Structural Characterization of a Blue Chromoprotein and Its Yellow Mutant from the Sea Anemone *Cnidopus Japonicus**

Mitchell C. Y. Chan†1, Satoshi Karasawa‡1, Hideaki Mizuno§1, Ivan Bosanac§2, Dona Ho§, Gilbert G. Privé‡, Atsushi Miyawaki§2, and Mitsuhiko Ikura∥2

From the †Division of Signaling Biology, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Toronto, Ontario M5G 1L7, Canada, ‡Laboratory for Cell Function and Dynamics, Advanced Technology Development Center, Brain Science Institute, RIKEN, Wako City, Saitama 351-0198, Japan, §Amalgaam Co. Ltd., 2-9-3 Itabashi, Itabashi-ku, Tokyo 173-0004, Japan, and ∥Medical & Biological Laboratories Co. Ltd., 3-5-10 Marunouchi, Naka-ku, Nagoya City 460-0002, Japan

Green fluorescent protein (GFP) and its relatives (GFP protein family) have been isolated from marine organisms such as jellyfish and corals that belong to the phylum Cnidaria (stinging aquatic invertebrates). They are intrinsically fluorescent proteins. In search of new members of the family of green fluorescent protein family, we identified a non-fluorescent chromoprotein from the Cnidopus japonicus species of sea anemone that possesses 45% sequence identity to dsRed (a red fluorescent protein). This newly identified blue color protein has an absorbance maximum of 610 nm and is hereafter referred to as cjBlue. Determination of the cjBlue 1.8 Å crystal structure revealed a chromoprotein comprised of Gln62, Tyr64-Gly65. The ring stacking between Tyr64 and His197 stabilized the cjBlue trans chromophore conformation along the Ca2–Cβ2 bond of 5-[(4-hydroxyphenyl)methylene]-imidazolinone, which closely resembled that of the “Kindling Fluorescent Protein” and Rtm5. Replacement of Tyr64 with Leu in wild-type cjBlue produced a visible color change from blue to yellow with a new absorbance maximum of 417 nm. Interestingly, the crystal structure of the yellow mutant Y64L revealed two His197 imidazole ring orientations, suggesting a flip-flop interconversion between the two conformations in solution. We conclude that the dynamics and structure of the chromophore are both essential for the optical appearance of these color proteins.

The green fluorescent protein (GFP) from Aequorea victoria has gained widespread interest as a biological reporter in living cells (1). Since its discovery, considerable efforts have been devoted to protein engineering, in conjunction with isolation of new GFP homologs, to expand the visible spectrum and properties of GFP protein family (1, 2). Characterized GFP protein family can be divided into two groups, the fluorescent proteins (FPs) and the non-fluorescent chromoproteins (CPs) (3, 4). The GFP chromophore arises through a unique autocatalytic post-translational modification of a tripeptide, usually X-Tyr-Gly, in the primary sequence. The conformation and interaction of the chromophore with its local environment determines the spectral properties of the protein. X-ray crystallographic studies (5–7) have revealed the general relationship between the trans non-co-planarity of chromophores found in CPs and the cis co-planarity of chromophores found in FPs, with the exception of eqFP611, which has a trans co-planar chromophore.

To date, four CPs from the Anthozaan species have been characterized: Rtm5 from Montipora efflorescens (8), gtCP from Goniopora tenuidens (9), aeCP597 from Actinia equine (10), and asFP595 from Anemonia sulcata (KFP) (5). Three-dimensional structures of Rtm5 (6) and KFP (5) have been solved previously, both of which show the same fold as GFP and contain an internal chromophore. Studied CPs have exhibited absorbance maxima in a confined range of 560–597 nm (11–14); no CP has been thus far found to absorb at absorbance maxima greater than 600 nm.

Here we present a new CP from the Cnidopus japonicus sea anemone species, which absorbs at 610 nm. We report the molecular cloning, characterization and structure determination of this blue CP, hereafter termed cjBlue. We have also generated a yellow mutant variant from cjBlue with a single mutation at the 64 position (Tyr to Leu) using semi-random mutagenesis. We discuss the structural basis for the blue chromophore formation of wild-type cjBlue and for the blue-to-yellow shift in cjBlue(Y64L) mutant, which lacks an aromatic amino acid in the tripeptide chromophore. Elucidation of the structural details of cjBlue and cjBlue(Y64L) helps to answer not only why cjBlue and cjBlue(Y64L) absorb different colors, but also contributes to our better understanding of why FPs can fluoresce having a structural architecture similar to that of CPs.

REFERENCES

1. This work was supported by Canadian Institutes of Health Research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2. The atomic coordinates and structure factors (codes 2IB5 and 2IB6) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/). The nucleotide sequence (s) reported in this paper has been submitted to the GenBank™EBI Data Bank with accession number(s) BD048947 and BD048956.

3. The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. 1 and 2.

4. The abbreviations used are: GFP, green fluorescent protein; KFP, kindling fluorescent protein; FP, fluorescent protein; CP, chromoprotein; TCEP, Tris(2-carboxyethyl)phosphine.
EXPERIMENTAL PROCEDURES

cDNA Cloning and Gene Construction—A sample of the C. japonicus was acquired from the ocean near the Uozu city in Toyama. Total RNA was isolated from the sea anemone by guanidine thiocyanate extraction. Synthesis, amplification using degenerate primers, and generation of full-length cDNA were performed as described previously (23) using the following degenerate primers: 5'-GAAGGRTGYGTCAYGGRCAY-3' and 5'-ACVGGDCCATYDGYVAGAARRTT-3' (R = Arg or G1y; Y = Cys or Thr; V = Arg, Cys, or G1y; D = Arg, G1y, or Thr). The cDNA encoding the protein-coding region was amplified using primers containing 5' BamHI and 3' EcoRI sites. The digested product was then cloned in-frame into the BamHI/EcoRI sites of pSETB (Invitrogen) for bacterial expression. Site-directed and semi-random mutations were introduced as described (19).

Protein Expression and Purification—CjBlue and cjBlue-(Y64L) were cloned into a pET28a expression vector. Seleno-l-methionine-labeled protein was produced using minimal M9 media and expressed with N-terminal His tag using B834 (DE3) Escherichia coli strain (Novagen). Cells were grown in a shaker incubator at 37 °C until an A600 of 1.20 was reached. Upon induction with 1 mM isopropyl 1-thio-galactopyranoside, the temperature was lowered to 20 °C, and the protein was allowed to express for 48 h. Protein containing the N-terminal His tag was subsequently removed using thrombin followed by size exclusion chromatography (Superdex 75, Amersham Biosciences) to achieve satisfactory levels of purity. Incorporation seleno-l-methionine was confirmed via electrospray mass spectrometry. The purified protein was concentrated to 25 mg/ml in cryotubes. Concentration of each sample was diluted from the stock 25 mg/ml solution to 1 mg/ml. 0.5 ml of diluted sample was gently transferred into a 1-cm path length quartz cuvette. Absorbance was measured using an Ultrospec 2000 UV-visible spectrometer (GE Healthcare). An absorbance scan was initiated from 250 to 750 nm at 1-nm increments.

RESULTS

cDNA Isolation and Protein Purification—Degenerate primers were employed to amplify cDNAs isolated from the sea anemone, C. japonicus. The primers covered several conserved amino acid sequences identified from among GFP-like fluorescent proteins found in other Anthozoa species. The missing 5' and 3' ends of the cDNA fragment were amplified using the rapid amplification of cDNA ends strategy. The resultant open reading frame coded 225 amino acids with high sequence homologies to KFP (5), hccP (15), eqFP611 (7), and Rtm5 (6) (Fig. 1). The full-length protein was expressed in E. coli with a His tag at the N terminus and purified using metal affinity chromatography. The protein referred as cJBlue is a chromoprotein, which does not fluoresce but is dark green in color. CjBlue at pH 7.4 displayed a major absorption wavelength maximum at 610 nm (ε = 66,700 M⁻¹ cm⁻¹) (Fig. 2) showing the longest wavelength absorption of all known CPs. The absorption spectrum of cJBlue showed high resistance to acidity (up to pH 4) (data not shown).

Structure of cJBlue—The crystal structure of cJBlue, solved by X-ray diffraction, belongs to the P2₁ space group. A final R_factor and R_free of 19.5 and 22.5%, respectively, were reached (Table 1). The final cJBlue model contained eight subunits (residues 5–232), 16 PO₄, and 1330 water molecules in an asymmetric unit. Further, the tertiary structure of cJBlue shares a similar fold to GFP (16), dsRed (17), KFP (5), and Rtm5 (6). Each subunit consists of β-strands 11 forming a β-barrel with a central helix running co-axially with the axes of the β-barrel (Fig. 3). The central helix of cJBlue connects the chromophore to the rest of the protein (Fig. 3).

The crystal structure of cJBlue revealed a pair of four chemically identical subunits that were intimately in contact with one another (Fig. 3A), in a manner similar to that observed for eqFP611 (7), Rtm5 (6), and dsRed (17). This molecular association is stabilized by 1) inter-barrel interface interactions and 2) association of the C terminus tails with β-strands 11 of adjacent pair of subunits (Fig. 3). Asn20, Asn21, Thr103, Gln105, and Arg179 are residues located at the inter-barrel interface forming hydrogen bonds between subunits. The C-terminal tail interaction involves hydrogen bonding between Ser227, His231, and Asn232 at one tail and Asp196, Arg198, Glu200, and Thr224 within β-strands 11 of an adjacent pair of subunit.

The cJBlue chromophore consists of a 5’-[4-(hydroxyphenyl)methylene]-imidazolinone group with an acylimine bond found between the Cα and nitrogen atom positioned at Gln63 (Figs. 3
Crystal Structures of Chromoproteins

and 4) (6). The additional double bond at Gln63 is comparable with the double bond found at Gln60 of dsRed (17, 18). Like other FP and CPs, the cjBlue chromophore is buried deeply within the β-barrel (Fig. 3) with substituents hydrogen bonded to neighboring residues and internal water molecules. These residues include Thr158 (2.5 Å), Glu145 (3.1 Å), and water molecule W1 (3.3 Å), which are hydrogen bonded to the oxygen of the p-hydroxyphenyl ring (Fig. 4). Remarkably, water molecules near the cjBlue chromophore were all found in the same positions as those seen in the crystal structure of Rtms5 (6). The imidazole ring of His197, located near the chromophore, shows a stacking orientation with the p-hydroxyphenyl ring of ~3.9 Å.

The cjBlue chromophore adopts a trans conformation between the Ca2–Cβ2 bond with noticeable bond angle distortion. Because of internal steric hindrance between O2 and C8–H, an increase in the C2–Ca2–Cβ2 bond angle (141°) and the Ca2–Cβ2–Cy2 bond angle (117°) was observed. Cys445 also appears to contribute to the trans conformation by sterically hindering the p-hydroxyphenyl ring from adopting a cis conformation.

Chromophores adopting a trans conformation, with the exception of eqFP611, lack planarity between the imidazolinone and p-hydroxyphenyl ring because of internal collision of O2 and C8–H (supplemental Fig. 1). For example, structures such as Rtms5 (6) and KFP (5) adopt a trans non-co-planar conformation, whereas eqFP611 (7) has a trans co-planar conformation. Chromophore planarity can be evaluated through the dihedral angles between the imidazolinone and p-hydroxyphenyl rings (Fig. 4). Chromophores with a cis conformation, such as dsRed, have a nearly perfect co-planarity (dihedral angle <1°). In contrast, Rtms5 (6) and KFP (5) have a trans non-co-planar conformation with a dihedral angle of 35° and 20°, respectively. Similarly the dihedral angle of cjBlue is 40°. A second assessment of chromophore planarity is the measurement of torsion angles between χ1 (C2–Ca2–Cβ2–Cy2) and χ2 (C8–C–Cβ2–Cy2). Rtms5 (6) and KFP (5) have a χ1 and χ2 of 169°, −136° and 171°, 163°, respectively, whereas the torsion angles of cjBlue are 178° and −141°.

Structural Comparison of cjBlue(Y64L) with Wild-type cjBlue—To examine site-specific roles of the chromophore-forming tripeptide, Gln–Tyr–Gly, we employed semi-random site-specific mutagenesis on cjBlue (19). Random substitutions of these and other amino acids surrounding the chromophore were simultaneously introduced into the protein. We found that Tyr64 could be replaced by some other amino acid without losing any light absorbing ability. For example, substitution of Leu only resulted in a blue shift of the absorption peak. This variant protein, cjBlue(Y64L), was yellowish in color and absorbed light maximally at 418 nm (Fig. 2). Most FPs/CPs have Tyr at this position, whereas blue- and cyan-emitting FPs derived from Aequorea GFP have His and Trp, respectively.

To investigate the structural properties of this aromaticless chromophore, we determined the crystal structure of cjBlue(Y64L) to 2.0 Å using molecular replacement and cjBlue as the search model. A final R factor and Rfree of 19.8 and 24.4%,
respectively, were reached. The overall quaternary and tertiary structure of cjBlue(Y64L) was found to be similar to cjBlue, but significant differences were observed in the chromophore structure and environment (Fig. 3).

In comparison with the wild-type, the overall size of the cjBlue(Y64L) chromophore is smaller than that of the cjBlue chromophore. The cjBlue(Y64L) chromophore consists of a single imidazolinone ring and has the wild-type tyrosine ring

### TABLE 1
Data collection and refinement statistics for cjBlue and cjBlue(Y64L)

| Sample/Data set | Wavelength | Resolution | Reflections (total/unique) | Completeness | \(R_{merge}\) | \((I/\sigma(I))^b\) |
|-----------------|------------|------------|---------------------------|--------------|----------------|----------------|
| cjBlue (Peak)   | 0.9793     | 50.0–1.6   | 8,835,277/335,346         | 99.3 (100)   | 7.7 (26.1)    | 12.0 (9.0)    |
| cjBlue(Y64L) (Native) | 1.5419 | 50.0–1.8   | 1,054,020/121,926         | 100 (100)    | 6.2 (38.1)    | 39.65 (3.0)   |

**Refinement statistics**

| Sample/Data set | Resolution | Reflections (working/test) | Total number of atoms | \(R_{cryst}\) | \(R_{free}\) | \((B)\) Value | r.m.s.\(^e\) deviations |
|-----------------|------------|---------------------------|----------------------|--------------|-------------|---------------|------------------------|
| cjBlue (Peak)   | 50.0–1.8   | 318,579/16,767            | 15,730               | 19.5/22.5    | 19.5        | 1.673         |
| cjBlue(Y64L) (Native) | 50.0–2.0  | 107,910/5,761            | 15,706               | 19.8/24.4    | 16.2        | 1.624         |

\(^a\) \(R_{merge} = \frac{\sum_i |I_i - \langle I_i \rangle|}{\sum_i I_i} \times 100\), where \(I_i\) is the intensity of \(i\) observations for reflection \(h\) and \(\langle I_i \rangle\) is the mean intensity of the reflection.

\(^b\) \((I/\sigma(I))\), mean intensity/mean standard deviation.

\(^c\) \(R_{cryst} = \frac{\sum |F_{o} - F_{c}|}{\sum |F_{o}|} \times 100\), where \(F_{o}\) and \(F_{c}\) are the observed and calculated structure factor magnitudes, respectively.

\(^d\) \(R_{free}\) as for \(R_{cryst}\) except calculated for 4.7% of the reflections not used for model refinement.

\(^e\) r.m.s., root mean square.

**FIGURE 3.** The four subunit association, monomer, and electron density map of the chromophore environment. The representative top view of the cjBlue four subunit arrangements is shown in A. The monomeric cjBlue subunit is shown in B. CjBlue 2\(F_o\) – \(F_c\) density map contoured at 1.0 \(\sigma\) is shown in C. cjBlue(Y64L) electron density 2\(F_o\) – \(F_c\) map contoured at 1.0 \(\sigma\) is shown in D.
replaced with an isobutyl moiety from leucine. This generates a new 5-isobutyl-imidazolinone group, which forms the cjBlue(Y64L) chromophore (Fig. 3). As compared with the cjBlue chromophore, the overall electron conjugation is reduced despite the presence of an acylimine bond between the Cα and nitrogen atom of Gln63.

Similar to cjBlue, the deeply buried chromophore in cjBlue(Y64L) forms extensive contacts with neighboring residues and internal water molecules (Fig. 4). The isobutyl moiety creates a new hydrogen bond network between the cjBlue(Y64L) chromophore and water molecules. Three water molecules are found in this network for chromophore stabilization within the β-barrel (Fig. 4). Water molecule W4 forms a hydrogen bond with O2 (3.2 Å) and N2 (3.5 Å) of the chromophore and a third hydrogen bond with W3 (2.4 Å), which in turn hydrogen bonds with W2 (2.8 Å) (Fig. 4). Completion of the bridging coordination between the chromophore and β-barrel is achieved by W2 hydrogen bonding to Thr158 (2.9 Å). The cjBlue(Y64L) water molecule (W1) is shifted from an equivalent water molecule (W1) position in cjBlue forming a new hydrogen bond with Glu145 (3.0 Å), Thr158 (3.0 Å), and Thr176 (2.7 Å). This point mutation also brings the chromophore into closer proximity with His197 (Fig. 5). Such proximity between His197 and the chromophore, not previously observed in cjBlue, appears to contribute to cjBlue(Y64L) absorbance. A striking difference between the cjBlue and cjBlue(Y64L) structures is that His197 assumes two different conformations at a nearly identical population within the cjBlue crystal structure. In conformation A, His197 N is 3.1 and 5.5 Å away from Cα2 and N2 of the chromophore, respectively. In conformation B, the distance of His197 N is 4.2 and 4.6 Å away from Cα2 and N2 of the chromophore, respectively. Conformation A, His197 has corresponding dihedral angles χ1 of 117° and χ2 of 51°. Although His197 in conformation B has dihedral angles χ1 of −64° and χ2 of −65°.

To assess the importance of His197 in the optic properties of cjBlue and cjBlue(Y64L), we generated cjBlue(H197S) and cjBlue(Y64L/H197S) mutants. Not surprisingly, both cjBlue(H197S) and cjBlue(Y64L/H197S) yielded a colorless protein with previous absorbance peaks abolished (Fig. 2). Proper protein folding of cjBlue(H197S) and cjBlue(Y64L/H197S) was confirmed through circular dichroism analysis (data not shown).
In this study, we determined the crystal structure of a blue CP, cjBlue, to 1.8 and 2.0 Å, respectively, and compared it with the crystal structures of Rtms5 (6) and dsRed (18). We suggest that the cjBlue chromophore follows a similar formation mechanism as suggested for Rtms5 (6, 8) and dsRed (18, 20) (supplemental Fig. 1). However, noticeable differences are seen in the planarity of the hydroxyphenyl ring among the non-fluorescent cjBlue, the weakly fluorescent Rtms5 (6), and the highly fluorescent dsRed (18). The cjBlue and Rtms5 chromophores adopt a trans non-co-planar conformation with the hydroxyphenyl ring rotated 40° and 35° out of plane, respectively (supplemental Fig. 2). In comparison, the dsRed chromophore adopts a cis co-planar conformation with a nearly perfect planarity (<1°). Another piece of information is that the chromophore of a highly fluorescent protein, eqFP611, has a trans co-planar conformation. Taken altogether, there is a correlation between the chromophore co-planarity and high fluorescence quantum yield.

Further inspection of the cjBlue crystal structure revealed a number of residues, which contributed to the trans non-co-planar conformation of the cjBlue chromophore. First, His<sup>197</sup> appears to stabilize chromophore conformation through parallel ring-stacking interaction with the hydroxyphenyl ring (Fig. 5). In Rtms5, Arg<sup>197</sup> is found in the same position as His<sup>197</sup> and favors the trans chromophore orientation through hydrogen bonding with the phenoxy group (6). Mutagenesis of His<sup>197</sup> to Ser<sup>197</sup> in cjBlue results in the loss of absorbance at 610 nm (Fig. 2), which suggests that His<sup>197</sup> is required for catalysis of the chromophore or that the imidazole ring sustains the trans non-co-planar conformation of the chromophore. Second, interactions between the phenoxy group of the chromophore with Glu<sup>145</sup> of β-strand 7 and Thr<sup>158</sup> of β-strand 8 stabilize the orientation of the chromophore (Figs. 1 and 4). Of note, Rtms5 has Glu<sup>148</sup> in β-strand 7 and Asn<sup>161</sup> in β-strand 8 found at the equivalent position (6). The interaction of these residues with the chromophore contributes to the chromophore conformation stability.

Through semi-random mutagenesis studies, we have identified a chromophore mutant, cjBlue(Y64L) in which the hydroxyphenol group of tyrosine is replaced with the isobutyl moiety of the leucine, resulting in a significant blue shift in the absorbance spectrum (610 – 417 nm). Visually this mutant appears yellow in color (Fig. 2). The same blue shift was achieved by the mutagenesis of Tyr<sup>64</sup> to Met. To investigate the structural basis for this color shift, the crystal structure of cjBlue(Y64L) was determined to 2.0 Å. The cjBlue(Y64L) chromophore structure comprises a single cyclical imidazolinone ring and forms a unique hydrogen bond network with its surrounding residues (Fig. 4); Glu<sup>145</sup> of β-strand seven hydrogen bonds with Arg<sup>147</sup> of β-strand 7 instead of the chromophore. Thr<sup>158</sup> of β-strand 8 no longer interacts with the chromophore but rather forms new hydrogen bonds with other neighboring residues.

Strikingly, the crystal structure of the cjBlue(Y64L) mutant revealed two alternative side-chain conformations of His<sup>197</sup> (Fig. 5, B and C), both of which increased proximity to the chromophore relative to that in the wild-type structure. In conformation A, the distance between the side chain of His<sup>197</sup> and the chromophore is 3.1–5.5 Å, whereas in conformation B the distance increases to 4.2–4.5 Å (Fig. 5). It is possible that these two conformations coexist in solution, thereby contributing to the blue-shifted absorbance of cjBlue(Y64L). Interestingly, the isobutyl moiety of the cjBlue(Y64L) chromophore appears to have missing electron density in our structure, suggesting that the conformational flexibility of His<sup>197</sup> is intimately coupled with the chromophore conformation. It remains to be deter-
mined what exact contributions each conformer has to generate the yellow appearance.

cjBlue and the cjBlue(Y64L) mutant set a new absorbance range (417–610 nm) for CPs. The structures of cjBlue and cjBlue(Y64L) provide valuable insights into the understanding of the non-fluorescent properties of this sea anemone protein. The cjBlue crystal structure provided a better understanding for the importance of the ring stacking effect between Tyr64 and His197. The cjBlue(Y64L) crystal structure revealed the interconversion between two His197 imidazole ring conformations on the CP optical behavior. We conclude that the dynamics and structure of the chromophore are both essential for the optical appearance of these color proteins. Future studies on the chromophore formation mechanism will further increase our knowledge of CPs and strengthen our understanding of their spectral properties.

Acknowledgments—We thank the staff of 19-ID beamline at Advanced Photo Source for help on data collection and Dr. Emil Pai of the University of Toronto for his encouragement. We also thank lab members of Drs. M. Ikura and E. Pai for helpful discussions.

REFERENCES

1. Tsien, R. Y. (1998) Annu. Rev. Biochem. 67, 509–544
2. Verkhusha, V. V., Chudakov, D. M., Gurskaya, N. G., Lukyanov, S., and Lukyanov, K. A. (2004) Chem. Biol. 11, 845–854
3. Matz, M. V., Lukyanov, K. A., and Lukyanov, S. A. (2002) BioEssays 24, 953–959
4. Labas, Y. A., Gurskaya, N. G., Yanushevich, Y. G., Fradkov, A. F., Lukyanov, K. A., Lukyanov, S. A., and Matz, M. V. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 4256–4261
5. Quillin, M. L., Anstrom, D. M., Shu, X., O’Leary, S., Kallio, K., Chudakov, D. M., and Remington, S. J. (2005) Biochemistry 44, 5774–5787
6. Prescott, M., Ling, M., Beddoe, T., Oakley, A. J., Dove, S., Hoeve-Guldberg, O., Devenish, R. J., and Rossjohn, J. (2003) Structure (Camb.) 11, 275–284
7. Petersen, J., Wilmann, P. G., Beddoe, T., Oakley, A. J., Devenish, R. J., Prescott, M., and Rossjohn, J. (2003) J. Biol. Chem. 278, 44626–44631
8. Beddoe, T., Ling, M., Dove, S., Hoeve-Guldberg, O., Devenish, R. J., Prescott, M., and Rossjohn, J. (2003) Acta Crystallogr. Sect. D Biol. Crystallogr. 59, 597–599
9. Martynov, V. I., Maksimov, B. I., Martynova, N. Y., Pakhomov, A. A., Gurskaya, N. G., and Lukyanov, S. A. (2003) J. Biol. Chem. 278, 46288–46292
10. Shkrob, M. A., Yanushevich, Y. G., Chudakov, D. M., Gurskaya, N. G., Labas, Y. A., Poponov, S. Y., Mudrik, N. N., Lukyanov, S., and Lukyanov, K. A. (2005) Biochem. J. 392, 649–654
11. Pollok, B. A., and Heim, R. (1999) Trends Cell Biol. 9, 57–60
12. Truong, K., Sawano, A., Mizuno, H., Hama, H., Tong, K. I., Mal, T. K., Miyawaki, A., and Ikura, M. (2001) Nat. Struct. Biol. 8, 1069–1073
13. Miyawaki, A., Llopis, J., Heim, R., McCaffery, J. M., Adams, J. A., Ikura, M., and Tsien, R. Y. (1997) Nature 388, 882–887
14. Chudakov, D. M., Belousov, V. V., Zaraisky, A. G., Novoselov, V. V., Staroverov, D. B., Zorov, D. B., Lukyanov, S., and Lukyanov, K. A. (2003) Nat. Biotechnol. 21, 191–194
15. Remington, S. J., Wachter, R. M., Yarbrough, D. K., Branchaud, B., Anderson, D. C., Kallio, K., and Lukyanov, K. A. (2005) Biochemistry 44, 202–212
16. Ormo, M., Cubitt, A. B., Kallio, K., Gross, L. A., Tsien, R. Y., and Remington, S. J. (1996) Science 273, 1392–1395
17. Wall, M. A., Socolich, M., and Ranganathan, R. (2000) Nat. Struct. Biol. 7, 1133–1138
18. Yarbrough, D., Wachtler, R. M., Kallio, K., Matz, M. V., and Remington, S. J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 462–467
19. Sawano, A., and Miyawaki, A. (2000) Nucleic Acids Res. 28, E78
20. Gross, L. A., Baird, G. S., Hoffman, R. C., Baldridge, K. K., and Tsien, R. Y. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 11990–11995
21. Deleted in proof.
22. Deleted in proof.
23. Karasawa, S., Araki, T., Yamamoto-Hino, M., and Miyawaki, A. (2003) J. Biol. Chem. 278, 34167–34171