A GUIDE TO CHOOSING FLUORESCENT PROTEINS pdf

1: Shaner Nature Methods - Choosing Fluorescent Proteins - [PDF Document]

A guide to choosing fluorescent proteins. Shaner NC(1), Steinbach PA, Tsien RY. Author information: (1)Department of Pharmacology, University of California at San Diego, Gilman Drive, La Jolla, CA , USA.

Fluorescence Microscopy and Live-Cell Imaging Fluorescent Proteins The discovery and development, over the past decade, of fluorescent proteins from a wide variety of marine organisms has initiated a revolution in the study of cell behavior by providing convenient markers for gene expression and protein targeting in living cells and organisms. The most widely used of these fluorescent proteins, the green fluorescent protein GFP first isolated from the jellyfish Aequorea victoria, can be attached to virtually any protein of interest and still fold into a fluorescent molecule. The resulting GFP fusion product can be used to localize previously uncharacterized proteins or to visualize and track known proteins to further understand cellular events. The use of fluorescent proteins as a minimally invasive tool for studying protein dynamics and function has been stimulated by the engineering of genetic variants with improved brightness, photostability and expression properties see Figure 1. Cells that express gene products tagged with fluorescent proteins can be imaged with low light intensities over many hours to provide useful information about changes in the steady-state distribution of a protein over time. Introduction to Fluorescent Proteins - The discovery of green fluorescent protein in the early s ultimately heralded a new era in cell biology by enabling investigators to apply molecular cloning methods, fusing the fluorophore moiety to a wide variety of protein and enzyme targets, in order to monitor cellular processes in living systems using optical microscopy and related methodology. When coupled to recent technical advances in widefield fluorescence and confocal microscopy, including ultrafast low light level digital cameras and multitracking laser control systems, the green fluorescent protein and its color-shifted genetic derivatives have demonstrated invaluable service in many thousands of live-cell imaging experiments. The Fluorescent Protein Color Palette - A broad range of fluorescent protein genetic variants have been developed over the past several years that feature fluorescence emission spectral profiles spanning almost the entire visible light spectrum. Extensive mutagenesis efforts in the original jellyfish protein have resulted in new fluorescent probes that range in color from blue to yellow and are some of the most widely used in vivo reporter molecules in biological research. Longer wavelength fluorescent proteins, emitting in the orange and red spectral regions, have been developed from the marine anemone Discosoma striata and reef corals belonging to the class Anthozoa. Still other species have been mined to produce similar proteins having cyan, green, yellow, orange, red, and far-red fluorescence emission. Developmental research efforts are ongoing to improve the brightness and stability of fluorescent proteins, thus improving their overall usefulness. We now have jellyfish proteins that span an nanometer portion visible spectrum from deep blue to yellow-green, providing a wide choice of genetically encoded markers for studies in cell biology. Most of the fluorescent proteins that are commonly used today have been modified through mutagenesis to optimize their expression in biological systems. Continued efforts using directed evolution approaches will no doubt improve the spectral characteristics, photostability, maturation time, brightness, acid resistance, and utility of the fluorescent protein tags for cellular imaging. Anthozoa Fluorescent Proteins - Although promising candidates are now available in every Anthozoa fluorescent protein spectral class, in most cases there remains no EGFP equivalent, in terms of photostability and other critical areas of performance with the exception of pH stability. New additions to the blue and cyan region feature substantially improved brightness and photostability, and any of the orange fluorescent proteins are excellent choices for long-term multicolor imaging. In addition, although brighter than EGFP, photostability is still suboptimal for the yellow fluorescent proteins, whereas the red and far-red variants are among the dimmest in all spectral classes. Further compounding the problem is the potential for aggregation artifacts due to poorly folding proteins, regardless of the spectral class or supposedly monomeric characteristics. Given that most of these proteins have only been introduced in the past couple of years, we remain optimistic that in the future, bright and photostable additions will become available for all spectral classes. Imaging Parameters for Fluorescent Proteins - The wide spectrum of fluorescent proteins and derivatives uncovered thus far are quite versatile and have been successfully employed in almost every

biological discipline from microbiology to systems physiology. These unique probes have proven extremely useful as reporters for gene expression studies in both cultured cells and entire animals. In living cells, fluorescent proteins are most commonly utilized to track the localization and dynamics of proteins, organelles, and other cellular compartments, as well as a tracer of intracellular protein trafficking. Quantitative imaging of fluorescent proteins is readily accomplished with a variety of techniques, including widefield, confocal, and multiphoton microscopy, to provide a unique window for exposing the intricacies of cellular structure and function. Optical Highlighter Fluorescent Proteins - Protein chromophores that can be activated to initiate fluorescence emission from a quiescent state a process known as photoactivation , or are capable of being optically converted from one fluorescence emission bandwidth to another photoconversion , represent perhaps the most promising approach to the in vivo investigation of protein lifetimes, transport, and turnover rates. Appropriately termed molecular or optical highlighters, photoactivated fluorescent proteins generally display little or no initial fluorescence under excitation at the imaging wavelength, but dramatically increase their fluorescence intensity after activation by irradiation at a different usually lower wavelength. Photoconversion optical highlighters, on the other hand, undergo a change in the fluorescence emission bandwidth profile upon optically-induced changes to the chromophore. These effects result in the direct and controlled highlighting of distinct molecular pools within the cell. Practical Considerations for using Fluorescent Proteins - Fluorescent proteins are quite versatile imaging probes and have been successfully employed in virtually every biological discipline ranging from microbiology to systems physiology. These ubiquitous probes are extremely useful as reporters for gene expression studies in cultured cells, excised tissues, and whole animals. In live cells, fluorescent proteins are most commonly used to track the localization and dynamics of proteins, organelles, and other cellular compartments, while they can also be used to assess protein-protein interactions through the use of resonance energy transfer techniques FRET. This review provides some general tips for the practical aspects of using and imaging enhanced green fluorescent protein EGFP and newer members of the color palette. Interactive Java Tutorials Choosing Filter Combinations for Fluorescent Proteins - Fluorescence filter combinations designed to image fluorescent proteins must be carefully chosen to maximize the level of emission intensity presented to the detector while simultaneously reducing the number of unwanted photons from autofluorescence or bleed-through by other fluorophores. The broad absorption and emission spectral profiles exhibited by most fluorescent proteins offer a wide range of choice in filters, which are usually optimized for use with a specific detection system human eye, digital camera, or photomultiplier. This interactive tutorial is designed to enable the identification of critical filter parameters, including the center wavelength, bandwidth region, and dichromatic mirror cut-on wavelength, which are necessary for imaging fluorescent proteins. Choosing Fluorescent Proteins for Dual Labeling Experiments - The broad excitation and emission spectral profiles exhibited by fluorescent proteins and their color-shifted genetic variants often require specialized considerations when designing live-cell imaging experiments using two or more of these unique probes simultaneously. Of primary concern are potential bleed-through artifacts resulting from the significant degree of emission spectral overlap usually exhibited by fluorescent protein combinations. This interactive tutorial explores matching fluorescent proteins for dual labeling investigations with regards to spectral bandwidth and overlap, excitation efficiency, emission window dimensions, and other parameters necessary to design logical experiments. Fluorescence Resonance Energy Transfer with Fluorescent Proteins - Fluorescent proteins are increasingly being applied as non-invasive probes in living cells due to their ability to be genetically fused to proteins of interest for investigations of localization, transport, and dynamics. Because energy transfer is limited to distances of less than 10 nanometers, the detection of FRET provides valuable information about the spatial relationships of fusion proteins on a sub-resolution scale. This interactive tutorial explores various combinations of fluorescent proteins as potential FRET partners and provides information about critical resonance energy transfer parameters, as well as suggestions for microscope optical filter and light source configuration. Optical Highlighter Fluorescent Proteins - Photoactivated fluorescent proteins generally display little or no initial fluorescence under excitation at the imaging wavelength, but dramatically increase their fluorescence intensity after activation by irradiation at a different usually lower wavelength. This interactive tutorial explores the optical conversion of several useful highlighter probes and simulates how

these proteins would be viewed in an actual confocal microscope. Fluorescent Protein Fluorophore Maturation Mechanisms - Autocatalytic formation of the fluorophore also referred to as a chromophore within the shielded environment of the polypeptide backbone during fluorescent protein maturation follows a surprisingly unified mechanism, especially considering the diverse natural origins of these useful biological probes. Shortly after synthesis, most fluorescent proteins slowly mature through a multi-step process that consists of folding, initial fluorophore ring cyclization, and subsequent modifications of the fluorophore. The spectral properties of fluorescent proteins are dependent upon the structure of the fluorophore as well as the localized interactions of amino acid residues in the immediate vicinity, and in some cases, residues far removed from the fluorophore. The interactive tutorials in this section explore fluorophore formation in a wide variety of spectrally diverse fluorescent proteins deduced from crystallographic studies. PA-GFP Chromophore Photoactivation - The highly unique photophysical properties of wild-type green fluorescent protein wild-type GFP were thoroughly investigated during the mids, and served as a foundation for the creation of the first useful optical highlighter designed specifically for photoactivation studies. Termed PA-GFP for Photo Activatable Green Fluorescent Protein , this optical highlighter was developed by improving the photoconversion efficiency of the native chromophore from a predominately neutral form to a species that is anionic in character. By replacing the threonine at position with a histidine residue TH in wild-type GFP, researchers produced a variant having negligible absorbance in the region between and nanometers, thus dramatically enhancing contrast between the non-activated and activated species. Dronpa Fluorescent Protein Chromophore Photoswitching - The most prominent and well-studied photoswitchable fluorescent protein is named Dronpa named after a fusion of the Ninja term for vanishing and photoactivation , which is a monomeric variant derived from a stony coral tetramer. Dronpa fluorescent protein exhibits an absorption maximum at nanometers arising from the anionic, deprotonated chromophore with a minor peak at nanometers from the neutral, protonated chromophore. The anionic chromophore emits green fluorescence with a maximum at nanometers and has a brightness level almost 2. Dronpa photoswitching occurs in part by interconversion between the deprotonated on state; bright and protonated off state; dark forms. Illumination at nanometers drives Dronpa to the dark species after which the fluorescent protein can be subsequently switched back on by brief illumination at nanometers. This cycle can be repeated several hundred times without significant photobleaching. Unlike photoactivatable fluorescent proteins, these probes are readily tracked and imaged in their native emission state prior to photoconversion, making it easier to identify and select regions for optical highlighting. The first report of a photoconvertable highlighter was a tetrameric fluorescent protein isolated from the stony Open Brain coral, Trachyphyllia geoffroyi, which can be photoconverted from green to red fluorescence emission by illumination with ultraviolet light. The discovery of this highlighter was serendipitous, as are many important discoveries. It occurred when the researchers accidentally left a test tube containing the protein on a laboratory bench near a window, and then astutely observed the shift from green to red. The unusual color transition prompted investigators to name the protein Kaede, after the leaves of the Japanese maple tree that turn from green to red in the fall. Excited-State Proton Transfer - Among the most unique features of wild-type green fluorescent protein wt-GFP is the fact that illumination with either ultraviolet or blue-cyan light gives rise to green fluorescence having a maximum wavelength at approximately nanometers. The bimodal absorption spectrum of wt-GFP features a large peak at nanometers the A band and a much smaller peak at nanometers the B band. The lesser B band corresponds to the anionic chromophore, which demonstrates normal photophysics, whereas the predominant A band corresponds to the neutral chromophore that would normally be expected to emit blue light peaking at approximately nanometers upon excitation in the ultraviolet. However, when excited with light ranging from to nanometers, the tyrosine residue in the neutral chromophore of wt-GFP becomes a strong acid and transfers a proton through a novel hydrogen bond network to generate an excited state anion a process known as excited-state proton transfer; ESPT. It is the anionic form of the chromophore that emits green light. Literature Sources General Fluorescent Protein References - The disciplines of cellular and molecular biology are being rapidly and dramatically transformed by the application of fluorescent proteins developed from marine organisms as fusion tags to track protein behavior in living cells. The most widely used of these probes, green fluorescent protein, can be

attached to virtually any target of interest and still fold into a viable fluorescent species. The resulting chimera can be employed to localize previously uncharacterized proteins or to visualize and track known proteins to further understand critical events at the cellular and molecular levels. This section features a bibliography of literature sources for review articles and original research reports on the discovery, applications, and continued development of fluorescent proteins. ZEISS Campus Fluorescent Protein Reference Library - Application of the growing class of fluorescent proteins capable of forming an intrinsic chromophore to the observation of living cells and animals has almost single-handedly launched and fueled a new era in biology and medicine. These powerful research tools have provided investigators with a mechanism of fusing a genetically encoded optical probe to a practically unlimited variety of protein targets in order to examine living systems using fluorescence microscopy and related technology. The references listed below point to review articles that should provide the starting point for a thorough understanding of fluorescent protein technology. Biosensor Fluorescent Protein References - By directly coupling the advanced microscopic technique of fluorescence resonance energy transfer FRET to the ubiquitous green fluorescent protein and its multispectral variants fused with selected biopolymers, ingenious researchers have created a new class of biosensors capable of elucidating mechanisms of signaling, enzymatic cleavage, and other critical functions in living cells. This section features a bibliography of literature sources for review articles and original research reports on the construction, applications, and continued development of biosensor fluorescent proteins. Optical Highlighter Fluorescent Protein References - The ability to selectively initiate or alter fluorescence emission profiles in photoconversion optical highlighter proteins renders these probes as excellent tools for exploring protein behavior in living cells. As the fluorescence intensity or color spectrum of highlighters occurs only after photon-mediated conversion, newly synthesized non-photoactivated protein pools remain unobserved and do not complicate experimental results. This section lists sources for review articles and original research reports on optical highlighter fluorescent proteins. Fluorescent Protein Photobleaching References - The field of cell biology is rapidly being transformed by the application of fluorescent proteins as fusion tags to track dynamic behavior in living cells. In this regard, fluorescence recovery after photobleaching FRAP is often employed to selectively destroy fluorescent molecules within a region of interest with a high-intensity laser, followed by monitoring the recovery of new fluorescent molecules into the bleached area over a period of time with low-intensity laser light. The resulting information can be used to determine kinetic properties, including the diffusion coefficient, mobile fraction, and transport rate of the fluorescently labeled molecules. The selected references in this section point to important literature sources for information on FRAP with fluorescent proteins. Fluorescent Protein FRET References - Understanding the dynamic interactions between proteins within living cells is fundamental to a basic knowledge of the underlying concepts that guide molecular and cellular biology. Over the past few years, the rapid development of fluorescent proteins and their application as fusion products and biosensors has significantly expanded the molecular toolkit available for probing the mysteries of cellular physiology and pathology. The references listed in this section highlight important literature sources for review articles and original research reports on the construction and applications of fluorescent proteins for resonance energy transfer experiments. Internet-Based Education on Fluorescent Proteins - Despite the explosive growth of the Internet in terms of the World Wide Web as an informational resource for the original scientific literature pertaining to fluorescent protein investigations, there remains an obvious void in educational Websites targeted at beginning students and novices in the field. To address this issue, educational sites dedicated to optical microscopy and digital imaging being constructed and hosted at The Florida State University are turning their attention to the increasing application of fluorescent proteins for live-cell imaging studies. The primary focus of this effort is to create new sections of the sites that address the structure and properties of fluorescent proteins as well as optimizing their utility in imaging experiments. Internet Resources Fluorescent Protein Vector Commercial Sources - A variety of fluorescent proteins, available as recombinant DNA plasmid vectors designed for transfection of mammalian cells or transformation of bacteria, are commercially available from a number of distributors. Most of the vectors containing fluorescent protein DNA sequences have been codon-optimized for expression in mammalian cells and contain antibiotic genes for selection of stable mutants having relatively constant expression levels. The

vectors often contain multiple cloning sequences that enable researchers to easily insert their gene of interest for fusion to the fluorescent protein. Other common features in fluorescent protein vectors include a human cytomegalovirus CMV promoter, a Kozak translation initiation site, an early mRNA polyadenylation signal, and a bacterial antibiotic gene. Live Cell Imaging - One of the foremost targets in the life sciences is to understand the structure, function, and behavior of living organisms, and with evolving advances in technology, such as the development of confocal microscopy and fluorescent probes, it has become possible to pursue this goal at the cellular and subcellular levels. Still, working with and imaging live cells can be a complex, if not daunting, task to microscopists unfamiliar with the techniques and tools that are available. The following is a compilation of resources that offer overviews, background information, interactive forums, frequently asked questions, protocols, and hints that should aid any microscopist attempting to enter into this important, burgeoning field. Microscopy Specimen Chambers - The demands of modern confocal microscopy, especially those involving imaging of living cells and tissues, require that researchers take special precautions with their specimens. Indeed, simple microscope slides are unsuitable for many applications, resulting in the development of a broad range of specimen chambers, which can often supply the flexibility a microscopist needs. The list of resources in this section exemplifies the great variety of specimen chambers that are available today and should help visitors locate the products that are best suited for their specific scientific pursuits. Fluorescence Filters - Fluorescence microscopy relies heavily on the ability to select a specific wavelength region for excitation of the specimen and gathering secondary emission during image formation.

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2: A Biologistâ€™s Guide to Choosing Your Fluorophore Palette - Bitesize Bio

Since the original green fluorescent protein (GFP) gene was cloned in 1, there has been an explosion in the variety of fluorescent proteins (FPs) available. They can be fused to a protein in transgenic cells or animals, conjugated to an antibody, or even used as a substrate in enzymatic reactions.

Oligomerization Generally, try to use a monomeric FP. To view the properties of some of the most popular fluorescent proteins, take a look at our quick reference card. Whether you choose to fuse your FP to the C or N terminal of your protein of interest largely depends on the protein itself: If you have the resources or your experiment is novel, it might be best to clone both C- and N-terminally tagged constructs to determine the best option. One research group found that more C-terminal fusion proteins localize to the intended subcellular compartment than N-terminally tagged fusion proteins2. However, it is important to stress that while C-terminally tagged proteins tend to localize and behave as expected, this is not always possible to predict. You should confirm fusion protein localization by using an antibody against the native protein. Immunofluorescence can be used to check that the fusion protein localizes correctly; an immunoblot will help to confirm the fusion protein is the correct size and expressed at the expected levels; and co-immunoprecipitation can help to assess how the fusion protein interacts with known substrates3. Excitation, emission, and brightness If you plan on using multiple FPs, it is important to choose FPs with distinct emission peaks â€” as well as excitation peaks that you can target with your available lasers. If the emission peaks overlap, it will be difficult, or possibly impossible, to differentiate them. You typically want the brightest FP within your available spectra in order to achieve a clear signal and overcome any potential background fluorescence. Maturation and bleaching Maturation defines how long it takes an FP to fold correctly, form the chromophore, and begin to fluoresce. For time-sensitive events in live cells, a short maturation time can be important. Bleaching is a measure of photostability, ie how long after excitation the chromophore loses the ability to emit light. If you plan on conducting lengthy time-lapse experiments, consider an FP with a high photostability. Environmental conditions Like most proteins, FPs are affected by pH, temperature, and oxygen levels. Depending on the environment you plan to use your FP in, you may need to either adjust the conditions slightly or select a more appropriate FP. The pH can affect excitation and emission peaks, and the majority of FPs are sensitive to acid. The pKa value is a good indicator of pH sensitivity: Temperature and oxygen levels both affect maturation times: Codon optimization As most FPs are derived from jellyfish or coral proteins â€” rather than something like the mammalian cells and tissues you are likely to use them in â€” there can be an interspecies difference in the amino acid codons used. This can lead to poor FP expression and therefore low signal. Fortunately, many of the newer versions of FPs have been codon-optimized to reflect mammalian cell preferences. Check to see if your FP sequence has been modified for use in a certain species. Many of the early FPs were prone to form oligomers, and oligomerization can affect the biological function of the fusion protein. EGFP, for example, is a monomer that can form dimers when used in high enough concentrations, which can distort subcellular organelles7 or disrupt experiments like FRET8. Truly monomeric FPs are recommended in the vast majority of cases. Primary structure of the Aequorea victoria green-fluorescent protein. Gene , â€” Investigation into the use of C- and N-terminal GFP fusion proteins for subcellular localization studies using reverse transfection microarrays. Genomics 5, â€” A guide to choosing fluorescent proteins. Methods 2, â€” A bilirubin-inducible fluorescent protein from eel muscle. Cell , â€” Codon usage limitation in the expression of HIV-1 envelope glycoprotein. Formation of stacked ER cisternae by low affinity protein interactions. Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells. Science , â€”6 Get resources and offers direct to your inbox Sign up A-Z by research area.

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3: A guide to choosing fluorescent proteins | Flavio Alves - www.enganchecubano.com

The recent explosion in the diversity of available fluorescent proteins (FPs) promises a wide variety of new tools for biological imaging. With no unified standard for assessing these tools.

With no unified standard for assessing these tools, however, a researcher is faced with difficult questions. Which FPs are best for general use? Which are the brightest? What additional factors determine which are best for a given experiment? Although in many cases, a trial-and- error approach may still be necessary in determining the answers to these questions, a unified characterization of the best available FPs provides a useful guide in narrowing down the options. We can begin by stating several general requirements for For example, the newly released DsRed-Monomer the successful use of an FP in an imaging experiment. Second, the FP should have several highly variable factors, including the intrinsic sufficient photostability to be imaged for the duration of brightness of the protein determined by its maturation the experiment. Third, if the FP is to be expressed as a speed and efficiency, extinction coefficient, quantum fusion to another protein of interest, then the FP should yield and, in longer experiments, photostability , the not oligomerize. Fourth, the FP should be insensitive optical properties of the imaging setup illumination to environmental effects that could confound quanti- wavelength and intensity, spectra of filters and dichroic tative interpretation of experimental results. Finally, in mirrors , and camera or human eye sensitivity to the multiple-labeling experiments, the set of FPs used should emission spectrum. Although these factors make it have minimal crosstalk in their excitation and emission impossible to name any one FP as the brightest over- channels. For more complex imaging experiments, such all, it is possible to identify the brightest protein in each as those using fluorescence resonance energy transfer spectral class when more than one protein is available , FRET 17 or selective optical labeling using photocon- as this depends only on the intrinsic optical proper- vertible FPs12,15, additional considerations come into ties of the FP. The brightest proteins for each class are play. General recommendations to help determine listed in Table 1, with greater detail on the properties of the optimal set of FPs in each spectral class for a given each listed protein available in Supplementary Table 1 experiment are available in Box 1, along with more detail online. As discussed below in relation to photostability, on each issue discussed below. Purely teins may fold more or less efficiently. Correspondence should be addressed to R. Indications of potential fold- at a much lower rate than many small-molecule dyes Table 1. For experiments requiring a limited number malian cell lines could have substantial influences on folding and of images around 10 or fewer , photostability is generally not a maturation efficiency. Briefly, aqueous droplets of purified in living organisms is the presence or absence of molecular oxygen. FPs at pH 7 were formed under mineral oil in a chamber that The requirement for O2 to dehydrogenate amino acids during chro- allows imaging on a fluorescence microscope. Droplets of volumes mophore formation has two important consequences. First, each comparable to those of typical mammalian cells were photobleached molecule of AFP should generate one molecule of H2O2 as part with continuous illumination while recording images periodically of its maturation process18, and the longer-wavelength FPs from to generate a bleaching curve. To account for differences in bright- corals probably generate two Even when anoxia initially tion coefficient and quantum yield of the FP, the emission spectrum prevents fluorophore maturation, fluorescence measurements are of the arc lamp used for excitation, and the transmission spectra usually done after the samples have been exposed to air Although it is not as bright as many shorter-wavelength options, it should be used when spectral separation from other FPs is critical, and it may give some advantage when imaging thicker tissues. Its predecessor mRFP1 is now obsolete. The tandem dimer tdTomato is equally photostable but twice the molecular weight of mCherry, and may be used when fusion tag size does not interfere with protein function. Each of these should perform well in most applications. Its photostability under arc-lamp illumination, however, is much lower than that of other CFP variants. This method of normalization pro- Fig. Because dimmer in its applicability to different imaging modalities, such as laser scan- proteins will require either higher excitation power or longer expo- ning confocal microscopy. Although we believe that our measure- sures, we believe this method of normalization provides a realistic ments are valid for excitation

light intensities typical of standard picture of how different FPs will perform in an actual experiment epifluorescence microscopes with arc lamp illumination up to imaging populations of FP molecules. Taking into account brightness and folding when excited at nm on a laser scanning confocal microscope. The red tandem dimer tdTomato is laser illumination on a confocal microscope3 but appears less photo- also highly photostable and may be used when the size of the fusion tag stable than ECFP with arc lamp illumination. Such inconsistencies is not of great concern. The relative photostability of proteins in each between bleaching behavior at moderate versus very high excita- spectral class is indicated in Table 1. Single-molecule illumination intensityâ€”dependent fast bleaching components, and so measurements will be even less predictable based on our population photobleaching curves were taken at lower illumination intensities measurements, because our extinction coefficients are averages that where this effect was less pronounced. The GFP variant Emerald dis- include poorly folded or nonfluorescent molecules, whereas single- played a very fast initial bleaching component that led to an extremely molecule observations exclude such defective molecules. But after this initial fast bleaching phase, its It is critical to choose filter sets wisely for experiments that require photostability decayed at a rate very similar to that of EGFP. All YFPs, long-term or intensive imaging. Choosing suboptimal filter sets will with the exception of Venus, have reasonably good photostability, and lead to markedly reduced apparent photostability owing to the need thus, YFP selection should be guided by brightness, environmental to use longer exposure times or greater illumination intensities to sensitivity or FRET performance see Box 1 for greater detail and for obtain sufficient emission intensity. Its photostability after the initial few seconds, however, is comparable to that of EGFP. Because tightly dimeric or tetrameric7,9â€”12,14, Many of these wild-type pro- of this, one should avoid using mOrange4, GFPs or YFPs for experi- teins, however, can be engineered into monomers or tandem dimers ments in which acid quenching could produce artifacts. Conversely, functionally monomeric though twice the molecular weight , which the pH sensitivity of these proteins can be very valuable to monitor can then undergo further optimization4,10,12, Thus, even though organellar luminal pH or exocytosis26, Although linear unmixing deleterious effects Thus, any of the recommended proteins in systems promise the ability to distinguish between large numbers of Table 1 should be capable of performing well in any application different fluorophores with partially overlapping spectra28, it is pos- requiring a monomeric fusion tag. Researchers should remain vigil- sible even with a simpler optical setup to clearly distinguish between iant of this issue, however, and always verify the oligomerization sta- three or four different FPs. Lack of visible pre- Table 2, one may image cyan, yellow, orange and red Cerulean or cipitates does not rule out oligomerization at the molecular level. To produce even cleaner spectral separa- ture, but care should always be taken to do the appropriate controls tion, one could image cyan, orange and far-red Cerulean or CyPet, when exploring new cell lines or tissues. In our hands, tetrameric proteins can Additional concerns for complex experiments be somewhat toxic to bacteria, especially if they display a substan- For more complex imaging experiments, additional factors come tial amount of aggregation, but monomeric proteins are generally into play when choosing the best genetically encoded fluores- nontoxic. It seems difficult or impossible to generate transgenic mice cent probe, many of which are beyond the scope of this perspec- widely expressing tetrameric RFPs, whereas several groups have suc- tive. The recent development of the FRET- When images must be quantitatively interpreted, it is critical that the optimized cyan-yellow pair CyPet and YPet holds great promise for fluorescence intensity of the protein used not be sensitive to factors the improvement of FRET sensitivity2, and it is the current favorite other than those being studied. Early YFP variants were relatively as a starting point for new FRET sensors but has yet to be proven chloride sensitive, a problem that has been solved in the Citrine and in a wide variety of constructs. For experiments requiring photo- Venus and likely YPet variants1,2, Most FPs also have some acid activatable or photoconvertible tags, several options are available, sensitivity. More acid- PA-mRFP 13, reversibly photoswitchable Dronpa29, the tetrameric sensitive FPs, however, may give poor results when targeted to kindling fluorescent protein KFP 9, and the green-to-red photo- acidic compartments such as the lumen of lysosomes or secretory convertible proteins KikGR14 and EosFP12 the latter is available as a bright tandem dimer and cyan-to-green photoconvertible mono- mer PS-CFP8. A more detailed but probably not exhaustive list of Table 2 Recommended filter sets options for

these more advanced applications of FPs are listed in Fluorescent protein Excitationa Emissiona Supplementary Table 3 online. Bandpass filters with the steepest possible cutoff are new development in the field of FPs and make an informed decision strongly preferred. Supplementary information is available on the Nature Methods website. Selective photolabeling of Thanks to S. Adams for helpful advice on choosing filter sets. Methods 32, â€" Hughes Medical Institute Predoctoral Fellow. This work was additionally supported The authors declare competing financial interests see Nature Methods website for The green fluorescent protein. USA 97, â€" Reprints and permissions information is available online at A variant of yellow fluorescent protein with fast and efficient â€" Rapid detection of a gfp-marked Enterobacter aerogenes under anaerobic conditions by aerobic fluorescence 2. Evolutionary optimization of fluorescent recovery. The molecular properties and 3. An improved cyan applications of Anthozoa fluorescent proteins and chromoproteins. Improved monomeric red, orange and yellow fluorescent lipid-modified monomeric GFPs into membrane microdomains of live cells. Science , â€" Genetic and spectrally 5. Evolution of new distinct in vivo imaging: USA , â€" Efficiently folding and circularly permuted Ubiquitous expression of mRFP1 in transgenic mice. G enesis variants of the Sapphire mutant of GFP. Visualizing secretion optical marker based on the UV-induced green-to-red photoconversion of a and synaptic transmission with pH-sensitive green fluorescent proteins. USA 99, â€" N ature , â€" Photoswitchable cyan fluorescent protein for protein Kindling fluorescent proteins for precise in vivo caspase activation during apoptosis. Multispectral imaging fluorescence Cyan-emitting microscopy for living cells. Reversible single-molecule photoswitching in the GFP- fluorescence resonance energy transfer. Fluorescent proteins from nonbioluminescent Anthozoa USA , ; advance online publication, 15 September doi: Conversion of the monomeric red fluorescent Far-red fluorescent proteins evolved from a blue protein into a photoactivatable probe. Semi- publication, 15 September doi: Fungia concinna 51, 0.

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4: Maturation time of tdTomato fluorescent prote - Bacteria Escherichia coli - BNID

A guide to choosing fluorescent proteins Nathan C Shaner 1,2, Paul A Steinbach 1,3 & Roger Y Tsien 1,3,4 The recent explosion in the diversity of available fluorescent proteins (FPs)

History[edit] Aequorea victoria 3D reconstruction of confocal image of VEGF-overexpressing neural progenitors red and GFP-positive control neural progenitor cells green in the rat olfactory bulb. RECApositive blood vessels - blue color. Wild-type GFP wtGFP [edit] In the s and s, GFP, along with the separate luminescent protein aequorin an enzyme that catalyzes the breakdown of luciferin , releasing light , was first purified from Aequorea victoria and its properties studied by Osamu Shimomura. Some of this luminescent energy is transferred to the GFP, shifting the overall color towards green. The lab of Martin Chalfie expressed the coding sequence of wtGFP, with the first few amino acids deleted, in heterologous cells of E. Researchers have modified these residues by directed and random mutagenesis to produce the wide variety of GFP derivatives in use today. Further research into GFP has shown that is resistant to detergents, proteases, guanidinium chloride GdmCl treatments, and drastic temperature changes. Due to the potential for widespread usage and the evolving needs of researchers, many different mutants of GFP have been engineered. This matched the spectral characteristics of commonly available FITC filter sets, increasing the practicality of use by the general researcher. Superfolder GFP, a series of mutations that allow GFP to rapidly fold and mature even when fused to poorly folding peptides, was reported in They exhibit a broad absorption band in the ultraviolet centered close to nanometers and an emission maximum at nanometers. BFPms1 have several important mutations including and the BFP chromophore Y66H ,YF for higher quantum yield, HG for creating a hole into the beta-barrel and several other mutations that increase solubility. Therefore, they can be used as Zn biosensor. Several additional compensatory mutations in the surrounding barrel are required to restore brightness to this modified chromophore due to the increased bulk of the indole group. These conformations both have a complex set of van der Waals interactions with the chromophore. The YA and HD mutations in Cerulean stabilize these interactions and allow the chromophore to be more planar, better packed, and less prone to collisional quenching. Genetically encoded FRET reporters sensitive to cell signaling molecules, such as calcium or glutamate, protein phosphorylation state, protein complementation, receptor dimerization, and other processes provide highly specific optical readouts of cell activity in real time. Semirational mutagenesis of a number of residues led to pH-sensitive mutants known as pHluorins, and later super-ecliptic pHluorins. By exploiting the rapid change in pH upon synaptic vesicle fusion, pHluorins tagged to synaptobrevin have been used to visualize synaptic activity in neurons. The redox state of the cysteines determines the fluorescent properties of roGFP. However, the same term is also used to refer to monomeric GFP, which is often achieved by the dimer interface breaking AK mutation. GFP is co-expressed with aequorin in small granules around the rim of the jellyfish bell. It is conserved in all three GFP isoforms originally cloned by Prasher. The precise mechanism of this sensitivity is complex, but, it seems, involves donation of a hydrogen from serine 65 to glutamate , which influences chromophore ionization. Thus, the jellyfish may change the color of its bioluminescence with depth. Other fluorescent proteins[edit] Different proteins produce different fluorescent colors when exposed to ultraviolet light. Picture taken by Erik A. These properties are so far unique to fluorescent proteins other than GFP derivatives. FMN-binding fluorescent proteins FbFPs were developed in [27] and are a class of small kDa , oxygen-independent fluorescent proteins that are derived from blue-light receptors. They are intended especially for the use under anaerobic or hypoxic conditions, since the formation and binding of the Flavin chromophore does not require molecular oxygen, as it is the case with the synthesis of the GFP chromophore. White light image, or image seen by the eye, of fluorescent proteins in image above. A review of new classes of fluorescent proteins and applications can be found in Trends in Biochemical Sciences. This process of post-translational modification is referred to as maturation. In addition to the auto-cyclization of the SerTyrGly67, a 1,2-dehydrogenation reaction occurs at the Tyr66 residue. The residues of Gln94, Arg96, and His are able to stabilize by delocalizing the chromophore charge. Arg96 is the most important stabilizing residue due to the fact that it prompts the

necessary structural realignments that are necessary from the HBI ring to occur. Any mutation to the Arg96 residue would result in a decrease in the development rate of the chromophore because proper electrostatic and steric interactions would be lost. Tyr66 is the recipient of hydrogen bonds and does not ionize in order to produce favorable electrostatics. Movie created by Erik A. GFP molecules drawn in cartoon style, one fully and one with the side of the beta barrel cut away to reveal the chromophore highlighted as ball-and-stick.

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5: Roger Y. Tsien - Wikipedia

In our hands, tetrameric proteins can Additional concerns for complex experiments be somewhat toxic to bacteria, especially if they display a substan- For more complex imaging experiments, additional factors come tial amount of aggregation, but monomeric proteins are generally into play when choosing the best genetically encoded fluores- nontoxic.

By Alex Chen You may notice that nature is full of vibrant, even fluorescent, colors. The human eye detects wavelengths ranging from nm and our perception of colors is actually a narrow part of the light spectrum. Other organisms can detect color from a wider spectrum. Why do colors exist? Compounds that can give off bright signals communicate tracking information to observing scientists. In the past years, scientists have found myriad ways to use fluorophores to label and characterize proteins or cells of interest. Today, I would like to walk you through the history of fluorophores and touch upon a few essential considerations in choosing the right one for your experiment, especially when combining multiple fluorophores simultaneously. A Short History of Fluorophores Fluorophores, as the name suggests, are compounds able to emit fluorescence. The fluorescent chemical compound absorbs light at one spectrum and emits light at a different spectrum. A fluorophore, or fluorochrome, typically contains multiple aromatic groups with conjugated electron systems. When you look at a class of fluorophoreâ€"be it organic, synthetic oligomer, protein, or multi-component systemâ€"there are certain distinguishing physical characteristics. On the other hand, the extinction coefficient indicates the quantity of absorbed light at a given wavelength. Another important parameter is the quantum yield, which gives you the efficiency of energy transfer from absorbed light to fluorescence. First Generation Fluorophores Fluorescein has an absorption max at nm and emission max at nm. Fluorescein can also be conjugated to nucleic acids for in situ hybridization. Figure 1 is a typical fluorescence microscopy image using FITC-labeled monoclonal antibodies. A dye commonly used in applications like flow cytometry with similar excitation as FITC is Alexa Nuclear speckles labeled by FITC. Second Generation Fluorophores Built upon the lessons learned from first generation of fluorophores, second generation fluorophores are often more photostable, brighter, and less pH sensitive. The following two fluorescence compounds are found in nature and play an important role in photosynthetic light harvesting! Phycoerythrin PE is isolated from red algae! It is composed of a peptide covalently linked to phycobilin chromophores. It has an absorption max at nm and emission max at nm, giving off a bright red color. Allophycocyanin APC is a protein from the light-harvesting phycobiliprotein family. It is an accessory pigment to chlorophyll. How cool is that? APC can be found in Cyanobacteria blue-green algae and red algae. It absorbs and emits red light at nm and nm, respectively. In this case, one fluorophore serves as the donor and the other as the acceptor. By carefully choosing the fluorophores for pairing, you are able to create unique emission spectra and, thus, increasing your color range. You should be aware of tandem dye stability, because the tandem dye could uncouple and result in unintended consequences like high background fluorescence. Latest Generation Fluorophores Quantum dots, or q-dots, are nano-crystals with amazing physical properties. Q-dots are made of semi-conductor materials, and one of the remarkable features is that q-dot size determines the emission color see figure below. Size, not structurally complex fluorophores, is the only concern with q-dots. In addition, q-dots are brighter with a high extinction coefficient and good quantum yield compared to traditional organic fluorophores. Being composed of inorganic crystals, q-dots are also more stable, which means less photo bleaching than traditional fluorophores. No wonder, then, that q-dots have become the new darling for many biological applications. Below is a picture showing that different size of q-dots gives off different colors Figure 2. Quantum dot q-dot Nano crystals generate different light emission spectra based on size. Next Generation Fluorophores Although technological advances in fluorophore design have steadily increased, current fluorophores still suffer from some common setbacks such as photobleaching, limited cell permeability and strong background autofluorescence. Conclusions To reiterate, one of the most important things when you are running experiments using multiple colors simultaneously is to pick your color palette carefully. Because of the potential emission spectrum overlap for each fluorophore, you need to choose fluors requiring the least

amount of signal compensation for your machine. You can minimize the time it takes to tweak the machine and background color as well. In addition, if you using tandem-dyes, run extra quality controls before planning an experiment to ensure the dyes have not degraded. Also make sure you go through instrument settings thoroughly to make sure it is equipped with proper lasers and optical filters for your experiment. Kudos to the creative minds!

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6: ZEISS Microscopy Online Campus | Fluorescent Protein References

This chapter provides a synopsis of fluorescent protein development, a list of commonly used fluorescent proteins, some practical considerations and strategies for detection, and examples of.

The immunofluorescence technique is a mixed method where fluorescence reflects the amount of target structure s measured by molecular probes. Phycobiliproteins, the brightest fluorescent probes applicable to the immunofluorescence assays, can become conjugated with biologically active molecules and proteins, such as antibodies. Quantum dots have provided a highly sensitive method for detection of biomolecules, favourably antibodies and key antigens. However, the main limitation of using quantum dots that restricts their application to clinical and in vivo settings is the potential toxicity of particles used in quantum dot synthesis. The family of Brilliant Violet fluorophores is a recently introduced product aimed at providing fluorescent dyes suitable for multilabel fluorescence. The conventional method of immunofluorescence is too costly in terms of the amounts of reagents and time required. In addition, its efficacy is influenced by many factors related to reagents and specimen. Key Concepts Photoluminescence, the process through which atoms emit light following light absorption, includes fluorescence and phosphorescence. Immunofluorescence, making major advances in the area of immunoimaging, is based on the application of immunological mediators to the fluorescence assay. Immunofluorescence is used to detect and identify the investigated biomolecules receptor molecules by the predefined biomolecules receptor probes. The factors that affect the efficiency of fluorophores include peak excitation wavelength, peak emission wavelength, quantum yield, brightness, water solubility, pH insensitivity and photostability, the spillover effect and autofluorescence effects. Fluorophores can be categorised according to their nature into three: There exist two major factors limiting the use of small organic dyes: Great care should be taken when applying quantum dots to clinical and in vivo settings because of the potential toxicity of particles such as cadmium used in quantum dot synthesis. The main obstacles that limit the use of immunofluorescence techniques are as follows: Current Opinion in Biotechnology 16 1: Brinkley M A brief survey of methods for preparing protein conjugates with dyes, haptens and crosslinking reagents. Bioconjugate Chemistry 3 1: Chalfie M Green fluorescent protein as a marker for gene expression. Trends in Genetics 10 5: Part A 81 6: Journal of Molecular Histology 40 4: Experimental Biology and Medicine 47 2: Davidson A and Diamond B Autoimmune diseases. New England Journal of Medicine 5: Journal of Biological Chemistry Chemical Society Reviews 38 Expert Review of Clinical Immunology 11 5: Glazer AN Phycobiliproteinsâ€"a family of valuable, widely used fluorophores. Journal of Applied Phycology 6 2: Chemical Society Reviews 43 1: Encyclopedia of Life Sciences. Heim R and Tsien RY Engineering green fluorescent protein for improved brightness, longer wavelengths and fluorescence resonance energy transfer. Current Biology 6 2: Journal of the American Chemical Society Journal of Immunological Methods 1: Part A 77 9: Malumbres M and Barbacid M Milestones in cell division: Nature Reviews Cancer 1 3: Journal of Investigative Dermatology 1: Journal of Cell Biology 93 3: Nature Protocols 2 9: Nature Methods 5 9: Lab on a Chip 12 2: Shimomura O, Johnson FH and Saiga Y Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. Journal of Cellular and Comparative Physiology 59 3: Angewandte Chemie International Edition 51 Tsien RY The green fluorescent protein. Annual Review of Biochemistry 67 1: Journal of the American Chemical Society 5: Nanoscale Research Letters 7 1: Cell and Tissue Research 1: Journal of Pharmaceutical Sciences 96 1: Nature Biotechnology 21 1: ACS Nano 7 8: Applied and Environmental Microbiology 70 1: Current Opinion in Chemical Biology 14 1: Analytical Biochemistry 1: Journal of Histochemistry and Cytochemistry 47 9: Current Opinion in Biotechnology 18 1: Nature Methods 2 Journal of Chemical Education 88 6: Weissleder R A clearer vision for in vivo imaging. Nature Biotechnology 19 4:

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7: Live Cell Imaging Facility

Addgene's plasmid repository contains a variety of fluorescent protein plasmids. Use this guide to learn more about the many applications of fluorescent proteins (FPs) and to find the plasmids that are available from Addgene's depositing scientists.

PAmCherry is nonfluorescent until activated by a short exposure to â€” nm light, while Dendra2 is a monomeric, green-to-red photoswitchable fluorescent protein. Once it is irreversibly switched to its red form, Dendra2 is highly photostable. Switch on these fluorescent proteins in a subset of cells, proteins, or organelles in order to track their movement. Use the Timer fluorescent protein to follow the on and off phases of gene expression. Initially, the Timer fluorescent protein emits green fluorescence, but as time passes, the fluorophore undergoes additional changes that shift its fluorescence to longer wavelengths. When fully matured, the protein is bright red. Fluorescent protein applications Fusions with red fluorescent proteins Monomeric fluorescent proteins are often ideal for fusions as they tend to be least disruptive to the function of the protein. In many cases, oligomers can also be effective choices. We offer N- and C-terminal vectors for creating fusion constructs. These fusions have also been used for quantitative imaging techniques including fluorescence resonance energy transfer FRET Anderson et al. HeLa cells were transiently transfected via a lipid-based method, with mammalian expression vectors encoding mCherry fused to either human cytoplasmic actin Panel A or tubulin Panel B. DsRed-Monomer is well-tolerated by mammalian cells and has been successfully used to create stably transfected clonal cell lines. The DsRed-Monomer chromophore matures rapidly and is readily detected 12 hours after transfection and the fluorescent protein is extremely stable, allowing you to monitor fluorescence over extended periods of time. An ideal fusion tag Ideally, when you label a protein of interest, the fluorescent tag itself should not interfere with the biological function of the target protein. If the fluorescent protein has a strong tendency to form oligomers, it is more likely to alter or hinder the original function of the tagged protein. Because DsRed-Monomer is a true monomer, it is the optimal choice for use as a red fluorescent fusion tag. When expressed in mammalian cells, the protein is highly soluble and homogeneously distributed within the cytosol, with no detectable aggregation Figure 3. DsRed-Monomer is soluble and displays even, consistent, and homogeneous distribution in HeLa cells. DsRed-Monomer has been expressed as a fusion with a large panel of diverse proteins with diverse functions and subcellular locations. The localization of the resulting tagged protein was monitored and all the tested proteins localized properly. For example, the DsRed-Monomer-Actin fusion protein correctly incorporates into the actin filament system of the cytoskeletal network, ruffling edges, and filipodia. A true monomer The monomeric nature of the DsRed-Monomer protein 28 kDa, calculated molecular weight based on amino acid sequence has been confirmed by two independent methods: FPLC gel filtration chromatography of recombinant DsRed-Monomer yields a single elution peak at a retention time consistent with a kDa molecular weight. The elution profile does not display higher molecular weight species and provides strong evidence that DsRed-Monomer is a true monomer. Pseudonative gel electrophoresis yields a fractionation profile that is consistent with a monomeric protein, and similar to the monomeric green fluorescent protein AcGFP1. Living Colors DsRed-Monomer is a monomeric protein. Overall absorbance A and chromophore excitation A of the eluted material were monitored simultaneously. DsRed-Monomer elutes from the column at a retention time 39 min corresponding to a molecular weight of 28 kDa. The calculated molecular weight of DsRed-Monomer is DsRed-Express is a tetrameric protein that elutes at an earlier retention time 33 min corresponding to a molecular weight of 89 kDa. Pseudonative gel analysis of proteins. Boiled and unboiled recombinant proteins 7. The unboiled nondenatured DsRed-Express runs at a much higher molecular weight than its boiled denatured counterpart due to its tetrameric structure. DsRed-Monomer has an excitation maximum of nm and an emission maximum of nm. Although DsRed-Monomer is somewhat less bright than DsRed-Express, it is nevertheless an excellent choice for fluorescence microscopy imaging and flow cytometry. A new configuration of the Zeiss LSM for simultaneous optical separation of green and red fluorescent protein pairs. A 69, â€”9 Labeling HIV-1 virions with two fluorescent proteins allows identification of virions that have

productively entered the target cell. Virology , â€”93 Targeted ablation of beta cells in the embryonic zebrafish pancreas using E. Polar positional information in Escherichia coli spherical cells. Multistep and multimode cortical anchoring of tea1p at cell tips in fission yeast. A noncytotoxic DsRed variant for whole-cell labeling. Methods 5, â€”7 Fusion with green fluorescent proteins AcGFP1: Its open reading frame has been human codon-optimized to increase the translational efficiency of the AcGFP1 mRNA, which results in higher expression in mammalian cells. The AcGFP1 protein is stable, allowing you to monitor fluorescence over extended periods of time. The chromophore matures rapidly and is readily detected 8â€”12 hours after transfection. As with all of our fluorescent proteins, AcGFP1 is well-tolerated by mammalian cells and has been successfully used to establish stably transfected clonal cell lines. AcGFP1 has an excitation maximum of nm and an emission maximum of nm. AcGFP1 can be easily detected via both Western blot and immunoprecipitation applications with our wide array of Living Colors antibodies. AcGFP1 is particularly suited for use in multicolor applications e. It also performs well in cell-based assays that monitor protein subcellular trafficking Figure 1, Panels A and B. Cells expressing AcGFP1 are easily detected and sorted by flow cytometry. Use of AcGFP1 for fusions and fluorescence microscopy applications. Panels A and B. Cells were induced with 1. Sucrose density gradient ultracentrifugation yields a fractionation profile consistent with a monomeric protein Figure 2, Panel B. Pseudonative gel electrophoresis of recombinant AcGFP1 protein in comparison to an oligomeric fluorescent protein supports the same conclusion Figure 2, Panel C. AcGFP1 is a monomeric protein. Overall protein absorbance A and chromophore excitation A of the eluted material were monitored simultaneously. AcGFP1 elutes from the column at a retention time corresponding to a molecular weight of 24 kDa. The calculated molecular weight of AcGFP1 is Recombinant AcGFP1 protein was analyzed by sucrose density ultracentrifugation using a continuous gradient. The unboiled native DsRed-Express runs at a much higher molecular weight than its denatured boiled counterpart due to its oligomeric structure. Molecular brightness characterization of EGFP in vivo by fluorescence fluctuation spectroscopy. A colourless green fluorescent protein homologue from the non-fluorescent hydromedusa Aequorea coerulescens and its fluorescent mutants. Oligomerization of green fluorescent protein in the secretory pathway of endocrine cells. The following four pairs have been reported in the literature to be suitable for FRET:

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8: Green fluorescent protein - Wikipedia

Fluorescent Proteins (FPs) were first discovered over 50 years ago, with the discovery of the Green Fluorescent Protein (GFP), a protein from the jellyfish Aequorea Victoria. Since that discovery, the family of FPs just keeps getting larger with hundreds of variants available.

Tsien was born in New York, in Hsue-Chu Tsien , his father, was a mechanical engineer had excelled academically and graduated at the top of his university class. Tsien suffered from asthma as a child, and as a result, he was often indoors. He spent hours conducting chemistry experiments in his basement laboratory. When he was 16, he won first prize in the nationwide Westinghouse Talent Search with a project investigating how metals bind to thiocyanate. He also developed fluorescent indicators of calcium ions and other ions important in biological processes. GFP Movie showing entire structure and zoom in to fluorescent chromophore. Movie created by Erik A. Tsien for the Birch Aquarium. In , Tsien was awarded the Wolf Prize in Medicine "for his seminal contribution to the design and biological application of novel fluorescent and photolabile molecules to analyze and perturb cell signal transduction. Typically, the gene coding for a protein of interest is fused with the gene for a fluorescent protein, which causes the protein of interest to glow inside the cell when the cell is irradiated with ultraviolet light and allows microscopists to track its location in real time. This is such a popular technique that it has added a new dimension to the fields of molecular biology, cell biology, and biochemistry. All these then largely amplified the practicality of using GFP by scientists in their research. Tsien mainly contributed to much of our understanding of how GFP works and for developing new techniques and mutants of GFP. Former trainees of Roger Y. Tsien include Atsushi Miyawaki and Alice Y. Timelines of GFP-development involved by Tsien: Tsien showed the mechanism that GFP chromophore is formed in a chemical reaction which requires oxygen but without help from the other proteins. Tsien and collaborators made various GFP mutants by genetic modification and structural tweaking. Newly created variants of GFP can shine more brightly and show different colours, such as yellow, cyan, and blue. Tsien produced monomeric variants of DsRED, which can glow in shades of red, pink, and orange. Remarkably, since then complicated marcromolecular networks of living organisms can be labelled or marked by using "all the colours of the rainbow". Other detailed highlights involved by Tsien: One extra double-bond in the chromophore of DsRed extends its conjugation thus causes the red-shift. New "fruit" FPs were generated by in vitro and in vivo directed evolutions. The new IFPs are developed from bacterial phytochromes instead of from multicellular organism like jellyfish. Under normal conditions, bacterial phytochromes absorb light for signaling instead of fluorescence, but they can be turned fluorescent after deleting some of the signaling parts by genetic means such as site-directed mutagenesis. In order to fluoresce, IFPs require an exogenous chromophore , biliverdin. He has also developed fluorescent indicators for other ions such as magnesium, zinc, copper, iron, lead, cadmium, aluminum, nickel, cobalt, and mercury. The peptides are used as probes, and are harmless to living tissues and organs. Their lifetime in the body is only 4 or 5 days. Clinical trials are awaited. In , Tsien co-founded the Aurora Biosciences Corporation, which went public in In , Aurora was acquired by the Vertex Pharmaceuticals. Similarly, Tsien was also a scientific co-founder of Senomyx in Tsien also promoted science education to promising young scientists through the first-ever San Diego Science Festival Lunch with a Laureate Program. His family belongs to the line of King Qian Hongzong. He died on August 24, Although the actual cause of death is yet to be determined, he seemed to have died while on a bike trail in Eugene, Oregon. Courage, determination, creativity and resourcefulness were hallmarks of his character. He will not be forgotten. Tsien has received numerous honors and awards in his life, including:

9: Fluorescent protein quick guide

The first step in choosing fluorescent proteins for your multi-color imaging experiment is to be aware of what fluorescent proteins are available. With new fluorescent proteins being published every month, deciding on the best protein for a given application is a challenge.

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Toefl ibt vocabulary list DNA stability and repair Malcolm F. White and Dennis W. Grogan Mastering Word 2000 JLA the Flashs book of speed Hymn and Sequence of the Holy Ghost 515 Initial D Volume 29 Participatory research in conservation and rural livelihoods Windows and Words: A Look at Canadian Childrens Literature in English (Reappraisals: Canadian Writers Ser Disability Rights Movement Cl La Lista de Salud False nation and its / French literary laughter Improving urban water and sanitation services : health, access and boundaries Kristof Bostoen, Pete Kolsk The closet gorilla The thrones, chairs, stools, and footstools from the tomb of Tutankhamun The Awakening West A narrative of the mutiny, on board His Majestys ship Bounty William Bligh Management of information and knowledge The audacity of hops Chicken external nesting box plans Risk management for meetings and events julia rutherford silvers Jews and Money; Towards a Metaphysics of Money Commutative Noetherian and Krull rings Cells And Systems (Life Processes/2nd Edition) Victim-oriented policing The papacy in the 19th century The reliques and ragges of popish superstition Diseases caused by immune responses: hypersensitivity and autoimmunity 1. El 2. 3. The 4. El 5. Susan Butcher Pupil Book What Was Happening? Foundations of psychological testing 5th edition torrent Order Doesnt Equal Understanding, 48 6. Discussion and Implications 128 Mimicry and the evolution of signals The street and the flower Indesign cs5 classroom in a book 2. The Yao Village of Khun Haeng: Some Impressions, Tan Chee Beng, 33 Star Wars Clone Wars Adventures 1 Musical growth in the elementary school