Project C7: Recognition of synthetic polymers by biological nanopores

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Current state of the research. It has been demonstrated in recent years that protein nanopores can be used as sensors for nonnatural analytes, and in particular for the detection of synthetic polymer chains. Single macromolecules entering into a nanoscopic pore by a random walk or by a drift in a force field induce blockades of the pore. When the pore forms the only connection between two electrolyte reservoirs in the presence of an electric field, these blockades can be detected as sudden drops in the resulting ionic current (resistive pulses). It has been shown using poly(ethyleneglycol) (PEG) as an analyte that, given appropriate conditions, this signal is exquisitely sensitive to the size of the polymer molecule. However, other neutral polymers and especially more complex structures, such as block-copolymers or sequence-defined polymers, have not been studied yet by this method. Also, the physicochemical basis of the high sensitivity is not well understood.

Contribution of the principal investigators. The Behrends group has studied the recognition of model PEG chains in nanopores based on resistive pulse single molecule sensing (see the Figure below) using an innovative microstructured recording device, the microelectrode cavity array (MECA), that allows the otherwise difficult and fragile experimental arrangement for nanopore single molecule detection to be produced very reproducibly and measurements to be performed with enhanced throughput and accuracy. The MECA technology therefore, for the first time, enables systematic, large-scale studies of the interaction of various polymer structures with biological nanopores.

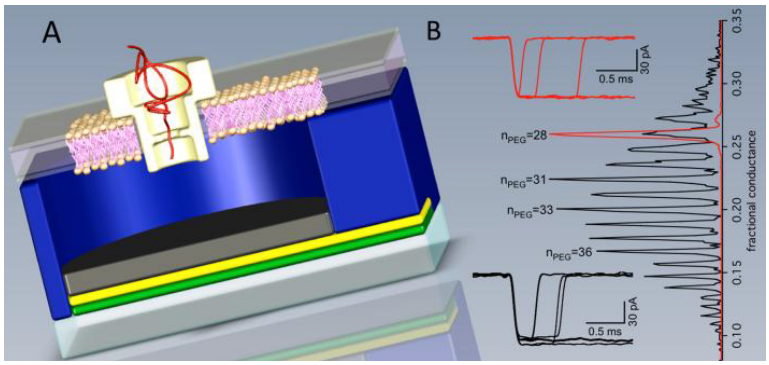

Figure: A) Schematic of nanopore-based resistive pulse sensing. Polymer (red) in a biological nanopore embedded in a lipid bilayer blocks the ionic current measured by the integrated microelectrode (grey). B) Examples: Individual blocking events and histograms of residual conductance in the presence of monodisperse (red) and polydisperse (black) PEG preparations. Note the single-monomer sensitivity of the resulting distributions.

In addition to size-sensitivity, preliminary collaboration between the Freiburg and the Strasbourg groups has shown that the resistive pulse signal is also strongly influenced by the macromolecular structure of the polymer, allowing, for instance, to differentiate between PEG and PDMA. Thus, beyond single molecule mass spectometry, nanopore-based single-molecule detection appears to have potential as a very powerful technique for polymer analysis. In particular, it is of great interest to extend the use of this principle to differentiate between different polymer structures. At present, most of the studies have focused on simple commercial PEG models. Regarding the mechanism of detection, it has been suggested that coordination of cations by PEG as well as salting-out in the pore contribute importantly. However, this has not been verified experimentally using other polymers. Testing a wide variety of water-soluble polymer structures, as envisaged in this project, will deliver important new information regarding the basic mechanisms of interaction. These polymers will be synthesized and characterized at the Institut Charles Sadron (ICS). This part of the project will be supervised by J.-F. Lutz who is the head of the research group *Precision Macromolecular Chemistry* (PMC). The PMC group has expertise in polymer chemistry,

solid-phase synthesis, automated chemistry and polymer characterization. In particular, J.-F. Lutz is a world pioneer in the field of sequence-controlled polymers. Thus, all necessary knowledge and expertise are available for the project.

Research project and collaborations. In this project, a wide variety of water-soluble polymer structures will be tested. As mentioned above, these polymers will be synthesized at the ICS. At first, different types of neutral (e.g. polyacrylamide derivatives) or charged homopolymers (e.g. polyanions) will be tested. Well-defined polymer samples with controlled average molecular weights and narrow molecular weight distributions will be synthesized by controlled radical polymerization (CRP). The next step in this study will be the analysis of more complex copolymer structures. For instance, water-soluble copolymers with controlled microstructures, such as, block, multiblock, gradient or periodic copolymers, will be synthesized and analyzed. All these structures can be prepared using CRP protocols. These types of models will be tested to assess the influence of comonomer composition and chain microstructure on pore/polymer interactions. For example, block copolymers, composed of two water-soluble segments with markedly different pore-responses (e.g. PEG and PDMA), will be synthesized and tested. The ultimate objective of this project is the pore-analysis of more complex sequence-defined macromolecules. During the last five years, the Lutz group has reported some efficient approaches for the preparation of sequence-controlled polymers. For instance, sequence-controlled water-soluble polymers can be synthesized by chain-growth copolymerization or using more complex iterative concepts (e.g. step-by-step solid-phase chemistry). These novel chemical routes will be used in the present project to design optimal water-soluble macromolecules for pore-analysis. One very important aspect of our strategy is the fact that the molecular structure of the polymeric analytes can be adapted and optimized for the analytical technique. Indeed, the pore-responses observed with model homopolymers and copolymers will help to imagine and construct tailor-made ‘readable’ analytes.

Work plan. Two doctoral researchers with complementary scientific profiles will be involved in the project (Strasbourg: polymer synthesis, Freiburg: nanopore analysis).
The model homopolymers will be synthesized during the first months of the project. The chemical protocols for preparing these polymers are already available. While they will be analyzed in nanopores (in Freiburg), more complex macromolecular architectures will be prepared (in Strasbourg). Block and gradient copolymers will be delivered after several months and monodisperse sequence-defined structures will be delivered at the beginning of the second year of the project.