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Discrimination of α- and γ-thrombin using aptamer-functionalized nanopore sensing

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Protein detection and identification at the single-molecule level is a major challenge in many biotechnological fields. Solid-state nanopores are label-free sensing tools that have raised attention as biosensors with high sensitivity. Nanopores can characterize single biomolecules such as DNA, RNA and proteins in solution[1,2]. The principle is based on the Coulter counter, a resistive-pulse technique that counts and sizes particles by monitoring the ionic change through a small aperture.

In this work, we aim to discriminate two closely related proteins, α- and γ-thrombin, using aptamer-functionalized nanopores[3]. The selected aptamer targets human α-thrombin, a spherical protein with a molecular weight of 36 kDa and a volume of 49.6 nm^3. Human γ-thrombin is a modified α-thrombin lacking the aptamer binding epitope with a similar molecular weight and volume.

We show that aptamer functionalization improves protein discrimination thanks to a significant difference in the relative current blockade amplitude. To enhance discrimination, we post-processed the signals using machine learning and training algorithms and we reached an accuracy of 98.8 % using 7 features and ensemble methods.

[1] K. Lee, *Adv. Mater. 93*, 1704680–28 (2018)
[2] J. W. F. Robertson, *Proteomics 18*, 1800026–14 (2018)
[3] D. Rotem, *J Am Chem Soc 134*, 2781–2787 (2012)

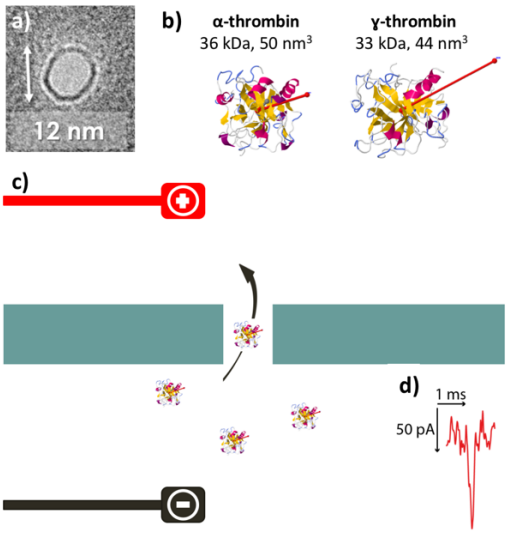

Figure 1 : a) Picture of a 12 nm nanopore drilled inside a transmission electron microscope b) structural view of α- and γ-thrombin with their dipole moment (red arrow) c) basic principle of nanopore sensing d) example of a single translocation event.