ACS chemical biology

Spotlight

Plant Chemical Biology Comes of Age

Current knowledge of the molecular underpinnings of development in higher plants has progressed greatly from genetic analyses using *Arabidopsis*. However, studies on this model plant often provide no more than a bare glimpse of how diverse plant species develop. To complicate matters further, it is not always possible to glean useful spatial and temporal information from gene knockouts. With the tools of chemical biology, plant biologists can gain new insights into essential cellular processes. Small-molecule probes allow researchers to "fine tune" biomolecular interactions inside cells, often in a reversible manner. Now, Hayashi *et al.* (*Proc. Natl. Acad. Sci.* 2008, *105*, 5632–5637) describe a major breakthrough in plant chemical biology. The authors report the rational design of small molecules that function as either activators or inhibitors of a protein receptor of the plant hormone auxin.

The most abundant auxin, indole 3-acetic acid (IAA), is an endogenously produced plant hormone central to plant growth and development. This small molecule influences the expression of hundreds of genes. Two landmark studies in 2005 showed that auxin binds to a receptor protein, TIR1, which then mediates the proteolysis of specific transcriptional repressor proteins. Recent structural studies pointed to auxin as enhancing this process by providing additional hydrophobic interactions in a pocket of TIR1. The authors of the current study have generated activators and inhibitors of TIR1 by introducing different α-alkyl chains in the α-position of IAA. Further, they demonstrate that these small molecules are specific to TIR1. X-ray crystallographic analysis of TIR1 in complex with three of these small molecules demonstrates that all of these lie in the auxin-binding pocket of the protein. What is perhaps most striking is that the authors were then able to use one of the synthesized inhibitors to block typical auxin responses in two other species, namely, rice, *Orzya sativa*, and the moss *Physcomitrella patens*. This observation indicates that these small molecules should be useful in studying auxin responses in a wide variety of plants. **Anirban Mahapatra, Ph.D.**

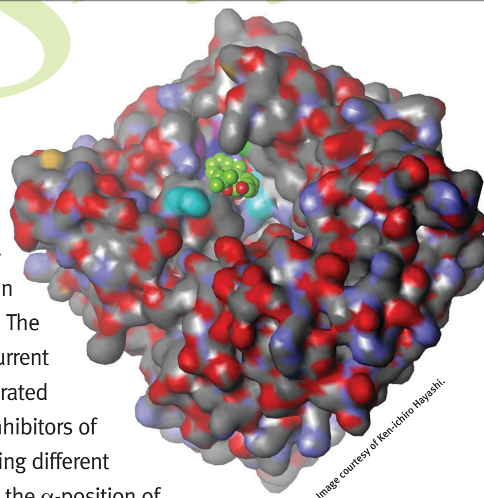
Image courtesy of Ken-ichiro Hayashi.

Visualizing Virions

Many approaches have contributed to our understanding of viruses, enabling specific characterizations of their attachment, entry, intracellular transport, and eventual commandeering of the cell. However, technical challenges have hampered our ability to watch the actual assembly of a viral particle, or virion, in real time. Now, Jouvenet *et al.* (*Nature*, published online May 25, 2008; DOI: 10.1038/nature06998) combine various fluorescence techniques to provide a new perspective of the biogenesis of individual HIV-1 virions in live cells.

The protein Gag is the main structural component of HIV-1, and its expression drives virion assembly. Expression of fluorescently tagged Gag molecules enabled monitoring of Gag from the initiation of the assembly process through fission from the plasma membrane. First, total-internal-reflection (TIR) fluorescent microscopy facilitated observation of Gag molecules at the plasma membrane, where virion assembly takes place, relative to those in the cytoplasm. These studies revealed that Gag molecules were detected in slowly appearing puncta that remained in the TIR field or as rapidly appearing and disappearing puncta that were associated with endosomes. Further experi-

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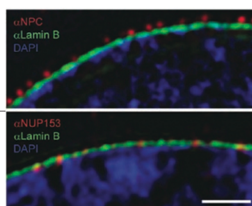
Spotlight

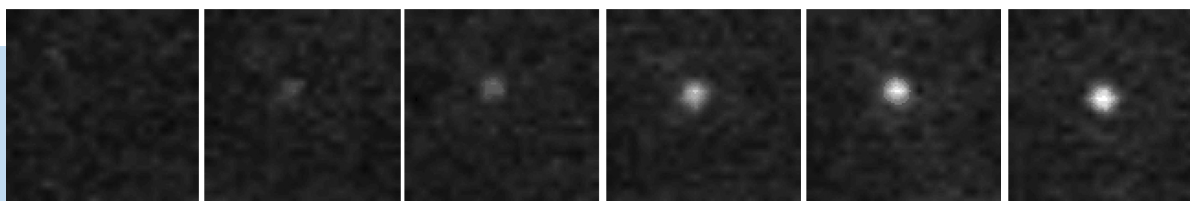


From Schermelleh, H., *et al.*, *Science*, June 6, 2008, DOI: 10.1126/science.1156947. Reprinted with permission from AAAS.

Beam Me Up, Three Times

Cell biologists have long sought to build the better mousetrap, or more appropriately, the better light trap, to take full advantage of the litany of fluorescence light microscopy techniques, but with higher resolution. Imaging fine subcellular structures with special tags usually yields beautiful multicolor pictures, but physics dictates a practical limitation. Light can only be focused on the sample in a disk shape that is approximately half of the wavelength of the incident light. This means that even the shortest wavelength of visible light can only resolve objects that are 200 nm apart. This makes fine spatial assignments difficult within a cell. Taking on this problem, Schermelleh *et al.* (*Science* 2008, *320*, 1332–1336) use a novel trick with the incident light to produce far more data, and then they let a computer process the final image.

The trick, termed 3D structured illumination microscopy, beamed the sample with three different incident light waves. These waves were focused so as to interfere with one another and create what is termed a moiré pattern. These patterns, usually a bane to digital image wranglers, are of particular interest to the software on the other end of the custom microscope. If something in the cell, such as a fluorescently labeled protein, stands in the way of the three waves, the pattern is disrupted, and the software pinpoints the culprit. The study used this technique to perform multicolor imaging of nuclei from a mouse myoblast cell line, and it yielded amazing pictures with a resolution that easily doubles that of standard techniques. For example, antibody stains against components of the nuclear pore complex could easily distinguish proteins that lie on the cytoplasmic face from those within the basket that resides inside the nuclear lamina. Also, the authors could actually visualize invaginations in the nuclear envelope as it disappears during mitotic spindle formation, a key phenomenon in the cell cycle that has largely eluded the camera's lens. This microscopy technique, combined with the rainbow of fluorescent molecules now available, will be of great assistance to an array of scientists. Those just seeking the cellular zip code for their favorite protein and those that study the dynamics of the cell's workings will both enjoy this evolution of resolution. **Jason G. Underwood, Ph.D.**



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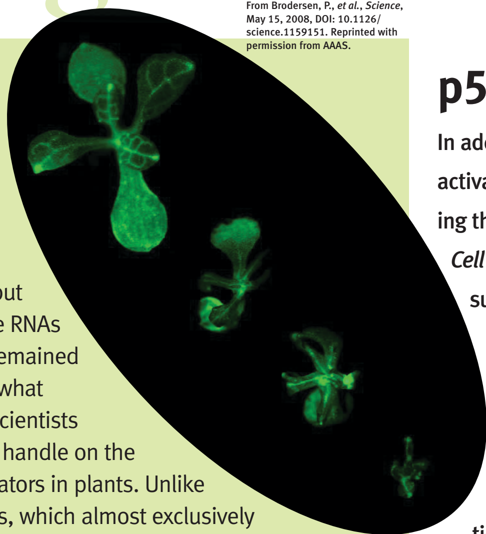
ments focused on the slowly appearing puncta, because those likely contained molecules in the process of virion assembly. FRET experiments indicated that during their emergence at the plasma membrane Gag molecules come into closer proximity with each other, as would be expected for molecules participating in the viral assembly process. In addition, experiments using fluorescence recovery after photobleaching supported the notion that the stable fluorescence signal achieved by the slowly appearing puncta is a result of a completed viral particle assembly whose Gag molecules are segregated from the cytoplasm. Finally, use of a pH-sensitive fluorescent tag supported the notion that the particle is not able to exchange any molecules with the cytoplasm after it progresses through the final step of fission from the cell membrane. The clever integration of these approaches enabled a unique view into HIV virion assembly not accessible through traditional techniques. **Eva J. Gordon, Ph.D.**



From Brodersen, P., *et al.*, *Science*, May 15, 2008, DOI: 10.1126/science.1159151. Reprinted with permission from AAAS.

Plants Get Repressed, Too

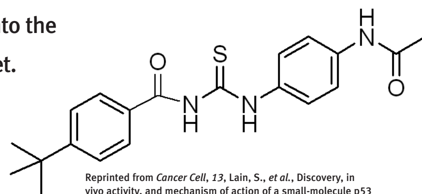
In the past 8 years, small interfering RNAs (siRNAs) and microRNAs (miRNAs) have emerged as key regulators of thousands of cellular events, but the mechanisms for how these RNAs function in animal cells have remained highly controversial and somewhat inconclusive. In comparison, scientists thought that they had a better handle on the mechanism of these tiny regulators in plants. Unlike their eukaryotic animal cousins, which almost exclusively show imperfect matches between small RNAs and mRNAs, the plants often display perfect or near-perfect base pairing between miRNAs and coding mRNAs. These perfect matches are thought to lead to cleavage of the mRNA, similar to the siRNA "knockdown" method that is used by many molecular biologists working with tissue culture cells. Now, a new study indicates that even the plants can be shifty when it comes to their mechanism of RNA interference.

Using a fluorescent reporter linked to a natural miRNA binding site with perfect complementarity, Brodersen *et al.* (*Science* 2008, *320*, 1185–1190) began a genetic screen for *Arabidopsis* mutants defective in the miRNA pathway. A wild-type plant would turn down the GFP by miRNA-mediated slicing of the mRNA, but a mutant would make the plant's leaves glow green. Several mutants were isolated and linked to known miRNA pathway genes, but a number of new miRNA action deficient (*mad*) genes also emerged. In the course of characterizing the *mad* mutants for their GFP mRNA and protein content, the authors noticed that several of the mutants displayed low GFP mRNA like the parent strain but showed high amounts of the protein. This meant that the RNA stability had not changed remarkably, but rather that the translational efficiency was up in these mutants. But was this just an artifact of the GFP transgene? They went on to test the mutants for many flavors of endogenous mRNAs with miRNA-binding sites in all possible positions in the transcript and with various degrees of complementarity, two features considered to play major roles in determining the outcome of a miRNA: target interaction in plants and animals. In all cases, the mRNAs showed just slightly lower accumulation in the mutants, but the protein was significantly higher. This indicates that plants do more than just slice their miRNA targets; rather, just like animals, they utilize translational repression as a widespread means to regulate genes. The authors suggest that this generalized phenomenon justifies a look back at previous results in plants and a re-evaluation of what genes may be targets for these fascinating, but crafty, small RNAs. **Jason G. Underwood, Ph.D.**

p53 Activators Take Action

In addition to being potential anticancer agents, small-molecule activators of the tumor suppressor p53 are valuable tools for probing the molecular mechanisms involved in cancer. Lain *et al.* (*Cancer Cell* 2008, *13*, 454–463) describe an effective approach for finding such small molecules and characterize the activity of a promising hit discovered in their screen for p53 activators.

The sequential, seven-step, forward chemical genetic approach involves the use of a high-throughput cell-based screen, various secondary assays to validate and examine hits, structure–activity studies for compound optimization, *in vivo* testing, genetic methods for target identification, biochemical assays for target verification, and finally, validation of the mechanism of action in cells. The high-throughput screen was designed to detect molecules that cause an increase in expression of a reporter construct under the control of a p53-dependent promoter. A compound referred to as tenovin-1 was identified from 30,000 compounds screened as capable of increasing p53 levels in mammalian cells. Various secondary assays validated tenovin-1 activity and suggested that it targets a factor upstream of p53. In order to effectively evaluate the compound *in vivo*, the authors created a more water-soluble derivative, tenovin-6. Indeed, tenovin-6 exhibited impressive antitumor activity in mice, prompting investigation into the identity of its cellular target. Exploiting yeast genetics and the knowledge that heterozygous strains are often hypersensitive to compounds that target the affected gene, they discovered that partial deletion of the *SIR2* gene, which encodes a NAD^+-dependent deacetylase, conferred high hypersensitivity to tenovin-6. Consistent with this finding, tenovin-6 was subsequently found to inhibit the deacetylase activity of SirT1 and SirT2 in mammalian cells. This effective drug discovery approach led to the successful identification of a promising anticancer agent, and importantly, outlines a target identification process to facilitate further development of similar compounds as therapeutic agents and biological tools. **Eva J. Gordon, Ph.D.**



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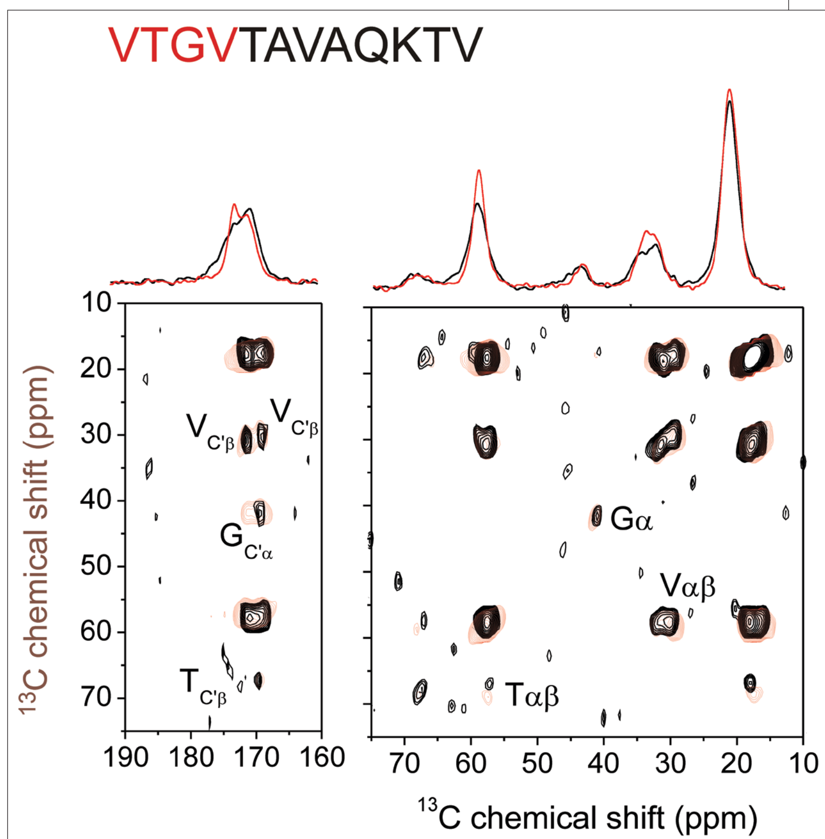
A Solid Strategy

Molecules that can prevent protein aggregation have therapeutic potential against a variety of neurodegenerative diseases. For example, compounds that inhibit aggregation of the protein α-synuclein, a key factor in the pathogenesis of Parkinson's disease, could lead to treatments for this devastating condition. Madine *et al.* (*J. Am. Chem. Soc.*, 2008, *130*, 7873–7881) combine solid-state NMR (ssNMR) and biochemical studies to identify key residues in α-synuclein-derived peptides that promote aggregation, guiding the generation of promising new α-synuclein aggregation inhibitors.



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Residues 71–82 of α-synuclein were selected as a starting point for the design of aggregation inhibitors because this peptide self-aggregates to form amyloid fibrils similar in morphology to those formed by the full length protein. ssNMR offers a simple, straightforward approach to probe fibril structure and was used to examine the structure of the peptide before and after aggregation. Prior to aggregation, the peptide existed as a random coil. However, after aggregation, the C-terminal resides adopted a highly ordered β-strand conformation, characteristic of those in amyloid fibrils, while the N-terminal residues remained in a disordered state. Dynamic light scattering experiments next revealed that a peptide consisting of just the six C-terminal residues aggregated in a similar fashion to the full-length peptide. N-Methylated analogs of this peptide were synthesized to explore whether the presence of the methyl group could disrupt the aggregation process. Indeed, methylation of either a valine or an alanine resulted in peptides with reduced aggregation potential. Importantly, dynamic light scattering, electron microcopy, and diagnostic dye analysis demonstrated that the peptide containing the N-methylated valine also inhibited aggregation of full-length α-synuclein. These studies provide a truly "solid" strategy for guiding the design of α-synuclein aggregation inhibitors and could enhance and accelerate the design of inhibitors for other aggregation-prone proteins as well. **Eva J. Gordon, Ph.D.**

The Path Less Traveled

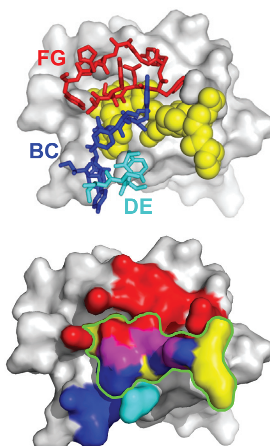
The pathogenicity of several protein misfolding disorders, including Parkinson's and Alzheimer's diseases, stems from the tendency of certain polypeptides to form toxic, β-sheet-rich polypeptide aggregates called amyloid fibrils. The molecular mechanisms behind the generation of these aggregates have remained elusive, but it is known that the presence of certain protein chaperones or small molecules can prevent fibril formation. Ehrnhoefer *et al*. (*Nat. Struct. Mol. Biol*., published online May 30, 2008; DOI: 10.1038/nsmb.1437) now explore how the polyphenol (–)-epigallocatechin gallate (EGCG) redirects the amyloidogenic polypeptides α-synuclein (αS) and amyloid-β (Aβ) onto a less traveled, or at least less hazardous, path leading to nontoxic, spherical oligomers.

An impressive combination of biochemical, biophysical, and cell biological methods was used to probe the mechanism behind EGCG activity. Using electron microscopy, size-exclusion chromatography, and a thioflavin T binding assay that enables quantification of fibril formation, the authors first demonstrated that the presence of EGCG reduced formation of αS fibrils and promoted the generation of highly stable, compact, spherical oligomers. Next, a nitroblue tetrazolium staining assay and NMR studies established that EGCG binds directly to the backbone of natively unfolded αS polypeptides, and circular dichroism studies provided evidence that the presence of EGCG prevents the transition of αS from a random coil to a β-sheet-rich structure. The thioflavin T binding assay was further used to demonstrate that EGCG-generated oligomers do not possess seeding capabilities and thus are unable to promote aggregation of soluble polypeptides. Finally, cellular toxicity studies revealed that EGCG-generated oligomers are less toxic than αS β-sheet-rich fibrils. Parallel experiments with Aβ demonstrated that EGCG also prevents Aβ amyloidogenesis, suggesting that EGCG acts as a chemical chaperone that can thwart aggregation of otherwise aggregation-prone polypeptide chains. **Eva J. Gordon, Ph.D.**

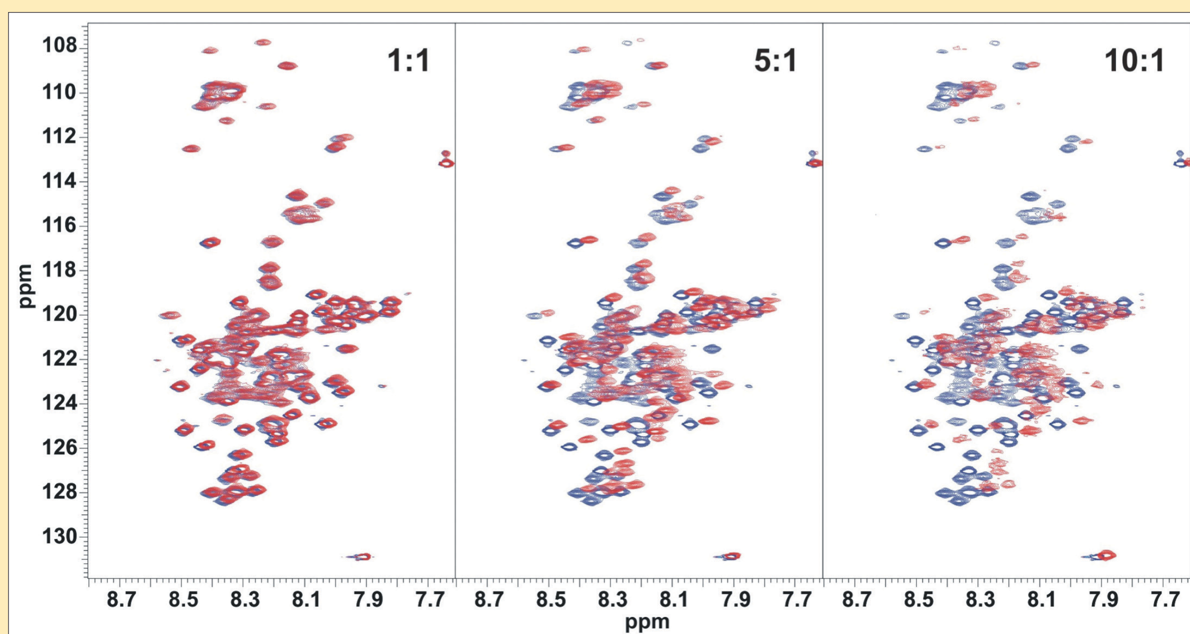
Leaping to New Protein Function

Directed evolution is a protein-engineering technique that mimics processes used in the natural evolution of proteins, such as point mutations and gene duplication, to create new functions. Evolutionarily, leaps in protein function are thought to have arisen from gene recombination events in which distinct protein domains came together to generate new active sites. Active sites are typically located at domain interfaces, but directed evolution has yet to be used to create new active sites by combining two evolutionarily unrelated domains. Now, Huang *et al*. (*Proc. Natl. Acad. Sci.* 2008, *105*, 6578–6583) describe use of a new technique called "directed domain interface evolution" to create a novel class of engineered proteins with distinct functions.

The researchers set out to generate what they termed "affinity clamps", or protein-based affinity reagents for short peptide motifs and predefined epitopes. To this end, a PDZ domain, which is a small globular domain that binds with low affinity to C-terminal sequences of target proteins, was joined with a specific domain from human fibronectin. The fibronectin domain was chosen because the group had already established that its three surface loops can be diversified to create novel binding surfaces. Based on this new two-domain protein, a combinatorial phage display library was created in which the three surface loops in the fibronectin domain were diversified, and the library was screened for binding to an eight-residue peptide derived from a known PDZ ligand. Two clones exhibiting high affinity for the peptide were selected for further characterization with surface plasmon resonance, NMR, and X-ray crystallography. Remarkably, the peptide bound to the optimized two-domain protein with substantially higher affinity and specificity than the PDZ domain alone, reminiscent of an antibody–antigen interaction. In addition, structural studies indicated that the complex adopted a clamshell architecture with a large binding interface in which the peptide resided. Finally, it was demonstrated that, like antibodies, the affinity clamps could be used as binding reagents in Western blotting and immunoprecipitation experiments. Moreover, they are stable, easy to produce and store, and can easily be modified with any number of tags for further applications, an indication of their value as innovative antibody alternatives. **Eva J. Gordon, Ph.D.**



Huang, J., *et al.*, *Proc. Natl. Acad. Sci., U.S.A.*, *105*, 6578–6583. Copyright 2008 National Academy of Sciences, U.S.A.



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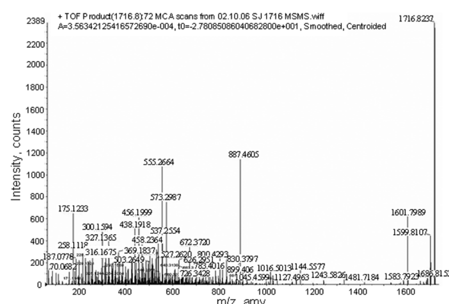
At the Heart of Marijuana Use

An astounding 4.3% of young adults report daily use of the illicit drug marijuana, known for its pleasurable psychoactive effects. However, marijuana use is also associated with various cognitive dysfunctions and increased risk of cerebral and cardiovascular disorders.



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To explore the molecular basis for these adverse physiological effects, Jayanthi *et al.* (*Mol. Psychiatry*, published online May 13, 2008; DOI: 10.1038/mp.2008.50) use a proteomic approach to search for proteins in the serum that may be affected by marijuana use.

The surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) ProteinChip facilitates analysis of overall protein expression in the plasma. The ProteinChip is composed of a chromatographic phase grafted to bind different serum proteins, depending on their biochemical properties. Proteins that bind to the surface are subsequently identified and characterized using a SELDI-TOF mass spectrometer. It was discovered that, compared with control groups, marijuana users exhibited significantly increased levels of a 9.4 kDa protein. Mass spectrometry and immunoaffinity capture experiments were used to determine that the protein was apolipoprotein C-III, which has been linked to cardiovascular disorders through its regulatory role in triglyceride metabolism. Coupled with other recent reports, this finding suggests that chronic marijuana use may lead to impaired cellular energetics and mitochondrial function, which in turn affect both the cardiovascular and cerebrovascular systems. The precise mechanism by which marijuana use causes these adverse effects, however, remains elusive. Notably, recent evidence implicating cannabinoid receptors, which bind to the active ingredient in marijuana, in signaling pathways involved in lipid parameters suggests that marijuana-induced activation of these receptors in the periphery is involved. Continued efforts to get to the heart of marijuana's biological activities could indeed lead to methods to prevent cardiovascular disease associated with marijuana use. **Eva J. Gordon, Ph.D.**