Play with HUMOS and answer the following questions.

MS1 scan mode:

1. Why has the spectrum of “Equimolar” mixture of peptides different intensities? What is the difference in observed peptide repertoire between the peptide distributions (equimolar, typical etc)?
2. What resolution do you use in standard “bottom-up” experiment and why? How higher resolution does influence on your experiment? What minimal resolution is needed to distinguish 127N and 127C TMT reporters?
3. How accumulation of more ions (higher AGC target) change the spectrum? (Hint: check overall “shape” of the spectrum and then focus on some low intensity ion cluster). In terms of observed ion range (difference in span of most and least abundant ions) could it be advantages to accumulate as many ions as possible?
4. Does AGC target influence mass accuracy? What might be the reason for this? Think of other possible complications and advantages of high AGC.

BoxCar scan mode:

1. How do peptide intensities change in the BoxCar mode compare to usual MS1 mode? Which peptide distribution leads to the most changes?
2. Test BoxCar mode with different ion distributions (equimolar, typical etc), AGC targets, and injection times. What do you observe? In which cases one can expect improvement? Which limitations of BoxCar can you observe?
3. Compare AGC and ion accumulation time for MS1 and BoxCar. What can you observe? Hint: does AGC and ion accumulation time reach target values? Can you come with explanation?
4. Compare instrument throughput (number of MS1 and MS2 scans) in BoxCar and MS1 mode? Test different TopN values. What consequences it might have?