ELECTRODE NANOGAP ENHANCED AND DIELECTROPHORESIS-ENABLED RAMAN SPECTROSCOPY OF SINGLE BIOMOLECULES WITH SIMULTANEOUS REAL-TIME ELECTRONIC MONITORING

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ABSTRACT

This work reports a versatile platform for manipulation and sensing of biological molecules based on electrode nanogaps, which function as dielectrophoresis-enabled molecular trapping templates as well as spectroscopic and nanoelectronic detection substrates. On a chip, an array was constructed of Ti/Al nanoelectrode pairs with gap dimensions ranging from few tens of nm to <10 nm. During the molecular trapping process, ionic current measurements across the nanoelectrodes were used to detect trapping of a protein in the gap. Concurrent recording of time-dependent Raman spectra provided evidence of molecule(s) appearing in the nanogap.

KEYWORDS

Multi-functional Molecular Analysis, Surface-enhanced Raman Spectroscopy, Nanoelectronics, Dielectrophoresis.

INTRODUCTION

Metallic electrode nanogaps, aided by the application of AC and DC electric fields in aqueous solutions, are able to generate a non-uniform electric field that exerts forces on polarizable dielectric particles, giving rise to the phenomenon of dielectrophoresis (DEP) [1,2]. DEP has been employed to attract, e.g., large biological molecules in the inter-electrode space. Trapping has been observed down to the level of a single/few molecule(s) [3]. On the other hand, electrode nanogaps are able to enhance optical near-fields, leading to a dramatic enhancement of Raman spectra of target molecules in the junctions [4]. This work demonstrates the use of metallic nanostructures to capture freely diffusing proteins present in an aqueous solution, and simultaneously characterize the capturing process both spectroscopically and electronically in a time-dependent manner.

EXPERIMENT

An array of 50 nm thick Ti/Al nanoelectrode pairs, with gap dimensions ranging from few tens of nm to <10 nm, were fabricated on an open top 7x7 mm^2 thermally oxidized Si chip with electron-beam lithography, e-gun metal evaporation followed by a lift-off process. The chip has predefined microelectrodes that were constructed on a wafer scale by means of positive-tone photolithography, thermal evaporation of a Ti/Au bilayer and liftoff with an acetone soak assisted by low-power sonication. Microelectrodes have contact pads for wire bonding interconnections to a printed circuit board for electric voltage application, current amplification and data acquisition (Figure 1).

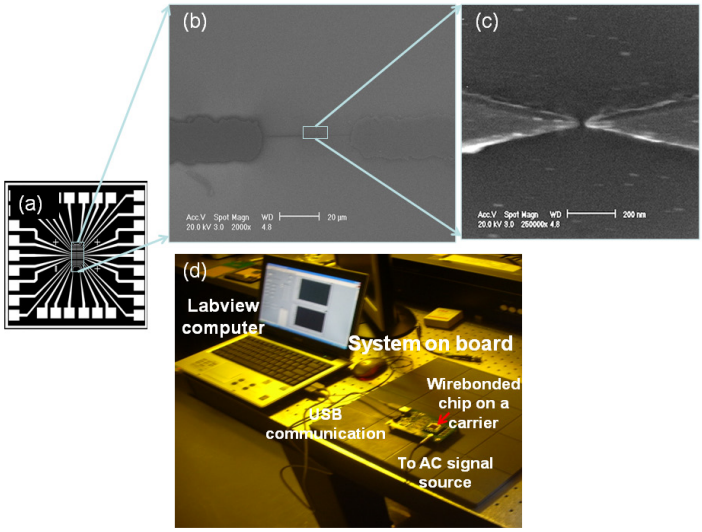

Figure 1. (a) Electrode nanogaps on a chip (scanning electron micrographs, b and c). (d) Computer-controlled integrated circuit board with field application, current amplification and data acquisition capabilities.

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R-phycoerythrin (RPE), a 240 kDa protein of a disk-like shape with a diameter of 11 nm and a thickness of 6 nm from red algae [3], was used for the trapping experiments. RPE was cassette dialyzed in PBS buffer, then diluted to nM and sub-nM concentrations. A small fluidic chamber cut out from a double-sided adhesive tape (70 µm thick) was used to encapsulate a 2 µL droplet of protein solution. The chamber was sealed against evaporation by a coverslip of 5 mm diameter.

Raman spectra were acquired using a Horiba Labram 2 confocal Raman microscope with a HeNe laser (633 nm) as light source.

RESULTS AND DISCUSSION

To identify a "hotspot" for easy sample scanning, Raman spectral maps were first acquired from across an extended region around the gap between the metal electrodes on grid shown in Figure 2(a). The integrated intensity of the Si peak at 520 cm^{-1} is shown in Figure 2(b). The position where the maximum intensity obtained was identified as a "hotspot", and was used for subsequent time-dependent measurements with loaded protein samples.

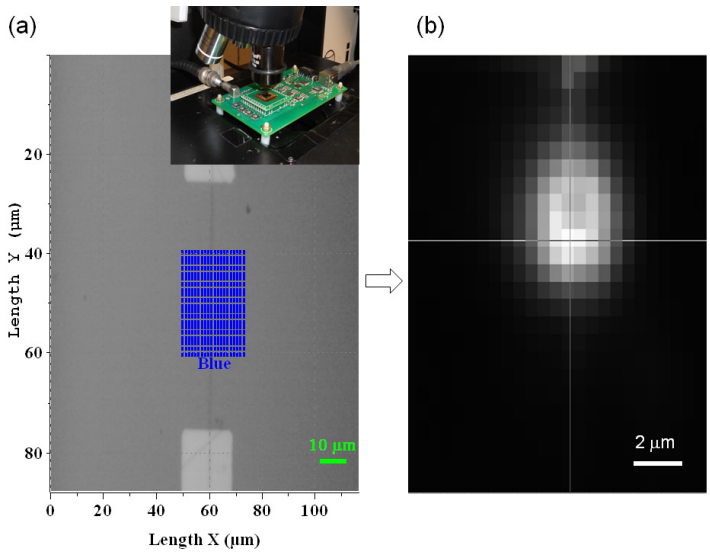

Figure 2. Raman spectral maps in the inter-electrode region (a: grid, b: integrated intensity between 500-550 cm^{-1}, inset: image of the board under a confocal Raman microscope).

Upon the application of a voltage across the nanoelectrodes and the subsequent onset of dielectrophoresis, an increase in current is observed (Figure 3), which is absent in experiments without proteins (negative controls). The increase in current is related to protein bridging of the gap during a "field-on" acquisition period. Due to the limited number of protein molecules in the vicinity of the trap at the concentrations used here one can infer that only few molecules are trapped, and that trapping occurs on a molecule-by-molecule basis.

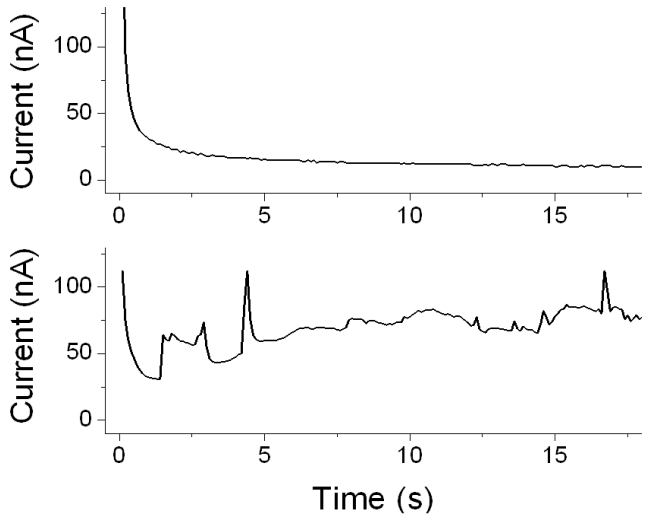

Figure 3. Negative control (upper) and electronic signature of RPE trapping across nanoelectrodes in PBS buffer (lower) with RPE concentration of 40 nM, DC bias 0.1 V, and AC signal of 1 Vpp at 1 MHz.

Time-dependent Raman spectra (Figure 4) show an increase in the fluorescence background, as well as the appearance of a number of vibrational modes concurrent to the increase in current. This observation confirms the presence of protein molecules in the hotspot region. The Raman spectra show several peaks which are characteristic for aromatic amino acid side chains [5], in particular tyrosin. In addition, a peak at ~1570 cm^{-1} is characteristic for carboxylate groups [6].

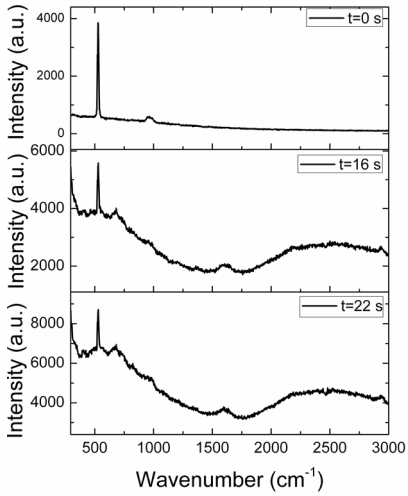

Figure 4. Time-lapse Raman spectroscopy from RPE in the vicinity of a nanogap during a trapping event. Appearance of peaks after few seconds of field application confirms protein trapping in the nanogap region.

Simultaneous fluorescence microscopy with electronic detection was performed in a later experiment to confirm the trapping of a small number of protein molecules under the same electric field conditions used to obtain the Raman results.

CONCLUSIONS

Trapping of a low number of protein molecules has been initiated by DEP. The time evolution of the trapping events has been traced by simultaneous electronic detection of the current and acquisition of Raman spectra. The nanoelectrode DEP-enabled system is suitable for multi-functional molecular analysis with simultaneous spectroscopic and electronic characterizations at single/few molecules level.

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