

Scientists on the Spot: Christoph Maack on how to measure mitochondrial parameters in cardiomyocytes

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Watch the interview here: <https://youtu.be/cUqvG8o1wv8>



Biography

Professor Christoph Maack is Chair of the Comprehensive Heart Failure Centre (CHFC) and Director of the Department of Translational Research at the University Clinic in Würzburg, Germany.

Albano Meli: Hi, my name is Albano Meli and I am a member of the Scientists of Tomorrow from the European Society of Cardiology (ESC). Today I am very pleased to welcome you to this *Cardiovascular Research Onlife* interview with Professor Christoph Maack.

Good afternoon, Professor Maack.

Christoph Maack: Good afternoon.

Albano Meli: Thank you for sharing your thoughts with us today. To start with, my first question is: what are the key mitochondrial parameters that we can experimentally measure in cardiomyocytes?

Christoph Maack: Before we discuss this, I would like to point out that, of course, one can always measure mitochondrial function in isolated organelles, in the isolated mitochondria. However, we know today that mitochondrial function is very tightly controlled by everything that is going on in the cytosol around it. And that is why we actually try to measure these parameters in working cardiac myocytes—just to get a more physiological approach to the whole thing.



Dr Albano Meli from Inserm interviews Prof Christoph Maack, Chair of the Comprehensive Heart Failure Centre (CHFC) in Würzburg, Germany.

So, in mitochondria, the Krebs Cycle is a key regulatory mechanism that produces NADH (nicotinamide adenine dinucleotide hydride) as an electron donor for the electron transport chain. That is already the first parameter that we can measure: NADH autofluorescence. As a second product of the Krebs Cycle, we have FADH₂ (flavin adenine dinucleotide) which also delivers electrons to the respiratory chain. That is the second thing that we can measure. Once the electron transport chain establishes a proton gradient, we can measure mitochondrial membrane potential—that would be the next important parameter for mitochondrial function. We then must consider that the Krebs Cycle is regulated by calcium. Therefore, it is quite important to measure fluxes of calcium into and out of the mitochondria, and how net concentrations change over the course of several different conditions. Finally, we have learned that a mismatch on the mitochondrial level between calcium and adenosine diphosphate, which controls respiration, can lead to a net oxidation of the redox state. This can lead to oxidative stress, thereby the emission of reactive oxygen species (ROS) from the mitochondria. So that would be the fourth important parameter one can measure.

Albano Meli: Okay, so how can we measure these parameters?

Christoph Maack: So, going back to NADH and FADH₂, this is actually a very nice thing because these have autofluorescence so that we do not need any extra dyes or anything, but we can just excite the myocytes at 340 nm and measure the emission of light at 450 nm—that is for NADH. For FADH₂, excitation is at 485 nm and emission at 525 nm. The nice thing is that we can do this in the very same cell, and upon oxidation, the fluorescence of NADH will decrease, and for FADH₂, it will increase. This means that we can use both parameters ratiometrically which allows us to get rid of movement artefacts and gives us a very sensitive and very well reproducible inert parameter of mitochondrial redox state.

Second is the mitochondrial membrane potential. This is actually easier to follow because you can use a fluorescent dye, either TMRM (tetramethylrhodamine, methyl ester) or TMRE (tetramethylrhodamine ethyl ester). There is also a dye called JC-1. We use TMRM and TMRE, which are excited at 540 nm with the light emission collected at 605 nm. This gives a very bright fluorescence which is easy to detect, but it is also a very stable parameter. There are few conditions that really change the mitochondrial membrane potential, because it is very well controlled by many things.

The third aspect, calcium, is probably the most challenging thing to do because it is difficult to locate calcium indicators specifically into the mitochondria. We have developed a technique during my time as a postdoc at Johns Hopkins University (MD, USA) with Brian O'Rourke, whereby we load cardiac myocytes with rhod-2-acetoxymethyl ester, which, due to its positive charge, locates primarily to the mitochondria but it can always leave traces in the cytosol. Therefore, we patch-clamp the myocytes and dialyze the cytosol to get cytosolic traces of rhod-2 out. At the same time, we put indo-1 as a salt in the pipette solution and wash this into the cytosol. So we end up with two different dyes in two different compartments, namely: rhod-2 in the mitochondria and indo-1 in the cytosol. Then, we can go through all sorts of protocols and measure the changes of mitochondrial and cytosolic calcium in the same cell. The downside of rhod-2 is that it is not ratiometric, so it is difficult to calibrate. As such, there are genetically encoded calcium probes that can be targeted to mitochondria which can be better calibrated, so quantitative estimations can be made easier. The downside of this is that you need to incubate cells with a virus for 1 or 2 days. This can lead to de-differentiation of the cardiac myocytes to some extent, which also means that the t-tubular structure can become impaired. Therefore, the processes of excitation-contraction could be changed slightly. So there is always pros and cons to each technique; but these are probably the most commonly used techniques for calcium.



Professor Christoph Maack: 'We now have a mouse that is stably expressing this reporter (roGFP2-Orp1) either in the mitochondria or the cytosol, and this reporter is quite specific and quite sensitive for hydrogen peroxide (H₂O₂). We hope therefore that with this new reporter, we can make many new important observations.'

Finally, for ROS, we have several fluorescent dyes that we can use. For instance, dichlorofluorescein diacetate is often used.

It is limited by the fact that it can undergo auto-oxidation and it is not very specific for hydrogen peroxide (H₂O₂), but also for hydroxyl radicals and peroxynitrite. However, in our hands, we also calibrate this fluorescent dye at the end of each experiment, and therefore, you can still get valuable information out of it. But it is important to keep the excitation energy as low as possible, because that would avoid auto-oxidation. A second dye, which is a little bit more specific, is called MitoSOX. This one will give you superoxide more specifically. There are also several genetically encoded ROS reporters as well. We are starting to use one that is called roGFP2-Orp1 by Leticia Roma, who established this mouse in Tobias Dick's lab in Heidelberg, then worked in my lab in Homburg for one year and is now an independent Junior professor in Homburg. We still collaborate very closely. We now have a mouse that is stably expressing this reporter either in the mitochondria or the cytosol, and this reporter is quite specific and quite sensitive for hydrogen peroxide (H₂O₂). We hope therefore that with this new reporter, we can make many new important observations. So these are the four main parameters and the four most important techniques to use—but, of course, there are many more that I just don't have the time to mention now.

Albano Meli: Thank you for the great answer. So can we target mitochondria for the prevention or treatment of heart failure?

Christoph Maack: Yes, there are actually approaches ongoing to do this. So maybe the most advanced one right now is a compound which is generically called SS-31 (d-Arg-2', 6'-dimethyltyrosine-Lys-Phe-NH₂). It's a Szeto-Schiller peptide, a four amino acid peptide which accumulates in the mitochondria and it is known that it binds to cardiolipin. Cardiolipin is a phospholipid in the inner mitochondrial membrane and the idea right now is that SS-31, or Elamipretide which is its name under clinical investigation, by binding to cardiolipin is thought to protect cardiolipin from oxidative damage and cardiolipin, in turn, is important to glue the complexes of the respiratory chain together. So the idea is that with this compound, the function of the respiratory chain per se, which is deteriorated in various cardiovascular diseases by oxidative and other damages, is kind of fixed and therefore electron flow along the respiratory chain is more intact. This would also avoid excessive emission or production of ROS in the first place. The compound is not a direct anti-oxidant, but it is, as I said, protecting cardiolipin from oxidative damage, and therefore, has secondary effects on reducing oxidative stress. This compound, Elamipretide, is currently in Phase 2

clinical trials in patients with systolic and also diastolic heart failure—and we are really keenly expecting the results at some point. I think it will be an important source of information as to whether targeting mitochondria directly in cardiac diseases is promising.

There also have been many other approaches, and still are. So for instance, it has been observed in a clinical trial of 500, or a couple of hundred patients, with heart failure that the randomized supplementation with Coenzyme Q, which is an over-the-counter drug, results in a surprisingly reduced mortality in these patients without grossly improving the functional status. But it improved survival in these patients. This is an interesting result that still needs to be verified by a larger trial that we hopefully are getting at some point. But for now, it has not found its way into the current guidelines because it's considered that the trial so far was underpowered. It is also not quite clear whether Coenzyme Q, if you give it like it is, will find its way into the mitochondria because Coenzyme Q is a component of the respiratory chain and accepts electrons; therefore it can, in a way, scavenge ROS as long as it is recycled and regenerated. There are approaches to locate Coenzyme Q more specifically to mitochondria and this compound is then called MitoQ, where Coenzyme Q

has been coupled to triphenylphosphonium which is a molecule that, due to its positive charge, is targeted to mitochondria. This compound, MitoQ, in turn, has been tested broadly experimentally. It has been developed by Mike Murphy and colleagues in Cambridge (UK) and there have even been some clinical trials involving it, although not in cardiovascular diseases, but in, I think, hepatitis. We don't have data in patients with heart failure or other cardiovascular diseases yet.

One last thing is that iron therapy is already accepted in heart failure and it's in the guidelines. It's been proven to improve symptoms but not mortality. One idea is that, of course, iron is important for many mitochondrial processes involving the electron transport chain but also the Krebs Cycle, and it could be that through iron therapy, mitochondrial function is improved. There are some data on that but it's not completely clear how the drug works and it's also not clear if this is an effect that takes place in the heart or in the skeletal muscle—or both. Probably both, I would say. But this is still not completely resolved yet.

Albano Meli: Thank you very much Professor Maack, and thank you for joining us for this Cardiovascular Research Onlife interview.