# "The avidin(streptavidin)–biotin-system: basics and potential applications in TASNANO"

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# Lecture outline

Biotin: structure & biological role
Avidin & relatives
Streptavidin
Basic configurations of biotin-avidin based detection systems
Basic configurations of biotin-avidin based immobilization systems
Biotinylation procedures
Biotinylated molecules and their applications
(Strept-)Avidin conjugates and their applications
Applications of biotin-avidin system in theTASNANO projec

# General

Avidin is a protein found in egg white.

Streptavidin is a protein found in *Streptomyces avidinii.*

Both proteins have the ability to bind with very high affinity the vitamin biotin.

This interaction represent a natural defense mechanism because the binding with avidin or streptavidin of biotinylated enzymes that participate in $CO_2$ transfer inactivates the enzymes and thus inhibits the growth of bacteria that depend on biotinylated enzymes.

# Biotin or Vitamin H

Biotin is a member of the B-vitamin family.

Biotin is an essential nutrient in human nutrition.

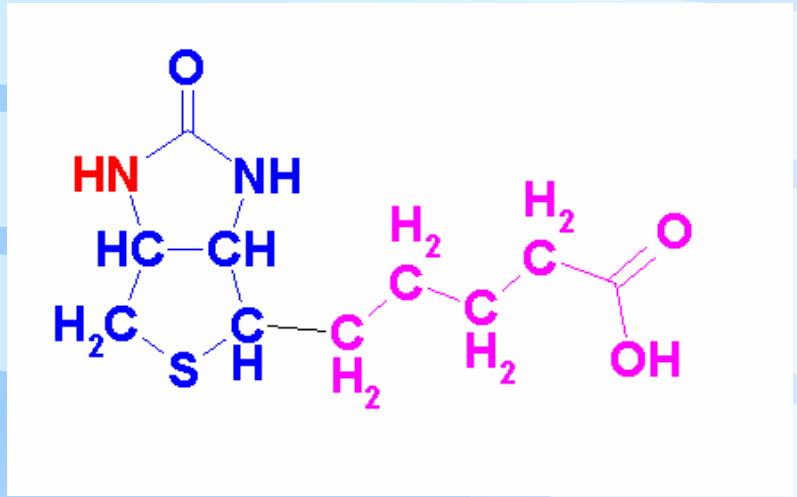
It is involved in the biosynthesis of fatty acids, gluconeogenesis, energy production, the metabolism of the branched-chain amino acids (L-leucine, L-isoleucine, L-valine) and the *de novo* synthesis of purine nucleotides.

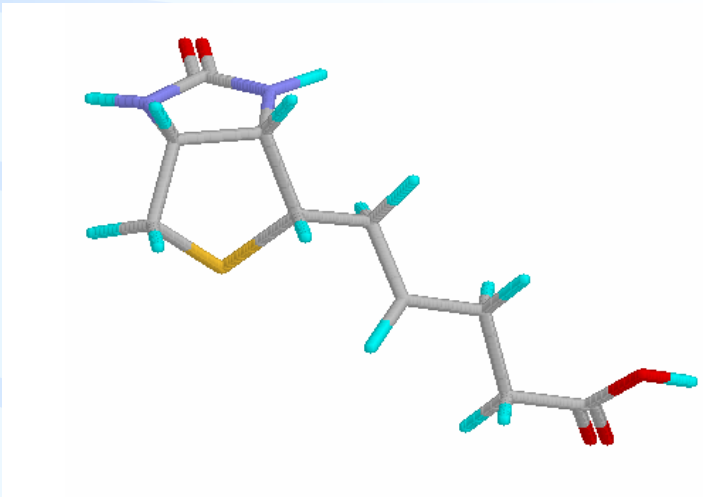
Recent research indicates that biotin plays a role in gene expression, both at the transcriptional and translational levels, and that it may also play a role in DNA replication.

# Structure of biotin

Biotin ($C_{10}H_{16}N_2O_3S$) MW = 244.31
is composed of

- an ureido ring fused with a tetrahydrothiophene ring
- a valeric acid substituent attached to 1 of the 2 carbon atoms of the tetrahydrothiophene ring



Chemical structure of d-(+)-biotin

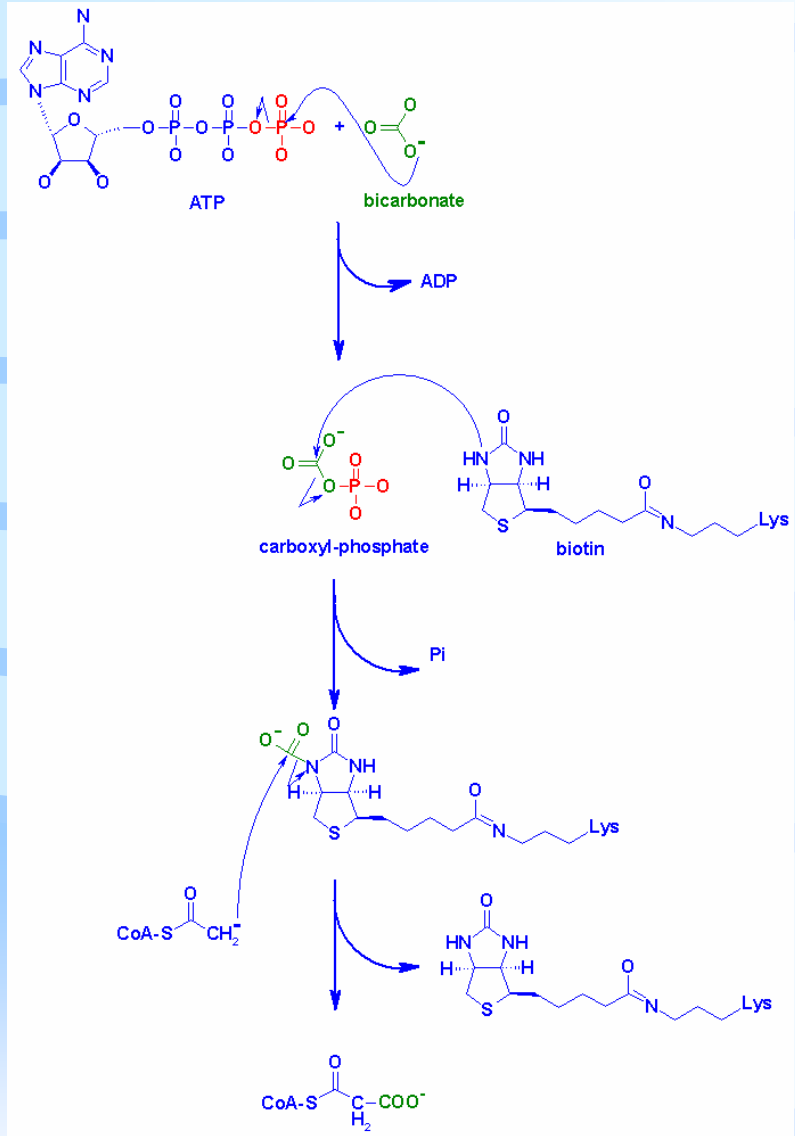
# Biological function of biotin

Through its carboxyl group, biotin is linked covalently to the e-amino group of lysine in four carboxylases that play critical roles in intermediary metabolism.

The 4 enzymes are:
propionyl coenzyme A (CoA) carboxylase (PCC),
pyruvate carboxylase (PC),
β-methylcrotonyl CoA carboxylase (β-MCC), and
acetyl CoA carboxylase (ACC).

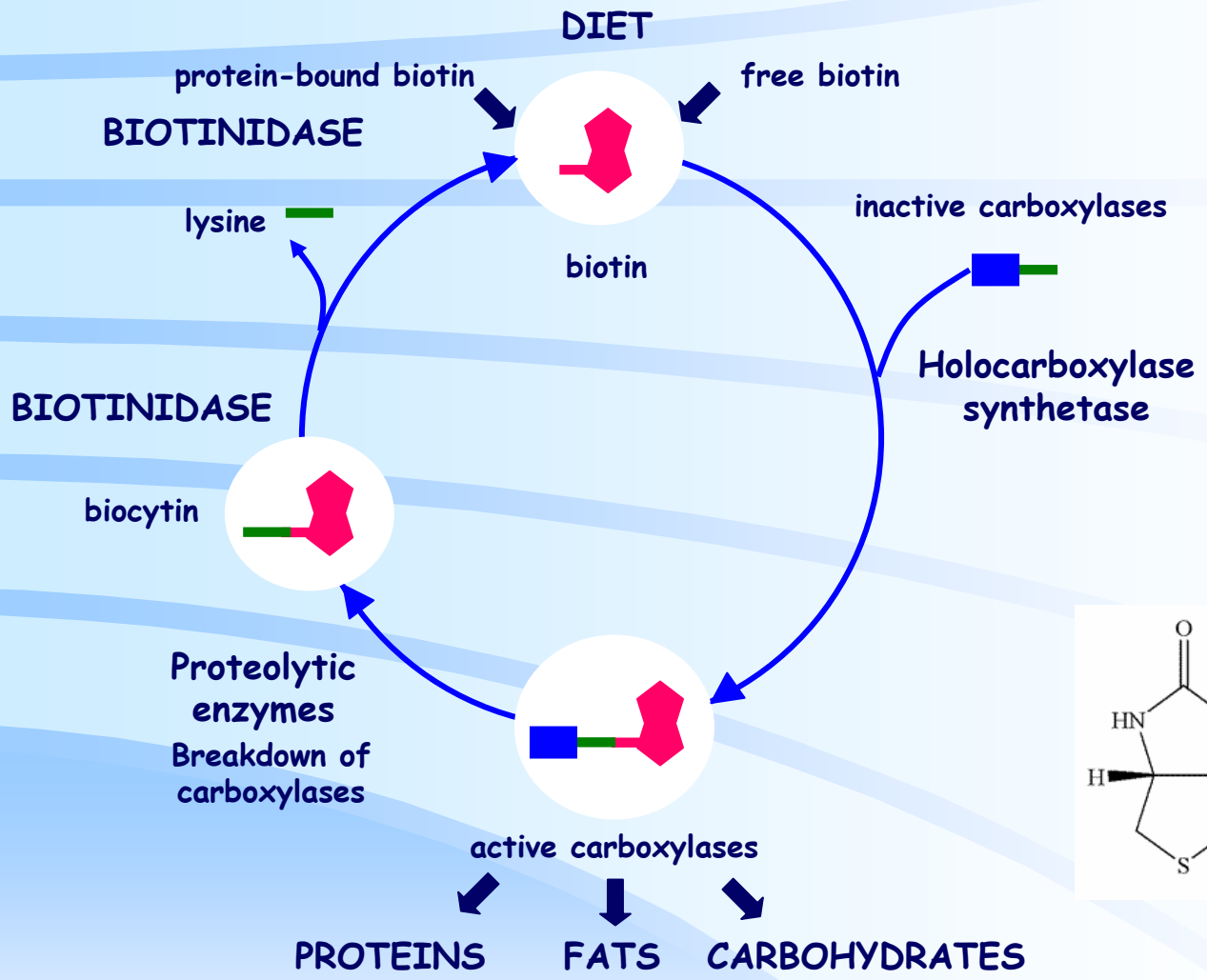
In all 4 carboxylases, biotin serves as a carrier for $CO_2$ in a multistep reaction.

# Biological function of biotin

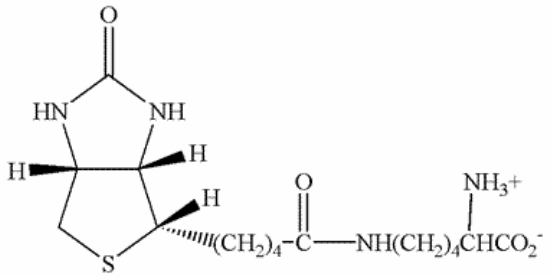



- ✓ The biotin moiety of a carboxylase is carboxylated at a nitrogen atom.
- ✓ The $CO_2$ moiety is transferred to the substrate (carboxylation).
- ✓ The original carboxylase is liberated intact, ready to perform another carboxylation.

# The Biotin Cycle

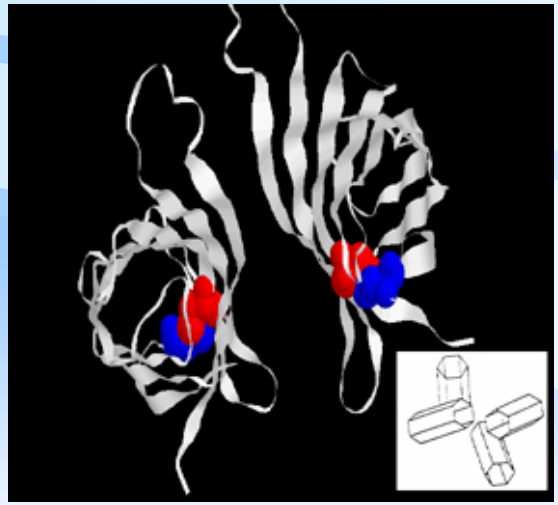


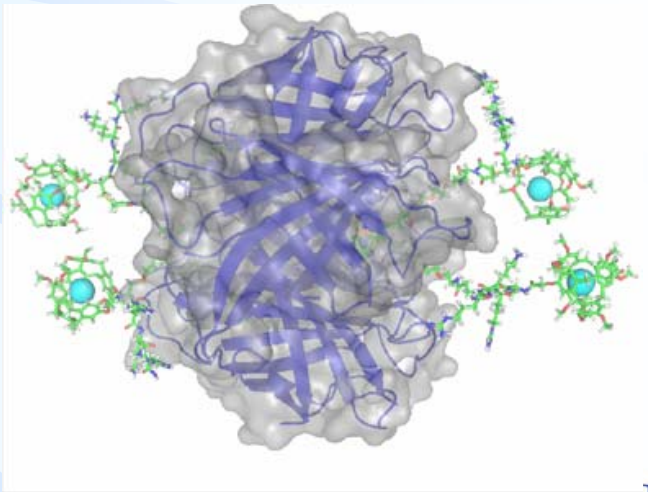

## Biocytin



# Avidin

- ✓ Is synthesized in the hen oviduct.
- ✓ Is a glycoprotein of MW 68,000 daltons which occupies about 0.05% (w/w) of the total protein content of the hen egg white.
- ✓ Is a tetrameric protein composed of four identical subunits of 128 amino acids.
- ✓ Each subunit is glycosylated at 17-Asparagine and has one binding site for d-biotin.
- ✓ Its affinity for d-biotin is $k_d = 10^{-15}$M.
- ✓ Is highly soluble in water or salt solution at physiological pH (50 mg of avidin per ml of solution).
- ✓ Is very stable against heat, pH changes and chaotropic reagents.





# Avidin purification

Avidin can not be purified by adsorption on an insoluble biotinylated matrix because of the very high affinity of the biotin-avidin complex, the avidin is not easily eluted.

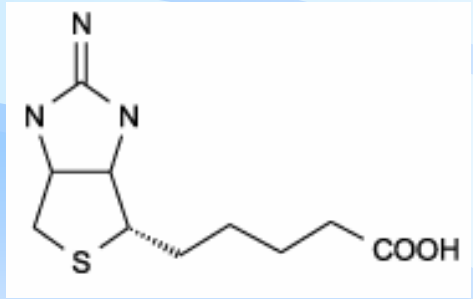
Affinity-purified avidin can be prepared by using insoluble matrices covalently linked with iminobiotin.

Iminobiotin is a biotin derivative containing a guanidinium group instead of a ureido group.

At relatively high pH (e.g., 11.0), when, the guanidinium group is not protonated, i.e., is iminobiotin binds strongly to avidin.

At low pH (e.g., 4.0), the guanidinium group is protonated, and imidobiotin does not bind to avidin.

Thus, avidin is adsorbed on an imino-biotin-matrix at pH 11.0 and eluted in pure form at pH 4.0.




Iminobiotin

***Units of Avidin activity: the amount of protein which will bind 1 mg of d-biotin***

# Avidin and its Relatives

NATIVE AVIDIN, **pI 10.5.**
Side reactions that lead to non-specific binding and high background levels.

DEGLYCOSYLATED AVIDIN, **pI 10.5**
Total removal of carbohydrate moieties that are recognized by lectins or other carbohydrate binding proteins by concanavalin-A affinity columns that adsorb only the glycosylated fraction or deglycosylation by enzymatic cleavage.

DEGLYCOSYLATED AVIDIN, **pI neutral**
A non-glycosylated Avidin with neutral isoelectric point arising from additional modifications of

- amino groups of lysine residues(formylation, acetulation, succinylation)
- arginine residues

RECOMBINANT AVIDINS

# Streptavidin

- Is synthesized by ***Streptomyces avidinii***.
- It consists of four identical subunits of 159 amino acids each.
- It does not contains carbohydrates.
- Its p1 is 5-6.
- Many regions of the molecule show significant homology with that of avidin.

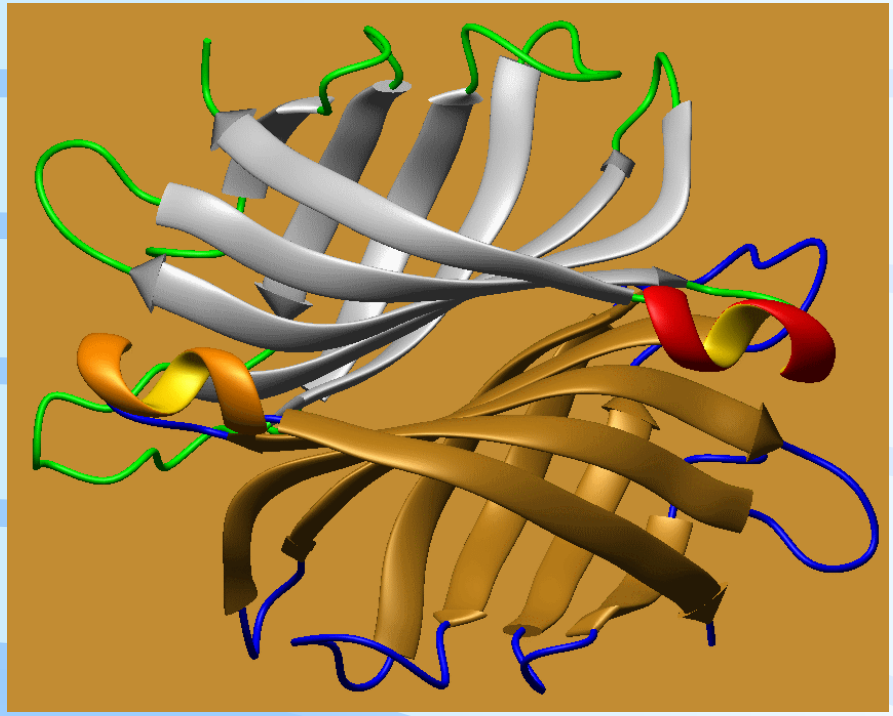
- Is isolated from the culture broth of S. *avidinii* by ammonium sulfate precipitation, ion-exchange chromatography, and crystallization and affinity chromatography using iminobiotin colurnns.

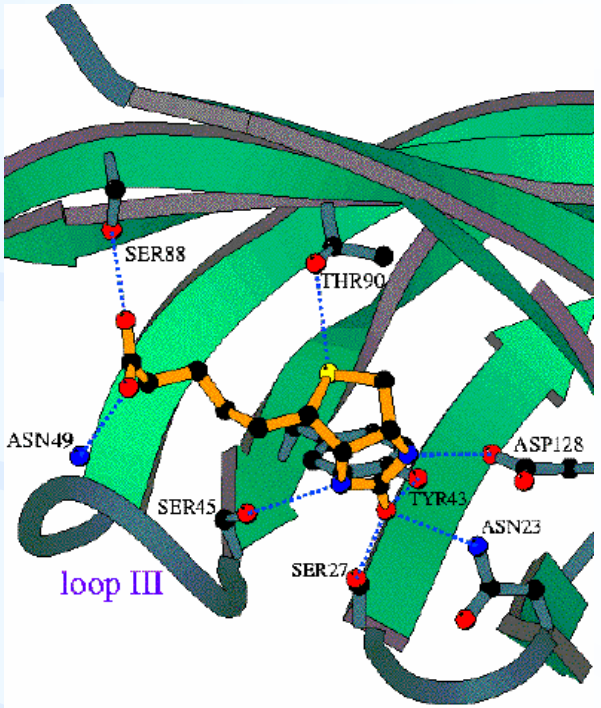
- The gene sequence predicts a mass of 66 kDa.
- A 75-kDa product was isolated on iminobiotin columns.
- The molecular mass of streptavidin product isolated by an initial ammonium sulfate precipitation was 60 kDa.

- Native streptavidin of relatively high mass (66-75 kDa) can be converted to a lower-mass form (i.e., 60 kDa) by proteolytic digestion at both the N and C termini.

- The lower MW streptavidin is called "core" or "truncated" streptavidin and has better biotin-binding characteristics than the native protein.

# Streptavidin structure






Weber et al. Science 243:85, 1989,
Weber et al., J. Am. Chem. Soc. 114:3197, 1992.

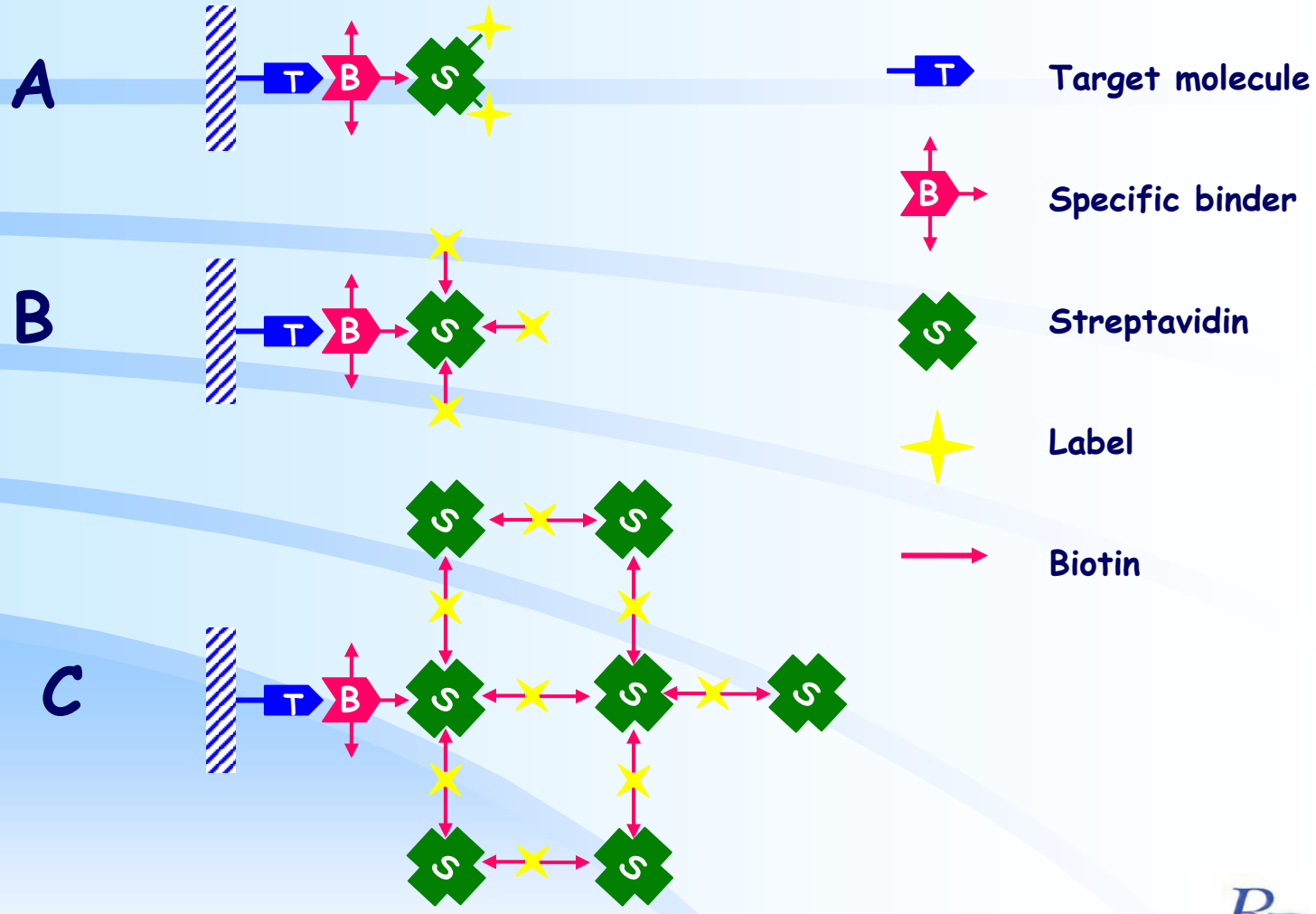
# Avidin or Streptavidin?

Both molecules can be used with equally satisfactory results

# Why the biotin-avidin system has found extensive application in bioanalytical methods

- ✓ The high affinity constant of interaction of avidin or streptavidin with biotin ($10^3$-$10^6$ times greater affinity than the interaction of ligands with the specific antibidies) that ensures that the complex is not disturbed by changes in pH, the presence of chaotropes, or manipulations (multiple washings, etc.).
- ✓ Avidin or streptavidin binding to biotin is specific enough to ensure that the binding is directed only to the target of interest.
- ✓ Biotinylation does not usually alter many properties of the molecules. This applies both to macromolecules (proteins) as well as to small molecules (mononucleotides, hormones, etc.)
- ✓ Streptavidin and avidin are exceptionally stable molecules and their biotin-binding activity can survive harsh reaction conditions and extensive derivatizations.
- ✓ Streptavidin and avidin possess four binding sites per molecule and in combination with multiply biotinylated moieties can create polymers of biotinylated moieties with avidin or streptavidin that could still have some free binding sites for biotin.

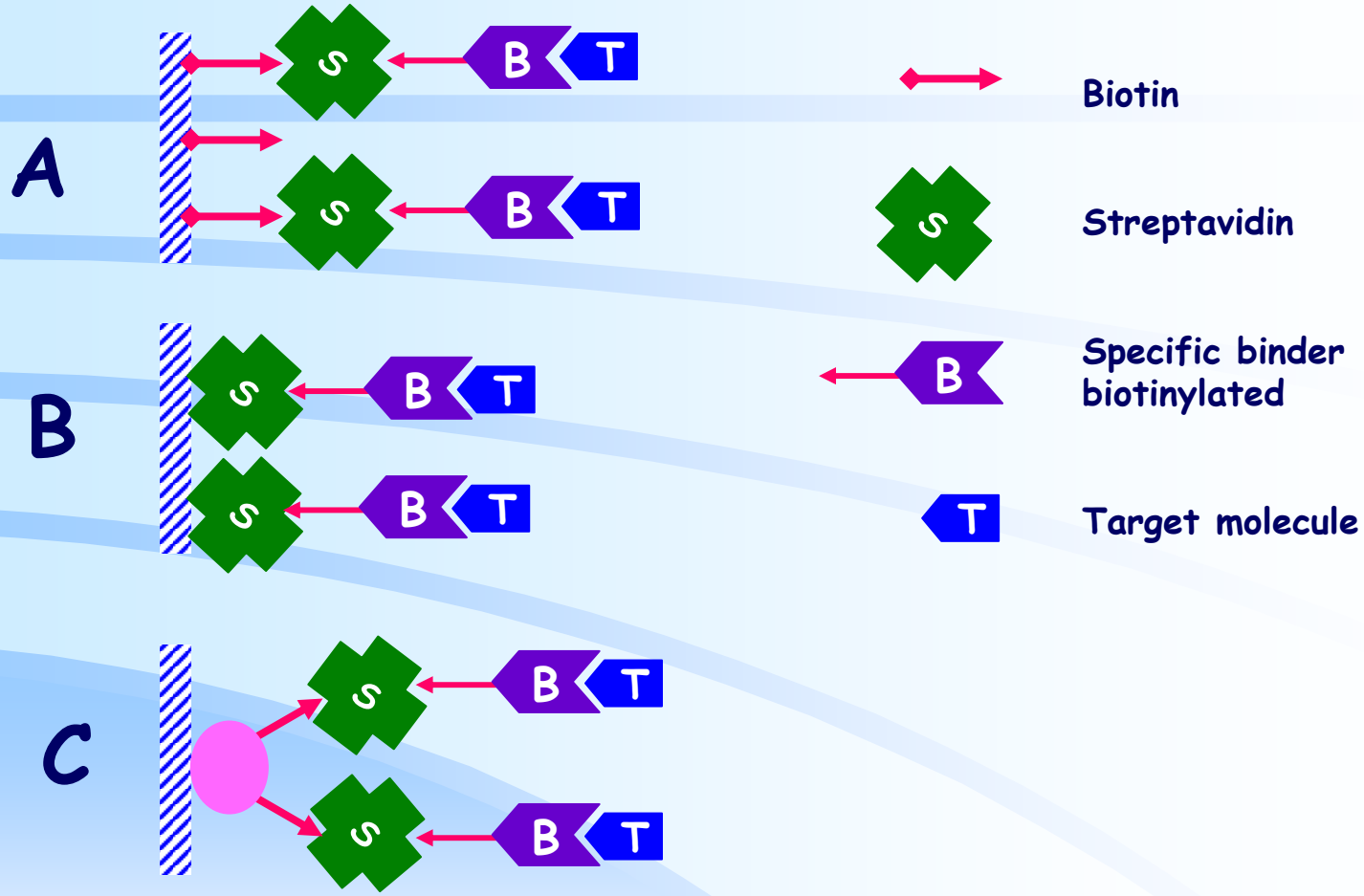
# Basic configurations of biotin-avidin based detection systems



# Advantages over other systems

- ✓ Signal amplification
- ✓ Increase in detection sensitivity
- ✓ Biotinylation does not disturb the activity of modified molecule
- ✓ General detection system can be used in combination with any biotinylated molecule

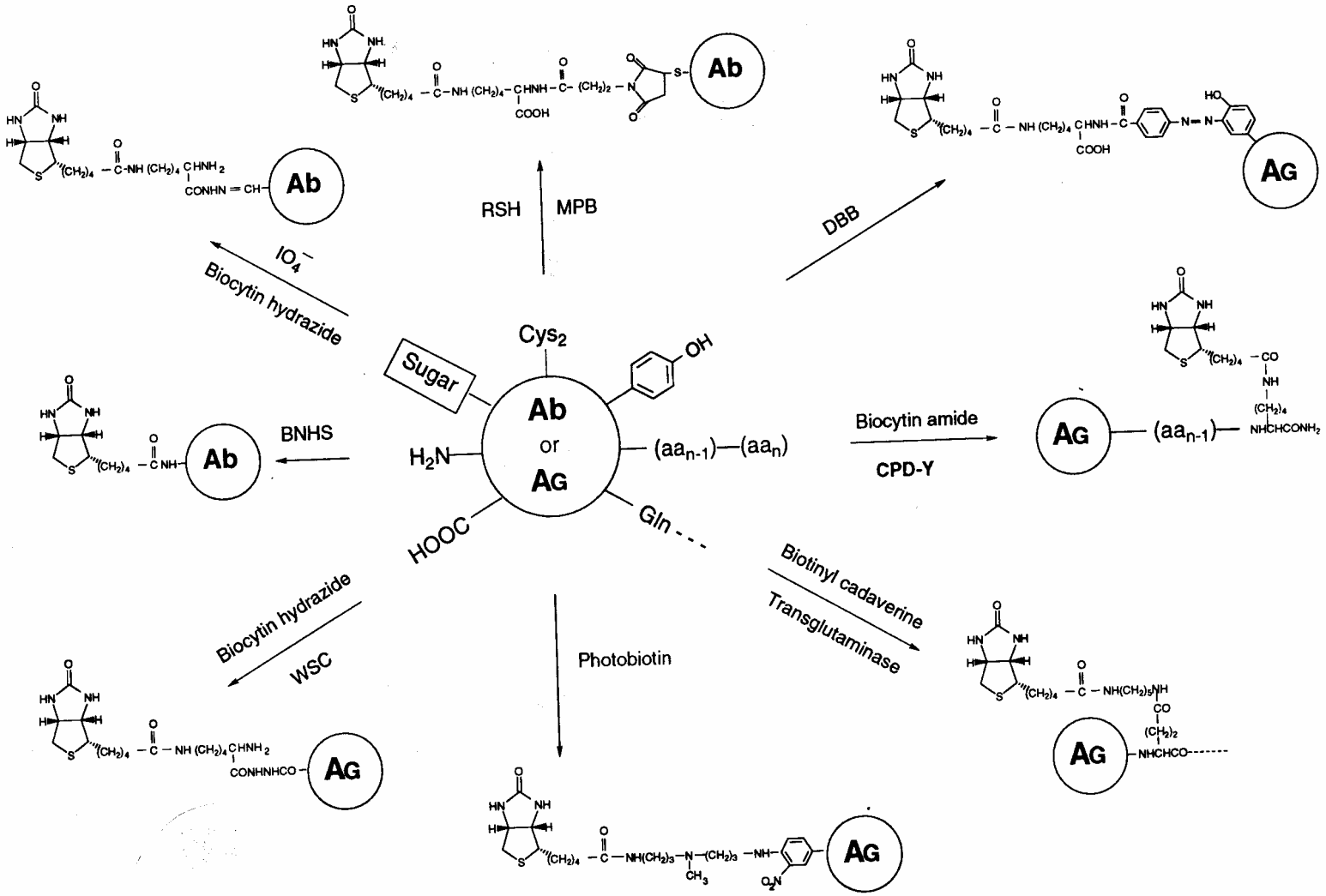
# Basic configurations of biotin-avidin based immobilization systems



# Advantages over other systems

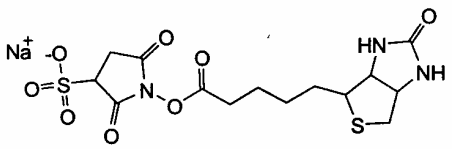
- ✓ Strong binding of biotinylated species onto solid supports
- ✓ Preservation of solid-phase reagent reactivity
- ✓ General immobilization system can be used in combination with any biotinylated molecule

# Biotinylation procedures

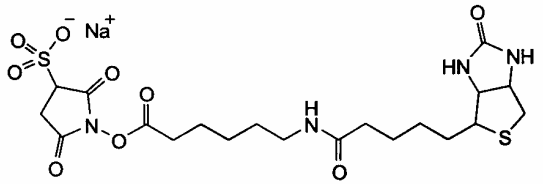


# Biotin coupling via amino-groups

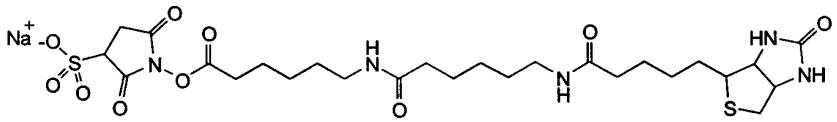
## Primary amines



Sulfo-NHS-Biotin

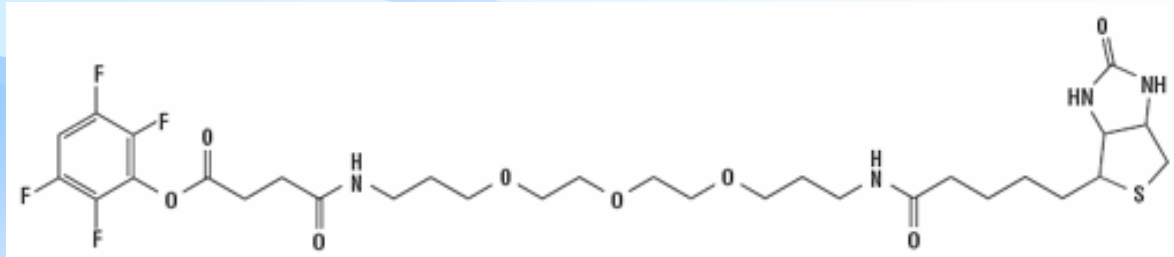


Sulfo-NHS-LC-Biotin



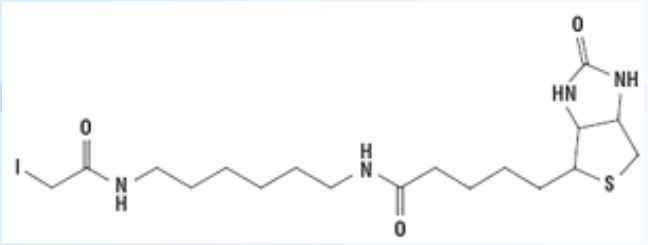
Sulfo-NHS-LC-LC-Biotin

## Primary & secondary amines

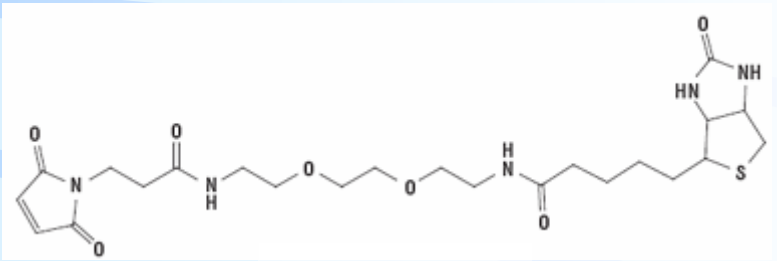


# Biotin coupling via thiol groups

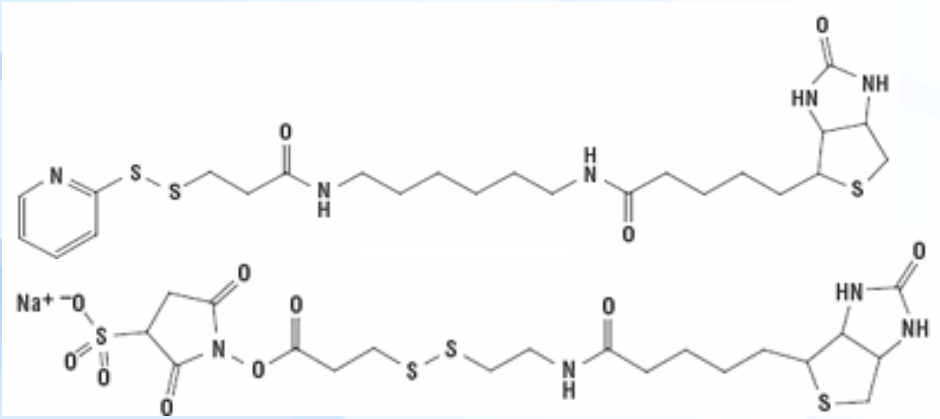
Iodoacetyl-biotin



Maleimide-biotin

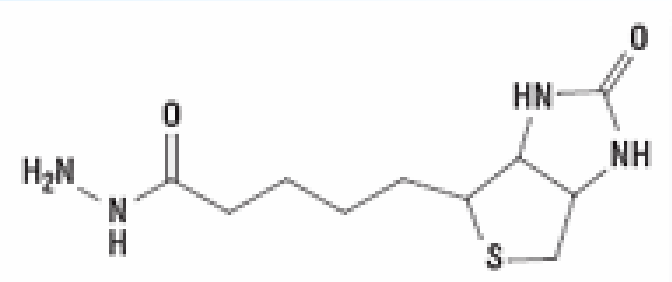


Dithiol-biotin

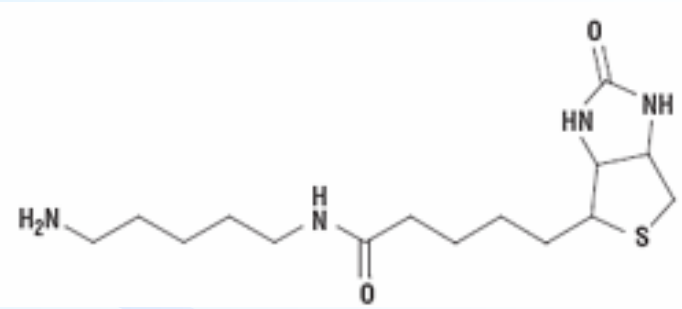


# Biotin coupling via other reactive groups

Carbohydrates ↔ Biotin-Hydrazide

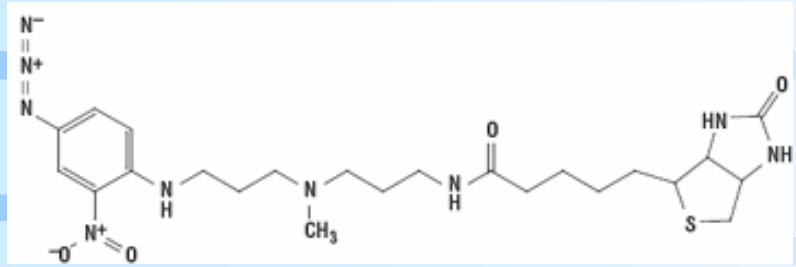



Carboxyl groups ↔ Biotin-Amine

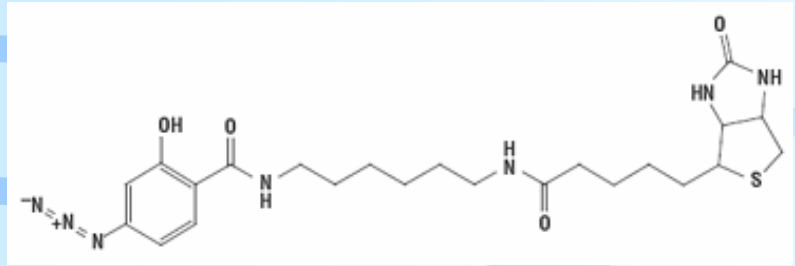


# DNA biotinylation reagents

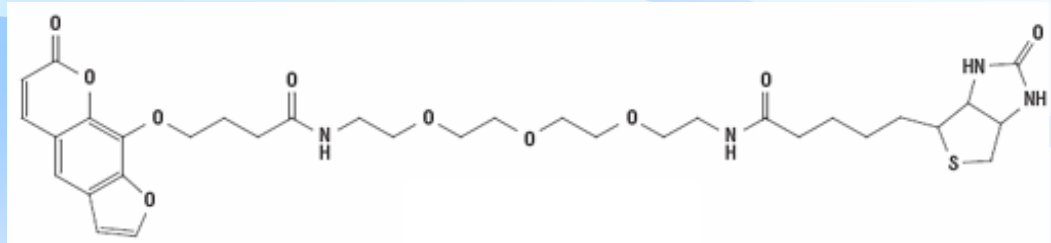
## Photoactivable biotin derivatives



Covalent attachment by exposure to UV light (350-370 nm)



Covalent attachment by exposure to UV light (365 nm)

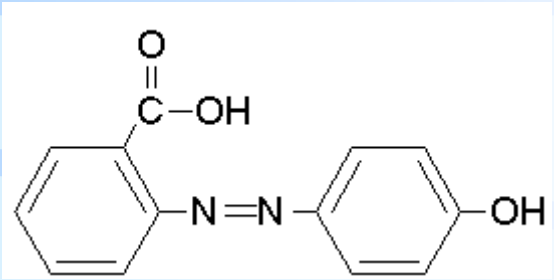


Covalent attachment in presence of UV light (~ 350 nm).

Psoralen moiety reacts through a cyclo-addition mechanism with the 5,6 double bond in thymine- and other pyrimidine-containing bases

# Determination of biotin to protein ratio

**HABA:** 2-(4'-HydroxyAzoBenzene)Benzoic Acid (MW 242.24)




The HABA dye binds to avidin to produce a yellow-orange colored complex which absorbs at 500 nm.

Free or conjugated biotin displace the HABA dye and cause the absorbance to decrease.

A standard curve is established using the free biotin to estimate the number of moles of biotin incorporated in biotinylated proteins.

# Biotinylated molecules and their applications

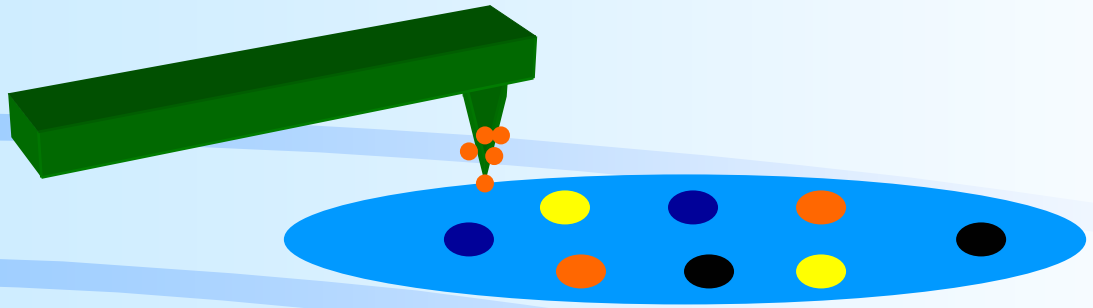
| Biotinylated moiety | Applications |
| --- | --- |
| Antibodies, anti-immunoglobulins, protein A, protein G | Immunoassays, immunohistochemistry, flow cytometry, cell sorting, Western blots |
| Lectins | Glycoconjugate studies, mitogenic stimulation studies |
| Anti-lectins | Localization of lectin receptors |
| Enzymes | Immunoassays, nucleic acid hybridisation |
| Ferritin, hemocyanin | Electron microscopy |
| Agarose, cellulose | Affinity chromatography |
| Anti-avidin, anti-streptavidin | Amplification assays |
| Nucleotides, DNA | Nucleic acid hybridisation, DNA sequencing |
| Hormones | Affinity chromatography, ligand-receptor interaction studies |
| Cells | Hybridoma production |

# (Strept-)Avidin conjugates and their applications

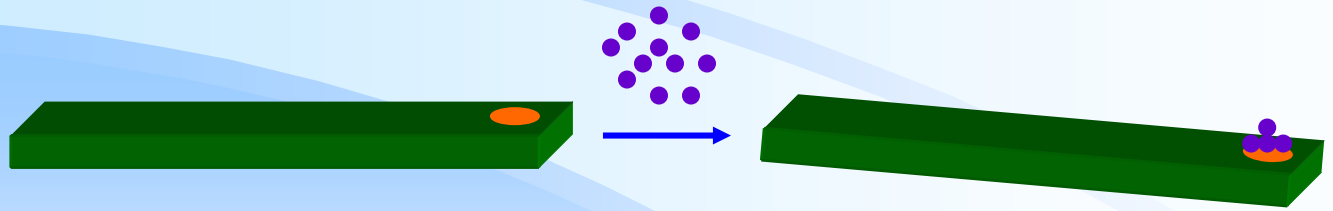
| Conjugated moiety | Applications |
| --- | --- |
| Enzymes, Fluorophores, Eu3+ chelates, Chemilumicsent labels | Immunoassays, Immunohistochemistry, Flow cytometry, Cell sorting, Western blots, Nucleic acid hybridisation |
| Ferritin, gold | Electron microscopy |
| Agarose | Affinity chromatography |
| Magnetic particles | Affinity chromatography, Nucleic acid hybridisation, DNA sequencing |
| Polystyrene | Immunoassays |

# Applications of biotin-avidin system in theTASNANO project

## Scanning probe



## Mass sensor



# Functionalization of tips/cantilevers

| Surface | Molecule to be immobilized |
| --- | --- |
| Silicon oxide | Biotinylated protein<br>✓ Adsorption<br>✓ Covalent |
| Gold | Biotin<br>Covalent |