S. Passamonti, S. Gustincich, T. Lah Turnšek, B. Peterlin, R. Pišot, P. Storici (Eds.)
CONFERENCE PROCEEDINGS with an analysis of innovation management and knowledge transfer potential for a smart specialization strategy
ISBN 978-88-8303-572-2 / e-ISBN 978-88-8303-573-9. EUT, 2014.

SERS-BASED NANOSENSORS FOR THE SENSITIVE DETECTION OF REACTIVE OXYGEN SPECIES

Stefano Fornasaro[1,2], Annalisa Vicario[3], Lovro Ziberna[2], Federica Tramer[2], Valter Sergo[3], Sabina Passamonti[2] and Alois Bonifacio[3]

[1] Graduate School of Nanotechnology, University of Trieste, Trieste
[2] Department of Life Sciences, University of Trieste, Trieste
[3] Department of Engineering and Architecture, University of Trieste, Trieste

Abstract — The unbalanced production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) has been implicated in the pathogenesis of several human diseases. Indeed, there is great interest in developing methods for measuring quantitatively and selectively the production of ROS/RNS in living cells. Here, we report a simple and sensitive method for the detection of ROS, based on surface-enhanced Raman scattering (SERS) spectroscopy.

Index Terms — ROS, SERS, gold nanoparticles, antioxidants

1 BACKGROUND

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are small-molecule reactive species associated with various cellular functions. Indeed, the unbalanced production of ROS/RNS have been implicated in the pathogenesis of several human diseases and conditions, including aging, chronic inflammation, neurodegeneration, cardiovascular diseases, and certain types of cancer. A high inter-reactivity and short life spans have made real-time monitoring of these compounds in cellular systems challenging. Thus, there is currently great interest in developing methods for measuring quantitatively and selectively the production of ROS/RNS in living cells.
Recently, surface-enhanced Raman spectroscopy (SERS) has proven to be an attractive technique for sensitive, ultra-fast and non-destructive determination of biological species in low-concentration.

2 OBJECTIVES

Development of a simple and sensitive method for detection and imaging of ROS/RNS, based on surface-enhanced Raman scattering (SERS) spectroscopy

3 APPROACH & METHODS

General approach

Modified metal nanoparticles are introduced into cells and used to directly monitor internal cellular molecules.

Methods

We developed nanosensors composed of a self-assembled layer of molecules susceptible to oxidation, adsorbed onto the surface of gold nanoparticles (Au NPs). The oxidation of these compounds induces a chemical change that is reflected in spectral differences. The spectral analysis permits the concentration of ROS/RNS to be monitored in real time and in a non invasive manner.

4 RESULTS

Various ROS/RNS can induce chemical changes in the structure of the adsorbed molecules, which is reflected in specific spectral differences. This allows the measurement of specific ROS/RNS under different experimental conditions. SERS imaging experiments also demonstrate that these nanosensors are capable of giving information on both supra-physiological and physiological oxidative stress in single living cells.

Our nanosensors showed interesting properties, such as good water solubility and biocompatibility, and low cytotoxicity. In particular, they exhibit selective and concentration-dependent signals upon exposure to certain ROS, especially superoxide anion radicals. Moreover, they are best excited with near-infrared excitation (785 nm), a particularly convenient feature when studying biological specimen (i.e. low fluorescence background, good tissue penetration).

5 POTENTIAL NEW PRODUCTS & SERVICES

Product: A refinement of this method may enable the multimodal analysis of a complex array of ROS/RNS in bio-systems. This can lead to an improved understanding of the oxidative stress related diseases at a cellular level.

Service: A high performance intracellular detection platform for the evaluation of the antioxidant activity of pure compounds (e.g. new molecular entities, drug candidates), natural extracts (foods, nutraceuticals), or endogenous compounds could be established.

6 CURRENT COLLABORATIONS

6.1 With other researchers

Mojca Kržan, Insitute of Pharmacology and Experimental Toxicology, Faculty of Medicine, University of Ljubljana;
Vanessa Nicolin, Department of Medicine, Surgery and Health Sciences, University of Trieste.

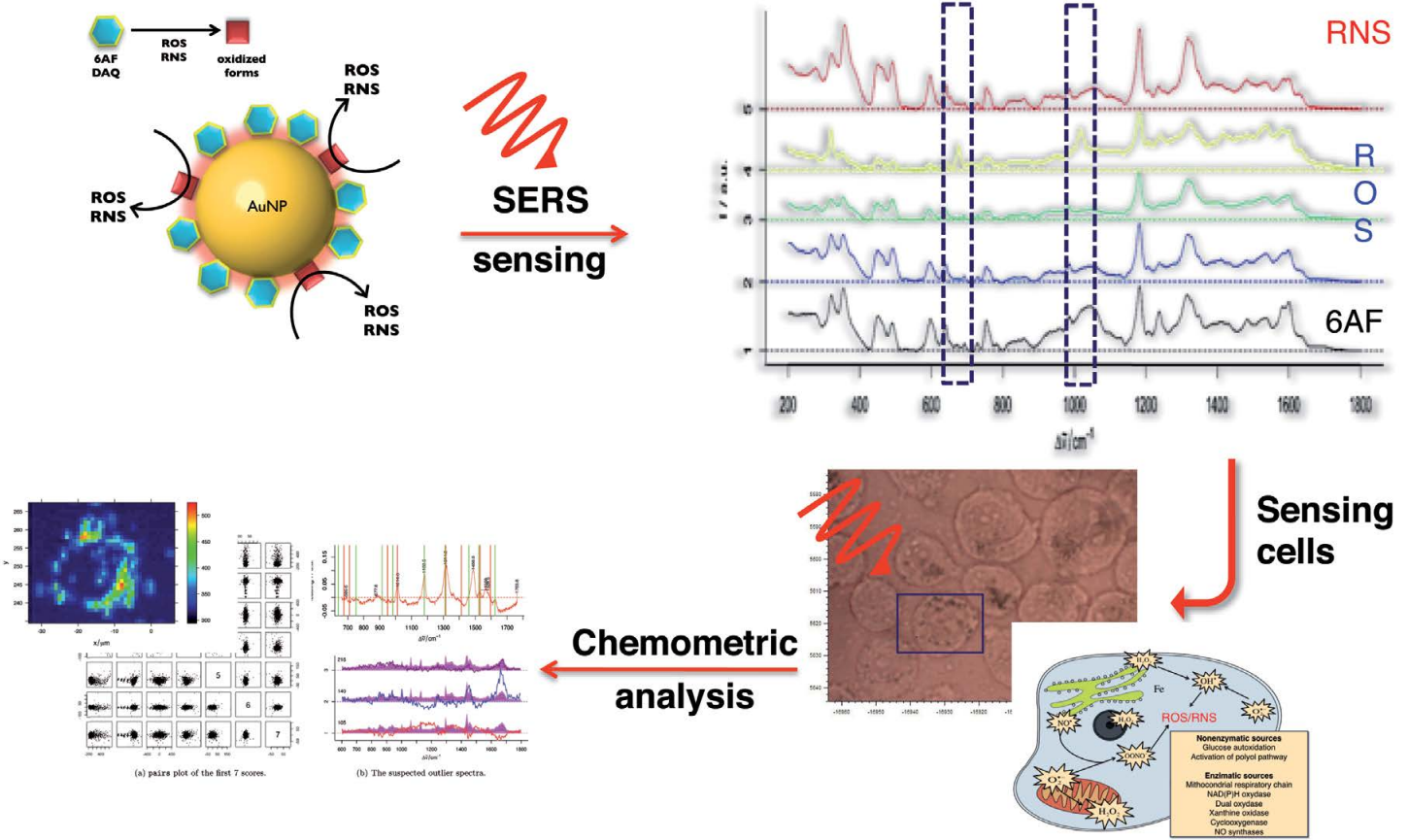

Figure 1: Graphical abstract

7 CONTACT OR COLLABORATIONS NEEDED

To conduct feasibility study (technical feasibility, method validation, prototype development), we are looking for experts in bioinformatics and engineers.
To develop business strategy (business model, market research and validation, sustainability strategy), we need business experts.

8 COMMUNICATION TOOLS

This method has been presented to the scientific community at the ET4H - 1° International Workshop on Protein Electron Transfer: from Fundamentals to Applications for Health, Modena (ITA) 29-30 October 2013. (http://www.et4health.unimore.it/site/home.html).

9 FUNDS NEEDED

9.1 For basic research (investigation of biological mechanisms): 60.000 €

9.2 For applied research (solutions for real-world problems): 100.000 €

9.2 For pilot & demonstrator activities (to develop a prototype): 500.000 €

10 CONCLUSION

We presented a novel SERS-based nanosensors, which are able to detect reactive oxygen species with high sensitivity and sensibility. They exhibit good biocompatibility, and low cytotoxicity. Furthermore, the combination of this sensing method with the advance of SERS imaging technology formed the basis for a high performance intracellular detection platform, which could lead to a greater understanding of oxidative stress-related diseases at a cellular level.

ACKNOWLEDGEMENT

This work was supported by the projects TRAN2CARE and AGROTOUR, which are funded by the European Regional Development Fund, Cross-Border Cooperation Programme Italy-Slovenia 2007-2013.