High Temperature Extends the Range of Size Discrimination of Nonionic Polymers by a Biological Nanopore

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Biological nanopores have recently been used to perform the size-discrimination of synthetic polymers[1, 2, 3] or short oligonucleotides[4] with a single monomeric unit resolution. To date, in the case of nonionic polyethylene glycol (PEG) polymers, the polymer-length range of size-discrimination was limited from $\approx$ 15 monomers (678 g/mol) to $\approx$ 48 monomers (2130 g/mol) depending on the nanopore nature and on the experimental conditions. In this work[5] we explore the effect of temperature on the interaction of polydisperse mixtures of PEG polymers of different average molar masses (1250, 1500, 2000, 3400, 6000 and 8000 g/mol) with the biological nanopore α-hemolysin. We find that increasing temperature extends the polymer-length range of application of nanopore-based single-molecule size-discrimination. Indeed, in the case of PEG 3400 g/mol, discrimination of individual molecular species of different monomer number is impossible at room temperature but is achieved when the temperature is raised to 45°C. In addition, and in contrast with what has been previously observed with various nanopores and analytes, we find that, for PEGs larger than a threshold molar mass (2000 g/mol), increasing temperature increases the duration of the PEG/nanopore interaction. In the case of PEG 3400 g/mol the duration increases by up to a factor of 100 when the temperature increases from 5°C to 45°C. We interpret our observations as the consequence of a decrease of PEG solubility and a collapse of PEG molecules with higher temperatures. As a perspective, we plan to extend the nanopore-based single-molecule size-discrimination to biomolecules such as peptides.

References

[1] Robertson, J. W. F. *et al.* Proc. Natl. Acad. Sci. U. S. A. **104**, 8207–8211 (2007).

[2] Angevine, C. E. *et al.* Anal. Chem. **86**, 11077–11085 (2014).

[3] Baaken, G. *et al.* ACS Nano **9**, 6443–6449 (2015).

[4] Cao, C. *et al.* Nat. Nanotechnol. **11**, 713–718 (2016).

[5] Piguet, F. *et al.* Sci. Rep. **6**, 38675 (2016).