Catalytic vs Affinity

Catalytic biosensors: kinetic devices that measure concentration of a formed/lost compound as a result of a biocatalytic reaction

What is monitored?	i) rate of product formation
	ii) disappearance of a reactant
	iii) inhibition of a reaction
Biocatalysts?	i) enzymes
	ii) microorganisms
	ii) organelles
	iv) tissue samples

Affinity biosensors: devices in which receptor molecules bind analyte molecules with a specific affinity causing a physicochemical change that is detected by a transducer

Receptor molecules:	i) antibodies
	ii) nucleic acids
	iii) hormone receptors

More generally:

Catalytic (Mostly Enzymatic)

The biological element converts the substrate into a product

$S \rightarrow P$

The transducer detects S or P

Affinity

The biological element binds specifically the analyte leading to a complex

$A + B \rightarrow AB$

The transducer reveals the complex

Catalytic Biosensors

i) enzymes (mostly)

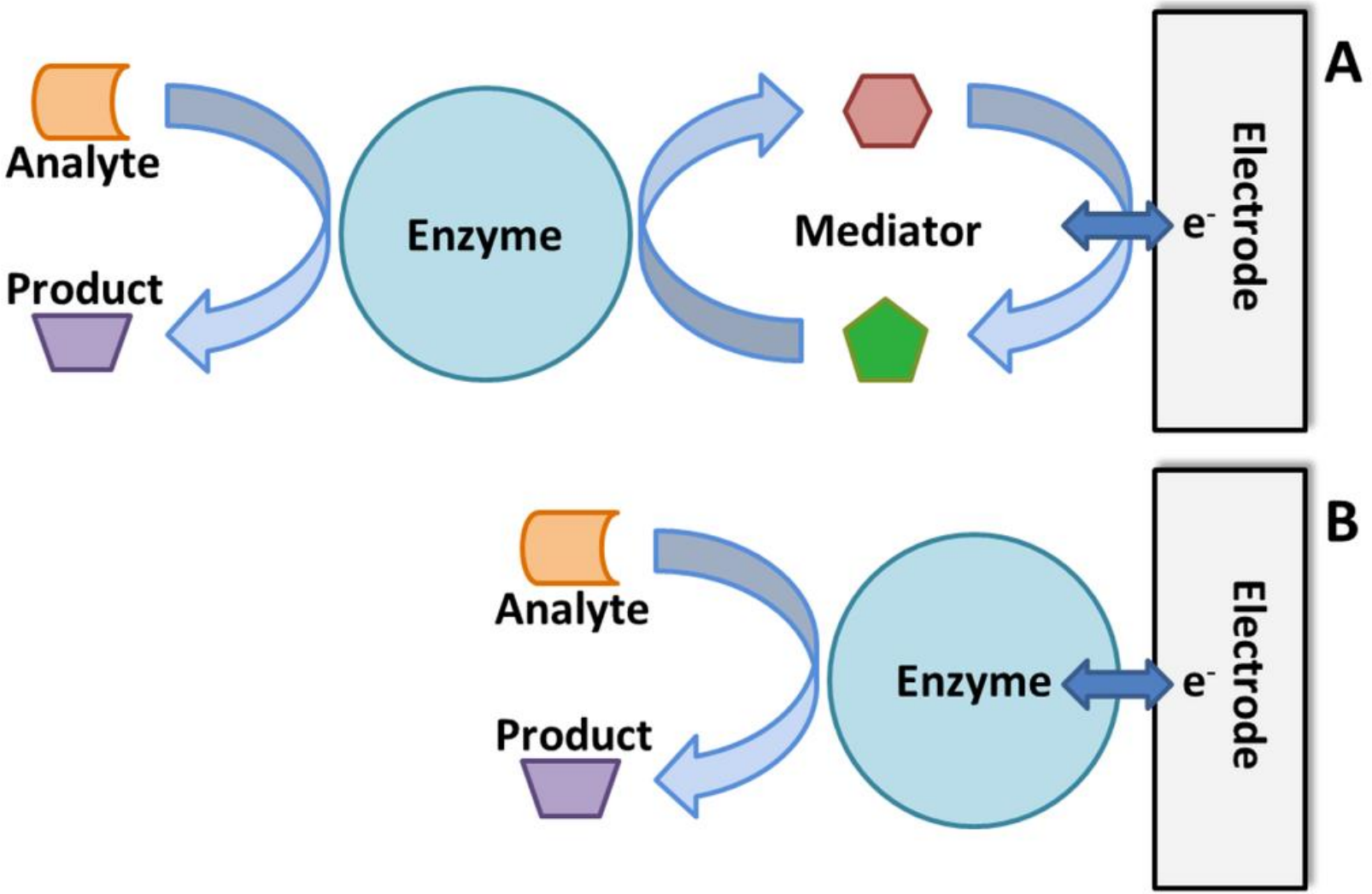
ii) whole cell biosensor (mostly m/o)

iii) organelles

iv) tissue samples

Toxicant detection via sentinel species. Coal miners formerly relied on caged canaries to detect occupationally hazardous levels of methane in mines. Canaries proved extremely sensitive and susceptible to methane, such that death of the canary alerted the miners to dangerous levels of methane.

enzymes (mostly)



Biosensors 2015, 5(3), 537-561; doi:10.3390/bios5030537

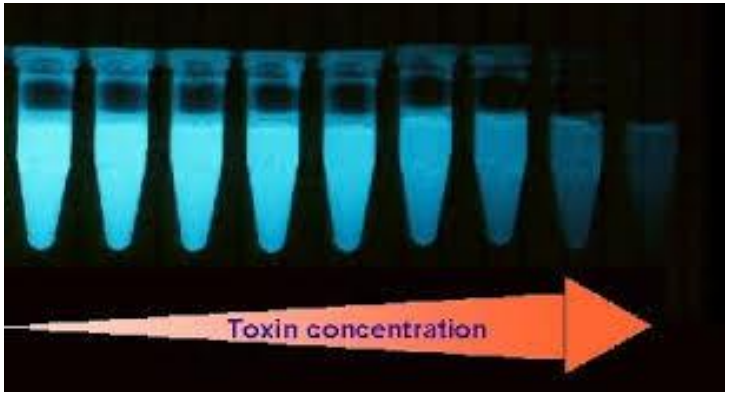
Ex: Enzymatic Glucose Sensors

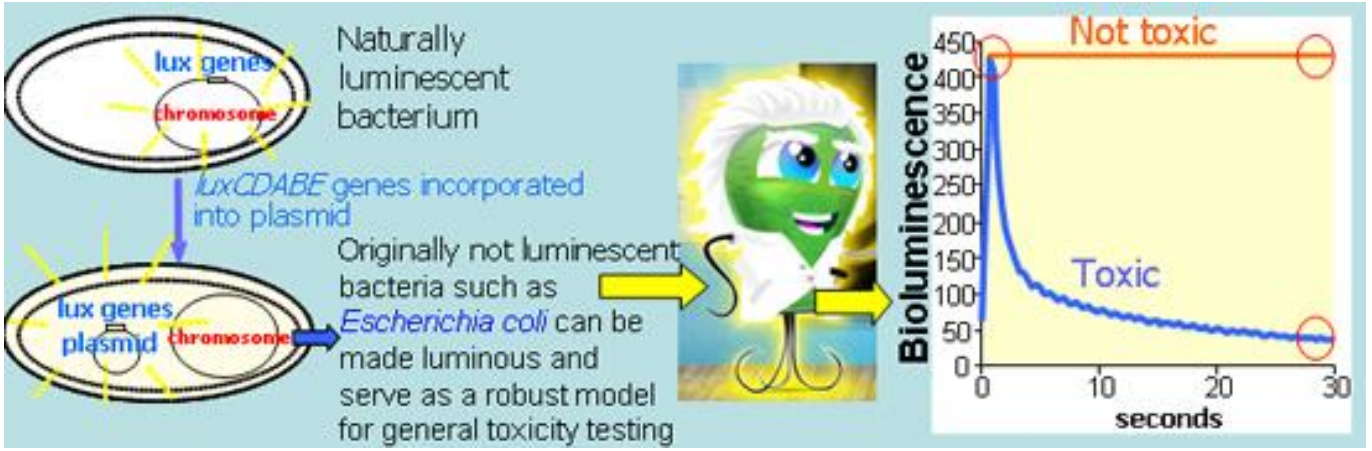
$$\text{GLUCOSE} + O_2 \xrightarrow[\text{Oxidase}]{\text{Glucose}} \text{GLUCONIC ACID} + H_2O_2$$

$$H_2O_2 \longrightarrow 2H^+ + O_2 + 2e^-$$

Whole Cell Biosensors

- Especially to demonstrate the toxicity of a variety of environmental media including soil, sediment, and water
- Genetically engineeried bacteria with a reporter gene that generates a signal with a contaminant-sensing component that responds to chemical or physical change, such as exposure to a specific analyte.





Whole Cell Biosensors

Such biosensors have been developed for various environmental contaminants (Review: *Su L., 2011*):

- heavy metals like arsenic, cadmium, mercury, and lead.
- pesticides
- various organo-phosphorous nerve agents
- mutagens and genotoxins
- general toxicity

A variety of well-characterized promoters are available for the construction of pollutant-reporting biosensors.

Promoter?

The Operon: a procaryote model

- series of genes and their shared regulatory elements

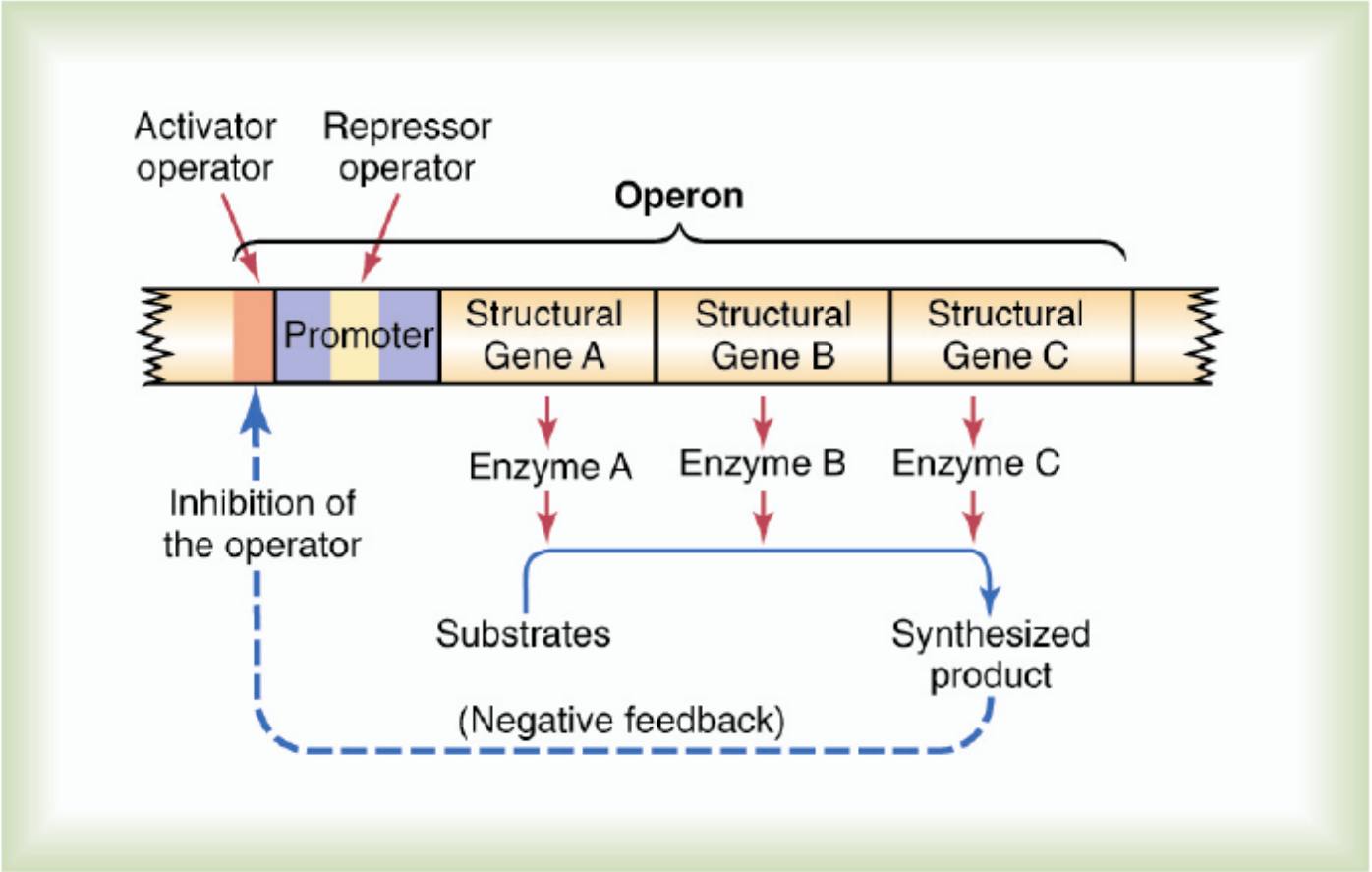


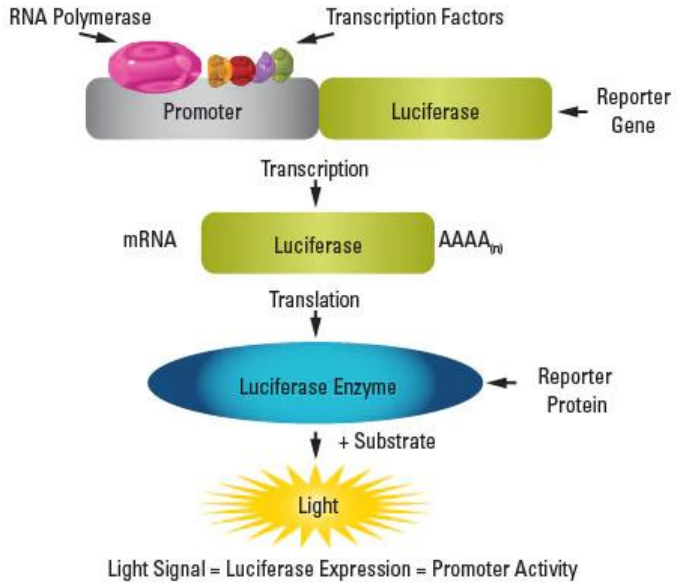
Figure 3-12 Function of an operon to control synthesis of a non protein intracellular product, such as an intracellular metabolic chemical. Note that the synthesized product exerts negative feedback to inhibit the function of the operon, in this way automatically controlling the concentration of the product itself.

Whole Cell Biosensors-Reporter Genes

Luciferase: The most commonly used reporter gene is the luc operon from the firefly.

- The luc operon produces the enzyme, luciferase, capable of generating the luminescence of the firefly.

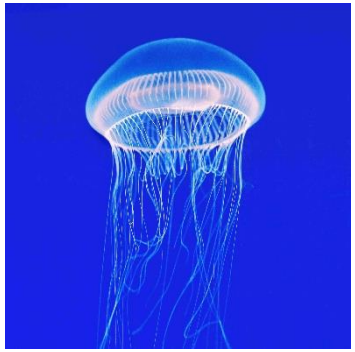
- This reporter gene has great versatility and can be mutated to produce enzymes that express a range of colors from green to red, which can then be independently controlled for multianalyte assays



Whole Cell Biosensors-Reporter Genes

Green fluorescent protein (GFP): from the jellyfish *Aequorea victoria*

- lower sensitivity compared to the luc operon
- Its stability and spectral properties could be altered to obtain mutant ones with superiour properties



A comparison: toluene* bacterial biosensors which comprised of two reporters, gfp and lux (bacterial):

- Luminescence biosensor allowed faster and more sensitive detection of toluene while the fluorescence biosensor strain was much more stable and thus more applicable for long-term exposure. (Li et al., 2008)

*

- Toluene is an aromatic hydrocarbon
- it is a colorless, water-insoluble liquid with the smell associated with paint thinners
- it can cause mild damage to the skin and the eyes
- Products containing toluene can produce dangerous fumes which can cause nausea, headaches, unconsciousness, and even death if inhaled

Whole-cell Biosensors

An example from EPA

Arsenic Contamination

Challange: Current risk assessments for evaluating the potential ecological or human health risks associated with arsenic exposure* rely on an analysis of the total arsenic content. BUT

- Methods for arsenic speciation offer a powerful analysis but require expensive, bulky equipment, high-grade analytical reagents, and specialized training

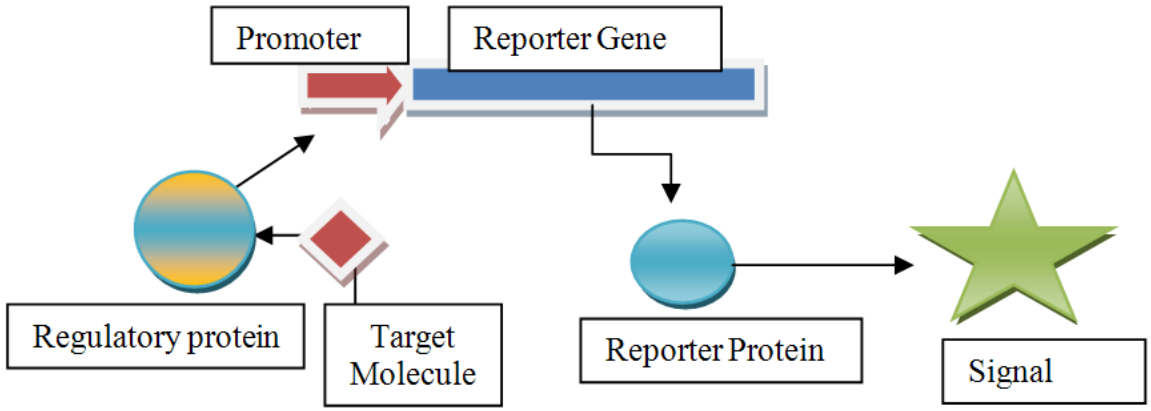
An Alternative: Genetically engineered bacteria that illuminates in the presence of bioavailable arsenic. These whole-cell bacterial biosensors are being developed to detect arsenic and provide at least qualitative information about the bioavailability of arsenic in the contaminated soil or water

* Humans may be exposed to arsenic (As) through food, water and air, and it may cause
  - Acute As poisoning
  - Nausea
  - Vomiting
  - Blood in the urine
  - Cramping muscle
  - Hair loss
  - Stomach pain
  - Organ failure

Whole-cell Biosensors

An example from EPA

- Arsenic resistance gene remains inactive with the absence of As(III) in the cell due to the binding of the ars operon repressor protein to the promoter region of the gene.
- As(III) activates the system by binding to the repressor protein and freeing the promoter region for transcription



Liu, X et al, Sensors, 2010

Whole-cell biosensors: Pros and Cons

Advantages:

- Inexpensive
- Produces real-time data
- Less labor intensive
- Suitable for field work

Disadvantages:

- Limited availability of strong promoters that respond only to relevant stimuli
- Short lifetime
- Limited understanding of applicability to higher organisms
- Performance dependent on environment of procedure

Tissue-based biosensors-*mammalian cells*

- Current technologies that detect and/or identify biological and chemical warfare (BCW) agents, environmental toxins and pollutants rely on molecular recognition strategies that detect only anticipated or known agents.
- Highly specific for limited sets of targets but provide no information as to the physiological effects of the materials

➢ **Detection of active compounds using excitable cells: Neuronal physiological cells and cardiomyocytes**

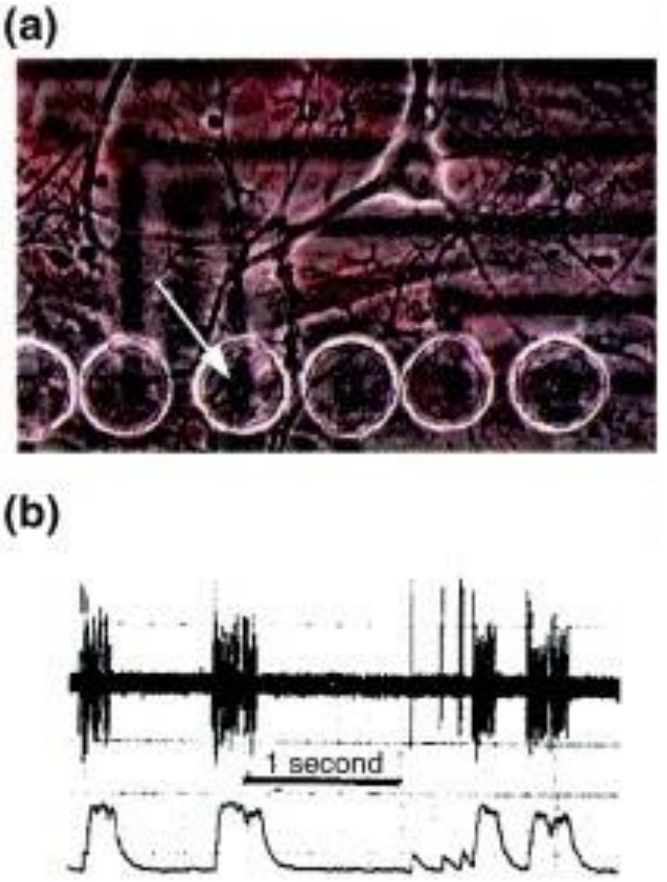
- ➢ Generate electric signals in a substance-specific and concentration-dependent manner
- ➢ Signals generated can be monitored by microelectrodes

Tissue-based biosensors-*Neural cells-1*

Neurotoxicant detection and classification using cultured neural networks.

(a) Mouse spinal cord neurons growing on a microelectrode array: Six microelectrode recording sites

(b) A comparison of action potentials (top) and associated 'bursts' (bottom), representing the collective behavior of a group of action potentials.



Stenger et al, 2001

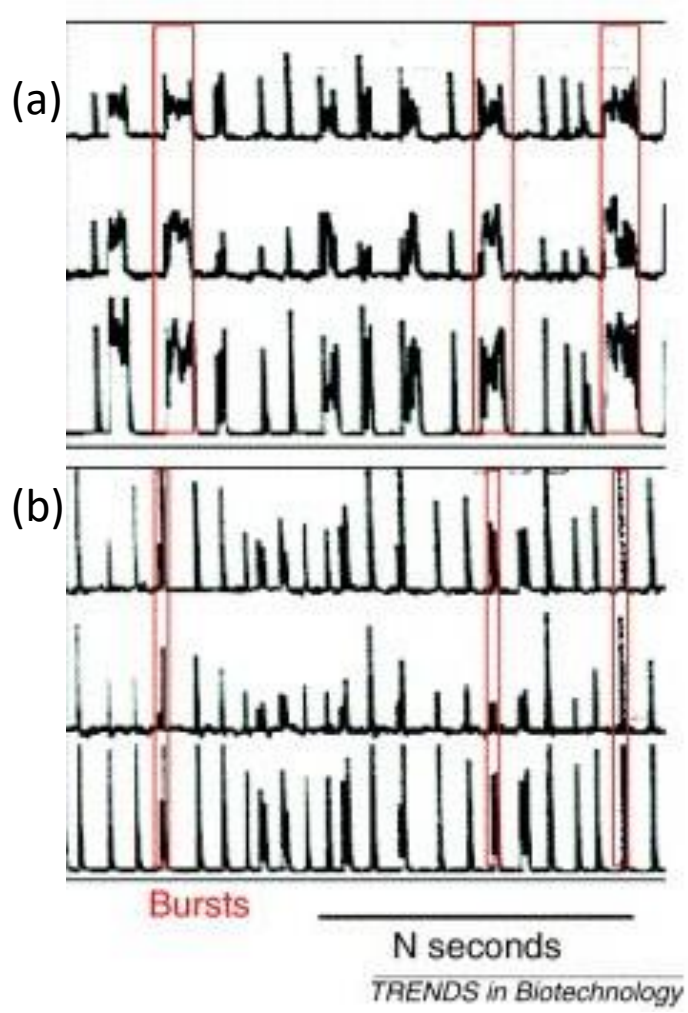
Tissue-based biosensors-*Neural cells-2*

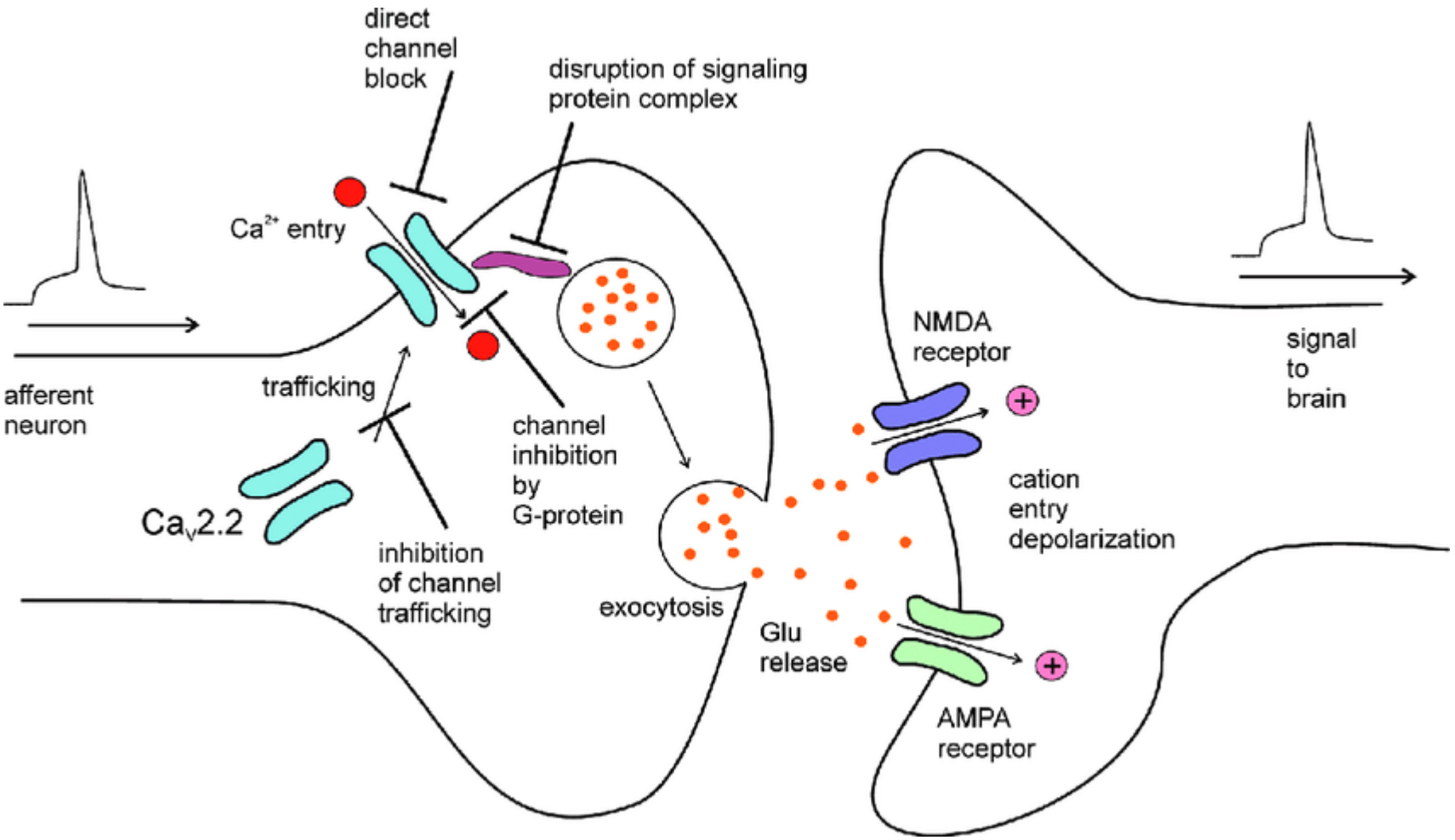
Burst profiles from three representative microelectrode array channels from a mouse frontal cortex culture;

(a) baseline activity

(b) modified activity following perfusion of trimethylolpropane phosphate (TMPP)

CONCLUSION: exposure to TMPP elicits an increase in burst frequency and amplitude, a decrease in burst duration and enhanced synchronization of bursts among channels. These observations are indicative of the disruption of inhibitory synaptic transmission.





Synaptic transmission

Tissue-based biosensors

Mushroom or plant tissue

For stable, low cost biosensors

- biosensor for phenol detection using lyophilized mushroom tissue (cube or powder) as the enzyme (tyrosinase) source (*Silva et al, 2014)*
- Mushroom homogenate as alcohol oxidase source for ethanol detection *(Akyılmaz & Dinçkaya, 2000)*
- Various plant tissue-based bioelectrodes were constructed for hydrogen peroxide (H_2O_2) monitoring. Examples are bioelectrodes based on grape tissue, horseradish root, pineapple, zucchini, asparagus, sweet potato etc. (*S Liawruangrath et al, 2001*)

Tissue-based biosensors-*Asparagus tissue-1*

FACTS

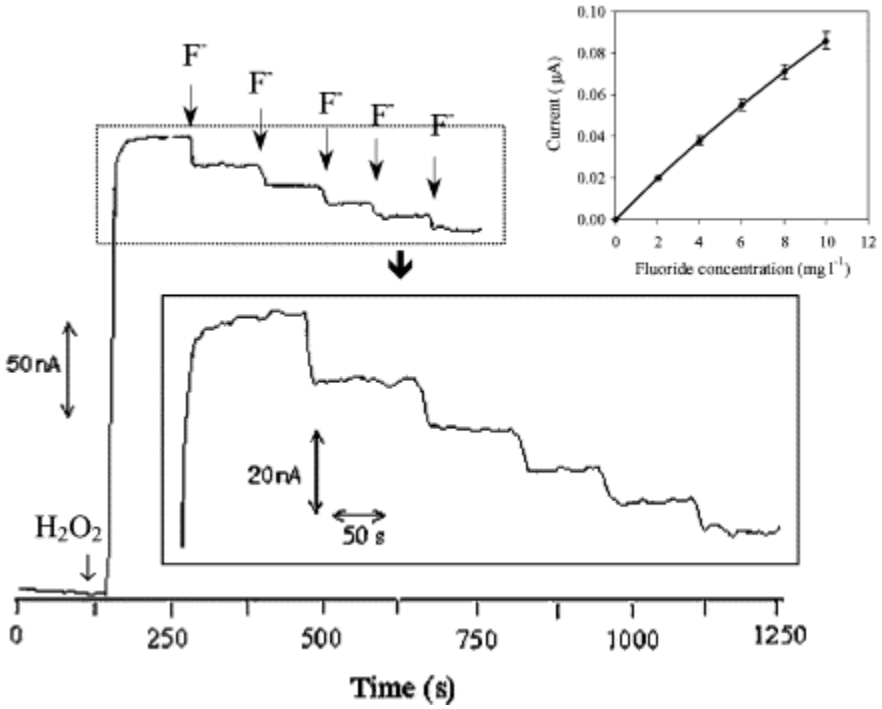
- Fluoride has beneficial effects on teeth and on bone structure in humans, at low levels. BUT moderate or high levels of fluoride in water cause toxicity and mottled teeth in humans.
- Fluoride is a peroxidase inhibitor
- The stem skin of asparagus has been reported to be rich in peroxidase activity but the enzyme from this plant is not yet commercially available.

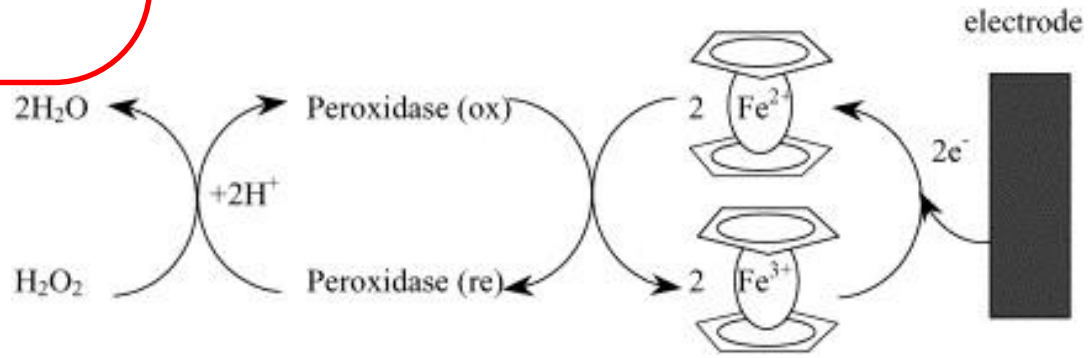
MeThoD of DETECTION

Determination of fluoride is based on the decrease in response of a peroxidase-modified electrode to H_2O_2 upon introduction of fluoride

Tissue-based biosensors-*Asparagus tissue-2*

- Ferrocene-modified carbon paste was prepared using graphite powder.
- This paste was mixed with ground tissue from the stem skin of asparagus stems.
- The peroxidase (re) reduces H_2O_2 to water, forming the oxidized species which then oxidizes the ferrocene used as a mediator to the ferricinium ion.
- The ferricinium ion is subsequently reduced back to ferrocene by rapid reaction involving the acceptance of an electron from the electrode





Tissue-based biosensors-Pros&Cons

☺ Cell-based sensors may utilize the ability of cells to respond to complex mixtures of signals in a unique way

☺ Receptors, channels, and enzymes maintained in a physiologically-relevant state by the machinery of the cell

☺ May provide alternatives to animal testing in the future

☹ Issues of maintaining cell viability and reproducibility in measurements

☹ Issues of cell sources

☹ Often require primary cells in current systems

Organelle biosensors??

Mitochondria is a critical target in the toxicity of a wide variety of agents, ranging from clinically-relevant drugs, to environmental poisons.

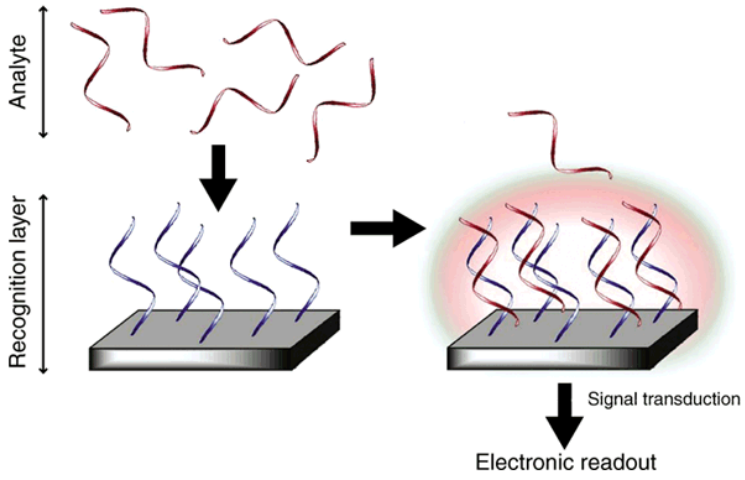
- Anthracyclines (eg Doxorubicin), important in cancer therapy BUT significant side effects, including cardiotoxicity: More evidence suggests that mitochondria is a critical target in the development of DOX-induced cardiomyopathy
- Nucleoside reverse transcriptase inhibitors (NRTIs), a class of anti-retroviral drugs, are specifically prescribed for AIDS. Several studies indicate that these drugs induce mitochondrial toxicity by interfering with mitochondrial DNA (mtDNA) synthesis
- Others: anti-diabetic drugs, anti-depressants, statins, environmental pollutants
- Mitochondria as a biosensor for drug-induced toxicity...

Affinity Biosensors

Based on Hybridization:

Target: DNA or RNA sequences

Probe: DNA oligonucleotides, PNAs

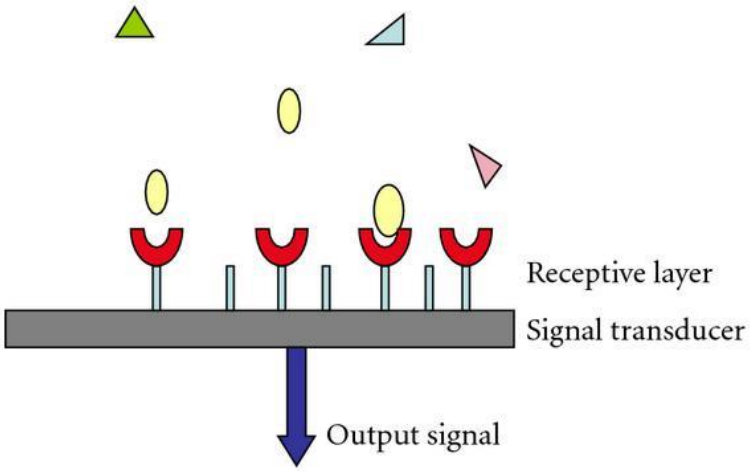


Based on Interaction:

Immuno- and apta- sensors or receptors

Target: hormones, proteins, toxins, vitamins, drugs, etc

Probe: Antibodies (Ab) and/or Aptamers; Receptors

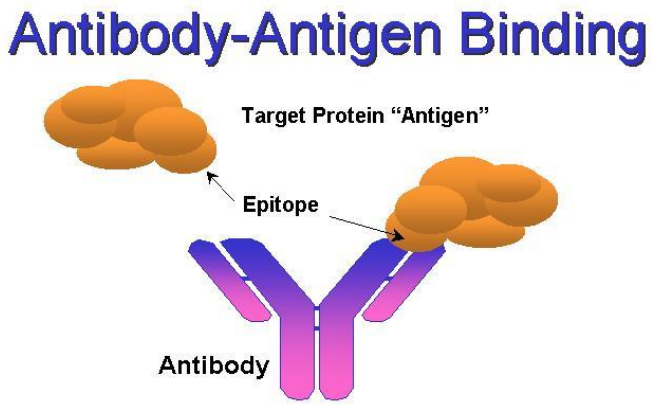


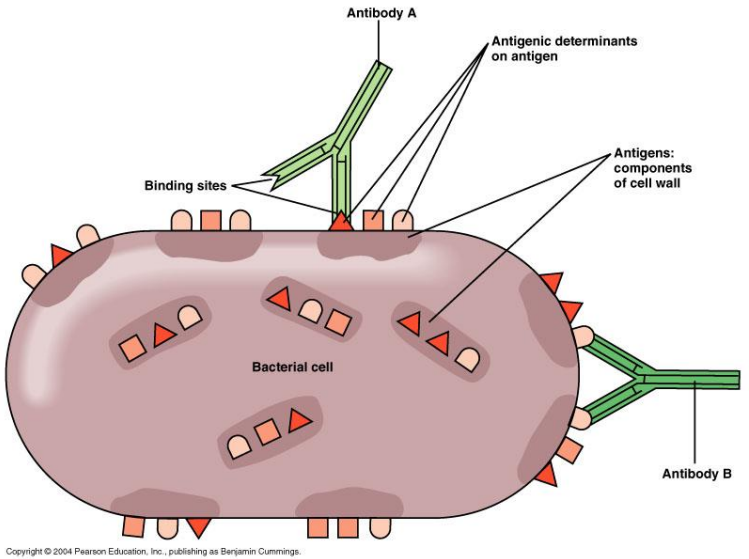
Affinity Biosensors-*Antibodies*

Basic Facts:

- Antigen: Chemical substances that demonstrate immunogenicity, stimulate formation of antibodies
- Most antigens are proteins
- Antigenic determinant groups (epitopes) - the specific regions on an antigen recognized by specific antibodies

➢ Specific interaction….





Affinity Biosensors-*Immunosensors*

- Immunoassay “Immuno” refers to an immune response that causes the body to generate antibodies.
- “Assay” refers to a test.
- Immunoassays are based on the ability of an antibody to recognize and selectively bind to an antigen, referred to as the analyte
- The high degree of selectivity and specificity of an antigen–antibody binding makes immunoassays the gold standard technique for the detection of presence, and measurement of the concentration of the analyte of interest
- Immunoassays may measure either the antigen or antibody.

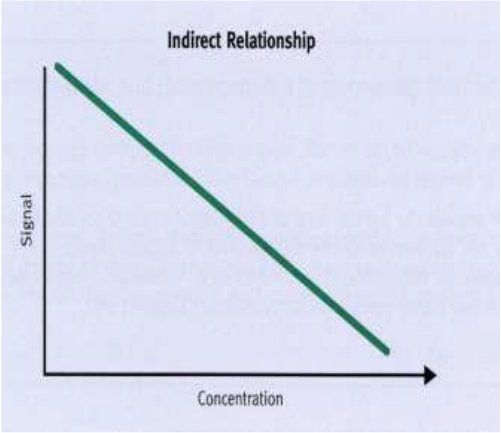
Affinity Biosensors-*Immunosensors*

Immunoassay techniques

- **Competitive assay**
- **Non-competitive (sandwich) assay**

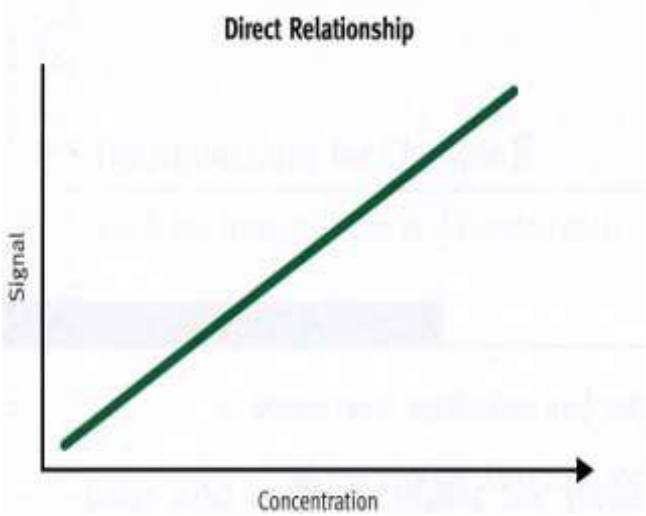
All immunoassays require the use of labeled material in order to measure the amount of antigen or antibody present.

- In a competitive format, unlabeled analyte in the test sample is measured by its ability to compete with the labeled antigen in the immunoassay.
- less label measured in the assay means more of the unlabeled (test sample) antigen is present.



Affinity Biosensors-*Immunosensors*

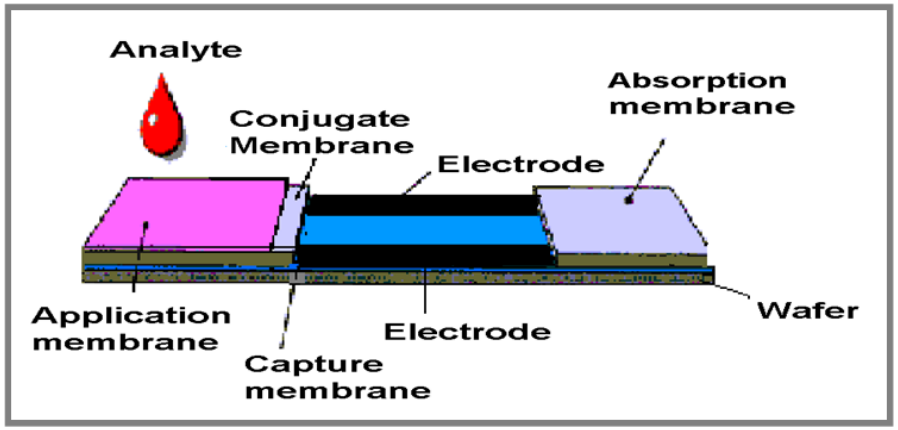
- Noncompetitive assay formats give the highest level of sensitivity and specificity.
- the measurement of the labeled analyte (usually the antibody) is directly proportional to the amount of antigen present in the sample



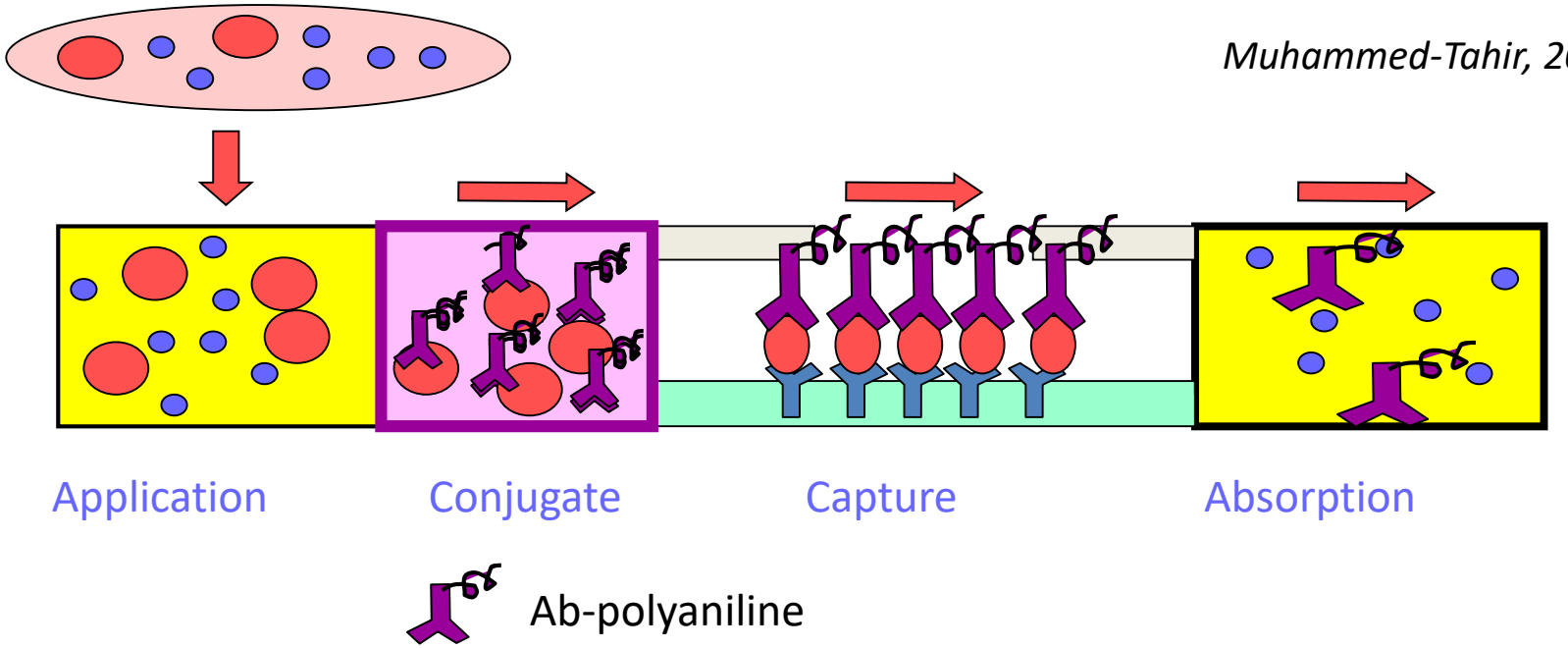
Affinity Biosensors-*Immunosensors*

Application in Food Industry

- Foodborn pathogen detection: in 6 min with high sensitivity
- Mycotoxins (*Vidal, 2013*)
- Pesticide residues



Muhammed-Tahir, 2002



Affinity Biosensors-*Immunosensors*

Medical Field:

- *Disease Biomarkers*: indicators of normal or disease processes and pharmacologic responses to therapeutic intervention
- Serum concentration of protein cancer biomarkers increases at the onset of cancer, even before tumor develops

➢ Cheap, simple, fast, accurate, multiplex point-of-care (POC) clinical assays are needed

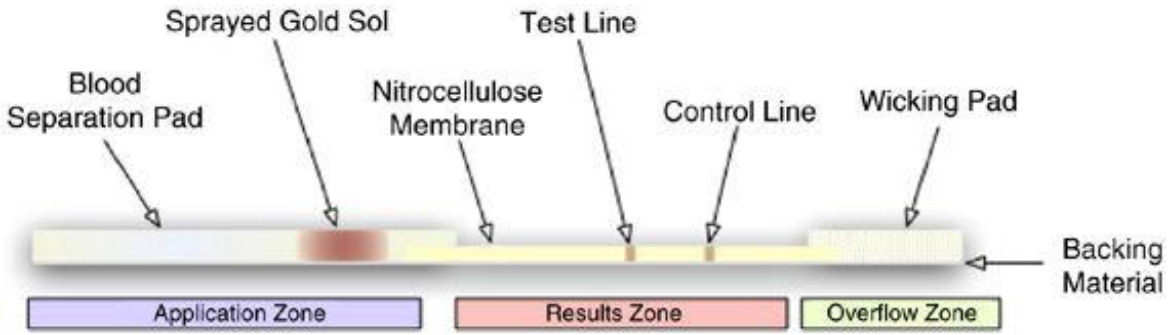
E.g. Prostate Specific Antigen

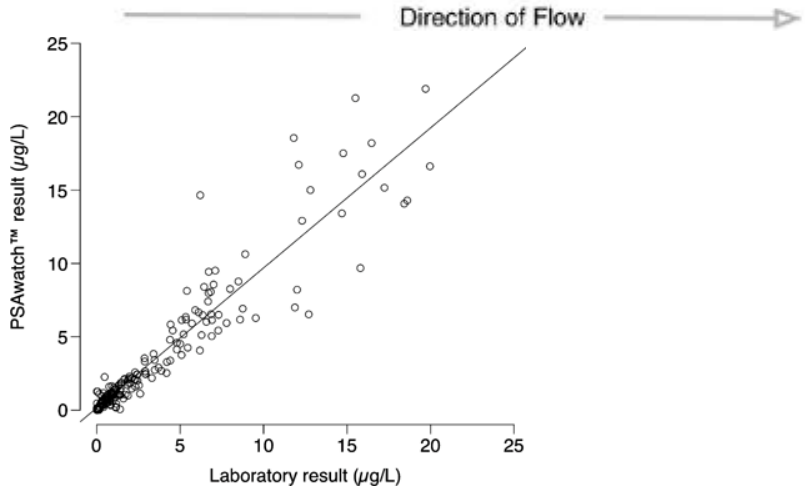
Original Article

Prostate Cancer and Prostatic Diseases (2007) **10,** 270–273; doi:10.1038/sj.pcan.4500962; published online 13 March 2007

Point-of-care PSA testing: an evaluation of PSAwatch

- PSA is a 32-kDa single-chain glycoprotein serine protease produced by the secretory epithelium of the prostate gland.
- PSA is the gold standard for the early detection and surveillance of carcinoma of the prostate (CaP)



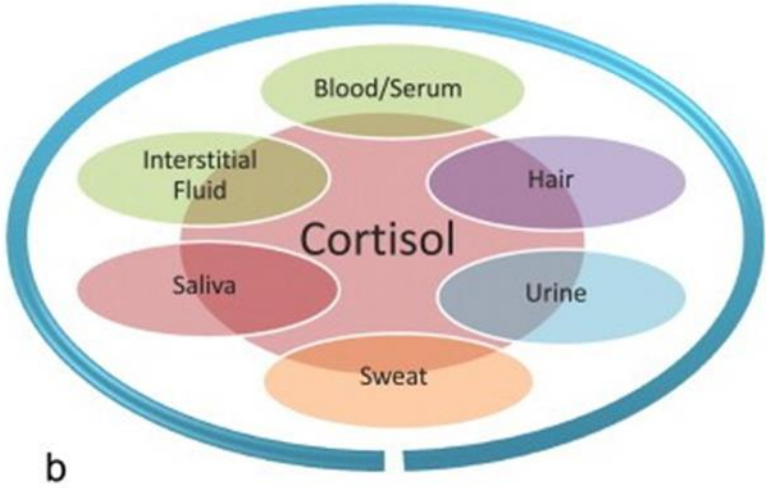


- a novel, quantitative, point-of-care PSA assay.
- PSAwatch has been specifically developed to work with no more than a drop of whole blood (35 ul).

Affinity Biosensors-*Immunosensors*

Medical Field: Cortisol Levels *(Kaushik, 2014)*

- Prolonged exposure to stress leads to the activation of signaling pathways from the brain that leads to release of cortisol.
- Various biomarkers have been affected by psychological stress, but cortisol is known as a potential biomarker.
- Cortisol secretion of individuals depends on day–night cycle and field environment hence its detection at point-of-care (POC) is essential to provide personalized healthcare.



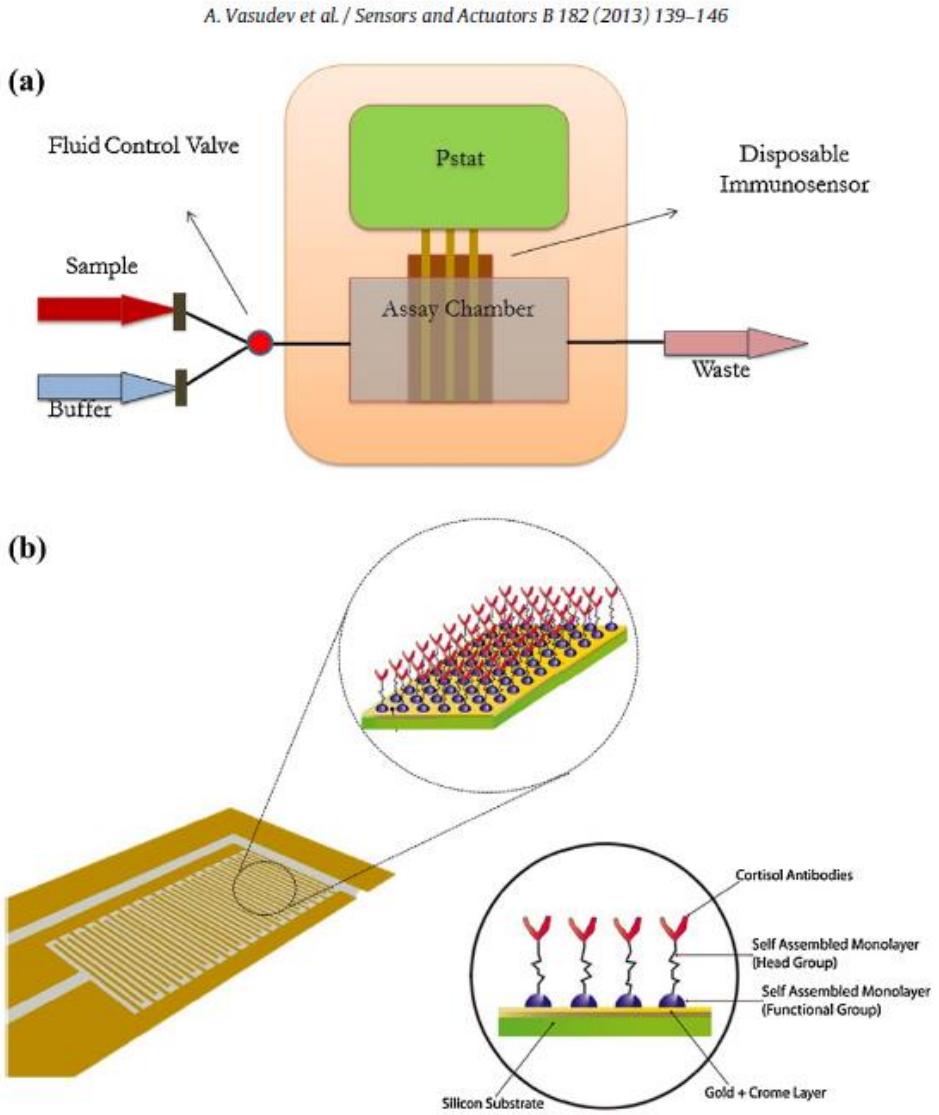
↑ cortisol levels inhibits inflammation, depresses immune system, increases fatty and amino acid levels in blood. It contributes to the development of Cushing's disease with the symptoms of obesity, fatigue and bone fragility

↓ cortisol levels lead to weight loss, fatigue, and darkening of skin folds and scars

Affinity Biosensors-*Immunosensors*

Medical Field: Cortisol Levels *(Kaushik, 2014; Vasudev, 2013)*

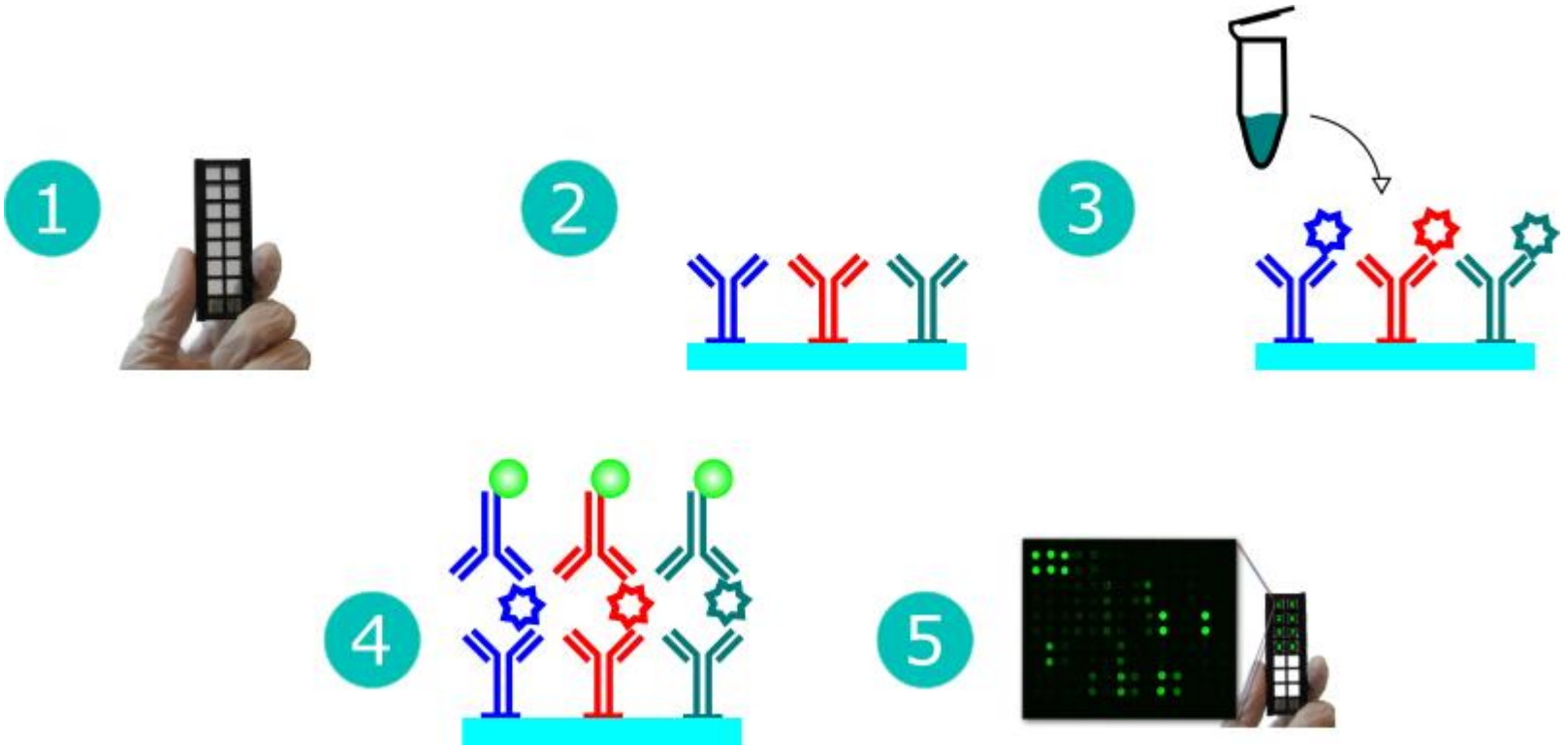
- Recently, miniaturized sensing devices have been explored in order to decrease the probability of human error and the sample volume required
- ➢ microfluidic systems: small sample volumes, precise control of fluidic routines, repeatable sensing protocols, controlled environment for biomolecule reaction, and application at point of care.



Affinity Biosensors-*Immunosensors*

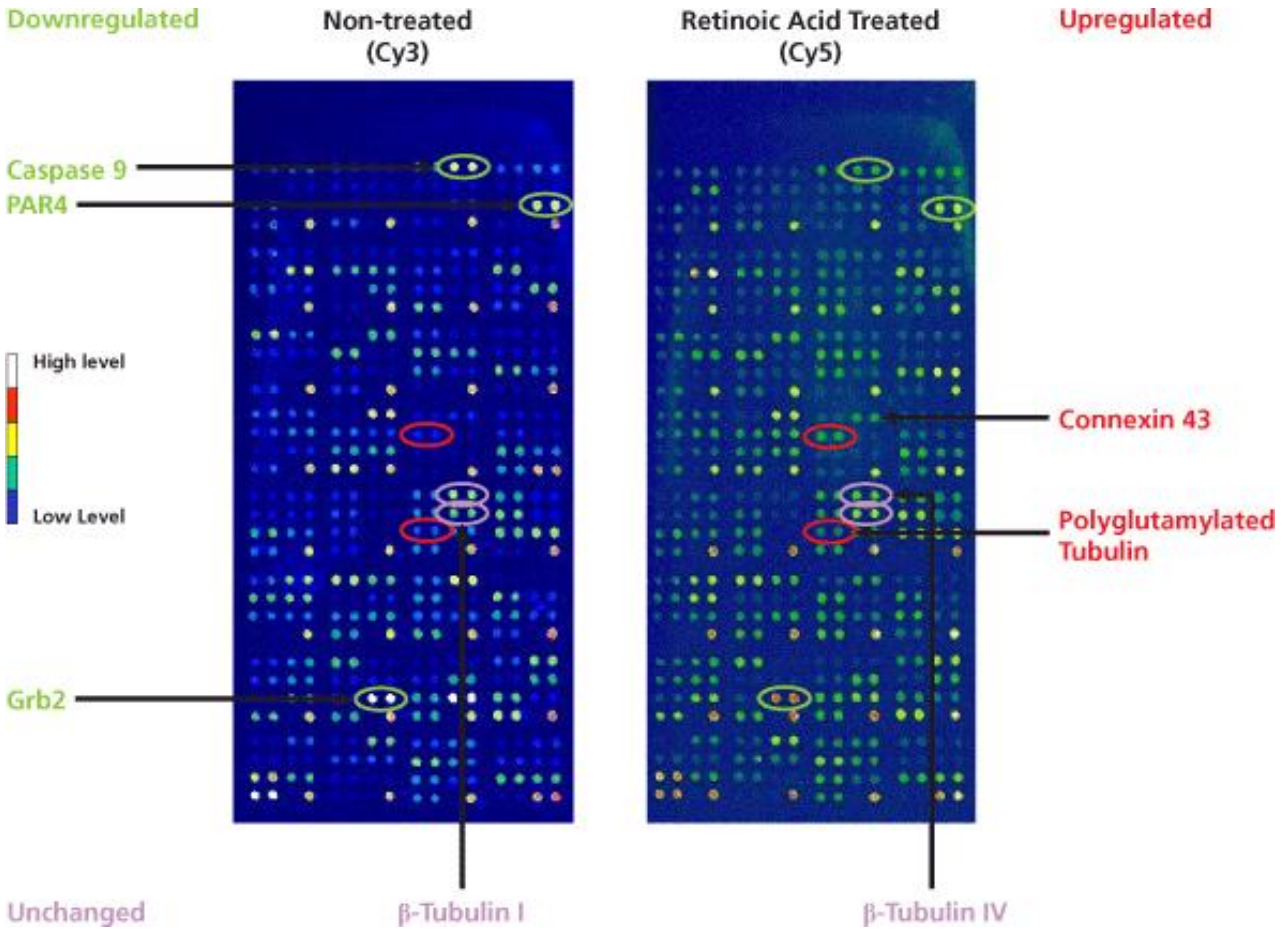
Antibody Arrays

a collection of capture antibodies are spotted and fixed on a solid surface such as glass, plastic, membrane, or silicon chip, and the interaction between the antibody and its target antigen is detected



Affinity Biosensors-*Immunosensors*

- A commercial example: Panorama Antibody Array Technology

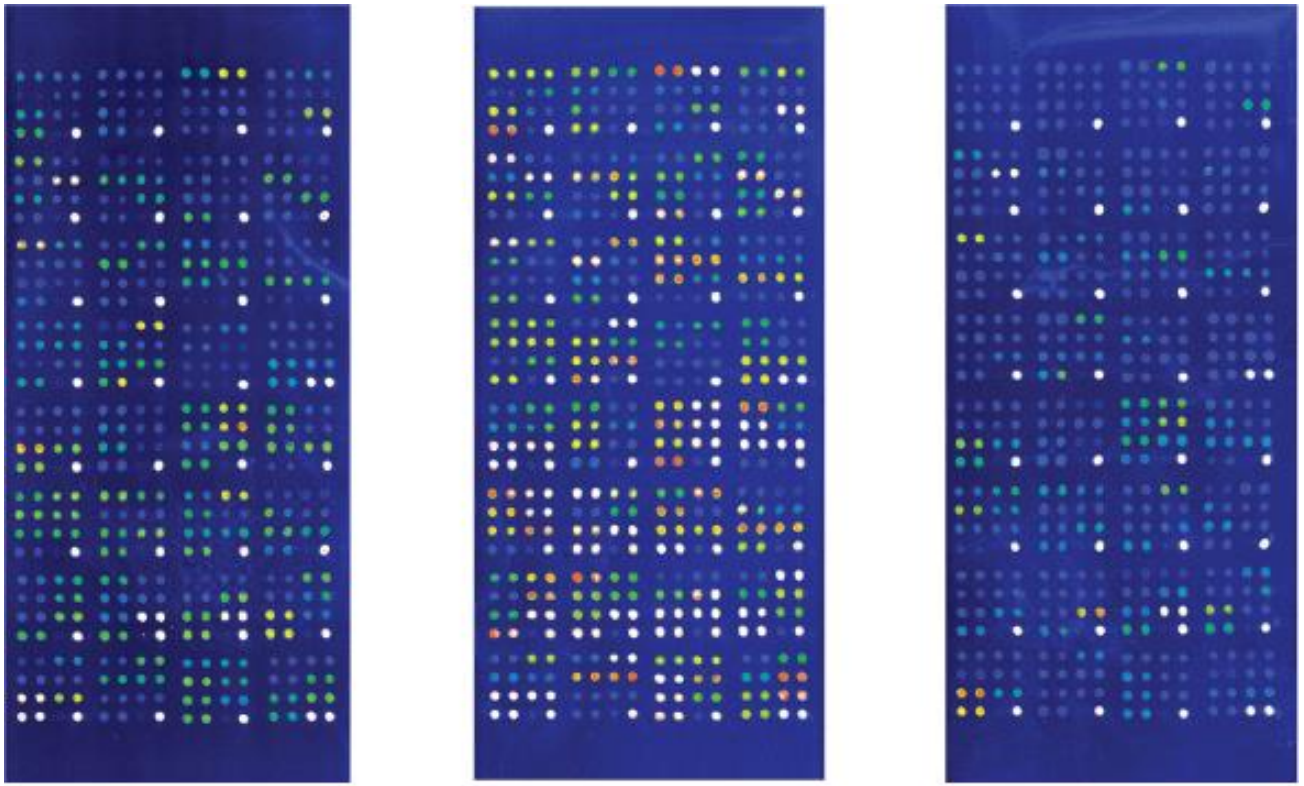


Protein Expression in F9 Mouse Stem Cells Induced by Retinoic Acid

Affinity Biosensors-*Immunosensors*

Differential Expression between Different Tissue Type

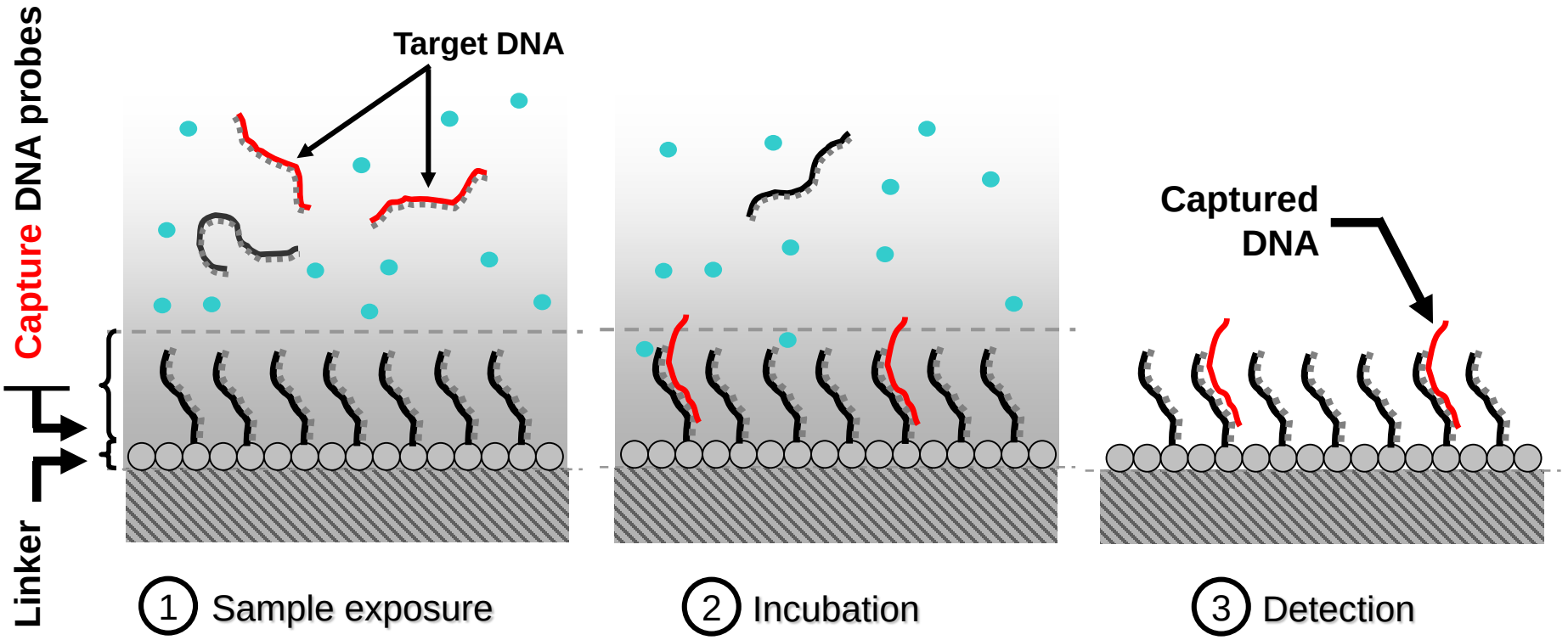
See more at: http://www.sigmaaldrich.com/life-science/cell-biology/protein-arrays/antibody-arrays.html#sthash.3fO6wTQM.dpuf



Affinity Biosensors-*DNA biosensors*

Basic Facts:

- DNA structure - double helix (complementary)
- Hydrogen bonding between base pairs – reversible
- Hybridization based on perfect match vs one or more mismatches



Affinity Biosensors-*DNA biosensors*

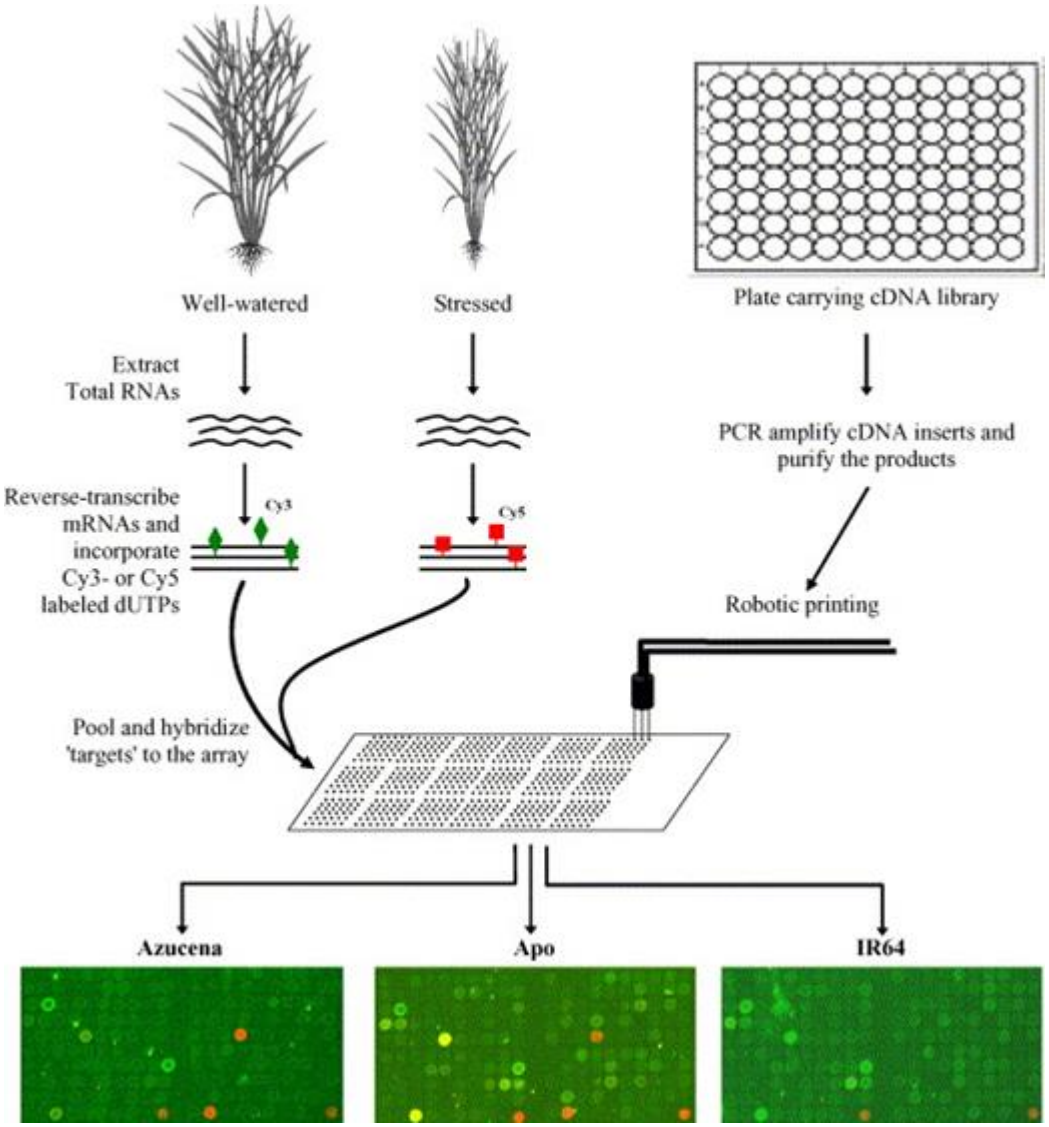
Applications

- Gene expression (Measuring protein levels would be more direct but more difficult).
  - Usually looking for RNA expression
  - Differences between cells and tissues
  - Differences in time
  - Expression during development
  - Differences between normal vs. diseased states
  - Analyze response of cells exposed to drugs or different physiological conditions
- Polymorphisms
- Diagnosis: chips have been designed to detect mutations in p53, HIV, and the breast cancer gene BRCA-1
- GMO Analysis

Affinity Biosensors-*DNA biosensors*

DNA Biosensors in gene expression

- Cells are different because of differential gene expression
- DNA → mRNA → protein
- Microarrays measure the level of mRNA expression by analyzing cDNA binding



Affinity Biosensors-*PNA biosensors*

Peptide nucleic acids (PNAs)

- PNAs are DNA mimics in which a peptide-like repeat of (2-aminoethyl) glycine unit
- Bind to their complementary nucleic acid sequences with higher thermal stability and specificity than the corresponding deoxyribooligonucleotides
- However, they are more destabilized by single-base mismatches than are DNA/DNA hybrids

➢ PNA biosensors in stead of DNA sensors for higher selectivity