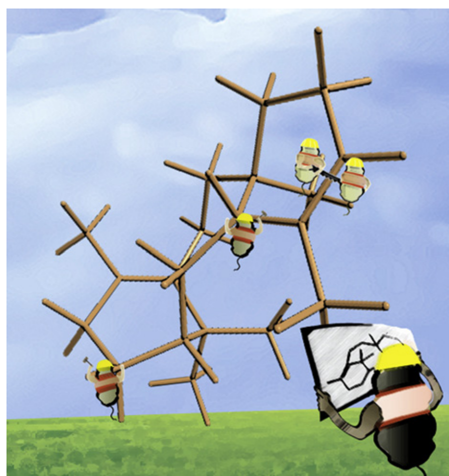
Select: Manipulating Cellular Machinery

Cells can be manipulated for any number of purposes, owing to the emergence of synthetic biology and bioengineering tools. Recent biotechnological advances have generated improved therapeutics, sophisticated biosensors, and new energy sources. The findings presented in this Select highlight innovative ways to both dissect and reconstruct various cellular machineries and components.



Microbes can be instructed to build complex chemical networks. Image courtesy of G. Stephanopoulos.

An Untaxing Way to Produce Taxol Precursors

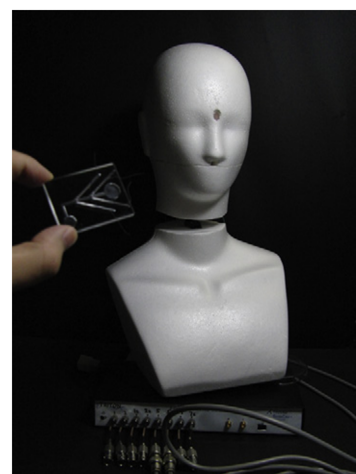
Taxol (paclitaxel) is a potent anticancer therapeutic typically used to treat breast, lung, and ovarian cancers. Historically it has been produced by synthetic or semisynthetic routes, requiring as many as 51 synthesis steps or the use of a plant-derived intermediate, baccatin III, respectively. Ajikumar et al. (2010) introduce a new approach to produce Taxol: engineering the isoprenoid pathway in *Escherichia coli*. There are two pathways to consider for Taxol biosynthesis: the native upstream isoprenoid pathway produces metabolic precursors, and the downstream taxadiene pathway has been reconstructed successfully in *E. coli*. To date, most groups have assumed that these pathways are additive, without paying due attention to toxic side effects of intermediate metabolites or competing pathways and metabolites that could inhibit the drug production. With traditional combinatorial approaches unavailable for taxadiene, the team undertook a "multivariate modular pathway engineering" approach, splitting the pathway into two individually addressable modules. The upstream module focused on the four rate-limiting genes of the methylerythritol phosphate pathway. The downstream module comprised the two-gene pathway, resulting in taxadiene. By tweaking various genetic components and expression levels in the modules, the authors were able to optimize taxadiene production to levels 15,000-fold greater than the control, yielding 1.02 g/l (to date, the highest reported titers from metabolically engineered microbes have been a modest ~10 mg/l). These yields notwithstanding, the key challenge in the generation of Taxol-producing microbes is the oxidation of taxadiene to taxadien-5α-ol via CYP450, a plant enzyme that is difficult to express in *E. coli*. By generating a chimeric CYP450, the authors are able to produce the alcohol but discover drastic reductions in taxadiene production, with concomitant increases in a pathway inhibitor, indole. Improvements to the CYP450-based oxidation chemistry that converts taxadiene to taxadien-5α-ol will open the door to commercial-scale synthesis of Taxol and other high-value terpenoids for use as chemicals or fuels.

*P.K. Ajikumar et al. (2010). Science **343**, 70-74.*

This Cell Can Smell

Whole-cell biosensors have attracted great interest in recent years, owing to their ability to detect environmental contaminants with high specificity and sensitivity. Misawa et al. (2010) now show that oocytes from *Xenopus laevis* can also be engineered for use as chemical sensors, detecting odorants under controlled conditions in a portable fluidic device. Because of their large size (~1 mm in diameter), oocytes have been used as model systems in electrophysiology experiments; and, like all living systems, they can respond to certain chemicals by converting chemical signals into an amplified current signal, which can be detected using capillary electrodes. The authors use microinjections to introduce RNAs encoding the odorant or pheromone receptors from moths or flies that detect bombykol, bombykal, Z11-16:Ald, or 2-heptanone. The oocytes are trapped within a microfluidic chamber under optimized temperature and flow conditions. The modified oocytes can "smell" odorants down to a concentration of 10 nM, with variation in sensing capabilities most likely due to variability in receptor expression levels. The live-cell sensor is also able to discriminate between bombykol and bombykal, which differ only by a terminal alcohol or aldehyde, respectively, thereby highlighting the sensor's inherent specificity in detecting slight differences in chemical structures. The bio-hybrid sensor is ultimately interfaced with a robot to demonstrate that it retains the capacity for chemical sensing under shaking conditions to mimic "real world" implementation of the technology.

*N. Misawa et al. (2010). Proc. Natl. Acad. Sci. **107,** 15340-15344.*

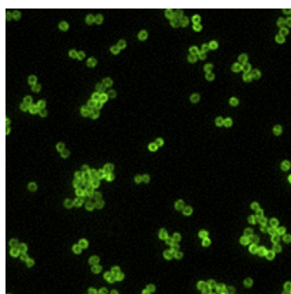


A portable fluidic device that detects odorants can be attached to a human-like robot. Image courtesy of S. Takeuchi.

Fast Track to Biofuels with Cellodextrin Transport

The plant cell wall is highly resistant to degradation, which poses a significant challenge to the bioethanol industry. *Saccharomyces cerevisiae* is currently the yeast-of-choice for fermenting sugars from cornstarch or sugarcane; but these yeasts are unable to naturally ferment cellodextrins derived from plant cell walls without the assistance of cellulase cocktails containing β-glucosidases, which release yeast-fermentable glucose. The degradation of cellodextrins exhibited by the cellulolytic fungus *Neurospora crassa* has inspired Galazaka et al. (2010) to create new yeast strains for enhanced growth and production of bioethanol. Two sugar transporters, CDT-1 and CDT-2, are important for plant-fungi symbiosis and plant degradation. Genes encoding intracellular β-glucosidases are also widespread in fungi, attesting to their importance for optimal growth of fungi on cellulose-derived sugars. Toward complete cellodextrin catabolism and production of lignocellulosic biofuels, the authors re-engineered an *S. cerevisiae* strain to contain the functional cellodextrin transport system from *N. crassa* (CDT-1, CDT-2, and a β-glucosidase, GH1-1). Simultaneous saccharification and fermentation (SSF) is typically limited by the need for complete extracellular conversion of cellodextrin to glucose, which is then transported into the cell. For SSF in yeast re-engineered to express *cdt-1* and *gh1-1*, the authors bypassed this extracellular step to allow the cell to take up cellodextrin via CDT, with intracellular conversion to glucose via β-glucosidases. The result is a biofuel-producing *S. cerevisiae* strain that sidesteps the fermentation bottleneck for lignocellulosic biomass, with yields comparable to those reported from the bioethanol industry.

*J.M. Galazka et al. (2010). Science **330**, 84-86.*



The *S. aureus* cell wall is visualized using a biosynthetically incorporated small molecule fluorophore. Image courtesy of D. Spiegel.

Redecorating the Walls

Antibiotic-resistant *Staphylococcus aureus* has emerged as a major public health threat, with the number of *S. aureus*-infection-related deaths on the rise. Nelson et al. (2010) present an innovative engineering approach to incorporate nonnative small molecules into the *S. aureus* cell wall—a technique that could have myriad implications for imaging bacteria, engineering organisms with novel functions, and, perhaps most importantly, discovering new therapeutics. To incorporate small molecules into the bacterial cell wall, small molecules conjugated to "cell wall sorting" peptides with the sequence LPETG were administered to *S. aureus*. These peptide sequences were recognized by the periplasmic enzyme sortase A, which covalently cleaves the peptide and attaches the new N terminus to lipid II in the cell wall. The authors confirm, using immunocryoelectron microscopy and mass spectrometry, respectively, that the peptides localize and covalently attach to the cell wall. Covalent attachment of small molecules then permits the incorporation of azido groups into the cell wall, which can undergo click reactions with alkyne groups in a manner that does not interfere with native cellular biochemistry. Thus, the authors demonstrate the ability to functionalize the bacterial surface with an array of reactive molecules, which could enable further perturbation and studies of bacterial process in vitro and in vivo.

J.W. Nelson et al. (2010). ACS Chemical Biology published online October 5, 2010. 10.1021/cb100195d.

High-Frequency Genes, High-Specificity Antibodies

Antigen-specific monoclonal antibodies are typically identified by screening immortalized B cells or recombinant antibody libraries. Both in vivo and in vitro methods suffer from complex design and technical drawbacks that limit their use for rapid and reliable antibody selection. In a recent report, Reddy et al. (2010) present an antibody isolation method that uses high-throughput DNA sequencing to analyze the *variable heavy* (V_H) and *light* (V_L) gene repertoires of antibody-secreting bone marrow plasma cells from immunized mice. Bioinformatic analysis then ranks the frequency of *V* genes, which permits the identification of highly expressed V_H and V_L genes whose products are the most likely to be antigen specific. *V* genes appearing at roughly the same frequency are reasoned to be expressed as a pair in the same plasma cell; these pairs are then synthesized and expressed as antibody fragments in *Escherichia coli* or as full-length immunoglobulin in mammalian cells. An antibody specific for the complement protein C1s isolated using this approach demonstrated subnanomolar binding affinity and functionality in ELISA and immunoprecipitation assays. Because this methodology relies on analysis of the *V* gene repertoire from bone marrow plasma cells, which produce the most abundant type of circulating antibodies, the authors hypothesize that this screening-free, rapid isolation approach could be used to generate therapeutic antibodies. In addition, this approach can be extended to other B cell populations and thus allow for the generation of antibodies that are tailored to an individual patient's immune system.

*S.T. Reddy et al. (2010). Nature Biotechnology **28**, 965-969.*

Megan Frisk