DNA clogging in hafnium oxide nanopores investigated for DNA sensing

Single-molecule analysis using nanopores has emerged as a promising method that can be employed across a range of disciplines from analytical chemistry to proteomics. Among these, solid-state nanopores (SSNPs)—which are fabricated from synthetic materials—offer a variety of sensing modalities, mechanical robustness, and stability. However, nonspecific interactions between biomolecules and the sidewalls of the nanopores remain a major limitation that leads to clogged pores in applications such as DNA sensing.

In a recent publication in *The Journal of Physical Chemistry B* (<https://doi.org/10.1021/acs.jpcc.0c07756>), researchers from Uppsala University, Sweden, investigated the clogging behavior in sub-20-nm hafnium oxide (HfO_2) nanopores using an optical sensing platform, focusing on its dependence on DNA length, applied voltage, and surface charge.

“The great advantage of HfO_2 is its excellent chemical stability compared to other materials, especially considering that SSNPs often need to be treated with aggressive cleaning processes. For commonly used SiN_x , SiO_2 , and Si membranes, many reports showed that they suffer slow erosion, which is detrimental for nanopore sensing requiring long-term or repetitive measurements, because the pore conductance will vary due to the pore expansion,” first author Shiyu Li tells *MRS Bulletin*.

The study demonstrated real-time visualization of DNA clogging in nanopore arrays with an average pore diameter of 15 nm using confocal fluorescence microscopy. The number of clogged pores increased with higher bias voltages. Also, clogging increased with increasing DNA length. “The dependence on DNA length can be accounted for by invoking an increased probability of knotting and folding with longer DNA strands, while that on bias is attributed to more frequent translocation events at higher voltage,” Li says.

A decrease in temporary clogging time was observed with increasing voltage. External forces such as electrophoretic force and electroosmotic flow (EOF) are likely responsible for the declogging of the DNA molecules. “ HfO_2 is slightly positive-charged at a physiological pH around 7, which may pose strong electrostatic interaction to negatively charged DNA molecules,” Li says.

The degree of clogging was also found to vary at different pH values, with a higher clogging percentage at low pH. “We showed that the surface charge on pore walls has

a prominent effect on the probability of DNA clogging through electrostatic attraction or induced EOF as a reinforcing or countering factor,” Li says. At low pH the surface is positively charged, and the EOF and electrophoretic force oppose each other, which slows down translocation, increasing the likelihood of pore-clogging.

Researchers also developed an analytical model to assist in their understanding of DNA clogging during translocation. The results not only provide a deeper understanding of the DNA clogging phenomenon, but also can serve as a guide to prevent pore clogging in SSNP-based sensing.

Ralph Scheicher of Uppsala University, Sweden, who was not involved in the study, tells *MRS Bulletin* that “nanopores getting clogged by DNA is a serious problem in the field. The dynamic properties of the clogging are not well understood yet, and this study ... [explored] clogging in nanopore arrays through a clever combination of ingenious experiments and sophisticated theoretical modeling. Crucially, the authors studied nanopores with a diameter smaller than 20 nm, which is a sufficiently narrow opening to obtain a signal-to-noise ratio that would allow one to sequence DNA.” Scheicher expects this study to “boost the applicability of nanopores in the field of rapid DNA sequencing and in the emerging area of single-molecule protein analysis.”

Li thinks these SSNPs can be employed to “pursue DNA sequencing on SSNPs in a similar manner as that achieved in biological pores. A motor enzyme, such as DNA polymerase, can be docked onto truncated pyramidal or funnel-like shape nanopores employed in this work. The wider region of the nanopore can host the enzyme to synthesize DNA while the narrow region can serve as the sensing region to identify the bases. Such efforts should be based on an accurate control of pore geometry and dimension, as well as precise positioning of the motor enzyme with retained activity.”

Xaimara Santiago-Maldonado

Schematic representation of the optical sensing of DNA translocation in HfO_2 -coated Si nanopores. Credit: *The Journal of Physical Chemistry B*.