DOI: 10.13208/j.electrochem.181054 **Artical ID**:1006-3471(2019)03-0374-12
Cite this: *J. Electrochem*. **2019**, *25*(3): 374-385 **Http**://electrochem.xmu.edu.cn

Single Particle Impact Electrochemistry: Analyses of Nanoparticles and Biomolecules

ZHANG Jian-hua, ZHOU Yi-ge*

(*Institute of Chemical Biology and Nanomedicine, State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha* 410082, *China*)

Abstract: Single particle impact electrochemistry (SPIEC) has grown rapidly in recent years and shown great promise in the analysis of nanoparticle properties as well as the detection of biomolecules including DNA, RNA, protein, enzyme, bacteria, virus, vesicles and others. This minireview summarizes recent advances in electroanalytical applications of SPIEC according to different analytical methods, i.e., direct electrolysis of nanoparticles or labeled nanoparticles, direct electrolysis of soft particles encapsulated redox molecule, indirect electrochemistry of particles, area and diffusion blocking, and changes in current magnitude and collision frequency.

Key words: single particles; impact; electrochemistry; analysis; biomolecules

CLC Number: O646 **Document Code:** A

Nanoparticles (NPs) have generated considerable interest in electrocatalysis and electroanalysis as a result of their unique physical and chemical properties, which significantly differ from their bulk counterparts. Traditional way of examining nanoparticle electrochemistry is based on averaging the electrochemical response which occurs over large ensembles of NPs. However, the unavoidable variations in nanoparticle size[1], shape[2], exposed crystal facets[3], aggregation status[4] and even the nanoparticle spacing[5] all impose negative impacts to the reliability of the electrochemical information obtained from the ensemble measurements. To address this challenge, Heyrovsky[6], Bard[7] and Compton[8] carried out single nanoparticle impact electrochemistry (SPIEC) on an ultramicroelectrode (UME) involving Faradaic charge transfer processes in 2006, 2007 and 2011, respectively, following the sporadically reported studies mainly on non-Faradaic charge transfer of single particles[9-10]. SPIEC has now become a booming area in both fundamental electrochemical studies and real-world applications, with more than 100 papers emerging so far.

SPIEC allows *in situ* electrochemical detection of solution-phase particles, which started from the studies in single metal NPs, such as PtNPs[11], AgNPs[8], and AuNPs[12]. The experimental principle is straightforward, where single-particle collision is by virtue of Brownian motion of individual NPs that randomly collide with an electrode held at a suitable potential. Under such potential, direct oxidation of the NPs themselves[8,12-18] or mediated reaction via electron transfer facilitated by the NPs[7,19] takes place, enabling the characterization, detection and evaluation of individual NPs, such as size distribution[8,12-13,19-21], concentration[18], aggregation/agglomeration state[14,22], and catalytic reactivity[7,23-26].

The study of single metal NPs has rapidly been extended to a broad range of other types of particles and species in the recent five to six years, including but not limited to metal oxide NPs[27-28], tagged NPs[29-30], organic NPs[31], emulsion droplets[32-34], vesicles[35-37], liposomes[38-39], DNA[40-41], RNA[42], protein[43-44], enzyme[45-48], virus[49-51], and even cells[52-54]. Accordingly,

Received: 2018-12-27, Revised: 2019-01-27 *Corresponding author, Tel: (86-731)88823902, E-mail: yigezhou@hnu.edu.cn

a number of other detections or analytical methods concerning single particle impacts are developed, especially after the most recent reviews of this field published in 2017[55-56]. To fill this gap, we will review in this paper the electroanalytic applications of SPIEC based on different analytical methods. Such perspective is not seen elsewhere.

1 SPIEC in the Analyses of Nanoparticles and Biomolecules

1.1 Direct Electrolysis of Nanoparticles or Labeled Nanoparticles

The direct electrolysis of nanoparticle was pioneered by Compton et al. in 2011 as a direct but destructive way to quantitatively measure the size of AgNPs (Figure 1)[8], which is named as anodic particle coulometry (APC). In this method, metal NPs are completely oxidized as they impact with an ultramicroelectrode held at a suitable potential, and therefore, produce oxidative charge which can be converted to the size of single NPs. APC method was subsequently applied for sizing of Au[12,57], Ni[18] and Cu NPs[15], sizing AgNPs in environmental media such as sea water[58-59] and tap water[60], determining unknown concentration of NPs[18], monitoring aggregation/agglomeration behaviors of NPs in solution[22,61] under the effects of capping agents[62], ionic strength[63] and pH[64]. The same principle also allows the sizing of metal oxide particles[27-28], characterization of core-shell Au-Ag NPs[65], determination of the aspect ratio of Au nanorod[66] and analysis of the composition of bimetallic Ag-Au NPs[67]. Furthermore, sizing of layer transition metal dichalcogenides (TMD) NPs (in a general form of MX_2) was also realized by direct oxidation of the NPs (from M^{4+} to M^{6+})[68].

Apart from obtaining information on NPs themselves, APC of labeled metal NPs provides a sensitive tool for biomolecule detections such as DNA[69], viruses[49] and bacterial[70-71]. Crooks et al. reported the individual collisions between a conjugate consisting of AgNPs linked to conductive magnetic microbeads via DNA hybridization and a magnetized ultramicroelectrode[69]. By electrochemically oxidizing the labeled AgNPs, DNA can be detected with a limit of detection (LOD) as low as 20 amol·L^{-1}. Such low LOD is achieved due to multiple AgNPs presented on each microbead and the increased rate of mass transport of the microbeads to the UME surface. In the meantime, Compton et al. demonstrated a proof-of-concept for the electrochemical detection of single *Escherichia coli* (*E. coli*) bacteria decorated with Ag NPs, which are directly oxidized during the collisions of single bacteria to an electrode[70]. Later on, they extended this working principle to the detection of single influenza viruses H1N1 by the oxidation of the adsorbed AgNPs on virus surface and achieved a sub pmol·L^{-1} level of detection[49]. The signal arising from individual species manifested the fact that viruses in general are at least

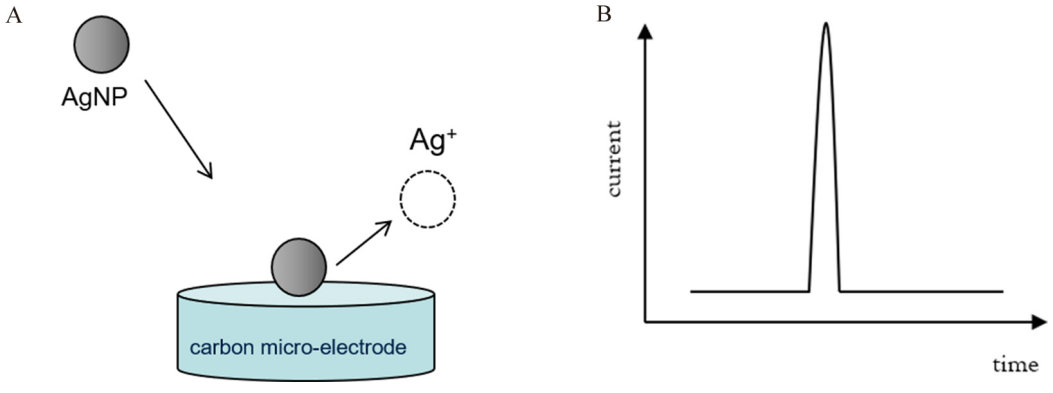

Fig. 1 Schematic illustration of direct oxidation of individual AgNPs. (A) Individual AgNP in electrolyte randomly collides with the surface of a carbon microelectrode from Brownian motion. If the microelectrode is held with a suitable potential, the AgNP can be oxidized into silver ions during the collision. (B) A typical "spike" like response recorded on the current-time profile corresponding to the direct oxidation of a single AgNP.

an order of magnitude smaller than bacteria. A recent study by Compton et al.[71] reported a label-free detection of *E. coli* bacteria in solution and a concentration dependence of bacteria impacts was found. In their strategy, N,N,N',N'-tetramethyl-para-phenylene-diamine was employed as a redox mediator, which interacts with bacterial cytochrome c oxidases, resulting in electrochemical current "on"-signals in the presence of *E. coli.* This strategy can minimize false positive signals from non-electroactive impurities.

After APC method which is capable of quantitatively sizing metal and metal oxide NPs by direct oxidation, cathodic particle coulometry (CPC) was immediately established to size impacting organic[31], metal oxide[27-28] and C_{60}[72] NPs by observing their reduction current upon contact with an electrode. Combining APC and CPC, particle coulometry has become a powerful technique to detect individual NPs that is not limited by nanoparticle types and electrode materials, which can achieve a large detection range of 5 nm[13] to 150 nm[14] in diameter. The currently widespread microscopic or spectroscopic technologies for nanoparticle detection, such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), and dynamic light scattering (DLS) all have disadvantages, e.g., expensive to acquire, extensive sample preparation, long processing time, limited detection range, difficult in real-time detection, prone to compromise samples when imaging is conducted. Particle coulometry, as an alternative, can provide a cost effective, efficient, and *in situ* characterization and detection of NP.

1.2 Direct Electrolysis of Soft Particles Encapsulated Redox Molecule

The direct electrolysis approach has recently been applied to soft particles such as liposomes[38-39], vesicles[37], micelles[73], emulsion[33-34,74], and even cells[37]. During the collision processes, the redox molecules inside those soft particles may be released to the electrode surface and get detected. Compton et al. [38-39] first reported the electrochemistry of ascorbate/glutathione-loaded liposomes as they impact an electrode, which can be used for sizing liposomes and the quantifying attomolar-scale drug content at the single liposome level (Figure 2)[38-39]. They proposed in their work a "full collapse fusion" process and a near-100% impacting liposomes undergoing reaction, however, the data shown in the work was not able to sufficiently support this. Ewing et al. later investigated the electrochemical response of single adrenal chromaffin vesicles filled with catecholamine hormones as they are adsorbed and rupture on a microelecrode[36]. In contrast to what was reported by Compton et al.[38], they found that only 86% of the single vesicles were observed to produce current transients under the given experimental conditions, allowing the quantification of the vesicular catecholamine content. In their parallel work, they employed a nanotip conical carbon-fiber microelectrode to achieve electrochemical quantification of the total content of electroactive neurotransmitters in individual vesicles in single PC12 cells[37]. They also emphasized that only part of the neurotransmitter is released during exocytosis, which supports the hypothesis that vesicles do not open all channels during the normal exocytosis, leading to incomplete release of vesicular contents. This work is also an example of expanding single particle impact electrochemistry to the realm of single cell detection.

The electrolyses of single emulsion[33-34, 74] and micelle[73] droplets collisions are also reported. Bard et al. demonstrated the reduction of nitrobenzene (NB) emulsion droplets and the selective reduction of 7,7,8,8-tetracyanoquinodimethane (TCNQ) in NB droplets, respectively, and discussed collision frequency, size distribution, current transient decay of the individual emulsion droplets[33]. Besides, they showed that the detections of nano- and micro-sized single attoliter emulsion droplet collisions can be achieved by not only electrochemistry, but also electrochemiluminescence (ECL) when the components within the droplet are an ECL luminophore[74]. Later on, Compton et al.[34] took toluene droplets as a model for artificial oxygen carriers to detect oxygen reduction within individual toluene droplets and achieved the quantifi-

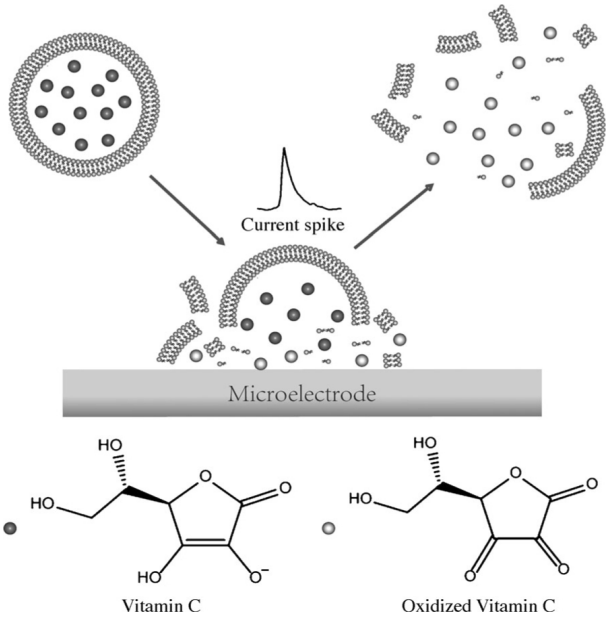

Fig. 2 Schematic illustration of a “nano-impact” experiment of single ascorbate-loaded liposome. The ascorbate-loaded liposomes fully collapse as they hit a microelectrode and subsequently release ascorbate that can be oxidized at the electrode to produce a current spike. Reprinted with permission from ref. 38. Copyright 2014 Wiley-VCH KGaA, Weinheim.

cation of attomole oxygen contents[34]. This strategy will have applications in the quantification of oxygen or oxygen related species in cells, vesicles, or artificial oxygen carriers at single carrier level. They further established the first example in the detection of single CTAB (cetyltrimethylammonium bromide) micelles by the oxidation of the bromide content, indicating that significant numbers of spikes are observed only when CTAB concentration is above the critical micelle concentration (CMC)[73].

Single cell analysis is a rapidly evolving field with applications such as cancer diagnostics and therapy, immune response, and others. Electrochemical devices feature in fast response, rapid diagnostics, and cost-effective materials, which show great potential in point-of-care detection. Dick et al.[53] for the first time applied single particle impact electrochemistry to the examination of single cancer cells and healthy cells by monitoring the redox-active molecules releasing from cells membrane in the presence of TX-100 during the collisions of cells to the electrode. Cancer cells and healthy cells can then be discriminated based on the fact that the electrochemically active metabolites, such as ROS and enzyme cofactors, are presented in different levels in cancerous versus healthy cells. Compton et al.[52] examined the concentration of red blood cells by studying the reduction currents of oxygen which relies on the catalytic activity of red blood cells from surface-induced haemolysis. They showed that the enhanced signal can be used to detect red blood cells at a single entity level, which is suitable for a point-of-care test device.

1.3 Indirect Electrochemistry of Particles: Electrocatalytic Amplification

Unlike direct electrochemistry of single NPs, in the model of indirect electrochemistry, NPs themselves are not electrolyzed, e.g., directly oxidized, but the electroactive species in solution react on the surfaces of single NPs during the collisions to an inert electrode, producing electrocatalytic current amplification. Indirect nanoparticle electrochemistry was first implemented by Bard et al.[7], where they observed transient currents of hydrogen evolution reaction[7] and hydrazine oxidation reaction[23] occurring on the surfaces of single PtNPs. They also attributed the staircase current response to nanoparticle sticking to the electrode upon collision, while the spike response to the transient stay of nanoparticle or poisoning of the nanoparticle surface during collision[7,23,75]. This method was immediately followed by research groups such as Compton[17,76-77], Crooks[78] and Koper[79], and widely utilized in studying size distributions[20,79], electron transfer kinetics[17], electroactive molecule tagged NPs[29-30], and various bioanalyses[40-41,80]. In the following text we will focus on reviewing the development of bioanalysis enabled by electrocatalytic amplification approach.

Electrocatalytic amplification method has been applied to the detection of biomolecules since 2012. Bard et al.[40] first reported the detection of DNA by recording the electrocatalytic signal in the presence of a target DNA that is hybridized with both a capture

probe DNA and a detection DNA modified to a single PtNP which catalyzes the oxidation of hydrazine in solution when it is made in contact with an Au UME after hybridization (Figure 3)[40]. The resulting LOD is 10 pmol·L^{-1}, which is not impressive due to the high background current level at a certain concentration of target DNA, making it difficult to distinguish the signal from the background level. In the following year Crooks et al. reported a real time electrocatalytic amplification detection of single DNA hybridization events at microfluidic microband electrode surface[80]. The current signal arises from the electrocatalytic oxidation of N_2H_4 at single PtNPs, which represents the hybridization of a single DNA target at electrode surface modified with ssDNA probe. Again, this work only presents a LOD of 25 pmol·L^{-1} target DNA due to the local dehybridization of dsDNA in hydrazine. In 2017, the same research group reported on the study of microRNA detection[42]. In their strategy, the modification of single-strand DNA (ssDNA) on PtNP surface forms PtNP@ssDNA, which passivates the activity of PtNP. When miRNA, which is complementary to the ssDNA, is presented in solution, the hybridization takes place and forms PtNP@ssDNA∶miRNA conjugate. After introducing duplex specific nuclease (DSN) to such conjugate, a fraction of the surface-bound DNA can be removed thereby exposing some of the PtNPs surface, and thus, the electrocatalytic properties of the PtNPs are reactivated. The corresponding LOD of such strategy is 100 pmol·L^{-1} for both miRNA-21 and miRNA-203.

Recently, current amplification through electron transfer or mediated electron transfer by single electroactive bio-species opens up a new route for the detection of biomolecules such as protein[43-44] and enzyme[45-48,81] with higher sensitivity. Detection of single or few freely diffusing biomolecules by electrochemical techniques is very challenging because a redox-active molecule contributes only one electron or a few electrons to the measured current with each encounter at the electrode. To address this challenge, Yang et al. reported on the detection of protein molecules (MP-11) via electrocatalytic current amplification of single entity[43]. They employed small graphene nanosheet to act as a vehicle to assemble a group of MP-11 molecules and then amplify the reduction current of MP-11 when graphene nanosheets collide with the electrode, showing that the number of the MP-11 molecules on a single grapheme nanosheet is in the range of 105±18. Later, Eriksson et al. reported for the first time the detection of single redox enzyme molecules (laccase) during their collisions to an UME by studying the electrocatalytic current produced from the reduction of oxygen by the active center of the enzyme molecule[45]. This new methodology monitors turnover number of the catalytic reaction at a single enzyme molecule level. Bard et al. soon detected GOx enzyme using impact electrochemistry by functionalizing thousands of enzymes to the surface of single murine cytomegalovirus, which bring enzymes to the vicinity of the electrode surface to realize the detection[50]. Similar to Yang's strategy, this work allows the preconcentration of enzyme molecules and hence greatly amplifies the signal of just one GOx enzyme, which exhibits a lowest concentration of virus particle from urine to be 30

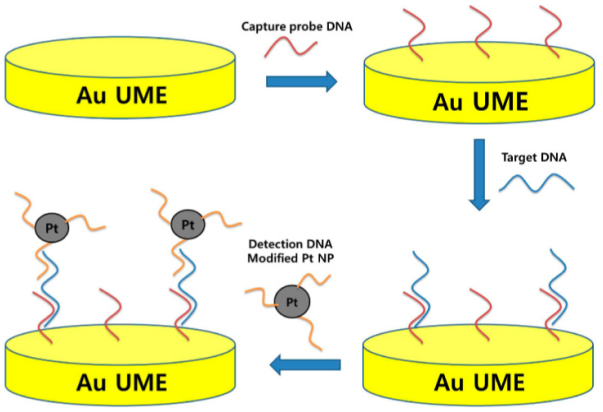

Fig. 3 Schematic illustration of a sandwich-type DNA sensor on an Au UME (radius 5 μm). The Au UME is modified with a capture probe DNA, and the PtNP is modified with a detection probe DNA. With the presence of a target oligonucleotide that hybridizes with both capture and detection probes, the PtNP is brought on the electrode surface and catalyzes an electrochemical reaction to produce a current response. Reprinted with permission from ref. 40. Copyright 2012 American Chemical Society.

fmol·L^{-1}. Similar researches into single enzyme molecule via current amplification method were also presented by Foord et al.[47] and Zhan et al.[48]. It is worth mentioning that Compton et al. combined theoretical study with experimental study of single catalase impact to achieve deep understanding in dynamic fluctuations of the catalytic ability of single catalase enzyme toward hydrogen peroxide decomposition[46].

1.4 Area and Diffusion Blocking

Area and blocking approach to detect insulating particles upon their collisions to an electrode surface was first implemented by Lemay et al. using a time-resolved electrochemical method[82]. The principle of this method can be put as follows[82-83]. The molecules in the solution where the insulated beads are presented are electrochemically active and will undergo diffusion-limited oxidation or reduction reactions on the surface of an electrode. When the beads impact on the electrode surface, they will hinder the diffusion of the redox molecules to the electrode and therefore lead to a decrease in the diffusional flow. Similar to the aforementioned Faradaic charge transfer in nanoimpact electrochemistry, when a bead hits and adsorbs on the electrode surface, a steady-state stepped current response will occur, enabling the detection and characterization of the insulated single particles.

Bard et al. in 2014 extended such area and diffusion blocking based impact electrochemistry to the detections of emulsion drops and biomolecules. Based on this method, they established a so-called emulsion droplet blocking (EDB) technique and obtained size distribution of toluene-in-water emulsion droplets from single emulsion droplet collisions by virtue of the drops blocking the electrochemical oxidation of $Fe(CN)_6^{4-}$ at the electrode surface[32]. They also examined the size distribution of single unilamellar vesicles by using similar principle[33]. Later on, the same group reported on the detection of single biomacromolecules by blocking a solution redox reaction when the molecules adsorb on the UME surface and block the active sites (Figure 4)[44]. The biomolecules cover a wide range of species such as plasmid DNA, catalase, horseradish peroxidase (HRP), glucose oxidase and mouse monoclonal antibody. This method demonstrated the feasibility of detecting single biomacromolecules, however, the difficulty in distinguishing electrochemical signal from the noise level fails to provide a low limit of detection.

Departing Bard's work, the area and diffusion blocking strategy was then applied to the detections of bacteria[84-85] and viruses[50]. Park et al. observed that when an *Escherichia coli* (*E. coli*) collides with and then attaches to the UME surface, the level of the steady-state current from the oxidation of $Fe(CN)_6^{4-}$ in solution decreases because the flux of $Fe(CN)_6^{4-}$ is blocked by the *E. coli*. Such label-free approach enables single *E. coli* detection with a fmol·L^{-1} level sensitivity[84]. Using similar method, Bard et al. examined single murine cytomegaloviruses (MCMVs) im-

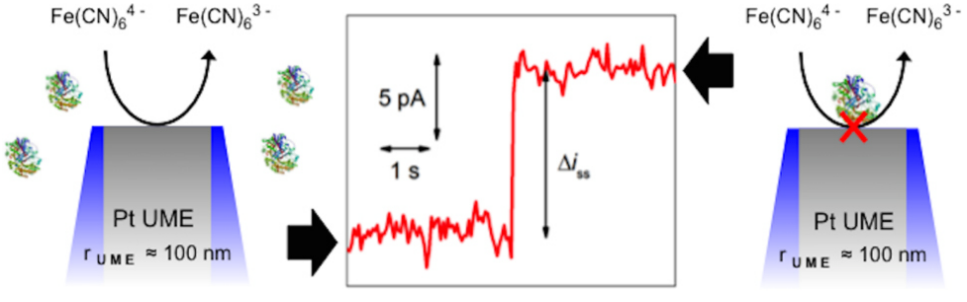

Fig. 4 Schematic illustration of the area and diffusion blocking experiment. An insulating molecule, such as protein, glucose oxidase, antibody or DNA, adsorbs on the surface of a Pt UME and blocks the diffusion of ferrocyanide to the electrode, leading to a staircase-shaped decrease in steady-state current with a magnitude of Δi_{ss}. The chronoamperometric curve represents the current decrease when a 150 nm radius Pt UME is partially blocked by a single glucose oxidase (GOx) molecule. Reprinted with permission from ref. 44. Copyright 2015 American Chemical Society.

pacting at a UME [50]. From the height of the decreased current step and the collision frequency, the concentration and size of MCMVs can be obtained.

1.5 Changes in Current Magnitude and Collision Frequency

When a molecule, especially a not very electrochemically active molecule, binds to a catalytic particle that collides to an electrode, the electrocatalytic current magnitude as well as the collision frequency will be changed. Looking at such differences provides a new route for the analysis of biomolecules. Andreescu et al. quantitatively measured ochratoxin A (OTA) using nanoimpact electrochemistry by looking at the collision frequency change before and after the binding of OTA to ssDNA-functionalized AgNPs [86]. The difference in collision frequency arises from the surface coverage of the NP by the ssDNA aptamers and subsequent conformational changes of the aptamer probe which affect the electron transfer between the NP and the electrode surface. This method achieved a limit of detection of 0.05 nmol·L^{-1}. Bard et al. utilized the same principle to the detection of single viruses by studying the differences in current step size and step frequency when the viruses are modified with specific antibody (Figure 5)[51]. The attachment of antibody to the viruses causes decreased collision frequency and larger current step size, due to rare collisions of larger aggregates.

2 Conclusions and Perspectives

Significant advances have occurred in the field of single particle impact electrochemistry (SPIEC) since the last review published 2 years ago[55], especially the development of new detection methods which feature the unique advantages of SPIEC technique for the analysis of various biomolecules. In this minireview, we have summarized recent developments in electroanalysis, especially electrochemical bioanalysis, categorized by different detection strategies. SPIEC in general is a powerful analytical tool that allows fast, convenient and more accurate detection at a single entity level to avoid ensemble averaging. Moreover, the electrocatalytic amplification strategy of SPIEC is promising to achieve more sensitive detection of biomolecules. We believe that SPIEC will drastically promote the development of analytical chemistry. However, there is still room to develop

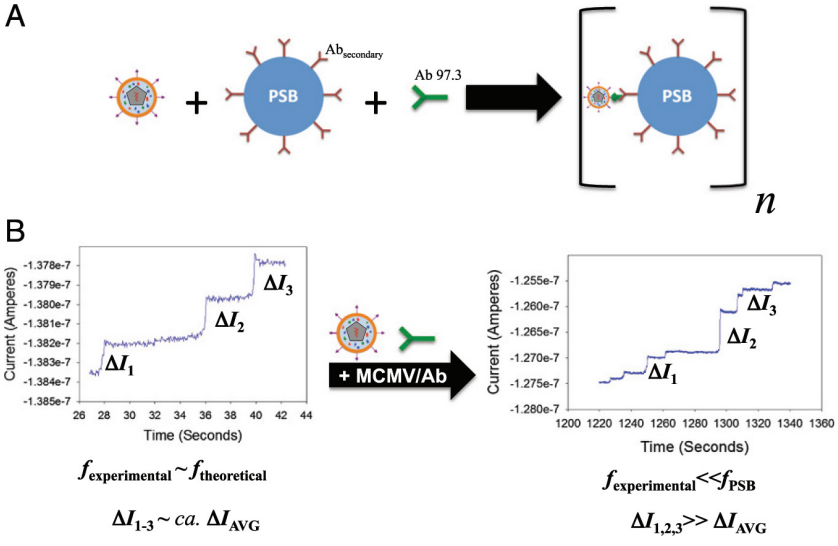

Fig. 5 Schematic illustration of the detection of single viruses from the response differences. (A) The polystyrene beads (PSBs) are functionalized with a secondary antibody (maroon Ys) that will specifically bind to the primary antibody (green Y). (B) Electrochemical responses with and without virus. Without the presence of virus, collisions of PSBs are observable with a characteristic current step height and frequency. Upon the addition of virus, aggregation of the PSBs will occur, resulting in decreased collision frequencies and larger current steps. Reprinted with permission from ref. 51. Copyright 2015 National Academy of Sciences.

SPIEC into a more mature stage.

1) Sensitivity of this technique is still not high enough. The high background current resulting from particle sticking and the electroactive non-target species may make the signal of single nanoparticles difficult to stand out. Therefore, electrode modification method and the selection of more specific electrode materials may be required.

2) Real applications of SPIEC should be further explored. In the first few years after the establishment of SPIEC, the main capability of this technique was to obtain a distribution of specific properties rather than averaged information from previous ensemble measurements. Although in recent years researchers already presented great progress in biodetection enabled by SPIEC, the limit of detection is still not qualified for clinically relevant detection. To find a particle tag with higher current amplification may be a potential solution to this challenge.

3) SPIEC of multiple particles might be worth looking into. Current studies still focus on the detection of one kind of molecules that are tagged to one type of particles. Simultaneous multi-target detection with multiple particles should be highly demanded in clinically related utilizations.

4) The significantly reduced reaction time of SPIEC can be made use of. Some features of SPIEC already manifested advantages in bioanalysis, such as to bring a redox molecules-containing bioentity to the electrode surface to avoid the diffusional loss of the redox molecules in solution. However, there is no study ever that utilizes the intrinsic feature of the significantly reduced reaction time scale during each transient collision of particles to an electrode, which may avoid the fatigue problem of catalytic particles fixed on an electrode under long-term electrochemical stress. Such feature may inspire a change in the working mode of reactions that are less efficient under continuous long-term operations.

5) Other analytical techniques should be combined with SPIEC to resolve more information. The lack of structural information is still an issue for SPIEC. If coupled with other techniques such as TEM, SPIEC is promising in providing a great deal of spatial and temporal information on single particles. Moreover, the plasmonic property of nanoparticles is now receiving tremendous attention. To combine SPIEC with such techniques as electrochemiluminescence, chemiluminescence, and surface plasmon resonance (SPR) will allow better understanding of plamonic nanoparticles and meanwhile benefit their utilization in photoelectrochemical applications at a single entity level.

Acknowledgements

This paper was supported by the grant from the National Natural Science Foundation of China (No. 21804036).

References:

[1] Bergamaski K, Pinheiro A L N, Teixeira-Neto E, et al. Nanoparticle size effects on methanol electrochemical oxidation on carbon supported platinum catalysts[J]. The Journal of Physical Chemistry B, 2006, 110(39): 19271-19279.

[2] Chen C, Kang Y J, Huo Z Y, et al. Highly crystalline multimetallic nanoframes with three-dimensional electrocatalytic surfaces[J]. Science, 2014, 343(6177): 1339-1343.

[3] Chen Q L(陈巧丽), Li H Q(李慧齐), Jiang Y Q(蒋亚琪), et al. Constructions of noble metal nanocrystals with specific crystal facets and high surface area[J]. Journal of Electrochemistry(电化学), 2018, 24(6): 602-614.

[4] Xiu L Y(修陆洋), Yu M Z(于梦舟), Yang P J(杨鹏举), et al. Caging porous Co-N-C nanocomposites in 3D graphene as active and aggregation-resistant electrocatalyst for oxygen reduction reaction[J]. Journal of Electrochemistry(电化学), 2018, 24(6): 715-725.

[5] Streeter I, Baron R, Compton R G. Voltammetry at nanoparticle and microparticle modified electrodes: theory and experiment[J]. The Journal of Physical Chemistry C, 2007, 111(45): 17008-17014.

[6] Korshunov A V, Heyrovsky M. Voltammetry of metallic powder suspensions on mercury electrodes[J]. Electroanalysis, 2006, 18(4): 423-426.

[7] Xiao X Y, Bard A J. Observing single nanoparticle collisions at an ultramicroelectrode by electrocatalytic amplification[J]. Journal of the American Chemical Society, 2007, 129(31): 9610-9612.

[8] Zhou Y G, Rees N V, Compton R G. The electrochemical detection and characterization of silver nanoparticles in aqueous solution[J]. Angewandte Chemie International Edi-

tion, 50(18): 4219-4221.

[9] Hellberg D, Scholz F, Schauer F, et al. Bursting and spreading of liposomes on the surface of a static mercury drop electrode[J]. Electrochemistry Communications, 2002, 4 (4): 305-309.

[10] Banks C E, Rees N V, Compton R G. Sonoelectrochemistry in acoustically emulsified media[J]. Journal of Electroanalytical Chemistry, 2002, 535(1/2): 41-47.

[11] Xiao X Y, Fan F R F, Zhou J P, et al. Current transients in single nanoparticle collision events[J]. Journal of the American Chemical Society, 2008, 130(49): 16669-16677.

[12] Zhou Y G, Rees N V, Pillay J, et al. Gold nanoparticles show electroactivity: counting and sorting nanoparticles upon impact with electrodes[J]. Chemical Communications, 2011, 48(2): 224-226.

[13] Batchelor-McAuley C, Ellison J, Tschulik K, et al. *In situ* nanoparticle sizing with zeptomole sensitivity[J]. Analyst, 2015, 140(15): 5048-5054.

[14] Rees N V, Zhou Y G, Compton R G. The aggregation of silver nanoparticles in aqueous solution investigated via anodic particle coulometry[J]. ChemPhysChem, 2011, 12 (9): 1645-1647.

[15] Haddou B, Rees N V, Compton R G. Nanoparticle-electrode impacts: the oxidation of copper nanoparticles has slow kinetics[J]. Physical Chemistry Chemical Physics, 2012, 14(39): 13612-13617.

[16] Zhou Y G, Haddou B, Rees N V, et al. The charge transfer kinetics of the oxidation of silver and nickel nanoparticles via particle-electrode impact electrochemistry[J]. Physical Chemistry Chemical Physics, 2012, 14 (41): 14354-14357.

[17] Zhou Y G, Rees N V, Compton R G. Electrochemistry of nickel nanoparticles is controlled by surface oxide layers [J]. Physical Chemistry Chemical Physics, 2013, 15(3): 761-763.

[18] Stuart E J E, Zhou Y G, Rees N V, et al. Determining unknown concentrations of nanoparticles: the particle-impact electrochemistry of nickel and silver[J]. RSC Advances, 2012, 2(17): 6879-6884.

[19] Rees N V, Zhou Y G, Compton R G. The non-destructive sizing of nanoparticles via particle-electrode collisions: Tag-redox coulometry (TRC)[J]. Chemical Physics Letters, 2012, 525: 69-71.

[20] Little C A, Li X T, Batchelor-McAuley C, et al. Particle-electrode impacts: Evidencing partial versus complete oxidation via variable temperature studies[J]. Journal of Electroanalytical Chemistry, 2018, 823: 492-498.

[21] Little C A, Xie R C, Batchelor-Mcauley C, et al. A quantitative methodology for the study of particle-electrode impacts[J]. Physical Chemistry Chemical Physics, 2018, 20(1): 13537-13546.

[22] Sokolov S V, Tschulik K, Batchelor-McAuley C, et al. Reversible or not? Distinguishing agglomeration and aggregation at the nanoscale[J]. Analytical Chemistry, 2015, 87(19): 10033-10039.

[23] Kwon S J, Fan F R F, Bard A J. Observing iridium oxide (IrO_x) single nanoparticle collisions at ultramicroelectrodes[J]. Journal of the American Chemical Society, 2010, 132(38): 13165-13167.

[24] Xiao X Y, Pan S L, Jang J S, et al. Single nanoparticle electrocatalysis: effect of monolayers on particle and electrode on electron transfer[J]. The Journal of Physical Chemistry C, 2009, 113(33): 14978-14982.

[25] Chen L F, Tanner E E L, Lin C H, et al. Impact electrochemistry reveals that graphene nanoplatelets catalyse the oxidation of dopamine via adsorption[J]. Chemical Science, 2018, 9(1): 152-159.

[26] Xiang Z P, Deng H Q, Peljo P, et al. Electrochemical dynamics of a single platinum nanoparticle collision event for the hydrogen evolution reaction[J]. Angewandte Chemie International Edition, 57(13): 3464-3468.

[27] Tschulik K, Haddou B, Omanović D, et al. Coulometric sizing of nanoparticles: cathodic and anodic impact experiments open two independent routes to electrochemical sizing of Fe_3O_4 nanoparticles[J]. Nano Research, 2013, 6 (11): 836-841.

[28] Zampardi G, Thoming J, Naatz H, et al. Electrochemical behavior of single CuO nanoparticles: implications for the assessment of their environmental fate[J]. Small, 2018, 14(32): 1801765.

[29] Zhao L J, Qian R C, Ma W, et al. Electrocatalytic efficiency analysis of catechol molecules for NADH oxidation during nanoparticle collision[J]. Analytical Chemistry, 2016, 88(17): 8375-8379.

[30] Zhou Y G, Rees N V, Compton R G. The electrochemical detection of tagged nanoparticles via particle-electrode collisions: nanoelectroanalysis beyond immobilisation[J]. Chemical Communications, 2012, 48(19): 2510-2512.

[31] Cheng W, Zhou X F, Compton R G. Electrochemical sizing of organic nanoparticles[J]. Angewandte Chemie International Edition, 2013, 52(49): 12980-12982.

[32] Kim B K, Boika A, Kim J, et al. Characterizing emulsions by observation of single droplet collisions attoliter electrochemical reactors[J]. Journal of the American Chemical Society, 2014, 136(13): 4849-4852.

[33] Kim B K, Kim J, Bard A J. Electrochemistry of a single

attoliter emulsion droplet in collisions[J]. Journal of the American Chemical Society, 2015, 137(6): 2343-2349.

[34] Feng A, Cheng W, Compton R G. Measuring the oxygen content of a single oil droplet[J]. Chemical Science, 2016, 7(10): 6458-6462.

[35] Lebègue E, Anderson C M, Dick J E, et al. Electrochemical detection of single phospholipid vesicle collisions at a Pt ultramicroelectrode[J]. Langmuir, 2015, 31(42): 11734-11739.

[36] Dunevall J, Fathali H, Najafinobar N, et al. Characterizing the catecholamine content of single mammalian vesicles by collision-adsorption events at an electrode[J]. Journal of the American Chemical Society, 2015, 137(13): 4344-4346.

[37] Li X C, Majdi S, Dunevall J, et al. Quantitative measurement of transmitters in individual vesicles in the cytoplasm of single cells with nanotip electrodes[J]. Angewandte Chemie International Edition, 54(41): 11978-11982.

[38] Cheng W, Compton R G. Investigation of single-drug-encapsulating liposomes using the nano-impact method[J]. Angewandte Chemie International Edition, 2014, 53(50): 13928-13930.

[39] Cheng W, Compton R G. Measuring the content of a single liposome through electrocatalytic nanoimpact "titrations"[J]. ChemElectroChem, 2016, 3(12): 2017-2020.

[40] Kwon S J, Bard A J. DNA analysis by application of Pt nanoparticle electrochemical amplification with single label response[J]. Journal of the American Chemical Society, 2012, 134(26): 10777-10779.

[41] Castaneda A D, Robinson D A, Stevenson K J, et al. Electrocatalytic amplification of DNA-modified nanoparticle collisions via enzymatic digestion[J]. Chemical Science, 2016, 7(10): 6450-6457.

[42] Castañeda A D, Brenes N J, Kondajji A, et al. Detection of microRNA by electrocatalytic amplification: a general approach for single-particle biosensing[J]. Journal of the American Chemical Society, 2017, 139(22): 7657-7664.

[43] Li D, Liu J Q, Barrow C J, et al. Protein electrochemistry using graphene-based nano-assembly: an ultrasensitive electrochemical detection of protein molecules via nanoparticle-electrode collisions[J]. Chemical Communications, 2014, 50(60): 8197-8200.

[44] Dick J E, Renault C, Bard A J. Observation of single-protein and DNA macromolecule collisions on ultramicroelectrodes[J]. Journal of the American Chemical Society, 2015, 137(26): 8376-8379.

[45] Sekretaryova A N, Vagin M Y, Turner A P F, et al. Electrocatalytic currents from single enzyme molecules[J]. Journal of the American Chemical Society, 2016, 138(8): 2504-2507.

[46] Lin C H, Sepunaru L, Kätelhön E, et al. Electrochemistry of single enzymes: fluctuations of catalase activities[J]. The Journal of Physical Chemistry Letters, 2018, 9(11): 2814-2817.

[47] Jiang L, Santiago I, Foord J. Observation of nanoimpact events of catalase on diamond ultramicroelectrodes by direct electron transfer[J]. Chemical Communications, 2017, 53(59): 8332-8335.

[48] Han L H, Wang W, Nsabimana J, et al. Single molecular catalysis of a redox enzyme on nanoelectrodes[J]. Faraday Discussions, 2016, 193: 133-139.

[49] Sepunaru L, Plowman B J, Sokolov S V, et al. Rapid electrochemical detection of single influenza viruses tagged with silver nanoparticles[J]. Chemical Science, 2016, 7(6): 3892-3899.

[50] Dick J E, Hilterbrand A T, Strawsine L M, et al. Enzymatically enhanced collisions on ultramicroelectrodes for specific and rapid detection of individual viruses[J]. Proceedings of the National Academy of Sciences, 2016, 113 (23): 6403-6408.

[51] Dick J E, Hilterbrand A T, Boika A, et al. Electrochemical detection of a single cytomegalovirus at an ultramicroelectrode and its antibody anchoring[J]. Proceedings of the National Academy of Sciences, 2015, 112 (17): 5303-5308.

[52] Sepunaru L, Sokolov S V, Holter J, et al. Electrochemical red blood cell counting: one at a time[J]. Angewandte Chemie International Edition, 55(33): 9768-9771.

[53] Dick J E. Electrochemical detection of single cancer and healthy cell collisions on a microelectrode[J]. Chemical Communications, 2016, 52(72): 10906-10909.

[54] Ho T L T, Hoang N T T, Lee J, et al. Determining mean corpuscular volume and red blood cell count using electrochemical collision events[J]. Biosensors and Bioelectronics, 2018, 110: 155-159.

[55] Peng Y Y, Qian R C, Hafez M E, et al. Stochastic collision nanoelectrochemistry: a review of recent developments[J]. ChemElectroChem, 2017, 4(5): 977-985.

[56] Anderson T J, Zhang B. Single-nanoparticle electrochemistry through immobilization and collision[J]. Accounts of Chemical Research, 2016, 49(11): 2625-2631.

[57] Qiu D F, Wang S, Zheng Y Q, et al. One at a time: counting single-nanoparticle/electrode collisions for accurate particle sizing by overcoming the instability of gold nanoparticles under electrolytic conditions[J]. Nanotechnology, 2013, 24(50): 505707.

[58] Stuart E J E, Tschulik K, Omanovic D, et al. Electrochemical detection of commercial silver nanoparticles: identification, sizing and detection in environmental media[J]. Nanotechnology, 2013, 24(44): 444002.

[59] Stuart E J E, Rees N V, Cullen J T, et al. Direct electrochemical detection and sizing of silver nanoparticles in seawater media[J]. Nanoscale, 2013, 5(1): 174-177.

[60] Li X T, Batchelor-McAuley C, Compton R G. Silver nanoparticle detection in real world environments via particle impact electrochemistry[J]. ACS Sensors, 2019, 4(2): 464-470.

[61] Ellison J, Tschulik K, Stuart E J E, et al. Get more out of your data: a new approach to agglomeration and aggregation studies using nanoparticle impact experiments[J]. ChemistryOpen, 2013, 2(2): 69-75.

[62] Toh H S, Jurkschat K, Compton R G. The influence of the capping agent on the oxidation of silver nanoparticles: nano-impacts versus stripping voltammetry[J]. Chemistry - A European Journal, 2015, 21(7): 2998-3004.

[63] Lees J C, Ellison J, Batchelor-McAuley C, et al. Nanoparticle impacts show high-ionic-strength citrate avoids aggregation of silver nanoparticles[J]. ChemPhysChem, 2013, 14(17): 3895-3897.

[64] Dasari R, Walther B, Robinson D A, et al. Influence of the redox indicator reaction on single-nanoparticle collisions at mercury- and bismuth-modified Pt ultramicroelectrodes[J]. Langmuir, 2013, 29(48): 15100-15106.

[65] Holt L R, Plowman B J, Young N P, et al. The electrochemical characterization of single core-shell nanoparticles[J]. Angewandte Chemie International Edition, 2016, 55(1): 397-400.

[66] Plowman B J, Young N P, Batchelor-McAuley C, et al. Nanorod aspect ratios determined by the nano-impact technique[J]. Angewandte Chemie International Edition, 2016, 55(24): 7002-7005.

[67] Saw E N, Grasmik V, Rurainsky C, et al. Electrochemistry at single bimetallic nanoparticles-using nano impacts for sizing and compositional analysis of individual AgAu alloy nanoparticles[J]. Faraday Discussions, 2016, 193: 327-338.

[68] Lim C S, Tan S M, Sofer Z, et al. Impact electrochemistry of layered transition metal dichalcogenides[J]. ACS Nano, 2015, 9(8): 8474-8483.

[69] Yoo J J, Kim J, Crooks R M. Direct electrochemical detection of individual collisions between magnetic microbead/silver nanoparticle conjugates and a magnetized ultramicroelectrode[J]. Chemical Science, 2015, 6(11): 6665-6671.

[70] Sepunaru L, Tschulik K, Batchelor-McAuley C, et al. Electrochemical detection of single *E. coli* bacteria labeled with silver nanoparticles[J]. Biomaterials Science, 2015, 3(6): 816-820.

[71] Couto R A S, Chen L, Kuss S, et al. Detection of *Escherichia coli* bacteria by impact electrochemistry[J]. Analyst, 2018, 143(20): 4840-4843.

[72] Stuart E J E, Tschulik K, Batchelor-McAuley C, et al. Electrochemical observation of single collision events: Fullerene nanoparticles[J]. ACS Nano, 2014, 8(8): 7648-7654.

[73] Toh H S, Compton R G. Electrochemical detection of single micelles through 'nano-impacts'[J]. Chemical Science, 2015, 6(8): 5053-5058.

[74] Dick J E, Renault C, Kim B K, et al. Simultaneous detection of single attoliter droplet collisions by electrochemical and electrogenerated chemiluminescent responses[J]. Angewandte Chemie International Edition, 2014, 53(44): 11859-11862.

[75] Bard A J, Zhou H, Kwon S J. Electrochemistry of single nanoparticles via electrocatalytic amplification[J]. Israel Journal of Chemistry, 2010, 50(3): 267-276.

[76] Stuart E J E, Rees N V, Compton R G. Particle-impact voltammetry: The reduction of hydrogen peroxide at silver nanoparticles impacting a carbon electrode[J]. Chemical Physics Letters, 2012, 531: 94-97.

[77] Kahk J M, Rees N V, Pillay J, et al. Electron transfer kinetics at single nanoparticles[J]. Nano Today, 2012, 7(3): 174-179.

[78] Alligrant T M, Anderson M J, Dasari R, et al. Single nanoparticle collisions at microfluidic microband electrodes: the effect of electrode material and mass transfer [J]. Langmuir, 2014, 30(44): 13462-13469.

[79] Kleijn S E F, Serrano-Bou B, Yanson A I, et al. Influence of hydrazine-induced aggregation on the electrochemical detection of platinum nanoparticles[J]. Langmuir, 2013, 29(6): 2054-2064.

[80] Alligrant T M, Nettleton E G, Crooks R M. Electrochemical detection of individual DNA hybridization events[J]. Lab on A Chip, 2013, 13(3): 349-354.

[81] Cheng W, Compton R G. Quantifying the electrocatalytic turnover of vitamin B12-mediated dehalogenation on single soft nanoparticles[J]. Angewandte Chemie International Edition, 2016, 55(7): 2545-2549.

[82] Quinn B M, van't Hof P G, Lemay S G. Time-resolved electrochemical detection of discrete adsorption events [J]. Journal of the American Chemical Society, 2004, 126(27): 8360-8361.

[83] Boika A, Thorgaard S N, Bard A J. Monitoring the elec-

trophoretic migration and adsorption of single insulating nanoparticles at ultramicroelectrodes[J]. The Journal of Physical Chemistry B, 2012, 117(16): 4371-4380.

[84] Lee J Y, Kim B K, Kang M, et al. Label-free detection of single living bacteria via electrochemical collision event [J]. Scientific Reports, 2016, 6: 30022.

[85] Guanyue G, Dengchao W, Brocenschi R F , et al. Toward the detection and identification of single bacteria by electrochemical, collision technique[J]. Analytical Chemistry, 2018, 90(20): 12123-12130.

[86] Karimi A, Hayat A, Andreescu S. Biomolecular detection at ssDNA-conjugated nanoparticles by nano-impact electrochemistry[J]. Biosensors and Bioelectronics, 2017, 87: 501-507.

单颗粒电化学：纳米颗粒及生物分子的分析检测

张建花，周一歌*

（化学生物传感与计量学国家重点实验室，湖南大学化学化工学院，化学生物学与纳米医学研究所，湖南 长沙 410082）

摘要：单颗粒碰撞电化学近年来已得到迅速发展并在纳米颗粒的性质分析及包括 DNA、RNA、蛋白质、酶、细菌、病毒、囊泡类物质等生物体的检测上展示出广阔的前景. 在这篇综述中，作者总结了近年来单颗粒碰撞电化学在电化学分析中的进展，按分析检测的策略不同分为以下几个部分阐述：纳米粒子或标记纳米粒子的直接电解；包含氧化还原活性分子的软颗粒的直接电解；颗粒的间接电化学行为；区域扩散阻塞效应；电流强度及碰撞频率的改变.

关键词：单颗粒；碰撞；电化学；分析；生物分子