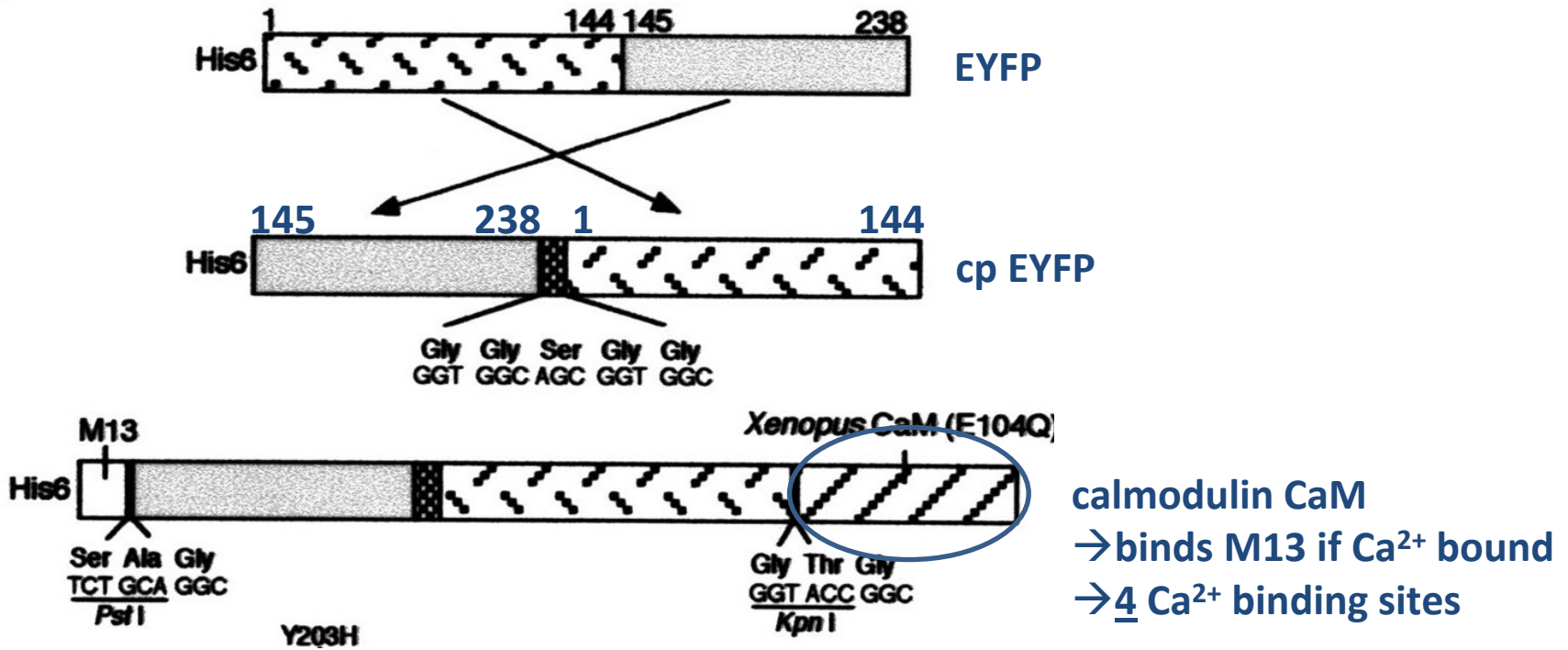


- **Announcements**
- **Pre-lab Lecture**
  - ❖ **Module 2: Design Overview**
  - ❖ **Primer design for mutagenesis**
  - ❖ **Intro to Restriction Enzymes**
  - ❖ **Today in Lab: M2D1**

# Announcements

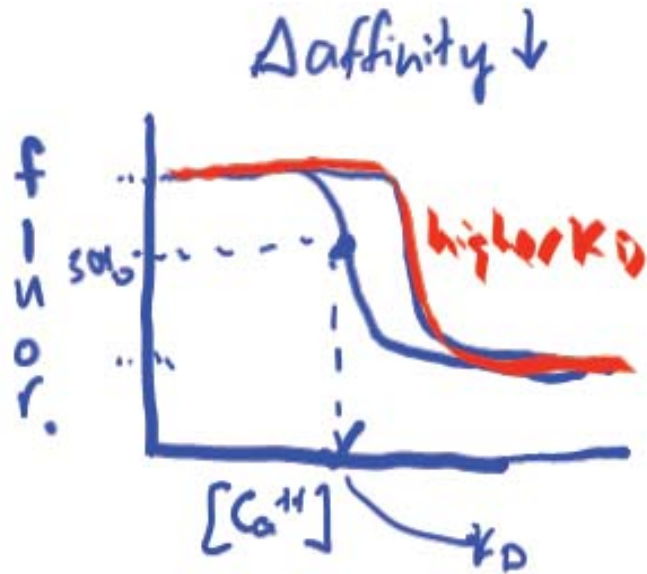
- iGEM competition
- Introducing... Xiaosai, your TA for Module 2

# Inverse Pericam



Courtesy of National Academy of Sciences, U. S. A. Used with permission.  
 Source: Nagai, T., et al. "Circularly Permuted Green Fluorescent Proteins Engineered to Sense Ca<sup>2+</sup>." *PNAS* 98, no. 6 (March 6, 2001): 3197-3202.  
 Copyright © 2001 National Academy of Sciences, U.S.A.

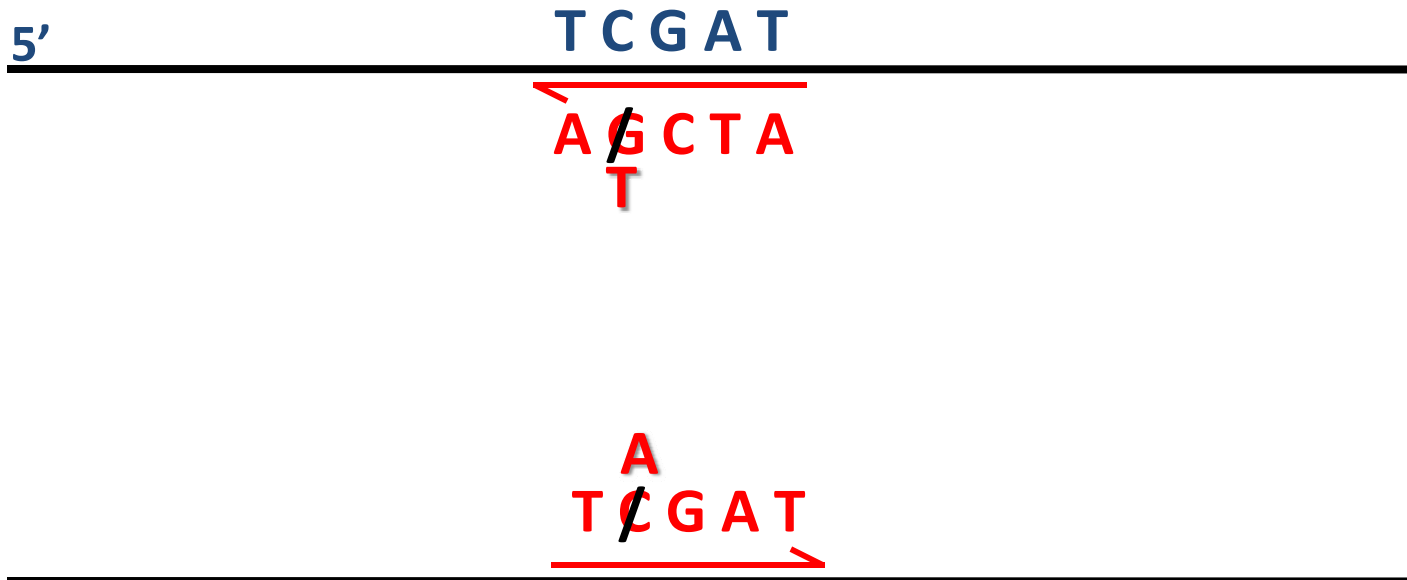
# Goal: Affect Binding Properties



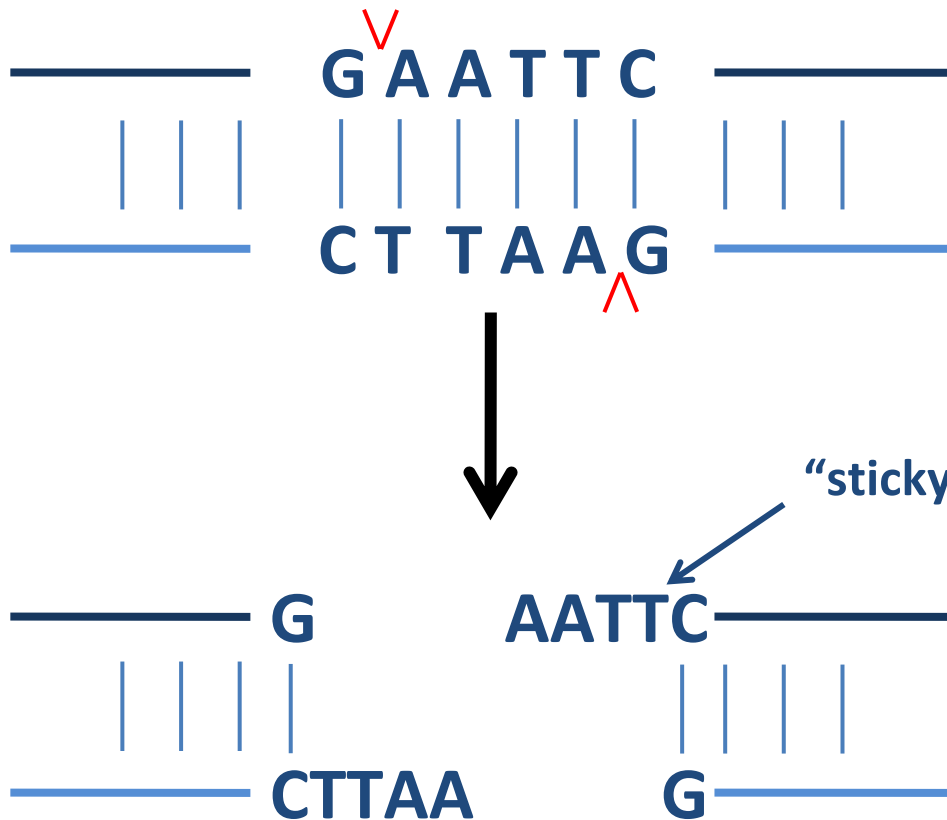
vary  $[Ca^{2+}]$ , keep  $[CaM]$  constant

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

# Designing Mutagenic Primers



# Intro to Restriction Enzymes



endonucleases  
→ cut DNA



“sticky ends” can be used in ligations

palindromic DNA  
also type 2  
leave blunt ends

# Today in Lab

- Study inverse pericam at multiple levels

- Design primers

- Amino acid change



- Silent change make new, unique restriction site



- For next time: begin reading two papers

- Focus on Nagai; other one is for some history/context

- Time in class on D2 to finish

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