I. PUBLISHABLE EXECUTIVE SUMMARY

VSNs Voltage-Sensitive Nanoparticles.

Consortium partners:

Technical University of Catalonia
University of Nottinhgam
Multichannel Systems GmbH
Ludwig-Maximilians-Universität München
Hebrew University of Jerusalem
Vrije Uni Amsterdam

Contact information: Dr. Enric Claverol-Tinturé
Technical University of Catalonia
C/ Baldiri i Reixach 15-21
Barcelona 08028
(34)934020209

Project website: http://www.eel.upc.edu/~claverol/VSNs/index.htm

The main goal of the project for the first 12 months has been to develop the experimental setups, protocols and tools, including ITO-based multielectrode arrays and thin multielectrode amplifiers, to support simultaneous electrical/optical recording of activity from nanoparticle-stained model cells, low-density neuronal cultures and brain slices. The consortium has achieved the synthesis of nanoparticles with a variety of capping layers towards electric-field sensitive plasmon-resonance. Single nanoparticle spectroscopy has been carried out under potentiostatic control as a first step to characterise the resonance shift as a function of voltage under cell-free conditions. A setup for dual electro/optical recording of neuronal activity in low-density cultures for long periods fo time (hours to days) has been established using 3D neurochip technology. Further, neurons within slices have been intracellularly stained with nanoparticles and activity has been recorded to validate the low toxicity associated with the procedure. Planar transparent (ITO) multielectrode arrays and thin multichannel amplifiers have been developed to enable multisite dual electrical/optical measurements.

The successful synthesis of a series of metal nanoparticles (NPs) and their characterization by absorption spectroscopy, TEM and SEM was accomplished as part of **workpackage 1**. Scheme 1 outlines the different functionalized NPs that were prepared.

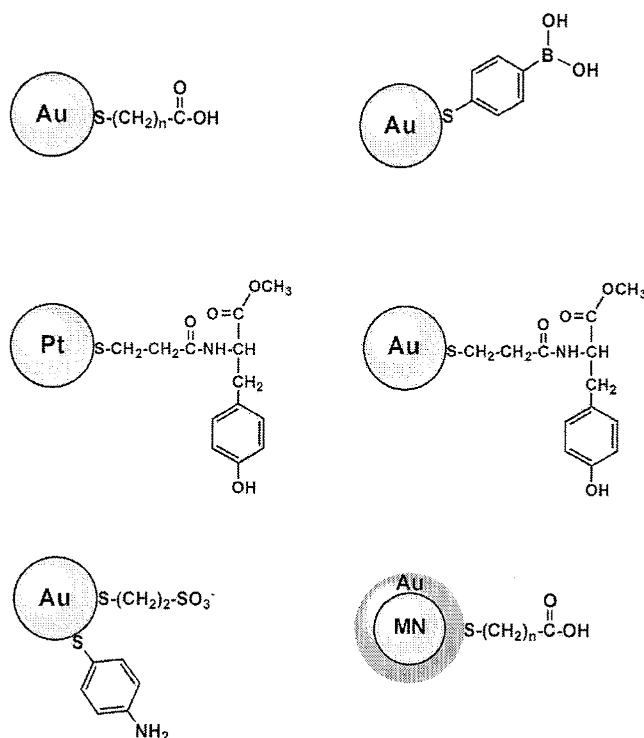

Figure 1.1

1. Nanoparticle plasmon resonane (NPPR) refers to the resonant collective oscillation of nanoparticle-confined electrons in response to light excitation. This phenomenon is responsible for the distinctive colour of a geometrically homogeneous set of nanoparticles when white light illumination is used.

1. Au NPs of different sizes (13 nm; 25-30 nm; 50 nm) were synthesized and capped with thiolated carboxylic acids (mercaptopropanoic acid; mercaptoheptanoic acid; mercaptoundecanoic acid). The different NPs were distributed to the partners for further investigations.
2. Au NPs capped with thiophenyl boronic acid for the specific binding to glycosylated membranes were synthesized.
3. Pt NPs and Au NPs modified with a tyrosine methyl ester capping layer were synthesized. The resulting NPs were reacted with tyrosinase to yield the respective L-DOPA derivatives. The Pt NPs were associated to a thiolated phenylboronic acid, as a model for membrane interactions with glycosylated membranes. The system was used to develop an electrochemical sensor for tyrosinase activity.
4. Au NPs modified with π-donor thioaniline capping units were prepared. These NPs were assembled on conductive electrode surfaces by electropolymerization.

The central objective of **workpackage 2** is to gain insight into the spectral changes of a nanoparticle plasmon resonances (NPPRs) of gold nanoparticles (AuNPs) bound to membranes as they occur when the voltage across the membrane is changed. Single AuNP spectroscopy is employed to measure voltage sensitive NPPR changes that would otherwise remain hidden behind inhomogeneously broadened ensemble spectra.

Within the first 12 months, the focus was on establishing single AuNP spectroscopy in the presence of membranes. Non-functionalised AuNPs were to be investigated under potentiostatic control (Deliverable D2.1). We have successfully carried out these experiments as outlined below. In addition, we have started to work on deliverable D2.2 (Effects of external parameters on the NPPR resonance) and to work on deliverable D2.3 (NPPR resonance shift with electrically tuneable dielectric coatings).

The central goal of **workpackage 3** is to correlate the optical signals, obtained from NP-stained neurons in dissociated cultures, with the electrical activity of the cells. This work is designed to

inform the consortium on the impact of NPs on the anatomy and function of the stained neurons. Should negative effects be observed, feedback is to be provided to HUJI in guiding the redesign of the NP capping layer. Further, optical signals obtained from NP-stained neurons could potentially be modulated by membrane potential, the ultimate goal of the project. Threrefore, dual optical/electrical recordings from dissociated cultures are necessary to validate progress towards this goal.

Over the first 12 months we have performed neuron staining using various NPs provided by HULJI. We have studied with TEM the possibility of particle internalisation and the effect of cell culture viability. We have observed little toxicity effects on the density of viable cells and have confirmed that NPs with some capping layers can be internalised. This observation suggests that NP attachment to substrates might be an appropriate strategy to ensure NP-to-membrane proximity and avoid internalisation. Such a strategy will be explore over the following phase of the project, should internalisation be observed for all capping layers.
We have also performed whole-cell recordings with conventional electrophysiology setups in preparation of future deliverables. These were carried out concomitandly with HeNe laser illumination of the neuronal membranes in order to study possible sources of optical noise associated with motion of the liquid-air interface or liquid-particles which could preclude future unaveraged observation of activity-induced changes in NP plasmon resonance. These preliminary experiments suggested that should long-term (>1 hour) recordings be necessary to average thousands of spikes in search for optical signatures in the NP resonance, multielectrode array (MEA) devices would be better suited as these potentially allow recordings over days. To this end we have started to set up a polymer-on-multielectrode array (PoM) setup to perform long-term recordings on identified axon bundles simultaneously with optical measurements.

As part of **workpackage 4,** CNCR tested the putative extent of toxicity and visualization properties of commercially available nanoparticles (ranging from 15-150 nm, uncoated), by perfusing them into cortical neurons in intact brain slices with intact synaptic connectivity. Recording and visualization was performed on a Leica Laser Scanning Microscope (LSM). For these experiments we used whole cell patch clamp technology and acute brain slices from Black6 inbred normal mice. Recordings were performed both in hippocampus (CA1 neurons) and in prefrontal cortex (layer V, medial prefrontal and infralimbic) slices (400 micrometer thick) of postnatal day 14 animals. In total during the last 10 months over 250 intracellular recordings were performed, and currently we can conclude that
a) NPs of variable diameter (15, 40 and even 150 nm commercially available) were perfused into functional neurons with producing any negative effects on their firing properties and/or synaptic input.
b) In two photon experiments using a 810 nm, low laser power (< 5%) and a microscope objective of 20 x (NA 0.5) NPs can be visualized (see fig 4.1)

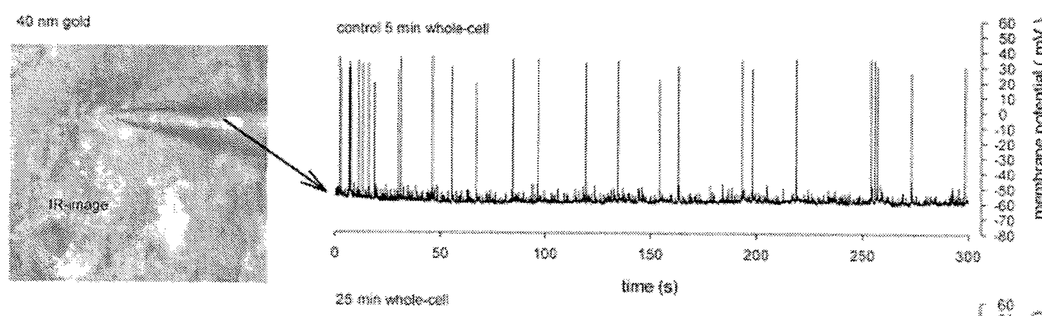

Fig. 4.1: Perfusion of 40 nm NPs does not produce harmfull effects on neuronal firing (n = 15)

c) Excitation NPs with low laser power (5%) does not significantly affect neuronal firing and/or synaptic activity, even when using a 40x objective (0.8 NA)
d) At (abnormal) high laser power of ~ 10%, NPs dialysed into intact neurons, induces only small excitations without being harmfull for the neuronal integrity (see fig. 4.2)

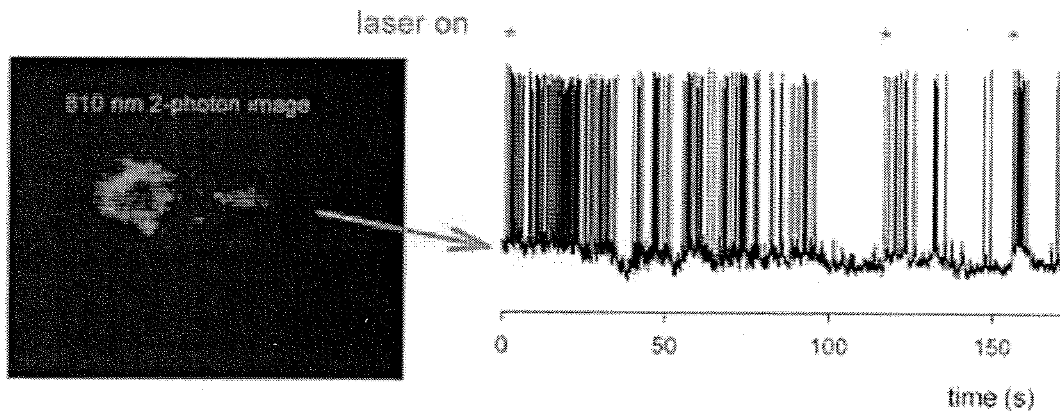

Fig. 4.2: Neuron shown in Figure 1, but here visualized with laser-light above 10% intensity. Small excitatory effects are observed, but the cell's integrity was not lost, given the fact that recording could be continued.

e) At ultra high laser powers of > 20-36 % intensity, recording were lost presumably due to apoptosis of the neurons. Hence instead of being toxic to neurons NPs appear inert, unless high laser intensities are used.

As for **workpackage 5**, UNOTT has explored optimal dark-field microscope (DFM) for NP/NR characterisation and optimisation of wavelengths for one or two-wavelength systems. Work is ongoing on a phase sensitive microscope for high SNR imaging of VSNs. We have designed and currently constructing a PTI microscope by making modifications to a commercial microscope body. We also plan to investigate the use of phase contrast, rather than interferometry to image the transient phase object created by the heated NP. We believe this will produce a more reliable instrument that is better suited for use by our partners in biological, rather than optical laboratories. Initial results and published literature indicate that analysis of NPs down to 10 nm or less should be possible with this instrument, even in cultured cells and possibly brain slices. The system is presently under construction and results from the technique will be reported in the next three months. We believe that is the most promising method since it is the only one that promises to visualize single particles in a scattering background with dimensions comparable to the thickness of the cell membrane ((to a factor of two or so).

Workpackage 6 has addressed the integration of VSN hardware/software with MCS's electrophysiology product range. Microelectrode arrays with transparent ITO electrodes have been developed and a multi channel amplifier has been modified to fit onto a microscope and to fulfill the requirements of the optics for VSNs imaging. Towards fully functional integration of electrical and optical measurements, the development of a microcontroller unit for camera control and synchronization of electrical and optical signals is ongoing.