A Dual Functional Electroactive and Fluorescent Probe for Coupled Measurements of Vesicular Exocytosis with High Spatial and Temporal Resolution

Xiaoqing Liu, Alexandra Savy, Sylvie Maurin, Laurence Grimaud, François Darchen, Damien Quinton, Eric Labbé, Olivier Buriez, Jérôme Delacotte, Frédéric Lemaître, et al.

International Edition: DOI: 10.1002/anie.201611145
German Edition: DOI: 10.1002/ange.201611145

A Dual Functional Electroactive and Fluorescent Probe for Coupled Measurements of Vesicular Exocytosis with High Spatial and Temporal Resolution

*Xiaoqing Liu, Alexandra Savy, Sylvie Maurin, Laurence Grimaud, François Darchen, Damien Quinton, Eric Labbé, Olivier Buriez, Jérôme Delacotte, Frédéric Lemaître, and Manon Guille-Collignon**

Abstract: *In this work, Fluorescent False Neurotransmitter 102 (FFN102), a synthesized analogue of biogenic neurotransmitters, was demonstrated to show both pH-dependent fluorescence and electroactivity. To study secretory behaviors at the single-vesicle level, FFN102 was employed as a new fluorescent/electroactive dual probe in a coupled technique (amperometry and total internal reflection fluorescence microscopy (TIRFM)). We used N13 cells, a stable clone of BON cells, to specifically accumulate FFN102 into their secretory vesicles, and then optical and electrochemical measurements of vesicular exocytosis were experimentally achieved by using indium tin oxide (ITO) transparent electrodes. Upon stimulation, FFN102 started to diffuse out from the acidic intravesicular microenvironment to the neutral extracellular space, leading to fluorescent emissions and to the electrochemical oxidation signals that were simultaneously collected from the ITO electrode surface. The correlation of fluorescence and amperometric signals resulting from the FFN102 probe allows real-time monitoring of single exocytotic events with both high spatial and temporal resolution. This work opens new possibilities in the investigation of exocytotic mechanisms.*

Vesicular exocytosis is a ubiquitous process for intercellular communication in living systems. During exocytosis, an intracellular vesicle fuses with the cell membrane and releases its contents to the extracellular space within a brief period of time (milliseconds to seconds), resulting in various physiological responses, such as insertion of receptors at defined areas of the plasma membrane, release of messengers, and removal of waste products from the cell.[1] Owing to its involvement in many normal and pathological events in living cells, exocytosis has attracted increasing research attention.

Amperometry and total internal reflection fluorescence microscopy (TIRFM) are two of the most used techniques for studying exocytosis. Amperometry provides quantitative information on the amount of secreted electroactive molecules, with sub-millisecond temporal resolution that matches the fast kinetics of exocytotic release.[2] However, this method is blind to vesicle behaviors before secretion and is unable to locate the releasing sites in living cells. In contrast, by virtue of visible fluorescence, TIRFM is capable of revealing the distribution of secretory vesicles within a single cell, and can track their motility during the whole exocytotic process in real-time.[3] Despite its excellent spatial resolution, this optical method lacks the temporal resolution to determine the kinetic parameters. Considering their complementary advantages, we successfully developed a technique combining these two analytical methods to study exocytosis by building a microchip with transparent indium tin oxide (ITO) electrodes.[4] In this new coupling method, vesicular secretions are simultaneously recorded as fluorescence intensity variations and oxidation current spikes. Characterization of single exocytotic events is thus achieved with both high spatial and temporal resolution by correlating optical and amperometric signals resulting from the same vesicle. However, the majority of reports so far have only used optical (GFP, lysotracker green, or acridine orange) and electrochemical (serotonin, catecholamine) probes that were independently loaded to accomplish coupling measurements.[4,5] Therefore, the difficulty of controlling the ratio of two decoupled probes and their absolute concentrations leads to an unsatisfactory coupling efficiency and relevant data analysis.

Herein, we develop a unique dual fluorescent and electroactive probe for coupling measurements. A synthesized analogue of biogenic neurotransmitters, previously termed as FFN102[6] by the Sames and Sulzer groups, was chosen as a potential candidate for the basis of the probe. This molecule was designed as a fluorescent substrate for cells expressing vesicular monoamine transporter (VMAT), and has been demonstrated to be able to specifically stain secretory vesicles in PC12 cells[7] or synaptic terminals of the dorsal striatum.[6a] Furthermore, this probe may be also electroactive due to the presence of a phenol group at the 7-position of the coumarin nucleus.[8] Indeed, exocytosis monitoring by amperometry is usually based on the electrooxidation of some neurotransmitters stored inside vesicles

[*] X. Liu, A. Savy, S. Maurin, L. Grimaud, D. Quinton, E. Labbé, O. Buriez, J. Delacotte, F. Lemaître, M. Guille-Collignon
Ecole normale supérieure
PSL Research University, UPMC Univ Paris 06, CNRS
Département de Chimie, PASTEUR
24, rue Lhomond, 75005 Paris (France)
and
Sorbonne Universités, UPMC Univ Paris 06, ENS, CNRS, PASTEUR
75005 Paris (France)
E-mail: manon.guille@ens.fr
F. Darchen
Laboratoire de Neurophotonique, CNRS UMR 8250
Université Paris Descartes
45, rue des Saints-Pères, 75006 Paris (France)

Supporting information for this article can be found under: http://dx.doi.org/10.1002/anie.201611145.

(serotonin[4b,9] or catecholamines like dopamine, norepinephrine, and epinephrine[2b,10]) due to their phenolate hydroxy groups. In this work, we successfully introduced FFN102 as a pH-responsive fluorescent/electroactive dual probe in a TIRFM/amperometry coupling method to obtain more information concerning exocytotic release mechanisms.

The photophysical and electrochemical properties of FFN102 (Figure 1a) were first investigated. The absorption,

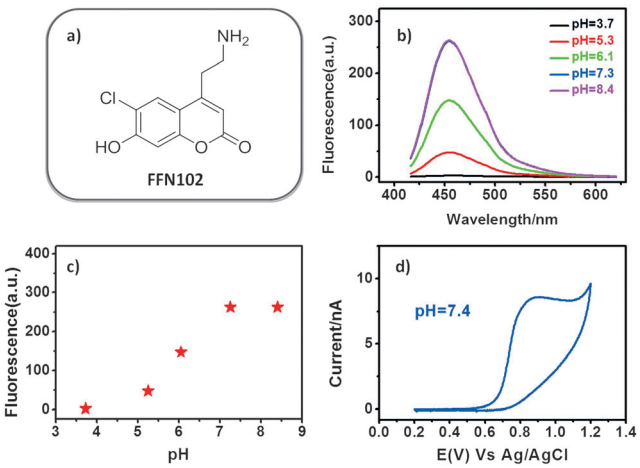
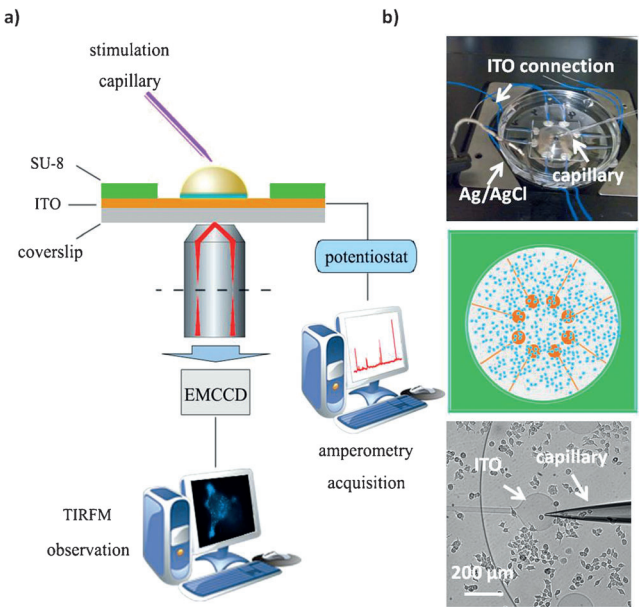


Figure 1. Photophysical and electrochemical properties of FFN102. a) Chemical structure. b) pH-dependent emission spectra with the excitation wavelength at 405 nm. c) Maximum emission intensities detected at different pH values. d) pH-responsive cyclic voltammograms (background-subtracted) of 100 μM FFN102 (in PBS) obtained on ITO microelectrodes (surfaces of two squares of 200-μm × 200-μm, scan rate: 20 $mV s^{-1}$).

excitation, and emission spectra (Figure S1) of this molecule are highly pH-dependent owing to the equilibrium between the protonated phenol and deprotonated phenolate forms, in accordance with previous publications.[6a,7] At physiological conditions (pH 7.4), optimal excitation and emission wavelengths of this probe lie at 371 nm and 456 nm, respectively. The excitation wavelength was set at 405 nm, which corresponds to the most appropriate laser source value of our TIRFM equipment. Fluorescence intensity greatly enhances with the increase of pH values, especially from pH 5.3 to pH 7.3 where the emission intensity increased by 4.6 times (Figure 1c). Taking into account the pH gradient between the vesicular lumen (pH 5–6) and the extracellular medium (pH 7.4), the fluorescence intensity is therefore expected to increase in the event of diffusion of the FFN102 from the acidic vesicle to the neutral medium.

FFN102 was then studied by cyclic voltammetry with an ITO microdevice (MD-A in Scheme S1) that shows this molecule is electroactive at extracellular pH (Figure 1d). As seen for other neurotransmitters (dopamine, serotonin), fouling of the electrode surface was also observed when FFN102 was oxidized on the ITO surface during voltammetric measurements. However, such passivation does not impede its application in amperometric investigation of exocytosis at the single cell level because the voltammetry conditions (temporal and spatial scales) are not comparable to those of the amperometry at the single cell level. For instance, serotonin and dopamine are two widely recognized neurotransmitters that easily foul the working electrodes (formation of insoluble polymer-like oxidation products at the electrode surface),[11] while they are the most extensively used probes for amperometric electrochemical detection of vesicular exocytosis at the single cell level. Therefore, FFN102 properties show it might work as an optical/electrochemical dual probe in TIRFM/amperometry coupling detection.

The implementation of TIRFM/amperometry coupling measurements relies mainly on the specific property of the working electrode, which allows both electrochemical recording and fluorescence imaging at the same time (Scheme 1a).



Scheme 1. a) Setup for TIRFM/amperometry coupling. b) Top: ITO microdevice embedded with eight independent ITO microelectrodes. Middle: Scheme of BON N13 cells adhered to the ITO device. Bottom: Single ITO microelectrode extracted from the upper device with 8 microelectrodes.

Accordingly, the material used for the working electrode must be transparent and electrically conductive.[12] In this context, a microdevice (Scheme 1b) with eight independent ITO electrodes was fabricated (Scheme S1). The individual ITO terminals were designed as a "lollipop" shape (a narrow microband connected with a wider round disk; Scheme 1b) in order to get remove the fluorescent interference of SU8 and to minimize the capacitive current noise that was found to be comparable to that of some reported devices for exocytosis investigation (around 2 pA).[13] Considering the inhomogeneity of the SU8/ITO/glass surface (Scheme 1b) and the fact that the environment must be adapted for living cell seeding, the microdevice surface was pre-coated with collagen IV. Importantly, this pretreatment did not change the electrical properties of the ITO electrodes (data not shown). For the coupled detection, only one of these eight microelectrodes is connected during all of the electrochemical measurements. The 8 electrodes can be used successively if the cell on the first electrode tested does not respond, and so on.

BON cells are derived from a carcinoid tumor and have been increasingly employed as a convenient model system for calcium-triggered exocytosis of secretory granules.[14] Here we used N13 cells, a stable clone of BON cells, to test the feasibility of employing FFN102 for exocytosis study by TIRFM and amperometry, first independently and then concomitantly.

By imaging with a confocal microscope, we examined the uptake and distribution of FFN102 in N13 cells (Figure S2). Only part of the N13 cells specifically recognized this probe by its ethylamino group (data not shown) and selectively accumulated them in their vesicular lumens. No difference was seen from 1 to 6 h of incubation, and the percentage of cells with fluorescent vesicles was independent with the exposure time to FFN102, always varying from 10 % to 40 % after 1 h of incubation.

For individual cells stained by FFN102, with the excitation of 405 nm laser at TIRFM configuration, their vesicles adjacent to the plasma membrane were directly visualized as bright and stable blue spots with high signal-to-noise ratio (Figures 2 b and S3), and their motility behaviors were monitored, showing that the 405 nm laser is a proper light source for fluorescence illumination.

Next, exocytotic secretions of FFN102 from the N13 cell was initiated by the application of a secretory stimulus (a calcium ionophore, 10 μM ionomycin), resulting in extinctions of fluorescent vesicles in the target cell (Figure 2 b). According to the sequential images in TIRFM, a great deal of information concerning an entire exocytotic event can be collected at the single-cell level with distinct spatial resolution, including the locations of releasing sites, vesicle motility before fusion, and the diffusion pathways of fluorescent dyes upon stimulation (see the video in the Supporting Information). For a single vesicle in the given example (Figure 2 b), the fluorescent signal transiently brightened during the release of the pH-responsive optical probe FFN102 from acidic vesicle to the neutral extracellular medium and then disappeared completely within 100–200 ms as the fluorophores diffused away. Note that the diffusion step could be visually missed if low amounts of the fluorescent dyes was packaged inside the vesicle, resulting in prompt extinctions of bright spots (Figure S4). Thus, FFN102 was shown to be a decent optical probe to directly visualize and locate individual exocytotic events by TIRFM.

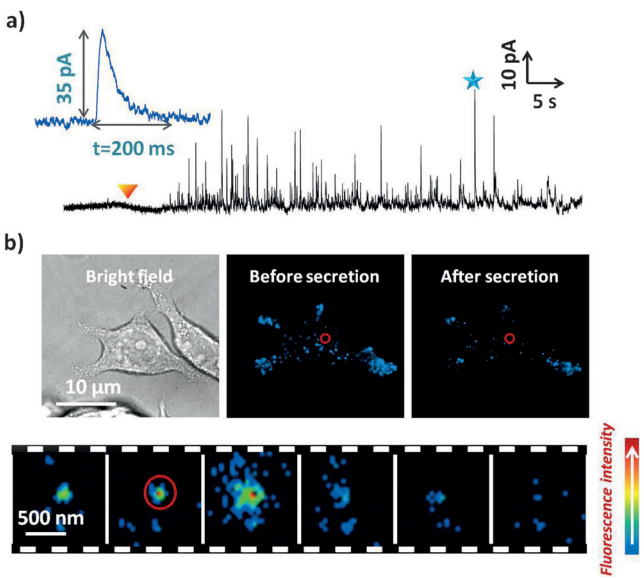


Figure 2. a) Typical amperometric traces obtained from FFN102-stained BON N13 cells where the red triangle indicates the moment at which the simulation is applied (the slight delay of the effect of stimulation is due to the distance between the stimulating capillary and the target cell). An amplified current spike (indicated by the blue star) is shown in blue. b) Top: TIRFM images of FFN102-stained BON N13 cells before and after secretion. Bottom: An individual exocytotic release from a single fluorescent vesicle indicated by the red circle in the upper cell (time interval is set at 35 ms between each pseudocolor image).

Considering the electroactivity of FFN102 on the ITO microelectrode (Figure 1 d), the capability of this molecule working as an electrochemical probe for exocytosis tracking was tested with N13 cells. A given amount of cells (3×10^5 cells) was first plated onto the assembled ITO microchip precoated with collagen. The cells appeared to settle randomly, and a few cells adhered to the ITO electrode surface after 24–36 h. Cells were then incubated in culture media supplemented with 20 μM FFN102 for 1 h. Target cells were manually selected thanks to the fluorescent character of FFN102, and only those cells with bright blue vesicles were chosen for electrochemical detection.

Amperometry was performed at a constant potential of + 900 mV vs. Ag/AgCl at the ITO working electrode to immediately oxidize FFN102 secreted during exocytosis. A glass capillary filled with a saline solution supplemented with 10 μM ionomycin was placed close to the target cell to trigger exocytotic releases (Scheme 1 b). Figure 2 a is a typical amperometric trace representing exocytotic release from FFN102-stained N13 cells. A succession of individual current spikes appeared and is attributed to the calcium-triggered sequential release of FFN102. The current spikes obtained here were shown to result from oxidation of FFN102 rather than residual serotonin. On one hand, this cell line has lost the ability to synthesize serotonin.[9] On the other hand, no spike was observed during control experiments performed at + 650 mV (data not shown). In the amperometric trace shown in Figure 2 a, each spike corresponded to an individual exocytotic release of a single secretory vesicle (Figure 2 a, blue spike). It is evident that compared with optical imaging by TIRFM (35 ms/frame), this technique exhibits extremely high temporal resolution ($\approx$ 25 μs for each acquisition), allowing study of exocytosis kinetics as well as the amount of FFN102 released (by considering the spike area and Faraday's law).

Independent optical and electrochemical measurements of exocytotic events were successfully achieved by using FFN102. We then characterized the feasibility of this molecule functioning as a fluorescent/electrochemical dual probe by conducting TIRFM/amperometry coupled detection of vesicular exocytosis in our ITO microdevice. The trigger of 405 nm laser light for FFN102-stained vesicle illumination in TIRFM was found to induce a sluggish variation of the amperometric current baseline. This effect is likely to be

negligible due to its much slower time course compared with that of the detected current spikes resulting from exocytotic release (Figure S5). Therefore, exocytotic release of the unique dual probe FFN102 from the vesicular lumen to the ITO microelectrode surface was depicted as oxidation current spikes accompanied by fluorescence spot extinctions.

Figure 3 shows a classic exocytotic secretion of an individual FFN102-stained vesicle detected by the TIRFM/

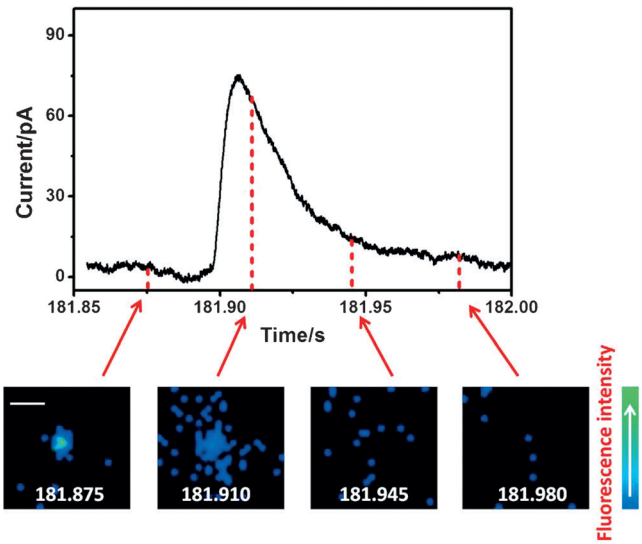


Figure 3. Correlation of amperometric and fluorescence information for a single exocytotic event of FFN102-stained BON N13 cells over an ITO microdevice. Top: An exocytotic event appeared as the current spike in electrochemical detection. Bottom: Sequential pseudocolor TIRFM images of a single exocytotic event viewed as a flash of fluorescence. Scale bar = 500 nm.

amperometry coupled measurement. The correlation of fluorescence variations and amperometric signals demonstrates that the frame in which the initial fluorescence enhancement (frame, $t = 181.910$ s) occurred was accompanied by the steep rise of the amperometric spike, indicating a maximum flux of the fluorescence/electrochemical dual probe FFN102. Then, the dispersion of FFN102 led to a lower concentration of fluorophore/weaker fluorescence (frame, $t =$ 181.945 s) on the ITO surface, associated with a slow decrease of the detected current. Finally, the disappearance of fluorescence (frame, $t = 181.980$ s) was tested by the regression of the current to the baseline. With the unique FFN102 dual probe, the optical observation by TIRFM allowed precise localization of the release sites in the secretory cell while electrochemical detection by amperometry provided quantitative information with unparalleled temporal resolution.

In fact, besides the coupled signals, "fluorescent orphan" (extinction of fluorescent vesicles without corresponding electrochemical signals) and "amperometric orphan" (oxidation current spikes without related secretory vesicles) were also detected during the coupled measurements.[4b] In this view, we compared the present results with our previous work in which GFP and serotonin were separately loaded as reporters at similar conditions (exposure time, ≈100 ms;

Table 1: Comparison of proportions of three kinds of signals detected from N13 cells labeled by FFN102 and GFP-transfected BC21 cells preloaded with serotonin.[4b]

	FFN102	GFP + serotonin
Coupled event	34%	22%[a]
Fluorescent orphan	42%	57%
Amperometric orphan	24%	21%

[a] Coupling efficiency in this case was recalculated using the same method as FFN102.

Table 1).[4b] First, the optical/amperometric coupling efficiency using FFN102 (34%) is optimized by 12% compared with GFP/serotonin conditions (22%). The "optical orphan" percentage significantly decreased according to a more efficient loading of the electroactive probe. Furthermore, the "amperometric orphan" percentage remains quite constant due to a similar time resolution in comparison with the GFP-serotonin work (80 ms in most of the cases in the present work and 100 ms for the previous dual probes loading strategy). Moreover, the amperometric spikes resulting from serotonin oxidation ranged from 0.2 to 3.5 pA in magnitude caused by its low accumulation inside the GFP-transfected vesicles. Such a low current signal makes it difficult to extract confident spikes thus requiring extra data treatments such as filtering. In contrast, the electrochemical signals coming from FFN102 oxidation seems much better ($I_{max} = 19.2 \pm 0.7$ pA, $n = 215$ events) because of its successful loading into the vesicles. Therefore, the strategy of using a unique probe for electrochemical and fluorescent detection of exocytotic release seems better adapted than previous works involving independent probes.

In summary, the unique FFN102 probe was demonstrated to show pH-dependent fluorescence and electrochemical properties. Using transparent ITO microelectrodes, exocytotic events of N13 cells stained by FFN 102 were simultaneously observed as fluorescence variations and oxidation current spikes with the TIRFM/amperometry coupled technique. Considering the excellent spatial and temporal resolution of the coupled method, analysis of the amperometric signals as a function of the zone of release will help to investigate the kinetic properties of the release sites and, in particular, the specificities of "hot spots".[15] Further studies will also examine the specific roles of regulatory proteins involved in the exocytotic process (for example, SNARE proteins and actin). Finally and most importantly, this work offers an innovative idea to develop other probes capable of acting as optical/electrochemical dual reporters in a coupled method, especially those with longer excitation/emission wavelengths, which will greatly contribute to an understanding of various regulating factors of exocytotic secretion.

Acknowledgements

This work has been supported in part by CNRS (UMR 8640), Ecole Normale Supérieure (PSL Research University), French Ministry of Research, Université Pierre & Marie Curie Paris 6 (Sorbonne Universités). MGC thanks "Emer-

gences Ville de Paris 2015" Grant and Institut Universitaire de France Fellowship Program. XL thanks Pr. C. Amatore for helpful suggestions and the China Scholarship Council for her financial support with a Ph. D. grant. Ms. Lihui Hu is gratefully acknowledged for discussions and her help in the experimental work.

Conflict of interest

The authors declare no conflict of interest.

Keywords: electrochemistry · exocytosis · indium tin oxides · neurotransmitters

[1] a) C. Amatore, S. Arbault, M. Guille, F. Lemaitre, *Chem. Rev.* **2008**, *108*, 2585 – 2621; b) R. Jahn, T. C. Sudhof, *Annu. Rev. Neurosci.* **1994**, *17*, 219 – 246; c) N. Vardjan, J. Jorgacevski, R. Zorec, *Neuroscientist* **2013**, *19*, 160 – 174; d) Z. P. P. Pang, T. C. Sudhof, *Curr. Opin. Cell Biol.* **2010**, *22*, 496 – 505; e) T. C. Normann, *Int. Rev. Cytol.* **1976**, *46*, 1 – 77; f) R. D. Burgoyne, A. Morgan, *Physiol. Rev.* **2003**, *83*, 581 – 632; g) A. I. Ivanov, *Exocytosis and endocytosis*, Springer, New York, **2008**.

[2] a) C. Amatore, M. Guille-Collignon, F. Lemaıtre, *Electrochemical Biosensors*, *Vol. 3*, PAN STANFORD, **2015**; b) T. J. Schroeder, J. A. Jankowski, K. T. Kawagoe, R. M. Wightman, C. Lefrou, C. Amatore, *Anal. Chem.* **1992**, *64*, 3077 – 3083; c) A.-S. Cans, A. G. Ewing, *J. Solid State Electrochem.* **2011**, *15*, 1437 – 1450.

[3] a) D. Toomre, D. J. Manstein, *Trends Cell Biol.* **2001**, *11*, 298 – 303; b) H. Schneckenburger, *Curr. Opin. Biotechnol.* **2005**, *16*, 13 – 18; c) D. Axelrod, T. P. Burghardt, N. L. Thompson, *Annu. Rev. Biophys. Bioeng.* **1984**, *13*, 247 – 268.

[4] a) K. Kisler, B. N. Kim, X. Liu, K. Berberian, Q. Fang, C. J. Mathai, S. Gangopadhyay, K. D. Gillis, M. Lindau, *J. Biomater. Nanobiotechnol.* **2012**, *3*, 243 – 253; b) A. Meunier, O. Jouannot, R. Fulcrand, I. Fanget, M. Bretou, E. Karatekin, S. Arbault, M. Guille, F. Darchen, F. Lemaitre, C. Amatore, *Angew. Chem. Int. Ed.* **2011**, *50*, 5081 – 5084; *Angew. Chem.* **2011**, *123*, 5187 – 5190.

[5] a) B. X. Shi, Y. Wang, T. L. Lam, W. H. Huang, K. Zhang, Y. C. Leung, H. L. W. Chan, *Biomicrofluidics* **2010**, *4*, 043009; b) C. Amatore, S. Arbault, Y. Chen, C. Crozatier, F. Lemaître, Y. Verchier, *Angew. Chem. Int. Ed.* **2006**, *45*, 4000 – 4003; *Angew. Chem.* **2006**, *118*, 4104 – 4107.

[6] a) P. C. Rodriguez, D. B. Pereira, A. Borgkvist, M. Y. Wong, C. Barnard, M. S. Sonders, H. Zhang, D. Sames, D. Sulzer, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 870 – 875; b) P. Merchant, D. Sulzer, D. Sames, *Neuropharmacology* **2015**, *98*, 90 – 94.

[7] M. Lee, N. G. Gubernator, D. Sulzer, D. Sames, *J. Am. Chem. Soc.* **2010**, *132*, 8828 – 8830.

[8] X.-R. Hu, J.-B. He, Y. Wang, Y.-W. Zhu, J.-J. Tian, *Electrochim. Acta* **2011**, *56*, 2919 – 2925.

[9] A. Meunier, M. Bretou, F. Darchen, M. Guille-Collignon, F. Lemaître, C. Amatore, *Electrochim. Acta* **2014**, *126*, 74 – 80.

[10] a) G. Chen, P. F. Gavin, G. Luo, A. G. Ewing, *J. Neurosci.* **1995**, *15*, 7747 – 7755; b) R. M. Wightman, J. A. Jankowski, R. T. Kennedy, K. T. Kawagoe, T. J. Schroeder, D. J. Leszczyszyn, J. A. Near, E. J. Diliberto, O. H. Viveros, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 10754 – 10758.

[11] a) A. N. Patel, P. R. Unwin, J. V. Macpherson, *Phys. Chem. Chem. Phys.* **2013**, *15*, 18085 – 18092; b) A. G. Güell, K. E. Meadows, P. R. Unwin, J. V. Macpherson, *Phys. Chem. Chem. Phys.* **2010**, *12*, 10108 – 10114; c) R. Trouillon, D. O'Hare, *Electrochim. Acta* **2010**, *55*, 6586 – 6595; d) D. P. Manica, Y. Mitsumori, A. G. Ewing, *Anal. Chem.* **2003**, *75*, 4572 – 4577.

[12] C. Amatore, J. Delacotte, M. Guille-Collignon, F. Lemaître, *Analyst* **2015**, *140*, 3687 – 3695.

[13] a) H. Zhao, L. Li, H. J. Fan, F. Wang, L. M. Jiang, P. G. He, Y. Z. Fang, *Mol. Cell. Biochem.* **2012**, *363*, 309 – 313; b) X. H. Sun, K. D. Gillis, *Anal. Chem.* **2006**, *78*, 2521 – 2525.

[14] a) B. M. Evers, J. I. N. Ishizuka, C. M. Townsend, J. C. Thompson, *Ann. N. Y. Acad. Sci.* **1994**, *733*, 393 – 406; b) V. S. Tran, S. Huet, I. Fanget, S. Cribier, J.-P. Henry, E. Karatekin, *Eur. Biophys. J.* **2007**, *37*, 55 – 69.

[15] P. Keller, D. Toomre, E. Diaz, J. White, K. Simons, *Nat. Cell Biol.* **2001**, *3*, 140 – 149.

Manuscript received: November 14, 2016
Revised: December 13, 2016
Final Article published: ■■ ■■, ■■■■

Spectroscopic Methods

X. Liu, A. Savy, S. Maurin, L. Grimaud, F. Darchen, D. Quinton, E. Labbé, O. Buriez, J. Delacotte, F. Lemaître, M. Guille-Collignon* ——— ■■■–■■■

A Dual Functional Electroactive and Fluorescent Probe for Coupled Measurements of Vesicular Exocytosis with High Spatial and Temporal Resolution

The synthetic neurotransmitter FFN102 is employed to monitor coupled amperometric and fluorescence microscopy measurements of individual exocytotic events. This method capitalizes on the strengths of both individual techniques, and opens the way for new approaches to study the kinetics and mechanisms of vesicular secretion.