

A versatile nanopore identifies four classes of analytes simultaneously

A versatile nanopore sensor, created by modifying a bacterial porin, enables the simultaneous identification of amino acids, nucleoside monophosphates, saccharides and peptides. Coupled with machine learning, it achieves 98.7% classification accuracy and enables the compositional analysis of native glycopeptides.

This is a summary of:

Yao, L. et al. An engineered nanopore identifies saccharides, amino acids, peptides and ribonucleotides. *Nat. Biotechnol.* <https://doi.org/10.1038/s41587-026-03308-9> (2026).

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The problem

Nanopore sensing based on single-molecule chemistry¹ enables fine discrimination of structurally similar analytes. A typical biological nanopore is a channel-forming protein inserted in a lipid bilayer membrane. When voltage is applied across the membrane, an ionic current (the nanopore signal) passes through the channel pore. The interaction of a molecule with the pore alters the current in a specific pattern that enables the identification of the molecule class (for example, amino acids, peptides, nucleotides or glycans), as well as the molecule itself.

Nanopore single-molecule chemistry-based sensing requires an adapter – a molecule suitable for conjugation to a pore constriction. Although various adapters have been previously reported, such as phenylboronic acid for saccharides² and nickel-nitrilotriacetic acid for amino acids³, simultaneous detection of diverse analyte classes using the same nanopore–adapter construct remains challenging. Consequently, because no single pore construct is universally applicable, prior knowledge of the analyte class under investigation is generally required before the measurement, and in practice each experiment is typically confined to only a single class of analyte. This limited measurement approach can easily miss the detection of many analytes when an inappropriate pore type is used. Moreover, nanopore signals that lack an exact match in a reference database are often impossible to assign to a specific analyte class, which further constrains the generality of nanopore-based analysis.

The solution

Mycobacterium smegmatis porin A (MspA) is a bacterial channel protein that can be used for nanopore sensing. We conjugated a custom-designed adapter, maleimido-C2-formylphenylboronic acid (FPBA), to the pore constriction of MspA, creating the engineered nanopore construct MspA–FPBA. We selected maleimido-C2-FPBA for its optimal size, analyte compatibility and ease of synthesis. Our aim was to find an adapter that would confer on MspA the capacity for reversible interactions with amino acids, nucleoside monophosphates (NMPs), saccharides and peptides. The resulting MspA–FPBA nanopore mediates reversible covalent binding to amino acids and their post-translational modifications (PTMs) and to saccharides, peptides and

NMPs (as well as their epigenetic variants) in a single assay (Fig. 1a). MspA–FPBA demonstrates broad analyte coverage, encompassing 21 proteinogenic amino acids, 3 amino acids bearing PTMs, 4 canonical NMPs, 3 epigenetically modified NMPs, 4 monosaccharides and 5 peptides.

Using a database-referenced (supervised) machine learning classifier trained on the signal patterns of known analytes, MspA–FPBA achieved an overall accuracy of 98.7%, underscoring the robustness of this approach for simultaneous analysis of a wide range of analytes within the same assay. We then leveraged the signal patterns of the analytes identified by MspA–FPBA, grouped into amino acids, NMPs, peptides or saccharides, to demonstrate that a few-shot learning strategy, trained on these previously identified molecules, enables the direct classification of previously unseen analytes into their corresponding categories, without requiring their inclusion in the training database (Fig. 1b). We further validated both classification strategies – database-referenced identification and few-shot learning-based classification – through nanopore analysis of a complex biological mixture, namely, yeast cell extract. MspA–FPBA also effectively characterized the composition of G2 glycopeptide, a complex-type N-glycopeptide, suggesting that it could be applied to the compositional analysis of glyco-RNAs and RNA-binding peptides.

Future directions

In principle, MspA–FPBA is also applicable to the analysis of D-amino acids, D-peptides, nucleotide sugars, oligosaccharides and oligonucleotides, thereby further expanding the scope of measurement and the corresponding database. As this database grows, the performance of the machine learning algorithm, including its few-shot learning capability, is expected to improve further, in preparation for the analysis of increasingly complex biological samples.

Looking ahead, by combining the FPBA adapter with our transient pore analyte looping technology⁴ and by integrating proteases, ribonucleases and glycosidases with MspA–FPBA for exogenous sequencing, we anticipate that the integration of multiple sequencing modalities (including nucleic acid, protein and glycan sequencing) into a single and versatile nanopore platform will be possible.

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EXPERT OPINION

"This paper describes a novel nanopore sensor capable of distinguishing 21 amino acids, 4 canonical nucleobases, 4 monosaccharides and several post-translational modifications. Additionally, the introduction of few-shot learning enhanced the detection of unknown or 'out-of-database' events and their corresponding

analytes. Although engineered heteromeric nanopores and the use of deep learning in single-molecule nanopore sensing are already well established, the unification of simultaneous sensing of multiple analytes is innovative." **Liviu Movileanu & Lauren Mayse, Syracuse University, Syracuse, NY, USA.**

FIGURE

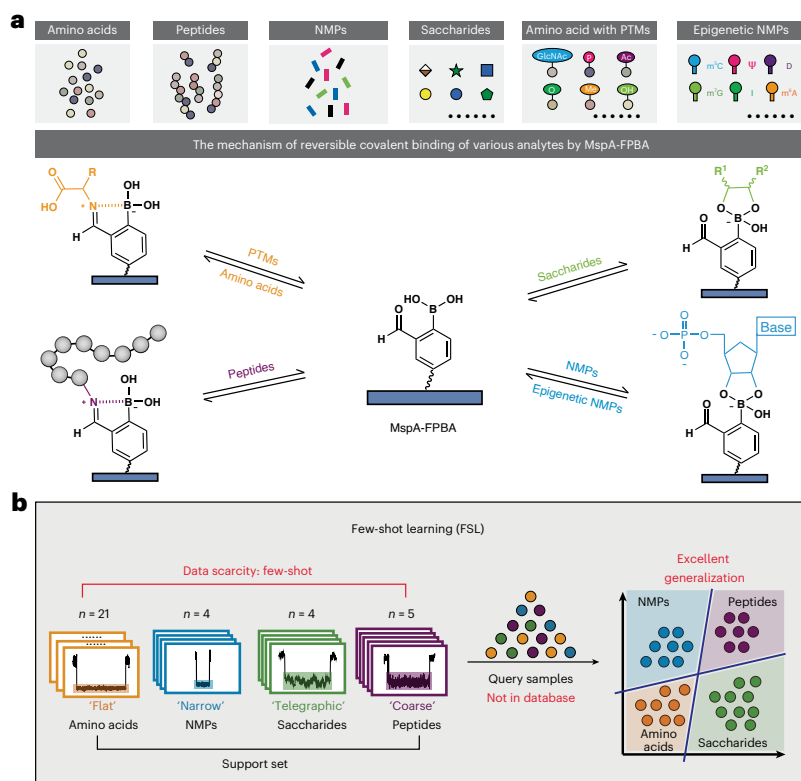


Fig. 1 | Sensing mechanism of MspA-FPBA and workflow of few-shot learning. **a**, Sensing mechanism of MspA-FPBA. MspA-FPBA reversibly interacts with a single analyte at a time, with a broad substrate range that spans from amino acids and their PTMs to peptides, saccharides and NMPs (and their epigenetic modifications). **b**, The workflow of few-shot learning for the classification of out-of-database analytes. © 2026, Yao, L. et al.

BEHIND THE PAPER

This study began with a bold question: can we turn a single nanopore into a versatile sensor capable of detecting nearly all analytes of interest in multi-omics studies, including amino acids, peptides, nucleotides and glycans? Nanopore single-molecule chemistry¹ pointed us toward a promising direction: chemically modifying the pore with a suitable adapter to enhance its resolving power. However, finding a single adapter molecule capable of taking on so many functions at once seemed,

at first, an impossible task. We turned to artificial intelligence to mine the vast body of existing literature for inspiration. This search helped us to narrow the search to several candidate molecules, among which FPBA stood out as the most promising⁵. Guided by this insight, we synthesized a maleimide-linked FPBA derivative tailored for nanopore conjugation, and subsequent nanopore testing confirmed its performance. **Z.W. & S.H.**

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This paper inspired the development of the maleimido-C2-FPBA adapter.

FROM THE EDITOR

"By engineering an *M. smegmatis* porin A nanopore with an adapter at its constriction, the authors develop a nanopore to sense proteins, RNAs and glycans simultaneously. This achievement has been technically challenging owing to the distinct physicochemical properties of these biomolecules." **Editorial Team, Nature Biotechnology.**