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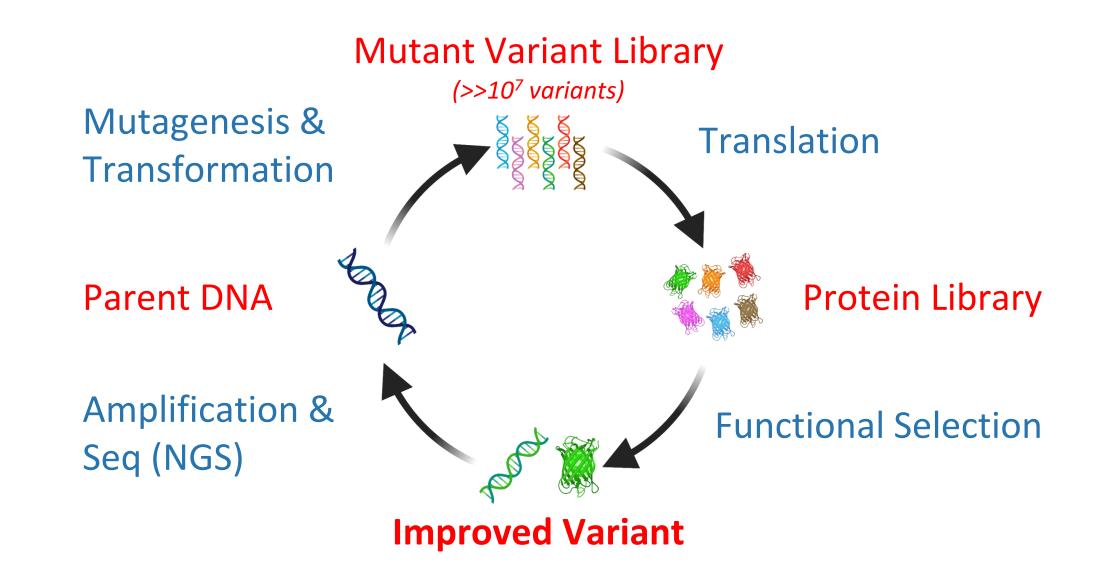
5 California NanoSystems Institute (CNSI), UCLA 6 Jonsson Comprehensive Cancer Center, UCLA

+Ca²⁺ buffer

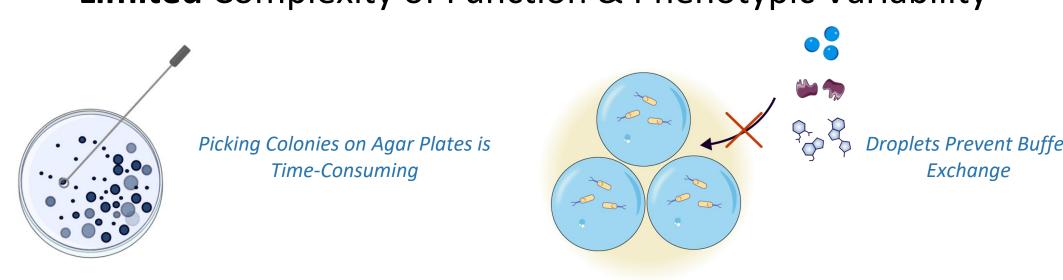


Background

- Deep Mutational Scanning of protein variants by:
- **Directed Protein Evolution**: screening novel proteins



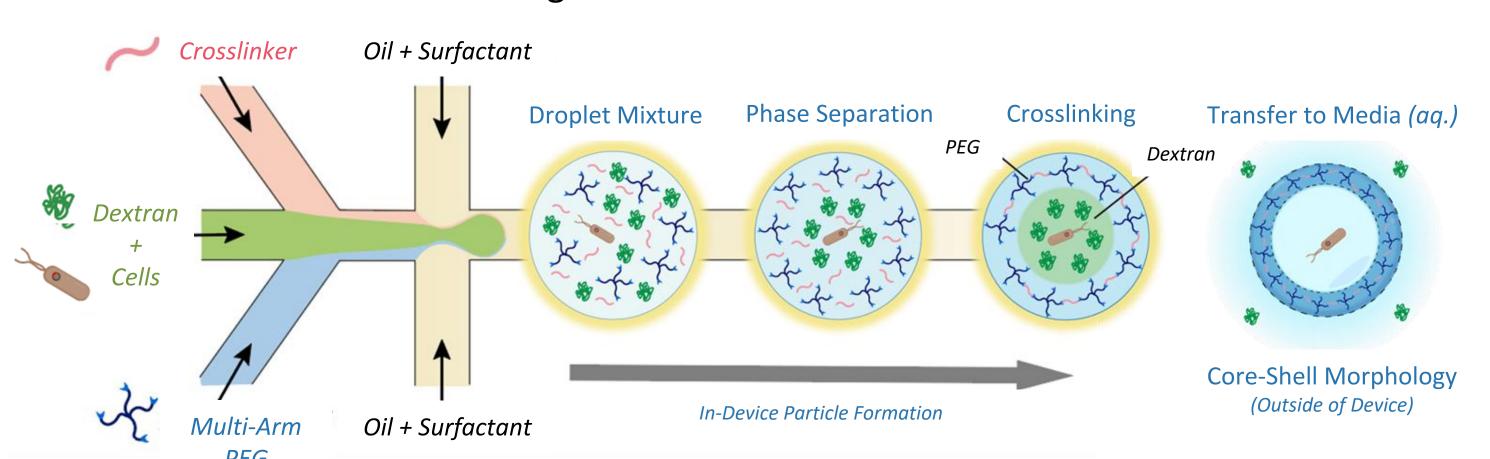
- Current Colony Screening is limited (Weeks to Months)
 - Low Throughput (~10³-10⁴ variants)
 - **Limited** Complexity of Function & Phenotypic Variability



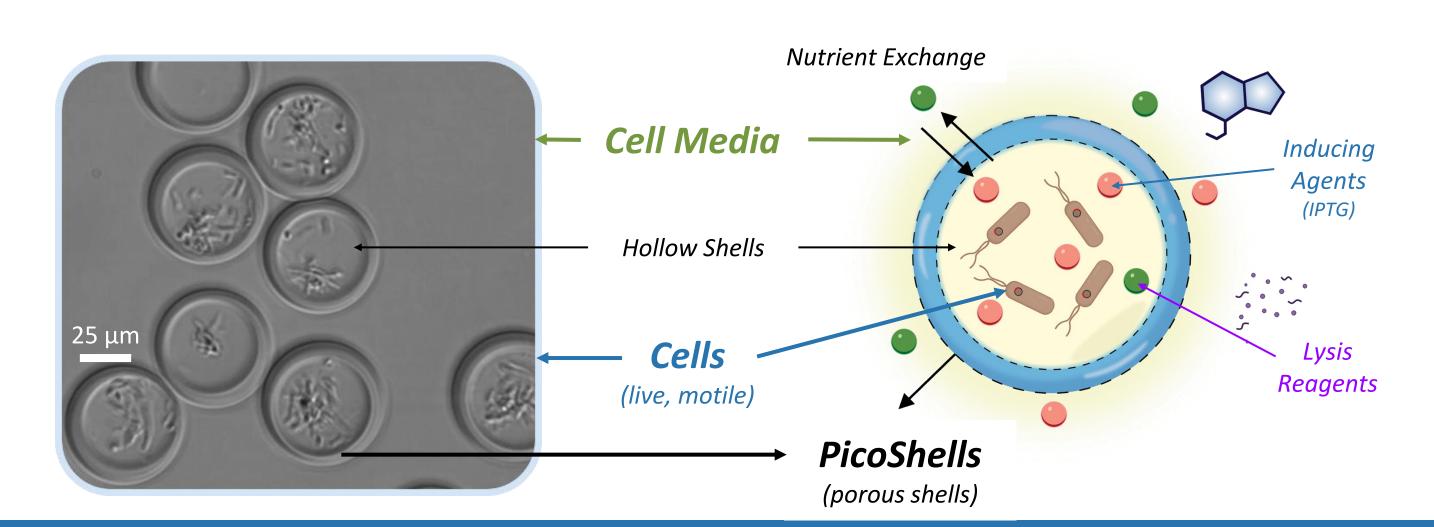
High-Throughput Accelerated Workflow (Days to Weeks)
 screening millions of variants for dynamic function

PicoShells: Lab on a Particle

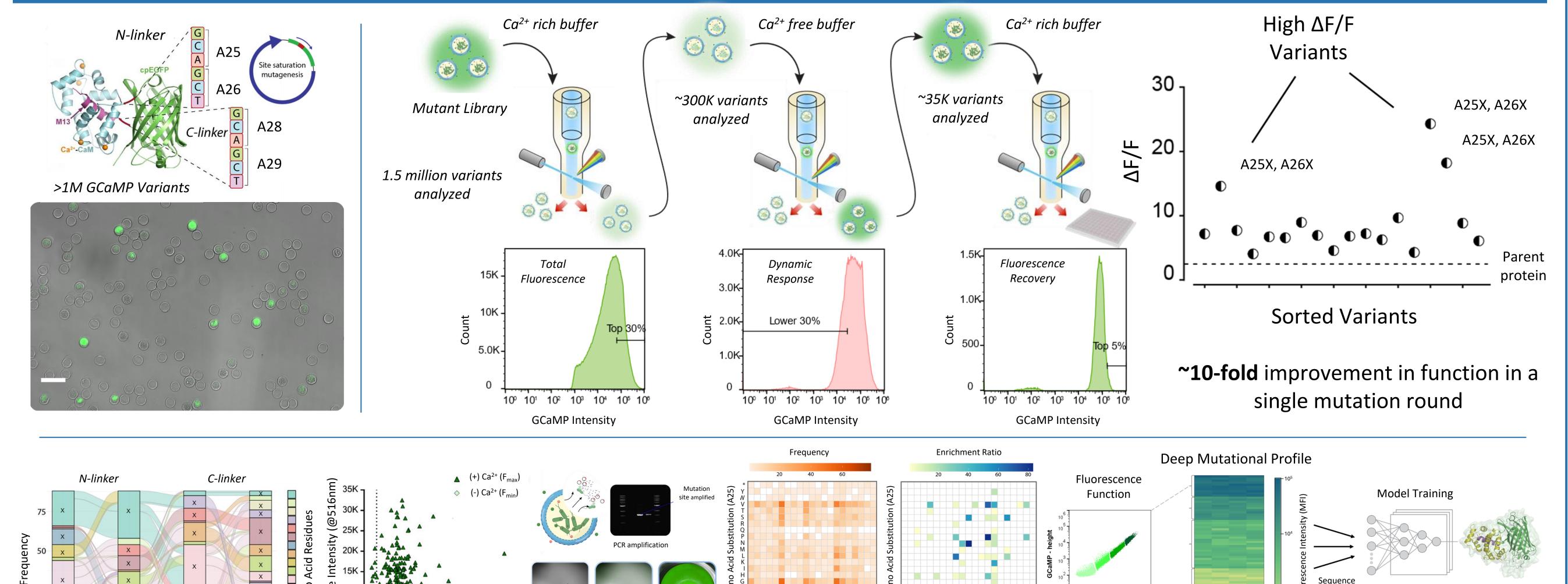
- PicoShells are **hollow hydrogel microparticles** enabling single-cell or colony encapsulation, allowing for analysis of **millions of clones** within a single day!
- Fabrication of PicoShells using Microfluidics:



- Enables Buffer/Solute Exchange & Growth in Media: Multi-Step Workflows

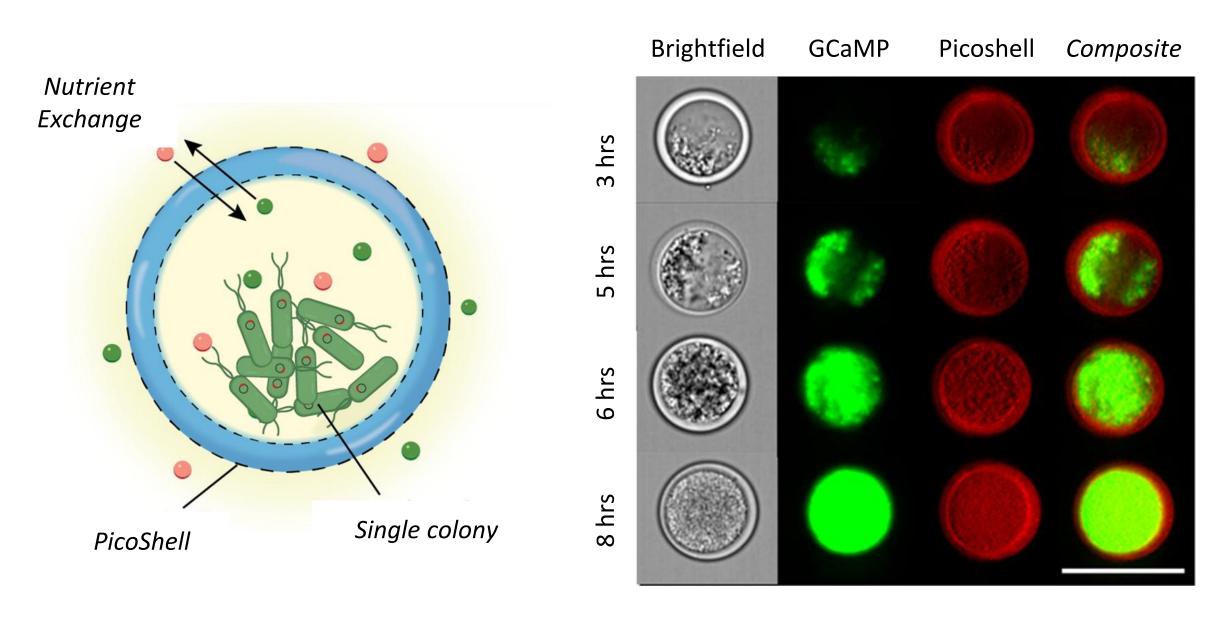


High-Throughput Functional-Based Screening Workflow

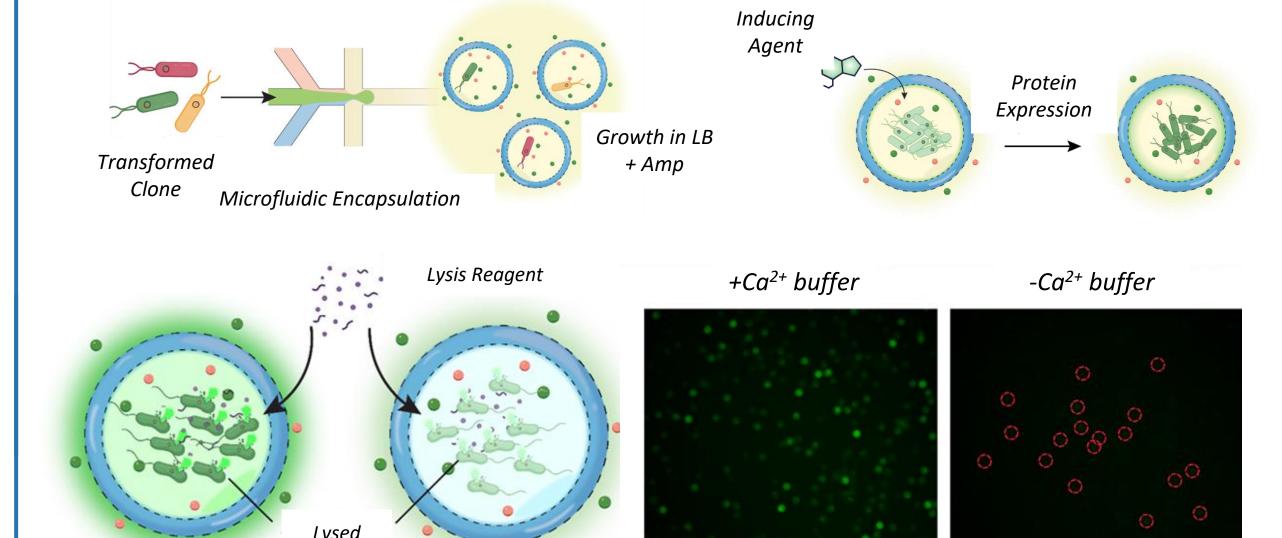


PicoShell Demonstration

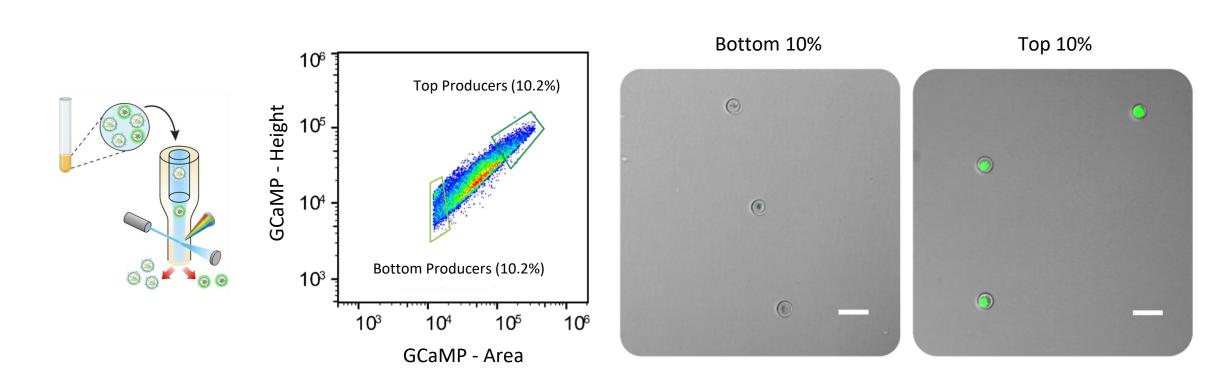
- PicoShell Clonal Population Growth (E. coli expressing GCaMP)

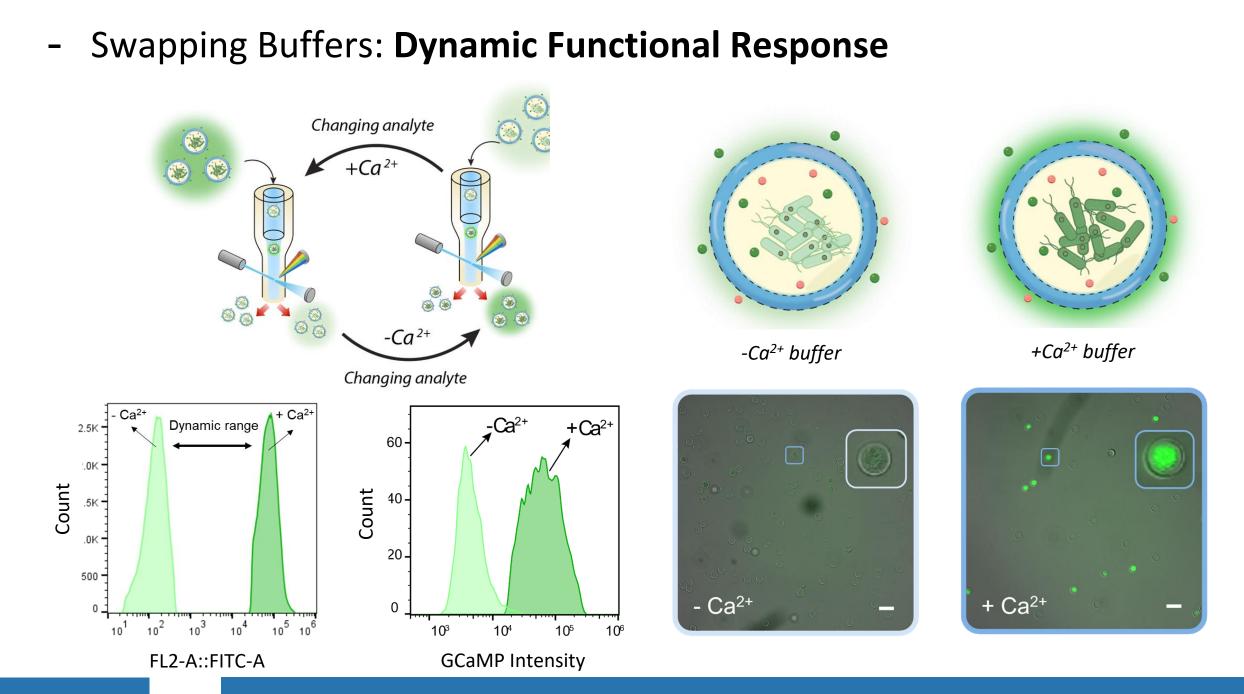


On-Demand Inducable Protein Expression



- Screening and Sorting individual PicoShells via FACS





Conclusions and Future Directions

requency of Amino Acids in the

Starting Library

Amino Acid Substitution (A26)

Enrichments of variants

after three-step screening

- PicoShells enable single-cell or colony screening workflows that can be leveraged for functional assays.
 PicoShells, combined with FACS, screened millions of clones in a single-day's workflow, significantly improving the directed protein evolution.
- This demonstrates the platform's efficiency at screening a mutagenized library and sorting/selecting bacteria producing fluorescent GCaMP.
- Antibiotic Susceptibility Testing (AST) on bacteria spiked in whole blood and isolated to encapsulate into PicoShells and screen for various MICs.

PCR Amplification of Mutatior

Site in PicoShell

- PicoShells and Nanovials can be leveraged to study cell-cell interactions or isolate RNA (scRNA-seq or bulk seq) and single-cell assays.
- We are also **optimizing** PicoShell porosity and quality of the hydrogels to improve the robustness in downstream workflows.

2 * 10⁴ cfu/mL Plasma Fraction Growth in + Amp

-function data

Predictive Modeling

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