7.17 SYNTHETIC BIOLOGY

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Synthetic biology takes its cue from the multiplicity of natural materials and architectures whose combination enables vital functions that lead to the living world as we know it. We can benefit from its approaches and engineering principles to develop active systems as dynamic and responsive materials with emerging and programmable functionalities. Biomaterials and bioinspired systems have great potential not only to uncover fundamental mechanisms behind complex biological functions, but also to drive progress in various fields, including medicine and environmental science.

We focus our efforts on the analysis of beating structures such as cilia or flagella isolated from biological microswimmers and used to develop synthetic swimming transporter. By using a few biological building blocks from flagella we show how synthetic cilia can be reconstituted. Furthermore, we show that synthetic tissues and cells can be assembled also by using non-biological materials with the aim of engineer materials characterized by the properties of biological systems such as self-regeneration, reactiveness and self-organization.

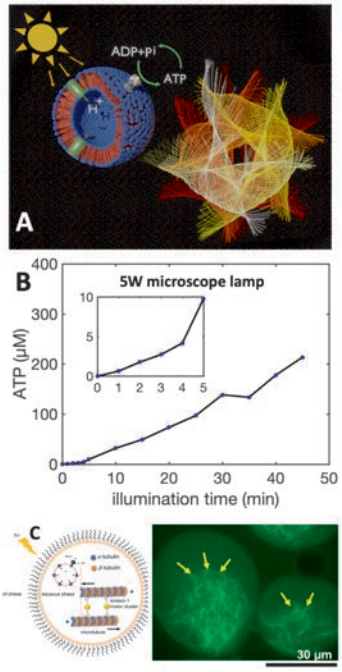

Figure 7.39: A-B) Integration of the isolated flagella from *Chlamydomonas reinhardtii* with the light-switchable energy module. To convert light into ATP, we co-reconstituted two purified transmembrane proteins namely, Bacteriorhodopsin (bR) and EF_OF_1-ATP synthase from *E. coli*. Upon illumination, Bacteriorhodopsin pumps proton into the vesicle's interior, establishing a proton motive force that drives ATP synthase to catalyze the conversion of ADP to ATP. C) Light-driven MTs contraction.

7.17.1 *Light-powered reactivation of flagella and contraction of microtubules network: towards building an artificial cell*

(A. Gholami, I. Guido) Artificial systems capable of self-sustained movement with self-sufficient energy are of high interest with respect to the development of many challenging applications including medical treatments but also technical applications. Our work demonstrates the biocompatibility and efficiency of an artificial light-driven energy module and a motility functional unit by integrating light-switchable photosynthetic vesicles with demembranated flagella that provide ATP for dynein molecular motors upon illumination. We engineered light-switchable photosynthetic liposomes (150 nm in diameter) as energy modules to generate ATP under illumination. The flagellar propulsion is coupled to the beating frequency and dynamic ATP synthesis in response to illumination allows us to control beating frequency of flagella in a light-dependent manner (Fig. 7.39A-B). In addition, we verified the functionality of light-powered synthetic vesicles in *in vitro* motility assays by encapsulating microtubules assembled with force-generating kinesin-1 motors and the energy module to investigate the dynamics of a contractile filamentous network in cell-like compartments

by optical stimulation. Functionalized artificial liposomes capable of continuous production of ATP in response to light, can serve as an efficient energy source for *in vitro* microtubule motility assays in which kinesin-1 motors are actively engaged in generating active stresses in the network (Fig. 7.39C) [1].

7.17.2 *Anomalous propulsion regime in axonemal-propelled cargoes*

(A. Gholami) In our work isolated flagella from green algae *Chlamydomonas reinhardtii* as an ATP-fueled bio-actuator for propulsion of micron-sized beads have been used. *Chlamydomonas* flagella have an asymmetric waveform, which can be described as a superposition of a static component corresponding to an arc-shaped intrinsic curvature, and a main base-to-tip traveling wave component. By applying resistive force theory, we performed numerical simulations and obtained analytical approximations for the mean rotational and translational velocities of a flagellum-propelled bead. Our analysis reveals the existence of a counter-intuitive anomalous propulsion regime where the speed of the flagellum-driven cargo increases with increasing cargo size. We also demonstrate that in addition to the intrinsic curvature and even harmonics, asymmetric bead-flagellum attachment also contributes in the rotational velocity of the micro-swimmer (Fig. 7.40) [2].

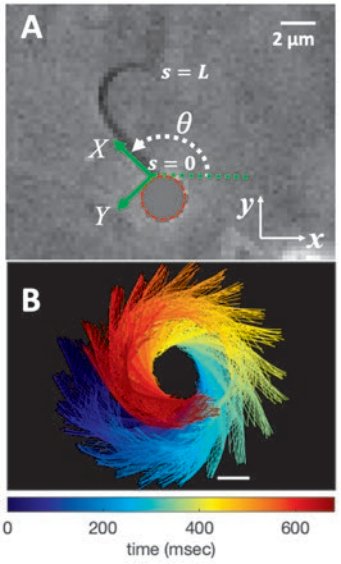

Figure 7.40: A-B) An axoneme attached to a 1 micron bead, swimming in a helical path.

7.17.3 *Bio-inspired active systems assembled from the bottom-up*

(I. Guido) In nature, the self-assembly of biopolymers and motor proteins leads to interesting emergent behaviour that is crucial for cellular organisation and motility. An example of such self-organisation is the rhythmic bending of cilia and flagella that promotes fluid transport or propels swimming organisms introduced in Sec. 7.17.2. Ciliary beating is powered by motor proteins, which drive a sliding motion of microtubule doublets. We investigated their mechanical interplay by analysing a minimal synthetic system that we assembled using one [3] or two microtubules and different types of motor proteins including axonemal dynein [4] (Fig. 7.41). The systems undergo rhythmic bending through cyclic association/dissociation that, despite their extreme simplicity, resembles the dynamics of more complex flagellar structures. Besides their potential for answering crucial questions about the active dynamics of ciliary beating, the synthetic cilia may encourage the technological development of molecular machines for fluid transport at micro- and nanoscale. Alongside the investigation of self-organising active systems at single filament level, we also focus on microtubule-motor protein active networks. We investigate how these active filamentous structures promote nonequilibrium processes induced by active stress at the microscale. By combining passive components that produce entropic forces and extensile and contractile forces exerted by motors, we show that the network exhibits nematic organization characterised by long-range orientational order [5, 6]. The evolution of the system over time is particularly interesting and unique. It undergoes a 3D to 2D transition by contracting into a sheet, expan-

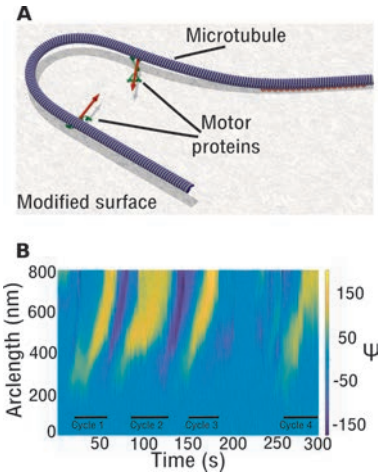

Figure 7.41: A) Clamped single microtubule and motors. B) Kymograph of tangent angle along the filament.

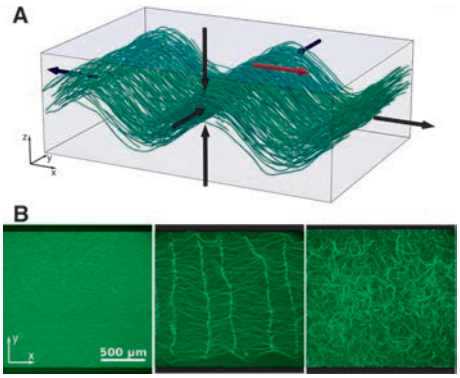

Figure 7.42: A) Schematic representation of wrinkle formation. B) Instability evolution.

sion in the direction perpendicular to the contraction, 3D wrinkling pattern formation, and finally, 3D active turbulence. These results show that these minimal synthetic structures can serve as new model systems for the quantitative understanding of fundamental questions about cytoskeletal self-organisation.

7.17.4 Synthetic cell fabrication with microfluidic system

(H. Kim) We develop stimulus-responsive minimal cell compartments which is in the form of thermoresponsive core–gap–shell (TCGS) microcapsules with microfluidic techniques using the thermo-responsive polymer, poly(N-isopropylacrylamide)(PNIPAm) (Fig. 7.43A). By leveraging the temperature dependent solubility of NIPAm, various bioactives are able to be directly loaded inside the cavity during the formation of the microcapsules. When temperature of external solution of the TCGS is higher/lower than lower critical solution temperature of PNIPAm (33 °C), the microcapsule is reversibly shrunken/swollen by extruding/absorbing water molecules. As an application, we developed a thermo-controlled glucose sensor which encapsulates silica nanocontainers with loaded GOX (Fig. 7.43B). According to the temperature of the external solution, the GOX reaction is suppressed or intensified. In this way, glucose detection can be controlled on-demand in our thermo-controlled microcapsule system. In the future, the proposed microcapsule with stimuli-responsiveness will provide more practical applications by incorporating various bioactive compounds [7].

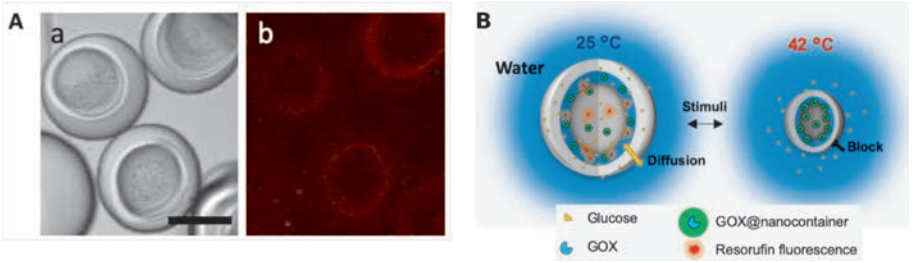

Figure 7.43: A) Nanoparticle encapsulation within TCGS microcapsules. Scale bar: 50 μm. B) Schematic of thermo-controlled glucose sensor developed with GOX@nanocontainers encapsulating TCGS microcapsule.

7.17.5 *Wound healing and active foams for hybrid tissues*

(H. Kim, M. Tarantola) Shape, dynamics, and viscoelastic properties of eukaryotic cells are primarily governed by a thin, reversibly cross-linked actomyosin cortex located directly beneath the plasma membrane, which needs to be fully described before successful reconstitution in synthetic models or application to wound healing. We extract time-dependent rheological responses of various cells from deformation-relaxation curves using atomic force microscopy (AFM). We access the dependence of cortex fluidity on prestress and introduce a viscoelastic model that treats the cell as a composite shell, assuming power-law-based relaxation of the cortex. It gives access to cortical prestress, area-compressibility, and cortex fluidity independent of indenter geometry and compression velocity. By interfering with myosin activity, we find that fluidity decreases with increasing intrinsic prestress and area-compressibility modulus, in accordance with synthetic biological findings for isolated actin networks subject to external stress [8].

Polymer networks in synthetic biological applications also encompass stimuli responsive coatings. We have characterized temperature dependent adhesive properties of PNIPAm microgel coated surfaces (PMS) using various AFM-based approaches: we imaged and quantified material properties of PMS upon temperature switching using quantitative AFM imaging but also employed single-cell AFM before and after decreasing the temperature to assess the forces and work of initial cell-PMS adhesion. We performed a detailed analysis of steps in force-distance curves and applied colloidal probe AFM to analyze the adhesive properties of two major components of the extracellular matrix to PMS under temperature control, namely collagen I (col) and fibronectin (FN). In combination with confocal imaging, we showed that these two ECM components differ in their detachment properties from PMS upon cell harvesting (Fig. 7.44) involving partial ECM dissolution. These tissues finally will help to create hybrid assemblies with polymerosomes or liposomes [9]. We also studied PNIPAm polymerosomes as potential carriers for encapsulating cytokines relevant to wound healing. Their Young's moduli were characterized via AFM upon swelling and TGF-beta encapsulation using fluorescence microscopy. Release of TGF-beta from polymerosomes to wound healing fibrotic cultures was quantified by impedance spectroscopy for various wound sizes and found to accelerate healing dynamics paving the way for their usage in hybrid tissues [10].

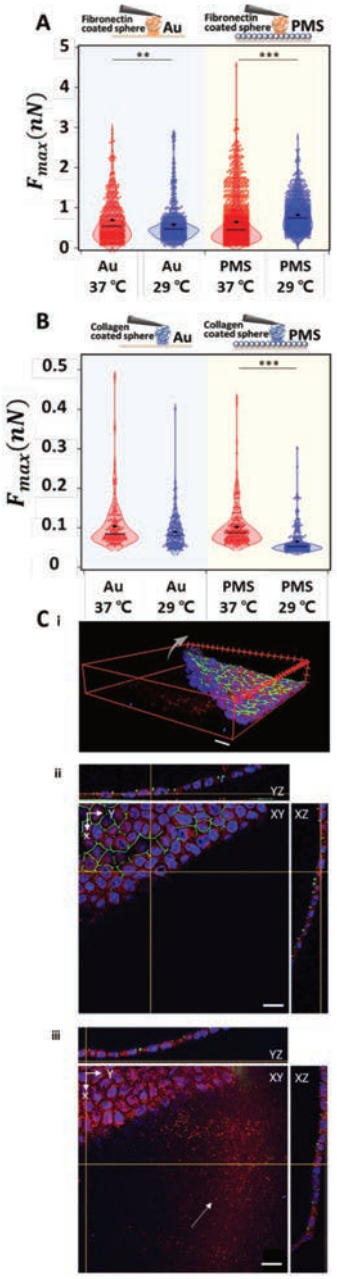

Figure 7.44: Adhesive forces $F_{\max}$ between ECM component FN (A) or col (B) and gold or PMS. C. Confocal images of MDCK II cell sheet (nuclear DNA (blue), tight junction (TJ, green), and ECM component FN (red) on PMS) with: i) 3D image upon detachment (arrow). ii) Substrate plane with remnant PMS-attached FN (arrow). iii) Apical cell membrane showing continuous TJ distribution. Scale bar: 20 μm.

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