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19th NSTDA Annual Conference
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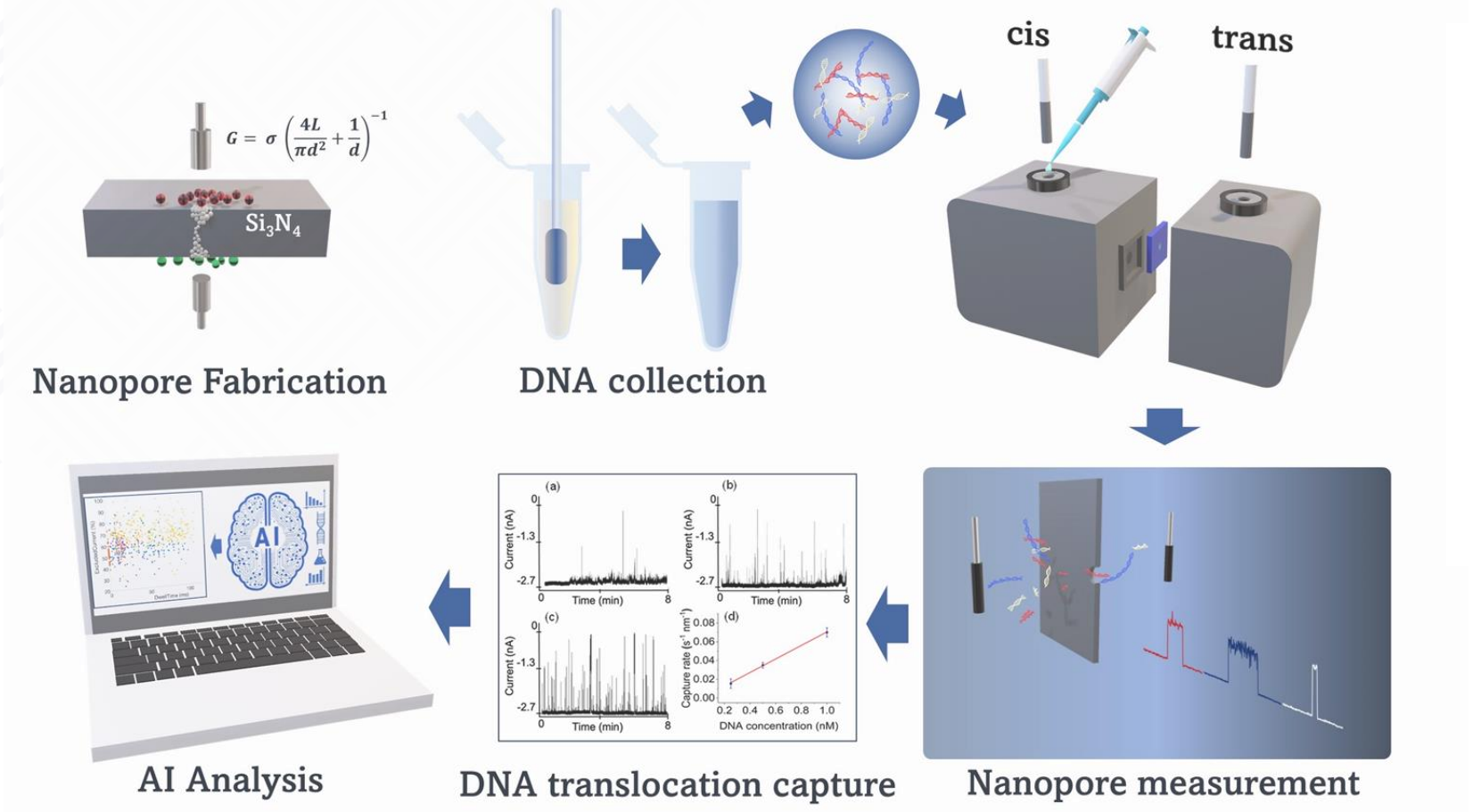
30 มีนาคม 2567

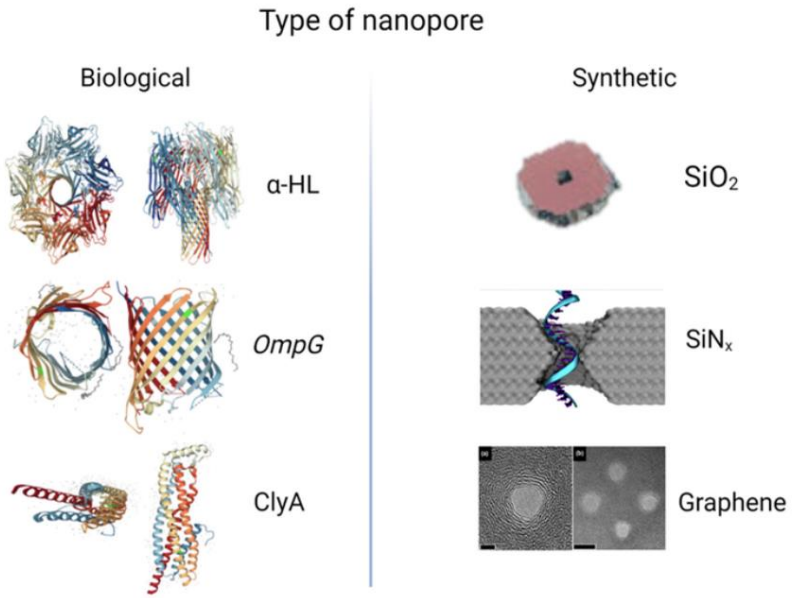
สำนักงานพัฒนาวิทยาศาสตร์และเทคโนโลยีแห่งชาติ

Advancements in Nanopore Technology: Interrogating DNA, Unveiling Proteins, and Predicting Disease with Machine Learning

Dr. Ibrar Alam
National Nanotechnology Center, NSTDA

Introduction to Nanopore





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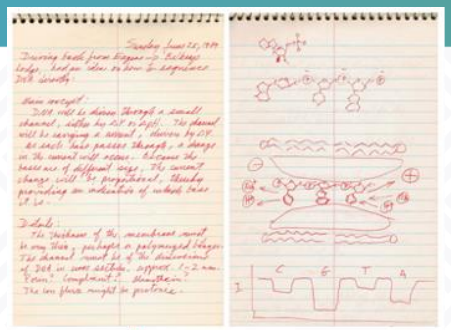
Alam, I., Boonkoom, T., Pitakjakpipop, H., Boonbanjong, P., Loha, K., Saeyang, T., Vanichtanankul, J. and Japrung, D., 2024. Single-Molecule Analysis of SARS-CoV-2 Double-Stranded Polynucleotides Using Solid-State Nanopore with AI-Assisted Detection and Classification: Implications for Understanding Disease Severity. *ACS Applied Bio Materials*, *7*(2), pp.1017-1027.

Brief History



1995 — Church, Deamer, and Branton file patent for Nanopore Sequencing

1996 — First DNA translocation through alpha-hemolysin nanopore

1999 — Purine and Pyramidine segments are distinguished in a single RNA molecules

2001 — Solid state nanopore are produced and used to detect DNA

2005 — Single nucleobase are distinguished

Oxford Nanopore Technology was founded

2008 — Single molecule of DNA was detected with MspA nanopore

2012 — DNA sequencing with Phi 29 polymerase and MspA nanopore

MinION device was developed

2014 — ONT releases MinION device for early users

2015 — E.Coli genome assembled denovo with 99.4% accuracy with MinION

2016 — CRISPR-Cas9 is combined with nanopore technology for precise DNA editing and sequencing.

2017 — Introduction of the PromethION, a high-throughput nanopore sequencer

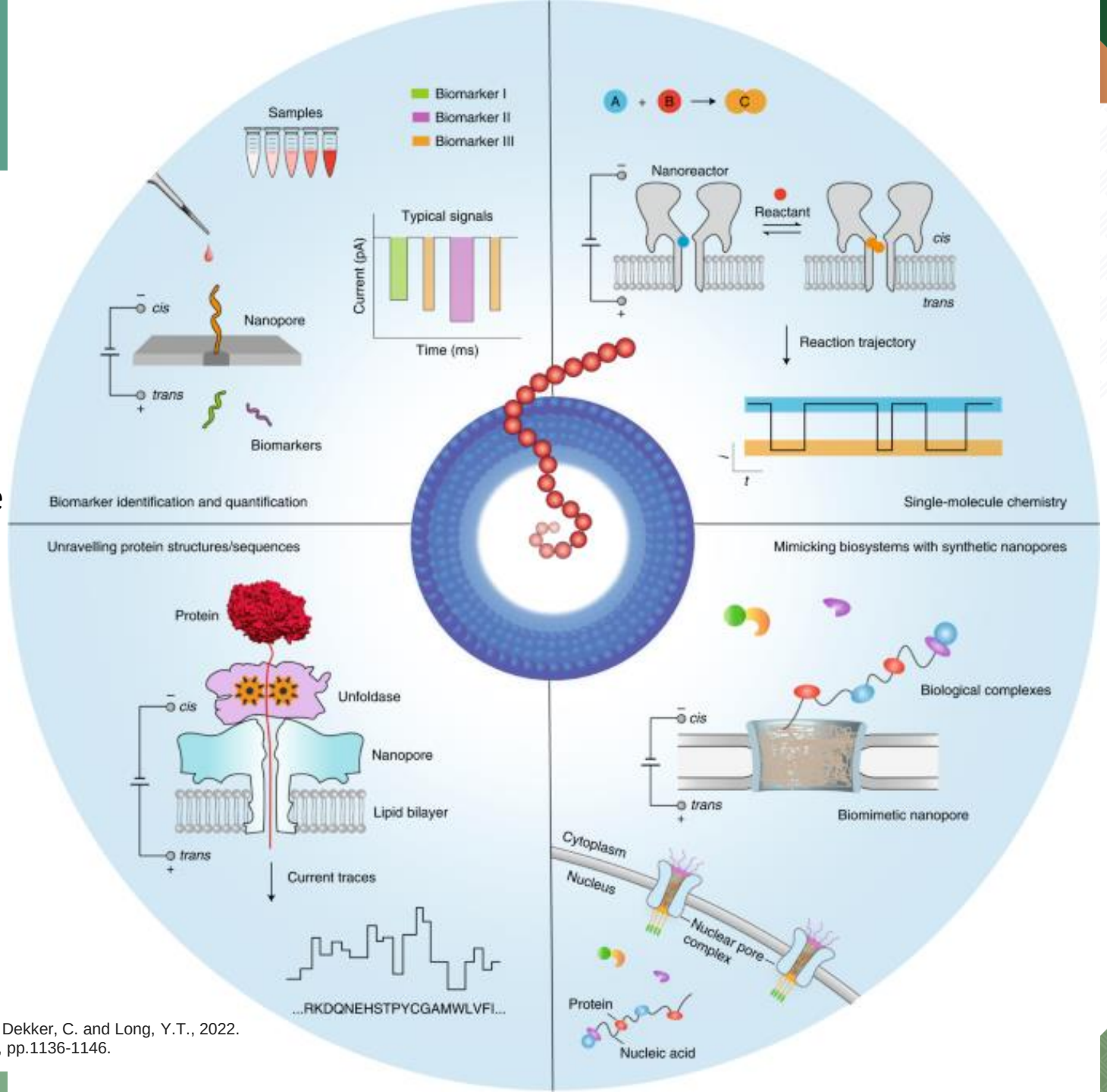
2019 — Development of nanopore-based diagnostic tests for infectious diseases

2020 — Advances in machine learning enable real-time base calling and analysis of nanopore sequencing data

Expanded Applications
Clinical Adoption
Miniaturization and Portability
Integration with Other Technologies

Importance in Biological Research

- Nanopore technology revolutionized DNA sequencing, enabling rapid and cost-effective analysis of DNA.
- It allows for real-time, single-molecule analysis, providing insights into biological processes at a level of detail previously unattainable.
- Nanopores have diverse applications beyond DNA sequencing, including protein analysis, drug discovery, and biomarker detection.



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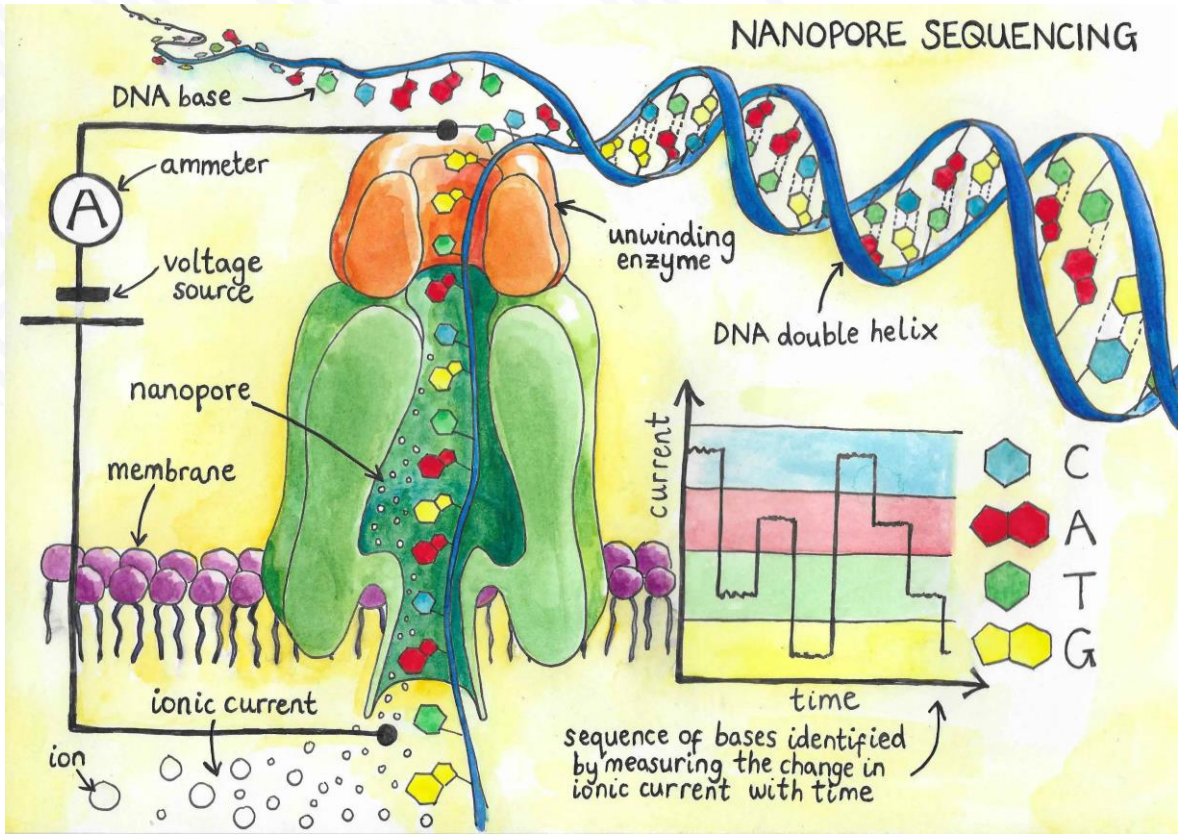
Nanopore Sequencing of DNA

Principle of DNA sequencing using nanopores



Advantages Over Traditional Sequencing Methods

Real-Time Analysis: Eliminating the need for time-consuming library preparation and post-sequencing processing.
Single-Molecule Sensitivity: Detection of rare mutations and structural variations that may be missed by bulk sequencing methods.
Portability and Accessibility: Expanding the reach of genomic research and diagnostics.
Long Read Lengths: Assembly of complex genomes and the analysis of repetitive regions.
Cost-Effectiveness: More accessible to researchers and clinicians with limited budgets.
Versatility: Amino acids, nucleic acids, including DNA, RNA, and modified bases, offering versatility for various research and diagnostic applications.

https://oxsci.org/pore-over-this-advances-in-dna-sequencing/

ACS APPLIED BIO MATERIALS

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Single-Molecule Analysis of SARS-CoV-2 Double-Stranded Polynucleotides Using Solid-State Nanopore with AI-Assisted Detection and Classification: Implications for Understanding Disease Severity

Ibrar Alam, Thitikorn Boonkoom, Harit Pitakjakpipop, Poramin Boonbanjong, Kawin Loha, Thanaya Saeyang, Jarunee Vanichtanankul, and Deanpen Japrung*

Cite This: https://doi.org/10.1021/acsabm.3c00998 Read Online

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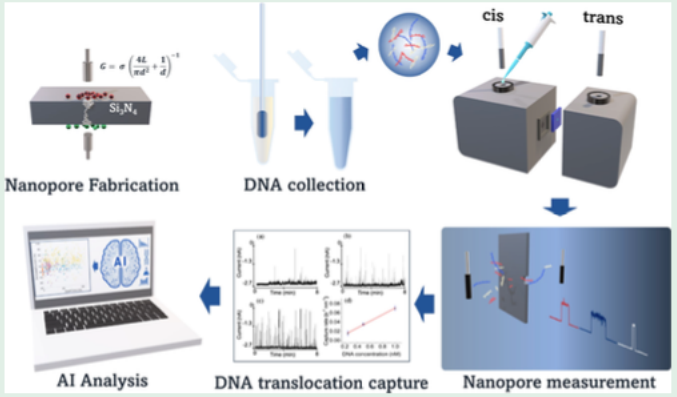
ABSTRACT: This study utilized solid-state nanopores, combined with artificial intelligence (AI), to analyze the double-stranded polynucleotides encoding angiotensin-converting enzyme 2, receptor-binding domain, and N protein, important parts of SARS-CoV-2 infection. By examining ionic current signals during DNA translocation, we revealed the dynamic interactions and structural characteristics of these nucleotide sequences and also quantified their abundance. Nanopores of sizes 3 and 10 nm were efficiently fabricated and characterized, ensuring an optimal experimental approach. Our results showed a clear relationship between DNA capture rates and concentration, proving our method's effectiveness. Notably, longer DNA sequences had higher capture rates, suggesting their importance for potential disease marker analysis. The 3 nm nanopore demonstrated superior performance in our DNA analysis. Using dwell time measurements and excluded currents, we were able to distinguish the longer DNA fragments, paving the way for a DNA length-based analysis. Overall, our research underscores the potential of nanopore technology, enhanced with AI, in analyzing COVID-19-related DNA and its implications for understanding disease severity. This provides insight into innovative diagnostic and treatment strategies.



KEYWORDS: *solid-state nanopore, SARS-CoV-2, single-molecule analysis, DNA translocation, capture rate, excluded current*

Highlights

- Nanopore technology and artificial intelligence (AI) used to investigate double-stranded polynucleotides of ACE2, RBD, and N protein
- Analysis of ionic current signals reveals interactions and structures of these DNA sequences
- AI enhances accuracy and depth of observations, determining prevalence of specific sequences
- Findings provide foundation for developing new diagnostic and treatment strategies for COVID-19

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Solid-state nanopore fabrication and DNA translocation experiment

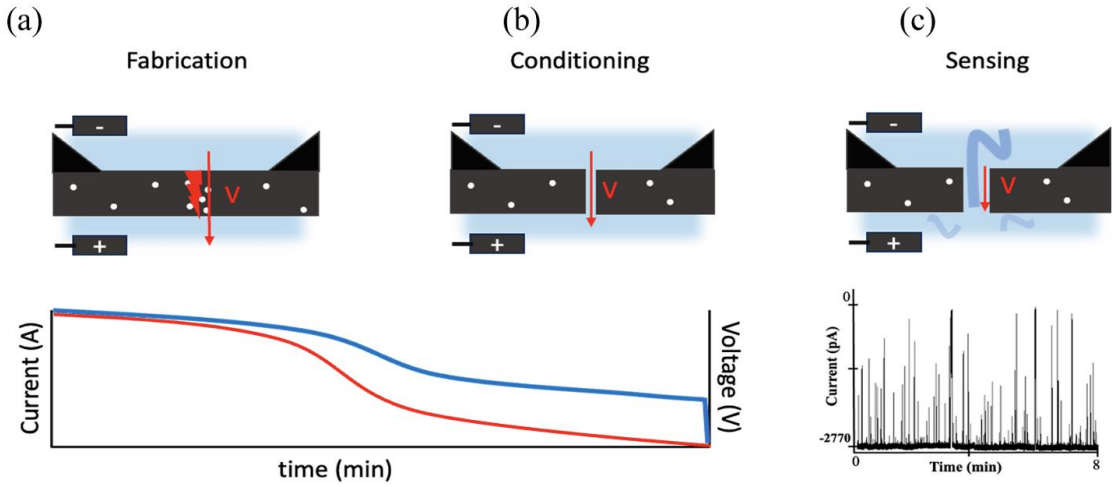
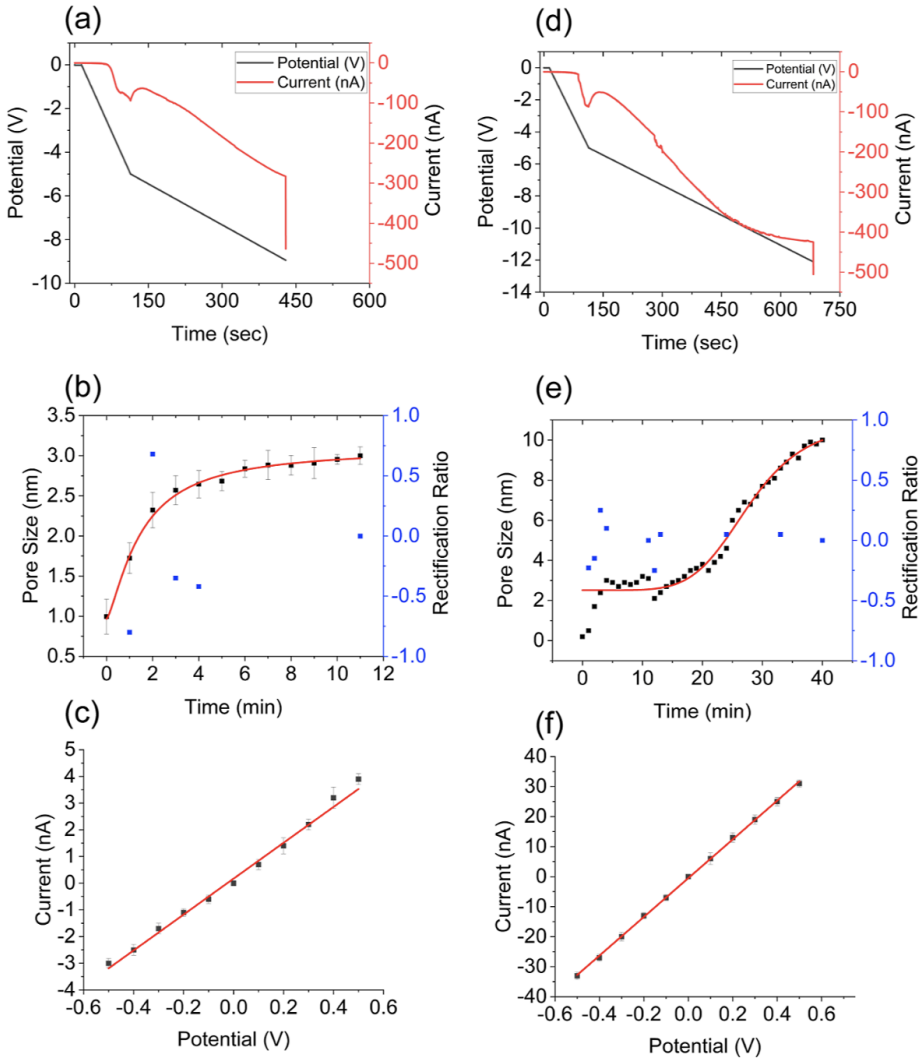


Table 1. Summary of Prepared Double-Stranded Polynucleotides for ACE2, RBD, and N Protein

DNA fragment	Encoded protein	Base pair length (bp)	% GC content
ACE2-PD	peptidase domain of ACE2 protein	1836	54.96
RBD	spike protein RBD	846	54.61
NTD	N-terminal domain of N protein	450	59.55
CTD	C-terminal domain of N protein	414	56.28



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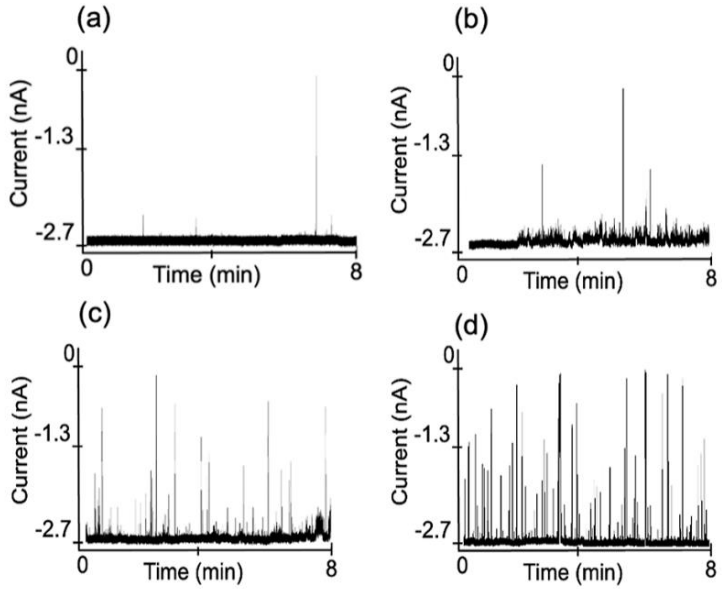
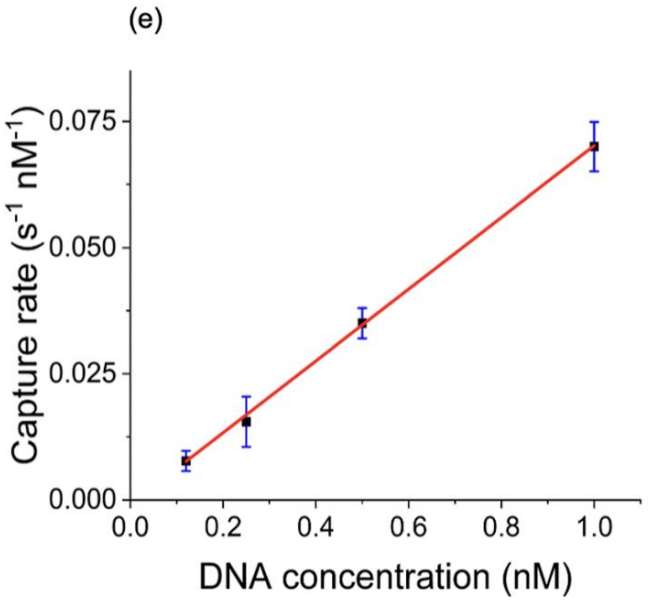
Nanopore Analysis of Double-Stranded Poly-nucleotides Encoded for ACE2, RBD, and N Protein

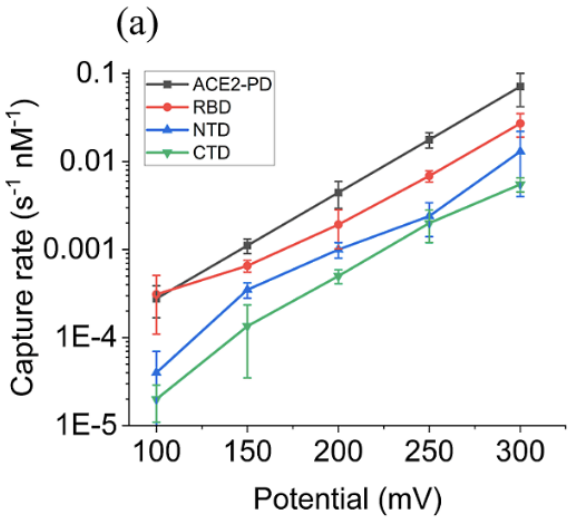


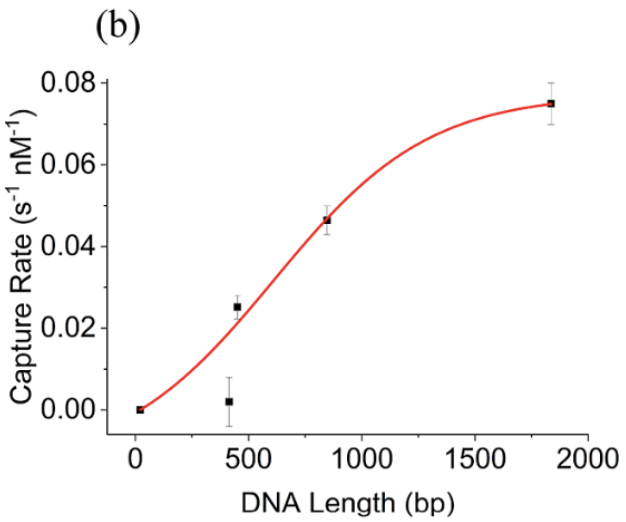


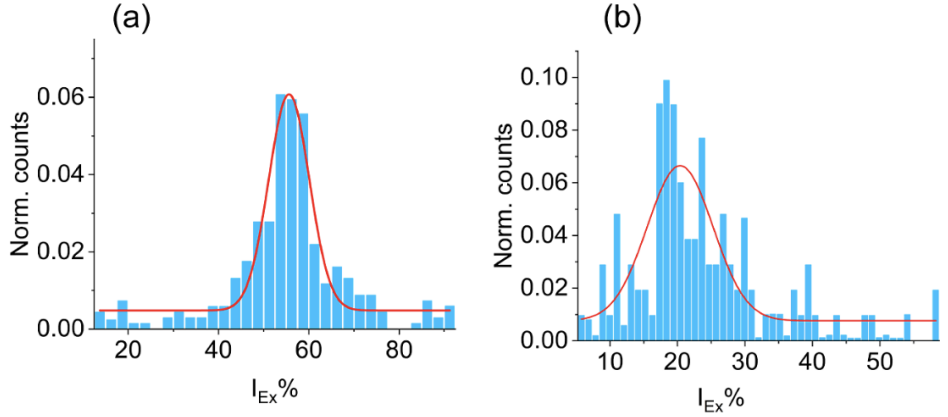
$$G = \sigma\left(\frac{4L}{\pi d^2} + \frac{1}{d}\right)^{-1}$$

$$\text{Capture rate} \propto e^{(\alpha V)}$$

$$\text{Capture rate} \propto e^{(\alpha V)} \times L^{\beta}$$







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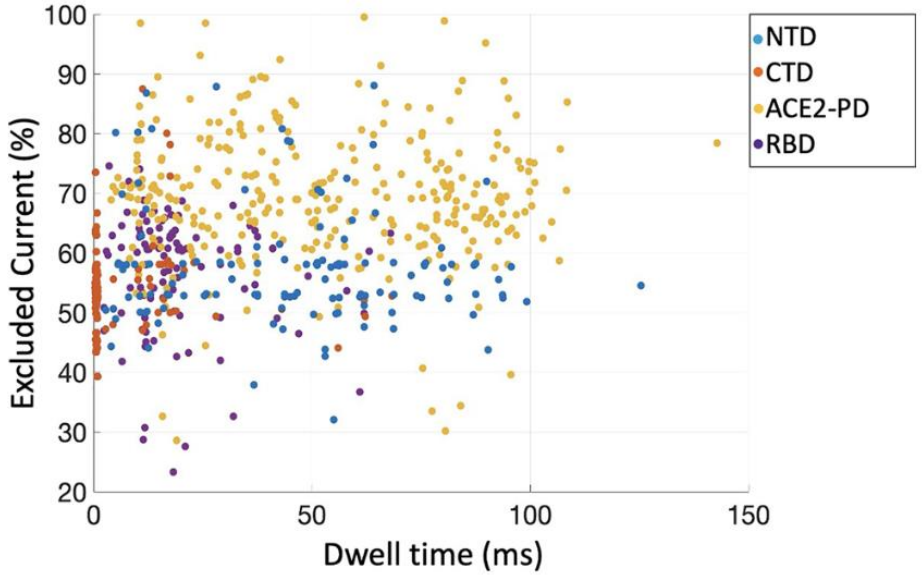
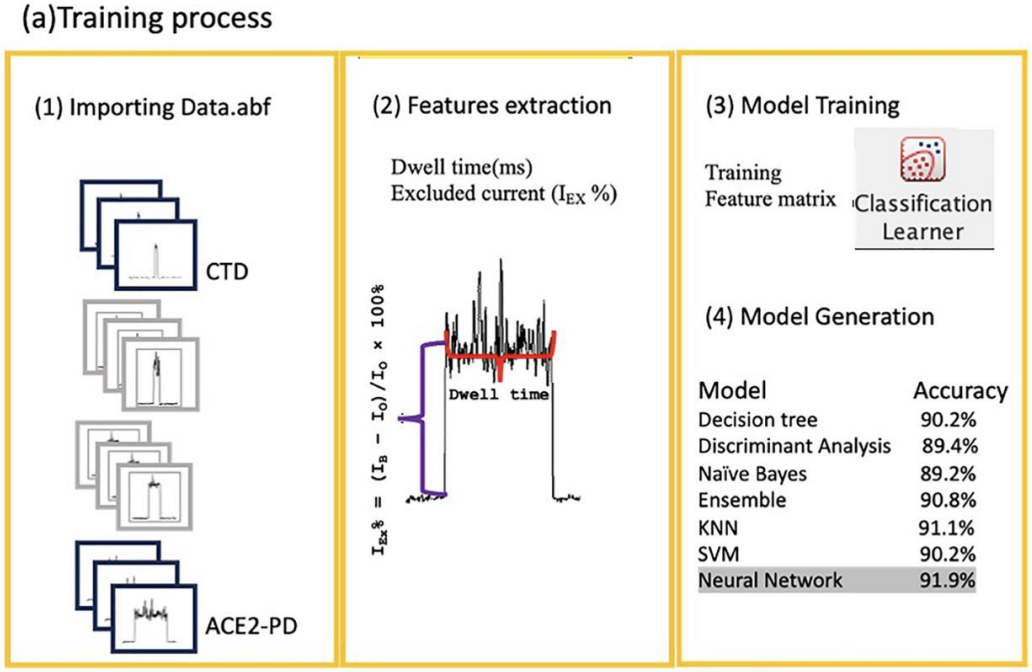
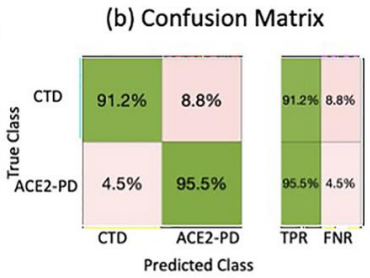
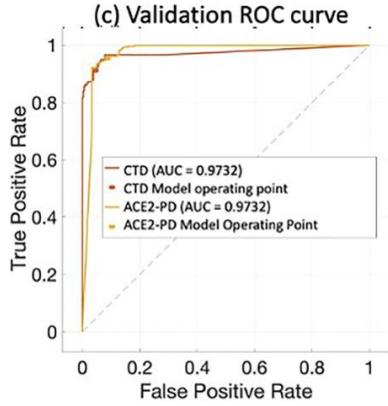
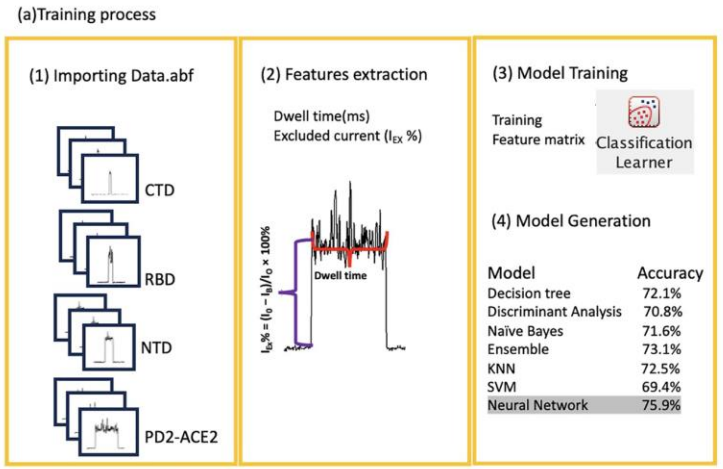
AI-Assisted Dwell Time Analysis of DNA Interactions and Translocations through the Nanopore.

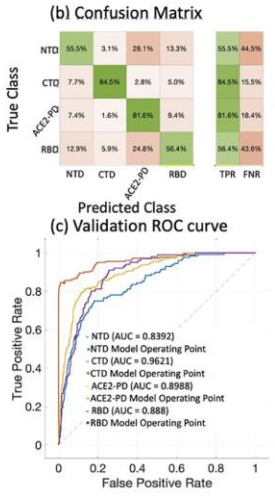












$$\text{Number of polynucleotide molecules} = \frac{\text{capture rate} \times \text{total number of molecules}}{\text{capture efficiency}}$$

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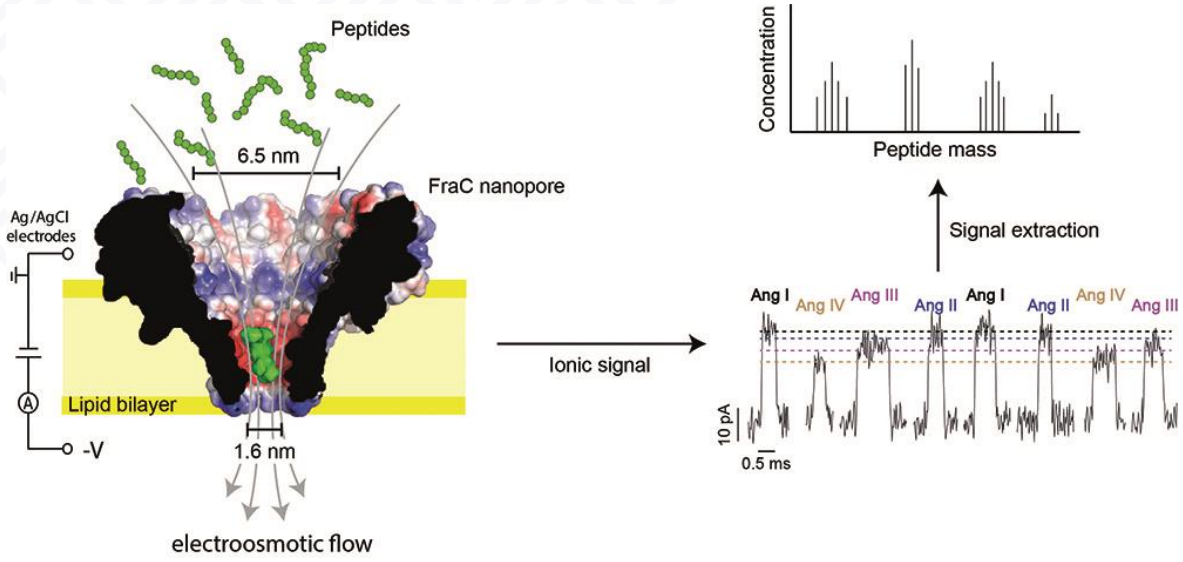
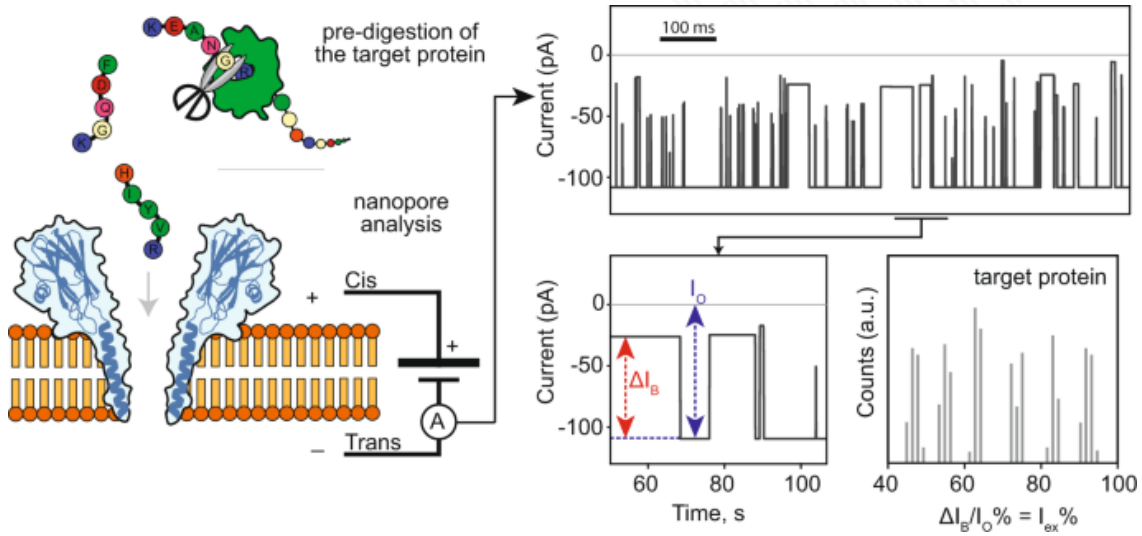
Protein Analysis with Nanopores





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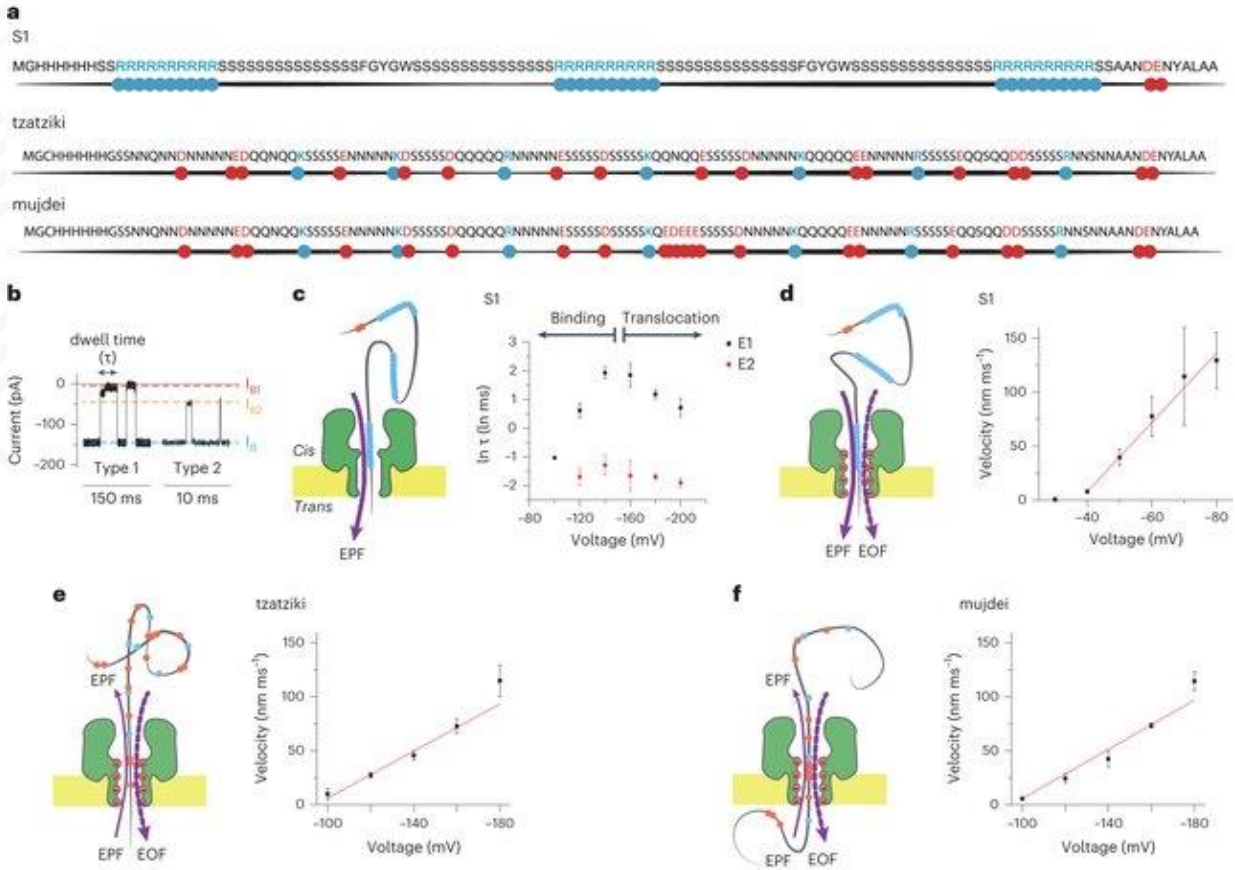

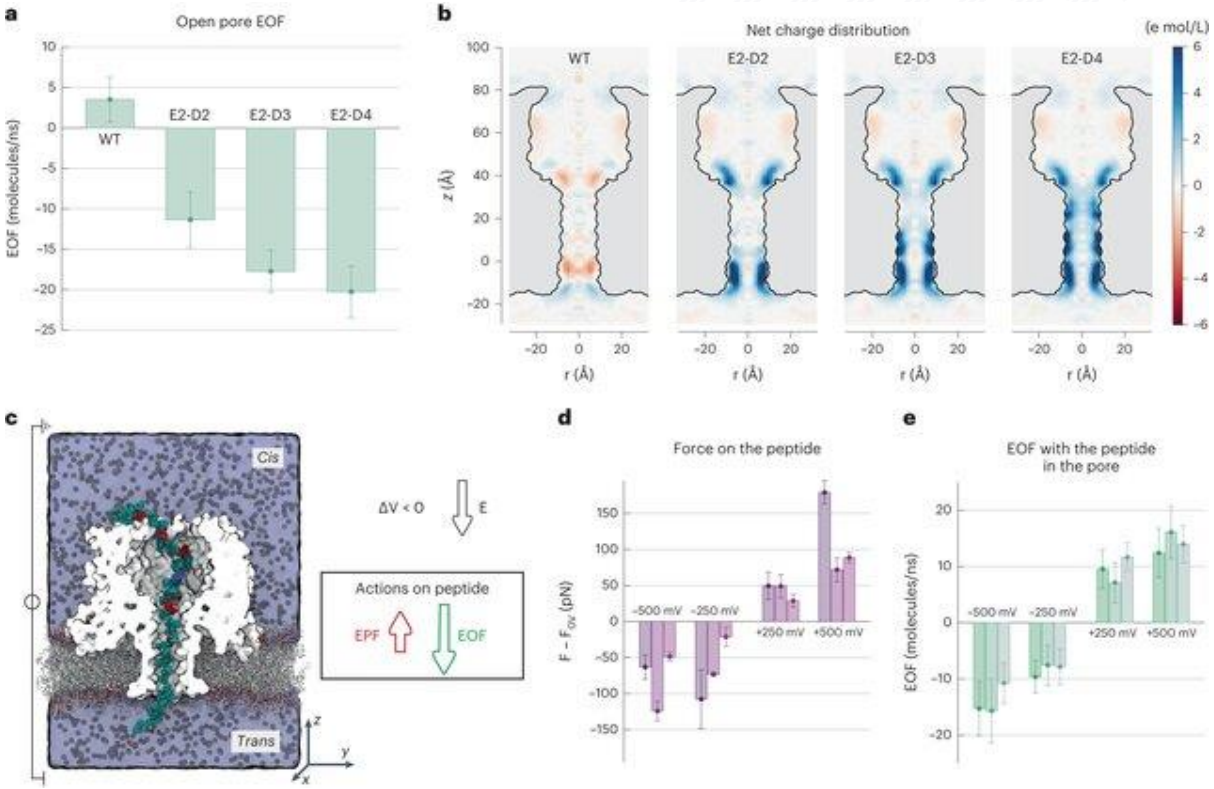

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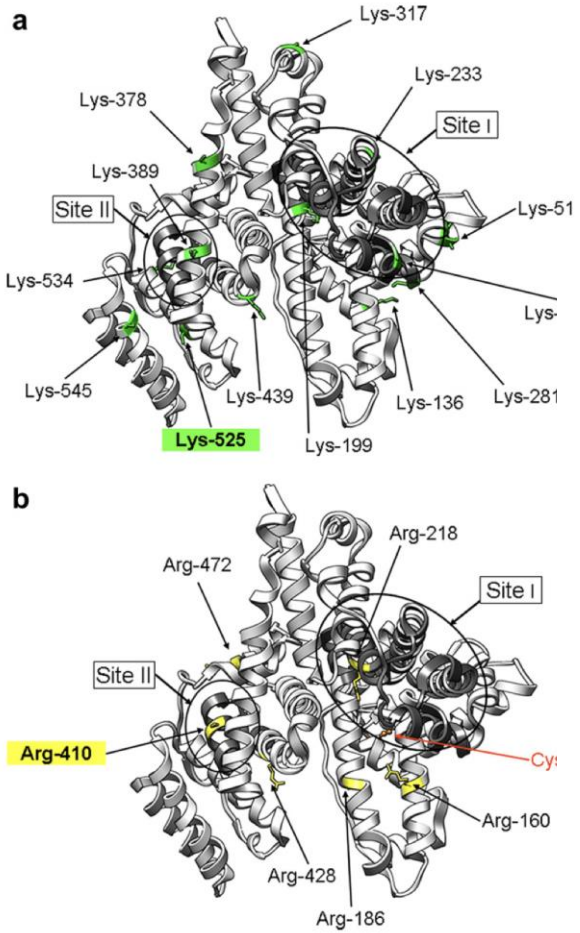
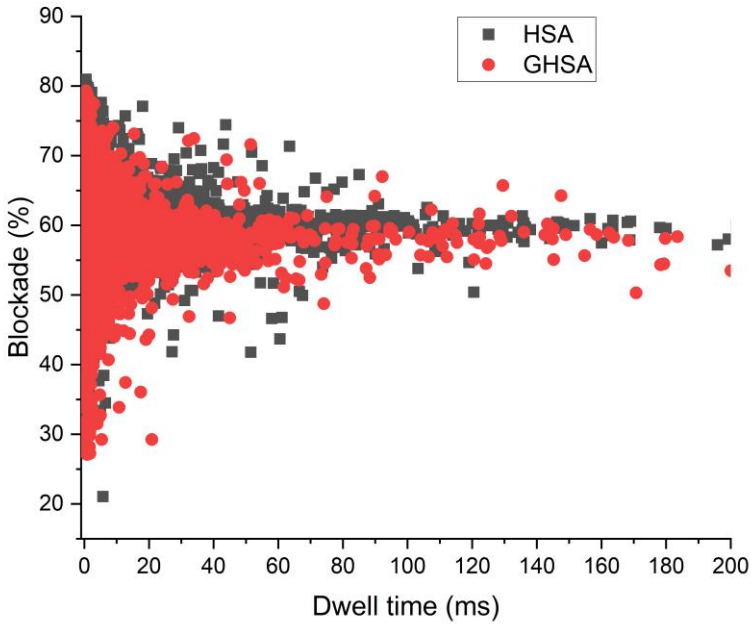
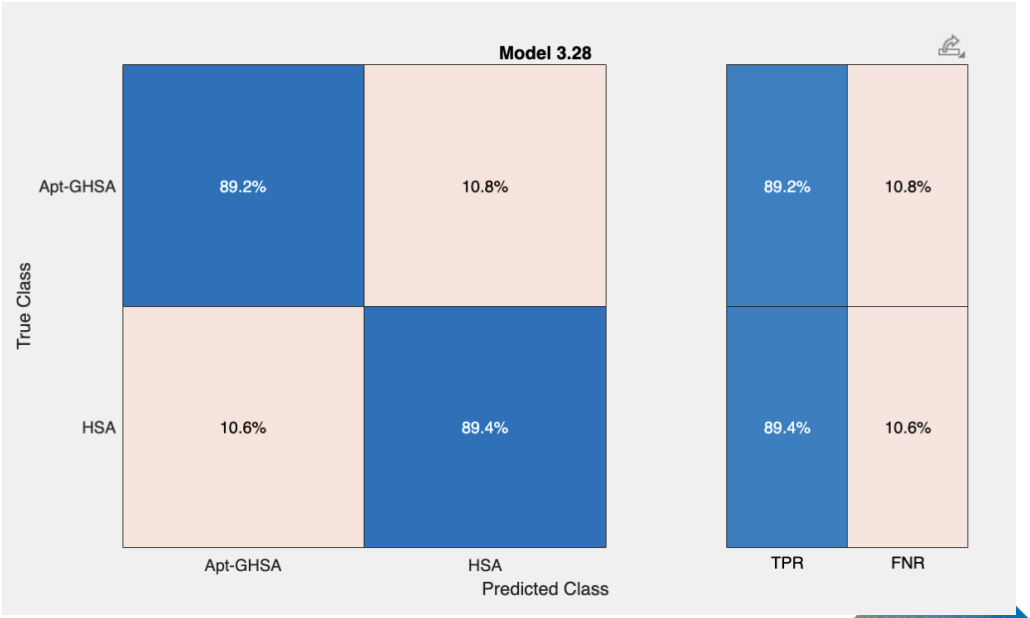
Protein Analysis with Nanopores

Solid-state nanopore analysis of Human Serum /Glycated Albumin.







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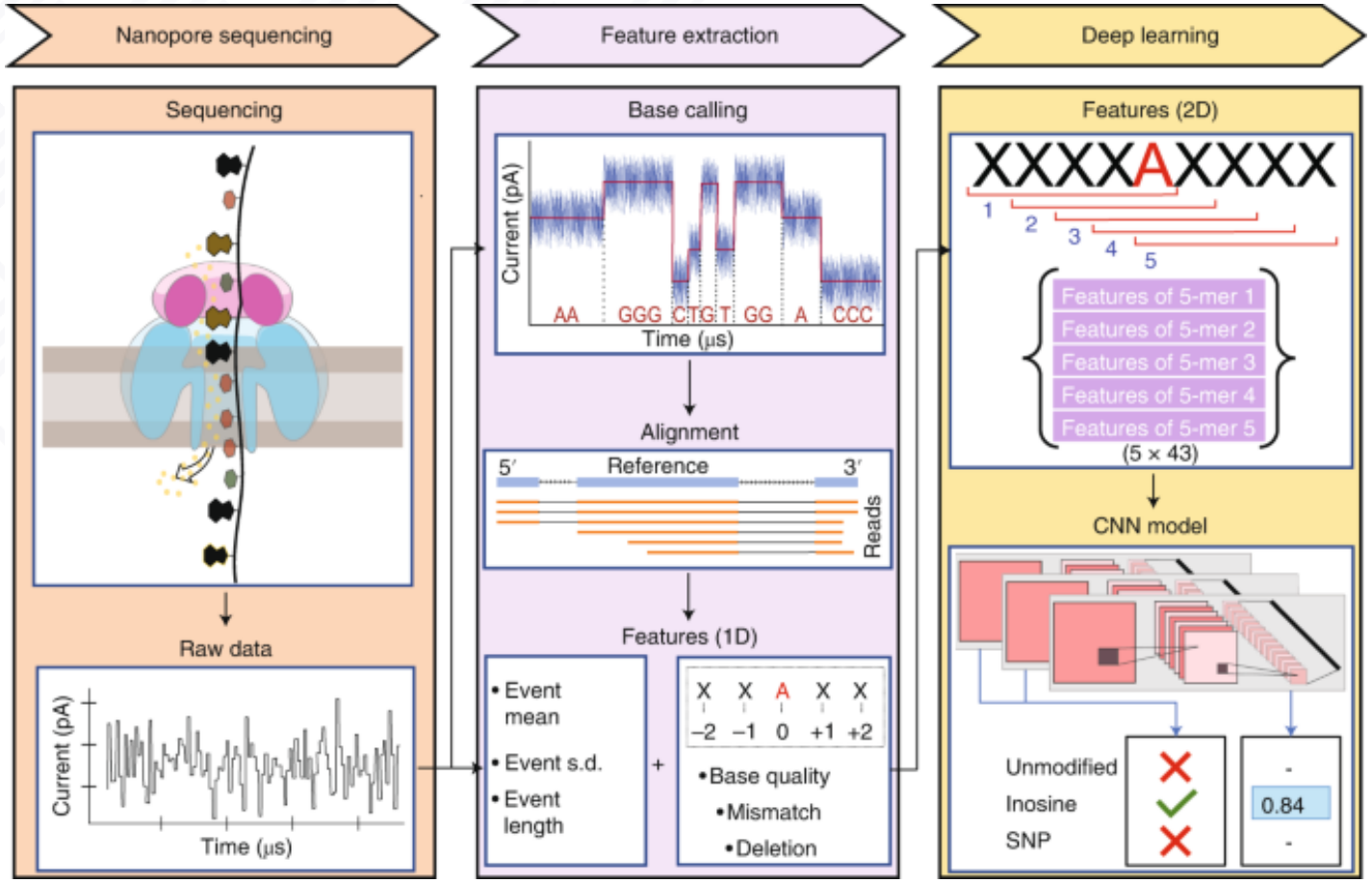
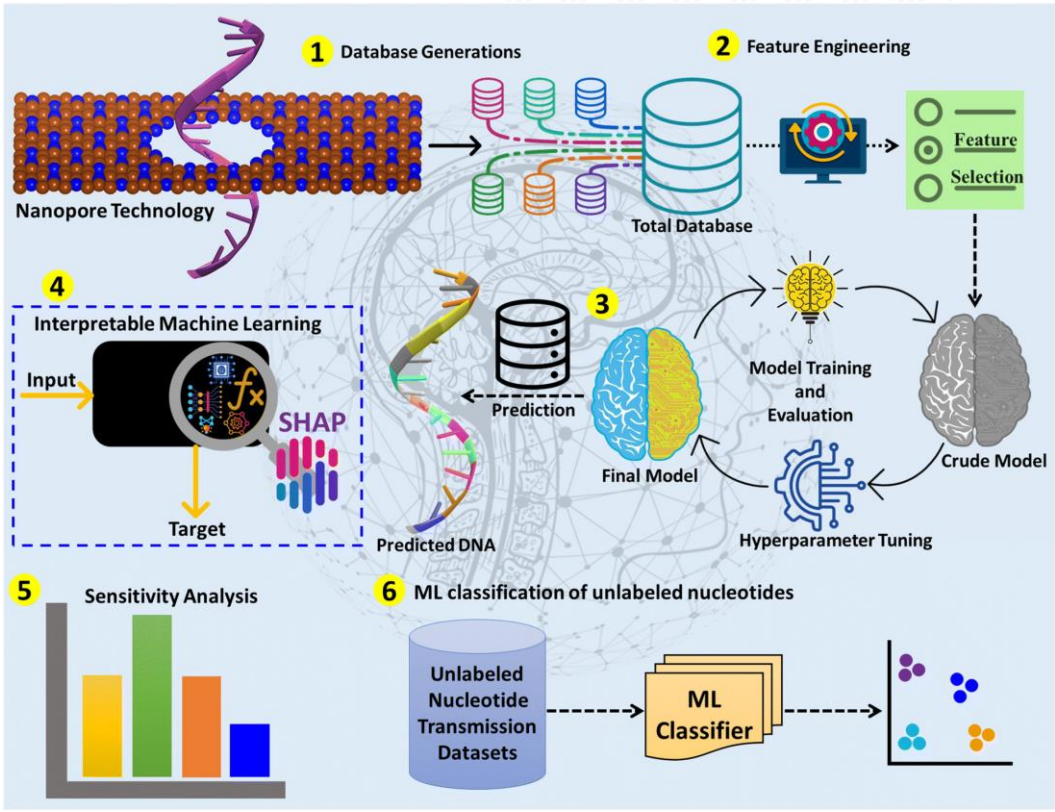
Machine Learning in Nanopore Technology





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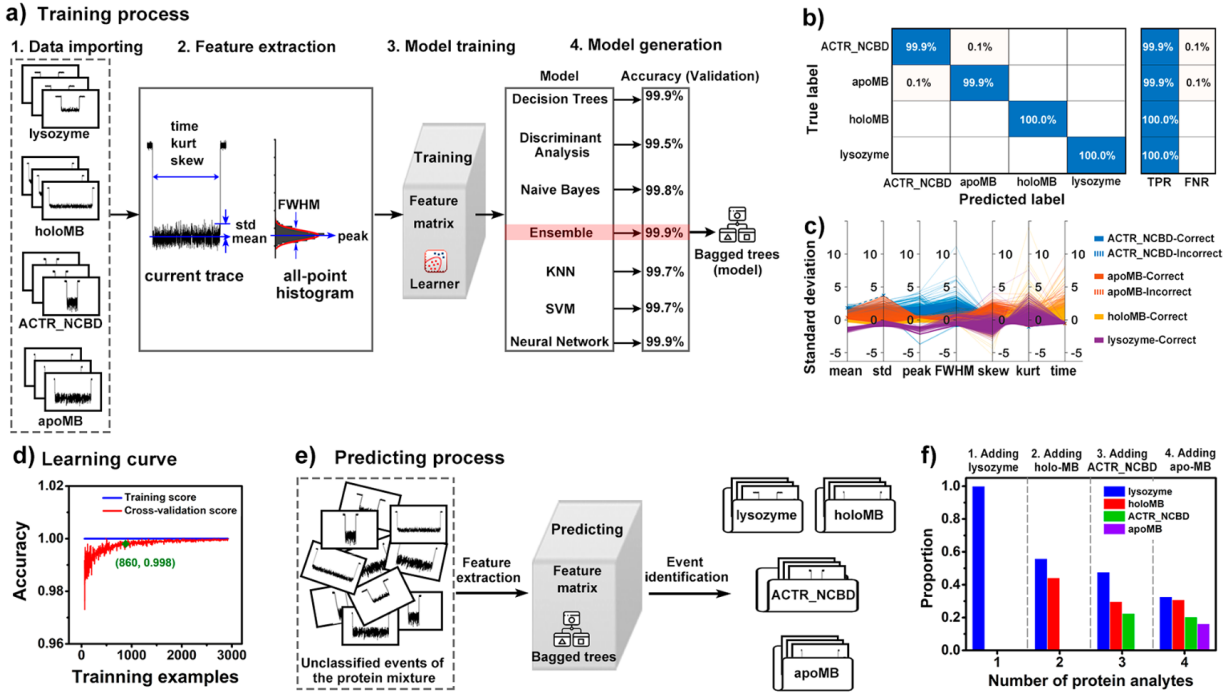

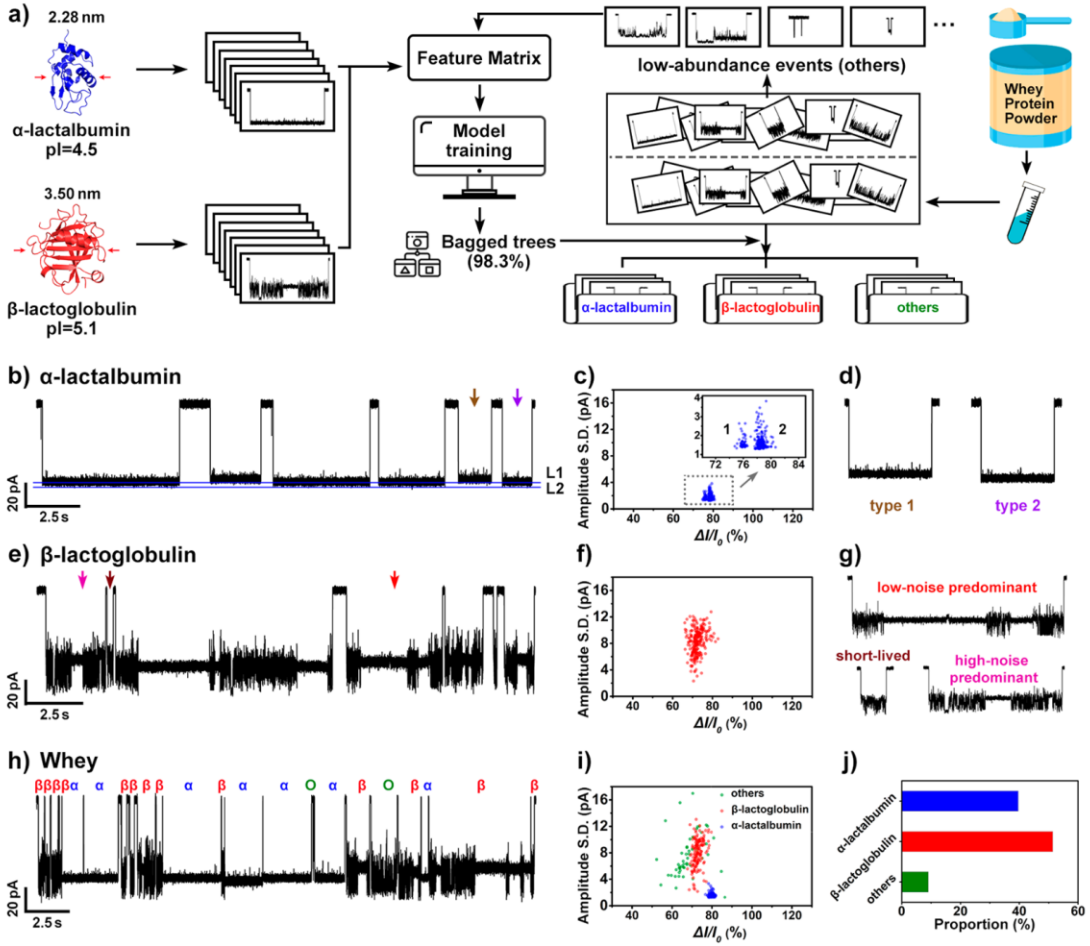

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Future perspective

Nanopore Arrays: Development of nanopore arrays for parallel sequencing, increasing throughput and reducing sequencing time.

Improved Base Calling: Advancements in base calling algorithms for higher accuracy and better handling of difficult sequences, such as repetitive regions.

Nanopore Protein Sequencing: Progress in nanopore technology for direct sequencing of proteins, enabling rapid and accurate protein analysis.

Nanopore-Based Epigenetic Analysis: Use of nanopores for studying epigenetic modifications, such as DNA methylation and histone modifications, providing insights into gene regulation.

Single-Molecule Imaging: Advancements in nanopore imaging techniques for real-time visualization of single molecules, expanding applications in structural biology and drug discovery.

Integration with Microfluidics: Further integration of nanopores with microfluidic devices for automated sample preparation and analysis, improving workflow efficiency.

Clinical Diagnostics: Continued development of nanopore-based diagnostic tools for personalized medicine, early disease detection, and monitoring of treatment responses.

Synthetic Biology Applications: Utilization of nanopores in synthetic biology for designing novel biosensors, gene editing tools, and bioinformatics applications.

Challenges and Limitations of Nanopore Technology

Current Challenges:

Accuracy: Nanopore sequencing still faces challenges in achieving the same level of accuracy as other sequencing methods, particularly in detecting repetitive sequences and base modifications.

Throughput: Despite improvements, nanopore sequencing throughput is lower compared to some high-throughput sequencing technologies, limiting its applicability in large-scale studies.

Cost: While nanopore sequencing is cost-effective for certain applications, the overall cost, including equipment and reagents, can be a barrier for widespread adoption.

Signal-to-Noise Ratio: Maintaining a high signal-to-noise ratio is crucial for accurate base calling, and noise levels can vary depending on the sample and experimental conditions.

Strategies for Overcoming Limitations:

Improving Base Calling Algorithms: Continued development of base calling algorithms to enhance accuracy, especially in challenging regions of the genome.

Enhancing Nanopore Technology: Research efforts focused on improving nanopore design and materials to increase throughput and reduce error rates.

Cost Reduction: Streamlining workflows and reducing the cost of reagents and consumables to make nanopore sequencing more affordable.

Noise Reduction: Developing methods to reduce noise levels and improve signal-to-noise ratio for more accurate sequencing results.

ขอขอบคุณผู้ให้การสนับสนุน Industrial Postdoc

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(e-ASIA Joint Research Program)

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