Six decades of research in physical biochemistry

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This summary of a 60-year research career in physical biochemistry begins with an admission that fate, rather than any detailed planning on my part, has played a large role in directing its pathway. In the first place, the decision to opt for higher education rather than life as a farmer reflected the unsuitability of the latter for a chronic asthmatic. Secondly, the possibility of majoring in physical chemistry only became an option at the University of Adelaide midway through undergraduate study with the arrival from the University of Nottingham of a new Head of Department, D. O. (Doj) Jordan, in 1954 as well as his former graduate student, J. M. (Mike) Creeth, a year later. The consequent location within the Department of Chemistry of a Spinco model E analytical ultracentrifuge (transferred from Nottingham) and a newly acquired Spinco model H electrophoresis–diffusion apparatus thus set the scene for my postgraduate entry as well as that of L. W. (Laurie) Nichol into the biophysical chemistry arena at the beginning of 1956.

Our introduction to research was the modification of the model H optical system to attain the level of precision in diffusion measurements required for the envisaged but unachieved goal of using deviations from Gaussian spreading behavior as the ultimate criterion of protein homogeneity with respect to size and shape. Instead, those studies on the A₁

component of isoelectric ovalbumin plunged us both unwittingly into the field of interacting systems—the need to consider the flux of buffer components as the result of solute diffusional flow even under conditions of constant solvent chemical potential. Those years with Mike Creeth and Doj Jordan provided the rigorous training in thermodynamics and macromolecular chemistry that has been a major contributing factor to our success as physical biochemists.

Appointment to the CSIRO Wheat Research Unit in 1960 heralded my introduction to the chromatographic fractionation of proteins. In that regard a decision to interrupt my 8-year employment there for a 12-month postdoctoral position with Harold A. Scheraga at Cornell University led to the development of frontal size-exclusion chromatography (gel filtration in those days) for the quantitative characterization of protein self-association—an endeavor that attracted international acclaim. My return to Sydney marked the recommencement of research collaboration with Laurie Nichol, by then a postdoctoral associate of A.G. (Sandy) Ogston at the Australian National University. Our extension of the Cornell development to studies of heterogeneous association soon revealed that the Gilbert–Jenkins treatment of the migration of interacting systems could be regarded as a manifestation of the Johnston–Ogston effect with solute–solute interactions now governed by the law of mass action—an observation that had the desired effect of tempting Sandy to participate in many subsequent collaborative studies.

Our ventures into studies of preferential ligand interaction with different oligomeric states of a protein acceptor led to the realization that the observation of allostery merely signified an interplay of acceptor–ligand interactions that needed to be identified and characterized for each given system. In that regard the decision to concentrate attention on associating acceptors rather than their isomeric counterparts reflected an ability to differentiate between

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pre-existence and ligand induction of the equilibrium between acceptor states as the source of the allostery.

Appointment to the University of Queensland in 1968 heralded my introduction to interactions of greater biological as well as physicochemical interest—a change in emphasis facilitated by affiliation with the Enzyme Biology Group headed by Colin J. Masters. Those studies of glycolytic enzymes and proteins associated with the actin filaments of muscle myofibrils involved the research programs of three of Colin's PhD students, Frank Clarke, Ross Brinkworth and Terry Walsh, as well as my first PhD student, Stephen Lovell.

The development of affinity chromatography as a means of protein (enzyme) purification raised the prospect of employing the same chromatographic matrix to characterize the affinity interactions responsible for solute purification in a procedure that became known as quantitative affinity chromatography. Despite our publication of an exhaustive set of equations in 1974 for situations with all solute–matrix, solute–ligand and matrix–ligand interactions univalent, a solution to the problem of solute (acceptor) multivalence in its interaction with matrix-bound affinity sites had to await the end of the decade. That development, which involved the application of Flory reacted-site probability theory, paved the way for deduction of the counterpart of the Scatchard equation for multivalent ligands and hence the characterization of interactions between glycolytic enzymes and the muscle myofibril system—a naturally occurring affinity matrix. Another decade elapsed before discovery of the binding expression of which the multivalent counterpart of the Scatchard equation was its linear transform.

Laurie Nichol's departure from the research scene in 1985 to become Vice-Chancellor of the University of New England [and then soon after Vice-Chancellor of the University of the Australian National University (ANU)] marked the beginning of an even longer collaborative venture with Peter R. Wills at the University of Auckland—a venture aimed at acquiring a quantitative understanding of the effects of thermodynamic nonideality on the characterization of protein interactions by equilibrium techniques. Peter's terrier-like tenacity in the approach to establishing a rigorous thermodynamic basis for the quantitative description of a physicochemical measurement finds parallel in his dogged pursuit of the facts in matters nuclear that caused so much anguish on the international as well as national (New Zealand) political scenes. A 4-year struggle with the deliberations of Terrell L. Hill led to recognition of a deficiency in the traditional text-book definition of solute chemical potential. In situations where solute chemical potential is being monitored under the constraints of constant temperature and solvent chemical potential, the thermodynamic activity is a molar quantity that is logically expressed in terms of a molar standard-state chemical potential and molar solute concentration. Under the more common constraints of constant temperature and pressure, the thermodynamic activity is a molal

parameter that is logically expressed in terms of a molal standard-state chemical potential and molal solute concentration.

The most significant outcome to emerge from that realization was the demonstration that conventional analyses of sedimentation equilibrium distributions yielded the molar thermodynamic activity of solute—a fortunate situation reflecting the pressure dependence of the centrifugal force (through radial distance and solution density) and the use of a molar (or g/L) concentration scale to define the opposing chemical potential gradient. Effects of thermodynamic nonideality in sedimentation equilibrium studies thus became amenable to rigorous description on the statistical–mechanical basis of excluded volume.

My retirement in 2000 resulted in expansion of the collaborative team to a foursome with the inclusion of Steve Harding and Dave Scott from the University of Nottingham. Prompted by reports of negative second virial coefficients for protein self-interaction from static light scattering and small-angle X-ray scattering measurements on solutions supplemented with high concentrations of small cosolutes, those collaborative studies have entailed detailed consideration of the effect of thermodynamic nonideality in experiments conducted under the constraints of constant temperature and pressure—situations where the small molecules must be regarded as additional non-scattering solutes. The reported negative second virial coefficients have been shown to reflect a necessarily positive contribution from protein self-interaction that is outweighed by larger negative contributions from protein–cosolute interactions.

I must also stress the importance of a 20-year collaboration with John R. Cann at the University of Colorado Health Sciences Center in Denver. His expertise in chromatographic simulation rendered possible the identification of the relevant migration behavior of an $A + B \rightleftharpoons C$ system for which the Gilbert and Jenkins theory had predicted two possible outcomes. Allowance for boundary spreading in those simulated migration patterns was simplified greatly by adapting the Glueckauf theoretical plate model of chromatography with continuous flow—a simplification that led to an experimentally realistic extent of boundary spreading as the result of a truncation error embodied in the substitution of a finite difference expression for the actual continuity equation describing migration. Mention also needs to be made of joyful ventures with Craig M. Jackson into the realm of interactions within the blood-clotting system—a collaboration initiated by a brief visit in 1984 to the American Red Cross Blood Services Laboratory in Detroit.

My involvement with the Department of Physical Biochemistry throughout most of its lifetime (1960–1985) has inevitably led to close affiliations with people there—either during their stay at ANU or after their attainment of employment elsewhere. Those with whom I have published include Peter Jeffrey, Peter Baghurst, Bill Sawyer, Mike Sculley,

Roland Siezen, Barry Preston and Geoff Howlett. Brief encounters during stopovers overseas have also led to publications with Helmut Cölfen, Chris Lowe, Gary Pielak, Natalia Chebotareva and Trushar Patel. In addition, collaborative ventures at the University of Queensland with Burt Zerner, John de Jersey, Paul Reilly, Peter Nixon, Sue Pond, Ross Smith and Paul Kroon have also been productive.

Finally, it gives me great pleasure to acknowledge Steve Lovell, Ross Tellam, Larry Ward, Chris Ford, Doug Bergman, Phil Hogg, Simon Harris, Keith Shearwin, Peter Munro, Mike Jacobsen, Damien Hall and Thierry Lonhienne—my 11 PhD students and sole postdoctoral research associate—for their help in maintaining my research into protein interactions at the scientific forefront for so long. My gratitude also extends to the Australian Research Grants Commission for continuous financial support over 33 years (1969–2001), and to the biophysical

chemistry community (Australian and International) for their fellowship and collegiality. As I look back over a research career spanning six decades I feel blessed to have been party to a number of significant developments in the study of protein interactions: although many developments are obvious in retrospect, it does take someone to point out the obvious in the first place.

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Compliance with ethical standards

Conflict of interest Donald J. Winzor declares that he has no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.