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Cluster of the future

nanodiagBW: using nanopores to create completely new diagnostic possibilities

Modern medicine has a wide range of molecular diagnostics at hand. In the next decade, this will increasingly be supplemented by prognostic methods. The BMBF Cluster of the Future finalist, nanodiagBW, is developing prognostic methods to identify epigenetic factors for diseases through a new type of bioanalytics – single molecule analysis in nanopores – which would make it possible to take personalised prevention approaches.

Nanopores are pores of nanometre size. When they are integrated into an electrically insulating membrane that separates two chambers filled with a conductive solution, they can be used to detect individual molecules. This works as follows: when a voltage is applied between the two chambers, ions will, driven by the electric field, migrate through the nanopore. The ionic current decreases while a molecule migrates through the pore. Detection of the ionic current allows conclusions to be drawn about the type of molecule migrating through the nanopore.

Using micrometre dimensions, a similar measurement principle has been used since the 1960s for counting or sizing cells; and, with the help of bacterial pore proteins, for individual molecules with nanometre dimensions since the mid-1990s. These protein pores led to a breakthrough in sequencing technology as recently as a few years ago. These third-generation sequencing methods (TGS) can be used for single-molecule analyses with enormous read lengths and have since opened up completely new possibilities, for example for sequencing entire genomes in a short time.

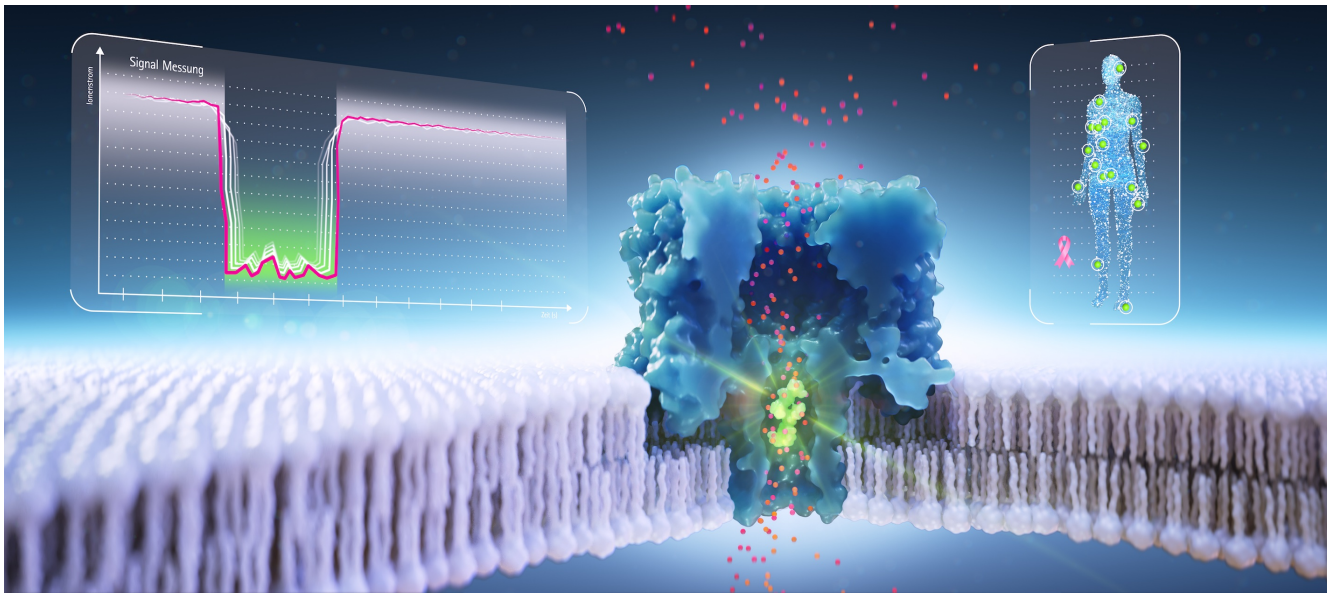
New nanopore method recognises all twenty proteinogenic amino acids

Until now, proteins have been sequenced using complex and expensive mass spectrometric methods in combination with chromatographic separation steps. Chemical modifications – often essential for protein function – can be detected but often not reliably localised.

This is set to change in the near future: Prof. Dr. Jan C. Behrends, a researcher from Freiburg, Germany, together with colleagues, recently developed a way to detect and characterise all twenty proteinogenic amino acids based on the ionic current of a biological nanopore.[1)] The technique could open up entirely new possibilities in the future. "We can't do de novo sequencing at the moment, but we have developed a proof of principle for a sequence recognition method," Behrends reports. "In addition, we can detect posttranslational modifications and even localise the precise amino acid where the change has occurred. Mass spectrometry struggles to do this and the accuracy is not great. With the help of the pores, we have also distinguished peptides with identical masses. So we now have a detector for the molecular shape available to us, so to speak."

Behrends and his research group at the Institute of Physiology at the University of Freiburg developed the technical requirements for this innovative protein analysis technology in cooperation with the spin-off company IONERA Technologies GmbH.[2)] Together with the Department of Microsystems Engineering – IMTEK – at the University of Freiburg and the Hahn-Schickard-Gesellschaft für angewandte Forschung e.V., initial steps were also taken to develop a suitable amplifier technology that can be technically integrated with the nanopore and membrane in a very small space.[3)]

Positionally accurate protein analysis detects influencing factors for diseases



Molecular biological diagnostics using nanopores is expected to open up completely new possibilities in medicine in the future. Researchers from a wide range of disciplines have joined forces in the nanodiagBW cluster to bring the technology to patients as quickly as possible.
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Leading international researchers from Baden-Württemberg saw the enormous potential for modern medicine and felt it had to be exploited as quickly as possible. So they sent a joint application to the BMBF in February 2021 for the establishment of the nanodiagBW cluster, with the intention of developing the method for the positionally accurate analysis of proteins and bringing it to application within the next few years.

Independent experts also believed that the transfer into application needed to proceed as quickly and effectively as possible. In spring 2021, a BMBF jury selected 15 from a total of 117 proposals submitted to the Clusters4Future ideas competition to receive funding for a conception phase; the selected proposals included nanodiagBW and another application from Baden-Württemberg.

Baden-Württemberg offers everything the cluster needs

"We have already been working hard on preliminary projects, and we will be ready to start the conception phase shortly," says Prof. Dr. Felix von Stetten, director of the Hahn-Schickard Institute of Microsystems Analysis in Freiburg and spokesperson of nanodiagBW. "Our networking activities bring together around 40 stakeholders, mostly from Baden-Württemberg."

Numerous other activities are to be added to nanodiag's research and development activities, for example graduate programmes, cooperation with other associations, specialist group events for medical professionals, patients and interested members of the public and start-up academies. "What we particularly like about Baden-Württemberg is that we have here all the technological components that we want to bring together in the cluster's innovative ecosystem in which we can make use of them to provide improved prognostics for patients," says von Stetten.

Prognostics is the medical future

"And the relevance for future medical applications is quite good as well," points out Behrends, who is deputy spokesperson for nanodiagBW. He continues: "The ability to detect epigenetic markers is an extremely hot field of research. The markers have been shown to be associated with many diseases of civilisation. For example, we know that certain histone acetylations play a role in cell growth, therefore also in the development of cancer or developmental disorders. Bacterial and viral genomes are also a worthwhile target. If we are able to detect such markers reliably, physicians will be able to work in a whole new dimension. We would then be talking 'prognostics' rather than 'diagnostics'.

Another aspect the nanodiag cluster is focused on is products that could emerge from the technology platform: "The cluster is intended to be a breeding ground for numerous spin-offs," says von Stetten. "Various assays that could be marketed as diagnostic products are possible. But the entire platform can also be turned into a diagnostic product, obviously once it has been developed further. Our vision is to be able to provide 'on-site tests' in the long term, but the first milestone will be a cost-effective benchtop system."

Medical milestone that can only be achieved synergistically

At the moment, the nanopore sensors are being manufactured in the institutes of the Baden-Württemberg Innovation Alliance and the University of Freiburg, but eventually an entire production line will be set up. At present, the technology is on the threshold of implementation, as von Stetten comments: "However, it is not yet possible to develop a product. To do this, we depend on synergies in the cluster and the opportunity to work with the same team over the next ten years." "Concrete applications are already in sight", he adds. The main focus will initially be on epigenetic markers for oncology, but accelerated vaccine development is also on the agenda.

Infobox: nanodiagBW

In the nanodiagBW cluster, players from basic science, applied research and industry are working together under the leadership of Hahn-Schickard Gesellschaft für angewandte Forschung. Based on the latest advances in single-molecule analysis, the cluster has the goal to develop and bring to application molecular diagnostic systems involving nanopores. This new type of biosensor technology should make it possible to determine for the first time epigenetic changes in proteins with positional accuracy using a small diagnostic device. These changes are causally associated with many diseases, such as cancer, diabetes, neurodegenerative diseases and hypertension. Being able to efficiently determine these post-translational modifications is expected to improve the prognosis of patients with such diseases in the future.

The potential of the technology for application in medical diagnostics is already internationally recognised, but not yet exploited. nanodiagBW therefore aims at technical sovereignty in this field, in which research groups from Baden-Württemberg are already active and successful in terms of internationally visibility and excellence – but not yet active enough when it comes to working synergistically. Examples of application scenarios can be found in epigenetics, cancer research, virology and microbiology.

The cluster's strategy is based on the already proven collaboration between application-oriented research institutes in the Innovation Alliance Baden-Württemberg and academic players in nanopore analytics, for which the state government already granted start-up funding of around 5 million euros over two years in 2020. The current aim of the cluster is to expand, intensify and stabilise these activities by involving many other players from the clinical and industrial sectors in order to consistently drive forward the concrete implementation of processes and products. An application will be made to the BMBF for 45 million euros in funding over a period of 9 years.

References:

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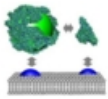
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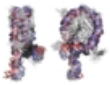
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Nanobiotechnology



Epigenetics – heritable traits without changing the DNA sequence

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Further information to the project:

Using nanopores to detect epigenetic factors influencing disease