DNA Template Synthesis

DNA template: each 10 nt region encodes different reagents

- hybridize
- coupling DCC/NHS
- affinity elute, streptavidin
- continue

PCR amplify/sequence
Biological Probes
- Ca\(^{2+}\) ~ important signaling messenger in the cell [Ca\(^{2+}\)]
- Develop Ca\(^{2+}\) sensors for live cell imaging
- Based on Ca\(^{2+}\) chelator, in which fluorescence $\lambda$ or $\Phi_F$
  is sensitive to [Ca\(^{2+}\)]

**FURA2** (by Tsien's lab): Ca\(^{2+}\) sensor

Ca\(^{2+}\) binding leads to shift in $\lambda$ from 385 nm to 350 nm
$K_d \sim 135$ nM
(close to physiological cellular Ca\(^{2+}\) concentration)

**Photocaged Ca\(^{2+}\) chelators:**
Release of Ca\(^{2+}\) with control over spatial and temporal distribution of Ca\(^{2+}\) in cell

**NITR-5**

hv leads to a 12,500-fold decrease in affinity for [Ca\(^{2+}\)]
Imaging agents for proteins

long wavelength fluorophores/chromophores to image or release in vivo

image agents for proteins
1. fixed cells
2. live cells

Snap-Tag

genetic fusion

label with fluorophore

182 residue protein
O-6-methyl-guanine DNA methyl transferase: suicide enzyme that dealkylates O-6-methyl-G

X = Tag, fluorophore, photoaffinity label
**Biotin Ligase**

- Biotin ligase
- Recognition sequence: 15 aa
- Biotin ligase mutant

**FLAsH**

- Free rotation leads to radiationless conversion, low $\Phi_F$
- Tetra Cys motif
- Radiationless conversion (heat)
- $\Phi_F$ increases when rotation is restricted

**SFP**

- Synthetase phosphopanethienyl transferase (Spf)
- 11 amino acid tag
- $R =$ fluorophore or other reagent

**Downsides:**
- Reacts with other thiols ~ some cytotoxicity

**Engineer target 4 Cys in $\alpha$-helix to react with FLAsH**

**WT and/or mutant enzymes can accept various R groups**
**GFP (Green Fluorescent Protein)**

Green Fluorescent Protein as a biological genetically encoded tag

- **GFP Tag**
  - Genetic fusion to N- or C-terminus
  - ![GFP Tag](image)

- **Tyr66**
  - ![Tyr66](image)

- **Gly67**
  - ![Gly67](image)

- **Ser65**
  - ![Ser65](image)

- **O2**
  - ![O2](image)

- **-H2**
  - ![H2](image)

- **Screen for mutants with altered photophysics**
  - Y66H Blue
  - Y66W Cyan
  - Thr203Y Yellow

**FRET (Förster Resonance Energy Transfer)**

Distance/orientation

- 436 nm
  - ![436 nm](image)

- 480 nm
  - ![480 nm](image)

- 514 nm
  - ![514 nm](image)

- 527 nm
  - ![527 nm](image)

- Efficiency
  - 1. distance between dyes
  - 2. orientation of dye dipoles
  - 3. spectral overlap of dye pairs

- **Efficiency**
  - ![Efficiency](image)

- **R0**
  - ![R0](image)

- **Protein-Protein Interaction**
  - ![Protein-Protein Interaction](image)

- **Cellular Localization**
  - ![Cellular Localization](image)

- **CaM :Ca2+ binding protein**
  - ![CaM :Ca2+ binding protein](image)

- **Ca2+ binding loop**
  - ![Ca2+ binding loop](image)

- **CaM changes conformation on Ca2+ binding**
  - ![CaM changes conformation on Ca2+ binding](image)
Phospho-Ser Binding Protein

- kinase
- phosphorylation leads to altered conformation
- CFP
- YFP
- Phospho-Ser binding protein
- phosphopeptide
- Lys/Arg rich

Serine Hydrolase

- serine hydrolase family
- reactivity based approach to protein family profiling
- F-P-O-NH-biotin
- streptavidin
- sequence protein

Kinases

- Protein
- Kinases
- Protein
- Phospho-Ser binding protein
- phosphopeptide

- v-Src (I338G)
- wt kinase
- mutant kinase
- selective inhibition for mutant kinase

- ATP is still a substrate
- IC_{50} WT/mutant (nM)

Enzyme families activities associated with disease states, which results in increased protein expression and band intensity.