

Vision-guided precision targeting for spatially controlled biolistic corneal delivery

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Millions of people worldwide experience visual impairment caused by diabetes mellitus and delayed corneal wound healing, highlighting the need for accessible, low-risk, and cost-efficient treatment strategies. The Kornfield lab has developed a biolistic delivery platform for targeted administration of therapeutic particles to the cornea. In this project, we integrated the delivery device with a robotic positioning stage, image processing, feature detection, and tracking to enable spatially controlled particle delivery with approximately 2 mm x 2 mm localization. Specifically, we tested our vision-guided targeting system with 2D images, 2D images embedded within a gel, and *ex vivo* porcine eyes. Particle delivery positions were validated with optical coherence tomography and overhead camera images and processed with Fiji and Python. With this system, the user is able to target specific ocular regions, allowing for potential applications targeting the limbus with regenerative therapeutics to promote corneal epithelial repair. Future work will focus on optimizing the system for clinical translation, including integrating targeting with corneal topography and real-time motion adaptations for *in vivo* deliveries.

Optimization of next generation lithium ion batteries with silicon graphite carbon nanotube composite as active material

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With the advent of ever intensifying climate change and global supply issues, a viable replacement for fossil fuels is needed for industries such as transportation. Lithium ion batteries stand as a clear contender to fill this role in technologies like electric vehicles, but currently are lacking in energy density and face long-term stability and safety issues. Silicon has long been identified as a contender for the active material of next generation battery anodes for its theoretical specific capacity of ~4,200 mAh/g, nearly ten times that of current generation graphite, but faces the debilitating issue of rapid expansion and rupturing, consuming capacity through the formation of excessive surface electrolyte interfaces. Carbon nanotubes have been introduced as both a conductive scaffold to physically restrain silicon particles from expanding and transport electrons between active materials. Ultimately, the combination of ultra fine >1 micrometer silicon and graphite particles bound in a carbon nanotube structure produces a battery with much of the capacity benefits of silicon while maintaining the longer cycle life, increased stability, and decreased costs of traditional graphite lithium ion batteries.

Optimizing *Azotobacter vinelandii* as a hydroponic biofertilizer

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With approximately 70 million people being born each year, the pressure on the agricultural industry to meet food demands is escalating. In response, farmers have resorted to excessive chemical fertilizer use. This has resulted in the depletion of non-renewable resources, damage of marine systems via eutrophication, and release of potent greenhouse gas emissions. One sustainable strategy to reduce agrochemical inputs to farms while maintaining yields is to harness the power of beneficial plant-microbe interactions. *Azotobacter vinelandii* is a plant growth-promoting rhizobacteria (PGPR) of significant interest due to its innate nitrogenase enzyme that fixes unusable atmospheric N₂ into accessible NH₃ for plants to uptake. In this project, we study and finetune *A. vinelandii*'s ability to fix nitrogen in hydroponic systems with *Brachypodium distachyon* and *Solanum lycopersicum*. We established reliable nitrogen-deprivation phenotypes in both plant species, identified the best-performing *A. vinelandii* strain and growth medium for maximal NH₄⁺ production, and optimized the physical hydroponic system (tube volume, powered aeration, volume) for maximal nitrogen fixation and plant benefit.

Decoding the lanthanome: Medium-throughput characterization of protein affinity and selectivity towards lanthanides

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The periplasmic protein CnrX in *Cupriavidus metallidurans* cells was engineered to have higher selectivity and binding affinity towards lanthanides for improved rare earth elements extraction. Specific residues in the binding pocket were mutated with MAX randomization to construct a saturation site mutagenesis library, and each variant was immobilized on a column with the SpyCatcher protein in 96-well plates. Through employing experimental techniques such as isothermal calorimetry and inductively coupled plasma mass spectrometry, the K_d constants and lanthanide selectivities of each variant were calculated. Using circular dichroism spectroscopy, nano differential scanning fluorimetry, and dynamic light scattering, the protein structure and dynamics were further studied. With this library, we aim to understand the energetics and thermodynamics of lanthanide binding interactions with proteins to develop a more sustainable approach to lanthanides mining.

Nanobody-mediated stabilization of human effectors enables antigen-dependent cell selection

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Isolating a target population of cells from a mixture based on an intracellular molecular marker, also known as selection, is a central challenge in cell engineering. DASIT (Destabilized-nanobody Antigen Selection and Identification Tool) achieves this by converting an intracellular antigen into a selectable marker. A nanobody carrying destabilizing framework mutations is degraded by the proteasome unless it binds to its target. Fusing it to an effector confers the same stability properties to the fusion protein, resulting in the effector accumulating only in antigen-positive cells. This enriches a population based on intrinsic cell state rather than delivery. Because the mechanism operates at the protein level, it needs no permanent genetic integration and supports transient recyclable delivery. DASIT's central limitation for translational applications is that it has only been shown to function with bacterial resistance markers, and foreign proteins provoke the rejection of the cells that are being selected. Here, we humanize DASIT by replacing the bacterial effector with a panel of human effector proteins that each turn antigen presence into a survival advantage through a distinct pathway, from suppressing stress-induced death with a dominant-negative p53 fragment to upregulating proliferative signaling through cytokine-receptor and STAT modules. Because these effectors function through different pathways, they are used to test whether antigen-based selection generalizes across effectors. The outlined validation spans immortalized lines to iPSCs, with primary T cells as the therapeutic endpoint. Together, this establishes antigen-dependent selection from clinically compatible parts, positioning the effector as a tunable input for selecting and enriching human cells on intracellular state.

Expanding the antigen repertoire and characterizing localization dependence of programmable nanobody circuits for selection

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Cell purification methods are a major bottleneck in advancing cell-based therapeutics. Conventional separation methods rely on flow-based or physical separation processes which reduce cell viability and scale poorly with cell numbers. DASIT (Destabilized-nanobody Antigen Selection and Identification Tool) operates at the protein level, fusing a destabilized nanobody to an antibiotic resistance protein to selectively provide antibiotic resistance to antigen-positive cells. While DASIT functions robustly with EGFP and ALFA nanobodies, not all nanobodies tolerate destabilization or domain fusion. Here, we evaluated FLAG and BC2 nanobodies, showing that wildtype nanobody constructs survived puromycin selection without enriching for antigen-positive cells. Additionally, both wildtype and destabilized BC2 constructs lost mRuby2 signal after lentiviral delivery despite apparent integration, implicating a transcript-level defect. We also evaluated DASIT's selection ability for nuclear-localized antigens in HEK293T cells. Enrichment was moderate in cases when both the antigen and the DASIT construct

were permanently co-localized, weakest when only the antigen was nuclear-localized, and strongest (85% EGFP+) when only DASIT was nuclear-localized. This result suggests that a post-translational binding period in the cytoplasm is critical to DASIT's success. In hiPSCs, we discovered that modRNA-delivered DASITEGFP-PURO enriched LAMP1-EGFP hiPSCs cells from ratios as low as 1:14 (LAMP to wt iPSCs) but failed to enrich SOX2-EGFP hiPSCs. Overall, these results show DASIT can be extended to EGFP antigens with different cellular localizations (lysosome, nucleus), but further work is needed to expand DASIT to other antigens and to generate rules for reproducible nanobody destabilization.

Engineering truly tiny, genetically encodable, acoustic actuators in mammalian cells

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Abstract withheld from publication at mentor's request.

Engineering carotenoid production in *Yarrowia lipolytica*

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Abstract withheld from publication at mentor's request.

Limitations of EC-labeled contrastive learning for reaction-enzyme retrieval

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Contrastive learning has emerged as a leading computational method for linking enzyme sequences to chemical reactions. However, it has not been assessed whether such a linkage is genuine or how well contrastive models can learn to represent the data. In this work, we compare multimodal models trained on EC-labeled reaction and sequence data to unimodal models trained only on EC-labeled reaction data across a diverse set of reaction encoders. We find that unimodal models convincingly outperform multimodal models, that bespoke reaction encoders have marginal to no benefit over general encoder architectures, and that contrastive models do not greatly improve upon untrained baselines, facing reproducible performance caps on out-of-distribution queries. This leads us to believe that EC-labeled contrastive models do not learn a meaningful linkage between sequence and reaction space. Finally, we hypothesize that EC-labeled contrastive models rely heavily on pattern matching and are fundamentally limited by the curation of the EC system and the model's inability to learn the underlying chemistry.

An automated approach to uniform latex film formation on former

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Natural rubber is a critical material used across industries, yet global production is heavily dependent on *Hevea brasiliensis*, creating vulnerabilities due to geographic concentration and disease susceptibility. Guayule (*Parthenium argentatum*), a drought-tolerant shrub native to the American Southwest, offers a promising domestic alternative, but requires optimized processing methods for consistent film fabrication. An automated dip-coating system was developed to control immersion, withdrawal, and rotational motion during latex film formation. A three-stage drying process was developed to improve film uniformity on glass slide formers. The parameters of withdrawal speed and angular speed were investigated and optimized based on hevea and guayule latex material properties. Optical Coherence Tomography (OCT) was used to characterize film thickness and uniformity. Future work will optimize withdrawal rates and other processing parameters to improve film uniformity and repeatability. Additional tensile testing will be conducted using smaller specimens to quantify mechanical properties.

Mechanical and rheological characterization of guayule latex systems

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Guayule (*Parthenium argentatum*) is a drought-tolerant shrub that produces hypoallergenic natural rubber (NR) and may act as a domestic hedge against global NR supply disruptions. During traditional guayule latex extraction, resin remains in the resulting latex and may affect its properties and the performance of films produced from it. The Kornfield lab developed a novel approach to extract resin-then-latex sequentially, and this project aims to investigate how the removal of resin affects the flow behavior and mechanical properties of guayule latex. Conventionally extracted guayule latex, which still contains resin, was used to develop procedures for characterizing the material prior to testing latex that went through the resin-then-latex extraction process. The flow behavior of the latex was measured over a range of strain rates and concentrations using a rheometer, while the mechanical properties of latex films were evaluated through tensile testing using an Instron machine. These experiments provide a framework to evaluate the quality of latex produced through sequential extraction processes, towards the larger goal of enhancing the overall value of guayule as a commercially viable crop.

Development of a biomimetic intervertebral disc implant using digital light processing 3D printing and wet-spinning

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Intervertebral discs (IVDs) are fibrocartilaginous joints located between adjacent vertebral bodies that provide mechanical support and spinal mobility. Each IVD consists of the nucleus pulposus, annulus fibrosus, and cartilaginous endplates. IVD degeneration is recognized as a leading cause of lower back pain. Current clinical treatments are largely limited to pain management or invasive surgical interventions, such as spinal fusion, which permanently alter spinal mechanics rather than restoring natural joint function. Furthermore, existing synthetic implants fail to replicate the complex architecture and biomechanical properties of a healthy disc. To address these limitations, we established a biomimetic approach to fabricate whole-disc implants. Utilizing methacrylated fibrinogen (FibMA) blended with polyethylene glycol diacrylate (PEGDA) and collagen fibers, we developed a fabrication strategy that combines digital light processing (DLP) and collagen fiber wet-spinning. This multimaterial fabrication approach enables control over scaffold architecture and mechanical properties to reproduce the distinct structural and functional characteristics of native IVD components within an assembled construct. Preliminary data indicate that these materials support cellular differentiation toward IVD-specific phenotypes. Together, this approach produces biomimetic whole-disc implants whose component-specific mechanics guide cell function and promote a native tissue phenotype.