Electrochemistry **Very Important Paper**

International Edition: DOI: 10.1002/anie.201501820
German Edition: DOI: 10.1002/ange.201501820

Oxygen Reduction Mediated by Single Nanodroplets Containing Attomoles of Vitamin B_{12}: Electrocatalytic Nano-Impacts Method**

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Abstract: *We report the use of single Vitamin B_{12} nanodroplets to mediate the reduction of oxygen in neutral buffer. Electron transfer to single Vitamin B_{12} nanodroplets is observed using the nano-impacts method and shown to be quantitative. The mechanism of mediated oxygen reduction by single VB_{12} droplets is revealed as via both Co^{II} and Co^{I} reduced from Co^{III} in VB_{12} through one or two electron transfer followed by the four-electron reduction of oxygen.*

The electrochemistry of single nanoparticles typically as a result of their random collision with a suitable electrode as a consequence of their Brownian motion to induce quantitative oxidation or reduction of, or catalytic reaction by, the nanoparticle, so called "nano impacts", has allowed new insights into charge transfer together as well as providing the opportunity to identify altered reactivity at the nanoscale with analytical methodology for single nanoparticle characterization.[1] Recent work has extended the scope of the nanoparticles that can be studied in this way to embrace liquid droplets[2] including nanoliposomes.[2e] Such studies are important because of the use of emulsions in electrosynthesis and the scope for avoiding environmentally non-benign non-aqueous solvents.[3] Moreover, by studying seemingly complex processes using single nanodroplets the very high rates of mass transport to and from the droplet may allow mechanistic simplification and corresponding meditation of catalytic processes.

One important biphasic system is that involving Vitamin B_{12} emulsions in water. Electron transfer mediated through this species has found diverse uses in synthesis;[4] the redox center in Vitamin B_{12} is Co^{III}. In addition electrodes modified with molecule Vitamin B_{12} catalyze the reduction of oxygen,[5] a process at the heart of energy transformation technologies. Here, we report the use of single nanodroplets of Vitamin B_{12} to mediate the low potential reduction of dioxygen. In particular the mechanistic simplification offered by the conditions of high mass transport prevailing during "nano impacts" allows insights into the key relevant steps involved in the reaction.

The structure of the Vitamin VB_{12} nanodroplet is illustrated in Figure S1 in the Supporting Information. Prior to investigating the reduction of oxygen mediated by VB_{12} nanodroplets, the electrochemical behavior of VB_{12} was investigated in an oxygen-free buffer solution. First, a glassy carbon (GC) macroelectrode (diameter = 3 mm) was modified with VB_{12} droplets and cyclic voltammograms recorded. Figure S2 shows the first three scans of this modified electrode immersed in phosphate buffered saline (PBS) buffer (pH = 6.9). Two reduction peaks were observed around −0.10 and −0.95 V versus the saturated calomel reference electrode (SCE). These two reductive peaks likely correspond to the two stages of each one-electron transfer between Co (^{III}) and Co^{II}, Co^{II} and Co^{I} respectively, which is consistent with the reported values for reduction of VB_{12} on a glassy carbon electrode.[5] This was further confirmed by the cyclic voltammograms recorded for VB_{12} dissolved in the buffer solution using a glassy carbon electrode, where similar two reductive peaks position were also measured (see Figure S3 in the Supporting Information).

Next, a carbon microelectrode was immersed in a N_2 degassed PBS buffer solution and a known concentration of solution dispersed VB_{12} droplets added.[6] Under potentiostatted conditions, clear reductive (Faradaic) current spikes at −0.30 V versus SCE were detected (Figure 1). This potential is significantly more negative than the first reduction potential of VB_{12}, ensuring the complete reduction of Co^{III} to Co^{II}. The onset of spikes were found to be dependent on the

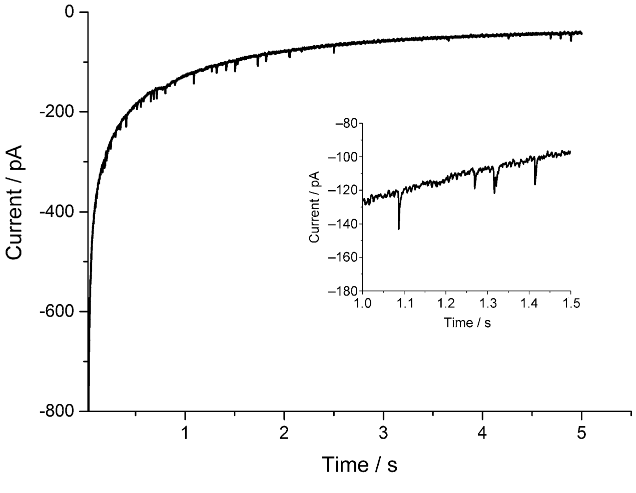

Figure 1. Chronoamperometric profiles showing Faradaic reduction spikes of single VB_{12} droplets in oxygen-free PBS buffer (100 mM; pH 6.9) at −0.30 V versus SCE.

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[**] The European Research Council under the European Union's Seventh Framework Programme (ERC Grant Agreement number 320403) is gratefully acknowledged for funding this work. We thank Dr. D Omanović (Center for Marine and Environmental Research Zagreb, Croatia) for developing Signal Counter software for data analysis.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201501820.

reductive potentials and no reductive spikes of VB_{12} droplets at the reductive potentials of +0.1 V or above, confirming the spikes correspond to Faradaic reduction of VB_{12} droplets. Another control experiment was conducted at the potential of −0.30 V with no droplets in the solution and no spikes were detected, further confirming that the occurrence of reductive spikes is due to the random collisions of droplets with the electrode.

The charge from the reduction of single droplets was calculated by integrating the area of each spike (Figure S5). To quantitatively characterize the reduction of single VB_{12} droplets, the modal size D_{np} of VB_{12} encapsulating droplets was estimated to be 615 nm through dynamic light scattering (DLS; Figure S6). By comparing the modal charge resulting from reduction of 146 single VB_{12} droplets Q_{y1} ($Q_{y1} = 0.033$ pC, Figure S7) from experimental nano-impact method with the theoretical estimated modal charge Q_1 ($Q_1 = 0.029$ pC) of single VB_{12} droplet indicates that there is effectively complete reduction of single VB_{12} droplets (see the Supporting Information).

A "voltammogram" of single droplet electrochemistry was measured reflecting the average charge transfer to the droplets as a function of potential, as illustrated in Figure 2. From Figure 2, the average charge was observed to reach a plateau at a higher potential and the average charge value Q_{av2} (0.35 ± 0.08 pC, where the error of the mean charge is the standard error of the mean given by $SD/\sqrt{n}$ where SD is the standard deviation and n is the sample number, in this case the number of spikes) is almost two times of Q_{av1} (0.17 ± 0.02 pC) measured at −0.3 V, confirming a second electron transfer resulted from subsequent reduction of Co^{II} to Co^{I} upon a droplet impacting at higher potential such as −1.25 V or more negative, as illustrated in Scheme S1 (see the Supporting Information).

Subsequently, voltammetric methods were used to study the electrochemical behavior of VB_{12} emulsion droplets in the presence of oxygen. When VB_{12} is absent in O_2 saturated buffer solution, the voltammogram (Figure 3) shows an irreversible peak at −0.80 V for the reduction of oxygen ($O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$). The CVs of a VB_{12} droplet-modified GC electrode (nanodroplets suspension was drop-casted on the electrode, see the Experimental Section) was also recorded in the presence of saturated oxygen, as shown in Figure 3, showing two irreversible waves which are due to the reduction of oxygen.[5]

It was found that the presence of VB_{12} in buffer solution decreases the overpotential of the oxygen reduction on GC electrode about 200 mV, with another reduction peak arising at −0.95 V. Comparing to voltammograms without oxygen (black line) or without VB_{12} (red line), this clearly demonstrates the mediating activity of VB_{12} droplets modified on the electrode for the reduction of oxygen.

Corresponding nano-impact experiments of single VB_{12} nanodroplets were conducted in an O_2-saturated PBS buffer. As before, the onset of spikes was found to be dependent on the reductive potentials, with clear spikes observed at −0.3 V or more negative potential. No spikes were seen at positive potential such as 0.1 V or above. The observation is consistent with the measurements in the N_2 degassed buffer, suggesting

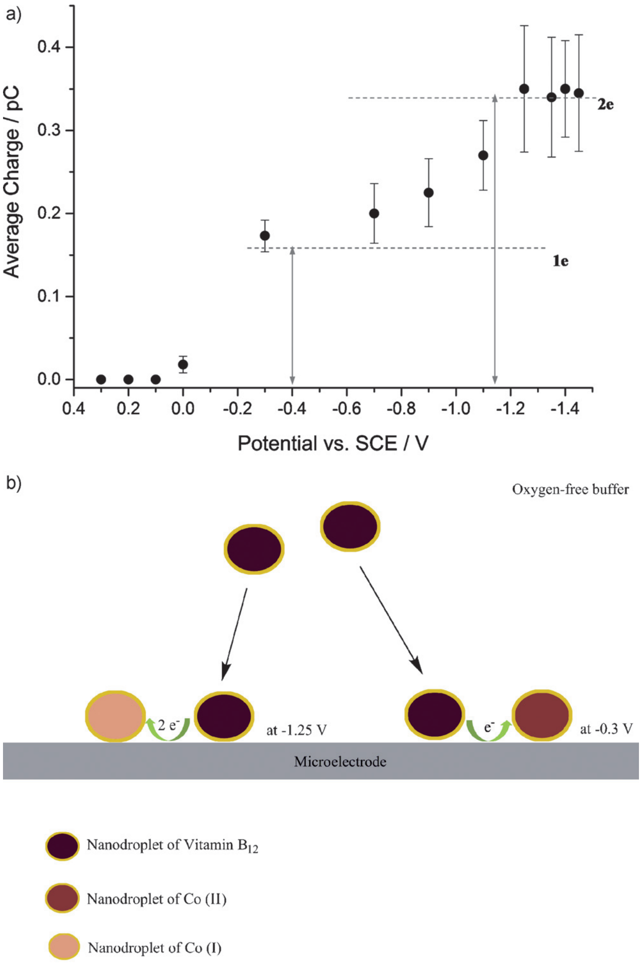


Figure 2. a) Average charge Q for the reduction (black dots) of individual VB_{12} droplet impact versus potential E. b) Likely mechanism of reduction of single VB_{12} nanodroplets.

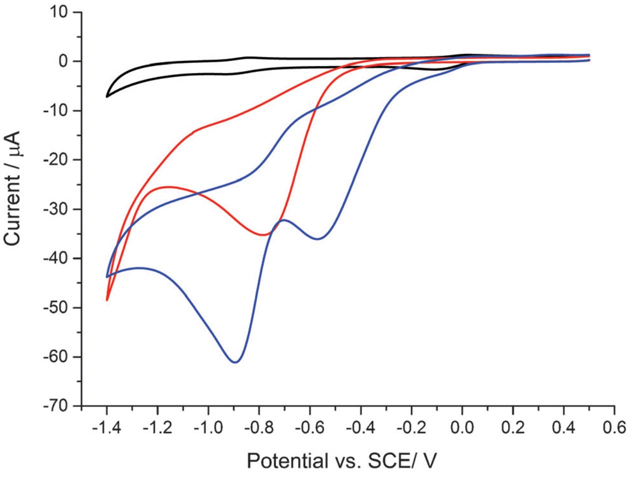


Figure 3. Voltammogram of VB_{12} droplet modified glassy carbon electrode in PBS buffer (pH 6.9) without oxygen presence (black line); voltammogram of bare glassy carbon electrode in oxygen saturated PBS buffer (pH 6.9; red line); voltammogram of VB_{12} droplet modified glassy carbon electrode electrode in oxygen saturated PBS buffer (pH 6.9; blue line).

that the observed spikes in an O_2 saturated PBS buffer are also associated with Faradaic reduction of VB_{12} droplets. However, when the potential was set more negative such as −1.25 V, the amplitude of the spikes significantly increased, as compared to those in the N_2 degassed buffer as shown in Figure 4a, indicating there is an oxygen reduction reaction mediated by VB_{12} when single VB_{12} nanodroplets collide with the electrode.

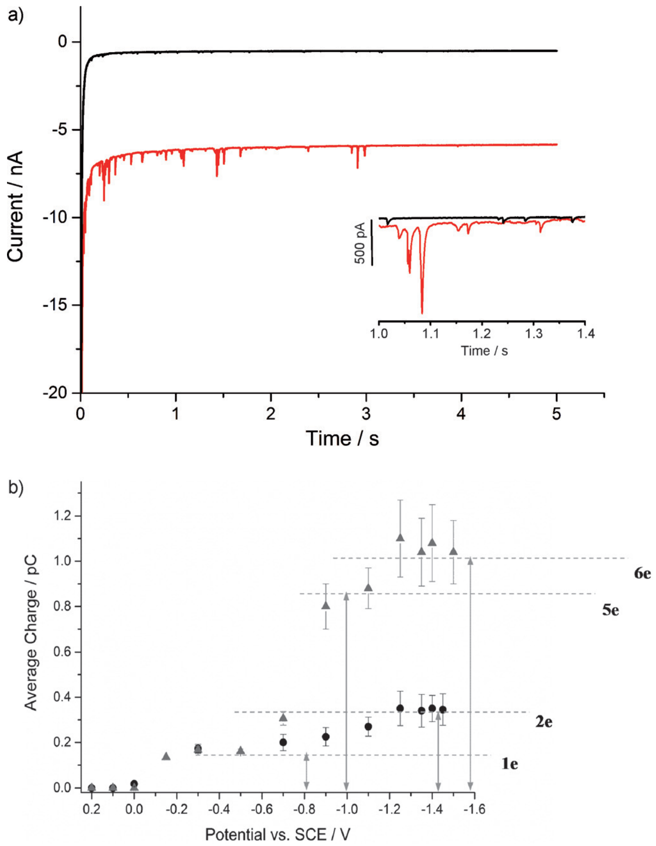
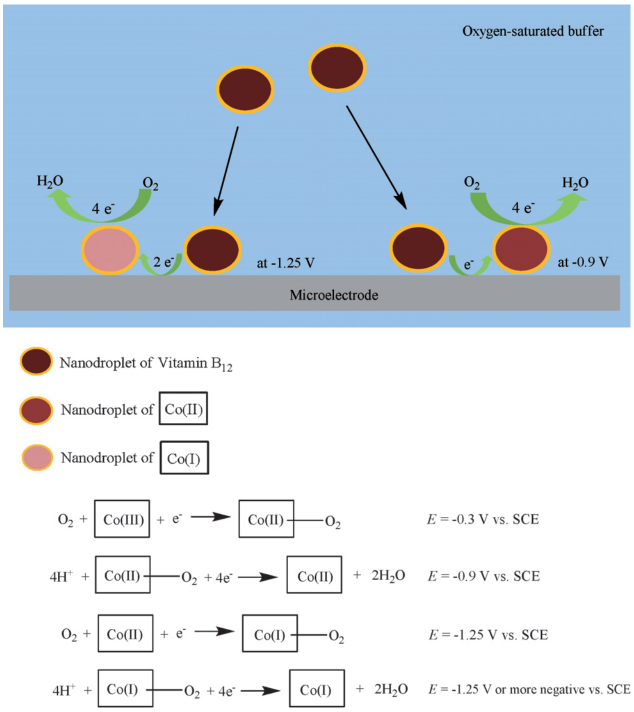


Figure 4. a) Chronoamperometric profiles showing Faradaic spikes of single VB_{12} droplets in oxygen-saturated PBS buffer (100 mM; pH 6.9; red line) or without oxygen (black line) at −1.25 V versus SCE. The inset showing the overlapped (background level adjusted) impact spikes. b) Average charge Q for individual VB_{12} droplets impacts versus potential E in PBS buffer with saturated oxygen (red triangles) and without oxygen (black dots). The labels show the number of electrons transferred at each step among stoichiometric reaction.

To verify this hypothesis, chronoamperograms were recorded at a range of electrode potentials between 0 and −1.5 V, the same potential range for chronoamperometric measurements without presence of oxygen such that the number of recorded impact events for each potential was greater than 150. By integrating the area of each spike as before, the average charge of individual spikes at a range of potentials was calculated and then was plotted as a function of potential, as illustrated in Figure 4b. Comparing this to the "voltammogram" of single droplets in the absence of O_2 (Figure 4b, black dots), the value of average charge of each spike potential is significantly larger at −0.9 V or more negative, indicating that at sufficient negative potentials there is an oxygen reduction accompanying the reduced of VB_{12} contributing to the injection of the charge measured at each spike when individual VB_{12} nanodroplets collide with the electrode.

The oxygen reduction can proceed by two main, parallel pathways, namely the peroxide pathway where oxygen undergoes a two-electron reduction to peroxide ($O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$) or a direct four electron pathway to give water ($O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$),[7] as shown in Scheme 1.



Scheme 1. Likely mechanism of oxygen reduction by single VB_{12} nanodroplets.

Whilst, the mechanism of the role of VB_{12} for oxygen reduction is still controversial. Qui and Dong concluded oxygen reduction via $\mathrm{Co^{II}}$ and $\mathrm{Co^{I}}$ reduced from $\mathrm{Co^{III}}$, both oxidative state are active for four electron reduction of oxygen,[5a] while Zagal et al. implied the role of dangling imidazole chain of $\mathrm{Co^{II}}$ with two- or four-electron reduction of oxygen.[5b] Other mechanisms have been discussed.[8]

In the present study, the value of the charge Q_{av3} and Q_{av4} was further found to be 0.8 ± 0.1 pC and 1.1 ± 0.17 pC at −0.9 and −1.25 V, respectively. The values are approximately 5 times and 6 times Q_{av1} at −0.3 V (0.17 ± 0.15 pC; Figure 4). The similar charge measured at −0.3 V at oxygen-saturated solution, as compared to oxygen-free condition suggests that no oxygen reduction is active at −0.3 V or below, while a reduction of VB_{12} from $\mathrm{Co^{III}}$ to $\mathrm{Co^{II}}$ likely dominates at such a low potential. The number of electrons transferred at different potentials could be inferred, dependent on the applied potentials, to be 5 and 6 electrons transferred at −0.9 V and −1.25 V, indicating the following likely mechanism of oxygen reduction by single VB_{12} nanodroplets

(Scheme 1). Multiple catalytic cycles, as seen with the droplet-modified electrode, are not seen with nano-impact method, probably because of the short duration of the impact times.[9]

To summarize, we have shown oxygen reduction mediated by single nanodroplets, in addition to demonstrating the quantitative measurements of the size and electron transfer of the reduction of VB_{12} within a single droplet. The likely mechanism of oxygen reduction mediated by single VB_{12} droplet is also proposed which is through the reduced couples of VB_{12}, namely Co^{II} or Co^{I} reduced from VB_{12} through one- or two-electron transfer, followed by four-electron reduction of oxygen when single nanodroplets impacting the electrode.

To our knowledge, this is the first time a mediated reaction has been seen at single droplets containing attomoles of molecules. This procedure facilitates understanding the mechanism of oxygen reduction. The strategy is general and can be applied to designing a wide range of aqueous droplets containing various redox molecules for diverse catalytic reactions. We believe this would open a new area and lead to greater understanding and implications of "soft" nanoparticles such as droplets, micelles, polymers or organic nanomaterials towards more affordable and natural non-metal catalysts, as an alternative to "hard" precious metal nanomaterials heavily used for current energy systems and fuel cells.

Keywords: catalytic reaction · electrochemistry · nanodroplets · oxygen reduction · soft nanoparticles

How to cite: *Angew. Chem. Int. Ed.* **2015**, *54*, 7082–7085
Angew. Chem. **2015**, *127*, 7188–7191

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[1] a) Y. G. Zhou, N. V. Rees, R. G. Compton, *Angew. Chem. Int. Ed.* **2011**, *50*, 4219–4221; *Angew. Chem.* **2011**, *123*, 4305–4307; b) W. Cheng, X.-F. Zhou, R. G. Compton, *Angew. Chem. Int. Ed.* **2013**, *52*, 12980–12982; *Angew. Chem.* **2013**, *125*, 13218–13220; c) E. J. E. Stuart, K. Tschilik, C. Batchelor-McAuley, R. G. Compton, *ACS Nano* **2014**, *8*, 7648–7654; d) W. Cheng, R. G. Compton, *Trac-Trend Anal. Chem.* **2014**, *58*, 79–89; e) X.-F. Zhou, W. Cheng, R. G. Compton, *Angew. Chem. Int. Ed.* **2014**, *53*, 12587–12589; *Angew. Chem.* **2014**, *126*, 12795–12797; f) M. Pumera, *ACS Nano* **2014**, *8*, 7555–7558; g) N. V. Rees, *Electrochem. Commun.* **2014**, *43*, 83–86; h) X. Y. Xiao, A. J. Bard, *J. Am. Chem. Soc.* **2007**, *129*, 9610–9612; i) X. Y. Xiao, F. R. F. Fan, J. P. Zhou, A. J. Bard, *J. Am. Chem. Soc.* **2008**, *130*, 16669–16677; j) A. J. Bard, H. J. Zhou, S. J. Kwon, *Isr. J. Chem.* **2010**, *50*, 267–276; k) R. Dasari, D. A. Robinson, K. J. Stevenson, *J. Am. Chem. Soc.* **2013**, *135*, 570–573; l) S. E. Fosdick, M. J. Anderson, E. G. Nettleton, R. M. Crooks, *J. Am. Chem. Soc.* **2013**, *135*, 5994–5997; m) J. M. Kahk, N. V. Rees, J. Pillay, R. Tshikhudo, S. Vilakazi, R. G. Compton, *Nano Today* **2012**, *7*, 174–179.

[2] a) C. E. Banks, N. V. Rees, R. G. Compton, *J. Electroanal. Chem.* **2002**, *535*, 41–47; b) C. E. Banks, N. V. Rees, R. G. Compton, *J. Phys. Chem. B* **2002**, *106*, 5810–5813; c) A. D. Clegg, N. V. Rees, C. E. Banks, R. G. Compton, *ChemPhysChem* **2006**, *7*, 807–811; d) J. E. Dick, C. Renault, B. K. Kim, A. J. Bard, *Angew. Chem. Int. Ed.* **2014**, *53*, 11859–11862; *Angew. Chem.* **2014**, *126*, 12053–12056; e) W. Cheng, R. G. Compton, *Angew. Chem. Int. Ed. Angew. Chem. Int. Edit.* **2014**, *53*, 13928–13930; f) B. K. Kim, A. Boika, J. Kim, J. E. Dick, A. J. Bard, *J. Am. Chem. Soc.* **2014**, *136*, 4849–4852; g) J. E. Dick, C. Renault, B. K. Kim, A. J. Bard, *J. Am. Chem. Soc.* **2014**, *136*, 13546–13549; h) B. K. Kim, J. Kim, A. J. Bard, *J. Am. Chem. Soc.* **2015**, DOI: 10.1021/ja512065n.

[3] a) C. E. Banks, T. J. Davies, R. G. Evans, G. Hignett, A. J. Wain, N. S. Lawrence, J. D. Wadhawan, F. Marken, R. G. Compton, *Phys. Chem. Chem. Phys.* **2003**, *5*, 4053–4069; b) J. D. Wadhawan, F. J. Del Campo, R. G. Compton, J. S. Foord, F. Marken, S. D. Bull, S. G. Davies, D. J. Walton, S. Ryley, *J. Electroanal. Chem.* **2001**, *507*, 135–143; c) J. D. Wadhawan, F. Marken, R. G. Compton, S. D. Bull, S. G. Davies, *Chem. Commun.* **2001**, 87–88; d) J. D. Wadhawan, F. Marken, R. G. Compton, *Pure Appl. Chem.* **2001**, *73*, 1947–1955; e) X. Z. Niu, F. Gielen, J. B. Edel, A. J. deMello, *Nat. Chem.* **2011**, *3*, 437–442; f) N. Gasilova, Q. L. Yu, L. Qiao, H. H. Girault, *Angew. Chem. Int. Ed.* **2014**, *53*, 4408–4412; *Angew. Chem.* **2014**, *126*, 4497–4501; g) A. J. deMello, *Nature* **2006**, *442*, 394–402; h) M. Pera-Titus, L. Leclercq, J.-M. Clacens, F. De Campo, V. Nardello-Rataj, *Angew. Chem. Int. Ed.* **2015**, *54*, 2006–2021; *Angew. Chem.* **2015**, *127*, 2028–2044.

[4] a) R. Scheffold, S. Abrecht, R. Orlinski, H. R. Ruf, P. Stamouli, O. Tinembart, L. Walder, C. Weymuth, *Pure Appl. Chem.* **1987**, *59*, 363–372; b) D. Lexa, J. M. Saveant, *Acc. Chem. Res.* **1983**, *16*, 235–243; c) J. Kim, C. Gherasim, R. Banerjee, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 14551–14554; d) K. A. P. Payne, C. P. Quezada, K. Fisher, M. S. Dunstan, F. A. Collins, H. Sjuts, C. Levy, S. Hay, S. E. J. Rigby, D. Leys, *Nature* **2015**, *517*, 513–516.

[5] a) Q. S. Qiu, S. J. Dong, *Electrochim. Acta* **1993**, *38*, 2297–2303; b) J. H. Zagal, M. J. Aguirre, M. A. Paez, *J. Electroanal. Chem.* **1997**, *437*, 45–52; c) P. Tomčik, C. E. Banks, T. J. Davies, R. G. Compton, *Anal. Chem.* **2004**, *76*, 161–165.

[6] The small current (usual pA) upon nanoparticle impacting is normally measured using a microelectrode because of its much reduced capacitance.

[7] S. J. Guo, S. Zhang, S. H. Sun, *Angew. Chem. Int. Ed.* **2013**, *52*, 8526–8544; *Angew. Chem.* **2013**, *125*, 8686–8705.

[8] a) S. T. Chang, C. H. Wang, H. Y. Du, H. C. Hsu, C. M. Kang, C. C. Chen, J. C. S. Wu, S. C. Yen, W. F. Huang, L. C. Chen, M. C. Lin, K. H. Chen, *Energy Environ. Sci.* **2012**, *5*, 5305–5314; b) G. Wu, K. L. More, C. M. Johnston, P. Zelenay, *Science* **2011**, *332*, 443–447; c) B. F. Cao, G. M. Veith, R. E. Diaz, J. Liu, E. A. Stach, R. R. Adzic, P. G. Khalifah, *Angew. Chem. Int. Ed.* **2013**, *52*, 10753–10757; *Angew. Chem.* **2013**, *125*, 10953–10957; d) M. Pumera, H. Iwai, *Chem. Asian J.* **2009**, *4*, 554–560.

[9] E. Kätelhön, R. G. Compton, *Chem. Sci.* **2014**, *5*, 4592–4598.

Received: February 25, 2015