The team

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Inspiration – Valencia 2009
Our idea

SOMY-LCD

super, optimized, modified, yeast - light-emitting, cell, display
Goals

- Blue $\rightarrow$ Multiple colors
- Aequorin $\rightarrow$ GECO
- Minutes $\rightarrow$ Milliseconds
- 96-well plate $\rightarrow$ Improved control device
Lab work
GECO

- Genetically encoded Ca$^{2+}$ indicators
- Detection of the intracellular Ca$^{2+}$ concentration
- R-GECO
- G-GECO
- B-GECO

(Campbell et al., Science 2011)
CCH1-MID1 channel

- Over expression
- Homologous to mammalian voltage-gated calcium channels
- Depolarization plasma membrane $\rightarrow$ calcium ions enter cytoplasm

(Iida et al., 2007)
Ideal yeast cell
Yeast

- Stable
- Simple eukaryote
- Native calcium channels
- Ideal homeostatic calcium concentration
- Environmental safety

- Shuttle vector
Approach
GECO characterization

R-GECO

Intensity (a.u.)

Wavelength (nm)

0 nM Ca
616 nM Ca
808 nM Ca
1000 nM Ca
1200 nM Ca
1500 nM Ca
1700 nM Ca
2000 nM Ca
2400 nM Ca
2900 nM Ca
3600 nM Ca
3900 nM Ca
Yeast characterization

Emission spectra of INVSc1 + GECO, CCH1, MID1 @ 565 nm excitation

Fluorescence intensity (a.u.)

Wavelength (nm)

R-GECO
G-GECO
B-GECO
R Ctrl.
G Ctrl.
B Ctrl.
Initial model

• Neuronal cell of bullfrog
• Parameter sensitivity analysis

• Conclusion:
  Model not fully applicable due to unknown yeast parameters

(Koch and Segev, 1989)
Combined models

- Calcium homeostasis in yeast
- Voltage-dependent calcium channels
- Kinetics of the GECO-proteins

(Cui et al., 2009)

(Zhao et al., 2011)
Combined models

\[
\frac{dCaM(t)}{dt} = k_{M}^{+}([CaM_{\text{total}}] - CaM(t)) \cdot Ca(t)^{3} - k_{M}^{-}CaM(t)
\]

\[
\frac{dCaN(t)}{dt} = k_{N}^{+}([CaN_{\text{total}}] - CaN(t)) \cdot CaM(t) - k_{M}^{-}CaN(t)
\]

\[
\frac{dCrz(t)}{dt} = d \cdot \phi(1/CaN(t)) \cdot (1 - Crz(t)) - f \cdot (1 - \phi(1/CaN(t))) \cdot Crz(t)
\]

\[
\frac{dCaGECO(t)}{dt} = k_{on}([GECO_{\text{total}}] - CaGECO(t)) \cdot Ca(t)^{n} - k_{off}CaGECO(t)
\]

\[
\frac{dm(t, V)}{dt} = \frac{m_{\infty}(V) - m(t, V)}{\tau_{m}(V)}
\]

\[
\frac{dCa(t)}{dt} = \frac{V_{2} \cdot [Ca_{ex}]}{K_{2} + [Ca_{ex}]} - \frac{Crz(t)\theta}{\text{Transporter X}} - \frac{V_{1} \cdot Ca(t)}{K_{1} + Ca(t)}
\]

\[
- \frac{1}{K_{2} + Ca(t)} \cdot \frac{V_{2} \cdot Ca(t)}{Pmc1} - \frac{1}{1 + k_{c}CaN(t)K_{3} + Ca(t)} \cdot V_{3} \cdot Ca(t)
\]

\[
- n(k_{on} \cdot CaGECO(t) - k_{off} \cdot Ca(t)^{n}([CaGECO_{\text{total}}] - CaGECO(t)))
\]

\[- \alpha Ca(t)\]
Calcium homeostasis

Conformational switch model
Calcium channels
GECO kinetics

[Diagram showing the interaction between X-GECO, Vacuole, Vcx1, Pmc1, Calmodulin, Calcinurin, Crz1, Pmr1, PMR1, PMC1, and Nucleus, with Transporter-X and Ca^{2+} in the center.]
Modelling results

80% decrease
Device
Specifications

• 16 pixels
• Moving image using one signal
  • Refresh time: 1 ms per pixel
• Maximum of 20 V
• Software can easily be extended
In use
Accomplishments

• Succeeded in an iGEM yeast-project
• CCH1/MID1/X-GECO in yeast
• BioBricks of red, green and blue GECO
• Working computational model
  • Basis for future iGEM teams
  • Easy to extend
• Working device and elaborate software
Human Interest

• Construct is suitable for calcium research
• Insights in calcium fluxes and pathways
• Insights in pathologies
Collaborations

• Two offers on ‘Matchmaker’
  [team: NTNU-Trondheim]
  • Modelling help
    [team: Queens-Canada] [team: Trieste]
• Responded to request on ‘Matchmaker’
  • Input ‘tips and tricks’ new teams
    [team: Cinvestav-IPN-UNAM]
• Two symposia (July 9th and October 3rd)
  [teams: Dutch teams]
Teaching 14 year-olds
Discovery Festival

• Art – Music – Science
• Synthetic biology workshop
• National effort:
  • Three locations
  • Four iGEM teams: Amsterdam, Groningen, TU-Eindhoven and Wageningen_UR

• Day: 300 high-school students
• Night: 5000+ visitors
Discovery Festival
Discovery Festival
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