Sensors and Actuators B 255 (2018) 1507–1516

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Research paper

Highly sensitive, selective and label-free protein detection in physiological solutions using carbon nanotube transistors with nanobody receptors

Marcin S. Filipiak [a,b], Marcel Rother [b], Nesha M. Andoy [a], Arne C. Knudsen [a], Stefan Grimm [b], Christopher Bachran [a], Lee Kim Swee [a], Jana Zaumseil [b], Alexey Tarasov [a,b,*]

[a] BioMed X Innovation Center, 69120 Heidelberg, Germany
[b] Institute for Physical Chemistry, Universität Heidelberg, 69120 Heidelberg, Germany

ARTICLE INFO

Article history:
Received 9 May 2017
Received in revised form 16 August 2017
Accepted 20 August 2017
Available online 24 August 2017

Keywords:
Single-walled carbon nanotube (SWCNT) networks
Field-effect transistor (FET)
Debye screening
Label-free immunosensing
Nanobodies (VHH)
Polyethylene glycol (PEG)

ABSTRACT

Nanomaterial-based field-effect transistors (FETs) have been proposed for real-time, label-free detection of various biological species. However, two major challenges have limited their use in physiological samples: screening of the analyte charge by electrolyte ions (Debye screening) and non-specific adsorption. Here, these challenges are overcome by combining highly stable FETs based on single-walled semiconducting carbon nanotube (SWCNTs) networks with a novel surface functionalization comprising: 1) short nanobody (VHH) receptors, and 2) a polyethylene glycol (PEG) layer. Nanobodies are stable, easy-to-produce biological receptors that are very small (~2–4 nm), thus enabling analyte binding closer to the sensor surface. Despite their unique properties, nanobodies have not been used yet as receptors in FET based biosensors. The addition of PEG strongly enhances the signal in high ionic strength environment. Using green fluorescent protein (GFP) as a model antigen, high selectivity and sub-picomolar detection limit with a dynamic range exceeding 5 orders of magnitude is demonstrated in physiological solutions. In addition, long-term stability measurements reveal a low drift of SWCNTs of 0.05 mV/h. The presented immunoassay is fast, label-free, does not require any sample pretreatment or washing steps.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Nanoelectronic biosensors based on field-effect transistors (FET) have received significant attention as highly sensitive transducers with potential applications in compact and inexpensive biosensing devices for diagnostics, environmental monitoring or screening. Over the past years, many different nanomaterials have been investigated as channel materials for FET fabrication, including semiconducting nanowires [1–3], carbon nanotubes [4–7], graphene [8–11], organic semiconductors [12,13] and other layered two-dimensional materials [14,15]. Among these different materials, carbon nanotubes combine excellent electronic and mechanical properties with the possibility of solution-based processing, rendering them useful e.g. for low-cost printed electronics [16] and sensors. Historically, CNTs have faced significant manufacturing challenges mainly because metallic and semiconducting nanotubes could not be reliably separated resulting in poor control over the device properties. With the recent progress in sorting of CNTs, high purity semiconducting nanotubes with excellent current on/off ratios and reproducible device characteristics – a prerequisite for high-performance transistors and sensors – have finally become available, and these applications seem to be within reach [7].

The basic sensing principle of FET sensors relies on adsorption of charged species on the sensor surface that cause a current change in the transistor channel *via* the field effect. However, two main issues have so far limited their use: 1. screening of the analyte charge by electrolyte ions (Debye screening) and 2. significant non-specific adsorption of other species present in complex physiological solutions. Debye screening is particularly severe as the effective distance for charge detection in physiological conditions (100–200 mM ionic strength) is on the order of 1 nm. This makes the direct detection of large analyte molecules such as proteins extremely difficult, given the fact that the size of typical anti-

Abbreviations: SWCNT, single-walled carbon nanotube; VHH, camelid single domain antibodies (nanobodies); GFP, green fluorescent protein; TEV, tobacco etch virus protease; BSA, bovine serum albumin; FET, field-effect transistor; QCM, quartz crystal microbalance; SPR, surface plasmon resonance; PEG, polyethylene glycol; PBA, pyrene butyric acid.

* Corresponding author at: BioMed X Innovation Center, 69120 Heidelberg, Germany.
E-mail address: tarasov@bio.mx (A. Tarasov).

http://dx.doi.org/10.1016/j.snb.2017.08.164
0925-4005/© 2017 Elsevier B.V. All rights reserved.

body receptor molecules is already 10–15 nm. Therefore, shorter receptors that enable analyte binding closer to the surface, such as antibody fragments or aptamers, have been proposed. Camelid heavy-chain VHH antibody fragments (also called "nanobody") are among the shortest available biological receptors (~13 kDa, <3 nm)[17] and are much smaller and structurally simpler than common whole antibodies (~150 kDa, ~15 nm), Fab fragments (~50 kDa, ~7–8 nm) [18,19] or single-chain variable fragments (scFv, ~27 kDa) [20]. In addition, the nanobodies are easily produced and stable in a range of different conditions [17]. Despite these advantageous properties, nanobodies have not been used yet as receptors for FET-based sensing. On the other hand, using short receptors alone may not be sufficient to achieve appropriate detection limits, as a large part of the analyte may be still screened by the electrolyte ions due to very short Debye length in high ionic strength solutions (<1 nm). As a result, additional strategies have been investigated to address this challenge. [11,21–26]. Unfortunately, most of these approaches require sample dilution, multiple washing steps or elaborate labelling schemes. A particularly interesting idea was developed by Kulkarni et al. [27], where high frequency AC measurements were performed to mitigate ionic screening and to sense analytes beyond the Debye length. However, more work is needed to test the viability of this method for detection of low protein concentrations. More recently, the addition of a polymer that can increase the effective Debye screening length has been proposed as a more general approach [11,28]. So far, specific label-free immunodetection over a wide analyte concentration range in high ionic strength solutions has not been shown.

In this work, we overcome the Debye length problem by developing a novel mixed surface modification consisting of very short nanobody receptors and a polyethylene glycol (PEG) layer. Using green fluorescent protein as a model antigen, we first systematically study the effect of PEG by comparing two different nanobody-coated surfaces, with and without the addition of PEG. The modification is carried out directly on the FET channels, made from high purity semiconducting carbon nanotube networks offering high on/off ratios [29–32], good device reproducibility, and scalable fabrication by solution processing. Interestingly, even without PEG, a significant signal can be measured which is attributed to the small size and random orientation of nanobodies on the surface. In addition, the signal can be strongly enhanced in the presence of PEG, resulting in a substantial sensor response even in high ionic strength buffer. With the PEGylated devices, highly sensitive, selective and label-free protein detection with a sub picomolar detection limit is demonstrated – at least 5 orders of magnitude lower than previous report on a similar PEGylation concept for sensing of prostate specific antigen (PSA) with graphene FETs and aptamer receptors [11]. Finally, the sensor signal versus time is measured and compared with other biosensing platforms, revealing superior stability of carbon nanotubes vs. conventional semiconductors.

2. Materials and methods

2.1. Preparation of SWCNT dispersions

Plasma torch SWCNTs (RN-220, 0.9 –1.9 nm of diameter, 0.3–4 μm of length, SWCNT content 60 – 70%, 70% semiconducting SWCNTs, produced by NanoIntegris, Inc.) were added to 2 mg/mL toluene solution of PF12 (poly(9-dodecyl-9-methyl-fluorene)) to achieve 1.5 mg/mL RN-220 content and bath sonicated for 90 min. Next, the dispersion was centrifuged for 45 mins at 60 000g to remove undispersed material. The supernatant was collected and centrifuged again at 268 000*g* for 60 min to remove bundles of SWCNTs. The supernatant was transferred to a fresh centrifuge tube and sonicated at 268 000 g for 16 h to remove excess polymer. After the solvent was removed, the formed pellet was washed with THF and stored dry until redispersed in pure toluene again just before the alignment step.

2.2. FET fabrication

Interdigitated bottom electrodes (channel width 2 mm, channel length 20 μm) were patterned by means of photolithography using double layer photoresist (MicroChem LOR5 B and Microposit S1813) and evaporated with an electron-beam evaporator (Ti/Au thickness 2 + 30 nm). After that, 1 mL of the redispersed SWCNTs was placed on top of the electrodes and an AC voltage was applied (80 V, 0.1 Hz). Substrates were then washed with THF to remove residual polymer. Atomic force microscopy (tapping mode, Bruker Dimension Icon) was used to determine the average SWCNT density: 5–6 μm^{-1}. Gold contacts were passivated in another photolithography step using SU-8 2005 (diluted 1:1 with cyclopentanone resulting in a thickness of approx. 1 μm) to reduce leakage current.

2.3. Liquid handling

The liquid cell was made from polydimethylsiloxane (PDMS, Dow Chemicals) slab, casted out of a CNC milled Teflon mold with defined microfluidic channels. Inlet and outlet PTFE tubing was introduced as well as a Ag/AgCl reference electrode (DRIREF-2, World Precision Instruments). The liquid cell was aligned on the chip and pressed with a cover. The liquids were introduced into the channel using a Harvard PhD Ultra syringe pump in withdrawal mode (25 μL/min, if not stated otherwise).

2.4. Expression of receptor and analyte molecules

VHH GFP enhancer (a camelid heavy chain VHH fragment specific to green fluorescent protein and additionally enhancing its fluorescence) [33], enhanced GFP (eGFP) and tobacco etch virus protease (TEV) were expressed recombinantly in *E. coli*. VHH GFP enhancer gene in plasmid pHEN (a kind gift of H. Ploegh, Whitehead Institute, MIT, USA) was transformed into *E. coli* strain WK6 for expression. eGFP was cloned into plasmid pET19b (Novagen) using NcoI and EcoRI sites. TEV (maltose binding protein fusion to TEV containing a S219 V point mutation, MBP-TEV S219 V, kindly provided by D. Waugh *via* Addgene, plasmid reference RK793) and eGFP plasmids were transformed into *E. coli* strain Rosetta (DE3) pLysS (Novagen). For all three proteins, protein expression was induced by 1 mM Isopropyl-β-D-thiogalactoside at an OD 600 nm of 0.8–1 for 3 h at 37 °C in a shaker. Cultures were centrifuged (4000*g*, 15 min, 4 °C), resuspended in phosphate-buffered saline (PBS) and sonicated (2 × 1 min at 100% intensity and 0.5 s duty cycle on a Sonopuls HD2070 (Bandelin) equipped with a MS 73 microtip). Samples were centrifuged (4000*g*, 15 min, 4 °C), supernatants applied to Ni-NTA Agarose-loaded columns (Macherey & Nagel), washed by 20 and 50 mM imidazole in PBS before elution in 250 mM imidazole in PBS. Eluted samples were concentrated in Amicon centrifugal filter devices with 3 kDa and 10 kDa cut-off for VHH GFP enh and eGFP/TEV, respectively (Millipore). Concentrates were dialyzed over night against PBS before concentrations were determined by A 280 nm measurements.

2.5. Surface functionalization

An ethanol solution of 1 mM 1-pyrenebutyric acid (PBA, Sigma-Aldrich) and 0.25 mM mPEG-pyrene (molecular weight = 10 kDa, Creative PEGWorks) to achieve heterogeneous self-assembled monolayer ("mixed SAM") or just 1 mM PBA to get homogeneous

Download English Version:

https://daneshyari.com/en/article/7141872

Download Persian Version:

https://daneshyari.com/article/7141872

Daneshyari.com