Bioconjugation Techniques

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References:

- "Bioconjugate Techniques", Greg T. Hermanson, Academic Press, 1996
- "Principles of Fluorescence Spectroscopy", Joseph Lakowicz, Third Edition, Springer, 2006
- "Current Protocols in Nucleic Acid Chemistry", Wiley, e-Book @ McGill
- Molecular Probes (Invitrogen) http://probes.invitrogen.com/
- Glen Research http://www.glenres.com/

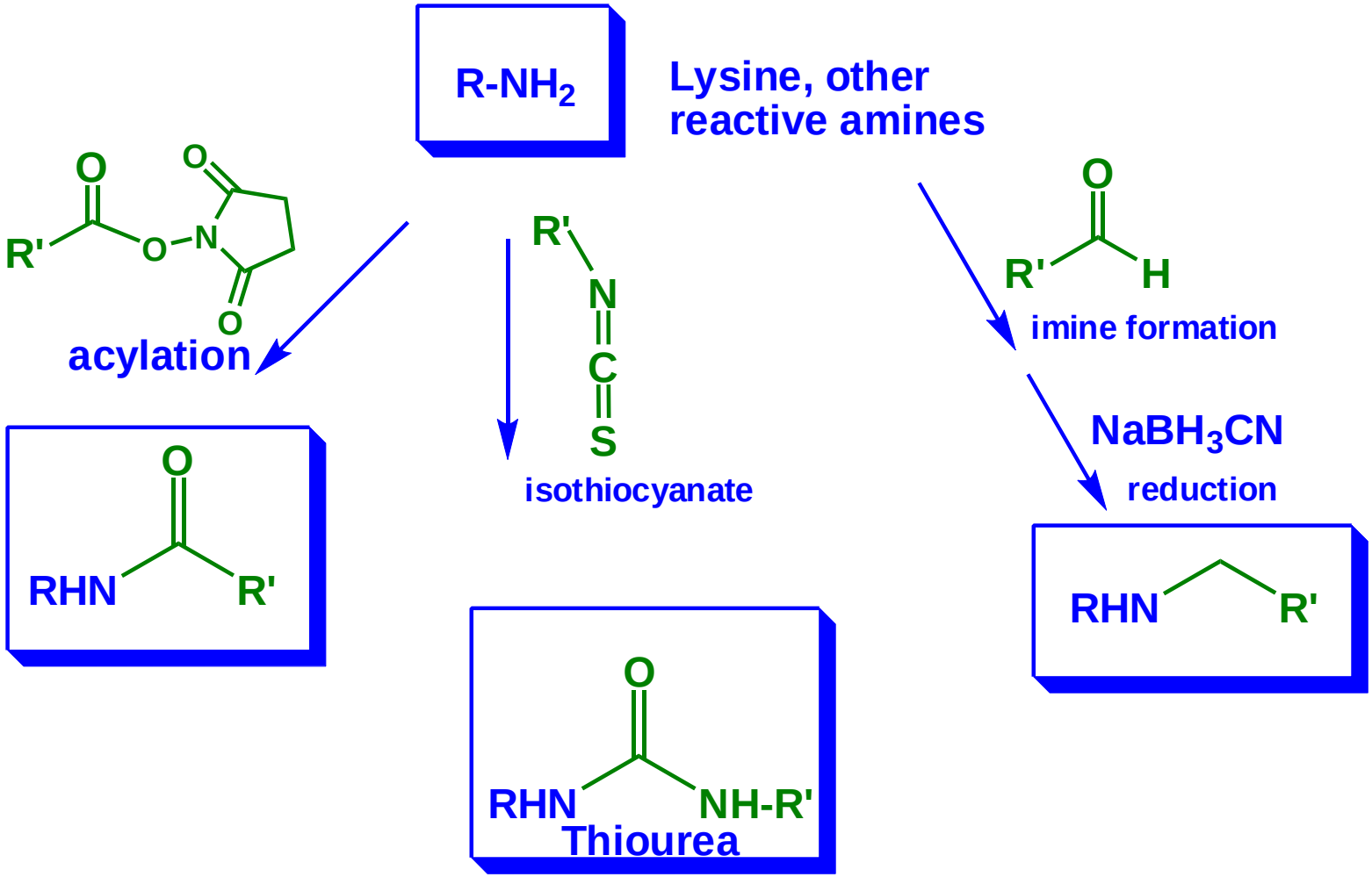
Attachment of Fluorescent Labels to Biological Molecules

- **Biological Assays:** Detection and monitoring of a protein or nucleic acid finding the function of genes/proteins,monitoring the progress of a disease, etc.

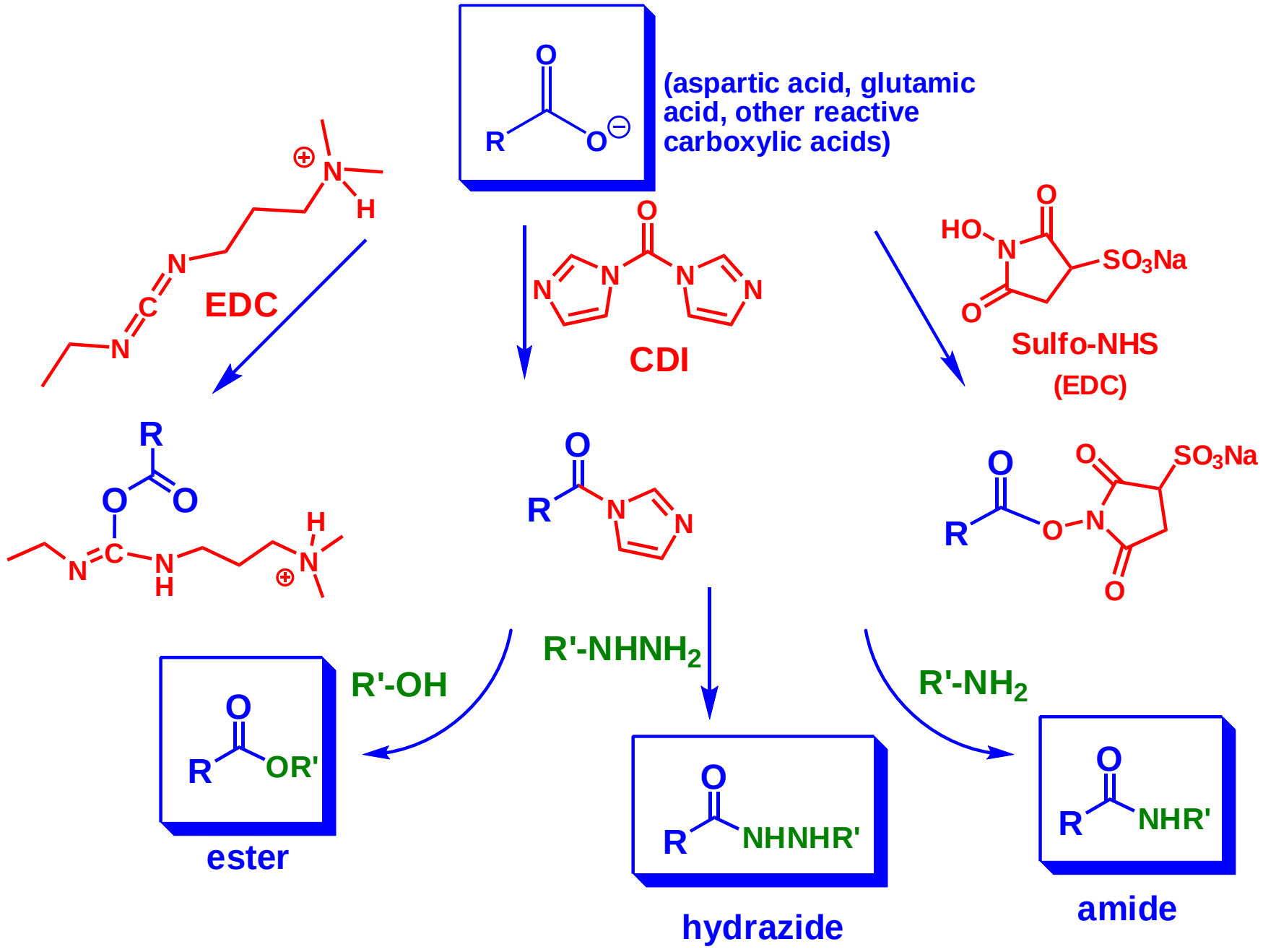
- **Cell Biology:** mechanistic studies of biological transformations in cells

- Genomics, Molecular Biology, Proteomics, Medical Diagnostics

Bioconjugation of Common Functional Groups



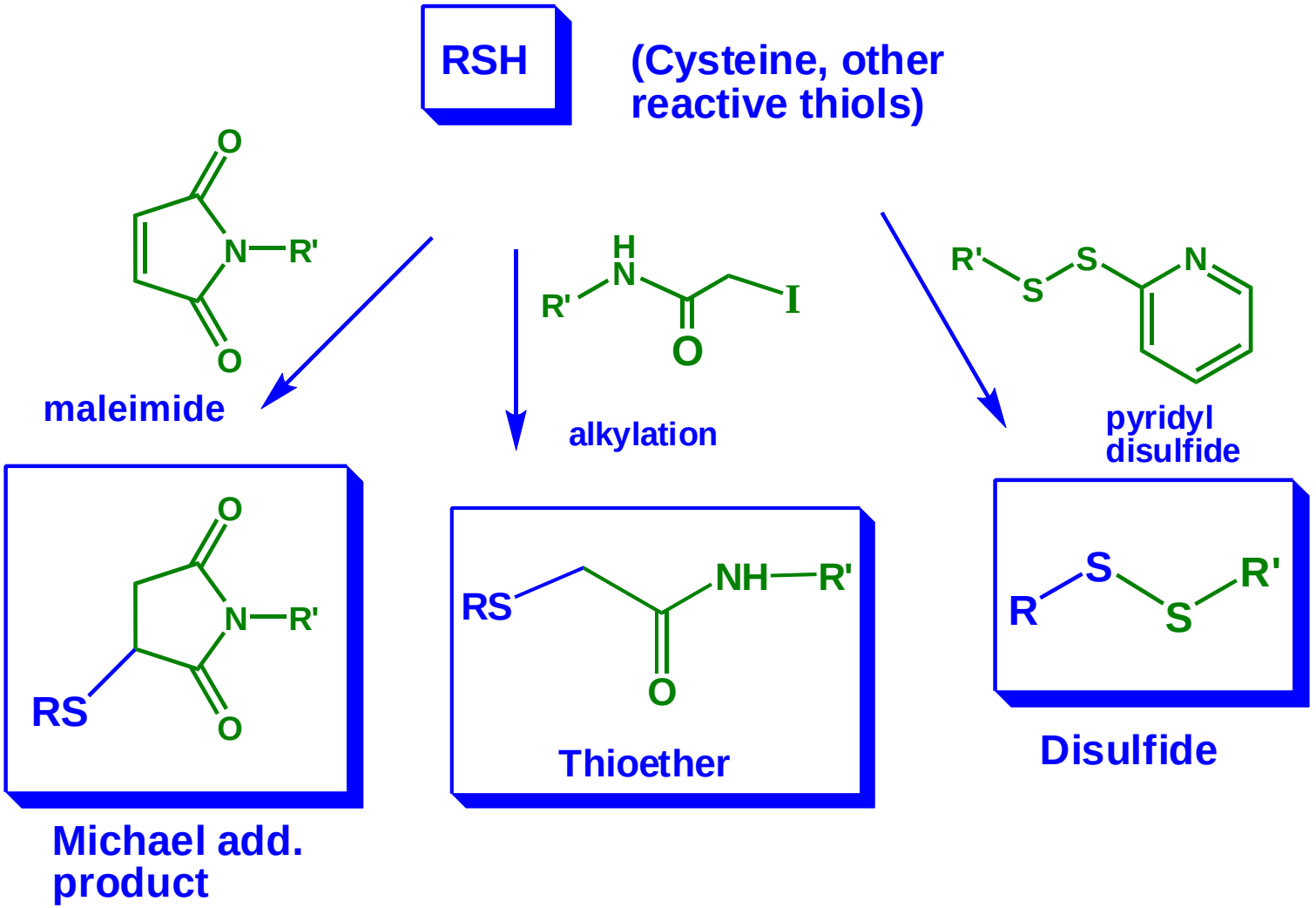
Bioconjugation of Common Functional Groups



Protocol (eg, for EDC coupling):

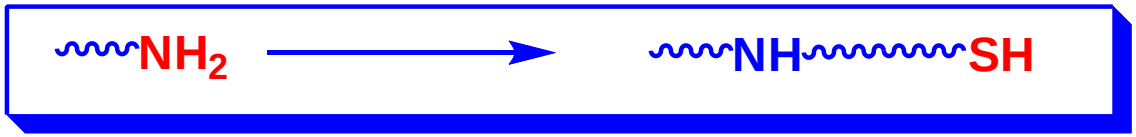
1. Dissolve the protein in buffer (no amines or carboxylate buffers)
2. Dissolve the molecule to be coupled in same buffer at 10-fold molar excess to protein
3. Add the molecule solution to the protein solution
4. Add EDC (concentrated solution in water, freshly prepared) to the protein and molecule; EDC should be 10-fold molar excess
5. React 2 hours at RT
6. Purify the conjugate by gel filtration or dialysis

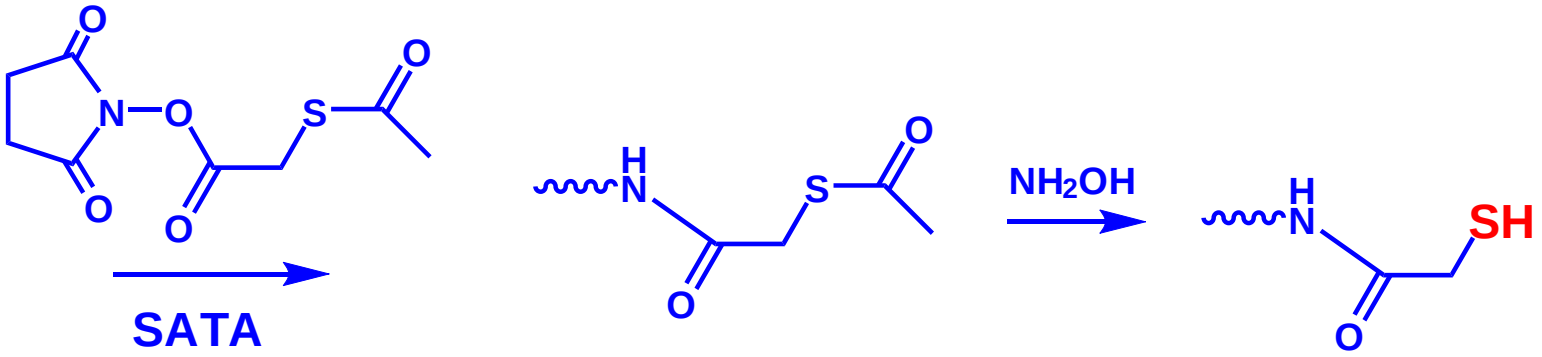
Bioconjugation of Common Functional Groups

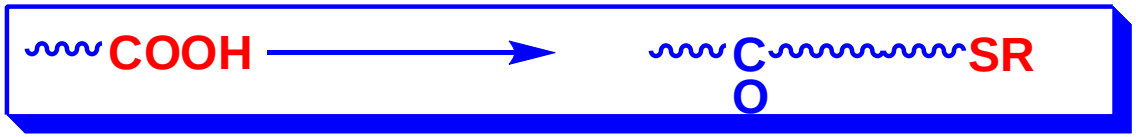


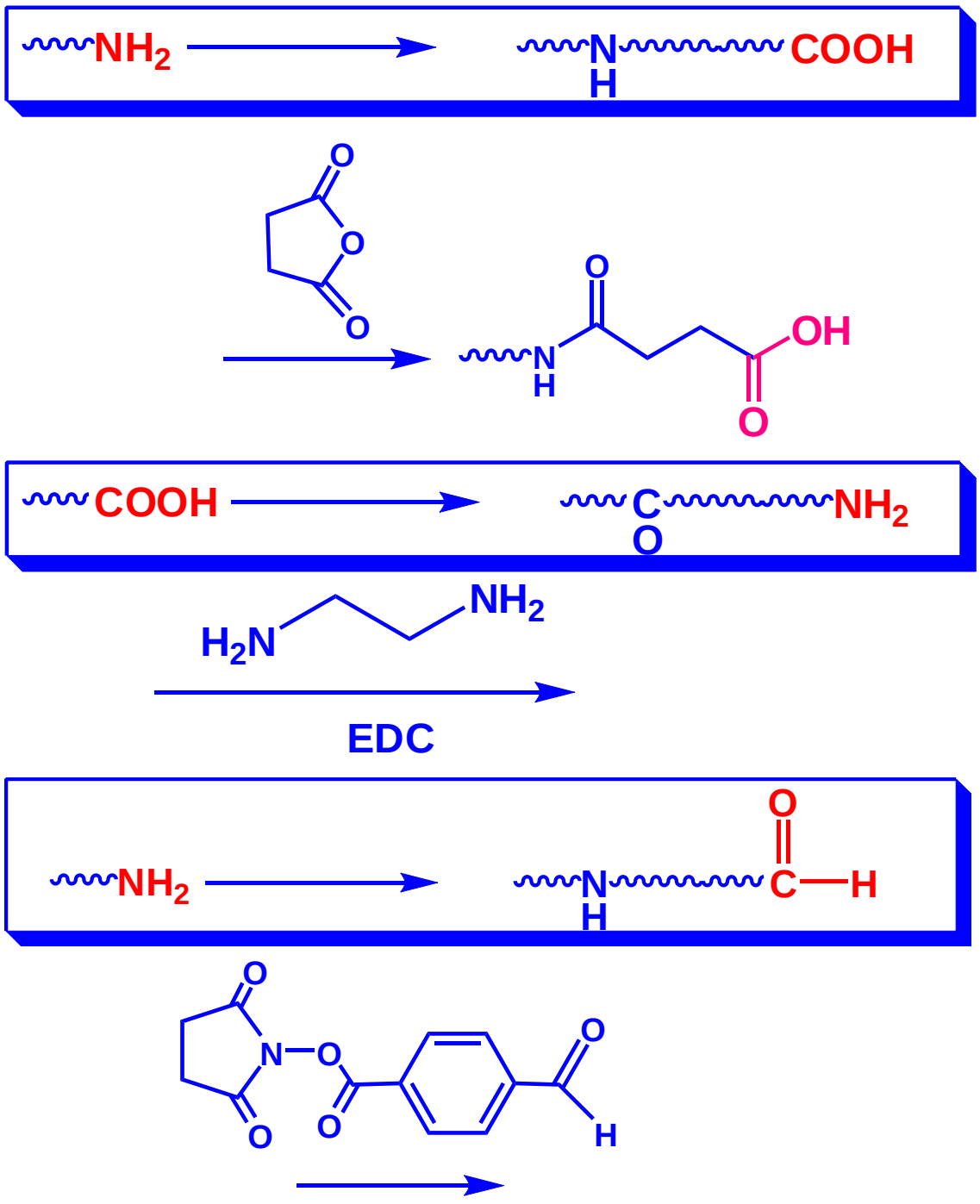
Cysteine/cystine buried inside protein, reduction may cause disruption of protein structure.

Creation of new groups

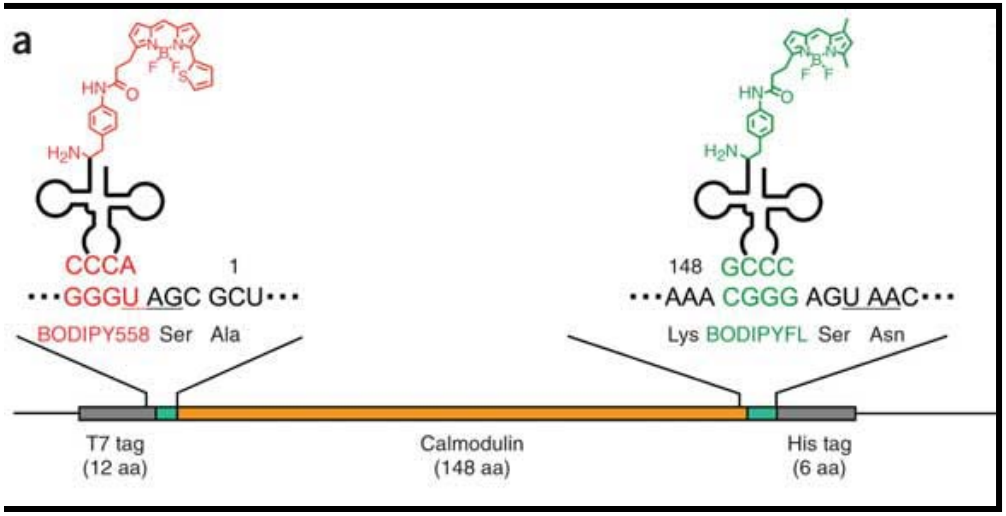




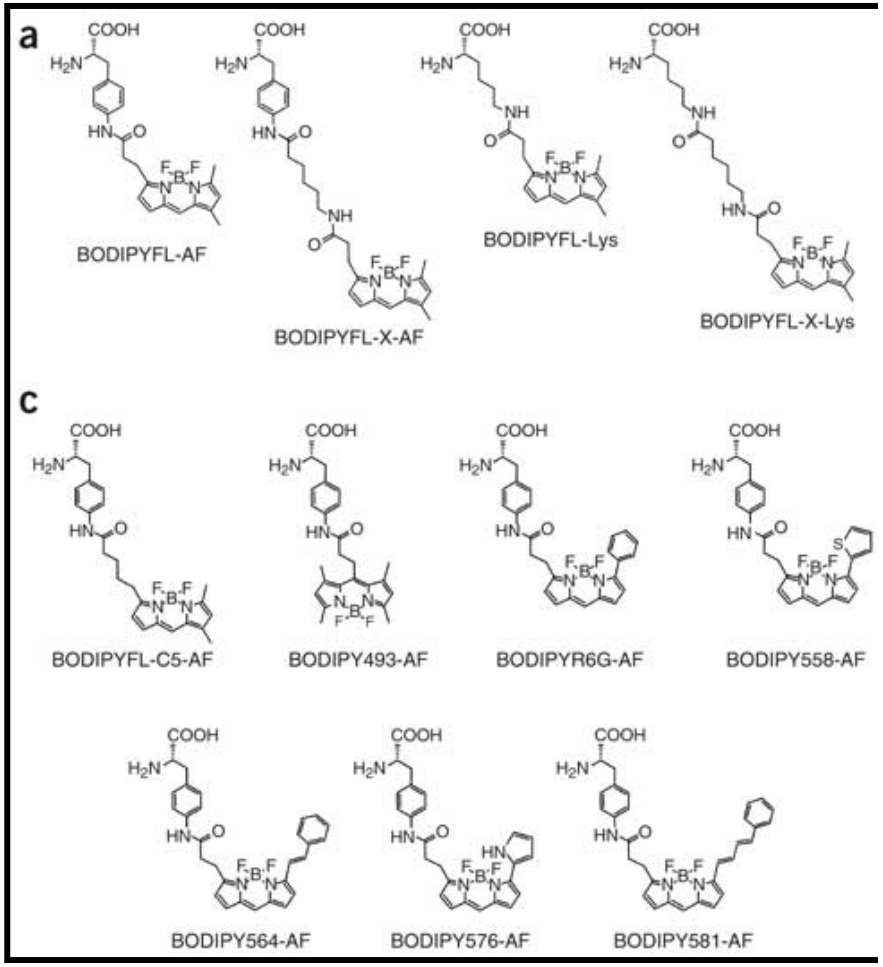



NH2
N
H
COOH
O
O
O
N
H
OH
O
COOH
C
O
NH2
H2N
NH2
EDC
NH2
N
H
O
C
H
O
N
O
O
O
O
H

Site-Selective incorporation of fluorophores to study protein conformation



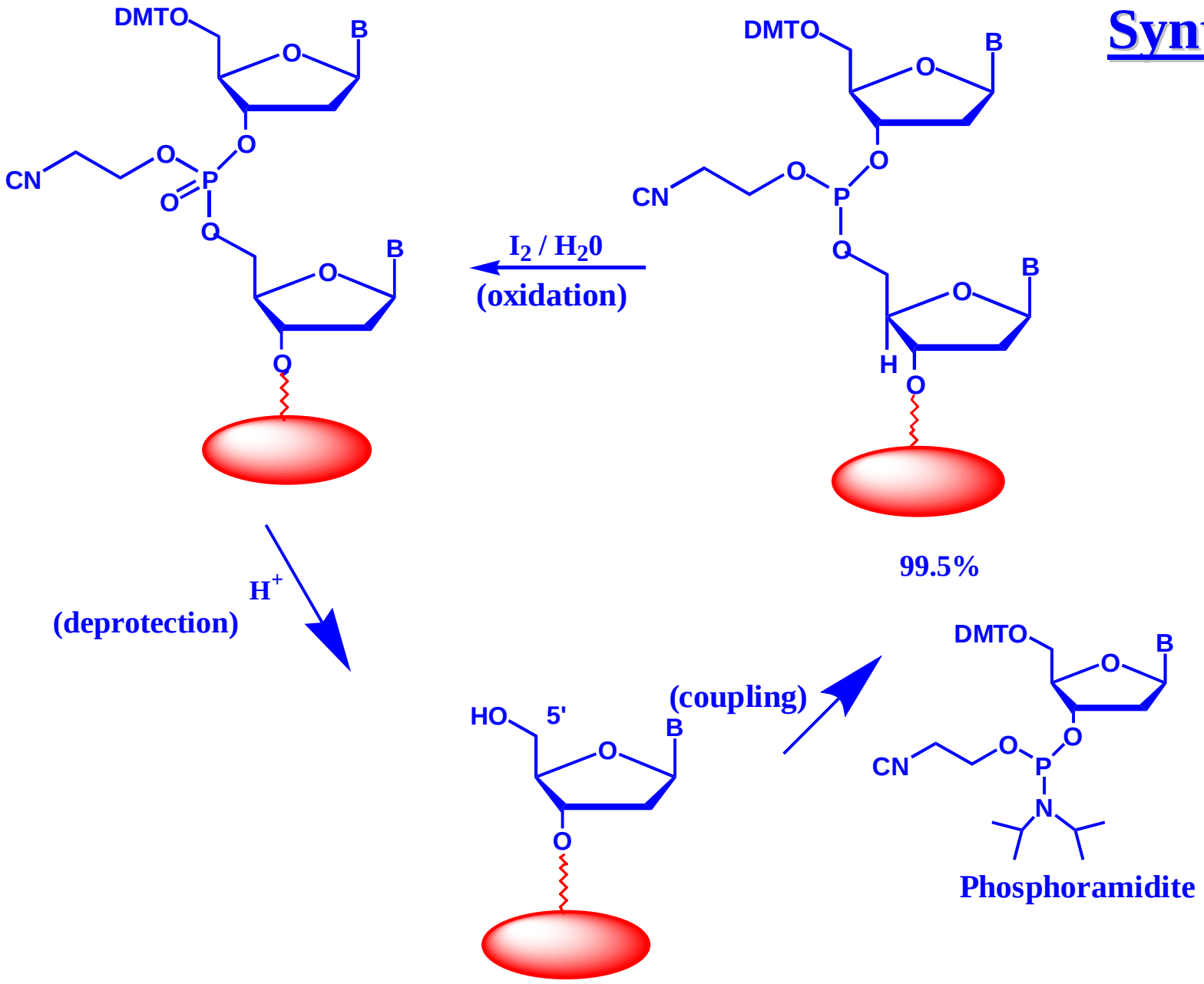
FRET pair



Hohsaka, Nature Methods, 2006, 923

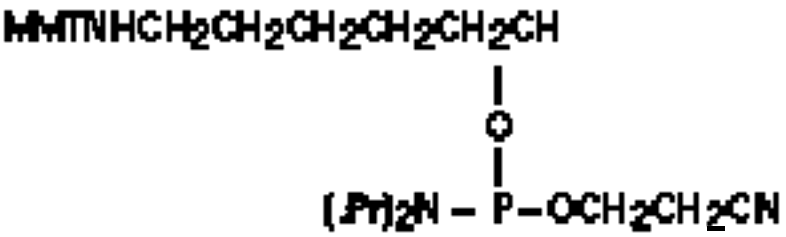
Nucleic Acid Biolabeling

DNA Synthesis

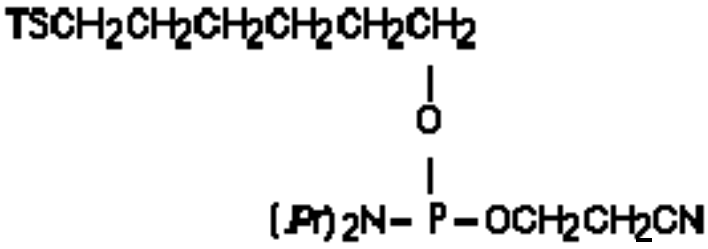


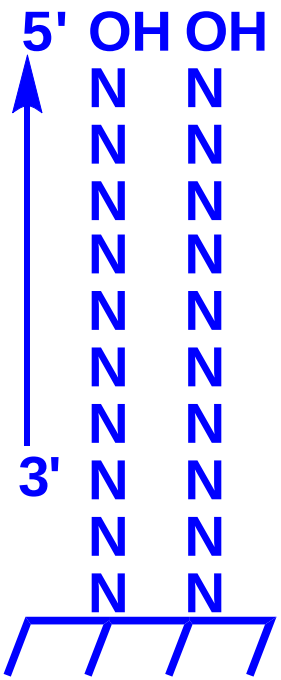
Nucleic Acid Biolabeling

5’-amino-DNA



5’-thio-DNA

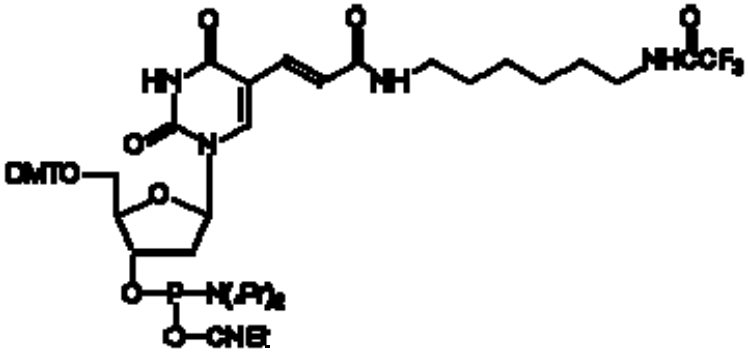




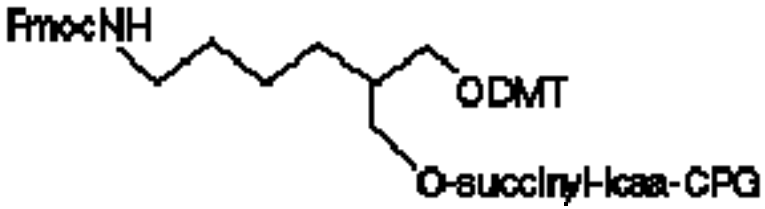
MMT : monomethoxytrityl

T : trityl

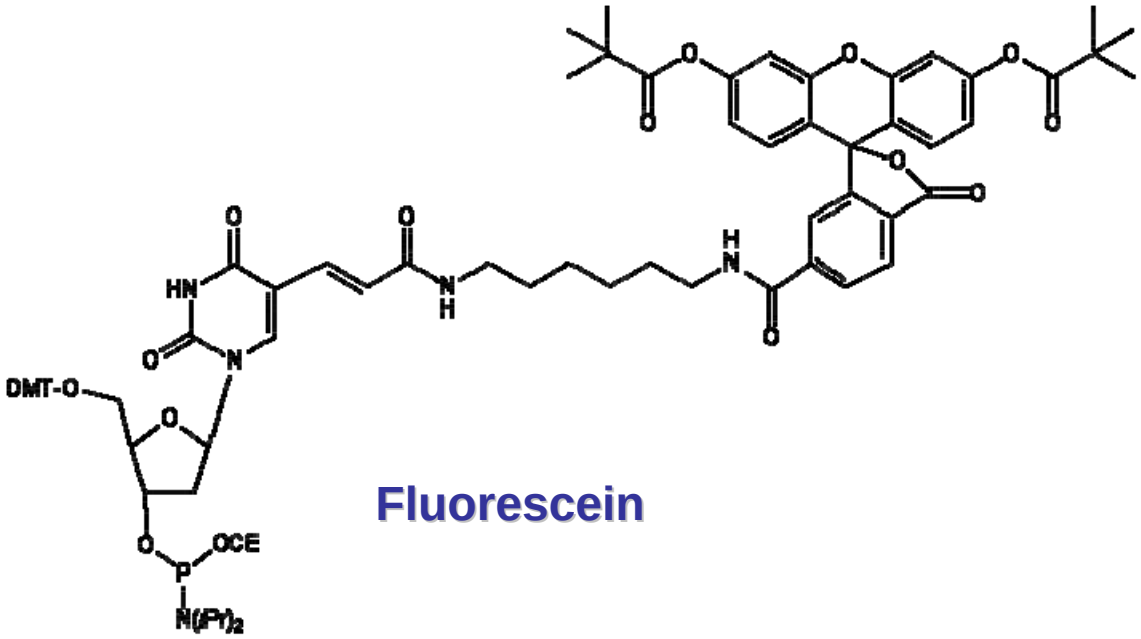
Sequence-labeled amino-DNA



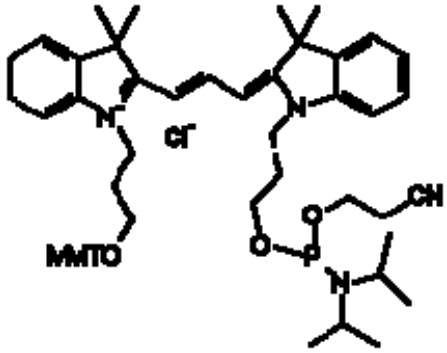
3’-amino DNA



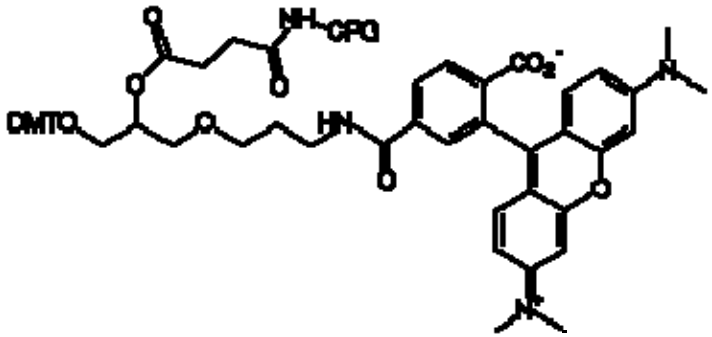
Nucleic Acid Biolabeling



Fluorescein

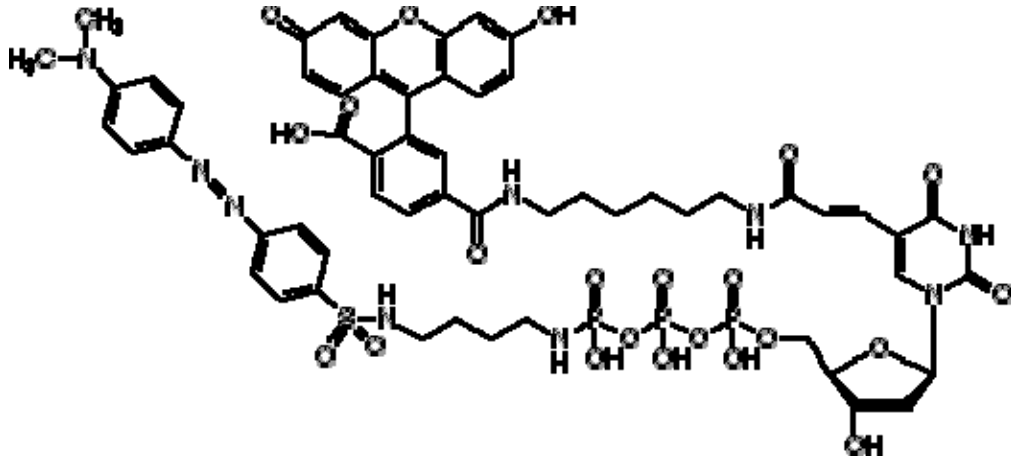


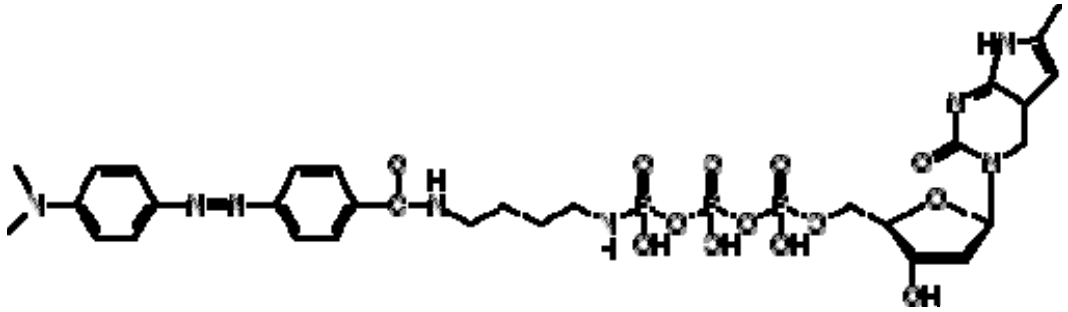
Cy3



TAMRA

Nucleic Acid Biolabeling –enzymatic incorporation





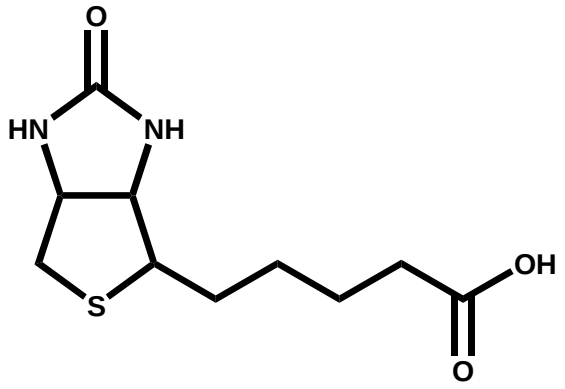
Glen Research

Biotinylation

The Biotin- Streptavidin Interaction

Biotin (Vitamin-H):

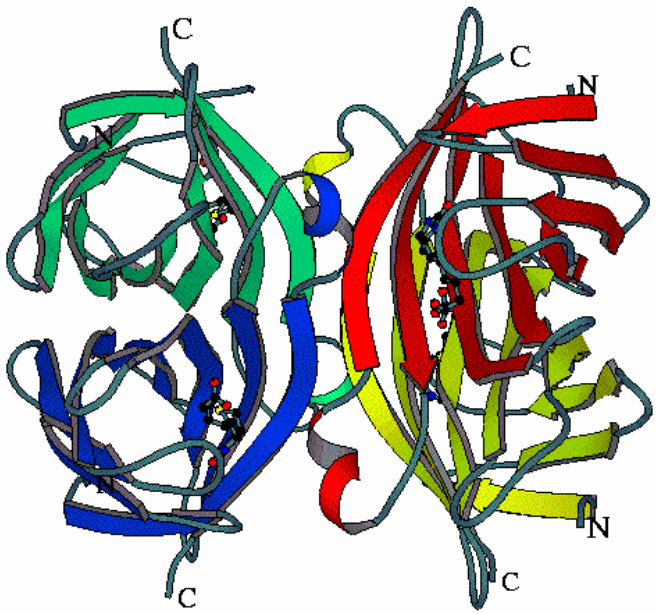
- Can be conjugated to different biomolecules
- Possesses one of the strongest binding interactions with avidin/streptavidin ($K_d \sim 10^{-15}$ M)



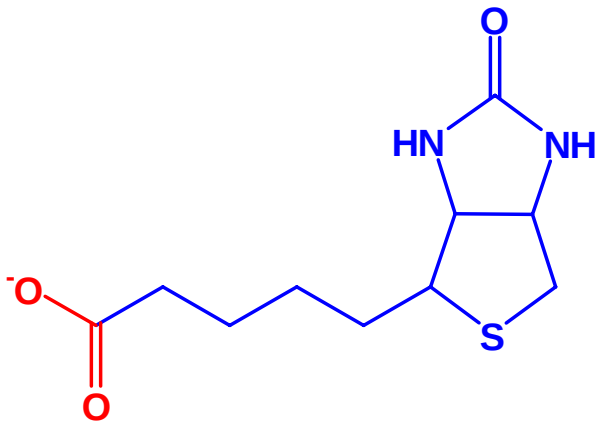
Biotin

avidin / streptavidin

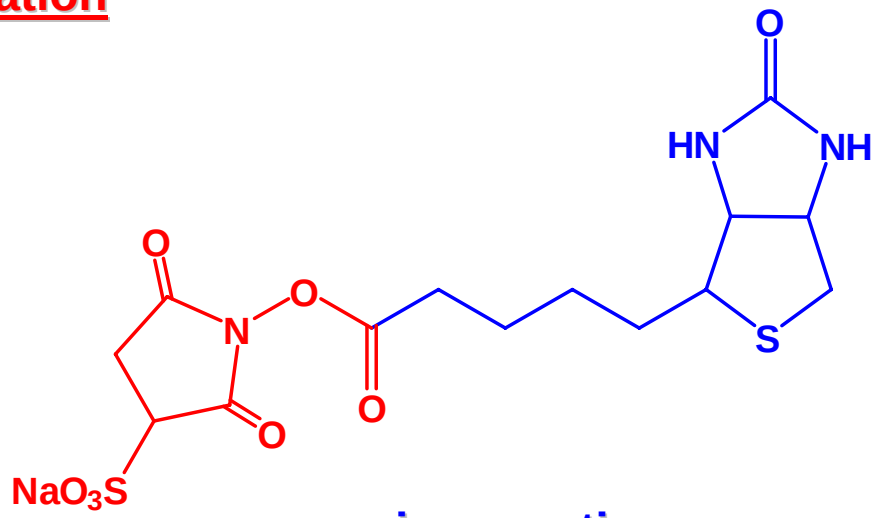
- Contains four identical binding sites for interaction with biotin



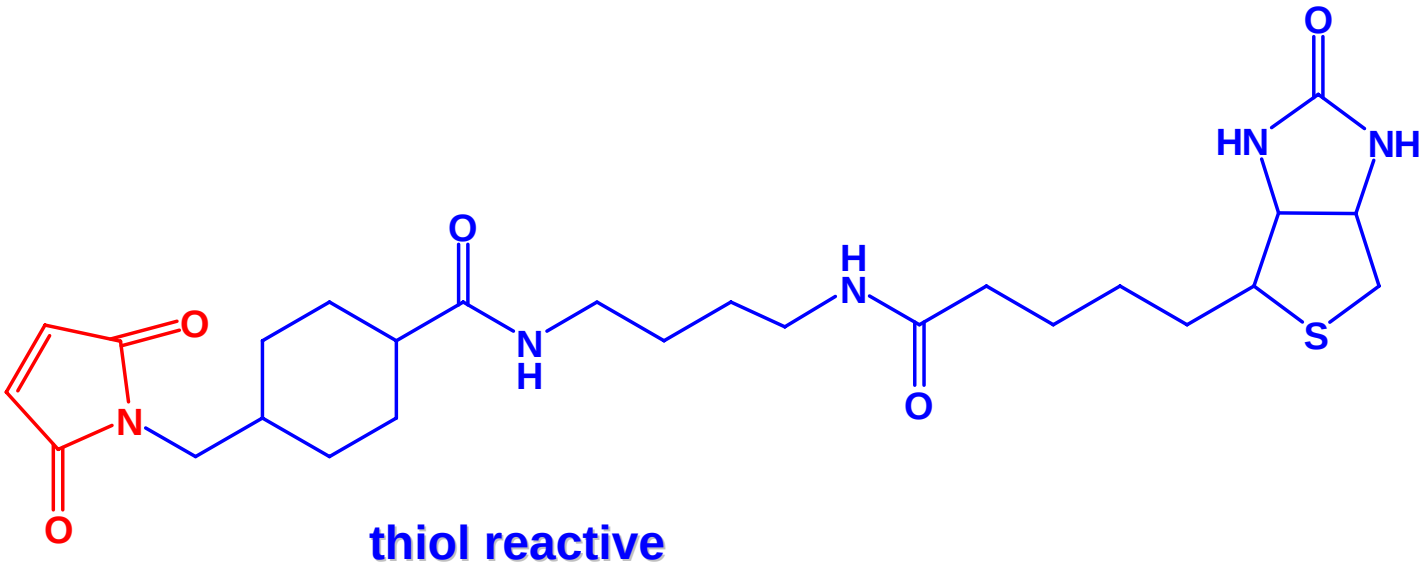
Biotinylation



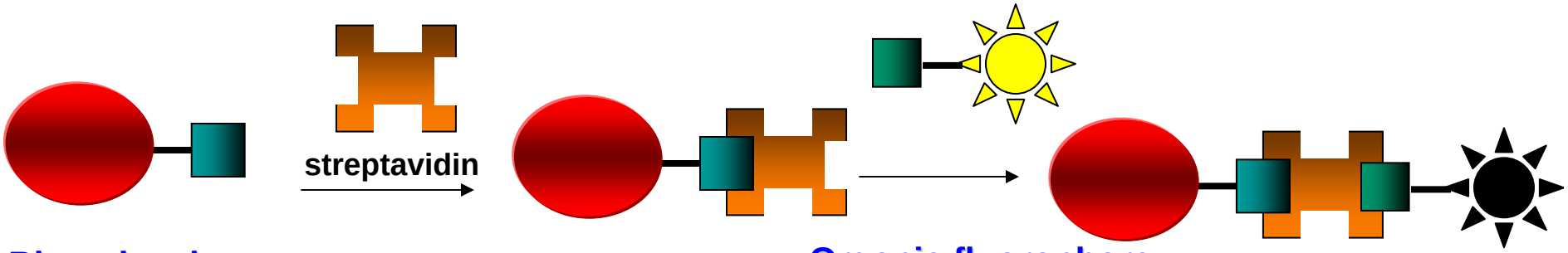
Biotin



amino reactive



thiol reactive

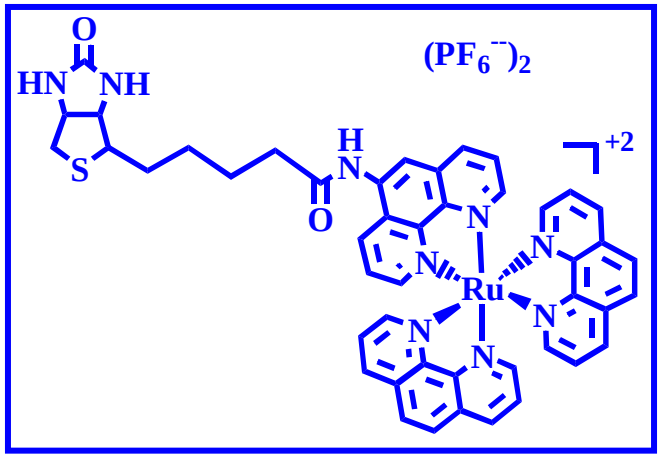


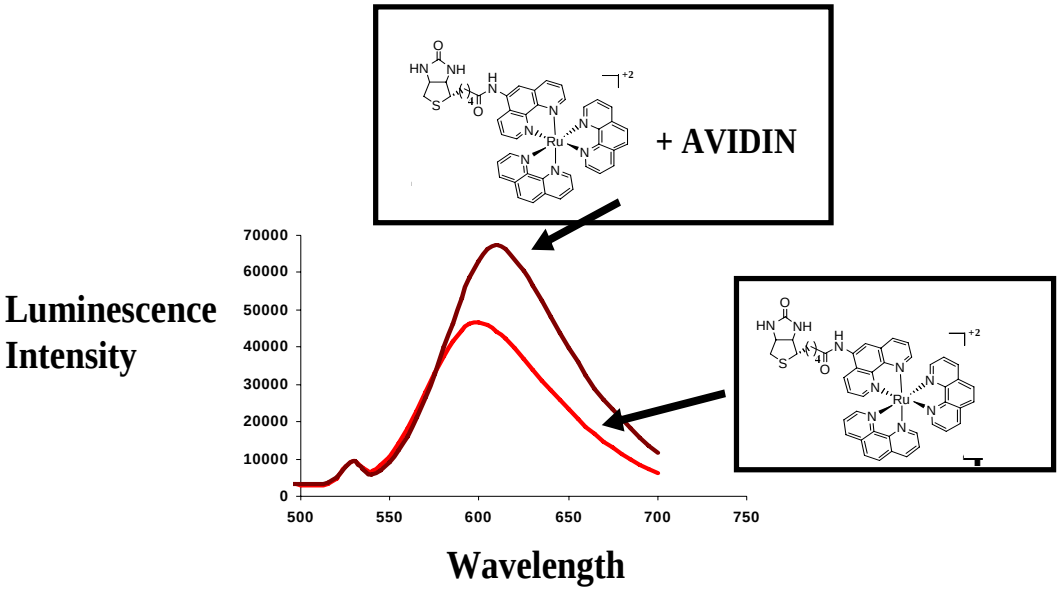
Biomolecule tagged with biotin

Organic fluorophore tagged with biotin

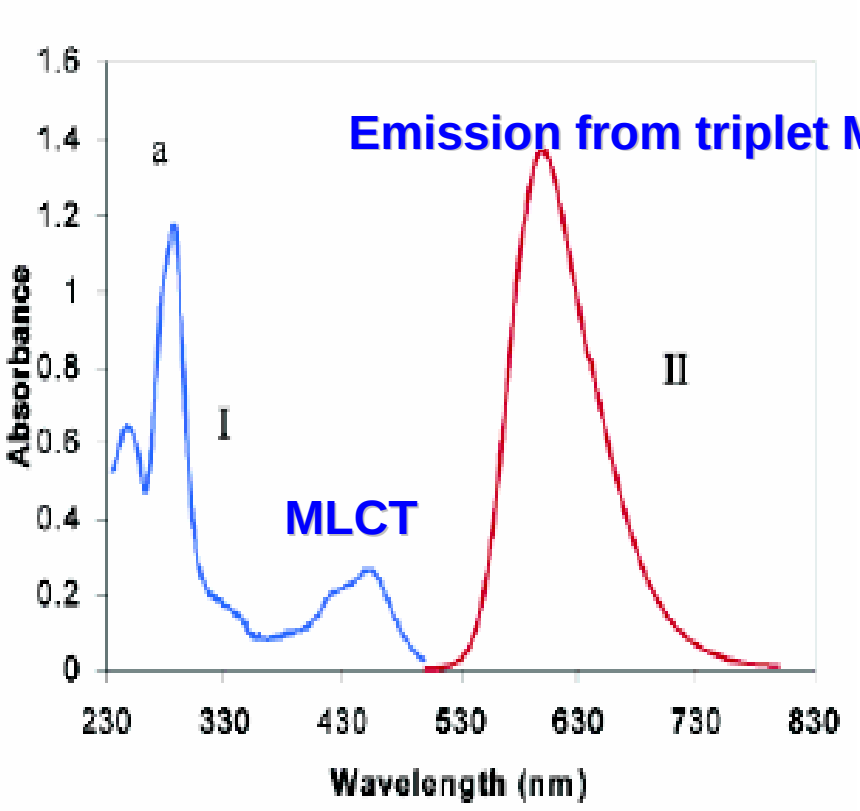
fluorescence quenched

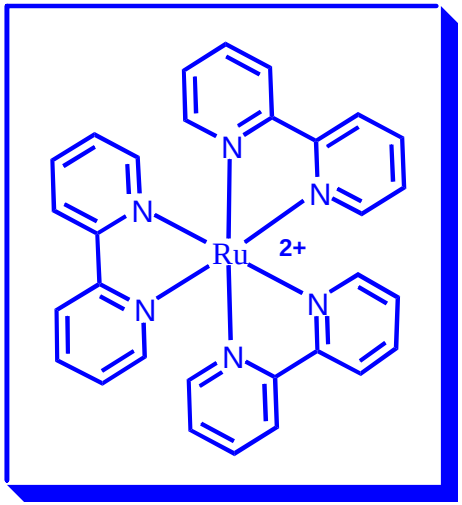
Mohamed Slim, H. F. Sleiman, Bioconjugate Chemistry, 2004, *15*, 949



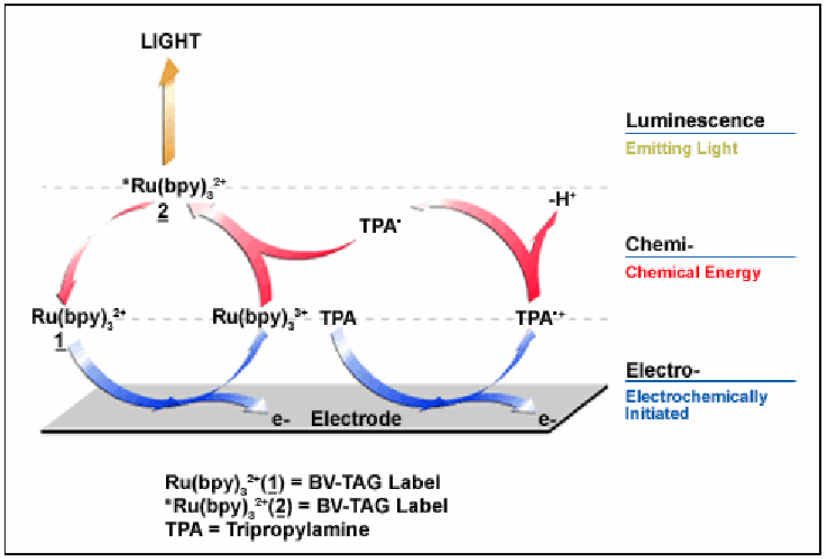


Ruthenium Bipyridine as Chromophore



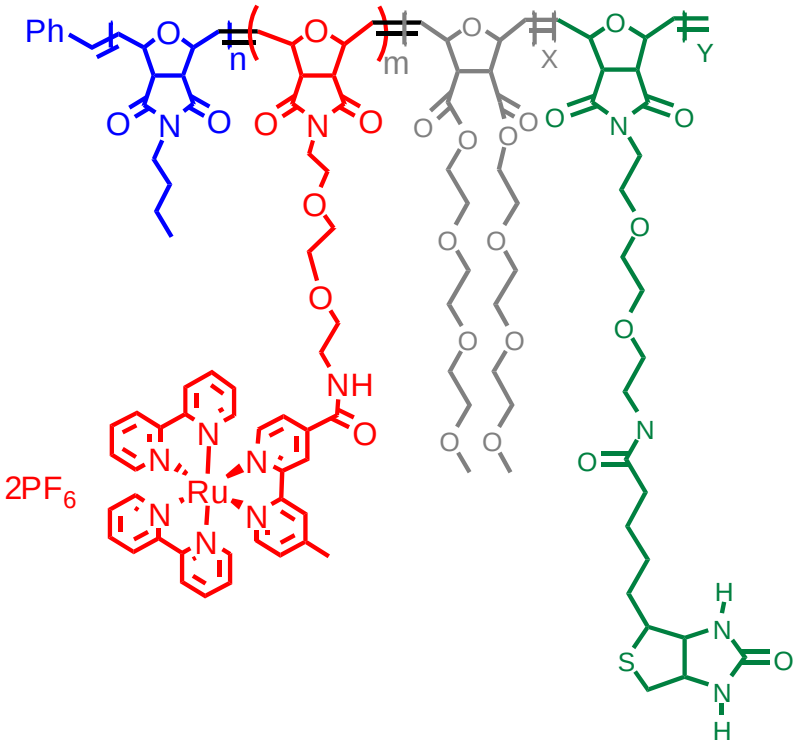


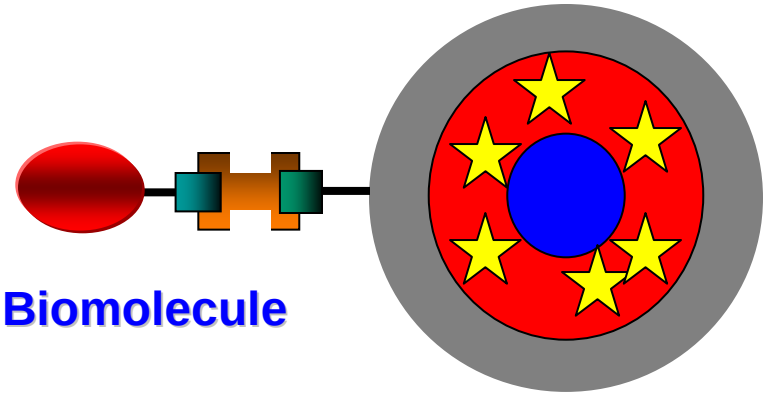
- Long lifetime ~ 1 μs
- Large Stokes Shift
- Resistance to photobleaching
- Electrochemiluminescence (generates light catalytically)



Chem. Rev. 2004, *104,* 3003-3036

Bingzhi Chen, Kim Metera, Rachel Nassif, N. Sankaran





Biomolecule

Micelle
~ 10,000 luminescent centers

B. Chen, K. Metera, H. F. Sleiman, Macromolecules, 2005, *38*, 1084-1090

B. Chen, H. F. Sleiman, Macromolecules, 2004, *37*, 5866

Fluorescence probe for DNA mismatches

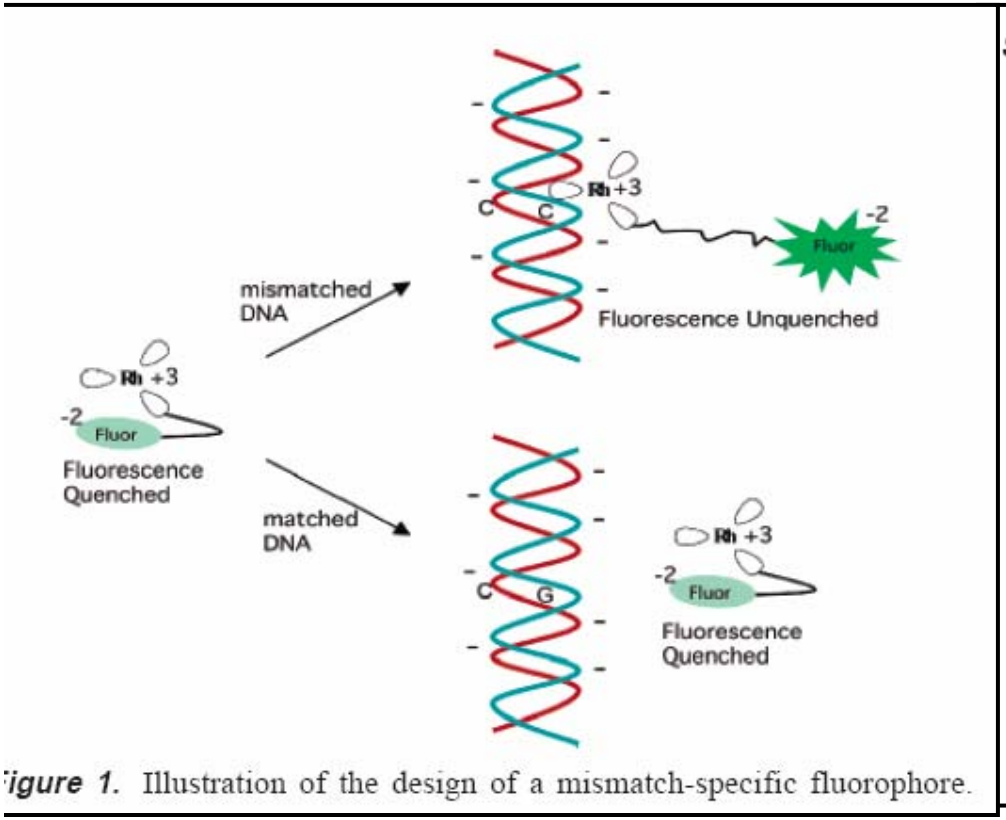
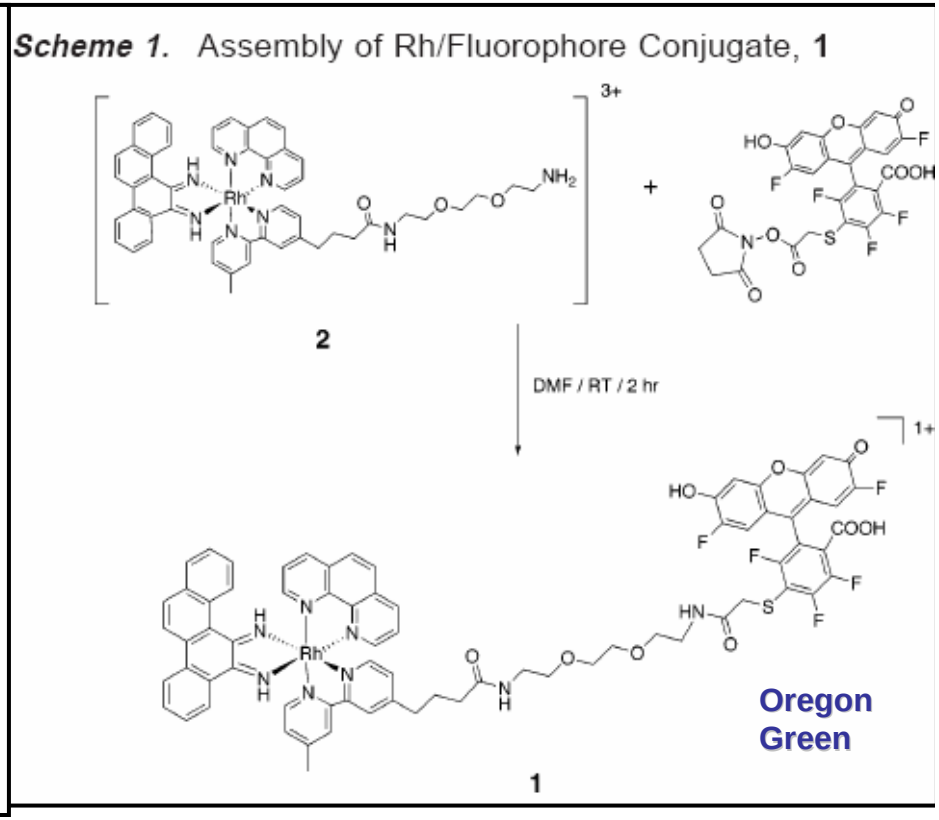
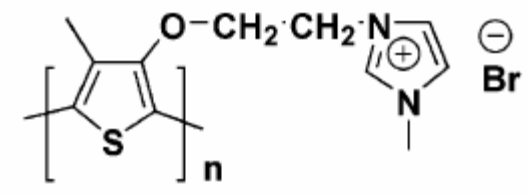


Figure 1. Illustration of the design of a mismatch-specific fluorophore.

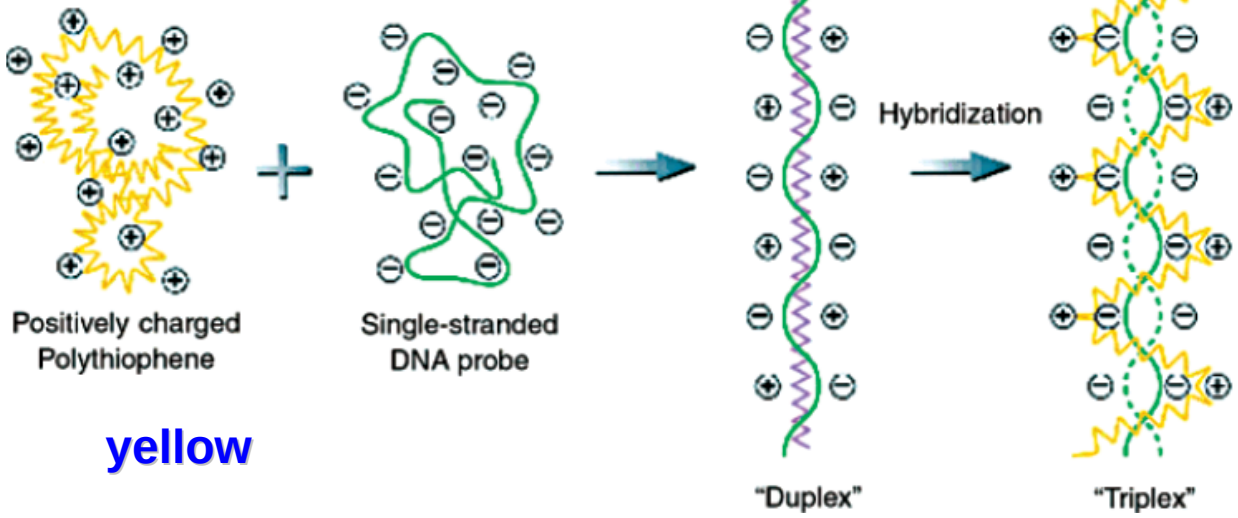


Barton, et al, JACS 2006

Is labeling of biomolecules always necessary?



cationic, conjugated polymer



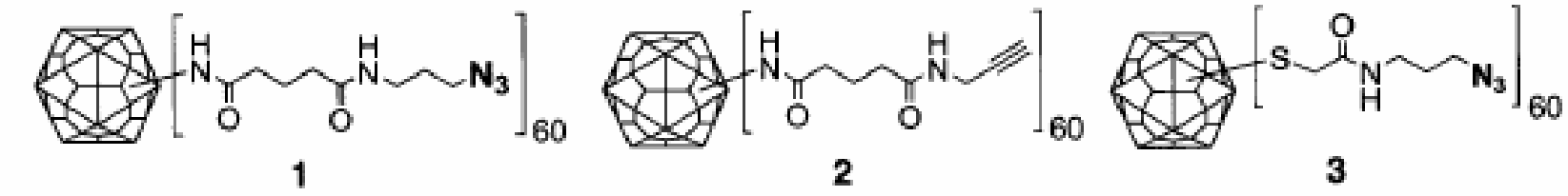
yellow

red **yellow**

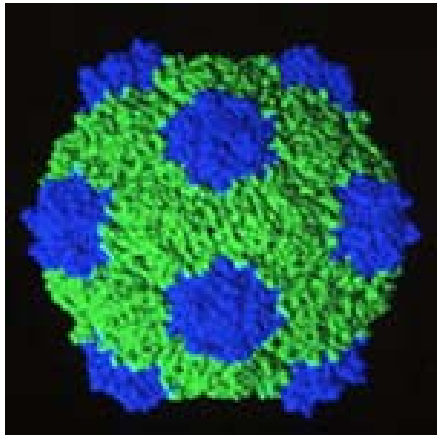
Leclerc, M.; Ho, H. A.; Boissinot, M. WO 02/081735 A2, 2002.

Gaylord, B. S.; Heeger A. J.; Bazan G. C. *Proc. Natl. Acad. Sci. U.S.A.* 2002, *99*, 10954.

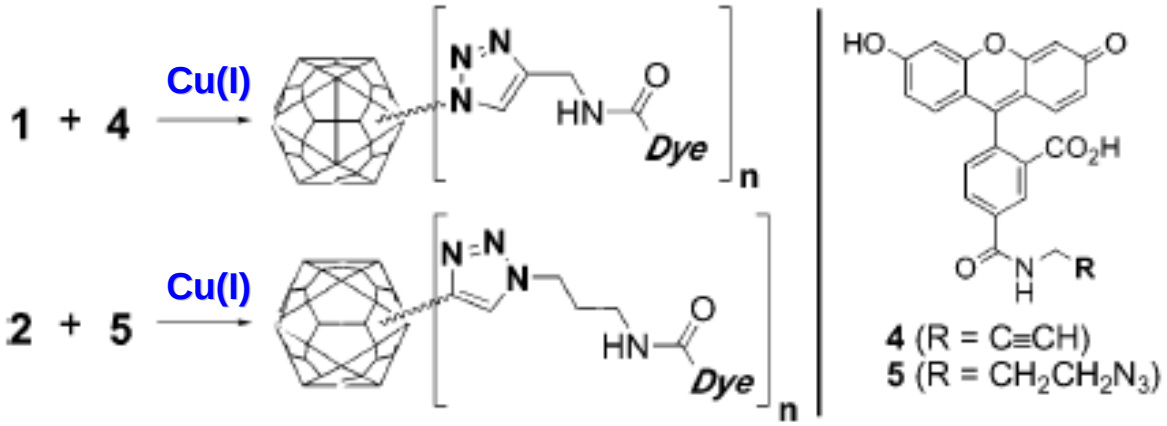
“Click Chemistry” – Labeling of virus at all 60 sites



Cowpea mosaic virus



Scheme 1



Finn, Sharpless, et al, J. AM. CHEM. SOC. 2003, 125, 3192

Staudinger Reaction: *in vivo* biological labeling

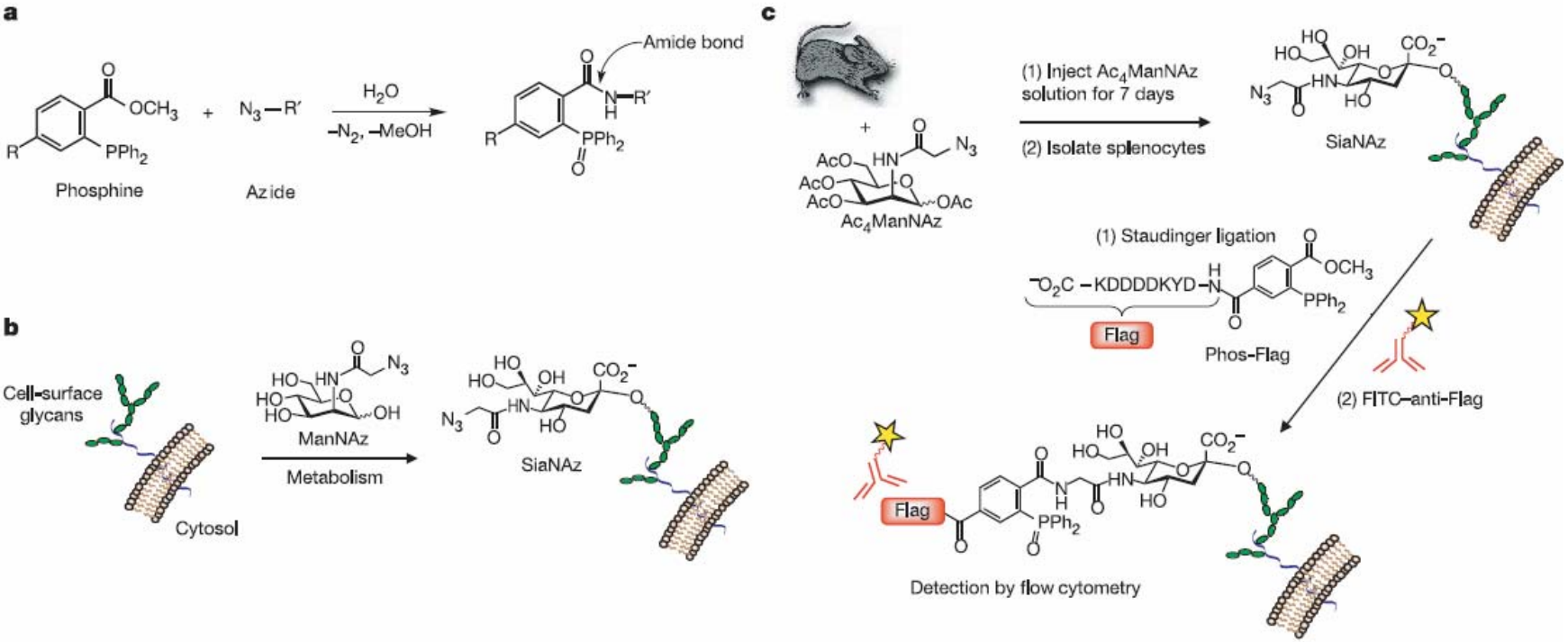


Figure 1 The Staudinger ligation and metabolic oligosaccharide engineering. **a**, The Staudinger ligation of an azide and functionalized phosphine results in the formation of an amide bond. **b**, Azides can be delivered to cell-surface glycoconjugates by metabolism of ManNAz to SiaNAz. **c**, Experimental overview for investigating the metabolic conversion of Ac_4ManNAz *in vivo*. Splenocytes from mice treated with the azido sugar were collected and probed for the presence of cell-surface azides using Phos–Flag. Labelled cells were treated with FITC–anti-Flag and analysed by flow cytometry.

Bertozzi, et al, Nature, 2004, 430, 873

Broad comments:

- The biolabeling “market” is lucrative; “kits” available for most jobs
- Beware of “black box” attitude; need to understand the chemistry
- Think outside the “Molecular Probes” box
- New fluorescent labels: metal centers, quantum dots, conjugated polymers, etc.
- New trends: *in vivo* labeling with bio-orthogonal chemistry