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Label-Free Immunodetection in High Ionic Strength Solutions Using Carbon Nanotube Transistors with Nanobody Receptors †

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Abstract: Nanomaterial-based field-effect transistors (FETs) have been proposed for real-time, label-free detection of various biological species. However, screening of the analyte charge by electrolyte ions (Debye screening) has so far limited their use in physiological samples. Here, this challenge is overcome by combining FETs based on single-walled semiconducting carbon nanotube networks (SWCNTs) with a novel surface functionalization comprising: (1) short nanobody receptors, and (2) a polyethylene glycol layer (PEG). Nanobodies are stable, easy-to-produce, short biological receptors (~2–4 nm) that enable analyte binding closer to the sensor surface. The addition of PEG 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 4 orders of magnitude is demonstrated in physiological solutions. The presented immunoassay is fast, label-free, does not require any sample pre-treatment or washing steps.

Keywords: single-walled carbon nanotube (SWCNT) networks; field-effect transistor (FET); Debye screening; label-free immunosensing; nanobodies (VHH); polyethylene glycol (PEG)

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], carbon nanotubes [2], graphene [3], organic semiconductors [4] and other layered two-dimensional materials [5]. 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 [6] and sensors.

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 antibody 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 fragment (also called nanobody) receptors are among the shortest available biological receptors (~13 kDa, <3 nm) [7] and are much smaller and structurally simpler than common whole antibodies (~150 kDa, ~15 nm) or Fab fragments (~50 kDa, ~7–8 nm) [8]. In addition, the nanobodies are easily produced and stable in a range of different conditions [7]. 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 [9–12]. Unfortunately, most of these approaches require sample dilution, multiple washing steps or elaborate labelling schemes. More recently, the addition of a polymer that can increase the effective Debye screening length has been proposed as a more general approach [3,13]. So far, specific label-free immunodetection over a wide analyte concentration range in high ionic strength solutions has not been shown.

2. Results

To study the effect of PEG on the signal due to GFP binding, VHH was immobilized on both the PBA (pyrene butyric acid) + PEG coated surface (Figure 1D) and on the control SWCNT samples modified with PBA only (Figure 1A). Both sensor surfaces were then exposed to various GFP concentrations dissolved in 1 mM and 100 mM Tris buffer. The measurements are shown in Figure 1E,F for the PEGylated surface and in Figure 1B,C for the non-PEGylated case. In both cases, the transfer curves shift to more positive values in response to increasing GFP concentration, with the PEGylated surface reacting more strongly (Figure 1B,E). Figure 1C,F compare the response of both sensors as a function of GFP concentration. Importantly, the signal of the PEGylated sensor exhibits a threefold enhancement in 100 mM buffer compared to the non-PEGylated surface (25 mV vs. 8 mV for 100 nM GFP). The observed signal enhancement in 1 mM is less drastic and amounts to an approximately twofold increase (47 mV vs. 25 mV for 100 nM GFP). These results clearly indicate that the PEG has a strong positive impact on the maximum achievable sensor response.

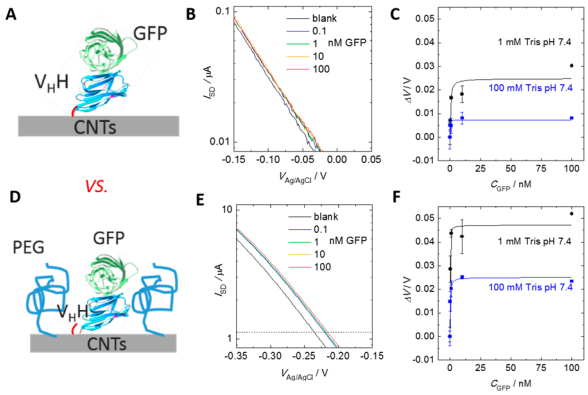

Figure 1. Comparison of GFP detection with non-PEGylated (**A**–**C**) and PEGylated SWCNT FETs (**D**–**F**). The surface of the SWCNTs was modified either with a mixture of pyrene butyric acid (PBA) with methyl PEGylated pyrene (**D**) or with PBA only (**A**). Camelid nanobodies (VHH), specific to green fluorescent protein (GFP), were then immobilized on both surfaces and exposed to GFP solutions to assess the VHH-GFP binding. (**B**,**E**) show the transfer curves measured in different concentrations of GFP in 100 mM Tris buffer. A shift to more positive potentials is visible in both cases with a stronger response in the PEGylated case (**E**). (**C**,**F**) summarize the potential shift ΔV obtained as a function of GFP concentration C_{GFP} in 1 mM (circles) and 100 mM (squares) ionic strength solutions. ΔV was read out at a constant I_{SD} value, as indicated by horizontal lines in (**B**,**E**). The signal in (**F**) is up to 3× larger than the signal in (**C**).

3. Discussion

For the first time, a transistor-based biosensor with short and highly stable nanobodies (VHH) as biological receptors is reported. The size of GFP specific VHH is only ~2 × 4 nm and the binding site of the GFP is located on the longer side of the nanobody [8]. Despite the very short Debye length of approximately 1 nm in 100 mM ionic strength, a small signal due to GFP binding was still observed even in high ionic strength buffers (Figure 1E,F). This behavior is attributed to the small size and the random orientation of the receptors on the sensor surface which can favor the binding of GFP at a distance within the Debye length.

Moreover, when carbon nanotubes were additionally PEGylated (10 kDa PEG, 6 nm length [3]), a substantial signal enhancement was achieved in 1 mM and 100 mM buffer (Figure 1D–F). A threefold signal increase in 100 mM buffer compared to non-PEGylated sensor is observed. The signal enhancement in 1 mM buffer is less pronounced (~2 x times), because the Debye length in this diluted buffer is already large (~10 nm) compared to 100 mM (~1 nm). In fact, only small signal enhancement is expected in 1 mM buffer, because the Debye length is similar to the size of the receptor-analyte complex studied here (5–7 nm). We believe that this additional and unexpected signal improvement in 1 mM buffer comes from PEG-induced stabilization and proper spacing of the immobilized biomolecules [14].

4. Conclusions

- Electrolyte-gated field-effect transistors based on high-purity semiconducting carbon nanotube networks were investigated as an immunosensing platform.
- A novel mixed surface functionalization was developed, consisting of short and stable nanobody receptors as well as a polyethylene glycol layer.
- Using green fluorescent protein (GFP) as a model analyte, the described surface modification proved to be highly effective for sensitive and selective protein detection over a large concentration range, even in physiological solutions with high ionic strength (100 mM).
- The proposed direct immunosensing concept eliminates the need for any sample dilution, labelling or washing steps, which significantly simplifies the workflow, reduces the cost and the time to result.

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