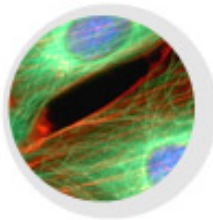

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Nanotech Tools To Address Biotech Problems

Nano Diagnostics in the Food Industry

Dr David Sarphie
CEO

Presentation to CIAA
Brussels
March 2010

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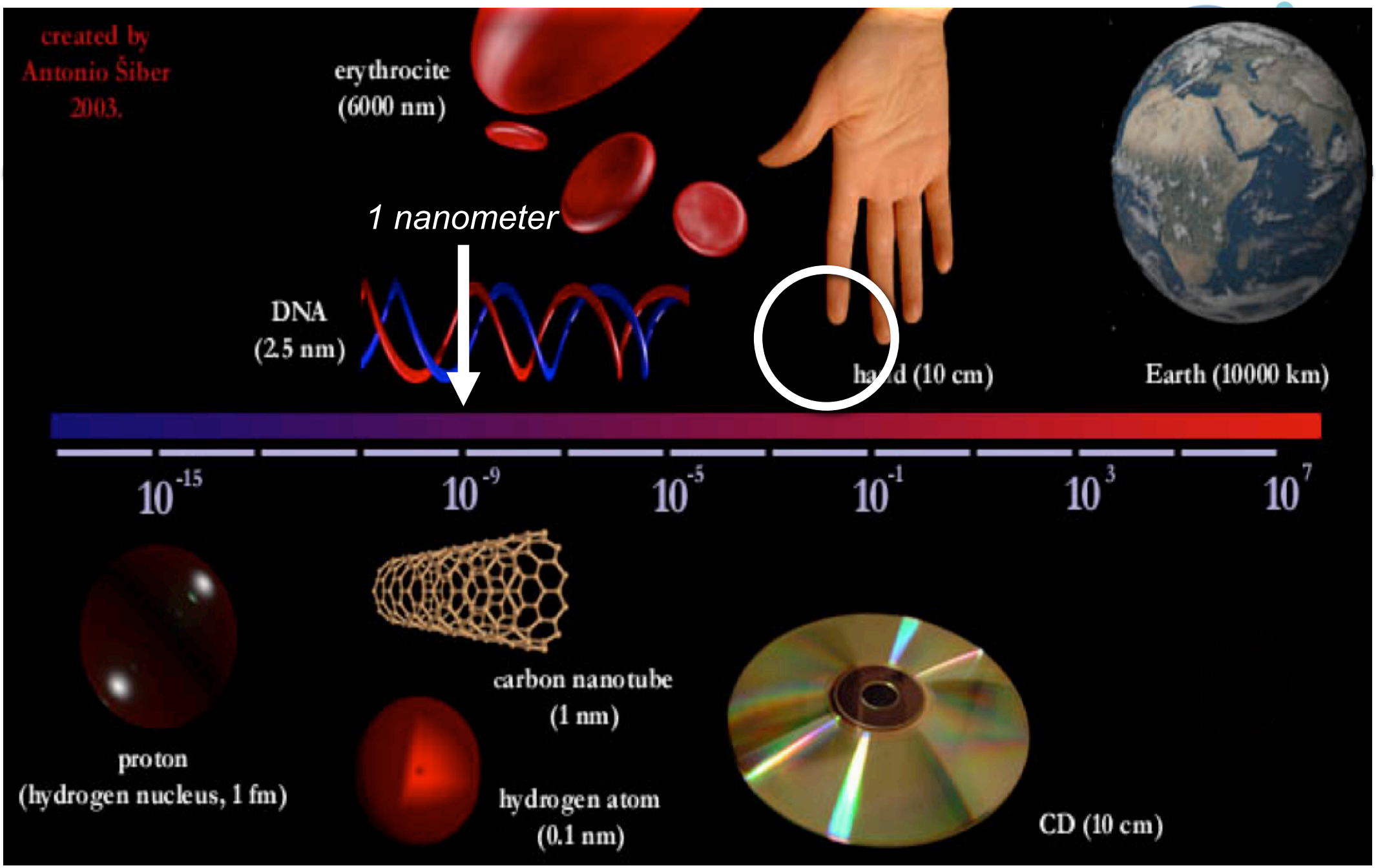
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Antonio Šiber
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10^{3}
10^{7}
carbon nanotube
(1 nm)
proton
(hydrogen nucleus, 1 fm)
hydrogen atom
(0.1 nm)
CD (10 cm)

Nanotechnology in food processing

Example from chocolate industry

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- *Early application of nanotechnology...*
- *Use of melanger to produce chocolate crystals of specific size*
- *Results in crystals of cocoa 6.5 nm in diameter*
- *Developed several centuries ago...*
- *...though they didn't call it **"nanotechnology"** then....*

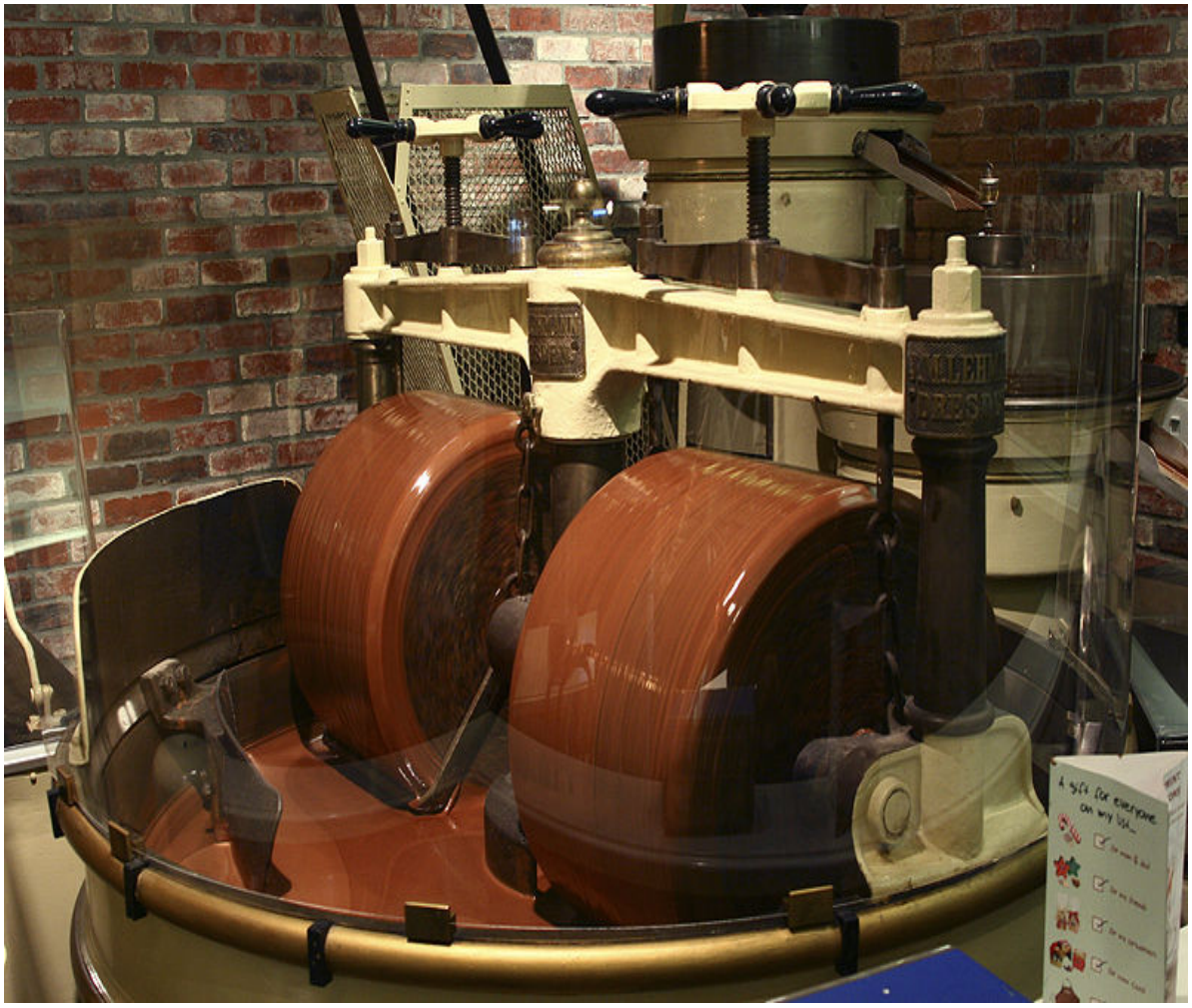

Photo courtesy of Sanjay Acharya

Nanotechnology in food processing

Example from ice cream industry

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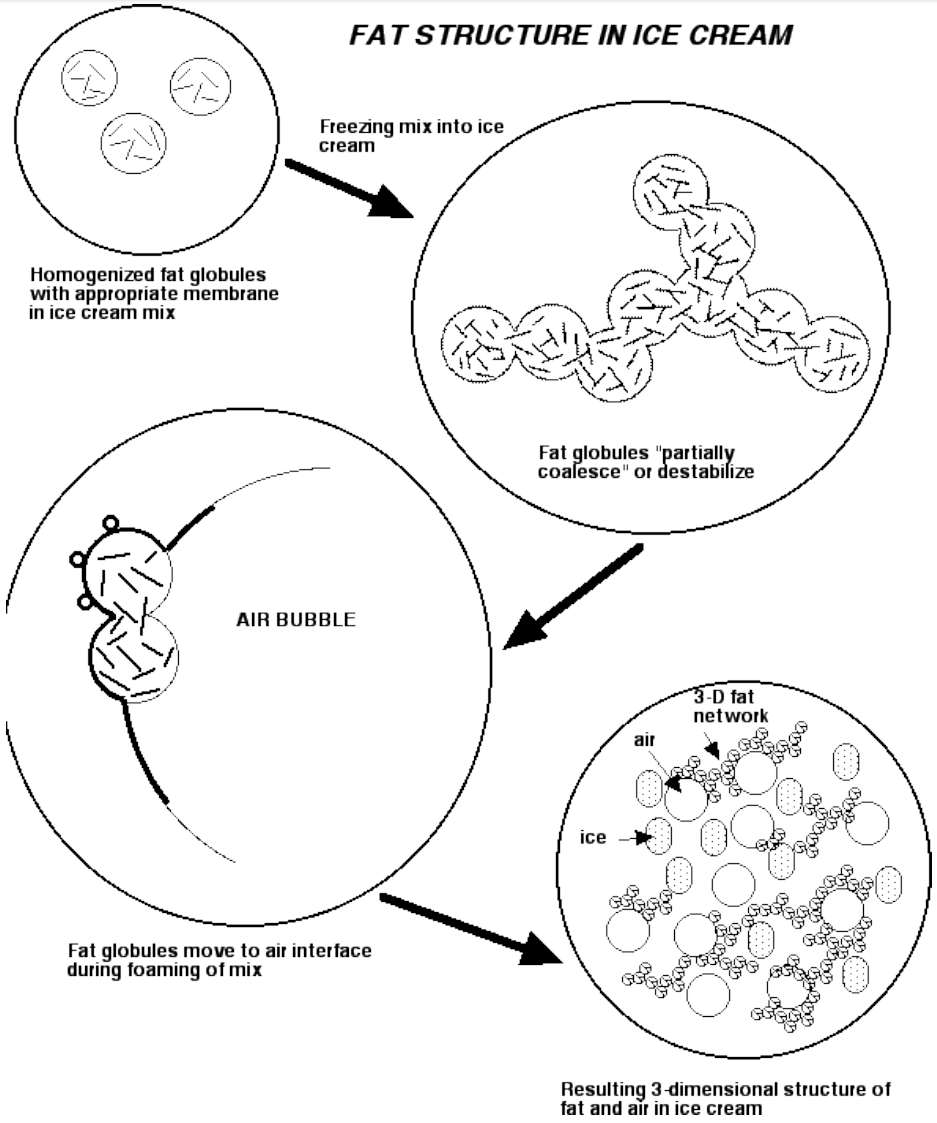

- *Addition of emulsifiers such as lecithin to ice cream*
- *Reduce amount of cream in the ice cream while keeping texture*

- ***courtesy of Professor H. Douglas Goff – University of Guelph***

Nano-rod–based biosensor
Detection of Salmonella

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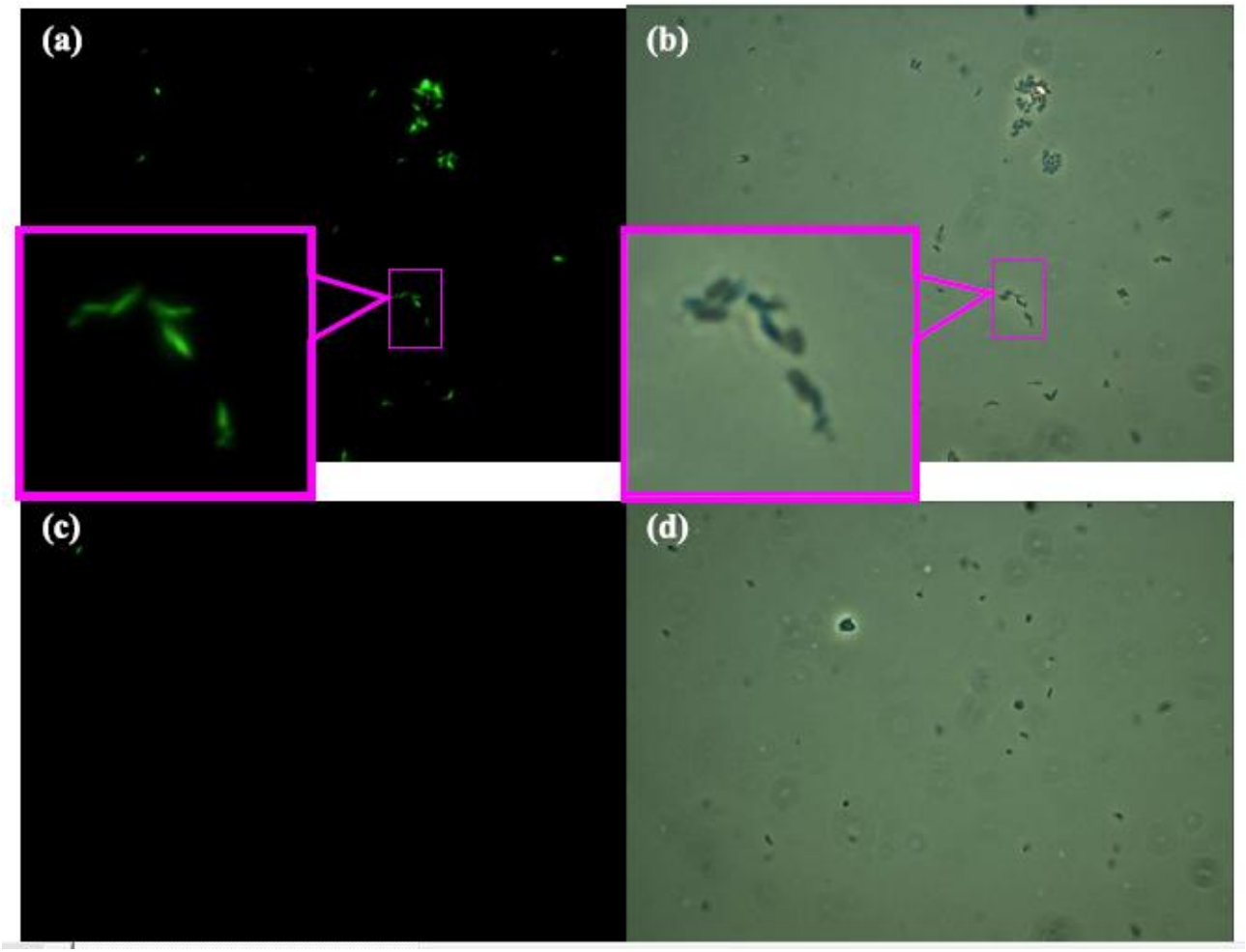

IOP Publishing Nanotechnology
Nanotechnology 19 (2008) 155502 (7pp) doi:10.1088/0957-4484/19/15/155502

An Au/Si hetero-nanorod-based biosensor for *Salmonella* detection

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Abstract

We present a novel and effective food-borne bacteria detection method. A hetero-structured silicon/gold nanorod array fabricated by the glancing angle deposition method is functionalized with anti-*Salmonella* antibodies and organic dye molecules. Due to the high aspect ratio nature of the Si nanorods, dye molecules attached to the Si nanorods produce an enhanced fluorescence upon capture and detection of *Salmonella*. This bio-functional hetero-nanorod detection method has great potential in the food safety industry as well as in biomedical diagnostics.

M Supplementary data are available from stacks.iop.org/Nano/19/155502

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Salmonella is one of the major causes of bacterial gastroenteritis in humans and a source of many food-related outbreaks, thus rapid and sensitive detection is important in the control of food safety. Various analytical methods have been developed to detect the *Salmonella*. The conventional microbiological technique, i.e. ISO method 6579, often requires up to 5–7 days to obtain a positive result [1–4]. These culture methods have been reported to show poor sensitivity for low-level contamination in samples [5]. To further improve the detection sensitivity, a number of investigators use fluorescent-antibody (FA) procedures for *Salmonella* detection [6–10]. Although FA procedures require less time and have higher sensitivity for detection over culture methods, the detection is still limited by the requirement for substantial numbers of bacteria present for detection to discriminate a fluorescent signal over background fluorescence. This is a relevant issue in food microbiology because the *Salmonella* numbers are usually low, thus it is often necessary to use enrichment culture techniques prior to immunofluorescence microscopy. Therefore the FA procedure in the detection of *Salmonella* has not been in routine use.

Several rapid detection methods have been developed over the past few years, such as enzyme-linked immunosorbent assay (ELISA) [11]. ELISA is based on the specific recognition of the antigen by antibodies and is measured with the aid of an enzyme–substrate reaction. Several different ELISA methods have been developed including competitive ELISA [12], PCR-ELISA [13] and Dot-ELISA [14, 15]. These ELISA methods are considered highly sensitive, and provide specific and rapid screening of a large number of samples for the presence of *Salmonella*. However, a disadvantage of the ELISA method is that it requires considerable amounts of antigen to be used for detection. Some research has reported that the detection sensitivity of ELISA was 10^5 colony-forming unit (CFU) of *Salmonella spp.* per ml of culture [11]. Another effective method for the detection and identification of *Salmonella* is the polymerase chain reaction (PCR), which is based on isolating the bacteria and exponentially amplifying a DNA fragment or sequence of interest via enzymatic replication [5, 16, 17]. Real-time PCR offers the advantage of semi-quantification of the bacterial DNA and no post-PCR handling of the sample, thus reducing the risk of false-positive results [3, 4, 18, 19]. Although PCR and its modifications may detect *Salmonella* within a relatively short time, issues remain such as primer design and PCR-inhibitory effects of complex food matrices that make PCR detection of *Salmonella* in food far from being a routine procedure [4, 20]. Electrochemical biosensing has also been listed as one of the

Fu et al. University of Georgia
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Development of biosensor
Pathogen detection

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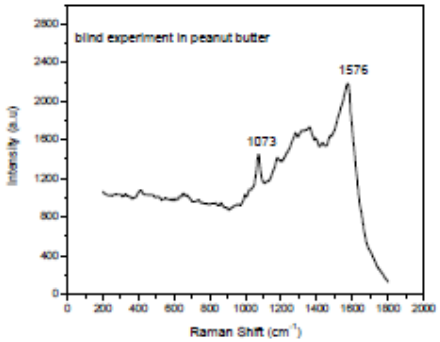
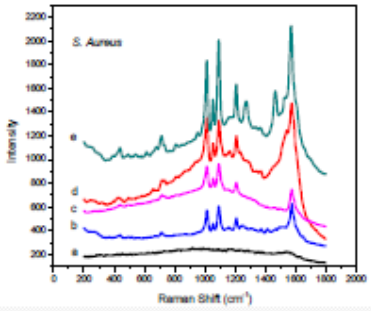
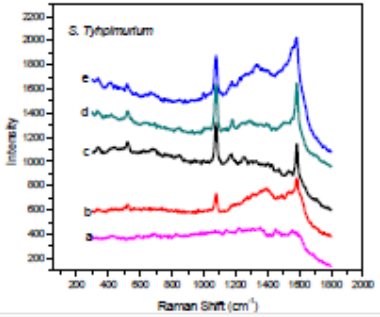
Detection in Peanut butter by SERS

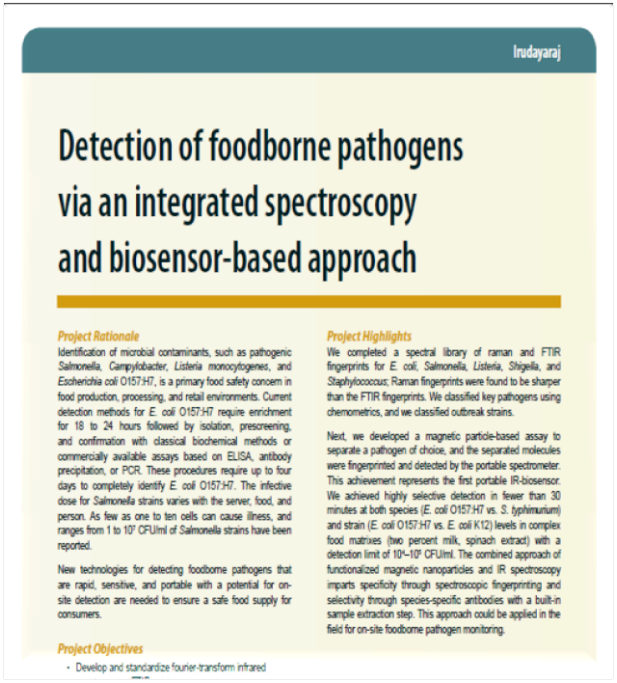


Blind experiment for detection in peanut butter. The spectrum indicates that the pathogen is *S. typhimurium.*



SERS spectra for *S. aureus* and *S. tyhpimurium* in peanut butter (a. 0 cfu/mL; b. 10^3 cfu/mL; c. 10^4cfu/mL; d. 10^5 cfu/mL; e. 10^6 cfu





Irudayaraj

Detection of foodborne pathogens via an integrated spectroscopy and biosensor-based approach

Project Rationale

Identification of microbial contaminants, such as pathogenic *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7, is a primary food safety concern in food production, processing, and retail environments. Current detection methods for *E. coli* O157:H7 require enrichment for 18 to 24 hours followed by isolation, prescreening, and confirmation with classical biochemical methods or commercially available assays based on ELISA, antibody precipitation, or PCR. These procedures require up to four days to completely identify *E. coli* O157:H7. The infective dose for *Salmonella* strains varies with the server, food, and person. As few as one to ten cells can cause illness, and ranges from 1 to 10^7 CFU/ml of *Salmonella* strains have been reported.

New technologies for detecting foodborne pathogens that are rapid, sensitive, and portable with a potential for on-site detection are needed to ensure a safe food supply for consumers.

Project Highlights

We completed a spectral library of raman and FTIR fingerprints for *E. coli*, *Salmonella*, *Listeria*, *Shigella*, and *Staphylococcus*; Raman fingerprints were found to be sharper than the FTIR fingerprints. We classified key pathogens using chemometrics, and we classified outbreak strains.

Next, we developed a magnetic particle-based assay to separate a pathogen of choice, and the separated molecules were fingerprinted and detected by the portable spectrometer. This achievement represents the first portable IR-biosensor. We achieved highly selective detection in fewer than 30 minutes at both species (*E. coli* O157:H7 vs. *S. typhimurium*) and strain (*E. coli* O157:H7 vs. *E. coli* K12) levels in complex food matrixes (two percent milk, spinach extract) with a detection limit of 10^1–10^2 CFU/ml. The combined approach of functionalized magnetic nanoparticles and IR spectroscopy imparts specificity through spectroscopic fingerprinting and selectivity through species-specific antibodies with a built-in sample extraction step. This approach could be applied in the field for on-site foodborne pathogen monitoring.

Project Objectives

- Develop and standardize fourier-transform infrared

Irudayaraj et al. Purdue University Internal Report 2009

Micro-cantilevers

Detecting pathogens in food

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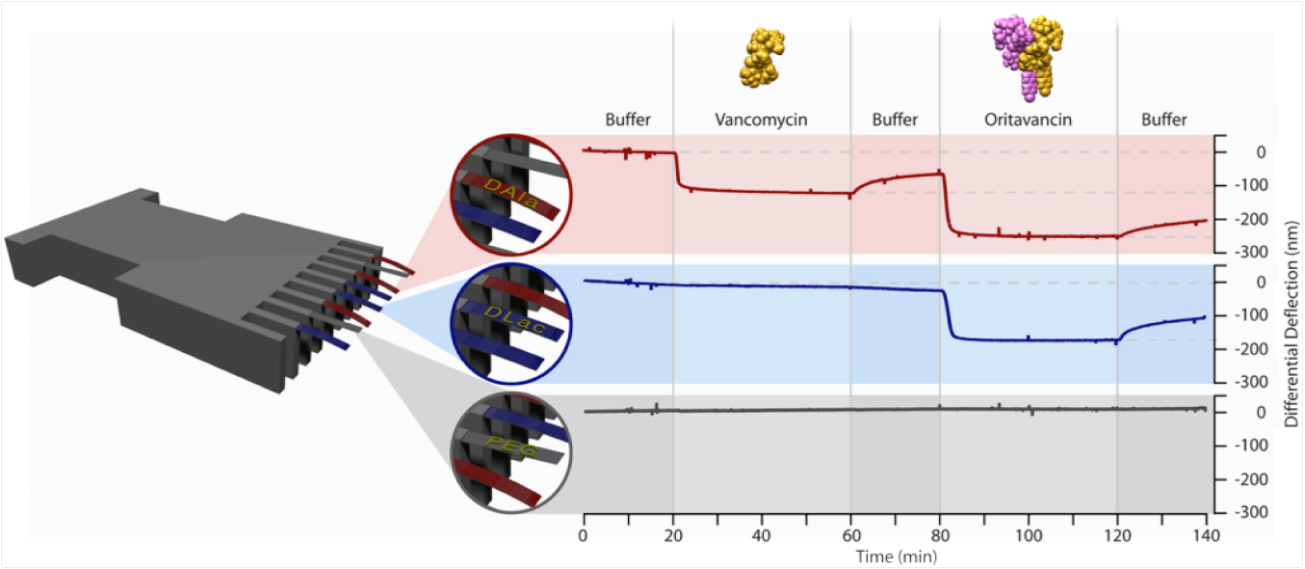

ARTICLES

Nanomechanical detection of antibiotic–mucopeptide binding in a model for superbug drug resistance

JOSEPH WAFULA NDIEYIRA[1,2†], MOYU WATARI[1†], ALEJANDRA DONOSO BARRERA[1], DEJIAN ZHOU[3,4], MANUEL VÖGTLI[1], MATTHEW BATCHELOR[5], MATTHEW A. COOPER[5], TORSTEN STRUNZ[1], MIKE A. HORTON[1], CHRIS ABELL[3], TREVOR RAYMENT[6], GABRIEL AEPPLI[1] AND RACHEL A. McKENDRY[1]*

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The alarming growth of the antibiotic-resistant superbugs methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) is driving the development of new technologies to investigate antibiotics and their modes of action. We report the label-free detection of vancomycin binding to bacterial cell wall precursor analogues (mucopeptides) on cantilever arrays, with 10 nM sensitivity and at clinically relevant concentrations in blood serum. Differential measurements have quantified binding constants for vancomycin-sensitive and vancomycin-resistant mucopeptide analogues. Moreover, by systematically modifying the mucopeptide density we gain new insights into the origin of surface stress. We propose that stress is a product of a local chemical binding factor and a geometrical factor describing the mechanical connectivity of regions activated by local binding in terms of a percolation process. Our findings place BioMEMS devices in a new class of percolative systems. The percolation concept will underpin the design of devices and coatings to significantly lower the drug detection limit and may also have an impact on our understanding of antibiotic drug action in bacteria.

When biochemically specific interactions occur between a ligand immobilized on one side of a cantilever and a receptor in solution, the cantilever bends due to a change in surface stress[1–4]. The general applicability of this novel nanomechanical biosensing transduction mechanism has been shown for sequence-specific DNA hybridization[5–8], single base mismatches[5], DNA quadruplex[9], protein recognition[1,4,7,8] and recently the detection of interferon-alpha-induced 1-8U gene expression in total human RNA, a potential marker for melanoma progression and viral infections[8]. However, to date, multiple cantilever arrays have not been applied to quantify drug–target binding interactions, despite offering considerable advantages. First, cantilevers require no reporter 'tags' or external probes, and biomolecules can therefore be detected rapidly in a single-step reaction. Second, cantilever arrays can screen multiple drug–target interactions and reference coatings in parallel and under identical experimental conditions. Third, we have previously shown that quantitative ligand–receptor binding constants can be measured on cantilever arrays[8]. Moreover, the nanomechanical signal is not limited by mass, in contrast to evanescent techniques such as surface plasmon resonance, which detects mass-related changes in the dielectric constant[10–13]. Cantilevers are therefore unique as probes of small-molecule drug-binding interactions and, by virtue of their miniaturized dimensions, they are amenable for parallelization[14,15] for high-throughput screening of thousands of drugs per hour.

Here we report the first quantitative differential nanomechanical investigation of drug–target binding interactions on multiple cantilever arrays focusing on the antibiotic vancomycin (Fig. 1). Today vancomycin is one of the last powerful antibiotics in the battle against resistant bacteria and the 'hospital superbug' methicillin-resistant *Staphylococcus aureus* (MRSA)[16–17]. It is a vital therapeutic drug used worldwide for the treatment of infections with Gram-positive bacteria, particularly those *Staphylococci* and *Enterococci* responsible for post-surgical infections. Vancomycin binds to the C-terminus of peptidoglycan mucopeptide precursors terminating in the sequence Lysine-D-Alanine-D-Alanine[1–4,18,19], as shown in Fig. 1. This interaction blocks the action of bacterial transpeptidases and transglycosylases, which catalyse the crosslinking of the growing bacterial cell wall, resulting in cell lysis[16–17]. Unfortunately, due to the overuse of antibiotics,

nature nanotechnology | VOL 3 | NOVEMBER 2008 | www.nature.com/naturenanotechnology 601

Ndieryira et al. University College London
Nature Nanotechnology 2008

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Micro-cantilevers

Useful for detection of pathogens

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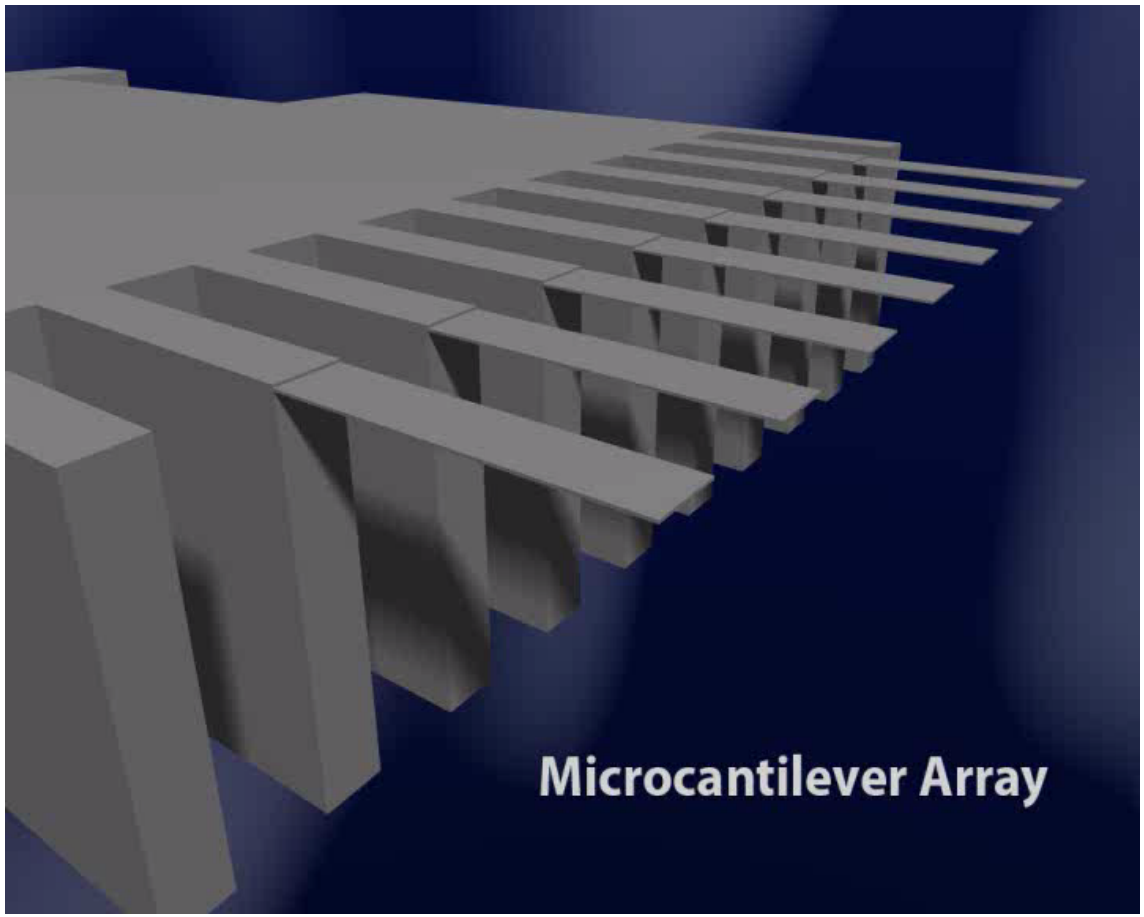

- *Demonstrated extremely high sensitivity*
- *Highly selective binding*
- *Can work in liquid and gas phase (i.e. as an electronic "tongue" or "nose")*

Case study:
Micro-cantilevers

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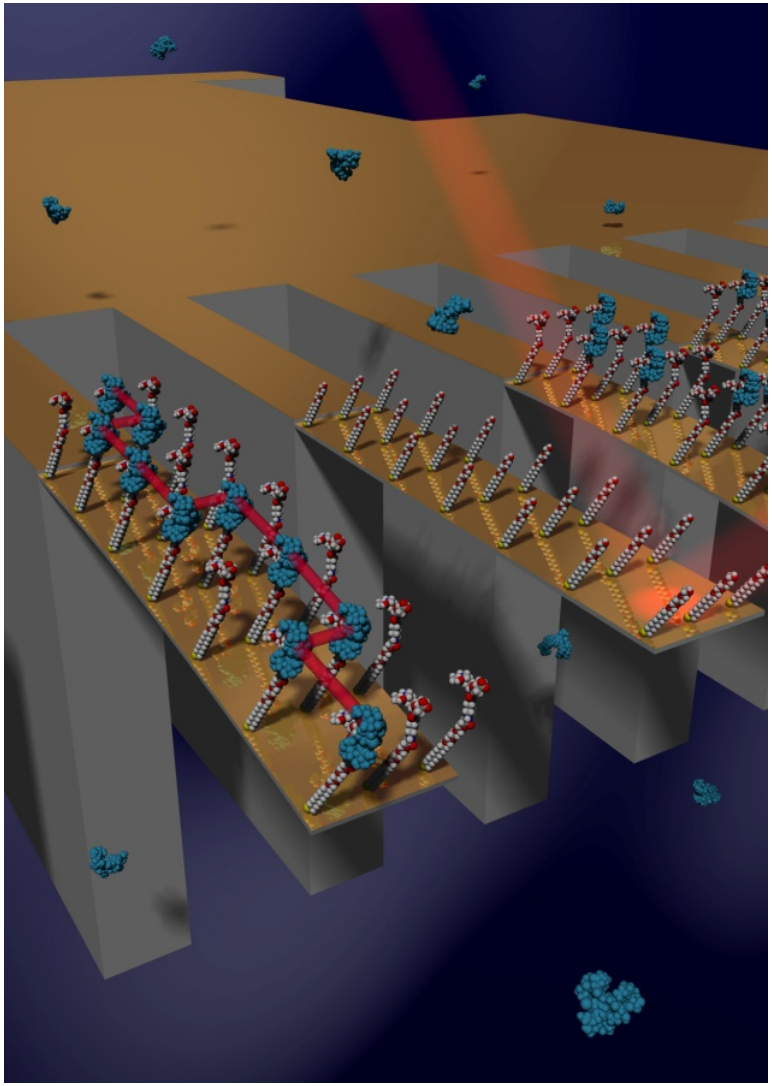
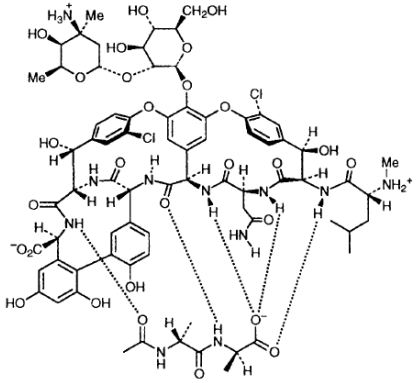


- Biosensors useful for detecting superbugs & developing superdrugs
- Highly sensitive
- Assess antibiotic binding



Vancomycin (soil actinomycetes)

Last line of therapeutics in battle against Gram positive bacteria

Micro-cantilevers

Useful for detection of pathogens

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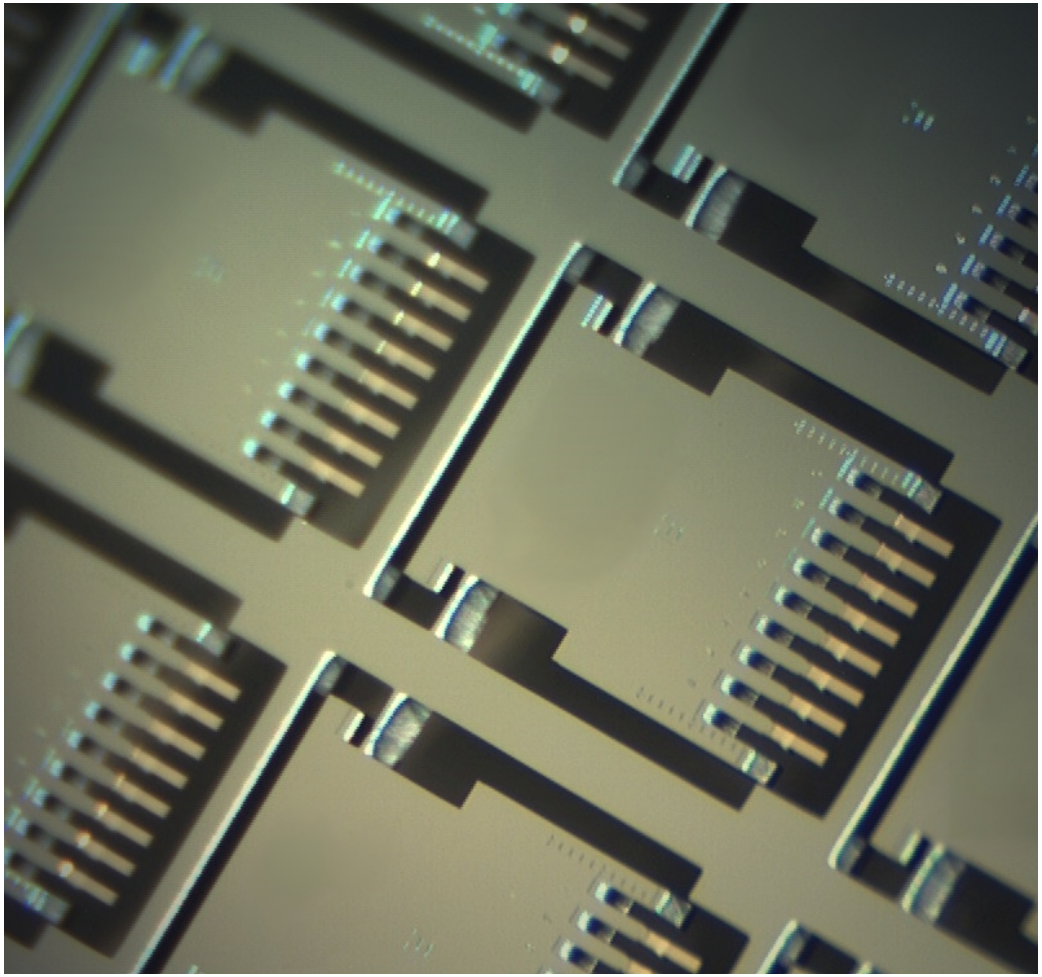

- *Originally developed in 80's by IBM-Zurich using methods from micro-electronics industry*

Silicon diving boards...

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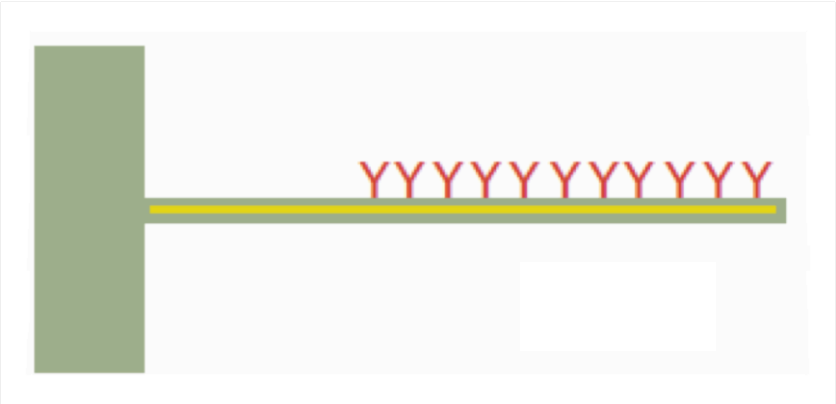
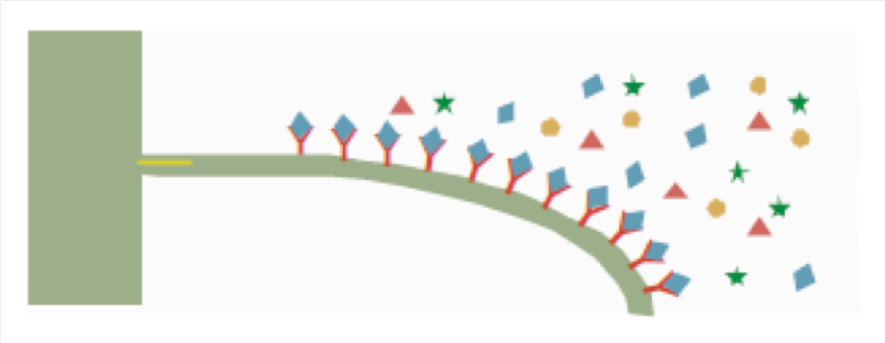


a.) functionalised cantilever before detection



b.) bending cantilever during binding

Dimensions:

Length:	500µm
Width:	100µm
Thickness:	< 1µm

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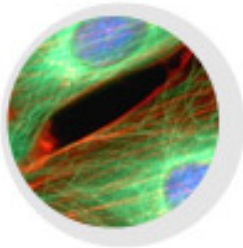
- Commercially-focused problem-solving consultancy across the life sciences & technology sectors



  - Product development
  - Strategic consultancy
  - Project management
  - Access to state of the art instrumentation and expertise

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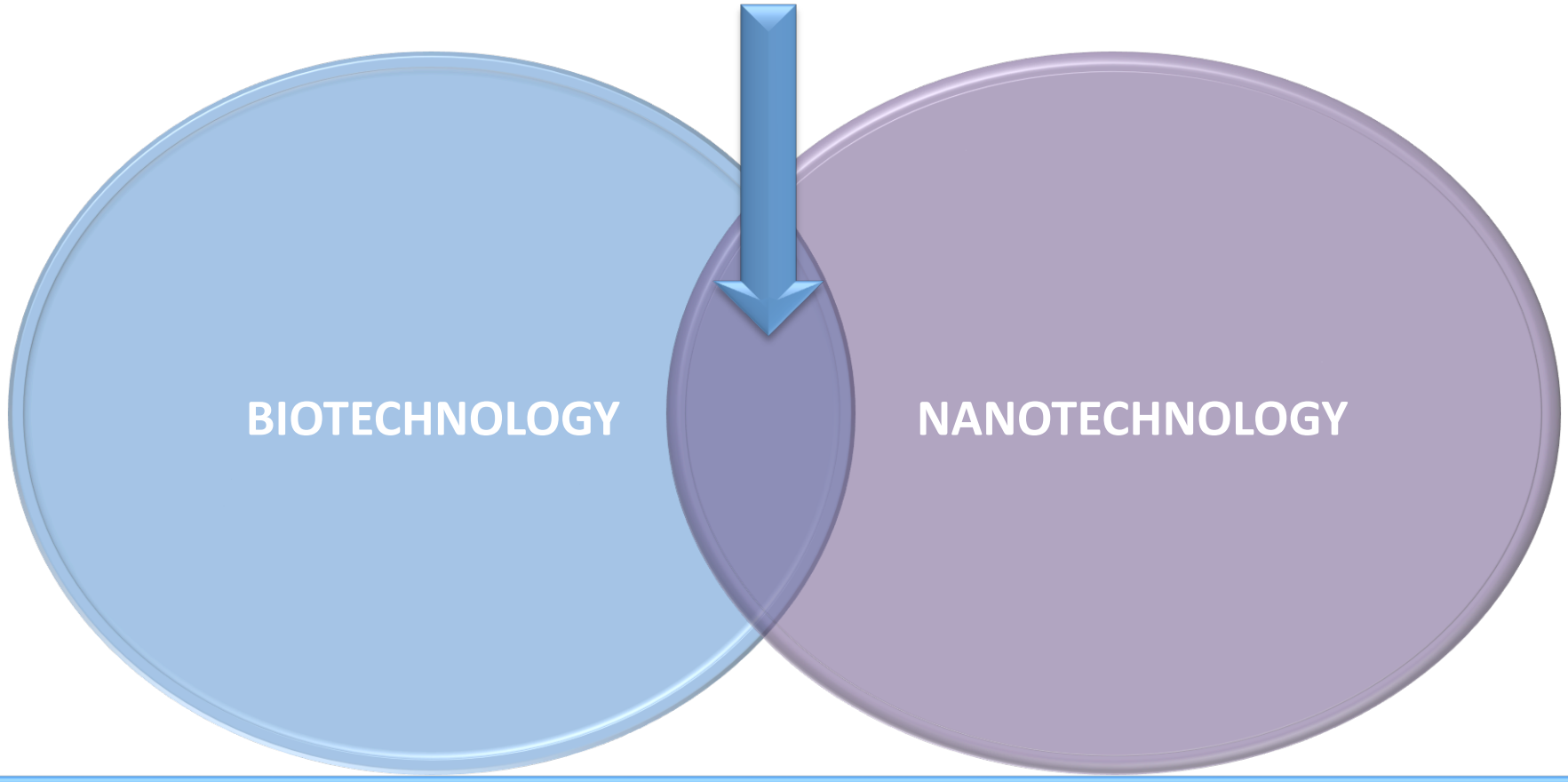

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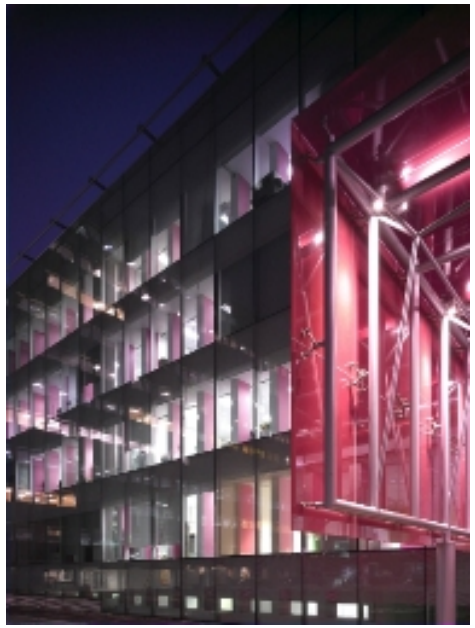

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BNC business model

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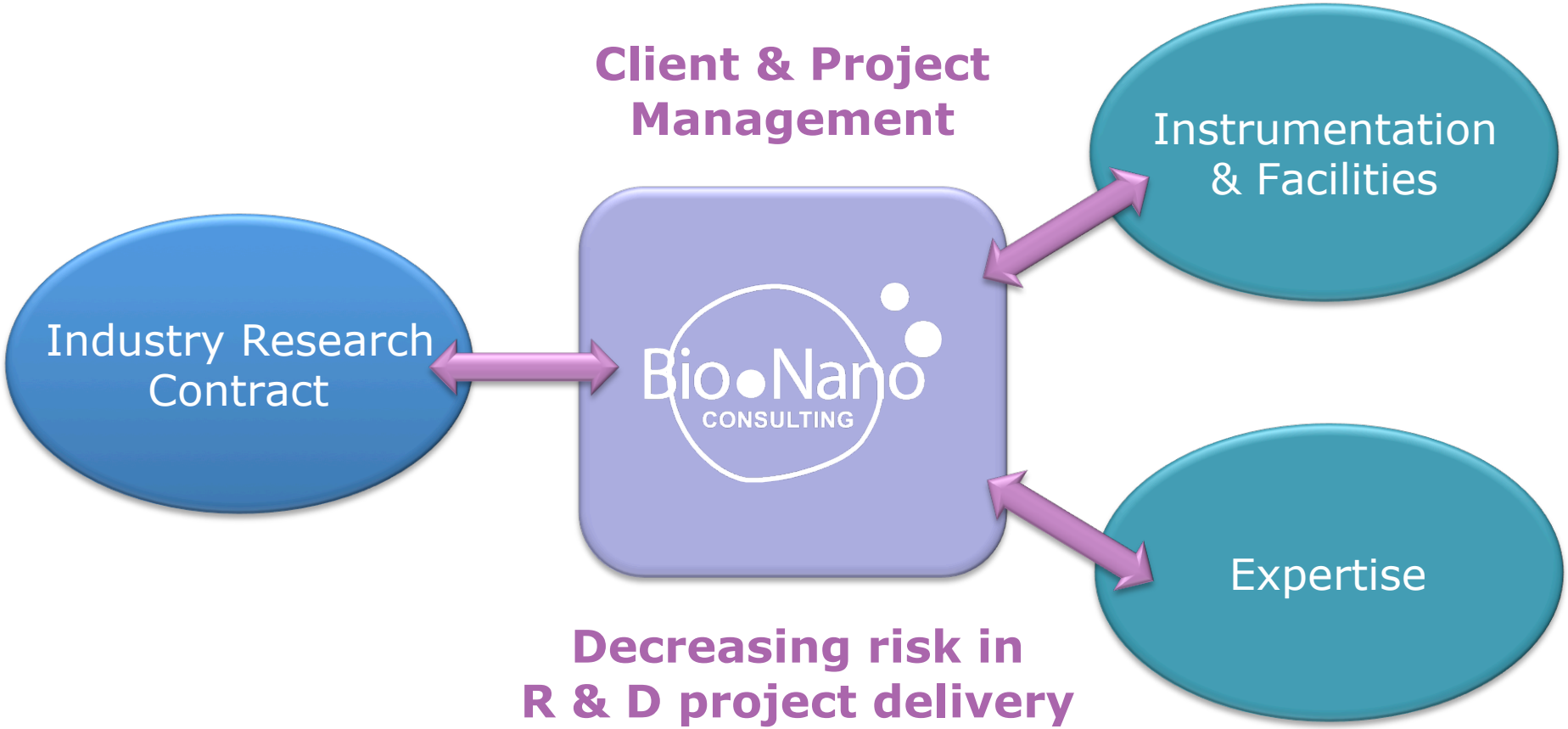

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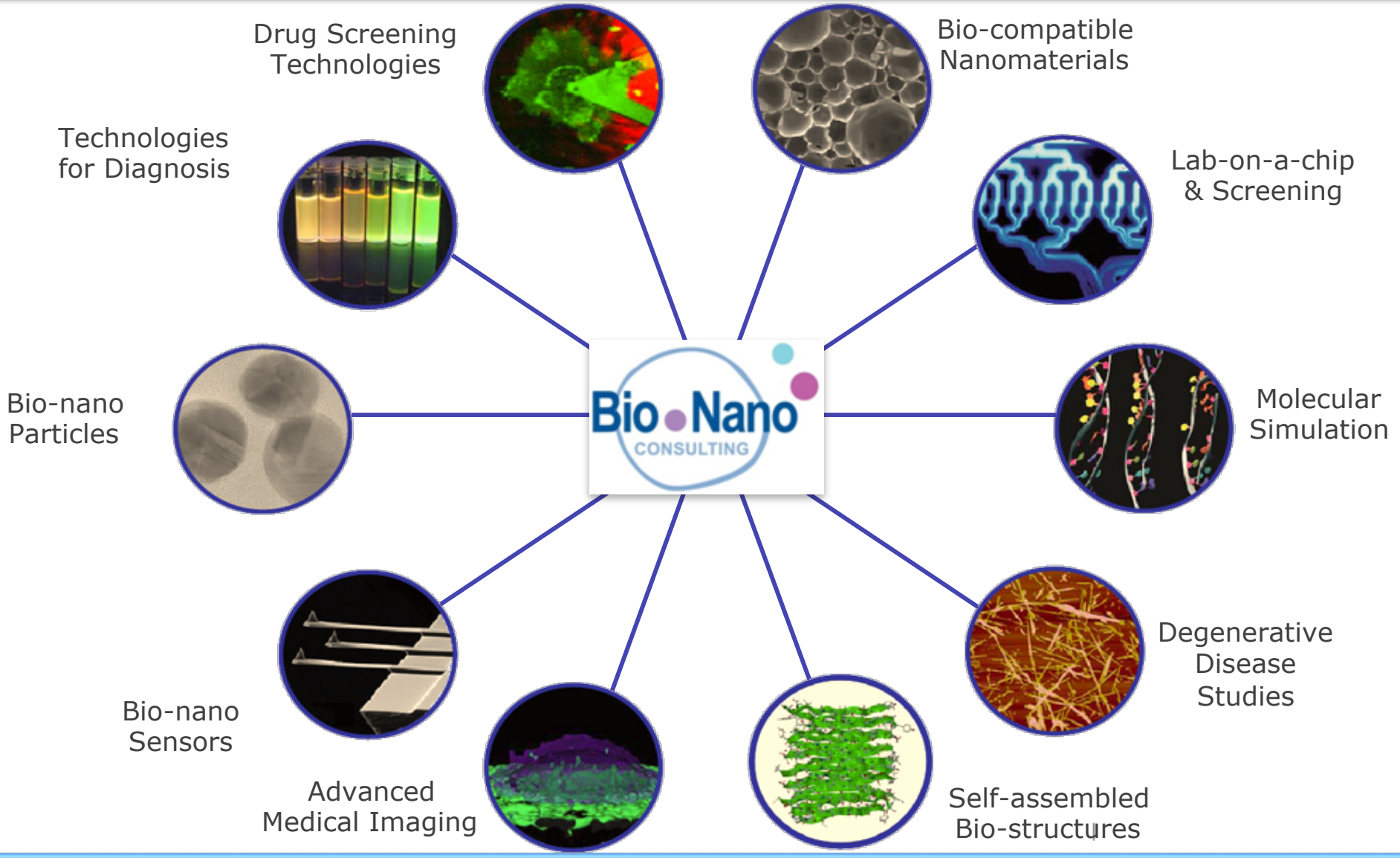

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Summary

Nanotech Knowledge Solutions™

- BNC is a dynamic problem-solving consultancy providing key service to industry
- Excellent links to UCL/LCN, Imperial College and NPL
- Exclusive access to state-of-the-art equipment and world-leading scientists

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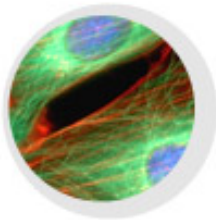

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