Welcome to Lecture 2

At the last lecture:

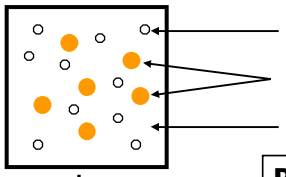
- Defined sensors and discussed the main components of typical biosensors and their basic characteristics
- Main areas of applications

Plan for today:

- Whole cells biosensors
- Performance requirements and characteristics
- Homework review

Homework review

Temperature abuse sensor



Molecules of substrate

Enzyme particles

Media: solid wax/paraffin with desired Mp

Temperature abuse

I was exposed to high T°C !

Principle of operation:

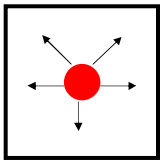
While the T stays below the Mp, the mix remains solid and no reaction takes place. However, as soon the T exceeds the target, the mixture melts and enzymatic (or chemical) reaction takes place leading to the coloration e.g. red

Hence, a detectable signal (color change) is generated as a result of the exposure to higher than desired temperature – SENSOR!

Physical T-abuse sensor

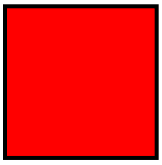
Design a **physical** (with no chemistry or biology) T-abuse sensor for the chicken i.e. VERY CHEAP and SIMPLE

1)



When T rises, the matrix melts and the dye diffuses out

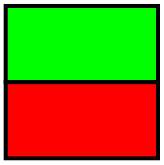
Now the whole sensor is colored



A "spot" of dye in a solid matrix

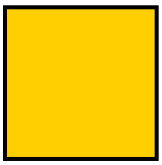
Another solution:

2)

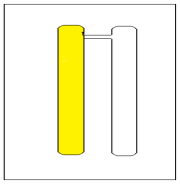


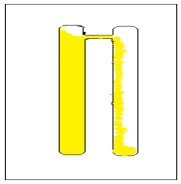
Two layer of the same material with two dyes of different color

If the product is exposed to T>Tm, the sensor turns GREEN



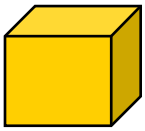
And yet another design





Two hollow tubes connected by a nozzle - when melted the liquid will flow into the empty one

My personal favorite:



Change of shape

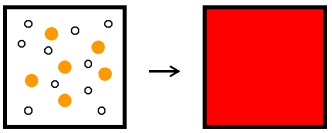
A distinct shape would be better - easier to see

Even better to combine a change of shape with colors

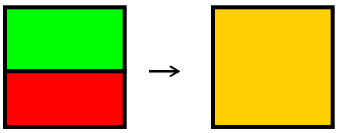
The bottom line:

There is more than one way to skin a cat

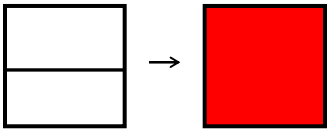
Enzymatic reaction



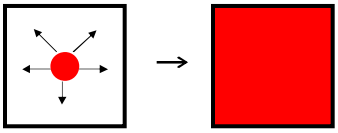
Color mixing



Chemical reaction



Diffusion



Commercialization? Think about it, guys. There must be something, where it would be REALLY REALLY useful

Change of shape

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
- **B&E students only**: estimate the market for your device (assume it can be built easily) in $$$

Stress-o-meter as an illustration

You have come up with some pretty cool ideas!

Homework review

Good news: plenty of cool ideas!

Medical applications were the most prevalent:

- Early detection of cancer: using specific biomarkers in blood (devices like glucose sensors) or in urine (like pregnancy kit)
- Heart attack detectors – mainly implants to measure ischemic biomarkers
- Biosensors for other medical conditions tuberculosis, anemia, etc, etc, etc

My personal biomedical favorites:

- Drug metabolites biosensor for side-effects monitoring
- A biosensor for detecting metastases in cancer patients
- Epilepsometer to detect epileptic sieges in advance

Cool sensors

- A biosensor for monitoring water quality in spaceships
- Allergy-meters
- Mood-meters
- Ambitiousness-meter (recommended for employers ☺)
- Sleepometers (2) – target melatonin (regulate circadian cycle)

Both guys provided a **compelling case** for their biosensors:
"drowsy driving" - 1,500 lives and 100,000 auto accidents a year

Personal cars market

Biosensor

Freight/Corporate customers

Combine the melatonin biosensor (enzyme or Ab) with physical sensors to measure the pulse and breathing frequency that tend to slow down during sleep

Detect why your baby is crying

"The sensor will measure the components in the tears and will <…> determine if the crying is caused by hunger, need of a diaper change, teething, or just by emotion (parent leaving the room). Monitoring why the baby cries can decrease the stress level of the parents as a baby's cries are not forgiving at 3:00 in the morning".

Why bio? "…because components in the tears (ie: protein, enzymes, antibodies, water, salt) can react with biological elements (ie: enzymes, antibodies, proteins) and produce a reaction" - results will be compared to a chart

+

Crying detector

➔

According to a paper by the Mercanti Group, an Investment Banking firm (2006), the market for baby and toddler spending (excluding diapers, food and apparel), is up to $40 bln/year. The crying detector will probably have a market of $50-100 million in the first year, and $500 million to $1 billion in the years to follow.

The Peanut Allergy Patch

"Peanut allergies are one of the most common allergies experienced by people today; about 1.5 million people in the US suffer from peanut allergies. Many of these allergic reactions (about 80%) are fatal or near fatal".

When an allergic person consumes peanuts, their immune system recognizes some of the peanuts' proteins as foreign: so the body responds by creating with antibodies that give rise to histamines, which cause the allergic reaction. To counteract an allergic reaction, the person must be administered a shot of epinephrine directly to the upper thigh.

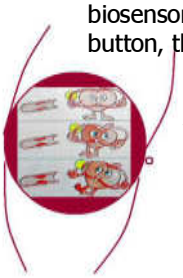
My biosensor is called the Allergy Patch. In the event that a person with a peanut allergy cannot receive a shot of epinephrine, they will have this patch

The Allergy Patch will have two parts – a sensor and a stored shot of epinephrine (contained by a polymer perhaps). The sensor will detect the presence of immunoglobulin E, <…> and after the IgE is detected, the epinephrine in the patch will be released into the body transdermally"

Another idea

Concept: "I am thinking of a nano sensor sitting on a transmitting RFID (Radio Frequency Identification) chip. This sensor will be injected in to the blood vessel and will be guided to coronary vessels. It will be able to sense the calcium and fatty substance deposition into the coronary vessel. Hence, we can call it a biosensor

"Now, we can design a wrist watch having corresponding frequency receiver RFID. The watch will have a button. Upon pressing the button, the watch will read the nano sensor and transmit the information to the watch. There has to be some kind of transducer which can convert the biosensor information to a readable format. Hence, upon pressing the button, the wrist watch will display something as below and alert you"



Go and see the cardiologist now!

"I know, the sensor would be quite complex and the manufacturing and implementing cost would be high. But, the outcome is priceless –

Nothing is more valuable than a human life!

Radio Frequency Identification

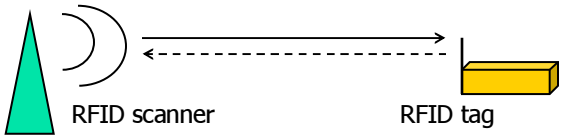
RFID is an identification method, relying on storing and remotely retrieving data using devices called RFID tags

Most **RFID tags** contain:

(1) a microchip for storing/processing information and (de)modulating radio signal (other functions can be added)

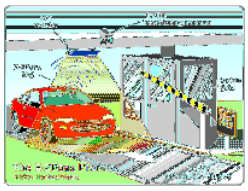
(2) an antenna for receiving/transmitting the signal

The tag's antenna picks up signals from RFID reader or scanner and the tag signals back with some additional data e.g. a serial number or any other customized information



RFID technology

There are two main varieties: active (require powers supply) and passive (no power needed but they work on much shorter distances)



Transport payment: road toll collection

New smart U.S. passports

Hitachi μ-chip (0.4 x 0.4mm)

New generation tags are even smaller - 50μm

Great technology for biosensors and an active research area – if we have time at the end we'll talk about it

Not so cool biosensors

Some biosensors were not sufficiently NOVEL or COOL, some had obvious practical drawbacks…

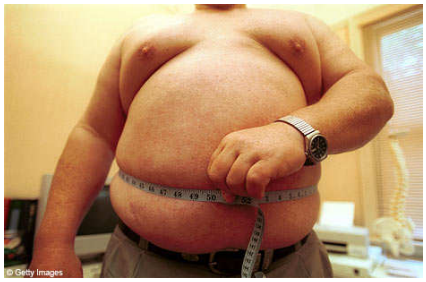
- Biosensors for detecting glucose (???) or cholesterol – both are commercial products
- Detection of microorganisms in food – mentioned in class…
- An easy way out – take a common clinical assay and make it into biosensor e.g. creatinine clearance. Justification?
- Alzheimer's and Parkinson's disease – measure β-amyloidal peptides or dopamine. These diseases develop slowly – would people want to drill holes in their heads for day to day monitoring?

A number of students attempted to find something suitable (or work out how certain commercial sensors work) rather then trying to **IMAGINE/CREATE** their own cool sensor…

Lipidometer

This biosensor measures the excess lipid level stored in the body, <u>especially in the waist part</u>

This belt generally measures lipid levels which is presents in the body. The sensors in the belt are specifically built to recognize these three lipid forms - LDL, VLDL and Cholesterol…



Isn't there an easier way to do this? ☺

There were also a hunger-o-meter and a headache-o-meter…

Do we really need biosensors for these?

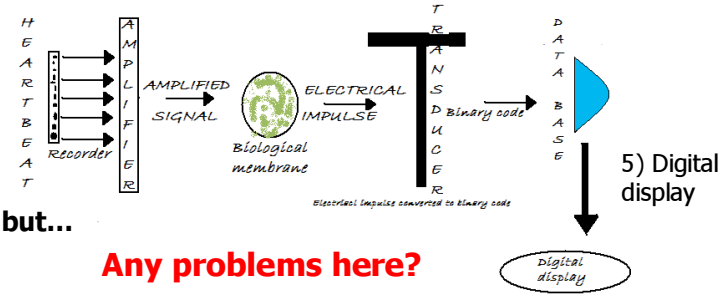
Love sensor

"The concept of love sensor is a idea developed based on my experiences. I always had a confusion to identify my love for things like cars, building, places. If I see 2 or more cars at a time I could not tell which one I like the most <…>

I always thought of one instrument which could really visualize what my heart says; just like a electronic monitor which can show what my heart feels. Hence the idea of LOVE sensor is developed"

Design of the love sensor

1) Recorder: records response of the heart and amplify the signal. The recorder is at the very end of the sensor so that it is in contact with left side of chest.
2) Biological membrane: The biological membrane used here is similar to the brain cell. The function of this membrane is to absorb the amplified signal and generate electric impulses.
3) Transducer: The function the transducer is to convert the electric signal into binary code and transfer it to the data analyzer.
4) A preloaded data base with information of responses of various people.



5) Digital display

Very cool but…

Any problems here?

A couple more designs

"Myocardial infraction is nothing but interruption of blood supply to heart, which occurs due to occlusion of coronary artery fallowing by atherosclerotic plague <…> This lead to increased arterial pulse pressure stretching and relaxation of arteries with each heart beats".

Solution: "My machine works on the basis of intravascular ultra sound. Injured heart tissue conducts electrical impulses more slowly than normal heart tissue. Machine distinguiesh conduction velocity between injured and uninjured tissue".

Any problem with this?

"Iodine is an essential trace element. Its main role <…> is as constituents of thyroxine (T4) and triiodothyronine hormones

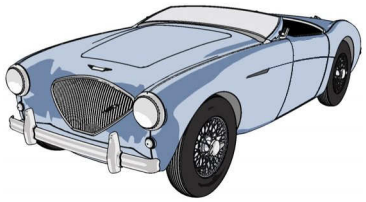
THYRO-CHECK is a small instrument like a wrist watch to be worn on patients' wrist. THYRO-CHECK would use radiowaves to check the blood levels of T4 <…> after the patient is administered radioactive iodine"

What about this one?

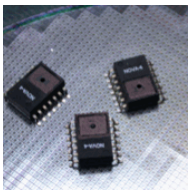
Both are NOT biosensors

Right sensors for the job

Are any of these biosensors? No, because there is NO NEED



- 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

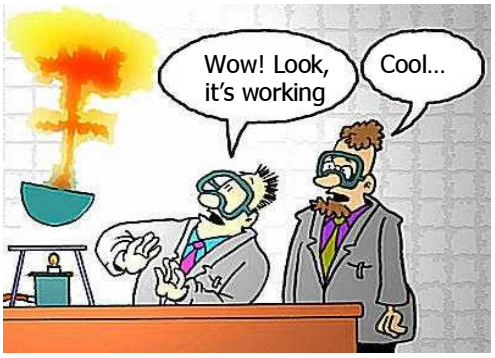
O2 sensors made modern electronic fuel injection and emission control possible. They determine the air fuel ratio exiting the engine and provide real time feedback to the fuel injector

Look for a practical solution!

Can one crack nuts with nuclear explosions?

Probably

but there is a better way ☺



The bottom line: It may well be a great piece of science but it won't work for entrepreneurs and engineers…

A VERY important message

If your design has NOT appeared on my slides – do **NOT** get upset!

My selection was not based entirely on quality and you may have done a great job. Also, I have seen only about a half of your designs

If your design appeared on the slides as "not so great" – do **NOT** get upset!

Presumably most of you attend because you want to learn more about biosensors. When people learn, they make mistakes – it's OK

Also, you will have another shot at it before too long ☺

A few pieces of homework were **too similar** to my liking.

I am NOT going to start any investigations but will disregard both

Is there anything to get upset about?

YES!

The last warning

Biosensor detecting pollutant in water, drinks and food:

"The agricultural use of Atrazine, and other herbicides based on a chemical substance called Triazine, often causes contamination both of underground water and over ground water. Similarly, antibiotics used to treat bacterial infections **and so on and so force**

Public release date: 17-May-2007

[Print Article | E-mail Article | Close Window]

Contact: Octavi López Coronado, Universitat Autonoma de Barcelona octavi.lopez@uab.es34-935-813-301

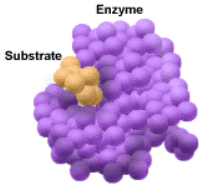
A new portable biosensor detects traces of contaminants in food more quickly and cheaply <…> The agricultural use of Atrazine, and other herbicides based on a chemical substance called Triazine, often causes contamination both of underground water and over ground water. Similarly, antibiotics used to treat bacterial infections

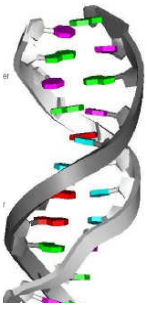
http://www.eurekalert.org/pub_releases/2007-05/uadb-anp051707.php

If in doubt what it means, consult the policy on plagiarism

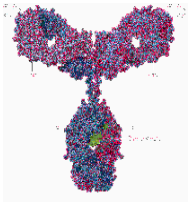
Type of biological receptors

What can be used in biosensors?





Nucleic acids

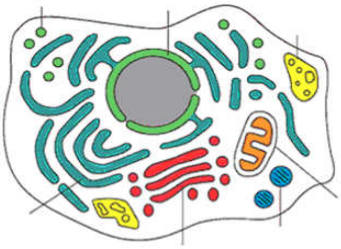


Antibodies

By definition anything "biological" but most frequently

Macromolecules: proteins and nucleic acids

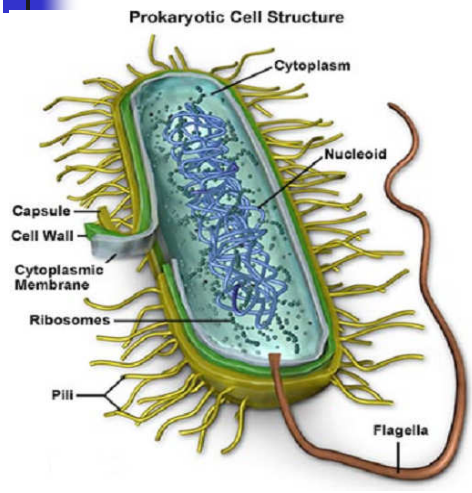
Whole cells: bacteria, plant and animal cells



Whole cells

Today we will take a look at cells

Microorganisms



Bacteria in sensors:

- Typically more stable than isolated macromolecules (e.g. proteins, receptors)
- Can be used to perform complex multi-step sensing reactions – an enzymatic microreactor
- Can be stabilized to work (or store) for a long time
- INEXPENSIVE!

Main drawbacks: can be slow, side-reactions, reproducibility

Biosensors for uranium

- There are ~120 uranium-contaminated sites in the US alone, containing more than 6 trillion liters of waste
- The uranyl ion (UO_2^{2+}) is the most water-soluble form of uranium; it spread easily through groundwater systems
- Sensitive detection is necessary to identify contaminated areas and to evaluate (bio)remediation efforts

Why do you need bugs/biosensors, when there are plenty of sensitive and selective methods available?

• Phosphorescence	• Low sample throughput
• Atomic emission	• Portability
• Mass spectrometry	• Measure total uranium

Bacteria-based biosensors can be used **directly on the site** detecting the presence of **bioavailable uranium** in situ

Bacteria can sense uranium

- *Caulobacter sp* can live in low-nutrient environments, including poor soil and contaminated ground and wastewater - just the habitat where uranium contamination may be present
- The bacterium is resistant to uranyl concentrations of up 1 mM and it has several genes that are significantly upregulated in response to uranium
- One gene (urcA) is upregulated up to 27 fold and it is NOT induced by other heavy metals (selectivity!)

OK, we have a gene (whatever it does) which is specifically upregulated when bacteria are exposed to uranyl cations but

How do we make a sensor from this?

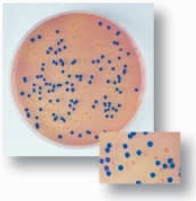
We need something to tell us that the expression of this gene is on e.g. a **reporter protein** that would generate a good signal…

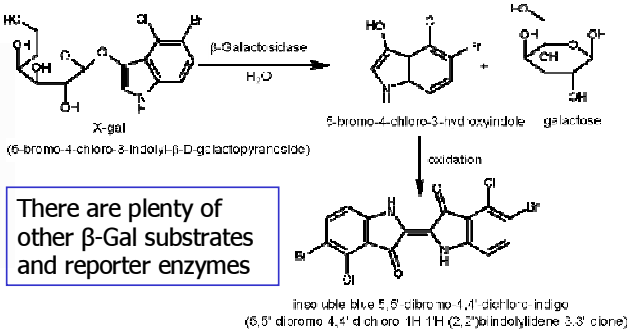
Reporter protein

What can this be?

An enzyme catalyzing a reaction, the product of which can be easily detected – it must be non-toxic and not naturally present in the cell in large quantities (better none at all)

β-Galactosidase (*lacZ*) is a classic reporter used in combination with growth media containing substrates that produce colored products





There are plenty of other β-Gal substrates and reporter enzymes

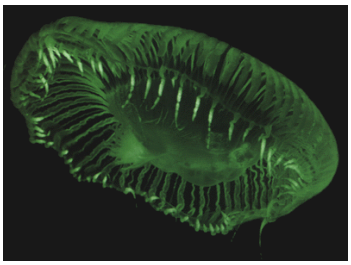
Non-enzymatic reporters

lacZ is great for lab work but may be problematic for some field sensors – need to supply a chromogenic substrate

Better option? A protein the presence of which can easily be detected e.g. a fluorescent protein

The green fluorescent protein (GFP) originally isolated from jellyfish Aequorea

Can be expressed in every cell of a mammal – needs illumination to see



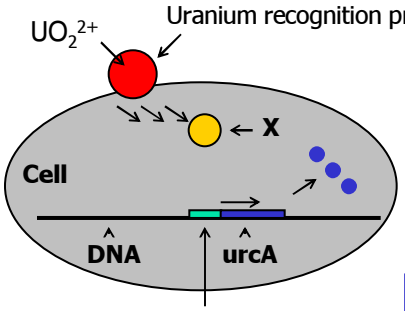
Easily detectable, non-toxic and not present in other cells or animals

One more problem to solve…

We have great reporters and we know that when the bacteria "see" uranium they will switch on the expression of urcA

How do we make a biosensor?

Put lacZ or gfp under the control of urcA promoter



Promoter that switch the gene on when bacteria senses Uranium

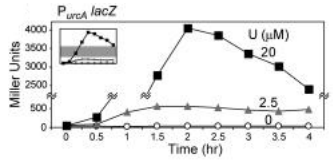
- Interaction of UO_2^{2+} with receptor would initiate a chain of intra-cellular events leading to the activation of transcription factor X
- It will then bind to the promoter and initiate the transcription of urcA protein – the cellular response

If lacZ/GFP are under urcA promoter, the cell will start making the reporter when exposed to uranium - SIGNAL

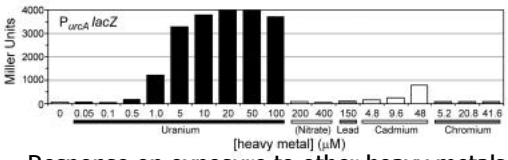
Let's see whether it works

Kinetics, sensitivity, specificity

lacZ under urcA promoter



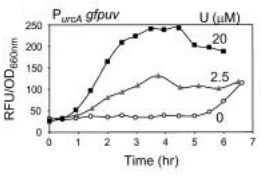
Response on exposure to UO_2^{2+}

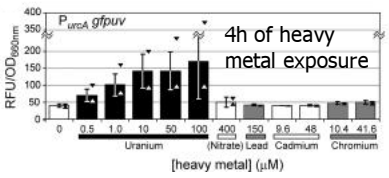


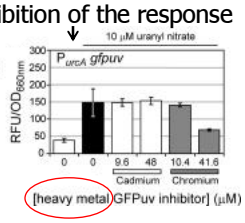
Response on exposure to other heavy metals

GFP under urcA promoter

and no inhibition of the response

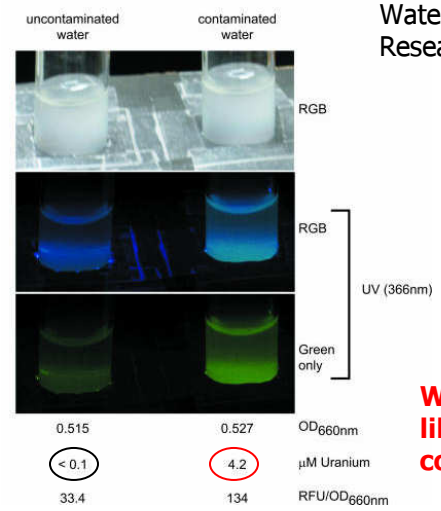






APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 2007, 73, 7615–7621

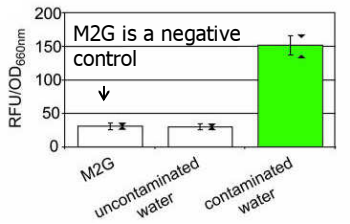
Analysis of ground water



Illuminated with daylight (top) and a hand-held UV lamp after 4 h of exposure

Water samples from Oak Ridge Field Research Center (contaminated field)

Easily quantifiable too

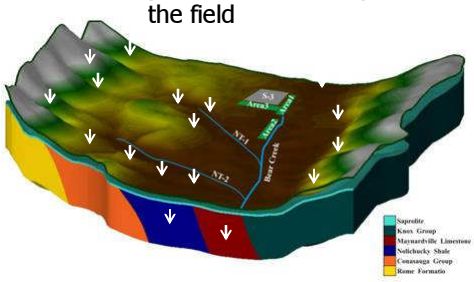


What can you do with technology like this that you cannot do with conventional chemical analysis?

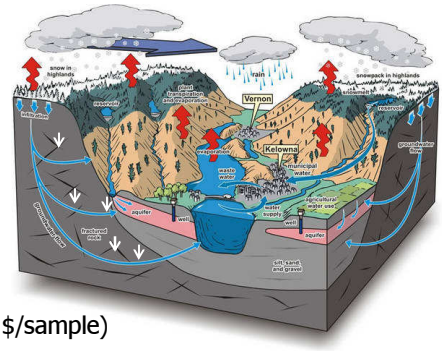
Hillson et al (2007) Appl Env Microbiol, 2007, 73, 7615

Unattended monitoring

Position as many sensor as you like in different parts of the field



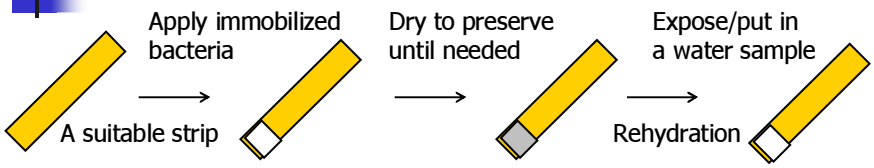
Continuous monitoring e.g. water supply (and not just for uranium)



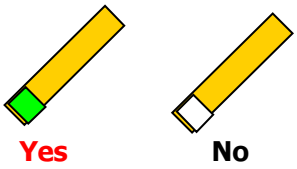
- Very high potential throughput (less $/sample)
- No need to collect samples and send them to the lab for analysis

...and this technology can be adapted for home use too

Adaption for consumers?



Is my water contaminated?



- Technology for making biosensors like this has been around for years
- Can be used for detecting practically any contaminants e.g. pesticides, etc
- Easy and cheap to manufacture

Problem?

Market?

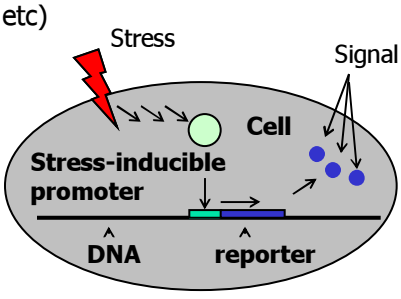
A more useful test

Overall quality of water

Any ides how to do this?

Bacteria respond to harmful compounds and conditions (stress) by activating cellular processes that protect them against the invoked stress (heat shock response, SOS – and *ada* in response to DNA damage, etc, etc, etc)

Hence, by placing a reporter gene under control of a stress-responsive promoter, bacteria will generate a warning signal when they are exposed to stress triggering condition e.g. DNA or protein damage, toxic chemicals, solvents, pH and others



Such tests have been used for years but with a different reporter

Yet another reporter

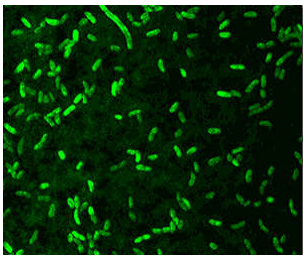
Luciferases are light emitting enzymes responsible for bio-luminescence in nature

1) luciferin + ATP → luciferyl adenylate + PPi

2) luciferyl adenylate + O_2 → oxyluciferin + AMP + light

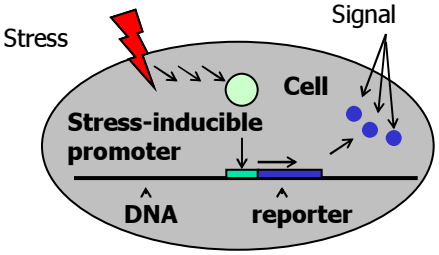
A great reporter

Similar gene is present in bacteria e.g. a gram-negative marine bacterium *Vibrio fischeri* and others (lux gene)



By putting this gene under the control of appropriate promoter, the bug will signal the sensing event by emitting light

Great principle but…



Stress response activated →

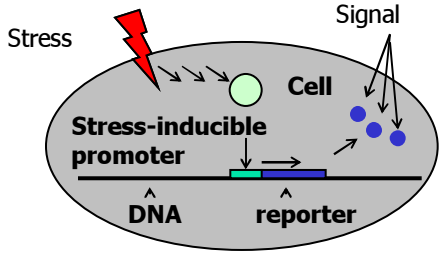
Bacteria "flashes" the warning

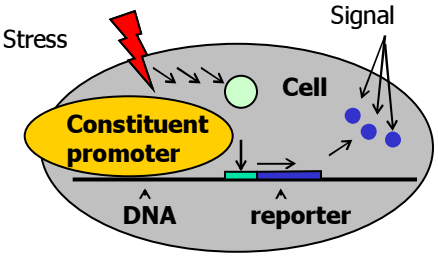
…there is a problem with this set up **Any ideas?**

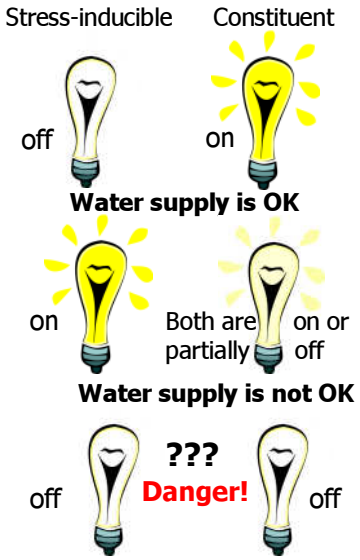
If the stress is too BIG bacteria are dead – there will be no warning!

Is there a way to control for such an event?

Yes, there is







Microtox® test

- A commercial test to monitor the quality of water supplies with bacteria constructively expressing the lux reporter gene
- An advantage of using constitutive promoters is a "self-testing" function – if the bacteria glow, the sensor is working OK
- Disadvantage – decreased sensitivity

Examples of practical use:

In many Iraqi cities during the Gulf war

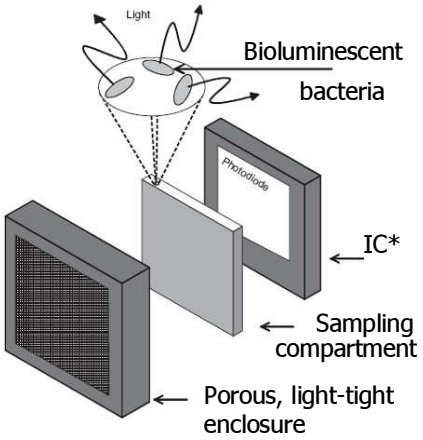
Olympic Games in Los Angeles (1984) and Atlanta (1996)

Political events e.g. the Democratic convention (2000)

The U.S. Army Corps of Engineers routinely monitor water supply to Pentagon

Numerous civilian installations in the U.S. and Europe

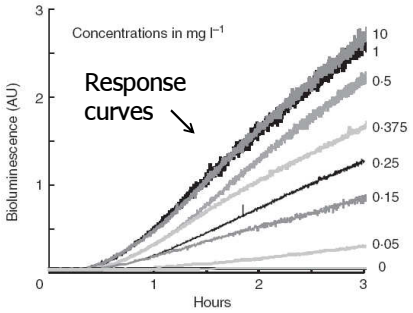
Integrated circuit device



Schematic diagram of bioluminescent integrated circuit biosensor

*IC - Integrated circuit

Detection of salicilates



The detection limit is ~0.05 mg/L for a 45 min exposure and the bioluminescent response saturates at a ~1 mg/L

Nivens et al (2004) Journal of Applied Microbiology 2004, 96, 33–46

Which reporter is the best?

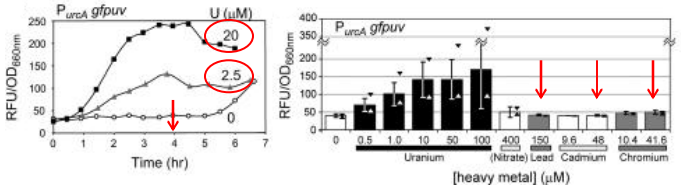
Gene/protein	Pros	Cons
lux (bacterial luciferase)	Ease of measurement; rapid response, high sensitivity	Unstable at elevated temperatures
luc (firefly luciferase)	Rapid response; high sensitivity, stable at elevated temperatures	Requirements for oxygen and for exo-genous substrate
gfp (green fluo-rescent protein	Autofluorescence – no substrate requirement	Lower sensitivity, slower response
lacZ (β-Gal)	Wide variety of detection methods (including naked eye)	Exogenous substrate requirements

No perfection in this world – make your pick ☺

Back to uranium

The uranium biosensor is cheap and detect bioavailable U

but how about disadvantages – **have you spotted any?**



Sensitivity is not great: conventional chemical methods enable the detection of uranium at a pmol level

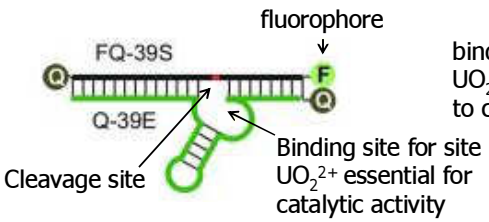
Selectivity is not great: conventional chemical methods are MUCH more specific

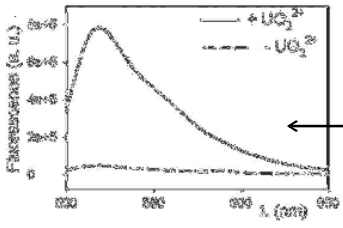
and it takes 4h to get a result: conventional chemical analysis can be completed in ~10-15 minutes

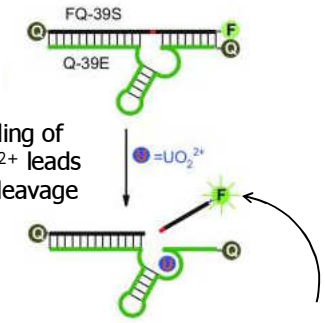
Is this a problem with biosensors in general or this one in particular?

Another way to skin this cat

A small catalytic DNA that can cleave itself but only in the presence of UO_2^{2+}







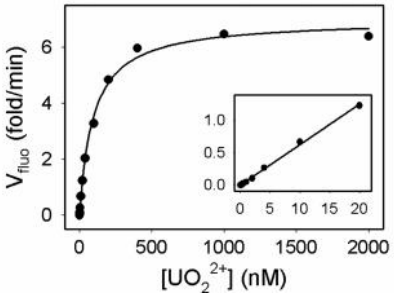
and the released of a piece of DNA with the fluorophore

The fluorophore is then detected by the sensor

Liu et al (2007) Proc Natl Acad Sci USA 104, 2056-2061

How does it compare?

- Detection limit: is 11 parts per trillion (45 pM)
- Dynamic range: up to 400 nM
- Selectivity: >1-million-fold over other metal ions
- Detection time: 4-5 min

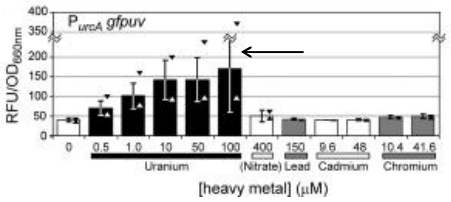


- This biosensor is on the par with the most sensitive analytical instruments for uranium detection, and its can detect uranium directly in contaminated soil samples

We will discuss how this type of biosensors work later in the course

One more point to note

These are pretty big strandard deviations



Why?

The error bars indicate one standard deviation from the mean; the triangles indicate the maximum and minimum observed fluorescence values

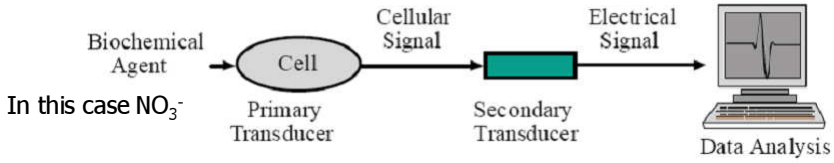
Variations are inherent to all living systems:

- Different levels of the reporter gene expression
- Different cell's sensitivity to UO_2^{2+}
- Subtle variation in growth conditions e.g. nutrient or O_2 availability

Let's look at this in more detail

Bacterial nitrate biosensor

To detect the availability of nitrate in soil around plants' roots

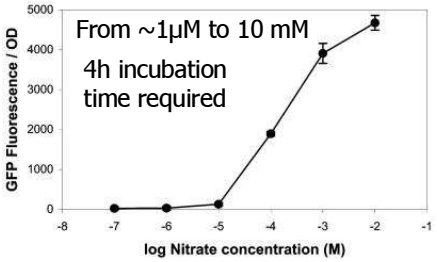


In this case NO_3^-

Biosensor: The reporter gene (gfp) is put under the control of a nitrate-inducible promoter in root-colonizing bacterium *E. cloacae*

Nice but not particularly exciting…

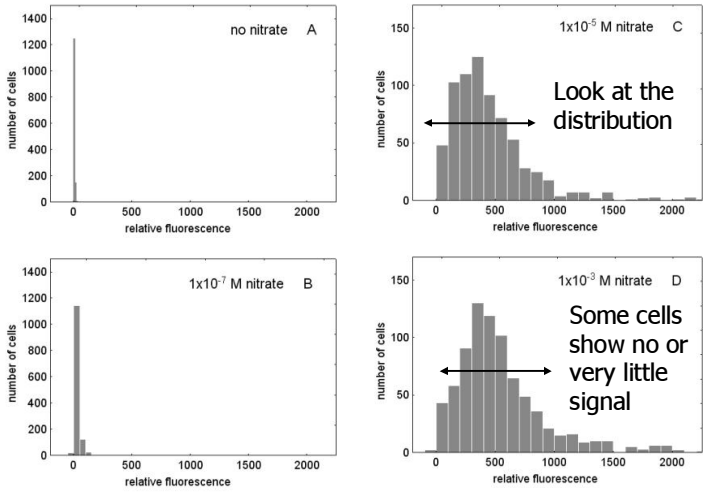
However, these guys did something else – they looked at fluorescence of **individual bacterial cells**



Appl Environ Microbiol. 2005 Dec;71(12):8537-47

Fluorescence of individual cells

Not great for quantitative analysis…



Appl Environ Microbiol. 2005 Dec;71(12):8537-47

Other cool applications

Novel Whole-Cell Antibiotic Biosensors for Compound Discovery▽

Andreas Urban,¶† Stefan Eckermann,¶§ Beate Fast,‖ Susanne Metzger, Matthias Gehling,‡ Karl Ziegelbauer, Helga Rübsamen-Waigmann,† and Christoph Freiberg*

Pharma Research & Development, Discovery Europe, Bayer HealthCare AG, D-42096 Wuppertal, Germany

Generated and validated a set of five antibiotic biosensors for comprehensive HTS-compatible mechanism-based screening:

1. DNA
2. RNA
3. proteins
4. cell wall
5. fatty acids

- Depending on the mechanism of action, different antibiotics trigger the expression of different bacterial genes
- By putting lux under the control of the respective promoters, these guys made bacteria that tell them HOW EXACTLY they are being killed

Validation: a subset of known antibiotics was correctly identified in a library of 14,000 pure natural products and new lead compounds were found too…

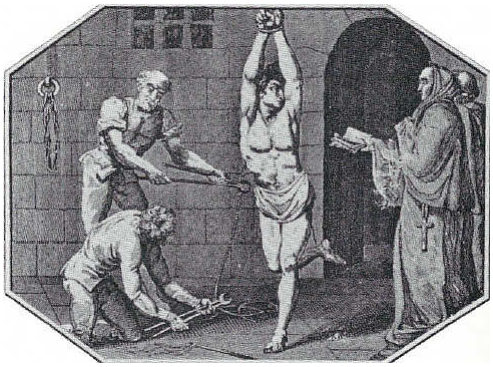
Appl Environ Microbiol 2007, 73, 6436–6443

Let's summarize

- Bacterial biosensors are mainly used for environmental monitoring but other applications are being developed too
- The most frequently used reporter genes are gfp (green fluorescent protein), lux (luciferase) and lacZ (β-galactosidase)
- The expression of a reporter gene can be triggered by binding to a specific receptor-protein or enzymatic reaction
- The reporter gene can also be placed under control of a stress-responsive promoter to turn it on when bacteria are exposed to stress triggering conditions - these sensors are "semi-specific" i.e. they do not identify what caused the stress
- When the reporter gene is constructively expressed, bacteria are used as non-specific sensors: e.g. monitor water quality, BOD
- Generally, drawbacks of bacterial sensors include long response time and relatively low sensitivity and/or selectivity

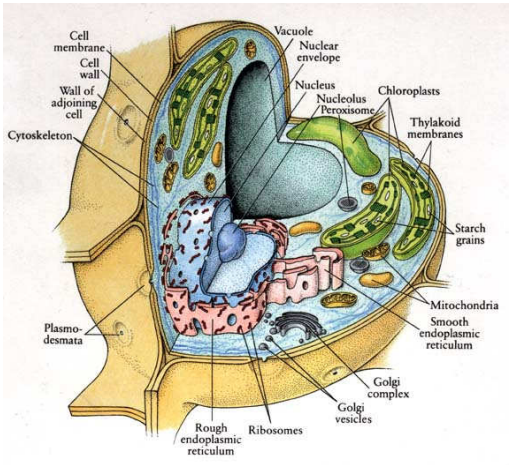
Now guess what's coming

The Quiz



Plant cells: more complex

not often used in biosensors

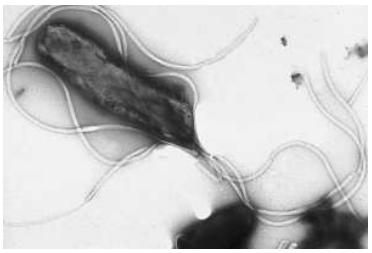


- Plants may look attractive – they can "feed" themselves but plant cells are rarely used in biosensors
- Expensive, can be slow (cell walls) and only useful for some very specialized applications

Plant cell-based sensor

Plants can detect potentially pathogenic microorganisms through the so-called general elicitors i.e. molecules that are characteristic of a whole class of microorganisms

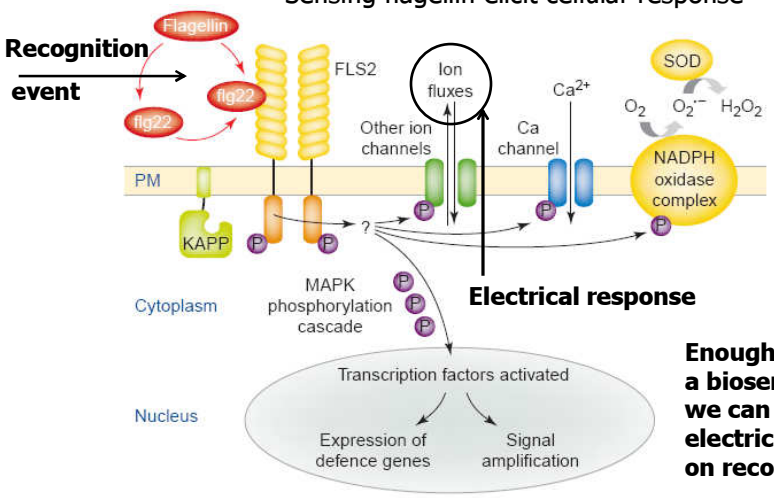
- Flagellin, a protein that makes up bacteria's propeller (flagella), is a typical such elicitor
- Plants recognize flagellin with the help of specific extracellular receptor, FLS2



Biochemistry of FLS2 is reasonably well understood

FLS2 – flagellin receptor

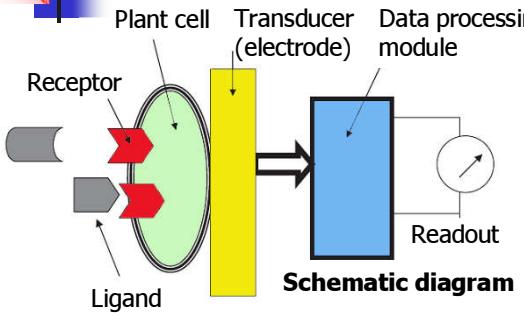
Sensing flagellin elicit cellular response



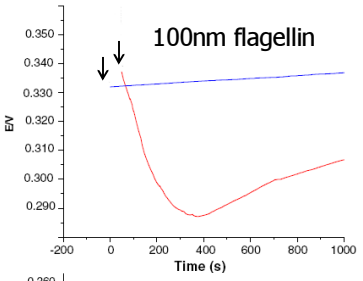
Enough to build a biosensor - we can get an electric signal on recognition

TRENDS in Plant Science (2002) 7, 251-256

Potentiometric biosensor



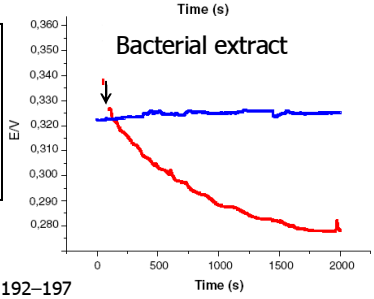
Biosensor's response



Is it a useful biosensor?

Probably not… e.g. no data on selectivity

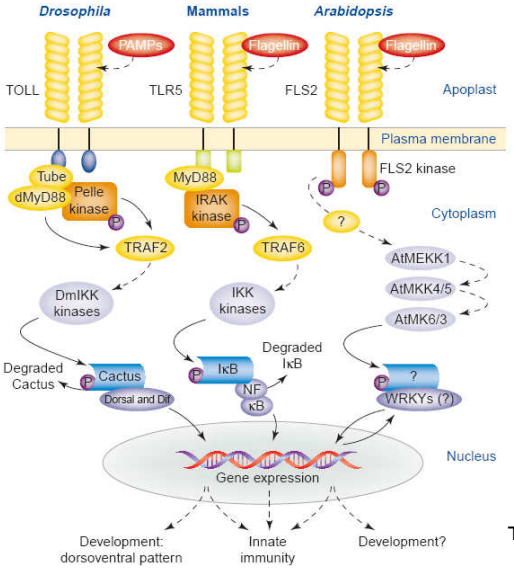
Would the same ion movement/electrical response occur on another stimulus? I bet



A reporter gene? Perhaps, but…

Bioelectrochemistry 70 (2007) 192–197

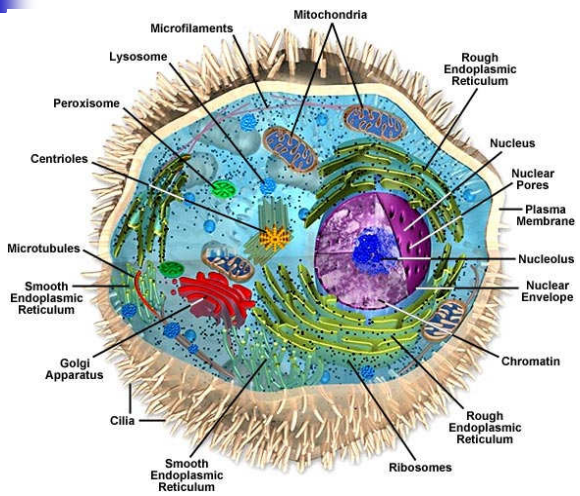
Why plant cells?



- Same receptors are present in insects and mammals as a part of innate immune system (recognition of pathogens in "generic" way rather than adaptively)
- If the purpose is to construct a flagellin biosensor why don't they use antibodies?
- Better to leave cells for jobs that only cells can do…

TRENDS in Plant Science (2002) 7, 251

Animal cells

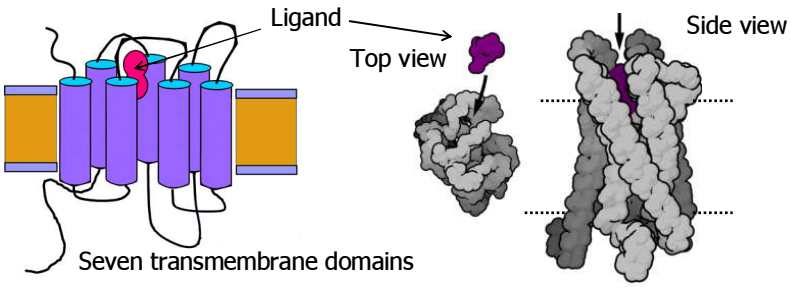


- Offer unique possibilities in biomedical analysis
- Used in specialized sensors as individual cells or tissues
- **Can be the ONLY option available**

Main drawback: Still in development and EXPENSIVE!

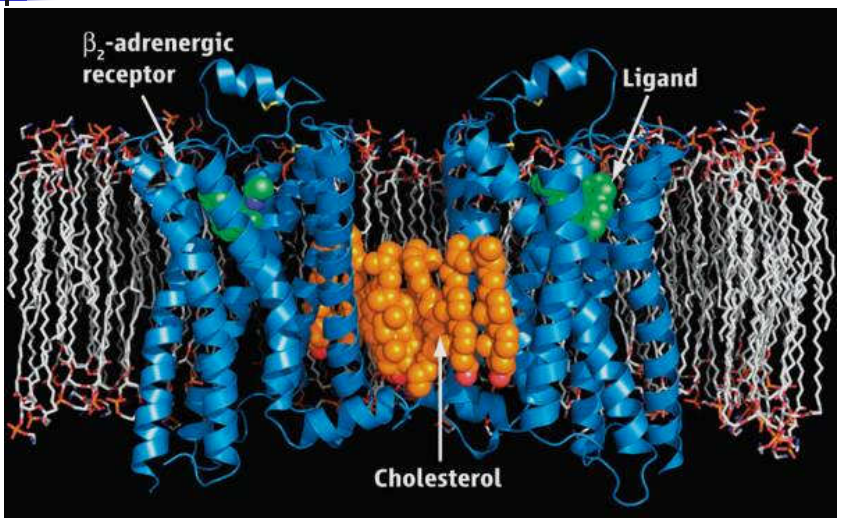
G protein-coupled receptors

G protein-coupled receptors (GPCRs) are a large family of transmembrane protein receptors that sense extracellular signals e.g. hormone, neurotransmitters, light, odors, etc



Once activated, GPCRs initiate intracellular signal transduction cascades, which ultimately lead to the cell's response

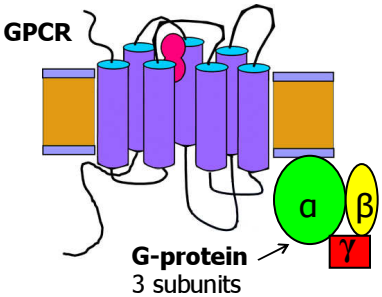
X-ray structure



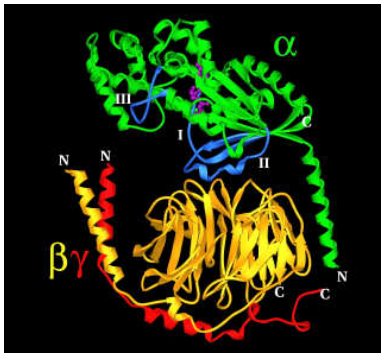
Cherezov et al., Science 318, 1258 -1265 (2007)

GPCRs signal *via* G-proteins

GPCRs signal through so-called G-proteins that are normally associated with the receptor in inactive state

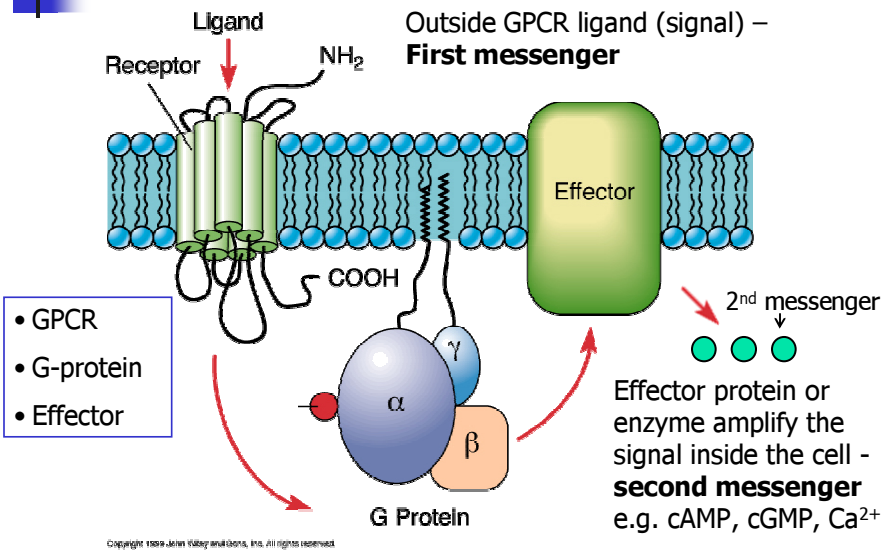


Binding of ligand (or other signal mediator) leads to conformational change in GPCR, causing activation of the G protein. Further effects depend on the type of G protein



G-protein X-ray structure (Lambright et al., 1996)

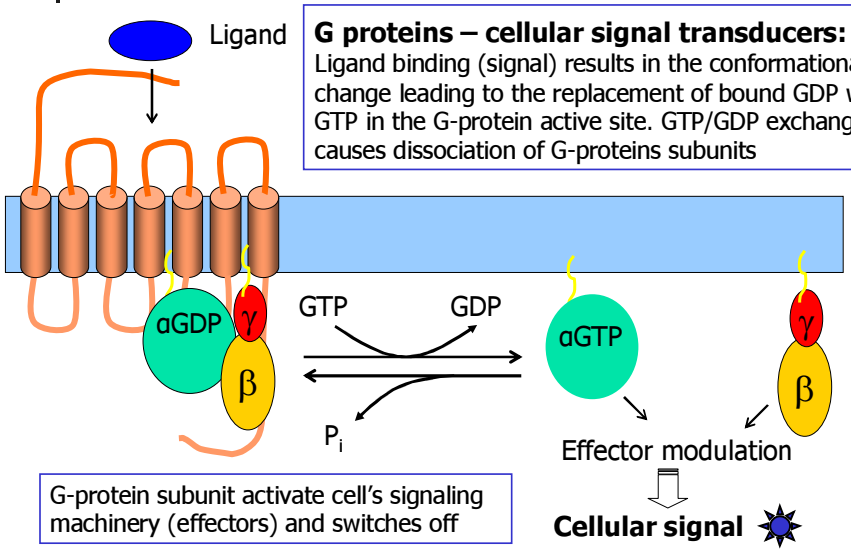
A three component system



Outside GPCR ligand (signal) – **First messenger**

Effector protein or enzyme amplify the signal inside the cell - **second messenger** e.g. cAMP, cGMP, Ca^{2+}

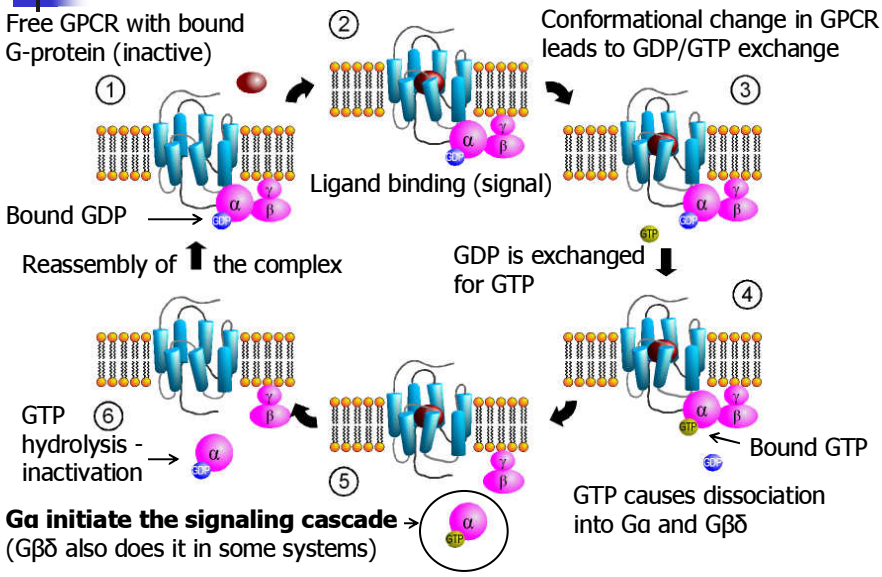
G-protein signaling



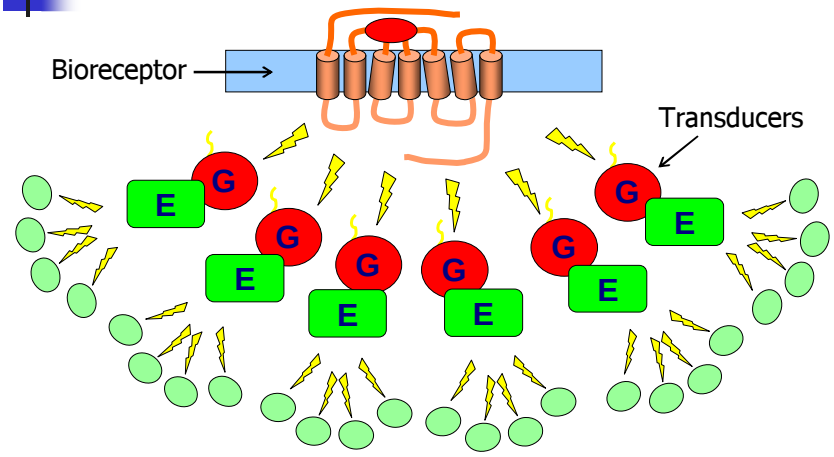
G proteins – cellular signal transducers: Ligand binding (signal) results in the conformational change leading to the replacement of bound GDP with GTP in the G-protein active site. GTP/GDP exchange causes dissociation of G-proteins subunits

G-protein subunit activate cell's signaling machinery (effectors) and switches off

G-protein cycle



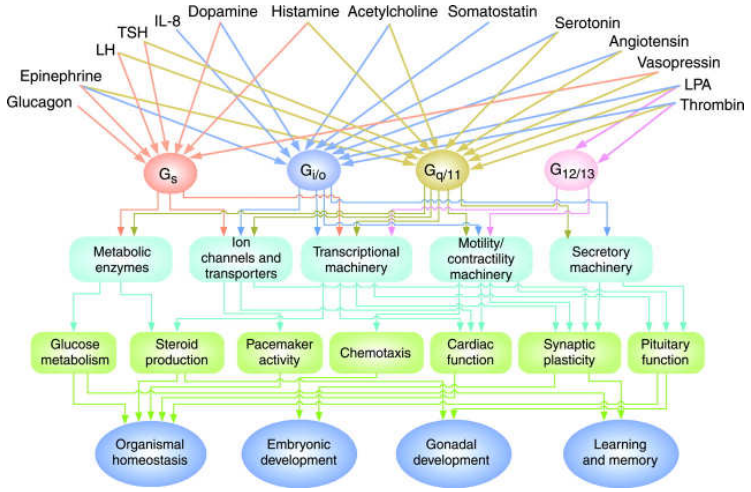
Cell's own biosensors



Second messenger: Signal amplification and "read-out"

Integration of cell signaling

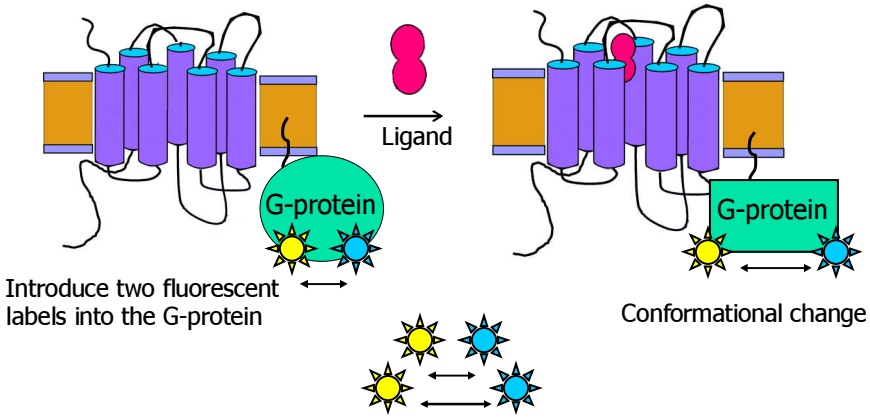
G-proteins unify and integrate intracellular signaling



Neves, Ram, and Iyengar *Science* (2002) **296**:1644

Hijacking cells' signaling

Can we get G-proteins to "talk" to us too?



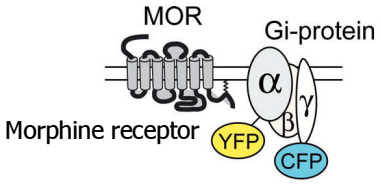
Introduce two fluorescent labels into the G-protein

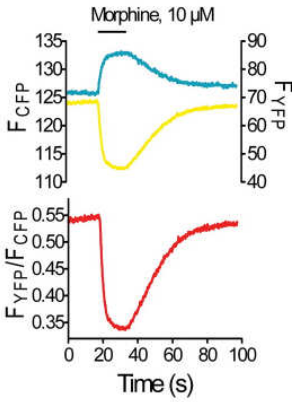
Conformational change

Note the change in distance between the two

Whole-cell G-protein sensor

Biosensor for monitoring morphine activation in living cells



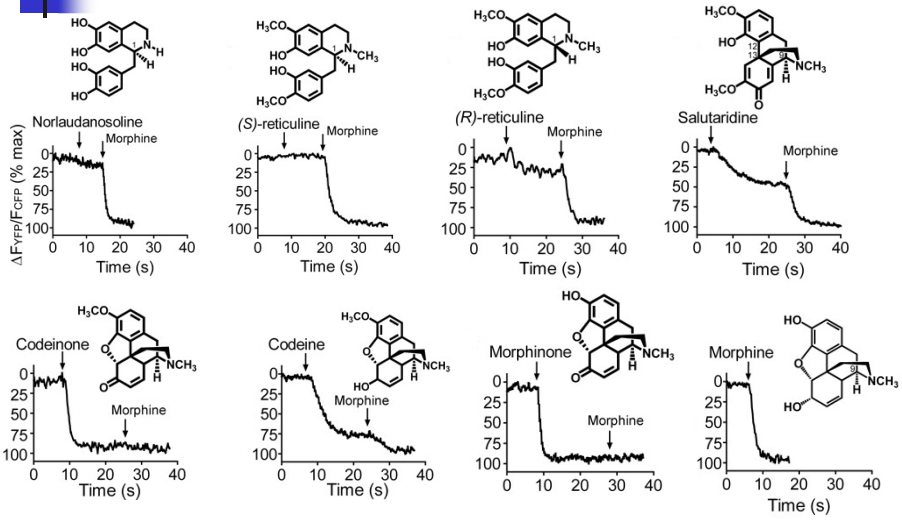


The conformational change results in the increase of "blue" signal and decrease in the "yellow" – plotting the ratio gives a VERY nice response

Control: the introduction of the labels does NOT affect biochemical events triggered by morphine binding to the GPCR

Nikolaev, V. O. et al. J. Biol. Chem. 2007;282:27126-27132

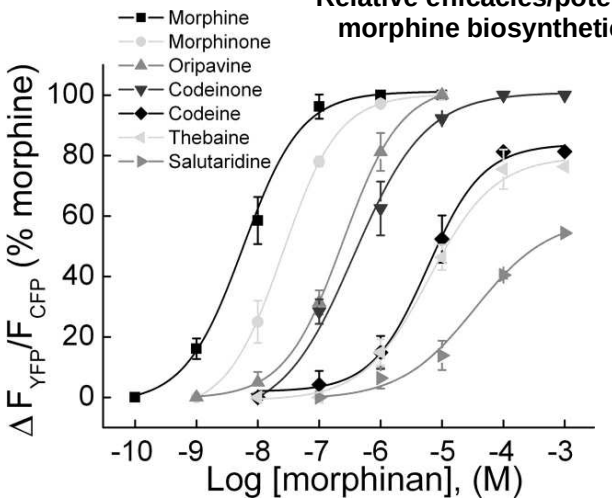
Study of morphine analogs



Nikolaev, V. O. et al. J. Biol. Chem. 2007;282:27126-27132

IC50 of morphine derivatives

Relative efficacies/potencies of major morphine biosynthetic precursors



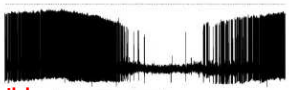
G-protein activation biosensors can be used to investigate the pharmacological properties of compounds acting on G-protein coupled receptors – very cool

Currently ~40% of drugs on the market target GPCR

Nikolaev, V. O. et al. J. Biol. Chem. 2007;282:27126-27132

Neurons for sensing

chemical warfare agents



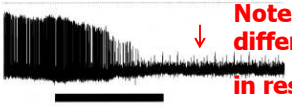
reversible

irreversible

10 μM VX

20 mV

2 min

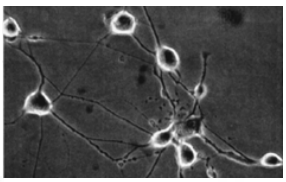


10 μM GD

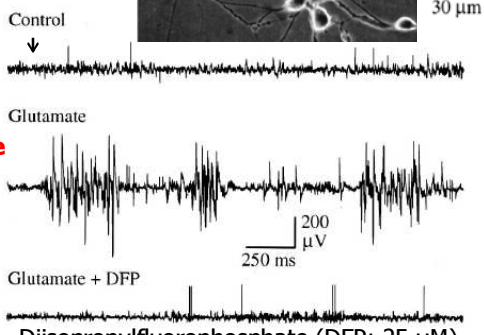
Note the difference in response

Effects of VX (ethyls-2-NN-diisopropylamin ethyl methylphosphonofluoridate) and GD (soman; O-pincolyl methylphosphono-fluoridate on spontaneous, rhythmic firing (measured with glass microelectrodes)

Neuroblastoma NG108-15 ⟶



30 μm

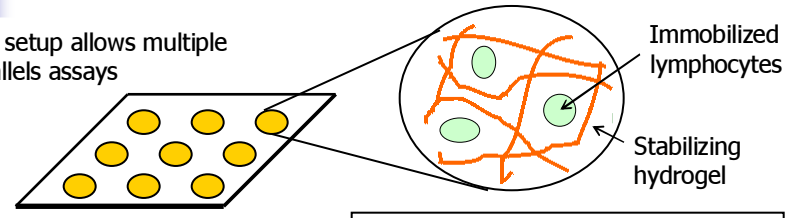


Diisopropylfluorophosphate (DFP; 25 μM) markedly inhibit spontaneous firing of spinal cord neurons

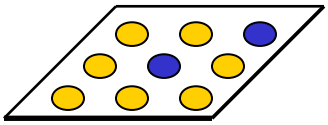
Annals of Biomedical Engineering, Vol. 27, pp. 697–711, 1999

Lymphocytes sense toxins

This setup allows multiple parallels assays



Exposure to potentially hazardous bacteria/toxins



Operating principle:

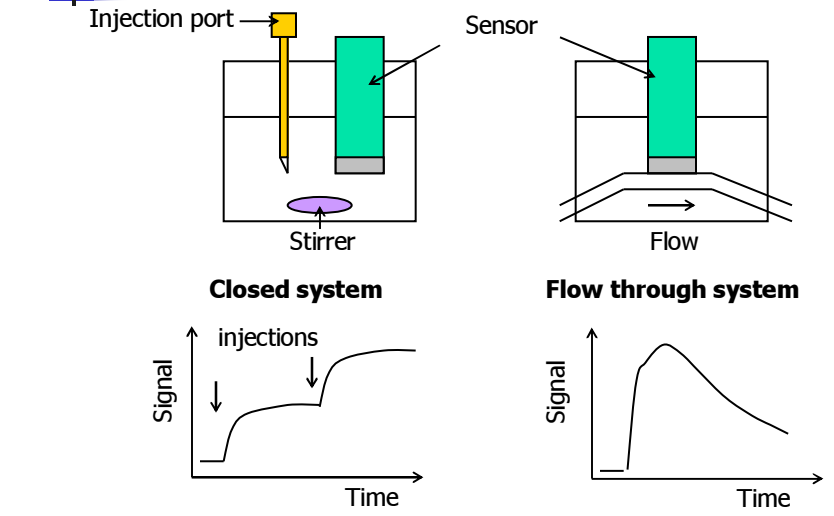
- Detects whether a toxin or bacteria have "broken" the lymphocytes
- If damaged, lymphocytes release alkaline phosphatase, which can be detected by a color reaction

- Unlike other biosensors, it only signals when bacteria or toxins pose a real threat to humans
- Biosensors based on mammalian cells can provide unique information

Bioreceptors: quick summary

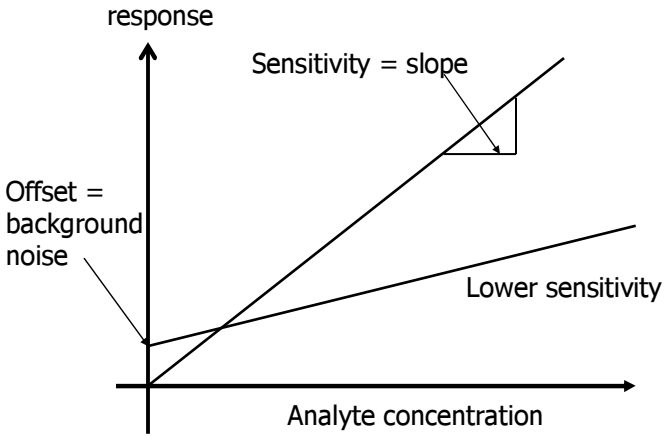
Bioreceptor	Advantage	Disadvantage
Macromolecules (proteins, DNA)	Sensitive, no side reactions	Need to be identified isolated or designed
Microorganisms	Multi-step reaction are possible, cheap	Low selectivity and sensitivity, slow
Eukaryotic cells (mammalian)	Enable to perform unique assays	Relatively expensive unstable
Tissues slices	Minimal preparation. cheap	Slow diffusion/side reactions
Plant cells	Only useful in some VERY special cases	Expensive (if cells), slow

Basic assay systems



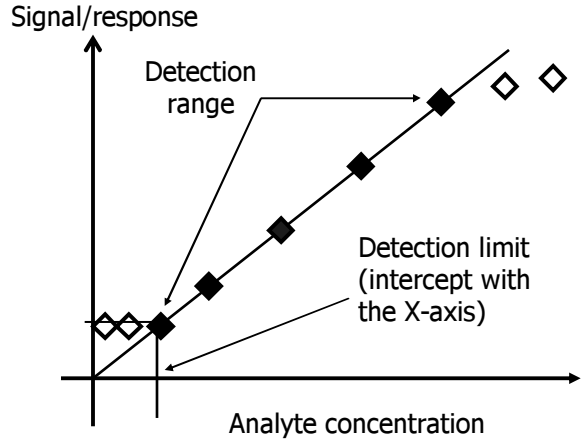
Typical response

Performance of different sensors can be compared by comparing the slopes



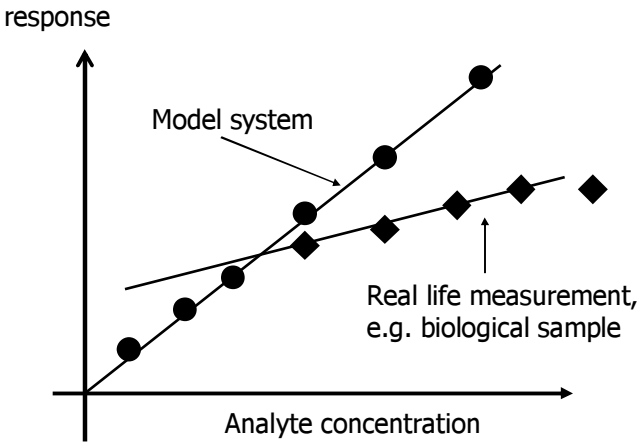
Detection limits

Often the lower, the better but not necessarily so…

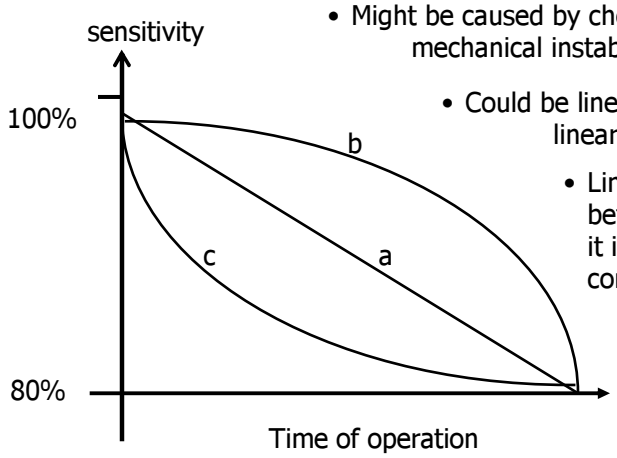


Selectivity

Dramatic loss of performance due to the presence of "competing" substances

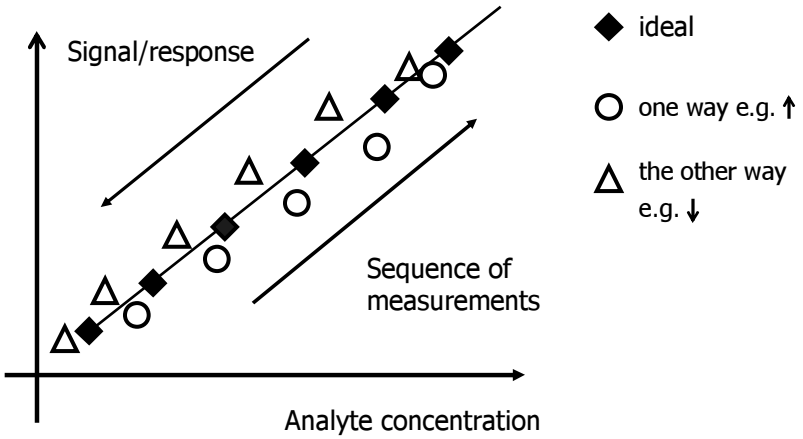


Stability



- Might be caused by chemical or mechanical instability
- Could be linear (a) or non-linear (b, c)
- Linear is MUCH better because it is easier to correct

Reproducibility



The effect known as hysteresis

Performance requirements

What characteristics would you expect from a great sensor?

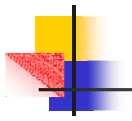
Selectivity – often the critical feature!
Sensitivity – typically 1µ-1mM range, often 1pm-1nm, sometimes down to fentomolar range 10^{-15} M
Stability, Reproducibility and Reliability
Accuracy – needs to be better than 5% (at least!)
Response/Recovery Time – often >30s (nerve agents?)
Measurement Range –susceptible to saturation?
Re-usability/Cost – e.g. antibody based biosensors are often not re-usable due to very tights interactions
Self-testing/Internal calibration
Physical robustness – doesn't break easily, long life time
Acceptability – ease of use and product safety!

Three critical "S" – selectivity, sensitivity and stability

Factors affecting performance

- Analyte concentration (too little or too much)
- Presence of interfering molecules (e.g. enzyme inhibitors)
- Denaturing agents (e.g. organic solvents and detergents)
- Unfavorable chemical or physical conditions (e.g. pH, temperature)
- Type of surfaces (e.g. hydrophobic surfaces are often subject to rapid bio-fouling)

All these factors can significantly affect the performance
Hence, it is important to think through what kind of samples (e.g. "crude" vs partially purified) and under what conditions (e.g. "field" vs "lab") will be analyzed and design the biosensor and sampling protocols accordingly



Home work

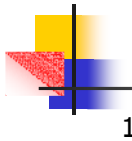
In the light of what we discussed today I would like you, guys, to take another shot at your cool sensor design

Point to ponder on:

- Do I want to make any amendments to make my sensor any better, cooler or more useful?
- What kind of bioreceptor would I use and why? You do NOT have to specify it now but may need later ☺
- Something else you might want to change

As before, the HW must be emailed to me by 10am next Thursday

If you are happy with your design – it's OK. There is **NO need to do anything**



A few tips on HW submission

1. Homework must be emailed to poly603@gmail.com
2. The deadline for submitting all the homework is Thursday, 10am – late submissions will be disregarded
3. Questions like "Professor, am I heading in the right directions?" on Wednesday night (or later!) will be disregarded
4. Multiple choice answers – Professor, pick the best - don't work
5. E-mails like "Professor, here is my homework" with no signature will be disregarded
6. It is better to put your answers in the main body of your email but if you have to use attachments (graphics, pp slides, etc)

   Don't forget to actually attach the file

   Name the file appropriately with your name, e.g. John_ Smith_homework.xyz, but not biosensor1.doc or hw.pdf

In conclusion

Today we

- Discussed whole cells biosensors
- Talked about some cool technology and applications
- Looked at sensors' performance characteristics

Any questions?

Have fun and see you next Thursday