Introduction

Materials and Methods

* Cell culture, strains
* Absorption measurements + normalization and confidence interval
* WB
  + FULL VERSION:
  + \noindent \textbf{Western Blot} Cultures were inoculated in \ac{bg11} without antibiotica at an \ac{od}\textsubscript{750} of 0.3 and grown over night. The next day, half of the cultures were harvested immediately and the other half was put under a 2h cold shock prior to harvest. Cells were harvested (2~min, 7500~g, 4°C) and the pellets were frozen in liquid nitrogen and resuspended in 400~\textmu L \ac{pbs} supplemented with protease inhibitors (100 \textmu g/mL \ac{aca}, 6.25 \textmu g/mL benzamidine, 2.5 \textmu g/mL \ac{aebsf}). Cell lysis consisted of three cycles in a bead beater (500~\textmu L 0.25 - 0.5 mm glass beads, 10~min, 4~°C, 30~Hz) with with 5~min breaks to avoid overheating. Beads and unbroken cells were separated from the cell extract (2~min, 500~g, 4~°C) and he membrane and cytosolic fractions were separated (30~min, 20.000~g, 4~°C). The membrane pellet was resuspended in 500~\textmu L \ac{pbs}. The protein concentration was determined by \textcolor{red}{DirectDetect system}. 9~\textmu g of protein in \ac{pbs} and loading dye \textcolor{red}{(reciepe)} in a final volume of 40~\textmu L were denaturated for 10~min at 95~°C and loaded on 10~\% \ac{sds} gels \textcolor{red}{(reciepe?)}. The gels were run at 85~V until the samples reached the separating gel, then the voltage was increased to 190~V. For blotting, the \ac{sds}-gels were equilibrated in cooled Towbin buffer \textcolor{red}{(reciepe)} for 10~min, then blotted on \textcolor{red}{(nitrocellulose ?)} membranes for 1h30 at 300~mA. The membrane was washed with \ac{tbst} for 10~min, blocked with 5~\% milk powder in \ac{tbst} for 45~min at room temperature washed three times with \ac{tbst} for 10~min, incubated over night with \textcolor{red}{anti-GFP (1:4000, Hersteller)}, washed three times with \ac{tbst} for 10~min and incubated 2~h at room temperature with \textcolor{red}{anti-rabbit (1:40000, Hersteller)}. The blots were developed using \ac{ecl} (kit from \textcolor{red}{Hersteller}).\\
* In depth analysis of DESeq (bei Ute’s paper schauen, wie sie es gemacht hat) + warum man welchen tool benutzt hat
  + GO enrichement analysis (function + parameter (LFC, padj) used) + cite!
  + Other bioinformatical analyses (parameters used)
  + Anno file (Phillipp): paper proteogenomics zitieren
* AlphaFold Multimer and ColabFold (parameters used,) + cite!
* FISH (tbd)

Results

* Phenotypical changes of Δrbp1 mutants and RBP1 complementants under cold shock
  + Peaks nennen und erklären, warum es 2 chlorophyll peaks gibt
* In depth analysis of DESeq2 dataset
  + Venn diagrams
  + GO enrichement analysis
* Protein interaction modelling using AlphaFold Multimer
* FISH (tbd)

Discussion