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Preface to Festschrift for Dave Yphantis

This special issue of *Biophysical Chemistry* has been organized to honor the outstanding contributions Dave Yphantis has made to the field of analytical ultracentrifugation (AUC) over the last 50 years. It reflects the great respect and admiration that his students and colleagues feel for Dave as a scientist, a colleague and a friend.

The Festschrift is organized into two parts. Part one includes four articles that describe the history of the AUC field as told from the perspectives of three of Dave's contemporaries and an ensemble of his students. Ken van Holde recounts the earliest work by Svedberg and collaborators in the 1920s and 1930s on the development of the sedimentation equilibrium technique, and the formative implications these experiments had for understanding the size and homogeneity of macromolecules (van Holde). Dave had an especially important role in advancing this technique, with the short column and meniscus depletion method, and both biospin graphical analysis and NONLIN direct global fitting to self-association models (see Correia, Laue, Johnson, Stafford and Williams). Howard Schachman outlines his own impressions of the important developments in the AUC field, emphasizing his own experiences, discoveries and graduate students, and how his career overlapped with Dave's (Schachman). It is an entertaining and enthusiastic journey, beginning with TMV, 70s ribosomes, the Johnston–Ogston effect, synthetic boundary cells, Rayleigh interferometric optics, photoelectric scanning absorbance optics, the H_2O/D_2O method for determining partial specific volume and ATCase. Peter von Hippel went to graduate school with Dave, and shares what he calls his failing memory and fond impressions of the 'good old days' in David Waugh's lab at MIT and during the early stages of their careers (von Hippel). Finally, five of Dave's students (Jack Correia, Tom Laue, Mike Johnson, Walter Stafford and Robley Williams) outline the entire history of the Yphantis lab, stressing both the influences Dave had on the field as well as on their own careers (Correia, Laue, Johnson, Stafford and Williams). A significant

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number of the current influential practitioners and developers in the AUC field come from Dave's lab or the UCONN campus. This influence continues unabated with the AUC workshop taught every year at the National Analytical Ultracentrifuge Facility at UCONN in Storrs, CT. Through their regular participation at the workshop, collaborations and fundamental investigations and development of software and new methods, Dave and his students continue to have a major influence on AUC. Howard Schachman predicts that 25 years from now, on the 100th anniversary of the analytical ultracentrifuge, the Yphantis influence on this indispensable tool will still be in effect.

Part two comprises 16 scientific contributions from Dave's students and colleagues. The topics cover a broad range of areas that for the most part, and not surprisingly, parallel Dave's own interests and contributions. Dave and his students were instrumental in developing software for AUC data analysis. Dennie Roark describes a new smoothing algorithm that will serve as the basis for a new generation of the graphical analysis routine BIOSPIN (Roark). This package is useful for analyzing molecular weight moments and non-ideality from sedimentation equilibrium data. Walter Stafford describes a new and powerful package Sedanal appropriate for the global analysis of sedimentation velocity experiments on heterogenous interacting species (Stafford and Sherwood). Chris Sontag, Walter and Jack Correia use this approach coupled to HYDRO frictional ratio predictions to investigate isodesmic self-association of tubulin by direct boundary fitting (Sontag, Stafford and Correia). Walter Stafford and Emory Braswell also describe a multiple speed wide distribution analysis method that is embodied in two software packages, DCDTWD and Sedanal, appropriate for extremely broad and complex mixtures of species (Stafford and Braswell). This is an extension and simplification of a technique originally developed by Dave, Tom Laue and Robley Williams for the investigation of 1000S neurofilament complexes.

Dave in collaboration with George Weiss at the NIH and numerous other students and colleagues developed methods for simulating solutions to the Lamm equation and explored numerous experimental designs and questions including approach to sedimentation equilibrium, pressure dependence and non-ideality. Two manuscripts by Peter Schuck extend our ability to solve the Lamm equation and simulate and interpret experimental data. Using the finite element solution developed by Claverie and greatly enhanced by himself, Peter first presents a new model for sedimentation in inhomogeneous media as based upon spatial and temporal variation of the density and viscosity (Schuck-I). This is shown to agree with experimental profiles in a cesium chloride gradient. In a second study Peter uses finite element solution of the Lamm equation to investigate the influence of compressibility on both aqueous and organic solvents (Schuck-II). Neglecting variation of density and pressure across the cell can lead to systematic errors in sedimentation coefficients, especially at high speeds. Both of these features have been incorporated into Sedfit.

Dave had a significant impact in the development of new instrumentation, cell components and methods for AUC (temperature controller, short column, six channel and external loader centerpieces, meniscus depletion, laser controller, television-based interferometer). Tom Laue and colleagues carry on this tradition by describing a fluorescence optical system for the XLA/XLI that is capable of equilibrium and velocity measurements down to the 500 pM concentration range (MacGregor, Anderson and Laue). A commercial version of this system (AFS or Aviv fluorescence system) is under development by Aviv Biomedical, Inc. Dave's interest in the development of transport theory for interacting systems, in this case for electrophoresis, is found in the paper by Tom Moody, a former student and current colleague of Tom Laue's (Moody and Shepard). Recent work from this group has demonstrated that the valence of proteins may be determined experimentally. For interacting systems, the energy change accompanying the valence change upon association is a component of the overall binding energy. However, it is likely that conservation of valence may not hold on association, making the analysis of steady-state electrophoresis data significantly different from the analysis of sedimentation equilibrium. The paper by Moody and Shepard develops the theory of steady-state electrophoresis of interacting

systems using the framework of non-equilibrium thermodynamics.

Dave was instrumental in developing computer methods for analysis of sedimentation equilibrium data, starting with the meniscus depletion approach, the BIOSPIN graphical approach with Dennie Roark, and later NONLIN and direct global fitting with Mike Johnson. Two contributions to this issue expand direct global fitting of equilibrium data to associating systems. John Philo and collaborators implement an analysis approach developed by John years ago to re-investigate the oligomerization state of MIF or macrophage migration inhibitor factor (Philo, Yang and LaBarre). The association is tight but requires experiments as a function of solution density (Schachman's H_2O/D_2O method) and thus measuring the partial specific volume of MIF to verify the molecular weight of the complex is a trimer. Jim Cole describes a new heterogeneous multiwavelength equilibrium fitter and its application to an overlapping non-specific interaction between protein kinase R and a small 20 basepair RNA construct (Ucci and Cole). This software package will be released on the RASMB and at the 2004 AUC workshop. Yujia Xu, Dave's last graduate student, presents her thesis work titled 'Characterization of Macromolecular Heterogeneity by Equilibrium Sedimentation Techniques' (Xu). This is a detailed extension of work Dave did in the 1970s with Gay-May Wu, Mike Johnson and Jack Correia on the presence of inactive monomers or aggregated polymers against a background of reversible association. The idea is simply to fit equilibrium data from different loading concentrations with NONLIN as separate reactions with independent *K*'s. Then depending upon the model for both reversible and irreversible association, the resultant *K*'s can be plotted to generate estimates for the true association constants and the amount of contaminant causing the heterogeneity. This approach can equally be applied to *K*'s derived from sedimentation velocity studies. In conjunction with the sedimentation velocity developments for homogeneous and heterogeneous systems described by Schuck and Stafford and colleagues, these approaches are expanding the power of AUC and re-imposing its appropriateness for quantitative studies of complex macromolecular interactions.

Allen Minton and collaborators have long standing interest in exploring the behavior of weakly self-associating systems at very high concentrations (up to 200 g/l) or in the presence of very high concentrations of inert molecules and macromolecules (Zorrilla, Jimenez, Lillo, Rivas and Minton). The general methodology of characterizing weak association and thermodynamic nonideality in concentrated solutions is extremely important for investigating the typical behavior of proteins in cellular environments. This will prove to be very important for the interpretation of XLF studies of fluorescently labeled macromolecules (MacGregor, Anderson and Laue) against the background of concentrated inert or cellular solutions. Karen Fleming has been extending AUC methodology to the association of proteins in a lipid environment. This requires the use of neutrally bouyant detergents so that only the protein gradient is visible in the signal. In this recent study Fleming and colleagues investigate glycophorin A transmembrane helix–helix dimerization as a function of C14 betaine micelles (Fleming, Ren, Doura, Eisley, Kobus and Stanley). This allows them to extrapolate to mole-fraction standard state conditions to determine the free energy of dimerization in C14 betaine. The ability to investigate quantitatively membrane association reactions is a major advance in the AUC arsenal.

Jim Lee and colleagues use spectroscopic and sedimentation techniques to investigate the self-association of HIV Rev (Surendran, Herman, Cheng, Daly and Lee). The studies reveal two consecutive progressive association schemes that include ring formation, filament formation and bundles of filaments. Spectroscopic and low concentration hydrodynamic studies indicate the presence of a molten-globule to folded monomer reaction that precedes the association steps.

Olwyn Byron's group presents a complex hydrodynamic investigation of a 2:1 p50-I$\kappa\beta\gamma$ transcription factor complex (Smolle, Hay and Byron). Hydrodynamic bead modeling is used in conjunction with the AUC data to determine that a p50 dimer in a closed conformation interacts with I$\kappa\beta\gamma$ monomer to form the inhibitory complex. Bead

modeling, as demonstrated in this manuscript and the isodesmic studies of Sontag et al. (Sontag, Stafford and Correia), is both a valuable complement to and a powerful verification of hydrodynamic data.

Finally, Sara Szuchet, once a postdoc in Dave's lab at Buffalo, and her colleagues present a characterization of a novel marker for brain cell lineage and development in selected glial cells (Szuchet, Plachetzki, Seeger, Domowicz and Szele). Structural and functional studies are now required to understand the role NOVOcan plays in development. Sara quotes Goethe in her dedication and credits her time in Dave's lab where she 'sharpened and perfected the theoretical understanding and application of biophysical techniques' that have been instrumental in her science and career. Derived from this understanding is confidence that leads to a willingness, a boldness in ones craft, and to take from Sara's dedication:

> Boldness has genius, power, and magic to it. *Goethe*

This series of manuscripts highlights not just Dave's career, but the continuing level of achievement and excellence that AUC practitioners and Dave's students carry forward into the new millennium. The continuing challenge is to bridge the gap between physical chemistry, cell biology and biological function and regulation. As analytical, instrumental and experimental AUC tools expand, can we meet the challenges that biological problems present to us? Do we dare, boldly and confidently, to continue on this quest for understanding and insight into the structure and function of the proteome? Will our influence be felt 25 years hence? If the answer is yes, then we can collectively thank Dave for his outstanding contributions and his encouragement, training and wisdom along the way. Thank you Dave, and may your influence and guidance be eternal.

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