M3D6: Transcript & Protein Assays, con't

- Final lab treat:
- **Module 3 report due M3D7, 5pm (before you leave lab)**
  - Work in groups.
  - No separate Methods section needed. Note important experimental conditions (your experimental design, cell counts, alginate %, etc).
  - Figures/Results/Discussion (all in one!) needed for: Cell Viability (+ stats!), qPCR, ELISA, and PG (if you obtained results)
  - **Note: Use T/R Green data for Cell Viability -- conclude if you believe their conditions resulted in a significant change**
- **Final project: Research Proposal Presentation M3D8**
  - Scope: Depth vs. Breadth -- not ok to focus only on one paper / variable, but balance with 'too ambitious'
  - Timing of idea choice -- dangers and pitfalls
  - How to find research gaps -- *Actual experts!* also, reviews or 'future work' sections of primary literature.
- Day 7 lecture: Atissa is back / Day 8 lecture: special topics in TE (may also be D7)

ELISA: Indirect

△ = CN

⅄ = Ab = ⅄

4 Wash w/ Tween-20

5 2° Ab + wash ● = AP alkaline phosphatase

6 Add AP substrate

△ color ⇒ plate reader @ 420 nm

★ ★ ★ ★

4 Washing technique

5 2° Ab α-Rabbit - AP

- amplification
- flexibility $$, multiples
- ↑ cross-reactivity

6 $pNPP^{*} + H_2O \xrightarrow{AP} pNP + P_i$

+ NaOH

yellow

blue → A_{420}

timing is critical

Short t → miss [CN II]

↓ dynamic range

Long t → Saturation

ELISA:

Outcome	Possible Explanations
High reading in "blank" samples	- bad washing - bad blocking - X-contamination
No signal at all (including standards)	- wrong 2°Ab - old/storage conditions 1° 2° - flipped plate Non-optimal incubation NPP
Saturated signal for some samples	- wrong [Abs]

Proteoglycan Assay:

- Measure GAGs with DMMB -- cationic dye

- Absorbance shift due to complex

A595

  - Note: fades very quickly! ← pipet upstairs

- Done at low pH to select for? sulfates vs. carboxyl (pH 1.5)

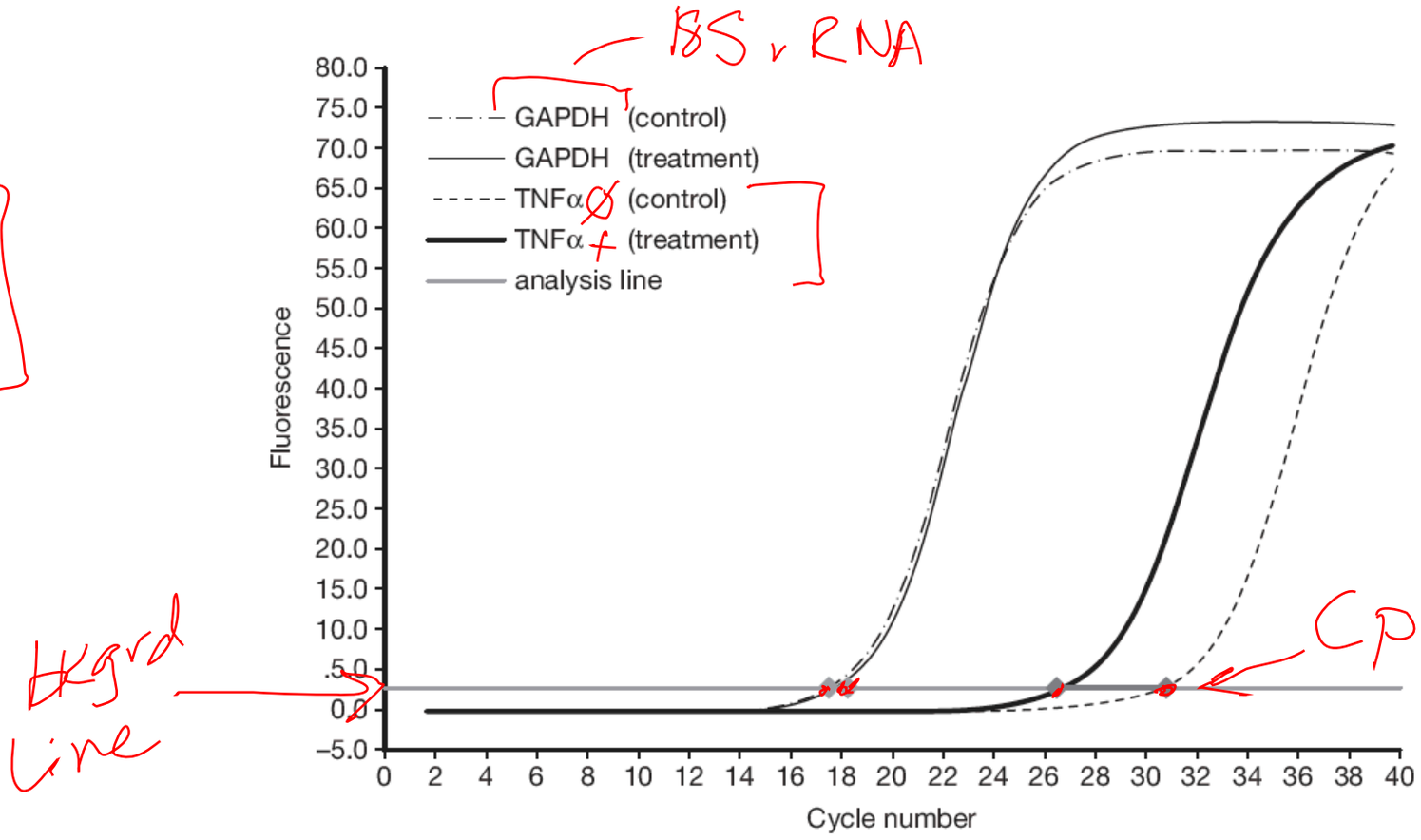
- this corrects for: Alginate

- Standard curve made with: chondroitin sulfate

- How to compare across conditions? — # of cells; Lysates concentration; Spike

qPCR:

Threshold Cycle (Ct) =
Crossing point (Cp)



$$\text{ratio} = \frac{(E_{\text{target}})^{\Delta C_P\,\text{target}\,(\text{control} - \text{sample})}}{(E_{\text{Ref}})^{\Delta C_P\,\text{Ref}\,(\text{control} - \text{sample})}}$$

$$\text{fold change} = \frac{2^5}{2^1} = \frac{32}{2} = 16\text{x}$$

Relative quantification

Equation 1 from M.W. Pfaffl, *Nucleic Acids Res* **29**:2002 (2001)

Michael W. Pfaffl *in: Real-time PCR. Published by International University Line (Editor: T. Dorak), p 63-82*

Today in lab:

- Finish ELISA -- includes a 90 min incubation

- Meanwhile:

  - DMMB assay -- 15-20 min of work (staggered)

  - qPCR analysis

  - viability analysis using T/R Green data

  - cross-group research discussion (see talk page)

  - (optional: clean up)