STRUCTURAL BIOLOGY

# Breaking the diffraction barrier

**Researchers exploit crystal imperfections to solve protein structures at resolutions beyond the X-ray diffraction limit.**

A little bit of a 'magic touch' is usually required for successful growth of high-quality protein crystals for X-ray diffraction. The goal is to produce regular arrangements of rigid molecules in order to obtain intense diffraction signals, called Bragg peaks, to solve a high-resolution protein structure. But very often proteins in crystals get a little bit out of line: they may exhibit conformational heterogeneity, or they may be rotated or translated from their ideal positions. Such imperfections are the bane of crystallographers, as they lead to vague diffraction patterns that cannot be interpreted.

As demonstrated by new work from Henry Chapman of DESY and the University of Hamburg in Germany, along with his colleagues, exploiting imperfections enabled the researchers not only to solve a structure from poor crystals but to do so at even higher resolutions than the X-ray diffraction limit would seem to allow.

When a crystal exhibits translational disorder—that is, when the protein molecules all have the same orientation but are slightly displaced from a regular arrangement—it gives rise to a vague, so-called continuous diffraction pattern. Chapman's team's insight was to exploit these data that are usually discarded. They first used the Bragg peaks that were available from the data to generate a low-resolution outline of the three-dimensional protein structure. Then they applied an algorithm to analyze the leftover continuous diffraction pattern to generate a three-dimensional reconstruction of the electron density, using the low-resolution structure outline as a modeling constraint.

Chapman's team used the method to solve the structure of the photosystem II membrane protein complex, using data collected by serial femtosecond crystallography (SFX) as carried out on an X-ray free-electron laser (XFEL). Their crystals diffracted to just 4.5 Å—relatively poor for crystallography. After applying their method to exploit the

GENOMICS

## CAS9 AND THE IMPORTANCE OF ASYMMETRY

**Short single-stranded DNA donors that asymmetrically span the Cas9 cut site show high efficiency in homology-directed editing.**

Among the many attractive features of the CRISPR–Cas9 system is its ability to replace a target sequence with a donor of choice. The efficiency of this homology-directed repair (HDR) varies widely depending on the cell type and whether long double-stranded DNA constructs bearing selection markers or short single-stranded oligonucleotides (ssDNA) are used to replace the target sequence. Jacob Corn at the Innovative Genomics Initiative and the University of California, Berkeley, is interested in the ssDNA approach because it allows scarless editing, but he notes that the pathways enabling such editing are still being worked out. "We wanted to understand how we can make this work best given a mechanistic understanding of the complex," he explains.

In work led by postdoctoral fellow Christopher Richardson, the team first determined the kinetics of the Cas9 nuclease *in vitro*. Cas9 stayed bound to a single target site for 5.5 hours, even after cutting the DNA. This long residency time surprised the researchers, but they found supporting evidence in the work of Jin-Soo Kim, who independently explored the time course of CRISPR editing in cells and found similar repair-time lengths. Thus the long residency time of Cas9 seen *in vitro* could determine *in vivo* rates of editing.

"We knew that Cas9 holds on to the duplex very tightly," says Corn, "but at first we thought it treated all strands equally." This turned out not to be the case. By labeling the ends of the four fragments that are created by Cas9 cleavage—proximal and distal to the PAM site on both the nontarget and the target (bound by the single guide RNA) strand—they saw that only the distal site on the nontarget strand was accessible to ssDNA binding. "It flaps out," as Corn graphically puts it. Even

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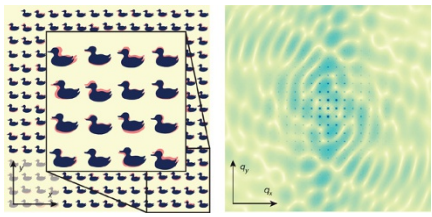

Translational disorder in a protein crystal (left) gives rise to a vague X-ray diffraction pattern (right) that can be exploited to solve structures at higher resolution.

continuous diffraction information, however, they solved the structure at 3.5 Å.

In terms of the data collection, "you don't really have to do anything different to what you usually do," Chapman points out. "It's all in the analysis." The method could therefore be immediately applied to interpret data sets from crystals that were deemed just not good enough for high-resolution structure determination. The method should be very robust, because the continuous diffraction pattern actually contains much more information than nice, sharp Bragg peaks, Chapman notes.

The method could potentially allow phase information to be directly retrieved without the need for any of the additional experimental manipulations that are typically required in crystallography. For this, however, "we still need to improve our method to deal with noise," says Chapman. As the approach requires crystals that are disordered in just the right way (that is, containing only translational disorder), reproducible methods for creating these perfectly imperfect crystals will be welcome. Chapman and his colleagues are also working on a user-friendly software tool to help others implement the approach.

Although the group's proof-of-principle demonstration was carried out using SFX data collected on an XFEL, Chapman believes that it should be possible to apply the method to data collected by conventional X-ray diffraction using a synchrotron. The approach could also potentially be used to help solve high-resolution structures of single particles, without crystallization, which would be very exciting for the structural biology field indeed.

**Allison Doerr**

**RESEARCH PAPERS**

Ayyer, K. *et al.* Macromolecular diffractive imaging using imperfect crystals. *Nature* **530**, 202–206 (2016).

catalytically inactive Cas9 (dCas9) and a Cas9 mutant that introduces single-strand breaks make the nontarget strand bulge; "even if it is not cut, it is accessible to annealing with oligos," says Corn. The team envisions that a 30-nucleotide stretch on the nontarget strand is looped out from the complex and that this loop is free to bind to ssDNA.

With this mechanistic insight, the researchers designed asymmetric ssDNA oligos that were complementary to the nontarget strand and spanned the cut site, overlapping the PAM-proximal site by 90 nucleotides but the PAM-distal site by only 30. They saw HDR frequencies of nearly 60%.

Even with dCas9, they were able to achieve HDR rates of up to 1% using ssDNA that annealed to the nontarget strand released by Cas9. With a strong selectable phenotype, this efficiency could be adequate to obtain edited cells. The advantage of dCas9 is that one does not have to worry about off-target cutting and editing. Although it is generally believed that nickases—enzymes that cleave only a single (target or nontarget) strand—do not introduce mutations at the nick site, the researchers did observe mutations that led to silencing of a reporter. They assessed editing only at the target site, but the observation that a single nick can be mutagenic should caution that the genome-wide off-targeting potential of nickases may need to be revisited.

One focus in the Corn lab is on introducing single-nucleotide polymorphisms at therapeutically relevant sites in primary human cells, and they recently succeeded in doing this using their asymmetric ssDNAs. Corn thinks that the discovery of Cas9's asymmetry and long residence time has implications beyond genome editing and could affect the use of dCas9 as a transcriptional regulator.

**Nicole Rusk**

**RESEARCH PAPERS**

Richardson, C.D. *et al.* Enhancing homology-directed genome editing by catalytically active and inactive CRISPR-Cas9 using asymmetric donor DNA. *Nat. Biotechnol.* **34**, 339–344 (2016).

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