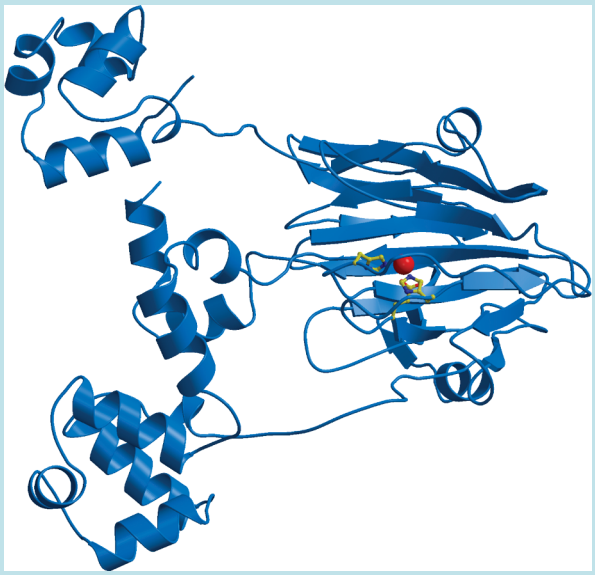
Spotlight

HEP to HMP Hype



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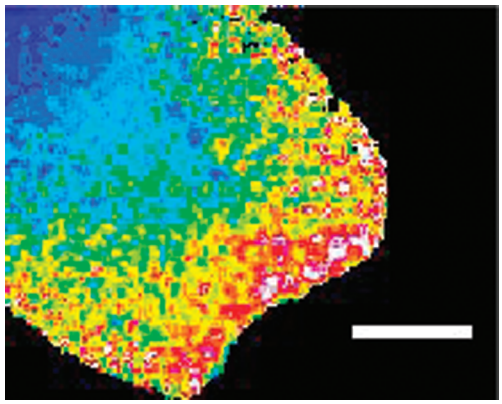
The natural product phosphinothricin tripeptide is a potent herbicide that contains an unusual functional group, a phosphinic acid. Investigations into the biosynthesis of this unique tripeptide have revealed a number of novel steps, including an unprecedented carbon bond cleavage. Specifically, the enzyme hydroxyethylphosphonate dioxygenase (HEPD) converts 2-hydroxyethylphosphonate (HEP) to hydroxymethylphosphonate (HMP), that is, it essentially extracts a single CH_2 group from the middle of the molecule. Cicchillo *et al.* (*Nature* 2009, *459*, 871−874) now report the mechanism of this reaction along with the structural characterization of the enzyme.

What makes this reaction unique is the oxidative cleavage of a carbon−carbon single bond that is lacking known activating groups such as aromatic, alkene, or1,2-dihydroxy functionalities. To determine the reaction mechanism, various isotope-labeled HEP derivatives were synthesized, and the reaction products were examined using ^{13}C and ^{31}P nuclear magnetic resonance spectroscopy and gas chromatography−mass spectroscopy. The data indicated that the carbon at position 2 is the target of oxidation, resulting in its excision from the molecule and conversion to formate. The structure of HEPD is composed of imperfect tandem repeats, each containing an α-helical domain linked to a β-barrel fold reminiscent of that in the cupin family of highly functionally diverse enzymes. Notably, HEPD does not require additional cofactors in order to carry out the oxidation, as do other members of the 2-His-1-carboxylate mononuclear non-haem iron family of enzymes. These insights into HEPD structure and function extend our understanding of the biosynthesis of this important compound and reveal novel chemistry achieved by this enzyme family. **Eva J. Gordon, Ph.D.**

H_2O_2 Healing

In response to a wound, white blood cells rush to the site of injury to begin the healing process. The molecules that orchestrate wound to leukocyte signaling are not definitively established, but the reactive oxygen species hydrogen peroxide (H_2O_2) is an intriguing candidate due to its ability to function as a signaling molecule, diffuse in tissues, and cross cell membranes. Using the zebrafish larval tail fin as a model system, Niethammer *et al.* (*Nature* 2009, *459*, 996−999) demonstrate that a rise in H_2O_2 concentration does occur in response to a wound, and the resulting H_2O_2 gradient is required for rapid leukocyte recruitment.

The zebrafish larval tail fin is an attractive model system for investigating wound healing, as leukocyte recruitment is easily imaged and the molecular dynamics of the tissue is readily manipulated. H_2O_2 concentration in the tail fin was monitored using HyPer, a genetically encoded H_2O_2 sensor that was introduced into the zebrafish embryos. After wound generation, the local concentration of H_2O_2 rapidly increased, peaking at about 20 min after the injury occurred and extending up to 200 μm away from the site of the



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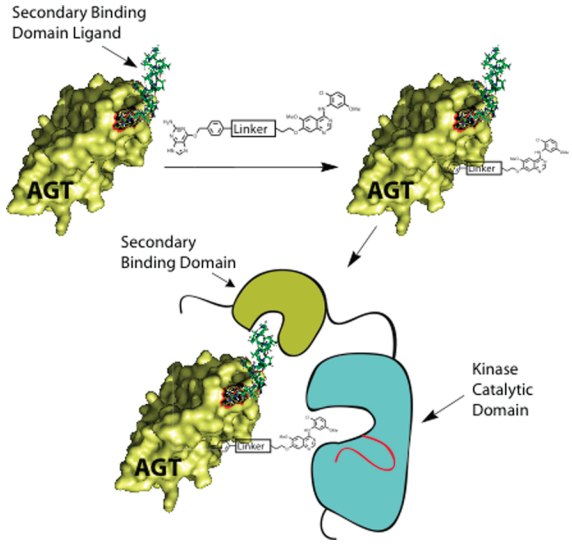
Published online July 17, 2009 • 10.1021/cb900153f CCC: $40.75

wound. Surprisingly, the source of the H_2O_2 was determined to be local epithelial cells, not the oxidative burst exhibited by activated leukocytes as might be expected. Moreover, a H_2O_2 concentration gradient was observed that extended from the wound toward the nearest blood vessel. The enzyme dual oxidase (Duox), which is expressed in tail-fin tissue, was identified as the enzyme responsible for H_2O_2 production. Furthermore, this enzyme was found to be required for leukocyte recruitment to the wound. The results suggest that H_2O_2, in addition to its activity as a reactive oxygen species, may serve as a chemotactic signal, or perhaps trigger the generation of a chemotactic signal, that directs leukocytes to the site of a wound. **Eva J. Gordon, Ph.D.**

Targeting Kinases, Two Sites at a Time

The thought of deciphering the functions of the hundreds of protein kinases that comprise the human kinome is mind-boggling to say the least. However, kinase function is intricately involved in cell signaling mechanisms, and understanding signaling pathways has critical implications in the search for drug targets for many diseases. The use of kinase inhibitors is integral toward decoding kinase function, but since most inhibitors target the highly conserved ATP binding site, designing compounds that are selective for specific kinases is notoriously difficult. Hill *et al.* (*J. Am. Chem. Soc.* 2009, *131*, 6686−6688) now present a chemical genetic approach for creating selective, bivalent kinase inhibitors.



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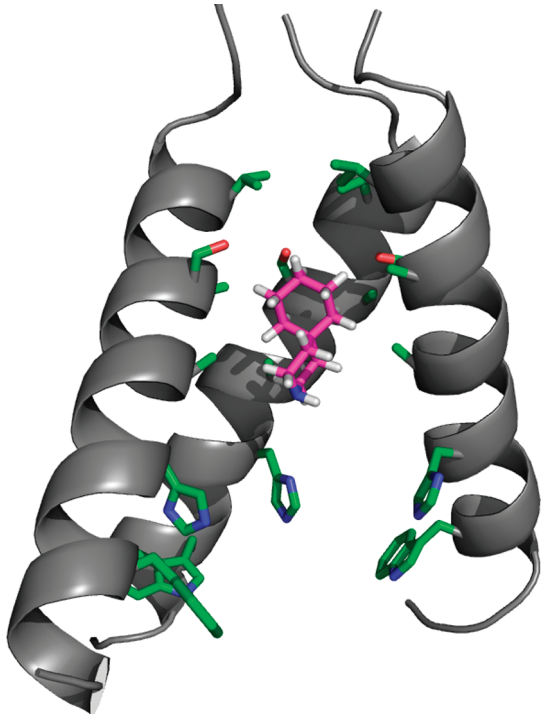
The approach relies on the increased affinity and selectivity that can be gained by targeting two binding sites on a given kinase, instead of just one. To this end, bivalent inhibitors were designed to contain a small molecule that targets the ATP site and a peptide containing apolyproline motif that targets the SH3 domain of either Src or Abl, two closely related tyrosine kinases. The small molecule and peptide components of the inhibitors were displayed on a protein scaffold by exploiting the reactivity of O^6-alkylguanine-DNA alkyltransferase (AGT), an enzyme that can transfer small molecules to its active site cysteine provided they are linked to its natural substrate O^6-benzylguanine (BG). Thus, fusion proteins containing AGT linked to specific polyproline motifs were expressed and reacted with BG-conjugated derivatives of the ATP-competitive inhibitor 4-anilinoquinazoline. The resulting protein-small molecule conjugates were tested against Src, Abl, and a series of other protein kinases and found to be potent inhibitors that were selective for their intended target. As most kinases are bisubstrate enzymes, this clever strategy can be extended to the design of selective, bivalent inhibitors for a large portion of the kinome. **Eva J. Gordon, Ph.D.**

Thwarting Influenza

The search for new, improved antiviral drugs to treat influenza remains a challenge in drug discovery. The H1N1 strain of influenza is now at pandemic levels and concerns linger about the deadly H5N1 strain that devastated poultry populations in 1997 and 2004−2005. Amantadine, a member of one of the two available classes of influenza drugs, targets the M2 channel of the influenza A virus. However, central nervous system side effects and the emergence of drug-resistant strains limit amantadine's use in the clinic. Now, Wang *et al.* (*J. Am. Chem. Soc.* 2009, *131*, 8066−8076) have reported a molecule in a new class of AM2 inhibitors that binds more potently and to a longer region of the transmembrane helix of the protein than amantadine.

The M2 proton channel facilitates the uncoating of viral proteins after the virus enters the host cells. The low pH within the endosome activates the channel, which leads protons to flood inside the virus to release its contents. Amantadine and related compounds block this acidification. A previous high-throughput screen had identified a new structure, a spirene guanidine analogue, as a novel M2 inhibitor.

In this study, researchers synthesized a library of compounds related to this novel inhibitor and tested them against AM2 channels expressed in *Xenopus* oocytes. The simple spiro-piperidine (3-azaspiro[5,5]undecane hydrochloride) bound most tightly to AM2 ($IC_{50} = 0.92 \pm 0.11$ μM), 45 times more tightly than amantadine. In docking studies, the spiro-piperidine bound to similar regions of the AM2 transmembrane region as amantadine. Solid-state NMR experiments showed that the spiro-piperidine interacted with the same residues in AM2 (L26, G34, A29, and I35) as amantadine. However, the spiro-piperidine affected a longer stretch of the helix and introduced greater conformational homogeneity into the protein

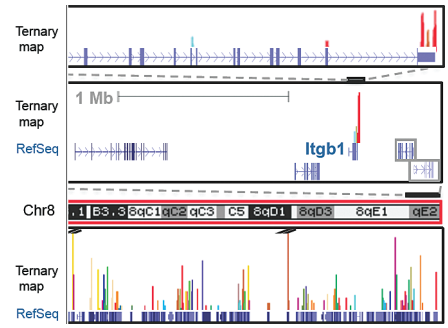


structure. This class of molecules represents a promising class for future study and could serve as a scaffold for inhibitors of mutant AM2 proteins. **Sarah A. Webb, Ph.D.**

News CLIP for miRNA

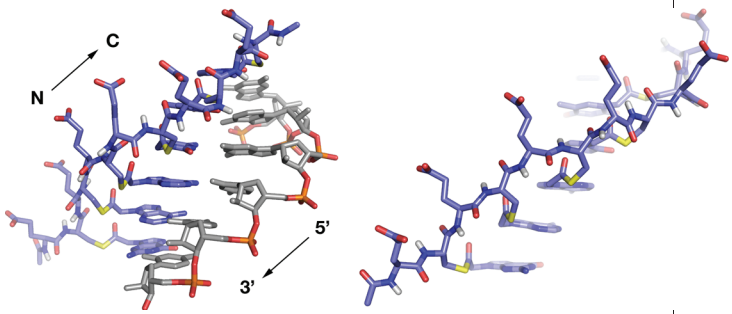
The recent discovery of hundreds of miRNAs uncovered tremendous potential for gene regulation, but validating which miRNAs tend to which mRNAs has remained difficult. Numerous computational methods search for miRNA targets *via* sequence complementarity and phylogenetic conservation of putative mRNA sites. For several years, a Venn diagram overlapping these predictions remained the state-of-the-art method for target prediction. Meanwhile, numerous laboratories worked on how to biochemically pursue legitimate interactions. Now, a new method (*Nature*, published online June 17, 2009, DOI: 10.1038/nature08170) paints the big picture of miRNAs and their mRNA targets within the mouse brain by taking advantage of Argonaute. This cellular protein resides at their intersection.

The group treated mouse brains with ultraviolet light to generate covalent cross-links between endogenous RNA-binding proteins and their targets. Then, after fragmenting the bound RNAs, an antibody specific for the Argonaute fished out the miRNAs that specifically interacted with this key player, and in the process, a surprising number of mRNAs were also found. This cross-linking-immunoprecipitation, or CLIP, followed by high-throughput sequencing unlocked an intimidating data set from which microRNAs could be matched to their mRNA target sites. The authors then went on to validate CLIP targets for one particular brain miRNA, miR-124, and found that many computational predictions were correct, but the CLIP set found additions and subtraction from their list. Analysis of the miR-124 targets uncovered enrichment for genes involved in neuronal differentiation and cytoskeletal dynamics. Clearly, the validation experiments just scratch the surface as to the potential of such an immense data set. This methodology points toward the new direction in miRNA research for those working on disease connections or in other model systems. Adding to the computational methods, physical methods such as CLIP can help define the strokes that miRNAs paint on the protein expression canvas within each cell. **Jason G. Underwood, Ph.D.**



Protein Shake for the RNA World

In a primordial soup, the first chemical reactions to pave the way toward biological systems must have involved information storage and replication. The ability of RNA to act as both information library and catalyst leads many to postulate that an RNA World might have bridged the gap between simple reactions and biological replicons. But with biological systems now relying heavily on proteins, were amino acids and simple peptides critical ingredients in the soup? A new study by Ura *et al.* (*Science*, published online June 11, 2009 DOI: 10.1126/science.1174577) experiments with this notion in an impressive hybrid approach.



Instead of a ribose sugar backbone, a peptide backbone was chosen with the reactive thiol of cysteine at alternating positions. Then, instead of nucleobase monomers that polymerize, thioester monomers of each nucleobase were synthesized to then react with the preexisting backbone to form peptide-nucleic acids. Homopolymers synthesized one nucleobase monomer that could bind to complementary RNA or DNA as shown by thermal denaturation. Also, two peptide-nucleic acids with complementary bases could pair with one another, a critical recognition characteristic that must have existed in early information systems. Finally, the authors used their new chemistry to ask an interesting *in vitro* evolution question. What would happen if the DNA or RNA was there first and then the peptide and monomers were added? The result demonstrated that the natural nucleic acid could act as an information template to direct the thioester reaction onto the peptide backbone, with the selectivity for the complementary nucleobase around 75−90%. Finally, the templated reaction could adapt to promote different thioester reactions if the template DNA sequence was changed during the time-course, an essential feature of selective genetic pressure. While this simple chemistry cannot approach the amazing precision and fidelity of the modern protein world, it does open up a new avenue of discovery for what reactions may have been cooking in a primordial soup. **Jason G. Underwood, Ph.D.**