

Making Pretty Pictures

Sure, the images Kit Pogliano takes of bacterial proteins are breathtaking, but the science is even more so.

By Karen Hopkin

She biked her way around Iceland. She was a certified ski instructor by the age of 17. But it was in the laboratory that Kit Pogliano really took off and showed what she was made of. At the University of Washington (UW) in Seattle in the 1980s, “she was this star undergraduate researcher who helped Steve Lory get off to a flying start,” recalls Kelly Hughes, now at the University of Utah. And before her association with Lory, Pogliano did research on capsid and pilus assembly in bacteriophage T4 with UW geneticist Gus Doermann. “She had great hands and was hard-working,” Hughes recalls.

Pogliano was so hardworking that she published a handful of papers on the work she did with Lory in the late 1980s, and she even wrote up the work she did with Doermann after she joined Jon Beckwith’s lab as a graduate student at Harvard Medical School in 1986. “She had done some very sophisticated phage genetic work with Doermann and then essentially wrote up the papers herself because he had retired and moved into a cabin way up in northern Canada,” says Beckwith. “Those were some pretty good papers.”

They were just the first of many “pretty good papers” she has put together so far in her career. Pogliano, now at the University of California, San Diego, has become a major contributor to the field of microbial biology, where she dissects the molecular machinery involved in cell division and spore formation in *Bacillus subtilis*. “To this day I think she’s one of the best cell biologists in the microbial field,” says Rich Losick of Harvard University, Pogliano’s postdoctoral advisor. Says Hughes, “I used to say Kit’s a rising star, but now she’s definitely risen.”

UNPACKING ENGULFMENT

After publishing another half dozen papers with Beckwith – including one in *Genetics* on the cold sensitivity of protein secretion in *Escherichia coli* that Beckwith says is still routinely cited – Pogliano moved across the Charles River to be a postdoc in the Losick lab. “She came in with a clear view about what she wanted to work on,” recalls Losick: a process called engulfment.

When faced with starvation, *B. subtilis* is capable of forming spores, the hardy cells that can withstand heat, desiccation, UV irradiation, and other harsh conditions. To produce a spore, a bacillus cell first divides asymmetrically, generating a small forespore (the cell that is destined to become the spore) and a larger “mother” cell, which nurtures the developing spore until it dies. At first the two cells lie side by side; over time, however, the membrane of the mother cell migrates around that of the forespore, eventually swallowing it up and then pinching off to generate

a cell-within-a-cell arrangement. The process looks similar to phagocytosis that allows an immune cell to engulf an invading bacterium. “But how bacteria carry out this process was really a complete puzzle,” says Pogliano.

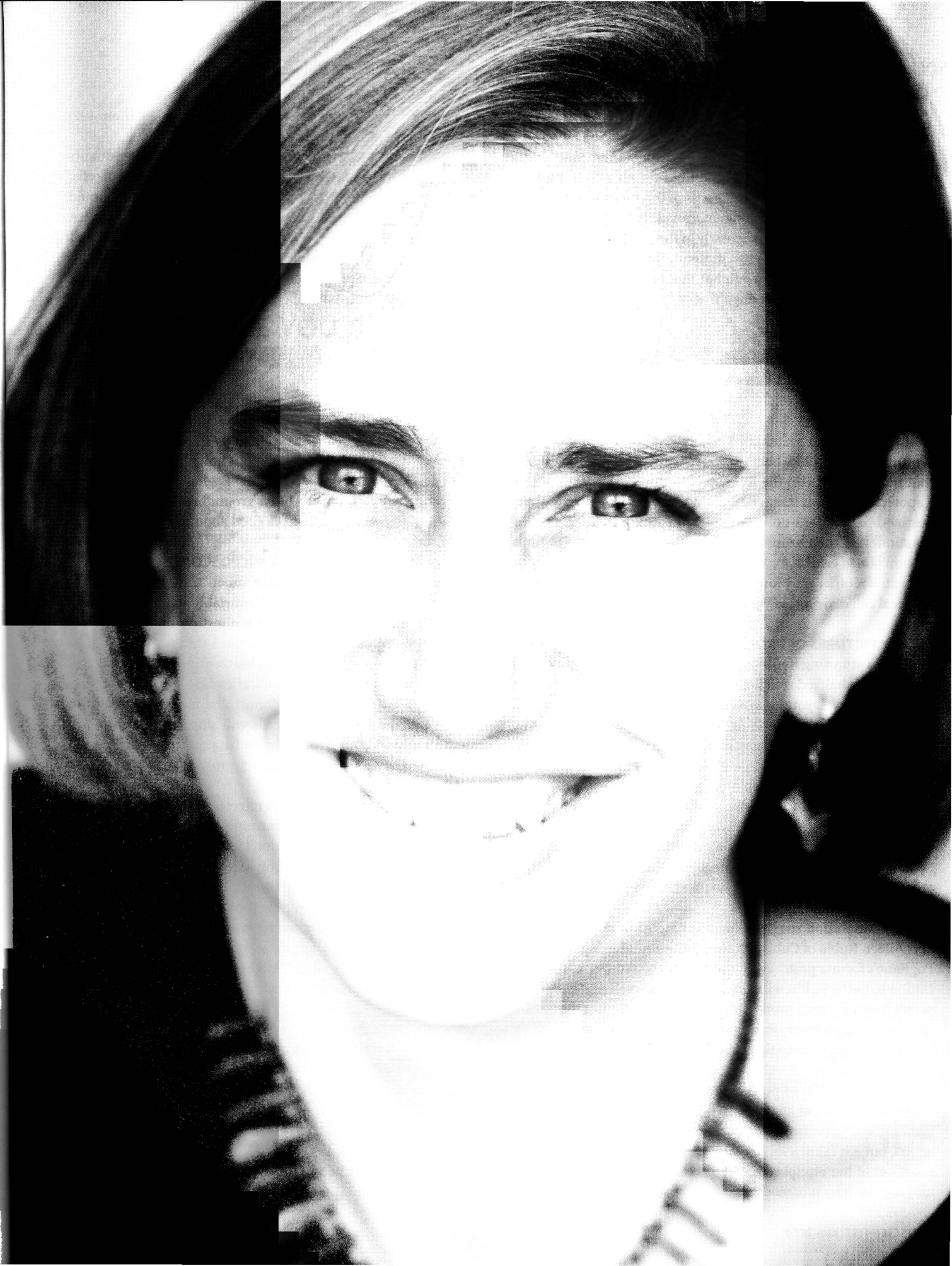
To begin to sort out the pieces, Pogliano had to develop some new ways to approach the problem. “One of the reasons no one had ever set out to understand engulfment is that they could only see it with electron microscopy,” she says. That technique produces only static images of dead bacteria, and it is tedious as well. As a postdoc she had tried to localize proteins involved in sporulation with immunoelectron microscopy. “But it was too slow for me,” she says. “I couldn’t feel it, and it took too long to get a result. That’s when I really started developing fluorescent tools to localize proteins.”

First, Pogliano perfected a system for using green fluorescent protein (GFP)-fusion proteins to visualize the proteins in bacterial cells. Her 1995 *Science* paper, which tracked the movement of a protein key to spore formation in *B. subtilis*, was, “the first example of using GFP in a bacterium,” Losick says. Pogliano also pioneered the use of time-lapse, immunofluorescence microscopy in *Bacillus*, which allowed (and still does) her to “follow the behavior of a single protein molecule inside a cell and see how it dances around and what it does, in a way that is totally convincing,” says Moselio Schaechter, a retired microbiologist who still holds adjunct positions at UCSD and San Diego State University.

“She not only transformed my lab, but had a wide impact in that field and beyond,” says Losick. Once Pogliano established her own lab, she further developed methods for using fluorescent molecules to stain bacterial cell membranes. Combined with the time-lapse microscopy, Pogliano could then watch engulfment happen in living cells in real time.

Part of the reason such techniques were in short supply is because “no one thought about ultrastructure in bacteria,” says Losick. “Until the early ‘90s, people thought bacteria were just these bags with proteins floating around inside.” That’s when Stanford’s Lucy Shapiro demonstrated that the proteins that direct bacterial chemotaxis cluster at one end of an *E. coli* cell.

Such localization, Pogliano has found, is also key to sporulation. While in Losick’s lab, she studied how one protein, called SpoIIE, gathers at the sites where cell division will occur. After the cells divide, the protein is present in higher concentrations in the forespore (because it’s so much smaller than the mother), which allows it to initiate the gene expression program that directs forespore development.



Pogliano has also made great strides in understanding the molecular mechanisms that underlie engulfment. She found that one pair of proteins actually reach through the septum that divides the mother from the forespore and hold tightly to one another. The contact anchors the proteins within the septum, where they promote the assembly of the signal transduction machinery that directs gene expression, particularly in the mother cell. Last year, Pogliano showed that these two proteins, zippered together in the septum, act as a molecular ratchet that keeps the mother's cell membrane moving during engulfment.

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In addition to watching proteins hold hands across the septum, Pogliano has been following the activity of a protein that pumps DNA into the forespore. When *B. subtilis* produces a spore, the septum closes down around the chromosome that's destined for the forespore. A DNA translocase then pumps the remainder of the chromosome home. Pogliano finds that this protein is also critical for the membrane fusion event that finally separates mother and forespore, and in the final steps of symmetrical cell division, as well.

PRETTY PICTURES OF PROKARYOTE PROTEINS

Pogliano's stunning fluorescence images have graced the covers of more than half a dozen journals, but making pretty pictures of proteins in prokaryotes is not as easy as it may seem. "You see all these beautiful images looking at localization in eukaryotes and it's spectacular," says Hughes. "But it's trivial. Compared to what Kit does, it's easy." Bacteria are a thousand times smaller than eukaryotic cells, he notes. "She's looking at things on the nano scale, and that's really hard. She's taken fluorescence microscopy as far as it can physically go, and she's fine-tuned her skills to see things nobody has been able to see before." Shapiro agrees. "We're right at the limit of detection and we have to be very creative with our microscopy – and she's very creative. She's a wonderful scientist."

"The resolution required for this kind of work is very high," says Aileen Rubio of Cubist Pharmaceuticals in Lexington, Mass., Pogliano's former postdoc. "Some people claim they can do localization in bacteria, but with the cell being as small as it is, you can pretty much say your protein is anywhere. But Kit has the resolution so you can see very clearly where things are going. There are very few people who do this as well as she does."

The technique Pogliano prefers, called deconvolution microscopy, is akin to performing a CAT scan on a bacterium – producing optical slices that can later be rebuilt into three-dimensional structures. The process, says Schaechter, "is a bit of an art, but Kit takes a lot of care to get it right." She also gets the most out of her equipment. "She doesn't just buy a microscope and use the lens it comes with. She demands that the company

gives her a choice of five objectives and she studies them very carefully and selects the one that works best."

But more than taking pretty pictures, Pogliano brings quantitation to her microscopy. "She makes you look at hundreds of cells," says Rubio. "And [she'll] say, of the thousand cells you counted, 90% are doing this, so this is the phenotype. The benefit is you have numbers behind the models that you're proposing." By all accounts, Pogliano is a master modeler. "She makes models in her head and she doesn't even know she's doing it," says Schaechter. "She's terrifically imaginative."

Beckwith agrees. "She's good at pretty quickly extending her results to come up with big ideas about how things are working. Lots of her papers have new concepts." Pogliano is "always at the cutting edge," adds Hughes. "Always groundbreaking, always new, and always seminal. That's what's good about her stuff."

MAKING MICROBES HAPPY IN A MICROSCOPE

These days, Pogliano doesn't have much time to work at the bench. "When I do an experiment I invariably abandon it halfway through when somebody asks me a question or they're doing something more exciting," she says. "So I think it's much more efficient for me to spend my time in lab training people how to do things and offering guidance and cheerleading." Pogliano's coaching includes suggestions about "how to be nicer to cells during microscopy," she says. "Lots of cells will stop performing if you slap them on a slide and trap them under a cover slip. You have to keep them happy. Maybe let them keep growing or keep sporulating. It's interesting."

In addition to making sure her microbes stay happy, Pogliano also pitches in to keep the lab running smoothly. "She loves to clean pipettes," laughs Rubio. "I don't know if she still does it, but when our dishwashers were out on break, you'd see her in these big rubber gloves, collecting pipettes."

Perhaps washing glassware is part of her problem-solving nature. "She has a knack for seeing everything that needs doing, and it gets done right, it gets done quick, and it gets done perfectly," says Hughes, who co-taught the Cold Spring Harbor advanced bacterial genetics course with Pogliano between 2001 and 2005. "When Kit showed up I could relax. I knew that all the problems were going to go away."

That same efficiency allows Pogliano to juggle life as a scientist, wife, and mother. "To be able to get tenure while you have two little children, to have a life and a successful marriage and run a successful lab takes a tremendous amount of intelligence and time-management skills," notes Rubio. "Everything in lab always seemed to get done, yet she also made time to be with her family. I'm still impressed by her ability to do it all." ■

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