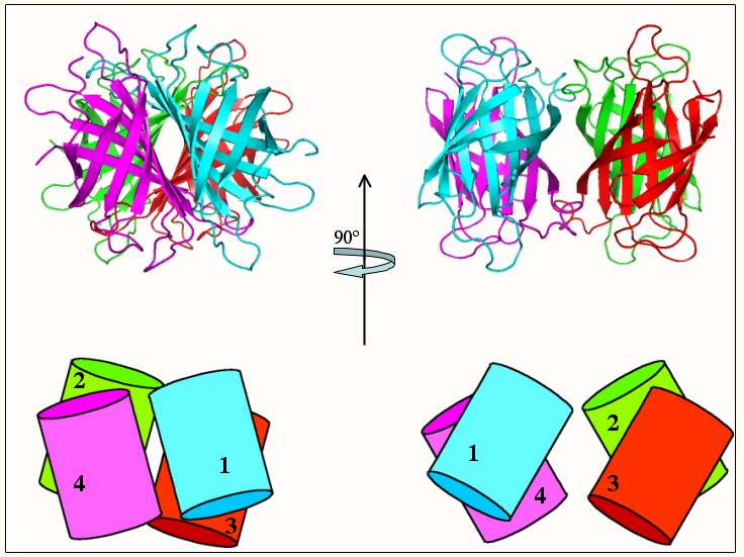
Avidine – biotin interaction: partners

- Avidin: 4 subunits, 16 400 Da → 66 000 Da glycoprotein
  - Green NM „Avidin" Adv Prot Chem 29 85 (1975)
  - biotin binding site/subunit
  - CH binding site/subunit
  - pI ~ 10
  - stability (8 M UREA, 3M Guanidin·HCl)
  - non-specific binding as well

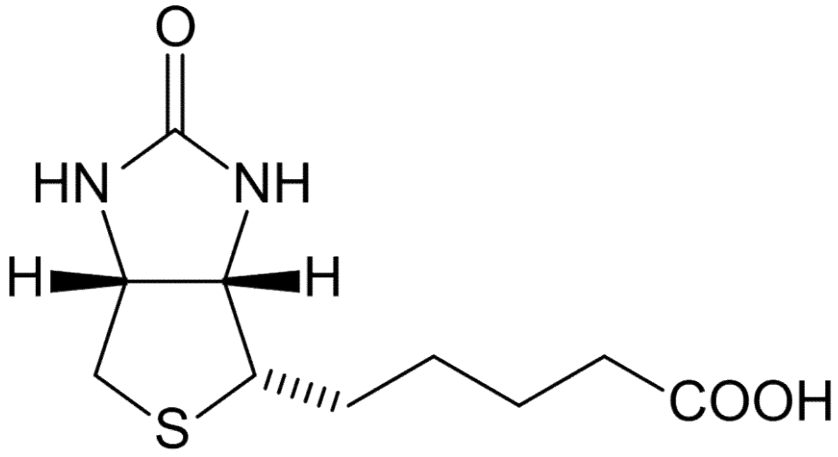
- Streptavidin „Streptomyces avidinii"
  4 subunits, 60 000 Da protein
  - biotin binding site/subunit
  - pI 5 – 6
  - lower level non-specific binding



www.weizmann.ac.il

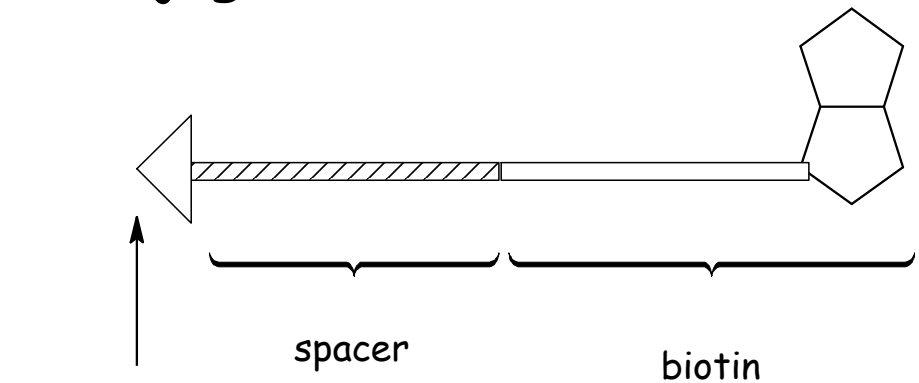
- Biotin (D-biotin, vitamin H, vitamin B_7, coenzyme R)

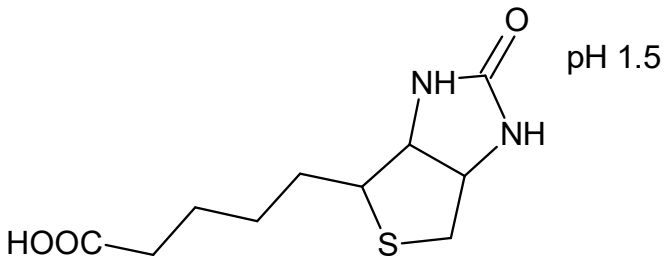
  Hexahydro-2-oxo-1H-thieno(3,4-d)imidazole-4-pentanoic acid



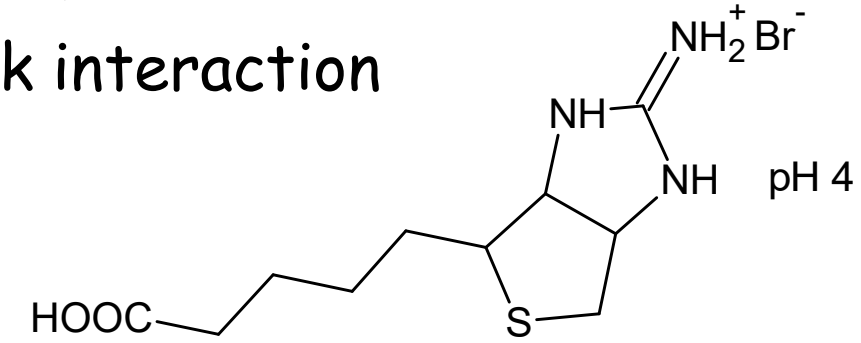
  - in all cells, small amount
  - cofactor R (CO_2 transport)
  - source/derivative: attached to ε-amino group of Lys ➡BIOCYTIN
  - deficiencies (1927, hair loss, dermatitis, conjunctivitis)

- Conjugates

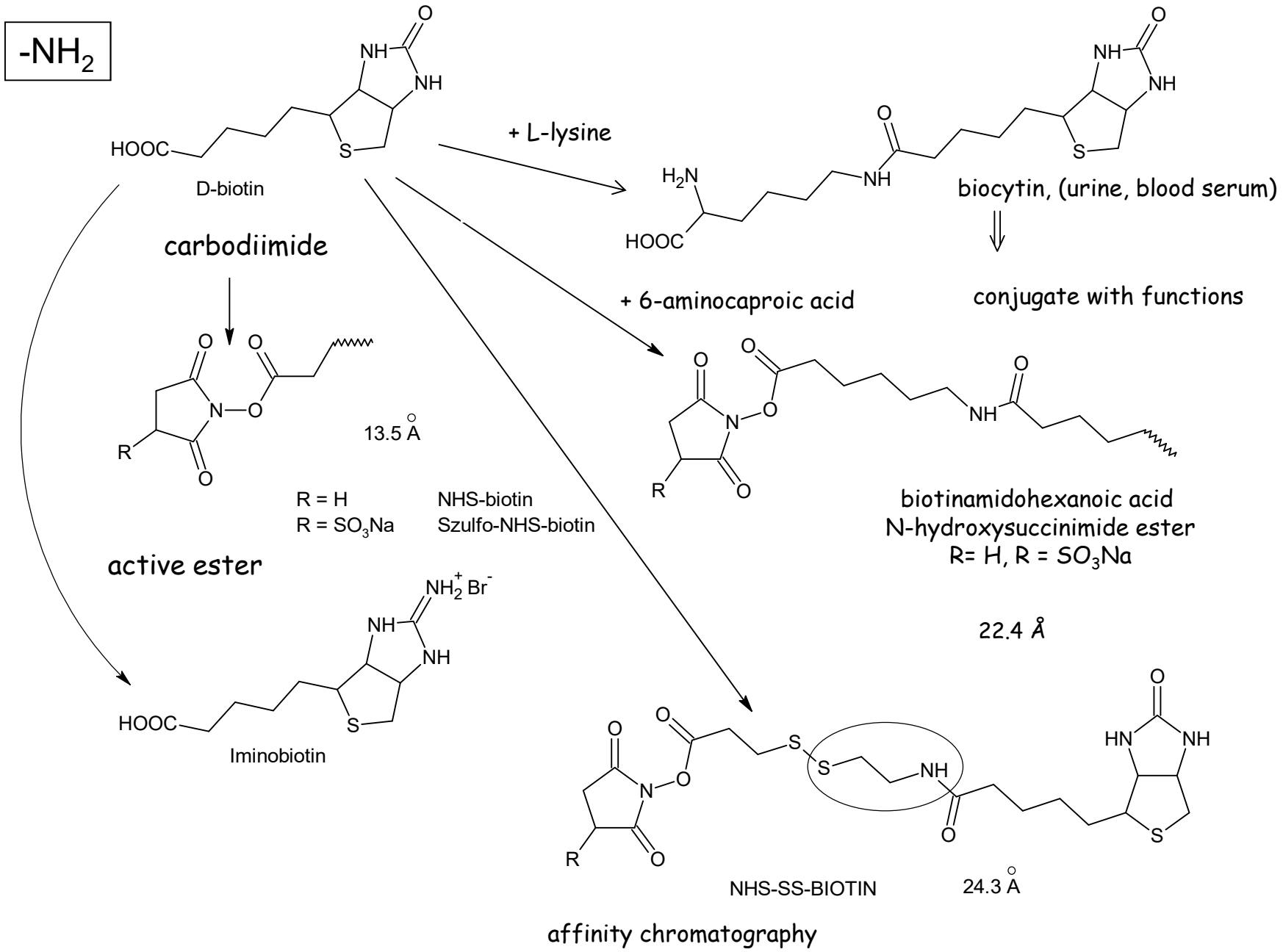




- Approaches
  - depth of the binding site: 9 Å
  - biotin derivatives → weak interaction
    - e.g. iminobiotin

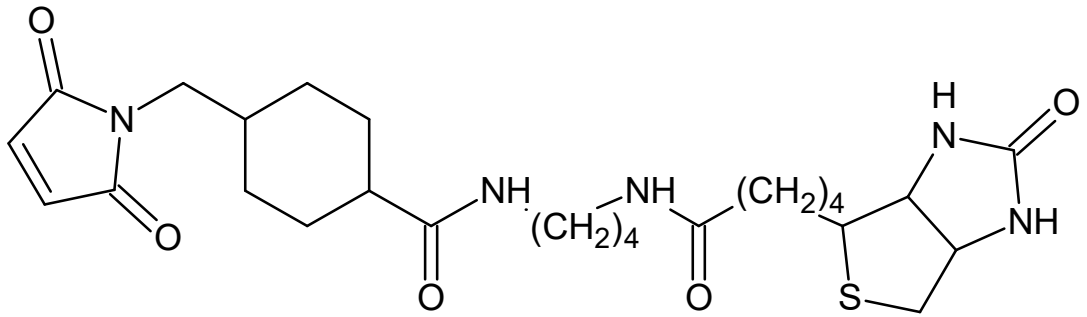


- Labelling with biotin
  - -NH_2, -SH, -C=O, -COOH, photoreactive group


-NH2
D-biotin
+ L-lysine
biocytin, (urine, blood serum)
conjugate with functions
carbodiimide
+ 6-aminocaproic acid
13.5 Å
R = H
R = SO3Na
NHS-biotin
Szulfo-NHS-biotin
active ester
biotinamidohexanoic acid
N-hydroxysuccinimide ester
R= H, R = SO3Na
22.4 Å
Iminobiotin
NHS-SS-BIOTIN
24.3 Å
affinity chromatography

-SH

a



MALEIMIDO — BUTANE — BIOTIN

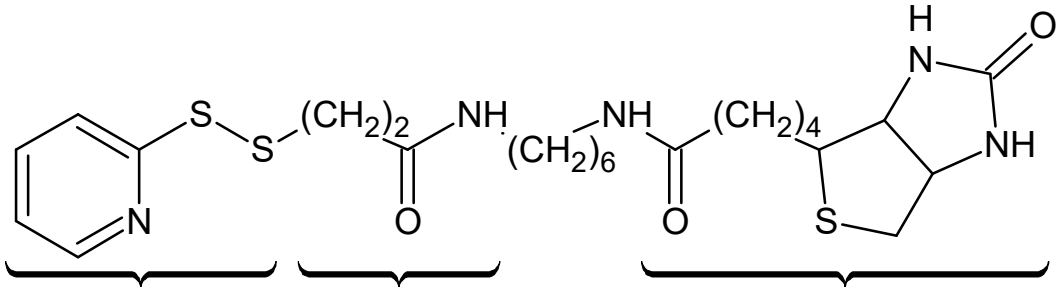
- pH 6.5-7.5
- DMF/DMSO

32.6 Å

BIOTIN-BMCC

thioether bond

b



2-pyridyl-dithio — propionic acid amide — Biotin

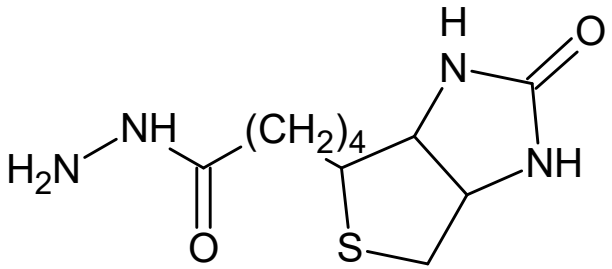
- pH 7 EDTA
- DMF/DMSO

29.2 Å

BIOTIN-HPDP

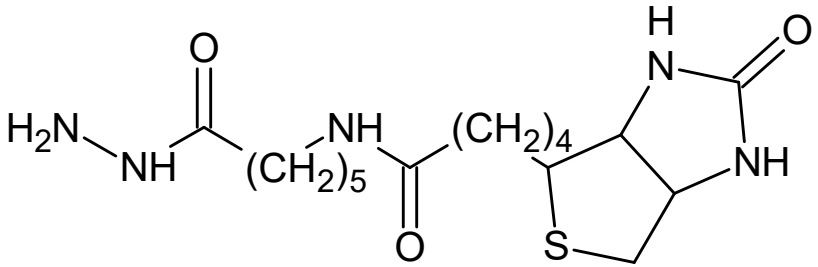
disulphide bond

-CHO



biotin hydrazide

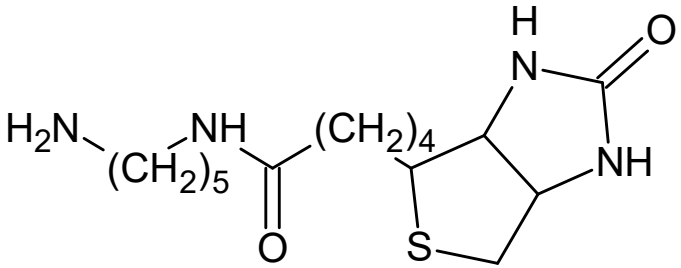
15.7 Å



biotin – LC - hydrazide

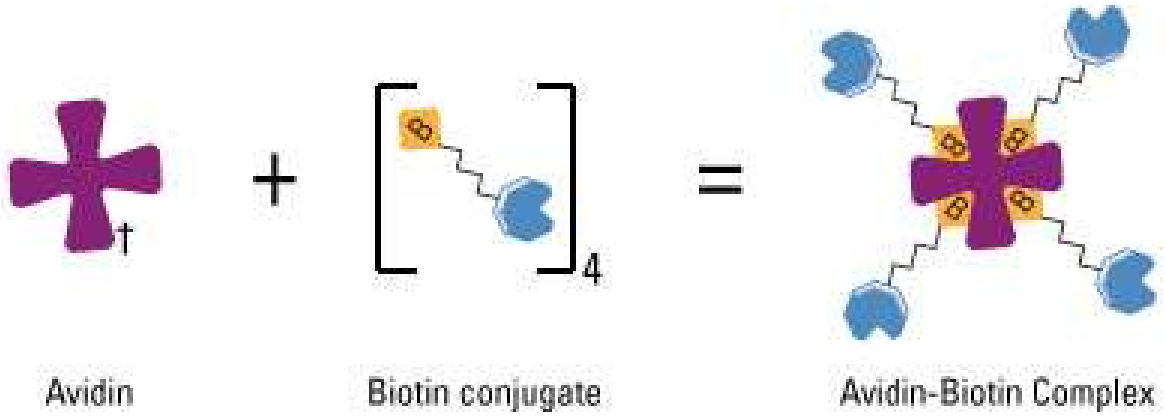
24.7 Å

-COOH



5-(biotinamido)pentylamine

Avidin – biotin complex



K_d = 1.3*10^{-15} M (Trp, Lys in the binding site)

www.thermofisher.com

Preparation and characterization of bioconjugates

A. Synthesis

1) Reactions

Single pot – single step

- two partners e.g. direct labelling
- two partners + coupling reagent e.g. homobifunctional reagent

Two-step

- two partners + coupling reagent:

  first step: partner No.1 + coupling reagent

  second step: „activated" partner No.1 + partner No.2

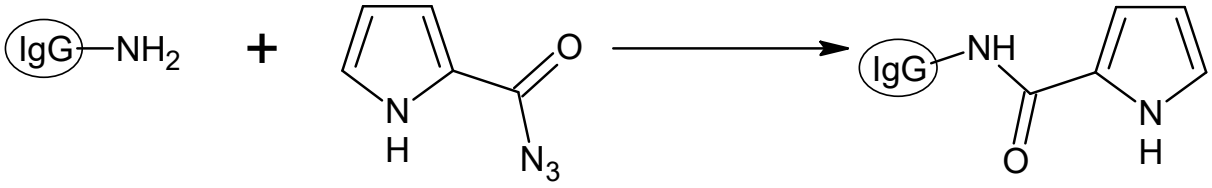
  e.g. heterobifuncitional reagent (MBS)

  homobifunctional reagent

  (toluol-2,4-diisocyanate, glutaraldehyde)

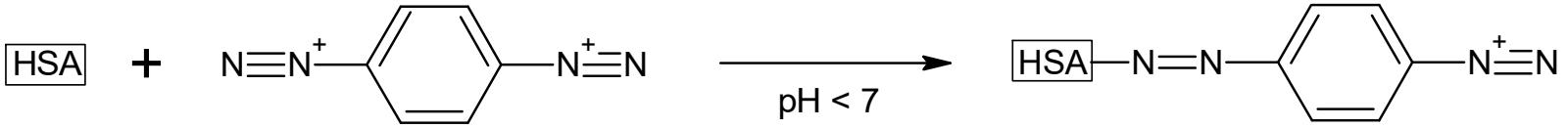
Three-step

1.



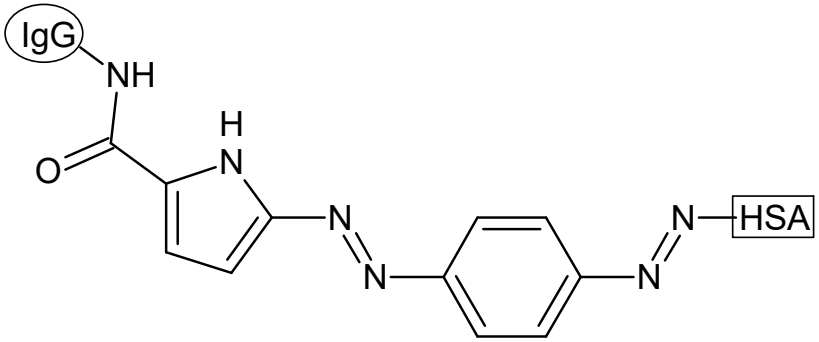
pyrrol-α-acylazide

2.



bis-diazo-p-phenylene diamine

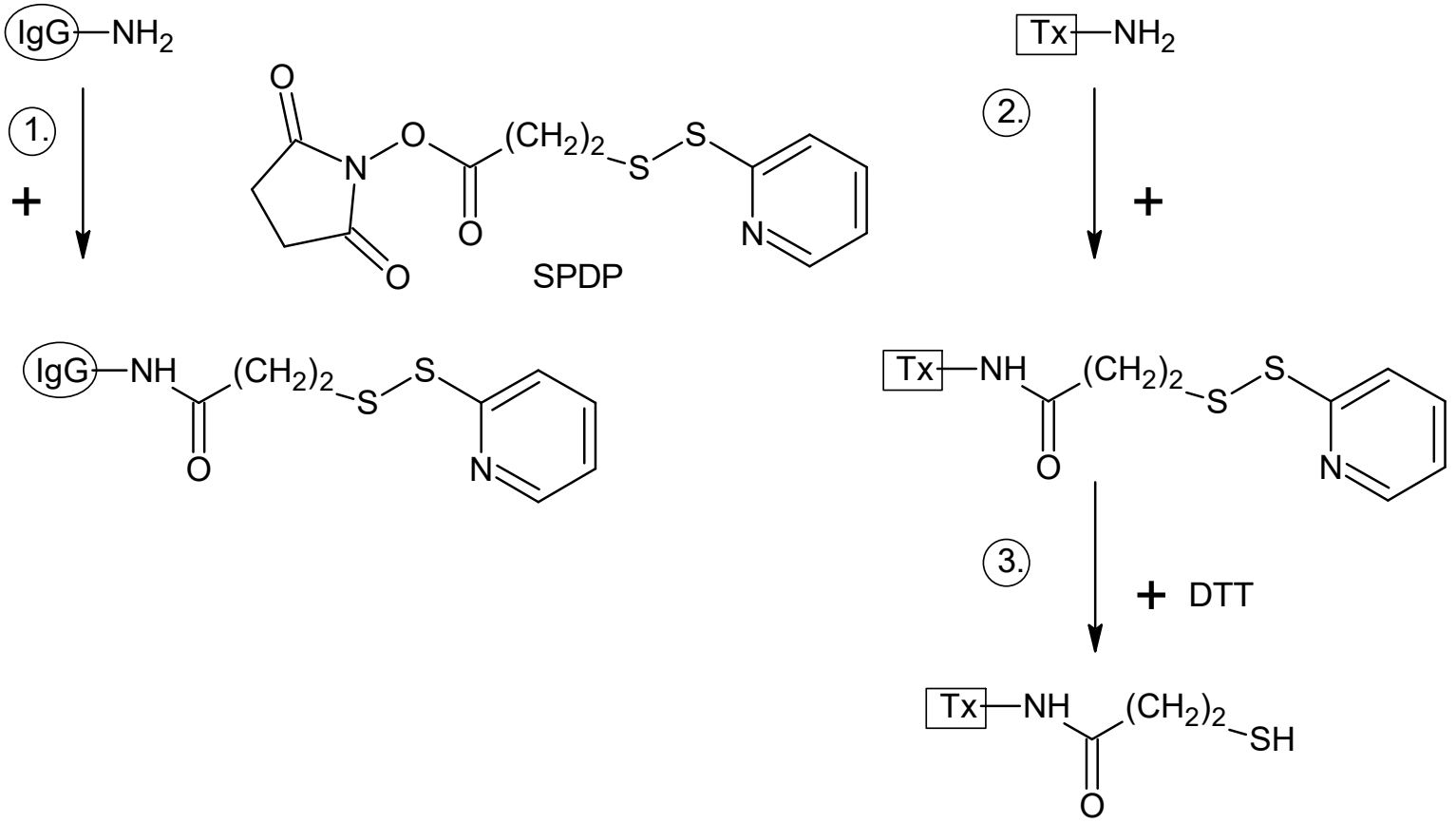
3.



Howard AN, Wild F: Br J Exp Pathol 38 640 (1957)

e.g. immunoconjugates, immunotoxins

Four-step


IgG—NH2
1.
+
SPDP
Tx—NH2
2.
+
IgG—NH (CH2)2 S S N
Tx—NH (CH2)2 S S N
3.
+ DTT
Tx—NH (CH2)2 SH
4.
Tx
HN
IgG—NH (CH2)2 S S (CH2)2 O

2) Reaction conditions

- choice of reagent / coupling agent
- pH [reagent, functional groups]
- reaction time [30 min – 24 h]
- light [UV – visible]
- temperature
- ionic strength [ionization degree, conformation]
- molar ratio
- concentration
- solubility
- solvent

⇓

Strategy

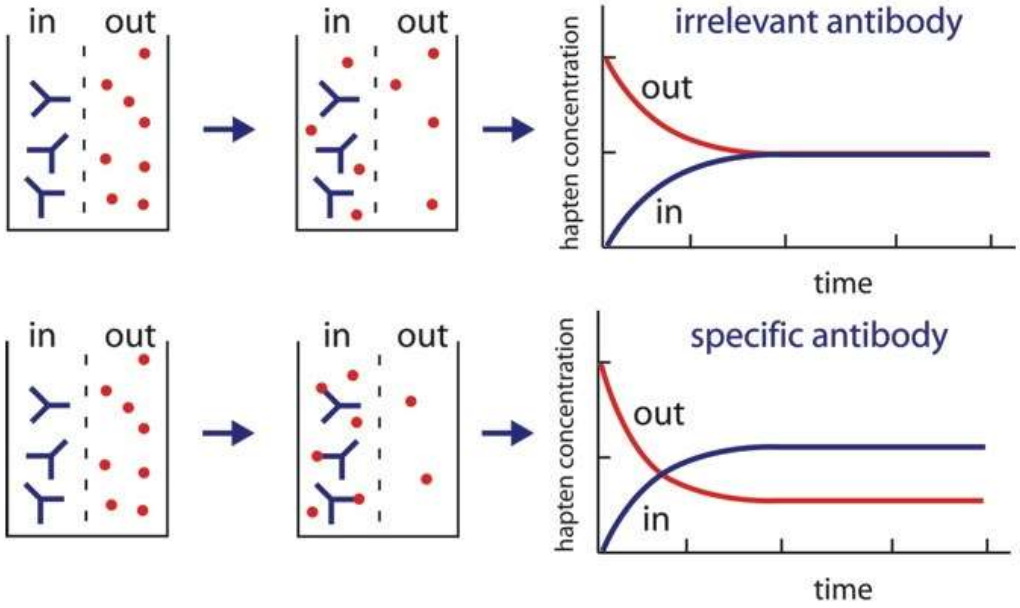
e.g. *N*-hydroxy succinimid – hydrolisis
– small dependence on temperature
– pronounced pH effect

10 min at pH 8.6
4-5 h at pH 7.0
– concentration 0.05-9 mM

B. Analytical methods [for small – big, big – big]

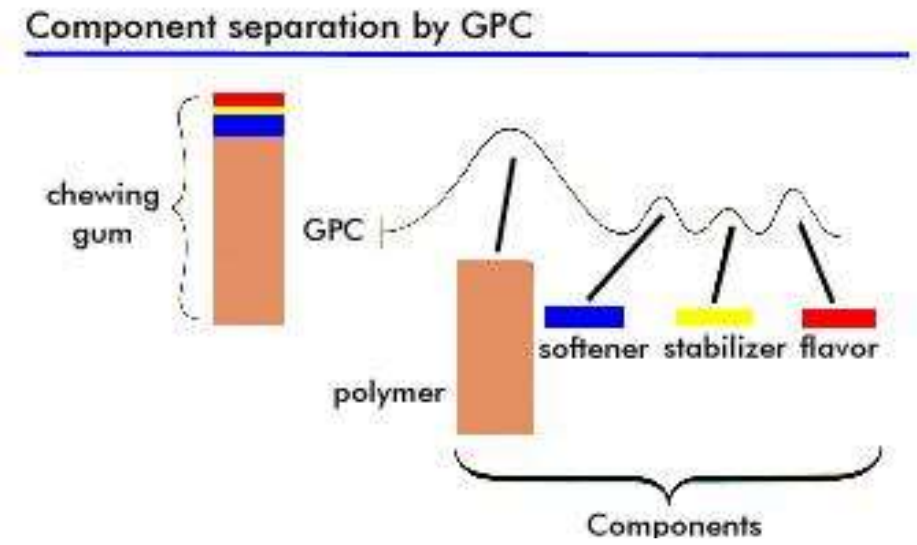
- dialysis (**dialysis**, meaning *dissolution*, **dia**, meaning *through*, and **lysis**, meaning *loosening or splitting*, Greek)
  - molecular mass – length of time
  - molecular size/shape – „change"
  - molecular charge – solvents

equilibrium dialysis



http://nfs.unipv.it/nfs/minf/dispense/immunology/lectures/files/antigens_antibodies.html

- UC – molecular size/shape – temperature

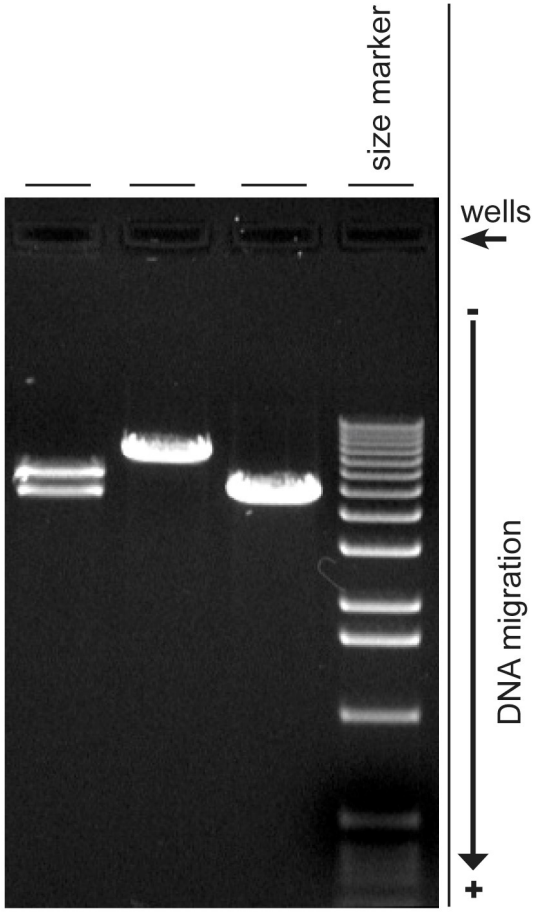


- Bio-Gel Permeation Chromatography (GPC)
  - molecular shape
  - detection method (cpm, UV etc.)
  - eluents (solubility, stability)
  - molecular size - standards

http://www.waters.com/waters/en_HK/GPC---Gel-Permeation-Chromatography-Beginner%27s-Guide/nav.htm?cid=10167568&locale=en_HK

- HPLC

- Electrophoresis
  - molecular mass/shape
  - molecular charge
  - detection – labelling
    - staining
    - cpm
    - UV, fluorescence
  - functional
    - enzyme activity
    - immunoreactivity



- forms
  - paper
  - gel (PAGE, AGAR)

} one/two dimension

c. Complications, problems

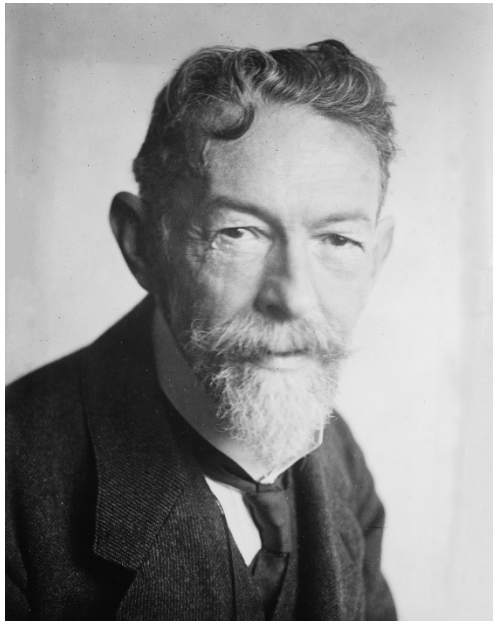
- heterogeneity – characterization / solubility
- reproducibility /specificity, selectivity/
- determination of the degree of substitution (spectroscopy, cpm, MS … etc.)
- stability („shelf", solution vs. time, conditions)
  - chemical (e.g. aggregation)
  - functional (e.g. enzyme)
- immunogenicity / allergizing effect
- denaturation
- biodistribution
- cellular uptake

A „golden" story

Zsigmondy R. (1905) colloid golden-sol (< 10 nm)

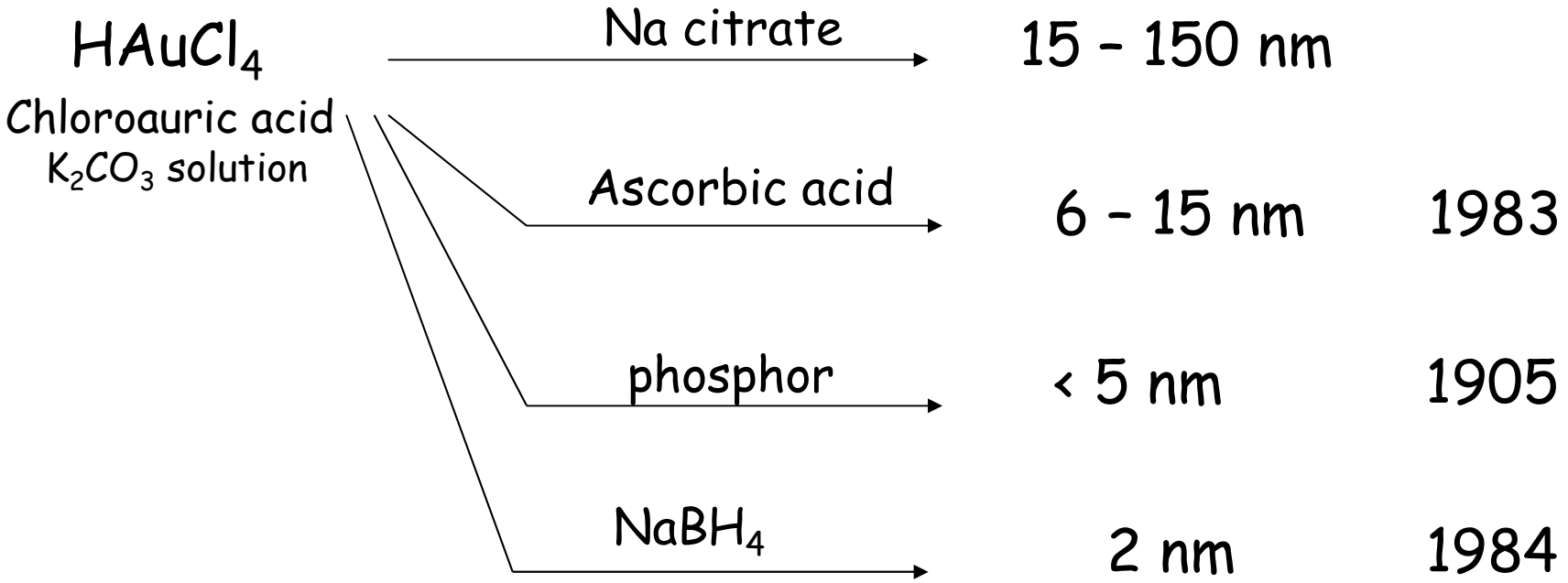
Faulk WP, Taylor GM (1971) „Immunogold" staining electronmicroscopy Immunochemistry 8 1081-1083

- blotting
- flow-cytometry
- hybridization (1990/1991)



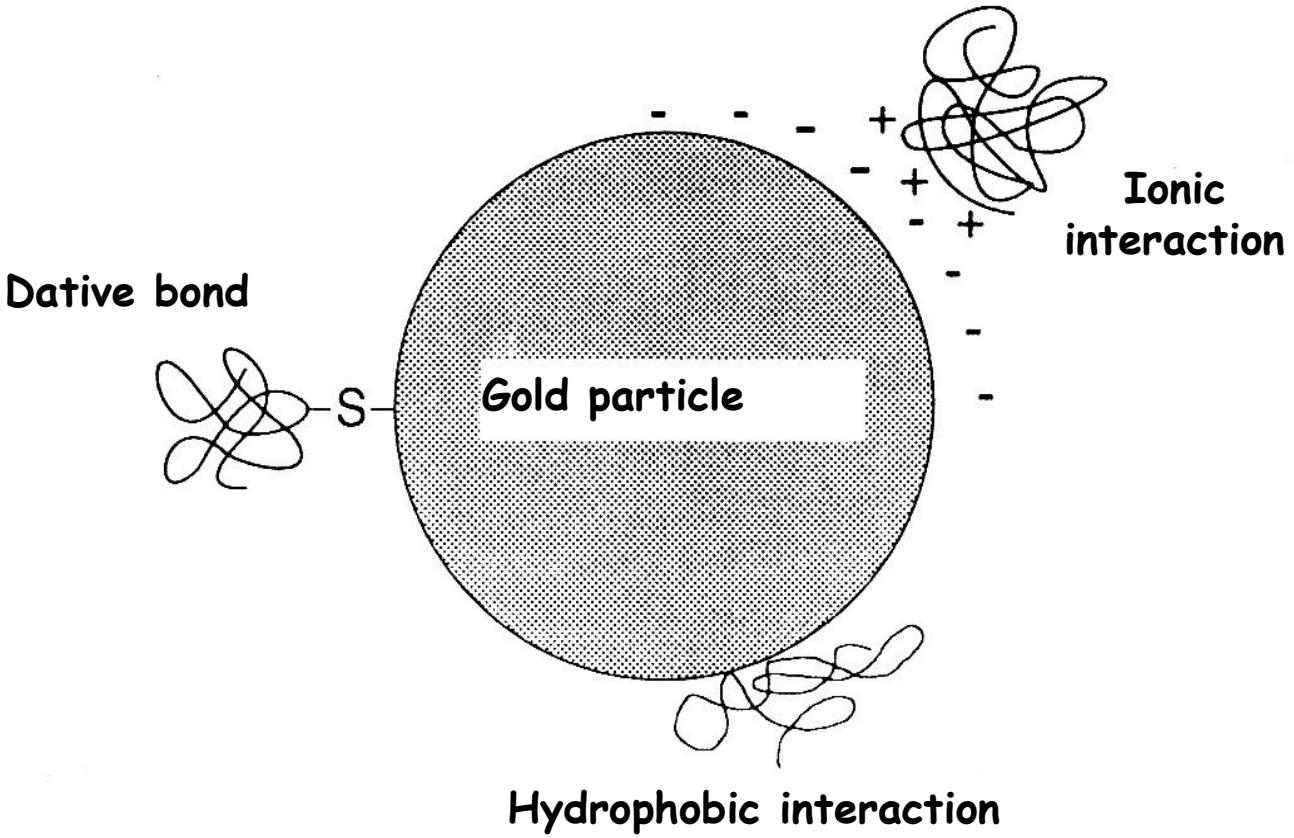
(Vienna, 1865 – Göttingen, 1929)
Nobel prize, 1925

1. Preparation of monodisperse gold suspension



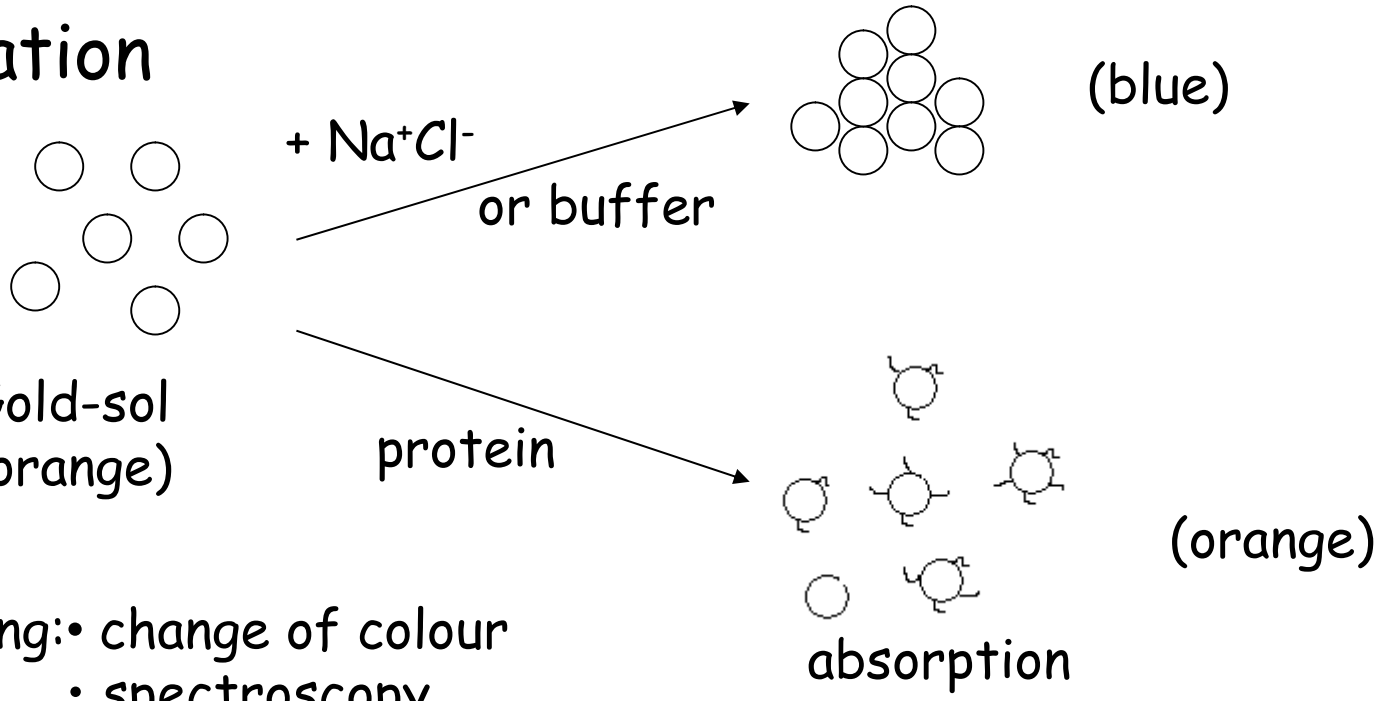
Σ Reduction: $Au^{3+} \rightarrow Au^{o}$

2. Protein – gold conjugate



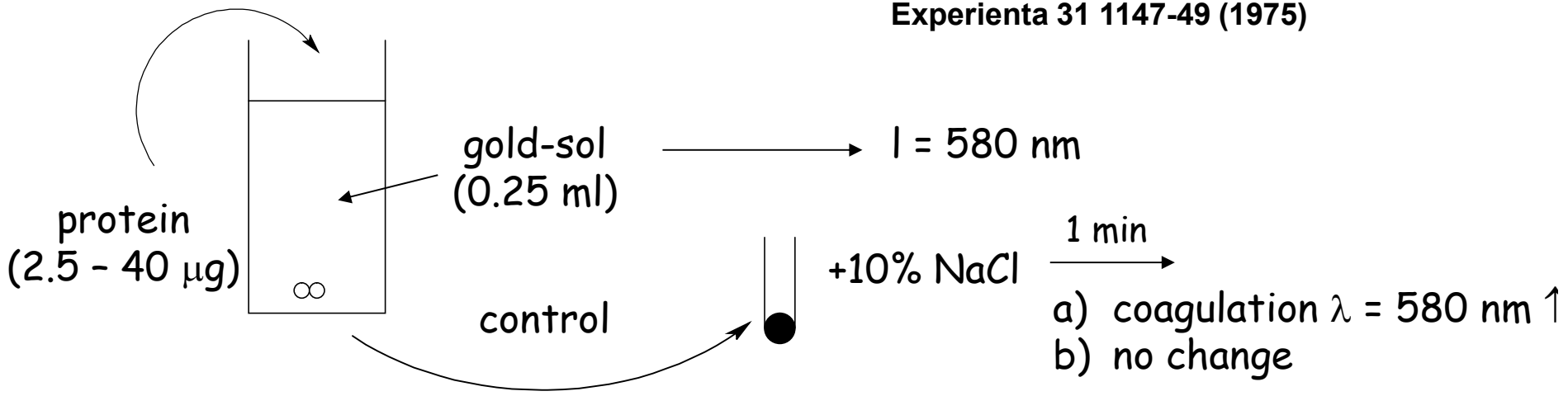
Deryagin BV, Landau L (1941) Acta Physiochim VRSS 14 633-662
Verwey EJW, Overbeck JTG (1948) „Theory of the stability of lyophobic colloids" Elsevie

Preparation



monitoring:• change of colour
• spectroscopy
at λ = 580 nm change

Horisberger M et al.
Experienta 31 1147-49 (1975)



Effect

- charge of the protein ➡ pI
- pH

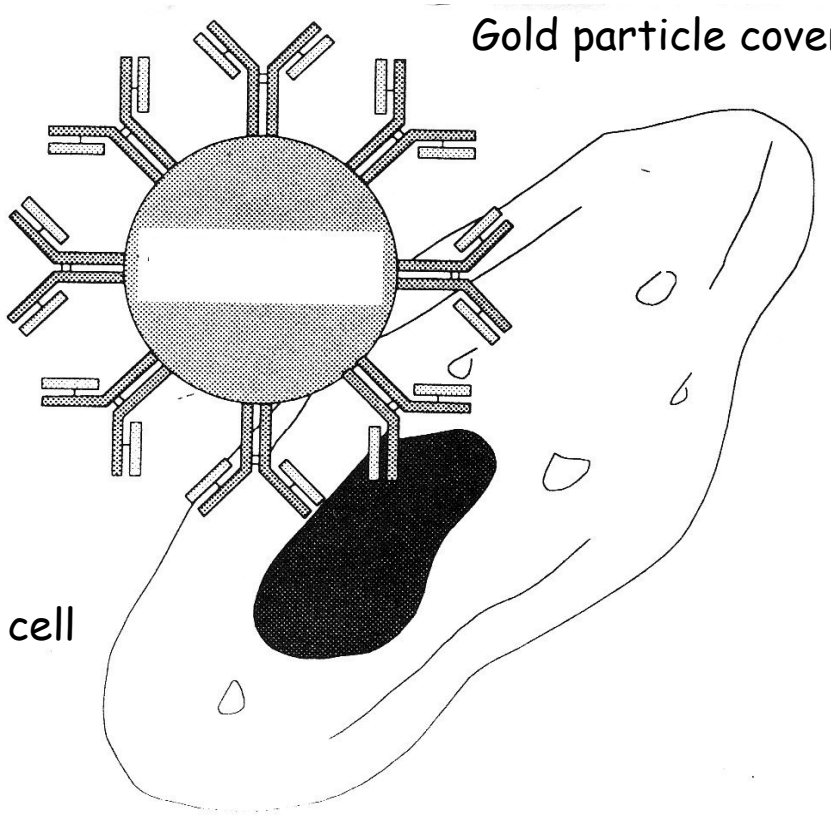
Practice

- probes → no universal protocol
- 10% extra of protein to use
- pH around should be used

Advantages

- visualisation / microscopy
- stable (vs. fluoropkore, chromophore)
- safety (vs. radioisotope)
- quantitative measurements

Gold particle covered by antibodies

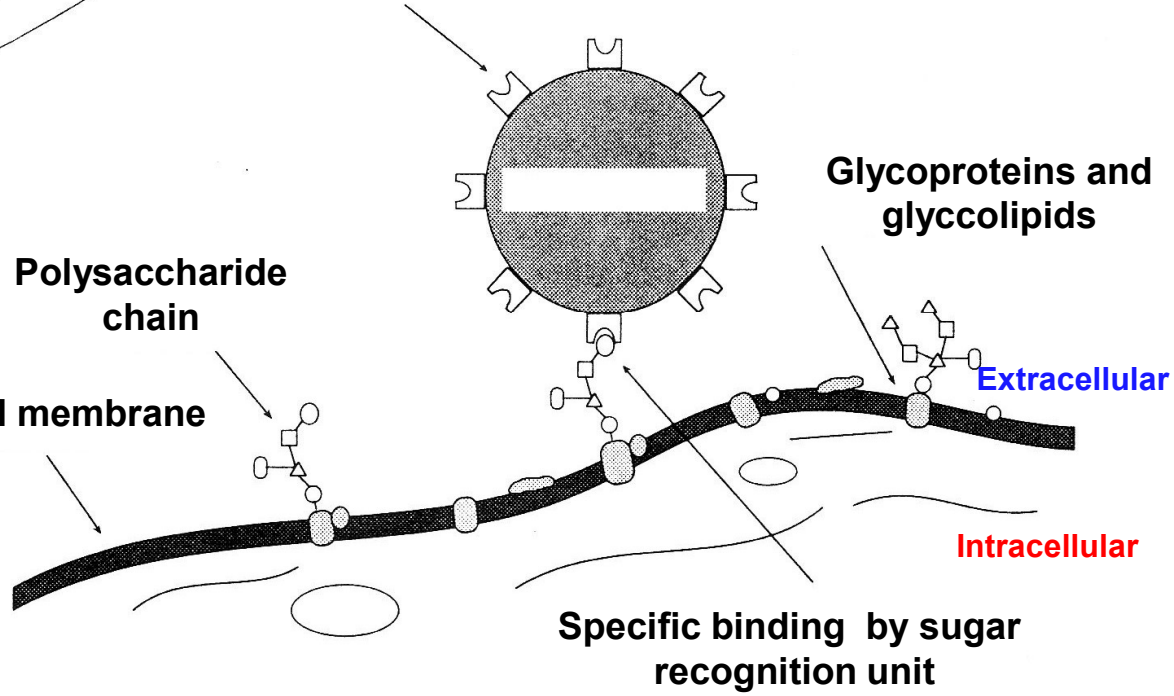


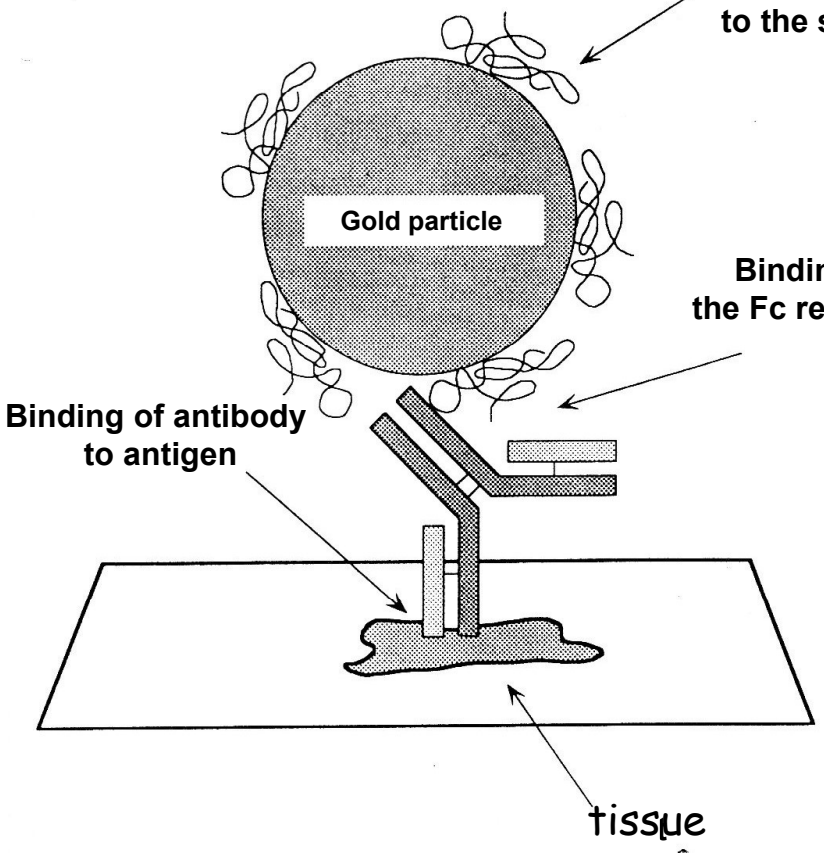
Target cell

Ellis IO et al.
J Histochem Cytochem 36 121-124 (1988)

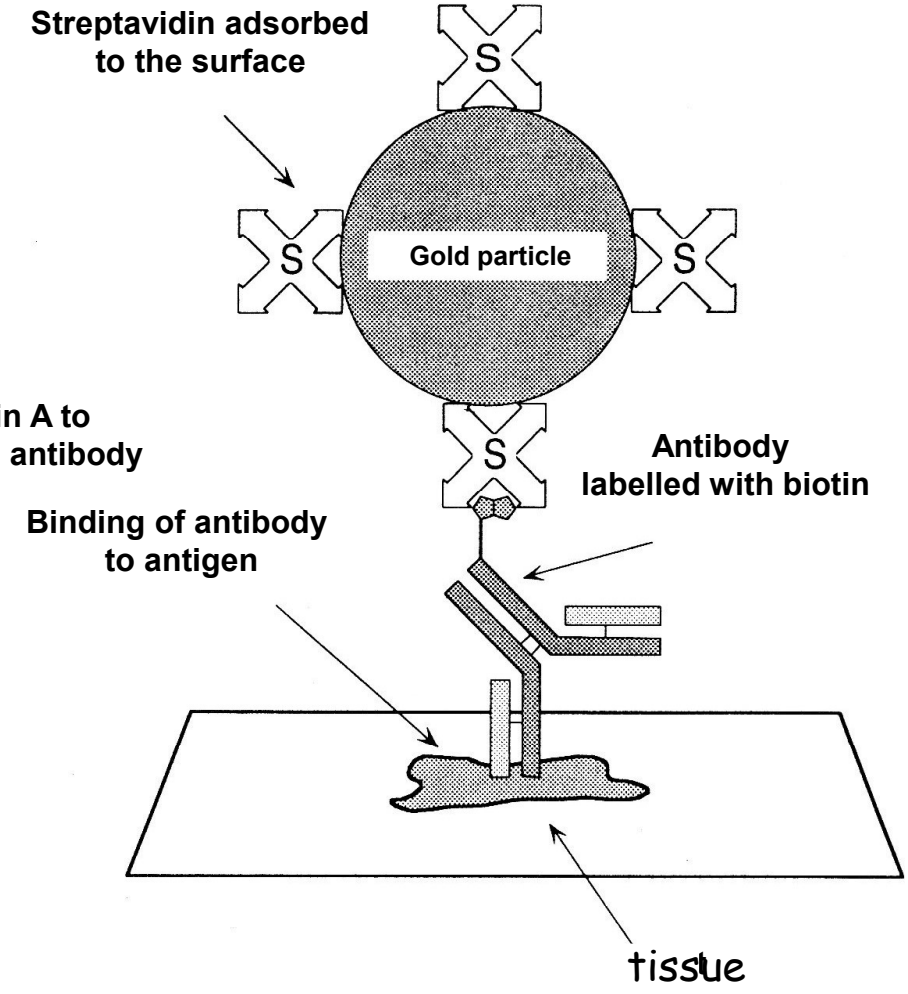
BOG-Hansen TC et al.
J Immunol Meth 22 293 (1978)

Gold particle covered by lectins





Jemmenson R, Agre M
J Histochem Cytochem 35 1277 (1987)



Moreis RE, Saelinger CB
J Histochem Cytochem 32 124-128 (1984)