Fibrinopeptide A Family Biomarker Identification at Single Molecule Level

Sebastien Balme

Fibrinopeptide A Family Biomarker Identification at Single Molecule Level

Sebastien Balme*

The identification of fibrinopeptide A family biomarkers using aerolysin: a first step toward point-of-care application.

The detection and analysis of peptide biomarkers is one of the most important tools for the early diagnosis of many diseases, which is a key element for personalized medicine. One of the main challenges in this field is the ability to identify small sequence modifications (length, phosphorylation, post-translational modification, and mutation) that are often associated with a given pathology. In this issue of *ACS Central Science*, Pelta, Cressiot, and co-workers report a breakthrough approach to detect and discriminate fibrinopeptide A (FPA) family biomarkers at the single molecule level.[1] Unphosphorylated and phosphorylated FPA are clinical biomarkers of the coagulation system, whose concentration in blood increases in the case of heart disease.[2] In addition, the N-terminal cleaved derivatives FPA-3 and FPA-6 are biomarkers of cancer.[3] These make this peptide a target of choice for the development of a point-of-care device as soon as a simple, accurate, and low-cost detection and analysis technique can be used.

Since its proof of concept 30 years ago,[4] nanopore technology has allowed considerable advances in biomolecule analysis. This technology is based on analysis of the current perturbation induced by the passage of a macromolecule inside a biological nanopore (a-hemolysin, aerolysin, etc.). It has the advantages of being inexpensive, accurate, and extremely reproducible, and has thus allowed the development of long-read DNA sequencing.[5] Following this, the analysis of protein and peptide sequences or their modifications is likely the most important challenge in the field of nanopore sensing.[6] Major recent advances in this field include homopolypeptide identification,[7] protein fragment identification after enzymatic degradation,[8] peptide reading at single amino-acid resolution,[9] and detection of familial mutation of Aβ peptide.[10]

The analysis of protein and peptide sequences or their modifications is likely the most important challenge in the field of nanopore sensing.

Pelta, Cressiot, and co-workers go further in the application of biological nanopores for the sensing of peptide biomarkers, aiming to discriminate FPA, its phosphorylated form (FPA-P), and two cleaved forms (FPA-3 and FPA-6). The team demonstrated that the amplitude of the current blockage is greater for phosphorylated than unphosphorylated FPA (Figure 1). The significant difference in the current blockade level allowed the authors to discriminate the two forms from a mixture by eye without the need for further statistical analysis or extensive signal processing. In addition, two discrete blocking levels were shown for the phosphorylated form relating two conformational states that could significantly improve the accuracy of the nanopore sensor. Using the same method, the team analyzed the truncations FPA-3 and FPA-6, showing as expected a decrease of the blocking amplitude with the size of the peptide (Figure 1). Here again, the precision of the nanopore allows a visual discrimination of the biomarker mixture by

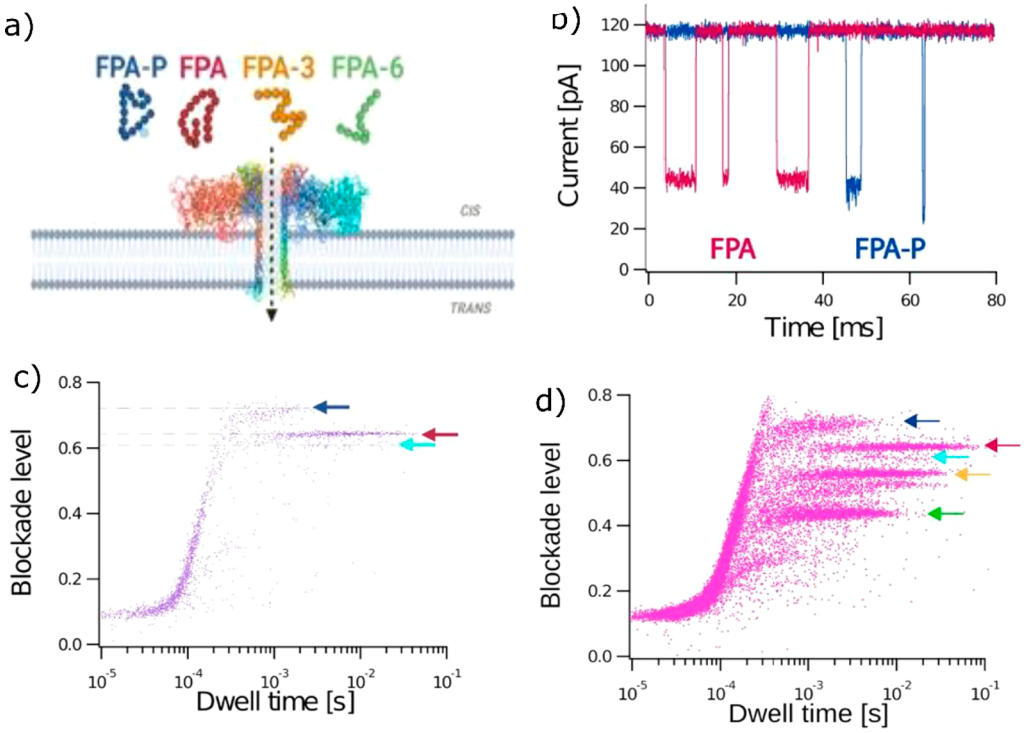

Figure 1. Scheme of FPA detection using the aerolysin nanopore. (b) Representative current traces recorded over 80 ms for FPA (red) and FPA-P (blue) showing the difference in the current blockade amplitude. (c) Scatter plots showing normalized blockade level, defined as (I0 − Ib)/I0, against the dwell time for the mixture of FPA and FPA-P. The arrows denote the different populations of FPA (red) and FPA-P (blue). (D) Scatter plots for the mixture of FPA, FPA-P, FPA-3, and FPA-6 and their associate population denoted by the red, blue, yellow, and green arrows, respectively.

plotting the histogram of the current blocking amplitudes, where each molecule induces a discrete level in the plot.

The team demonstrated that the amplitude of the current blockage is greater for phosphorylated than unphosphorylated FPA.

By using commercial peptides and showing that it is possible to easily discriminate among the different forms of FPA, Pelta, Cressiot, and their team have taken an important step toward the use of aerolysin in point-of-care devices. Indeed, this proof of concept could be extended to a wide range of peptide biomarkers, since it does not involve the use of a specific target. Obviously, several developments will be required to make it a real diagnostic device. A fine analysis of the signal will be essential to achieve an accurate sensor, using a methodology that selects only the translocation events and removes the bumping. This will certainly involve the use of machine learning for both statistical analysis and signal processing. It will also be necessary to demonstrate whether this approach is quantitative to determine the concentration of each biomarker and their ratio. This will involve an understanding and consideration of the energetic barriers of entry of the peptide. Finally, strategies for the analysis of its markers in complex biofluids should also be considered in the future.

By using commercial peptides and showing that it is possible to easily discriminate among the different forms of FPA, Pelta, Cressiot, and their team have taken an important step toward the use of aerolysin in point-of-care devices.

Corresponding Author

Sebastien Balme − *Institut Européen des Membranes, UMR5635 UM ENCSM CNRS, 34095 cedex 5 Montpellier, France;* orcid.org/0000-0003-0779-3384;
Email: sebastien.balme@umontpellier.fr

REFERENCES

(1) Stierlen, A.; Greive, S. J.; Bacri, L.; Manivet, P.; Cressiot, B.; Pelta, J. Nanopore discrimination of coagulation biomarker derivatives

and characterization of a post-translational modification. *ACS Central Science* **2023**, in press. DOI: 10.1021/acscentsci.2c01256

(2) Chapin, J. C.; Hajjar, K. *A.* Fibrinolysis and the control of blood coagulation. *Blood reviews* **2015**, *29*, 17.

(3) Bhalla, S.; Verma, R.; Kaur, H.; Kumar, R.; Usmani, S. S.; Sharma, S.; Raghava, G. P. S. CancerPDF: A repository of cancer-associated peptidome found in human biofluids. *Sci. Rep* **2017**, *7*, No. 1511.

(4) Kasianowicz, J. J.; Brandin, E.; Branton, D.; Deamer, D. W. Characterization of individual polynucleotide molecules using a membrane channel. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 13770.

(5) Clarke, J.; Wu, H.-C.; Jayasinghe, L.; Patel, A.; Reid, S.; Bayley, H. Continuous base identification for single-molecule nanopore DNA sequencing. *Nat. Nanotechnol.* **2009**, *4*, 265.

(6) Alfaro, J. A.; Bohländer, P.; Dai, M.; Filius, M.; Howard, C. J.; van Kooten, X. F.; Ohayon, S.; Pomorski, A.; Schmid, S.; Aksimentiev, A.; Anslyn, E. V.; Bedran, G.; Cao, C.; Chinappi, M.; Coyaud, E.; Dekker, C.; Dittmar, G.; Drachman, N.; Eelkema, R.; Goodlett, D.; Hentz, S.; Kalathiya, U.; Kelleher, N. L.; Kelly, R. T.; Kelman, Z.; Kim, S. H.; Kuster, B.; Rodriguez-Larrea, D.; Lindsay, S.; Maglia, G.; Marcotte, E. M.; Marino, J. P.; Masselon, C.; Mayer, M.; Samaras, P.; Sarthak, K.; Sepiashvili, L.; Stein, D.; Wanunu, M.; Wilhelm, M.; Yin, P.; Meller, A.; Joo, C. The emerging landscape of single-molecule protein sequencing technologies. *Nat. Methods* **2021**, *18*, 604.

(7) Ouldali, H.; Sarthak, K.; Ensslen, T.; Piguet, F.; Manivet, P.; Pelta, J.; Behrends, J. C.; Aksimentiev, A.; Oukhaled, A. Electrical recognition of the twenty proteinogenic amino acids using an aerolysin nanopore. *Nat. Biotechnol.* **2020**, *38*, 176.

(8) Afshar Bakshloo, M.; Kasianowicz, J. J.; Pastoriza-Gallego, M.; Mathé, J.; Daniel, R.; Piguet, F.; Oukhaled, A. Nanopore-Based Protein Identification. *J. Am. Chem. Soc.* **2022**, *144*, 2716.

(9) Brinkerhoff, H.; Kang, A. S. W.; Liu, J.; Aksimentiev, A.; Dekker, C. Multiple rereads of single proteins at single−amino acid resolution using nanopore. *Science* **2021**, *374*, 1509.

(10) Xin, K.-L.; Hu, Z.-L.; Liu, S.-C.; Li, X.-Y.; Li, J.-G.; Niu, H.; Ying, Y.-L.; Long, Y.-T. 3D Blockage Mapping for Identifying Familial Point Mutations in Single Amyloid-β Peptides with a Nanopore. *Angewandte Chemie (International ed. in English)* **2022**, *61* (44), No. e202209970.