

Fluorescent nanoparticles: Sensing pathogens and toxins in foods and crops

Kellie P. Burris^{a,b} and
C. Neal Stewart Jr.^{a,b,*}

^aDepartment of Plant Sciences, University of Tennessee, Knoxville, 252 Ellington Plant Sciences, 2431 Joe Johnson Drive, Knoxville, TN 37996-4561, USA

^bDepartment of Food Science and Technology, University of Tennessee, Knoxville, 252 Ellington Plant Sciences, 2431 Joe Johnson Drive, Knoxville, TN 37996-4561, USA
(Tel.: +1 865 974 7324; fax: +1 865 974 1947; e-mail: nealstewart@utk.edu)

Emerging tools in molecular nanotechnology will be useful to monitor and quantify pathogen levels and toxin production inter-and intra-cellularly and on the surface of foods. Recent developments in the manufacture and modification of fluorescent nanoparticles, quantum dots (QD), in conjunction with established molecular beacon (MB) technology, have demonstrated potential for on-the-food detection and quantification of pathogens and toxins harmful to human health. Recent research has demonstrated QDs effective use for *in vivo* cellular imaging, demonstrating its potential use in detecting live bacterial contamination in foods and crops. We envisage food protection systems using automated sampling of food products with near instantaneous detection and reporting of food contaminants using molecular nanotechnologies. We also address the potential limitations of these emerging technologies and how shortcomings can be addressed.

Introduction

Foodborne illnesses are a continuous threat to public health with increasing costs to society. One recent estimate is that the 31 major foodborne pathogens account for nearly 9.4 million people becoming sick, more than 55,961 hospitalizations, and 1351 deaths each year in the United States (Scallan *et al.*, 2011) with an estimated economic cost of \$77.7 billion annually (Scharff, 2012). Federal agencies such as the US Department of Agriculture (USDA), Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) have responsibility to protect US citizens from such preventable illness and mortality and are, therefore, striving to adopt better monitoring technologies and systems; such is the case around the globe in various countries.

Recent pathogenic outbreaks via food supplies and post-September 11 threats of bioterrorism have prompted the need for better detection methodologies for foodborne pathogens and toxins that go beyond the normal USDA, FDA and EPA concerns and into the realm of defense and homeland security. Further, food safety concerns are not confined to only the US, but are a global priority. There are a number of current standard methods for detecting and quantifying microorganisms in foods, however, these methods are laborious and time-consuming, have limited specificity and sensitivity, and require longer periods of time for identification. Nanotechnology can enable rapid detection and monitoring of pathogen and toxin contamination at various steps in food supply chains, thereby potentially drastically reducing costly recalls and human health costs including deadly foodborne disease. If a positive sample is identified through rapid screening, then standard microbiological techniques would still be required to confirm the presence of potential pathogens and/or toxins. Rapid, sensitive testing would also increase business efficiency in food industries that produce perishable products (i.e. eggs, meats, vegetables) by sending product out faster without having to wait for long, laborious testing to determine its safety. While only select types of foods are held until results are known, sensors that can detect contaminated batches quickly will enable raw materials or products to be rapidly removed from the food chain. Besides investigative sampling, these rapid methods can also be used to verify the efficacy of food safety systems such as HACCP and substantiate the safety of end-product.

* Corresponding author.

In addition to significant time delay between sampling and assay results, these methodologies all suffer from a common deficiency: they rely on destructive sampling of food products rather than an on-the-food, which could be powerful to non-destructively assure safety prior to food leaving the farm or processing facility, especially in instances where homogenization is not practical. However, when pathogens are distributed within foods (e.g. ground beef) then there is no option but to perform destructive testing. The only way to say that a food is 100% free from pathogens is to utilize the entire food for testing, which has been considered impractical, if not impossible.

MB technology utilizing organic fluorophores (see Table 1 for definitions) is established for the detection and quantification of foodborne pathogens *in vitro* (Hadjinicolaou, Demetriou, Emmanuel, Kakoyiannis, & Kostrikis, 2009; Hadjinicolaou, Demetriou, Hezka, *et al.*, 2009; Liming & Bhagwat, 2004; Patel, Bhagwat, Sanglay, & Solomon, 2006), in conjunction with q-PCR; however, newer fluorescent structures, QDs, are becoming of greater interest and been found to be more sensitive and stable than other fluorophores; able to detect multiple pathogens within a single sample. Several reviews have addressed the use of other nanoparticle-based biosensors, such as metallic and magnetic materials, for the detection and imaging of pathogenic bacteria (Duncan, 2011; Heo & Hua, 2009; Sanvicens, Pastells, Pascual, & Marco, 2009; Tallury, Malhotra, Byrne, & Santra, 2010); however, this article more specifically addresses the benefits of the molecular micro- and nanotechnologies, such as molecular beacons (MBs) and quantum dots (QDs), as emerging tools to protect the food supply chain from the farm to consumer.

Chemistry and bioconjugation of QDs

QDs are spherical, semiconducting nanoparticles that are composed of elements from groups II–VI, III–V or IV–VI of the periodic table (Klostranec & Chan, 2006; Smith & Nie, 2004). QDs are semiconducting materials in the nanoscale dimension, confining their electrons to a small space. Their electronic characteristics are closely

associated to their particle diameter and shape and of which their color closely correlates. The smaller their size, the greater amount of energy required for excitation and therefore, the more blue-shifted the emitted fluorescence. Elements commonly used include Cd, Zn, Se, Te, In, P or As (Klostranec & Chan, 2006; Smith & Nie, 2004). QDs utilized in many sensing applications consist of a semiconductive core surrounded by a shell, enhancing optical properties and a cap for improving its solubility in aqueous solutions (Ghasemi, Peymani, & Affi, 2009). For biological applications, QDs composed of CdSe/ZnS (core/shell) are used most often (Ghasemi *et al.*, 2009). QDs are almost an order of magnitude larger than many of the conventional organic dyes in use (Michalet *et al.*, 2005).

CdSe QDs can be size-tuned to emit in the 450–650 nm range, while CdTe can emit in the 500–750 nm range. The larger size may be beneficial for many applications, allowing for multiple biomolecules to be attached to a single QD surface. These size and chemical compositional differences provide multicolor QDs to be simultaneously excited with a single light source, allowing for numerous uses in biomolecular sensing and tissue imaging (Ghasemi *et al.*, 2009; Klostranec & Chan, 2006).

There are three basic methods used for conjugating biomolecules to QDs: (1) covalent modification of either the amine, thiol or carboxyl groups found on the QD surface by cross-linking, (2) direct attachment of biomolecules to the atoms on the surface of the QD, using either thiol bonding or metal affinity, or (3) use of electrostatic interactions between the surfaces of the QD surfaces and oppositely charged proteins or other biomolecules (Ozkan, 2004). The method of conjugation used is most dependent upon the biomolecule chosen as the target for QD detection.

Organic versus QD fluorophores

Organic fluorescent dyes have been used extensively and are well-established for use in PCR-based applications (Resch-Genger, Grabolle, Cavaliere-Jaricot, Nitschke, & Nann, 2008). However, most of these molecules are sensitive to photobleaching, unstable in complex mixtures, and

Table 1. Glossary of terms used.

Term	Definition
Blinking (Fluorescence intermittency)	Randomly switching fluorescence emission on (bright) and off (dark) under continuous excitation.
Fluorescence	Emission of light by a compound that has absorbed light.
Fluorescence lifetime	Average time an excited fluorophore remains in an excited state before it emits a photon and returns to ground state.
Fluorescence quenching	Any process that causes a decrease in fluorescence intensity. Examples of quencher molecules include black hole quencher.
Fluorophore	A molecule capable of fluorescence.
Molar absorption coefficient	Measurement of how strongly a chemical molecule absorbs light at a given wavelength.
Photobleaching	Photochemical destruction of fluorescence when the fluorophore is exposed to the light necessary for its excitation.
Quantum yield	Ratio of emitted to absorbed photons.
Stokes shift	Difference (in wavelength or frequency units) between positions in the maxima of the absorption and emission spectra within the same electronic transition.

are limited in biological applications (Ghasemi *et al.*, 2009). Organic fluorophores commonly used in conjunction with MBs and their excitation and emission wavelengths are listed in Table 2. Each fluorophore has unique properties that can affect its detection limit, sensitivity for a specific target, and ability to detect multiple targets in a single sample (Resch-Genger *et al.*, 2008). Some fluorophores are more sensitive to assay conditions, such as variation in pH or temperature, which can result in a decrease in the quantum yield, and thus decreasing the fluorescence intensity (Marras, 2008).

QDs have emerged as a better choice over organic fluorophores for their photostability, suppression of photo-oxidation, sustained fluorescence, larger effective Stokes shifts, and narrow emission and wider excitation bandwidths (Resch-Genger *et al.*, 2008). The suppression of photo-oxidation and enhancement of photostability make QDs more advantageous over organic fluorophores for imaging applications that require intensive laser excitation for potentially long periods of time. The thermal stability of QDs is much higher than for organic fluorophores and are dependent upon biomolecule and shell construction (Resch-Genger *et al.*, 2008). The fluorescence lifetime for organic dyes are 1–10 ns while QDs have sustained fluorescence for 10–100 ns (Resch-Genger *et al.*, 2008). QDs allow target fluorescence to be clearly separated from background fluorescence (Yang *et al.*, 2000). Their higher molar extinction coefficients at excitation wavelength than organic fluorophores result in higher fluorescence quantum yield, which are important when monitoring in such complex systems as plants and foods, by allowing the visualization of fluorescence through tissues or other interfering compounds (Marras, 2008). Further, cross-talk between various fluorescent molecules can occur due to the lack of separation between absorption and emission spectra as observed with organic fluorophores (Resch-Genger *et al.*, 2008).

Biomolecules as targets for QD detection

Fluorescent assays have been developed using a variety of biomolecules or biorecognition agents for use in

foodborne pathogen and toxin detection; however, each biomolecule has certain advantages and disadvantages for its use in specific targeting. The ability for each biomolecule to detect a specific bacterial target can be highly dependent upon the food matrix and the growth of the bacteria. Food matrixes are complex and their components may cause degradation or non-specific binding of the biomolecule, resulting in false-positives or false-negatives respectively. Therefore, the choice of biomolecule to attach to the QD fluorophore is critical and can be dependent upon sample and target bacteria or toxin. There are many different biomolecules that can be attached to water-soluble QDs for specific biological targeting (Table 3), and here we focus on the use of nucleic acids, antibodies, and carbohydrates.

Nucleic acids can be single stranded DNA or RNA molecules or sequences and are often used in specific targeting. Molecular beacons are nucleic acid probes, consisting of between 15 and 25 nucleotides, with a fluorescent molecule at one end and a quencher molecule at the opposite end (Tyagi & Kramer, 1996). When unbound to their targets, molecular beacons have quenched fluorescence because of a hairpin structure that allows close proximity of the fluorophore to the quencher molecule (Fig. 1A). When in the presence of the target molecule (template), the molecular beacon binds to its complementary nucleic acid sequence and fluorescence is triggered, allowing measurable detection (Fig. 1B). Molecular beacons were first developed by Tyagi & Kramer, 1996 for use in identifying the presence of specific nucleic acid sequences in homogeneous solutions. Since then, modifications to this technology have enabled the detection of foodborne pathogens *in vitro* (Hadjinicolaou, Demetriou, Emmanuel, *et al.*, 2009; Hadjinicolaou, Demetriou, Hezka, *et al.*, 2009; Liming & Bhagwat, 2004; Patel *et al.*, 2006), in conjunction with q-PCR. However, *in vivo* monitoring has only been demonstrated in detecting mRNA in living cells (Bratu, Cha, Mhlanga, Kramer, & Tyagi, 2003; Santangelo, Nix, Tsourkas, & Bao, 2004; Wu, Yang, Moroz, & Tan, 2008). Bratu *et al.* (2003) were the first to visualize the movement of native mRNA in living cells through the use of MBs. Liming and Bhagwat (2004) were the first to report using an MB-based method to detect a single *Salmonella* species in fresh fruits and vegetables. Further, the generation of fluorescent signal using MBs was quite rapid (18 h *versus* 3–4 days) and sensitive (4 CFU per 25 g produce, same as standard microbiological methods). Xi, Balberg, Boppart, and Raskin (2003) demonstrated that MBs can generate a specific fluorescent signal for detecting rRNA from whole bacterial cells in minutes.

However, there are limitations in using MBs to monitor for pathogens in complex systems, because of interacting or inhibitory compounds. It has been shown that when monitoring for the presence of pathogens using these PCR-based technologies, a false-negative signal may result from the presence of inhibitory compounds derived from plants,

Table 2. Overview of available organic fluorescent dyes.

Organic fluorophore	Excitation (nm)	Emission (nm)
Cy3	550	570
Cy5	650	670
FAM	495	515
Fluorescein	500	541
HEX	535	555
LC red 640	625	640
LC red 705	680	710
Nile Red	552	636
ROX	575	605
TAMRA	554	573
TET	525	540
Texas Red	585	605
TMR	555	575

Adapted from Marras (2008) and (Resch-Genger *et al.*, 2008).

Table 3. Examples of nanotechnology sensors coupling QDs used for foodborne pathogen detection.

Sensor type	Pathogen(s)	Target biomolecule	Detection level	Food matrix	Detection time	Reference
Nucleic acid	<i>S. Typhimurium</i>	Nucleic acid	10 pM DNA	N/A*	N/D**	(Vasudev <i>et al.</i> , 2011)
	<i>E. coli</i> O157:H7	Nucleic acid	9.72 fM DNA	N/A	N/D	(Kim & Son, 2010)
Immunoassay	<i>E. coli</i> O157:H7	Antibody	7.3 log CFU/ml	N/A	N/D	(Hahn, Tabb, & Krauss, 2005)
	<i>E. coli</i> O157:H7	Antibody	<10 CFU/ml	N/A	2 h	(Sanvicens <i>et al.</i> , 2011)
	<i>E. coli</i> O157:H7	Antibody	3 log CFU/ml	N/A	<2 h	(Su & Li, 2004)
	<i>E. coli</i> O157:H7	Antibody	4 log CFU/ml	N/A	<2 h	(Yang & Li, 2006)
	<i>S. Typhimurium</i>	Antibody	5.5 log CFU/ml	Milk (diluted)	<2 h	(Zhao <i>et al.</i> , 2009)
	<i>S. Typhimurium</i> , <i>S. flexneri</i> , and <i>E. coli</i> O157:H7		3 log CFU/ml	Apple juice	<2 h	
Phage-based	<i>E. coli</i>	Protein	20 cells/ml	Water	<1 h	(Edgar <i>et al.</i> , 2006)
PCR	<i>E. coli</i>	Lectin	4 log CFU/ml	N/A	N/D	(Mukhopadhyay <i>et al.</i> , 2009)
Flow cytometry	<i>E. coli</i> O157:H7	Antibody	6 log CFU/ml	Water	N/D	(Hahn, Keng, & Krauss, 2008)
Impedimetric	<i>S. enteritidis</i>	Antibody	4 log CFU/ml	PBS	3 min	(Kim <i>et al.</i> , 2007)
			5 log CFU/ml	Milk	3 min	

*N/A: not applied to a food system; **N/D: Not determined.

such as polysaccharides, phenolic compounds, other secondary metabolites or other DNA polymerase inhibitors (Bhagwat, 2003; Liao & Shollenberger, 2003; Liming & Bhagwat, 2004; Shearer, Strapp, & Joerger, 2001). Chen, Behlke, and Tsourkas (2007) found that false-positives

occur as a result of non-specific binding in the nucleus of the cell. However, when linking MBs to QDs, these false-positive signals are reduced to an insignificant level when monitoring mRNA synthesis in the cytoplasm of living cells. A second problem with the MB technology utilizing

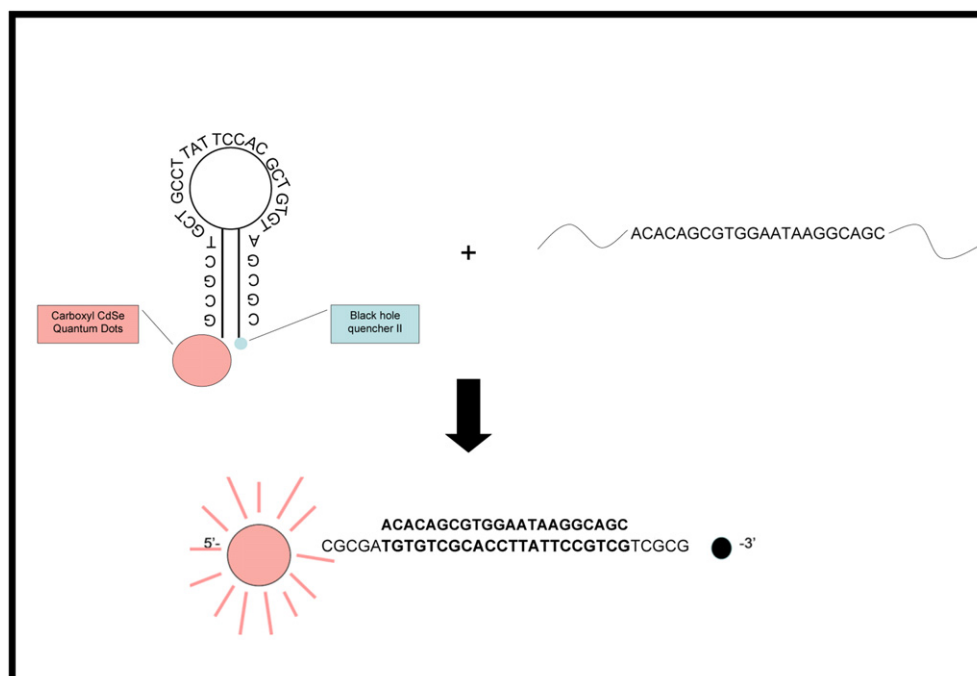


Fig. 1. Schematic of molecular beacon technology. Molecular beacons are nucleic acid probes with a fluorescent molecule at one end (such as a QD) and a quencher molecule (such as black hole quencher II) at to opposite end. When unbound to their targets, molecular beacons have quenched fluorescence because of a hairpin structure that allows close proximity of the fluorophore to the quencher molecule. When in the presence of the target molecule (complementary nucleic acid sequence), the loop of the MB binds to its complementary nucleic acid sequence (ACACAGCGTGGAATAAGGCAGC) and fluorescence is triggered, allowing measurable detection.

organic fluorophores is the low signal to background ratio. One way to address this problem is to utilize fluorophores, such as QDs, with increased levels of sustained fluorescence, where fluorescence from a single QD molecule is equivalent to that of twenty individual organic fluorophores (Kim, Sohn, & Tan, 2008). A third problem when using nucleic acids as the biomolecule for detection is the ability to distinguish between live and dead cells. Cells that have been lysed or have membrane damage may expose their DNA and cause a false-positive.

A second group of biomolecules that have been coupled with QDs for use in pathogen detection are antibodies. Antibodies are one of the most widely used biomolecules for the detection and separation of bacteria and toxins. There are two main categories of antibodies used in immunoassays: polyclonal and monoclonal. Polyclonal antibodies are typically produced *in vivo* and consist of a group of antibodies that bind to a multiple number of epitopes on the antigen. Monoclonal antibodies are produced *in vitro* and consist of an identical group of antibodies that bind to a single epitope on an antigen, making monoclonal antibodies more specific than polyclonal antibodies. Disadvantages involved with the use of antibodies for pathogen detection include low affinity of the antibody to the bacteria or toxin and interference from contaminants (Meng & Doyle, 2002), non-specific binding, sensitivity to pH and temperature, variations in antibodies from batch to batch, sensitivity to chemical treatments, the inability to distinguish live from dead cells, high costs, and their limited shelf life (Jayasena, 1999). Despite these limitations, antibodies are frequently selected as the biomolecule for the detection of a broad range of microorganisms and are advantageous for their ability to be combined with magnetic beads for simultaneous detection and separation of toxins (Schlosser *et al.*, 2007) and bacteria (Hibi *et al.*, 2006; Jung, Frank, & Brackett, 2003; Su & Li, 2004). Kim, Om, and Mun (2007) used anti-*Salmonella* antibody conjugated nanoparticles to detect 4 log CFU/mL and 5 log CFU/mL of *Salmonella enteritidis* in phosphate buffered saline and milk, respectively, with a detection time of 3 min. Su and Li (2004) found the detection limit determined for *Escherichia coli* O157:H7 was ca. 3 log CFU/mL with high specificity, where 6 log CFU/mL of *E. coli* K12 and *Salmonella* Typhimurium did not interfere with detection. The total detection time, from adding a sample solution to obtaining the final result, was rapid (less than 2 h).

A third group of biomolecules that are important in binding to and detecting pathogens and their toxins are carbohydrates (oligosaccharides or polysaccharides). Carbohydrates can bind to proteins (glycoproteins) and/or lipids (glycolipids and/or lipopolysaccharides). Pathogens and their toxins are able to recognize and attach to specific carbohydrate sequences on epithelial cells, a mechanism of bacterial adhesion that can be manipulated as a means for detecting target pathogens and their toxins (Sharon, 2006). In addition, some bacteria form surface structures,

such as fimbriae or pili that can also bind to carbohydrates (Mouricout, 1997). Carbohydrates have an affinity for a wide range of organisms and/or target proteins giving them broad specificity. This broad specificity can be useful by allowing for the detection of target organisms that may have undergone mutations from the original target. Unlike antibodies, carbohydrates do not denature and therefore, do not lose their activity when exposed to extreme temperature or pH changes, and could be used in those conditions found in food matrices for pathogen and toxin detection (Vikesland & Wigginton, 2010). El-Boubbou, Gruden, and Huang (2007) were able to use magnetic glyconanoparticles to quickly (within 5 min) detect *E. coli* with up to 88% capture efficiency. Mukhopadhyay, Martins, Karamanska, Russell, and Field (2009) used carbohydrate functionalized QDs to target mannose-specific lectin that *E. coli* uses for adhesion to host cells and were able to detect *E. coli* at levels of 4 log *E. coli* cells per mL. However, while carbohydrates are stable, the actual interactions with the target are pH dependent. Disadvantages involved with the use of carbohydrates for pathogen detection include their low affinity (De la Fuente & Penades, 2006), broad specificity and difficulty in synthesis (Haseley, 2002). Increased specificity may be achieved when coupling the carbohydrate to a lectin or type of protein that is able to bind to specific parts of the carbohydrate molecule (Vikesland & Wigginton, 2010). Carbohydrate-protein interactions are also several factors weaker than the protein–protein interactions (Haseley, 2002).

Sensors using QDs for food safety applications

Standard microbiological methodologies to detect and quantify levels of bacteria within a single sample in foods require at least 24 h due to time-consuming enrichment procedures. QD-based sensors require less time (<2 h) (Table 3), but still require enrichment to differentiate live from dead cells and to obtain high enough levels of contamination for detection. PCR methodologies have been used to detect *Salmonella* from fruits and vegetables at a level of 4 CFU/25 g produce (Liming & Bhagwat, 2004) and from chicken rinse or spent irrigation water at a level of 2.2 CFU/100 mL (Wolffs, Glencross, Thibaudeau, & Griffiths, 2006). However, these techniques still require time-consuming enrichment and access to q-PCR instruments and specialized methodologies. QDs are highly suitable for the practical applications of detecting foodborne pathogens and toxins by being able to be excited and emit signal through complex food components (Bruno, Phillips, Carrillo, & Crowell, 2009). Several researchers have demonstrated detection of a variety of foodborne and human pathogens, including *Cryptosporidium parvum* (Lee *et al.*, 2004; Zhu, Ang, & Liu, 2004), *E. coli* (Mukhopadhyay *et al.*, 2009; Yang & Li, 2006; Zhao *et al.*, 2009), *Giardia lamblia* (Zhu *et al.*, 2004), *Listeria monocytogenes* (Tully, Hearty, Leonard, & O’Kennedy, 2006), *Salmonella* Typhimurium (Yang & Li, 2006; Zhao

et al., 2009), *Shigella flexneri* (Zhao *et al.*, 2009), *Bacillus anthracis* (Ho, Kung, Yang, & Wang, 2005), *Campylobacter jejuni* (Bruno *et al.*, 2009), and enteric viruses (Rawsthorne, Phister, & Jaykus, 2009) utilizing QDs as the fluorophore. Vasudev *et al.* (2011) have demonstrated the ability to monitor for the *invA* gene of *Salmonella* Typhimurium DT104 in real-time using QDs coupled with MB probes. It has also been demonstrated that QDs can be used for the simultaneous detection of pathogens, *E. coli* and *S. Typhimurium* (Yang & Li, 2006) and *C. parvum* and *G. lamblia* (Zhu *et al.*, 2004). Using QD fluorophores, the signal to noise ratio was determined to be 17, demonstrating better photostability and increased fluorescence as compared to commercial kits (Zhu *et al.*, 2004). While the sensitivity was low with regard to novel detection technologies, Mukhopadhyay *et al.* (2009) were able to detect *E. coli* at levels of 4 log *E. coli* cells per ml using mannose-specific CdS QDs. These carbohydrate functionalized QDs were used to target a mannose-specific lectin that *E. coli* uses for adhesion to host cells (Mukhopadhyay *et al.*, 2009). Ho *et al.* (2005) were able to use oligonucleotide functionalized QDs to detect pathogenic strains of *B. anthracis*. Bruno *et al.* (2009) demonstrated that QD-aptamers can be used to detect very low levels of live and dead *C. jejuni* in buffer and various food systems (chicken broth, ground beef wash, and 2% milk) without using any type of wash step. Washing to remove part of the food complex in place was actually shown to displace assay materials causing a decrease the detection limit due to material losses. Detection limits in diluted food matrices were found to be between 10 and 250 CFU per ml for both live and dead *C. jejuni*. QDs were advantageous due to their ability to absorb energy from the high energy excitation and emit a signal that could be observed through the food components.

While great focus has been placed on the utilization of QDs for pathogen detection, toxins have also been sensed and quantified using QDs (Goldman *et al.*, 2002; Warner *et al.*, 2009). Many cases of foodborne illnesses can be attributed to toxins that have been produced by the bacteria during their growth into the foods, with the leading cause of intoxication due to *Staphylococcus aureus* enterotoxins (Balaban & Rasooly, 2000). The traditional method for the detection of microbial toxins in foods is the microslide gel double diffusion technique, approved by the AOAC and the current standard for microbial toxins, and requires a minimum of 30–60 ng of enterotoxin per gram of food. This method requires considerable sample preparation and clean-up prior to testing to reduce and/or remove any interfering compounds from the sample food matrix (Bennett, 1998; Bennett & Hait, 1998). Latex agglutination test is also widely used and commercially available for toxin detection (D'Aoust, Sewell, & Greco, 1991). These traditional methods have detected toxins in the range of 30–60 ng. However, more modern testing utilizes high-throughput methods, such as Enzyme-Linked Immunosorbent Assay (ELISA), reversed

passive latex agglutination, and PCR and can detect toxins at less than 1 ng per ml (Cremonesi *et al.*, 2007; Loncarevic, Jørgensen, Lovseth, Mathisen, & Rorvik, 2005; Monday & Bohach, 1999; Tkacikova, Tesfaye, & Mikula, 2003). Warner *et al.* (2009) developed a fluorescence sandwich assay using QD and high affinity antibodies to detect and quantify botulinum neurotoxin serotype A at a level of 5–31 pM in PBS buffer and fetal bovine serum. Goldman *et al.* (2002) used multiplexing fluorescent immunoassay with CdSe/ZnS (core/shell) QDs to detect cholera toxin, ricin, Shiga-like toxin and staphylococcal enterotoxin B with detection limits as low as 3 ng per ml for staphylococcal enterotoxin B.

On-the-plant use of QDs

While many researchers have used QDs as the fluorophore of choice for *in vitro* assays, such as q-PCR, to detect foodborne pathogens, to date, no one has been able to use them in on-the-plant monitoring *in vivo*. Developing and implementing such a technology is somewhat futuristic, but nonetheless important because of the rising number of foodborne illness deriving from vegetables and fruits in recent years and increasing threats of bioterrorism to our crop and food supply.

While *in vitro* techniques coupling QDs to MBs have been well-established, the possibility to monitor specific pathogens and transgenes within or on the surface of plants using these nanoparticles has not been fully achieved, but is necessary. While it would be more pragmatic to use rinse solutions to screen for pathogens on fresh produce as opposed to attempting to perform direct detection, *E. coli* O157:H7 cells have been shown to penetrate into the stomata and junction zones of cut lettuce leaves 20–100 μ m below the surface of the cut, where they are protected from sanitation practices, such as chlorine washes (Seo & Frank, 1999; Takeuchi & Frank, 2000), making detection within the plant necessary. Recently, Hu *et al.* (2010) have demonstrated high efficiency transport of QDs into maize (*Zea mays*) roots with the aid of silwet L-77 (Fig. 2), demonstrating the translocation of QDs to tissues throughout a plant where pathogens may colonize and be detected. Fluorescence emitted by QDs was only observed in the epidermis after 4 h incubation with QDs alone (Fig. 2B) (Hu *et al.*, 2010). With the addition of the surfactant, silwet L-77, a greater penetration of the QDs into the root was observed (Fig. 2C) and with increased incubation time to 12 h, almost the entire root was infiltrated with the QDs (Fig. 2D) (Hu *et al.*, 2010). When a maize root was soaked in the QD solution for 16 h, Hu *et al.* (2010) observed QDs upstream of the incubation solution within only the stele of the root, indicating the ability of the QDs to be translocated to other tissues above the soaking medium with the aid of a surfactant (Fig. 2E). While advancements have been made with using QDs in plant tissues, the use of biomolecules in conjunction with QDs to target the presence or absence of specific transgenes or

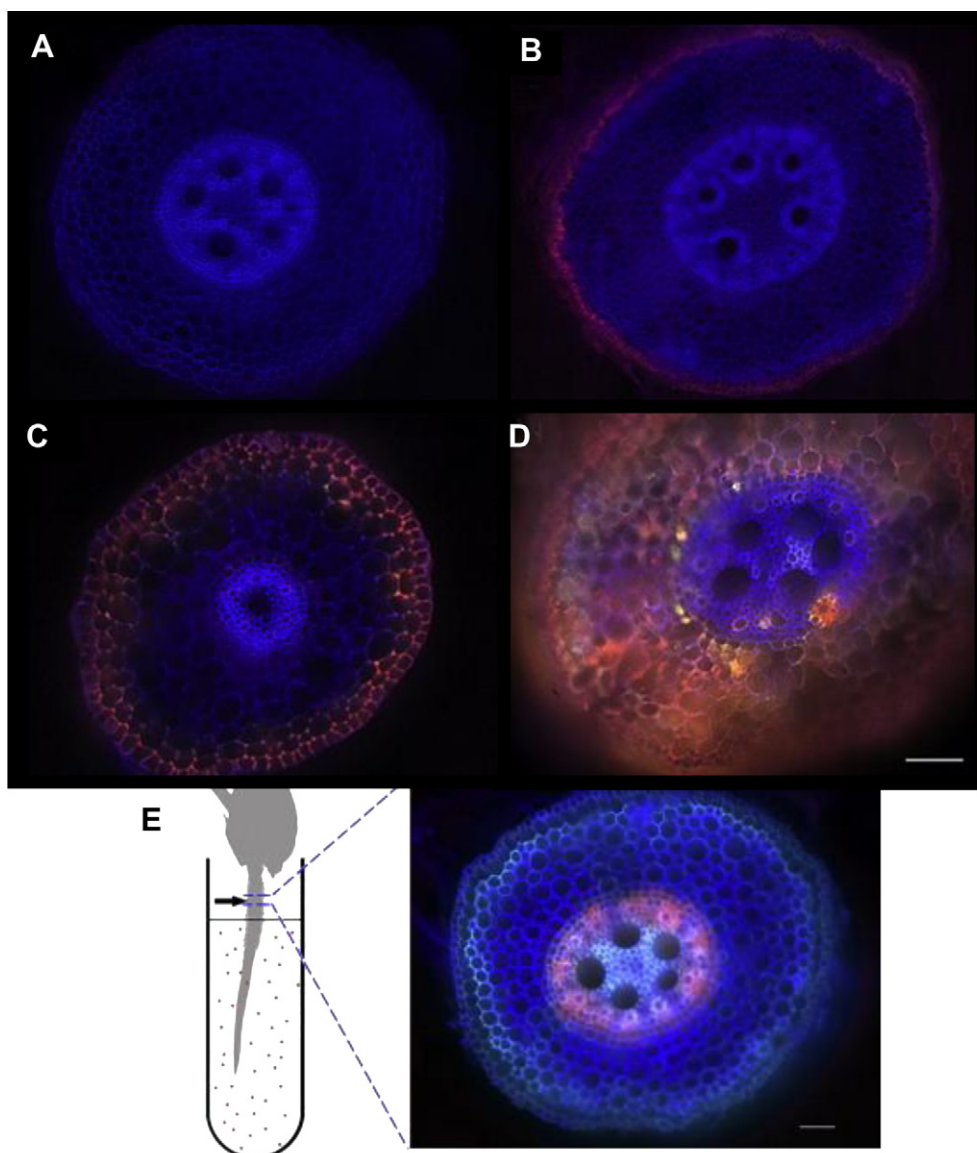


Fig. 2. *In vivo* cross-section fluorescent images of maize roots (A) control (blue fluorescence) and infiltrated with QDs (red fluorescence) (B) without and (C) with the aid of silwet L-77 in infiltration solution for 4 h and (D) 12 h and (E) above infiltration solution after 16 h. Red fluorescence is indicative of QD presence while blue fluorescence is emitted by the control. The use of a surfactant allowed for greater penetration of the QDs into the root, demonstrating the translocation of QDs to tissues throughout a plant where pathogens may colonize and be detected. Scale bars are 50 μm .

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pathogens within and on the surface of fresh produce has not been realized. When visualizing pathogens within plant tissues, sensitivity and specificity, in addition to contacting the target are some of the problems encountered. However, further advancements have confirmed the possibility of on-the-plant detection. We have recently demonstrated observable fluorescence, under UV light, using an MB designed for the detection of the gene, *cryIAC*, and co-infiltrating *Arabidopsis thaliana* (belonging to the Brassicaceae (mustard or crucifer) family) with genomic DNA derived from *Bacillus thuringiensis* and its respective MB (Burris *et al.* unpublished data). Since these QDs can be excited using

UV light and fluorescence can be visualized with the naked eye, this technology can be implemented into the field for immediate use. While there are specific sensitivity and specificity problems associated with crop plants, they are not that different from problems associated with pathogen and toxin detection within complex food matrices, as limitations are described in more detail in subsequent sections.

Limitations of QDs for *in vivo* monitoring of pathogens and transgenes

QDs have been used as fluorescent labels in a variety of assays for many biological and clinical applications

(Azzazy, Mansour, & Kazmierczak, 2007). However, there are several disadvantages relating specifically to the use of QDs in these types of applications. While the size of QDs could be advantageous by allowing for the binding of multiple biomolecules, their size may also limit their ability to be up taken into cells and tissues, particularly when bound to multiple biorecognition elements. Further, reproducibility of photophysical properties may vary from one manufacturer to another or from batch to batch. Other disadvantages include blinking effects (Table 1) (Lee & Osborne, 2009), degradation of the surface over time, solubility, expense, and aggregation which can cause photo-oxidation resulting in a decrease in overall stability (Hoshino *et al.*, 2004).

These and other problems relating to interfering substances, toxicity, aggregation, and non-specific binding must be addressed before their full potential and use as biosensors can be recognized. Further, because of their potential toxicity, regulations regarding the use of QDs in food applications will most likely be under scrutiny.

One of the main challenges for pathogen detection in foods is adequate sensitivity for the detection of bacteria of interest within the complex food matrix. Ideally, there would be implementation of techniques that are able to detect low levels of pathogens without destroying the food product for consumption and without the need for enrichment. Other challenges of monitoring for foodborne pathogens within food systems are their complexity (pH, interfering substances, non-pathogenic bacteria, etc) causing degradation, false-negatives or false-positives. Methods, such as filtration (Stevens & Jaykus, 2004; Wolffs *et al.*, 2006) and centrifugation (Lantz, Stålhandske, Lundahl, & Rådström, 1999; Uyttendaele, Van Hoorde, & Debevere, 2000; Wang, Cao, & Johnson, 1992), have been established to provide a means of detecting low numbers of pathogens from foods; however, more research needs to be done for individual pathogens, toxins and foods.

Conclusions

Food safety is an ever-growing concern for regulators, consumers and the food industry. The ability to detect foodborne pathogens and toxins in low concentrations is highly needed to ensure the production and distribution of safe food products. Conventional microbiological methods are deficient in sensitivity and specificity, and are often slow due to laborious, time-consuming protocols that are constrained to current technology. Coupling QDs to nucleic acid specific MBs or other biomolecules allows for higher specificity and for the accurate detection of target pathogen. QDs have emerged as a leading choice for their photostability, large effective Stokes shifts, sustained fluorescence, and narrow emission and wider excitation bandwidths.

Research is just beginning for the use of fluorescent nanoparticles as fluorophores for detecting foodborne pathogens and toxins on and in foods and plants. Work must continue for the optimization of assays to obtain an

accurate signal for low levels of pathogens in complex systems, whether they are food, plants or mammalian cells. The opportunities are endless for the potential nanotechnology has for food safety applications, the field is ever-expanding and food scientists are trying to keep up with the latest technologies that can be used to protect our food supply.

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References

- Azzazy, H. M., Mansour, M. M., & Kazmierczak, S. C. (2007). From diagnostics to therapy: prospects of quantum dots. *Clinical Biochemistry*, 40, 917–927.
- Balaban, N., & Rasooly, A. (2000). Review staphylococcal enterotoxins. *International Journal of Food Microbiology*, 61, 1–10.
- Bennett, R. W. (1998). *Bacillus cereus* diarrheal enterotoxin. In *Bacteriological analytical manual* (8th ed). Washington, D.C.: Food and Drug Administration. Revision A, Chapter 15.
- Bennett, R. W., & Hait, J. M. (1998). Staphylococcal enterotoxins: micro-slide double diffusion and ELISA-based methods. In *Bacteriological analytical manual* (8th ed). Washington, D.C.: Food and Drug Administration. Revision A, Chapter 13A.
- Bhagwat, A. A. (2003). Simultaneous detection of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* strains by real-time PCR. *International Journal of Food Microbiology*, 84, 217–224.
- Bratu, D. P., Cha, B.-J., Mhlanga, M. M., Kramer, F. R., & Tyagi, S. (2003). Visualizing the distribution and transport of mRNAs in living cells. *Proceedings of the National Academy of Sciences USA*, 100, 13308–13313.
- Bruno, J. G., Phillips, T., Carrillo, M. P., & Crowell, R. (2009). Plastic-adherent DNA aptamer- magnetic bead and quantum dot sandwich assay for *Campylobacter* detection. *Journal of Fluorescence*, 19, 427–435.
- Chen, A. K., Behlke, M. A., & Tsourkas, A. (2007). Avoiding false-positive signals with nuclease-vulnerable molecular beacons in single living cells. *Nucleic Acids Research*, 35, e105.
- Cremonesi, P., Perez, G., Pisoni, G., Moroni, P., Morandi, S., Luzzana, M., et al. (2007). Detection of enterotoxigenic *Staphylococcus aureus* isolates in raw milk cheese. *Letters in Applied Microbiology*, 45, 586–591.
- D'Aoust, J.-Y., Sewell, A. M., & Greco, P. (1991). Commercial latex agglutination kits for the detection of foodborne *Salmonella*. *Journal of Food Protection*, 54, 725–730.
- De la Fuente, J. M., & Penades, S. (2006). Glyconanoparticles: types, synthesis and applications in glycoscience, biomedicine and material science. *Biochimica et Biophysica Acta (BBA) – General Subjects*, 1760, 636–651.
- Duncan, T. V. (2011). Applications of nanotechnology in food packaging and food safety: barrier materials, antimicrobials and sensors. *Journal of Colloid and Interface Science*, 363, 1–24.

- Edgar, R., McKinstry, M., Hwang, J., Oppenheim, A. B., Fekete, R. A., Giulian, G., et al. (2006). High-sensitivity bacterial detection using biotin-tagged phage and quantum-dot nanocomplexes. *Proceedings of the National Academy of Sciences USA*, 103, 4841–4845.
- El-Boubbou, K., Gruden, C., & Huang, X. (2007). Magnetic glycol-nanoparticles: a unique tool for rapid pathogen detection, decontamination, and strain differentiation. *Journal of the American Chemical Society*, 129, 13392–13393.
- Ghasemi, Y., Peymani, P., & Afifi, S. (2009). Quantum dot: magic nanoparticle for imaging, detection and targeting. *Acta Bio-Medica: Atenei Parmensis*, 80, 156–165.
- Goldman, E. R., Anderson, G. P., Tran, P. T., Mattoussi, H., Charles, P. T., & Mauro, J. M. (2002). Conjugation of luminescent quantum dots with antibodies using an engineered adaptor protein to provide new reagents for fluoroimmunoassays. *Analytical Chemistry*, 74, 841–847.
- Hadjinicolaou, A. V., Demetriou, V. L., Emmanuel, M. A., Kakoyiannis, C. K., & Kostrikis, L. G. (2009). Molecular beacon-based real-time PCR detection of primary isolates of *Salmonella* Typhimurium and *Salmonella enteritidis* in environmental and clinical samples. *BMC Microbiology*, 9, Article No.: 97.
- Hadjinicolaou, A. V., Demetriou, V. L., Hezka, J., Beyer, W., Hadfield, T. L., & Kostrikis, L. G. (2009). Use of molecular beacons and multi-allelic real-time PCR for detection of and discrimination between virulent *Bacillus anthracis* and other *Bacillus* isolates. *Journal of Microbiological Methods*, 78, 45–53.
- Hahn, M. A., Keng, P. C., & Krauss, T. D. (2008). Flow cytometric analysis to detect pathogens in bacterial cell mixtures using semiconductor quantum dots. *Analytical Chemistry*, 80, 864–872.
- Hahn, M. A., Tabb, J. S., & Krauss, T. D. (2005). Detection of single bacterial pathogens with semiconductor quantum dots. *Analytical Chemistry*, 77, 4861–4869.
- Haseley, S. R. (2002). Carbohydrate recognition: a nascent technology for the detection of bioanalytes. *Analytica Chimica Acta*, 457, 39–45.
- Heo, J., & Hua, S. Z. (2009). An overview of recent strategies in pathogen sensing. *Sensors*, 9, 4483–4502.
- Hibi, K., Abe, A., Ohashi, E., Mitsubayashi, K., Ushio, H., Hayashi, T., et al. (2006). Combination of immunomagnetic separation with flow cytometry for detection of *Listeria monocytogenes*. *Analytica Chimica Acta*, 573–574, 158–163.
- Ho, Y. P., Kung, M. C., Yang, S., & Wang, T. H. (2005). Multiplexed hybridization detection with multicolor colocalization of quantum dot nanoprobe. *Nano Letters*, 5, 1693–1697.
- Hoshino, A., Fujioka, K., Oku, T., Suga, M., Sasaki, Y. F., Ohta, T., et al. (2004). Physicochemical properties and cellular toxicity of nanocrystal quantum dots depend on their surface modification. *Nano Letters*, 4, 2163–2169.
- Hu, Y., Li, J., Ma, L., Peng, Q., Feng, W., Zhang, L., et al. (2010). High efficiency transport of quantum dots into plant roots with the aid of silwet L-77. *Plant Physiology & Biochemistry*, 48, 703–709.
- Jayasena, S. D. (1999). Aptamers: an emerging class of molecules that rival antibodies in diagnostics. *Clinical Chemistry*, 45, 1628–1650.
- Jung, Y. S., Frank, J. F., & Brackett, R. E. (2003). Evaluation of antibodies for immunomagnetic separation combined with flow cytometry detection of *Listeria monocytogenes*. *Journal of Food Protection*, 66, 1283–1287.
- Kim, G., Om, A. S., & Mun, J. H. (2007). Nano-particle enhanced impedimetric biosensor for detection of foodborne pathogens. *Journal of Physics: Conference Series*, 61, 555.
- Kim, Y., Sohn, D., & Tan, W. (2008). Molecular beacons in biomedical detection and clinical diagnosis. *International Journal of Clinical and Experimental Pathology*, 1, 105–116.
- Kim, G.-H., & Son, A. (2010). Quantitative detection of *E. coli* O157:H7 *eaeA* gene using quantum dots and magnetic particles. *Biotechnology and Bioengineering*, 15, 1084–1093.
- Klostranec, J. M., & Chan, W. C. W. (2006). Quantum dots in biological and biomedical research: recent progress and present challenges. *Advanced Materials*, 18, 1953–1964.
- Lantz, P.-G., Ståhlhandske, F. I. T., Lundahl, K., & Rådström, P. (1999). Detection of yeast by PCR in sucrose solutions using a sample preparation method based on filtration. *World Journal of Microbiology and Biotechnology*, 15, 345–348.
- Lee, L. Y., Ong, S. L., Hu, J. Y., Ng, W. J., Feng, Y., Tan, X., et al. (2004). Use of semiconductor quantum dots for photostable immunofluorescence labeling of *Cryptosporidium parvum*. *Applied and Environmental Microbiology*, 70, 5732–5736.
- Lee, S. F., & Osborne, M. A. (2009). Brightening, blinking, bluing and bleaching in the life of a quantum dot: friend or foe? *ChemPhysChem*, 10, 2174–2191.
- Liao, C. H., & Shollenberger, L. M. (2003). Detection of *Salmonella* by indicator agar media and PCR as affected by alfalfa seed homogenates and native bacteria. *Letters in Applied Microbiology*, 36, 152–156.
- Liming, S. H., & Bhagwat, A. A. (2004). Application of a molecular beacon – real-time PCR technology to detect *Salmonella* species contaminating fruits and vegetables. *International Journal of Food Microbiology*, 95, 177–187.
- Loncarevic, S., Jørgensen, H. J., Lovseth, A., Mathisen, T., & Rorvik, L. M. (2005). Diversity of *Staphylococcus aureus* enterotoxin types within single samples of raw milk and raw milk products. *Journal of Applied Microbiology*, 98, 344–350.
- Marras, S. A. (2008). Interactive fluorophore and quencher pairs for labeling fluorescent nucleic acid hybridization probes. *Molecular Biotechnology*, 38, 247–255.
- Meng, J., & Doyle, M. P. (2002). Introduction microbiological food safety. *Microbes and Infection*, 4, 395–397.
- Michalet, X., Pinaud, F. F., Bentolila, L. A., Tsay, J. M., Doose, S., Li, J. J., et al. (2005). Quantum dots for live cells, *in vivo* imaging, and diagnostics. *Science*, 307, 538–544.
- Monday, S. R., & Bohach, G. A. (1999). Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in staphylococcal isolates. *Journal of Clinical Microbiology*, 37, 3411–3414.
- Mouricout, M. (1997). Interactions between the enteric pathogen and the host. An assortment of bacterial lectins and a set of glycoconjugate receptors. *Advances In Experimental Medicine and Biology*, 412, 109–123.
- Mukhopadhyay, B., Martins, M. B., Karamanska, R., Russell, D. A., & Field, R. A. (2009). Bacterial detection using carbohydrate-functionalised CdS quantum dots: a model study exploiting *E. coli* recognition of mannosides. *Tetrahedron Letters*, 50, 886–889.
- Ozkan, M. (2004). Quantum dots and other nanoparticles: what can they offer to drug discovery? *Drug Discovery Today*, 9, 1065–1071.
- Patel, J. R., Bhagwat, A. A., Sanglay, G. C., & Solomon, M. B. (2006). Rapid detection of *Salmonella* from hydrodynamic pressure-treated poultry using molecular beacon real-time PCR. *Food Microbiology*, 23, 39–46.
- Rawthorne, H., Phister, T. G., & Jaykus, L. A. (2009). Development of a fluorescent *in situ* method for visualization of enteric viruses. *Applied and Environmental Microbiology*, 75, 7822–7827.
- Resch-Genger, U., Grabolle, M., Cavaliere-Jaricot, S., Nitschke, R., & Nann, T. (2008). Quantum dots versus organic dyes as fluorescent labels. *Nature Methods*, 5, 763–775.
- Santangelo, P. J., Nix, B., Tsourkas, A., & Bao, G. (2004). Dual FRET molecular beacons for mRNA detection in living cells. *Nucleic Acids Research*, 32.
- Sanvicens, N., Pascual, N., Fernández-Argüelles, T., Adrián, J., Costa-Fernández, J. M., Sánchez-Baeza, F., et al. (2011). Quantum dot-based array for sensitive detection of *Escherichia coli*. *Analytical and Bioanalytical Chemistry*, 399, 2755–2762.

- Sanvicens, N., Pastells, C., Pascual, N., & Marco, M.-P. (2009). Nanoparticle-based biosensors for detection of pathogenic bacteria. *Trends in Analytical Chemistry*, 28, 1243–1252.
- Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., et al. (2011). Foodborne illness acquired in the United States - major pathogens. *Emerging Infectious Diseases*, 17, 7–15.
- Scharff, R. L. (2012). Economic burden from health losses due to foodborne illness in the United States. *Journal of Food Protection*, 75, 123–131.
- Schlosser, G., Kacer, P., Kuzma, M., Szilagy, Z., Sorrentino, A., Manzo, C., et al. (2007). Coupling immunomagnetic separation on magnetic beads with matrix-assisted laser desorption/ionization-time of flight mass spectrometry for detection of staphylococcal enterotoxin B. *Applied and Environmental Microbiology*, 73, 6945–6952.
- Seo, K. H., & Frank, J. F. (1999). Attachment of *Escherichia coli* O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scanning laser microscopy. *Journal of Food Protection*, 62, 3–9.
- Sharon, N. (2006). Carbohydrates as future anti-adhesion drugs for infectious diseases. *Biochimica et Biophysica Acta*, 1760, 527–537.
- Shearer, A. E. H., Strapp, C. M., & Joerger, R. D. (2001). Evaluation of a polymerase chain reaction-based system for detection of *Salmonella enteritidis*, *Escherichia coli* O157:H7, *Listeria* spp., and *Listeria monocytogenes* on fresh fruits and vegetables. *Journal of Food Protection*, 64, 788–795.
- Smith, A. M., & Nie, S. (2004). Chemical analysis and cellular imaging with quantum dots. *Analyst*, 129, 672–677.
- Stevens, K. A., & Jaykus, L. A. (2004). Direct detection of bacterial pathogens in representative dairy products using a combined bacterial concentration-PCR approach. *Journal of Applied Microbiology*, 97, 1115–1122.
- Su, X. L., & Li, Y. (2004). Quantum dot biolabeling coupled with immunomagnetic separation for detection of *Escherichia coli* O157:H7. *Analytical Chemistry*, 76, 4806–4810.
- Takeuchi, K., & Frank, J. F. (2000). Penetration of *Escherichia coli* O157:H7 into lettuce tissues as affected by inoculum size and temperature and the effect of chlorine treatment on cell viability. *Journal of Food Protection*, 63, 434–440.
- Tallury, P., Malhotra, A., Byrne, L. M., & Santra, S. (2010). Nanobioimaging and sensing of infectious diseases. *Advanced Drug Delivery Reviews*, 62, 424–437.
- Tkacikova, L., Tesfaye, A., & Mikula, I. (2003). Detection of the genes for *Staphylococcus aureus* enterotoxin by PCR. *Acta Veterinaria*, 72, 627–630.
- Tully, E., Hearty, S., Leonard, P., & O'Kennedy, R. (2006). The development of rapid fluorescence-based immunoassays, using quantum dot-labelled antibodies for the detection of *Listeria monocytogenes* cell surface proteins. *International Journal of Biological Macromolecules*, 39, 127–134.
- Tyagi, S., & Kramer, F. R. (1996). Molecular beacons: probes that fluoresce upon hybridization. *Nature Biotechnology*, 14, 303–308.
- Uyttendaele, M., Van Hoorde, I., & Debevere, J. (2000). The use of immuno-magnetic separation (IMS) as a tool in a sample preparation method for direct detection of *L. monocytogenes* in cheese. *International Journal of Food Microbiology*, 54, 205–212.
- Vasudev, M., Wu, T.-C., Dutta, M., Strosio, M. A., Guthrie, S., Reed, M., et al. (2011). Optoelectronic signatures of DNA based hybrid nanostructures. *IEEE Transactions on Nanotechnology*, 10, 35–43.
- Vikesland, P. J., & Wigginton, K. R. (2010). Nanomaterial enabled biosensors for pathogen monitoring – a review. *Environmental Science & Technology*, 44, 3656–3669.
- Wang, R. F., Cao, W. W., & Johnson, M. G. (1992). 16S rRNA-based probes and polymerase chain reaction method to detect *Listeria monocytogenes* cells added to foods. *Applied and Environmental Microbiology*, 58, 2827–2831.
- Warner, M. G., Grate, J. W., Tyler, A., Ozanich, R. M., Miller, K. D., Lou, J., et al. (2009). Quantum dot immunoassays in renewable surface column and 96-well plate formats for the fluorescence detection of botulinum neurotoxin using high-affinity antibodies. *Biosensors & Bioelectronics*, 25, 179–184.
- Wolffs, P. F. G., Glencross, K., Thibaut, R., & Griffiths, M. W. (2006). Direct quantitation and detection of salmonellae in biological samples without enrichment, using two-step filtration and real-time PCR. *Applied and Environmental Microbiology*, 72, 3896–3900.
- Wu, Y. R., Yang, C. J., Moroz, L. L., & Tan, W. H. (2008). Nucleic acid beacons for long-term real-time intracellular monitoring. *Analytical Chemistry*, 80, 3025–3028.
- Xi, C., Balberg, M., Boppart, S. A., & Raskin, L. (2003). Use of DNA and peptide nucleic acid molecular beacons for detection and quantification of rRNA in solution and in whole cells. *Applied and Environmental Microbiology*, 69, 5673–5678.
- Yang, M., Baranov, E., Jiang, P., Sun, F. X., Li, X. M., Li, L., et al. (2000). Whole-body optical imaging of green fluorescent protein-expressing tumors and metastases. *Proceedings of the National Academy of Sciences USA*, 97, 1206–1211.
- Yang, L., & Li, Y. (2006). Simultaneous detection of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium using quantum dots as fluorescence labels. *Analyst*, 131, 394–401.
- Zhao, Y., Ye, M., Chao, Q., Jia, N., Ge, Y., & Shen, H. (2009). Simultaneous detection of multi-food-borne pathogenic bacteria based on functionalized quantum dots coupled with immunomagnetic separation in food samples. *Journal of Agricultural and Food Chemistry*, 57, 517–524.
- Zhu, L., Ang, S., & Liu, W. T. (2004). Quantum dots as a novel immunofluorescent detection system for *Cryptosporidium parvum* and *Giardia lamblia*. *Applied and Environmental Microbiology*, 70, 597–598.