Programmable Fabrication of Biohybrid Microswimmers: Templated Spore Outgrowth using Polar-Localized Membrane-Nanobody Fusion Proteins

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Biohybrid microswimmers integrate the innate motility and sensing strategies of microorganisms with the versatile properties of artificial cargo, offering a sophisticated platform for targeted microscale transport. To overcome the challenges of stochastic assembly [1] and low attachment yields [2], we utilize sequential capillarity-assisted particle assembly (sCAPA)[3]. This technique allows for the precise, highly reproducible control of construct geometry by using *B. subtilis* endospores as a resilient biological starting material. By incorporating these spores into the colloidal fabrication process, we harness their inherent resistance to unfavorable conditions, such as heat and desiccation, without compromising the eventual physiological performance of the living actuator.
In this novel approach, the spores are outgrown directly on a functionalized PDMS template, facilitating a transition into vegetative cells that integrate seamlessly with the pre-assembled cargo (Fig. 1a). To ensure a robust and oriented mechanical link, we have genetically engineered the *B. subtilis* cells to display a nanobody fusion-protein with a polar localized expression. This biological affinity is matched by modifying the artificial particles with a green-fluorescent protein, creating a high-specificity polar bond (Fig. 1b). This programmable interface ensures that the cellular actuator is positioned optimally for propulsion, effectively bridging the gap between synthetic architecture and biological machinery.
The success of this nanobody-mediated attachment demonstrates the profound versatility of the spore-templated sCAPA platform. Beyond mere motility studies, this approach provides a universal framework for the fabrication of complex biohybrid microswimmers, where both the cargo and the cellular interface can be genetically and chemically tailored. By enabling the precise marriage of engineered biology and colloidal science, this platform opens new avenues for creating autonomous, multi-functional microsystems capable of navigating and interacting with complex biological environments.

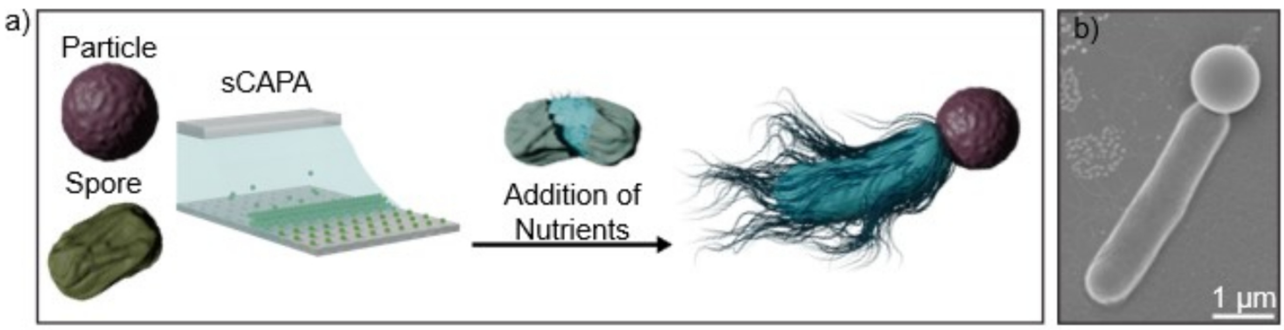

Figure 1:Biohybrid microswimmer fabrication using capillary assisted particle assembly. a) Sequential deposition of colloids and spores on PDMS. Upon addition of nutrients the spores germinate, attach to the particles and form biohybrid microswimmers. b) SEM image of a biohybrid microswimmer.

References:

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