PROFESSOR STEPHAN GRISSMER

Passing through the eye of the needle

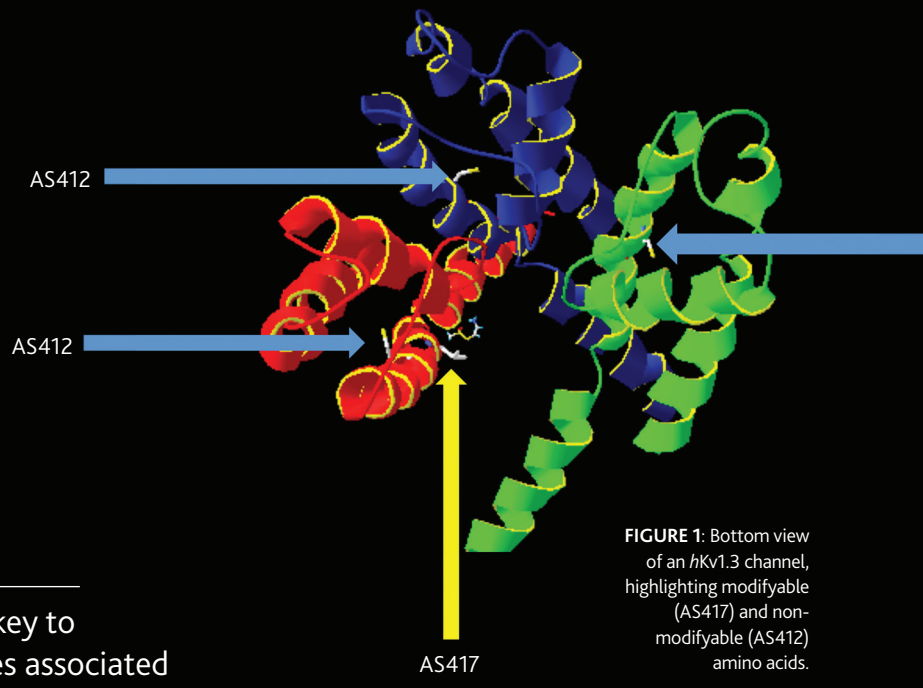


FIGURE 1: Bottom view of an *h*Kv1.3 channel, highlighting modifyable (AS417) and non-modifyable (AS412) amino acids.

Understanding ion channels may hold the key to unlocking the secrets of a variety of diseases associated with their dysfunction. **Professor Stephan Grissmer** is a leader in this research field, and here provides an exclusive snapshot into his latest work

To begin, could you explain the background to your research and what inspired you to conduct this study?

My overall research interests are ion channels and their functions, and the molecular mechanisms by which these functions can be modulated. There are numerous diseases that are associated with ion channel dysfunction, for example skeletal muscle diseases like myotonies, central nervous system diseases like familial hemiplegic migraine, or cardiac arrhythmias. Repairing the dysfunction of ion channels might be a way of treating these diseases. The dysfunction can be regulated by substances that will either enhance the function of the ion channel (openers), or reduce the function (blockers). Since these ion channels are expressed in a vast variety of tissues, it is very important that the above-mentioned substances be very specific for the ion channel in question. Therefore the specific feature of any given ion channel can be used to find a substance that will only be specific for this channel.

Can you offer an insight into the electrophysiological approaches that you have employed to characterise the structural change in the mammalian potassium channel *h*Kv1.3?

We have mainly used the whole-cell patch-clamp method to measure the flow of potassium ions through the *h*Kv1.3 channels. These measurements can give us information on how these ion channels work. In principle, a feedback amplifier (FBA) measures and amplifies the actual current between two electrodes. One electrode is suspended in solution in a glass pipette connected to the interior of a cell through a small opening. The other electrode is suspended in the fluid surrounding the cell. Usually, ions can only pass the membrane

through ion channels. Therefore, if there are no or only a few ion channels located in the cell membrane, only very few or no ions can flow from the inside to the outside of the cell. If there are many ion channels present in the cell membrane, a high current can flow from the interior to the exterior electrode. Since the feedback amplifier can also control the membrane potential, we can not only measure the size of the current but also the time course of the current in response to a short or longer depolarisation. Therefore, we are able to study the time course of the structural changes in the *h*Kv1.3 channel associated with the opening and the C-type inactivation of the channel.

Moreover, what makes these techniques so unique and applicable in this setting?

The above described method allows, in principle, the measurement of the flow of ions through a single ion channel. One can directly observe the result of the movement of a single molecule: current flow if the channel is open, and no current flow if it is closed. In addition, the setting allows for the measurement of current in the absence or presence of channel modulators. This approach has several advantages: first, one can get information about the structure of the channel by using the known structure of peptide toxins, originally isolated from scorpion venoms, as a negative imprint. Moreover, we can test potential new modulators to check for an improved affinity or specificity for the ion channel in question.

What has been the greatest success of your research to date?

I was involved in research involving a voltage-gated potassium channel in T lymphocytes. My main contribution in this regard was the identification of the channel gene encoding this channel (Kv1.3) and that in T lymphocytes this channel is expressed as a tetramer of four identical units. Normally, voltage-gated potassium channels are made out of four subunits. These subunits may be identical or different. It is obvious that the subunit composition of the functional channel influences the characteristic properties of the channel including the binding properties of possible modulators. Therefore it was essential for the development of Kv1.3-specific modulators to know the subunit composition. In addition, the identification of the channel genes and the expression of these genes in cell lines allows for modulator screens without having to isolate the specific tissue in which the channel is normally expressed.

AS412

Crossing the channel

Ion channels exist in all cells, and play a crucial role in cellular function. Understanding their structure and physiology could pave the way for new therapies for a whole host of diseases caused by their dysfunction

ION CHANNELS ARE crucial to cellular function through their role in regulating the electrical voltage gradient (potential) across the cells' plasma membranes. These microscopic proteins are embedded in nearly all cell membranes and their organelles, and control the flow of charged ions in and out. While ubiquitous and essential for functions such as secretion, absorption, electrolyte transport, and cell division and proliferation, their role is especially pertinent in neurons (nerve cells), which allow all sensory and motor functions to occur, including brain activity, muscle contraction and the generation of each heartbeat.

Neurons relay information by transmitting electrical impulses through their cell bodies and extensions (axons) to adjacent neurons across synapses (the junction between these cells). The charge inside the neuron is usually negative relative to the outside of the cell, with the difference known as the membrane potential. When stimulated, sodium ion (Na^+) channels open to allow an influx of positively-charged Na^+ into the cell, causing the membrane potential to drop. This process is known as depolarisation. When a section of the membrane is depolarised, local voltage-gated (activated by a change in voltage) potassium ion (K^+) channels open, leading to an exodus of positively-charged K^+ and subsequent repolarisation. With a strong enough stimulus, the influx of Na^+ outnumbers the K^+, resulting in a positive feedback loop that propagates the signal along the length of the axon. At the peak of this process, known as the action potential, the polarity of the plasma membrane reverses, causing the Na^+ channels to shut, while the K^+ channels stay open till hyperpolarisation (when the charge inside the cell drops below its resting potential) occurs, hence limiting the duration of depolarisation.

FIGURE 2: Top view of an *h*Kv1.3 channel interacting with a peptide toxin (green).

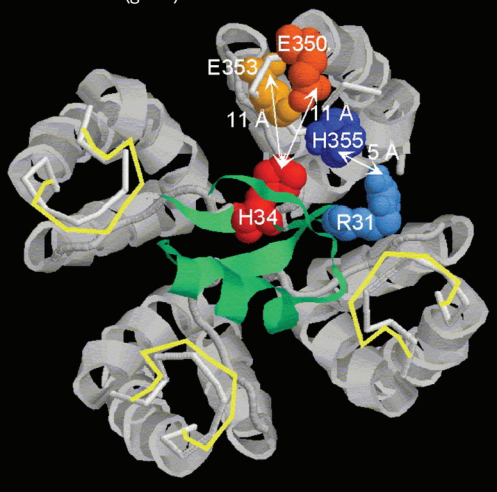
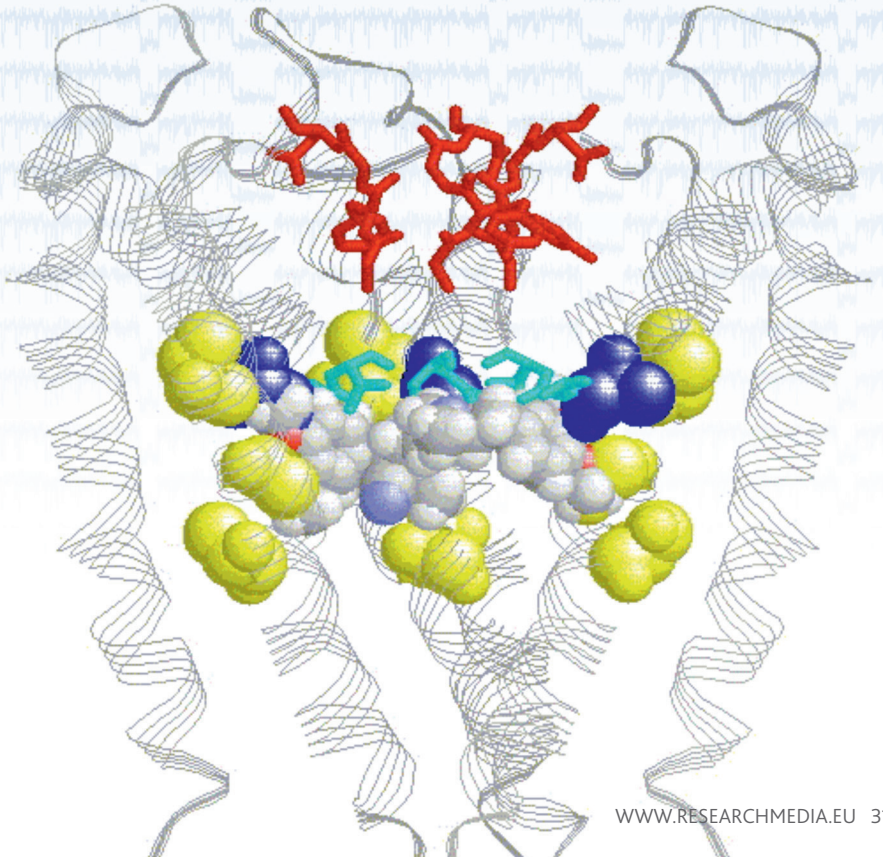


FIGURE 3. Side view of an *h*Kv1.3 channel interacting with verapamil (white balls), interacting amino acids in the channel in yellow balls.



Pathological functioning of ion channels can lead to a variety of human diseases that affect the central nervous, musculoskeletal, and cardiac systems

STUDIES ON THE *h*Kv1.3-SPECIFIC C-TYPE INACTIVATION

OBJECTIVES

The idea behind the project is the development of specific drugs that modify the function of specific ion channels, in this case the *h*Kv1.3 potassium channels. Since these channels are present in T lymphocytes, *h*Kv1.3 channel modulators could be used as immunosuppressants or for the treatment of some autoimmune diseases.

KEY COLLABORATORS

Former collaborators include:

Dr John Adelman (Oregon Health & Science University, Portland, USA), **Dr Michael D Cahalan** (University of California, Irvine, USA), **Dr K George Chandy** (University of California, Irvine, USA), **Dr Hubert Kerschbaum** (University of Salzburg, Austria), **Dr Eva Küppers** (University Tübingen, Germany), **Dr Anil K Lala** (IIT Mumbay, India), **Dr Andre Menez** (CEA, Saclay, France), **Dr Jean-Marc Sabatier** (CNRS, Marseille France), **Dr Heike Wulff** (University of California, Davis, USA), **Dr Boris Zhorov** (McMaster University, Hamilton, Ontario, Canada), **Pfizer Inc** (Groton, USA), **Pfizer Ltd** (Sandwich, UK), and **4SC AG** (Martinsried, Germany)

Current collaborator:

Dr Rüdiger Ettrich (University of South Bohemia, Czech Republic)

FUNDING

DFG – German Research Foundation

CONTACT

Professor Dr Stephan Grissmer

Institute of Applied Physiology
Ulm University
Albert-Einstein-Allee 11
D-89081 Ulm
Germany

T +49 731 500 23253
F +49 731 500 23260
E stephan.grissmer@uni-ulm.de

www.uni-ulm.de/uni/fak/medizin/grissmer

PROFESSOR GRISSMER obtained a Biology Diploma (1982) and a PhD in Physiology (1985) from the University of the Saarland in Homburg, Germany. For almost 10 years he conducted research at the University of California, Irvine, before becoming a Professor at Ulm University, Germany in 1994 where he has been based ever since.

STEMMING THE FLOW – C-TYPE INACTIVATION OF *h*Kv1.3

Pathological functioning of ion channels can lead to a variety of human diseases that affect the central nervous, musculoskeletal, and cardiac systems, and as such, understanding these mechanisms is the focus of much research globally. Professor Stephan Grissmer is the leader of a research group at Ulm University that is investigating the physiology of a specific mammalian K^+ channel named *h*Kv1.3. *h*Kv1.3 is found exclusively in T cells in the form of a membrane protein consisting of four identical units. Grissmer's team has been attempting to elucidate the mechanisms of the C-type inactivation of this ion channel – a state in which it changes its structural formation to prevent the outflow of K^+ and hence allow a more prolonged depolarisation – essential for some forms of neural functioning. This reformation is different from the normal closed position of the channel when the membrane is polarised, and is instead a temporary holding mechanism suited for this specific purpose.

By understanding the molecular mechanisms of *h*Kv1.3, Grissmer hopes its function can be modulated and lead to new therapies for conditions caused by its dysfunction. The key to this, he believes, is identifying the specific amino acids responsible for the transformation of the channel during the C-type activation, and those that act synergistically to stabilise this temporary structure. In order to examine the unique characteristics of *h*Kv1.3 in this state, the project scientists have employed some ingenious methods to grow the channel in isolation. They have transfected the *h*Kv1.3 gene into cells that do not normally contain this channel in their membranes, resulting in its transcription by these cells when they are cultured in an incubator that mimics the conditions of the human body. The constituent protein of this ion channel, consisting of several hundred amino acids, is embedded into the cell membrane automatically, and can be modified genetically through altering the gene to enable different amino acid expression. This flexibility has allowed the researchers to be able to create bespoke versions of the channel with slightly different electrophysiological characteristics according to the particular functions of each amino acid in that section of the molecule, as Grissmer explains: "We could conclude whether the changed amino acids in the channel are involved in toxin binding or opening of the channel or the C-type inactivation".

SHIFTING STRUCTURES

In the course of their research, the project collaborators were able to identify a network of amino acids that are likely candidates for maintaining the structural stability of the C-type inactivated state of the *h*Kv1.3 ion channel. They have engineered modified versions of the channel in which specific amino acids have been replaced, for example with cysteine, which can be modulated by methanethiosulfonate (MTS) reagents. Using the whole-cell patch-clamp method to assess K^+ flow through the *h*Kv1.3 channels, they have been able to determine whether modulating the cysteines led to a change in current flow, and hence whether cysteine itself was accessible for modification *in vivo*. These techniques have allowed them to identify the specific amino acids in the ion channel that change position during the C-type inactivation, and whether they are susceptible to manipulation by MTS reagents in this state. However, it is important to note that *h*Kv1.3 is, unsurprisingly, structurally very similar to other K^+ channels, and that substances that bind to and modulate one type could also affect the other. This is why they have consciously focused on the C-type state, as this is a unique feature of *h*Kv1.3 that is not found in the other K^+ channels. Grissmer believes that those amino acids that are only exposed during the C-type state are the best candidates for modification, and it may be that this degree of specificity paves the way for therapies that have few side effects due to their pinpoint accuracy in targeting this group of proteins only.

Grissmer's findings are still a work in progress, with additional confirmation needed to provide solid evidence of the role these amino acids play when changing position during C-type inactivation. As he states: "Our current model predicts several hypotheses, which need to be tested, verified or falsified". During their preliminary studies they also discovered an additional current pathway in one of their modified *h*Kv1.3 channels that was permeable to Na^+. The region of this leakage corresponded to an area that is commonly mutated in other ion channels, and this is thought to be the cause of a variety of diseases, such as hemiplegic migraines, in which extended depolarisations are believed to occur due to the unstemmed influx of Na^+. This is a source of potential future research for Grissmer and his team, who have already achieved so much towards enhancing our understanding of how microscopic ion channels function, and, importantly, how their dysfunction might be treated in the future.

ulm university universität ulm