# Welcome to Lecture 1

**Introduction to Biosensors**

# Bioterrorism

**Terrorism** is violence against civilians to achieve political or ideological objectives

**Bioterrorism** is an intentional release of biological warfare agents (e.g. micro-organisms, viruses, toxins) with the aim to harm and scare people

# Bioterrorism in history

**6th Century BC:** Assyrians poisoned the wells of their enemies with rye ergot (fungus infecting rye - causes convulsions, seizures and spasms, diarrhea, itching, headaches, nausea and vomiting

**1346:** During the siege of Kaffa, the Tartar army catapulted plague-ridden corpses over the walls of the city to force the defenders to surrender

**1767:** During the French- Indian War, the English general Jeffrey Amherst distributed blankets laced with smallpox to Indians loyal to the French - the epidemic decimates the tribes

**1797:** Napoleon attempted to force the surrender of Mantua by infecting the citizens with swamp fever

**1914-1917:** Allegations were made against the Germans that during WWI they attempted to spread cholera in Italy

# Modern bioterrorist incidents

**1984 Rajneeshee *salmonella* attack**

In 1984, followers of the Bhagwan Shree Rajneesh attempted to control local election by infecting salad bars in 10 restaurants with Salmonella typhimurium. The attack caused about 751 people to get sick (no fatalities). This incident was the first known bioterrorist attack in the United States in the 20th century.

**2001 *anthrax* attacks**

In September/October of 2001, several cases of anthrax broke out in the United States. These were deliberate attacks (act of bioterrorism) that motivated efforts to define and put in place National Biodefense and Biosecurity policies

**2003 *ricin* incidents**

Ricin was detected in the mail at the White House in Washington, D.C. in November of 2003**.** Nobody developed any medical problems.

# Anthrax attack 1

**September 17 or 18: Attack 1**

**September 22–October 1**: Nine people contracted anthrax but they were not correctly diagnosed at this time

**October 1**: American Media mail clerk Ernesto Blanco was hospitalized and diagnosed with pneumonia; in fact, it was inhalation of anthrax

**October 4**: Robert Stevens was publicly confirmed to have inhaled anthrax - the first known case of anthrax inhalation in the US since 1976

**October 5**: Robert Stevens (63) died; he was the first fatality in the anthrax attacks

**October 7**: Anthrax spores were found on Robert Stevens's computer keyboard. The American Media building was closed and workers were tested for exposure

# Anthrax attack 2

**October 6–October 9**: **Attack 2** — two more anthrax letters were mailed

**October 12**: The (already opened) anthrax letter to NBC News was found and turned over to the FBI

**October 13**: The NBC News letter was tested positive for anthrax

**October 15**: The letter to Senator Daschle was opened. The anthrax in the letter was described as a "fine, light tan powder" which easily flew into the air…

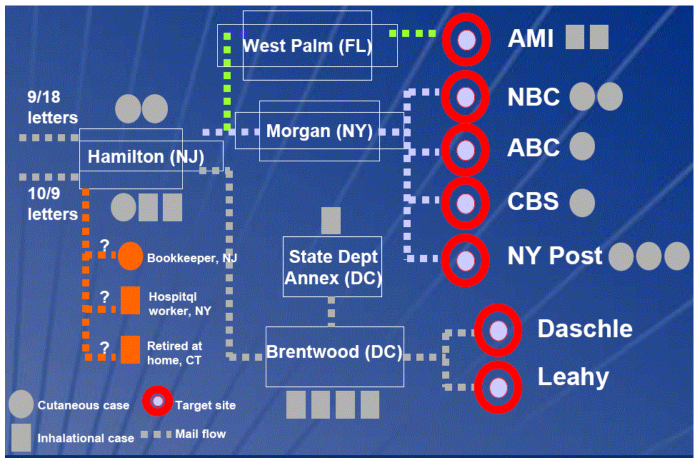
**October 17**: 31 Capitol Hill workers were tested positive for the presence of anthrax. The House of Representatives announced it will adjourn in response to the threat

**October 19**: Unopened New York Post anthrax letter was found

**October 19**: Governor Thomas Ridge, Director of Homeland Security, briefed the media on "potential" anthrax threats

# Anthrax attacks at a glance

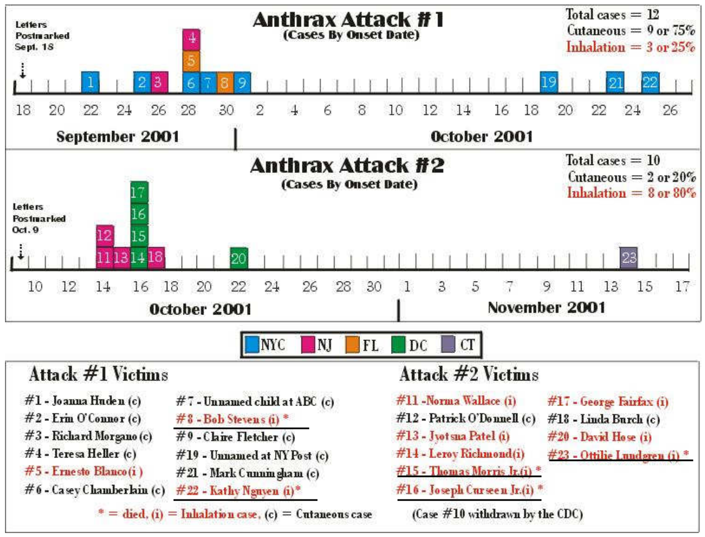
Letters containing anthrax were mailed to several news media offices and two U.S. Senators




# Letters were posted from NJ

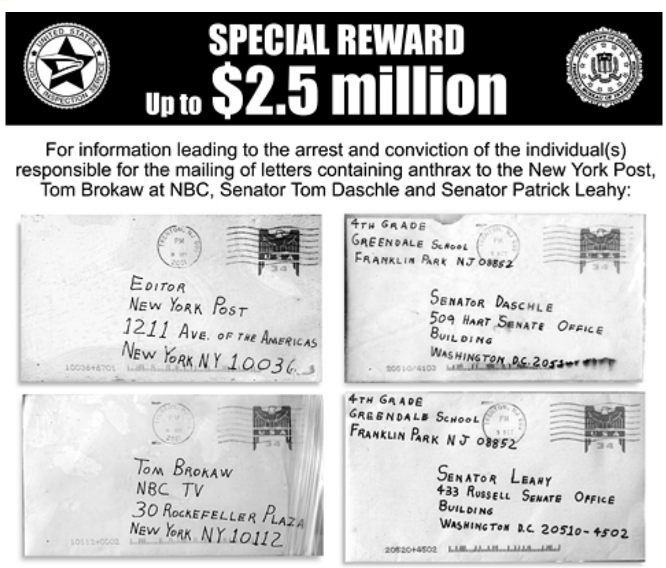

Princeton, NJ

# Victims of the Anthrax attacks




**Anthrax letters infected people in 5 states; 5 people died**

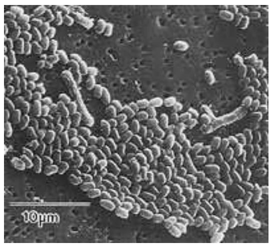
# The crime remains unsolved



# What is Anthrax?

**Anthrax is rare disease caused by *Bacillus anthracis*,** a large Gram-positive, spore-bearing bacteria, which normally causes disease in animals

**Anthrax produces exotoxins with enzymatic activity**




**spores**

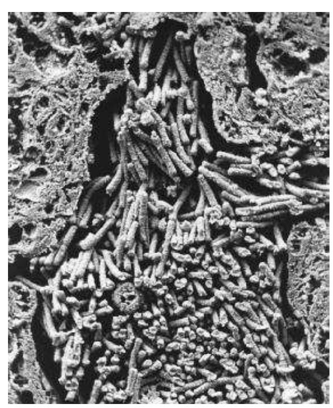
**bacteria**

Typically, from 8,000 to 50,000 spore is sufficient to cause infection by inhalation; the illness is caused by Anthrax bacteria

# Anthrax: modes of infection

Anthrax can enter the human body through the lungs (inhalation), intestines (ingestion), or skin (cutaneous); it is non-contagious and is unlikely to spread from person to person

- The respiratory infection initially leads to the development of cold or flu-like symptoms (several days), followed by severe respiratory collapse
- If not treated soon after exposure, anthrax inhalations are highly fatal - nearly 100% mortality
- A lethal dose of anthrax is reported to result from inhalation of 10,000-20,000 spores



Anthrax in lungs

# Antrax: modes of infection

**Gastrointestinal anthrax**

- Gastrointestinal infection often presents with serious gastrointestinal disorder, severe diarrhea, blood vomiting, acute inflammation of the intestinal tract, and loss of appetite
- Mortality from untreated intestinal infections 25-65%

**Let's do a calculation**

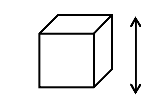
**Cutaneous infections** generally appear within 1-2 weeks after the exposure; form large, necrotic ulcers at the site of infection

This is the least fatal form anthrax but, if allowed to progress without treatment, it can result in death (~20% of the skin infection)

# Do these guys need a lot?

For simplicity let's assume that Anthrax spores are 1μM cubes

If so, the volume of 1 spore V= $1\mu m^3 = 10^{-18} m^3$



$1\mu m = 10^{-6} m$

Let's further assume that the spore density is ~1g/mL ($H_2O$) or $10^6 g/m^3$

If so, a single spore would weight m = Vxd = $10^{-18} m^3 \times 10^6 g/m^3 = 10^{-12} g$

Let's assume 100,000 = $10^5$ spore of anthrax is enough to infect a person

**1g**

**How much does a bioterrorist need to attack NYC?**

Assuming there are 10mln = $10^7$ people in the city the answer is:

$10^5$ spore x $10^{-12}$g x $10^7$ people = 1g(!)

**Oversimplication, of course, but you can see much damage can be done in contained area e.g. subway stations, etc**

# Political effects

- The anthrax attacks (and the attacks of September 11, 2001) have spurred significant increases in U.S. government funding for biological warfare research and preparedness
- For example, biowarfare-related funding at the National Institute of Allergy and Infectious Diseases (NIAID) increased by US$1.5 billion in 2003

**A great chunk of this money went into biosensors research**

# Rapid detection is vital!

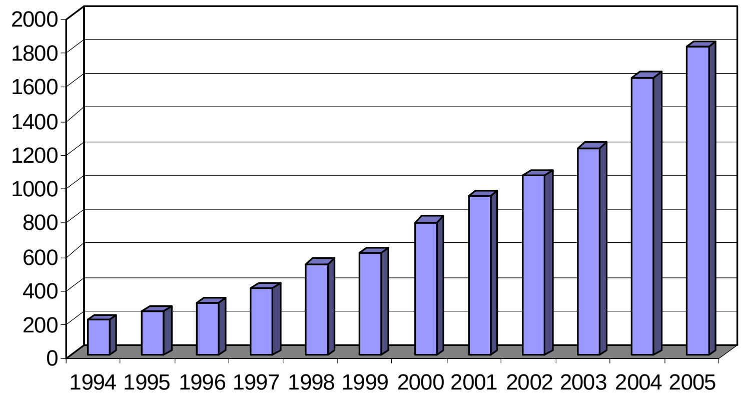
Another example of a handheld detector for biological agents, including anthrax. This unit uses magnetic beads and fluorescence to separate and detect anthrax spores

and we'll talk about this unit later in the course

**Early detection**: Portable mini laboratory enables a fully automatic recognition of selected biological agents in less than 30 mins

Can be used by a fire brigade or trained volunteers on the spot e.g. subway, etc

# Biosensors papers




This is a pubmed search; the number of paper published in ALL relevant journals is significantly higher

# Department of Homeland Security

**DHS** is a Governmental agency set up to prevent terrorist attacks within the U.S.

One of six technology division in DHS is S&T Chemical/Biological Division, which " seeks out science the science needed to reduce probability and potential consequences of a biological pathogen or a chemical attack on the nation's civilian population, its infrastructure or its agricultural system"

**2008 priority area in science and technology** (among others)

- Detection paradigms and systems for improved, emerging and novel biological threats
- Handheld rapid biological and chemical detection systems
- Improved Chem-Bio forensic analysis

**Any questions so far?**

**Source**: DHS High Priority Technology Needs (released in June, 2008)

# Biosensors and Biochips

**Evgeny Vulfson**
Chemical and Biological Sciences Department (RH-801)

Office: RH-612
Phone: (718) 260 3096
E-mail: evulfson@poly.edu

**Office hours**:
Tuesdays and Thursdays from 10.00-18.30 but it would be **much better to email me for appointment**

Lectures will be given in this very room every Thursday at 3pm; there will be no lecture on Thursday, November, 27 2008 (Thanksgiving)

# Outline and objectives

- "Biosensors and Biochips" is one of the most exciting, complex and fast growing areas of biotechnology today
- The course will cover both conventional biosensors (e.g. enzymatic glucose monitoring) and newer and emerging technologies related to the design, fabrication and applications of multi-array biochips and micro-fluidic systems (lab-on-the-chip)
- It is impossible to cover ALL aspects of such a multi-disciplinary topic in depth; hence we will aim at developing a general understanding of the technology, e.g. by working with representative examples
- **The main goal** is to familiarize students with basic principles of modern biosensors' design and applications

## Syllabus: Main Topics

- **Terminology, Definitions and Performance Characteristics**
- **Recognitions Elements and Selectivity:** enzymes, antibodies, nucleic acids, whole cells and biomimetics
- **Transducers and Sensing Elements:** electrochemical, optical, EFT, and piezoelectric sensors)
- **Positioning and Attachment of Bioreceptors to the Surface**
- **Working Principles and Applications of Enzyme- and Ab-based Sensors:** health care (e.g. glucose monitoring) and other biomedical and industrial applications
- **Modern Biosensors Technologies:** Surface Plasmon resonance, Quartz Crystal Microbalances, FRET-based sensors, etc
- **Biochips and Microarray Technologies:** design and applications of modern biochips, including microfabrication and miniaturization techniques
- **Nanotechnology, MEMS and Lab-on-the-chip**

## Requirements and Arrangements

**Lectures:**

- INFORMAL and INTERACTIVE! You can interrupt with comments and questions at ANY TIME. Do not save it to the end! We will normally take ONE 10-15 min break
- I hope we would have a couple of guest speakers but I have not yet made any firm arrangements

**Attendance**:

- It is up to you whether you attend lectures or not
- However, there is no textbook for this course and your class work (e.g. quiz) counts toward your final grade. Thus, you would be better off attending ☺

# Course Materials

**Books and Notes:**

- For the preparation of this course I used a dozen of books and numerous original papers and reviews and you can borrow some of these books to decide whether you want to get your own copy
- NO SINGLE BOOK will be sufficient in the preparation for your final exam. Hence, once again, I would recommend attending lectures
- After every lecture I will post print outs of all the slides in a pdf format and additional/supporting materials on Poly's Blackboard - **there is no need to copy slides!**
- Don't hesitate to email me with <u>any questions</u> regarding the course

# Homework

- There will be homework after some (not all) lectures. Unless agreed otherwise, it must be submitted **by email** before 10am on the day of the next lecture
- My inbox is NOT UNLIMITED and, hence, I set up a special email address for **your homework** on THIS COURSE only: poly603@gmail.com
- **All other correspondence** should be sent to my Poly address: evulfson@poly.edu
- Innovative and diligent homework will be appreciated and earn bonus points
- Late homework will be disregarded. In exceptional circumstances I may extend the deadline (NO PROMISES!) but you must request it in advance

# Grading and exams

### Mid-Term exam

The good news - there will be none!

### The bad news – there will be a regular quiz instead

We will have a 10-15 min quiz just before the break. Typically, I will distribute a page with 4-5 questions based on the earlier lectures and supporting materials. The answers should be reasonably brief, to the point, and preferably in your own words. I am looking for understanding, not a mechanical recitation of the slides

**Each quiz will be scored separately on a scale from 0 to 100**

| Quiz 1 | Quiz 2 | Quiz 3 | Quiz 4 | Quiz 5 | Quiz N | Final |
|---|---|---|---|---|---|---|
| 100 | 60 | 80 | 70 | 90 | 65 | **80** |

# How does grading work?

**Weighting**

| | |
|---|---|
| Home work: | 0.1 |
| Quiz: | 0.2 |
| Final Exam | 0.7 |
| | 1.00 |

**Hypothetical Score**

| | |
|---|---|
| Home work: | 85% |
| Quiz: | 80% |
| Final Exam: | 95% |

**For example:**

**Student 1:** 85%*0.1 (HW) + 80%*0.2(Q) + 95%*0.7(FE) = 91 ← **A**

**Student 2:** 85%*0.1 (HW) + 60%*0.2(Q) + 95%*0.7(FE) = 87 ← **B**

**Student 3:** 85%*0.1 (HW) + 60%*0.2(Q) + 100%*0.7(FE) = 90 ← **A**

Grades awarded: A (90-100), B (80-90) and C (70-80)

**Anything below 70% is UNSATISFACTORY**

## More on grading

Can a student completely neglect his/her homework and still get an A? **Yes**

**Final sore:** 0%*0.1(HW) + 100%*0.2(Q) + 100%*0.7(FE) = **90**

Can a student who has NOT taken a single Quiz get an A? **No**

**Final sore:** 100%*0.1(HW) + 0%*0.2(Q) + 100%*0.7(FE) = **80**

Hence, if you don't want to attend lectures it is advisable to pop in for an occasional quiz ☺

Can a student get an A with less than 90 at the final exam? **Yes**

**Final sore:** 100%*0.1 (HW) + 100%*0.2(Q) + 85%*0.7(FE)=**89.5**

**The bottom line:** The harder/more diligently you work during the semester, the easier it will be for you to prepare for the final exam. Also, you will need to do "less" at the exam to get an A

## Other points to note

- To graduate from the Biotechnology & Entrepreneurship Program students must get at least a B grade for **ALL** their required (core) courses, while Biotech students only need to get an average B in the core courses
- You can withdraw from the course without any penalty by Tuesday next week; you can also withdraw with a W grade by November, 11 2008
- Many of you have a Scholarship or other financial aid package from Poly. To continue receiving the aid you must meet average GPA requirements at the end of the semester
- "Professor can I have an A because otherwise I would lose $$$" **does not work**! There will be no re-examination

## Academic Dishonesty Policy

**1. <u>DEFINITION</u>:** Academic dishonesty is an act of fraud, which may include misrepresentation, deceit, falsification, or trickery of any kind that is done by the student with the purpose, intent, or expectation of influencing a grade or other academic evaluation. <…> Common examples of academically dishonest behavior include, but are not limited to, the following:

**1.1 Cheating**—intentionally using or attempting to use unauthorized materials, information, or study aids in any academic exercise; copying from another student's examination <…>

**1.2 Fabrication**—intentional and unauthorized falsification or invention of any information or citation in an academic exercise

**1.3 Facilitating academic dishonesty**—intentionally or knowingly helping or attempting to help another person to commit an act of academic dishonesty

**1.4 Plagiarism**—intentionally or knowingly representing the words or ideas of another as one's own in any academic exercise <…>

## Academic Dishonesty Policy

**4. <u>SANCTIONS</u>:** The purpose of sanctions is to provide a fair educational opportunity for all found responsible for their actions. Sanctions may include any combination of Sections 4.1- 4.7

**4.2 Rejection of the assignment, examination, or project** — with the requirement that the student complete compensatory work

**4.4 Grade of F for the course or other academic requirement**

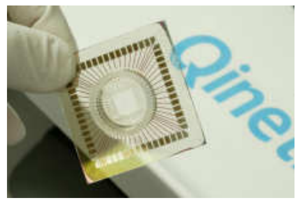
**4.6 Suspension** from the University for a period not to exceed one year with notation on the transcript during the suspension period.

In all the above cases, a record of the sanction will be retained in the student's file in the Department of Student Development and in the student's academic department file

**Any questions?**

# Introduction

- What are biosensors?
- Biosensor components
- Performance characteristics
- Areas of application



***Additional materials:***

***Chemical Sensors and Biosensors***
BR Eggins, chapter 1 (will be posted as pdf)

# Sensors: Definitions

**A sensor** is a physical device (or biological organ ☺) that detects or measures i.e. "*senses*" a physical condition or a chemical substance or other signals, and responds in such a manner that it can be measured, recorded or otherwise indicated

**Sensors can be divided into three main groups:**

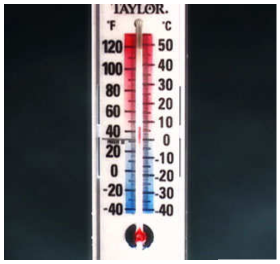
**1. Physical sensors** are devices for measuring physical parameters such as mass, temperature, pressure, etc

**2. Chemical sensors** are devices that **detect** chemical substances e.g. concentration of an analyte or a specific component in a complex sample or its total composition, and **transform** this chemical information into an analytically useful **signal**

**3. Biosensores** are a subset of sensors (mainly chemical but can be physical) that use a biological sensing element for detection

# Physical sensors

Thermometer



Odometer

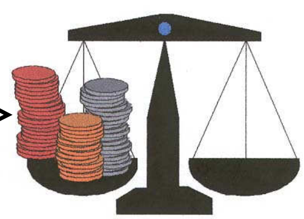
Pressure Gauge/monitor

**Examples?**

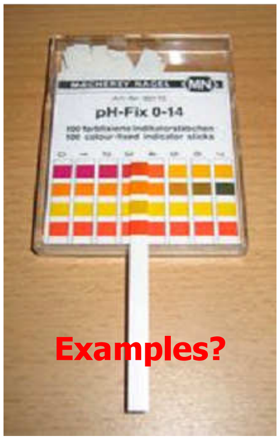
Weighing scales

# What's the difference?

**Never mind the appearance!**



# Chemical sensors




**Examples?**

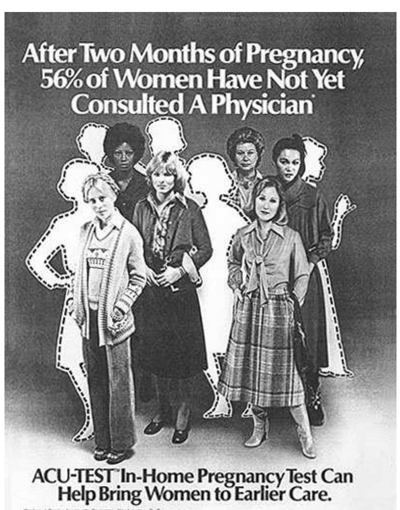
**A pH indicator** is a compound (a weak acid/base) that changes color when added to solution with different pH

| Indicator | Color Low pH | Transition range | Color High pH |
|---|---|---|---|
| Methyl yellow | red | 2.9-4.0 | yellow |
| Bromophenol blue | yellow | 3.0-4.6 | violet |
| Congo red | blue | 3.0-5.2 | red |
| Methyl orange | red | 3.1-4.4 | yellow |
| Methyl orange | purple | 3.2-4.2 | green |
| Litmus (Azolitmin) | red | 4.5-8.3 | blue |
| Phenol red | yellow | 6.6-8.0 | red |
| Neutral Red | red | 6.8-8.0 | yellow |
| Phenolphthalein | colorless | 8.2-10.0 | pink |

Hence, a pH indicator strip is a **chemical sensor** for hydronium ions ($H_3O^+$); [$H^+$] cuases the color change we sense - **optical detection**

# Biosensors

## A home pregnancy test




**Examples?**

The first home pregnancy test kit ("Early Pregnancy Test") was approved by FDA in 1976 and the commercial products were launched in the U.S. in 1977

By the mid-nineties there were about twenty different products on the market with combined annual sale of about $200mln

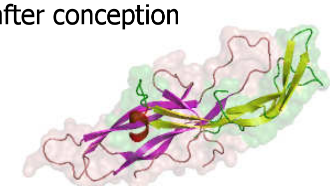
# Home pregnancy test

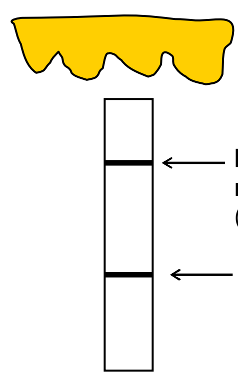
- It enables women to determine **quickly**, **easily** and **reliably** whether they are pregnant or not
- The test is an immunoassay for measuring Human chorionic gonadotropin (hCG), a hormone secreted in urine during pregnancy. hCG in urine can be detected as early as a week (typically two) following conception
- A little urine is applied to the test strip and, if the urine contains hCG, a reaction occurs resulting in a part of the stick to change color, thus signaling that the hormone is present and, hence, the woman is pregnant

**Let's see how it works…**

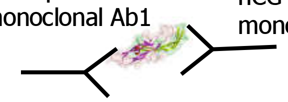
# How does it work?

hCG is a 244 amino acid glycoprotein (MW ~37kDa) produced by the embryo soon after conception






**Sandwich assay:**




If Ab1-hCG complex present in the urine (pregnant), it will get stuck just where Ab2 is located

But if hCG is not present (not pregnant), hCG-specific monoclonal Ab1 will go straight through

# How can this be used in a sensor?

**Suppose now we "paint" Ab1 (red)**

hCG-specific monoclonal Ab1

hCG-specific monoclonal Ab2

Enclose the test strip in an opaque matrix and leave a transparent "window"

just where the Ab2 is immobilized

**Note that I put a dash line here because we cannot actually see it**

If Ab1-hCG complex is present in the urine (pregnant) we will see **WHAT?**

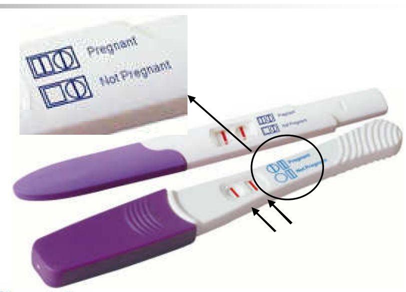
**A red band:** Ab1-hCG-Ab2 complex formed and the red Ab1 will get stuck exactly where Ab2 is located

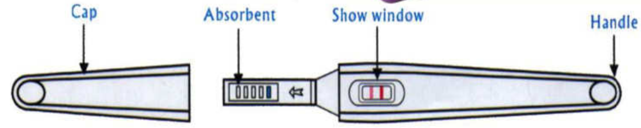
**And what will we see if she is not pregnant?**

**Nothing!** because Ab1 on its own won't bind to Ab2- it will just go through

**Any questions?**

# The actual device

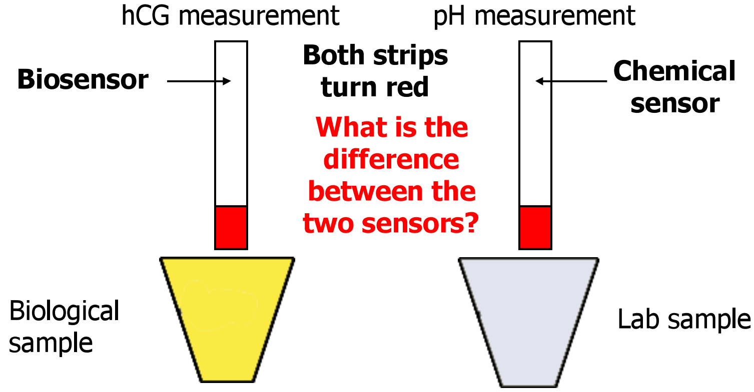




Note that there are two bands rather than one; we will discuss the function of the 2nd one a little later

**How else can you analyze for hCG?**

**Plenty of lab methods e.g. electrophoreses but ~$150 vs $1-2**

# Let's compare two assays




It is not what you measure (hCG vs $H^+$) or where you measure it (a body fluid vs chemical solution); it is **HOW** you measure it - one sensor relies on a biological sensing element (Ab) and the other one does not

# pH meter

**The pH probe** measures pH as the concentration of $H^+$ surrounding a thin-walled glass bulb at its tip

The probe produces a small voltage (about 0.06 volt per pH unit) and the pH meter displayes the measured voltage as pH units.

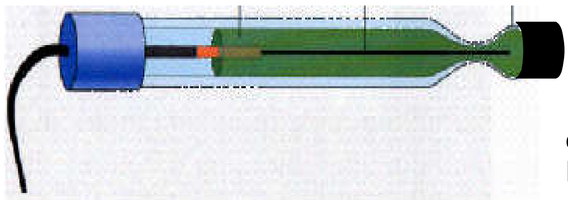
Basically, the meter circuitry is no more than a voltmeter that displays measurements in pH units rather than volts

**pH meter is a typical electrochemical sensor**

<u>Many other chemical</u> sensors (e.g. various ion selective electrodes) and <u>biosensors</u> use exactly the same or similar principle of operation

# Chemical sensor to biosensor

How to convert a conventional pH electrode in a Biosensor?



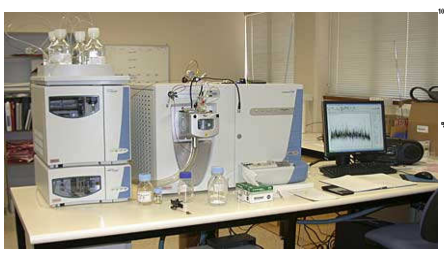
… let's add a little extra and call it a black box for now

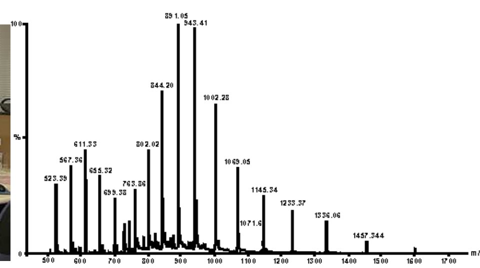
If the black box happens to contain an enzyme and the enzyme activity is converted in a detectable signal i.e. $H^+$

**WE HAVE A BIOSENSOR!**

# Mass spectrometer

## What kind of sensor is it?





**You can measure mass –** hence, it's a **physical sensor**

**or**

You can use it as an analytical tool for the identification of **chemical species –** hence, it is **chemical sensor** ☺

## Temperature abuse sensor

There are instances when a substance/product must be stored or shipped at certain temperature e.g. below a certain critical value; otherwise it would go off

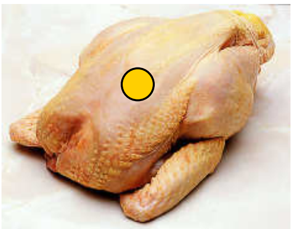
**Examples?**

**Drugs, food, temp-sensitive chemicals, etc**

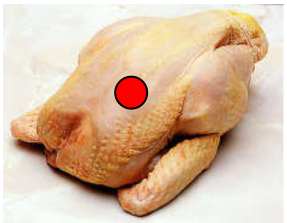
## Temperature abuse sensor

If such product is exposed to temperature above some specified value (let's call it "abuse temperature"), it may no longer be safe for human consumption or efficacious

**Let's try to construct a <u>simple</u> T abuse BIOSENSOR**



Exposure to higher than desired T°C →



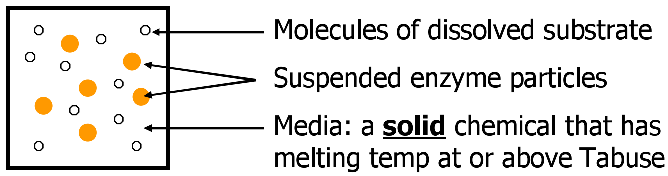
Good chicken i.e. fit for human consumption ☺

Temp-abused chicken

**ANY ideas?**

# Temperature abuse biosensor

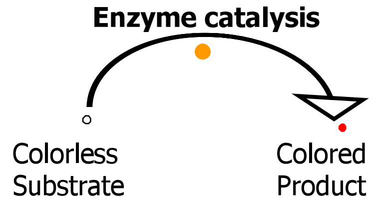
**Enzyme are INACTIVE in a solids**




The sensor is fabricated as a little drop or strip and attached to the packaging

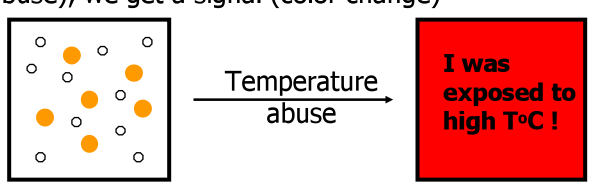
However, when the T°C exceeds the Mp, the mix melts and the enzyme become active!

While the T stays below the Mp, the mix remains solid and no reaction takes place

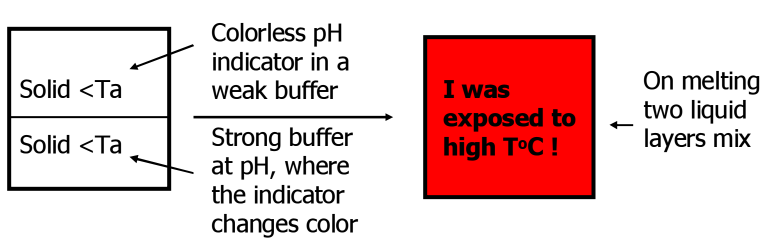



# Temperature abuse biosensor

As a result of exposure to higher than desired T (temp abuse), we get a signal (color change)




**<u>Question</u>:** Can you design an inexpensive temperature abuse chemical sensor i.e. based on chemical reaction with no "bio"?



# "No-chemistry" Tabuse sensor

Easy – any kind of thermometer would work…

**Which technology is better?**

We have looked at three types of devices: physical, chemical and biological sensor, each capable of doing the same job

It depends on requirements: if everything else is equal - the cheapest ☺

## Think it over

Design a **physical** (with no chemistry or biology) T-abuse sensor for the chicken i.e. VERY CHEAP and SIMPLE

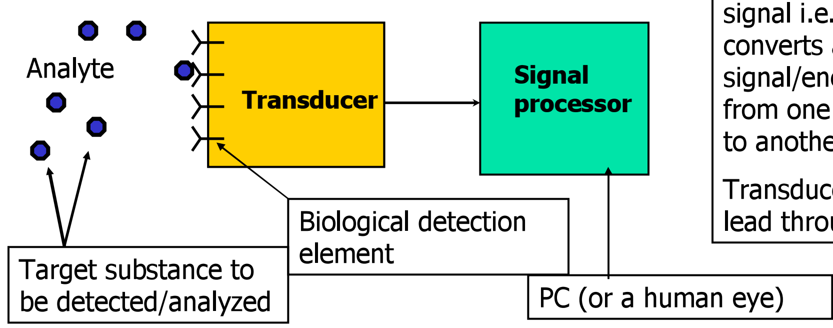
And, please, do **NOT** stick mercury thermometers on food

We will discuss your ideas in the next class – there will be a bonus for the best design ☺

# A generalized biosensor

Broadly defined "biosensor" is a measuring device that contains a biological sensing (recognition) element

**Biosensor Function:** To selectively respond to an analyte through a biochemical reaction and to enable qualitative or quantitative determination of this analyte




Transducer converts a biological response into a measurable signal i.e., it converts a signal/energy from one form to another

Transduce = lead through

# More definitions

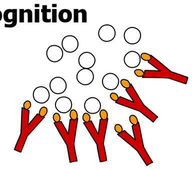
**Biological Recognition element:**
Typically, a macromolecule, cell or even tissue that recognizes and bind or transform the target substrate/analyte

**Transducer:**
Device that converts the biological recognition event into a measurable signal or observable event (e.g. electric current, electromagnetic radiation, etc)

**Processor:**
Converts the measured signal into a signal that can be interpreted by the user (e.g. a number, color, analog or digital readout)

**Three key processes:**

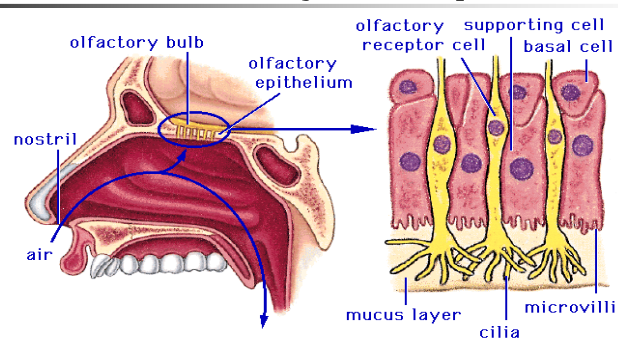
**1) Recognition**



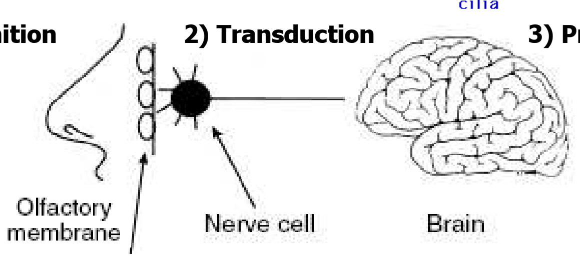
**2) Transduction**

**3) Processing**

# Olfactory reception



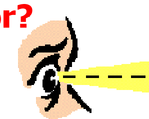

**1) Recognition** **2) Transduction** **3) Processing**



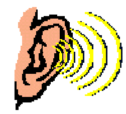
# Human senses: how many?

**Are these chemical or physical sensor?**

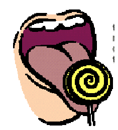
Sight or vision is the ability to detect electro-magnetic waves within the visible range (light)

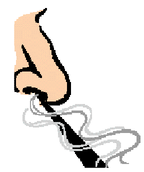


Hearing - the sense of sound perception



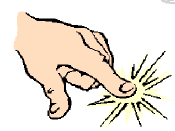
Sense of taste
**is one of the two main "chemical" senses**





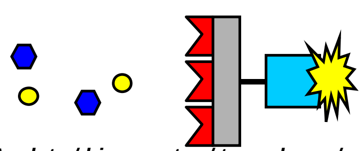
Olfaction - the sense of smell
**is the chemical sense too**

Tactile perception - the sense of touch



# What's the difference?

**Biosensor**

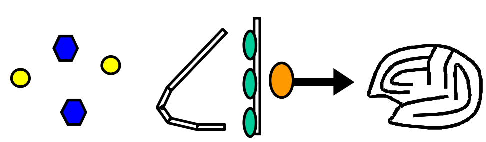


***Analyte / bioreceptor / transducer / processor***

**The human nose and eye are biosensors**

**Nose**

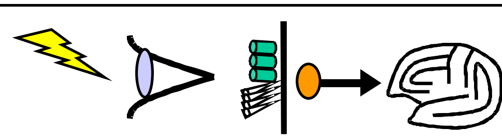
chemical



***Small molecules / olfactory membrane proteins / nerve cells / brain***

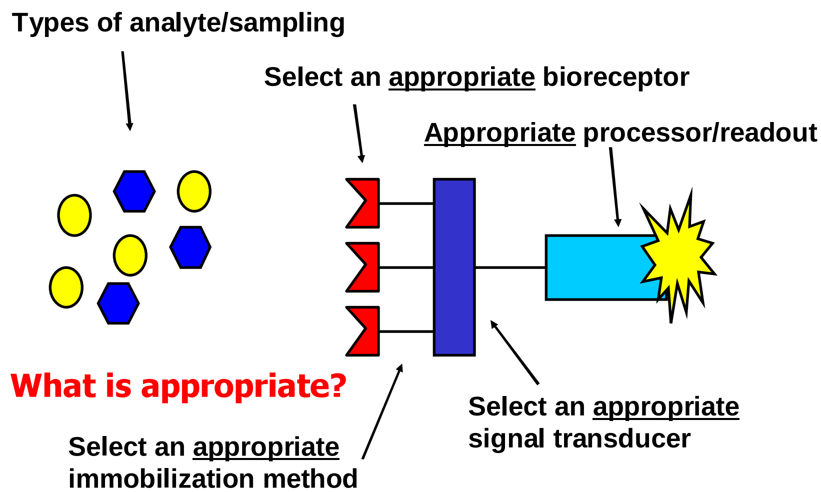
**Eye**

physical



***Visible light / rods and cones (proteins) / nerve cells / brain***

# What does it take to make it?




**"Appropriate" means the best suited for the requirements**

# Type of Analytes

What can biosensors measure?

***Small molecules***

glucose, cholesterol, urea, drugs and drug metabolites (medical), pesticides, dioxins, TNT (environment), nerve agents (defence), alcohol and narcotics (forensic)

***Bio-macromolecules***

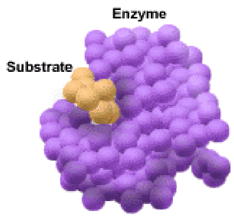
enzymes, proteins, hormones, DNA, RNA, viruses (medical, defence)

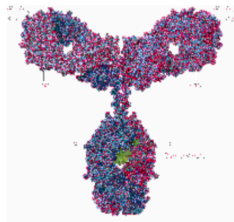
***Micro-organisms***

anthrax and the like (defence), *E. coli*, *Candida* (medical, food safety)

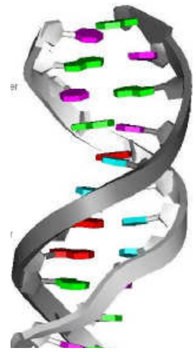
## Type of biological receptors

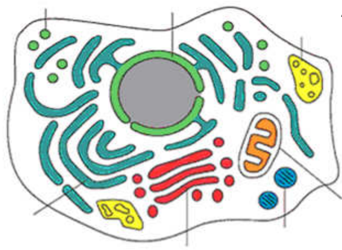
What can be used in biosensors?



Antibodies





Whole cells

Nucleic acids

**Basically, anything biological even a whole animal…**

## Animal-based biosensors

In Japan many people in earthquake-prone areas keep goldfish in a bowl. If the fish starts swimming around frantically, it is believed to be a signal for an approaching earthquake

Miners inspecting a canary in the cage

Coal miners used to take canaries in the mines as "biosensors" for detecting the build-up of methane gas and CO. Canaries are especially sensitive to these gases – if they stopped singing, there could be a problem. A dead canary would be signal for immediate evacuation…

## Bioreceptors: not all equal…

***Enzymes:*** Commonly used in biosensors. Enzymes are catalysts i.e. a chemical reaction takes place that can be measured directly or through a coupled reaction to give a colored product. Hence, there is a "built-in" amplification

***Antibodies***

- Highly selective interactions and very tight binding
- Antibodies can be raised against almost any antigen
- Often need to be linked to another probe for detection

***Receptors Proteins***

- Many highly selective receptor proteins are membrane-bound e.g. on cells' surface
- Difficult to isolate and/or handle (labile) may necessitate the use of whole cells; typically require signal amplification

## Immobilization technologies

**Bioreceptor molecules have to be immobilized on or near the surface of the transducer**

- There are numerous immobilization methods (e.g. physical adsorption and covalent chemical attachment) providing various degrees of stability and shelf-life from hours to years
- There are methods to immobilize bioreceptors on a transducer' surface as single monolayer with a high degree of orientation i.e. active/binding site "looking" out
- When biological fluids are analyzed proteins can deposit on the surface of a transducer and this would result in fouling (biocompatibility issues) – surface modification chemistry

**Whatever the method, the recognition capability or catalytic activity must be preserved!**

## Main types of transducers

### Electrochemical Transducers

**Potentiometric:** These involve the measurement of the emf (potential) of a cell at zero current. The emf is proportional to the Log C of the substance being determined

**Voltammetric:** An increasing (decreasing) potential is applied to the cell until oxidation (reduction) occurs and there is a sharp rise (fall) in the current to give a peak current which is measured

**Conductometric:** Most reactions involve a change in the composition of the solution. This will normally result in a change in the electrical conductivity of the solution, which can be measured electrically.

**FET-based sensors:** Miniaturization can often be achieved by constructing one of the above types of electrochemical transducers (mainly potentiometric sensors) on a silicon chip-based field-effect transistor (**FET -** field-effect transistor).

## Main types of transducers

### Other Major Types

**Optical Transducers:**

The techniques used include absorption spectroscopy, fluorescence spectroscopy, luminescence spectroscopy, internal reflection spectroscopy, surface plasmon spectroscopy and light scattering

**Piezo-electric Devices:**

These devices involve the generation of electric currents from a vibrating crystal, the frequency of which is affected by the mass of material adsorbed on its surface

**Thermal Sensors:**

All chemical and biochemical processes involve the production or absorption of heat. This heat can be measured by sensitive thermistors and related to the amount of substance to be analyzed

# Processor/read out

***Accuracy***

e.g. litmus paper/pH indicator/pH meter

***Usability***

- e.g. analog vs digital
- other issues: too small LCD display in first glucose biosensors

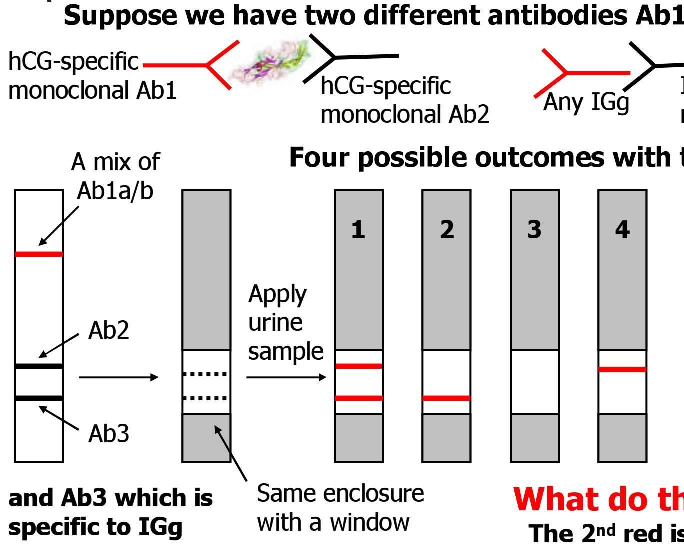
***Control***

e.g. self-testing function – does the sensor work properly?

**Let's briefly discuss self-testing using the Pregnancy Test as an example**

# Control: does it work properly?

**Suppose we have two different antibodies Ab1a and Ab1b**




**Four possible outcomes with two red Abs**

1. Pregnant
2. Not pregnant
3. Something is wrong – the sensor doesn't work properly
4. As 3 but ???

**and Ab3 which is specific to IGg**

**What do they mean?**

**The 2nd red is a self-test**

## Why is it important?


**And this is precisely what we saw**

In a sensors like this Abs are likely to be the most expensive component. By using two pairs of Abs manufacturers have almost doubled the production cost

**Why???**

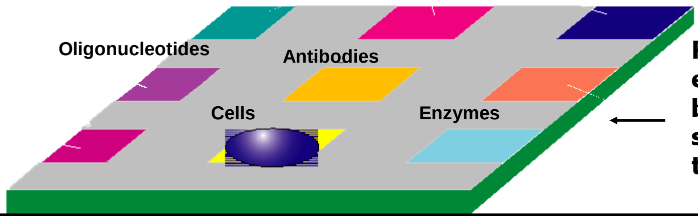
Manufacturing costs are still a fraction of the retail price and RELIABILITY is very IMPORTANT!

**Any questions?**

## What are biochips?

A biochip is a collection of miniaturized test sites (e.g. bio-sensors) arranged as micro-arrays on a solid substrate enabling to run many tests in parallel to achieve high (or VERY high) throughput


- Often, a biochip's surface area is no larger than a fingernail
- Like a computer chip that can perform millions of mathematical operations in one second, a biochip can perform thousands of biological reactions, such as decoding genes, in a few seconds




**Recognition elements must be spatially separated on the biochip**

# Microarrays

**Microfabricated two-dimensional structures for massively parallel analysis**

- Possibility to attach, localize and/or address receptors on the chip in a very precise way and at very high density
- Development driven by innovation in biotechnology, micro-electronics technology and nanotechnology

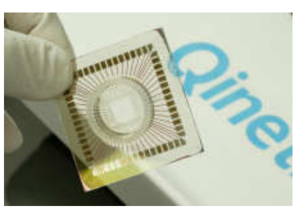
**Multi-site detection:** fast and simultaneous detection of multiple analytes

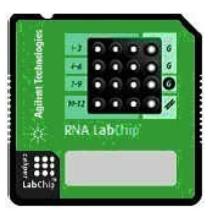
**Miniaturization:** less sample quantity and reagent cost, mass production

# Lab-on-a-chip devices

## and Micro Total Analysis Systems (µTAS)

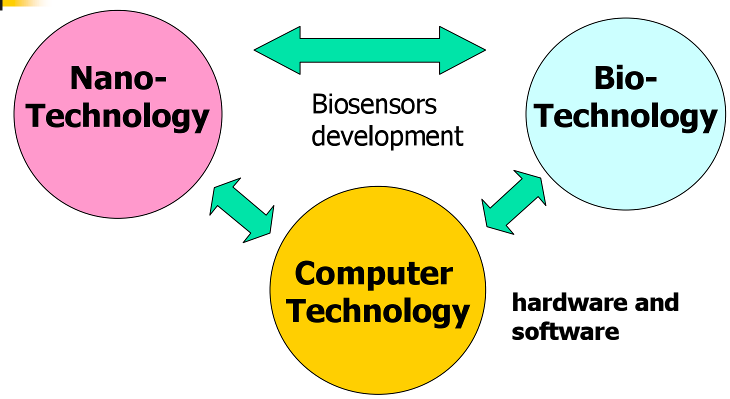
These are devices that integrate (multiple) laboratory functions on a single chip, often only a few square centimeters in size, capable of handling extremely small fluid volumes very rapidly





"Lab-on-a-Chip" generally indicates the scaling of single or multiple lab processes down to a chip-format, whereas "µTAS" is dedicated to the integration of the total sequence of lab processes to perform chemical analysis

# Highly multi-disciplinary space

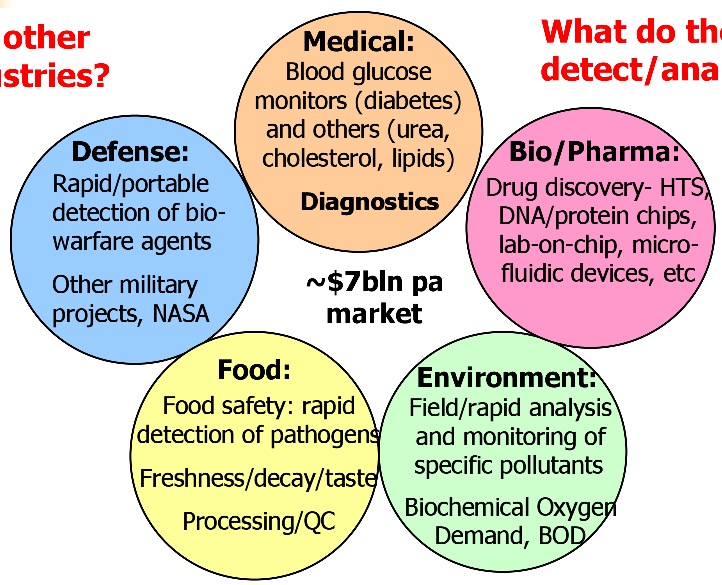



**Biosensor technology** has undergone a dramatic transformation in the past decade due to the introduction of multi-array formats, great advances in microelectronics and nano-technology, and adaptation of the methods well established in the microelectronics industry.

# Biosensors: market segments

**Any other industries?**

**What do they detect/analyze?**



## Application areas

***Medical - health monitoring and diagnostics***
various blood and tissues metabolites, proteins, hormones, DNA, RNA, antibiotics, drugs and drug metabolites

***Industrial bio-processes***
amino acids, yeast, lactic acid, ethanol, etc

***Environmental***
pesticides, fertilizers, oestrogenic substances

***Food and beverage industry***
microbial pathogens (e.g. salmonella, e. coli), food quality

***Defence/forensic***
anthrax, small pox, nerve agents, TNT, abuse substances

## Biosensors in Heath Care

- Medical diagnostics and monitoring is by far the biggest (>90%) area of biosensors' application
- It is expected to have the most profound effect on the provision of health care in the XXI century
- Glucose biosensors for diabetics are the greatest success story so far…

**Why diabetes?**

Because there are more than 20 million children and adults in the US alone (7% of the population) and monitoring provides great benefits

# Commercial glucose monitors


LifeScan by Johnson & Johnson


Therasense/Abbott Laboratories


TrackEase and Prestige by Home Diagnostics Inc

Ascencia by Bayer

Accu-check by Roche Diagnostics

# The bad news …

Adjustable Lancing Device for obtaining blood sample

**INVASIVE!**


**And the good!**

Glucowatch Biographer™ enables non-evasive glucose sampling through the skin

# Government agencies

**A very big customer too…**

- The US Defense agencies have biosensors that can detect Anthrax and other biohazards
- The US Navy has created underwear woven from conductive polymers that can provide data on the wearer's injuries
- The US Army (apparently) built a pill-sized computer that soldiers can swallow. The computer tracks the health of the soldiers
- NASA are planning biochips and micro-array technology to conduct chemical experiments in space (look for extraterrestrial life? ☺)
- Police and forensic scientists are getting biosensors for detecting narcotic substances

# Portable cocaine sensor

**A real-time sensor for detecting cocaine** – made with inexpensive, off-the-shelf electronics – has been developed at University of California, Santa Barbara

Currently the cocaine sensor used by police are (i) dogs (biosensors ☺) and (ii) chemical tests e. g. the Scott test – color development. Unfortunately, there are ways around this test e.g. adding chemicals that block the development of color…

**The CA sensor can detect cocaine no matter** what was added or what it was cut with - powdered sugar, flour, or coffee that is sometimes used to mask the smell from dogs

Other potential applications of this technology include bioterrorism and various medical applications

**We'll talk about how it works later in the course but now…**

# Home Work

**Come up with a "cool", new biosensor**

**The rules of the game:**

- It has to be something NEW and COOL so that people may really want to use it (or better buy!)
- You need to come up with a concept/idea – no technical details are necessary
- However, you have to (1) describe what your biosensor does, (2) why it is useful and (3) specify why the "bio" as a recognition element is required
- **<u>B&E students only</u>**: estimate the market for your device (assume it can be built easily) in $$$

**Warning:** Take it seriously! Don't cook something up in 5 min – there will be a follow up to this HW based on what you submitted

# Imaginary sensor: example

**Stressometer - a "cool" new biosensor**



There are specific chemicals in human plasma, which are released when people are stressed up

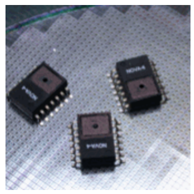
The blood analysis can be performed non-invasively through the skin (technology for doing this exists and we'll talk about it)

The biorecognition elements (e.g. enzymes or Abs) are incorporated into a skin patch to detect the appearance of these chemicals – tough to measure any other way; hence "bio" is needed

The patch containing the biosensor and transducer is built into a watch-like device (biosensor is on the skin) that takes periodic measurement, converts the signal into a "readable" format and displays your stress level

# Sensors are everywhere

- The oxygen sensor
- The air pressure sensor
- The air temperature sensor
- The engine temperature sensor
- The knock sensor



Low-cost tire pressure sensor from GE transmit signal to driver's dashboard

O2 sensors made modern electronic fuel injection and emission control possible. They determine the air fuel ratio exiting the engine and provide real time feedback to the fuel injector

**There are plenty of things folks need to measure, BUT it does NOT necessarily have to be a BIOSENSOR**

# Biosensor: market dynamics

- The global market for biosensors and other bioelectronics is projected to grow to $8-9 billion in 2009
- Biosensors, particularly glucose sensors, account for the majority of the market. However, sales of other bioelectronic devices are expected to increase significantly over the next five years
- The market for biosensors is well-established and is likely to continue to experience steady growth. There is a strong probability that the market for bioelectronic devices other than sensors will develop and grow substantially
- Biomedical and life sciences applications dominated the biosensor and bioelectronics sales in 2003, accounting for almost 95% of the market. However, biomedical and life sciences' share of the market is projected to slip slightly due to the emergence of other applications

Source: Report by BCC research

# In conclusion

**The first look at biosensors:**

- Definitions
- Basic characteristics
- Main areas of applications

**Have fun and see you next Thursday**

# The end of show