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MALDI TOF AND AFM STUDIES OF DNA/SWNT HYBRIDS

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Abstract
A primer is a strand of nucleic acid that serves as a starting point for DNA synthesis. DNA, as a large molecule, is very difficult for analysis by means of mass spectrometry. However, primer, as a short sequence of nucleic acid, is much more convinient for e.g. MALDI TOF MS analysis. Mass spectrometric experiment resulted in quality MALDI TOF spectra of 3 primers. Compound 3-HPA (3-hydroxypicolinic acid) showed the best results as matrix. AFM studies were also conducted, for both pure primer samples and hybrids of primer molecule with single wall carbon nanotubes (SWNT). Thus, obtained functionalisation of SWNT with DNA primers was confirmed by AFM imaging. AFM images clearly showed wrapping of DNA structures around nanotube „template". Functionalisation of SWNT is very important for potential applications of nanotubes in biomedical field. MALDI TOF mass spectrometry, in combination with AFM imaging, proved its great potential in analysis of short DNA sequences, and indicated the possibility of investigating more complex DNA structures.

Introduction
One of the most commonly used strategies to render carbon nanotubes (CNT) soluble in aqueous media, and therefore, potentially useful to biomedical applications, is through their surface functionalization (f-CNT). Functionalization of carbon nanotubes can be achieved either by covalent or noncovalent methodologies [1]. Noncovalent association of DNA with CNTs is an effective way to disperse individual CNTs in aqueous solution and has already been used to investigate nanotube optoelectronic and spectroscopic properties and classification of nanotubes according to their length and chirality. Aim of this work is functionalisation of CNTs with DNA primers and AFM characterisation of obtained hybrids. These DNA/SWNT hybrids may have better solubility, compared to „raw" CNTs and this property will facilitate applications of carbon nanotubes. Another aim is to develope a protocol for MALDI TOF and AFM studies of oligonucleotides and hopefully, longer DNA sequences.

Results and discussion
Samples of DNA primers and primer/SWNT hybride were studied by Atomic Force Microscopy (AFM) and matrix assisted laser desorption/ionisation mass

spectrometry (MALDI TOF MS). Primer length was in range from 21 to 32 nucleobases.SWNT were functionalized in advance by amide groups, followed by a reaction with DNA primers. Matrix for MALDI TOF MS was 3-hydroxypicolinic acid (3-HPA). Figure 1. presents MALDI TOF MS spectrum of GSTT1R primer. Spectrum was recorded in linear positive mode. GSTT1R primer is a sequence: 5'CAGCTGCATTTGGAAGTGCTC 3', with theoretical molecular mass M = 6437.2 g/mol. Dominant peak in shown primer mass spectrum is on mass 6519.57 Da, and can be assigned to the molecular ion.

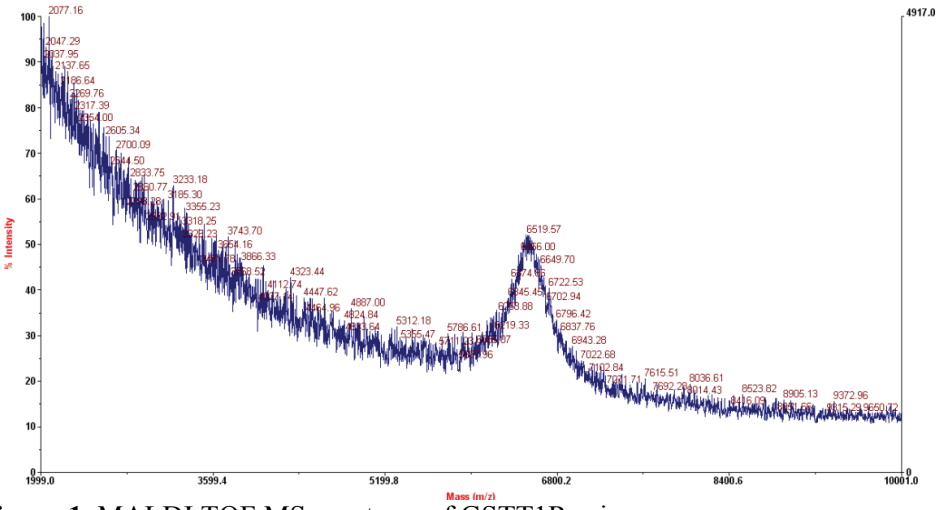

Figure 1. MALDI TOF MS spectrum of GSTT1R primer.

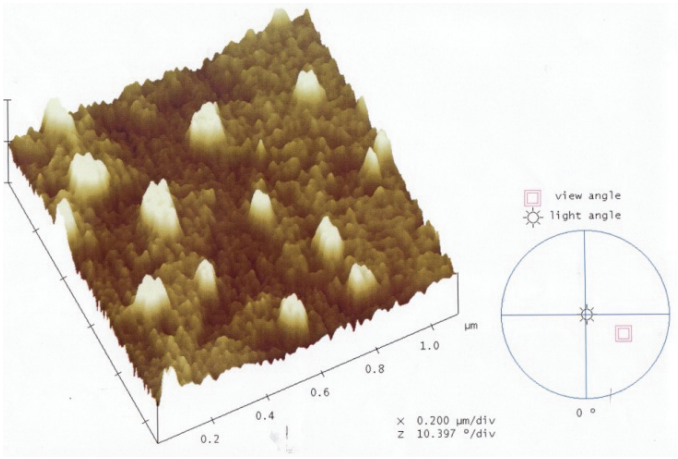

Figure 2. AFM imaging of ApoEF primer (5'TAAGCTTGGCACGGCTGTCCAAGGATAAGCTT-3') on HOPG (highly ordered pyrolytic graphite).

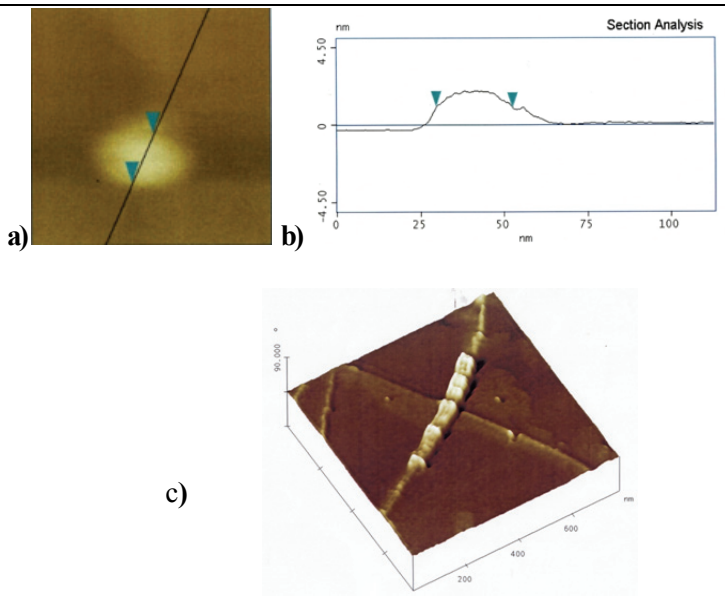

Figure 3. Image of part of GSTT1R primer (a), its dimensions (b) and decoration of SWNT with primer (c).

Figure 2. is AFM image of GSTT1R primer with „caps“, which can be attributed to small fragments within primer strand. Dimension of single fragment is shown in figures 3a and 3b, so observed length was 23.062 nm. Figure 3c shows image which indicate that primer (DNA oligomer) wrap in several layers around the nanotube, forming a strand-like spindle. DNA sequences form compact structures near the tube surface due to the formation of self-assembled structures consisting of a few DNA fragments.

Conclusion

In this work, we developed method for MALDI TOF MS analysis of short DNA sequences, known as DNA primers. AFM studies enabled us to observe dimensions of small fragments within primer samples. Single wall carbon nanotubes (SWNT) were functionalised by primers, and this was confirmed by AFM technique. AFM images confirmed assumption that layers of DNA will wrap around nanotube backbone and this is very improtant for future investigations of enhancing the solubility of carbon nanotubes.

Acknowledgment

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