B. Executive Summary

B.1. Specific Aims & Significance

Our long-term goal is to understand the fundamental design principles of cellular control systems and to apply these principles to engineer "smart" cells or cell-like devices with novel therapeutic functions. This goal is extremely challenging, as cells are built from large numbers of molecules that interact with one another to form complex, dynamically regulated, self-organizing systems. Our ability to engineer cells and their constituent molecular systems is extremely primitive. Our goal is to develop the foundation for precision cellular engineering, analogous to the foundations that already exist in other highly developed engineering disciplines.

In the long-term, the ability to precisely manipulate cells or synthesize molecular assemblies with cell-like behaviors would have revolutionary therapeutic potential. Engineered therapeutic cells, much like endogenous immune cells, would be "smart" — they could diagnose a lesion or threat, move to that site, and respond with a precise, feedback controlled treatment. Smart cells could hunt and destroy microscopic cancers or cardiovascular lesions. Their responses could be complex: they might directly release a therapeutic agent, or they might act as intermediaries, mobilizing and redirecting the immune system. The designed cells could act as a network, with distinct cells carrying out specific steps in a complex therapeutic cascade. Engineered cells with sophisticated homeostatic control circuits could precisely readjust hormonal and metabolic imbalances. Engineered control of cell motility could be used for repair of nerve and other tissue damage. Modified cells could be from the patient, or from compatible matches, thus eliminating or alleviating problems of immune response. The range of therapeutic potential would be staggering. In the shorter-term, this center may attempt to generate cells that mimic the regulated shape change of platelets or cells that can move in response to heterologous inputs.

To achieve our goal, we are initially focusing on actin-based cell motility as a testbed system. Many cells have the remarkable ability to detect environmental cues and to precisely move or alter their shape through spatiotemporal regulation of the actin cytoskeleton. Our primary goal is to learn how to program molecular systems to achieve this type of precise morphological control. Development of a fundamental cellular engineering framework within this testbed will then allow expansion and application to engineering other cellular behaviors. We have formed an interdisciplinary Center which we refer to as the ***Cell Propulsion Lab (CPL).***

To understand how cell motility systems are built, our team is focusing on two engineering ***Grand Challenges***:

1. Characterize and reprogram cell guidance/signaling systems (e.g., convert a non-motile cell into a motile cell)
2. Characterize and build alternative force generating polymer systems that can perform work in a cell (either from non-actin polymers or from nanomaterials). Use these to build synthetic cell-like assemblies capable of induced shape change or movement

B.2. Organization of the Center

We have made significant progress in the organization of our center. The full center (12 Pis) meets once a month, with various subgroups meeting twice a month. This high level of communication has led tot the establishment of an extensive network of highly integrated interlab collaborations. Our lab website and wiki sites are at: www.qb3.org/CPL. The Center has served as a highly effective means

to attract outstanding new graduate students and postdocs from diverse scientific backgrounds who are excited to be part of this highly integrated and creative network.

B.3. Research Progress

In our second year we have made significant progress towards these aims.

Re-programming cell guidance circuits: basic mechanisms and toolkit to control GTPases. One significant area of progress involves efforts to manipulate and engineer Rho family GTPase signaling, master regulators of the actin cytoskeleton and cell morphology. The state and extent of GTPase activity is controlled in large part by a set of upstream activators known as guanine nucleotide exchange factors (GEFs). A major goal of our center is to learn how GTPase components are wired in in motile cells, resulting in complex spatial self-organization such as cell polarization. A complementary goal is to learn how to engineer regulatory control and output of GEF proteins to that we can confer motility to non-motile cells or tune cells to show novel morphological responses. We have made progress in characterizing, reconstituting, and modeling Rho GTPase circuits that control cell polarization. Focusing on the DH-PH family of GEF proteins, we have demonstrated that we can use modular regulatory domain recombination to engineer GEFs that show novel input gating. We have also demonstrated that these synthetic proteins can be used to activate the Rho family GTPases Cdc42 and Rac in vivo, thereby generate new morphological regulatory pathways in living cells. They can also be used

In addition we have used a combined computational and experimental approach to successfully redesign the interface of a GEF-GTPase complex. This has allowed us to design new GEF-GTPase pairs that are "orthogonal" -- they only interact with a mutant partner and not with endogenous GTPases. Thus these orthogonalized proteins can be used as new control channels to mediate new, highly specific outputs. These early findings suggest that it is possible to rationally alter some of the signaling linkages that control cell morphology through cellular systems engineering.

Characterization, Reconstitution, and Engineering of Force Generating Systems. Another significant area of progress is in understanding the regulatory and force generating behavior of non-actin polymer systems. We have extensively characterized the nucleation/polymerization behavior of the bacterial actin homology ParM, which is normally used to segregate drug resistance plasmids to the opposite poles of bacteria. We demonstrate that we can reconstitute ParM nucleation in vitro (using the native nucleator, a plasmid binding protein called ParC), and that this system can be used to move loads such as polystyrene beads in solution or in microfabricated chambers, if a nucleation complex is properly tethered to the beads. Specific dynamic instability properties of the ParM polymer (both ends need to be attached to a nucleator complex to get stable polymer growth) allow it to push two objects to the long axis of an exclosed space – explaining how it is used to segregate two copies of a plasmid in a dividing bacteria. We have recently shown that ParM polymerization can occur when the protein is microinjected into mammalian cells. We are now attempting to couple ParM to tetrapod nanoparticles to build hybrid materials with regulatable self-organizing properties. We also hope to use the ParM polymer as an alternative system to perform new motility functions in these mammalian cells. The ParM polymer system should be completely orthogonal to the host cytoskeleton -- it should not interfere with actin and other endogenous cytoskeletal systems.

We have also made significant progress in developing methods to load or encapsulate vesicles with actin and other regulatory components, in an effort to reconstitute minimal morphological control systems. These are early models for artificial systems that show synthetic morphological control, such as an artificial platelet or particles capable of simple search and delivery functions.