Tuning Electrostatic Gating of Semiconducting Carbon Nanotubes by Controlling Protein Orientation in Biosensing Devices

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Abstract: The ability to detect proteins through gating conductance by their unique surface electrostatic signature holds great potential for improving biosensing sensitivity and precision. Two challenges are: (1) defining the electrostatic surface of the incoming ligand protein presented to the conductive surface; (2) bridging the Debye gap to generate a measurable response. Herein, we report the construction of nanoscale protein-based sensing devices designed to present proteins in defined orientations; this allowed us to control the local electrostatic surface presented within the Debye length, and thus modulate the conductance gating effect upon binding incoming protein targets. Using a β-lactamase binding protein (BLIP2) as the capture protein attached to carbon nanotube field effect transistors in different defined orientations. Device conductance had influence on binding TEM-1, an important β-lactamase involved in antimicrobial resistance (AMR). Conductance increased or decreased depending on TEM-1 presenting either negative or positive local charge patches, demonstrating that local electrostatic properties, as opposed to protein net charge, act as the key driving force for electrostatic gating. This, in turn can, improve our ability to tune the gating of electrical biosensors toward optimized detection, including for AMR as outlined herein.

The construction of nanoscale field effect transistors (FETs) for sensing, whereby the gating voltage is replaced by a biomolecular event, offers huge potential for building high sensitivity, target-specific, miniaturized, and label-free biosensing devices. Protein surfaces are decorated with charged residues, whose distribution and area varies between proteins and within a protein, so generating a specific electrostatic signature. Therefore, there is great interest in developing systems that can sense and importantly differentiate these surfaces. Protein-protein interactions are widespread in nature driving many important biological processes and form the basis for many diagnostic approaches. These highly specific interactions can in principle be used to electrostatically gate conductance far more effectively than they currently do by defining the interface between protein and the transducer, so forming the basis of greatly improved electrical-based biosensors.

While various semiconducting materials have been used in FET sensing devices, one-dimensional (1D) materials offer advantages in terms of high surface area and restricted conduction pathways. Among 1D nanomaterials for sensing, single walled carbon nanotubes (SWCNTs) have emerged as excellent candidates, due to their high aspect ratios, restricted conductance pathways, appropriate size compatibility with biological analytes, the different strategies available for their functionalization, and the ease of integrating them into electronic circuits. CNTs have been interfaced to different biomolecules with a particular focus on nucleic acids (e.g., DNA/aptamers) and proteins (e.g., antibodies); this allowed biomolecular events to be transduced into measurable changes in CNT conductance.

Despite previous work on FET biosensors, including CNT-based ones, the approaches developed so far for the assembly of protein hybrids in device configurations typically suffer from the drawback of lacking control over protein interface orientation (Figure 1a). This is particularly critical, as it does not allow us to fully take advantage of the unique surface distribution of electrostatic features of a protein, nor optimize communication between the protein(s) and the CNT. Furthermore, non-specific attachment generates multiple orientations that can compromise sensing capability by, for example, sterically blocking access to a binding site.
Those orientations that retain access to protein ligand binding blocking by the SWCNT will prevent protein ligand binding; protein interaction surfaces areas are larger, and any steric attachment site need to be measured (Figure 1a). Protein-protein interactions need to be monitored. This is a far more biosensing, restricted to events close to the attachment residue of localized static signature driving gating upon sensing protein targets. In this regard, the lack of geometric control is particularly important for nanoscale sensors where proteins constitute the sensing element and individual protein attachment variations can lead to major functional differences between devices. It is therefore of paramount importance to control the protein’s site/residue that interfaces with the FET in order to define and understand the unique surface electrostatic signature driving gating upon sensing protein targets.

Previous work on attachment of proteins at defined residues demonstrated that CNT conductance can respond to local electrostatic changes close to the tube surface as a result of localized intra-molecular changes, even down to the single-molecule level. However, these approaches were restricted to events close to the attachment residue of a protein directly attached to the SWCNT; for use in biosensing, inter-molecular events such as protein-protein interactions need to be monitored. This is a far more challenging proposition, as electrostatic events far from the attachment site need to be measured (Figure 1a). Protein-protein interaction surfaces areas are larger, and any steric blocking by the SWCNT will prevent protein ligand binding; those orientations that retain access to protein ligand binding placing the incoming ligand beyond the Debye field length, or even destructively interfering by presenting differently charged surfaces. In this regard, the lack of geometric control is particularly important for nanoscale sensors where proteins constitute the sensing element and individual protein attachment variations can lead to major functional differences between devices. It is therefore of paramount importance to control the protein’s site/residue that interfaces with the FET in order to define and understand the unique surface electrostatic length upon this behavior is interpreted as due to changes of the local charge profiles should be sampled (Figure 2). Gly49 lies at the opposite to that of residue 213. Electrostatic surface model residues 41 lies on one side of the cone-like BLIP2 structure, residues demonstrated that CNT conductance can respond to the clinical prevalent BL, TEM-1 shown in red, white and blue shades). Defined attachment using bioorthogonal ncAAs (bottom). The dashed purple line represents a putative Debye field length. b) Selected residues for replacement with AzF (see Supporting Scheme SI-1 for details on incorporation) (PDB code 1jtd). The AzF models were built as described previously. (AzF) at four different designed positions in BLIP2 [see Scheme SI-1 in the supporting information (SI)] we define the single-site attachment of BLIP2 to the CNTs, in order to sample different electrostatic surfaces of an incoming BL (Figure 1b). The benefit of using AzF is that multiple routes become available for tethering proteins to CNTs in a highly precise manner, including direct photo-chemical attachment to the CNT sidewall and click chemistry to a pyrene adduct. Here we use the click approach as unlike photo-chemical attachment, it does not introduce defects into the CNT; furthermore, our click chemistry approach has the advantage that any surface residue can be engineered to act as the interface site in one simple mutagenesis step, thiol-based approaches may require further protein engineering to existing cysteine residues.

Structural analysis of BLIP2 led to the selection of four residues being chosen to introduce AzF so as to investigate different facets of the protein-protein interaction that gate SWCNTs conductance in response to the clinical prevalent BL, TEM-1: Ala41, Ser43, Gly49 and Thr213 (Figure 1b). Residues 41 lies on one side of the cone-like BLIP2 structure, opposite to that of residue 213. Electrostatic surface modeling of BLIP2 complexed to the BL TEM-1 demonstrates that by placing AzF at residues 41 or 213, very distinct surface charge profiles should be sampled (Figure 2). Gly49 lies at the BL binding interface so introduction of AzF and subsequent site may result in the measured binding event being beyond the Debye field. This is particularly limiting in biological systems where high salt buffers may be needed so reducing Debye field distance. Thus, the initial choice of attachment residues becomes critical.

To address the challenge of monitoring inter-molecular protein-protein interactions through device response by sampling distinct electrostatic-surface gating, we systematically tested how protein orientation dictates current response through a SWCNT-FET device by defining the interface site on the capture protein. This is especially important given that most protein-CNT interfacing approaches are essentially random, which leads to a heterogenous system comprised of non-productive, non-optimal and even mutually destructive orientations. Crucially, the device conductance increased or decreased depending on the selected designed orientation; this behavior is interpreted as due to changes of the local electrostatic surface presented within the Debye length upon binding and can support the identification of preferred proteins orientations for optimal sensing.

We have fabricated CNT-protein FET biosensors with control over protein orientation in device configuration, focusing on the detection of a major cause of antimicrobial resistance (AMR), class A β-lactamases (BLs). The BLs target and deactivate β-lactam antibiotics (e.g. penicillin, ampicillin, amoxicillin), which are the mostly widely prescribed and utilized class of antibiotics. Thus, there is a real need to develop real time sensors for the presence of BLs in clinical samples, which will enable more appropriate and effective antibiotic at earlier stages of infection. We employed the BL inhibitory protein, BLIP2, that binds clinically prevalent class A BLs such TEM-1 used here. By placing the non-canonical amino acid (ncAA) p-azido-L-phenylalanine (AzF) at four different designed positions in BLIP2 [see Scheme SI-1 in the supporting information (SI)] we define the single-site attachment of BLIP2 to the CNTs, in order to sample different electrostatic surfaces of an incoming BL (Figure 1b). The benefit of using AzF is that multiple routes become available for tethering proteins to CNTs in a highly precise manner, including direct photo-chemical attachment to the CNT sidewall and click chemistry to a pyrene adduct. Here we use the click approach as unlike photo-chemical attachment, it does not introduce defects into the CNT; furthermore, our click chemistry approach has the advantage that any surface residue can be engineered to act as the interface site in one simple mutagenesis step, thiol-based approaches may require further protein engineering to existing cysteine residues.

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CNT binding should abolish any BL-dependent conductance; this equates to a non-productive interface configuration. Ser43 allows us to assess how even small changes to the attachment position can influence binding-dependent conductance. BL enzyme inhibition assays revealed BLIP2 \(^{41\text{AzF}}\) and BLIP2 \(^{213\text{AzF}}\) retained near wild type, picomolar binding affinity, with BLIP2 \(^{23\text{AzF}}\) and BLIP2 \(^{9\text{AzF}}\) having attenuated affinity in the low nanomolar range [see the SI for discussion, and Figure SI-1].

CNT-FETs were fabricated by casting solutions of sodium dodecyl sulfate (SDS) dispersed enriched semiconducting SWCNTs (sSWCNTs) on pre-patterned electrodes pairs on doped silicon wafers, and immobilizing the nanotubes between 300 nm gap electrodes via dielectrophoresis (DEP): see the SI, and Figure SI-2. \(^{10}\) Typical transfer characteristics of the device are shown in Figure SI-3, demonstrating that these sSWCNT-based FETs are p-type.

The pristine sSWCNTs where then coated in a 3:1 mixture of \(\pi\)-stacking molecules pyrene-butanol and dibenzylcyclooctyne (DBCO) modified pyrene-amine. \(^{11,12}\) The pyrene butanol acts as a spacer, reducing BLIP2 density and minimizing non-specific adsorption, while the DBCO allows attachment of BLIP2 via biocompatible strain-promoted azide-alkyne cycloaddition (SPAAC) [see Figure SI-4 and Figure 3a]. \(^{13,14}\) Without altering the electronic properties of the nanotubes by direct covalent attachment, tethering of the BLIP2 variants to the FET devices, via the DBCO-pyrene modified SWCNTs (sSWCNTs). \(^{10}\) This means only limited surfaces of TEM-1 enter the sensor field configuration before and after attachment of BLIP2 \(^{41\text{AzF}}\). Additional height analysis of BLIP2 \(^{41\text{AzF}}\)-sSWCNT is presented in Figure SI-5.

We then successfully demonstrated protein surface electrostatic gating of the p-type SWCNT using our device configuration. Real-time conductance measurements were performed for the four different attachment sites (and hence protein’s orientations), monitoring current dependent changes upon addition of TEM-1. Measurements were carried out in physiologically relevant, high ionic strength Dulbecco’s phosphate buffered saline (DPBS) as a stringent test for our sensing configuration. The high ionic strength of DPBS restricts the Debye length \(l_\text{D}\) to circa 0.7 nm [see the SI]. \(^{16,17}\) which is smaller than the size of TEM-1 \(\approx 3–4\) nm (Figure 3c), in line with the structure of BLIP2. Analysis of the other BLIP2 variants (Figure SI-5) also showed nanotube transverse height increases of \(3–6\) nm upon protein attachment, again in line with what was expected based on the structure of BLIP2. AFM topographical analysis allowed us to estimate the average number of proteins attached to the nanotubes in each device to be ca. 40 to 80 proteins (see the SI and Figure SI-6). No height increase was observed with the wild type protein (BLIP2\(^{\text{WT}}\)) confirming the requirement of AzF for attachment (Figure SI-7); moreover, no BLIP2-AzF protein bound when nanotubes were coated with just pyrene-butanol, hence ruling out off-pathway attachment routes (Figure SI-8). These results strongly suggest that our AzF-containing BLIP2 variants attached to SWCNT-FETs as designed.

Figure 3. Attachment of BLIP2 to SWCNTs. BLIP2 \(^{41\text{AzF}}\) is shown as a representative example with data for other BLIP2-AzF variants in the Supporting Information (Figure SI-8). a) Schematic of nanopatterned electrodes bridged by SWCNTs and attachment of BLIP2 variants to the sidewall of the nanotubes with defined orientation. The upper panel show nanotubes decorated with DBCO pyrene (orange) followed by attachment of BLIP2 (green). b) Representative AFM topographical image of the electrode with the yellow line indicating the axis across which height measurements were taken. c) AFM height profiles in device configuration before and after attachment of BLIP2 \(^{41\text{AzF}}\). Additional height analysis of BLIP2 \(^{41\text{AzF}}\)-sSWCNT is presented in Figure SI-5.
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to identify specific analytes in complex biological solutions. For example, with regards to BLs this can potentially allow the rapid identification of which BLs are present in a sample, and therefore inform on suitable antibiotic treatments; such information is not currently accessible by existing biochemical methods, even using ultrasensitive chemoluminescence approaches.[20]

In summary, we fabricated SWCNT-FETs to investigate the influence of a protein’s controlled orientation and electrostatic surface features in the electrical detection of an incoming ligand, namely the sensing of a β-lactamase enzyme involved in AMR. Four distinct BLIP2 variants were engineered to contain bioorthogonal reaction handles at specific residues allowing proteins to be tethered to SWCNTs in defined orientations. Devices functionalized with different BLIP2 variants successfully detected the β-lactamase TEM-1 through changes in conductance, with device performance dependent on BLIP2 attachment site, and hence orientation. Presentation of different TEM-1 electrostatic surfaces within the Debye length led either to increase or decrease in conductance; this allowed biosensing of less than 100 molecules through electrostatic surface profiling of protein-protein interactions. The strategy presented here is of general applicability for the control and detection of protein-protein interactions in nanoscale device configurations. Defined and homogenous attachment allows distinctive conductance profiles to be sampled based on the unique electrostatic features of individual proteins, and can support the identification of preferred proteins orientations for optimal sensing. By avoiding random/uncontrolled orientations we can minimize non-productive and destructive interactions, and therefore consistently fabricate biosensors with defined response. With regards to AMR detection, this has significant potential as BLIP2 binds a range of BL enzymes, each with their own specific electrostatic profile; this may in turn open up new possibilities to identify specific electrostatic profile; this may in turn open up new opportunities for the development of AMR-related diagnostic devices that can be used to quickly detect the presence of resistance biomarkers and so more effectively utilize appropriate antibiotics to treat bacterial infections. The ability to quantify BL levels will also allow us to more accurately probe any link between enzyme levels and AMR, something which microbiological and genetic testing approaches cannot currently achieve.

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Conflict of Interest

The authors declare no conflict of interest.

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Communications

Biosensors


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Nanoscale protein-based sensing devices designed to present proteins in defined orientations allowed the control of local electrostatic surface presented within the Debye length, and thus modulation of the conductance gating effect upon sensing protein targets.