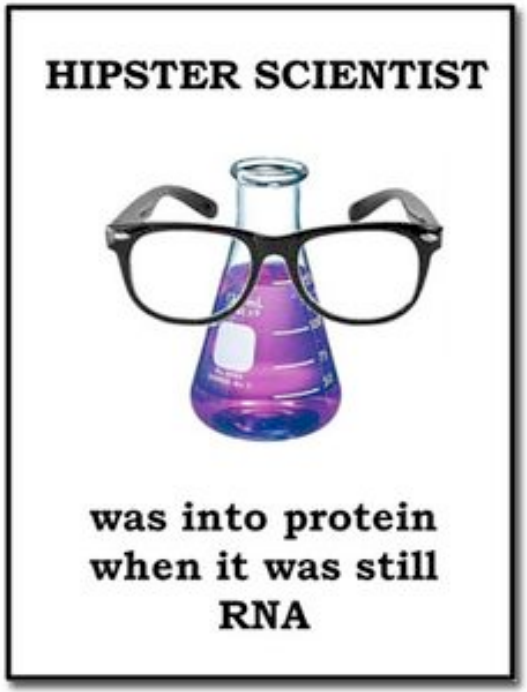
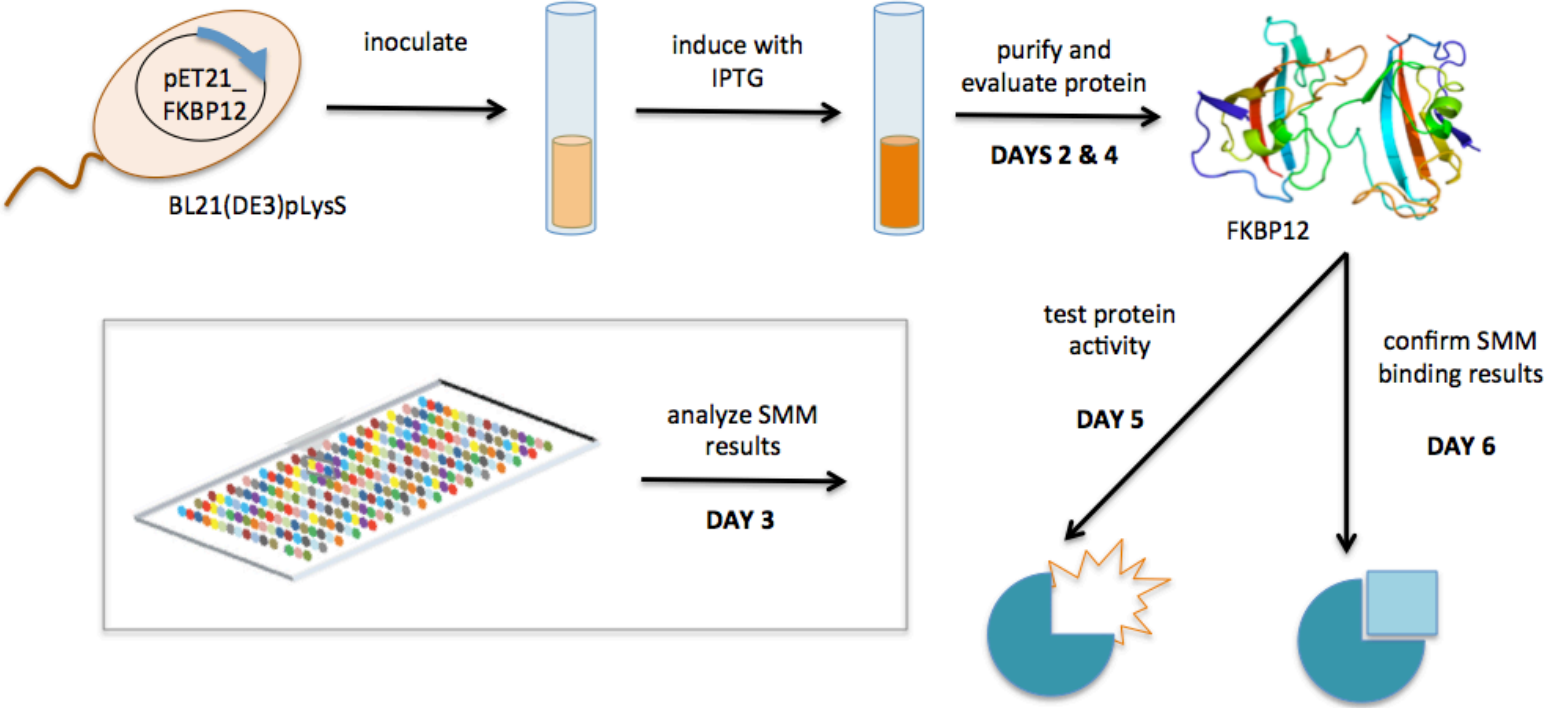
M1D5: Test protein activity using peptidyl-prolyl *cis-trans* isomerase assay

Abby McGee mcgeea@mit.edu

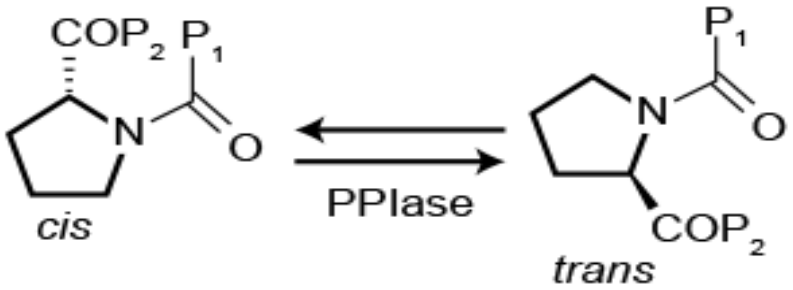
1. Prelab discussion
2. Prepare ‘master mixes’ for PPIase assay
3. Load and Measure PPIase samples



Overview of Mod 1 experiments



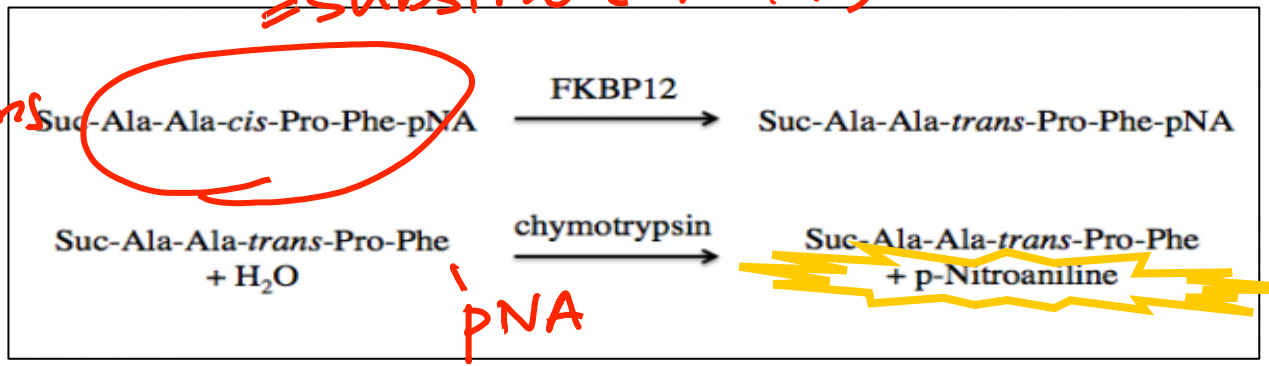
FKBP12 protein has peptidyl-prolyl *cis-trans* isomerase (PPIase) activity



- PPIase Catalyzes *cis-trans* isomerization of peptide bonds N-terminal to proline residues

- Isomerization is rate limiting step in protein folding

PPIase assay measures conversion from *cis* to *trans* isomer



- *trans* form of suc-AAFP-pNA substrate cleaved by chymotrypsin
- pNA is chromogenic; absorbs at A_{405} (appears yellow)
- PPIase assay measures rate of pNA conversion ($\Delta A/\Delta t$)

PPIase assay used to test / confirm activity of purified FKBP12

- Is the protein you purified active?

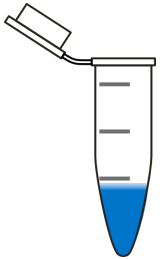
↑ A_{405} (yellow)

- How will you know your protein is active?

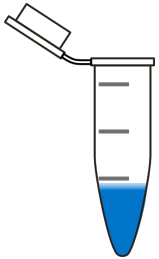
FKBP12
substrate
chymotrypsin

± FKBP12
comparison

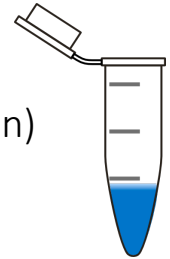
Each team will test 4 conditions



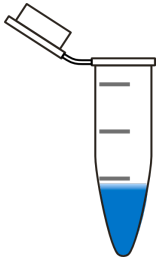
Condition #1:
(NO substrate)



Condition #2:
(NO chymotrypsin)



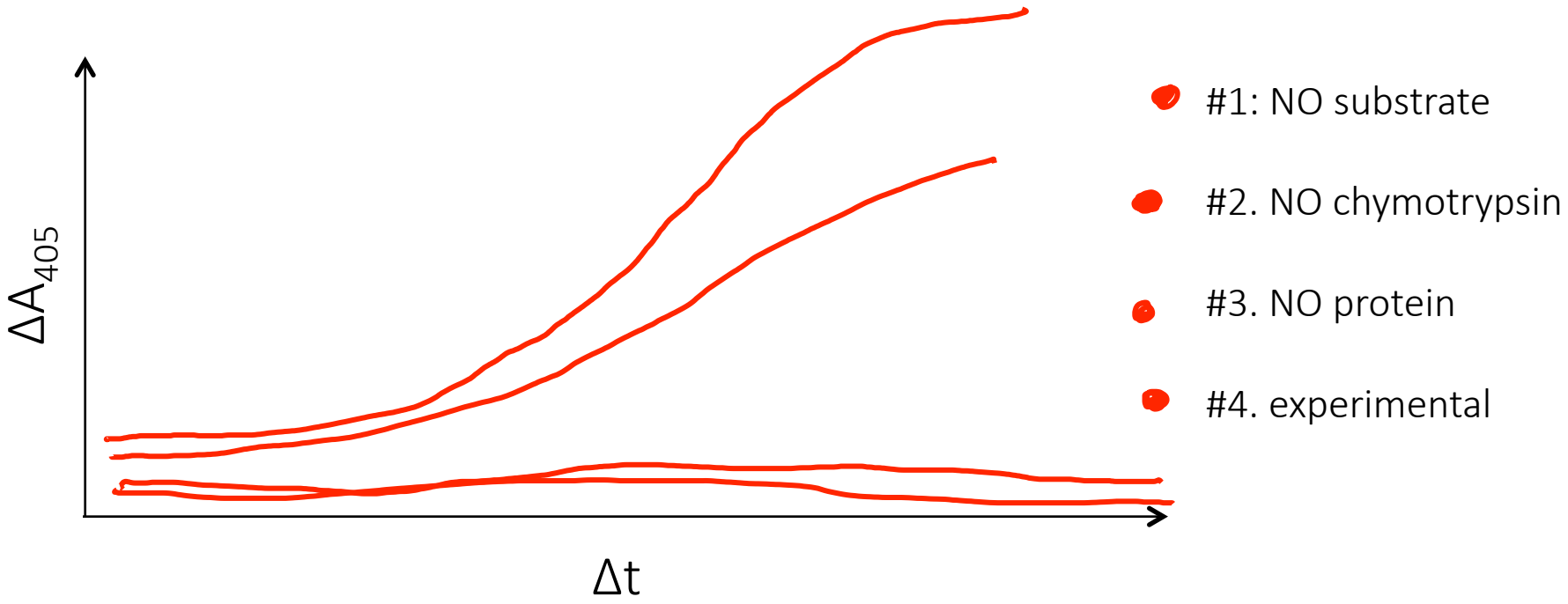
Condition #3:
(NO protein)



Condition #4:
(Experimental)

- Total reaction volume = 200 µL
  - Prepare 3.25 reaction volumes for each condition
- All reactions will include assay buffer: 200 mM Tris-HCl, pH=8 with 0.2% DMSO
- Need to calculate volumes needed for each additional component
  - substrate: 20 µL; final concentration = 5 mM
  - chymotrypsin: final concentration = 20 nM
  - protein: total amount = 1 µg

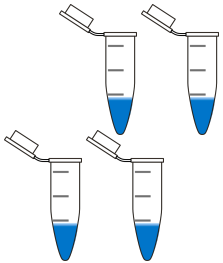
What are the expected results?



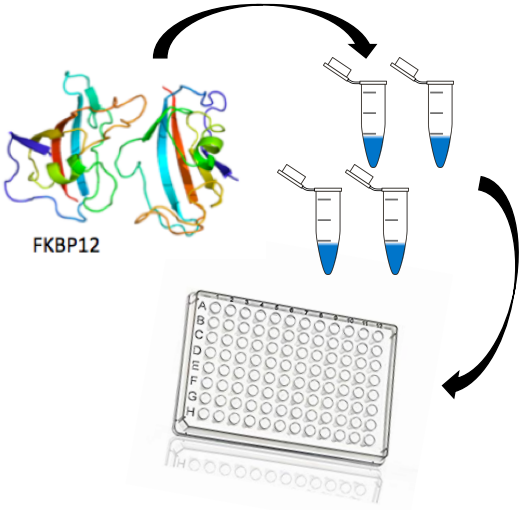
Workflow for PPIase experiment

calculate volumes for each component in each condition, confirm with Instructor

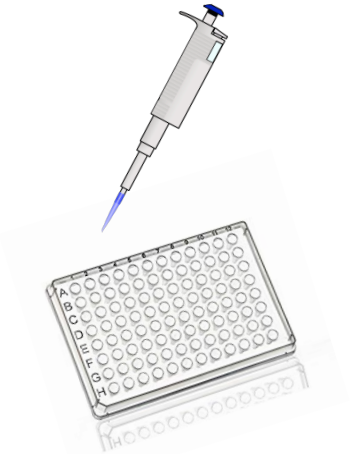
add assay buffer, water, and chymotrypsin (to appropriate tubes!)



when told to do so, add protein (to appropriate tubes!) and load samples into plate



immediately prior to measuring A_{405} values, add substrate (to appropriate wells!)

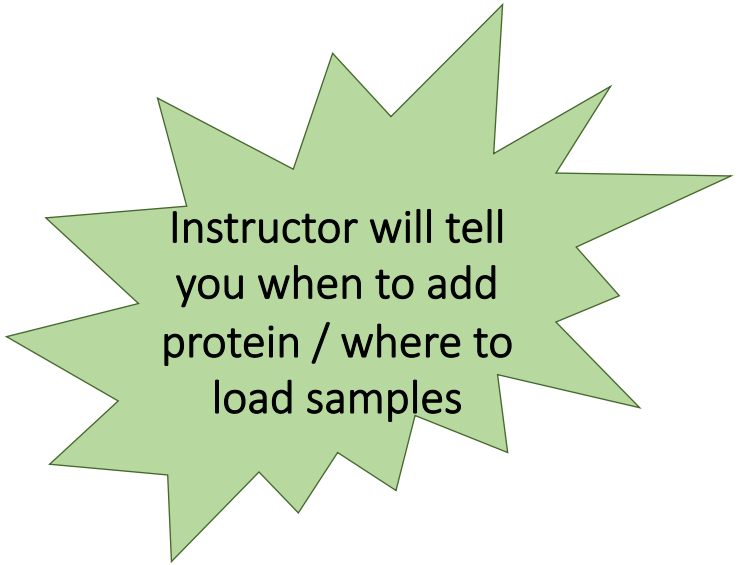


completed by Instructor

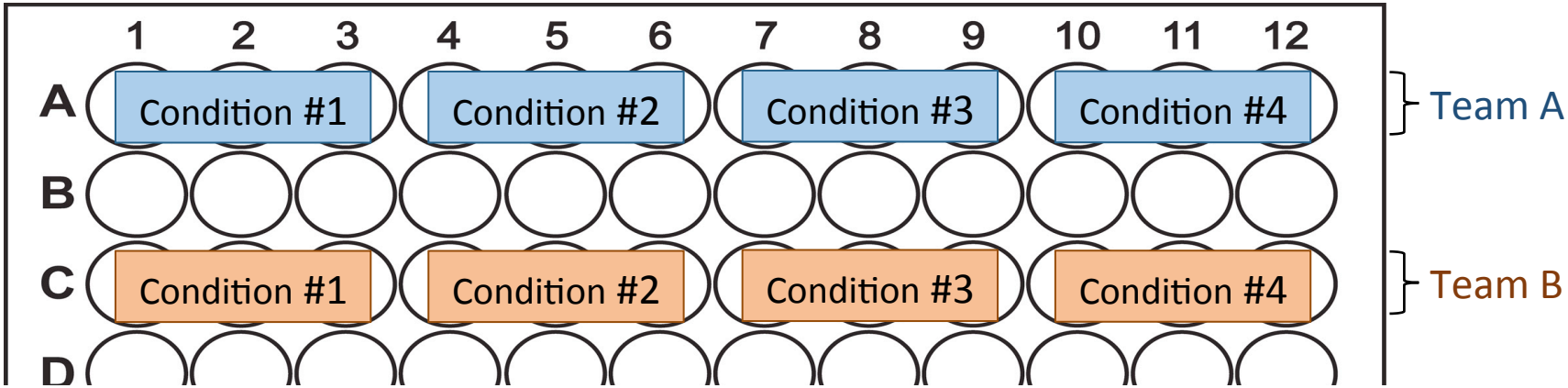
collect data and calculate specific activity for protein

PPlase assay is very time sensitive!!!

- Will prepare master mixes in shifts:
  - Red, Orange, Yellow (at ~2:45p)
  - Green, Blue (at ~3:15p)
  - Pink, Grey (at ~3:45p)
- At your bench, prepare assay buffer
- At front bench, add protein then immediately load samples
- Instructor will add suc-AAFP-pNA



Be careful to load samples into correct wells!!



- A_{405} measured every minute for 30 minutes; total of 31 readings (including t = 0)
- Samples maintained at 25 °C and shaken immediately prior to reading

Quantify the specific activity of FKBP12

$$\text{Specific activity} = \frac{(\Delta A_{405\_Test}/\#\text{min} - \Delta A_{405\_Blank}/\#\text{min})(\text{rxn volume})}{(\text{volume of FKBP12})(\varepsilon_{pNA})}$$

ε_{pNA} (extinction coefficient for pNA) ~ 9.3 mM^{-1}

- Convert FKBP12 volume to mg using known concentration
- Units for specific activity = nmol substrate/min/mg of protein

For today…

- Be very mindful of timing for PPIase assay
  - Read ahead and confirm calculations
  - Listen for instructions on when to begin sample preparation

For M1D6…

- Craft outline for Mini-presentation

Notes on Mini-presentation…

- Bullet / outline format
- Follow time and content guidelines:
  - Introduce yourself and your research project
  - Clearly state hypothesis to identify main question
  - Be quantitative when stating results (NOT “this was more/less than…”)
  - For now, use placeholder statements for key findings

Category	Approximate worth	Elements of a strong presentation
Content	50%	• Did you introduce your research? • Did you include the key findings (and the techniques used to gather these results, if necessary)? • Was the importance of your project clear?
Organization	25%	• Is the presentation logical and easy-to-follow? • Are the main points emphasized? • Did you include transition statements such that the presentation 'flows' and is easily followed/understood?
Delivery	25%	• Do you show confidence and enthusiasm? • Did you use appropriate language (technical or informal, as appropriate)? • Is your speech clear?