# Application of in-silico and in-vitro optogenetic tools to cardiac arrhythmia research

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Chapter 6

# Summary, Conclusions and Future perspectives

Chapter I, the general introduction of this thesis, begins with the description of the general mechanisms of cardiac arrythmias and of the computational modelling tools to study these mechanisms. Next, recent optogenetic studies dedicated to deepen the mechanistic understanding of cardiac arrhythmias are highlighted. **Chapter II** described the fundamental and clinically relevant hypothesis of "trapped" reentrant waves, which is proven by *in-vitro* and *in-silico* optogenetics experiments corroborated by the full 3D anatomical modelling of human atria. In this chapter, we showed that regions of dense fibrosis can form non-conducting pathways where reentry is originated by ectopy and becomes "trapped" by a local conduction block. Light-gated depolarizing ion channels and patterned illumination were employed for mimicking such non-conducting pathways in silico and in vitro. We demonstrated that a reentrant arrhythmia trapped inside a non-conducting pathway can coexist with normally propagation of sinus rhythm in the surrounding tissue. We also showed that through a change in gap junctional or sodium channel conductance, the reentrant wave can escape from the non-conducting pathway, thus transforming a local dormant arrhythmic source into a global arrhythmic driver. In **chapter III**, the generation of ectopic waves by creating oxidative damage in a cardiac tissue model was studied with the aid of miniSOG, a small light-activated singlet oxygen generator[1] . This led to the novel mechanistic insight that ectopic waves preferentially emanate from convex, high-curvature areas (e.g. corners) of the damaged region, a result that was reproduced in silico. These findings defy a widely accepted paradigm for wave propagation, which postulates that waves should arise not from corners but from the middle of a tissue region. Using a semianalytical analysis, and by drawing a mathematical analogy from quantum mechanics, we found that high-curvature areas lend themselves to oscillatory instability in the transition zone between normal and depolarized tissue. These oscillations overcome the passive electrical load from the surrounding normal tissue and generate propagating ectopic waves. Due to generality of our computer model, we would expect similar effects to be found in other areas of biology, chemistry, and physics. Although we reconstructed the phenomenon in complex detailed models, in our research we have purposefully given emphasis to a simplified computer model to link the effect to the most fundamental properties of cardiac excitation and to carry out a mathematical analysis of the dynamics. Also, it is important to emphasize that the aforementioned mechanism is purely collective and cannot be explained by typical single-cell mechanisms of ectopic activity such as early or delayed afterdepolarizations. In **chapter IV**, another purely collective phenomenon of frequency-dependent transition to ectopic activity is described. To demonstrate it in vitro, we performed experiments in monolayers of neonatal rat ventricular cardiomyocytes (NRVMs) expressing the light-sensitive depolarizing ion channel CheRiff. This

channel was employed to achieve different levels of depolarization within a region of a monolayer. By doing so, we observed a bifurcation pattern in the transition to ectopic activity and performed detailed experimental analysis of the onset of ectopic activity under pointwise periodic external stimulation. The external stimulation mimics periodic excitation waves emanating from the sinus node. Depending on the stimulation pattern, we found that our system underwent phases of bistability (involving transitions between a (nonarrhythmic) quiescent state and ectopic activity) and multistability (with transitions between a (nonarrhythmic) quiescent state, sustained ectopic activity and transient ectopic activity). Excitingly, the possibility to transition to ectopic activity depended on the frequency of external stimulation in a resonant manner, i.e. transition frequencies form continuous band in the spectrum in which stimulation of lower or above the band frequencies do not induce ectopy. These results were reproduced *in-silico* in a detailed physiological model for thorough investigation of the mechanism(s) involved. Particularly, the onset of ectopic activity jointly depended on the degree of optogenetic depolarization and the number and frequency of the waves propagating through the tissue. As a consequence of the resonance phenomenon, we found both *in-silico* and *in-vitro* that ongoing ectopic activity can also be eliminated by external stimulation of higher than the resonant frequency. To generalize our results, we were able to reproduce all the phenomena pertaining to the detailed physiological model in a simplified three-variable reaction-diffusion system. This allowed us to propose a general mechanism explaining the resonance phenomenon as the coupling between an excitable and a monostable region of the medium. Here, in a phenomenological language of excitable systems, the monostable region modeled the optogenetically depolarized area and the excitable region corresponded to non-depolarized part in *in-vitro* experiments. The emergence of ectopic activity needed accumulation of multiple waves to cross the transition threshold thereby excluding lower frequencies. The cutting off of high frequencies was a consequence of wave amplitude decrease in the monostable zone and activation of amplitude-sensitive dynamics in the monostable units. This amplitude-sensitive dynamics is explained by the phenomenon of "hidden" bistability, which is introduced by us in the chapter. "Hidden" bistability is a novel general phenomenon in excitable systems. It permits coexistence of an excitable and bistable regime in an excitable element (single cell). Conversion between the regimes depends on the amplitude of external stimulation. "Hidden" bistability phenomenondoes not depend on the precise details of the system thus ensuring wide applicability of collective resonance mechanisms.

Finally, **chapter V** was devoted to the description of fiber meshes from recombinant analogs of spider proteins, spidroins, as a platform for cardiac tissue engineering. In this chapter, we showed that NRVMs in principle can attach to 3 different types of recombinant spidroin constructs without the need of additional attachment factors such as fibronectin. NRVMs can grow and form confluent cell sheets on spidroin meshes despite the fact that number of cardiomyocytes attached three different types of spidroin meshes was 10%, 60% and 50% lower than the amount of cardiomyocytes attached to fibronectin-coated glass and fibronectin-coated fibrous polycaprolactone meshes. The presence of fibronectin-related RGDS motifs only improved initial cell adherence to the spidroin meshes, while the functional and morphological properties of the cellularized constructs became undistinguishable after 3-5 days of cultivation. This was jointly proven by immunostaining, optical mapping measurements and phase-contrast mi-

croscopy. The latter technique showed coherence of the contractions throughout the tissue sheet thus demonstrating an important property of properly functioning cardiac tissue, i.e. coordinated contraction. The optical mapping of excitation waves further proved that the cells grown on spidroin meshes constitute a functional syncytium, where electrical excitation pulses travel uniformly and are coordinating subsequent contractions.

In conclusion, studies presented in this thesis demonstrate the strength of combining *in-silico* studies and theory with *in-vitro* experiments to increase the understanding of the mechanisms of cardiac arrythmias in particular and functioning of excitable media in general. Optogenetics served as an important bridge between theory and experiments, since it allowed control of multiple parameters in otherwise inextricably complex biological experiments. In particular, the versatility of optogenetics allowed us to i) demonstrate the phenomenon of "trapped" reentry by asserting control over dynamics of shaped depolarization patterns, ii) show and explain the violation of the classical sink-source mismatch principle by precisely defining the shape of the depolarized region in virtual and true monolayers of ventricular cells, iii) discover a novel resonance phenomenon during the induction of ectopic activity by regulating the strength of depolarization.



## Perspectives of combined *in-vitro*, *in-silico* and theoretical mechanistic research powered by optogenetics

As demonstrated in this thesis, application of optogenetics has added value for the mechanistic understanding of arrhythmias and overall theory of excitable media.

## Future for technical aspects of optogenetics

After more than a decade since its inception, the field of optogenetics has gained huge momentum from a technical point of view by the development of a versatile palette of light-sensitive proteins and the application of state-of-the-art opto-electronic hardware. Mechanistic cardiac and excitable media research can greatly benefit from these and future technical advances. At the protein engineering side, optogenetics can provide tools to modulate other parameters of the cell than transmembrane potential. For example, optogenetic tools can be used to influence subcellular development and biochemical processes by modulating G protein-coupled receptor or receptor tyrosine kinases signaling within the cell, organelle placement[2], cellular traction and intercellular tension[3]. Optogenetic modulation of cellular traction and intercellular tension would be interesting to utilize in conjunction with the recently developed joint mathematical model of cardiac morphogenesis and electrophysiology[4]. Also, optogenetics can help to directly regulate cellular metabolism by depolarizing the transmembrane potential of mitochondria[5]. From the side of advances in opto-electronic techniques, cardiac optogenetic experiments can benefit from holographic light projections[6], which allow focusing of light in 3D thus permitting local optical stimulation in the depth of cardiac tissue at a very high spatial resolution . By combing such deep focused stimulation with recently introduced 3D ultrasound mapping technique[7], fundamental theories about scroll waves might be probed. Also, infrared light stimulation together with upconver-

sion nanoparticles might be employed for optogenetic stimulation in the entire depth of tissues[8]. 2D resolution and temporal fidelity of light stimulation can be also augmented by employing microLED arrays both for fundamental *in-vitro* research[9] and for translational *in-vivo* studies[10]. LED arrays permit direct regulation of the light output of individual LEDs in contrast to the binary on/off technique commonly used for patterned illumination by digital micromirror devices40. Thus, by using microLED arrays, an extra parameter can be explored and adjusted both in fundamental cardiac arrhythmia research and in arrhythmia termination applications. In addition, more exotic optical features of coherent light beams such as polarization[11] and quantum coherence[12] can be leveraged to further improve light sensitivity and the specificity of light stimulation.

## Complex models, simple models and theories

In this thesis, we put specific emphasis on simplified low-dimensional models of cardiac dynamics, since they allow mathematical analysis to obtain general behavior, which later can be found in different species . In the future, this simplification approach can be done more rigorously and automatically using free of preset equation models extracted directly from experimental data using neural networks[13] or reservoir computing[14]. It might deliver a low-dimensional representation of the dynamics, which can be further analyzed mathematically. Such a low-dimensional representation is in the spirit of the widely accepted neural manifold hypothesis[15] for motor control in neuroscience, which states that most of the relevant dynamics is concentrated on a low dimensional manifold disregarding all complexity of the underlying neuronal dynamics. Also, the results of this thesis provide predictions and raise some questions regarding the further development of theoretical analysis. Proposed in chapter IV of this thesis, the theory of "hidden" bistability might explain arrhythmia termination after multiple beats in whole heart optogenetic experiments[16]. Also this thesis exposes the need to develop or borrow from other fields of research, new theoretical tools for studying cardiac excitation and impulse propagation. For example, currently there are no convenient mathematical tools to study limit cycles (i.e. ectopic activity) in reaction-diffusion models[17]. The only available tool is Conley index[17], which is a complex tool requiring knowledge of algebraic topology and needs to be further adapted and simplified for the use in complex situations.

## Strategies for future cardiac control

Knowledge gained from models and theories can help to control or terminate cardiac arrhythmias in a time- and energy-efficient manner. This task can be achieved by closed-loop feedback systems , which are already utilized in optogenetic setups for research purposes[18] and for termination of arrhythmias[19]. Presently employed closed-loop control strategies can be further advanced using reinforcement learning[20]. Future reinforcement learning-based systems can possibly be designed without complex readouts, which drain the battery and require high processing power. This is in contrast to a recently proposed universal and effective mechanism of cardiac arrhythmia termination[21], which relies on detailed spatiotemporal information about the arrhythmia. Future reinforcement learning-based algorithms can be implemented in a variety of situations if trained

on a sufficiently vast number of diverse samples . A reinforcement learning-based technique has been already theoretically proposed for the suppression of Parkinson disease-associated synchronization in neuronal ensembles[22]. Also, this type of technique can be implemented without the explicit goal to terminate cardiac arrhythmias. The goal might be set to simply achieve some target values from limited readouts (cardiac ejection fraction , ECG). This might be done akin to works dedicated to optimal steering of fluid flow in turbulent condition[23] and control of turbulent flow[24], where only limited characteristics such as drag force and speed of flow are optimized thereby adjusting to unpredictable external conditions of turbulence without stopping the turbulence. To conclude, the studies presented in this thesis elucidated novel mechanisms of cardiac arrhythmias and excitable media in general with the aid of optogenetics, modelling and theory. These insights might contribute to the development of new precise and cost-effective mechanistically driven therapies for heart rhythm disturbances.

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