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Novel GFP-like proteins from reef corals with unique light-inducible properties highly suited for fluorescence imaging technologies.

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In recent years, there has been a dramatic development of the use of the green fluorescent protein (GFP) homologues, discovered in reef corals and related anthozoans [1], as reporters for the detection of events in living cells and tissues. Numerous GFP-like proteins have been cloned from marine organisms and their expanded use, together with the genetically modified GFPs from the jelly-fish *Aequorea,* has been paralleled by the development of various photolabeling techniques and of advanced fluorescence-based imaging technologies [3]. Photolabeling includes the use of the photoactivatable PA-GFP [3]; greening properties of DsRed by photobleaching [4]; green to red photoconverting coral protein, Kaede [5]; and kindling of the non-fluorescent anemone protein to red fluorescent state [6]. Research of the diversity, optical properties and biological functions of the GFP-like proteins of the Great Barrier Reef (GBR) corals [7] identified the existence of spectrally diverse blue to red GFP-like proteins in numerous species, many possessing unique photo-inducible properties [8]. Previous research of their localization in coral cells found heterogeneous mixtures of different GFP spectral variants present, functioning in photoprotection by dissipating excessive sunlight via energy transfer cascades [7]. Photoactivation of GFPs in tissue samples of various Great Barrier Reef coral species were tested by exposing frozen or live, small coral samples to epifluorescent light. *Acropora millepora*, was found to be one of species whose GFP-pigmented tissues exhibited photoactivation. To determine the photoinducible properties of its individual GFP-like proteins, cloned and purified proteins (amilFP513 & amilFP593 - provided by M. Matz, University of Florida) in phosphate buffer were dried onto glass slides and exposed to various wavelengths (1 min) at epifluorescent or confocal (458, 488, 543 nm) irradiation. They were next imaged and spectrally scanned (obj. PL APO 40.0x0.75) by Leica TCS SP2 confocal microscope.

Photoactivation occurred in amilFP513, with green emissions increasing by ~2 fold following UVA epifluorescent irradiation for 2 min , with no further increase after 4 min (Fig. 1). Spectral scanning at 488 nm excitation caused quenching of fluorescence observable as only slight emission increase at 513 nm after UVA exposure (Fig. 1d). Bleaching by 488 nm for 1 min caused a dramatic photoconversion of the red amilFP594, with a reduction of 594 nm peak and an increase of 535 nm yellow-green peak (Fig. 2). Thus, UV photoactivation of amilFP513 appears similar to the UV photoactivation of PA-GFP, genetically modified for this purpose [4]. Photoconversion of amilFP594 appears to be similar to “greening” of the DsRed coral protein [4], which has emission maxima at 509 (weak) and 583 nm (strong) [1]. In DsRed, greening is caused by the selective bleaching of the red species of the tetramere by multi-photon wavelengths, causing the fluorescence of the green species, normally quenched by Forster resonance energy transfer, to be enhanced [4]. Photoactivation of PA-GFP and DsRed’s “greening” are used as powerful techniques for regional optical marking of cells and proteins and for analyzing protein kinetics within cells [3,4]. The discovery of the Great Barrier Reef GFP-like proteins with novel optical properties expands the scope of the available bio-imaging techniques for studying cellular and protein dynamics and in their applications in biotechnology.

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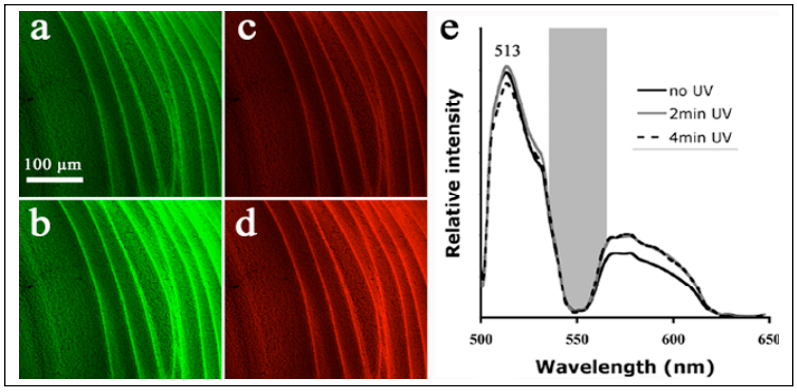

FIG. 1. Photoactivation of green GFP-like coral protein by UVA (360-390 nm). Green emission at 500-520 nm imaged in PMT1 at excitation by 488 nm laser line (a, b); red emissions at 570-620 nm imaged in PMT2 at excitation by 543 nm laser line (c, d). Emissions before (a, c) and after (b, d) UVA irradiation for 2 min. (d) Emission spectra before and after 2 & 4 min UV exposure, spectrally scanned at 488 nm excitation which appeared to quench UVA-induced fluores-cence amplification (Shaded area indicates excitation 488/543/633 beam splitter position on spectrum)

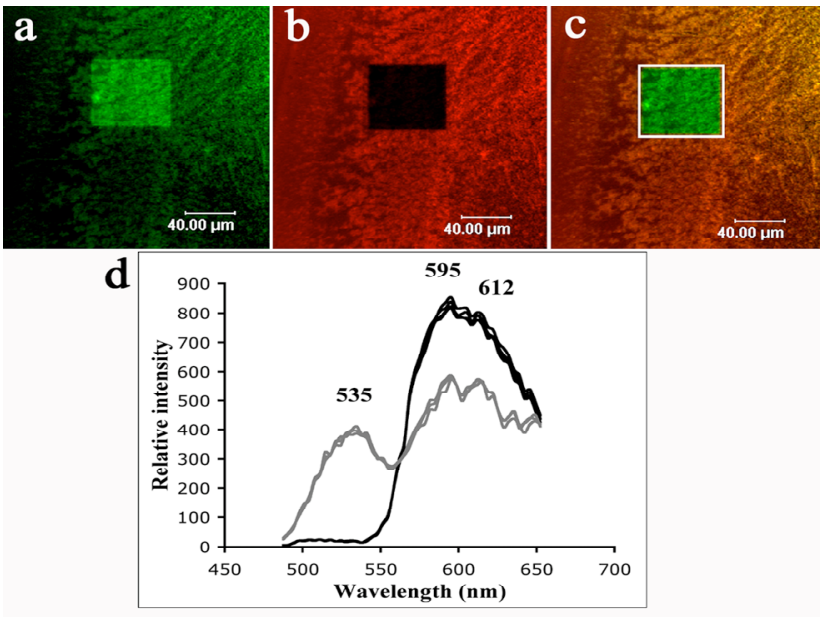

FIG. 2. Photoconversion of red GFP-like protein amilFP594 in selected AOI to yellow-green color by 488 nm laser line. Bleaching achieved by zooming to 18µm x 18µm area, scanning for 1 min and zooming out to perform imaging and spectral collection. (a) Emissions imaged at 500-540 nm by 488 nm excitation; and (b) and at 580-650 nm by 543 nm excitation, showing increase of yellow-green and loss of red emissions in AOI; (c) over-layed a and b. (d) Spectral scan at 2-photon 925 nm excitation shows the appearance of 535 nm peak in the bleached area (grey line) compared to unbleached area (black line) .

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