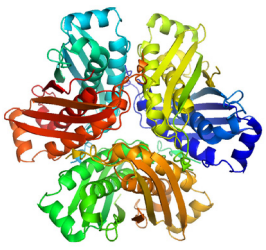
Single-molecule Protein Sequencer changes the paradigm of proteomics

Recent breakthroughs in molecular biology have started the era of genomics and proteomics. This has brought hope to diagnose diseases such as cancer at the gene and protein level. However, personal diagnosis remains challenging because protein expression profiles vary widely among individual human beings and protein expression changes rapidly in time.

A team of TU Delft scientists is developing a new proteomics tool, which is based on single-molecule detection. This highly sensitive proteomics tool will enable medical doctors to analyze even a small number of protein molecules from a patient's tissue or body fluid.
A proteomics tool for medical diagnosis should have high fidelity, use only a small quantity of sample and be able to detect minor protein species. Current protein sequencing techniques, mainly based on mass spectrometry, have such fundamental shortcomings that they cannot fulfill these three criteria all together.
First, with the mass spectrometry analysis, only segments of proteins can be sequenced (about 10-20 amino acids), which sets the upper limit of the prediction fidelity. Secondly, the mass spectrometry requires a large quantity of protein samples. However, if a sample is obtained from a patient's tissue, this demand is hard to fulfill. Thirdly, in the spectral analysis, it is difficult to identify minor species that are embedded among other dominant species.



Therefore, it remains a challenge to analyze cellular proteins that are intrinsically heterogeneous and exist over a wide dynamic range.

High fidelity

A team of TU Delft scientists is developing novel sequencing methods, using single-molecule fluorescence and nanopore technologies. Individual protein molecules will be threaded into a nanochannel, and their sequences will be read from one end to the other in a linear manner. The read of a full sequence will bring about high prediction fidelity. Since all the protein molecules will be read one by one, the single-molecule sequencing scheme will cover a complete protein population, including low-abundance proteins. Furthermore, the high sensitivity of single-molecule fluorescence and nanopore will require only femtomolar level samples for analysis.

Fingerprinting approach

Single-molecule protein sequencing is challenged by the fact that proteins are composed of 20 amino acids. Unlike single-molecule DNA sequencing, in which 4 nucleotides (A,G,C,T) can be distinguished with four different colors or electric signals, it is impossible to distinguish twenty different amino acids at the single-molecule level, using existing technology. Therefore, the sequencers of the team of TU Delft scientists are based on a fingerprinting approach. Their computational analysis, using a human proteomic database, suggests that this fingerprinting approach will be highly accurate in prediction and robust against any errors.

Development

Both a measuring device for scientific research and a measuring device for accurate protein analysis for medical purposes will be developed.

Foto: Sander Foederer

As clinical applications, one may think of accurate protein analysis of human tissues and serums, as well as a preventive test for colon cancer detection in identified risk groups. Therefore, this novel sequencing approach, that will change the paradigm of proteomics, is very interesting for biological researchers, medical professionals as well as for diagnostic companies.

Advantages

- A small amount of sample (e.g. brain tissues) required: applicable to medical diagnosis
- An entire population probed: suitable for proteomic analysis
- A full length sequenced in real time: less prone to errors
- Compatible with a mixed protein population

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