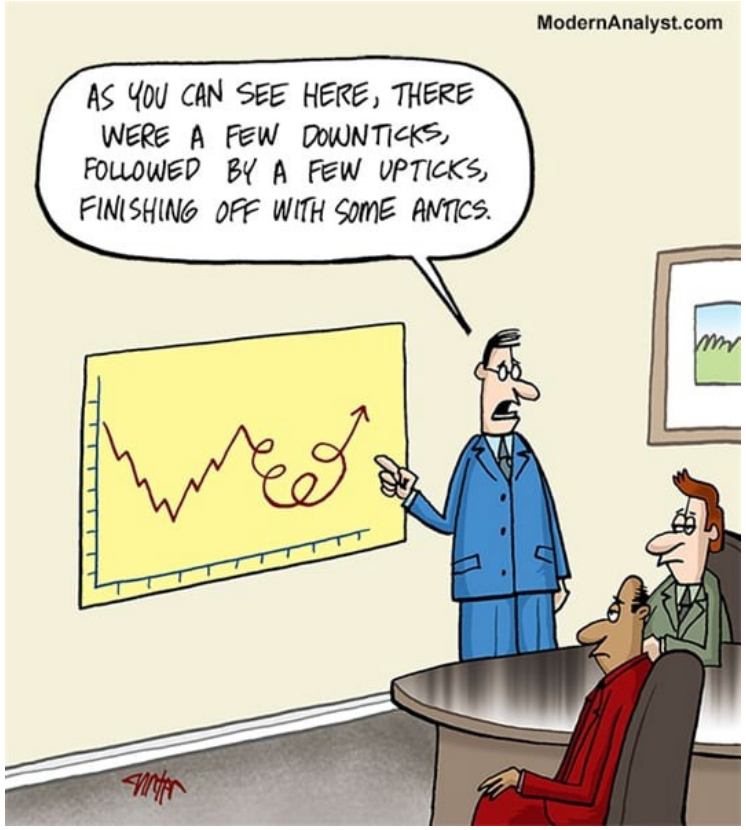
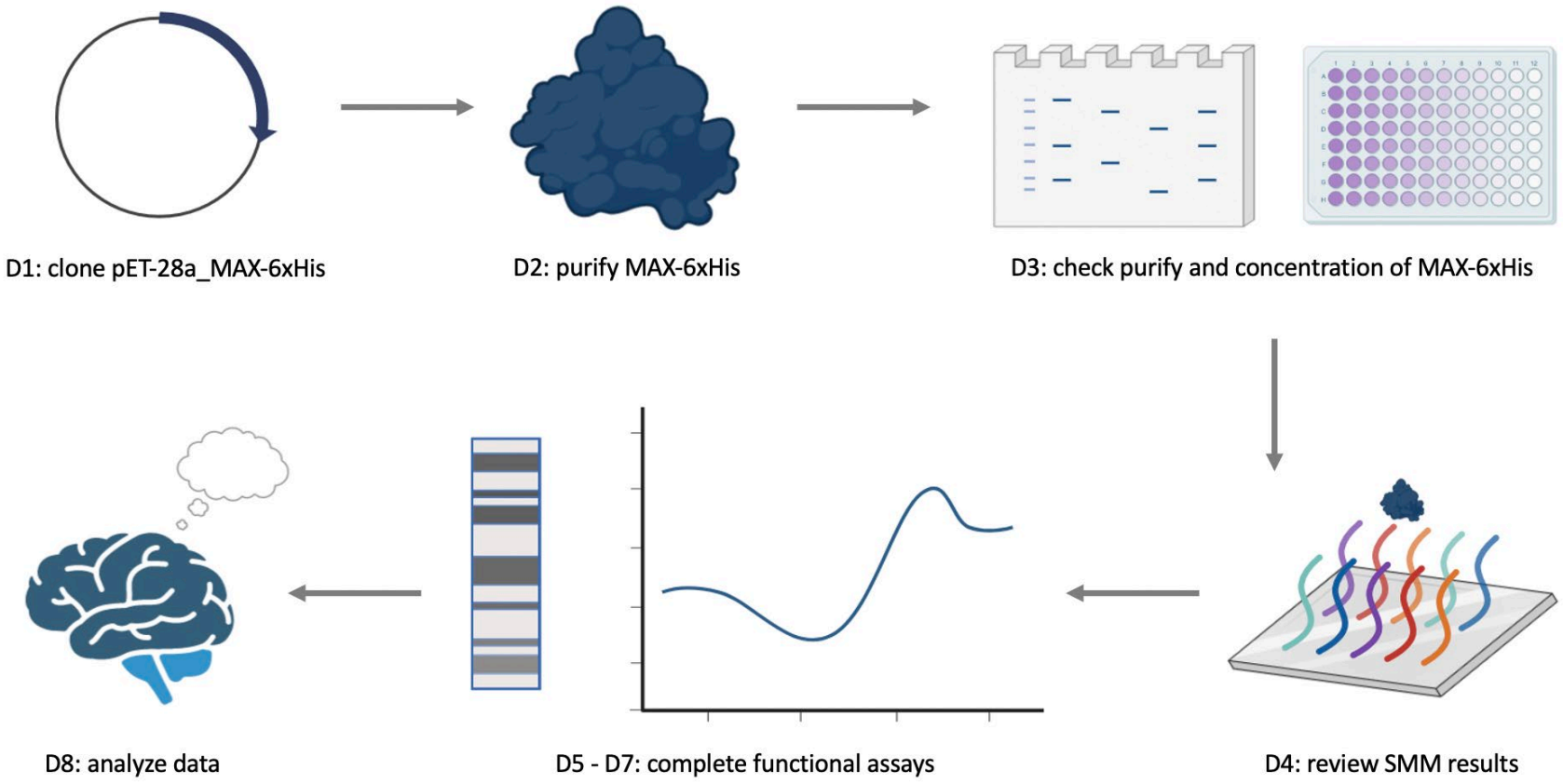
M1D6: Prepare cells for electromobility shift assay (EMSA)

1. Harvest nuclear proteins from treated cells
2. Plot data from DSF experiment



Overview of Mod 1 experiments:



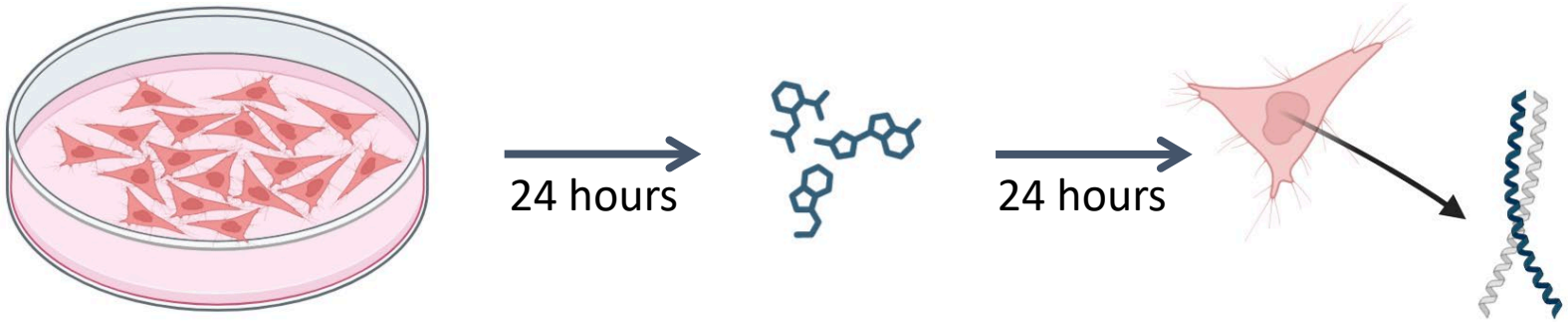
Workflow for secondary assays

	M1D5	M1D6	M1D7	M1D8
DSF	prepare samples and setup assay run DSF experiment	plot data to identify shifts in melting temperature		apply statistics to data interpret results
EMSA	seed cells	extract nuclear proteins	complete electrophoresis and transfer nuclear proteins onto membrane	image EMSA experiment to assess binding interpret results

What are we testing with each experiment?

Preparing cells for EMSA experiment

- Why were the HeLa cells seeded 24 hours prior to small molecule treatment?
- Why were the small molecules added 24 hours prior to nuclear extraction?



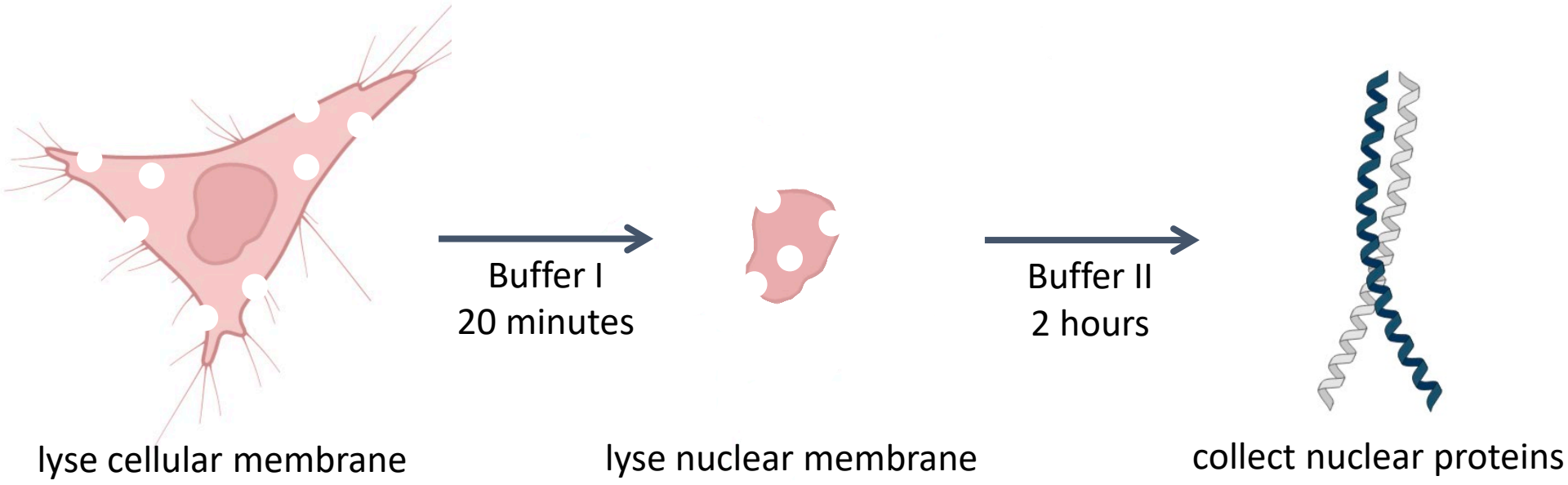
M1D5: seeded HeLa cells

added small molecule

M1D6: complete nuclear extraction

Nuclear extraction uses two-step lysis protocol

- Why do we need to perform a nuclear extraction for the EMSA experiment?

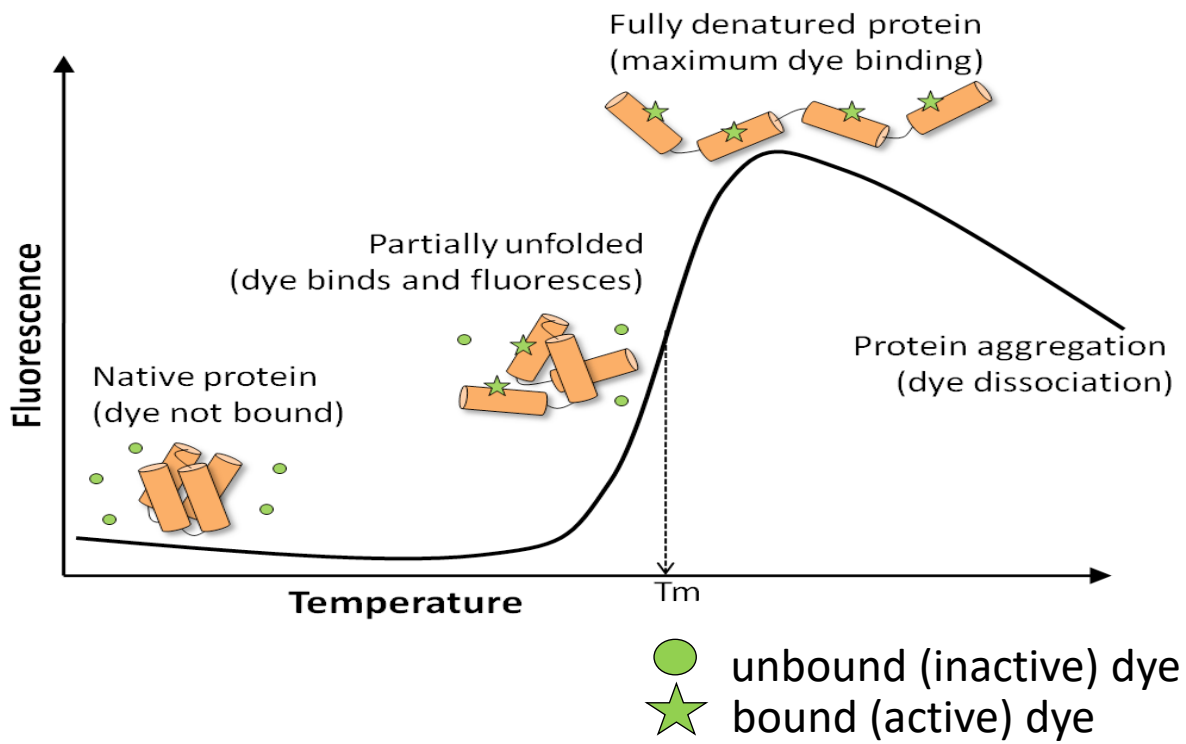


Data analysis for DSF experiment

1. Each group has two .xml format files
   - Melt Curve
   - T_m Calling

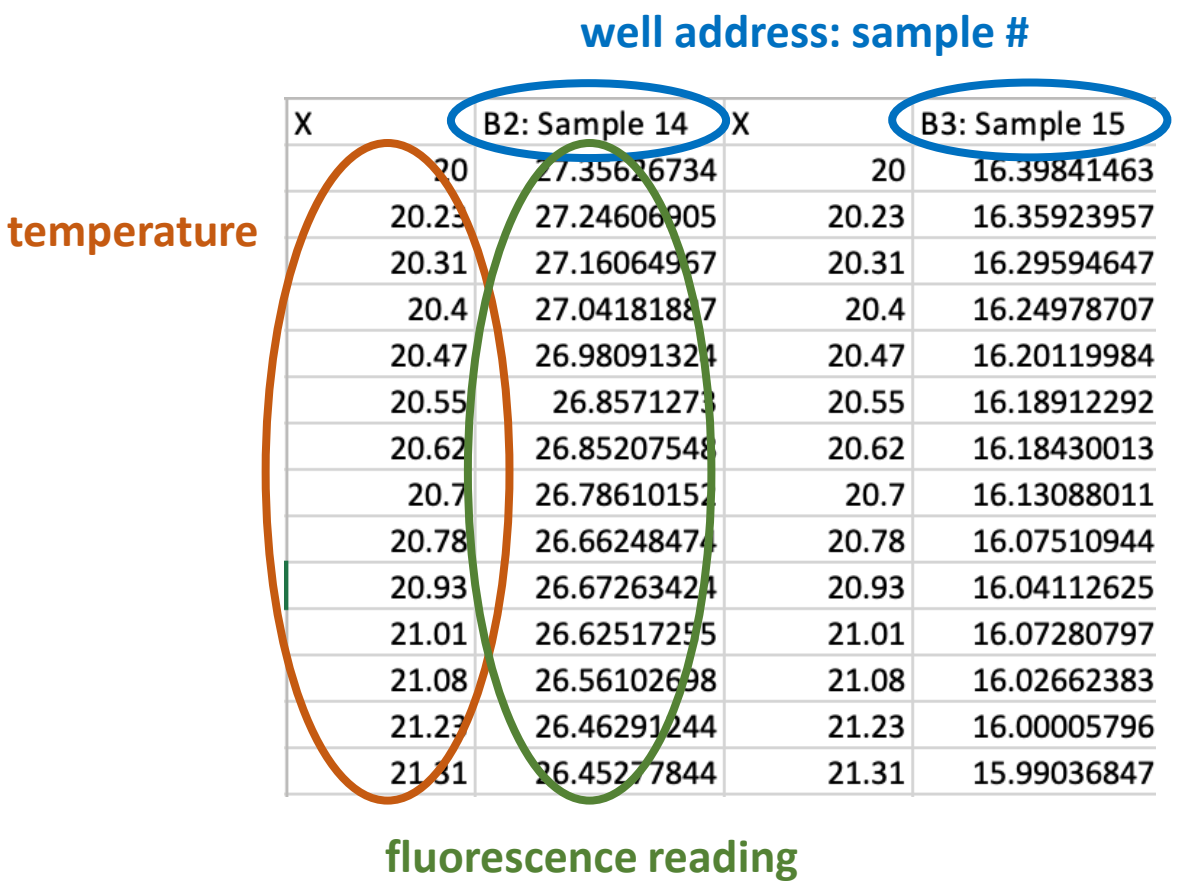
2. Open files with excel

3. Plot your data according to the wiki instructions!



What information is included in the .xml files?

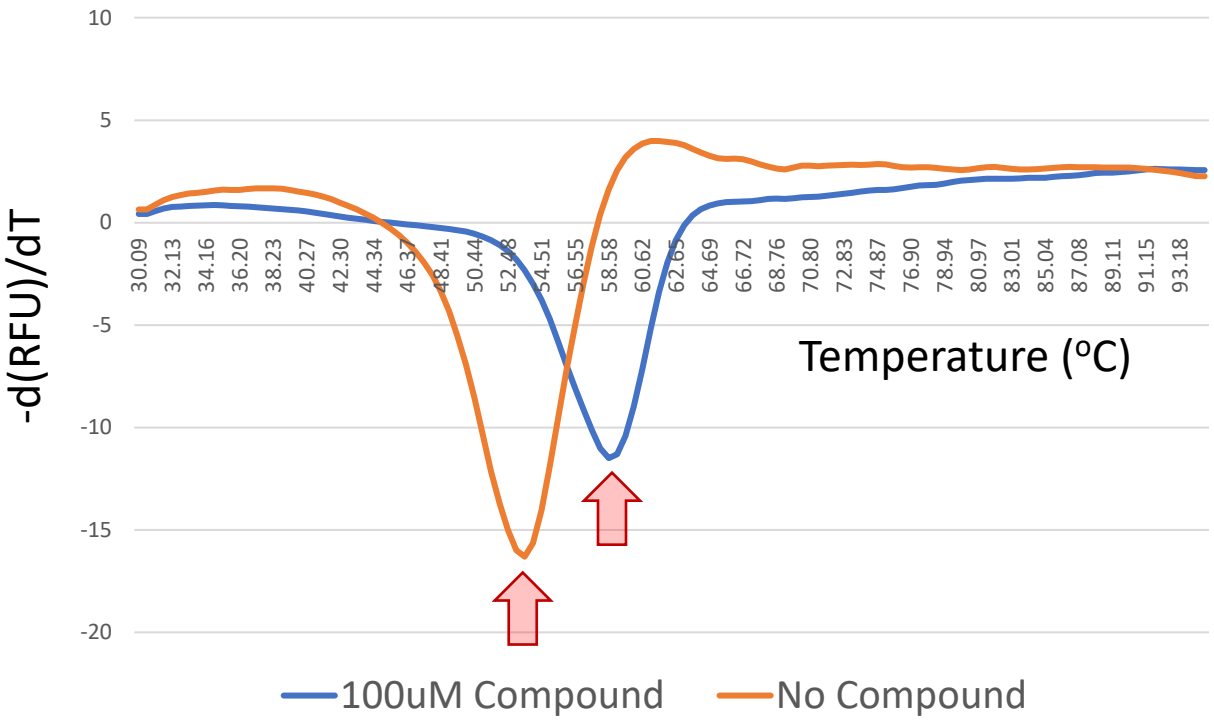
- Use plate map to determine which wells contain your samples
- Format spreadsheet such that temperature is in left most column
- Include sample data in subsequent columns
- Plot data with temperature on x-axis



X	B2: Sample 14	X	B3: Sample 15
20	27.35626734	20	16.39841463
20.23	27.24606905	20.23	16.35923957
20.31	27.16064967	20.31	16.29594647
20.4	27.04181887	20.4	16.24978707
20.47	26.98091324	20.47	16.20119984
20.55	26.8571273	20.55	16.18912292
20.62	26.85207548	20.62	16.18430013
20.7	26.78610152	20.7	16.13088011
20.78	26.66248474	20.78	16.07510944
20.93	26.67263424	20.93	16.04112625
21.01	26.62517255	21.01	16.07280797
21.08	26.56102698	21.08	16.02662383
21.23	26.46291244	21.23	16.00005796
21.31	26.45277844	21.31	15.99036847

Negative first derivative of fluorescence/time used to determine T_m

- Record temperature at the inverse peak
  - These are your T_m values!
- ΔT_m = T_m of (+) small molecule – T_m of (-) small molecule
- Report T_m values for your group on the Class Data page of the Wiki



RFU= relative fluorescence units

For today…

- Use incubation time to complete DSF data analysis
- Extra time can be used to work on your Research talk ☺ (Due Mon, 10 PM)

For M1D7…

- Draft page using SDS-PAGE and BCA results for Data summary
  - Use homework feedback to edit data figures, title, caption
  - Add text for results / interpretations
- Read paper for in-class discussion during next laboratory session

What to include for Results & Interpretations

- State the goal / intent / purpose of experiment in the first bullet
- What you did:
  - What are the experimental conditions?
  - What are the controls?
  - What are the expectations for the controls / conditions that were tested?
- What you found: quantitatively describe your result, referring to the figure (”Figure 1a shows a % increase of…”
- What does this indicate: interpret your results, what does it mean?
- What does this motivate you to do next: transition to next experiment

Figure	Result	Interpretation
	“A band of ~50 kDA was purified”	POI was purified

Template:

Image **should not** be the entire page

- Only needs to be large enough to be clear / visible

Title **should** be conclusive

- Don't state what you did, rather state what you found (take home message)

Caption **should not** detail the methods or interpret the data

- Define abbreviations, symbols, etc.
- Include details needed to "read" figure

Bullet points **should** present and interpret the data

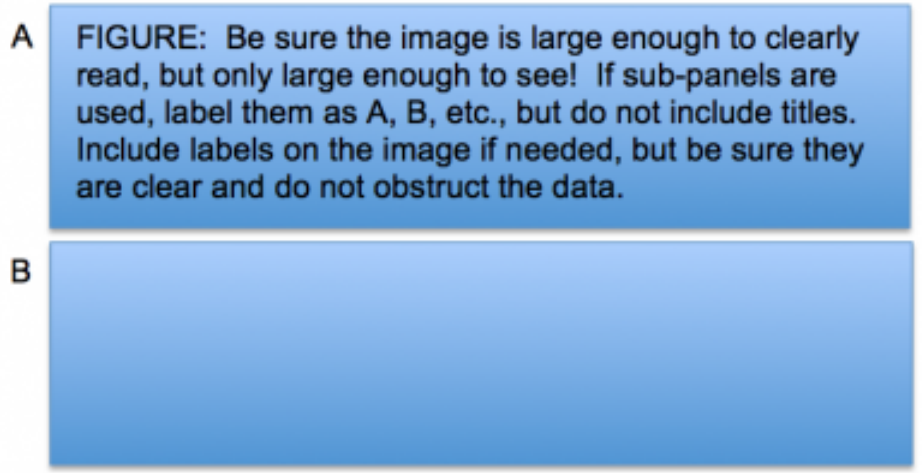


FIGURE TITLE: This should state the conclusion of the figure in very brief and precise language. CAPTION: Start with a topic sentence that introduces the figure or sub-panel. Provide all of the information that the reader needs to interpret the figure (define abbreviations, explain labeling scheme, differentiate 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?