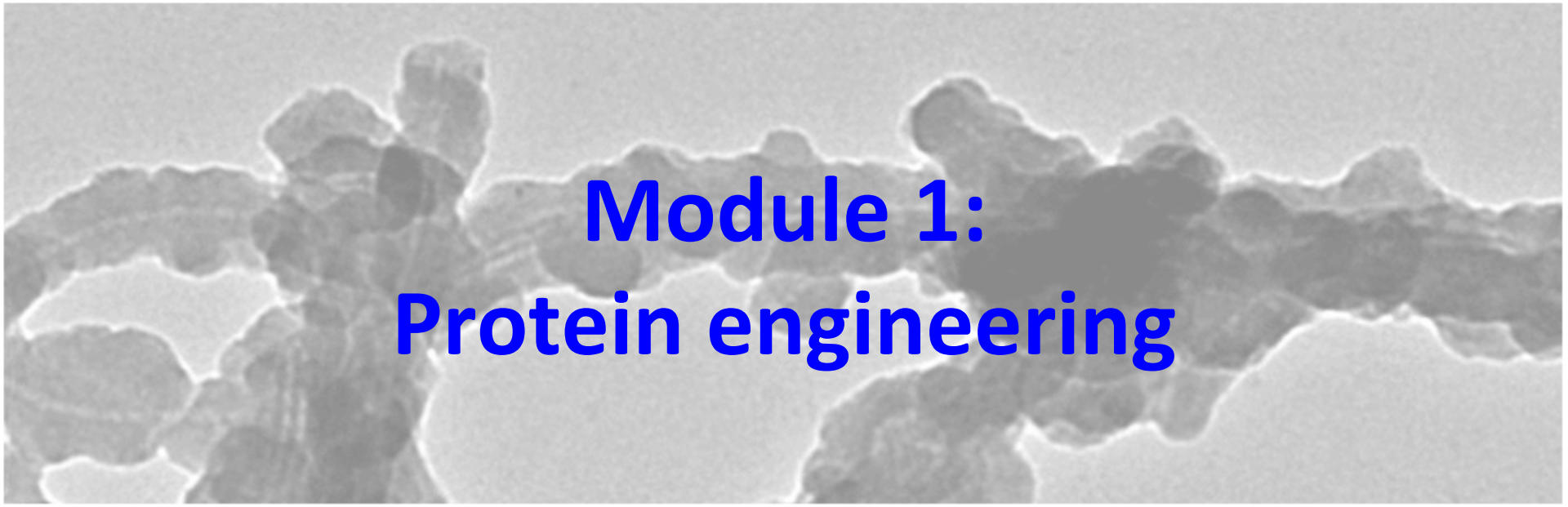


Module 1:
Protein engineering

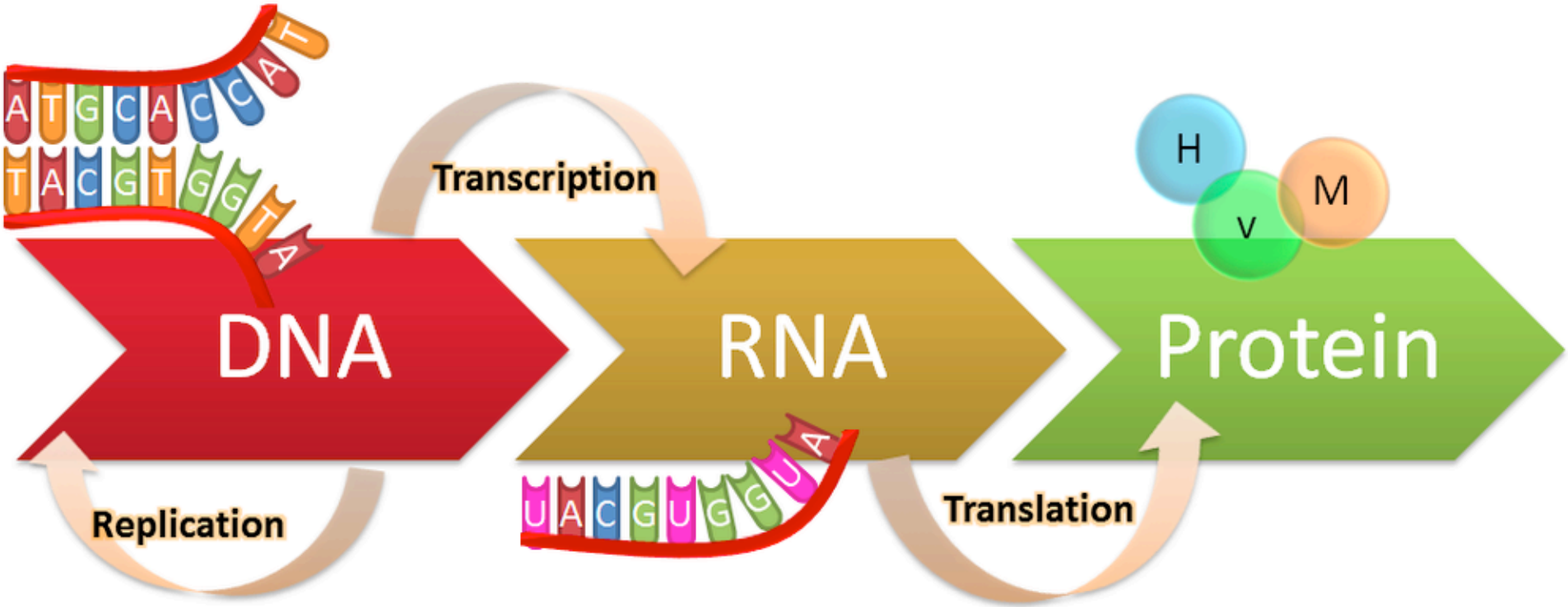
I Calcium signaling

II Calmodulin

III Protein engineering

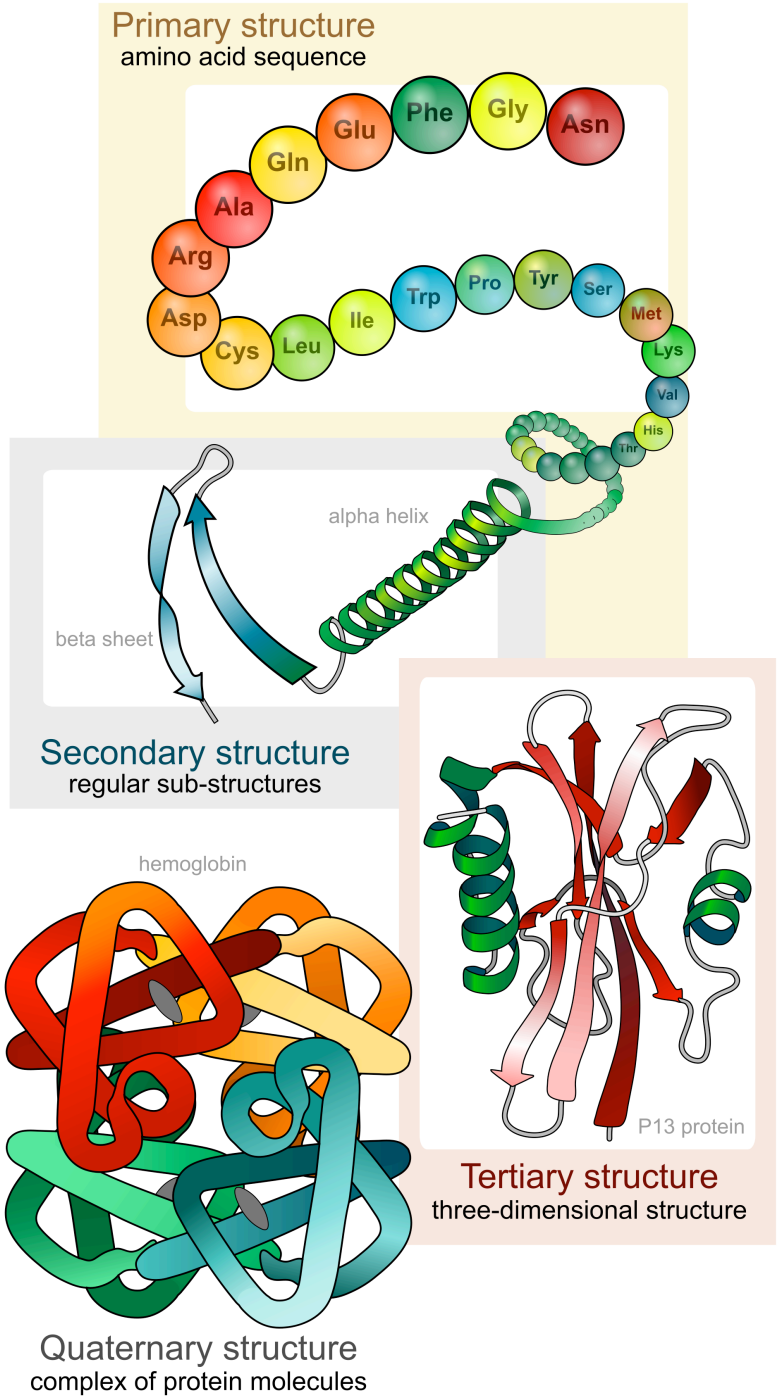
2/9/16

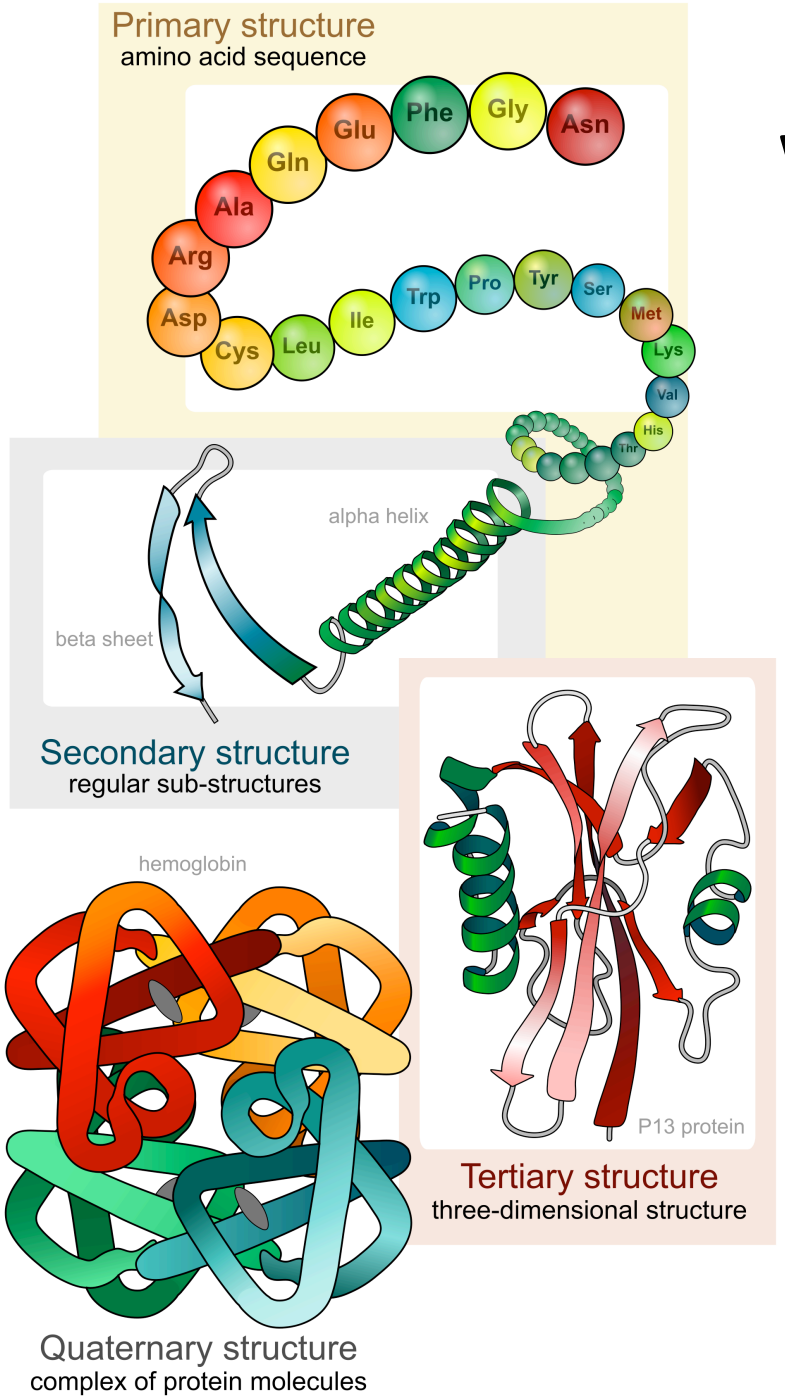
The central dogma



http://genius.com/Biology-genius-the-central-dogma-annotated

What are proteins?





What are proteins?

- Large macromolecules consisting of one or more long chains of amino acids
- Function related to conformation
  - H-bonding
  - Ionic interactions
  - Van der Waals forces
  - Hydrophobic packing

Why do cells (we) need proteins?

Why do cells (we) need proteins?

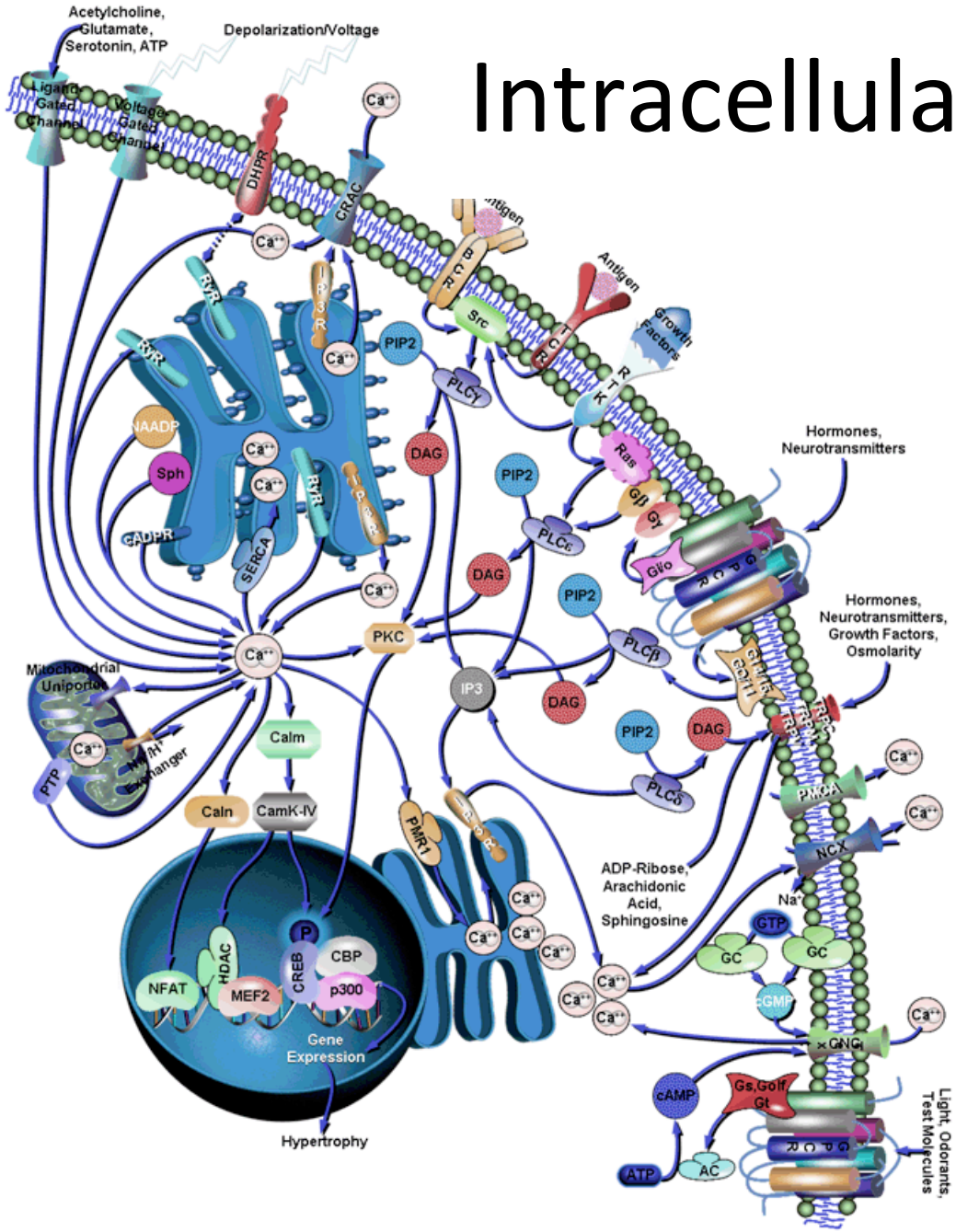
- Catalyze metabolic reactions
- DNA synthesis and maintenance
- Transport of molecules
- Structural and mechanical functions
- Propagation of and response to stimuli
- …and all other cellular processes

- Food

Your engineering task in Mod 2:

Alter Ca^{2+} binding properties of inverse pericam protein

Intracellular Ca^{2+} signaling

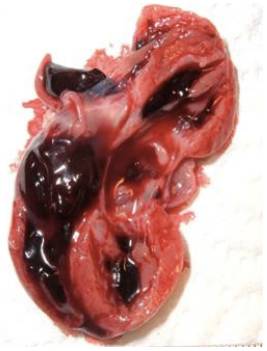


- Binding triggers change in protein charge and shape
- 20,000-fold gradient between intracellular and extracellular concentrations

Ca^{2+} is not just a structural element

Sidney Ringer, 1835-1910

= Contractions



≠ Contractions

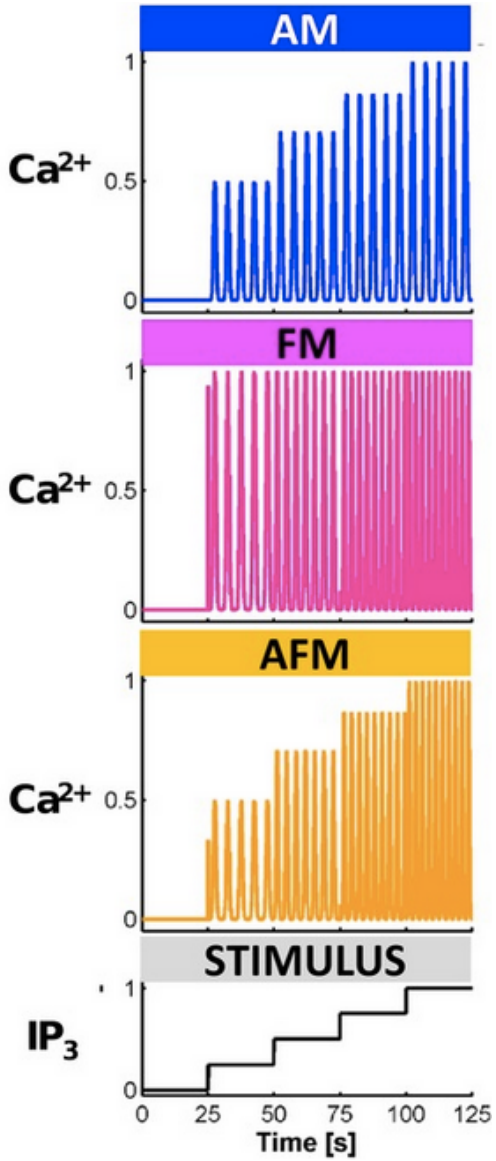
Calcium is an essential messenger

- Contracts frog muscle fibers
- Activates ATPase activity of myosin
- Binding to myofibrils activates actomysin
- Accumulated in sarcoplasmic reticulum vesicles
- Receptor, troponin C, mediates myofibrillar contractions
- EDTA chelation relieves muscle contractions

Calcium signal is tightly controlled

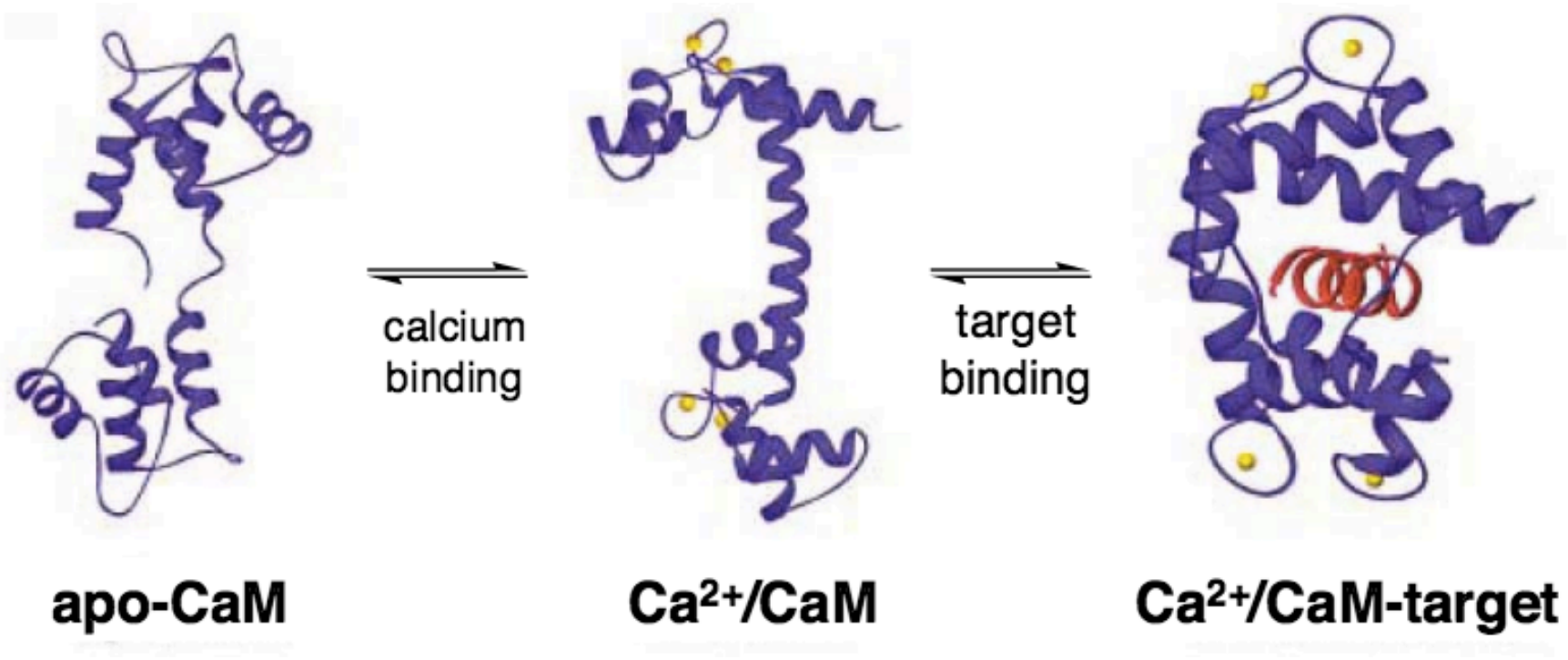
- Regulated by channels and transporters
  - Maintain low cytostolic concentrations
  - Enable dynamic and rapid changes in stores
- Transduces signals from extracellular sources: hormones and growth factors
- Amplitude and frequency code for the signal
  - AM, FM, or AFM

How is the Ca^{2+} signal coded?



- AM (amplitude modulations)
- FM (frequency modulations)
- AFM (amplitude and frequency modulations)

Calmodulin (CaM) translates Ca^{2+} code



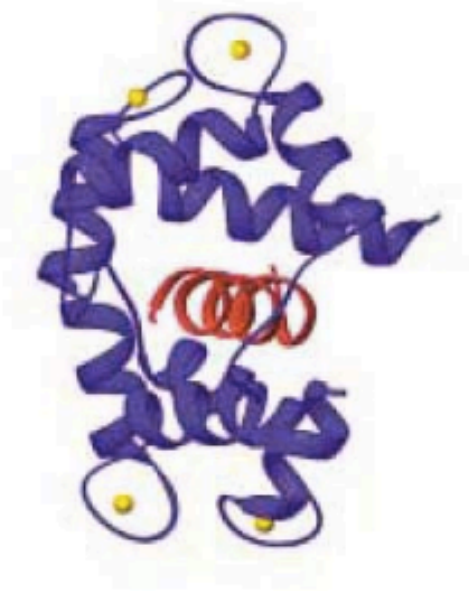
Vetter *et al*. (2003) *Eur J Biochem* 270:404-414.

Ca^{2+}/CaM binding is dynamic

- Apo-CaM N-terminal domain in ‘closed’ conformation
- Apo-CaM C-terminal domain in ‘semi-open’ conformation
- C-terminal domain 3-5x higher affinity for Ca^{2+}
- Ligands coordinate Ca^{2+} binding
- Cooperative binding of Ca^{2+}

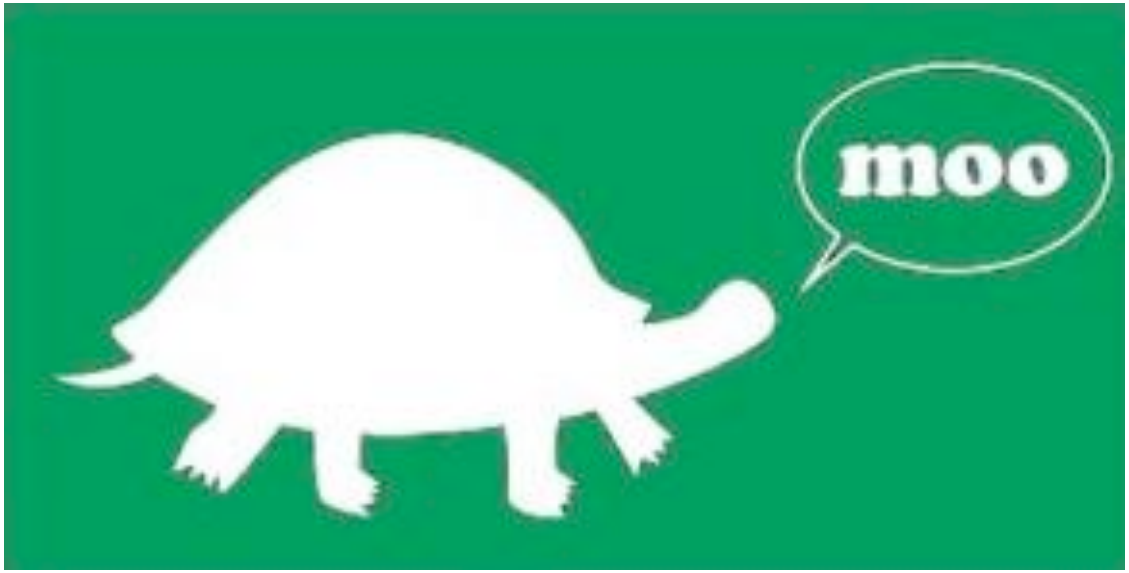
Ca^{2+}/CaM binds peptides

- Relieve autoinhibition
- Remodel active sites
- Promote dimerization



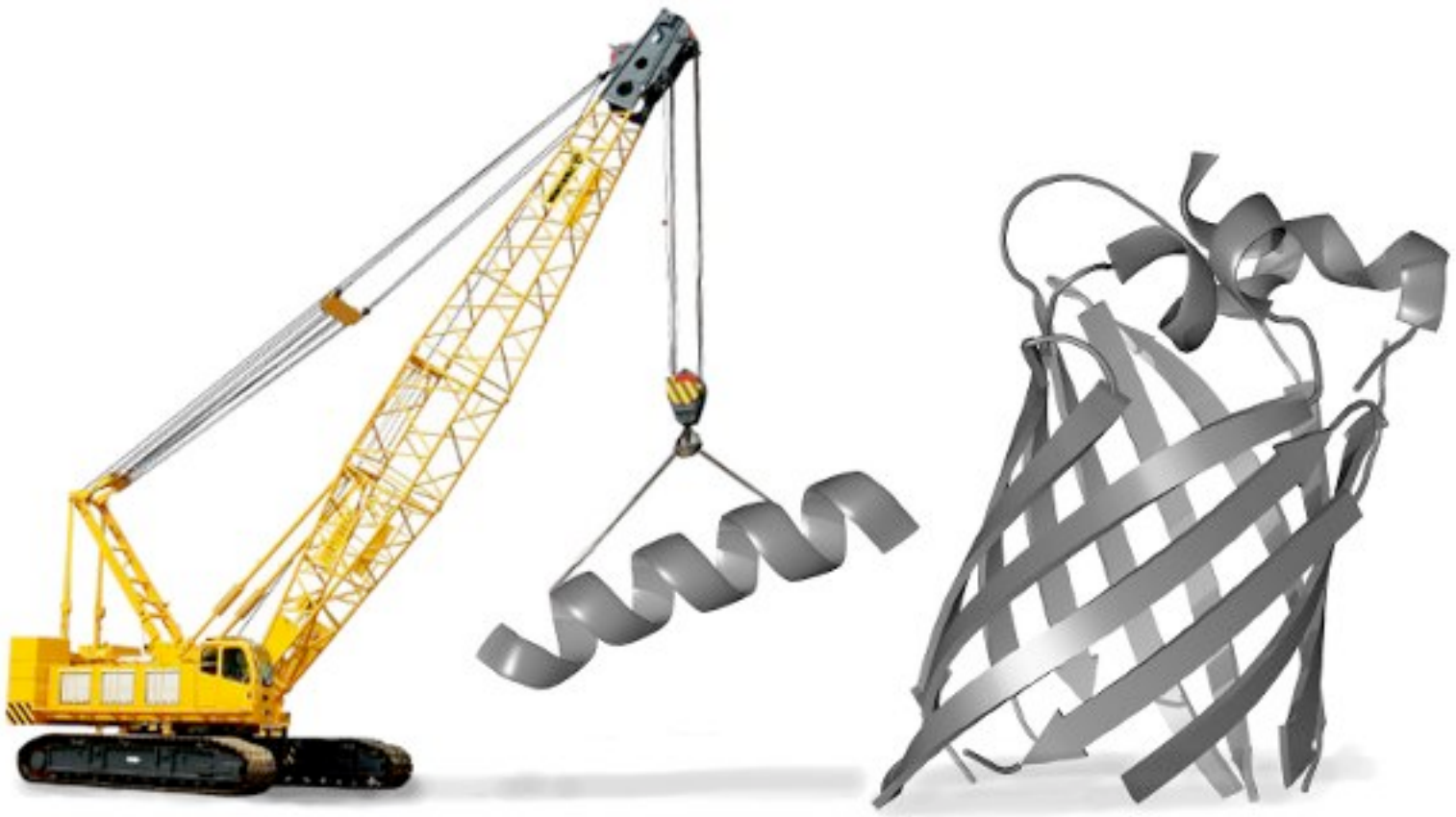
Ca^{2+}/CaM-target

So what. Now what?



We are biological *engineers*!

Protein engineering

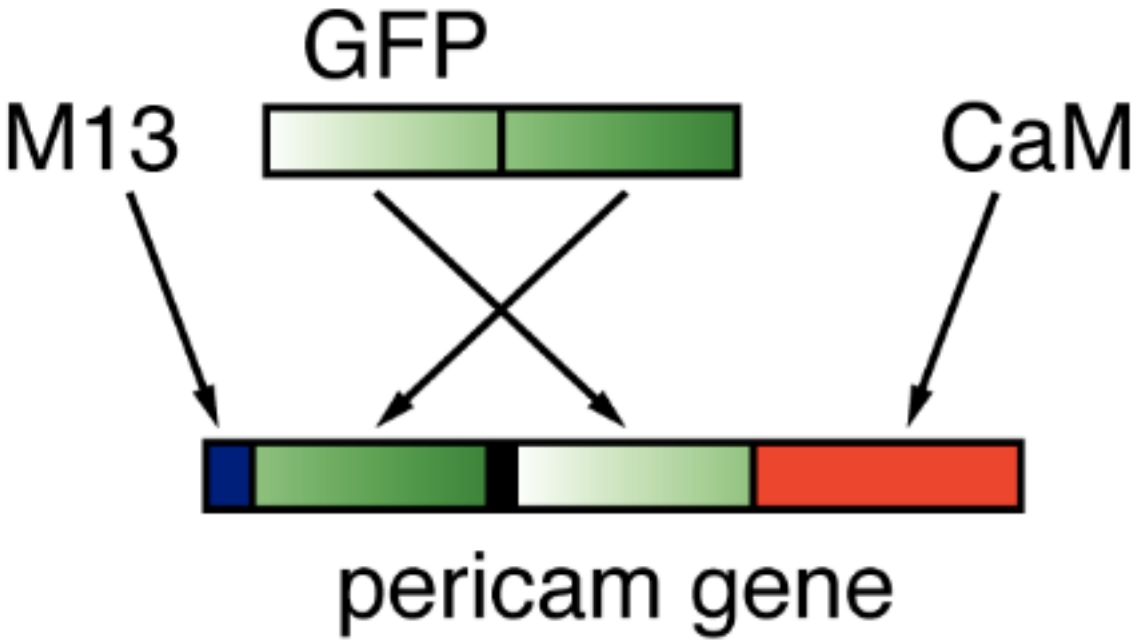


Mechanisms for engineering proteins

- Rational design
- Random library screens
- Directed evolution

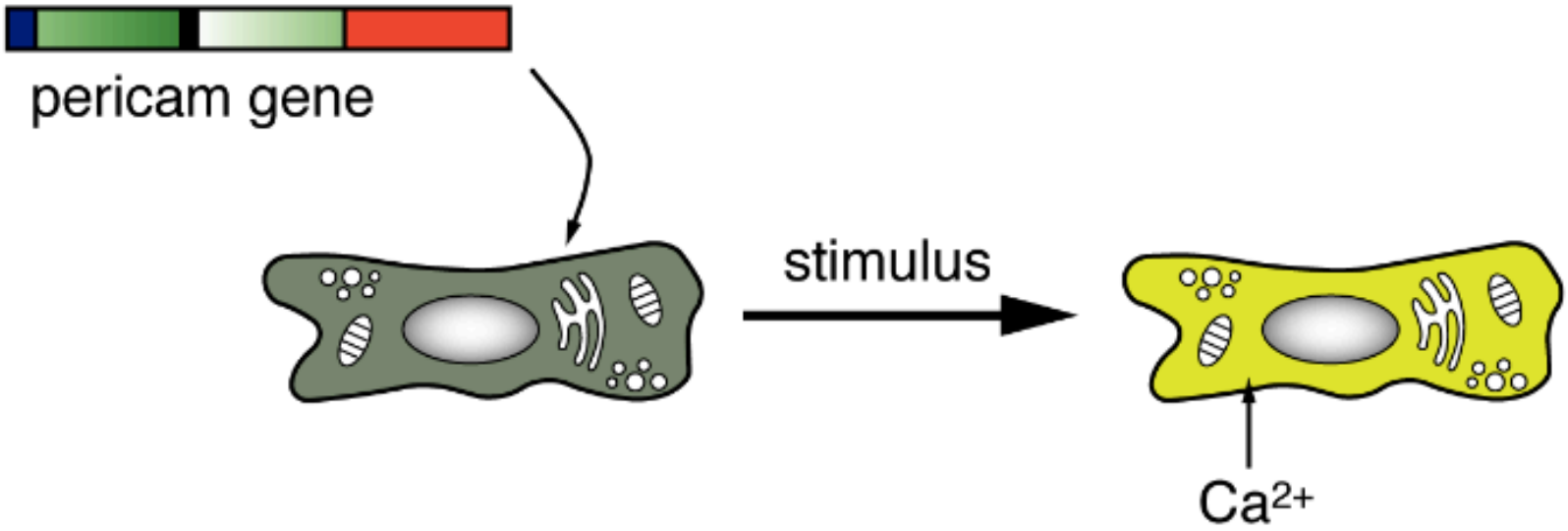
What if you engineered a Ca^{2+} sensor?

Pericam design strategy



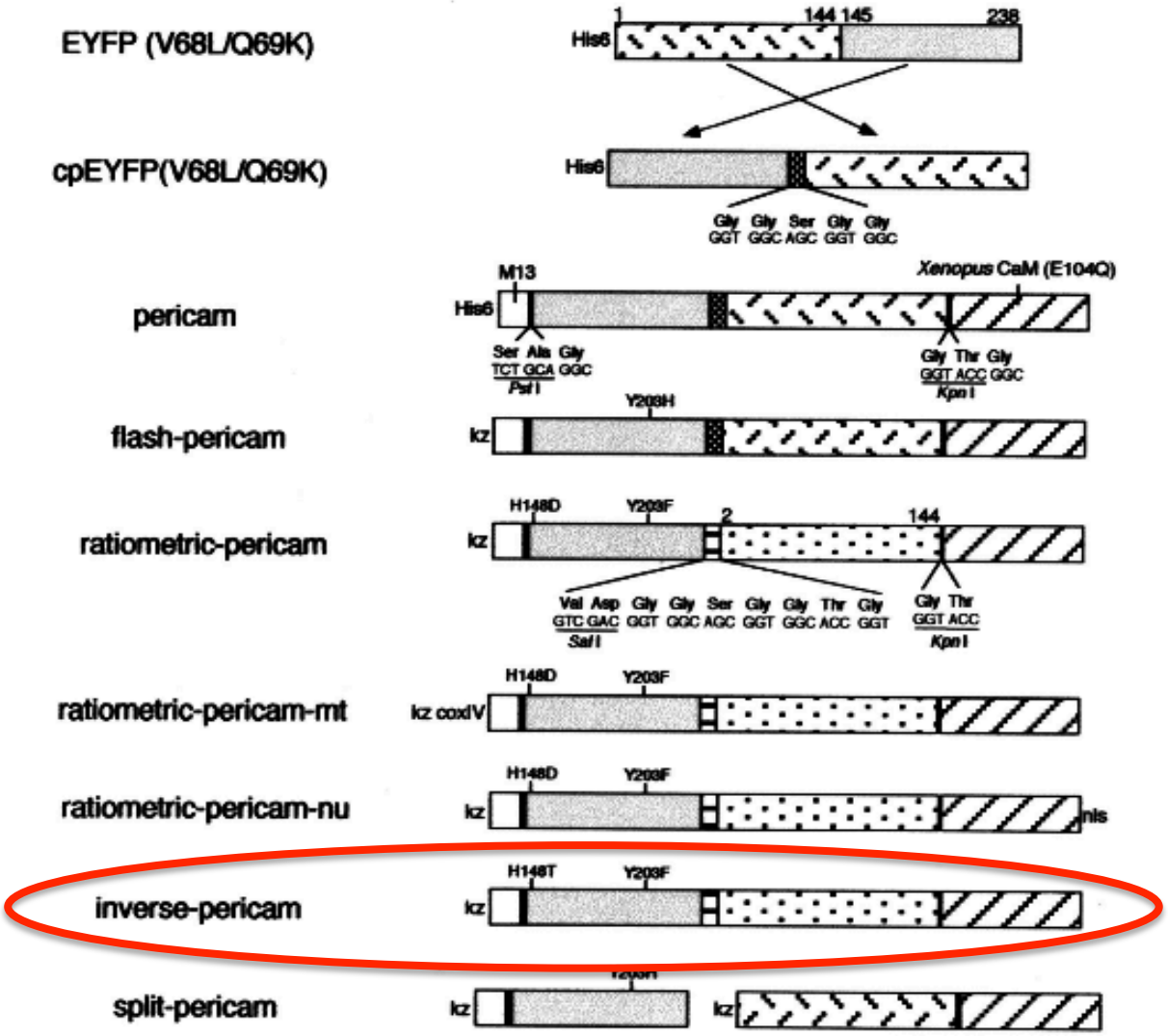
- GFP = green fluorescence protein
- CaM = calmodulin
- M13 = CaM binding peptide of cellular kinase

Pericam protein monitors free Ca2+



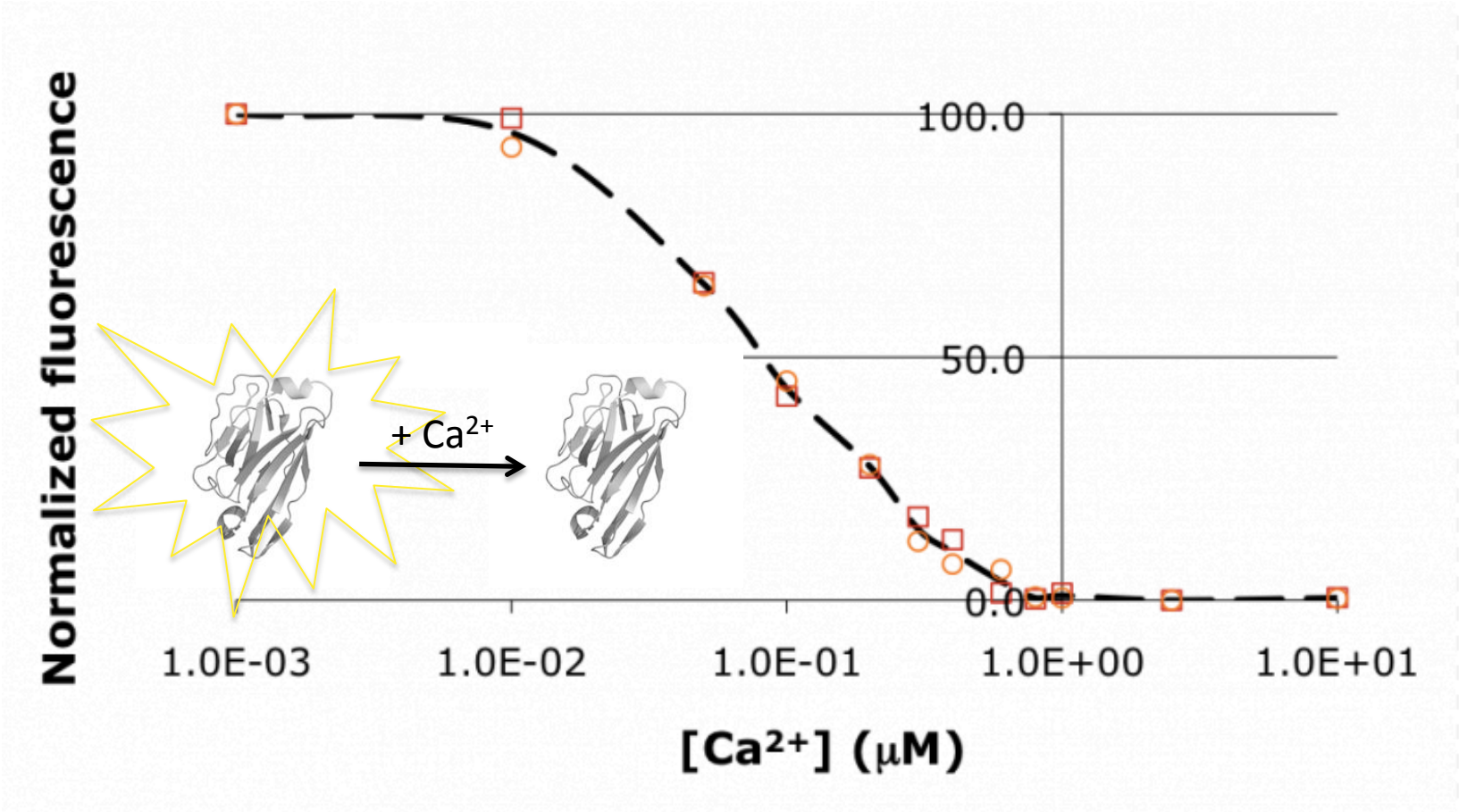
Protein-based machine for measuring [Ca^{2+}]

Suite of pericam constructs



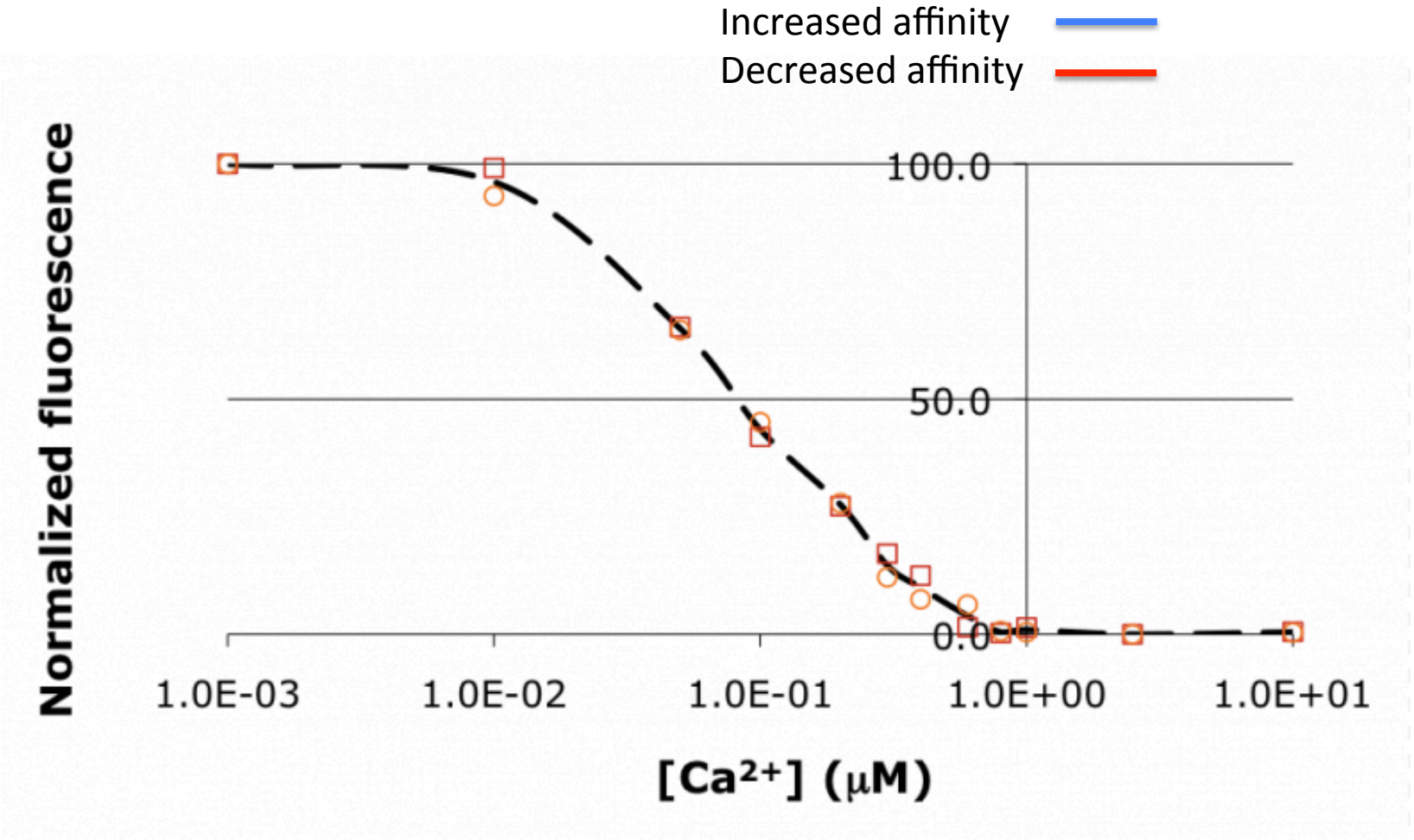
Nagai *et al*. (2001) *PNAS* 98:3197-3202.

Inverse pericam (IPC) dims with Ca^{2+}

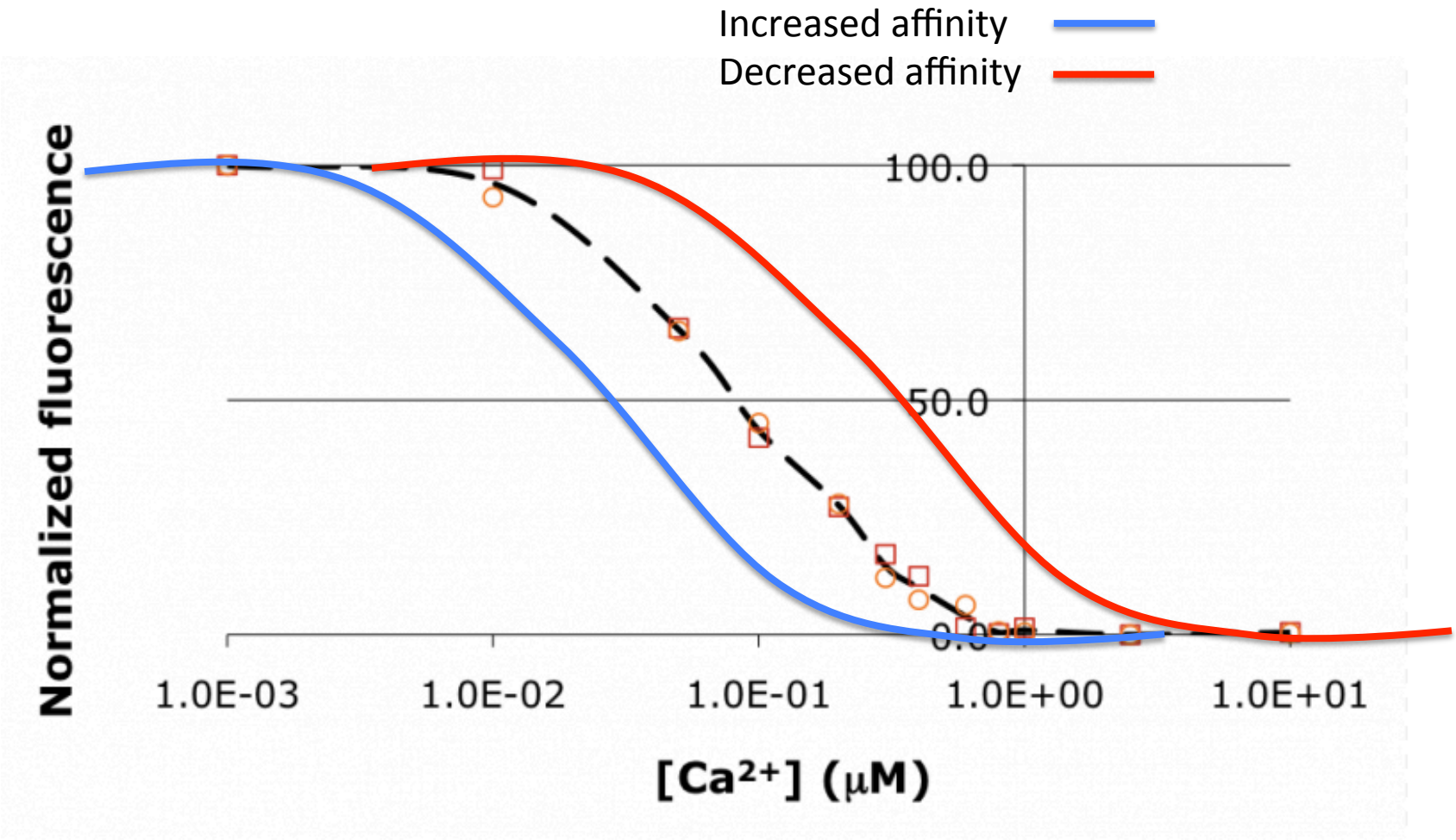


What parameters are we changing?

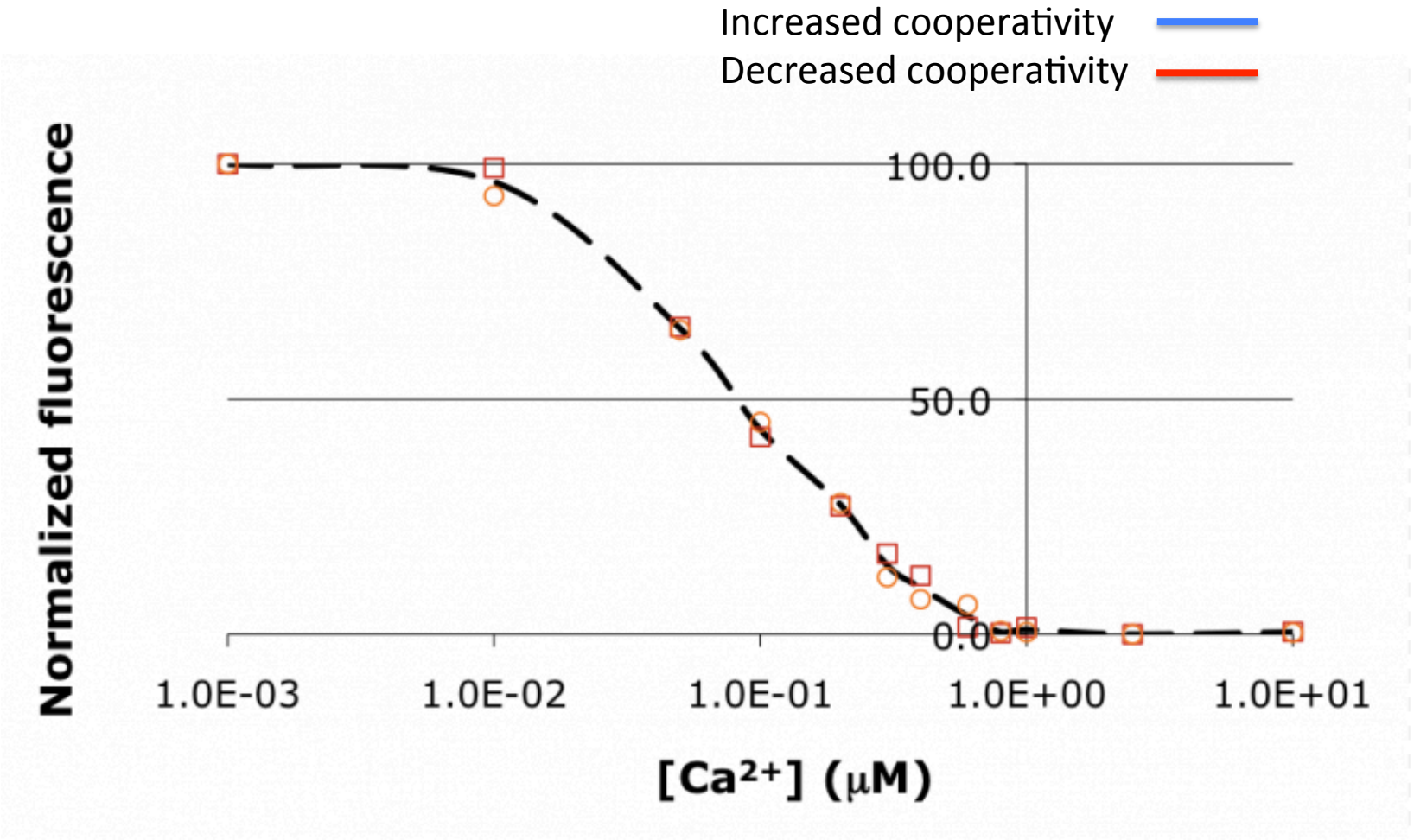
How do our mutations alter binding?



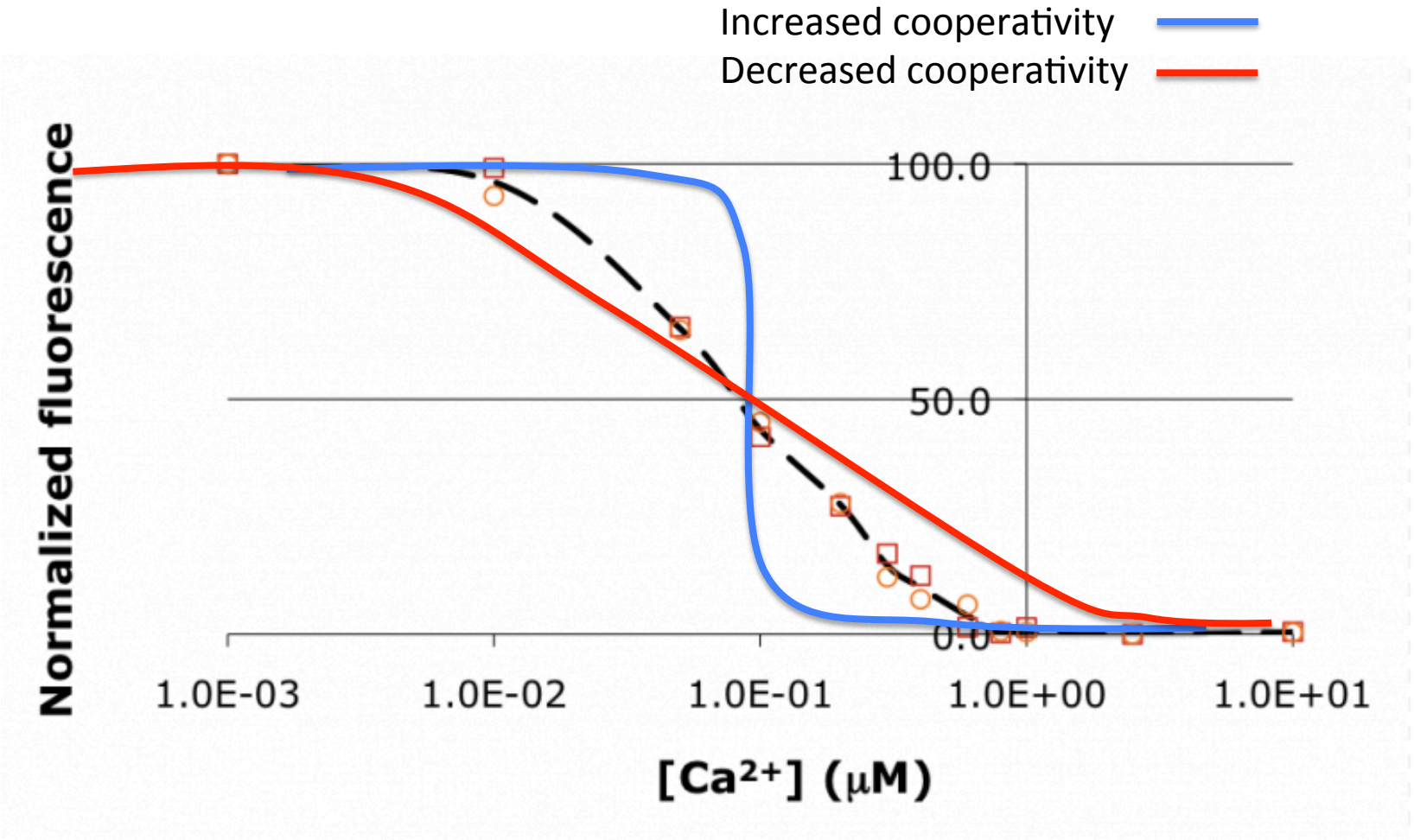
How do our mutations alter binding?



How do our mutations alter binding?

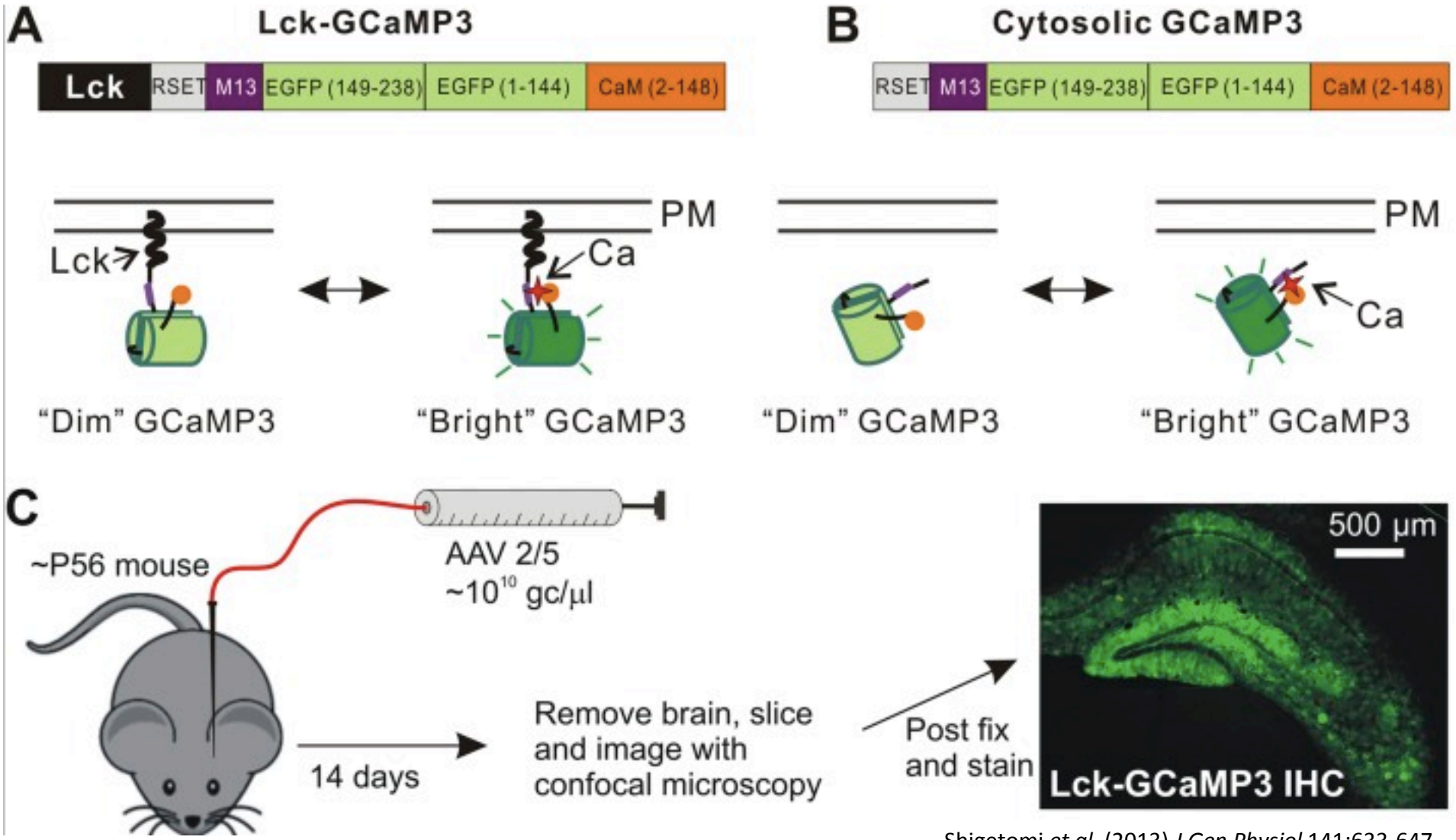


How do our mutations alter binding?



Why engineer a Ca^{2+} sensor?

Astrocytes propagate Ca^{2+} signals to neurons and blood vessels

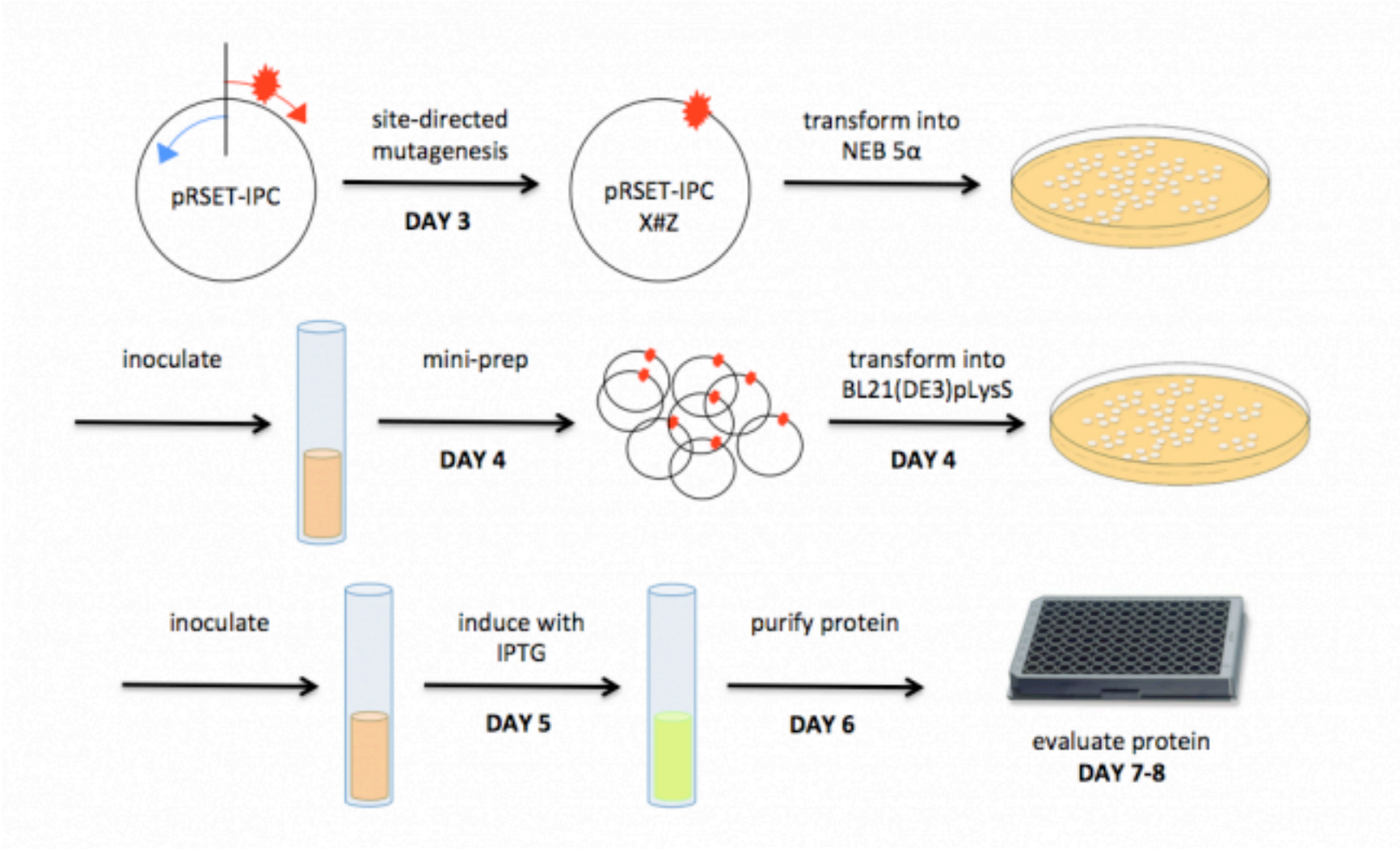


Shigetomi *et al*. (2013) *J Gen Physiol* 141:633-647.

Which brings us back to Mod 2…

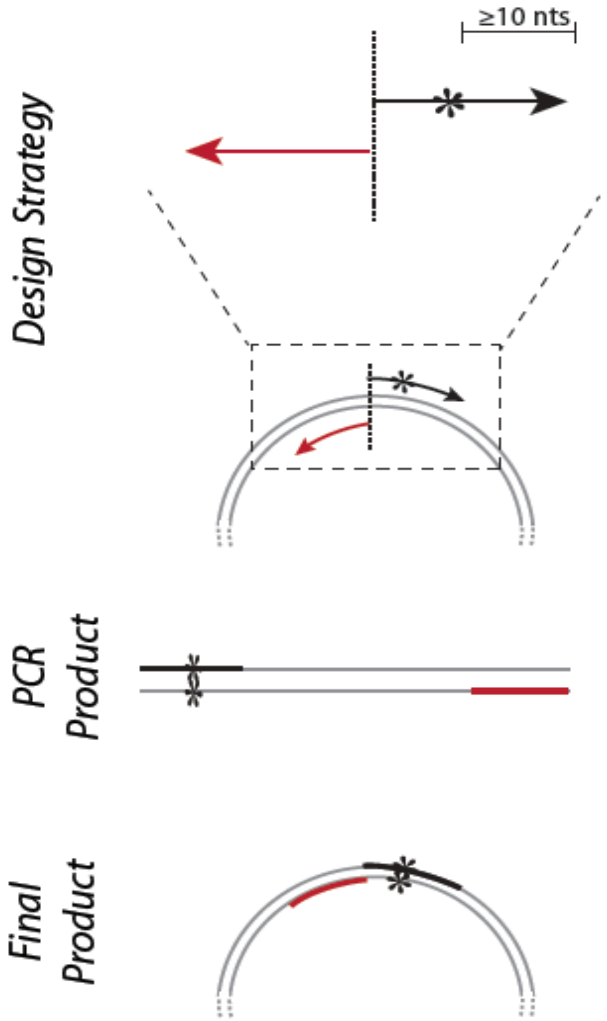
- Aim: alter binding affinity and/or cooperativity
- Skills:
  - Use computational tools to rationalize protein engineering strategy
  - Perform mutagenesis reaction to alter protein sequence
  - Purify protein
  - Assess calcium binding behavior with titration assay

Mod 2 overview



How do we engineer a Ca^{2+} sensor?

Site-directed mutagenesis



- Can mutate 1-3 bp
  - 1-2 bp is ideal
- Mutation located in center of forward primer
- Forward and reverse primer anneal ‘back-to-back’

In the laboratory…

- Familiarize yourself with IPC sequence
- Identify mutagenesis site
- Design mutagenesis primers

- For next time…

Circularly permuted green fluorescent proteins engineered to sense Ca^{2+}

Takeharu Nagai, Asako Sawano, Eun Sun Park, and Atsushi Miyawaki*

Laboratory for Cell Function and Dynamics, Advanced Technology Development Center, Brain Science Institute, Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako, Saitama, 351-0198, Japan

Communicated by Roger Y. Tsien, University of California, San Diego, CA, December 30, 2000 (received for review December 11, 2000)