European Society of Cardiology
Att. Susan Del Gaiso
2035, Route des Colles
Les Templiers – BP 179
06903 Sophia Antipolis
France

Dear Council members,

First of all, I would like to sincerely thank the European Society of Cardiology for the generous support and for allowing me to establish a strong and cooperative relationship with research groups sharing mutual scientific interest. The ESC first contact initiative grant provided me with a great opportunity to obtain a research visit to Dr. R. Gourdie's lab (Centre for Heart and Regenerative Medicine, Virginia Tech Carilion Research Institute, Virginia, USA) from March. 24th - April 9th. The research activities conducted during my stay are summarized as follows:

The host institution, Dr. R. Gourdie's lab (Center for Heart and Regenerative Medicine, Virginia Tech Carilion Research Institute, Virginia, USA) has a long standing interest in the contribution of connexin-interacting proteins and connexins to electrical conduction in the heart. Dr. Gourdie's group has recently characterized a 'perinexus' zone in which unapposed Cx43 hemichannels reside at the gap junction plaque periphery. Highlight of the work is that interaction between Cx43 and scaffolding Zonula occludens-1 (ZO-1) dynamically controls the transitions of hemichannels from unapposed to apposed forms. A peptide mimicking the carboxyl terminus (CT) of Cx43 has been developed to disrupt Cx43/ZO-1 interaction, leading to restored gap junction coupling and reduced Cx43 remodelling in diseased heart. Upon my arrival, I presented one of my research projects consisting of a Cx43 targeting peptide that selectively inhibits Cx43 hemichannels without downregulating junctional coupling. Interestingly, this peptide interacts with the Cx43CT tail, the same domain for the binding of ZO-1 and in fact slightly elevates the junctional communication after long-term exposure (>24h). Therefore, one of the objectives of my visit was to verify whether the peptide disrupts the Cx43/ZO-1 interaction and sequentially affects the 'perinexus'

Faculteit Geneeskunde & Gezondheidswetenschappen, Vakgroep Medische Basiswetenschappen, Fysiologie Groep, De Pintelaan 185 (Blok B, lok. 304), B-9000 Gent. Tel.: +32 9 332 39 38
E-mail: Nan.Wang@Ugent.be

organization/size of the gap junction plaques in addition to its inhibitory action on hemichannel activities. To study this, I incubated cells overexpressing Cx43 with the peptide for 24 h, and detected the total population of Cx43 proteins using immunofluorescence labelling. Under the supervision of Dr. R. Gourdie, we captured z-series of optical sections and analysed gap junction length distribution in cell cultures treated with or without the peptide.

The second aim of my visit was to learn a novel technique employed by Dr. R. Gourdie's group for studying the spatial organization of the perinexus. Due to the low density of Cx43 protein embedded in the perinexus, conventional immunostaining approach suffers from poor signal intensity and resolution. However, an *in situ* proximal ligation assay Duolink detecting the subcellular interaction between Cx43 and ZO-1 can markedly amplify the fluorescent signals of the unapposed hemichannel fraction, giving rise to an enhanced spatial resolution. With the assistance of other lab members, I examined antibodies targeting different domains of Cx43 protein for the Duolink assay. We further optimised the hybridization, ligation and amplification steps to improve the performance of the system. Since my return, I've been adapting the same technique to characterize hemichannel distributions in adult heart slices from several animals models.

In addition, I was fortunate to attend a research seminar on the role of Cx43 in T cell development given by Dr. P. Kraj (Georgia Health Sciences University, USA).

Overall, my experience was most fulfilling. Thanks to the first contact initiative grant, I was able to further my understandings of the new techniques under specialized supervision. More importantly, the visit to Dr. R. Gourdie's lab offers great networking opportunities. I have gained helpful feedback on my own research and was able to initiate new collaborations. I would therefore like to thank the European Society of Cardiology for their part in making it possible for me to obtain this invaluable experience.

Sincerely

Nan Wang

June 5, 2014

Faculteit Geneeskunde & Gezondheidswetenschappen, Vakgroep Medische Basiswetenschappen, Fysiologie Groep, De Pintelaan 185 (Blok B, lok. 304), B-9000 Gent. Tel.: +32 9 332 39 38
E-mail: Nan.Wang@Ugent.be