# Unveiling the causes of cardiac conditions

**Professor Marcel Egger** discusses the excitements and challenges of working at the cutting edge of cellular physiology research and the innovative techniques he and his team are developing to make new insights in this field

**How has your academic background led you to your current area of research, and what motivates you in your work?**

As with many things in life, it was quite serendipitous. My thesis advisor, Professor Edwin Donath, became both my mentor and friend, triggering my interest in the biophysical aspects of cellular signalling. Some years later, I met Professor Ernst Niggli in Bern and took the opportunity to join his team. At that time, some fundamental new findings had revolutionised the field of excitation-contraction coupling of cardiac cells – and I was utterly fascinated from the beginning. For the past 20 years, our teams have collaborated to try to understand the regulatory mechanisms of cardiac muscle contraction.

As to what motivates me, I could give a simple answer: I like what I'm doing and it is always exiting to discover something new and understand nature a little better. However, the real reason is a lot more philosophical and complex, concerning questions about the inherent driving force for curiosity and human creativity. Under this view, science and art are twins. I could say a lot more, but I feel that such a complex discussion would go beyond the scope of the question!

**What knowledge gaps in the pathogenesis of cardiac diseases is your research attempting to address?**

The excitation-contraction coupling mechanism is the translation of an electrical stimulus to the mechanical response of a cardiac cell finally resulting in contraction of the heart muscle. The general concept of excitation-contraction coupling is well established; however, details of the process are still unclear. For example, the mechanisms of calcium release, which cause the cardiac muscle tissue to contract, are not conclusively explained, especially under pathophysiological stress.

The aim of our recent research is to understand how, and to what extent, the signalling link is impaired between two intracellular calcium release channels in cardiac cells in conditions such as arrhythmias, where the heart beats irregularly. We would like to understand how these intracellular calcium release mechanisms interact to affect and to modulate calcium homeostasis in cardiac muscle cells.

**Do you use any interesting or innovative methods in your studies on the two calcium release channels?**

Our expertise are functional assays at the level of a single cell and the subcellular scale to examine excitation-contraction coupling in isolated cardiomyocytes under a variety of physiological and pathophysiological conditions. The methodology combines several state-of the-art biophysical techniques in an integrative way, including laser-scanning confocal microscopy with UV-flash and two-photon excitation photolysis of caged compounds, with whole-cell voltage clamp techniques. We are able to perform all of these techniques simultaneously.

We are also using carefully selected animal models, including, for example, a newly developed transgenic mouse model of hypertrophic cardiomyopathy.

**Can you outline some of the main challenges you face in your work and how you are seeking to overcome them?**

Investigating calcium signalling from two separate calcium release mechanisms is challenging for several reasons. Compared to large calcium-induced calcium release signals mediated by ryanodine receptors (RyRs), the smaller calcium release signals induced by an intracellular signalling molecule called inositol trisphosphate ($InsP_3$) are more difficult to identify and discern within the overall calcium response. Furthermore, revealing the specific contribution of $InsP_3$ is hampered by the lack of adequately selective pharmacological tools. A fast fluorescent $InsP_3$ indicator is not yet available, which makes it difficult to examine the intracellular $InsP_3$ production and its subcellular distribution.

To solve the first problem, we developed an image analysis tool with a pixel-wise fitting algorithm applied for the event analysis on full frame confocal imaging. This provides a method to separate calcium released via RyRs and $InsP_3$ receptors with much higher precision, which was urgently needed. These experiments are extremely difficult to perform successfully. The person performing the experiment requires a great deal of training and experience, as well as lots of patience and tolerance!

**Where do you see your research going in future?**

We still need more functional data on a cellular level, which in the future will be combined with reproducible genetic models such as patient-specific induced pluripotent stem cell-derived cardiomyocytes and gene-targeted animal models. Calcium-dependent cardiac dysfunctions like arrhythmias are the product of complex electromechanical phenomena, emerging only at higher levels of biological organisation. An in-depth approach to investigating these phenomena at a high level will require collaboration. From this point of view, the pressure to establish synergistic national or international research cooperations will probably increase even more in the near future.

# The pulse of calcium signalling

Defects in calcium signalling underlie many heart problems. Groundbreaking new research from the **University of Bern**, Switzerland, is uncovering the mechanisms driving disruptions to calcium signalling and revealing new therapeutic targets for common cardiac conditions like arrhythmia

**A HEART BEATS** about 2 billion times during the average human lifespan. If this rhythmical beating is disrupted, the consequences can be lethal; in fact, sudden cardiac arrest is the largest cause of death in European adults – an event that usually results from uncoordinated contractions of the heart known as ventricular fibrillation. Moreover, cardiovascular disease accounts for over half of all deaths in Europe each year.

The cardiac muscle is stimulated to contract by an electrical signal in a process known as excitation-contraction coupling. This electrical signal, called action potential, is generated in an autorhythmic manner by a group of cells known as the sinus node – located in the right atrium of the heart – and it spreads across the organ to excite the heart's muscle cells (known as 'cardiomyocytes'), to contract and pump blood.

Calcium plays an extremely important role in excitation-contraction coupling – and calcium ion ($Ca^{2+}$) release within cells is vital for the muscle cells to contract. Recent studies have revealed that aberrant $Ca^{2+}$ signalling could play a critical role in several cardiac conditions in which the heart does not beat in a regular pattern, including tachycardia, atrial and ventricular fibrillation, and palpitations, as well as heart failure and myocardial ischaemia.

Clearly, there is an urgent need to find out more about the underlying causes of these deadly conditions – and this is where the work of Professor Marcel Egger comes in. Based at the University of Bern, Egger is leading a team of researchers using state-of-the-art biophysical techniques to achieve a better understanding about the regulation of $Ca^{2+}$ signalling within cardiac muscle and identify new targets for therapeutic strategies for cardiac pathologies.

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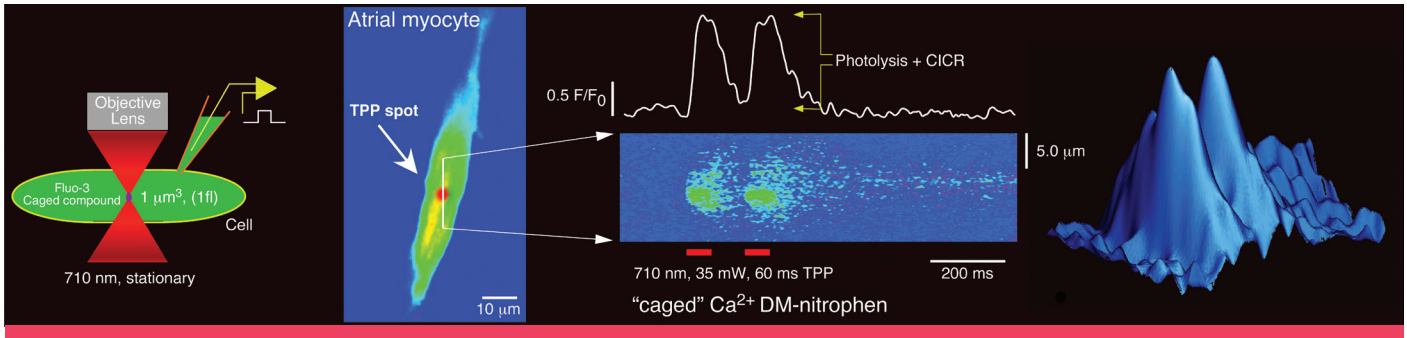
## CALCIUM-INDUCED CALCIUM RELEASE

Cardiomyocytes contract when an electrical stimulus passes through the tissue and causes the myocyte membrane to depolarise. In turn, this depolarisation causes dihydropyridine receptors – namely, voltage-dependent calcium channels on the membrane – to open and allow a small amount of $Ca^{2+}$ to enter the cell. This influx of $Ca^{2+}$ is amplified by a mechanism called calcium-induced calcium release and this calcium is then sufficient to initiate contraction of the cardiac myocyte. The global calcium transient is the spatio-temporal summation of many localised intracellular $Ca^{2+}$ release events from intracellular ryanodine receptors (RyRs) known as a '$Ca^{2+}$ spark'. By recruiting $Ca^{2+}$ sparks calcium-induced calcium release can be regulated and graded on the cellular level while being regenerative at the same time.




TPP method

Egger and his team are particularly interested in the crosstalk between calcium-induced calcium release and another more enigmatic $Ca^{2+}$ release mechanism that occurs within channels sensitive to the intracellular secondary messenger known as inositol-1,4,5-triphosphate ($InsP_3$). This mechanism is initiated by several hormones. The $InsP_3$ receptor functions as a $Ca^{2+}$ channel that leads to smaller, localised releases of $Ca^{2+}$. These localised releases are called $Ca^{2+}$ 'puffs'. While $Ca^{2+}$ sparks and puffs can occur simultaneously in cardiomyocytes, very little is known about how the two release pathways interact. "We are especially keen to understand the contribution of $InsP_3$-induced $Ca^{2+}$ release in the initiation, propagation and amplification of the calcium-induced calcium release mechanism," Egger says.

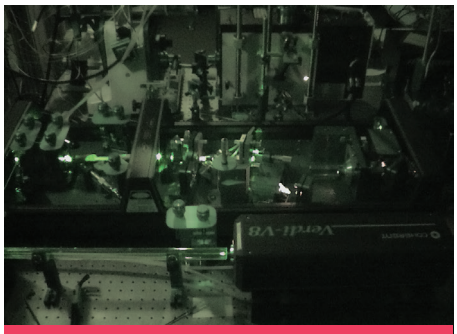
## GROUNDBREAKING DISCOVERIES

In order to determine the relative importance of each $Ca^{2+}$ release pathway, Egger and his team have designed a novel approach using a unique combination of innovative biophysical techniques. They began by isolating atrial myocytes from adult mice and applying 'caged' $InsP_3$ to the cells. (Caging the $InsP_3$ means rendering it biologically inert by binding its active site to a photo-removable protecting group). Upon UV treatment, the cage is removed and the $InsP_3$ is activated, allowing the researchers to assess its subsequent effects.

"Using caged compounds means that we can produce global or very local intracellular concentration jumps of $Ca^{2+}$ or $InsP_3$ extremely rapidly," explains Egger. "This enables us to probe the function of $InsP_3R$ or RyRs directly and mimic several temporal and spatial features, for example from a physiological excitation-contraction response."

As expected, the $InsP_3$ activation led to significant $Ca^{2+}$ release. However, following treatment with a RyR inhibitor, the $InsP_3R$ $Ca^{2+}$ release did not occur. This was because there were may not enough functional $InsP_3$ receptors to enable $Ca^{2+}$ puff formation. However, at the same time, the researchers observed an increase in the occurrence of $Ca^{2+}$ sparks, suggesting that even a small $InsP_3$-induced $Ca^{2+}$ releases are able to facilitate and boost RyR function. Through their analytical approach – which uses 2D confocal imaging – Egger and his team were able to directly observe and verify that $InsP_3$-induced $Ca^{2+}$ release triggers RyR-mediated $Ca^{2+}$ release and modulates the calcium-induced calcium release mechanism.

The team also discovered a slow but significant $Ca^{2+}$ leak from the sarcoplasmic reticulum, even in the presence of the RyR inhibitor – an action that was not seen when $InsP_3R$ activity was blocked. There is also some evidence that $Ca^{2+}$ spark activity may help trigger or boost the $InsP_3R$-mediated $Ca^{2+}$ release. "These results strongly support the idea that both $Ca^{2+}$ release channels coordinate their activities, suggesting



TPP setup

a regulatory determinant of $Ca^{2+}$-dependent cardiac arrhythmogenicity," Egger points out. "$InsP_3$-induced $Ca^{2+}$ release may change the RyRs $Ca^{2+}$ sensitivity, potentially leading to pro-arrhythmic effects". Thus the next step for Egger and his colleagues is to assess whether these findings can be translated from atrial myocytes to the heart as an entire organ.

## TOWARDS THERAPEUTIC TARGETS

Intriguingly, RyRs could operate as potential drug targets that could alleviate chronic anti-arrhythmic conditions, as well as other muscular and neurological disorders. To this end, Egger and his team are particularly interested in two cardiac arrhythmia diseases: catecholaminergic polymorphic ventricular tachycardia (CPVT) and hypertrophic cardiomyopathy. They believe that $Ca^{2+}$ release-targeting therapeutics could help treat both of these conditions. "We postulate that life-threatening rhythm disturbances in the vast majority of CPVT cases, and a large portion of hypertrophic cardiomyopathy cases, emanate from excessive $Ca^{2+}$ release due to RyR2 hyperactivity and a modulatory impact of $InsP_3$-induced $Ca^{2+}$ release in that process," says Egger. "Both receptors are potential anti-arrhythmic drug-targets."

A deeper understanding of the $Ca^{2+}$ release mediated by crosstalk between RyRs and $InsP_3R$ is an important goal for Egger's team. Once this has been achieved, the researchers plan to identify both novel and established characterised drugs that could restrict the activity of these receptors without significant side effects. This poses several challenges, not least because RyRs are found in many different types of excitable cells, including muscles and neurons, while $InsP_3R$ is vital in a wide range of cellular processes. However, a compound called Rycal has recently been pinpointed as a possible drug for RyRs, which acts by preventing $Ca^{2+}$ from leaking out of cardiomyocytes. Meanwhile, finding ways to target $InsP_3R$ remains a little more elusive. Egger and his team certainly have their work cut out for them when it comes to gaining a clearer perspective of potential useful therapeutic approaches – but ultimately it is hoped that their dedication and ingenuity will pay off.

### CARDIAC EXCITATION-CONTRACTION COUPLING

#### OBJECTIVES

To elucidate the interplay of two $Ca^{2+}$ release mechanisms in cardiac myocytes and understand their functional crosstalk in physiology and pathophysiology.

#### KEY COLLABORATORS

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**Professor Héctor H Valdivia**, Frank N Wilson Professor of Cardiovascular Medicine & Professor of Internal Medicine Professor of Molecular & Integrative Physiology University of Michigan, USA

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#### FUNDING

Swiss National Science Foundation (SNF)

NOVARTIS Foundation for Medical-Biological Research

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**http://onlinelibrary.wiley.com/doi/10.1002/cbic.200500345/full**

**http://www.nature.com/ncb/journal/v1/n6/full/ncb1099_323.html**

**MARCEL EGGER** focuses on cardiovascular health and key cellular mechanisms of heart muscle contraction. He is particularly interested in uncovering details about a lesser-known mechanism of calcium-induced calcium release and hopes that this will lead to a better understanding of how to tackle heart disease in the future. He received his PhD in Biophysics from Humboldt University Berlin and is currently an Associate Professor for Physiology at the University of Bern.