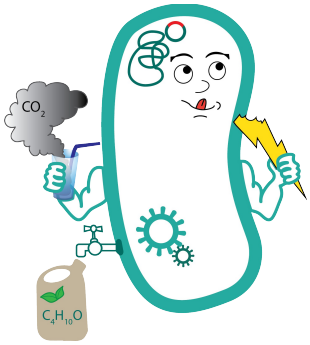
His Trap FF purification

- Column preparation
  - 1 h Incubation with 1 M NaOH
  - Wash with 10 ml Binding Buffer
  - Wash with 10 ml distilled water
  - Strip the column with 10 ml Stripping Buffer
  - Wash with 10 ml Binding Buffer
  - Wash with 10 ml distilled water
  - Recharge with 2.5 ml 0.1 M $NiSO_4$
  - Wash with 5 ml distilled water
  - Wash with 5ml Binding Buffer
- Perform a blank run:
  - Wash with 5 ml distilled water (After Ethanol storage)
  - Wash with 5 ml Binding Buffer
  - Wash with 5 ml Elution Buffer 5
  - Equilibrate with 10 ml Binding Buffer
  - Store in 20% ethanol
- Sample preparation:
  - Add 5 ml Binding Buffer to each gram of pellet
  - Add 0.2 mg/ml lysozyme, 20 µg/ml DNAse, 1 mM $MgCl_2$, 1 mM PMSF
  - Stir for 30 min at +4 °C up to +20 °C (depending on the protein)
  - Centrifuge 30 min at 4 °C
  - Adjust pH to 7.4 – 7-6
  - Apply the lysate on the column immediatly



- Sample run:
  - Wash with 5 ml distilled water
  - Equilibrate with 5 ml Binding Buffer
  - Apply the lysate on the column
  - Wash with Binding Buffer until the absorbance reaches a steady baseline (10 ml)
  - Elute with 5 ml Elution Buffer gradient (Elution Buffer 1 – 5)