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Louise N. Johnson 1940–2012

David Barford & David I Stuart

Louise Johnson was a leading architect of modern-day protein crystallography. She pioneered the application of the technique to understand how enzymes function at the molecular level. Much of our current knowledge of how enzymes catalyze chemical reactions with high specificity and how their activities are regulated, especially by reversible protein phosphorylation, have their origins in Louise's research on lysozyme, glycogen phosphorylase and protein kinases. Her delight in science and kindness toward her colleagues were an inspiration to those who knew her.

Louise graduated with a degree in physics from University College London and, in 1962, started her PhD research at the Royal Institution, also in London, whose director was Sir Lawrence Bragg. While Cavendish Professor in Cambridge, Bragg had overseen John Kendrew's and Max Perutz's crystallographic studies of myoglobin and hemoglobin that, in the late 1950s, had revealed the first structure of a protein. By the 1960s, it was time to move on to enzymes. Louise joined a team, led by David Phillips, that was investigating the crystal structure of lysozyme. To identify the enzyme's catalytic site, Louise incubated lysozyme crystals with the inhibitor tri-*N*-acetylglucosamine and bacterial cell wall–derived oligosaccharides and used difference Fourier analysis[1]. On the basis of Louise's structures, model building and carbohydrate chemistry, Phillips deduced the first stereochemical description of an enzymatic mechanism, demonstrating the enormous explanatory power of protein crystallography and founding the field of structural enzymology.

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David Phillips and Louise Johnson at the Ciba Foundation in 1991. (Ad Bax is in the background.)

In 1966, Louise moved to Yale University for postdoctoral research in the group of Frederic Richards. There, she contributed to the structure determination of RNase S (ref. 2). Louise returned to the UK a year later, joining the newly created Laboratory of Molecular Biophysics, headed by David Phillips and based in Oxford University's Zoology Department. The head of the department, John Pringle, had a remarkable vision of integrating biology from molecule to animal. The Phillips laboratory was key to establishing a major research program to understand the structural and evolutionary relationships of enzymes of the glycolytic pathway. The grant award system at that time allowed Louise freedom to explore a variety of projects, and these included crystallographic studies of aldolase and triose phosphate isomerase.

In 1971, Louise initiated her groundbreaking studies on glycogen phosphorylase, which would remain the focus of her laboratory for nearly 30 years. Phosphorylase controls glycogen metabolism. In muscle, the enzyme regulates production of glucose-1-phosphate to fuel muscle contraction, whereas the liver enzyme functions to control blood glucose homeostasis.

Muscle glycogen phosphorylase is a fascinating protein, and Louise's analysis of its various structural states provided remarkable insights into its regulatory mechanism. The enzyme integrates signals by metabolites that act as allosteric effectors according to the energy needs of the cell with signals triggered by adrenaline and neurons. The latter effects are exerted by phosphorylase kinase, which activates phosphorylase by modifying a single serine residue on the enzyme. The discovery of the control of phosphorylase by reversible protein phosphorylation, made in 1955 by Edmond Fischer and Edwin Krebs, was the first description of the control of protein function by a reversible covalent modification[3]. By the early 1970s, such a regulatory mechanism had only been uncovered for a few biological systems. Regulation by tyrosine phosphorylation had yet to be discovered. Louise's choice of glycogen phosphorylase to understand the structural consequences of protein phosphorylation was an inspired and fortunate one. By 1991, structures of the various functional states of phosphorylase had been determined, including contributions from Robert Fletterick and colleagues, allowing an understanding of the regulation of the enzyme by allosteric effectors and phosphorylation[4–9]. In the late 1990s, Louise's team also answered the long-standing question of how phosphorylase recognizes oligosaccharides at the catalytic site, and the enzyme's complete catalytic mechanism was defined[10].

Research on phosphorylase was a massive challenge. In the 1970s, the protein was by far the largest crystallized at that time, and the determination of its structure was dependent on technical advances and serendipity. Although initial crystallographic studies of phosphorylase were performed on monoclinic crystals, their weak diffraction and large unit-cell dimensions precluded high-resolution analysis. It was the discovery of a new tetragonal crystal form of phosphorylase b (the unphosphorylated form of the enzyme), requiring IMP for its growth, that facilitated determination of the phosphorylase b structure[11]. The presence of IMP was unintentional. Contaminating AMP deaminase in the phosphorylase preparation had converted the activator AMP into IMP. Technical advances for data collection were also crucial, including the development of the Arndt-Wonacott oscillation camera and the intense and highly collimated X-ray beams afforded by synchrotron radiation. Louise was one of the first to realize the potential of synchrotron sources as a tool for protein crystallography, and her group members (notably Keith Wilson) were early users, initially at the LURE synchrotron in Paris and then at the first dedicated synchrotron, at Daresbury in the UK.

David Barford is at the Institute of Cancer Research, Chester Beatty Laboratories, London, UK. David I. Stuart is at the Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK and the Diamond Light Source, Oxfordshire, UK.

Her group exploited the bright synchrotron radiation to conduct time-resolved studies to visualize the enzymatic reaction catalyzed within phosphorylase crystals[12].

Control of glycogen phosphorylase by allosteric effectors conforms to the two-state model of concerted allosteric transitions proposed by Monod, Wyman and Changeux[13]. Carl and Gerty Cori discovered that phosphorylase b catalytic activity had an absolute requirement for AMP[14]. Phosphorylase a (the phosphorylated form of the enzyme) is fully active without AMP. The structures of phosphorylase in different functional states revealed that communication between the AMP allosteric site and the catalytic site—some 40 Å away—could be explained by the coupling of tertiary and quaternary conformational changes. The phosphorylase studies were among the first descriptions of how an enzyme is controlled by allosteric transitions, and the regulatory mechanisms demonstrated by phosphorylase shared the same molecular principles of allostery first revealed from structures of T and R states of hemoglobin[15].

Louise's work on phosphorylase provided the first molecular explanation for the control of a protein activity by phosphorylation. The phosphorylation event converted the N terminus of the enzyme into an allosteric effector that bound at the dimer interface, stabilizing the same activated tertiary and quaternary conformations as those promoted by AMP. This beautifully explained how distinct signaling mechanisms invoke the same biological output. The phosphorylase study anticipated the tremendous capacity of a reversibly attached phosphate group to mediate diverse structural changes of proteins and multiprotein complexes, rationalizing why phosphorylation is involved in virtually every regulated biological process.

In the 1990s, Louise's research interests had grown to encompass studies to understand the molecular mechanisms underlying protein phosphorylation—how protein kinases are regulated and how they identify their substrates. Her group solved the first serine/threonine protein kinase in complex with a peptide substrate[16], the structure of the phosphorylase kinase catalytic subunit in complex with a phosphorylase-like peptide. More recently, her group used single-particle EM to investigate how the phosphorylase kinase holoenzyme interacts with the intact phosphorylase dimer[17]. Studies of CDK2–cyclin A explained the molecular basis for the substrate specificity of CDKs and how protein-recruitment sites on cyclins influence CDK specificity[18]. Her work on protein-kinase regulation had a major impact on the field because it recognized the central role of the activation segment in controlling kinase activity[19]. Louise's insight also had important implications for understanding how oncogenic mutations activate protein kinases and how drugs inhibit their activity. Using techniques that traced back to her ligand-binding studies with lysozyme, Louise's group applied structure-based design approaches for the development of CDK inhibitors as anticancer therapies[20].

Louise spent over 40 years in Oxford, with 'great contentment'[21]. In 1967, she was appointed as a University Demonstrator in the Department of Zoology and as the Janet Vaughan Lecturer at Somerville College, rising to become David Phillips Professor of Molecular Biophysics within the Department of Biochemistry and Professorial Fellow at Corpus Christi College in 1990. In 2003, Louise was appointed Life Sciences Director at the Diamond Light Source that was to be built near Oxford, while maintaining her research group at Oxford University. She was a powerful and enthusiastic advocate of new techniques and instrumentation to advance the scope and capacity of structural biology to address increasingly ambitious questions. In recent years, Louise had become intrigued by the prospects of X-ray imaging of whole cells. She had championed the X-ray tomography beamlines at Diamond and performed her own experiments with live cells on the X-ray free-electron laser (FLASH) in Hamburg.

Louise had a strong commitment to education and training. Through her wise guidance and support, she mentored and influenced the careers of many graduate students and postdoctoral fellows and was generous in acknowledging their contributions to work from her laboratory. With Tom Blundell, she wrote the classic and influential textbook *Protein Crystallography*. Louise also had a deep interest in encouraging science in the Islamic world and advised on the construction of the SESAME synchrotron in Jordan. Her achievements were recognized by numerous awards and honors, including election to the Royal Society in 1990, appointment as Associate Fellow of the Third World Academy of Sciences in 2000, election as a Foreign Associate Member of the US National Academy of Sciences in 2011 and receipt of the Novartis Medal and Prize of the Biochemical Society.

Louise was fond of quoting Humphrey Davy who, when asked to define his most important discovery, named Michael Faraday. Louise herself wrote, "In many ways I feel that my most important discoveries have been those with whom I have worked and trained."[21]. This captures something of her unassuming modesty and generosity which, along with a clear mind and firm conviction, allowed her to influence and inspire those people for the better.

In 1962, the year Louise started her PhD research, her interest in science and world affairs took her to the Pugwash conference in London, where Bertrand Russell spoke, and where she met Abdus Salam, the Pakistani physicist and future Nobel laureate. They were married in 1968 and had two children.

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