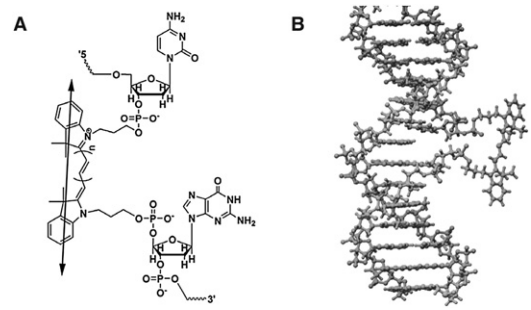
the LBT motif and the probability of water molecules interacting with the Tb^{3+} ion as a function of the applied force is used as a parameter related to the observed quenching of the emission.
Supported by AHA 07257632(WS) and by NIH GM30376(FB) and GM68044(AMC).

2072-Pos Board B42
Random Insertion of Split-can Venus into Kv1.4 Yields Voltage Sensitive Fluorescent Probes
Lei Jin[1], Bradley Baker[1], Lawrence Cohen[1], Carolyn Roman[1], Vincent Pieribone[1], Arnd Pralle[2], Ehud Isacoff[3], Robbie Mealer[4], Thomas Hughes[4].
[1]Yale University, New Haven, CT, USA, [2]The State University of New York, Buffalo, NY, USA, [3]University of California, Berkeley, CA, USA, [4]Montana State University, Bozeman, MT, USA.
In order to increase the optical signal of genetically encoded voltage sensors, we have utilized a transposon reaction to randomly insert the front and back halves of venus into separate subunits of a voltage gated potassium channel, Kv1.4. This split fluorescent protein approach was used with the rationale that only properly folded subunits will produce a functional, fluorescent channel at the cell surface.
We tested 27 combinations for optical signals using voltage clamp fluorometry in HEK293 and NIE115 (mouse neuroblastoma) cell lines. 14 combinations show fluorescence only on the plasma membrane and achieve the goal of the split can design. The best sensitivity is -0.9% in $\Delta F/F$ for a 100 mV depolarization. The on rate during depolarization is on the scale of ms, but the off rate during repolarization is very slow, on the scale of 100 ms.
One combination yielded a surprising optical signal upon depolarization in NIE115 cells. The fluorescence decreased at the edge of the cell, but increased at the cell top and bottom. This phenomenon provides a clue for us to further study the mechanism of the probe's voltage sensitivity. (Funded by NIH grant 1U24NS057631-01A1)

2073-Pos Board B43
Use of Fluorescence Anisotropy to Explore the Subunit Composition of Ca2+/Calmodulin Protein Kinase II Holoenzymes
Srinagesh V. Koushik, Christopher Thaler, **Steven S. Vogel**.
NIAAA/NIH, Rockville, MD, USA.
Ca/calmodulin dependent protein kinase II (CaMKII) is a serine/threonine kinase which is highly enriched in the CNS and thought to be important in the development of long term potentiation. Several variants of CaMKII exist but alpha and beta subunits predominate in brain tissue. Though it is commonly believed that alpha and beta subunits combine to form heteromeric holoenzymes comprised of 12 subunits, this has not been directly demonstrated in vivo. Therefore, we used FRET imaging to directly examined the proximity of alpha and beta isoforms when they assemble to form heterologous holoenzymes. Specifically we used both time-resolved and steady-state fluorescence anisotropy measurements. Energy migration FRET (emFRET; FRET between like fluorophores) is observed in holoenzymes in which the C termini have been tagged with Venus. Fluorescence anisotropy is one of the few techniques which can detect emFRET and provide information of the number of fluorophores undergoing energy transfer. Venus tagged alpha isoforms were coexpressed with Amber (a point mutation in Venus that destroys the fluorophore) tagged beta isoforms and energy migration was seen to decrease indicating that holoenzymes are composed of both alpha and beta subunits in close proximity. Fluorescence anisotropy signatures of Venus tagged holoenzymes were invariant across a broad range of expression levels suggesting that inter-holoenzyme emFRET was not occurring. We used anisotropy ''standards'' consisting of concatamers of Venus and Amber to facilitate the interpretation of anisotropy curves in terms of the number of fluorescent proteins participating in emFRET. Together, these experiments indicate that alpha and beta subunits can coexist in the same holoenzyme. The availability of emFRET ''standards'' to the general scientific community should aid in the interpretation of anisotropy decay data.

2074-Pos Board B44
Backbone Fluorescent DNA Modifications: Reducing Uncertainties In FRET
Suman Ranjit, Marcia Levitus.
Arizona State University, Tempe, AZ, USA.
The kappa square factor is one of several uncertainties that preclude FRET from being a true quantitative technique. Fluorescent backbone modification of DNA structure can constrain the rotational flexibility of the dyes and the orientation can be predicted from the DNA structure. These constraints also reduce the dye-DNA interaction and the uncertainties associated with measuring donor acceptor distances for flexible linkers. In this work we show that the



FRET can be observed at very long distances because of favorable orientation which would have been impossible for freely rotating donor-acceptor pair and also that the assumption that kappa square = 2/3 may lead to large errors in distance measurements.

2075-Pos Board B45
In The Quest Of The Best Fluorescent Protein Couple For Quantitative Fret-flim
Sergi Padilla-Parra, Hervé Lalucque, Marie-Jo Masse, Jean Claude Mével, Nicolas Audugó, Maité Coppey-Moisan, Marc Tramier.
Institut Jacques Monod, Paris, France.
Quantification of protein-protein interactions in living cells by using a fluorescent-protein based FRET approach is a powerful method, especially combined with Fluorescence Lifetime Imaging Microscopy (FLIM), since the fluorescent lifetime does not depend on fluorophore concentration or the excitation light path. In that respect, it is convenient to use a donor protein with a single exponential decay with no detectable photo-conversion. Single lifetime decays allow simplifying the calculation of the fraction of interacting donor-protein (fD). Moreover, in the case of single exponential donor fluorophores the lifetime is not influenced by light induced changes, i.e. photo-bleaching. At first, Green Fluorescent Protein (GFP) was taken as an appropriate donor because of its fluorescence decay can be convincingly fitted to a single exponential model and no photo-conversion was observed. GFP-Red tandems (GFP-mRFP, GFP-mStrawberry, GFP-HaloTag (TMR) and GFP-mCherry) were quantitatively studied by FRET-FLIM obtaining fD values far from the ideal 100%. The maximum value of FRET percentage (fD), was only around 50% for the couple GFP-mCherry. This relatively low percentage could be due to the dark states of the acceptor and/or misfolding of the red proteic domain as well as cleavage in the tandem. Aiming to improve the amount of donor protein engaged in FRET we have tested mTFP1 as a single exponential donor. mTFP1 lifetime remained constant when performing light induced fluorescent changes and no photo-conversion was detected. The percentage of FRET when combined to YFP as an acceptor turned out to be around 70%. Although higher percentages would be desirable mTFP1-YFP stands alone as the best FRET-FLIM standard because of its high transfer percentage (fD) and the single exponential behavior of mTFP1 as a donor.

2076-Pos Board B46
Improved FRET Sensing Of Membrane Voltage With Voltage Sensitive Phosphatase And New Coral Fluorescence Proteins
Hidekazu Tsutsui[1], Satoshi Karasawa[2], Atsushi Miyawaki[2], Yasushi Okamura[1].
[1]Osaka University, Suita, Japan, [2]RIKEN, Wako, Japan.
Ciona voltage sensitive phosphatase (Ci-VSP) is an unique enzyme discovered first in asicdian genome, in which phosphatase activity is regulated by transmembrane potential. Ci-VSP consists of a phosphatase domain and a preceding voltage sensing domain (VSD) which is homologous to the S1-S4 transmembrane domain found in conventional voltage-gated ionic channels. Possible mechanism of Ci-VSP should be that changes in transmembrane potential elicit conformational changes in the VSD, which then induce conformational changes in the phosphatase domain, regulating enzymatic activity. Analogously, by replacing the phosphatase domain with two fluorescent proteins that act as fluorescence resonance energy transfer (FRET) donor and acceptor, it is expected that transmembrane potential can be optically probed as FRET readout. Using two new coral fluorescent proteins, we developed such a membrane potential reporter, named Mermaid, that displays 40% changes in emission ratio per 100 mV change, allowing for visualization of spatiotemporal dynamics in electrical activities of excitable cells. Notably, Mermaid has fast on-off kinetics at warm temperatures and can report voltage spikes comparable to action potentials.