Bioconjugate Chemistry on Proteins

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Carell Group, ChemBio Lecture
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Bioconjugate Chemistry

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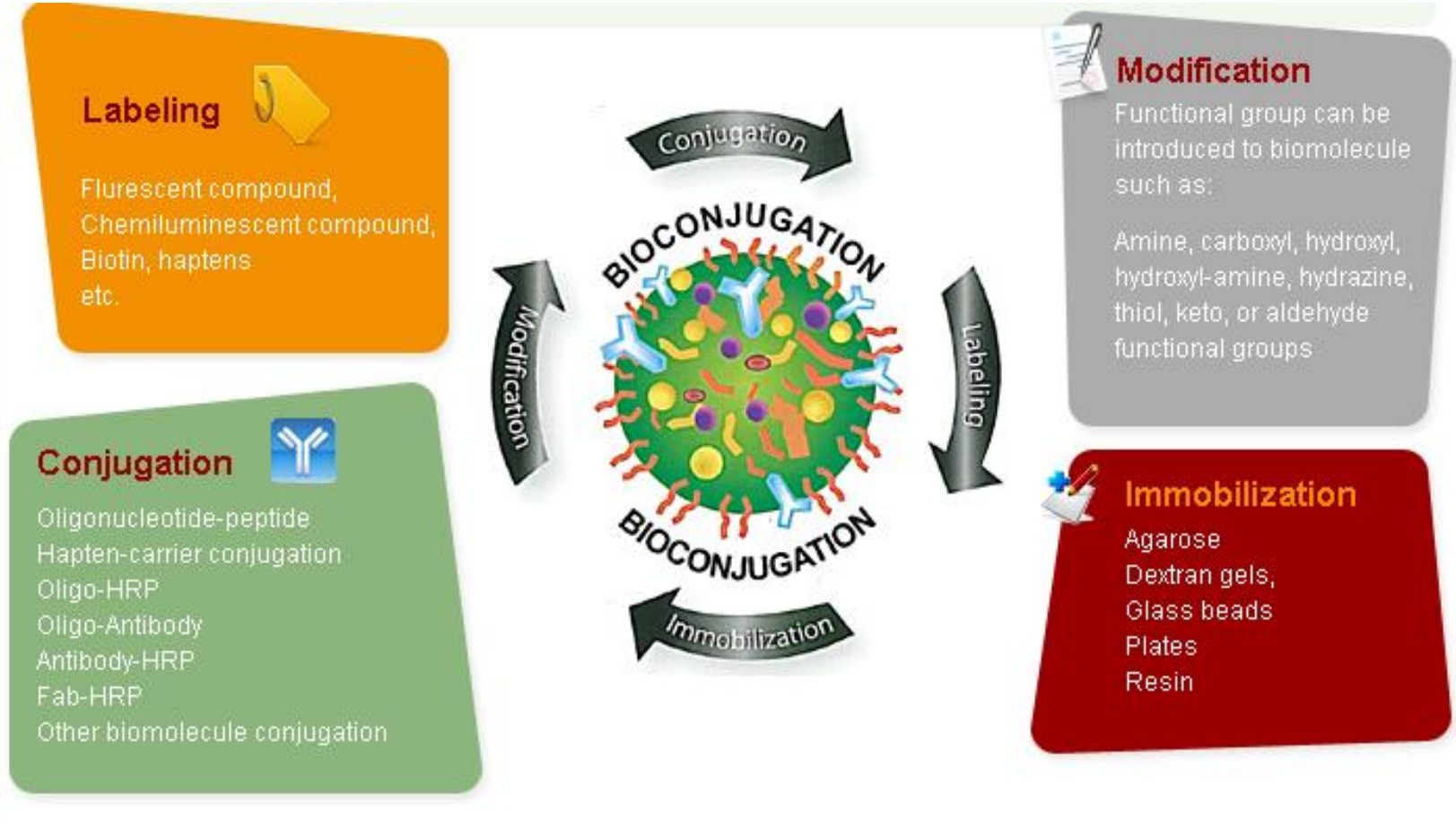
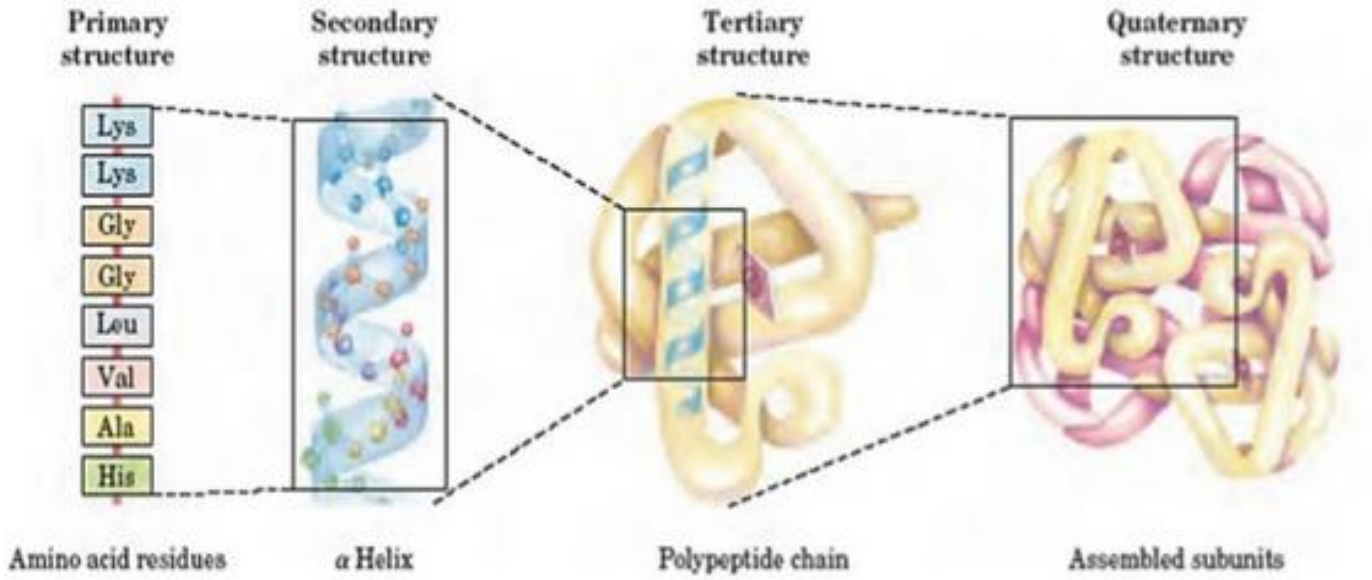
joining of small molecules to other biomolecules and polymers by chemical or biological means

Bioconjugate Chemistry



Challenge: Chemistry on Proteins

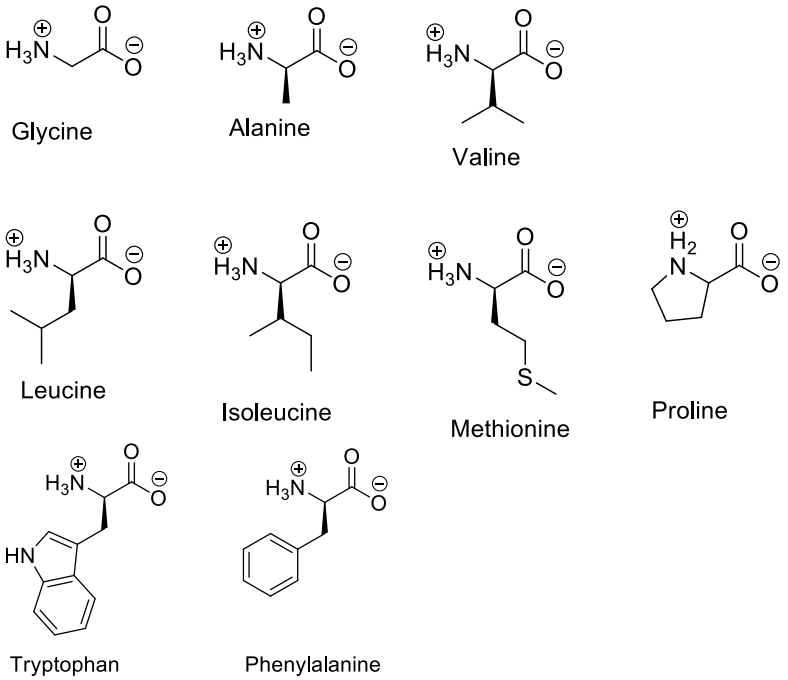


Challenges:

- proteins are very **complex** molecules with different functional groups
- chemistry has to be site specific and can not be denaturing in order to keep biomolecules functional
- Reactions have to proceed at low temperatures and in water at pH = 6 - 9

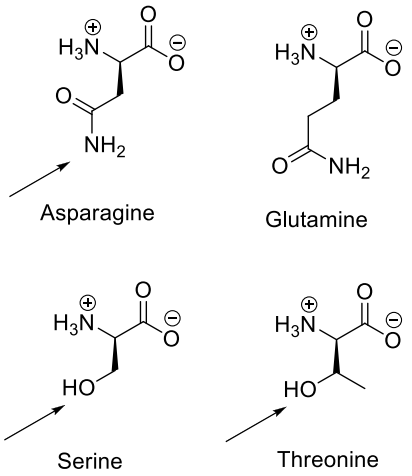
Amino Acids

aliphatic & aromatic AA



- Hardly accessible because their hydrophobic core locates them inside the protein
- No reactive groups that can be derivatized

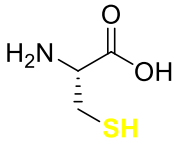
polar AA



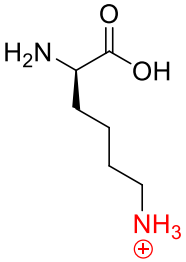
- Hydrophilic and usually near the surface which makes them accessible
- Often post-translationally modified, comparable nucleophilicity as water

Amino Acids

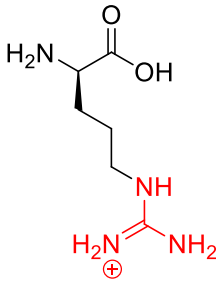
ionizeable AA



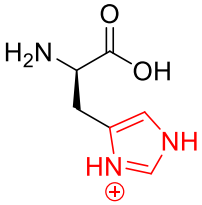
Cystein, pKa = 8.8-9.1



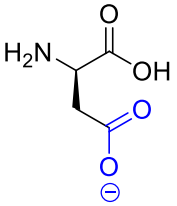
Lysine, pKa = 9.3-9.5



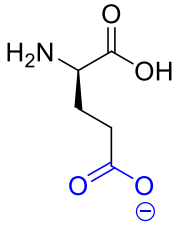
Arginine, pKa > 12



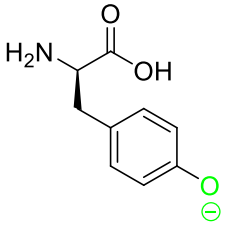
Histidine, pKa = 6.7-7.1



Aspartic Acid,
pKa = 3.7-4.0



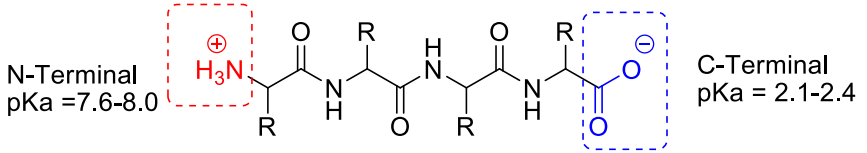
Glutamic Acid,
pKa = 4.2-4.5



Tyrosine,
pKa = 9.7-10.1

chemistry possible through reactive groups:

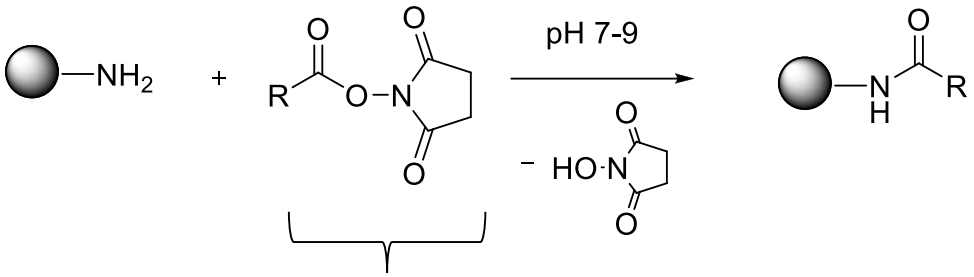
- free cystein rarely occurs on protein surface making it a great target for site specific tagging
- nucleophilic lysine residue commonly used for reaction with or cyanates/isocyanates (pH = 8-9



Polypetide

Chemistry on Lysines

Chemistry on Lysines



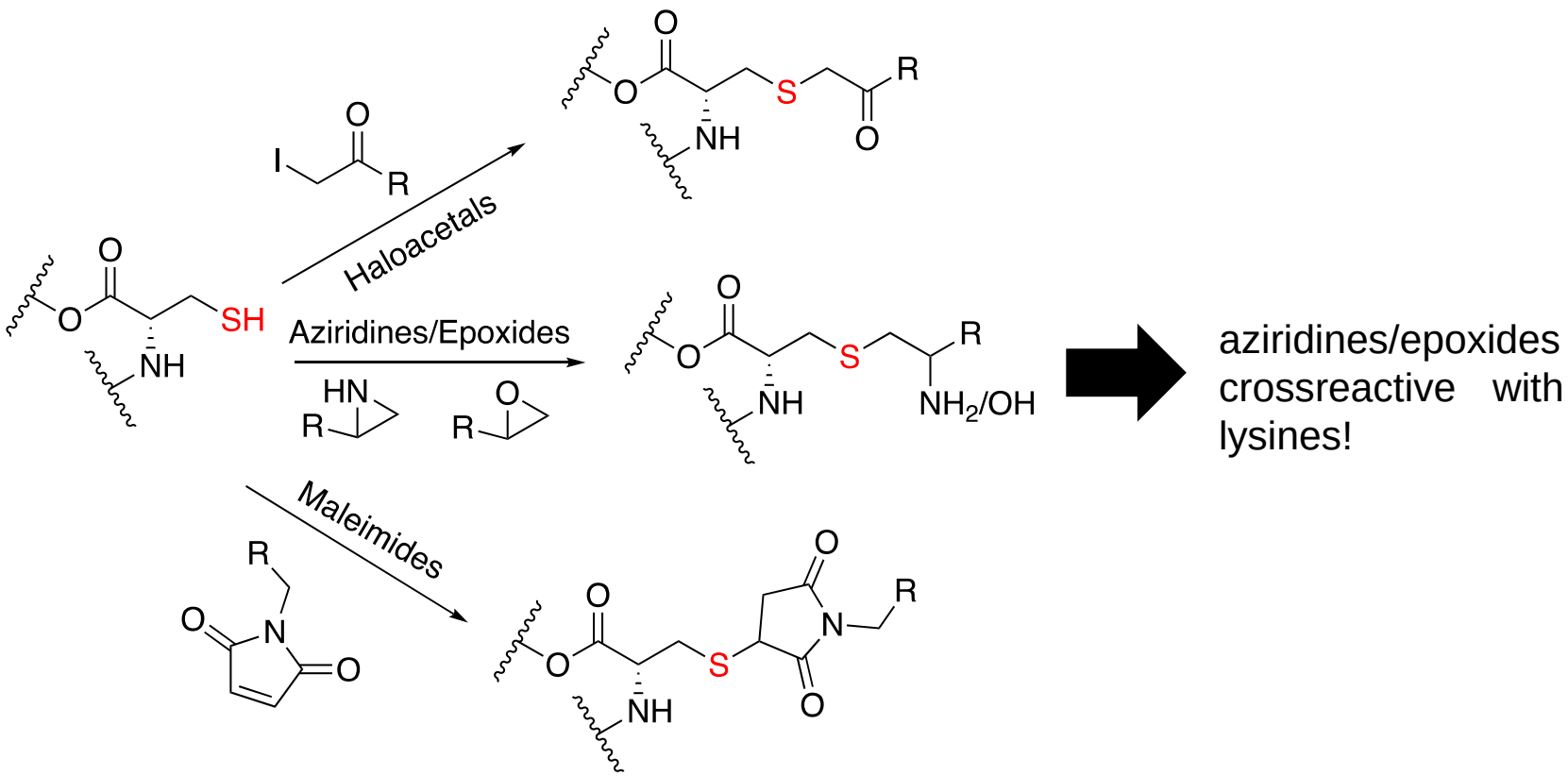
NHS-Ester

***N*-Hydroxysuccinimide**

- NHS-esters are easily accessible through reacting carboxylic acids with NHS-TFA
- activated carboxylic acids then readily react with amine groups of lysines under various conditions (pH = 7-9, T = 4 ° C to rt, reactions usually fast)
- various buffer systems can be used (phosphate, bicarbonate, HEPES, borate) except for buffers that contain amines (such as TRIS)

Chemistry on cysteins

Chemistry on Cysteins

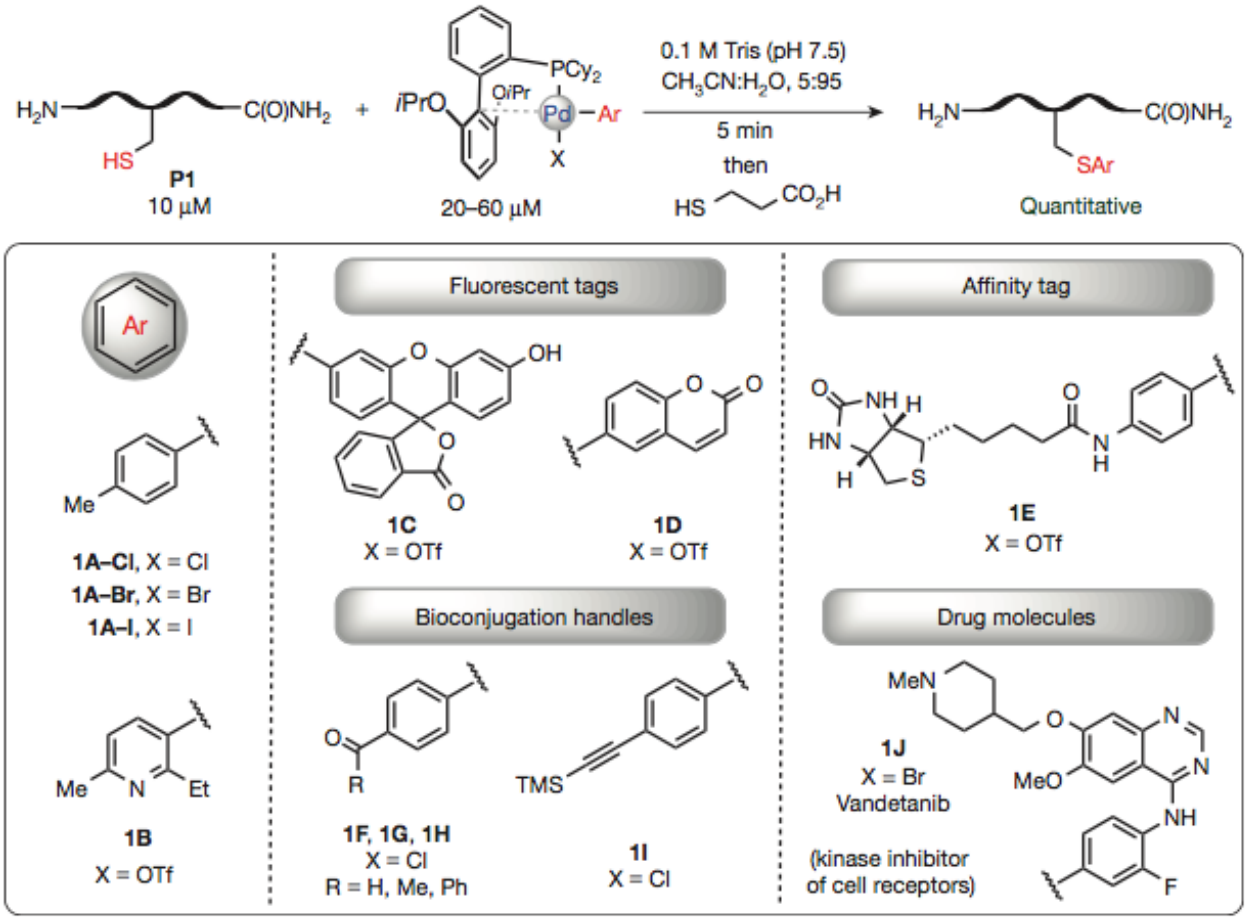


aziridines/epoxides crossreactive with lysines!

--> many tools to derivatize cystein residues!

Chemistry on Cysteins

Buchwald type coupling on peptides



- Fast, reliable, versatile, broad pH range, big substance scope

Chemistry on Cysteins

π-Clamp mediated cystein conjugations

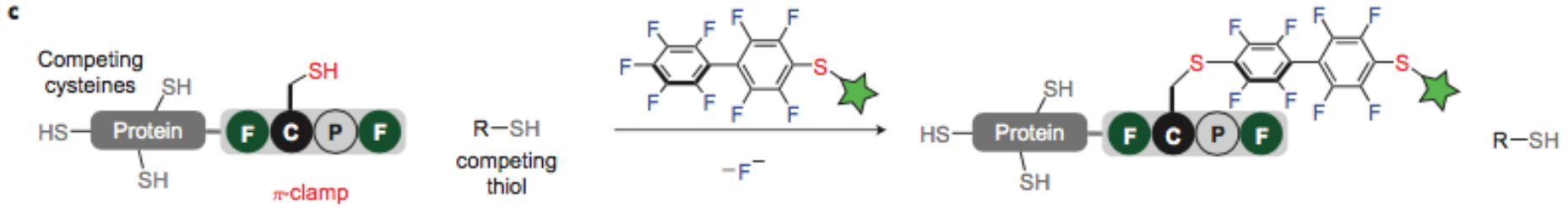
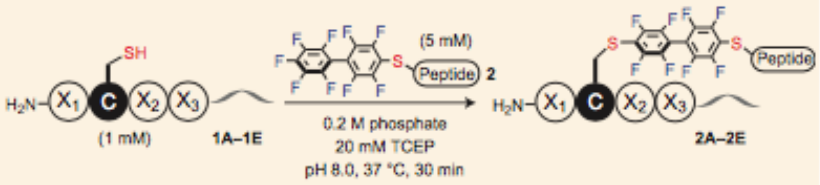
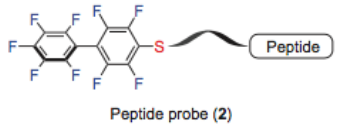


Table 1 | Mutation studies show that Phe-1, Pro-3 and Phe-4 are required for the observed reactivity.

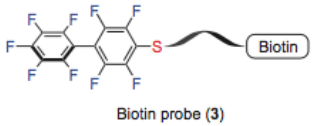


Entry	Peptide	X_1	X_2	X_3	k_2 (M^{-1} s^{-1})	Yield (%)
1	1A	Gly	Pro	Gly	N/A	<1
2	1B	Phe	Pro	Gly	N/A	<1
3	1C	Gly	Pro	Phe	0.09	50
4	1D	Phe	D-Pro	Phe	0.05	30
5	1E	Phe	Pro	Phe	0.73	>99

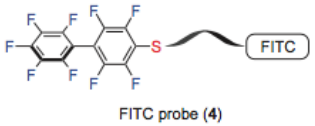
TCEP, tris(2-carboxylethyl)phosphine. Yields shown are from LC-MS analysis of the crude reactions at 30 min.



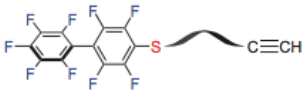
Peptide probe (**2**)



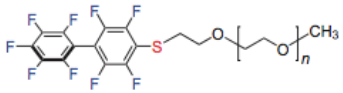
Biotin probe (**3**)



FITC probe (**4**)



Alkyne probe (**5**)



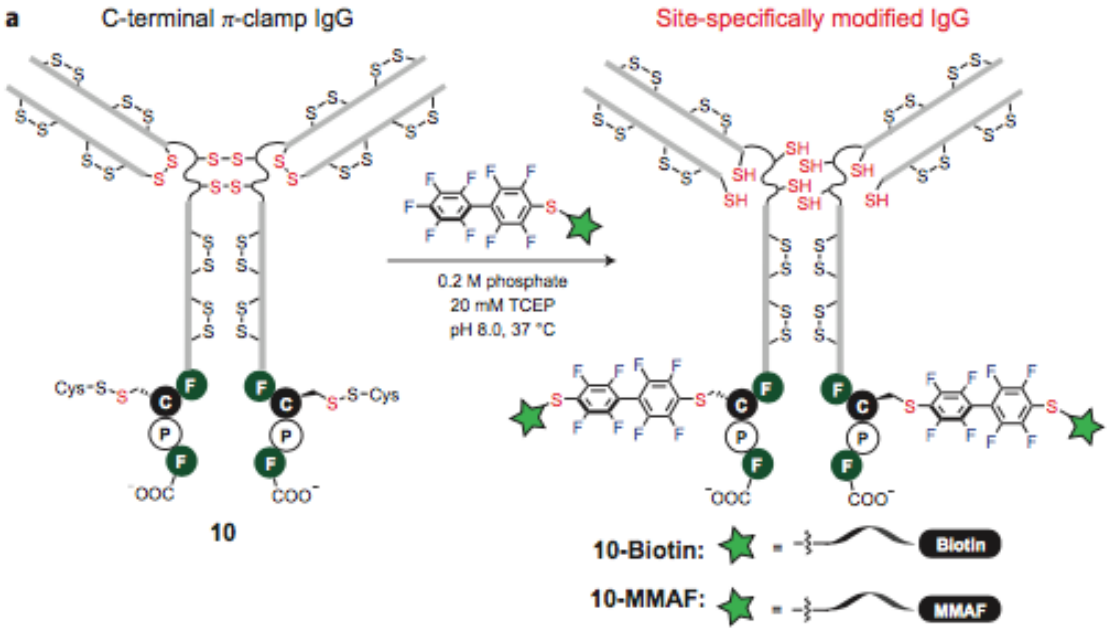
PEG probe (**6**)

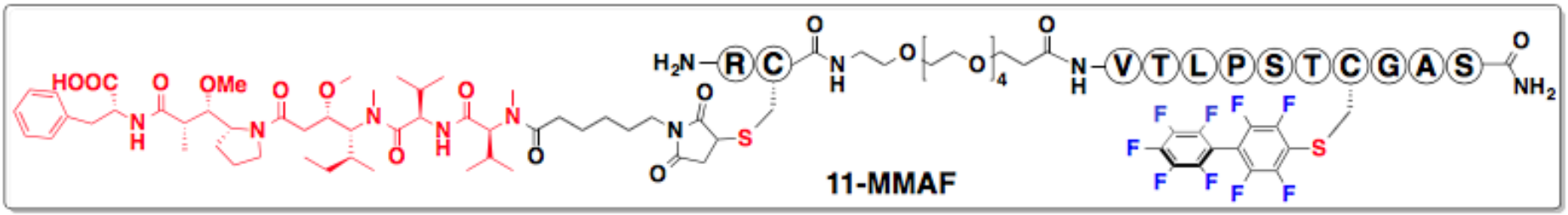
- Perfluoroaryls can be prepared easily → modular approach, many different probe molecules possible

- Very site specific reaction, only reacts with cystein in Phe-Pro-Phe sequence environment, other thiols are completely outcompeted

Chemistry on Cysteins

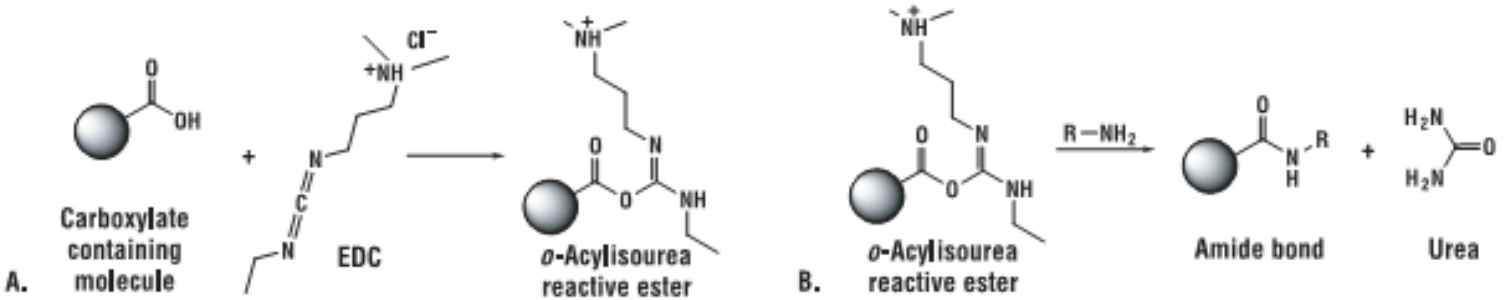
π-Clamp-mediated cysteine conjugation





Conjugation with carboxylic acids

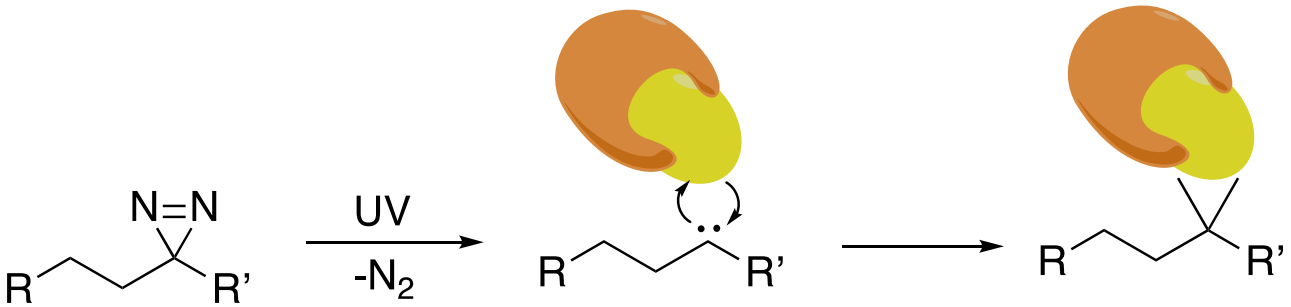
Preparation of Carboxylic Acids for Conjugation



- like in peptide synthesis, unreactive carboxylic acids must be activated prior to coupling
- EDC coupling is a fast, reliable and convenient method, that proceeds at room temperature and at pH = 6-7
- all components (peptide, carboxylic acid and EDC + buffers) are mixed together, the activation of the carboxylic acid proceeds *in situ* and is readily trapped with e.g. a lysine

Unspecific conjugation techniques

Photoreactive Reagents

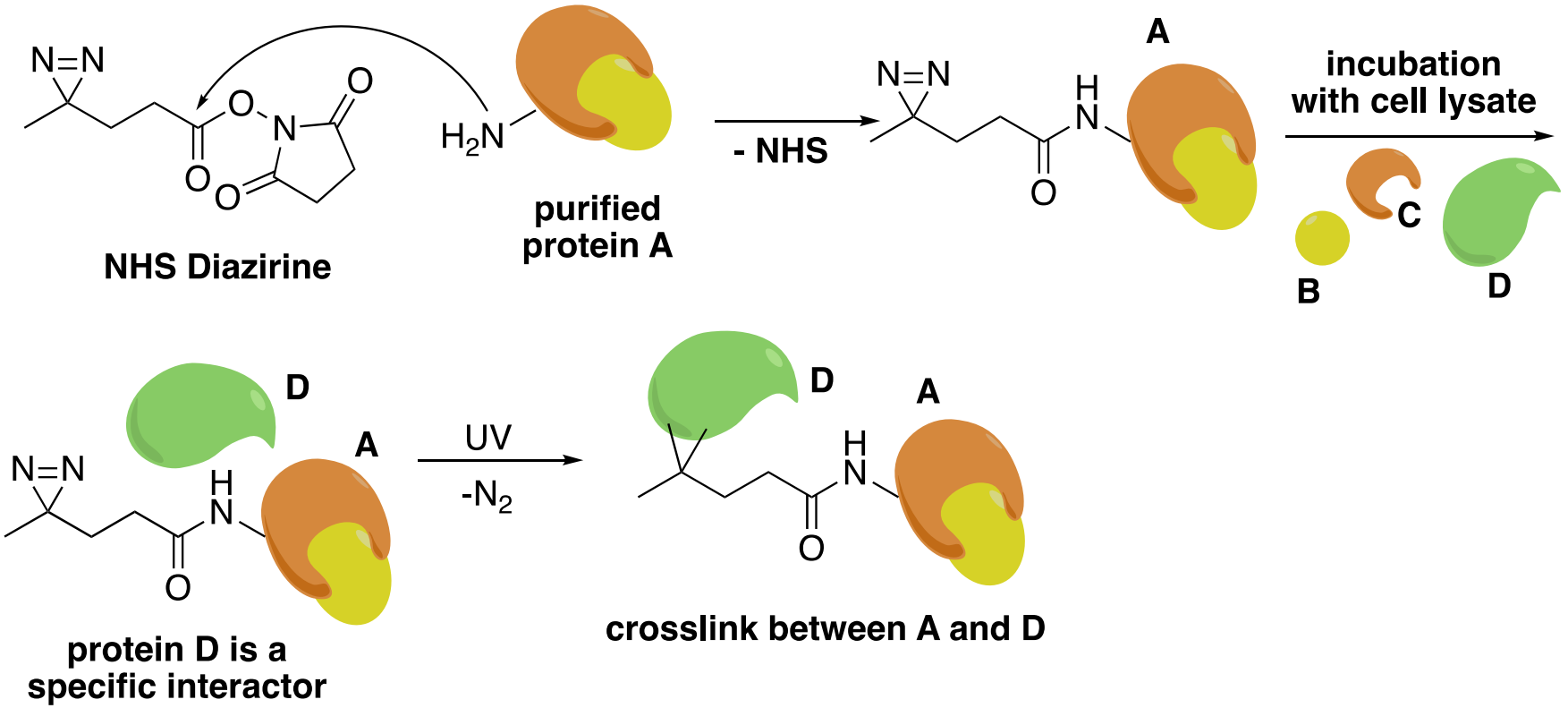


diazirines

UUU: UV-light, **U**nspecific, **U** can decide when!

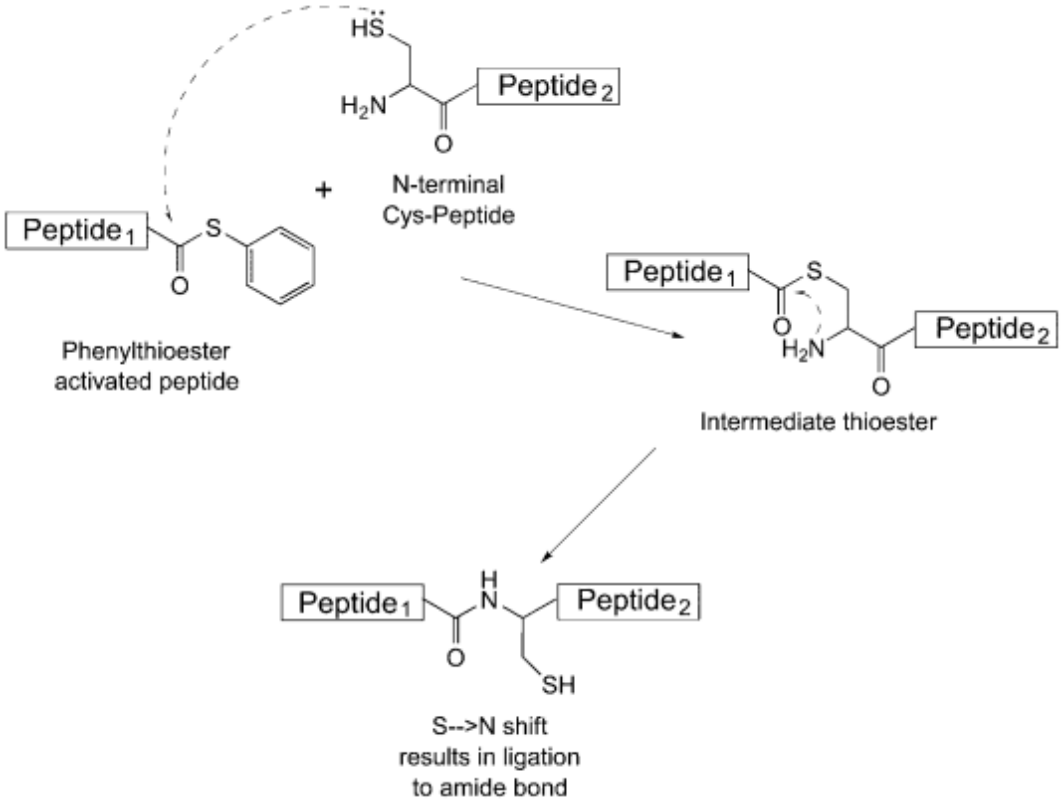
→ **mostly used for crosslinking of proteins**

Photoreactive Reagents



Native Chemical Ligation

Native Chemical Ligation



- No protecting groups necessary
- Large constructs possible (>300 AA)
- Chemoselective

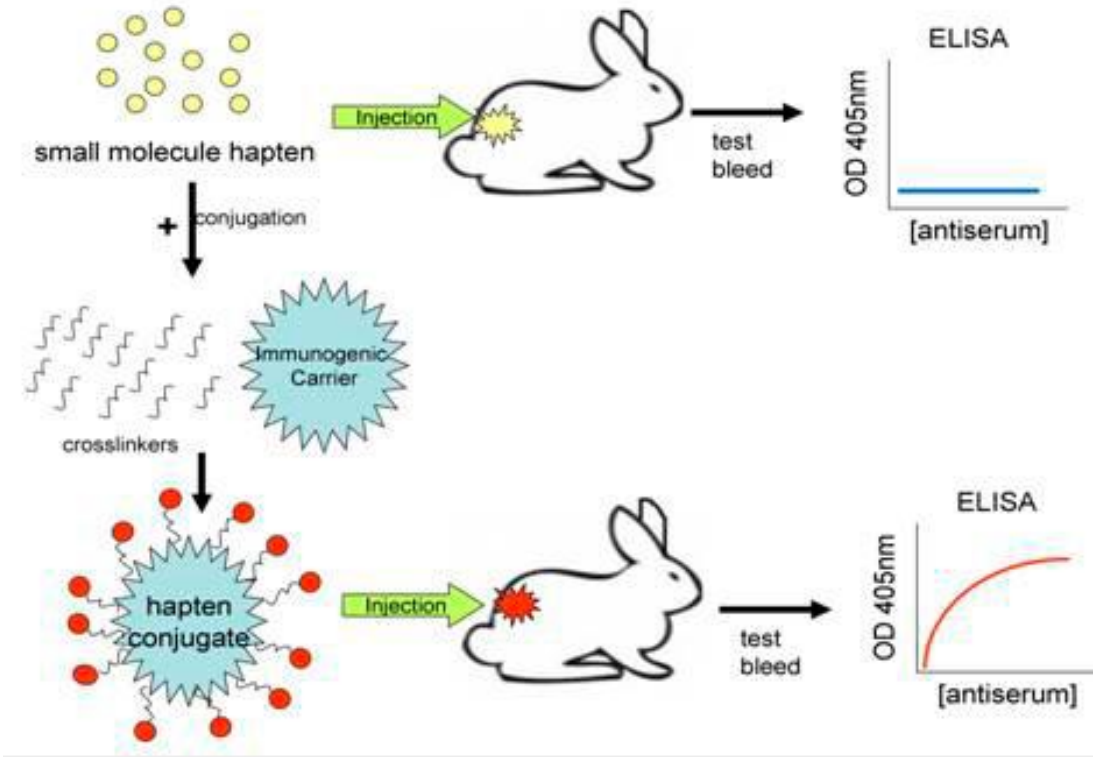
Applications of Conjugation Techniques

Applications

- Antigen preparations for small molecules such as drugs, nucleosides, peptides, sugars
- Antibody-drug conjugates (targeted cancer treatment)
- PEGylation of proteins
- Crosslinking for structural and interactional proteomics

Antibody Production

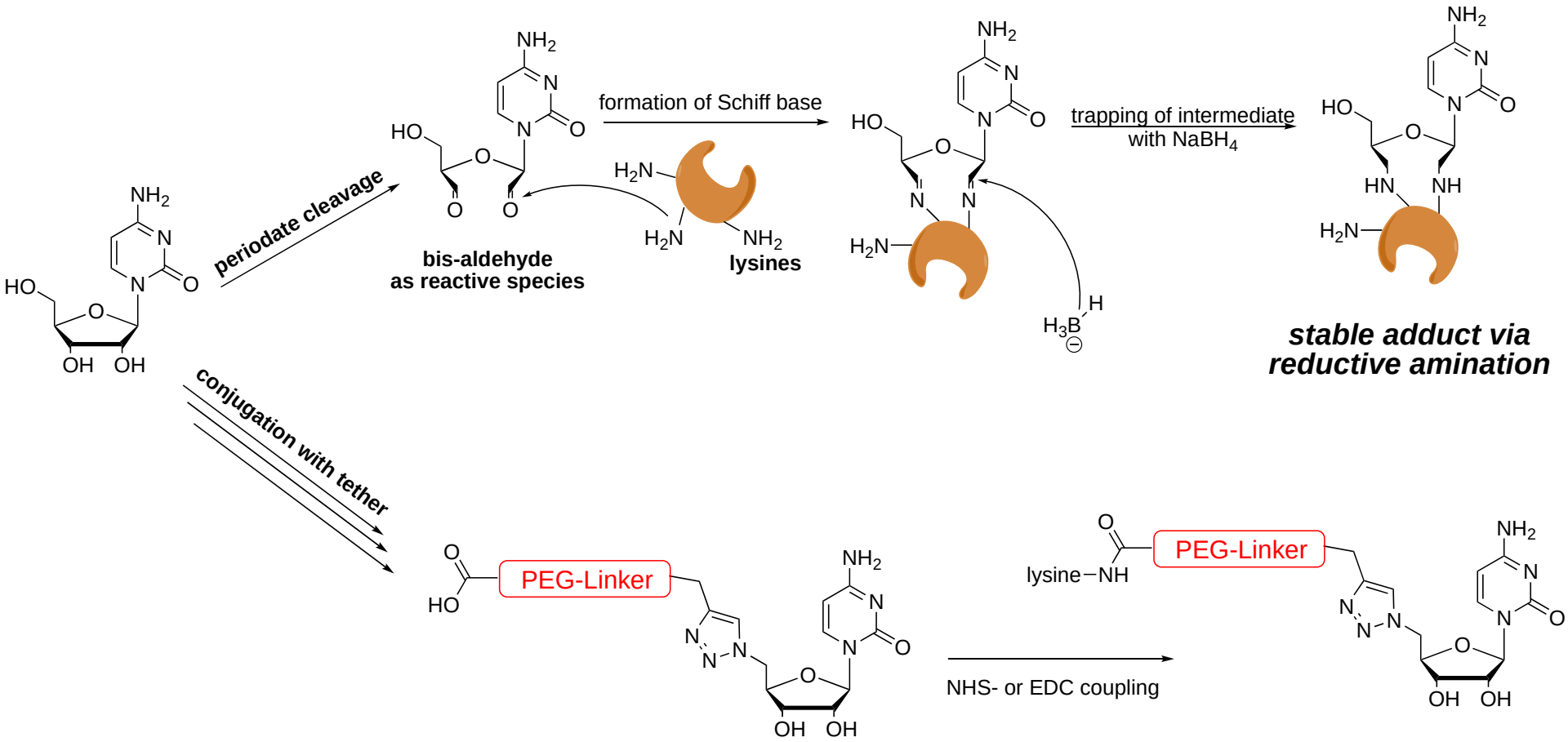
Applications: Antibody Production



- Rabbits can generate antibodies against haptens that are conjugated to carrier proteins (such as OVA, BSA, KLH)

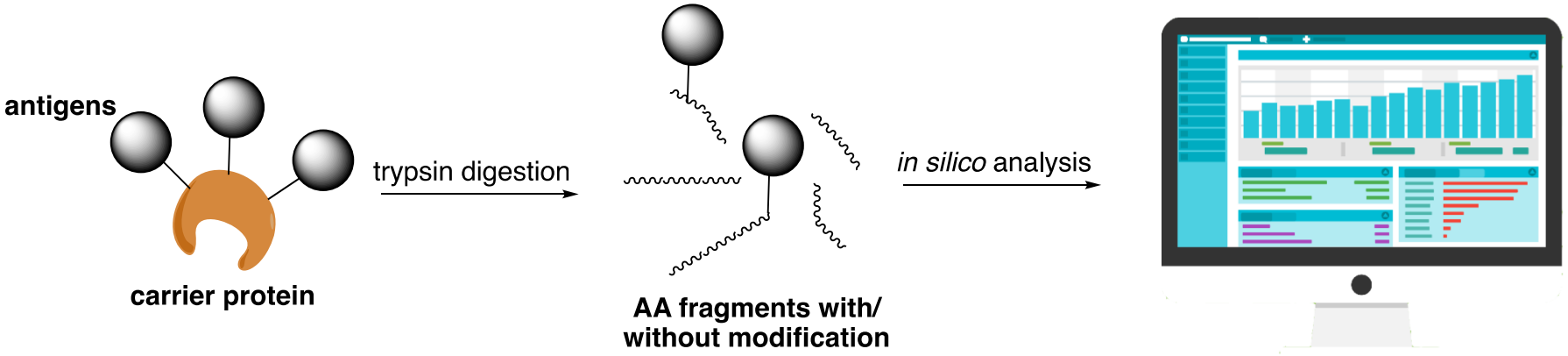
Applications: Antibody Production

Examples to generate haptens/antigens

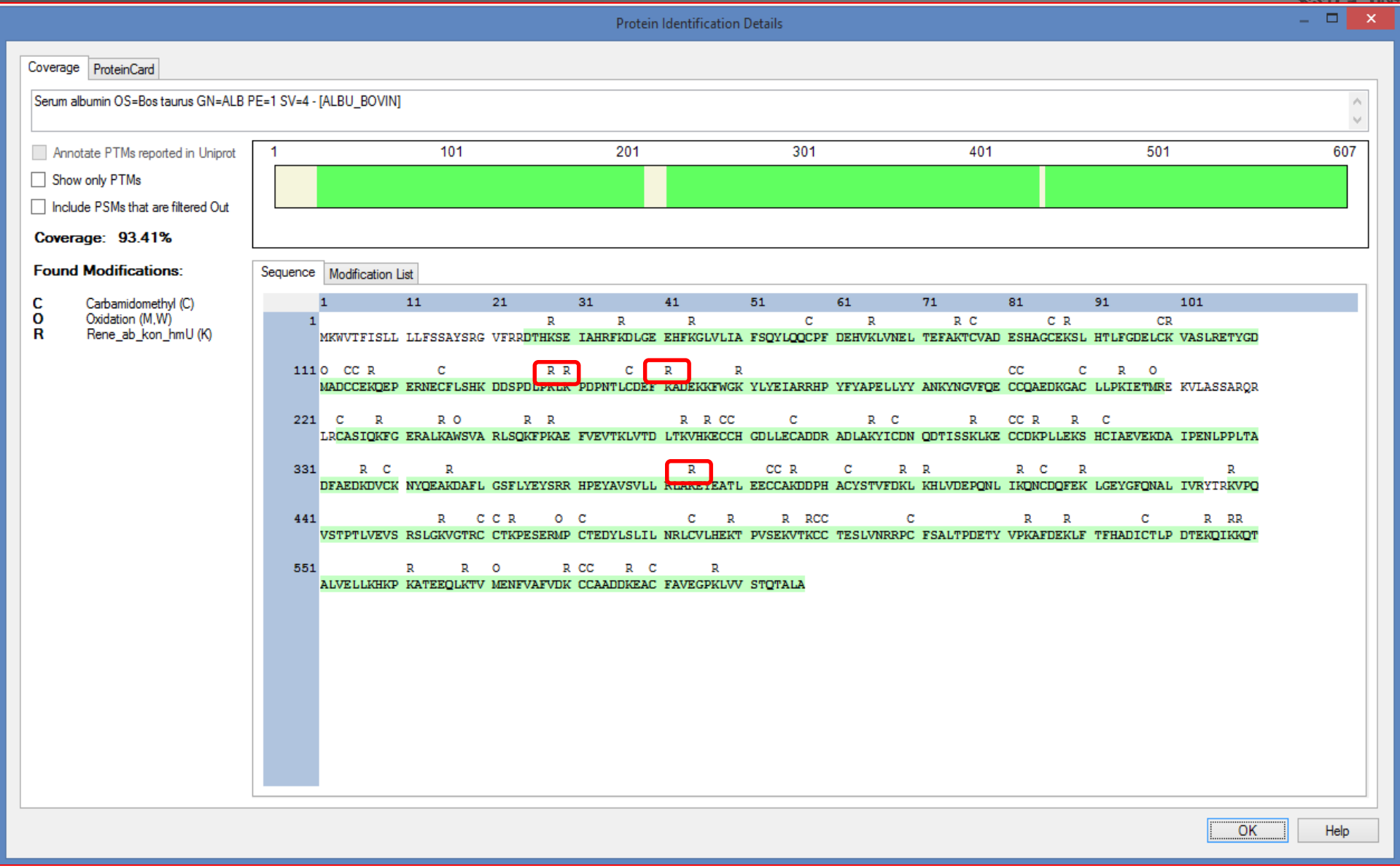


Applications: Antibody Production

HR-MS analysis allows to identifiy derivatized sites

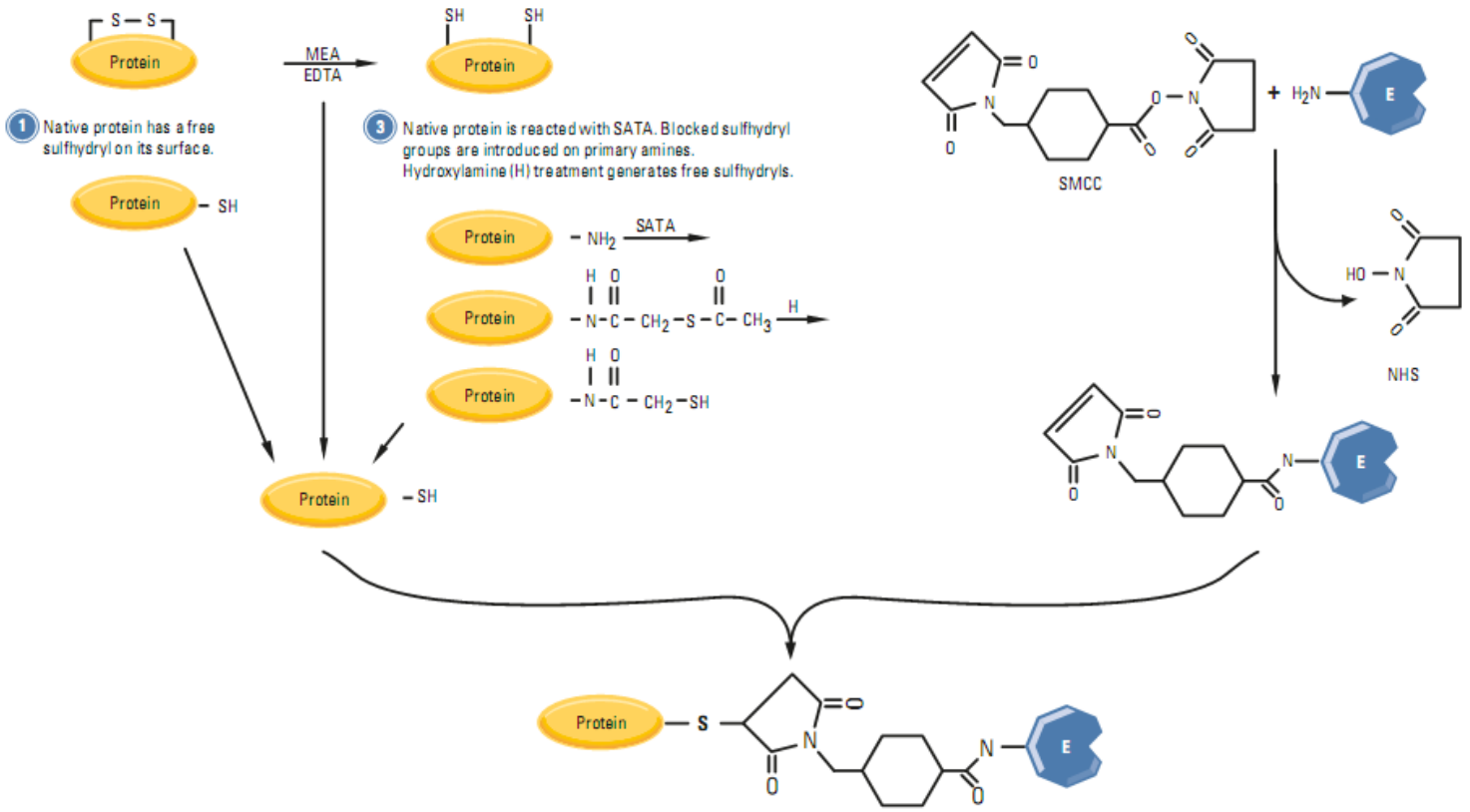


Applications: Antibody Production



Applications: Antibody Production

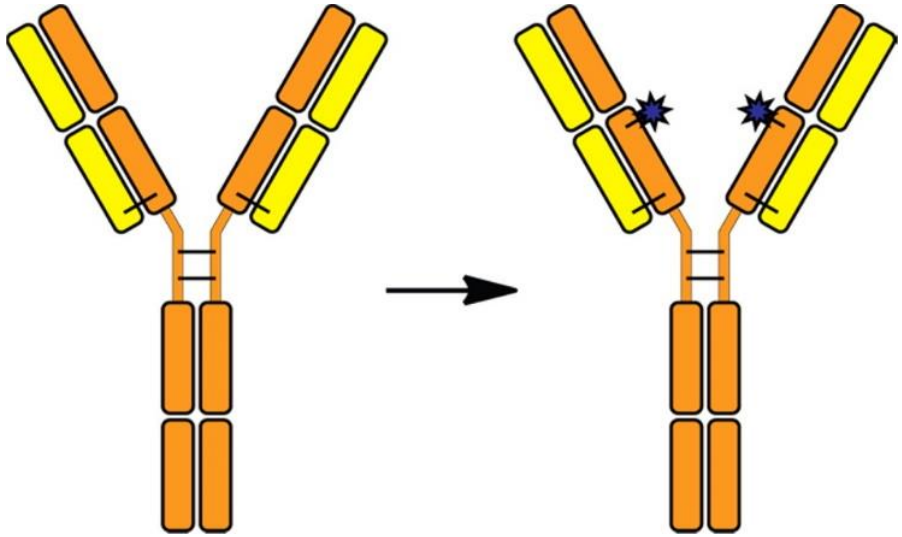
Maleimide method



- High yields and easy to prepare (maleimide carriers are commercially available)

Antibody-Drug Conjugates

Applications: Antibody-Drug Conjugates



combining the specificity of antibodies with the potency of small molecules to create targeted drugs!

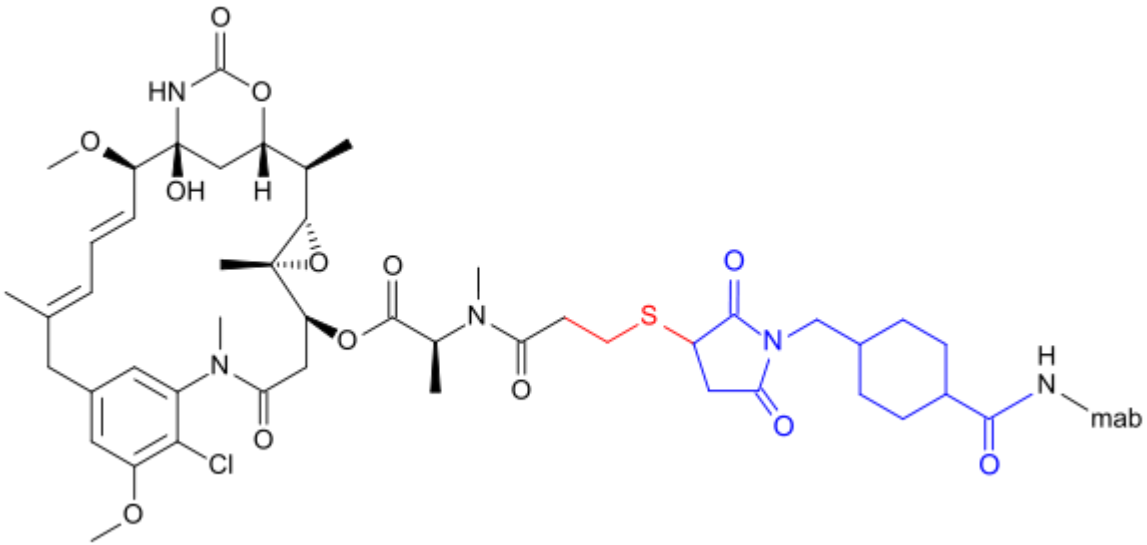
Applications: Antibody-Drug Conjugates

Trastuzumab emtansine (T-DM1)

- Trastuzumab is a monoclonal antibody which interferes with HER2/neu receptor
- HER protein binds to human EGFs and promotes cell proliferation
- Especially in breast cancers, HER2 is over expressed which causes cancer through the loss of cell proliferation control
- Trastuzumab can interfere at this stage by inducing p27

➢ **Trastuzumab can be used for targeted cancer treatment in breast cancer!**

Applications: Antibody-Drug Conjugates



Conjugated DM1 (maytanside)

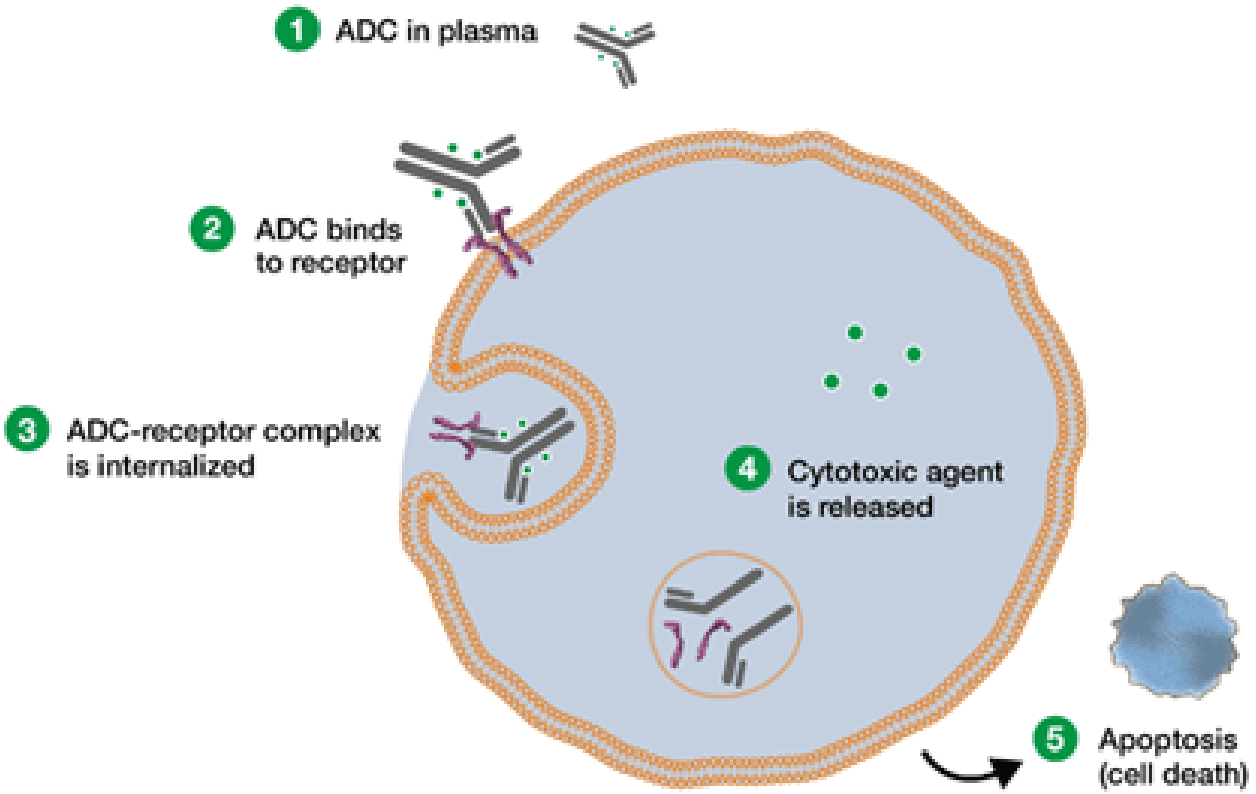
Conjugate tether for drug

Trastuzumab (antibody)

- Several molecules of the DM1, which is a maytansine derivative are conjugated per antibody via a maleimide conjugation
- DM1 binds to plus end of microtubules and inhibits cell division in the targeted tumor cells

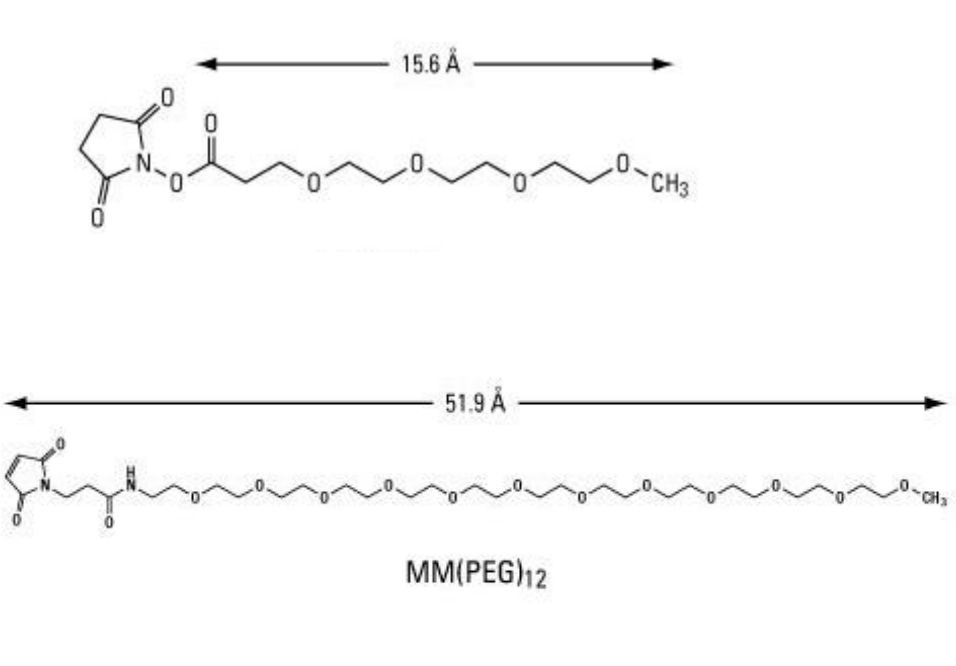
Applications: Antibody-Drug Conjugates

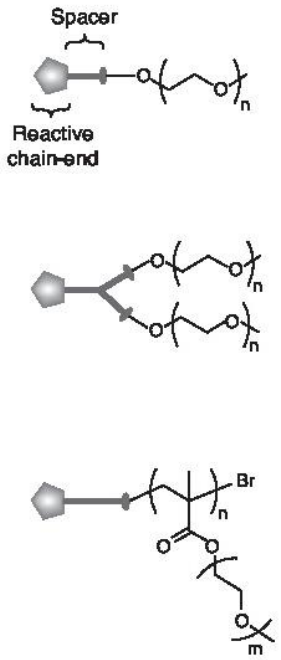
Mode of action

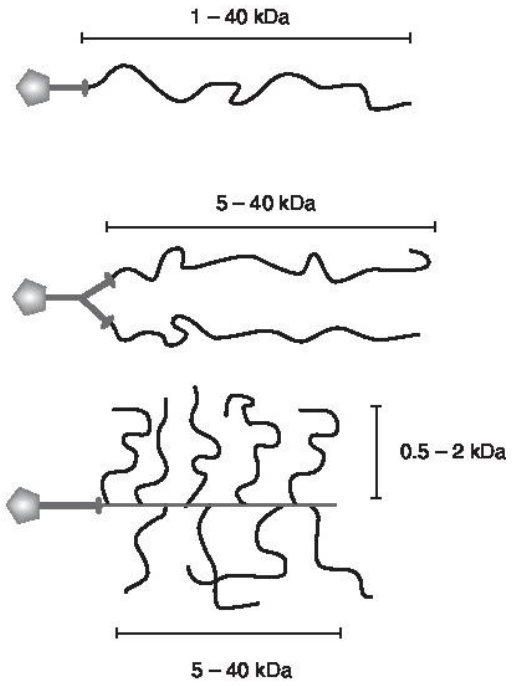


PEG-ylation of Proteins

Applications: PEG-ylation of Proteins

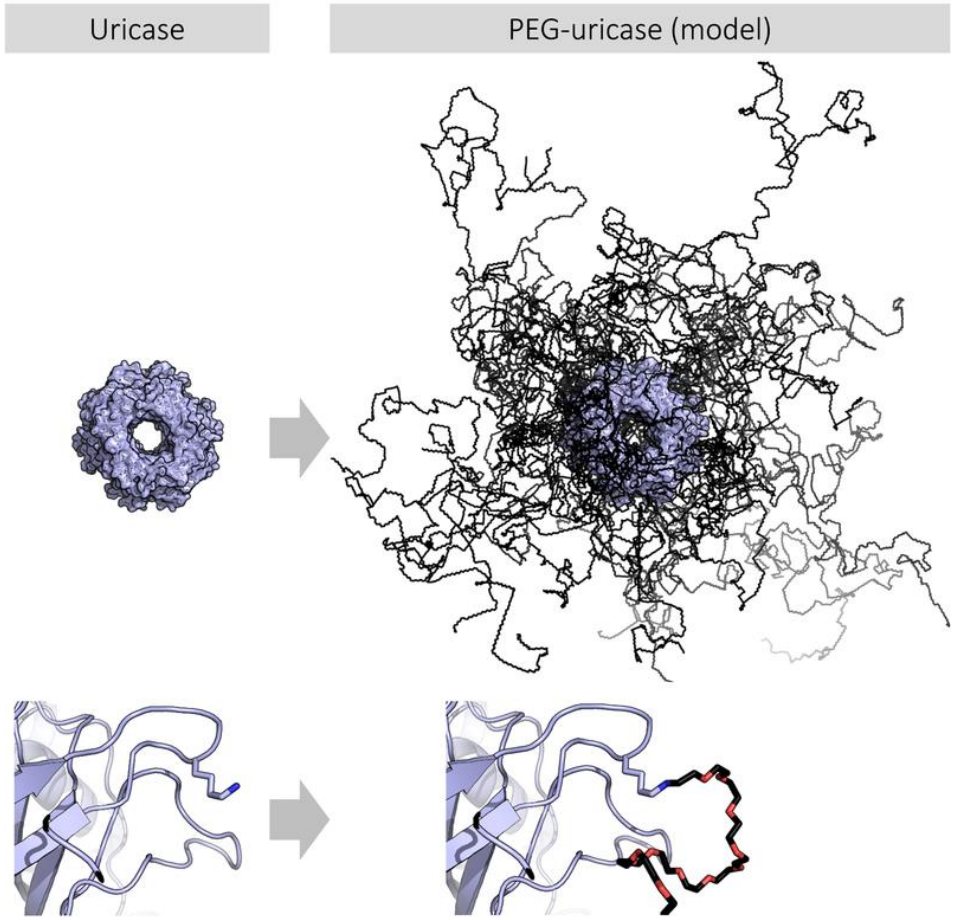






- Prolonged half-life
- Higher stability
- Water solubility
- Lower immunogenicity / antigenicity

Applications: PEG-ylation of Proteins



Pegloticase

- Uricase is used as treatment against gout (*Gicht*)
- Uricase metabolizes uric acid to allantonin (more soluble than uric acid)
- PEG-ylation increases the half life from 8 hours to 10 – 12 days!
- Immunogenecity is greatly decreased

→ Suitable for long term treatment

- Approx. 9 out of 30 lysinses are conjugated to PEG-chains (225 ethyleneglycol units each)

Bioorthogonal Chemistry

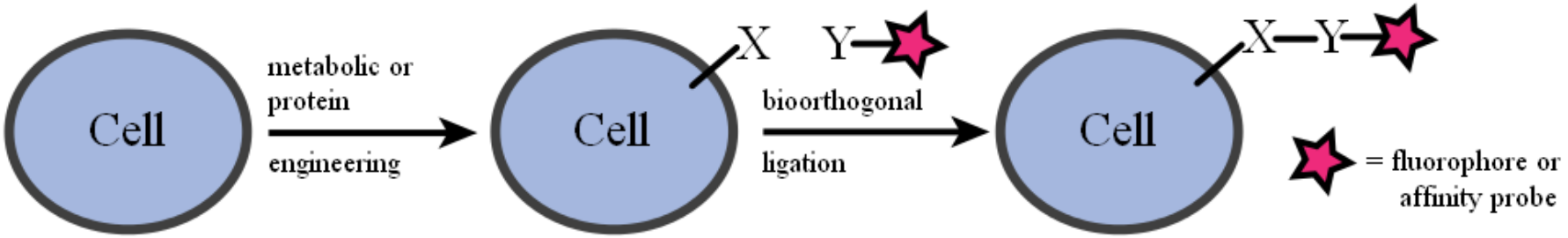
Bioorthogonal Chemistry

Bioorthogonal chemistry = chemical reactions that neither interact nor interfere with a biological system

Bioorthogonal Chemistry

→ **Real time studies in living systems without cellular toxicity**

- **Cellular system is modified with a bioorthogonal functional group (chemical reporter) and introduced into the cell**
- **Probe containing the complimentary functional group is introduced to react and label the substrate**



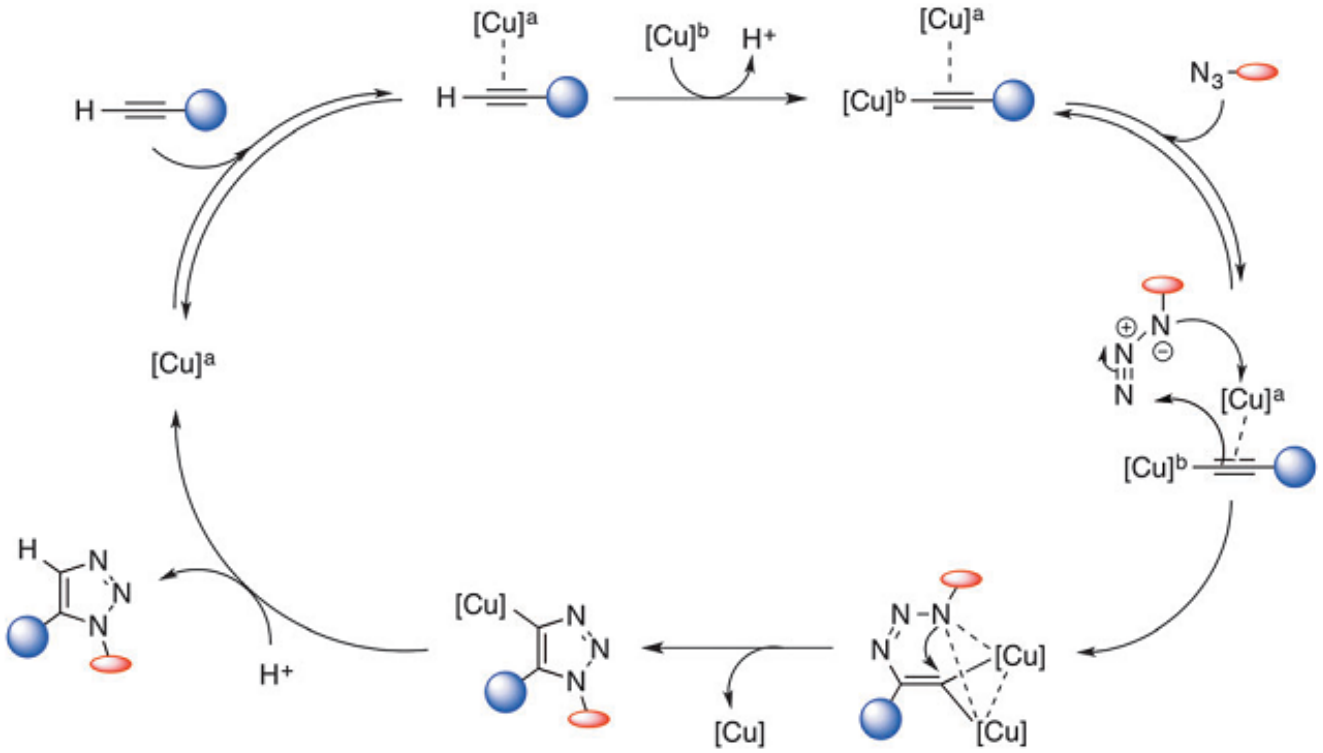
X = bioorthogonal group not present in the biological system
Y= complementary group, reacts in a bio-compatible way with X

Bioorthogonal Chemistry Strategies

- Cu-catalyzed Azide-Alkyne Cycloaddition „Click“
- Strain-promoted „Click“-Reaction
- Staudinger Ligation
- Tetrazine Ligation
- Photo-induced Tetrazole-Alken Cycloaddition
- Norbonene System
- Strain-promoted Alkyne-Nitrone Cycloaddition

Copper Catalyzed Click Chemistry

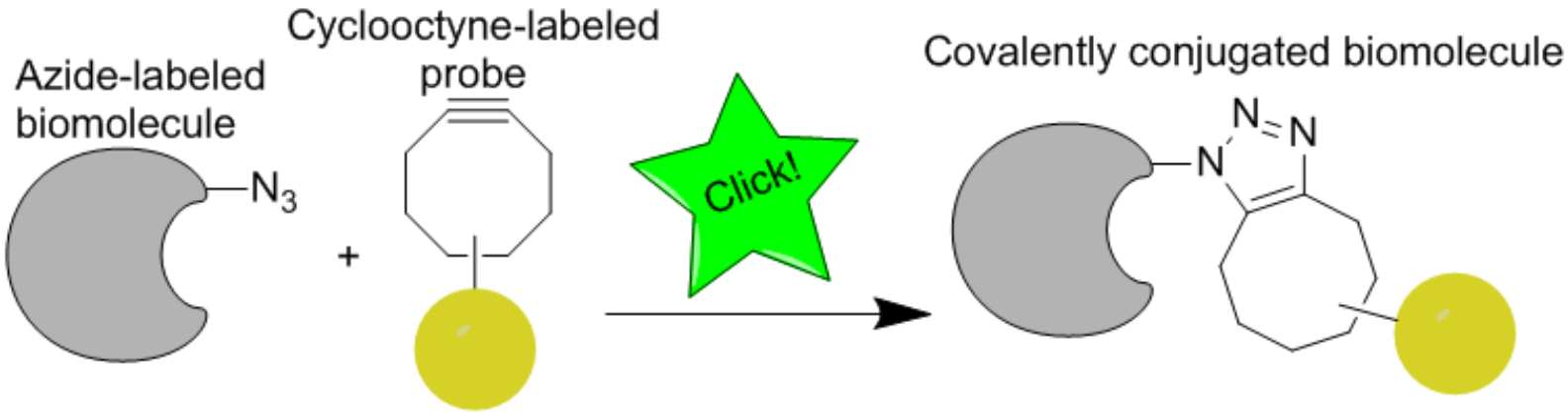
copper catalyzed azide alkyne cycloaddition



- Initially developed by Rolf Huisgen (LMU) and further developed by Barry Sharpless (Cu-catalysis)
- Extremely versatile reaction, broadley applicable, easy to prepare

Copper Free Click Chemistry

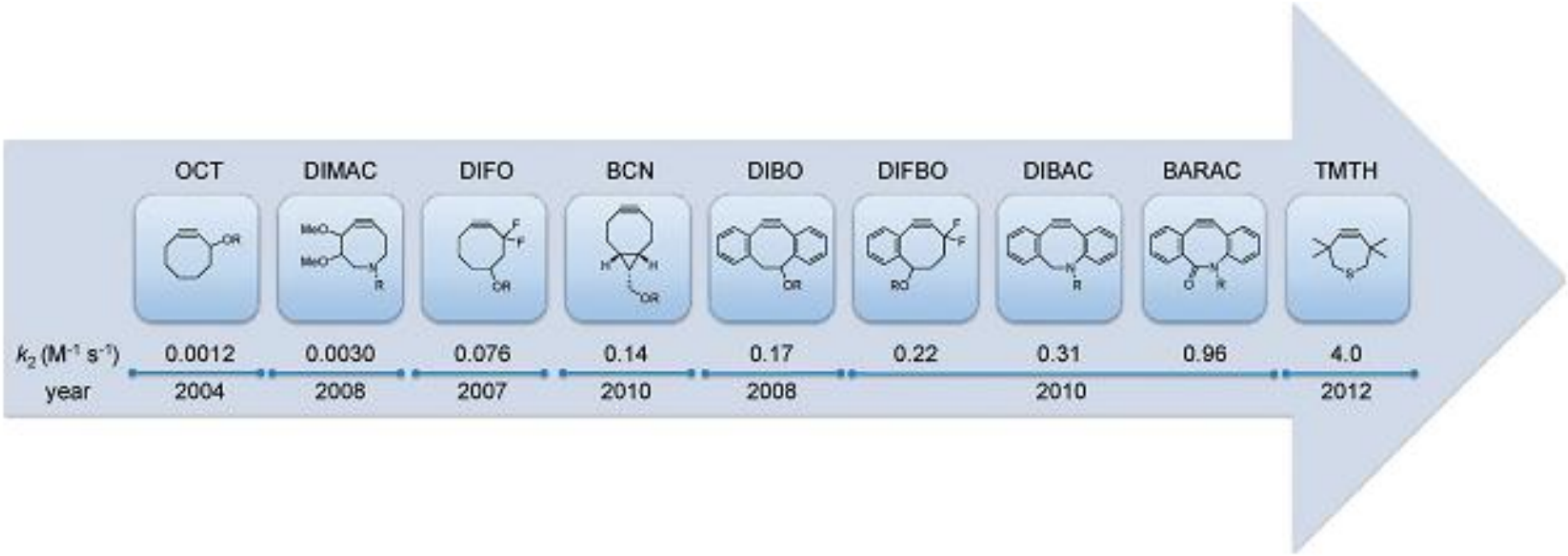
copper free click promoted through strained alkynes (Bertozzi Lab)



→ Click-Chemistry possible in living systems! (Cu click would be toxic)

→ Bioorthogonal Chemistry (actually Bertozzi introduced the term)

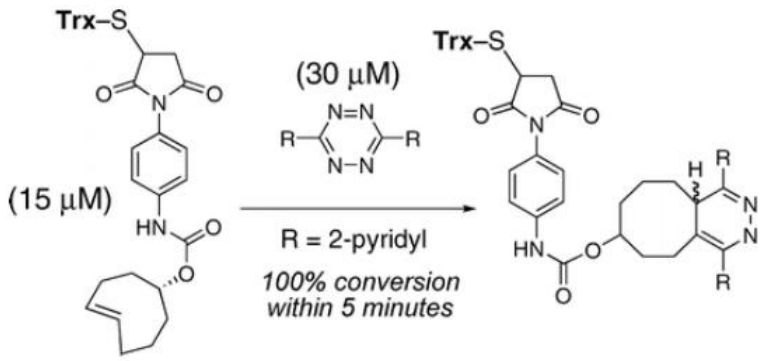
Copper Free Click Chemistry

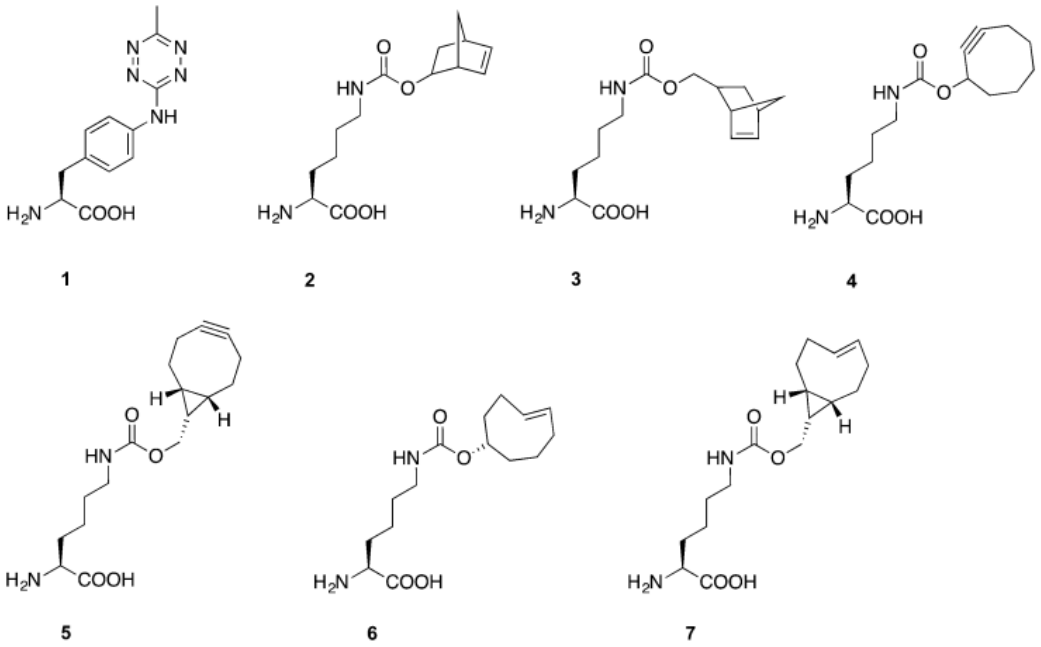


Copper Free Click Chemistry

Tetrazine Ligation

Inverse/retro Diels Alder Reaction

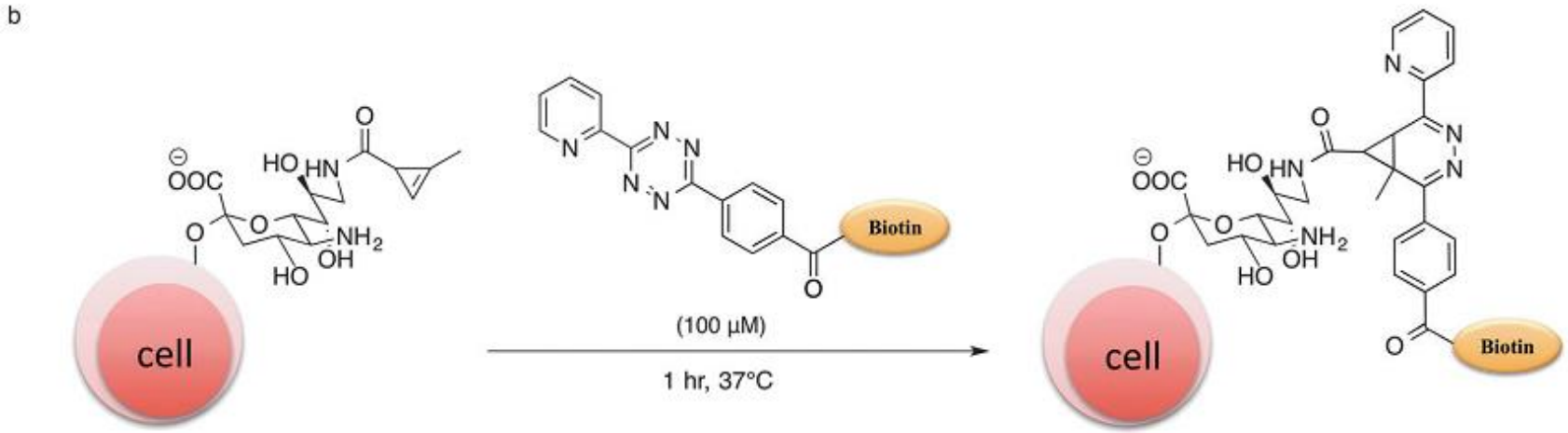




- Reactions proceed smoothly at physiological conditions
- Many different tethers are available and can be introduced e.g. through amber suppression

Tetrazine Ligation

Inverse/retro Diels Alder Reaction



- Many different reporters can be introduced

M.Fox, *J.Am.Chem.Soc.* 2008, *130*,13518–13519.

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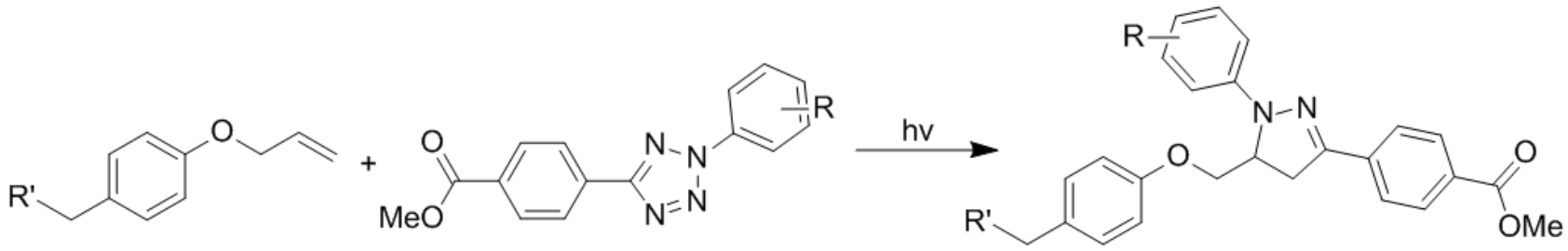
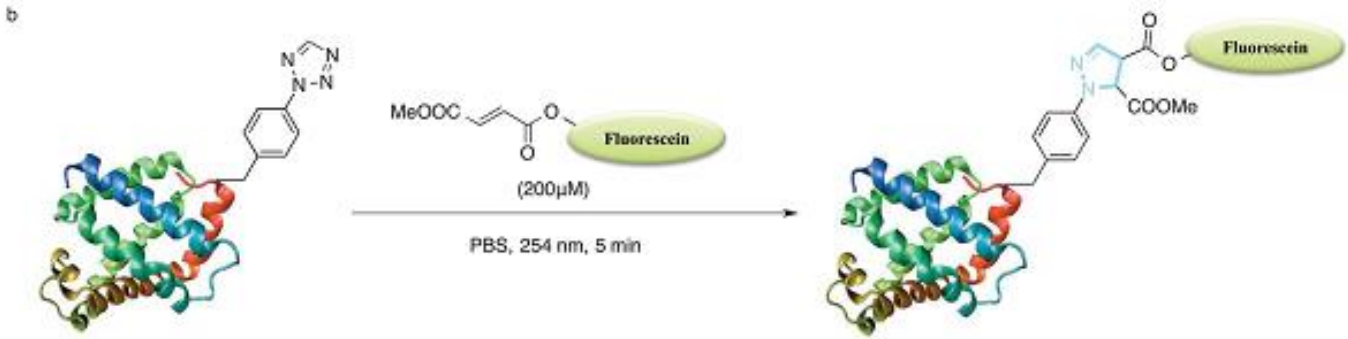
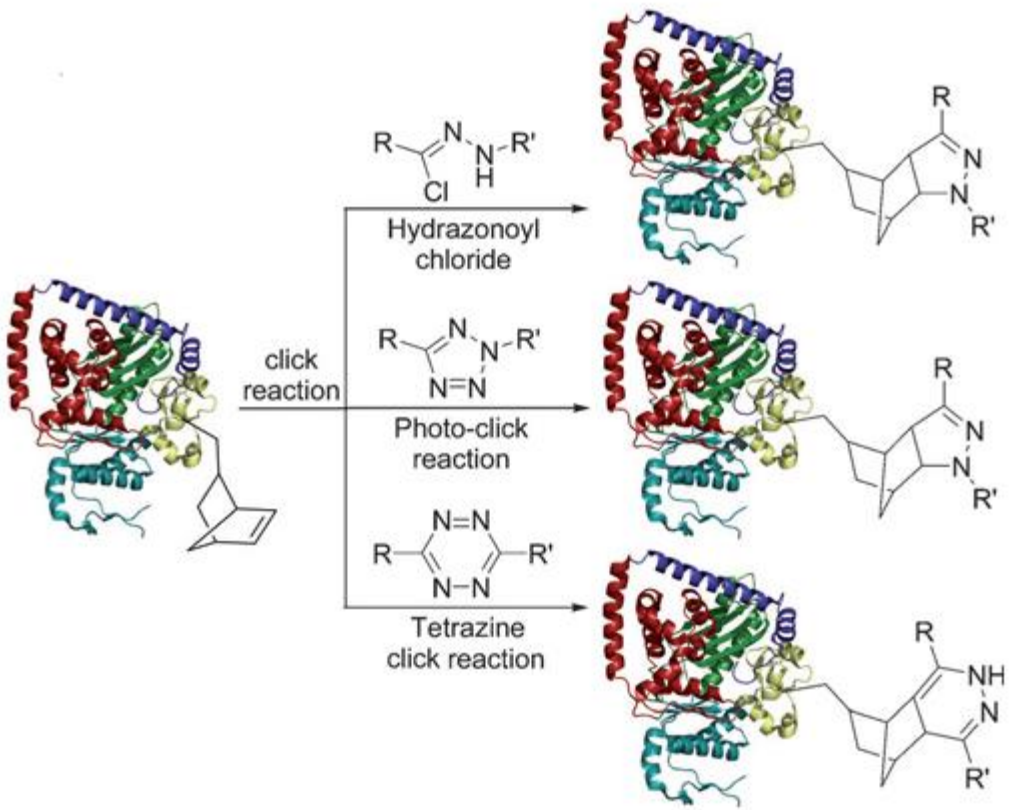
Inverse/retro Diels Alder Reaction



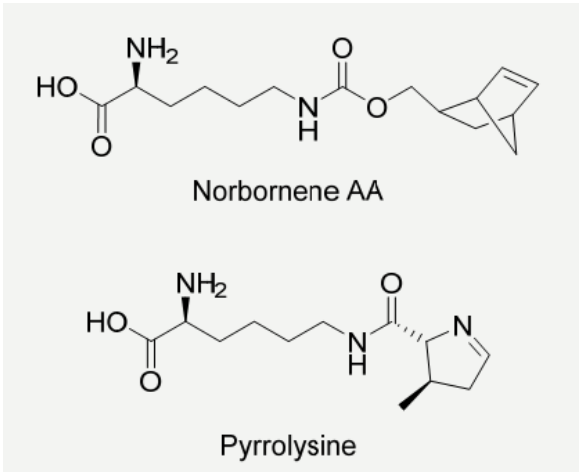


- Temporal control possible through photoinducible tetrazole alkene cycloaddition

Norbonene Click



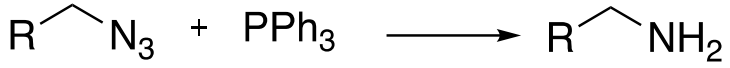
- Incorporation via pyrrolysine system (Praktikum!)
- balance between strain-promoted reactivity and stability

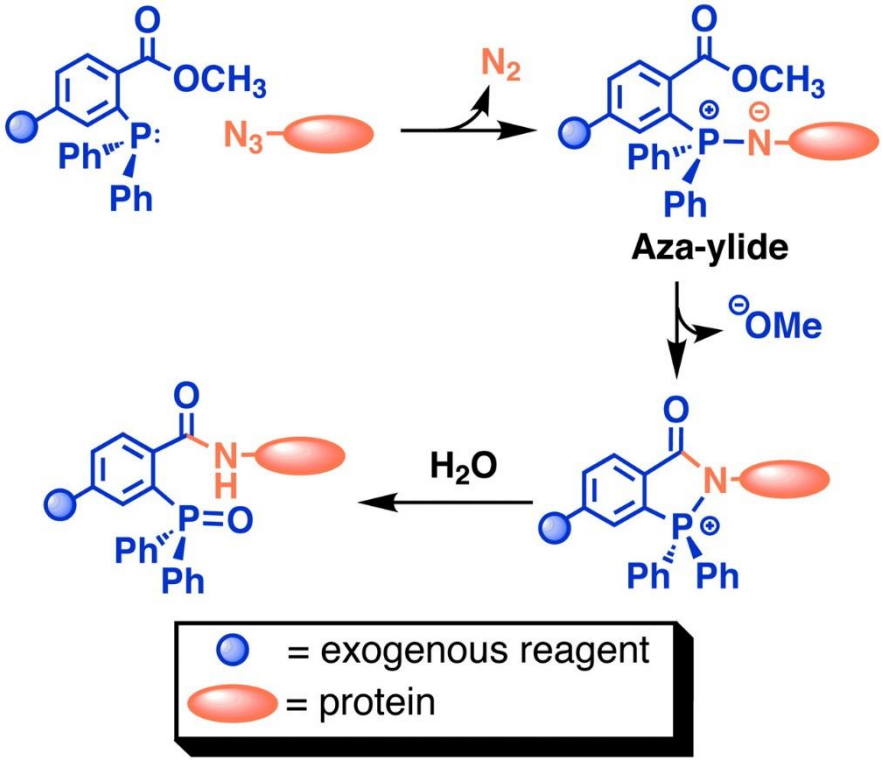


Staudinger Ligation

Staudinger Ligation

Based on classic Staudinger reduction:





- Ester on the phosphine acts as electrophilic trap
- The aza-ylide intermediate is therefore intramolecularly trapped prior to hydrolysis with water

→ Coupled product as a result, different reporter molecules can be attached to the exogeneous reagent

→ BUT: slow kinetics, oxidation of the phosphine before the coupling is also a problem (unreactive!)