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REVIEW PAPER

Applications, techniques, and microfluidic interfacing for nanoscale biosensing

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Abstract Biosensors based on nanotechnology are rapidly developing and are becoming widespread in the biomedical field and analytical chemistry. For these nanobiosensors to reach their potential, they must be integrated with appropriate packaging techniques, which are usually based on nano/microfluidics. In this review we provide a summary of the latest developments in nanobiosensors with a focus on label-based (fluorescence and nanoparticle) and label-free methods (surface plasmon resonance, micro/nanocantilever, nanowires, and nanopores). An overview on how these sensors interface with nano/microfluidics is then presented and the latest papers in the area summarized.

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1 Introduction

Nanotechnology-based sensing methods have developed rapidly and have been used to elucidate a wide spectrum of genetic and proteomic information with improved selectivity and sensitivity when compared to traditional methods. For example, nanotechnology-based, single-cell detection methods have been used to investigate how a cell responds to environmental changes, interacts with neighboring cells, and expresses specific genes as it responds—activities enabled by the small scale of the sensors and impossible with traditional techniques (Fu et al. 2007; Jaiswal et al. 2004). Nanotechnology methods for biomedical applications, with challenging requirements regarding dependability, cost, measurement precision, speed, and sample throughput, are likewise experiencing rapid development (Cheng et al. 2006; Kewal 2005b; Murphy 2006). By introducing advanced materials and devices, the nanotechnology-based platforms have the potential to achieve these requirements and will enable better biomolecule research tools, real-time environmental monitoring, and point-of-care diagnostic systems, but only if they are combined with appropriate fluid handling, molecular recognition elements, and reporter molecules, in some cases. Thus, the purpose of this review paper is to explore some of the recent developments in nanosensors along with their integration with these three supporting areas: molecular recognition elements, reporter elements, and microfluidics, to create novel nanotechnology-based sensing platforms, and to preview where this research area may be headed in the near future. But before

looking at the specific developments in this field, a brief overview of the field is helpful.

For the purposes of this review, nanoscale sensors have critical structural dimensions of less than 100 nm. Nanoscale transduction mechanisms are typically classified into two categories: label-based and label-free. Label-based transduction mechanisms typically rely on the presence of an added labeling molecule or structure. Traditional label-based methods typically rely on fluorescent dyes made up of organic or synthetic dye molecules or radioactive labels made up of unstable isotopes. More recently developed methods consist of discreet, inorganic nanoparticles that can be transduced using optics, magnetics, electronics, or other means. For example, gold nanoparticles (Fig. 1c) are now often used in DNA hybridization assays to detect a desired DNA sequence with improved sensitivity and specificity compared to traditional fluorescence assays. Quantum dots (Fig. 1b) are another important nanoscale element that is applicable to biosensing. Quantum dots can be excited using broadband radiation and yet provide high sensitivity and stability compared to fluorescence (Jamieson et al. 2007). To increase the efficiency of nanoparticle methods, magnetic nanoparticles have been developed and used for applications such as finding the telomerase activity in a biological sample (Fortina et al. 2007; Kewal 2005a). One of the potential challenges of label-based methods is the conformational change that may occur due to labeling. This may induce changes in the binding affinity as well as the function of the protein (Kewal 2003).

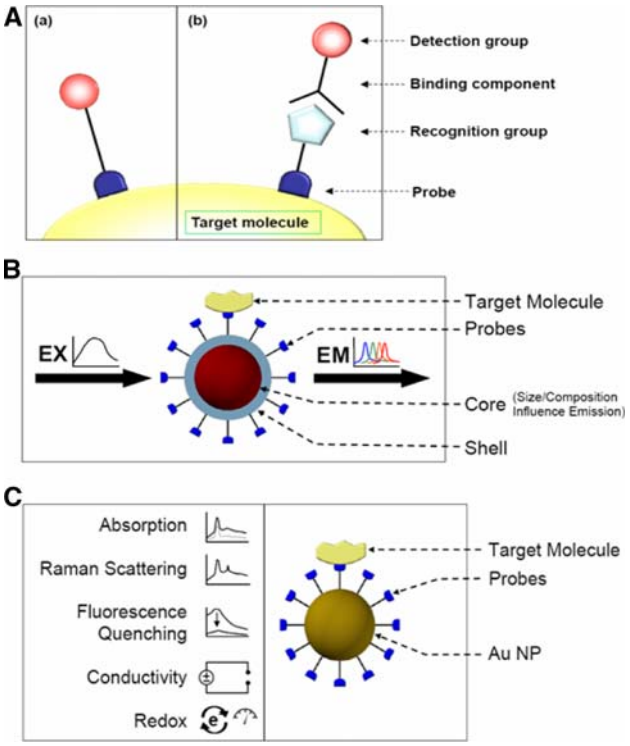

Fig. 1 **a** Schematic diagram of two approaches to label-based biomolecule detection. (a) Direct method. (b) Indirect method. **b** Schematic diagram of nano-shell (quantum dot) detection by attaching probe moledules to the nanoparticle surface. **c** Schematic diagram of using a gold nanoparticle as a label and possible detection methods

An advantage of many of the new nanoscale biosensors, such as micro-cantilevers, nanowires, and resonators, is that they are label-free (Englebienne et al. 2003; Gupta et al. 2006; Wang et al. 2005), which is the second category of nanosensors. For example, a cantilever-based system can be used to detect DNA hybridization by measuring the change in bending or resonant frequency of a cantilever as molecules bind to the cantilever. This method has the potential to serve as a platform for multiplexed biosensing (Cheng et al. 2006). Another label-free method is the use of nanowires that register a conductance change when a molecular target binds to the nanowires. Using a microfluidic chip integrated with capture-DNA coated nanowires, researchers have measured DNA hybridization kinetics (Nie et al. 2007). Another commonly used label-free method is surface plasmon resonance (SPR), which measures a change in the angle of optical reflection as surface binding occurs. This technique has been used to detect protein quantity in microarray locations (Yu et al. 2006) and can be used to detect single binding events for large molecules (Englebienne et al. 2003; Nguyena et al. 2007).

In addition to the basic transduction mechanisms, which are in this case typically nanoscale devices, molecular recognition, and packaging or the transducer interface are crucial elements to be considered in biosensor design (Turner 2000). Molecular recognition is typically achieved by the binding of targets with specific probes, such as antibodies, or complementary oligonucleotides. Recent developments in molecular design and in vitro screening technologies can provide new probe molecules, such as double-stranded DNA probes (Meserve et al. 2008; Wang et al. 2008), molecular aptamers (Osborne et al. 1997) and ribozymes (Ellington et al. 1997) for specific interactions with a variety of biomolecules. Packaging is focused on how the nanosensor is interfaced to the real world, which may include how the nanosensor receives samples, is protected from the outside world, and how the outside world is protected from the nanosensor. Many nano-biosensor concepts have been invented, but the commercial applications have been limited, primarily by limitations in packaging and interfacing. To maximize the value of nanotechnology-based biosensors, it is necessary to integrate these sensors with appropriate micro/nanofluidic systems that can deliver appropriate samples to the sensing platform. Micro/nanofluidic approaches provide one of the most promising strategies to interface nanoengineered biosensors in a wide spectrum of clinical and biomedical applications. Most biomedical samples are naturally in a liquid environment,

so the sensors must be combined with compelling fluid handling systems. Appropriate microfluidic delivery systems can be used to eliminate contamination, minimize analysis times, and enable portable systems.

In this article, we review the recent advancements of several nanotechnology-based biomolecule detection methods and assign them to one of the two following categories: label-based and label-free methods. Fluorescence, nanoparticles, quantum dots, and magnetic beads are introduced as label-based techniques, and methods such as micro-SPR, micro/nano-cantilevers, nanowires, and nanopores are discussed as label-free techniques. We then discuss how micro/nanofluidics is enabling these biosensing techniques to be implemented in a practical way. Finally, we discuss how these technologies need to be able to work together to produce useful biosensing systems.

2 Label-based methods

Biomolecules such as proteins and nucleic acids often cannot be recognized directly due to their small size. To track these biomolecules and their activity, probes for these target molecules or the target biomolecules themselves can be labeled by conjugation with a detectable agent, commonly a fluorophore or an enzyme. Labeling methods allow high sensitivity and these approaches are developed to the point that they give reproducible results. These agents for labeling proteins, nucleic acids, and other molecular probes are called tags. These tags have unique detectable properties such as radioactivity, chromogenicity, fluorescence, or magnetism. Additionally, electrical and electrochemical principles, based on the properties of labeled probes have been developed to establish corresponding detection methods through a target binding technique (Fig. 1). Having a uniquely detectable property, most tags can be functionalized to link to a specific molecular probe. In a related approach, instead of having a detectable group directly attached to a probe molecule, a recognition reagent having strong affinity for a secondary probe can be used to detect the target molecules. A variety of interaction pairs, such as biotin-avidin, hapten-antibody, and DNA–RNA hybrids, etc., are already in use (Kessler 1992). This two-stage detection scheme can be utilized when a primary-labeled probe is not available. A schematic diagram for both labeling approaches is shown in Fig. 1a. In the past, scientists mostly relied on radioactive probes to detect samples. However, safety and convenience concerns spurred the development of alternative techniques (Gary 1993). Among a range of options, current biomolecule detection methods have mainly employed fluorescent labels, quantum dots, or heavy atom complex nanoparticle labels. Chromogenic labels are also available, but they have been replaced with fluorescent labels which give larger quantum emission yield upon excitation resulting in better detectability. Bioluminescence, which generates detectable light as a result of biochemical reactions, is another popular technique for biodetection. Other detection methods are Au nanoparticle and magnetic nanoparticle labels. These techniques are emerging as better substitutes in terms of simplicity, sensitivity, specificity, and reliability for the current standards, e.g., PCR and ELISA.

2.1 Fluorescent labels

Fluorescent molecules contain fluorophores capable of being excited, via absorption of light energy at a specific wavelength, and subsequently emitting at a longer wavelength. Common organic fluorophores are derivatives of fluorescein, rhodamine, coumarin, and cyanine. Despite their considerable advantages in biomolecular imaging, there are some limitations, such as photobleaching (Benchaib et al. 1996), pH-sensitivity (Nakamura et al. 1991), and loss of fluorescence when they are conjugated to biomolecules (Valdes-Aguilera and Neckers 1989). It should be noted that the pH-sensitive character is problematic for general quantitative analysis but very useful as a pH indicator (Briggs et al. 2000; Jayaraman et al. 2001) or for preparing pH-sensitive conjugates (Watkins et al. 1992). Great efforts have been made to create better fluorophores that overcome the aforementioned drawbacks along with the development of highly sensitive fluorescence detection techniques (Enderlein et al. 1999; Haustein and Schwille 2007). Some examples are Alexa Fluors (Panchuk-Voloshina et al. 1999) and DyLight Fluors (Sun et al. 2007), newer generation fluorophores, which are synthesized by sulfonating common fluorophores (such as fluorescein). Increased fluorescence has been achieved from fluorophore-protein-coated fractal-like silver, which has been termed “metal-enhanced fluorescence” (Geddes et al. 2003). Combined with nanotechnology, fluorescent core-shell nanoparticle labels offer favorable characteristics, because many dye molecules are encapsulated in nano-sized particles that also shield them from photobleaching (Hun and Zhang 2007; Karst 2006). Very recently, a novel biarsenical derivative of fluorescein has been specially designed to investigate protein-protein interactions via “label transfer” chemistry, in which a tag is transferred from one protein to partner proteins(Liu et al. 2007). Nanodiamonds can also serve as fluorescent labels in biosensing (Fu et al. 2007; Yu et al. 2005). Details of fluorescent labels and their biological applications have been reviewed extensively (Albani 2007; Mason 1999) and the conjugation protocol for producing fluorescently labeled molecules has been reviewed as well (Hermanson 1996).

2.2 Quantum dots

Quantum dots (QDs) have provided a great breakthrough in many biological labeling applications (Alivisatos 2004; Bruchez et al. 1998; Chan and Nie 1998; Jamieson et al. 2007; Larson et al. 2003; Tansil and Gao 2006; Wang et al. 2006b; Zhang et al. 2005) with their typical core size of 1–10 nm and outstanding fluorescence compared to typical organic fluorophores (Fig. 1b). QD fluorescence comes about as a result of quantum effects from the three-dimensional spatial confinement of the QD's core semiconductor atoms. This confinement limits the core atoms' electron excitation states with the end result being that discrete fluorescence emission is produced with broad excitation. This unique type of fluorescence produces a fluorophore that is bright, photostable, has sharp fluorescence peak, and has colors that are controllable by varying size and composition of the core atoms. These properties have in turn enabled several novel uses of quantum dots in bio-detection methods. Among those uses is multicolor bio-labeling (Chan et al. 2002; Ho et al. 2005; Jaiswal et al. 2003), achieved by simultaneous use of different quantum dots as well as the use of different QD color and intensity combinations. Water-soluble quantum dots (Larson et al. 2003) have also been developed to improve biocompatibility allowing long-term multicolor imaging of live cells and fluorescence correlation spectroscopy (Yeh et al. 2006a, b; Zhang et al. 2005). Encapsulation within polymeric or lipid based layers (Dubertret et al. 2002) and coating with a short chain of peptides (Jaiswal et al. 2004) has also helped to disguise QDs as similar sized biomolecules like proteins or nucleic acids. QDs have also been used in combination with fluorescence energy transfer (FRET) for such applications as signal amplification during DNA sensing and detection of molecular orientation, size and binding (Ho et al. 2006; Medintz et al. 2003; Medintz et al. 2004; Zhang et al. 2005).

2.3 Au (Gold) nanoparticles

Since unique optical properties for an aggregate of Au nanoparticles and oligonucleotides were reported in 1996 (Mirkin et al. 1996), Au nanoparticles have attracted the attention of many researchers, which has led to Au nanoparticles becoming one of the most commonly used nanoscale materials in molecular diagnostics (Daniel and Astruc 2004; Tansil and Gao 2006). Their versatility has been proven with a variety of detection approaches, such as optical absorption (Storhoff et al. 1998), fluorescence (Maxwell et al. 2002), Raman scattering (Mulvaney et al. 2003; Vo-Dinh et al. 2005), electrical conductivity (Park et al. 2002), and an electrochemical redox property (Ozsoz et al. 2003). Moreover, Au nanoparticles generate highly efficient multi-photon absorption induced luminescence without significant blinking, suggesting they are viable alternatives to fluorophores or semiconductor nanoparticles for biological labeling (Farrer et al. 2005). Other advantages are easy preparation and conjugation to biomolecules, very low toxicity, and operation at a safe wavelength of the laser light used to visualize the particles (Kewal 2005b). To amplify signals and improve sensitivity, catalytic deposition of Ag on Au nanoparticle labels has been performed extensively for the various detection methods (Cai et al. 2002; Nam et al. 2004; Park et al. 2002). The Ag-enhancement scheme has been utilized in the recent bio-barcode approach (Cheng et al. 2006) and provided the lowest detection limit to date for both DNA (500zM, PCRless) (Nam et al. 2004)and protein targets (3–30 aM) (Nam et al. 2003). The bio-barcode assay utilizes oligonucleotide-modified Au nanoparticles for signal amplification and magnetic microparticles, described in the next section, for quick separation of the reacted elements. The use of the Au nanoparticles can also likely be expanded further with developments in chemistry allowing additional shape (Perez-Juste et al. 2005) and functionality control (Huo and Worden, 2007).

2.4 Magnetic nanoparticles

Magnetic particles, which respond to an external magnetic field, have been used extensively for separation and preconcentration purposes in optical (Dubus et al. 2006) and electrochemical biosensors (Hsing et al. 2007). Their unique properties allow magnetic particle-conjugated molecules to be quickly agglomerated or resuspended in the medium according to the external magnetic force, thus making them suitable for purifying biologically active compounds, such as nucleic acids (Obata et al. 2002), proteins (Safarik and Safarikova 2004), and cells (Xia et al. 2006). Besides being a universal separation tool, magnetic particles can also serve as magnetic labels for detecting biomolecules and enzymatic activity due to their innate magnetic properties (Perez et al. 2002, 2004). They are stable and safe over time, inexpensive, and the analysis procedure of the magnetic tags-generated signal is rapid. In addition, magnetosensors detecting magnetoresistance (MR) (Li et al. 2003), giant magnetoresistive effect (GMR) (Li et al. 2006), anisotropic magnetoresistive rings (Miller et al. 2002), and other techniques are often sensitive enough to detect a single particle. The magnetic core generally consists either of magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3) with size-dependent magnetic properties (superparamagnetic, 1–10 nm; ferromagnetic, in μm) (Gupta and Gupta 2005; Lefebure et al. 1998). In particular, the iron oxide-based nanoparticles provide a high surface area due to their tiny size and possess superparamagnetism, which has no magnetic memory,

resulting in particles being redispersed easily by removing the external magnetic field. Moreover, the synthesis procedures are relatively easy and inexpensive compared to other magnetic materials. Additionally, their particle size can be adjusted to match the biomolecules with which they interact (protein, 5–50 nm; virus, 20–450 nm; cell, 10–100 μm)(Ngomsik et al. 2005). With the aforementioned advantages, magnetic nanoparticles provide a highly sensitive and reliable detection technology for sensing biomaterials by being involved as separation tools combined with the various detection methods or labels.

3 Label-free detection methods

Label-free methods have emerged as a potential way to avoid possible structural and functional alterations of target molecules while providing acceptable sensitivity and selectivity. Assay protocol simplification, which facilitates portable or point-of-care sensing, is an additional expected advantage. With recent advances in micro and nanotechnologies, label-free biosensors have achieved attogram sensitivity and tremendous high-throughput analysis capabilities. Here we describe the current state of the art in label-free detection techniques, including surface plasmon resonance, surface- enhanced raman scattering, micro/nano-cantilevers, nanowires, and nanopores.

3.1 Surface plasmon resonance (SPR)

SPR is an affinity-based optical detection method that senses the binding between molecules through local changes in the refractive index on a thin (about 50 nm) metallic film (Fig. 2a). Numerous studies have been completed for monitoring the thermodynamics and kinetics of the binding processes of biomolecules (Willets and Van Duyne 2007). Using the SPR technique, the affinity of various biomolecules has been measured, such as nuclear receptor–DNA interactions, DNA hybridization, small molecule-DNA interactions, quantitative immunoassays, drug-protein interactions, protein-ligand interactions, and antibody-antigen interactions (Englebienne et al. 2003; Nguyena et al. 2007). To put this information into practical use, researchers have developed high-throughput systems, such as the Biacore Flexchip and an associated microfluidic system (Lee et al. 2007a; Natarajan et al. 2008; Rich et al. 2008). A portable SPR system was recently developed as a point-of-care system and used for immunoassays and as a bio-toxin detection system (Chinowsky et al. 2007; Feltis et al. 2008; Suzuki et al. 2002). Nanolithography techniques have recently been used to improve the sensitivity of SPR techniques. For example, localized surface plasmon resonance (LSPR) has been demonstrated at the nanoscale (Fig. 2a). While SPR relies on the propagation of plasmon along the metal surface, LSPR measures the absorption and scattering of incident light on a nano-patterned substrate. To create LSPR substrates, a nanostructure is fabricated on the substrate and then coated with a functional group to provide a binding site for biomolecules. To perform measurement in LSPR, a known wavelength of light is applied and the incident photon wavelength is shifted due to different dielectric constant of the substrate. Binding of target biomolecules causes an additional shift in the the dielectric constant and an accompanying shift in the measured wavelength, allowing binding to be detected. The sensitivity of this technique is highly correlated with the LSPR shift induced by the extinction maximum (λ_{max}) of the nanostructure coated substrates (Zhao et al. 2006). Using LSPR sensors, we can decrease the sample volume and tune the wavelength to the changing characteristics of the interrogated nanoparticles like size, shape, and material. To generate the desired size and shape of the nanostructures, nanoimprinting, electron-beam lithography, film over nanosphere (FON), and nanosphere lithography (NSL) are usually used (Ormonde et al. 2004; Stewart et al. 2008; Willets and Van Duyne 2007; Yan et al. 2006). Among these techniques, NSL is the most common, because it is cost-effective, rapid, and easily generates an array; however, the shape of the nanostructure created is limited. To get non-spherical nanostructures, a variety of approaches have been used. For example, starting with silver pyramidal nanostructures fabricated using NSL, the size and shape can be modified by electrochemical etching. Depending on the degree of etch, the λ_{max} can be shifted from 654 to 506 nm (Zhang et al. 2005). To find the effect of different nanostructure materials, nano-disks of Ag, Au, Pt and Pd were fabricated by hole-mask colloidal lithography. In Pt and Pd nano-disks, absorption is the dominant factor causing extinction. In Ag and Au, scattering is the main cause of extinction for 110 and 150 nm diameter disks (Lee and El-Sayed, 2006). Research continues to focus on how to maximize the sensitivity in LSPR based on the optimal material and geometrical shape. Several researchers have demonstrated LSPR in practical applications. A valuable clinical application demonstrated using LSPR involved a method for diagnosing Alzheimer‘s disease. Using the sandwich assay approach, the LSPR nanostructure was functionalized to detect amyloid-derived diffusible ligand (ADDL), which is known as a potential biomarker for Alzheimer’s (Fig. 3a). Using a cerebrospinal fluid sample from an Alzheimer’s patient, the λ_{max} value shifted from 751.6 to 758.3 nm (6.7 nm shift), while samples from normal patients caused a shift of only 0.1 nm (Haes et al. 2004, 2005) To detect the Tau protein, another known biomarker for Alzheimer’s, a gold-capped nanoparticle array was functionalized using a thiol- self assembled monolayer (SAM), followed by protein G, and an antibody for the Tau

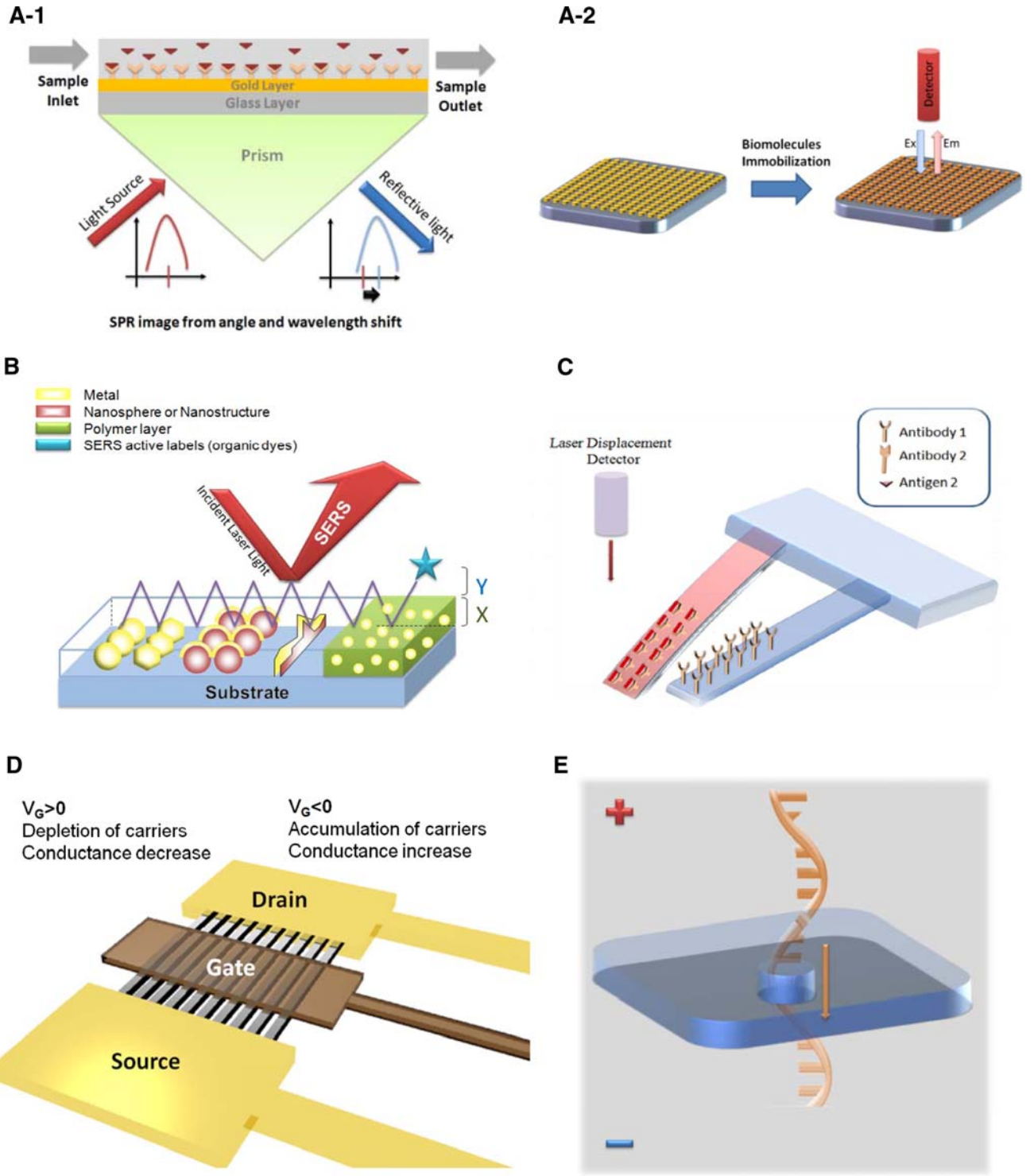

Fig. 2 **a** The principle of SPR and LSPR. In SPR (A-1), the incident light propagates through the metal-coated layer and in LSPR (A-2), it absorbs or scatters due to the nanostructure-coated substrate. **b** Schematic diagram of SERS showing several types of SERS active substrates. (Various shapes of metal nanoparticle colloids or films, metal-coated nanospheres, metal-coated random nanostructures, or metal nanoparticle-embedded polymer coatings). **c** The working principle of cantilever-based sensors. **d** Schematic of a regular p-type FET device. S and D correspond to source and drain. **e** The principle of gene sequencing from translocation of DNA strands through a nanopore

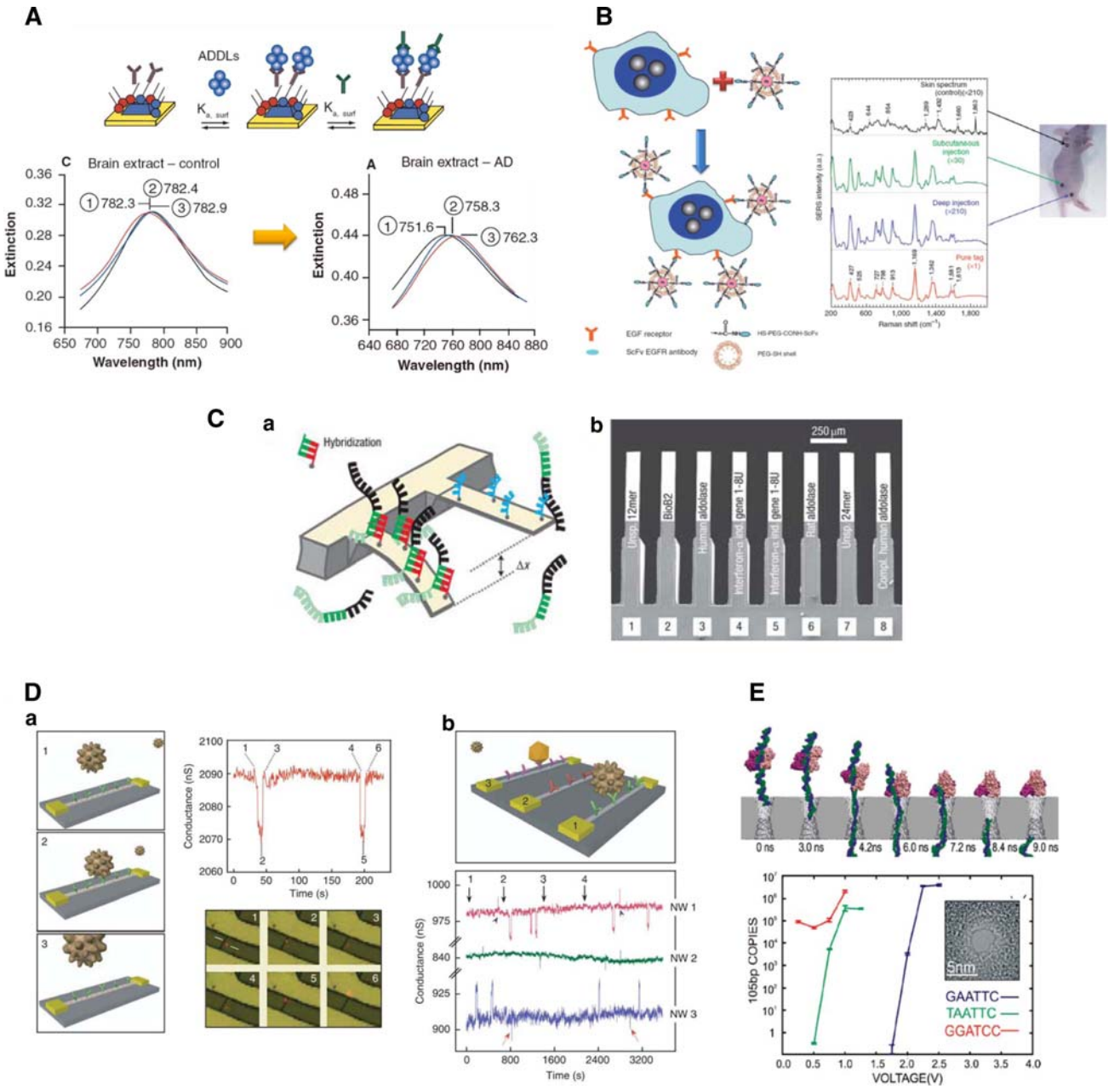

Fig. 3 **a** The detection of Alzheimer's disease using the ADDL biomarker (Haes et al. 2005) and surface plasmon resonance. **b** Application of SERS for cancer targeting (Qian et al. 2008). **c** Nanomechanical detection of the up-regulation of a gene. (C-a) Setup showing sensor and reference cantilevers and (C-b) a bio-functionalized cantilever array (Zhang et al. 2006a). **d** (D-a) Schematic illustration of a single virus binding and unbinding to the surface of a silicon nanowire device modified with antibody receptors. (D-b) Schematic of multiplexed single-virus detection (Patolsky et al. 2007). **e** Using a nanopore, 3 different amplicons were successfully discriminated by measuring the transition time and current blockage in the nanopore (Zhao et al. 2007)

protein. This LSPR immuno-chip was able to be detect the Tau proteins in a 10 pg/ml CSF sample (Vestergaard et al. 2008). Using similar functionalization techniques, a LSPR array was fabricated to detect various proteins such as: immunoglobulins (IgA, IgD, IgG, IgM), C-reactive protein, and fibrinogen. This LSPR chip had 300 nanospots and the minimum detectable concentration was 100 pg/ml for each of the proteins (Endo et al. 2006).

3.2 Surface enhanced Raman scattering (SERS)

Raman spectroscopy is a well-known technique, which can be used to provide information about molecular vibration frequencies—a molecular fingerprint—without prior labeling (Raman and Krishnan 1928). In spite of its specificity, its practical uses have been significantly limited because the Raman scattering (RS) signal is intrinsically

weak, compared to most fluorescent signals. Surface-enhanced Raman scattering (SERS) provides a method for improving sensitivity and signals by factors of up to about 1015 (Fig. 2b) (Qian and Nie 2008) enabling many applications, from detection of biomolecules, such as DNA (Braun et al. 2007), DNA/RNA mononucleotides (Bell and Sirimuthu 2006), and proteins (Bizzarri and Cannistraro 2007) to recognition of diverse cellular (Yu et al. 2007) and molecular events (Bonham et al. 2007).

SERS, a surface-sensitive phenomenon showing enhanced Raman scattering, occurs when molecules reside at or near the surfaces of certain nanostructure materials and allows single-molecule level detection (Qian and Nie 2008). It was first observed by Fleischmann and coworkers in 1974 (Fleischmann et al. 1974). Three years later, two different research groups reported the enhanced signal, which is not dependent on the concentration of scattering species, and proposed mechanisms to explain SERS enhancement that are still generally accepted: (1) there is an electromagnetic contribution based on plasmon excitation through laser light on the metal surface, which leads to a large localized electromagnetic field, also known as a "hot spot" (Jeanmaire and Duyne 1977) and (2) a chemical interaction associated with charge transfer or bond formation between the adsorbed molecules and the metal surface (Albrecht and Creighton 1977) that allows the molecules to highly scatter the signal. Although still debated, the electromagnetic effect broadly covers all cases. The design of efficient and reliable SERS active substrates have been actively investigated since the substrate strongly affects the strength of the RS enhancement. The first SERS substrate was electrochemically roughened silver (Fleischmann et al. 1974), achieving enhancement factors of about 106 (Jeanmaire and Duyne 1977). Now SERS substrates are often created by metal nanoparticle colloids made up of various metals and different shapes. The junctions formed between nanoparticles, a mechanical break, or electro-migrated metal gaps serve as hot spots amplifying the Raman signal. Au and Ag nanoparticles are still the most popular, but the spherical shape is no longer attractive due to its smaller Raman scattering enhancement. Beyond spheres, current nanofabrication techniques make it possible to generate a variety of nanostructures, such as prisms (Jin et al. 2003), octahedra (Zhang et al. 2006b), and crescents (Liu et al. 2005a). In addition to the shape-selective synthesis methods, lithographic techniques have been employed to produce certain nanostructures such as optical arrays (Driskell et al. 2008). A simple schematic diagram of SERS and some of the various SERS active substrates that have been created are shown in Fig. 2c. The invention of the SERS tags, called extrinsic Raman labels or SERS dots, is a remarkable advancement and is especially important for biomedical applications (Doering et al. 2007). The SERS tags are considered beneficial primarily because their signal enhancements are reproducibly controlled by the size and shape of the nanoparticle tags, while providing full spectroscopic information, and also because the SERS tags have higher signal intensities than quantum dots in the near-infrared spectral window. They also minimize the distance between the particle surface and reporters in order to maximize the enhancement of the electric field and have shown 10–100 times narrower bandwidths than fluorescence techniques, which facilitates multiplexing. The most recent generations of the SERS tags (Kim et al. 2006; Qian and Nie, 2008; Qian et al. 2008) share four key components: (1) a metal nanoparticle core for optical enhancement (2) a Raman reporter molecule (SERS active label in Fig. 2c) providing a spectroscopic signature (3) a silica or polymeric shell for shielding and easy conjugation, and (4) a surface-conjugated or -coated antibody for targeting specific biomarkers. Of the available organic dyes, aromatic compounds like Crystal Violet and Nile Blue have been chosen as reporter molecules due to their intrinsically strong Raman scattering. SERS has been used for the targeted detection of biomarkers such as cancer antigens, found in blood or on a cell surface, using immunoassay approaches for in vitro cancer diagnosis (Porter et al. 2008; Sha et al. 2007) and in vivo cancer targeting and imaging (Qian et al. 2008), respectively (Fig. 3b). Reflecting increased attention and effort on SERS research, recent progress and the impact of SERS on various research fields has been thoroughly reviewed with an emphasis on the influence of nanotechnology enhancements (Banholzer et al. 2008; Hering et al. 2008; Kiefer, 2007; Porter et al. 2008; Qian and Nie, 2008; Stiles et al. 2008).

3.3 Cantilever-based biosensors

Micro/nano-cantilevers act as a force transducer for biosensing applications and function by detecting changes in cantilever bending or vibration frequency (Ziegler 2004) when molecules bind to the surface of the cantilever (Fig. 2c). Characterization of the forces and dynamics of biomolecular interactions with cantilever-based sensors offers an opportunity for the development of highly sensitive, miniaturized, parallel, and label-free biological sensors. Advancement in microelectromechanical systems (MEMS) technology over the past decade has led to cost-effective, miniaturized cantilevers with low spring constants with a high sensitivity to applied forces, or high resonance frequencies for faster response times (Hansen and Thundat 2005). The classic perception that smaller sensors are more sensitive (sensitivity $\approx -0.5\omega_r/m_c$, where ω_r is the resonant frequency and m_c is the mass of the cantilever) has motivated scaling of biosensors to nanoscale dimension (Gupta et al. 2006). Accordingly, efforts have been made to develop

smaller cantilevers with higher frequencies and better mass resolution. The recent development of nanoscale cantilevers with frequencies in the MHz range and an integrated electronic displacement transducer has scaled down the technology further and increased the capability for rapid, ultrasensitive, and selective detection of captured biomolecules (Fig. 3c) (Li et al. 2007; Masmanidis et al. 2007; Zhang et al. 2006a). For example, a cantilever sensor array with a pitch of 250 nm and individual cantilevers of 500 nm × 100 nm × 20–500 nm dimension operating a 2–4 kHz allowed for real-time monitoring of lipid bilayer formation on the cantilever and fast microorganism detection (Li et al. 2007). An oligonucleotide detection system with an ability to identify for label-free genes within a complete genome or an unlabeled gene in total RNA, was also created (Fig. 3c) (Zhang et al. 2006a). Such nanomechanical biosensors can be used for applications in ultrasensitive detection of: DNA hybridization (Biswal et al. 2006; Fritz et al. 2000), biomarker transcripts in human RNA (Zhang et al. 2006a), and viruses (Gupta et al. 2006). In addition, cantilever-based force spectroscopy has emerged as a powerful tool for characterizing spatiotemporal dynamics of the interaction between individual ligands and receptors, either on isolated molecules or on cellular surfaces (Hinterdorfer and Dufrene 2006), such as VEGFR2 (Lee et al. 2007b) or integrin $\alpha 2\beta 1$ (Taubenberger et al. 2007). The cantilever-based biosensors can also be utilized for early detection and clinical diagnosis of cancer (Cross et al. 2007; Wu et al. 2001). Further advancement to a high degree of parallelization with nanomechanical biosensors may eventually allow for high-throughput screening for disease diagnosis and drug discovery. Several challenges still remain before cantilever array sensors can be used as one of the primary, next-generation diagnostic tools. For example, efficient immobilization techniques are required to functionalize the large number of cantilevers in an array. Damping, viscosity, and thermal fluctuations also combine to make precise measurements in fluids difficult. Advances in integration of electric, mechanical and fluidic designs are required to accelerate the design of fully integrated cantilever-biosensor devices. It is envisioned that nanomechanical label-free detection has the potential to detect disease states by providing a promising approach to a completely new class of high-throughput, ultrasensitive diagnostic tools in the near future.

3.4 Nanowires

Silicon nanowire (SiNW) biosensors along with nanotubes (Kong et al. 2000) and conducting-polymer nanowires (Ramanathan et al. 2005) are promising label-free electronic biosensors (Fig. 2d). The most important and powerful advantage of SiNW sensors is the possibility of multiplexed, real-time detection. The progress in nanofabrication techniques allows us to make an array of identical structures, which leads to massively parallel measurements. As most nanofabrication techniques originated in microelectronics, they can be easily scaled-up and transferred to a mass production line with high reliability. The underlying mechanism of nanowire sensors is based on the principle of field-effect transistors (FETs). Figure 2d illustrates a p-type SiNW FET and its response to different gate voltages. In the case of a p-type semiconductor, a positive gate voltage depletes carriers and reduces the conductance, while a negative gate voltage leads to an accumulation of carriers and an increase in the conductance. For biosensors, binding of a charged species on the surface of the SiNWs is analogous to applying a gate voltage. By monitoring the conductance change, the binding of targets to probe molecules can be detected on the Si surface. Several research groups have already demonstrated the successful solution-phase SiNW sensing of DNA (Bunimovich et al. 2006), viruses (Patolsky et al. 2004), small molecules (Wang et al. 2005) and proteins (Stern et al. 2007a; Zheng et al. 2005). The current detection sensitivity is about fM range, which is several orders of magnitude more sensitive than a conventional ELISA assay. For most of those experiments, however, low salt buffer solutions were used to avoid the screening effect associated with solution counter ions (Patolsky et al. 2004; Stern et al. 2007a; Wang et al. 2005; Zheng et al. 2005). The charge of target molecules is screened by the counter ions in solution and effective only on the scale of the Debye length. Several research groups recently focused on the importance of the Debye length in this application (Stern et al. 2007b; Zhang et al. 2008a). The Debye length for a 0.1 M solution is about 1 nm and biologically relevant media is typically a 0.14 M electrolyte. Since the salt concentration and pH are important factors for the binding between biomolecules, it is necessary to find an alternative way of overcoming the charge screening to perform an ideal sensing measurement with NW-FETs (Huan-Xiang 2001). For DNA sensing, the charge screening can be overcome by electrostatically immobilizing ssDNA on the SiNW surface (Bunimovich et al. 2006). With antibodies, however, the biomolecular recognition event occurs ~10 nm away from the wire due to the antibody's large size. In biological media, the binding event usually takes place farther away than the Debye screening length, so alternative small capture agents are required to bring the binding event closer to the nanowire. Thus, finding small molecules that have the same specificity for proteins as antibodies and that can distinguish between slightly different proteins is critical. Due to their small scale, high sensitivity and real-time detection capability, nanowire based sensors could be used to study single cells.

Table 1 Comparison of nanobiosensor technique based on analyte type and sensitivity

Sensing technique	Analyte type	Limit of detection	References
Quantum dot	Oligonucleotides	~2 zM	Yeh et al. (2006a)
LSPR	Tau protein in CSF	10 pg/ml	Vestergaard et al. (2008)
SERS	Oligonucleotides	100 fM	Qin et al. (2007)
Cantilever	1,1-difluoroethane gas	Less than 1 attogram	Li et al. (2007)
Nanowire	Streptavidin	~70 aM	Stern et al. (2007a)
Nanopore	SsDNA	DNA sequencing	

Moreover, recent articles which reported the theoretical analysis of this complex system should help researchers to understand the underlying mechanism of SiNW-FETs and develop the device functionality (Nair and Alam 2006; Squires et al. 2008; Wunderlich et al. 2007). A report of using SiNWs to detect, stimulate, and inhibit nerve signals along the axons and dendrites of live mammalian neurons shows another interesting application of SiNWs (Patolsky et al. 2006). Viruses can also be detected using these methods as shown in Fig. 3d (Patolsky et al. 2006).

3.5 Nanopores

Biological nanopores have existed for a long time, but with the development of nanolithography techniques, it has been possible to create engineered nanoscale pores and holes. Researchers have also found that nanopores can be used for single molecule detection, especially with oligonucleotides and protein molecules (Fig. 2e). The first demonstration of this technique was to use α- hemolysin(HL), a biologically-based nanopore, to identify a DNA sequence (Dekker 2007). In comparison to solid state nanopores, a biological nanopore is usually cheaper, but it is difficult to generate precise pore sizes using biological techniques, which leads to a non-uniform signal due to the lack of stability. Another disadvantage is that biological nanopore works in a limited range of pHs and temperatures (Griffiths, 2008). Thus, a solid-state nanopore is more stable, flexible and robust for identifying nucleic acid and proteins (Dekker, 2007; Hosokawa et al. 2004; Martin and Siwy, 2007). Solid-state nanopore has been developed by using forced-ion-beams, micromolds, e-beam, and TEM techniques on SiO_2 or Si_3N_4 thin layers. The pore size of the biological nanopore in α-HL is fixed at 1.5 nm, but, a wide range of pore sizes (1–80 nm) are possible using these fabrication techniques (Lanyon et al. 2007; Rhee and Burns 2007). The working principle of the nanopore technique as a sensor is to measure the current when molecules pass through the nanopores. As a molecule passes through a nanopore, the current is temporarily blocked. The current pulse profile is then recorded to determine when a molecular-translocation event has occurred. To increase the sensitivity and selectivity of the nanopore sensor, the surface of nanopores can be modified by a specific protein or nucleic acid. For example, the surface of a solid-state nanopore was functionalized with a protein binding molecular-recognition agent (MRA) and an oligonucleotide binding MRA to increase the selectivity of the nanopore (Martin and Siwy 2007). In another case, hair-pin loop DNA was immobilized on the nanopore wall to increase the selectivity of single stranded DNA (Iqbal et al. 2007). Recently, this technique was used for investigating the mutation of a histidine-containing protein. With a biological nanopore, such as α-HL and aerolysin (ARL), the current blockage, duration, and number of events were measured. Based on the number of events and the measured current profile, mutant proteins could be identified. Even when it was difficult to know the exact sequence of the protein, this technique was able to detect the structural change of the protein (Stefureac et al. 2008). In a more practical application, it would be possible to detect and diagnose a disease caused by a misfolded protein, such as bovine spongiform encephalopathy (mad cow disease), Alzheimer's or Parkinson's disease. Furthermore, this technique allows biophysical experiments to measure the electrophysical characteristics of DNA, RNA and protein in a single molecule unit (Keyser et al. 2006).

Table 1 provides a summary of some of the nanobiosensing techniques as well as some of the best reported results for these techniques in terms of sensitivity. Note that several of these techniques are beginning to approach single molecule sensing for small samples.

4 Micro/nanofluidics integrated with nanobiosensors

Nanoengineered elements have significantly benefited microfluidic biosensing systems. In particular metal nanoparticles, and especially gold nanoparticles (Fig. 1c), with their superior optical, electrical, and thermal properties, have been applied to enhance the functionality of various microfluidic biosensing platforms. Several optical detection schemes based on metal nanoparticles, such as surface plasmon resonance and colorimetric assays, have been incorporated into microfluidic systems (Hosokawa et al. 2004; Park et al. 2006; Puleo et al. 2008; Sato et al. 2008).

For instance, Au nanoparticle-labeled antibodies were used to amplify the signal of DNA-encoded antibody libraries (DEAL) for multiplexed cell sorting and detection (Bailey et al. 2007). The amplification allows the limit of detection of interleukin-2 to be less than 10 fM, which is several orders of magnitude better than conventional ELISA. Metal nanoparticles have also been extensively applied in microfluidic platforms electrochemically (Tang et al. 2007; Yeung et al. 2006). The opto-thermal properties of gold nanoparticle have also enabled on-chip lysing of pathogenic agents by transforming infrared energy into thermal energy (Cheong et al. 2008). Metal nanoparticles can also serve as functional materials for enhancing the performance of a microfluidic platform. For instance, metal nanoparticles have been directly incorporated into or coated onto the channel material (e.g., poly(dimethylsiloxane)(PDMS)) to serve as surface-enhanced Raman scattering substrates (Giesfeldt et al. 2005), to enhance electrophoretic performance (Wang et al. 2006a), to bind bio-molecular recognition elements (Luo et al. 2005), and to develop immobilized enzyme reactors (Zhang et al. 2008b). Furthermore, gold nanoparticles self-assembled on a substrate have been demonstrated for coupling of localized surface plasmon energy for thermal mixing (Miao et al. 2008) and manipulation of bio-particles (Miao and Lin 2007a, b).

Quantum dots (QDs) represent another promising technology for nano-engineered, microfluidic-based biosensing platform. The QDs' inherent characteristics offer several benefits to microfluidic based detection schemes, such as allowing for increased sensitivity and multiplexed detection. Recently, QDs have been used in microchannel assays including antigen-antibody binding for microbe and virus detection (Liu et al. 2005b; Lucas et al. 2007; Zhang et al. 2006c), microbead- based system for signal enhancement and detection of hepatitis and tetanus (Riegger et al. 2006). They have also been used for sensing single molecules via detection of a pair of differently colored quantum dots or a paired quantum dot-fluorescent molecule combination bound to the target molecule (Agrawal et al. 2006; Stavis et al. 2005; Yeh et al. 2004). QDs have also been used in combination with fluorescence energy transfer (FRET) for applications such as signal amplification during DNA sensing and detection of molecular orientation, size and binding (Ho et al. 2006; Medintz et al. 2003; Medintz et al. 2004; Zhang et al. 2005). Microfluidic control provides enhanced capabilities for QD biosensors. A recalculating microfluidic system has recently been coupled with a confocal fluorescence detection system (Puleo et al. 2008). The inclusion of the circulating channel reduces the volume requirement per measurement nearly three orders of magnitude compared to conventional approaches. The systematic interrogation of complex biology events can be monitored with high spatiotemporal resolution by exploiting the parallel processing abilities of a microfluidic platform. As mentioned before, the SPR provides a great opportunity to detect biomolecules without any tags, but it still needs to be improved for use with real samples in the field. In general, most microfluidic biosensors are moving toward a high-throughput configuration and SPR techniques follow this trend after being combined with micro/nano-fluidic systems. Recently, a protein microarray spotting technique performed with microfluidics was introduced. Using a U-shape microfluidic channel, protein and lipid spots are constructed on the substrate by contacting the tip of a microfluidic channel. This microfluidic spotter has progressed to a high-throughput and sensitive system, and has also shown the possibility of integration with SPR systems (Chang-yen et al. 2006; Natarajan et al. 2008) as shown in Fig. 4a. Using this system, a high-throughput sandwich assay for real-time immunoassays is possible. With a more complicated microfluidic system, an SPR-integrated microfluidic layout was implemented for immunoassays. To control the flow of each inlet, micr-ovalves were fabricated from multi-layers of PDMS on a gold patterned substrate. The binding kinetics was tested by flowing Anti-biotin Ab across a biotin-BSA-coated gold spot. An additional experiment showed low detection limits using anti-IgG labeled gold nanoparticles with SPR images (Luo et al. 2008). Both systems were able to reduce the reaction time and sample amount, and they are very useful for multi-samples screening from SPR sensing. Another valuable demonstration of SPR sensing with microfluidics involved the use of digital microfluidic techniques. Digital microfluidics uses a droplet to minimize volumes, reduce the reaction time and solve the adherence and adhesion problems associated with solid surfaces. A streptavidin droplet was positioned on the surface acoustic wave (SAW) to layer enhance the interaction with the self assembled monolayer (SAM) surface. SPR substrate was coupled with a droplet on the SAW layer and compared with static mode (without the wave). The droplet on the SAW layer presents a higher binding reaction due to the eddie flow within droplet. SPR integrated with digital microfluidics offers a way to implement a high-throughput screening system and to study reliable quantification (Galopin et al. 2007).

The SERS technique holds considerable promise for analytical analysis due to its label-free detection mechanism, comparable sensitivity with fluorescence measurement, and narrow signal bands enabling multiplex detection (Chen and Choo, 2008). Since SERS detection in a microfluidic channel would be under flowing conditions, quantitative measurement with faster and more reproducible results than conventional SERS detection under static conditions can be achieved. This benefit occurs because the flowing system allows homogeneous mixing of analytes and metal nano-colloids providing consistent sampling and heat dissipation.

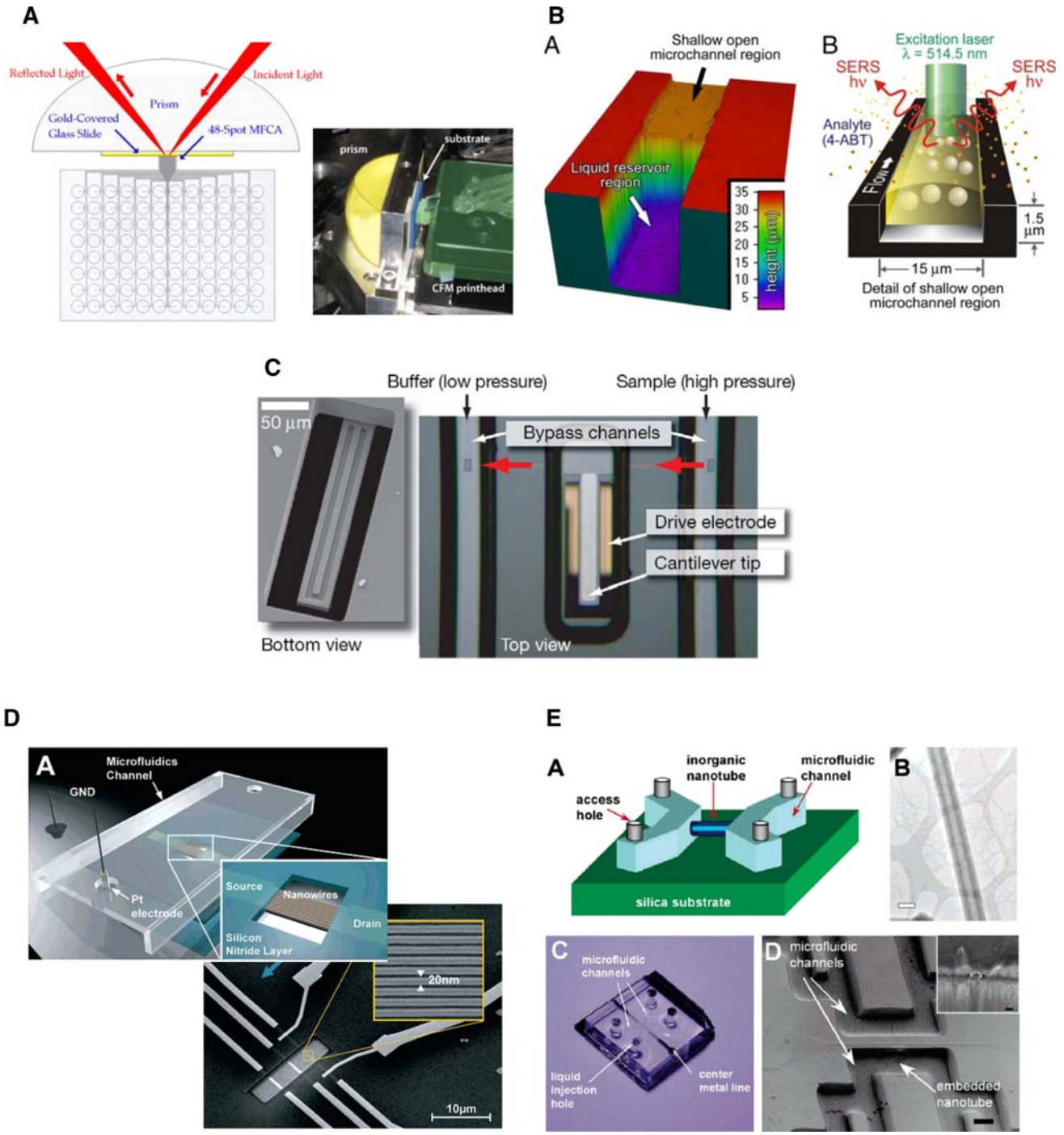

Fig. 4 Nanobiosensors integrated with microfluidic systems. **a** A highly parallel microfluidic system was integrated with SPR to measure binding kinetics in a protein microarray. (Natarajan et al. 2008). **b** A SERS sensing device that is designed for free-surface fluidics. The free-surface liquid/atmosphere interface allows analyte absorption and subsequent optical stimulation for SERS detection (Piorek et al. 2007). **c** A microcantilever containing a microfluidic channel was developed for measuring the weight of single cells and single nanoparticles (Burg et al. 2007). **d** A microfluidic channel integrated with three groups of 10 SiNWs for the real-time measurement of DNA hybridization (Bunimovich et al. 2006). **e** A nanopore (nanofluidic) device was developed by using inorganic nanotubes connecting two microfluidic channels. With this device, when DNA translocation event occurred through the nanopore, a transient change of ionic current was measured successfully (Fan et al. 2005)

It should be noted that the mixing is highly dependent on the channel structure, so the packaging and flow cell design are critical to success of assays in SERS and similar systems. By shaping simple channels into more complex channels with zigzag structures, staggered-herringbones with grooves, or alligator teeth-forms, chaotic advection is generated with

higher mixing efficiencies than can be accomplished through simple diffusion (deMello 2006). Among the chaotic channels, the alligator teeth-shaped channel was the best and may prove valuable when integrated with SERS and SPR type systems. A challenge associated with mixing small samples in single phase microfluidic systems is that of minimizing dispersion during fast mixing due to the difference between fluid velocity at channel walls and at the center. Recently, Ackermann et al. have reported that two-phase liquid/liquid segmented flow systems can overcome the common adhesion problem of analyte/nanocolloid aggregates within the channel that can limit sensitivity in SERS microfluidic systems. This SERS system performed quantitative online monitoring of drug concentrations over many hours without the need for aggressive chemicals to wash the channel (Ackermann et al. 2007). Two phase systems also reduce the dispersion challenge.

High sensitivity SERS detection may be possible by combining SERS with other techniques beyond microfluidics, and several efforts have been made to move in this direction, though most efforts are still limited in the number of channels. In combined SERS/microfluidic systems, the majority of the background noise is produced by PDMS, which is a widely used material in the construction of microfluidic channels. The noise occurs because PDMS is a Raman active polymer that generates its own Raman signals. To overcome some of these limitations, Park et al. investigated a confocal SERS microfluidic chip for detecting duplex dye-labeled DNA oligonucleotides (Park et al. 2005). The confocal microscope combination increases signal-to-noise ratio by excluding background signals not associated with the specimen features. In this study, concentrations down to 10^{-11} M were observable in the alligator teeth-shaped flow channel, so that immobilization steps and signal amplification were not necessary. Dootz et al. created a microfluidic system combined with small angle X-ray microdiffraction and Raman microscopy to study the dynamics of DNA condensation by dendrimers. They demonstrated that the additional structural information obtained from X-ray scattering measurement offers a detailed insight into the molecular interactions of DNA with dendrimers monitored by Raman imaging (Dootz et al. 2006). More recently, Zhang et al. successfully performed cellular assays with SERS in a microfluidic chip. They characterized cellular drug response of a single live Chinese hamster ovary (CHO) cell (Zhang et al. 2008c). Raman active CHO cells were prepared by incubating them with gold nanoparticles, which were uptaken by cells via pinocytosis. Beyond liquid phase analytes, Pioreck et al. reported that airborne molecules can be detected with a SERS based gas sensor equipped with an open microchannel, which was designed for confining a silver nanoparticle solution flow at a constant rate by surface tension (Piorek et al. 2007). A flow through the open channel with a large surface-area-to-volume ratio, which authors call free-surface fluidics, controls the exposure time of nanoparticles to an analyte absorbed through the free-surface (Fig. 4b). They showed the system allows absorption of a gas-phase 4-aminobenzenethiol into a silver colloidal fluid and enables subsequent detection of the analyte and simultaneous monitoring of the nanoparticle aggregation process with the analyte by SERS. To conclude, the SERS, in combination with a lab-on-a-chip technology, is expected to be a great tool for conducting fast and sensitive bio-analysis.

Arrays of cantilever-based sensors also have been integrated in individually accessible, parallel microfluidic channels (Aubin et al. 2007; Lechuga et al. 2006). A microfluidic -based multiplexed assay format enables faster sample delivery to the integrated cantilevers due to the small volume of the channels and facilitates the monitoring of cantilever bending in parallel. Each chamber can be also addressed independently by different analyte liquids. This enables functionalization of cantilever-based sensors separately by flowing different solutions through each chamber. For instance, Lechuga et al. developed a portable biosensor microsystem able to detect nucleic acid hybridization with high sensitivity. It comprises an array of 20 micromechanical cantilevers, a polymer microfluidic system for delivery of the samples, an array of 20 vertical cavity surface emitting lasers (VCSELs), and chips with the photodetectors and the CMOS circuitry for signal acquisition and conditioning. It is also capable of measuring the cantilever deflection with subnanometer resolution (Lechuga et al. 2006). On the other hand, cantilever-based sensors offer a simple and nonintrusive way to detect changes in solvent type, temperature, and pH, promising great potential for sensing applications in microfluidic devices. For example, cantilever-based sensors were used in microchannels to measure the viscosity and flow rate of ethylene glycol mixtures in water over a range of concentrations, as well as of low viscosity, biologically relevant buffers with different serum levels (Quist et al. 2006). A fiber-tip cantilever has been shown to transduce an ultrawide dynamic range of microfluidic flow rates (up to 1,500 μl/min) to an optical signal readout, suitable for high throughput applications, such as flow cytometry and particle sorting/counting (Lien and Vollmer 2007). Manalis' group went in a unique direction when they successfully fabricated a microfluidic channel on top of a microcantilever (Fig. 4c). It was demonstrated that integration of a micro/nanocantilever with a microfluidic channel gives access to intriguing biomedical applications, such as mass-based flow cytometry and the direct detection of pathogens with high sensitivity biodetection (Burg et al. 2007).

Integrating nanowire sensors with microfluidics allows improved use of the sensor. The high sensitivity of the nanowires can be most effective when it is difficult to obtain

a large amount of sample. Thus, to fully utilize the advantages of the nanowires as an active sensing material, it is necessary to manipulate a sample with small volume. Owing to the small size of the microfluidic channel, it was possible to detect target molecules with only 10–100 μl of sample (Zheng et al. 2005). Another advantage of utilizing both nanowires and microfluidics is real-time and rapid sensing. Since the nanowire signal is an electrical readout and the microfluidics can deliver target molecules to the active sensing zone through a constant flow, it is possible to monitor the binding of target molecules in a real-time manner. Most results from nanowire-based biosensors have been focused on their high sensitivity. Sensing target molecules of only ~fM concentration is very attractive but a quantitative analysis is also an important aspect of sensors. Quantitative analysis is possible through a deep understanding of the sensing mechanism of nanowires and the transport of target molecules to the active sensing zone. Bunimovich et al. reported that it is possible to quantitate the analyte concentration with silicon nanowires fabricated using a top-down approach in microfluidics (see Fig. 4d) (Bunimovich et al. 2006). They compared the results with SPR, which is a more standardized microfluidics-based sensing platform. Manalis et al. recently reported the effect of fundamental quantities, such as fluxes on the transport of target molecules and the reaction limits of microscale as well as nanowire sensors (Squires et al. 2008). Due to the extremely small size of the nanowires and the complex fluidic dynamics in microfluidics, it is necessary to put more effort on understanding mechanisms for both nanowire sensing and target molecule transport in microfluidics. Based on these understandings, it can open up a new avenue for the practical use of the nanowire-based biosensors. Likewise, micro/nanofluidic technique can improve the addressability and applicability of nanopore sensors. The simplest way to integrate with solid-state nanopores is to use multilayer microfluidic channels. Using the multilayer technique, biomolecules and particle were separated and trapped on nanopore substrate successfully (Kuo et al. 2003; Zhou et al. 2008). Using a similar technique, a single nanopore was integrated with a microfluidic device to understand the ion depletion and sample stacking effect around the nanopore (Zhou et al. 2008). Based on this technique, nanopore sensing is easily integrated within a micro/nanofluidic system. Another integration method was to use a nanotube to fluidically connect microfluidic channels. Instead of using an etching technique to make a nanopore system, the inorganic nanotube functioned as a bridge between the microfluidic channels. Under different ionic concentrations, λ-DNA translocation was successfully implemented and measured by the current blockage (Fig. 4e). As compared with solid-state nanopore systems, the nanotube-embedding technique makes it possible to fabricate the high-aspect ratio component and apply it to a planar system (Fan et al. 2005).

5 Perspectives

Nanotechnology has quickly transitioned from its early conceptual days to numerous commercial successes in a wide variety of fields including advanced materials, surface science, biophysics, and biosensor development. Advances in nanotechnology-based platforms, which interact with biological matter in novel ways, have led to the discovery of new, previously unknown labeling and therapeutic agents with improved performances. Thus it is clear that with the aid of these new nanotechnologies, unprecedented progress is now possible towards high-throughput multiplexing analyses of nucleic acids, and proteins, resulting in advances in medical diagnostics.

The progression of nanotechnology-based sensors is likely to move in several directions. The first general direction benefits from the small scale and low cost of nanobiosensors and is applied towards highly sensitive and inexpensive sensing platforms for use in point of care diagnostics and low-cost systems for use in remote, poor areas where medical care is lacking and full-scale labs are not available. These systems fall into the "lab-on-a-chip" category, but may have different goals, such as the need for taking one sample and measuring many things about it, as will likely occur in hospitals, as compared to other applications where only one, very inexpensive measurement is made, which is more likely in field facilities for human testing, as well as testing of animals, food, and water sources. For these applications, simple assays requiring minimal user intervention will be required. These simple devices can be implemented by developing new assays with few steps than can be visually understood, or by developing instruments that remove the complexity from the user and provide a digital or quantitative readout. Both directions are being rapidly pursued at the present time.

The small scale of nanobiosensors will also feed the development of sensing platforms that rely on large-number arrays, such as DNA microarrays that have become ubiquitous in nucleic acid research. These DNA arrays, which use a single "detector" (CCD camera) and typically only one "sample," will eventually lead to sensing arrays where the sensor itself is nanoscale, but there are large numbers (large numbers mean different things in different fields, anywhere from hundreds to billions) of sensing locations that report their results independently. In these arrays, each nanoscale sensor might report results for one sample, as may be the case in large centralized labs, and there will be multiple samples tested on one nanoscale platform. Arrays of sensors are also beginning to meet an array of samples

(an array of arrays), where multiple samples are being tested for multiple characteristics. The primary challenge in these systems is not the sensors themselves, but the connections and interfaces to the outside world, both with regard to fluidics (sample handling) and electronics or optics (signal output). The high-density electronics and optics appear available, but high-density microfluidic systems are just beginning to come online. Overall, these types of arrangements are likely to lead to inexpensive screening for multiple diseases or biomolecule states in a simultaneous fashion, which should quickly change the way in which medicine is practiced, possibly leading to a prevention mindset rather than a response after a diagnosis. In any case, the impact of nanoscale sensors will have a profound effect on medical, food, and environmental testing.

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