Experimental Design

*We first came up with the following plan, however on review, it seems, that the second plan (New Plan after looking more at the existing data) is more reasonable. This should serve only as a discussion base, of course.*

# Original Rationale

We know, that at 15' the concentration inside the cell is still declining, so something must still be happening. On the other hand the major conversion should be happening in the first few minutes, but always re-adding MG might always disturb the cell

## With MG

* 0' Add MG
  + 12C
    - 15' harvest
    - 15' "change" media to 12C containing 7 µm MG
    - 17' harvest
    - 20' harvest
  + 13C
    - 15' change media to 13C containing 7 µm MG
    - 17' harvest
    - 20' harvest

## Without MG

* 12C
  + 0' "change" media to 12C containing no MG
  + 2' harvest
  + 5' harvest
* 13C
  + 0' "change" media to 13C containing no MG
  + 2' harvest
  + 5' harvest

9 harvests 5 tecreps= 45

## Original Plan

* Adding MG will change the pool sizes dramatically, so we need matched 12C and 13C time points
* For us the early time points will be the most interesting, but to have the controls, we also need samples for the later time points (17', 20') where we trace with 13C-Glucose
* 12C time points: 0', 1', 2', 5', 10', 15', 17', 20'
* 13C time points: 1', 2', 5', 10', 15', 17', 20'
* This means with n=4 plates per condition: 60 samples

## Checking 13C-MG:

* 1 media sample directly after media preparation
* 1 media sample of an empty plate at latest time point (20'), to make sure that e.g. labelled pyruvate we measure is not actually somehow forming from processes in the solution

# New Plan after looking more at the existing data

## Where does MG interfere with "normal" central carbon metabolism?

* Add MG (or just change media without MG as control) to let the cells switch their metabolism
* Add MG (or no MG) with 13C Glucose trace for 1', 2', 3' minutes (this will show us, where MG regulates metabolism)
* Obtain the same values, where Glucose stays 12C (but still with and without MG)
* This means with n=5 plates per condition: 60 plates/samples + the ones to check cell number

## Where does MG go (what happens with its carbon backbone)?

* Add labelled MG, harvest after 1', 2', 3', 5' (samples from unlabelled controls from experiment above could be used, but it's often better to have cells seeded on the same day etc

What happens likely to MG

* For a certain part: MG evaporates, MG has a similar vapour pressure and boiling point as ethanol and a petri dish presents a lot of open surface (concentration seems to decay in a first order kinetic, to make more reliable statements about this, we would need [MG] measurements
  + at earlier time points (anyways interesting)
  + Time curve of media with MG on a plate without cells (obligatory control)
  + 🡪So we definitely need more measurements of MG amounts (one should also think about why there is a loss of 75-80% already at *t* = 0
* MG binds unspecific to proteins: this might explain differences in decay on different dishes. Albeit HeLas are extremely quick, the dishes likely all had the same amount of media, however cell size and protein content differed between the cell types in the experiment. This might also create a buffer system of free and bound MG
  + 🡪how would the data look, if normalised to protein content and not cell number?
* MG moves into the cell and also binds to proteins, but also might be metabolised
  + We see a rise and decay of intracellular MG, the buffers within the cell might overflow with time (inconsistent between cell types), while, when the extracellular [MG] falls below a certain level, MG is either not passing the membrane anymore, or the cell can clear MG at higher rate than it can permeate the membrane. So it also might make sense to trace with 13C-glucose (and a known amount MG respective to the [MG] in the media) and also 13C-MG at a later time, e.g. 20 minutes, so also adding MG at time -adjusted concentration, where the system should be stable but still regulated by MG