Profile of Dame Carol Robinson

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Over a quarter century ago, chemist Carol Robinson led research that yielded the first mass spectra of molecular chaperones in complex with protein ligands. The achievement, which defied early scientific dogma concerning the theorized limits of mass spectrometry, inspired a discipline: gas-phase structural biology. Since then, the work of this first female professor of chemistry at Oxford University, who was formerly the first female professor of chemistry at Cambridge University, has advanced mass spectrometry's utility in uncovering the 3D architecture of macromolecular complexes. Appointed Dame Commander of the Order of the British Empire in 2013, Robinson was elected as a foreign associate of the National Academy of Sciences in 2017. The following year, Robinson and her team accomplished their goal of ejecting protein complexes directly from native membrane fragments into a mass spectrometer. Her Inaugural Article (1) details her path toward this feat, one that holds promise for improving the assessment of numerous therapeutics.

Gas Liquid Chromatographer at Age 16

Born in the county of Kent in Southeast England, Robinson lived with her family in London until they moved to the port town of Folkestone when she was 10-years-old. She says, "I remember being struck by the fact that there was so much wildlife around. I started a nature collection of wildflowers, frogspawn, and stick insects." She also was fascinated from an early age by the periodic table, which led to an early interest in chemistry.

After taking preuniversity O-level science courses and passing the 11-plus examination administered to some students in their last year of primary education, in 1972, at age 16, Robinson left school. Shortly thereafter, she accepted a laboratory technician position with the pharmaceutical firm Pfizer. She was proud of her title, gas liquid chromatographer, but did not initially enjoy the work. "My role as a technician, however, involved rotations around all the laboratories, and it was when I reached the mass spectrometry laboratory that I felt most at home," she says. "I really took to it and made it my career."

Mentors Guide Work, Education Advancement

Robinson's first supervisor at Pfizer, Trevor Kemp, noticed her skills and offered guidance. "He instilled

Dame Carol Robinson. Image courtesy of Robert Taylor (taylor-photo.co.uk).

my passion for mass spectrometry, and I am still in touch with him to this day. He also encouraged me to aim high. Slightly horrified that I had left school so early, he suggested that I get more qualifications. . . at the local college," she says. Following his advice, Robinson did 4 years of part-time study at the Canterbury College of Technology, leading to certifications in chemistry. She then undertook part-time studies at Medway College of Technology to become a Graduate of the Royal Society of Chemistry before leaving Pfizer to attend the University of Wales, where Robinson earned a Master's degree in 1980.

Robinson next applied to Cambridge University, where she met chemist Dudley Williams. "He told me

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that he had heard that I had 'green fingers' on the mass spectrometer and so would overlook my non-traditional qualifications." With the support of Williams, Robinson was accepted to Cambridge University and earned her doctorate in chemistry in 1982. A fellowship at the University of Bristol Medical School followed before Robinson took an 8-year break to raise her three children.

Robinson resumed studies in 1991, attending the University of Keele for a postgraduate diploma in information technology. She was accepted as a post-doctoral research fellow and later as a Royal Society research fellow at the University of Oxford, where Robinson met chemist Christopher Dobson. He became another important mentor. "During the many times in my career that I doubted my abilities, and focused on the things that I didn't know, he would always point out all the things that I could do, such as my unusual aptitude for mass spectrometry," says Robinson.

Mass Spectra Recording of a Molecular Chaperone

With Dobson and colleagues, in 1994, Robinson used mass spectrometry to capture protein folding in the presence of the molecular chaperone GroEL (2). The seminal accomplishment provided evidence that at least some aspects of protein secondary structure could be preserved in the gas phase. Employing electrospray ionization, her team next investigated noncovalent interactions. They observed that cofactors surprisingly adhered to proteins in the waterless gas phase. The researchers concluded that such binding requires partially folded proteins (3).

Two years later, Robinson proposed a mass spectrometry design to Micromass UK Ltd. that would enable the transmission and analysis of macromolecular complexes. The proposed modifications were made, such that she and coauthor Adam Rostom applied mass spectrometry to the 800-kilodalton GroEL chaperone and successfully projected it into the gas phase (4). Robinson says, "Given its mass, the survival of this 14-mer was surprising but also, and perhaps more importantly, it could be disassociated into 7-mers, paving the way for applications wherein the disruption of complexes could inform models of protein assemblies."

Elucidating Protein Topology and Stoichiometry

In 2001, Robinson moved to Cambridge, where she broke a glass ceiling by becoming the first female professor in Cambridge University's department of chemistry. Two years later, she became a senior research fellow at Cambridge's Churchill College and was awarded the 2003 Biemann Medal from the American Society for Mass Spectrometry.

During this time, Robinson and others made improvements to the application of ion mobility mass spectrometry for protein complexes, which differentiates gas phase ions on a millisecond timescale. This permitted transmission of an 11-mer protein (5). Its collision cross-section was in agreement with the protein complexes retaining a ring-shape. Because this protein and the others that she had studied were recombinant

complexes, Robinson next sought to apply her mass spectrometry approaches to ribosomes and other heterogeneous assemblies. Ribosomes from the bacterium *Thermus thermophilus* yielded a high mass spectral resolution, such that their charge states were resolved and the ribosomes' novel heptameric stalk stoichiometry was revealed for the first time (6).

Projection of Membrane Proteins into the Gas Phase

In 2006, the Royal Society granted Robinson a funded research professorship. The year also marked an early success in her team's quest to define the structural organization of protein subunits using only mass spectrometry restraints. The research involved piecing together mass spectra-generated data to form a model of a yeast exosome (7). X-ray crystallography by another team validated the model a few years later (8).

Another quest concerned membrane proteins. Robinson and her colleagues studied complexes extracted from membranes. While the researchers had achieved success in projecting soluble complexes into the gas phase, membrane protein complexes proved to be more of a challenge until a 2008 breakthrough (9). Says Robinson, "We were able to project an entire membrane protein complex into the gas phase of a mass spectrometer by maintaining its structure in a detergent micelle." The proteins and micelle jointly formed a membrane mimetic that exhibited characteristics of native membrane protein complexes.

Uncovering Roles of Lipids in Membrane Proteins

While still under the Royal Society professorship, which ended in 2016, Robinson left Cambridge for Oxford to accept both an Exeter College fellowship and her current position as Dr. Lee's Professor of Physical and Theoretical Chemistry. Continuing research on membrane protein complexes, Robinson studied rotary ATPase enzymes, which are molecular "motors" involved in biological energy conversion. Her team maintained them intact in a mass spectrometer (10). Robinson says, "Projecting an intact rotary ATPase into the gas phase of a mass spectrometer was a significant milestone. Interestingly, it also revealed the presence of specific lipid binding in the rotary ring."

Other studies on lipids followed. For example, Robinson and her team used mass spectrometry to identify the lipids that interact with a membrane protein of the ammonia channel (AmtB) to change its function and structure (11). Investigating lipid-mediated oligomerization, Robinson and her colleagues calculated interface strengths and showed how some lipid binding stabilizes weak interfaces (12). The researchers speculated that some G protein-coupled receptors might contain lipid-mediated interfaces. A second study confirmed that supposition, and also revealed a previously unknown lipid-mediated interaction in which a specific lipid-binding event enhances the coupling between G protein-coupled receptors and downstream signaling proteins (13).

Protein Assemblies from Native Membranes

Despite her team's success in projecting membrane mimetics into a mass spectrometer, Robinson wished to eject protein complexes directly from native membrane fragments and without being encapsulated in detergent micelles or nanodiscs. She finally achieved the goal in 2018 (14). Robinson says, "By sonicating lipid vesicles and then exposing them to high fields in the mass spectrometer, we showed that we can release complexes, in the absence of detergent or other chemicals, leading to new interactions and subunit stoichiometries."

The achievement, described in her Inaugural Article (1), produced complex mass spectra that Robinson and her colleagues are continuing to interpret. She has already determined that the spectra captured a translocator passing metabolites. Her team's methods are expected to transform the way in which numerous diseases, such as cancer, metabolic, and heart diseases, are studied because the techniques permit analysis of cell membrane proteins in situ.

Since founding OMass Technologies Ltd. in 2016, Robinson has started to form partnership agreements with pharmaceutical and biotechnology companies to tackle challenging targets using mass spectrometry approaches. "My future plans involve adapting some of the recent mass spectrometry approaches we've

developed to study protein complexes within their native environments for drug discovery," Robinson says. "There are a lot of opportunities to apply our approaches to different membrane targets. I am excited about the possibilities."

Promoting Women in Science

For leading the development of advanced mass spectrometry, Robinson has received several awards. They include the 2017 Hans Krebs Medal awarded by the Federation of European Biochemical Societies, the 2018 Field and Franklin Award from the American Chemical Society, and the 2015 L'Oreal-UNESCO for Women in Science Award, which reflects not only her research achievements, but also her teaching and mentorship.

Robinson has supervised more than 35 doctoral graduates, most of whom are women. She has also supervised more than 50 postdoctoral fellows, of whom over half were women. Robinson says, "I take an active role in mentoring younger women through the tenure process and encouraging them to stay in science. My research group has included many scientists with children, many of whom have gone on to achieve high profile academic jobs elsewhere."

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