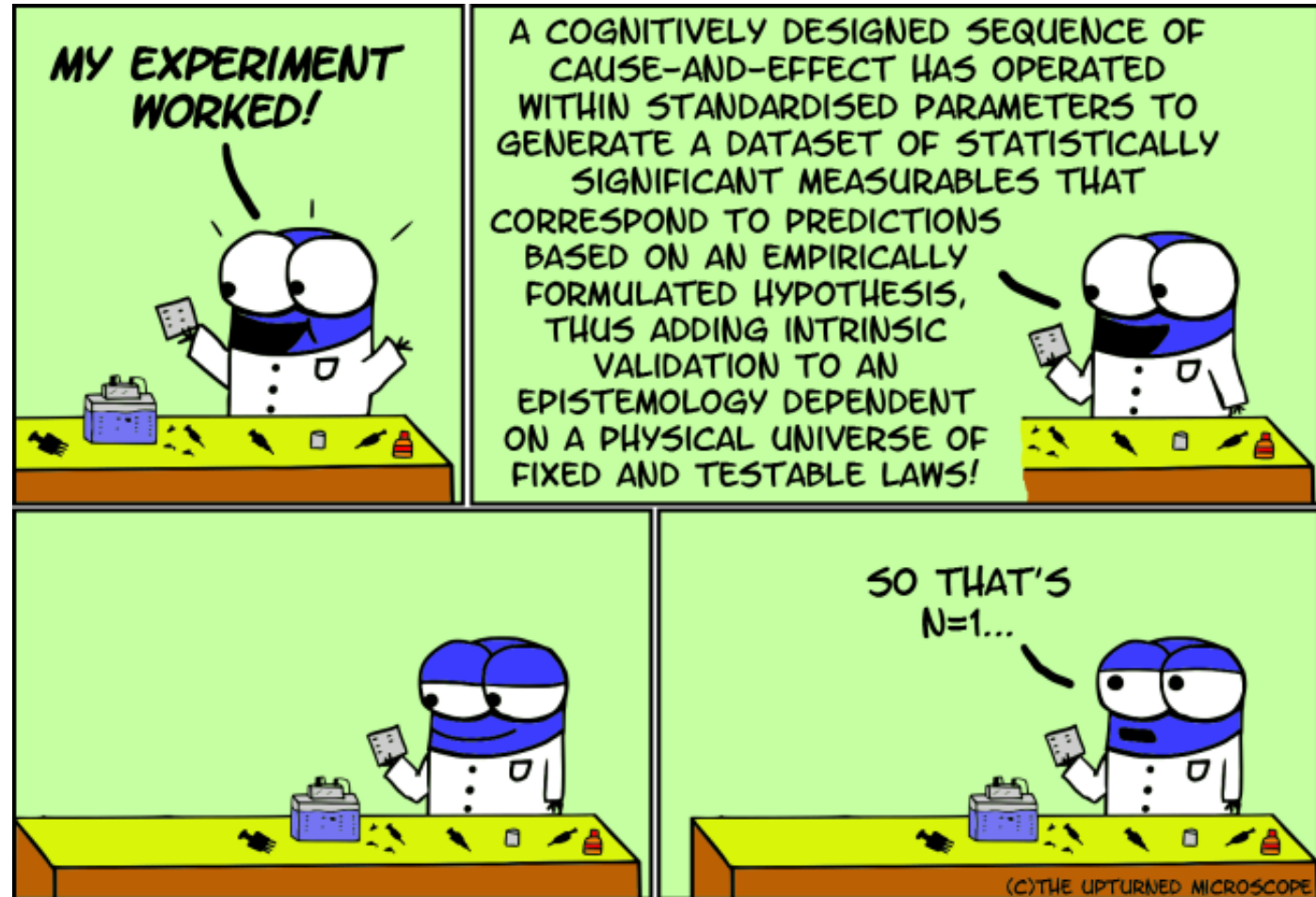


# Start Aggregation assay (Part 3)!

- **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

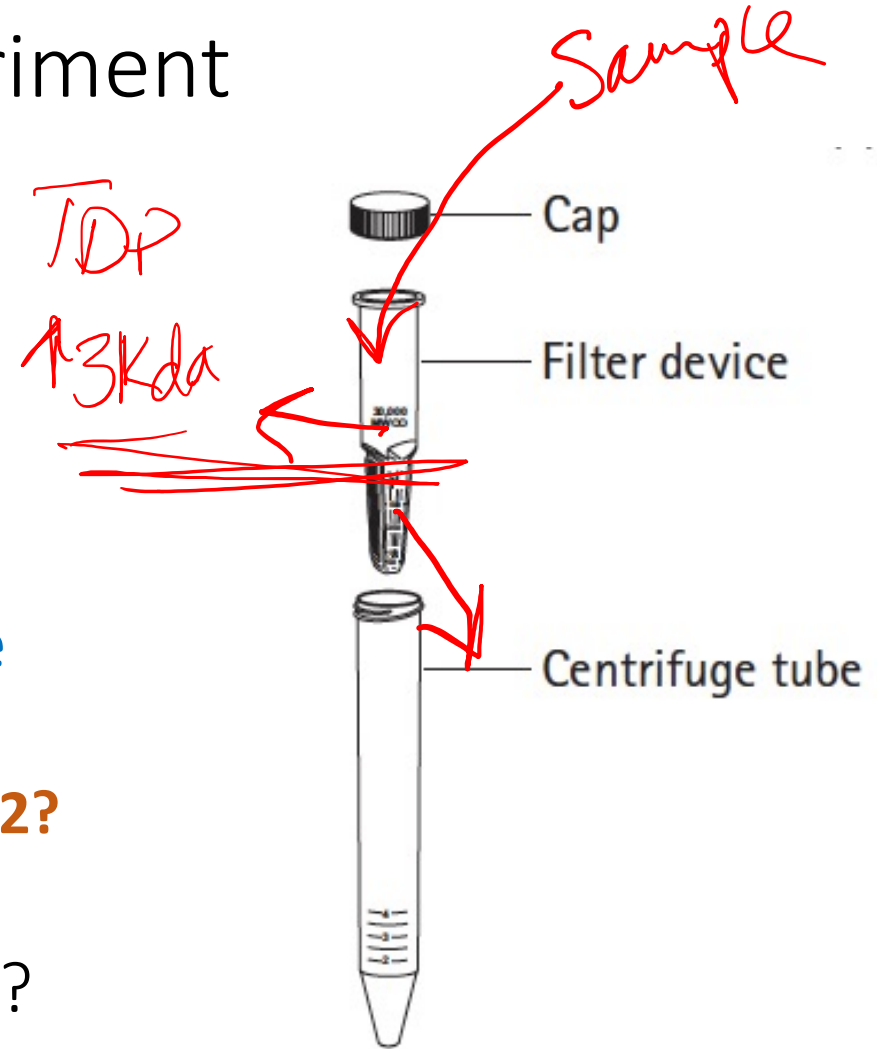
# M1D5: Perform aggregation assay using TDP43 protein and draft data slide for Data Summary

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



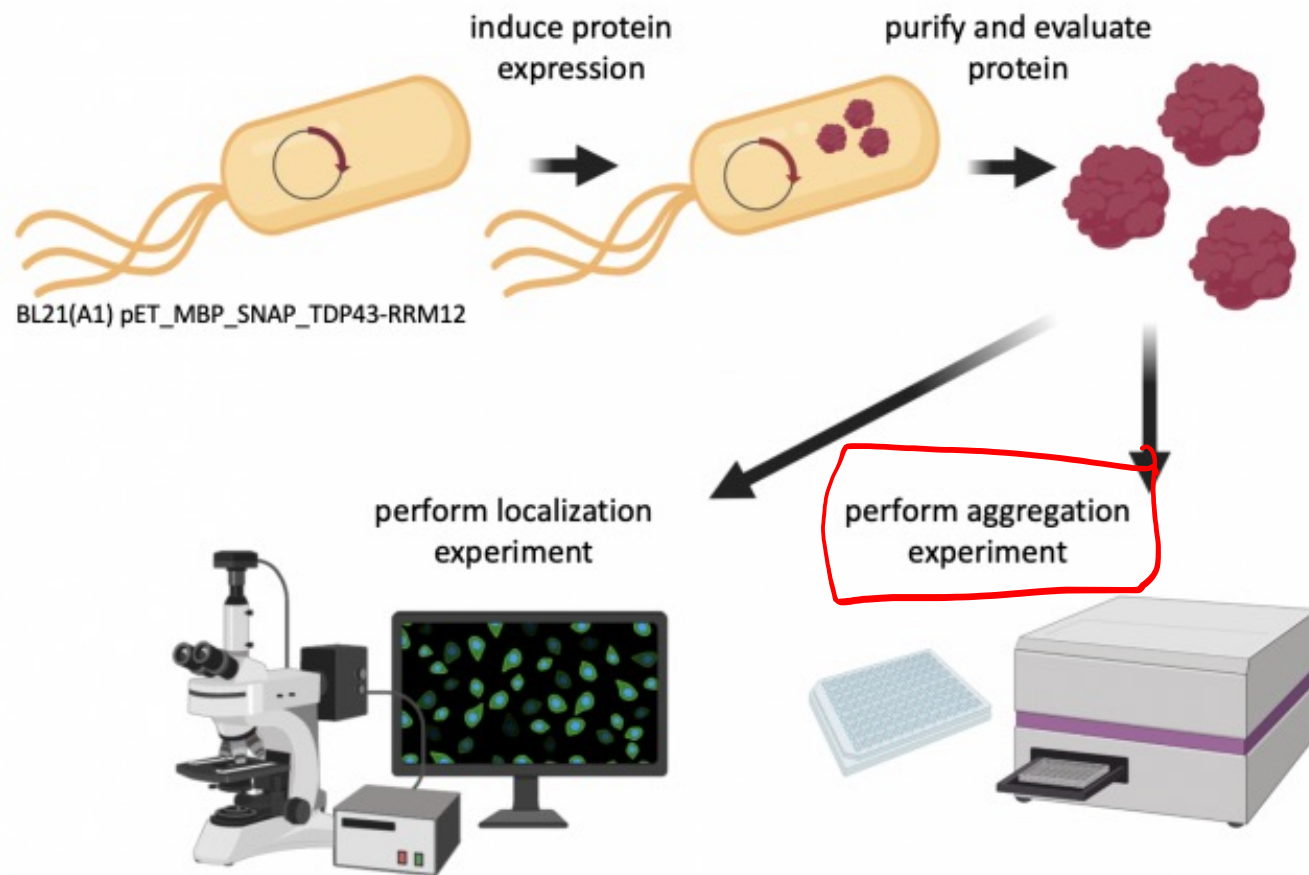
# Concentrate protein before experiment

- 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**

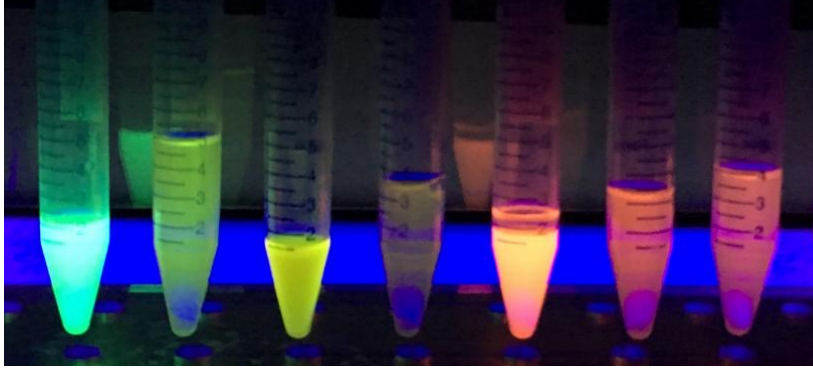


Types of protein aggregation:

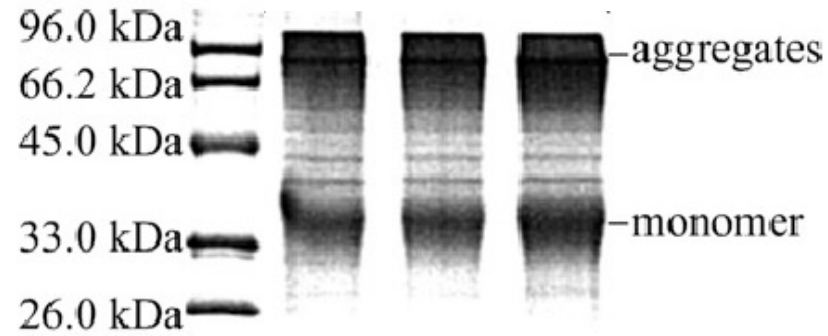
Amorphous aggregation  
Amyloid fibrils  
Oligomers

# Multiple read-outs for protein aggregation

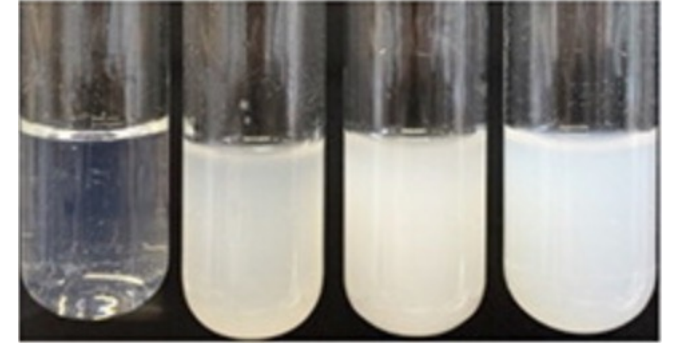
Fluorescent Dyes



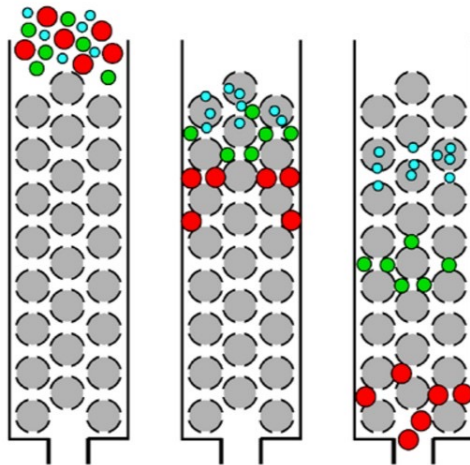
Protein gel



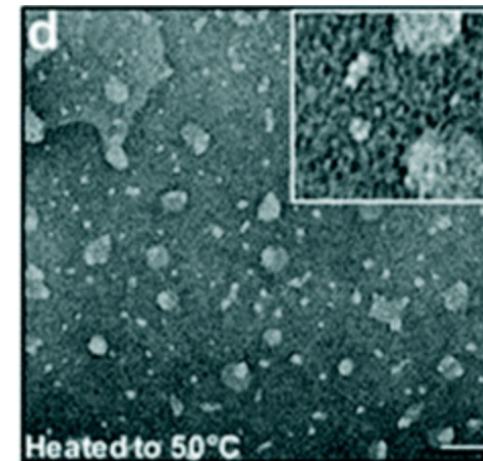
Turbidity



Size exclusion chromatography



Electron Microscopy





# Fluorescent aggregation dyes and antibodies

## Congo Red, Thioflavin T, etc...

→ Fluor. 485

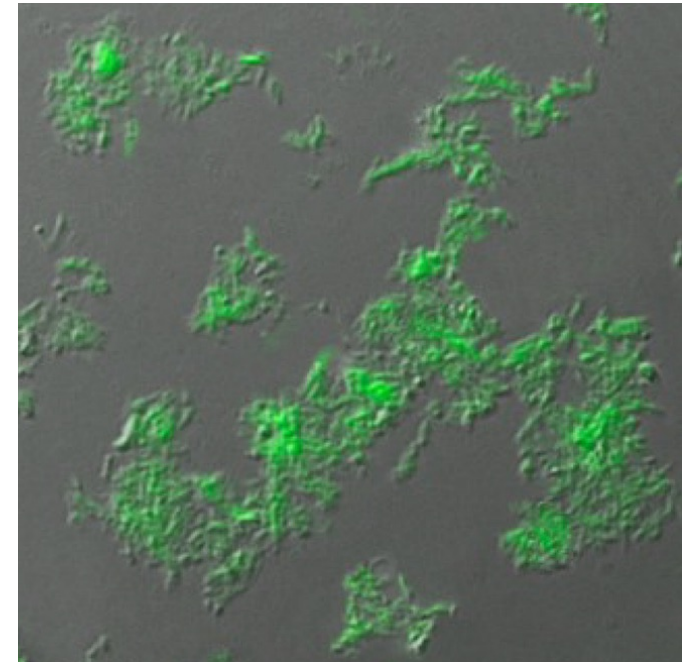
- Traditionally used to identify amyloid fibrils
  - Contain beta sheets
- • Literature on their use for TDP43 is mixed
  - Effective in some papers, but weak or no signal in others
- Can be used in microscopy or with a plate reader

## → Aggregation antibodies (fluorescent conjugated)

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

→ AT8 - hyperphos. Tau

Thioflavin T aggregates  
(visualized with microscopy)



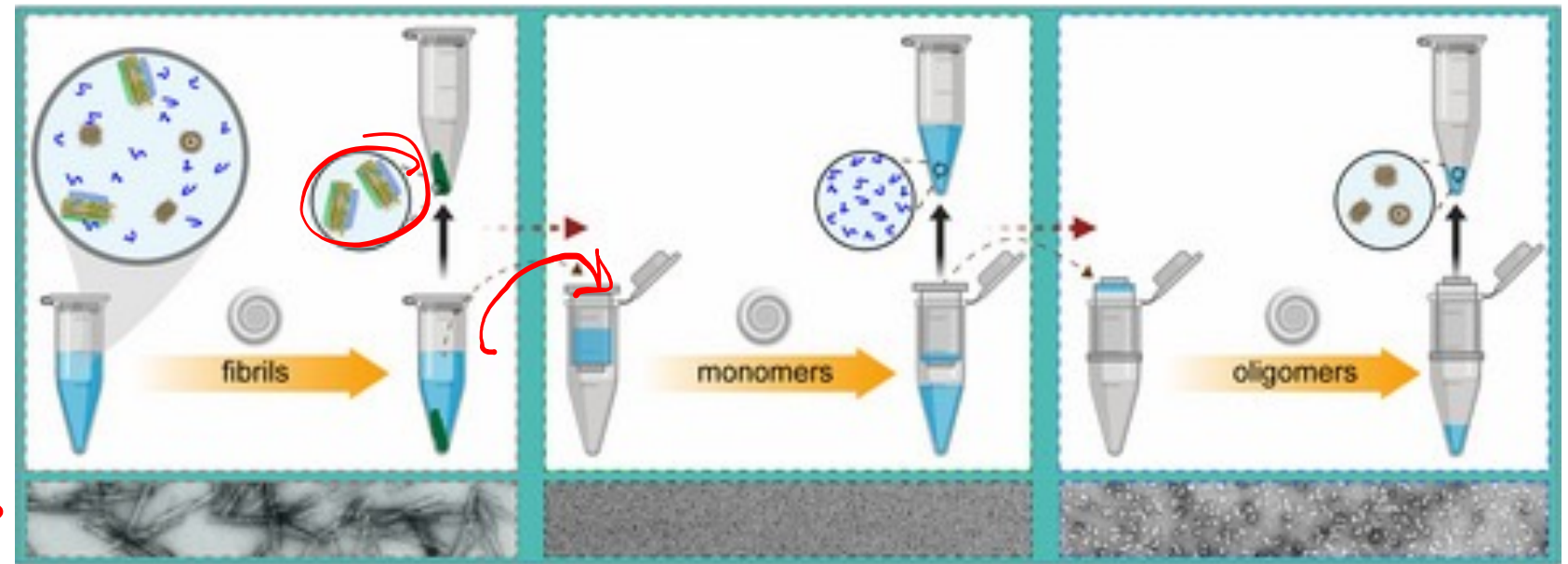
Sulatskaya et al, 2018

# Fractionation and protein gels to separate aggregates

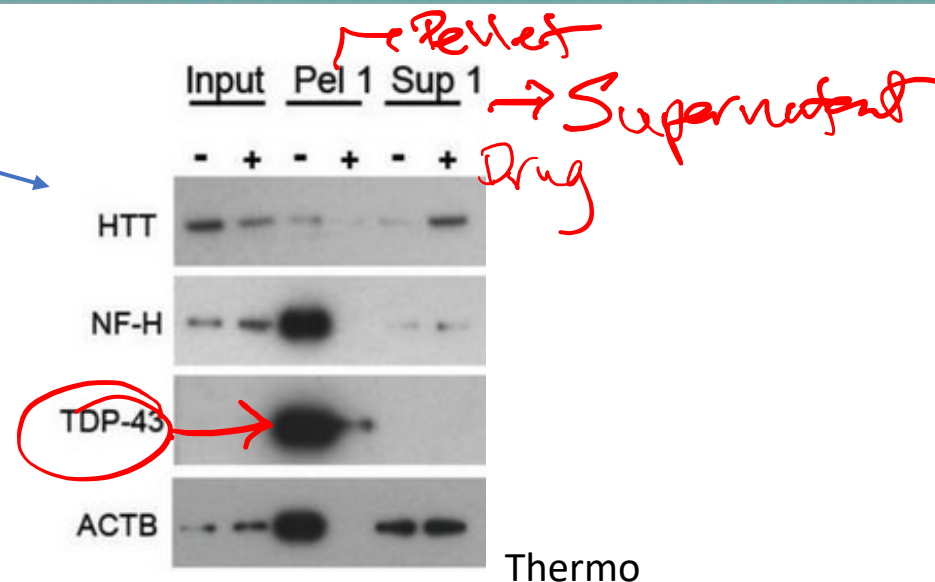
- Physically separate insoluble aggregates from soluble monomers

- Electrophorese these "fractions" on a protein gel

- Can use non-reducing protein gel to look at protein-protein interactions to identify oligomers



EM →

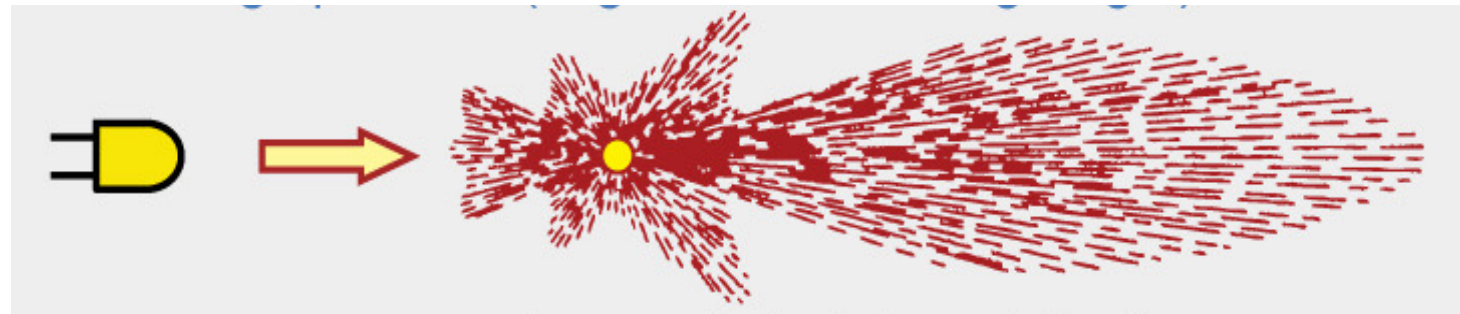


Kumar et al, 2020

# Turbidity as a measure of protein aggregation

405 nm

- 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





# Experimental groups

- Buffer A
- TDP43\_RRM12 + DMSO
- TDP43\_RRM12 + 3  $\mu$ M SM
- TDP43\_RRM12 + 30  $\mu$ M SM

*Blank*

*- (→) control  
- SM solvent*

*> ~~A~~ Angela Koehler  
approved*

# 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
  - only have to show the SDS-PAGE
  - Can show BCA graph if you want
- Submit a paragraph on your Comm lab visit
  - MUST report [ ] from BCA

# 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

1/3-  
1/2

A FIGURE: Be sure the image is large enough to clearly read, but only large enough to see! If sub-panels are used, label them as A, B, etc., but do not include titles. Include labels on the image if needed, but be sure they are clear and do not obstruct the data.

B

**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 your 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?

# Mini-presentation due Wednesday, Feb 23

- Prepare a video of you verbally discussing your research
  - Use any device or Zoom
  - No visuals / slides
  - Do not edit / splice the video
- **Submit to Gmail account!**
  - bioeng20.109@gmail.com
  - Remember to follow file name guidelines



# Presentation should be 3 min (+/- 15 sec)

- Introduce yourself
- Provide important background information
- Describe key results
  - Briefly describe critical methods used to generate important data
  - Use quantitative descriptions when discussing results
- Highlight the take-home message



# What data / results should be included?

- Protein purification
- Protein purity and concentration
- Aggregation assay results

# Review assignment description on wiki

Category	Elements of a strong presentation	Weight
Introduction	<ul style="list-style-type: none"><li>• Introduce yourself and the research</li><li>• Summarize the background information necessary to understand the research</li><li>• State the research question</li></ul>	25%
Methods & Data	<ul style="list-style-type: none"><li>• Provide ONLY the method information necessary to understand the results</li><li>• Give complete and concise explanations of the results</li><li>• Relate the results to the central question</li></ul>	25%
Summary & Conclusions	<ul style="list-style-type: none"><li>• Highlight the key finding(s) relevant to the central question / hypothesis</li></ul>	25%
Organization	<ul style="list-style-type: none"><li>• Give a logical, easy-to-follow narrative</li><li>• Include transition statements</li></ul>	15%
Delivery	<ul style="list-style-type: none"><li>• Show confidence / enthusiasm and speak clearly</li><li>• Use appropriate language (technical or informal, as appropriate)</li><li>• Be mindful of the time limit (3 minutes +/- 15 seconds!)</li></ul>	10%

The Research talk will be graded by Dr. Noreen Lyell with input from Dr. Becky Meyer and Jamie Zhan.