

John Schellman and his scientific work

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In 1955 John Schellman wrote a paper estimating the stability of the α -helix in aqueous solution [1]. Four years earlier Pauling and Corey had proposed that the α -helix should be a major structural unit in proteins, but no one had yet seen an α -helix. Only in 1958, when Kendrew and co-workers determined the structure of the first protein (sperm whale myoglobin) at 0.6 nm resolution, could α -helices begin to be seen. So John's paper was on the cutting edge, to put it mildly. His was also the first modern paper on the energetics of protein folding: Kauzmann's landmark paper on the energetic importance of burying non-polar side chains in the interior of proteins appeared 4 years later in 1959.

John's classic paper was only part of the post-doctoral work he accomplished at the Carlsberg Laboratory from 1953–1955. It was a glorious time both for John personally and for Linderstrøm-Lang and the group of visitors to the Carlsberg Laboratory. Many notable figures in protein chemistry came to the Carlsberg in the 1950's, including Chris Anfinsen, Charlotte Green (she married John), Bill Harrington (then a postdoctoral fellow, like John, who was closely associated with John at the Carlsberg), Walter Kauzmann (John's Ph.D. adviser at Princeton), Fred Richards and Harold Scheraga. The group at Carlsberg had a strong sense of optimism that the secrets of protein folding would soon be solved, and Linderstrøm-Lang would play a leading role. John and Charlotte have

described Linderstrøm-Lang's own scientific achievements and his personal gaiety and charisma [2,3], and Fred Richards has described [4] how enjoyable life was at the Carlsberg in those days.

In his 1955 paper John proposed that the α -helix is stabilized in water by the net enthalpy of the peptide H-bond [1], in other words that the helix backbone is responsible for whatever stability an isolated helix in water possesses. John's proposal that the enthalpy of the peptide H-bond is favorable for helix folding became the center of an active controversy in the early 1960s, as a result chiefly of work from Irving Klotz' laboratory on the dimerization of amides. John hates controversy and he said nothing more on this subject for a long time. In his companion 1955 paper [5], he estimated the net enthalpy of the peptide H-bond in water to be -6.3 kJ/mol, quite close to the modern value (~ -4 kJ/mol) for peptide helices. John's value was based on interpreting the heat of dilution of urea solutions as arising from the dissociation of H-bonded urea dimers [5]. John also proposed that the major factor opposing helix formation is the loss of backbone conformational entropy upon folding. He gave plausible upper and lower bounds for the number of backbone conformations per peptide unit and in this way obtained for the entropy loss an upper limit of 29 J/mol K and a lower limit of 13 J/mol K. With the upper limit, the α -helix backbone is marginally unstable in water whereas with the lower limit it is margin-

ally stable. Determination of the actual value of the entropy loss on helix formation is still under study today.

Moreover, in his 1955 paper John analyzed the end effect on helix stability which arises both from the non-H-bonded groups at the two ends of the helix, the 4 peptide NH groups at the N-terminal end and the 4 peptide CO groups at the C-terminal end, and from the increased freedom of a backbone bond at each end of the helix. In 1958 John showed how his model could be used to describe the peptide helix-coil transition [6], by using statistical thermodynamics to include fluctuations. His helix end effect is related to the helix nucleation constant in helix-coil theory, and John's analysis of the stability of an α -helix was followed by Zimm and Bragg and by Lifson and Roig in their theories of the peptide helix-coil transition based on statistical mechanics. The modern search for helix-capping motifs was motivated by the fact that 4 NH and 4 CO groups at either end of an α -helix are not H-bonded.

Thus, John laid out a work program in his 1955 paper that is still ongoing today, for measuring, analyzing and understanding the basic thermodynamic parameters of peptide helix-coil theory and for understanding the properties of the helix-coil transition. In a separate influential paper from their Carlsberg stay, Bill Harrington and John considered oxidized ribonuclease A as a possible model for an isolated α -helix in water [7]. They found it does not form a stable helix and they concluded there must be energetically important interactions in proteins besides forming α -helices and β -sheets. An important Carlsberg-related contribution was the advice John gave to Aase Hvidt as she pondered the meaning of the pH dependence of exchange experiments on proteins, and considered that it might result from an equilibrium between open and closed forms of the protein [8], a suggestion made originally by Linderstrøm-Lang. John showed her how to solve the basic kinetic equation, whose solution shows that the exchange rate is controlled by one of two mechanisms: either the commonly occurring, pH-dependent EX2 mechanism, in which the exchange rate depends on the equilibrium constant for interconversion between open and closed forms of the protein, or

by the pH-independent EX1 mechanism, in which exchange depends on the rate constant for opening the closed form to an exchange-susceptible form.

John took his Ph.D. in 1951, in three years, in theoretical chemistry with Walter Kauzmann at Princeton. His first scientific paper [9] was on the dielectric polarization of ice, which appeared in *Physical Reviews* in 1951, with Walter Kauzmann as co-author. John found time during his Ph.D. to take part [10] in Kauzmann's experimental program of determining the physico-chemical basis of protein denaturation. This program led to the modern understanding that protein denaturation is protein unfolding.

John's main postdoctoral project at the Carlsberg Laboratory was to develop optical rotatory dispersion (ORD) into a tool for detecting and quantitating protein secondary structures. He and Charlotte made extensive studies of the ORD spectra of various proteins, which were published in 1958 as an opus magnum of 7 papers, 3 years after they left the Carlsberg Laboratory. The first of the seven papers was a description of the theory and methods of studying optical rotation by John [11] and the last paper was a summary of the field by Charlotte and John [12]. By the time their papers appeared the field had shot off in a new direction. First there was a paper by Yang and Doty on α -helix formation by poly- γ -benzyl-L-glutamate, plus observation of its helix-coil transition in mixed organic solvents, and then came theories of the optical rotatory properties of the α -helix by Moffitt and by Fitts and Kirkwood.

John and Charlotte returned to the US in the Chemistry Department of the University of Minnesota at the suggestion of Rufus Lumry, with whom John had worked for a year before going to the Carlsberg Laboratory. They stayed in Minneapolis from 1956–1958 but could not resist an invitation to move to the Chemistry Department of the University of Oregon in 1958. John became one of the early members of Aaron Novick's new Institute of Molecular Biology at Eugene. They were soon joined, both in the Chemistry Department and the Institute, by Peter von Hippel, a close scientific colleague and personal friend of John. Together John and Pete taught a course on biophysical chemistry which was the envy of many

universities and which greatly influenced the students and fellows of the Institute. Bill Harrington visited John in Eugene not long after John and Charlotte moved there. I asked Bill how John was settling in. ‘Well, I don’t know about John,’ Bill replied, ‘he spends his evenings reading Einstein’s papers in the German.’

At Oregon, John pushed ahead vigorously with the projects he had started at the Carlsberg Laboratory. John’s 1958 discussion of the theory and uses of optical rotation [11] had already pointed out that circular dichroism (CD) would be a better tool than optical rotatory dispersion for studying an asymmetric structure such as the α -helix, and John planned to build an instrument that could measure CD. He spent a sabbatical year in Paris in 1963–1964 at the Laboratoire des Hautes Pressions investigating topics such as the birefringence of quartz in the Schumann region, preliminary to building a CD instrument. The commercial importance of a CD instrument was obvious to several people by this time, however, and a commercial instrument became available before John finished building his, so he abandoned the project. He and his family spent a wonderful year in Paris, nevertheless, and they had no regrets about being there, especially from a cultural and culinary viewpoint. Before John went to Paris, he and Patrick Oriol discovered that the $n\text{-}\pi^*$ transition of the peptide group is responsible for the Cotton effect (or the 220 nm CD band) used to quantitate α -helix formation [13]. Bob Woody and Nacho Tinoco also found this result in independent work.

John undertook the study of the CD spectra of diamides as models for the peptide group, with backbone conformations localized in all the well-populated sections of the Ramachandran map. Then he also undertook development of CD theory appropriate to interpreting the results. He completed most of this project, but I understand that a few CD spectral results still resist interpretation. Nevertheless, a lot is now known about this subject through his efforts. A 1972 paper by Vincent Madison and John summarizes much of this work [14]. A CD paper by John [15] became a citation classic: ‘Symmetry rules for optical rotation.’

In 1969–1970 John took a Guggenheim Fellowship to the Weizmann Institute in Israel, where he shared the scientific companionship of Shneior Lifson. It was a particularly profitable and enjoyable year, a rare opportunity to discuss and compare theoretical approaches in biophysical chemistry with a master of the craft. John was deeply interested in the Lifson–Roig approach to the problem of the polypeptide helix–coil transition, which uses the backbone phi and psi angles as key variables. Later John and Hong Qian [16] showed how the parameters of the Zimm–Bragg theory could be calculated from those of the Lifson–Roig theory, or vice versa. Their paper solved the problem for experimentalists of how to translate experimentally measured parameters of one theory into those of the other.

In the 1970s John began a systematic study of certain physical properties of DNA, while continuing his work on polypeptides and proteins. He provided a theoretical framework in terms of statistical polymer theory for interpreting studies of DNA flexibility and he also made flexibility measurements by flow dichroism. At the same time, he showed how the measured optical properties depend on orientation of the macromolecule: see the paper by Jim Hofrichter and John [17]. A surprising offshoot of the work on DNA flexibility was the discovery by Leonard Gosule and John [18] that spermidine causes the DNA of phage lambda to condense into a compact form, with dimensions small enough for packaging inside the phage head. With his good friend Dirk Stigter [19], John began a long-term study of DNA electrostatics and the interpretation of measurable quantities that depend on electrostatic properties.

Also in the 1970s, a project inspired by the late George Streisinger came into being at the Institute: a study of mutants of Streisinger’s protein, the lysozyme of phage T4. Using independent approaches appropriate to each one’s laboratory, some members of the Institute, including John and Charlotte, Brian Matthews and Rick Dahlquist, went to work on characterizing T4 lysozyme mutants. John showed how to interpret thermodynamic data on their unfolding reactions and his procedures are widely used today: see the summary

by Wayne Becktel and John [20]. John went on to study the kinetics of the unfolding reactions and his methods for analyzing kinetic data have also been influential.

At the end of the 1970s John returned to a problem he had already considered briefly in his 1955 paper [1]: how do protein denaturants work? The standard explanation had been that denaturants bind at sites exposed in the unfolded protein and pull the unfolding reaction, and the binding reaction is of the standard site-binding type, like the binding of protons to glutamate side chains. In 1955 John gave the equation for binding to multiple, equivalent sites [1], an equation that afterwards was used generally to interpret denaturation experiments. However, in 1974 Nick Pace found that the free energy of the unfolding reaction depends directly on the denaturant concentration, whereas the site-binding equation predicts a dependence on the logarithm of the denaturant concentration. In 1978 John showed that the observed dependence is a natural consequence of the interaction between denaturant and protein being of the weak interaction type, rather than the site-binding type [21]. In the following years, both John and Serge Timasheff probed ever more deeply into the physical chemistry of the denaturation reaction, and showed the importance of an exchange between the solvent (water) and the denaturant for understanding the measured properties. Recently, John interpreted the changes in excluded volume that occur in the denaturation reaction [22].

Recognition of John's splendid contributions to biophysical chemistry has come in several forms, including election to the National Academy of Science in 1982 and of Arts and Sciences in 1983, and designation as a Fellow of the Physical Society in 1984 and of the Biophysical Society in 2001. John received honorary doctorates from Chalmers University (Sweden) in 1984, and from the University of Padua (Italy) in 1990. The high esteem of his peers can be illustrated by the following anecdote. When John was approaching retirement, he decided to turn in one last, short, research grant proposal to the National Institutes of Health. It said in essence: 'I have been collecting significant

unsolved problems in biophysics. Ten of them are described below. If you award me this grant, I will try to solve some of them.' His proposal received a top priority score, and of course he solved several of the problems.

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