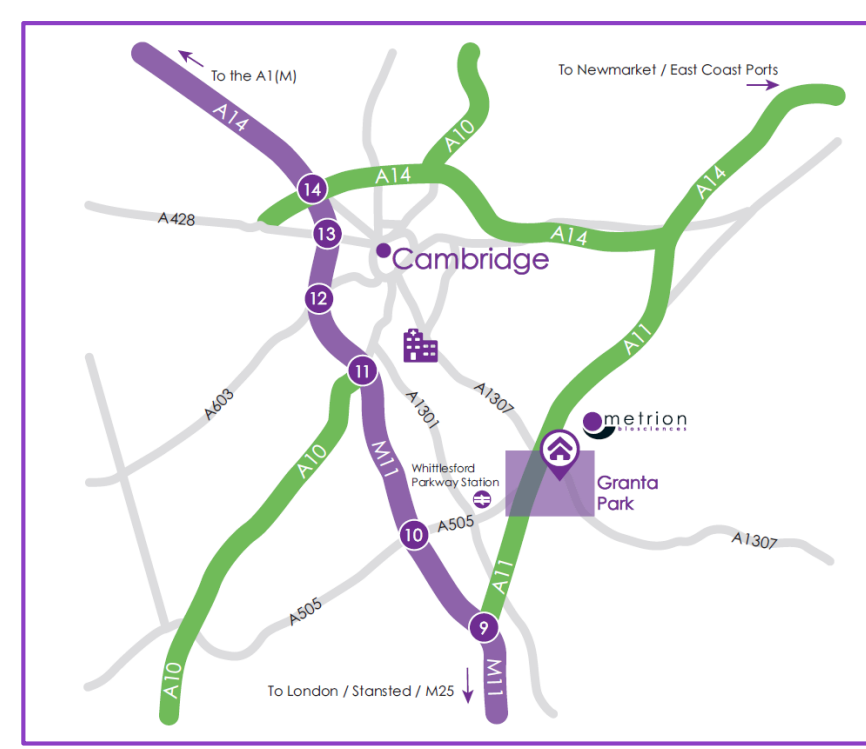


Metrion Biosciences: Experts in Ion Channel Drug Discovery Services

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Introduction

Metrion Biosciences is a UK based CRO, located at Granta Park in Cambridge. Our team has substantial expertise in providing research services to deliver preclinical and clinical stage drug candidates, and has a proven track record of providing high quality drug discovery services to our clients for ion channel targets on a fee-for-service or collaboration basis. The Metrion team takes pride in providing a knowledgeable, collaborative and flexible service to all clients, whether for small stand alone projects or fully integrated drug discovery programmes.



Ion Channel Screening

Metrion offers high quality ion channel screening services using a variety of **electrophysiology**, **label free** and **fluorescence-based platforms**. Our assay technologies include **QPatch 48** and **Patchliner** automated electrophysiology, 'gold standard' **manual patch clamp** electrophysiology, **impedance/microelectrode array** techniques and **FlexStation** assays.

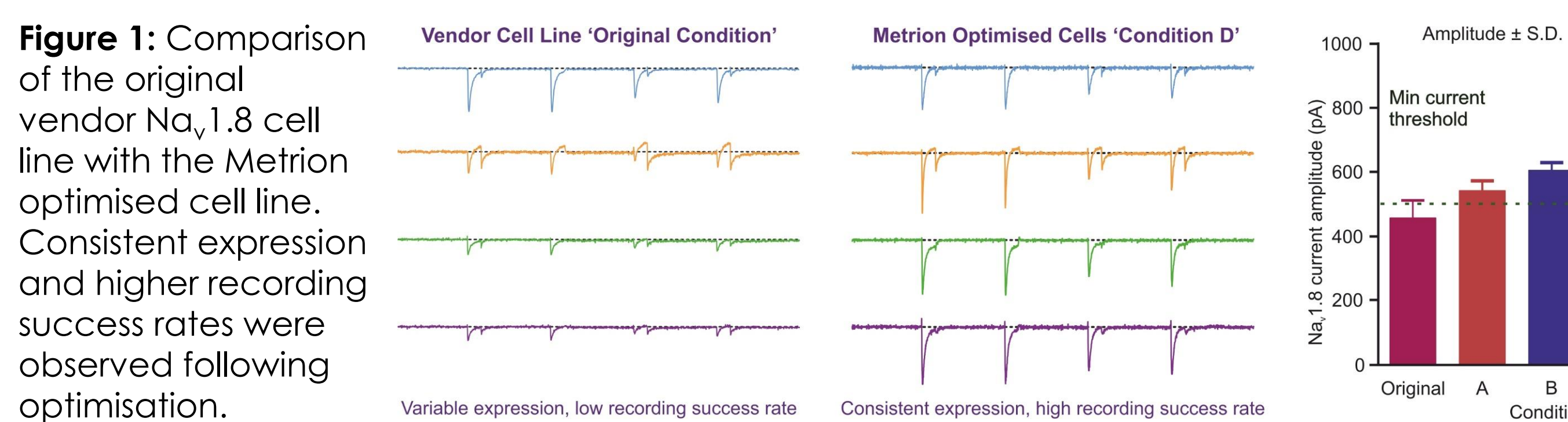


Cell line optimisation for Ion Channel Screening

Metrion has extensive experience of cell line generation, assay development and validation in order to help develop robust screening assays.

Case Study: development of a Na_v1.8 assay on the Patchliner

- To overcome the poor expression associated with a vendor-provided Na_v1.8 recombinant cell line, Metrion developed and refined a Na_v1.8 assay on the Nanion Patchliner platform.
- Metrion optimised the cell line handling and tissue culture conditions to increase Na_v1.8 expression levels, allowing the development of a highly efficient SAR profiling assay.



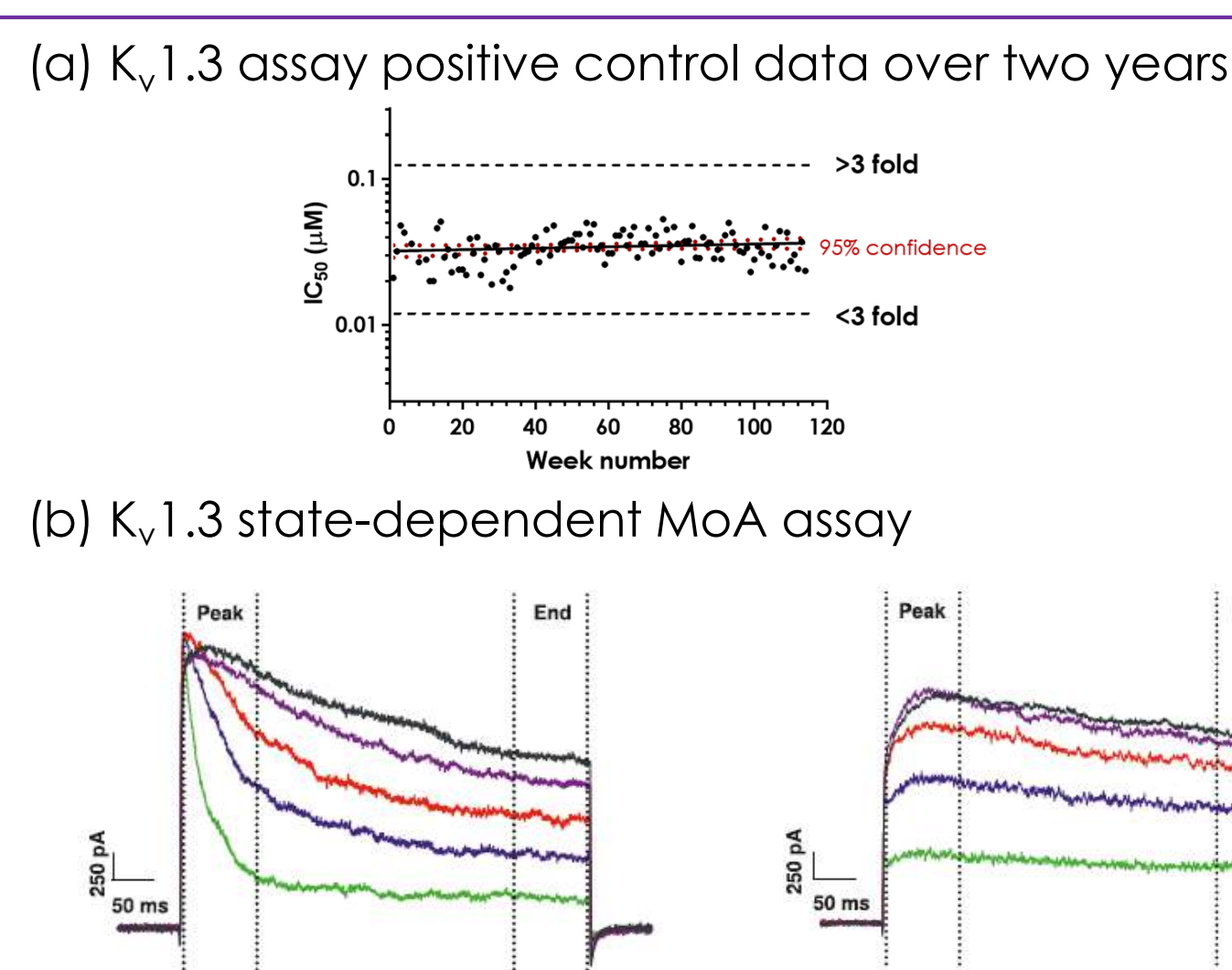
Ion Channel Assay Optimisation and Consistency

- For over a decade, Metrion's scientists have been involved in developing, optimising and validating automated and manual patch clamp assays for ion channel screening.
- We can fully customise ion channel assays at different stages of the screening cascade, from hit finding and medium throughput structure-activity studies, through to specialised biophysical and mechanism-of-action studies of lead compounds.

Case Study: consistent behaviour of a state-dependent K_v1.3 assay on the QPatch

Metrion developed a human K_v1.3 assay on QPatch 48 with the ability to provide an early Indication of mechanism of action (MoA) of compound activity. This assay format was successfully applied to a rat K_v1.3 channel and the full human K_v1.x selectivity panel.

Figure 2: Consistent behaviour of a state-dependent K_v1.3 QPatch 48 assay over two years (a). Example MoA traces (b).



Compound Screening

- Single concentration point and cumulative concentration-response formats.
- Using either Metrion stable cell lines, client cell lines, or transiently transfected cells.
- Detailed biophysical studies to characterise compound MoA.
- Validated assays for a wide range of voltage- and ligand-gated ion channels.
- CiPA-compliant cardiac safety panel.
- Industry-standard neurotoxicology assay.
- Translational native cell and human iPSC assays.

Case Study: a natural product screen in collaboration with Venomtech to find novel toxin modulators of the K_v1.3 channel

- Metrion undertook a screen of phylogenetically diverse animal toxins using its validated human K_v1.3 cell line assay.
- QPatch 48 platform used for a high quality primary screen.
- Fractions screened at n=1/2; 20 - 200 nM concentration range.
- Threshold for hit identification was set at >51% block.
- All positive controls were identified correctly.
- Negative controls yielded an average inhibition of 9.5 ± 15.3%.
- Seven scorpion species samples were confirmed active.

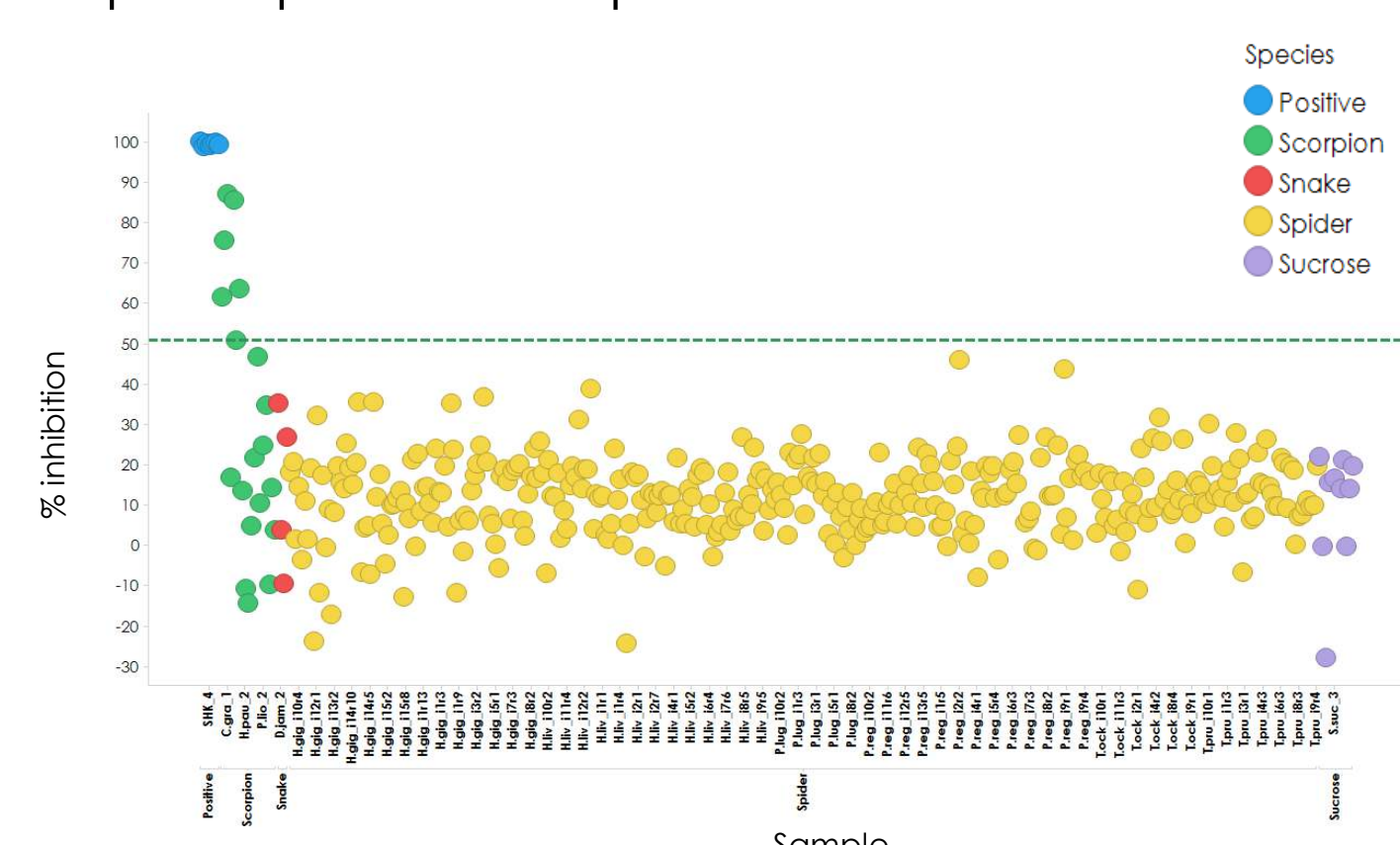


Figure 3: Summary of toxin species activity against the K_v1.3 channel

Cardiac Safety Profiling

Metrion provides cardiac safety screening services including assays incorporating all components of the FDA's **Comprehensive in vitro Proarrhythmia (CiPA)** initiative. This includes:

- Automated electrophysiology screening against an expanded panel of six cardiac ion channels, including hERG.
- In silico* modelling using electrophysiology data from the expanded panel.
- Confirmation of *in silico* predictions using translational assays employing human iPSC-derived cardiomyocytes.



Metrion is a member of the HESI Cardiac Safety committee and a key data provider in the CiPA HTS sub-team employing automated patch clamp validation data.

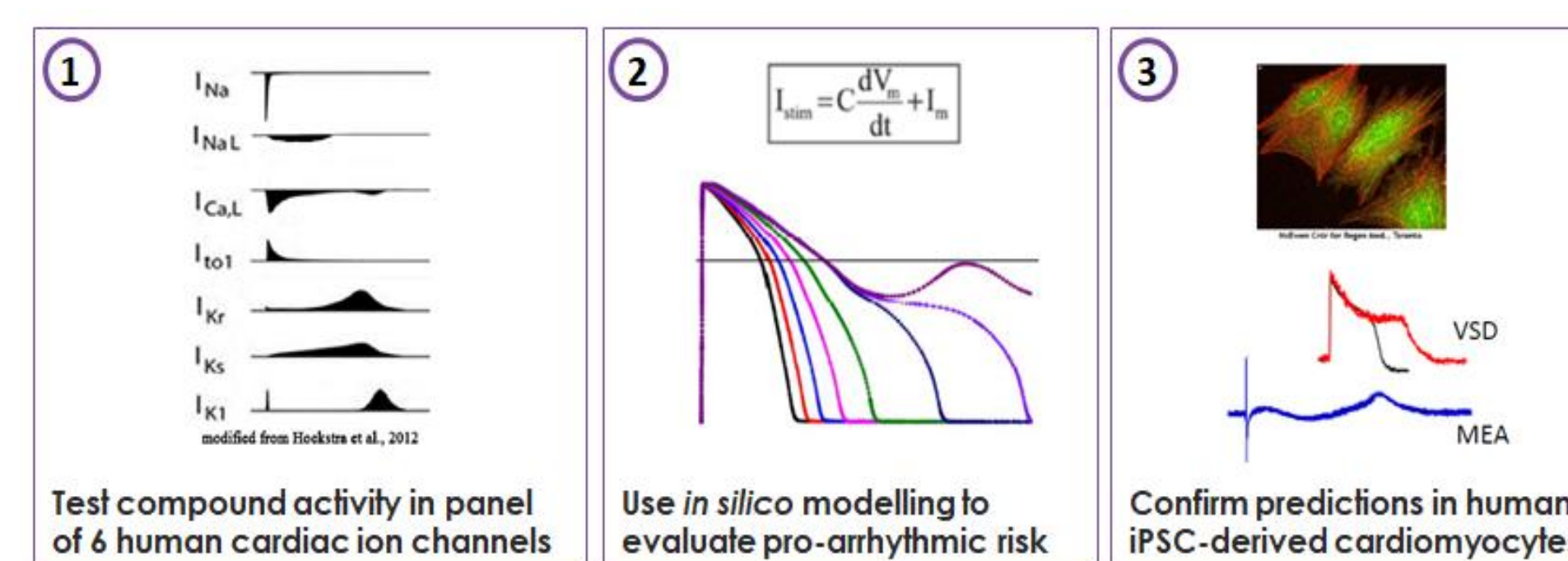


Figure 4: CiPA *in vitro* and *in silico* pillars for integrative assessment of cardiac arrhythmia risk. Graphic adapted from CiPA publications (Gintant 2015).

Figure 5: Metrion's panel of CiPA-ready cardiac ion channel safety assays – the **core cardiac panel** for balanced cardiac safety assessment and the **expanded cardiac panel** which meets or exceeds that offered by other CROs.

CiPA Panel	Target	hERG	Na _v 1.5	Ca _v 1.2	KLQT1	K _v 2.1	K _v 4.3
Core	hERG						
	hNa _v 1.5						
	hCa _v 1.2						
Expanded	hK _v 2.1						
	hK _v 4.3_KChIP						
	hK _v LQT1_minK						
Other	hHCN4						
	hK _v 1.5						

hERG Screening

- Metrion offers gigaseal quality non-GLP **hERG screening services** using either manual patch clamp or the QPatch 48 automated patch clamp platform.
- We offer **four-point** and **eight-point concentration response hERG screening assays**.
- Compounds are applied in **cumulative concentration-response format**, with double additions of compound at each concentration to ensure assessment of activity at steady-state.
- A **concentration-response curve** is also provided using the positive control reference compound, verapamil.

Neuroscience Services and Translational Assays

Translational Assays - Neuroscience Models

Manual Patch Clamp: In-depth Studies

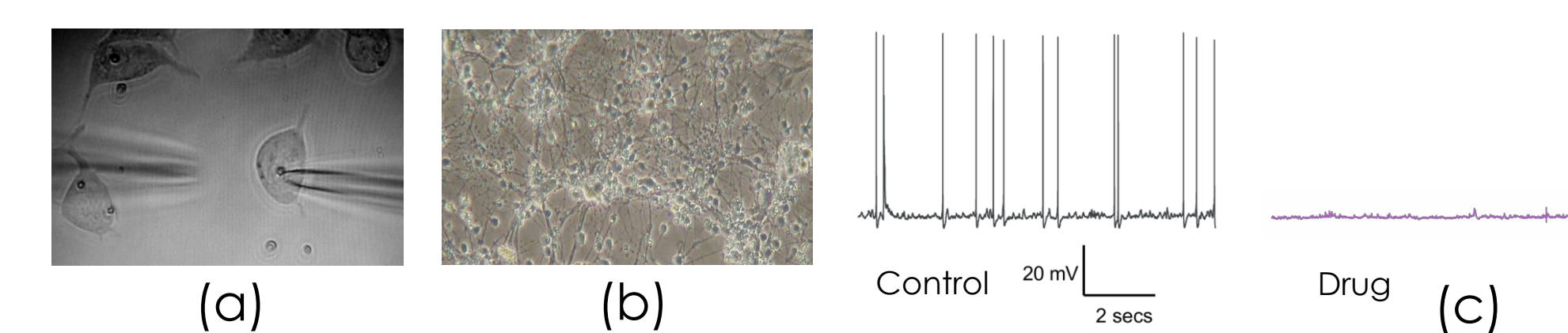


Figure 6: Native neurons

- Manual patch electrophysiology.
- Rat cortical neuronal networks.
- Spontaneous action potential recordings (control and in presence of drug).

Multi-Electrode Array (MEA) : Network firing behaviour



Figure 7: MEA assays

- Electrode array.
- Axion Maestro MEA System.
- Extracellular potentials.
- Activity heatmap (control and in presence of drug).

Metrion's neuroscience services include:

- Isolation of rat dorsal root ganglion (DRG neurons), access to cryopreserved hippocampal and cortical neurons.
- Neuroscience focused assay development services.
- Compound screening against ligand gated and voltage gated targets – staff have extensive experience with wide range of targets (TRPx, GluR, ASICs, etc) includes portfolio of TRPA1 assays and IP for pain and other therapeutic indications.

Integrated Drug Discovery

Metrion is also able to offer a fully integrated drug discovery service, combining expertise from our carefully selected partners: including AMRI, Concept Life Sciences and Assay.Works. We offer a streamlined service, fully managed by our expert team.

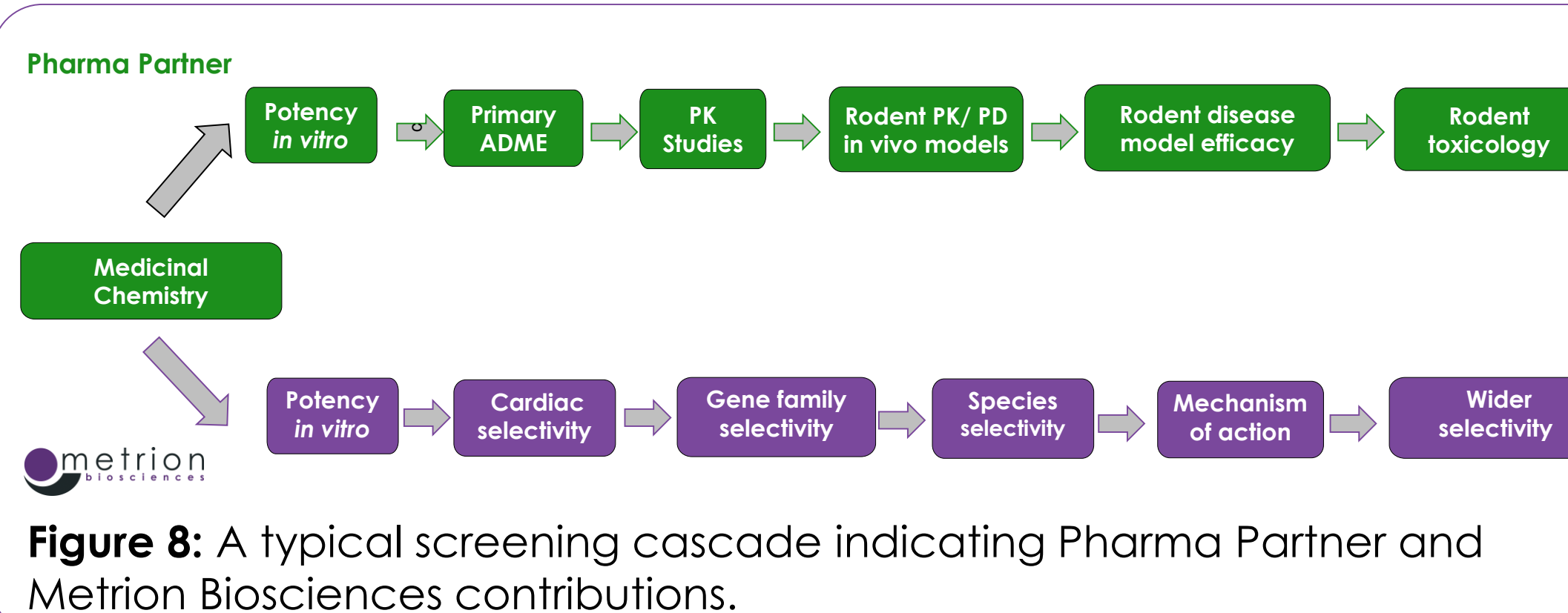
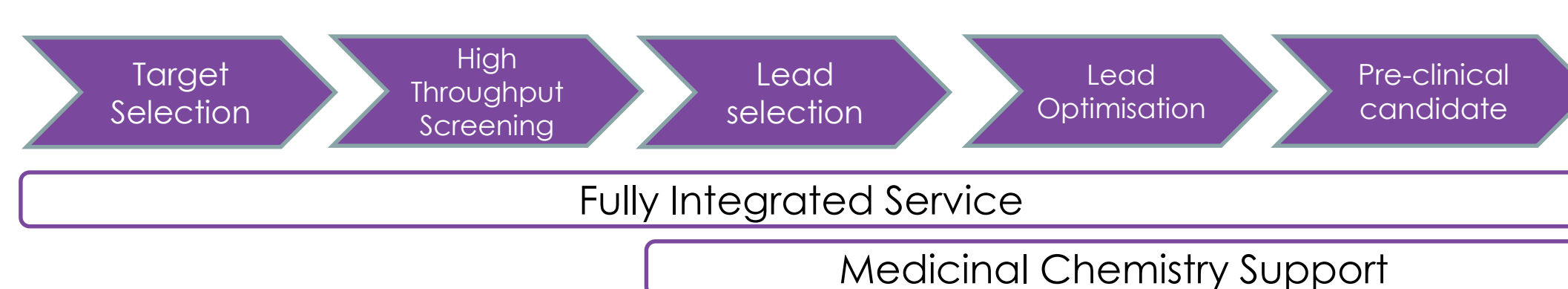


Figure 8: A typical screening cascade indicating Pharma Partner and Metrion Biosciences contributions.

Combining expertise in:

- Ion Channel Biology.
- Assay Development.
- High Throughput Screening.
- Medicinal and Computational Chemistry.
- Translational Biology.
- ADMET & DMPK.
- Toxicology.
- Project Management.