Describe analysis:
Visually carve out what were doing computationally
Lot of manual clicking data integrated from different files and depending on the molecule there's different steps

Adobe illustrator
"Tiff pool analysis" (tumor interstitial) isn't always what we're measuring pooled standard analysis. Trying to quantity the absolute concentrations of metabolites in interstitial fluid

Analyse samples on a mass spectrometer but also analyse these samples that are mixtures of standards
A standard is a store bought molecule
Like a store bought glucose. So we know exactly the amount of it whereas in biological samples we don't know the exact amound in there. A lot of ways to think of abuncdacne and a lot of ppl get away with relative abundnae (comparing glucose in molecules for example, not exact so not micro or mili molar concentration)

The whole point is to run mixtures standards (both glucose and 100s of others). Do it in pools, not individually. Weigh out each metabolite standard and dilute and mix it into different combos. 7 pools of chemical standards we usually use. Representation of how it looks on a mass spec (is why we split them into 7 different pools) and we want our molecules to be able to be measured individually without interference from another.

Every pool undergoes multiple dilutions that we run
We are measuring standards and at different dilutions
Idea here is that we will have a signal value for different standards at different concentrations

- Image example here (peak area from mass spec integration)

The complicated nature is that we are imperfect humans and so when prepping these mixtures it might not be exactly 5 mM of glucose (for example). It'll be close but there'll be error that we account for in this analysis.

Prep for mass spec analysis
Idea

1) Mix 5 micro L of each Standard Pool dilution with 45 micro L of extraction buffer + Internal Standard (Internal Standard is another set of standards that we add into the standard mix but they differ in that they're isotopically labeled meaning they look like these standards but they have chemical modifications that change their mass yet they're chemically identical)
    a) Standard = glucose; internal standard = [U-13C] Glucose. Once we prep __ it's going to include both standards. That internal standard has heavy isotope is able to tell the internal standard from glucose standard. Always room for error so

reintegrate these things to account for variance for absolute quantification of biological samples
    i) If i analyze a molecule the presence of other molecules can influence the detectability of that molecule
    ii) Hypothetically if i inject the same amount for all of these things U13
    iii) Ion suppression and signal variation can happen during a mass spec run. If there's a fluctuation in u13 glucose we want to account for that bc we want to make sure the changes are caused by dilution and not by signal variation caused by some other error.
  b) Extraction buffer and internal standard are the same across every rim
  c) Not an internal standard for every standard it would be better if we did
    i) Internal (heavy isotopes) Glucose is normalized to labeled glucose
  d) Deuteron
  e) Peak area of the internal standard should be relatively the same
  f) Self normalization: (creatine with creatine internal standard)
  g) Non self normalization: Lactate to normalize 3 hydroxybuterate
2) We want to calculate a response ratio for every molecule. that the standard molecule peak area / the internal standard molecule peak area
  a) Calculated for both normalization
  b) This value is very important
3) Create two datasets: first contains molecules self normalized, 2nd is non self normalized
  a) Bc we analyze these normalizations slightly differently

<u>Looking at smaller dataset of glucose and lactate (prob self norm.):</u>

She had 14 separate concentrations / dilutions and calculated the response ratio for these dilutions

- First calc is taking the theoretical molarity of the molecule (defined by conc) and dividing that by the response ratio of the corresponding molecule. TM/RR.
- In an ideal scenario TM/RR in each dilution should be similar/the same
- molarity / rr : we want to look at where there's similar values across a range. They're supposed to be similar bc of this **** relationship here where the intensity of molecule correlates with amount of molecule in that sample and where that occurs is where they should all be same value (those plus the TM/RR value)
- She chooses a range (range is like the TM/RR from concentrations 1000-20,000 for example and ignored the rest) and averages the TM/rr value and that is the ***conversion factor***
- Obtained conversion factor (mili of micro molar)
- Take CF and go back to original response ratio and multiply each by the conversion factor = accepted off???
  - Idea here is that this gives u the actual concentration in the standard pool
  - Actual concentration value for each standard in each pool (specifically for self normalized samples)

<u>Nonself normalized:</u>

- Take the RR and concentration to find a LOBF / slope

        - Calc a y = mb+b eqn and want it to be highly linear and prob dont want to include values where the LOBF looks off
        - What is p quality
    - Slope, intercept, R^2 value should be as close to 1 as possible, and note the max value used in the range (for theoretical concentration so the highest TC) (TC is defined by how much we believe we added)\
    -

M b R^2 is a measure of linearity
Do calculations and organize data better

Measure absolute concentrations in biological fluids
Real reason is brain fluids?
Whole idea is unders molecular epigenetic features of brea brain metastasis
Measure metabolite profiles in brain interstitial fluids in different places (BIF outside and inbetwene cells derived by whats secreted from other cells and blook cells? Do these standard pool analyses to determined absolute concenrtrations)
Manipulate csvs datatables how to plot certain things
Learn r taking the peak