Domainex
Enrich your Medicines Pipeline

Cell Accumulation and Fraction Unbound Bridge the Gap Between Enzyme and Cellular Assay Disconnects

White Paper

Introduction

The ability to quantify total intracellular compound exposure as well as the unbound compound fraction ($f_{u,cell}$) can greatly enrich the understanding of cellular efficacy, disposition and toxicity.[1] It is particularly useful in probing the apparent disconnect between the biochemical, enzyme and cellular activities often observed in *in vivo* and/or *in vitro* models.

At Domainex, our *Analytical Chemistry* experts have established cell accumulation assays that deliver a concentration profile over time, capturing rate of penetration, AUC and C_{max} parameters. This helps explain why compounds with similar enzyme activities behave differently in cellular environments. It can reveal highly accumulating compounds, which might cause off-target effects and cytotoxicity, as well as non-penetrating compounds, which cannot reach intracellular targets. This can help guide *Medicinal Chemistry*, for instance by prioritising certain molecular scaffolds over others. Cell accumulation can be particularly informative when used with modified cell lines to inform on the impact of drug transporters and drug-metabolising enzymes.

Often, cell accumulation is supplemented by measurements of intracellular compound binding, giving insight into effective concentrations within the intracellular matrix. This measure has the capacity to not only explain why compounds with similar cell penetration abilities have different efficacies, but also to clarify the disparity between the intracellular and extracellular concentrations of compounds that rely on uptake/efflux transporters for distribution. For a comprehensive understanding of intracellular and extracellular bioavailabilities, it is recommended to determine both $f_{u,cell}$ and $f_{u,medium}$.

Both the data from cell accumulation and the fraction unbound can be combined to obtain the unbound drug accumulation ratio (Kp_{uu}). This can be utilized as a powerful tool in probing the disconnect between the enzyme and cellular assays as well as aiding medicinal chemists with compound selection by ranking the chemical probes according to their intracellular target engagement.

Methods

Intracellular Drug Accumulation is determined in live cells after incubation with test compound at 37 °C. The assay is carried out typically over the course of 24 hours but can be extended for longer periods, if required. The reaction is terminated by removing the media and washing the cells with ice cold phosphate-buffered saline (PBS) to remove any compound that is not associated with the cells. Both the media and the cell pellets are extracted via protein precipitation and sonication. The drug concentrations in the cells and incubated solution are quantified using Ultra Performance Liquid Chromatography (UPLC)-MS/MS analysis.

Intracellular Fraction of Unbound Drug is determined in a cell homogenate by equilibrium dialysis using a Rapid Equilibrium Dialysis (RED) device. The test compound (typically at a concentration of 0.5 µM) is added to a cell homogenate and dialysed against modified Hank's balanced salt solution (HBSS). After dialysis, the concentrations in the buffer and in the cell homogenate are quantified by UPLC-MS/MS analysis.

Example Data

A brief example of the application of cell accumulation/penetration data to explain the disconnect between the enzyme activity and potency as shown in figure 1, below:

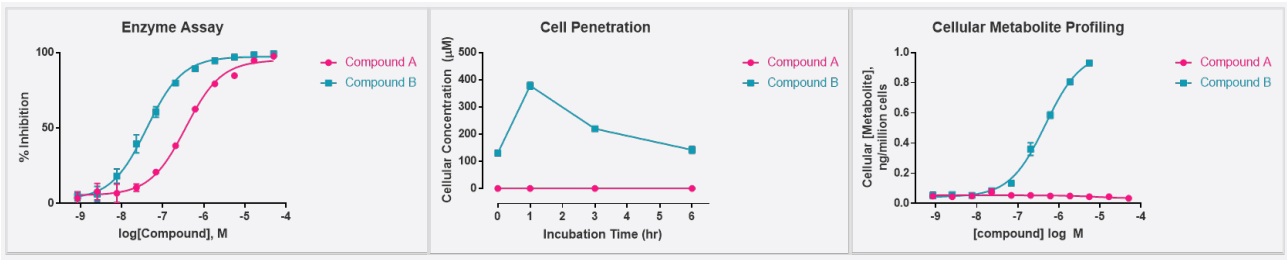


Figure 1: Structurally similar compounds show comparable enzyme activities but contrasting intracellular levels, which explain the differences observed in *in vitro* models.

It can be clearly seen from this example (panel 2, figure 1) that compound B has superior cell penetration compared to compound A and that assessing cell accumulation via this assay format is an extremely beneficial additional tool for prioritising compounds through any screening cascade and into *in vivo* assessment. In addition, the assessment of inhibitors utilising the unbound drug ($f_{u,cell}$) in different cell types (figure 2) is an accepted way of relating potency to efficacy.

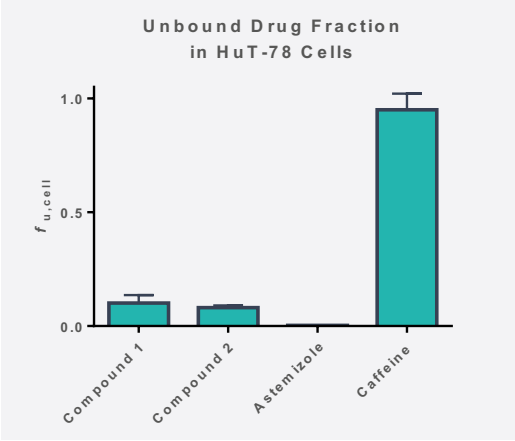


Figure 2: Unbound drug fraction can be applicable to multiple targets, cell types and therapeutic areas.

Reference

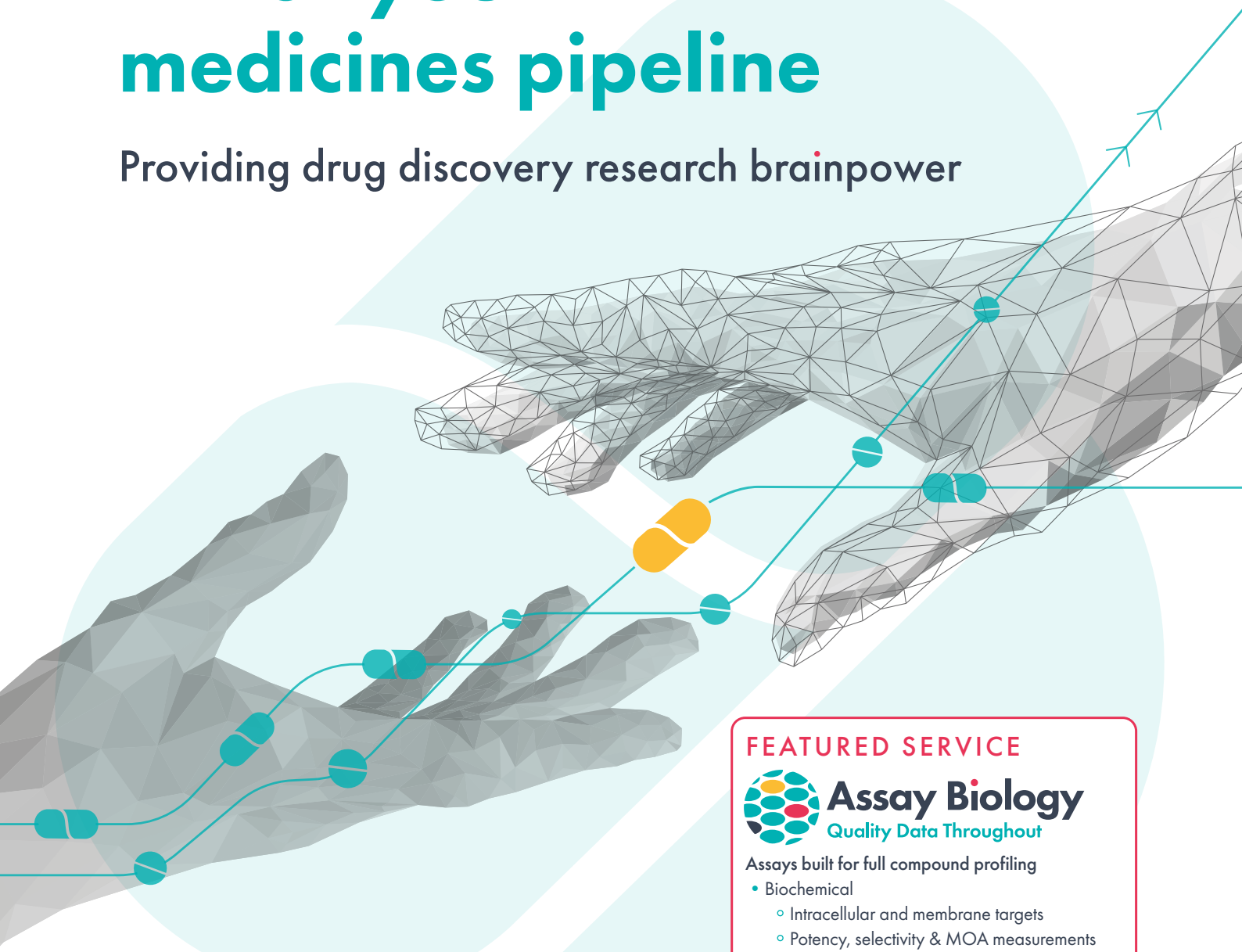
1. Rapid Measurement of Intracellular Unbound Drug Concentrations. *Mol. Pharmaceutics* **2013**; 10: 2467-2478.

Domainex

domainex.co.uk

Enrich your medicines pipeline

Providing drug discovery research brainpower



- Tailored multidisciplinary expertise
- Forming dynamic relationships
- Innovative outcomes for our partners

FEATURED SERVICE

Assay Biology

Quality Data Throughout

Assays built for full compound profiling

- Biochemical
  - Intracellular and membrane targets
  - Potency, selectivity & MOA measurements
- Biophysical:
  - Full suite (MST, GCI/SPR, DSF/nanoDSF, NMR, X-ray crystallography)
  - Affinity, kinetic & structural determinations
- Cellular
  - Cell lines and primary cells
  - Signalling & reporter-based assays
  - On and off target assessments

Based in the Cambridge UK Biocluster

Established in 2001

Contact us today!

enquiries@domainex.co.uk