AD are still an unknown. Especially, the beta-amyloid protein is well known for the main cause of AD, which is detected e by observing manifestation state of cell bound with fluorescent material or using DNA test. However, such diagnosis methods have some disadvantages such as long time testing process and limitation for mass production of diagnosis kits. Therefore, we suggest a new technical concept that is capable of evaluating as small as a few Aβ peptides by using a photo-sensitive FET (p-FET) integrated with a selectively optical-transmissible filter. Selenium filter has optically adequate properties for the quantum dots for labeling Aβ peptides, which reflects the unwanted range (<600 nm) of the excitation light and is only able to transmit the emitted light (>650 nm). We measured the photo-currents generated from the transmitted fluorescent beam through the selenium filter when the quantum dots conjugated with Aβ on the filter were excited at 550 nm and emitted at 655 nm. Moreover, we amplified the output signal using an amplifier circuit and lock-in amplifier (LIA) because the output signal by the fluorescent is very small around a few nA level. This study showed that with even slightly small amount of Aβ peptides the selenium-filtered p-FET is simply applicable to differentiating the optically tenuous fluorescent beam. In addition, the correlation between the photo-currents and the various amounts of the quantum dots represented a linearity which showed a good conceptual agreement with the theoretically induced equation, consequently assuming that the photo-current would potentially evaluate the small quantity of Aβ peptides.

2111-Pos Board B841
Photochromic FRET Sensors to Monitor Heme Protein Dynamics
Halil Bayraktar[1], Selen Manioglu[2].
[1]Chemistry, Koc University, Istanbul, Turkey, [2]Chemical and Biological Enginering, Koc University, Istanbul, Turkey.
The dynamic imaging of redox proteins is crucial for understanding their role in cell signaling. The spatiotemporal monitoring would provide an insight on regulation as well as determining endogenous factors directly affecting oxidation and reduction. However, limited number of probes and methods are available to measure dynamics with high specificity and sensitivity in living species. C-type cytochromes (Cyt c) are an important class of redox proteins whose function plays a key role for electron transfer in mitochondria and triggering apoptosis. Here we developed a new protein biosensor using Cyt c that is linked to a fluorescent reporter protein. The redox state of Cyt c was measured reversibly by photochromic fluorescence resonance energy transfer (pcFRET). The changes in the absorption of the endogenous heme cofactor bound to Cyt c covalently can modulate the fluorescence emission of the reporter. The multiple redox cycle of Cyt c was clearly monitored by pcFRET. We also demonstrated that the reporter could be used to monitor redox activity of Cyt c in living E. coli.

2112-Pos Board B842
An Engineered Palette of Metal Ion Quenchable Fluorescent Proteins
Xiaozhen Yu[1], Marie-Paule Strub[1], Travis J. Barnard[2], Nicholas Noinaj[2], Grzegorz Piszczek[1], Susan K. Buchanan[2], Justin W. Taraska[1].
[1]NHLBI, National Institutes of Health, Bethesda, MD, USA, [2]NIDDK, National Institutes of Health, Bethesda, MD, USA.
Many fluorescent proteins have been created to act as genetically encoded biosensors. With these sensors, changes in fluorescence report on biochemical states within living cells. Transition metal ions such as copper, nickel, and zinc are crucial in many physiological and pathophysiological pathways. Here, we engineered a spectral series of optimized transition metal ion-binding fluorescent proteins that respond to metals with large changes in fluorescence intensity. These proteins are useful as metal biosensors or tunable imaging probes. Each protein is uniquely modulated by four different metals (Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+}). Crystallography revealed the geometry and location of metal binding to the engineered sites. When attached to an extracellular membrane protein VAMP2, dimeric pairs of the sensors could be used as in vivo ratiometric probes for metals. Thus, these engineered probes act as sensitive transition metal ion-responsive genetically encoded probes that span the visible spectrum.

2113-Pos Board B843
Nabi, a Novel FRET-Based Voltage Sensor Protein
Uhna Sung[1], Masoud Allahverdizadeh[1,2], Lei Jin[2], Thomas Hughes[3], Lawrence B. Cohen[1,2], Bradley J. Baker[1].
[1]Center for Functional Connectomics, KIST, Seoul, Republic of Korea, [2]Department of Molecular and Cellular Physiology, Yale University, New Haven, CT, USA, [3]Department of Cell Biology and Neuroscience, Montana State University, Bozeman, MT, USA.
FRET (fluorescence resonance energy transfer) has been used to develop genetically encoded biosensors. FRET-based voltage sensors can be useful to monitor change of membrane potential and neuronal activity because the ratio signal removes noise from a common source such as heart beat artifacts. Positions of fluorescent donor and acceptor in voltage sensor proteins may affect plasma membrane expression, signal intensity, and response time constant of FRET. We developed "Nabi" voltage sensors by using the voltage-sensing domain of CiVSP (*Ciona intestinalis* voltage sensitive phosphatase) as a backbone. We aimed at improving FRET performance of voltage sensors by optimizing interactions between fluorescent donor and acceptor. For this effort, we designed a series of constructs with insertion of two fluorescent proteins (FPs) at different locations in the CiVSP backbone. We engineered a total of 40 different combinatorial constructs to evaluate 6 different locations for one FP and 10 different locations for the other FP of FRET pairs. By examining FRET signals of these voltage sensors in response to changes in membrane potential in HEK293 cells, we identified amino acid residues of CiVSP at which insertion of FP pairs can generate efficient FRET signals. Nabi1 series of probes contained UKG and mKO (Tsutsui H et al., 2008) as FRET donor and acceptor. Nabi2 series contained Clover and mRuby2 (Lam AJ et al., 2012) as FRET pairs. Nabi voltage sensors had improved signal size (up to 15% ΔF/F) and time constant compared to the previously reported FRET based voltage probes. Nabi2 was able to respond to single action potentials in primary cultured neurons. Supported by US NIH Grants DC005259 and grant WCI 2009-003 from the National Research Foundation of Korea.

Biomaterials

2114-Pos Board B844
One-Dimensional and Two-Dimensional Alignment of Gold-Nanoparticles Coated with Amyloidogenic Protein of α-Synuclein and their Applications
Seung R. Paik, Jae Hyung Park, Unkyu Paik, Daekyun Lee.
School of Chemical and Biological Engineering, College of Engineering, Seoul National University, Seoul, Republic of Korea.
Alignment of noble-metal nanoparticles has been challenged to develop optoelectric response system. By employing an amyloidogenic protein of α-synuclein, a major constituent of Lewy bodies in Parkinson's disease, gold-nanoparticles (AuNPs) were aligned into either anisotropic one-dimensional (1-D) chain or tightly packed monodispersed two-dimensional (2-D) array on a glass support. Assembly units prepared with AuNPs encapsulated by two different mutant forms of cysteine-containing α-synuclein (A53C and Y136C) were manipulated with hexane or pH to induce subtle structural rearrangement within the protein layer in the presence and absence of the glass support, which led to the instantaneous alignment of AuNPs in 1-D and 2-D, respectively. The resulting peapod-type AuNP chains embedded within the amyloid protein nanofibrils were capable of exhibiting photoconductance with visible-light essential for the development of subwavelength-size light guiding system. With the 2-D array, surface-enhanced Raman scattering (SERS) was achieved for a chemical ligand of α-synuclein, phthalocyanine tetrasulfonate (PcTS), which was shown to be capable of detecting copper and zinc ions to sub-ppm level via the *in situ* chemical reaction of metal chelation by PcTS. Not only these approaches, therefore, offer a facile and general way to align noble-metal nanoparticles into either 1-D or 2-D structures, but also the properties like specific chemical ligand binding, electrical conduction, rich chemistry for modifications, and biocompatibility provided by the protein sheath make the resulting nanostructures the multifunctional photoelectric fusion nanomaterials suitable for applications in the area of future nanobiotechnology.

2115-Pos Board B845
Modular Stitching to Image Single-Molecule DNA Transport
Juan Guan, Bo Wang, Sung Chul Bae, Steve Granick.
Materials Science, UIUC, Urbana, IL, USA.
For study of time-dependent conformation, all previous single-molecule imaging studies of biopolymer transport involve fluorescence labeling uniformly along the chain, which suffers from limited resolution due to the diffraction limit. Here we demonstrate the concept of submolecular single-molecule imaging with DNA chains assembled from DNA fragments such that a chain is labeled at designated spots with covalently-attached fluorescent dyes and the chain backbone with dyes of different color. High density of dyes ensures good signal-to-noise ratio to localize the designated spots in real time with nanometer precision and prevents significant photobleaching for long-time tracking purposes. To demonstrate usefulness of this approach, we image electrophoretic transport of λ-DNA through agarose gels. The unexpected pattern is observed that one end of each molecule tends to stretch out in the electric field while the other end remains quiescent for some time before it snaps forward and the stretch-recoil cycle repeats. These features are neither predicted by prevailing theories of electrophoresis mechanism nor detectable by conventional whole-chain labeling methods, which demonstrate pragmatically the usefulness of modular stitching to reveal internal chain dynamics of single molecules.