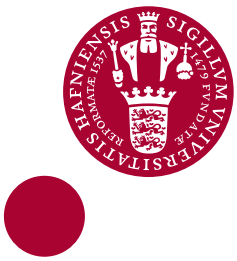

A Semi-Synthetic Approach to Engineer Ligand- and Voltage-Gated Ion Channels in Live Cells

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particles as well as the friction coefficient of BD particles. Integration of the methods shows little impact on the time-to-solution compared to a pure RDME simulation. A proof of principle is presented using the particle densities and geometry of a 500-nm diameter minimal cell. This minimal cell, named JCVI-syn3A, with a 543-kbp genome and 493 genes, provides a versatile platform to study the basics of life. This computational methodology will become a major component of the goal to simulate JCVI-syn3A at a spatially resolved, stochastic level.

1346-Pos

Optogenetic Delineation of Receptor Tyrosine Kinase Subcircuits in PC12 Cell Differentiation

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Nerve growth factor elicits signaling outcomes by interacting with both its high-affinity receptor, TrkA, and its low-affinity receptor, p75NTR. Although these two receptors can regulate distinct cellular outcomes, they both activate the extracellular-signal-regulated kinase pathway upon stimulation with ligand. To delineate TrkA subcircuits in PC12 cell differentiation, we developed an optogenetic system where light was used to specifically activate TrkA signaling in the absence of nerve growth factor. By using tyrosine mutants of the optogenetic TrkA in combination with pathway-specific pharmacological inhibition, we find that Y490 and Y785 each contribute to PC12 cell differentiation through the extracellular-signal-regulated kinase pathway in an additive manner. Optogenetic activation of TrkA eliminates the confounding effect of p75NTR and other potential off-target effects of the ligand. This approach can be generalized for the mechanistic study of other receptor-mediated signaling pathways.

1347-Pos

The Proposed Mechanism Behind Lyse-It$^{(R)}$: A Rapid Sample Preparation Technique

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Lyse-It® is a microwave-based platform that focuses microwaves for cellular lysis, DNA/ RNA/ protein extraction and fragmentation, and nuclease degradation and inactivation. DNA/RNA and protein fragmentation cannot be simply explained by rapid focused heating alone; therefore, we have investigated reactive oxygen species, ROS, as contributing factors.

The mechanism starts with the rapid heating and lysis of the sample which releases intact intracellular components such as genomic DNA/RNA and proteins. Subsequently, at higher and longer both microwave powers and times, DNA/RNA and proteins get fragmented into tunable fragment sizes which get progressively further fragmented over time. We postulate that this fragmentation is due to the generation of ROS, which in the literature, has been shown to cause oxidative damage, such as DNA fragmentation and protein degradation. In this paper, we present data demonstrating the release and fragmentation of DNA and proteins and the generation of ROS using both fluorescent probes and metal complexes. Additionally, we demonstrate that the released and fragmented DNA is viable for Polymerase Chain Reaction (PCR).

1348-Pos

Spy and Snoop Superglues Enhance Anchoring and Team-Building in Biophysics and Synbio

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Biophysicists often need robust linkage or anchoring of component parts. Even the best non-covalent interactions may break in milliseconds with force, so irreversible interaction systems bring important advantages. We previously engineered a genetically-encoded unbreakable interaction based on the pathogenic bacterium *Streptococcus pyogenes*: a peptide tag (SpyTag) upon mixing forms an irreversible amide bond to its protein partner (SpyCatcher). This protein padlock has been applied for single-molecule AFM in mechanobiology, creating living biomaterials with amyloids, and making ultra-stable enzymes for nutrition and bioremediation. We now have other peptide/protein tags forming unbreakable linkages, including SnoopCatcher and SnoopTag/DogTag/SnoopLigase. We also have new generations of SpyTag/SpyCatcher from Rosetta-based design, along with evolution on phage or intimin-display on *E. coli*. We will describe their use for programmable synthesis of polyprotein teams for modulation of signal transduction on cancer cells and how nanoassembly stimulates the immune system against malaria. The simple modular nature of these synthetic linkages should be a powerful tool for biophysical analysis and extend the possible architectures in synthetic biology.

1349-Pos

A Semi-Synthetic Approach to Engineer Ligand- and Voltage-Gated Ion Channels in Live Cells

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Site-directed insertion of non-canonical amino acids (ncAAs) or post-translational modifications (PTMs) is often challenging, particularly in large membrane proteins, such as ion channels. Amber-codon suppression mutagenesis is routinely used for this purpose. However, this approach has limitations regarding the side chain identity that can be introduced, as well as the efficiency of incorporation. For this reason, it would be valuable to develop a method that overcomes these issues. Here, we present the development of a intein-based approach for the incorporation of synthetic peptides into ion channels expressed in live cells. The approach utilizes split inteins, which can seamlessly join selected protein segments, to replace selected peptide segments within ion channels with synthetic peptides carrying the desired ncAAs and/or PTMs. We demonstrate the successful implementation of this approach by inserting non-canonical lysine analogs into the extracellular binding pocket of P2X2 receptors and by inserting PTMs into intracellular linkers of the cardiac voltage-gated sodium channel, Nav1.5. Correct reconstitution of full-length ion channels and the impact of the mutations on channel function were verified by Western blots and electrophysiology. The technology has the potential to complement existing ribosome-dependent methods to incorporate ncAAs and PTMs, especially for those that cannot currently be incorporated using existing methods. Additionally, the approach offers a unique way to introduce combinations of multiple ncAAs and/or PTMs, thus enhancing the precision with which we can study ion channel function and pharmacology.

1350-Pos

Removal of Colour from Textile Industrial Effluent using Modified (Epoxidized) and Unmodified Rubber (Hevea Brasiliensis) Latex

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Discharge of colored effluent from textile industries interfere with the photosynthetic activities of aquatic life. A large number of dyes and their metabolites have been reported to be toxic and carcinogenic to humans and other living things. The removal of this color dye from waste effluents becomes environmentally important. The purpose of this research is to investigate how to remove color from textile industrial effluent using modified and unmodified natural rubber latex. Epoxidized rubber latex (ERL) and unmodified rubber latex (URL) were the absorbents used on textile industry effluent. Adsorption experiments were carried out on the adsorbent and adsobate, while varying its contact time between 30 and 180 minutes in 30-minute increments. The acidity of the solution varied from pH of 2 to 12. The effect of the pH showed that adsorption of dyes was pH dependent. Better adsorption was obtained in an acidic medium as compared to an alkaline medium. It was also noticed that adsorption decreased as contact time increased. The kinetics study revealed pseudo 1st order and 2nd order and temkin showed that adsorption process took place. The results showed that adsorbents prepared from rubber latex could be good adsorbents for the removal of dye from textile industrial effluent.

1351-Pos

Controlled Stirring of Biological and Bio-Mimetic Microdoplets

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Producing and manipulating droplets of biological and bio-mimetic fluids in microfluidic platforms is a versatile tool for Synthetic Biology. Here we report on an approach to achieve a controlled stirring of aqueous microdroplets by encapsulating magnetic nano stir bars. The nanometric diameters were produced from 30 nm iron oxide beads aligned in a magnetic field and strengthened by a silica coating, the final cylindrical structures reaching lengths in the range 1-20 μm. They were then included in microdroplets with sizes in the range 10-100 μm and varying composition, produced at a PDMS microfluidic junction within a hydrophobic continuous phase. Proper stirring upon applying an external rotating magnetic field was monitored via quantitative microscopy. In addition, the impact of stirring both on the diffusional transport at small Peclet numbers and on the formation of a mitotic spindle in Xenopus egg extract was studied.