Predicting Cell-Specific Intracellular Binding from Phospholipid Affinity and Membrane Composition

Hinnerk Boriss

Sovicell, Friedrich Ebert Str 16, 04109 Leipzig, Germany, hbo@sovicell.com

Why Intracellular Binding Matters

Only the unbound intracellular drug fraction is available to interact with intracellular targets and therefore contributes directly to pharmacological activity. Differences in intracellular binding can substantially alter the relationship between total cellular drug concentration and effective target-site exposure. Phospholipid membrane affinity is a major determinant of intracellular binding and provides a mechanistic basis for predicting cell-specific $f_{u,cell}$ (Treyer et al., 2018; Treyer et al., 2019).

Homogenate Dilution Amplifies Error

Conventional estimates of intracellular unbound fraction ($f_{u,cell}$) are commonly obtained by measuring $f_{u,hom}$ in diluted cell homogenates and extrapolating back to the intact cell. This approach becomes increasingly unreliable as $f_{u,hom}$ approaches 1, because the measurable binding signal, 1-$f_{u,hom}$, becomes small relative to analytical error. With only 5% CV in each LC-MS/MS concentration measurement, the propagated CV of $f_{u,hom}$ is approximately 7.1%, while the uncertainty of the corresponding bound/free ratio (RBF) increases steeply: from ~28% at $f_{u,hom}$=0.75 to 71% at 0.90 and 354% at 0.98. Maintaining a sufficiently large binding signal therefore requires highly concentrated homogenates, which, depending on membrane affinity and cellular phospholipid content, may require hundreds of millions to billions of cells per mL.

The minimum cell concentration required to keep the homogenate binding signal within a defined precision limit can be calculated from the analytical variability of $f_{u,hom}$, the tolerated RBF uncertainty τ, the compound's membrane affinity (MA), and the phospholipid volume per cell:

$$C_{\min} = \frac{CV_{f_{u,hom}}}{(\tau - CV_{f_{u,hom}})MAv_{PL,cell}}$$

Applying this criterion reveals how rapidly the required cell concentration increases as membrane affinity decreases. Moderate-affinity compounds may require tens to hundreds of millions of cells per mL, while weakly membrane-binding compounds can require experimentally impractical cell concentrations.

	C_{min}			
cell type	**MA = 10**	**MA = 100**	**MA = 1,000**	**MA = 10,000**
	[×10⁶ cells/mL]	[×10⁶ cells/mL]	[×10⁶ cells/mL]	[×10⁶ cells/mL]
Human hepatocytes	291.0	29.1	2.9	0.3
Caco-2	629.0	62.9	6.3	0.6
A549	995.0	99.5	10.0	1.0
K562	1186.0	118.6	11.9	1.2
HEK293	2056.0	205.6	20.6	2.1
HL-60	2804.0	280.4	28.0	2.8

Mechanistic Model for $f_{u,cell}$

Measure the binding driver directly

Rather than inferring strong intracellular binding from the small residual binding signal left after homogenate dilution, the underlying interaction can be measured directly. Phospholipid membrane affinity (MA) captures the compound-specific tendency to partition into cellular membranes; when combined with the cell-specific phospholipid phase fraction, it provides a mechanistic route to predict intracellular binding and $f_{u,cell}$ without relying on error-amplified back-extrapolation from $f_{u,hom}$.

Separate compound and cell properties

Intracellular phospholipid binding can be separated into two independent components: a compound-specific membrane affinity (MA) and a cell-specific effective phospholipid phase fraction φ_{PL}. MA describes the compound's tendency to partition into phospholipid membranes, whereas φ_{PL} reflects the amount of phospholipid membrane phase available for binding in a given cell type. This separation allows the same experimentally determined MA to be combined with different cellular parameters to predict cell-specific intracellular binding.

The intracellular bound/free ratio can therefore be expressed as the product of the compound-specific membrane affinity (MA) and the cell-specific effective phospholipid phase fraction φ_{PL}:

$$RBF_{cell} = MA \cdot \phi_{PL} \quad \text{with} \quad \phi_{PL} = \frac{V_{PL}}{V_{aq}}$$

Predict intracellular binding directly

Once the intracellular bound/free ratio is predicted from MA and the cell-specific phospholipid phase fraction, the corresponding unbound intracellular fraction follows directly from the relationship between RBF and f_u:

$$f_{u,\text{cell}} = \frac{1}{1 + \text{MA}\phi_{\text{PL}}}$$

This converts the mechanistic binding inputs into a direct prediction of fu,cell without back-extrapolation from diluted homogenates.

Predicted vs. Measured Intracellular Binding

We compared published intracellular unbound fractions ($f_{u,cell}$) for 42 distinct compound/cell-type combinations with TRANSIL-based predictions derived from compound affinity for phosphatidylcholine-coated beads and cell-specific phospholipid content. This comparison tests how well phosphatidylcholine membrane affinity captures published intracellular binding estimates. Predictions are shown both without adjustment and with empirical membrane-composition correction factors to assess systematic differences between the mechanistic model and literature-derived $f_{u,cell}$ values.

Predictions were calculated from PC membrane affinity and the cell-specific phospholipid phase fraction. To assess systematic cell-type-dependent deviations, corrected predictions were additionally calculated as:

$$f_{\text{u,cell}} = \frac{1}{1 + K\,MA_{\text{PC}}\,\phi_{\text{PL}}}.$$

For HEK293 and MDCK, the observed systematic offsets were approximated by K_{HEK293}=2.5 and K_{MDCK}=4, respectively; human-hepatocyte predictions remained uncorrected.

Tab. 1: Literature $f_{u,cell}$ values compared with predictions from phosphatidylcholine membrane affinity and the cell-specific effective phospholipid phase fraction, φ_{PL}. Corrected predictions apply empirical composition factors of K_{HEK293}=2.5 and K_{MDCK}=4; human hepatocyte predictions are shown uncorrected..

#	compound	cell type	ϕ_{PL}	$f_{u,cell}$ literature	$f_{u,cell}$ predicted	$f_{u,cell}$ predicted (K_{HEK293} = 2.5, K_{MDCK} = 4)
1	Caffeine	HEK293	0.0132	0.74 [1]	0.975	0.939
2	Carbamazepine	HEK293	0.0132	0.22 [2]	0.323	0.16
3	Diazepam	HEK293	0.0132	0.033 [2]	0.0888	0.0375
4	Diclofenac	HEK293	0.0132	0.11 [1]	0.0981	0.0417
5	Diclofenac	HEK293	0.0132	0.059 [2]	0.16	0.0707
6	Fluvastatin	HEK293	0.0132	0.017 [2]	0.0162	0.00653
7	Imipramine	HEK293	0.0132	0.017 [1]	0.0372	0.0152
8	Imipramine	HEK293	0.0132	0.018 [2]	0.0149	0.006
9	Imipramine	HEK293	0.0132	0.018 [2]	0.0124	0.005
10	Indomethacin	HEK293	0.0132	0.098 [1]	0.502	0.287
11	Indomethacin	HEK293	0.0132	0.053 [2]	0.131	0.057
12	Ketoconazole	HEK293	0.0132	0.01 [2]	0.0292	0.0119
13	Ketoconazole	HEK293	0.0132	0.01 [2]	0.0314	0.0128
14	Propranolol	HEK293	0.0132	0.032 [2]	0.0392	0.0119
15	Propranolol	HEK293	0.0132	0.032 [2]	0.0174	0.00705
16	Repaglinide	HEK293	0.0132	0.016 [2]	0.0567	0.0235
17	Repaglinide	HEK293	0.0132	0.016 [2]	0.0564	0.0234
18	Carbamazepine	MDCK	0.0185	0.22 [3]	0.254	0.0785
19	Diazepam	MDCK	0.0185	0.021 [3]	0.065	0.0171
20	Diclofenac	MDCK	0.0185	0.033 [3]	0.119	0.0328
21	Fluvastatin	MDCK	0.0185	0.0099 [3]	0.0116	0.00292
22	Imipramine	MDCK	0.0185	0.00089 [3]	0.0107	0.00269
23	Imipramine	MDCK	0.0185	0.00089 [3]	0.00888	0.00224
24	Indomethacin	MDCK	0.0185	0.025 [3]	0.0973	0.0262
25	Ketoconazole	MDCK	0.0185	0.0042 [3]	0.021	0.00535
26	Ketoconazole	MDCK	0.0185	0.0042 [3]	0.0226	0.00574
27	Propranolol	MDCK	0.0185	0.0037 [3]	0.021	0.00535
28	Propranolol	MDCK	0.0185	0.0037 [3]	0.0125	0.00315
29	Repaglinide	MDCK	0.0185	0.0069 [3]	0.0411	0.0106
30	Repaglinide	MDCK	0.0185	0.0069 [3]	0.0409	0.0106
31	Diclofenac	Human hepatocytes	0.0488	0.83 [4]	0.0286	0.0286
32	Erythromycin	Human hepatocytes	0.0488	0.0444 [5]	0.452	0.452
33	Fluvastatin	Human hepatocytes	0.0488	0.013 [6]	0.00444	0.00444
34	Imipramine	Human hepatocytes	0.0488	0.0036 [6]	0.00408	0.00408
35	Imipramine	Human hepatocytes	0.0488	0.0036 [6]	0.00339	0.00339
36	Indomethacin	Human hepatocytes	0.0488	0.048 [6]	0.0393	0.0393
37	Ketoconazole	Human hepatocytes	0.0488	0.0061 [6]	0.0081	0.0081
38	Ketoconazole	Human hepatocytes	0.0488	0.0061 [6]	0.0087	0.0087
39	Omeprazole	Human hepatocytes	0.0488	0.93 [7]	0.131	0.131
40	Propranolol	Human hepatocytes	0.0488	0.013 [6]	0.0081	0.0081
41	Propranolol	Human hepatocytes	0.0488	0.013 [6]	0.00478	0.00478
42	Roxithromycin	Human hepatocytes	0.0488	0.0241 [5]	0.167	0.167

Sources for $f_{u,cell}$ literature values:

[1] Mateus et al. (2013)
[2] Mateus et al. (2017), Scientific Reports 7, 43047; Supplementary Table 2
[3] Mateus et al. (2017), Scientific Reports 7, 43047; Supplementary Table 3
[4] Human hepatocyte donor study (2021)
[5] Filppula et al. (2019), Scientific Reports 9, 5850; Table 2
[6] Mateus et al. (2017), Scientific Reports 7, 43047; Supplementary Table 4
[7] Human hepatocyte donor study (2021); Table 3

Conclusion

Measure the binding driver—not the diluted signal

(1) Homogenate dilution can severely amplify uncertainty in $f_{u,cell}$. When $f_{u,hom}$ approaches 1, small analytical errors translate into large errors in the inferred bound/free ratio, and reliable measurements may require impractically high cell concentrations..

(2) Intracellular binding can be expressed mechanistically from separable compound and cell properties. Compound-specific membrane affinity (MA) and the cell-specific effective phospholipid phase fraction φ_{PL} provide a direct route to predict $f_{u,cell}$ without back-extrapolation from diluted homogenates.

(3) Membrane composition matters; PC-only MA introduces systematic cell-type-dependent bias.

(4) The mechanistic model is supported by independent literature data. Across 42 HEK293, MDCK, and human-hepatocyte observations, composition-corrected predictions gave a log-scale R^2 of about 0.63, despite uncertainty in both cellular parameters and the literature-derived $f_{u,cell}$ values.

(5) A defined membrane-affinity assay provides a practical alternative to cell-intensive homogenate studies. TRANSIL-derived Intracellular Binding Kit measurements can supply the compound-specific input for scalable, cell-free prediction of intracellular binding.

Mechanistic Composition Correction

Treyer et al. showed that affinities to individual phospholipid classes contribute approximately additively. Thus, the effective membrane affinity can in principle be calculated from phospholipid composition:

$$MA_{\text{eff}} = \sum_i x_i MA_i$$

and

$$K = \frac{MA_{\text{eff}}}{MA_{\text{PC}}}$$

where x_i is the cellular fractional abundanceof each phospholipid class. The empirical K values used here therefore approximate the membrane-composition contribution missing from the PC-only MA measurement. Ultimately, K should be predicted from phospholipid-class affinities and cell-specific membrane composition rather than fitted empirically.

Figure 1 shows the resulting composition-corrected predictions for HEK293 and MDCK; human hepatocyte predictions remain uncorrected. Notably, the strongest outliers originate from literature $f_{u,cell}$ values back-calculated from diluted-homogenate $f_{u,hom}$ measurements and fall in the regime where our error analysis predicts >300% CV in RBF. Thus, part of the apparent model deviation likely reflects uncertainty in the reference values, suggesting more consistent performance of the mechanistic membrane-affinity approach than the raw comparison alone implies.

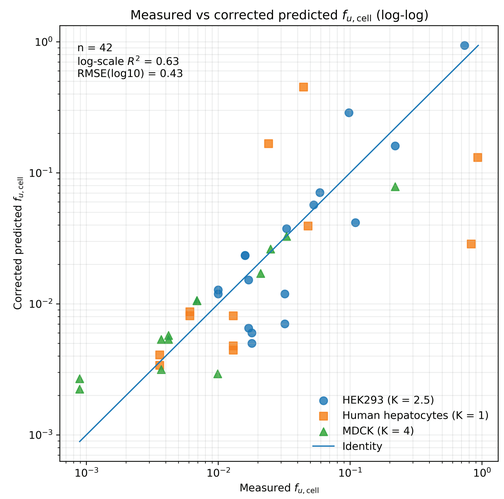


Fig. 1: Measured versus mechanistically predicted intracellular unbound fraction ($f_{u,ell}$) for HEK293, MDCK, and human hepatocytes. Predictions were calculated from phosphatidylcholine membrane affinity (MA_{PC}) and the cell-specific effective phospholipid phase fraction (φ_{PL}). To approximate membrane-composition effects not captured by PC-only MA measurements, empirical correction factors of K_{HEK293}=2.5 and K_{MDCK}=4 were applied; human hepatocyte predictions remained uncorrected. Across 42 literature observations, corrected predictions showed a log-scale (R^2=0.63). The remaining deviations likely reflect compound-specific phospholipid selectivity, uncertainty in cellular parameters, and experimental uncertainty in the literature-derived $f_{u,cell}$ values. Several of the strongest outliers derive from diluted homogenate-based literature estimates and lie in the high-error regime predicted by our uncertainty analysis, suggesting that part of the residual scatter reflects uncertainty in the reference $f_{u,cell}$ values rather than the mechanistic model itself.