Chiara Florindi - Final Report for the Scientific Exchange Grant from the European Working Group on Cardiac Cellular Electrophysiology

Light-driven modulation of cardiac conduction by an intramembrane molecular photoswitch

Host: Professor Jan P. Kucera, Department of Physiology, University of Bern, Switzerland.

Exchange period: May 1 to 31, 2025

Background: Recently, my home laboratory proposed Ziapin2, a newly synthetized photosensitive molecule, as an efficient tool for light-driven modulation of membrane potential and excitation-contraction coupling in human-induced pluripotent stem cells (hiPSC)–derived cardiomyocytes[1,2]. To fully explore the potential of this approach, the biophysical mechanisms underlying the effects of Ziapin2 were then investigated in murine adult cardiac myocytes. These experiments elucidated the processes driving light-induced modulation of cellular excitability in a mature cellular model, providing a better understanding of how such molecules interact with the cell membrane and influence electrophysiological behavior. Ziapin2 partitions into the sarcolemma and operates via an optomechanical mechanism. In the dark, the compound dimerizes within the membrane and induces an increase in membrane capacitance (C_m) by decreasing membrane bilayer thickness. When exposed to visible light, the molecules de-dimerize following photoisomerization, which relaxes membrane bilayer thickness, decreases C_m and thus modulates membrane potential, ultimately inducing light-driven action potentials.[3] My hypothesis is that these C_m modifications influence conduction velocity (CV) in cardiac tissue[4], highlighting the potential of Ziapin2 for light-controlled regulation of cardiac impulse conduction.

Aim: The aim of my project was to investigate the effects of Ziapin2 in presence vs. absence of light on CV in cultured cardiomyocyte cell strands using microelectrode arrays (MEAs).

Scientific experience: As part of my research experience, I was trained in the preparation of patterned cardiomyocyte cultures on MEAs, a technique developed and routinely used in Prof. Kucera's laboratory[5]. Through this process, I learned to isolate and culture neonatal wild-type murine cardiomyocytes and to pattern the growth of these cultures into strands aligned over the MEAs. After 2 to 3 days in culture, each preparation was electrically paced at a basic cycle length (BCL) of 300 ms. Baseline CV was recorded without Ziapin2, both in the dark and upon specific illumination protocols (with a LED having a spectrum peaking at 470 nm). These protocols were then repeated in the presence of Ziapin2 (25 µmol/L). During my stay in Bern, I acquired hands-on experience in experimental protocol execution, data acquisition, and analysis, and I deepened my understanding of cardiac electrophysiology at the tissue level.

Results: In the dark, Ziapin2 significantly reduced CV in cardiomyocyte strands by 50-70%, a finding compatible with an increase in C_m. Intriguingly, both continuous (6 seconds) and pulsed (5–20 ms) light stimulation in the presence of Ziapin2 also led to a reduction in CV. While continuous light caused only a slight decrease in CV by about 1%, pulsed light produced a more prominent, but only transient reduction. Specifically, switching on the light during the passage of the action potential wavefront caused measurable conduction delays, evidenced by a distinct shift in activation times along the strand just after the application of light, or even conduction block. This strong modulatory effect on cardiac conduction is likely due to a very rapid C_m relaxation, whose primary effect is to cause a transient hyperpolarization during diastole, as predicted by cable theory and as observed *in vitro* in isolated murine cardiomyocytes and hiPSC-derived cardiomyocytes[2,3]. This

hyperpolarization then depresses conduction by increasing the charge necessary to bring membrane potential to threshold.

Conclusions: These findings provide valuable insights into the dynamic tissue-level effects of Ziapin2-mediated photostimulation, supporting its potential as a novel tool for light-based modulation of cardiac electrophysiology.

Future perspectives: This grant permitted me to explore cardiac impulse propagation in tissue with state-of-the-art techniques. The data generated represent a meaningful advancement in the application of molecular photoswitches in cardiac research. These results will be presented at national and international conferences and will serve as the foundation for a future scientific publication. Moreover, this exchange also allowed me to build a fruitful collaboration with the University of Bern, paving the way for future joint research initiatives.

Acknowledgments: I sincerely thank Professor Jan P. Kucera for the opportunity to visit his laboratory and for his invaluable guidance throughout my stay. I am also grateful to the entire lab team for their support, collaboration, and for teaching me the necessary techniques. Finally, I extend my gratitude to the European Working Group on Cardiac Cellular Electrophysiology for funding this exchange.

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Director of the host institution

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Exchange grant awardee

References:

1. Mattia Lorenzo Di Francesco, Francesco Lodola, Elisabetta Colombo, Luca Maragliano, Mattia Bramini, Giuseppe Maria Paternò, Pietro Baldelli, Mauro Dalla Serra, Lorenzo Lunelli, Marta Marchioretto, Giorgio Grasselli, Simone Cimò, Letizia Colella, Daniele Fazzi, Fausto Ortica, Vito Vurro, Cyril Giles Eleftheriou, Dmytro Shmal, José Fernando Maya-Vetencourt, Chiara Bertarelli, Guglielmo Lanzani and Fabio Benfenati., Neuronal firing modulation by a membrane-targeted photoswitch. Nat. Nanotechnol. 15, 296–306 (2020).

2. Vito Vurro, Beatrice Federici, Carlotta Ronchi, Chiara Florindi, Valentina Sesti, Silvia Crasto, Claudia Maniezzi, Camilla Galli, Maria Rosa Antognazza, Chiara Bertarelli, Elisa Di Pasquale, Guglielmo Lanzani, and Francesco Lodola., Optical modulation of excitation-contraction coupling in human-induced pluripotent stem cell-derived cardiomyocytes. iScience. 26, 106121(2023).

3. Chiara Florindi, Vito Vurro, Paola Moretti, Chiara Bertarelli, Antonio Zaza, Guglielmo Lanzani and Francesco Lodola., Role of stretch-activated channels in light-generated action potentials mediated by an intramembrane molecular photoswitch. J Transl Med. 22:1068 (2024).

4. Stefano Andrea De Simone, Sarah Moyle, Andrea Buccarello, Christian Dellenbach, Jan Pavel Kucera, Stephan Rohr., The role of membrane capacitance in cardiac impulse conduction: an optogenetic study with non-excitable cells coupled to cardiomyocytes. Front. Physiol. 11:194 (2020).

5. Aleksandar A Kondratyev, Julien G C Ponard, Adelina Munteanu, Stephan Rohr, Jan P Kucera., Dynamic changes of cardiac conduction during rapid pacing. Am J Physiol Heart Circ Physiol. 292(4):H1796-811 (2006).