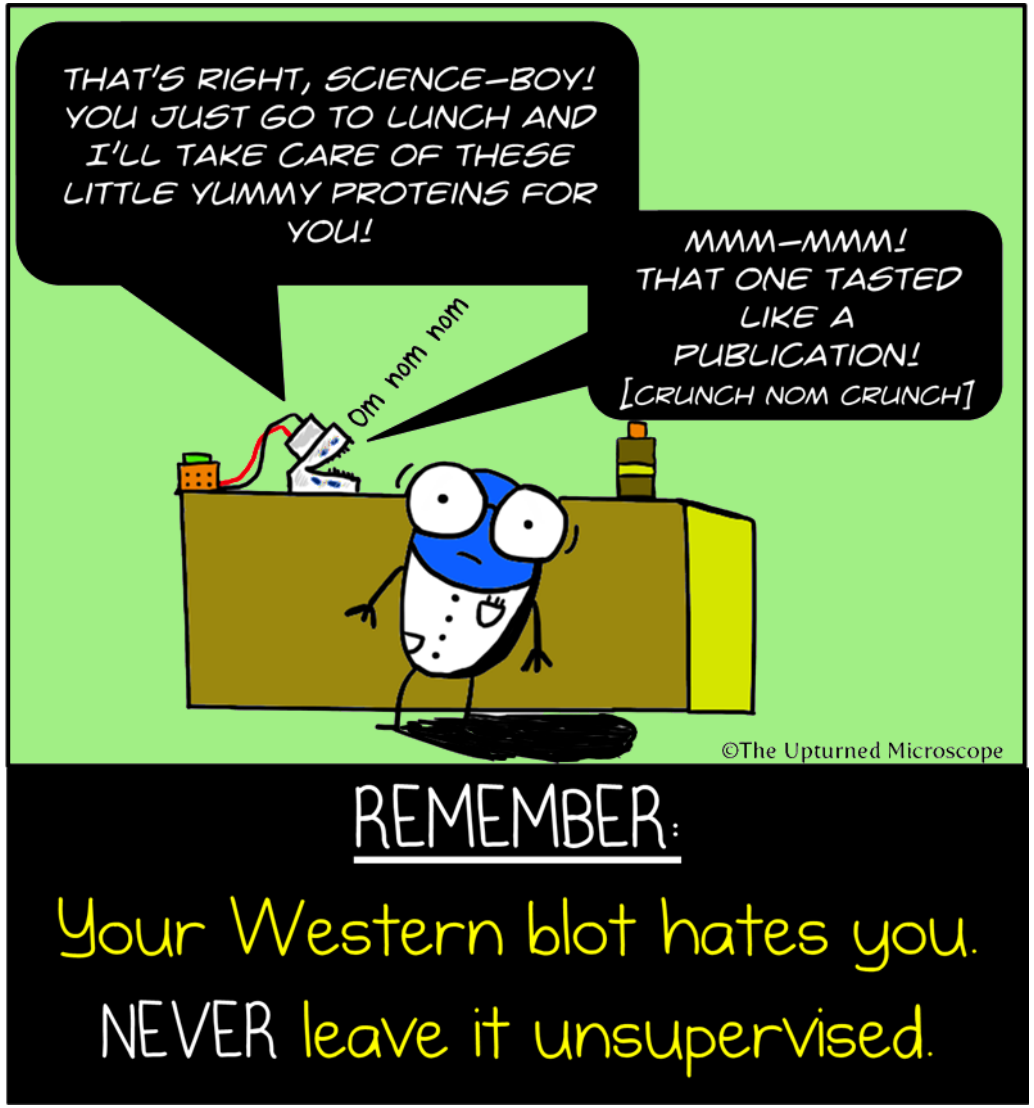
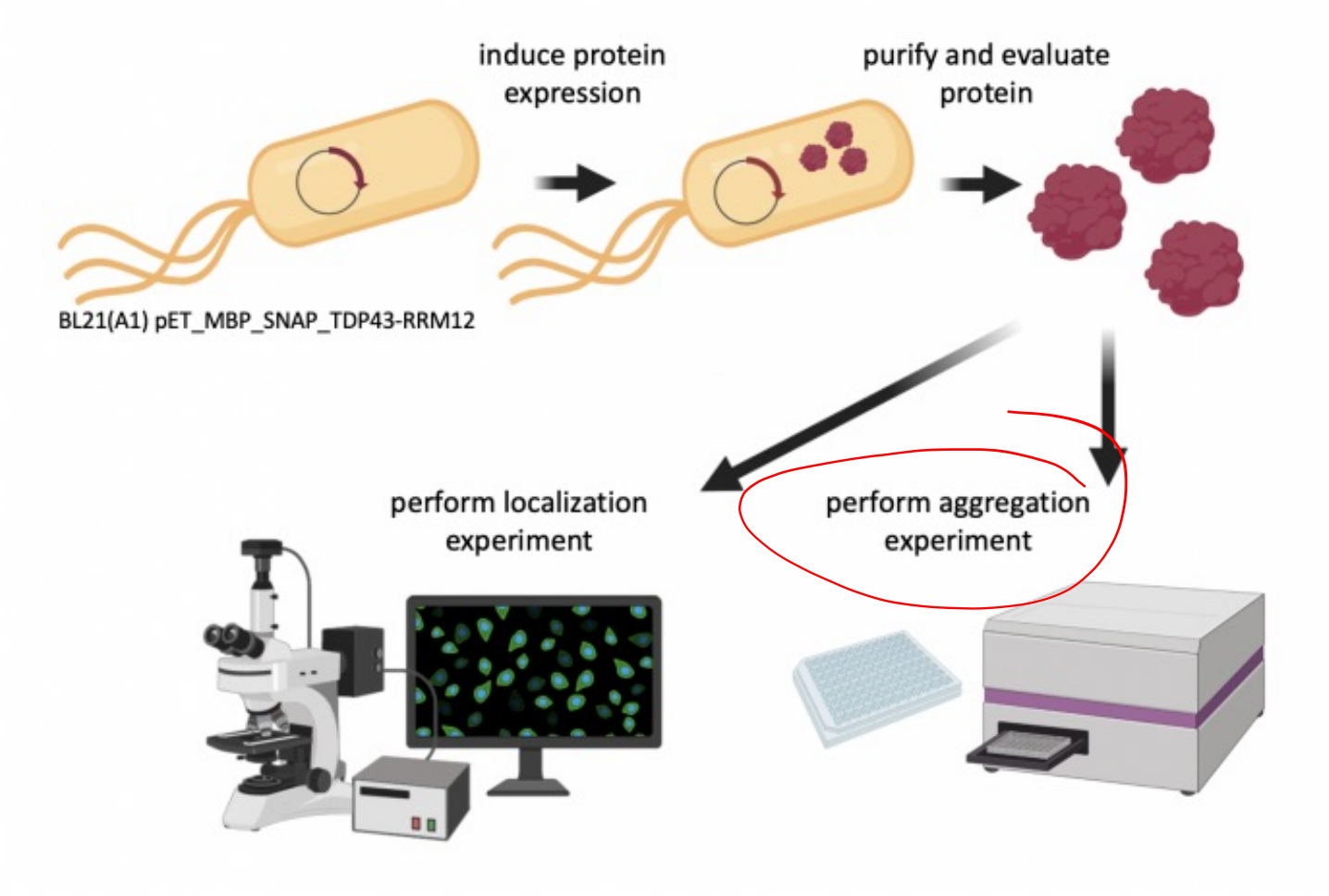
M1D4: Assess purity and concentration of purified protein

1. Comm Lab
2. Quiz
3. Prelab discussion
4. Visualize protein purity with SDS-PAGE
5. Measure protein concentration with BCA assay



Overview of Mod 1 experiments

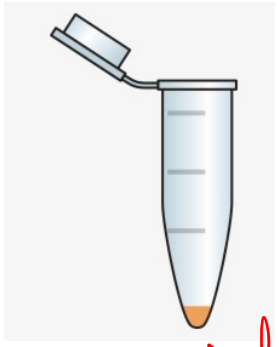
Research goal: Use functional assays to characterize ligands identified as binders to TDP43 from SMM technology



Protein purification review

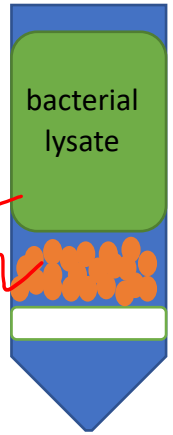
- Why this step? → for you later

Pellet



bacterial proteins

Lysate



TDP 43

Flowthrough

TDP 43

other proteins (Dorings)

Wash

TDP 43

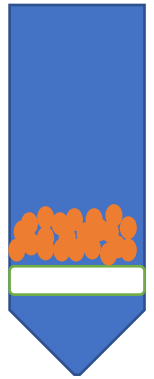
Non specific proteins

Elution

Imidazole

TDP43!

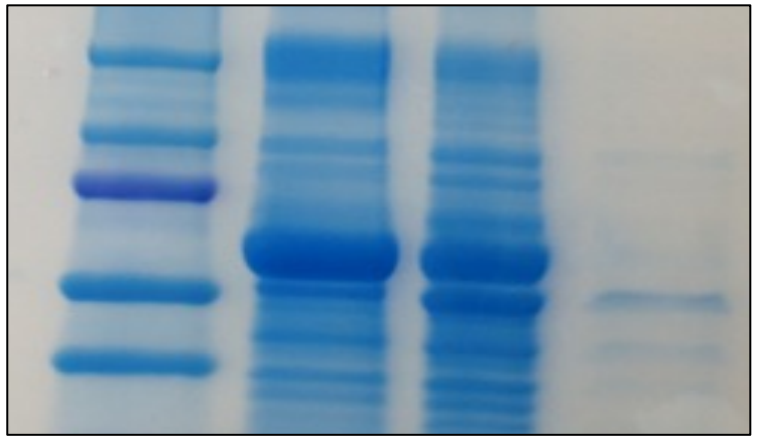
Slurry

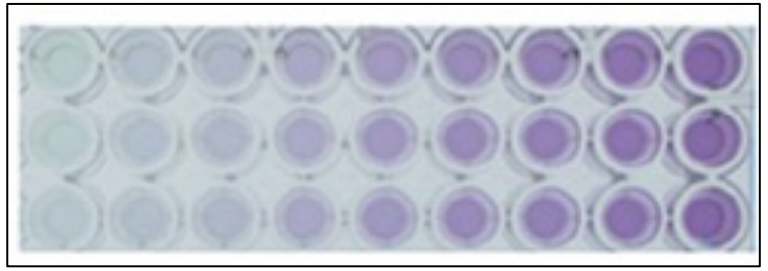


- What's on the resin?
- What's in the expelled liquid?

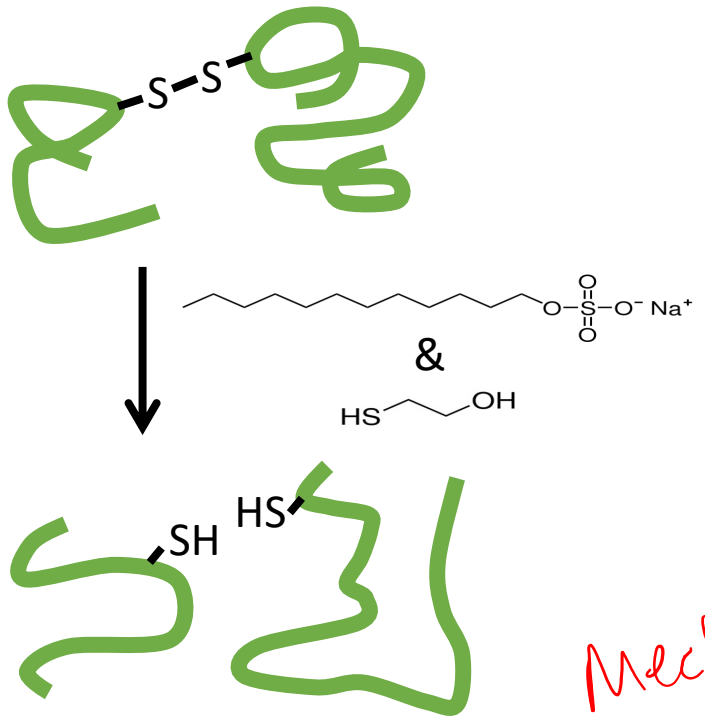
How will you assess purity and concentration?

- Check purity using SDS-PAGE
  - Visual detection of all proteins in sample
  - Identifies purity of sample at multiple stages of purification
- Measure concentration using BCA assay
  - Colorimetric assay
  - Calculate concentration from standard curve





Purity: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)



Chemical processing

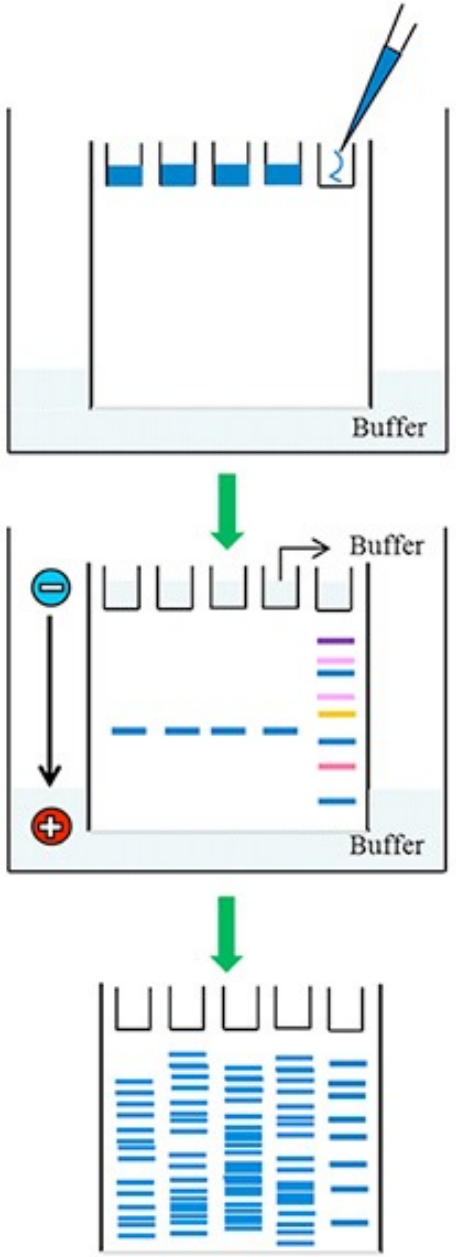
- Laemmli sample buffer / loading dye:
  - SDS — detergent (surfactant) - denature, (–) charge
  - β-mercaptoethanol (BME) - breaks disulfide bonds
  - bromophenol blue - tracking proteins
  - glycerol — weight sample in well
- Boiling: - denature

Mechanical

How are proteins separated?

- Laemmli buffer and boiling results in denature _____ and (−) _____ charged proteins
- SDS-PAGE separates proteins by size _____
- Electrophoresis completed in TGS buffer – Running buffer
  - Tris-HCl
  - SDS
  - Glycine

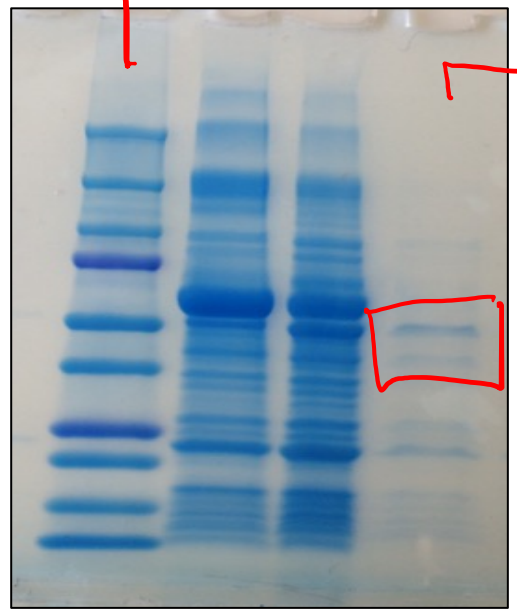
Buffers: pH control, ions

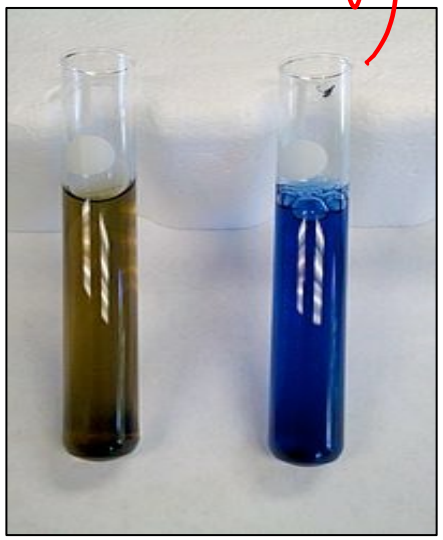


How are proteins visualized?

Coomassie brilliant blue G-250 dye used to stain gel after electrophoresis

- Red if unbound (cationic form)
- Blue if bound to protein (anionic form)
- Hydrophobic and electrostatic interactions with basic residues
  - Arg (also His, Lys, Phe, Trp)





Be mindful when assessing SDS-PAGE protein samples

Consider the order of your samples:

1. molecular weight ladder
2. pellet
3. lysate
4. flow-through
5. wash
6. elution
7. resin

- Figure will be included in your Data Summary!

→ Elution

Concentration: Bicinchoninic acid (BCA) protein assay

Step 1: Chelation of copper with protein, reduction of copper sulfate to copper ion

Peptide bonds

+ Cu^{2+} —(OH^-, Temperature-dependent)→ Cu^{1+}

Step 2: BCA complexes with cuprous cation

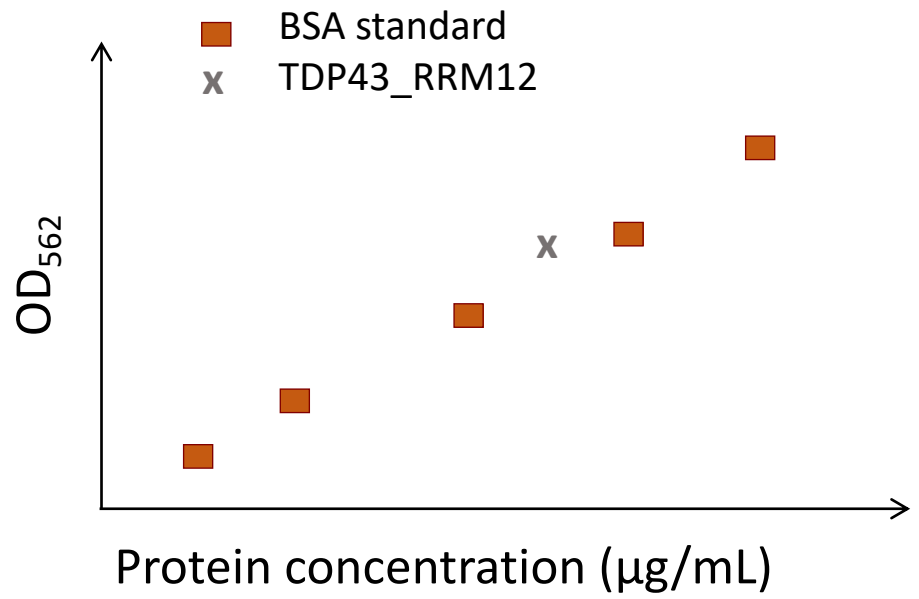
Cu^{1+} + 2 BCA → $BCA\text{-}Cu^{1+}$ complex (λ = 562 nm, violet)

measure absorbance

BCA/Cu^{1+} absorbance proportional to protein concentration

Standard curve generated using serial dilutions of bovine serum albumin (BSA)

- Use fresh tips between tubes
- Mix well between dilutions
- Be mindful of volumes



For today…

- Complete the purity and concentration assessments
  - It's good to divide the work load here!
  - Start by deciding who will be in charge of the two different techniques today

For M1D5…

- Create a data figure of your purification results
  - must include SDS-PAGE gel
- Outline your Research Talk
  - See Assignments page for details and Homework page for checklists
  - Focus is aggregation experiment, just put a placeholder for actual results

Data figure example

- Image should not be the entire page
  - Only needs to be large enough to be clear / visible
- Title should be conclusive
  - Don't include 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.
  - Info needed to "read" figure

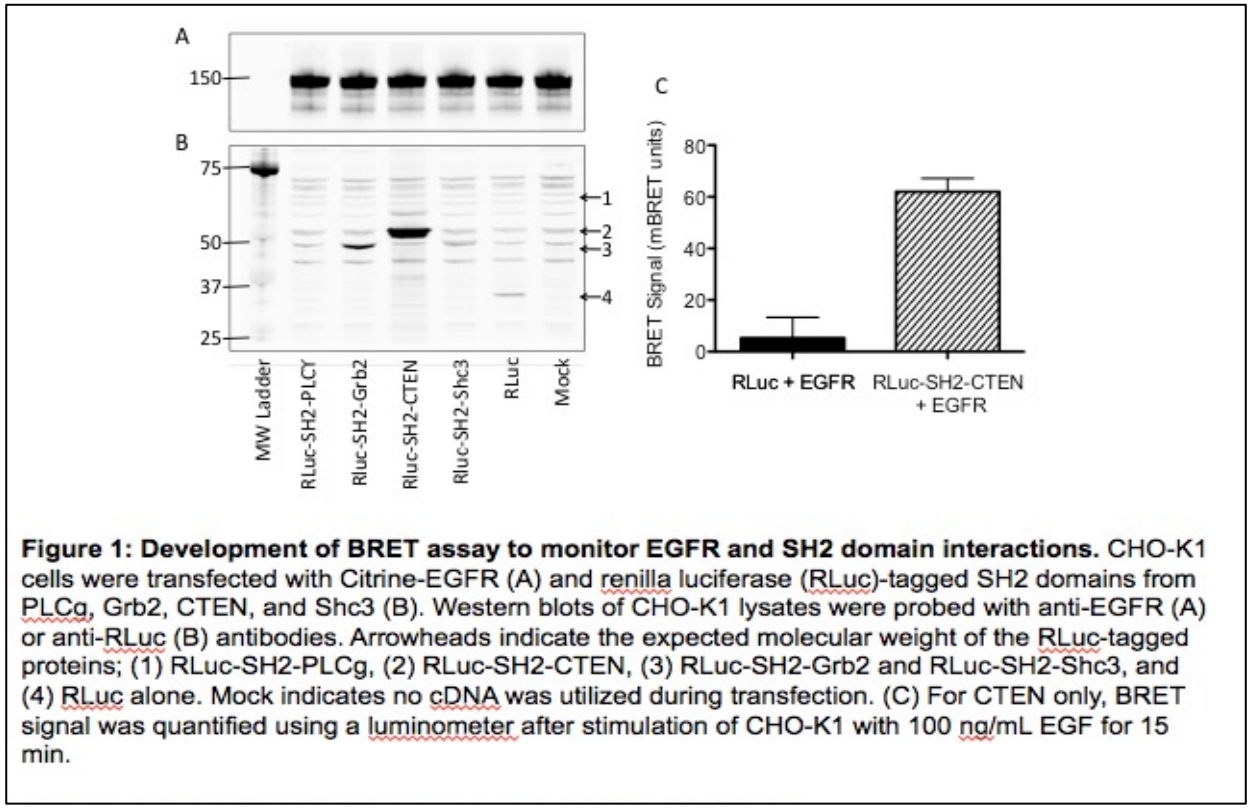


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies. Arrowheads indicate the expected molecular weight of the RLuc-tagged proteins; (1) RLuc-SH2-PLCg, (2) RLuc-SH2-CTEN, (3) RLuc-SH2-Grb2 and RLuc-SH2-Shc3, and (4) RLuc alone. Mock indicates no cDNA was utilized during transfection. (C) For CTEN only, BRET signal was quantified using a luminometer after stimulation of CHO-K1 with 100 ng/mL EGF for 15 min.

Notes on the Research Talk

- Individual assignment
- Three (3) minute video of you talking directly into the camera
- No visual aids allowed
  - Introduce yourself and your project
  - Highlight key results with quantitative information
  - Place your work in the scope of the larger field
- No need to state you are doing a class project or anything about 20.109

- Homework = outline
  - Introduction of your project
  - Key results from your research (including a statement as to the method(s) used to generate data)
  - Take-home message