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Fluorescent Proteins For Color-Conversion Light-Emitting Diodes

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Light emitting diodes,
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Color conversion

Abstract: Biologically derived fluorescent proteins are an attractive alternative to current color-conversion materials for solid state lighting applications, such as inorganic phosphors, organic dyes and nanocrystal quantum dots. Despite extensive research into fluorescent proteins for biological applications, they were not explored to be used for color-conversion for light-emitting diodes. In this study we investigate the in-solution characteristics of enhanced green fluorescent protein (eGFP) and monomeric cherry (mCherry) for LED applications. We demonstrate that these proteins can be used to completely convert the electroluminescence of a pump LED to photoluminescence. We show that up to typical pump driving current (150 mA) there is no saturation or bleaching of the proteins. Moreover, we compare the performance of in-solution unpurified and purified proteins as color convertors for LEDs and we show that unpurified fluorescent proteins in solution are suitable for color conversion applications.

Renk Dönüşüm Işık Yayan Diyotlar İçin Floresan Proteinleri

Keywords
Işık yayan diyotlar,
Floresan proteinler,
Fotolüminesans,
Renk dönüşümü

Özet: Biyolojik olarak elde edilen floresan proteinleri günümüzde inorganik fosfor, organik boyalar ve nanokristal kuantum noktaları gibi renk dönüşüm malzemelerinin katıhal aydınlatma uygulamalarında ilgi çekici bir alternatif olmuşlardır. Floresan proteinleri ile biyoloji uygulamları yönü ile ilgili büyük araştırmalar yapılmasına rağmen, ışık yayan diyot (LED) için renk dönüştürücü olarak araştırılmamıştır. Bu çalışmada sıvı içerisinde gelişmiş yeşil floresan proteinler (eGFP) ve monomerik cherry (mCherry)'nin LED (ışık yayan diyot) uygulamaları için özellikleri incelenmiştir. Bu çalışmada proteinlerin elektroışımayı tamamen fotoışımaya çevirdiklerini gösterdik. Bunun yanında, normal pompalama akımı seviyesine kadar (150 mA) proteinlerin doyumlarını ya da ağarmalarını gözlemlemedik. Ayrıca, solüsyon içerisinde saflaştırılmış ve saflaştırılmamış florasan proteinlerin performansını renk dönüşüm LED'leri için karşılaştırdık ve solüsyon içerisinde bulunan saflaştırılmamış floresan proteinlerin renk dönüşüm LED uygulamaları için kullanılabileceğini gösterdik.

1. Introduction

Naturally occurring fluorescent proteins had been studied as early as the 1960s, but it wasn't until the 1990s that their full potential began to be exploited [1]. Fluorescent proteins are now available in many different colors, from blue to deep red, and are a ubiquitous tool in molecular biology [2]. The unique structure of biologically derived fluorescent proteins, with their active chromophore protected by a barrel like protein sheath make them ideal candidates for photon and electro-photonic applications [3]. For example, in recent years green fluorescent protein (GFP) has been use as an optical gain material in both conventional type lasers and also to make cell lasers [3,4].

Solid state lighting (SSL), and in particular the light emitting diode (LED), is one of the most important modern technologies. They are replacing existing lighting technologies such as incandescent and fluorescent bulbs for both general lighting and high luminescence applications [5]. The majority of LEDs are based on inorganic semiconductor materials. The common white LEDs are coated with the inorganic phosphor Ce:YAG as a color-conversion layer. The Ce:YAG phosphor absorbs a portion of the blue electroluminescence from the inorganic semiconductor LED and emits a broad yellow

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photoluminescence; the combination of transmitted blue light and emitted yellow give the color-conversion LEDs a white color [6]. Even though these phosphor-based color-conversion LEDs have been technologically successful, they also have their intrinsic material-originated limitations. Firstly, the broad emission spectrum of the inorganic phosphor limits their spectral tunability, which restricts the sensitive adjustment of photometric properties such as color temperature, color rendering index, etc. of LEDs. Moreover, the granule size of the phosphor increases the scattering [6]. In addition, there are concerns about the global supply of the rare earth elements required for the phosphor [7].

Alternative SSL have been extensively investigated, such as organic light emitting diodes (OLEDs) and quantum dot light emitting diodes (QD-LEDs). In these devices the inorganic semiconductor material is replaced with an alternative electroluminescent material; they have shown promise in overcoming the "green-gap" problem [6]. In addition, quantum dots have been investigated as an inorganic color-conversion layer [8]. Organic dye molecules suffer from self-quenching at high concentrations. Favourably fluorescent proteins with their protected chromophore have enhanced protection against self-quenching, which makes them promising materials for color conversion [3]. Unlike quantum dots, fluorescent proteins are prepared at low temperatures and do not require toxic or rare materials. In a recent study, artificial and synthetic rubber polymers are used as a matrix for fluorescent proteins [9].

Large scale production of biologically derived proteins is well understood; however the current methods for production of pure fluorescent proteins require chromatography to separate the desired protein. These results in increase of the production cost (hundreds of dollars per gram) and time (3 days more per gram) for these materials. The unpurified fluorescent proteins may also offer a color-converter for LEDs. For that, in this study we investigate the color-conversion properties of two different fluorescent proteins expressed in E.coli, one of the most common expression systems. We compare the performance of in-solution unpurified and purified proteins as color convertors for LEDs. Our results suggest that unpurified fluorescent proteins are suitable for color conversion applications.

2. Material and Method

2.1. Fluorescent protein purification

The fluorescent protein fusion vectors eGFP-GST and mCherry-GST were cloned into a pGEX and pDEST vectors, respectively. The fluorescent proteins were expressed in E.coli BL-21 Rosetta cells, grown to OD600 = 0.6 and induced with 1mM IPTG for 2 days. After expression, the bacteria were pelleted and lysed by freeze-thaw and sonication with 1 mg/ml lysozyme in glutathione S-transferase (GST)–binding lysis buffer (PBS [137 mM NaCl, 2.7 mM KCl, 8 mM Na2HPO4, 1.46 mM and KH2PO4] pH 7.4 with 250 mM KCl, and protease inhibitors 10 µg/ml of aprotinin, leupeptin, pepstatin, and 1 mM phenylmethylsulfonyl fluoride). The lysate was centrifuged to remove cell particulates and insoluble proteins, and either used directly or purified further.

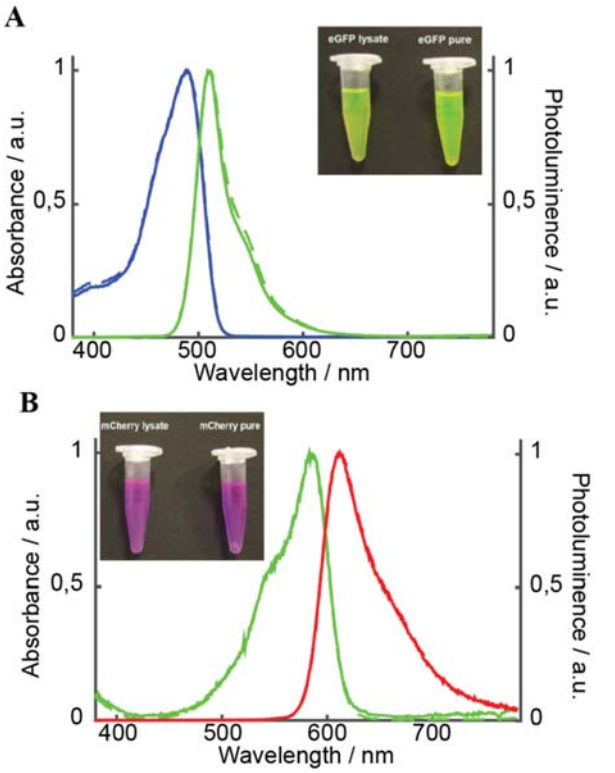

Figure 1. Absorbance and photoluminescence spectra. A. eGFP spectra. Absorbance blue and photoluminescence green. Solid line pure fluorescent protein solution and dashed line lysate. The insert show lysate (left) and pure solution (right) under white light illumination. B. mCherry spectra. Absorbance gren and photoluminescence red. Solid line pure fluorescent protein solution and dashed line lysate. The insert show lysate (left) and pure solution (right) under white light illumination.

The lysis was purified by affinity chromatography using glutathione–sepharose beads (Thermo Scientific). The fluorescent proteins were eluted from the beads with elution buffer (50 mM Tris pH 8.0, 10 mM glutathione, 10 mM DTT supplemented with protease inhibitors). The eluates were run through the 10000 NMWL centrifugal filters (Amicon® Ultra-4) for buffer exchange and concentration. The expression of fluorescent proteins was confirmed by Coomassie Blue gel staining. Spectroscopy absorption and photoluminescence spectra were acquired with a Fluoromax 3 (HORIBA, Ltd; Kyoto, Japan).

2.2. Spectroscopy

For the measurement of color conversion LED spectra metal tubes, with internal diameters of 5 mm and length 50 mm, were glued over blue and green

LEDs. Aliquots of the fluorescent protein solutions were loaded into the metal tube. A diffusor was placed directly over the open end of the metal tube and a fiber optic cable placed above the diffusor. The fiber optic cable was couple to a CCS200 Compact Spectrometer (Thorlabs; Newton, NJ). To avoid thermal damage to the proteins, the blue or green pumps LEDs were turned on for approximately 2 seconds during which time the spectrum were collected. All data was analyzed in Matlab R2014B (MathWorks; Natick, MA).

3. Results

The absorption and photoluminescence spectra of pure eGFP and mCherry fluorescent proteins were compared with those of lysate in Figure 1A and 1B, respectively. The eGFP lysate had a total protein concentration of 24.3 mg/ml, of which 16.2 mg/ml was eGFP-GST; the net percentage of fluorescent protein was 66 % (by weight). The mCherry lysate had a total protein concentration of 25.1 mg/ml, of which 12.5 mg/ml was mCherry-GST; the net percentage of fluorescent protein was 50 % (by weight). Noticeably, no difference was observed between the absorption and photoluminescence spectra for either eGFP or mCherry pure solution and lysis. The root-mean-square deviation (RMSD) between pure solution and lysate were 0.72 %, 1.58 %, 1.35 % and 0.61 % for eGFP absorption, mCherry absorption, eGFP photoluminescence and mCherry photoluminescence, respectively. This suggested that the presence of other proteins does not affect the absorption and emission spectra of in-solution fluorescence proteins.

We tested pure fluorescent protein solutions and lysate for in-solution color conversion. 1000 µl of 2.44 mg/ml (43.6 µM) pure eGFP solution was placed over a blue pump LED (λc = 456 nm; λFWHM = 16 nm) in 40 µl aliquots (Figure 2a). Each aliquot increased path length by 0.204 cm. The color went from pure blue ([x,y] = 0.145,0,036) to pure green, ([x,y] = 0.385,0.598). For comparison, 600 µl of 4.05 mg/ml (72.3 µM) eGFP lysate was placed over the same blue pump LED in 40 µl aliquots (Figure 2b). Each aliquot increased path length by 0.204 cm. The color shifted from pure blue to pure green, ([x,y] = 0.374,0.591). The LER of the final in-solution color conversion LEDs were 588 lm/W and 571 lm/W, for eGFP solution and lysate respectively. The RMSD between the normalized final spectra was 3.6 %.

Next, in-solution color conversion with mCherry pure solution and lysate was investigated. 800 µl of 3.47 mg/ml (65.8 µM) pure mCherry solution was placed over a green pump LED (λc = 544 nm; λFWHM = 39 nm) in 40 µl aliquots (Figure 2c). Each aliquot increased path length by 0.204 cm. The color went from pure green ([x,y] = 0.327,0.655) to pure red, ([x,y] = 0,701,0.276). For comparison, 680 µl of 4.16 mg/ml (78.9 µM) mCherry lysate was placed over the same green pump LED in 40 µl aliquots (Figure 2d). Each aliquot increased path length by 0.204 cm. The color went from pure green to pure red, ([x,y] = 0.682,0.296). The LER of the pure mCherry solution and lysate final in-solution color conversion LEDs were 36.4 lm/W and 45.7 lm/W, respectively. The RMSD between the normalized final spectra was 3.7 %.

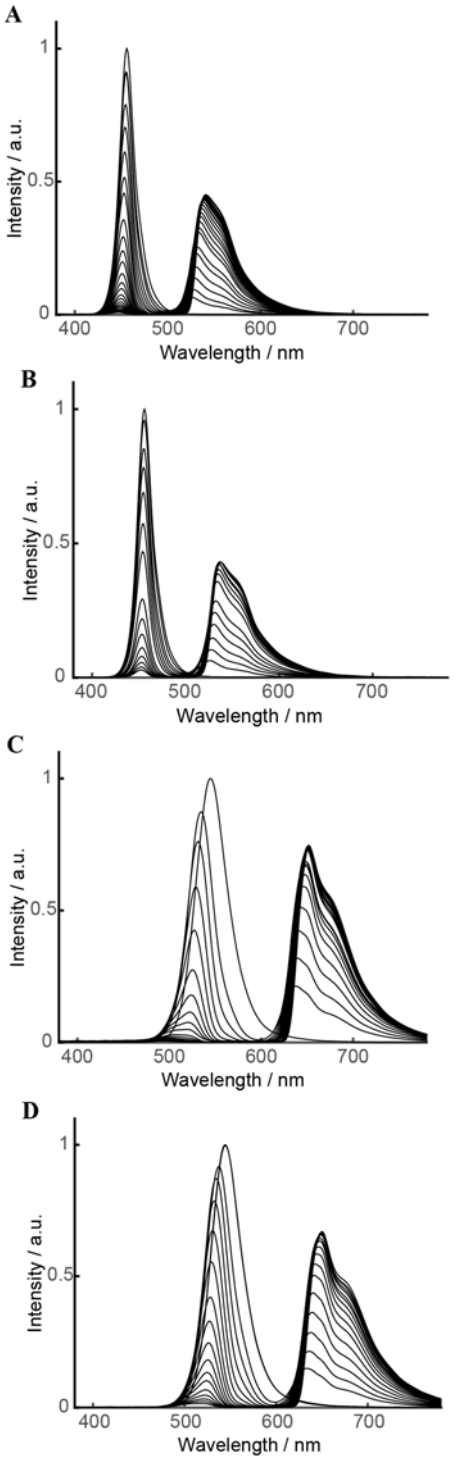

Figure 2. Color conversion spectra. A. Pure eGFP solution over a blue pump LED; from 0 – 1000 µl of solution in 40 µl aliquots. B. eGFP lystate over a blue pump LED; from 0 – 600 µl of solution in 40 µl aliquots. C. Pure mCherry solution over a green pump LED; from 0 – 800 µl of solution in 40 µl aliquots. D. mCherry lystate over a green pump LED; from 0 – 680 µl of solution in 40 µl aliquots.

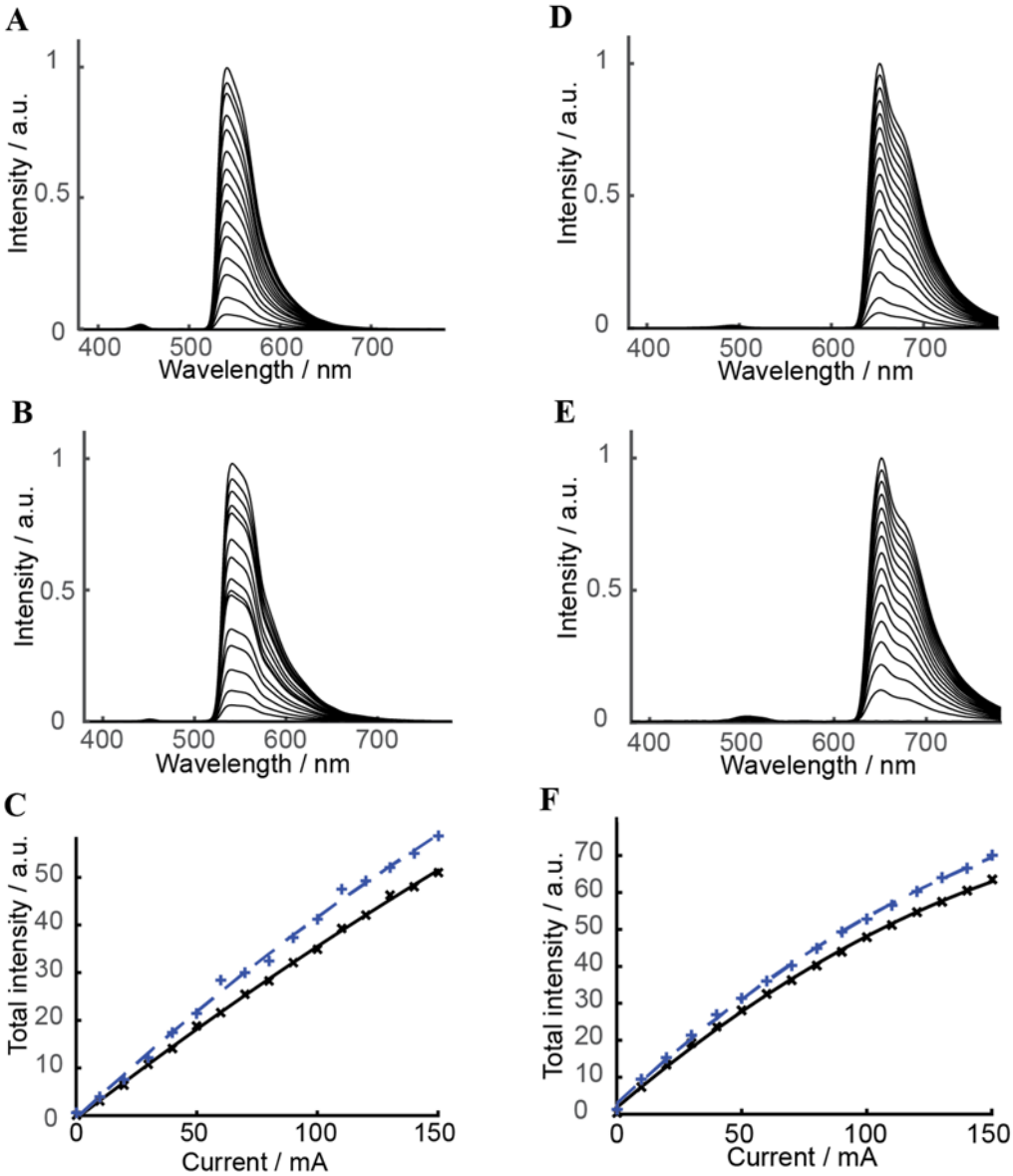

Figure 3. Spectra under different pump LED current bias. A. Pure eGFP solution from 0-150 mA in 10 mA steps. B. eGFP lysate from 0-150 mA in 10 mA steps. C. Total integrated intensity for pure eGFP solution (black crosses; black solid line is quadric fit) and eGFP lysate (blue pluses; blue dashed line is quadratic fit). D. Pure mCherry solution from 0-150 mA in 10 mA steps. E. mCherry lysate from 0-150 mA in 10 mA steps. F. Total integrated intensity for pure mCherry solution (black crosses; black solid line is quadric fit) and mCherry lysate (blue pluses; blue dashed line is quadratic fit).

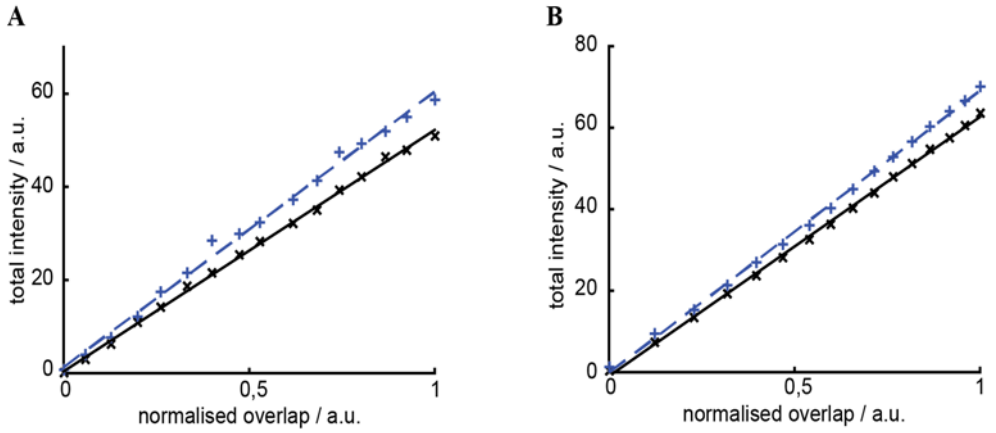

Figure 4. Total integrated intensity as a function of spectra overlap of the pump and fluorescent protein absorption spectra. A. Pure eGFP solution (black crosses) and eGFP lystate (blue pluses) and linear fits (pure eGFP solution is black line; eGFP lystate is blue dashed line). B. Pure mCherry solution (black crosses) and mCherry lystate (blue pluses) and linear fits (pure mCherry solution is black line; mCherry lystate is blue dashed line).

The current intensity relationships were investigated for the in-solution color conversion LEDs. In-solution color conversion layers were prepared from pure eGFP solution and lysate with total path lengths of 5.09 cm and 4.07 cm respectively, and the spectra of these layers pumps with a blue LED was measured at different current injection levels (Figure 3A-B). The total intensity of the collected light strongly correlated with the LED current bias up to 150 mA (ρ = 0.999 and ρ = 0.997 for pure and lysate respectively). The relationship was almost linear with deviations of 11.6 % and 16.8 % for pure solution and lysate, respectively (Figure 3c). The small deviations from linearity were due to non-linearity in the blue pump LED and not due to saturation or bleaching of the fluorescent proteins.

Similarly, in-solution color conversion layers were prepared from pure mCherry solution and lysate with total path lengths of 4.07 cm. The spectra of these were collect by pumping with a green LED with different current injections up to 150 mA (Figure 3D-E). As with eGFP color conversion layers the total intensity of collected light strongly correlated with the LED bias current (ρ = 0.993 for both pure and lysate. The deviation from linearity was greater for the mCherry in-solution color conversion LEDs; 27.9 % and 27.5 % for pure solution and lysate respectively (Figure 3F). This is due to greater non-linearity and spectral changes in the green pump LED. After correcting for total effective overlap of the green LED spectrum with the mCherry absorption spectrum, the relationships become linear (Figure 4).

4. Discussion and Conclusion

Organic dyes would be promising materials for color conversion application because of their low toxicity and relative ease of production; however most suffer from their strong static and dynamic quenching preventing their use at high concentration. Biologically derived fluorescent proteins also have low toxicity and their production, although currently expensive, is simple, safe, green and scalable. eGFP, the most popular and extensively used fluorescent protein, has recently been shown to be an interesting optical material [3,4]. In 2015 Weber et. al. demonstrated fluorescent proteins chemically bonded to an organic polymer matrix could be used to produce color-conversion layers for white LEDs [9]. We have demonstrated that in solution both eGFP and mCherry can be used as optical wavelength down-convertors for color conversion application, such as color-conversion layers for LEDs.

Our work suggests that the presence of other protein impurities in solution does not affect the spectrum quality of color-conversion layers produced from fluorescent proteins. The purification of fluorescent proteins by GST binding affinity chromatography adds between €250-750 per gram of pure protein as well as increasing the time for production by 2-3 days, removing this step greatly increases the possible throughput for fluorescent protein production. Proteins are typically not stable at elevated temperatures; however the possibility of mixing active proteins, such as fluorescent proteins, with inactive proteins or polysaccharides may improve their stability.

Research and development into biologically derived fluorescent proteins is focused on improving brightness, removing the need for oligomerization, for single molecule resolution fluorescence microscopy, and control bleaching [1-2]. The result is that, although there is a large library of fluorescent proteins none have been optimized for traditional optical applications, such as color conversion or lasing. Due to their low stokes shift, fluorescent proteins are already promising candidates for color-conversion materials; low stokes shift results in less energy losses during absorption and emission of photons, this lead to less heat loss and higher efficiencies. The other critical value for efficiency is quantum yield. The highest know quantum yield of a fluorescent protein is currently 0.93 [10]; however most fluorescent proteins, especially those with emissions between 500-700 nm have quantum yields below 0.8. There is no theoretical limitation to the generation of high quantum yield low stokes shift fluorescent proteins.

Currently, research into the use of fluorescent proteins in lighting application is in its infancy; however these bio-friendly, flexible and renewable materials already show great promise for SSL. In addition, devices build around fluorescent proteins could be used for biomedical application where, correctly designed devices would simply dissolve into the body after its useful lifetime with no harmful or toxic bio-products. We believe that fluorescent proteins show great promise for future solid-state lighting technologies.

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