FACULTY OF MEDICINE AND HEALTH SCIENCES

DEPARTMENT OF BASIC AND APPLIED MEDICAL SCIENCES
PHYSIOLOGY GROUP

Maarten De Smet, MD
PhD student

E MaartenA.DeSmet@UGent.be
T +32 9 332 00 34

Ghent University, University Hospital
Campus, Block B, 3rd floor, Room 011
Corneel Heymanslaan 10 – entrance 36
B-9000 Ghent
Belgium

www.ugent.be

The European Heart House
European Society of Cardiology
2035 Route des Colles - Les Templiers
06903 Sophia Antipolis
France

Report First Contact Initiative Grant 2018

Dear ESC Council on Basic Cardiovascular Science,

First of all, I would like to sincerely thank the European Society of Cardiology and the Council for their generous support, allowing me to establish a strong research collaboration between research groups sharing mutual scientific interest. The ESC first contact initiative grant 2018 provided me with a great opportunity to visit Dr. Mario Delmar's lab (Leon H. Charney Division of Cardiology, NYU School of Medicine, New York, USA) from August 27 until September 16. Dr. Delmar's lab has a longstanding interest in cardiac gap junctions and their relation to arrhythmogenic mechanisms. It is the long-term objective of his laboratory to gain knowledge on how disruption of mechanical function can alter the electrical stability of the heart. Therefore, their research focuses on the interactions occurring at the intercalated disc. Specifically, they study the cross-talk between molecules involved in mechanical coupling (desmosomes; adherens junctions) and those involved in electrical excitability and in the propagation of electrical signals between cells (gap junctions; sodium channels; cardiac Ca^{2+} handling). It is their understanding that these players interact closely with each other (termed the cardiac connexome), rather than being independent, so that loss of mechanical integrity can lead to electrical dysfunction and arrhythmias.

We were happy to notice the convergence of Dr. Delmar's research interest with our research in the lab of Dr. Luc Leybaert (my mentor; Physiology group, Department of Basic Medical Sciences, Ghent University, Belgium). The lab of Dr. Leybaert has a longstanding interest in elucidating mechanisms of intercellular communication and to unravel its intricate linkage to signaling processes in the brain and cardiovascular system, more specifically Ca^{2+} signalling. Our work over the past few years has been directed at understanding the role and regulation of Cx-based intercellular communication and developing peptide tools with improved selectivity towards hemichannels composed of Cx43. Work with these tools has demonstrated that Cx43 hemichannels can be considered as novel targets for therapeutically influencing diseases in the

brain and the cardiovascular system. In the present work, peptides identical to intracellular Cx43 domains were found to significantly suppress Cx43 hemichannel activation in ventricular cardiomyocytes, as well as preventing altered cardiac Ca^{2+} handling with implications towards arrhythmogenesis.

Given our mutual interests, we joined forces to describe the relation of Cx43 to specialized structures involved in cardiac Ca^{2+} handling. Dr. Delmar's laboratory has implemented a number of sub-diffraction limit methods of microscopy that are directly applicable to the proposed studies. As such, I had a unique opportunity to train on state-of-the-art methods with direct impact to the research that we are conducting. The research activities conducted during my stay are summarized as follows:

1. Dr. Delmar's laboratory has previously described a close relationship of the cardiac intercalated disc with structures consistent with sarcoplasmatic reticulum, including longitudinal tubular structures originating from the intercalated disc (Leo-Macias et al., 2016). We therefore hypothesized that Cx43 would occur in close approximation to key proteins of cardiac dyads. We used direct Stochastic Optical Reconstruction Microscopy (dSTORM, a super-resolution fluorescence technique that bypasses the diffraction limit and allows to resolve single molecules with a spatial resolution of ~20 nm; in collaboration with the lab of Dr. Eli Rothenberg, Department of Biochemistry and Molecular Pharmacology, NYU School of Medicine, New York, USA) to probe nanoscale distribution of Cx43 in relation to RyR2, Pospholamban (PLN), Cav1.2 and NCX in isolated adult murine left ventricular cardiomyocytes. Indeed, a large fraction of Cx43 occurs within 20 nm of these proteins, confirming that a fraction of Cx43 is localized to dyadic structures at the intercalated disc. We also started optimizing the antibodies for super-resolution immunohistochemistry.
2. Given that a fraction of Cx43 localizes to a specific signaling nanodomain, we hypothesized that single channel currents could be recorded from specific dyadic nanodomains at the cell ends of isolated left ventricular murine cardiomyocytes. We therefore used Scanning Ion Conductance Microscopy (SICM, a non-contact scanning probe microscopy technique, with a resolution up to ~20 nm, based on the principle that the flow of ions through the tip of a nanopipette filled with electrolytes decreases when the pipette approaches the surface of the sample) to generate a three dimensional topography image which, when combined with cell-attached patch-clamp, allowed to perform single-channel recordings from a specific nanodomain at the intercalated disc.
3. I also received an introduction to segment dyadic structures at the intercalated disc in 3D reconstructions of volume electron microscopy techniques.

Finally, I was fortunate to attend journal clubs and lab meetings from both the lab of Dr. Delmar and Dr. Rothenberg that provided both theoretical and practical insight in the use of super-resolution techniques for answering various research questions. Additionally, I had the opportunity to present our data and get valuable insight from our peers. I also attended the First Grand Round of the Department of Medicine at NYU on "Precision Medicine in the Era of Big Human Data" by Dr. Frederick Dewey.

I would like to thank the European Society of Cardiology and the Council on Basic Cardiovascular Science again for giving me the opportunity to work in an international

collaboration, receive an introduction to super-resolution techniques and successfully apply these techniques to our own research. All involved parties have agreed to continue the collaboration in the near future.

I would like to thank Dr. Mario Delmar for accepting my request to stay in his lab for an introduction to super-resolution techniques. Finally, I would also like to thank Dr. Mario Delmar, Dr. Marta Pérez-Hernández Duran and Dr. Xianming Lin for their expert supervision, resulting in an efficient, fulfilling and invaluable experience which will be incorporated in our ongoing research manuscripts.

Most sincerely,

Maarten De Smet