ACA: The Structural Science Society

RefleXions

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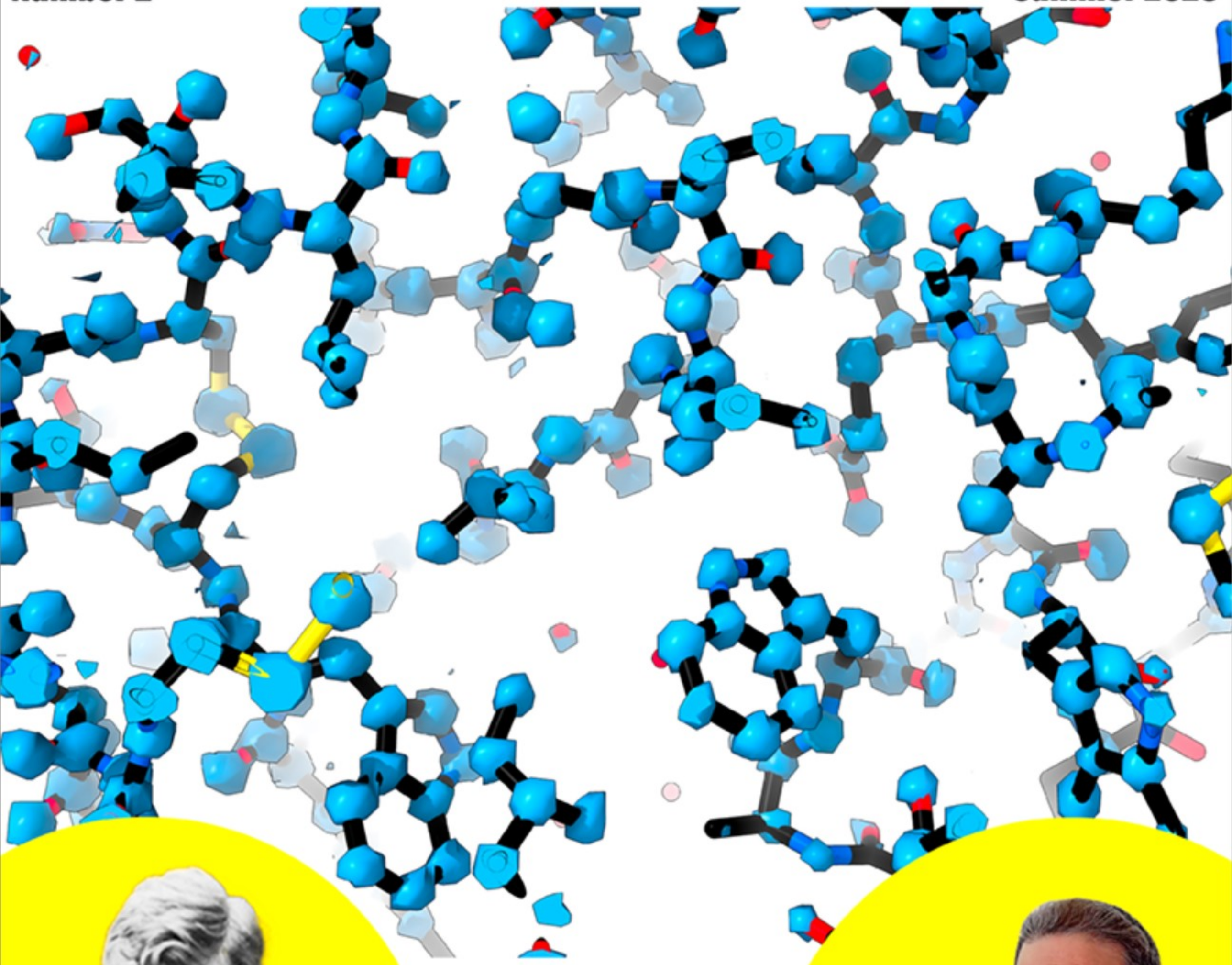

Tamir Gonen receives prestigious A. L. Patterson Award for his pioneering work on MicroED

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About the Cover

An example of a protein solved at subatomic resolution *ab initio* using MicroED. The 0.87 Å resolution map is shown in blue. Continuous rotation MicroED is an effective method for structure determination of macromolecules and small molecules or materials using crystals a billionth the size needed for X-ray crystallography.

Reflections on two decades of MicroED development

Tamir Gonen

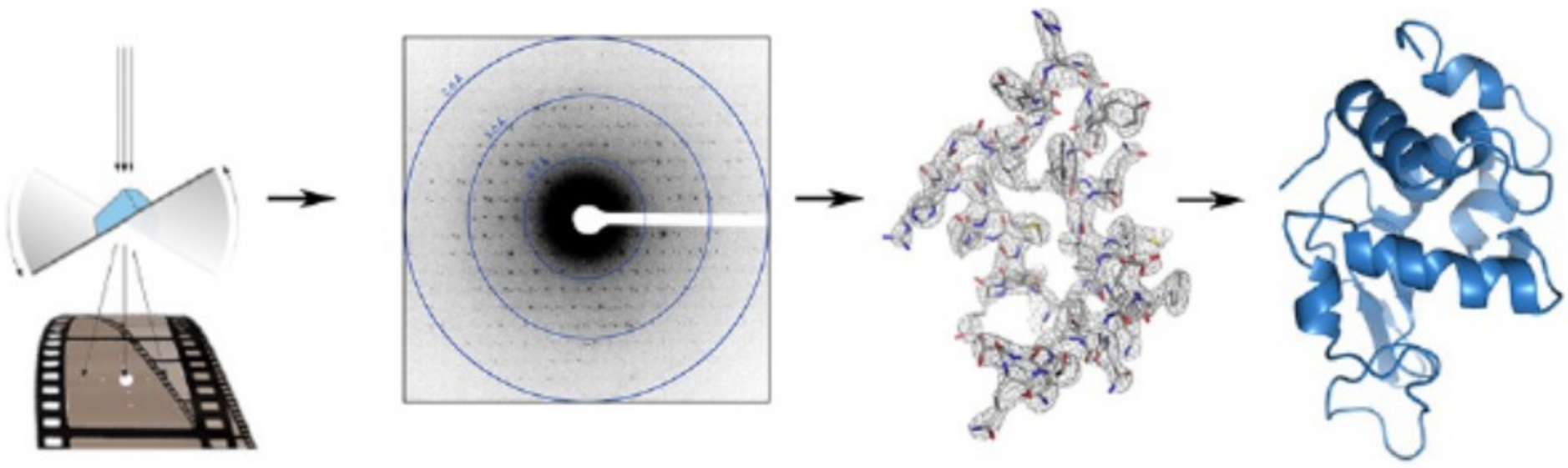
For years, my fascination has centered on understanding how membrane proteins promote cellular homeostasis, particularly the structure of specific membrane proteins and how their structural characteristics relate to their functionality. To that end, I have dedicated my scientific career to examining the structure and function of membrane proteins. Although I was working alongside a prominent X-ray crystallographer (E.N. Baker) as a graduate student, I was more drawn to electron-based techniques than X-ray methods. Opting for electron crystallography over other structural biology approaches was a daring move as an aspiring student. At that time, X-ray studies had already exceeded the atomic resolution benchmark, whereas electron crystallography of biological materials had only achieved a resolution of around 3Å for a handful of samples, and single particle reconstructions were generally unimpressive, just blobs ranging from 15-20Å at best. Despite these shortcomings, I was convinced that with further improvements in methodology, electron crystallography could unlock new, critical insights into how membrane proteins are constructed and how they operate, even uncovering information that other methods couldn't provide.

As such, my professional journey has always centered around pushing the boundaries of electron crystallography and the development of new methodologies aimed at investigating the structure of membrane proteins critical for cellular homeostasis. The breakthrough moment came when I determined the structure of the aquaporin-0 (AQP0)-mediated membrane junction to 1.9Å resolution using electron diffraction from ultra-thin 3D crystals (which we called double-layered 2D crystals). For CryoEM this represented the first instance of atomicity demonstrated in macromolecular electron diffraction, where we observed holes in aromatic residues and saw water molecules, as well as a complete lipid membrane surrounding the channel and it was done a decade before the "resolution revolution" in cryoEM. To achieve this milestone, I developed various data collection and processing procedures, as well as figuring out molecular replacement and structure refinement procedures using electron diffraction data, which included creating an electron scattering library for refinement in CNS. Additionally, the libraries required for modeling the lipids surrounding AQP0 had to be written for manipulation in "O", among other things. These advancements, initiated in the early 2000s, established the foundation for what later evolved into Microcrystal Electron Diffraction or MicroED.

It is worth highlighting that during that period, electron diffraction from biological materials was regarded as an obsolete method, and funding for such studies was practically non-existent. The scientific community was more preoccupied with dynamical scattering, and literature was abound with anecdotes claiming that it would cause significant errors and invalidate any electron diffraction experiment. In material science procedures for precession were established but those are cumbersome and do not work for proteins. With the backing of HHMI and the seclusion of the Janelia Research Campus, we continued developing procedures for determining structures through electron diffraction of 3D crystals, shielded from traditional funding agencies and peer review. In 2013, we unveiled the MicroED method in eLife, demonstrating that it was indeed practical to use electron diffraction for structure determination from 3D crystals. We used standard cryoEM and crystals that were a billionth of the size required for X-ray crystallography. In 2014, we further improved the method by introducing continuous rotation, which is now the standard method of modern electron diffraction studies conducted worldwide.

Continuous rotation MicroED revolutionized electron diffraction by allowing data processing using X-ray

software, making it more powerful and accessible. While other electron diffraction methods were developed, only MicroED could utilize X-ray reduction software, which had been refined over 30 years. This approach has already yielded unprecedented structures in various fields, including macromolecular structural biology, natural product research, materials science, chemistry and analytics. The biggest impact will likely be in analyzing small membrane proteins that are difficult for X-ray crystallography and single particle analysis. The success of MicroED would not have been possible without the contributions of many graduate students, postdoctoral fellows, and researchers, as well as collaborators who made the research exciting and rewarding. Looking ahead, the next decade of MicroED research promises to be just as exciting and groundbreaking.



Tamir Gonen is a membrane biophysicist and an expert in crystallography and cryo-EM. Gonen is a professor of Biological Chemistry and Physiology at the David Geffen School of Medicine of the University of California, Los Angeles, an Investigator of the Howard Hughes Medical Institute, and a Member of the Royal Society of New Zealand. He is the 2023 winner of the ACA A. L. Patterson Award and will give an award presentation at the upcoming 2023 ACA meeting in Baltimore.

The Gonen laboratory at Crystal Cove State Park, CA, June 16th, 2021.