



Carbon nanotubes-based chemiresistive immunosensor for small molecules: Detection of nitroaromatic explosives

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ABSTRACT

In recent years, there has been a growing focus on use of one-dimensional (1-D) nanostructures, such as carbon nanotubes and nanowires, as transducer elements for label-free chemiresistive/field-effect transistor biosensors as they provide label-free and high sensitivity detection. While research to-date has elucidated the power of carbon nanotubes- and other 1-D nanostructure-based field effect transistors immunosensors for large charged macromolecules such as proteins and viruses, their application to small uncharged or charged molecules has not been demonstrated. In this paper we report a single-walled carbon nanotubes (SWNTs)-based chemiresistive immunosensor for label-free, rapid, sensitive and selective detection of 2,4,6-trinitrotoluene (TNT), a small molecule. The newly developed immunosensor employed a displacement mode/format in which SWNTs network forming conduction channel of the sensor was first modified with trinitrophenyl (TNP), an analog of TNT, and then ligated with the anti-TNP single chain antibody. Upon exposure to TNT or its derivatives the bound antibodies were displaced producing a large change, several folds higher than the noise, in the resistance/conductance of SWNTs giving excellent limit of detection, sensitivity and selectivity. The sensor detected between 0.5 ppb and 5000 ppb TNT with good selectivity to other nitroaromatic explosives and demonstrated good accuracy for monitoring TNT in untreated environmental water matrix. We believe this new displacement format can be easily generalized to other one-dimensional nanostructure-based chemiresistive immuno/affinity-sensors for detecting small and/or uncharged molecules of interest in environmental monitoring and health care.

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1. Introduction

2,4,6-Trinitrotoluene (TNT) is a dual use compound that has found applications both for peaceful industrial and military/terrorist purposes. It is used as ammunition/explosive and in the manufacturing of dyes, plasticizers, herbicides, etc. Because of its persistence, traces of this harmful chemical can be found in soil and groundwater in the vicinity of manufacturing plants and areas with past or current military activities for a long time. TNT contamination has a serious adverse effect on all life forms of our ecosystem (Pennington and Brannon, 2002; Shriver-Lake et al., 1997, 2002). In humans, TNT can cause anemia, abnormal liver function, skin irritation and weakened immune system and it has been classified as a potential carcinogen by the U.S. Environmental Protection Agency (EPA) (Roberts et al., 1993). Therefore, there is a great need for on-site, rapid, reliable, inexpensive and sensitive detection of TNT

in groundwater and soil. Various analytical methods, such as gas chromatography (GC), high performance liquid chromatography (HPLC), mass spectroscopy (MS), X-ray imaging have been utilized for monitoring TNT (Halasz et al., 2002; Walsh, 2001; Yinon and Zitrin, 1993). Although highly sensitive, these techniques require bulky and expensive analytical instruments, sample pretreatment and trained technicians, and cannot be field-deployed (Shankaran et al., 2005).

Immunosensor/affinity-biosensor is a promising technology that is rapidly gaining recognition as an important analytical tool for rapid, on-site/point-of-care trace level monitoring of complex biological and environmental samples. These sensing devices are constructed by combining the specificity/affinity of antibody-antigen reaction with the signal transducing and processing capability of competent electrical and/or optical components. The first generation immunosensors employed the sandwich format with radionuclide, enzyme, fluorophore or redox labeled secondary antibody for signal generation. The lack of an ideal label and the relatively slow speed of detection are the limitations of label-based immunosensors. The second generation immunosensors circumvent these shortcomings by directly measuring the

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physical changes induced by the formation of the complex such as refractive index using surface plasmon resonance (SPR), mass by quartz crystal microbalance (QCM), and piezoelectric or surface acoustic wave (SAW) transducers (Elkind et al., 1999; Larsson et al., 2006; Shankaran et al., 2005).

One-dimensional (1-D) nanostructure based field-effect transistor (FET)/chemiresistor, with carbon nanotubes, silicon nanowires, conducting polymer nanowires, III–V semiconductor, metal oxide, etc. is rapidly gaining recognition as a powerful transducer in label-free monitoring of antigen and antibody binding (Bangar et al., 2009; Wanekaya et al., 2006; Allen et al., 2007; Patolsky et al., 2006; Hangarter et al., 2010; Kim et al., 2007; Li et al., 2005). Besides label-free detection, 1-D nano-FET/chemiresistor advantages include, extremely high sensitivity (potentially down to single molecule), ease of miniaturization, low power requirement and development of high density arrays that will allow simultaneous analysis of a range of different species in extremely small sample volume and reduce false negatives/positives due to massive redundancy. The resistance/conductance of these devices is extremely sensitive to any surface adsorption/perturbation and is a function of the analyte charge. Because of this charge dependence of the sensor sensitivity, successful demonstrations of 1-D nanostructure-based FET immunosensor have been limited to targets with large charges such as proteins, DNA, RNA, viruses, spores and cells. Thus the use of 1-D chemiresistor/FET immunosensor for highly sensitive and selective detection of small and/or weak-/un-charged molecules, important in environmental monitoring, such as TNT, and health care, remains a challenge.

The objective of this study is to develop, characterize and evaluate a 1-D nanostructure based chemiresistive immunosensor for label-free and highly sensitive detection of TNT, a small molecule. In order to achieve this goal, we developed a displacement mode/format nano-immunosensor (Fig. 1A) in which a network of single-walled carbon nanotubes (SWNTs) forming the conduction channel between the source and drain electrodes of the nano-chemiresistive was first modified with trinitrophenyl (TNP), an analog of the target analyte TNT, and then ligated with a highly charged anti-TNP single chain antibody (scAb). The introduction of the target analyte, TNT, that has a higher or comparable binding affinity to anti-TNP scAb resulted in the displacement of the attached scAb producing a large change, several folds higher than the noise, in the nano-chemiresistive resistance/conductance giving an excellent limit of detection, sensitivity and selectivity. Using the displacement principle, we were able to detect 0.5–5000 ppb TNT in buffer and also demonstrated the utility of the sensor for monitoring TNT in untreated water samples with adequate reliability.

2. Experimental

2.1. Reagents

2,4,6-Trinitrophenyl (TNP) coupled with ovalbumin (OVA) was purchased from Biosearch Technologies (Novato, CA, USA). 2,4,6-Trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB), 2-amino-4,6-dinitrotoluene (2A-4,6-DNT), 2,4-dinitrotoluene (2,4-DNT),

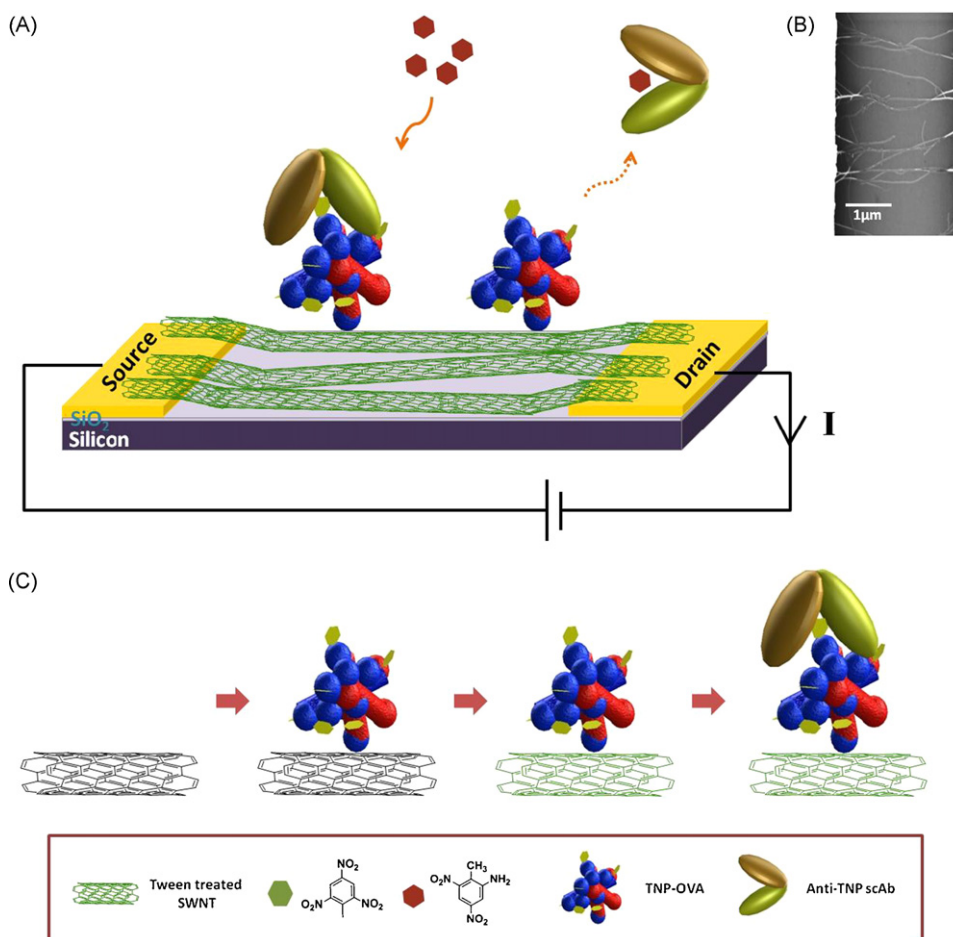


Fig. 1. (A) Schematic diagram of the SWNT immunosensor to detect TNT. Anti-TNP scAb is leaving from the sensor platform due to displacement by TNT. (B) SEM image of aligned SWNTs between two gold electrodes. (C) Sequential modification of the sensor.

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