Biography of Martha L. Ludwig

In 1965, lysozyme became the first three-dimensional enzyme structure to be solved with x-ray crystallography (1). At the time, Martha L. Ludwig was a young research fellow at Harvard University (Cambridge, MA) attempting to solve an enzyme structure of her own, that of carboxypeptidase A. Her results were soon forthcoming (2–5). Ludwig recalls that event fondly, although she now finds the thought of hand-contouring the structures on sheets of paper "unfathomable." These days, of course, the image would be compiled in seconds on a computer screen. Over the next three and a half decades Ludwig's career continued to flourish alongside the rapidly evolving field of x-ray crystallography. For her lifetime of accomplishments Ludwig was elected to the National Academy of Sciences in 2003.

In her Inaugural Article, published in this issue of PNAS, Ludwig and colleagues describe their more recent studies of the crystal structures of the N-terminal substrate-binding modules of methionine synthase (MetH) from *Thermotoga maritima* in complex with its substrates homocysteine and methyltetrahydrofolate and its cofactor cobalamin (vitamin B_{12}) (6). Importantly, Ludwig and colleagues found that the substrate-binding domains are intimately associated with $(\beta\alpha)_8$ barrels and that the two active sites are separated by ≈50 Å. The arrangement of the barrels suggests that the cobalamin-binding domain must swing back and forth to reach the two active sites. This motion is in contrast to the typical movements within enzymes, which more often involve swivels or rotations. According to the researchers, the unusual and dramatic swinging movements that occur during the activation of methionine synthase may be a general paradigm for the molecules of signaling pathways in which domains interact with multiple targets.

Visually Inclined

Ludwig has built a long and accomplished career on her analysis of crystal structures. Born in Pittsburgh in 1931, Ludwig completed her undergraduate degree in chemistry at Cornell University (Ithaca, NY) in 1952. She went on to obtain her master's degree in biochemistry at the University of California, Berkeley, and then a Ph.D. degree in biochemistry at Cornell University Medical College in 1956. Ludwig subsequently held postdoctoral positions at Harvard and the Massachusetts Institute of Technology (Cambridge). While in these positions, she used classical techniques of biochemistry, such as protein purification and chromatography, to study interactions between proteins. It was in 1962, when great strides were being made in x-ray crystallography, that Ludwig became interested in the field. "It was the realization that we could finally look at where the atoms were. I became an early convert of three-dimensional structures," she said. "I'm always delighted to look at a new structure. The visual part of it is personally satisfying, and many of the basic hypotheses about how enzymes work have come directly from observing structures."

Martha L. Ludwig

As a result of her interest in 3D structures, Ludwig returned to Harvard in 1962 to study under the mentorship of William N. Lipscomb. During this time, she helped solve the structures of carboxypeptidase A (2–5), a feat she still considers one of her greatest. In 1967, Ludwig moved to the University of Michigan (Ann Arbor) to study flavodoxins under the direction of Vincent Massey (7). She has remained at the university since, ultimately serving as chair of the biophysics research division of the Institute of Science and Technology.

Structurally Speaking

Over the years, Ludwig's work has delved into the structures of several enzymes, including the flavin-dependent hydroxylases, thioredoxin reductase, and, most recently, methionine synthase. Importantly, defects in methionine synthase may result in elevated homocysteine levels, a suspected risk factor for heart disease; thus, the study of this enzyme may have important implications for the treatment of heart disease.

Specifically, Ludwig has attempted to define the significance of conformational changes and the way in which the reactivity of bound cofactors, such as flavin, cobalamin, and transition metals, influences proteins interactions. In a group of enzymes known as flavodoxins, for example, Ludwig's work established the relationship between the enzyme flavodoxin and its cofactor flavin mononucleotide (FMN), which acts as an electron carrier (8, 9). In addition, studies of mutant flavodoxins by Ludwig and Vincent Massey, her colleague at the University of Michigan, have documented the roles of hydrogen bonding, electrostatic interactions, and peptide "flips" in controlling the redox potential of bound FMN. The researchers have also examined the gating functions performed by flavin, the equilibria between conformations, and the way in which NADPH is bound (10–12).

Ludwig and her colleagues have studied another enzyme, thioredoxin reductase (TRR), which catalyzes the transfer of electrons from NADPH to thioredoxin. The previously solved structure of TRR indicated that two domains were positioned for the half reaction in which flavin reduces the active site disulfide of the enzyme (13). With their efforts, Ludwig and her colleagues revealed the existence of an alternate domain arrangement that permits reduction of FAD by NADPH and oxidation of the enzyme dithiol by the protein substrate thioredoxin (14). Furthermore, the x-ray analysis of this complex confirmed that switching between the two conformations entailed a large and unusual ball-and-socket rotation of 67°.

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This is a Biography of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 3729.

Much of Ludwig's work has focused on the enzyme methionine synthase. Previously, Ludwig and colleagues described the N-terminal modules of methionine synthase (2) and isolated other fragments of the enzyme, such as the adenosylmethionine binding module (15) and the C-terminal domains arranged for reactivation (16). The most recent work in Ludwig's laboratory, and the subject of her Inaugural Article, suggests that the methylated cobalamin form of methionine synthase exists as an ensemble of interconverting conformational states (17). In addition, differential binding of substrates and products appears to alter the distribution of conformers, suggesting that the methylation state of the cobalamin influences the distribution of conformers during turnover. Finally, analysis of the cobalamin-binding fragment of methionine synthase in Ludwig's laboratory (18) provided the first structure of B_{12} bound to a protein, which is accepted as an important contribution to knowledge of vitamin B_{12} chemistry.

On Being a Scientist

According to Ludwig, many people have helped guide her career, but three people stand out as being her most influential mentors. One of them is Academy member Howard K. Schachman, whose course in physical biochemistry she took while at the University of California, Berkeley. "It totally changed what I decided I wanted to do in research," she stated. The other two, also Academy members, are William N. Lipscomb and Vincent Massey. "I joined Lipscomb's group to learn crystallography and to work on carboxypeptidase, whereas Massey was a senior faculty person when I came to the University of Michigan, and I began my work here by collaborating with him," she said. "Massey had enormous impact on studies of flavoproteins and on a number of research groups here at Michigan," she added.

Although Ludwig entered science at a time when women were more likely to work inside rather than outside the home, she maintains that being female has never been a "big issue" for her. "Through all of my training I thought 'I'm going to go out there on a level playing field with all the other folks,' although admittedly more of them are male than female." In addition, when she was at Cornell and the University of California, Berkeley, a fair number of women were majoring in chemistry and biochemistry, she notes. "I've found over the years that both male and female scientists have been good models and mentors." Ludwig's significant female mentors include Dorothy Hodgkin, who has been dubbed a founder of the science of protein crystallography and who held a position at Oxford University, and Mildred Cohn, a professor at the University of Pennsylvania (Philadelphia) who pioneered the use of stable isotopes to study metabolic processes and enzymatic reactions. "If I had been male, maybe I would have started with physics, but I probably would have ended up in the field I'm in now. It has lots of personal attraction for me," she explained.

Ludwig, aged 72, says she is considering retiring but hasn't quite reached that point yet. "I've enjoyed my work so much, I question 'what would I do?'" She speculates that she might enjoy teaching elementary school students to become more excited about science. "But at some point, especially with computer advances, one begins to feel that one isn't productive enough," she said, pointing out that the field now requires collaboration with scientists from a spectrum of specialties, including mathematics and computational analysis.

Ludwig hopes that future research in her field will connect the plethora of structural information (she estimates ≈20,000 structures) to energetics and dynamics. By far the most important factor in the success of x-ray crystallography, Ludwig says, is the ability to produce and purify proteins from recombinant DNA technology. "This technology lets us solve x-ray structures of practically any molecule we would like to target," she noted. Ludwig remembers a time when she worried about what was going to happen when all the "low-hanging fruit" had been harvested. "And then, this enormous change occurred because we were able to clone and express DNA sequences of very rare proteins," she said. "The field has come a long way from the days when I sketched out structures on sheets of paper."

Emma Hitt, *Freelance Science Writer*

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