

## Task Group Summary 4

### *Can engineering systems and control approaches generate new strategies for altering imbalanced macrophage profiles in human disease?*

#### Challenge Summary

Biological organisms are complex systems whose design has been shaped by billions of years of evolution. We are now at the threshold of identifying the entire “parts list” (genes, proteins, metabolites, etc.) for many organisms including humans. The enormous task confronting us currently is to understand how these parts work together in complex hierarchies of systems within systems. The only analogous systems whose complexity we can claim to understand are complex engineered systems.

The reductionist approaches of cell and molecular biology have been spectacularly effective in revealing the elements of biological design, but these approaches are incapable of providing the quantitative and integrative techniques that are required to reveal the design principles of the intact system, the repertoire of its dynamic behaviors, and its pathologies.

There is an emerging focus of activity on discovering biological design principles that draws upon our experience with complex engineered systems. This experience is providing a useful guide for this discovery process. With this understanding one can begin to envision rational strategies for reengineering biological systems for therapeutic purposes. For some time complex biological systems will receive the most benefit of this focused activity at the interface between biology and engineering. However, the design principles of biological systems are likely to suggest new ways of providing robust control of complex distributed systems that will also benefit the design of complex engineered systems. The specific challenge for this task group is the increasing recognition that immune responses involving macrophage cells play important roles both in protection against disease and in the promotion of disease (DeNardo & Coussens, summary in Fig.3, 2007). Characterizing the phenotype, molecular signaling, and therapeutic opportunities associated with these warrior cells has great relevance in the diagnosis and treatment of conditions ranging from vascular disease to cancer. Two distinct phenotypes have emerged in the systems analysis of cancer—an “M1” phenotype associated with acute inflammation and tumor rejection and an “M2” phenotype associated with chronic inflammation and tumor progression. The distinct phenotypes can be identified by their specific signals: secretion of anti-tumor cytokines in M1 and secretion of tumor-promoting growth factors in M2.

#### Key Questions

The challenge to the working group is to come up with an engineering analysis, design and control protocol to address the “M1–M2” phenomenon in human disease. Therapeutic strategies that are incapable of distinguishing between these phenotypes and therefore destroy all immune function are suboptimal. Therefore, important goals include the minimally invasive characterization of the phenotype and the creation of therapies designed to shift the macrophage phenotype from M2 to M1—preserving the important role of the macrophages in the control and elimination of disease.

Elements of a possible approach include:

- Development of an overall strategy for the engineered design process.

- In addition to the overall strategy you will need a process of subdividing the design tasks and then integrating them. Consider the following required subsystems.
    - o Sensors for the relevant biological signals
    - o Logic processor for integration of input signals
    - o Signal processing for a relevant set of actuators
    - o Design and optimization of a control strategy
    - o Consider fault detection and plan an abort module to cover unintended consequences
    - o Integrate testing and validation of all subsystem models as the overall design progresses
- An example that is already being developed (Anderson et al., 2005; 2007) is the engineering of a vehicle (bacterial) that can deal with the trade-offs of evading the host immune system long enough to be effective and delivering with specificity toxins to kill only tumor cells.

### **Required Reading**

- Anderson JC, Clarke EJ, Arkin AP, Voigt CA. Environmentally controlled invasion of cancer cells by engineered bacteria, *J Mol Biol* 2005;355:619-627.
- DeNardo DG, Coussens LM. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. *Breast Cancer Research* 2007;9:212-222.
- de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. *Nature Reviews Cancer* 2006;6:24-37.

### **Suggested Reading**

- Anderson JC, Voigt CA, Arkin AP. Environmental signal integration by a modular AND gate. *Mol Syst Biol* 2007;3:133.
- Cold Spring Harbor Laboratory Proceedings from recent conferences and workshops on Engineering Design Principles in Biology. [Accessed online July 31, 2008: <http://meetings.cshl.edu/meetings/engine08.shtm/engine08.shtml>.]
- Recent Conference on Synthetic Biology. [Accessed online July 31, 2008: <http://syntheticbiology.org/> and <http://sb4.biobricks.org/series/>.]

### **TASK GROUP MEMBERS**

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### **TASK GROUP SUMMARY**

*By Hadley Leggett, Graduate Science Writing Student, University of California, Santa Cruz*

“Men at some time are masters of their fates. The fault, dear Brutus, is not in our stars, but in ourselves.”

—*Cassius, from Julius Caesar by William Shakespeare*

When Shakespeare said that men are the masters of their own fates, he almost certainly wasn't talking about controlling destiny on a cellular level. But significant recent advances in the understanding of human biology have led scientists to wonder whether it might soon be possible to affect a person's future by controlling the fate of his or her individual cells.

By coaxing cells down a specific developmental path, scientists might be able to grow new pancreatic cells for a diabetic, or cure a patient's cancer by forcing wayward cells to stop dividing. The potential applications are endless, but the challenge of actually accomplishing this enticing goal is incredibly difficult.

At the 2008 meeting of the National Academies Keck *Futures Initiative* Conference, a Task Group (4) including biochemists, physicists and engineers met to wrestle with the enormity of the challenge.

“How do we cope with the huge size of networks that we find in biology?” asked Dr. Herbert Sauro, an associate professor of bioengineering at the University of Washington. “Estimates for different kinds of proteins in a single human cell may be up to 100,000,” he said. “And who knows, the number of connections among those proteins could be much bigger.”

To approach the problem, the group started with a specific example of regulating cell fate in the immune system of a cancer patient. Research has shown that the immune system plays a paradoxical role in cancer development: Some types of immune cells fight cancer, while others promote tumor growth. The group's task was to apply engineering-control models to create a cancer-fighting environment, rather than a tumor-promoting one.

### **How to Control a Macrophage**

As described in the Challenge Summary to the group, immune cells called macrophages secrete specific signaling proteins depending on their phenotype. “Good” macrophages, called M1s, produce anti-tumor cytokines, while “bad” macrophages, called M2s, secrete growth factors that increase inflammation and encourage tumor growth.

Designing a macrophage control system to regulate cell phenotype means making a series of engineering decisions applicable to an active biological system. First, the group discussed general strategy. Should the focus be on changing M2 macrophages back into M1s, or preventing the development of M2 macrophages in the first place? Would simply eliminating all M2 macrophages create a tumor-fighting phenotype, or should the control system also generate more anti-tumor M1s?

A control system must also address specific engineering questions, such as whether to exert control from inside the cell—by altering gene expression, for example—or from outside, by changing the chemical landscape around the cell. Table 1 presents a list of specific design questions necessary for creating a macrophage control system.

Another important consideration is the need to anticipate unintended consequences. For instance, although M2 macrophages promote tumor growth, they also encourage wound healing and kill parasites. Some group members worried that eliminating M2 macrophages could slow tumor growth but create other problems, such as decubitus ulcers or chronic parasitic infections.

Essentially, the group decided the control system itself would need to be controlled. Instead of an open-loop system that operates without feedback, the system would need built-in

monitoring and some kind of “volume knob,” adjustable to maintain an ideal balance between M1 and M2 cells.

### A Possible Solution: A Well-tailored Virus

One group member proposed a solution that could potentially encompass all of the design principles described in Table 1. Leor Weinberger, a virologist at the University of California, San Diego, suggested engineering a virus to infect fledgling immune cells and force them down the pathway of M1 development.

TABLE 1 Design Characteristics and Possible Solutions

Characteristics	Possible Solutions
Mechanism	Revert M2s to M1s
	Prevent M2 development
	Eliminate M2s
Application	Internal (e.g., altering gene expression)
	External (e.g., changing the microenvironment)
Range	Local (just around the tumor)
	Systemic (everywhere)
Timing	Continuous
	On-demand
Control	Open-loop (no control)
	Feed-forward (anticipatory control)
	Feed-back (outcome-based control)
Delivery method	Bacterial
	Viral
	Systemic injection with or without targeting

The virus would carry a set of genes that favored M1s and discouraged or destroyed M2s. It could exert internal control on infected cells by making them express M1 genes and secrete M1-friendly proteins. This would in turn exert external control on neighboring cells, by altering their chemical environment and encouraging them to become M1s as well. For instance, the virus might make infected cells secrete one protein to kill M2 macrophages and another to stimulate growth of M1s. Best of all, Weinberger suggested, the virus could control expression of these genes by carrying an “inducible promoter”—essentially, a switch that turns on the genes only when there’s an overabundance of M2s.

Unfortunately, using viruses to package and ship genes into cells is still difficult to do with the precision and safety needed for human medicine. To minimize risk, a virally delivered system would need to be carefully monitored and controlled.

In response to unintended side effects, one could keep adding more genes to the viral package so that each addition exerts a greater level of control over the system. However, a single virus can only fit a certain number of genes. “If you have a 64-kilobase plasmid, how many genes can you really control?” asked John Wikswo, a professor of biomedical engineering, molecular physiology and biophysics, and physics at Vanderbilt University.

By the second day of the conference, the group realized that many of their questions could only be answered through direct experimentation in the lab—either the tailored virus strategy would work, or it wouldn’t. This is an experiment worth doing, and the group plans to explore this strategy after the conference.

### Creating a General Model

For the rest of the weekend, the group focused on applying engineering principles to controlling cell fate in a general context, not specific to immunology. Like any good control system, a system to control cell fate would require sensors, actuators and mathematical models. The sensors would need to detect what’s going on both inside and outside of the cell, and they might take advantage of recent advances in silicon technology. The actuators would do the real

“work” of the system—in other words, they would take information provided by the sensors and translate it into whatever action would stabilize the system.

For instance, in the example of macrophage phenotypes, the sensors might detect an overabundance of M2 cells, and the actuators (in this case, a virus) would respond by turning on genes to correct the balance between M1 and M2 cells.

But to know what action to take in a given situation, a system to regulate the phenotype of a cell would need accurate mathematical models describing cell behavior. Creating these models could present a huge hurdle: Given the hundreds of thousands of proteins in a cell, it would be extraordinarily difficult to accurately describe every interaction. For the present, the group recommended a “black box” model that would deal only with the level of detail necessary to achieve a given outcome. Basically, learn only what you need to know, and dump everything else in a black box.

Creating that kind of model would require what engineers call a “system decomposition”—essentially, breaking down cellular function into discrete modules and identifying specific inputs and outputs.

Once again, this task requires venturing back into the lab, where researchers could perform many experiments in parallel, making small adjustments to see which changes made a difference in the stability of the system. In Task Group Four’s final presentation, Wikswo from Vanderbilt left conference participants with a final, somewhat disturbing thought: In the end, it’s quite possible that systems to control cell fate might turn out to be just as complex as the organisms they’re meant to control. He compared controlling cell fate to trying to fly the X-27 fighter jet, a highly complex experimental plane that never made it past the mock-up phase. “You might have to turn a lot of knobs at once,” he said, “to keep the system in the air.” The biological challenge is to identify strategies that control the system on a coarse-grained scale and don’t require turning the 100,000 or more knobs within each cell. Obviously many single-target drugs can control some cells. What’s needed now is to address distributed, multitarget cell sensing and control.