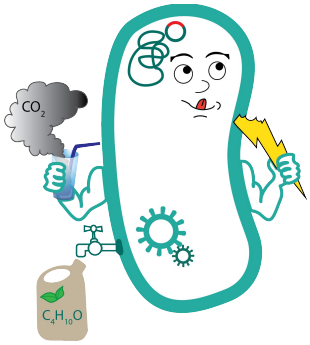
MALDI-TOF

- <u>Tryptic digest of gel lanes for analysis with MALDI-TOF:</u>
  - Make sure to work under a fume hood
  - Do not work with protective gloves to prevent contamination of your sample with plasticizers
  - Reaction tubes have to be cleaned with 60 % (v/v) CH_3CN and 0.1 % (v/v) TFA. Afterwards the solution has to be removed completely followed by evaporation of the tubes under a fume hood. Alternatively microtiter plates from Greiner® (REF 650161) can be used without washing
  - Cut out the protein lanes of a Coomassie-stained SDS-PAGE using a clean scalpel. Gel parts are transferred to the washed reaction tubes. If necessary cut the parts to smaller slices
  - Gel slices should be washed two times. Therefore add 200 µl 30 % (v/v) acetonitrile in 0,1 M ammonium hydrogen carbonate each time and shake lightly for 10 minutes. Remove supernatant and discard to special waste
  - Dry gel slices at least 30 minutes in a Speedvac
  - Rehydrate gel slices in 15 µl Trypsin-solution followed by short centrifugation
  - Trypsin-solution: 1 µl Trypsin + 14 µl 10 mM NH_4HCO_3
  - For this solution solubilize lyophilized Trypsin in 200 µl of provided buffer and activate Trypsin for 15 minutes at 30 °C. For further use it can be stored at -20 °C
  - Gel slices have to be incubated 30 minutes at room temperature, followed by incubation at 37 °C over night
  - Dry gel slices at least 60 minutes in a Speedvac
  - According to the size of the gel slice, add 5 - 20 µl 50 % (v/v) ACN / 0,1 % (v/v) TFA
  - Samples can be used for MALDI measurement or stored at -20 °C



- Preparation and Spotting for analysis of peptides on Bruker AnchorChips:
  - Spot 0,5 - 1 µl of sample aliquot
  - Add 1 µl HCCA matrix solution to the spotted sample aliquots. Pipet up and down approximately five times to obtain a sufficient mixing. Be careful not to contact the AnchorChip. Note: Most of the sample solvent needs to be gone in order to achieve a sufficiently low water content. When the matrix solution is added to the previously spotted sample aliquot at a too high water content in the mixture, it will result in undesired crystallization of the matrix outside the anchor spot area
  - Dry the prepared spots at room temperature
  - Spot external calibrants on the adjacent calibrant spot positions. Use the calibrant stock solution (Bruker's "Peptide Calibration Standard II", Part number #222570), add 125 µl of 0.1 % TFA (v/v) in 30 % ACN to the vial. Vortex and sonicate the vial
  - Mix the calibrant stock solution in a 1:200 ratio with HCCA matrix and deposit 1 µl of the mixture onto the calibrant spots
  - Analyze samples in ultrafleXtreme by Bruker Daltonics