Design of Light-regulated Proteins

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Time-resolved Crystallography – Need of a Trigger

Need trigger to initiate and synchronize dynamics within crystal:

Properties of a good trigger for time-resolved crystallography:
• fast
• spatially uniform
• reversible

→ use light as a trigger
Light – A Trigger with Exquisite Spatiotemporal Control

Use of light as trigger with high spatiotemporal control:
- pulse duration: femto- to picoseconds
- focal spot size: ≤ micrometers

Use in time-resolved X-ray crystallography ... and in other applications

Phototropism in Arabidopsis thaliana:

(Blue light from the left, 5 hrs real time)

Rajagopal et al. (2005) Structure 13, 55-63. (Hangarter lab, Indiana University)

→ but most systems do not naturally respond to light!

Photolabile Chemically Caged Compounds

- versatile
- small
- reversible?
  → data accumulation on single crystal
- fast?
  → photochemistry should not be rate-limiting
- side reactions?
  → heterogeneity, damage to crystal
- diffusion?
  → restrict photoexcitation to small volume
- in vivo?
  → application in living organisms

→ alternatives, improvements?
→ "naturally caged compounds"?
“Naturally Caged Compounds”: Photoreceptors

- examples: photoactive yellow protein (PYP)
  visual pigment rhodopsin
  plant phototropins

- detection of light by non-protein chromophores

- modular composition: sensor and effector domains

→ Can we design artificial photosensors?
→ Can we make genetically encoded caged compounds? (analogy to GFP)

Light-oxygen-voltage (LOV) Proteins

- flavin-binding blue light sensors that form a subset of the Per-Arnt-Sim (PAS) family

- absorption of blue light promotes formation of thioether bond
  → long-lived signaling state: change of protein activity

- LOV domains naturally coupled to highly civerse effector domains including kinases, transcription factors, phosphodiesterases
**LOV Domain Structure of Bacillus subtilis YtvA**

- YtvA mediates stress response in *B. subtilis* following blue light absorption.
- flavin mononucleotide (FMN) cofactor
- C-terminal helix Ja extending from core
- upon light absorption formation of covalent bond between cysteine 62 and C4a atom of FMN
- small light-induced quaternary structure changes


**Structural Similarity of YtvA-LOV to PAS H Domain of FixL**

Two-component system histidine kinase FixL from *Bradyrhizobium japonicum*:

- PAS H domains binds heme and detects oxygen.

Bradyrhizobium japonicum FixL/FixJ two-component system

Regulation of nitrogen metabolism in response to oxygen levels.

Can We Reprogram FixL by Fusion with LOV Domain?

Modular architecture of YtvA and FixL:

Fusion site within Jα helix according to structure-based sequence alignment:
**YF1 Kinase Activity Assays**

Kinase activity of YF1 was determined in turnover assays

\[
\text{ATP} \rightarrow \text{YF1} \rightarrow \text{FixJ} \rightarrow \text{FixJ-P} \rightarrow \text{H}_2\text{O} \rightarrow \text{P}_i
\]

Reaction aliquots are separated on SDS-PAGE and visualized by \(^{32}\text{P}\)-radiography

- **Dark**: max. turnover: \(56.4 \pm 2.8 \text{ h}^{-1}\)
- **Light**: < 0.04 \text{ h}^{-1}

→ YF1 is a light-inactivated histidine kinase

**YF1 Retains Catalytic Efficiency of FixL**

Substrate affinities for ATP and FixJ are comparable in YF1 and FixL

<table>
<thead>
<tr>
<th></th>
<th>(K_m^{\text{ATP}}) ((\mu\text{M}))</th>
<th>(K_m^{\text{FixJ}}) ((\mu\text{M}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>YF1</td>
<td>33 ± 2</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>FixL</td>
<td>206 ± 13</td>
<td>1.6 ± 0.4</td>
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</tbody>
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**Michaelis-Menten** \(K_m\)-values
Light Regulation of YF1 is Mediated by Its LOV Domain

YF1:

YF1 C62A:

But What Happens in the Light?

→ rapid decay of phospho-FixJ after illumination

→ YF1 is a light-activated phosphatase
**YF1 Linker Variants**

Does any fusion between YtvA LOV and FixL histidine kinase work?

→ alter linkage between sensor and effector domain

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**Kinase Activity of YF1 Linker Variants**

Heptad periodicity of kinase activity and regulation by light.

e.g.  
- YF1Δ275-277 and YF1 L4
- YF1Δ276-277 and YF1 L5
- YF1 and YF1 L7
- YF1 L1 and YF1 L8
Heptad Periodicity of Kinase Activity

<table>
<thead>
<tr>
<th>YF1 Δ274-277</th>
<th>YF1 Δ275-277</th>
<th>YF1</th>
<th>YF1 Δ1277</th>
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<tr>
<td>turn over (s⁻¹)</td>
<td></td>
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<td>0.04</td>
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<tr>
<td>10</td>
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</tbody>
</table>

Δresidues: -4 -3 -2 -1 0 1 2 3 4 5 6 7 8
helix rise (Å): -6 -4.5 -3 -1.5 0 1.5 3 4.5 6 7.5 9 10.5 12
helix angle (°): -60 60 -160 100 -160 -60 40 140 -120 -20 80

Helix rise and angle calculated according to canonical α-helix conformation.

Model for Kinase Regulation by Light

Activity vs. helix angle:

Light-induced rotation of linker helices by about 40-60°:

[Diagram of gear rotation from dark to light]
Design Strategy Applicable to Histidine Kinases...

Analysis of >3000 PAS histidine kinases:

- PAS
- Jα
- DHp

A0X4D6 220  NQPHREnovationEIRREIENIQGQ--------EVAVRKEHEIIEIREIENIQGQ   258
A0ljL6 499  NFTMFYLVAYEGDELTEEAI--------SAVNRSVARTRARTVERP   537
Q2NMA5 481  PASGHRVETTEDTROLLOQ--------RAQANDVARRARTVERP   519
A0XMP1 482  DTIRYETIERTYATIKATQ--------RAQANDVARRARTVERP   571
A1PRM1 314  GTIYIQRENDQTSAPITQGQQ--------HEAVGCIAADAPVTP   159
A6PSM4 257  RFAKRIRHYLDEPVRMGSRSE--------PEAVLGRASITASSMQL   322
A5UXG0 245  GGOGLITLAVORDKNERGERGRES--------NEGVGLOGGATFDMNL   250
Q1ITG2 424  QNMEIDPTQGVRRTSGGQRQ--------KEAVGLOGGATFDMNL   469
A2PG48 210  QPFAALSNQYDKRALKVARHRSQASAKHLNSGQAVLTSABAGQ   262
A6GQY9 148  GLGCCTTQRENDKNERGERGERS--------GEAVGLOGGATFDMNL   220
Q1NMA5 291  DGRALICVFDPFRKZNCQKLNTKDFQPCRSALGGRASITASSMQL   343
P23222 244  GGRYPSTQGRTKDHERCITGQDNEKVLHNLGAGGNGASITASSMQL   296

Conservation

Avg. Hydrophathy


... and Other Proteins

PAS and LOV domains naturally coupled to wide variety of effector domains.

- histidine kinase
- bacteriophytochrome
- adenylate/acylase/lyase/lipase/luteinase
- transcription factor
- anti-sigma antagonist
- potassium channel
- chemotaxis protein

Recently, also regulation of other effector domains by fusion with LOV domains:


→ dihydrofolate reductase (Lee et al. (2008) Science 322, 438-442.)
Summary

- **Genetically Encoded Caging**
  - modularity of natural and synthetic photoreceptors: sensors and effectors
  - light regulation *in situ* by fusion with LOV photosensor domain

- **Design of Light-regulated Histidine Kinases**
  - switch histidine kinase from oxygen control to light control
  - retain catalytic efficiency of parent enzyme
  - activity and light regulation both *in vitro* and *in vivo*

- **Rotary Signaling Mechanism**
  - length of helical domain linker determines activity and regulation by light
  - regulation of activity by light could involve rotation of linker helices

Use in Time-resolved Crystallography

- **Applications**
  - bestow light sensitivity on otherwise light-inert systems
  - reversibly trigger and synchronize reactions within crystal

- **Properties and Requirements**
  - photochemistry suitable for kinetics on microseconds timescale (e.g. enzymes):

  \[
  \text{hv} \quad \rightarrow \quad \begin{array}{c}
  \mu s \\
  \geq s
  \end{array}
  \]

  - how to couple LOV photocycle to change in protein activity?
    - link LOV domain N-terminally to effector domain
    - helical domain linkers
    - in oligomeric proteins: effect quaternary structure changes
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