M2D1: Design IPC Mutant

3/13/13

Announcements

- Mod2 TA: Hi Mark!

- Mod2 Quiz days: M2D3, M2D6 & M2D8

- Module 1 report is due right now. Submit to 20109.submit@gmail.com.
  - Drafts will be returned to you **Friday, March 22**
  - Revisions are due **Friday, April 6 by 11AM**
  - Opportunity for a 1.33 grade improvment
  - Indicate in your revision where you made changes/improvements

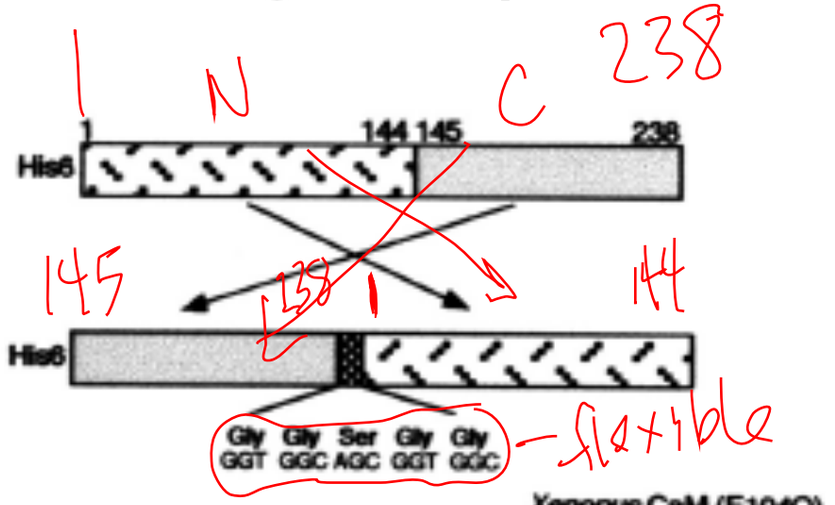
- Primer design summaries are due ~~Tuesday,~~ Wed 11AM

- Time management

Protein Engineering -- Experimental Overview

EYFP (V68L/Q69K)

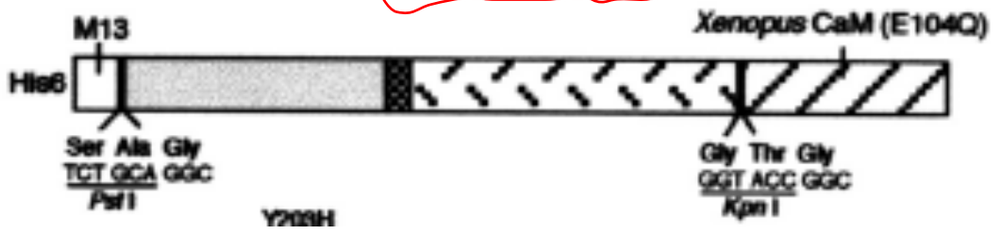
cpEYFP(V68L/Q69K)



eYFP

cp(eYFP)

pericam



inverse-pericam

M13

Myosin Light Chain Kinase

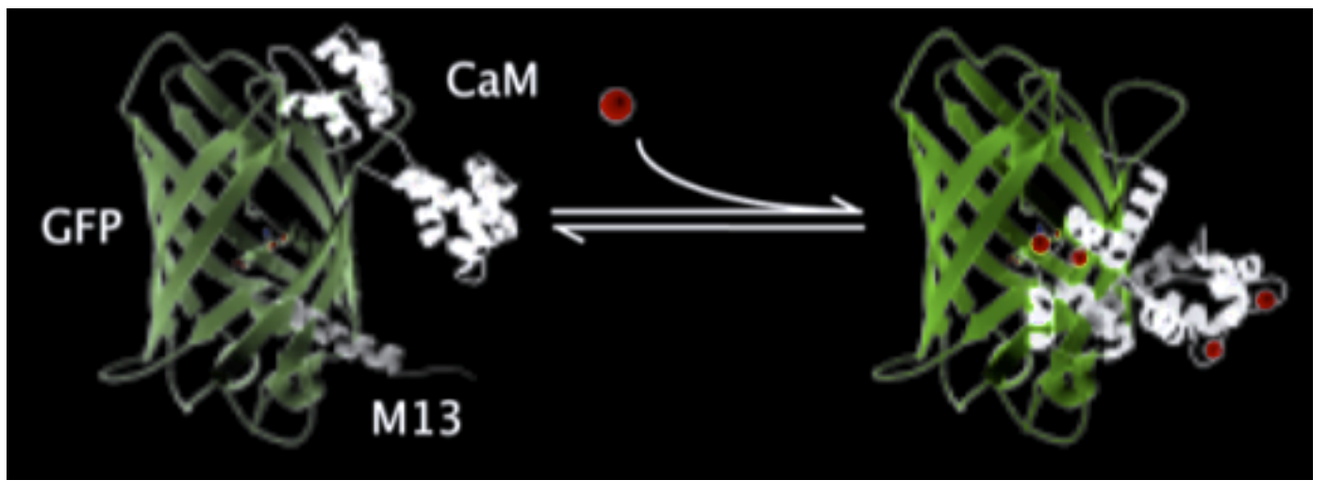
Calmodulin (CaM)

4 Ca^{2+} → E-F hand

Moderate $[Ca^{2+}]$

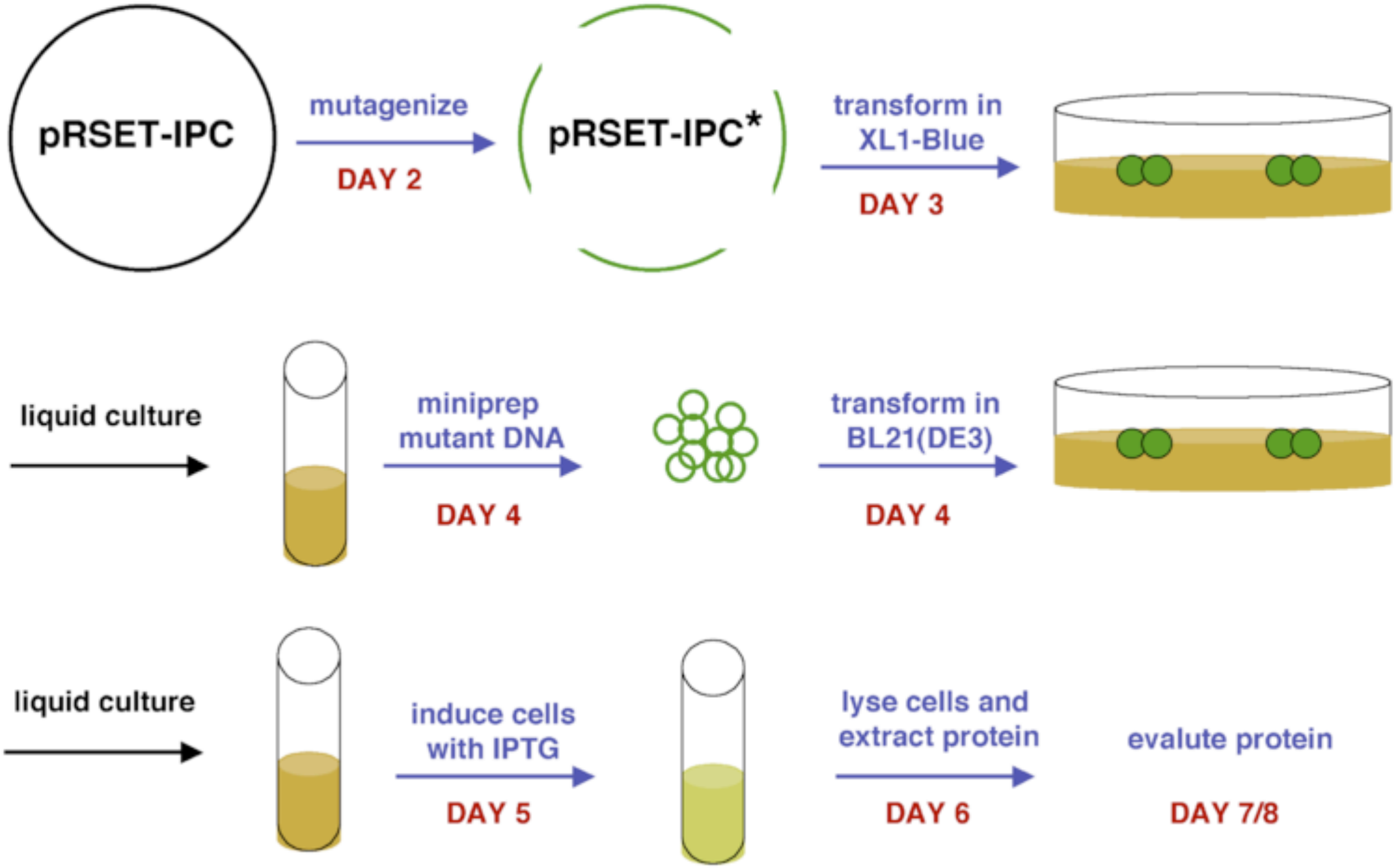
CaM binds M13

Pericam



optogenetics.org

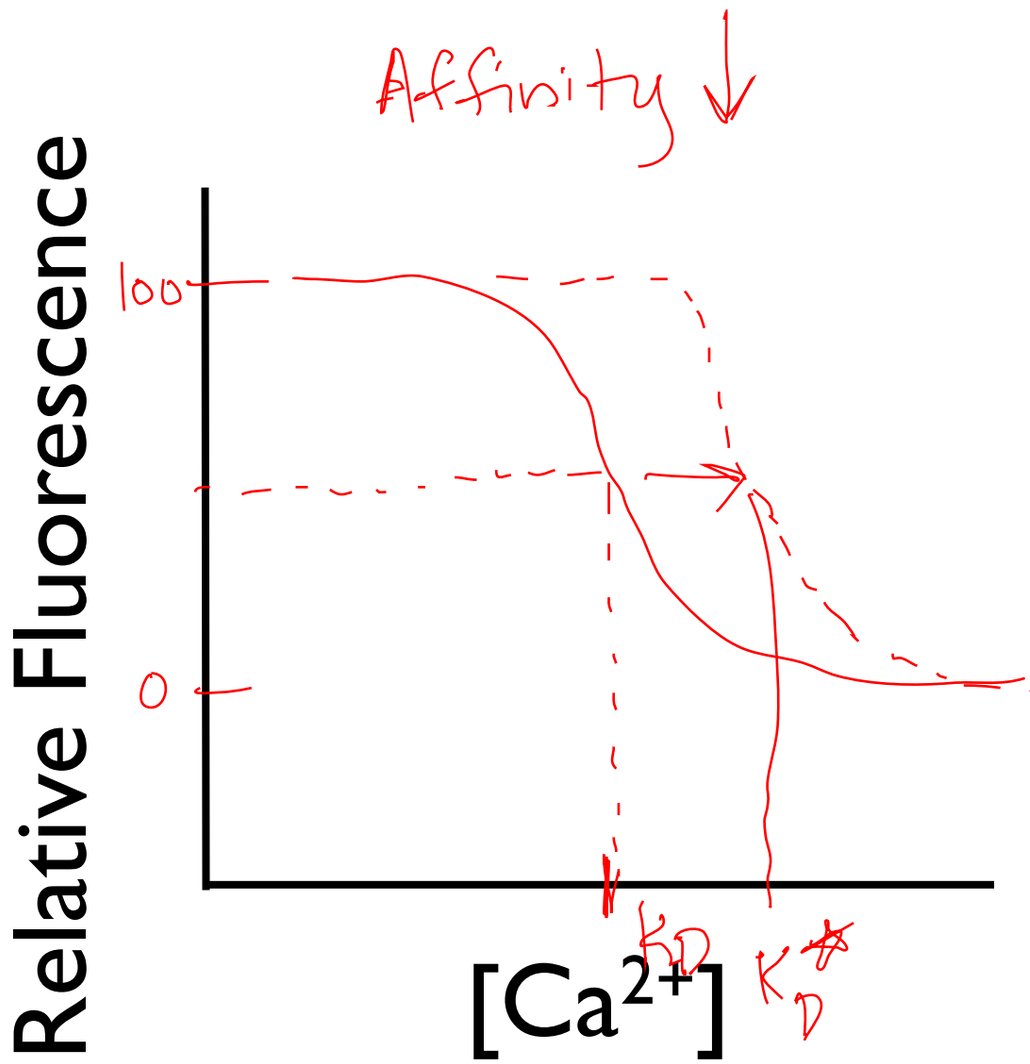
Protein Engineering -- Experimental Overview

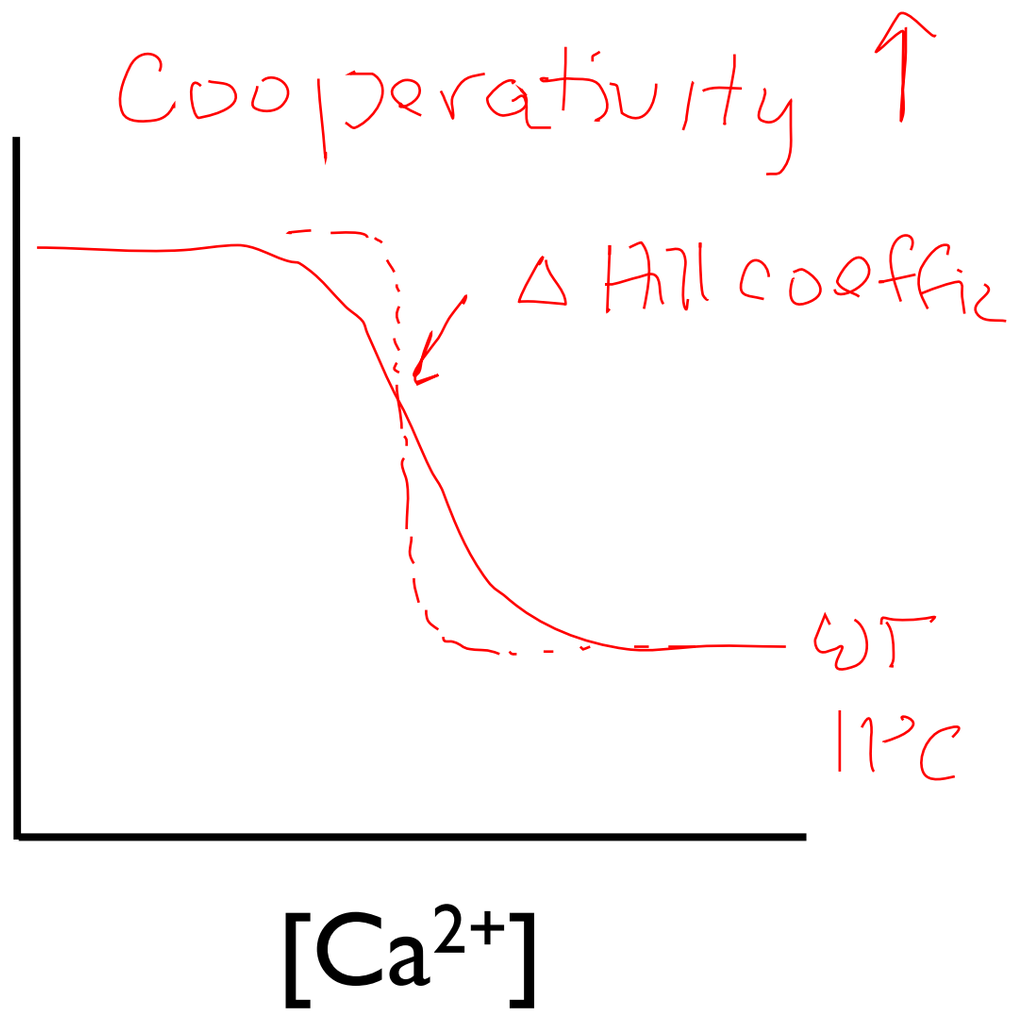


Protein Engineering -- Experimental Overview

Goal: Modulate binding of Ca^{++}

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





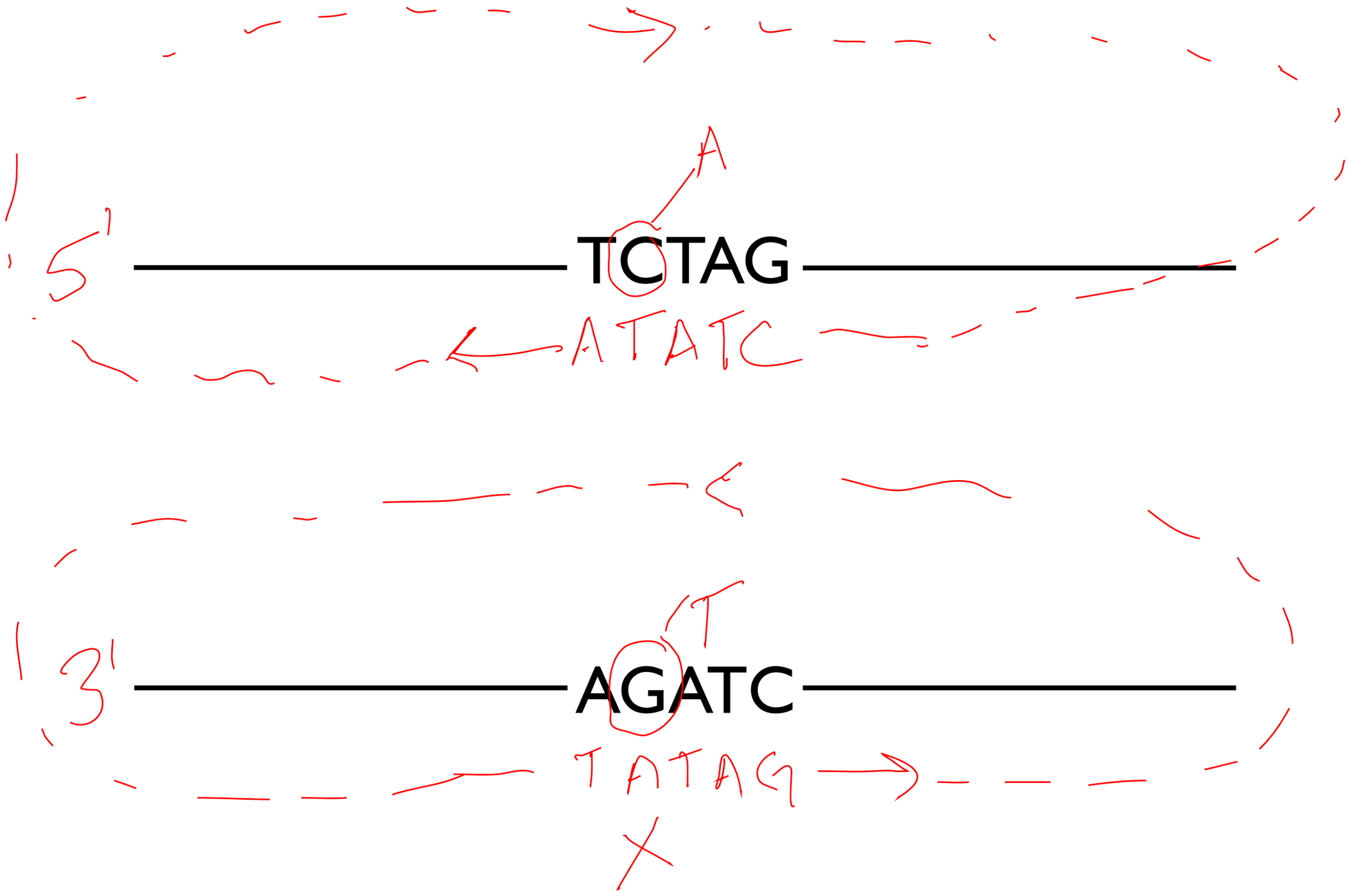
$Flour \propto \frac{1}{[Ca^{2+}]}$

Designing your primers for mutagenesis:

TCT → TAT or TAC

First Position (5' end)	Second Position: U			Second Position: C			Second Position: A			Second Position: G			Third Position (3' end)
U	UUU	Phe		UCU	Ser		UAU	Tyr		UGU	Cys		U
	UUC			UCC			UAC			UGC			C
	UUA	Leu		UCA			UAA	Stop		UGA	Stop		A
	UUG			UCG			UAG	Stop		UGG	Trp		G
C	CUU	Leu		CCU	Pro		CAU	His		CGU	Arg		U
	CUC			CCC			CAC			CGC			C
	CUA			CCA			CAA	Gln		CGA			A
	CUG			CCG			CAG			CGG			G
A	AUU	Ile		ACU	Thr		AAU	Asn		AGU	Ser		U
	AUC			ACC			AAC			AGC			C
	AUA	Met		ACA			AAA	Lys		AGA	Arg		A
	AUG			ACG			AAG			AGG			G
G	GUU	Val		GCU	Ala		GAU	Asp		GGU	Gly		U
	GUC			GCC			GAC			GGC			C
	GUA			GCA			GAA	Glu		GGA			A
	GUG			GCG			GAG			GGG			G

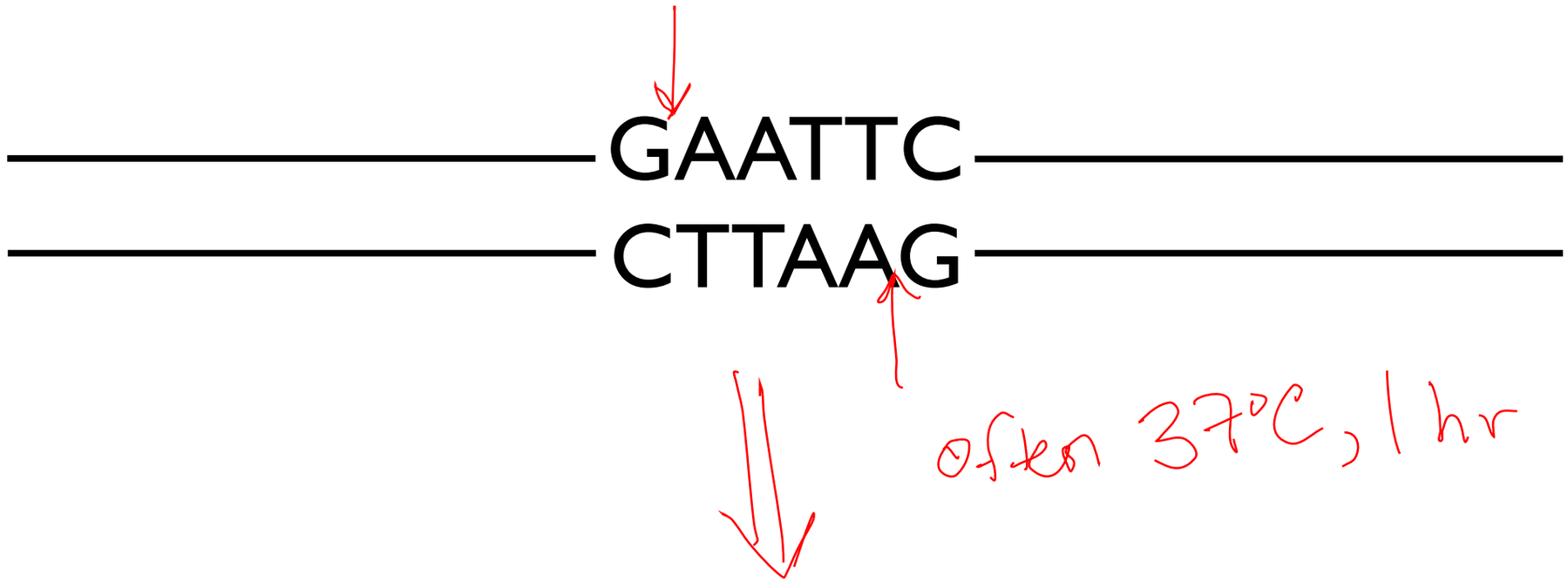
Designing your primers for mutagenesis:



Adding a Restriction Enzyme site for:

endonucleases: cuts DNA

EcoRI site



often 37°C, 1 hr

sticky ends

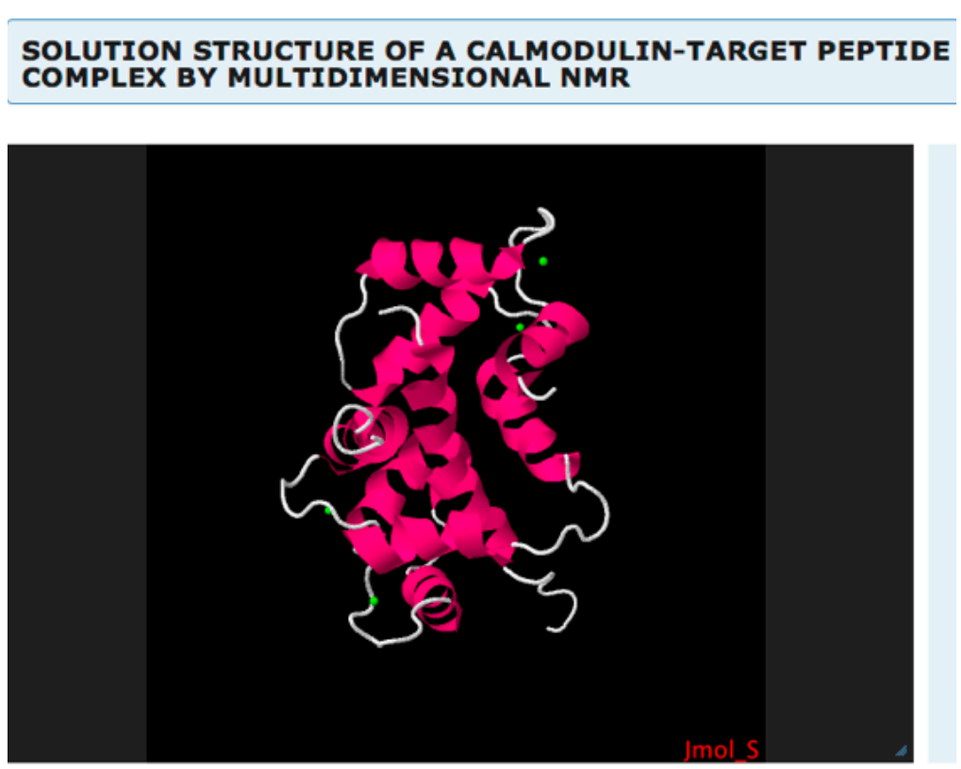
why?

1. Selection/Screening — time, $$
2. cut & paste - recombinant DNA
3. Sequencing

Today in lab:

1. Explore inverse pericam
   - Gene/Protein sequence
   - PDB visualization
   - pick mutation site

2. Design mutagenesis primers
   - Single aa mutation
   - Silent mutation to insert RE site



3. Pick a reference mutant based on your rational design: See Talk page

FNT: Read journal articles by 1) Heim, Prasher, and Tsien and 2) Nagai et al. -- linked in introduction on M2D2

Part 3 of M2D2 -- you will have some time to re-read your section on Friday.