Foreword to ‘Quantitative and analytical relations in biochemistry’—a special issue in honour of Donald J. Winzor’s 80th birthday

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Abstract The purpose of this special issue is to honour Professor Donald J. Winzor’s long career as a researcher and scientific mentor, and to celebrate the milestone of his 80th birthday. Throughout his career, Don has been renowned for his development of clever approximations to difficult quantitative relations governing a range of biophysical measurements. The theme of this special issue, ‘Quantitative and analytical relations in biochemistry’, was chosen to reflect this aspect of Don’s scientific approach.

Keywords Donald J Winzor · Physical chemistry · Analytical ultracentrifugation · Quantitative and analytical relations in biochemistry

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In this special issue, Don’s colleagues, scientific collaborators and former students have contributed an array of articles concerned with biosensor analysis (Karlsson 2016), analytical ultracentrifugation (Harding et al. 2016), dynamic light scattering (Stetefeld et al. 2016), turbidimetric analysis (Zhao et al. 2016), protein–ribonucleic acid interaction (Jones 2016), computer modelling of amyloid formation (Schor et al. 2016), protein denaturation (Chebotareva et al. 2016), statistical mechanics (Wills 2016), structural phage biology (Arisaka et al. 2016), neutron scattering (Scott 2016), nuclear magnetic resonance analysis of mass transfer (Shishmarev and Kuchel 2016), electrophoresis (Laue et al. 2016), thermodynamic nonideality (Rao et al. 2016) and protein–DNA interactions (Munro et al. 2016). Prefatory comments have also been added by two long-time research collaborators and fellow passengers through time, Professor Emeritus William H. Sawyer (Sawyer 2016) and Dr. Allen P. Minton (Minton 2016).

As Editors of the special issue, we would like to thank all of the contributors for their efforts in meeting the special issue deadline and to Don for his help in filling in the gaps with information about his life and career. To one of us (DH), Don was a PhD supervisor (1995–2000)¹, whilst to the other (SH), Don has been a long-time scientific collaborator²; however, to both, Don has been a stalwart companion, good friend and genuinely interesting character (Fig. 1). In this introduction, we describe a roughly chronological account of Don’s scientific history and finish with a few personal recollections about Don’s life and career (Winzor 2016).

Fig. 1 Example of an ideal interaction. Don Winzor (*left*) enjoying a beer with Allen Minton (*middle*) and Stephen Harding (*right*) in Nottingham

Early life and university

Don was born in 1935 in Gawler, South Australia, and, as the only son, seemed destined to follow the family tradition of becoming a farmer. However, his completion of primary education as dux of the school led Don's parents to consider higher education as an alternative career pathway. Excellent results at high school propelled him into science at the University of Adelaide, from which Don graduated in 1956 with First Class Honours in Physical Chemistry. The award of a CSIRO postgraduate scholarship then paved the way for PhD studies (1957–1959) with J. M. (Mike) Creeth on the physico-chemical characterisation of ovalbumin by diffusion, sedimentation velocity and moving boundary electrophoresis. The award of a PhD in 1960 was followed by a DSc in 1976.

Time at the CSIRO Wheat Research Unit (1960–1967)

On completing his PhD studies in 1959, Don moved from Adelaide to Sydney to take up an appointment as Research Scientist in the newly formed CSIRO Wheat Research Unit.

¹ Don's undergraduate lecturing and postgraduate supervision were notable for a number of things. The lecture courses were quite hard; indeed, upon receiving a B-grade for one of Don's undergraduate courses, DH was congratulated on achieving the highest mark in four years (we note that such a situation would not be allowed in today's universities). As a postgraduate supervisor, Don was unusual in his tendency to only take on one student at a time, his encouragement/requirement of students to sit postgraduate coursework and the enjoyable Friday lab meetings at the staff club, nearly always accompanied by a jug of beer or two (when Don was not travelling overseas - indeed see Fig. 1 for an example of both).

² The origins of the relationship between SH and Don stems from the common link of Mike Creeth, Don's PhD supervisor at the University of Adelaide and SH's postdoctoral mentor at the Lister Institute of Preventive Medicine in London. Mike was the nexus of their mutual interest in analytical ultracentrifugation. A nice exploration of this linkage can be found in their joint essay on his life (Harding and Winzor 2010).

His first 2 years there introduced him to the fractionation of gluten proteins by cellulose ion-exchange chromatography, and also to the librarian, Felicity, who became his wife. A honeymoon in Europe preceded their arrival at Cornell University for Don to take up a 12-month research associate-ship with Harold Scheraga in the Department of Chemistry. The year at Cornell resulted in the development of frontal size-exclusion chromatography for the quantitative characterisation of protein self-association (Winzor and Scheraga 1963, 1964). That work, a logical extension of his classical training in ultracentrifugation and electrophoresis, gained a degree of international acclaim for Don as a biophysical chemist at the age of 28 years old. Upon his return to Sydney, Michael Tracey, Leader of the Wheat Research Unit, encouraged Don to continue basic research into the characterisation of interacting systems. Those academic pursuits, performed in conjunction with Laurie Nichol, continued to the end of 1967 (Nichol and Winzor 1964; Winzor and Nichol 1965; Nichol et al. 1967a, b, c), at which stage Don moved to Brisbane to take up an appointment as Senior Lecturer in the Department of Biochemistry at the University of Queensland.

Transfer to academia

For someone faced with the prospect of presenting the first lecture in biochemistry that he had ever attended, the transition to academia posed considerable challenges. On the research side, it was facilitated by an affiliation with the Enzyme Biology Group headed by Colin Masters, who introduced Don to interactions involving glycolytic enzymes and proteins associated with the actin filaments of muscle myofibrils. At around this time publications with his first PhD student (Steven Lovell) began to appear (Masters et al. 1969; Clarke et al. 1970, 1976; Masters and Winzor 1971; Lovell and Winzor 1974, 1976, 1977). These efforts were rewarded with promotion to Reader in Biochemistry in 1972, a position held until Don was appointed to a Personal Chair in 1992. Upon retirement in 2000, he was granted Professor Emeritus status.

Characterisation of protein interactions

Collaborative studies with Laurie Nichol continued, with demonstrations of ways in which to unravel and quantify the interactions responsible for various types of self-association (Nichol et al. 1984) and binding behaviour (Baghurst et al. 1971; Nichol et al. 1972, 1973; Winzor et al. 1977; Tellam et al. 1978; Ward and Winzor 1982; Nichol and Winzor 1985; Cann and Winzor 1987; Gow et al. 1990; Wilson et al. 1997; Winzor et al. 1998). The effect of ligand-induced self-association on the size of acceptor species (Nichol and Winzor 1976) was used to explore

the crosslinking theory of lymphocyte activation (Sawyer and Winzor 1976), as well as to characterise an antigen–antibody interaction from the precipitin curve (Winzor et al. 1989). Sigmoidal binding behaviour reflecting self-association of the ligand (rather than the acceptor) was also demonstrated (Sculley et al. 1981; Cann et al. 1981; Hogg and Winzor 1984a), as was the need to consider kinetic as well as thermodynamic consequences of the parking problem in the characterisation of non-specific protein interaction with linear polymer chains (Munro et al. 1998, 2000; Winzor 2013; Hall and Winzor 1999). Although most physico-chemical characterisations of interactions refer to *in vitro* systems, results from a study of the redistribution of desipramine in rats following the administration of a desipramine-specific monoclonal antibody allowed for estimation of the operative binding constant for the antigen–antibody interaction *in vivo* (Pond et al. 1991).

A 12-month sabbatical in Oxford

Sabbatical leave with Alexander (Sandy) Ogston for the 1974 calendar year in the Department of Biochemistry at Oxford University proved to be an extremely productive research experience for Don, providing as it did the first opportunity for direct collaboration (rather than by correspondence). The first problem tackled was the development of a set of quantitative expressions for the characterisation of all possible combinations of solute–matrix, solute–ligand and matrix–ligand interactions by quantitative affinity chromatography (Nichol et al. 1974), a technique introduced by Don in conjunction with Pat Andrews (University of Reading) the year before. A solution to the problem of allowance for the effects of osmotic gel shrinkage on the characterisation of protein self-association by gel chromatography then followed (Baghurst et al. 1975). This period also involved Don's introduction to the statistical-mechanical treatment of thermodynamic nonideality on the basis of excluded volume (Ogston and Winzor 1975) a point of view which was later used to invalidate the Adams–Fujita approach to nonideality in the characterisation of protein self-association by equilibrium techniques (Scott and Winzor 2009).

Quantitative affinity chromatography

A noted odd feature of early quantitative affinity chromatography studies was the seeming conformity of experimental data with the behaviour predicted for a univalent solute (acceptor), despite the presence of multiple acceptor sites for interaction with matrix affinity sites (Brinkworth et al. 1975). Initially, this behaviour was attributed (somewhat intuitively) to spatial considerations, which suggested that further

acceptor attachment after the initial event was unlikely to occur (Nichol et al. 1974). This potential paradox was resolved by quantitative solution to the problem of acceptor multivalency in its interaction with matrix-bound affinity sites (Nichol et al. 1981; Winzor et al. 1982), a problem solved initially by means of Flory reacted-site probability theory but later handled using a different approach (Lollar and Winzor 2014). That development then led to a counterpart of the Scatchard equation for a multivalent ligand (Hogg and Winzor 1984b, 1985); a mathematical statement of the condition under which the affinity chromatographic behaviour became symptomatic of a univalent solute (Kalinin et al. 1995); and to the binding equation of which the multivalent counterpart of the Scatchard equation was its linear transform (Harris et al. 1995). Around this time, some potential breakthroughs for the study of high-affinity interactions were also developed by Don, including the refinement of a recycling affinity chromatography procedure (Hogg et al. 1991) and the derivation of analytical expressions in terms of total ligand concentration (Winzor et al. 1992). The feasibility of measuring rate constants by affinity chromatography was also demonstrated (Winzor et al. 1991; Munro et al. 1993, 1994). Solution of the solute multivalency problem in quantitative affinity chromatography paved the way for partition equilibrium studies of interactions of glycolytic enzymes with muscle myofibrils, which established their significance at physiological ionic strength (Kuter et al. 1983). However, the results of these studies cast doubt over the existence of glycolytic enzyme complexes on the myofibrillar matrix as a means of enhancing glycolysis by direct metabolite transfer between enzymes. This doubt arose from the demonstration of active-site involvement in the aldolase–myofibril interaction (Harris and Winzor 1987) resulting from competition between some glycolytic enzymes for the myofibrillar matrix (Harris and Winzor 1989a), and by the demonstration that calcium ions were a non-competitive inhibitor of aldolase adsorption, with a binding constant corresponding to that for the Ca^{2+} –troponin interaction (Harris and Winzor 1989b).

ELISA and biosensor studies of interactions

Don used his experiences gained from characterising solute partition in affinity chromatography to improve the quantification of antigen–antibody interactions by ELISA (Hogg et al. 1987; Winzor 2011), as well as by the biosensor technologies that emerged in the early 1990s (Ward et al. 1995; O'Shannessy and Winzor 1996; Hall and Winzor 1997, 1999; Hall et al. 1997; Edwards et al. 1998), methods in which the measured binding constant was previously based on 1:1 reaction stoichiometry, and referred to the solute–matrix interaction rather than that between reactants in the solution phase.

Effects of thermodynamic nonideality

Don performed a series of studies in which the effects of thermodynamic nonideality were either taken into account on the statistical-mechanical basis of excluded volume (Nichol et al. 1978, 1979; Siezen et al. 1981) or used for the detection of conformational changes in enzyme kinetic and ligand binding reactions (Nichol et al. 1983, 1985; Ford et al. 1983; Winzor et al. 1984; Shearwin and Winzor 1988a, b). Additional methods for evaluating second virial coefficients were also illustrated (Van Damme et al. 1989; Shearwin and Winzor 1990a). Excluded volume considerations were used to justify interpretation of the acid expansion of ribonuclease and serum albumin in terms of displacement of an isomerisation equilibrium between native and expanded enzyme states (Shearwin and Winzor 1990b). Another finding of significance was the demonstration of pre-existence of the isomerisation equilibrium as the source of allosteric effects for rabbit muscle pyruvate kinase, the only system for which unequivocal distinction between the Monod and Koshland models of allostery had, up till that point, been achieved (Harris and Winzor 1988). Indeed, to the present day such non-ideal experiments are one of only a few experimental techniques available for uncovering the possible existence of protein isomerisation equilibria (Bergman and Winzor 1989a, b; Bergman et al. 1989; Lonhienne and Winzor 2001; Lonhienne et al. 2003a, b), which otherwise remain merely theoretical postulates.

The Wills–Winzor collaboration

In the midst of the above investigations of thermodynamic nonideality, the departure of Laurie Nichol from the research scene to take on the Vice-Chancellorship of The Australian National University (1985) heralded the commencement of an equally fruitful collaborative effort in which Don was joined by Peter Wills from the University of Auckland, with a goal of gaining a deeper understanding of thermodynamic nonideality and, thereby, of improving the rigour of procedures for incorporating it into the quantitative characterisation of protein interactions. In that regard, the early demonstration that the displacement of protein equilibria, by small as well as macromolecular cosolutes, found rational explanation in terms of excluded volume (Winzor and Wills 1986) suggested that molecular crowding and protein solvation could be considered as the same phenomena. However trying to establish an equivalence of the quantitative expressions for the two phenomena required a return to earlier theoretical treatments (Wills 2016). The outcome of those revisionary deliberations was the realisation that, for highly concentrated systems, differing analytical results would be gained based on the choice of concentration scale (molar or molal) to define the system thermodynamics. Under constraints of constant temperature and solvent chemical potential, the

chemical potential of a solute is a molar quantity, whereas it is a molal parameter under constraints of constant temperature and pressure (Wills and Winzor 1992; Wills et al. 1993; Wills and Winzor 1993; Winzor and Wills 1995a).

Another problem addressed was the nature of thermodynamic activity being monitored in sedimentation equilibrium distributions. Those studies led not only to correction of the basic sedimentation equilibrium equation for a single solute (the density parameter is that of the solvent instead of the solution density), but also to identification of the thermodynamic activity as a molar quantity, a finding of considerable significance in that the statistical-mechanical treatment of thermodynamic nonideality in terms of excluded volume refers specifically to molar thermodynamic activity (Wills and Winzor 1992; Wills et al. 1993; Wills et al. 2000a).

Solution to the question of the identity of the nature of the thermodynamic activity being monitored in a given experiment prompted a series of investigations with thermodynamic nonideality as the central theme (Wills et al. 1995; Hall et al. 1995). Notable outcomes included: (i) a reinterpretation of the concept of osmotic stress (Winzor and Wills 1995b; Poon et al. 1997), (ii) development of a direct analysis of sedimentation equilibrium distributions for self-associating proteins that takes account of thermodynamic nonideality on the statistical-mechanical basis of excluded volume (Wills et al. 1996), (iii) a demonstration that sedimentation equilibrium distributions for a non-interacting protein in the presence of a small cosolute provides a simpler way of obtaining information analogous to that derived from the Eisenberg treatment of partial specific volume measurements (Jacobsen et al. 1996), (iv) an analysis of sedimentation distributions for non-interacting proteins that quantifies second virial coefficients for solute self-interaction (Wills et al. 2000a; Winzor et al. 2001) and (v) development of a global analysis of sedimentation equilibrium distributions reflecting nonideal association of dissimilar reactants (Wills et al. 2000b).

Research in retirement

The downsizing of research resources that comes with retirement has slowed down Don's research efforts somewhat. Since retiring, Don has received funding for a number of short-term visits to the University of Nottingham in a Biotechnology and Biological Sciences Research Council (UK) grant to the National Centre for Macromolecular Hydrodynamics. In retirement, the Wills–Winzor team has expanded to include Steve Harding, Dave Scott and Trushar Patel. During this period, Don's major research theme has, once again, swung to thermodynamic nonideality (Chebotareva et al. 2001, 2005; Wills and Winzor 2001, 2002, 2009; Winzor and Wills 2003, 2006, 2007; Winzor et al. 2007a; Wills et al. 2012). Methods for the prediction of the thermodynamic osmotic 2nd virial coefficient from

structure and charge information were developed with the routine COVOL (Harding et al. 1998, 1999) and the scope expanded to cover the determination of second virial coefficients from static light scattering measurements. Results from those methods (Deszczynski et al. 2006; Winzor et al. 2007b) as well as spectral studies (Wills and Winzor 2011) have allowed the testing of theory associated with the correct definition of virial coefficients (Wills et al. 2015). Attention has also turned to the analogous situation in small-angle X-ray scattering studies (Scott et al. 2010, 2011, 2013), which have been used to deduce the structure of components of the basement membrane protein system (Patel et al. 2011, 2012, 2014). Of very late, in a return to his research starting point as a PhD student, Don has been investigating the concentration dependence of diffusion coefficients for globular proteins, extending theories for Brownian motion in dynamic light scattering (Harding and Johnson 1985a, b) and boundary spreading in the analytical ultracentrifuge (Scott et al. 2014), along with the possible use of an approximate steady-state condition in sedimentation velocity experiments (Creeth 1964) to measure the diffusion coefficients of proteins exhibiting a sufficiently large negative concentration dependence of the sedimentation coefficient (Scott et al. 2015).

Concluding remarks

Don's research style has been marked by a number of notable features. One has been his prodigious output, involving around ten papers a year for the last 60 years. Another is his tireless work ethic, which may/may not have its origins in his farming upbringing. Don's always eager and energetic contributions, whether it be by simple discussion, conference organisation, taking on reviewing jobs or the critiquing of a colleague's or student's written work, has impressed all those who have experienced it. Balancing this zealous work ethic has been a healthy dose of humanity, which has shown itself in the form of kindness to workmates and colleagues in times of need. Indeed, with regards to this last point, we suspect that there are many working scientists today who, in part, owe their research success/tenured position/visa status/society membership to a well written letter of support, carefully crafted piece of advice or incisive critique written by Professor Donald J. Winzor. Third and fourth on the list of Don's admirable qualities have been his very real enthusiasm and passion for his topic of physical biochemistry and his courage to, when necessary, dig in and argue heatedly over a point of science or conduct that he believed to be incorrect. Indeed, in a scientific world increasingly defined by slick operators who focus more on the psychology of collaboration and academic progression than the actual topic of scientific investigation, this last quality will be sorely missed when Don eventually decides to retire (from his semi-retirement).

In closing, we note that Don and his wife Felicity have three daughters (Catherine, Christine and Carol), along with six grandchildren. We (DH and SH), along with all the contributors to this special issue, wish Don and his family the very best for the future and congratulate him on his just passed 80th birthday. We hope that this special issue acts as a suitable commemoration to a scientific career defined by a desire to understand biochemical phenomenon in a quantitative and analytical fashion.

All the best Don in the years to come.

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Compliance with ethical standards

Conflict of interest Damien Hall declares that he has no conflicts of interest. Stephen E. Harding 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.

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