Module 2 overview

*lecture*

1. Introduction to the module
2. Rational protein design
3. Fluorescence and sensors

*lab*

1. Start-up protein eng.
2. Site-directed mutagenesis
3. DNA amplification

**PRESIDENT’S DAY**

4. Review & gene analysis
5. Protein expression
6. Purification and protein analysis
7. Binding & affinity measurements
8. High throughput engineering

4. Prepare expression system
5. gene analysis & induction
6. Characterize expression
7. Assay protein behavior
8. Data analysis
Lecture 1: Introduction to the module

I. Engineering proteins

II. Pericam: an engineered protein sensor
   A. Imaging calcium signaling
   B. Calmodulin and GFP
   C. Pericam variants

III. Reengineering Pericam: experimental overview
   A. Structure-based design
   B. Protein expression and purification
   C. Measurements and analysis

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Pericam: a protein-based machine for measuring $[\text{Ca}^{2+}]$
Calcium indicators can be used to detect signaling in individual cells and multicellular ensembles. Two purposes:
  • learn what stimuli trigger calcium fluctuations and how calcium behaves in context of an organism or system
  • use calcium as a “handle” on cell-cell interaction (e.g. neural activity)
Calcium is important to cellular signaling in the immune system. Activation of B-cells can be detected by calcium imaging in lymph nodes (Qi et al., 2006, Science).
Pericam is a second generation calcium sensor, based on design strategies originally developed by Roger Tsien and colleagues. Tsien won a 2008 Nobel Prize for engineering novel forms of GFP.
Calmodulin (CaM) facts and figures

• 16-18 kD (depending on species), ~20 x 40 Å protein
• highly conserved among eukaryotes (vertebrate and yeast calmodulin are functionally interchangeable)
• binds four Ca\(^{2+}\) ions using EF hand amino acid sequence motifs
• Ca\(^{2+}\)-CaM binds short segments of target proteins, modulates activity
EF hand binding motif named for E & F helices of the calcium-binding protein parvalbumin; example of helix-loop-helix structure, with calcium bound in the loop
Ca\(^{2+}\)-saturated CaM binds to peptides by “grasping” target sequences, in helical conformation, between N- and C-terminal domains. In many cases, this activates an enzyme by sequestering an inhibitory domain (e.g. M13 from MLCK). Interactions between CaM and targets involve hydrophobic contact area and charge-charge interactions.

\[ \text{Ca}_{2+} \text{-saturated CaM} \]

Green Fluorescent Protein (GFP)

from the jellyfish *Aequoria victoria* is a protein fluorophore and component of genetically-encoded calcium indicators. The molecular structure (1996) shows a chromophore formed by spontaneous cyclization and oxidation of three amino acids (Ser/Thr65, Tyr66, and Gly67).

parent construct
dynamic range
ratiometric $\Delta F$
mitoch. localization
nuclear localization
inverse $\Delta F$
fun
Mutations can also affect calcium sensitivity; both $K_d$ (affinity) and cooperativity (slope/shape of transition) can be affected. Miyawaki et al. engineered calcium sensitivity of CaMeleons, a related type of engineered protein calcium sensor:

In this module, our goal will be to influence the calcium sensitivity of "inverse pericam."
Step 1: Design and implement mutations to affect inverse pericam’s calcium sensitivity.

**Skills:**
- Use computational tool to look closely at protein structures
- Design primers to make site mutations in the pericam gene
- Perform mutagenesis using PCR
Step 2: Express and purify mutant inverse pericams for analysis.

Skills:
- Transform plasmid DNA into *E. coli*
- Induce protein expression using IPTG
- Purify mutant pericams using affinity-based separation
- Assay protein expression and purity using SDS-PAGE
Step 3: Analyze calcium titration behavior of mutant pericams.

Skills:

• Perform fluorescence assays to measure calcium binding
• Use software to extract binding parameters from the data
• Pool data from across the class to observe patterns
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