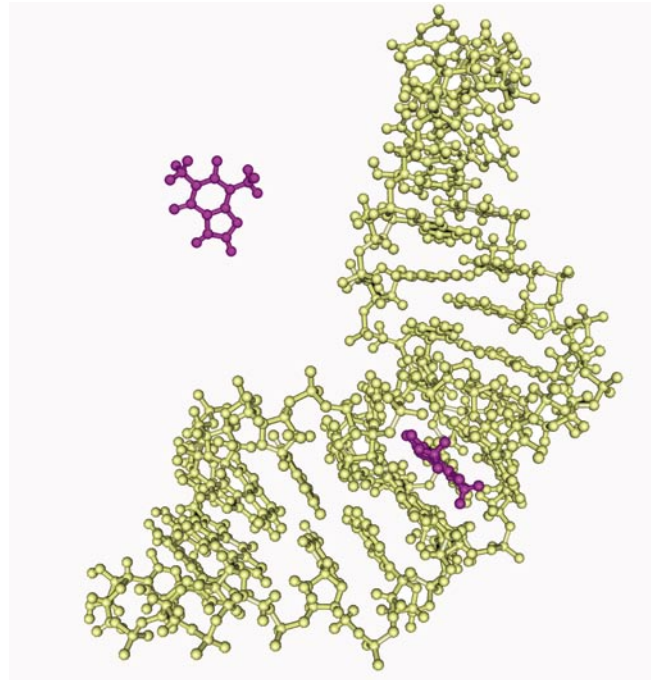


## Technical Highlights of the Recent Literature

## Flip the (RNA) Switch

Riboswitches are regulatory elements occurring naturally in certain mRNAs, usually those of microbial origin. In each case, the relevant sequence forms a structure that acts as a binding pocket for a particular metabolite; the presence of this ligand stimulates a conformational change that dampens translation or increases transcription termination. Though designed for natural feedback loops, these genetic switches are also attractive for artificial regulation of gene expression. To date, transcript-based gene regulation in mammalian cells has relied upon incorporation of aptamer or ribozyme sequences into a transcript's 5' UTR. Alternatively, regulated expression of shRNA can be used to tune levels of the targeted transcript, although the inducible promoters used in these approaches require *trans*-acting factors that might trigger undesirable immune reactions if used in a gene therapy scenario. An et al. combine the best of both worlds by constructing an aptamer-shRNA fusion transcript. In the presence of the aptamer ligand, no silencing occurs—apparently as a result of interference with Dicer-mediated cleavage. The authors' test system, which consists of a theophylline aptamer and a GFP-targeted shRNA, works both in vitro and in the HEK293 cell line. The exquisite specificity of the aptamer appears to be preserved in the hybrid transcript, as theophylline's close structural relative, caffeine, does not affect silencing. Overall, this strategy represents an attractive way of regulating RNA silencing. Unlike a related chemical biology approach that used ATP analogues to downregulate RNAi, theophylline is expected to act specifically on the aptamer-shRNA transcript rather than globally affecting the silencing pathway. Although further work is necessary to confirm the flexibility and applicability of this approach, aptamer-shRNA constructs appear to be an extremely promising means of regulating both exogenous and endogenous genes. —ND



An et al. 2006. Artificial control of gene expression in mammalian cells by modulating RNA interference through aptamer-small molecule interaction. *RNA* 12:710-716.

## Detecting Deviant Behavior

The chemical attraction that sperm have for oocytes is well known; however, questions still remain about the exact nature of the chemotactic appeal, such as whether all sperm types are equally attracted to the egg and exactly which molecules released by the oocyte are most potent and alluring. The role sperm chemotaxis may play in infertility also warrants more careful study; with it exists the potential of developing better fertility treatments and new birth control products. A facile means to study sperm chemotaxis in a controlled environment would therefore greatly enhance our ability to examine these issues and better characterize the biochemical pathways involved, as well as methodically test compounds suspected of being involved. To this end, a recent paper from scientists at Indiana University presents a new microfluidic system for studying sperm movement in response to chemical gradients. Although a number of devices have been used successfully in the past, there are advantages to the new apparatus that give it a clear edge. Most importantly, the gradient between buffer and test reagent can be precisely generated and controlled, providing a very stable experimental microenvironment. The authors engineered a six-chamber system with three inputs for sperm, buffer, and test substance, feeding into a single mixing chamber, plus three waste chambers. An inverted microscope equipped with a camera was used to monitor, electronically capture, and analyze sperm movement in response to different concentrations of added oocyte extract. By selection and positioning of the three input chambers, the attraction of the sperm (placed in the center chamber) to the oocyte extract (left chamber) could be measured relative to the buffer (right chamber). Some design flaws still exist that need to be ironed out, but results obtained thus far agree well with previous studies. The device may prove to be a promising new tool for examining the taxis of sperm in response to chemical signals, with the potential for expansion to other motility and chemokinesis studies. —SS

Koyama et al. 2006. Chemotaxis assays of mouse sperm on microfluidic devices. *Analytical Chemistry* [Epub ahead of print, April 8, 2006].

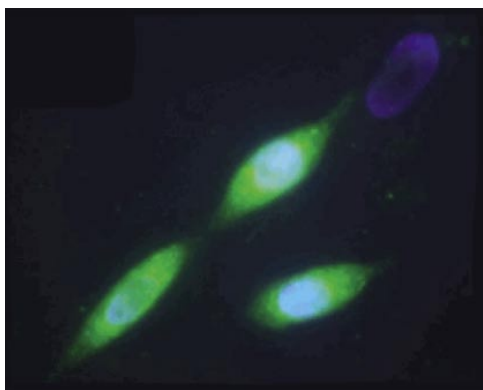
## Technical Highlights of the Recent Literature

## Credit Card Application

Chemical biology-based strategies for disrupting molecular interactions promise to enhance understanding of cellular processes and may ultimately yield small molecule therapeutics. While interfering with protein-ligand interactions presents substantial challenges, the problem of blocking protein-protein binding is particularly arduous. Unlike the tight binding pockets that characterize protein-ligand docking, protein-protein interactions tend to involve expansive surface areas and multiple stabilizing forces. Nevertheless, binding energies are not uniformly distributed, and binding "hot spots" are frequently present. Intriguingly, the layout of these hot spots often resembles the slot of a credit card reader device. If compounds could mimic credit cards and enter the slot reader, moieties that disrupt the protein-protein interface might be identifiable. With that premise in mind, Xu et al. developed an aromatic, hydrophobic scaffold, whose planar nature allows it to serve as a chemical credit card. To test this credit card library approach, the authors synthesized a family of 285 compounds based on a naphthyl scaffold containing four points of chemical diversity. They then examined whether any member of this library could prevent the oncogenic factor Myc from binding Max, the heterodimeric partner Myc requires for DNA binding and transcriptional activation. The screen was a FRET-based assay involving a Myc domain fused to CFP and a Max domain-YFP fusion; the readout for disruption was a reduction in fluorescence. Forty promising candidates survived the initial screen and were analyzed in more detail by EMSA (lack of DNA binding is a proxy of failed heterodimerization). Four of the most promising compounds were then tested in a chicken fibroblast cell line that was infected with oncogenic retroviruses expressing the Myc oncoprotein. Two compounds reduced Myc-induced oncogenic transformation in this cell culture system, demonstrating that the credit card library approach can be applied to identify inhibitors of protein-protein interactions. Although small molecule inhibitors of Myc/Max binding are not themselves new, the fact that potent inhibitors could be identified from such a small library bodes well for the credit card approach, and makes it an appealing strategy for disrupting other protein-protein interactions. –ND

Xu et al. 2006. *A credit-card library approach for disrupting protein-protein interactions. Bioorganic and Medicinal Chemistry* 14:2660-2673.

## Undercover Nanotubes



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In yet another (of the increasingly frequent) meeting of diverse minds and disciplines, nanotechnology has once more entered into a partnership that holds promise for a number of medical and scientific fields. In a recent paper from a collection of groups at the University of California, Berkeley, the successful marriage of glycobiology and nanotech has engendered a means to address the important and previously intractable problem of the toxicity of carbon nanotubes in biological systems. Led by HHMI investigator Carolyn Bertozzi, researchers established a methodology for hiding carbon nanotubes under a coat of glycans, enabling the nanotubes to impersonate glycoproteins found on cell surfaces. Glycans play an integral part in the cell recognition functionality required for numerous biological processes such as immune regulation, cell signaling, and pathogen detection. In the experiments described, multiple  $\alpha$ -N-acetylgalactosamine ( $\alpha$ -GalNAc) residues were attached to a polymer backbone to create a molecule that closely mimics the O-linked glycans found on mucin glycoproteins. Using a specific  $\alpha$ -GalNAc-binding lectin as a bridging molecule, these

coated nanotubes could be targeted to cells with the same  $\alpha$ -GalNAc on their surface, and their effect on cell viability could be determined. The data clearly showed no detrimental effect using the coated molecules, while the uncoated equivalent caused either an abrogation of cell division or apoptosis. Unique glycan epitopes have been identified associated with particular cell types and organelles, as well as distinct disease states. Biomimicry of these molecules by functionalization of nanotubes with different glycoforms can take advantage of very specific glycan-receptor binding interactions. Although the authors did not make direct use of receptors, but rather utilized an intermediary to bind the coated nanotubes to live cells, this paper lays the groundwork for the addition of a broad range of carbohydrates—or any other ligand—to carbon nanotubes, enabling a myriad of experiments incorporating highly specific cell targeting. Thus, the toxicity of the carbon nanotubes, as well as the typical non-specificity of their interactions with cells, can be overcome using this methodology. –SS

Chen et al. 2006. *Interfacing carbon nanotubes with living cells. Journal of the American Chemical Society. [Epub ahead of print, April 21, 2006].*

Selected and written by the editors.