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PROFILE

Joshua Coon: Retooling Chemical Biology

Profiles provide insights into the lives, backgrounds, career paths, and futures of scientists who serve as Experts on *ACS Chemical Biology*'s online Ask the Expert feature. Coon will begin answering your questions in mid-May, 2008. Readers are encouraged to submit their questions to the Experts at www.acschemicalbiology.org. The editors will post the most interesting exchanges on the web site.

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To make groundbreaking discoveries in any scientific field, researchers need more than just keen observational skills; they need a suite of powerful tools. Among the standard tools kept in many laboratories is the mass spectrometer (MS), a machine that is been cast in various forms since the early 1900s to suit researchers' changing needs. Joshua Coon, assistant professor of chemistry and biomolecular chemistry at the University of Wisconsin—Madison, has made the venerable MS the focus of his burgeoning career. Coon's laboratory centers its efforts on solving problems in proteomics, juggling both basic and applied projects. He and his colleagues have reinvented tandem MS into an incarnation especially useful for breaking proteins into manageable peptide segments while preserving post-translational modifications, which could provide useful functional clues. Along with collaborators at the University of Wisconsin, an institution renowned for its work in stem cell biology, Coon is using this new tool to identify and quantify the array of proteomic changes that take place in stem cells as they progress along the path to differentiation. By retooling chemical biology, Coon's work is opening the door to any number of exciting future discoveries.

From Hobby to Career. Coon was born in 1976 in Michigan, the only child of his father, a high school woodshop teacher, and his mother, a secretary. Rather than steer their child toward a particular hobby or career path, Coon's parents encouraged his many interests, especially his interest in building. As a child, he enjoyed constructing model airplanes, later taking his hobby into bigger structures, including wooden fishing boats.

He remembers having a competitive streak from an early age. "I didn't know what I wanted to be other than good at what I did," says Coon. He recalls going to a fishing shop with his father and talking with the proprietor about a special type of boat necessary to fly fish on a nearby river. Coon and his father chatted about building the distinctive boat, but the proprietor told them that it would be impossible for amateurs like them to construct. Taking it as a challenge, Coon and his father built three of the boats back to back. "If someone said that you can't do something, that it's too hard, my father helped me think that you can do anything you want to do," he says.

A scheduling mishap introduced him to higher-level chemistry in high school. Before the advent of advanced-placement classes, Coon received special permission from his school to take math at the local college about 20 miles away. However, when he went to register, the math class he was qualified to take was full. However, a chemistry class still had some openings. Coon began attending the class, and after a year, he recalls, he was "hooked". When the time came to choose a college, he chose another local school—Central Michigan University in Mount Pleasant, about 30 miles from his home—and selected chemistry as his major.

Because the school was so close by, Coon lived at home and commuted a half-hour to college, staying on campus to read and study between classes. "Since I was an atypical student, I learned to treat college like a job," he says. Every day, Coon recalls, he showed up at school around 7:00 a.m. and left at about 6 p.m. "I keep the same schedule now—it's the same schedule that I've had since I was a freshman in undergrad," says Coon.

Midway through college, Coon got a campus job running the chemistry department's MS. Students and faculty dropped off

samples of molecules they synthesized, and he made measurements and sent them results. Coon's job was purely analytical, he recalls—he did not alter the machine—yet, he became more and more interested in how he might improve MS and develop new MS technology. "It played into the sorts of things I liked to do for a hobby," he says. "The idea of building a new machine tapped into the idea of working with my hands. I was really excited about the prospect of doing this for a career."

Coon had also become interested in teaching after admiring the work of his Central Michigan professors. To go into teaching and research at a competitive school, he knew that he needed to select a graduate school carefully. By the time he completed his undergraduate degree in 1998, Coon had been accepted into a doctoral program at the University of Florida in Gainesville. Besides having a top-rated analytical chemistry program, Coon says, the school had the added advantage of being in a warm place. "I was tired of the cold weather, which made Florida even more attractive," he quips.

Big Gamble Pays Off. Early in graduate school, Coon says he became intrigued with the idea of working on uncovering the vast amount of information locked in the human proteome, the natural extension of the human genome project. "MS was the device that seemed best for doing the sequencing. This field was wide open for technology development," Coon recalls.

He eventually began working with William Harrison, an analytical chemist who did elemental MS, on a project with a biological bent. Coon's work focused on taking a sample, such as a peptide or protein, then using a laser to put the sample in the gas phase in front of the machine's inlet. An ion source nearby the sample generated ions that reacted with the substance of interest, giving it a charge. Coon then used the MS to measure the sample's characteristics.

Working with Harrison, Coon says, he published four papers on this new technique within a year and a half (*1*–*4*). Though it has never been widely adopted, the work served as an important proof of principle that such a technique was feasible for measuring biological samples.

Harrison encouraged Coon's interest in an academic career, urging him to aim for the most ambitious appointment he could achieve. "His advice was to start as high as you could get—it's always easier to go down than up," recalls Coon. Knowing that he'd need a unique postdoctoral fellowship to stay competitive, Coon searched for laboratories developing innovative ways to use MS for biological applications. He found the perfect fit with Donald Hunt, a leader in biological MS at the University of Virginia in Charlottesville.

At the time, Hunt was working with an unconventional graduate student, John Syka, an engineer who had decided to get his doctorate after spending decades in a career at Thermo Fisher Scientific. Prior to Coon's arrival, Hunt and Syka had already developed an idea for sequencing peptides by reacting peptide cations with anions in an MS, causing them to fall apart in a way that preserved the proteins' post-translational modifications. Other more typical techniques for breaking up peptides for MS analysis stripped these modifications from proteins, removing potentially useful information about how proteins are regulated inside a cell.

He points out that the idea of reacting anions with cations on peptides was not novel; researchers had been trying to do similar experiments for the previous decade, but they hadn't been successful. Rather than successfully fragmenting peptides, the added anions instead abstracted hydrogen from the cations, leaving the peptides intact. "The literature said that the reaction we wanted to happen wouldn't work," Coon recalls (*5*). However, he and his colleagues were not deterred by the challenge. "We thought that if we chose the anion appropriately and treated it gently, we might get it to work. It was a big gamble."

Coon and Syka spent the next 10 months modifying an existing MS machine to try their experiment, then testing various classes of small-molecule anions for the best candidate for their experiment. Coon recalls that he and Syka tested the modified machine and their best candidate molecule, a polyaromatic hydrocarbon, on a Saturday. Their first impression was that the experiment did not work. "We were down as low as you can get," Coon says. However, after averaging the series of scans the following Monday morning, the researchers found hints of evidence that their experiment was successful after all.

Coon and his colleagues spent the next several months optimizing the chemical reaction and characterizing their new technique, which they named electron transfer dissociation (ETD). Two months after publishing his first papers on this topic (*6*–*8*), Coon began applying for jobs. Within months, he was offered a position at the University of Wisconsin—Madison.

Tooling Up as a Team. Coon says that school offered him an ideal combination of characteristics to match his goals: running a laboratory that would have a biology focus but still doing instrumentation and methods development. He notes that Wisconsin—Madison was unusual for having both top-rated biology and analytical chemistry programs. In additional, Coon says, he was impressed by the extremely collegial and collaborative atmosphere at the university, which he'd need to rely on to further his biological work. "All my knowledge of biology is self-taught. I can talk the talk a little bit, but to do good science, I rely on good collaborations," he says.

These attributes sealed his decision; he accepted a position in the school's department of chemistry and began setting up his laboratory in the fall of 2005.

The past two-and-a-half-years have been "a lot of fun," Coon says. During that time,

he has recruited eight graduate students, a postdoctoral fellow, and a laboratory manager, who also happens to be his wife. The time has also been a productive one for Coon and his new colleagues. "Things have moved along pretty fast, and I think it's because of the high-quality graduate students here at Wisconsin," he says. "Everything we've published has really been on the shoulders of the first- and second-year students."

On the basic side, Coon is maintaining his long-term objective of creating cutting-edge instruments to eventually sequence the entire human proteome. Last summer, he and his colleagues published a paper detailing an extension to the ETD technology he'd worked on with Hunt and Syka (*9*). The new work involved modifying an Orbitrap MS machine to enable ETD chemistry, giving measurements with better accuracy and resolving power. He and his co-workers licensed the new technology to Thermo Fisher, which plans to sell Orbitrap machines modified for ETD this summer.

In more recent work, the researchers have further optimized the Orbitrap, giving it a physical source of ions on the back of the machine. They plan to publish on this new technology soon.

In his laboratory's applied projects, Coon is particularly proud of his collaboration on stem cell research with Wisconsin scientist James Thomson, a pioneer in isolating and culturing both nonhuman primate and human embryonic stem cells. Early in Coon's career at Wisconsin, Thomson approached him about using ETD to help decipher the factors that steer these undifferentiated "blank slate" cells into blood, skin, liver, and other committed cell lineages.

The pair and their colleagues have since published a paper detailing the use of Coon's advanced MS technology to identify and quantify various post-translational modifications to histones, the chemical spools that cell nuclei engage to package long strands of DNA (*10*).

The tails of histones can have several possible modifications, which serve to influence which genes on nearby DNA are activated and which are silenced. Previously, researchers have probed for these modifications with antibodies, which can readily locate individual modifications on histone tails. However, notes Coon, it is difficult to employ antibodies to identify combinations of modifications, an important clue for discovering how these modifications change gene activity. "Each modification is like a word in a sentence," explains Coon. "If you know all of the modifications, you can read the sentence. But if you just know individual words, you're missing the context."

In their paper, Coon, Thomson, and their colleagues identify 74 patterns of modifications present in stem cells. Strikingly, Coon notes, proteomic analysis showed that these modification patterns shift in characteristic ways as cells move from an undifferentiated state toward committed lineages. He and Thomson plan to explore and extend this finding in future research.

Though these two areas of research consume most of Coon's time at the moment, he hopes eventually to tackle a host of other biochemical questions. For example, MS can currently detect typical proteins in minute amounts, but the technology often cannot detect proteins that are considered aberrant, for example, those that are the results of alternative splicing or SNPs. Coon plans to develop new technology aimed specifically at detecting these aberrant proteins as well as developing new bioinformatics tools to identify and characterize what researchers will find.

Though he's expecting plenty of challenges in his future research, Coon predicts that he and his laboratory will have lots of fun along the way. "There's not a single part about this job that I don't like," he says.

—Christen Brownlee, Science Writer

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