**A PhotoGENEic Approach to Biosynthesis**

Ellen Cliff, Conor Horgan, Richard Kong, Henry Orton, Jaselle San Juno, Victor Wang, Laura Wey, Matthew Whitney
Colin Jackson (Research School of Chemistry), Spencer Whitney (Research School of Biology), The Australian National University, Canberra, Australia

As 2015 is the International Year of Light, we sought to adapt the light dependent CRY2/CIB1 molecular interaction occurring in plants as a blue light inducible protein biosynthesis switch in *Escherichia coli*.

---

**Introduction**

In plants CRY2 is a cryptochrome (a photoreceptor protein) that upon blue light irradiation can interact with a certain regulatory protein to activate its function. Such proteins include the transcription factor activator CIB1. Formation of the CRY2-CIB1 dimer inhibits DNA binding by CIB1.

Optogenetic studies using the blue light specific CRY2-CIB3 interaction has been applied in a number of eukaryotic expression systems to regulate the activity of split effector proteins [1-7].

**Experimental Objectives**

We sought to develop a light-inducible recombination system using CRY2-CIB1 from the model plant Arabidopsis thaliana that was suitable for controlling the biosynthesis of toxic metabolites and/or proteins in E. coli. Our experimental concept was as follows:

**Aim 1. Can we detect CRY2-CIB1 function in *E. coli?***

**Approach** – Tether the yellow (YFP) and cyan (CFP) fluorescent proteins separately to the minimal interaction domains of CRY2 and CIB1 and test for evidence of blue light induced dimerization using fluorescence Resonance Energy Transfer (FRET).

**Aim 2. Can we adapt CRY2-CIB1 to trigger gene recombination?***

**Approach** – Expose the 34-bp LoxP sequence specificity of Cre recombinase to drive blue light induced recombination by tethering the N-terminal (CreN) and C-terminal (CreC) domains separately to CRY2 and CIB1.

**Aim 3. Exploiting CRY2-CIB1 triggered recombination to enable toxic metabolite biosynthesis.***

**Approach** – As a proof of concept our goal was to expand Aim 2 to demonstrate the feasibility of high yield NAD production in *E. coli* at levels normally toxic to cell viability. The design strategy was to overexpress NADP (producing the non-toxic intermediate α-iminopimelate) before blue light induction recombinatorial excision of NAD to facilitate NADP expression thus allowing production of the NAD precursor quinolinate.

**Methods and Results**

**AIM 1**

Initially we cloned the following reporter construct pETFRET2 for co-expressing the fusion proteins CRY2-YFP and CIB3-CFP in E. coli BL21 (DE3).

The fusion proteins contained 13 amino acid SGGSGSGSGSGG linker sequences and either Myc or 6x histidine (6H) epitope tags.

**OSD PAGE analysis** (Figure 1) indicated:

- the full length Cry2-CFP (and some degradation products) are produced in *E.coli* but are largely (>95%) insoluble.
- a series of truncated, soluble CIB1-Cre fusion protein products were detected that suggests the CIB1 domain is susceptible to proteolysis in *E.coli*.

We re-designed the reporter constructs on separate plasmids to individually and expressly isolate H-tagged CFP-Cry2 and CIB1-YFP proteins from E. coli BL21 (DE3) using immobilized metal affinity chromatography.

**AIM 2**

The following co-expression reporter constructs were made to assay for blue light induced Cre-recombinase functionality in *E. coli* BL21 (DE3).

**AIM 3**

**Conclusion**

Functionality of the light-activated CRY2/CIB1 system may be limited in *E. coli* due to challenges associated with insolubility of the CRY2-fusion proteins and an apparent sensitivity of the Lysine rich CIB1 protein to proteolysis.

**Application of light-inducible expression systems**

We considered a number of aspects associated with applying light-activated systems such as the CRY2/CIB1 module. These included:

- the design of photo-bioreactors for large scale and industrial biosynthesis.
- biocontainment strategies using genetically recoded organisms (GROs) dependent on synthetic amino acids.
- a simple method to create a cheap, customisable blue-light source for small scale testing (Figure 4).

**Human Practices**

We undertook a range of exciting outreach activities:

- **Student Outreach**: We ran workshops with a range of student groups at Melba Copland Secondary School in which we explored the ethics of synthetic biology and the philosophy of scientific research– while amazing them with some captivating examples of science in action.
- **Science in Action**: Over a two day period we used simple, visual analogies to explain synthetic biology concepts to school groups and the general public. Over 2000 people attended the event.
- **Questacon SciNight**: The national science and technology culture (Questacon) invited the team to display our “optogenetic research” as part of their spectacular smorgasbord of sound and light activities to kick off National Science Week. This event attracted over 680 attenders. [http://www.questacon.edu.au/events/scinight](http://www.questacon.edu.au/events/scinight)
- **University Open day**: We prepared a number of hands on activities associated with explaining the role of light in various biological reactions and the potential for integrating these natural processes in synthetic biology applications.

[@anu_igem](http://anu.igem.org/)

@anu_igem@gmail.com

http://2015.igem.org/Team/ANU-Canberra

---

**References**