from carbon fixation and hydrocarbon synthesis to antibiotic resistance and sensitivity remain undiscovered. My team has developed Knockout Sudoku: a new technique that democratizes the creation of the most important genetic characterization tool for any organism: a collection of single-gene knockout mutants for each of the thousands of non-essential genes in an organism's genome (a whole genome knockout collection). Knockout Sudoku uses probabilistic inference algorithms to replace complex robotics to increase the speed of constructing whole genome knockout collections by 100× and reduces the cost by 30× compared to the next comparable method. It brings this modern genomic tool to many more organisms at price that almost any investigator can afford. This technique now allows my team to learn how to apply biology to challenges across sustainable energy from enhancing the efficiency of photosynthesis for biofuels to the safe use of nuclear power and enabling the widespread use of batteries.

Posters: Neuroscience: Experimental Approaches and Tools

3310-Pos Board B518
The Position and Dynamics of Glutamate Receptors Measured by Brightness- and Size-Equalized Small Quantum Dots

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Quantum dots have been widely used for biomedical imaging due to their brightness and extraordinary photo-stability. Recently, they have also been used to study neuronal receptors. However, commercially available quantum dots (cQD) tend to be large, 25 ~ 35 nm in diameter, which is problematic because steric hindrance prevents their entry into the ~ 30 nm synaptic cleft. In this study, we develop new and small quantum dots that are brightness- and size-equalized (12 nm in hydrodynamic diameter), called BSE-QD. We developed two different-colors BSE-QDs (605 nm and 725 nm) to label and observe two different neuronal receptors, AMPARs and NMDAR, in the synapses in live neurons. They were each able to enter the synaptic cleft, allowing us to observe and measure diffusion. We find that the receptors were in constrained ''nanodomains''. Through cross-correlation of the two receptors, we found that the nanodomains of AMPAR and NMDAR are colocalized in the range of 100 nm ~ 400 nm, implying that excitatory synapses need their cooperative interaction to be effective.

3311-Pos Board B519
Diffusive Dynamics of NMDA Receptors in Live Neurons using Super-Resolution Imaging and Tracking

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N-methyl-D-aspartate receptors (NMDA receptors or NMDARs) are ionotropic glutamate receptors (iGluRs) located on the membrane of neuronal cells. Their location and dynamics are strongly associated with synaptic plasticity. However, we know little about the distribution between extra- and intra-synaptic locations and dynamics for two important NMDAR subunits, GluN2A and GluN2B. Results have been presented using large quantum dots (~20 nm in diameter), making it inconclusive about their synaptic localization. Here we use three dimensional super-resolution fluorescent microscopy to quantify the diffusion properties of these two subunits by tracking them with conjugated organic dyes (~4 nm in diameter) and small quantum dots (sQDs, ~10 nm in diameter). More than 70% of these two subunits were found to diffuse in constrained sub-synaptic regions with similar diffusion constant regardless of development stage of neurons. This suggests that majority of both GluN2A- and GluN2B-containing NMDARs are synaptic in early and late stage of neuronal development, their diffusion constant is a function of location rather than subunit type.

3312-Pos Board B520
Two-Photon Absorption Analysis of Red Fluorescent Genetically-Encoded Calcium Ion Indicators

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Genetically-encoded calcium ion indicators (GECIs) illuminate brain activity in model organisms. The basic design consists of a Ca2+ binding domain attached to a fluorescent protein in such a way that the binding of Ca2+ modulates its fluorescence. The current favorite GECI is the green GCaMP6, due to the large increase of fluorescence upon binding Ca2+ (up to a 50-fold change). However, red fluorescence scatters less than green fluorescence, which is especially desirable for deeper imaging in tissue. Although there are red fluorescent GECIs available, they are less popular for reasons that include a smaller change in fluorescence. Three factors can contribute to the change in fluorescence: 1) different quantum yields of the Ca2+-bound and Ca2+-free forms; 2) different extinction coefficients or cross sections, in one- or two-photon imaging, respectively; and 3) a redistribution of the neutral and anionic forms of the chromophore in the presence of Ca2+. The third factor was previously shown to be the predominant mechanism for modulating the fluorescence of GCaMP6. We present a thorough analysis of the one-photon and two-photon properties of several red GECIs and use this data to determine the main factor(s) contributing to the increase in fluorescence upon binding Ca2+, particularly under two-photon excitation. In most cases we observe both the change of the maximum two-photon cross section and the shift of equilibrium between the neutral and anionic forms of the chromophore upon binding Ca2+. Quantitative analysis of the shapes of the two-photon absorption spectra provides us with clues regarding changes in the electrostatic environment of the chromophore and can potentially direct mutagenesis efforts towards creating red GECIs with a larger Ca2+-dependent fluorescence increase.

3313-Pos Board B521
Potassium Channel-Based Two Component Optogenetic Tool for Silencing of Excitable Cells

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Opsin-based optogenetic tools still lack a light-gated potassium channel for cell inhibition. Silencing of excitable cells had been achieved so far by the use of light driven pumps for protons and chloride as well as light-gated chloride channels. Unfortunately, changes on pH or Cl^- concentration could lead to undesirable effects on synaptic transmission limiting the use of these tools for neuronal silencing. We have developed a potassium channel-based inhibitory tool combining the small cAMP-gated potassium channel SthK (from *Spirochaeta thermophile*) and a photoactivated adenylyl cyclase. From all the PACs tested we found that bPAC (from the bacterium *Beggiatoa)*, and TpPAC (from bacterium *Turneriella parva*) produced the best results. Characterization of both optogenetic-tools in ND7/23 cells showed that application of short blue light pulses to cells transfected with SthK and a PAC, produced high-amplitude potassium outward currents that are dependent of the light intensity. The TpPAC-based construct needed 10 times more light to reach the maximum current compared to bPAC. However, this is an advantage in biological systems where a lower production of cAMP is needed or where the ubiquitous phosphodiesterase activity is very low. Two construct configurations were made. In the first configuration the SthK channel and the PAC are expressed as a fused protein (PAC-SthK) allowing localized expression of cAMP at the plasma membrane. In the second configuration both proteins are separated by a P2A site (SthK-P2A-PAC) and the PAC is expressed in the cytoplasm. The fused constructs produced an increase on the current's latency and a decrease on the current density. Nevertheless, both configurations were capable of inhibiting neuronal action potential firing in cultures of hippocampal neurons. Cyclic nucleotide-gated potassium channel - photoactivated cyclese based constructs are a very promising tool for acute optogenetic inhibition.