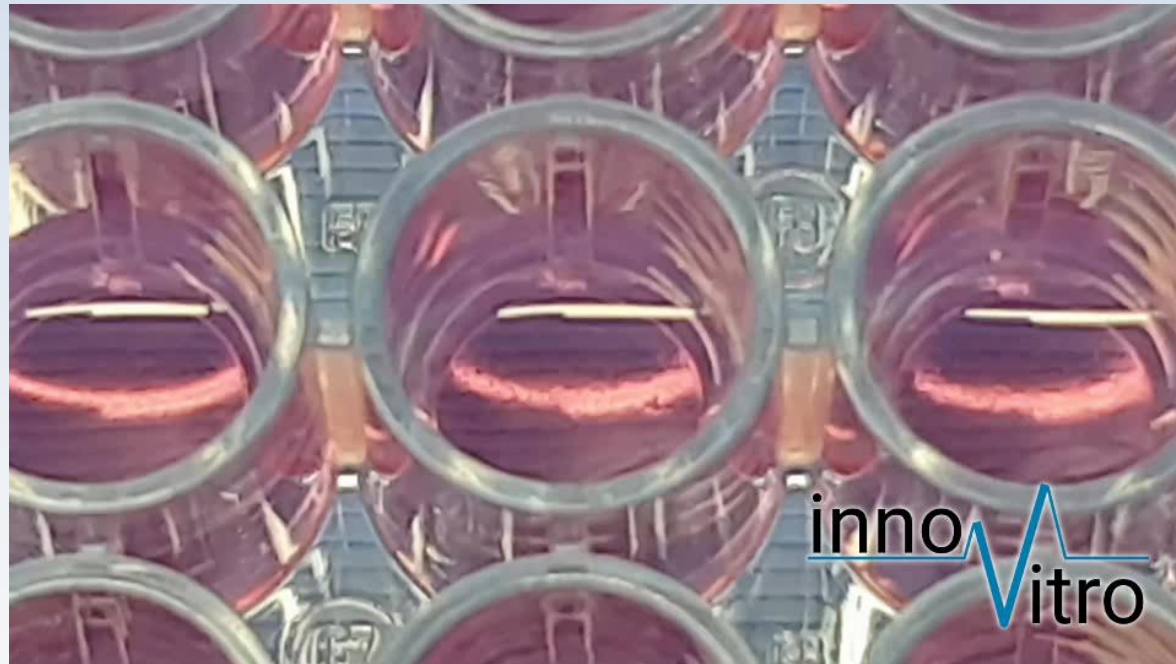


Mechanobiology of *in vitro* assays:

Tackling prevailing challenges in pre-clinical drug development



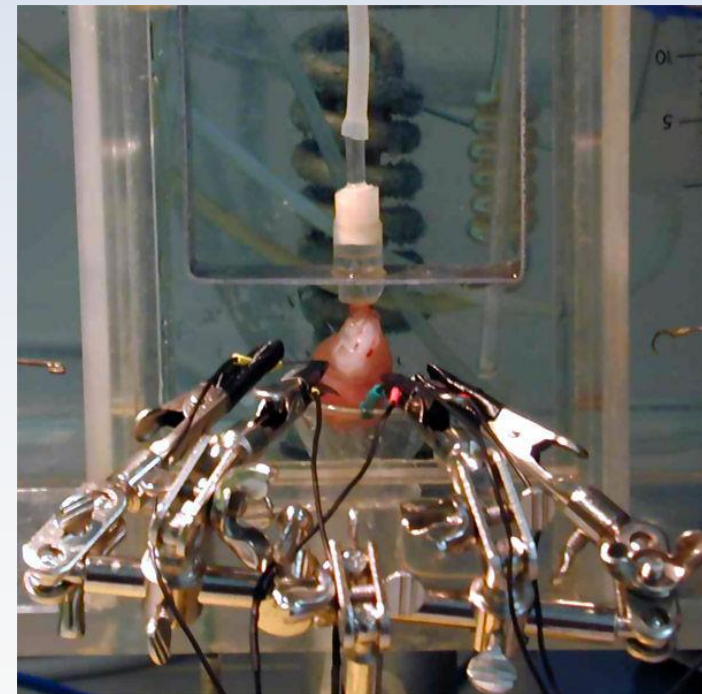
Dr. Matthias Goßmann

2020 Annual Meeting of the Biophysical Society, San Diego

February 17th, 2020

State-of-the-Art

- The Langendorff Heart (1895) and related *ex vivo* techniques are still the „gold standard“ for contractility measurement
 - Drawbacks:
 - Low predictivity
 - Low throughput
 - Labor-intensive
 - Advantage:
 - Physiologic responses
- Natural mechanical environment



Mechanosensitivity

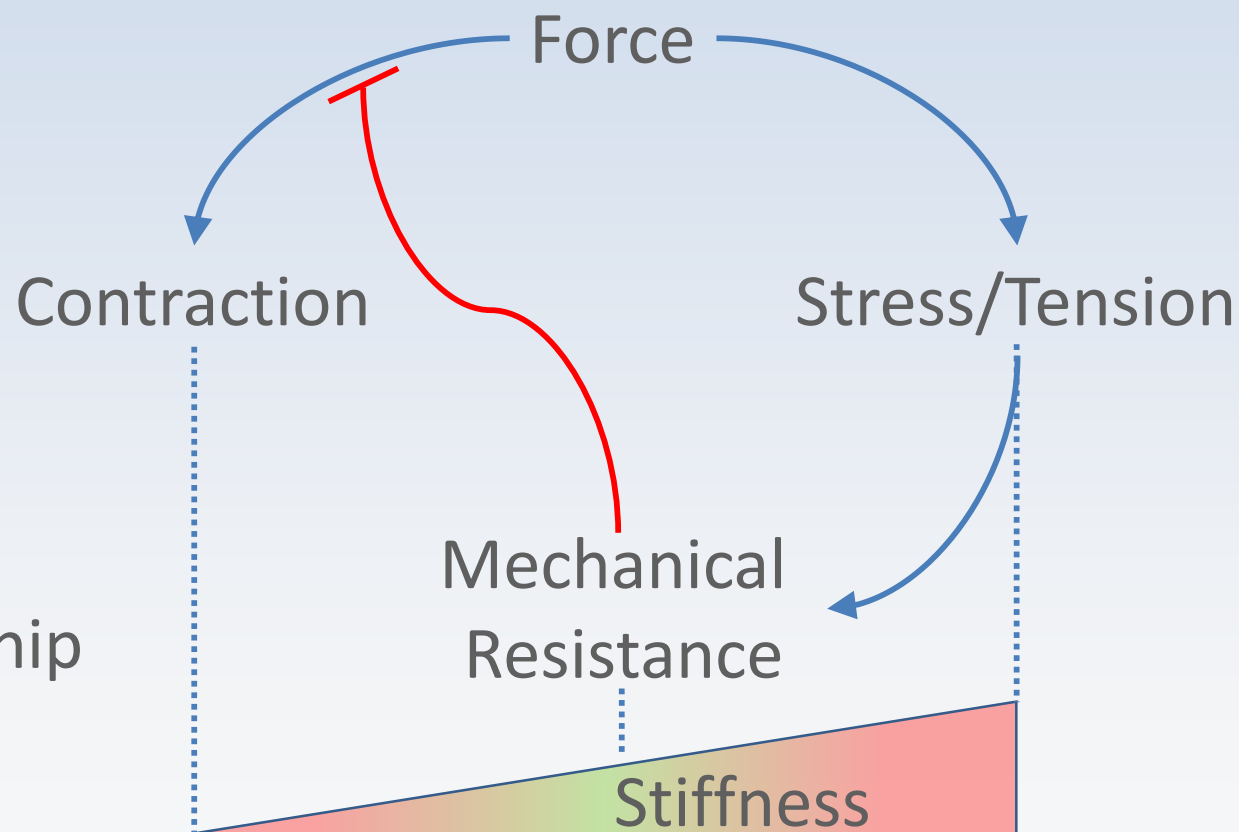
- Isotonic: Max Contraction



- Isometric: Max Stress



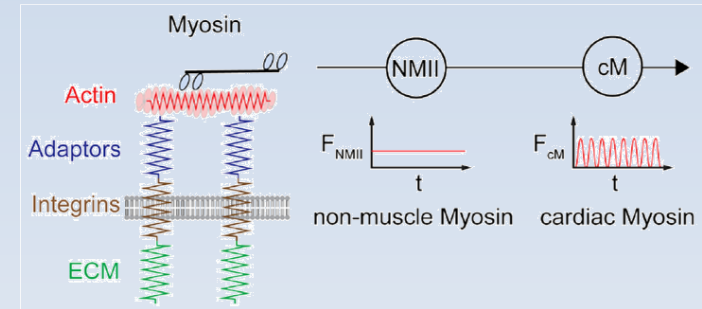
- Auxotonic: Physiological Relationship



Mechanosensitivity

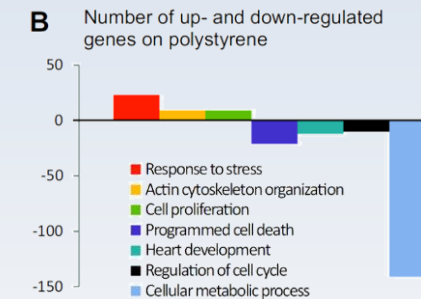
- Cardiomyocytes use deformable adaptor proteins to **sense** contraction

Pandey et al.: Cardiomyocytes Sense Matrix Rigidity through a Combination of Muscle and Non-muscle Myosin Contractions. *Developmental Cell* 44 (2018) 326-336



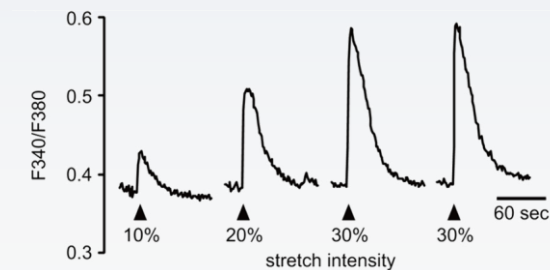
- Cardiomyocytes **respond** to unphysiologic matrix stiffness with drastic transcriptional de-regulation, especially metabolic de-regulation

Heras-Bautista et al.: Cardiomyocytes facing fibrotic conditions re-express extracellular matrix transcripts. *Acta Biomaterialia* 89 (2019) 180-192



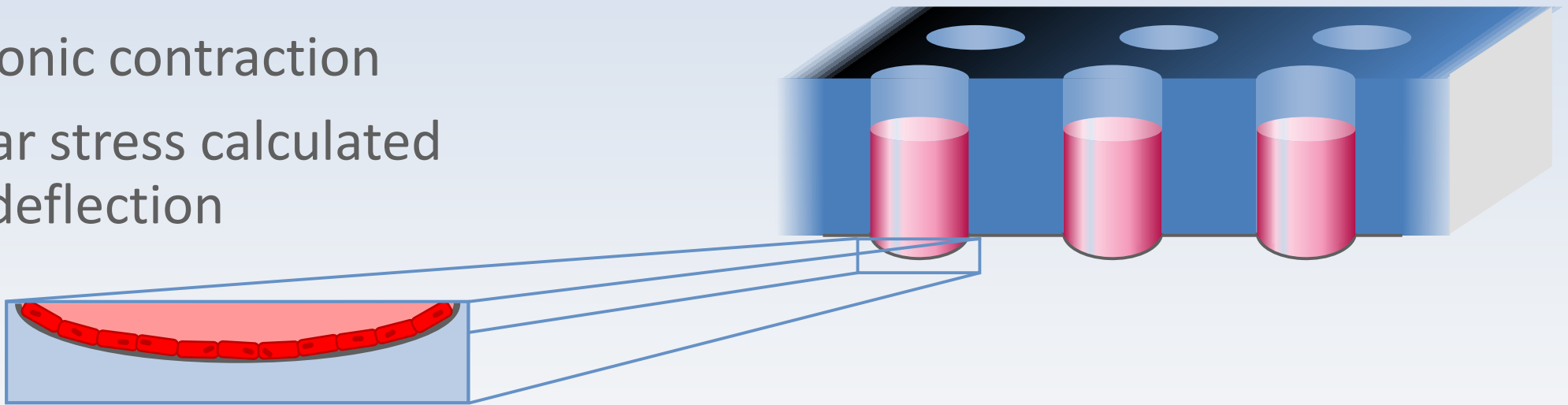
- Crucial ion channels are sensitive to mechanical stress (e.g. L-type Ca^{2+} channels)

Takahashi et al.: L-type calcium channel modulates mechanosensitivity of the cardiomyocyte cell line H9c2. *Cell Calcium* 79 (2019) 68-74



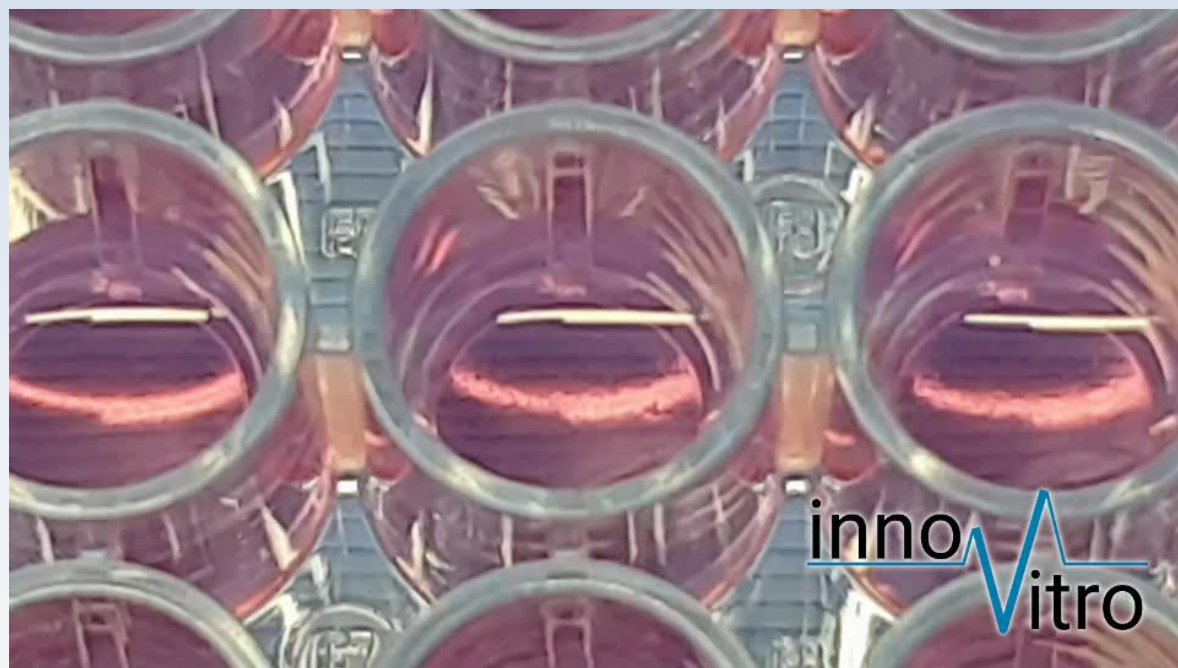
The FLEXcyte 96 Technology

- Ultra-thin silicone substrate
- Adjustable mechanical environment
- Auxotonic contraction
- Cellular stress calculated from deflection

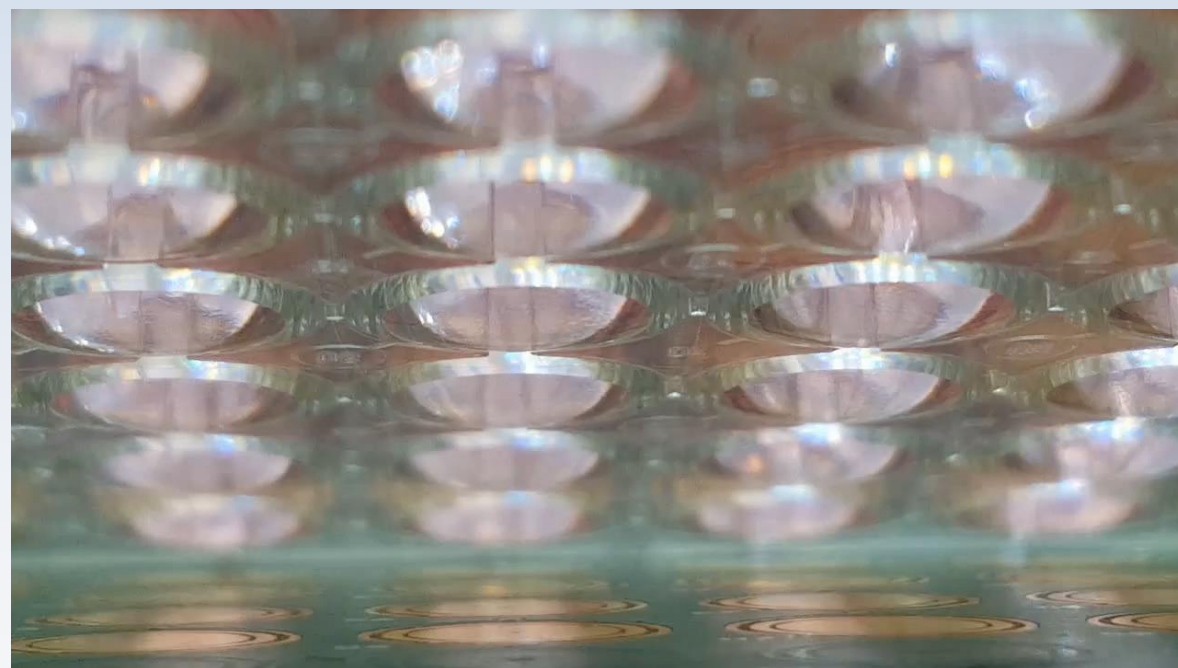


$$\sigma = \frac{p}{h} \cdot \frac{r^2}{4s} \cdot \left(1 + \frac{h^2}{r^2} \right)$$

The FLEXcyte 96 Technology



Top View

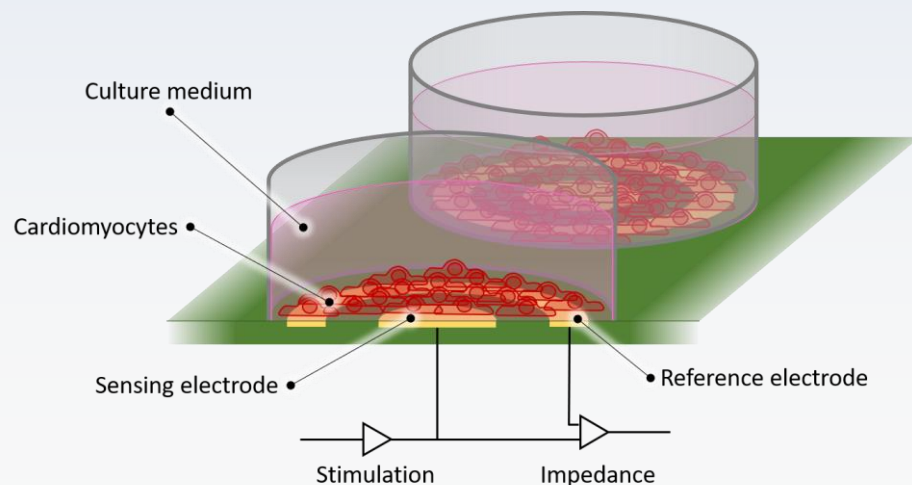


Bottom View

The FLEXcyte 96 Technology

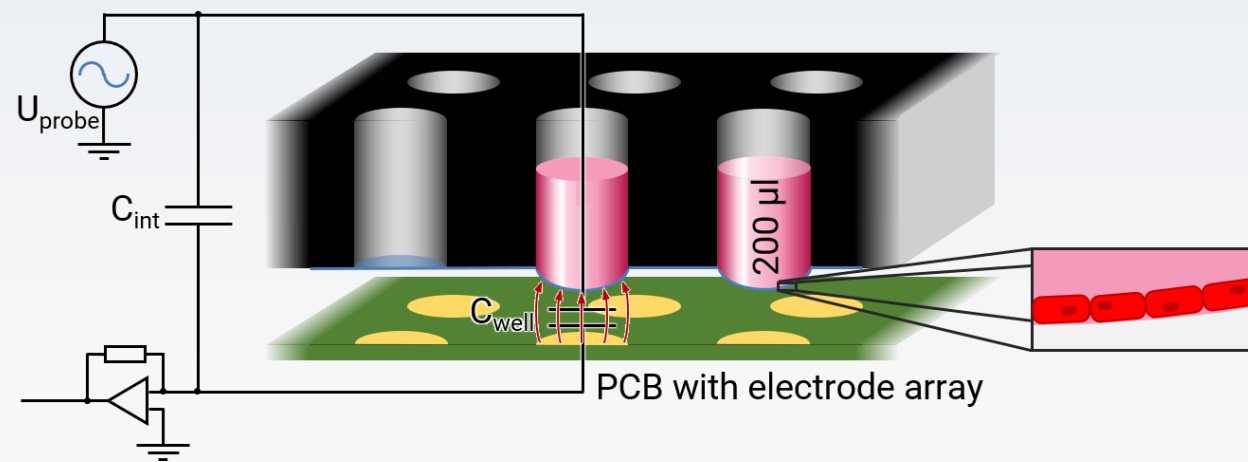
Impedance

- Infinitely stiff substrate
- Isometric contraction
- Parameter: Cellular shape



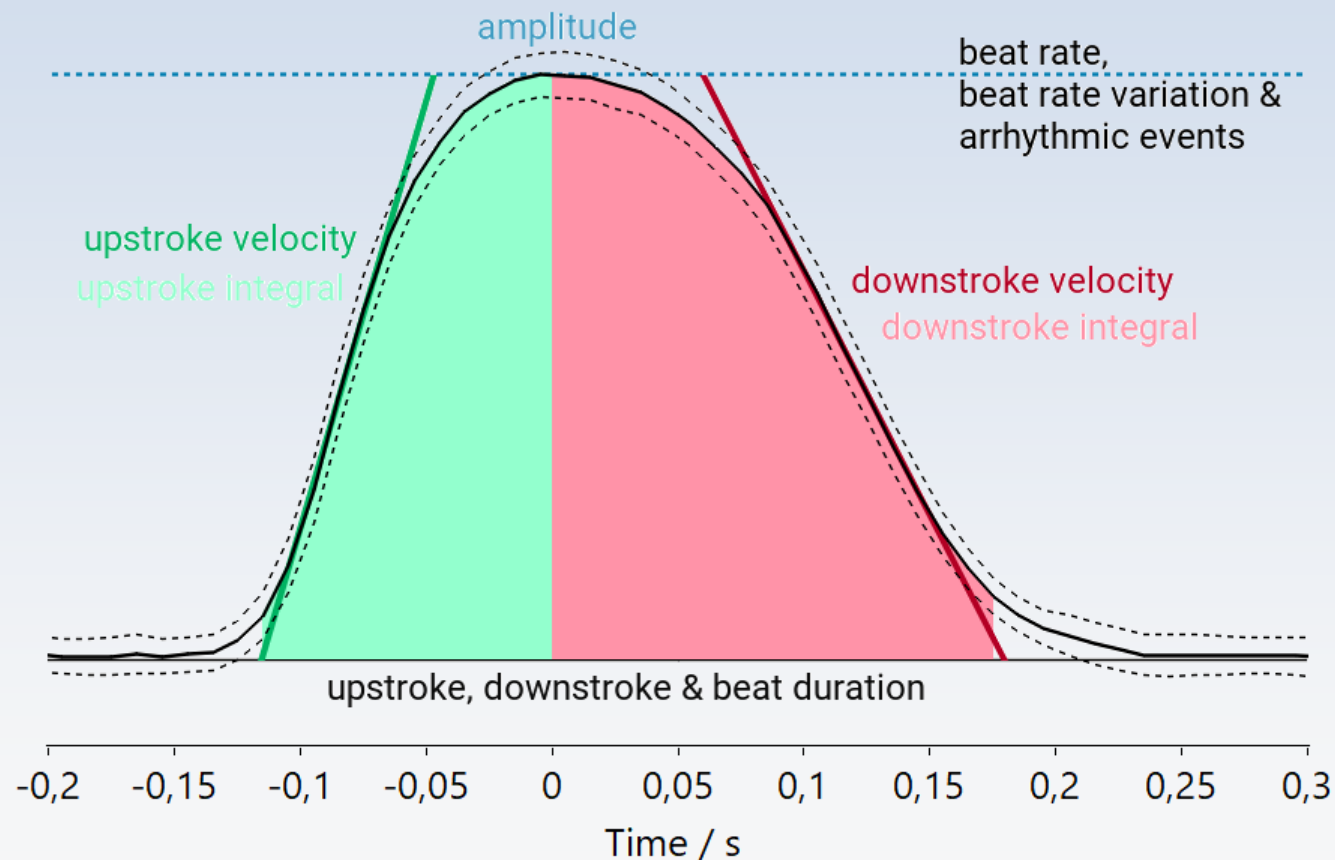
FLEXcyte 96

- Physiologic stiffness
- Auxotonic contraction
- Parameter: Contraction



The FLEXcyte 96 Technology

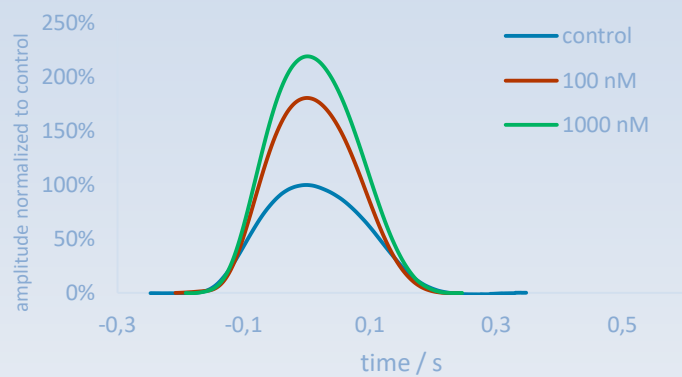
- Amplitude
- Durations
- Slopes
- Integrals
- Frequency
- Arrhythmic Events



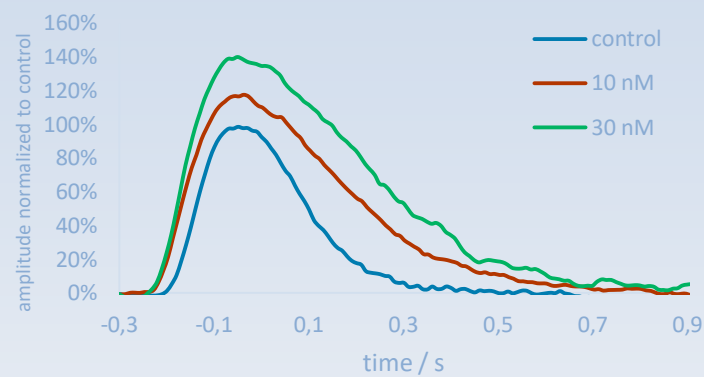
Acute Compound Application

Positive Inotropes

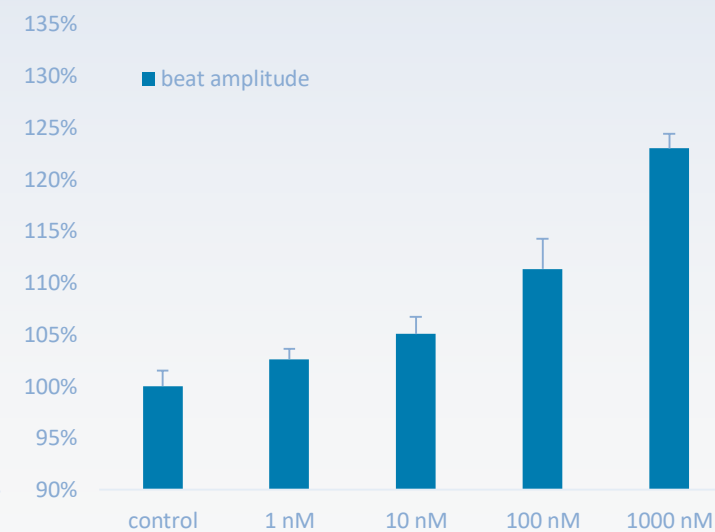
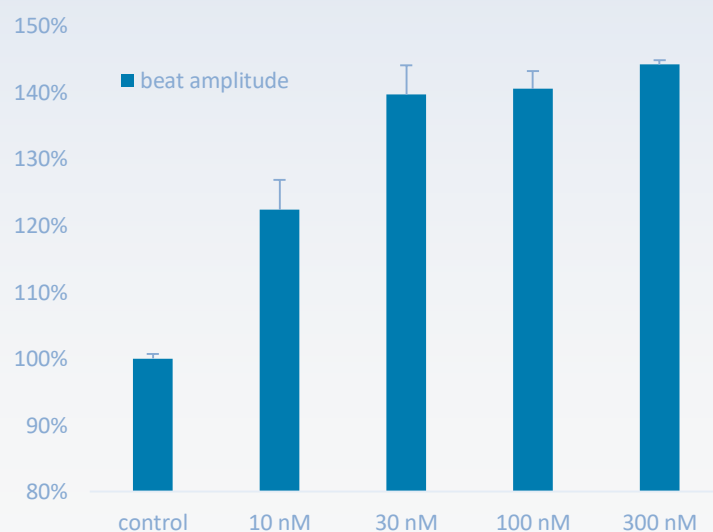
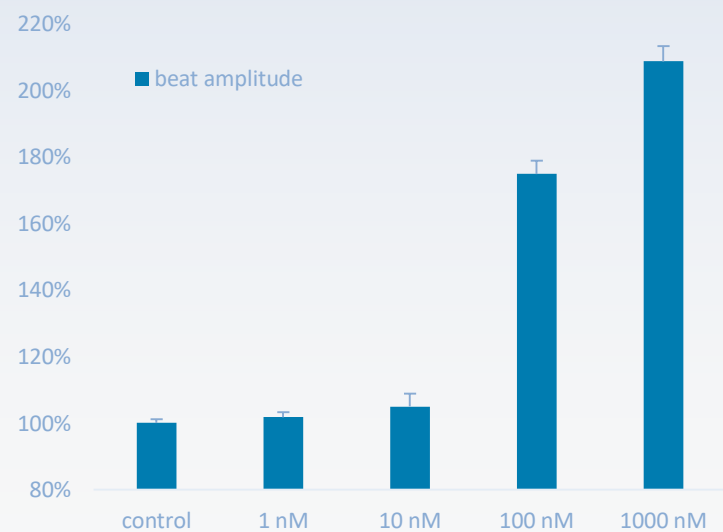
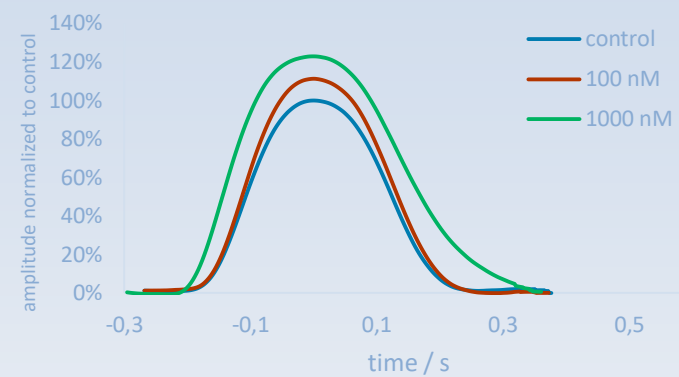
isoproterenol



S-BAY K8644

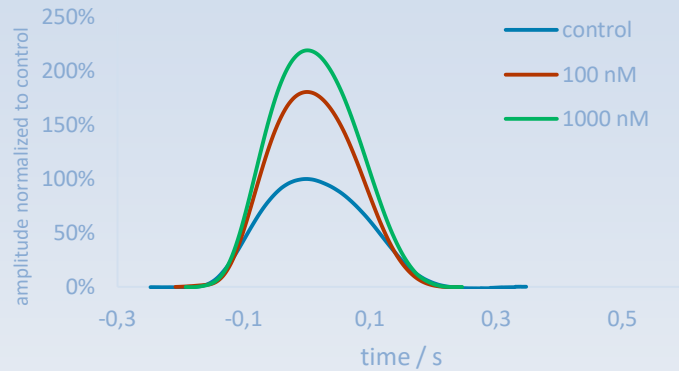


omecantiv mecarbil

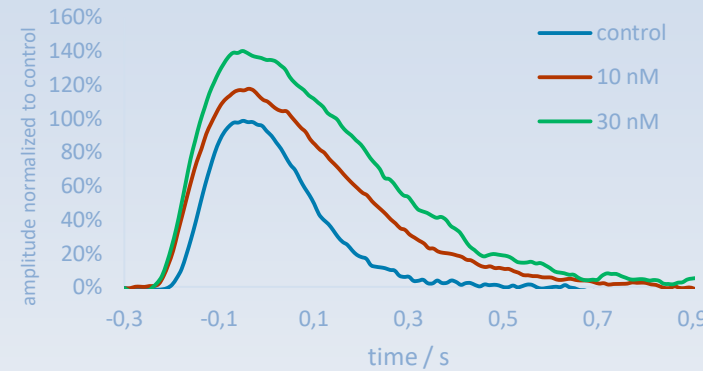


Positive Inotropes

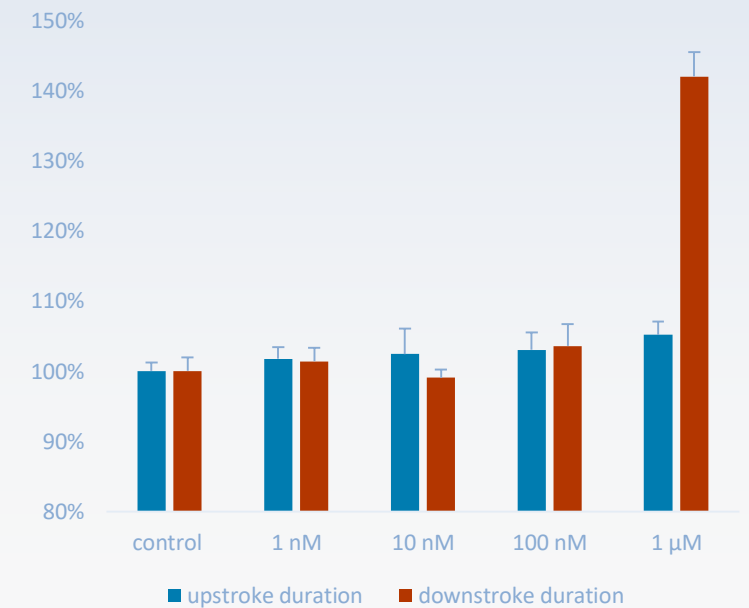
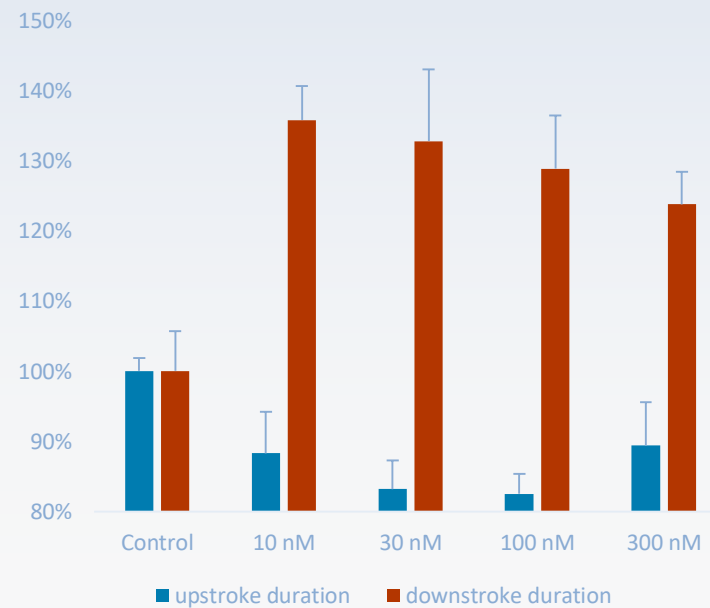
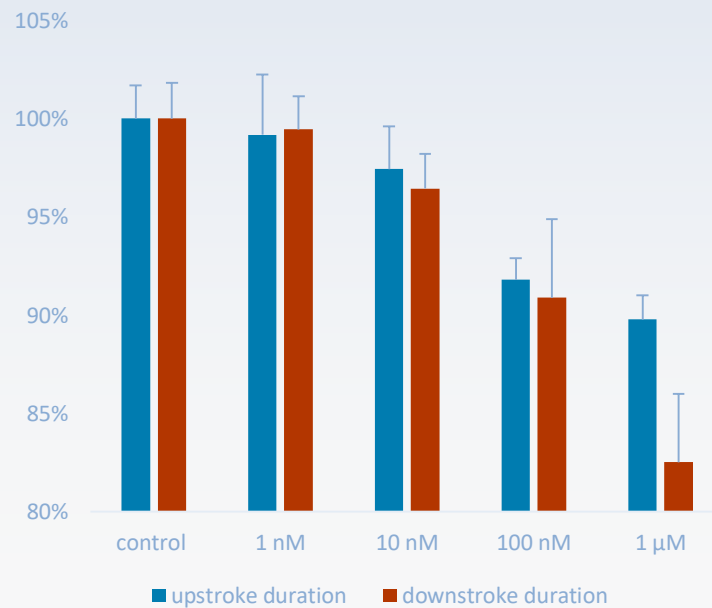
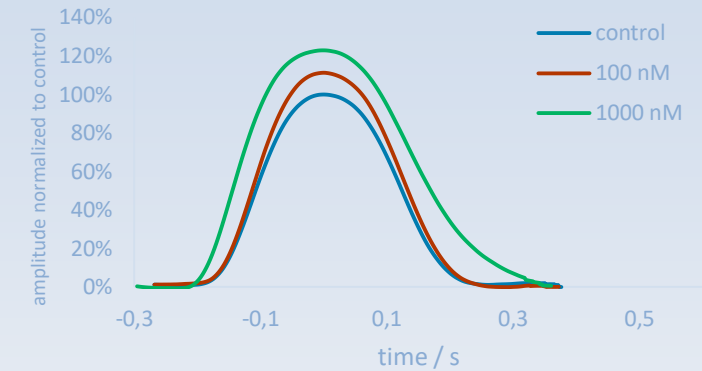
isoproterenol



S-BAY K8644



omecantiv mecarbil



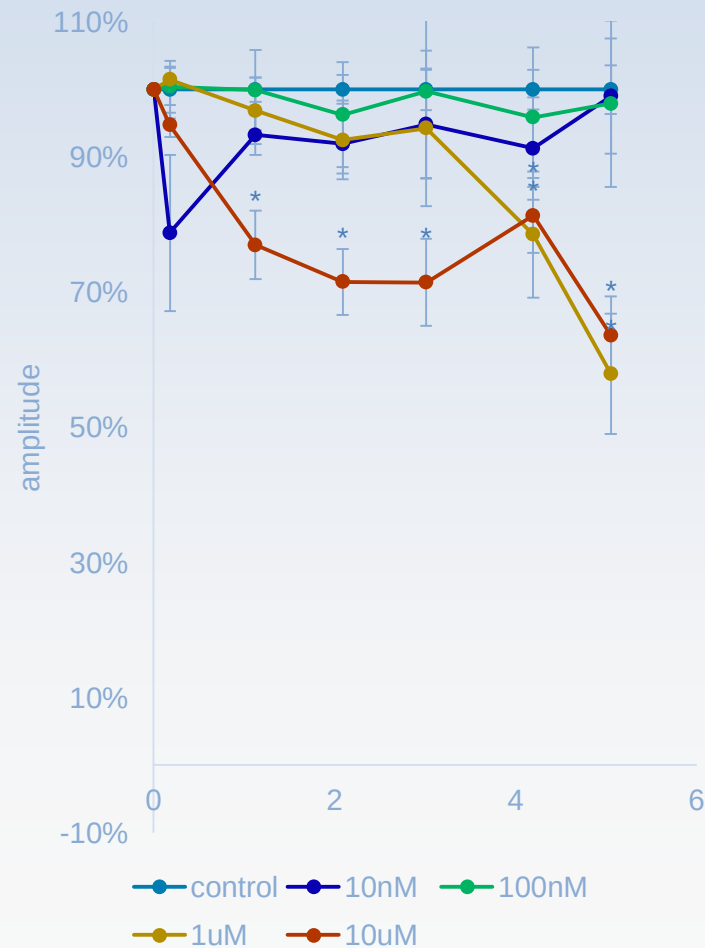
Chronic Compound Application

Kinase Inhibitors / Anthracyclines

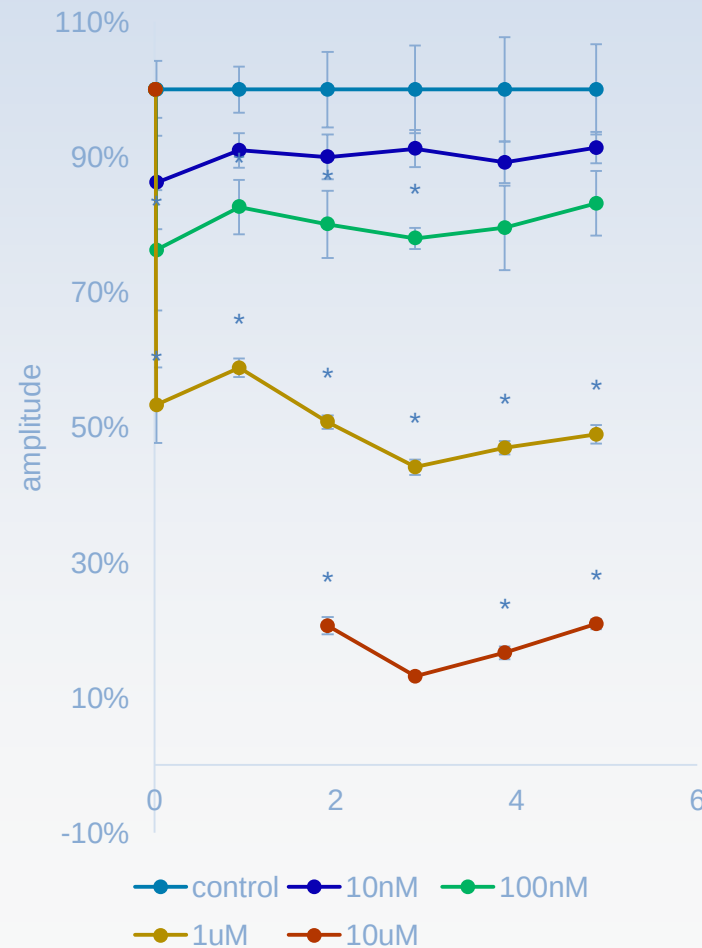
- Structural and/or functional toxicity
- Days and weeks until onset of adverse effects
- Compound incubation: 5 days

Kinase Inhibitors / Anthracyclines

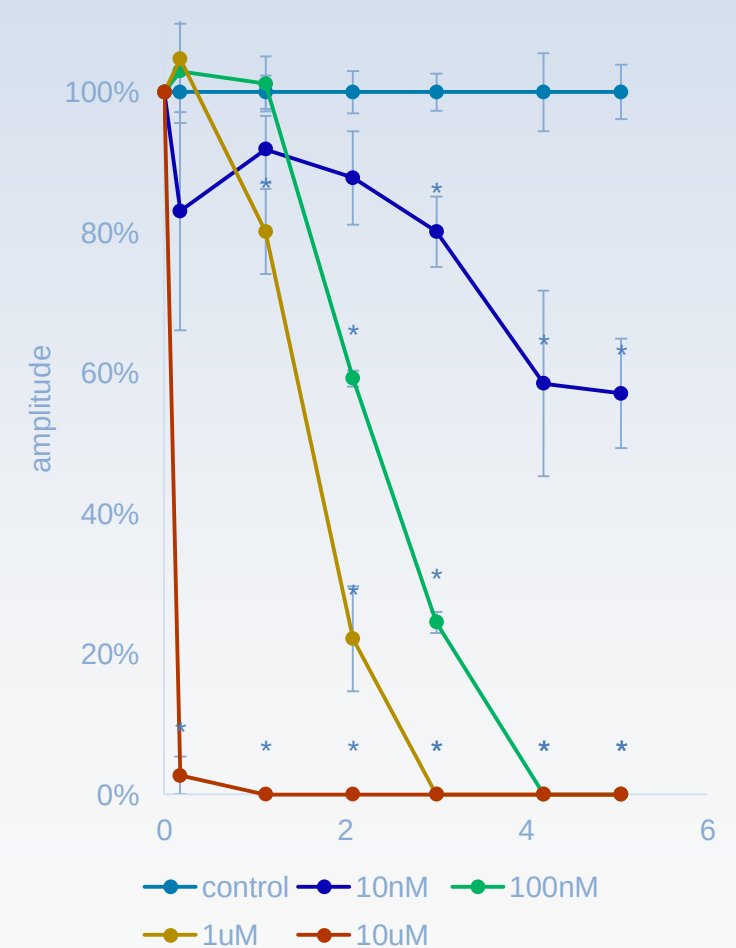
Erlotinib



Vandetanib



Idarubicin



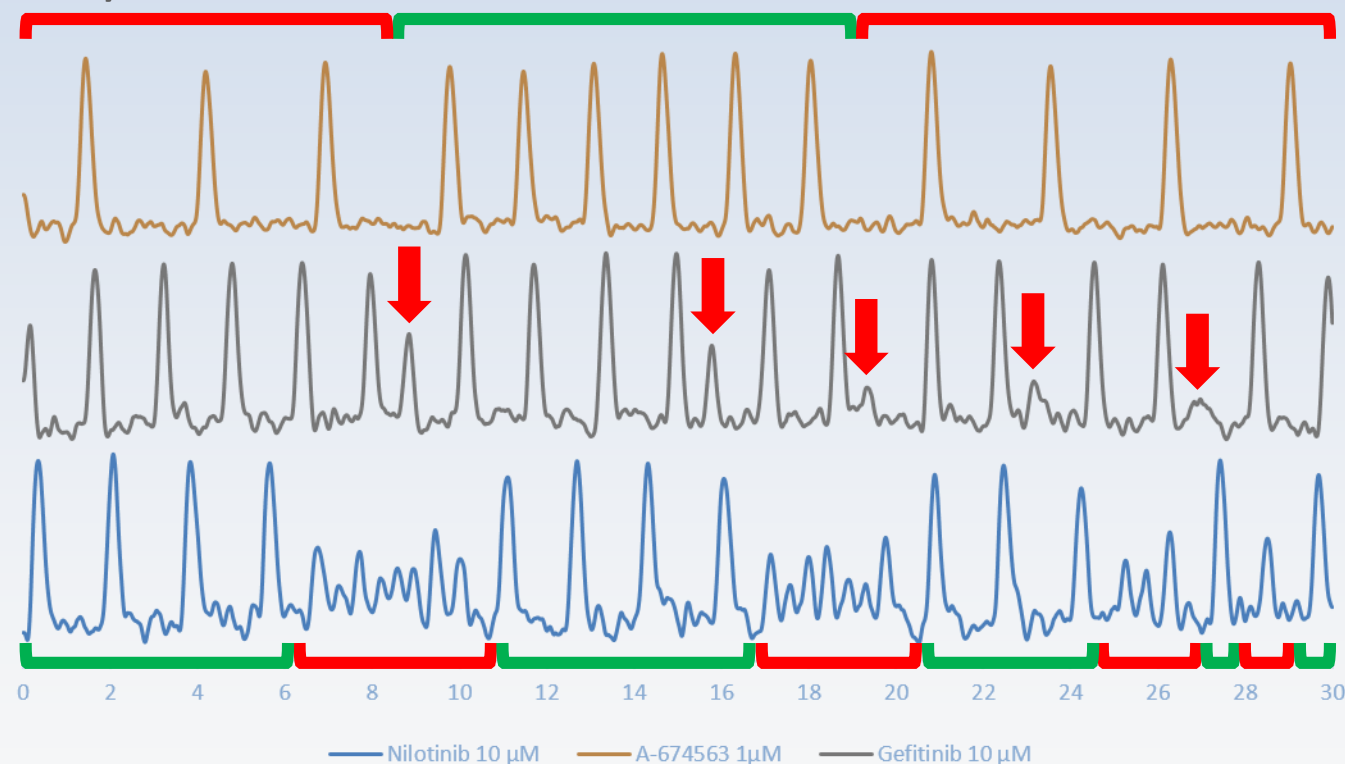
Kinase Inhibitors / Anthracyclines

- Arrhythmogenic effects (24 h):

- Beat rate irregularity

- Aftercontractions

- Fibrillation

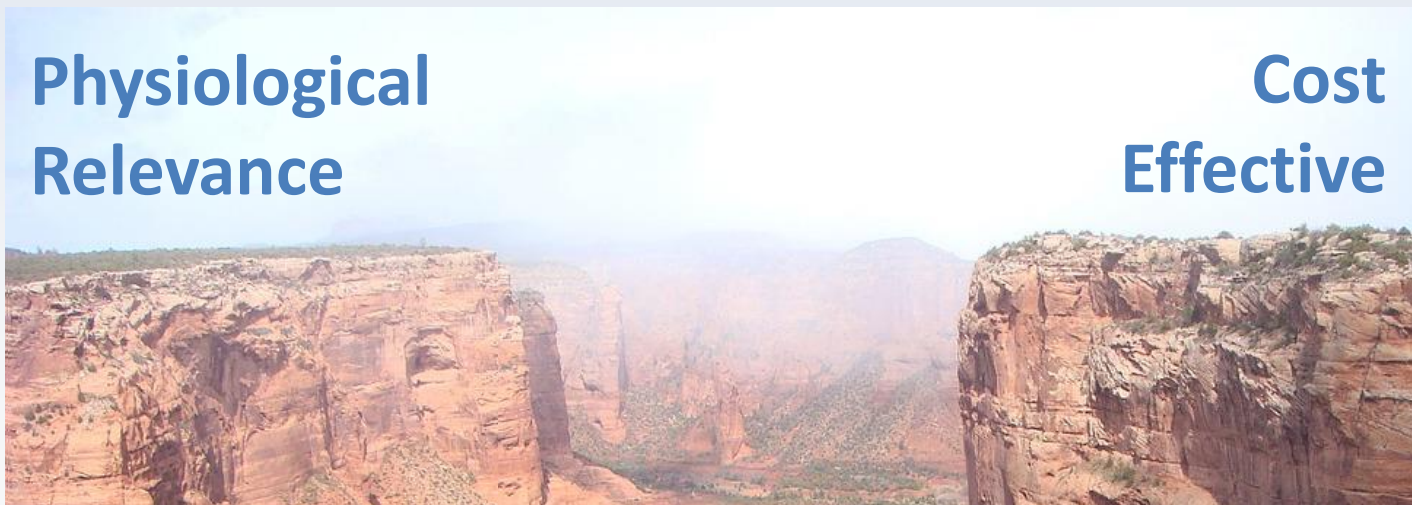


Conclusion

- 96-well format
- Auxotonic environment
- Acute and sub-chronic compound effects

**Physiological
Relevance**

**Cost
Effective**



Thank you!



nanji[on

- Ulrich Thomas
- Sonja Stölzle-Feix
- Elena Dragicevic

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UNIVERSITY OF APPLIED SCIENCES



- Alexander Jung
- Manfred Staat
- Aravind Hariharan Raman

