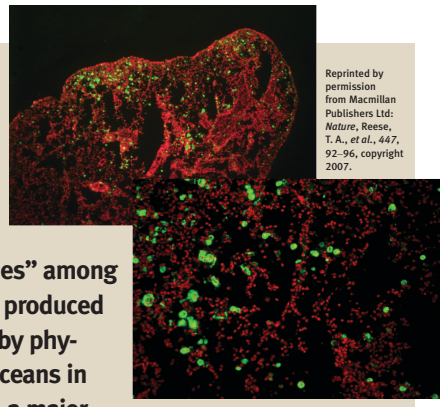
Spotlight

Fighting Chitin



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The many known functions of chitin, a biopolymer of glucosamine, make it a sort of "jack of all trades" among biopolymers. Chitin is produced in massive quantities by phytoplankton and crustaceans in the oceans and is also a major component of the cell wall of fungi and the exoskeletons of arthropods, insects, and parasites. In addition to its natural biological roles, chitin has found several industrial applications as well, including its use in water purification, as a food additive, and as a surgical thread. Reese *et al.* (*Nature* 2007, *447*, 92–96) now report that chitin may also alert our immune system to fight off parasitic infections and allergens.

The observation that mice infected with a parasitic worm exhibited increased expression of acidic mammalian chitinase (AMCase), an enzyme that degrades chitin, led the researchers to speculate that chitin might serve as a recognition element in the immune system. Indeed, injection of chitin in the lungs of mice resulted in recruitment of eosinophils and basophils, two types of white blood cells that play important roles in the innate immune response to allergens and parasitic infections. It is important to note that, when mice overexpressing AMCase were challenged with chitin, the recruitment of eosinophils and basophils was markedly attenuated. In addition, treatment with chitin of mice lacking the receptor for leukotriene B4, which is a potent chemoattractant for eosinophils, also resulted in significantly reduced eosinophil and basophil recruitment to the lung. The authors further demonstrated that macrophages also respond to chitin and play an important role in leukotriene B4-mediated eosinophil recruitment. Taken together, the data suggest that chitin triggers the innate immune response to allergens and parasites by inducing the recruitment of specific innate immune cells and that AMCase facilitates an important feedback attenuation mechanism to help mediate the response. Eva J. Gordon, Ph.D.

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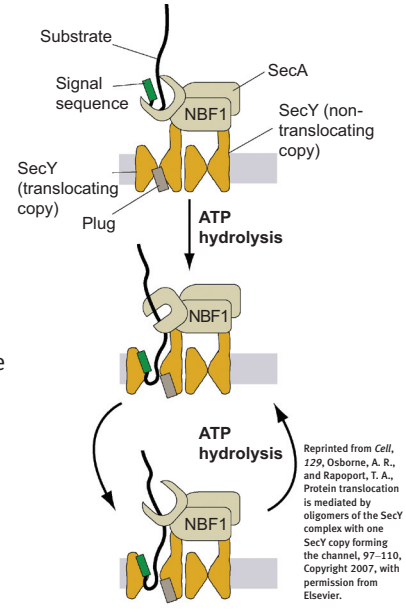
Translocation Teamwork

The exceedingly complex process of transporting proteins across membranes is critical for important cellular events such as protein translation and secretion. Bacteria use the heterotrimeric membrane protein complex SecY as a channel for translocation, but additional components, such as the cytoplasmic ATPase SecA, are required to help the process along. Though many of the players on the protein translocation team have been identified, the complexity of the process along with inherent challenges associated with studying membrane proteins has hampered elucidation of the architecture of, and the cooperation among, the translocation components. Osborne and Rapoport (*Cell* 2007, *129*, 97–110) present a new model for protein translocation that implicates oligomers of the SecY complex as critical components of the translocation machinery.

The authors explore the translocation process in bacteria using purified SecY complexes reconstituted into proteoliposomes and purified SecA. Using disulfide cross-linking and protease digestion experiments, they determined that a single SecY complex forms the pore of the translocation channel. However, use of covalently coupled SecY complexes where one copy contained a mutation that rendered it incapable of functioning as a channel indicated that SecY oligomers are necessary to mediate translocation. They further demonstrated that SecA interacts with one copy of the SecY complex to anchor itself to the membrane while using multiple cycles of its ATPase activity to propel the translocating protein through the neighboring SecY channel. The authors propose that the translocation model presented here likely represents protein translocation mechanisms in all organisms.

Eva J. Gordon, Ph.D.



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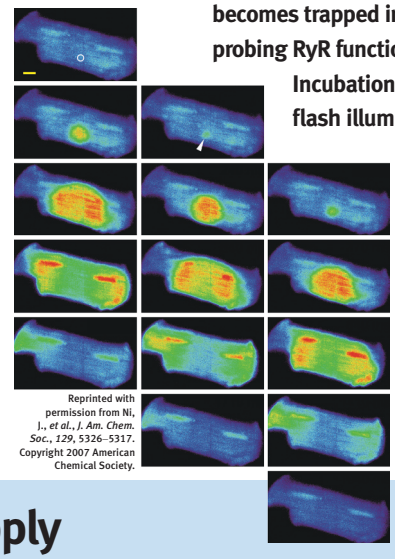
Controlled Channeling

Electrically excitable cells, namely, nerve and muscle cells, are highly regulated by intracellular calcium ion concentration. Ryanodine receptors (RyRs) are channels that mediate Ca^{2+} release from intracellular stores; the released Ca^{2+} controls critical functions such as the contraction of muscle cells and the excitation of nerve cells. RyRs are activated by Ca^{2+} ions entering the cell through voltage-gated channels, in a process called Ca^{2+}-induced Ca^{2+} release (CICR), but RyR regulation is difficult to study with available methods. Now, Ni *et al.* (*J. Am. Chem. Soc.* 2007, *129*, 5316–5317) report on the regulation and effects of Ca^{2+} release from RyRs in a controlled fashion by designing and synthesizing a photoactivatable RyR agonist—an inert molecule that is transformed into an active RyR agonist by photolysis.

An ideal RyR agonist for this application would be water soluble, membrane permeant, and amenable to chemical modifications that block its activity in a photoreversible fashion. The authors chose paraxanthine, a close structural relative of caffeine. Alkylation of paraxanthine with a photolabile nitrobenzyl group containing two carboxylic acid functions yielded the photoactivatable agonist, which they named BiNiX. Protection of the carboxyl groups as labile acetoxymethyl esters bestows lipophilicity and, thus, membrane permeation on the ester. Upon entering a cell, the ester is hydrolyzed by esterases to afford the anionic form of BiNiX, which becomes trapped inside the cell. The utility of BiNiX for probing RyR function was tested in heart muscle cells.



Incubation of the cells with BiNiX, followed by flash illumination of a 5-μm spot in the middle of the cell, caused local Ca^{2+} release, which was amplified through CICR to trigger contraction of the cell. Further experiments with BiNiX could help discern how RyRs are regulated independently of other calcium sources in the cell and enable mapping of RyR distribution in structurally complex cells. Eva J. Gordon, Ph.D.

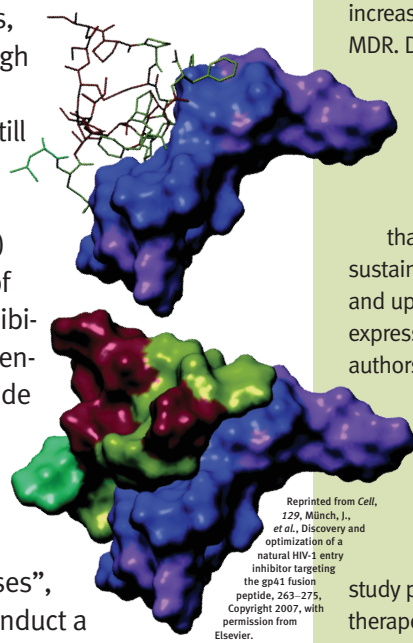
A Universal Blood Supply

The discovery of the ABO blood group system over a century ago provided the key to performing safe blood transfusions. The different types of red blood cells, A, B, AB, and O, differ in the terminal carbohydrate residues present on their cell surface glycoproteins and glycolipids. The A antigen terminates in an α-1,3-linked *N*-acetylgalactosamine, the B antigen ends with an α-1,3-linked galactosamine, and the O antigen contains neither, instead ending in α-1,2-linked fucose residues. These differences cause an immune response in individuals with incompatible blood types, but type O cells are considered "universal" donors because they lack the antigens that would trigger the response. The idea of using enzymes to remove A and B antigens from red blood cells to yield type O blood is not new, because the continual shortage of group O units available for transfusions, coupled with the dangers associated with transfusion of mismatched blood types, is high incentive for the development of such methods. However, attempts to find enzymes with sufficient efficiency and specificity for this important job have not been successful. Liu *et al.* (*Nat. Biotech.* 2007, *25*, 454–464) now report the discovery of two bacterial glycosidases that have proven to be up to the task.

A screen of >2500 bacteria and fungi led to the isolation of two previously unknown bacterial glycosidases: an α-*N*-acetylgalactosaminidase from *Elizabethkingia meningosepticum* and an α-galactosidase from *Bacteroides fragilis*. The enzymes bear no sequence homology with known glycosidases, and structural analysis of the α-*N*-acetylgalactosaminidase revealed that, unlike most glycosidases, the enzyme uses a nicotinamide adenine dinucleotide cofactor and an unusual mechanism for achieving glycosidic bond cleavage. However, these enzymes were highly effective at converting type A and type B blood cells to type O, as assessed by routine licensed blood typing reagents and highly sensitive fluorescence-activated cell sorting. Further development of this method in a clinical setting could provide an exciting new source for type O blood. **Eva J. Gordon, Ph.D.**

Battling HIV from Within

Sometimes the very thing we are looking for might be right before our eyes, but if we do not have the right glasses, we will never see it. For more than two decades, researchers have used countless approaches in the search for successful drugs against HIV-1. Novel, structurally unique, small-molecule inhibitors have been designed against various potential HIV targets in the hopes of finding effective drugs that are not outsmarted by this notoriously devious, self-mutating virus. Although much progress has been made, a desperate need still exists for new, more effective drugs. Münch *et al.* (*Cell* 2007, *129*, 263–275) now report the discovery of a promising new HIV-1 inhibitor that they found by screening the molecules that reside in human blood.



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Technological advances in small-molecule purification techniques provided the necessary "glasses", enabling the authors to conduct a systematic screen of the >1 million circulating compounds that are <30 kDa found in human hemofiltrate. A 20-amino-acid fragment of the serine protease inhibitor α1-antitrypsin, termed VIRIP, was found to specifically inhibit infection by various HIV-1 subtypes as well as variants resistant to protease, reverse transcriptase, and fusion inhibitors. VIRIP was shown to bind to the highly conserved HIV-1 gp41 fusion peptide, thereby preventing HIV-1 entry into its target cell. Structure–activity analysis enabled the creation of various VIRIP derivatives with enhanced potency and minimal toxicity. NMR and surface plasmon resonance studies of the interaction between the VIRIP derivatives and the gp41 fusion peptide revealed that the peptides have highly complementary hydrophobic surfaces, explaining the remarkable specificity of their interaction. Although several HIV-1 entry inhibitors are known, the compounds described here prevent HIV-1 entry by a distinct mechanism that appears to render them impervious to known and likely drug-resistant HIV-1 variants. These compounds, which this study has auspiciously brought into focus, offer an exciting starting point for a new class of HIV-1 drugs. **Eva J. Gordon, Ph.D.**

Porins and Pumps

Bacteria have developed several mechanisms to resist the effects of antibiotics. Some particularly effective methods lead to multidrug resistance (MDR), in which bacteria simultaneously outmaneuver a wide variety of potential chemical assassins by decreasing the permeability of their membranes and increasing the activity of their efflux pumps. Viveiros *et al.* (*PLoS ONE* 2007, *2*, e365) investigate the genetic and functional bases behind the remarkable and notoriously challenging phenomenon of MDR.

A variety of genes and proteins are known to regulate MDR in *Escherichia coli*. Membrane permeability is largely mediated by porin proteins, which are outer membrane proteins that control the transport of small molecules into the cell. In addition, at least nine different multiprotein efflux pump systems can evict multiple foreign substances from the periplasm back into the environment. To explore the molecular details of the acquisition of an MDR phenotype, the authors exposed an *E. coli* strain to increasing concentrations of the antibiotic tetracycline to induce MDR. During this process, they used quantitative real-time reverse-transcriptase polymerase chain reaction and Western blot detection to analyze the activities of various genes and proteins. Initially, the expression of several stress response regulators increased, as did the activity of various membrane and periplasmic proteases that regulate membrane permeability. Subsequently, a more sustained response ensued that led to down-regulation of porins and up-regulation of efflux pumps. It is notable that increased expression of an outer membrane protein was also observed. The authors propose that the abundance of this protein overburdens the system, impairing the normal assembly of porins. Subsequent degradation of unfolded porin monomers will occur, resulting in decreased porin protein levels. Thus, in addition to regulation at the transcriptional level, post-translational regulation of certain proteins also appears to play an important role in MDR. This study provides intriguing details about MDR that will help guide therapeutic strategies to combat it. **Eva J. Gordon, Ph.D.**

Immunity Gets Help from a Tiny RNA

The immune system in mammals acts as a complex computer that calculates the nature of invading pathogens and responds with the appropriate antibody for the job. Input in the form of extracellular signals is downloaded into immune cells *via* surface receptors. Under the influence of these signals, cells can reprogram their gene expression circuits and enter an activated state. Upon activation, multiple levels of gene expression are altered, ranging from transcription and RNA decay to protein modification and stability. Now, a new pair of studies by Thai *et al.* and Rodriguez *et al.* (*Science* 2007, *316*, 604–608 and 608–611) takes aim at an additional level of control during the immune response. Both studies demonstrate that a single microRNA (miRNA) gene, miR-155, is an important regulator of proper immune cell differentiation and activation. Both studies use transgenic mouse techniques more commonly used to study protein-coding genes. In fact, these papers are among the first examples of miRNA knockout mice, animals deficient in functional miR-155.

In the study by Thai *et al.*, the researchers created the knockout of the miR-155 locus and also a second mutant mouse that constitutively makes the miRNA, but only in mature B-cells. When they tested wild-type B-cells, they found that miR-155 expression was induced during activation by extracellular stimuli. Interestingly, the miR-155 was switched on particularly in B cells undergoing germinal center (GC) maturation. GCs are distinguishable areas of lymphoid tissues, where antibody affinity is tuned to a particular antigen during a T-cell-induced immune response. When the researchers tested the mouse deficient in miR-155, they found that the number of B cells present in these GCs was significantly lower. The cells harboring a transgene that makes increased miRNA in the B cell were rescued and actually showed increased numbers localized to GCs. These results indicated that miR-155 may act during activation at GCs. To test this further, the researchers injected the mice with an antigen to initiate a GC response. Amazingly, the mice with no miR-155 showed almost 5-fold lower antibody titers than the wild-type mice, and these knockout mice also showed lower numbers of GCs. Finally, the researchers show that particular cytokines and lineage commitments are altered in the transgenic mice.

In a parallel study, Rodriguez *et al.* made mutant mice that lacked functional miR-155 and found another interesting effect of this tiny regulator. The lungs of mice deficient in miR-155 showed interesting lung changes as the mice aged. The collagen thickness and smooth muscle area around the bronchiolar openings were significantly thicker in the knockout mice, an indication that these mice were prone to autoimmunity. These researchers also focused on the immune system from these animals and found that mice deficient in the miRNA had a harder time fighting off a bacterial infection. Even immunizing the deficient mice to the bacterial pathogen did not render them competent to fight off the invader. Complementary to the other study's GC results, this infection-challenge experiment shows that the miRNA probably takes part in adaptive immune responses. Finally, this second study embarked on a molecular fact-finding mission to see what genes may be regulated by miR-155. Using a DNA microarray, the researchers examined messenger RNAs (mRNAs) from T cells of deficient animals, and they singled out the up-regulated ones. These mRNAs were then scanned for the hallmarks of a miRNA binding site, and a majority of these mRNAs did contain miR-155 sites. Although the authors went on to confirm that just one of these genes, c-Maf, is a *bona fide* target of the miR-155, the array data foreshadow that this tiny RNA casts a regulatory shadow on many genes in the immune system. Given that such a profound immune defect resulted from erasing one small RNA from the organism, this pair of studies also gives us a preview of what is to come for the miRNA field.

Jason G. Underwood, Ph.D.

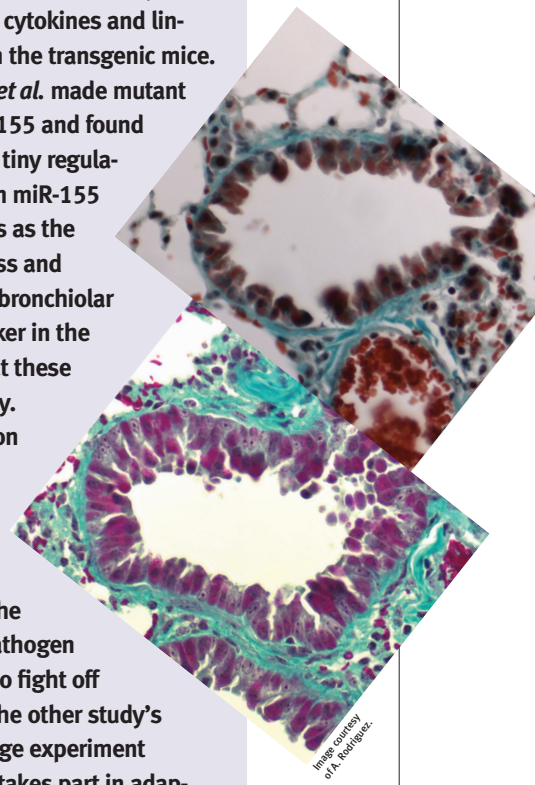


Image courtesy of A. Rodriguez.

Jurassic Park Protein

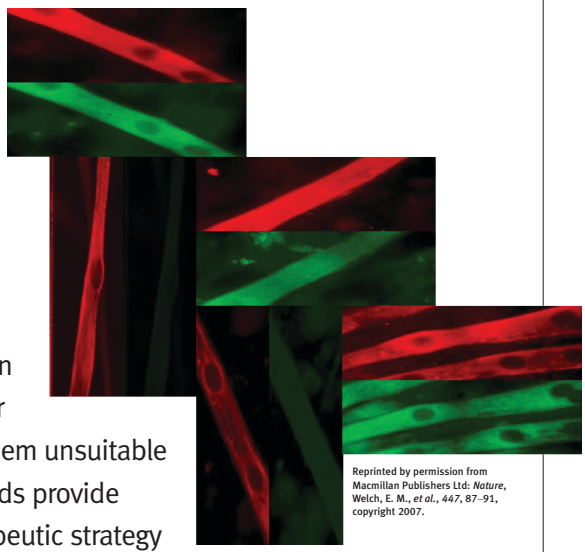
The lives and sudden extinction of the dinosaurs have long been the subject of literature, from children's book reports to doctoral dissertations. Unfortunately, much of the information about their existence died with them. Millions of years of decay left precious little material for analysis and reconstruction. Fossilized bones of the towering beasts adorn our museums and tantalize the minds of the curious, but any molecular information about these animals has remained elusive. Now, a pair of studies by Asara *et al.* and Schweitzer *et al.* (*Science* 2007, *316*, 277–280 and 280–285) use ultrasensitive modern techniques on a particularly well-preserved *Tyrannosaurus rex* unearthed in a Montana dinosaur graveyard. Four years ago, a bone from this dinosaur was broken during removal from its sandstone resting place. Inside the bone of the dinosaur, now known as Museum of the Rockies 1125, was a surprising discovery. The bones showed evidence of something rare in ancient samples, soft tissues. The two new studies use sophisticated methods to learn more about this tissue from the big beast that once wandered the earth.

Alan Male, Dorling Kindersley RF, Getty Images.

Schweitzer *et al.* took demineralized bones from the *T. rex* and compared the surface texture of the bone with that from a large living bird species, the emu. Using atomic force microscopy, the researchers observed a pattern with a repeat periodicity of ~70 nm in the bone, consistent with collagen, a triple-helical protein. To check whether some partially intact collagen was present in the sample, the researchers used antibodies raised against chicken collagen on thin sections of the dinosaur bone. Sensitive visualization methods revealed tiny speckles of antibody on the sections. This result was specific, because pre-blocking of the antibody with chicken collagen or pre-digestion of the bone with collagenase made the signal disappear. Using another powerful technique, time-of-flight secondary ion mass spectrometry (TOF-SIMS), the researchers assayed the amino acid composition of the bone. Again, collagen seemed to be the predominant player: ~33% of the detected amino acid was glycine and 10% alanine. These percentages are consistent with the collagen of all known living species. Finally, the authors postulate that iron species detected by TOF-SIMS may indicate a possible mechanism for the unique tissue preservation. They speculate that the metal ion microenvironment catalyzed free radical reactions to form inter- and intramolecular cross-links in the protein. This in turn stabilized the protein, leaving it immune to degradative reactions.

In a complementary study on the same dinosaur, Asara *et al.* used another ultrasensitive MS method capable of obtaining peptide sequence information from subpicomolar amounts of starting material. Detective work on proteins by MS fragmentation normally requires a complete genome of the organism to match up the peptide ions with plausible sequences. To get around the lack of ancient genomic information, the researchers used for comparison the protein sequences of collagens from birds, the nearest living relative to the dinosaurs, and also factored in adjacent branches on the tree of life. Computational tricks looked at many collagens at once and found which amino acids were always conserved and which ones could drift to form new polypeptides. This resulted in a reasonable list of peptide ions that might be found in a collagen sample from an organism where no genomic information is available. As a proof of principle, this method was applied to the ostrich, a modern bird whose genome has not been sequenced. After identifying many peptides from the ostrich collagen, the authors used synthetic peptides in parallel MS experiments to validate the experimental samples. They then took a step back in time by sampling bone remains from a 160,000–600,000-year-old mastodon, an extinct elephant-like creature. Impressively, the method identified peptides from more than a dozen different collagen family members. Finally, the group stepped back in time to that 68-million-year-old *T. rex*. A remarkable seven collagen peptides were determined in the dinosaur sample. They closely match the sequence of bird collagen, confirming the prediction that the feathery fliers are the closest living relative to dinosaurs. **Jason G. Underwood, Ph.D.**

No-Nonsense Drug



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Many inherited diseases are caused by nonsense mutations, or mutations within messenger RNA that change the original codon to a stop codon, causing premature termination of protein translation. For example, in the case of muscular dystrophy, a nonsense mutation in the dystrophin gene causes reduced levels of the dystrophin protein and leads to the muscle abnormalities that characterize the disease. Some aminoglycoside antibiotics are known to promote read-through of premature nonsense mutations, but their lack of potency and undesirable pharmacological properties make them unsuitable as treatments for these types of disorders. However, these compounds provide compelling evidence that small molecules may be an effective therapeutic strategy for treating such genetic disorders. Welch *et al.* (*Nature* 2007, *447*, 87–91) have started to make sense of nonsense mutations and now describe the therapeutic potential of the small molecule PTC124 for the treatment of diseases caused by nonsense mutations.

Screening of ~800,000 small molecules for the ability to suppress a nonsense mutation and subsequent chemical optimization of the lead scaffold led to the identification of PTC124, an orally available oxadiazole. Human cells from muscular dystrophy patients along with a mouse model of muscular dystrophy, called *mdx*, were used to test the efficacy of PTC124 in promoting read-through of the dystrophin gene. Upon treatment with PTC124, human dystrophic muscle cells exhibited increased levels of appropriately localized dystrophin, and *mdx* mice were protected from contraction-induced injury, the major functional deficit in dystrophic muscles. It is important to note that PTC124 is specific for suppressing premature termination and, thus, eliminates the likely adverse effects that read-through of normal termination codons would present. PTC124's unique mechanism of action holds much promise, not only for its use against muscular dystrophy, but also for the treatment of other genetic disorders caused by premature termination, such as cystic fibrosis and β-thalassemia. **Eva J. Gordon, Ph.D.**

UPCOMING CONFERENCES

Bioorganic Chemistry
June 10–15, 2007
Andover, NH

21st Annual Symposium of the Protein Society
Saturday, July 21–25, 2007
Boston, MA

Molecular Membrane Biology
Sunday, July 8–13, 2007
Andover, NH

2007 ACS Fall National Meeting
Sunday, August 19–23, 2007
Boston, MA

Physics and Chemistry of Microfluidics
Sunday, July 15–20, 2007
Waterville Valley, NH

Conference on Protein Synthesis and Translational Control
Wednesday, September 12–16, 2007
Heidelberg, Germany