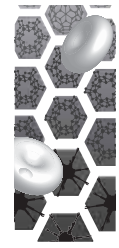
REVIEW



Nanowire sensors for medicine and the life sciences

Fernando Patolsky, Gengfeng Zheng & Charles M Lieber†

†Author for correspondence Harvard University, Department of Chemistry and Chemical Biology, and Division of Engineering and Applied Sciences, 12 Oxford Street, Cambridge, MA 02138, USA Tel.: +1 617 496 3169; Fax: +1 617 496 5442; E-mail: cml@cmliris.harvard.edu

The interface between nanosystems and biosystems is emerging as one of the broadest and most dynamic areas of science and technology, bringing together biology, chemistry, physics and many areas of engineering, biotechnology and medicine. The combination of these diverse areas of research promises to yield revolutionary advances in healthcare, medicine and the life sciences through, for example, the creation of new and powerful tools that enable direct, sensitive and rapid analysis of biological and chemical species, ranging from the diagnosis and treatment of disease to the discovery and screening of new drug molecules. Devices based on nanowires are emerging as a powerful and general platform for ultrasensitive, direct electrical detection of biological and chemical species. Here, representative examples where these new sensors have been used for detection of a wide-range of biological and chemical species, from proteins and DNA to drug molecules and viruses, down to the ultimate level of a single molecule, are discussed. Moreover, how advances in the integration of nanoelectronic devices enable multiplexed detection and thereby provide a clear pathway for nanotechnology, enabling diverse and exciting applications in medicine and life sciences, are highlighted.

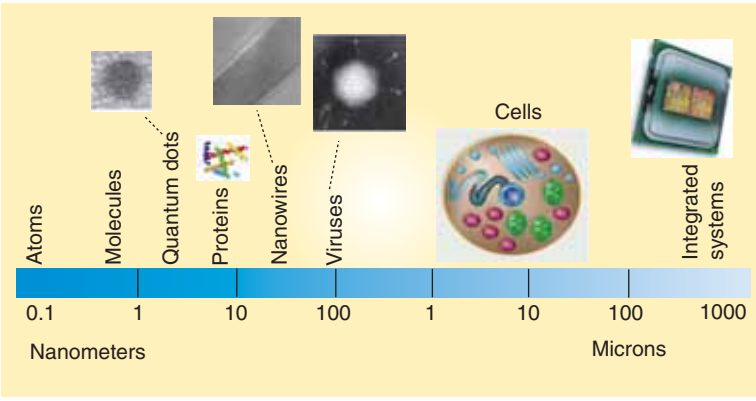
Keywords: biomarkers, biosensors, cancer detection, diagnosis, drug discovery, field-effect-transistor, label-free detection, nanowires, silicon, virus detection

future medicine

Nanotechnology is a broad area of science and technology devoted to studies of the synthesis, properties and applications of structures and materials with at least one critical dimension less than the scale of approximately 100 nm. A strong motivating force driving interest in nanotechnology in the physical sciences has been the long-standing recognition that the physical and chemical properties of synthetic materials can significantly improve or radically change as their size is reduced to the nanometer regime due, for example, to the effects of quantum confinement [1,2]. More importantly, it is now becoming increasingly well recognized that the emerging concepts and applications of nanotechnology are not limited to the physical sciences and, indeed, can be applied to and represent a rich area intersecting with the life sciences and medicine. The natural connection between nanotechnology and the life sciences can be understood in many ways, although perhaps the most straightforward is to consider the size and organization of common structures, as shown in Figure 1. Here, we see that quantum dots, nanowires and proteins, which are built from atoms, have similar average diameters. These basic structures or building blocks can be further organized into large structures, such as viruses and nanoelectronics circuits, and ultimately further elaborated into functional systems, such as cells and computer chips.

The similarity in sizes of synthetic and natural nanostructures makes nanotechnology an obvious choice for creating probes and other tools that can enable detection and treatment of disease in powerful new ways, an intersection between nanotechnology and medicine that we and others refer to as nanomedicine. Detection and quantification of biological and chemical species is central to many areas of medicine, ranging from diagnosing disease to the discovery and screening of new drug molecules. Nanostructures, such as nanowires [3–15] and carbon nanotubes [16,17] as well as nanoparticles [18–39], offer new and sometimes unique opportunities for this key task. Inorganic nanowires, nanocrystals and carbon nanotubes exhibit unique electrical, optical and magnetic properties that can be exploited for sensing and imaging [3–39]. For example, colloidal gold and semiconductor nanocrystals have been used as labels for the detection of disease markers and other biological species critical to understanding diseases [18,19,31–39]. In addition, iron oxide nanocrystals with superparamagnetic properties and semiconductor nanocrystals with size-tunable emissive properties are being developed as specific contrast agents in magnetic resonance and optical imaging, respectively, capable of wide-ranging applications, including the imaging of cancer tumors in live animal models [20–27].

Figure 1. Comparison of the sizes of biological, chemical and nanoscale structures, assemblies and systems.



The reproducible and tunable conducting properties of semiconducting nanowires combined with surface binding provide a very different and powerful approach to nanomedicine. Specifically, nanowires enable a detection and sensing modality – direct and label-free electrical readout – that is exceptionally attractive for many applications in medicine and the life sciences [40–45]. Electronic nanowire devices are readily integrated into miniaturized systems and, moreover, direct electrical detection dispenses with time-consuming labeling chemistry. These characteristics, together with ultrahigh sensitivity, suggest that nanowire devices could revolutionize many aspects of sensing and detection medicine, ranging from the diagnosis and monitoring of disease treatment to the discovery and screening of new drug molecules. While similar concepts are possible with carbon nanotubes, the lack of control of the electronic properties of this nanomaterial make it much less attractive for the development of electronic nanosensors compared with semiconducting nanowires. Here, the authors provide an introduction to the underlying nanowire nanotechnology and then illustrate the diverse and exciting applications of this technology in nanomedicine.

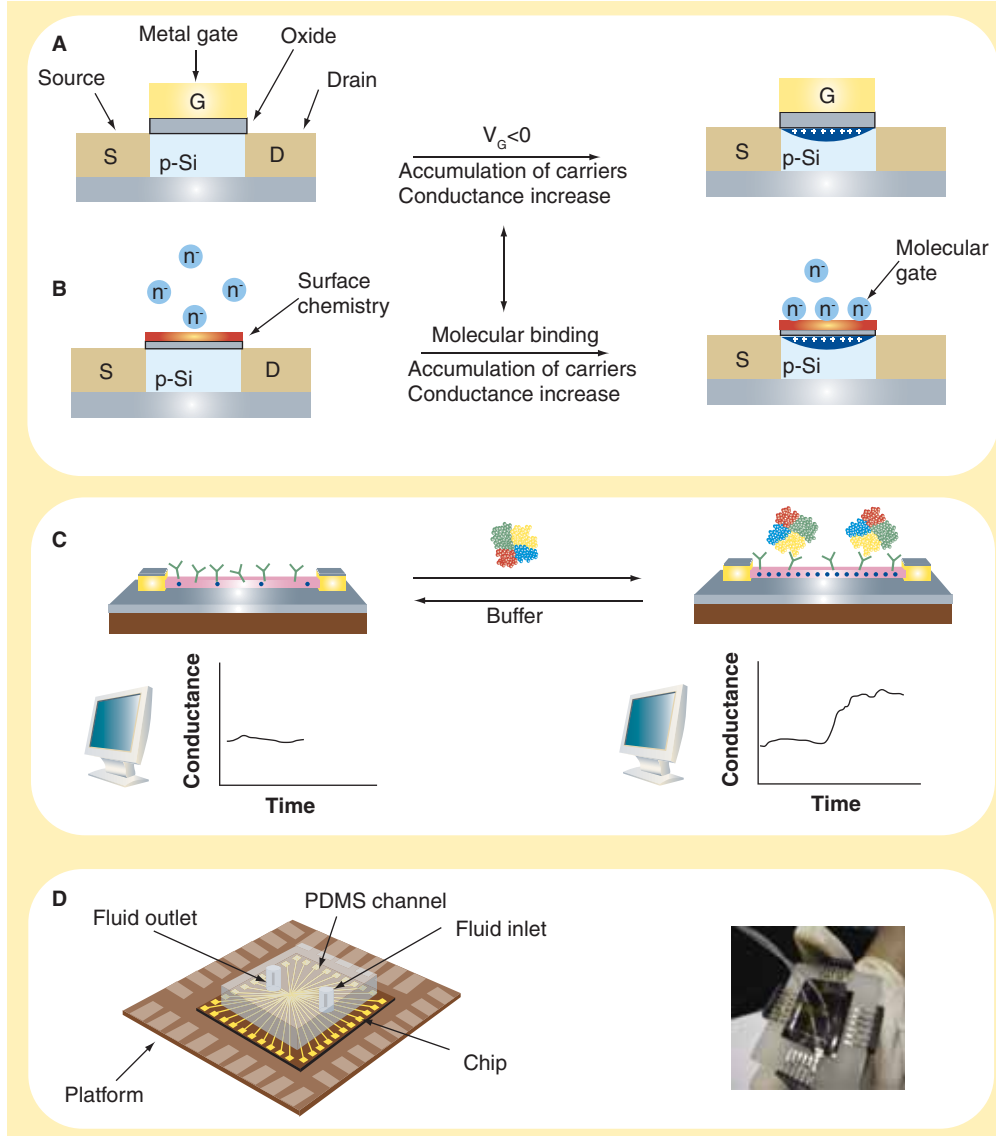
Nanowire field-effect sensors

Underlying detection using semiconductor nanowires is their configuration as field-effect transistors (FETs), which will exhibit a conductivity change in response to variations in the electric field or potential at the surface of the nanowire FET [5,40]. In a standard FET (Figure 2A), a semiconductor, such as p-type silicon (p-Si), is connected to metal source and drain electrodes, through which a current is injected and collected, respectively. The conductance of the semiconductor between source and drain is switched on and off by a third gate electrode capacitively coupled through a thin dielectric layer [46]. The gate voltage can be applied in a conventional manner using the degeneratively doped silicon substrate as a back-gate or by an external electrode immersed in the aqueous solution as a water gate. In the case of a p-Si or other p-type semiconductor, applying a negative gate voltage, which leads to negative charges at the interface between the gate electrode and dielectric, leads to an accumulation of carriers (positive holes) and a corresponding increase in conductance, as shown in Figure 2A. However, applying a positive gate voltage to a p-type device, which leads to positive charges at the interface between the gate electrode and dielectric, will deplete carriers in the device and lead to a decrease in the conductance.

The dependence of the conductance on gate voltage and corresponding charge at the gate electrode–dielectric interface makes FETs natural candidates for electrically based sensing because the binding of a charged or polar biological or chemical species to the gate dielectric is analogous to applying a voltage using a gate electrode. For example, binding of a protein with net negative charge to the surface of a p-Si FET will lead to an accumulation of positive hole carriers and an increase in device conductance (Figures 2B,2C). This idea for sensing with FETs was introduced several decades ago [47,48], although the limited sensitivity, which usually does not allow sensing of a biological analyte with planar FET sensors of previous planar devices, has precluded them from having a large impact as chemical or biological sensors.

Semiconductor nanowires composed of silicon and other materials can also function as FET devices [5,9,11–15]. The structure of one of the best characterized examples of semiconducting nanowires, silicon nanowires, can be prepared as single-crystal structures with diameters as small as 2–3 nm [49,50]. The Si nanowires can be prepared as both p- and n-type materials and configured as FETs that exhibit performance characteristics comparable to or better than the best achieved in the microelectronics industry for planar silicon devices [11,13,14]. These attractive performance characteristics are also achieved with high reproducibility [13]; that is, the electronic characteristics of the nanowire are well controlled during

Figure 2. Nanowire field-effect transistor sensors.



(A) Schematic of a field-effect transistor (FET) device, where S, D and G correspond to source, drain and gate metal electrodes, respectively. **(B)** Schematic of electrically based sensing using FET devices, where S and D correspond to source and drain electrodes, respectively, and the binding of a 'charged or polar' biological or chemical species to the chemically modified gate dielectric is analogous to applying a voltage using a gate electrode as shown in Figure 2A. **(C)** Schematic of a nanowire device configured as a sensor with antibody receptors (green), and binding of a protein with net negative charge yields an increase in the conductance. **(D)** Schematic and photograph of a prototype nanowire sensor biochip with integrated microfluidic sample delivery.

growth, in contrast to carbon nanotubes. The high performance switching characteristics of silicon nanowires are important to us since these are factors that affect sensitivity. More important than overcoming the sensitivity limitations of previous planar FET sensors is the one-dimensional nanoscale morphology of these structures, because binding to the surface of a nanowire leads to depletion or accumulation of carriers in the 'bulk' of the nanometer diameter structure (versus only a small region of the surface region of a planar device) [40]. This unique feature of

semi-conductor nanowires can lead to sufficient sensitivity to enable the detection of single viruses [44] and make the detection of single molecules in solution possible.

Nanowire-based sensing devices can be configured from high-performance field-effect nanowire transistors [40,42–45,51] by linking recognition groups to the surface of the nanowire (Figure 2C). Silicon nanowires with their native oxide coating make this receptor linkage straightforward since extensive data exist for the chemical modification of silicon oxide or glass surfaces from research on planar chemical and biological arrays [52]. When the sensor device with surface receptors is exposed to a solution containing macromolecule species, such as a protein, which has a net negative (positive) charge in aqueous solution, specific binding will lead to an increase (decrease) in the surface negative charge and an increase (decrease) in conductance for a p-type nanowire device (Figure 2C). An important point about this detection process, which is distinct from common optically based assays, is that it is real-time and the binding process can literally be viewed as it occurs on a computer logging the conductance of one or more devices. Practically, the authors have developed a reliable and flexible integrated nanowire sensor platform capable of single or multiplexed detection (Figure 2D) [44,45]. The platform incorporates silicon nanowires with well defined p- or n-type doping, addressable source drain electrodes that are insulated from the aqueous environment so that only processes occurring at the silicon nanowire surface contribute to electrical signals, integrated electrical interconnects that enable standard interface to data logging electronics and a mated microfluidic channel for delivery of sample solutions.

A tool for drug discovery

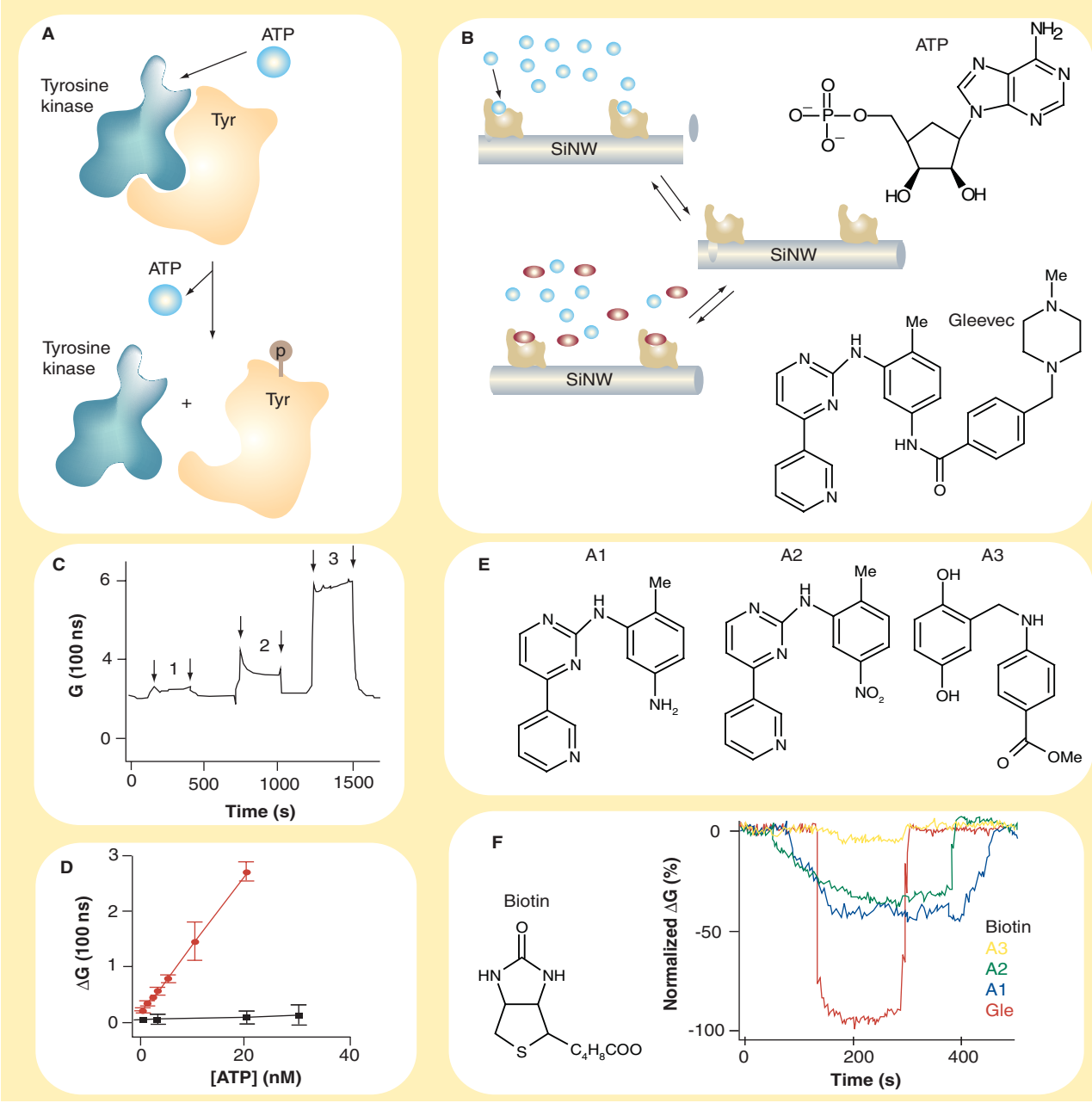
The discovery and/or identification of organic molecules that bind specifically to proteins is central to the discovery and development of new pharmaceuticals and to chemical genetic approaches for elucidating complex pathways in biological systems [53]. This thus represents an important area in which the unique capabilities of new nanosensors could impact. A broadly representative example in this area has been the identification of molecular inhibitors to tyrosine kinases, which are proteins that mediate signal transduction in mammalian cells through phosphorylation of a tyrosine residue of a substrate protein using adenosine triphosphate (ATP) (Figure 3A) [54]. Deregulation of the phosphorylation process has been linked to a number of diseases, including cancer [54]. To configure nanowire sensor devices for screening small molecule inhibitors to tyrosine kinases, the authors linked the kinase Ab1 to the surface of silicon nanowire FETs and investigated the binding of ATP and competitive inhibition of ATP binding with organic molecules, such as the drug Gleevec™ (Figure 3B) [43]. In this configuration, binding or inhibition of binding of the negatively charged ATP to Ab1 linked at the silicon nanowire surface can be detected in a real-time, quantitative manner as an increase or decrease in the conductance of the p-type nanowire device. The direct yet simple nature of this approach, enabled by nanotechnology, contrasts conventional indirect assays used to study kinases [55,56].

Data recorded from Ab1-modified p-type silicon nanowire devices have been found to exhibit reversible, concentration-dependent increases in conductance upon introducing solutions containing ATP (Figures 3C,3D), where the increases in conductance are consistent with the binding of negatively charged ATP to Ab1 [43]. Of perhaps greater importance has been the ability to quantify inhibition of ATP binding by Gleevec and other small molecules, such as the analogs shown in Figure 3E. Plots of the normalized conductance recorded from Ab1-modified p-type silicon nanowire devices (Figure 3F) exhibited reversible decreases in conductance due to competitive inhibition of ATP binding by the different small molecules. Notably, the conductance decreases at constant small molecule concentration depends strongly on molecular structure with Gleevec > A1 > A2 > A3; the control biotin shows essentially no change above background, as expected [43]. These studies demonstrate the substantial advantages of nanowire detectors over existing methods for rapid, direct and high-sensitivity analysis of binding and inhibition using minimal protein receptor and thus suggest great potential as a new 'nano' technology platform for drug discovery.

Detection of DNA & DNA enzymatic processes

Biological macromolecules, such as nucleic acids and proteins, are generally charged in aqueous solution, and as such can be readily and selectively detected by nanowire sensors when appropriate receptors are linked to the nanowire active surface. The authors first examined studies addressing this key capability in the context of sequence-specific detection of DNA [42] and the monitoring of the enzymatic elongation of DNA [45].

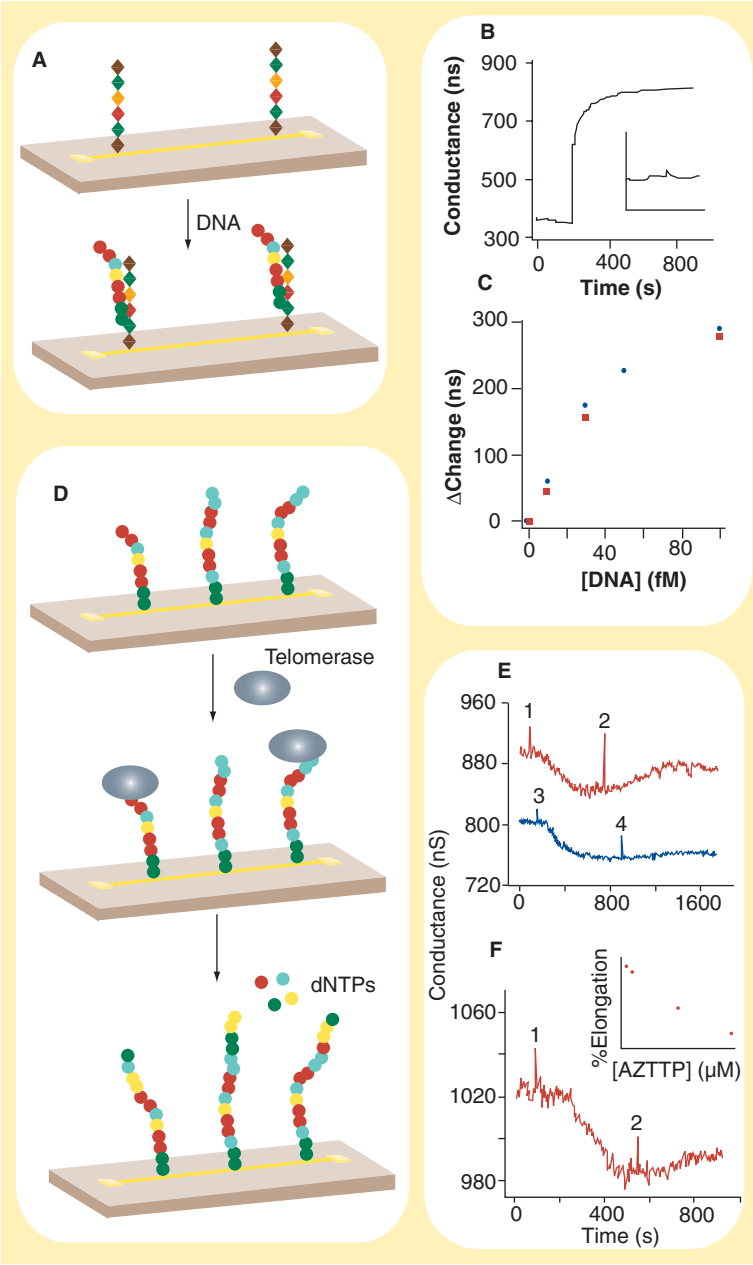
Figure 3. Nanowire sensors for drug discovery.



(A) Illustration of tyrosine kinase phosphorylation of a tyrosine (Tyr) residue of the substrate protein. **(B)** Detection of ATP binding and small molecule inhibition using a silicon nanowire sensor device functionalized with the tyrosine kinase Ab1. **(C)** Time-dependent conductance (G) curve at different ATP concentrations for a nanowire modified with Ab1. Regions 1, 2 and 3 correspond to 0.1, 3 and 20 nM ATP, respectively. Arrows indicate the points where the solution is changed. **(D)** Change in conductance (ΔG) versus ATP concentration for Ab1-modified SiNW (red) and SiNW without Ab1 (black). **(E)** Structures of small molecules investigated for inhibition of ATP binding to Ab1 and **(F)** normalized conductance data showing the relative inhibition of the different compounds.

Silicon nanowire field-effect devices have been used for the detection of single-stranded DNA [40,57], where the binding of this negatively charged polyanionic macromolecule to p-type nanowire surfaces leads to an increase in conductance. Recognition of the DNA target molecule was carried out using complementary single-stranded sequences of peptide nucleic acids (PNAs) (Figure 4A) [58]. PNA was used as the receptor for DNA detection in this work since

Figure 4. Real-time detection of DNA and DNA reactions.



(A) Schematic of silicon nanowire sensor surface modified with PNA receptor before and after duplex formation with target DNA. **(B)** Silicon nanowire DNA sensing, where the arrow corresponds to the addition of a 60 fM complementary DNA sample and the inset shows the device conductance following addition of 100 fM mutant DNA. **(C)** Conductance versus DNA concentration for two independent (red and blue) devices. **(D)** Schematic representation of the telomerase binding and activity assay. **(E)** Conductance versus time data recorded for oligonucleotide-modified p-silicon nanowire devices, following the introduction of solutions containing (1,3) extract from 100 HeLa cells, (2) all four dNTPs and (4) dCTP only. **(F)** Conductance versus time data from device following the introduction of solutions containing (1) extract from 100 HeLa and (2) all four dNTPs each at 0.1 mM and 20 μM azido thymidine triphosphate (AZTTP). Inset: plot of the inhibition of elongation versus AZTTP concentration.

the uncharged PNA molecule has a greater affinity and stability than corresponding DNA recognition sequences at low ionic strength where nanowire sensitivity is greater [58]. Studies of p-type silicon nanowire devices modified with a PNA receptor designed to recognize wild-type versus the EF508 mutation site in the cystic fibrosis transmembrane receptor gene showed that the conductance increased following addition of a 60 femtomolar wild-type DNA sample solution (Figure 4B). The increase in conductance for the p-type silicon nanowire device is consistent with an increase in negative surface charge density associated with binding of negatively charged DNA at the surface and, moreover, careful control experiments showed that the binding response was specific to the wild-type sequence and that a sample sequence with the EF508 mutation site did not show this stable change in conductance (inset, Figure 4B) [42]. The sequence specificity in these experiments is a critical first step towards the development of the nanowire devices for genetic-based disease detection.

In addition, there are several other features of the nanowire-based DNA sensors that deserve mention. First, the studies of the conductance change versus target sequence concentration demonstrated that direct electrical detection was possible down to at least the 10 femtomolar level (Figure 4C). Significantly, this current detection limit is substantially better than that demonstrated by existing real-time measurements, including surface plasmon resonance (SPR) [58], nanoparticle-enhanced SPR [59] and quartz-crystal microbalance [60] for DNA detection. Second, Figure 4C also illustrates that the DNA detection data obtained from independent silicon nanowire devices exhibit very similar changes in conductance with increasing DNA concentration. Device to device reproducibility is an important validation of the potential of the silicon nanowires for development as integrated sensors, which could enable high-throughput, highly sensitive DNA detection for basic biology research and genetic screening.

The potential breadth of nanowire arrays as broad-based diagnostic tools can be seen in an orthogonal nucleic acid-based marker assay involving the detection of activity and inhibition of telomerase [45], a eukaryotic ribonucleoprotein (RNP) complex that catalyzes the addition of the telomeric repeat sequence TTAGGG to the ends of chromosomes [61]. Telomerase is inactive in most normal somatic cells but active in at least 80% of known human cancers [62], and thus is a

potential general marker and therapeutic target for cancer detection and treatment, respectively. The nanowire telomerase assay illustrated in Figure 4D is remarkably simple yet powerful. First, this assay detects the presence or absence of telomerase simply by monitoring the nanowire conductance following delivery of a sample cell extract to the device array that has been modified with the telomeric repeat sequence. Moreover, subsequent addition of deoxynucleotide triphosphates (dNTPs) also enables monitoring of telomerase activity through an increase in conductance owing to the incorporation of negatively charged nucleotides near the nanowire surface.

Conductance data recorded from oligonucleotide primer-modified p-type silicon nanowires show well defined conductance decreases following delivery of the HeLa cell extract (Figure 4E, points 1, 3), which corresponds to the selective binding of the positively charged telomerase at the surfaces of the nanowires in the array [45]. Notably, concentration-dependent studies showed that binding was readily detectable to at least the 10 cell level without amplification, while no signal was observed from as many as 100,000 normal human fibroblast cells or heat-denatured HeLa cell extracts. The primer-modified nanowire arrays were also effective in monitoring activity (Figure 4E, point 2) where addition of dNTPs, following initial telomerase binding, showed an increase in the device conductance that corresponds to incorporation of negatively charged nucleotide units on the nanowire surface during the telomerase catalyzed process.

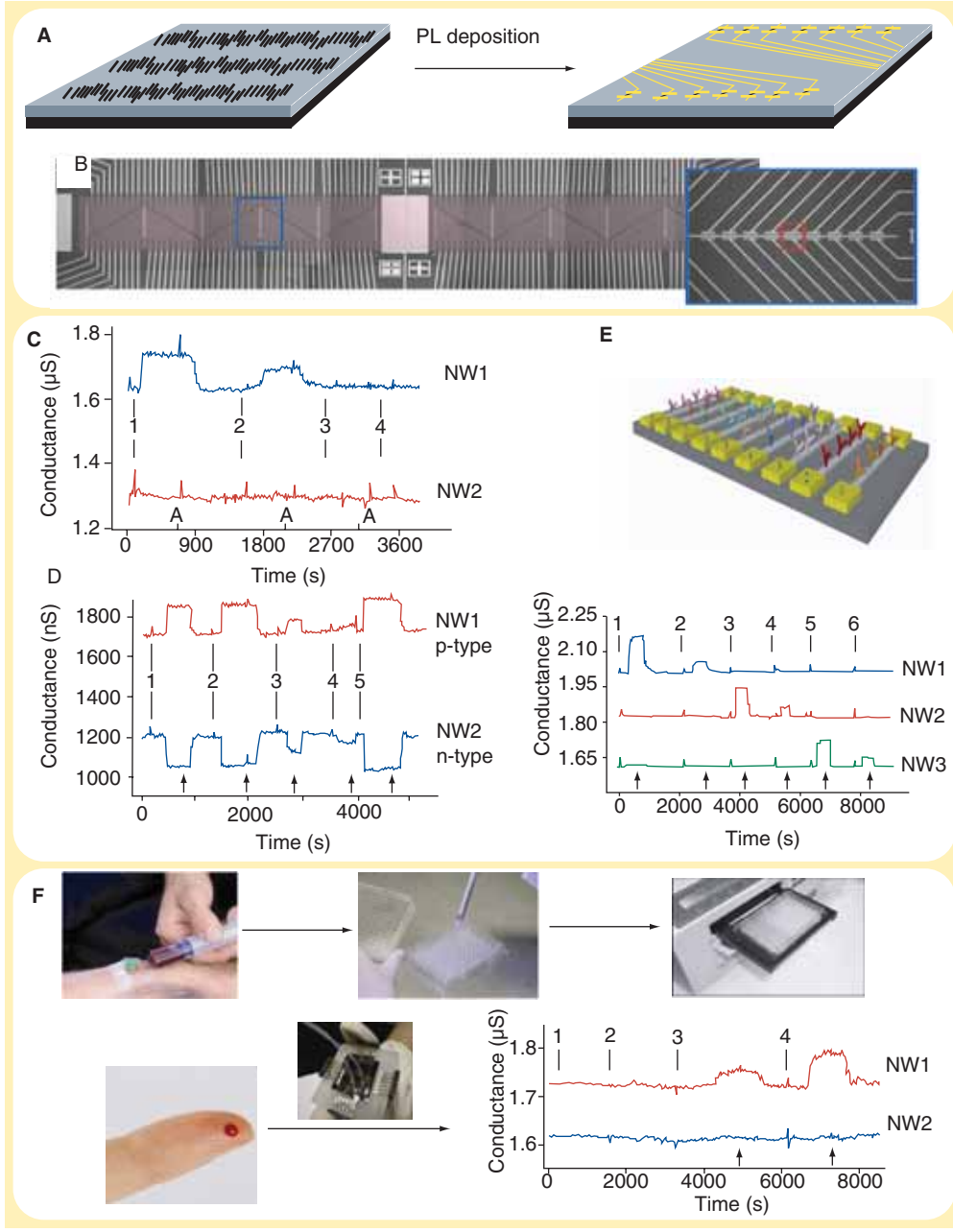
This conclusion is supported strongly by a number of control experiments [45], including the fact that no significant conductance increase was observed after the telomerase-binding step in the absence of dNTPs (Figure 4E, point 4). Significantly, the authors' telomerase activity measurements are distinct and advantageous compared with current approaches based on variations of telomeric repeat amplification protocol (TRAP) [63,64] since we do not require PCR amplification to achieve high sensitivity. In addition, the versatility of nanowire detectors and this telomerase assay can be recognized by the fact that telomerase inhibitors, which might serve as therapeutic agents, can be screened directly. This point was demonstrated clearly through investigations of the inhibition of telomerase elongation activity in the presence of azido thymidine triphosphate (AZTTP), a known reverse transcriptase inhibitor [65], as shown in Figure 4F [45]. Taken together, these studies have shown clearly the sensitivity and selectivity of nanowire sensors for telomerase detection and activity from common biological cell samples, and thus suggest substantial promise both as a novel diagnostic tool and as a method for screening for potential drugs.

Multiplexed real-time, label-free detection of proteins

The first example of electrical detection of proteins in solution using nanostructures was reported by the authors' group using p-type silicon nanowire devices in 2001 [40]. In these studies, biotin, which binds with high selectivity to the protein streptavidin, was linked to the oxide surface of the nanowires and used as a binding receptor. When solutions of streptavidin protein were delivered to nanowire sensor devices modified with biotin receptors, the authors found that the conductance increased rapidly to a constant value and that this conductance value was stable after the addition of pure buffer solution [40]. These results were consistent with the net negative charge on streptavidin at the pH of these experiments (i.e., causing accumulation of carriers in the p-silicon nanowires) and the very small dissociation rate of the streptavidin–biotin system [66], respectively. This initial work provided clear indication that this approach could lead to sensor devices with unique value for nanomedicine.

More recently, the authors developed the use of nanowire devices for the detection of multiple disease marker proteins simultaneously in a single, versatile detection platform [45]. Electrically addressable arrays are fabricated by a process that uses fluid-based assembly of nanowires to align and set the average spacing of nanowires over large areas [5,13,67,68], and then photolithography and metal deposition to define interconnects to a large number of individual nanowires in parallel (Figure 5A). A key feature of this approach is that the metal electrodes defined by conventional lithography do not need to be registered to individual nanowires in an array to achieve a high yield of devices; only the position of the electrodes relative to a group of aligned nanowires needs to be fixed. A state-of-the-art nanowire sensor array fabricated in this way and containing more than 100 independently addressable elements is shown in Figure 5B. In this array, the active nanowire sensor devices are confined to a central rectangular area on the device chip that

Figure 5. Nanowire arrays for multiplexed protein sensing.



(A) Illustration of the nanowire array fabrication. **(B)** Optical image of a portion of a nanowire array, where the inset shows one row of individually addressable elements and the red box highlights a single device. **(C)** Data recorded simultaneously from two p-silicon nanowire devices, where NW1 was functionalized with prostate-specific antigen (PSA) Ab1 and NW2 was modified with ethanolamine. Vertical lines correspond to times when solutions of (1) 9 pg/ml PSA, (2) 1 pg/ml PSA, (3) 10 μg/ml BSA or (4) a mixture of 1 ng/ml PSA and 10 μg/ml PSA Ab1 were added. Black arrows correspond to the points where the solution was switched buffer. **(D)** Complementary sensing of PSA using p-type (NW1) and n-type (NW2) nanowire devices. Vertical lines correspond to addition of PSA solutions of (1,2) 0.9 ng/ml, (3) 9 pg/ml, (4) 0.9 pg/ml and (5) 5 ng/ml. **(E)** Schematic of array detection of multiple proteins. Simultaneous detection of PSA, carcinoembryonic antigen (CEA) and mucin-1 using NW1, NW2 and NW3 functionalized with antibodies for PSA, CEA and mucin-1, respectively. Protein solutions of (1,2) PSA, (3,4) CEA and (5,6) mucin-1 were delivered sequentially to the array. **(F)** Photographs of blood samples from finger and arm. Conductance-versus-time data recorded for the detection of PSA in donkey serum samples with (1) buffer, (2) serum or (3,4) serum and PSA. NW1 was functionalized with PSA Ab1 and NW2 was passivated with ethanolamine.

overlaps with the microfluidic sample delivery channel. Critical to the success of any integrated nanoelectronic array is the reproducibility of the device elements within the array and, significantly, measurements made on nanowire FET arrays have demonstrated very reproducible, high-performance properties [13,45,68].

Device arrays prepared in this way offer unique opportunities for label-free multiplexed detection of biological species and protein markers in particular. An important result of ongoing genomics and proteomics research is the elucidation of many new biomarkers that have potential for greatly improving the diagnosis of diseases [69]. The availability of multiple biomarkers is believed to be especially important in the diagnosis of complex diseases, such as cancer [70], where disease heterogeneity makes single marker tests, such as the analysis of prostate-specific antigen (PSA), inadequate. The analysis of a pattern of multiple cancer markers might, however, provide the information necessary for robust diagnosis of any person within a population [71] and, moreover, detection of markers associated with different stages of disease pathogenesis could further facilitate early detection, which is especially important for successful cancer treatment.

The development of silicon nanowire sensor arrays for cancer protein marker detection has been carried out by attaching monoclonal antibodies to the nanowire elements following device fabrication. The linkage chemistry is similar to that described previously for protein microarrays [72,73] and silicon nanowire sensors for viruses and small molecules [43,44], and involves three key steps. First, aldehyde propyltrimethoxysilane (APTMS) is coupled to oxygen plasma-cleaned silicon nanowire surfaces in order to present terminal aldehyde groups at the nanowire surface. Second, the aldehyde groups are coupled to the monoclonal antibodies and, third, unreacted free aldehyde groups are blocked by reaction with ethanolamine. The basic array design enables incorporation of different types of addressable nanowires, for example, different receptor antibodies printed on elements in a nanowire device array to enable selective multiplexed protein detection. Sensitivity limits for cancer marker protein detection using this new generation of silicon nanowire device array was determined by measuring conductance changes as the solution concentration of PSA was varied, where the devices were modified with monoclonal antibodies for PSA (PSA-Ab1). Figure 5C (blue curve) shows a well defined conductance increase and subsequent return to baseline when PSA solution (Figure 5C points 1 and 2) and pure buffer, respectively, are delivered alternately through the microfluidic channel to the devices. Notably, these data show that direct label-free detection of PSA is achieved routinely with signal to noise of more than 3 for concentrations down to 75 fg/ml or approximately 2 fM [45].

In addition, the authors investigated details of the modification chemistry to define limits for high-sensitivity detection of cancer marker proteins using these silicon nanowire field-effect devices. Specifically, atomic force microscopy measurements of the initial aldehyde-silane layer thickness on single nanowires demonstrate a systematic thickness increase with modification time. This thickness increase is consistent with previous studies showing that similar silane reagents can form multilayers [45,74,75]. Significantly, measurements of the nanowire device sensitivity show that the sensor response decreases rapidly for initial reaction times of more than 30 min. The observed decrease in sensitivity is consistent with expectations for a field-effect sensing device and, moreover, shows that the surface modification chemistry must be controlled in order to achieve reproducible high-sensitivity devices [45].

The reproducibility and selectivity of the nanowire devices was further demonstrated through to competitive binding experiments (Figure 5C, points 3 and 4) that showed no conductance changes following delivery of concentrated bovine serum albumin (BSA) solutions or PSA-Ab1 pre-blocked PSA solutions. In addition, a second control nanowire element, which was a p-type silicon nanowire device passivated with ethanolamine, was monitored in parallel (NW2, Figure 5C). Significantly, simultaneous measurements of the conductance of NW1 and NW2 show that well defined concentration-dependent conductance increases are only observed in NW1 on delivery of PSA solutions with no response observed for NW2. This simple implementation of multiplexing represents one highly robust means for discriminating against false positive signals arising from either electronic noise or nonspecific binding and represents a powerful advantage of this nanotechnology [45].

The unique multiplexing capabilities of nanowire device arrays have been exploited in several distinct ways. First, distinct p- and n-type nanowire device elements were incorporated in a

single sensor chip and data recorded simultaneously from the p-type nanowire (NW1, Figure 5D) and n-type nanowire (NW2, Figure 5D) showed a conductance increase and decrease, respectively, when PSA solution was delivered and subsequent return to baseline following introduction of buffer. The complementary electrical signals provide a simple yet robust means for detecting false positive signals from either electrical noise or nonspecific binding of protein; that is, real and selective binding events must show complementary responses in the p- and n-type devices. The presence of correlated conductance signals in both devices (Figure 5D), which occur at points when buffer and PSA/buffer solutions are changed, illustrates clearly how this multiplexing capability can be used to distinguish noise from disease marker protein-binding signals [45].

More generally, multiplexed detection of distinct disease marker proteins, which will facilitate pattern analysis of existing and emerging markers for robust diagnosis [71], can be carried out with high sensitivity and selectivity using nanowire arrays modified with distinct antibody receptors, as shown in Figure 5E. This critical capability was demonstrated in reported studies of PSA, carcinoembryonic antigen (CEA) and mucin-1 detection using silicon nanowire devices functionalized with monoclonal antibody receptors for PSA (NW1), CEA (NW2), and mucin-1 (NW3) [45]. Conductance measurements recorded simultaneously from NW1, NW2 and NW3 as different protein solutions were sequentially delivered to the device array (Figure 5E) demonstrated clearly multiplexed real-time, label-free marker protein detection with sensitivity to the femtomolar level and essentially 100% selectivity.

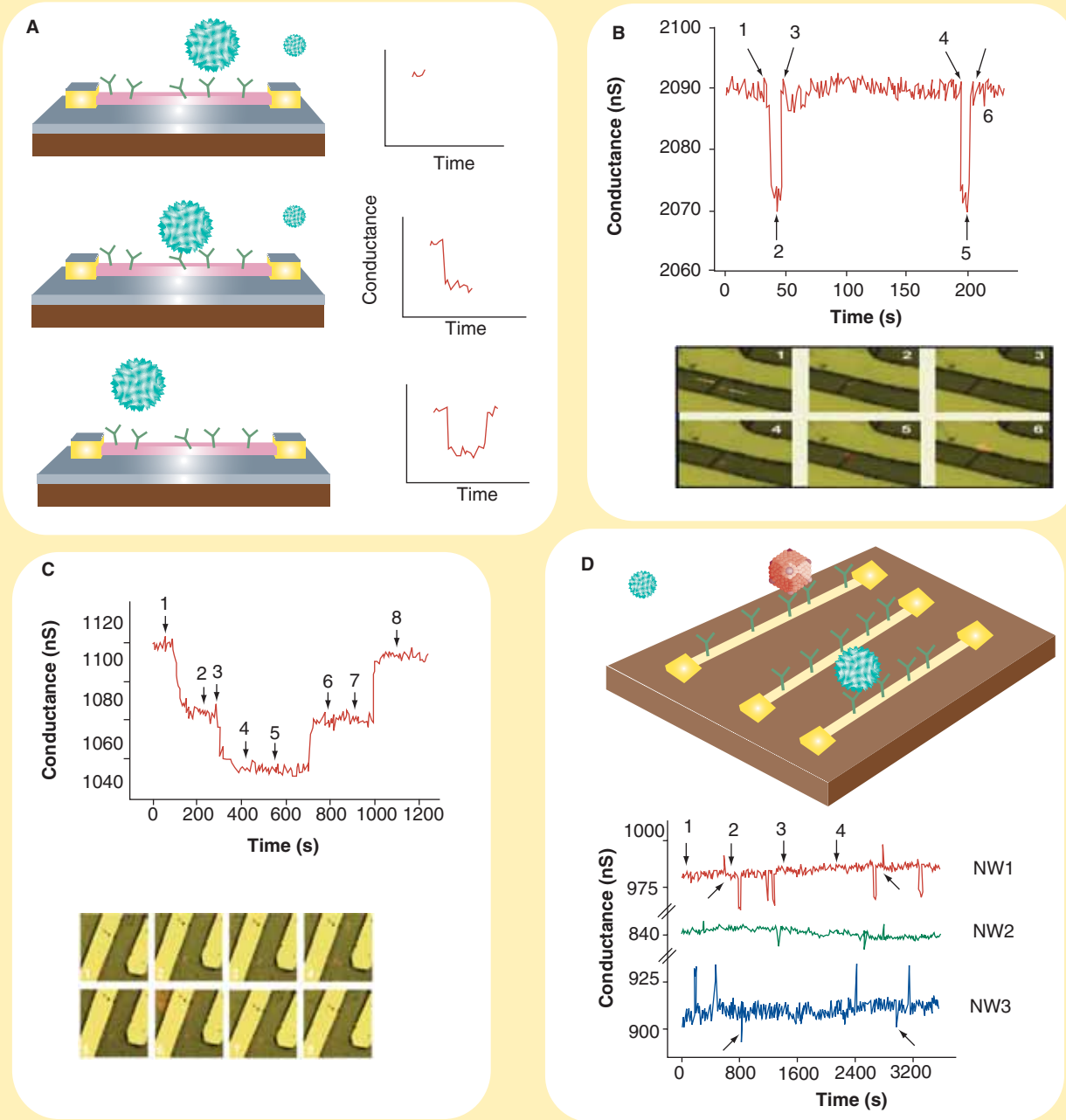
Effective cancer diagnosis with a new technology will require rapid analysis of clinically relevant samples, such as blood serum. A unique feature of our nanotechnology is that analysis of one or many markers can be achieved on literally a drop of blood, much like a simple glucose test, in contrast to standard analysis serum analysis today requiring milliliters of blood and extensive laboratory work-up, as illustrated in Figure 5F. The authors demonstrated this key advance in nanomedicine using the nanowire arrays to detect PSA in undiluted serum samples that were desalted in a rapid and simple purification step [45]. Notably, conductance versus time data recorded simultaneously from NW1, which was modified with PSA-Ab1 receptor, and NW2, which was passivated with ethanolamine, show that donkey serum containing 59 mg/ml total protein did not lead to an appreciable conductance change relative to the standard assay buffer, while serum containing PSA led to concentration-dependent conductance increases only for NW1 (Figure 5F). Well defined conductance changes were observed for PSA concentrations as low as 0.9 pg/ml, which corresponds to a concentration of approximately 100-billion times lower than that of the background serum proteins. This sensitivity limit is approximately an order of magnitude better than a recent paper reporting the detection of PSA in human plasma by surface plasmon fluorescence spectroscopy [76], where labeling of the 'sandwich' immunoassay was required and represents an additional step costing time and money beyond that of the authors' approach. These results demonstrate unambiguously that nanowire sensor arrays can be used to detect multiple cancer markers rapidly with high sensitivity and selectivity in undiluted human serum and, moreover, it should be noted that the detection can be carried out on as little as a drop of blood versus the several milliliters for current laboratory analysis – a clear advantage of this nanotechnology and a potential breakthrough for the field of nanomedicine.

Pushing sensitivity limits: detection of single viruses

The studies reviewed here demonstrate some of the exciting capabilities of nanowire sensors relevant to medicine. While these studies implicitly show exquisite sensitivity, indeed unmatched by existing label-free sensor devices, they do not define the ultimate sensitivity of the nanowire FET devices. To address this critical issue, the authors' group recently carried out studies on the detection of viruses [44], which are among the most important causes of human disease [77] and an increasing concern as agents for biological warfare and terrorism [78], with the goal of determining whether the ultimate limit of one single entity could be detected reliably.

The underlying concept of these experiments is illustrated schematically in Figure 6A. When a virus particle binds to the antibody receptor on a nanowire device, the conductance of that device will change from the baseline value, and when the virus unbinds, the conductance will return to the baseline value. Significantly, delivery of highly dilute influenza A virus solutions, of the order of 80 attomolar (10^{-18} M) or 50 viruses/ml, to p-type silicon nanowire devices modified with monoclonal antibody for influenza A produced well defined, discrete conductance changes (Figure 6B) that are characteristic of

Figure 6. Detection of single viruses.



(A) Schematic of a single virus binding and unbinding to the surface of a silicon nanowire device modified with antibody receptors,and the corresponding time-dependent change in conductance. **(B)** Simultaneous conductance and optical data recorded for a silicon nanowire device modified with a low density of antibody receptor units after introduction of influenza A virus solution. **(C)** Simultaneous conductance and optical versus time data recorded from a single nanowire device with a high density of anti-influenza type A antibody. Influenza A solution was added before point 1 and the solution was switched to pure buffer between points 4 and 5 on the plot. **(D)** (top) Schematic of multiplexed single virus detection. (bottom) Conductance versus time data recorded simultaneously from three nanowire elements, where NW1 was modified with antibody for influenza A (red data), NW2 was modified with ethanolamine only (green data) and NW3 was modified with antibody for adenovirus (blue data). Black arrows 1–4 correspond to the introduction of adenovirus, influenza A, pure buffer and a 1:1 mixture of adenovirus and influenza A.

binding and unbinding of single positively charged influenza viruses [44]. Definitive proof that the discrete conductance changes observed in these studies were due to detection of single virus binding and then unbinding was obtained from simultaneous optical and electrical measurements using fluorescently labeled influenza viruses. The optical and electrical data in Figure 6B show that, as a virus diffuses near a nanowire device, the conductance remains at the baseline value and only after binding at the nanowire surface does the conductance drop in a quantized manner, similar to that observed with unlabeled viruses; as the virus unbinds and diffuses from the nanowire surface, the conductance returns rapidly to the baseline value. These parallel measurements also showed that a virus must be in contact with the nanowire device to yield an electrical response, thus suggesting that it will be possible to develop ultra-dense nanowire device arrays without crosstalk in the future, where the minimum size scale is simply set by that of the virus.

Executive summary

- Silicon nanowires (SiNWs) are readily synthesized with controlled diameters (2–20 nm) and doping properties (variable doping ratio and dopant type) to yield electronically-reproducible p- and n-type nanowires.
- SiNWs are used as building blocks for the fabrication of arrays of individually addressable nanoscale field-effect-transistor devices using standard microfabrication techniques.
- Chemical modification of SiNW device surfaces enables the development of ultra sensitive sensors for electrical, real-time and label-free sensing of chemical and biological species.
- SiNW devices are used for ultra-sensitive detection of DNA sequences down to femtomolar concentrations.
- SiNW devices are used to detect the binding of ATP to protein kinases and the inhibition of ATP binding by small molecules, a topic of great importance in drug discovery.
- Arrays of approximately 200 addressable nanowire-devices are readily prepared. These arrays are used for multiplexed simultaneous detection of multiple analytes, including proteins and viruses.
- SiNW large-scale arrays are applied in the highly-selective detection of multiple protein biomarkers for cancer detection, with detection limits down to 3 femtomolar for proteins of interest.
- SiNW arrays are used for the detection of protein biomarkers in serum samples after a simple sample desalting step, with discrimination of 100-billion to one versus background serum proteins.
- SiNW devices are used for highly-selective detection of single viral particles, and simultaneous detection of two distinct viruses at single virus level.

Selective detection, the ability to specifically distinguish one type of virus from another, is crucial for exploiting the high sensitivity of these nanowire devices in most medical and bio-threat applications. Selectivity was first investigated by characterizing how variations in the density of the influenza A antibody receptors affect the binding/unbinding properties. For example, simultaneous conductance and optical data recorded on devices with average antibody coverage 10-times higher than shown in Figure 6B (Figure 6C), show sequential binding of virus particles without unbinding on a 5- to 10-min time scale (vs unbinding on a 20-s time scale in Figure 6B). Slow sequential unbinding of the virus particles is observed after introducing pure buffer solution. These data show that the unbinding kinetics can be substantially slowed/controlled through increases/changes in the density of specific antibodies and provide strong evidence for selective binding of influenza A; that is, the unbinding kinetics should be slowed as the number of specific antibody–virus contact points increases.

More significantly, the authors have demonstrated clear selectivity in multiplexing experiments that could enable powerful advance in rapid medical diagnosis. Specifically, p-type silicon nanowire sensor elements in an array were modified with monoclonal antibody receptors specific for influenza A (NW1) and adenovirus (NW3) and, in addition, a control nanowire element (NW2), which was passivated with ethanolamine, was included. Simultaneous conductance measurements were obtained when adenovirus, influenza A and a mixture of both viruses were delivered to the device array (Figure 6D) and demonstrate several significant points. First, delivery of adenovirus, which is negatively charged at the pH of the experiment [44], to the device array yields positive conductance changes for NW3 with an on time similar to the selective binding/unbinding in single device experiments. Well defined binding/unbinding events are not observed from the nanowire device modified with the influenza virus receptor. Second, delivery of influenza A solutions yields negative conductance changes for NW1 similar to single device measurements of Figure 6B, while well defined binding/unbinding is not observed on NW3. In both cases, no evidence of binding/unbinding was found in the control element, NW2. Last, delivery of a mixture of both viruses demonstrates unambiguously that selective

binding/unbinding responses for adenovirus and influenza A can be detected in parallel by NW3 and NW1, respectively, at the single virus level and, moreover, combined with the control NW2 enables highly robust assignment of positive signals at this ultimate level of detection. Significantly, the simplicity, single viral particle sensitivity and capability of selective multiplexed detection shows that nanowire sensors could serve as the key element in powerful viral sensing devices for medical and bioterrorism applications in the future.

Conclusions

In this review, we have illustrated how nanowire-based field-effect sensor devices and device arrays modified with specific surface receptors represent a powerful nanotechnology-enabled diagnostic/detection platform for medicine and the life sciences. These nanowire sensor devices have a number of key features, including direct, label-free and real-time electrical signal transduction, ultra-high sensitivity, exquisite selectivity and potential for integration of addressable arrays on a massive scale, which sets them apart from other sensor technologies available today. The examples described here illustrate unique capabilities for multiplexed real-time detection of proteins, single viruses, DNA, DNA enzymatic processes and small organic molecule-binding to proteins. These examples show clearly the potential to impact significantly on disease diagnosis, genetic screening and drug discovery, as well as serve as powerful new tools for research in many areas of biology.

Future perspective

In the near future, we argue that these advances could be developed at the commercial level in simple nanowire sensor devices that would represent a clear application of nanotechnology and, more importantly, a substantial benefit to humankind. Looking to the longer term, we believe that the future is exciting from both science and technology perspectives. For example, we believe that advances in capabilities of assembling larger and more complex nanowire sensor arrays and integrating them with first conventional and later nanoscale electronics for processing will lead to exquisitely powerful sensor systems that help to enable the dream of personalized medicine. Moreover, recognizing the fact that these nanowire sensors transduce chemical/biological-binding events into electronic/digital signals suggests the potential for a highly sophisticated interface between nanoelectronic and biological information processing systems in the future.

Acknowledgments

The authors graciously thank the scientists cited in this review for their contributions to the research. Charles Lieber acknowledges generous support from the Defense Advanced Research Projects Agency, National Cancer Institute, Applied Biosystems and the Ellison Medical Foundation.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Poole CP: *Introduction to Nanotechnology.* John Wiley & Sons, Hoboken, New Jersey, USA (2003).
2. Roco MC, Williams RS, Alivisatos P: Biological, medical and health applications. In: *Nanotechnology Research Directions.* Kluwer Academic Publishers, Boston, USA (2000).
3. Morales AM, Lieber CM: A laser ablation method for the synthesis of crystalline semiconductor nanowires. *Science* 279, 208–211 (1998).
4. Hu JT, Odom TW, Lieber CM: Chemistry and physics in one dimension: synthesis and properties of nanowires and nanotubes. *Acc. Chem. Res.* 32, 435–445 (1999).
5. Lieber CM: Nanoscale science and technology: building a big future from small things. *MRS Bull.* 28(7), 486–491 (2003).
6. Wang ZL: Nanostructures of zinc oxide. *Mater. Today* 7(6), 26–33 (2004).
7. Patolsky F, Lieber CM: Nanowire nanosensors. *Mater. Today* 8, 20–28 (2005).
8. Duan XF, Huang Y, Cui Y, Wang JF, Lieber CM: Indium phosphide nanowires as building blocks for nanoscale electronic and optoelectronic devices. *Nature* 409, 66–69 (2001).
9. Cui Y, Lieber CM: Functional nanoscale electronic devices assembled using silicon nanowire building blocks. *Science* 291, 851–853 (2001).
10. Gudiksen MS, Lauhon LJ, Wang J, Smith DC, Lieber CM: Growth of nanowire superlattice structures for nanoscale photonics and electronics. *Nature* 415, 617–620 (2002).
11. Cui Y, Zhong ZH, Wang DL, Wang WU, Lieber CM: High performance silicon nanowire field effect transistors. *Nano Lett.* 3, 149–152 (2003).
12. Thelander C, Martensson T, Bjork MT *et al.*: Single-electron transistors in heterostructure nanowires. *Appl. Phys. Lett.* 83, 2052–2054 (2003).
13. Jin S, Whang DM, McAlpine MC, Friedman RS, Wu Y, Lieber CM: Scalable interconnection and integration of nanowire devices without registration. *Nano Lett.* 4, 915–919 (2004).
• First demonstration of large scale organization, fabrication and properties of reproducible nanowire device arrays.
14. Wu Y, Xiang J, Yang C, Lu W, Lieber CM: Single-crystal metallic nanowires and metal/semiconductor nanowire heterostructures. *Nature* 430, 61–65 (2004).
15. Zheng GF, Lu W, Jin S, Lieber CM: Synthesis and fabrication of high-performance n-type silicon nanowire transistors. *Adv. Mater.* 16, 1890–1894 (2004).

16. Chen RJ, Bangsaruntip S, Drouvalakis KA *et al.*: Noncovalent functionalization of carbon nanotubes for highly specific electronic biosensors. *Proc. Natl. Acad. Sci. USA* 100, 4984–4989 (2003).
17. Chen RJ, Choi HC, Bangsaruntip S *et al.*: An investigation of the mechanisms of electronic sensing of protein adsorption on carbon nanotube devices. *J. Am. Chem. Soc.* 126, 1563–1568 (2004).
18. Tyagi S, Kramer FR: Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* 14, 303–308 (1996).
19. Taton TA, Lu G, Mirkin, CA: Two-color labeling of oligonucleotides array via size-selective scattering of nanoparticle probes. *J. Am. Chem. Soc.* 123, 5164–5165 (2001).
• Excellent example of the use of metal nanoparticles for the detection of biological species.
20. Jaffer FA, Weissleder R: Seeing within – molecular imaging of the cardiovascular system. *Circ. Res.* 94, 433–445 (2004).
21. Perez JM, Josephson L, Weissleder R: Use of magnetic nanoparticles as nanosensors to probe for molecular interactions. *Chem. Bio. Chem.* 5, 261–264 (2004).
22. Saleh A, Schroeter M, Jinkmanns C, Hartung HP, Modder U, Jander S: *In vivo* MRI of brain inflammation in human ischaemic stroke. *Brain* 127, 1670–1677 (2004).
23. Dousset V, Ballarino L, Delalande C *et al.*: Comparison of ultrasmall particles of iron oxide (USIOP) –enhanced T2-weighted, conventional T2-weighted, and gadolinium enhanced T1-weighted MR images in rats with experimental autoimmune encephalomyelitis. *Am. J. Neuroradiol.* 20, 223–227 (1999).
24. Harisinghani MG, Barentsz J, Hahn PF *et al.*: Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. *N. Engl. J. Med.* 348, 2491–2499 (2003).
25. Dardzinski BJ, Schmithorst VJ, Holland SK *et al.*: MR imaging of murine arthritis using ultrasmall superparamagnetic iron oxide particles. *Magn. Res. Imaging* 19, 1209–1216 (2001).
26. Ruehm SG, Corot C, Vogt P, Kolb S, Debatin JF: Magnetic resonance imaging of atherosclerotic plaque with ultrasmall superparamagnetic particles of iron oxide in hyperlipidemic rabbits. *Circulation* 103, 415–422 (2001).
27. Kooi ME, Cappendijk VC, Cleutjens KBJM *et al.*: Accumulation of ultrasmall superparamagnetic particles of iron oxide in human atherosclerotic plaques can be detected by *in vivo* magnetic resonance imaging. *Circulation* 107, 2453–2458 (2003).
28. Watson A, Wu X, Bruchez M: Lighting up cells with quantum dots. *Biotechniques* 34, 296–303 (2003).
29. Chan WC, Maxwell DJ, Gao X, Bailey RE, Han M, Nie S: Luminescent quantum dots for multiplexed biological detection and imaging. *Curr. Opin. Biotechnol.* 13, 40–46 (2002).
30. Jaiswal JK, Mattoussi H, Mauro JM, Simon SM: Long-term multiple color imaging of live cells using quantum dot bioconjugates. *Nat. Biotechnol.* 21, 47–51 (2003).
31. Alivisatos P: The use of nanocrystals in biological detection. *Nat. Biotechnol.* 22, 47–52 (2004).
• Provides very good overview of the use of nanostructures for detection and sensing i n biology.
32. Larson DR, Zipfel WR, Williams RM *et al.*: Watersoluble quantum dots for multiphoton fluorescence imaging *in vivo*. *Science* 300, 1434–1436 (2003).
33. Dubertert B, Skourides P, Norris DJ, Noireaux V, Brivanlou AH, Libchaber A: *In vivo* imaging of quantum dots encapsulated in phospholipid micelles. *Science* 298, 1759–1762 (2002).
34. Ballou B, Lagerholm BC, Ernst LA, Bruchez MP, Waggoner AS: Noninvasive imaging of quantum dots in mice. *Bioconjugate Chem.* 15, 79–86 (2004).
35. Gao X, Cui Y, Levenson RM, Chung LWK, Nie S: *In vivo* cancer targeting and imaging with semiconductor quantum dots. *Nat. Biotechnol.* 22, 969–976 (2004).
• Important example demonstrating the power of quantum dots for *in vivo* targeting and imaging.
36. Akerman ME, Chan WCW, Laakkonen P, Bhatia SN, Ruoslahti E: Nanocrystal targeting *in vivo*. *Proc. Natl. Acad. Sci. USA* 99, 12617–12621 (2002).
37. Voura EB, Jaiswal JK, Mattoussi H, Simon SM: Tracking metastatic tumor cell extravasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy. *Nat. Med.* 10, 993–998 (2004).
38. Dahan M, Levi S, Luccardini C, Rostaing P, Riveau B, Triller A: Diffusion dynamics of glycine receptors revealed by single-quantum dot tracking. *Science* 302, 442–445 (2003).
39. Wu X, Liu H, Liu J *et al.*: Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. *Nat. Biotechnol.* 21, 41–46 (2003).
40. Cui Y, Wei Q, Park H, Lieber CM: Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species. *Science* 293, 1289–1292 (2001).
•• First demonstration of label-free electronic detection of biological species in solution using nanowire elements.
41. Comini E, Faglia G, Sberveglieri G, Pan ZW, Wang ZL: Stable and highly sensitive gas sensors based on semiconducting oxide nanobelts. *Appl. Phys. Lett.* 81, 1869–1871 (2002).
42. Hahm J, Lieber CM: Direct ultrasensitive electrical detection of DNA and DNA sequence variations using nanowire nanosensors. *Nano Lett.* 4, 51–54 (2004).
43. Wang WU, Chen C, Lin KH, Fang, Y, Lieber CM: Label-free detection of small-molecule-protein interactions by using nanowire nanosensors. *Proc. Natl. Acad. Sci. USA* 102, 3208–3212 (2005).
44. Patolsky F, Zheng GF, Hayden O, Lakadamyali M, Zhuang XW, Lieber CM: Electrical detection of single viruses. *Proc. Natl. Acad. Sci. USA* 101, 14017–14022 (2004).
•• First demonstration of single viral particle detection using nanowire devices.
45. Zheng GF, Patolsky F, Cui Y, Wang WU, Lieber CM: Multiplexed electrical detection of cancer markers with nanowire sensor arrays. *Nat. Biotechnol.* 23, 1294–1301 (2005).
•• Shows selective detection of multiple cancer marker proteins, down to femtomolar concentrations, and direct telomerase activity and inhibition assays.
46. Sze SM: *Physics of Semicondutor Devices.* Wiley, New York, USA, 431 (1981).
47. Bergveld P: Development, operation, and application of ion-sensitive field-effect transistor as a tool for electrophysiology. *IEEE Trans. Biomed. Eng.* BME-19, 342 (1972).
• Good introduction to electronic sensing using semiconductor microelectronic devices.
48. Blackburn GF: In: *Biosensors: Fundamentals and Applications.* Turner APF, Karube I, Wilson GS (Eds), Oxford University Press, Oxford, UK, 481 (1987).
49. Wu Y, Cui Y, Huynh L, Barrelet CJ, Bell DC, Lieber CM: Controlled growth and structures of molecular-scale silicon nanowires. *Nano Lett.* 4, 433–436 (2004).
50. Ma DDD, Lee CS, Au FCK, Tong SY, Lee ST: Small-diameter silicon nanowire surface. *Science* 299, 1874–1877 (2003).

51. Li C, Curreli M, Lin H *et al.*: Complementary detection of prostate-specific antigen using In_2O_3 nanowires and carbon nanotubes. *J. Am. Chem. Soc.* 127, 12484–12485 (2005).
52. Bartlett PN: In: *Handbook of Chemical and Biological Sensors.* Taylor RF, Schultz JS (Eds), IOP Publishing, Philadelphia, USA, 139 (1996).
53. Strausberg RL, Schreiber SL: From knowing to controlling: a path from genomics to drugs using small molecule probes. *Science* 300, 294–295 (2003).
54. Becker J: Signal transduction inhibitors – a work in progress. *Nat. Biotechnol.* 22, 15–18 (2004).
55. Peck SC: Analysis of protein phosphorylation: methods and strategies for studying kinases and substrates. *Plant J.* 45, 512–522 (2006).
56. Tagliati F, Bottoni A, Bosetti A, Zatelli MC, Uberti ECD: Utilization of luminescent technology to develop a kinase assay: Cdk4 as a model system. *J. Pharmaceut. Biomed.* 39, 811–814 (2005).
57. Li Z, Chen Y, Li X, Kamin TI, Nauka K, Williams RS: Sequence-specific label-free DNA sensors based on silicon nanowires. *Nano Lett.* 4, 245–247 (2004).
58. Jensen KK, Orum H, Nielsen PE, Norden B: Kinetics for hybridization of peptide nucleic acids (PNA) with DNA and RNA studied with the BIAcore technique. *Biochemistry* 36, 5072–5077 (1997).
59. He L, Musick MD, Nicewarner SR *et al.*: Colloidal Au-enhanced surface plasmon resonance for ultrasensitive detection of DNA hybridization. *J. Am. Chem. Soc.* 122, 9071–9077 (2000).
60. Hook F, Ray A, Norden B, Kasemo B: Characterization of PNA and DNA immobilization and subsequent hybridization with DNA using acoustic-shear-wave attenuation measurements. *Langmuir* 17, 8305–8312 (2001).
61. Moyzis RK, Buckingham JM, Cram LS *et al.*: A highly conserved repetitive DNA-sequence, (TTAGGG)N, present at the telomeres of human-chromosomes. *Proc. Natl. Acad. Sci. USA* 85, 6622–6626 (1988).
62. Kim NW, Piatyszek MA, Prowse KR: Specific association of human telomerase activity with immortal cells and cancer. *Science* 266, 2011–2015 (1994).
63. Szatmari I, Tokes S, Dunn CB, Bardos TJ, Aradi J: Modified telomeric repeat amplification protocol: a quantitative radioactive assay for telomerase without using electrophoresis. *Anal. Biochem.* 282, 80–88 (2000).
64. Wege H, Chui MS, Le HT, Tran JM, Zern MA: SYBR Green real-time telomeric repeat amplification protocol for the rapid quantification of telomerase activity. *Nucleic Acids Res.* 31(2), e3 (2003).
65. Mitsuya H, Yarchoan R, Broder S: Molecular targets for AIDS therapy. *Science* 9, 1533–1544 (1990).
66. Wilchek M, Bayer EA: Biotin-binding proteins – overview and prospects. *Methods Enzymol.* 184, 49–51 (1990).
67. Huang Y, Duan XF, Wei QQ, Lieber CM: Directed assembly of one-dimensional nanostructures into functional networks. *Science* 291, 630–633 (2001).
68. Whang D, Jin S, Lieber CM: Large-scale hierarchical organization of nanowires for functional. *Jap. J. Appl. Phys.* 43, 4465–4470 (2004).
69. Etzioni R, Urban N, Ramsey S *et al.*: The case for early detection. *Nat. Rev. Cancer* 3, 1–10 (2003).
70. Wulfkuhle JD, Liotta LA, Petricoin EF *et al.*: Proteomic applications for the early detection of cancer. *Nat. Rev. Cancer* 3, 267–275 (2003).
71. Sidransky D: Emerging molecular markers of cancer. *Nat. Rev. Cancer* 2, 210–219 (2002).
72. MacBeath G, Schreiber SL: Printing proteins as microarrays for high-throughput function determination. *Science* 289, 1760–1763 (2000).
73. Arenkov P, Kukhtin A, Gemmell A, Voloshchuk S, Chupeeva V, Mirzabekov A: Protein microchips: use for immunoassay and enzymatic reactions. *Anal. Biochem.* 278, 123–131 (2000).
74. Pomerantz M, Segmuller A, Netzer L, Sagiv J: Coverage of Si substrates by self-assembling monolayers and multilayers as measured by IR, wettability and x-ray diffraction. *Thin Solid Films* 132, 153–162 (1985).
75. Heiney P A, Gruneberg K, Fang JY, Dulcey C, Shashidhar R: Structure and growth of chromophore-functionalized (3-aminopropyl)triethoxysilane self-assembled on silicon. *Langmuir* 16, 2651–2657 (2000).
76. Yu F, Persson B, Lofas S, Knoll W: Surface plasmon fluorescence immunoassay of free prostrate-specific antigen in human plama at the femtomolar level. *Anal. Chem.* 76, 6765–6770 (2004).
77. Stadler K, Masignani V, Eickmann M, Becker S: SARS – beginning to understand a new virus. *Nat. Rev. Microbiol.* 1, 209–218 (2003).
78. Atlas RM: Bioterrorism and biodefence research: changing the focus of microbiology. *Nat. Rev. Microbiol.* 1, 70–74 (2003).