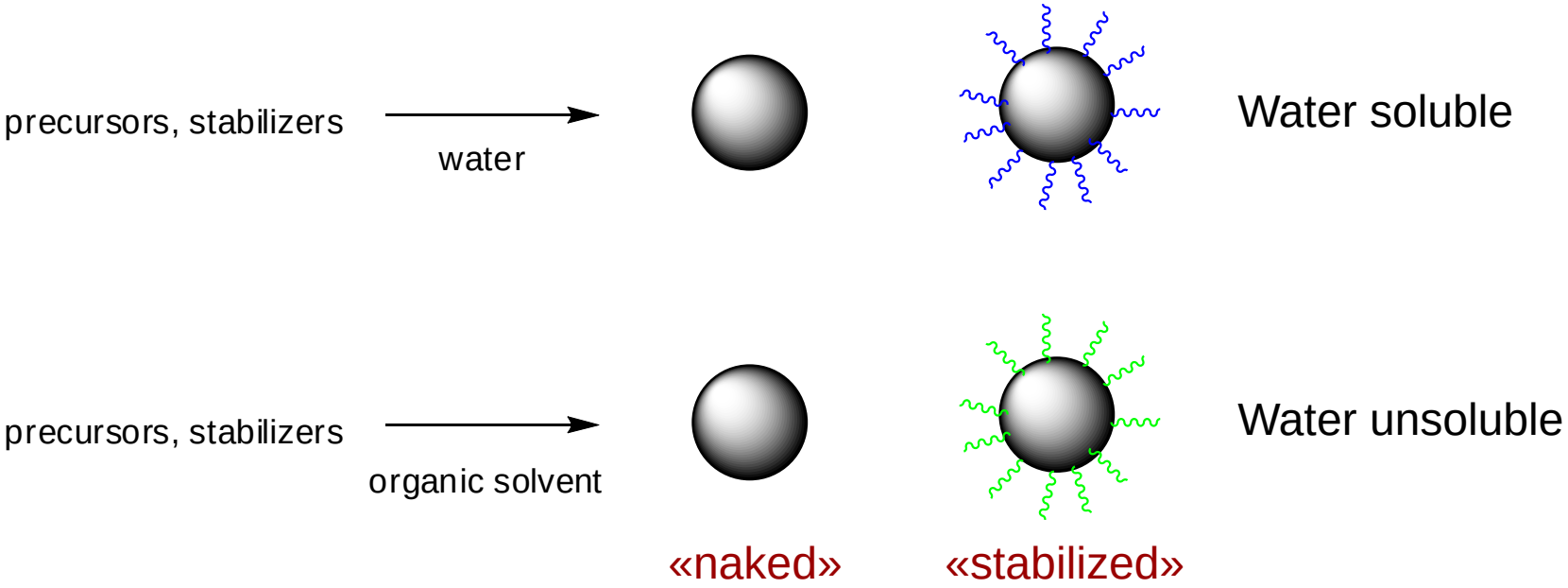
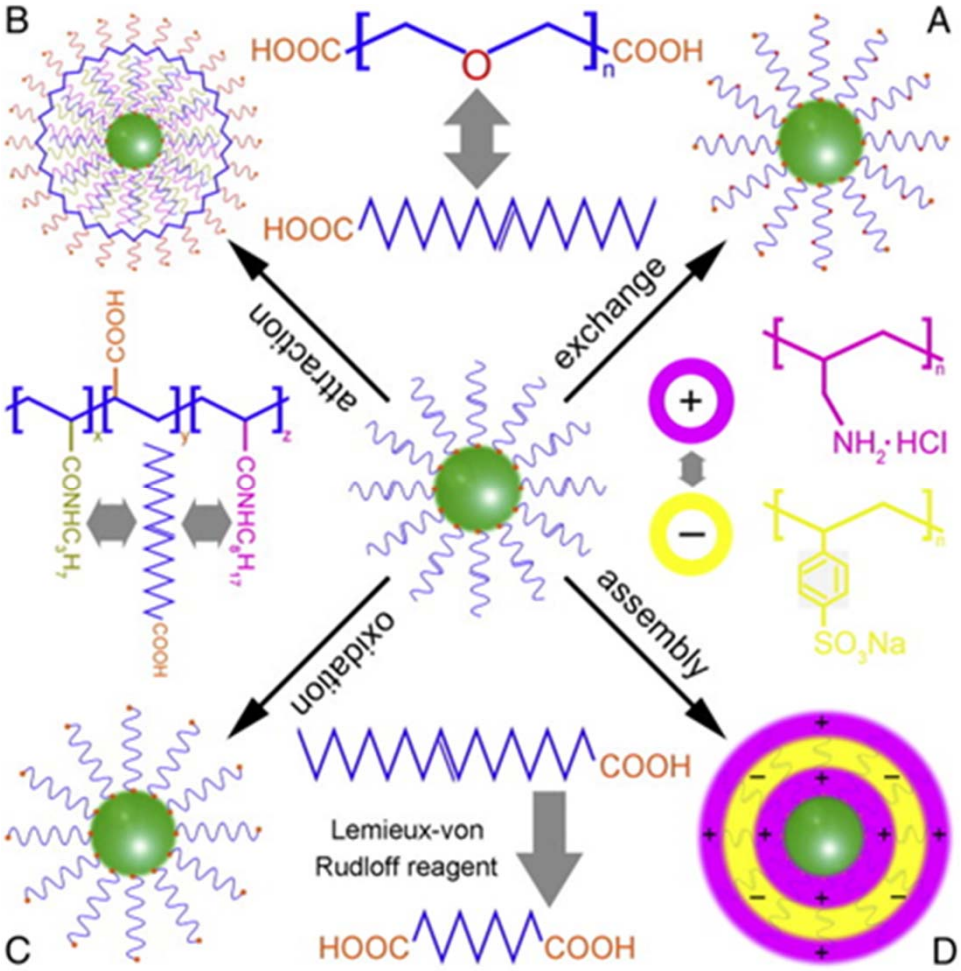
Nanoparticles synthesis



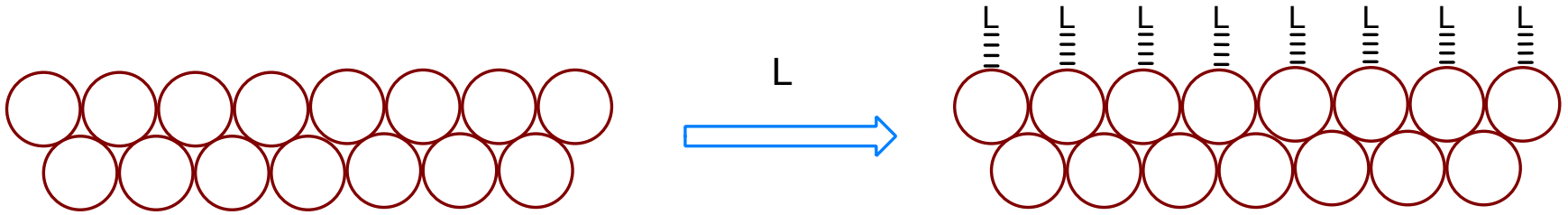
- Synthetic methods in organic solvents are more widely used, as they allow for a better control of the reaction (size, dispersion).
- «Naked» or «weakly stabilized» nanoparticles require stronger stabilization to grant colloidal stability.
- It is difficult to introduce complex functional groups during the nanoparticles formation.

⇨ Functionalization

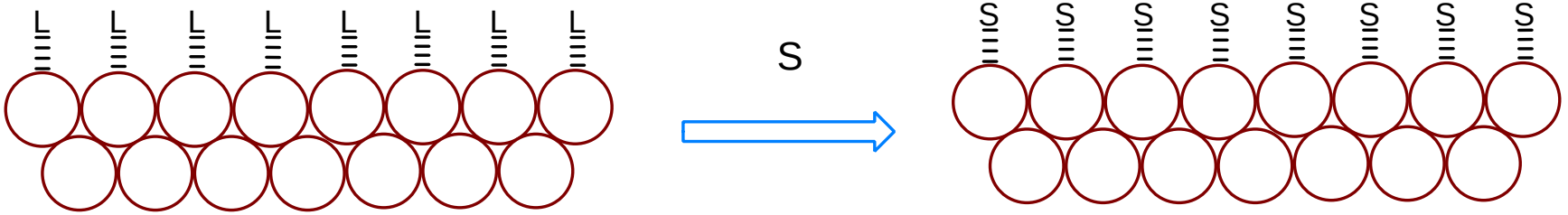
Nanoparticles functionalization



Nanoparticles functionalization

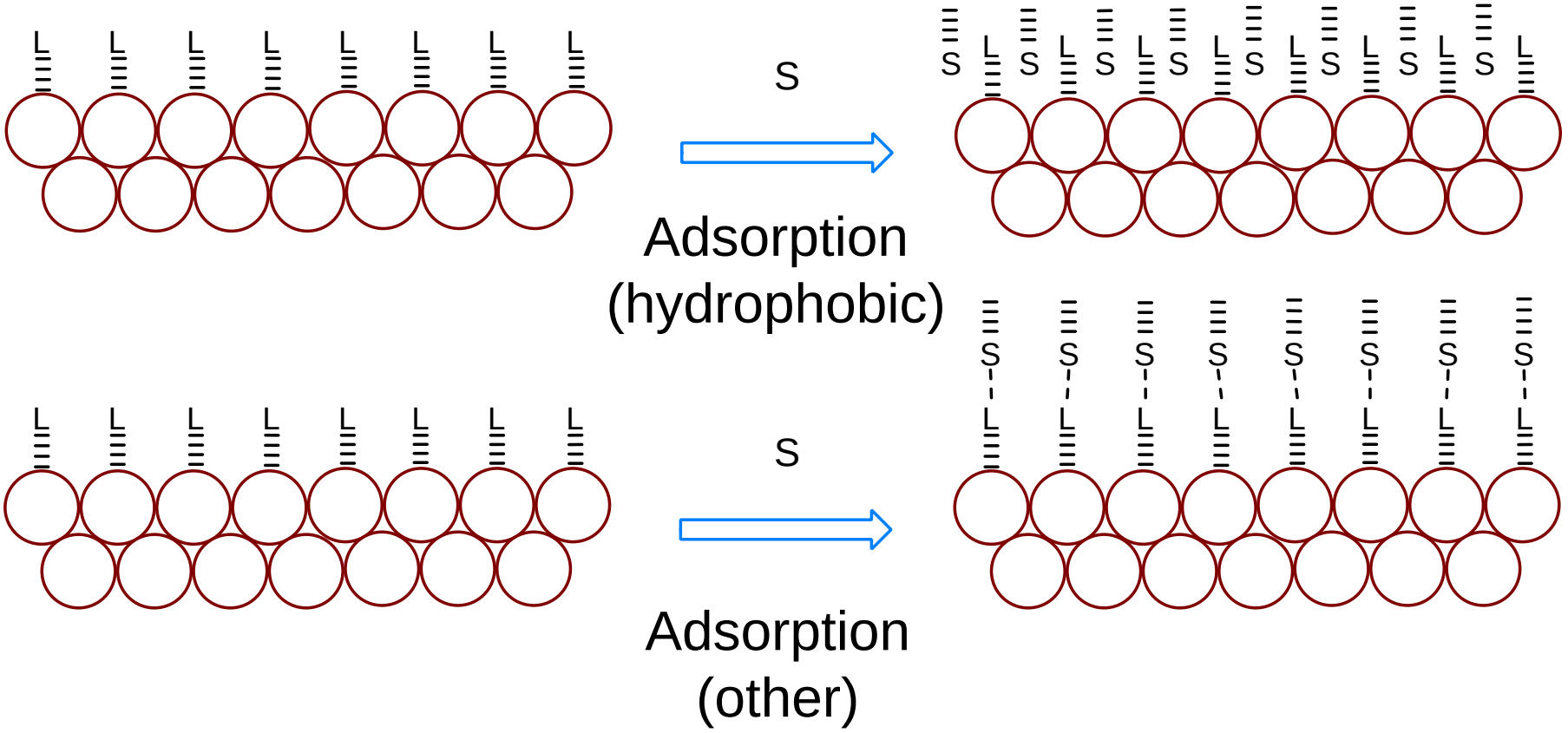


Chemisorption



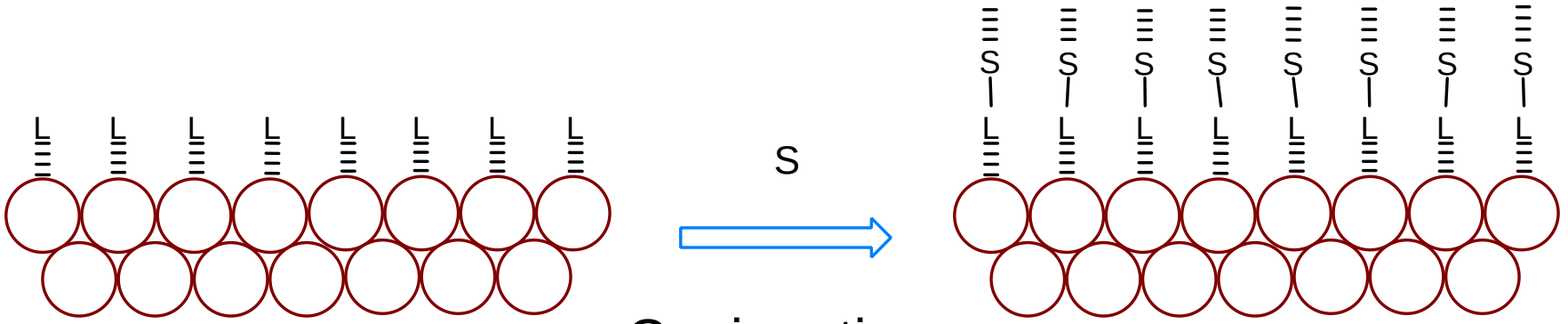
Substitution

Nanoparticles functionalization



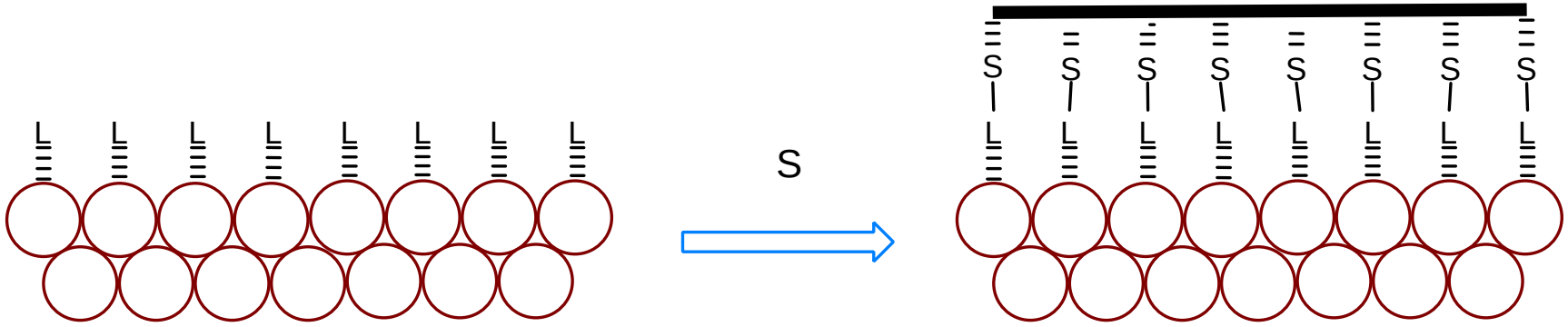
Corona!!

Nanoparticles functionalization



Conjugation

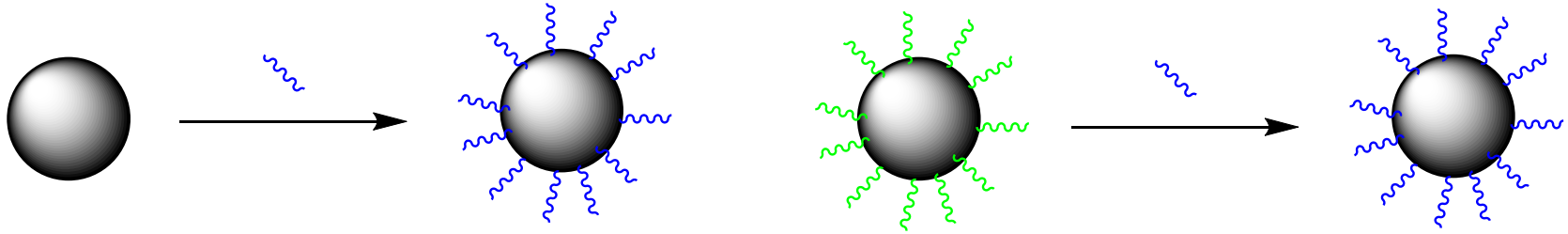
Note that reactive group may belong either to ligands or to nanoparticle surface.



Functionalization effectiveness is always enhanced by cooperation/multivalency, usually this is not necessary in case of conjugation

Nanoparticles functionalization

Ligand exchange/addition



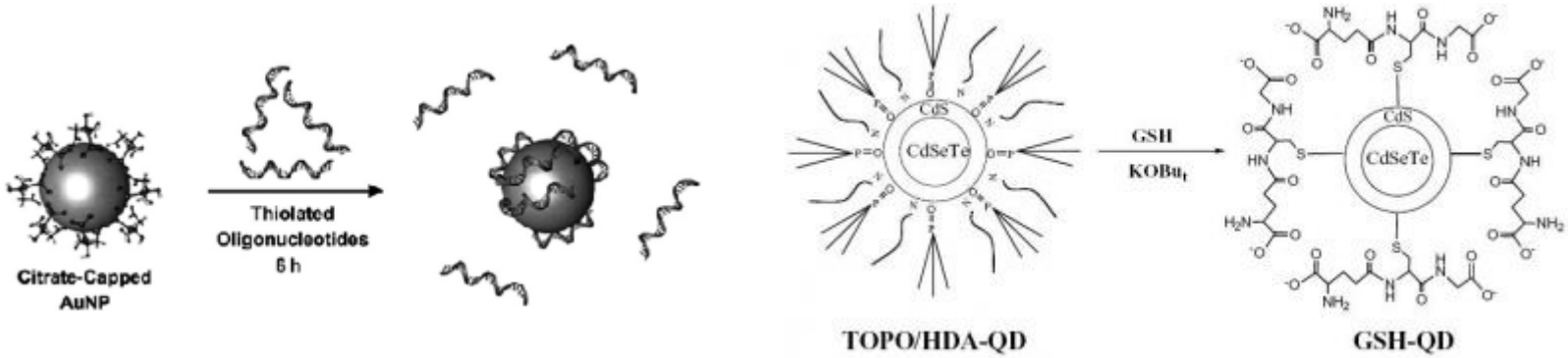
Non-stabilized nanoparticles (silica, titania...) or **weakly stabilized nanoparticles** (gold-citrate, gold-amines, semiconductors-amines, semiconductors-phosphine oxide, iron oxide carboxylates, …) can be functionalized by the addition of a stronger ligand that cover the surface or displace the weakly bound one.

Exchange reactions are possible also in the case of strongly stabilized nanoparticles (gold-thiols, …), but the final composition is likely an equilibrium mixture.

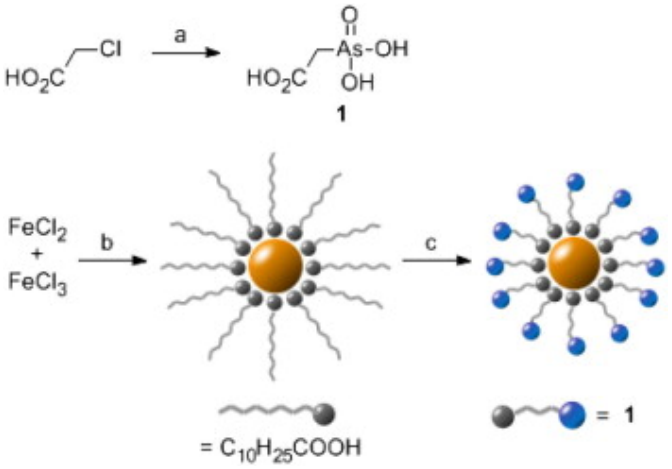
Nanoparticles functionalization

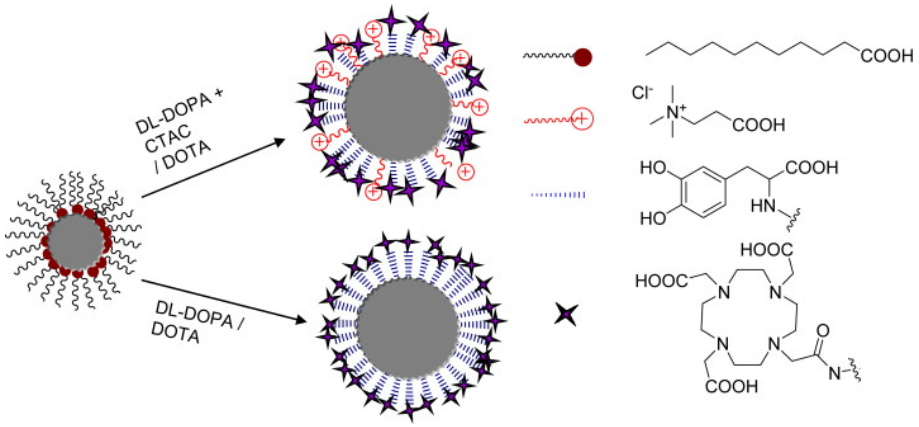
Ligand exchange/addition

- Metal and semiconductors: thiols



- Iron oxide: phosphates, arsenates and catechols

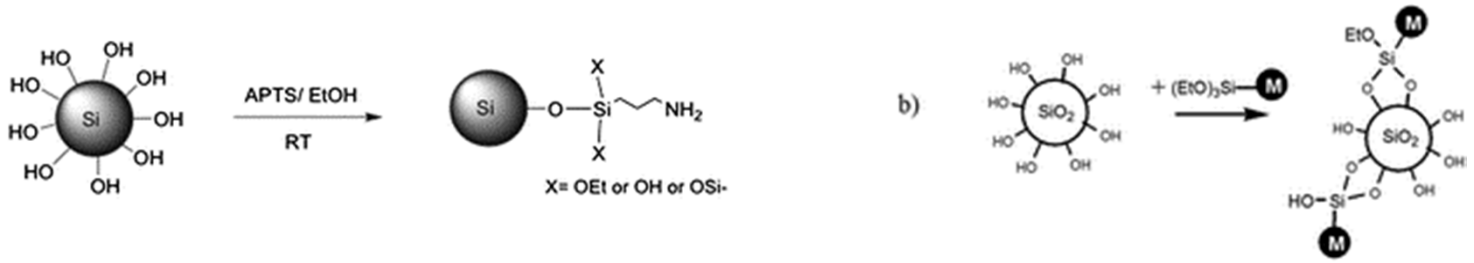




Nanoparticles functionalization

Ligand exchange/addition

- Silica: trialkoxysilanes



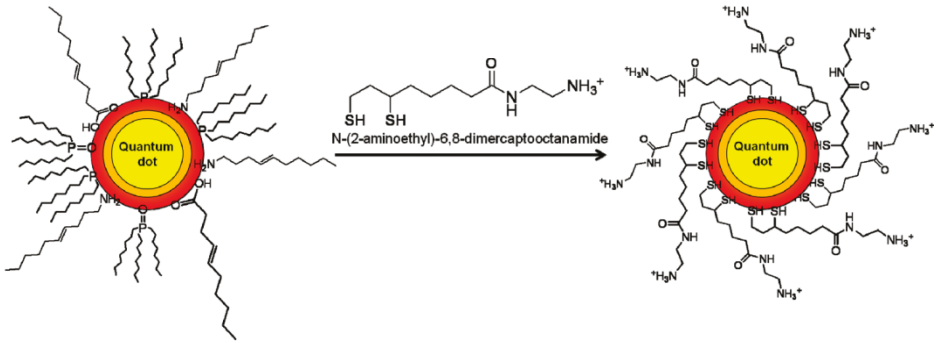
- Easy for naked/weakly stabilized nanoparticles
- Difficult to control for strongly stabilized nanoparticles
- Particle aggregation is possible
- May require strong excess of the incoming ligand

- May result into poorly stabilized particles (if strong ligands are not available)
- Solution -> **Cooperativity**

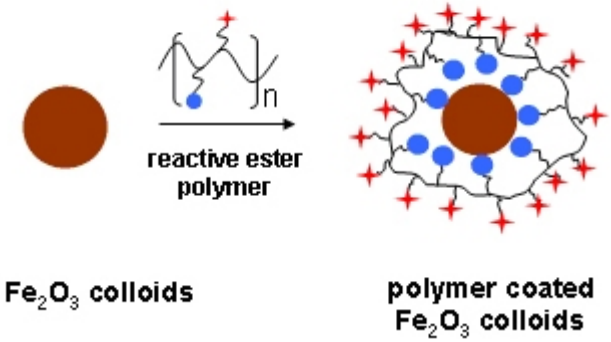
Nanoparticles functionalization

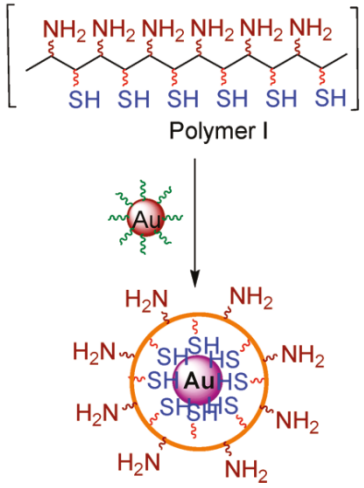
Solutions for ligands exchange:

- Polydentate ligand: increased stability due to cooperation



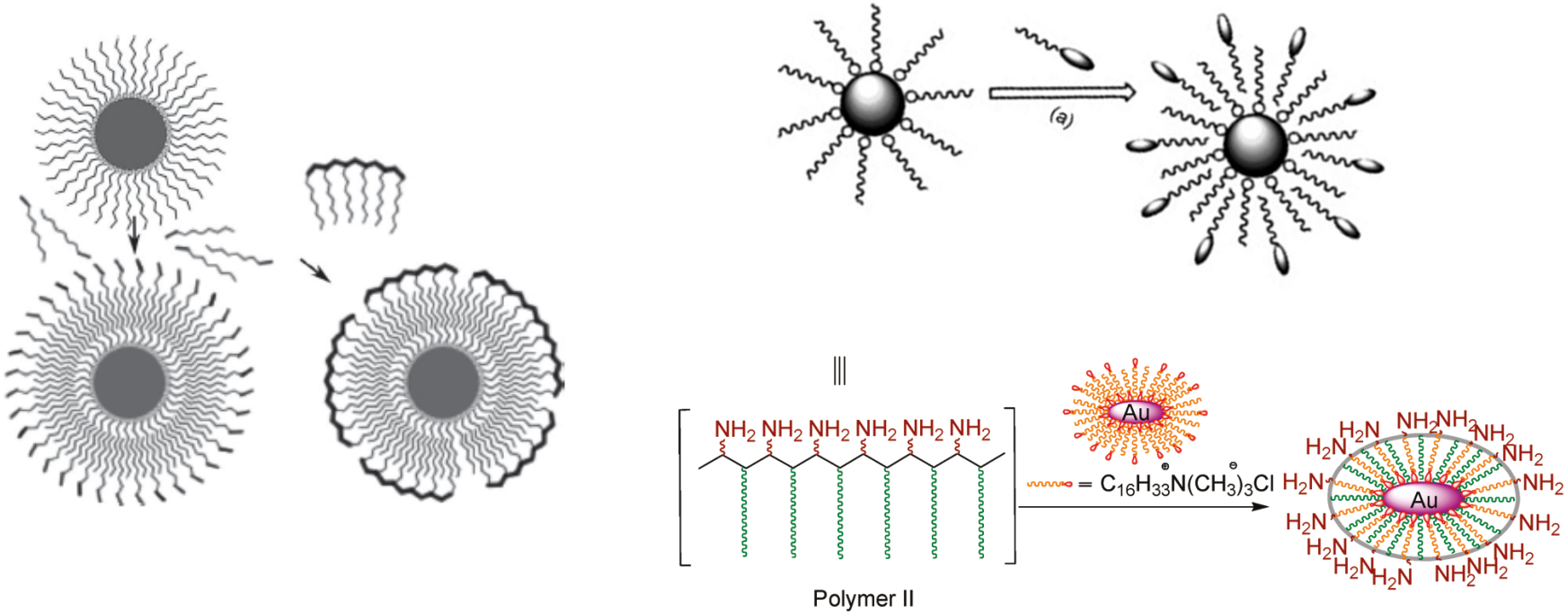
- Polymers:





Nanoparticles functionalization

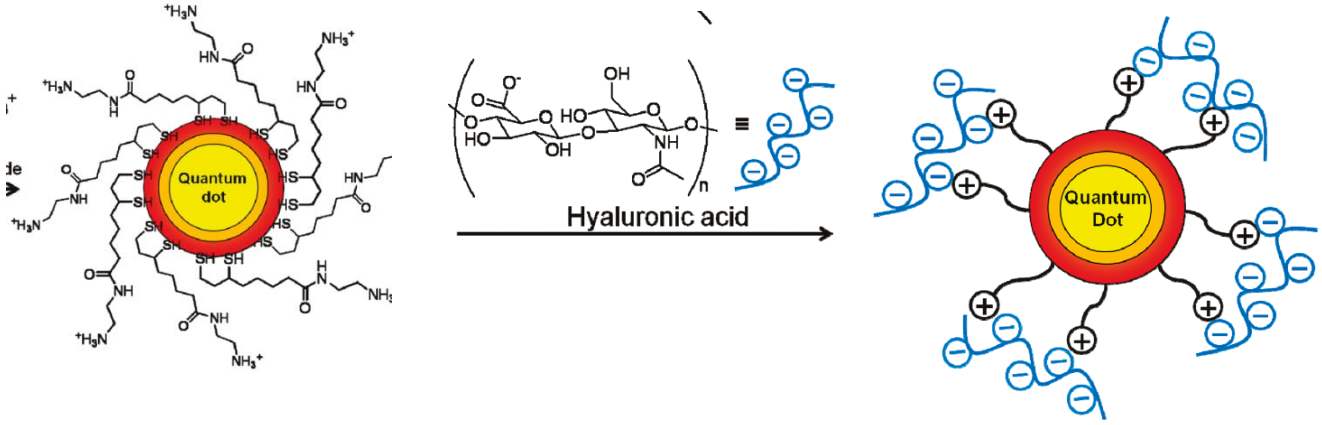
Adsorption by formation of an interdigitated layer

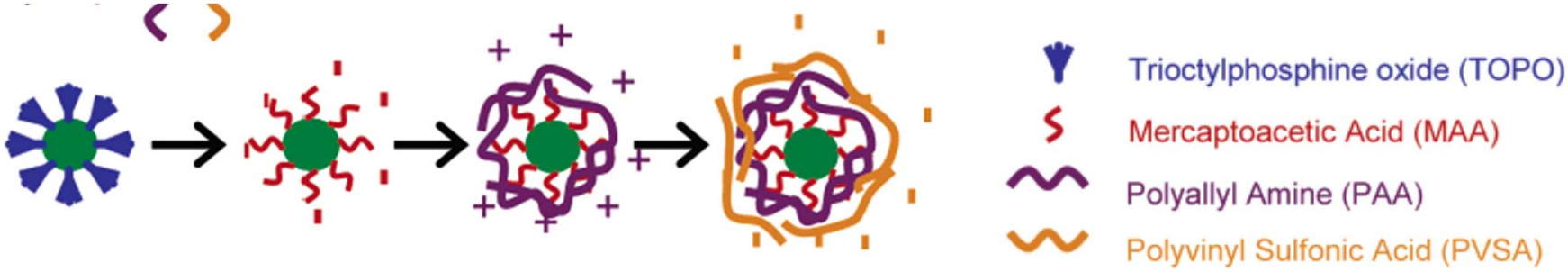


- Allow for the use of **commercially** available surfactant and phospholipids
- Strength of interaction can be controlled by interdigitating chain length/number
- Non-covalent interaction based on hydrophobic interaction: liphophylic compartments in biological environments may interphere

Nanoparticles functionalization

Adsorptio by non-covalent interaction: Layer by layer coating

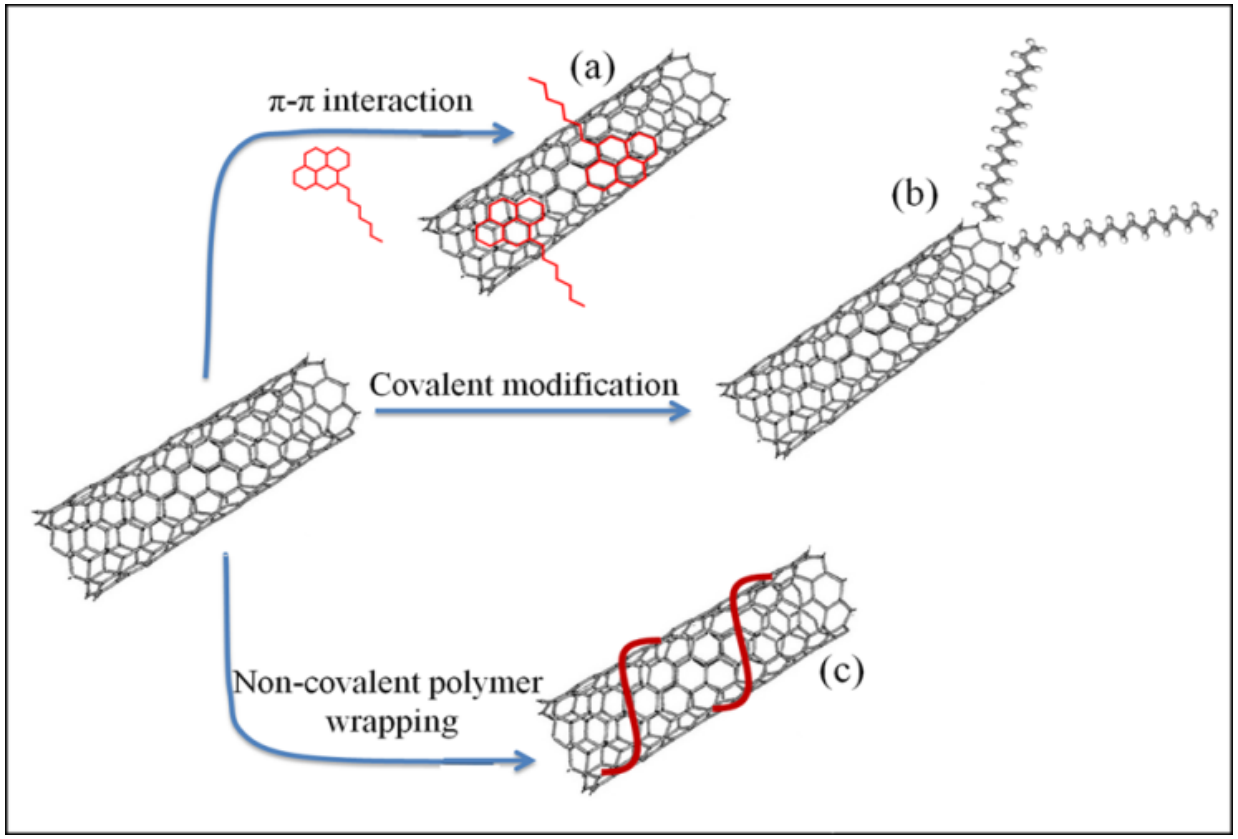


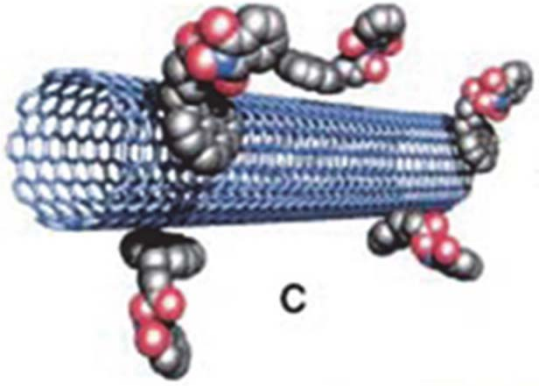


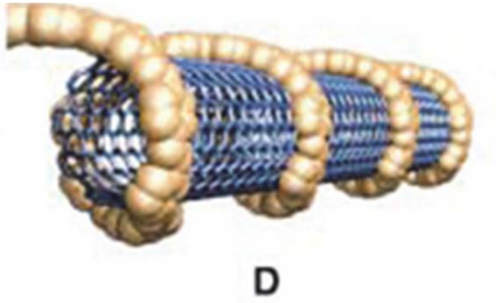
- Allow for the realization of thick shells
- Thickness of the coating can be easily controlled
- Problems in high ionic strength media

Nanoparticles functionalization

Adsorption by non-covalent interaction: hydrophobic/stacking

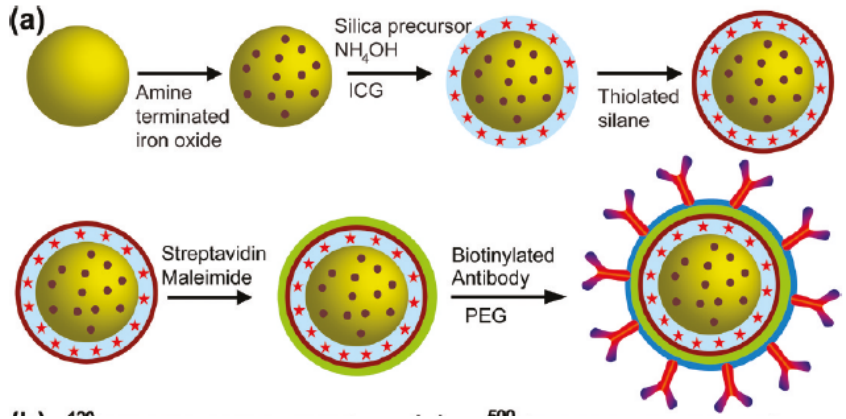


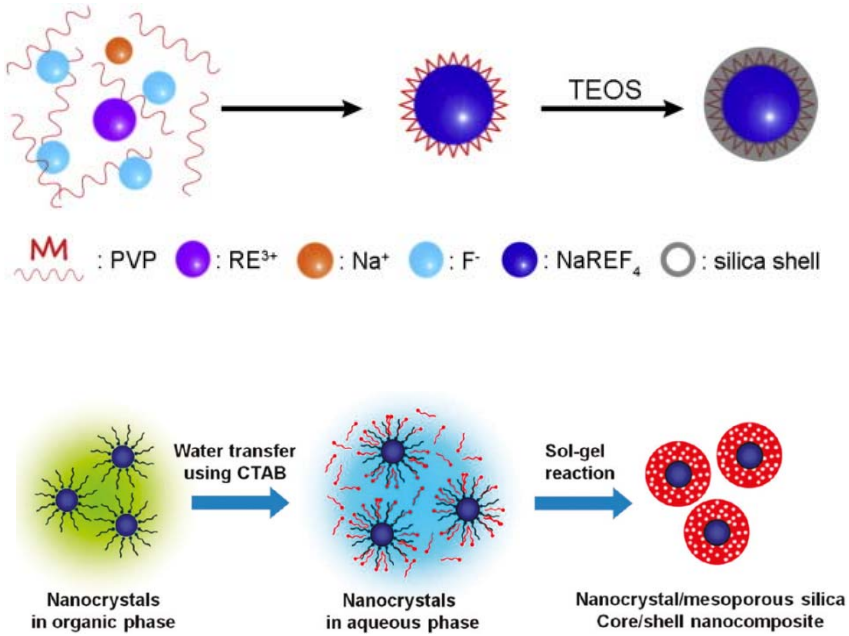




Nanoparticles functionalization

Silica coating

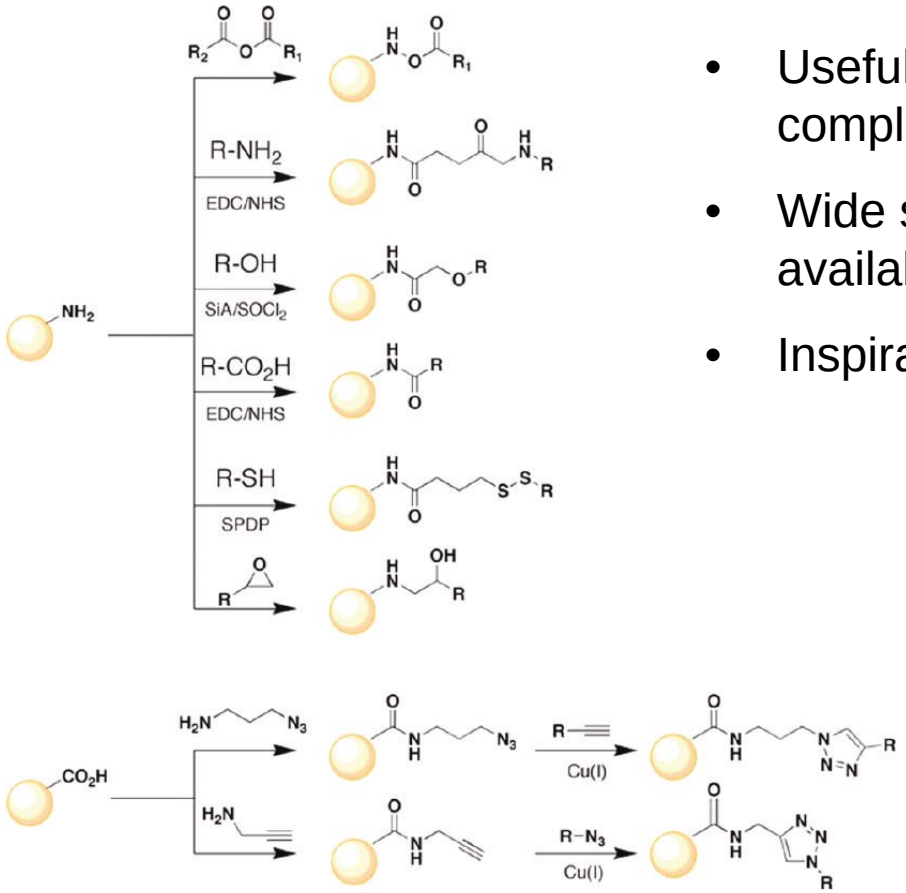




- Protecting shell: stable (?), biocompatible (?), porous, easily modified.
- Several trialkoxysilanes commercially available
- Active species can be entrapped also “within” the silica shell (bulk and poress).
- The core can be removed by chemical reactions with suitable reagents
- Require water/ethanol soluble nanoparticles

Nanoparticles functionalization

Reactions at the surface



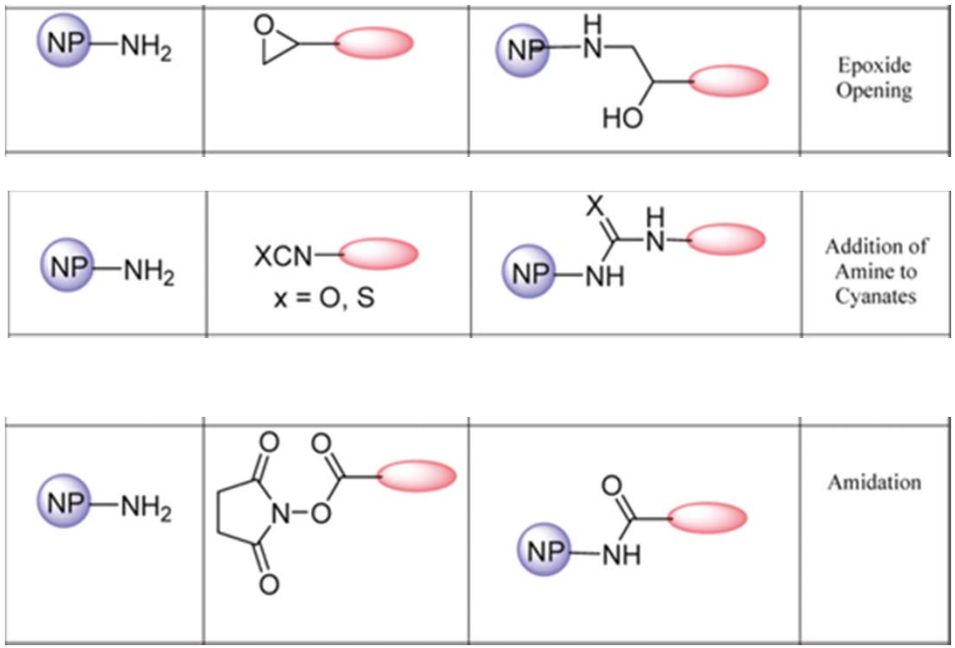
- Covalent binding ensures stability
- Useful/necessary for the introduction of complex functional groups/active species
- Wide selection of conjugation reactions available
- Inspiration from bioconjugation chemistry

Requisites

- Must form stable bonds
- High yield to avoid excess of reactants (expensive)
- Mild conditions
- Better if feasible in water
- Better if side products are not produced (click chemistry)

Nanoparticles functionalization

Reactions with amino groups



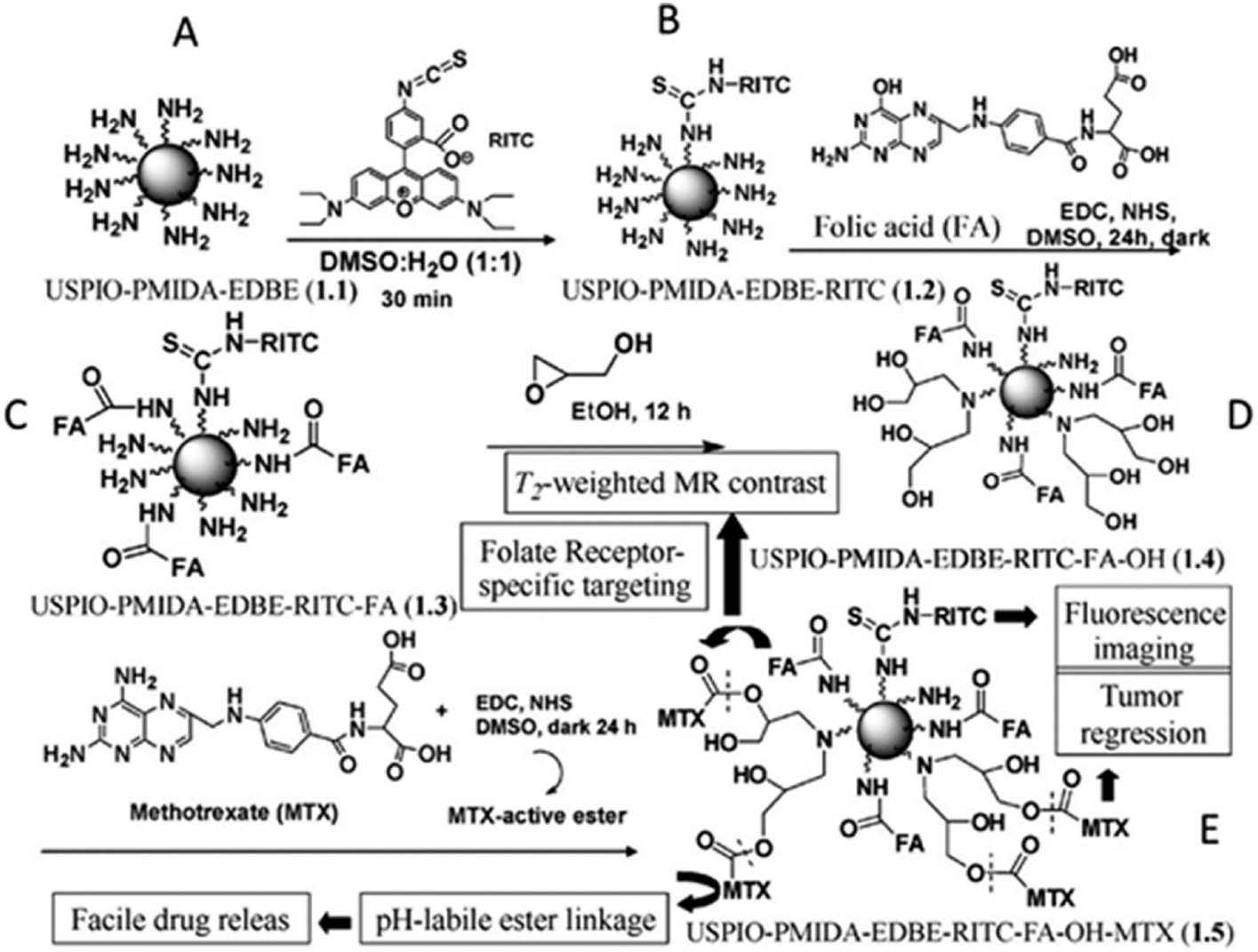
Click reactions

N-hydroxysuccinimide is being replaced by 2,3,5,6-tetrafluorophenol

- Amines are easily introduced in the nanoparticles and stable
- Amines are protonated below pH 9
- Water (and hydroxide) competes
- Other reactions: sulphonyl clorides (-SO_2Cl), reductive ammination with aldeides, *in situ* amidation with coupling reagents

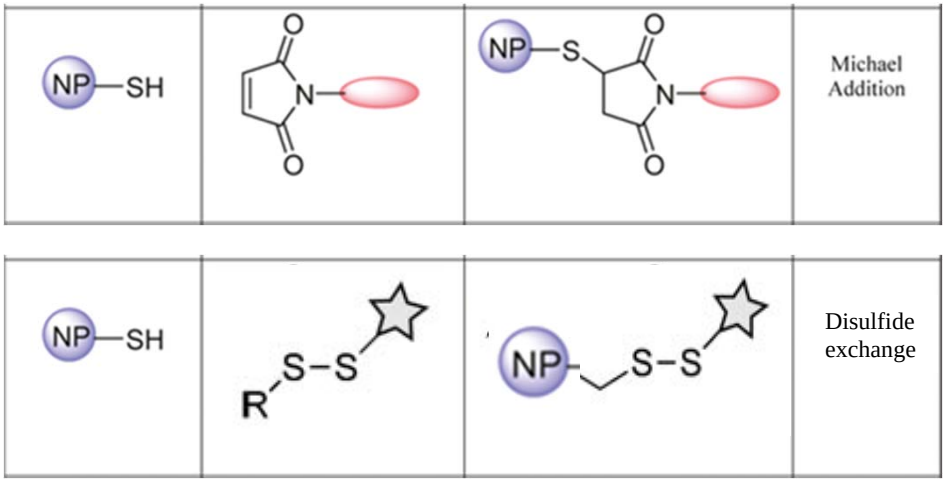
Nanoparticles functionalization

Reactions with amino groups



Nanoparticles functionalization

Reactions with thiols groups

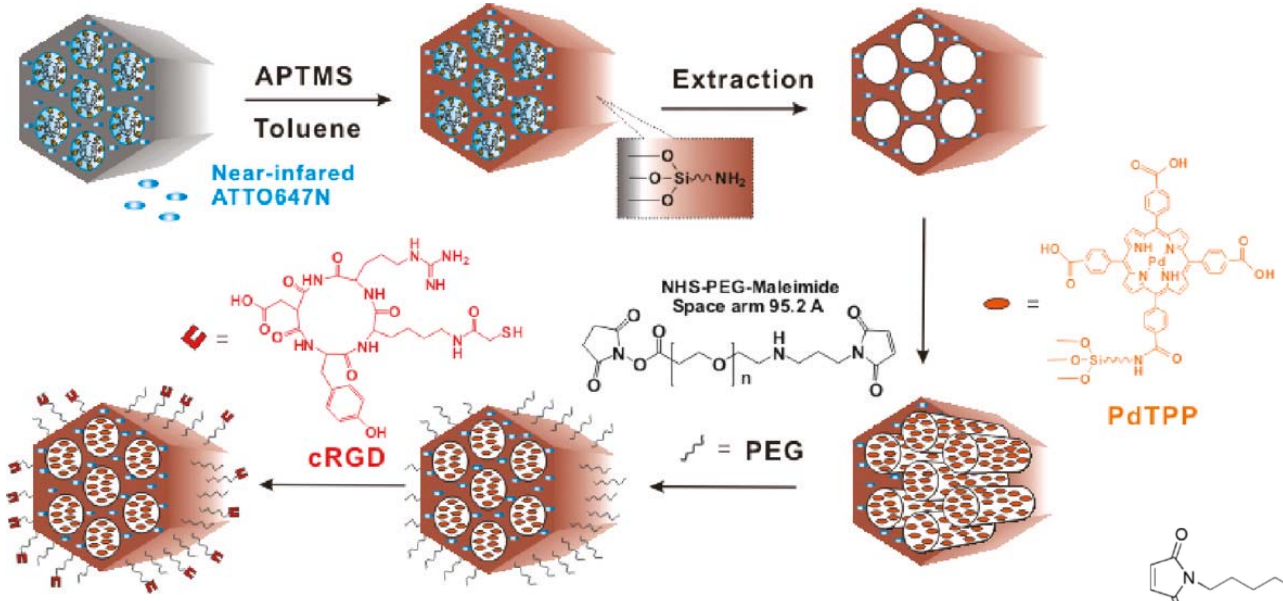


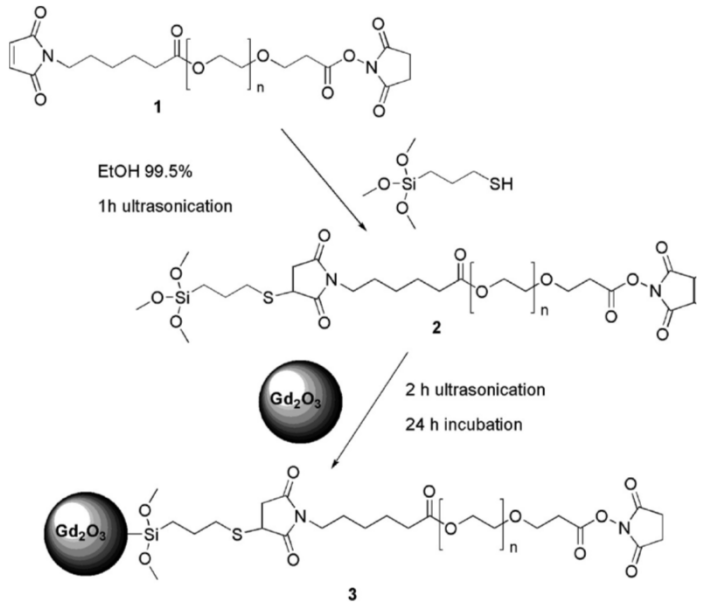
Click reaction

- Fast and quantitative
- No water competition
- Thiols are widespread in biological molecule (cysteine)
- Introduction of thiols in nanoparticles may be difficult
- Cross-linking reagents available

Nanoparticles functionalization

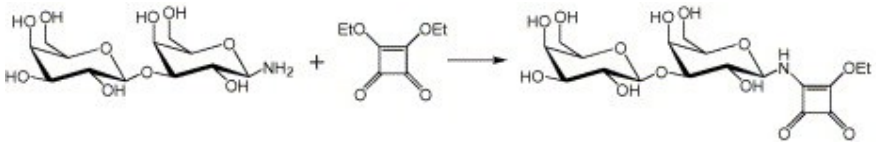
Reactions with thiols groups

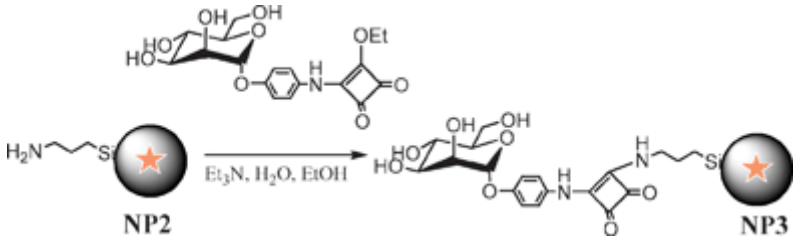




Transforming functional groups

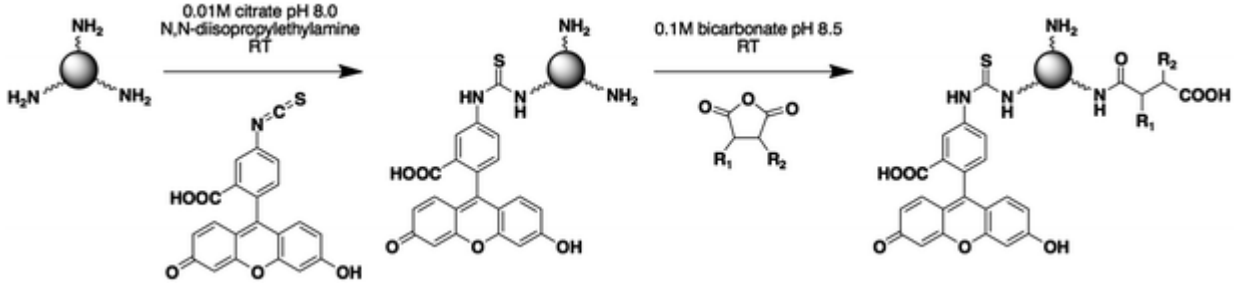
- Amines into active esters: diethyl squarate





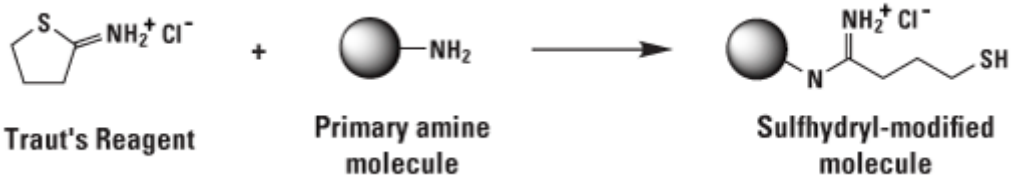
It is easy to fine amines both in the nanoparticle and in the molecule to conjugate. Diethyl squarate allows conjugating amines with amines

- Amines into carboxyl acids: anhydrides

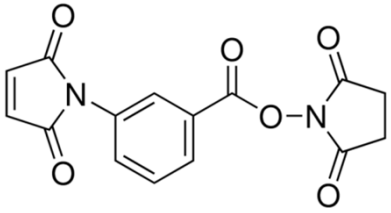


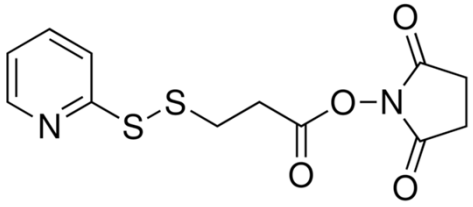
Transforming functional groups

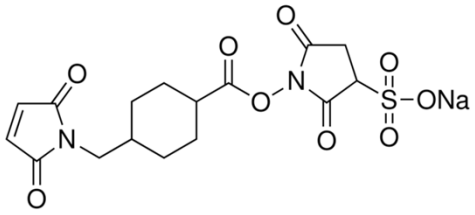
- Amines into thiols: imminothiolane



- Crosslinkers

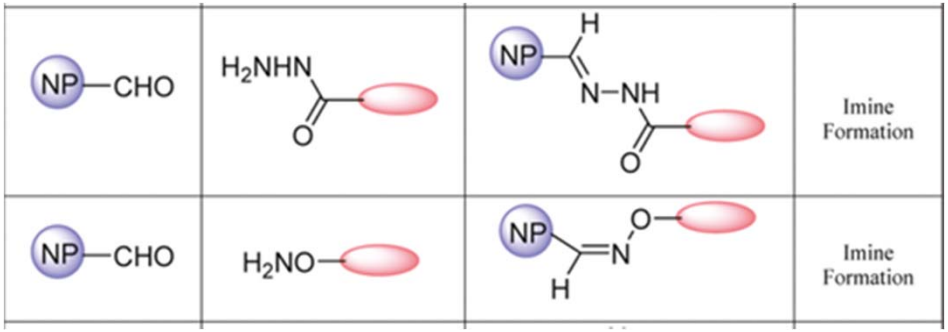






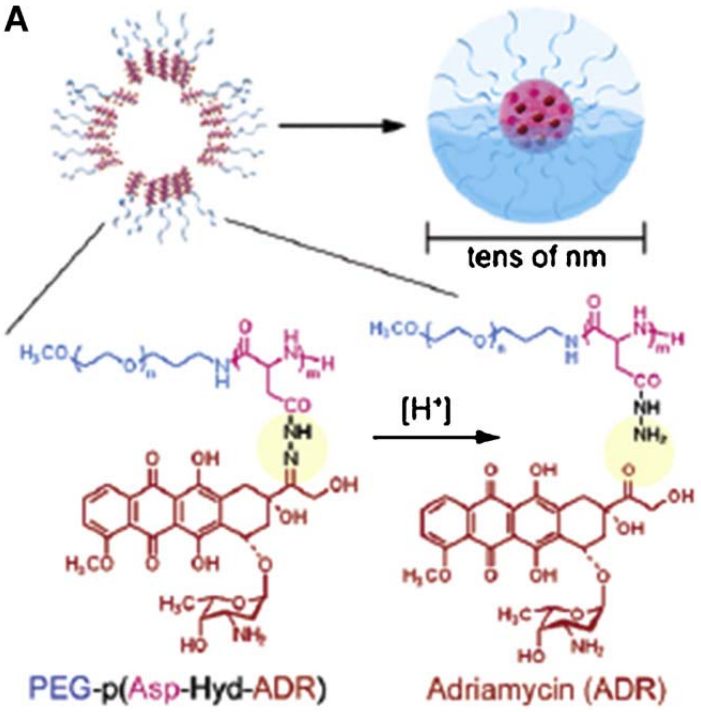
Nanoparticles functionalization

Reactions with aldeide groups



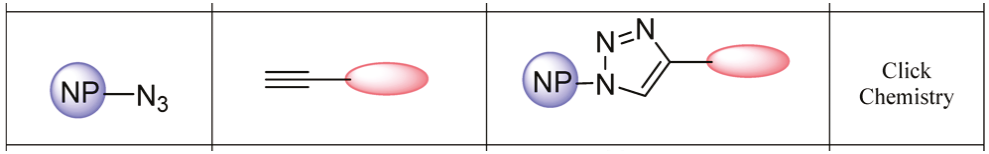
- Fast and quantitative
- Reversible!
- No water competition
- By-product is water
- Difficult to introduce in nanoparticles

Quasi-click reaction



Nanoparticles functionalization

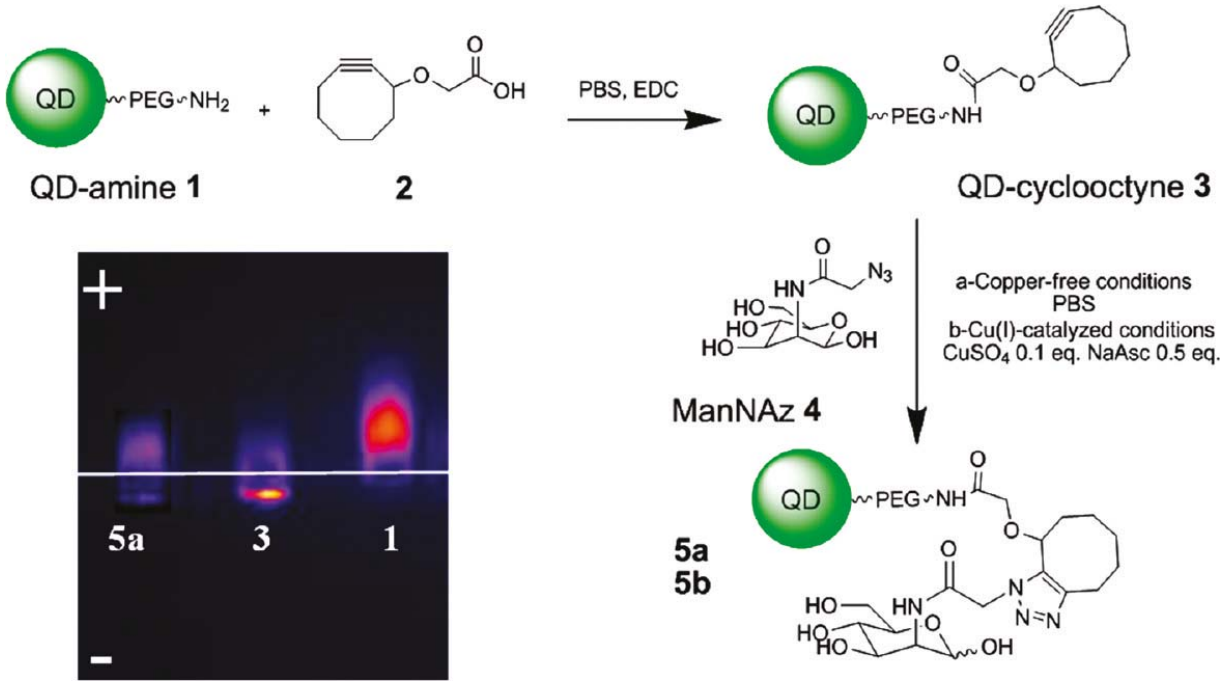
Reactions azide/alkine



3+2 cycloaddition

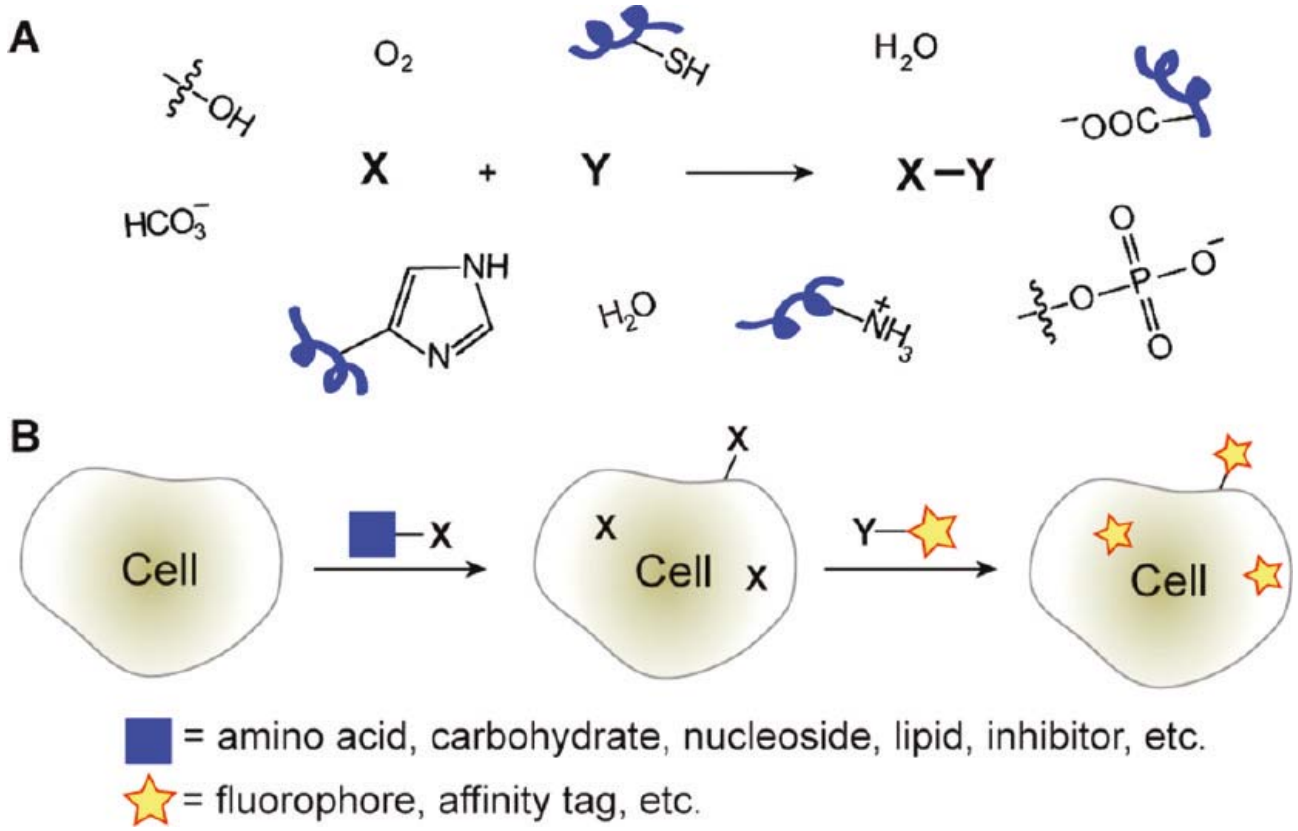
"Huisgens reactions"

- Fast and quantitative (with Cu(I) or high temperature)
- No water competition
- No by products
- Stable functional groups



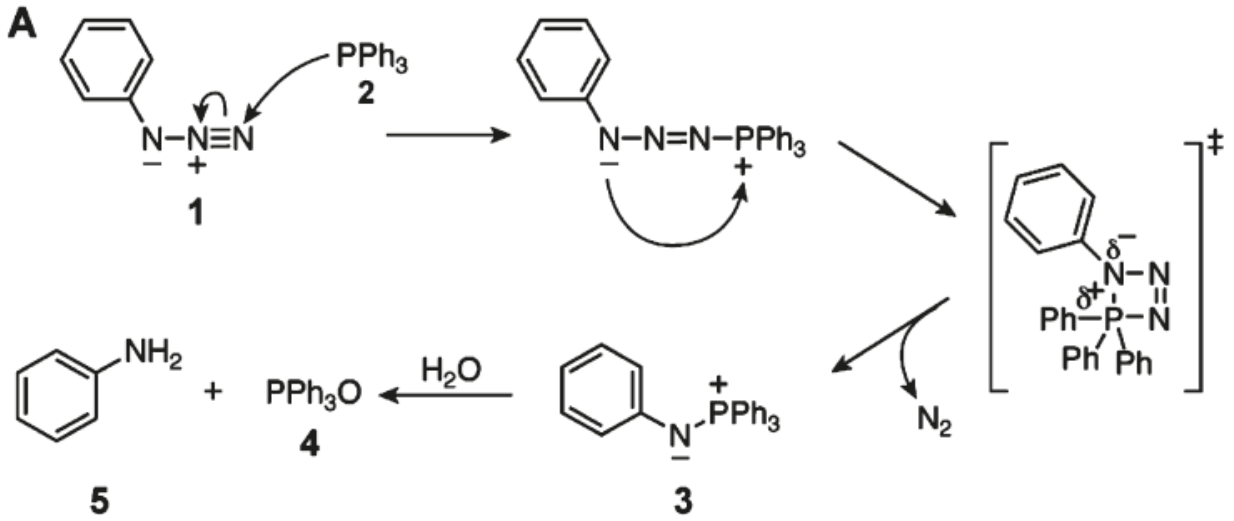
Bioortogonal chemistry

Bioorthogonal reactions are chemical reactions that neither interact with nor interfere with a biological system.



Bioorthogonal chemistry can be used to probe biomolecules in living systems

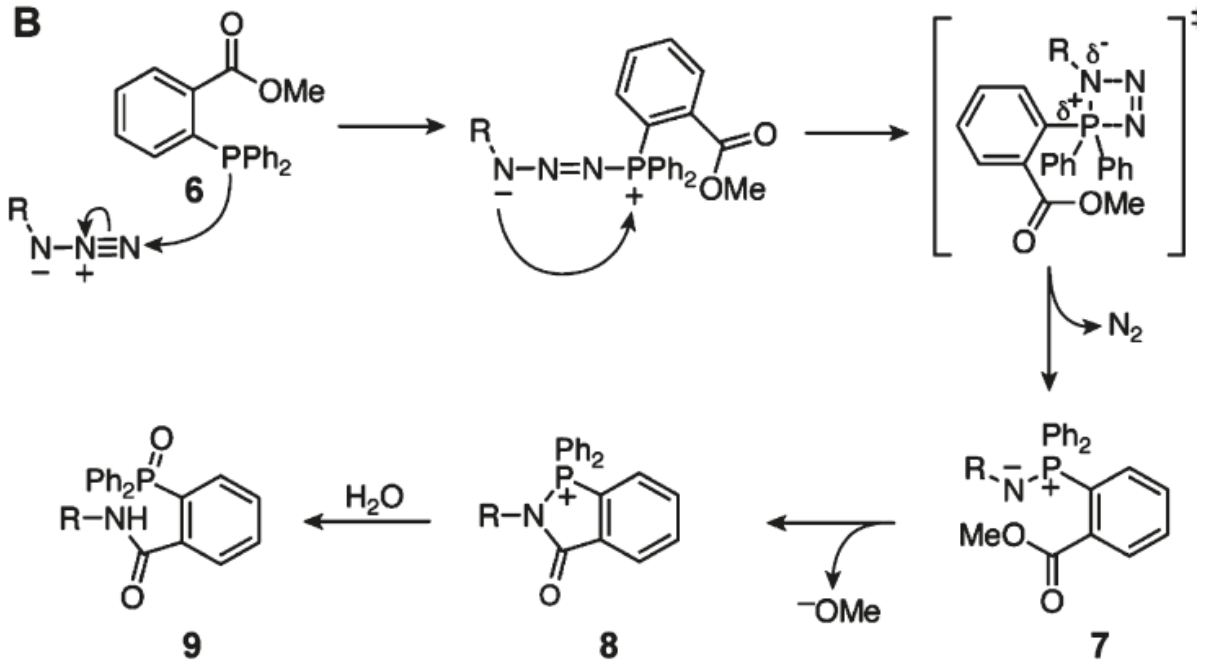
The Staudinger reduction



- Used to reduce azides to amines in mild conditions.
- Water tolerant
- Soft electrofiles/nucleophiles.
- Azide is stable, present in many drugs, small
- Azide and phosphine are absent in living organisms

In its first steps, the reaction forms a bond between the two molecules, but the reactive aza-ilide (**3**) is cleaved by water.

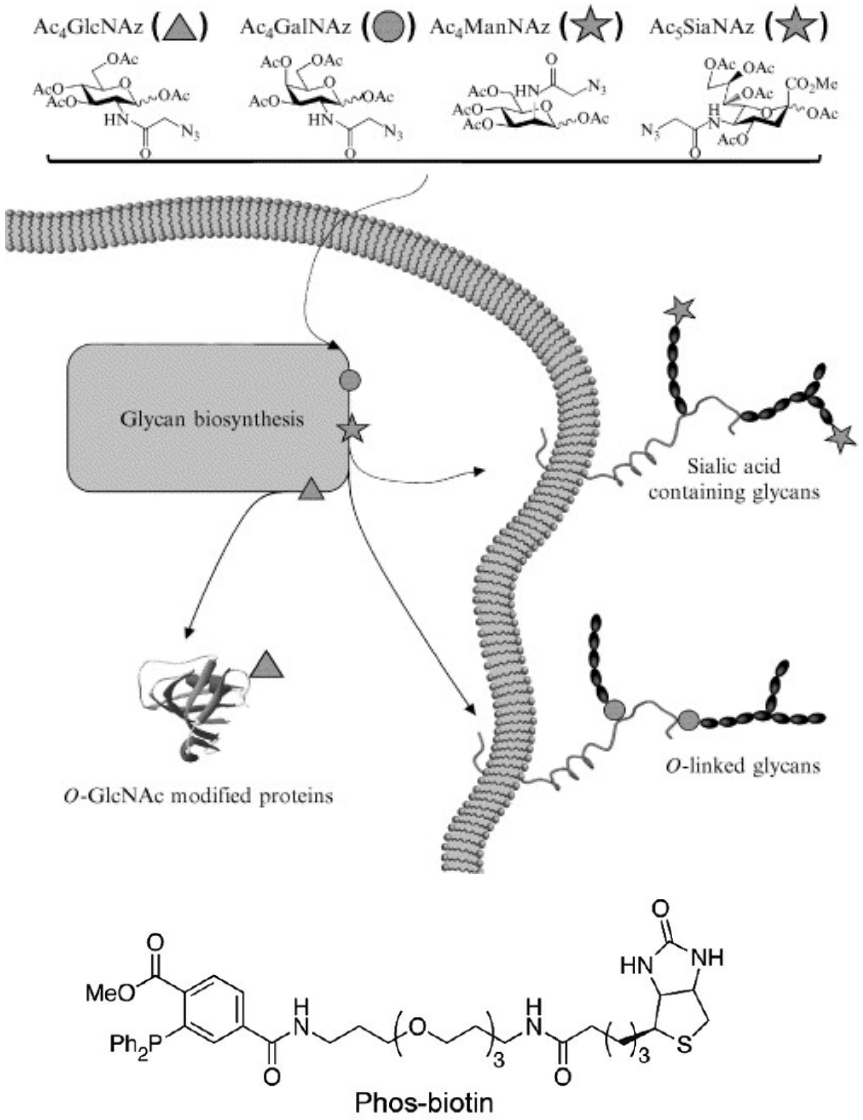
The Staudinger ligation

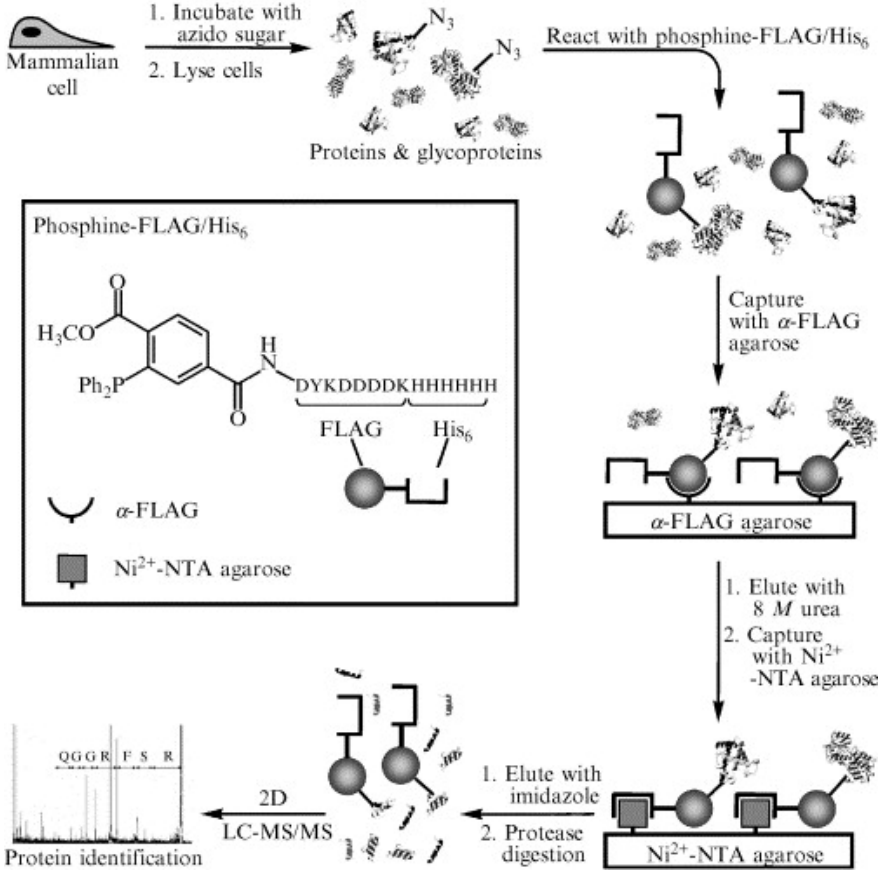


- The ester group in the orto position is attached by the nucleophilic nitrogen of the aza-ilide group.
- A stable imide bond is formed

The Staudinger ligation

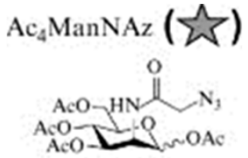
Metabolic labeling of glycans

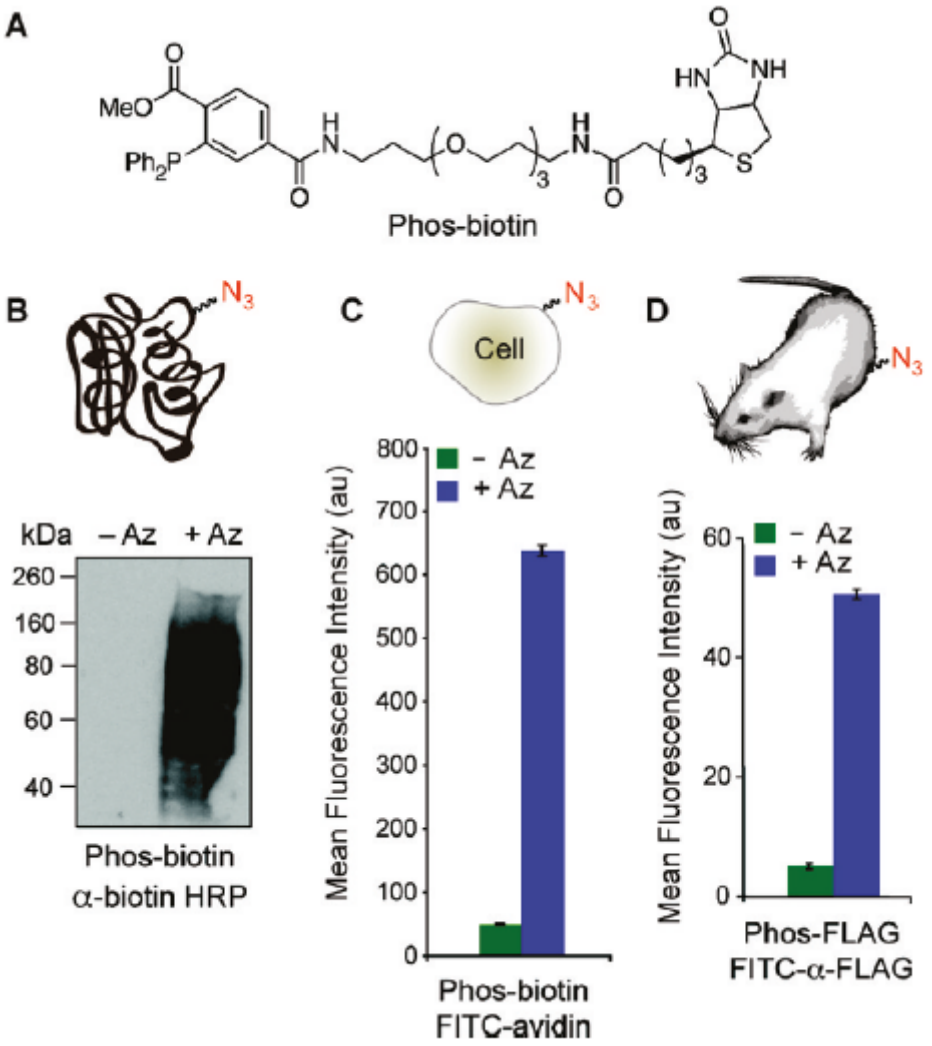




The Staudinger ligation

Metabolic labeling of glycans

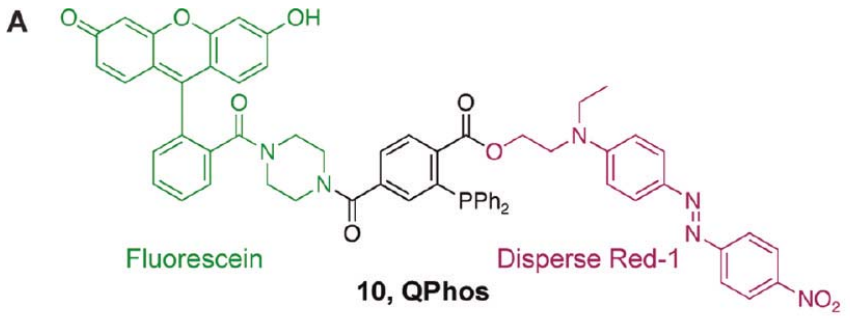


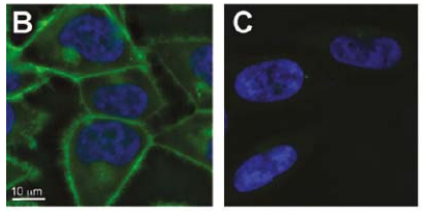


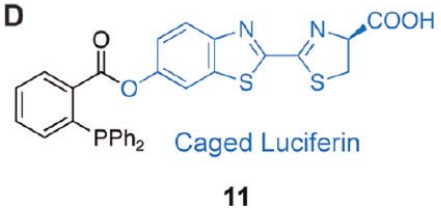
The Staudinger ligation

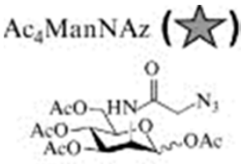
Drawbacks

- Phosphines are oxidized by air and citochrome P450
- The reaction is slow (0,002 M^{-1} s^{-1}): high concentrations of phosphine are needed.
- In case of fluorescent labeling, background emission from unconjugated dye is a problem
- Increasing the reactivity of phosphine by inserting electron-donating groups results also in increased oxidation



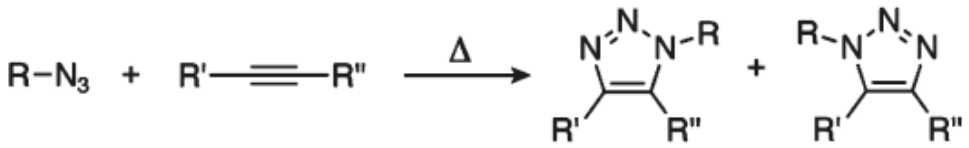




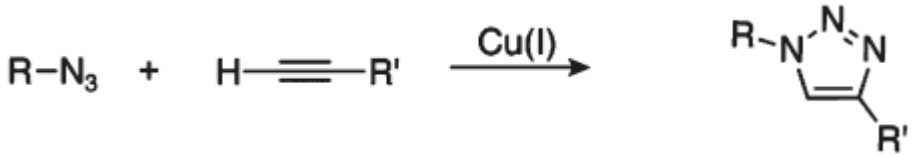


The Huisgen reaction

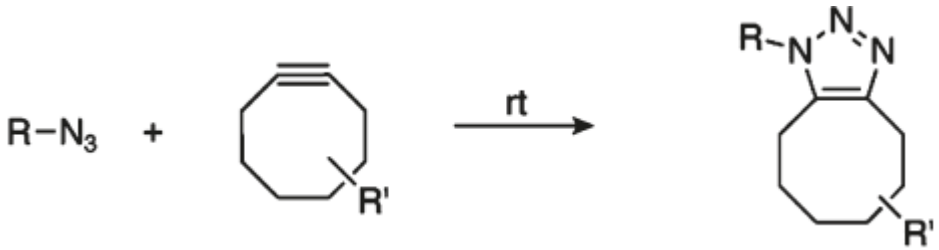
History



- Reported by Michael in 1890s
- Studied in detail by Huisgen in the 50s.
- Water compatible, quatititative
- Slow (high T and P)



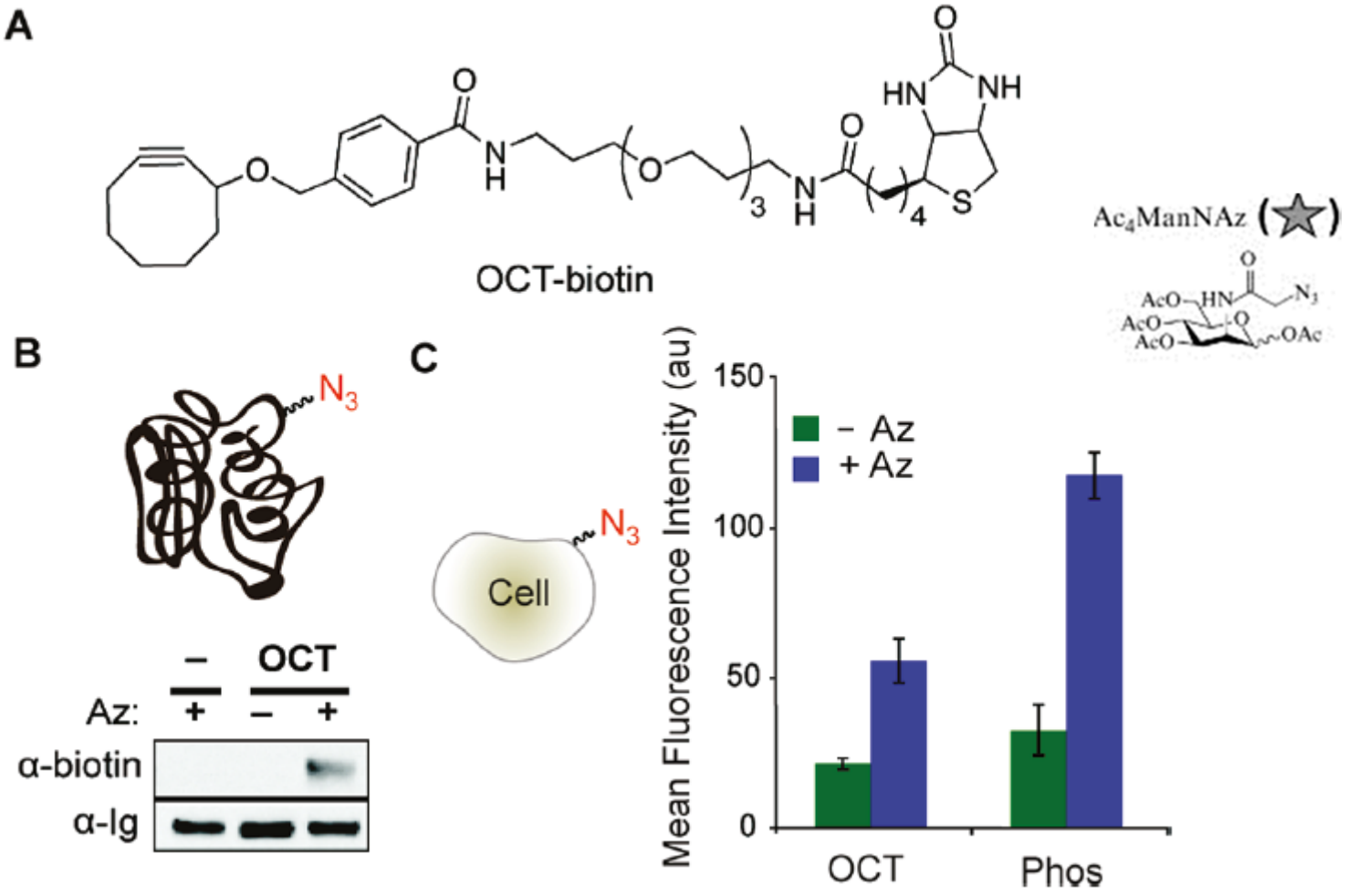
- Sharpless and Medals in early 2000s discover the Cu(I) catalysis
- High rate and selectivity
- Use of a transition metal difficult *in vivo*



- Reported by Wittig and Krebs to occur “like an explosion” in 1961
- Release of ring strain

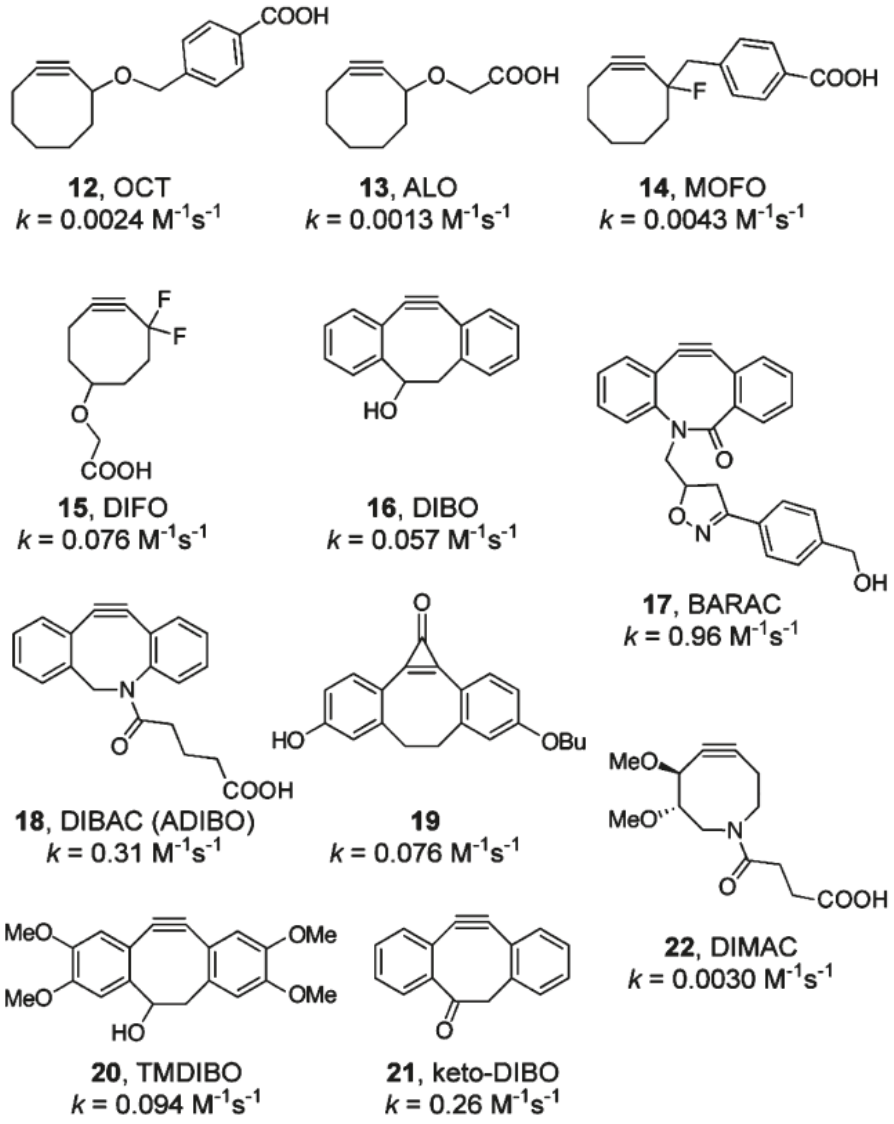
The Huigen/Bertozzi ligation

Metabolic labeling of glycans



The Huigen/Bertozzi ligation

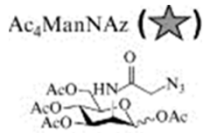
Optimization

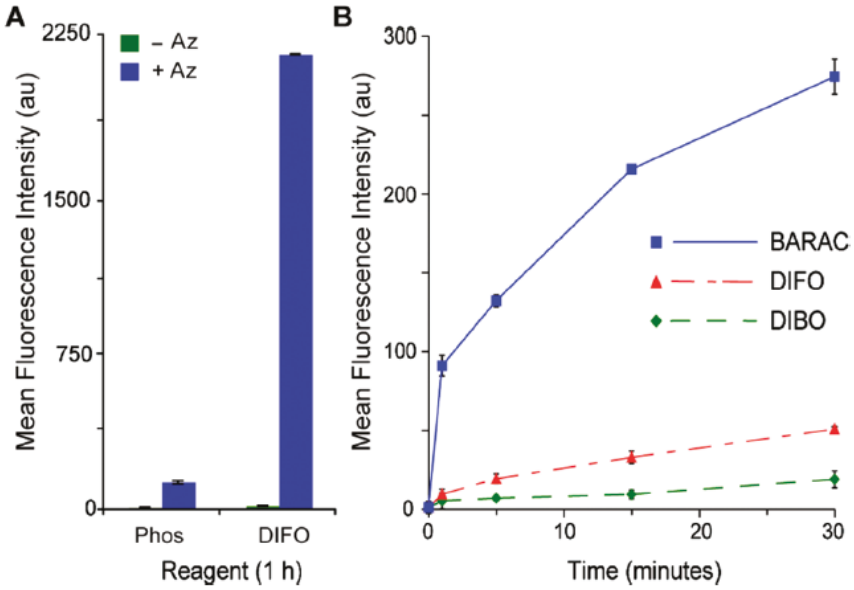


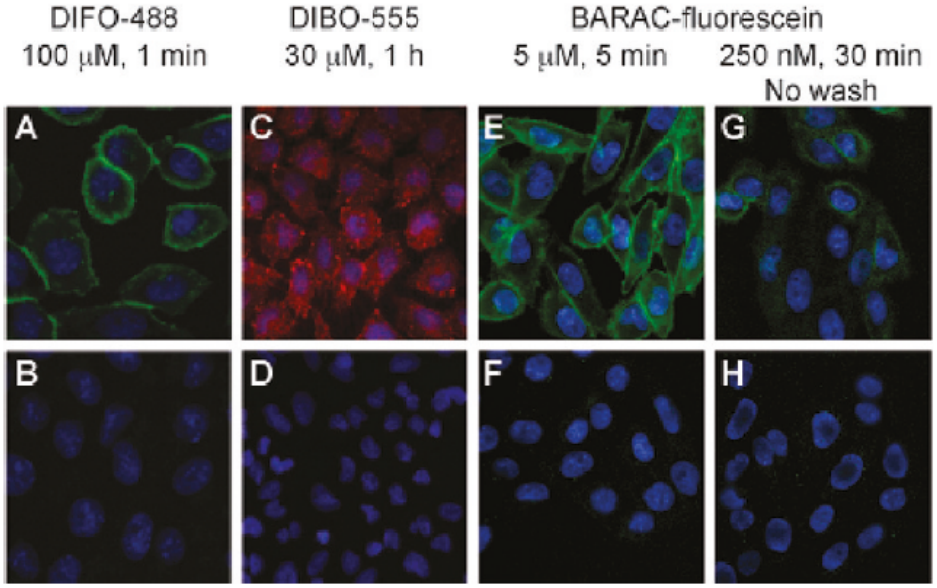
- Low solubility of **2**
- Rate not different form the Staudinger ligation
- Removal of phenyl ring improves solubility (**13**)
- Insertion of F in position 3 increases the rate (**14-15**), 2 much better
- Increase cyclooctane stain increase the rate (**16-18**).
- Many other derivatives prepared (**19-21**)

The Huigen/Bertozzi ligation

Optimization







The Huigen/Bertozzi ligation

Optimization