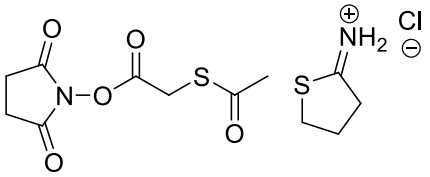
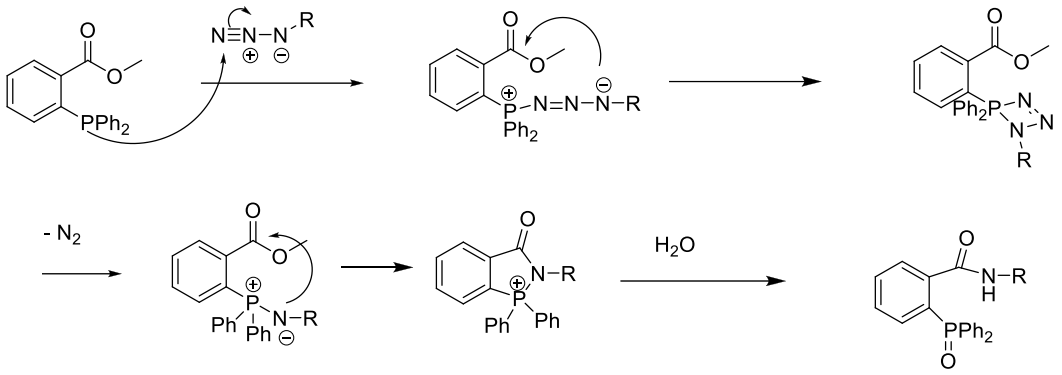
Beispielfragen und Lösungen T1OM-M „Basics of Cloning and Proteomics“

1. Which reaction is catalyzed by pyruvate carboxylase (what is the name of the product) and which coenzyme is required? Depict the reaction mechanism. (4 points)
2. What happens with the product of the previous reaction in the next step of sugar biosynthesis? What is the reaction partner? (4 points)
3. Name four features of a plasmid for high level protein production in *E.coli*. (2 points)
   Promoter / Selection marker / Repressor / Origin of Replication / Multiple Cloning Site / Encoded Affinity Tag (0.5 Pt. pro Feature)
4. What is the function of phosphatase treatment in molecular cloning? Name a method to avoid it. (2 points)
   Reduce re-ligation of the plasmid. (1 Pt.) Use of incompatible ends (1 Pt.)
5. Name an alternative method of molecular cloning that circumvents ligation. Describe the key feature. (2 points)
   Ligation independent cloning (1): generation of long sticky overhangs by exonuclease function of T4 DNA polymerase (1)
   Gateway (or recombination cloning): homologous recombination
6. Name three different methods of liquid chromatography and sketch the principle. (3 points)
   Affinity / ion exchange / hydrophobic interaction / size exclusion / reverse phase / chromatofocussing (0.5 Pt. per method, 0.5 Pt. per sketch)
7. Which interaction principle is the His-tag technology based on? Which eluent is commonly used? Name one alternative elution method. (3 points)
   Complex formation with Ni / Co / Zn (1), Imidazole (1), EDTA / low pH / Histidine (1)
8. When you compare classic Sanger and third generation SMRT sequencing, how do the fluorescent nucleoside triphosphates used in both methods differ with respect to dye attachment? (2 points)
   Sanger: attachment at the base (1), SMRT: at the gamma phosphate (1)
9. What is the advantage of the pyrrolysine system over conventional orthogonal tRNA/AARS pairs (name two)? (2 points)
   No mutation of the tRNA required / no selection against natural AA required / wide substrate spectrum of pylRS / no SECIS element required
10. How can premature termination in amber suppression systems be reduced? (2 points)
    Deletion of release factor 1
11. You need to modify a lysine-rich protein to contain thiol functionality. How would you accomplish that (name or structure of reagent)? (2 points)
    transforming Aminogroups to Sulfurylgroups is possible for example by using Traut`s reagent or SATA



SATA Traut`s Reagent

12. Which modification was developed for the Staudinger reaction to allow bioconjugation (mechanism)? (4 points)



Ester-Derivative traps intermediate prior to hydrolysis → conjungation possible

13. For the given peptide NH_2-ANDENTPGLVR-COOH, note the sequence of the y2 and the b5 ions. With which protease the peptide was most likely generated? Explain. (4 points)
    a. y2: NH_2-VR-COOH (1) und b5 NH_2-ANDEN-COOH (1) b. Trypsin (1), schneidet spezifisch nach Arginin und Lysin (1)
14. Explain how ionization in MALDI and ESI is achieved. Spell out the abbreviations. (4 points)
    Matrix Assisted Laser Desorption and Ionization (1+0.5 Bonuspunkt, wer LASER auch noch ausschreibt!) The sample is embedded in the matrix, which absorbs the LASER energy (1)
    Electron Spray Ionization: (1) An der Nadel (Auslass der LC oder Spritzenpumpe) bildet sich ein Tropfen/Hohe Temperatur und Trockengas führen zu Evaporation, sodass die Tropfen immer kleiner werden und sich die Ladung dadurch verdichtet/die angelegte elektrische Spannung führt zur Explosion der Tropfen (überschreiten des Rayleigh limits führt zur Coulombexplosion) in kleinere Tröpfchen und es wiederholt sich (1)
15. Bonus: The coding sequence of the yellow fluorescent protein ends with a TAA stop codon: GGC ATG GAC GAG CTG TAC AAG TAA. The multiple cloning site of pET28a allows C-terminal fusion with a 6xHis tag (CAC coding for His): GCGGCCGC CTC GAG CAC CAC CAC CAC CAC CAC TGA. Design a reverse primer sequence for the amplification of YFP to result in a C-terminal His-tag, using the restriction enzyme NotI (GCGGCCGC). Write down the first 12 bases of the primer sequence (5'->3').
    Sense: AAG NGC GGC CGC Reverse: GCG GCC GCN CTT
    Kritisch: Frame erhalten (1 Base zusätzlich erforderlich) und Stop codon entfernen