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Zinc Oxide Nanomaterials for Biomedical Fluorescence Detection

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Abstract

One-dimensional zinc oxide nanomaterials have been recently developed into novel, extremely effective, optical signal-enhancing bioplatfroms. Their usefulness has been demonstrated in various biomedical fluorescence assays. Fluorescence is extensively used in biology and medicine as a sensitive and noninvasive detection method for tracking and analyzing biological molecules. Achieving high sensitivity via improving signal-to-noise ratio is of paramount importance in fluorescence-based, trace-level detection. Recent advances in the development of optically superior one-dimensional materials have contributed to this important biomedical area of detection. This review article will discuss major research developments that have so far been made in this emerging and exciting topical field. The discussion will cover a broad range of subjects including synthesis of zinc oxide nanorods (ZnO NRs), various properties differentiating them as suitable optical biodetection platforms, their demonstrated applicability in DNA and protein detection, and the nanomaterial characteristics relevant for biomolecular fluorescence enhancement. This review will then summarize the current status of ZnO NR-based biodetection and further elaborate future utility of ZnO NR platforms for advanced biomedical assays, based on their proven advantages. Lastly, present challenges experienced in this topical area will be identified and focal subject areas for future research will be suggested as well.

Keywords

Zinc oxide nanorod sensor; Enhanced fluorescence detection; Biosensor; Nanosensor; Zinc oxide nanorod bioplatfrom

INTRODUCTION

Zinc oxide (ZnO) has proven to be extremely useful in a wide variety of every day applications, ranging from more traditional areas of additives and coatings to more high tech sectors in electronics, optoelectronics, and photonics.^{1–5} Traditional applications typically involve ZnO of bulk and two-dimensional (2D) forms, whereas more advanced high tech applications of the material often rely on one-dimensional (1D) or zero-dimensional (0D) forms of ZnO. A wealth of literature exists in these aforementioned areas, demonstrating and exploiting many attractive physical, electrical, and optical properties of ZnO.^{6–25} Examples of ZnO in traditional application fields can be found in rubber production, food additives, pigmentary components, cosmetic ingredients, and medical products.^{2,10,26–29} ZnO is used in processing rubber goods such as car tires to promote effective dissipation of heat during

their manufacturing and road-driving conditions.³⁰ ZnO is added in some food products as a source of zinc, a nutritive substance.³¹ ZnO is also used as a pigment in paints and as a coating material in papers.²⁶ ZnO serves as an active ingredient in some skin lotions and creams to provide protection against harmful ultraviolet (UV) rays.²⁷ In addition, the antibacterial and antifungal property of ZnO is put to use as topical ointments.³² More related to applications in high tech industry, ZnO is used to achieve higher blocking voltages, switching frequencies, operating temperature, efficiency, and reliability of devices.^{1–5,10,33,34} ZnO has a wide, direct band gap of 3.37 eV and a large exciton binding energy of 60 meV at room temperature.^{1–5} The direct bandgap of ZnO enables more efficient absorption and emission of light. The large exciton binding energy of ZnO permits lower temperature operation of devices, unlike most other semiconductor materials. ZnO has large refractive index and is known to guide and amplify light effectively through the material. In addition, ZnO exhibits high chemical and thermal resistance as well as high electromechanical coupling efficiency.^{35–37} For these reasons, a broad range of ZnO applications in photonic and electronic areas include short-wavelength light-emitters,^{7,11,12} field-emitters,¹³ wave-guides,^{18–20,38} solar cells,^{15–17} piezoelectrics,^{23,33} and lasers.^{7,20–22,39,40} Furthermore, the electrical and optical properties of ZnO have been utilized in chemical, mechanical, and photon sensing.^{24,41,42} For these aspects, many research efforts have been made to demonstrate the capability of ZnO as pH,^{43,44} gas,^{23,45} temperature,⁴⁶ and light sensors.⁴⁷ Figure 1 summarizes these various fields for which ZnO materials, especially nanoscale ZnO, are effectively applied.

Very recently, the novel use of ZnO with reduced dimensions has been successfully extended to *in vivo* and *in vitro* biomedical detection.^{8,9,48–56} Specifically, this review will focus on the recent development of one-dimensional (1D) ZnO in fluorescence-based *in vitro* biodetection. Fluorescence is one of the most widely used detection mechanisms in many fields such as biology, biophysics, biochemistry, genomics, proteomics, drug discovery, disease diagnostics and environmental analysis. Therefore, the utility of 1D ZnO in enhanced biodetection is expected to be high and its future application in biomedical detection is likely to grow continuously and its impact to the field is also anticipated to be significant. This review summarizes the current status of 1D ZnO in this *in vitro* framework as biodetection supports and further provides discussion on present challenges and future outlook on their application as enhanced biomedical detection platforms.

DESIRED PROPERTIES OF ZINC OXIDE

With regards to their use in biomedical detection, nanoscale ZnO materials offer many advantages such as ease of synthesis, biocompatibility, and desirable optical properties. Nanometer scale ZnO can be easily synthesized into controlled dimensions and shapes through many established processing routes. Gas-phase growth approaches for synthesizing nanoscale ZnO structures include physical vapor deposition, pulsed laser deposition, chemical vapor deposition, metal-organic chemical vapor deposition, and vapor-liquid-solid epitaxy.^{33,57–64} Solution-based growth methods include homogeneous precipitation and hydrothermal decomposition processes.^{65–70} When using appropriate growth methods, ZnO nanomaterials can be successfully synthesized to exhibit well-defined, single-crystalline, atomic defect-free structures. At the same time, their synthesis can be directed to show

desired optical properties such as displaying no autofluorescence and having no overlap in spectroscopic properties with those of common fluorophores.^{48–52} Figure 2 displays such an example of ZnO NR platforms whose size, shape, morphology, crystalline structure, and photoluminescence property of ZnO NRs were precisely controlled during vertical NR growth into periodic square patterns in an array format. The surfaces of ZnO nanomaterials have chemical functionality needed for covalent derivatization to allow linking of specific biomolecules and to increase specificity of biomolecular interaction.^{71,72} In addition, ZnO is biocompatible and biosafe.^{73–75} As evidenced by their current applications in food, cosmetics, and medical products; ZnO can be used in applications directly as-grown without any post-synthetic modification such as adding a protective coating layer.

In the burgeoning area of the life sciences, new demands have been identified in conventional fluorescence detection platforms. In biology and medicine, fluorescence-based detection is considered to be the technique of choice as fluorescence provides reasonably high sensitivity to target components in complex biomolecular assemblies, versatility in accommodating a range of sample types, and modest instrumentation requirements for signal detection.⁷⁶ However, the major challenge of improving signal-to-noise ratio still largely exists in many fluorescence-based techniques. Overcoming this challenge will mark a breakthrough in biology and medicine as it will advance key development areas such as population-level genetic screening, system-wide study of proteins, and early disease diagnosis.^{77–81} Therefore, biomedical communities have long sought novel methods enabling rapid, high-throughput, specific, and ultrasensitive fluorescence detection.

With regards to meeting these demands, the recent applications of as-grown ZnO nanorods (ZnO NRs) for improved fluorescence detection involving a variety of biological systems have led to much enthusiasm. By simply replacing conventional platforms of glass and polymers, high-quality ZnO NRs used in these applications were proven to increase considerably the detection capability of biomolecular signal with no further improvements required for fluorophores, filter sets, signal processing software, or instrumentation. Enhanced detection in this case was realized with minimal alternation to the existing fluorescence detection systems, making the newly developed system more attractive as a next-generation biodetection platform. The next section highlights such research efforts demonstrating promising roles of ZnO NRs in advanced fluorescence detection.

ENHANCED BIOMOLECULAR FLUORESCENCE ON ZnO NRs

Based on the abovementioned potential of the materials, the suitability of ZnO NRs as optical signal enhancing platforms was initially assessed. The test support of ZnO NR platforms was evaluated in fluorescence detection of single-layer adsorbed DNA and proteins and, subsequently, its performance was compared to various control supports ranging from bulk, 2D, and 1D materials.⁴⁹ The control supports included silicon NRs, ZnO thin films, as well as conventional biosupport materials of glass, quartz, silicon, and polymers. When treated by the same biodeposition conditions yielding no measurable fluorescence signal on various control supports, intense fluorescence signal was still clearly observable from fluorophore-coupled proteins on ZnO NR platforms. This effect was seen more dramatically in quantitative comparison assays involving varying protein

concentrations. Much stronger fluorescence signal was obtained from ZnO NR platforms, although the concentration of proteins was two or three orders of magnitude lower on ZnO NR platforms than that on control supports. Figures 3(A and B) display fluorescence signal obtained from ZnO NR platforms versus other polymeric control systems using two model proteins of immunoglobulin G antibody and tumor necrosis factor α .^{48,49} In another study, results from direct comparison of fluorescence signal from biomolecules on the two different 1D systems of comparably-sized ZnO NRs versus SiNRs showed extremely strong signal from biomolecules on ZnO NRs versus negligible biomolecular fluorescence on SiNRs. When 2D ZnO thin films were used as supports, higher fluorescence signal from proteins was detected on the thin film when compared to all other control supports. However, the signal increase on ZnO thin films was only about 20% of that detected using 1D ZnO NR platforms. Figure 3(C) summarizes the quantitative comparison between fluorescence signal obtained from the same biomolecules on the test and control platforms. These initial results demonstrated that both the reduced dimensionality of the platform (comparison study of ZnO NRs versus ZnO thin film) and the chemical make-up of the platform (comparison study of ZnO NRs versus SiNRs) play a role in fluorescence signal detection. Overall, when compared to various control platforms, application of ZnO NR platforms in biomolecular fluorescence detection effectively led to two or three orders of magnitude higher sensitivity.

ZnO NR-BASED ENHANCED FLUORESCENCE ASSAYS

Based on the encouraging evidence that the use of ZnO NR platforms may facilitate highly sensitive detection of biomolecular fluorescence, the NR platforms have been evaluated for enhanced fluorescence assays involving single-component biological systems of DNA and proteins. Possible applicability of ZnO nanomaterials in highly sensitive detection of DNA duplex formation has been first demonstrated.⁵² In this experiment, fully complementary or non-complementary probe oligonucleotides (5'-AGTGCGCGAGGAGCCT-3' and 5'-GTTACGGAAAGAACCA-3') were linked to ZnO NRs either through noncovalent physical adsorption or a covalent derivatization of ZnO NR surfaces⁸²⁻⁸⁴. DNA analytes (3'-TCACGCGCTCCTCGGA-5'), preconjugated with 6-carboxyfluorescein, were subsequently introduced to the ZnO NR platforms for hybridization reactions to occur. The covalent linking scheme using glycidoxylpropyltrimethoxysilane (GOPS) allowed genetic sequence detection at concentration levels down to a few femtomolar range, whereas the detection limit of ZnO NR platforms using a non-covalent scheme was in the tens of nanomolar range. Figure 4(A) displays these results from DNA hybridization reactions carried out on a ZnO NR platform. The use of elastomeric microfluidic chambers enabled simultaneous detection of multiple DNA hybridization reactions on the same NR platform.

Further, the capability of ZnO nanomaterials for fluorescence detection of interacting proteins was also tested through multi-layer biodeposition processes.⁵⁰ In these experiments, different pairs of model proteins are sequentially introduced to ZnO NR platforms and then screened for fluorescence to identify the presence/absence of protein-protein interactions in both direct and sandwich assays. Example systems include well-known interacting protein pairs, such as biotinylated bovine serum albumin (BBSA)/dichlorotriazinylaminofluorescein (DTAF-streptavidin) and immunoglobulin G (IgG)/fluorescein-conjugated IgG antibody

(FITC-antiIgG), as well as non-interacting protein pairs. The outcomes of these studies further confirmed that ZnO NRs exhibit an optical property useful for effectively monitoring fluorescence signal from simple biological systems of DNA and proteins, even at ultratrace concentrations. However, the model biological systems tested up to this point involved purified DNA and proteins where biomolecular interaction is limited to the same type of biomolecules. Before long, it was realized that ZnO NR platforms needed to be further assessed in more biologically complex and clinically relevant systems to prove their true potential in biomedical and clinical applications.

In more complex biological assays of multi-component systems, ZnO NR platforms have demonstrated their impact on highly sensitive fluorescence detection of cancer and kidney disease biomarkers of telomerase and cytokines, respectively. A new telomeric repeat elongation (TRE) assay was developed based on ZnO NR platforms and they have been used for a fast and straightforward assay for determining telomerase activity.⁵¹ TRE assays are useful since they can eliminate polymerase chain reaction (PCR)-related artifacts as well as post-PCR procedures such as separating PCR products by gel electrophoresis and evaluating them by phosphorimager or densitometry. The experimental design of the newly developed TRE analysis relied on the conserved physical integrity and intact biological functionality of diverse bioconstituents such as telomerase-expressing HeLa cell lysates, proteins, oligonucleotides, and deoxyribonucleotide triphosphate (dNTPs) on ZnO NR platforms. Results from these assays qualitatively showed that ZnO NR platforms allowed biofunctionality of all TRE assay components, effectively permitting fluorescence-based monitoring of telomerase activity.

These promising results led to further demonstration of ZnO NR platforms in quantitative cytokine detection in biological fluids. It has been reported that elevated levels of cytokines in body fluids may serve as markers of either disease severity or diagnosis for acute kidney injury. However, these proteins are very difficult to detect in early stages of disease when their concentrations are well below the detection level of conventional enzyme linked immunosorbent assay (ELISA)-based detection platforms. In order to assess the feasibility of ZnO NR platforms as an alternative, highly sensitive cytokine screening system, they have been employed in various assays involving primary antibodies, cytokines spiked in urine, and fluorophore coupled secondary antibodies.⁴⁸ Examples of cytokines used with ZnO NR platforms in the initial tests were interleukin-18 (IL-18) and tumor necrosis factor- α (TNF- α). ZnO NRs showed exquisite detection sensitivity much higher than conventional platforms. For example, the IL-18 detection sensitivity of ZnO NRs was down to protein concentrations of several fg/ml, whereas commercial ELISA-based IL-18 detection kits showed sensitivity of tens of pg/ml. Figure 5 displays these comparison results of IL-18 carried out on ZnO NR platforms versus on a commercial ELISA kit.

In many of these studies, it has also been shown that the ZnO NR platform-enabled enhancement is not dependent on the spectroscopic characteristics of fluorophores. For example, two different dyes of Alexa 488 and phycoerythrin were successfully used in the above mentioned cytokine analysis. The use of common fluorophores such as FITC, DTAF, tetramethyl rhodamine isothiocyanate (TRITC), and indocarbocyanine (Cy3) on ZnO NR platforms has revealed an increased fluorescence detection capability of ZnO NRs. Many

biologically relevant fluorophores have absorption and emission bands in the visible and near infra-red (NIR) range. As high quality ZnO NRs do not exhibit absorption or emission in this electromagnetic spectrum range and, at the same time, enable optical enhancement of many fluorophores in the visible and NIR range, their application can be extended to multiplexing, i.e. simultaneous detection of multiple analytes by coupling different fluorophores to different proteins. In addition, the synthesis of ZnO NRs can be tailored to offer an attractive platform that can be seamlessly integrated into existing fluorescence array scanners and plate readers.^{8,9,50,52} High density arrays of ZnO NRs can be straightforwardly fabricated by controlling the dimensions of the surface-printed catalyst patterns during ZnO NR synthesis^{48,50,52} which, in turn, can be used for more demanding, multiplexed, and high throughput biological assays.

To recap, the above-discussed studies have demonstrated that engineered nanoscale ZnO can serve as ideal optical signal enhancing platforms for identifying and screening DNA and protein analytes in both simple and complex biological reactions. Versatile and highly sensitive optical detection of many commonly used fluorophores spanning all visible wavelengths can promote the use of ZnO NR platforms in multiplexed detection. Extended target and signal amplification steps, typically required by other traditional detection methods, are not necessary for achieving high detection sensitivity of biomolecules when using ZnO NR platforms. In addition to their exquisite sensitivity, other key advantages of ZnO NR platforms include ease of array synthesis/fabrication, mechanical and chemical robustness, no autofluorescence, and direct correlation of observed signal to protein concentration. Unlike other commonly used biosupport materials which may display varying degrees of autofluorescence depending on excitation wavelength, this unique property of ZnO NRs exhibiting no spectral overlap with fluorophores can be conveniently used in fluorescence data analysis. Fluorescence signal in the ZnO NR-assisted assays can be correlated directly to interactions of target biomolecules, which differs from the signal monitoring scheme used in ELISA where signal from interacting target analytes is indirectly obtained by measuring enzyme-substrate reactions instead. These combined advantages suggest that ZnO NR platforms can be efficiently used for rapid identification of interacting protein pairs in an array format, especially for screening large libraries of protein molecules and undertaking biochemical studies of multiple protein activities.

PROPERTIES OF ZnO NRs USED IN ENHANCED FLUORESCENCE DETECTION

Optical and electronic studies of ZnO NRs provide insight into the useful properties attributing to the enhanced fluorescence detection capability of ZnO NRs. In optical and optoelectronic fields, light-guiding and light-amplifying properties of ZnO NRs have been extensively demonstrated in their high tech applications such as lasers and light emitters.^{11,12,18–23,25,85–89} A study using metal oxide nanoribbons coupled with a nanorod laser has shown that a larger fraction of light is guided outside the core.^{18,19,88} The increased fraction of light outside enhances the intensity of an evanescent field and its penetration depth into the surroundings which, in turn, leads to more powerful excitation of nearby molecules.^{19,87,88,90} Figure 6 displays examples of such guided wave modes in ZnO

NRs as well as metal oxide NR-based fluorescence/absorbance detection. In theoretical simulations based on MIT Photonic Bands (MPB) program and Finite Difference Method (FDM), the penetration depth of the evanescent field around a hexagonal ZnO NR with a diameter of 500 nm was predicted as approximately 125 nm when excitation of 761 nm was used.⁹¹ Figure 7 summarizes these simulation results on the evanescent field of ZnO NR. The outcomes corroborate with the observation of a relatively large decay length of fluorescence enhancement (much longer than 100 nm) in ZnO NR biodetection systems. In an earlier theoretical work involving a metal oxide-incorporated waveguide, the use of metal oxide in waveguides has been predicted for enhancing evanescent wave fields up to 1500 times higher than a waveguide with no metal oxide.⁹² The diameter of ZnO NRs used in the aforementioned biomolecular fluorescence detection is commensurate to the predicted dimensions of ZnO NRs to guide complex modes of visible light effectively. When calculating the diameter for single mode cutoff in a fiber waveguide,

$\lambda = \pi d_{\text{cutoff}} (n_{\text{NR}}^2 - n_{\text{vacuum}}^2)^{1/2} / 2.405$ using λ of 500 nm,⁹³ d_{cutoff} is estimated to be 220 nm. As the diameter of ZnO NRs in the aforementioned fluorescence detection ranges a few to several hundreds of nanometers, they can effectively support both single mode and higher modes of waves.

At the same time, biomolecules coupled directly on top of ZnO NRs do not suffer from fluorescence quenching and no effective resonance energy transfer was observed between fluorophores and ZnO NRs. In systems utilizing Ag and Au nanoparticles, fluorophores should be strategically placed between 5–20 nm away from the metal surface to capitalize on the metal enhanced fluorescence (MEF) effect.^{94–98} ZnO NRs, however, behave differently than the metal nanoparticles and show no such distance-sensitive effect or spectroscopic overlap between the nanomaterials and fluorophores commonly used in bioassays. Hence, ZnO NR platforms can be conveniently used for ultrahigh sensitivity fluorescence measurements involving biomolecules located directly on top of as well as many bilayers away from the NR surface. From these combined aspects, the unique optical properties of ZnO NRs to effectively guide and enhance light on and along the material surface may best explain the remarkable fluorescence sensitivity observed in ZnO NR-enabled biodetection.

From the viewpoint of photostability, a recent study involving nanostructures on a ZnO thin film provided insight from electronic states of fluorophores and ZnO.⁹⁹ The single molecule fluorescence and time-correlated single photon counting study demonstrated a possible role of electron transition between the lowest unoccupied molecular orbital (LUMO) of a Cy5 molecule and the conduction band (CB) of ZnO in increased fluorescence. In this configuration, electrons move from $\text{LUMO}_{\text{fluor}}$ to CB_{ZnO} and, subsequently, fluoresce through a relaxation process from CB_{ZnO} to the highest occupied molecular orbital, $\text{HOMO}_{\text{fluor}}$. This process in ZnO-fluorophore systems may eliminate electrons in their excited levels being self-quenched in non-radiative trap sites.

CONCLUDING REMARKS AND OUTLOOK

ZnO NR platforms have demonstrated promise for rapid, low-cost, multiplexed, high-throughput, and highly sensitive biomedical detection in both laboratory and clinical

settings. The main advantage of the NR platforms lies in their capability of increased fluorescence detection while using conventional fluorophores, optics, and instrumentation. While not interfering with the absorption and emission profiles of common fluorophores in bioassays, the presence of ZnO NRs leads to an increase in detection sensitivity to several orders of magnitude when compared to the same biomolecules on conventional substrates made out of glass and polymers. As ZnO NR synthesis can be easily configured to provide vertically grown NRs into patterned platforms, they have the potential to impact areas exploiting conventional fluorescence array scanners and plate readers as detectors. With such advantages of ZnO NR platforms, their future application will continue to yield new development of additional biomarker assays beyond the previous examples of telomerase and cytokines. In particular, the high detection sensitivity of ZnO NRs may impact the field of early detection in clinical research and testing which often profiles low-abundance biomarker levels in physiological fluids.

The size of biodetection platforms and devices is rapidly shrinking for low-cost, small-volume analyses of DNA and proteins. Therefore, assessing similar signal enhancing capability on individual ZnO NRs, instead of ensembles of ZnO NRs, may be particularly useful in their future applications in miniaturized detection. Development of clinical assays on single ZnO NRs may facilitate high sensitivity, *in vitro* screening of low-abundance bioanalytes in a rapid and cost-effective manner beyond what ensemble ZnO NR platforms can presently offer. Additionally, the unique detection capability of ZnO NRs may be extended to *in vivo* testing to probe locally hard-to-detect, low signal-emitting bioconstituents. In this context, further elucidation of light-matter interaction property on both single and a collection of NRs will deepen current understanding of the ZnO NR-enabled capability of enhanced fluorescence detection. Although some research efforts have been made on light interaction behavior on and around one-dimensional NR systems, the majority of these studies involve bandgap emission of the material.^{20,100,101} More in-depth studies are warranted for a systematic investigation of ZnO NR-light interaction as well as fluorescence detection of biomolecule-coupled ZnO NRs involving polarization dependence, fluorescence lifetime, and photostability measurements.

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Biography

Professor Hahm received her B.S. in Chemistry from Seoul National University and Ph.D. in Physical Chemistry at the University of Chicago. She then completed her postdoc in the Department of Chemistry at Harvard University. Her first faculty appointment was in the Department of Chemical Engineering at Pennsylvania State University. She then moved to the Department of Chemistry at Georgetown University where she currently serves as an Associate Professor. She is a recipient of 2013 Rising Star Award, 2008 Progress/Dreyfus Lectureship Award, and 2007 WISE Lectureship Award from the American Chemical Society.

Figure 1. Various application fields of ZnO nanomaterials. The red arrow highlights the focal area of this review as *in vitro* applications of ZnO NRs as optical signal-enhancing biodetection platforms.

Figure 2.

(A–C) Scanning electron microscopy (SEM) images of (A) $70 \times 70 \mu\text{m}^2$, (B) $850 \times 850 \text{ nm}^2$, and (C) $400 \times 400 \text{ nm}^2$, clearly show assembled ZnO NRs of square patterns, vertically grown ZnO NRs inside each pattern, and the hexagonal geometry of individual ZnO NRs, respectively. (D) X-ray diffraction (XRD) spectra of ZnO NRs on a silicon substrate confirm the single crystalline nature of wurtzite ZnO NRs whose predominant growth is along the c-axis of the crystal. (E) Typical room-temperature photoluminescence (PL) spectrum of ZnO NRs produces an extremely strong peak at 390 nm, originating from the material's band-gap emission for $\lambda_{\text{excitation}} = 335 \text{ nm}$. Reprinted with permission from Ref. #39, C. Zhang, F. Zhang, S. Qian, N. Kumar, J.-i. Hahm, and J. Xu, *Appl. Phys. Lett.*, 92, 233116 (2008), Copyright@ American Institute of Physics.

Figure 3.

Enhanced fluorescence detection facilitated by the use of ZnO nanoplatforms: single-type protein systems. (A) Strong fluorescence signal was observed from fluorescein-conjugated IgG antibody that was deposited on regularly-patterned ZnO nanoplatforms. The repeat spacing of the underlying ZnO nanoplatforms is 20 μm (shown in the inset SEM image) and the concentration of the biomolecules used in the confocal measurement was 200 $\mu\text{g/ml}$. (B) The plot of fluorescence intensity versus protein concentration displays the detection sensitivity limit of (left) fluorescein-conjugated IgG antibody and (right) tumor necrosis factor- α molecules on ZnO nanoplatforms using a conventional confocal microscope.

Fluorescence data from protein molecules of the same composition and concentration on patterned polymeric platforms are plotted for comparison. ZnO NR platforms demonstrate much improved detection capability of the emission from model proteins than control polymeric platforms in both cases. **(C)** Relative fluorescence intensity from fluorescein-conjugated IgG antibody molecules prepared on various control substrates such as PMMA film, quartz, glass, Si, ZnO thin film, and SiNRs. The plot displays fluorescence intensity measured from 200 µg/ml FITC-antiIgG on these control substrates which was normalized with respect to that on ZnO NR platforms. Reprinted with permission from Refs. #48 & #49, V. Adalsteinsson, O. Parajuli, S. Kepics, A. Gupta, W. B. Reeves, and J.-i. Hahm, *Anal. Chem.*, 80, 6594 **(2008)** & A. Dorfman, N. Kumar, and J. Hahm, *Langmuir*, 22, 4890 **(2006)**, Copyright© American Chemical Society.

Figure 4.

(A) Detection scheme to identify interacting DNA strands using ZnO NR platforms: Polydimethylsiloxane (PDMS) chambers were used in order to carry out simultaneous hybridization reactions on the same ZnO NR platform containing regularly patterned ZnO stripes with a repeat spacing of 20 μm . Oligonucleotide probes of *bce* (5'-GTTACGGAAAGAACCA-3') and *bas* (5'-AGTGC GCGAGGAGCCT-3') were first introduced to the reaction chambers 1 and 2, respectively. Subsequently, fluorescein modified *basr* (3'-TCACGCGCTCCTCGGA-5') strands were added to both chambers and

allowed to form DNA duplexes under the same hybridization conditions. Confocal images taken from these samples showed clear fluorescence emission from chamber 2 in contrast to no discernable fluorescence signal from chamber 1. The insets in the upper left corners of the confocal images are the corresponding bright field images taken from each chamber after the duplex formation reaction. Distinctive fluorescence emission monitored from chamber 2 is due to DNA duplex formation between fully complementary strands of *bas* and *basr* whereas the lack of duplex formation between mismatching sequences of *bce* and *basr* led to no observable fluorescence in chamber 1. **(B)** Fluorescence emission monitored from covalently bound *bas* strands and the fully complementary *basr* strands on an open square ZnO array. The concentrations of the probe and target strands were the same at 20 μ M. The underlying ZnO nanostructures as well as confocal fluorescence patterns exhibit open squares of 10 μ m in length with a repeat spacing of 10 μ m, demonstrating easy synthetic capability to fabricate ZnO NR arrays for use with conventional fluorescence instrumentations. Reprinted with permission from Ref. #52, N. Kumar, A. Dorfman, and J. Hahm, *Nanotech.*, 17, 2875 (2006), Copyright@ IOP Publishing.

Figure 5.

(A–B) Fluorescence obtained from $5 \mu\text{m}$ -period, square-array ZnO NR platforms after carrying out IL-18 assays in urine. Panels (A) $140 \times 140 \mu\text{m}$, and (B) $60 \times 60 \mu\text{m}$ display fluorescence images obtained from a sandwich assay involving 20 fg/ml of IL-18 in urine. Brightness and contrast in the fluorescence panels are adjusted in order to show the images more clearly. (C) After performing the cytokine assays on ZnO NR platforms, the measured fluorescence signals at various IL-18 concentrations in urine were normalized with respect to the fluorescence intensity detected when using 0.2 ng/ml of IL-18. The normalized fluorescence intensity is plotted against the logarithmic value of the IL-18 concentration. Assay results from two independent runs on ZnO NR platforms are displayed. (D) ELISA assay of urine containing the indicated amounts of IL-18. The measured absorbance signals at various IL-18 concentrations were normalized with respect to the absorbance detected using 1 ng/ml IL-18. Reprinted with permission from Ref. #48, V. Adalsteinsson, O. Parajuli, S. Kepics, A. Gupta, W. B. Reeves, and J.-i. Hahm, *Anal. Chem.*, 80, 6594 (2008), Copyright@ American Chemical Society.

Figure 6.

(A) Magnified view of the conical emission of a low-order guided mode from a ZnO nanowire. The emission angle is approximately 90° (dotted lines). (B) 2D finite difference time domain (FDTD) calculation of the square of the electric field of a light pulse emitted from a nanowire of diameter d . (C–F) Fluorescence and absorbance detection of rhodamine 6G (R6G) with a ribbon cavity. (C) Fluorescence image of a droplet of 1 mM R6G in 1,5-pentanediol excited by blue light from a ribbon waveguide (240 nm $\times$ 260 nm $\times$ 540 μm). The nanoribbon crosses the frame from upper left to lower right. A notch filter was used to block the excitation light. (Left Inset) A dark-field image showing the droplet and the bottom half of the ribbon. (Right Inset) A magnified ($\times 2.5$) view of the droplet emission, showing the light cone and evanescent pumping of the dye along the ribbon length. (D) Spectra taken of the droplet region (direct) and the fluorescence coupled back into the ribbon (guided). The redshift of the guided emission is a microcavity effect. (E) Dark-field image of the ribbon with a droplet deposited near its middle (absorbance geometry). The ribbon was UV-pumped on one side of the droplet and probed on the other side as indicated. (F) Spectra of the guided PL without liquid present and with droplets of pure 1,5-pentanediol and 1 mM R6G. Losses caused by the presence of the pure solvent droplet were negligible. The arrow indicates the absorption maximum of R6G. Reprinted with permission

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Figure 7.

(A–C) Three-dimensional model of the absolute value of the electric field $|E|$ of ZnO NRs with diameters (A) $D = 300$ nm, (B) $D = 600$ nm, and (C) $D = 1000$ nm at $\lambda = 761$ nm. (D) Normalized maximum magnitude of the evanescent field E_{max} as a function of NR diameter. (E) Penetration depth obtained by MPB and FDM as a function of diameter for $\lambda = 761$ nm. The inset shows schematically the used cut across the fundamental mode field distribution. Reprinted with permission from Ref. #91, S. Börner, C. E. Rüter, T. Voss, D. Kip, and W. Schade, *Phys. Stat. Sol. (a)* 204, 3487 (2007), Copyright@WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.