Comprehensive *in vitro* Proarrhythmia Assay (CiPA) Using Cor.4U Cardiomyocytes with the FDSS in a Calcium Transient Assay

Dr. Ralf Kettenhofen

09.06.2016 Hamamatsu User Meeting

Barcelona, Spain

Content

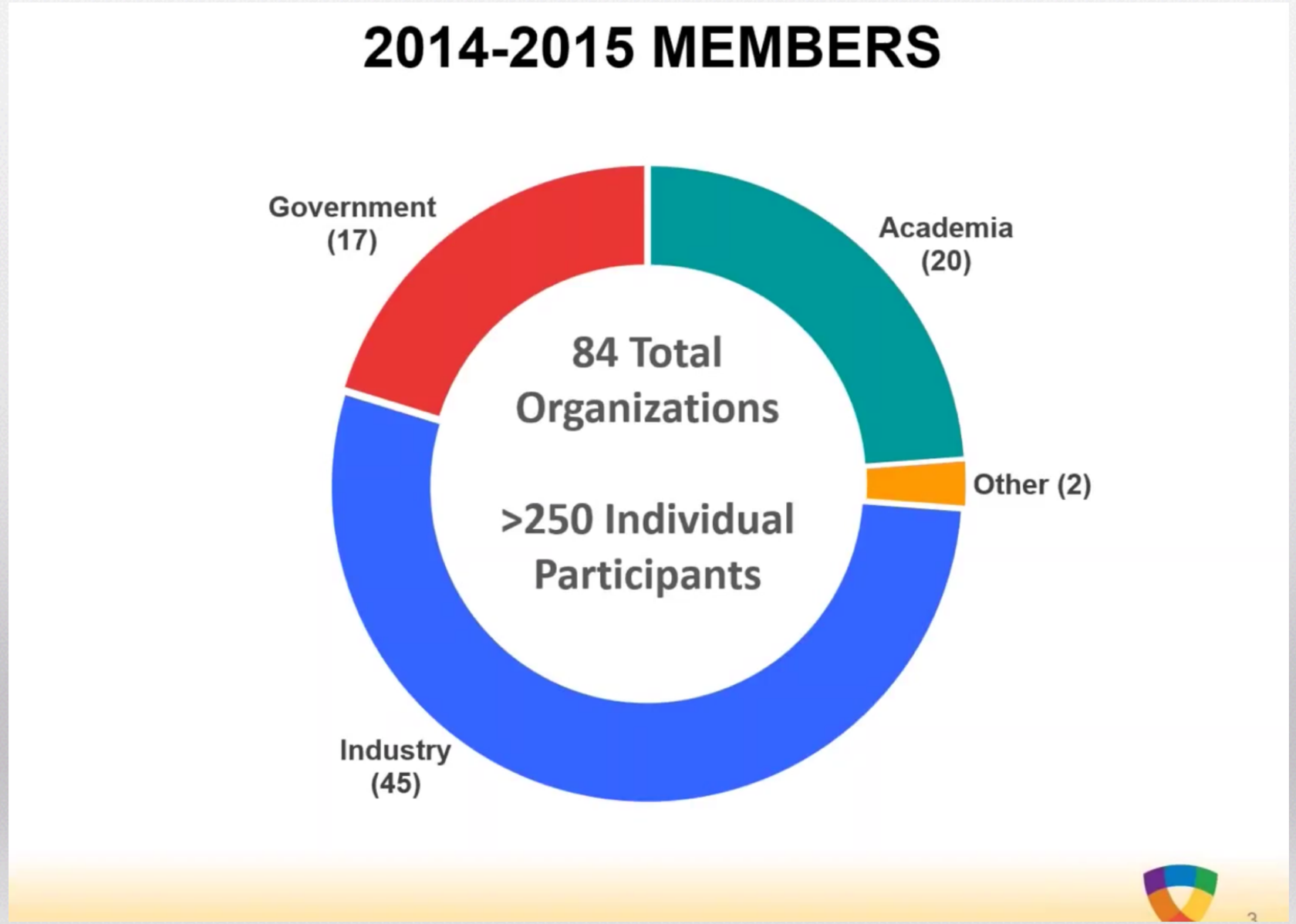
- The CiPA Initiative - Short Introduction
- Factors Influencing the Calcium Transient Assay
- Customer Report - Drug Development Support

Comprehensive in vitro Proarrhythmia Assay

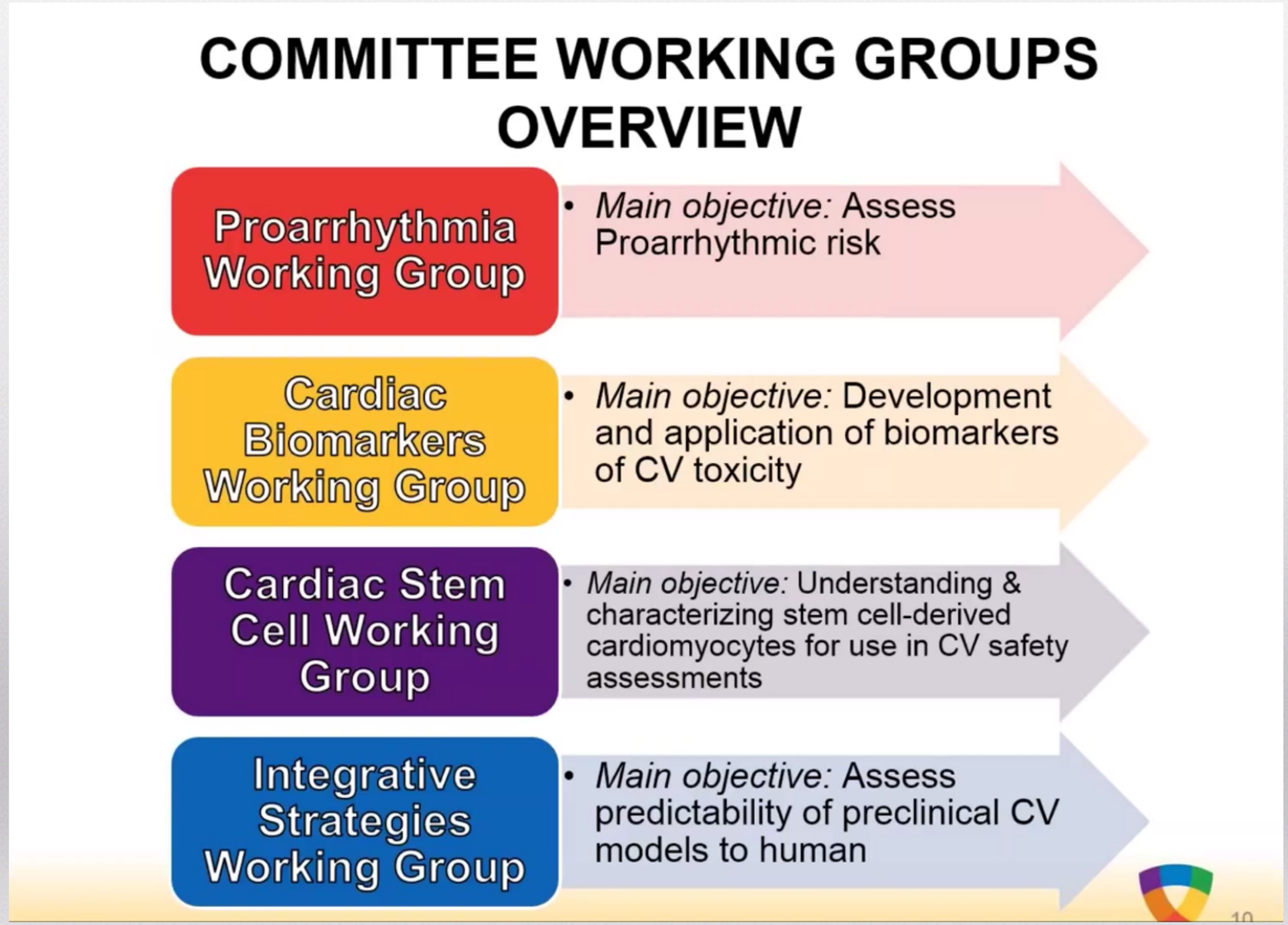
CiPA - Initiative

•Presented for:

CiPA Members



CiPA - Overview of Working Groups



CiPA Phase 1 - Pilot Study

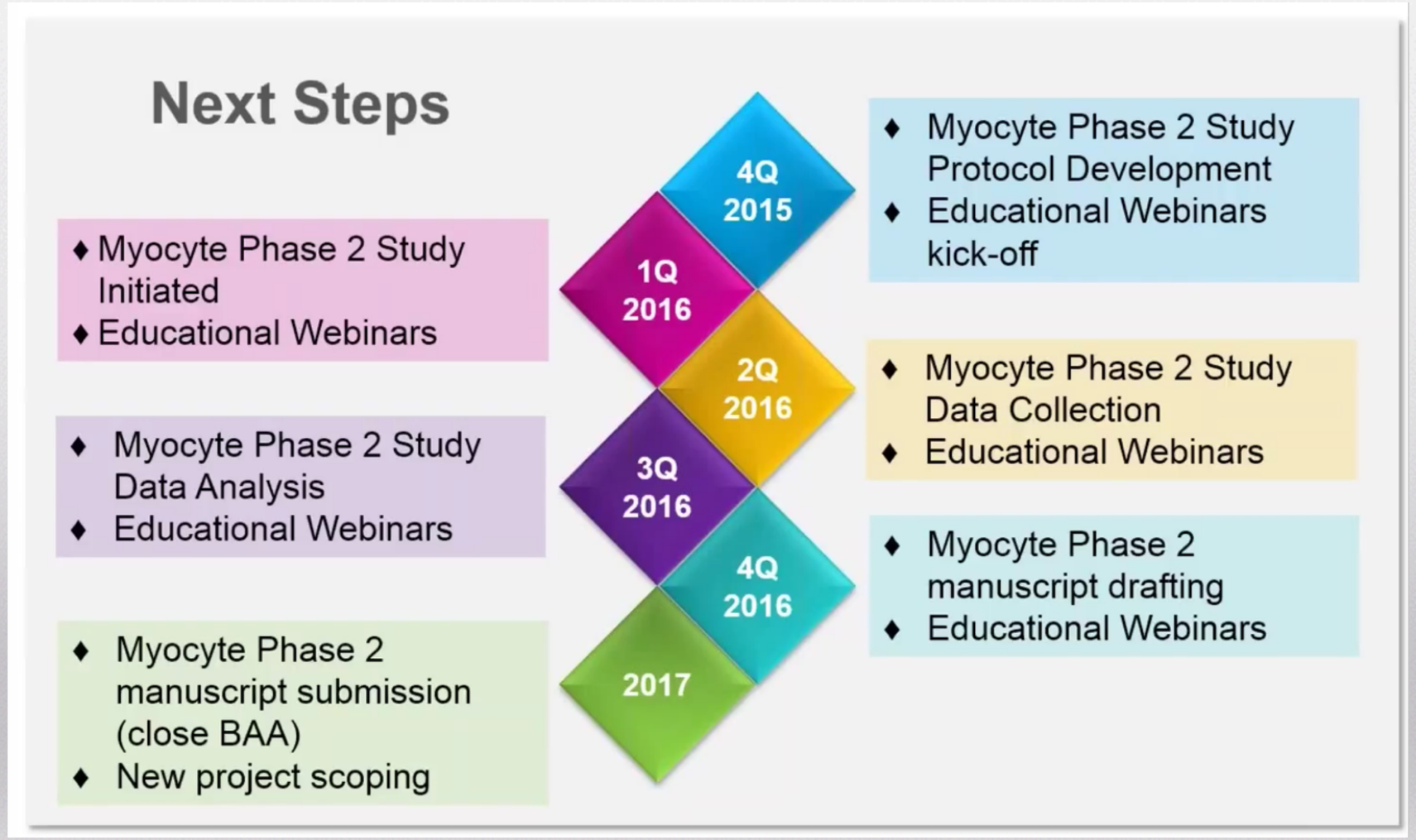
- 3 Providers of pluripotent stem cell-derived cardiomyocytes
- 16 Volunteer sites
  - 12 sites; 3 microelectrode array platforms
  - 4 sites; 4 Voltage-sensing-optical (VSO) platforms
  - 8 blinded test compounds; 4 concentrations, 3 triplicates

- Study was accomplished End 2014
- Manuscript for publication is under discussion

CiPA Phase II - Validation Study

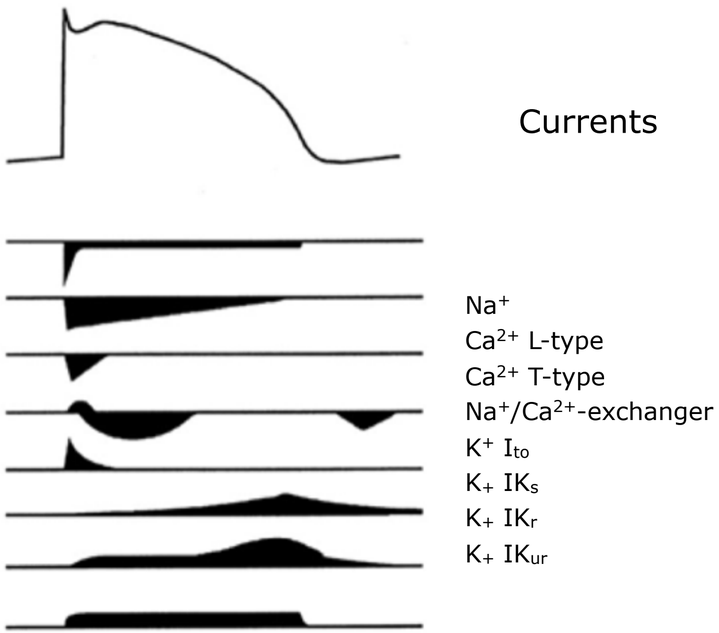
- 2 Providers of pluripotent stem cell-derived cardiomyocytes
- 5 core sites (funded by FDA grant)
  - 2 sites; 4 microelectrode array platforms
  - 3 sites; 3 Voltage-sensing-optical (VSO) platforms
  - Calcium Transient Assay (potential backup assay)
    - 3 sites: Janssen, Axiogenesis, Merck (USA)
  - Compounds:
    - 28 blinded test compounds; 4 concentrations, 6 replicates
    - 4 calibration compounds
- Volunteer non-core test sites:
    - 12 blinded test compounds + 4 calibration compounds

CiPA Phase II - Validation Study

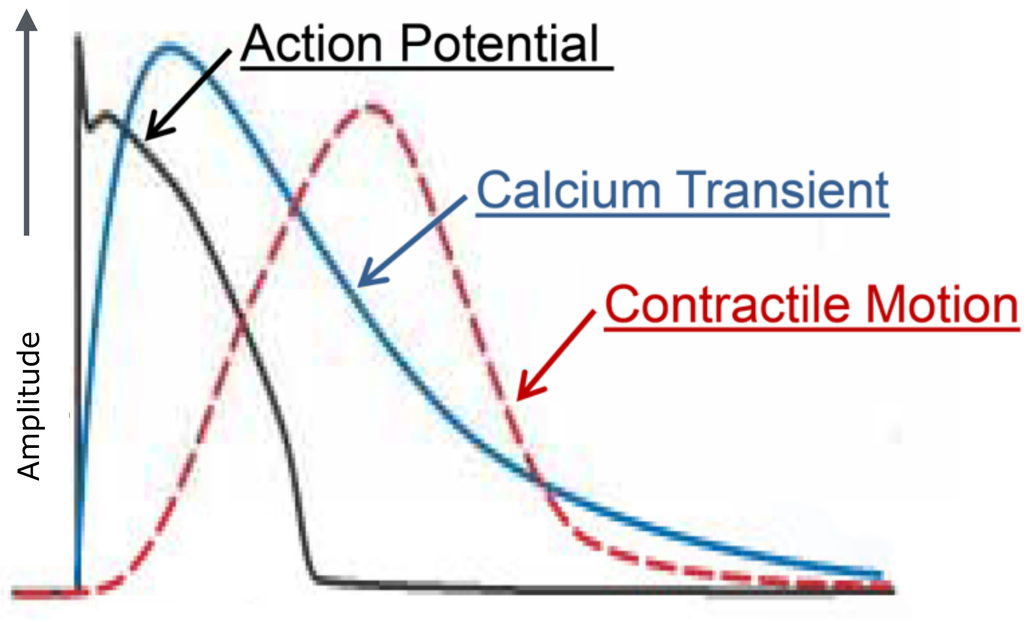


Excitation-Contraction Coupling

A)



B)

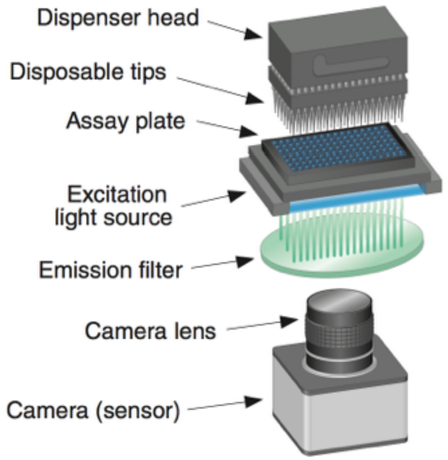


High Throughput Kinetic Plate Reader Assays

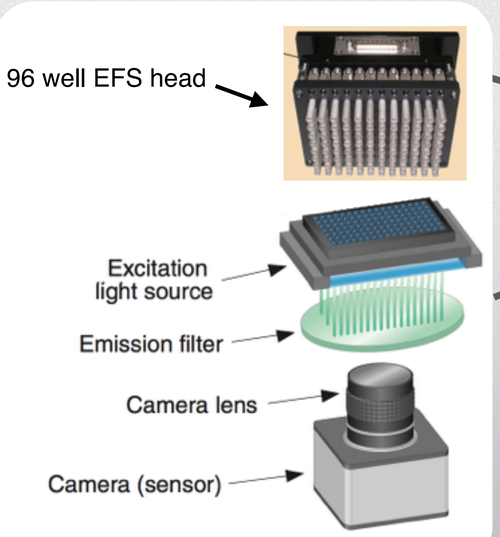
•Presented for:

Plate Reader System - Hamamatsu

Setup A
Pipettor Head



Setup B
EFS Head



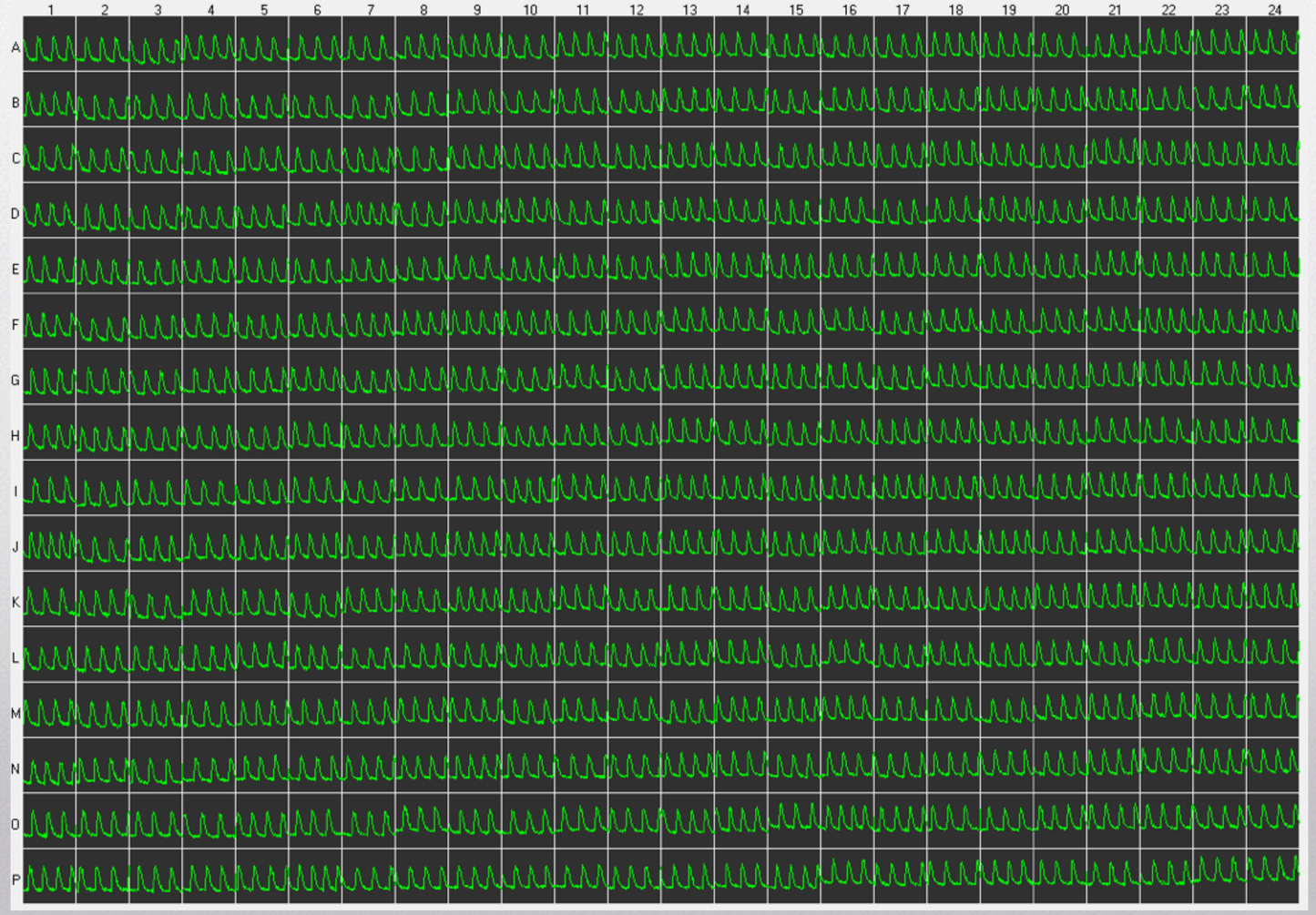
Hamamatsu
FDSS µCell

Hamamatsu
FDSS 7000EX

HAMAMATSU
Data generated in collaboration with Hamamatsu

Both systems can be equipped with a temperature control

Plating Efficiency of Cor.4U Cardiomyocytes on a 384 Well Plate



HAMAMATSU
Data generated in collaboration with Hamamatsu

Recording of Cor.4U cardiomyocytes with the FDSS 7000EX using Cal520 dye (AAT Bioquest).

axio GENESIS

Assay Optimisation

Important Factors Influencing the Calcium Transient Assay with hiPSC-derived Cardiomyocytes

•Presented for:

Calcium Transient Assay - Important factors

- The calcium dyes
- Dye loading time
- Assay stability over time (assay window)
- Wash vs. non-wash
- Signal to noise ratio
  - Medium / buffer
  - Quencher
- Addition of organic anion transporter (e.g. probenecid)

Dye-induced Morphological Differencesand Changes of Cor.4U Cardiomyocytes' Calcium Transients

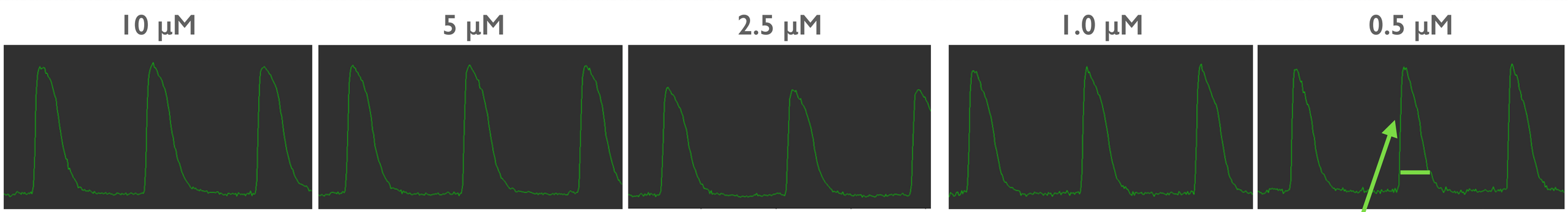
Cal520 (AAT Bioquest)

Calcium 5 Assay Kit (Molecular Devices)

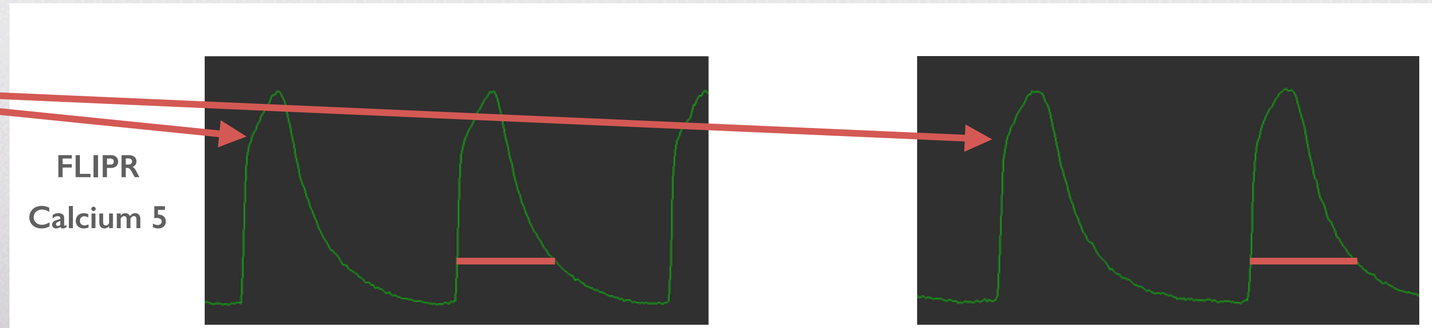
ACTOne (Codex)

Results - Cal-520

30 min



Bent fast rise of the calcium transient and prolonged peak width



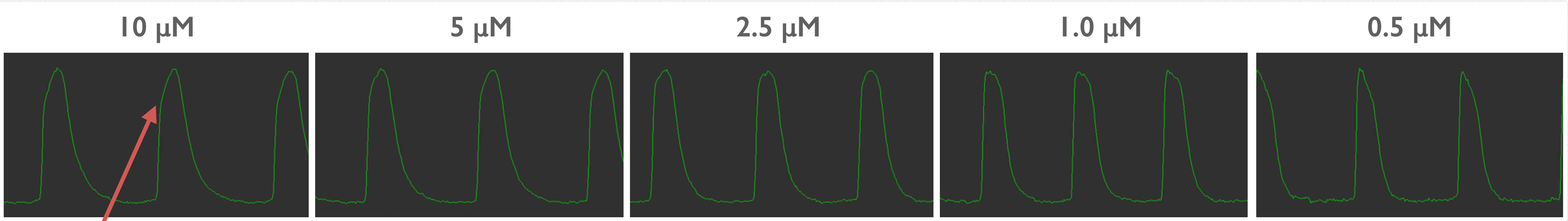
time windows: 4s

Physiological fast rise of the calcium transient and short peak width

- FLIPR Calcium 5 and Codex ACTOne reveal an slowed rise of the calcium transients from 80% to 100%.
- There is obviously a changes in calcium transients which potentially indicates the start of toxic events at an early time point.
- Calcium transient durations are increased with the FLIPR Calcium 5 dye and the ACTOne dye at concentrations tested compared to Cal-520 dye (see also quantitative analysis).

Results - Cal-520

90 min



Bent fast rise of the calcium transient

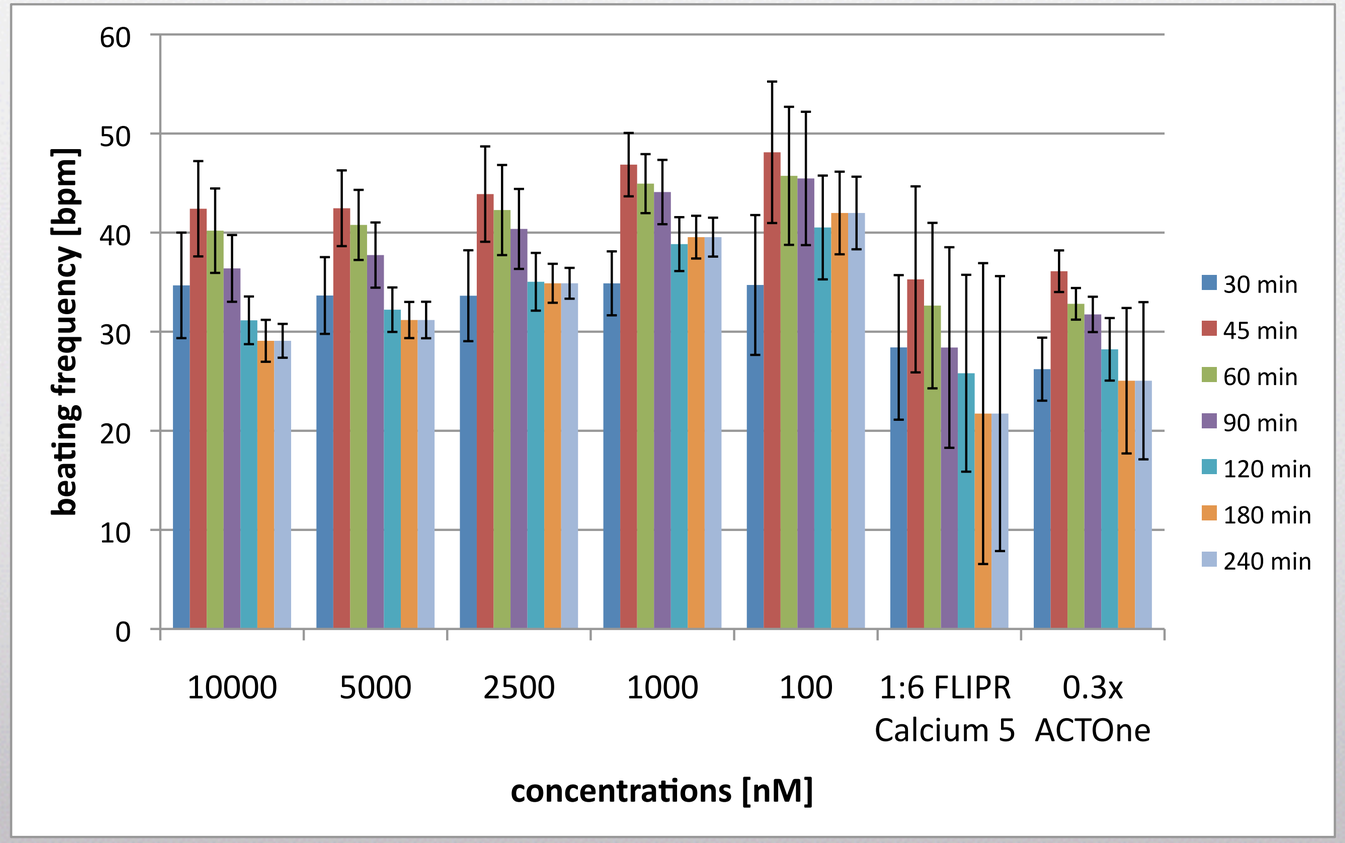
time windows: 4s

At higher concentrations of Cal-520 the slope at 80% to 100% starts to slow as well.

Quantitative Analysis of Non-Wash Cal-520 Calcium Transients Recorded from Cor.4U Cardiomyocytes

Results - Cal-520

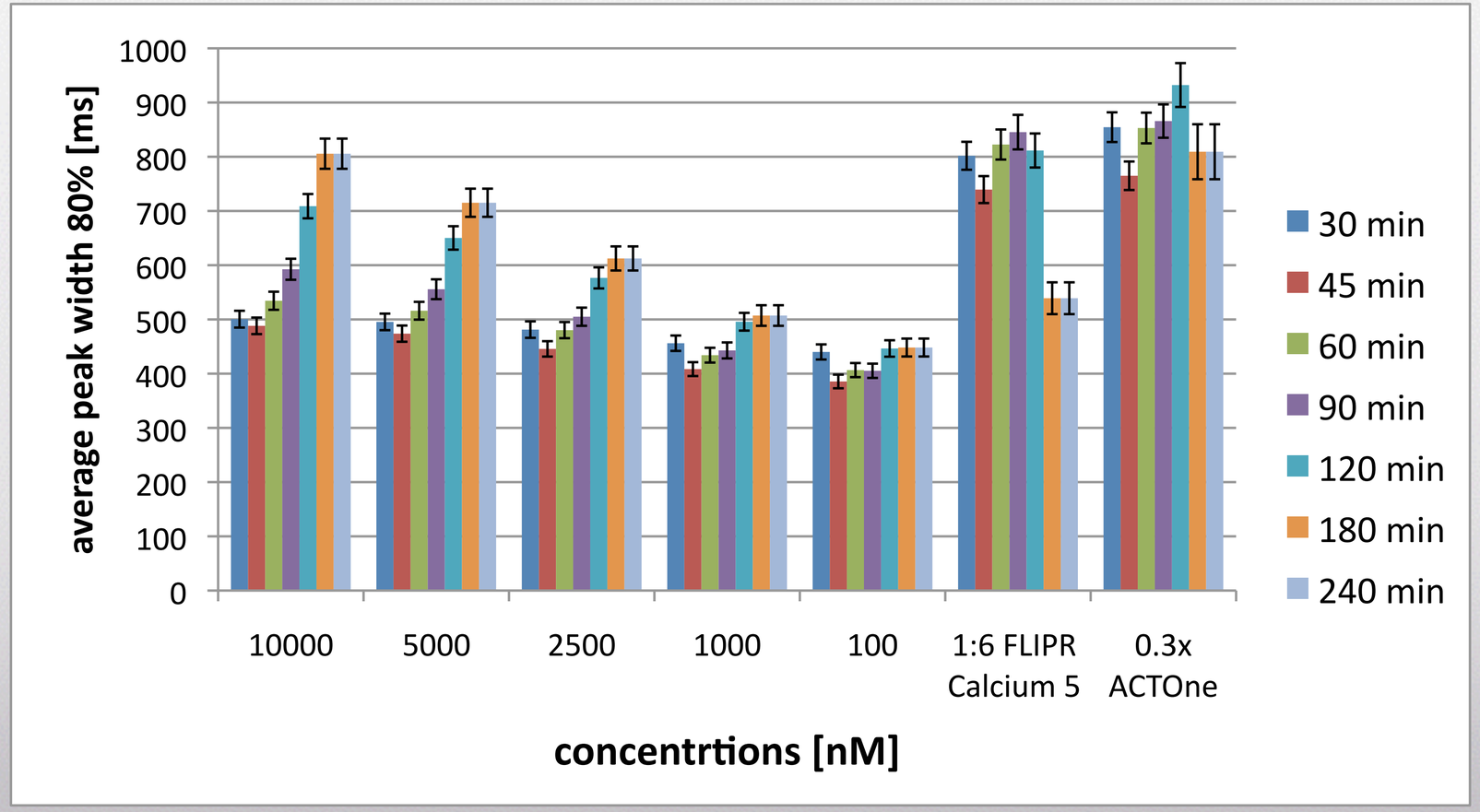
Beat Rate



- Beat rate is higher in Cal-520 Assay compared to the both other dyes, especially at the lowest dye concentration.
- Beat rate decreases with increasing dye concentrations.

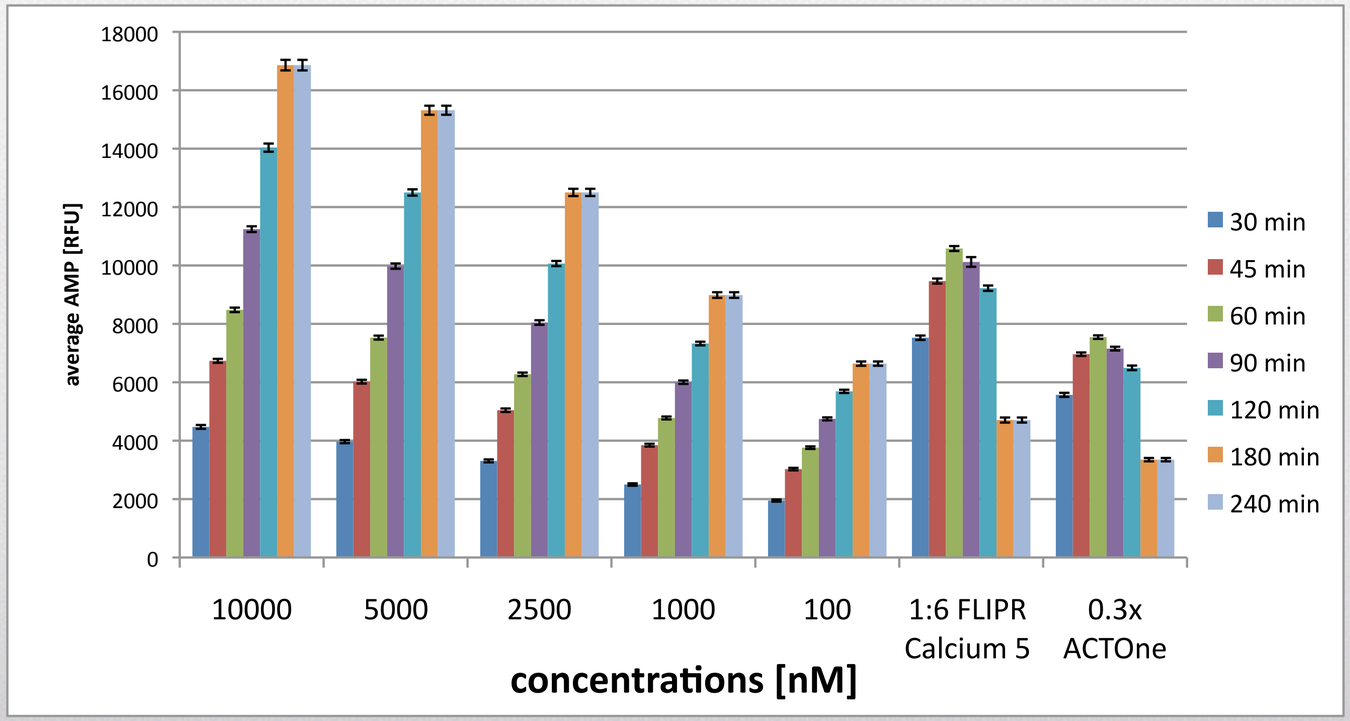
Results - Cal-520

Peak Width (PW) 80%



- Cal-520 calcium transient PW30% and 80% increase over time in the highest concentrations (toxic effect?).
- FLIPR Calcium 5 and ACTOne dye PW80% values are almost twice as high compared to the lowest Cal-520 concentration (=> toxic or unphysiological?).

Results - Cal-520



- Calcium Transient amplitudes from Cal-520 increase over time (max after 3 hours) although no probenecid was added.
- FLIPR Calcium 5 and ACTOne dye amplitudes reach a maximum after 60 min.

Wash Assay Using

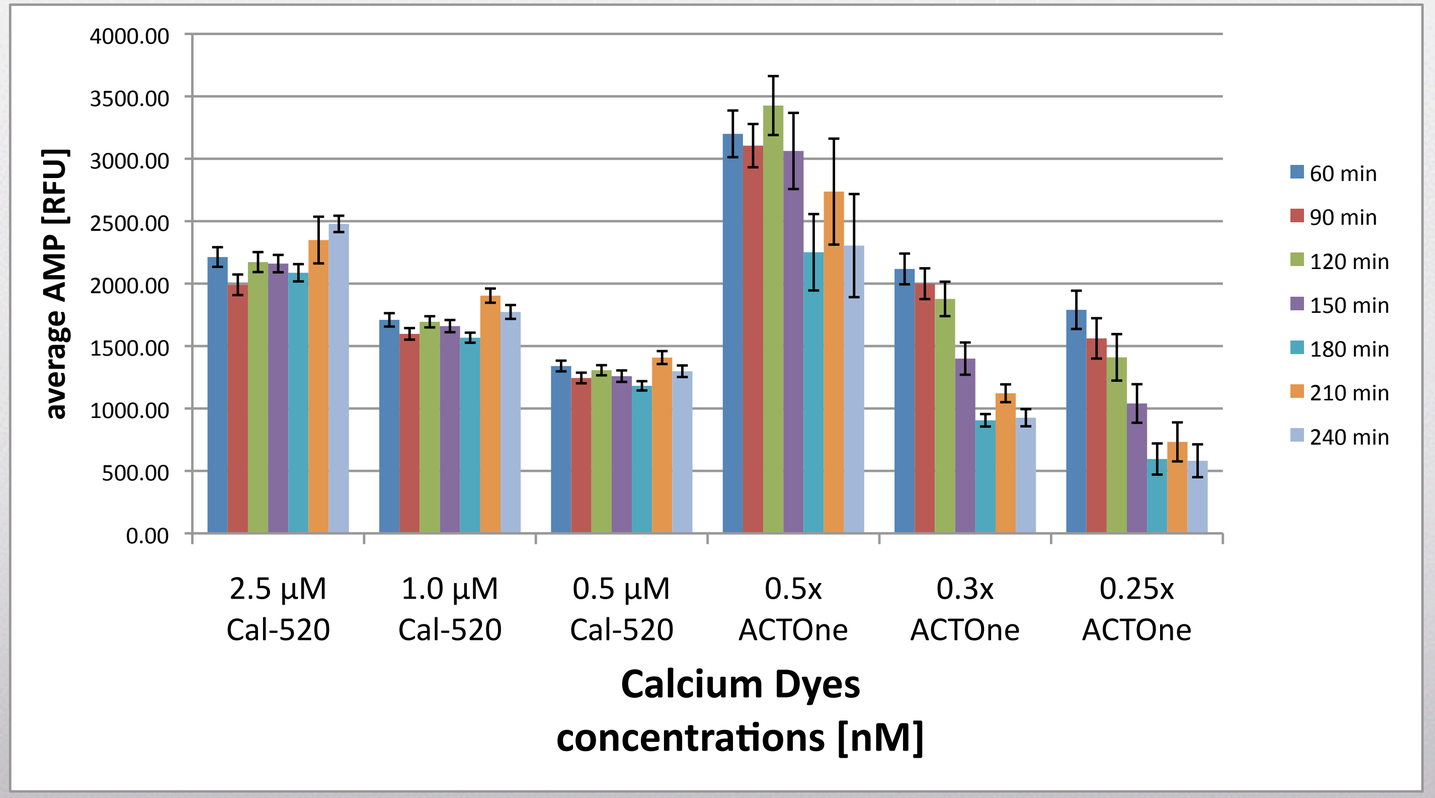
Cal-520™ , AM (AAT Bioquest)

and

ACTOne (Codex)

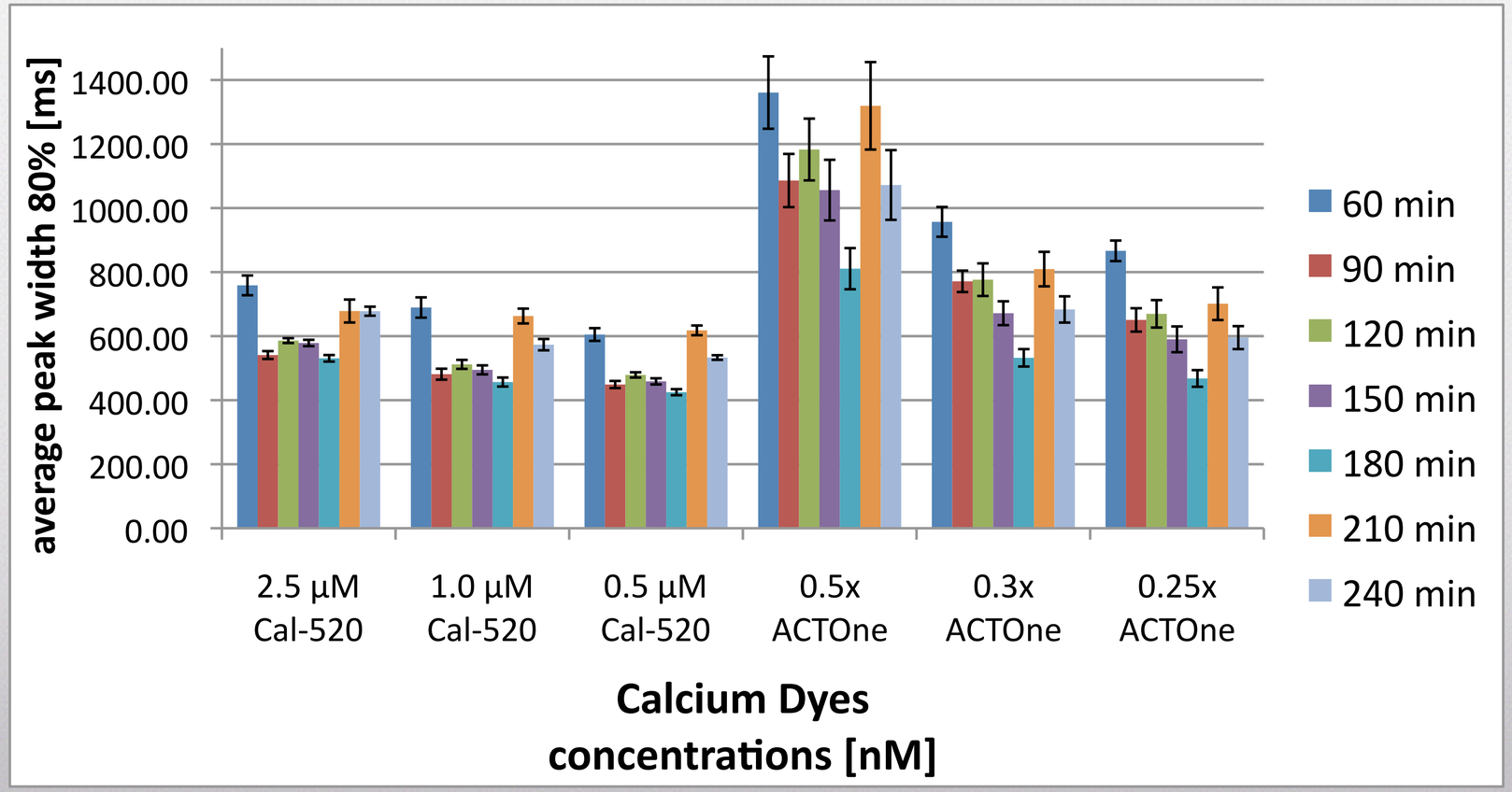
Results

Calcium Transient Amplitude



- Amplitude of Cal-520 calcium transients is absolutely stable during after 4 hours.
- ACTOne amplitudes are decreased after 3 hours.

Results

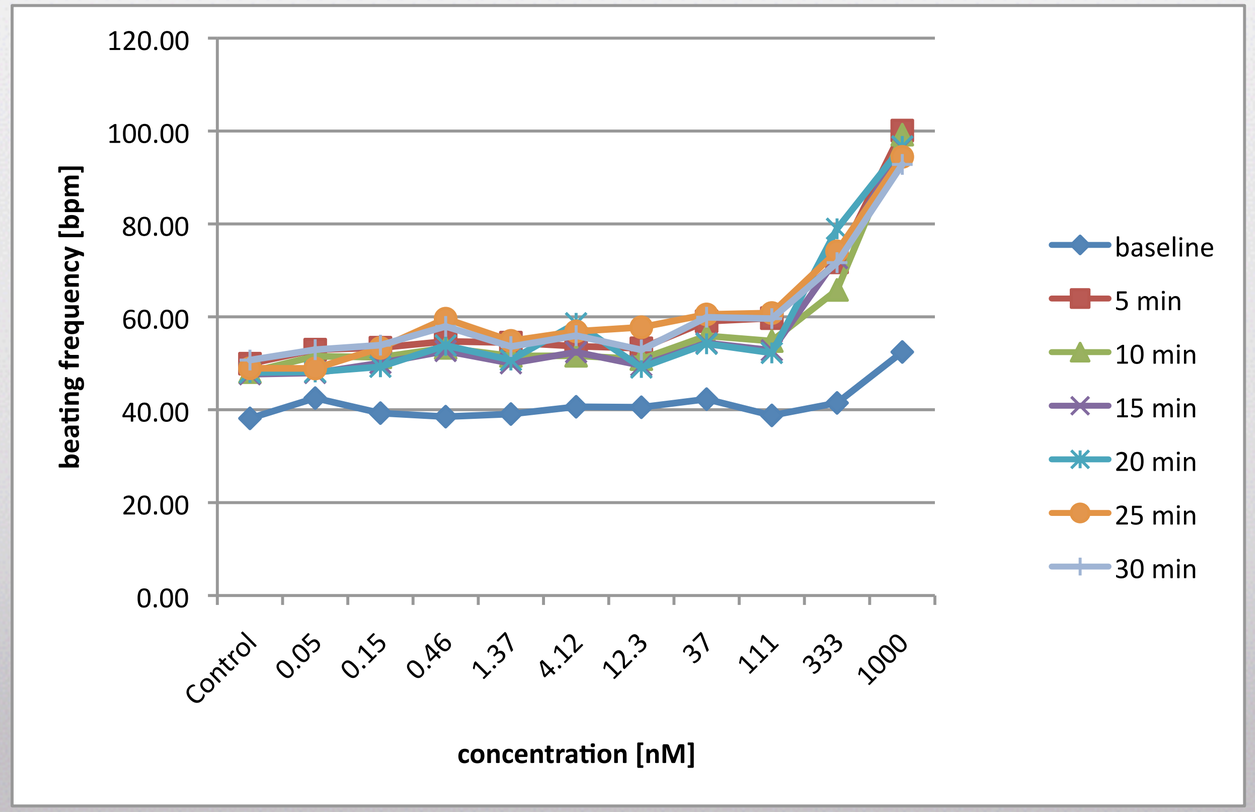


- 0.5x ACTOne peak width at 80% are doubled compared to 2.5 µM Cal-520 (and also 0.25x ACTOne) and almost 3x the values of 0.5 µM Cal-520.

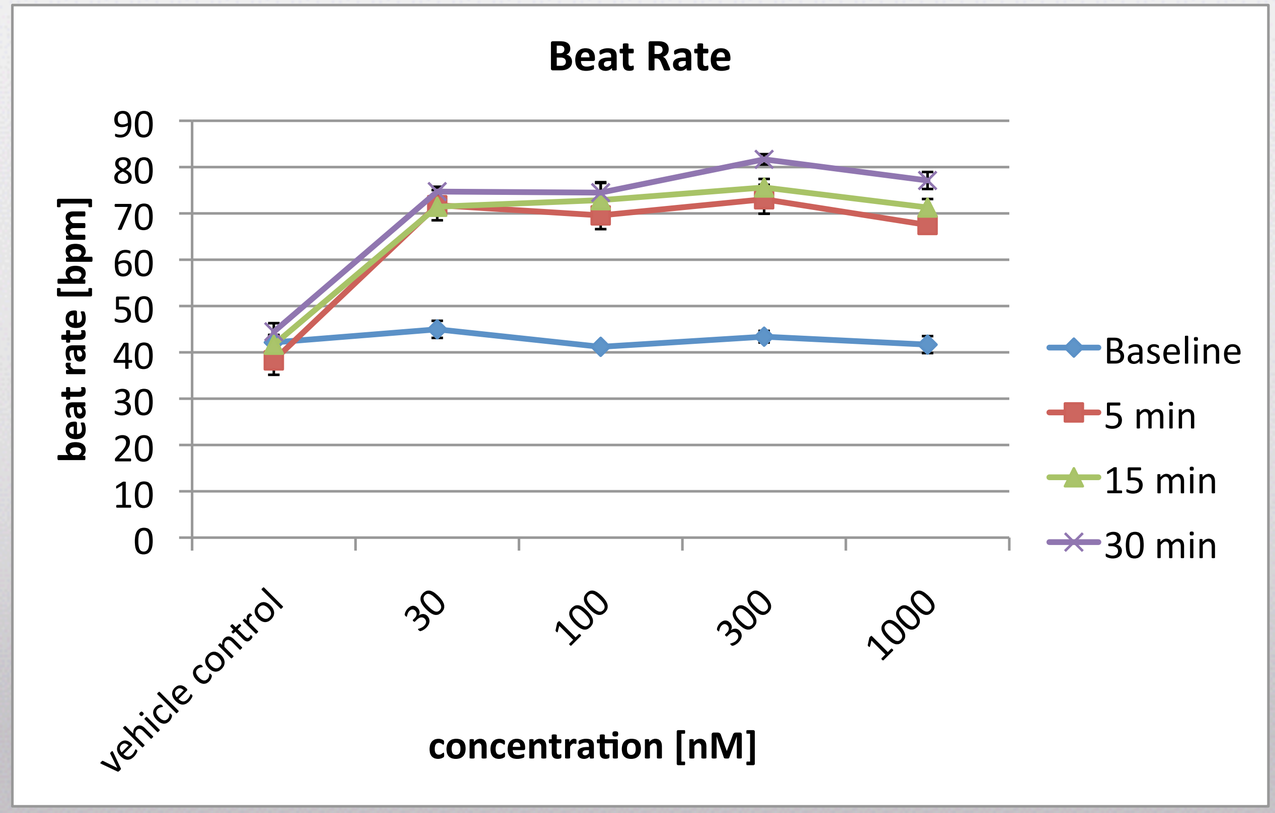
Dye Effect on GPCR Agonist Pharmacology with Cor.4U Cardiomyocytes

Results

Calcium 5 Assay Kit Dye



Cal520



- Right shift of isoproterenol increased beat rate with the Calcium 5 Assay Kit dye
- Cal520: More physiological isoproterenol effect

Conclusion

- Choice of the right calcium dye is important
- Cal520 at low concentrations revealed to be the most physiologic dye
  - Long-term stability (assay window)
  - calcium transient and beating parameters
- No quencher is required for Cal520 when the right assay medium/ buffer is chosen
- Washout is required for Cal520

Support of Pharma Drug Development

Dr. Thomas Licher, Sanofi Frankfurt, Germany

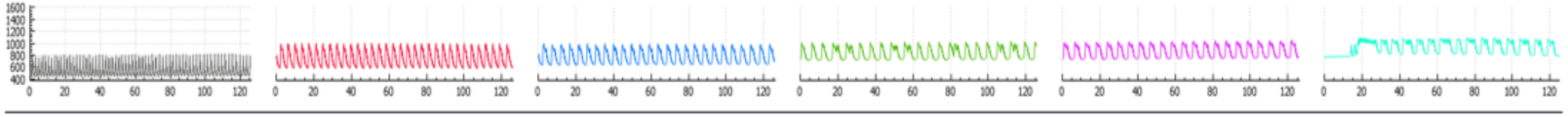
http://axiogenesis.com/resources/presentations/webinar.html

10.02.2016 Axiogenesis webinar

•Presented for:

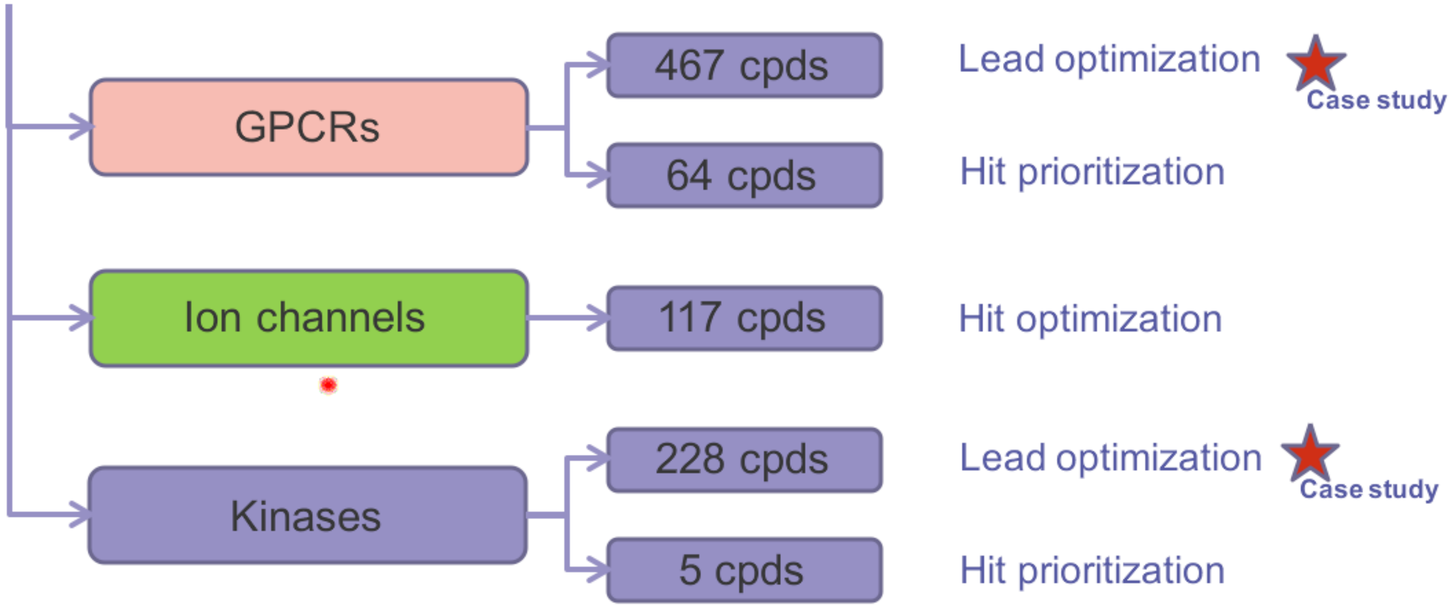
Internal software tool

- In-House software tool to calculate the relevant parameters for the “ Cardioscore”
  - Visualization of all time points at once
  - Combination of raw data, calculated data andfluorescence traces



Cardio-SAR support

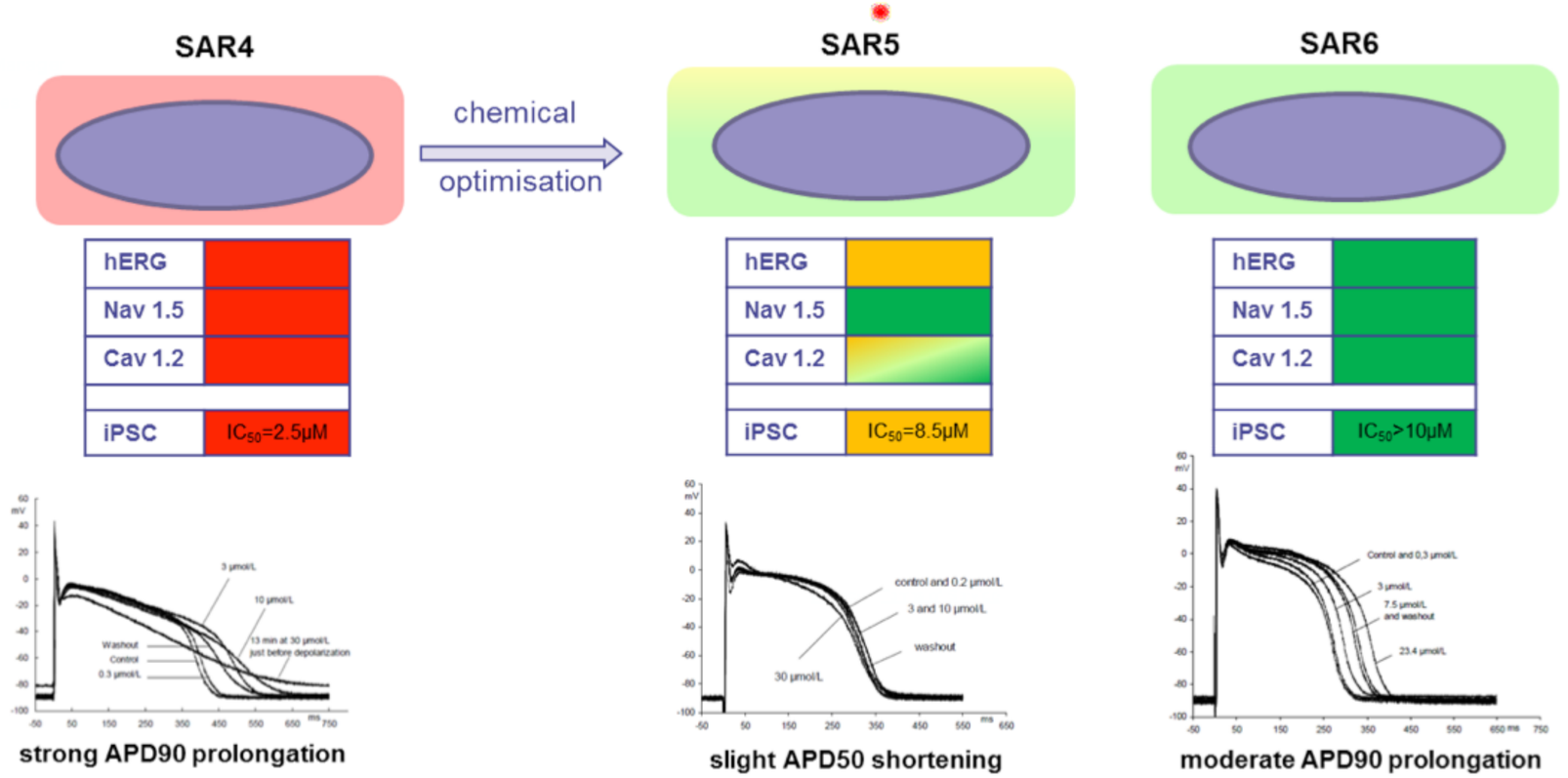
- Project support
  - 2 GPCRs, 2 Kinase, 1 Ion channel - till now, more coming



  - 881 compounds were tested in single dose @10 µM
  - Used as series prioritization, SAR optimization tool and filter for Purkinje fiber experiments

Case study I: "Cardiotox" measurements – SAR for Kinase

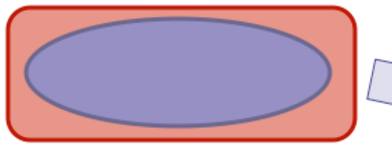
- Good correlation between ion channels activity and iPSC
- For SAR6, better correlation between ion channels and iPSC than FIP



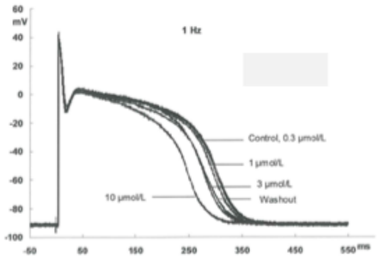
Case study II: "Cardiotox" measurements generate comprehensible SAR and guide selection of GPCR agonist with reduced AP impact

- **Objective:** Identify compounds early in the chemical optimization to reduce "cardiotox" risk.
  - Lead compound SAR1 showed undesiredeffect in Purkinje fiber study (APD_{50} shortening)

SAR1



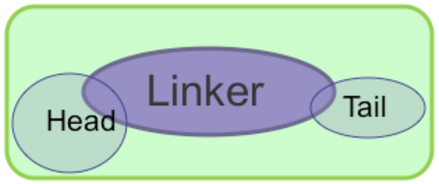
Add Polarity



AP shortening in Purkinje fiber (10 µM)

Linker region

Linker1 | Linker2



RA2

Tail Groups

Tail1

Tail2

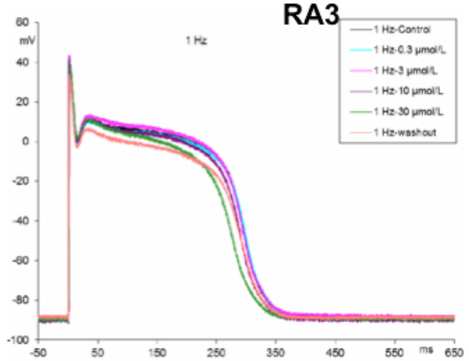
Heat map Cardioscore

EC_{50} >10 µM
EC_{50} ~10 µM
EC_{50} <10 µM

Head Groups

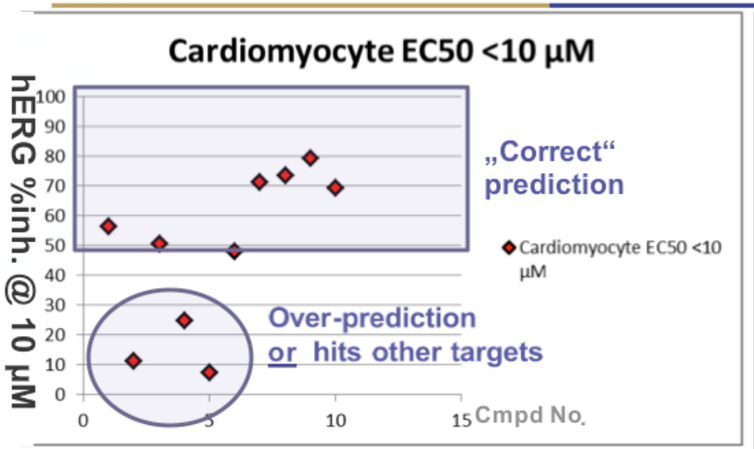
- Acids, high polarity
- Weak Bases
- Bases, lipophilicity

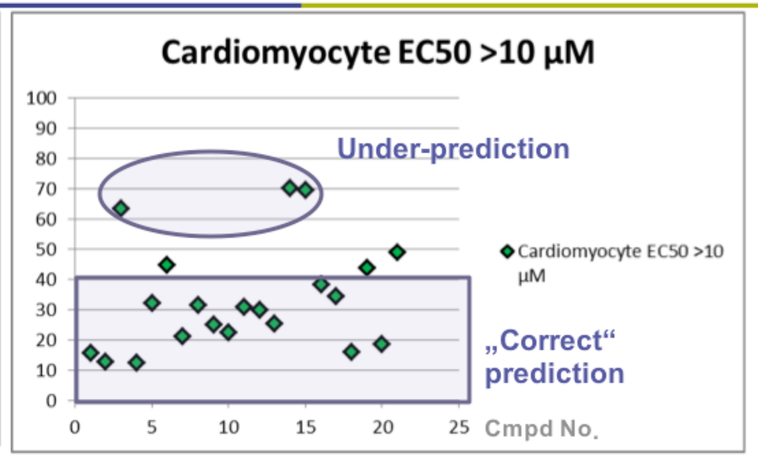
RA3

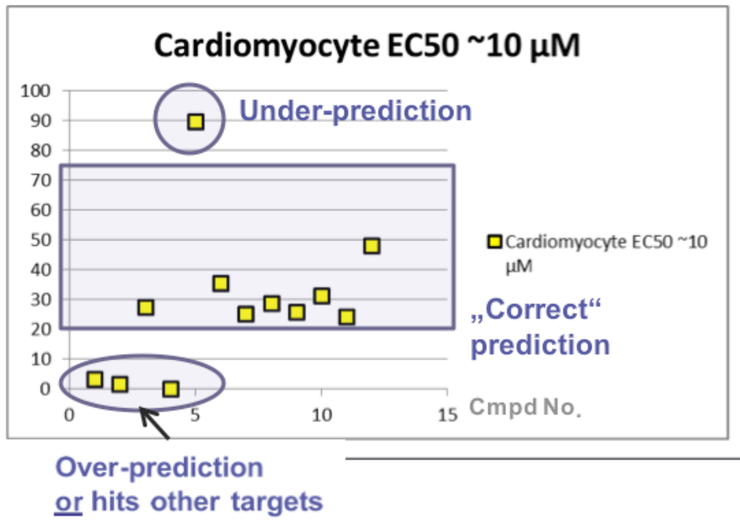


- **No marked AP impact (30 µM)**
- **Pre-candidate nomination planned for February 2016**

Case study II: Cardiomyocyte EC50 vs. hERG-Inhibition (patch clamp) Analysis for 43 GPCR agonists



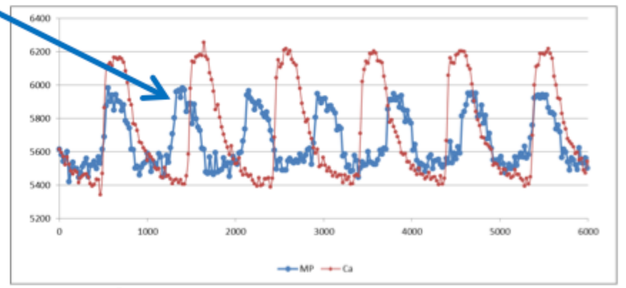




- Reasonable correlation of cardiomyocyte EC50 with % hERG-inhibition
- Only 4/43 hERG-blockers under-predicted
- Some „over-prediction“ may indicate response to non-hERG affinities, which would add value to the assay

Summary and Next Steps

- Summary of results
  - Measurement of the Ca^{2+}-oscillations of spontaneously beating hiPS-derived cardiomyocytes in 384 format with « standard » fluorescence imaging reader.
  - Identification of Na^{+}-, Ca^{+}-, hERG and mixed channel blockers
  - Single dose data for more than 850 compounds available
  - Good correlation with Purkinje experiments and hERG testing
  - Establishment of ”cardiomyocyte” SAR
- Plans for the next 6 months
  - Further support projects with cardiomyocyte SAR
  - Development of a fluorecence-based membrane potential assay as an “action potential”-readout
  - Extend knowledge of the MoA of tool compounds
    - Evaluate effects of a set of 48 “CIPA” compounds
- Critical issues and general points
  - Pharmacological QC in house is a must
  - Stem cell assays are cost intensive: miniaturization very important



Thank you!

Axiogenesis AG
Nattermannallee 1
50829 Cologne
Germany

www.axiogenesis.com
info@axiogenesis.com
order@axiogenesis.com

tel: +49 221 99 88 18 - 0
fax: +49 221 9988 18 -10