THE AUTHOR FILE

Bridget Carragher

Speeding up spot-to-plunge in cryo-EM and how to keep a lab talking.

"Cryo-EM should be for everyone, not somebody who wants to spend a whole PhD lifetime on it," says Bridget Carragher, who co-directs the Simons Electron Microscopy Center. The center is part of the New York Structural Biology Center (NYSBC), a consortium of nine universities and academic research institutions.

Carragher joined NYSBC in 2015 from Scripps Research Institute, along with her co-director Clint Potter. She received her PhD in biophysics from the University of Chicago and moved to Scripps after gaining academic and industry experience. With Potter, she co-leads the National Resource for Automated Molecular Microscopy (NRAMM), which focuses on cryo-EM methods development, and she will co-direct a new center entirely devoted to cryo-EM access and training. While at Scripps, she and Potter spun out NanoImaging Services to work with biopharmaceutical companies on cryo-EM projects.

Her latest project involves adapting a robot that she previously co-developed to address a headache in cryo-EM sample prep. Spotiton dispenses sample in picoliter volumes so it can be rapidly cooled for imaging in an electron microscope. But it takes too long to go from spotting the sample on a grid to plunge-freezing it. Also, says Carragher, some proteins in a sample take on a "pathologically preferred orientation" that offers too limited structural data. "You have to have more than one view of something in order to really understand its three-dimensional shape," she says. Carragher and her team showed experimentally that the bad poses are due to the interaction of proteins at the hydrophobic air–water interface. Some proteins disintegrate at this interface, others misbehave, she says. They can stay stuck in one orientation as if the protein were saying, "I'd like to just sit here in this position."

By adapting Spotiton and using a nanowire grid, she and her team reduced the 'spot-to-plunge' time from 500 milliseconds and longer to around 100 milliseconds. "I'd really like to see 20 milliseconds, 10 milliseconds," she says. That speed might have its own challenges, "but that's OK, we don't mind solving practical problems." The acceleration achieved gives a protein less time at the air–water interface and the protein remains in more varied orientations and delivers improved structural information. The team tested the approach with several proteins and it worked. Some, like apoferritin, readily deliver three-angstrom maps, but hemagglutinin is a known "problem child" that prefers a particular orientation on the grid.

Bridget Carragher (Credit: S. Bradlow)

Originally, Nobel laureate Jacques Dubochet developed the widely used approach of adding two to three microliters of sample to an EM grid and wicking away all but a few nanoliters with blotting paper. The sample spreads as a thin film across tiny holes in the grid through which the proteins can be imaged. The grid is plunged into ethane or another cryogen to vitrify the sample. These steps often fail. As Carragher sometimes says, "everybody using this facility is one grid away from their perfect three-angstrom structure." For years Carragher and her team explored other approaches, and eventually they came across a way to grow "a lovely lawn of nanowires" on a grid surface. The grid becomes self-wicking. "It's as if we build blotting paper into the grid," she says.

Today's researchers take automation in cryo-EM for granted, says Eva Nogales, a Howard Hughes Medical Institute investigator at the University of California, Berkeley, researcher at the Lawrence Berkeley National Laboratory and longtime NRAMM advisory board member. Carragher has pioneered automation in the face of resistance from labs worried that it would reduce their process control. Yet, says Nogales, without automated data collection, today's resolution gains in cryo-EM would be inconceivable. "Bridget, always with a disarming sense of humor, pushed forwards and was a true trendsetter," she says. "With a unique knack for recognizing technical bottlenecks and tackling them head on, she is again leading the pack with her efforts to improve sample preparation."

In the lab, Carragher fosters a collaborative spirit. Monday meetings give everyone a rundown of how others in the 25-member lab are handling engineering, software, hardware or biology-related issues. Graduate students and postdocs are "the communication devices," she says. They are go-betweens to an external lab needing cryo-EM help.

> "I get people to talk to other people".

Generally, she says, labs needn't be daunted by technical challenges. Unless researchers bang up against a fundamental physics hurdle, engineering issues are solvable with time, effort and energy. Instead of talking oneself out of something, she recommends: "Give it a shot." As Carragher roams the lab, she listens and might suggest someone touch base with another lab member. A colleague tells her, "That's my superpower, I get people to talk to other people," she says. "That's the thing that makes collaboration work, it's making sure that people don't live in their silos."

Outside of the lab, Carragher is an avid swimmer. "I love open-water swimming, and it's the biggest thing I miss about San Diego," she says. She used to swim across La Jolla Cove enjoying how the ocean waves, visibility, animal life change from one swim to the next. On a recent visit, she swam La Jolla Cove while solving a technical problem in her head. She had swum a quarter of a mile before realizing it. ❐

Vivien Marx

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Reference

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