Evaluating *a priori* fraction unbound predictions against brain homogenate data: Accounting for the experimental bias

Elena Righetti[1,2], Aleksandr Petrov[3,4], Charlotte Kloft[4,5], Andreas Reichel[6], Wilhelm Huisinga[3,4]

1 - Fondazione the Microsoft Research-University of Trento Centre for Computational and Systems Biology (COSBI), Rovereto, Italy
2 - Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Trento, Italy
3 - University of Potsdam, Institute of Mathematics, Potsdam, DE
4 - Graduate Research Training Program PharMetrX, Berlin/Potsdam, DE
5 - Frei Universität Berlin, Institute of Pharmacy, Berlin, DE
6 - Bayer AG, DMPK M&S, Berlin, DE

elena.righetti-1@unitn.it

Rationale & Objectives

- Unbound brain exposure is a critical factor in CNS drug development
- *A-priori* mechanistic frameworks predict fraction unbound in intact brain tissue (**fuT**)
- Brain homogenate assays disrupt cell membranes and pH gradients → mismatch between experimental data $fuH_{exp(u)}$ and predicted fuT

Objective. To derive a homogenate-specific metric **fuH_{tis}** by adapting Rodgers & Rowland's approach to mechanistically relate *a priori* predictions to experimental homogenate data

Drug partitioning in intact and homogenate brain tissue

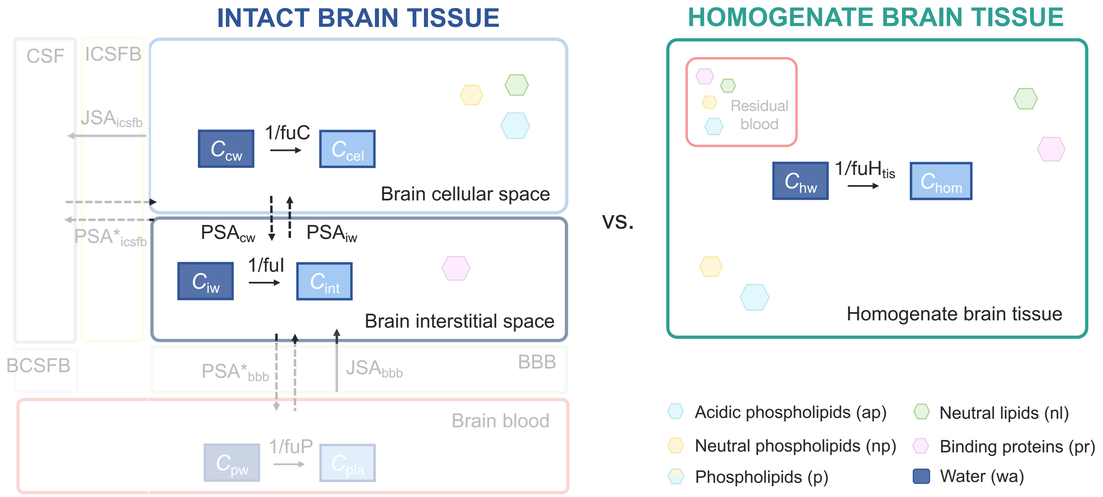


Figure 1. Conceptual representation of drug distribution in intact versus homogenate brain tissue, based on the brain model in Gaohua et al.[10]

Physiological parameters

Physiological parameters	
V_{cel}/V_{tis}	0.75
V_{int}/V_{tis}	0.25
V_{iw}/V_{isf}	1
pH_{cw}	7.0
pH_{iw}	7.3
pH_{rw}	7.22
pH_{pw}	7.4
PR_{tis}/PR_{pla}	0.048 (albumin) 0.041 (lipoproteins)

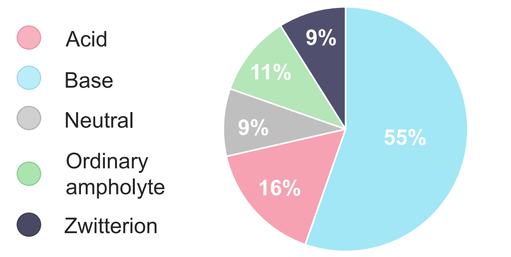
Species-specific parameters	Rat	Human
Brain weight (g)	0.1425	1450
V_{cw}/V_{tis}	0.591	0.5853
V_{iw}/V_{tis}	0.162	0.1604

Tissue lipid composition	Rodgers2006	Xu2020	Poulin2002
$V_{nl(c)}/V_{tis}$	0.391	0.0001831	0.509
$V_{p(c)}/V_{tis}$	0.0533	0.0206	0.0564
$V_{ap(c)}/V_{tis}$	0.0004	0.0022441	[0.7, 12.5]% $V_{p(c)}/V_{tis}$

Table 1. Human and rat fractional volumes of tissue constituents and other relevant physiological parameters from the literature[1,2,3,4,5]

Lack of consensus on brain lipid composition

Small molecule selection



Abundance of bases aligning with the standard percentages of acid–base compound classes within CNS drugs

Results

Identified a suitable metric for the fraction unbound in homogenized tissue and compared it with experimental data to gain mechanistic insights into discrepancies between *a priori* fuT predictions and data

1. Rodgers & Rowland-based derivation of fraction unbound in brain tissue

INTACT TISSUE

$$\frac{1}{fuT} = \frac{C_{tis}}{C_{iw}} = \frac{V_{int}}{V_{tis}}\frac{1}{fuI} + \frac{V_{cel}}{V_{tis}}\frac{1}{fuC}\frac{fn_{iw}}{fn_{cw}}$$

$$\frac{1}{fuT} = \frac{V_{iw}}{V_{tis}} + \frac{V_{iw}}{V_{isf}} K_{A,pr(p)}\, PR_{pla}\,(1-fcat_{pw})\,\frac{1-fcat_{iw}}{1-fcat_{pw}}\,\frac{PR_{tis}}{PR_{pla}} + \frac{V_{cw}}{V_{tis}}\frac{fn_{iw}}{fn_{cw}} + \frac{V_{nl(c)}}{V_{tis}} P_{o:w}\, fn_{iw} + \frac{V_{np(c)}}{V_{tis}}(0.3\,P_{o:w}+0.7)\, fn_{iw} + K_{A,ap(r)^-}\, AP^-_{tis}\, fcat_{cw}\frac{fn_{iw}}{fn_{cw}}$$

HOMOGENATE TISSUE

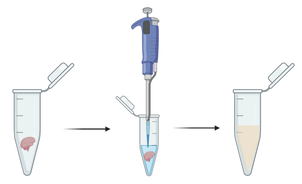
negligible residual blood

$$\frac{1}{fuH_{tis}} = \frac{C_{hom}}{C_{hw}} = \frac{V_{int}}{V_{hom}}\frac{1}{fuH_{int}} + \frac{V_{cel}}{V_{hom}}\frac{1}{fuH_{cel}} + \frac{V_{res,blo}}{V_{hom}}\frac{1}{fuH_{blo}}$$

$$\frac{1}{fuH_{tis}} = \frac{V_{iw}}{V_{hom}} + \frac{V_{hw}}{V_{htf}} K_{A,pr(p)}\, PR_{pla}\,(1-fcat_{pw})\,\frac{1-fcat_{hw}}{1-fcat_{pw}}\,\frac{PR_{hom}}{PR_{pla}} + \frac{V_{cw}}{V_{hom}} + \frac{V_{nl(c)}}{V_{hom}} P_{o:w}\, fn_{hw} + \frac{V_{np(c)}}{V_{hom}}(0.3\,P_{o:w}+0.7)\, fn_{hw} + K_{A,ap(r)^-}\, AP^-_{hom}\, fcat_{hw}$$

EXPERIMENTAL HOMOGENATE TISSUE

Experimental fraction unbound in undiluted homogenate tissue



$$fuH_{exp(u)} = \frac{1}{1 + DF\left(\frac{C_{hom}}{C_{buffer}} - 1\right)}$$

$$pH_{hw} = f(pH_{iw}, pH_{cw}, pH_{buffer}, DF)$$

Reference	Brain homogenate assay	Solution	DF	pH_{buffer}	pH_{hw}
Di et al. 2011	✓	DPBS	5	7.4*	7.3067
Summerfield et al. 2008	✓	PBS	3	7.4	7.2540
Fridén et al. 2011	✓	PBS	4	7.4	7.2862
Longhi et al. 2010	✓	CSF surrogate	2	7.4	7.1961
Culot et al. 2013	✓	PBS	10	7.4*	7.3508

Fraction unbound in homogenate tissue can be derived directly from intact tissue eq. by adjusting interstitial & cellular pH to homogenate pH:

$$\frac{1}{fuH_{tis}} = \frac{1}{fuT(pH_{hw})} = \frac{V_{int}}{V_{hom}}\frac{1}{fuI(pH_{hw})} + \frac{V_{cel}}{V_{hom}}\frac{1}{fuC(pH_{hw})}$$

yet, remaining fundamentally distinct from fuT due to irreversible structural disruptions that cannot be corrected by tuning dilution factor (DF) and pH_{buffer}

2. Evaluation of fuH_{tis} and fuT predictions against brain homogenate data

- (Unexpected) comparable prediction accuracy
- Largest differences between fuH_{tis} and fuT observed for moderate-to-strong bases (e.g., amantadine)

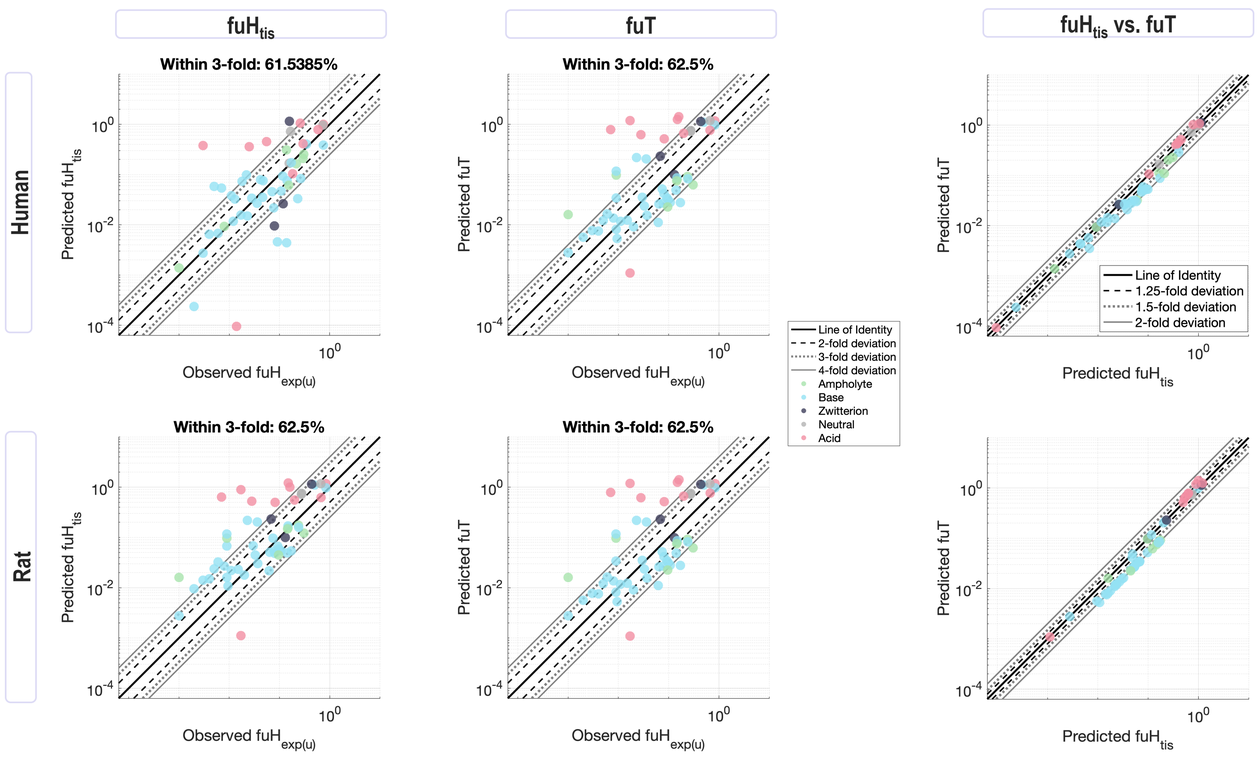


Figure 2. fuH_{tis} and fuT prediction vs. experimental data. Rat and human physiological parameters from Table 1; lipid content from Xu et al. 2020[3]. Relationship between predicted fuH_{tis} and fuT and experimentally observed fraction unbound for 56 and 52 small molecules (color-coded for drug type) for rats and humans[6,7,8], respectively. Dashed lines represent factor 2, 3, 4 either side of the line of unity for predicted vs. observed values, whereas they represent factor 1.25, 1.5, and 2 for predicted fuH_{tis} vs. fuT

3. Impact of tissue constituent fractional volumes on fuH_{tis} and fuT

- Prediction accuracy is highly sensitive to uncertainties in tissue composition (e.g., acidic phospholipids)
- fuH_{tis} improves with increasing $V_{ap(c)}/V_{tis}$ despite reduced accuracy in absolute terms at the upper bound

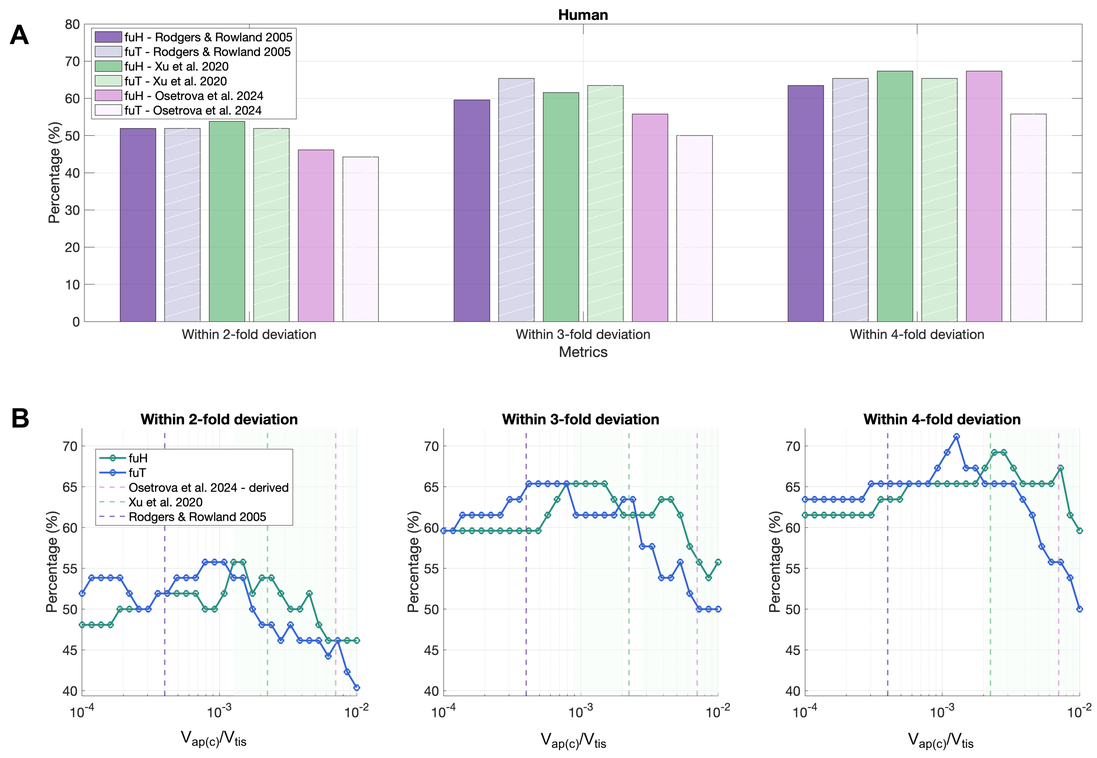


Figure 3. Evaluation of human fuH_{tis} and fuT prediction accuracy by systematic variation of acidic phospholipid fractional volume. Physiological parameter setting from Table 1. A) Comparison between three different $V_{ap(c)}/V_{tis}$ from the literature[1,3,9]. B) Sensitivity analysis of $V_{ap(c)}/V_{tis}$ within plausible ranges, with thresholds representing the different $V_{ap(c)}/V_{tis}$ employed in (A); green shaded area corresponds to fuH_{tis} better performance

Take home message & Perspectives

- fuH_{tis} is mechanistically suited for brain homogenate data, as it incorporates pH gradient and cell membrane disruption
- fuH_{tis} – particularly for highly basic drugs - could serve as a *sentinel probe* for acidic phospholipid content
- Need for more accurate measurements of brain lipid composition to improve fraction unbound predictions

References

1. Rodgers, T., Leahy, D. & Rowland, M. *J Pharm Sci* **94**, 1259–1276 (2005)
2. Rodgers, T. & Rowland, M. *J Pharm Sci* **95**, 1238–1257 (2006)
3. Xu, L., *et al. International Journal of Analytical Chemistry* 2020.**1**, 8819437 (2020)
4. Poulin, P. & Theil, F. *J Pharm Sci* **91**, 129–156 (2002)
5. ICRP Publication 89. *Ann ICRP* **32**, 5–265 (2002)
6. Summerfield, S. G. *et al. Xenobiotica* **38**, 1518–1535 (2008)
7. Di, L. *et al. Drug Metabolism and Disposition* **39**, 1270–1277 (2011)
8. Fridén, M., *et al. Drug Metabolism and Disposition* **39.3,** 353-362 (2011)
9. Osetrova, M., *et al. Nature Communications* **15.1**, 4455 (2024)
10. Gaohua, L., *et al. Drug Metabolism and Pharmacokinetics* **31.3**, 224-233 (2016).

Acknowledgments

We thank Prof. Ralf Engbert, Prof. Michael Rapp, and Prof. Daniel Schad for their valuable support to this project

Contacts