ACS chemical biology

Douglas Weibel: Using Microfluidics for Microbiology

The ubiquity of microorganisms is unparalleled in any other known organism. These creatures surround our outsides and colonize our insides, a fact that has been known for centuries. However, despite their prevalence and long study, many of their characteristics still remain largely unexplained, including how proteins organize within microbial cells and how microbes interact with each other and with their environments. Many of the techniques used to study microorganisms are nearly as old as the knowledge of microorganisms themselves. Seeking new ways to look at microbiology, Douglas Weibel, Ph.D., an assistant professor of biochemistry at the University of Wisconsin-Madison, turned to chemistry. He and his colleagues are using novel microfluidic methods to develop new ways to culture bacteria and small molecules to control the function of proteins *in vivo*. By combining chemistry with microbiology, Weibel and his team hope to shine new light on this old field.

Chemistry in the Family. Weibel was born in 1971 in Philadelphia to a father who is a chemist and a mother who raised Weibel, his older brother, and two younger sisters. "We actually grew up in the lab," he says, remembering years of spending time with his siblings in his father's workplaces, first as a professor of biochemistry at the University of Pennsylvania and later directing research in food chemistry and biochemistry for Watson, Inc., a food company based in West Haven, Connecticut.

At the age of 7, Weibel and his family moved to Connecticut to accommodate his father's new job at Novo Nordisk. He remembers a childhood full of fun, nonacademic pursuits, including spending plenty of time outdoors, fishing, skateboarding, and snowboarding. His parents encouraged him and his siblings to find their own way, supporting them in any pursuits they found interesting. By the time Weibel was a teenager, he spent more time in the lab helping out his father, working on various projects for several chemistry-related companies his father had founded. Weibel notes that his father's entrepreneurship has probably played a part in his own path through life. "I think I have the same entrepreneur spirit running deep in my veins, even though I don't have the desire to start any companies myself," he says. "Maybe it's lowered the threshold for trying new things or jumping across fields."

Image courtesy of Douglas Weibel

By the time he graduated from high school, Weibel knew that he'd eventually go to college, but he did not yet have a course of study in mind. Though he had plenty of experience in chemistry at that point, Weibel was not initially interested in pursuing chemistry himself. Remembering many enjoyable summers spent at his grandparents' farm in Iowa, he decided to work for a year on a farm in southern Indiana owned by a couple that he became close friends with. Though the 75-acre farm was a busy, vibrant place during the growing season, hosting a program for disabled adults, Weibel recalls a sense of extreme isolation during the rest of the year.

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"For many months, I was the only person there—it was too solitary," he says.

Seeking direction, Weibel headed back to Connecticut and worked for the next two years doing food biochemistry research at Watson with his father. During that time, "a switch was flipped," Weibel says. Though he had done similar work as a teenager, Weibel explains that he was never curious or particularly interested in chemistry before. But during his time at Watson, "I started getting really into it. Something changed over those years, and I realized I really wanted to be a scientist," he says.

With a clearer course in mind, Weibel accompanied his brother on a trip back home to the University of Utah, where his brother attended graduate school in chemistry. "I just fell in love with it," he says. Attracted to the natural beauty of the Salt Lake City area and the ample opportunities to run, bike, snowboard, and simply enjoy the outdoors, he decided to apply to the University of Utah. Soon after, Weibel received his acceptance and started classes the following fall.

Needing to declare a major early on, Weibel decided on chemistry. He found an undergraduate research position in a lab right away, working with enzymologist and biochemist C. Dale Poulter. Weibel continued to work with Poulter during the entirety of his undergraduate years, concentrating on two different projects. The first involved cloning the gene for farnesyl disphosphate synthetase, a protein essential for isoprene biosynthesis, from an archaebacterium known for its ability to survive extreme environments. Poulter, Weibel, and their colleagues hoped that understanding the role this gene played in constructing the archaebacterium's membrane might eventually shed light on the organism's hardy nature.

For the second project, Weibel applied the skills he learned in a graduate-level synthesis class he took during his second year at Utah. "I had to get permission to take it, and I think the professor who granted it was hesitant at first," he says. Nevertheless, Weibel "loved it" and was able to use what he learned to assist Poulter and his colleagues in synthesizing competitive inhibitors of the protein RAS farnesyltransferase, an enzyme important for distributing lipids in cell membranes.

Weibel shortened his stay at Utah by attended college year-round, spending his summers in classes and performing research in Poulter's lab. In his third year, as he neared graduation, he applied and was accepted for graduate school at Cornell University in Ithaca, New York. It was the third school he visited, but his favorite right away. Weibel recalls that after he saw the campus and met with potential advisors, he felt so secure that Cornell was the right choice that he sent in his acceptance letter and canceled the rest of his scheduled trips to other schools.

However, rather than attend Cornell immediately following graduation from Utah, Weibel deferred for a year to follow another opportunity. During the course of his years at Utah, Weibel had befriended synthetic inorganic chemist Goji Kodama, a Japanese-American professor who kept the same hours as Weibel and his labmates, often working through the night on his own research. At the time, Weibel was taking Japanese to fulfill his language requirement and often practiced speaking with Kodama at early morning breakfasts after a long stint at the lab. The two frequently spoke about Japanese life and culture, which fascinated Weibel. Sensing that Weibel might be interested in visiting Japan himself, Kodama suggested that he apply for a Fulbright scholarship to continue his chemistry training there. "I didn't think it would pan out, but it did. It was luck," Weibel says.

Several months after he graduated from Utah, Weibel flew to Sendai, Japan, and worked for a year on projects involving organometallic chemistry. In the lab of Yoshinori Yamamoto and under the direction of Vladimir Gevorgyan, a then-international associate professor who is now a professor at the University of Illinois at Chicago, Weibel developed new or more efficient reactions to make small molecules. Weibel had become interested in organic chemistry during his undergraduate years, and he welcomed the opportunity to work in an area of this discipline that was not biologically relevant. Weibel remembers this time as "fantastic" for several reasons: the cultural and language opportunities, as well as the science.

Eclectic Interests. Upon returning to Japan, Weibel married his wife, Gina, and the couple left for Cornell together, where Gina had also been accepted for a graduate program in engineering. Soon after he arrived, he had the chance to meet with Jerrold Meinwald, a chemical ecologist whose papers Weibel had often perused as an undergrad. Meinwald's work held a longstanding appeal with him and his chemistry-major friends. Even the names of compounds that Meinwald had studied were fascinating to Weibel and his classmates. "He'd worked out the structure of a natural product called grasshopper ketone—we thought that was the coolest thing ever," Weibel says.

When Weibel had a chance to talk with Meinwald in person, "something just clicked and seemed right," he says. He quickly asked Meinwald if he could join his lab. For the next five years, Weibel worked with Meinwald and his colleagues on deriving the structure and synthesis of members of a family of lipid-derived small molecule compounds that insects use for defense (*1*–*6*). "These small molecules are what many types of insects use to communicate—chemistry is the language they speak," he explains. One of the compounds that Weibel studied in particular was secreted in tiny amounts, on the level of micrograms, through the hollow hairs of moth larvae.

One summer during his graduate studies, Meinwald arranged a visiting scientist position for him at the Max Planck Institute for Chemical Ecology in Jena, Germany. The

opportunity allowed Weibel to accompany his wife to Germany, where she was spending the summer working with a collaborator on her own semiconductor research. Weibel's project there involved deriving the biosynthetic pathway for compounds called iridoids, monoterpenes used as a deterrent by some plant and animal species (*7*). Weibel harvested these compounds from glands in beetles that he captured live in the field weekly. "To get these beetles, I had to crawl around in cemeteries," he recalls. "I used to get the weirdest looks."

Working with miniscule amounts of the compounds he studied, Weibel became interested in microfluidics, the interdisciplinary field focused on studying the behavior and precise control of very small amounts of fluids. He reasoned that this area of science might be relevant to his own work, so he began reading up on current microfluidics research. This education came in handy during another summer of his graduate studies, when a grant funding his work, obtained through the National Institutes of Health Chemistry and Biology Interface Training Program, required that he do an internship at a company. Weibel chose to work with Orchid Biosciences, a company that used microfluidics as a tool in much of their research. His project there involved developing a microfluidic platform for genotyping. "It was totally different from anything I had done before," he says.

The work was a learning opportunity not just in a new area of science, but also with working for a small biotechnology company. During this time, Weibel was continually vacillating over what his next move would be: academia or industry? "Every week, it was a different thing. My life was like a sawtooth plot," he recalls. Though his experience at Orchid was a good one, it helped him decide that he was not interested in following an industry path. It also helped him focus on where to look for postdoctoral fellowships. "Because of my work with microfluidics, my interest was really expanding toward materials science," he says.

Weibel had read several papers by George Whitesides, a Harvard University chemist whose own interests were broad and eclectic, ranging from surface chemistry to cell biology. Whitesides' group had also published several papers on microfluidics. Weibel saw an opportunity to combine his own eclectic interests in Whitesides lab, mixing chemical biology, materials science, and microfluidics. "I knew that his group was very multidisciplinary. He took people who were organic chemists or biologists or physicists, and he gave them the flexibility to retrain and learn new areas," Weibel says.

Hoping to become a part of this diverse group himself, Weibel applied for a postdoctoral fellowship in Whitesides' lab toward the fourth year of his doctoral work and was accepted. He stayed an additional year to wrap up projects and allow his wife to continue her doctoral work. At the end of his fifth year, the couple moved together to Boston.

"I had never met George, never talked with him face-to-face, never seen the lab before I was locked in to this postdoc," Weibel remembers. One of his first conversations with Whitesides revolved around what projects Weibel would pursue. Whitesides encouraged him to jump into whichever ongoing projects he was interested in joining, as well as start new projects whenever he had an interesting idea. "I was all for doing as many different things as possible, which fit in really well with this group," Weibel says.

With a lab of 45 people at the time, Whitesides' group gave Weibel a number of good opportunities to follow his own interests and pick up new ones. "I figured out what people were doing and which people I clicked well with. People knew what expertise I had, and I started joining projects people were on and picking up projects from people who were leaving," he says. Over the course of the four years he spent there, Weibel worked on projects ranging from developing fuel cells to using purified cellular organelles as chemical sensors (*8–12*).

He also gained additional experience working with microfluidics. Weibel notes that he ended up inheriting the desk of Rustem Ismagilov, a postdoctoral fellow, now a professor at the University of Chicago, who left just before Weibel arrived in the lab. During his time working with Whitesides, Ismagilov laid the foundation for using microfluidics to create very tiny droplets and using each as a femtoliter-sized reactor. Weibel continued this project, working on developing ways to use chemistry to control the size and shape of droplets. He also worked on other microfluidics projects that involved developing tiny valves and hardware to control microfluidic flows and channels (*13–17*).

Weibel also used microfluidics as a tool in other projects involving the study of microbes, an area of research that he had only limited experience with previously, but which would soon become the focus of research in his own lab. He, Whitesides, and their colleagues used microfluidic channels as passageways for swimming *E. coli*, showing in one paper that the bacteria, which rotate while swimming, preferentially "drive on the right" in channels due to their clockwise motion (*18*). In another paper, the researchers showed that they could use surface chemistry to attach polystyrene "microloads" to microorganisms and coax them into pulling the loads through microfluidic channels with the aid of phototaxis (*19*). "It was really as a postdoc that I fell in love with microbes and started working on them," Weibel says.

Channeling Microbiology. As he wrapped up his postdoctoral fellowship nearly four years later, Weibel began looking for an academic position where he could continue to use a multidisciplinary approach as the leader of his own lab. With the diverse skill set he'd developed in his postdoc, Weibel

was able to interview for positions in several different departments at universities, including chemistry, biochemistry, biomedical engineering, and chemical engineering. "I was interested in being in a place where not only one area of science was strong but all of science and engineering was outstanding, and for them to have a strong interdisciplinary and collaborative research culture," he says. At this point, Weibel was also looking for a university in a family friendly town with a good school system. He and his wife had three small children at the time; they recently added a new baby to the mix.

Weibel found everything he was looking for at the University of Wisconsin-Madison (UW-Madison). In the fall of 2006, he moved there with his family and started his own lab.

When he arrived at Madison, Weibel says that he was daunted with the prospect of having to focus his lab on specific projects. "I thought, wow, now everything really counts," he says with a laugh. After recruiting a few graduate students and postdocs, Weibel thought at first that he and his new colleagues could delve into studying the physiology and behavior of one particular microbe at a time using chemical biology and material science, starting with *E. coli*. "That was totally naïve," he says. He and his colleagues soon realized that the research questions they hoped to pursue would best be studied in different organisms.

Eventually, the researchers decided to pursue several different areas using multiple species of microbes. "We started working on several different projects thinking that whatever worked out would become the focus of the lab. A bunch of these things worked out, so our focus has not narrowed," Weibel says.

Nowadays, Weibel and his lab, currently composed of three postdoctoral researchers, eight graduate students, five undergraduate students, and a handful of high school students, among others, work on a variety of projects ranging from studying homologues of mammalian proteins in bacterial cells to learning how bacteria interact with different surfaces to developing new materials for microbial studies. Microfluidics plays a pivotal role in much of this work, though the focus is very different from typical microfluidic studies. "Many people take advantage of pushing liquids through microfluidic channels, but most of the stuff we do uses totally quiescent fluid," he explains.

In a recent study, Weibel and his colleagues developed a new way to study microbes using small molecules with extremely limited availability. Several projects in his lab use a chemical biology approach to better understand microbial homologues of mammalian cytoskeletal proteins similar to actin, tubulin, and intermediate filaments. Using compounds pulled from a library, Weibel's team screens small molecules for their activity to perturb these homologues, as well as to search for mutant bacteria resistant to this activity. The traditional way to do similar studies involves infusing the medium in many Petri dishes with the compound of interest and isolating resistant mutants that survive. However, Weibel explains that only small amounts of these compounds are available from libraries. "We want to be able to do lots of studies quickly without taking six months to make more compounds," he says.

To work around this conundrum, Weibel's team developed a microfluidic solution (*20*). The researchers forced together streams of oil and warm agarose infused with small amounts of the compound of interest, along with a precisely calculated titer of fluorescent bacteria, into a microfluidic channel. "It is an expensive way of making the equivalent of salad dressing," Weibel says. As the agarose hits the oil, it forms small droplets, encapsulating single bacterial cells into the droplets, which then cool and form gel microparticles. After allowing the bacteria to incubate in these micromedia, the researchers can use cell sorters to isolate mutants based on their optical properties.

Such an approach allows Weibel's team to maximize scarce amounts of compounds. "It takes about a thousand times less material," he says. It also saves the researchers time, since the bacteria need only incubate for a short period to develop the optical characteristics necessary for cell sorting.

In another project, Weibel and his colleagues are using microfluidic channels to gain insight into how bacterial proteins localize to different parts of the cell (*21, 22*). Recent research by other investigators has shown that in rod-shaped bacteria, such as *E. coli*, many proteins localize to the poles. However, it is unclear what landmark steers the proteins to this place. To better understand this phenomenon, Weibel's team constructed a gel media embossed with thousands of V-shaped channels of several different angles. The researchers applied a suspension of bacteria over this gel, which pulled the microbes inside the channels by capillary pressure. Once the bacteria were confined to the channels, the researchers allowed them to grow normally for a period of time, then used a small molecule to prevent cell division. Without the ability to separate into daughter cells, the bacteria grew into long filaments, which wrapped around the apex of each V. Weibel and his colleagues found that proteins that normally migrated to the poles often collected at the new apex, suggesting that curvature may be the cue to create these microdomains.

Weibel and his team continue to build on these projects while pursuing a variety of other research questions, such as using bacteria to build new materials and screening small molecules to serve as starting points for next-generation antibiotics. Weibel also spends a significant amount of his time on teaching, mentoring, and outreach to the community. In 2007, he and a group of like-minded colleagues at UW-Madison founded MicroExplorers, an educational program for K-12 students in Madison

schools and surrounding areas. Using their own funds, along with recent grants, Weibel and his collaborators are putting together kits including microscopes and other equipment, along with curricula to help young students explore phenomena at the microscale.

Weibel notes that these young students, along with the students and postdoctoral researchers he continues to train in his own lab, will eventually be able to build on the broad questions on microbial physiology, organization, and behavior that he and his colleagues are currently working on answering. "There's still so much that needs to be discovered," he says.

—Christen Brownlee, Science Writer

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