Start Aggregation assay (Part 3)!

- **Collect** the following from the front bench:
  - Concentrated purified TDP43 protein
  - Buffer A
  - DMSO
  - Your small molecule (check the Wiki data page– the **BOLD** SM for your group)

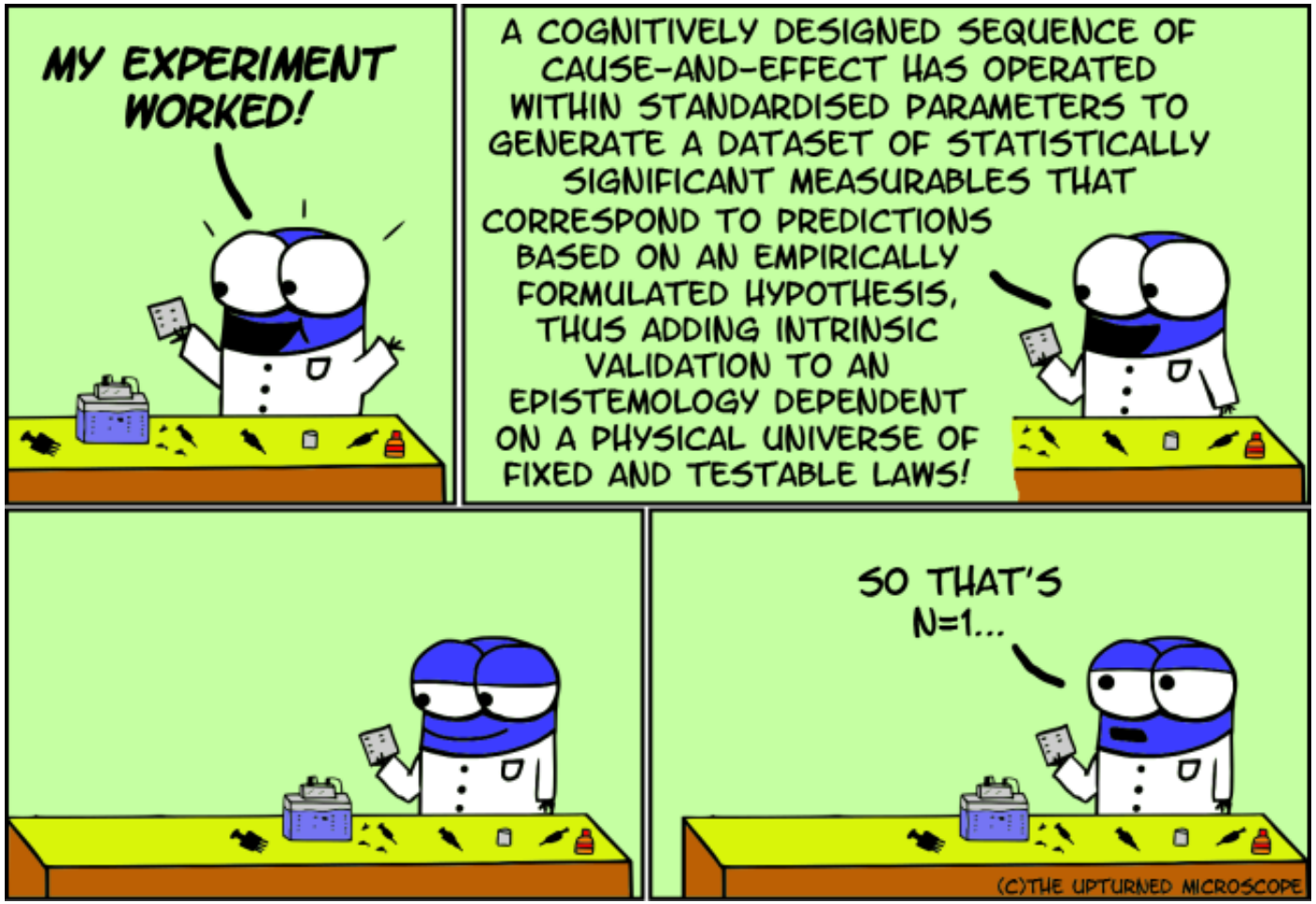
- **Calculate** volume of small molecules for each condition

- **Pipette** your samples into snap-cap tubes

- **Bring** tubes up to front bench to load into 96 well plates
  - If 2 group are ready at the same time, you'll share a plate to keep everything moving

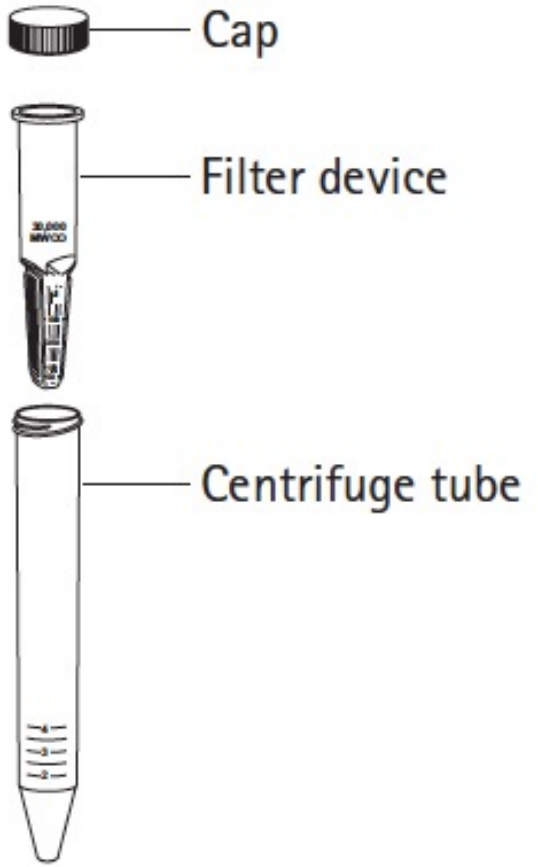
M1D5: Perform aggregation assay using TDP43 protein and draft data slide for Data Summary

- Comm Lab
- Pre lab discussion
- Aggregation assay
- Research Talk discussion with Noreen
- Draft data slide of purification results



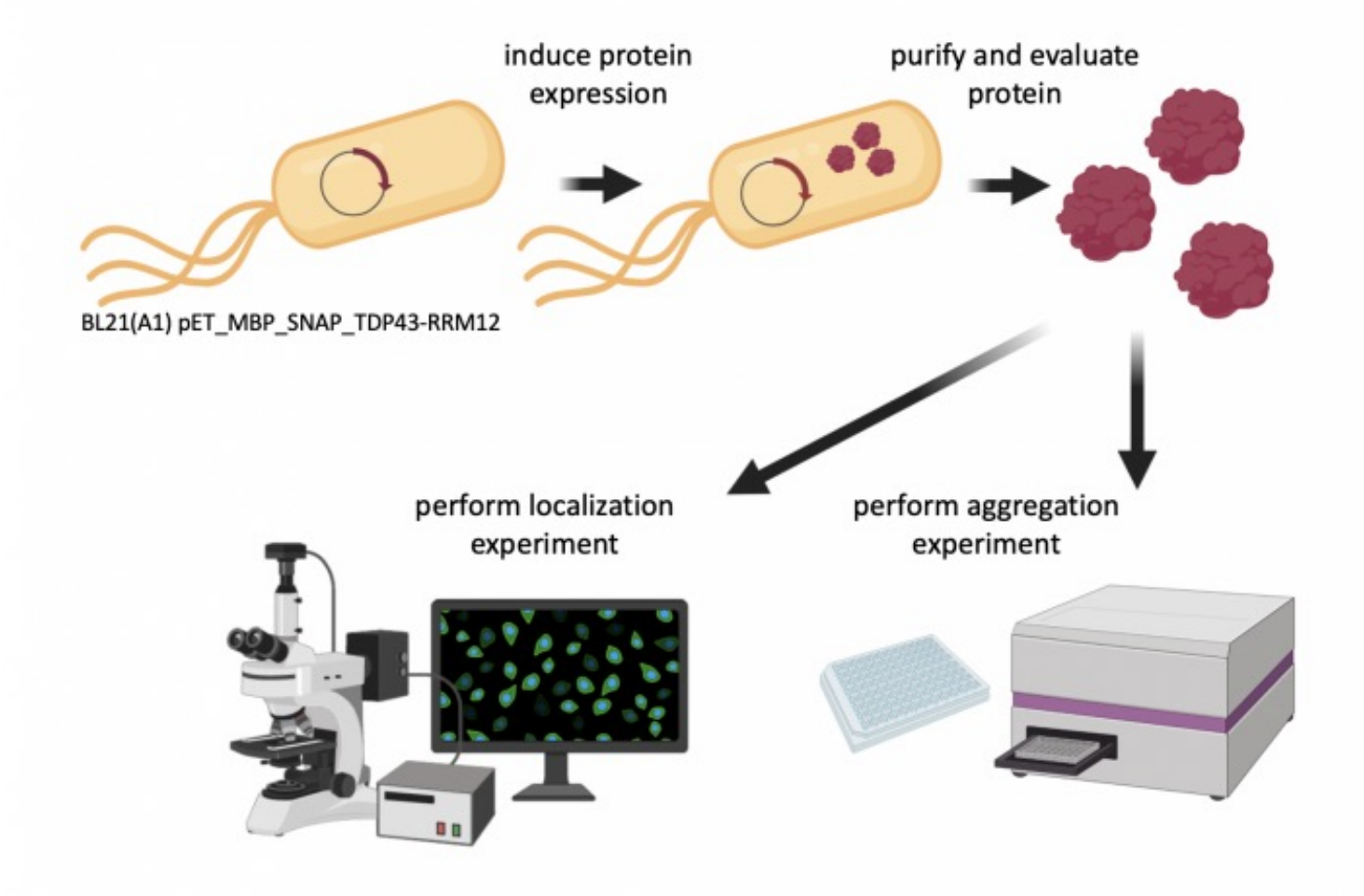
Concentrate protein before experiment

- Filter device sits within centrifuge tube…add protein to filter device for centrifugation
- Filter device has MW cutoff of 3 kDa …protein is retained in the filter device during centrifugation
  - **Is that a problem for your TDP43_RRM12?**
- How does this concentrate the protein?
- How did we remove imidazole?



Overview of Mod 1 experiments

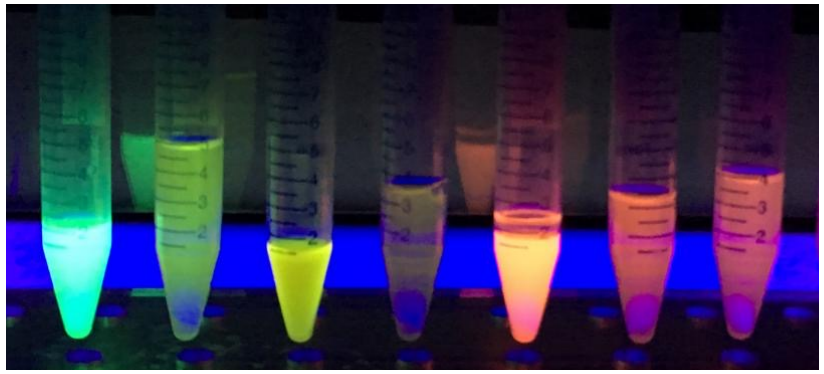
Research goal: Use functional assays to characterize ligands identified as binders to TDP43 from SMM technology



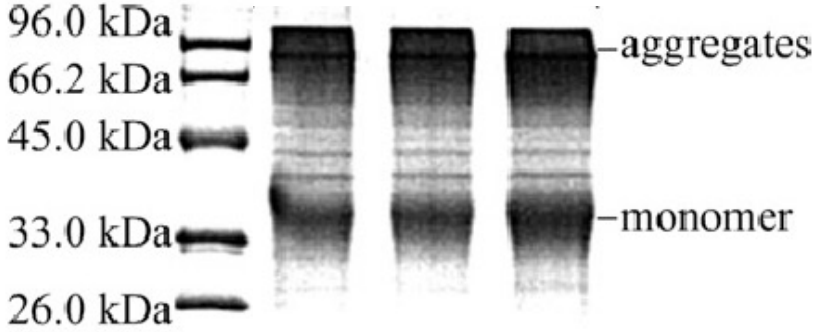
<u>Types of protein aggregation:</u>
Amorphous aggregation
Amyloid fibrils
Oligomers

Multiple read-outs for protein aggregation

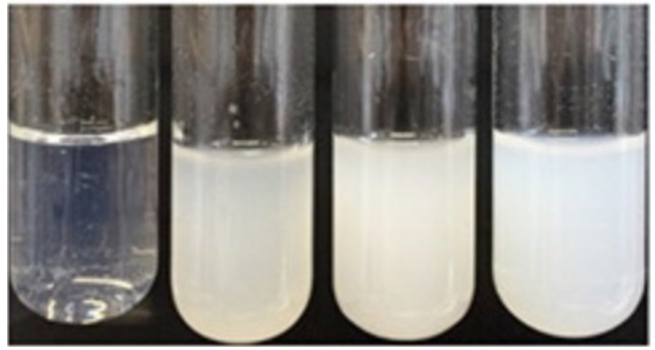
Fluorescent Dyes



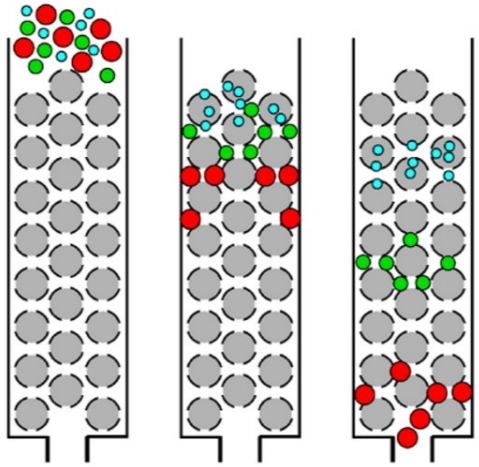
Protein gel



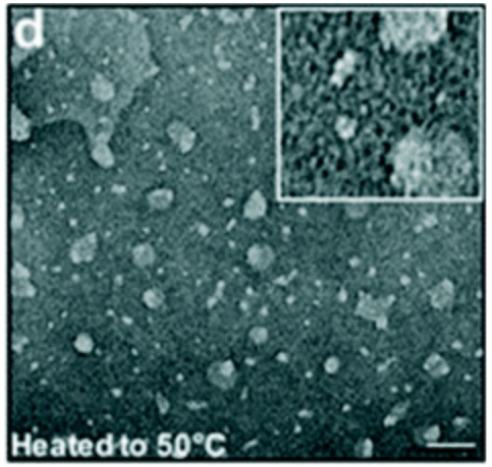
Turbidity



Size exclusion chromatography



Electron Microscopy

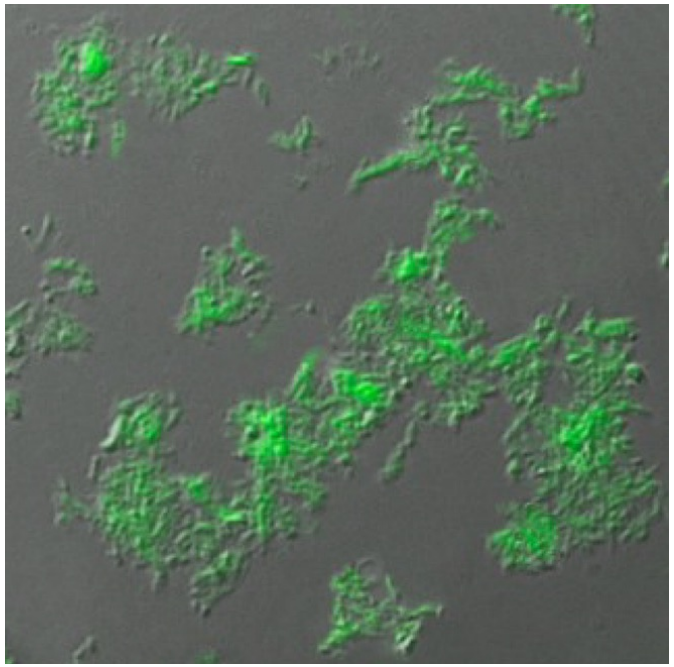


Fluorescent aggregation dyes and antibodies

<u>Congo Red, Thioflavin T, etc…</u>

- Traditionally used to identify amyloid fibrils
  - Contain beta sheets
- Literature on their use for TDP43 is mixed
  - Effective in some papers, but weak or no signal in others
- Can be used in microscopy or with a plate reader

Thioflavin T aggregates
(visualized with microscopy)



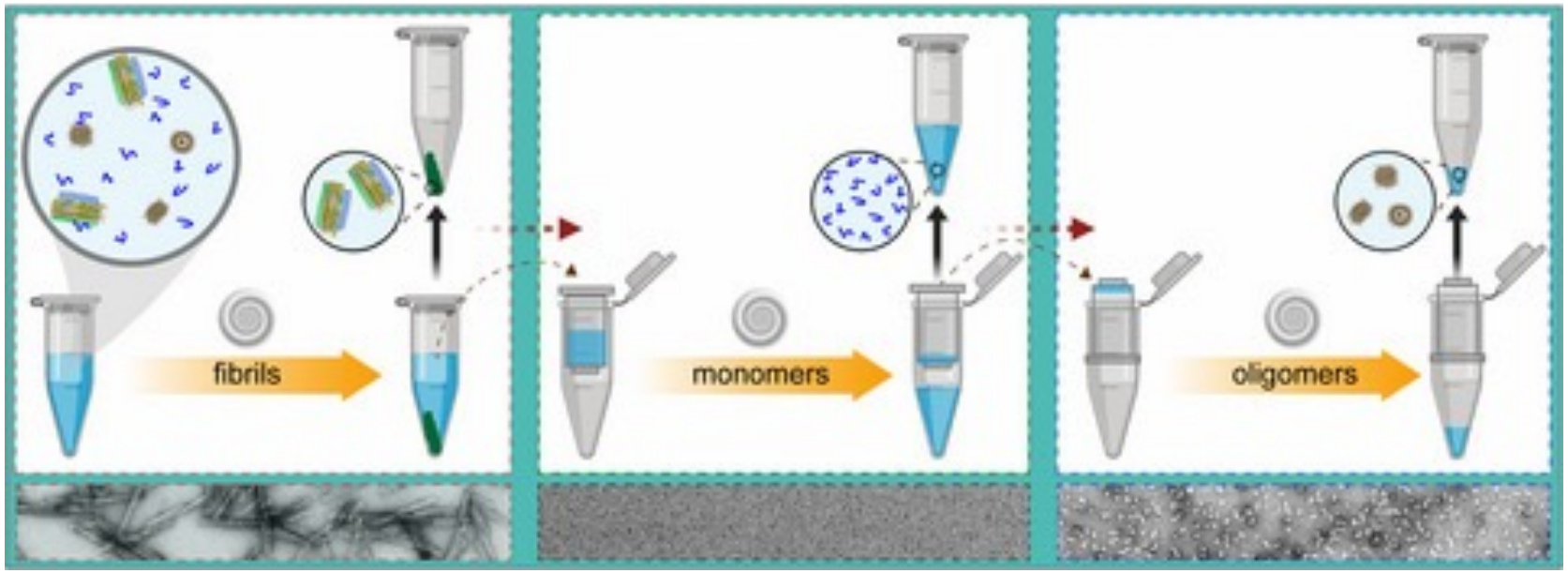
Sulatskaya et al, 2018

<u>Aggregation antibodies (fluorescent conjugated)</u>

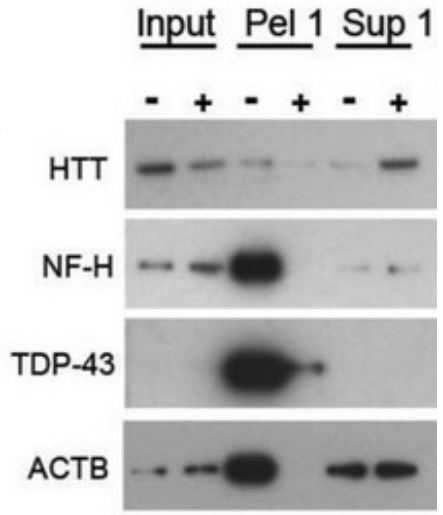
- Well characterized proteins
- Conformation specific
- Can also use antibodies that recognize other protein states that are aggregation-prone

Fractionation and protein gels to separate aggregates

- Physically separate insoluble aggregates from soluble monomers
- Electrophorese these ”fractions” on a protein gel
- Can use non-reducing protein gel to look at protein-protein interactions to identify oligomers



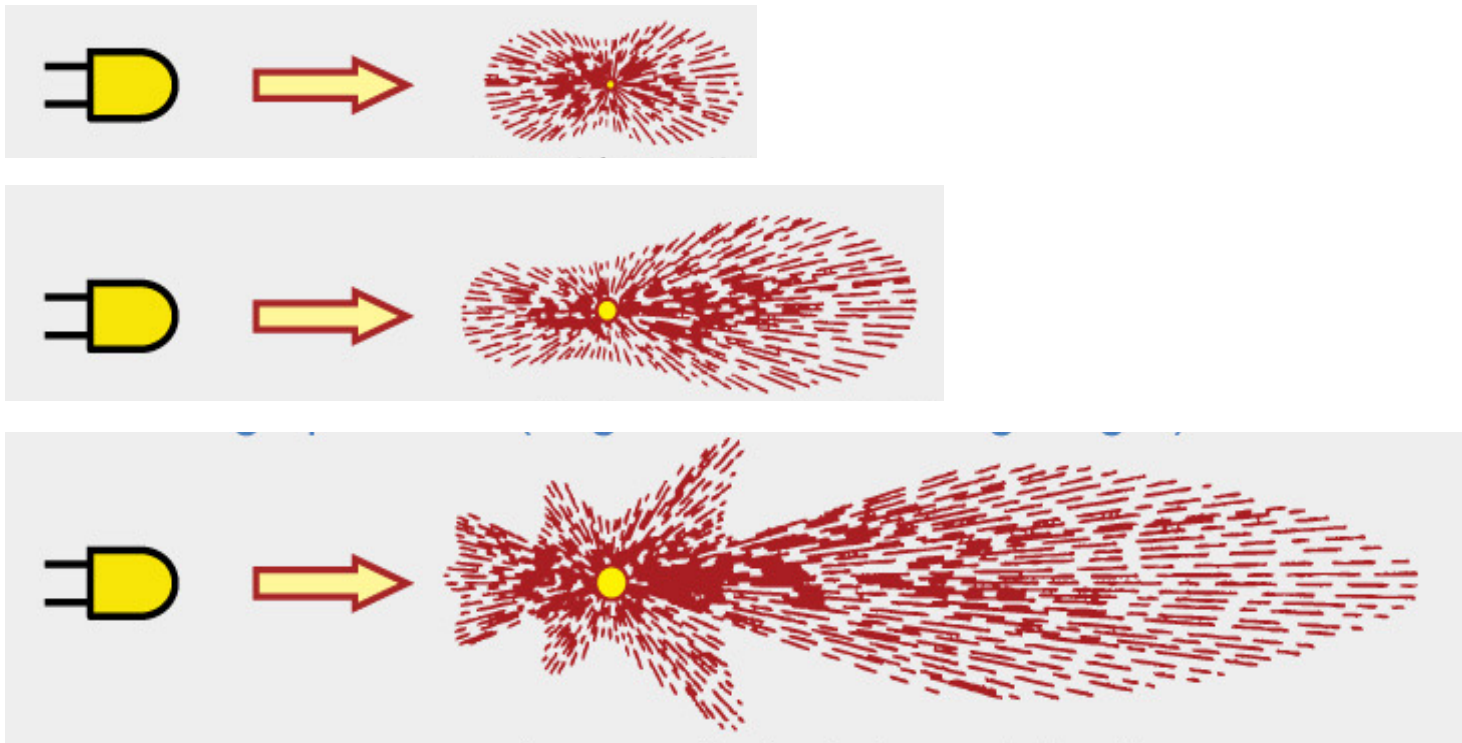
Kumar et al, 2020



Thermo

Turbidity as a measure of protein aggregation

- Protein aggregates are physically larger than soluble protein
  - Greater scatter of visible light
- Light scattered is a function of both size and shape of aggregate
- Straightforward and reliable measure of universal aggregation



Kemtrak

Experimental groups

- Buffer A
- TDP43_RRM12 + DMSO
- TDP43_RRM12 + 3 μM SM
- TDP43_RRM12 + 30 μM SM

For Today

- Aggregation assay with multiple time points
- Work on Data Summary data slide with your lab partner
- Resubmit your data figure by 10pm today if you would like an updated version graded!

For M1D6 (Thursday, 2/24)

- Submit a completed data slide (1 per group) of the purity and concentration data
- Submit a paragraph on your Comm lab visit

During incubation times: draft data slide for Data Summary

- Using insight from Comm Lab workshops, work on the draft of your data figure with your lab partner

- Use the protein purification data figure you did for homework to create the data slide
  1. Format
  2. Refine/edit
  3. Write a Results and Interpretation section in bullet points

Use wiki guidance!

- Format powerpoint slides to 8.5” x 11” in portrait-mode
- Work on figure arrangement so that figure and text all fit on same slide
- Make sure you discuss all the data in the figure

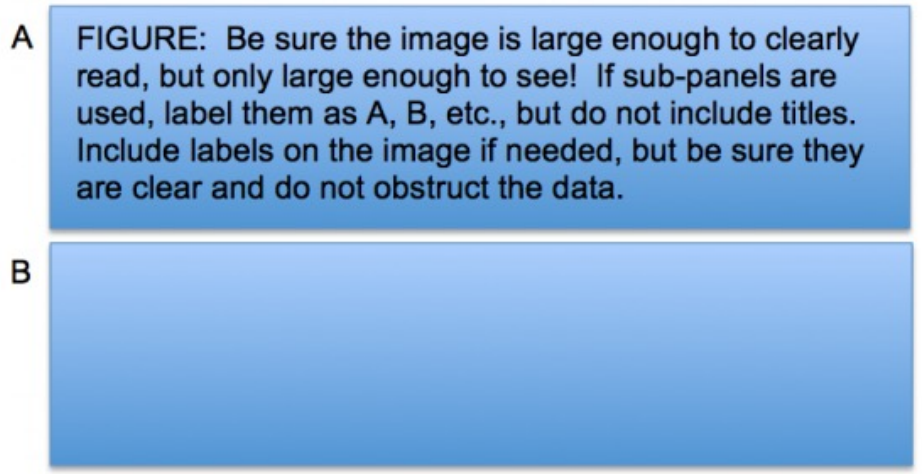


FIGURE TITLE: This should state the conclusion of the figure in very brief and precise language. CAPTION: Start with a topic sentence that introduces the figure or sub-panel. Provide all of the information that the reader needs to interpret the figure (define abbreviations, explain labeling scheme, differentiate between sub-panels A, B, etc.). You should not interpret the figure or give minor methods details.

RESULTS SECTION TITLE: This should state a conclusion concerning what you now know given the information provided on this slide…if there is more than one conclusion, consider separating the information into more than one slide.

RESULT(S)/INTERPRETATION(S): Use the questions below to guide the information you provide in your concise bullets.

- What is the overall goal of your experiment?
- What was your expected result according to your hypothesis?
- What evidence do you have that you result is ‘correct’ or ‘incorrect’?
  - What controls did you include and for what did these control?
  - Did the controls work as expected?
- What was the result?
  - Was the result expected?
- In sum, what do these data suggest or indicate?