M2D5: Purify Protein

4/5/13

1. Reflections on your ~~journal club~~ Mod 1 Report re-writes -- send them to me today or sometime this weekend.

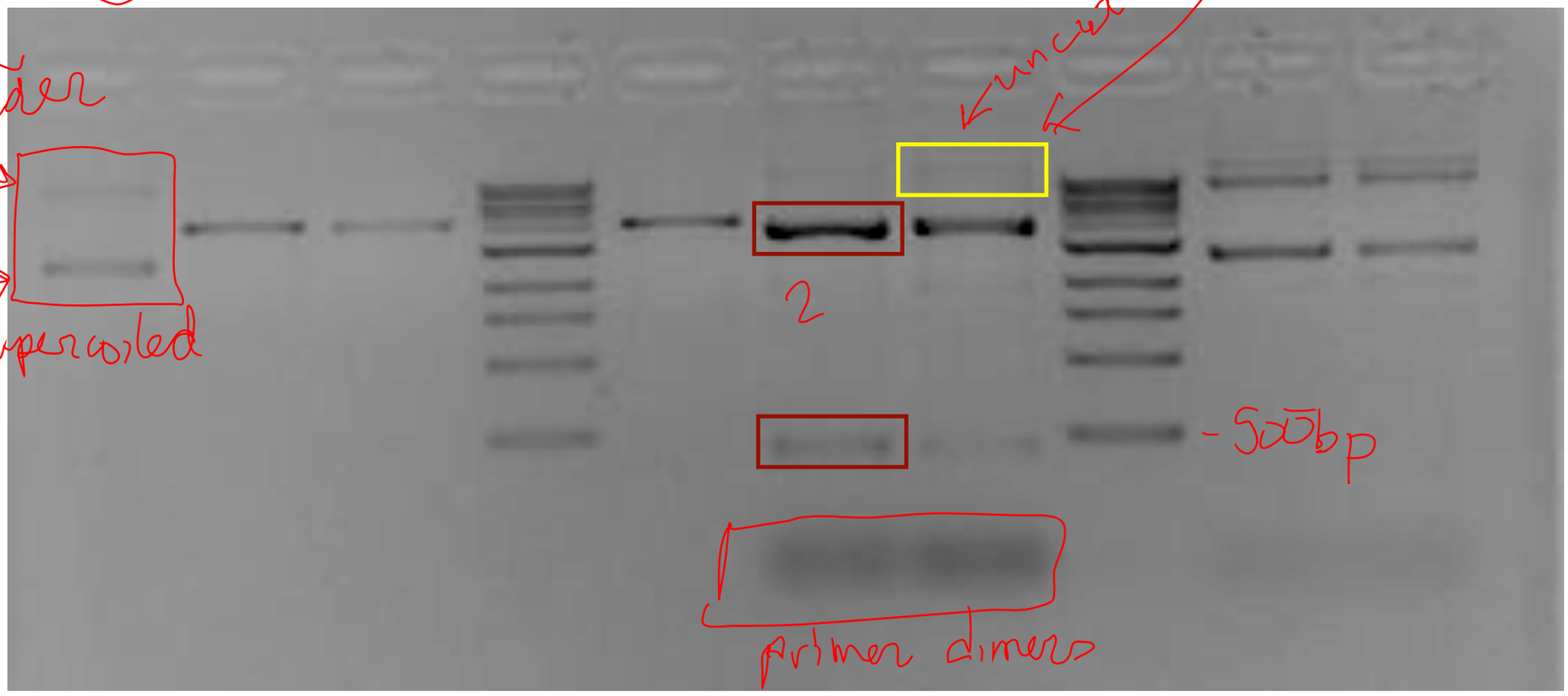
2. Lab treat.

3. More happy things:

Diagnostic Gels: How did you do?

- religation v. low
- inefficient rxn

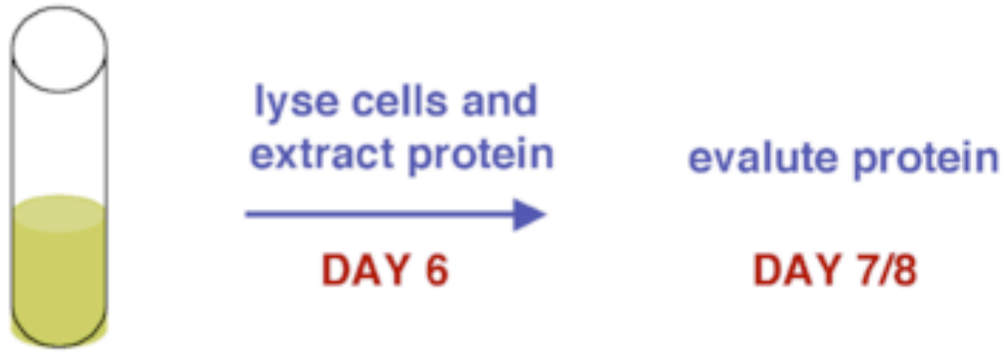
IPC WT | IPC Kpn I | D24H Kpn I | 1 Kb Ladder | IPC Sty I | G25S #1 Sty I | G25S #2 Sty I | 1 Kb Ladder | G25S #1 | G25S #2



1. Extra bands?
2. Unaccounted for bands? contamination ★activity random mutation

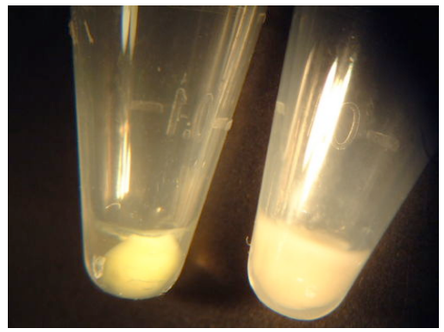
Pink Team!

Protein purification: Protocol overview



Protein purification: Protocol overview

*brown
*checked on microscope



lysis

evalute protein

DAY 7/8

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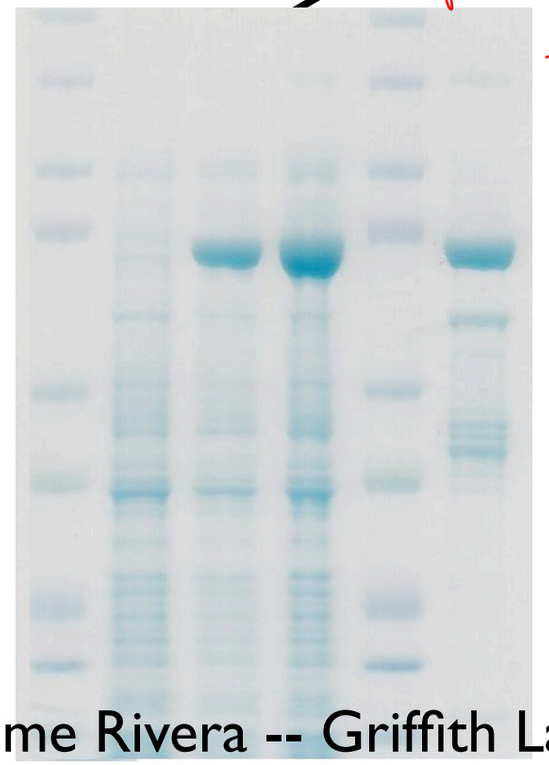


This is the most important step!

de-salt

Jaime Rivera -- Griffith Lab

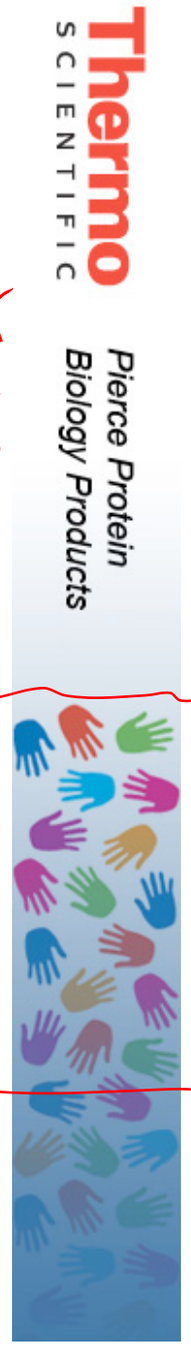
affinity purify

Protein purification: Get it out of there! ← lyse

Lysis Buffer: BPER from Pierce

+ Lysozyme → blow apart bacteria

+ DNASE I → chew up DNA

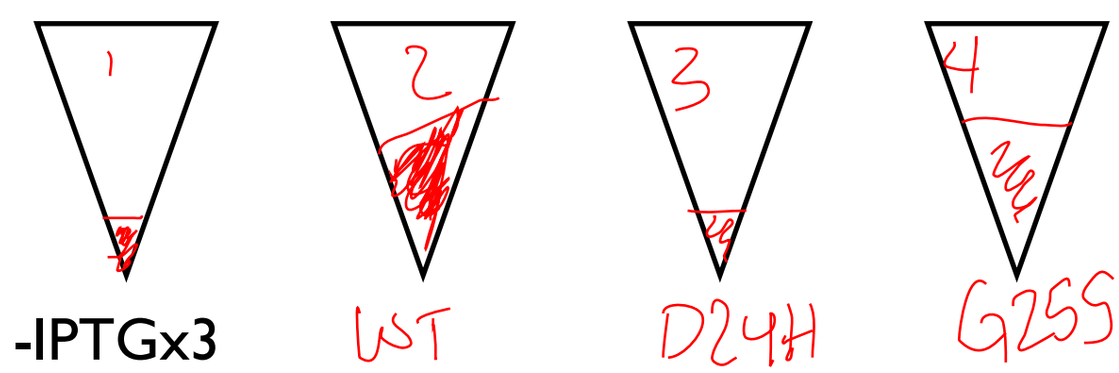


Prepare samples for SDS-PAGE analysis

OD600

Normalize samples by cell number. Why? - compare across samples

OD600 = 0.5 1 0.75 1



-IPTGx3 WT D24H G25S

$V_t = 15\mu L$

$V_{S1} = 15\mu L$ lysate

$V_{S2-S4} = 7.5\mu L$ lysate + $7.5\mu L$ H_2O

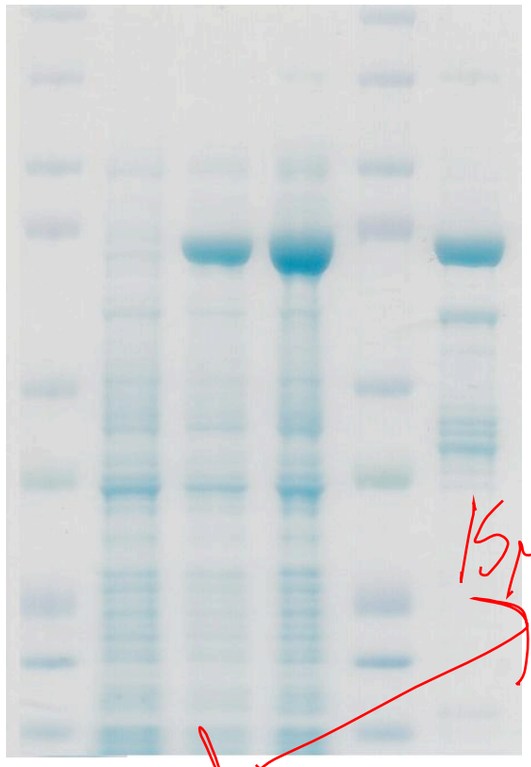
Get your cell counts off the M2D5 talk page (pick your clone! 3x)

Thanks Agi!!

$V_{S3} = 10\mu L$ lysate + $5\mu L$ H_2O

Protein purification: SDS-PAGE Analysis

size
charge



Hood

Some details:

1. Polyacrylamide is Toxic – Neurotoxin

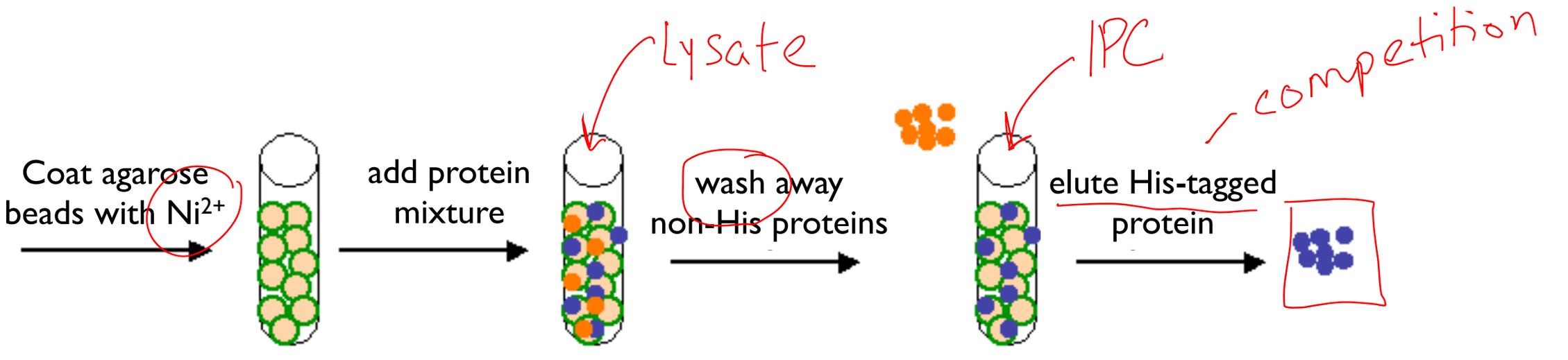
2. What is in the loading buffer? Why?
   - SDS: Neg charge coating
   - βME: breaks S-S bonds (denature)
   - Glycerol: hold sample in well
   - Dye: visualize migration

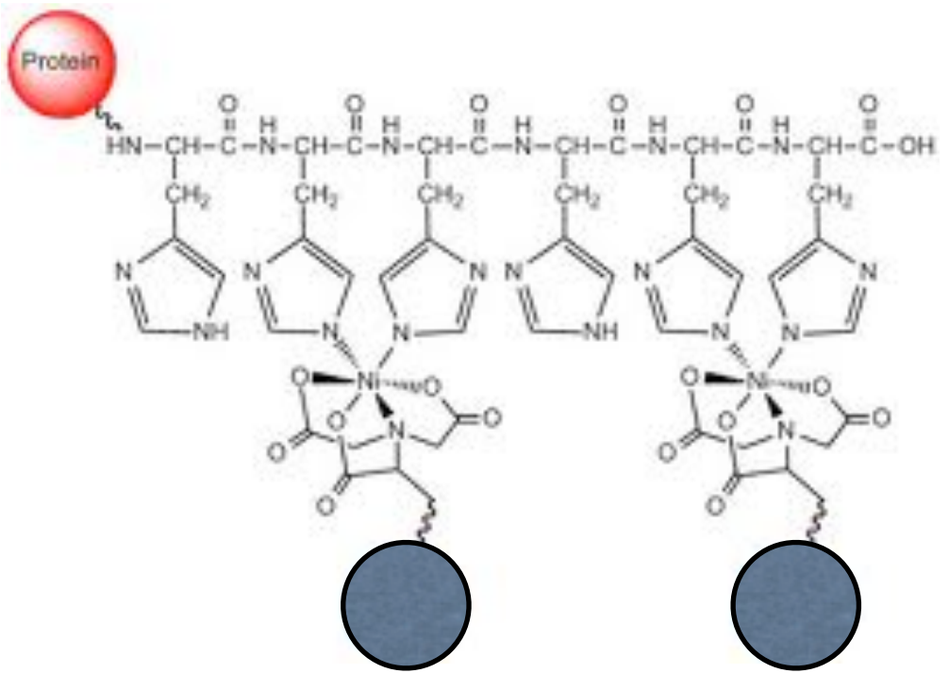
15μL

Last step:

Next time → Boil it!

Protein purification: Affinity purification + desalting



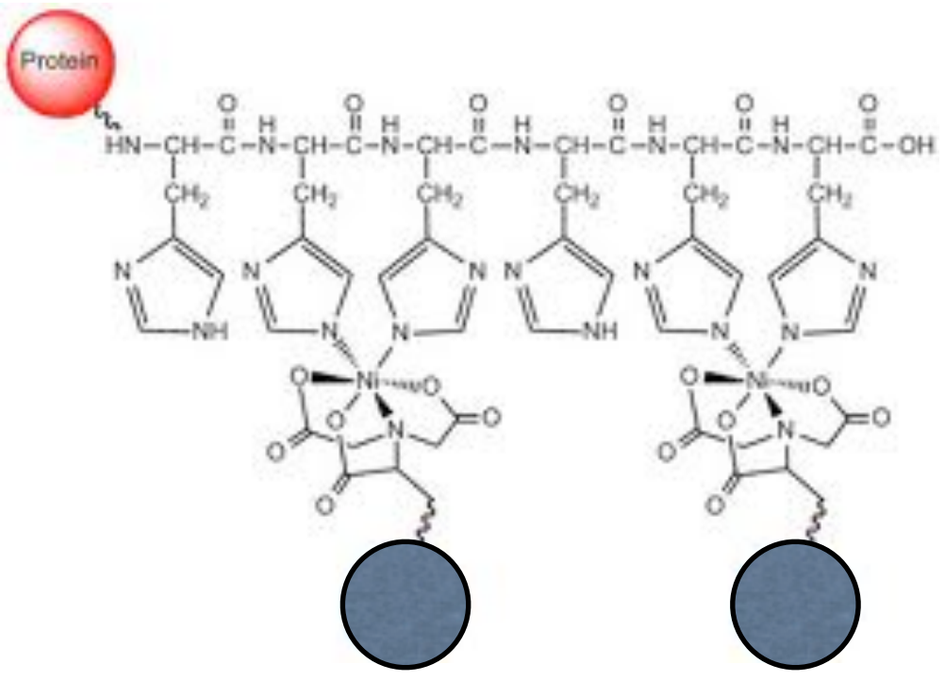


Elute with excess imidazole --

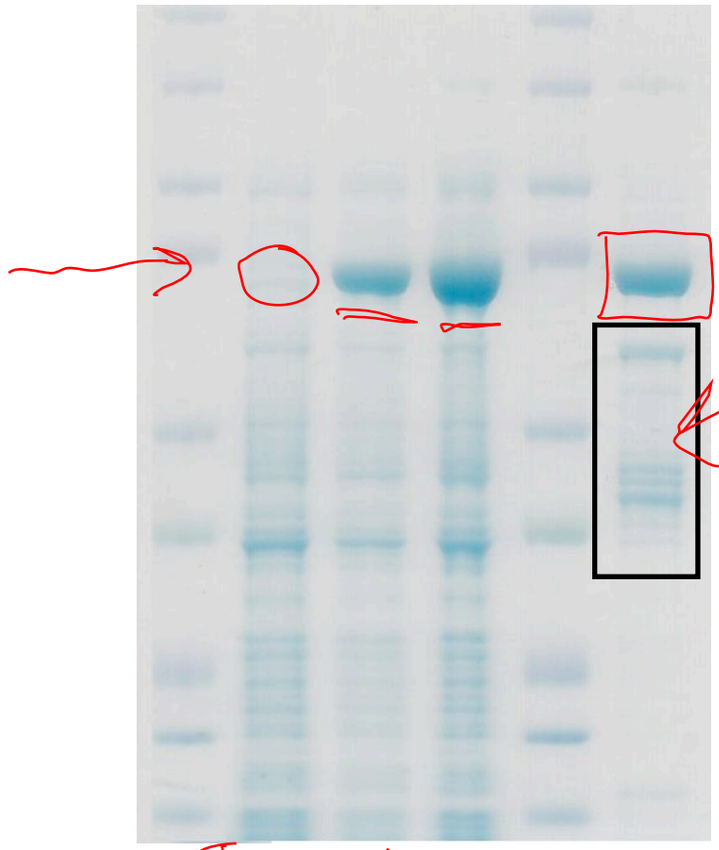
But -- binding and wash buffer also have imidazole. Why?

- weak binding of off-target proteins
- increases purity of His-tagged protein

Protein purification: Affinity purification + desalting



*Do not use sepharose beads!



The CRAPome: A Contaminant Repository for Affinity Purification Mass Spectrometry Data; Alexey Nesvizhskii[1]; Dattatreya Mellacheruvu [1]; Zachary Wright[1]; Amber Couzens[2]; Jean-Philippe Lambert[2]; Nicole St-Denis[2]; Tuo Li[3]; Yana Miteva[3]; Simon Hauri[4]; Mihaela Sardiu[5]; Teck Low[6]; Vincentius Halim[6]; Richard Bagshaw[2]; Nina Hubner[7]; Abdallah al-Hakim[2]; Annie Bouchard[8]; Denis Faubert[8]; Damian Fermin[1]; Wade Dunham[2]; Marilyn Goudreault[2]; Zhen-Yuan Lin[2]; Beatriz Gonzalez Badillo[2]; Tony Pawson[2]; Daniel Durocher[2]; Benoit Coulombe[8]; Ruedi Aebersold[4]; Giulio Superti-Furga[9]; Jacques Colinge[9]; Albert Heck[6]; Hyungwon Choi[10]; Matthias Gstaiger[4]; Shabaz Mohammed[6]; Ileana Cristea[3]; Keiryn Bennett[9]; Michael Washburn[5]; Brian Raught[11]; Robert Ewing[12]; Anne-Claude Gingras[2]; *[1]University of Michigan, Ann Arbor, MI; [2]Samuel Lunenfeld Research Institute, Totonto, Canada; [3]Princeton University, Princeton, NJ; [4]Institute of Molecular Systems Biology, ETZ, Zurich, Switzerland; [5]Stowers Institute for Medical Research, Kansas City, MO; [6]Utrecht University, Utrecht, The Netherlands; [7]Radboud University, Nijmegen, The Netherlands; [8]Institut de recherches cliniques de Montréal, Montréal, Canada; [9]CeMM Research Center for Molecular Medicine, Vienna, Austria; [10]National University of Singapore, Singapore, Singapore; [11]Ontario Cancer Institute, Toronto, Canada; [12]Case Western Reserve University School of Medicine, Cleveland, OH*

Today in lab:

- ☆ Pick your clone! Lyse samples with BPER + enzymes.
- ☆ Prepare samples for SDS-PAGE
- ☆ Affinity purify your samples (this will take awhile)
- ☆ De-salt. Why? – to get rid of imidazole + Ca^{2+} binding
- ☆ Immediately save 10 uL for the Bradford Assay
- ☆ Add BSA to the rest as a carrier protein. Why?

do not add ☆BSA until you take sample for ~~SDS-PAGE~~ Bradford

- Sleep.