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Jeffries Wyman: Scientist, Philosopher and Adventurer

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Jeffries Wyman, to whom this issue of *Biophysical Chemistry* is dedicated, is a man deeply rooted in his New England origins, yet very much a citizen of the world. He has lived abroad, mostly in Europe, for nearly forty years, but has never lost touch with his New England roots. I cannot write of him with detachment, for we are linked by a friendship that goes back to our student days, seventy years ago. I have told elsewhere in more detail the story of our two interacting lives [1]; here I focus more sharply on the special quality of his achievements and his outlook on the world.

His ancestors have been New Englanders from a long way back. His great grandfather, Rufus Wyman, was a psychiatrist of the early nineteenth century, and a pioneer in the humane treatment of the mentally ill. Rufus's son, the first Jeffries Wyman (1814–1874), was a great comparative anatomist, and probably the foremost American physical anthropologist of his time; he was a founding member of the U.S. National Academy of Sciences. He was a gentle and modest man, universally admired and beloved [2]. His son, the second Jeffries, was a businessman, not a scientist; it is *his* son, the third Jeffries, born in 1901, whom we honor in this special issue.

Entering Harvard College in 1919, Jeffries devoted himself at first to philosophy. His tutor in the philosophy department, Professor Raphael Demos, influenced him greatly and became a lifelong friend. The appeal of biology, physics and mathematics, however, drew Jeffries away into the sciences; but the spirit of philosophical inquiry remains a part of his nature. He has always asked, and still asks, deep questions about the nature of the world, and is not satisfied with easy answers. P.W. Bridgman's course in advanced thermodynamics was a major experience for him as a graduate student. In the course of time this was to lead him back to the work of Willard Gibbs, and on to his own major contributions to the thermodynamics of multicomponent systems.

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1. Early days abroad: Cambridge and London

In the autumn of 1924 Jeffries and I came to Cambridge, England, and began the Part II Biochemistry course in the new Institute, where for the first time Sir Frederick Hopkins had a building of his own to accommodate the throngs of young biochemists who wanted to work in his Department. Hopkins himself, a gentle and modest man, was a great leader. The Reader, next in command, was the unforgettable J.B.S. Haldane, powerfully built, immensely learned, and with a thunderous voice. He had learned from his famous father, John Scott Haldane, to do all sorts of experiments on himself, as the best way to solve some important problems. David Keilin, in the Molteno institute close by, was just beginning his great work on the cytochromes, and was full of kindness and encouragement for young people like Jeffries and me.

For us G.S. Adair was also an important influence. His beautiful osmotic pressure studies on

hemoglobin had just shown the molecule to be four times as large as most people had thought; so there were four oxygen-binding centers, not just one. Adair saw clearly that the cooperative interactions in oxygen binding could be understood if the first oxygens that were bound somehow enhanced the oxygen affinity of the still unoccupied sites. He formulated the basic equations to describe the interaction, but of course its underlying mechanism remained unknown. Still Adair had made a major advance. He was also probably the first biochemist to study carefully the thermodynamics of Willard Gibbs, and he pointed out that Gibbs had actually formulated the Donnan equilibrium relations, 35 years before Donnan. Jeffries could hardly have known at that time what a central role hemoglobin was to play in his scientific life, but those conversations with Adair certainly planted ideas in his mind that were to come to fruition many years later.

In another Cambridge laboratory, Hamilton Hartridge and F.J.W. Roughton, studying the reaction of hemoglobin with its ligands, found that they could get reliable values for the rates of reactions a thousand times faster than any measured before. The two reacting solutions flowed rapidly together into a special mixing chamber, where they mixed completely in less than a millisecond. The mixture then flowed down an observation tube, along which the observer could follow the course of the reaction spectroscopically. With this technique, the rates of binding and release of hemoglobin ligands thus became known, with important implications for the rate-limiting reactions in the circulation of the blood.

However, Cambridge biochemistry, Jeffries found, was not what he really wanted. The work that A.V. Hill was doing in London, on the mechanical and thermal events associated with muscular contraction and relaxation, appealed to him much more. After one term in Cambridge he left for London, though he and I continued to see much of each other. The rigorous training and mental stimulation that he got in Hill's laboratory, he always considered, provided a splendid preparation for the rest of his career, though he was never to work on muscle again.

2. Return to Harvard; studies on dielectric constants of very polar systems

Returning to Harvard in 1927 with a Ph.D. from London, Jeffries became an instructor in the Biology Department. However, he also became deeply involved in the work of the Department of Physical Chemistry at Harvard Medical School, headed by Edwin J. Cohn. This laboratory was dedicated to the physical chemistry of proteins, and of the amino acids and peptides of which proteins are built up. Cohn had drawn around him a group of young investigators, including T.L. McMeekin, Arda A. Green, Jesse P. Grenstein, and myself; later J.L. Oncley and J.D. Ferry joined the group. Cohn's intimate friend George Scatchard, at M.I.T., took a deep interest in the problems we were studying, and supplied essential insight. Soon his brilliant young colleague, John G. Kirkwood, also at M.I.T., became involved. Jeffries played a major role, and made a crucial contribution, that opened new vistas on the electrical properties of proteins and amino acids. A major problem in those days concerned the implications of the highly polar structure proposed for the amino acids by E.Q. Adams, and later independently by Niels Bjerrum. If they were right, and they had strong arguments, isoelectric glycine in water was almost entirely $^{+}H_3NCH_2COO^{-}$, not H_2NCH_2COOH, as most people had believed before. The same would be true of other α-amino acids. That is, there were essentially two ionic groups of opposite charge, about 3 Å apart, in the molecule. This would imply a dipole moment of some 14 Debye units, much higher than that of any molecule previously studied by the methods of Debye. A peptide, with a longer chain of atoms between the charges, would be expected to have a correspondingly higher moment. Amino acids and peptides, of course, were practically insoluble in nonpolar solvents; they had to be studied in aqueous solution, and there was no theory then available to deduce the dipole moment of the solute from the dielectric constant measurements, in such a highly polar system.

Jeffries set out to develop a new method of measuring dielectric constants in such solutions,

for the existing methods were unsuitable for aqueous solutions of such relatively high conductivity. Within a year or two he had solved that problem, and, with T.L. McMeekin in Cohn's laboratory, studied dielectric properties of amino acids and peptides. The results were dramatic. Even though water has one of the highest dielectric constants known for any liquid (78.5 at 25 °C), any added α-amino acid increased this value greatly, to about 102 for a molar solution. A simple linear equation described the data: the dielectric constant, ϵ, was a linear function of the molar concentration, c, so that $\delta = d\epsilon/dc$, was a constant over the wide range of concentration studied. The value of δ for α-amino acids was close to 23; for dipeptides near 70, for tripeptides near 113. Each extension of the peptide chain increased the value of δ by about 45. For lysylglutamic acid, with two positive and two negative charges well separated on the molecule, the value was a spectacular 345.

Realizing that Debye's treatment for less polar systems could not possibly explain these data, Jeffries developed a new interpretation, in which he concluded that the dielectric increment should be approximately proportional to the molar polarization, and hence to the square of the electric moment. The electric moments so inferred turned out to be close to those indicated by studies of molecular models for the actual distances between the charged amino and carboxyl groups, if one assumed that the actual molecules were not fully extended, but somewhat coiled. The dipolar ion structure of Adams and Bjerrum was completely vindicated. These studies by Jeffries (see ref. 3 for a review) provided the stimulus for Lars Onsager [4] to develop the first significant theory for the dielectric constants of polar liquids.

Jeffries also was perhaps the first investigator to determine the dielectric increment, and the rotary diffusion coefficient, of a protein, namely, zein in an ethanol-water mixture [5]. Some years later, with H.O. Marcy, working with improved apparatus, he studied the dielectric increment of myoglobin and its dispersion over a wide range of frequencies [6]. He was well aware of the power of this method for obtaining information on rotary diffusion coefficients of macromolecules, in conjunction with other measurements, such as coefficients of sedimentation, linear diffusion, specific viscosity, among others, and in 1943 he published a comprehensive nomographic treatment for coordinating such data, to calculate the best available values for the size and shape of proteins and other macromolecules [7].

3. Early studies on hemoglobin

Jeffries had long been deeply interested in the properties of the hemoglobin molecule, with its extraordinary adaptation for the efficient transportation of oxygen and carbon dioxide in blood. He had discussed hemoglobin in detail with his students in the course on biophysical chemistry (in those days it had another name) that he regularly gave at Harvard. His first publication on hemoglobin came in 1937, in work with a gifted undergraduate, Bernard German [8]. They studied the pH titration of horse hemoglobin in the deoxy and the oxy forms. Baird Hastings and D.D. Van Slyke, at the Rockefeller Institute, had published important work on this problem in 1924. At that time pH measurements had required the use of a hydrogen electrode, which naturally could not be used in a solution of oxyhemoglobin; so they had equilibrated the solutions with CO_2 at defined pressures, and calculated the pH from the ratio of dissolved CO_2 to bicarbonate. They could work only over a limited pH range by this method, but did show accurately the upward shift in acid strength of the group (or groups) that gave rise to the Bohr effect between pH 6 and 9, in which range oxygenation increases the acid strength of certain 'heme-linked' acid groups in the protein.

German and Wyman in 1937 had at their disposal the glass electrode, which was well developed by that time. With this, it was just as easy to titrate the oxy as the deoxy form of hemoglobin, and the measurements could cover a much wider pH range. Their work on the Bohr effect accorded well with that of Hastings and Van Slyke. They also discovered a 'negative Bohr effect', previously unknown, in solutions between pH 6 and about 4.3. In this region the deoxy form is a stronger acid than the oxy, in contrast to the Bohr effect, of opposite sign, at higher pH. Jeffries also made use

of reciprocal thermodynamic relations to calculate how the oxygen affinity of blood varies with pH. This was only the first hint of what was to come. Within the next few years he went on to study the heat of oxygenation of hemoglobin as a function of pH, by titration studies over a range of pH and temperature; and he then dealt with the more complex problem of correlating the pH dependence of the oxygenation process with that of the oxidative interconversion of hemoglobin and methemoglobin. However, these elegant studies were only the beginning of his later powerful and increasingly subtle analysis of linkage relations in multicomponent systems containing macromolecules.

The entry of the United States into the war was to draw him into work with the Navy, at the Woods Hole Oceanographic Institute, and later, in the Pacific, to the development of better smoke screens for the protection of warships in action. On returning to Harvard he resumed his research on hemoglobin, and I, as an editor of *Advances in Protein Chemistry*, was fortunately able to persuade him to write a comprehensive review of the whole subject of heme proteins. The review took a long time to materialize, but it was well worth waiting for [9]. It covered what was then known about hemoglobin and other heme proteins, in searching detail. It was also highly original in its analysis of thermodynamic linkage phenomena, including their application to the Bohr effect, to oxidation-reduction processes, to the form of the oxygen-binding curve, to the combination of competitive and cooperative interactions that are found when hemoglobin is exposed to a mixture of oxygen and carbon monoxide, and to a variety of other phenomena. His treatment of cooperative interactions in binding of ligands was an advance on previous work, though his major treatment of that great problem was still to come.

Indeed, his most important single flash of insight came some two years later, when he took leave from Harvard and spent six months in Japan as a scientific visitor, sponsored by the State Department. He moved from place to place, often on foot, accompanied by Japanese colleagues, and established numerous friendships, while he was lecturing and carrying on personal discussions. A sudden sense of illumination came to him one day, as he has told me, after he had paid a visit to the famous Zen Garden in Kyoto, with its stones and raked sand. He suddenly realized that both the cooperative interactions in ligand binding, and the Bohr effect in hemoglobin, might be due to a change in conformation of the molecule, induced by ligand binding. He lectured in Japan on this new idea, and indeed published a paper on it in the Japanese journal *Kagaku*.

After his return to Harvard he developed the idea systematically, in a paper with David W. Allen [10]. It had been known since the late nineteenth century that, for a given hemoglobin, crystals of the oxy and the deoxy forms often belonged to quite different crystallographic classes. The early work of Max Perutz on hemoglobin crystals, even though it had not yet led to a molecular structure, had already indicated that the oxy and deoxy molecules must differ significantly in conformation [11]. Jeffries developed his argument primarily on thermodynamic grounds, noting that the differences in the free energy of oxygen binding, for the successive steps in oxygenation, were primarily due to entropy effects, and therefore were to be interpreted in terms of changes in the conformational order of the molecular structure. The argument was subtle, intricate, and tentative in tone. This was apparently the first proposal of what Monod later termed an allosteric transition, as an explanation of cooperative interactions and the role of effectors (in this case protons) in modifying the interactions. The implications of these ideas were indeed far-reaching, but this paper appeared to have had little impact at the time on the thinking of others.

4. A new life in Europe, Africa and Asia

However, Jeffries chose, shortly after this, to leave Harvard, and he embarked on a quite different career, as Science Attaché in the United States Embassy in Paris. He clearly enjoyed this very different experience. He took a very active interest in establishing contacts with French scientists, visiting not only famous laboratories, but also others, less known, where his appearance might

come as a surprise. It was 1952 when he began, and the anti-Communist hysteria in Washington, driven by the reckless accusations of Senator Joe McCarthy, caused many foreign scientists to have serious difficulties in getting permission to visit the United States. Jeffries, with tact and skill, managed to straighten out such problems for a number of French colleagues.

After three years in Paris he embarked on a very different enterprise, as Director of the UNESCO Science Office for the Middle East: a large domain for him indeed, for it apparently included the entire belt of countries from Morocco to Pakistan. His headquarters were in Cairo, but his work naturally involved numerous and sometimes extended visits to many countries within his realm of responsibility.

It was during this time that he and I together managed to finish the first and (as it turned out) the only volume of our treatise on *Biophysical Chemistry* [12]. We had begun it much earlier, as an outgrowth of the course that first he, and later both of us together, had given for years to our students at Harvard. I carried on at Harvard with the writing and the teaching, while Jeffries found time, in the midst of other activities, to write his portion of the book. Occasionally we could get together, on one side or the other of the Atlantic, and settled down for intensive sessions of discussion and revision, generally with some long walks together in the surrounding countryside. I have told more elsewhere of the book and its history (ref. 1, pp. 148–149).

5. Return from administration to research: the years in Italy

Jeffries had been away from research, in diplomacy and administration, for nearly nine years. Few scientists, after so long an interval, manage to return to research at all, or can resume work at the forefront of knowledge if they do return. After his experience with UNESCO had ended, Jeffries was inclined to think that his research career was over, and was uncertain what to do. However, the young Eraldo Antonini, full of enthusiasm for what his group was doing on hemoglobin in Rome, sought him out, and pleaded eloquently that Jeffries should come to Rome and spend a year with them. What they were doing, he said, was a direct continuation of what Jeffries had done at Harvard. Jeffries accepted, for a year, and in the event he stayed for 24 years.

Distinguished as his work had been at Harvard, he surpassed it during the years in Rome. On the one hand he was a member of the group, with Antonini and his still younger associates, later joined by Maurizio Brunori, who were constantly planning new experiments, debating what they signified, after doing them, and planning still further experiments to test their ideas. Jeffries was deeply involved in all this. I had the pleasure of spending three months in the Rome laboratory in 1963, and participating in the lively discussions that went on within the group. The results of those years of very fruitful research, along with an account of much other research done elsewhere, are to be found in the book by Antonini and Brunori [13].

On the other hand, Jeffries pursued several major lines of thought, largely on his own, but often with essential stimulation from experimental work in other laboratories. This was true, for instance, of his analysis of the principles governing the process of facilitated diffusion, in which the diffusion of a ligand (such as oxygen) across a pressure (or concentration) gradient can often be greatly increased in the presence of a protein that combines rapidly and reversibly with the ligand. The phenomenon was discovered independently by P.F. Scholander and by J.B. Wittenberg. The latter was in Rome for a time, and Jeffries first learned of it from him, and developed an effective theoretical treatment [14]. However, there were some mathematical difficulties involved, which Jeffries could not resolve. He turned to his friend, J.D. Murray of Oxford, a mathematician deeply concerned with problems of biology, who found a solution [15]. Then, in a joint paper, Murray and Wyman [16] applied Murray's analysis to explain why, as experimenters had found, hemoglobin did not facilitate the transport of carbon monoxide, although it greatly facilitated the transport of oxygen. Qualitatively the reason was simple; there had to be a significant gradient of ligand binding

by the protein, as well as a gradient of free ligand activity, across the boundary, to achieve *facilitated* diffusion. In HbCO solutions, the CO was so tightly bound by the hemoglobin, and its rate of release was so slow, that virtually all the hemoglobin was in the form of HbCO, across the entire boundary. The only concentration gradient across the boundary layer was that of free CO in solution; there was essentially no gradient of HbCO concentration adjoining the boundary, and therefore no facilitated diffusion.

6. Linked functions and allosterism in multicomponent systems of macromolecules

However, the central problem with which Jeffries has continued to grapple, over the last quarter century, has been the deeper understanding of thermodynamic linkage relations in multicomponent systems containing biological macromolecules. His concern has not been restricted to equilibrium thermodynamics. In a series of papers he has considered systems in a steady state, and has ventured some preliminary analysis of irreversible phenomena. However, in this brief discussion, I cannot take up such matters, and will speak only of his equilibrium studies. In 1964, after a few years in Rome, he presented a larger and deeper view of linkage relations than he had given before [17] but this was only an early stage in an unceasing exploration that has continued for a quarter century. However, in the autumn of that same year, there was a major new development of the earlier ideas that he had expressed in his paper with David Allen [10].

This happened on a visit to Paris, when Jacques Monod asked him to give a seminar on his views concerning conformation changes in hemoglobin on ligand binding, and their relation to cooperativity and the action of modifying effectors. By this time the work of Max Perutz and his collaborators had shown striking differences between the structures of deoxyHb and of oxyHb. The concept Jeffries had put forward tentatively, though with considerable confidence, in 1951 [10] now had much evidence in its favor. Arthur Pardee, H.E. Umbarger, and others, had discovered feedback controls on enzymes involved in the first steps of metabolic sequences, and their relation to the phenomena observed in hemoglobin was apparent. Monod had become deeply involved in the study of such phenomena, and the ideas that Jeffries put forward in his lecture aroused him to formulate a new model for such allosteric systems. Soon he (and J.-P. Changeux) had produced a draft manuscript, with a mathematical analysis of the proposed system, and various applications to experimental data. Jeffries, in his personal recollections of Jacques Monod [18], has described the critical review that their paper received, before publication, in a long intensive personal discussion with the molecular biologists at the MRC laboratory in Cambridge, England. It finally emerged as the famous MWC model of a macromolecule existing in two conformational states, of widely different ligand affinity, in which increase of ligand binding produces a progressive shift from the low-affinity to the high-affinity state, as the ligand activity increases [19]. The model predicted both homotropic and heterotropic interactions, to use the terms that Jeffries has introduced. Oversimplified as the model necessarily was, it proved capable of fitting a remarkable range of experimental data, on a variety of allosteric systems, as many other workers soon proceeded to demonstrate.

Two years later Jeffries produced a searching and comprehensive paper on 'Allosteric Linkage' [20], bringing to bear the full power of linkage theory to the study of allosteric systems. Parts of this paper are by no means easy reading for most biochemists, but it brings together a series of important propositions, applying linkage relations to allosteric systems that can exist in two or more conformations, between which reversible transitions can occur. Such systems display cooperative free energy in ligand binding; his analysis contrasts them with analogous systems that likewise may consist of several different conformational isomers, in which however transitions between one conformation and another are forbidden, so that cooperative interactions with ligands cannot occur. In the following year he incorporated these considerations into a broader interpretation of regulation in macromolecules, with emphasis on

hemoglobin [21]. The steady growth of his conceptual scheme resulted, sixteen years later, in his discussion of 'linkage graphs', with a depth of analysis that indeed goes far beyond what that simple title indicates. [22].

For the last fifteen years or more, Jeffries has been closely involved with Stanley Gill, and for years used to spend every May with Gill at the University of Colorado, where he also collaborated with Paul Phillipson in the physics department. The Wyman-Gill collaboration was notable for a series of studies on sickle cell hemoglobin, in which they applied linkage theory to the phase relations between solutions and fibers of the deoxy form of this protein. For years they have been working on a comprehensive treatment of the whole domain of linkage theory and allosteric phenomena, in their applications to biological macromolecules. Beginning with elementary considerations, they have built up the treatment, step by step, until it covers the advances of recent years, including both equilibrium and steady state phenomena, with applications not only to hemoglobin but to a number of other allosteric proteins [23]. With Gill and Enrico Di Cera, Jeffries has continued to push on into more powerful mathematical approaches to linkage theory, as for instance in a recent canonical formulation that provides a broader understanding of generalized binding phenomena [24].

7. Jeffries Wyman as adventurer

Jeffries has been a great traveler, and he has traveled many times alone, in very independent fashion, and often on foot. He has ventured far from the well trodden highways, seeking shelter for the night in houses of the local peasants. He went once, on foot and alone, from India into Wakan, a region of Afghanistan normally closed to foreigners; and indeed he was evicted on orders from the local chiefs, who sent him back to India with all due respect, accompanied by a bodyguard, and riding on a yak. He has lived in the summer, for two months, with a tribe of Eskimos in northern Alaska, who apparently took good care of him and made him feel at home. He has walked alone in the Atlas Mountains of North Africa, and, with a small party of friends, and some luggage bearers, he took a fairly extensive journey in the Sudan, on foot. His prowess as a walker was legendary, and during his Italian years he took many friends, including me, on walks that brought us to quite wild and beautiful places that few travelers see. Those days are now in the past for him, since he is physically crippled and must walk with effort and for short distances.

In mind as in body he has been an adventurer, especially in his pursuit of linkage theory in all its ramifications. Following his own path, he has explored mathematical territory known to relatively few – certainly to few biologists – and developed it into a powerful tool for the understanding of important biochemical systems. When he has needed help in strange mathematical country, he has sought it out from others who knew the country better than he. He has been often a helper, stimulator and guide to fellow scientists, and not infrequently an inspiration for the young. Though chronic illness limits his physical activities today, his mind is still lively with the eager spirit of inquiry that has been his throughout life.

References

1 J.T. Edsall: Jeffries Wyman and Myself: a Story of Two Interacting Lives, in: Comprehensive Biochemistry 36, eds A. Neuberger, L.L. van Deenen and G. Semenza (Elsevier, Amsterdam, 1986) p. 99.
2 T.A. Appel, in: Science at Harvard University: Historical Perspectives, eds C.A. Elliott and M.W. Rossiter (Lehigh University Press, Lehigh, PA, 1990) in the press.
3 J. Wyman, Chem. Rev. 19 (1936) 213.
4 L. Onsager, J. Am. Chem. Soc. 58 (1936) 1486.
5 J. Wyman, J. Biol. Chem. 90 (1931) 443.
6 H.O. Marcy, III and J. Wyman, J. Am. Chem. Soc. 64 (1942) 638.
7 J. Wyman and E.N. Ingalls, J. Biol. Chem. 147 (1943) 297.
8 J. Wyman and B. German, J. Biol. Chem. 117 (1937) 533.
9 J. Wyman, Adv. Protein Chem. 4 (1948) 407.
10 J. Wyman and D.W. Allen, J. Polym. Sci. 7 (1951) 499.
11 M.F. Perutz, Proc. Roy. Soc. Lond. A195 (1949) 474.
12 J.T. Edsall and J. Wyman, Biophysical chemistry, vol. 1 (Academic Press, New York, 1958).
13 E. Antonini and M. Brunori, Hemoglobin and myoglobin in their reactions with ligands (North Holland, Amsterdam, 1971).

14 J. Wyman, J. Biol. Chem. 241 (1966) 115.
15 J.D. Murray, Proc. Roy. Soc. Lond. B178 (1971) 95.
16 J.D. Murray and J. Wyman, J. Biol. Chem. 246 (1971) 5903.
17 J. Wyman, Adv. Protein Chem. 19 (1964) 223.
18 J. Wyman, in: Origins of Molecular Biology: a Tribute to Jacques Monod, eds A. Lwoff and A. Ullmann (Academic Press, New York, 1979) p. 221.
19 J. Monod, J. Wyman and J.-P. Changeux, J. Mol. Biol. 12 (1965) 88.
20 J. Wyman, J. Am. Chem. Soc. 89 (1967) 2202.
21 J. Wyman, Regulation in Macromolecules as Illustrated by Hemoglobin: Q. Rev. Biophys. 1 (1968) 35.
22 J. Wyman, Q. Rev. Biophys. 17 (1984) 453.
23 J. Wyman and S.J. Gill, Binding and linkage: the functional chemistry of biological macromolecules (University Science Books, Mill Valley, CA, 1990) in the press.
24 E. Di Cera, S.J. Gill and J. Wyman, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 5077.