Nozomi Ando
2020 Margaret C. Etter Early Career Award
Structure Matters

Nozomi Ando Wins NSF Early Career Award

Nozomi Ando was selected by the American Crystallographic Association (ACA) to receive the 2020 Margaret C. Etter Early Career Award (see Winter 2019 RefleXions). The exceptional potential that the ACA honored has been recognized by the National Science Foundation (NSF) and Nozomi has just won an NSF Faculty Early Career Development (CAREER) award.

This program “emphasizes the importance [NSF] places on the early development of academic careers dedicated to stimulating the discovery process in which the excitement of research is enhanced by inspired teaching, enthusiastic learning and disseminating new knowledge.”

The research work that Nozomi will be pursuing under this grant will apply her interdisciplinary skills, which lie at the intersection of x-ray physics and enzymology, to the challenge of truly understanding the central question of structural biology: how sequence gives rise not just to structure but also to function. The techniques she will use come from many fields, including crystallography, chemistry, biology, physics and statistics.

Her study will be carried out on the ribonucleotide reductase (RNR) family, which performs an essential step in DNA synthesis. This family is of particular interest because it has evolved multiple levels of complex allostery while simultaneously conserving a catalytic mechanism that pre-dates the oxygenation of the Earth.

The goal of Nozomi’s educational plan is to promote innovative thinking both within academic research and beyond. She will be doing so in a two-pronged way: she will develop a career-focused seminar series and a modern structural biology course that is focused on filling an educational need. The typical student comes to such a course without familiarity with foundational mathematical concepts. To address this, the new course will foster active learning of both theory and practice.

Nozomi Ando - Thinking Outside the Lattice

Thinking Outside the Lattice

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It’s an honor to receive the 2020 Margaret C. Etter Early Career Award from the ACA. Since 2003, this award has recognized outstanding achievement and exceptional potential in crystallographic research demonstrated by a scientist at an early stage of their independent career. I am grateful to the ACA and my colleagues for this recognition and to my mentors who paved a path before me. I am especially grateful to my own mentees for believing in my vision and bringing their talent, creativity, and dedication. My career would not exist without them. This piece, which I am delighted to share as part of ACA’s celebration of International Women’s Day, is for them and for all young scientists.

A New Hope: The Rise of Diffuse Scattering

There were several pioneering studies on the topic of macromolecular diffuse scattering, but a particularly significant one was that of Caspar, et al. (Nature 1988). Using X-ray film, the scientists exposed an insulin crystal long enough to reveal features that were weaker than the bright spots that we commonly associate with diffraction images. These included the broad ring around 3 Å that is often seen with hydrated protein crystals as well as halo-like features emanating from diffraction spots. After subtracting the strongest of these features from the image, a blobby, cloudy pattern emerged. The scientists attributed this signal to the internal motions of the protein molecules by invoking a simple model known as the liquid-like model. The details of the model do not matter as they are not meant to be realistic, but the significance of their work was in establishing a new hope - that we might learn about protein dynamics from crystallography.

To understand what diffuse scattering is, it’s instructive to go back to the basics. The goal of crystallography is to determine the position of every atom in a molecule, and we do this by measuring the intensities of the bright spots known as Bragg diffraction. However, we have known for a long time that crystals fluctuate and are imperfect. The probability that an atom is at its average location is in part described by the temperature factor, or B-factor. In last year’s spring issue, Eaton Lattman from the Hauptman-Woodward Medical Research Institute wrote a delightfully intuitive way to understand B-factors. In his description, the instantaneous scattering from a fluctuating atom gives rise to a “tipsy walk” in the so-called Argand diagram. Importantly, the end-to-end distance of such a path is shorter than one constructed from a perfectly straight path. The consequence of this is that the amplitude of the scattering is diminished when atoms fluctuate, and from experience, we know this to be true: disorder in a crystal leads to the loss of diffraction spots starting at the outer edges. In other words, the B-factor describes the loss of the Bragg signal due to disorder.

What happens then to the diverted X-rays? For a typical protein crystal, it turns out that only about half of the scattered photons go into the Bragg diffraction pattern. The remainder scatters in all directions, giving rise to a diffuse pattern that is spread out throughout reciprocal space. The Bragg signal, as we know well from crystallography, provides atomic coordinates and B-factors. However, its twin, the mysterious diffuse scattering signal, holds the secrets to how atomic displacements are correlated. Understanding how different parts of a protein communicate is exactly what we so often seek in biochemistry. It was this promise of diffuse scattering that led to a series of attempts to understand this elusive signal. But this proved to be very difficult. Highlights from this period include the work of Wall, et al. (PNAS 1997), which was the first study to draw attention to the importance of how we measure this signal.
The Death Star: The Attack of the Phonons

In 2012, a few days before the ACA Meeting in Boston, a number of scientists gathered in Buffalo, NY for the Biodynamics@Buffalo conference. Those who attended may remember why this meeting took place. What mattered to me is that I happened to be thinking about a crystal structure with a striking B-factor pattern from Catherine Drennan’s group at MIT (Kung, et al. Nature 2012), and immediately after my talk, Sol Gruner from Cornell University gave a talk reminding the community that pixel-array detectors were now available for launching the next assault on diffuse scattering. This was around the time that similar detectors were about to catalyze a revolution in the cryo-electron microscopy field. Structural biology had just been equipped with our newest technology.

Having spent many years in the small-angle X-ray scattering (SAXS) field, the idea of a scattering signal that is orientation-dependent and even harder to measure than solution scattering was extremely appealing to me. When I began my independent career in 2014 at Princeton University, I was lucky that the like-minded Steve Meisburger had just defended his PhD. Based on our interactions at the Cornell High Energy Synchrotron Source (CHESS), I offered him a postdoc position without an interview, and he accepted the offer without even knowing what the job entailed. The essential part of this story is that the Force was strong in Steve, and like me, he came from a rigorous training in SAXS.

Soon after, we had succeeded in collecting data on crystals of interest. The diffuse signal, however, looked like a mess in 2-D diffraction images. They were smearable and uninterpretable. Were they even real? To address this, Steve mapped the pixel intensities in 3-D reciprocal space. What emerged was a 3-D map that showed that there is indeed information there (Fig. 1a). The messy diffuse signal was connected in 3-D reciprocal space, forming patterns and even displaying symmetry. We named the map, “the Death Star”. But we also noticed that a better approach was needed. Clear artifacts could be seen in the map, such as the shadow of the beamstop, which is what made the map look like a Death Star in the first place.

Our years of SAXS training told us what we needed to do: start over from scratch, focus on collecting the cleanest possible data, and write new data processing software. Steve went onto write an impressive software suite that borrowed techniques from both crystallography and SAXS. It was an incredible mountain to climb, and even then, we faced the problem of interpreting the signal when we would eventually arrive at the summit. Thus, I enlisted the help of Dave Case at Rutgers University who had initially met at the fateful Biodynamics meeting. In my final year at Princeton, Dave began a year-long sabbatical in my lab and performed a series of large molecular simulations of protein crystals, which simulated many important discussions.

The end result of this productive year was the 3-D diffuse scattering map of triclinic lysozyme collected at room temperature (Fig. 1b). It was the most detailed map that the field had seen. However, through its beauty, something was glaring at us. Halos. Our experience with SAXS means that we would obsess over the small-angle features near the diffraction spots, and with the fine detail in Steve’s map, we could clearly see halos emanating with a power-law dependence. This was the first sign that the diffuse signal was dominated by thermally excited lattice vibrations, known as acoustic phonons. The phonons cast doubt on our plans. Was there no information about protein dynamics in diffuse scattering? Was all hope lost?

It was time to move, however. The synchrotron had been calling, and so after four great years at Princeton, I moved my lab to Cornell University.

The Return of the Protein

In the summer of 2018, my lab regrouped in a new location on the beautiful Cornell campus. Sitting among boxes, we resumed research. The initial goal of the diffuse scattering field was to extract information about protein dynamics, but we had not yet succeeded. Steve had shown that the halo scattering had distinct 3-D shapes, and he performed simulations to confirm what we had feared: the phonons appeared to explain most of the diffuse scattering intensities. We now had data that showed us the strength of numbers that is, the number of unit cells. Lattice vibrations meant that atoms separated by many unit cells were correlated, and the signal arising from such correlations was amplified by the large number of unit cells. The contribution of phonons to diffuse scattering was actually something that the field was concerned about, and Peter Moore at Yale University had warned us about the possibility (Polikonov & Moore, Acta Cryst D 2015).

Although phonons were not what we were looking for, it was still a major victory that Steve was able to explain most of the diffuse scattering signal in a vibrational model. No other model had come so close to explaining this signal before. Moreover, the closest data points that we could measure next to each diffraction spot told us that atoms were correlated over at least 10 unit cells, and the existence of such long-ranged correlations has significance in the context of protein-protein interactions. This work also told us that strong features like halos should not be subtracted from the Death Star. They must be accounted for but not removed because the act of removing these features corrupts the diffuse scattering signal, and this was in fact how the diffuse signal had evaded us for so many decades.

At this point, we had come a long way, but we had not yet reached the true summit - there was another mountain to climb. Steve planned for his next battle by checking his calculations against another set of data. He calculated the B-factors that we would expect from the lattice vibrations and compared them to the B-factors we obtained from the Bragg data. What he saw was that although lattice vibrations accounted for a large amount of the atomic motions implied by the B-factors, there was still a gap. Could this gap be due to protein motions?

To test this, Steve performed a simulation treating the protein as an elastic network and fit the model to the residual B-factors. Then, he asked whether this model could explain the diffuse scattering. However, we already knew that the diffuse intensities are dominated by the contributions from lattice vibrations. How can we place protein motions and lattice motions on the same playing field? The trick was to return to a fundamental concept in structural biology: the

Figure 1. a) Death Star I in 2015. b) Death Star II a few years later (Meisburger, et al., bioRxiv 2019).
Fourier Transform. The Fourier Transform, as students learn in my class, tells us how much a certain component contributes to a signal. Hence, Steve carefully calculated the Fourier Transform of the diffuse intensities and produced the diffuse Patterson map, or 3D-ΔPDF as it is known in the materials field. The diffuse Patterson represents the autocorrelation of the difference electron density as a function of distances within the crystal. The key takeaway is that it allows us to detect the contribution of short-ranged correlations that are intrinsic to protein motions over all other correlated motions in a crystal.

The cover art of this issue depicts the prize after the long road to the summit: the experimentally derived diffuse Patterson from our triclinic lysozyme dataset. Shown as a topology map, dark red corresponds to strongly positive values, and dark blue corresponds to strongly negative values. Features near the origin corresponding to the shortest length scales have the largest amplitudes. In agreement with our B-factor analysis, we found that lattice vibrations fail to explain the full amplitudes near the center of the diffuse Patterson map. Steve then calculated the diffuse Patterson from the elastic network model of protein motions, which was fit to the residual B-factors. Remarkably, much of the missing short-ranged correlations appeared. Although there is much more to this story, this was the key finding that we had been waiting for. It was the return of protein dynamics.

Finally, I was asked to write a message for aspiring scientists in celebration of International Women’s Day. I am no expert on how to succeed, but I can offer some thoughts:

1. Think big, think outside the box. Be glad to be different. Use feelings of being “different” to propel you to be unique in science. Develop a vision for the future of science.
2. Learn to share and when you’re in a position to do so, pay forward. In the wise words of Sol Gruner, “By sharing, you lose some, but you gain more.” Use experiences of hardships to help others avoid the same. You can’t change the past, but you can change the future. In the end, we all benefit.
3. Do everything in your power to maintain your health – both physical and mental. My current method of choice is hot yoga, which I find more enjoyable than room-temp yoga. Find an activity that works for you, and remember that there is no shame in taking your mental health seriously.
4. Find humor in daily experiences. Maybe write about your day to day experiences in terms of an epic story, like Star Wars.

Nozomi Ando

References
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The main governing body of the American Crystallographic Association is the Council. The Council, which meets two to three times per year, sets policy and has the ultimate responsibility for the actions of the association. It is composed of a president, vice president, past president, Canadian representative, secretary and treasurer. The membership elects these officers and terms commence on January 1st. The president is elected and serves for three one year terms of vice president, president, and past president, sequentially and is a member of the ACA Council during each one year term. The Canadian representative, the secretary and treasurer are elected for three year terms.