M3D5: Transcript & Protein Assays

✱ Notebooks due today – D

Announcements

No Off on Monday
Sunday 6:30 – 7:30 pm

- Final quiz and final FNT **--** next lab (Tuesday)

- **Module 3 report due M3D7 (5/8), 5pm**

  - work in groups (tell me otherwise)

  - less formal, but clear and concise

- **Final project: Research Proposal Presentation M3D8 (5/13)**

  - More on next slide

- Day 7 lecture: Atissa is back (be there! **--** presenting with a partner)

- Final lecture (5/15): class discussion/evaluation + Party!

Final project: Research proposal

Manipulate → Model → Make

Appropriate subject matter:

3 modules 10g → DNA/RNA Engineering → Synthetic Biology
→ Detection/Aptamers
Systems → Systems Biology, HTS technol.
Biomaterial/Cells → drug delivery
→ scaffolds T.E.

What is biological engineering?

Biology Question:

What Kinase phosphorylates protein X ?

- Novel screening method
- How can we quantify the rxn kinetics?
- Can we predict the phosphorylation?
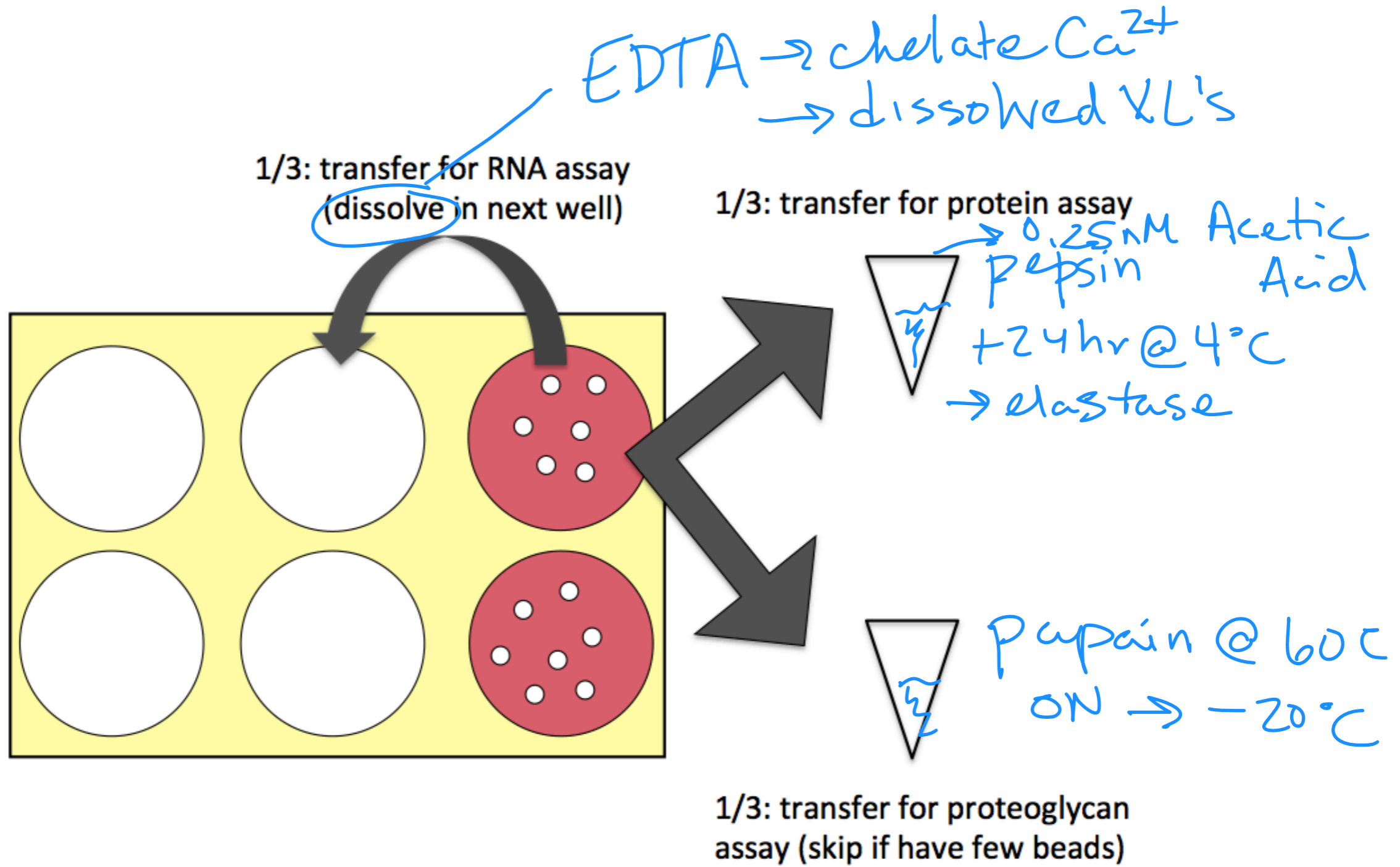(modeling → prediction)

Appropriate scope:
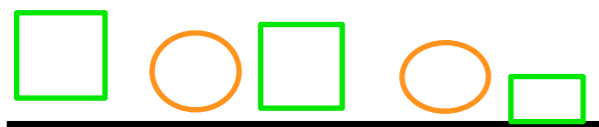
think years, not months (2-3 yrs)

think big

think outside the 10g lab → talk to faculty
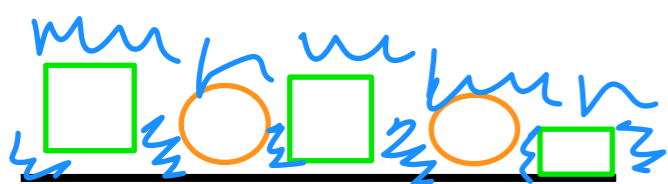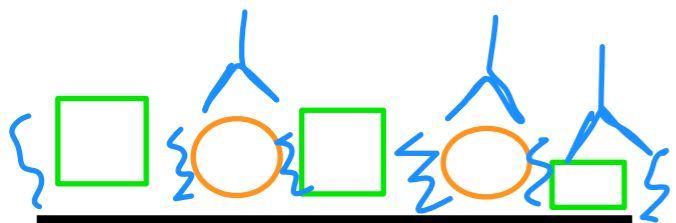
Review what we did last time:

EDTA → chelate Ca^{2+}
→ dissolved XL's

1/3: transfer for RNA assay
(dissolve in next well)

1/3: transfer for protein assay

→ 0.25mM Acetic Acid
Pepsin
+24hr @ 4°C
→ elastase

Papain @ 60 C
ON → -20°C

1/3: transfer for proteoglycan
assay (skip if have few beads)
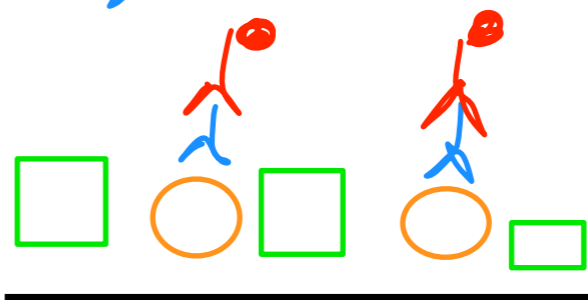
ELISA:

1 adsorb proteins to the plate
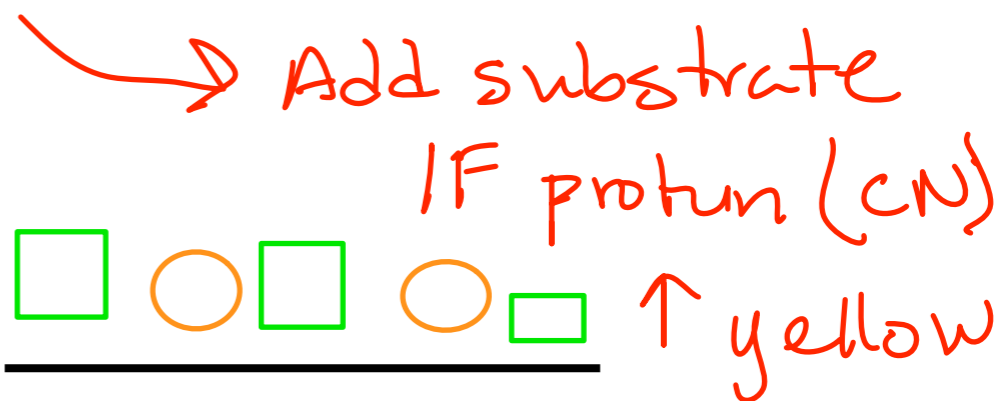

2 Wash/block → Milk

3 Wash/Add α-CN Ab

□ protein X

○ CN   Y —antibody

4 Wash to remove non-specific binding
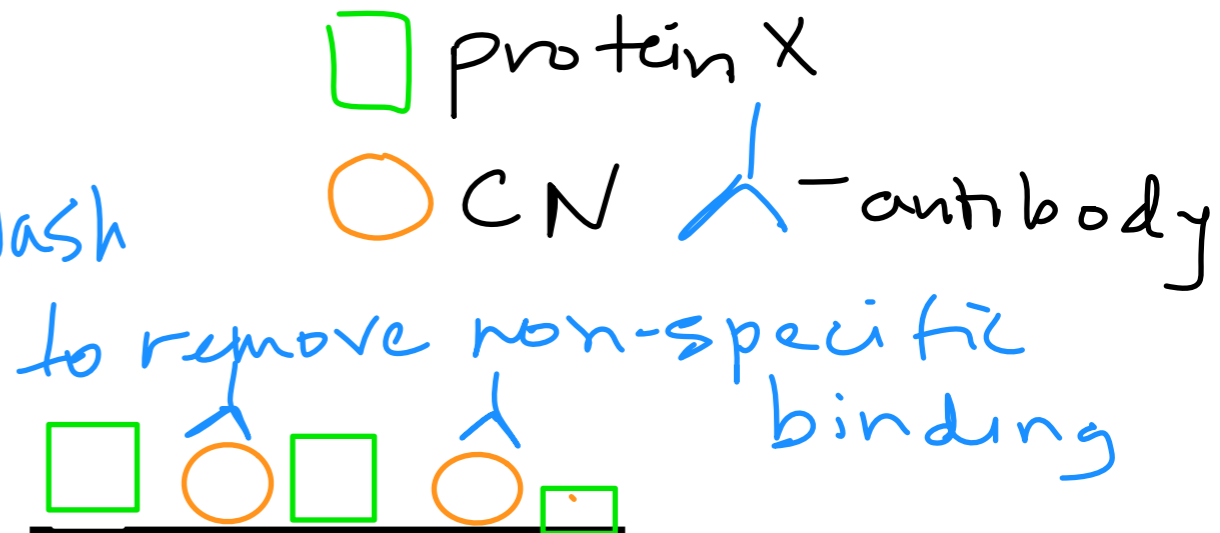
5 Wash/Add 2-Ab α-Rb—AP

6 → Add substrate IF protein (CN) ↑ yellow
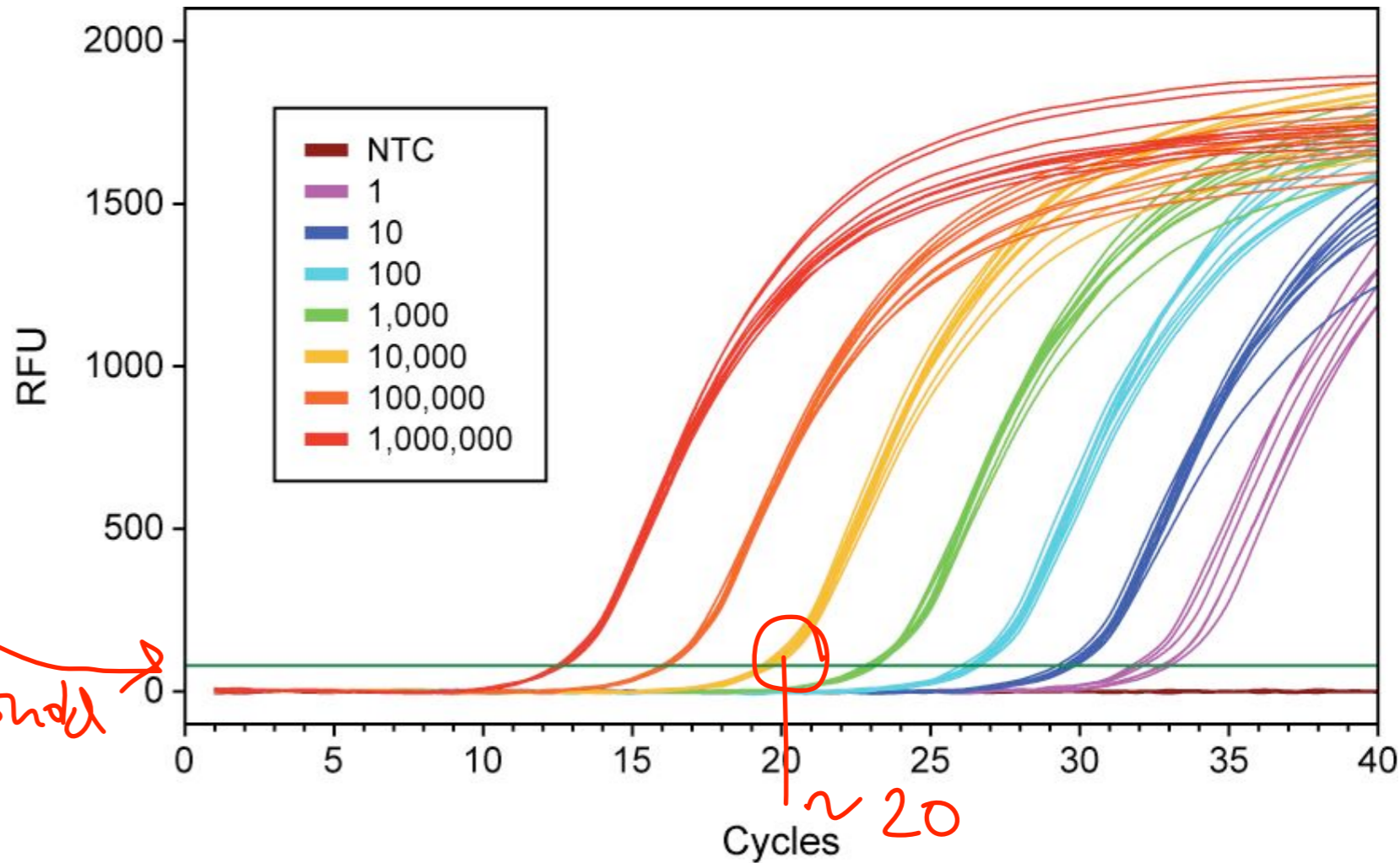
qPCR:

mRNA $\rightarrow$ cDNA

Sybr Green Dye

Real time tracking [DNA].

Threshold Cycle (Ct)

$\downarrow C_t \Rightarrow \uparrow$ product

CNI ⎫
CNII ⎬ 2 conditions
18S RNA ⎭ 2 - (duplicate) threshold

$\rightarrow$ 12

~ 20

Be *very* careful pipetting your samples today!