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The cellular power station of the cholera pathogen – from the structure to new antibiotics

The bacterium Vibrio cholerae causes cholera, a severe disease that affects up to 3.5 million people a year. A team of scientists from the universities of Freiburg, Hohenheim and Konstanz have now gained new insights into the way the bacterium produces energy. They have elucidated the structure and function of the bacterium's energy-production machinery. The research results provide new insights into biochemical energy production and the development of new antibiotics that specifically target V. cholerae and other pathogenic bacteria.

A team in private and professional life: Prof. Dr. Julia Fritz-Steuber und Dr. Günter Fritz.

The bacterium's "power plant", i.e. its energy production machinery, is a potential target in the search for new drugs to combat V. cholerae. V. cholerae and most other living organisms generate their energy by way of the so-called respiratory chain, along which electrons are transferred by a series of proteins or protein complexes. The respiratory chain of V. cholerae is a sodium ion pump that builds up an ion gradient across the cell membrane, which in turn drives other cellular processes. This sodium pump is the Achilles heel of the cholera pathogen. "We know from sequence comparisons that the sodium pump is prevalent among pathogenic bacteria, but that it is completely different from the one that humans have. This increases the chance that we will be able to specifically interfere with the bacterial pump," explains Dr. Günter Fritz, head of the Protein Biophysics and Biochemistry group at the Freiburg University Medical Centre. Working with microbiologist Prof. Dr. Julia Fritz-Steuber from the University of Hohenheim and bioinformatician Prof. Dr. Kay Diederichs from the University of Konstanz, Fritz successfully elucidated the structure and function of the bacteria's sodium pump.

The long road from protein to crystal structure

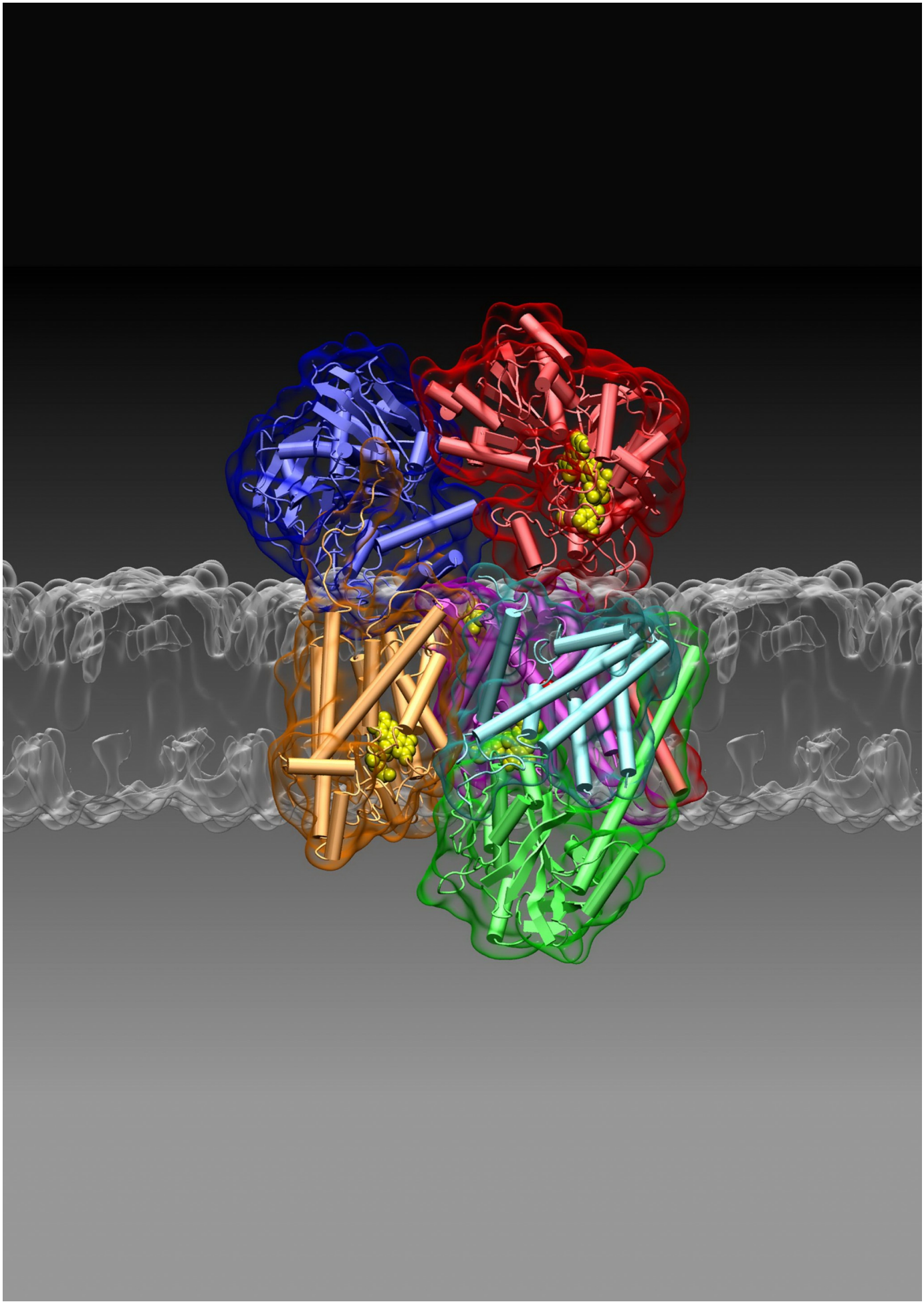
Bacterial sodium ion pumps have been the subject of extensive study. However, although details about the composition of the protein subunits and characteristics of individual cofactors were known, nothing was previously known about how individual components interacted and formed a functional system. Julia Steuber studied the bacterial sodium pump and its role in the physiology of V. cholerae from a microbiological perspective for many years, and was eventually able to isolate the V. cholerae sodium pump, without which it would have been impossible to study the pump's structure in detail. "Julia's work provided us with enough highly pure material to do the first investigations," says Günter Fritz who was in charge of crystallising the protein complex and optimising and analysing the crystals, which is a time-consuming process. Fritz also coordinated the joint project and successfully pooled the expertise of the partners involved.

"There is only one method to analyse the structure of such a large macromolecule at the atomic level. And this is a method known as protein crystallography," says Kay Diederichs who specialises in challenging X-ray structure analyses and has a great deal of experience of membrane proteins. Together with Günter Fritz, Diederichs has identified the optimal combination of methods for analysing the structure of the V. cholerae sodium pump. It was particularly helpful that Diederichs, who has co-authored a computer programme that is used for the majority of X-ray structure analyses around the world, is also a specialist in the analysis of crystallographic data.

The investigation of the pump's structure and function gave rise to a number of technical difficulties. "Crystal cell parameters that differ greatly between measurements, many reflexes of low resolution only, the absence of symmetric structures in the molecule and the absence of related structures meant that the structural analysis of the sodium pump presented a real challenge," explains Diederichs.

The success of the project depended on more than just the expertise and efforts of the cooperation partners and the long-term funding from Baden-Württemberg Stiftung. "Without the geographic proximity and easy access to the Swiss Light Source synchrotron, technical improvements in the field of X-ray detectors in recent years and the development of new crystallography programmes, our work would not have been possible," says Diederichs.

The three scientists, who know each other from their university and research days in Konstanz, have now succeeded where others have failed. "We have been able to describe the molecular structure of the protein complex with unprecedented accuracy," says Fritz. The detailed study of the protein



The “power plant” of the cholera pathogen deciphered by a team of researchers from Freiburg, Hohenheim and Konstanz is one of the largest membrane protein complexes known to date.

complex's structure also enabled the researchers to explain the function and interaction of the individual components. They were able to elucidate in detail how the ions are transported across the membrane. "Our data suggest that we have discovered a previously unknown pumping mechanism and, in the future, we hope to be able to understand how it works and how it is regulated," says Fritz.

New antibiotics without adverse effects

The team of researchers was able to provide detailed insights into the evolutionarily conserved, and hence essential, components of the sodium pump. They have contributed to a better understanding of the V. cholerae sodium pump as well as of that of other pathogenic bacteria. "Data about the pump's structure have already given us an idea about the site where potential inhibitors might be able to bind. We hope that these data will help us identify inhibitors that selectively inhibit the V. cholerae sodium pump, but which are not associated with adverse effects for humans," says Fritz.

In addition to using the structural data for developing new antibiotics, the information about the function of the bacteria's sodium pump is also of major importance for basic research applications. "It is a model system that can be used to answer fundamental questions on biological energy conversion, by which I mean the conversion of chemical energy into electric energy," says Fritz. The new investigation strategies and protocols developed in the cooperative project can also be used for similar systems. "As far as our research is concerned, the results will definitely contribute to obtaining successful results in projects of a similar degree of complexity much more quickly," concludes Fritz.

Original publication:

Steuber J, Vohl G, Casutt MS, Vorburger T, Diederichs K, Fritz G. Structure of the V. cholerae Na+-pumping NADH:quinone oxidoreductase. Nature 2014 Dec 4;516(7529):62-7. doi:10.1038/nature14003

Further information:
Dr. Günter Fritz
Protein Biophysics and Biochemistry
Institute of Neuropathology
University of Freiburg
E-mail: guenter.fritz(at)uniklinik-freiburg.de

Prof. Dr. Kay Diederichs
Molecular Bioinformatics
Department of Biology
University of Konstanz
E-mail: kay.diederichs(at)uni-konstanz.de

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Bettina Baumann
BioLAGO