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Solution-based functionalization of gallium nitride nanowires for protein sensor development

Elissa H. Williams [a,b,c], Albert V. Davydov [a,*], Vladimir P. Oleshko [a], Kristen L. Steffens [a], Igor Levin [a], Nancy J. Lin [a], Kris A. Bertness [d], Amy K. Manocchi [e], John A. Schreifels [b], Mulpuri V. Rao [c]

[a] Material Measurement Laboratory, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899, USA
[b] Department of Chemistry and Biochemistry, George Mason University, 4400 University Drive, Fairfax, VA 22030, USA
[c] Department of Electrical and Computer Engineering, George Mason University, 4400 University Drive, Fairfax, VA 22030, USA
[d] Physical Measurement Laboratory, National Institute of Standards and Technology, 325 Broadway, Boulder, CO 80305, USA
[e] Sensors and Electronic Devices Directorate, Army Research Laboratory, 2800 Powder Mill Road, Adelphi, MD 20783, USA

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ABSTRACT

A solution-based functionalization method for the specific and selective attachment of the streptavidin (SA) protein to gallium nitride (GaN) nanowires (NWs) is presented. By exploiting streptavidin's strong affinity for its ligand biotin, SA immobilization on GaN NWs was achieved by exposing the GaN NW surface to a 3-aminopropyltriethoxysilane (APTES) solution followed by reaction with biotin. Functionalization of the NWs with APTES was facilitated by the presence of an ≈1 nm thick surface oxide layer, which formed on the NWs after exposure to air and oxygen plasma. Biotinylation was accomplished by reacting the APTES-functionalized NWs with sulfo-N-hydroxysuccinimide-biotin at slightly alkaline pH. It was determined that the biotinylated GaN NW surface was specific towards the binding of SA and demonstrated no affinity towards a control protein, bovine serum albumin (BSA). There was however, evidence of non-specific, electrostatic binding of both the SA protein and the BSA protein to the APTES-coated NWs, revealing the importance of the biotinylation step. Successful SA immobilization on the biotinylated GaN NW surface was verified using fluorescence microscopy, field-emission scanning electron microscopy, high-resolution transmission electron microscopy, atomic force microscopy, and X-ray photoelectron spectroscopy. The functionalized GaN NWs demonstrate potential as biosensing platforms for the selective detection of proteins.

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1. Introduction

The detection of biological species in gasses and in liquids is necessary for homeland security, medical diagnoses, environmental monitoring, as well as food safety [1,2]. Current biosensing techniques, such as the enzyme-linked immunosorbent assay, particle-based flow cytometric assays, gas chromatography, chemiluminescence, fluorescence, and high density peptide arrays, suffer from recurring false positive responses, display variations in sensitivity limits, and do not meet the requirements of portable sensors for real-time monitoring. Consequently, there has been a shift to develop semiconductor thin film and nanowire based conductometric biosensors. These thin film/nanowire based biosensors can detect a biomolecule binding event through resistivity changes and have the advantages of a small footprint, a high sensitivity, and low power consumption [1–4].

Gallium nitride (GaN) is an attractive semiconductor for such biosensors since it exhibits excellent transport properties, such as high electron mobility and saturation velocity, as well as biocompatibility, non-toxicity, and stability under physiological conditions [1,4,5]. GaN thin film high-electron-mobility transistors (HEMTs) have proven ability to electrically detect proteins, antibodies, small molecules such as glucose, and strands of DNA with selectivity and high sensitivity [1,4]. Compared to their thin film counterparts, GaN nanowire (NW) based devices can display an even higher relative resistance change upon exposure to analytes due to their high surface-to-volume ratio [1,3]. In addition, several analyte specific nanowire sensors can be integrated on a single chip enabling a multiplexed mode of detection. GaN NW based chemical sensors have recently demonstrated a ppb level detection of aromatics [3] and alcohols [6–8] in air. GaN NW based biosensors are also emerging [9,10], as functionalization techniques for specific biomolecule attachment to GaN NWs are developed [11,12].

Abbreviations: APTES, 3-aminopropyltriethoxysilane; AFM, atomic force microscopy; BSA, bovine serum albumin; EDXS, energy dispersive X-ray spectroscopy; EELS, electron energy loss spectroscopy; FESEM, field-emission scanning electron microscopy; GaN, gallium nitride; HEMT, high-electron-mobility transistor; HRTEM, high-resolution transmission electron microscopy; NW, nanowire; SA, streptavidin; XPS, X-ray photoelectron spectroscopy.

* Corresponding author. Tel.: +1 301 975 4916; fax: +1 301 975 4553.
E-mail addresses: ehwill@nist.gov (E.H. Williams), albert.davydov@nist.gov (A.V. Davydov), vladimir.oleshko@nist.gov (V.P. Oleshko), kristen.steffens@nist.gov (K.L. Steffens), igor.levin@nist.gov (I. Levin), nancy.lin@nist.gov (N.J. Lin), bertness@boulder.nist.gov (K.A. Bertness), amy.manocchi.ctr@mail.mil (A.K. Manocchi), jschreif@gmu.edu (J.A. Schreifels), rmulpuri@gmu.edu (M.V. Rao).

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In this paper, we present a 2-step, solution-based sequential layer functionalization method for streptavidin (SA) immobilization on GaN NWs for protein sensor applications. This is an extension of our recently developed protocol for specific SA functionalization of silicon and silicon carbide NWs [13]. This approach performs all functionalization steps in solution thus enabling the assembly of multiple protein-specific NWs on a single biosensing platform. A variety of spectroscopic and microscopic techniques were utilized to verify each functionalization step on the NW surface.

2. Material and methods

2.1. Experimental design for protein selective sensing

The streptavidin (SA) protein exhibits a strong, non-covalent interaction with its ligand biotin and hence biotinylated GaN NWs should enable the specific immobilization of SA. The biotin ligand itself, however, does not bind to the GaN surface and thus a linker molecule is necessary. 3-Aminopropyltriethoxysilane (APTES) is a small organic silane that has the ability to covalently attach to oxidized or hydroxylated surfaces as well as amine reactive biomolecules (i.e. modified forms of biotin) [4,13,14]. APTES is one of the most explored coupling agents in the functionalization of semiconductor materials, including GaN. The covalent attachment of APTES to GaN thin films has been well characterized and demonstrated as an initial step for the immobilization of biomolecules [4,5,14]. Kang *et al.* proved that APTES functionalization, followed by biotinylation resulted in the electrical detection of the streptavidin protein on AlGaN/GaN thin film devices [4]. To date, no studies have been performed to functionalize the GaN NW surface with APTES nor its subsequent biotinylation and streptavidin immobilization. Consequently, in this manuscript, we investigate the APTES functionalization, biotinylation, and streptavidin immobilization on GaN NWs for biosensing applications and employ our own developed protocol in which all steps are performed in solution.

For the experiment as demonstrated in Fig. 1, surface oxidized GaN NWs were exposed to APTES, followed by sulfo-N-hydroxysuccinimide-biotin, with each functionalization step studied by surface characterization techniques. The biotinylated NWs were then exposed to a SA solution, binding of which was confirmed using various methods. Control tests were also conducted to examine the specificity of the functionalized GaN NW surfaces towards SA. Bovine serum albumin (BSA) is a protein that has no affinity for biotin or biotinylated surfaces and hence was chosen as the control protein [4,13]. The biotinylated GaN NWs were exposed to a mixture of SA and BSA, with each protein containing a different fluorophore. Fluorescence microscopy was then used to determine if the biotinylated NWs were indeed specific to only SA. The SA/BSA mixture was also exposed to the APTES-coated GaN NWs and the cleaned (oxidized) GaN NWs (see Fig. 1) and studied using fluorescence microscopy to determine if these surfaces were prone to the non-specific adsorption of SA or BSA.

2.2. Nanowire growth and functionalization

GaN NWs were grown on Si(111) substrates by molecular beam epitaxy, via the catalyst-free vapor-solid growth mechanism, as described elsewhere [15]. The as-grown NWs on Si were oxygen plasma cleaned in a 20% oxygen/80% argon gas mixture for 5 min and subsequently exposed to laboratory air. For field-emission scanning electron microscopy/energy dispersive X-ray spectroscopy (FESEM/EDXS) analyses, the cleaned NWs were mechanically scraped off the Si substrates and placed onto 1 cm × 1 cm Au-coated Si pieces. For high-resolution transmission electron microscopy/electron energy loss spectroscopy (HRTEM/EELS), the cleaned NWs were mechanically scraped off the substrates and placed onto standard 300 mesh lacey-carbon TEM grids. For fluorescence microscopy and X-ray photoelectron spectroscopy (XPS), the cleaned NWs were placed in a vial containing toluene and ultrasonically agitated to detach the NWs from the Si substrate and form a suspension. Drops of the suspended NWs in toluene were then placed onto 1 cm × 1 cm Si pieces (for fluorescence microscopy) and 1 cm × 1 cm Au-coated Si pieces (for XPS) and allowed to air dry.

APTES functionalization of the NWs was achieved by placing the cleaned NWs in a vial containing a 2% APTES in toluene solution. The NWs in the APTES solution were ultrasonically agitated to detach the NWs from the Si substrates and form a suspension. After a 30 min exposure to the APTES solution, the NWs were sedimented by centrifugation for 2 min at 10,000 rpm (4 cm centrifuge rotor radius) and rinsed with toluene. The APTES-functionalized NWs were re-suspended in toluene with subsequent placement onto 1 cm × 1 cm Si pieces for FESEM/EDXS, XPS, and fluorescence microscopy, as well as TEM grids for HRTEM.

Biotinylation of the GaN NWs was achieved by adding 0.5 mL of sulfo-N-hydroxysuccinimide-biotin, at a concentration of 5 mg/mL in 0.01 mol/L phosphate buffer (pH = 7.4), to the vial containing the rinsed and sedimented, APTES-coated GaN NWs. The vial was sonicated to bring the NWs back into suspension followed by a 2 h exposure to the biotin solution. The biotinylated NW suspension was centrifuged, followed by the removal of residual biotin solution from the vial. After rinsing in phosphate buffer, drops of the biotinylated NW suspension were deposited onto Si pieces for FESEM, XPS, and fluorescence microscopy, as well as TEM grids for HRTEM, and allowed to dry.

2.3. Protein immobilization

The biotinylated NWs, adhered to the uncoated or Au-coated Si pieces and TEM grids (as described in Section 2.2), were exposed to a 0.058 mg/mL streptavidin, labeled with cyanine-3, in 0.01 mol/L phosphate buffer (pH = 7.4) solution for 2 h, followed by a brief sonication,

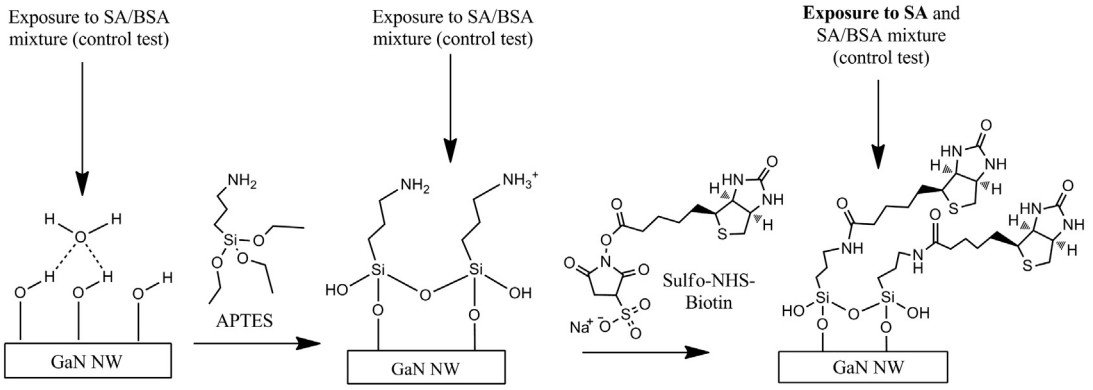

Fig. 1. Experimental reaction scheme.

a rinse in buffer, and drying in a N_2 flow. The SA immobilized NW samples were then analyzed using atomic force microscopy (AFM), FESEM, XPS, and HRTEM.

For the control experiments on the specificity of SA binding, the cleaned, the APTES-coated, and the biotinylated NWs (each adhered to Si pieces as described in Section 2.2) were placed in a mixture of 0.058 mg/mL streptavidin, labeled with cyanine-3, and 0.058 mg/mL bovine serum albumin, labeled with fluorescein isothiocyanate, in 0.01 mol/L phosphate buffer (pH = 7.4) solution for 2 h, followed by a brief sonication, a rinse with buffer, and drying in a N_2 flow. Fluorescence microscopy was then utilized to study the binding of each protein (SA and BSA) to the cleaned, the APTES-coated, and the biotinylated GaN NWs.

2.4. Characterization methods

The morphology, dimensions, and elemental composition of the cleaned, APTES-coated, biotinylated, and SA immobilized GaN NWs were characterized using a Hitachi-4700[1] FESEM equipped with an 80 mm^2 Oxford Instruments X-Max silicon drift EDX detector.

The NW morphology and microstructure, including thickness and composition of organic layers, were examined by an FEI Titan 80-300 HRTEM operated at a 300 kV accelerating voltage, equipped with S-TWIN objective lenses, Gatan's Enfina electron spectrometer, and an EDAX 30 mm^2 Si/Li EDX detector. Low intensity illumination conditions and beam blanking were used to minimize possible radiation damage of the organic layers.

The surface topography and morphology of the functionalized GaN NWs were investigated with a Veeco DI Dimension AFM on a 1 μm × 2 μm scale in tapping mode. Images were analyzed using WSxM v5.0 software [16].

For the XPS study, the cleaned and functionalized GaN NWs on Au-coated Si pieces were analyzed in a Kratos Analytical Axis Ultra DLD XPS instrument with a monochromated Al Kα X-ray source at 150 W (10 mA, 15 kV). X-rays were collected at a 0° angle from the surface normal on an area of 300 μm × 700 μm. The amount of nanowires in the probing area was estimated to be between 10,000 and 15,000, which was sufficient for detecting the Ga and N related peaks from GaN, as well as C, O, N, and Si peaks from the functional organic layers. Low resolution survey scans (160 eV pass energy, 0.5 eV step size) and high resolution narrow scans (40 eV pass energy, 0.1 eV step size) of Au 4f, O 1s, N 1s, C 1s, Si 2p, and Ga $2p_{3/2}$ were obtained. The binding energy scale was calibrated to the Au $4f_{7/2}$ peak at 84.0 eV. Charge neutralization was not necessary during sample analysis due to the calibration to the Au $4f_{7/2}$ peak.

Fluorescence microscopy was performed on the NWs using a Nikon Eclipse TE300 inverted epifluorescence microscope with a Plan Apo 60× (N.A. 1.4) oil immersion objective. SA was imaged using a Nikon EF.4 filter cube with an excitation of 515 nm to 565 nm at a 1 s exposure time. BSA was imaged using a Nikon B-2E/C filter cube with an excitation of 465 nm to 495 nm at a 3 s exposure time; the longer exposure time of 3 s was necessary to enhance the weak fluorescence signal of BSA.

3. Results and discussion

3.1. APTES functionalization, biotinylation, and SA immobilization

A cross-section FESEM image of the as-grown GaN NW batch is shown in Fig. 2. The NW dimensions ranged from 8 μm to 12 μm in length and 70 nm to 300 nm in diameter. The NWs, with wurtzite crystal structure, were shaped as hexagonal prisms with six equivalent {10-10} side facets growing along the [0001] direction [15].

[1] Commercial equipment and material suppliers are identified in this paper to adequately describe experimental procedures. This does not imply endorsement by NIST.

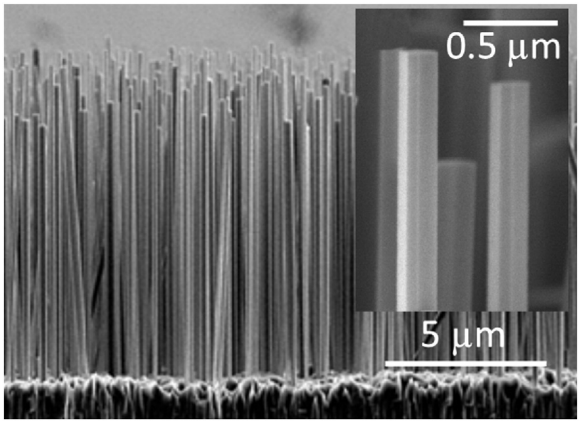

Fig. 2. A cross-section FESEM image of GaN NWs grown on a Si(111) substrate. The magnified image in the inset shows hexagonal faceting of NW sidewalls.

A FESEM image of the faceted NW sidewalls after exposure to oxygen plasma is shown in Fig. 3A. A corresponding HRTEM image (Fig. 4A) reveals an ≈1 nm thick semi-amorphous layer on the NW surface. The EELS analysis in high-resolution TEM identified the composition of this surface layer as a mixture of Ga, N and O, indicating a surface gallium oxide or oxynitride. It is likely that formation of this native oxide was initiated upon exposure of the NWs to air after NW growth [17,18], and was made both thicker and denser with the oxygen plasma treatment. More details concerning the chemical composition and microstructure of the oxygen plasma cleaned GaN NW surface can be found in the Supplementary section.

APTES functionalization of the cleaned NW surfaces produced an ≈5 nm thick amorphous coating, as seen in Fig. 4B (also, see the Supplementary data for an EDXS analysis of the chemical composition of this layer). A follow up biotinylation step is shown in Figs. 3C (FESEM) and 4C (HRTEM); the combined biotin/APTES layer is also amorphous and slightly thicker than the APTES layer with some local thickness variations. The final step of SA conjugation to the biotinylated NW surface caused the thickness of the organic layer to increase to 15 nm to 20 nm as shown in the HRTEM image in Fig. 4D. The FESEM image in Fig. 3D shows that the SA immobilized NW surface is covered with small hemispherical agglomerates that are likely composed of SA molecules (see the Supplementary data for a dimensional analysis of the agglomerates using AFM).

Successive deposition of the APTES, biotin, and SA layers on the NW surface was chemically validated using an XPS analysis. The N 1s and Ga $2p_{3/2}$ narrow scans for the oxygen plasma cleaned NWs prior to the functionalization steps are shown as spectra (1) in Fig. 5A and B, respectively. In Fig. 5A (spectrum 1), a N 1s signal is centered around 398.5 eV and corresponds to N atoms bound to Ga in GaN [19]. In Fig. 5B (spectrum 1), a Ga $2p_{3/2}$ signal appears at 1119 eV, indicative of Ga atoms bound to O and/or Ga atoms bound to N, i.e. Ga*–O and/or Ga*–N [19,20]. Unfortunately, it was not possible to distinguish between oxidized Ga and GaN itself, as the similar electronegativity of the N and O elements induces about the same binding energy shift in the Ga peak [20]. In spite of this uncertainty, the XPS data still conform to the TEM/EELS results, which unambiguously show the presence of a thin oxide layer on the NW surface. The cleaned GaN NWs also demonstrate clear C 1s and O 1s peaks (spectra not shown). However, because the NWs were moderately dispersed on the substrate, the C 1s and O 1s peaks were complicated by carbon and oxygen present on the Au-coated surface.

Upon APTES functionalization, there is the appearance of a Si 2p peak (see spectrum 2, Fig. 5C) which was absent on the cleaned NWs (spectrum 1). The Ga $2p_{3/2}$ peak on the cleaned NWs (spectrum 1, Fig. 5B) has disappeared following APTES functionalization (spectrum 2, Fig. 5B) indicating that the NWs have been fully coated with APTES.

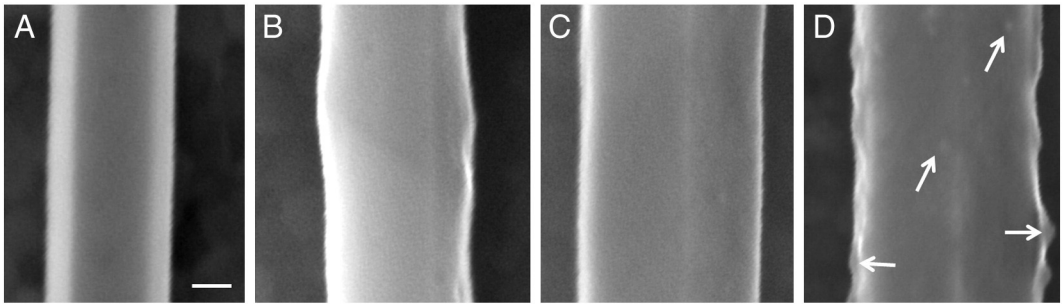

Fig. 3. FESEM images of the GaN NW sidewalls after: A) oxygen plasma cleaning, B) APTES functionalization, C) biotinylation, and D) SA immobilization. The hexagonal faceting of the clean NW in image A) gradually disappears upon the B), C), and D) functionalization steps as surface roughening increases. The arrows in D) point to hemispherical agglomerates, likely made of SA molecules. Note: the initial diameters of the NWs in A)–D) are not the same and therefore, the NW diameter in A) cannot be subtracted from the NW diameters in B)–D) to quantitatively determine organic layer thicknesses. The 50 nm scale bar in A) applies to all images.

The fact that a Ga peak is not seen with XPS signifies that the APTES layer thickness must be ≥5 nm, which is in agreement with the measured thickness of APTES using HRTEM. The absence of a Ga $2p_{3/2}$ peak and the presence of a Si 2p peak suggest that the N 1s peak of the APTES-coated NWs (spectrum 2, Fig. 5A) results from the amino group of APTES. The N 1s peak of the APTES-coated NWs appears at 399 eV with a shoulder at 402 eV. The peak at 399 eV is the NH_2 form of the amino group and the peak at 402 eV is the NH_3^+ or hydrogen bonded NH_2 form of the amino group that is present in APTES [13,14]. Feasibility of both forms of the APTES amino group to co-exist is schematically shown in Fig. 1 (see the APTES coating step).

The Si 2p peak that was present following APTES functionalization (spectrum 2, Fig. 5C) has disappeared post biotinylation (spectrum 3, Fig. 5C). In addition there is no Ga $2p_{3/2}$ peak (spectrum 3, Fig. 5B). The biotinylated NWs do display a N 1s peak (spectrum 3, Fig. 5A) at 399 eV. Since there is neither Si nor Ga present for the biotinylated NWs, the N 1s signal must be fully attributed to the NH amino groups contained within the ureido ring of biotin (see Fig. 1). The XPS data suggests that the APTES-functionalized NWs must be fully coated with biotin molecules.

The SA immobilized NWs displayed neither a Ga $2p_{3/2}$ peak (spectrum 4, Fig. 5B) nor a Si 2p peak (spectrum 4, Fig. 5C). There was however, a very strong N 1s peak at 400 eV with a shoulder at 398 eV. These N 1s peaks are consistent with typical protein N 1s binding energies [21]. In addition, the C 1s spectrum for the SA immobilized NWs (not shown) demonstrated very strong C*–O, C*–N, and C*═O components

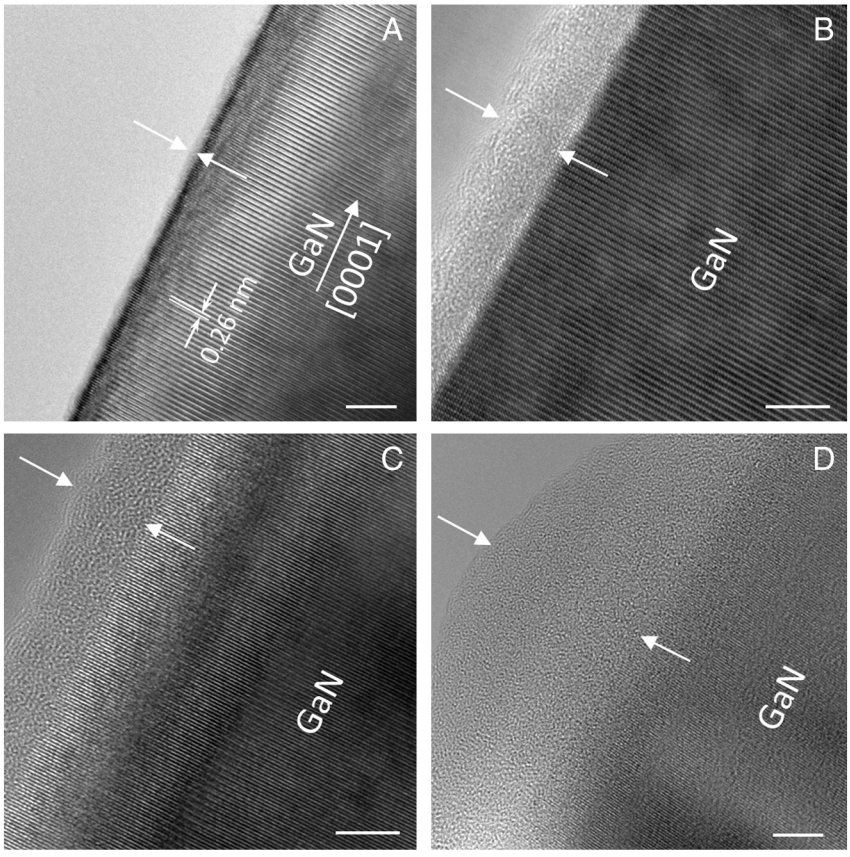

Fig. 4. HRTEM images of near edge regions of a GaN NW after: A) oxygen plasma cleaning, B) APTES functionalization, C) biotinylation, and D) SA immobilization. The 0.26 nm GaN lattice fringes, clearly evident in A)–C), indicate the [0001] c-axis NW growth direction. Arrows point out the surface layers of: A) the surface oxide, B) APTES, C) APTES/biotin, and D) APTES/biotin/SA. Note: with successive organic layer accumulation, the interface between the edge of the NW and the deposited organic layers, as well as the GaN lattice fringes, become barely visible. The scale bar in the right corner of each image is 5 nm.

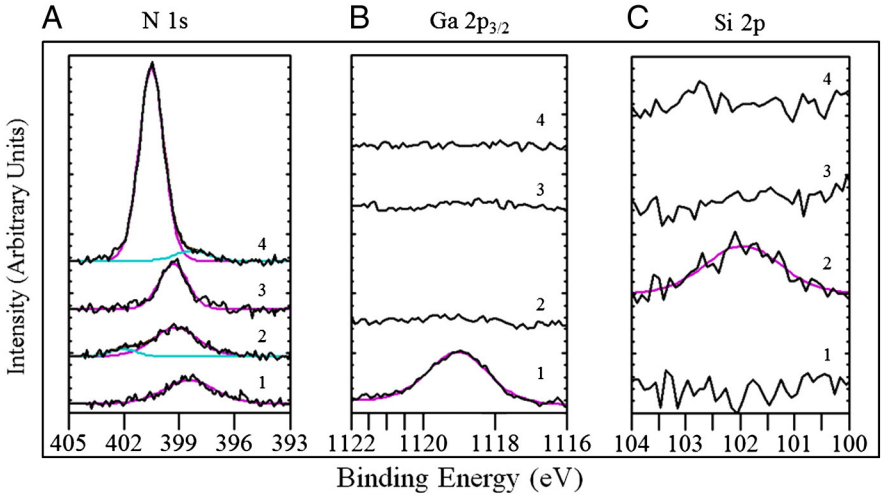

Fig. 5. XPS narrow scans: A) N 1s scan, B) Ga $2p_{3/2}$ scan, and C) Si 2p scan. For all three scans, (1) is the oxygen plasma cleaned NWs, (2) is the APTES-coated NWs, (3) is the biotinylated NWs, and (4) is the SA immobilized NWs.

that were substantially more pronounced than in the case of the cleaned, APTES-coated, and biotinylated NWs that were dominated by carbon from the underlying Au substrate.

It should be noted that the functionalization of the NWs, including SA binding, was repeated many times to ensure reproducibility of the solution-based functionalization technique and the results discussed above were observed in the numerous functionalization trials conducted.

3.2. Control (specificity) tests

To further confirm SA binding, and to prove specificity towards the SA protein, the biotinylated NWs were exposed to a mixture of red-fluorescent SA and green-fluorescent BSA and then analyzed using fluorescence microscopy. Fig. 6A demonstrates the fluorescence microscopy image of a biotinylated NW after exposure to the SA/BSA mixture. The bright red fluorescing NW in image A confirms the presence of SA. Fig. 6B demonstrates the fluorescence microscopy image of the same NW imaged in A, but under a different fluorescence filter cube. Since there is no detectable green fluorescing NW in image B (a white, dashed oval marks the location of the NW), it can be concluded that BSA did not bind to the biotinylated NWs, demonstrating specificity of the biotinylated GaN NWs towards the SA protein.

Additional control tests were performed to determine if there was any non-specific binding of the SA and BSA proteins to the cleaned and APTES-coated GaN NWs. The cleaned GaN NWs were exposed to a mixture of SA/BSA and then analyzed using fluorescence microscopy. There was neither red (from SA) nor green (from BSA) fluorescence detected under the respective fluorescence filter cubes (images not shown). Consequently, there was little, if any, non-specific attachment of the SA and the BSA proteins to the cleaned NW surface. This may be attributed to the fact that the native oxide on the GaN NW surface, existence of which was proven using HRTEM, EELS, and EDXS, likely carries some negative charge in the form of O^- and HO^- species. The surface negative charge repels the SA and BSA proteins which are also negatively charged at the working pH of 7.4 [22,23]. On the other hand, APTES-

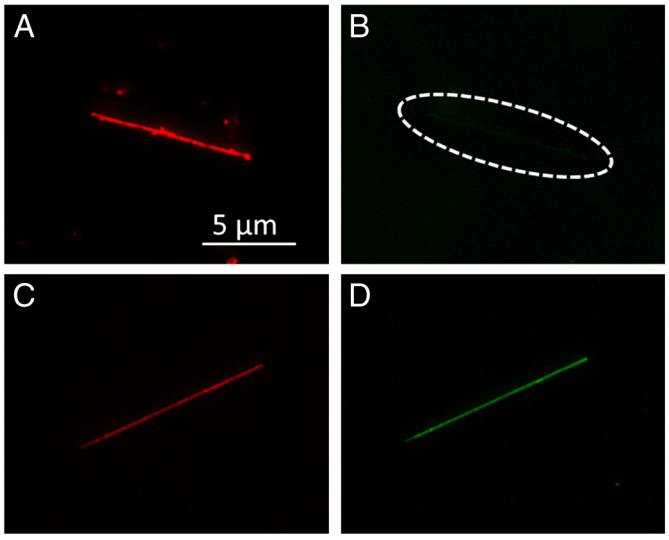

Fig. 6. A) and B): a biotinylated GaN NW after exposure to the SA/BSA mixture under the EF.4 fluorescence filter cube for SA detection (A), and the B-2E/C fluorescence filter cube for BSA detection (B). Note: the dashed line oval in image B) marks the location of the non-fluorescing NW. C) and D): an APTES-coated GaN NW after exposure to the SA/BSA mixture under the EF.4 fluorescence filter cube for SA detection (C), and the B-2E/C fluorescence filter cube for BSA detection (D). Note: images A) and C) were taken with a 1 s exposure time, while B) and D) with a 3 s exposure time. The 5 µm scale bar in A) applies to all images. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

coated GaN NWs exposed to the SA/BSA mixture demonstrated both red fluorescence from SA and green fluorescence from BSA. Fig. 6C shows a red fluorescing APTES-coated GaN NW after exposure to the SA/BSA mixture and Fig. 6D shows the same NW fluorescing green under a different filter cube. Therefore, there was non-specific attachment of both SA and BSA to the APTES-coated GaN NWs. This non-specific attachment of both proteins could be explained by the fact that the terminal amino group of APTES can exist in a positively charged, protonated state (as schematically shown in Fig. 1 and confirmed with XPS in Fig. 5) [11,24]. The SA and BSA proteins, both of which are negatively charged at the working pH, are likely electrostatically bound to the APTES-coated GaN NWs resulting in non-specific attachment [24]. Notably, comparing Fig. 6A and C, the specifically bound SA on the biotinylated NWs is significantly more fluorescent than the non-specifically bound SA on the APTES-only coated NWs. Therefore, there are likely a greater number of specifically bound SA molecules on the biotinylated NWs as compared to the APTES-coated NWs. Nonetheless, the fact that there was a detectable amount of both proteins on the APTES-coated NWs reveals the importance of the biotinylation step in order to inhibit non-specific SA/BSA binding and to result in specific SA binding only.

4. Conclusions

A solution-based functionalization method for SA immobilization on GaN NWs was presented for potential protein sensor applications. The protocol included the initial formation of an oxide layer on the as-grown NWs, which was facilitated by exposure to air and oxygen plasma cleaning. Specifically, the oxide layer on the NWs was estimated to be about 1 nm thick by HRTEM. Following surface oxidation, the next step of the protocol was achieved by reacting the oxidized NW surfaces with APTES, which led to formation of an $\approx$5 nm thick amine terminated APTES layer. The final step of the protocol for enabling SA immobilization employed the biotinylation of the APTES-coated NWs through reaction with an amine-reactive molecule, sulfo-N-hydroxysuccinimide-biotin. SA immobilization on the biotinylated GaN NWs was achieved and was demonstrated by FESEM, AFM, XPS, HRTEM, and fluorescence microscopy. Using these techniques, on average, a 15 nm to 20 nm thick organic layer, consisting of APTES, biotin, and SA, was present on the NW surface. A fluorescence microscopy analysis of the biotinylated NWs further proved SA binding. It also confirmed that SA binding was specific; the biotinylated surfaces demonstrated no affinity towards BSA, a control protein. On the other hand, the APTES-coated NWs demonstrated non-specific (likely electrostatic in nature) binding of both SA and BSA, revealing the importance of the biotinylation step in order to limit non-specific protein binding.

This study has demonstrated that GaN NWs are suitable for protein sensing and for biosensing applications in general. By employing this solution-based functionalization technique with other chemistries, the multiplexed detection of a wide range of biomolecules would be possible on a single biosensing chip.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.susc.2014.04.010.

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Selected Data on Microstructure, Composition, and Topography of the GaN NW Surface before and after Functionalization

1. HRTEM and EELS Analysis of the GaN NW Surface Oxide

The oxygen plasma cleaned GaN NWs were analyzed using HRTEM and EELS as demonstrated in Figure S1. The top left picture shows a HRTEM image of a NW edge with a semi-amorphous ≈ 1 nm thick outer layer (indicated by arrows). The EELS analysis (Figure S1, spectrum ② on the right) determined the presence of Ga, N, and O elements in this layer, indicating the formation of surface gallium oxide or oxynitride. Formation of this surface layer (hereafter and in the manuscript to be referred to as the "surface oxide" or "native oxide") was likely initiated upon a post-growth air exposure of the NWs (see references [17] and [18] in the manuscript), which became more pronounced after the oxygen plasma treatment step [1-3].

It is important to point out that as the NW surface further interacts with air, some hydroxyl groups are formed through the reaction of the surface oxide with water vapor [4,5]. This oxidized and partially hydroxylated NW surface is necessary for the covalent attachment of APTES to the NW. APTES hydrolysis and condensation reactions must occur with the hydroxyl groups on the NW surface for successful APTES functionalization [1,4,6].

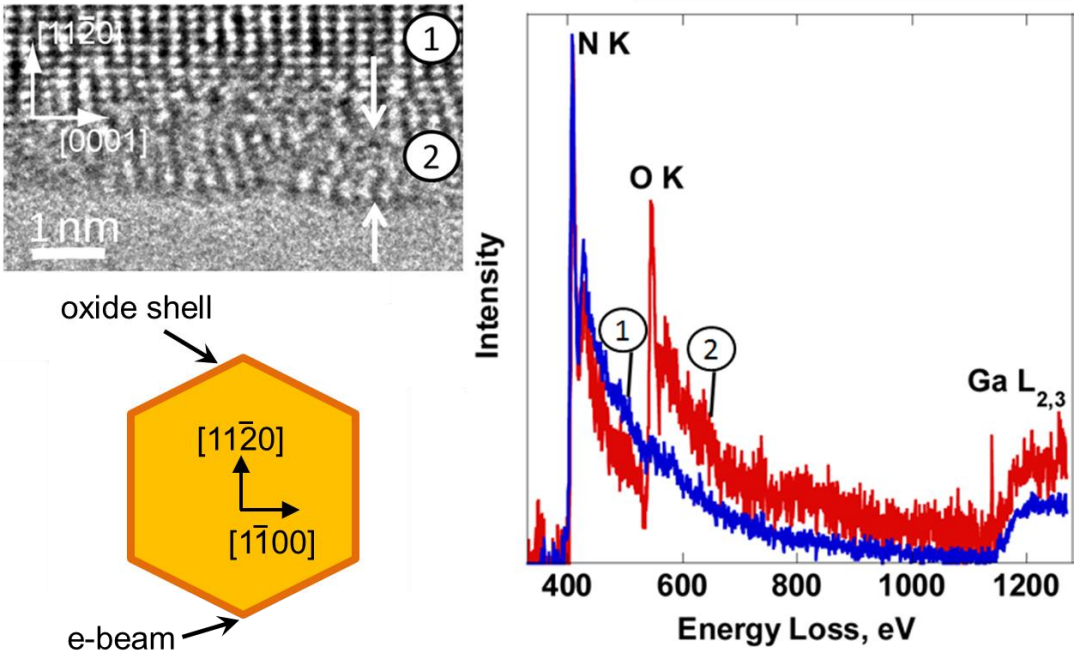

Figure S1. *Top left image:* a HRTEM image of a GaN NW edge. Region ① marks the NW interior and region ② is an amorphous-like surface oxide about 1 nm thick (marked with two

vertical arrows). The [0001] growth direction of the NW is indicated. *Bottom left picture:* a schematic view of the imaging geometry of the NW. *Right spectra:* representative EELS spectra of the NW interior ① (blue spectrum) and surface oxide ② (red spectrum). The Ga/N ratio in region ② (not shown) is lower than its stoichiometric value in the NW interior, which is consistent with the formation of a gallium oxide or oxynitride surface layer.

2. Analysis of the Oxygen Plasma Cleaned and APTES-coated GaN NWs Using FESEM/EDX

The EDX spectrum of a cleaned GaN NW deposited on an Au-coated Si substrate is shown in Figure S2 (red spectrum). The spectrum reveals the typical N and Ga peaks from GaN as well as an Au peak from the underlying substrate. A small carbon signal on the left shoulder of the N peak is attributed to unavoidable hydrocarbon contamination. A small oxygen signal on the right shoulder of the N peak is attributed to the surface oxide on the NW.

EDX of the APTES-coated NWs is shown in Figure S2 as the gray-shaded spectrum. Compared to the data for cleaned GaN NW sample, a Si peak emerges and the C and O peaks intensify. This is consistent with the expected coating of the NW surface with APTES, which contains carbon, oxygen, and silicon.

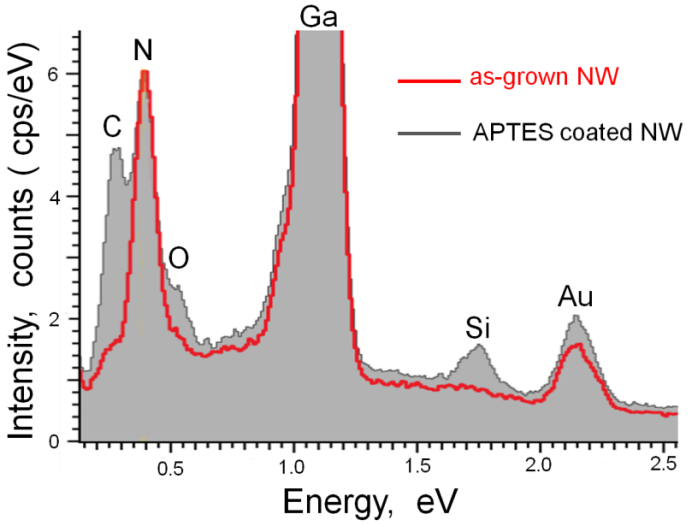

Figure S2. EDX spectrum of a cleaned GaN NW before (red spectrum) and after (gray-shaded spectrum) APTES functionalization. The appearance of a Si peak and an increase in C and O peak intensity indicate successful binding of APTES molecules to the NW surface. Note: The Au signal arises from the underlying Au-coated substrate.

3. AFM Analysis of Agglomerates on the SA conjugated GaN NW Surface

To further characterize agglomerates that appeared on the NW surface after the final SA conjugation step (indicated by arrows in Figure 3D of the main manuscript) which were likely a product of aggregated protein molecules, their dimensions were analyzed with AFM. The inset in Figure S3 shows a profile for a typical agglomerated cluster measuring ≈100 nm in the lateral and ≈16 nm in the vertical directions. The heights of similar agglomerates rarely exceeded 20 nm.

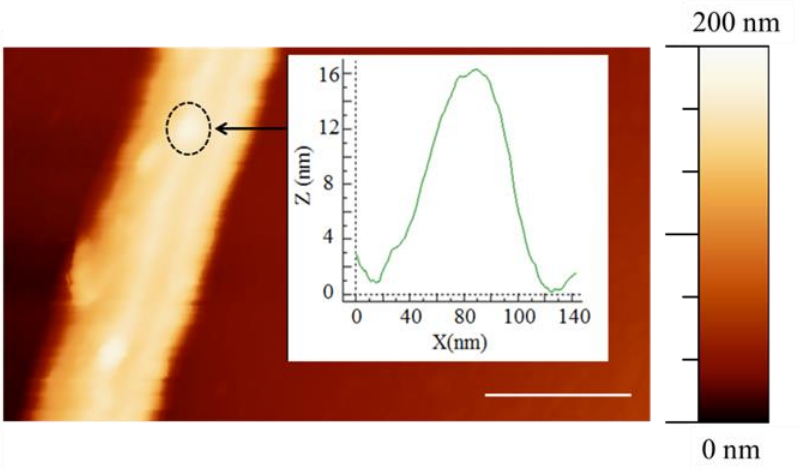

Figure S3. An AFM image of an SA immobilized GaN NW. The vertical bar on the right is the 200 nm color coded Z-scale and the horizontal bar in the lower right corner is the 400 nm X-scale. The inset shows a line scan across a typical protein agglomerate taken along the NW growth axis.

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