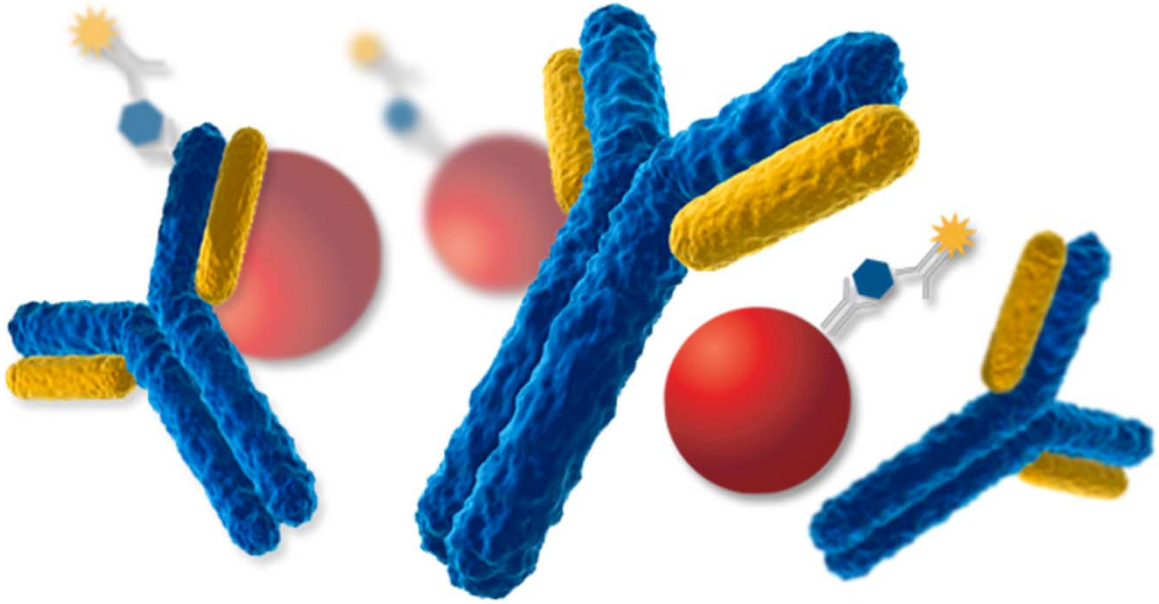


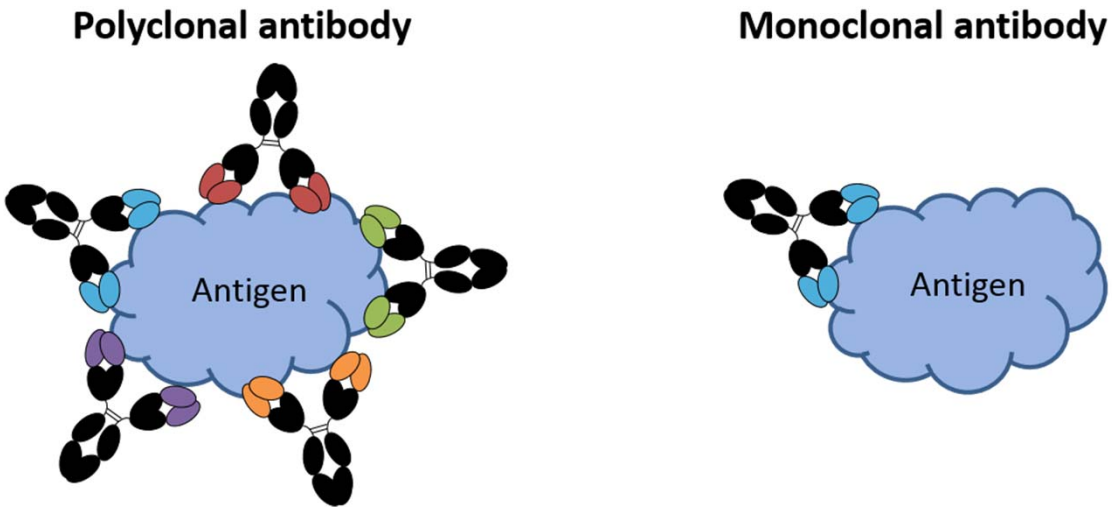
Immunoassays

Terms

- **Affinity: Binding force between one antigen determinant (epitop) and one binding site of an antibody**
- **Avidity: summary of binding forces measured on several sites**
- **Antibody titer: the last dilution with detectable interaction between antigen and antibody**

Terms

- **Antibodies:**

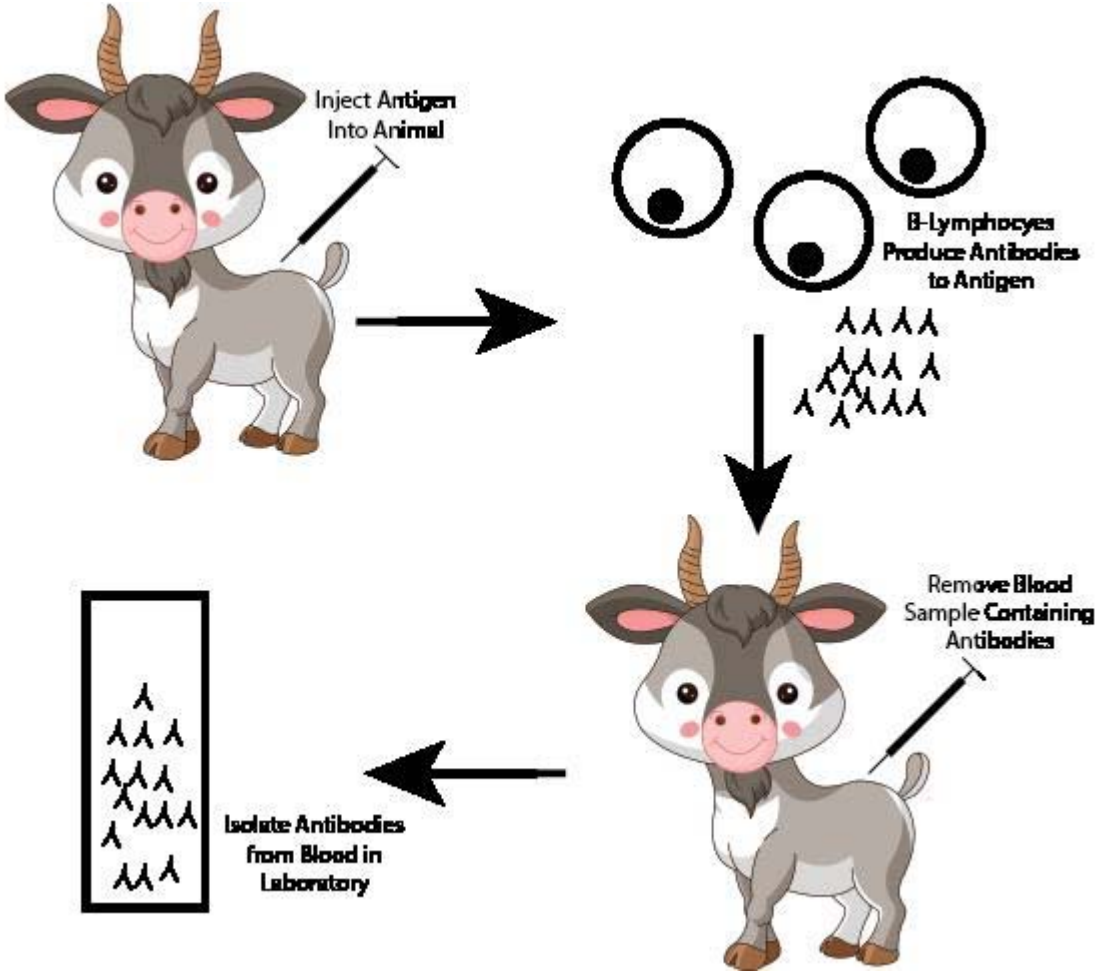


Used as secondary antibody

Used as primary antibody

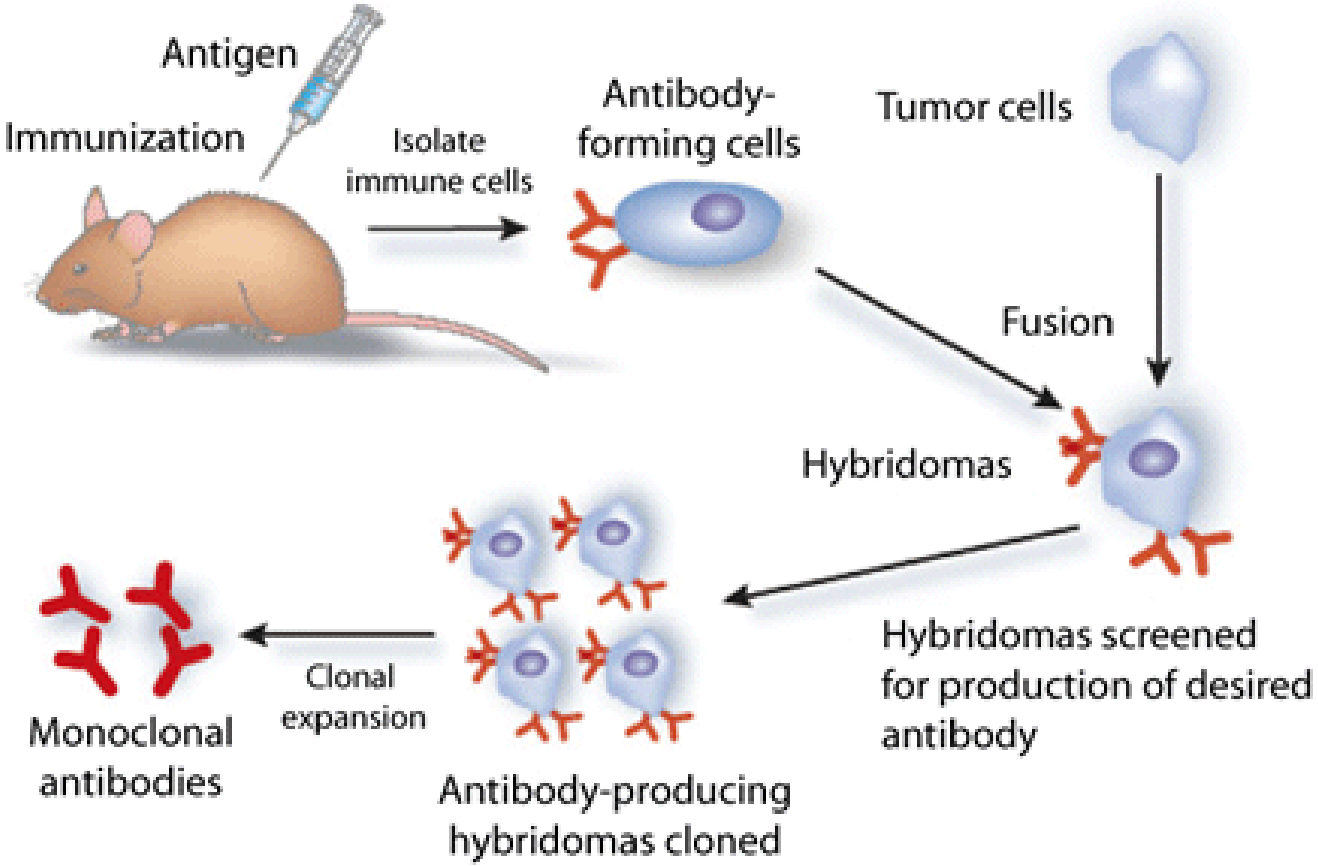
Terms

- **Polyclonal antibody**



Terms

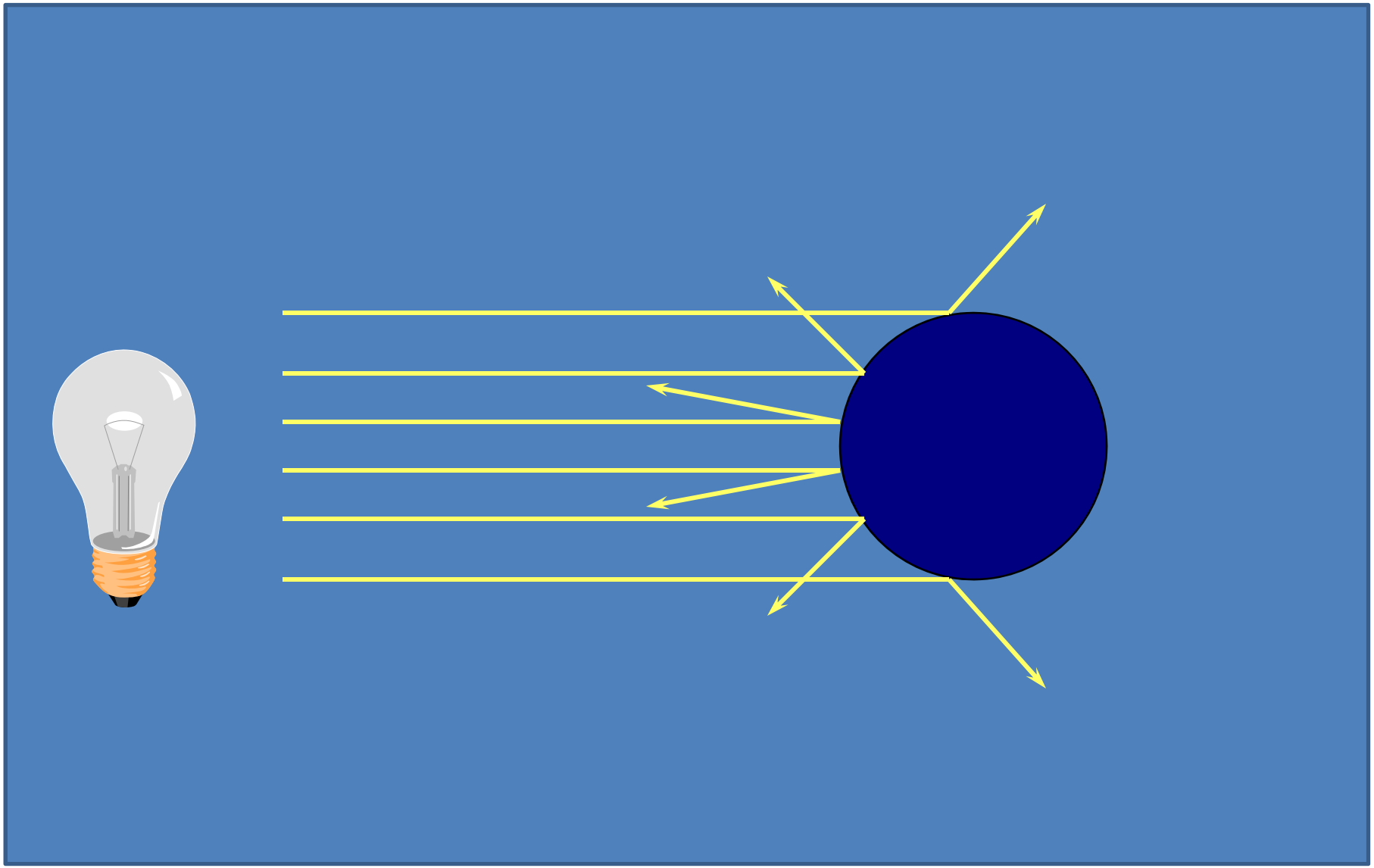
- **Monoclonal antibody**



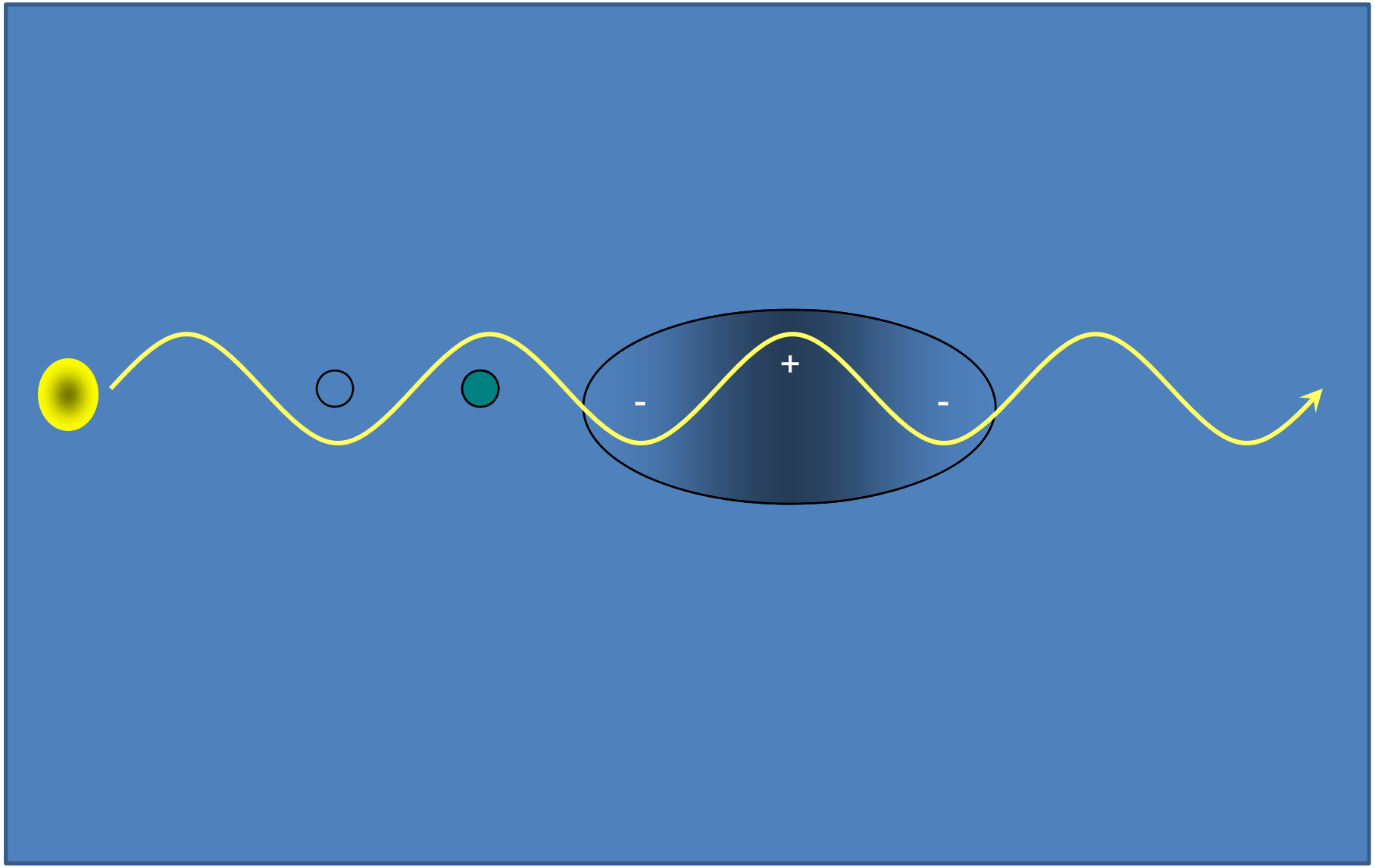
Particle methods involving soluble complexes

- The key physical property is still *size*
- Measurement is based on how the large antibody/antigen complexes interact with light
- The fundamental principle upon which the measurement is made is *light scattering*
- Two analytical methods are based on light scattering: *Nephelometry and Turbidimetry*

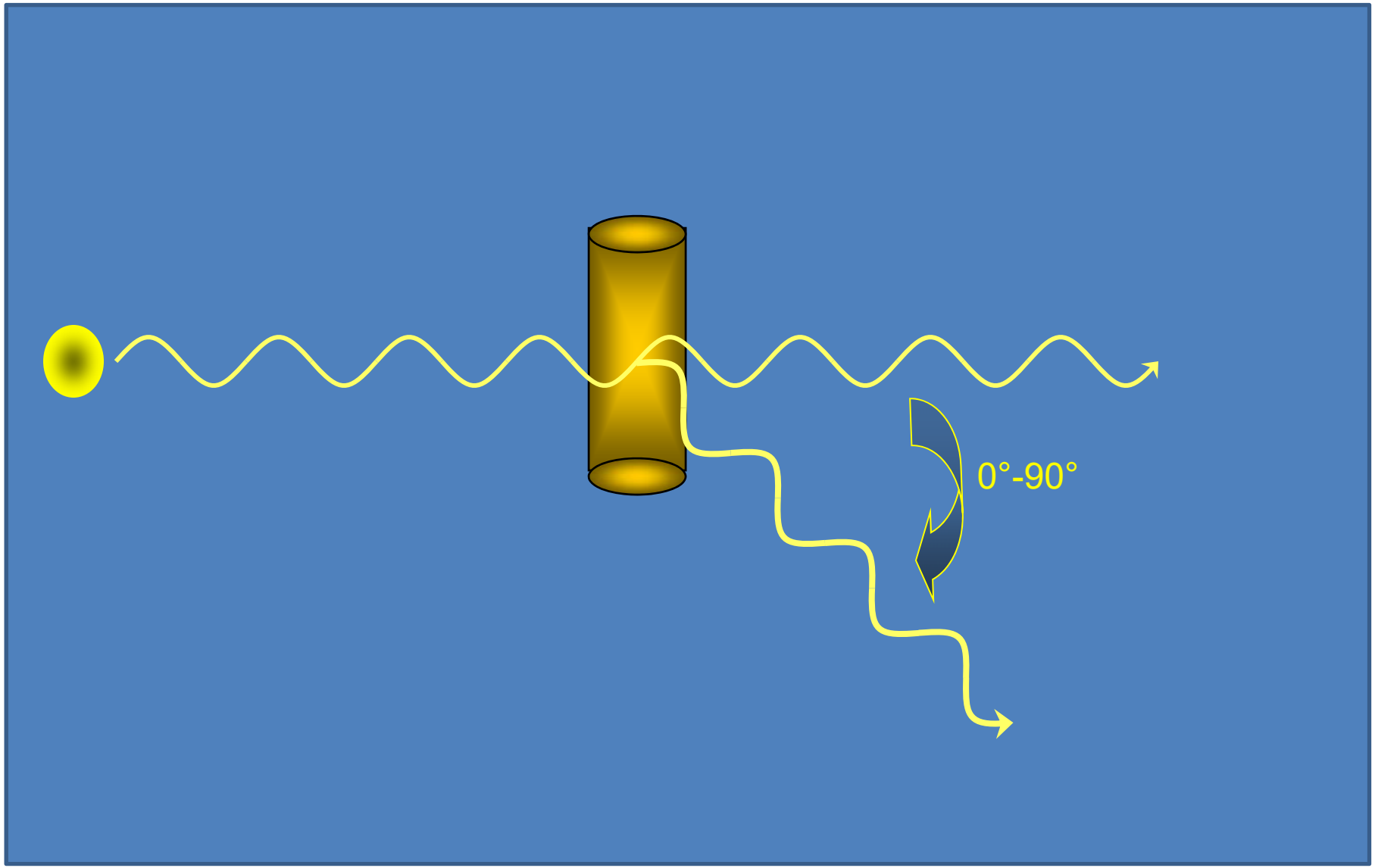
Light reflection



Molecular size and scattering



Nephelometry vs. Turbidimetry



Turbidimetry and nephelometry in lab

- **turbidimetry: applied on clin chem analyzers**

- relatively large concentrations(CRP, IgG, IgA, IgM, ferritin, AST, RF, transferrin)

- **nephelometry: applied on clin chem analyzers**

- Smaller levels (IgG subclasses, CHD-transferrin, kappa-lambda chains)

Immunoassay

- Since ’60s: radioisotopes
- Since ’70s: switched to other markers:

-enzymes

-fluorescent molecules

-chemiluminescent substances

} ”cold” immunoassay

Types of immunoassays

- **According to antigen-antibody reaction:**

  -non-competitive (immunometric)

  -competitive

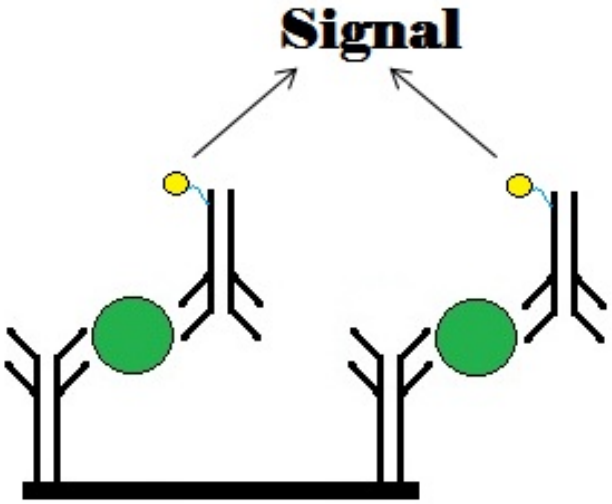
- **According to the principle of detection (whether antigen-antibody complex should be separated).**

  -homogenous assay (not required)

  -heterogenous assay (required) = solid phase assay

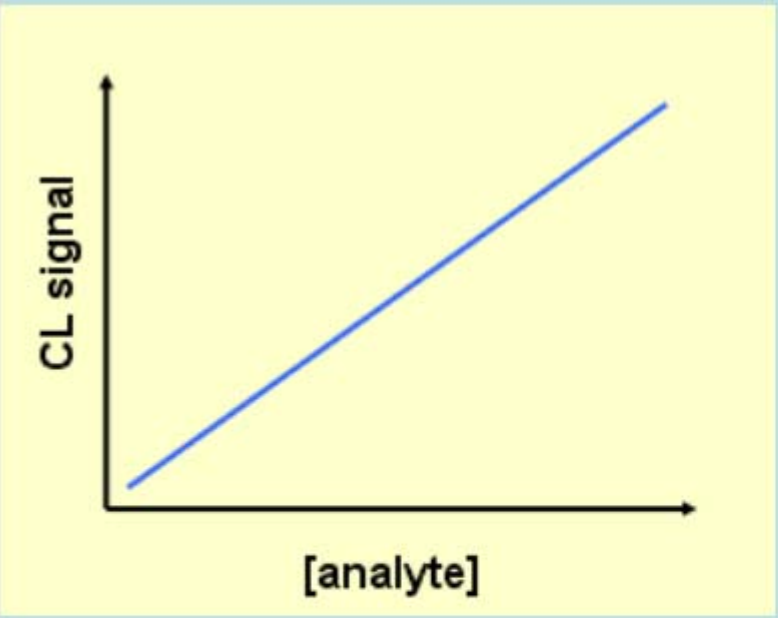
Non competitive immunoassay

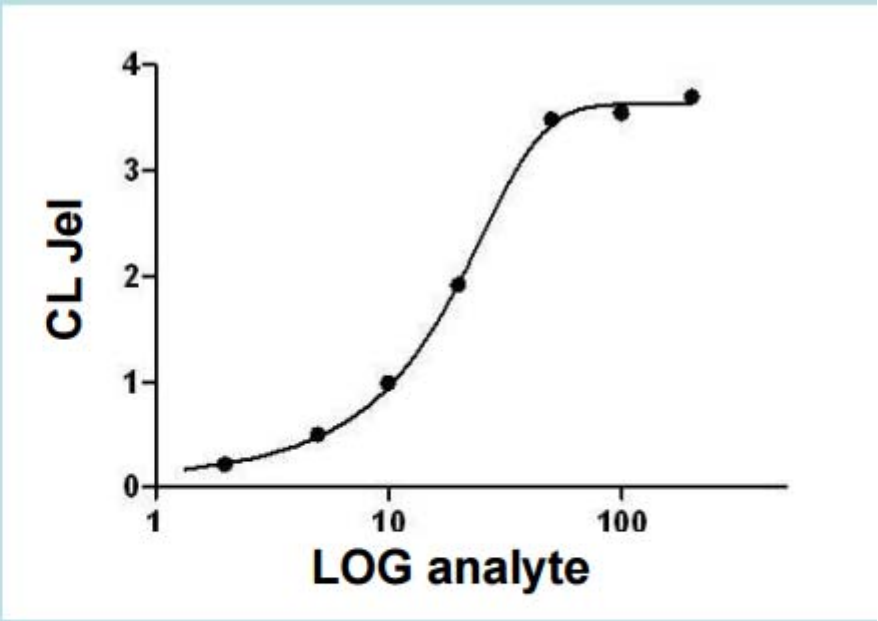
- labelled Ab: present in multifold concentration compared to the analyte
- quick binding, large range of detection
- signal is proportional to analyte level



”sandwich method módsze

Non competitive immunoassay





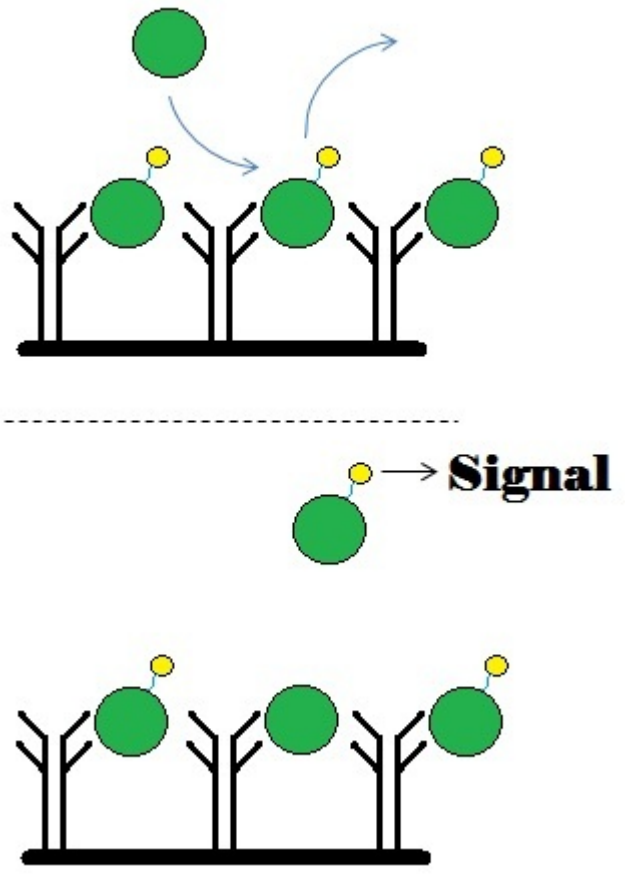
Linear regression fitting

Multiparametric fitting

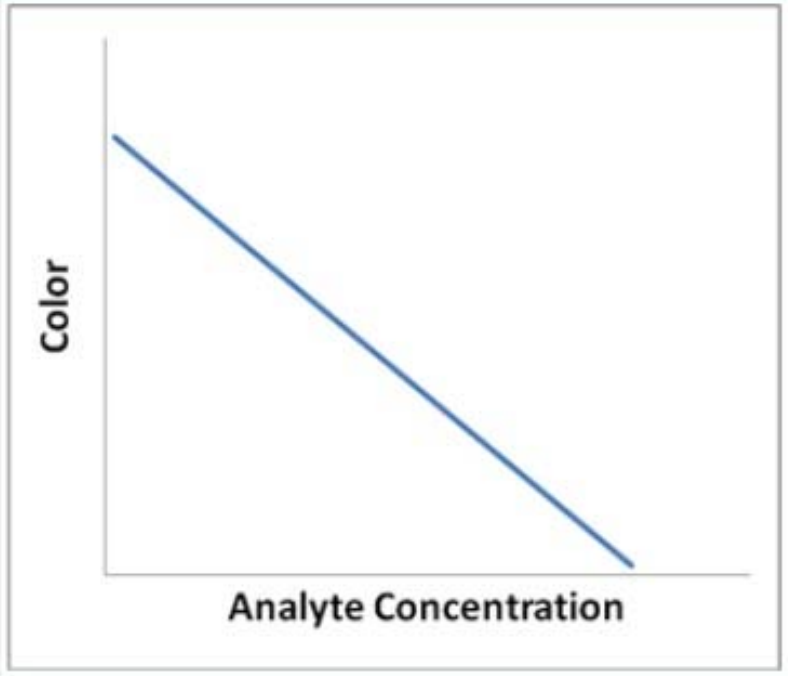
Competitive immunoassay

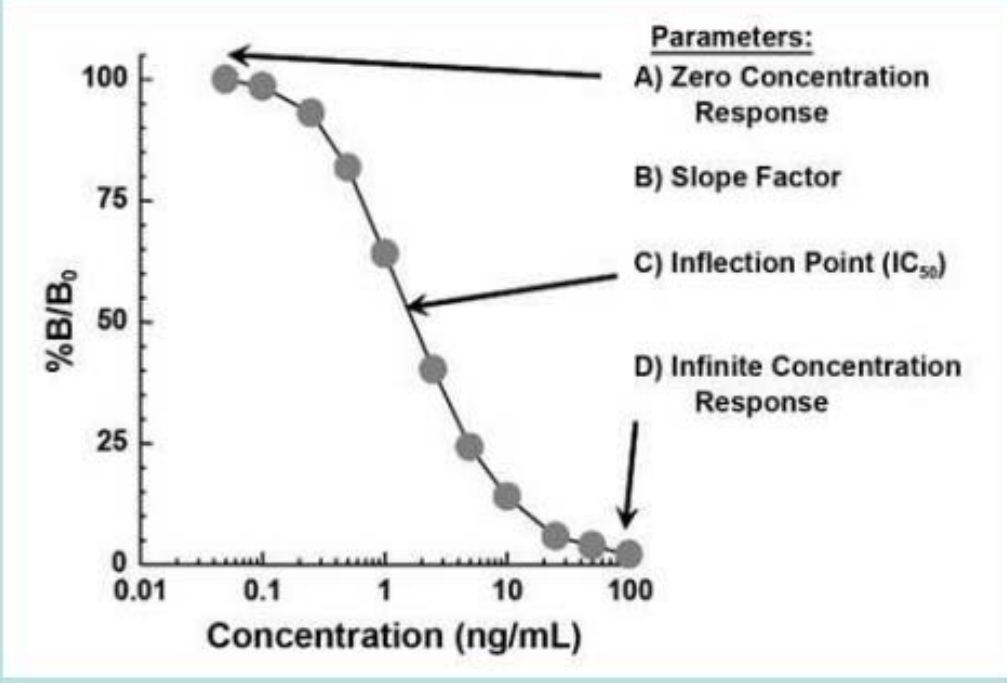
• Labelled Ag and Ab are present in a complex (Ab and Ag are negligible)

- Analyte is added; it replaces labelled Ag-decreaseing the amount of labelled immune complexes
- Signal is inversely proportional to the analyte



Competitive immunoassay





Separation of immune complexes

A key point ot separate immune complex from free Ab

-adsorption (medicinal charcoal, other adsorbent)

-precipitation (polyethylene-glycol)

- binding of one of the components to the solid phase

Radioimmunoassay (RIA)

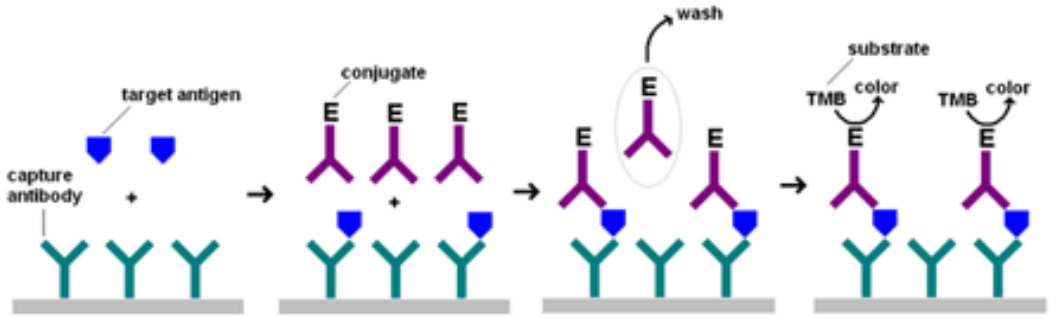
- Yalow and Berson 1959 (1977 Nobel-díj)
- ^{125}I →half life 57 days, hard γ-radiation (easy to detect)
- ^{3}H (tritium) → half life 12,7 év, soft β radiation (harder to detect)
- Nowadays ^{125}I is used for R&D or when the levels are in the range of attomol/L =10^{-18} mol/L)

Enzyme Immunoassay

• enzymes: -HR peroxidase

-alkaline phosfatase

-β-D galactosidase



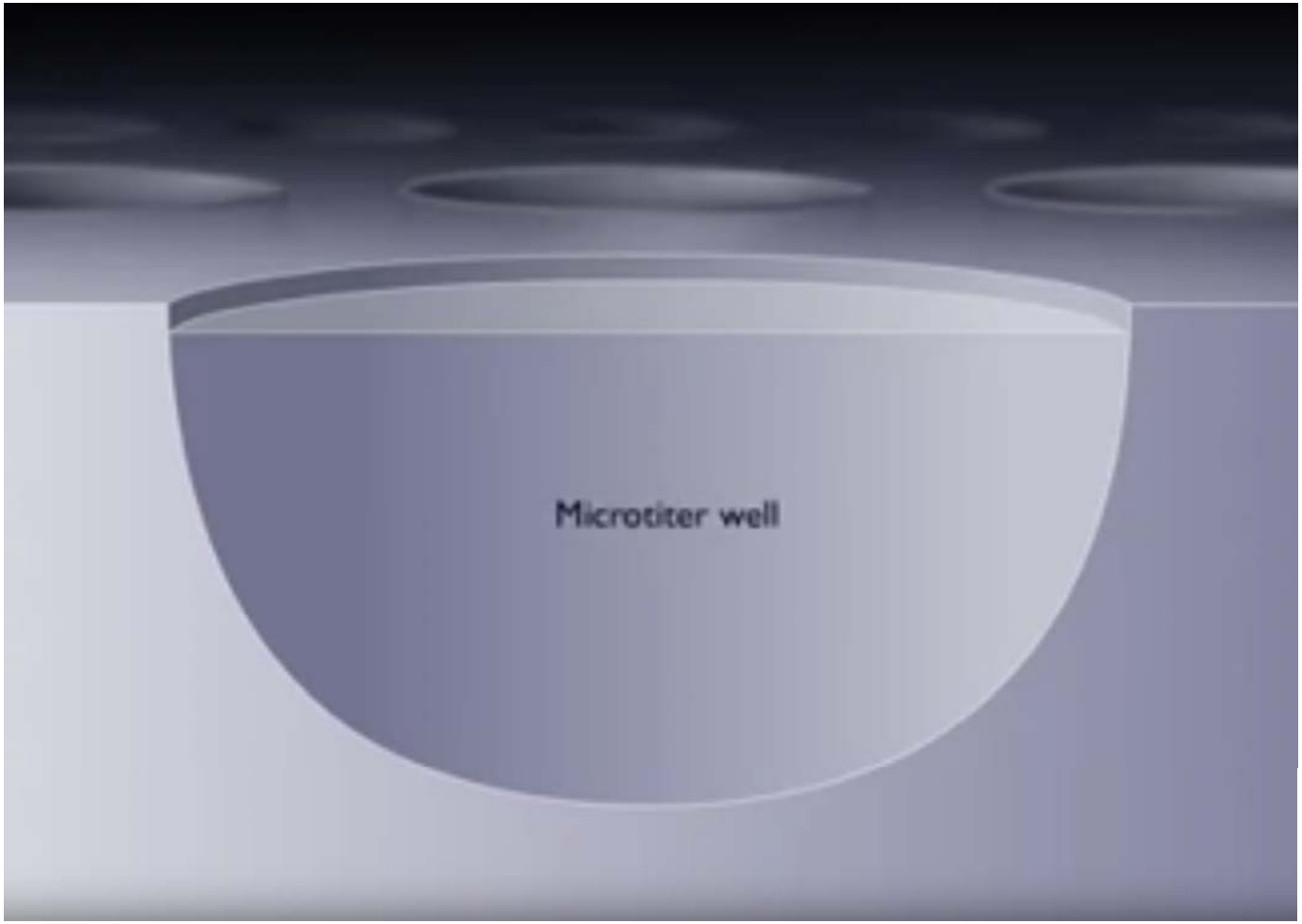
• enzyme activity → color reaction → photometry

•ELISA (IS=immunosorbent)

• initially: labelled molecules are attached to the tube wall or to polystriol beads. Large volume of reagent (1-2 ml) = ”macro” ELISA

• now: microtitrating polystirol plates (300 ul)=”micro” ELISA (specific device is required with appropriate photometer)

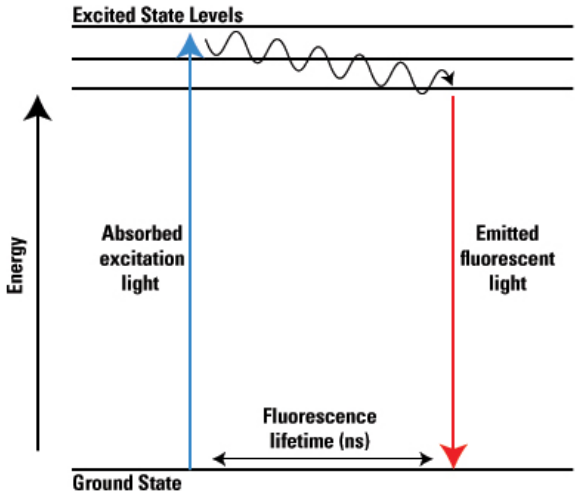
Principle of ELISA



https://www.youtube.com/watch?v=IUWpWKVcmc4

Fluorescent Immunoassay

- Similar sensitivity to RIA



- 2 major methods:

-FIA (Fluorescence Immunoassay): labelling with fluorescent molecules

-FPIA (Fluorescence Polarization IA): fluorescent light is separated with polarization screens. Signals over the optical plane (Ags not binding analytes) are not detected

- Both methods may be competitive and non-competitive

Luminescent Immunoassay (LIA)

- Luminophor substances does not require exogenous energy to produce light; they emit light upon dissociation (eg. oxidation)
- may be competitive and non-competitive
- Luminophor = luminol or isoluminol

Luminescent Immunoassay (LIA)

- **Luminofor cannot be bound directly to proteins (impaired light emission).**
- **Aminobuthyl is attached= N-aminobuthyl-ethyl-isoluminol (ABEI)**

Electrochemiluminscence technique (ECL)

- Half-life of substances used for LIA is short (shelf-life is short)
- In ’90s: light emission is reached by electrooxidation instead of chemical reaction
- Luminescent substance: organic ruthenium substance:

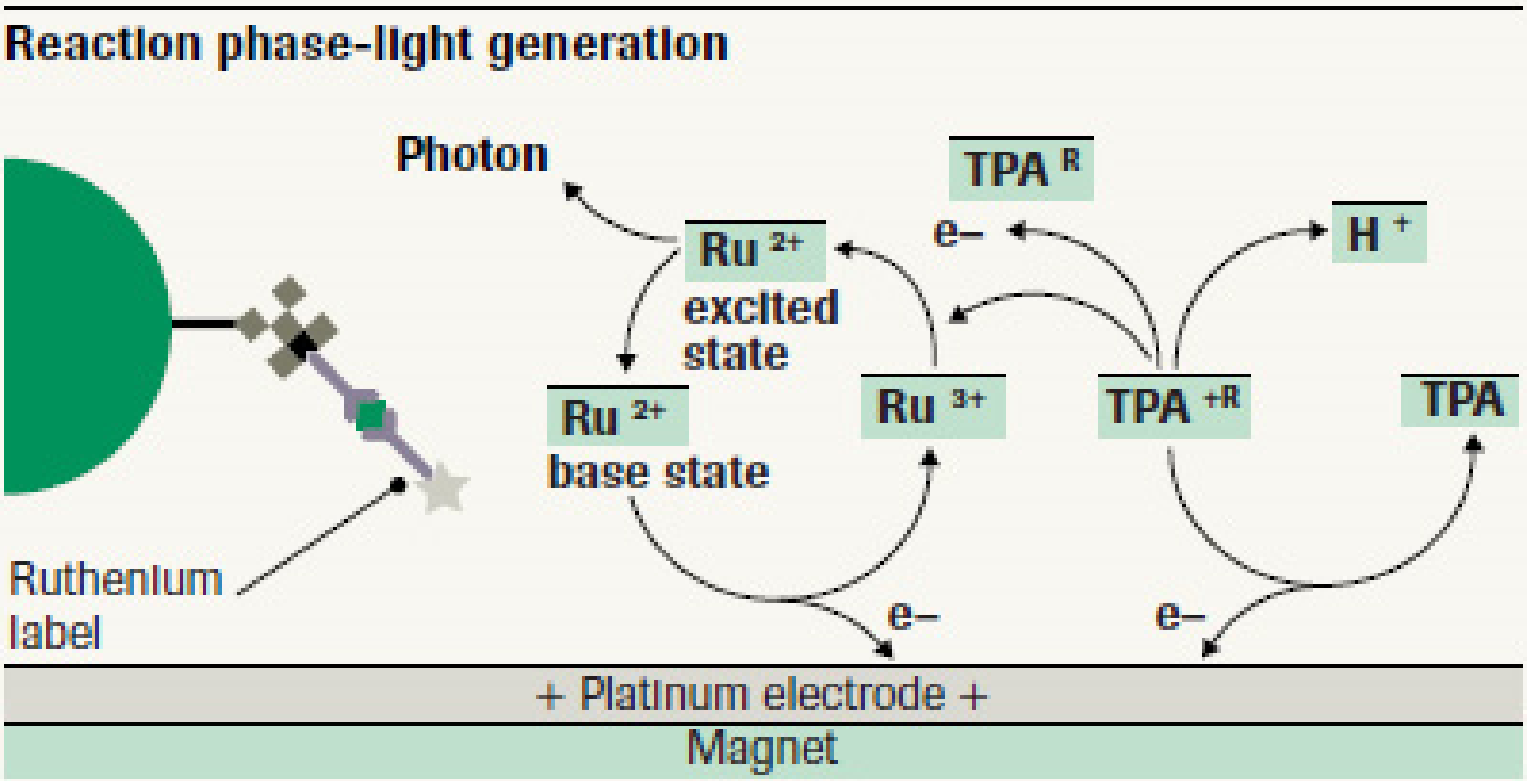
Ruthenium (II)-tris (2,2’-bipyridil)-N-hydroxy-succinimide esther

luminofor | Binding to protein

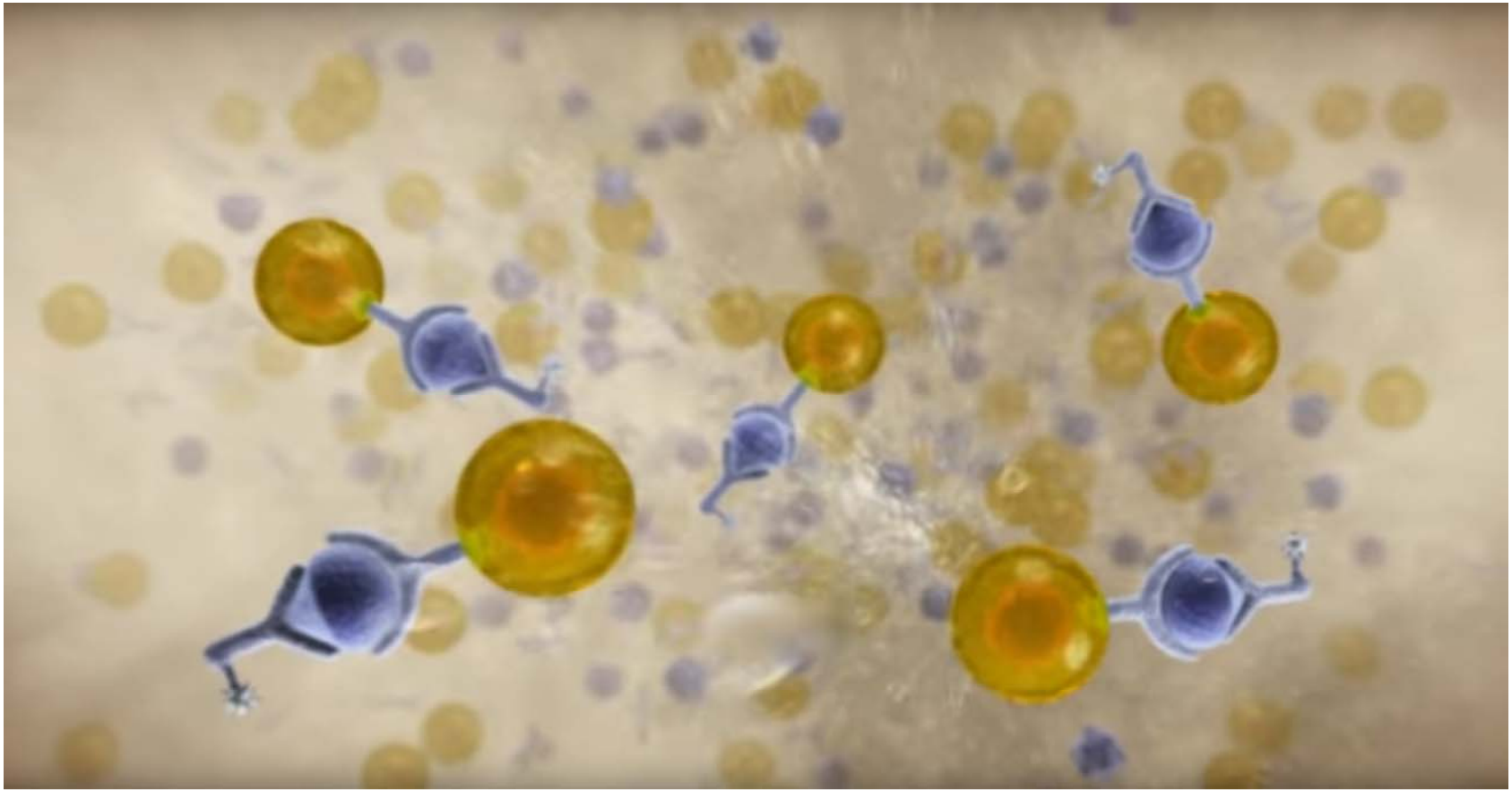
Electrochemiluminescence (ECL)

- A ruthenium (II) is a bivalent kation that is oxidized to a trivalent kation on the surface of a katode = ruthenium (III)
- Other component of reagent solution: tripropylamine. On katode surface a tripropylamine kation is generated that is converted to labile free radical. This reduces ruthenium (III) to (II).
- The excited molecule returns to baseline energy level emitting red light (detected at 620nm).

Electrochemiluminescence (ECL)



How Electrochemiluminescence (ECL) Works



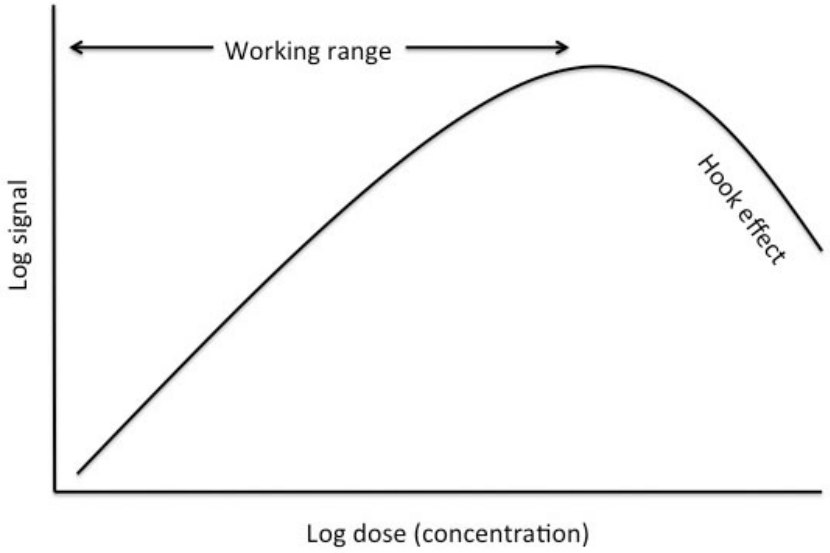
https://www.youtube.com/watch?v=PHbcvBGfzMM&list=PLRtEjgHlVIxFBqrdTltyK6_CQYf_wuTY-

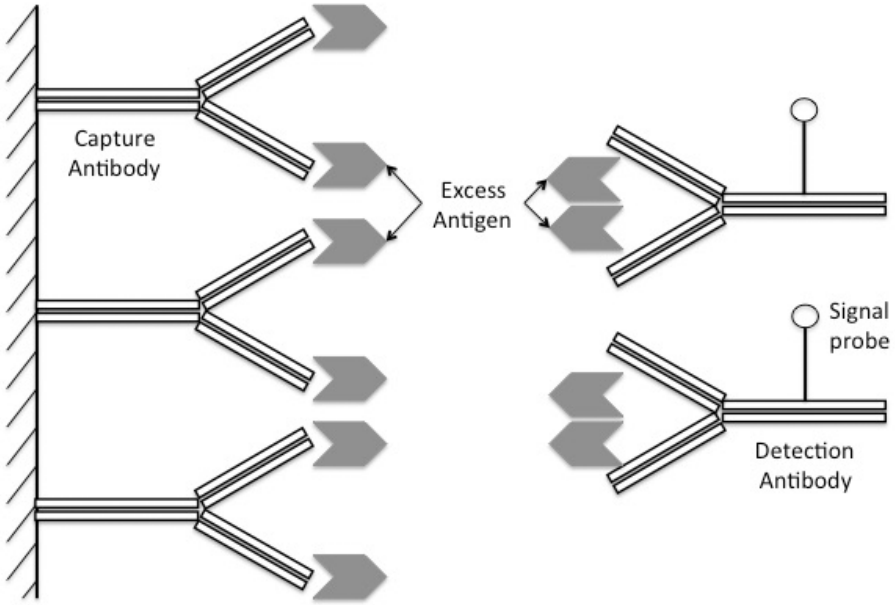
Electrochemiluminescence (ECL)

- Available assays are non-competitive
- solid phase = paramagnetic beads
- bind streptavidin (covalently), biotinylated Ab-s are attached

Interfering factors:
2 major comments

Hook effect


Working range
Hook effect
Log signal
Log dose (concentration)


Capture
Antibody
Excess
Antigen
Signal
probe
Detection
Antibody

Human anti-animal antibodies

- Humans exposed to animals can produce antibodies to animal immunoglobulins
  - Heterophilic antibodies
  - Anti-isotypic
  - Anti-idiotypic
- Human anti-mouse antibodies (HAMA) are most common
- Anti-animal antibodies can cross-link capture and detection reagent antibodies