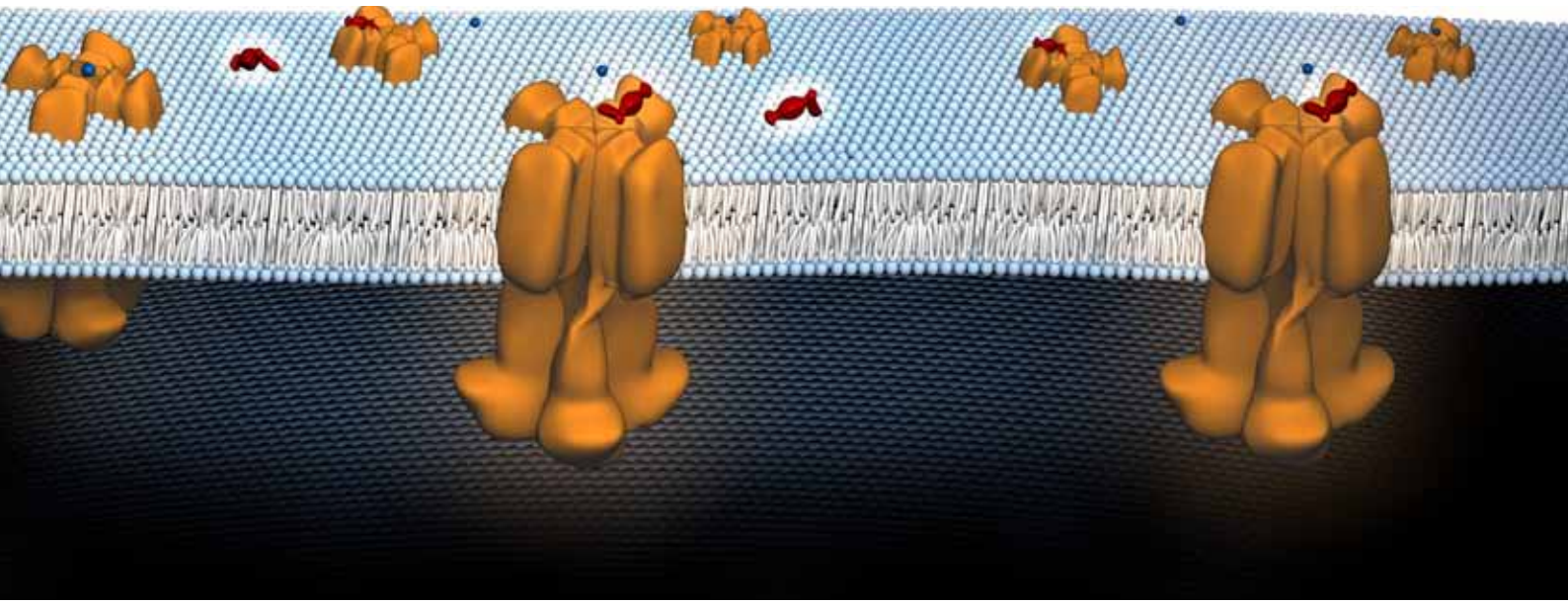
NMI TT
Pharmaservices

Pharmaservices



NMI TT Pharmaservices

The **NMI TT GmbH** is a service provider for the pharmaceutical industry as well as biotechnology companies in the fields of

- Preclinical Drug Screening and Development
- Microdevice Production for Life Science Applications
- Medical Product Testing

The **NMI TT GmbH** was founded in 2003 as a subsidiary of the NMI Natural and Medical Sciences Institute.

In our

Pharmaservice Department

we offer high quality services for

- Ion Channel Drug Discovery
- *in vitro* and *ex vivo* Safety Pharmacology

Ion Channels

Ion channels are critical to diverse physiological functions in both excitable and non-excitable cells. They are implicated in a variety of disorders, in particular in the central nervous and cardiovascular system. Moreover, ion channels may be unintended targets of new chemical entities that can result in adverse side effects.

Ion channels are thus an important target class in drug discovery and safety pharmacology. Primary research is continually uncovering potential new ion channel targets in a variety of indications such as cancer, pain or neurological disorders.

To help customers in their drug discovery efforts we offer

- electrophysiological assays in manual and automated mode
- hERG GLP services
- customer-specific assay development
- our expertise in electrophysiology and automation for your explorative projects

Our models range from cellular assays to complex organ preparations including stem-cell based approaches to test for

- ion channel activity under physiological and pathophysiological conditions
- drug-induced alterations of cellular network activity
- qualitative and quantitative description of compound interactions with ion channels and receptor subtypes
- adverse side effects of potential drugs on ion channel activity in early drug discovery
- interaction of compounds with cardiac and other safety-relevant ion channels including hERG
- functional relevance of hERG binding
- probability of compounds to induce QT prolongation
- risk of possible generation of torsade de pointes arrhythmia

Models available

- manual and automated assays for hERG and other ion channels
- GLP services
- automated oocyte voltage clamp assays
- QT prolongation assays on microelectrode arrays (MEA) in native and stem-cell derived cardiomyocytes (Cardiosensor)
- other MEA-based assays
- papillary muscle
- Langendorff heart
- isolated organs

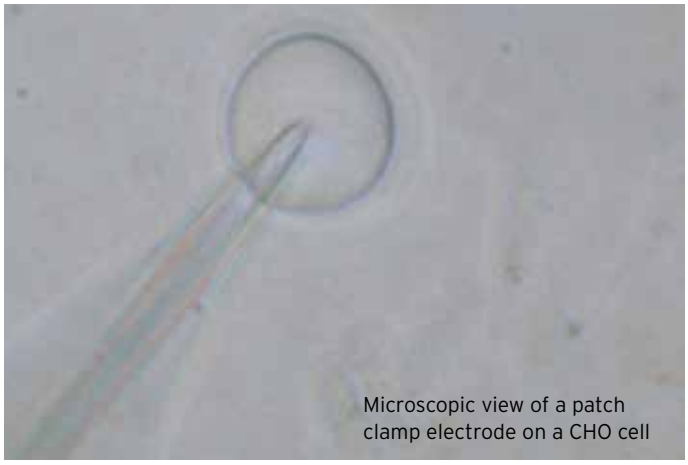
Benefits

- high quality services for pharmaceutical and medical industries as well as academic partners
- in accordance to FDA and EMA guidelines
- custom-tailored solutions

Patch Clamp Services

We offer patch clamp assays in manual and automated mode on cell lines, primary and stem-cell derived cells, cellular networks and tissue slices.

We work in close cooperation with our customers to develop individual solutions to meet their requirements.



Microscopic view of a patch clamp electrode on a CHO cell

hERG Assay

Many compounds bind to cardiac hERG channels and increase the probability of QT-interval prolongation with a risk of inducing a torsade de pointes arrhythmia.

Our manual patch clamp assay reveals interactions of compounds with cardiac hERG channels. This methodology is the "gold standard" and in accordance to ICH guideline 7B.

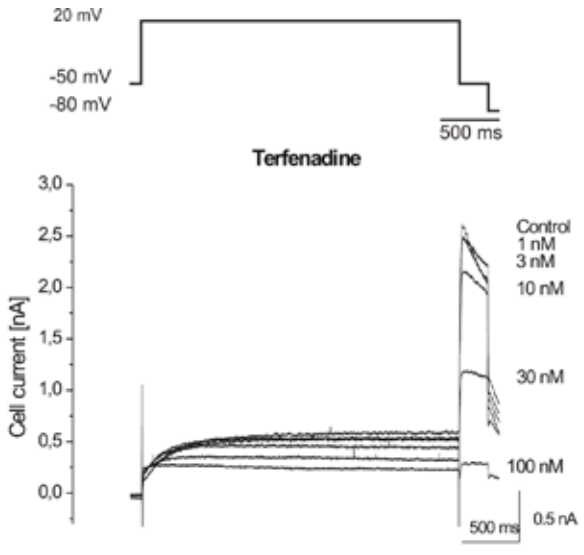
As a standard, hERG currents are recorded from stably transfected CHO cells.
Protocols are available for single dose testing and dose-response curves.

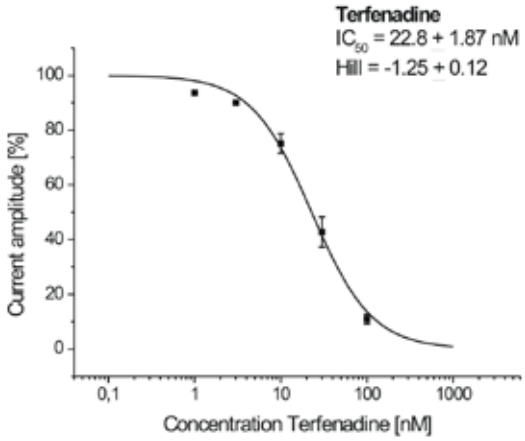
Other ion channel targets and assay development on request.

GLP hERG

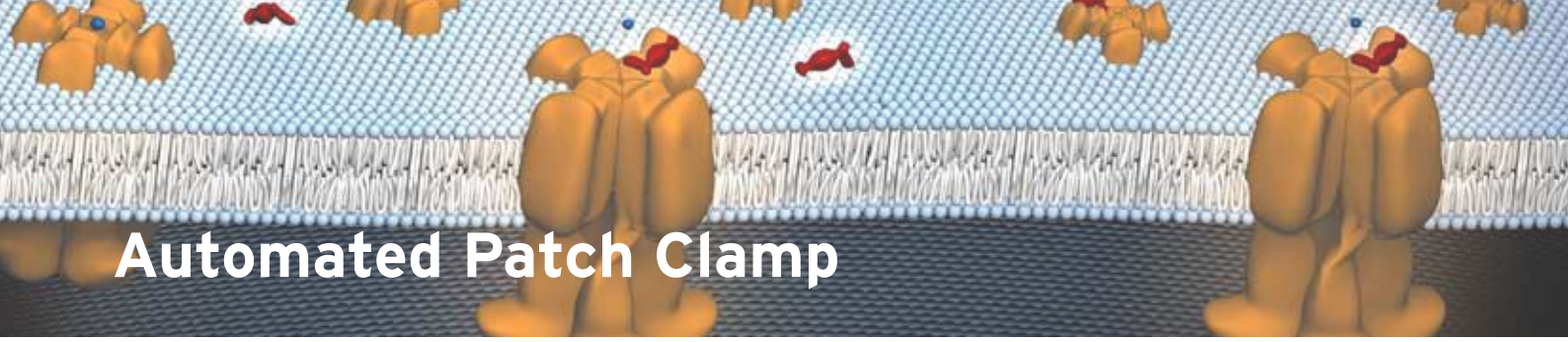
According to ICH S7B guideline, all compounds under consideration for Investigational New Drug (IND) applications need to be tested for hERG interaction in compliance with GLP principles.
We offer complete GLP IC_{50} determination (standard: c=4; n=3).

Please contact us to initiate your hERG GLP study.





Effect of Terfenadine application on hERG channel currents in CHO cells



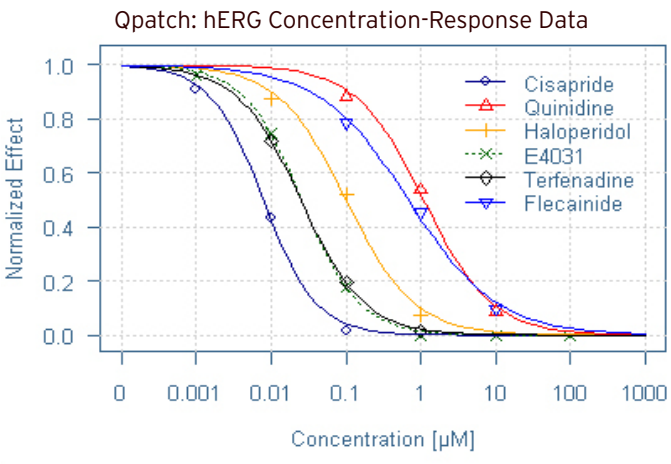
Automated Patch Clamp

Meeting the higher throughput demands of ion channel screening and early hERG risk assessment, automated patch clamp is an important part of our services. Based on Sophion's QPatch technology, combined with our long-standing expertise in ion channel research and automation of electrophysiological assays, we offer high quality ion channel screening.

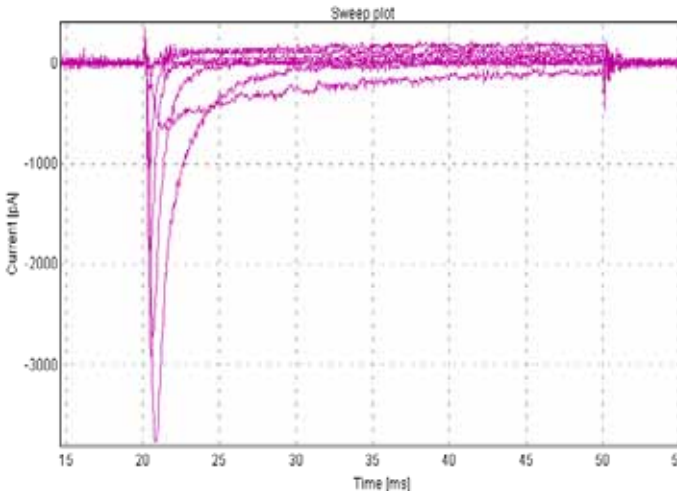
Applications

- **screening on other voltage-gated and ligand-gated targets**
  rely on our long-standing expertise in ion channel research and automation of ion channel assays
- **hERG screening service**
  detect hERG-related safety issues at early stages in the drug development process
- **QPatch™ outsourcing service**
  if you have established QPatch assays but limited instrumental capacity, do not hesitate to contact us. Our outsourcing service will help you to balance your work load.

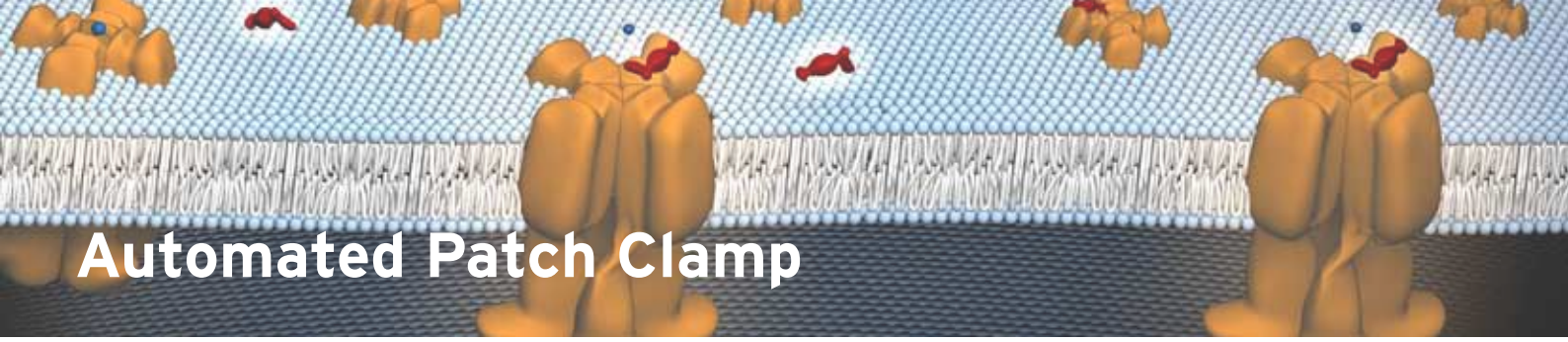
The QPatch™, our instrument for high throughput automated patch clamp services



Concentration-response curves (standard: c=4, n=3). Single and double point assays for cost efficient higher throughput demands



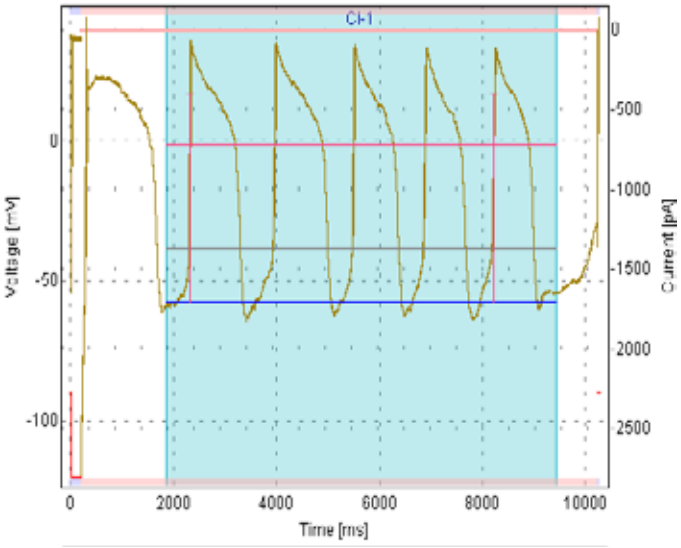
The cardiac sodium channel NaV1.5 expressed in CHO cells and measured with the QPatch (voltage clamp data)



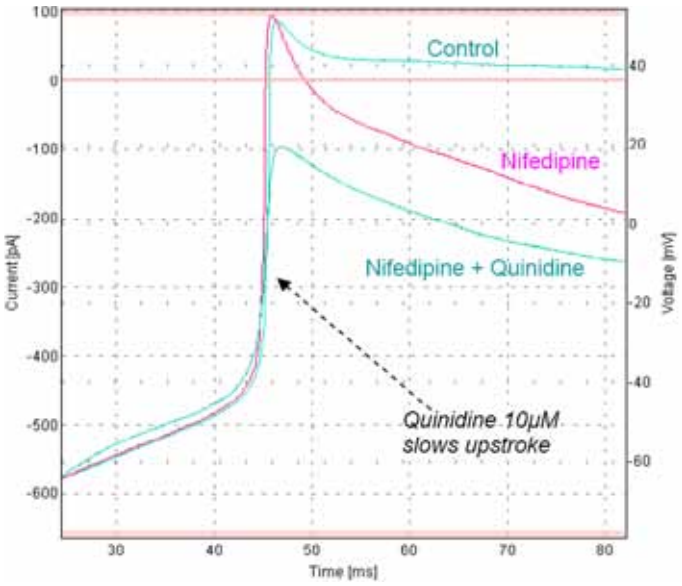
Automated Patch Clamp

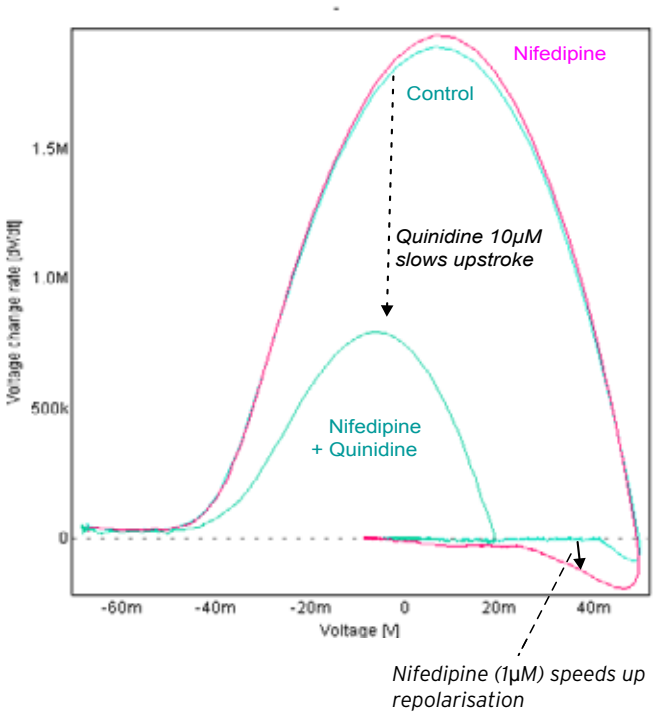
Current clamp assays on QPatch

Automated recordings of such action potentials is technically challenging and requires advanced „current clamp“ capabilities of the automated patch clamp instrument.
NMI TT has recently worked with Sophion to beta-test the brand new current clamp feature of the QPatch Instrument. We are now one of the first CROs offering this exciting new feature which helps improving cardiomyocyte and neurobiological research.



QPatch current clamp data featuring action potentials from a stem cell derived cardiomyocyte





Nifedipine (1µM) speeds up repolarisation

Effects of calcium and sodium channel blockers on cardiac action potentials recorded in current clamp mode.
On the left panel, the onset of action potential is shown. The calcium channel blocker Nifedipine speeds up repolarisation while the sodium channel blocker Quinidine slows the upstroke and reduces the amplitude. The right panel shows the same data analysed with a so called „phase plot“, an advanced analysis method that helps detecting changes in upstroke velocity, so that the quinidine effect is much more prominent

MEA-based assays

Cardiosensor

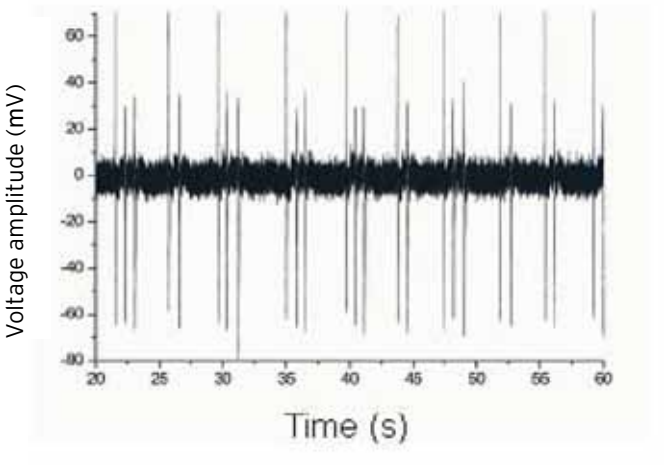
The MEA Cardiosensor is a fast, easy to handle and efficient test system for monitoring drug effects on cardiac activity *in vitro* using native or human stem-cell derived cardiomyocytes.

The system is based on microelectrode array (MEA) technology that allows multi-site recordings of extracellular field action potentials (fAPs). fAP duration correlates well with the QT interval in electrocardiograms.

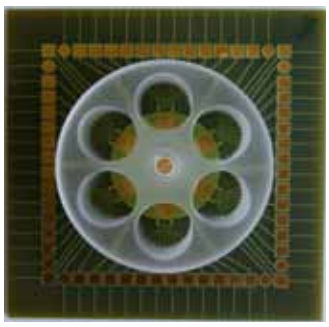
The MEA Cardiosensor can be used to

- screen drugs for potential prolongation of fAPs
- detect spatial and temporal properties of cardiac excitation
- determine conduction velocities of cardiac activity

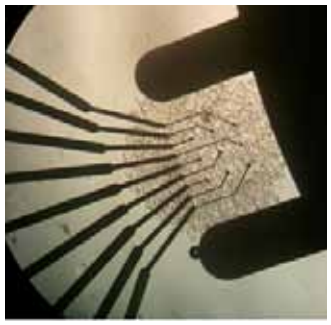
In order to increase the throughput of analysis, a multi-well-MEA system is routinely used to run 24 independent experiments in parallel.



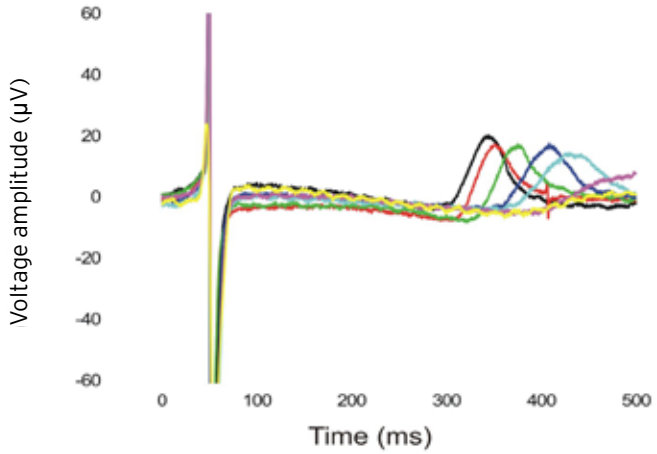
Example of drug-induced arrhythmic activity of native cardiomyocytes measured with the Cardiosensor



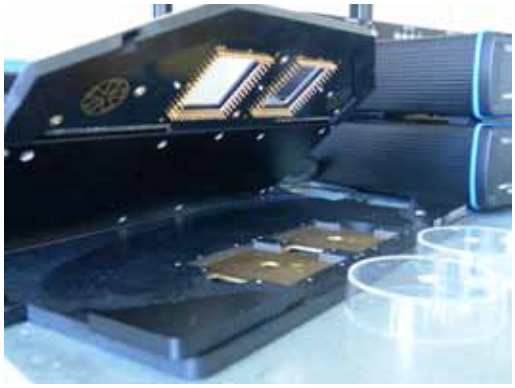
Multiwell-MEA (fabricated in house)



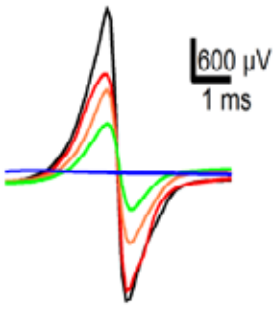
Monolayer of cardiomyocytes on a MEA chip (black: MEA electrodes)



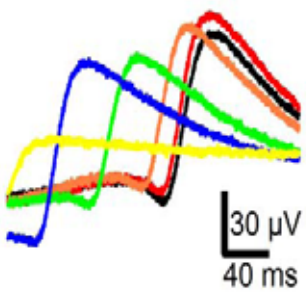
Dose-dependent prolongation of the fAP induced by quinidine measured with the Cardiosensor (black control; red 0.3 µM; green 1 µM; dark blue 3 µM; light blue 10 µM; violet 30 µM; yellow 100 µM)



MEA platform for multiwell-MEA (fabricated by MCS)



Lidocaine reduces the Na^+ component of fAP of stem-cell derived cardiomyocytes concentration-dependently (black control; red 3 µM; orange 10 µM; green 30 µM)



Verapamil reduces the fAP duration in human stem-cell derived cardiomyocytes (black control; red 0.1 µM; orange 0.3 µM; green 1 µM; blue 3 µM; yellow 10 µM)

MEA-based assays

DRG neurons on MEAs

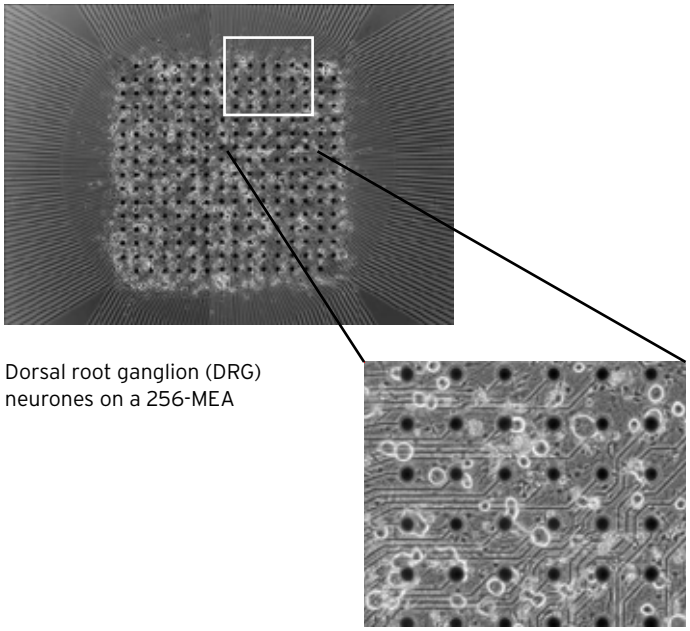
DRG neuronal cultures grown on high-densitiy MEAs allow a high-throughput assessment of drug effects on DRG neuron activity.

Compared to conventional patch-clamp analysis the MEA-based approach offers the following advantages

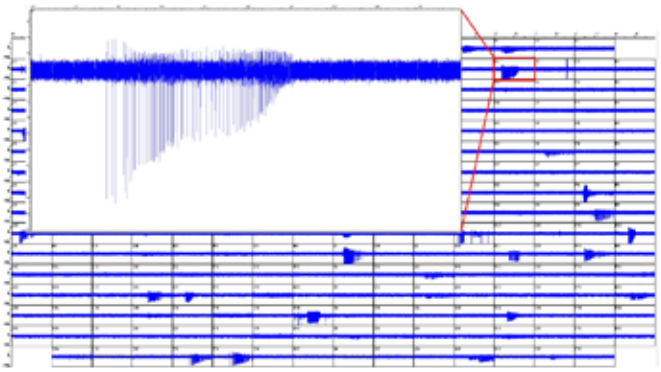
- recording of all responses becomes much faster, speeding up the drug screening process (thousands of cells per day)
- recordings can last hours / days as they are not invasive which will facilitate the study of modulators having long-term effects, like mediators of inflammation.

This approach can be easily combined with other methods such as

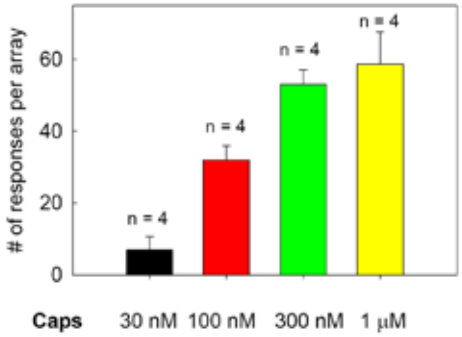
- transfection methods
- RNA interference
- immunoassays.



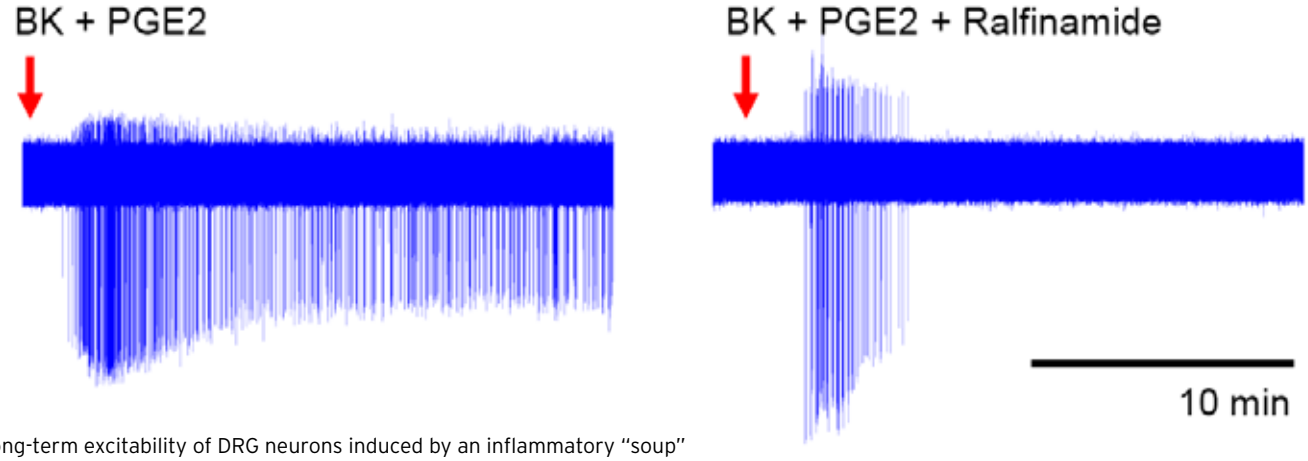
Dorsal root ganglion (DRG) neurones on a 256-MEA



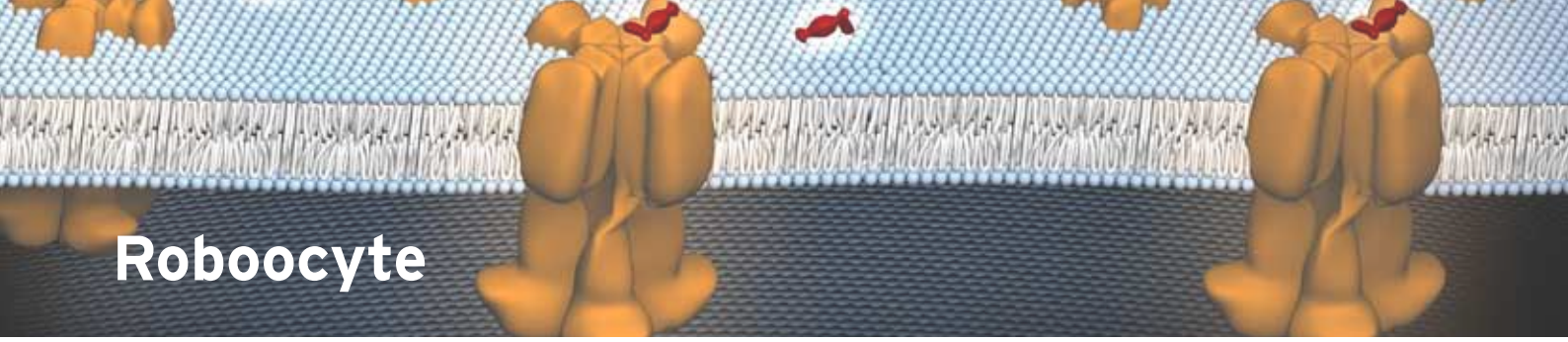
Capsaicin-induced excitability in primary DRG neurons recorded in parallel with a 256-MEA



Increasing concentration of capsaicin recruit an increasing number of DRG neurons



Long-term excitability of DRG neurons induced by an inflammatory "soup" (bradykinin 100 nM + PGE2 500 nM) and blocked by ralfinamide, use-dependent sodium channel blocker



Roboocyte

Automated Oocyte Recordings

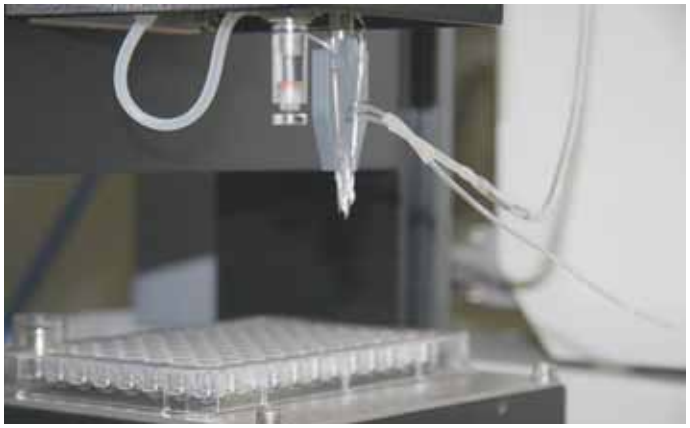
Automated Xenopus oocyte assays, implemented on the Roboocyte™ platform, offer high quality voltage clamp electrophysiology for drug screening and compound profiling on ion channel targets.

- The Roboocyte™ provides flexible assays with short development time for drug screening and compound profiling.
- We offer a broad range of ion channel assays, addressing hERG, SCN5A, P_2X, $GABA_A$ and many potassium channels as well as customer-defined targets.
- Two fully automated Roboocyte™ units are available, all equipped with liquid handling robots for higher throughput.

Experimental set up of the Roboocyte™

Standard assays

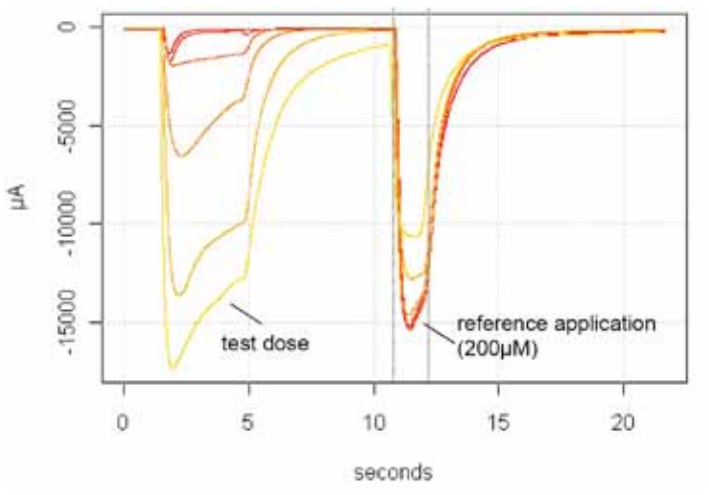
These assays are routinely available and can be started at any time. They generally test for potency of compounds in blocking or modulating the target ion channel. Using one oocyte per data point and sufficient compound incubation time, high data quality is guaranteed. Dose response curves are carried out by testing each data point in an individual oocyte
(for a list of standard assays please refer to our homepage www.nmi-tt.de).



Detailed view of the Roboocyte™

Custom-specific solutions

Many ion channels have already been successfully used in Roboocyte assays at our site. In order to transfer our expertise with these channels to new customers, the particular Roboocyte™ assay can be easily adapted to the customers needs. The price per data point depends on the details of this assay specification.



Kinetic of α4β2 AChR expressed in Xenopus oocytes stimulated with acetylcholine

Isolated organs

Schematic drawing of the experimental set up for measuring isometric contractions and field stimulation

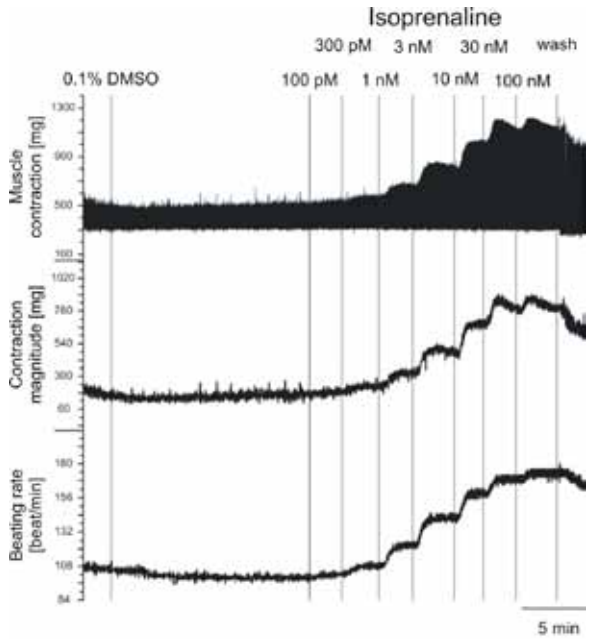
Isolated organs are effective models for testing the functional relevance of compound interaction with receptor subtypes.

- Dose-response curves are derived from smooth muscle contractions during cumulative agonist application
- These models allow the qualitative and quantitative discrimination of competitive and noncompetitive antagonism and unspecific effects

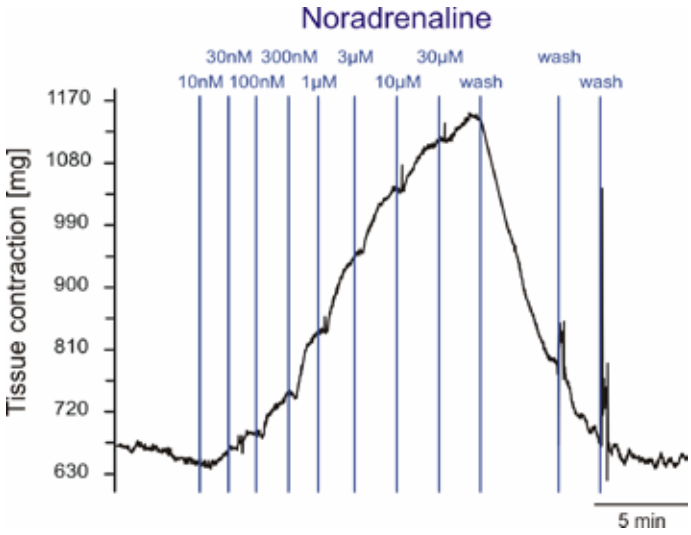
We offer several assays for receptor subtypes and ion channels

- Adenosine: A1, A2A, A2B
- Adrenoceptors: a1A, a1B, a1D, a2A, β1, β2
- Dopamine: D2
- $GABA_A$
- Histamine: H1, H2
- Muscarine: M1, M2, M3
- Serotonin: 5-HT2B
- L-type Ca^{2+}

in various organ preparations taken from guinea pigs, rats, mice and rabbits (for a list of standard assays please refer to our homepage www.nmi-tt.de).



Positive inotropic and chronotropic effect of isoprenaline in isolated guinea pig right atria preparations suspended in organ bath systems



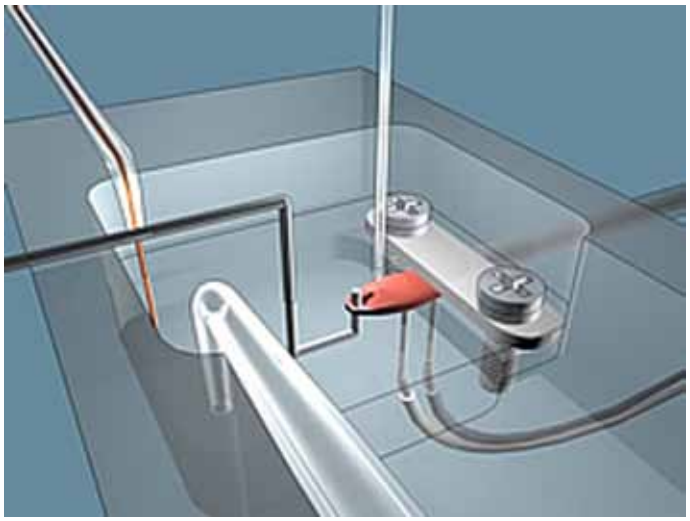
Guinea pig spleen preparation during cumulative noradrenaline application

Papillary muscle

The papillary muscle is an ideal organ model for analyzing the influence of compounds on the cardiac action potential as well as muscle force.

It allows us to test for the functional relevance of an hERG blockade, as demonstrated by an increase in action potential duration.

As an additional advantage, compound effects on Na^+ or Ca^{2+} channels can be detected as well.



Schematic drawing of the experimental chamber of the papillary muscle set up

The following parameters are analyzed

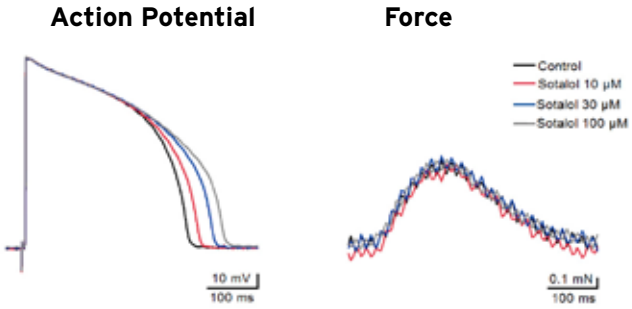
- action potential

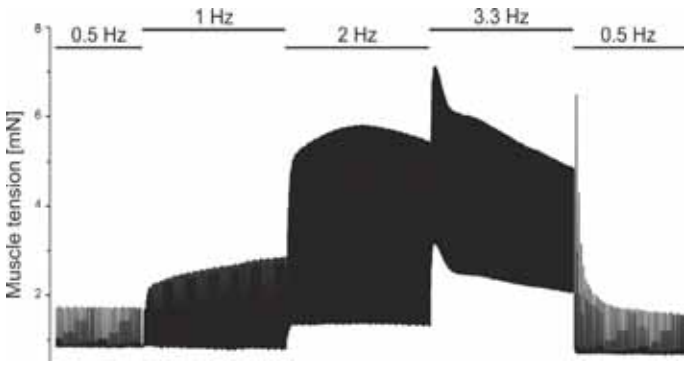
- resting membrane potential
- amplitude
- maximum upstroke velocity
- duration: 30%, 60%, 90%, APD30/APD90

- muscle contraction

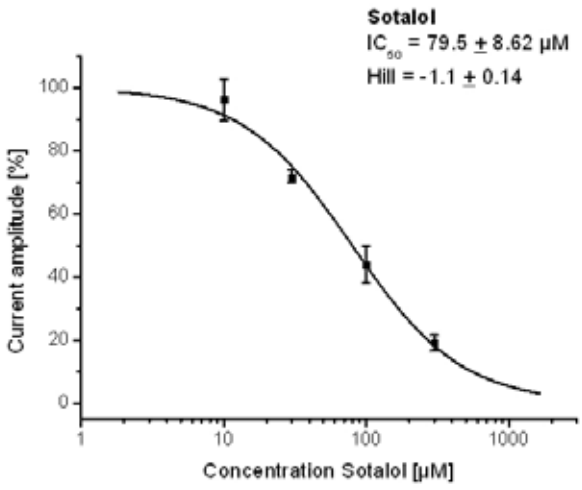
- resting tension
- force magnitude

Typically, four compound concentrations are tested per preparation, in addition to a solvent and positive control or washout.





Muscle contractions of rabbit papillary muscle during electrical stimulation at different frequencies



Influence of sotalol on the electric stimulated papillary muscle. The isometric muscle contraction and the action potential are presented in the absence and presence of sotalol

Langendorff Heart

The Langendorff heart is the best system currently available to determine the influence of compounds on cardiac parameters without interaction with the cardiovascular system. Additionally, this model allows to estimate the risk of compounds to induce torsade de pointes arrhythmia.

Two models are available

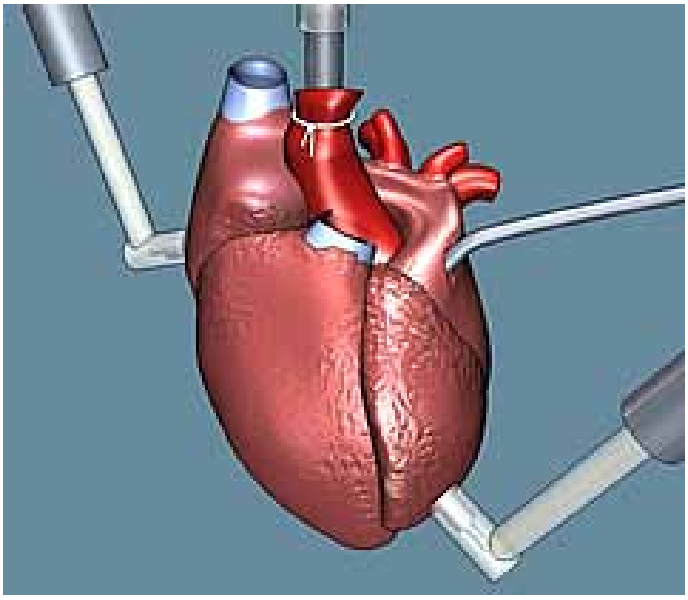
- standard model
- proarrhythmic model

The following electrophysiological and haemodynamic parameters are analyzed

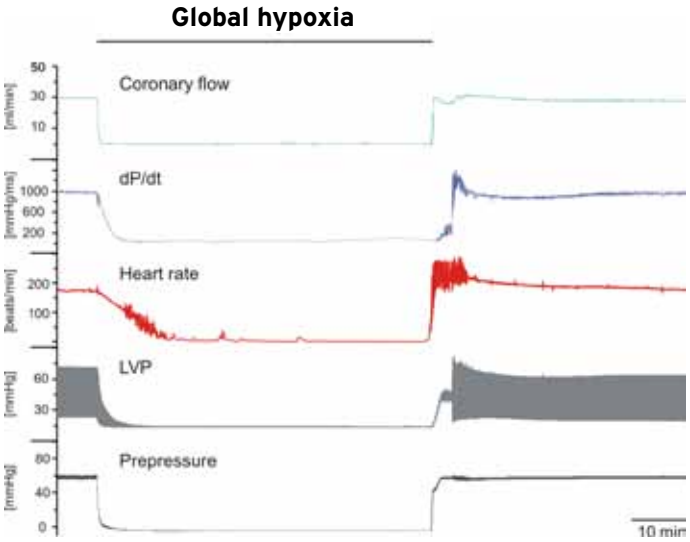
- left ventricular pressure
- maximum pressure rise
- heart beat
- coronary flow
- perfusion pressure
- QT time with QTc (according to Bazett) or atrial and ventricular focal field potentials (optional)

Poincaré analysis can be carried out upon request.

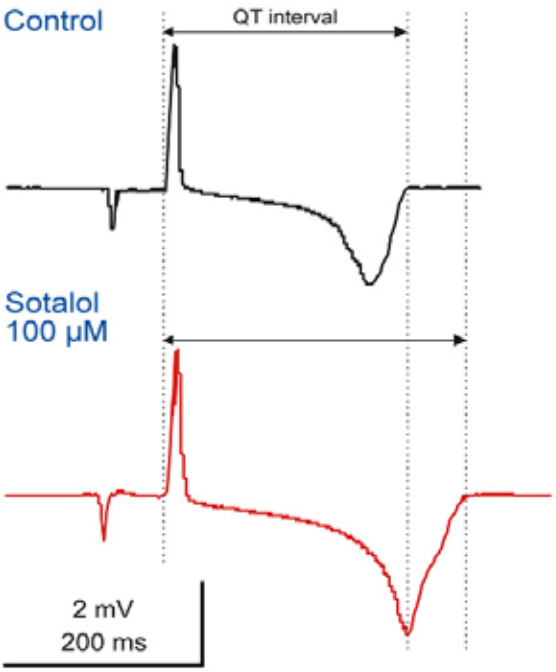
Typically, four compound concentrations are tested per preparation, in addition to a solvent control and positive control or washout.



Constant pressure-perfused guinea pig Langendorff heart model



Cardiac parameters during and after (ischemic reperfusion) global hypoxia induced in isolated Langendorff-perfused guinea pig hearts



Influence of sotalol on the QT-interval

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