Analyst

View Online
Dynamic Article Links

Cite this: *Analyst*, 2011, **136**, 1539

www.rsc.org/analyst

MINIREVIEW

Microcantilever biosensors for chemicals and bioorganisms

Koutilya R. Buchapudi,[a] Xin Huang,†[b] Xin Yang,[a] Hai-Feng Ji*[b] and Thomas Thundat[c]

Received 16th December 2010, Accepted 1st February 2011
DOI: 10.1039/c0an01007c

In the last fifteen years, microcantilevers (MCLs) have been emerging as a sensitive tool for the detection of chemicals and bioorganisms. Because of their small size, lightweight, and high surface-to-volume ratio, MCL-based sensors improve our capability to detect and identify biological agents by orders of magnitude. A biosensor is a device for the detection of an analyte that combines a biological component with a physicochemical detector component. The MCL biosensors have recently been reviewed in several papers. All of these papers were organized based on the sensing biological elements (antibody, enzyme, proteins, *etc.*) for recognition of analytes. In this review, we intend to summarize the microcantilever biosensors in a format of each specific chemical and bioorganism species to make information on individual biosensors easily accessible. We did this to aid researchers to locate relevant references.

1. Introduction

Recently, micro-electromechanical systems (MEMS) have been emerging as a platform for the development of miniature sensors with extremely high sensitivity. It is estimated by 2010 about 1000 papers on microcantilevers sensors had been published. Micromachined silicon cantilevers are the simplest MEMS sensors that can be micromachined and mass-produced. Microcantilever (MCL) sensor technology is an upcoming sensing technique with extremely high sensitivity and with broad applications in chemical, physical, and biological detection.[1,2] With their compactness and potential low cost, silicon-based MCLs provide a clear path for the development of miniaturized sensors for detection of chemical and biological agents. In liquid phase applications, the MCL technology has the potential for biodetection applications, such as detection of toxins and selective detection of pathogens *via* immunological techniques. Because of their small size, lightweight, and high surface-to-volume ratio, MCL-based sensors have unprecedented sensitivity for detection of biological analytes (potentially detecting as little as a single entity of an agent).

A SEM picture of a MCL is shown in Fig. 1. The MCL sensors have several advantages over the other sensor technologies, including faster response time, lower cost of fabrication, the possibility of sensor arrays with small overall dimensions, the ability to explore

[a]*Institute for Micromanufacturing, Louisiana Tech University, Ruston, LA, 71272, USA*
[b]*Department of Chemistry, Drexel University, Philadelphia, PA, 19104. E-mail: hj56@drexel.edu; Fax: +1 215 895 1265; Tel: +1 215 895 2562*
[c]*Chemical and Materials Engineering Department, University of Alberta, Edmonton, Canada, AB, T6G2V4*
† Current address: Institute of Animal and Plant Quarantine, Chinese Academy of Inspection and Quarantine, Beijing, 100029, P. R. China.

Koutilya R. Buchapudi

Mr Koutilya R. Buchapudi obtained his master degree in Molecular Science and Nanotechnology from Louisiana Tech University in 2008. He is currently pursuing his PhD degree in biomedical engineering at Louisiana Tech University. He is presently working on developing fluorescent sensors for real-time measurement of cell metabolism.

Xin Huang

Dr Xin Huang received his PhD in Physical Chemistry from Tsinghua University, China, in 2007. He has published more than 20 peer-reviewed journal papers and he is currently an associate professor at Chinese Academy of Inspection and Quarantine, Institute of Animal and Plant Quarantine. His research interest is rapid and sensitive detection of Exit-Entry agriculture products.

microenvironments, and improved portability for field applications. The MCL biological sensors are summarized briefly in this review.

The MCL sensor responses, such as resonance frequency, deflection, Q-factor, and amplitude, undergo changes due to adsorption of molecules on the cantilever surface or extreme changes in the cantilever environment, for example, density and viscosity. In theory, the MCLs could be modified and optimized for sensitive and interference-free detection of chemicals and physical quantities.

1.1. Resonance frequency

The resonance frequency, f, of an oscillating cantilever can be expressed as

$$f = \frac{1}{2\pi}\sqrt{\frac{K}{m^*}} \quad (1)$$

where K is the spring constant of the lever and m^* is the effective mass of the microcantilever. The effective mass can be related to the mass of the beam, m_b, through the relation: $m^* = nm_b$, where n is a geometric parameter. It is clear that the resonance frequency can change due to changes in mass as well as changes in spring constant.

1.2. MCL bending

The variation in resonance frequency of a MCL can be used for sensitive detection of adsorption of chemical and biological species from air. However, the frequency change due to mass loading on the MCL is highly dampened in aqueous medium. One of the unique characteristics of MCLs is that the device can be made to undergo bending due to molecular adsorption by confining the adsorption to one side of the MCL. This cantilever bending is due to adsorption-induced differential surface stress on the MCL. Using Stoney's formula,[3] the radius of curvature of bending of the MCL due to adsorption can be written as:

$$\frac{1}{R} = \frac{6(1-\nu)}{Et^2}\delta s \quad (2)$$

where R is the radius of curvature for the MCL, ν and E are Poisson's ratio and Young's modulus for the substrate, respectively, and t is the thickness of the MCL and δs is the film stress.

Microcantilevers have been modified by metal, metal oxides, self-assembled monolayers, self-assembled multilayers, surface conjugation chemistries, polymer coatings and polymer brushes. Functionalizing monolayers was one of the first surface modification approaches developed for microcantilever sensors. Differential surface stress between the two surfaces of a microcantilever is usually accomplished by previous deposition of a thin gold film on one surface of the microcantilever. The gold surface can be selectively functionalized by adsorption of a monolayer of thiol compounds. The receptors can then be crosslinked on the monolayer surface. However, the reported surface stresses of these sensors are in general quite small, due to both the poor characteristics of the gold surface and the surface chemistries, and thus surface modification is critical for

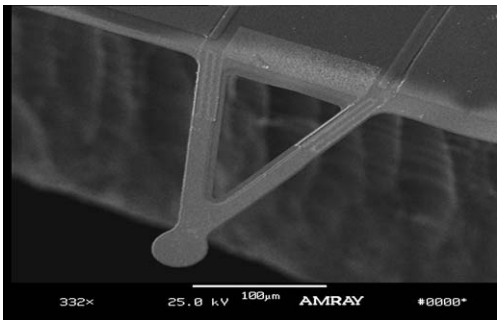

Fig. 1 SEM picture of a 200 µm long microcantilever.

Xin Yang

Dr Xin Yang received her PhD from Hunan University, China, in 2000. She has published more than 30 peer-reviewed journal papers and she is currently an associate professor in Beijing Institute of Pharmacology and Toxicology.

Hai-Feng Ji

Dr Hai-Feng Ji received his PhD degree of chemistry from Chinese Academy of Science, China, in 1996. He is currently an associate professor of Department of Chemistry, Drexel University. His research interests focus on MEMS devices, surface modification for sensors, and nanoassembly of organic molecules. He is currently a co-author of 100 peer-viewed journal articles and book chapters.

Thomas Thundat

Dr Thomas Thundat received his PhD degree of Physics from State University of New York, Albany. He is currently the Canada Excellence Research Chair in Oil Sands Molecular Engineering at University of Alberta. He had published more than 300 peer-reviewed papers and 30 patents. He has received many awards, including Nano50 award, Discover Magazine award, R&D 100 award, U.S. Department of Energy Young Scientist award, etc.

developing sensitive and reliable microcantilever sensors. The multilayer approach is one of the newer approaches for modifying microcantilever surfaces. The surface of the cantilever is modified through a positive and negative charge distribution technique. Microcantilevers that are modified with polyelectrolyte multilayers generate more surface stress than the corresponding monolayer films. There is a tremendous advantage in the use of multilayer structures in environmental remediation, and the LbL fabrication method affords a marvelous versatility of composition, size, and shape. However, so far, only enzymes have been used for the fabrication of microcantilever biosensors. Receptor-containing polyelectrolytes are not commercially available so far. Polymer brushes have the potential to provide an even higher signal-to-noise ratio. Polymer-brush-modified microcantilevers have recently been reported. They exhibited significant bending and unbending in response to alternate exposures to pure water and analytes. The amplified bending increased the signal-to-noise ratio, thereby increased the sensitivity of the microcantilever sensor. However, a nontrivial synthetic work is needed in order to prepare brushes that contain receptors for selectivity. These have been summarized in a recent review paper.[4]

A biosensor is an analytical tool consisting of biochemical recognition or active components (receptor) used in close conjunction with a platform (transducer) that convert the biochemical recognition into an electric signal. Biosensors can be classified by the receptors, such as antibodies (as in immunosensors), enzymes, DNA, membrane, or microorganisms, and transducers, such as electrochemical, optical, acoustic, or mechanical biosensors. The MCL biosensors have been reviewed in several papers.[5–9] All of these papers were classified by the receptors. This review describes individual microcantilever biosensors, ordered according to the analyte for which they have been developed. This review is intended to document all of these biosensors up to 2010, which makes it possible for the interested reader to quickly find references to specific sensors. For this purpose we have searched the literature published between 1994 and 2010 using the Science Citation Index. Physical sensors and chemical sensors without biological components are not a topic of this review. Some of the summaries are short to save space for a mini review.

2. Biosensors for toxic chemicals and heavy metal ions

2.1. Organophosphorus (OP) compounds

All nerve agents belong to the family of organophosphorus (OP) compounds, which are among the most toxic of known substances. The toxicity of these compounds arises from their irreversible binding to acetylcholinesterase (AChE) that is essential to nerve impulse responses.[10] Besides nerve gases, many pesticides also belong to the organophosphorus compound group. In an effort to feed the growing world population, the agriculture industry has increasingly taken the assistance of pesticides to increase the crop yield by fending off pest infestation. Their widespread use in agriculture may contaminate drinking water. Accordingly, there are considerable interests in the development of reliable devices for the sensitive detection of organophosphates.

Enzymes have been used for the detection of organophosphorus compounds. An AChE modified MCL was firstly used for such a purpose.[11] The gold surface of a MCL was modified first with a thiol layer and then exposed to glutaraldehyde, which acts as a crosslinker to link the AChE on the MCL surface. For different concentrations of paraoxon the bending of the MCLs varied. The MCL underwent a maximum of 7 nm bending due to the inhibition of AChE by paraoxon that slightly changed the conformation of AChE. The detection limit was approximately 10^{-7} M.

In another work, Chandana *et al.* modified MCLs with organophosphorus hydrolase (OPH)[12] by using a layer-by-layer technique for the detection of OP compounds. OPH is an enzyme that can be used for continuous monitoring of OPs in the environment. The MCL bending amplitude at equilibrium was a function of the concentration of paraoxon with the dynamic range extending from 10^{-7} to 10^{-3} M. The lower detection limit of approximately 10^{-7} M for paraoxon was an order of magnitude lower than the OPH-based potentiometric and optical biosensors based on a pH modulation. There was a good measurement-by-measurement (Fig. 2 left) and an acceptable MCL-by-MCL reproducibility as evidenced by the standard errors of 5% and 15%, respectively. OPs measured using this technique included parathion and diisopropyl fluorophosphate (DFP) in the order of sensitivity, paraoxon > DFP > parathion (Fig. 2 right). The conformational change of the OPH was most likely the main origin of MCL bending.

2.2. Dichlorodiphenyltrichloroethane (DDT)

DDT is a chlorinated compound with insecticidal properties that has been used worldwide for controlling insect pests. However, it is highly hydrophobic with great stability to physical, chemical, and biological degradation, which has resulted in the accumulation of its residues in animal and human tissues, as well as in the environment. The surface modification of a MCL was carried out in a flow-cell (Fig. 3) by exposing to cystamine and glutaraldehyde solutions, followed by a DDT hapten derivative (DDT5-BSA).[13] A bending of the MCL was observed on exposure to DDT showing the interaction between DDT and DDT5-BSA.

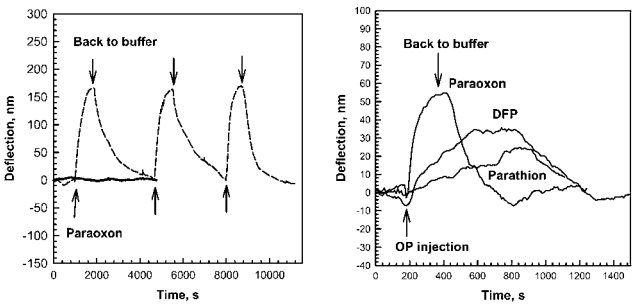

Fig. 2 Left: three replicates (dashed line) of bending responses as a function of time for a $(OPH/PSS)_3$ multilayer modified MCL to a 10^{-3} M paraoxon. Right: bending responses as a function of time for a $(OPH/PSS)_3$ modified MCL upon exposure to 10^{-3} M paraoxon, parathion, and DFP. Reprinted from ref. 11 with permission from Elsevier B.V.

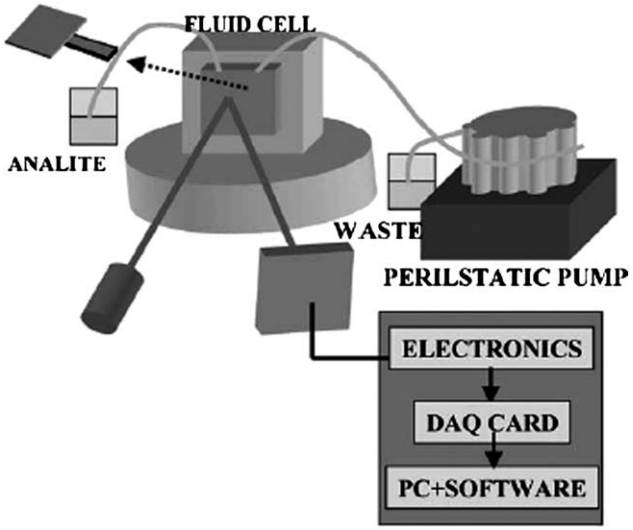

Fig. 3 Scheme of the experimental set-up. Reprinted from ref. 12 with permission from Elsevier B.V.

A concentration of 4 μg ml^{-1} of DDT has been detected using this method.

2.3. Endocrine disrupting chemicals (EDCs)

EDC can adversely affect the health of human, domestic, and wildlife species by altering or inhibiting the function of the endocrine system. Since a wide range of biological processes can be influenced and impaired by EDCs, it is crucial to screen and detect them. EDCs include a wide range of naturally occurring and synthetic chemicals. These chemicals and/or their byproducts include but are not limited to pesticides, plasticizers, detergents, pharmaceuticals, and biological compounds excreted by animals and plants. MCL sensors have been reported for the detection of EDC by using estrogen receptor and monoclonal antibodies.[14] In this study estrogen receptor alpha (ER-α), estrogen receptor beta (ER-β), and monoclonal antibodies (Ab) have been used to modify the MCLs for the detection of a particular EDC. Various EDCs have been used as below. The binding strength of EDCs with ER-β is in the order of diethylstilbestrol (DES) > 17-β-estradiol > 17-α-estradiol > 2-OH-estrone > bisphenol A > *p*,*p*′ 9-dichlorodiphenyldichloroethylene (*p*,*p*′ 9-DDE). A comparison of responses of three EDCs, which include 17-β-estradiol, 17-α-estradiol, and 2-OH-estrone, with ER-β and ER-α illustrates which estrogen receptor subtype provides the greatest sensitivity. Calibration plots for a MC functionalized with anti-17-β-estradiol Ab show responses in the range of 1×10^{-11} through 1×10^{-7} M for 17-β-estradiol with a linear portion extending over two orders of magnitude in concentration.

2.4. 2-Chloro-4-ethylamino-6-isopropylamino-*s*-triazine (atrazine)

The use of atrazine, an agricultural based pesticide, has been increasing at an alarming rate and a detection method using MCL has been reported by Suri *et al.* at very low concentration.[15]An antibody–antigen based detection method was used and a thiolated anti-atrazine antibody was immobilized on the MCL surface. This MCL was exposed to atrazine at various concentrations and it was found that the deflection of the MCL was proportional to the concentration of atrazine. The detection limit for atrazine is 4.65 pM, which is lower than the level required for agricultural products. 2,4-Dichloroacetic acid was used for a control experiment to verify the specificity of the MCL sensor and no deflection of the MCL was observed upon exposure to 2,4-dichloroacetic acid.

2.5. Trimethylamine (TMA)

A piezoresistive MCL has been used for the detection of TMA in both gaseous and liquid phases.[16] The Au side of the MCL was functionalized with a self-assembled monolayer of 11-mercaptoundecanoic acid (11-MUA). TMA binds to 11-MUA on the surface of the MCL through hydrogen bonding. The deflection of the microcantilever depends on the concentration of TMA, and the lowest detection limit is 10 mg l^{-1} for liquid TMA and 1.65 g l^{-1} for gas TMA. The selectivity of TMA with respect to ethanol, acetone, and butanone is also investigated.

2.6. Hydroflouric acid (HF)

HF is a strong acid used in applications including the petroleum industry, semiconductor processing, pharmaceutical, glass, hospital, and nuclear industries. Humans exposed to HF undergo extreme burns of the skin, although the accompanying high levels of pain may not be felt for up to 24 hours. Timothy *et al.* coated keratin on piezoresistive MCL sensors for the detection of poisonous HF gas in air.[17] The sensing material is thiolated gold nanoparticles in a keratin matrix. The whole experiment was carried out in a cell where there was no external force and liquid HF was introduced to vaporize naturally in the hydrogel. The bending of the MCLs was induced from the keratin disulfide bond broken. 2300 ppm of HF could be detected using this method.

2.7. Hg^{2+} and Zn^{2+}

Pollution of ground water and soil with toxic heavy metals like mercury, cadmium, lead, *etc.* poses a serious health risk. Because metals are non-degradable, they tend to bioaccumulate as they move up the food chain. MCLs modified by a metal-binding protein, AgNt84-6, have been used to detect a variety of heavy metals like Hg^{2+} and Zn^{2+}.[18] The modified MCLs bent on exposure to $HgCl_2$ or $ZnCl_2$ solutions. The MCLs did not respond to Mn^{2+}. A SDS-PAGE experiment was carried out to confirm the interaction between AgNt84-6 and Hg^{2+} and Zn^{2+} but not with Mn^{2+} ions. The detection limit was not reported.

2.8. Cd^{2+}

Heavy metal ions pose a major threat to nature and mankind. Cadmium, lead, and mercury metal ions are foremost among the highly dangerous environmental and occupational hazards. Cadmium is believed to have a biological half-life of greater than 10 years in the human body. Sreepriya *et al.* reported a MCL based biosensor for the detection of Cd(II) using an antigen–antibody based method.[19] The gold surface of the MCL was

modified with a multilayer of 2A81G5 antibody (Fig. 4). When the MCL was exposed to Cd(II)–EDTA–BSA antigen complex bending of the MCL was observed and the bending amplitude was proportional to the concentration of the BSA antigen complex. Selectivity experiments showed no bending of the MCL had been observed on exposure to Hg(II), Pb(II) and Mg(II) at low concentrations. A lowest detection limit for this biosensor is 10^{-9} M of Cd(II).

3. Biosensors for biochemicals

3.1. Myoglobin

Creatin kinase and myoglobin are two important cardiac biomarker proteins. As the development or absence of these proteins strongly predicts the individual mortality risk of a patient and has immediate therapeutic implications, continuous monitoring of a combination of these markers in real time would be very attractive. Arntz *et al.* developed a continuous label-free detection method for these two cardiac biomarker proteins using an array of microfabricated MCLs functionalized with covalently anchored anti-creatin kinase and anti-myoglobin antibodies.[20] The results showed that the sensitivity achieved for myoglobin detection is below 20 μg ml^{-1}. Both myoglobin and creatin kinase could be detected independently using cantilevers functionalized with the corresponding antibodies, in unspecific protein background. In another work, the anti-Myoglobin antibody (MAb) modified MCLs have been kept in a sucrose solution that can maintain the antibody's stability to up to 7 weeks.[21] In a comparative Enzyme Linked Immunosorbent Assay (ELISA) work, the MCL was coated with a fluorescein isothiocyanate (FITC) labeled secondary antibody. The fluorescent changes demonstrated the binding between myoglobin and the MAb used. Control experiments showed that BSA modified MCLs did not bend on exposure to myoglobin. Kang *et al.* showed a detection limit of 1 μM was achieved using MAb modified MCLs for recognition of myoglobin.[22] A site-directed biotinylated MAb was used in place of a randomly biotinylated MAb to improve the sensitivity of the sensor. A piezoelectric MCL modified by site-directed antibody showed a 10 fold increase in the sensitivity than those modified by the randomly bound antibodies.

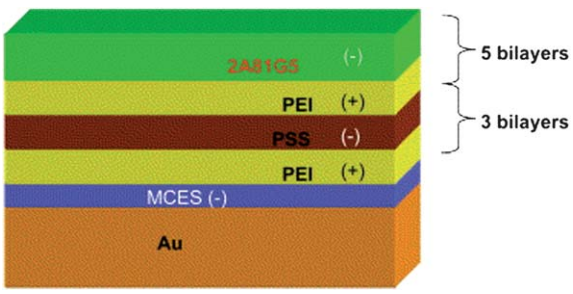

Fig. 4 LbL nanoassembly with intercalated antibody on the MCL surface. Reprinted from ref. 18 with permission from Elsevier B.V. PEI is Polyethylenimine, MCES represents 2-mercaptoethane sulfonic acid, and PSS refers to poly(sodium 4-styrenesulfonate).

3.2. Glucose

Diabetes is among the most prevalent and costly diseases in the world. Diabetes and its associated complications are leading causes of death and disability in the world. Approximately 17 million people in the United States, or 6.2% of the population, have diabetes. The diagnosis and management of diabetes require daily monitoring of blood glucose levels. Glucose oxidase (GOx) was used to develop MCL based biosensors for glucose detection. The MCL-based glucose biosensor was modified by poly-L-lysine or glutaraldehyde with GOx.[23,24] The MCL bent upon exposure to glucose solutions. A theoretical study has been made to study the effect of interaction and heat on the bending of the MCL and the work suggested that the bending was caused by the interaction of glucose with GOx enzyme. Another extensive study further proved the formational change of the proteins has the main contribution to the MCL bending.[25] In this work, the GOx was immobilized on the MCL by a layer-by-layer multilayer approach. The flow rate, concentration of glucose, reproducibility, selectivity, effect of pH, and effect of H_2O_2 have been studied. This multilayer film approach results in a 10% reproducibility for glucose measurement (Fig. 5 and 6). Selectivity experiments showed the MCLs did not respond to the same concentrations of mannose, fructose and galactose. It is noteworthy that the back side of the MCL was modified by a layer of perfluorocarbons silane (tridecafluoro-1,1,2,2-tetrahydrooctyl) triethoxysilane (TTS), to avoid the accumulation of GOx and polyelectrolytes on the backside of the MCL.[26] Another GOx based MCL sensor was modified by bovine serum albumin (BSA), glutaraldehyde (GA) and glucose oxidase (GOx). All these MCL sensors could measure glucose concentration in the range of 0.5 to 10 mM, which is of clinical interest.

In a recent study, poly(*N*-isopropylacrylamide)-*co*-poly(acrylic acid)-(3-aminophenyl-boronic acid) (PNIPAAM-*co*-PAA-PBA), glucose responsive polymer brushes were immobilized on gold substrates and microcantilever arrays for glucose sensing. The work demonstrates that stimulus-responsive polymer brushes on micromechanical cantilevers have a significantly larger bending response than that of self-assembled monolayers.[27] Huang *et al.* reported[28] a microcantilever sensor based on a similar glucose-specific polymer, poly(acrylamide-*ran*-3-acrylamidophenylboronic acid) (PAA-*ran*-PAAPBA). Glucose binds reversibly to the phenylboronic acid moiety of the polymer (Fig. 7). This results in a viscosity change of the sensing solution, which is obtained by measuring the damped cantilever vibration. The glucose response time constant of the sensor is approximately 3 min, which is shorter than a 5 min response time of commercially available continuous glucose monitoring sensors such as Guardian REAL-Time Continuous Glucose Monitoring System, Dexcom SEVEN Plus, and MiniMed Paradigm® REAL-Time System.

3.3. Fructose

Baker *et al.* showed that microcantilevers that are modified by a thiolated phenylboronic acid derivative deflected when exposed to D-(−)-fructose in aqueous buffer and the surface stress was linear in analyte concentration over the 0–25 mM range, suggesting potential applications in biomass process monitoring and glucose assay.[29]

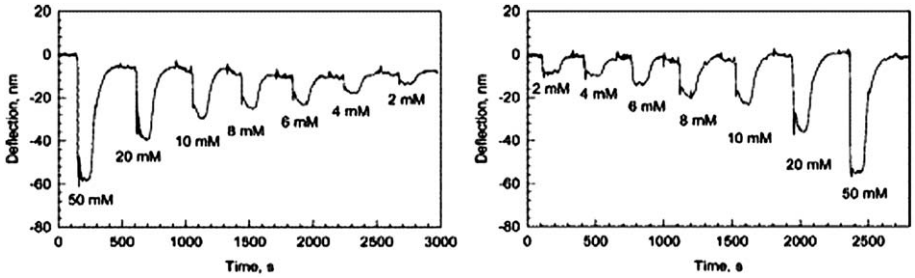

Fig. 5 Bending response of a $(PEI/GOx)_3$-modified MCL to various concentrations of glucose in a 0.01 M NaCl solution. Reprinted from ref. 24 with permission from American Chemical Society.

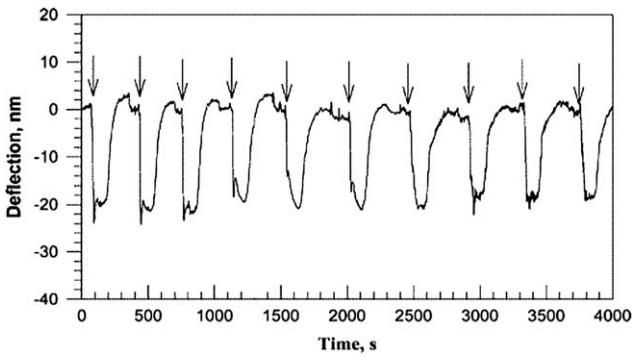

Fig. 6 Ten replications of bending responses as a function of time for a $(GOx/PEI)_3$ multilayer-modified MCL following injection of a 10 mM glucose concentration in 0.01 M NaCl solution (the injection point is indicated with arrows). Reprinted from ref. 24 with permission from American Chemical Society.

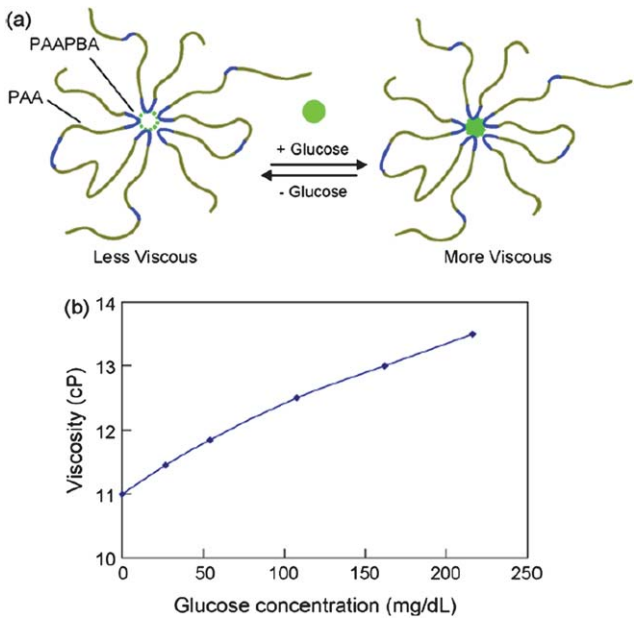

Fig. 7 Biocompatible, glucose-sensitive polymer poly(acrylamide-*ran*-3-acrylamidophenylboronic acid) (PAA-*ran*-PAAPBA). (a) The polymer composition and mechanism of interaction with glucose. (b) Glucose-induced viscosity change of a 1.9% PAA-*ran*-PAAPBA solution in PBS buffer (pH 7.4). Reprinted from ref. 27 with permission from Elsevier B.V.

3.4. C-reactive protein (CRP)

A highly sensitive CRP biosensor could provide a powerful method to predict risk of heart attack. All the developed MCL biosensors for CRP are based on antibody–antigen interaction. Lee *et al.* fabricated the CRP biosensors based on monolithic SiO_2/Ta/Pt/PZT/Pt/SiO_2 or SiO_2/Th/Pt/PZT/Pt/SiO_2 MCLs showing nanogram level per millilitre sensitivity by measuring the resonant frequency shift.[30,31] The resonance frequency change of the piezoelectric MCLs was due to a combination of mass loading and spring constant variation arisen from antigen–antibody interaction of CRP. The experimentally measured resonant frequency shift was larger than that of theoretically calculated resonant frequency by two orders of magnitude due to a compressive stress arising from CRP antigen–antibody interaction. In several other reports, Kwon *et al.* reported a CRP biosensor by using a piezoelectric thick film MCL, which exhibits the high quality factor ($Q = 15$) in a viscous liquid at a viscosity that mimics that of the human blood serum.[32] Wee *et al.* used a piezoresistive approach to detect CRP.[33] Their work demonstrated the bending and resistance change of the MCL upon its exposure to CRP solutions. Besides CRP for cardiac disease, their MCL biosensors modified by other antibodies were used for the detection of prostate specific antigen (PSA), which is a specific marker of prostate cancer and cardiac disease.

3.5. Bovine serum albumin (BSA)

Serum albumin, often referred to simply as albumin, is the most abundant plasma protein in humans and other mammals. Albumin is essential for maintaining the osmotic pressure needed for proper distribution of body fluids between intravascular compartments and body tissues. It also acts as a plasma carrier by non-specifically binding several hydrophobic steroid hormones and as a transport protein for hemin and fatty acids. Adsorption of IgG (one type of immunoglobulins produced by plasma cells) and Bovine Serum albumin (BSA) on the MCL surface was studied Moulin *et al.*[34] A very slow microcantilever bending response upon antibodies injection occurs over more than 10 h for both antibodies. This slow process was found to be not associated with adsorption of additional proteins. Two explanations included the expansion of the protein after surface adsorption and the proteins rearrangement caused by attractive (hydrophobic) protein–protein interactions. Piezoresistive and piezoelectric approaches have also been applied to study the interaction of anti-bovine serum albumin antibody (a-BSA) with

bovine serum albumin (BSA). A thin layer of BSA and 3 : 1 BSA : PEO attached to a glass slide of a MCL was used for the detection of a-BSA in solution and in an aerosol delivery, respectively. The MCL has a large and consistent deflection when exposed to the analyte, which was measured by a resistance change of the piezoresistors within the MCL. In another work, measurement of BSA was used as a model system to evaluate the effect of antibody immobilization of sensor performance by using a piezoelectric excited cantilever.[35]

3.6. Calcium (Ca^{2+})

Calcium is a biologically important ion, and its detection is important in clinical applications. Current detection techniques have limited sensitivity and dynamic range. Since only trace amounts of calcium exist in some organs (such as lungs), sensors with better sensitivity are required. A monolayer of bis(11-mercaptoundecyl) phosphate modified MCL selectively responded to Ca^{2+} with a 10^{-11} M detection limit.[36] Selective recognition of Ca^{2+} has also been realized by Calmodulin (CaM) modified MCLs with a detection limit at 10^{-7} M.[37,38] In these works, the cantilever deflection amplitudes were different under different ionic strengths, indicating different degrees of the conformational change of the proteins in these conditions. These results suggest that the conformational changes of proteins may be used to develop MCL biosensors, and the MCL system has potential for use in label free, proteins–analytes screening applications. Recently,[39] Gao *et al.* have improved the surface modification method for Ca^{2+} detection by introducing CF_3COOH in the formation of a 11-mercaptoundecanoic acid monolayer on the gold surface.

3.7. Iron (Fe^{3+})

Fe^{3+} is one essential element in biological systems. The adsorption of dopamine (DA) molecules on gold and their interactions with Fe^{3+} were studied by a MCL in a flow cell.[40] The interaction process between DA adsorbates and Fe^{3+} resulted in a deflection of the MCL. One-step and two-step reactions were observed in the profiles at low and high concentrations of Fe^{3+}, respectively. X-ray photoelectron spectroscopy and cycling voltammetry results provided complementary evidence for the result of MCL. As low as 5×10^{-10} M Fe^{3+} was detected by DA modified microcantilever with a good selectivity over other common metal ions including Zn^{2+}, Cu^{2+}, Ca^{2+}, and Mg^{2+}.

3.8. Human growth hormone (hGH)

The misuse of hGH in sport is deemed to be unethical and dangerous because of various adverse effects. Thus, it has been added to the International Olympic Committee list of banned substances. The very low concentration of hGH in the urine made its measurement difficult using classical methodology. A monoclonal hGH antibody modified MCL biosensor has been developed for the detection of hGH.[41] These MCLs were fabricated in the photoresist SU-8 and sensitized with hGH antibody. The thermal and mechanical properties of the chosen materials overcame the main limitations of gold-coated silicon cantilevers. The sensitivity of the developed polymeric nanomechanical sensor is demonstrated by real-time detection of the hGH with sensitivity in differential surface stress of about 1 mN m^{-1}. No detection limit was reported, however.

3.9. Biotin and streptavidin

Biotin is necessary for cell growth, the production of fatty acids, and the metabolism of fats and amino acids. It plays a role in the citric acid cycle, which is the process by which biochemical energy is generated during aerobic respiration. Biotin not only assists in various metabolic reactions, but also helps to transfer carbon dioxide. Biotin is also helpful in maintaining a steady blood sugar level. Shu *et al.* studied biotin–streptavidin binding interaction using MCLs.[42] A symmetric cantilever construction is employed to minimize the effects of thermal drift and the control of surface chemistry on the backside of the cantilever is demonstrated to reduce the effects of non-specific binding interactions on the cantilever. Three structurally different biotin modified cantilever surfaces are used as a model system to study the binding interaction with streptavidin. The lowest detection limit of streptavidin is found to be between 1 and 10 nM. Chen *et al.* also investigated streptavidin–biotin reaction by using their silicon piezoresistance encapsulated SiO_2 cantilever (Pr-OxiLever) and the sensors have been used for detection of avidin at 10^{-11} mol ml^{-1}.[43]

3.10. Hydrogen peroxide (H_2O_2)

Detection of hydrogen peroxide is both of industry interests and of biological importance. Yan *et al.* developed[44,39] MCL sensors for H_2O_2 detection. The MCLs were modified by horseradish peroxidase (HRP) through a layer-by-layer nanoassembly technique. These enzyme functionalized microcantilevers deflected in response to hydrogen peroxide concentrations in the nanomolar level. Lock *et al.* reported another approach[45] to modify the microcantilever for trace detection of peroxide vapors. In this method, the microcantilevers were modified by a self-assembled monolayer that undergoes chain polymerization in the presence of peroxide radicals, causing a deflection of the cantilever. The generation of radicals using a heated filament, and the resulting surface polymerization reaction, is based on initiated chemical vapor deposition chemistry.

3.11. Acetylcholine

Acetylcholine binding protein (AChBP) protein is a naturally occurring, soluble homolog of the amino terminal region of the nicotinic acetylcholine receptors (nAChR) and can be relatively easily expressed and purified. The crystal structure of this protein has been used extensively for developing homology models of Ligand gated ion channels (LGICs) and their interaction with receptor ligands. The LGICs are involved in a wide range of physiological processes including nerve conduction, regulation of blood flow, fluid balance and gastric motility. Biosensors based on the AChBP and its derivatives would have applications for *in vivo*, *in vitro* and environmental monitoring of chemicals that interact with acetylcholine, GABA (γ-Aminobutyric acid), histamine and serotonin receptors. Recently, Gao *et al.* have showed preliminary results by immobilizing the AChBP on the MCL surface for studying its interaction with acetylcholine.[39]

3.12. Protein kinase (PKA)

PKA is a protein kinase that modifies other proteins by chemically adding phosphate groups to them. Assessment of activated PKA holds a great promise in analytical applications and clinical medicine. A highly sensitive MCL biosensor assay based on an electrical measurement has been developed for detecting activated PKA.[46] The MCL surface was modified by a heat-stable protein kinase inhibitor, PKI-(5–24) peptide, for capturing PKA (Fig. 8). An increase in the resonant frequency shift was observed when the PKA binds to PKI-(5–24). Synergistic interactions of adenosine triphosphate (ATP) and the peptide inhibitor with the kinase were also investigated by a solution phase capillary electrophoretic assay, and by surface plasmon resonance technology. The detection limit can be as low as 6.6 pM, exhibiting much higher sensitivity and wider dynamic range than the conventional activity assay.

3.13. Prostate specific antigen (PSA)

PSA that is detectable in serum has proved to be a useful marker for early detection of prostate cancer and in monitoring patients for disease progression and the effects of treatment. Wu *et al.* have used a polyclonal anti-PSA antibody on the MCL[47] to detect free PSA (fPSA) concentrations from 0.2 ng ml^{-1} to 60 μg ml^{-1}, which includes the clinically relevant diagnostic PSA concentration range. The sensor could be able to detect fPSA even against the simulated background "noise" of unrelated human serum proteins such as HP and HSA or nonhuman serum protein such as bovine serum albumin (BSA), which was present at concentrations as high as 1 mg ml^{-1} (Fig. 9 and 10). In another work, a resonant cantilever sensor system for liquid-phase applications is presented.[48] The monolithic system consists of an array of four electromagnetically actuated cantilevers with transistor-based readout, an analog feedback circuit, and a digital interface. A package, which protects the electrical components and the associated circuitry against liquid exposure, allows for a stable operation of the resonant cantilevers in liquid environments. The device is operated at the fundamental cantilever resonance frequency of ~200 kHz in water with a frequency stability better than 3 Hz. The use of the integrated CMOS resonant cantilever system as a biosensor for the detection of biomarkers, such as PSA, is demonstrated. By functionalizing the MCLs with anti-PSA, the PSA has been detected at concentration levels as low as 10 ng ml^{-1} in a sample fluid.

In a recent work, Lee *et al.* enhanced the sensitivity by applying PSA polyclonal antibody (PSA pAb) as a surface stress inducer and PSA polyclonal antibody-conjugated silica nanoparticles (pAb-SiNPs) as mass inducers have been applied to the PSA-captured microcantilevers.[49] They have confirmed the sensitivity enhancement effects (2–4 times enhanced at the same concentrations) enough to detect PSA at low picogram levels (LOD of 1 pg ml^{-1} or below).

3.14. Human serum albumin (HSA)

Measurement of HSA has been used as model systems to study antibody–antigen or protein-surface interactions. Stolyarova *et al.* demonstrated that composite porous silicon-crystalline MCLs provided an excellent biocompatible material for immobilization of a wide variety of biological materials, resulting in enhanced sensitivity as demonstrated on the covalently immobilized antibody binding its complementary antigen.[50] In another work, Campbell and Mutharasan demonstrated the HSA adsorption decreased in the order of CH_3 > COOH > OH on these surfaces by using resonating MCLs.[51]

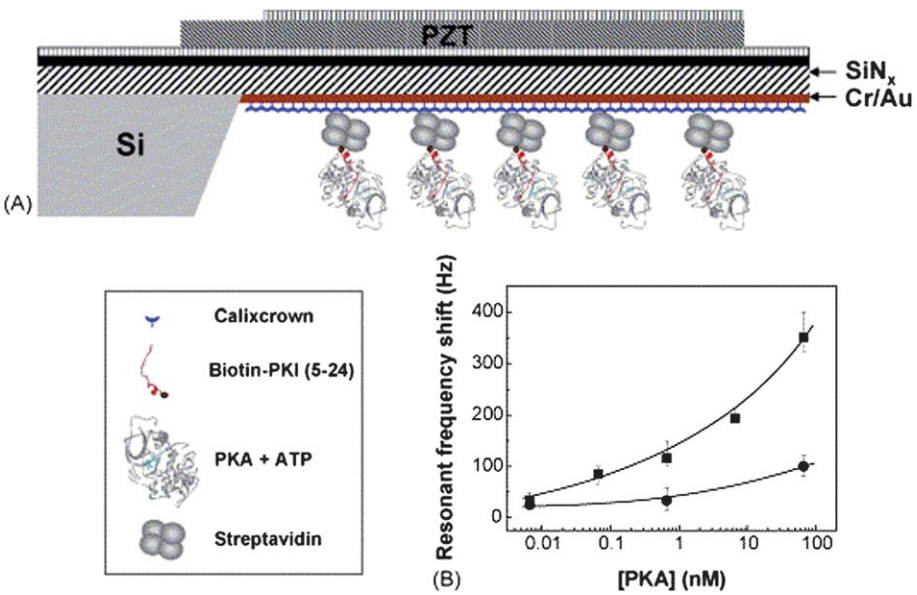

Fig. 8 Nanomechanical detection of PKA catalytic subunit on a functionalized cantilever. (A) Schematic representation of the binding on the Au surface of the PZT cantilever functionalized with biotin–PKI-(5–24) *via* the biotin–streptavidin interaction. (B) The resonant frequency was measured after incubation with varying concentrations of PKA in the presence (■) or absence of 100 μM ATP (●), and the resonant frequency shift subtracted from the frequency of the negative control cantilever was plotted as a function of the concentration of PKA catalytic subunit. Nanomechanical PZT cantilever devices (5 cm × 5 cm) with 12 arrays of 50 μm × 150 μm were used. Reprinted from ref. 45 with permission from Elsevier B.V.

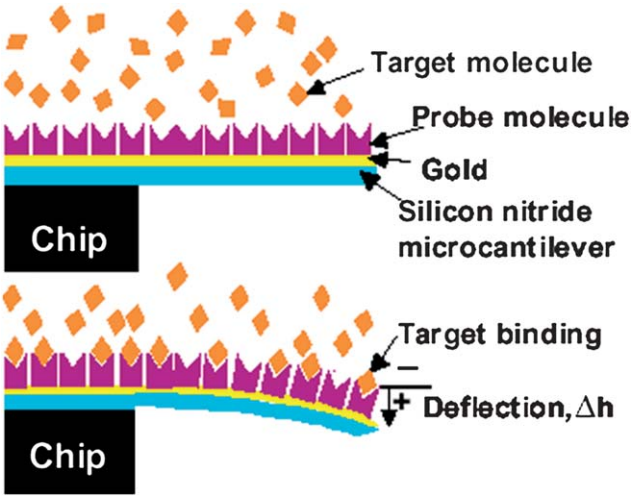

Fig. 9 Diagram of interactions between target and probe molecules on a MCL. Specific biomolecular interactions between target and probe molecules alter the intermolecular nanomechanical interactions within a self-assembled monolayer on one side of a MCL. This can produce a sufficiently large force to bend the cantilever beam and generate motion. Reprinted from ref. 46 with permission from Nature Publishing Group.

3.15. Single-chain Fv (scFv)

Single-chain Fv (scFv) is a fragment of an antibody with a molecular mass of 28 kDa and is the smallest antibody entity comprising of an intact antigen-binding site. Backmann *et al.* reported a MCL based immunosensor for the detection of different antigens using single-chain Fv (scFv) antibody fragments as a receptor.[52] The MCL was treated in such a way that one side of the MCL is a protein repellant and the other side is coated with the antibody. AR-GCN4, an antigen created by the genetic fusion of the antigenic peptide GCN4(7P14P) to an ankyrin (AR) MBP13-6, was used as a antigen to bind to the scFv on the MCL surface. The sensitivity of the sensor is ~1 nM.

3.16. Antibodies

Typically, the MCL surfaces were modified by antibodies to recognize antigens of interest. It is obvious that antigens modified MCLs can be used to detect antibodies as well, which is an indirect way to detect substances on a surface. One such example is the interaction of monoclonal antibody (mAb) in solution with a herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) immobilized on a MCL surface.[53] The sensitivity obtained was 5 μg ml^{-1}.

3.17. Cytokine

Cytokines are a category of signaling proteins and glycoproteins that, like hormones and neurotransmitters, are used extensively in cellular communication. Dutta *et al.* reported cytokine detection using nanostructured MCLs. The MCLs were functionalized using anti-human interleukin-1 beta (anti HIL 1-β) and then exposed to different concentrations of cytokine HIL 1-β in the ppb to ppm ranges.[54] A detection limit of 0.5 ppm molecules of HIL 1-β was achieved. This type of MCLs arrays functionalized with antibodies can be used in the label-free analysis of multiple proteins in a single step.

3.18. Human immunodeficiency virus (HIV)

HIV-1 is a lentivirus (a member of the retrovirus family) that can lead to acquired immunodeficiency syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections. Lam *et al.* reported monoclonal antibodies (mAbs) A32 or T8 modified MCL biosensors for detecting human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein (Env) gp120 from solution.[55] Subsequent exposure to mAb 17b, a known substrate to bind on gp120, further increased deflection of A32- but not T8-presenting MCLs. The detection limits were 8 μg ml^{-1} gp120 and 0.17 mg ml^{-1} 17b.

3.19. Human oestrogen receptor protein

Human oestrogen receptor beta protein was found in a well defined set of breast cancers. The significance of oestrogen receptor beta protein expression in breast cancers to therapy remains to be determined but the availability of detecting oestrogen receptor beta in archive material will facilitate the process. A piezoresistive MCL was reported for the detection of specific

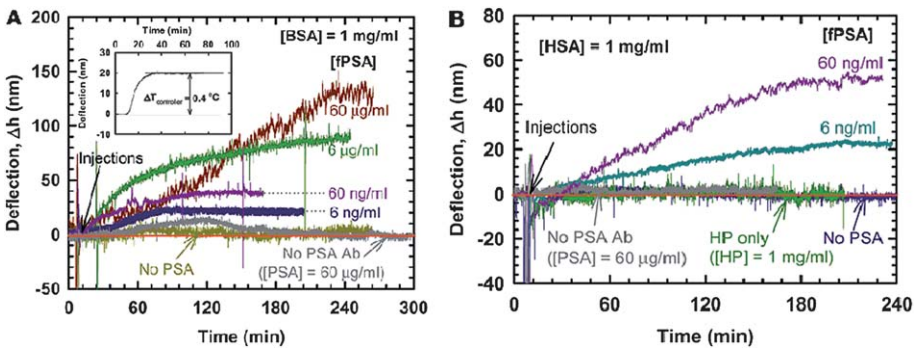

Fig. 10 Detection of free PSA (fPSA). (A) Cantilever deflection *versus* time for fPSA detection sensitivity against a background of 1 mg ml^{-1} of BSA using 200 μm long and 0.5 μm thick silicon nitride microcantilevers. fPSA detection was feasible over a concentration range 6 ng ml^{-1} to 60 μg ml^{-1} using this cantilever geometry. (B) Specificity of fPSA detection against a high background of human serum proteins, namely, human serum albumin (HSA) and human plasminogen (HP). Reprinted from ref. 46 with permission from Nature Publishing Group.

protein conformations.[56] In this work, oestradiol (E2) was pre-bound on a human oestrogen receptor (EPα-LBD) protein, which changes the conformation of the protein. The EPα-LBD and EPα-LBD-E2 complex were distinguished by the MCLs modified by conformation-specific peptides α/βI (Ser-Ser-Asn-His-Gln-Ser-Ser-Arg-Leu-Ile-Glu-Leu-Leu-Ser-Arg), which recognizes EPα-LBD-E2 complex and α/βII (Ser-Ala-Pro-Arg-Ala-Thr-Ile-Ser-His-Tyr-Leu-Met-Gly-Gly), which recognizes EPα-LBD (Fig. 11). This is an indirect and complementary approach for studying conformational change in proteins. It could be used when the conformational change of the protein is too small and does not produce detectable bending of the MCL. A sensitivity of 2.5 nM has been obtained using this method.

3.20. Lipid bilayer

Pera *et al.* demonstrated that MCLs can sense the formation of supported phospholipid bilayers on a surface and can monitor changes in mechanical property of lipid bilayers.[57] The formation of bilayers led to a bending of the MCLs of 70–590 nm comparable to a surface stress of 27–224 mN m^{-1}. Physisorption of bilayers of 1,2-dioleoyl-*sn-glycero*-3-phospho-choline (DOPC) on the silicon oxide surface of cantilevers led to a tensile bending of about 70 nm. The formation of chemisorbed bilayers of mixed thiolated 1,2-dipalmitoyl-*sn-glycero*-3-phosphothioethanol (DPPTE) and DOPC on the gold side of cantilevers led to a compressive bending of nearly 600 nm depending on the ratio of DPPTE to DOPC. The results demonstrate that MCL sensors with immobilized bilayers can be used as model systems to investigate mechanical properties of cellular membranes and may be used for screening of membrane processes involving modification, lateral expansion, or contraction of membranes.

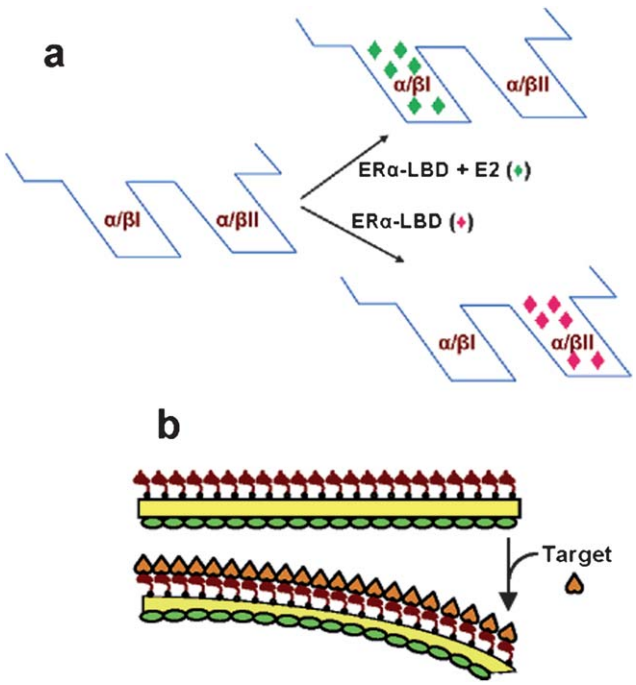

Fig. 11 (a) Schematic drawing showing a two-cantilever configuration, the α/βI attached on one cantilever and the α/βII on the other, and the preferential binding of ERα-LBD, E2-bound or free, onto the α/βI and α/βII, respectively. (b) A cartoon showing the sensor layer on top of the surface and the blocking layer at the bottom surface of a cantilever and its bending upon target binding onto the top sensing surface. Reprinted from ref. 55 with permission from American Chemical Society.

3.21. Low density lipoproteins (LDL)

It is of clinic interest to detect and differentiate between low-density lipoproteins (LDL) and their oxidized form (oxLDL). This is because their uptake from plasma, principally favored to the oxidized form, is believed to be responsible for the accumulation of cholesterol in the aortic intima and is associated with the first stage of coronary heart disease. A LDL and oxLDL differentiation by MCL sensors as shown in Fig. 12.[58]

3.22. α-Amino acid and peptides

The α-amino acids represent one of the most important classes of substances in nature that incorporate a stereogenic center and, therefore, exemplify an excellent model system to demonstrate chiral discrimination. Enantioselective antibodies modified cantilevers have been investigated for their stereoselective detection of trace amounts of an important class of chiral analytes, the α-amino acids.[59] This is the first demonstration of chiral discrimination using highly scalable microelectromechanical systems. The antibodies used were raised in such a way that they selectively bind to either D- or L-*R*-amino acids. The temporal response of the cantilever (Δdeflection/Δtime) is linearly proportional to the analyte concentration and allows the quantitative determination of enantiomeric purity up to an enantiomeric excess of 99.8%. Besides amino acid, the interaction of peptides with antibodies was also studied.[60]

3.23. Cyclin-dependent protein kinase (CDK2)

CDK2 is an important indicator for the cellular decision making for proliferation, during cancer. A MCL based sensor was developed for the detection of human CDK2.[61] The Au surface of the MCL was modified with Stefin A Triple Mutant (STM) which is known to interact with CDK2. The cell lysate that constitutes CDK2 was directly exposed to the MCL surface and a lowest concentration of 80 nM CDK2 was detected.

3.24. Clenbuterol and chloramphenicol

Tan *et al.* developed a MCL sensor for the detection of β-adrenergic agonist clenbuterol and the antibiotic chloramphenicol.[62] The Au side of the MCL was modified with protein A and antibodies for clenbuterol and chloramphenicol. These MCLs were exposed to various concentrations of clenbuterol and chloramphenicol and a detection limit of 0.1 ng ml^{-1} for clenbuterol and 0.2 ng ml^{-1} for chloramphenicol was observed. Such LODs were better than that of the corresponding direct competitive enzyme-linked immunosorbent assay (dcELISA). The results suggest that microcantilever immunosensors are suitable for detection of small molecules, and the assay sensitivity is mainly related to the quality and activities of the antibodies.

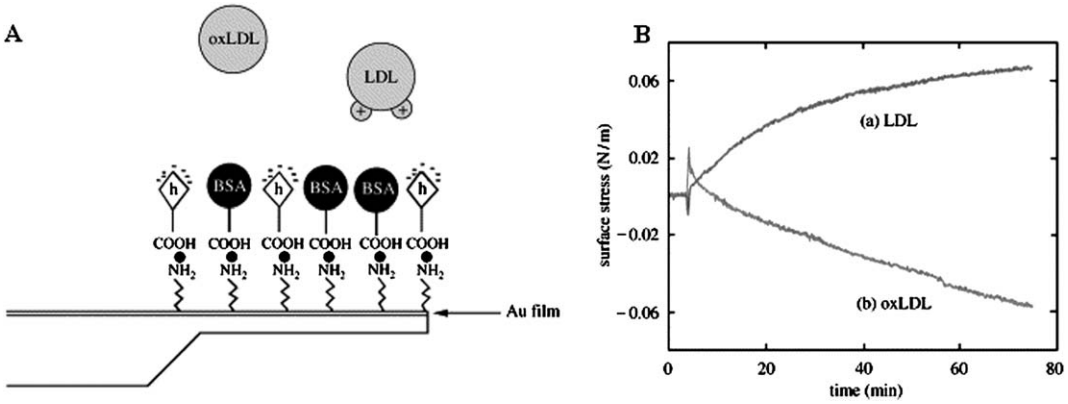

Fig. 12 A) Schematic of the functionalization of the cantilever top surface with heparin. (B) Comparison of the effects triggered on the cantilever by an 10 μl injection of (a) 3.5 mg ml^{-1} LDL and (b) 0.3 mg ml^{-1} oxLDL. Reprinted from ref. 57 with permission from Elsevier B.V.

3.25. Human epidermal growth factor receptor 2 (Her2)

Her2 is an antigen that is over expressed in 20–25% of invasive breast cancers. The Her2 concentration is 2–15 ng ml^{-1} in normal mammalians and 15–75 ng ml^{-1} in breast cancer patients. Capobianco *et al.* reported[63] the detection of human epidermal growth factor receptor 2 (Her2) with piezoelectric MCL sensors in a background of 1 mg ml^{-1} bovine serum albumin. The MCLs were modified with single-chain variable fragment (scFv). A detection limit of 5 ng ml^{-1} was obtained.

3.26. Immunoglobulin G (IgG)

IgG is a one type of immunoglobulins produced by plasma cells. In the early study of MCL sensors, Moulin *et al.* investigated the MCL responses on the adsorption of IgG on MCL.[64] Hill *et al.* reported the effect of concentration, reaction time, and pH for these reagents on the magnitude of the MCL responses using an anti-immunoglobulin G (anti-IgG) receptor. They also report the application of optimum and non-optimum conditions to detect thyroid disrupting chemicals (TDCs) using MCLs functionalized with the transport protein thyroxine-binding globulin. Selectivity patterns are reported for several TDCs and sensitive detection of thyroxin at sub-nM levels is demonstrated.[65] Recently, detection of IgG was used as a model system to show the enhancement of capture antibody immobilization under an electric field (Fig. 13)[66] and biofunctional polymer coatings.[67]

Characterization and control of proteolysis of peptides by specific cellular protease are *a priori* requisite for effective drug discovery. Kwon *et al.* reported the *in situ* monitoring of proteolysis of the peptide chain attributed to protease (Cathepsin B).[68] Specifically, a peptide chain polyethylene glycol-tetrapeptide GlyPheLysGly (PEG-GFLG) was immobilized on the surface of the MCL (Fig. 14) using the cross-linker EDC-NHS and the detection is based on the measurement of resonant frequency shift arising from proteolysis of peptides. Cathepsin B Protein (CTSB) catalytic sites Cys25 and His159 cleave PEG-GLFG peptide chain, leading to proteolysis of GFLG. This reduces the mass on the MCL surface and in turn a change in the resonant frequency. Different concentrations of CTSB were used to check for detection limit and it was found to be 0.28 μm. The author mentioned that this implies that the nanomechanical biosensor enables the characterization of specific cellular protease such as its kinetics.

3.27. Alpha-fetoprotein (AFP)

AFP is a marker for hepatocellular carcinoma (HCC). AFP levels of 10–1000 ng ml^{-1} in the blood serum can be related to early stage of HCC cancer. Liu *et al.* developed a sensor for early stage prognosis and disease diagnosis of HCC based on

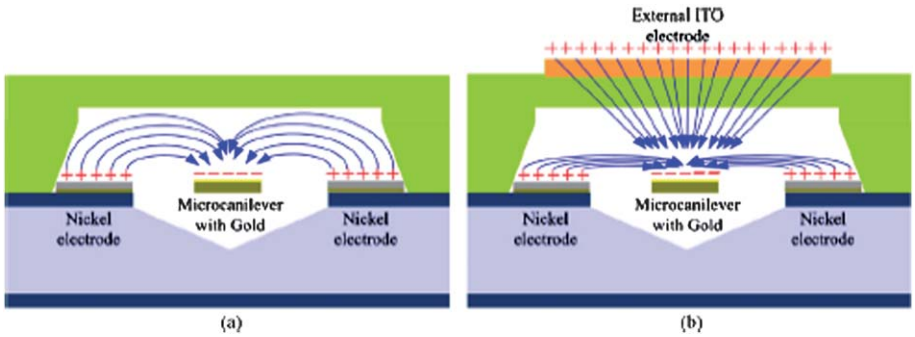

Fig. 13 (a) In-plane electric field from the nickel electrodes to the gold layer of the sensing microcantilever. (b) An additional external electric field in case (a), as an electric potential is applied between an outer ITO electrode and a gold layer of the microcantilever. Reprinted from ref. 65 with permission from Elsevier B.V.

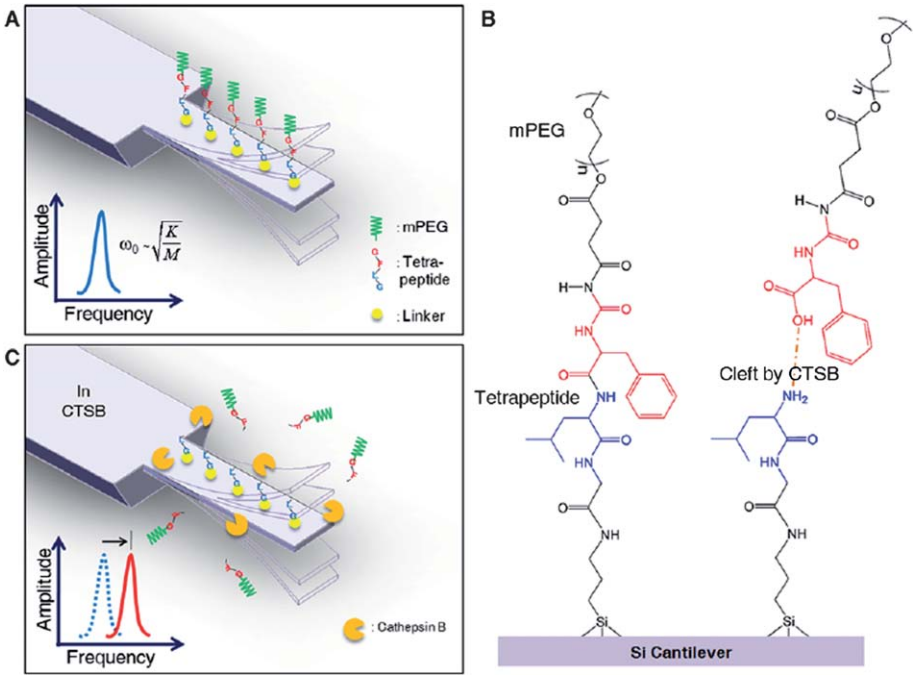

Fig. 14 Schematic illustration of nanomechanical, *in situ* monitoring of proteolysis of peptide chains on the surface by using resonant microcantilever immersed in a liquid. (A) A microcantilever was functionalized by peptide chains (PEGylated GFLG) through aminated cantilever surface. (B) Chemical structure of PEGylated GFLG (GlyPheLysGly) chains on a cantilever and proteolyzed peptides by protease (*i.e.* PEG-GF and LG sequence immobilized on a cantilever). (C) When GFLG peptides immobilized on a cantilever was exposed to protease (CTSB) in acidic medium, catalytic Cys25 and His159 of CTSB induce the successful cysteine protease of GFLG, leading to proteolysis of GFLG. Such proteolysis phenomenon reduces the overall mass of a cantilever, and consequently, the increase of the fundamental resonance. Reprinted from ref. 67 with permission from PLoS ONE.

a resonant MCL.[69] The anti-AFP-modified microcantilever is operated in a rotating resonance mode to improve sensitivity and resolution to specific mass adsorption. The detection limit is 2 ng ml^{-1}.

3.28. Angiopoietin-1 (Ang-1)

Ang-1 is a possible marker in tumor progression. Ricciardi *et al.* reported a microcantilever sensor to detect Angiopoietin-1 masses of the order of few hundreds of pictograms with less than 0.5% of relative uncertainty.[70] The microcantilevers were modified by receptors to study receptor–ligand and antibody–antigen interactions. They showed that the evaluation of the protein surface density (number of molecules per cm^2) could reveal interesting features concerning the multimerization state of the targeted protein.

3.29. Activated leukocyte cell adhesion molecule (ALCAM)

ALCAM is a model marker for tumor progression. The physiological concentration of 10–1000 ng ml^{-1} is found in mammalian serum. von Muhlen *et al.* demonstrated that a suspended micro-channel resonators based microcantilever can sense ALCAM in undiluted serum with a limit of detection of 10 ng ml^{-1}.[71] The MCLs were modified with a carboxybetaine-derived polymer, which acts as ultra low fouling and functionalizable surface coatings (Fig. 15).

4. Biosensors for microorganisms detection

4.1. Vaccinia virus

Vaccinia virus is well known for its role as a vaccine that eradicated the smallpox disease, making it the first human disease to be successfully eradicated by mankind. An embedded piezoresistive MCL has been used for the detection of aerosol and solution based vaccinia virus (Fig. 16).[72] The MCL was modified by a composite consisting of poly(ethylene oxide) (PEO), combined with vaccinia antibody as the active sensing material. The interaction of vaccinia virus with the antibody resulted in a swelling in the polymer layer and the subsequent changes in the resistance, which is proportional to the concentration of the vaccinia virus. A single virion can be detected using this method.

4.2. Aspergillus niger

Airborne fungus *Aspergillus niger* is gaining importance as a spoilage agent, main air contaminator in industry, and serious pathogen of humans. Nugaeva *et al.* demonstrated a MCL biosensor for detection of vital fungal spores of *Aspergillus niger*.[73] The biosensor is based on anti-*Aspergillus niger* polyclonal antibodies modified silicon MCL arrays operated in dynamic mode. The change in resonance frequency of the sensor is a function of mass binding to the cantilever surface. The detection limit of the sensor is 10^3 CFU ml^{-1}. Mass sensitivity of the cantilever sensor is 53 pg Hz^{-1}.

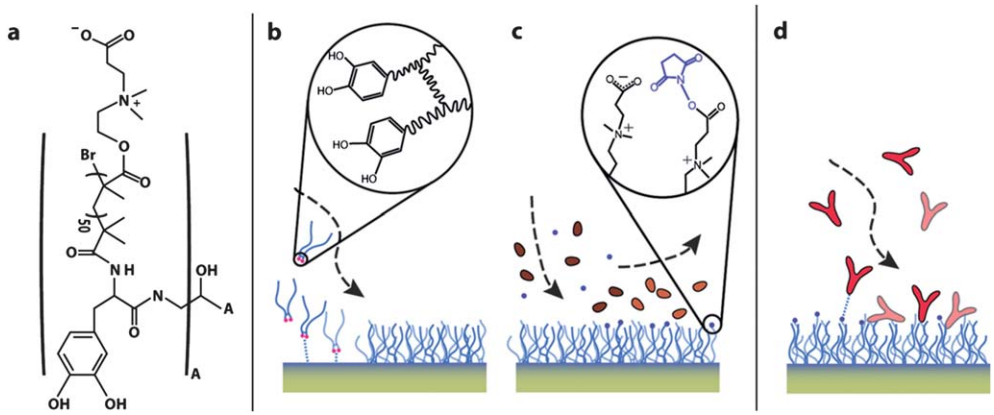

Fig. 15 Antibody functionalization schematic. (a) The structure of the carboxybetaine-derived polymer is shown; "A" represents the structure inside the large parentheses, two of which are connected by an R–COH–R bridge. The polymer adsorbs directly onto the thin SiO_2 layer of the Si microcantilever surfaces (b), immobilizing a monolayer of 275–360 ng cm^{-2}. (c) Terminal carboxylic acids are transformed to reactive NHS esters by injection of a mixture of NHS and EDC. (d) NHS esters react with primary amines on IgG antibodies to covalently bind them to the surface. Reprinted from ref. 70 with permission from American Chemical Society.

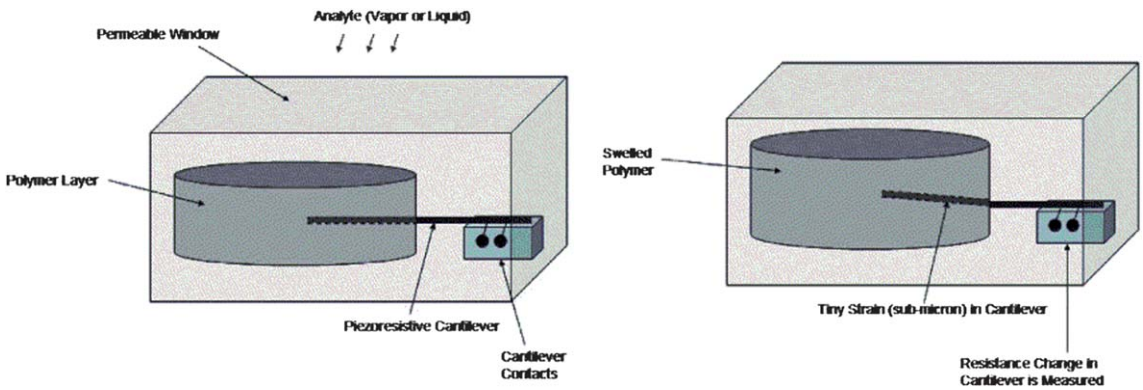

Fig. 16 Basic operation of EPM sensor. Exposure to analyte causes volumetric change in sensing material. This change is measured by embedded microcantilever. Reprinted from ref. 71 with permission from Elsevier B.V.

4.3. Baker's yeast

Baker's yeast is the common name for the strains of yeast commonly used as a leavening agent in baking bread and related products, where it converts the fermentable sugars present in the dough into carbon dioxide and ethanol. Li *et al.* developed a novel magnetostrictive MCL sensor concept.[74] The detection model is baker's yeast. The results demonstrate the feasibility of this sensor as a high performance biosensor platform. The oscillation of the magnetic material is wirelessly detected by a pickup coil. The oscillation amplitude is correlated to the resonant frequency. Compared to traditional MCL, the new sensor design has several advantages, including: (1) remote/wireless driving and sensing; (2) easy to fabricate. More importantly, it is experimentally found that the quality merit factor (Q value) of MSMC can reach more than 250, which is much higher than other cantilevers. A concentration of 1 mg ml^{-1} of yeast cells has been detected using this method.

4.4. Bacillus anthracis

Detection of airborne *Bacillus anthracis* spores an etiology agent of anthrax has gained significant interest since the anthrax spore mailing. Several MCL based sensors have been developed for detecting *Bacillus anthracis*. Fu *et al.* continued their magnetostrictive MCLs (MSMC) for detection of *Bacillus anthracis* spores.[75] The MSMC surface was modified with a biological phage having peptide EPRLSPHS on the surface to recognize *Bacillus anthracis*. By monitoring the shift in the resonance frequency of the MSMCs, the spores were detected in a real-time manner and a detection limit of 10^5 spores ml^{-1} was obtained for the MSMCs used in this research. Higher sensitivity is expected for the MSMCs with smaller size.

Davila *et al.* demonstrated an antibody modified MCL for detection of *Bacillus anthracis* Sterne spores in air and liquid.[76] They demonstrate that as few as 50 spores on the MCLs can be detected in water using the thermal noise as excitation source. Measurement sensitivity of 9.23 Hz fg^{-1} for air and 0.1 Hz fg^{-1} for water was obtained. McGovern *et al.* also further studied antibodies modified MCLs for detection of *Bacillus anthracis* spores.[77] They demonstrated specific detection of *Bacillus anthracis* (BA) spores from that of close relatives, such as *B. thuringiensis* (BT), *B. cereus* (BC), and *B. subtilis* (BS) by varying the flow speed of the sampling liquid over the surface of a piezoelectric MCL sensor. Spore binding to the anti-BA spore IgG coated MCL surface is determined by monitoring the

resonance frequency change in the sensor's impedance *versus* frequency spectrum. Their work showed that the change of resonance frequency from increasing to decreasing occurred at a lower fluid speed for the spores of BT, BC, and BS than BA. This trend reduces the cross-reactivity ratio of BC, BS, and BT to the anti-BA spore IgG immobilized PEMS. This cross-reactivity ratio of 0.05 was essentially negligible considering the experimental uncertainty. No detection limit was mentioned. Campbell and Mutharasan also reported the detection of BA from a mixture of BA, BT, BC, and BS by using their PZT-anchored piezoelectric excited millimetre-sized cantilever (PAPEMC) (Fig. 17).[78] The surface of PAPEMC was modified by a rabbit polyclonal antibody (anti-BA). They have detected BA from a concentration of 1 : 1000 ratios (BA: BT + BC). The detection limit was 38 BA spores l^{-1} of air in near real-time with an estimated lower limit of detection of ~5 spores l^{-1} of air in the configuration tested.[79]

4.5. Bacillus subtilis

B. subtilis may contaminate food but rarely causes food poisoning. *B. subtilis* produces the proteolytic enzyme subtilisin. *B. subtilis* spores can survive the extreme heating that is often used to cook food, and it is responsible for causing *ropiness*—a sticky, stringy consistency caused by bacterial production of long-chain polysaccharides—in spoiled bread dough. Dhayal *et al.* demonstrated a short peptide ligand modified MCL for efficient capture of *Bacillus subtilis* (a simulant of *Bacillus anthracis*) spores in liquids.[80] On an eight-MCL array chip, four MCLs were coated with binding peptide (NHFLPKV-GGGC) and the other four were coated with control peptide (LFNKHVP-GGGC). The four MCLs with binding peptide showed a substantial change in the deflection and resonance frequency as compared to that for control peptide MCLs. Further confirmation was obtained by subsequent examination of the microcantilever arrays under a dark-field microscope. Applications of this technology will serve as a platform for the detection of pathogenic organisms including biowarfare agents. A BS spore concentration of 1×10^5 spores ml could be detected using this method.

4.6. Enterohemorrhagic *Escherichia coli* serotype (*E. coli*)

E. coli O157 : H7, first recognized in 1982 in the United States, is an epidemiologically significant cause of food borne disease worldwide. *E. coli* O157 : H7 can readily contaminate ground beef, raw milk, poultry products, fresh apple cider, cold sandwiches, vegetables, and drinking water supplies. It can be transmitted efficiently from person to person not only *via* contaminated food, but also by sharing contaminated facilities. Zhang and Ji developed the first silicon microcantilever sensor for the detection of *Escherichia coli* (*E. coli*) O157 : H7.[81] The microcantilever was modified by anti-*E. coli* O157 : H7 antibodies on the silicon surface of the cantilever. When the aquaria *E. coli* O157 : H7 positive sample is injected into the fluid cell where the microcantilever is held, the microcantilever bends upon the recognition of the *E. coli* O157 : H7 antigen by the antibodies on the surface of the microcantilever. The detection limit of the sensor was 1×10^6 cfu ml^{-1} when the assay time was <2 h. Using a self-excited piezoelectric MCL, Campbell *et al.* showed the frequency method could improve the sensitivity by detecting a low concentration of 700 bacteria ml^{-1}.[82] The sensitivity has been further improved to a detection limit of 10 cells ml^{-1} and 1 cell ml^{-1}.[83]

Gfeller *et al.* investigated the *E. coli* bacterial growth on a MCL by using the oscillating mode of the MCL.[84] The MCL was coated with a layer of common nutritive layer. The basis of the detection scheme is the resonance frequency change as a function of the increasing mass during the cell growing process. The detection limit is 100 *E. coli* cells and the sensor detected active growth of *E. coli* cells within 1 h. Selectivity can be achieved by adding antibiotics to the nutritive layers. This new sensing method for the detection of selective bacterial growth

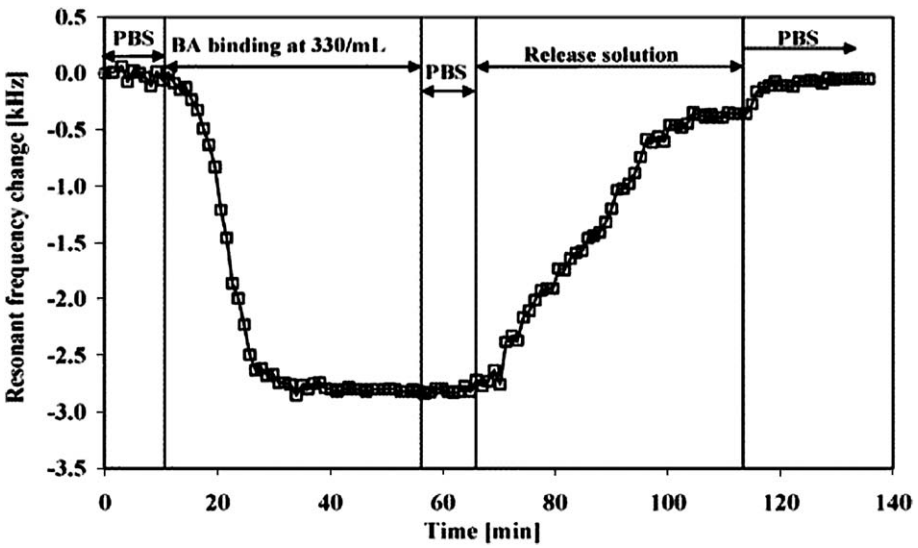

Fig. 17 Transient frequency response of PAPEMC sensor to the binding of 333 BA spores ml^{-1} and the release of the bound spores by exposure to low pH buffer, pH 1.85. Reprinted from ref. 77 with permission from American Chemical Society.

allows future applications in, *e.g.*, rapid antibiotic susceptibility testing.

Capobianco *et al.* examined a piezoelectric coating PbMg1/$3Nb_2/3O_3$ 0.63–$PbTiO_3$ 0.37 PMN-PT/tin to on a MCL sensor for *in situ* recognition of *E. coli* O157 : H7.[85] The mass detection sensitivity $m/f = -3 \pm 2 \times 10^{-12}$ g Hz^{-1} and the concentration sensitivity is better than 100 cells ml^{-1} in liquid.

Fu *et al.* developed a sensor (Fig. 18) for *E. coli* by using a magnetostrictive microcantilever (MSMC).[86] The MSMC surface was modified by polyclonal antibody and the detection limit is 10^5 cfu ml^{-1}.

4.7. Salmonella typhimurium

Salmonella typhimurium multiplies in the gastrointestinal tract of many animal species where it usually causes no disease, but in humans its growth causes gastroenteritis. Recently, Zhu *et al.* demonstrated an antibody modified MCL for *in situ* recognition of *Salmonella typhimurium*.[87] The MCL was coated with a lead zirconate titanate (PZT) and partially dipped in the suspensive without electrically insulating the PZT. A mass detection sensitivity of $\Delta m/\Delta f = -5 \times 10^{-11}$ g Hz^{-1} was achieved and the concentration sensitivities were 1×10^3 and 500 cells ml^{-1} in 2 ml of liquid with a 1 and 1.5 mm dipping depth, respectively. It is more than two orders of magnitude lower than the infectious dose and more than one order of magnitude lower than the detection limit of a commercial Raptor sensor.

A new type of cantilever has been reported for the *in situ* detection of various compounds or microorganisms. *Salmonella typhimurium* (ST) has been used as an example for this purpose. The cantilever surface was modified in such a way that it had the *Salmonella typhimurium* antibody on its surface and then it was dipped in a solution containing of *Salmonella typhimurium* up to 0.8 mm depth. The mass detection sensitivity of the cantilever observed was $\Delta m/\Delta f = -5$ to 6×10^{-11} g Hz^{-1} and the number of cells captured on the surface was also counted.

4.8. Ricin

Ricin, a biotoxin extracted from the seeds of the castor bean plant (*Ricinus communis*), is poisonous to people, animals, and insects. A few milligrams can kill an adult human being. Ricin is a potent cytotoxin that works by getting inside cells and preventing them from making essential proteins. Ricin can poison people by inhalation or ingestion. The symptoms of human poisoning, abdominal pain and severe dehydration, occur within hours of ingestion, and death occurs within 36 to72 hours. The results showed that a detection limit of 40 parts per trillion for ricin has been achieved by using the deflection mode of the MCLs.[88]

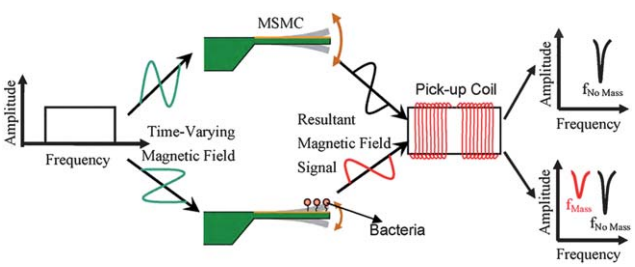

Fig. 18 Schematic illustration of the operation principle of MSMC as transducer for biosensor. Reprinted from ref. 85 with permission from Elsevier B.V.

4.9. Tularemia

Tularemia (*Francisella tularensis*) is a small (0.2 μm × 0.2–0.7 μm), pleomorphic, poorly staining, nonmotile, Gram-negative aerobic coccobacillus. It is one of the most infectious pathogenic bacteria known. *Francisella tularensis* biovar *tularensis* (type A) may be highly virulent in humans and animals, produces acid from glycerol, demonstrates citrulline ureidase activity, and is the most common biovar isolated in North America. Virulent, streptomycin-resistant *F. tularensis* strains have been examined in biowarfare agent studies. Yan *et al.* have demonstrated MCL sensors for the detection of tularemia (*F. tularensis*) by using the deflection approach.[88] The MCLs on which antibodies were immobilized were used for both experiments. A detection limit of less than 1×10^3 organisms ml was determined for *F. tularensis* after exposure at room temperature.

4.10. Cryptosporidum

Cryptosporidium parvum oocyst is a waterborne parasitic protozoan that can cause severe illnesses in humans called cryptosporidiosis. Campbell and Mutharasan demonstrated the detection of *Cryptosporidium parvum* using the piezoelectric-excited millimetre size cantilever.[89] The cantilever was functionalized with immunoglobulin M (IgM). The detection of 100, 1000, and 10 000 oocysts ml^{-1} was achieved with a positive sensor response in less than 1 min.

Besides these sensors, there are also MCL based biosensors for physical properties. One such example is MCLs modified bacteriorhodopsin (BR) for light detection. This device can be used to directly convert the solar energy to mechanical energy. In one report,[90] purple membranes from *Halobacterium salinarum* were deposited electrophoretically on platinum-coated MCLs. By illuminating the bacteriorhodopsin (BR)-containing purple membranes, the protein undergoes its photochemical reaction cycle, during which a conformational change occurs in the protein, changing its shape and size. The on–off change occurs in millisecond. Using polarized light, the orientation of the motion was detected, relative to the transition moment of the retinal. In air, the smaller dilatation of the protein could be explained as a smaller conformational change than that in water because the dried protein is more rigid. The average energy per BR molecule contributing to MCL bending was estimated to be 195 kT (in terms of the Boltzmann energy at 295 K). This calculated energy provides an estimate of the order of magnitude and compares to the energy of a photon of 84 kT with a wavelength of 580 nm, which triggers the photocycle of BR.

In another paper,[91] the same BR protein model was used for the photocycle and the author attributed the MCL bending to proton release caused by the conformational change in BR (Fig. 19).

4.11. Bacterial virus T5

Membrane proteins are central to many biological processes, and protein receptors–ligands interactions are of fundamental

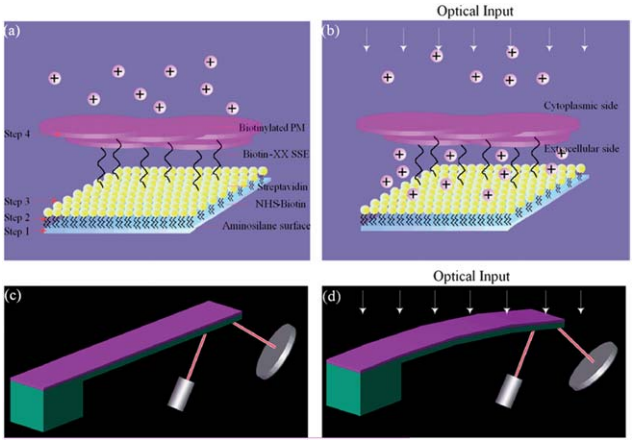

Fig. 19 Schematic illustration of assembling the purple membrane on the microcantilever surface and the experimental system. (a) Procedures for assembling the purple membrane on the solid surface. (b) Illuminated by visible light, bR pumps the proton across the membrane to the interface between the microcantilever surface and the purple membrane. (c) The optical beam deflection is used to measure the deflection of the microcantilever. (d) Illuminated by visible light, the beam bends away from the purple membrane side. Reprinted from ref. 90 with permission from Institute of Physics Publishing.

importance in medical research. Braun *et al.* reported the detection of bacterial virus T5 at subpicomolar concentrations with a microcantilever that is modified by a protein receptor, the FhuA receptor of *E. coli*.[92] A detection limit of 300 fM was observed with good repeatability. These experiments demonstrate the potential of resonating microcantilevers for time-resolved detection of membrane protein–ligand interactions in a micro-array format.

4.12. Severe Acute Respiratory Syndrome associated coronavirus (SARS-CoV)

SARS is a viral respiratory disease. Velanki and Ji demonstrated[93] the feasibility of detecting Severe Acute Respiratory Syndrome associated coronavirus (SARS-CoV) using microcantilever technology by showing that Feline Coronavirus (FIP) type I virus can be detected by a microcantilever modified by Feline coronavirus (FIP) type I anti-viral antiserum. A microcantilever modified by FIP type I anti-viral antiserum was developed for the detection of FIP type I virus. The detection limit of the sensor was 0.1 μg ml^{-1} when the assay time was <1 h.

5. Conclusion

To make information on individual biosensors easily accessible, the biosensors in this review are classified according to the analytes. We did this to aid researchers to locate relevant references. Biosensors based on microcantilevers have been described for nearly 50 analytes. In some cases, several different receptors are used for the same analyte. This number of analytes reveals the success of the microcantilever approach to chemical and biological sensing. However, it is too early to summarize and compare the selectivity, detection range, lifetime of the sensors since many publications did not provide the information. This may be related to the fact that the microcantilever-based sensors have not been commercialized. The development and improvement of microcantilever sensors that will allow measurements of analytes in fields and in complex real-life samples will remain a central issue in the development of such sensors.

Acknowledgements

We would like to thank National Institutes of Health (NIH) Grant Number 1R01NS057366 and National Natural Science Foundation of China (NSFC) 20728506/B05.

References

1 J. K. Gimzewski, C. Gerber, E. Meyer and R. R. Schlittler, Observation of a chemical reaction using a micromechanical sensor, *Chem. Phys. Lett.*, 1994, **217**, 589–594.
2 T. Thundat, R. J. Warmack, G. Y. Chen and D. P. Allison, Thermal and ambient-induced deflections of scanning force microscope cantilevers, *Appl. Phys. Lett.*, 1994, **64**, 2894–2896.
3 G. G. Stoney, The transition of metallic films deposited by electrolysis, *Proc. R. Soc. London*, 1909, **82**, 172–175.
4 H. F. Ji and B. Armon, Approaches to increasing surface stress for improving signal-to-noise ratio of microcantilever sensors, *Anal. Chem.*, 2010, **82**, 1634–1642.
5 X. Yan, H.-F. Ji and T. Thundat, Microcantilever (MCL) biosensing, *Curr. Anal. Chem.*, 2006, **2**, 297–307.
6 L. G. Carrascosa, M. Moreno, M. Alvarez and L. M. Lechuga, Nanomechanical biosensors: a new sensing tool, *TrAC, Trends Anal. Chem.*, 2006, **25**, 196–206.
7 D. Chen and C. Ziegler, Cantilever-based sensors, *Encycl. Nanosci. Nanotechnol.*, 2004, **1**, 499–516.
8 N. V. Lavrik, M. J. Sepaniak and P. G. Datskos, Cantilever transducers as a platform for chemical and biological sensors, *Rev. Sci. Instrum.*, 2004, **75**, 2229–2253.
9 K. M. Goeders, J. S. Colton and L. A. Bottomley, Microcantilevers: Sensing Chemical Interactions via Mechanical Motion, *Chem. Rev.*, 2008, **108**, 522–542.
10 L. E. Tammelin, Methyl-Fluorophosphorylcholines. Two synthetic cholinergic drugs and their tertiary homologues, *Acta Chem. Scand.*, 1957, **11**, 859–865.
11 X. Yan, T. Tang, H.-F. Ji, Y. Lvov and T. Thundat, Detection of organophosphates using an acetylcholinesterase (AChE) coated microcantilever, *Instrum. Sci. Technol.*, 2004, **32**, 175–183.
12 K. Chandana, D. Hongwei, H.-F. Ji, X. Xiaohe, Y. Lvov, A. Mulchandani, P. Mulchandani and W. Chen, Organophosphorus hydrolase multilayer modified microcantilevers for organophosphorus detection, *Biosens. Bioelectron.*, 2007, **22**, 2636–2642.
13 M. Alvarez, A. Calle, J. Tamayo, L. M. Lechuga, A. Abad and A. Montoya, Development of nanomechanical biosensors for detection of the pesticide DDT, *Biosens. Bioelectron.*, 2002, **18**, 649–653.
14 P. Dutta, K. Hill, P. G. Datskos and M. J. Sepaniak, Development of a nanomechanical biosensor for analysis of endocrine disrupting chemicals, *Lab Chip*, 2007, **7**, 1184–1191.
15 C. R. Suri, J. Kaur, S. Gandhi and G. S. Shekhawat, Label-free ultrasensitive detection of atrazine based on nanomechanics, *Nanotechnology*, 2008, **19**, 1–6.
16 R. Yang, X. Huang, Z. Wang, Y. Zhou and L. Liu, A chemisorption-based microcantilever chemical sensor for the detection of trimethylamine, *Sens. Actuators, B*, 2010, **145**, 474–479.
17 T. L. Porter, V. Vail, J. Reed and R. Stewart, Hydrogel based composite materials for chemical, biological and medical sensing applications, *Proc. Mater. Res. Soc.*, 2007, **1010-V08-07**.
18 S. Cherian, R. K. Gupta, B. C. Mullin and T. Thundat, Detection of heavy metal ions using protein-functionalized microcantilever sensors, *Biosens. Bioelectron.*, 2003, **19**, 411–416.
19 S. Velanki, S. Kelley, T. Thundat, D. A. Blake, and H.-F. Ji, Detection of Cd(II) using antibody-modified microcantilever sensors, *Ultramicroscopy*, 2007, **107**, 1123–1128.

20 Y. Arntz, J. D. Seelig, H. P. Lang, J. Zhang, P. Hunziker, J. P. Ramseyer, E. Meyer, M. Hegner and C. Gerber, Label-free protein assay based on a nanomechanical cantilever array, *Nanotechnology*, 2003, **14**, 86–90.

21 C. Grogan, R. Raiteri, G. M. O'Connor, T. J. Glynn, V. Cunningham, M. Kane, M. Charlton and D. Leech, Characterization of an antibody coated microcantilever as a potential immuno-based biosensor, *Biosens. Bioelectron.*, 2002, **17**, 201–207.

22 G. Y. Kang, G. Y. Han, J. Y. Kang, I.-H. Cho, H. Park, S. H. Paek and T. S. Kim, Label-free protein assay with site-directly immobilized antibody using self-actuating PZT cantilever, *Sens. Actuators, B*, 2006, **117**, 332–338.

23 A. Subramanian, P. I. Oden, S. J. Kennel, K. B. Jacobson, R. J. Warmack, T. Thundat and M. J. Doktycz, Glucose biosensing using an enzyme-coated microcantilever, *Appl. Phys. Lett.*, 2002, **81**, 385–387.

24 J. Pei, F. Tian and T. Thundat, Glucose biosensor based on the microcantilever, *Anal. Chem.*, 2004, **76**, 292–297.

25 X. Yan, K. X. Xu and H.-F. Ji, Glucose oxidase multilayer modified microcantilevers for glucose measurement, *Anal. Chem.*, 2005, **77**, 6197–6204.

26 X. Yan, H.-F. Ji and Y. Lvov, Modification of microcantilevers using layer-by-layer nanoassembly film for glucose measurement, *Chem. Phys. Lett.*, 2004, **396**, 34–37.

27 T. Chen, D. P. Chang, T. Liu, R. Desikan, R. Datar, T. Thundat, R. Berger and S. Zauscher, Glucose-responsive polymer brushes for microcantilever sensing, *J. Mater. Chem.*, 2010, **20**, 3391–3395.

28 X. Huang, L. Siqi, J. S. Schultz, Q. Wang and Q. Lin, A MEMS affinity glucose sensor using a biocompatible glucose-responsive polymer, *Sens. Actuators, B*, 2009, **140**, 603–609.

29 G. A. Baker, R. Desikan and T. Thundat, Label-free sugar detection using phenylboronic acid-functionalized piezoresistive microcantilevers, *Anal. Chem.*, 2008, **80**, 4860–4865.

30 J. H. Lee, K. H. Yoon, K. S. Hwang, J. Park, S. Ahn and T. S. Kim, Label free novel electrical detection using micromachined PZT monolithic thin film cantilever for the detection of C-reactive protein, *Biosens. Bioelectron.*, 2004, **20**, 269–275.

31 J. H. Lee, T. S. Kim and K. H. Yoon, Effect of mass and stress on resonant frequency shift of functionalized $Pb(Zr_{0.52}Ti_{0.48})O_3$ thin film microcantilever for the detection of C-reactive protein, *Appl. Phys. Lett.*, 2004, **84**, 3187–3189.

32 T. Y. Kwon, K. Eom, J. H. Park, D. S. Yoon, T. S. Kim and H. L. Lee, *In situ* real-time monitoring of biomolecular interactions based on resonating microcantilevers immersed in a viscous fluid, *Appl. Phys. Lett.*, 2007, **90**, 223903.

33 K. W. Wee, G. Y. Kang, P. J. Jaebum, J. Y. Kang, D. S. Yoon, J. H. Park and T. S. Kim, Novel electrical detection of label-free disease marker proteins using piezoresistive self-sensing micro-cantilevers, *Biosens. Bioelectron.*, 2005, **20**, 1932–1938.

34 A. M. Moulin, S. J. O'Shea, R. A. Badley, P. Doyle and M. E. Welland, Measuring surface-induced conformational changes in proteins, *Langmuir*, 1999, **15**, 8776–8779.

35 D. Maraldo and R. Mutharasan, Optimization of antibody immobilization for sensing using piezoelectrically excited millimeter sized cantilever (PEMC) sensors, *Sens. Actuators, B*, 2007, **123**, 474–479.

36 H.-F. Ji and T. G. Thundat, *In situ* detection of calcium ions with chemically modified microcantilevers, *Biosens. Bioelectron.*, 2002, **17**, 337–343.

37 S. Cherian, A. Mehta and T. Thundat, Investigating the mechanical effects of adsorption of Ca^{2+} Ions on a silicon nitride microcantilever surface, *Langmuir*, 2002, **18**, 6935–6939.

38 X. Yan, K. Hill, H. Gao and H.-F. Ji, Surface stress changes induced by conformational changes of proteins, *Langmuir*, 2006, **22**, 11241–11244.

39 H. Gao, K. R. Buchapudi, A. Harms-Smyth, M. K. Schulte, X. Xu and H.-F. Ji, Improved surface modification approach for micromechanical biosensors, *Langmuir*, 2008, **24**, 345–349.

40 Y. Xu, B. Zhang, S. Wu and Y. Xia, The adsorption of dopamine on gold and its interactions with iron(III) ions studied by microcantilevers, *Anal. Chim. Acta*, 2009, **649**, 117–122.

41 M. Calleja, J. Tamayo, M. Nordström and A. Boisen, Low-noise polymeric nanomechanical biosensors, *Appl. Phys. Lett.*, 2006, **88**, 113901.

42 W. Shu, E. D. Laue and A. A. Seshia, Investigation of biotin–streptavidin binding interactions using microcantilever sensors, *Biosens. Bioelectron.*, 2007, **22**, 2003–2009.

43 Y. Chen, P. Xu, M. Liu and X. Li, Bio/chemical detection in liquid with self-sensing Pr-Oxi-Lever (piezo-resistive SiO_2 cantilever) sensors, *Microelectron. Eng.*, 2010, **87**, 2468–2474.

44 X. Yan, X. Shi, K. Hill and H.-F. Ji, Microcantilevers modified by horseradish intercalated nano-assembly for hydrogen peroxide detection, *Anal. Sci.*, 2006, **22**, 205–208.

45 J. P. Lock, E. Geraghty, L. C. Kagumba and K. K. Mahmud, Trace detection of peroxides using a microcantilever detector, *Thin Solid Films*, 2009, **517**, 3584–3587.

46 H. S. Kwon, K. C. Han, K. S. Hwang, J. H. Lee, T. S. Kim, D. S. Yoon and E. G. Yang, Development of a peptide inhibitor-based cantilever sensor assay for cyclic adenosine monophosphate-dependent protein kinase, *Anal. Chim. Acta*, 2007, **585**, 344–349.

47 G. Wu, R. H. Datar, K. M. Hansen, T. Thundat, R. J. Cote and A. Majumdar, Bioassay of prostate-specific antigen (PSA) using microcantilevers, *Nat. Biotechnol.*, 2001, **19**, 856–860.

48 C. Vanura, L. Yue, J. Lichtenberg, K.-U. Kirstein, A. Hierlemann and F. Josse, Liquid-phase chemical and biochemical detection using fully integrated magnetically actuated complementary metal oxide semiconductor resonant cantilever sensor systems, *Anal. Chem.*, 2007, **79**, 1646–1654.

49 S.-M. Lee, K. S. Hwang, H.-J. Yoon, D. S. Yoon, S. K. Kim, Y.-S. Lee and T. S. Kim, Sensitivity enhancement of a dynamic mode microcantilever by stress inducer and mass inducer to detect PSA at low picogram levels, *Lab Chip*, 2009, **9**, 2683–2690.

50 S. Stolyarova, S. Cherian, R. Raiteri, J. Zeravik, P. Skladal and Y. Nemirovsky, Composite porous silicon-crystalline silicon cantilevers for enhanced biosensing, *Sens. Actuators, B*, 2008, **131**, 509–515.

51 G. A. Campbell and R. Mutharasan, Use of piezoelectric-excited millimeter-sized cantilever sensors to measure albumin interaction with self-assembled monolayers of alkanethiols having different functional headgroups, *Anal. Chem.*, 2006, **78**, 2328–2334.

52 N. Backmann, C. Zahnd, F. Huber, A. Bietsch, A. PluCkthun, H. P. Lang, H. J. Guntherodt, M. Hegner and C. Gerber, A label-free immunosensor array using single-chain antibody fragments, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 14587–14592.

53 R. Raiteri, G. Nelles, H. J. Butt, W. Knoll and P. Skladal, Sensing of biological substances based on the bending of microfabricated cantilevers, *Sens. Actuators, B*, 1999, **61**, 213–217.

54 P. Dutta, J. Sanseverino, P. G. Datskos and M. J. Sepaniak, Nanostructured cantilevers as nanomechanical immunosensors for cytokine detection, *Nanobiotech.*, 2005, **1**, 237–244.

55 Y. Lam, N. I. Abu-Lail, M. S. Alam and S. Zauscher, Using microcantilever deflection to detect HIV-1 envelope glycoprotein gp120, *Nanomedicine*, 2006, **2**, 222–229.

56 R. Mukhopadhyay, V. V. Sumbayev, M. Lorentzen, J. Kjems, P. A. Andreasen and F. Besenbacher, Cantilever sensor for nanomechanical detection of specific protein conformations, *Nano Lett.*, 2005, **5**, 2385–2388.

57 I. Pera and J. Fritz, Sensing lipid bilayer formation and expansion with a microfabricated cantilever array, *Langmuir*, 2007, **23**, 1543–1547.

58 A. M. Moulin, S. J. O'Shea and M. E. Welland, Microcantilever-based biosensors, *Ultramicroscopy*, 2000, **82**, 23–31.

59 P. Dutta, C. A. Tipple, N. V. Lavrik, P. G. Datskos, H. Hofstetter, O. Hofstetter and M. J. Sepaniak, Enantioselective sensors based on antibody-mediated nanomechanics, *Anal. Chem.*, 2003, **75**, 2342–2348.

60 B. H. Kim, O. Mader, U. Weimar, R. Brock and D. P. Kern, Detection of antibody peptide interaction using microcantilevers as surface stress sensors, *J. Vac. Sci. Technol., B: Microelectron. Nanometer Struct.–Process., Meas., Phenom.*, 2003, **21**, 1472–1475.

61 W. Shu, S. Laurenson, T. P. J. Knowles, P. Ko Ferrigno and A. A. Seshia, Highly specific label-free protein detection from lysed cells using internally referenced microcantilever sensors, *Biosens. Bioelectron.*, 2008, **24**, 233–237.

62 W. Tan, Y. Huang, T. Nan, C. Xue, Z. Li, Q. Zhang and B. Wang, Development of protein A functionalized microcantilever immunosensors for the analyses of small molecules at parts per trillion levels, *Anal. Chem.*, 2009, **82**, 615–620.

63 J. A. Capobianco, W. Y. Shih, Q.-A. Yuan, G. P. Adams and W.-H. Shih, Label-free, all-electrical, *in situ* human epidermal growth receptor 2 detection, *Rev. Sci. Instrum.*, 2008, **79**, 076101–076103.

64 A. M. Moulin, S. J. O'Shea, R. A. Badley, P. Doyle and M. E. Welland, Measuring surface-induced conformational changes in proteins, *Langmuir*, 1999, **15**, 8776–8779.

65 K. Hill, P. Dutta, A. Zareba, M. L. Eldridge and M. J. Sepaniak, Morphological and chemical optimization of microcantilever surfaces for thyroid system biosensing and beyond, *Anal. Chim. Acta*, 2008, **625**, 55–62.

66 Y.-K. Yen, C.-Y. Huang, C.-H. Chen, C.-M. Hung, K.-C. Wu, C.-K. Lee, J.-S. Chang, S. Lin and L.-S. Huang, A novel, electrically protein-manipulated microcantilever biosensor for enhancement of capture antibody immobilization, *Sens. Actuators, B*, 2009, **141**, 498–505.

67 G. Oliviero, P. Bergese, G. Canavese, M. Chiari, P. Colombi, M. Cretich, F. Damin, S. Fiorilli, S. L. Marasso, C. Ricciardi, P. Rivolo and L. E. Depero, A biofunctional polymeric coating for microcantilever molecular recognition, *Anal. Chim. Acta*, 2008, **630**, 161–167.

68 T. Kwon, J. Park, J. Yang, D. S. Yoon, S. Na, C.-W. Kim, J.-S. Suh, Y.-M. Huh, S. Haam and K. Eom, Nanomechanicalin situ monitoring of proteolysis of peptide by Cathepsin B, *PLoS One*, 2009, **4**, e6248.

69 Y. Liu, X. Li, Z. Zhang, G. Zuo, Z. Cheng and H. Yu, Nanogram per milliliter-level immunologic detection of alpha-fetoprotein with integrated rotating-resonance microcantilevers for early-stage diagnosis of hepatocellular carcinoma, *Biomed. Microdevices*, 2009, **11**, 183–191.

70 C. Ricciardi, S. Fiorilli, S. Bianco, G. Canavese, R. Castagna, I. Ferrante, G. Digregorio, S. L. Marasso, L. Napione and F. Bussolino, Development of microcantilever-based biosensor array to detect Angiopoietin-1, a marker of tumor angiogenesis, *Biosens. Bioelectron.*, 2010, **25**, 1193–1198.

71 M. G. Von Muhlen, N. D. Brault, S. M. Knudsen, S. Jiang and S. R. Manalis, Label-free biomarker sensing in undiluted serum with suspended microchannel resonators, *Anal. Chem.*, 2010, **82**, 1905–1910.

72 R. L. Gunter, W. G. Delinger, K. Manygoats, A. Kooser and T. L. Porter, Viral detection using an embedded piezoresistive microcantilever sensor, *Sens. Actuators, A*, 2003, **107**, 219–224.

73 N. Nugaeva, K. Y. Gfeller, N. Backmann, M. Düggelin, H. P. Lang, H. J. Güntherodt and M. Hegner, An antibody-sensitized microfabricated cantilever for the growth detection of *Aspergillus niger* spores, *Microsc. Microanal.*, 2007, **13**, 13–17.

74 S. Q. Li, L. Orona, Z. M. Li and Z.-Y. Cheng, Biosensor based on magnetostrictive microcantilever, *Appl. Phys. Lett.*, 2006, **88**(1–3), 073507.

75 L. Fu, S. Li, K. Zhang and Z. Y. Cheng, Detection of *Bacillus anthracis* spores using magnetostrictive microcantilever-based biosensor, *Mater. Res. Soc. Symp. Proc.*, 2007, **951**, 0951-E05-04.

76 A. P. Davila, J. Jang, A. K. Gupta, T. Walter, A. Aronson and R. Bashir, Microresonator mass sensors for detection of *Bacillus anthracis* Sterne spores in air and water, *Biosens. Bioelectron.*, 2007, **22**, 3028–3035.

77 J.-P. McGovern, W. Y. Shih, R. Rest, M. Purohit, Y. Pandya and W.-H. Shih, Label-free flow-enhanced specific detection of *Bacillus anthracis* using a piezoelectric microcantilever sensor, *Analyst*, 2008, **133**, 649–654.

78 G. A. Campbell and R. Mutharasan, Method of measuring *Bacillus anthracis* spores in the presence of copious amounts of *Bacillus thuringiensis* and *Bacillus cereus*, *Anal. Chem.*, 2006, **79**, 1145–1152.

79 G. A. Campbell, D. deLesdernier, and R. Mutharasan, Detection of airborne *Bacillus anthracis* spores by an integrated system of an air sampler and a cantilever immunosensor, *Sens. Actuators, B*, 2007, **127**, 376–382.

80 B. Dhayal, W. A. Henne, D. D. Doorneweerd, R. G. Reifenberger and P. S. Low, Detection of *Bacillus subtilis* spores using peptide-functionalized cantilever arrays, *J. Am. Chem. Soc.*, 2006, **128**, 3716–3722.

81 J. Zhang and H.-F. Ji, An anti *E. coli* O157:H7 antibody-immobilized microcantilever for the detection of *Escherichia coli* (*E. coli*), *Anal. Sci.*, 2004, **20**, 585.

82 G. A. Campbell and R. Mutharasan, Detection of pathogen *Escherichia coli* O157:H7 using self-excite PZT-glass microcantilevers, *Biosens. Bioelectron.*, 2005, **21**, 462–473.

83 G. A. Campbell and R. Mutharasan, A method of measuring *Escherichia coli* O157:H7 at 1 cell/mL in 1 liter sample using antibody functionalized piezoelectric-excited millimeter-sized cantilever sensor, *Environ. Sci. Technol.*, 2006, **41**, 1668–1674.

84 K. Y. Gfeller, N. Nugaeva and M. Hegner, Rapid Biosensor for detection of antibiotic-selective growth of *Escherichia coli*, *Appl. Environ. Microbiol.*, 2005, **71**, 2626–2631.

85 J. A. Capobianco, W. Y. Shih and W. H. Shih, Methyltrimethoxysilane-insulatedpiezoelectric microcantilevers for direct, all-electrical biodetection in buffered aqueous solutions, *Rev. Sci. Instrum.*, 2006, **77**(1–6), 125105.

86 L. Fu, K. Zhang., S. Li, Y. Wang, T.-S. Huang, A. Zhang and Z.-Y. Cheng, *In situ* real-time detection of *E. coli* in water using antibody-coated magnetostrictive microcantilever, *Sens. Actuators, B*, 2010, **150**, 220–225.

87 Q. Zhu, W. Y. Shih and W.-H. Shih, *In situ*, in-liquid, all-electrical detection of *Salmonella typhimurium* using lead titanate zirconate/gold-coated glass cantilevers at any dipping depth, *Biosens. Bioelectron.*, 2007, **22**, 3132–3138.

88 X. Yan, J. Zhang, H.-F. Ji and T. Thundat, Molecular recognition of biowarfare agents using micromechanical sensors, *Expert Rev. Mol. Diagn.*, 2004, **4**, 859–866.

89 G. A. Campbell and R. Mutharasan, Near real-time detection of *Cryptosporidium parvum* oocyst by IgM-functionalized piezoelectric-excited millimeter-sized cantilever biosensor, *Biosens. Bioelectron.*, 2008, **23**, 1039–1045.

90 Z. Balint, G. A. Vegh, A. Popescu, M. Dima, C. Ganea and G. Varo, Direct observation of protein motion during the photochemical reaction cycle of bacteriorhodopsin, *Langmuir*, 2007, **23**, 7225–7228.

91 Q. Ren, Y. P. Zhao, L. Han and H. B. Zhao, A Nanomechanical device based light-driven proton pumps, *Nanotechnology*, 2006, **17**, 1778–1785.

92 T. Braun, M. K. Ghatkesar, N. Backmann, W. Grange, P. Boulanger, L. Letellier, H.-P. Lang, A. Bietsch, C. Gerber and M. Hegner, Quantitative time-resolved measurement of membrane protein–ligand interactions using microcantilever array sensors, *Nat. Nanotechnol.*, 2009, **4**, 179–185.

93 S. Velanki and H.-F. Ji, Detection of Feline coronavirus using microcantilever sensors, *Meas. Sci. Technol.*, 2006, **17**, 2964–2968.

