

Electrochemical Detection Approaches Targeting Single Intact Virions

Róbert E. Gyurcsányi^{§,*}

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Abstract: Beyond emerging diagnostic applications, intact-virus detection is essential for understanding viral pathogenesis, host–pathogen interactions, and vaccine development. This review highlights in a timeline-based perspective the progress in electrochemical detection of intact virions, focusing on single-virion sensitivity. By directly probing intact viral particles rather than molecular surrogates, nanopore-based resistive-pulse sensing and nano-impact electrochemistry have emerged as versatile virus-counting strategies, enabling selective identification, quantitation, and characterization of morphological and chemical heterogeneity without ensemble averaging. Their potential for calibration-free quantitation, integration with microfluidics, *in situ* preconcentration, advanced signal processing, and artificial intelligence positions electrochemical platforms as powerful tools for virus analysis.

Keywords: Intact virus detection · Nano-impact electrochemistry · Resistive pulse sensing · Single virion counting



Róbert Gyurcsányi is the Head of the Department of Inorganic and Analytical Chemistry at Budapest University of Technology and Economics (BME), where he leads the Chemical Nanosensors Research Group. He received his PhD in Chemistry at BME, with further training at Duke University. Following postdoctoral appointments in Biomedical Engineering at the University of Memphis and in analytical

chemistry at ETH Zürich, he returned to BME in 2002. His research focuses on chemical sensing, with particular emphasis on selective synthetic ligands, solid-state nanopore sensing, chemical imaging, ion sensing, and single-species detection. Since 2018, he has served as co-Editor-in-Chief of *Sensors and Actuators B: Chemical*.

1. Introduction

Virus detection is essential across diverse fields, including fundamental virology, clinical diagnostics, agriculture, veterinary medicine, environmental surveillance, vaccine development, biosafety, and biodefence. Analytical requirements differ substantially among these applications in terms of sample complexity, sensitivity, selectivity, multiplexing capability, portability, and speed. Detection strategies can broadly be classified as indirect or direct. Indirect approaches rely on the host immune response by detecting virus-specific antibodies but may be limited by delayed seroconversion and reduced applicability in immunocompromised or hemodialyzed patients.^[1] Consequently, contemporary diagnostics for early-stage infection predominantly employ direct methods targeting viral nucleic acids or protein antigens. Nucleic acid-based assays, particularly quantitative polymerase chain reaction (qPCR), remain the benchmark owing to their exceptional analytical sensitivity, while antigen tests offer advantages

in analysis speed and deployability. However, both approaches interrogate molecular surrogates rather than intact viral particles. Viral genomes and proteins may persist^[2] after viral inactivation, degradation, or loss of structural integrity, such that their abundance does not necessarily correlate with the concentration of infectious virions.^[3] Therefore, methods capable of detecting intact virus particles are of particular interest, as they directly probe the infectious unit and may provide insight into infectivity, transmissibility, and biological activity. Beyond diagnostics, intact-virus detection is highly relevant for elucidating viral pathogenesis, evaluating host–pathogen interactions, and supporting vaccine development. Among these approaches, virus-counting methods are especially informative because they inherently achieve single-virion sensitivity while providing structural, morphological, and phenotypic information on individual particles, including size distributions and particle heterogeneity without ensemble averaging. This review takes a timeline-based perspective on the development of electrochemical approaches for intact-virus detection, tracing their progression over time and highlighting the key concepts that have enabled the field to advance, with particular emphasis on methods capable of single-virion sensitivity.

2. Resistive Pulse Sensing of Viruses

2.1 A Historical Overview

The first report describing resistive pulse sensing (RPS) of viruses was published by DeBlois and Wesley in the 1970s.^[4] This work was fundamentally based on the Coulter counter principle, originally patented by Wallace H. Coulter in 1953,^[5] which was developed for counting and sizing micrometre-sized particles suspended in electrolyte solutions. In its simplest configuration, a pore or aperture made in a membrane separates two electrolyte-filled compartments, each interfaced with an electrode to apply a transmembrane voltage while monitoring the resulting ionic

*Correspondence: Dr. R. E. Gyurcsányi, E-mail: gyurcsanyi.robert@vbk.bme.hu

Department of Inorganic and Analytical Chemistry, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Műegyetem rkp. 3, Budapest, H-1111

current (Fig. 1A). During RPS, individual particles are detected electrically as they translocate through the electrolyte-filled pore. The passage of a particle transiently increases the pore resistance by displacing an equivalent volume of highly conductive electrolyte, producing a characteristic resistive pulse, *i.e.* a transient current decrease (Fig. 1B). In classical Coulter counters, pore diameters are typically in the micrometre range, while detectable particle diameters span approximately 2–60% of the pore diameter. Because viruses predominantly fall within the lower nanometre size regime (typically 20–300 nm), achieving sufficient pulse amplitude and signal-to-noise ratio generally requires nanopores with dimensions comparable to those of the target virions. Consequently, virus analysis by RPS largely relies on single nanopores. The current–time signal obtained in RPS is primarily characterized by three parameters (Fig. 1B): (i) pulse frequency indicative of the concentration of translocating viruses; (ii) pulse amplitude, which is governed predominantly by particle size and shape; and (iii) pulse width, which reflects translocation velocity and particle–pore interactions. Beyond size, RPS enables access to additional physicochemical properties, including particle

deformability under sub-size confinement^[6] and surface charge derived from electrophoretic mobility during translocation.^[7–9]

At the time of the pioneering work by DeBlois and Wesley, single nanopore technology was far from its current level of sophistication. Nevertheless, a compelling proof of concept was achieved using remarkably simple experimental means.^[10] A single pore was manually isolated by blocking all but one pore within an otherwise highly porous track-etched membrane, a platform originally introduced by Fleischer and coworkers for the filtration of biological materials.^[11] Using an approximately 400 nm diameter cylindrical pore, Type C oncornaviruses and bacteriophage T2 particles in the ~100–150 nm size range were successfully counted and sized, thereby establishing the feasibility of extending the Coulter principle from microscale particles to nanoscale single-virus analysis.^[4] It also represented a major advance by enabling rapid quantitative analysis and determination of virus size distributions in their native aqueous environment, beyond the traditional confines of electron microscopy.

Remarkably, nearly three decades elapsed after DeBlois's pioneering feasibility study before resistive pulse sensing of viruses began to attract broader attention. The renewed efforts initially relied on relatively large single nanopores (~650 nm diameter) for the detection of comparatively large viruses approaching 200 nm in equivalent diameter, exemplified by *Paramecium bursaria* Chlorella virus 1 (PBCV-1).^[12] These larger pores could be conveniently fabricated in thin borosilicate cover slides using a femtosecond pulsed laser (Fig. 2). Very importantly, in addition to counting and sizing the virions, Mayer's group has shown that resistive pulse sensing can be adapted to monitor the formation of virus–antibody complexes in real time. It further provided the number of antibodies bound to a virus in its native conformation in solution (*ca.* 4,200), without the need for immobilization, modifications, or labelling, allowing prediction of the antibody's suitability for immunoprecipitation. This study also marked the transition from cylindrical pores, characteristic of Coulter counters, to conical nanopores, which are, in fact, truncated-cone-shaped nanopores.

The significant decrease in the tip diameters to *ca.* 40 nm, as shown by Jacobson and coworkers,^[13] by using conical polyethylene terephthalate nanopores, could already resolve hepatitis B virus (HBV) capsids (3 MDa T = 3 and 4 MDa T = 4 HBV capsids), *i.e.* 31 and 36 nm, respectively. However, reducing pore dimensions to a few tens of nanometres is not merely a straightforward geometric downscaling, but may have major

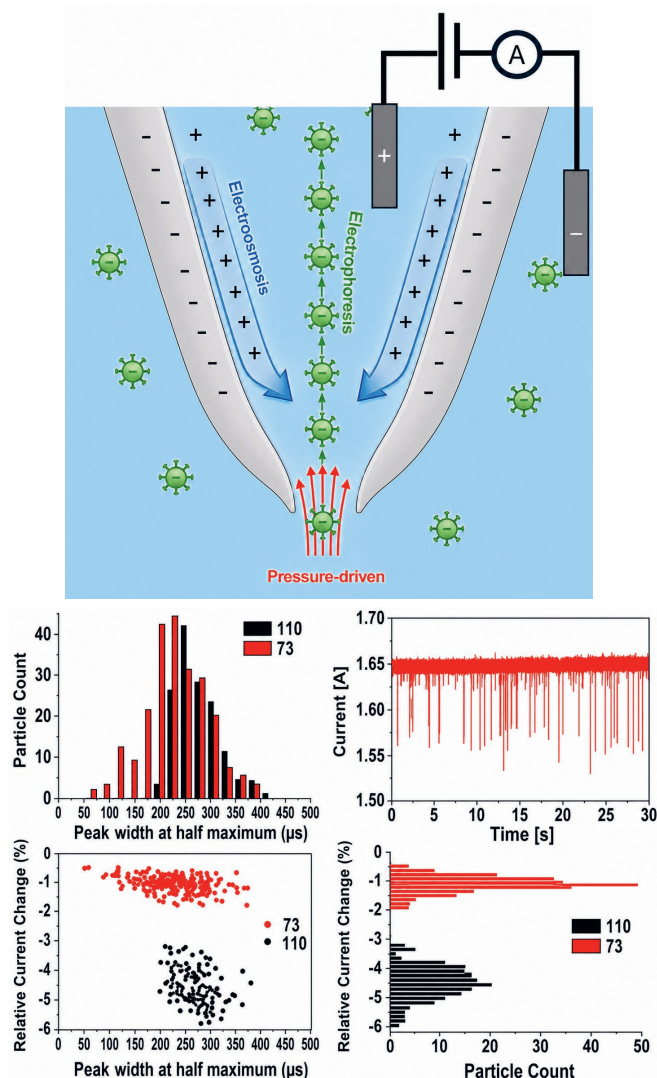


Fig. 1. Schematic illustration of a conical nanopore showing the principal directed transport mechanisms: pressure-driven flow, electrophoretic transport of charged viruses or particles, shown here as negatively charged, and electroosmotic flow (A). Representative resistive-pulse sensing current transients obtained for two sizes of nanoparticles (73 and 110 nm) during their pressure-driven translocation through the nanopore, together with the corresponding pulse-width and pulse-amplitude histograms and the resulting amplitude–width scatter plot (B). (Adapted with permission from Ref. [23]. Copyright 2014 American Chemical Society.).

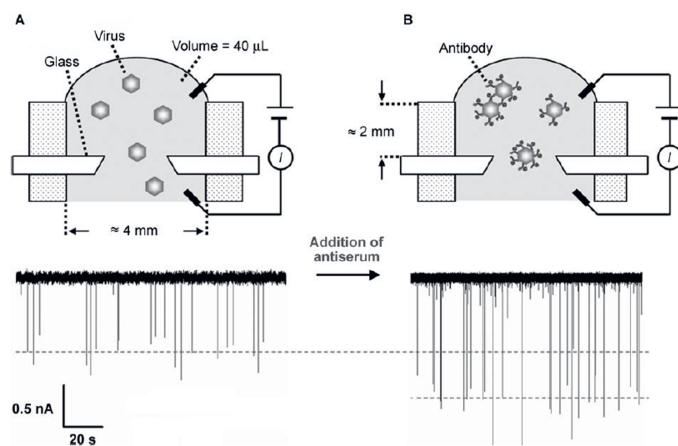


Fig. 2. Schematics of the experimental setting of nanopore-based RPS of antibody binding to PBCV-1 viruses and the relevant current signals before (A) and *ca.* 10–15 min after addition of diluted antiserum (B). The increased pulse after antibody binding reflects the increased volume of the virus particles (Reproduced with permission from Ref. [12]. Copyright 2006 Wiley-VCH GmbH).

consequences on mass transport and target-pore interaction, *e.g.* the inherently negatively charged pore surface had to be covalently modified with triethylene glycol to suppress electroosmotic flow by reducing the surface charge density and also to minimize undesired adsorption of the virus capsids. It was subsequently demonstrated for the same HBV capsids that the signal-to-noise ratio and the number of detectable translocation events could be significantly improved by employing a two-pore configuration in an electron-beam lithography–fabricated nanochannel device, in which the two nanopores were positioned in series at a 2 μm separation (Fig. 3 (4)).^[14] In this arrangement, a single capsid generates two consecutive resistive pulses as it traverses the two pores. Importantly, the time delay between these pulses provides additional information regarding the particle's electrokinetic mobility. This configuration also marked the emergence of in-plane nanopore sensors for virus analysis,^[15] an architecture inherently compatible with microfluidic integration. Such compatibility enables compact incorporation of nanopores and nanochannels into microfluidic platforms, thereby substantially enhancing the versatility and compactness of RPS devices. An early demonstration of the sophistication achievable with this approach was reported the same year, showing rapid size determination and titre quantification of unlabelled bacteriophage T7 in both saline media and complex biological matrices, such as mouse blood plasma.^[16]

Although the feasibility of virus detection by nanopore-based RPS was evident from the strong size dependence of pulse amplitude and the linear dependence of event frequency on particle concentration (at sufficiently low concentrations),^[17] it also became clear that obtaining quantitative size and concentration information for viruses in the lower nanoscale regime is far from

trivial. Indeed, in contrast to the established Coulter counters, at the lower nanoscale regime, RPS measurements may stop at reporting current amplitude distributions and translocation event frequencies, while still providing valuable qualitative information. Calibration using reference standards for size and concentration measurements is, in principle, possible.^[18] However, obtaining accurate and traceable reference materials for viruses – and even for synthetic nanoparticles in this size range – remains challenging. Notably, it has been reported that electrolyte-filled capsids exhibit lower pulse amplitudes than solid synthetic nanoparticles of the same diameter, limiting the accuracy of such calibration-based sizing.^[19] Nevertheless, RPS can, in principle, provide absolute virion concentrations in a calibration-free manner, provided that the number of detected resistive pulses, each corresponding to a single-particle translocation, can be rigorously related to the interrogated sample volume. Given the multitude of possible mass transport mechanisms – including, in practice, electrophoretic, electroosmotic, hydrodynamic, and diffusive contributions (Fig. 1A) – rigorous quantitative interpretation of RPS signals is inherently complex. Hydrodynamic flow provides the most direct basis for relating translocation events to a well-defined probed sample volume. Fortunately, for nanopores larger than approximately 10 nm – most relevant for virus measurements – pressure-driven hydrodynamic transport can become dominant, as the mean flow velocity increases approximately with the square of the pore diameter. In contrast, for smaller pores, translocation is often governed by voltage-driven electrophoretic and/or electroosmotic transport, depending on the surface charges of both the pore wall and the particle. However, the pressure-driven volumetric flow rates through single nanopores with diameters in the tens-of-na-

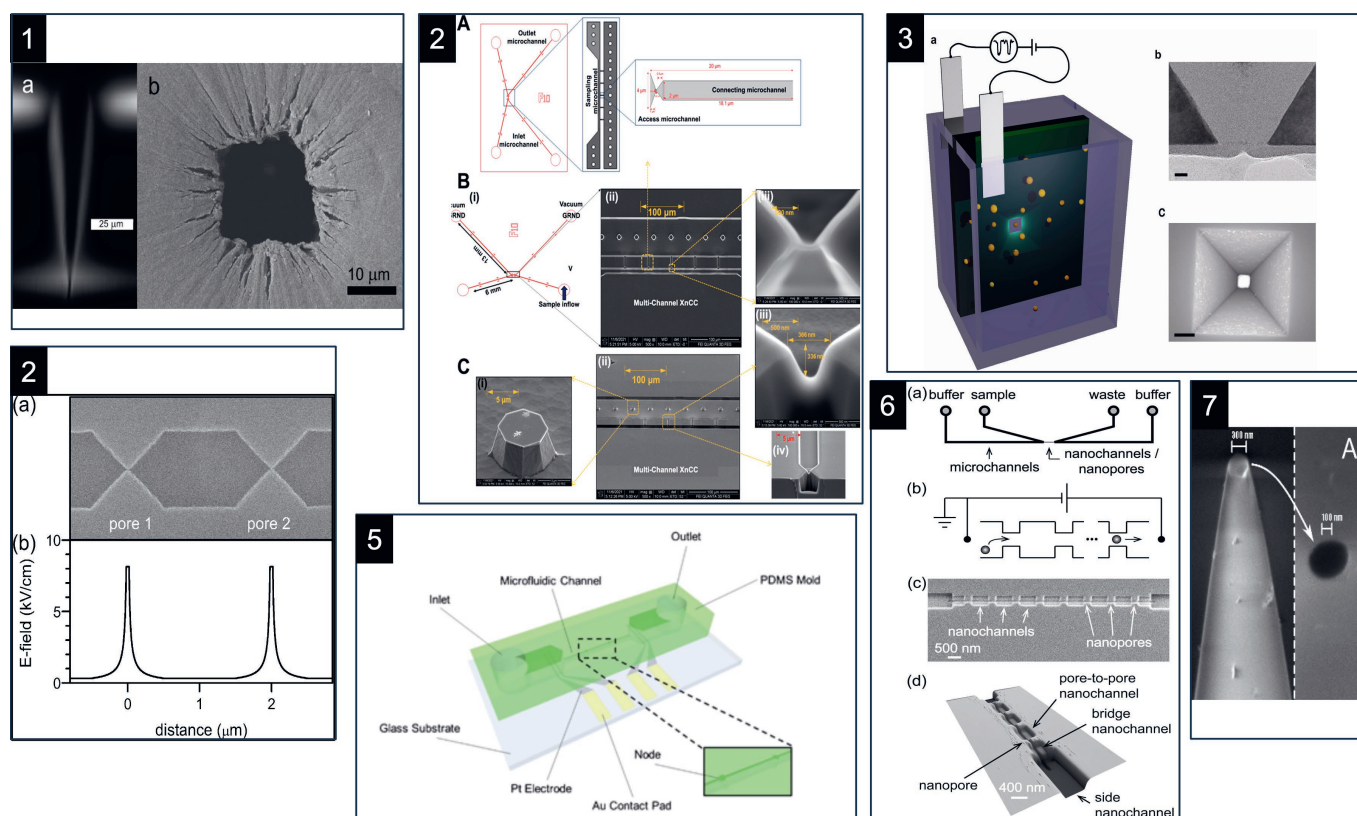


Fig. 3. In-plane (2,4,5,6) and out-of-plane nanopores (1,3,7) and nanochannels constructions used for resistive pulse sensing of viruses: tuneable size elastomeric nanopores (1) (Adapted with permission from Ref. [18]. Copyright 2011 American Chemical Society); 5 parallel nanopores (2) (Adapted with permission from Ref. [20]. Copyright 2023 American Chemical Society); sharp-edged silicon pyramidal nanopores (3) (Adapted with permission from Ref [7]. Copyright 2012 American Chemical Society);^[7] nanopores in series (2-nanopores (4) (Adapted with permission from Ref. [14]. Copyright 2011 American Chemical Society.) and 8-nanopores (6) (Adapted with permission from Ref. [21]. Copyright 2017 American Chemical Society.); node based nanopores in series (5) (Used with permission of the Royal Society of Chemistry, from Ref. [22], permission conveyed through Copyright Clearance Center, Inc.); nanopipettes (7) (Adapted with permission from Ref. [23]. Copyright 2014 American Chemical Society).

nometres range typically in the pL min⁻¹ range or lower; too small to be accurately measured by conventional means. Nevertheless, the volumetric flow rate (Q) can be inferred indirectly from the applied pressure (P) and the hydrodynamic resistance of the pore (R_H) according to $Q = \frac{P}{R}$, provided that the pore geometry is well defined. Under these assumptions, the virion concentration can be calculated from the event frequency as $c_{vir} = \frac{f_{event}}{Q}$.

The first attempt at calibration-free concentration measurements of viruses was made at larger pore dimensions for a target size range of 0.2 to 1 μm using tuneable elastomeric nanopores (Fig. 3 (1)).^[24] Notably, the pore size of these elastomeric nanopores can be dynamically adjusted *via* mechanical stretching, thereby extending the detectable size range.^[25] With larger nanopores where hydrodynamic transport clearly dominates, similarly to Coulter counters, this approach is relatively straightforward. However, as pore dimensions decrease to the tens-of-nanometres range, the validity of this assumption breaks down, and the contribution of electrokinetic effects becomes significant. To address this challenge, simple and cost-effective nanopores in the form of quartz nanopipettes fabricated by capillary pulling were employed, with geometries that could be accurately characterized and reproducibly fabricated (Fig. 3 (7)).^[23] The presence of transport mechanisms in addition to pressure-driven flow can be experimentally identified by detecting particle translocation events at zero applied pressure. Accordingly, plotting the translocation event frequency as a function of applied pressure yields a linear relationship, where the slope reflects the pressure-driven contribution, provid-

ing the particle concentration: ($c_{part} = \frac{\Delta f_{event}}{\Delta P} R_H$), while the intercept at zero pressure identifies the electrokinetic transport contributions. Similarly, the combination of Nernst–Planck/Poisson modelling with accurately characterized nanopipette geometries enabled calibration-free determination of synthetic nanoparticle and virus sizes, with measurements validated against high-resolution electron microscopy, even for small viruses such as the icosahedral poliovirus ($\sim 26 \pm 2$ nm).^[23] Of note, although not yet applied to viruses, nanopipettes have also emerged as a versatile platform for multipass resistive pulse sensing, in which particles are repeatedly translocated back and forth through the same nanopore to improve sizing accuracy, either by alternating the direction of the applied pressure^[26] or by reversing the voltage polarity.^[27] By repeatedly measuring the same particle, thereby eliminating contributions from particle-to-particle dispersity, these studies also enabled quantification of the intrinsic uncertainty of nanopore sensing. Remarkably, for spherical nanoparticles with diameters of several tens of nanometres, the inherent sizing uncertainty was found to be as low as ~ 0.3 nm. The size resolution can also be further improved by using multiple nanopores in series, as demonstrated with up to eight sequential pores (Fig. 3 (6)).^[21] Combined with rapid acquisition times, these configurations enable real-time analysis of HBV capsid assembly dynamics. Moreover, the unidirectional flow inherent to such multipore devices offers improved compatibility with microfluidic integration compared with the back-and-forth operation required in multipass RPS configurations.

Beyond size and concentration, RPS also enables the determination of the virus surface charge, inferred from the zeta potential. One possibility to determine the zeta potential is based on measuring the translocation velocity of particles under electrophoretic conditions, as shown by Arjmandi and coworkers^[7] by using single sharp edged silicon pyramidal nanopores (20–500 nm) made by e-beam lithography (Fig. 3(3)), *i.e.* the zeta potential can be calculated from the pulse duration measured accurately, and independently of the amplitude of the translocation pulse and the

particle size. This allowed determination of the zeta potentials of human immunodeficiency virus and Epstein-Barr virus with less uncertainty (± 2 mV) than dynamic light scattering (DLS, ± 5 mV). Other approaches are also possible, *e.g.* in variable-pressure RPS,^[9] the zeta potential is determined by varying the applied pressure until pressure-driven and electrokinetic transport forces balance, minimizing the particle translocation rate. The pressure at this minimum blockade frequency is then used to calculate the nanoparticle surface charge.

2.2 Limitations, Solutions and Opportunities

2.2.1 Limit of Detection

While RPS has single-species detection capability, this does not necessarily translate into an excellent limit of detection (LOD) in terms of concentration at reasonable analysis times. The worst limiting situation is the undirected, Brownian motion of a single particle in a volume V , that requires an excessively long mean encounter time of ($t_e = \frac{V}{2Dd_{pore}}$) (D is the diffusion coefficient of

the particle, d is the pore diameter) to approach the sensing zone of the nanopore.^[28] Although directed transport, *e.g.* hydrostatic pressure, can significantly reduce the mean time for a translocation event to occur, practical constraints remain. In particular, as the pore size decreases (and R_H increases), the volumetric flow rate through the nanopore decreases substantially.^[29] As a consequence, the LODs achievable with micrometre-sized pores in Coulter counters (approaching *ca.* 10² particles mL⁻¹) are not attainable using nanopore-based RPS under comparable measurement times. A promising strategy to overcome this limitation is *in situ* virus preconcentration prior to nanopore analysis. This approach has been demonstrated for human influenza virus (H1N1), where virions were selectively captured using 6'-sialyllactose-functionalized probes immobilized on a gold electrode surface.^[30] Following electrochemical release, the enriched viral population was subsequently detected using nanopore-based sensing. This concept not only improves the effective LOD but also introduces an additional layer of selectivity by the use of specific affinity ligands. Another approach enabled by in-plane pores integrated into a microfluidic platform is the use of parallel nanopore architectures. Even without individual electrical addressability, 5 parallel nanopores of 350 nm fabricated in cyclic olefin polymer *via* nanoinjection moulding within an optimized microfluidic setting (Fig. 3 (2)) achieved an impressive improvement in the LOD of 5.5×10^3 particles mL⁻¹.^[20] This corresponded to 1,100-fold improvement compared to a single in-plane pore device. Interfaced with an affinity-enrichment microfluidic chip^[20] comprising a high density of pillars modified with photocleavable aptamers for virus preconcentration and release, the parallel nanopore platform (called the extended nano-Coulter counter (XnCC)) could be used to detect SARS-CoV-2 viral particles in saliva samples. Of note, aptamers are short single-stranded DNA or RNA molecules selected against a given target from large random-sequence libraries, often containing up to 10¹⁵ variants, through an *in vitro* selection process that enriches high-affinity sequences capable of folding into specific three-dimensional structures for selective target binding.

2.2.2 Size Resolution

Nanopore-based RPS sensors exhibit excellent size resolution because the pulse amplitude scales approximately with particle volume and therefore with the third power of the particle diameter. Since the current blockade primarily arises from the displacement of the highly conductive electrolyte by the comparatively low-conductivity virus particle, the exact signal response depends on the particle-to-pore volume ratio ($V_{particle}/V_{pore}$) as well as on the

geometry of both the pore and the particle. Numerous theoretical models describing the resistance change induced by particle translocation have been developed for cylindrical pores and are applicable to different $d_{\text{part}}/d_{\text{pore}}$ regimes.^[10, 31–32] In contrast, no general simple analytical expression for pulse amplitudes in conical pores is available.^[23] The translocation through a conical pore can generate pulse amplitudes several times larger than in a cylindrical pore of identical minimum diameter and length. This is because the resistance of a conical pore is concentrated near the narrow tip, whereas in a cylindrical pore it is distributed more uniformly along the pore length. Most importantly, RPS is particularly well-suited to resolving minute size variations with high sensitivity. However, this sensitivity comes at the expense of a relatively narrow dynamic size range for a given pore, typically spanning roughly one order of magnitude. This limitation becomes evident when considering that a 10-fold decrease in particle diameter results in an approximately 1,000-fold reduction in pulse amplitude, rapidly compromising detectability. One strategy to overcome this limitation is the use of elastomeric nanopores, whose dimensions can be reversibly tuned by biaxial mechanical stretching to adjust the sensing aperture for different particle size ranges.^[24]

Another innovative strategy employs in-plane channels incorporating a series of nodes (enlarged cross-sections) along the channel length (Fig. 3 (5)).^[22] As particles traverse these structures, they generate characteristic electronic signatures that facilitate their discrimination from background noise. These periodic signal patterns can be transformed into enhanced fast Fourier transform (FFT) power spectra, enabling rapid and robust particle detection. Using this approach, measurements can be performed at particle-to-pore volume ratios ($V_{\text{particle}}/V_{\text{pore}}$) as low as 1.2×10^{-3} , greatly extending the dynamic range accessible to nanopore-based RPS sensing. The method was successfully applied to detect human immunodeficiency virus (HIV) particles (approximately 100–200 nm in diameter) in plasma samples.

2.2.3 Selectivity

Beyond physical descriptors, the selectivity of virus detection, as discussed before, can be enhanced either by adding specific affinity ligands to the virus containing solution to induce an effective size increase (manifested as an increased pulse amplitude),^[12] or by capturing viruses *via* cleavable affinity ligands followed by their release for subsequent RPS analysis.^[20, 30] A third strategy involves functionalizing the nanopore itself with affinity ligands and exploiting interaction-induced modulation of the translocation signal for selective recognition. This concept has been demonstrated by immobilizing peptide recognition probes derived from complementarity-determining regions of the anti-hemagglutinin antibody CT149 on a gold-coated nanopore surface.^[33] When oligopeptides with moderate affinity toward influenza A (H1N1) were employed, H1N1 virions exhibited longer dwell times (and apex bluntness) within the nanopore compared to influenza B, enabling feature-based single-virus discrimination. While unambiguous identification of viruses in complex samples remains highly challenging when relying solely on physical descriptors, such as size and surface charge–modulated signal features, there is growing evidence that machine learning and artificial intelligence may dramatically facilitate the recognition process.^[34] In this context influenza virus subtypes (three influenza subtypes, A (H1N1, H3N2) and B) have been identified at the single-virus level with 68% accuracy using pattern classification of ionic current signatures.^[35] For this purpose, 50 nm-thick Si_3N_4 membranes fabricated on Si wafers with a single 300 nm nanopore were employed. Notably, although viruses were driven electrophoretically through the pore, the negatively charged pore walls induced electroosmotic counterflow (see Fig. 1A), effectively reducing the passage of many impurities and acting as an intrinsic filtration mechanism. A similar approach has been reported for single-par-

ticle-level detection and discrimination of five respiratory tract viruses,^[36] including influenza A virus, influenza B virus, respiratory syncytial virus (RSV), adenovirus, and coronavirus, as well as for differentiation among similarly sized coronaviruses (HCoV-229E, SARS-CoV, MERS-CoV, and SARS-CoV-2).^[37] In addition, SARS-CoV-2 has been shown to be detectable in saliva within a 5-minute measurement, with reported sensitivity and specificity values of 90% and 96%, respectively. These results demonstrate that higher-dimensional machine learning analysis of RPS signals can enable label-free viral identification approaching clinical translation,^[38] with clear potential for detecting emerging strains yet lacking selective affinity ligands.

Beyond virus identification, it is important to distinguish infectious from non-infectious viral particles. As proof of concept, this was demonstrated by using DNA aptamer-modified solid-state nanopores for both enveloped (SARS-CoV-2) and non-enveloped viruses (human adenovirus).^[39] Essentially, aptamers were selected against the infectious virus, while applying counter-selection against the viruses rendered noninfectious by free chlorine treatment or UV. This strategy enriches aptamers that preferentially recognize structural or conformational features preserved in infectious particles but altered or lost upon inactivation.

3. Nano-impact Electrochemistry of Viruses

By nano-impact electrochemistry (NIE), we refer here broadly to electroanalytical methods in which individual nano- or micro-scale entities are detected and characterized through stochastic collisions with an electrode surface. Most implementations use ultramicroelectrodes (UMEs) with micrometre-scale dimensions and monitor faradaic current transients. Depending on whether the impacting entity remains on the electrode or interacts only transiently, collisions generate either stepwise or peak-shaped current responses. Owing to their relatively large size, planar geometry, and compatibility with established microfabrication methods, particularly photolithography, UMEs offer practical advantages over nanopore-based sensors. The conscious development of this electroanalytical methodology by Bard and Xiao^[40] led to a wide variety of direct and indirect detection mechanisms for synthetic and biological nanoparticles. However, before the emergence of faradaic NIE, Lieber and coworkers reported the use of semiconducting silicon nanowire field-effect transistors for the detection of individual virus particles, including influenza A.^[41] In this approach, the interaction of charged virions with antibody-functionalized nanowires produced discrete conductance changes, enabling selective single-particle detection through virus-specific surface recognition. Appealingly, this method offers substantial potential for massive parallelization; however, because signal transduction relies on electrostatic field effects, it is intrinsically sensitive to ionic strength, pH, virus surface charge, and the effectiveness of surface blocking against nonspecific adsorption.

The first application of faradaic NIE to virus detection was reported by Dick and coworkers^[42] for the detection of individual murine cytomegalovirus (MCMV) particles using a 10 μm -diameter Pt UME. The assay relied on stochastic adsorption of virions onto the electrode, which partially blocked ferrocyanide diffusion and produced discrete chronoamperometric current steps. Under diffusion-controlled transport, the expected collision frequency is: $f_D = 4DCr_eN_A$, where D and C are the diffusion coefficient and the concentration of the target particle, respectively, r_e is the radius of the electrode, and N_A is Avogadro's number. Binding of monoclonal antibodies against the MCMV envelope glycoprotein B altered both collision frequency and step magnitude, enabling discrimination between free and antibody-bound virions. Selectivity was further confirmed using secondary-antibody-functionalized polystyrene beads: MCMV bridged the beads into aggregates, decreasing collision frequency and increasing current-step amplitudes, whereas murine gammaherpesvirus 68 controls produced

no aggregation. Dick and Bard subsequently introduced an enzyme-amplified NIE assay for MCMV detection.^[43] Virions were selectively labelled with glucose oxidase (GOx)-conjugated antibodies and detected at a 1 μm -radius Pt UME in deoxygenated ferrocene methanol/glucose solution. At an oxidative potential, ferrocene methanol was converted to ferrocenium methanol on the UME, which served as the electron acceptor for GOx-catalyzed glucose oxidation. Regeneration of ferrocene methanol produced catalytic current steps when GOx-labelled virions collided with the electrode. The high enzyme loading on each virion, estimated at *ca.* 4,000 GOx molecules per particle, provided substantial amplification and enabled detection down to approximately 30 fM.

A more direct NIE strategy was implemented by Compton and coworkers^[44] who detected influenza viruses by tagging them with *ca.* 9 nm silver nanoparticles. The method exploits the spontaneous adsorption of Ag nanoparticles onto virions, a process also implicated in their antiviral activity. During stochastic collisions with an 8 μm carbon microelectrode held at 0.6 V vs. SCE, oxidation of the Ag-tags on the virus produced discrete current spikes. Signal generation required preconcentration of multiple Ag nanoparticles on each virion, as individual Ag nanoparticle impacts were not directly resolvable. Consequently, the assay was separation-free, allowing Ag-labelled viruses to be detected without removing excess unbound nanoparticles, with a reported detection limit of approximately 0.2 pM.

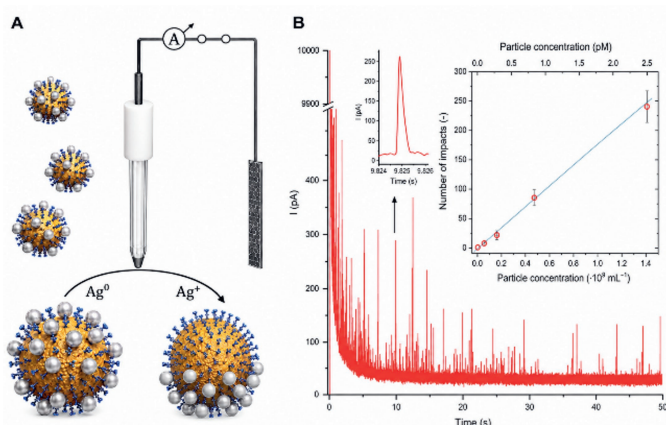


Fig. 4. Detection scheme for Ag-nanoparticle-labelled Influenza virus by NIE (A) and the corresponding current transient and the concentration dependence of the frequency of collision events (current spikes) (B). (Used with permission of the Royal Society of Chemistry, from Ref. [45]; permission conveyed through Copyright Clearance Center, Inc.).

To reduce the uncertainty associated with direct Ag nanoparticle labelling, *in situ* Ag deposition was introduced.^[45] Although not established for virus sensing, surface encapsulation of biological entities such as bacteria and cells has gained interest for imparting magnetic, conductive, or fluorescent properties.^[46] For nanoscale virions, an *in situ* Ag coating strategy was developed by activating the virus surface with a tannic acid–Sn²⁺ complex, followed by surface-catalysed reduction of silver from [Ag(NH₃)₂]⁺. The process produced Ag nanoparticle-decorated, rather than continuously Ag-coated, influenza A (H3N2) virions, enabling their detection by NIE (Fig 4). The more uniform Ag distribution considerably improved quantitative reliability relative to direct Ag nanoparticle tagging. However, because the coating is non-specific and was designed as a general nanoparticle modification method, its use is mainly restricted to relatively pure virus preparations, such as vaccines.

Most recently, Lemay and coworkers reported proof-of-concept, label-free detection of individual SARS-CoV-2 particles using CMOS-based nanocapacitor arrays and stochastic electrochemical impedance.^[47] The platform comprised 65,536 individually addressable electrochemical transducers functionalized with thiolated aptamers against the spike S1 protein, enabling virus detection in cell culture medium at physiological salt concentration. The massively parallel architecture allowed high-frequency impedance acquisition and statistical single-particle analysis, highlighting the potential of this approach for scalable, real-time virus sensing.

4. Conclusions

Both resistive-pulse and nano-impact electrochemical approaches for sensing intact virions leverage mature nanopore, microelectrode, nanoelectrode, and semiconductor fabrication technologies while they are compliant with advanced microfluidics. RPS excels at providing a wealth of information through current transients, enabling even calibration-free sizing and concentration measurements. Emerging signal-processing and artificial-intelligence methods are increasingly capable of extracting discriminatory information from these current signatures, although robust virus identification will likely benefit from using specific affinity ligands. Among these, nucleic acid aptamers are especially promising because they can be selected and tailored for optimal affinity^[48–50] relatively rapidly, which may be advantageous for tracking viral evolution and emerging variants. Important limitations of RPS, including restricted dynamic size range and insufficient limits of detection, are being addressed through tuneable or multiplexed nanopore designs, nanopore arrays, and *in situ* preconcentration strategies. These size-range constraints are generally less severe for NIE, although sensitivity and selectivity remain central challenges. Overall, combining nanofabrication, microfluidics, affinity recognition, signal processing, scalable semiconductor technologies, and parallelization is expected to advance both RPS and NIE.

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