Lecture 18: Biosensors

Last time: engineering intracellular delivery
Drug targeting

Today: biosensor device classes
Detection methods

Overview of biosensor technology

Classes of biosensor devices

External analysis/detection
- Large instruments
- Objectives
  - Maximum sensitivity
  - Highest throughput
- Samples probed
  - Biochemical
  - Cell populations
  - Intracellular (single cells)

Field detection
- Usually simpler, need to be more robust

***In vivo* detection**
- Usually catheter/needle-based for minimal invasiveness, with detector outside body

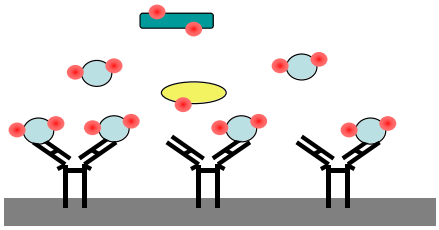
Classes of sensor mechanisms[1]

Capture-based
- Binding of labeled analyte to capture reagent
- Binding of unlabeled analyte to capture reagent triggers detectable signal

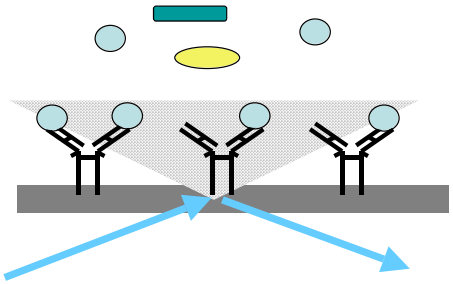
Catalysis[2]
- Enzymatic reaction generates a detectable product
  - Change in proton concentration, release of O_2, NH_3, CO_2
  - Release of metals, halides
  - Ion/electron transfer
  - Change in optical properties (e.g. production of a colored product)

Cell-based
- Single-cell-based
  - Binding of analyte to cell surface receptor triggers detectable signal
- Tissue-based biosensors[3]
  - Binding of analyte to one cell type triggers cell-cell interactions and signaling cascades that can be detected

Capture-based

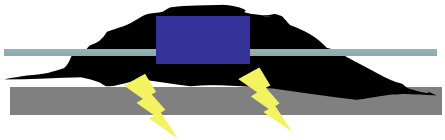


Binding of labeled analyte to capture reagent

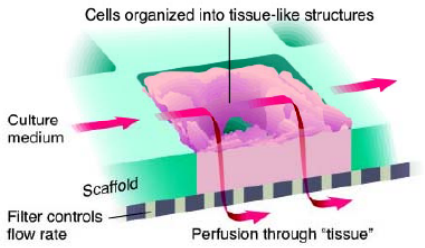


Binding of unlabeled analyte to capture reagent triggers detectable signal

Cell-based



Single-cell: Binding to cell surface receptor triggers signal



(Griffith and Naughton, 2002)

Tissue-based: Binding to multiple cells triggers cell-cell interacdtions

Applications

- Microanalysis
  - Small sample sizes, high throughput
- Toxicology and drug testing
  - Testing drug safety
  - Screening libraries of candidate drug compounds
- Toxin and pathogen detection

Detection Elements

Optical

- Concept
  - Capture analyte and detect binding by optical tag or binding-sensitive optical phenomenon
- Capture
  - Surface-immobilized capture molecules
    - E.g. single-stranded DNA (DNA), antibodies (target antigens)

identification based on x-y location of capture agent

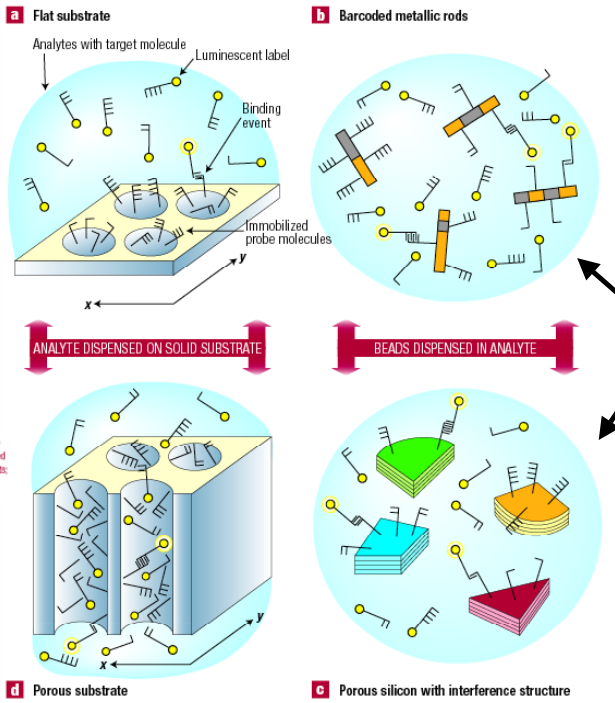


Figure 1 Probes for tracking biomolecular binding events can be identified by **a**, an *x*,*y* position on a flat substrate; **b**, using barcoded metallic rods; **c**, the interference colour of porous silicon fragments; or **d**, an *x*,*y* position in a porous substrate.

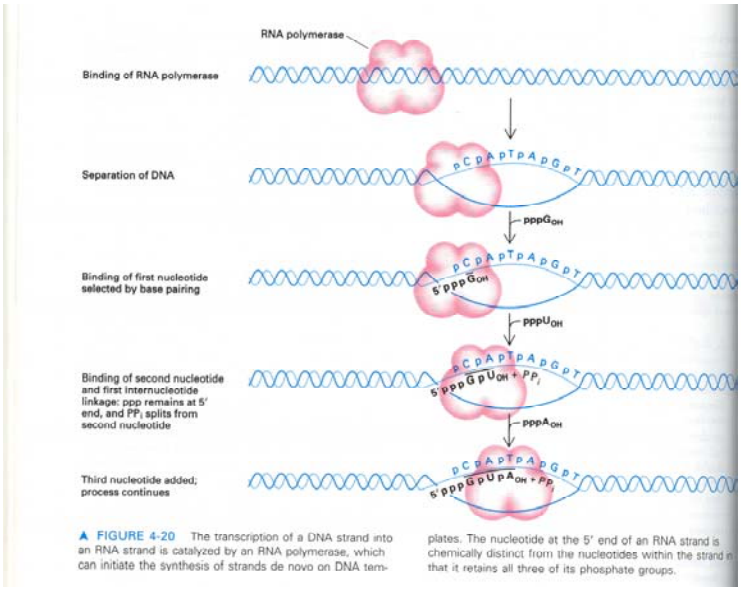
(Lehmann, 2002)

Optical bar-coding

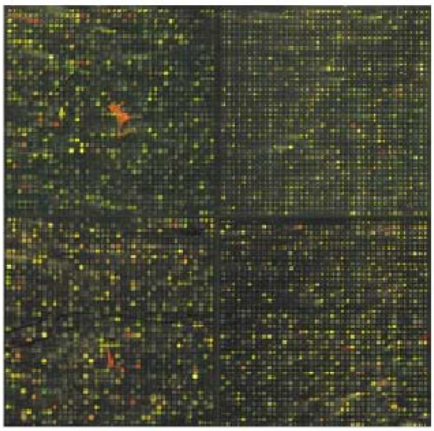
Capture agent uniquely marked for identification

- Detection surface can either be planar or composed of capture particles
  - Planar surface:
    - Identification based on x-y location of tag
  - Particle-based detection:
    - Faster kinetics of binding due to reduced distances to be traveled by analyte
    - Identification based on particle-specific labeling (challenge)
- Commercial technology example of planar detection surface: gene chips

- Composition of arrays:
  - Oligonucleotides
    - Each 'spot' composed of ~40 oligos 25 base pairs long and a matching control with one central base changed
      - Need different permutations for each gene to account for redundancy in short probe sequences
    - Must know gene sequence to prepare appropriate oligos
  - CDNA-sized fragments
    - Usually produced by PCR
    - Long fragments where each fragment uniquely identifies a gene
    - Can pack all 6000 yeast genes onto a 1.28 cm x 1.28 cm glass slide
    - Random cDNA clones can be used
- Application
  - Label mRNA from cell sample, apply to chip and allow to hybridize
  - Scan chip for bound fluorescence
- Gene chips can detect mRNA present at < 1 molecule in 100,000 (equivalent to detecting one transcript per 20 yeast cells)
- Entire yeast genome can be put on a chip



▲ FIGURE 4-20 The transcription of a DNA strand into an RNA strand is catalyzed by an RNA polymerase, which can initiate the synthesis of strands de novo on DNA templates. The nucleotide at the 5′ end of an RNA strand is chemically distinct from the nucleotides within the strand in that it retains all three of its phosphate groups.



A DNA fragment array of all ~ 6000 yeast genes probed with labeled cDNA made from galactose- and glucose-grown cells. Each spot (element) on the array contains a cDNA-sized DNA fragment representing one yeast coding sequence. mRNA from galactose-grown cells was converted to red-labeled cDNA (using dUTP labeled with the fluorescent dye Cy3); mRNA from glucose-grown cells was converted to green-labeled cDNA (with the dye Cy5). These two preparations of labeled cDNA were mixed and used to probe the array. Red spots bind only galactose-grown cDNA, and thus represent genes expressed only in galactose-grown cells; green spots bind only cDNA from glucose-grown cells, and therefore represent genes expressed only in glucose-grown cells. Spots containing genes expressed under both conditions hybridize to both cDNAs, and thus appear yellow. The intensity of the color of each spot (from red to green) reveals the relative expression level of genes under the two conditions. (Figure courtesy of Joe DeRisi, Vishy Iyer, and Pat Brown; for more of these images, see [17].)

(Johnston, 1998)

References

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