From Field Potential Waves to Ionic Current Knowledge by Leveraging Mathematical Modeling

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Objectives

- The combined use of :
  - microelectrode arrays (MEAs)
  - induced pluripotent stem cells cardiomyocytes (hiPSC-CMs)

  allows high-throughput screening on human-derived cells.
- But the field potential (FP) signals acquired by MEAs are difficult to analyze
- Objectives:
  - **Direct problem**: propose a mathematical model of MEA signals
  - **Inverse problem**: use this model to identify channel activities
  - **Classification**: use a hybrid modeling/machine learning approach to classify compounds

Direct problem: method

- Bidomain equations:

$$\begin{cases} A_{\mathrm{m}} C_{\mathrm{m}} \dfrac{\partial V_{\mathrm{m}}}{\partial t} + A_{\mathrm{m}} I(V_{\mathrm{m}}, \boldsymbol{g}) - \mathrm{div}(\boldsymbol{\sigma}_{\mathrm{i}} \boldsymbol{\nabla} u_{\mathrm{i}}) = A_{\mathrm{m}} I_a \\ \mathrm{div}(\boldsymbol{\sigma}_{\mathrm{e}} \boldsymbol{\nabla} u_{\mathrm{e}}) + \mathrm{div}(\boldsymbol{\sigma}_{\mathrm{i}} \boldsymbol{\nabla} u_{\mathrm{i}}) = 0 \\ \dfrac{\partial \boldsymbol{g}}{\partial t} + G(V_{\mathrm{m}}, \boldsymbol{g}) = 0 \end{cases}$$

  V_{m}: transmembrane potential
  u_{e}: extracellular potential
  $\boldsymbol{g}$: gating variables and ionic concentrations
  Fiel potential: obtained from u_{e} (electrode model)
  G and I: ionic model

- Ionic models:
  - Stem cells (Paci *et al.* Annals biomed Engng 2013)
  - Minimal Ventricular (MV) (Bueno-Orovio *et al.*, J Theo Bio 2008)
  - O'Hara-Rudy (O'Hara *et al.*, PLOS Comp Biol 2011)

- Population of cells can be homogeneous or with different phenotypes. In the latter case, several arbitrary configurations are averaged.

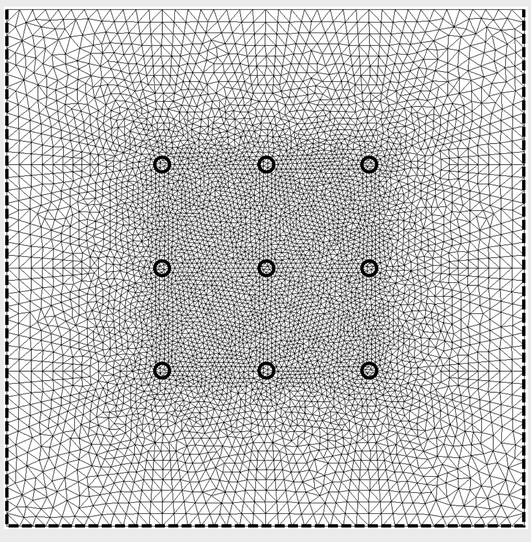
- Electrode model:

  I_{el}^k is the electric current measured by the k^{th} electrode

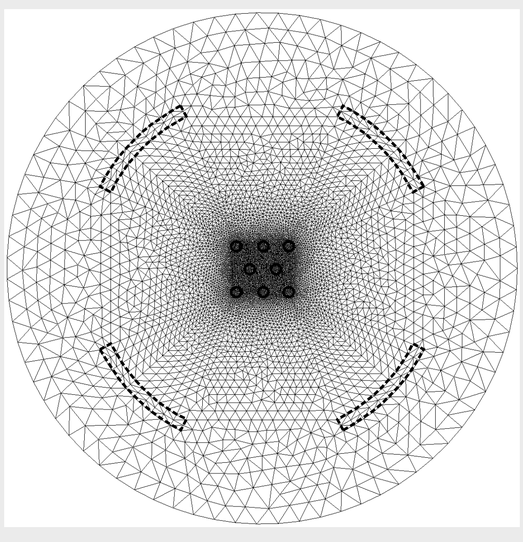
$$\frac{dI_{el}^k}{dt} + \frac{I_{el}^k}{\tau} = \frac{C_{el}}{\tau}\frac{dU^k}{dt} \qquad \text{with } U^k = \frac{1}{|e_k|}\int_{e_k} u_e,\ \boldsymbol{\sigma}_e \nabla u_e \cdot \mathbf{n} = \frac{I_{el}^k}{|e_k|}$$

  where $\tau = (R_i + R_{el})C_{el}$
  R_{el} and C_{el}: resistance and capacitance of the electrode
  R_i: inner resistance

- Devices



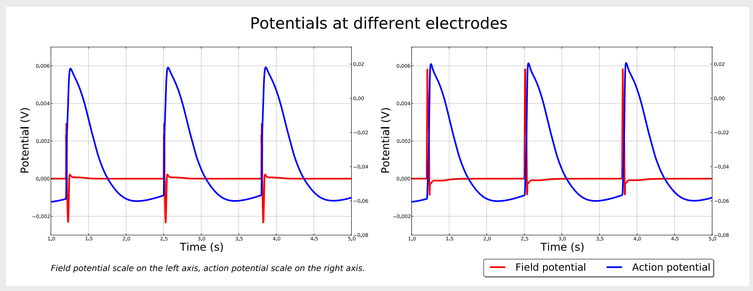
6-well of 9 electrodes (MSC)



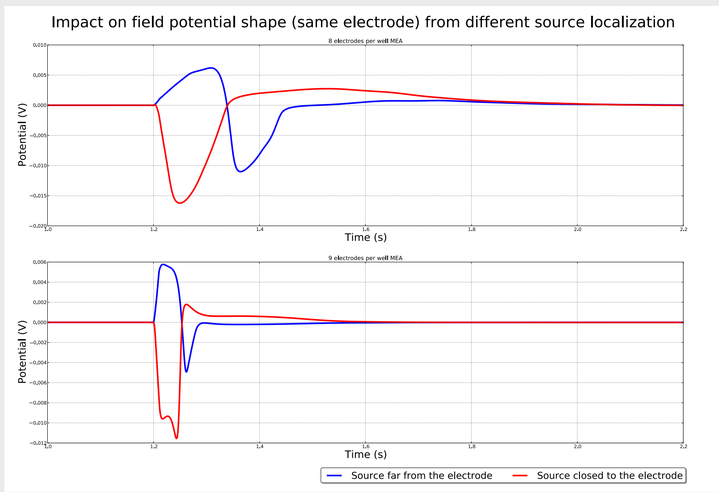
96-well of 8 electrodes (Axion)

Direct problem: results

- **Example 1: potential variability with homogeneous cells.**
  Variations of amplitude and orientation of FP are linked to:
  - MEA size, interelectrode distance, position of the ground
  - relative position of the initial stimulation

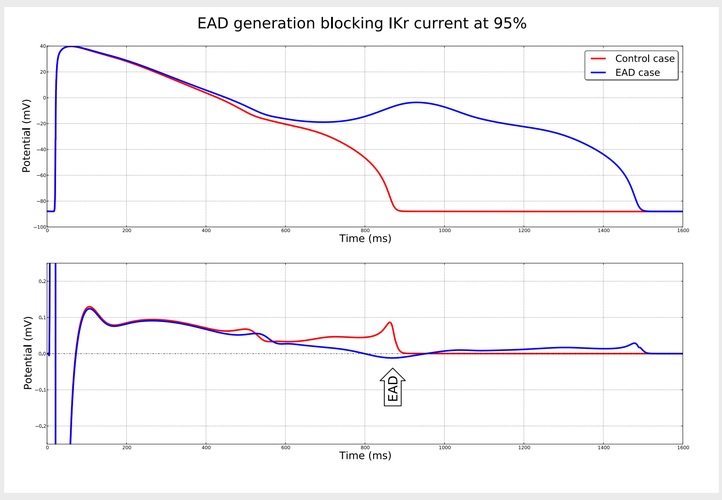


➡ same action potential, but different field potentials on different electrodes.



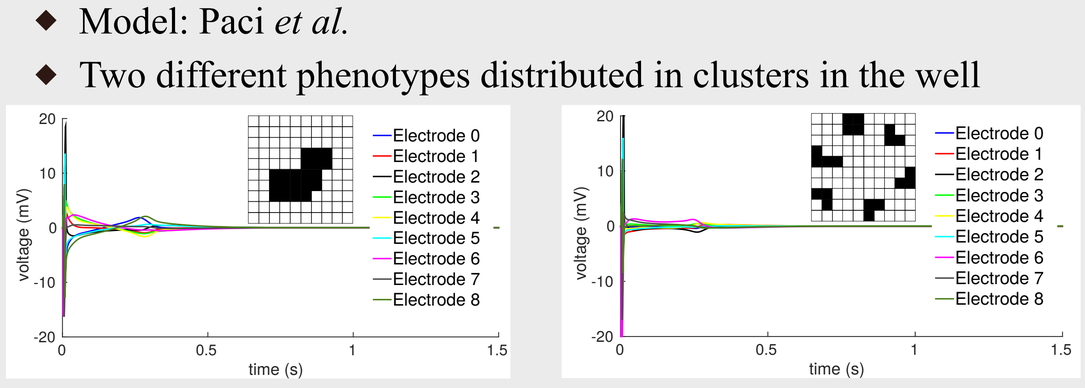
➡ different field potentials for different locations of the initial activation.

- **Example 2: an Early After Depolarization simulation.**
  - Model: O'Hara-Rudy

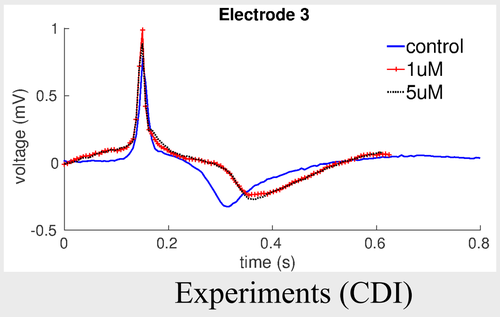


➡ good agreement with experimental results (K. Asakura *et al.* JPTM 2015)

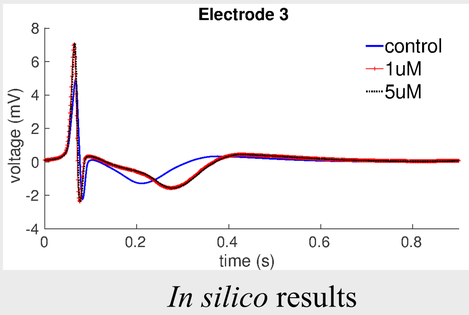
- Example 3: heterogeneous configurations of cells
  - Model: Paci *et al.*
  - Two different phenotypes distributed in clusters in the well



➡ different signals with different heterogeneous cell populations



Experiments (CDI)

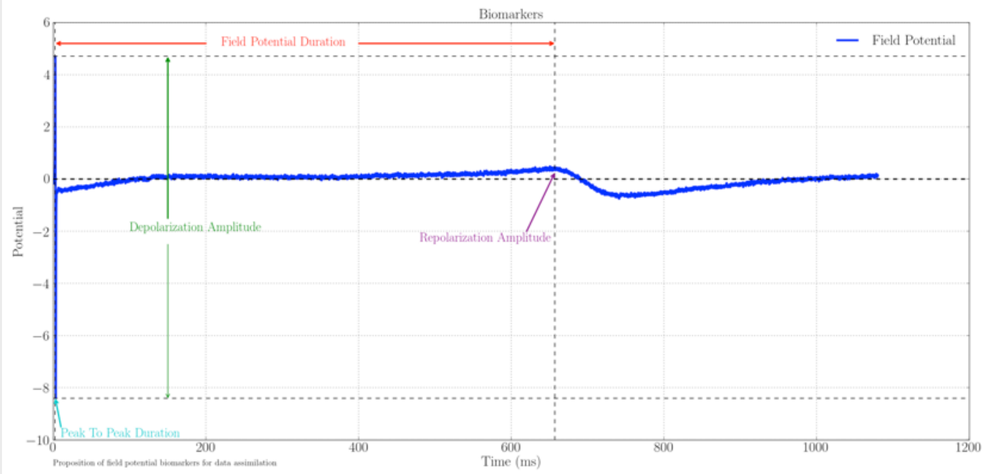


In silico results

➡ Effect of BayK, with heterogeneous cells

Inverse problem: method

- **Goal**: from measured FP signals, identify the channel conductances.
- Extraction of 3 biomarkers from **real signals** with NOTOCORD-fps: depolarization amplitude (DA), repolarization amplitude (RA) and field potential duration (FPD).



- Identification of channel conductance by minimizing the cost function:

$$J(\theta) = \sum_{k=1}^{N_{elec}} \left(\frac{\mathrm{DA}^k_{\mathrm{meas}}}{\mathrm{DA}^k_{\mathrm{c,meas}}} - \frac{\mathrm{DA}^k(\theta)}{\mathrm{DA}^k_{\mathrm{c}}} \right)^2 + \left(\frac{\mathrm{RA}^k_{\mathrm{meas}}}{\mathrm{RA}^k_{\mathrm{c,meas}}} - \frac{\mathrm{RA}^k(\theta)}{\mathrm{RA}^k_{\mathrm{c}}} \right)^2 + \left(\frac{\mathrm{FPD}^k_{\mathrm{meas}}}{\mathrm{FPD}^k_{\mathrm{c,meas}}} - \frac{\mathrm{FPD}^k(\theta)}{\mathrm{FPD}^k_{\mathrm{c}}} \right)^2$$

X^k_{meas} : experimental biomarker at electrode k (with compound)

$X^k(\theta)$: *in silico* biomarker at electrode k with channel conductances θ

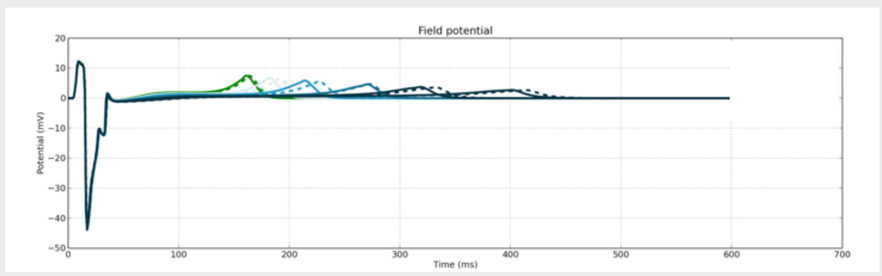
$X^k_{c,meas}$: control experimental biomarker at electrode k (without compound)

X^k_c : control *in silico* biomarker at electrode k

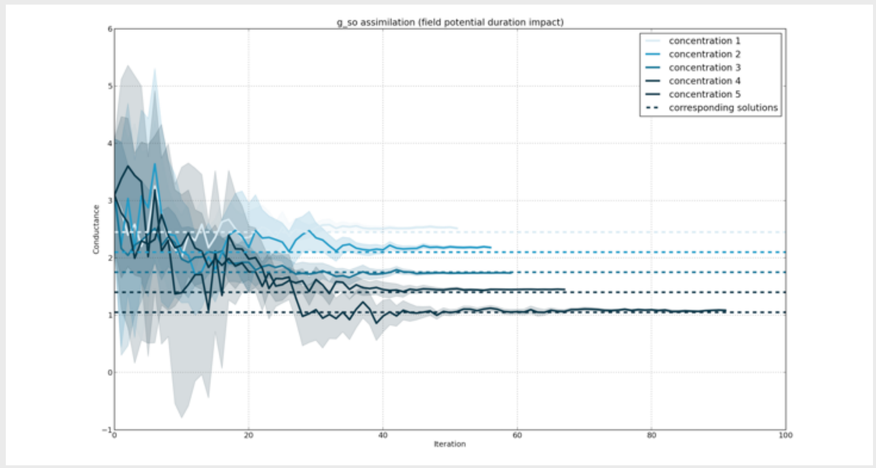
with X = DA, RA or FPD

Inverse problem: results

- **Example 1:** verification with synthetic data
  (i.e. data generated by the model + noise)
  - Model: MV.
  - Data: 1 control and 5 levels of inhibition of the outward currents channel.
  - Goal: Identification of the conductance of the outward currents.



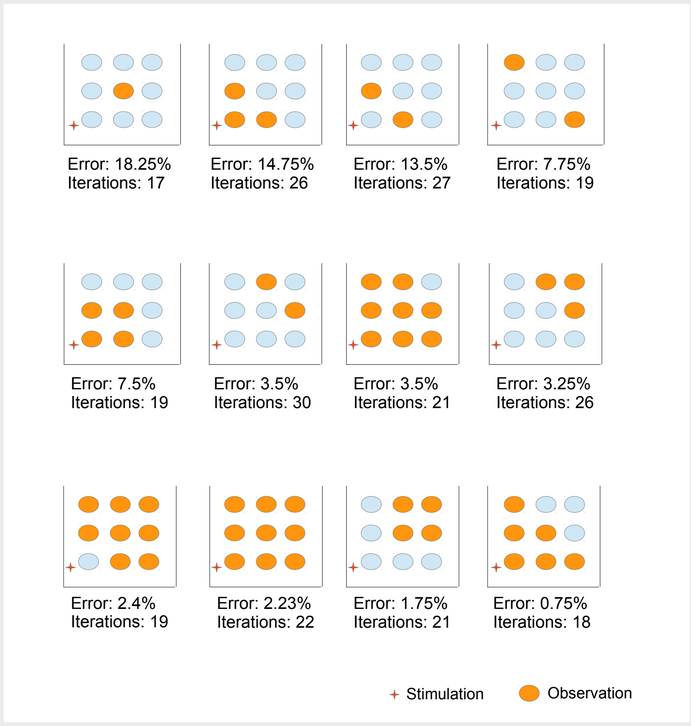
Dotted lines: synthetic data - Continuous lines: result of the identification



Identification of the values of the conductances

➡ the correct values of the conductances are recovered

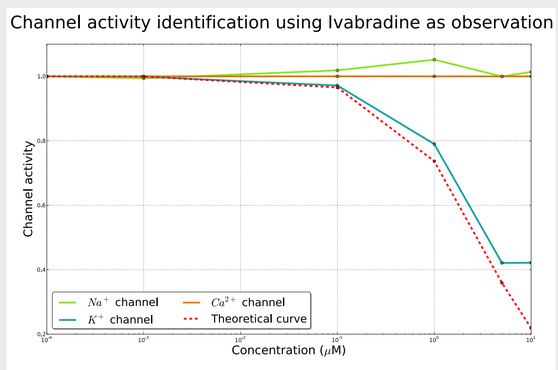
- **Example 2:** Is it worth keeping all the electrodes?
  - Model: MV.
  - Data: synthetic.
  - Identification of the conductance of the fast inward currents.
  - By observing 1, 2, 3, 4, 6, 8 or 9 electrodes.



➡ the error on the identification of the conductance depends on the relative positions of the electrodes and of the initial activation.
➡ it is worth keeping all the electrodes.

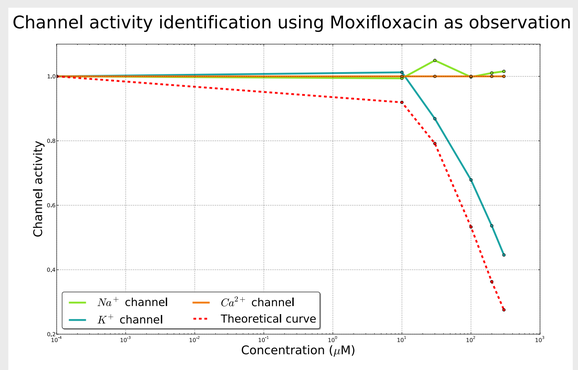
- **IC50 estimation of real data with the inverse problem approach**
  - Model: MV.
  - Data: real experiments (CDI) processed with NOTOCORD-fps.
  - Identification of 3 channel conductances:
    fast inward, slow inward and outward currents
    (denoted "Na^+", "Ca^{2+}", "K^+" for simplicity).
  - Theoretical curves obtained with the published IC_{50} and the function:

$$f(c) = \left(1 + \frac{c}{IC_{50}}\right)^{-1}$$



Ivabradine

IC_{50} identified *in silico* $\approx 3.7\mu M$
Theoretical curve with $IC_{50}(I_{Kf}) = 2.8\mu M$
(Shattock et al.- British J Cardiology, 2006)

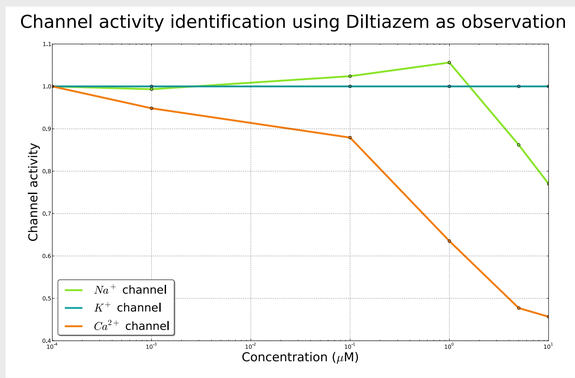


Moxifloxacin

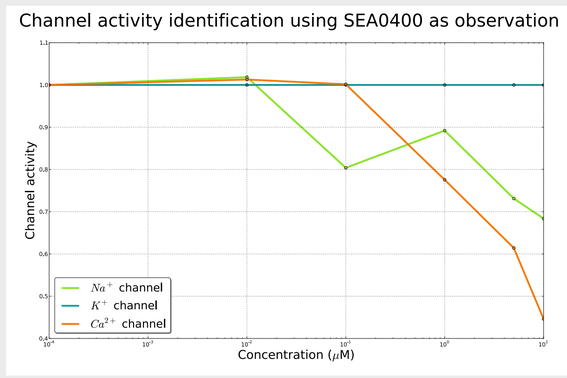
IC_{50} identified *in silico* $\approx 220\mu M$
Theoretical curve with $IC_{50}(I_{Kr}) = 114\mu M$
(Alexandrou et al., British J Pharmacol. 2006)

➡ realistic values of IC_{50} found by the inverse problem approach.

- Results with other compounds:



Diltiazem



SEA0400

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Classification: methods

- Model MV. 3 channels: fast inward, slow inward and outward currents (denoted “Na+”, “Ca2+”, “K+”for simplicity).
- Real data provided by CDI, processed with NOTOCORD-fps.
- One experiment – real or in silico – consists of 5 different sets of values of channel conductances. For each set of values, the ratio of the 3 biomarkers to a control is computed. The resulting 15 values are labelled (Na antagonist, K antagonist, Ca antagonist, Ca agonist, etc.).
- A machine learning classifier – Support Vector Machine (SVM) – is trained with real and/or in silico data.
- Then, 8 electrodes in 2 wells of a real experiment are tested, i.e. automatically classified between the different labels, with a probability.

Classification: results (preliminary)

- **Training with real experimental data**

Classification probabilities obtained with SVM using experimental data* for training

Means (8 electrodes tested per drug)

	Ivabradine	Mexiletine	Moxifloxacin	Diltiazem	JNJ303	Dofetilide	BayK**	SEA0400	Ranolazine	Nimodipine
Na antagonist	0.0	**0.98**	0.04	**0.48**	0.16	0.0	0.0	0.04	**0.38**	0.03
K antagonist	**1.0**	0.0	0.0	0.0	0.0	**1.0**	0.0	0.0	0.0	0.0
Ca antagonist	0.0	0.01	**0.66**	0.43	0.41	0.0	0.1	**0.86**	0.33	**0.92**
Ca agonist	0.0	0.01	0.3	0.08	**0.43**	0.0	**0.9**	0.1	0.29	0.05

*Ivabradine, Mexiletine, BayK and SEA0400 (as a Calcium blocker). **4 electrodes tested for the prediction.

Best probability / Expected solution

➡ Moxifloxacin, Diltiazem, JNJ303 are not correctly classified

- **Training with 4096 in silico experiments (results of simulation of a simplified model)**

Classification probabilities obtained with SVM using MV model (4K samples) for training

Means (8 electrodes tested per drug)

	Ivabradine	Mexiletine	Moxifloxacin	Diltiazem	JNJ303	Dofetilide	BayK*	SEA0400	Ranolazine	Nimodipine
Na antagonist	0.0	**0.99**	0.0	0.01	0.22	0.0	0.1	0.0	0.39	0.0
Na agonist	0.05	0.0	0.14	0.0	0.0	0.0	0.12	0.0	0.0	0.01
K antagonist	0.0	0.0	**0.8**	0.0	**0.77**	0.0	0.0	0.0	**0.61**	0.0
K agonist	0.0	0.0	0.0	0.22	0.0	0.0	**0.39**	0.42	0.0	**0.68**
Ca antagonist	0.0	0.0	0.01	**0.77**	0.0	0.0	0.0	**0.58**	0.0	0.31
Ca agonist	**0.95**	0.0	0.05	0.0	0.01	**1.0**	**0.39**	0.0	0.0	0.0

*4 electrodes tested for the prediction.

Best probability / Expected solution

➡ Ivabradine, Dofetilide, Ranolazine, Nimodipine are not correctly classified

- **Training with the real experimental data and the 4096 in silico experiments**

Classification probabilities obtained with SVM
using MV model (4K samples) and experimental data* for training

Means (8 electrodes tested per drug)

	Ivabradine	Mexiletine	Moxifloxacin	Diltiazem	JNJ303	Dofetilide	BayK**	SEA0400	Ranolazine	Nimodipine
Na antagonist	0.0	**0.96**	0.0	0.0	0.19	0.0	0.01	0.0	**0.51**	0.01
Na agonist	0.0	0.0	0.16	0.0	0.0	0.0	0.0	0.0	0.0	0.0
K antagonist	**0.83**	0.0	**0.6**	0.0	**0.73**	**0.98**	0.0	0.0	0.48	0.0
K agonist	0.0	0.0	0.0	0.12	0.0	0.0	0.0	0.45	0.0	0.48
Ca antagonist	0.0	0.03	0.05	**0.87**	0.01	0.01	0.0	**0.54**	0.01	**0.51**
Ca agonist	0.17	0.0	0.19	0.0	0.06	0.01	**0.99**	0.0	0.0	0.0

*Ivabradine, BayK and SEA0400 (as a Calcium blocker). **4 electrodes tested for the prediction.

Best probability / Expected solution

➡ By mixing real and *in silico* data for the training, all the compounds are correctly classified

Conclusion

- We proposed a mathematical model of MEA (direct and inverse problems) and we trained a machine learning algorithm with real and in silico signals.
  - Direct problem: allow to reproduce *in silico* observed phenomena and better understand some features of the signals (variability, …).
  - Inverse problem: identify the channel activity from synthetic and real signals, determine IC_{50}.
  - Promising preliminary results to characterize the impact of compounds on ionic channel activity.
- Limitations and future work
  - The cell model used for the inverse problem should be replaced by a more comprehensive one.
  - To improve inverse problems and classification, additional biomarkers should be identified on the FP.
  - Machine learning algorithm has to be trained with more experimental and synthetic signals.
  - The overall methodology has to be tested with more compounds.

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