**Questions:**

Here is a set of questions that should be addressed.

For calculations if possible use the data from all groups, exchange the data.

Make a supplement data excel sheet including all your data and calculations (in a readable manner).

**Introduction**

* Proteomics workflow for targeted proteomics
  + Principle of DDA, MRM, DIA
    - Role of masses, fragmentation energy and retention time
  + Specificity of the methods
  + Prerequisites of the methods
  + Advantages and disadvantages
* Experimental quantification strategies
  + Explain different label strategies
  + Proteomics workflow
  + Compare when the labeled species are added how they are added and what different error sources occur?
  + What is label efficiency and why is it important? How is it for SILAC and 15N metabolic labeling?
* Protein and protein complexes
  + Stoichiometry
  + Role of RuBisCO in CO2 fixation
  + Pyrenoid formation in Chlamy
  + Pathways and stoichiometry of proteins within the pathway, stoichiometry of pathways
  + Regulation of pathways / direction of metabolic processes
* Questions asked / goal of the experiments

**Methods**

Note only the deviations from the script

**Results**

* Physiological parameters of the chlamy cultures
  + Cell number/size distribution
  + Protein per cell
  + Chlorophyll per cell
  + Growth curve and doubling time
* QProt
  + Concentration of aliquots, differences between methods
  + Did you get all the protein purified, what is the loss?
  + Picture(s) of isotopic cluster(s) for the Chlamy and Q-protein peptide(s) used, what is the app. label efficiency, explain the isotopic cluster.
* Western Blot quantification
  + Quantification of the epitope vs. BSA
  + What is the concentration of the epitope?
  + µg rbcL per µg total cell protein, mole rbcL per µg total cell protein, molecules rbcL per cell, estimated concentration of RuBisCO in the chloroplast stroma?
  + Compare with literature
  + Picture of the western blots
  + Graph of the resulting intensities / linearity
  + Background subtraction?!
  + Picture of background subtraction? if necessary
* BlueNative
  + Why Blue Native and not SDS-Page? What do you separate here?
  + Pictures of your gel, what was green? Possible assignment of band to cplx?
  + What did you cut out?
  + Which proteins did you detect in the different lanes?
* Quant Bands (MS1 or MS2)
  + SDS gels stoichiometry of rbcL vs RBCS
  + BN gels stoichiometry of rbcL vs RBCS
  + Absolut amount of rbcL and RBCS in the cells (e.g. fmol / cell, fmol/µg total protein, conc in stroma (for comparison with paper))
* Quant WC (MS1)
  + Stoichiometry of rbcL vs. RBCS
  + Absolut amount of rbcL, RBCS in the cells (e.g. fmol / cell, fmol/µg total protein (for comparison with paper))
  + Quantitation of the linear electron transfer chain and the Calvin-Benson-Bassham cycle, table with numbers (amount, conc) for all proteins / peptides / pathways over the different strains.
  + How is the ratio and linearity over the 15N peptides / fragments for the two different mixing ratios?

**Discussion**

* Data analysis
  + Calculation on proteins, peptides, size distributions, etc.
  + Possible peptides for quantitation
  + How would you improve the peptides / qConCat Proteins?
  + Can you say something about reproducibility (between groups within groups?)
* MS quantification
  + How did you quantify the proteins / peptides? How did you set the retention times to integrate the areas?
  + Which data did you use? Which not and why not (noise, etc.)? What quality control did you implement?
  + Are the intensities of the Chlamy peptides the same in all runs? (should be because there was same µg of Chlamy each each)
  + If not, why not? And how would that translate in label-free quantification where only the intensities over the runs are compared?
  + Compare your results with the literature. Discuss differences.
* Biology
  + What are the amounts of rbcL and RBCS, compare your results with the literature. Discuss differences.
  + Can you find other absolute amounts of the proteins investigated in literature?
  + What is the stoichiometry of the different proteins / complexes / pathways in the different strains, what are the implications
  + Where are the pathways regulated?