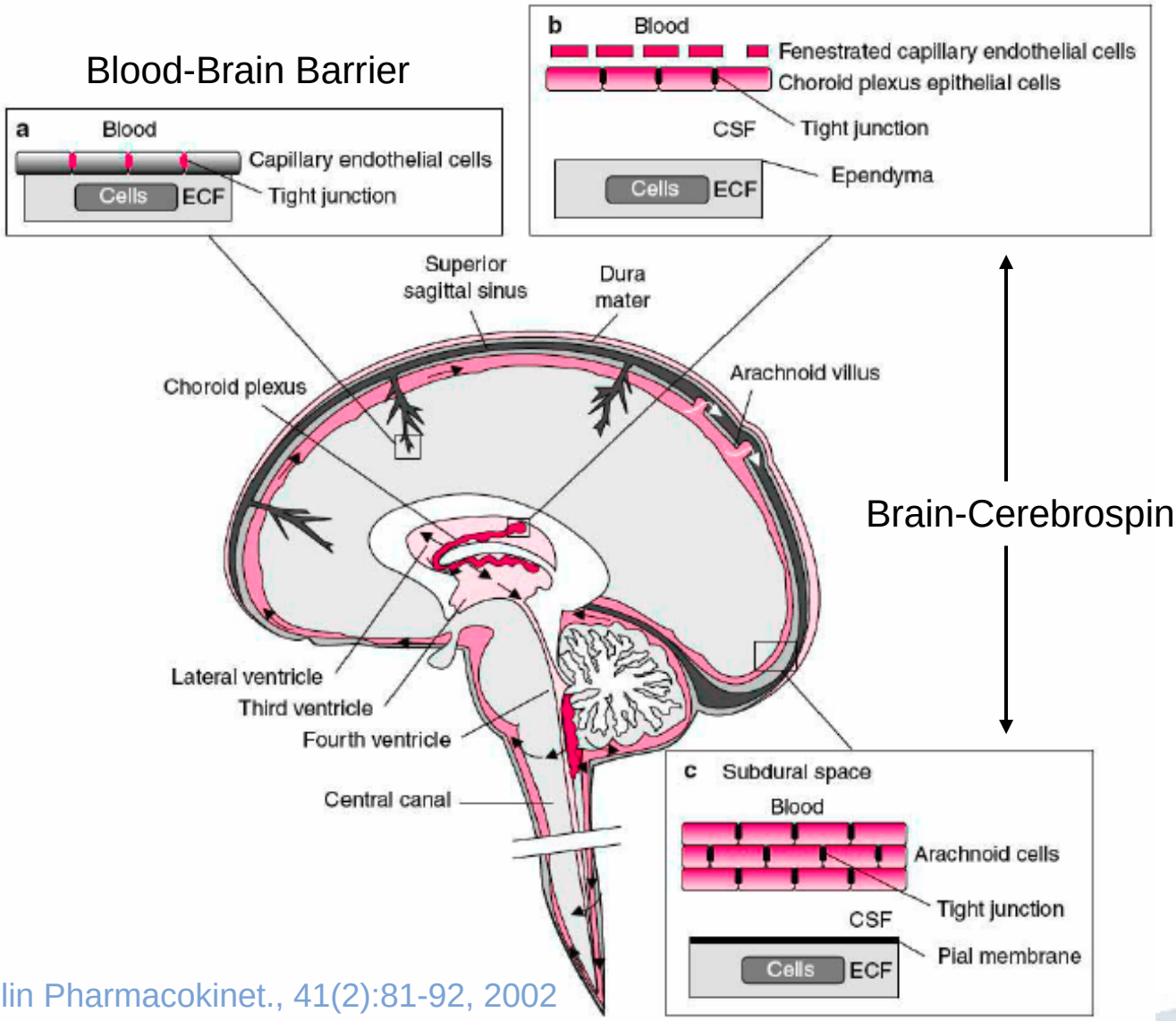
TRANSIL Brain Absorption
Key Features

- Prediction of brain availability (logBB 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

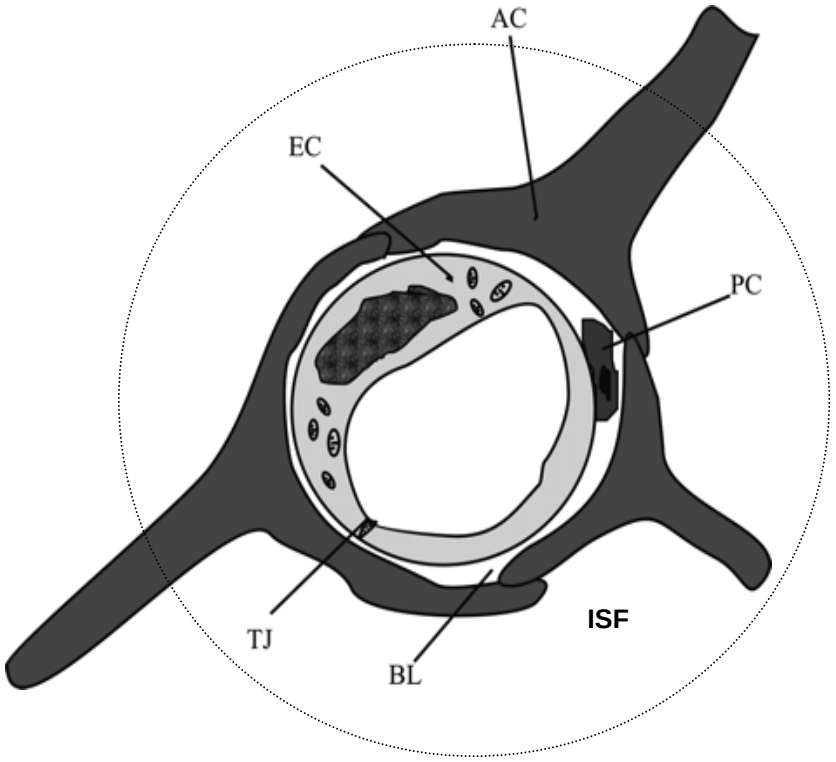
- Blood Brain Barrier protects the brain from xenobiotics while maintaining metabolic functions
- Significant hurdle for CNS targeted drugs
- Important protection from drug side-effects
- CNS-accessible chemical space much smaller in comparison to other organs

Brain Barriers



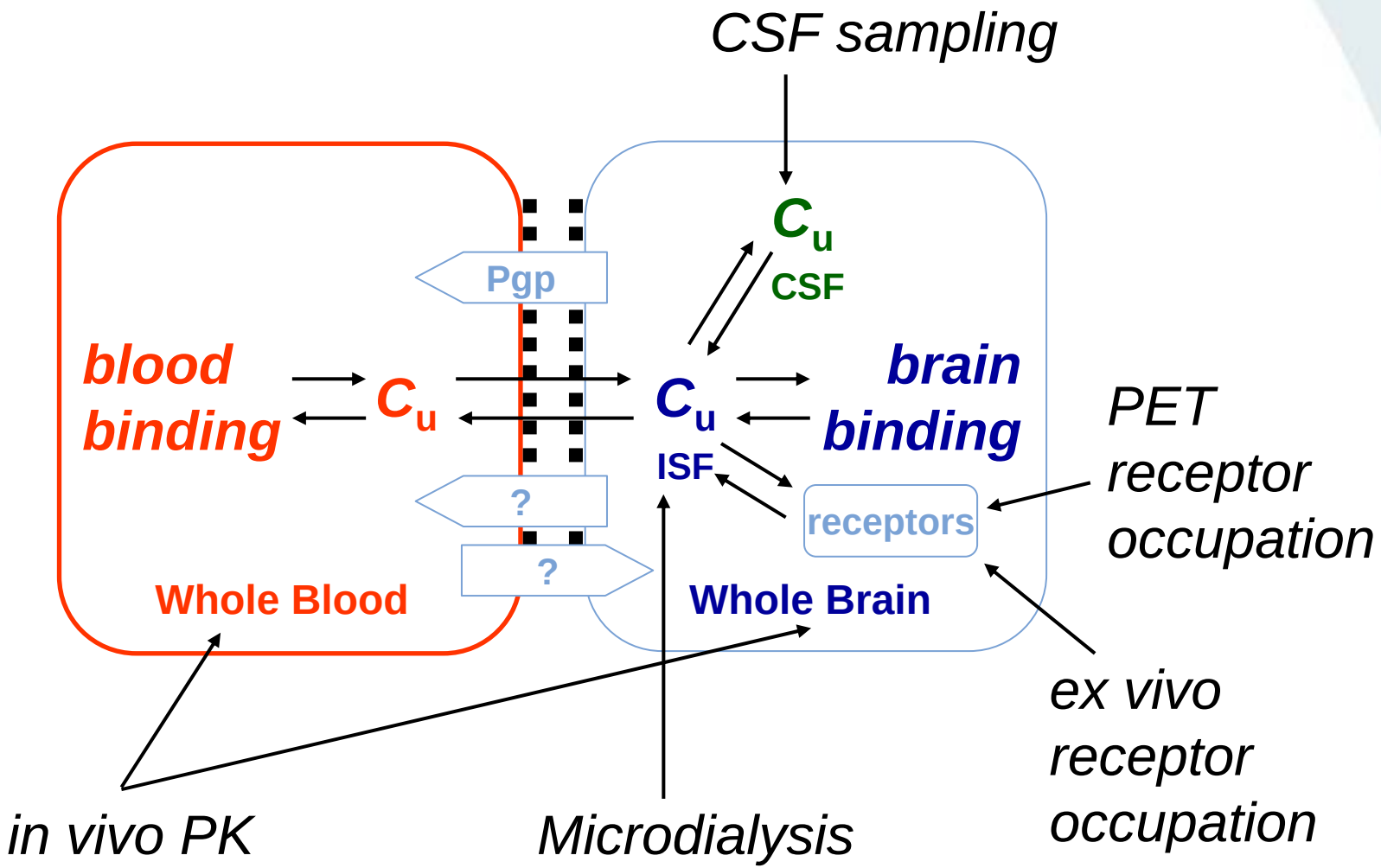
Taylor, Clin Pharmacokinet., 41(2):81-92, 2002

Neurovascular Unit



- Endothelial Cells
  - Interface btw blood and brain
  - Single cell encloises 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

$$ISF \approx C_u brain = C_t brain \times f_u brain$$

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)		
– *in vivo*	+	-
– IAM	+	-
• Uptake rates		
– PAMPA	0	-
– MDCK, caco-2, BCEC, etc	0	-
• PS-product		
– *in situ* perfusion	0	-
• Brain free fraction		
– *in situ* 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

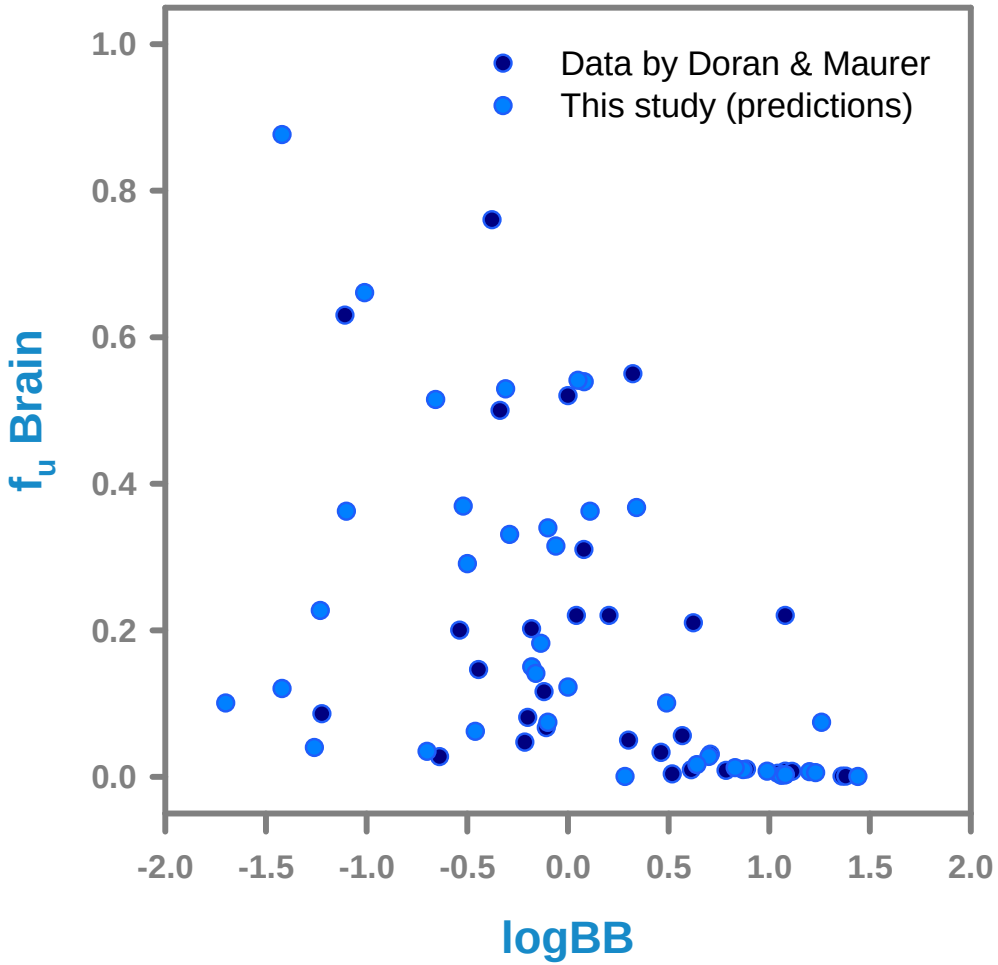
Assay	CNS+
MDR1-MDCKII	$P_{app} > 14*10^{-6}$ cm/s
PAMPA-BBB	$P_e > 4*10^{-6}$ cm/s
in vivo B/P ratio	B/P > 0.3
in vivo perfusion	Log PS > -2.5
CNS rules	PSA < 60 - 70Å^2 $1 < logD_{7.4} < 3$ MW < 450

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

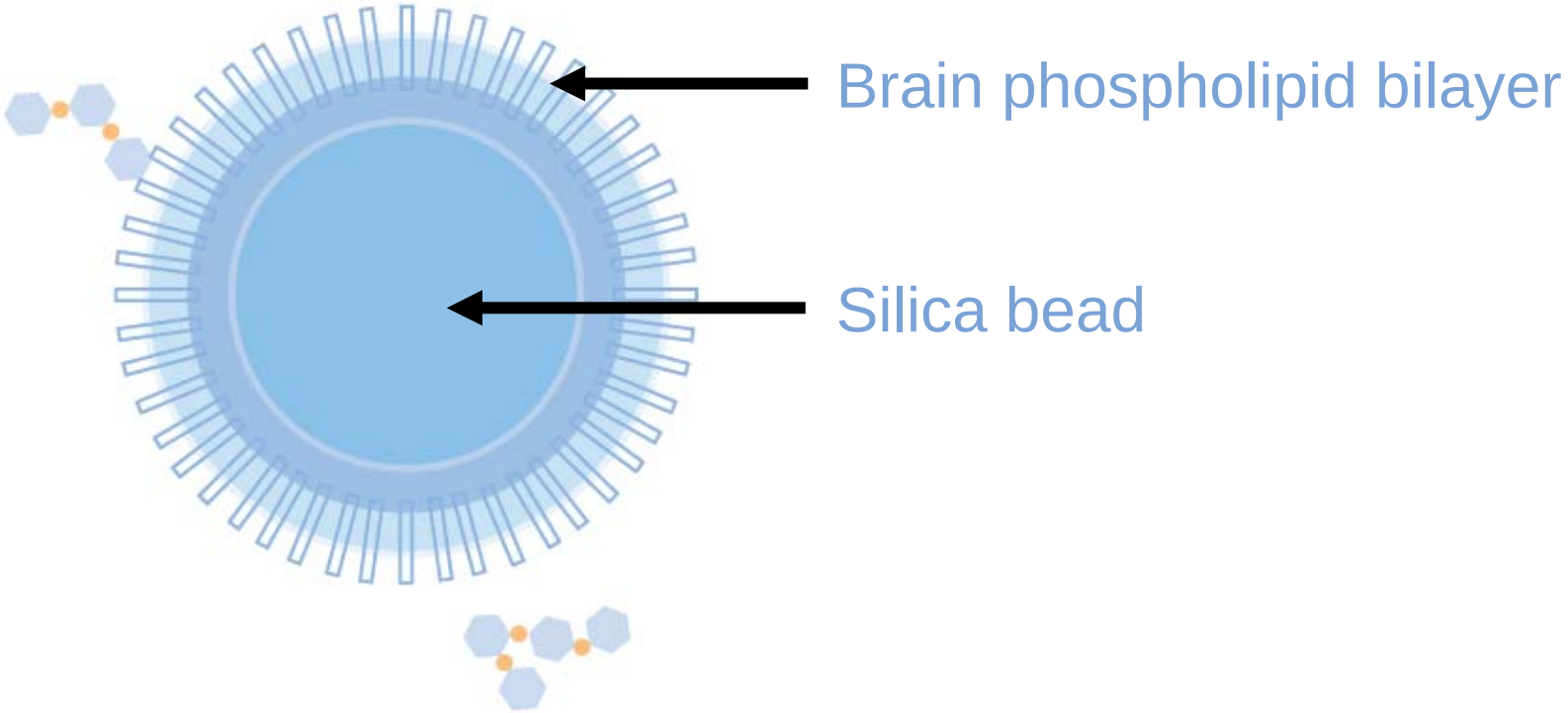
Brain Free Fraction and logBB



BBB Models

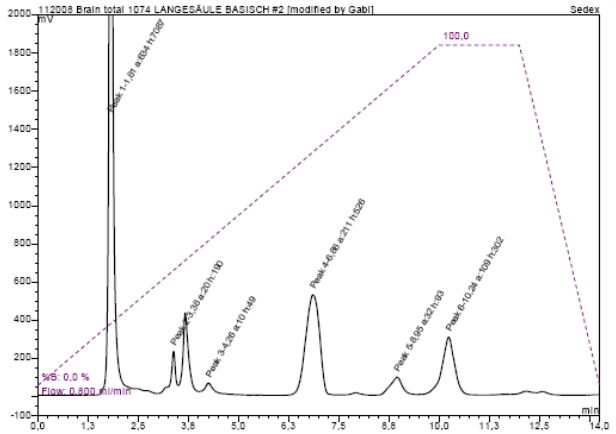
Assay	Permeability		Equil. Dist.	f_u
	passive	active		
• In silico	☹	-	😐	-
• MDCK cells	☹	😐	-	-
• BCEC/astrocyte co-culture	😐	☺	-	-
• PAMPA	😐	-	-	-
• TRANSIL Brain Absorption	☺	-	☺	☺

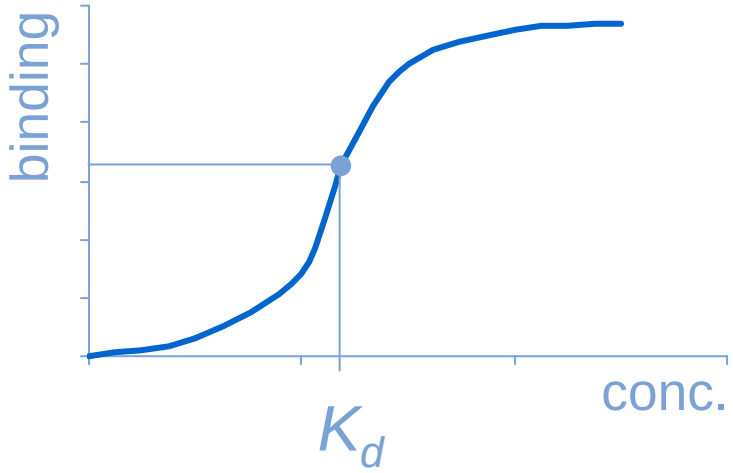
TRANSIL Brain Absorption I

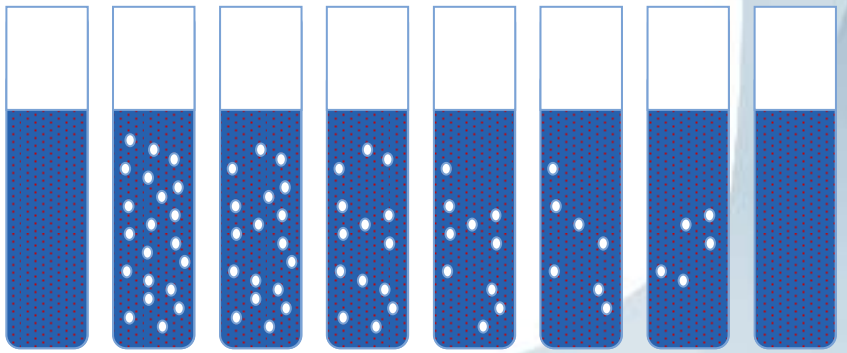


TRANSIL Brain Absorption II

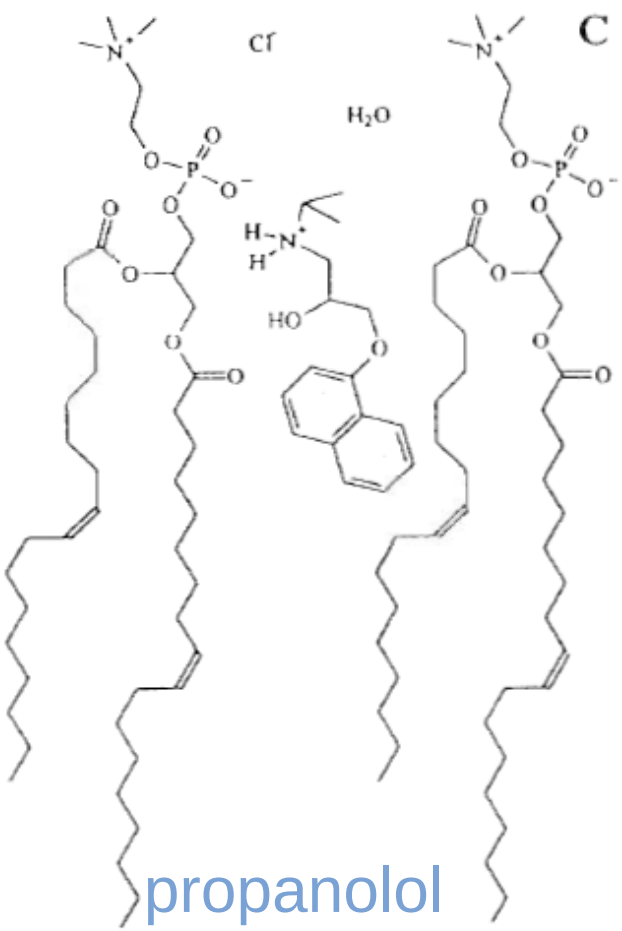
- Porcine brain lipids
- Reconstituted membrane vesicles on 10 µm beads
- Titration of membrane affinity



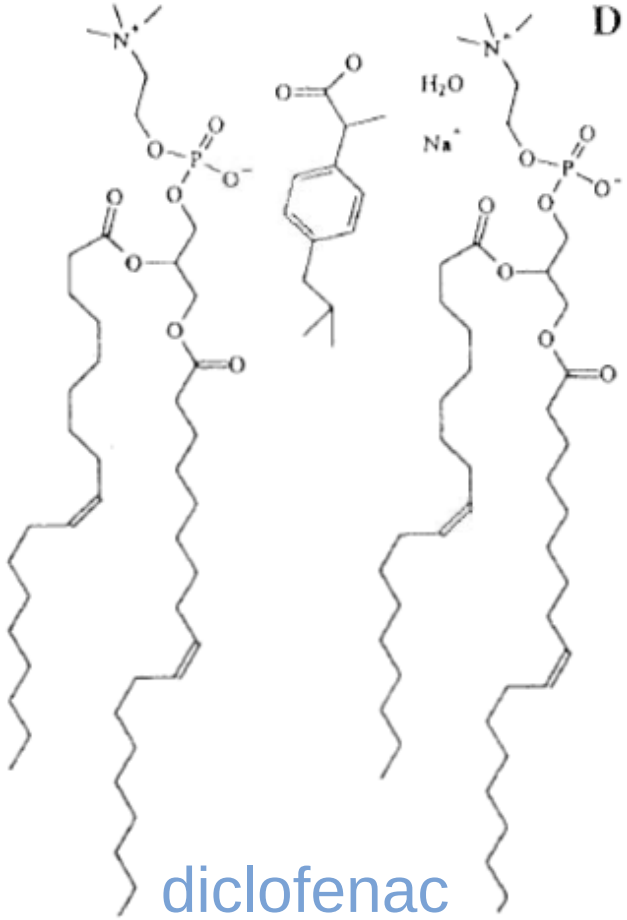




Membrane – Small Molecule Interaction



propanolol



diclofenac

TRANSIL Brain Absorption
A Hybrid Model

Prediction based on:

- Brain membrane affinity
- Polar surface area

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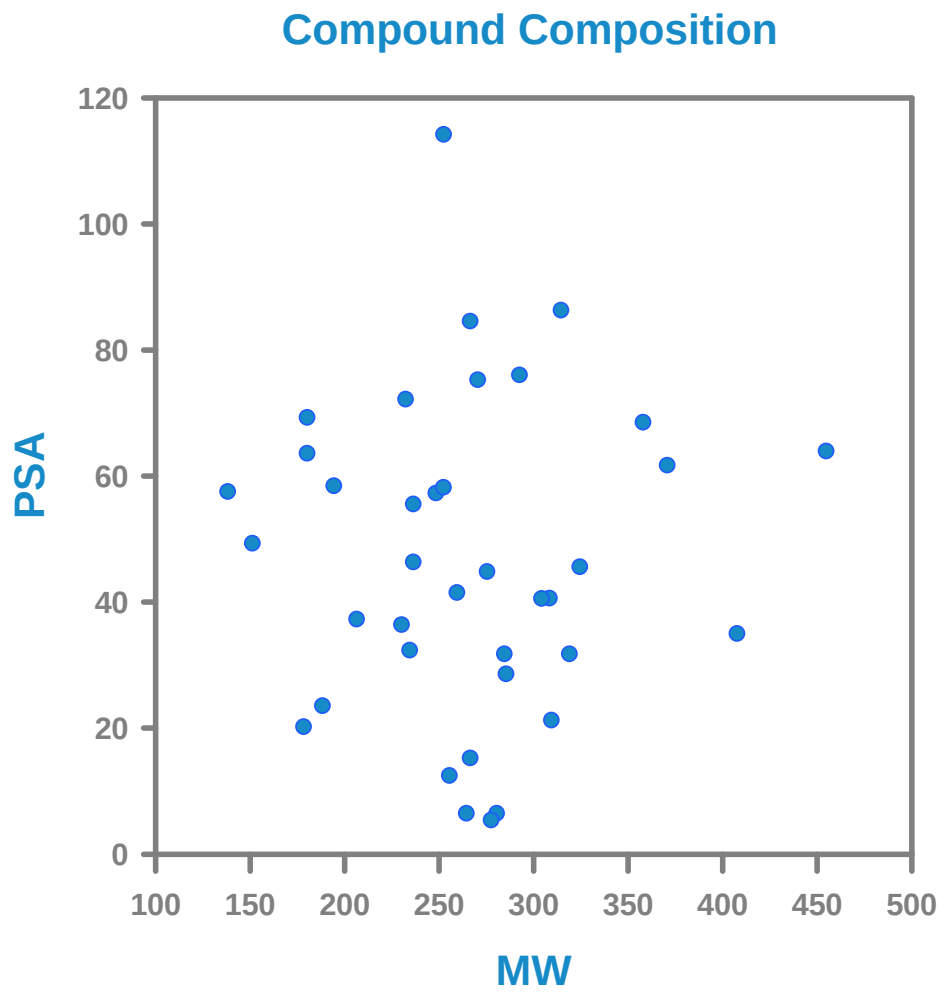
- Protein binding

f_u → permeability → equilibrium distribution

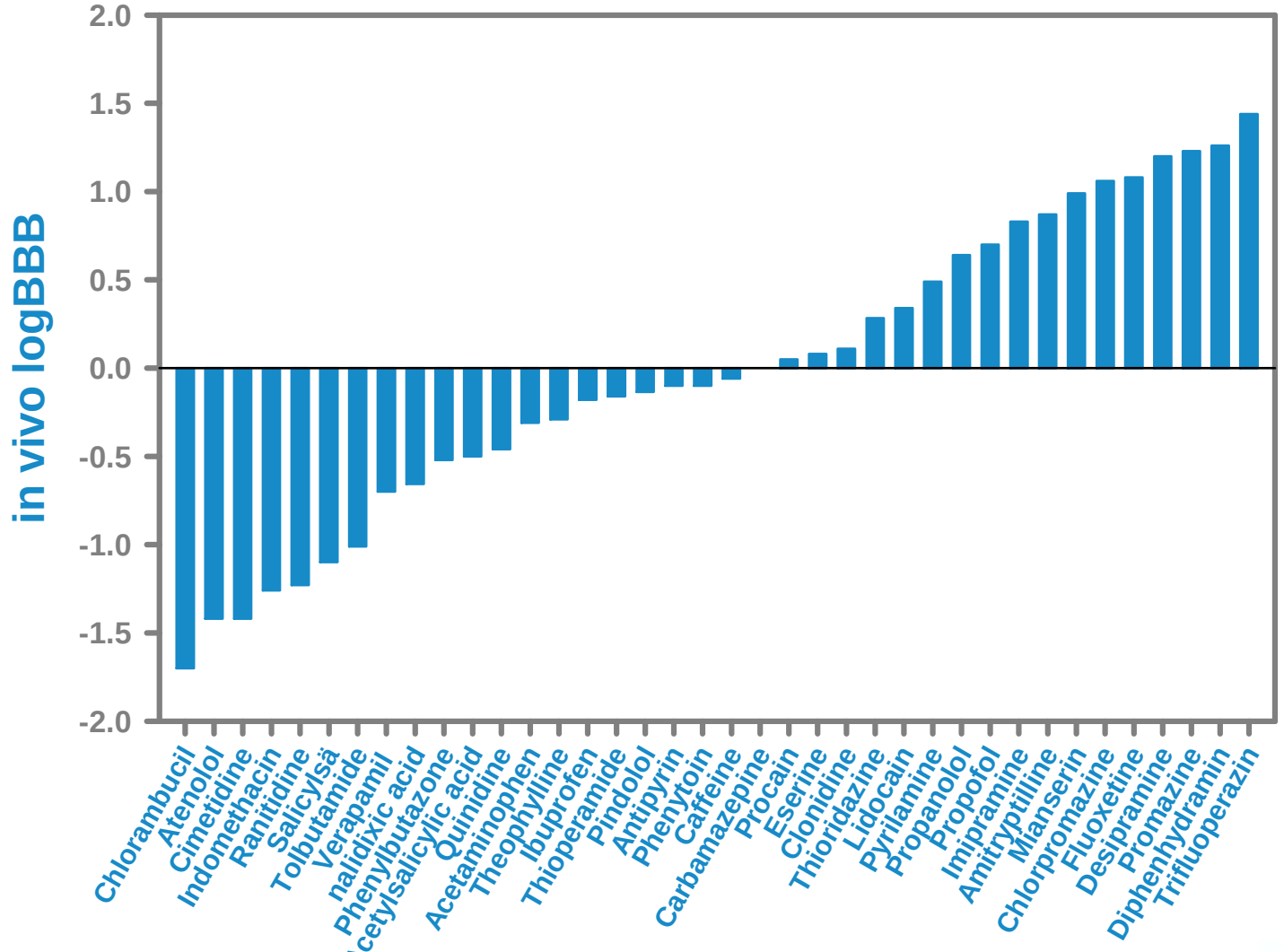
Validation Data



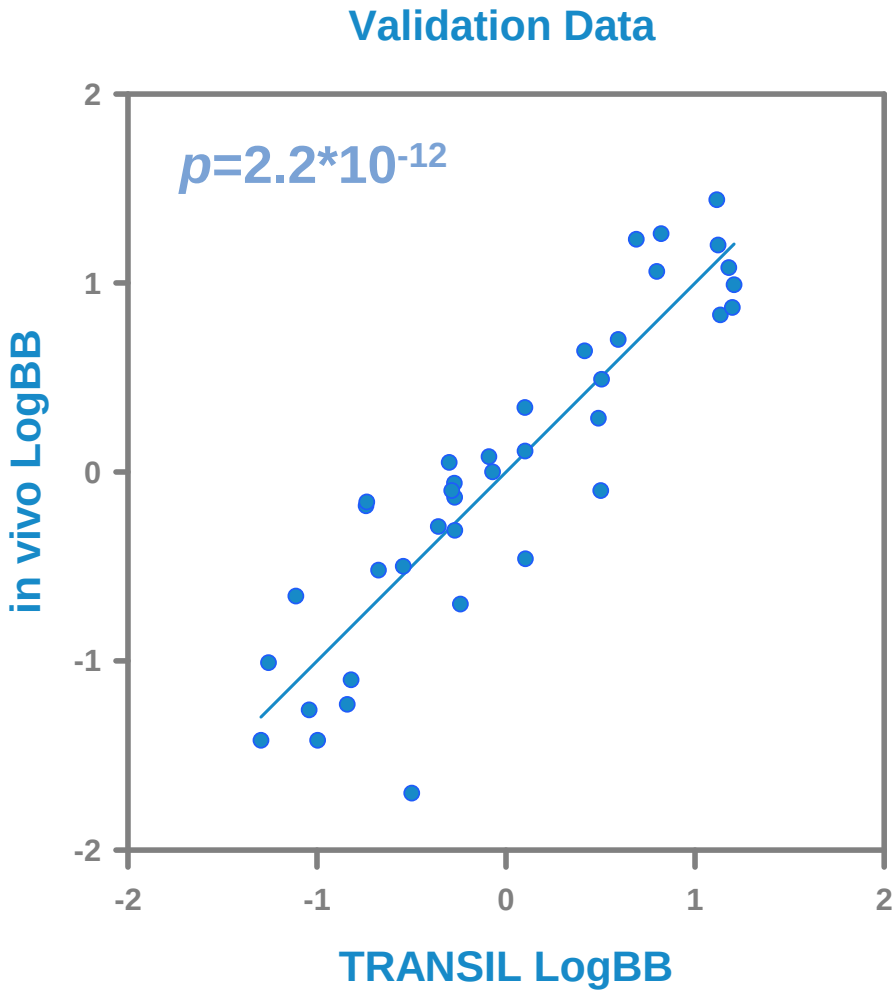
Atenolol
Chlorambucil
Cimetidine
Clonidine
Ibuprofen
Indomethacin
Lidocain
Nalidixic acid
Phenylbutazone
Pindolol
Procaine
Propranolol
Pyrilamine
Quinidine
Ranitidine
Salicylic acid
Theophylline
Tolbutamide

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



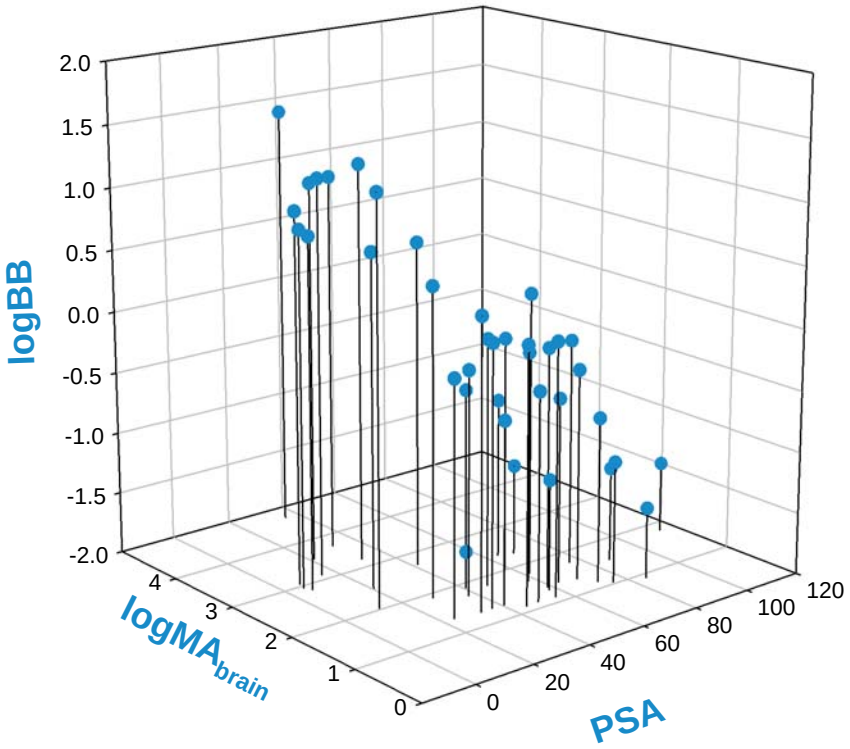
Linear Model of Validation Data

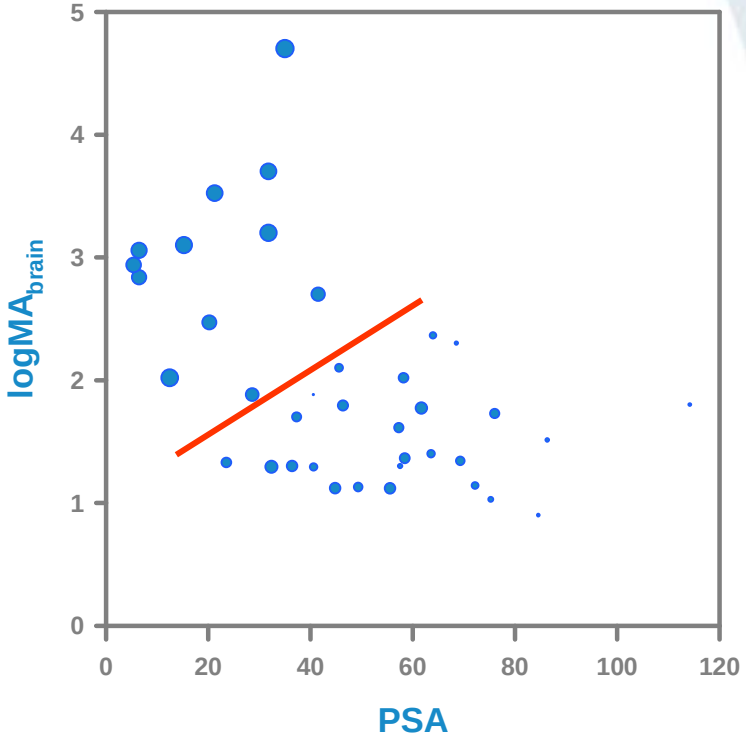


Variable	*p*-value
$logMA_{brain}$	$6.9*10^{-7}$
$logK_{B/F}$	$2.1*10^{-4}$
PSA	$1.2*10^{-8}$

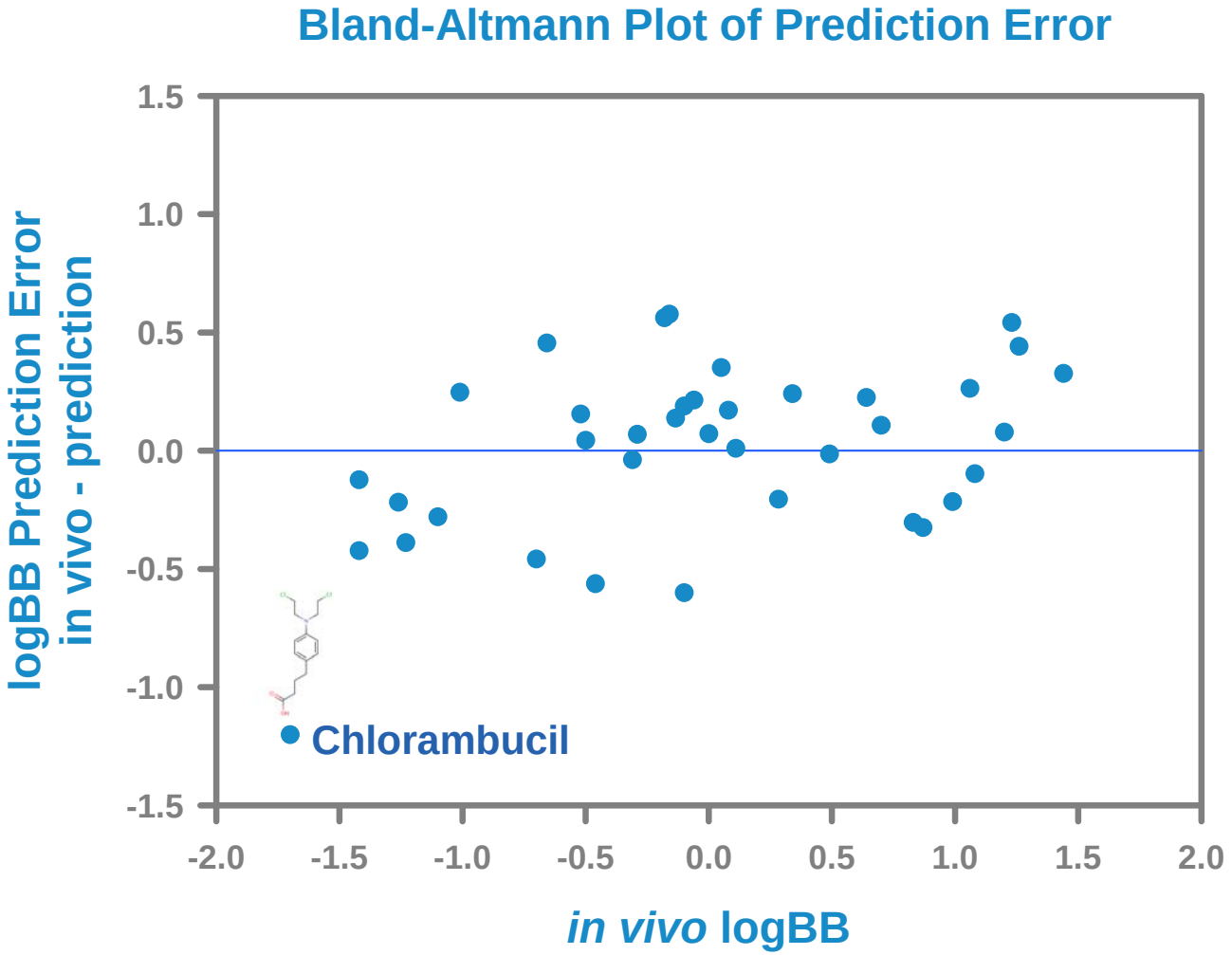
$$logBB = a*logMAbrain - b*PSA - d*logK^{B/F} + const$$

Key logBB Predictors: $logMA_{brain}$ and PSA



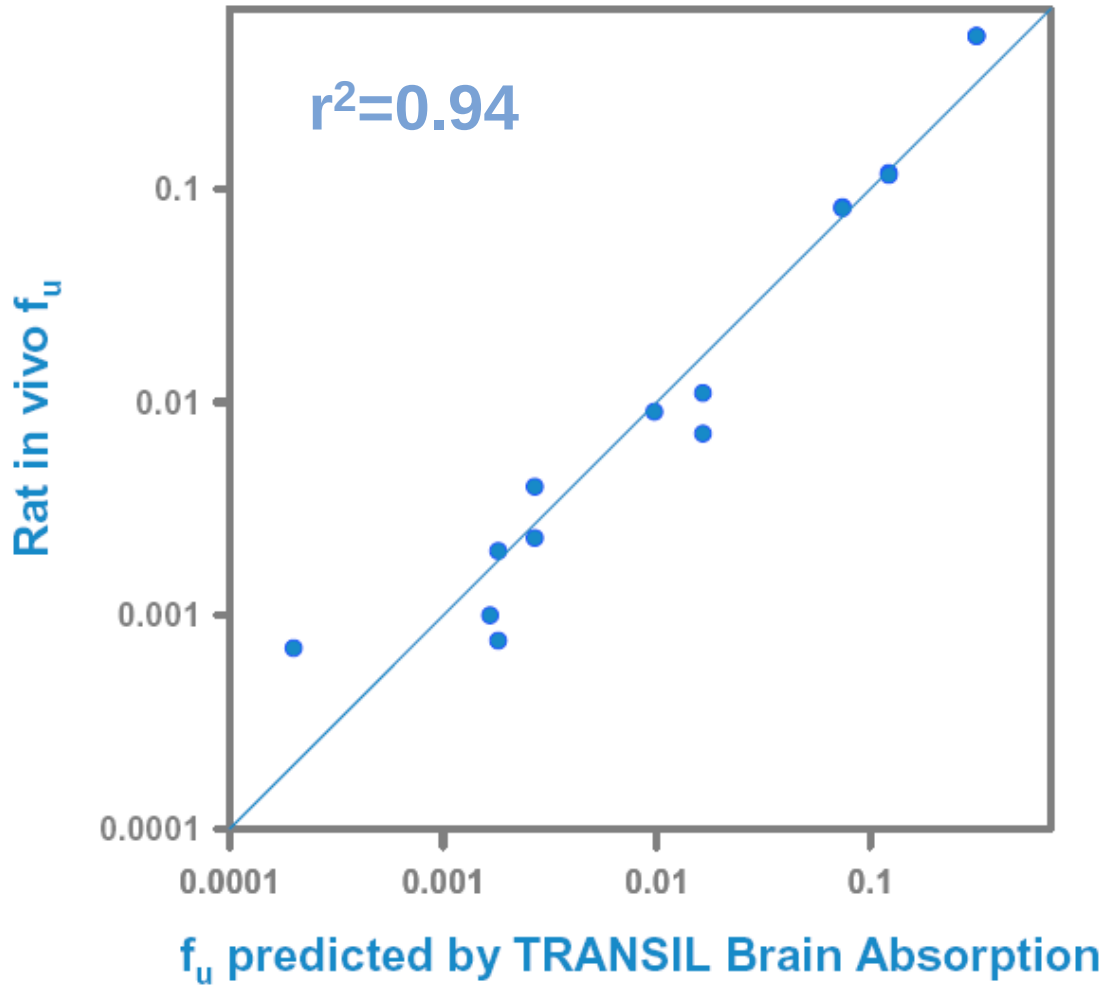


Leave One Out Cross Validation

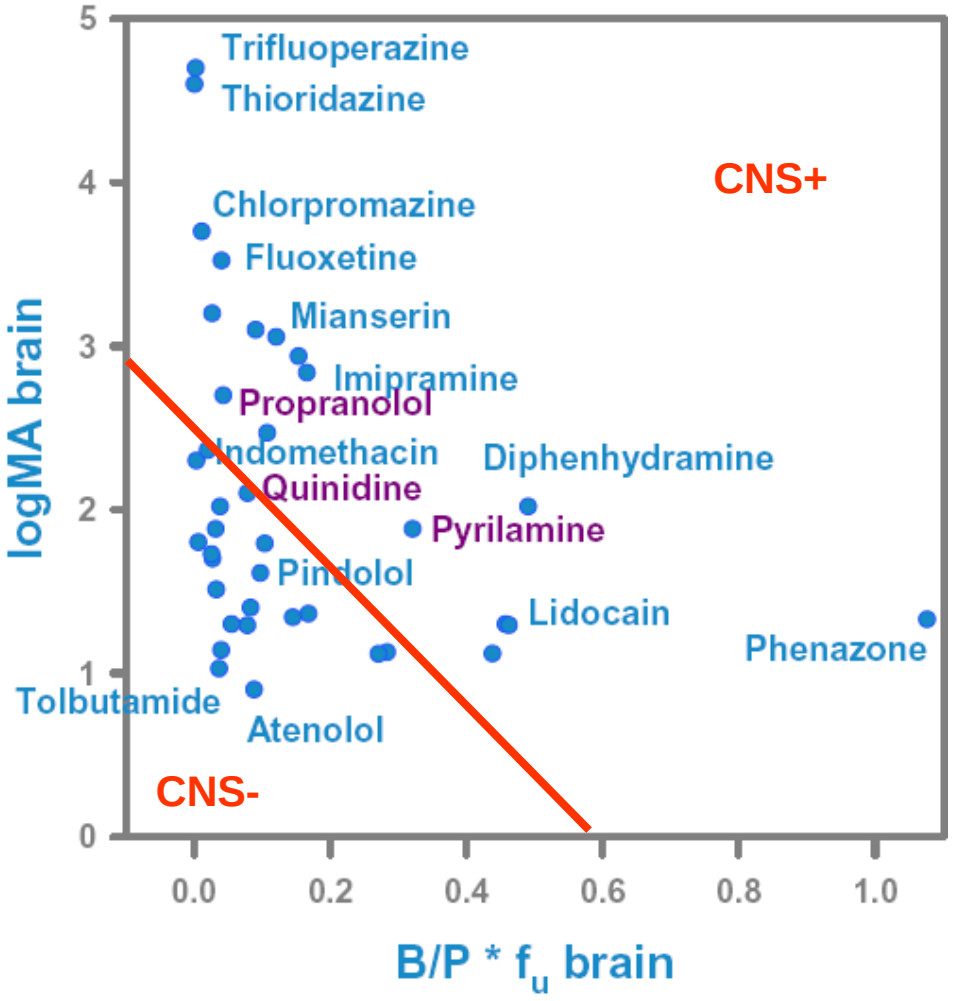


median prediction error: 0.2

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 (logBB 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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