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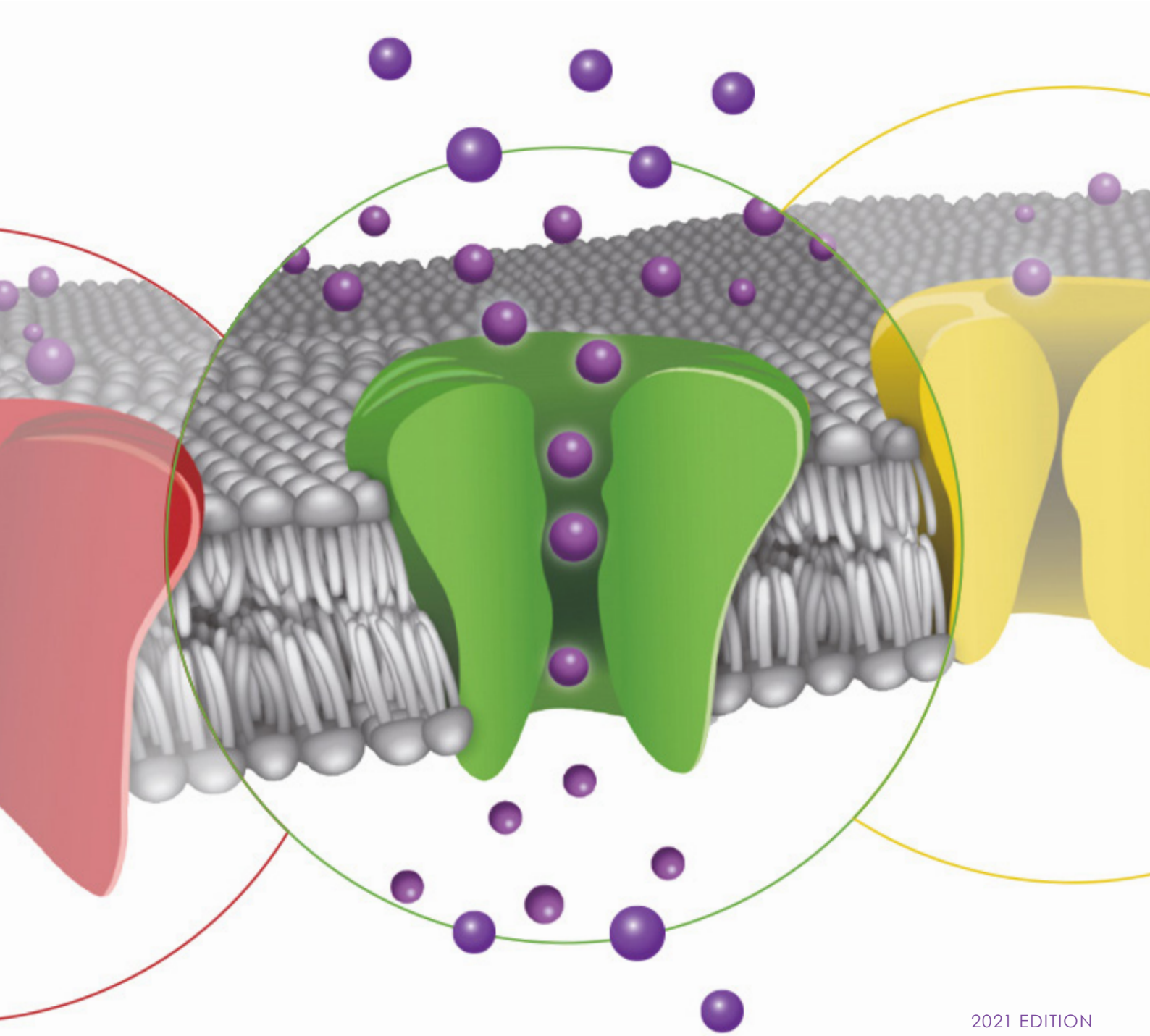
METRION BIOSCIENCES

Company Brochure



2021 EDITION

Welcome.

Welcome to Metrion Biosciences, leaders in ion channel drug discovery.

Metrion Biosciences is a preclinical ion channel contract research organisation formed in September 2015.

The Metrion team has substantial experience of providing high quality drug discovery services for ion channel targets to clients on a fee-for-service or collaboration basis.

Metrion is a leading provider of safety pharmacology services including cardiac safety profiling and neurotoxicology testing, as well as offering cardiac and neuronal translational assays using native cells and iPSC models. Our state of the art laboratory facility is located at Granta Park, the largest research park in Cambridge (UK), and the Metrion team takes pride in providing a knowledgeable, collaborative and flexible service to our clients.

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Our Services

Ion channel screening.
Metrion has developed validated screening assays against an extensive panel of ion channel cell lines using a variety of high quality ion channel screening platforms.

Cardiac ion channel screening.
Metrion offers screening services against a premium panel of validated Comprehensive In Vitro Proarrhythmia Assay (CiPA) compliant human cardiac ion channel screening assays.

Neuroscience ion channel screening.
Metrion offers a range of neuroscience related ion channel screening assays and platforms, including native tissue and species selectivity testing.

Translational Assays.
Metrion are developing phenotypic assays to aid the translation of in vitro cardiac safety and neuroscience data to the pre-clinical stage.

Integrated drug discovery.
Our highly experienced interdisciplinary team provides clients with a fully integrated drug discovery service by bringing together experts in ion channel biology, medicinal chemistry, specialist chemistry, translational biology, ADMET & DMPK.

What we do

Metrion staff offer proven ion channel electrophysiology expertise and reliable assays for our clients. Our services include:

- High quality, cost-effective compound screening assays
- Detailed characterisation of lead compounds in human cells and native tissue
- Confirmation of efficacy in stem cell and other phenotypic models
- Rapid reporting and data interpretation by our experienced ion channel team
- A dedicated, flexible service tailored to your requirements

Who we work with

From our Granta Park base in Cambridge, UK, we work with scientists and researchers from biotech and pharmaceutical companies, research institutions, disease charities and start-up companies worldwide to enable them to study this fascinating class of membrane proteins with confidence and insight.

We currently work with clients located in over 20 countries across five continents, many of whom are looking to validate, develop or de-risk ion channel modulators as they progress towards a nomination for clinical development.

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Why choose Metrion?

- **Highly experienced and diligent team with over 100 years combined knowledge of automated patch clamp (APC) and experience within academia, the pharmaceutical industry, contract research organisations and biotech companies.**
- **High quality data, with knowledgeable interpretation, within the defined timeframe.**
- **Fee for service and collaboration project options available.**
- **Client testimonials available.**
- **Flexible support for assay development, primary target screening, hit confirmation, lead optimisation and SAR, mechanistic and phenotypic studies.**

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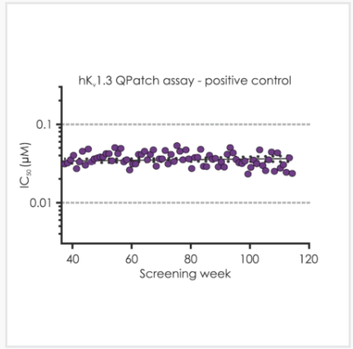
Ion Channel Screening.

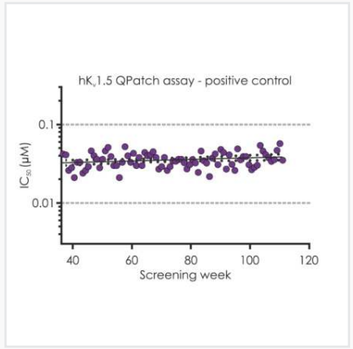
Metrion Biosciences ion channel and drug discovery screening expertise enables us to create and provide efficient assays that deliver reliable, high-quality data to accelerate and validate your drug discovery programmes. Metrion specialises in delivering high-quality ion channel screening services using electrophysiology, label-free and fluorescence-based platforms.

Our assay technologies include QPatch (Sophion) and Patchliner (Nanion) automated electrophysiology, conventional manual patch clamp, plate-based impedance and multi-electrode array techniques, and plate-based imaging using the FlexStation.

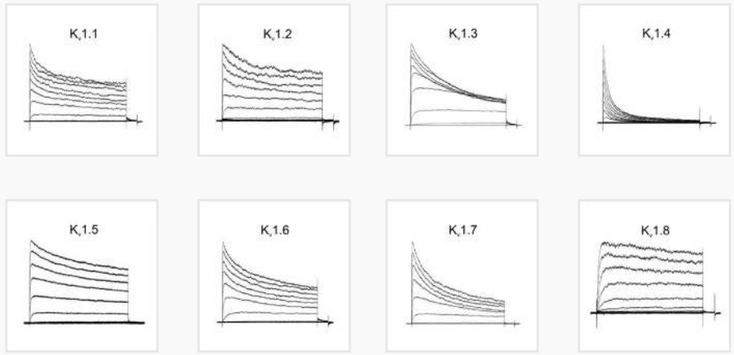
K_V1.3 Ion Channel Screening Case Study

Ion channel assays used for selectivity and SAR screening need to be stable over the entire duration of a drug discovery project, which can last for months or even years. In the example *below,* we show the exceptional reliability of the optimised primary target K_V1.3 (**below, left**) and gene family selectivity K_V1.5 counter-screening assays (**below, right**) used as part of a long-term pharma collaboration, plotting the positive control IC_{50} over the course of two years.



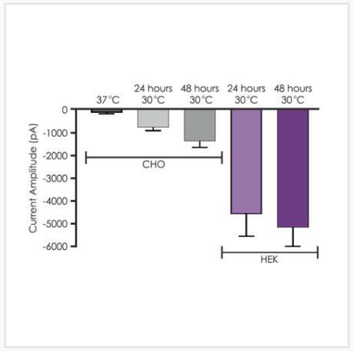


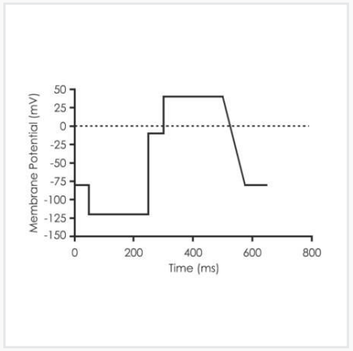
Metrion scientists also developed a complete gene family selectivity panel (**below**) of human K_V1.x ion channel cell lines and assays to complement the primary K_V1.3 screening assay. These assays were critical in supporting this long-term drug discovery project and in successfully identifying selective small molecule modulators able to treat auto-immune disease.



$Na_v1.5$ LQT3 cardiac cell line

During our work with the FDA's CiPA cardiac safety testing consortium it became apparent that a 'late' $Na_v1.5$ assay would be an important part of their selectivity panel to help predict the safety of new drug candidates.





We therefore created stable CHO and HEK cell lines expressing the DKPQ LQT3 mutant protein (**above, left**) and assessed their utility as reagents for automated patch clamp assays that would be compliant with CiPA HTS sub-team requirements (**above, right**).

Assay ready cells for APC

Biological reagents are inherently variable and it is typical for the performance of ion channel assays to fluctuate during extended passaging of stable cell lines. To minimise this variation during extended SAR screening campaigns, Metrion scientists created so-called 'assay ready' cells that are grown under conditions optimised for automated patch clamp assays and then frozen down to fix their state for later use. On the day of an experiment, cells are thawed and used directly on APC platforms to guarantee optimum performance, provide consistent screening results, and increase workflow flexibility to run multiple ion channel assays at short notice.

Cell line and assay optimisation

In some cases, cell reagents are not optimal for electrophysiology assays and need improved 'patchability' and expression of the protein of interest in a functional state at the cell surface.

Metrion's scientists have over a decade of experience in developing, optimising and validating automated and manual patch clamp assays for ion channel targets. We can fully customise 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.

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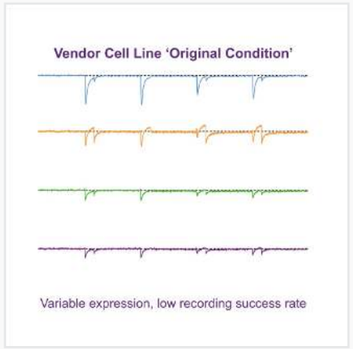
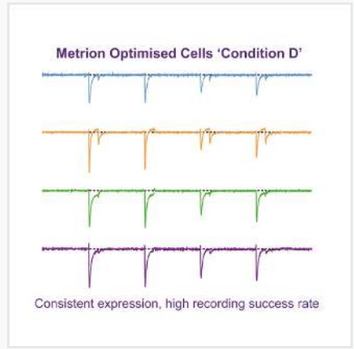
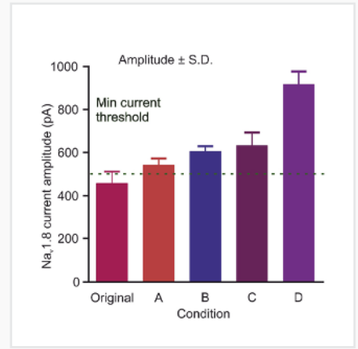
Na_v 1.x cell line optimisation

A neuronal Na^+ ion channel used as the primary counter-screening target in a major pharma drug discovery collaboration is notoriously difficult to express in heterologous cells, and under standard conditions the original cell line yielded a low success rate assay (**far left, below**). We tested a number of different cell culture, cell biology and experimental conditions (**far right, below**) to develop an optimised assay on the Patchliner (Nanion) automated patch clamp platform.





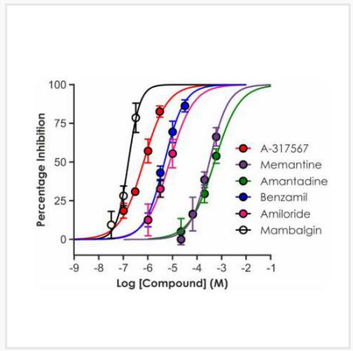


Our efforts significantly improved the low expression seen in the original reagent without affecting 'patchability', yielding a highly efficient gene family selectivity assay (**middle, above**).

Assay Development and Validation

Ion channels are large, complex transmembrane proteins that can be difficult to express with the correct folding and associated auxiliary subunit and scaffolding protein complexes, making it essential to validate cell line reagents and assays before they are used for drug discovery screening. Along with sequence verification, Metrion scientists determine the functional biophysical and pharmacological profile of each cell line reagent on our assay platforms prior to their use in drug discovery screening cascades.

ASIC1a QPatch assay



We recently validated a cell line expressing human ASIC1a, a ligand-gated receptor which is implicated in stroke and ischemia. Assay development efforts concentrated on creating a stable assay on the QPatch automated patch clamp platform and confirming the correct agonist and antagonist pharmacology using a selection of reference and literature compounds (**left**). More details on this assay validation effort is available in our ASIC1a application note which can be found on our website.

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Cardiac Safety Screening.

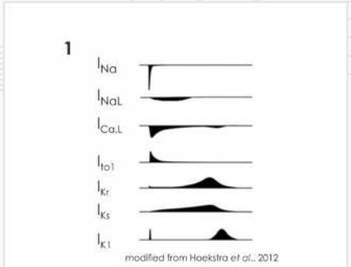
Metrion offers a range of high quality Cardiac Safety Screening assays, including hERG profiling and components of the Comprehensive *In Vitro* Proarrhythmia Assay (CiPA) initiative. This include assays that evaluate the proarrhythmic liabilities of compounds using:

- **A extensively validated panel of cardiac ion channels, including the six CiPA ion channels, using gigaohm seal quality automated electrophysiology devices.**
- **Action potentials recorded from human iPSC-derived cardiomyocytes using multi-electrode array and manual patch clamp platforms.**
- **The viability of human iPSC-derived cardiomyocytes using an impedance platform.**

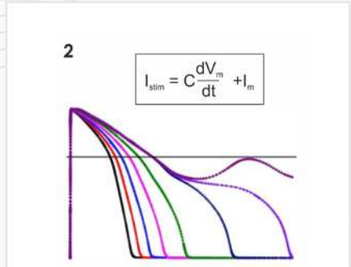
What is CiPA?

The International Council on Harmonization (ICH) S7B and E14 regulatory guidelines were introduced in 2005 to evaluate the proarrhythmic liability of new drugs. It was discovered that inhibition of a cardiac potassium channel (encoded by hERG) is associated with prolongation of the QT interval and a potentially deadly arrhythmia, Torsades de Pointes.

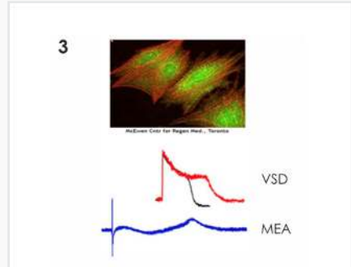
The guidelines utilise hERG inhibition and QT interval prolongation as surrogate markers of proarrhythmic liability, which are highly sensitive and effective at preventing proarrhythmic drugs from reaching the market. However, these markers have low specificity, with only a modest correlation between hERG inhibition, QT prolongation and proarrhythmic liability.



Compounds evaluated against panel of 6 cardiac ion channels



Use *in silico* modelling to evaluate proarrhythmic risk



Confirm predictions in human iPSC-derived cardiomyocytes

Therefore, to address these limitations, the **Comprehensive *In Vitro* Proarrhythmia Assay (CiPA)** initiative was launched by the FDA in July 2013 and aims to improve the accuracy and reduce the cost of predicting cardiac liability using three 'pillars' (**above**):

1. Compounds will be profiled against a panel of human ventricular ion channels
2. This *in vitro* data will be incorporated into an *in silico* model of a human action potential to provide a proarrhythmic risk classification
3. Compounds will be tested using human induced pluripotent stem cell-derived cardiomyocytes to confirm the risk classification derived from the *in silico* model

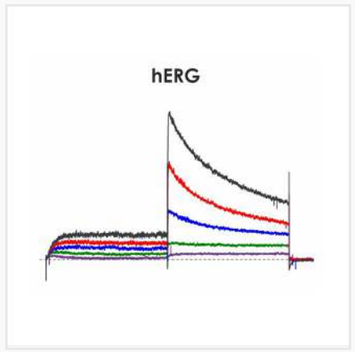
Step 1: Comprehensive ion channel panel

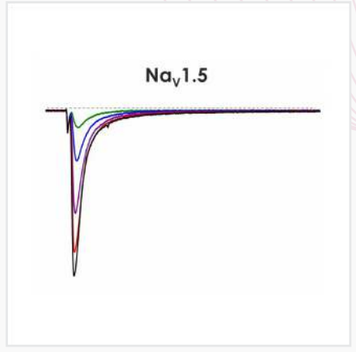
Metrion is an active participant in the CiPA ion channel HTS sub-team and on the Health and Environmental Sciences Institute (HESI) cardiac committee. We work closely with HESI to help reduce data variability between screening sites and provide validation data.

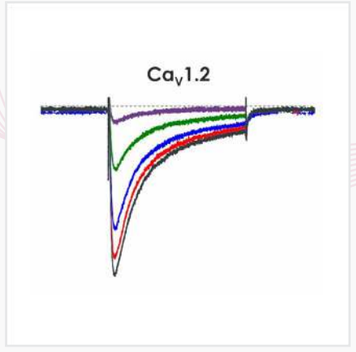
Metrion has validated assays for the full CiPA panel (below, right) (hERG, peak and late $Na_v1.5$, $Ca_v1.2$, KCNQ1/KCNE1, $K_{ir}2.1$ and $K_V4.3$). Metrion also offers screening services against HCN4 and $K_v1.5$, plus supplementary assays including dynamic hERG and a LQT3 late $Na_v1.5$.

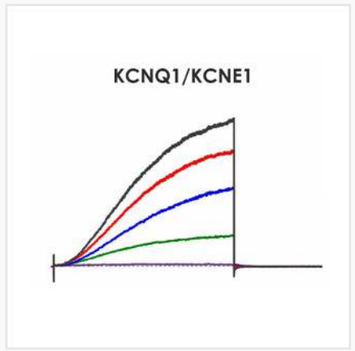
Metrion provides potency assessment against each of these channels using single-point or four-point concentration-response assays on the QPatch48.

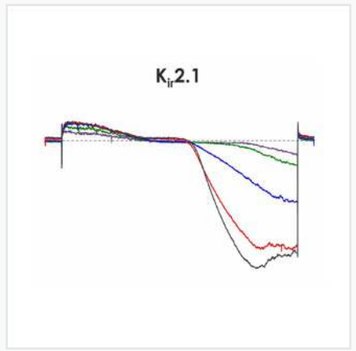
Compounds can also be screened using manual patch clamp methodology.

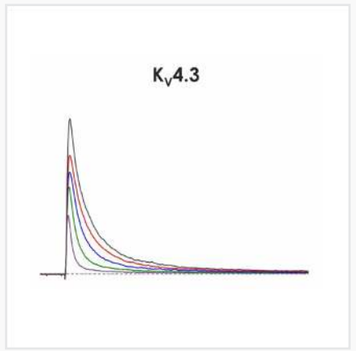












Step 2: *In Silico* Model

The high quality IC_{50} values derived from these high fidelity platforms are suitable for use in *in silico* action potential models, which is the second 'pillar' of the CiPA initiative.

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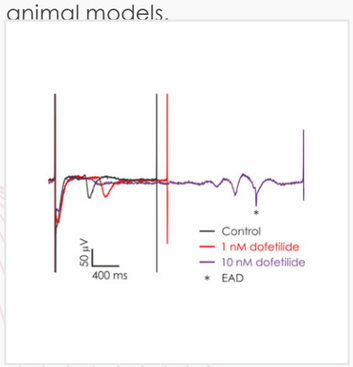
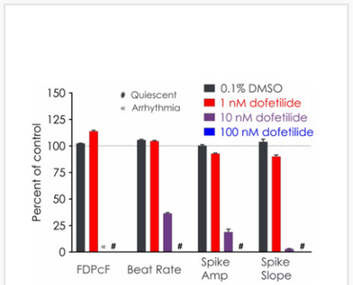
Step 3: Proarrhythmic assessment using human iPSC-derived cardiomyocyte assays

Metrion has developed a range of high quality assays that evaluate the effect of compounds on the electrophysiological properties of **human iPSC-derived cardiomyocytes.**

This includes a **multi-electrode array (MEA) assay** that non-invasively detects action potentials as fluctuations in the extracellular field potential. Metrion has validated its MEA assay using different commercially available iPSC-derived cardiomyocytes and the **CiPA toolbox**, which is composed of 28 compounds that are separated into high, medium and low categories of proarrhythmic risk.

Metrion's standard assay assesses the effect of compounds over a thirty minute application period. Data generated using this assay format yielded sensitivity and specificity values of 83.3% and 88.9% respectively, which exceeds those obtained from commonly used animal models.





Above: The effect of three concentrations of dofetilide on a range of electrophysiological parameters. Data could not be collected for the two highest concentrations, as early after depolarisations (EADs) were observed after five minutes of applications, which later degenerated into fibrillation at the top screening concentration.

Custom MEA assay service

Metrion can provide **custom MEA assays** to evaluate the proarrhythmic liability of compounds. For example, due to the non-invasive nature of the MEA system, the application duration of compounds can be extended to hours or days. This allows for the detection of compounds affecting ion channel function or expression over hours, rather than minutes.

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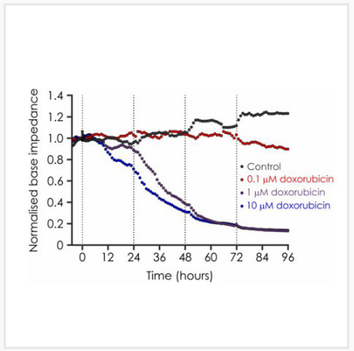
iPSC derived cardiomyocyte screening

Metrion can evaluate the effect of compounds on action potentials recorded from **iPSC-derived cardiomyocytes** using conventional manual patch clamp methodology. Spontaneous or evoked action potentials can be recorded and used to determine the effect of compounds on a range of action potential parameters. The manual patch clamp assay generates high fidelity recordings that allow the detection of even subtle changes to the action potential waveform. This helps to successfully discern between compounds with low, medium and high proarrhythmic risk profiles.

Chronic cardiotoxicity assessment using human iPSC-derived cardiomyocytes

Base impedance (an indicator of cell viability) can be used to non-invasively identify structural and functional cardiotoxicity over a chronic time course. Metrion has developed a validated **chronic cardiotoxicity assay** using human iPSC-derived cardiomyocytes. The adjacent example features doxorubicin, a member of the anthracycline family used to treat cancer. It is associated with a number of cardiac side effects, including acute atrial and ventricular arrhythmias and congestive heart failure.



Above: Metrion's chronic cardiotoxicity assay recapitulates doxorubicin's cardiotoxic effect by producing a concentration-dependent decrease of base impedance that develops following a 24 hour exposure period.

Neuroscience Services.

Metrion offers a range of peripheral and central nervous system (CNS) neuronal assays utilising native rodent tissue and human iPSC-derived neurons.

Our neuroscience assays are used for two main drug discovery purposes:

- **Neurotoxicity screening**
- **Confirmation of compound effects in native neuronal systems**

Phenotypic assays using native neurons offer an important translational step to confirm that client compounds are effective in native tissues that express a wide range of membrane proteins, scaffolding complexes and intracellular signalling cascades. In this way we can confirm that the desired potency and mechanism-of-action are seen in rodent species that are typically used in preclinical animal models. Similarly, the use of human iPSC-derived neurons can help confirm the efficacy of test compounds prior to testing in rare human tissue and give confidence that lead compound candidates may work in human patients as part of clinical trials.

We offer a range of translational CNS and peripheral neuron assays to support our client's drug discovery efforts including neuronal ion channels assays and translational neuroscience assays.

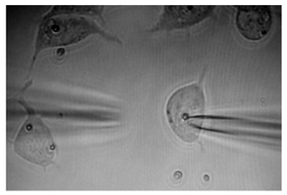
These neuronal assays are not limited to testing ion channel modulators, as they are also suitable for assessing the efficacy of ligands directed against GPCRs, kinases, enzymes, intracellular signalling and homeostatic pathways. Our neuroscience assays are well suited to validating drugs designed to treat pain, epilepsy and a variety of CNS diseases, and we can design custom neuronal assay and cell formats for more specific client needs.

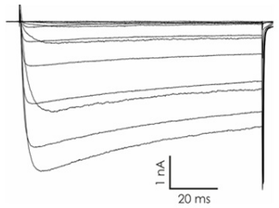
Ion Channel Assays - DRG sensory neuron assays

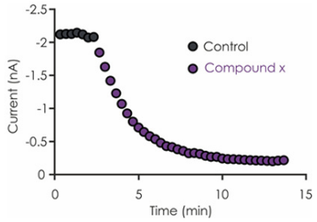
Dorsal root ganglion (DRG) neurons are the most accessible and efficient source of large numbers of peripheral neurons from both rodents and humans.

They are a major target of morphological toxicity deficits and functional manifestation of inflammatory and neuropathic pain.

Peripheral DRG sensory neurons are a workhouse of neuroscience drug discovery and Metrion scientists have proven expertise in creating assays to study voltage- and ligand-gated ion channels using manual patch clamp and multi-electrode array (MEA) biophysical techniques.







Above: a recording of Ca^{2+} currents in single rodent DRG neurons exposed to a test compound. This data is from an 8 year drug discovery collaboration with a global pharma company client that successfully delivered novel chemical matter and an IND candidate.

Neuroblastoma cell lines

Immortalised rodent and human neuroblastoma cell lines provide a useful alternative to native peripheral neurons as they endogenously express many relevant receptors and channels.

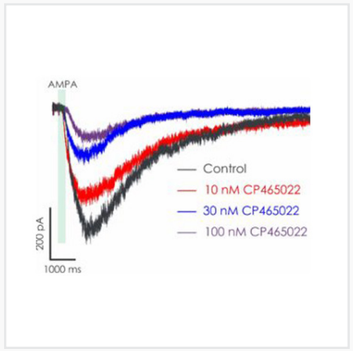
They can be used to address translational challenges by confirming compound efficacy in preclinical species and native human neuronal systems.

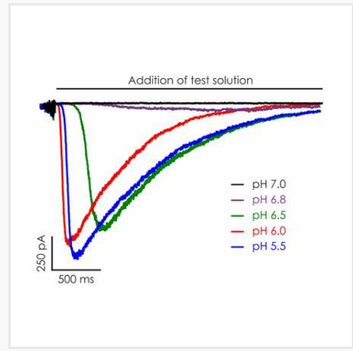
Metrion scientists have extensive experience using neuroblastoma cell lines for electrophysiological study of native and expressed ion channels.

CNS ion channel targets

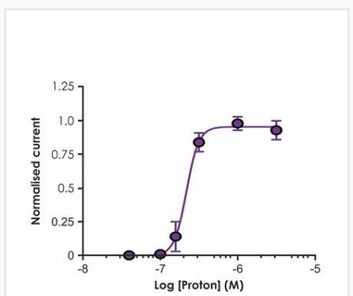
Metrion have to date worked on ligand-gated GluR (such as AMPA **shown below**), nAChR, P2XR and GABA-A receptors, voltage gated Na^{+}, K^{+} and Ca^{2+} channels and two-pore domain (K2P) ion channels. In many cases, work is conducted using heterologous cell lines on manual or automated patch clamp platforms. Metrion also offer manual patch and MEA recordings from native rodent CNS neurons.

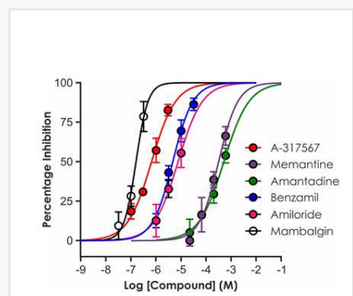
In this example, we show human GluR receptor currents recorded by manual patch (**A, shown below**) and on the automated Qpatch platform from Sophion (**B, shown below**). These assays can establish EC50 and IC_{50} concentrations of agonists and antagonists and investigate the action of negative and positive allosteric modulators.





Metrion's recent application note (ASIC1a-ligand-gated-ion-channel-assay-v1.3, available from the website) illustrates another example of an CNS ion channel assay, describing the optimisation and pharmacological validation of a QPatch automated patch clamp assay for the ASIC1a ligand-gated receptor which is implicated in stroke and ischemia.





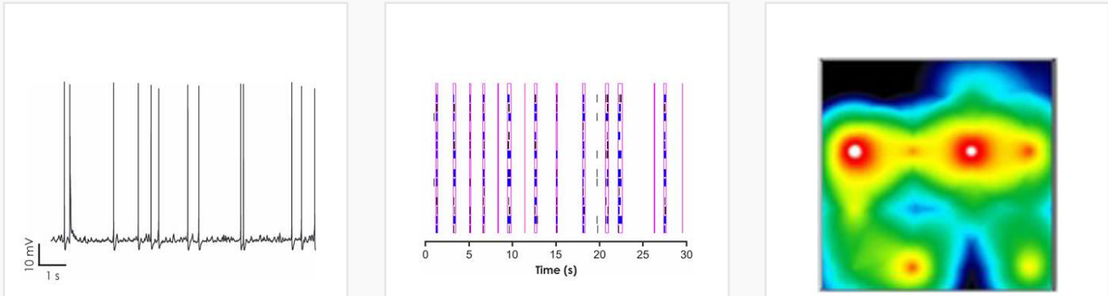
Please visit the Translational Assays section overleaf for more information about our neuroscience services.

Translational Neuronal Assays.

Metrion has a range of phenotypic assay platforms to enable its customers to validate the efficacy and mechanisms of their compounds in rodent and human neurons to help translate them towards preclinical testing and eventually into clinical trials. These assays use technologies such as manual patch clamp and multi-electode array (MEA) recordings to offer a direct read-out of physiological function in native tissue or human stem cell-derived neurons.

Central Neurons

Central neuron phenotypic assay platforms at Metrion include manual patch-clamp and MEA techniques, which can be used to validate compound target efficacy, establish target engagement in native tissue, and explore species selectivity. Access to CNS neurons from different brain regions and developmental stages also allows for a comparison of compound effects on cells with different functional profiles, as well as the potential to test compounds on native and iPSC-derived neurons from different, genetically validated, disease states.



Physiological activity is monitored from native CNS neurons such as rodent cortical neurons by measuring their firing behaviour with manual patch clamp (**left**) and MEA electrophysiology platforms (**centre/right**), where single cell excitability as well as metascale network bursting can be visualised with heat maps (**right**) and other sophisticated analysis and visualisation tools.

Current Clamp Recording

This technique allows recordings from individual neurons to measure changes in membrane potential or firing behaviour in response to compound application or current input.

Recordings can be used to compare firing characteristics from different cell types, verify findings from other sources (such as MEA) and to ascertain the MOA of compounds.

At Metrion, we are highly experienced at recording from native rodent neurons and are currently developing further translational assays such as recordings from stem cell derived neurons and human dorsal root ganglion neurons.

Multi-electrode array (MEA)

MEA enables simultaneous recording of the physiological activity in multiple peripheral neuronal cells.

Extracellular field potentials are recorded in a non-invasive manner to characterise neuronal firing before and after compound addition.

These techniques can be used to examine cells in sensory pathways, such as rodent dorsal root ganglia neurons, and their response to ligand application.

The electrophysiological behaviour of populations of disease modified neurons or cells from different sources can also be compared with relative ease.

Human iPSC-derived neurons

Metrion has experience utilising human iPSC-derived neurons in translational assays.

We work closely with leading commercial iPSC providers to evaluate and validate their neuronal reagents in our own laboratory, and also collaborate with academics and clients to profile bespoke iPSC neuron assay formats and reagents such as patient-derived neurological disease models.

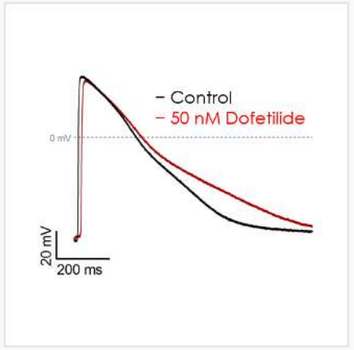
Translational Cardiac Assays.

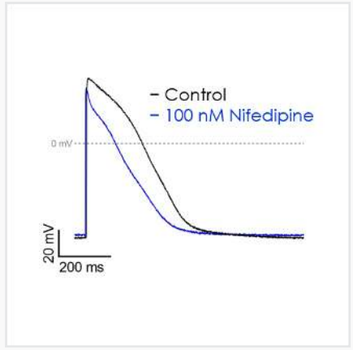
High quality cardiac toxicity data generation and interpretation is vital to the efficient progression of a drug discovery campaign. Metrion offers a range of translational cardiac assays and platforms that have been validated using commercially available iPSC-derived cardiomyocyte cell lines.

Using technologies such as manual patch clamp electrophysiology, multi-electrode array (MEA; Maestro platform) or dual MEA/impedance readouts (CardioExcyte96), Metrion can offer a direct readout of physiological function in the iPSC-derived cardiomyocyte cell line of your choosing.

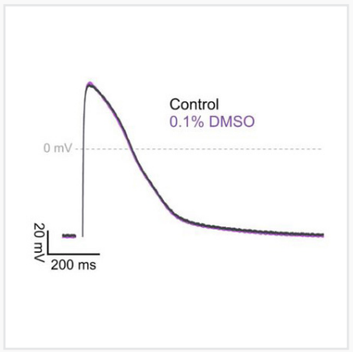
Manual Patch Assays

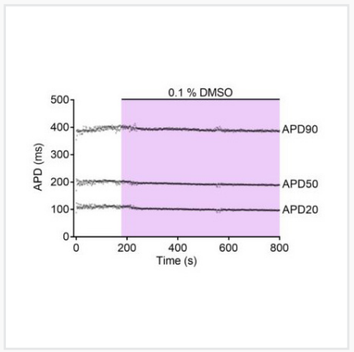
Metrion has considerable expertise working with iPSC-derived cardiomyocytes on the manual patch clamp platform and has characterised various commercially available cell lines. Metrion offers services to screen compounds against action potentials and membrane currents recorded from iPSC-derived cardiomyocytes.

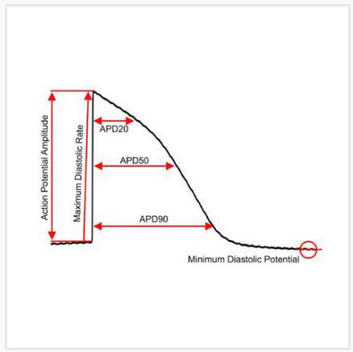




Furthermore, we provide a cell line characterisation service for companies and vendors looking to commercialise their cell lines. Stable action potentials can be recorded from both spontaneously beating or stimulated cardiomyocytes. Compound effects (**above**) are quantified against key action potential parameters, such as action potential duration (APD) which can be a surrogate marker for prolongation of the *in vivo QT interval*.







Stimulated Action Potentials

Stable action potential recordings can be taken for up to one hour using stimulation via a recording bath field potential or current injection through the recording pipette. This enables application of multiple concentrations of a compound during a single experiment (**above**). Frequency-dependent effects on action potential parameters can also be investigated.

METRION BIOSCIENCES – LEADERS IN ION CHANNEL DRUG DISCOVERY

Spontaneous action potential recordings

iPSC-derived cardiomyocytes possess intrinsic pacemaker activity, resulting in spontaneous action potential firing. This allows the Determination of the effect of a test compound on action potential (AP) firing frequency and to identify compounds with the potential to slow (bradycardia) or increase (tachycardia) heart rate *in vivo*. Metrion has tested a toolbox of compounds with cardiac activity and confirmed their anticipated effect on spontaneous action potential parameters, which were consistent with those determined from stimulated cells. For example, the hERG blocker dofetilide significantly prolonged the AP duration of iPSC-derived cardiomyocytes and induced EADs (**below**).

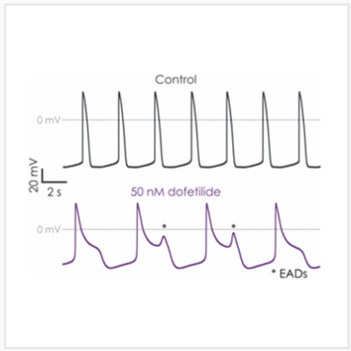


Plate based Assays (MEA and Impedance Platforms)

Metrion has developed plate-based iPSC-derived cardiomyocyte assays using MEA and impedance platforms to add further depth to our range of CiPA-compliant assays. Phenotypic readouts can be correlated with modelling predictions and electrophysiology readouts as part of an integrated CiPA approach.

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Integrated Drug Discovery.

Our highly experienced interdisciplinary team provides clients with a fully integrated drug discovery service by bringing together experts in:

- **ion channel biology**
- **medicinal chemistry**
- **specialist chemistry**
- **translational biology**
- **ADMET**
- **DMPK**

This integrated service spans initial assay development, custom assay validation and high throughput screening in 384-well plate-based assays. Metrion provides high quality electrophysiology-based support for medicinal chemistry optimisation of compounds, including an industry-leading suite of cardiac safety profiling assays. We also offer phenotypic assays on MEA and impedance platforms for target validation and mechanism-of-action studies in native rodent and human IPSC cell-based assays to facilitate the successful translation of lead compounds into preclinical and clinical testing.

Experienced Integrated Drug Discovery Team

Alongside our partners, Metrion Biosciences has the experience to steer your research programme to a successful, and cost effective, conclusion. We offer wide ranging expertise and our suite of industry leading validated assays includes:

- Voltage- and ligand-gated ion channels
- Multimodal transient receptor potential (TRP) channels
- Two-pore domain 'leak' channels
- Inward rectifier channels
- Hyperpolarisation-activated cyclic nucleotide gated ion channels

High Quality Small Molecule Library

Through Metrion Biosciences and our partners, clients can access a high quality library of 150,000 small molecules, a range of stable cell lines and validated assays, plus a choice of assay platforms to suit the requirements of individual customers.

Clients can access expert computational chemists for knowledge-based selection of a screening deck or we can perform diverse screens using the Assay.Works library alone or supplement this with client molecules or libraries accessed via compound vendors. Our team can either provide advice and build appropriate screening cascades for clients who do not possess significant experience of the ion channel target class or work together with experienced clients to maximise their chances of success for this challenging target class.

Metrion's Integrated Drug Discovery Service

Our integrated drug discovery service involves:

- Extensive interaction with clients
- Closely defining the profile of compounds most appropriate to the therapeutic focus
- Outlining individual drug-like properties to maximise efficacy and limit side effects,
- Optimisation of each assay in the screening cascade to produce the most relevant data
- Maximising the efficiency of the design and synthesis of new analogues to enable rapid structure-activity relationship (SAR) determination at an early stage
- High quality assays to drive enhancement of compound potency and selectivity properties
- Early assessment of ADME properties and ranking of hit compounds/ hit series
- Access to expert toxicologists able to design and perform experiments to characterise potential (for example on-target) or observed toxicity issues.
- Advice regarding appropriate biomarker assays to provide reliable biological translation for the discovery programme.

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