calculator in the context of nanopore sequencing where DNA is drawn through a MspA pore protein.
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4087-Pos Board B815
Real Valued Sequence Alignment using Adapted Smith Waterman Algorithms
Henry Brinkerhoff, Brian Ross, Ian M. Derrington, Andrew H. Laszlo, Jens H. Gundlach.
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Smith-Waterman is a dynamic programming algorithm that locally aligns sequences of discrete values by rewarding matched elements and penalizing mismatched elements. We present an adapted algorithm that aligns sequences of real-valued vectors, applied in our case to the output of nanopore DNA sequencing experiments using MspA. We choose each step of the algorithm to correspond to a driving behavior, such as a polymerase's synthesis and proofreading, and each penalty to be the logarithm of the probability that the step occurs. This allows us to interpret the total score of an alignment as its probability of being the true alignment. Given a known sequence but unknown kinetics, optimizing the score with respect to the penalties will set the penalties to the probabilistic values. This is a useful source of information about molecular motor kinetics. The algorithm's modularity and direct relation to physical behavior make it potentially useful for any physical probes of discrete time series. This work was supported by NIH/NHGRI grant R01HG005115 and R01HG006321.

4088-Pos Board B816
Since the DNA Molecule Returns from the Transcriptional Process Exactly as it was before the Process, the DNA → Protein Production Can Be Seen as a Series of Eigenvalue Problems
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Sequence of operators acts on the linear DNA-eigenfunction and gradually transforms it into the 3D protein-eigenvalue. The eigenvalue equation for the DNA → Protein production is Protein (DNA) = RxMxSxTxUtw (DNA).
The operation of splicing of an exon (S) is studied in the presented work: mRNA = S (preRNA). Here preRNA = TxUtw (DNA).
For illustration, the human beta globin gene cluster located on chromosome 11 is applied. The β- and δ-globin genes are expressed during adult life. The ε- and γ-globins are expressed in early embryonic and fetal erythroid tissues. The relative positions of the nucleic acid content centers for each globin gene before and after the splicing determine the initial and final 4x4 matrices. The eigenvalue equation of the preRNA → mRNA transformation is:
g u a c	·····	g c a u
c g a u	= S x	g c u a
c a g u	·····	c g a u
g a c u	·····	c g u a
By solving this equation one finds the matrix operator of exon splicing, S =
u g c a
a a u u
g u a c
c c g g
Is the process of exon splicing a simple removal of the mass? Is it rather a selective removal of specific nucleic acids? The latter changes the flexibility and the strength of the polypeptide chain, and is driven by electrostatic interactions. The former is an important component in defining the final structures and the spatial stability of the protein.

Biosensors II

4089-Pos Board B817
From One, Many: Modified Fluorogens Interact with a Fluorogen Activating Protein for Multicolor Cell Labeling
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Genetically targeted fluorescent labeling of proteins in living cells has enabled a wide range of biophysical and cell-biological studies. Fluorogen activating proteins (FAPs) increase the fluorescence of otherwise dark dyes (fluorogens) thousands-fold upon binding and represent a new class of fluorescent sensors with context-specific activation. These sensors have been applied for selective detection of the cell-surface pool of proteins,detection of surface protein pH, kinetic analysis of beta 2-adrenergic receptor (β2AR) internalization and other assays[1,2,3]. The malachite green (MG)-dL5 pair posesses high affinity, resistance to photobleaching and high brightness. We modified the core structure of the MG chromophore while maintaining the binding ability with dl5 to achieve a series of MG analogs with distinct spectral properties, in both cell-permeant and cell-excluded form (Figure 1).
These dyes enable simultaneous labeling of the surface and intracellular pools with distinct colors in living cells, and provide a real-time readout of biosynthetic trafficking to the cell surface.
Reference:
1: Szent-Gyorgyi C et al. Nat Biotechnol 2008.
2: Grover A et al. Angew. Chem. Int. Ed. 2012.
3: Fisher G W et al. J Biomol Screening 2010.

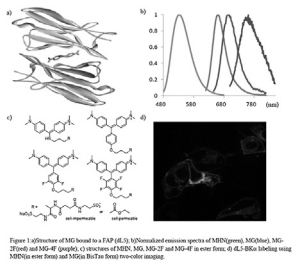

Figure 1:a)Structure of MG bound to a FAP (dL5); b)Normalized emission spectra of MBN(green), MG(blue), MG-2F(red) and MG-4F (purple); c) structures of MBN, MG, MG-2F and MG-4F in ester form; d) dL5-BSA labeling using MBN(in ester form) and MG(in BisTau form) two-color imaging.

4090-Pos Board B818
Mute-Kars: Silent Kinase Activity Reporters Useful for Co-Imaging
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Contemporary kinase activity reporters are sensitive molecular tools that unfortunately occupy a large portion of the visible spectrum. This precludes their simultaneous use with many subcellular translocation markers or single-color biosensors. We present a new, generalizable design of kinase reporters that permits co-imaging with a single-color biosensor of overlapping emission spectrum. We demonstrate the ability to monitor PKA and calcium signaling simultaneously using only two color channels. This work promotes multi-parameter imaging, which has become useful in many cell-signaling investigations.

4091-Pos Board B819
Designing a Thermostable Switch-Based Biosensor
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There is a critical need for developing new diagnostic chemical reporter tools that are stable, cost effective, and capable of sensitive detection. Fluorescent protein biosensors relay changes in fluorescence based on the physical interaction between a protein and the ligand. The thermostable arginine binding protein (ArgBP) from the Gram-negative, hyperthermophilic bacterium, *Thermotoga maritima*, has been modified to create a reagentless fluorescent biosensor. Typically, single Cys mutants are constructed and covalently modified with environmentally-sensitive fluorescent probes. However, no theoretical framework exists to accurately model placement of the probe to the protein sensor. In this work, site-directed mutations were engineered whereby a significant fluorescence quenching (>80%) is observed based on a photo-induced electron transfer (PET) mechanism, which occurs after *Tm*ArgBP binds with increasing concentrations of arginine. This method gave significantly better fluorescence response to arginine and responded in a predictable distance-dependent manner when compared with construction of single Cys mutants of *Tm*ArgBP. Using the PET method, a dissociation constant of 0.86 microMolar was determined to be consistent with previously published data for this protein. Time-resolved fluorescence lifetime measurements indicated that fluorescence deactivation of the *Tm*ArgBP biosensor is a result of static quenching, which is consistent with the formation of ground state complexes between the fluorescent probe and an internal quencher. In addition to the development of a thermostable fluorescent protein sensor, this investigation provides a generalizable spectroscopic tool for the study of conformational movements within a single polypeptide chain.

4092-Pos Board B820
The Two-Photon Bazooka: A New Way of Optically Screening Randomly Mutagenized Libraries of Fluorescent Proteins
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Two-photon (2P) microscopy is the preferred method for imaging fluorescent proteins and biosensors in living, thick tissues. Over the past decade, many groups have worked to improve single photon (1P) properties of fluorescent proteins, but little has been done to improve their 2P properties. This is important because 2P properties can be quite different from the 1P properties. Our