Enzyme Biosensors

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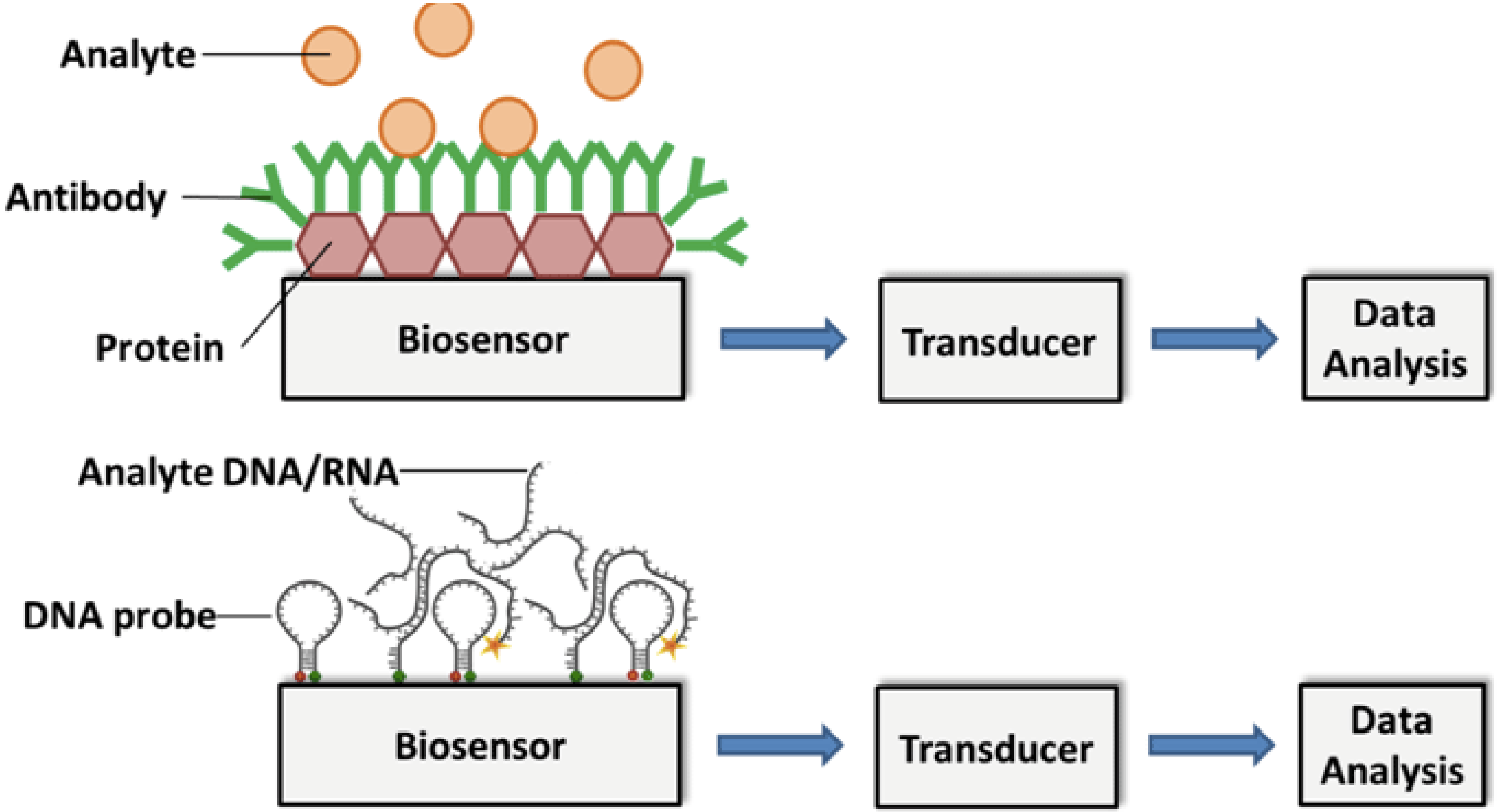
Brief history of Biosensors

- 1916 First report on immobilization of proteins : adsorption of invertase on activated charcoal
- 1922 First glass pH electrode
- 1956 Clark published his definitive paper on the oxygen electrode.
- 1962 First description of a biosensor: an amperometric enzyme electrodre for glucose (Clark)
- 1969 Guilbault and Montalvo – First potentiometric biosensor:urease immobilized on an ammonia electrode to detect urea
- 1970 Bergveld – ion selective Field Effect Transistor (ISFET)
- 1975 Lubbers and Opitz described a fibre-optic sensor with immobilised indicator to measure carbon dioxide or oxygen.

Structure of a Biosensor



A biosensor is an analytical device which is used to determine the presence and concentration of a specific substance in a biological analyte

http://dx.doi.org/10.15226/2471-3627/4/1/00116

What are different types of Biomarker ?

Samples :

1. Blood
2. Urine
3. Fecal
4. Nasal swab
5. Body fluids
6. Tissue samples
7. Environmental sample
8. Breath
9. Food

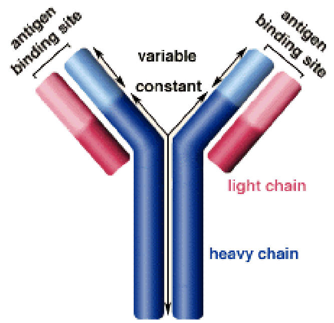
What could be biomarkers??

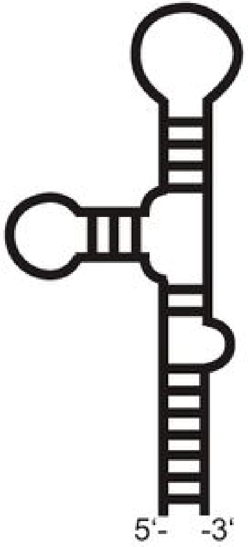
1. Protein
2. DNA/RNA
3. Lipids
4. Metabolites
5. Ions/metals

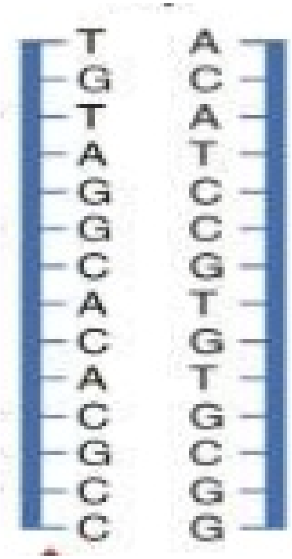
Recognizing element

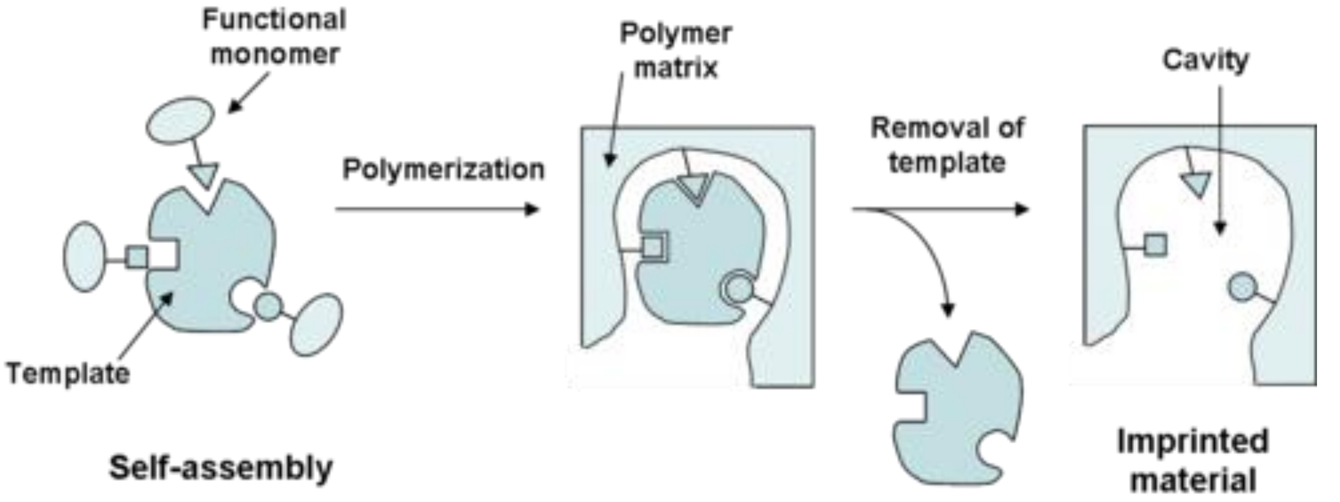
Bioreceptor

- Antibody
- Enzyme
- Nucleic Acid (DNA)
- Cell
- MIP
- ⋮

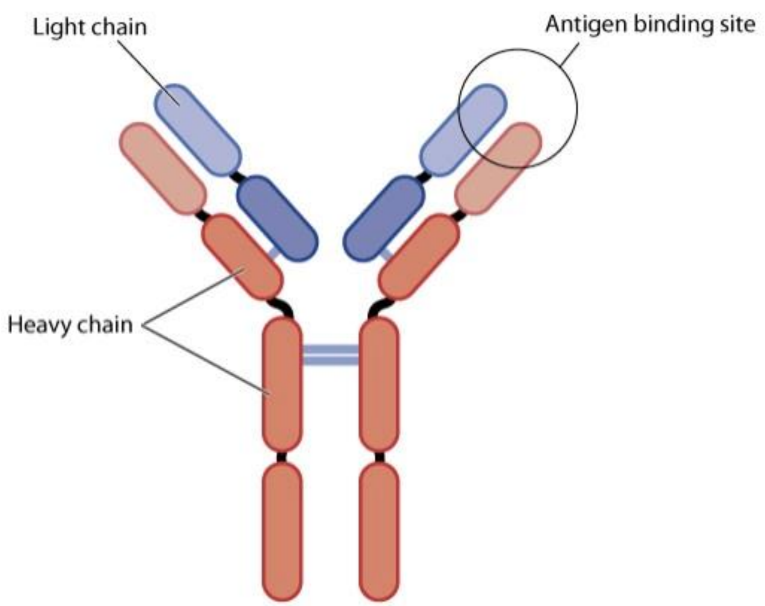




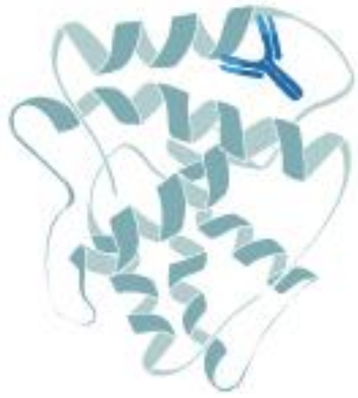




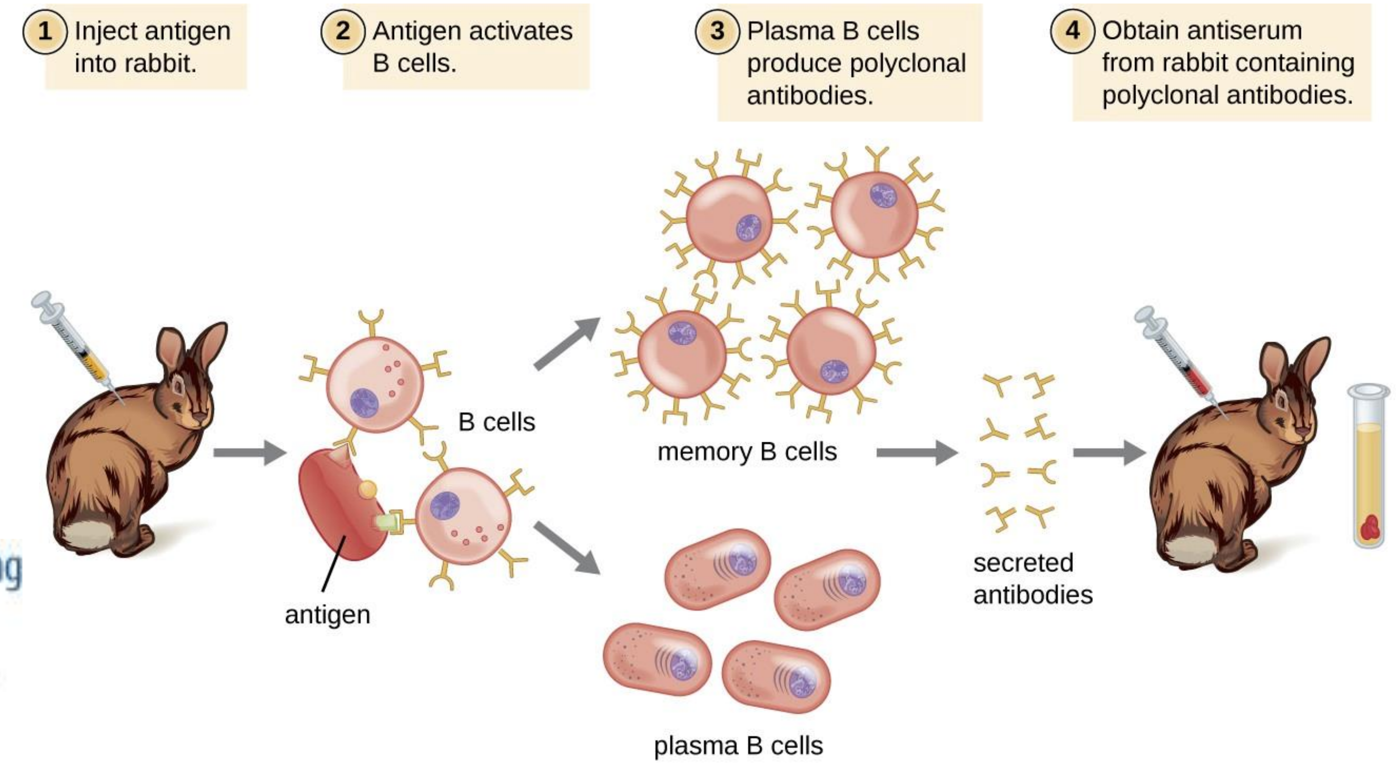
Bioreceptor: Antibody



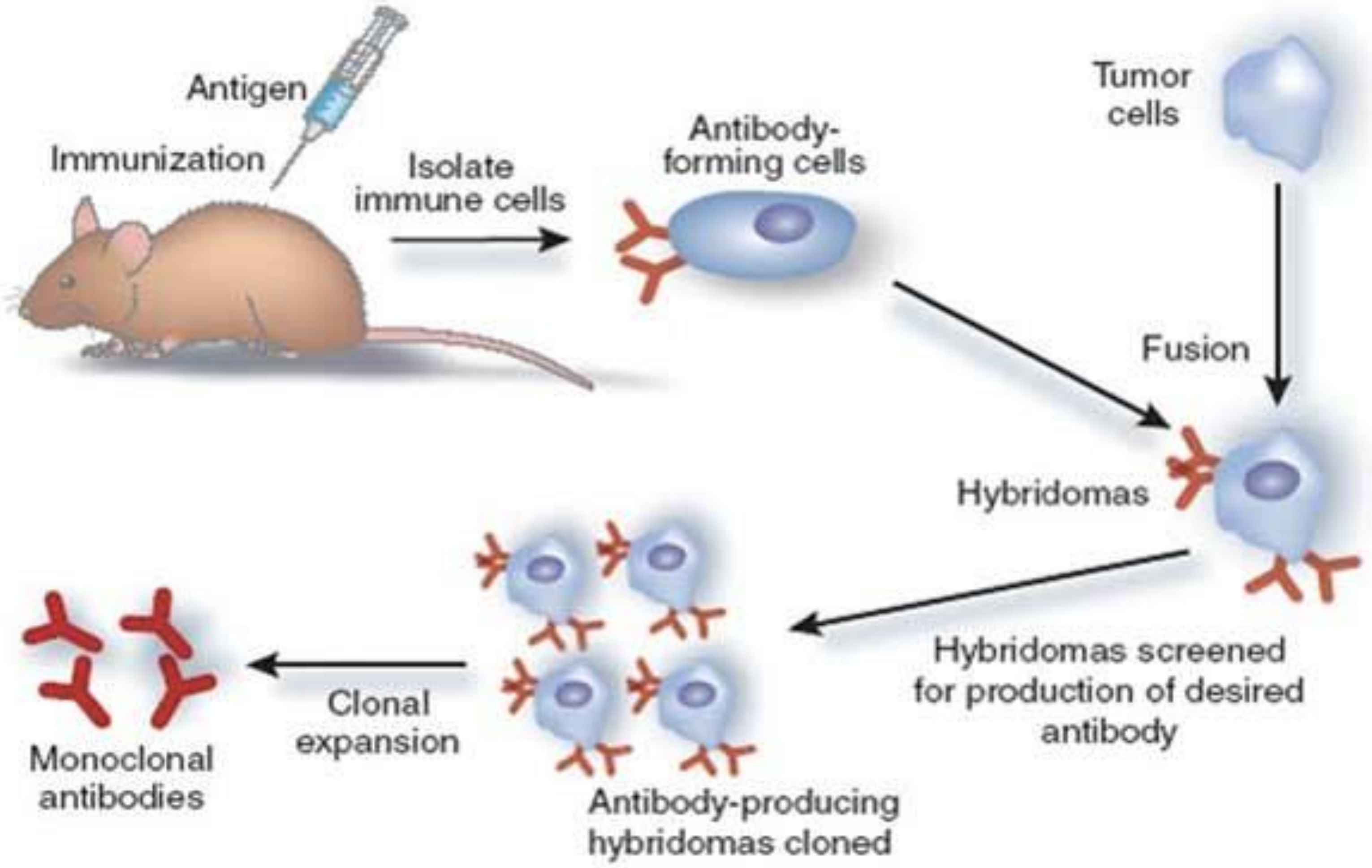
Monoclonal Antibody Binding



Polyclonal Antibody Binding

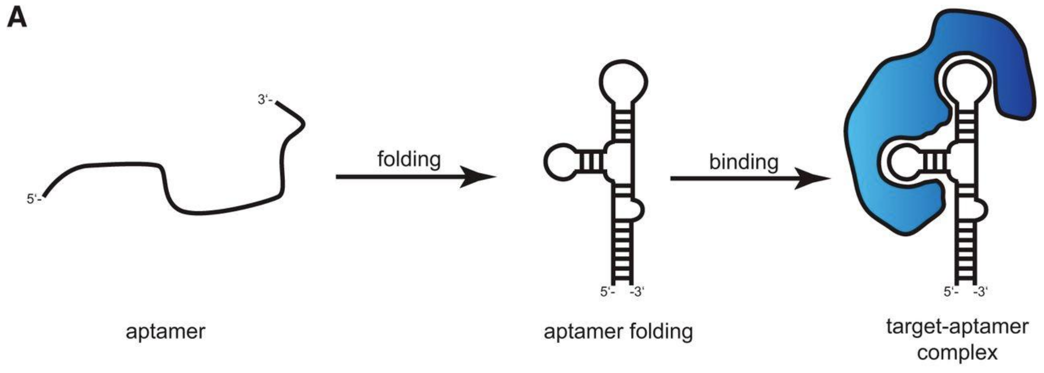


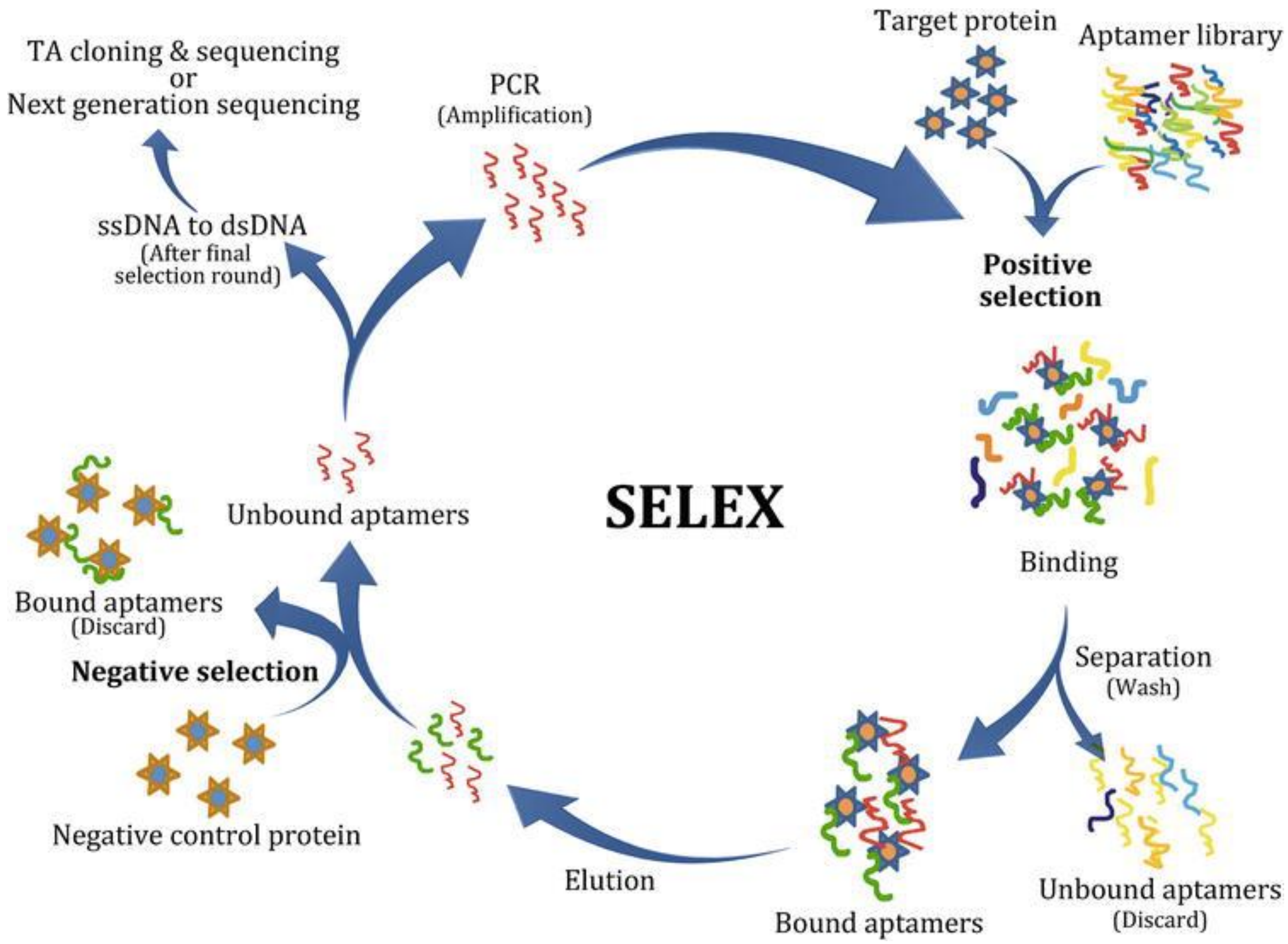
Bioreceptor: Monoclonal Antibody



https://microbenotes.com/monoclonal-antibodies-types-uses-and-limitations/

Bioreceptor: Aptamers





https://doi.org/10.1523/JNEUROSCI.1969-16.2017

https://doi.org/10.1007/s00216-007-1346-4

Course evaluation plan

So far….

Minor 1 - 20 marks
Quiz - 10 marks
Lab components - 10 marks

Lecture plan:

Biosensor complete – 13th June
Bio fuels complete - 20th June (2 lectures)
Techno-commercial – 27th June (2 lectures)

For graduating students

Do it from home Minor 2 – 15 marks

Oral viva - 10 marks

Lab notebook - 10 marks

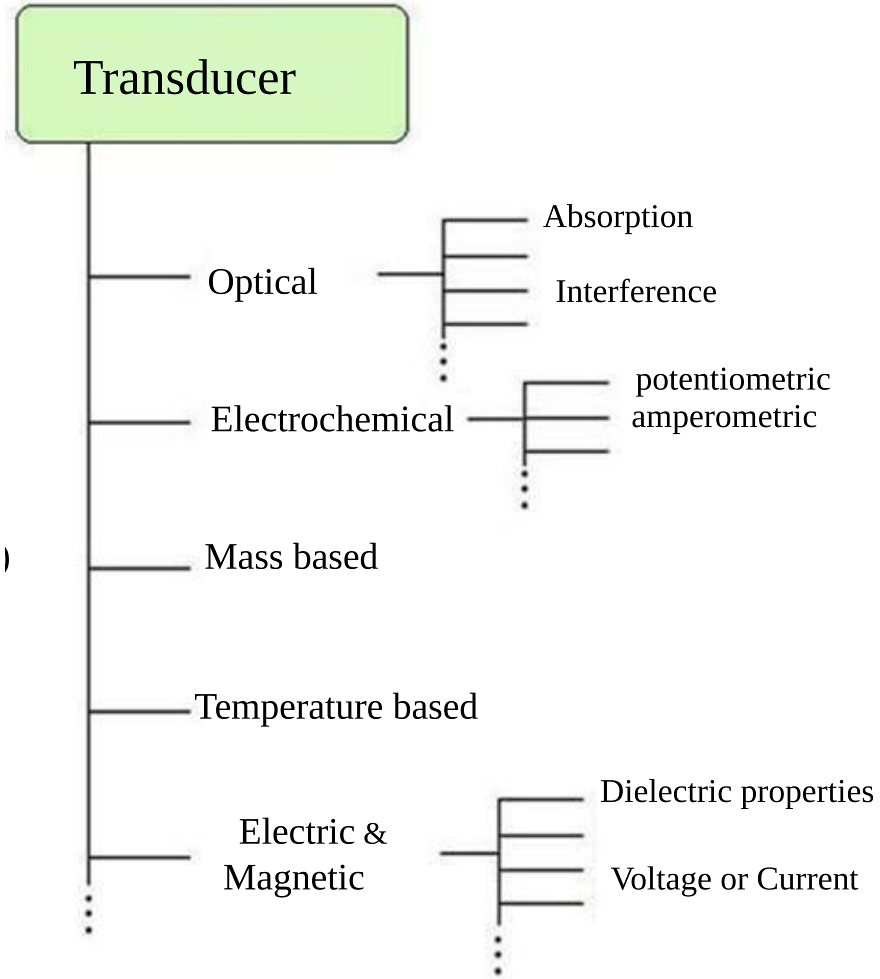
Business plan/Term paper – 25 marks

All lecture components will be completed before 30th June!

Request all the graduating students to complete submission by 5th July

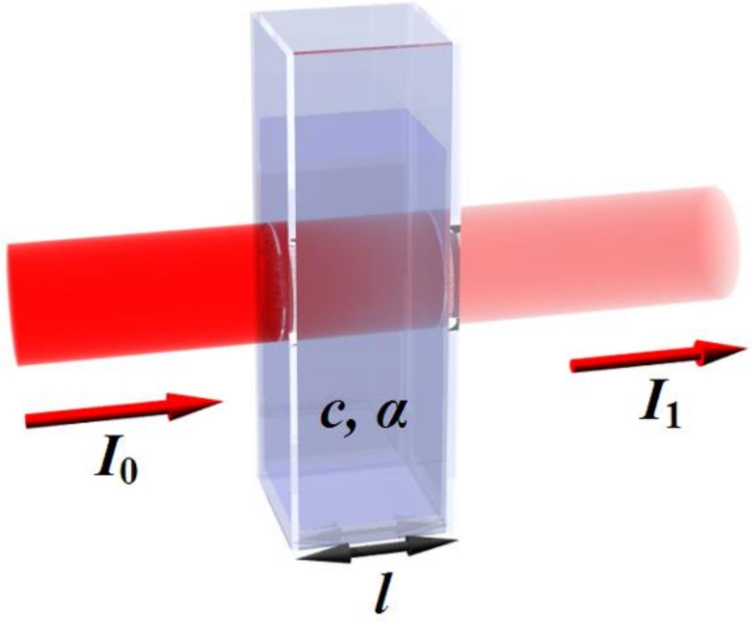
Request all the others students to complete submission by 15th July

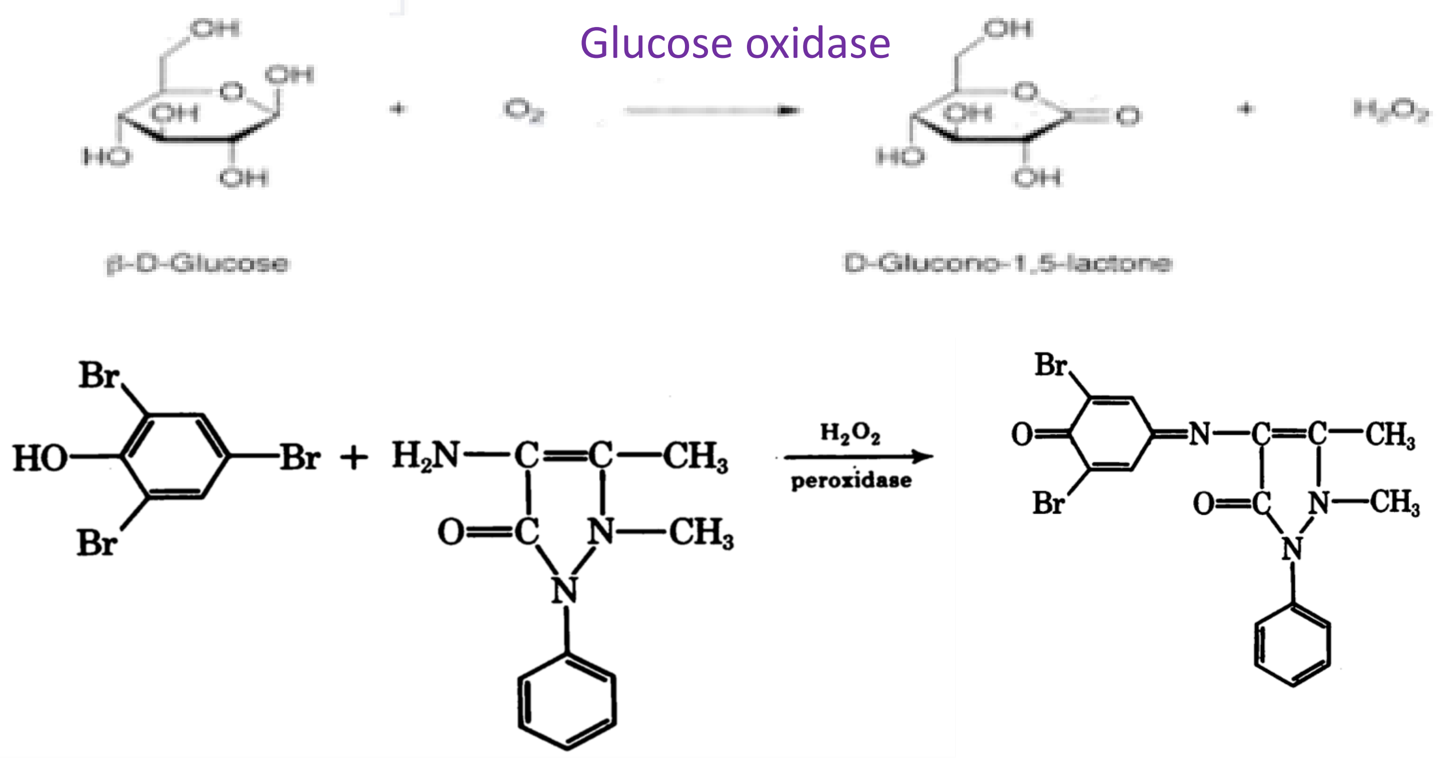
Transducer



Transducer
Absorption
Optical
Interference
potentiometric
Electrochemical
amperometric
Mass based
Temperature based
Dielectric properties
Electric &
Magnetic
Voltage or Current

Optical transducer: Absorption



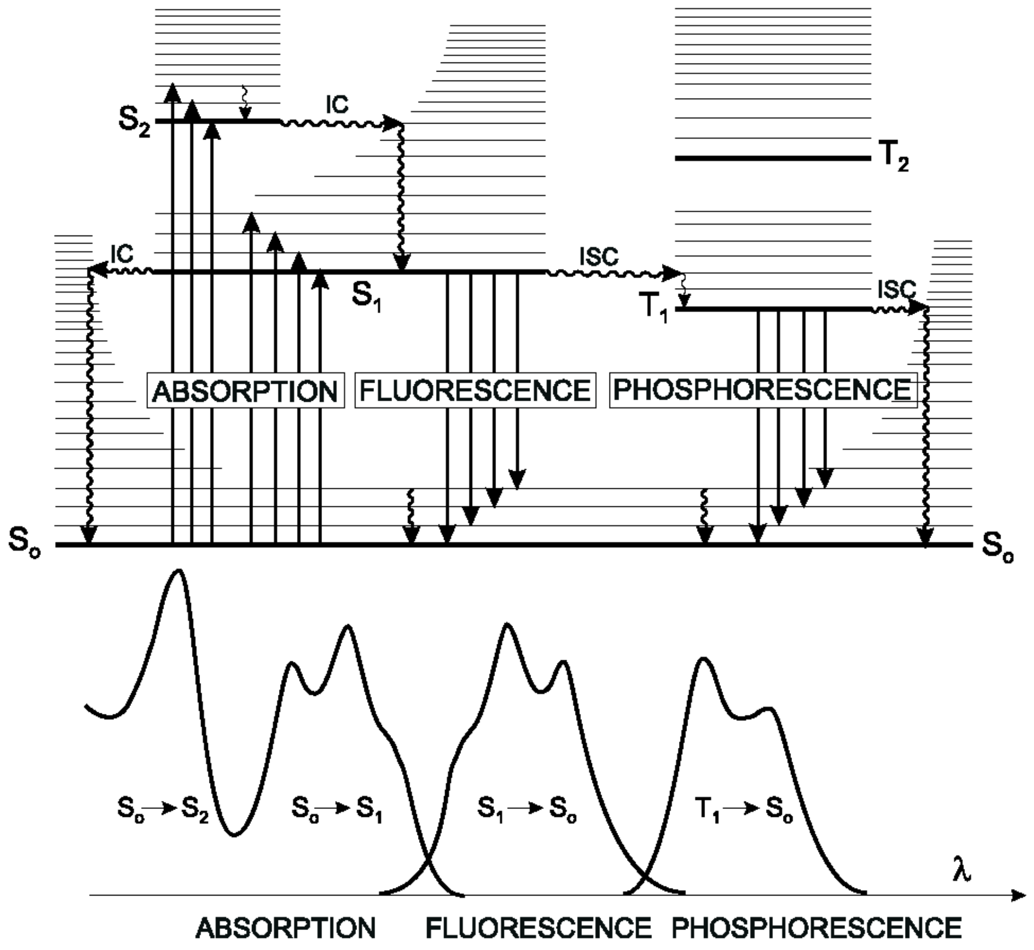


Color reaction

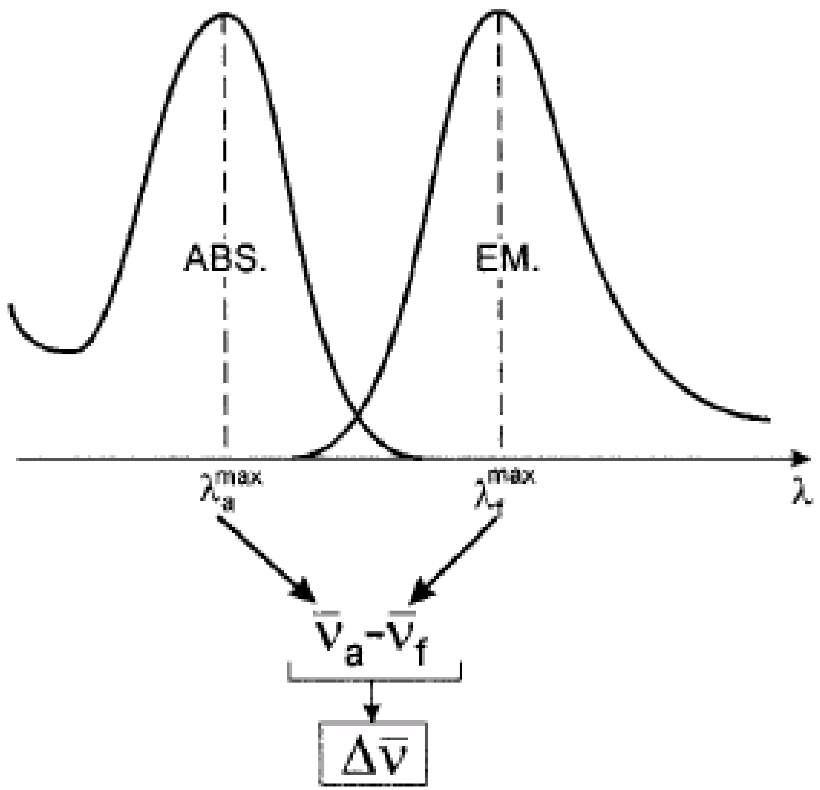
$$I_1/I_0 = e^{-\alpha l c}$$

l is the pass length
C is the concentration of absorbing material
α is the absorption coefficient

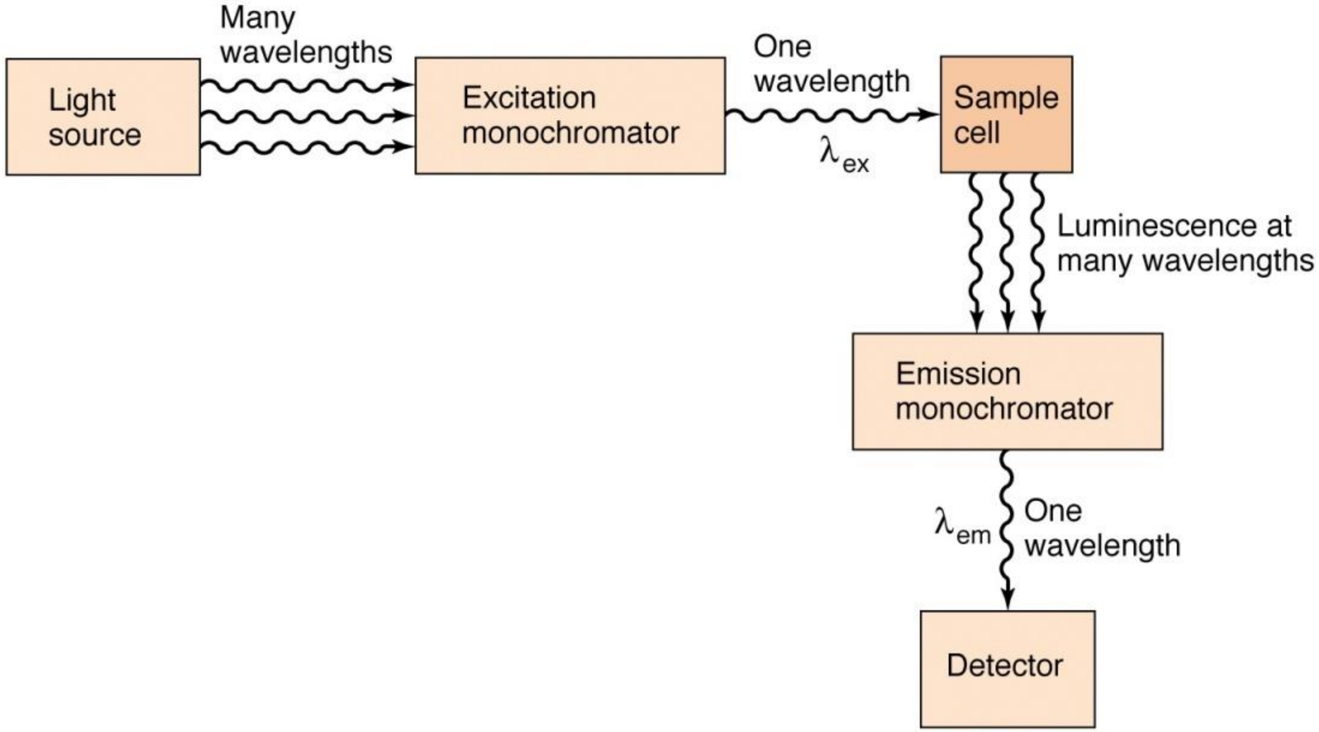
Optical transducer: Fluorescence

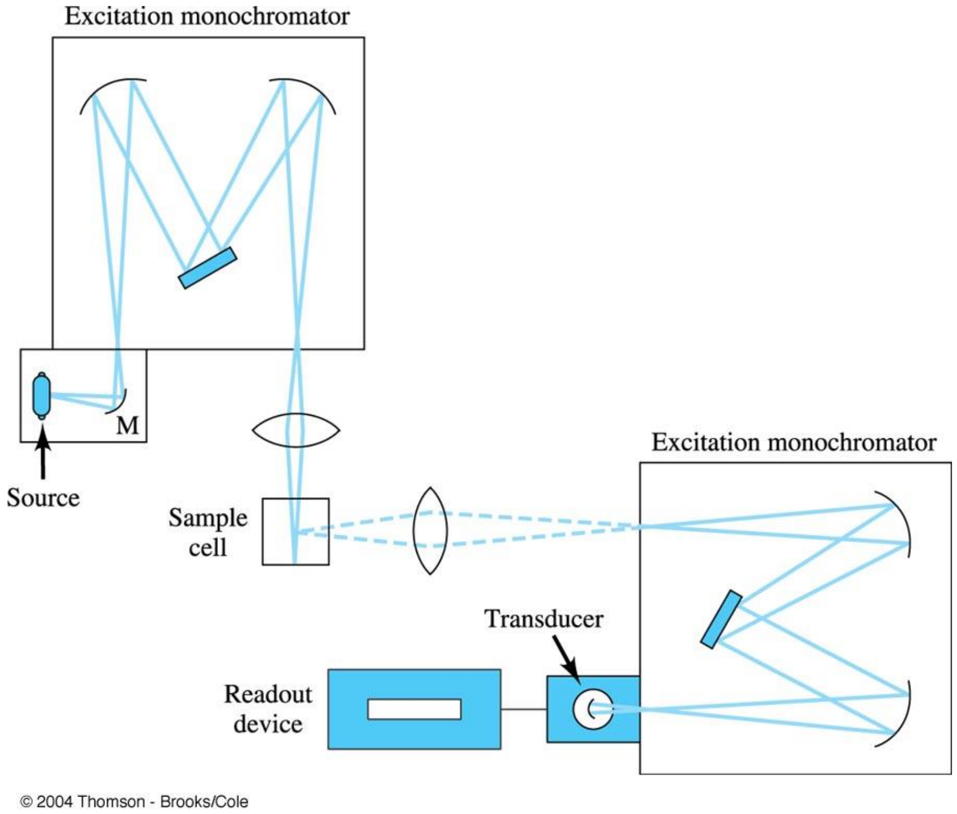


An optical system for
Florescence measurement



Optical transducer: Fluorescence





Optical transducer: Fluorescence probes

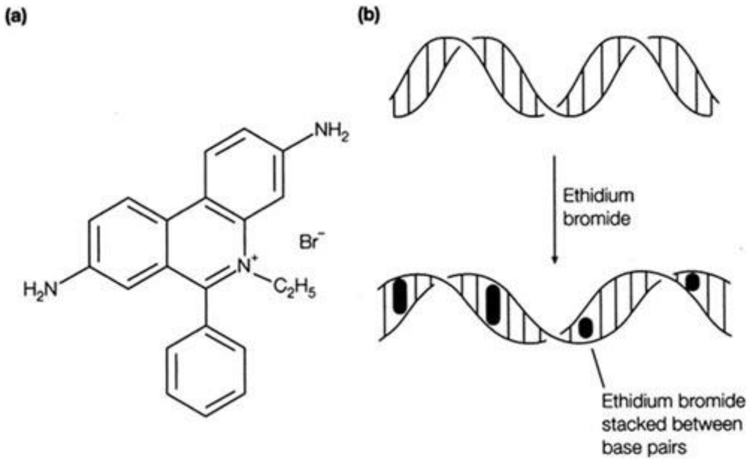


Fig. 3. (a) Ethidium bromide; (b) the process of intercalation, illustrating the lengthening and untwisting of the DNA helix.

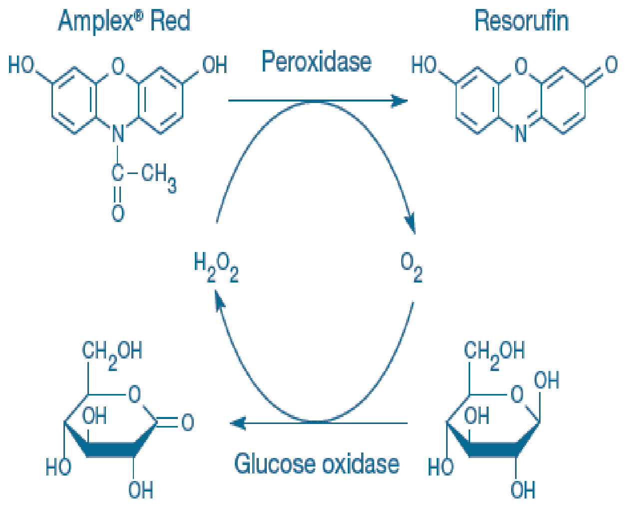


Figure 10.2.15 Principle of coupled enzymatic assays using our Amplex® Red reagent. Oxidation of glucose by glucose oxidase results in generation of H_2O_2, which is coupled to conversion of the Amplex® Red reagent to fluorescent resorufin by HRP. The detection scheme shown here is used in our Amplex® Red Glucose/Glucose Oxidase Assay Kit (A22189).

Optical transducer: Fluorescence probes

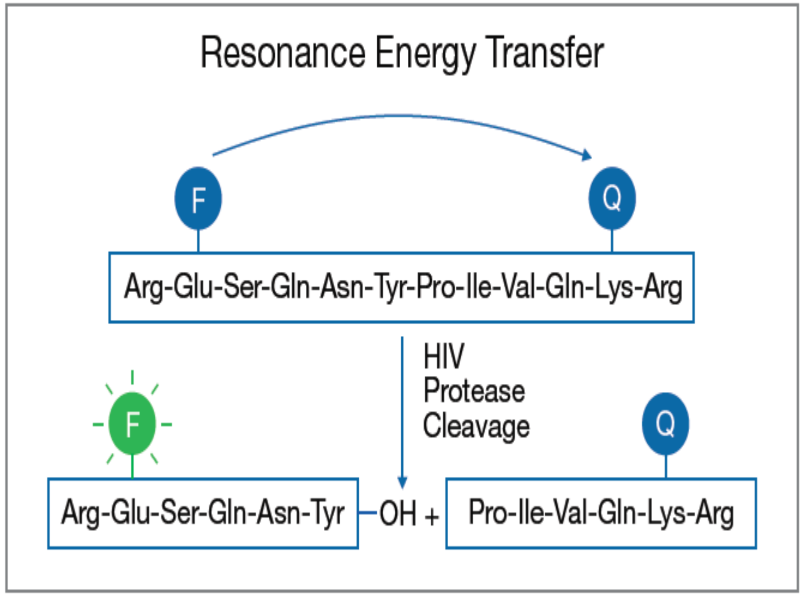
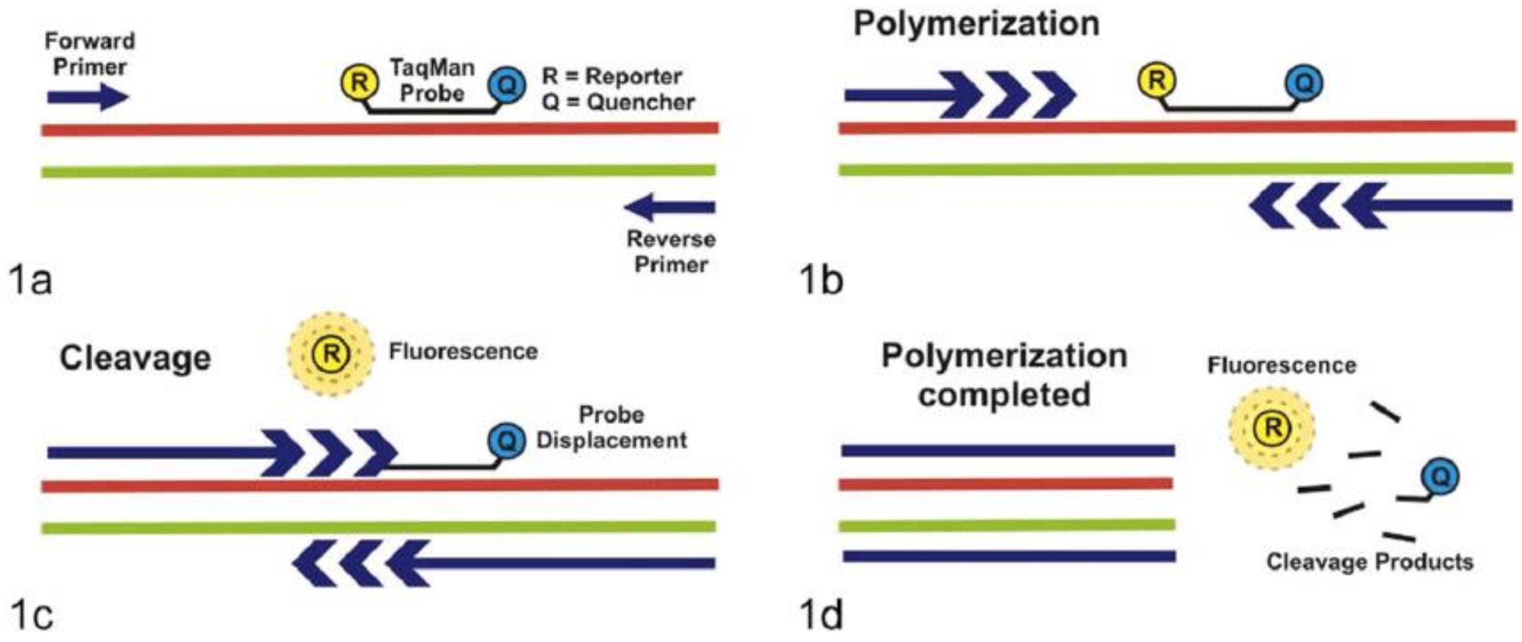


Figure 10.1.16 Principle of the fluorogenic response to protease cleavage exhibited by HIV protease substrate 1 (H2930). Quenching of the EDANS fluorophore (**F**) by distance-dependent resonance energy transfer to the dabcyl quencher (**Q**) is eliminated upon cleavage of the intervening peptide linker.

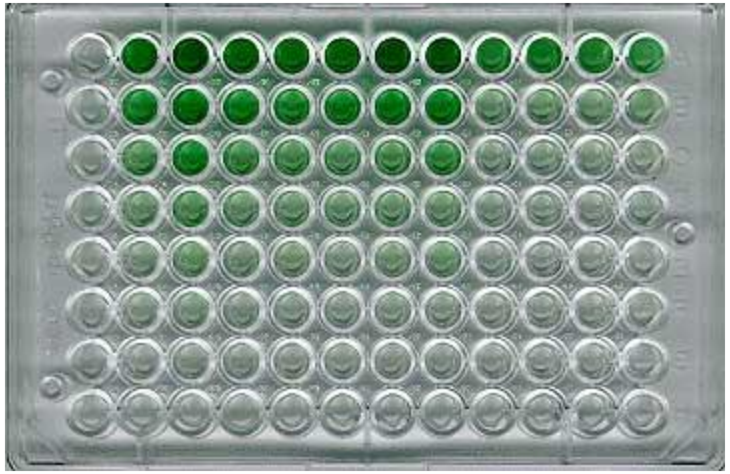


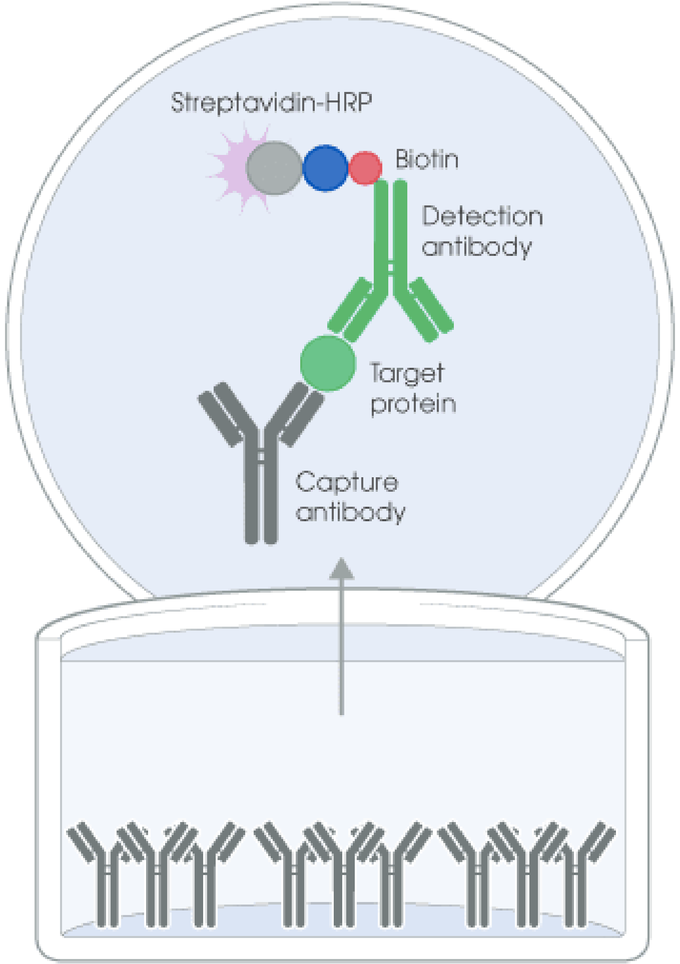
FRET pairs: Peptide

FRET pairs: Taqman probe

Reference: Thermofisher.com

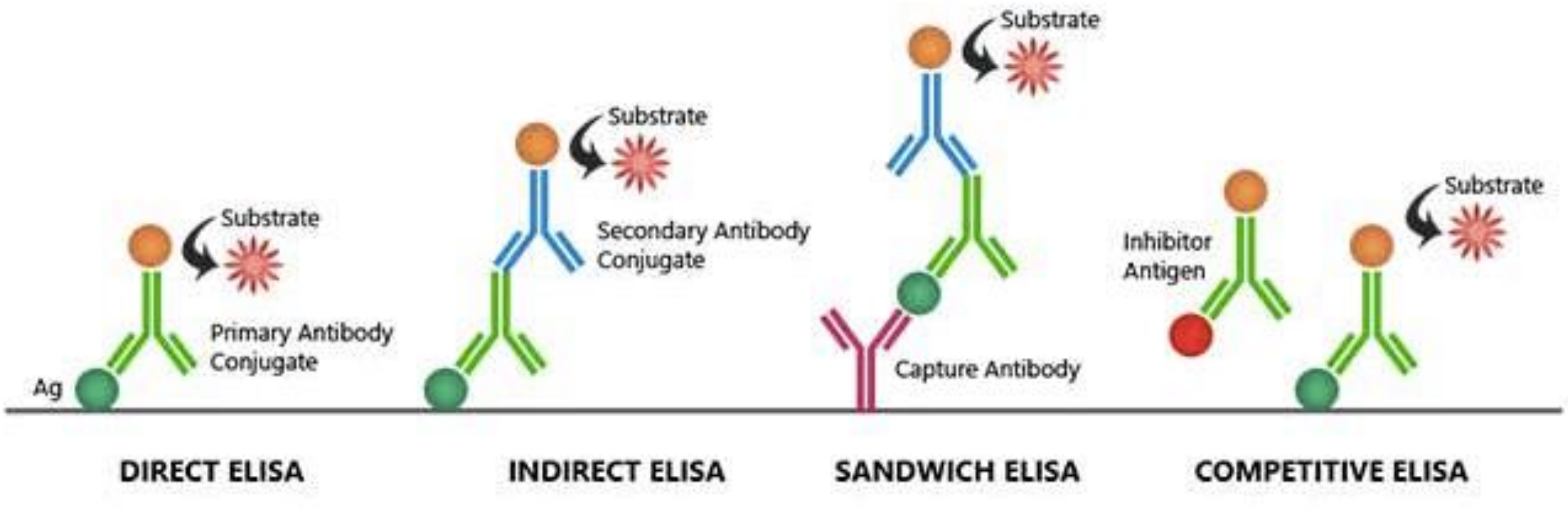
ELISA





https://www.abcam.com/kits/elisa-principle

Types of ELISA



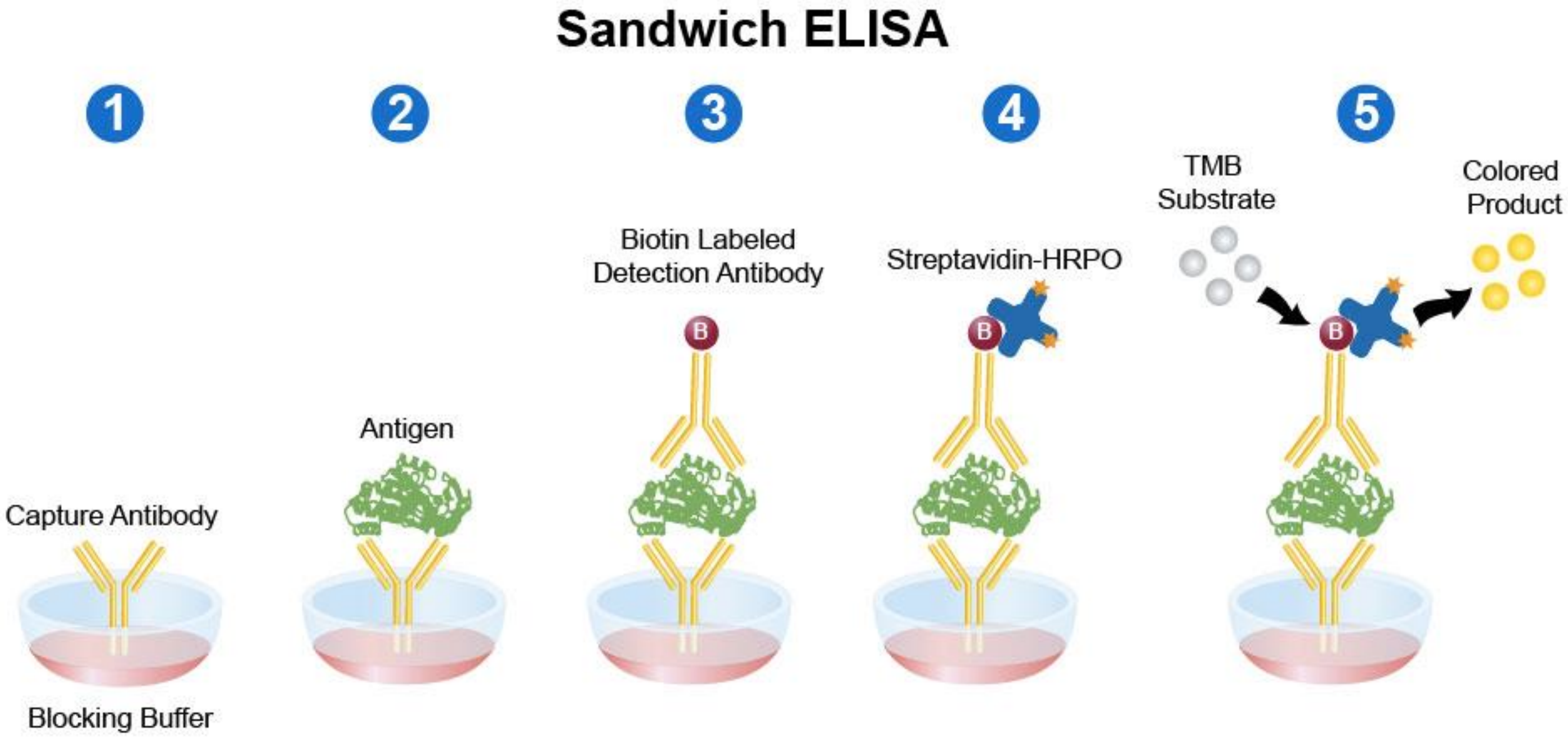
COVID Virus Dx test
Simultaneous absorbtion

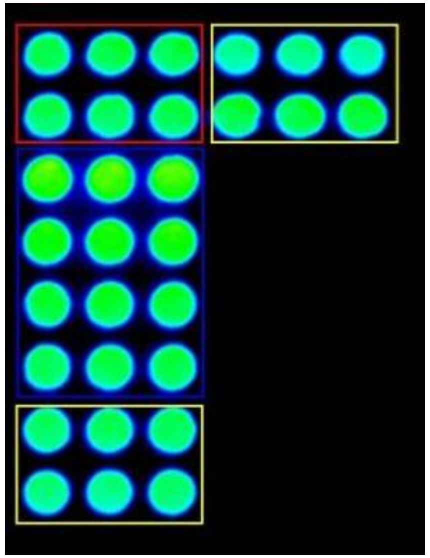
COVID Antibody test

COVID Virus Dx test

COVID Virus Dx test,
Sites have competitor molecules

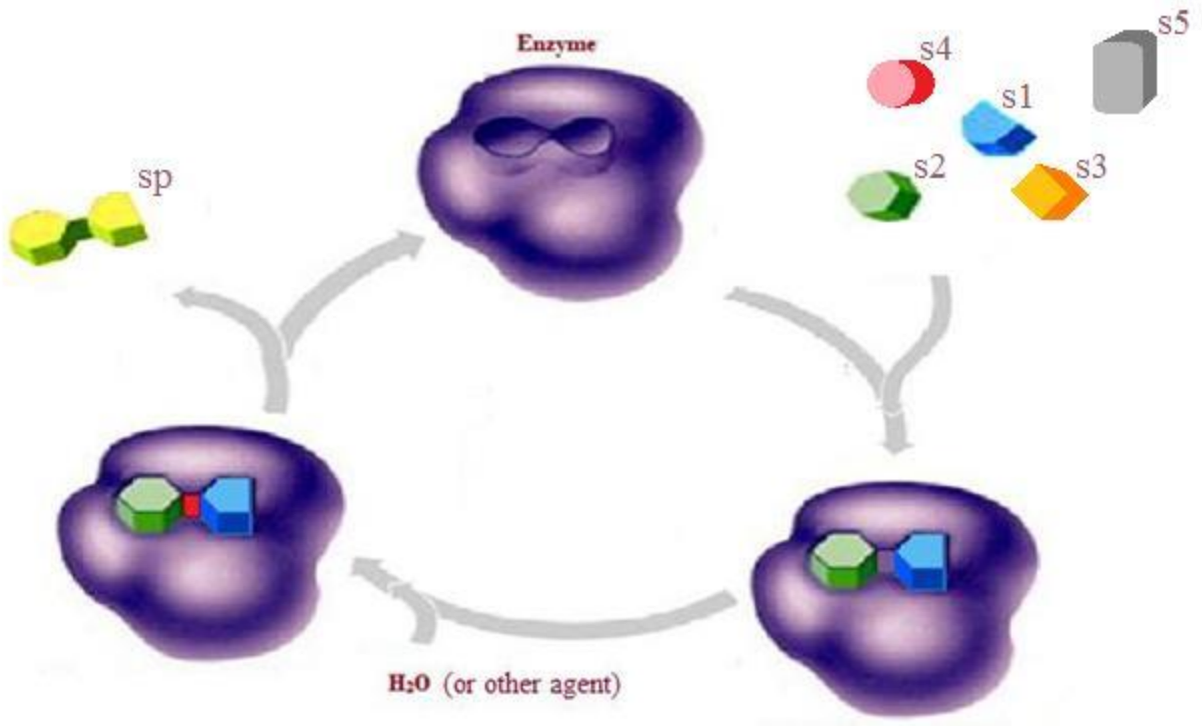
Basic principles of ELISA

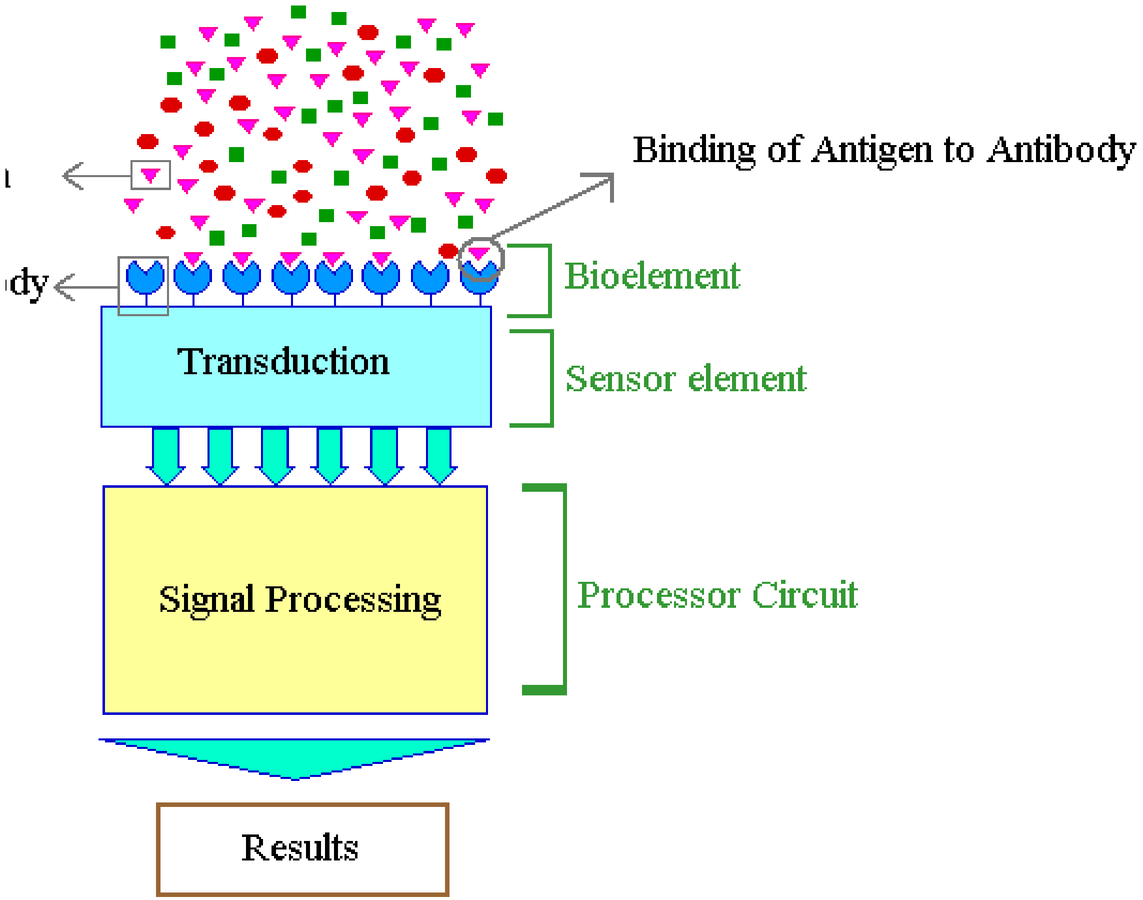




- the antibodies fixed to a solid surface, such as the inner surface of a test tube;
- a preparation of the same antibodies coupled to an enzyme.

Amplification effect of Enzyme in biosensing





High sensitivity: K_{cat} >100 s^{-1}: results are amplified!
High specificity: Enzyme have high selectivity

Case study 1: Enzyme sensor for cancer detection

click for updates

PNAS

Point-of-care diagnostics for noncommunicable diseases using synthetic urinary biomarkers and paper microfluidics

Andrew D. Warren[a,b,1], Gabriel A. Kwong[a,b,1], David K. Wood[c,1], Kevin Y. Lin[b,d], and Sangeeta N. Bhatia[a,b,e,f,g,h,2]

[a]Harvard–Massachusetts Institute of Technology Division of Health Sciences and Technology, Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA 02139; [b]David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139; [c]Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455; [d]Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139; [e]Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, MA 02139; [f]Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115; [g]Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA 02139; and [h]Howard Hughes Medical Institute, Cambridge, MA 02139

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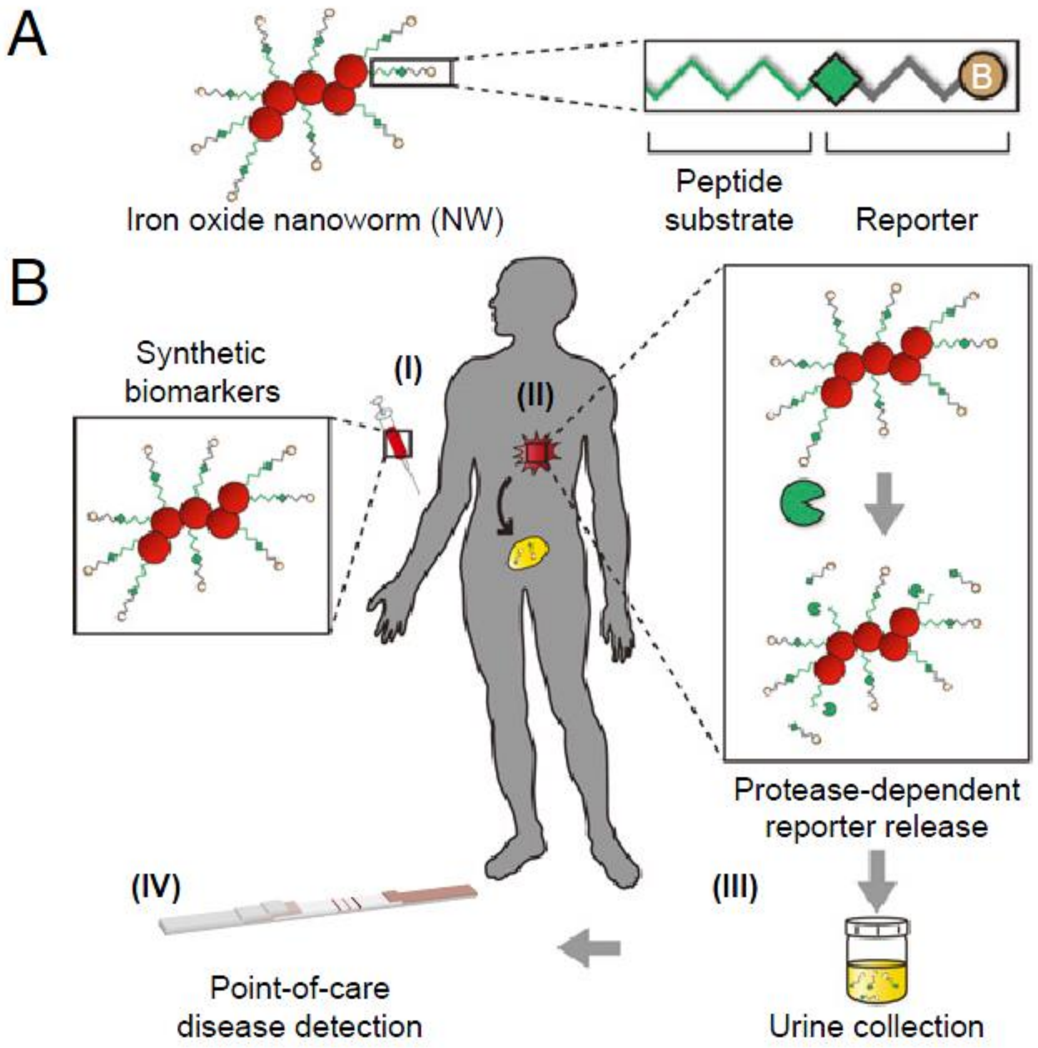
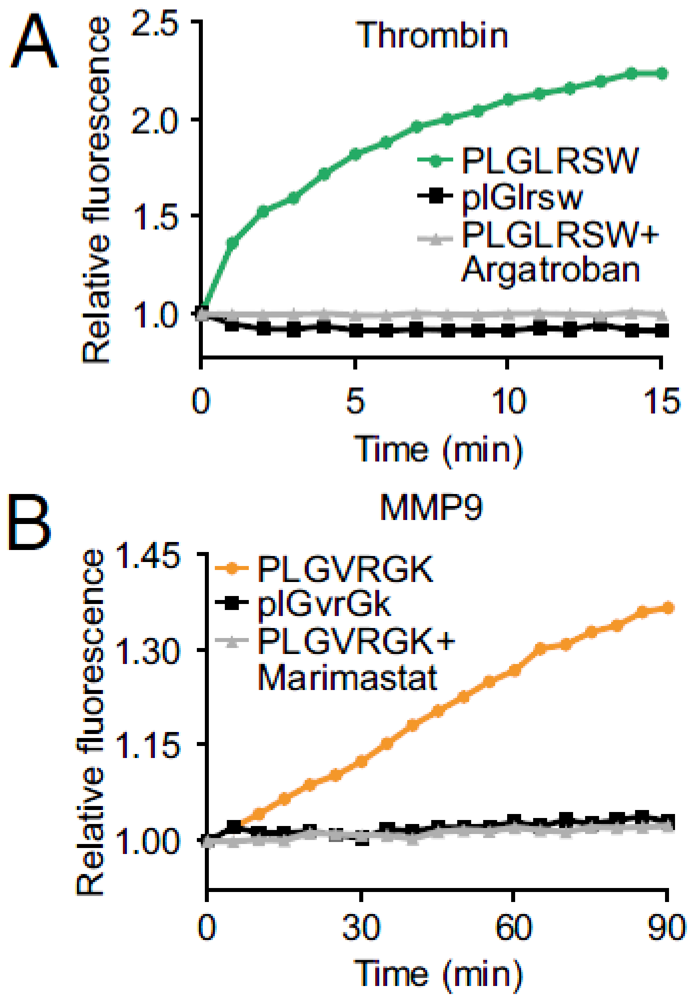
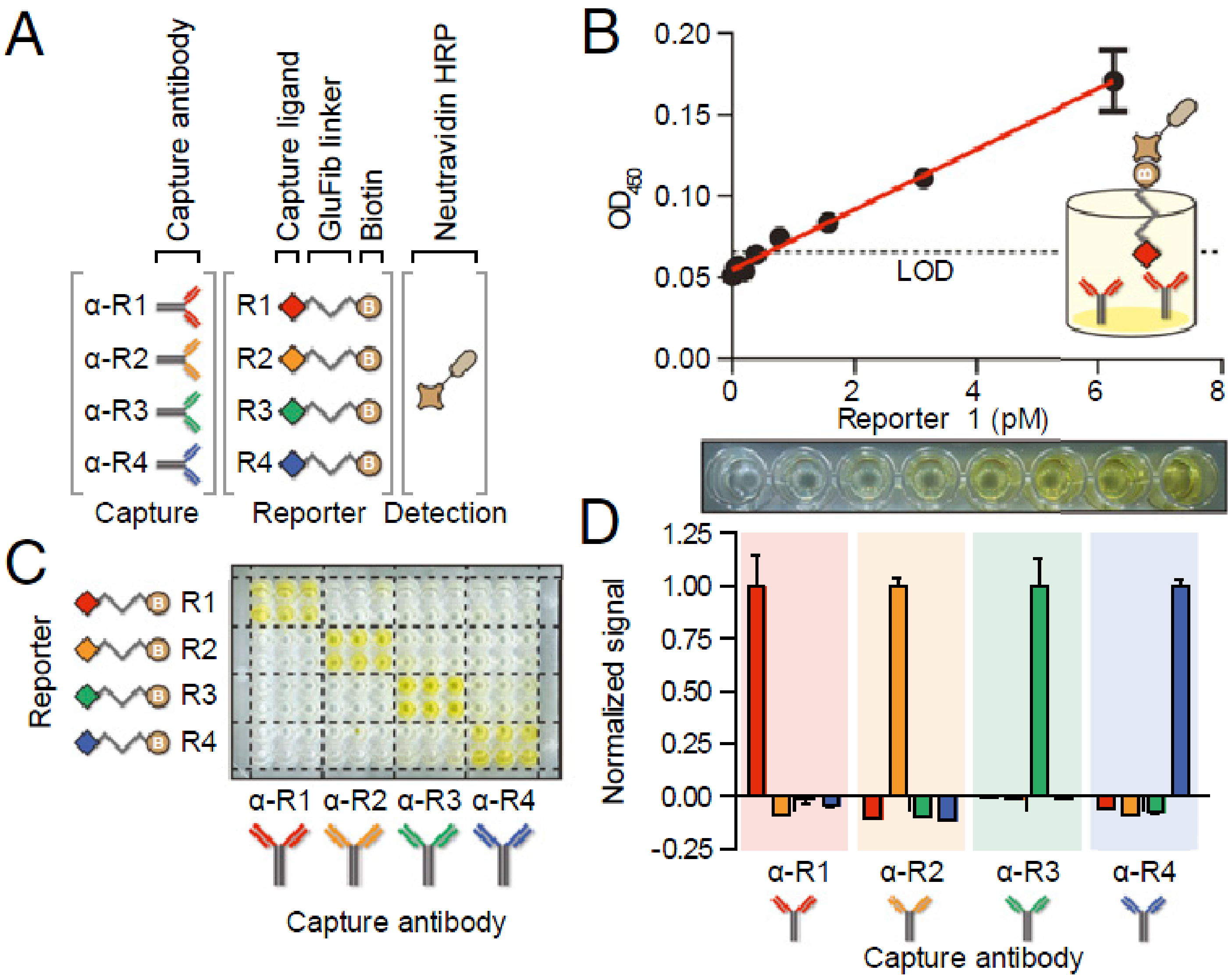
Case study 1: Enzyme sensor for cancer detection





Case study 1: Enzyme sensor for cancer detection



Case study 2: Enzyme sensor for TB detection

ARTICLES
PUBLISHED ONLINE: 2 SEPTEMBER 2012 | DOI: 10.1038/NCHEM.1435
natur
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Rapid point-of-care detection of the tuberc pathogen using a BlaC-specific fluorogenic

Hexin Xie[1†], Joseph Mire[2†], Ying Kong[3†], MiHee Chang[3], Hany A. Hassounah[3], Chris N. T
James C. Sacchettini[2], Jeffrey D. Cirillo[3] and Jianghong Rao[1*]

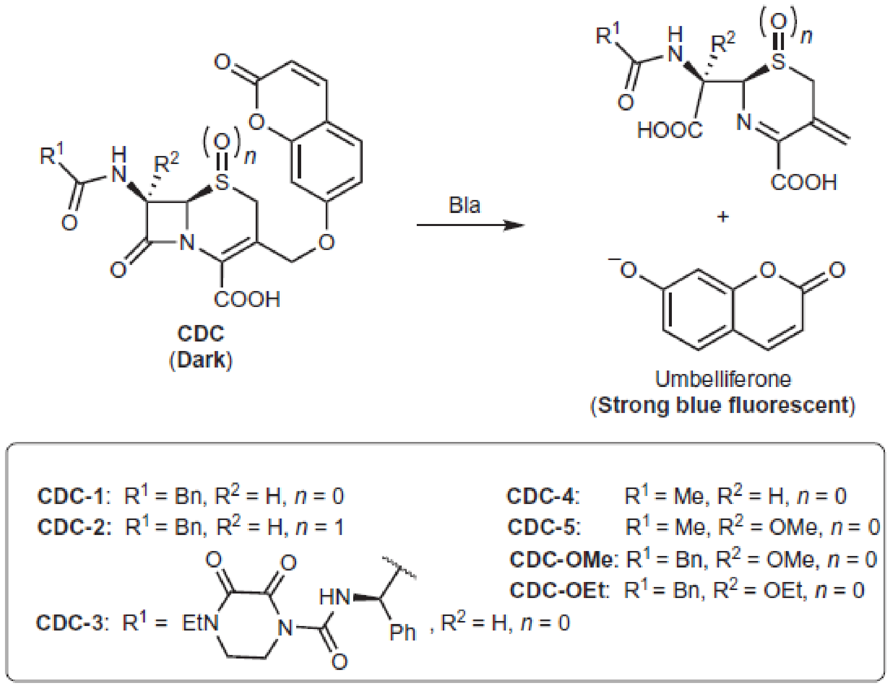
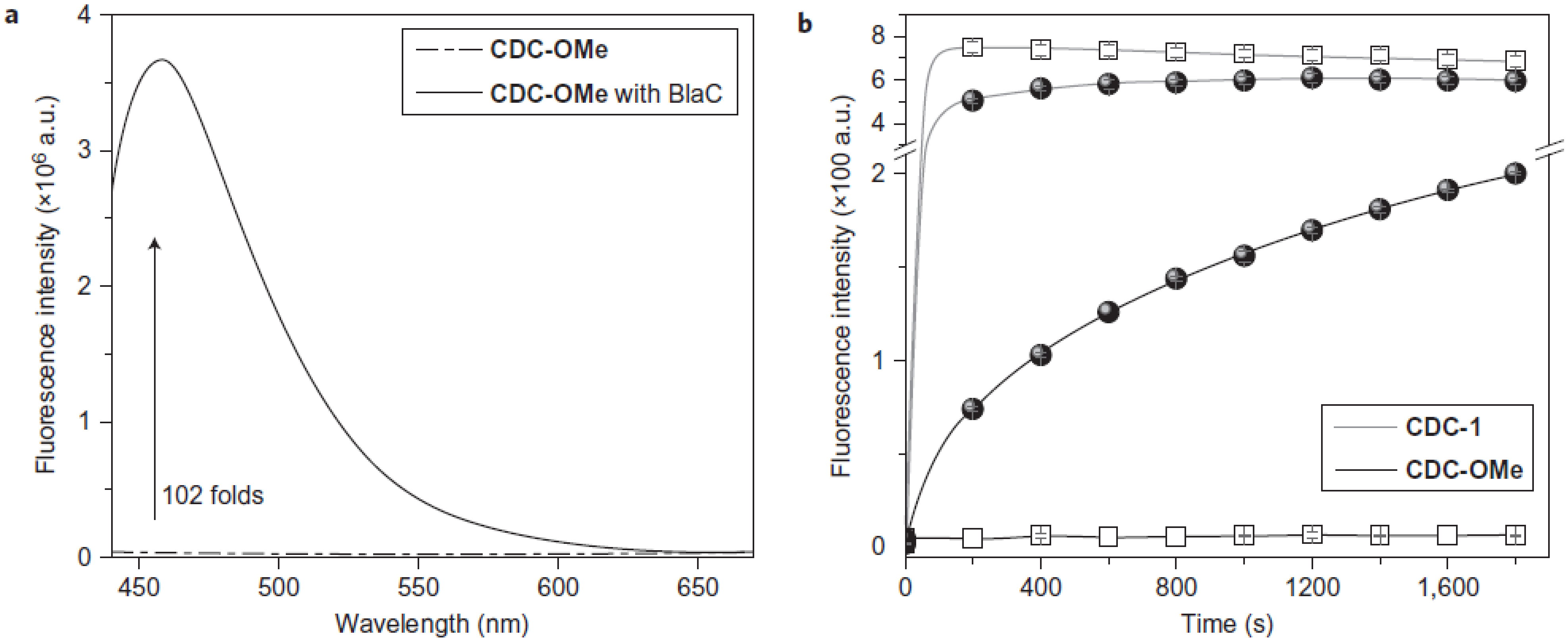


Figure 1 | General structures of blue fluorescent probes and their hydrolysis by β-lactamase (Bla), which triggers the release of umbelliferone and turns on fluorescence.

These probes showed an enhancement by 100–200 times in fluorescence emission on BlaC activation and a greater than 1,000-fold selectivity for BlaC over TEM-1 b-lactamase

Case study 2: Enzyme sensor for TB detection



Thank you