ACS Partner Journal
PRECISION CHEMISTRY
pubs.acs.org/PrecisionChem

Review

Single-Molecule Bioelectronic Sensors with AI-Aided Data Analysis: Convergence and Challenges

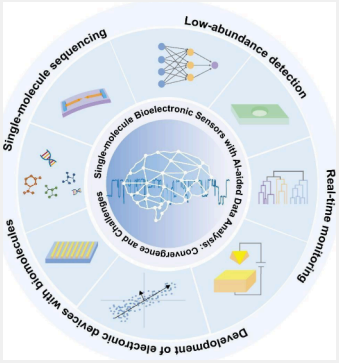
Yuxin Yang, Yueqi Li, Longhua Tang,* and Jinghong Li*

Cite This: *Precis. Chem.* 2024, 2, 518−538

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Single-molecule bioelectronic sensing, a groundbreaking domain in biological research, has revolutionized our understanding of molecules by revealing deep insights into fundamental biological processes. The advent of emergent technologies, such as nanogapped electrodes and nanopores, has greatly enhanced this field, providing exceptional sensitivity, resolution, and integration capabilities. However, challenges persist, such as complex data sets with high noise levels and stochastic molecular dynamics. Artificial intelligence (AI) has stepped in to address these issues with its powerful data processing capabilities. AI algorithms effectively extract meaningful features, detect subtle changes, improve signal-to-noise ratios, and uncover hidden patterns in massive data. This review explores the synergy between AI and single-molecule bioelectronic sensing, focusing on how AI enhances signal processing and data analysis to boost accuracy and reliability. We also discuss current limitations and future directions for integrating AI, highlighting its potential to advance biological research and technological innovation.



KEYWORDS: *single-molecule bioelectronic sensing, artificial intelligence, machine learning, signal processing, biosensors*

VOCABULARY SECTION

Bioelectronics: Bioelectronics is an interdisciplinary field that combines biology and electronics to study and manipulate biological systems using electronic devices and technologies.

Single-molecule bioelectronic sensors: Single-molecule bioelectronic sensors are devices that can detect and analyze the electrical behaviors of individual biological molecules such as proteins, enzymes, and DNA at a very detailed level.

Artificial Intelligence (AI): Artificial Intelligence refers to the simulation of human intelligence processes by computer systems, including learning, reasoning, and decision-making, to analyze and interpret complex data.

Single-molecule detection: The process of identifying and measuring the presence of individual molecules at the single-molecule level, allowing for sensitive detection and analysis of biological interactions and functions.

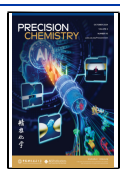
Single-molecule sequencing: A high-resolution method in bioelectronics that involves the direct analysis of individual molecules, typically DNA, RNA, or proteins, to determine their nucleotide or amino acid sequence. This technique provides detailed information about the genetic material at the molecular level, enabling precise identification and analysis of genetic variations.

Molecular electronics: Molecular electronics focuses on the development of electronic devices and circuits at the molecular scale, utilizing individual molecules as building blocks for innovative high-performance technologies.

1. INTRODUCTION

Bioelectronics, an interdisciplinary fusion of biology and electronics, has garnered significant attention for its transformative impact on biological research. The advancement of single-molecule bioelectronic techniques has overturned our traditional understanding of the fundamental material world and biosystems, which provides an approach to exploring and manipulating the electrical behaviors of biomolecules, including proteins, enzymes, and DNA, at an unprecedented level of detail, enabling a deeper understanding of their structures, dynamics, interactions, and functions from a perspective beyond ensemble systems.[1−3] Over the years, remarkable progress has been made in developing sensing devices with exceptional sensitivity, and temporal and spatial resolution, such as tunneling sensors with nanogapped electrodes, nanopores, and single-molecule FETs. Single-molecule bioelectronic technologies exhibit substantial potential across diverse applications, particularly in medicine, healthcare, and environmental management. By directly probing biological systems at the molecular level, these sensors enable timely and precise biomarker detection, enhancing early disease diagnosis.[4−8] They also play a pivotal role in drug screening by

Received: June 21, 2024
Revised: August 9, 2024
Accepted: September 9, 2024
Published: September 16, 2024



https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

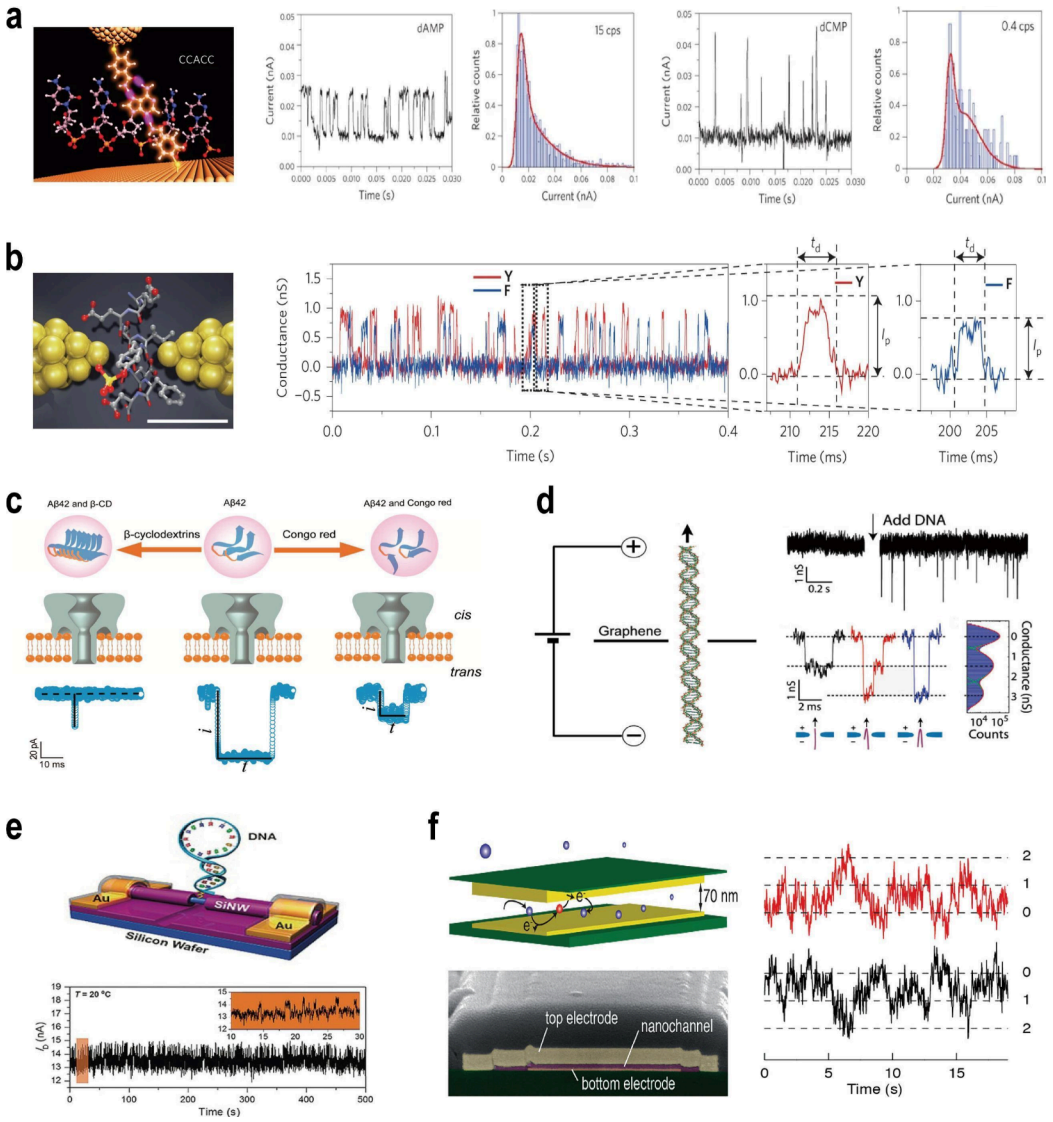


Figure 1. Single-molecule bioelectronic sensors. (a) STM and tunneling signals of a single nucleotide.[20] Reproduced with permission from ref 20. Copyright 2010, Springer Nature Limited (b) Fixed-nanogap electrodes and measurement of tyrosine (Y) and phenylalanine (F).[21] Reproduced with permission from ref 21. Copyright 2014, Springer Nature Limited. (c) Biological nanopore and electrical signals of polypeptide.[22] (d) Solid-state nanopore and its measurement of DNA.[23] (e) Silicon nanowire FET and its monitoring of DNA folding/unfolding process.[24] Reproduced with permission from ref 24. Copyright 2016 Wiley VCH Verlag GmbH & Co. KGaA, Weinheim. (f) The active molecules undergo oxidation and reduction through the top (red) and bottom (black) electrodes, generating an electric current.[25]

allowing real-time monitoring of molecular interactions, thereby accelerating the identification of effective drug candidates and fostering the development of targeted therapies.[9,10] Moreover, the platform's high sensitivity and selectivity make it an invaluable tool for detecting and quantifying pollutants and environmental toxins, contributing to the protection of water and air quality, and overall environmental health.[11] The advancement of single-molecule bioelectronics also drives the frontier of molecular electronics and device development, serving as the building blocks for innovative, high-performance molecular-scale electronic devices and circuits.[12]

Despite the remarkable sensitivity achieved by single-molecule bioelectronic sensing, several challenges persist in the measurement and data analysis process. Specifically, factors like temperature fluctuations and electromagnetic interference introduce noise, complicating signal interpretation.[13] On the other hand, the weak signals generated by biomolecules often pose a challenge in differentiating them from background noise.[14,15] Additionally, accurately interpreting these signals can be difficult due to their rich information and complex transformations, thus requiring sophisticated data analysis techniques. To address these complexities, Artificial Intelligence (AI), which emulates human intelligence through computer systems and involves advanced algorithms for learning, reasoning, and decision-making,[15−19] has emerged as a powerful facilitator in this field. AI excels in processing the massive, intricate data generated by single-molecule sensors, enabling efficient extraction of insights such as biomolecule identification, interaction characterization, and molecular structure analysis. Furthermore, AI algorithms optimize sensor design and performance by predicting optimal configurations and experimental conditions, thereby boosting the reliability of single-molecule measurement.

This review aims to delve into the integration of AI with single-molecule bioelectronic sensing, providing a comprehensive overview of the current landscape, challenges, and

https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

prospects. We discuss various single-molecule bioelectronic sensors, with a particular emphasis on how AI-driven methods enhance detection accuracy through enhanced signal processing and data analysis. By examining the interplay between AI techniques and single-molecule bioelectronic sensing, we seek to illuminate advancements, potential applications, and the remaining challenges that must be overcome to fully harness the power of AI in this rapidly evolving research domain.

2. DEVICES AND PRINCIPLE OF SINGLE-MOLECULE BIOELECTRONIC SENSING

Single-molecule bioelectronic sensing techniques have significantly transformed the realm of nanotechnology and bioelectronics by enabling the nanoscale detection and analysis of individual molecules. The fundamental principle lies in constructing nanoconfinement devices that convert molecular changes into detectable electrical signals at the molecular scale. This section will explore three primary types of these devices: tunneling sensors with nanogapped electrodes, nanopore sensors with monitoring ionic current, and the others, which are classified based on the different types of detected currents. Nanogapped electrodes, such as those in Scanning Tunneling Microscope (STM) (Figure 1a) and fixed-nanogap setups (Figure 1b), leverage the tunneling current, which is highly sensitive to both the gap size and the molecules nearby. Nanopores, including biological nanopores (Figure 1c) and solid-state nanopores (Figure 1d), function as nanoscale conduits for molecule passage, where monitoring ionic current fluctuations reveals information about size, shape, and charge properties. In addition, single-molecule field-effect transistors (smFETs) (Figure 1e) detect molecular interactions by measuring conductance variations in nanowires or nanotubes. For electrochemically active analytes, their molecular reactions can be harnessed to generate measurable currents by integrating them with external electronic circuits. (Figure 1f) Furthermore, innovative combinations of sensor components and advanced nanofabrication techniques have expanded the capabilities of single-molecule bioelectronic sensing, offering unprecedented precision and control in studying and manipulating individual molecules.

2.1. Tunneling Sensors with Nanogapped Electrodes

Tunneling is a fundamental concept in quantum mechanics and refers to the phenomenon where electrons can pass through a potential barrier that would be classically insurmountable. This occurs due to the wave-like nature of electrons, which allows them to exist in all regions of space simultaneously. The probability of tunneling is governed by the transmission coefficient, which depends on the barrier height, width, and the energy of the electrons. In a tunneling sensor, electrodes are separated by a very small gap, typically on the order of a few nanometers. The presence of a molecule between these electrodes can serve as the bridge for electron transport. The Landauer-Buttiker formula, derived from quantum transport theory, provides a quantitative description of the tunneling current. It states that the tunneling current I is proportional to the transmission probability $T(E)$ of electrons across the barrier and the applied voltage V, given by[26,27]

$$I = \frac{e}{h}\int_{-\infty}^{+\infty} T(E)[f_L(E) - f_R(E)]dE \quad (1)$$

where e is the electron charge, h is Planck's constant, and f_L and f_R are the Fermi−Dirac distribution functions for the left and right electrodes, respectively. This formula highlights the dependence of tunneling current on the molecule's properties, as changes in the molecule's energy levels or electronic structure can alter the transmission probability. The sensitivity of the tunneling current to the presence and properties of molecules lies in the fact that any change in the molecule's electronic structure, such as an alteration in its chemical bonding, charge state, or size, can influence the energy levels and thus change the tunneling probability. This can lead to a detectable variation in the current. In tunneling sensors, this sensitivity is exploited to detect and quantify the presence of specific molecules or molecule-binding events with high precision.

Various techniques have emerged for establishing tunneling interfaces between molecules and electrodes, with two prominent examples being top-contact junctions typified by Scanning Tunneling Microscope (STM) and planar fixed-nanogap electrodes. STM detects tunneling current between the sharp metal tip and substrate, analyzing individual molecules by monitoring current fluctuations during scanning of the tip.[28] When maintaining a constant tunneling current through feedback control, STM can perform high-resolution imaging and characterization of atomic and molecular features.[29,30] A notable technique is the STM break junction (STM-BJ), where the tip is repetitively withdrawn and repositioned to create single-molecule junctions, as illustrated in Figure 2a. This method permits controlled manipulation of molecules and offers insights into their electronic and mechanical properties.[31,32]

By comparison, fixed-nanogap electrodes construct contact of a molecule with two nanoelectrodes, facilitating integration and miniaturization of devices.[28] The fabrication of these electrodes involves diverse techniques, with a primary focus on creating uniform nanometer-sized gaps, which can be attained through three principal methods: addition, subtraction, and splitting.[33] Addition obtains nanogaps by increasing electrode materials from bottom to top and gradually reducing the interval, such as electrochemical deposition, which involves controlled electrochemical reactions to deposit gold particles in a nanogap configuration. Gold deposition is achieved by applying a voltage bias between electrodes in an electrolyte solution containing gold ions, while size adjustment and shape control can be performed based on the feedback of tunneling current between the formed electrodes, as shown in Figure 2b.[34] Besides, the template methods employ masks with predefined structures to form nanogaps, such as single-walled carbon nanotubes. (Figure 2c)[35] Subtraction creates nanogaps by removing electrode materials, which is typically achieved through high-energy ion or electron etching.[36,37] Focused ion beam etching (FIB) uses the ion beam, typically gallium, to etch material with atomic-level precision, allowing for the formation of subnanometer gaps. Ions are accelerated and scanned across the substrate, removing material layer by layer, as displayed in Figure 2d. Electron beam lithography (EBL) employs a highly energetic electron beam, which interacts with a photosensitive resist layer. When exposed to light, the resist becomes sensitive, and subsequent development removes the unexposed areas, leaving behind the desired pattern and further obtaining nanogaps through etching. (Figure 2e) The splitting approach obtains nanogapped electrodes by tearing electrode materials, such as the mechanically controllable break junction (MCBJ)[38−40] and electromigration.[41−43] In MCBJ, a metallic wire, usually gold or platinum, is clamped and stretched until it fractures, creating a precisely controllable nanogap, as depicted in Figure 2f. Electromigration involves high current densities that induce atomic rearrangement and gap

https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

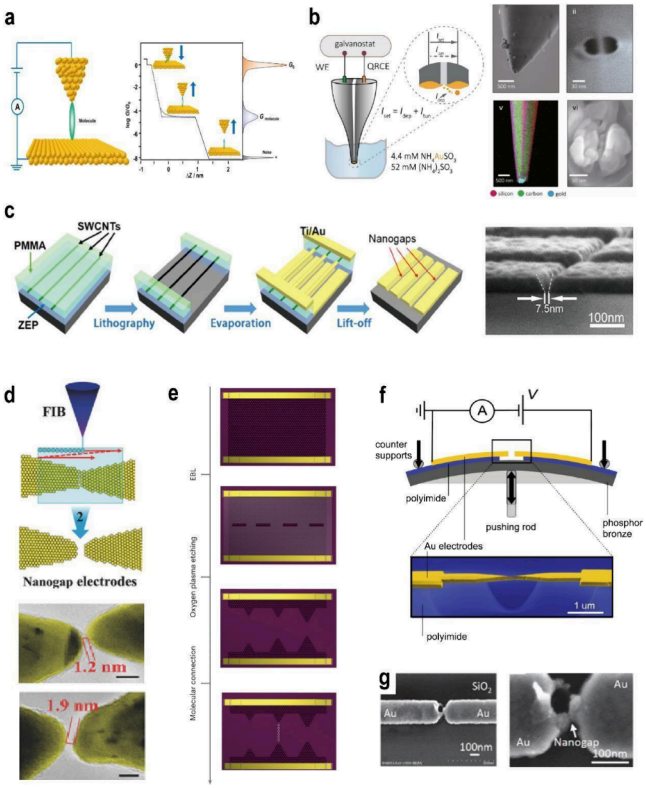


Figure 2. Construction of nanogapped electrodes. (a) STM-BJ.[31] (b) Electrochemical deposition.[34] (c) Manufacturing nanogaps using single-walled carbon nanotubes as masks.[35] (d) FIB and its generated nanogaps.[36] Reproduced with permission from ref 36. Copyright 2015 Wiley VCH Verlag GmbH & Co. KGaA, Weinheim. (e) Preparation of the graphene single-molecule device by EBL and oxygen plasma etching.[43] Reproduced with permission from ref 43. Copyright 2023, Springer Nature Limited. (f) MCBJ.[44] (g) Nanogapped electrodes prepared by electromigration.[42]

formation due to electron-metal atom momentum transfer. (Figure 2g) These nanofabrication techniques have led to highly sensitive tunneling sensors capable of detecting individual molecules with exceptional resolution, allowing for the study of charge transfer dynamics.

2.2. Nanopore Sensors with Monitoring Ionic Current

The foundation of nanopore technology dates back to the mid-20th century with the development of Coulter counting and single-channel current recordings.[45] In its typical configuration, nanopores are immersed in an electrolyte solution, such as potassium chloride (KCl), as depicted in Figure 3a. By positioning electrodes, often Ag/AgCl, on either side of the nanopore and applying an external voltage, ions are driven to traverse the pore, undergoing oxidation−reduction reactions on the electrode surfaces (as per eqs 2 and 3), resulting in a stable ionic current.

$$Cathode: AgCl + e^- \rightarrow Ag + Cl^- \quad (2)$$

$$Anode: Ag + Cl^- \rightarrow AgCl + e^- \quad (3)$$

Translocation of biomolecules through nanopores will induce transient current fluctuations, reflecting their physical properties like charge, shape, and interactions.[46] This process is influenced by competing forces, including diffusion, electrophoresis (EP), and electroosmotic flow (EOF). Diffusion is determined by the concentration gradient of molecules in the solution, and the EP force is closely related to the molecular charge, driving molecules to move toward electrodes with opposite polarity. In addition, the EOF plays an undeniable role, mainly due to local electrostatic interactions caused by surface charges of the nanopore. Free ions carrying opposite charges in the electrolyte will be electrostatically absorbed onto the charged solid surface, forming an electric bilayer system. When molecules approach the surface of charged nanopores, the electric double layer interferes with the movement of molecules with EOF.[47] The charge carried by molecules, as well as electrolyte concentration and pH, can affect the magnitude of EP and EOF forces, thereby modulating the process of molecular translocation.[45]

Nanopore sensors can be classified into two primary categories: biological nanopores and solid-state nanopores. Biological nanopores (Figure 3b), exemplified by α-hemolysin[46,48,49] and MspA,[50,51] are naturally occurring proteins or lipid membrane channels, offering high sensitivity and reliability due to their consistent structure. These pores can be harnessed for selective molecule binding, facilitating controlled molecular translocation. However, they are limited by stability concerns and stringent environmental requirements. In contrast, solid-state nanopores, including those fabricated from materials like silicon nitride, quartz, metals, and graphene,[45,52] are created through advanced nanofabrication techniques like focused ion beam or electron beam etching (Figure 3c),[53,54] with pore sizes below 5 nm can be obtained. However, these methods often involve expensive and complex procedures.[55] Alternative approaches, such as controlled dielectric breakdown (Figure 3d)[56] and chemical etching (Figure 3e),[57,58] apply high electric fields or use photolithography to pattern and etch substrates, respectively. Laser-assisted drawing[59] offers a low-cost, rapid route by heating and stretching capillary glass tubes, benefiting from the low capacitance noise of glasses due to their high dielectric constant.[60] Nevertheless, uniformity in shape and size of generated nanopores remains a significant challenge.

Solid-state nanopores exhibit several key advantages, including exceptional mechanical and thermal stability, tunable pore dimensions and shapes, and compatibility with microfluidic integration. Despite these advancements, practical implementation remains hindered by challenges such as limited temporal resolution due to rapid molecular translocation and unpredictable signals resulting from nonspecific adsorption on the pore surface.[52] Several surface modification strategies have been explored, such as the functionalization of specific functional groups, lipid bilayer coating to simulate biological environments, or surfactant modification to reduce adsorption.[45] Notably, molecular sensing with carriers like DNA has been proven to be effective in enhancing specificity in solid-state nanopore detection. By using DNA as a carrier and incorporating recognition elements like aptamers and antibodies, the selective capture of target molecules, such as cancer markers in serum, has been demonstrated. (Figure 3f)[61−63]

2.3. The Others

Transistors play a crucial role in electronic circuits, in which the current between the source and drain is controlled through the voltage applied to the gate. The interaction of a single molecule with the transistor channel can disrupt charge distribution, altering the leakage current. In 2000, the first smFET was achieved utilizing a solitary C_{60} molecule. (Figure 4a) In this pioneering work conducted by Park et al., a C_{60} molecule is connected to the source and drain electrodes, while the gate is positioned beneath them.[64] When a voltage is applied to the

https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

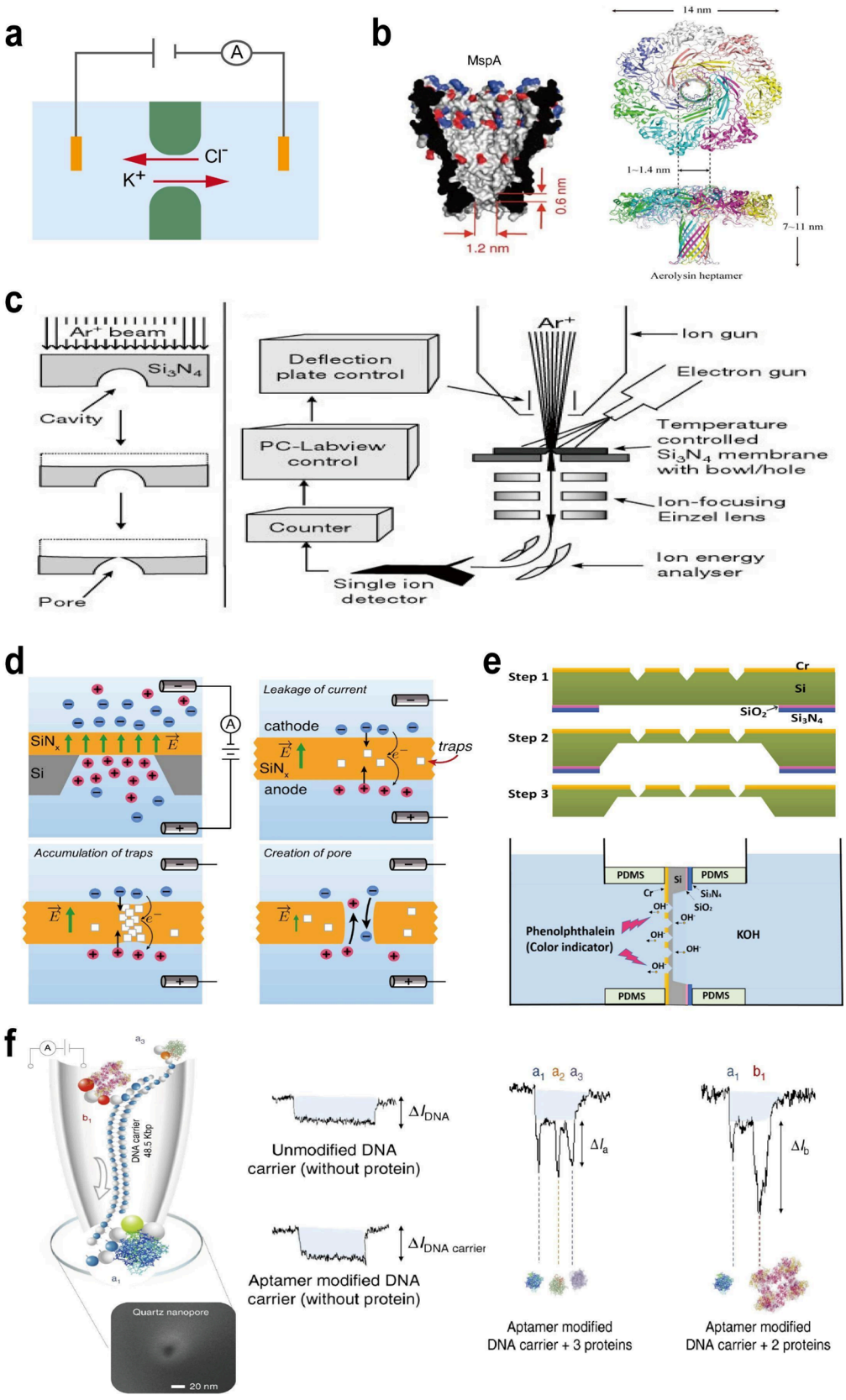


Figure 3. Nanopore sensors. (a) Generation of the ionic current. (b) Biological nanopores. MspA[51] and α-hemolysin.[46] Reproduced with permission from ref 51. Copyright 2012, Springer Nature America, Inc. (c) Focused ion beam etching.[53] Reproduced with permission from ref 53. Copyright 2001, Macmillan Magazines Ltd. (d) Controlled dielectric breakdown.[56] (e) Chemical etching.[57] Reproduced with permission from ref 57. Copyright 2018 IOP Publishing Ltd. The size of the nanopores in c, d, and e can be controlled by monitoring the number of ions or the magnitude of ion current through a feedback system. (f) Specific detection with DNA carriers through capillary nanopore. The binding of target molecules can be identified through the secondary spike of nanopore signals.[63]

https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

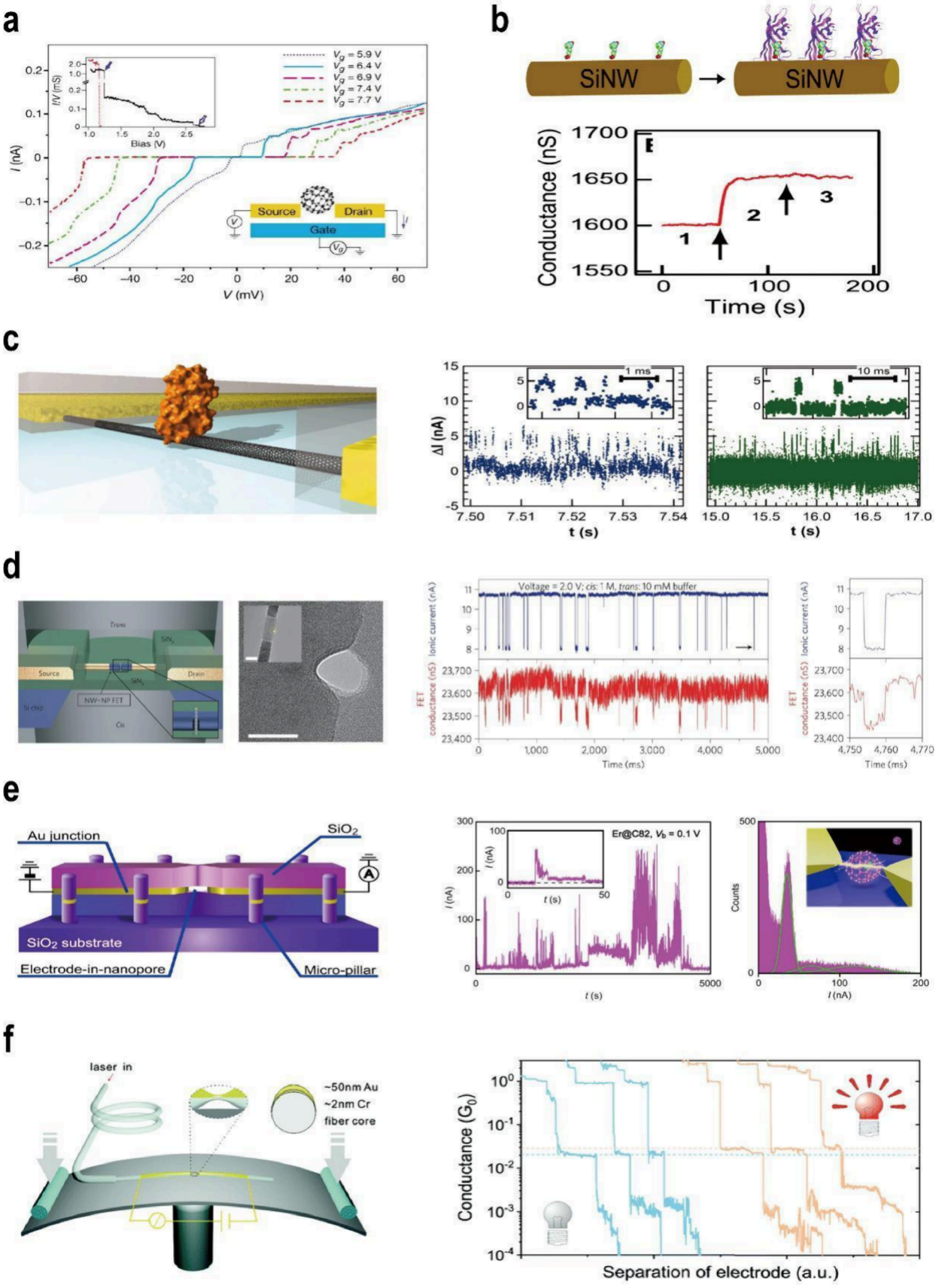


Figure 4. Other approaches of single-molecule bioelectronic sensing. (a) The first C_{60} FET.[64] Reproduced with permission from ref 64. Copyright 2000, Macmillan Magazines Ltd. (b) Silicon nanowire FET, whose surface is modified with biotin, with an increase in conductance at '2' indicating the binding of streptavidin.[67] Reproduced with permission from ref 67. Copyright 2001, The American Association for the Advancement of Science. (c) smFET constructed by single-walled carbon nanotube, which is utilized for monitoring lysozyme kinetics. The rapid (blue) and slow (green)

https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

Figure 4. continued

oscillations of the *I(t)* signal correspond to the nonproductive binding and catalytic events of lysozyme, respectively.[75] Reproduced with permission from ref 75. Copyright 2012, The American Association for the Advancement of Science. (d) The integration of nanopore and FET and the observed synchronous electrical signals.[70] Reproduced with permission from ref 70. Copyright 2011, Springer Nature Limited. (e) The integration of nanopore and tunneling electrode. The sudden jump of the current indicates the capture of molecules into the nanogap.[72] Reproduced with permission from ref 72. Copyright 2011, The Author(s). (f) Fiber-based MCBJ and conductance traces of single-imidazole junction measurements with logarithm coordinates in the absence (sky blue) and presence (sand) of light illumination.[73] Reproduced with permission from ref 73. Copyright 2016 Royal Society of Chemistry

gate, it modulates the electrostatic potential of the molecules, leading to energy displacements and offering valuable molecular information.[65]

Semiconductor nanowires (NWs) (Figure 4b) and carbon nanotubes (CNTs) (Figure 4c) are widely employed in the fabrication of highly sensitive FETs due to their molecular-scale dimensions.[1,66] NW-FETs consist of nanoscale semiconductor wires, such as silicon, gallium arsenide, or indium phosphide, typically synthesized through bottom-up methods like chemical vapor deposition or vapor−liquid−solid growth.[67] Carbon nanotubes, cylindrical carbon structures with exceptional electronic properties and a large specific surface area,[68] are produced using techniques like chemical vapor deposition or arc discharge, often resulting in aligned arrays or individual tubes. The conductance of smFETs can be modulated by molecular interactions, including charge scattering, transfer, and surface charge-induced gating.[1,68] However, despite their high carrier mobility, which enables strong signal generation, smFETs face the major challenge of relatively high noise levels, hindering their performance in demanding applications.[1,66]

Single-molecule bioelectric sensing can also be achieved through redox cycles or electrocatalysis. Introducing molecules with electrochemical activity into circuits can monitor the electron transfer of individual molecule redox reactions or study the mechanism characteristics of individual catalytic molecules.[66] Moreover, some new concepts have been proposed to combine different single-molecule bioelectronic sensors to further enhance their advantages. Combining nanopores with smFET (Figure 4d)[69,70] or integrating nanopores with tunneling sensors (Figure 4e)[71,72] can enhance the control and sensitivity of single-molecule detection, and improve the accuracy of signal measurement. Introducing additional incentives can also enhance the manipulation of single-molecule behavior.[40,73,74] Especially, light, as a noninvasive signal, can be combined with the break junction techniques. Imidazole molecules exhibit enhanced conductivity under optical stimulation (Figure 4f),[73] providing a new approach for constructing novel molecular optoelectronic devices.

3. INTEGRATION OF AI IN SINGLE-MOLECULE BIOELECTRONIC SENSING

The thorough and accurate comprehension of single-molecule bioelectronic sensing data is pivotal aspect of single-molecule research, while this often presents challenges. These data, derived from high-resolution experiments, are voluminous and characterized by low signal-to-noise ratios. The intricate nature of the signals, resulting from the dynamic behavior of individual molecules, necessitates advanced analytical techniques to decode the underlying information. The inherent complexity, with its potential for subtle variations, has yet to be fully harnessed or understood. Traditionally, analyzing such data has been a labor-intensive process, often relying on human intuition and expertise. However, the advent of Artificial Intelligence (AI) has revolutionized the way with its strength of processing and analyzing large, noisy data sets, detecting patterns that may be elusive to human observation. In the following section, we will give a brief introduction to AI and delve into the application of AI in enhancing the capabilities of single-molecule bioelectronic sensing.

3.1. Synopsis of AI

Artificial Intelligence (AI) aims to develop intelligent machines that can emulate human cognitive capabilities. Machine learning,[18,76] a subset of AI (Figure 5a), is further classified

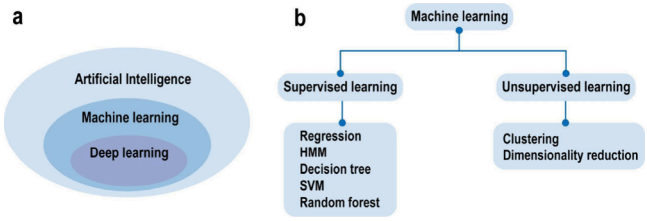


Figure 5. Synopsis of AI. (a) Relationship between artificial intelligence, machine learning and deep learning. (b) Classification of machine learning and related algorithms.

into supervised learning and unsupervised learning.[77,78] (Figure 5b) Supervised learning, with algorithms like linear regression, Hidden Markov Model (HMM), decision tree, Support Vector Machine (SVM), and random forest, relies on labeled data to construct a mapping function from inputs to outputs and is often applied in tasks like regression and classification. Unsupervised learning, which mainly includes clustering and dimensionality reduction, focuses on discovering hidden patterns within unlabeled data. Deep learning[79,80] has seen significant growth driven by the advancement of high-performance computing hardware. By creating neural network architectures with multiple layers and different computational logics, it can accomplish various tasks. These networks facilitate nonlinear relationship modeling, enhance sensitivity and accuracy, and handle high-dimensional and noisy data, thus enabling information extraction and pattern recognition in intricate data sets.[14] Machine learning and deep learning have shown considerable potential and advantages in single-molecule bioelectric sensing, with intelligent models being created, that could tackle tasks like automatic feature extraction, molecular difference recognition, and signal enhancement. Furthermore, they facilitate modeling and simulation analysis, providing supplementary insights for experimental design and aiding in optimizing sensors to improve data quality.

3.2. AI-Enhanced Single-Molecule Bioelectronic Sensing

3.2.1. Feature Extraction. A prominent benefit of AI is to perform efficient feature extraction on complicated and variable sensor data. Single-molecule bioelectronic sensing measurement data typically consists of one-dimensional temporal electrical

https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

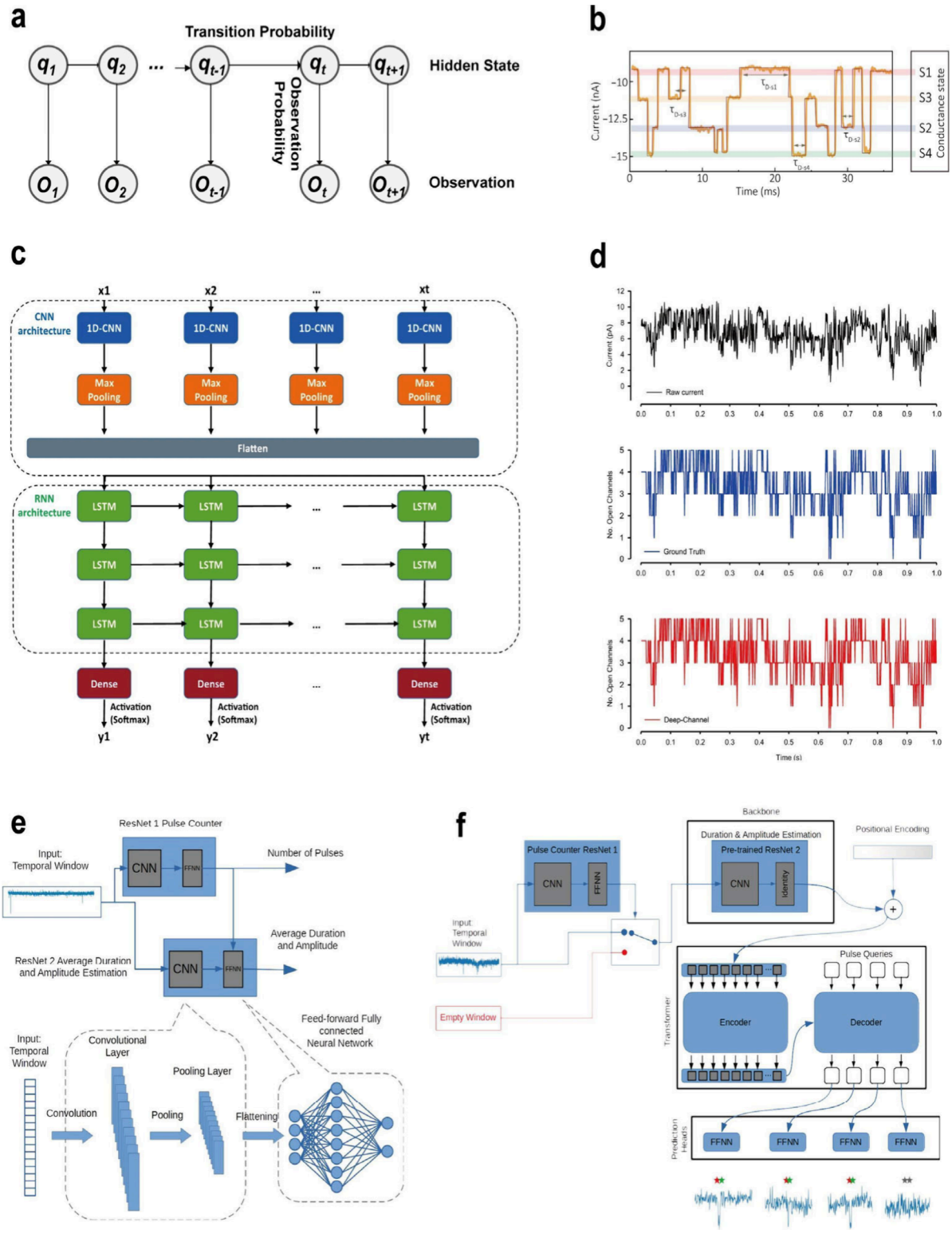


Figure 6. AI for feature extraction. (a) Principle of HMM. (b) Multilevel signals fitted by HMM.[90] (c) Composition of deep-channel and (d) its reduced single-molecule measurement signals.[85] (e) The B-Net architecture, comprising of two ResNets, each consisting of a CNN and FFNN.[86] (f) The PETR in which an input temporal window can be classified into pulse (colored) or no-pulse (gray).[89]

traces and changes in electrical signals reflect molecular information. However, due to the interference of background noise and the dynamic and random behavior of molecules, these signals take on complex forms. In traditional methods, it is necessary to define a threshold to separate the signal and noise, and to fit the changes in the signal through precise algorithm design, which concerns several limitations. On the one hand, the threshold setting is inherently subjective and can significantly impact the accuracy of the analysis. On the other hand, the custom-designed algorithms can be time-consuming and may not be universally applicable to all types of signals. In contrast, AI-based approaches offer a more robust and efficient solution.

https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

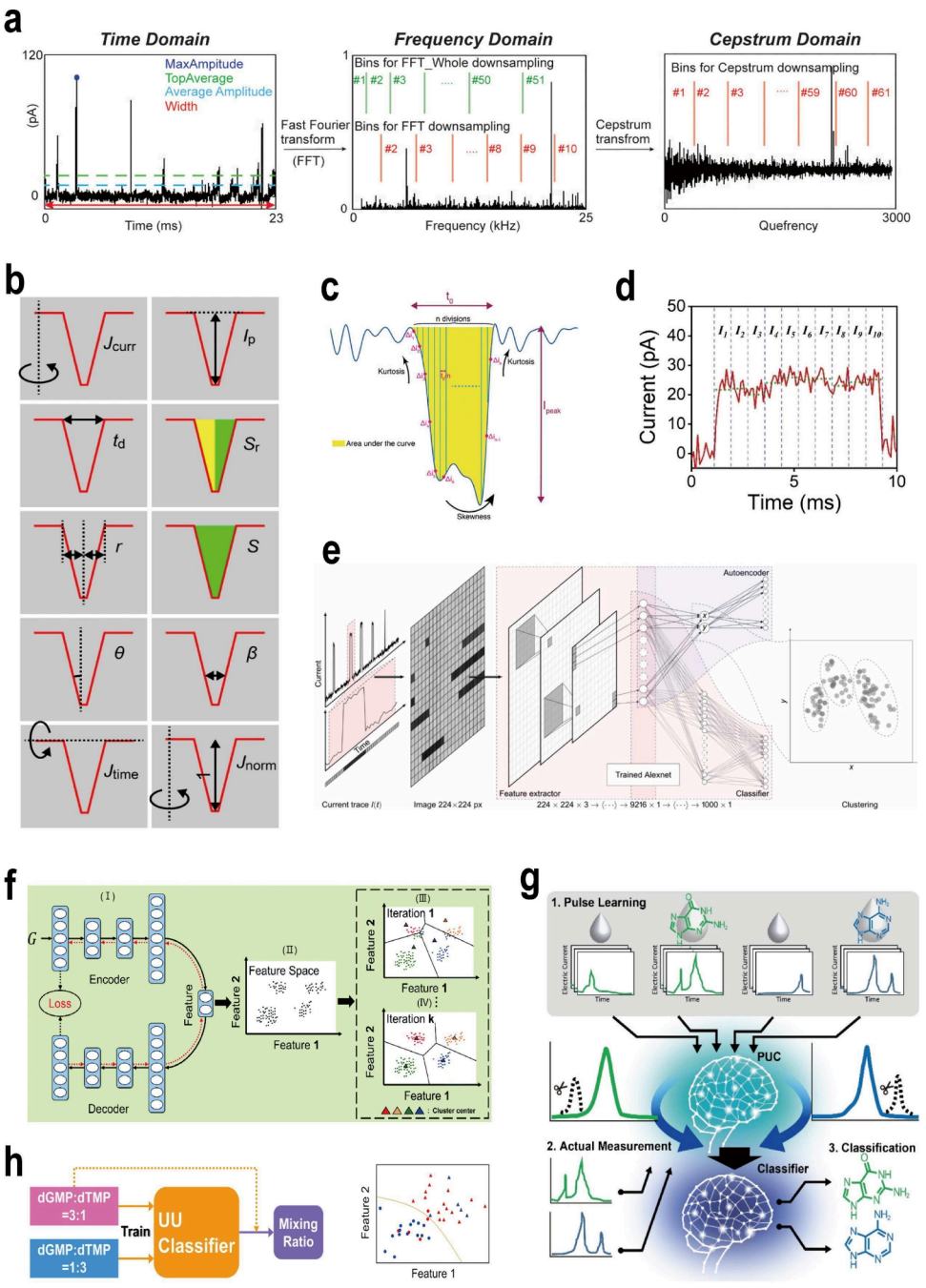


Figure 7. AI for high precision differentiation in single-molecule bioelectronic sensing. (a) Features in time, frequency, and cepstrum domain.[97] (b) Features about a single electrical pulse.[99] (c) Extraction of the kurtosis and the skewness.[4] Reproduced with permission from ref 4. Copyright 2023 The Authors. Small Methods published by Wiley VCH GmbH. (d) The pulse is divided into ten equal parts and combined with the maximum current, average current, and duration time to form a 13-dimensional feature vector.[104] Reproduced with permission from ref 104. Copyright 2022 Wiley VCH GmbH. (e) Conver-sion of the temporal signal into a two-dimensional image, extraction of features through a pretrained AlexNet network, and implementation of classification through clustering algorithms.[113] (f) Feature extraction through an Autoencoder.[114] Reproduced with permission from ref 114. Copyright 2020 Royal Society of Chemistry. (g) Removal of noise and classify through PUC.[105] (h) Direct classification of mixed samples based on the probability density of feature space.[116]

The Hidden Markov Model (HMM)[81,82] has been utilized for the idealization of electrical signals from single-molecule bioelectric sensing. The HMM, which is characterized by hidden states, observations, transition probabilities, and observation probabilities, postulates a sequence of unobservable hidden states with transition probabilities governing state shifts. These hidden states generate observable outputs, establishing a link between observed and hidden states. In practical implementation, the electrical signal represents the observation, with different electrical levels indicating distinct hidden states. The HMM is trained using known category data to estimate model parameters, therefore enabling signal prediction and computation of characteristic parameters based on the identified hidden states and observations, as depicted in Figure 6a, b.

Several types of neural networks have also been successfully applied. For example, Long Short-Term Memory (LSTM)

https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

networks[83] are well-suited for processing sequential data, allowing for the capture of long-term dependencies in sensor responses. Convolutional Neural Networks (CNNs)[84] excel in exploring the local spatial correlation in the data, which is crucial in detecting subtle changes. Celik et al. proposed a deep learning model[85] that combines CNN and LSTM. As illustrated in Figure 6c, d, the model first conducts one-dimensional convolution on the input temporal current data to extract crucial features, followed by feeding it into the LSTM architecture, which captures comprehensive information in sequence. Subsequently, the model generates predictions for each data category. This approach enables automatic and precise idealization of complex single-molecule activities in a swift manner, without the need for predefined parameters.

Besides, neural networks can also improve signal separation from background noises. Dematties et al. introduced the bipath neural network (B-Net) (Figure 6e) specifically tailored for identifying pulse signals in nanopore measurements.[86] The B-Net architecture is inspired by the ResNet,[87] which is designed for image processing and modified to adapt to one-dimensional data. It involves ResNet 1 predicting pulse count and ResNet 2 determining the average amplitude and dwell time of pulses within a specified temporal window. Furthermore, the pulse detection transformer (PETR) is proposed to extract pulse segments from traces, consisting of a pulse counter, backbone, transformer,[88] and feed-forward network. (Figure 6f)[89] ResNet 1 from the B-Net functions as the pulse counter, and ResNet 2 serves as the backbone network with convolution to extract essential features. The transformer employs a self-attention mechanism to capture dependencies within the input sequence, while the feed-forward network predicts the presence and timing of pulses in the input window. Both models are tested on synthetic data sets with varying signal-to-noise ratios and experimental data on DNA and protein translocation, demonstrating their robustness and versatility.

3.2.2. High-Precision Differentiation. Due to the similarity in structures or internal properties of certain molecules,[91,92] similar electrical signals are often generated, presenting challenges in distinguishing between molecules using statistical approaches. Machine learning and deep learning provide a promising solution to this problem by comparing large amounts of data and analyzing their differences comprehensively. Generally, this is achieved by training a classifier, which assigns input molecular features to corresponding categories. Classic supervised machine learning, such as SVM[93,94] and random forests[7,95] have been widely applied. SVM aims to find the optimal hyperplane to separate data from different groups.[96] The advantage is that it can effectively process high-dimensional data. For nonlinear problems, data is usually mapped to a high-dimensional space through specific kernel functions to construct linearly separable scenarios. Random Forest is an ensemble learning algorithm that combines multiple decision trees, each is trained on a subset of the data and then summarizes the results to obtain a final prediction. As the combination of multiple decision trees helps generalize well to unseen data, it is robust against overfitting.

Notably, these models which we usually define as shallow machine learning, often require manual extraction of data features, which reflect key information on individual molecules and can realize differentiation accordingly. Typical features to characterize molecules in single-molecule bioelectronic sensing include amplitude, dwell time, and signal frequency. In addition, multidimensional parameters are developed to improve the accuracy of recognition. Transforming time-domain electrical signals into the Fourier frequency domain and cepstral domain can extract a range of characteristic parameters. (Figure 7a)[6,97,98] However, due to the nonlinear and nonstationary nature of signals from single-molecule bioelectronic sensing, Fourier transform alone may not be sufficient for accurate signal analysis, hence methods like variational mode decomposition and Hilbert transform are employed to enhance the feature information.[91] Factors such as pulse area, kurtosis, and skewness[5,99−102] that describe the detailed shape of pulses are also taken into consideration. (Figure 7b, c) Moreover, each signal can be divided into multiple equal parts (Figure 7d), and from each the electrical component can be extracted to create a feature matrix.[4,103−105] It should be noted that the choice of feature combinations can significantly impact the final prediction accuracy of the model.[4] Principal Component Analysis (PCA)[106] has been suggested to identify the most relevant feature parameters.[107] The main principle of PCA is to transform a set of correlated variables into a new set of uncorrelated variables called principal components. It is achieved by identifying the eigenvectors and eigenvalues of the covariance matrix of the data, with the eigenvectors representing the directions of maximum variance and the eigenvalues indicating the magnitude of variance along these directions. Then, according to the size of the eigenvalues, the eigenvector corresponding to the eigenvalue with the highest variance is called the first principal component, and its corresponding variance is the variance of the data in the direction of the first principal component. By retaining a subset of the most significant principal components, PCA can reduce the dimensionality of the data while preserving the most important information.

Another approach is neural networks.[108−110] Compared to shallow machine learning like SVM and Random Forest, the advantage of neural networks is their ability to directly process large data sets. Automatic feature extraction can be performed on raw data to reduce the complexity of manual operations, while effectively obtaining important information that is overlooked due to human definition. Additionally, a large number of high-performance two-dimensional data neural network architectures have been designed based on the purpose of image processing. A feasible solution for one-dimensional single-molecule electrical data is to convert it into a two-dimensional form (Figure 7e),[111−113] thus networks for the image can be transplanted to facilitate noncomputer professionals to quickly process and analyze data.

Given that many models rely on labeled data for training, which may increase manual operation costs, some unsupervised machine learning methods have also been developed. Huang et al. utilized an Autoencoder for automatic feature extraction, as shown in Figure 7f.[114] Autoencoder is a type of unsupervised neural network, typically consisting of an encoder and a decoder. The encoder compresses input data to extract essential features or structures, and the decoder reconstructs the original input. The extracted features serve as the foundation for characterizing data similarities, which can then be classified using clustering algorithms. Clustering is a data mining technique used to group similar data points into clusters based on their inherent characteristics. The mostly utilized *k*-means clustering algorithm[115] starts by randomly selecting *k* initial centroids and assigning each data point to the nearest centroid. The centroids are then updated by recalculating the mean of the data points in each cluster, and the process iterates until centroids converge.

https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

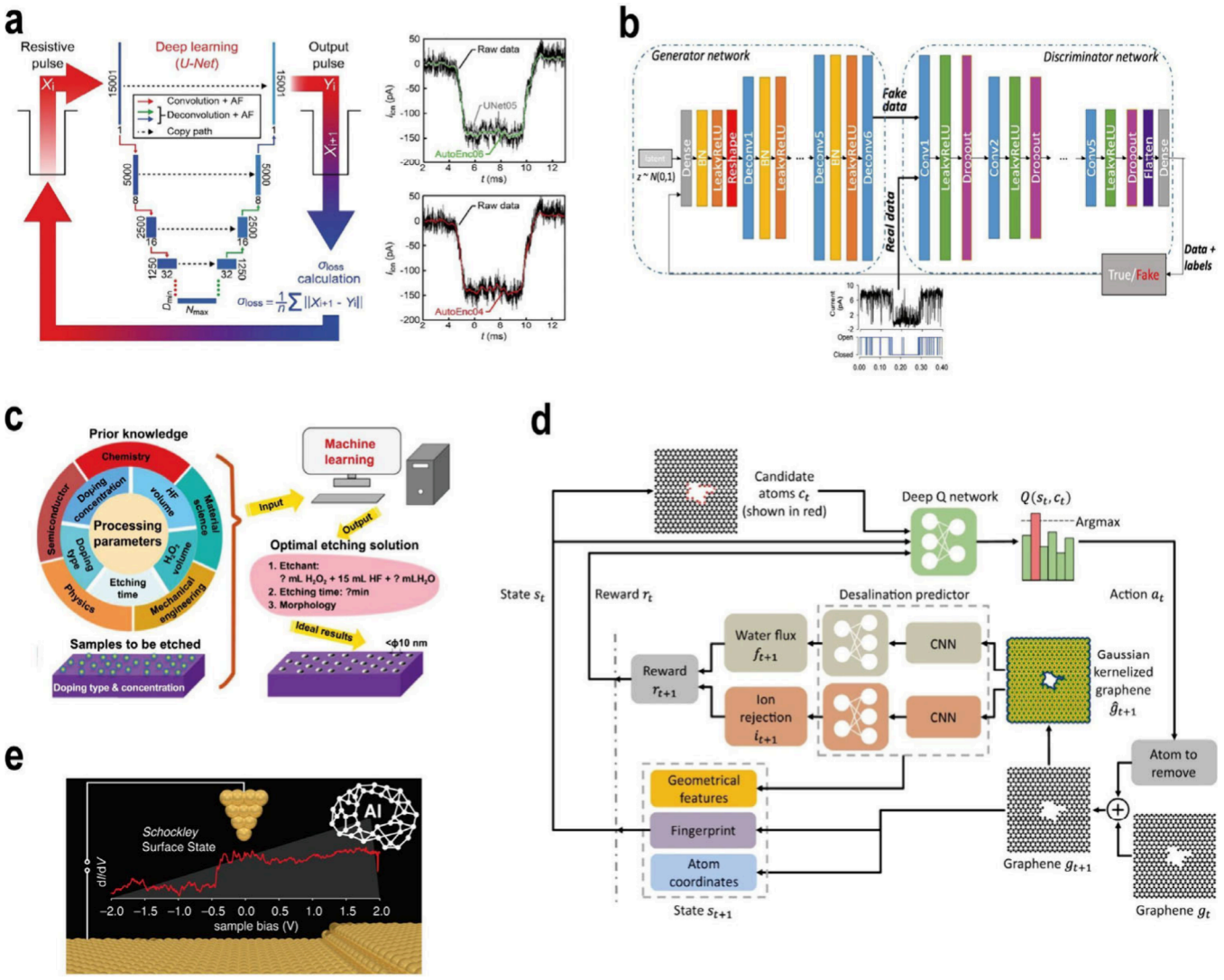


Figure 8. AI for simulation and optimization. (a) Neural network for denoising ionic current. The core information on the signal is preserved through convolution and pooling.[117] Reproduced with permission from ref 117. Copyright 2021 Wiley VCH GmbH. (b) Neural network for generating single molecule simulation data set.[118] (c) Optimize the most suitable conditions for nanopore array etching through machine learning.[119] (d) AI-assisted nanopore design. At each time step, the network will decide whether to remove carbon atoms and which atoms to remove to achieve the best performance of the pore.[120] (e) Quality control of STM tips based on machine learning.[121]

Besides, the concept of positive and unlabeled data classification (PUC) was introduced.[105] PUC first takes samples containing signals as unlabeled data, while background noise signals are positively labeled to train a two-class classifier. Next, classification is performed on the data set containing the target signal, and the noise is recognized as positive while the target signal is in negative groups. (Figure 7g) This method can effectively remove noise interference from measurement data. Ryu et al. proposed a method for directly classifying unlabeled data. (Figure 7h)[116] Signal features are extracted and the probability density functions of the feature space are computed. Classification is achieved based on the high probability density regions due to aggregation of parameters of similar molecules in the feature space, which can be used to distinguish molecules in mixed samples and estimate their concentration ratio.

3.2.3. Signal Enhancement. Noises inevitably exist in single-molecule bioelectric sensing systems, especially high-frequency noises generated by the coupling between the device capacitance and the voltage noise in current amplifiers, which are difficult to remove through ordinary low-pass filters.[117] Tsutsui et al. introduced a neural network based on the calculation of convolution for signal denoising, as depicted in Figure 8a. The method methodology revolves around treating signals as essential features and background noise as nonessential information. Through iterative convolution operations and dimensionality reduction from the autoencoder, significant features are retained, while the irrelevant noise is efficiently eliminated.[117]

3.2.4. Modeling and Simulation. To enhance model performance, training with diverse data sets is imperative. In this regard, artificially generated data sets based on deep neural networks provide a reliable approach. Ball et al. developed a model based on a Generative Adversarial Network (GAN), which only uses a small amount of real experimental data to generate an infinite amount of simulated data and can automatically add data labels, as shown in Figure 8b.[118] In addition, machine learning and deep learning can also help optimize the design and characteristics of sensors, improving the sensitivity and stability of systems. In order to efficiently prepare sub-10 nm solid-state nanopore arrays with controllable

https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

Table 1. Applications of AI-Integrated Single-Molecule Bioelectronic Sensing

Sensors	AI Techniques	Applications	Refs
		Single-molecule sequencing	
Nanopore	HMM, CNN, LSTM	Base calling	122−126
Nanopore	SquiggleNet	Selective sequencing	127
Nanopore	Sturgeon	Sequencing and real time classification	128
Combined nanopore and quantum tunneling	Regression	Prediction of nucleotide transfer functions	129, 130
		Low-abundance detection	
Nanopore	Classification	Detection of virus	95, 99
Nanopore	SVM	Quantification and identification of glycosaminoglycans	6
Nanogapped electrode	Random forest	Tracking of cyclic adenosine monophosphate	7
Nanopore	SVM	Identification of acidic catecholamine metabolites	5
Single-walled CNT	Neural network	Recognition of the mixed ammonia/amine gases	131
Nanopore	Classification	Differentiation of protein markers	4
smFET	PCA, k-NN	Early diagnosis of pancreatic cancer precursors	132
Nanopore	Classification	Monitoring of particulates in the air	11
		Real-time monitoring	
Nanogapped electrode	PUC, clustering	Counting of base−ligand interactions	10
Nanogapped electrode	PUC, XGBoost	Resolution of neurotransmitters	103
		Development of electronic devices with biomolecules	
Break junction	Clustering	Single-molecule conductance analysis	12, 133, 134

morphology through metal-assisted chemical etching technology, Chen et al. utilized SVM to establish a relationship model between the nanopore structure and preparation conditions such as etching time, doping type, and concentration, thus obtaining a process parameter window for generating regular nanopore arrays on silicon wafers with different doping types and concentrations. (Figure 8c)[119] Wang et al. designed a reinforcement learning framework aimed at designing the optimal geometry of graphene nanopores with high throughput and high ion suppression under certain external pressures for efficient seawater desalination (Figure 8d), and CNN is introduced to predict the performance of a given nanopore. Irregular shape with rough edges geometry of AI-created pores is found to be the key factor to achieve efficient seawater desalination. The network can design the shape of nanopores with atomic accuracy.[120] In addition, the quality of the STM tips is crucial for subsequent measurements. The commonly used method for preparing traditional STM tips is to repeatedly poke the tip into the metal substrate to obtain a thin layer of substrate atoms. Then, the dI/dV spectra of the substrate will be used as a reference to determine whether the tip is suitable for tunneling measurement. Due to the unpredictable geometric changes of the needle tip during the pressing process, needle tip adjustment is usually slow. A machine learning-based tip automatic control model has been proposed, which can autonomously analyze the surface morphology of metal substrates to find a sufficiently large flat substrate area, which is conducive to tip formation and can continuously monitor and feedback until the tip quality meets the requirements. (Figure 8e)[121]

4. APPLICATIONS OF AI-INTEGRATED SINGLE-MOLECULE BIOELECTRONIC SENSING

The fusion of AI with single-molecule bioelectronic sensing offers a plethora of advantages and promising transformative potential in the realm of biosciences and biotechnologies. It capitalizes on the inherent strengths of single-molecule bioelectronic sensors, which boast exceptional sensitivity, unparalleled temporal resolution, and the capability to be seamlessly integrated into various platforms, with the integration of artificial intelligence has significantly enhanced the processing of single-molecule bioelectrical sensing data, boosting the efficiency of data analysis. These intelligent bioelectronic sensors demonstrate extraordinary potential, including advance high-throughput and accurate sequencing, provide the ability to detect in low-abundance and capture real-time changes, as well as promote the development of electronic devices with biomolecules, as summarized in Table 1.

4.1. Single-Molecule Sequencing

Single-molecule sequencing technology, also known as third-generation sequencing, is a revolutionary approach that has significant importance in the field of genomics.[135] Unlike traditional techniques, it directly fetches the individual DNA or RNA molecules without the need for amplification or fragmentation. The advantage of single-molecule sequencing lies in its ability to generate long reads, enabling the assembly of complex genomes and the identification of structural variations that were previously challenging to detect. It provides a holistic view of the genome, including repetitive regions and epigenetic modifications.[136−138] Nanopore sequencing has experienced significant development over the years, especially since the successful inception of the first-generation Oxford Nanopore MinION in 2014.[139] The nanopore acts as a molecular-sized gateway and DNA helicase has been introduced as a translocation controller. When a strand of DNA passes through the pore, it induces a change in the electrical current flowing through the pore, which varies due to different bases (Figure 9a).[140]

Nanopore sequencing generates large amounts of data with high complexity, and solutions with AI techniques are crucial to efficient information extraction. HMM[122,123,125] has been used for base sequence recovery. Assuming a nucleic acid moves through the pore one nucleotide at a time and there are k nucleotides present in the nanopore at the same time. The ionic current signals generated by k nucleotides are considered a series of observable events, while the corresponding nucleotide sequence is regarded as the hidden state of HMM. Due to the overlap between the first nucleotide of each state and the last nucleotide of the previous state, the joint probability of the nucleotide sequence can be calculated, and the path with the highest total joint probability represents the final predicted sequence. Furthermore, deep learning models based on CNN or

https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

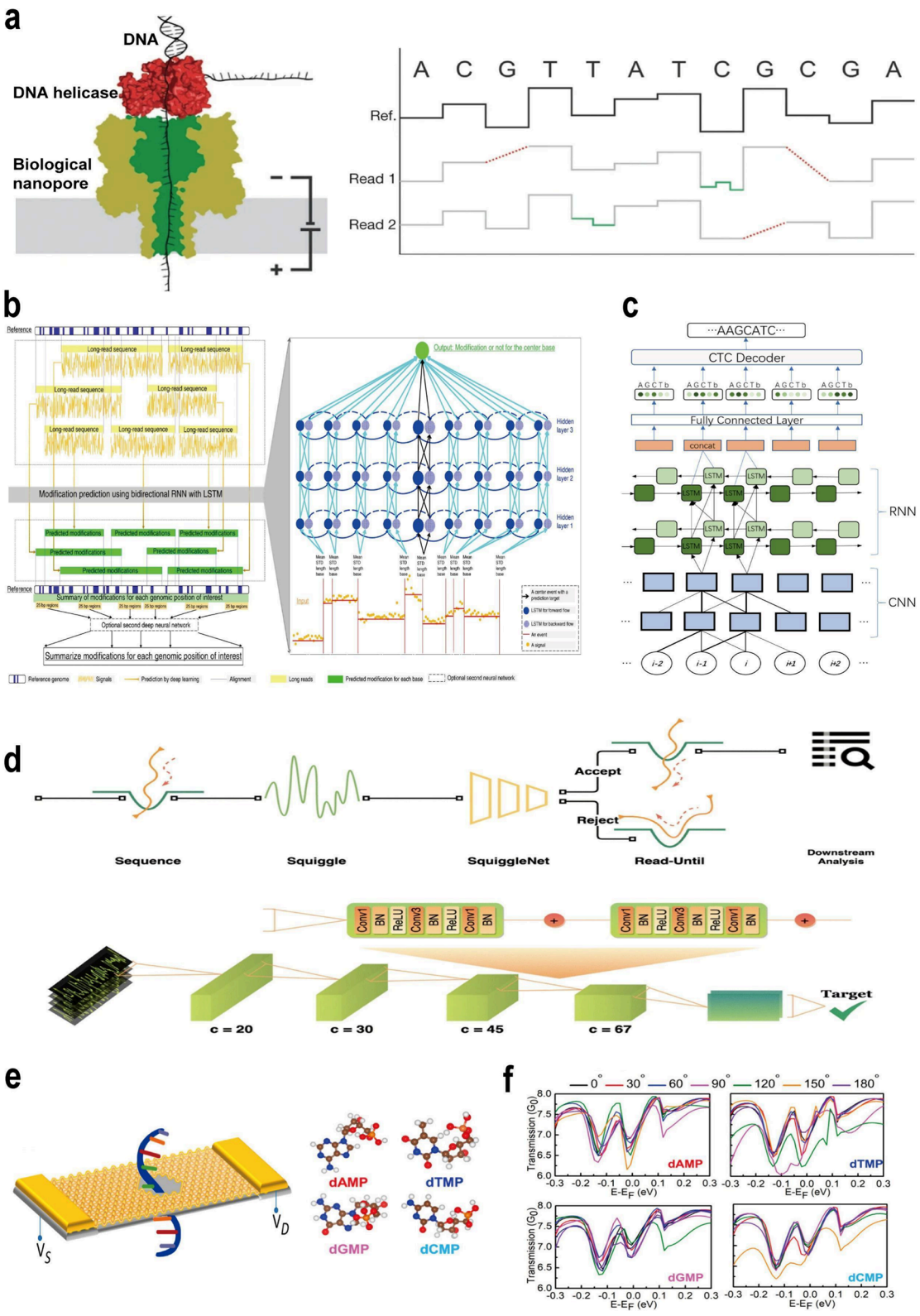


Figure 9. AI-integrated single-molecule bioelectronic sensing for sequencing. (a) Scheme of nanopore sequencing and the base sequence recovered by HMM.[125] Reproduced with permission from ref 125. Copyright 2015, Springer Nature America, Inc. (b) The flowchart of a deep neural network for the detection of DNA base modification.[124] (c) The neural network architecture for gene sequence restoration. The circles at the bottom represent the time series of raw signal input data. A CNN discriminates local pattern information from the input, which is then fed into a LSTM to capture long-range interaction information. A FC layer is employed to obtain base probabilities from the LSTM output. These probabilities are used by a CTC decoder to generate the nucleotide sequence.[126] (d) The SquiggleNet, employs 1D-ResNet-styled bottleneck blocks with increasing numbers of filters. The final fully connected layer and average pooling are applied after the last convolutional block.[127] (e) Schematic model of a monolayer gold nanopore prototype device for transverse tunneling conductance-based DNA sequencing and atomic structure of four DNA nucleotides. (f) Changes in transmission spectra due to in-plane rotation.[129]

https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

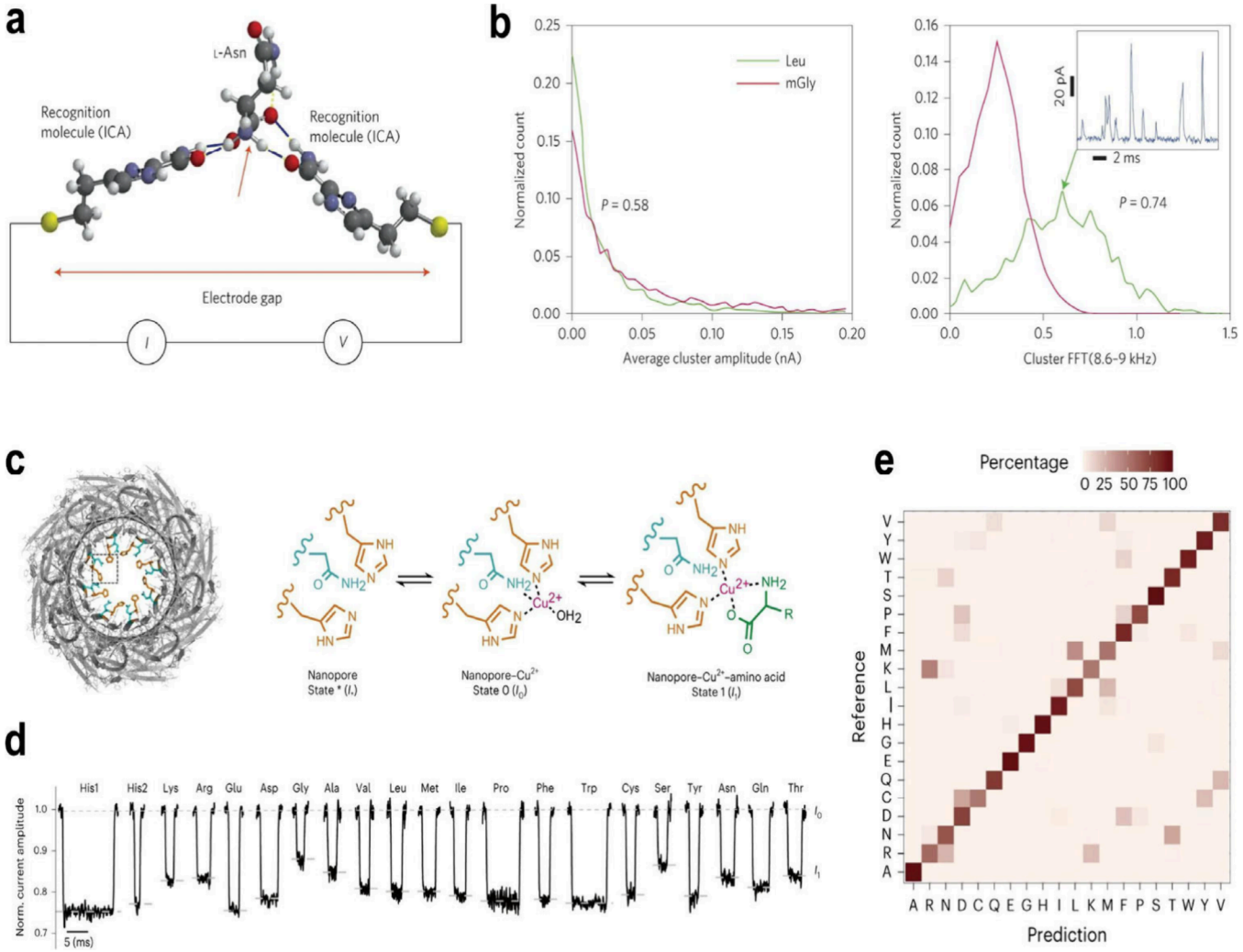


Figure 10. Progresses in protein sequencing. (a) The nanogapped-electrode with ICA functionalization. (b) Features of the amino acid signal clusters.[98] Reproduced with permission from ref 98. Copyright 2014, Springer Nature Limited. (c) The functionalized biological nanopore. (d) Representative signals of current blockade events of 20 amino acids. (e) The confusion matrix for classifying amino acids through machine learning.[142]

LSTM[124,126] extract meaningful features, which are then transformed into a sequence of probabilities, where each probability corresponds to a specific nucleotide. The model is trained on large data sets of known sequences and their corresponding electrical signals, learning to associate signal patterns with specific bases. Finally, the model predicts the most likely base at each time step based on the current signal, considering the context provided by the previous bases. The use of machine learning and deep learning can greatly improve the efficiency of nanopore sequencing data analysis while reducing the error rate in base calling (Figure 9b, c). Besides, the benefits of using deep neural networks lie in their ability to process information at high speeds. Bao et al. proposed a SquiggleNet to distinguish the DNA of humans and bacteria (Figure 9d). SquiggleNet runs faster than DNA passes through pores, allowing for real-time classification and targeted sequencing.[127] Besides, a Sturgeon network classifier has been introduced for analyzing sparse sequencing data in the early stages of surgery, helping doctors judge tumors and assist in intraoperative decision-making.[128]

A highly promising new idea proposed is to combine solid-state nanopores with quantum tunneling sequencing, where nanopores constrain an individual molecule and tunneling currents provide higher spatial resolution.[26,45] The nanoelectrodes of tunneling sensors are usually modified by specific recognition molecules, and when a single nucleotide or amino acid is captured between the electrodes, it can be recognized through tunneling signals.[93,97,98] Jena et al. proposed a graphene and gold nanopore quantum tunneling model (Figure 9e) and used density functional theory and nonequilibrium Green's function (DFT-NGF) method to calculate the transfer function of nucleotides. Due to the changes and oscillations in the orientation of nucleotides within the nanopores during the translocation process, the position of atoms relative to the edge of the pores changes, resulting in a change in the coupling strength between nanopores and nucleotides, which further affects the transmission performance of the device (Figure 9f). Using machine learning regression models, three other nucleotides were successfully predicted through the transfer function of a single nucleotide, demonstrating the predictive ability for unknown nucleotides. This will provide a reference value for fast and more accurate sequencing.[129,130]

Proteins, as the fundamental facilitators of biological processes, are defined by their primary amino acid sequences, which are pivotal in determining protein functions and reflecting genetic and disease signatures.[141−144] The advent of single-

https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

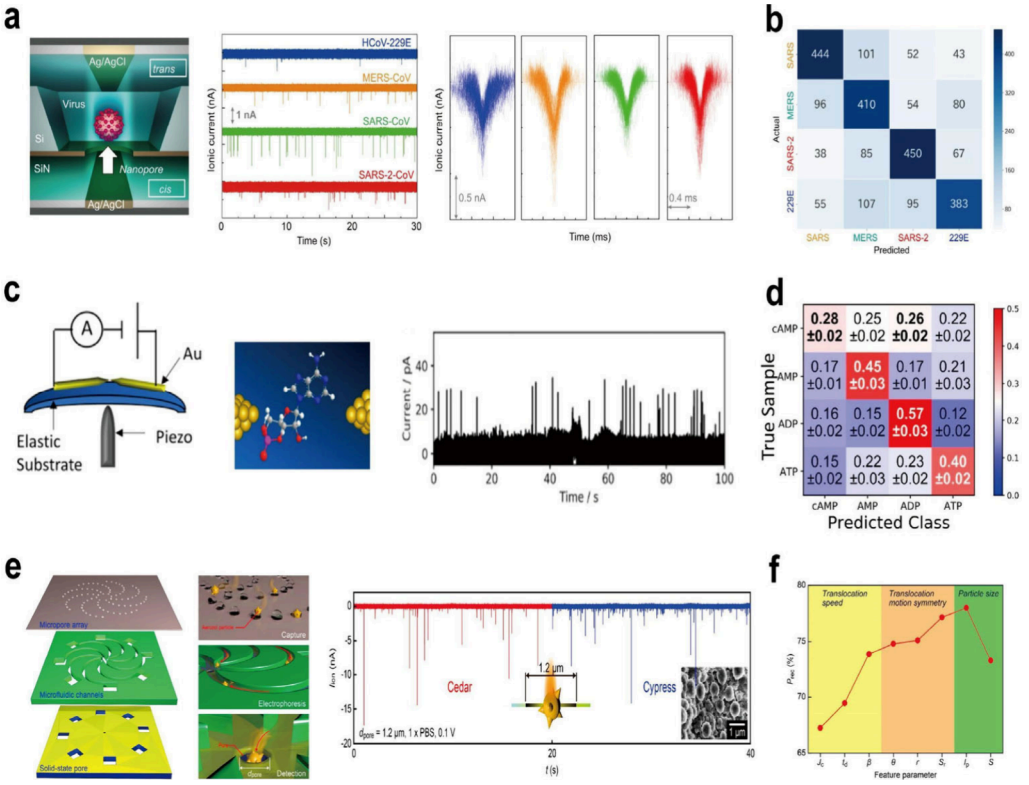


Figure 11. AI-integrated single-molecule bioelectronic sensing in low-abundance detection. (a) Nanopore for virus detection. (b) Confusion matrix obtained by machine learning corresponding to the four types of viruses.[95] (c) Single-molecule measurement of nucleotide-type second messenger which is relevant to the intracellular signal transduction, with nanogapped electrode fabricated by MCBJ. (d) Classification of cyclic adenosine monophosphate and its similar nucleic acid molecules.[7] (e) A 3D-integrated pore sensor to capture particles in air and ionic current traces of cedar and cypress. (f) Recall P_{rec} of pollen differentiation based on the characteristic parameters of each pulse.[11]

molecule protein sequencing technology promises to revolutionize proteomics by deepening our comprehension of life systems. However, proteins exhibit higher complexity compared to DNA or RNA. With proteins comprising 20 different amino acids, each differing by as little as 0.001 nm^3, detecting 20 distinguishable signals poses a significant challenge.[141] In 2014, Lindsay et al. made a pioneering contribution by employing tunneling current to measure amino acids, using a tunneling electrode functionalized with 4(5)-(2-mercaptoethyl)-1H-imidazole-2-carboxamide (ICA). By extracting 106 features in both temporal and spectral domains and integrating them with SVM model, they successfully classified three groups of amino acids with similar structures but minute differences (Figure 10a, b).[98] Kawai et al. used small gap electrodes at 0.7 and 0.55 nm for direct measurement, where 12 amino acids produced distinguishable signals.[21] Benefiting from the breakthrough and success of nanopore DNA sequencing technology, research on nanopore-based protein sequencing has also garnered significant attention.[145,146] The advancements in nanopore DNA sequencing technology have catalyzed interest in nanopore-based protein sequencing. Recent studies, employing functionalized biological nanopores in conjunction with machine learning algorithms, have exhibited high accuracy in identifying not only the 20 amino acids but also their post-translational modifications. (Figure 10c, d, e)[94,142]

4.2. Low-Abundance Detection

The detection of low-abundance biomolecules is a significant challenge in the field of molecular biology and biomedicine. Traditional detection methods often suffer from low sensitivity, making it difficult to identify rare biomolecules that are crucial for understanding biological processes and diagnosing diseases. Significantly, the advent of AI-integrated single-molecule bioelectronic sensing has revolutionized this field by offering unprecedented sensitivity and specificity. One of the key applications of this technology is in the identification of viruses with distinct allotypes. By utilizing nanopores in single-molecule bioelectronic sensing (Figure 11a, b),[95,99] it is possible to distinguish between viruses based on subtle differences in size and surface charge. This capability is particularly important for emerging infectious diseases, where the ability to quickly identify and track different strains of a virus can be critical for public health. Furthermore, the detection of molecular markers and metabolites, which play crucial roles in regulating human physiological and pathological processes, has been greatly enhanced by AI-integrated single-molecule bioelectronic sensing. By analyzing the subtle differences in signal features, these technologies can detect similar molecular markers and track changes in specific molecules (Figure 11c, d).[5−7,131] This has significant implications for early disease diagnosis, which can be crucial for recovery.[4,132] Besides, AI-integrated single-molecule bioelectronic sensing has also been applied to environmental monitoring. A three-dimensional solid-state nanopore has been developed for the identification and analysis of small and medium-sized molecular particles in the air (Figure 11e, f).[11] This breakthrough paves the way for effective environmental monitoring and provides a viable solution for assessing air quality.

https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

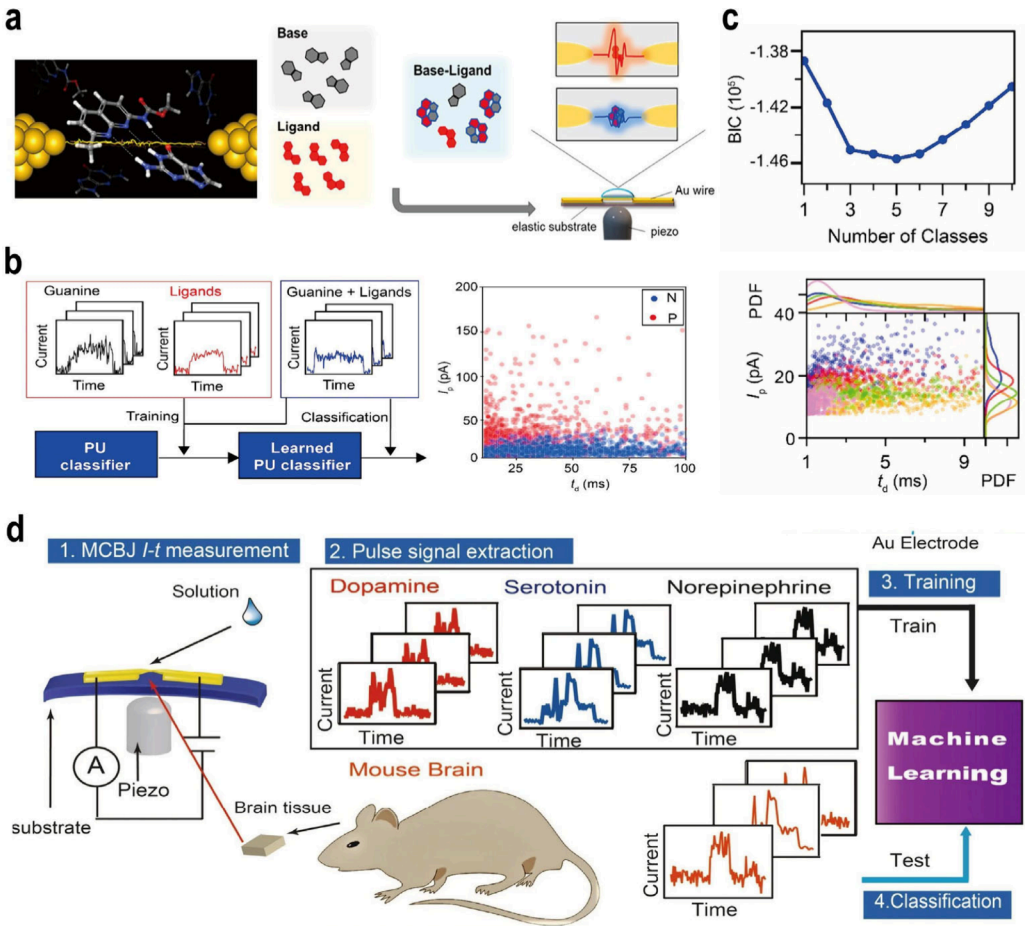


Figure 12. AI-integrated single-molecule bioelectronic sensing for real-time monitoring of molecules and molecular interactions. (a) Nanogapped electrode for the detection of base-ligand combination. (b) Recognition of base-ligand combination by PUC. (c) Classification of five hydrogen bonding modes in base-ligand combination based on clustering. The optimal number of clusters is determined by the Bayesian Information Criterion (BIC), which shows a minimum of five clusters.[10] (d) Utilizing nanogapped electrodes for time-resolved neurotransmitter detection and classification through supervised machine learning.[103]

4.3. Real-Time Monitoring

The ability to monitor biological processes in real time is also a key advantage of single-molecule bioelectronic sensing. This technology offers extremely high time resolution, allowing for the detection of molecular dynamics with high sensitivity. This capability is particularly valuable in drug discovery, where the ability to monitor interactions between biomolecules can significantly enhance the efficiency and success rate of drug development.[9] By combining the high sensitivity and resolution of single-molecule bioelectronic sensing with state-of-the-art machine learning algorithms, it is possible to significantly reduce the time and cost of drug research and optimize drug efficacy. For example, Takashima et al. employed nanogapped electrodes to detect nucleic acid-small molecule binding and introduced AI algorithms to evaluate the binding effects of different ligands with nucleic acid molecules. (Figure 12a, b, c)[10] This approach has the potential to accelerate the discovery of new drugs by identifying potential drug candidates with high binding affinity and specificity. Besides, the high time resolution of single-molecule bioelectronic sensing has been used to study the interactions between neurotransmitters and their receptors, contributing to a better understanding of brain function and the diagnosis of brain diseases. Komoto et al. utilized nanogapped electrodes to study the interactions between neurotransmitters and their receptors, providing valuable insights into the mechanisms of neurotransmitter action and the development of new treatments for brain diseases. (Figure 12d)[103] Furthermore, single-molecule bioelectronic sensing also enables the monitoring of conformational changes in biomolecules like enzymes,[147] providing crucial insights into their functions within organisms.

4.4. Development of Electronic Devices with Biomolecules

The integration of biomolecules into ultrasmall electronic devices represents a promising direction in bioelectronic research, with the potential to revolutionize the fields of information and computing. By leveraging the unique characteristics of biomolecules, these devices could play a pivotal role in information storage and signal processing, offering new paradigms for electronic devices.[148] A key technique in this context is the break junction, which enables the repeated study of individual molecules, yielding a plethora of conductance traces. Machine learning algorithms, particularly those capable of handling high-dimensional data, play a pivotal role in enhancing the analysis's efficiency and in revealing hidden patterns within the data.[12,133,134] By integrating theoretical calculations of molecules with machine learning techniques, our comprehension of molecules and their electronic properties can be profoundly enhanced, potentially leading to significant technological advancements in electronics and sensing, and facilitating the design of functional molecular devices.[149,150]

https://doi.org/10.1021/prechem.4c00048
Precis. Chem. 2024, 2, 518−538

5. SUMMARY AND FUTURE PROSPECTS

The integration of single-molecule bioelectric sensing and advanced AI technologies has opened up a new perspective for us to understand the micro world. In this review we introduce highly sensitive biosensors such as nanogapped electrodes, nanopores, and single-molecule FETs, detailing their principles, fabrication methods, and recent advancements. Comprehensive analysis and understanding of the data gathered by single-molecule bioelectric sensors are essential but face challenges due to large data volumes, noise interference, and low signal-to-noise ratios. As a result, we have meticulously summarized the enhancements brought about by AI algorithms in data processing, encompassing automatic feature extraction, precise interclassification differentiation, and noise reduction techniques. AI also plays a crucial role in enhancing the stability and reliability of experimental measurements and elevating data quality through sensor design optimization. Moreover, we explore the broad spectrum of applications of AI-integrated single-molecule bioelectric sensing, including enabling efficient and precise single-molecule sequencing, achieving early disease diagnosis and environmental monitoring through low-abundance detection capabilities, real-time monitoring of subtle molecular changes and interactions for high-throughput drug screening, in-depth investigation and comprehension of enzyme, protein, and organism structure and function, and fostering the future development of novel molecular electronic devices.

The collaborative optimization of single-molecule bioelectric sensing and AI technology in the future holds the potential to unlock further application capabilities. This relies on the continuous enhancement of single-molecule bioelectric sensing devices to improve their stability. Concurrently, emphasis should be placed on how AI algorithms can uncover overlooked rich information. A thorough understanding of noise sources and signal generation mechanisms in the sensor measurement process can facilitate more precise signal-to-noise separation. Additionally, appropriate data transformations could be a viable solution for subtle data differences in one-dimensional space. To enhance model generalization capabilities, it is imperative to evaluate their adaptability across various scenarios, with publicly available data sets serving to foster the development of universal algorithms. Furthermore, the introduction of cutting-edge AI technologies and algorithms will further promote the development of single-molecule bioelectric sensing, such as natural language processing, computer vision, and robotic process automation, may also find applications in single-molecule bioelectric sensors. As these technologies evolve, their integration into single-molecule bioelectric sensing could further enhance our capabilities in data analysis, interpretation, and sensor design. In conclusion, the fusion of AI and single-molecule bioelectric sensing represents a transformative frontier, offering both new opportunities and challenges. By continually refining our sensors and AI algorithms, we can expand the possibilities for unraveling the mysteries of the molecular world, paving the way for innovative molecular electronics and a deeper understanding of biosystems.

AUTHOR INFORMATION

Corresponding Authors

Longhua Tang − *State Key Laboratory of Extreme Photonics and Instrumentation, College of Optical Science and Engineering, Zhejiang University, Hangzhou 310027, China; Nanhu Brain-Computer Interface Institute, Hangzhou, Zhejiang 311100, China*; Email: lhtang@zju.edu.cn

Jinghong Li − *Department of Chemistry, Center for BioAnalytical Chemistry, Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology, Tsinghua University, Beijing 100084, China; Beijing Institute of Life Science and Technology, Beijing 102206, China; New Cornerstone Science Institute, Beijing 102206, China; Center for BioAnalytical Chemistry, Hefei National Laboratory of Physical Science at Microscale, University of Science and Technology of China, Hefei 230026, China*; orcid.org/0000-0002-0750-7352; Email: jhli@mail.tsinghua.edu.cn

Authors

Yuxin Yang − *State Key Laboratory of Extreme Photonics and Instrumentation, College of Optical Science and Engineering, Zhejiang University, Hangzhou 310027, China; Nanhu Brain-Computer Interface Institute, Hangzhou, Zhejiang 311100, China*

Yueqi Li − *Center for BioAnalytical Chemistry, Hefei National Laboratory of Physical Science at Microscale, University of Science and Technology of China, Hefei 230026, China*; orcid.org/0000-0001-7003-1978

Complete contact information is available at: https://pubs.acs.org/10.1021/prechem.4c00048

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Funding: The authors are grateful for support from the National Natural Science Foundation of China (grant nos. 62127818, 22374129), Natural Science Foundation of Zhejiang Province (grant no. LR22F050003), Fundamental Research Funds for Central Universities, and Zhejiang Key R&D Program of China under grant (grant no. 2023C03053). Author contributions: Y.Y., Y.L., J.L. and L.T. cowrote the manuscript, and the authors discussed the results and commented on them.

REFERENCES

(1) Gu, C.; Jia, C.; Guo, X. Single-Molecule Electrical Detection with Real-Time Label-Free Capability and Ultrasensitivity. *Small Methods* **2017**, *1* (5), 1700071.

(2) Sun, R.; Lv, J.; Xue, X.; Yu, S.; Tan, Z. Chemical Sensors Using Single-Molecule Electrical Measurements. *Chem. - Asian J.* **2023**, *18* (15), e202300181.

(3) Xu, M.; Fujita, D.; Hanagata, N. Perspectives and Challenges of Emerging Single-Molecule DNA Sequencing Technologies. *Small* **2009**, *5* (23), 2638−2649.

(4) Dutt, S.; Shao, H.; Karawdeniya, B.; Bandara, Y. M. N. D. Y.; Daskalaki, E.; Suominen, H.; Kluth, P. High Accuracy Protein Identification: Fusion of Solid State Nanopore Sensing and Machine Learning. *Small Methods* **2023**, *7* (11), 2300676.

(5) Hu, C.; Jia, W.; Liu, Y.; Wang, Y.; Zhang, P.; Chen, H.; Huang, S. Single-Molecule Sensing of Acidic Catecholamine Metabolites Using a Programmable Nanopore. *Chem. - Eur. J.* **2022**, *28* (44), e202201033.

(6) Im, J.; Lindsay, S.; Wang, X.; Zhang, P. Single Molecule Identification and Quantification of Glycosaminoglycans Using Solid-State Nanopores. *ACS Nano* **2019**, *13* (6), 6308−6318.

(7) Komoto, Y.; Ohshiro, T.; Taniguchi, M. Development of Single-Molecule Electrical Identification Method for Cyclic Adenosine Monophosphate Signaling Pathway. *Nanomaterials* **2021**, *11* (3), 784.

(8) Rauf, S.; Zhang, L.; Ali, A.; Liu, Y.; Li, J. Label-Free Nanopore Biosensor for Rapid and Highly Sensitive Cocaine Detection in Complex Biological Fluids. *ACS Sens.* **2017**, *2* (2), 227−234.
(9) Jeong, K.; Kim, J.; Dhanasekar, N. N.; Lee, M.; Chi, S. Application of Nanopore Sensors for Biomolecular Interactions and Drug Discovery. *Chem. - Asian J.* **2022**, *17* (19), e202200679.
(10) Takashima, Y.; Komoto, Y.; Ohshiro, T.; Nakatani, K.; Taniguchi, M. Quantitative Microscopic Observation of Base-Ligand Interactions via Hydrogen Bonds by Single-Molecule Counting. *J. Am. Chem. Soc.* **2023**, *145* (2), 1310−1318.
(11) Tsutsui, M.; Yokota, K.; Yoshida, T.; Hotehama, C.; Kowada, H.; Esaki, Y.; Taniguchi, M.; Washio, T.; Kawai, T. Identifying Single Particles in Air Using a 3D-Integrated Solid-State Pore. *ACS Sens.* **2019**, *4* (3), 748−755.
(12) Cabosart, D.; El Abbassi, M.; Stefani, D.; Frisenda, R.; Calame, M.; Van Der Zant, H. S. J.; Perrin, M. L. A Reference-Free Clustering Method for the Analysis of Molecular Break-Junction Measurements. *Appl. Phys. Lett.* **2019**, *114* (14), 143102.
(13) Yuan, S.; Gao, T.; Cao, W.; Pan, Z.; Liu, J.; Shi, J.; Hong, W. The Characterization of Electronic Noise in the Charge Transport through Single-Molecule Junctions. *Small Methods* **2021**, *5* (3), 2001064.
(14) Albrecht, T.; Slabaugh, G.; Alonso, E.; Al-Arif, S. M. R. Deep Learning for Single-Molecule Science. *Nanotechnology* **2017**, *28* (42), 423001.
(15) Wen, C.; Dematties, D.; Zhang, S.-L. A Guide to Signal Processing Algorithms for Nanopore Sensors. *ACS Sens.* **2021**, *6* (10), 3536−3555.
(16) Bro-Jørgensen, W.; Hamill, J. M.; Bro, R.; Solomon, G. C. Trusting Our Machines: Validating Machine Learning Models for Single-Molecule Transport Experiments. *Chem. Soc. Rev.* **2022**, *51* (16), 6875−6892.
(17) Cui, F.; Yue, Y.; Zhang, Y.; Zhang, Z.; Zhou, H. S. Advancing Biosensors with Machine Learning. *ACS Sens.* **2020**, *5* (11), 3346−3364.
(18) Greener, J. G.; Kandathil, S. M.; Moffat, L.; Jones, D. T. A Guide to Machine Learning for Biologists. *Nat. Rev. Mol. Cell Biol.* **2022**, *23* (1), 40−55.
(19) Taniguchi, M. Combination of Single-Molecule Electrical Measurements and Machine Learning for the Identification of Single Biomolecules. *ACS Omega* **2020**, *5* (2), 959−964.
(20) Huang, S.; He, J.; Chang, S.; Zhang, P.; Liang, F.; Li, S.; Tuchband, M.; Fuhrmann, A.; Ros, R.; Lindsay, S. Identifying Single Bases in a DNA Oligomer with Electron Tunnelling. *Nat. Nanotechnol.* **2010**, *5* (12), 868−873.
(21) Ohshiro, T.; Tsutsui, M.; Yokota, K.; Furuhashi, M.; Taniguchi, M.; Kawai, T. Detection of Post-Translational Modifications in Single Peptides Using Electron Tunnelling Currents. *Nat. Nanotechnol.* **2014**, *9* (10), 835−840.
(22) Wang, H.-Y.; Ying, Y.-L.; Li, Y.; Kraatz, H.-B.; Long, Y.-T. Nanopore Analysis of *β*-Amyloid Peptide Aggregation Transition Induced by Small Molecules. *Anal. Chem.* **2011**, *83* (5), 1746−1752.
(23) Schneider, G. F.; Kowalczyk, S. W.; Calado, V. E.; Pandraud, G.; Zandbergen, H. W.; Vandersypen, L. M. K.; Dekker, C. DNA Translocation through Graphene Nanopores. *Nano Lett.* **2010**, *10* (8), 3163−3167.
(24) He, G.; Li, J.; Ci, H.; Qi, C.; Guo, X. Direct Measurement of Single-Molecule DNA Hybridization Dynamics with Single Base Resolution. *Angew. Chem. Int. Ed.* **2016**, *55* (31), 9036−9040.
(25) Zevenbergen, M. A. G.; Singh, P. S.; Goluch, E. D.; Wolfrum, B. L.; Lemay, S. G. Stochastic Sensing of Single Molecules in a Nanofluidic Electrochemical Device. *Nano Lett.* **2011**, *11* (7), 2881−2886.
(26) Albrecht, T. Electrochemical Tunnelling Sensors and Their Potential Applications. *Nat. Commun.* **2012**, *3* (1), 829.
(27) Cui, X. D.; Zarate, X.; Tomfohr, J.; Sankey, O. F.; Primak, A.; Moore, A. L.; Moore, T. A.; Gust, D.; Harris, G.; Lindsay, S. M. Making Electrical Contacts to Molecular Monolayers. *Nanotechnology* **2002**, *13* (1), 5−14.
(28) Li, T.; Hu, W.; Zhu, D. Nanogap Electrodes. *Adv. Mater.* **2010**, *22* (2), 286−300.
(29) Hla, S.-W. Scanning Tunneling Microscopy Single Atom/Molecule Manipulation and Its Application to Nanoscience and Technology. *J. Vac. Sci. Technol. B* **2005**, *23* (4), 1351−1360.
(30) Hou, J. G.; Zhao, A. Detecting and Manipulating Single Molecules with STM. *Nano* **2006**, *01* (01), 15−33.
(31) Lv, S.-L.; Zeng, C.; Yu, Z.; Zheng, J.-F.; Wang, Y.-H.; Shao, Y.; Zhou, X.-S. Recent Advances in Single-Molecule Sensors Based on STM Break Junction Measurements. *Biosensors* **2022**, *12* (8), 565.
(32) Xu, B.; Tao, N. J. Measurement of Single-Molecule Resistance by Repeated Formation of Molecular Junctions. *Science* **2003**, *301* (5637), 1221−1223.
(33) He, Q.; Tang, L. Sub-5 nm Nanogap Electrodes towards Single-Molecular Biosensing. *Biosens. Bioelectron.* **2022**, *213*, 114486.
(34) Tang, L.; Nadappuram, B. P.; Cadinu, P.; Zhao, Z.; Xue, L.; Yi, L.; Ren, R.; Wang, J.; Ivanov, A. P.; Edel, J. B. Combined Quantum Tunnelling and Dielectrophoretic Trapping for Molecular Analysis at Ultra-Low Analyte Concentrations. *Nat. Commun.* **2021**, *12* (1), 913.
(35) Xiao, X.; Chen, M.; Zhang, J.; Zhang, T.; Zhang, L.; Jin, Y.; Wang, J.; Jiang, K.; Fan, S.; Li, Q. Sub-10 nm Monolayer MoS_2 Transistors Using Single-Walled Carbon Nanotubes as an Evaporating Mask. *ACS Appl. Mater. Interfaces* **2019**, *11* (12), 11612−11617.
(36) Cui, A.; Liu, Z.; Dong, H.; Wang, Y.; Zhen, Y.; Li, W.; Li, J.; Gu, C.; Hu, W. Single Grain Boundary Break Junction for Suspended Nanogap Electrodes with Gapwidth Down to 1−2 nm by Focused Ion Beam Milling. *Adv. Mater.* **2015**, *27* (19), 3002−3006.
(37) Fischbein, M. D.; Drndić, M. Nanogaps by Direct Lithography for High-Resolution Imaging and Electronic Characterization of Nanostructures. *Appl. Phys. Lett.* **2006**, *88* (6), 063116.
(38) Wang, L.; Wang, L.; Zhang, L.; Xiang, D. Advance of Mechanically Controllable Break Junction for Molecular Electronics. *Top. Curr. Chem.* **2017**, *375* (3), 61.
(39) Xiang, D.; Jeong, H.; Lee, T.; Mayer, D. Mechanically Controllable Break Junctions for Molecular Electronics. *Adv. Mater.* **2013**, *25* (35), 4845−4867.
(40) Zhao, X.; Zhang, X.; Yin, K.; Zhang, S.; Zhao, Z.; Tan, M.; Xu, X.; Zhao, Z.; Wang, M.; Xu, B.; Lee, T.; Scheer, E.; Xiang, D. In Situ Adjustable Nanogaps and In-Plane Break Junctions. *Small Methods* **2023**, *7* (4), 2201427.
(41) Esen, G.; Fuhrer, M. S. Temperature Control of Electromigration to Form Gold Nanogap Junctions. *Appl. Phys. Lett.* **2005**, *87* (26), 263101.
(42) Morita, A.; Sumitomo, T.; Uesugi, A.; Sugano, K.; Isono, Y. Dynamic Electrical Measurement of Biomolecule Behavior via Plasmonically-Excited Nanogap Fabricated by Electromigration. *Nano Ex.* **2021**, *2* (1), 010032.
(43) Yang, C.; Yang, C.; Guo, Y.; Feng, J.; Guo, X. Graphene-Molecule-Graphene Single-Molecule Junctions to Detect Electronic Reactions at the Molecular Scale. *Nat. Protoc.* **2023**, *18* (6), 1958−1978.
(44) Perrin, M. L.; Martin, C. A.; Prins, F.; Shaikh, A. J.; Eelkema, R.; Van Esch, J. H.; Van Ruitenbeek, J. M.; Van Der Zant, H. S. J.; Dulić, D. Charge Transport in a Zinc-Porphyrin Single-Molecule Junction. *Beilstein J. Nanotechnol.* **2011**, *2*, 714−719.
(45) Xue, L.; Yamazaki, H.; Ren, R.; Wanunu, M.; Ivanov, A. P.; Edel, J. B. Solid-State Nanopore Sensors. *Nat. Rev. Mater.* **2020**, *5* (12), 931−951.
(46) Cao, C.; Long, Y.-T. Biological Nanopores: Confined Spaces for Electrochemical Single-Molecule Analysis. *Acc. Chem. Res.* **2018**, *51* (2), 331−341.
(47) Firnkes, M.; Pedone, D.; Knezevic, J.; Döblinger, M.; Rant, U. Electrically Facilitated Translocations of Proteins through Silicon Nitride Nanopores: Conjoint and Competitive Action of Diffusion, Electrophoresis, and Electroosmosis. *Nano Lett.* **2010**, *10* (6), 2162−2167.
(48) Bayley, H.; Cremer, P. S. Stochastic Sensors Inspired by Biology. *Nature* **2001**, *413* (6852), 226−230.
(49) Zhang, L.; He, P.; Chen, H.; Liu, Q.; Li, L.; Wang, X.; Li, J. Real-Time Identification of Multiple Nanoclusters with a Protein Nanopore in Single-Cluster Level. *Nano Res.* **2024**, *17* (1), 262−269.

(50) Butler, T. Z.; Pavlenok, M.; Derrington, I. M.; Niederweis, M.; Gundlach, J. H. Single-Molecule DNA Detection with an Engineered MspA Protein Nanopore. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105* (52), 20647−20652.
(51) Manrao, E. A.; Derrington, I. M.; Laszlo, A. H.; Langford, K. W.; Hopper, M. K.; Gillgren, N.; Pavlenok, M.; Niederweis, M.; Gundlach, J. H. Reading DNA at Single-Nucleotide Resolution with a Mutant MspA Nanopore and Phi29 DNA Polymerase. *Nat. Biotechnol.* **2012**, *30* (4), 349−353.
(52) Lee, K.; Park, K.; Kim, H.; Yu, J.; Chae, H.; Kim, H.; Kim, K. Recent Progress in Solid-State Nanopores. *Adv. Mater.* **2018**, *30* (42), 1704680.
(53) Li, J.; Stein, D.; McMullan, C.; Branton, D.; Aziz, M. J.; Golovchenko, J. A. Ion-Beam Sculpting at Nanometre Length Scales. *Nature* **2001**, *412* (6843), 166−169.
(54) Storm, A. J.; Chen, J. H.; Ling, X. S.; Zandbergen, H. W.; Dekker, C. Fabrication of Solid-State Nanopores with Single-Nanometre Precision. *Nat. Mater.* **2003**, *2* (8), 537−540.
(55) Chen, Q.; Liu, Z. Fabrication and Applications of Solid-State Nanopores. *Sensors* **2019**, *19* (8), 1886.
(56) Kwok, H.; Briggs, K.; Tabard-Cossa, V. Nanopore Fabrication by Controlled Dielectric Breakdown. *PLoS One* **2014**, *9* (3), e92880.
(57) Chen, Q.; Wang, Y.; Deng, T.; Liu, Z. Fabrication of Nanopores and Nanoslits with Feature Sizes down to 5 nm by Wet Etching Method. *Nanotechnology* **2018**, *29* (8), 085301.
(58) Tsujino, K.; Matsumura, M. Morphology of Nanoholes Formed in Silicon by Wet Etching in Solutions Containing HF and H_2O_2 at Different Concentrations Using Silver Nanoparticles as Catalysts. *Electrochim. Acta* **2007**, *53* (1), 28−34.
(59) Piper, J. D.; Clarke, R. W.; Korchev, Y. E.; Ying, L.; Klenerman, D. A Renewable Nanosensor Based on a Glass Nanopipette. *J. Am. Chem. Soc.* **2006**, *128* (51), 16462−16463.
(60) Bafna, J. A.; Soni, G. V. Fabrication of Low Noise Borosilicate Glass Nanopores for Single Molecule Sensing. *PLoS One* **2016**, *11* (6), e0157399.
(61) Cai, S.; Pataillot-Meakin, T.; Shibakawa, A.; Ren, R.; Bevan, C. L.; Ladame, S.; Ivanov, A. P.; Edel, J. B. Single-Molecule Amplification-Free Multiplexed Detection of Circulating microRNA Cancer Biomarkers from Serum. *Nat. Commun.* **2021**, *12* (1), 3515.
(62) Ren, R.; Cai, S.; Fang, X.; Wang, X.; Zhang, Z.; Damiani, M.; Hudlerova, C.; Rosa, A.; Hope, J.; Cook, N. J.; Gorelkin, P.; Erofeev, A.; Novak, P.; Badhan, A.; Crone, M.; Freemont, P.; Taylor, G. P.; Tang, L.; Edwards, C.; Shevchuk, A.; Cherepanov, P.; Luo, Z.; Tan, W.; Korchev, Y.; Ivanov, A. P.; Edel, J. B. Multiplexed Detection of Viral Antigen and RNA Using Nanopore Sensing and Encoded Molecular Probes. *Nat. Commun.* **2023**, *14* (1), 7362.
(63) Sze, J. Y. Y.; Ivanov, A. P.; Cass, A. E. G.; Edel, J. B. Single Molecule Multiplexed Nanopore Protein Screening in Human Serum Using Aptamer Modified DNA Carriers. *Nat. Commun.* **2017**, *8* (1), 1552.
(64) Park, H.; Park, J.; Lim, A. K. L.; Anderson, E. H.; Alivisatos, A. P.; McEuen, P. L. Nanomechanical Oscillations in a Single-C_{60} Transistor. *Nature* **2000**, *407* (6800), 57−60.
(65) Perrin, M. L.; Burzurí, E.; Van Der Zant, H. S. J. Single-Molecule Transistors. *Chem. Soc. Rev.* **2015**, *44* (4), 902−919.
(66) Rosenstein, J. K.; Lemay, S. G.; Shepard, K. L. Single-molecule Bioelectronics. *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.* **2014**, *7* (4), 475−493.
(67) Cui, Y.; Wei, Q.; Park, H.; Lieber, C. M. Nanowire Nanosensors for Highly Sensitive and Selective Detection of Biological and Chemical Species. *Science* **2001**, *293* (5533), 1289−1292.
(68) Guo, X. Single Molecule Electrical Biosensors Based on Single-Walled Carbon Nanotubes. *Adv. Mater.* **2013**, *25* (25), 3397−3408.
(69) Traversi, F.; Raillon, C.; Benameur, S. M.; Liu, K.; Khlybov, S.; Tosun, M.; Krasnozhon, D.; Kis, A.; Radenovic, A. Detecting the Translocation of DNA through a Nanopore Using Graphene Nanoribbons. *Nat. Nanotechnol.* **2013**, *8* (12), 939−945.
(70) Xie, P.; Xiong, Q.; Fang, Y.; Qing, Q.; Lieber, C. M. Local Electrical Potential Detection of DNA by Nanowire-Nanopore Sensors. *Nat. Nanotechnol.* **2012**, *7* (2), 119−125.
(71) Ivanov, A. P.; Freedman, K. J.; Kim, M. J.; Albrecht, T.; Edel, J. B. High Precision Fabrication and Positioning of Nanoelectrodes in a Nanopore. *ACS Nano* **2014**, *8* (2), 1940−1948.
(72) Tsutsui, M.; Rahong, S.; Iizumi, Y.; Okazaki, T.; Taniguchi, M.; Kawai, T. Single-Molecule Sensing Electrode Embedded in-Plane Nanopore. *Sci. Rep.* **2011**, *1* (1), 46.
(73) Zhao, Z.; Guo, C.; Ni, L.; Zhao, X.; Zhang, S.; Xiang, D. In Situ Photoconductivity Measurements of Imidazole in Optical Fiber Break-Junctions. *Nanoscale Horiz.* **2021**, *6* (5), 386−392.
(74) Zhang, S.; Guo, C.; Ni, L.; Hans, K. M.; Zhang, W.; Peng, S.; Zhao, Z.; Guhr, D. C.; Qi, Z.; Liu, H.; Song, M.; Wang, Q.; Boneberg, J.; Guo, X.; Lee, T.; Scheer, E.; Xiang, D. In-Situ Control of on-Chip Angstrom Gaps, Atomic Switches, and Molecular Junctions by Light Irradiation. *Nano Today* **2021**, *39*, 101226.
(75) Choi, Y.; Moody, I. S.; Sims, P. C.; Hunt, S. R.; Corso, B. L.; Perez, I.; Weiss, G. A.; Collins, P. G. Single-Molecule Lysozyme Dynamics Monitored by an Electronic Circuit. *Science* **2012**, *335* (6066), 319−324.
(76) Janiesch, C.; Zschech, P.; Heinrich, K. Machine Learning and Deep Learning. *Electron. Markets* **2021**, *31* (3), 685−695.
(77) Badillo, S.; Banfai, B.; Birzele, F.; Davydov, I. I.; Hutchinson, L.; Kam-Thong, T.; Siebourg-Polster, J.; Steiert, B.; Zhang, J. D. An Introduction to Machine Learning. *Clin. Pharmacol. Ther.* **2020**, *107* (4), 871−885.
(78) Greene, C. S.; Tan, J.; Ung, M.; Moore, J. H.; Cheng, C. Big Data Bioinformatics. *J. Cell. Physiol.* **2014**, *229* (12), 1896−1900.
(79) Sarker, I. H. Deep Learning: A Comprehensive Overview on Techniques, Taxonomy, Applications and Research Directions. *SN Comput. Sci.* **2021**, *2* (6), 420.
(80) Shrestha, A.; Mahmood, A. Review of Deep Learning Algorithms and Architectures. *IEEE Access* **2019**, *7*, 53040−53065.
(81) Qin, F. Restoration of Single-Channel Currents Using the Segmental k-Means Method Based on Hidden Markov Modeling. *Biophys. J.* **2004**, *86* (3), 1488−1501.
(82) Zhang, J.; Liu, X.; Ying, Y.-L.; Gu, Z.; Meng, F.-N.; Long, Y.-T. High-Bandwidth Nanopore Data Analysis by Using a Modified Hidden Markov Model. *Nanoscale* **2017**, *9* (10), 3458−3465.
(83) Zhao, Z.; Chen, W.; Wu, X.; Chen, P. C. Y.; Liu, J. LSTM Network: A Deep Learning Approach for Short Term Traffic Forecast. *IET Intel. Trans. Sys.* **2017**, *11* (2), 68−75.
(84) Li, Z.; Liu, F.; Yang, W.; Peng, S.; Zhou, J. A Survey of Convolutional Neural Networks: Analysis, Applications, and Prospects. *IEEE Trans. Neural Netw. Learn. Syst.* **2022**, *33* (12), 6999−7019.
(85) Celik, N.; O'Brien, F.; Brennan, S.; Rainbow, R. D.; Dart, C.; Zheng, Y.; Coenen, F.; Barrett-Jolley, R. Deep-Channel Uses Deep Neural Networks to Detect Single-Molecule Events from Patch-Clamp Data. *Commun. Biol.* **2020**, *3* (1), 3.
(86) Dematties, D.; Wen, C.; Pérez, M. D.; Zhou, D.; Zhang, S.-L. Deep Learning of Nanopore Sensing Signals Using a Bi-Path Network. *ACS Nano* **2021**, *15* (9), 14419−14429.
(87) Shafiq, M.; Gu, Z. Deep Residual Learning for Image Recognition: A Survey. *Appl. Sci.* **2022**, *12* (18), 8972.
(88) Xu, P.; Zhu, X.; Clifton, D. A. Multimodal Learning with Transformers: A Survey. *IEEE Trans. Pattern Anal. Mach. Intell.* **2023**, *45* (10), 12113−12132.
(89) Dematties, D.; Wen, C.; Zhang, S.-L. A Generalized Transformer-Based Pulse Detection Algorithm. *ACS Sens.* **2022**, *7* (9), 2710−2720.
(90) Tang, L.; Yi, L.; Jiang, T.; Ren, R.; Paulose Nadappuram, B.; Zhang, B.; Wu, J.; Liu, X.; Lindsay, S.; Edel, J. B.; Ivanov, A. P. Measuring Conductance Switching in Single Proteins Using Quantum Tunneling. *Sci. Adv.* **2022**, *8* (20), eabm8149.
(91) Fu, X.; Wan, Y.; Li, X.; Ying, Y.; Long, Y. Analysis and Classification of Nanopore Data Based on Feature-Level Multi-Modality. In *2020 13th International Congress on Image and Signal Processing, BioMedical Engineering and Informatics (CISP-BMEI)*; IEEE:

Chengdu, China, 2020; pp 692−698 DOI: 10.1109/CISP-BMEI51763.2020.9263687.
(92) Wei, Z.-X.; Ying, Y.-L.; Li, M.-Y.; Yang, J.; Zhou, J.-L.; Wang, H.-F.; Yan, B.-Y.; Long, Y.-T. Learning Shapelets for Improving Single-Molecule Nanopore Sensing. *Anal. Chem.* **2019**, *91* (15), 10033−10039.
(93) Chang, S.; Huang, S.; Liu, H.; Zhang, P.; Liang, F.; Akahori, R.; Li, S.; Gyarfas, B.; Shumway, J.; Ashcroft, B.; He, J.; Lindsay, S. Chemical Recognition and Binding Kinetics in a Functionalized Tunnel Junction. *Nanotechnology* **2012**, *23* (23), 235101.
(94) Wang, K.; Zhang, S.; Zhou, X.; Yang, X.; Li, X.; Wang, Y.; Fan, P.; Xiao, Y.; Sun, W.; Zhang, P.; Li, W.; Huang, S. Unambiguous Discrimination of All 20 Proteinogenic Amino Acids and Their Modifications by Nanopore. *Nat. Methods* **2024**, *21* (1), 92−101.
(95) Taniguchi, M.; Minami, S.; Ono, C.; Hamajima, R.; Morimura, A.; Hamaguchi, S.; Akeda, Y.; Kanai, Y.; Kobayashi, T.; Kamitani, W.; Terada, Y.; Suzuki, K.; Hatori, N.; Yamagishi, Y.; Washizu, N.; Takei, H.; Sakamoto, O.; Naono, N.; Tatematsu, K.; Washio, T.; Matsuura, Y.; Tomono, K. Combining Machine Learning and Nanopore Construction Creates an Artificial Intelligence Nanopore for Coronavirus Detection. *Nat. Commun.* **2021**, *12* (1), 3726.
(96) Noble, W. S. What Is a Support Vector Machine? *Nat. Biotechnol.* **2006**, *24* (12), 1565−1567.
(97) Im, J.; Sen, S.; Lindsay, S.; Zhang, P. Recognition Tunneling of Canonical and Modified RNA Nucleotides for Their Identification with the Aid of Machine Learning. *ACS Nano* **2018**, *12* (7), 7067−7075.
(98) Zhao, Y.; Ashcroft, B.; Zhang, P.; Liu, H.; Sen, S.; Song, W.; Im, J.; Gyarfas, B.; Manna, S.; Biswas, S.; Borges, C.; Lindsay, S. Single-Molecule Spectroscopy of Amino Acids and Peptides by Recognition Tunnelling. *Nat. Nanotechnol.* **2014**, *9* (6), 466−473.
(99) Arima, A.; Tsutsui, M.; Harlisa, I. H.; Yoshida, T.; Tanaka, M.; Yokota, K.; Tonomura, W.; Taniguchi, M.; Okochi, M.; Washio, T.; Kawai, T. Selective Detections of Single-Viruses Using Solid-State Nanopores. *Sci. Rep.* **2018**, *8* (1), 16305.
(100) Liu, Y.; Wang, K.; Wang, Y.; Wang, L.; Yan, S.; Du, X.; Zhang, P.; Chen, H.-Y.; Huang, S. Machine Learning Assisted Simultaneous Structural Profiling of Differently Charged Proteins in a Mycobacterium Smegmatis Porin A (MspA) Electroosmotic Trap. *J. Am. Chem. Soc.* **2022**, *144* (2), 757−768.
(101) Meyer, N.; Janot, J.-M.; Lepoitevin, M.; Smietana, M.; Vasseur, J.-J.; Torrent, J.; Balme, S. Machine Learning to Improve the Sensing of Biomolecules by Conical Track-Etched Nanopore. *Biosensors* **2020**, *10* (10), 140.
(102) Tsutsui, M.; Tanaka, M.; Marui, T.; Yokota, K.; Yoshida, T.; Arima, A.; Tonomura, W.; Taniguchi, M.; Washio, T.; Okochi, M.; Kawai, T. Identification of Individual Bacterial Cells through the Intermolecular Interactions with Peptide-Functionalized Solid-State Pores. *Anal. Chem.* **2018**, *90* (3), 1511−1515.
(103) Komoto, Y.; Ohshiro, T.; Yoshida, T.; Tarusawa, E.; Yagi, T.; Washio, T.; Taniguchi, M. Time-Resolved Neurotransmitter Detection in Mouse Brain Tissue Using an Artificial Intelligence-Nanogap. *Sci. Rep.* **2020**, *10* (1), 11244.
(104) Ryu, J.; Komoto, Y.; Ohshiro, T.; Taniguchi, M. Single-Molecule Classification of Aspartic Acid and Leucine by Molecular Recognition through Hydrogen Bonding and Time-Series Analysis. *Chem. - Asian J.* **2022**, *17* (13), e202200179.
(105) Taniguchi, M.; Ohshiro, T.; Komoto, Y.; Takaai, T.; Yoshida, T.; Washio, T. High-Precision Single-Molecule Identification Based on Single-Molecule Information within a Noisy Matrix. *J. Phys. Chem. C* **2019**, *123* (25), 15867−15873.
(106) Bro, R.; Smilde, A. K. Principal Component Analysis. *Anal. Methods* **2014**, *6* (9), 2812−2831.
(107) Greive, S. J.; Bacri, L.; Cressiot, B.; Pelta, J. Identification of Conformational Variants for Bradykinin Biomarker Peptides from a Biofluid Using a Nanopore and Machine Learning. *ACS Nano* **2024**, *18* (1), 539−550.
(108) Cao, C.; Magalhães, P.; Krapp, L. F.; Bada Juarez, J. F.; Mayer, S. F.; Rukes, V.; Chiki, A.; Lashuel, H. A.; Dal Peraro, M. Deep Learning-Assisted Single-Molecule Detection of Protein Post-Translational Modifications with a Biological Nanopore. *ACS Nano* **2024**, *18* (2), 1504−1515.
(109) Fu, T.; Zang, Y.; Zou, Q.; Nuckolls, C.; Venkataraman, L. Using Deep Learning to Identify Molecular Junction Characteristics. *Nano Lett.* **2020**, *20* (5), 3320−3325.
(110) Misiunas, K.; Ermann, N.; Keyser, U. F. QuipuNet: Convolutional Neural Network for Single-Molecule Nanopore Sensing. *Nano Lett.* **2018**, *18* (6), 4040−4045.
(111) Díaz Carral, Á.; Ostertag, M.; Fyta, M. Deep Learning for Nanopore Ionic Current Blockades. *J. Chem. Phys.* **2021**, *154* (4), 044111.
(112) Li, S.; Li, X.; Wan, Y.; Ying, Y.; Yu, R.; Long, Y. SmartImage: A Machine Learning Method for Nanopore Identifying Chemical Modifications on RNA. *Chem. - Asian J.* **2023**, *18* (3), e202201144.
(113) Vladyka, A.; Albrecht, T. Unsupervised Classification of Single-Molecule Data with Autoencoders and Transfer Learning. *Mach. Learn.: Sci. Technol.* **2020**, *1* (3), 035013.
(114) Huang, F.; Li, R.; Wang, G.; Zheng, J.; Tang, Y.; Liu, J.; Yang, Y.; Yao, Y.; Shi, J.; Hong, W. Automatic Classification of Single-Molecule Charge Transport Data with an Unsupervised Machine-Learning Algorithm. *Phys. Chem. Chem. Phys.* **2020**, *22* (3), 1674−1681.
(115) Sinaga, K. P.; Yang, M.-S. Unsupervised K-Means Clustering Algorithm. *IEEE Access* **2020**, *8*, 80716−80727.
(116) Ryu, J.; Komoto, Y.; Ohshiro, T.; Taniguchi, M. Direct Biomolecule Discrimination in Mixed Samples Using Nanogap-Based Single-Molecule Electrical Measurement. *Sci. Rep.* **2023**, *13* (1), 9103.
(117) Tsutsui, M.; Takaai, T.; Yokota, K.; Kawai, T.; Washio, T. Deep Learning Enhanced Nanopore Sensing of Single-Nanoparticle Translocation Dynamics. *Small Methods* **2021**, *5* (7), 2100191.
(118) Ball, S. T. M.; Celik, N.; Sayari, E.; Abdul Kadir, L.; O'Brien, F.; Barrett-Jolley, R. DeepGANnel: Synthesis of Fully Annotated Single Molecule Patch-Clamp Data Using Generative Adversarial Networks. *PLoS One* **2022**, *17* (5), e0267452.
(119) Chen, Y.; Chen, Y.; Long, J.; Shi, D.; Chen, X.; Hou, M.; Gao, J.; Liu, H.; He, Y.; Fan, B.; Wong, C.-P.; Zhao, N. Achieving a Sub-10 nm Nanopore Array in Silicon by Metal-Assisted Chemical Etching and Machine Learning. *Int. J. Extrem. Manuf.* **2021**, *3* (3), 035104.
(120) Wang, Y.; Cao, Z.; Barati Farimani, A. Efficient Water Desalination with Graphene Nanopores Obtained Using Artificial Intelligence. *npj 2D Mater. Appl.* **2021**, *5* (1), 66.
(121) Wang, S.; Zhu, J.; Blackwell, R.; Fischer, F. R. Automated Tip Conditioning for Scanning Tunneling Spectroscopy. *J. Phys. Chem. A* **2021**, *125* (6), 1384−1390.
(122) David, M.; Dursi, L. J.; Yao, D.; Boutros, P. C.; Simpson, J. T. Nanocall: An Open Source Basecaller for Oxford Nanopore Sequencing Data. *Bioinformatics* **2017**, *33* (1), 49−55.
(123) Landry, M.; Winters-Hilt, S. Analysis of Nanopore Detector Measurements Using Machine-Learning Methods, with Application to Single-Molecule Kinetic Analysis. *BMC Bioinformatics* **2007**, *8* (S7), S12.
(124) Liu, Q.; Fang, L.; Yu, G.; Wang, D.; Xiao, C.-L.; Wang, K. Detection of DNA Base Modifications by Deep Recurrent Neural Network on Oxford Nanopore Sequencing Data. *Nat. Commun.* **2019**, *10* (1), 2449.
(125) Szalay, T.; Golovchenko, J. A. De Novo Sequencing and Variant Calling with Nanopores Using PoreSeq. *Nat. Biotechnol.* **2015**, *33* (10), 1087−1091.
(126) Teng, H.; Cao, M. D.; Hall, M. B.; Duarte, T.; Wang, S.; Coin, L. J. M. Chiron: Translating Nanopore Raw Signal Directly into Nucleotide Sequence Using Deep Learning. *GigaScience* **2018**, *7* (5), giy037.
(127) Bao, Y.; Wadden, J.; Erb-Downward, J. R.; Ranjan, P.; Zhou, W.; McDonald, T. L.; Mills, R. E.; Boyle, A. P.; Dickson, R. P.; Blaauw, D.; Welch, J. D. SquiggleNet: Real-Time, Direct Classification of Nanopore Signals. *Genome Biol.* **2021**, *22* (1), 298.
(128) Vermeulen, C.; Pagès-Gallego, M.; Kester, L.; Kranendonk, M. E. G.; Wesseling, P.; Verburg, N.; De Witt Hamer, P.; Kooi, E. J.; Dankmeijer, L.; Van Der Lugt, J.; Van Baarsen, K.; Hoving, E. W.; Tops,

B. B. J.; De Ridder, J. Ultra-Fast Deep-Learned CNS Tumour Classification during Surgery. *Nature* **2023**, *622* (7984), 842−849.
(129) Jena, M. K.; Roy, D.; Pathak, B. Machine Learning Aided Interpretable Approach for Single Nucleotide-Based DNA Sequencing Using a Model Nanopore. *J. Phys. Chem. Lett.* **2022**, *13* (50), 11818−11830.
(130) Jena, M. K.; Pathak, B. Development of an Artificially Intelligent Nanopore for High-Throughput DNA Sequencing with a Machine-Learning-Aided Quantum-Tunneling Approach. *Nano Lett.* **2023**, *23* (7), 2511−2521.
(131) Yan, M.-Z.; Chen, J.-M.; Wang, B.; Xu, W.; Cao, H.-M.; Fu, Y.-Y.; He, Q.-G.; Cheng, J.-G. High-Sensitivity Sensor Array Base on Molecular Design and Machine Learning for Amine Differentiation in Exhaled Vapor. *Chin. J. Anal. Chem.* **2022**, *50* (3), 100059.
(132) Genco, E.; Modena, F.; Sarcina, L.; Björkström, K.; Brunetti, C.; Caironi, M.; Caputo, M.; Demartis, V. M.; Di Franco, C.; Frusconi, G.; Haeberle, L.; Larizza, P.; Mancini, M. T.; Österbacka, R.; Reeves, W.; Scamarcio, G.; Scandurra, C.; Wheeler, M.; Cantatore, E.; Esposito, I.; Macchia, E.; Torricelli, F.; Viola, F. A.; Torsi, L. A Single-Molecule Bioelectronic Portable Array for Early Diagnosis of Pancreatic Cancer Precursors. *Adv. Mater.* **2023**, *35* (42), 2304102.
(133) Lemmer, M.; Inkpen, M. S.; Kornysheva, K.; Long, N. J.; Albrecht, T. Unsupervised Vector-Based Classification of Single-Molecule Charge Transport Data. *Nat. Commun.* **2016**, *7* (1), 12922.
(134) Wu, B. H.; Ivie, J. A.; Johnson, T. K.; Monti, O. L. A. Uncovering Hierarchical Data Structure in Single Molecule Transport. *J. Chem. Phys.* **2017**, *146* (9), 092321.
(135) Mardis, E. R. A Decade's Perspective on DNA Sequencing Technology. *Nature* **2011**, *470* (7333), 198−203.
(136) Ni, P.; Huang, N.; Nie, F.; Zhang, J.; Zhang, Z.; Wu, B.; Bai, L.; Liu, W.; Xiao, C.-L.; Luo, F.; Wang, J. Genome-Wide Detection of Cytosine Methylations in Plant from Nanopore Data Using Deep Learning. *Nat. Commun.* **2021**, *12* (1), 5976.
(137) Wang, Y.; Zhang, S.; Jia, W.; Fan, P.; Wang, L.; Li, X.; Chen, J.; Cao, Z.; Du, X.; Liu, Y.; Wang, K.; Hu, C.; Zhang, J.; Hu, J.; Zhang, P.; Chen, H.-Y.; Huang, S. Identification of Nucleoside Monophosphates and Their Epigenetic Modifications Using an Engineered Nanopore. *Nat. Nanotechnol.* **2022**, *17* (9), 976−983.
(138) Xu, L.; Seki, M. Recent Advances in the Detection of Base Modifications Using the Nanopore Sequencer. *J. Hum. Genet.* **2020**, *65* (1), 25−33.
(139) Rusk, N. MinION Takes Center Stage. *Nat. Methods* **2015**, *12* (1), 12−13.
(140) Deamer, D.; Akeson, M.; Branton, D. Three Decades of Nanopore Sequencing. *Nat. Biotechnol.* **2016**, *34* (5), 518−524.
(141) Hu, Z.-L.; Huo, M.-Z.; Ying, Y.-L.; Long, Y.-T. Biological Nanopore Approach for Single Molecule Protein Sequencing. *Angew. Chem., Int. Ed.* **2021**, *60* (27), 14738−14749.
(142) Zhang, M.; Tang, C.; Wang, Z.; Chen, S.; Zhang, D.; Li, K.; Sun, K.; Zhao, C.; Wang, Y.; Xu, M.; Dai, L.; Lu, G.; Shi, H.; Ren, H.; Chen, L.; Geng, J. Real-Time Detection of 20 Amino Acids and Discrimination of Pathologically Relevant Peptides with Functionalized Nanopore. *Nat. Methods* **2024**, *21* (4), 609−618.
(143) 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* (6), 604−617.
(144) Restrepo-Pérez, L.; Joo, C.; Dekker, C. Paving the Way to Single-Molecule Protein Sequencing. *Nat. Nanotechnol.* **2018**, *13* (9), 786−796.
(145) Zhang, Y.; Yi, Y.; Li, Z.; Zhou, K.; Liu, L.; Wu, H.-C. Peptide Sequencing Based on Host-Guest Interaction-Assisted Nanopore Sensing. *Nat. Methods* **2024**, *21* (1), 102−109.
(146) Yu, L.; Kang, X.; Li, F.; Mehrafrooz, B.; Makhamreh, A.; Fallahi, A.; Foster, J. C.; Aksimentiev, A.; Chen, M.; Wanunu, M. Unidirectional Single-File Transport of Full-Length Proteins through a Nanopore. *Nat. Biotechnol.* **2023**, *41* (8), 1130−1139.
(147) Li, F.; Fahie, M. A.; Gilliam, K. M.; Pham, R.; Chen, M. Mapping the Conformational Energy Landscape of Abl Kinase Using ClyA Nanopore Tweezers. *Nat. Commun.* **2022**, *13* (1), 3541.
(148) Yoon, J.; Lee, T.; Choi, J.-W. Development of Bioelectronic Devices Using Bionanohybrid Materials for Biocomputation System. *Micromachines* **2019**, *10* (5), 347.
(149) Blaschke, M.; Pauly, F. Designing Mechanosensitive Molecules from Molecular Building Blocks: A Genetic Algorithm-Based Approach. *J. Chem. Phys.* **2023**, *159* (2), 024126.
(150) Lanzillo, N. A.; Breneman, C. M. Predicting Single-Molecule Conductance through Machine Learning. *J. Appl. Phys.* **2016**, *120* (13), 134902.