Photomodulation of proteins in living cells and animals

Chemical caging

Optogenetics – channel proteins

Dimerization
  Phy-PIF
  Cryptochrome
  LOV
Steric blocking with LOV
Chemical caging

$$\text{CNB} = \begin{array}{c}
\text{O} \\
\text{C} \text{--OH} \\
\text{CH}^- \\
\text{NO}_2
\end{array}$$

$$\text{NPE} = \begin{array}{c}
\text{CH}_3 \\
\text{CH}^- \\
\text{NO}_2
\end{array}$$

$$\text{DMNB} = \begin{array}{c}
\text{CH}_3 \\
\text{O} \\
\text{CH}_3 \\
\text{CH}_2^- \\
\text{NO}_2
\end{array}$$

$$\text{DMNPE} = \begin{array}{c}
\text{CH}_3 \\
\text{O} \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}^- \\
\text{NO}_2
\end{array}$$

$$\text{CMNB} = \begin{array}{c}
\text{O} \\
\text{HO-C-CH}_2^- \\
\text{NO}_2
\end{array}$$

Chemical caging:

$$\text{Nitrophenyl ester} \xrightarrow{\text{hv}} \text{Nitroxide} \xrightarrow{\text{hv}} \text{Unprotected peptide}$$

Caged glutamate:

$$\text{Nitrophenyl ester} \xrightarrow{\text{hv}} \text{Nitroxide} \xrightarrow{\text{hv}} \text{Unprotected peptide}$$

Photocleavage:

$$\text{Nitrophenyl ester} \xrightarrow{\text{hv}} \text{Nitroxide} \xrightarrow{\text{hv}} \text{Unprotected peptide}$$
Acetoxy methyl ester (AM ester) cell loading
A New Strategy for Caging Proteins Regulated by Kinases
Mousumi Ghosh, Ilia Ichetovkin, Xiaoyan Song, John S. Condeelis, * and David S. Lawrence*
JACS 124, p2440.

**site-specific protein labeling**
- cysteine
- unnatural amino acid mutagenesis
- semisynthesis – peptide ligation

**cell loading**
- microinjection
- mechanical loading (bead, scrape, syringe…)
- electroporation
- import peptides

**in vivo labeling**
- unnatural amino acid mutagenesis
- labeling *in vivo*
photoactivatable rapamycin

- Hahn and Deiters – JACS 2010
- Inoue – Nature Meth 2011
Optogenetics with channelrhodopsins

channelrhodopsin2  halorhodopsin

retinol  halorhodopsin


• Deisseroth
• Ehrenberg/Huisken
• Hegemann/Moeglich
and many more
Photosensors in Plants

http://www.unil.ch/cig/page64757_en.html
Phytochrome – PIF interaction

Whitelam et al., *Curr Biol.* (1999)
Yang et al., *PNAS* (2009)
Controlling membrane dimerization using Phytochrome – Pif dimerization
Levskya, Weiner, Lim and Voigt; Nature 2009b

- precise spatial control – 2 wavelengths
- exogenous cofactor
- complexity in animals
Manipulating actin dynamics using Phytochrome – Pif dimerization
Rosen et al. PNAS 2008
Activation of protein splicing with light in yeast

Amy B Tyszkiewicz & Tom W Muir

Figure 1 | The light-activated protein splicing system. (a) In this system PhyB and PIF3 are reversibly dimerized by 660 nm and 750 nm light. The split S. cerevisiae VMA intein (I° and I°) is thus reconstituted, and the N and C exetins are spliced via a peptide bond. (b) Domain organization of PIF3 and PhyB from A. thaliana (left). The PhyB photosensing domain is attached to a tetrapyrole chromophore (red) through Cys357 via a thioether linkage. NLS, nuclear localization sequence; bHLH, basic helix-loop-helix domain; HKLD, histidine-like kinase domain. Also shown are the conditional protein splicing constructs used in this study (right). Phy, photosensing domain of PhyB. MBP and Flag are model exetins.
Voit et al.
Blue light induced FKF1 and GIGANTEA (GI) proteins interaction

Yazawa et al., *Nat Biotech* 2009
Dimerization via Cryptochrome2 – CIB1 interaction
Kennedy, Tucker et al.
Nature Methods 2010
Photomanipulation of GTPase activity
Light-mediated steric occlusion

Yi Wu
U. Connecticut

Angelika Jaehrig
LOV (light oxygen voltage) domain of phototropin

phototropism

LOV domain

Flavin cofactor

β Sheet (LOV2 core)

Jα Helix

hv

dark
Mechanism of light-induced conformational change:

Salomon, Briggs et al., 2000

Harper, Gardner et al., 2004

Swartz, et al., 2001

Salomon, Briggs et al., 2000
Caging Rac1 – mediator of protrusion, ruffling ...
An effective LOV-Rac linkage
PA-Rac1 reversible protrusion - moving irradiation spot 30x real time
A reversible caged protein
Are rates of diffusion and reversibility compatible with repeated local activation?

FRAP
(YFP-PA-Rac1, 515 nm, Φ=10 μm)

Photoactivatable-GFP
(405 nm and 488 nm, Φ=10 μm)
Rac1 activity gradient generates prolonged cell movement

10μ circle

protrusion
retraction

PA-Rac1
PA-Rac1-T17N
Rac and Rho control actin structure and dynamics

Adapted from Raftopoulou & Hall, 2004
Directing neutrophil migration in living zebrafish
Path controlled by front edge illumination of cells expressing PA-Rac

Sa Kan Yoo, Qing Deng, Anna Huttenlocher  
U Wisconsin - Madison

Developmental Cell, 18: 226-236, 2010
Migrating border cells in live Drosophila ovary
Xiaobo Wang, Li He, Denise J. Montell - Johns Hopkins University

Extension to other proteins: PA-Rac crystal structure

LOV

Rac

Daniel Frey
Max Planck Heidelberg

Ilme Schlichting
Rosetta modeling of the LOV-Rac and LOV-Cdc42 interface - mutation of Cdc42 for effective caging

Rac-LOV interface

PA-Rac W56

PA-Cdc42 F56

Pak pulldown of occluded conformation

Pak

Cdc42

PA-Cdc42
PA-Cdc42 W
PA-Cdc42 W I539E

PA-Cdc42 W
PA-Rac1

Brian Kuhlman

Oana Lungu
Extending caging to other GTPases via addition of docking sequences

PAK fragment on Cdc42

PA-Cdc42

Cdc42
filopodia

Rac
protrusion
# Salt Bridge Mutation

<table>
<thead>
<tr>
<th>LOV-IPA Mutant</th>
<th>$k_{\text{on}}$ (M$^{-1}$ s$^{-1}$)</th>
<th>$k_{\text{off}}$ (s$^{-1}$)</th>
<th>$K_D$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L514K L531E Dark</td>
<td>$4.5 \pm 1.5 \times 10^2$</td>
<td>$1.1 \pm 0.4 \times 10^{-4}$</td>
<td>$245 \pm 5$</td>
</tr>
<tr>
<td>L514K L531E Blue Light</td>
<td>$2.5 \pm 0.1 \times 10^3$</td>
<td>$1.3 \pm 0.1 \times 10^{-5}$</td>
<td>$5.0 \pm 0.1$</td>
</tr>
</tbody>
</table>
NIH, Leukemia and Lymphoma Society, American Cancer Society, Deutsche Forschungs Gemeinschaft