SUPPLEMENTARY MATERIAL

FORMATION OF FLUORESCENT PROTEINS BY NON-ENZYMATIC ATTACHMENT OF PHYCOERYTHROBILIN TO R-PHYCOERYTHRIN ALPHA AND BETA APO-SUBUNITS

Dragan Isailovic[1], Ishrat Sultana[2], Gregory J. Phillips[2], and Edward S. Yeung[1]

[1]Ames Laboratory-USDOE and the Department of Chemistry, and
[2]Department of Veterinary Microbiology and Preventive Medicine,
Iowa State University, Ames, Iowa, 50011

Running Title: Attachment of Phycoerythrobilin to R-Phycoerythrin Subunits

Address correspondence to: Edward S. Yeung, Ames Laboratory-USDOE and Department of Chemistry, Iowa State University, 1161 Gilman Hall, Ames, Iowa, 50011-3111, Tel. 515-294-8062; Fax: 515-294-0266; E-mail: yeung@ameslab.gov

Supplementary Table 1. Spectroscopic characteristics of holo-subunits formed after attachment of PEB to recombinant R-PE apo-subunits *in vivo*. From these values and referenced literature [23] it was concluded that attachment of PEB to cells containing R-PE apo-subunits yields holo-subunits containing both urobilin (UB) and PEB.

Phycobilin present in holo-subunit	UB	PEB	
Absorption maximum (nm)	496.0	552.0	
Excitation fluorescence maximum (nm)	495.0	542.5	569.5
Emission fluorescence maximum (nm)	506.5	573.0	581.5

Supplementary Table 2. Average fluorescence intensities of cells containing holo-subunits and control cells measured by flow cytometry. Fluorescence intensities of 10,000 cells were analyzed. All cells were incubated with PEB under the same conditions, but only α_{ind} and β_{ind} cells expressed apo-alpha and apo-beta subunits of R-PE, respectively. These cells had, on average, several times higher fluorescence compared to cells without expressed apo-subunits (α_{con}, β_{con}, BL21(DE3)).

Cells	α_{ind}	α_{con}	β_{ind}	β_{con}	BL21(DE3)
I_f	15.1	2.9	7.9	3.0	3.1

Supplementary Figures

Supplementary Figure 1. A, Photograph of *E. coli* cells from left to right: 1. BL21(DE3) cells that neither contained plasmids bearing subunit genes nor were treated with IPTG, 2. Control cells that were not induced for expression of apo-beta subunit of R-PE, 3. Cells that were induced for expression of apo-beta subunit of R-PE, 4. Control cells that were not induced for expression of apo-alpha subunit of R-PE, and 5. Cells that were induced for expression of apo-alpha subunit of R-PE; B, Photograph of holo-alpha subunit solution isolated in the denaturing buffer (right) compared to the pure denaturing buffer (left). Pink color of holo-subunits in cells and after isolation in denaturing buffer can be seen.

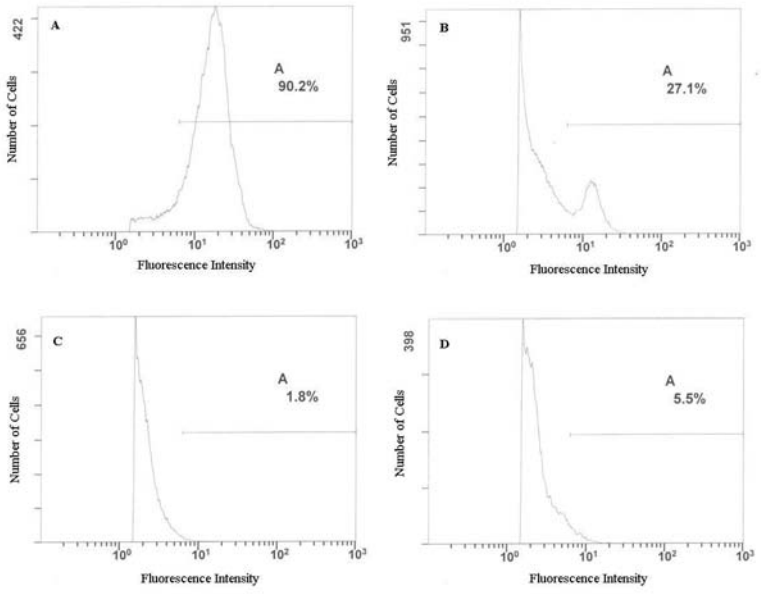
Supplementary Figure 2. Photographs comparing *E. coli* cells expressing periplasmic MBP-R-PE alpha subunit fusion protein and control cells after incubation of cells with PEB chromophore. Vials from left to right contained the following cells incubated with PEB: 1. Control cells that were not induced for expression of periplasmic MBP-R-PE alpha subunit fusion, 2. Cells that were induced for expression of periplasmic MBP-R-PE alpha subunit fusion. A, Daylight colors of cells. Pink color of induced cells can be seen. B, Colors of cells under UV illumination. Orange fluorescence of induced cells can be seen. Results indicate formation of fluorescent fusion proteins that are pink in color and show orange fluorescence.

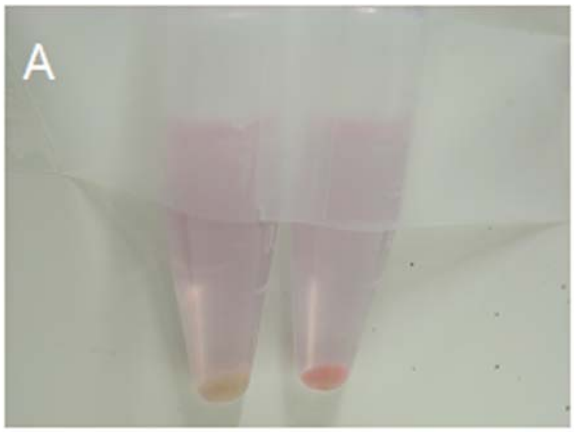
Supplementary Figure 3. MBP-R-PE subunit solutions after attachment of PEB compared to solution of free PEB. Vials from left to right contained the following

solutions: 1. cytoplasmic MBP-R-PE alpha subunit and PEB in maltose elution buffer, 2. PEB in maltose elution buffer. 3. cytoplasmic MBP-R-PE beta subunit fusion and PEB in maltose elution buffer, A, Daylight color of solutions. Solutions are pink in color due to the same color of free PEB and PEB bound to fusion proteins. B, Color of solutions under UV illumination. Orange fluorescence from solutions containing fluorescent subunit fusions is seen. These images demonstrate the large increase in fluorescence of PEB chromophore after attachment to soluble apo-subunit fusion proteins.


A


B


A
422
Number of Cells
A
90.2%
10^0 10^1 10^2 10^3
Fluorescence Intensity
B
951
Number of Cells
A
27.1%
10^0 10^1 10^2 10^3
Fluorescence Intensity
C
656
Number of Cells
A
1.8%
10^0 10^1 10^2 10^3
Fluorescence Intensity
D
398
Number of Cells
A
5.5%
10^0 10^1 10^2 10^3
Fluorescence Intensity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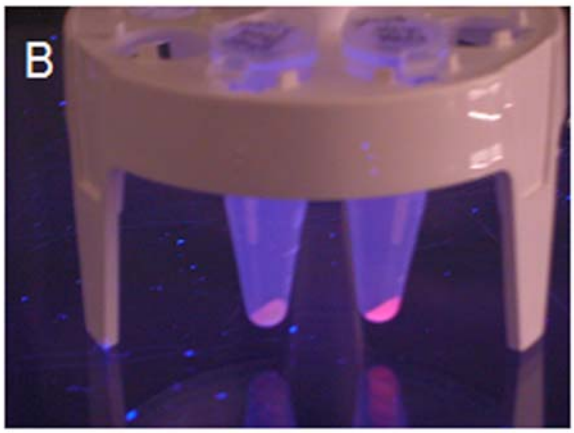
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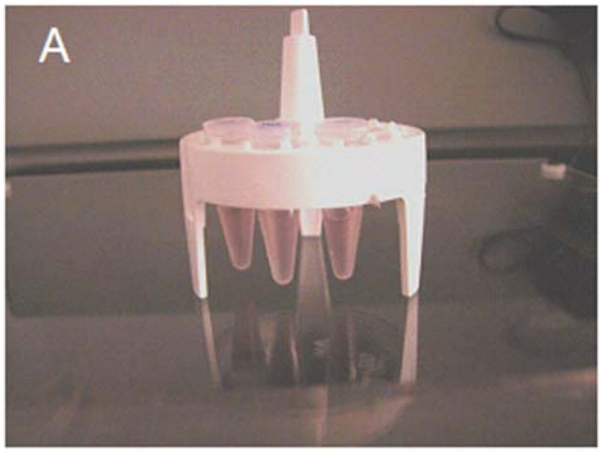
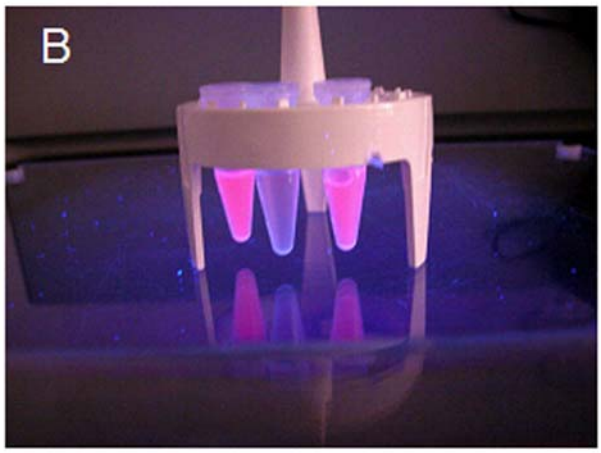

A


B