PNAS QNAS

# QnAs with Yifan Cheng

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Cryogenic electron microscopy (cryo-EM) has emerged as a powerful technique to study the three-dimensional structures of organic materials, such as protein complexes. University of California, San Francisco (UCSF) biophysicist and structural biologist Yifan Cheng leverages his training in electron optics and physics to improve the technique and solve the challenging structures of biological macromolecules, such as membrane proteins and multiple-subunit protein machines. His work has advanced the technology of single-particle cryo-EM and led to the determination of the atomic structure of a membrane protein, the TRPV1 ion channel, which is activated by heat and capsaicin, the compound found in chili peppers. Cheng was elected to the National Academy of Sciences in 2020. PNAS recently spoke to him about his current research.

**PNAS:** Your Inaugural Article details a method to purify endogenous proteasomes, which are cells' waste recycling factories, for structural analysis by cryo-EM (1). Why was it necessary to devise such a method?

**Cheng:** My group has been heavily involved in methodology development for cryo-EM for a long time. We have a broad interest in all aspects of cryo-EM–related technology, particularly those dealing with major bottlenecks in the structure determination pipeline, one of which is sample preparation.

In the past, it was very tricky and often very tedious to produce sufficient quantities of purified samples from endogenous sources for structural studies. You often had to develop purification methods that were specific to the sample. Later, recombinant technologies became available. They allowed you to overexpress proteins in various expression systems, ranging from bacterial or yeast cells to insect or mammalian cell lines. These recombinant proteins were often expressed with affinity tags to enable affinity-based purification. This method worked well for many types of proteins or subunits, but it did not work or was not efficient for many large and multiple-component protein complexes. In addition, recombinant proteins could emerge aggregated or misfolded if they weren't produced in the correct cell type or with the necessary accessory factors. Thus, having an efficient method to purify endogenous proteins or protein complexes is very beneficial to many structural targets.

**PNAS:** You developed a bipartite technique using the CRISPR-Cas9 gene editing system combined with fluorescence cell sorting to tag and purify endogenous proteosome complexes in a human cell line. How did you arrive at this method?

**Cheng:** We wanted to develop a broadly applicable method for purifying endogenous proteins for structural analysis by cryo-EM. Other groups had been using CRISPR-Cas9 to knock-in proteins with affinity tags for specific

**Yifan Cheng. Image credit: Alexa Rocourt (University of California, San Francisco).**

samples, and we thought about how this system could be used in a more general way.

One limiting factor was that after knocking in a tag by CRISPR-Cas9, you had to identify and isolate the successfully knocked-in cells and generate stable cell lines. It's quite a tedious process. We thought we could extend a method original developed by biophysicist Bo Huang's [laboratory] at UCSF, in which they combined CRISPR-Cas9 with split-GFP [green fluorescent protein] to identify successfully knocked-in cells by fluorescence. With Huang's laboratory, we started working on applying this approach to study structures of endogenous proteins from HEK cells.

We used CRISPR-Cas9 to knock in an affinity tag and one strand of a florescent protein, such as a GFP, to a subunit of a proteasome complex. The cells were then transiently transfected with the complementary section of the fluorescent protein. Only cells that contained both parts of the fluorescent protein would fluoresce. The tag knocked in to the target gene perturbed the cells minimally and let us identify the knocked-in cells efficiently by fluorescence cell sorting. We could then purify endogenous proteasomes in these established cell lines. It's a proof-of-principle



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experiment, and the same approach can be used to study other proteins or complexes.

Our long-term vision is to combine this approach with other technology, such as affinity grids. This combination would especially aid in the structural determination of low-abundance proteins or complexes without intensive purification.

**PNAS:** Why did you decide to tag proteasomes, and what did you learn about them?

**Cheng:** We chose to work with proteasomes because they're a model system, our [laboratory] has worked with them in the past, and the structure is relatively well-studied. We know, for example, that we can insert affinity tags in places where they won't interfere with protein function. In fact, we tagged three different components of the proteasome in parallel both to pull down individual components and to see which one had a higher success rate.

One thing that we discovered about proteasome structure that was a bit different from previous studies had to do with the PA28 regulatory particle. PA28 is known to be a heptamer, containing seven subunits. Work with PA28 derived from recombinant sources suggested that it is comprised of four α- and three β-subunits, while our work with endogenous PA28 showed the opposite subunit composition: three α and four β. This difference may be meaningful because our endogenous PA28 particle was very stable and the structural resolution very high, while recombinant PA28 is not well resolved, likely because the complex is not stable.

**PNAS:** Your academic training was in solid-state physics. How did you become interested in visualizing biological samples?

**Cheng:** I arrived at structural biology through electron microscopy. The discovery of quasicrystals in 1984 happened just as I entered graduate school; that drove me to electron microscopy. It was a very exciting period: quasicrystals were controversial, and there was a lot of discussion in the field as to whether they were real. They were a purely scientific discovery with no apparent applications.

Unfortunately, once the heat died down, I realized that it was very hard to actually find a job working on quasicrystals. I needed a change, and cryo-EM was just being developed at that time. It fascinated me, so I made a complete change from solid-state physics to biology. I entered biology from scratch, never having had any formal training. So I would like to thank my mentors, collaborators, and colleagues for helping me along the way. I particularly want to thank UCSF, which has allowed [me] a chance to flourish.

1. J. Zhao *et al.*, Structural insights into human PA28-20S proteasome enabled by efficient tagging and purification of endogenous proteins. *Proc. Natl. Acad. Sci. U.S.A.*, 10.1073/pnas.2207200119 (2022).