

- **Announcements**

- **Pre-lab Lecture**

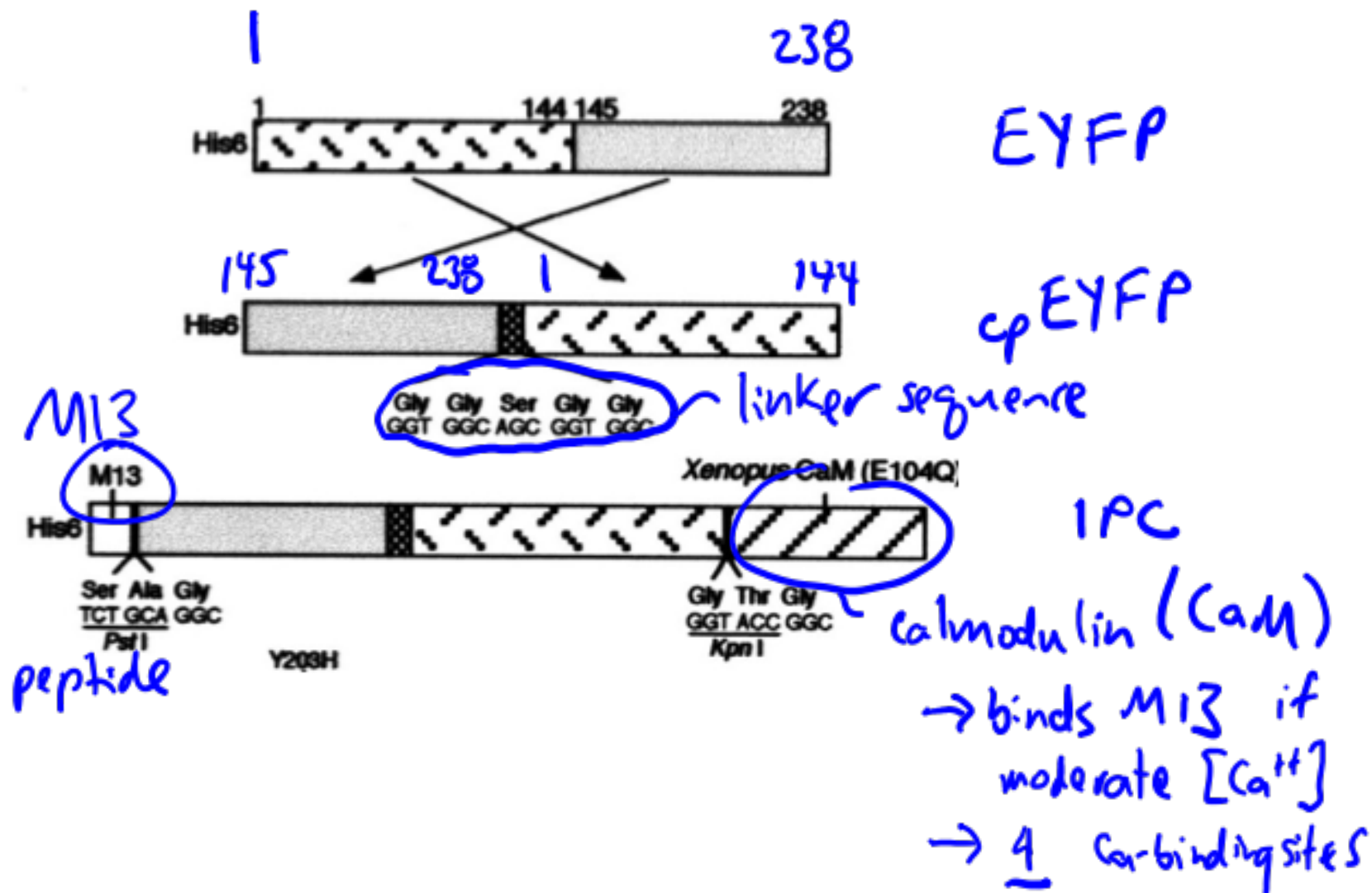
- ❖ **Module 2: design overview**
- ❖ **Primer design for mutagenesis**
- ❖ **Intro to restriction enzymes**
- ❖ **Today in Lab: M2D1**

Announcements

- Module 1 report due tomorrow by 1 PM, to 20109.submit@gmail.com
 - Module 1 drafts returned Fri March 22nd
 - Revisions due April 6th (Fri) by 11 AM
 - Draft letter grade may increase by up to 1½
 - Indicate (highlight, etc.) revisions made
- Primer design summary due Tuesday by 11 AM
- Introducing... Mark, your TA for Module 2

M2 quizzes: D3, D6, D8

Inverse pericam composition

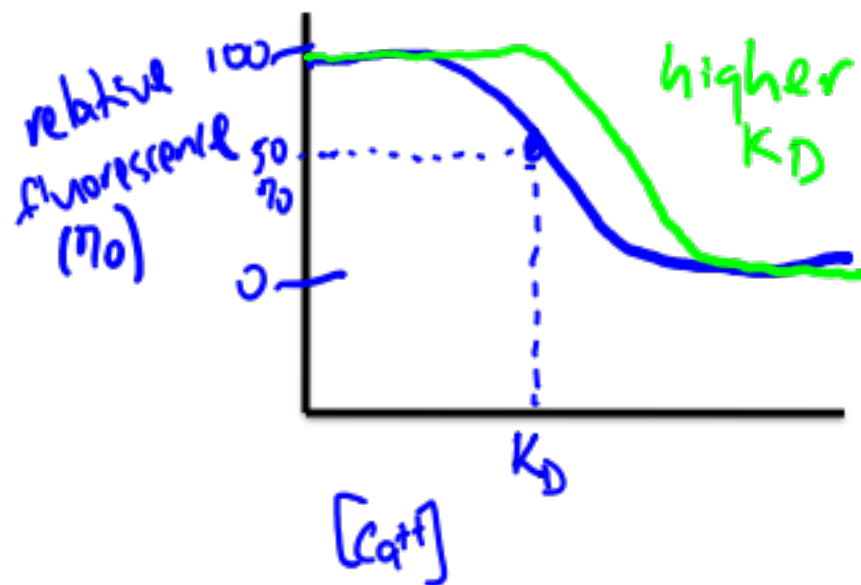


Goal: affect binding properties

vary $[Ca^{2+}]$, keep $[IPC]$ constant

fluorescence \propto $\frac{1}{\text{binding}}$

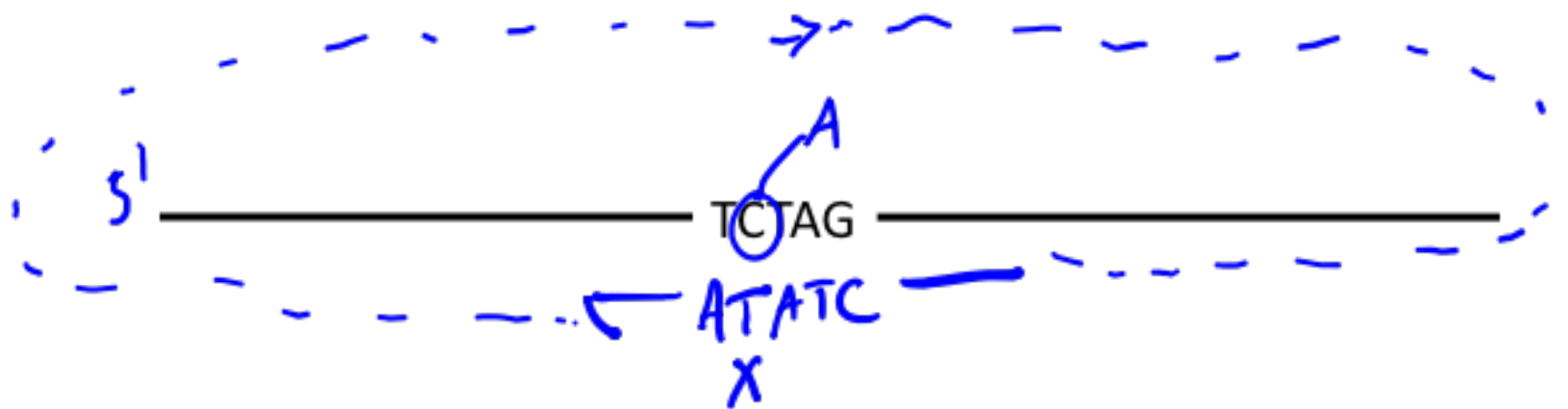
affinity \downarrow



cooperativity \uparrow



Designing mutagenic primers



Intro to restriction enzymes

EcoRI site



EcoRI enzyme



"sticky" ends / overhangs

endonucleases
→ cut DNA

can be used in
- cloning (directional)
- analysis

also blunt cut
eg. TACCA
ACGCT

palindromic sites

Today in Lab: M2D1

- Study inverse pericam at multiple levels
- Design primers
 - Amino acid change

* read the wiki
 * don't limit to 9 loops

- Silent change
- make new unique (or low copy)
 restriction site

same AT



- Pick reference mutant: D24H, E67K, T79P, M124S
- For next time: start reading paper for discussion
 - time in class on D2 to re-read your assigned section