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Spinning with Dave: David Yphantis's contributions to ultracentrifugation

John J. Correia[a,*], Michael L. Johnson[b], Tom Laue[c], Walter F. Stafford[d], Robley C. Williams[e]

[a]*Department of Biochemistry, University of Mississippi Medical Center, 2500 North State St., Jackson, MS 39216, USA*
[b]*Center for Biomathematical Technology, University of Virginia Health System, Charlottesville, VA, USA*
[c]*Center of Advance Molecular Interaction Science, University of New Hampshire, Durham, NH, USA*
[d]*Analytical Ultracentrifugation Research Laboratory, Boston Biomedical Research Institute, Watertown, MA, USA*
[e]*Department of Molecular Biology, Vanderbilt University, Nashville, TN, USA*

Abstract

For nearly 50 years David Yphantis has helped advance analytical ultracentrifugation, promoted rigor in the thermodynamic analysis of biochemical data and encouraged students and colleagues to look for the deepest possible understanding of science. This article, written by five of Daves's students, presents some of the impressions he has made over the years.

Keywords: Ultracentifugation; Model E, XLA/XLI ultracentrifuges; National analytical ultracentrifugation facility (NAUF)

1. Introduction

Analytical ultracentrifugation has been an important biophysical technique for over 75 years. For the past five decades David A. Yphantis has been a creative and innovative force in the field. His first ultracentrifuge paper, written with David Waugh, his Ph.D. mentor, was published in 1952. Entitled 'Rotor Temperature Measurement and Control in the Ultracentrifuge' [1], it served as the foundation for development of the radiometric detector used in most modern centrifuges (but not the Model E), and launched Dave's lifelong passion for redesigning and building improvements for the Model E and the current XLA/XLI.

The Model E ultracentrifuges in Dave's lab were subjected to constant modification and redesign, especially the optical system, to the point that we are not sure if any of us ever knew what a stock Model E looked like. Two phrases we have all regularly experienced in Dave's presence were 'We can do better!' typically delivered with a smile and a wink, and 'It is only work!', a maxim intended to encourage the flagging student. Throughout our careers Dave's optimism and expectations have been both a guiding light and a source of motivation.

Dave has been devoted to the spirit of quantitative description, contributing both to theory and

*Corresponding author. +1-601-984-1522; fax: +1-601-984-1501.
E-mail address: jcorreia@biochem.umsmed.edu (J.J. Correia).

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experiment. His experimental work was guided by a full grasp of the physical principles and mathematics used to describe it. It is ironic that Dave's research on instrument development often was dismissed by reviewers as simply being 'methods development' and not worthy of the same esteem as hypothesis-driven scientific research. What many of the reviewers failed to appreciate was that having a profound grasp of the theory (often well beyond their own) was essential to the success and insightful application of his designs.

2. The early years

Born in Boston July 14, 1930, Dave spent many of his early years in Greece. He left Greece in 1942 with his immediate family during the World War II occupation of Greece by Germany in an exchange of diplomatic personnel. He graduated magna cum laude from Harvard University with an A.B. in physics in 1952. Crucial to the direction of his career was the effects of an incident at the freshman-dining hall. At the time, dining hall policy required that coat and tie be worn by all male diners. On a hot spring evening Dave decided to forego shirt and trousers and dine wearing a bathing suit along with the requisite coat and tie. The official reaction that followed led to the loss of his Harvard National Scholarship and made it necessary to find employment so as to stay in school. Fortunately an appropriate position was found taking care of the animal room for the Biology Department at MIT. The position was shortly expanded to include an apprenticeship under David Waugh on the use of one of the first Model E ultracentrifuges that had just been installed. This led to a research project to determine the molecular weight of the active principle of ACTH using the available impure preparations: transient sedimentation of the hormone was followed by biological assay of two fractions removed from modified analytical centerpieces (dubbed separation cells).

After graduation from Harvard, Dave entered the Massachusetts Institute of Technology to work with David Waugh on the continuation of this project. His thesis was entitled 'Determination of sedimentation coefficients by assay of intrinsic chemical or biological activity'. He received his Ph.D. in Biophysics in 1955. (Peter von Hippel was a contemporary and graduated the same year from the same lab. Pete was an usher at Dave and Lorna's wedding, and remains a close friend. See his comments later in this issue [von Hippel].) With his mentor David Waugh, Dave published five papers from 1952 to 1957 on ultracentrifuge instrumentation, theory, data analysis and protein characterization [1–5]. Available to them was the MIT Differential Analyzer #2, an analog computer they employed for integrations of the Lamm equation, the differential equation describing sedimentation and diffusion in a centrifugal field. Their 1956 papers, 'Ultracentrifugal Characterization by Direct Measurement of Activity' [3,4], described procedures and approaches to estimate size and shape based completely on biological assays (and complimented the rigorous theoretical work of Goldberg [6]). This work was aided by the development of separation cells allowing the fractionation of the cell contents at the end of the run [4,7]. The several areas covered in this thesis work would be focal topics for the rest of his career.

After a short postdoc with Waugh, Dave spent 2 years at the Argonne National Laboratory before going to Rockefeller University as an assistant professor. There he studied the sedimentation behavior of proteins of interest to many investigators, especially those connected with Lyman C. Craig's group—notably Guido Guidotti and T.P. King. His 1959 work on the Archibald technique, 'Ultracentrifugal Molecular Weight Averages During the Approach to Equilibrium' [8], was a preface to weightier stuff. In 1960, Dave combined his insight for experimental design with his first major development in accessory applications by describing short column centerpieces [9]. This development took advantage of the relationship described by van Holde and Baldwin a few years earlier [10] that time to equilibrium was proportional to the square of the column height; therefore four times shorter allowed the achievement of equilibrium in $1/16$ of the typical time. Dave and Jack Correia showed that the loss of information content due to fewer points and a narrower data spread can be partially recovered by spinning at higher speeds (shown in a reinvestigation of the

technique years later; [11]). The development of new centerpiece designs, already facilitated by Nils Jernberg and the skilled machinists of the Rockefeller instrument shop, continued with the development of the six-channel centerpiece for equilibrium runs. This appeared in Dave's classic 1964 Biochemistry paper [12] that also introduced the 'meniscus depletion' (a.k.a. 'high speed' and 'Yphantis') technique. This method required spinning the sample at a rotor speed high enough to deplete the meniscus of macromolecules. Quantitatively, this speed can be described by a parameter coined the reduced molecular weight, or $\sigma = M(1-\nu\rho)\omega^2/\mathrm{RT}$, which reflects the effective curvature of the pattern. Sigma values of >4 cm^2 would result in meniscus depletion, which then allowed one to fix the meniscus concentration to zero and then mathematically propagate fringe displacement directly into solute concentration and, by moment analysis, to appropriate molecular weight averages. Without depletion the uncertainty of the meniscus concentration propagated as error into the MW calculations. This method became the gold standard for all AUC experimentalists, and this publication was listed by E. Garfield [12] as one of the 100 most cited papers in the scientific literature. While this paper still is frequently referenced, it is unlikely that it is actually read as frequently as it is cited. It is a dense work, full of mathematics covering thermodynamics, optics and sedimentation. Howard Schachman, who served on the editorial board of Biochemistry at that time, commented that he had to argue vehemently with the rest of the board to have the paper published in toto; he alone realized the full import of the presentation [see Schachman later in this issue]. It was in this paper that Dave pointed out that computers could be used to fit sedimentation data to first-principle equations, thus foreshadowing the subsequent development of all of the curve fitting programs that are at the heart of modern ultracentrifugation and biophysics.

The development of centerpieces culminated 6 years later with the description and testing of the six-channel external loading centerpieces [13]. They were designed with double filling holes for each solvent and solution channel so that all compartments could be cleaned by flushing using a 'hexapus' (six pieces of plastic tubing connected to a rubber stopper for attachment to a side arm flask) between data collection on the samples and pre- and post-baselines. Once assembled and brought up to speed a few times to stabilize the window stress, they could be used for years without disassembly. The major advantage was the accurate collection of reproducible baselines. Every external loader used in Dave's lab through the 1980s was constructed (fitting of plastic into aluminum rings, cutting of the channels with a special broach, drilling and tapping of the filling holes) by the instrument shop at Rockefeller University. Raymond Kikas, Dave's lab technician for over 30 years, did the final polishing of the centerpiece on ground glass, as well as the actual assembly of casing, centerpiece, and windows. In the 1990s, the National Analytical Ultracentrifuge Facility at the University of Connecticut started producing external loaders. Finally, in 1999, Beckman started production of these six-channel external loaders, although the first generation cells contained only one filling hole so they are not as easy to clean between scans. Beckman has recently released a two-filling-hole double sector version of the standard double sector assembly that also allows for flushing without disassembly (part #s 392772 or 392773).

In 1965, Dave and George Weiss at the National Institutes of Health began a series of what would eventually be 14 papers [14–27] on solutions to the Lamm equation, investigating the behavior of macromolecules during sedimentation. The series spanned from 1965 to 1977, and the topics spanned the full breadth of the field and included velocity and equilibrium methods, band sedimentation, density gradient centrifugation, pressure effects, concentration dependence, the Johnson–Ogston effect, and methods to extrapolate to equilibrium distributions. These studies were conducted with IBM mainframe computers (at the time the fastest processors available) that typically required 12 h to perform a single set of calculations corresponding to one velocity run. Subsequent finite element solutions by Claverie [28] (see Sedfit, SedAnal) and approximate analytical solutions (see SVEDBERG, DCDT+) have now been implemented by many software developers and are routine compo-

nents of fitting algorithms run on desktop computers. The major developments today are in the area of direct fitting of a reaction boundary profile (absorbance or fringe displacement vs. time) to extract composition, mechanism and energetics information, areas that were also inspired and influenced by Dave's early developments [see articles by Schuck and Stafford later in this issue].

During the years 1958–1965 at Rockefeller University (then the Rockefeller Institute for Medical Research) Dave commuted to Manhattan from New Jersey, where he and Lorna were increasing the size of their family. Raymond Kikas, an Estonian who came to the US in 1959, and in his first US position, joined the laboratory as senior technician and stayed with Dave until retirement in 1990. In addition to his considerable machinist skills, Raymond often brought in homemade European delicacies (e.g. apple-wood smoked eel). He also served as a conscience for the laboratory: his WW II experiences made him acutely aware of human rights and he was an early supporter of Amnesty International. At Rockefeller, Dave was part of the larger laboratory group of Lyman Craig, with whom he published papers on bovine and human plasma albumin [29,30]. When graduate students came to Rockefeller, Dave began teaching a didactic and laboratory course in biophysical chemistry that covered both Edsall and Wyman's Biophysical Chemistry and Tanford's Physical Chemistry of Macromolecules. This was an extremely rigorous course on equilibrium and non-equilibrium thermodynamics that was an intense rite of passage for any student making passage through Dave's lab. Intrigued by the synergy of quantitative description and biological subject matter revealed in that course, Robley Williams, Jr. came to the lab in 1963, starting his dissertation research on the association in solution of the cyclic decapeptide tyrocidine B [31]. Dave's lab in those years was located in the basement of Founder's Hall, in the three small rooms Sinclair Lewis described in his novel Arrowsmith. Dave cheerfully shared the lab with Robley and with Allen Ansevin, who did postdoctoral studies of improved methods for characterizing DNA and later worked with Dave on the design of the external loader centerpiece [13]. The protein physical chemists Lewis Longsworth and Gertrude Perlman occupied the laboratories across the hall, constituting a small group with allied interests. When, in 1965, Dave decided to go to SUNY Buffalo, Robley stayed at the Rockefeller and completed his thesis with direct supervision from Lyman Craig, commuting up to Buffalo every few months to confer with Dave.

Early on protein chemists recognized that solubility was a problem for many proteins. At SUNY Buffalo, Dave and Sara Szuchet explored the use of organic acids to improve protein solubility and enhance dissociation into subunits. Later on at UCONN, another graduate student, Carl Paul, looked for ways to synthesize detergents that was neutrally buoyant in standard solvents. Sara's work turned into an exploration of charge-related thermodynamic non-ideality [32–34], while Carl became intrigued with the notion of using lasers as the light source for the Rayleigh interferometric optics [35,36].

Todd Schuster, then an assistant professor at SUNY Buffalo, occupied a neighboring laboratory. His deep involvement with the cooperativity of hemoglobin produced long and productive discussions of physical techniques and experimental strategy. Robley arrived in Buffalo in the fall of 1967 to do a postdoc with Dave, joining Dennie Roark, Sara Szuchet, Carl Paul and Gay-May Wu in the laboratory. ('He was my adviser and so I decided it would be nice to actually work with him for awhile,' Robley recalls.) Dave stayed at Buffalo for only 2 years. Just as Robley arrived, Dave, who had been made department chairman at Buffalo and loathed the time lost from science, moved to the University of Connecticut at Storrs. Dave made this change in part because of the recent developments in the field of biophysics and ultracentrifugation at UConn. Emory Braswell, a physical chemist, arrived at the UConn chemistry department in 1962, fresh from a postdoc position with Geoffrey Gilbert, a well-known contributor to transport theory and molecular interaction during velocity sedimentation, at the University of Birmingham, England. Emory was contemplating a move to Buffalo, coincidentally, but Heinz Herrmann, a developmental biologist at UConn, persuaded him to transfer to the biology group as

Fig. 1. Dave at the University of Connecticut ~1968, in a usual repose, with pipe, oscilloscope and playing with the Model E. The tie is very unusual for Dave, although he kept a clip-on tie in his office for the rare occasion when he needed one. This photo was taken from the Biochemistry and Biophysics Department brochure, 1971.

the first biophysicist (in 1964) and gave him a recently purchased Beckman Model E. At that time, biology at UConn was going through rapid growth and reorganization and everyone was told to go out and recruit the best scientists possible. Emory convinced Gerson Kegeles to join the newly formed biochemistry and biophysics section of the very large biology group. Gerson agreed to come but only if he could bring two or three additional biophysicists. His first choice was Dave Yphantis (Fig. 1).

3. University of Connecticut

Coincidentally, part of Dave's attraction to Storrs was that Gerson Kegeles had also agreed to come there. 'Keg' had been at Clark University in Massachusetts and was renowned in the ultracentrifuge and diffusion fields for his insightful and thoughtful approach to science. He began his career working with Louis Gosting on the theory of and measurements with a Gouy diffusiometer. Later Keg did extensive ultracentrifugation, countercurrent distribution and pressure jump studies on reacting systems; developing optical systems; describing the application of flotation sedimentation; the significance and use of weight average values; and incorporating pressure and kinetically mediated effects into the analysis [37,38]. From the late 1960s through the 1970s, both Keg and Dave worked on hemocyanin, the blue blood of lobster, *Homarus americanus* (Keg), and horseshoe crab, *Limulus polyphemus* (Dave). (Since Keg and Dave are allergic to many types of seafood, the choice of species was not a culinary decision.) Hemocyanin is a complex nested, allosteric O_2 transport system composed of heterogeneous subunits and numerous intermediate

assembly states, making it perfect for ultracentrifuge analysis and still used as a model system at the NAUF workshop in Storrs. Extraction of the bright blue blood is a simple matter of tapping through a membrane in the animal's midsection; once the blood is withdrawn, the animal is returned to the tank or the ocean. Hemocyanin extracted in this manner is essentially pure and is commonly used without further purification.

Dave requested that Todd Schuster come with him to Storrs. A bit later, James Knox came as the first protein X-ray crystallographer at UConn to essentially complete the biophysics group, although his position was through the Institute of Material Sciences. Thus the biophysics group at UConn in 1970 consisted of Keg, Dave, Todd, Jim and Emory. Robley followed Dave again, via Woods Hole, but spent only 6 months in Storrs, leaving for an assistant professorship at Yale in 1968.[1]

Another attraction to UConn for Dave was that he was promised he would never be made department chairman. Dave's chairmanship in Buffalo had spawned a lifetime's disdain of administrative work and the lab group often noticed that the mimeograph-blue notices of various faculty meetings were a favored source of scrap paper. Dave's real love and talent is doing science and teaching students to do science. Dave's view was that if the student was going to use an instrument, then the student absolutely must know the 'ins and outs' of how the instrument worked. This was especially true of the analytical ultracentrifuge. The Beckman service people loved working on Dave's Model E because they would bring the parts and then watch while Dave and the students serviced the machine, sometimes rejecting several drive motors at a time because of excess precession.

One of the great moments for Dave was when he learned that his National Science Foundation grant made him eligible to bid on surplus government equipment. The surplus lists were studied to see if anything could be used for the instrumentation projects. At times the stacks of surplus equipment occupied almost all the space within the lab. Once a group of IBM circuit boards were found that looked interesting, so a phone call was placed to the IBM contact person to ask what the circuit boards were. It caused quite a ruckus because they were classified top-secret components of the guidance computers from an ICBM. IBM wanted to know how we even knew the circuit boards existed. The lab never got these circuit boards because they were immediately removed from the surplus list.

Many of the lab's computers were also obtained from surplus. For example, one of the computers that operated the automatic photographic plate reader (discussed below) was a PDP-8I that formerly operated a US radar installation in Iran. Another computer was cylindrically shaped so that it would fit through the entrance of a submarine. Several Bell X-15 flight computers were obtained. With the help of Dave Rhodes, these were revamped and put to use. The great coup, however, came at the termination of Apollo project. In a flurry of phone calls, Dave managed to get much of the LEM project surplus of integrated circuits, small hardware items, several hundred square feet of copper-clad circuit board and approximately a half-ton of liquid mercury. While the Model E was famous for using mercury for electrical connections to the thermistor, a few ounces of mercury would suffice for several years. Many plans for its use were discussed including ballast for driving over the snowy Connecticut roads or a pool supporting an optical track to damp vibrations. Eventually, OSHA was created and the mercury was disposed of appropriately.

During this time, Dave also participated in the physiology course (summers, 1968–1973) at the Marine Biological Laboratory in Woods Hole, Mass., replacing Ken van Holde as an instructor

[1] When Dave first went to Storrs, he spent a few months building an electronically controlled double-distilled water purification system, an effort that Robley said astonished him. This was in the days of transistors and other discrete electronic components, before integrated circuit chips came into wide use. The still became a teaching device that lead to many blackboard discussions in which they analyzed current–voltage relationships for various (in those days) exotic devices, such as uni-junction transistors and zener diodes. Years later, when Jack Correia went to Robley's Vanderbilt lab as a postdoc, there on the upper shelf in the main lab was an electronically controlled double-distilled water purification system built by Robley.

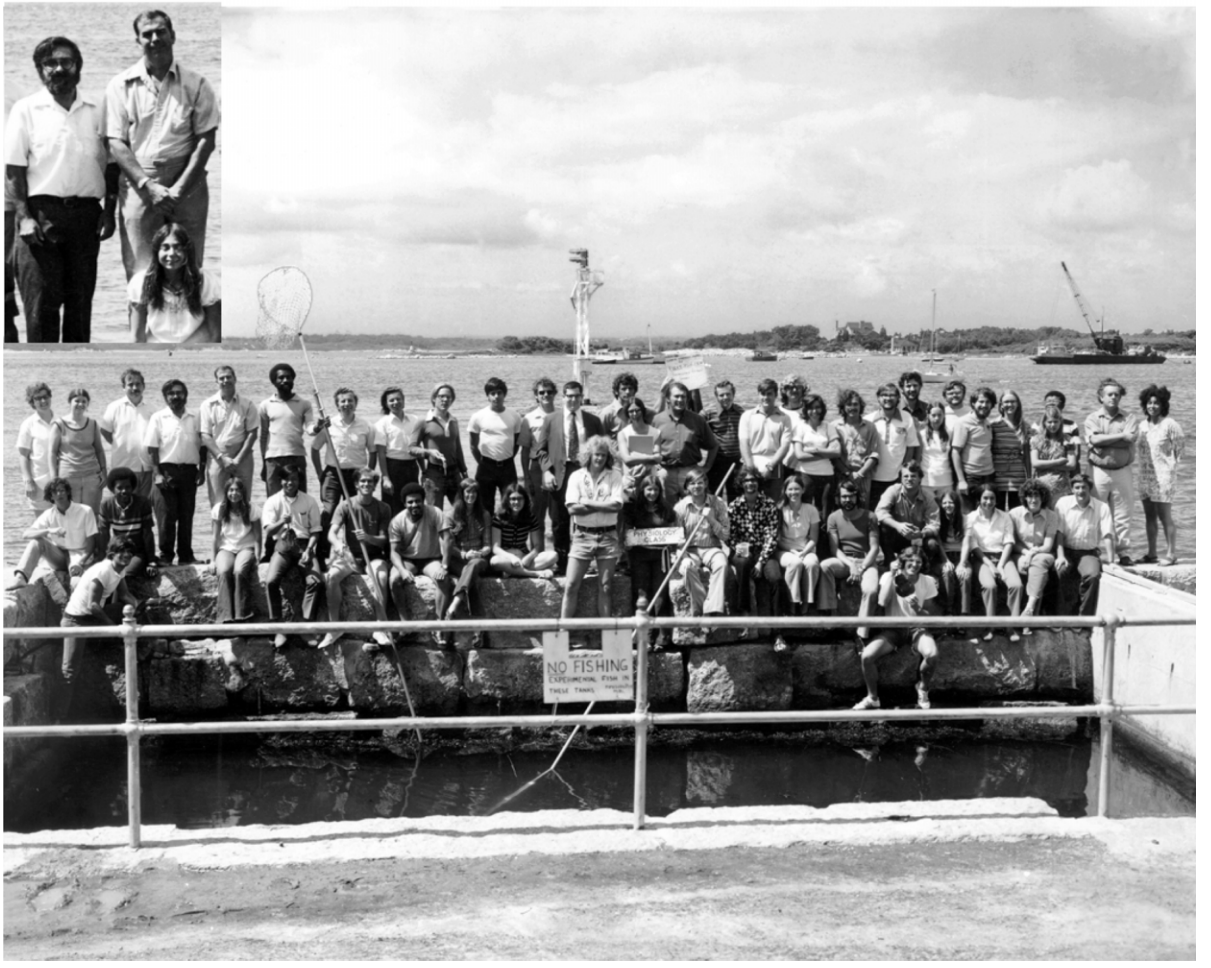

Fig. 2. The 1973 class at the physiology course at the Marine Biological Laboratory, Woods Hole, Mass. This class included Tom Laue, front right with a horseshoe crab, and Brad Chaires, just to Tom's right, with a beer in hand. Dave is in the back row, fourth from the left, and Carl Paul is third to the right of the fishing net. Insert: Dave and to the lower right, Sandy Yphantis, Dave's oldest daughter.

(Fig. 2). Ken was still spending summers at Woods Hole working on squid hemocyanin with, among others, Mike Johnson, who was an undergraduate technician. Mike's main job that summer was to learn how to operate the Model E scanner because Ken had one on order. It was the experiences of the summer of 1968 that convinced Mike Johnson that he wanted to become one of Dave's students. In addition to Mike Johnson, Ken also was responsible for contributing his cat to Dave and Lorna's growing menagerie. Ken was convinced that the cat, Charlot, was too old and infirm, or at least too indisposed to travel, to make the trip back to Oregon from Woods Hole. As it was Charlot joined the other 12 cats, three dogs and five kids (Sandy, Peter, Susan, Kim and Diana) at Dave and Lorna's house, where he served as chief curmudgeon for the next 17 years.

Walter Stafford also met Dave the summer of 1968 while attending the Woods Hole Physiology Course. He had just completed his courses and passed his Ph.D. qualifying exam in biochemistry

at Northwestern University and was ready to start his research. But after spending time with Dave he realized that a working knowledge of both physics and mathematics would be important for understanding the way the world works. To thrive under Dave's influence one must acquire basic attitudes and approaches to solving problems. Using both example and coercion, Dave was able to impress upon his students a need to understand and unravel problems by starting at the most fundamental and basic levels. Walter decided to continue his Ph.D. with Dave, and his deficiencies in physics and math disappeared dramatically.[2] That was also the summer that Robley was helping Dave with the physiology course. Late in the summer, though, Mike introduced Robley to his future wife, June, after which Robley was seldom seen.

Walter and Mike were teaching assistants for the Woods Hole physiology course, starting in 1969. In the late 1960s, this was an 18-hour-day, seven-day-a-week course. Things began at 08.00 h, getting the experiments started before the formal lecture part of the course. After lecture it was off to the beach or the Captain Kidd Restaurant and Bar for lunch. By mid-afternoon we were back in the lab doing science until well after midnight. Of course, a drink or two was consumed in the early morning hours. Dave was always the experimentalist. Once he started drinking a terrible India pale ale at a lab party. It was not because he liked the IPA; it was because he wanted to see if the students would start drinking the same terrible IPA. Most did. After that, Ballantine IPA became a staple of the lab parties.

[2] Walter recalls, one of his earliest 'assignments' (a challenge, actually): 'After arriving in Storrs I was issued a request by Dave on a Friday afternoon to derive a set of equations describing the astigmatic optics of the Model E analytical ultracentrifuge. The idea that I might not be able to succeed filled me with fear and trembling all weekend. This challenge required mustering my entire and rather inadequate arsenal of mathematical tools. But the idea that this might actually be possible gave me the energy to forge ahead. On Monday I appeared in the lab with a set of equations, which seemed to work. (I still use these equations and found them quite handy when designing a real-time CCD optical system for a Model E for use with time derivative analysis.) What I learned that weekend was what I needed to succeed in Dave's lab.'

This era in Dave's lab coincided with the graduate and post-doctoral careers of Robley Williams, Dennie Roark, Gay-May Wu, Carl Paul, Walter Stafford and Mike Johnson. Most took and/or taught the physiology course in Woods Hole. Dennie established himself as a remarkable theoretician. His work included application of the two-species plot, a complete derivation of the Donnan effect in sedimentation equilibrium, and development of a graphical analysis method for sedimentation equilibrium data, BioSpin [39–42]. As history often arranges, graphical approaches were simultaneously being developed by Dave Teller in Howard Schachman's lab at the University of California, Berkeley [43]. BIOSPIN is a graphical analysis approach that extracts both molecular weight averages and non-ideality information from equilibrium data. This approach is now mostly applied by the practitioners of the Omega function [44], although Yphantis students still use the original FORTRAN (now in DOS) version of BioSpin. In fact, in 1980 Walter Stafford published an impressive application of graphical methods to the analysis of non-ideal discrete and indefinite associating systems [45]. (A Windows graphical user interface to BIOSPIN is being updated and developed by Roark and Stafford; see Roark later in this issue.)

Walter Stafford spent most of his time in Dave's lab isolating muscle from clams [46] and waiting for equilibrium time on the Model E, which (even before Tom Laue's arrival) was often undergoing installation of new lasers and laser controllers by Carl Paul. The basic ideas for the time derivative or DCDT method [47] came from discussions with Dave while Walter was writing his thesis in 1973. These discussions germinated the idea that it might be possible to separate the time-independent components that were not of much interest in sedimentation velocity experiments from the time-dependent components that contained the interesting information. Mike Johnson was nominally studying *Limulus* hemocyanin [48], but spent virtually all of his time working with the computers in the laboratory. Mike was involved in the numerical solutions to the Lamm equation project with George Weiss [25,26] and was developing the non-

linear least-squares data analysis approaches (NONLIN) that remained a focal point of Dave's research in subsequent years [11,42,48–50]. (Mike has contined this tradition and is currently editing his fourth volume of Methods in Enzymology on Numerical Computer Methods.)

It was an exciting time—Dave was dramatically improving the quality of the data collected, but even more important was his work to automate the process of reading the photographic plates on which the interference patterns were collected. (It is worth noting that Gerson Kegeles taught a remarkable course on refractometric optics that biophysics graduate students recall with great fondness, not only for its clarity and precision, but mostly for the fact that Keg never used notes—he just went to the board and derived everything. Modern users of the XLI would no doubt prosper from a similar course (for the inquiring student: [51].) The plate reader was driven in those days by a PDP-8, a primitive computer with 8 kb of memory (12 bits segmented into two 'banks' of 4 K each) and an ASR-33 teletype for input and output. Yet, it spared all of us countless hours of tedium on the manually operated Gaertner plate reader. Mike Johnson had read one glass plate manually and decided it was much easier to program a computer to read the plates than it was to do it himself. Two great days for the lab were when we got the nine-track tape drive and floppy disk drive for the PDP-8. What is most remarkable is that when these items were first installed, the PDP-8 had no provisions for either piece of hardware. In true Yphantis form, the students built the digital interfaces to operate the hardware, and helped him write the software to operate them.

In order to take advantage of the precision of the interference optics automation had become imperative. David Teller and Glen Richards had demonstrated different ways to improve acquisition from interferograms [52,53]. Dave Yphantis wanted his plate reader automated. Over the next few years, Mike Johnson, Dick Domanik and Tom Laue worked on ways to do this [54,55]. Finally, they built a system based on a linear photodiode array that could help align the photograph, and then acquire fringe displacement data at three to five times the point density and better precision than manual measurements [56,57], and all of this in approximately 5–10 min. This acquisition system was the first major step towards the automatic image collection and analysis so prevalent in the XLA/XLI hardware and software today.

The photographic plate reader evolved into a camera that replaced the glass plates, first with rolls of film, and then with a diode array camera. Initially this was a linear diode [56] used by Robley, Tom and Dave to investigate the rapid sedimentation (1000 S) of neurofilament and microtubule solutions; [58]).[3] Later rectangular diode arrays and TV monitors were used [57,60–62]. A major hurdle in these 'real-time' systems was synchronizing a laser pulse with the spinning rotor so that a single rotor hole was illuminated. (To take full advantage of this double slit interference pattern, the lower window holder has a double slit mask limiting the illumination area. In the XLI, this double slit mask should be placed on the upper window holder.) A circuit based on the then-new phase-lock-loop technology had been described [60,61], but the implementation in Dave's lab proved to be very touchy in use. Eventually, an all-digital solution based on the 'rate-multiplier' was developed. This circuitry, which took advantage of the differences between sequences and frequencies, turned out to be stable and robust. Still, there remained a subtle problem caused by the propagation time of the signal from the centrifuge to the computer (the rotor continues to spin after the signal is first produced, so that the angular position when the laser needs to be

[3] Since design of the optical system at the time used a one-dimensional array, the data were collected across the interference envelope at a single radial position. To interpret the sedimentation distribution of the boundary, the data was converted to $g(s)$ and presented on a log scale. This represents the first experimental application of the gravity sweep method (for biological systems). This approach is also used for synthetic polymer distributions, although in this case the centrifuge was altered to collect data while undergoing continuous but slow acceleration [59]. This wide distribution technique can now also be used in the XLA/XLI by analyzing data from multiple speeds with DCDTWD [see Stafford and Braswell later in this issue]. The gravitational sweep or wide distribution method has also been incorporated into SedAnal [see Stafford and Sherwood in this issue].

fired appears to be dependent on the rotor speed). Eventually this problem was overcome in a simple fashion, though it took over a year to determine the source of the problem and several months after that to find a simple fix [62]. The design of this all-digital timing system is still used on the XLI although the timing pulse is now synchronized with a magnet embedded in the rotor. What became standard practice on the XLA/XLI in 1992 had been developed in Dave's lab in 1979 [63]. These developments have continued into the 1990s [64–67]. A common topic of discussion at the NAUF workshop is how to improve the current XLI optics[4]. Each year an evening session is presented by either Dave or by Jeff Lary, a former student of Emory Braswell and a former collaborator with Dave, and one of the most knowledgeable persons about the intricacies of the XLI, interference optics and sedimentation analysis. Jeff is now the facility scientist at the NAUF.

The laboratory computers available at that time did not have the power to simultaneously operate the data acquisition system and perform the Walsh transform analysis, a square wave approach to doing Fourier analysis of fringe displacement. Consequently, two computers on different parts of the campus were linked by a 2-mile-long dedicated line, and hardware was constructed to send and receive signals over the wires. Small, fast algorithms were written to synchronize the two computers so that data could be transferred at high speed. The depth of understanding of computer operations instilled in Dave's students was profound, though none of us fully understood that at the time—it was just fun to make things work.[5]

Concurrent with the development of automated data acquisition was the development of NONLIN for direct fitting of equilibrium data to association schemes by Mike Johnson [42,49]. This was a revolutionary idea, complete with proper error analysis by F-statistic. This program was heavily used and tested in the lab before its description, or any data, were published [42,48–50]. It is now included on all Beckman XLA computers and has been the gold standard (with apologies to down under) for the analysis of equilibrium data on self-associating systems. Maybe the most intensive ultracentrifuge study ever done was Gay-May Wu's work on insulin association [68]. Insulin associates into a hexameric ring structure in the presence of Zn^{2+}, and can undergo an indefinite association in its absence. Gay-May could detect 0.1% heterogeneity developing after purification and assigned it to non-catalytic deamidation reactions. It is worth noting that the XLI optics are still not as good as the Model E optics in Dave's lab in the 1970s, largely because of the signal/noise of the television camera used (See footnote 4).

Mike Johnson also applied NONLIN to the analysis of stopped flow and T-jump data in Todd's lab [69]. Like equilibrium data, kinetic data are nothing but sums of exponentials. Mike has produced a generic version of NONLIN that has been recoded for fitting binding data, melting data, and hosts of other techniques popular in the thermodynamics community [70–72]). Also working in Todd's lab were Steve Shire, John Steckert and

[4] Two major issues that limit the precision of the interference optics are the dimensions of the camera's diode array and flexing of the thermoelectric heat sink under vacuum thus shifting the focal plane of the optics. The vertical (across the fringes) extent of the diode array is short (96 pixels) so that any imperfection or dirt on the array results in errors in the Fourier transform, thus inducing error in the fringe displacement. A larger array (1024 vertical pixels) collects data across a sufficient number of fringes to minimize the impact of a small region containing imperfections. Flexing of the heat sink is induced by heating and cooling and leads to drifts in the fringe pattern. Changes have been made to the light source mounting (Laue, personal communication) that reduces the shifts by an order of magnitude.

[5] These topics, seeming quite ancillary to biophysics in many cases, were all internalized and applied, often many years later, in the development of laser light sources and the real time laser CCD camera based optics for the Model-E and the newer XL centrifuges. Both Robley at Yale and Vanderbilt and Walter at Boston Biomedical Research Institute had laser light sources on their Model Es. To get the most out of the time derivative method, Walter designed a real-time optical system with a CCD camera to replace the standard optical system. One of the requirements was that it could acquire single images of the entire cell in one frame. The background in math, optics, programming and electronics acquired so many years before would play an important role in the development of both the optical system and the method.

John Philo. All three have gone off to industry (Shire at Genentech, John Steckert at Wyeth, and Philo originally at Amgen and now Alliance Protein Laboratories) where they continue to this day to use analytical ultracentrifugation to characterize proteins and biotechnology products. At Storrs, Steve and John Steckert both did extensive sedimentation velocity work on the phase diagram for tobacco mosaic virus (TMV) assembly [73], one of Todd's major projects along with hemoglobin binding kinetics. It is worth noting that during this period at UConn there were four investigators who worked extensively with the Model E: Dave, Keg, Todd and Emory. Consequently every student and postdoc was regularly exposed to intensive discussions and presentations of centrifuge data and their analysis. John Philo did not do AUC work at Storrs, but he obviously was listening because he has proven to be one of the most innovative software developers (XLAGraph, SVEDBERG, DCDT+) and fundamental practitioners in the field [[74,75], and see Philo, Yang, LaBarre later in this issue].

The last year Dave taught at Woods Hole, 1973, both Tom Laue and Brad Chaires took the physiology course. Brad, like Walter, liked working with Dave so much that he transferred from Wesleyan University to Storrs as a graduate student. Brad would eventually do a thesis with Keg (and Tony Infante at Wesleyan University) on the thermodynamics and kinetics of ribosome assembly. Tom took a leave of absence from the University of Virginia to work with Dave in the fall of 1973 before deciding his true calling was disassembling and rebuilding the Model E optical system. After a break from school, he arrived at Storrs in 1975, following Jack Correia who had arrived in 1973, and preceding Dave Rhodes who arrived in 1976. Tom and Jack displayed a contrast that continues to this day. Tom designed and built numerous laser controller data acquisition systems for the Model E optics, including cameras with rolls of film, linear and rectangular diode arrays, and TV monitors. Tom is still building optical systems and now, like his mentor, also designs new instrumentation including a fluorescence optical system for the XLA/XLI and devices for analytical electrophoresis [[76,77], see MacGregor, Anderson, Laue in this volume]. Jack initially worked on the Lamm solution project [26,27] and the testing of NONLIN [42,49], and eventually collaborated with Steve Shire and Todd Schuster on short-column tobacco mosaic virus studies that established the helical character of TMV rings [11,50]. His lab focuses on the ligand-induced self-association of tubulin and, in collaboration with Walter Stafford, the heterogeneous interaction of tubulin with regulatory proteins and drugs (see Sontag, Stafford, Correia in this volume). The development of analytical methods for the analysis of sedimentation velocity data for interacting system is a major advance in the field and essential for undertaking these studies.

The transition into the 1980s proved to be a transition to fewer students for Dave. After Tom and Dave Rhodes graduated [78–80], Dave focused on collaborations [81–83], particularly with Tsutomu Arakawa [84–91]. This was greatly facilitated by the arrival of Jeff Lary into the lab in 1988. Jeff trained with Emory and brought a profound understanding of ultracentrifuge theory and practicality to the group. The focus on instrumentation and development has continued in the 1990s and the new millennium [92,93]. Dave's final student Yujia Xu worked on an old problem, detection of heterogeneity in self-associating systems [42,94,95]. This involves a combination of NONLIN analysis and graphical techniques to extract the intrinsic association properties in the presence of non-interacting species or aggregation. Her thesis work appears in this volume as a tribute to Dave's career [see Xu in this volume].

4. The National analytical ultracentrifugation facility (NAUF) years

In the late 1970s and early 1980s it became painfully apparent that analytical ultracentrifugation was coming to an equipment-induced crisis. The antique parts for the Model E were becoming harder and harder to find. Fewer labs had the shops available to maintain and improve their instruments, and a new generation of researchers who did not have time to be bothered with operating non-automated instruments had become the scientific leaders. Worst of all, fewer scientists were

being schooled in quantitative biophysical methods. One of the methods hardest hit by this ignorance was analytical ultracentrifugation. David, Todd and Emory recognized that several solutions were needed to address this problem.[6] One solution would require new, better-automated instrumentation and more accessible software, and it was questionable whether Beckman was going to develop a successor to the 'E' which it had just declared obsolete. At a workshop ('The Future of Analytical Ultracentrifugation') held during the 1986 annual Biophysical Society meeting in San Francisco, the point was made strongly by van Holde and others that Beckman had a moral obligation to develop a new model. At that meeting Todd and Emory talked separately to John Wooley, a program manager for the National Science Foundation, pointing out that about half the people at the meeting (approx. 60) had been to UConn at some time in their careers. They also emphasized the need for an entirely new instrument designed along the lines Dave had developed. In addition, until one was manufactured there would be a grave hiatus in the field. In this case, the only way to preserve and develop the 'art' would be to fund a center, which would continue the design (supplying plans, software, and some hardware to other investigators), offer research collaborations and, through publications and an annual workshop, effectively communicate the technology transfer. Dr Wooley was very interested and encouraged Todd and Emory to write a 'seed' grant which allowed the addition of nine more model Es (largely scavenged for parts) to the four operating machines already there. It was an impressive sight to see four of these mighty machines lined up looking like they were presenting themselves for military review. Each machine was outfitted with the optics and computers, which Dave and his students developed to make them fully automatic. After the seed grant, a large NSF grant provided funding for the National analytical ultracentrifuge facility (NAUF) to be housed at UConn with Dave, Emory, Todd and John Philo as principle investigators. (An advisory committee for the facility was headed by Bill Harrington and included Guido Guidotti and Jonathan King as members. After Bill Harrington died, Howard Schachman became the head of the committee.) Since it was rare for the NSF to set up such facilities, this support speaks well of John Wooley and his interest in the field. (During these developments, another workshop ('A New National Ultracentrifugation Facility at the University of Connecticut') was held at the 1989 Biophysical Society meeting and included a series of presentations with Beckman engineers to discuss the design of the XLA.) In 1990 a small laboratory and office building was dedicated. This building became the Biotech Center, directed by Todd, which housed the NAUF and several other facilities. Later, the grant was renewed and with other sources of funding kept the facility well funded until 2002. The NSF finally claimed that it had never been its policy to establish such facilities, but that once it was going, they thought it was necessary and important.

Dave, Emory and Todd were an ideal team during these years. Dave continued his development of hardware and software, and his reluctance to chair any committees. Emory was the head of the facility and did much of the collaborative research. Todd was the director of biotechnology, in charge of the workshop and technology transfer and remained active in the center after his retirement in 1998. Sadly he passed away in 2000, and Dave lost a friend of many years. Dave retired in 2001 and Emory in 2002. Both are still contributing to the workshop and the publication list of the facility. Now, Jim Cole, for many years a research scientist at Merck, and a NAUF workshop participant in 1992, heads the NAUF and organized the 12th workshop, presented in 2003. In the 15 years since the center began in April 1988, over 45 academic collaborations and a similar number of corporate collaborations have been conducted leading to 42 publications. The facility continues to actively engage in collaborative research and software development projects and is working to expand the applications of analytical ultracentri-

[6] A parallel realization occurred in the thermodynamics field, where Gary Ackers, Jim Lee, Ernesto Friere, Stan Gill and Wayne Bolen met to organize the Gibbs Conference on Biothermodynamics [96]. Regularly attended by the authors of this article, the 17th annual meeting will be held in 2003. Herb Halvorson and Gary Ackers affectionately referred to Model E users during this time as an antique car club.

Fig. 3. The first workshop held at the NAUF (National Analytical Ultracentrifuge Facility in 1992. Attendees and instructors; kneeling: (left to right): Henry Havel, Jia-Wen Wu, Donald McRorie, Ronald 'Skip' Cole, John Steckert. Row one, standing (left to right): Jeff Lary, Carl Burke, Shoku Sarrafzadeh, Jim Cole, Gay-May Wu, Gaston Daumy, Steven Shire, Allen Pekar, Jacob 'Jack' Lebowitz, Tom Laue, David Yphantis, John Philo, Siddarth Advant, Walter Stafford, Dan Zhu, Chaoying 'Cindy' Zhang, Paul Voelker, Emory Braswell, Mark Vieira. Back row (left to right): Yujia Xu, Todd Schuster, Saleh Darawshe, Dan Sackett, German Rivas.

fugation and related methods to study biomolecular interactions.

At about the time that the facility was founded, Beckman Instruments realized the need for analytical ultracentrifugation was not about to go away. To its credit, Beckman devoted considerable resources to the development of the XLA, even though the size of the market (currently approx. 400 instruments in the field) hardly justified the effort. The first of three XLAs were delivered shortly after the NAUF had moved into the new building. This was followed by the interference upgrades (XLI), though it took lots of convincing (and nagging) of Beckman as to the merits of the refinement. Gradually, with sadness, the Es were phased out as research instruments at the NAUF but were kept, until recently, as reminders of the long path to the XLA/XLI. The original facility adjacent to the Life Sciences Annex has been torn down, and the facility is now located in the new Biological Sciences and Physics Building.

In addition to producing and distributing useful computer programs, and performing collaborative research, in 1992 the NAUF started its annual workshop on the use of the analytical ultracentrifuge (Fig. 3). This workshop, modeled on the Woods Hole concept of combining lectures each morning with hands-on practical training including experiments and data analysis the rest of the day, has been a huge success. As of 2003 more than

260 students, postdocs, and PI's have been trained and many have gone on to make significant contributions to the field. During each workshop the 24 participants[7] work in three groups, and over the course of three days they rotate through instruction from three teams: A) Tom Laue and Dave Yphantis on sedimentation equilibrium techniques, including the fitting of data with NONLIN; B) Walter Stafford and John Toedt [Todd's last student and a collaborator of Dave's; [83]] on the analysis of sedimentation velocity data with DCDT and his new heterologous association routine SedAnal; and C) Peter Schuck on the analysis of sedimentation velocity data with Sedfit and global analysis of data obtained with multiple techniques using Sedphat. Jeff Lary and Jim Cole oversee the running of experiments on hemocyanin, the availability of workstations and the distribution of CDs containing the newest versions of each software package with the data sets analyzed during the course. The level of the workshop is extremely high and the participants are challenged to absorb all of the information that is presented in 3 days. Often, the course catalyzes ongoing interactions among the participants that continue the learning experience that started at UConn. The workshop also brings out collegial competitions between the instructors that have encouraged them to produce new advances in software packages every year. Walter handed out preprints of his first DCDT paper at the 1992 workshop. Both Sedphat and SedAnal were introduced to the course in 2002. Jim Cole will be releasing a new heterogeneous fitter for sedimentation equilibrium data next year [see Ucci and Cole this volume]. The fourth day is devoted to a symposium that covers academic and industrial applications of the XLA/XLI. Dave, Tom, Walter, Peter Schuck, John Philo, Steve Shire, Jack Correia and Jeff Lary regularly contribute along with previous course members and other centrifuge experts. Over the years Howard Schachman, Al Holtzer, Allen Minton, Henryk Eisenberg, Geoff Howlett, Preston Hensley, Jeff Hansen, Karen Fleming, and Olwyn Byron have spoken.

[7] In the small Life Science Annex facility this number was limited to 21. The expanded facility in the Biological Sciences and Physics building is now able to comfortably accommodate 24 participants.

A complement to the NAUF was developed in Australia by Don Winzor, Geoff Howlett, Allen Minton and Greg Ralston when they put together a meeting on Reversible Associations in Structural and Molecular Biology (RASMB). At the first meeting, held in Melbourne, Australia in 1994, it was suggested that a web-based group be established to exchange ideas and address questions about quantitative methods for characterizing molecular interactions. Walter agreed to set up the host site, and the RASMB came into existence. It quickly became the site for exchanging news and information about analytical ultracentrifugation. The warm and welcoming atmosphere of this site has helped many novices become comfortable with using analytical ultracentrifugation routinely to answer questions. A great deal of the atmosphere of the NAUF workshop and the RASMB site can be traced to the generous and enthusiastic character of David Yphantis and his students.

At the 1995 Biophysical Society meeting, held in San Francisco, Dave received the Elisabeth Roberts Cole Award, known today as the Founders Award. This award is for outstanding achievement in any area of biophysics, but uses as a critical measure acceptance and use of a device or method by others in the field, either promptly or over a period of years. The period of time during which Dave influences the field of analytical ultracentrifugation actively continues. In 1996 at the 40th annual meeting of the Biophysical Society, in Baltimore, a workshop symposium entitled 'Advances in Sedimentation Velocity Analysis' was organized by Tom Laue and held in honor of Dave's 65th birthday [97]. The next year, in November 1997, Dave was honored with the Svedberg Award at the First Beckman Symposium on Solution Interactions of Macromolecules, in Galveston Texas. 'This award acknowledges an established investigator for sustained and outstanding contributions to the field of analytical ultracentrifugation. By example and achievement the honoree has pioneered the advancement of instrumentation, data analysis or understanding of solution interactions, thereby providing leadership in the understanding of hydrodynamic and thermodynamic properties of molecules'.

5. Postscript

Perhaps Dave's most outstanding achievement has been the teaching of students. He rarely sat in his office or had conferences with students. He was in the lab working with the students. He helped us with everything. He helped us run the centrifuge, he helped us locate and replace the faulty transistors in the Beckman Model E scanner, and he helped us prepare the samples. Once when Mike Johnson's reel-to-reel tape drive failed to record Dave spent 12 h on a Sunday working with him to trace and adjusting the tape drive circuits. We learned that if something is worth doing, then it is worth doing right, and that there is no place for compromise. In many ways his actions and attitudes were far above and beyond that of a scientific adviser. He was also an older brother and a father to us. In an era when PIs typically no longer do bench science, each of us still carries on the tradition of being in the lab, at the bench, discussing and teaching. With a smile and sometimes a wink we all repeat the phrase, 'We can do better!' at every available opportunity, and hope that someone else is listening. Thanks, Dave.

6. Biography of David A. Yphantis

Born:
– 14 July 1930 in Boston, Massachusetts.

Education:
– A.B. in Physics, 1952, Harvard University (Magna cum Laude).
– Ph.D. in Biophysics, 1955, Massachusetts Institute of Technology.

Professional positions:
– Fellow of the American Cancer Society and Research Associate, 1955–1956, Massachusetts Institute of Technology.
– Assistant Biophysicist, 1956–1958, and Associate Biophysicist, 1958, Argonne National Laboratory.
– Assistant Professor, 1958–1964, and Associate Professor, 1964–1965, The Rockefeller Institute/Rockefeller University.
– Consultant, 1958–1962, and Visiting Investigator, Summers, 1959–1961, 1966–1967, Division of Biological and Medical Research, Argonne National Laboratory.
– Visiting Investigator, Summer 1962, Biology Division, Brookhaven National Laboratory.
– Professor of Biology, 1965–1968; Professor of Biophysics, 1967–1968; Chairman, Department of Biology, 1967–1968; The State University of New York at Buffalo.
– Consultant, 1967–1980, Division of Computer Research, National Institutes of Health.
– Instructor in Physiology, Summers 1968–1973, The Marine Biological Laboratory, Woods Hole.
– Professor of Biochemistry and Biophysics/Molecular and Cell Biology, 1968–1999; and Professor Emeritus, 1999–, The University of Connecticut.
– Consultant, 1982–1987, Xenogen, Inc., Mansfield, CT.
– Visiting Professor, 1986–, The University of Crete.
– Instructor, Workshop on Analytical Ultracentrifugation: Theory and Practice, 1992–, Analytical Ultracentrifuge Facility, Biotechnology Center, University of Connecticut.

Member:
– American Chemical Society.
– American Society for Biochemistry and Molecular Biology.
– Biophysical Society.
– Editorial Board, Biophysical Journal, 1962–1968.
– Editorial Board, Analytical Biochemistry, 1968–1980.
– Editorial Board, Archives of Biochemistry and Biophysics, 1974–1986.
– NIH Study Section, BBCA, 1970–1974.
– SPIE (Society of Photo-Optical Instrumentation Engineers) 1998–.

Honors and Awards:
– Westinghouse Science Talent Search, 1948–1952.
– Harvard National Scholarship, 1948–1949, 1950–1952.
– Co-chairman, Conference on Advances in Ultracentrifugal Analysis, New York Academy of Sciences, 1968.

– Council, Biophysical Society, 1968–1971, 1975–1978.
– Consulting Editor, Annals of the New York Academy of Sciences, 1969.
– Chairman, Nominating Committee, Biophysical Society, 1969, 1976.
– Chairman, Kendall Award Symposium, American Chemical Society, 1970.
– Executive Council, Biophysical Society, 1975, 1977.
– Keynote Speaker of Conference: 'Ultra sensitive Clinical Laboratory Diagnostics', Biomedical Optics Society Meeting, S.P.I.E., January 1994.
– Elisabeth Roberts Cole Award, Biophysical Society, February 1995. The award is for outstanding achievement in any area of biophysics. A test of significance is the acceptance and use of a device or method by others in the field, either promptly, or over a period of years.
– Elected to Connecticut Academy of Science and Engineering, 1995.
– Workshop symposium 'Advances in Sedimentation Velocity Analysis' was held in honor of 65th birthday at the 40th Annual Meeting of the Biophysical Society, on 20 February 1996, Baltimore, MD. (See T.M. Laue. 1997. *Biophysical J.* **72**: 395–6.)
– Svedberg Award, November 1997, First Beckman Symposium on Solution Interactions of Macromolecules, Galveston Texas. This award acknowledges an established investigator for sustained and outstanding contributions to the field of analytical ultracentrifugation. By example and achievement the honoree has pioneered the advancement of instrumentation, data analysis or understanding of solution interactions, thereby providing leadership in the understanding of hydrodynamic and thermodynamic properties of molecules.

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