Backing out of the nanotunnel: New method for nucleic acid analysis

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Nanopores provide a versatile tool for probing molecular structures. A new German study shows that one can obtain more detailed information about the dynamic behavior of nucleic acids during passage through nanopores by directing them to asymmetric pores for the return journey.

In the world of biomolecules such as proteins and the hereditary nucleic acids DNA and RNA, three-dimensional structure determines function. Analysis of the passage of such molecules through nanopores offers a relatively new, but highly promising, technique for obtaining information about their spatial conformations. However, interactions between the test molecules and the proteins used as pores have so far hindered quantitative analysis of the behavior of even simply structured molecules within nanopores. This problem must be solved before the technique can be routinely used for structure determination. In a project carried out under the auspices of the Cluster of Excellence "Nanosystems Initiative Munich" (NIM), researchers led by LMU physicist Professor Ulrich Gerland and Professor Friedrich Simmel (Technical University of Munich) have developed a new method that depends on the analysis of reverse translocation through asymmetric pores, which minimizes the interference caused by interactions with the pore material itself. This approach has enabled the team to construct a theoretical model that allows them to predict the translocation dynamics of nucleic acids that differ in their nucleotide sequences.

The nucleic acids RNA and DNA both belong to the class of molecules known chemically as polynucleotides. Both are made up of strings of

four basic types of building blocks called nucleotides, which fall into two complementary pairs. In their single-stranded forms, DNA and RNA can fold into what are called secondary structures, as complementary nucleotides in the sequence pair up, forcing the intervening segments to form loops. If the single-stranded loop is very short, the secondary structure is referred to as a hairpin. As in the case of proteins, the secondary structures of nucleic acids influence their biochemical functions. The elucidation of the secondary structure of nucleic acid sequences is therefore of great interest.

"Nanopores are increasingly being employed to investigate the secondary structures of RNA and DNA," Gerland points out. "Passage through narrow nanopores causes the sequence to unfold, and the dynamics of translocation provide insights into the structural features of the molecules, without the need to modify them by adding a fluorescent label. The technique is relatively new, and its potential has not yet been fully explored."

In the new study, he and his collaborators used a new experimental procedure, which allowed them to quantitatively describe the passage of simply structured polynucleotide sequences through nanopores, and develop a theoretical model that accounts for their findings. This level of understanding has not been achieved previously, because complicating factors such as interactions between the protein nanopore and the polynucleotide have had a significant influence on the measurements and made it difficult to predict the behavior of the test molecules.

Thanks to a clever change in experimental design, the impact of these factors has now been minimized. The trick is to perform the measurements on molecules as they translocate through the pore in reverse. First, the polynucleotide of interest is forced through the conical orifice from one side under the influence of an electrical potential. This causes its secondary structure to unfold and, as it emerges, the molecule

refolds. An anchor at the end of the polynucleotide chain prevents it from passing completely through the pore onto the other side. For the return journey the potential is reversed, so that the process of unfolding now begins at the narrow end of the pore, and at this point the analysis is initiated.

"In contrast to the situation during forward translocation, no significant interactions appear to take place during the reverse trip," says Simmel.

On the basis of their experimental measurements, the researchers went on to construct a theoretical model that enabled them to predict the translocation dynamics of various hairpin structures with the aid of thermodynamic calculations of so-called "free-energy landscapes".

"This model could in the future provide the foundation of a procedure for the elucidation of the secondary structures of complex polynucleotides," says Gerland.

More information: "Quantitative Analysis of the Nanopore Translocation Dynamics of Simple Structured Polynucleotides" S. Schink, S. Renner, K. Alim, V. Arnaut, F.C. Simmel, U. Gerland *Biophysical Journal* Vol. 102, January 2012, pp 1-11. doi: 10.1016/j.bpj.2011.11.4011

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