Cardiac Research Activities at Cellular and Molecular Engineering Laboratory (CMEL)

Department of Electronics, Computer Science and Systems
University of Bologna, Cesena, Italy

Associated to the Italian National Institute for Cardiovascular Research

Ing. Stefano Severi

Manchester, November 11-12, 2005

Outline

1. Research group at CMEL

2. Past research activities on cardiovascular modelling
   - System modelling
     - Hemodynamics
     - Baroreflex regulation and heart rate variability genesis
     - Cardiovascular response to hemodialysis-induced hypovolemia
   - Single Cell simulations
     - Sinoatrial node (application to hemodialysis)
   - Single Channel modelling
     - Deterministic Model of Ion Channel Flipping

3. Present (in progress...) activities

- Computational
  - o Single Cell simulations
    - ✓ Ventricular cells (application to inherited diseases)
  - o Ion current modelling
    - ✓ Markov model of Na current
  - o Single Channel modelling
    - ✓ Molecular dynamics and electrodiffusion modelling of K channels

- Experimental
  - o Gene expression studies
  - o Expression studies in eterologous cell lines
  - o Whole cell electrophysiology in cardiomyocites
  - o Single channel recording in artificial bylayer
  - o Cellular and molecular imaging

3. Examples of research projects

- Modulation of ventricular AP by hemodialysis-induced NO overload
- Macrolides effects on CFTR and repolarization

Research group at CMEL

Prof. Silvio Cavalcanti	Biomedical engineering
Prof. Marco Tartagni	Microelectronics, Biosensors
Dr. Emanuele Giordano	Biochemistry, Molecular biology
Dr. Paolo Iorio	Physician
Ing. Stefano Severi	Biomedical engineering
Ing. Eleonora Grandi	Biomedical engineering
Ing. Simone Furini	Biomedical engineering
Ing. Francesco Lodesani	Electronic engineering
Ing. Andrea Ciandrini	Biomedical engineering
Dr. Marco Govoni	Biochemistry, Molecular biology

Research group at CMEL

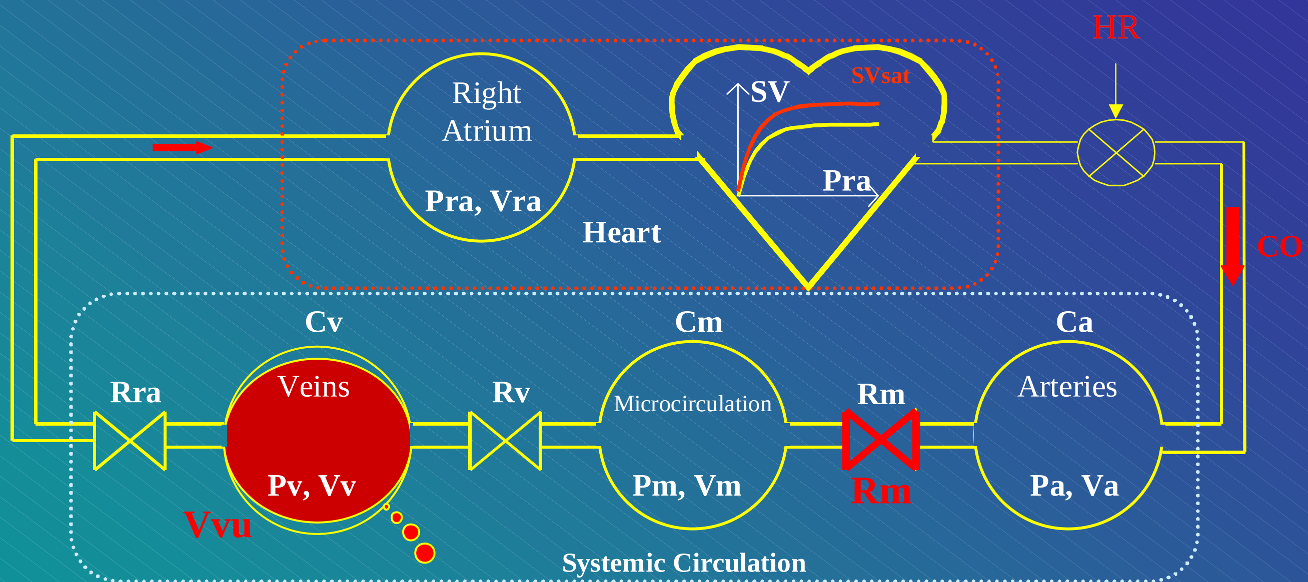
Past: System modelling

- Hemodynamics
  - Cavalcanti S Hemodynamics of an artery with mild stenosis. *J Biomech*. 1995
  - Cavalcanti S et al Analysis by mathematical model of haemodynamic data in the failing Fontan circulation. *Physiol Meas*. 2001
  - Tura A and Cavalcanti S Numerical simulation of flow oscillations in stenotic arterial segment.*Comput Biol Med*. 2001

- Baroreflex regulation and heart rate variability genesis
  - Cavalcanti S and Belardinelli E: Modeling of cardiovascular variability using a differential delay equation. *IEEE Trans Biomed Eng*. 1996
  - Cavalcanti S Arterial baroreflex influence on heart rate variability: a mathematical model-based analysis. *Med Biol Eng Comput*. 2000

Past: System modelling

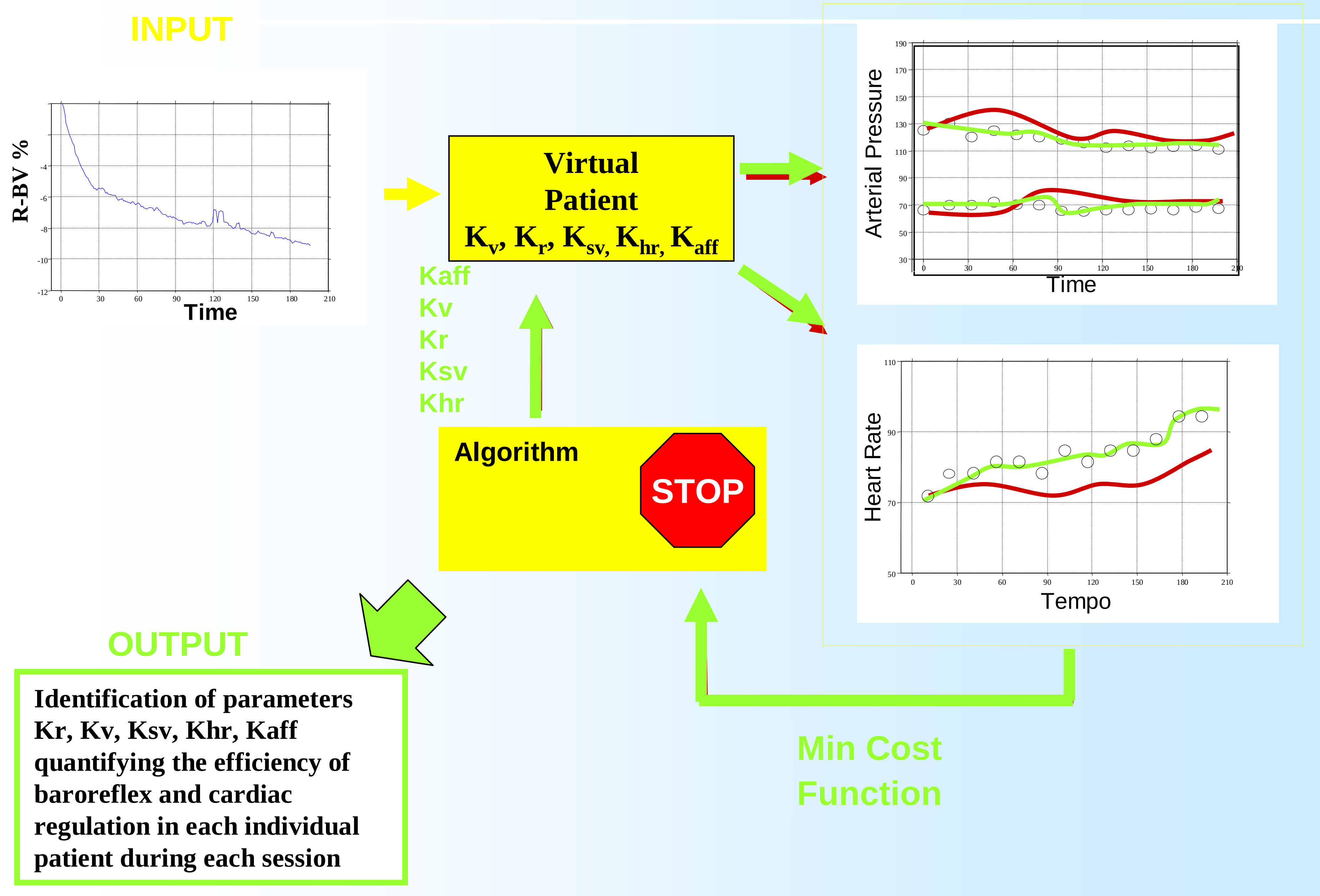
- Cardiovascular response to hemodialysis-induced hypovolemia



Compensatory Mechanisms to the blood volume reduction implemented in DialSim:

1. Increase of vascular resistance (**Rm**)
2. Decrease of venous capacity (**Vvu**)
3. Increase of stroke volume (**SVsat**)
4. Increase of heart rate (**HR**)

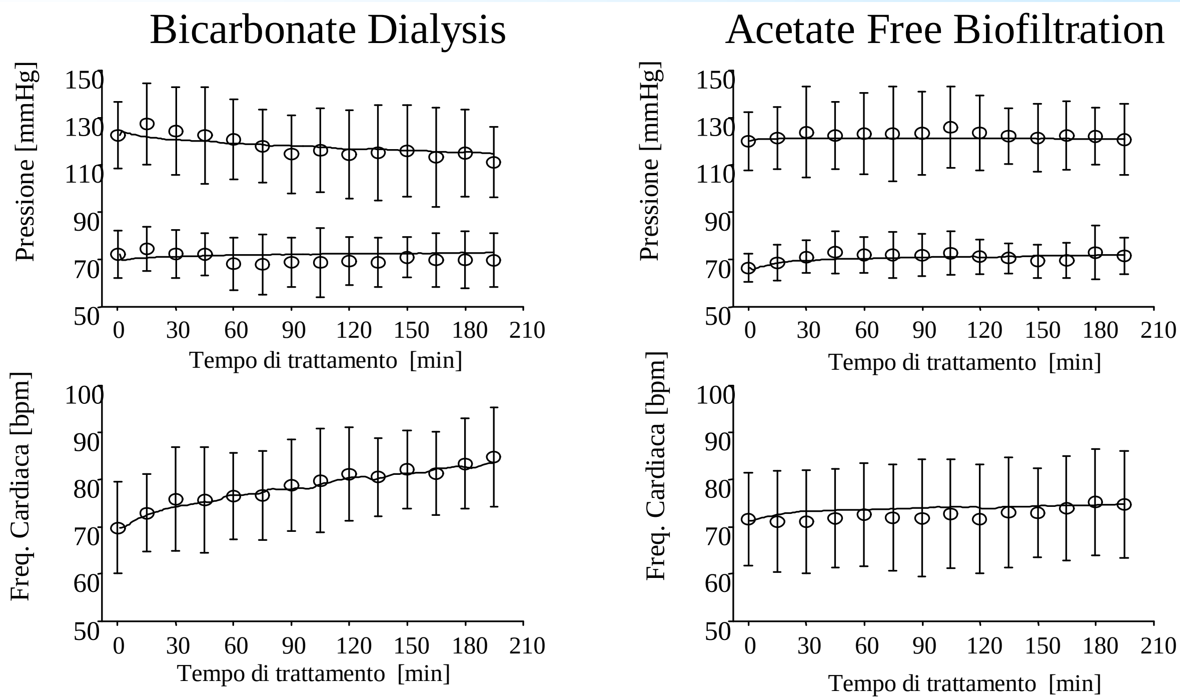
DEIS Università di Bologna



Cavalcanti et al. Role of short-term regulatory mechanisms on pressure response to hemodialysis-induced hypovolemia *Kidney Int*. 2002

DEIS Bologna University

Application: assessment of the differential cardiovascular impact of different hemodialysis techniques



Kr = 0.24 Ksv = 0.21

Kr = 0.90 Ksv = 0.73

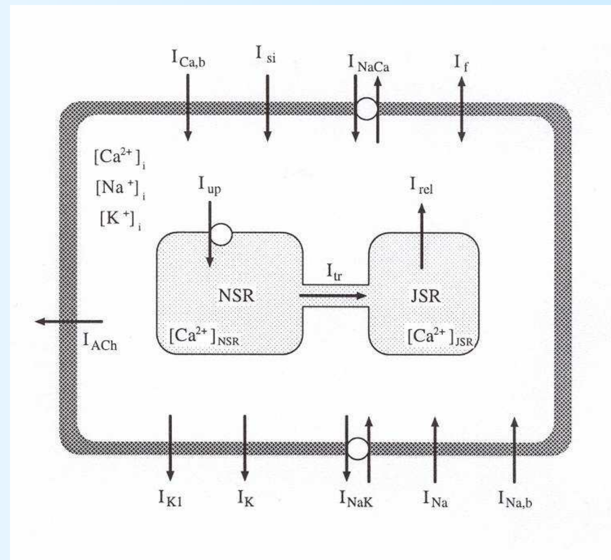
S Cavalcanti et al. Model-based study of the effects of the hemodialysis technique on the compensatory response to hypovolemia. *Kidney International*, 2004

A Ciandrini et al. Effects of dialysis technique on the acute hypotension: a model-based study. *Cardiovascular Engineering*, 2004.

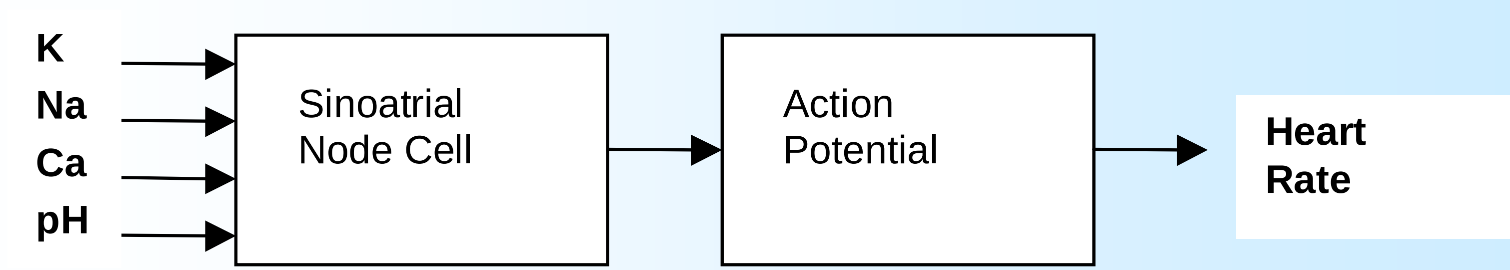
Past: Single Cell simulations

Sinoatrial node (application to hemodialysis)

• Di Francesco-Noble model



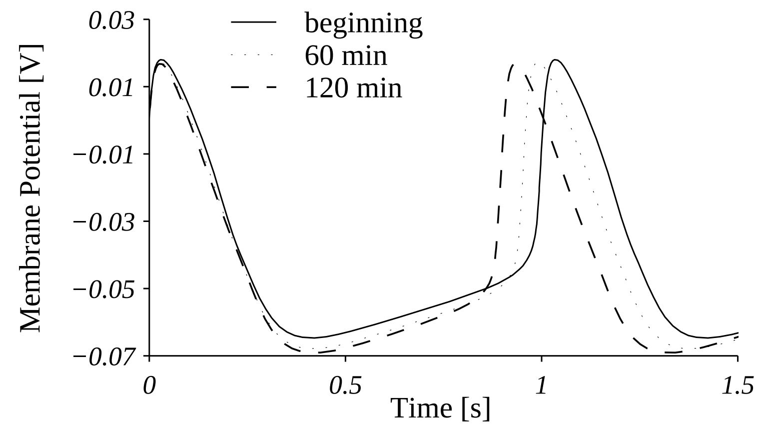
• Time-varying input (recorded during hemodialysis sessions)



Past: Single Cell simulations

Sinoatrial node (application to hemodialysis)

- Effects of hemodialysis-induced changes in electrolytes on sinusal heart rate



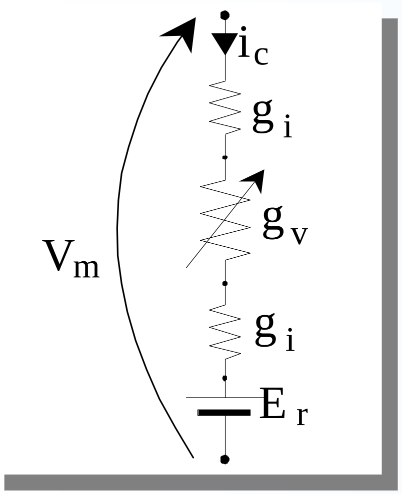
Severi S and Cavalcanti S: Electrolyte and pH dependence of heart rate during hemodialysis: a computer model analysis, *Artificial Organs*, 2000.

Severi S et al: Heart rate response to hemodialysis-induced changes in potassium and calcium levels , *Journal of Nephrology*, 2001.

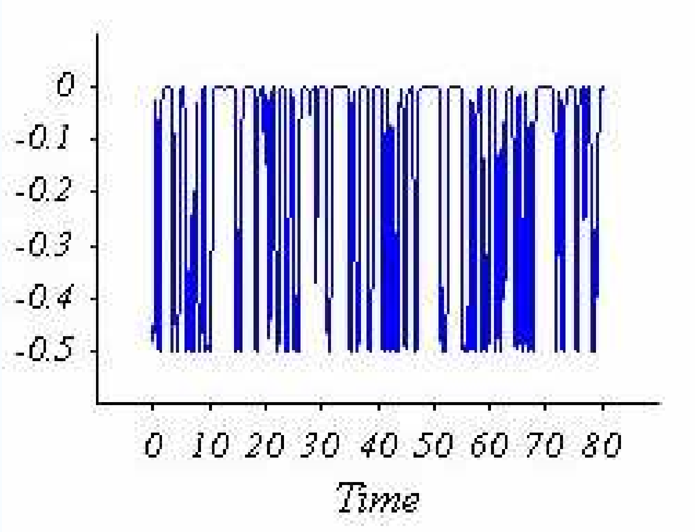
Severi S et al: Effect of electrolyte and pH changes on the sinus node pacemaking in humans, *Journal of Electrocardiology*, 2002.

Past: Single Channel modelling

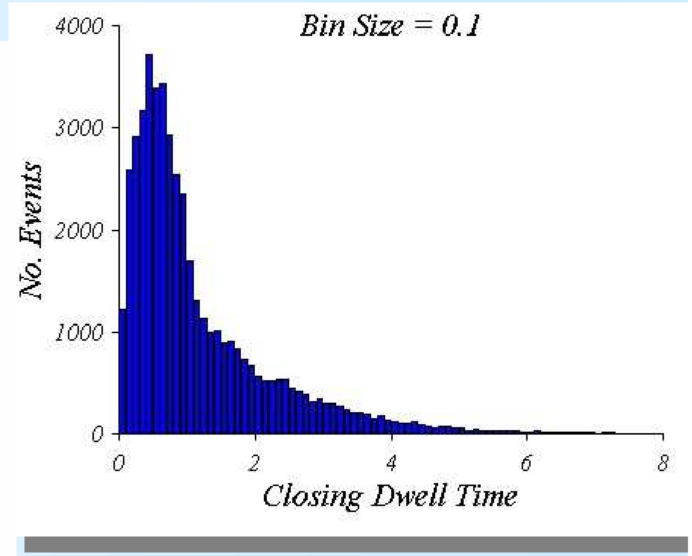
Deterministic Model of Ion Channel Flipping



Lumped parameters model



Simulated channel flipping



Dwell time distribution

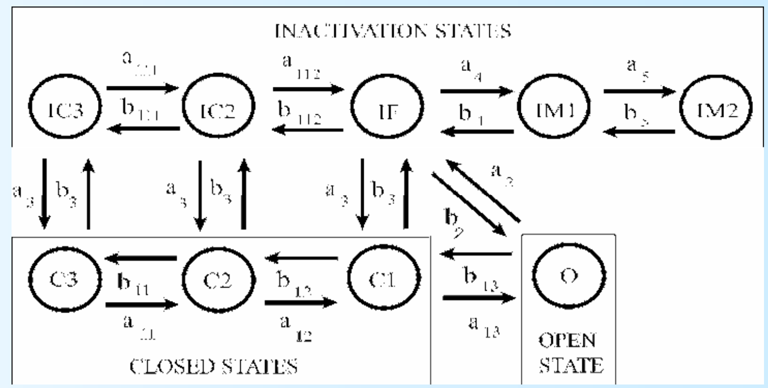
Cavalcanti S and Fontanazzi F: Deterministic model of ion channel flipping with fractal scaling of kinetic rates. *Ann Biomed Eng*. 1999

Present and Future

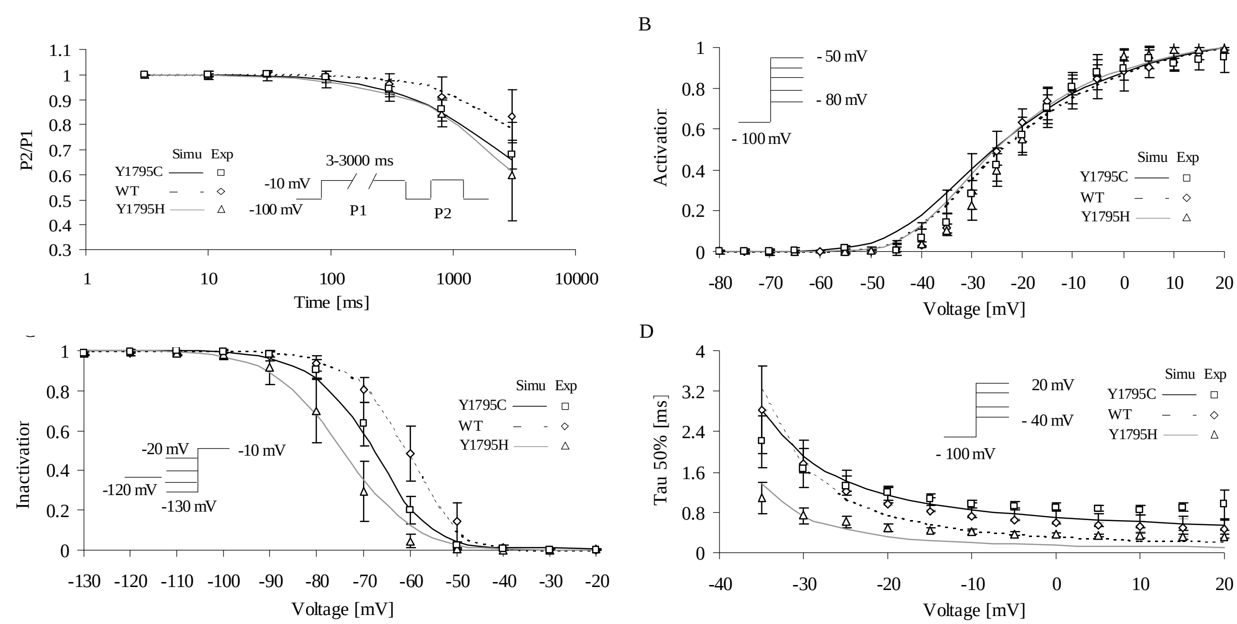
Computational: Ion current modelling

Markov model of WT and mutant Na currents

- Nine states model (mostly based on Clancy-Rudy model)



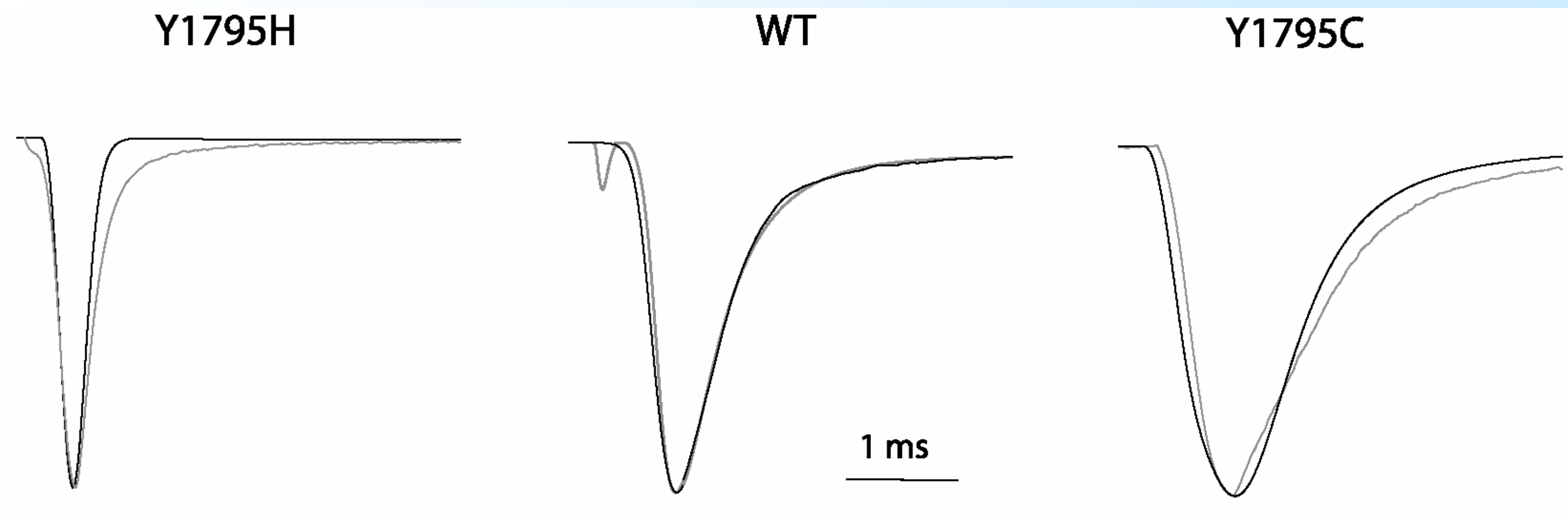
- Identification on voltage clamp measurements on wild type and mutant channels expressed in eterologous cells



Computational: Ion current modelling

Markov model of Na current

• Validation on Action Potential clamp experiments performed at the Molecular Cardiology Laboratory, Fondazione Salvatore Maugeri, Pavia – Italy

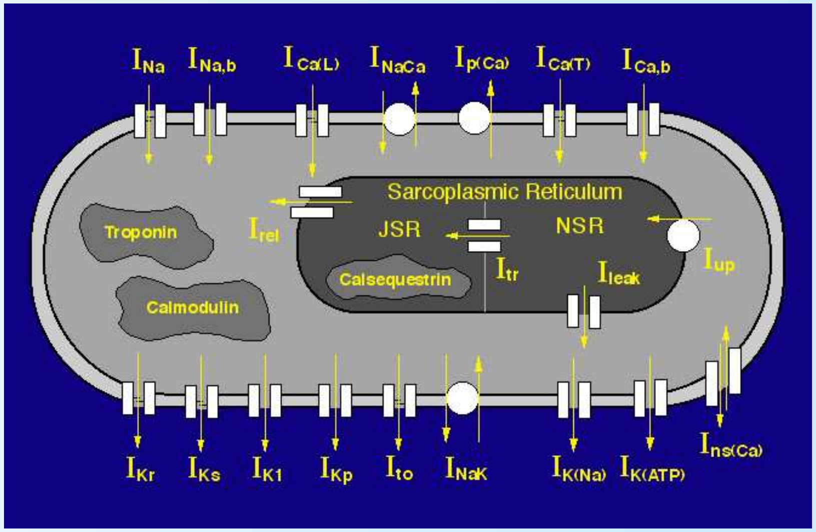


S Vecchietti, I Rivolta, S Severi, C Napolitano, SG Priori, S Cavalcanti. Computer Simulation of the Na+ Current in Wild-Type and Mutant Human Na+ Cardiac Channels. Under revision in *Med Biol Eng Comput*

Computational: Single Cell simulations

In silico assessment of SCN5A mutations causing Long QT and Brugada syndromes

- LRd model
- Markov model of Na current
- Matlab Simulink as simulation envirinment



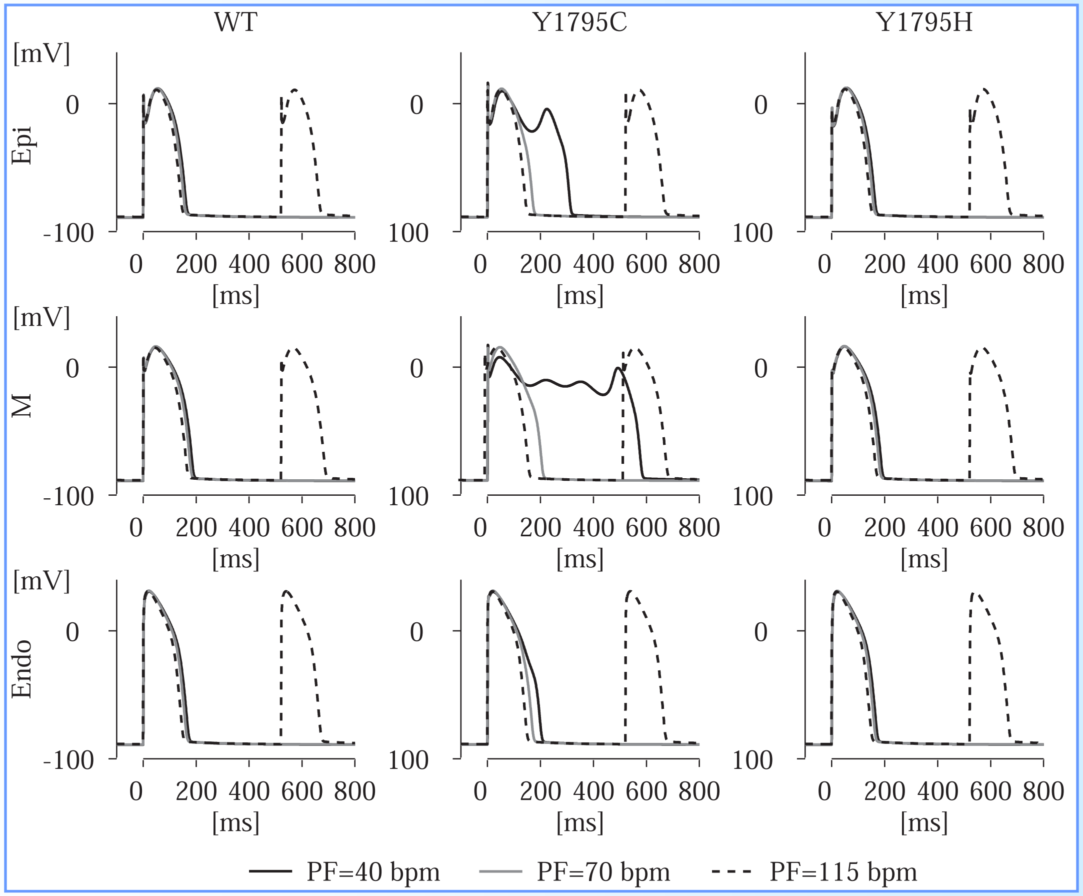
Faber GM and Rudy Y *Biophys J* 2000

Cooperation with Molecular Cardiology Laboratory, Fondazione Salvatore Maugeri, Pavia – Italy

Funded by Italian Health Ministry

Computational: Single Cell simulations

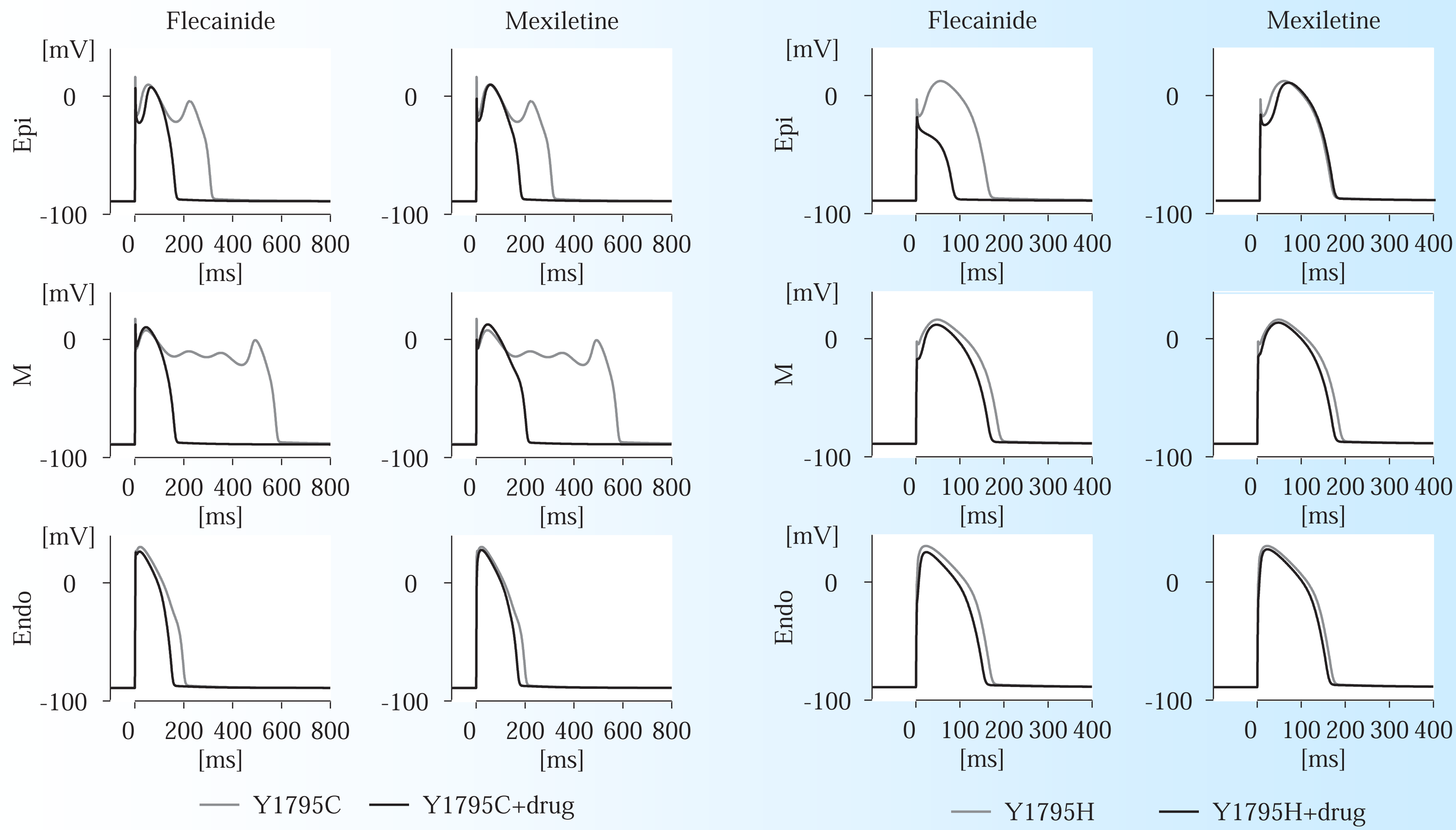
Analysis of the effects of mutations on AP



S Vecchietti, E Grandi, S Severi, I Rivolta, C Napolitano, SG Priori, S Cavalcanti In silico assessment of SCN5A mutations. Implication for inherited arrhythmogenic syndromes. Submitted to *Circulation Research*

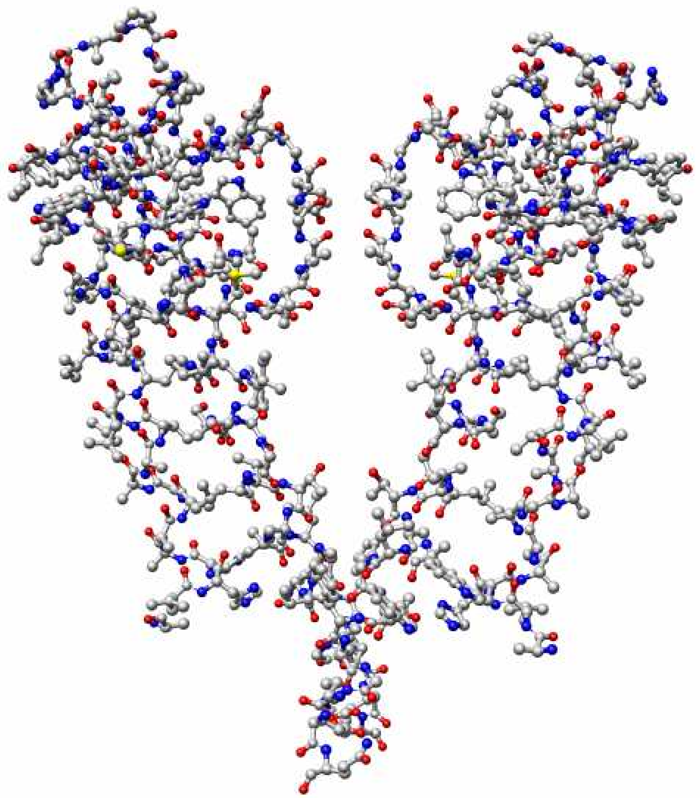
Computational: Single Cell simulations

Analysis of the effects of antiarrhythmic drugs



S Vecchietti, E Grandi, S Severi, I Rivolta, C Napolitano, SG Priori, S Cavalcanti In silico assessment of SCN5A mutations. Implication for inherited arrhythmogenic syndromes. Submitted to *Circulation Research*

Computational: Single channel modeling



KcsA Potassium Channel

Thanks to x-ray crystallographic experimental data it is now possible to develop channel models based on the protein atomic structure

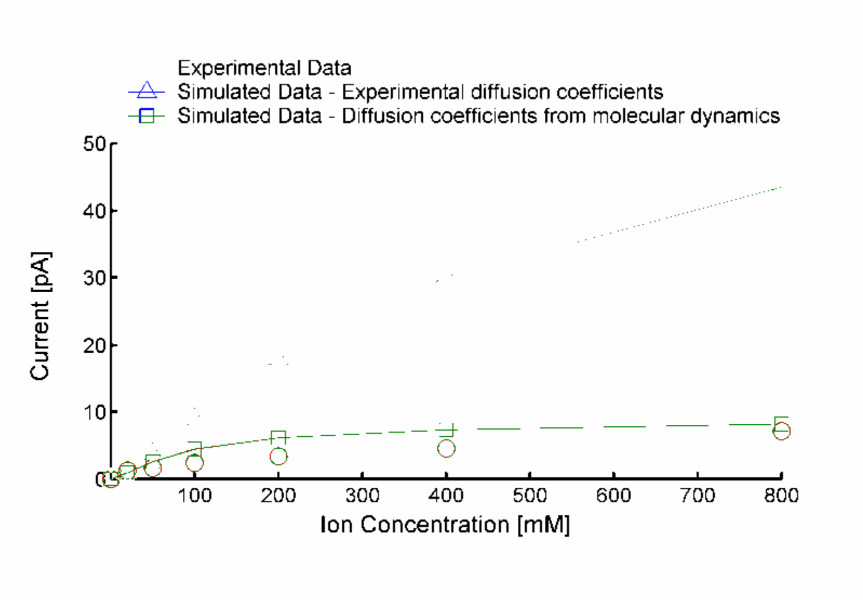
The high number of atoms limits the temporal scale of Molecular Dynamic simulations

To compute channel currents a simplified mathematical model of ion conduction is needed → Poisson-Nernst-Planck Theory

Computational: Single channel modeling

Continuum – Stationary theory to model ion conduction

- Numerical algorithm to solve the PNP differential equation set in 3D with space dependent diffusion coefficients has been developped
- Molecular Dynamic simulations were performed to compute diffusion coefficients inside the channel



- The model will be applied to the analysis of the effects of mutations on channel currents

Furini S et al: Application of the Poisson-Nerst-Plank theory with space-dependent Diffusion coefficients to the KcsA potassium channel. Submitted to *Biophysical Journal*

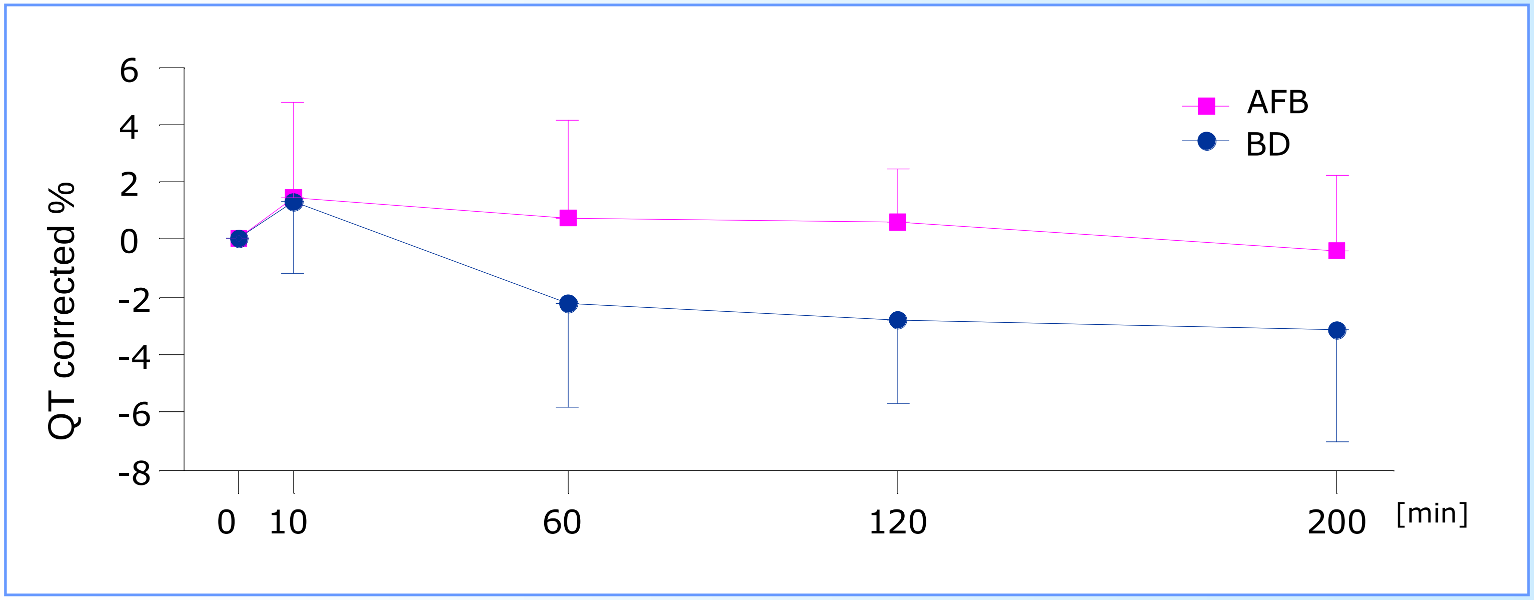
Research Projects integrating computational and experimental approaches:

- Modulation of ventricular AP by hemodialysis-induced NO overload

- Macrolides effects on CFTR and repolarization

Modulation of ventricular AP by hemodialysis-induced NO overload

- Macroscopic clinical observation: Different trend of QT interval durations in patients when treated with different hemodialysis technique



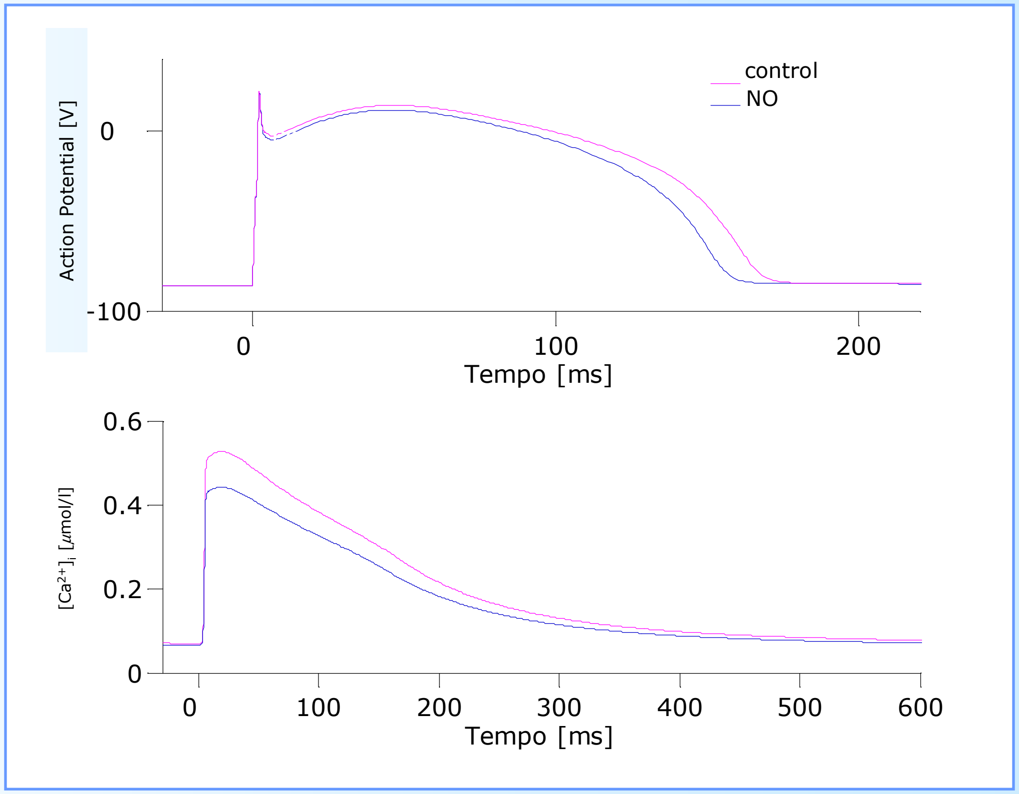
- HP: dif|ferences at cellular level because of different NO overload

Modulation of ventricular AP by hemodialysis-induced NO overload

- Simulation: single cell electrical activity with different NO-induced effects

- LRd model
- Currents modulation by NO (mainly ICa)

Result: NO reduces APD and Ca transient

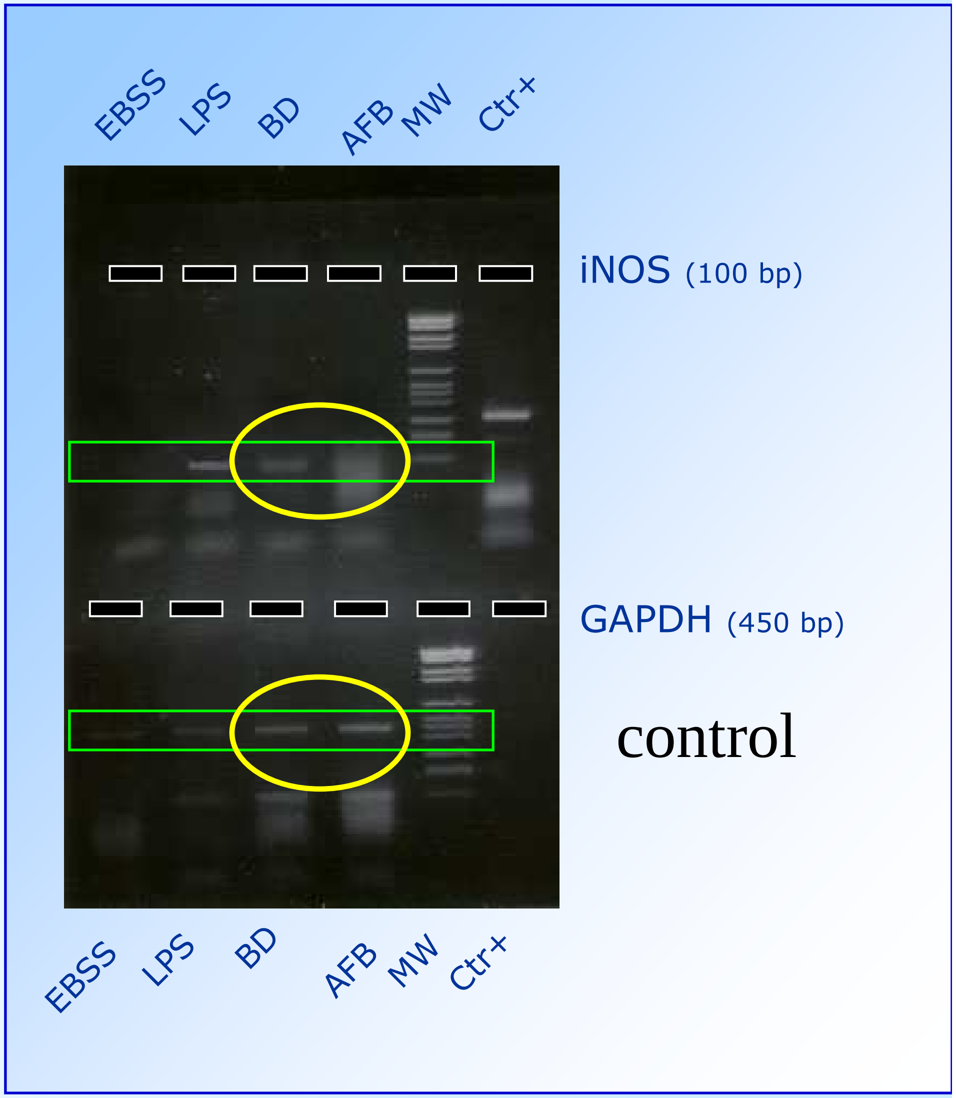


E. Grandi et al. *Effects of β-adrenergic stimulation on the ventricular action potential: a simulation study*. In *Modelling in Medicine and Biology VI* , WIT press, Southampton, 2005

Modulation of ventricular AP by hemodialysis-induced NO overload

- Expression study of the iNOs gene in rat cardiomyocites treated with differrent bath solutions:
  - Cardiomyocite extraction
  - 8 hours in the dialysis bath
  - RNA extraction
  - PCR to amplify the gene of interest
  - Electrophoresis to detect the expression of the gene

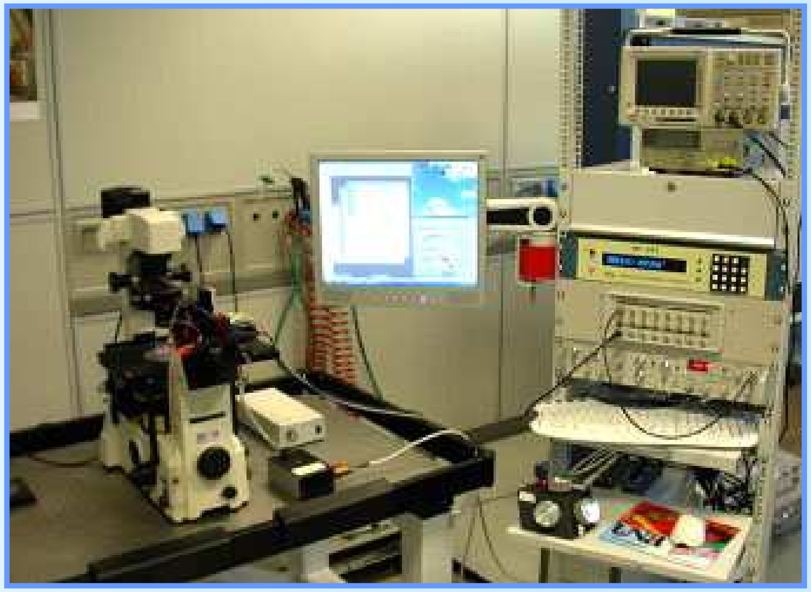
Result: BD induces iNOS expression, hence NO sinthesis in the cell



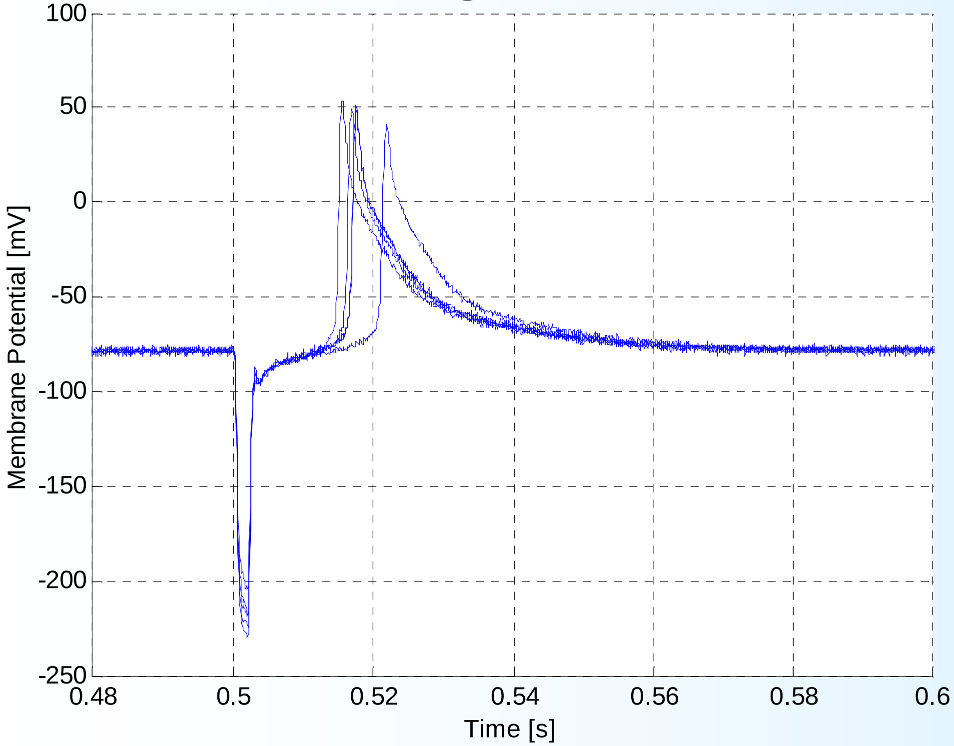
Whole cell electrophysiology in cardiomyocytes

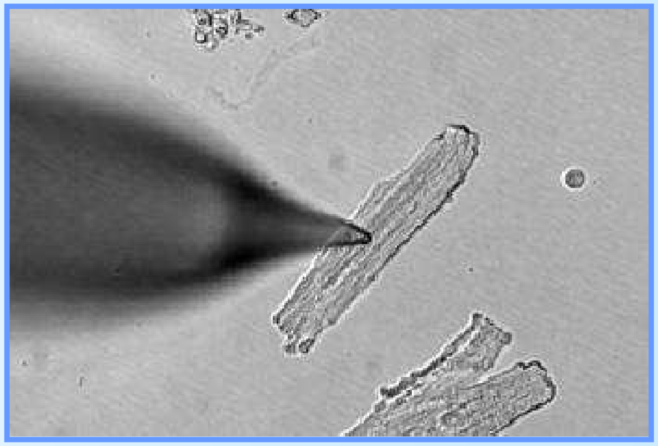
- Do bath solutions also influence APD?

Work in progress...



Our first AP recordings:





Calcium imaging

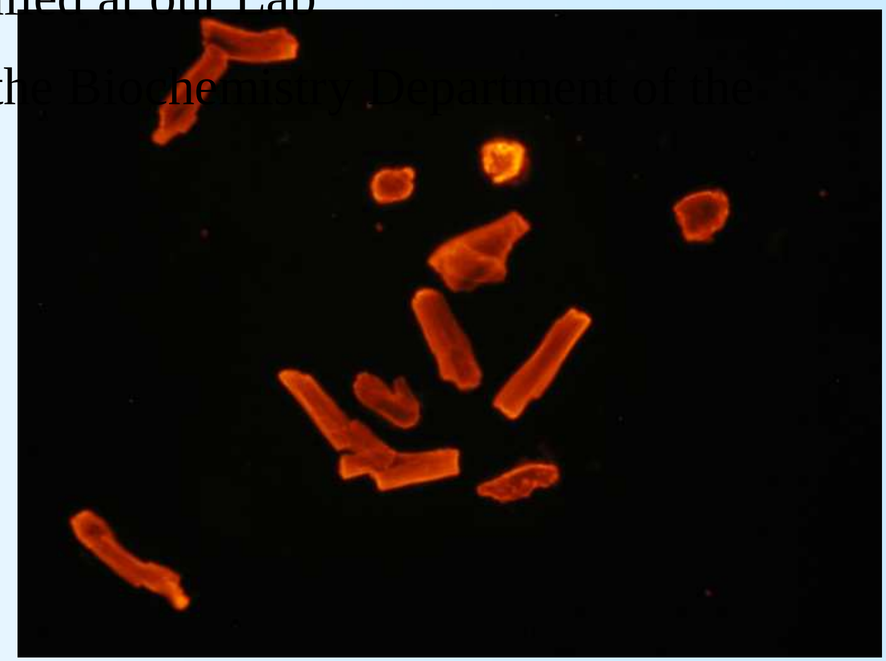
- Can the different intracellular calcium kinetic be experimentally confirmed?

Work in progress...

Goal: dynamical recording of intracellular calcium through a fluorescent marker

Calcium imaging facilities are being installed at our Lab

Preliminary experimental experiences at the Biochemistry Department of the University of Bologna



Fluorescence microscopy images of cardiomyocites with a fluorescent marker for troponin

Macrolides effects on CFTR and repolarization

Objectives

- To evaluate the potential effect of macrolide antibiotics on cystic fibrosis transmenbrane conductance regulator (CFTR) chloride channel.

- The working hypothesis is to test if
  - macrolide antibiotics can alter cardiomyocyte action potential (as suggested by Milberg 02)
  - CFTR chloride currents may participate in the anticipated proarrhythmic profile (AP prolongation)

Funded by the Italian Cystic Fibrosis Foundation

Macrolides effects on CFTR and repolarization

Project description

a) Action potential recordings in WT and mutant cardiomyocytes using a selective blocker of CFTR current. A set of macrolide antibiotics will be tested

b) WT and mutant CFTR single channel will be studied *in vitro* using:

patch clamp analysis of an heterologous expression system (293 HEK cells);

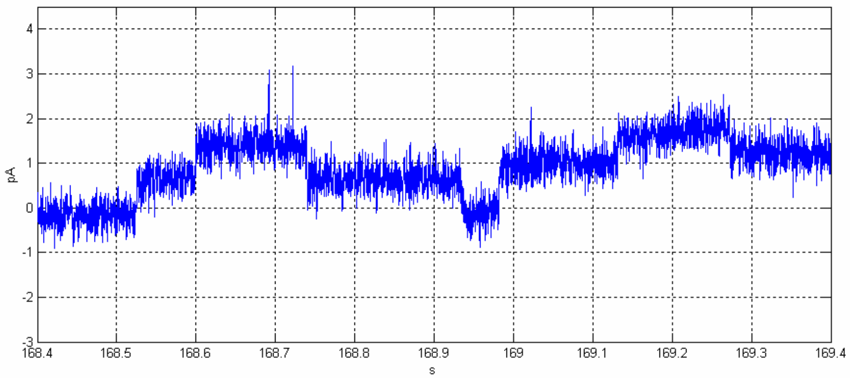
current recordings from channels incorporated into a planar lipid bilayer formed into a Warner® workstation.

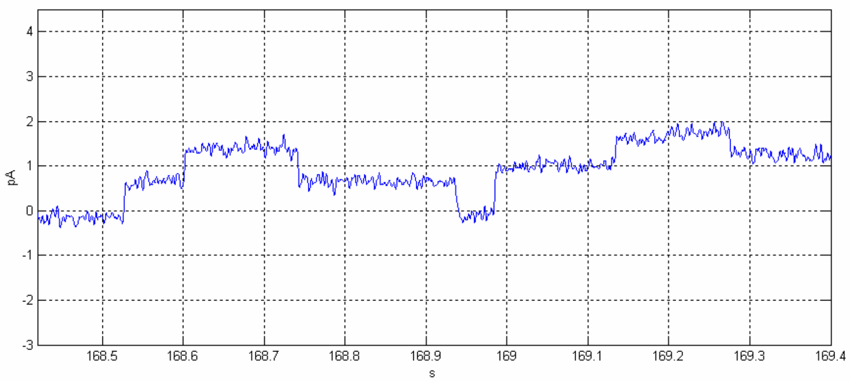
c) *In silico* modelling will be used to infer the effect of wt and mut chloride currents on the pathophysiology of the cardiac muscle cell.

Macrolides effects on CFTR and repolarization

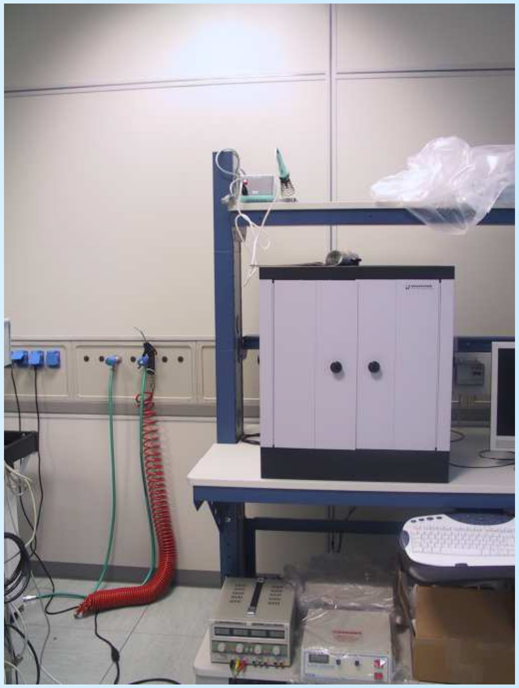
- Single channel recording in artificial bilayer

Work in progress...





- First recordings on gramicidin channel



Summary

- Good experience in system and single cell/single channel modelling (not in tissue, propagation, reentry…)
- Strong interest for clinical applications and collaborations
- Great experience in the study of cardiac response to hemodialysis therapy (hemodialysis as in vivo “experimental setup” in humans)
- Great effort towards integration of computational and experimental approaches
- Interdisciplinary staff