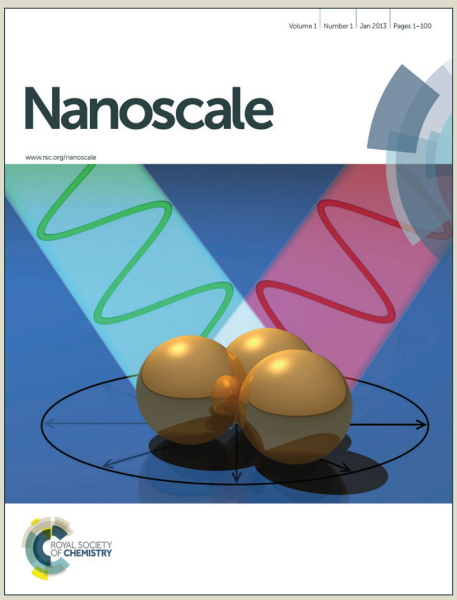
Nanoscale

Accepted Manuscript






ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/nanoscale

Journal Name

RSCPublishing

ARTICLE

# Annexin-V/Quantum dot probes for multimodal apoptosis monitoring in living cells: improving bioanalysis using electrochemistry

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Helena Montón,[a,b] Claudio Parolo,[b] Antonio Aranda-Ramos,[a] Arben Merkoçi,*[b,c] and Carme Nogués*[a]

There is a great demand to develop novel techniques that allow useful and complete monitoring of apoptosis, which is a key factor of several diseases and target of drug development. Here, we present the use of a novel dual electrochemical/optical label for the detection and study of apoptosis. We combined the specificity of Annexin-V for phosphatidylserine, a phospholipid expressed in the outer membrane of apoptotic cells, with the optical and electrochemical properties of quantum dots to create a more efficient label. Using this conjugate we addressed three important issues: i) we made the labeling of apoptotic cells faster (30 min) and easier; ii) we fully characterized the samples with common cell biological techniques (Confocal Laser Scanning Microscopy, Scanning Electron Microscopy and Flow Cytometry); and iii) we developed a fast, cheap and quantitative electrochemical detection of apoptotic cells with results in fully agreement with those obtained by flow cytometry.



## Introduction

The development of new techniques to detect and study the apoptosis is crucial to fully understand how its deregulation leads to disorders such as cancer,[1,2] neurodegenerative diseases[3] and myocardial infarction,[4] among others. Nowadays, most techniques used to detect apoptotic cells aim to label phosphatidylserine (PS), a negatively charged aminophospholipid, whose translocation from the inner to the outer plasma membrane is a distinctive hallmark of early stages of apoptosis.[5–8] Methods based on PS recognition are useful clinical tools for early diagnosis, evaluation of diseases progression, and monitoring therapy efficacy, besides providing excellent quantitative analysis in living cells and new potential targets for drug discovery.[9,10] To date, Annexin-V (AnnV), a phospholipid-binding protein with high affinity for PS,[11] has been widely used as an imaging tool to monitor the apoptosis progression when conjugated to a fluorescent dye.[12–15] Nevertheless, its applicability is restrained by the properties of common fluorescent dyes, which have a limited photostability and a tendency to photobleach.[16,17]

Considering this framework, CdSe/ZnS Quantum Dots (QDs) are excellent tools to overcome organic dye inconveniences;[18] in fact, they have size-dependent tunable narrow fluorescence emission spectra, high-resistance threshold to chemical and photo degradations, high extinction coefficient and high quantum yield.[19,20] They are already widely used in fluorescence based technologies[21,22] such as fluorescence microscopy and confocal laser scanning microscopy (CLSM),[23–26] flow cytometry,[27] spectroscopy[28,29] and microarrays[30–32]. Another unique characteristic of QDs, over dyes and enzymes, is that their high electron density allows for their use also as electron microscopy labels. Although fluorescence-based and electron microscopy methods provide an accurate detection of apoptosis, most of them are time-consuming, troublesome, expensive and require qualified staff to analyze the results. A complementary and quantitative electrochemical detection of apoptosis would solve those inconveniencies. Several works reported the use of voltammetry to detect QDs as electrochemical labels, taking advantage of their redox properties.[33–36]

Herein, we describe the use of AnnV-QDs conjugates as dual optical/electrochemical labels for fully monitoring apoptosis in living leukemia-derived THP1 cells (Fig. 1). Using this label, we achieved to: i) make the labeling process easier and faster (30 min) than most commercially available kits, ii) study the same sample with classical cell biology techniques (CLSM, Scanning Electron Microscopy (SEM) and flow cytometry) obtaining a deep overview of the cell status, and iii) develop a cheap, easy-to-use, fast and quantitative electrochemical technique, which results are in fully agreement with those obtained by flow cytometry.

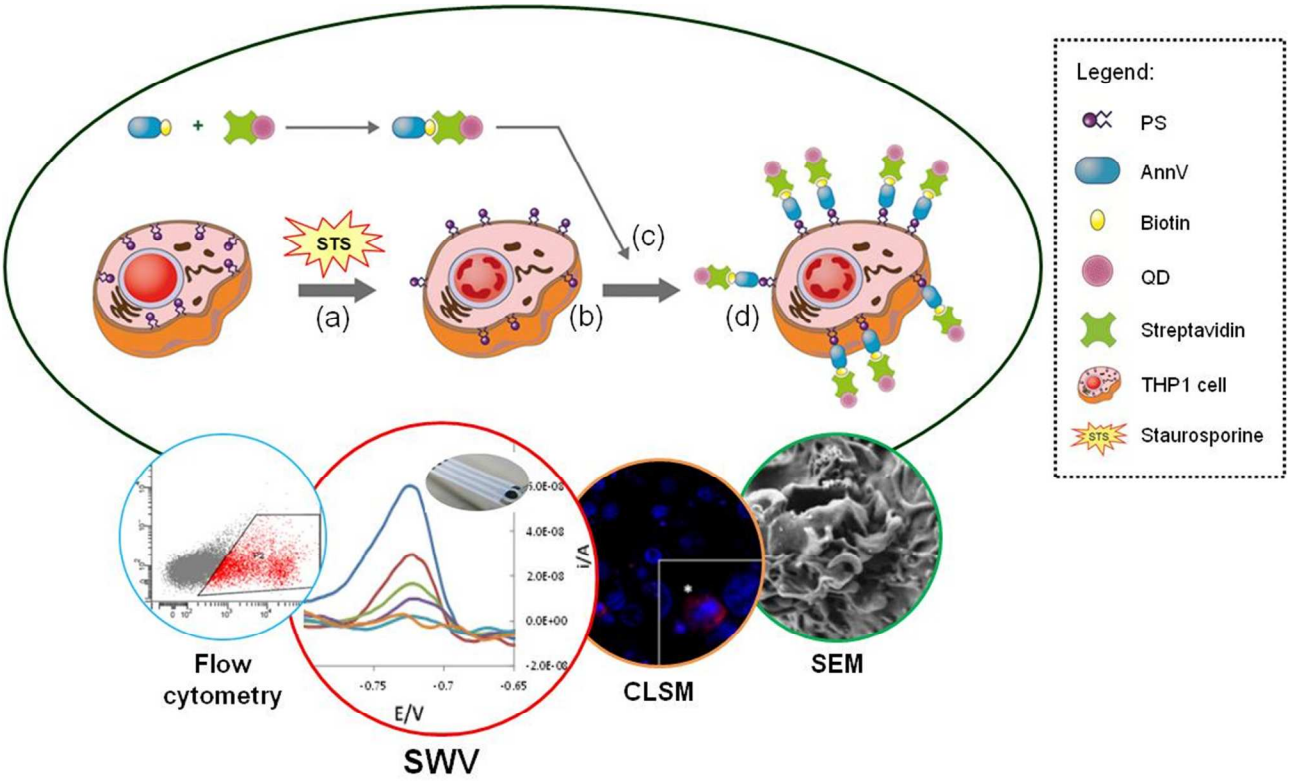



**Fig. 1 Use of Annexin-V/Quantum dot probes for optical and electrochemical detection of apoptotic cells.** THP1 cell cultures are incubated with Staurosporine (STS) (a), a pro-apoptotic drug, and as a consequence phosphatidylserine (PS) is externalized (b). AnnV-QD conjugates previously prepared are added to the cell cultures (c) and bound to PS expressed in the outer membrane of apoptosis induced THP1 cells (d). Due to the properties of QDs (they are fluorescent, electroactive and electron dense) diverse analyses can be performed.

## 2. Experimental

### 2.1 Reagents and instruments

RPMI-1640 (Gibco), Foetal Bovine Serum (FBS) (Gibco) and L-Glutamine (Gibco), for cell cultures, and Qdot® 655 Streptavidin Conjugates (Invitrogen), dimer EthD (Invitrogen) and Hoechst 33342 (Invitrogen), for labeling the cells, were purchased from Life Technologies S.A. (Spain). T-25 and T-75 flasks (Nunc) were purchased from Thermo Scientific and the hemocytometer with a Bürker grid was purchased from Brand Gmbh + Co KG (Germany). A HERAcell 150 incubator (Heraeus, Spain) and an Eppendorf Centrifuge 5804 R (Eppendorf Ibérica S.L. Spain) were used for cell culture maintenance and in some steps of the experimental procedure.

Staurosporine (STS) and Dimethyl sulfoxide (DMSO) for the apoptosis induction step were purchased from Sigma (Spain) and Annexin-V biotin (Biovision Inc.) was purchased from Deltaclon S.L. (Spain). Sample shaker used for preparation of the conjugate was a TS-100 Thermo shaker (Spain).

Reagents for PBS and AnnexinV binding buffer preparation were purchased from Sigma, Aldrich or Fluka. All the solutions were prepared using mQ water, produced using Milli-Q system (>18.2MΩcm$^{-1}$) purchased from Millipore (Sweden).

### 2.2 Cell culture and induction of apoptosis

The human monocytic THP-1 cell line (ATCC® TIB-202TM) was maintained at a density of 5-8 x $10^5$ cells/mL in 75 cm$^2$ flasks in RPMI-1640 medium supplemented with 2 mM L-Glutamine and 10% FBS in standard conditions (5% $CO_2$ at 37 ºC).

For apoptosis induction, 2 mL of cell suspension at a concentration of 5 x $10^5$ cells/mL were transferred to 25 $cm^2$ flasks where 3 µL of 0.5 mg/mL of STS diluted in DMSO were added into the flasks (induced cultures). The equivalent volume of DMSO was added into the control flasks (control cultures). Cultures were kept in standard conditions for 6 h. Two washing cycles with PBS were performed in order to remove any presence of STS in the cell cultures transferring cell suspensions to centrifuge tubes and spinning at 300 x g for 5 min. Final pellets were resuspended in 200 µL PBS.

During the incubation with STS, THP-1 cell cultures were checked using an inverted microscope with a phase-contrast objective. We took images of control and apoptosis-induced cell cultures in order to observe changes occurring in the cells due to the effect of STS such as cell shrinkage and membrane blebbing (Fig. SI 1).

### 2.3 AnnexinV-QD conjugates preparation

AnnV-QD conjugates were prepared according to the manufacturer's protocol. Briefly, 3 µL of 2 µM streptavidin-QDs solution were incubated with 187 µL of QD conjugation buffer and 10 µL of biotinylated Annexin-V for 45 min in a thermo-shaker at 500 rpm at 25ºC and stored at 4ºC until used.

### 2.4 Simple labelling protocol for apoptosis

A simple labelling procedure was carried out by adding 200 µL of AnnV-QD conjugate and 200 µL of $Ca^{2+}$ containing binding buffer to the cell suspensions (prepared in 200 µL PBS). Then it was incubated at room temperature (RT) in a rocker shaker for 30 min. Thereafter, in order to eliminate the unbound AnnV-QD conjugates two washing cycles with PBS were performed.

### 2.5 Confocal Laser Scanning Microscopy

A 10 µL drop of the labelled cell suspension was placed in a bottom glass petri dish for confocal imaging. Images were captured with a CLSM Leica TCS-SP5 AOBS spectral (Leica Microsystems Heidelberg GmbH; Mannheim, Germany) using Plan-Apochromatic 20× and 40× objectives (NA 0.9 and NA 1.2 respectively).

Simultaneous excitation of the PS-expressing cells (labeled with AnnV-QD) and their nuclei (Hoechst staining) was performed using a 405 nm blue laser diode and simultaneous detection was done using two different photomultipliers (PMT), one per each corresponding spectral range. CLSM software (Leica LAS AF) was used to obtain overlapped images of nuclei and AnnV-QD labeling. Stacks of XY images at different Z planes were processed with ImageJ (NIH, Maryland, USA) and Imaris 3D rendering software (Bitplane AG, Switzerland). In order to perform semiquantitative analyses, fluorescence intensity data from the images were obtained with ImageJ extracting the sum of intensities of all pixels for the nuclei (FIN) and the QD (FIQD) labeling, separately for each given image. Average intensities of 3 pair of images, from different areas, were calculated and the average ratio FIQD/FIN was used to quantify the cell death.

Further projections and 3-D views of apoptotic cells were performed using Imaris software.

### 2.6 Sample preparation for SEM analysis

Labeled-cell suspensions were washed in 0.1 M cacodylate buffer for 1 min and then fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, during 1 h at RT. Cells were incubated again with 0.1 M cacodylate buffer for 1 min and then dehydrated sequentially in increasing concentrations of cold ethanol (50, 70, 90% during 8 min each). To complete the dehydration process samples were incubated two times in 100% ethanol (8 min each, 25ºC) and finally resuspended in hexamethyl disilazane solution (Sigma-Aldrich) for 15 min at RT. A 50 µL of each sample was placed in separate glass coverslips and let dry for 15 min at RT. SEM samples were kept in a desiccator at 25 ºC until analysis.

The volume of all reagents used in the sample preparation was 1 mL and after each of the above-mentioned steps cells were recovered by centrifugation (300 x g, 5 min).

Prior to SEM analysis (Magellan®FE-SEM, FEI), glass coverslips were placed over a typical SEM sample holder and the edges of the coverslips were painted with silver ink in order to increase their conductivity and facilitate the analysis of the samples.

This protocol avoids the use of metallization steps keeping intact the cellular structure and allows a direct visualization of small metallic nanoparticles such as QD on the cell surface.

### 2.7 Flow Cytometry

AnnV-QD labeled cells (20,000 cells) were analyzed to quantify the number of apoptotic cells in both induced and control cultures using a FACSCanto II flow cytometer (BD Biosciences, Franklin Lakes, NJ) equipped with the BD Biosciences FACSDiva™ software.

### 2.8 Electrochemical detection of apoptotic cells

The electrochemical detection of apoptotic cells was done using mercury-modified screen printed electrodes (from ItalSens, acquired through PalmSens) placing 30 µL of the AnnV-QD labeled cells onto the working electrode. Apoptotic cells were detected electrochemically though the presence of Cd ions (as defects at Cd QDs surface) contained in the QDs structure by direct voltammetric detection. This methodology has been previously reported by our group[33] and applied now with some minimal modifications. Briefly, the Cd(II) contained in the QDs was reduced to Cd(0) applying a potential of -1.1V for 5 min. After that, a square wave voltammetry

(SWV) was performed scanning the potential from -1.1 to -0.4 V (step potential 10 mV, modulation amplitude 30 mV, frequency 15 hz) and measuring the current value (analytical signal) due to the oxidation of Cd(0) to Cd(II). The oxidation process generates a peak of current at −0.8 V which intensity is related with the quantity of QDs in contact with the working electrode surface. The electrochemical detection in the samples was directly performed in PBS pH 7.4.

Different concentrations of cells ranging from 20,000 to 1,250 were measured and three measures using three different electrodes (represented as error bars in the graphs) were performed per each number of cells.

## 3. Results and Discussion

### 3.1 Optimization of apoptosis induction and labelling with AnnV-QD probes

In this study, we used a human monocytic leukemia cell line, THP-1 as target of Staurosporine (STS), a commonly used drug to induce apoptosis in cell-based assays.[37,38] Although several works report the apoptotic effect of STS, concentrations and incubation times change depending on the cell line and specific assay requirements.[39,40] We observed that 5 µg/mL of STS during 6 hours provoke the expected effect in THP-1 cell cultures by exhibiting early apoptotic symptoms such as cell shrinkage or membrane blebbings (Fig. S-1 in the Electronic Supplementary Material (ESM)). We also optimized the labelling steps in order to make the method faster and easier than the use of Annexin-V-biotin kits in conjunction with conventional streptavidin or avidin-dye reagents. In fact in these procedures, cells are first incubated with Annexin-V-biotin followed by a second incubation with a fluorescently labelled streptavidin; besides, after each incubation step several washing steps are necessary, making the overall labelling process at least longer than 1 h. In the proposed method the Annexin-V-biotin is pre-incubated with streptavidin-QDs and the resulting conjugates can be stored at 4ºC, protected from light, until use. In this way we minimized the number of steps, decreasing the overall assay time to 30 min.

### 3.2 In vitro CLSM monitoring of apoptosis in THP-1 cell cultures

In order to study the efficiency and specificity of AnnV-QDs conjugates to detect apoptotic cells, we analyzed the sample both with CLSM and SEM. In particular, taking advantage of the fluorescent emission of QDs, we used CLSM to obtain qualitative information of the assay by comparing images of the control and apoptosis-induced samples (Fig. 2a). Images in Fig. 2a show the differences in the amount of AnnV-QD labeled-cells (in red) between the two samples. Nuclear staining (in blue; using Hoechst 33342 dye) confirms the apoptotic status of cells in the induced cultures, where DNA condensation and nuclear fragmentation are evident (insert box in Fig. 2a). We used ImageJ software to analyze three random areas in both samples (control and induced), to attain the integrated fluorescence intensity of nuclear (FIN) and QDs (FIQD) staining. The histogram in Fig. 2b displays the FIQD/FIN ratio of control and induced samples, respectively 0.022±0.001 and 0.06±0.001 A.U.. The higher ratio observed in the induced sample indicates a prevalence of apoptotic cells in the culture incubated with STS. Furthermore, by scanning the sample along the Z axis, we obtained information of the whole-cell labeling. A cross section of a selected stack of an apoptotic cell is shown in Fig. 2c. XZ and YZ plane projections show the relative localization of AnnV-QD labeling, which is restricted to the periphery of the cells, as expected, and also the fragmentation of the nucleus. Finally, this peripheral localization can also be seen in a surface-rendered 3-D reconstruction of an apoptotic cell, which gives a more complete volumetric view of the same labeling information (Fig. 2d).

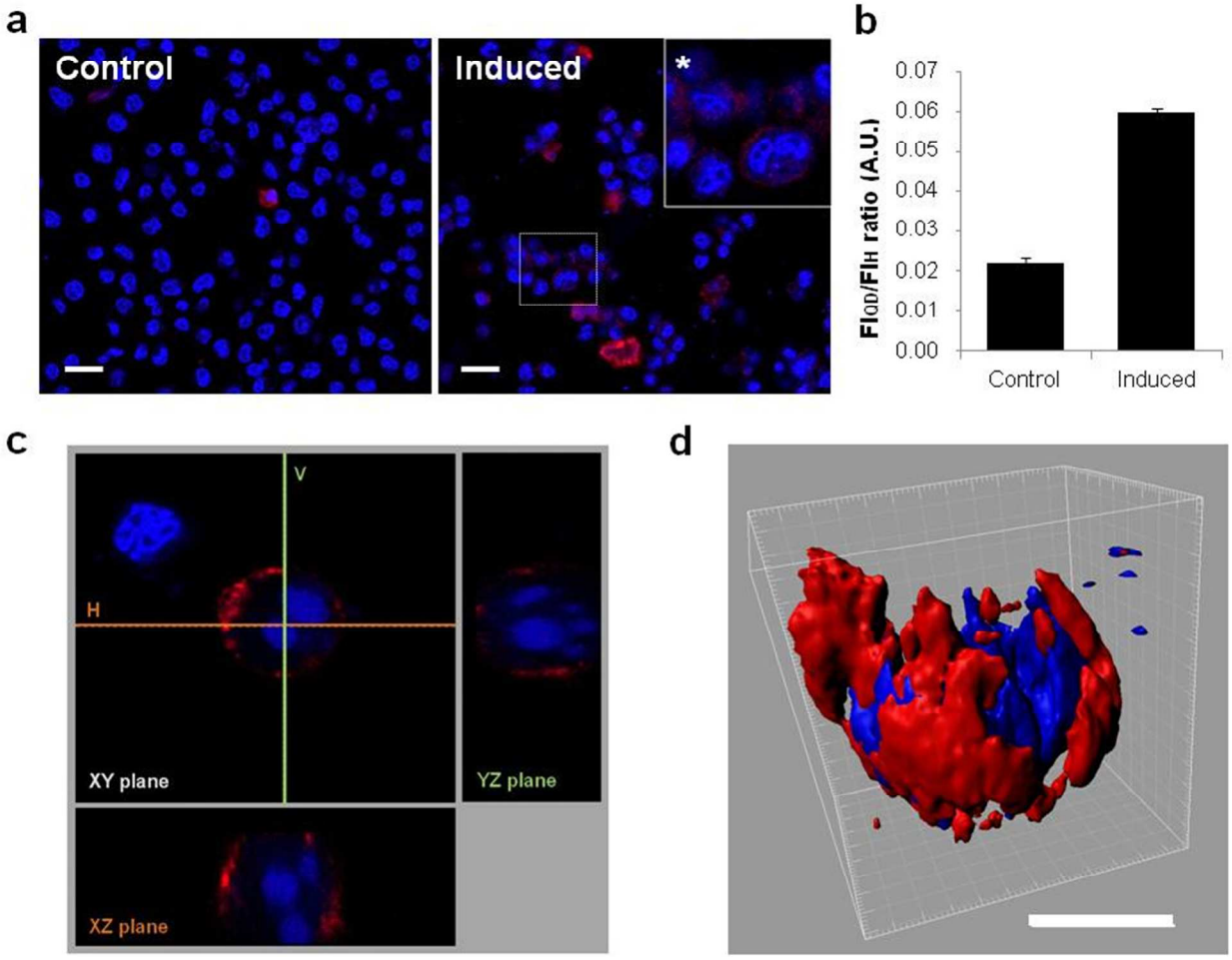



**Fig. 2 CLSM analysis.** (**a**) Images of THP1 cells after AnnV-QD labeling (red) show few apoptotic cells in control cultures and many in induced ones. Nuclei were counterstained with Hoechst (blue). A zoom in a selected region (dashed line) is displayed in a box for more detail (*). Scale bars 20 μm. (**b**) Apoptosis in control and induced cultures was established from the images acquired. Fluorescence intensities of nuclei ($FI_{H,}$ Hoechst stain) and of AnnV-QD ($FI_{QD,}$ QDs) bound to cells were quantified and the ratio $FI_{QD}/FI_{N}$ was calculated. This ratio was twice in induced cultures compared to control ones. (**c**) A cross section projection allows the visualization of an XZ plane (horizontal -H- section) and a YZ plane (vertical -V- section), where DNA condensation and nuclear fragmentation can be clearly observed. (**d**) 3-D rendered projection shows the peripheral localization of the AnnV-QDs in a volumetric view. Scale bar 5 μm.

### 3.3 SEM analysis of apoptotic THP-1 cells and AnnV-QD labeling at the plasma membrane

We performed SEM analysis both to see in detail the external morphology of the cells, and to evaluate the presence or absence of QDs in the plasma membrane surface (CdSe/ZnS QDs can be visualized by SEM thanks to their electron dense composition). Fig. 3a and 3b show respectively non-apoptotic and apoptotic cells at 20,000X magnification: whereas non-apoptotic cells (Fig. 3a) had usual morphologies, apoptotic cells (Fig. 3b) decreased in size (shrinkage) and presented membrane blebbings (pointed out in the picture by yellow arrows), which are typical of apoptotic processes. Moreover at 50,000X magnification, the presence of QDs was evaluated: no QDs were observed in non-apoptotic cells (Fig. 3a'). However, in apoptotic cells, QDs (displayed as bright dots in the image) were present in their membranes (yellow arrowheads in Fig. 3b'), corresponding to what was expected, due to the interaction of AnnV-QD with the externalized PS.

Journal Name RSCPublishing

ARTICLE

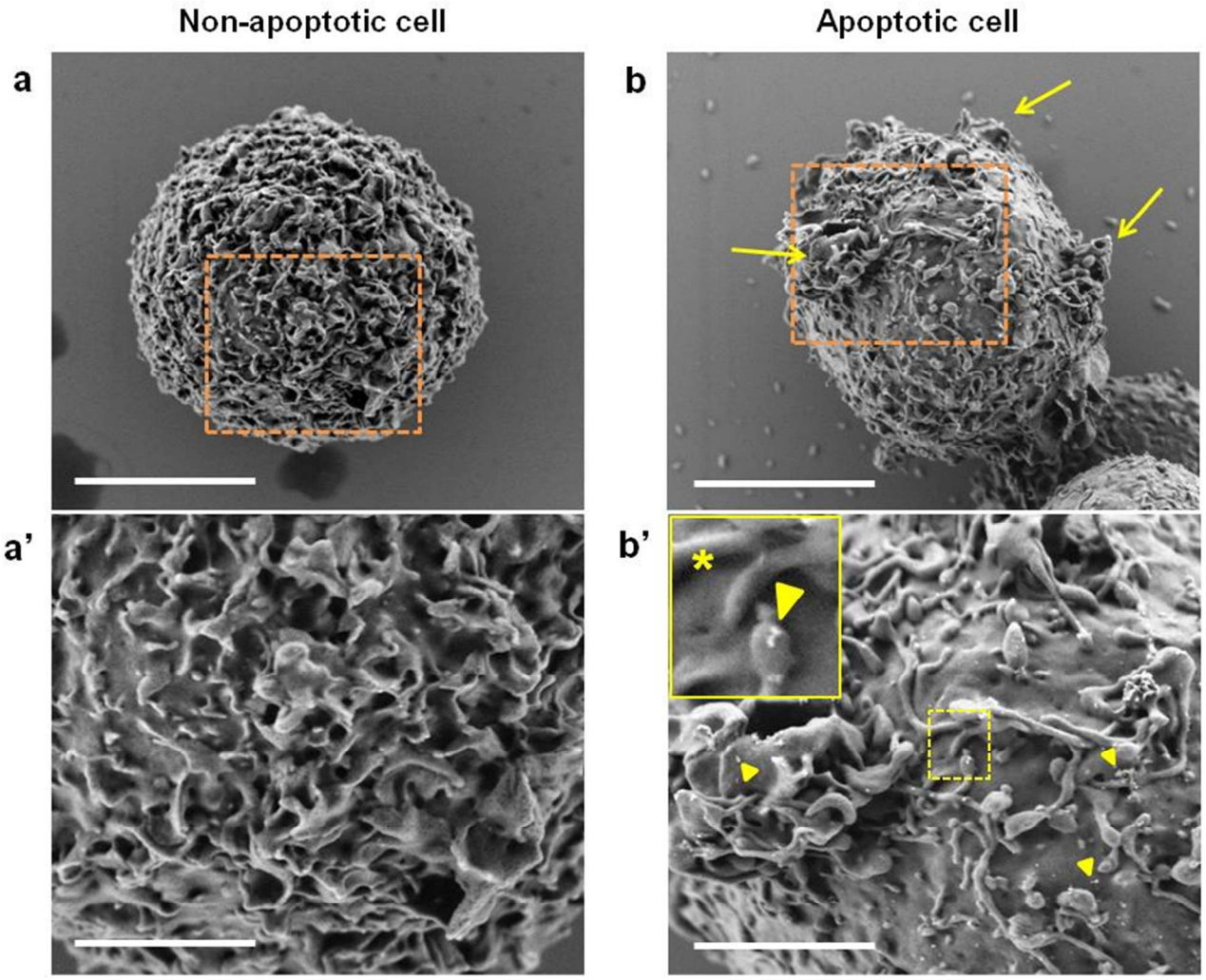



**Fig. 3 SEM analysis.** Morphology of control and apoptotic cells and presence of QDs in the outer membrane of cells were evaluated by SEM imaging. Images a and b were taken at 20,000X to check the size and morphology of cells, scale bar 5 μm. Apoptotic cells (b) shown to be smaller in size (shrinkage) and presented also big evaginations or blebbings (indicated by arrows), typical of apoptotic processes. Images a' and b' were taken at 50,000X in a selected area (dashed square in top images) in order to evaluate the presence of QD in the membrane (bright dots indicated by arrowheads), scale bar 2 μm. No QDs were present in non-apoptotic cells (a') whereas in apoptotic cells (b') dispersed or groups of QDs were observed. In the inset (*) a detail of a selected area (dashed square) is displayed and two QDs in the membrane of the cell can be clearly observed.

### 3.4 Quantification of AnnV-QD labelled apoptotic THP-1 cells by Flow Cytometry

We also used flow cytometry to analyze the same samples used for CLSM and SEM; in this way we could prove both, the versatility of AnnV-QD labels using a different technique, and the acquisition of quantitative data to be used for evaluating the performances of the new electrochemical detection. In particular, for each culture, 20,000 cells were analyzed and representative plots were obtained for each sample. Figure 4 shows the distribution of the total cell culture population. The red colored area corresponds to the apoptotic cell

population labelled with AnnV-QD, which was determined according to the negative control analyzed under the same conditions (Fig. S-2 in the ESM). Comparing both plots, a more condensed red area is observed in the apoptosis induced culture than in the control one, as expected. We extracted numerical data from the plots and calculated the percentage of apoptosis for each sample. Apoptotic cells constituted 5% of the control sample, as usual in normal healthy or untreated cell cultures [37], whereas for induced cultures it was 17.1%. These results indicate that apoptosis was successfully induced by STS and that AnnV-QD label method allowed the specific detection of apoptotic cells via flow cytometry.

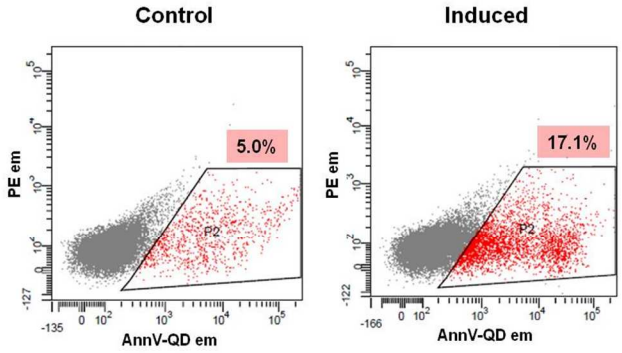



**Fig. 4 Flow cytometry analysis.** A total of 20,000 cells from control and induced cultures were analyzed and the number of cells emitting fluorescence (AnnV-QD labelled cells) were quantified (P2 area; red dots). The population of AnnV-QD labelled cells (P2) increased substantially in induced cell cultures (17.1% in front of 5.0% of the control). AnnV-QD em = fluorescence intensity emitted at 655 nm. PE em = fluorescence intensity emitted at 585 nm (used as control).

### 3.5 Electrochemical stripping detection of apoptotic THP-1 cells through QDs

Finally, we developed an electrochemical method, based on the stripping voltammetry of QDs (Fig. 5a), to detect apoptotic cells. It is based on the redox properties of the CdSe/ZnS QDs, which produced a characteristic oxidative peak at -0.720 V. The peak intensity can be used to estimate the amount of QDs in the sample that in turn can be related to the quantity of apoptotic cells present. The stripping voltammetry was performed in PBS using disposable screen printed electrodes and a potentiostat, as shown in Fig. 5b. In this way, an entire population of cells can be screened, checking the effect of the drug upon their viability. In Fig. 5c, the histogram displays the peak values obtained by electrochemical detection of serial dilutions of control and apoptotic-induced cells. Considering the same number of cells, the apoptotic-induced cells generated higher peaks compared to control samples, due to the higher amount of QDs present in their plasma membrane, indicating the specific binding of AnnV-QDs to the externalized PS. The difference is observable in a range from 20,000 to 1,250 cells (inset of Fig. 5c shows a classical read-out of the electrochemical stripping detection of different dilutions of apoptotic cells). Control cell cultures, as observed by CLSM and flow cytometry, also gave low intensity signals due to the basal apoptotic activity of a normal cell culture. This electrochemical method allows quantitatively compare the effect of a drug between an induced sample and a control one, but, when used in correlation with flow cytometry data, allows to estimate the number of apoptotic cells in the sample. In the present case, the flow cytometry results indicate that 17% of cell analyzed in the induced sample are apoptotic cells, thus, we can assume that in the smallest concentration used in the electrochemical detection, consisting of 1,250 cells, about 212 are apoptotic cells.

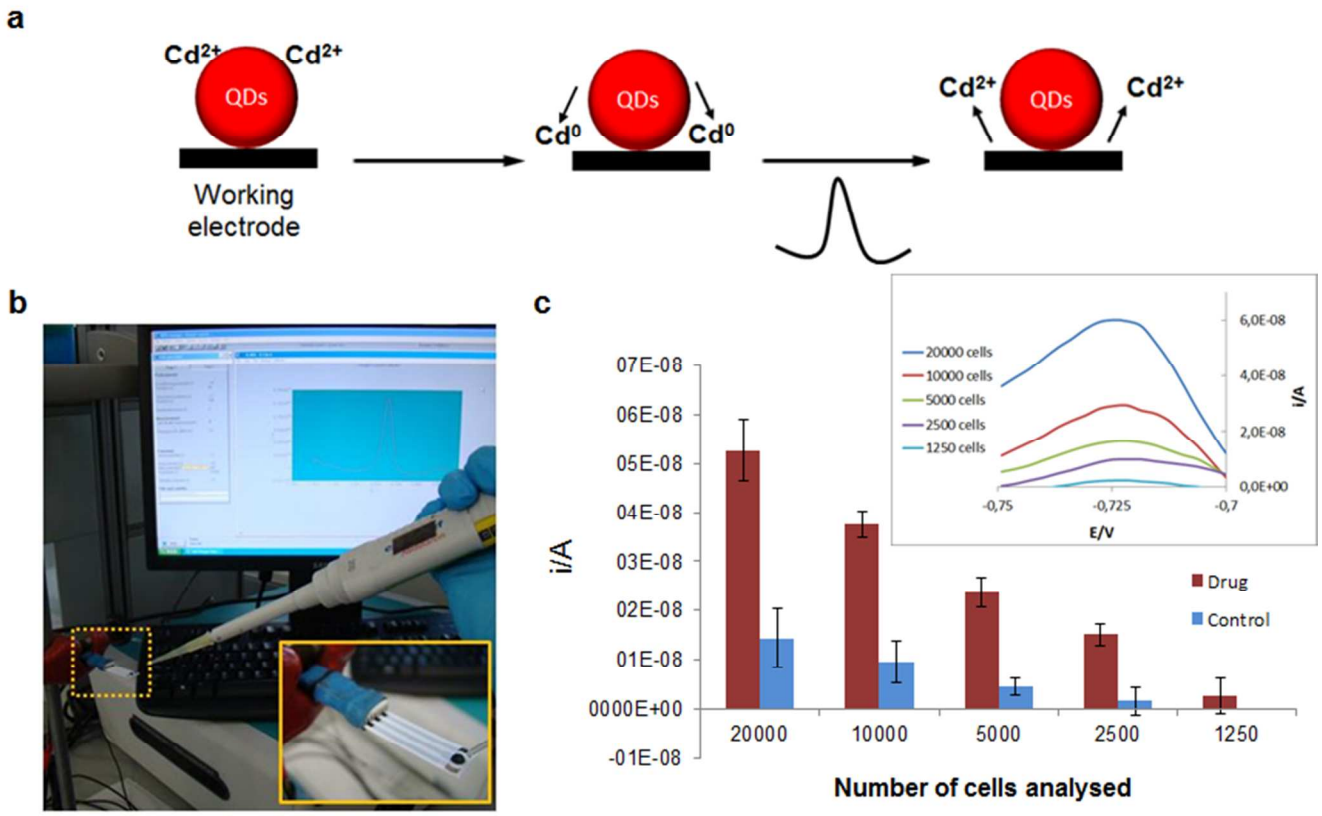



**Fig. 5 Electrochemical detection.** (**a**) Schematic of the electrochemical detection by SWV of QDs. First Cd ions (as defects at CdSe QDs surface) are reduced to $Cd^0$ and the subsequent oxidation to $Cd^{2+}$ generates a peak, the intensity of which corresponds to the amount of QDs present on the electrode surface. (**b**) Photo of the set-up for the electrochemical detection. (**c**) Electrochemical stripping peak currents obtained measuring different amounts of induced-apoptotic cells (red columns) and control cells (blue columns). In the inset typical electrochemical stripping curves obtained for the apoptotic cells are shown.

### 3.6 Correlation between electrochemistry and other quantitative techniques

We compared the results obtained analyzing 20,000 cells by flow cytometry and electrochemistry, as well as with those from CLSM (Table 1). Data collected showed an excellent correlation (~97.7%) between flow cytometry and electrochemistry, with almost the same value of fold-increase between the control and apoptosis induced sample, respectively 3.42 and 3.50. The 2.72 fold increase obtained with CLSM is also in a good agreement with the previous ones (~78%) (although it cannot be used as a quantitative result, due to the lack of statistical significance of the technique). This new electrochemical method can be considered as a faster (readout obtained in less than 5 minutes), cheaper and easier-to-use alternative or complementary method to flow cytometry. Unlike other electrochemical methods for heavy metal detection (such as those incorporating QDs) all measurements are carried out in a cell-friendly saline solution instead of using strong acidic conditions. Other advantages of this method include the fact that it can be used by untrained personnel, that is easily miniaturizable and that does not limit the user to working with samples containing high number of cells.

**Table 1.** Apoptosis analysis. Correlation between electrochemistry, flow cytometry and confocal laser scanning microscopy (CLSM) measures.

| Technique | Control cells | Induced cells | Fold increase |
|---|---|---|---|
| Electrochemistry (nA) | 15.0 | 52.50 | 3.50 |
| Flow Cytometry (%) | 5.0 | 17.10 | 3.42 |
| CLSM (A.U.) | 0.022 | 0.06 | 2.72 |

## 4. Conclusions

We have explored the use of AnnV-QD probes as optical and electrochemical labels to easily carry out correlative studies of the same sample using different techniques such as voltammetry, flow cytometry, CLSM and SEM. The complementary information obtained from the different methods allows for greater insight into the apoptotic process, compared to data obtained from single techniques using other dyes or enzymes. The proposed quantitative, electrochemical-based method is faster, cheaper and more versatile than most of those currently in use and can be easily miniaturized into a point-of-care, micro-fluidic device, making it useful for both research and industrial settings. We expect this technique to be extended not only to other cell toxicity studies with interest for clinical industries, but also to the detection of any disease-biomarker expressed in the plasma membrane, with clear relevance for diagnostic applications.

## Acknowledgements

The authors thank MICINN (project MAT2011-25870 and TEC2011-29140-C03-03), SMS (EU FP7 project) and the Generalitat de Catalunya (project 2014SGR-524) for supporting of this project.
We also would like to thank Paula Llergo-Noel (Cell Biology Unit, UAB, Barcelona) for the maintenance of cell cultures, Manuela Costa (Service of Cell cultures, Antibody production and Cytometry, UAB, Barcelona) for the advice in Flow Cytometry analysis, Marcos Rosado (Electron Microscopy Division, ICN2, Barcelona) for the help with SEM imaging, Dámaso Torres (Webmaster and Graphic Designer, ICN2, Barcelona) for the help in drawing schematics and Dr. Eleanor Gray (London Centre for Nanotechnology, UCL, London) for helping during the revision of the manuscript.

## Notes and references

[*a] Departament de Biologia Cel•lular, Fisiologia i Immunologia, Universitat Autònoma de Barcelona, Campus UAB-Facultat de Biociències, 08193 Bellaterra (Barcelona), Spain. E-mail: carme.nogues@uab.cat
[b] Nanobioelectronics & Biosensors Group, Institut Català de Nanociència i Nanotecnologia ICN2, Campus UAB, 08193 Bellaterra (Barcelona), Spain. E-mail: arben.merkoci@icn.cat
[c] ICREA - Institució Catalana de Recerca i Estudis Avançats, 08010 Barcelona, Spain.

Electronic Supplementary Information (ESI) available: optical microscope images of apoptotic induced cell cultures at different times and negative control of flow citometry. See DOI: 10.1039/b000000x/

1 J. F. Kerr, C. M. Winterford and B. V Harmon, *Cancer*, 1994, **73**(12), 3018.

2 S. MacEwan and A. Chilkoti, *Nano Lett.*, 2014, **14**, 2058–2064.

3 M. P. Mattson, *Nat. Rev. Mol. Cell Biol.*, 2000, **1**, 120–129.

4 G. Takemura and H. Fujiwara, *J. Cell. Mol. Med.*, 2006, **10**, 56–75.

5 S. J. Martin, C. P. Reutelingsperger, A. J. McGahon, J. A. Rader, R. C. van Schie, D. M. LaFace and D. R. Green, *J. Exp. Med.*, 1995, **182**, 1545–1556.

6 G. Rimon, C. E. Bazenet, K. L. Philpott and L. L. Rubin, *J. Neurosci. Res.*, 1997, **48**, 563–570.

7 S. M. Van den Eijnde, L. Boshart, C. P. Reutelingsperger, C. I. De Zeeuw and C. Vermeij-Keers, *Cell Death Differ.*, 1997, **4**, 311–316.

8 P. A. Leventis and S. Grinstein, *Annu. Rev. Biophys.*, 2010, **39**, 407–427.

9 K. Schutters and C. Reutelingsperger, *Apoptosis*, 2010, **15**, 1072–1082.

10 H. Shi, R. T. K. Kwok, J. Liu, B. Xing, B. Z. Tang and B. Liu, *J. Am. Chem. Soc.*, 2012, **134**, 17972–17981.

11 H. A. Andree, C. P. Reutelingsperger, R. Hauptmann, H. C. Hemker, W. T. Hermens and G. M. Willems, *J. Biol. Chem.*, 1990, **265**, 4923–4928.

12 I. Vermes, C. Haanen, H. Steffens-Nakken and C. Reutelingsperger, *J. Immunol. Methods*, 1995, **184**, 39–51.

13 H. van Genderen, H. Kenis, P. Lux, L. Ungeth, C. Maassen, N. Deckers, J. Narula, L. Hofstra and C. Reutelingsperger, *Nat. Protoc.*, 2006, **1**, 363–367.

14 D. M. Monsalve, T. Merced, I. F. Fernández, S. Blanco, M. Vázquez-Cedeira and P. Lazo, *Cell Death Dis.*, 2013, **4**, e513.

15 L. Quinti, R. Weissleder and C.-H. Tung, *Nano Lett.*, 2006, **6**, 488–90.

16 U. Resch-Genger, M. Grabolle, S. Cavaliere-Jaricot, R. Nitschke and T. Nann, *Nat. Methods*, 2008, **5**, 763–775.

17 H. Montón, C. Nogués, E. Rossinyol, O. Castell and M. Roldán, *J. Nanobiotechnology*, 2009, **7**, 4.

18 O. Kovtun, X. Arzeta-Ferrer and S. J. Rosenthal, *Nanoscale*, 2013, **5**, 12072–81.

19 A. P. Alivisatos, W. Gu and C. Larabell, *Annu. Rev. Biomed. Eng.*, 2005, **7**, 55–76.

20 R. Gill, M. Zayats and I. Willner, *Angew. Chem. Int. Ed. Engl.*, 2008, **47**, 7602–25.

21 R. Freeman, J. Girsh and I. Willner, *ACS Appl. Mater. Interfaces*, 2013, **5**, 2815–34.

22 S. Jiang, K. Y. Win, S. Liu, C. P. Teng, Y. Zheng and M.-Y. Han, *Nanoscale*, 2013, **5**, 3127–48.

23 S. Le Gac, I. Vermes and A. van den Berg, *Nano Lett.*, 2006, **6**, 1863–9.

24 Y. Wang and L. Chen, *Nanomedicine*, 2011, **7**, 385–402.

25 X. Wu, H. Liu, J. Liu, K. N. Haley, J. A. Treadway, J. P. Larson, N. Ge, F. Peale and M. P. Bruchez, *Nat. Biotechnol.*, 2003, **21**, 41–46.

26 J. Dimitrijevic, L. Krapf, C. Wolter, C. Schmidtke, J.-P. Merkl, T. Jochum, A. Kornowski, A. Schüth, A. Gebert, G. Hüttmann, T. Vossmeyer and H. Weller, *Nanoscale*, 2014, **6**, 10413–22.

27 P. K. Chattopadhyay, S. P. Perfetto, J. Yu and M. Roederer, *Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology*, 2010, **2**, 334–348.

28 E. Petryayeva, W. R. Algar and I. L. Medintz, *Appl. Spectrosc.*, 2013, **67**, 215–52.

29 Y. Luo, C. Wang, T. Jiang, B. Zhang, J. Huang, P. Liao and W. Fu, *Biosens. Bioelectron.*, 2014, **51**, 136–142.

30 G. Rousserie, A. Sukhanova, K. Even-Desrumeaux, F. Fleury, P. Chames, D. Baty, V. Oleinikov, M. Pluot, J. H. M. Cohen and I. Nabiev, *Crit. Rev. Oncol. Hematol.*, 2010, **74**, 1–15.

31 E. Morales-Narváez, H. Montón, A. Fomicheva and A. Merkoçi, *Anal. Chem.*, 2012, **84**, 6821–7.

32 E. Morales-Narváez, A. R. Hassan and A. Merkoçi, *Angew. Chemie - Int. Ed.*, 2013, **52**, 13779–13783.

33 S. Marin and A. Merkoçi, *Nanotechnology*, 2009, **20**, 055101.

34 M. Medina-Sánchez, S. Miserere, S. Marín, G. Aragay and A. Merkoçi, *Lab Chip*, 2012, **12**, 2000–5.

35 M. Amelia, T. Avellini, S. Monaco, S. Impellizzeri, I. Yildiz, F. M. Raymo and A. Credi, *Pure Appl. Chem.*, 2011, **83**, 1–8.

36 J. Wang, G. Liu and A. Merkoçi, *J. Am. Chem. Soc.*, 2003, **125**, 3214–5.

37 Y. Wu, D. Connors, L. Barber, S. Jayachandra, U. M. Hanumegowda and S. P. Adams, *Toxicol. Vitr.*, 2009, **23**, 1170–1178.

38 W.-T. Li, H.-W. Tsao, Y.-Y. Chen, S.-W. Cheng and Y.-C. Hsu, *Photochem. Photobiol. Sci.*, 2007, **6**, 1341–1348.

39 A. H. Heussner and D. R. Dietrich, *Open J. Apoptosis*, 2013, **02**, 25–30.

40 N.-S. Chang, *BMC Cell Biol.*, 2002, **3**, 8.

## TOC

A novel-electrochemical AnnexinV-QD conjugate, which can be also used as classical-optical label, is applied for apoptosis detection in THP-1 cell cultures. The electrochemical detection of AnnV-QD labeled cells gives qualitative and quantitative results in a fast and cost/effective way.