Nanopore Single-molecule Analysis of Biomarkers: Providing Possible Clues to Disease Diagnosis

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Abstract: Biomarker detection has attracted increasing interest in recent years due to the minimally or non-invasive sampling process. Single entity analysis of biomarkers is expected to provide real-time and accurate biological information for early disease diagnosis and prognosis, which is critical to the effective disease treatment and is also important in personalized medicine. As an innovative single entity analysis method, nanopore sensing is a pioneering single-molecule detection technique that is widely used in analytical bioanalytical fields. In this review, we overview the recent progress of nanopore biomarker detection as new approaches to disease diagnosis. In highlighted studies, nanopore was focusing on detecting biomarkers of different categories of communicable and noncommunicable diseases, such as pandemic Covid-19, AIDS, cancers, neurologic diseases, etc. Various sensitive and selective nanopore detecting strategies for different types of biomarkers are summarized. In addition, the challenges, opportunities, and direction for future development of nanopore-based biomarker sensors are also discussed.

Keywords: Nanopore; Biomarkers; Analytical bioanalytical methods; Single-molecule analysis; Human diseases; Lab assays and diagnosis

1. **Introduction**

Diseases have a substantial influent on global demography. Compared to the year of 2000, the overall number of deaths in 204 countries and territories has notably risen (Demographic Analysis for the Global Burden of Disease Study 2019)[1]. World Health Organization (WHO) reported that more than 92% of deaths were accounted for diseases, among which noncommunicable diseases were responsible for 71% of all deaths[2]. On the other hand, as to communicable diseases, the current outbreak of unexpected COVID-19 severely crushed the medical system all over the world, and an estimated 18.2 million people died due to the COVID-19 pandemic during the period from Jan. 2020 to Dec. 2021, which makes it as the world's number one public health emergency.[3] The diseases (either acute and curable ailments or chronic and incurable health problems) are aggravating the economic and social instability, which in turn impacts the human civilization development. Therefore, the prevention, diagnosis, and treatment of diseases are critical issues that humans have to confront in the past, today, and will continue to face in the future.

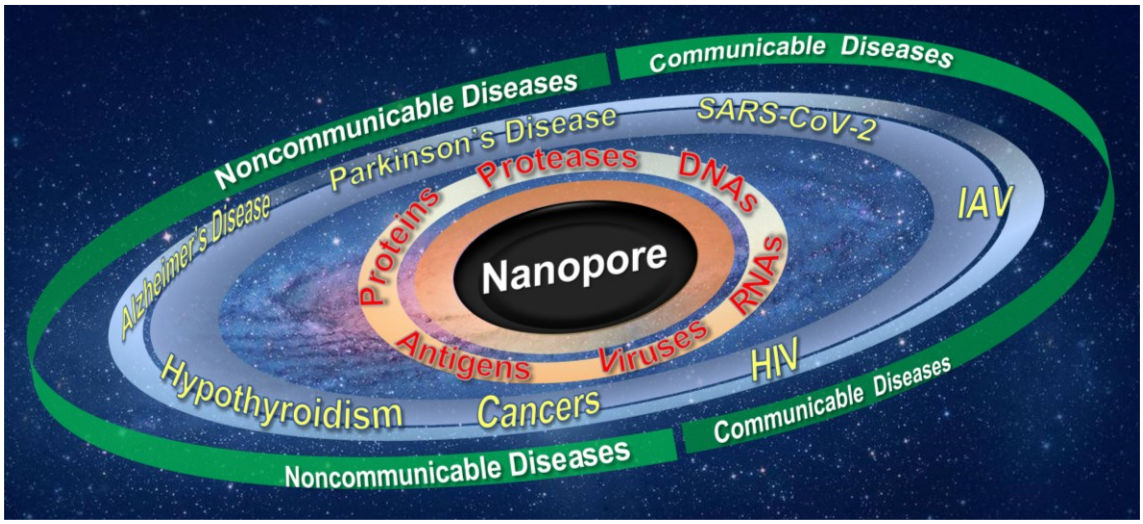
Recent developments in medicine promote comprehending of the underlying mechanisms of diseases and subsequently motivating feasible disease-related interventions. For instance, body vital signs (*e.g.,* level of blood sugar & oxygen, and other metabolites) are gathered to evaluate personal physiological state[4],[5],[6], bioinformatic techniques are applied to reveal the genetic behavior (DNAs, RNAs, Proteins) behind diseases[7],[8],[9], and extensive body fluid testing (*e.g.*, blood, saliva, and urine analysis) is developed as a quantifiable measure of a person's overall health[10],[11]. Apparently, the above intervention strategies require the measurement of molecules, products, or biological reaction cascades, and the corresponding results are notable for objectively assessing the physiological responses, cellular processes, function of metabolisms, as well as therapeutic intervention in clinical diagnosis and prognosis. Those substances or processes are defined by WHO as biomarkers thereof, which could be used to predict the incidence of diseases[12],[13],[14],[15].

Assessment of biomarkers in body fluids is important and necessary in disease diagnosis and prognosis. Thus far, clinical disease diagnosis involves using various analysis strategies and

principles such as radiology, pathological assay, and histological examinations. Although these tests provide professional, systematic, and reliable results, they suffer from various limitations, including the need of specially trained personnel and the requirement of sophisticated instruments and invasion tests, which is not appropriate to be used as tools for self-measurements, point-of-care tests, and remote disease monitoring[16],[17],[18],[19]. Consequently, novel techniques, such as wearable devices, smartphone-based systems, and electronic skins, are invented to satisfy the purpose of healthcare monitoring wherever and whenever it is feasible. Other detection techniques, such as fluorescence, chemiluminescence, and enzyme-linked immunosorbent assay (ELISA), also made remarkable contributions to biomarker detection for disease diagnosis[20],[21],[22]. There are, of course, strengths and limitations in each method.

The development of single entity-based analytical methods enables the monitoring of individual biomarkers within complex biological systems, providing information on molecular diverisities [23]. As an innovative single entity method, nanopore sensing is a pioneering single-molecule detection technique and can detect various substances with high sensitivity and selectivity[24],[25]. After decades of development, nanopore technology has become a versatile tool for exploring a wide variety of applications, such as pharmaceutical development and environmental surveillance[26],[27],[28],[29],[30],[31]. Meanwhile, numerous research efforts on nucleic acid, proteins, and polysaccharides have also boosted nanopores' application in life science and disease diagnostics[32],[33],[34]. In terms of life science research, nanopore has made a great contribution to nucleic acid sequencing[35],[36],[37],[38],[39],[40] and offers the potential for protein sequencing[41],[42],[43]. Furthermore, nanopore technology was also utilized to identify other biological molecules like saccharides and glycosaminoglycans[44],[45]. Moreover, nanopore provides a label-free strategy to reveal the molecular dynamics of molecules, enabling the study of molecule-molecule interactions, investigation of dynamic conformational transformation, and transition state monitoring[46],[47],[48],[49]. Many good reviews have been written on these aspects of using nanopore sensing technology in life science exploration[50],[51],[52],[53].

Sequencing approaches are recognized as the most straightforward ways to identify genetic biomarkers for human diseases[54],[55],[56]. However, there is no doubt that the development of non-sequencing analytical methods for biomarker detection to predict the incidence of diseases is also of great importance. In this review, we will overview the recent advances in nanopore detection of biomarkers, and highlight the potential application of nanopores in disease prevention, diagnosis, and treatment (Scheme).



Scheme. Concept of the nanopore-based method for biomarker detections that provide possible clues to disease diagnosis. In the highlighted works, the nanopore (either biological or solid-state) technique is utilized for practical applications of detecting biomarkers of different categories of communicable and noncommunicable diseases, such as pandemic SARS-CoV-2, AIDS, Cancers, HIV, Neurologic diseases, etc. Meanwhile, in terms of biomarkers of distinct species, those of proteins, nucleic acids, and viruses that may be used as indicators to spot diseases were involved in nanopore detection, showing high sensitivity and selectivity. Nanopore biomarker detection: a method of choice for disease diagnosis, not only accessible to the precise detection of a series of biomarkers in laboratories, but also capable to realize the most significant breakthroughs in compatible performance for clinical application.

2. Noncommunicable diseases

Noncommunicable diseases (NCDs), also known as chronic diseases, happen to people of all

ages, religions, and countries, and are top challenges for public health. Approximately 71% of global deaths (~41 million) were accounted for NCDs by 2016[57],[58]. NCDs are the result of a combination of genetic, physiological, environmental, and behavioural factors and often create a need for long-term treatment and care. The main risk factors contributing to NCDs include unhealthy diets, physical inactivity, tobacco use, and alcohol misuse. Since NCDs eventually progress in early life due to lifestyle aspects, most of them are preventable as long as they can be detected at the early stage. To this end, there is a need for sensitive and accurate NCDs diagnostic methods. In this section, we will summarize various nanopore detection strategies on a series of NCDs-related biomarkers including those of cancers, neurologic diseases, and endocrine diseases.

2.1. Cancers

Cancer is the result of a human body being invaded by cancerous tumors. It typically happens due to the uncontrollable outgrowth of a clonal population of cells, that can intractably start almost anywhere in a living system. To date, the predominance of cancer is growing continuously which will result in the number of victims approximately reaching 29.4 million by 2040[59]. It is believed that the reduction of mortality of cancer is notably achievable *via* timely screening and earlier detection of biomarkers[60]. Thus far, for the convenience of cancer researches, various databases of cancer biomarkers have been established, including genes, proteins, and related epigenetic modifications. In this context, a variety of innovative biomarker detection strategies have been developed, using either a biological ion channel or a nanoscale-sized pore fabricated in a solid-state membrane.

2.1.1 Detecting proteinic biomarkers *via* antigen-antibody interaction

Proteins are functionally informative molecules. They participate in the dynamics of cellular status and mediate normal functions of metabolism. So far, many proteinic biomarkers related to malignant tumors have been identified. One example is Alpha-fetoprotein (AFP). It is a tumor-associated fetal protein with a normal concentration of 5-8 ng/mL in healthy humans[61]. It has

been reported that AFP participates in the development of several malignant tumors such as hepatocellular carcinoma, yolk sac tumor, and gastric cancer[62],[63]. To treat those tumors, identification of appropriate molecular targets is highly important. One popular strategy used for molecular target determination involves antigen-antibody interaction. Other interactions can additionally be coupled with the antigen-antibody interaction to further improve the performance (e.g., sensitivity, accuracy, etc.) of the molecular diagnosis. For example, Zhang *et al.* developed an innovative click-chemistry reaction-assisted biological nanopore detection method for AFP. The click chemistry reaction (herein refers to copper-catalyzed azide-alkyne cycloaddition click reaction), is a canonical chemical reaction that has frequently been studied in recent years[64],[65]. As shown in Fig. 1, the click reaction-assisted nanopore detection consists of three essential steps: biomarker ligation, Cu^{2+} release, and click reaction. To be more specific, first, AFP served as a bridge to ligate the antibody-modified magnetic beads and CuO nanoparticles, thus constructing a sandwich structure. Then, the tethered CuO nanoparticles would release Cu^{2+} ions under the treatment of hydrochloric acid. Finally, the external probes were harvested for nanopore detection *via* the Cu^{2+}-activated click reaction by an alkyne-containing DNA and an azide-containing ferrocene⊂cucurbit[7]uril. By using an α-hemolysin (αHL) nanopore, femtomolar concentrations of AFP could be detected within minutes. Using a similar sensing strategy, other cancer proteinic biomarkers such as carcinoembryonic antigen (CEA) and ferritin were also successfully identified[66]. It is worth mentioning that, the click reaction-assisted antigen-antibody interaction-based nanopore detection strategy offers several advantages, including (1) removing interferences from serum, (2) concentrating the target analytes, and (3) improving the resolution between reacted DNA and unreacted DNA. As an important aside, the click reaction could be used for metal ion detection as well[67],[68].

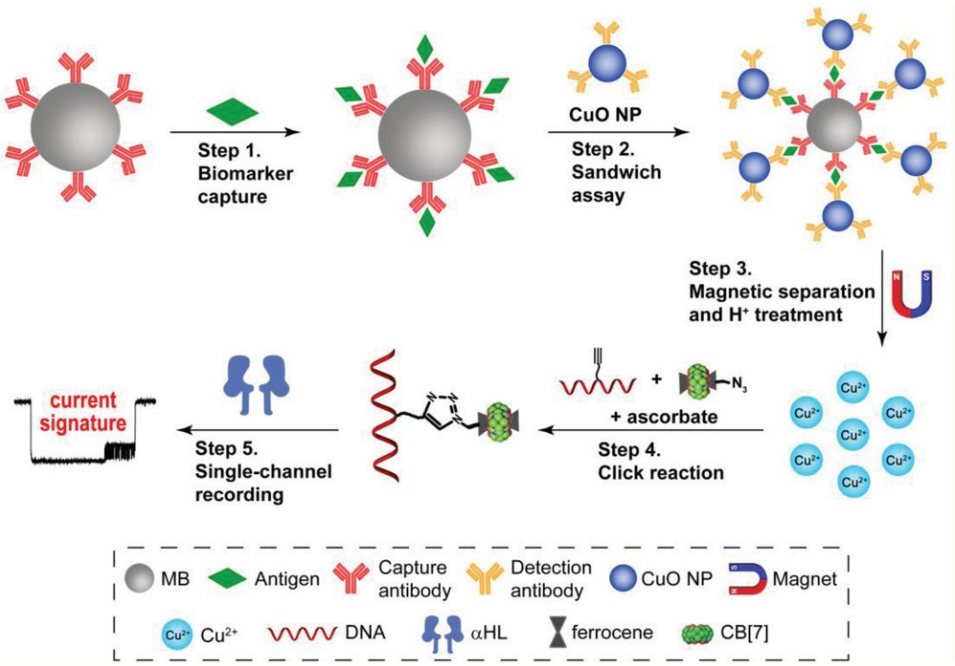


Figure 1. Schematic illustration of nanopore-based strategy for the detection of biomarkers with sandwich assay and click chemistry. (i) A sandwich assay between antibody-modified magnetic beads (MBs) and copper oxide nanoparticles (CuONPs) was employed to capture the biomarkers. (ii) CuONPs were treated with hydrochloric acid to release Cu^{2+} and activate click reaction. (iii) The conjugated DNA probes processed single-channel recording in nanopore. Adapted with permission from Ref.[66].

It should be noted that a disadvantage of using protein ion channels as nanopore sensing elements lies in their small and fixed pore sizes, which make the detection of bulky analytes (*i.e.,* antibodies) difficult. However, solid-state nanopores with customized pore diameters could overcome this limitation. For example, in order to capture AFP efficiently, Ying *et al* attached antibodies to the inner surface of a solid-state nanopore[69]. In their experiment, antibodies were covalently coupled to the lumen of a gold-coated quartz nanopore (~40 nm) *via* EDC/NHS modification. The purpose of the modification was to produce a strengthened nanopore electric signal shift (*i.e.,* residence time, amplitude) in current-voltage responses. As expected, the residence time of the nanopore signals corresponding to AFP capture in the modified nanopore was ten times larger than those detected using a bare nanopore (2.81 ms *vs.* 0.26 ms at 400 mV).

The experimental results demonstrated that the antigen-antibody interactions indeed provided an improved resolution to AFP detection than the direct translocation strategy. Due to the flexible diameter of a solid-state nanopore, various other large molecules could be detected similarly.

In another study, Chuah et al. reported a magnetic field-assisted ultra-sensitive nanopore sensor for detection of prostate specific antigen (PSA), the specific biomarker of prostate cancer with a level between 4.0-10.0 ng/mL in healthy humans[70]. As shown in Fig. 2, the silicon nitride (SiN) nanopore and the magnetic nanoparticles were modified with PSA antibodies (epitope 1 and epitope 5, respectively). The function of the antibodies on magnetic nanoparticles was to drag the motion of PSA under the guidance of the magnetic field, while those on nanopores aimed to immobilize PSA, causing a current blockage. After a steady-state process, with the application of *trans*-magnet, the unbonded nanoparticles would leave while the bonded ones remained in the pores. Accordingly, PSA could be quantified by statistically analyzing the fluctuations in current blockages under different magnetic fields[71]. Compared with the traditional nanopore detection, the advantage of this sensing strategy was that no translocation procedure was required. In the follow-up study, by using gold metalized nanopores instead of the naked SiN_x nanopores to analyze PSA, a much better detection limit was obtained (80 aM vs. 0.8 fM)[72].

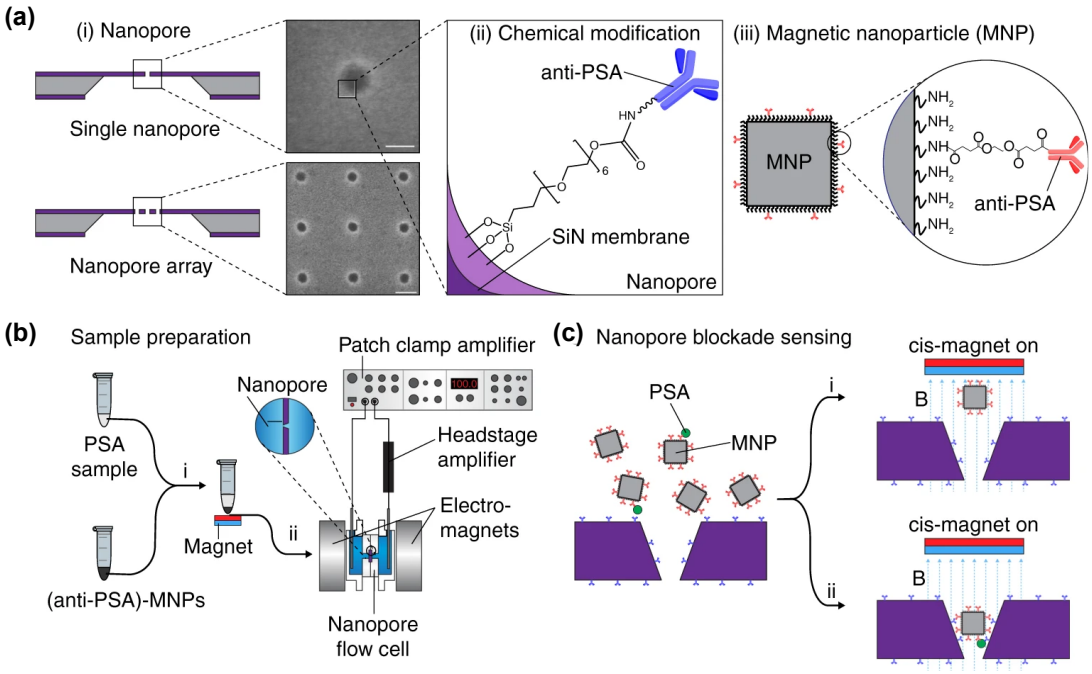


Figure 2. Nanopore blockade sensor. (a) (i) Schematics and scanning electron micrographs of (*top*) a solid-state nanopore; (*bottom*) a nanopore array in SiN membrane, inset: a 3 × 3 array of nanopores with 0.5 μm spacing between each pore, scale bar: 200 nm. (ii) Illustration of a chemically modified SiN nanopore with silane-EG6-(anti-PSA) self-assembled monolayer (SAM). (iii) Illustration of an (anti-PSA)-conjugated MNP used in this work. Inset: chemical structure of the (anti-PSA)-EG6 immobilized onto the amine-rich PEI coating of the MNP. (b) Flow chart illustrated the sample preparation steps for detection of PSA in a sample. (c) Active filtering of non-specific nanopore blockade events can be achieved by applying a magnetic field from the *cis*-side. Adapted with permission from Ref.[71].

2.1.2 Detecting proteinic biomarkers *via* peptide-antibody affinity

Inspired by immunoassay, functionalized bio-nanopores, which integrate peptide fragments with specific affinity to certain proteinic biomarkers, were developed for disease diagnosis. As a noted example, Movileanu and colleagues fabricated a truncated version of ferric hydroxamate uptake component A (t-FhuA) nanopore which could reversibly capture and release an 89-residue contained barstar protein with the assistance of RNase barnase. This reversible action (protein-

protein interactions in principle) could be monitored *via* tiny current amplitude variations (~10 pA) produced by the t-FhuA nanopore with high selectivity and sensitivity and even in serum[73]. Building on this study, they further equipped the t-FhuA nanopore with a 14-residue peptide motif (derived from mixed lineage leukemia 4 methyltransferase, hereafter termed as $MLL4_{Win}$), instead of RNase barnase, for specific recognization of WDR5 protein, which is overexpressed in prostate cancer and promotes the proliferation of bladder cancer cells[74]. The typical nanopore signals demonstrated the effective capture and release of WDR5-$MILL4_{win}$ in a WDR5 concentration-dependent manner. In addition, this nanopore assay could identify three binding modes of $MLL4_{Win}$-WDR5 complexes with an ultra-high sensitivity through accurate analysis of the different residence time values of the signals (~12 ms, ~120 ms, and ~1370 ms at -20 mV) (Fig. 3a). These results demonstrate that nanopore analysis offers the great potential in precise detection of WDR5 cancer biomarkers and uncovering the groove-containing protein-protein (protein-peptide) binding system in biophysical research[75].

In a separate study, Wang and collaborators arrested Epithelial Cell Adhesion Molecule (EpCAM) antibody by adopting the strong binding affinity of the engineered phi29 DNA packaging nanomotor (an 18 amino-acid containing peptide specific to EpCAM) (Fig. 3c). Unlike αHL, which is derived from a transmembrane protein channel, phi29 is a viral packing connector protein[76]. The presence of EpCAM antibody resulted in the transient molecular binding events (~30 ms) as well as the permanent binding events with the same amplitude blockage at ~35 %. The engineered phi29 nanopore didn't mute to the signals of serum (30.4±1.5% blockage) and the nonspecific antibody (28.8±2.7% blockage); furthermore, the presence of serum matrix would make the amplitude of the EpCAM antibody blockage events slightly shifted (37.4±1.9% with serum vs. 38.9±2.3% without serum). In addition, this nanopore sensor could detect the EpCAM antibody with its concentration at as low as 4 ng/μL, indicating the high sensitivity of the engineered nanopore[77].

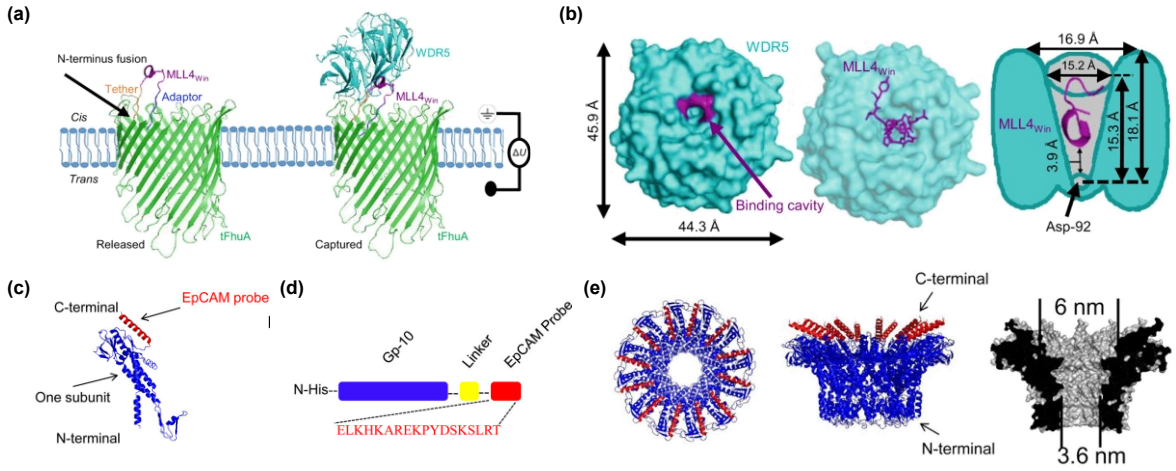


Figure 3. (a) An MLL4$_{Win}$tFhuA protein nanopore reconstituted into a planar lipid membrane. This protein comprised a tFhuA protein nanopore (green), a flexible Gly/Ser-rich hexapeptide tether (yellow), a 14-residue MLL4Win peptide ligand (magenta), and a peptide adaptor (blue). The left- and right-hand cartoons showed the protein nanopore in the released and WDR5-captured substrates, respectively. (b) On the left side, a top-view cartoon of WDR5 (cyan) showed the binding cavity. Phe-133, Cys-261, and Ser-91 were colored in magenta. The central cartoon was the WDR5-MLL4Win complex. MLL4Win (magenta) occupied the cavity of WDR5. On the right side, there was a cross-section schematic of the conical WDR5 cavity (gray). MLL4Win (magenta) partitions into the WDR5 cavity. Adapted with permission from Ref.[75]. Illustration of the phi29 connector channel structure (c) Structure of one subunit showing the location of the EpCAM probe (red); (d) Construction of the modified gp10 gene by insertion of His tag at the N-terminus, 6-Gly linker, and EpCAM probe at the C-terminus; (e) Top view, side view, and section view of the phi29 connector. Adapted with permission from Ref.[77].

2.1.3 Detecting proteinic biomarkers *via* electro-osmotic flow

It should be noted that the nanopore integrated with peptide fragments uses an out-pore strategy to significantly enhance the sensor selectivity and sensitivity, while introduction of new functional groups in the interior wall of the protein pore by site-directed mutagenesis is considered as an on-pore method to improve the performance and resolution of the nanopore for protein sensing, thus

facilitating disease detection[78]. For example, Huang *et al* demonstrated that modification of the internal surface of a protein pore would affect electro-osmotic flow (EOF), thus having an impact on its identification of protein biomarkers. Basically, ions would transport through the surface of pores under the driven of the electric field[79], while EOF is the directional flux of water across the nanopore, which is induced by the fixed charges in the internal wall of the nanopore[80],[81]. EOF would create an effective absorbing field around the entry of nanopores, which promotes nanopores to capture analytes against the electrophoretic field[82]. In their study, protein biomarkers were detected using two types of nanopores: wild-type FraC (fragaceatoxin C, WTFraC) and mutant FraC (D10R, K159E) (ReFraC) nanopores. Theoretically, ReFraC possesses a constriction with more positively charged than WTFraC under acidic conditions. After systematically comparing the detection of endothelin 1 and chymotrypsin in the wild-type FraC (WTFraC) and mutant FraC (D10R, K159E) (ReFraC) nanopores with various voltage biases and pH values (Fig. 4), two conclusions were drawn: (1) the ion selectivity of the nanopore could be adjusted by changing the potential at the center of the constriction, which caused EOF changes; and (2) proteins could enter the nanopore when the translocation direction was the same as the direction of EOF, whatever the charge of the biomarkers or the voltage bias was applied. Those conclusions were validated by analyzing other biomarkers, including β2-microglobulin, human EGF, and angiotensin I, which are all closely associated with colorectal cancer[83],[84],[85]. The combined results implied that, if the target proteins have comparative sizes to that of the nanopore, a process of deformation would occur in the transmembrane region of the nanopore during the protein translocation. Note that EOF provided the nanopore with a remarkable sensitivity to identify protein isomers (endothelin 1 and endothelin 2) differing only by a single amino acid[86].

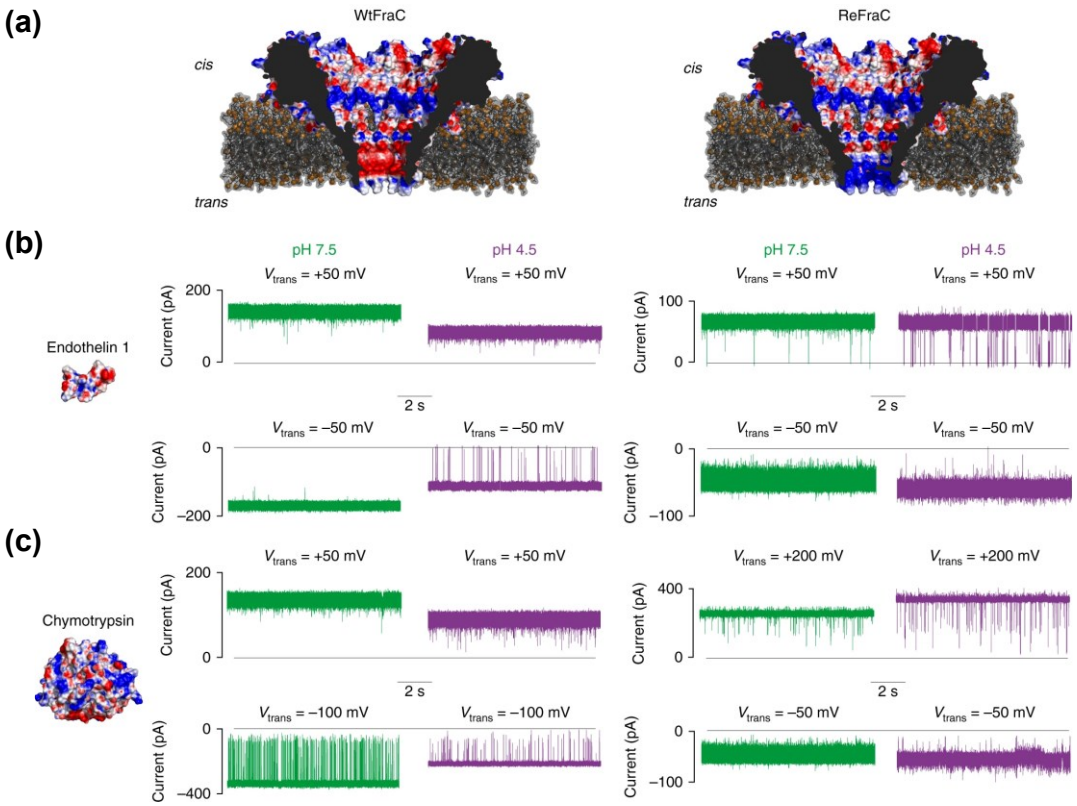


Figure 4. The capture of endothelin 1 and chymotrypsin with two FraC variants at two different pH conditions. (a) Cross sections of wild-type FraC (WtFraC, PDB: 4TSY) and D10R-K159E-FraC (ReFraC). Representative traces induced by 1 μM endothelin 1 (b) and 200 nM chymotrypsin (c) to WtFraC (left) and ReFraC (right). Chymotrypsin (PDB: 5CHA) and human endothelin 1 (PDB: 1EDN) were shown as surface representations. Endothelin 1 and chymotrypsin entered WtFraC under negative applied potentials, while they entered ReFraC under positive applied potentials. Chymotrypsin blockades to WtFraC were also observed under −50 mV at pH 7.5 and 4.5; however, the applied potential was increased to −100 mV to obtain a sufficient number of blockades. At pH 7.5, blockades to ReFraC by chymotrypsin under positive applied bias require higher potential than to WtFraC under negative applied bias. Endothelin 1 and chymotrypsin were added into the *cis* compartment. Adapted with permission from Ref.[86].

2.1.4 Detecting proteinic biomarkers *via* protease activity

Some protein biomarkers such as proteases may catalyze the proteolytic process toward

proteins, providing a useful strategy to detect enzyme biomarkers[87],[88]. For example, a disintegrin and metalloproteinase (ADAM) enzymes are implicated in key signaling pathways in many tumors. Among them, ADAM-9 is observed in human liver metastases, ADAM-17 is overexpressed and associated with human colon carcinoma, while ADAM-12 influences the development and progression of prostate cancer in the mouse model[89]. As a proof-of-concept purpose, we developed an activity-based nanopore sensing method to detect ADAM-17 (Fig. 5a)[90]. Briefly, the peptide substrate of ADAM-17 (sequence: LAQAVRSSSARLVFF) was incubated in the absence and presence of ADAM17, followed by analysis with an αHL nanopore. Experimental results (in 3 M/1 M NaCl buffer solution) showed that the substrate alone produced only one major type of events in the nanopore, with the normalized event mean residual current of 30.7 ± 0.3 % of full channel block. In contrast, in the presence of ADAM17, a new type of events appeared (the normalized event residual current of 48.6 ± 0.1% of full channel block). By taking advantage of the new events, selective detection of ADAM proteinic biomarkers was successfully accomplished. Furthermore, it was found out that salt gradient provided a better nanopore detection performance than the symmetric electrolyte solutions. In addition, the ADAM-17 sensor showed good selectivity and high sensitivity (LOD: 0.15 ng/mL), offering the potential for clinical sample analysis. In the follow-up study, we pioneered a multiplexing nanopore platform in a non-array format for the concurrent measurement of two protein biomarkers (ADAM-10 and ADAM-17) by employing a single substrate with two cleavage sites (Fig. 5b). The multiplexing sensing strategy developed in this work could further be coupled with the conventional sensor array to improve the multiplexing capability of the sensor system[91]. Clearly, the developed activity-based nanopore protease assay could be used to detect a variety of other cancer-related proteases, such as kallikreins (KLKs) and metalloproteinases (MMPs), which are the biomarkers of prostate, oral, and lung cancers[92],[93]. Regarding other disease diagnosis, our group used a similar strategy to detect trypsin, a marker of exocrine pancreatic function. Similar to the ADAMs sensor described above, our developed trypsin sensor was highly sensitive with the detection limit of 1.4 ng/mL, comparable with those of the current commercial detection kits[94]. Note that peptides are not the

only candidate to serve as the substrate of proteases. For example, as a non-peptide based substrate, *N*α-benzoyl-L-arginine ethyl ester was used to trigger and monitor trypsin activity[95].

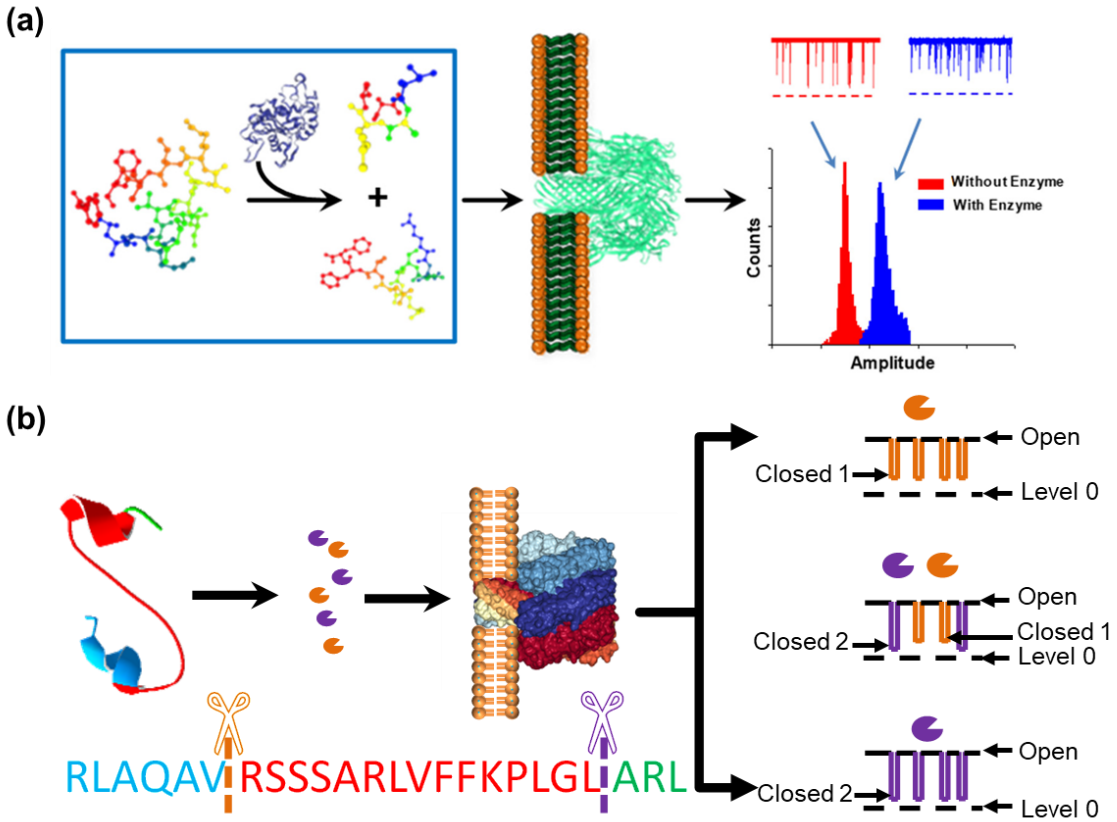


Figure 5. (a) Schematic representation of the principle of nanopore detection of ADAM-17. αHL nanopore presented distinctive repulsive events in the absence/presence of AMAD-17. Adapted with permission from Ref.[90]. (b) Simultaneous detection of ADAM-10 and ADAM-17 using a single nanopore and a single substrate. The substrate contained both digestion sites of ADAM-10 and ADAM-17, which enabled recognize two proteases through the different digestion fragments. The enzymatic digestion of ADAM-10 produced a typical blockage event (closed 1), while that of ADAM-17 produced another type of blockage events (closed 2). Adapted with permission from Ref.[91].

2.1.5 Detecting proteinic biomarkers *via* DNA aptamer-protein affinity

Using biorecognition molecules as an external probe to detect analytes *via* coordination

interaction has attracted great attention in recent years since ligands could coordinate to a central atom or molecule with specific selectivity[96],[97],[98]. Using a similar strategy, nanopore sensing has achieved detection of various carcinomatous biomarkers. For example, Zhang *et al*. used a DNA aptamer-assisted strategy to determine the quantity of platelet-derived growth factor (PDGF) by taking advantage of a catch-and-release mechanism (strand displacement). Since PDGF participates in upregulating Mcl-1 and protecting prostate cancer cells from apoptosis, its levels are aberrant in individuals with prostate cancer[99],[100]. In their design, the DNA aptamers served as molecular hooks, causing two probes to approach each other and release Probe C (Fig. 6a). Although Probe A, Probe B, and Probe C produced current modulation events with similar blockage amplitudes (~90% of full channel block), their event mean residence time values were quite different (~7 ms, ~22 ms, and ~0.16 ms, respectively), allowing qualitative and quantitative detection of PDGF-BB (LOD=500 fM). In addition to the high sensitivity, this sensor was also selective: other nonspecific proteins in serum did not interfere with its detection[101].

It is worth emphasizing that cancer is a multistage process that often involves defects and/or alterations in many cellular pathways. Since one biomarker only represents one aspect of carcinogenesis, individual biomarkers have very limited indicative value. Accordingly, no cancer marker identified to date is sufficiently sensitive or specific to be used on its own to screen for cancer. Hence, there is growing recognition of the need for a panel of cancer markers instead of one specific biomarker for early cancer detection and diagnosis. As a multi-biomarker detection example, Fang and co-workers developed a nanopore sensor for simultaneous quantitation of three lung cancer biomarkers (i.e., vascular endothelial growth factor, thrombin, and PDGF-BB[102]) by taking advantage of morphology-dependent and length-dependent aptamer probes. In their study, three probes (H12, H25, and H16-fork) were designed, which could form a furcation structure by a short oligonucleotide laterally linked to the H16 strand. In the presence of the target protein biomarkers, the aptamer would firmly be bonded to the target analytes, and hence the probe would be released for nanopore detection. Since three different length capture arms (H12, H16-fork, and H25) were used, three types of current blockage events could be identified (amplitude:

80-95% channel block, residence time: 0.3-2 ms; amplitude: > 95% channel block, residence time: 1-30 ms; amplitude: 80-95% channel block, residence time: 5-1000 ms), enabling the discrimination among different protein biomarkers (Fig. 6b)[103].

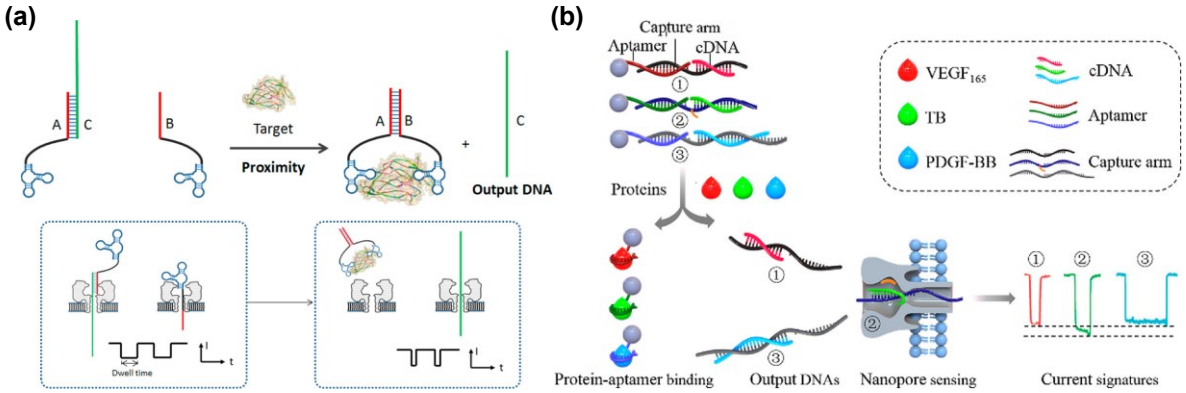


Figure 6. Label-free nanopore proximity bioassay for platelet-derived growth factor detection: schematic diagram of (a) the nanopore proximity bioassay for protein biomarker detection by binding-induced DNA strand displacement. Single stranded probe C was compatible with the pore size, resulting in a short current blockade. Instead, probe AC and probe B were either a double stranded DNA or an aptamer with complex secondary structure, generating long-lived blocks. Adapted with permission from Ref.[101]. (b) The nanopore assay used for simultaneous detection and discrimination of multiple protein biomarkers with a series of dsDNA-based probes. A series of double-stranded DNA-based probes, which consisting of a capture arm, a target-specific aptamer, and a complementary DNA molecule, were constructed. In the presence of target proteins, the individual capture arm-cDNA hybrids (H12, H16-folk, and H25) would release and scan in nanopore, enabling simultaneous discrimination among multiple protein biomarkers. Adapted with permission from Ref.[103].

Another study on aptamer-assisted nanopore-sensitive detection worth mentioning is performed by Yousefi et al., where mucin 1 protein (MUC1) was investigated (Fig. 7). The abnormal level of MUC1 is associated with multiple adenocarcinomas (*e.g.* breast cancer and ovarian cancers)[104]. The detection consisted of two important steps, i.e., analyte-triggered signal conversion and cascaded amplification. Briefly, to accurately capture MUC1 proteins in circulating tumor cells,

an aptamer-cross-linked DNA hydrogel was employed. In the presence of MUC1 proteins, the hydrogel would collapse and release AgNPs. Under the treatment of nitric acid, AgNPs would release Ag^{+}, which then oxidized CuS NPs to produce a large amount of Cu^{2+}, which activated the DNAzyme cleavage reaction. After the cascaded reaction, a large amount of triplex DNA molecules was produced, which could be detected by the engineered *Mycobacterium smegmatis porin* A (MspA) nanopore[105]. Similar to other nanopore-based protein sensors, the MUC1 sensor was highly sensitive with a detection limit of ~0.1 fg/mL.

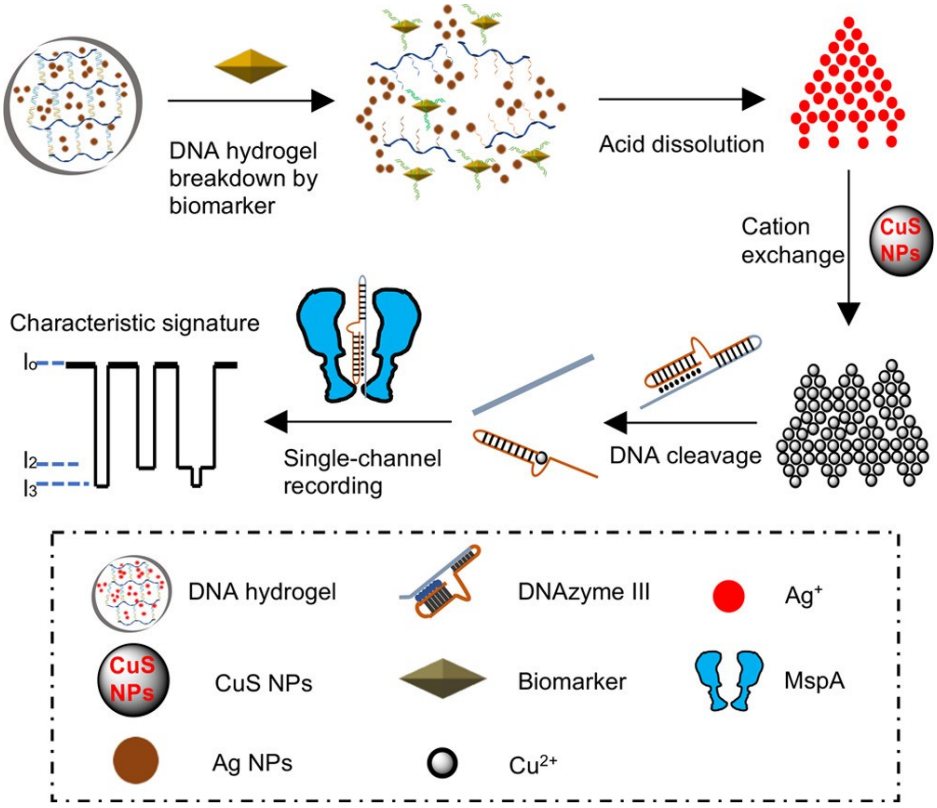


Figure 7. Schematic diagram of the nanopore sensing of MUC1 and breast cancer circulating tumor cells (CTCs) integrating cascaded amplification, signal conversion, and triplex-DNA beacon. Firstly, the aptamer-cross-linked DNA hydrogel was employed to specifically capture the MUC1 protein or breast cancer CTCs and release AgNPs. Then, the Ag NPs released Ag^{+} under the treatment of nitric acid to generate Cu^{2+}. Finally, the presence of Cu^{2+} activated triplex DNAzyme cleavage reaction, which accessed the engineered *Mycobacterium smegmatis porin* A (M2MspA) nanopore to indirectly inflect the amount of MUC1 and CTCs. Adapted with

permission from Ref.[105].

In addition to biological nanopores, solid-state nanopores have also been used to detect protein biomarkers in the presence of DNA aptamers. For instance, DNA aptamers can be designed with high affinity toward carcinoembryonic antigen (CEA) and human C-reactive protein, which could then be detected by nanopore[106],[107]. DNA aptamers could also be coupled with gold nanoparticles to further improve the selectivity, sensitivity, and resolution of the protein biomarker sensor[108]. In addition, similar to antibodies, DNA aptamers could also be anchored inside the lumen of the nanopore as immobile probes for protein detection, as reported by Ren *et al*[109].

2.1.6 Detecting nucleic acid biomarkers *via* artificial intelligence (AI), electro-optical sensing method, and beyond

Other biomarkers, such as DNA/RNA methylation and microRNA, were equally accessible candidates for the early detection of cancers. As an important epigenetic biomarker, DNA/RNA methylation can be detected by nanopore sequencing[110]. For short sequence biomarkers like microRNA, specific polyethylene glycol (PEG)-barcoded DNA probes, peptide nucleic acid (PNA) probes, and locked nucleic acid (LNA) probes were utilized to form duplex structures with microRNAs for sensitive detection[111],[112],[113],[114]. In terms of practical application, Gu's group demonstrated a higher level of DNA-microRNA duplexes observed in lung cancer patients[33]. Assisted with artificial intelligence (AI), the typical multi-level current signatures of DNA-microRNA duplexes were obtained in serum samples of colorectal cancer patients, which enabled the efficient processing of large-scale nanopore data in clinical diagnosis[115]. In a separate study, Cai *et al* introduced an electro-optical nanopore sensing method to detect multiple microRNAs from the serum of prostate cancer patients (Fig. 8). The optical signal was utilized to confirm the ligation of microRNAs to the molecular beacon, while the electrical signal aimed to identify microRNAs. In the presence of the target microRNAs, the molecular beacon would open, thus producing fluorescence. To sense multiple microRNAs, molecular beacon probes with

different lengths (5.6, 10, and 38.5 kbp) were designed, and significantly different nanopore signals were produced accordingly (e.g., the mean residence time values: 0.45 ± 0.19, 1.1± 0.3, and 5.0 ± 4.1 ms; the current blockages: 9.3 ± 5.5, 26.9 ± 7.1, and 189.7 ± 123.5 fAs, respectively). Using this method, the detection limit of miR-375-3p and miR-141-3p, whose upregulations were observed in the cancerous tissue and were evident in prostate cancer[116], could be greatly improved from 0.13 pM and 0.1 pM to 8 fM and 5 fM, respectively. Furthermore, the developed nanopore sensor was sensitive enough to identify different stages of prostate cancer patients (e.g., in remission, advanced localized, and advanced metastatic)[117].

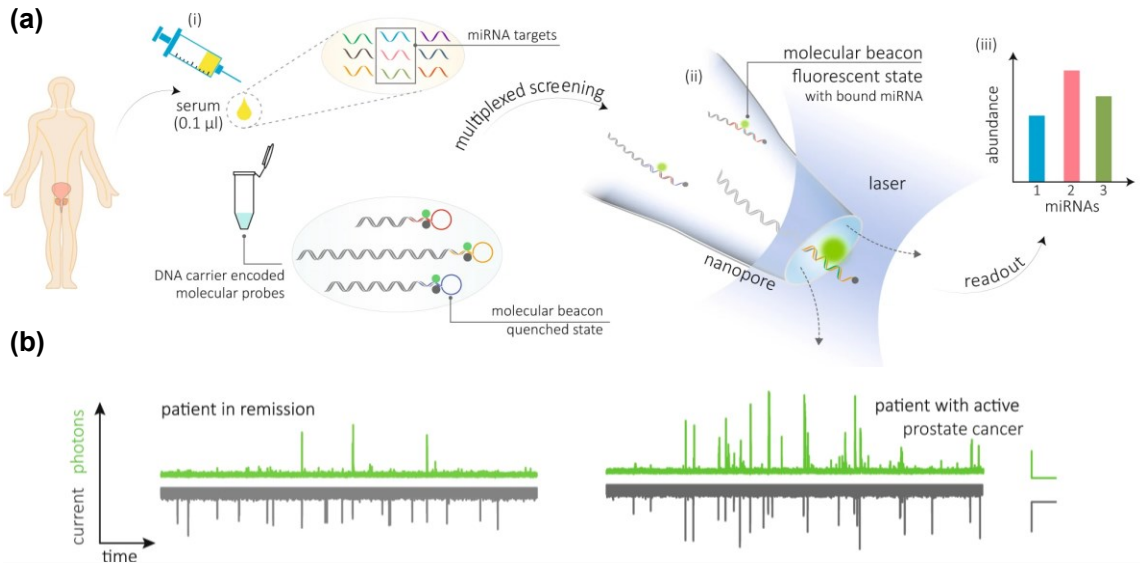


Figure 8. (a) Workflow for detection of miRNAs directly from patient serum. (i) Serum from patients was incubated with length-encoded molecular probes consisting of a DNA carrier and molecular beacon (MB). (ii) Electro-optical sensing was then performed, and (iii) the miRNA expression levels were determined. (b) Representative photon and current time traces for simultaneous detection of miR-141-3p and miR-375-3p from a patient in remission and a patient with active prostate cancer. Traces were recorded using asymmetric KCl buffer conditions (inside/outside nanopipette, 40/400 mM) at −300 mV bias and a laser power of 90 ± 4 µW. Scale bar of photon trace (green): vertical, 100 counts/ms, horizontal, 2.5 s. Scale bar of current trace: vertical, 30 pA, horizontal, 2.5 s. Adapted with permission from Ref.[117].

2.2. Neurologic diseases

The voluntary and involuntary activities of individuals are under the regulation of the neurological system. Dysfunctions of the neurological system are ubiquitous and may probably influence memory, cognition, language, personality, and skilled movements, further resulting in the incidence of diseases like Alzheimer's disease (AD), Parkinson's disease (PD), etc.[118] Due to the increasing, unprecedented number of elderly people globally, neurological illness debuted on the list of top ten causes of death in 2016[119],[120]. Since then, early detection and diagnosis of AD and PD have been in strong demand. In this section, we will briefly summarize nanopore-based detection of various neurological-related biomarkers, in particular, those related to AD and PD.

2.2.1 Detecting biomarkers of Alzheimer's disease

AD attacks brain cells, causing the progressive dysfunction and degeneration of neurons in the neocortex, limbic system, hippocampus, and various subcortical regions of the brain, which leads to memory deficits, cognitive impairments, and personality changes[121]. Although there is currently no cure for AD, appropriate medications and therapies will temporarily postpone the symptom development. Therefore, it is important to efficiently detect AD biomarkers in the earlier stage. The AD biomarkers could be easily harnessed from the genome (*e.g. APOEε4* allele, *BIN1*, and *CLU*), the cerebrospinal fluid (*e.g.* Aβ peptides, Tau protein, and neurogranin), and the blood (*e.g.* ICAM-1, α-2-macroglobulin, and miRNA-34c), of the patients[122]. Thus far, nanopore sensing has achieved great accomplishments in the detection of those biomarkers both theoretically and experimentally.

There is a shared awareness that a panel of biomarkers, rather than a single biomarker, is recommended for disease diagnosis and prognosis in order to improve precision and efficiency. For this purpose, Zou *et al.* detected three AD biomarkers (alpha-1 antitrypsin, Tau 381, and β-site amyloid precursor protein cleaving enzyme 1) using an aerolysin (AeL) nanopore array (Fig. 9a). The reason why aerolysin is used as the nanopore sensing element is that this nanopore features a

narrow sensing constriction (~1.3 nm), which offers high sensor sensitivity and selectivity[123],[124],[125]. Briefly, a target-specific aptamer and stem-forming oligonucleotides were formulated to construct a triple-helix molecular switch (TMS). In principle, TMS would open and release the oligonucleotides (7 bases, 10 bases, and 13 bases, respectively) as signal reporters in the presence of the corresponding target analytes. Experimental results demonstrated that the three biomarkers could be accurately detected at femtomolar levels[126].

In another study, Huo *et al* utilized an engineered aerolysin nanopore (T232K) to identify post-translational modifications (PTM) in Tau306-316 fragments (acetylated, phosphorylated, di-modified, and non-modified). Note that PTM influences the function of Tau protein in AD pathogenesis or progression. The four Tau peptides produced typical blockades with comparable residual current blockages (I/I_o=0.43 ± 0.01, I/I_o=0.55 ± 0.01, I/I_o=0.45 ± 0.01, and I/I_o=0.48 ± 0.01, respectively), while the events of the modified Pep-Ac, Pep-P and Pep-P-Ac had 4–15 folds increase in the residence time compared to those of the non-modified Pep (Fig. 9b). The results showed the potential applications of nanopore proteomics in disease diagnosis[127]. Other post-translational modifications such as glycosylation, phosphorylation, and ubiquitination were identified by Restrepo-Perez and Wloka with a FraC nanopore and a Cytolysin A nanopore, respectively[128],[129].

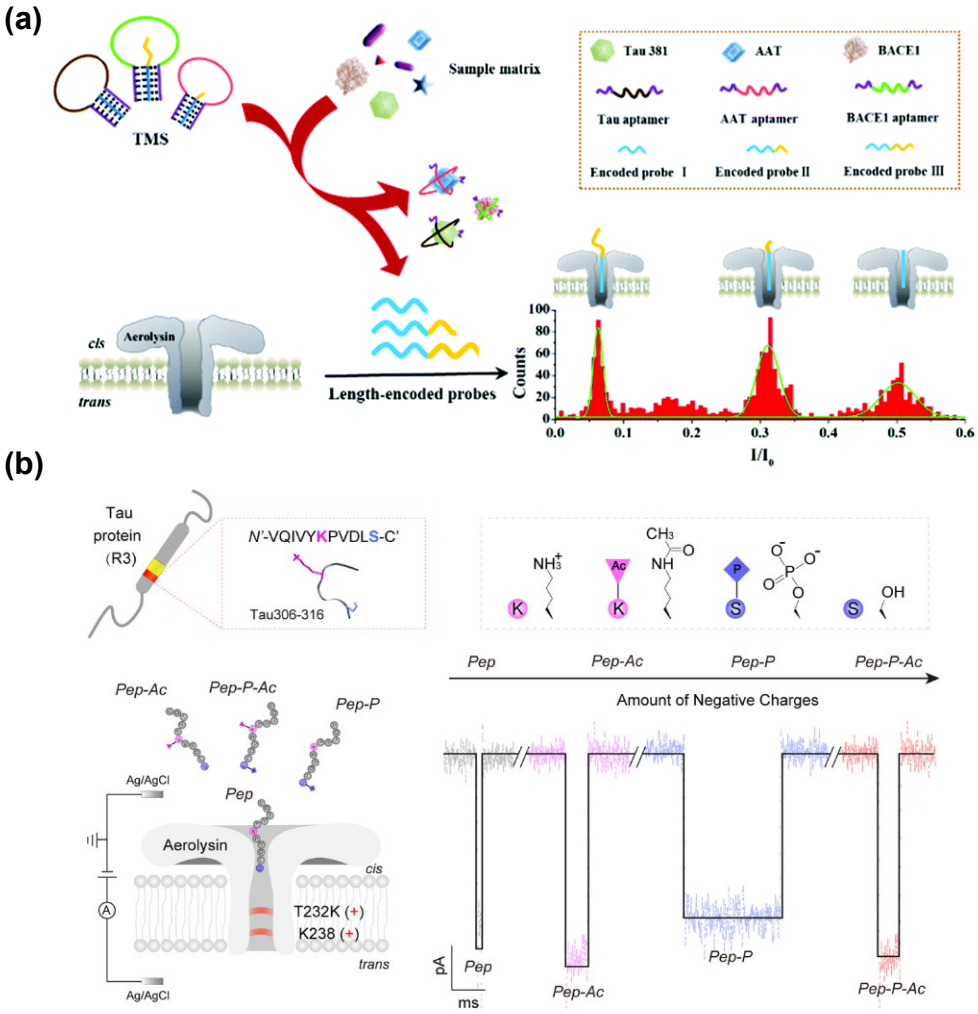


Figure 9. (a) Schematic illustration of multiplexed biomarker discrimination with the aerolysin nanopore and the corresponding characteristic current events induced by different biomarkers. The two compartments of the bilayer cell were termed *cis* and *trans*, and the length-encoded TMS probe was presented in the *cis* solution. Adapted with permission from Ref.[126]. (b) Schematic representation of the detection of Tau acetylation and phosphorylation with an engineered T232K AeL nanopore. The control model peptide with the sequence of N'-VQIVYKPVDLS-C' (Tau306-316) was a selected fragment in the R3 region of human Tau microtubule binding domain, which contained two potential posttranslational modification sites of K311 and S316. The non-modified Pep, acetylated Pep-Ac, phosphorylated Pep-P, and di-modified Pep-P-Ac exhibited increasing negative net charges and distinctly different current blockages at +120 mV. Adapted with permission from Ref.[129].

In addition to protein nanopores, solid-state nanopores have also been used for detection of AD biomarkers. For example, amyloid β (Aβ) is a significant AD biomarker[130]. It has been well documented that Aβ pathophysiology regulates the upstream pathophysiological events in AD and may activate downstream molecular pathways, including Tau misfolding, Tau-mediated toxicity, accumulation in tangles, and Tau spreading, which leads to cortical neurodegeneration[131]. Meyer *et al.* fabricated three PEG-functionalized nanopores (5 kDa PEG-modified conical nanopore, 5 kDa PEG-modified bullet nanopore, and 20 kDa PEG-modified conical nanopore) to detect Aβ fibrils. All the three nanopores showed satisfactory selectivity toward Aβ fibrils. In particular, the bullet-shaped and crowded nanopores offered remarkable performance toward fibrils detection. The results suggested that the geometry and crowding situation of nanopores had an impact on AD detection[132].

It should be noted that other readout methods were also developed instead of the conventional repulse-resistant method for Aβ detection. For example, Tabrizi and colleagues introduced interferometric reflectance spectroscopy (IRS) into the nanopore field. In their study, anodic alumina nanopores were sequentially modified by amine groups, glutaraldehyde, the amino-terminal aptamer probe, and methylene blue (MB). MB possessed a high absorption coefficiency but had a weaker affinity for the guanine-rich aptamer than Aβ. Therefore, in the presence of Aβ, MB would be detached from the aptamer, resulting in an increase in the reflective intensity[133]. With this strategy, Aβ was sensitively detected (LOD=0.02 μg/mL).

2.2.2 Detecting biomarkers of Parkinson's disease

Parkinson’s disease (PD) is another common neurodegenerative ailment. Previous studies demonstrated that PD causes a drop in dopamine levels in patients. In addition, it was reported that the conformations of α-synuclein (αS) aggregation may affect dopamine metabolism, causing the selective degeneration of dopamine neurons. This gradual degeneration of dopamine neurons in the substantia nigra has a close relationship with slowed movements, stiffness, and tremor in PD

patients[134]. Hence, α-synuclein (αS) is recognized as a noteworthy biomarker of PD[135]. Nanopore sensing allows to reveal molecular dynamics at the single-molecule level, and is useful to study the pathology of PD and perform PD diagnosis testing. As one noted example, quartz nanopores (34±3 nm) were fabricated to monitor αS aggregation process at different time scales (t=0, 30, 60, 90, and 120 min). With an increase in the incubation time, subtle changes in the blockage amplitudes and event frequencies of αS were observed, indicating the dynamic changes of αS aggregation. More importantly, this sensor could reveal the real-time aggregation information including the different stages of αS fibril formation (monomer, lag phase, and elongation phase), αS mutation in the cross-seeding aggregation, and the concentration-dependent quantitative information on aggregation[136].

Besides αS, another potential biomarker for the early diagnosis and prognosis of PD is acetylcholinesterase (AChE), which catalyzes the breakdown of acetylcholine and some other choline esters that function as neurotransmitters which are involved in synaptic transmission. To detect AChE using a solid-state nanopore, Sze *et al.* designed an aptamer-modified λ-DNA carrier which enabled the hybridization of complementary oligonucleotides to further access a second complementary sequence and an extended aptamer (Fig. 10). In the absence of AChE and other target proteins, translocation of the aptamer-modified DNA carrier in the nanopore produced one major type of events with short duration and small blockage amplitude. In contrast, in the presence of AChE, significantly larger blockage amplitude events were observed due to the AChE / aptamer interaction[137].

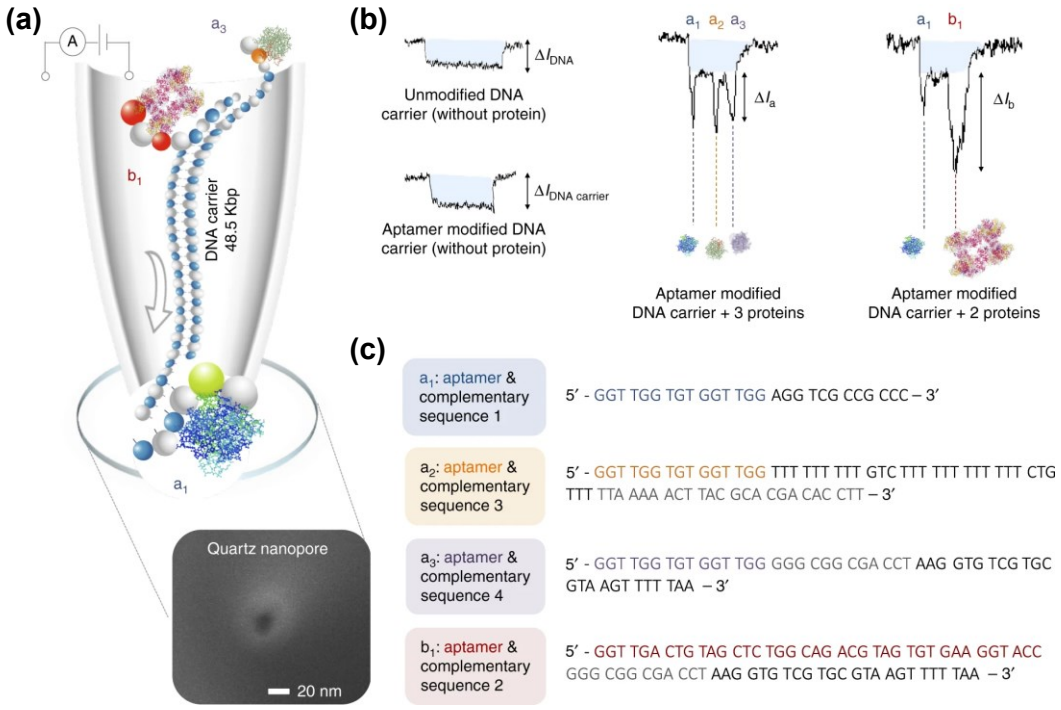


Figure 10. The design concept for using DNA aptamers and a carrier for protein sensing. (a) Schematic representation of a DNA carrier (48.5 kbp) engineered to contain aptamer sequences (a_1, b_1, a_3) that binded to three proteins translocating through a nanopore driven by the electric field. Translocations took place from inside the nanopipette to the outside. (b) Examples of translocation events of unmodified and aptamer-modified carriers. Also shown were example translocations for the detection of 3 and 2 proteins bound to the aptamers on the DNA carrier. Importantly, the sub-levels could be used to differentiate between proteins both in terms of location and magnitude. (c) Aptamer sequences were used for the detection of thrombin (a_1, a_2, a_3) and AChE (b_1). Adapted with permission from Ref.[137].

In addition to the detection of protein biomarkers, nanopore sequencing was used to detect the methylation of mitochondrial DNA, which was closely associated with pathological and physiological conditions in neurodegenerative diseases[138], and was a valuable molecular indicator in the detection of PD[139].

2.3 Detecting biomarkers of endocrine dysfunctions

The endocrine system comprises a complex network of various glands that releases hormones to regulate numerous bodily processes, including metabolism, growth and development in tissue, sexual and reproductive functions[140]. The body's homeostasis balance will be broken and result in endocrine dysfunctions when a gland produces an endocrine hormone at either a high or low level[141]. Among the patients who have endocrine diseases, up to 5% of them are tortured by hypothyroidism. Although the classic symptoms (e.g., weight gain, fatigue, poor concentration, depression, and menstrual irregularities) of hypothyroidism are mild, if they are ignored, patients will develop cardiovascular diseases later on and mortality will rise[142]. Therefore, it is important to detect hypothyroidism at the early stage. As one of such examples, He and colleagues adopted the sandwich immunoassay and release-capture mechanism for solid-state nanopore detection of thyroid-stimulating hormone (TSH), a staple biomarker of hypothyroidism[143]. THS was isolated from serum by taking advantage of the sandwich complex formed by the interaction among antibody-coated magnetic beads, target proteins, and secondary detection antibodies. It should be noted that the secondary detection antibodies were modified by streptavidin so that 50 nt biotinylated ssDNA with a photocleavable linker would be attached to the sandwich structure. Under the exposure of UV, the ssDNA would first separate from the structures and then served as junction strands to instantly connect two star-like DNA nanostructures, forming dumbbell structures, which produced two deep blockages separated by a shallower blockage level in the nanopore (Figure 11). This nanopore sensor was sensitive, with the limit of detection (LOD) reaching as low as ~20 pM. By employing antibodies-modified gold nanoparticles that contained hundreds of junction strands, the LOD might be further improved to the femtomolar level[144].

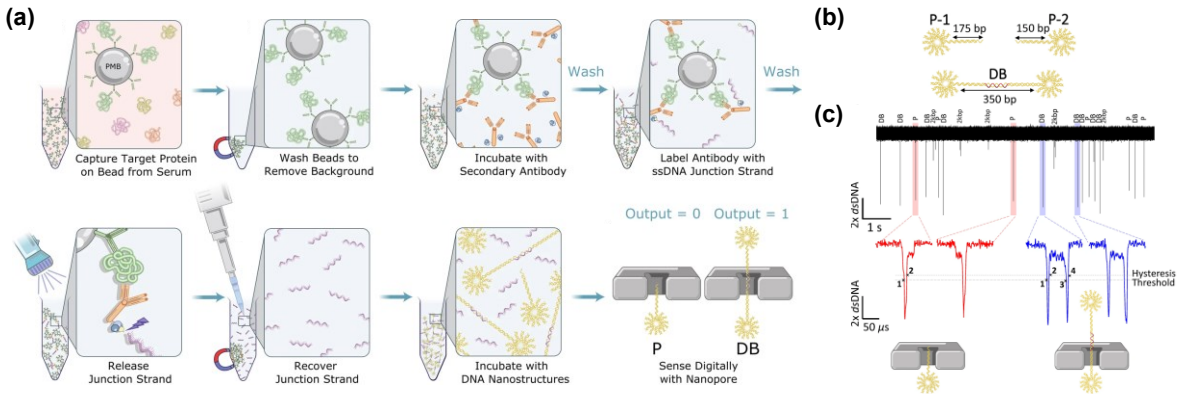


Figure 11. (a) Schematic illustration of the digital immunoassay workflow with nanopore electrical detection. (i) Paramagnetic beads (PMBs) conjugated with antibodies efficiently capture specific target protein in serum sample. (ii) PMBs were pelleted and immobilized with a magnet and supernatant was removed to eliminate unbound molecules. (iii) PMBs were resuspended and incubated with secondary antibody conjugated with streptavidin. (iv) Following a wash, the immuno-sandwich structure was incubated with biotinylated ssDNA junction strand. (v) Following another wash, the solution was exposed to UV light to release the junction strand. (vi) PMBs were pelleted and immobilized with a magnet and the supernatant containing the junction strand was recovered with a pipette. (vii) Shooting star-like DNA probes were added to the solution containing recovered junction strand leading to assembly of a dumbbell-like DNA nanostructure. (viii) Digital nanopore sensing to determine the fraction of probes to dumbbells. (b) Artistic representation of the shooting star probes (P-1 and P-2) and the dumbbell (DB). (c) 10 s current trace of a mixed population of both DNA nanostructures and 2 kbp dsDNA calibrator, with representative current traces showing individual translocation events corresponding to probes (left, red) and dumbbells (right, blue). Reproduced with permission from reference. Adapted with permission from Ref.[144].

3. Communicable diseases

Communicable diseases are caused by pathogenic microorganisms and can spread from person to person, with a new infection even spreading to the entire continents within days or weeks after first appearing. Communicable diseases are so intractable that the detection of related biomarkers

makes a great contribution to the prevention, diagnosis, and treatment. In this section, we will briefly summarize nanopore-based approaches to detect three important communicable diseases: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A viruses (IAV), and human immunodeficiency viruses (HIV), as they are the top global emergency case, the prototypical example of reemerging infection, and the deadliest communicable disease, respectively.

3.1 Detecting biomarkers of SARS-CoV-2

The emergency of SARS-CoV-2 results in a pandemic in humans, from the alpha variant to the omicron, with tens of millions of victims suffering from fever, cough, fatigue, *etc*.[145] SARS-CoV-2 was presumed to be originated from bats, but after the transmission from the intermediary hosts, it is able to transmit among humans mainly through respiratory droplet transmission. The invasion of SARS-CoV-2 principally depends on its spike proteins, which would aim at ACE2 receptors, fuse membranes, and inject the viral ribonucleoprotein.[146] Therefore, nucleic acids, viruses, and proteins are all ideal biomarkers to detect SARS-CoV-2. However, reverse transcription-polymerase chain reaction (RT-PCR) currently remains the gold standard for SARS-CoV-2 testing. Several nanopore-based studies have demonstrated the feasible application of nanopores in the surveillance of the pandemic with different biomarkers.

3.1.1 Detecting nucleic acids biomarkers

As one of such examples, Guan and colleagues developed a poly-(ethylene terephthalate) film (PET)-based nanopore platform for proof-of-concept detection of SARS-CoV-2. Due to its commercial availability, low cost, and high stability, PET has been utilized to construct nanopore sensors for various applications, including DNA sensing, metal detection, and protein binding[147],[148],[149]. In the developed SARS-CoV-2 sensor, a conical nanopore was fabricated by irradiating the PET film with single swift heavy ions, followed by asymmetric etching. The base diameter of the PET nanopore was about 1000 ± 80 nm, while its tip diameter

reached ~19.7 nm, which enabled the translocation of bare gold nanoparticles (with a diameter of 3.0 nm) and their variants. As illustrated in Fig. 12, nanopore detection of the target short length SARS-CoV-2 nucleic acid (NA) was achieved by using two DNA hybridization probes, one of which (P1) was immobilized on the surface of AuNPs, while the other (P2) was free in solution. Experimental results showed that AuNPs, P1, P2, and NA alone rarely produced any observable current modulation events in the nanopore. In contrast, frequent current modulations were observed when the ssDNA immobilized AuNPs (AuNP-P1) were present in the solution. When the solution also contained NA, in addition to the AuNP-P1 events (mean blockage amplitude: 53 ± 5 pA; residence time: 0.24 ± 0.08 ms), a new type of larger blockage amplitude and residence time events (amplitude: 110 ± 10 pA; residence time: 2.1 ± 0.4 ms) appeared due to the hybridization between AuNP-P1 with NA (i.e., the AuNP-P1-NA complex). Furthermore, when the second DNA probe P2 was additionally added to the AuNP-P1-NA solution, another event with quite different translocation signatures (blockage amplitude: 150 ± 8 pA; residence time: 2.4 ± 0.3 ms) was observed, further supporting the identification of SARS-CoV-2. Using the two DNA probe strategy, PET nanopore detection of SARS-CoV-2 could be achieved at a concentration of as low as ~9.7 nM under the symmetric electrolyte condition. The detection limit could be improved to 0.5 nM under an asymmetric condition[150].

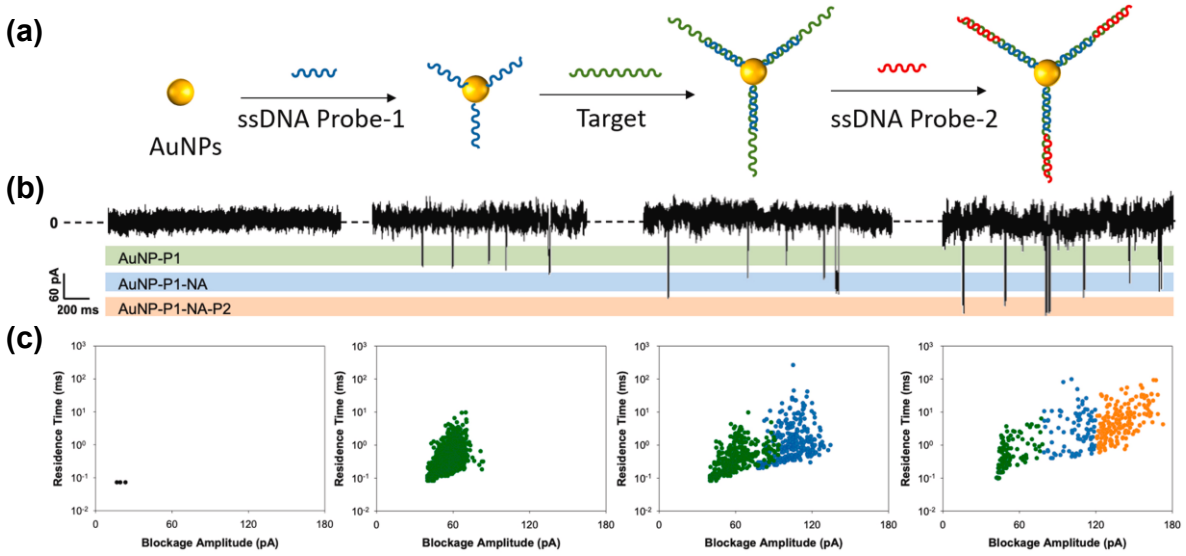


Figure 12. (a) Schematic illustration of the method and procedure of detecting target ssDNA in a large-size solid-state nanopore. (b) Typical trace segments of different species. (c) The corresponding scatter plots of event residence time *vs.* current blockage amplitude. The experiments were performed at +800 mV using a 20-nm diameter PET nanopore in a symmetric buffer condition with both the base and tip compartments filled with 0.5 M KCl and 10 mM Tris (pH 8.0). Adapted with permission from Ref.[150].

It should be noted that the nanopore sensor sensitivity could be significantly improved by coupling solid-state nanopores with other techniques. For example, recently, Nouri *et al* reported a CRISPR-Cas12a-assisted solid-state nanopore strategy to detect SARS-Cov-2 at 13.5 copies/μL (i.e., 22.5 aM), where three steps were involved, including reverse transcription and amplification, Cas12 assay, and nanopore-based molecule classification and counting[151]. In another study, RT-LAMP was coupled with a glass nanopore for the successful detection of SARS-CoV-2 RNA at as low as 65 copies/μL[152].

3.1.2 Detecting pathologic coronaviruses

As one of such examples, Taniguchi *et al* developed an effective and sensitive method (LOD = 2.5 pfu/μL in a 15 min assay) for detection of SARS-CoV-2 coronavirus using solid-state

nanopores. By taking advantage of artificial intelligence, the nanopores successfully identified four types of coronaviruses (HCoV-229E, SARS-CoV, MERS-CoV, and SARS-CoV-2). Furthermore, high sensitivity (90%) and specificity (96%) of SARS-CoV-2 detections were reported in saliva sample analysis[153]. In addition to the repulse-resistant-based detection, nanopore could identify analytes through steady-state I-V measurements. For example, Peinetti integrated the aptamers of SARS-CoV-2 into PET nanopores. The aptamer was selected from the DNA library by systematic evolution of ligands by exponential enrichment (SELEX). This nanopore sensor provided a comparable detection sensitivity toward nucleic acid (LOD = 1×10^4 copies/mL) to those of other methods. Moreover, due to the extraordinary specificity of the DNA aptamer, this sensor could differentiate between infectious and noninfectious SARS-CoV-2, which enabled asymptomatic diagnosis[154].

3.1.3 Detecting proteinic biomarkers

As an alternative to the nucleic acids-based detection, serologic assays to detect the presence of SARS-CoV-2 antibodies were also studied. For example, Zhang *et al* developed a nanopore sensor for assessment of IgG and IgM of SARS-CoV-2. Since the diameters of IgG and IgM are larger than the constriction of the αHL nanopore, they could not pass through the nanochannel, so that only the bumping events could be observed[155]. To detect IgG and IgM, external probes were used as reporters for nanopore analysis. As shown in Fig. 13, two types of nanoparticles were prepared: (1) magnetic beads (MBs) were functionalized by N protein, and (2) gold nanoparticles (AuNPs) were modified by IgG/IgM antibodies and probe DNA. In the presence of the target antibodies, two types of functionalized nanoparticles would form sandwich structures. After being subjected to high-temperature treatment (63 ºC), the probe DNAs would depart from the sandwich structures, which could then be detected by the nanopore sensor. By taking advantage of two different DNA reporters, which were obtained by covalently attaching adamantane-cucurbituril[6] to the different places of the probe, simultaneous detection of IgG (Signal A: I_1/I_0=98.1 ± 0.6% and I_2/I_0=71.6 ± 0.1%; τ_1=300.89 ± 30.1 ms and τ_2=20.17 ± 1.3 ms) and IgM (Signal B: I_1/I_0=79.5

± 0.3% and I_2/I_0=98.9 ± 0.4%; τ_1=244.36 ± 22.3 ms and τ_2=877.16 ± 287.8) were successfully achieved. This nanopore sensor was highly sensitive (LoD_{IgG}=10 ng/mL and LoD_{IgM}=50 ng/mL, respectively) and had a wide dynamic range (up to μg/mL level)[156]. In another noteworthy project, Asandai *et al* explored αHL nanopore to identify the collision events of SARS-CoV-2 spike S1 protein region-binding domains and their complexes with corresponding antibodies, which provided a comparative performance to other label-free detection techniques in SARS-CoV-2 detection[157].

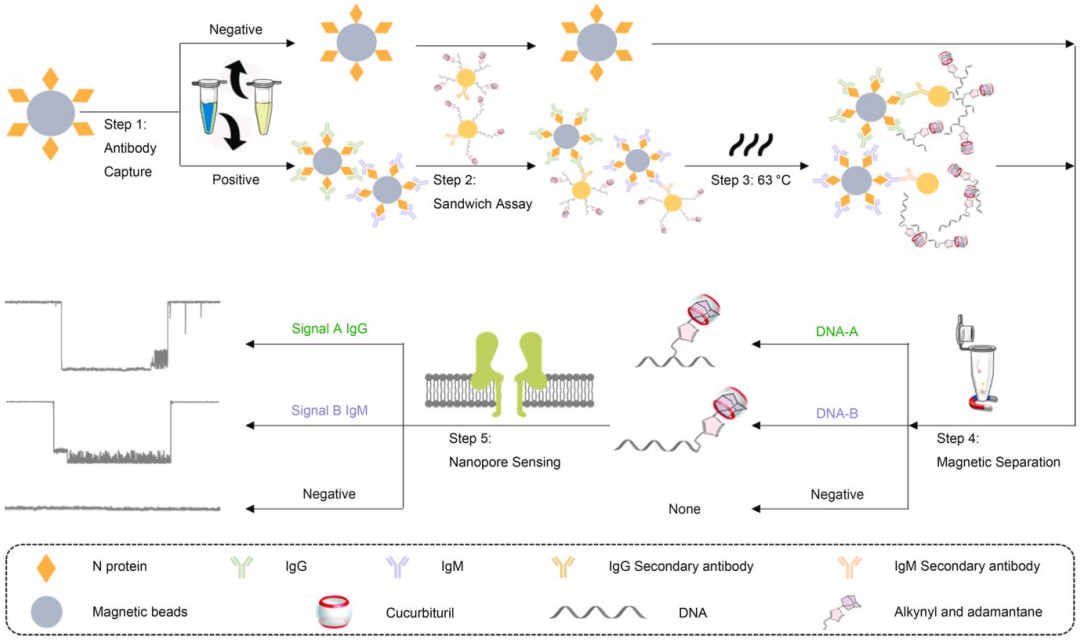


Figure 13. Schematic representation of the DNA-assisted nanopore assay for multiplex quantification of SARS-CoV-2 antibodies. Step 1: IgG and IgM captured by the N-protein modified MBs. Step 2: Formation of the sandwich structure between MBs, IgG or IgM antibody, and probe DNA modified AuNPs. Step 3: Dehybridization of the probe DNAs from the AuNPs. Step 4: Magnetic separation of probe DNAs from the remaining sandwich complex. Step 5: Quantification of probe DNAs to derive the concentration of IgG and IgM, respectively. Adapted with permission from Ref.[156].

3.2 Detecting biomarkers of Influenza A viruses (IAV)

IAV could cause respiratory infection that ranges from asymptomatic to deadly. Neuraminidase

(NA) is a vital glycoside hydrolase on the surface of IAV. It functions for proper budding and releasing of progeny virions from the host cell surface *via* digesting the sialic acid-containing receptors of the host viral membranes[158]. Furthermore, previous reports demonstrated that the NA activity was also involved in neurodegenerative diseases, cardiovascular diseases, and cancers[159].

A worth-mentioning example of NA detection was reported by Kwak *et al*., who used Cytolysin A (ClyA) nanopore to monitor the activity of IAV NA. In this investigation, ClyA nanopore was used as the sensing element because its cavity (with a width of 3.8-7 nm) is large enough to reversibly trap the proteins inside the pore, thus revealing the conformational dynamics, ligand bound, and catalytic states of proteins[160],[161],[162]. As shown in Fig. 14, the hydrolysis of glycan was initiated by NA. In the absence of galactose, when D-glucose/D-galactose binding protein (GBP) entered the lumen of the nanopore, two types of events were observed with the normalized current blockage of $I_{res\%L1}$= 69.1 ± 0.8% pA and $I_{res\%L2}$=67.4 ± 0.7% pA, respectively, while their relative event durations were 28% (L1) and 72% (L2), respectively. In contrast, in the presence of galactose, the high affinity between GBP and galactose induced the conformational change of GBP, resulting in an increase in the relative event duration of L1. Using this nanopore platform, NA could be detected with a LOD of 38 nM, which was 40-80 times better than the commercial kits[163]. Note that this sensing platform can serve as a model system to study protein folding and misfolding in the presence of sugar.

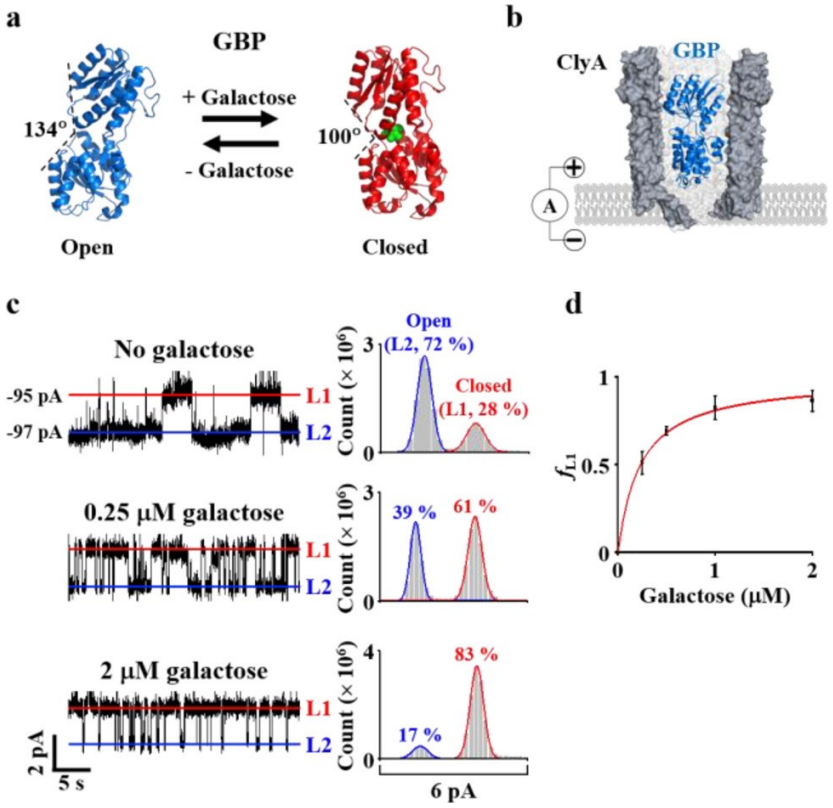


Figure 14. Analysis of galactose binding using the GBP-trapped ClyA nanopore. (a) Illustration of GBP in the ligand-free (open) and ligand-bound (closed) states. (b) Surface depiction of the GBP-trapped ClyA nanopore. (c) Electric current traces in the presence of increasing concentrations of galactose (left). Histograms of event durations of closed (L1) and open (L2) conformations in 30 s of current traces (right). (d) Fractional times of L1 (f_{L1}) versus concentrations of galactose fitted to a Hill function with the coefficient set to 1 ($f_{L1}=\frac{x-x_{min}}{0.92-x_{min}}$, where x_{min} is the closed faction of the ligand-free GBP, and 0.92 is the closed fraction at saturating galactose concentrations). Adapted with permission from Ref.[163].

Unlike the strategy used by Kwak et al., which was focused on the surface proteins of IAV, Oh *et al* detected IAV *via* its conserved RNA promoter. In their investigation, a specific DNA probe was designed to hybridize to the RNA promoter. When the hybridized complex molecules translocated through the αHL nanopore, ionic blockage events with characteristic signatures were produced. Using the DNA probe/IAV RNA promoter binding interaction strategy, the IAV RNA

promoter could be detected at concentration of 2 nM in the presence of 1 M KCl electrolyte, while the dynamic range of the sensor was from 5 pM to 500 nM at high salt conditions[164]. A similar DNA probe-based strategy was utilized to detect infectious anthrax as well[165]. Furthermore, solid-state nanopores were used as downstream sensors to monitor the PCR amplicons of group A streptococcus due to their flexible customized dimensions[166].

3.3 Detecting biomarkers of human immunodeficiency virus

Human immunodeficiency virus (HIV) infection is another noteworthy communicable disease, which can cause acquired immunodeficiency syndrome (AIDS). HIV infection would also increase the risk of developing cardiovascular disease, bone disease, renal and hepatic dysfunction, and several other common morbidities[167]. HIV-1 protease is a primary biomarker of HIV infection due to its crucial role in processing the precursor proteins Gag and Gag-Pol into functional mature proteins[168]. Thus far, several nanopore-based HIV infection detection methods have been reported. For example, HIV infection could be identified using enzymatic digestion and aptamer binding strategies[169],[170].

Another work worth mentioning was reported by Zhang et al., where PET nanopore was utilized for direct detection of HIV-1 protease rather than relying on the enzymatic reaction. In order to reduce the entropic barrier for HIV-1 protease to enter the nanopore and to improve the interactions between the pore and the protease, the interior wall of the PET nanopore was functionalized with amine groups (Fig. 15). This functionalization enabled the nanopore to identify and differentiate HIV-1 PR from multiple spiked proteins (trypsin, HSA, and BSA)[171]. The developed PET nanopore sensing strategy provides a general platform not only for rapid detection of proteins at the single-molecule level but also for exploring fundamental protein dynamics and opening new avenues toward constructing novel biomimetic nanopore systems.

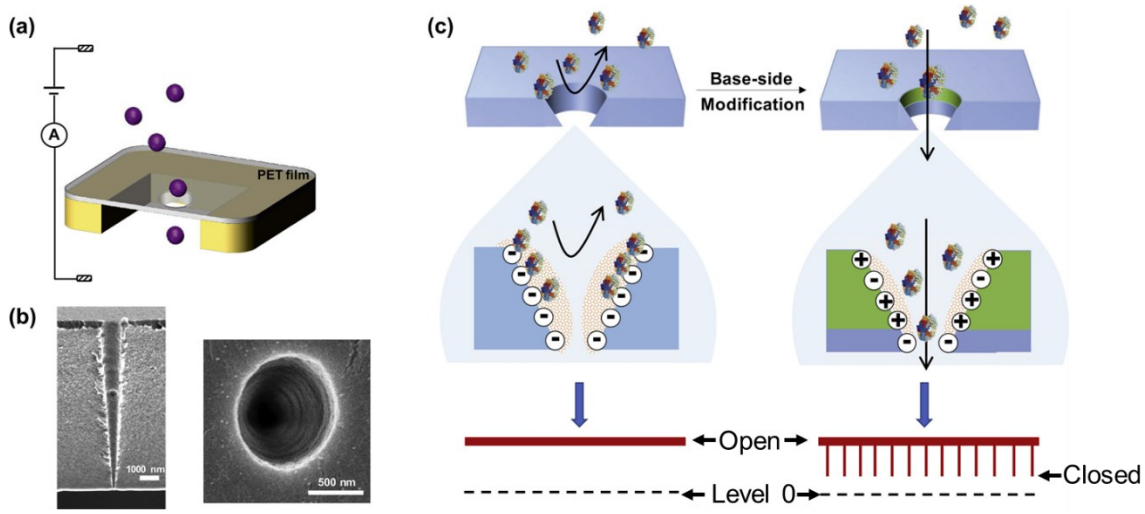


Figure 15. The principle of a modified single conical PET nanopore for detection of proteins. (a) Schematic representation of te PET nanopore sensing system. (b) Representative cross-sectional SEM image and top view of a conical nanopore. (c) Before modification of the PET nanopore, protein molecules could not pass through the nanopore due to the large energy barrier, and some protein molecules might be absorbed on the interior wall of the nanopore by electrostatic and hydrophobic interactions. In contrast, asymmetric modification of the nanopore base produced a new type of surface and property in the interior wall of the nanopore, which reduces the energetic barrier for proteins' entrance into the pore and slows down the velocity of ion flux, resulting in ionic current blockage events. Adapted with permission from Ref.[171].

Besides protein detection, Sethi *et al* developed another approach for HIV infection analysis by locating the conserved sequence of HIV-1B RNA. In their design, the DNA probes, which were specific to the conserved HIV RNA sequence, were modified with a biotin moiety so that they could construct the nucleoprotein complexes with monovalent streptavidin to enlarge the valid sterical volume and stimulate of nanopore response. It should note that mung bean nuclease was used to digest the unannealed genomic regions. As the nucleoprotein complexes were mobilized on the center of DNA probes and subjected to enzymatic digestion, the leaving target/probe duplexes evoked nanopore signals. This nanopore sensor was able to detect HIV-1B RNA at 1 nM[172].

4. Conclusions, challenges, and future outlooks

It is apparent that the nanopore-based biomarker detection indeed makes a great contribution to the early diagnosis and prognosis of a wide variety of human diseases, enabling timely and effective disease treatment and prevention. One major challenge in clinical sample analysis is matrix effect. In order to improve the performance of biomarker detection for noncommunicable and communicable disease diagnostics, numerous innovative nanopore sensing strategies have been developed, where various auxiliary materials (*i.e.,* magnetic beads, aptamers, and antibodies) have been used based on the intrinsic properties of biomarkers (*i.e.*, nucleic acids, proteins, proteases, antigens, *etc*.). For example, DNA probes, aptamers, and antibodies present high selectivity to nucleic acid / protein biomarkers. When combined with magnetic beads, they are able to extract and concentrate target biomarkers from clinical samples for nanopore sensing. To improve the diagnosis accuracy, a panel of biomarkers instead of one specific species could be used.

Although nanopore offers a promising potential in disease diagnosis and prognosis, some challenges still remain. First, the fixed internal diameter of a biological nanopore limits its on-pore (in situ) detection when encountering bulky biomarkers, especially proteins. Second, the sensitivities of nanopore sensors need further improvement to satisfy the requirement for accurate detection of some ultra-low concentrations of biomarkers in clinical samples. Third, concurrently screening multiple biomarkers is difficult to be achieved using a single nanopore because of the potential event signature overlap. To address these issues, several tactics could be attempted. For example, instead of direct detection of bulky protein analytes, nanopore can be used to detect nucleic acids. By analyzing the features of genomes at sequence level and studying differences among individuals by comparing the whole genomic sequences, clinical evaluation could be obtained[173],[174]. On the other hand, the performance of nanopore analysis could be improved by EOF rectification (*i.e.,* by taking advantage of ionic strength, salt gradient, and voltage bias), testing for correlations between different sets of combined electrical and optical signals, and by

means of enzyme-hybridized strategies. In addition, machine learning can be utilized for more complicated data analysis, which makes disease diagnosis and prognosis more accurate and valuable[175].

Although the past biomarker-related research efforts were mainly focused on cancer, neurologic diseases, endocrine dysfunction, and infectious diseases, clearly, the various nanopore-based detection strategies summarized in this review can be used for early diagnosis and prognosis of numerous other diseases by analyzing their corresponding biomarkers. Given the advantages of label-free analysis, ease of operation, high sensor sensitivity and selectivity, and instrument simplicity and portability, it is expected that nanopore technique will be a method of choice for disease diagnosis. Furthermore, nanopore technique is also promising to be utilized for determining sample composition and purity, studying dynamics and folding/unfolding of biomolecules, and investigation of protein-nucleic acid interactions[176],[177],[178],[179]. In conclusion, nanopore technology provides an avenue for biology and medical research both theoretically and experimentally.

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Notes

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Reference

[1] GBD 2019 Demographics Collaborators, Global age-sex-specific fertility, mortality, healthy life expectancy (HALE), and population estimates in 204 countries and territories, 1950-2019: a comprehensive demographic analysis for the Global Burden of Disease Study 2019, Lancet. 396 (2020) 1160–1203. https://doi.org/10.1016/S0140-6736(20)30977-6.

[2] T. Chikowore, A.B. Kamiza, O.H. Oduaran, T. Machipisa, S. Fatumo, Non-communicable diseases pandemic and precision medicine: Is Africa ready?, EBioMedicine. 65 (2021) 103260. https://doi.org/10.1016/j.ebiom.2021.103260.

[3] H. Wang, K.R. Paulson, S.A. Pease, S. Watson, H. Comfort, P. Zheng, A.Y. Aravkin, C. Bisignano, R.M. Barber, T. Alam, J.E. Fuller, E.A. May, D.P. Jones, M.E. Frisch, C. Abbafati, C. Adolph, A. Allorant, J.O. Amlag, B. Bang-Jensen, G.J. Bertolacci, S.S. Bloom, A. Carter, E. Castro, S. Chakrabarti, J. Chattopadhyay, R.M. Cogen, J.K. Collins, K. Cooperrider, X. Dai, W.J. Dangel, F. Daoud, C. Dapper, A. Deen, B.B. Duncan, M. Erickson, S.B. Ewald, T. Fedosseeva, A.J. Ferrari, J.J. Frostad, N. Fullman, J. Gallagher, A. Gamkrelidze, G. Guo, J. He, M. Helak, N.J. Henry, E.N. Hulland, B.M. Huntley, M. Kereselidze, A. Lazzar-Atwood, K.E. LeGrand, A. Lindstrom, E. Linebarger, P.A. Lotufo, R. Lozano, B. Magistro, D.C. Malta, J. Månsson, A.M. Mantilla Herrera, F. Marinho, A.H. Mirkuzie, A.T. Misganaw, L. Monasta, P. Naik, S. Nomura, E.G. O'Brien, J.K. O'Halloran, L.T. Olana, S.M. Ostroff, L. Penberthy, R.C. Reiner Jr, G. Reinke, A.L.P. Ribeiro, D.F. Santomauro, M.I. Schmidt, D.H. Shaw, B.S. Sheena, A. Sholokhov, N. Skhvitaridze, R.J.D. Sorensen, E.E. Spurlock, R. Syailendrawati, R. Topor-Madry, C.E. Troeger, R. Walcott, A. Walker, C.S. Wiysonge, N.A. Worku, B. Zigler, D.M. Pigott, M. Naghavi, A.H. Mokdad, S.S. Lim, S.I. Hay, E. Gakidou, C.J.L. Murray, Estimating excess mortality due to the COVID-19 pandemic: a systematic analysis of COVID-19-related mortality, 2020–21, The Lancet. 399 (2022) 1513–1536. https://doi.org/10.1016/S0140-6736(21)02796-3.

[4] P.C. Crepaldi, T.C. Pimenta, R.L. Moreno, E.C. Rodriguez, A Low Power CMOS Voltage Regulator for a

Wireless Blood Pressure Biosensor, IEEE Trans. Instrum. Meas. 61 (2012) 729–739. https://doi.org/10.1109/TIM.2011.2172121.

[5] M. Rothmaier, B. Selm, S. Spichtig, D. Haensse, M. Wolf, Photonic textiles for pulse oximetry, Opt. Express. 16 (2008) 12973. https://doi.org/10.1364/OE.16.012973.

[6] A. Salim, S. Lim, Recent advances in noninvasive flexible and wearable wireless biosensors, Biosensors and Bioelectronics. 141 (2019) 111422. https://doi.org/10.1016/j.bios.2019.111422.

[7] J. Li, Y. Liang, J. Fan, C. Xu, B. Guan, J. Zhang, B. Guo, Y. Shi, P. Wang, Y. Tan, Q. Zhang, C. Yuan, Y. Wu, L. Zhou, W. Ci, X. Li, DNA methylation subtypes guiding prognostic assessment and linking to responses the DNA methyltransferase inhibitor SGI-110 in urothelial carcinoma, BMC Med. 20 (2022) 222. https://doi.org/10.1186/s12916-022-02426-w.

[8] X. Yao, Z. Lu, Z. Feng, L. Gao, X. Zhou, M. Li, S. Zhong, Q. Wu, Z. Liu, H. Zhang, Z. Liu, L. Yi, T. Zhou, X. Zhao, J. Zhang, Y. Wang, X. Huang, X. Wang, J. Liu, Comparison of chromatin accessibility landscapes during early development of prefrontal cortex between rhesus macaque and human, Nat Commun. 13 (2022) 3883. https://doi.org/10.1038/s41467-022-31403-3.

[9] Y. Hou, S. Zhao, Q. Liu, X. Zhang, T. Sha, Y. Su, W. Zhao, Y. Bao, Y. Xue, H. Chen, Ongoing positive selection drives the evolution of SARS-CoV-2 genomes, Genomics Proteomics Bioinformatics. (2022) S1672-0229(22)00079–1. https://doi.org/10.1016/j.gpb.2022.05.009.

[10] M. Comabella, X. Montalban, Body fluid biomarkers in multiple sclerosis, The Lancet Neurology. 13 (2014) 113–126. https://doi.org/10.1016/S1474-4422(13)70233-3.

[11] L. Guo, C. Wang, C. Chi, X. Wang, S. Liu, W. Zhao, C. Ke, G. Xu, E. Li, Exhaled breath volatile biomarker analysis for thyroid cancer, Translational Research. 166 (2015) 188–195. https://doi.org/10.1016/j.trsl.2015.01.005.

[12] N. Yuan, Y. Chen, Y. Xia, J. Dai, C. Liu, Inflammation-related biomarkers in major psychiatric disorders: a cross-disorder assessment of reproducibility and specificity in 43 meta-analyses, Transl Psychiatry. 9 (2019) 233. https://doi.org/10.1038/s41398-019-0570-y.

[13] K. Strimbu, J.A. Tavel, What are biomarkers?:, Current Opinion in HIV and AIDS. 5 (2010) 463–466. https://doi.org/10.1097/COH.0b013e32833ed177.

[14] R. Huss, Biomarkers, in: Translational Regenerative Medicine, Elsevier, 2015: pp. 235–241. https://doi.org/10.1016/B978-0-12-410396-2.00019-0.

[15] N. Rifai, M.A. Gillette, S.A. Carr, Protein biomarker discovery and validation: the long and uncertain path to clinical utility, Nat Biotechnol. 24 (2006) 971–983. https://doi.org/10.1038/nbt1235.

[16] Y.Y. Broza, X. Zhou, M. Yuan, D. Qu, Y. Zheng, R. Vishinkin, M. Khatib, W. Wu, H. Haick, Disease Detection with Molecular Biomarkers: From Chemistry of Body Fluids to Nature-Inspired Chemical Sensors, Chem. Rev. 119 (2019) 11761–11817. https://doi.org/10.1021/acs.chemrev.9b00437.

[17] T. Mishra, M. Wang, A.A. Metwally, G.K. Bogu, A.W. Brooks, A. Bahmani, A. Alavi, A. Celli, E. Higgs, O. Dagan-Rosenfeld, B. Fay, S. Kirkpatrick, R. Kellogg, M. Gibson, T. Wang, E.M. Hunting, P. Mamic, A.B. Ganz, B. Rolnik, X. Li, M.P. Snyder, Pre-symptomatic detection of COVID-19 from smartwatch data, Nat Biomed Eng. 4 (2020) 1208–1220. https://doi.org/10.1038/s41551-020-00640-6.

[18] A.J. Bandodkar, P. Gutruf, J. Choi, K. Lee, Y. Sekine, J.T. Reeder, W.J. Jeang, A.J. Aranyosi, S.P. Lee, J.B. Model, R. Ghaffari, C.-J. Su, J.P. Leshock, T. Ray, A. Verrillo, K. Thomas, V. Krishnamurthi, S. Han, J. Kim, S. Krishnan, T. Hang, J.A. Rogers, Battery-free, skin-interfaced microfluidic/electronic systems for

simultaneous electrochemical, colorimetric, and volumetric analysis of sweat, Sci. Adv. 5 (2019) eaav3294. https://doi.org/10.1126/sciadv.aav3294.

[19] D. Liu, P. Zhu, F. Zhang, P. Li, W. Huang, C. Li, N. Han, S. Mu, H. Zhou, Y. Mao, Intrinsically stretchable polymer semiconductor based electronic skin for multiple perceptions of force, temperature, and visible light, Nano Res. (2022). https://doi.org/10.1007/s12274-022-4622-x.

[20] Y. Zhang, X. Chen, S. Yuan, L. Wang, X. Guan, Joint Entropy-Assisted Graphene Oxide-Based Multiplexing Biosensing Platform for Simultaneous Detection of Multiple Proteases, Anal Chem. 92 (2020) 15042–15049. https://doi.org/10.1021/acs.analchem.0c03007.

[21] S. Ghosh, K. Aggarwal, V.T. U., T. Nguyen, J. Han, C.H. Ahn, A new microchannel capillary flow assay (MCFA) platform with lyophilized chemiluminescence reagents for a smartphone-based POCT detecting malaria, Microsyst Nanoeng. 6 (2020) 5. https://doi.org/10.1038/s41378-019-0108-8.

[22] R. de la Rica, M.M. Stevens, Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye, Nature Nanotech. 7 (2012) 821–824. https://doi.org/10.1038/nnano.2012.186.

[23] K. Ven, B. Vanspauwen, E. Pérez-Ruiz, K. Leirs, D. Decrop, H. Gerstmans, D. Spasic, J. Lammertyn, Target Confinement in Small Reaction Volumes Using Microfluidic Technologies: A Smart Approach for Single-Entity Detection and Analysis, ACS Sens. 3 (2018) 264–284. https://doi.org/10.1021/acssensors.7b00873.

[24] R. Kawano, T. Osaki, H. Sasaki, M. Takinoue, S. Yoshizawa, S. Takeuchi, Rapid Detection of a Cocaine-Binding Aptamer Using Biological Nanopores on a Chip, J. Am. Chem. Soc. 133 (2011) 8474–8477. https://doi.org/10.1021/ja2026085.

[25] S. Zhang, W. Shi, K.-B. Li, D.-M. Han, J.-J. Xu, Ultrasensitive and Label-Free Detection of Multiple DNA Methyltransferases by Asymmetric Nanopore Biosensor, Anal. Chem. 94 (2022) 4407–4416. https://doi.org/10.1021/acs.analchem.1c05332.

[26] D.-K. Kwak, H. Chae, M.-K. Lee, J.-H. Ha, G. Goyal, M.J. Kim, K.-B. Kim, S.-W. Chi, Probing the Small-Molecule Inhibition of an Anticancer Therapeutic Protein-Protein Interaction Using a Solid-State Nanopore, Angew. Chem. 128 (2016) 5807–5811. https://doi.org/10.1002/ange.201511601.

[27] O. Tavassoly, J. Kakish, S. Nokhrin, O. Dmitriev, J.S. Lee, The use of nanopore analysis for discovering drugs which bind to α-synuclein for treatment of Parkinson's disease, European Journal of Medicinal Chemistry. 88 (2014) 42–54. https://doi.org/10.1016/j.ejmech.2014.07.090.

[28] F. Zhang, Y. Sun, D. Tian, H. Li, Chiral Selective Transport of Proteins by Cysteine-Enantiomer-Modified Nanopores, Angew Chem Int Ed Engl. 56 (2017) 7186–7190. https://doi.org/10.1002/anie.201701255.

[29] G.M. Roozbahani, X. Chen, Y. Zhang, R. Xie, R. Ma, D. Li, H. Li, X. Guan, Peptide-Mediated Nanopore Detection of Uranyl Ions in Aqueous Media, ACS Sens. 2 (2017) 703–709. https://doi.org/10.1021/acssensors.7b00210.

[30] G.M. Roozbahani, Y. Zhang, X. Chen, M.H. Soflaee, X. Guan, Enzymatic reaction-based nanopore detection of zinc ions, Analyst. 144 (2019) 7432–7436. https://doi.org/10.1039/c9an01784d.

[31] G. Wang, L. Wang, Y. Han, S. Zhou, X. Guan, Nanopore detection of copper ions using a polyhistidine probe, Biosens Bioelectron. 53 (2014) 453–458. https://doi.org/10.1016/j.bios.2013.10.013.

[32] M. Wanunu, T. Dadosh, V. Ray, J. Jin, L. McReynolds, M. Drndić, Rapid electronic detection of probe-specific microRNAs using thin nanopore sensors, Nat Nanotechnol. 5 (2010) 807–814. https://doi.org/10.1038/nnano.2010.202.

[33] Y. Wang, D. Zheng, Q. Tan, M.X. Wang, L.-Q. Gu, Nanopore-based detection of circulating microRNAs in lung cancer patients, Nat Nanotechnol. 6 (2011) 668–674. https://doi.org/10.1038/nnano.2011.147.

[34] L. Chalupowicz, A. Dombrovsky, V. Gaba, N. Luria, M. Reuven, A. Beerman, O. Lachman, O. Dror, G. Nissan, S. Manulis-Sasson, Diagnosis of plant diseases using the Nanopore sequencing platform, Plant Pathol. 68 (2019) 229–238. https://doi.org/10.1111/ppa.12957.

[35] S. Benner, R.J.A. Chen, N.A. Wilson, R. Abu-Shumays, N. Hurt, K.R. Lieberman, D.W. Deamer, W.B. Dunbar, M. Akeson, Sequence-specific detection of individual DNA polymerase complexes in real time using a nanopore, Nature Nanotech. 2 (2007) 718–724. https://doi.org/10.1038/nnano.2007.344.

[36] E.A. Manrao, I.M. Derrington, A.H. Laszlo, K.W. Langford, M.K. Hopper, N. Gillgren, M. Pavlenok, M. Niederweis, J.H. Gundlach, Reading DNA at single-nucleotide resolution with a mutant MspA nanopore and phi29 DNA polymerase, Nat Biotechnol. 30 (2012) 349–353. https://doi.org/10.1038/nbt.2171.

[37] Y. Qing, S.A. Ionescu, G.S. Pulcu, H. Bayley, Directional control of a processive molecular hopper, Science. 361 (2018) 908–912. https://doi.org/10.1126/science.aat3872.

[38] Y. Qing, H. Bayley, Enzymeless DNA Base Identification by Chemical Stepping in a Nanopore, J Am Chem Soc. 143 (2021) 18181–18187. https://doi.org/10.1021/jacs.1c07497.

[39] Y. Wang, S. Zhang, W. Jia, P. Fan, L. Wang, X. Li, J. Chen, Z. Cao, X. Du, Y. Liu, K. Wang, C. Hu, J. Zhang, J. Hu, P. Zhang, H.-Y. Chen, S. Huang, Identification of nucleoside monophosphates and their epigenetic modifications using an engineered nanopore, Nat. Nanotechnol. (2022). https://doi.org/10.1038/s41565-022-01169-2.

[40] W. Li, Y. Wang, Y. Xiao, M. Li, Q. Liu, L. Liang, W. Xie, D. Wang, X. Guan, L. Wang, Simultaneous Dual-Site Identification of 5mC/8oG in DNA Triplex Using a Nanopore Sensor, ACS Appl Mater Interfaces. (2022). https://doi.org/10.1021/acsami.2c08478.

[41] H. Brinkerhoff, A.S.W. Kang, J. Liu, A. Aksimentiev, C. Dekker, Multiple rereads of single proteins at single-amino acid resolution using nanopores, Science. 374 (2021) 1509–1513. https://doi.org/10.1126/science.abl4381.

[42] S. Yan, J. Zhang, Y. Wang, W. Guo, S. Zhang, Y. Liu, J. Cao, Y. Wang, L. Wang, F. Ma, P. Zhang, H.-Y. Chen, S. Huang, Single Molecule Ratcheting Motion of Peptides in a Mycobacterium smegmatis Porin A (MspA) Nanopore, Nano Lett. 21 (2021) 6703–6710. https://doi.org/10.1021/acs.nanolett.1c02371.

[43] Z. Chen, Z. Wang, Y. Xu, X. Zhang, B. Tian, J. Bai, Controlled movement of ssDNA conjugated peptide through Mycobacterium smegmatis porin A (MspA) nanopore by a helicase motor for peptide sequencing application, Chem Sci. 12 (2021) 15750–15756. https://doi.org/10.1039/d1sc04342k.

[44] S. Zhang, Z. Cao, P. Fan, Y. Wang, W. Jia, L. Wang, K. Wang, Y. Liu, X. Du, C. Hu, P. Zhang, H. Chen, S. Huang, A Nanopore-Based Saccharide Sensor, Angew Chem Int Ed. (2022). https://doi.org/10.1002/anie.202203769.

[45] J. Im, S. Lindsay, X. Wang, P. Zhang, Single Molecule Identification and Quantification of Glycosaminoglycans Using Solid-State Nanopores, ACS Nano. 13 (2019) 6308–6318. https://doi.org/10.1021/acsnano.9b00618.

[46] D.-H. Lee, S. Oh, K. Lim, B. Lee, G.-S. Yi, Y.-R. Kim, K.-B. Kim, C.-K. Lee, S.-W. Chi, M.-K. Lee, Tertiary RNA Folding-Targeted Drug Screening Strategy Using a Protein Nanopore, Anal. Chem. 93 (2021) 2811–2819. https://doi.org/10.1021/acs.analchem.0c03941.

[47] X. Li, K.H. Lee, S. Shorkey, J. Chen, M. Chen, Different Anomeric Sugar Bound States of Maltose Binding

Protein Resolved by a Cytolysin A Nanopore Tweezer, ACS Nano. 14 (2020) 1727–1737. https://doi.org/10.1021/acsnano.9b07385.

[48] J. Fu, L. Wu, G. Hu, F. Li, Q. Ge, Z. Lu, J. Tu, Solid-state nanopore analysis on the conformation change of DNA polymerase I induced by a DNA substrate, Analyst. 147 (2022) 3087–3095. https://doi.org/10.1039/d2an00567k.

[49] S. Schmid, P. Stömmer, H. Dietz, C. Dekker, Nanopore electro-osmotic trap for the label-free study of single proteins and their conformations, Nat Nanotechnol. 16 (2021) 1244–1250. https://doi.org/10.1038/s41565-021-00958-5.

[50] L. Wang, H. Wang, X. Chen, S. Zhou, Y. Wang, X. Guan, Chemistry solutions to facilitate nanopore detection and analysis, Biosens Bioelectron. 213 (2022) 114448. https://doi.org/10.1016/j.bios.2022.114448.

[51] I.M.F. Tanimoto, B. Cressiot, S.J. Greive, B. Le Pioufle, L. Bacri, J. Pelta, Focus on using nanopore technology for societal health, environmental, and energy challenges, Nano Res. (2022) 1–15. https://doi.org/10.1007/s12274-022-4379-2.

[52] X. Tan, C. Lv, H. Chen, Advances of nanopore-based sensing techniques for contaminants evaluation of food and agricultural products, Crit Rev Food Sci Nutr. (2022) 1–14. https://doi.org/10.1080/10408398.2022.2085238.

[53] M. Cui, Y. Ge, X. Zhuge, X. Zhou, D. Xi, S. Zhang, Recent Advances in Nanopore Sensing†, Chin. J. Chem. 39 (2021) 2035–2043. https://doi.org/10.1002/cjoc.202000721.

[54] J.E. Valle-Inclan, C. Stangl, A.C. de Jong, L.F. van Dessel, M.J. van Roosmalen, J.C.A. Helmijr, I. Renkens, R. Janssen, S. de Blank, C.J. de Witte, J.W.M. Martens, M.P.H.M. Jansen, M.P. Lolkema, W.P. Kloosterman, Optimizing Nanopore sequencing-based detection of structural variants enables individualized circulating tumor DNA-based disease monitoring in cancer patients, Genome Med. 13 (2021) 86. https://doi.org/10.1186/s13073-021-00899-7.

[55] C.F. Minervini, C. Cumbo, P. Orsini, L. Anelli, A. Zagaria, G. Specchia, F. Albano, Nanopore Sequencing in Blood Diseases: A Wide Range of Opportunities, Front. Genet. 11 (2020) 76. https://doi.org/10.3389/fgene.2020.00076.

[56] L.L. Zhang, C. Zhang, J.P. Peng, Application of Nanopore Sequencing Technology in the Clinical Diagnosis of Infectious Diseases, Biomed Environ Sci. 35 (2022) 381–392. https://doi.org/10.3967/bes2022.054.

[57] D.J. Hunter, K.S. Reddy, Noncommunicable Diseases, N Engl J Med. 369 (2013) 1336–1343. https://doi.org/10.1056/NEJMra1109345.

[58] L. Sun, Y. Zhou, M. Zhang, C. Li, M. Qu, Q. Cai, J. Meng, H. Fan, Y. Zhao, D. Hu, Association of Major Chronic Noncommunicable Diseases and Life Expectancy in China, 2019, Healthcare (Basel). 10 (2022) 296. https://doi.org/10.3390/healthcare10020296.

[59] C.P. Wild, The global cancer burden: necessity is the mother of prevention, Nat Rev Cancer. 19 (2019) 123–124. https://doi.org/10.1038/s41568-019-0110-3.

[60] M.H. Ebell, T.N. Thai, K.J. Royalty, Cancer screening recommendations: an international comparison of high income countries, Public Health Rev. 39 (2018) 7. https://doi.org/10.1186/s40985-018-0080-0.

[61] A.T. Kal-Koshvandi, Recent advances in optical biosensors for the detection of cancer biomarker α-fetoprotein (AFP), TrAC Trends in Analytical Chemistry. 128 (2020) 115920. https://doi.org/10.1016/j.trac.2020.115920.

[62] A. Mohammadinejad, R. Kazemi Oskuee, R. Eivazzadeh-Keihan, M. Rezayi, B. Baradaran, A. Maleki, M. Hashemzaei, A. Mokhtarzadeh, M. de la Guardia, Development of biosensors for detection of alpha-fetoprotein: As a major biomarker for hepatocellular carcinoma, TrAC Trends in Analytical Chemistry. 130 (2020) 115961. https://doi.org/10.1016/j.trac.2020.115961.

[63] Y.J. Lee, S.-W. Lee, Regression of hepatocarcinoma cells using RNA aptamer specific to alpha-fetoprotein, Biochem Biophys Res Commun. 417 (2012) 521–527. https://doi.org/10.1016/j.bbrc.2011.11.153.

[64] Q. Li, G. Li, L. Fan, Y. Yu, J. Liu, Click reaction triggered turn-on fluorescence strategy for highly sensitive and selective determination of steroid hormones in food samples, Food Chem. 374 (2022) 131565. https://doi.org/10.1016/j.foodchem.2021.131565.

[65] T. Yamamoto, M. Akahori, K. Natsui, T. Saitoh, Y. Einaga, Controlled decoration of boron-doped diamond electrodes by electrochemical click reaction (e−CLICK), Carbon. 130 (2018) 350–354. https://doi.org/10.1016/j.carbon.2017.12.098.

[66] Z. Zhang, T. Li, Y. Sheng, L. Liu, H.-C. Wu, Enhanced Sensitivity in Nanopore Sensing of Cancer Biomarkers in Human Blood via Click Chemistry, Small. 15 (2019) e1804078. https://doi.org/10.1002/smll.201804078.

[67] M.M. Haugland, S. Borsley, D.F. Cairns-Gibson, A. Elmi, S.L. Cockroft, Synthetically Diversified Protein Nanopores: Resolving Click Reaction Mechanisms, ACS Nano. 13 (2019) 4101–4110. https://doi.org/10.1021/acsnano.8b08691.

[68] L. Liu, Z. Fang, X. Zheng, D. Xi, Nanopore-Based Strategy for Sensing of Copper(II) Ion and Real-Time Monitoring of a Click Reaction, ACS Sens. 4 (2019) 1323–1328. https://doi.org/10.1021/acssensors.9b00236.

[69] Y.-L. Ying, R.-J. Yu, Y.-X. Hu, R. Gao, Y.-T. Long, Single antibody-antigen interactions monitored via transient ionic current recording using nanopore sensors, Chem Commun (Camb). 53 (2017) 8620–8623. https://doi.org/10.1039/c7cc03927a.

[70] A. Partin, M. Brawer, E. Subong, C. Kelley, J. Cox, D. Bruzek, J. Pannek, G. Meyer, D. Chan, Prospective evaluation of percent free-PSA and complexed-PSA for early detection of prostate cancer, Prostate Cancer Prostatic Dis. 1 (1998) 197–203. https://doi.org/10.1038/sj.pcan.4500232.

[71] K. Chuah, Y. Wu, S.R.C. Vivekchand, K. Gaus, P.J. Reece, A.P. Micolich, J.J. Gooding, Nanopore blockade sensors for ultrasensitive detection of proteins in complex biological samples, Nat Commun. 10 (2019) 2109. https://doi.org/10.1038/s41467-019-10147-7.

[72] Y. Wu, Y. Yao, S. Cheong, R.D. Tilley, J.J. Gooding, Selectively detecting attomolar concentrations of proteins using gold lined nanopores in a nanopore blockade sensor, Chem Sci. 11 (2020) 12570–12579. https://doi.org/10.1039/d0sc04552g.

[73] A.K. Thakur, L. Movileanu, Real-time measurement of protein–protein interactions at single-molecule resolution using a biological nanopore, Nat Biotechnol. 37 (2019) 96–101. https://doi.org/10.1038/nbt.4316.

[74] X. Chen, W. Xie, P. Gu, Q. Cai, B. Wang, Y. Xie, W. Dong, W. He, G. Zhong, T. Lin, J. Huang, Upregulated WDR5 promotes proliferation, self-renewal and chemoresistance in bladder cancer via mediating H3K4 trimethylation, Sci Rep. 5 (2015) 8293. https://doi.org/10.1038/srep08293.

[75] L.A. Mayse, A. Imran, M.G. Larimi, M.S. Cosgrove, A.J. Wolfe, L. Movileanu, Disentangling the recognition complexity of a protein hub using a nanopore, Nat Commun. 13 (2022) 978.

https://doi.org/10.1038/s41467-022-28465-8.

[76] S. Wang, Z. Zhao, F. Haque, P. Guo, Engineering of protein nanopores for sequencing, chemical or protein sensing and disease diagnosis, Curr Opin Biotechnol. 51 (2018) 80–89. https://doi.org/10.1016/j.copbio.2017.11.006.

[77] S. Wang, F. Haque, P.G. Rychahou, B.M. Evers, P. Guo, Engineered nanopore of Phi29 DNA-packaging motor for real-time detection of single colon cancer specific antibody in serum, ACS Nano. 7 (2013) 9814–9822. https://doi.org/10.1021/nn404435v.

[78] Y. Wang, Y. Zhang, X. Chen, X. Guan, L. Wang, Analysis with biological nanopore: On-pore, off-pore strategies and application in biological fluids, Talanta. 223 (2021) 121684. https://doi.org/10.1016/j.talanta.2020.121684.

[79] X. Ding, Z. Wu, Z. Li, X. Xia, Electric Field Driven Surface Ion Transport in Hydrophobic Nanopores †, Chin. J. Chem. 39 (2021) 1511–1516. https://doi.org/10.1002/cjoc.202000730.

[80] M. Firnkes, D. Pedone, J. Knezevic, M. Döblinger, U. Rant, Electrically facilitated translocations of proteins through silicon nitride nanopores: conjoint and competitive action of diffusion, electrophoresis, and electroosmosis, Nano Lett. 10 (2010) 2162–2167. https://doi.org/10.1021/nl100861c.

[81] M. Boukhet, F. Piguet, H. Ouldali, M. Pastoriza-Gallego, J. Pelta, A. Oukhaled, Probing driving forces in aerolysin and α-hemolysin biological nanopores: electrophoresis versus electroosmosis, Nanoscale. 8 (2016) 18352–18359. https://doi.org/10.1039/c6nr06936c.

[82] A. Asandei, I. Schiopu, M. Chinappi, C.H. Seo, Y. Park, T. Luchian, Electroosmotic Trap Against the Electrophoretic Force Near a Protein Nanopore Reveals Peptide Dynamics During Capture and Translocation, ACS Appl Mater Interfaces. 8 (2016) 13166–13179. https://doi.org/10.1021/acsami.6b03697.

[83] C. Bossard, S. Bézieau, T. Matysiak-Budnik, C. Volteau, C.L. Laboisse, F. Jotereau, J.-F. Mosnier, HLA-E/β2 microglobulin overexpression in colorectal cancer is associated with recruitment of inhibitory immune cells and tumor progression, Int J Cancer. 131 (2012) 855–863. https://doi.org/10.1002/ijc.26453.

[84] S. Siena, A. Sartore-Bianchi, S. Marsoni, H.I. Hurwitz, S.J. McCall, F. Penault-Llorca, S. Srock, A. Bardelli, L. Trusolino, Targeting the human epidermal growth factor receptor 2 (HER2) oncogene in colorectal cancer, Ann Oncol. 29 (2018) 1108–1119. https://doi.org/10.1093/annonc/mdy100.

[85] H. Kuniyasu, Multiple roles of angiotensin in colorectal cancer, World J Clin Oncol. 3 (2012) 150–154. https://doi.org/10.5306/wjco.v3.i12.150.

[86] G. Huang, K. Willems, M. Soskine, C. Wloka, G. Maglia, Electro-osmotic capture and ionic discrimination of peptide and protein biomarkers with FraC nanopores, Nat Commun. 8 (2017) 935. https://doi.org/10.1038/s41467-017-01006-4.

[87] H. Kim, S. Park, I.G. Jeong, S.H. Song, Y. Jeong, C.-S. Kim, K.H. Lee, Noninvasive Precision Screening of Prostate Cancer by Urinary Multimarker Sensor and Artificial Intelligence Analysis, ACS Nano. 15 (2021) 4054–4065. https://doi.org/10.1021/acsnano.0c06946.

[88] E. González-Fernández, M. Staderini, N. Avlonitis, A.F. Murray, A.R. Mount, M. Bradley, Effect of spacer length on the performance of peptide-based electrochemical biosensors for protease detection, Sensors and Actuators B: Chemical. 255 (2018) 3040–3046. https://doi.org/10.1016/j.snb.2017.09.128.

[89] G. Murphy, The ADAMs: signalling scissors in the tumour microenvironment, Nat Rev Cancer. 8 (2008) 932–941. https://doi.org/10.1038/nrc2459.

[90] X. Chen, Y. Zhang, G.M. Roozbahani, X. Guan, Salt-Mediated Nanopore Detection of ADAM-17, ACS Appl Bio Mater. 2 (2019) 504–509. https://doi.org/10.1021/acsabm.8b00689.

[91] X. Chen, Y. Zhang, X. Guan, Simultaneous detection of multiple proteases using a non-array nanopore platform, Nanoscale. 13 (2021) 13658–13664. https://doi.org/10.1039/d1nr04085e.

[92] M. Paliouras, C. Borgono, E.P. Diamandis, Human tissue kallikreins: The cancer biomarker family, Cancer Letters. 249 (2007) 61–79. https://doi.org/10.1016/j.canlet.2006.12.018.

[93] Y. Feng, Q. Li, J. Chen, P. Yi, X. Xu, Y. Fan, B. Cui, Y. Yu, X. Li, Y. Du, Q. Chen, L. Zhang, J. Jiang, X. Zhou, P. Zhang, Salivary protease spectrum biomarkers of oral cancer, Int J Oral Sci. 11 (2019) 7. https://doi.org/10.1038/s41368-018-0032-z.

[94] S. Zhou, L. Wang, X. Chen, X. Guan, Label-free nanopore single-molecule measurement of trypsin activity, ACS Sens. 1 (2016) 607–613. https://doi.org/10.1021/acssensors.6b00043.

[95] M. Li, A. Rauf, Y. Guo, X. Kang, Real-Time Label-Free Kinetics Monitoring of Trypsin-Catalyzed Ester Hydrolysis by a Nanopore Sensor, ACS Sens. 4 (2019) 2854–2857. https://doi.org/10.1021/acssensors.9b01783.

[96] M. Kimoto, R. Yamashige, K. Matsunaga, S. Yokoyama, I. Hirao, Generation of high-affinity DNA aptamers using an expanded genetic alphabet, Nat Biotechnol. 31 (2013) 453–457. https://doi.org/10.1038/nbt.2556.

[97] E.B. Porter, J.T. Polaski, M.M. Morck, R.T. Batey, Recurrent RNA motifs as scaffolds for genetically encodable small-molecule biosensors, Nat Chem Biol. 13 (2017) 295–301. https://doi.org/10.1038/nchembio.2278.

[98] A. Minopoli, B. Della Ventura, B. Lenyk, F. Gentile, J.A. Tanner, A. Offenhäusser, D. Mayer, R. Velotta, Ultrasensitive antibody-aptamer plasmonic biosensor for malaria biomarker detection in whole blood, Nat Commun. 11 (2020) 6134. https://doi.org/10.1038/s41467-020-19755-0.

[99] S. Iqbal, S. Zhang, A. Driss, Z.-R. Liu, H.-R.C. Kim, Y. Wang, C. Ritenour, H.E. Zhau, O. Kucuk, L.W.K. Chung, D. Wu, PDGF upregulates Mcl-1 through activation of β-catenin and HIF-1α-dependent signaling in human prostate cancer cells, PLoS One. 7 (2012) e30764. https://doi.org/10.1371/journal.pone.0030764.

[100] A. Skarmoutsos, I. Skarmoutsos, I. Katafigiotis, E. Tataki, A. Giagini, C. Alamanis, I. Anastasiou, A. Angelou, M. Duvdevani, N. Sitaras, C. Constantinides, Detecting Novel Urine Biomarkers for the Early Diagnosis of Prostate Cancer: Platelet Derived Growth Factor-BB as a Possible New Target, Curr Urol. 12 (2018) 13–19. https://doi.org/10.1159/000447225.

[101] L. Zhang, K. Zhang, G. Liu, M. Liu, Y. Liu, J. Li, Label-free nanopore proximity bioassay for platelet-derived growth factor detection, Anal Chem. 87 (2015) 5677–5682. https://doi.org/10.1021/acs.analchem.5b00791.

[102] N. Razmi, B. Baradaran, M. Hejazi, M. Hasanzadeh, J. Mosafer, A. Mokhtarzadeh, M. de la Guardia, Recent advances on aptamer-based biosensors to detection of platelet-derived growth factor, Biosensors and Bioelectronics. 113 (2018) 58–71. https://doi.org/10.1016/j.bios.2018.04.048.

[103] Z. Fang, L. Liu, Y. Wang, D. Xi, S. Zhang, Unambiguous Discrimination of Multiple Protein Biomarkers by Nanopore Sensing with Double-Stranded DNA-Based Probes, Anal Chem. 92 (2020) 1730–1737. https://doi.org/10.1021/acs.analchem.9b02965.

[104] M. Yousefi, S. Dehghani, R. Nosrati, H. Zare, M. Evazalipour, J. Mosafer, B.S. Tehrani, A. Pasdar, A.

Mokhtarzadeh, M. Ramezani, Aptasensors as a new sensing technology developed for the detection of MUC1 mucin: A review, Biosensors and Bioelectronics. 130 (2019) 1–19. https://doi.org/10.1016/j.bios.2019.01.015.

[105] K. Sun, P. Chen, S. Yan, W. Yuan, Y. Wang, X. Li, L. Dou, C. Zhao, J. Zhang, Q. Wang, Z. Fu, L. Wei, Z. Xin, Z. Tang, Y. Yan, Y. Peng, B. Ying, J. Chen, J. Geng, Ultrasensitive Nanopore Sensing of Mucin 1 and Circulating Tumor Cells in Whole Blood of Breast Cancer Patients by Analyte-Triggered Triplex-DNA Release, ACS Appl Mater Interfaces. 13 (2021) 21030–21039. https://doi.org/10.1021/acsami.1c03538.

[106] H. Tang, H. Wang, C. Yang, D. Zhao, Y. Qian, Y. Li, Nanopore-based Strategy for Selective Detection of Single Carcinoembryonic Antigen (CEA) Molecules, Anal Chem. 92 (2020) 3042–3049. https://doi.org/10.1021/acs.analchem.9b04185.

[107] J. Wu, L. Liang, M. Zhang, R. Zhu, Z. Wang, Y. Yin, B. Yin, T. Weng, S. Fang, W. Xie, L. Wang, D. Wang, Single-Molecule Identification of the Conformations of Human C-Reactive Protein and Its Aptamer Complex with Solid-State Nanopores, ACS Appl Mater Interfaces. 14 (2022) 12077–12088. https://doi.org/10.1021/acsami.2c00453.

[108] X. Lin, A.P. Ivanov, J.B. Edel, Selective single molecule nanopore sensing of proteins using DNA aptamer-functionalised gold nanoparticles, Chem Sci. 8 (2017) 3905–3912. https://doi.org/10.1039/c7sc00415j.

[109] R. Ren, X. Wang, S. Cai, Y. Zhang, Y. Korchev, A.P. Ivanov, J.B. Edel, Selective Sensing of Proteins Using Aptamer Functionalized Nanopore Extended Field-Effect Transistors, Small Methods. 4 (2020) 2000356. https://doi.org/10.1002/smtd.202000356.

[110] J. Zhang, S. Xie, J. Xu, H. Liu, S. Wan, Cancer Biomarkers Discovery of Methylation Modification With Direct High-Throughput Nanopore Sequencing, Front Genet. 12 (2021) 672804. https://doi.org/10.3389/fgene.2021.672804.

[111] X. Zhang, Y. Wang, B.L. Fricke, L.-Q. Gu, Programming nanopore ion flow for encoded multiplex microRNA detection, ACS Nano. 8 (2014) 3444–3450. https://doi.org/10.1021/nn406339n.

[112] K. Tian, Z. He, Y. Wang, S.-J. Chen, L.-Q. Gu, Designing a polycationic probe for simultaneous enrichment and detection of microRNAs in a nanopore, ACS Nano. 7 (2013) 3962–3969. https://doi.org/10.1021/nn305789z.

[113] D. Xi, J. Shang, E. Fan, J. You, S. Zhang, H. Wang, Nanopore-Based Selective Discrimination of MicroRNAs with Single-Nucleotide Difference Using Locked Nucleic Acid-Modified Probes, Anal Chem. 88 (2016) 10540–10546. https://doi.org/10.1021/acs.analchem.6b02620.

[114] L. Mereuta, A. Asandei, I. Dragomir, J. Park, Y. Park, T. Luchian, A Nanopore Sensor for Multiplexed Detection of Short Polynucleotides Based on Length-Variable, Poly-Arginine-Conjugated Peptide Nucleic Acids, Anal Chem. 94 (2022) 8774–8782. https://doi.org/10.1021/acs.analchem.2c01587.

[115] J.-H. Zhang, X.-L. Liu, Z.-L. Hu, Y.-L. Ying, Y.-T. Long, Intelligent identification of multi-level nanopore signatures for accurate detection of cancer biomarkers, Chem Commun (Camb). 53 (2017) 10176–10179. https://doi.org/10.1039/c7cc04745b.

[116] E. Samami, G. Pourali, M. Arabpour, A. Fanipakdel, S. Shahidsales, S.A. Javadinia, S.M. Hassanian, S. Mohammadparast, A. Avan, The Potential Diagnostic and Prognostic Value of Circulating MicroRNAs in the Assessment of Patients With Prostate Cancer: Rational and Progress, Front Oncol. 11 (2021) 716831. https://doi.org/10.3389/fonc.2021.716831.

[117] S. Cai, T. Pataillot-Meakin, A. Shibakawa, R. Ren, C.L. Bevan, S. Ladame, A.P. Ivanov, J.B. Edel, Single-

molecule amplification-free multiplexed detection of circulating microRNA cancer biomarkers from serum, Nat Commun. 12 (2021) 3515. https://doi.org/10.1038/s41467-021-23497-y.

[118] D.L. Price, New order from neurological disorders, Nature. 399 (1999) A3–A5. https://doi.org/10.1038/399a003.

[119] D.C. Bergen, D. Silberberg, Nervous System Disorders: A Global Epidemic, Arch Neurol. 59 (2002) 1194. https://doi.org/10.1001/archneur.59.7.1194.

[120] R. Peters, N. Ee, J. Peters, N. Beckett, A. Booth, K. Rockwood, K.J. Anstey, Common risk factors for major noncommunicable disease, a systematic overview of reviews and commentary: the implied potential for targeted risk reduction, Therapeutic Advances in Chronic Disease. 10 (2019) 204062231988039. https://doi.org/10.1177/2040622319880392.

[121] D.L. Price, R.E. Tanzi, D.R. Borchelt, S.S. Sisodia, Alzheimer's disease: genetic studies and transgenic models, Annu Rev Genet. 32 (1998) 461–493. https://doi.org/10.1146/annurev.genet.32.1.461.

[122] R.A. Huynh, C. Mohan, Alzheimer's Disease: Biomarkers in the Genome, Blood, and Cerebrospinal Fluid, Front. Neurol. 8 (2017). https://doi.org/10.3389/fneur.2017.00102.

[123] B. Cressiot, H. Ouldali, M. Pastoriza-Gallego, L. Bacri, F.G. Van der Goot, J. Pelta, Aerolysin, a Powerful Protein Sensor for Fundamental Studies and Development of Upcoming Applications, ACS Sens. 4 (2019) 530–548. https://doi.org/10.1021/acssensors.8b01636.

[124] M.-Y. Li, Y.-L. Ying, S. Li, Y.-Q. Wang, X.-Y. Wu, Y.-T. Long, Unveiling the Heterogenous Dephosphorylation of DNA Using an Aerolysin Nanopore, ACS Nano. 14 (2020) 12571–12578. https://doi.org/10.1021/acsnano.0c03215.

[125] H. Ouldali, K. Sarthak, T. Ensslen, F. Piguet, P. Manivet, J. Pelta, J.C. Behrends, A. Aksimentiev, A. Oukhaled, Electrical recognition of the twenty proteinogenic amino acids using an aerolysin nanopore, Nat Biotechnol. 38 (2020) 176–181. https://doi.org/10.1038/s41587-019-0345-2.

[126] Z. Zou, H. Yang, Q. Yan, P. Qi, Z. Qing, J. Zheng, X. Xu, L. Zhang, F. Feng, R. Yang, Synchronous screening of multiplexed biomarkers of Alzheimer's disease by a length-encoded aerolysin nanopore-integrated triple-helix molecular switch, Chem Commun (Camb). 55 (2019) 6433–6436. https://doi.org/10.1039/c9cc02065a.

[127] M. Huo, Z. Hu, Y. Ying, Y. Long, Enhanced identification of Tau acetylation and phosphorylation with an engineered aerolysin nanopore, Proteomics. 22 (2022) 2100041. https://doi.org/10.1002/pmic.202100041.

[128] L. Restrepo-Pérez, C.H. Wong, G. Maglia, C. Dekker, C. Joo, Label-Free Detection of Post-translational Modifications with a Nanopore, Nano Lett. 19 (2019) 7957–7964. https://doi.org/10.1021/acs.nanolett.9b03134.

[129] C. Wloka, V. Van Meervelt, D. van Gelder, N. Danda, N. Jager, C.P. Williams, G. Maglia, Label-Free and Real-Time Detection of Protein Ubiquitination with a Biological Nanopore, ACS Nano. 11 (2017) 4387–4394. https://doi.org/10.1021/acsnano.6b07760.

[130] T.A. Enache, A.M. Oliveira-Brett, Alzheimer's disease amyloid beta peptides in vitro electrochemical oxidation, Bioelectrochemistry. 114 (2017) 13–23. https://doi.org/10.1016/j.bioelechem.2016.11.003.

[131] H. Hampel, J. Hardy, K. Blennow, C. Chen, G. Perry, S.H. Kim, V.L. Villemagne, P. Aisen, M. Vendruscolo, T. Iwatsubo, C.L. Masters, M. Cho, L. Lannfelt, J.L. Cummings, A. Vergallo, The Amyloid-β Pathway in Alzheimer's Disease, Mol Psychiatry. 26 (2021) 5481–5503. https://doi.org/10.1038/s41380-021-01249-

0.

[132] N. Meyer, N. Arroyo, J.-M. Janot, M. Lepoitevin, A. Stevenson, I.A. Nemeir, V. Perrier, D. Bougard, M. Belondrade, D. Cot, J. Bentin, F. Picaud, J. Torrent, S. Balme, Detection of Amyloid-β Fibrils Using Track-Etched Nanopores: Effect of Geometry and Crowding, ACS Sens. 6 (2021) 3733–3743. https://doi.org/10.1021/acssensors.1c01523.

[133] M. Amouzadeh Tabrizi, J. Ferré-Borrull, L.F. Marsal, Highly sensitive aptasensor based on interferometric reflectance spectroscopy for the determination of amyloid β as an Alzheimer's disease biomarkers using nanoporous anodic alumina, Biosens Bioelectron. 137 (2019) 279–286. https://doi.org/10.1016/j.bios.2019.04.050.

[134] C.W. Olanow, W.G. Tatton, Etiology and pathogenesis of Parkinson's disease, Annu Rev Neurosci. 22 (1999) 123–144. https://doi.org/10.1146/annurev.neuro.22.1.123.

[135] E. Maries, B. Dass, T.J. Collier, J.H. Kordower, K. Steece-Collier, The role of α-synuclein in Parkinson's disease: insights from animal models, Nat Rev Neurosci. 4 (2003) 727–738. https://doi.org/10.1038/nrn1199.

[136] N. Meyer, J.-M. Janot, J. Torrent, S. Balme, Real-Time Fast Amyloid Seeding and Translocation of α-Synuclein with a Nanopipette, ACS Cent. Sci. (2022) acscentsci.1c01404. https://doi.org/10.1021/acscentsci.1c01404.

[137] J.Y.Y. Sze, A.P. Ivanov, A.E.G. Cass, J.B. Edel, Single molecule multiplexed nanopore protein screening in human serum using aptamer modified DNA carriers, Nat Commun. 8 (2017) 1552. https://doi.org/10.1038/s41467-017-01584-3.

[138] V. Iacobazzi, A. Castegna, V. Infantino, G. Andria, Mitochondrial DNA methylation as a next-generation biomarker and diagnostic tool, Mol Genet Metab. 110 (2013) 25–34. https://doi.org/10.1016/j.ymgme.2013.07.012.

[139] T. Lüth, K. Wasner, C. Klein, S. Schaake, R. Tse, S.L. Pereira, J. Laß, L. Sinkkonen, A. Grünewald, J. Trinh, Nanopore Single-Molecule Sequencing for Mitochondrial DNA Methylation Analysis: Investigating Parkin-Associated Parkinsonism as a Proof of Concept, Front. Aging Neurosci. 13 (2021) 713084. https://doi.org/10.3389/fnagi.2021.713084.

[140] F. Maqbool, S. Mostafalou, H. Bahadar, M. Abdollahi, Review of endocrine disorders associated with environmental toxicants and possible involved mechanisms, Life Sciences. 145 (2016) 265–273. https://doi.org/10.1016/j.lfs.2015.10.022.

[141] S. Hiller-Sturmhöfel, A. Bartke, The endocrine system: an overview, Alcohol Health Res World. 22 (1998) 153–164.

[142] L. Chiovato, F. Magri, A. Carlé, Hypothyroidism in Context: Where We've Been and Where We're Going, Adv Ther. 36 (2019) 47–58. https://doi.org/10.1007/s12325-019-01080-8.

[143] D.S. Ross, Hyperthyroidism, Thyroid Hormone Therapy, and Bone, Thyroid. 4 (1994) 319–326. https://doi.org/10.1089/thy.1994.4.319.

[144] L. He, D.R. Tessier, K. Briggs, M. Tsangaris, M. Charron, E.M. McConnell, D. Lomovtsev, V. Tabard-Cossa, Digital immunoassay for biomarker concentration quantification using solid-state nanopores, Nat Commun. 12 (2021) 5348. https://doi.org/10.1038/s41467-021-25566-8.

[145] D. Wu, T. Wu, Q. Liu, Z. Yang, The SARS-CoV-2 outbreak: What we know, International Journal of Infectious Diseases. 94 (2020) 44–48. https://doi.org/10.1016/j.ijid.2020.03.004.

[146] M.M. Lamers, B.L. Haagmans, SARS-CoV-2 pathogenesis, Nat Rev Microbiol. 20 (2022) 270–284. https://doi.org/10.1038/s41579-022-00713-0.

[147] D. Kaya, A. Dinler, N. San, K. Kececi, Effect of Pore Geometry on Resistive-Pulse Sensing of DNA Using Track-Etched PET Nanopore Membrane, Electrochimica Acta. 202 (2016) 157–165. https://doi.org/10.1016/j.electacta.2016.04.014.

[148] M. Ali, I. Ahmed, P. Ramirez, S. Nasir, S. Mafe, C.M. Niemeyer, W. Ensinger, Lithium Ion Recognition with Nanofluidic Diodes through Host-Guest Complexation in Confined Geometries, Anal Chem. 90 (2018) 6820–6826. https://doi.org/10.1021/acs.analchem.8b00902.

[149] M. Bernhard, M. Diefenbach, M. Biesalski, B. Laube, Electrical Sensing of Phosphonates by Functional Coupling of Phosphonate Binding Protein PhnD to Solid-State Nanopores, ACS Sens. 5 (2020) 234–241. https://doi.org/10.1021/acssensors.9b02097.

[150] Y. Zhang, X. Chen, C. Wang, H.-C. Chang, X. Guan, Nanoparticle-assisted detection of nucleic acids in a polymeric nanopore with a large pore size, Biosens Bioelectron. 196 (2022) 113697. https://doi.org/10.1016/j.bios.2021.113697.

[151] R. Nouri, Y. Jiang, Z. Tang, X.L. Lian, W. Guan, Detection of SARS-CoV-2 with Solid-State CRISPR-Cas12a-Assisted Nanopores, Nano Lett. 21 (2021) 8393–8400. https://doi.org/10.1021/acs.nanolett.1c02974.

[152] Z. Tang, R. Nouri, M. Dong, J. Yang, W. Greene, Y. Zhu, M. Yon, M.S. Nair, S.V. Kuchipudi, W. Guan, Rapid detection of novel coronavirus SARS-CoV-2 by RT-LAMP coupled solid-state nanopores, Biosens Bioelectron. 197 (2022) 113759. https://doi.org/10.1016/j.bios.2021.113759.

[153] M. Taniguchi, S. Minami, C. Ono, R. Hamajima, A. Morimura, S. Hamaguchi, Y. Akeda, Y. Kanai, T. Kobayashi, W. Kamitani, Y. Terada, K. Suzuki, N. Hatori, Y. Yamagishi, N. Washizu, H. Takei, O. Sakamoto, N. Naono, K. Tatematsu, T. Washio, Y. Matsuura, K. Tomono, Combining machine learning and nanopore construction creates an artificial intelligence nanopore for coronavirus detection, Nat Commun. 12 (2021) 3726. https://doi.org/10.1038/s41467-021-24001-2.

[154] A.S. Peinetti, R.J. Lake, W. Cong, L. Cooper, Y. Wu, Y. Ma, G.T. Pawel, M.E. Toimil-Molares, C. Trautmann, L. Rong, B. Mariñas, O. Azzaroni, Y. Lu, Direct detection of human adenovirus or SARS-CoV-2 with ability to inform infectivity using DNA aptamer-nanopore sensors, Sci Adv. 7 (2021) eabh2848. https://doi.org/10.1126/sciadv.abh2848.

[155] Q. Liu, Y. Wang, Y. Liu, H. Wang, W. Li, P. Tang, T. Weng, S. Zhou, L. Liang, J. Yuan, D. Wang, L. Wang, Reduction chemistry-assisted nanopore determination method for immunoglobulin isotypes, Nanoscale. 12 (2020) 19711–19718. https://doi.org/10.1039/d0nr04900j.

[156] Z. Zhang, X. Wang, X. Wei, S.W. Zheng, B.J. Lenhart, P. Xu, J. Li, J. Pan, H. Albrecht, C. Liu, Multiplex quantitative detection of SARS-CoV-2 specific IgG and IgM antibodies based on DNA-assisted nanopore sensing, Biosens Bioelectron. 181 (2021) 113134. https://doi.org/10.1016/j.bios.2021.113134.

[157] A. Asandei, L. Mereuta, I. Schiopu, J. Park, C.H. Seo, Y. Park, T. Luchian, Non-Receptor-Mediated Lipid Membrane Permeabilization by the SARS-CoV-2 Spike Protein S1 Subunit, ACS Appl Mater Interfaces. 12 (2020) 55649–55658. https://doi.org/10.1021/acsami.0c17044.

[158] R.A. Medina, A. García-Sastre, Influenza A viruses: new research developments, Nat Rev Microbiol. 9 (2011) 590–603. https://doi.org/10.1038/nrmicro2613.

[159] C. Albrecht, A.S. Kuznetsov, A. Appert-Collin, Z. Dhaideh, M. Callewaert, Y.V. Bershatsky, A.S. Urban, E.V. Bocharov, D. Bagnard, S. Baud, S. Blaise, B. Romier-Crouzet, R.G. Efremov, M. Dauchez, L. Duca, M.

Gueroult, P. Maurice, A. Bennasroune, Transmembrane Peptides as a New Strategy to Inhibit Neuraminidase-1 Activation, Front Cell Dev Biol. 8 (2020) 611121. https://doi.org/10.3389/fcell.2020.611121.

[160] F. Li, M.A. Fahie, K.M. Gilliam, R. Pham, M. Chen, Mapping the conformational energy landscape of Abl kinase using ClyA nanopore tweezers, Nat Commun. 13 (2022) 3541. https://doi.org/10.1038/s41467-022-31215-5.

[161] K. Willems, D. Ruić, A. Biesemans, N.S. Galenkamp, P. Van Dorpe, G. Maglia, Engineering and Modeling the Electrophoretic Trapping of a Single Protein Inside a Nanopore, ACS Nano. 13 (2019) 9980–9992. https://doi.org/10.1021/acsnano.8b09137.

[162] N.S. Galenkamp, A. Biesemans, G. Maglia, Directional conformer exchange in dihydrofolate reductase revealed by single-molecule nanopore recordings, Nat. Chem. 12 (2020) 481–488. https://doi.org/10.1038/s41557-020-0437-0.

[163] D.-K. Kwak, J.-S. Kim, M.-K. Lee, K.-S. Ryu, S.-W. Chi, Probing the Neuraminidase Activity of Influenza Virus Using a Cytolysin A Protein Nanopore, Anal Chem. 92 (2020) 14303–14308. https://doi.org/10.1021/acs.analchem.0c03399.

[164] S. Oh, M.-K. Lee, S.-W. Chi, Single-Molecule-Based Detection of Conserved Influenza A Virus RNA Promoter Using a Protein Nanopore, ACS Sens. 4 (2019) 2849–2853. https://doi.org/10.1021/acssensors.9b01558.

[165] L. Wang, Y. Han, S. Zhou, G. Wang, X. Guan, Nanopore biosensor for label-free and real-time detection of anthrax lethal factor, ACS Appl Mater Interfaces. 6 (2014) 7334–7339. https://doi.org/10.1021/am500749p.

[166] S. King, K. Briggs, R. Slinger, V. Tabard-Cossa, Screening for Group A Streptococcal Disease via Solid-State Nanopore Detection of PCR Amplicons, ACS Sens. 7 (2022) 207–214. https://doi.org/10.1021/acssensors.1c01972.

[167] S.G. Deeks, J. Overbaugh, A. Phillips, S. Buchbinder, HIV infection, Nat Rev Dis Primers. 1 (2015) 15035. https://doi.org/10.1038/nrdp.2015.35.

[168] J.M. Louis, R. Ishima, D.A. Torchia, I.T. Weber, HIV-1 Protease: Structure, Dynamics, and Inhibition, in: Advances in Pharmacology, Elsevier, 2007: pp. 261–298. https://doi.org/10.1016/S1054-3589(07)55008-8.

[169] L. Wang, Y. Han, S. Zhou, X. Guan, Real-time label-free measurement of HIV-1 protease activity by nanopore analysis, Biosens Bioelectron. 62 (2014) 158–162. https://doi.org/10.1016/j.bios.2014.06.041.

[170] D.J. Niedzwiecki, R. Iyer, P.N. Borer, L. Movileanu, Sampling a biomarker of the human immunodeficiency virus across a synthetic nanopore, ACS Nano. 7 (2013) 3341–3350. https://doi.org/10.1021/nn400125c.

[171] Y. Zhang, X. Chen, C. Wang, G.M. Roozbahani, H.-C. Chang, X. Guan, Chemically functionalized conical PET nanopore for protein detection at the single-molecule level, Biosens Bioelectron. 165 (2020) 112289. https://doi.org/10.1016/j.bios.2020.112289.

[172] K. Sethi, G.P. Dailey, O.K. Zahid, E.W. Taylor, J.A. Ruzicka, A.R. Hall, Direct Detection of Conserved Viral Sequences and Other Nucleic Acid Motifs with Solid-State Nanopores, ACS Nano. 15 (2021) 8474–8483. https://doi.org/10.1021/acsnano.0c10887.

[173] X. Yue, Z. Xie, M. Li, K. Wang, X. Li, X. Zhang, J. Yan, Y. Yin, Simultaneous profiling of histone modifications

and DNA methylation via nanopore sequencing, Nat Commun. 13 (2022) 7939. https://doi.org/10.1038/s41467-022-35650-2.

[174] J.T. Simpson, R.E. Workman, P.C. Zuzarte, M. David, L.J. Dursi, W. Timp, Detecting DNA cytosine methylation using nanopore sequencing, Nat Methods. 14 (2017) 407–410. https://doi.org/10.1038/nmeth.4184.

[175] Y. Liu, S. Zhang, Y. Wang, L. Wang, Z. Cao, W. Sun, P. Fan, P. Zhang, H.-Y. Chen, S. Huang, Nanopore Identification of Alditol Epimers and Their Application in Rapid Analysis of Alditol-Containing Drinks and Healthcare Products, J Am Chem Soc. 144 (2022) 13717–13728. https://doi.org/10.1021/jacs.2c04595.

[176] S. Schmid, C. Dekker, The NEOtrap - en route with a new single-molecule technique, IScience. 24 (2021) 103007. https://doi.org/10.1016/j.isci.2021.103007.

[177] Q. Li, Y.-L. Ying, S.-C. Liu, Y. Lin, Y.-T. Long, Detection of Single Proteins with a General Nanopore Sensor, ACS Sens. 4 (2019) 1185–1189. https://doi.org/10.1021/acssensors.9b00228.

[178] A.K. Thakur, L. Movileanu, Single-Molecule Protein Detection in a Biofluid Using a Quantitative Nanopore Sensor, ACS Sens. 4 (2019) 2320–2326. https://doi.org/10.1021/acssensors.9b00848.

[179] X. Shi, Q. Li, R. Gao, W. Si, S.-C. Liu, A. Aksimentiev, Y.-T. Long, Dynamics of a Molecular Plug Docked onto a Solid-State Nanopore, J Phys Chem Lett. 9 (2018) 4686–4694. https://doi.org/10.1021/acs.jpclett.8b01755.

Table 1. Summarized list of diseases and their corresponding biomarkers reviewed in this review.

	Diseases	Biomarkers	Nanopore Types	Detection limit
Noncommunicable Diseases	Cancers	AFP	αHL	1 fM[66] and 10 fg/mL[103]
		PSA	SiNx	0.8 fM[71] and 80 aM[72]
		WDR5	t-FhuA	N/A[75]
		β2-microglobulin	FraC/ReFraC	N/A[86]
		Human EGF	FraC/ReFraC	N/A[86]
		Angiotensin I	FraC/ReFraC	N/A[86]
		ADAM-17	αHL	0.15 ng/mL[90] and 1.5 ng/mL[91]
		ADAM-10, ADAM-17	αHL	23.0 ng/mL[91]
		PDGF	αHL	500 fM [101] and 35.1 fM[103]
		VEGF	αHL	40.6 fM[103]
		TB	αHL	3.11 fM[103]
		CEA	Quartz	0.6 ng/mL[106]
		Human C-reactive protein	SiNx	0.3 ng/μL[107]
		TB	Quartz	N/A[137]
		AChE	Quartz	N/A[137]
	Breast cancer	MUC1	M2MspA	~0.1 fg/mL[105]
	Prostate cancer	miR-375-3p	SiNx	8 fM[117]
		miR-141-3p	SiNx	5 fM[117]
	Lung cancer	miR-155	αHL	N/A[33]
	Alzheimer's disease	alpha-1 antitrypsin	Aerolysin	77.9 fM[126]
		Tau 381	Aerolysin	6.79 fM[126]
		BACE1	Aerolysin	86.4 fM[126]
		Post-translocation modifications	Aerolysin T232k	N/A[127]
		Amyloid β	Anodic alumina	0.02 μg/mL[133]

Communicable Diseases	Parkinson's disease	α-synuclein	Quartz	N/A[136]
	Hypothyroidism	TSH	SiNx	20 pM[144]
	SARS-CoV-2	mRNA	PET	0.5 nM[150]
			SiNx	13.5 copies/μL[151]
		Coronavirus	SiNx	2.5 pfu/μL[153]
			PET	10 copies/μL[154]
		IgG and IgM	αHL	10 ng/mL and 50 ng/mL[156]
	Influenza A viruses	Neuraminidase	ClyA	38 nM[163]
		RNA promoter	αHL	2 nM[164]
	Human immunodeficiency virus	HIV-1 protease	αHL	0.47 ng/mL[169]
			PET	2.27 ng/mL[171]
		HIV-1B RNA	SiNx	1 nM[172]

Graphical abstract