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Soldiers of Science: A Profile

PROFESSOR M. VIJAYAN : GREAT SCIENTIST, TEACHER AND HUMANIST

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Abstract: This essay presents a brief outline of the seminal contributions of Professor M. Vijayan in the area of structural biology and attempts to describe his prominent role as a teacher, administrator and mentor in nurturing macromolecular crystallography in India. He is and will remain in the years to come a role model for crystallographers and will be remembered by his students, peers and colleagues not only as an outstanding scientist but also as a warm, generous and compassionate human being.

Keywords: M Vijayan; macromolecular crystallographer; teacher; lectin; insulin

The Beginning

Professor Mamannamana Vijayan was born in Cherpu, Kerala in the year 1941. He graduated in physics from Sree Kerala Varma College, Trichur and completed his M.Sc. degree from Allahabad University in 1963. Thereafter, he enrolled as a PhD student at the Department of Physics, Indian Institute of Science specializing in the x-ray crystallography of small molecules, under the supervision of Professor M. A. Viswamitra. Upon the successful completion of his PhD degree he proceeded to Oxford University to join the group of Professor Dorothy Hodgkin as a post-doctoral fellow (1968).

Dorothy Hodgkin and Insulin

It is probably difficult to convey in words the intangible influence of one human personality on another, yet without any doubt Dorothy made a profound and lasting impression on all those who came in contact with her. In several of his reminiscences, Vijayan would warmly recount the enormous debt he owed to her both as a scientist and as a person. In his own words (Vijayan, 1994), '*To her students and colleagues, she was a teacher, mother, friend and guide rolled into one…. Among her peers in the scientific community there would have been others who were respected as much as Dorothy was but perhaps none who was loved more than she was*'. Vijayan's entry into Dorothy's research group (Figure 1) was at a crucial and exciting period. A problem very close to Dorothy's heart was the crystallography of insulin, which she first subjected to x-rays in the 1930s (Crowfoot, 1935). It will be recalled that insulin is a peptide hormone secreted by the pancreas to maintain homeostasis in blood sugar levels and also to enable cells to absorb sugar from the blood stream. A couple of decades had passed and when Vijayan joined the group the insulin work had truly entered a critical phase. Rotation and translation functions applied to one of the forms of insulin crystals (2Zn) showed the insulin hexamer to posses 32 (D_3) symmetry and after fairly intense efforts only a single heavy atom derivative had been obtained.

That was where things stood in 1968, and one of the first tasks assigned to Vijayan was to recollect accurate data from native and heavy atom lead soaked crystals. Gradually things fell

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Figure 1: **Photograph taken in the late sixties showing (from left) David Phillips, Dorothy Hodgkin, Tony North, Thomas Hodgkin, M. Vijayan, Tom Blundell, Ted Baker and Eleanor Dodson. The last four were members of the insulin group. Guy Dodson, whose contribution to the structural work on insulin was next only to Dorothy Hodgkin, is not in the picture.**

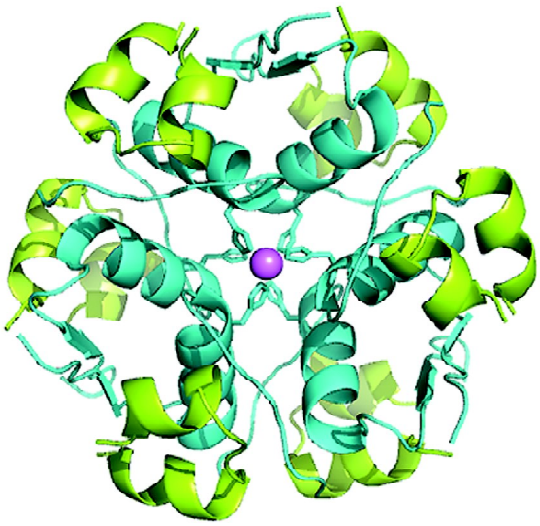

Figure 2: **Ribbon diagram of the insulin hexamer. The zinc ion (magenta sphere) is located at the centre of the hexamer (PDB Code: 4INS).**

into place as Dorothy's team consisting of Vijayan, Ted Baker, Tom Blundell, Guy and Eleanor Dodson obtained five more derivatives and successfully combined the methods of multiple isomorphous replacement and anomalous dispersion in solving the phase problem. At long last an interpretable 2.8 Å electron density map was obtained and Vijayan recalls (Vijayan, 2002), '*…it was with great excitement and anticipation that we went through the final stages of the preparation of the map. The day on which we finally stacked the map sections, Dorothy happened to be away from Oxford. Guy and I were the first to examine the map. Neither of us had any experience in interpreting protein electron density maps. However, we had no difficulty in recognizing a helical stretch in the map …..For the next few days, our almost exclusive occupation was the building of the model.*' As the saying goes, the rest is history and the crystal structure of insulin is now textbook material (Adams *et al.*, 1969; Blundell *et al.*, 1971; Baker *et al.*, 1988). Very briefly, the overall symmetry of the (2Zn) insulin molecule is 32 and consists of six polypeptide chains, organized around the three fold axis. Thus, the hexamer (Figure 2) consists of three dimers, the monomers in each of which are related by a two fold axis perpendicular and passing through the three fold axis. Coordination of the subunits to the Zn atoms located on the three fold axis stabilizes the entire molecule. Vijayan was also involved in the subsequent refinement of the insulin structure and its extension to a resolution of 1.5 Å.

After cutting his protein crystallography teeth in Oxford, Vijayan returned to India in 1971 and joined the Physics department in the Indian Institute of Science, Bangalore. He moved to the newly established Molecular Biophysics Unit at the Institute in 1974.

Crystalline complexes involving amino acids and peptides: Implication to the origin of life

Vijayan's dreams to drive Indian science to the very frontiers were many, but he was forced to reconsider the material resources at his disposal which were probably not commensurate with his dreams. But what could be done? How could one still pursue excellence in science despite being handicapped by limited resources? Vijayan decided to study the fundamental aspects of molecular interactions utilizing x-ray crystallography as a tool. Basically, the work involved crystallizing heterogeneous complexes of dissimilar molecules and probing their atomic interactions upon the determination of their three-dimensional crystal structures. Preparation of crystals involving more than one compound can prove to be quite difficult, yet work was initiated on two systems - one involving anti-inflammatory drugs (also referred to as aspirin like drugs) which

inhibits prostaglandin biosynthesis (Singh and Vijayan, 1973; Singh and Vijayan, 1977) and the other focused on amino acid complexes. In this period he was joined by extremely capable students Tej Pal Singh and T. N. Bhat (both of whom have gone onto become distinguished scientists), which gave an added boost to the projects. The second project, initiated by T. N. Bhat, was also highly relevant within the context of structural biology, as in those days very high resolution protein crystal structures were rare and complexes of peptides/amino acids gave an opportunity to study the geometrical features of non-bonded interactions which plays a crucial role in the structure and function of proteins. Several structures (L - lysine : L- aspartate; L – arginine : L – glutamate; L – histidine : L – aspartate; L – ornithine : L-aspartate) were solved in quick succession (Bhat *et al.,* 1976 ; Bhat *et al.,* 1977; Bhat *et al.,* 1978; Sudhakar *et al.,* 1980; Sudhakar *et al.,* 1980a; Salunke andVijayan, 1983; Salunke and Vijayan, 1984, Salunke and Vijayan, 1984a) and as the work progressed another extremely interesting aspect began to emerge.

The least understood or in any case the least studied area related to the emergence of life on this planet is the era pertaining to chemical evolution which can be subdivided into (i) abiotic organic synthesis (ii) sequence specific polymerization (iii) chiral selection and finally (iv) the emergence of the first self – replicating systems. The famous Urey – Miller experiment and others of the same genre unambiguously demonstrated the spontaneous generation of organic compounds (including amino acids) from a simulated non – oxygenous atmosphere with energy inputs such as UV radiation or electric discharge. The next step would be the sequence specific non – enzymatic condensation of these monomeric units to form polymers, which should require the proximity and favorable juxtaposition of the individual units. Analysis of several crystal structures consisting of amino acids of identical chirality showed that the molecules invariably self – assembled in a head – to – tail fashion of the type NH_3^+ - CHR – COO^- …….. NH_3^+ - CHR – COO^- …….. NH_3^+ - CHR – COO^- …….. , as if to promote polymerization. Such a pattern observed in crystals also occurs in solution and the propensity for this form of aggregation might have played an important role enabling polymeric condensation in the prebiotic milieu (Vijayan, 1980; Suresh and Vijayan, 1983; Suresh and Vijayan, 1985; Suresh and Vijayan, 1985a). Furthermore, in crystals of mixed chirality consisting of both D and L amino acids (Soman *et al.,* 1990; Sridhar Prasad and Vijayan, 1993; Suresh and Vijayan, 1995; Venkatraman *et al.,* 1997; Ravishankar *et al.,* 1998; Saraswati *et al.,* 2001) this regular arrangement in head to tail fashion appears to get disrupted by other interactions (cross connections) which could hinder polymerization as a linear chain (Figure 3). Vijayan summarizes the extensive study (Vijayan, 2009), '*Thus functional groups in biomolecules and biological monomers have propensities to form specific interactions and characteristic interaction patterns. The propensities could be weak, but they exist all the same. It is a combination of weak propensities that leads to subtlety and complexity in multi-molecular systems which often self assemble.*'

Another area Vijayan worked on in collaboration with K.R.K. Easwaran and P. Balaram, pertains to ionophores and related compounds (Nair and Vijayan, 1980; Devarajan *et al.,* 1980; Francis *et al.,* 1983).

Water mediated transformation in proteins

Although, Vijayan worked extensively in the area of small molecule crystallography, proteins were never really far from his mind. He next turned his attention to a fundamental aspect of protein structure which he could address, given the scarce resources at his disposal. Globular proteins are surrounded by a ring of water molecules referred to as their hydration shell, essential for both stability and function. For example, it had been previously demonstrated in lysozyme, that the molecule must be surrounded by at least 0.22 g of water per gram of the protein in order to be active. However, the water structure around proteins can be studied in an ingenious fashion by means of techniques employed in protein crystallography. For the collection of diffraction data, protein crystals are mounted in glass capillaries submerged in a thin layer of mother liquor. A few drops of mother liquor are also inserted at one end of the capillary prior to sealing the tube. By changing the ionic strength of this

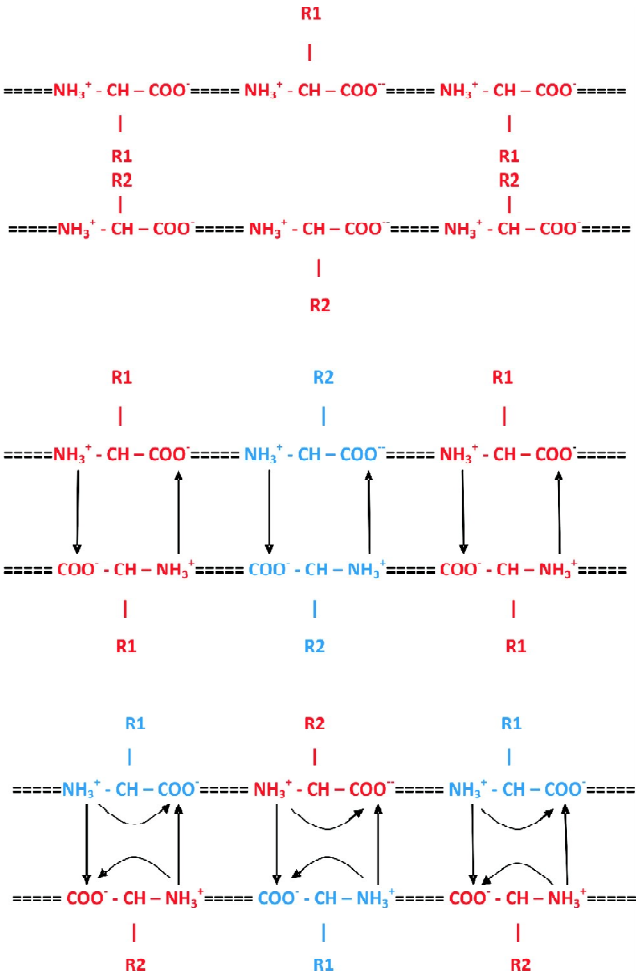

Figure 3: **Schematic representations of the aggregation patterns in the LL, LD and DL-DL complexes between arginine and glutamic acid. L and D molecules are coloured differently and the interactions indicated by arrows and broken lines.**

solution the relative humidity inside the sealed tube can be regulated, which in turn leads to 'water – mediated' transformations of the crystals. These transformations include not only perturbations in the hydration shell but also subtle structural changes in the protein molecule itself, as evidenced by changes in the diffraction patterns (Figure 4) at different levels of relative humidity (Salunke *et al.*, 1984b).

Two well characterized proteins lysozyme and ribonuclease A (Salunke *et al.*, 1985) were first selected for these studies. One of the most interesting result to emerge was that structural changes of proteins due to distortions in their hydration shells (at different relative humidities) were similar to those that occur in native protein

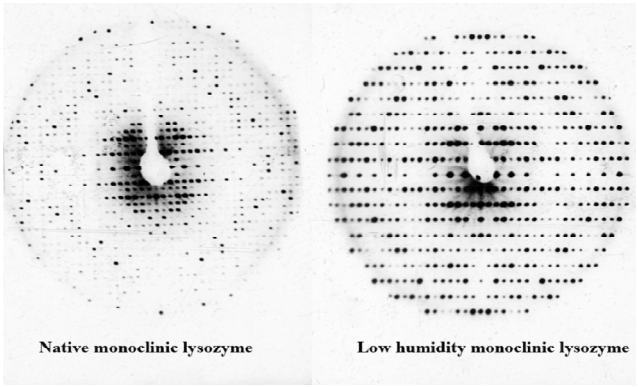

Figure 4: **Comparable diffraction patterns from the native and the low humidity forms of monoclinic lysozyme.**

function, identified by comparing the crystal structures of the native protein with its inhibitor bound form (Kodandapani *et al.*, 1990; Nagendra *et al.*, 1996; Radha Kishan *et al.*, 1995). The experimental method also clearly delineated the conformationally flexible from the rigid regions of the molecule (Biswal *et al.*, 2000; Sadasivan *et al.*, 1998). A low humidity form of lysozyme crystals (Figure 4) with solvent content as low as 9.5% had been obtained (Nagendra *et al.*, 1998), whose crystal structure exhibited a substantially collapsed water network in the active site due to severe dehydration, accompanied by overall contraction of the active site itself, with respect to the native structure. Another important case study concerned β-lactoglobulin (Kontopidis *et al.*, 2002; Wu *et al.*, 1990; Qin *et al.*, 1998) which is a dimeric barrel like protein, consisting of a hydrophobic pocket which binds hydrophobic molecules such as fatty acid and retinol. A loop at the entrance of the pocket exhibits dual conformation which controls access to the site; closed at acidic and open at basic pH. Loop movements are also accompanied by slight alterations in the quaternary structure of the molecule. Upon controlled dehydration, the loop conformation was found to be dissimilar in both subunits (almost open in one and half closed in the other), thus unusually rendering the dimeric molecule asymmetric (Vijayalakshmi *et al.*, 2008). Last but not the least, water mediated transformations were studied in hemoglobin, where incidentally the phenomenon had been first observed by Perutz. The monumental work by Perutz provided structural rationale to the allosteric behavior of hemoglobin (Perutz, 1970;

Perutz *et al.,* 1998) as a oxygen carrier, which is based on the equilibrium between a relaxed state (R) and an unliganded tense state (T). The structural difference between the two states lies primarily in their quaternary structure; that is the relative geometry between the subunits of this tetrameric molecule (Baldwin *et al.,* 1979), though changes in the vicinity of the oxygen binding site and the switch region are also known to occur. Subsequently, another conformational state (R2) was characterized, even more distant structurally from the T state than the R state (Silva *et al.,* 1992). Crystal structure of human methemoglobin (ferric iron in the heme group) in Vijayan's laboratory exhibited a quaternary structure intermediate between the R and R2 states (Biswal *et al.,* 2001) and what is more interesting deviation from the T state was also observed upon reduction in relative humidity for oxyhemoglobin (Biswal *et al.,* 2002; Sankaranarayanan *et al.,* 2005; Kaushal *et al.,* 2008). It was thus established for the first time that rather than discrete R and T states, hemoglobin has access to an ensemble of states.

Lectin crystallography

As these exciting developments were in progress, Vijayan had simultaneously begun the painstaking work of building a world class laboratory to address problems in protein crystallography, at just about any level of difficulty. Prof. A. Surolia joined the Molecular Biophysics Unit (MBU) in 1981, which initiated, what was to become a long and fruitful collaboration between them. Induction of Prof. MRN Murthy and Prof. Suguna in MBU made it a veritable power house of macromolecular crystallography and in 1983 the 'Bangalore group' was recognized by governmental agencies as the nucleus to foster the growth of the subject in the country. During this period Vijayan and Surolia began their study of lectins and in these early days progress in macromolecular crystallography in India was most probably concomitant with the progress in the crystallography of lectins.

As is well known lectins are defined as multivalent carbohydrate binding proteins of non-immune origin which specifically bind to different carbohydrates and their biological role in symbiosis, host defense, cell-cell adhesion, innate immunity etc. are contingent on this singular ability. Research in lectins received a boost when it was recognized that most of the molecular recognition processes at least on cell surfaces are mediated by carbohydrates. The first lectin to be identified for structural studies by Vijayan and Surolia was a molecule from peanut (hence the name peanut agglutinin: PNA) a non-glycosylated homo tetrameric protein of molecular weight 1,10,000 which is specific to galactose at the monosaccharide level (Salunke *et al.,* 1982). PNA is homologous to another tetrameric lectin concanavalin A (ConA), whose crystal structure was available at the time and hence it was presumed that ConA should serve as an effective search model for a molecular replacement solution for PNA (Salunke *et al.,* 1985a; Mande *et al.,* 1988). However, despite repeated attempts it soon became clear that the molecular replacement calculations were not yielding the expected results. The group then successfully solved the structure *de novo* by the multiple isomorphous replacement method and on retrospect the reasons for the failure of the earlier molecular replacement calculations became quite clear.

The individual subunit for both the ConA or PNA tetramer is the classical legume lectin fold (Figure 5) consisting primarily of two beta sheets, a flattened six membered sheet at the 'back' packed against a seven membered curved sheet system in 'front' of the molecule. In addition, a much smaller two stranded sheet is located on the 'top' of the two main sheet systems. The rest of the structure consists of loops which in effect interconnect the individual β strands. However, the primary interest in the PNA structure does not lie in the canonical fold of its individual subunit but rather in its quaternary arrangement which is not only wholly different from ConA but apparently violates even the received wisdom with regard to the general principles underlying quaternary association in proteins (Monod *et al.,* 1965; Cornish-Bowden *et al.,* 1971). The overwhelming majority of oligomeric proteins self-assemble with closed point group symmetries so as to effectively seal inter-subunit association sites and thereby prevent the non-specific aggregation of the monomeric units. In case of any tetrameric protein, 222 (D_2) and 4 (C_4) are the only

possible point groups which can accomplish this structural 'closure'. For PNA (Banerjee *et al.*, 1994; Ravishankar *et al.*, 2001; Natchiar *et al.*, 2004) it was found that the overall symmetry was neither of the two point groups mentioned above but a peculiar assembly involving three non-orthogonal two folds, one of which relates two halves of the whole molecule (AD and BC) while the other two dyads are internal to subunits A, D on one hand and B, C on the other. This was the first time that such an open quaternary arrangement had been observed in a tetramer (Figure 6). The issue of oligomerization in legume lectins was further elaborated in two more crystal structures of glycosylated WBAI **(**Figure 7**)** and WBAII (Prabu *et al.*, 1998; Manoj *et al.*, 2000) basic and acidic lectins from winged bean respectively, whose individual subunits (as they are dimers) again belonged to the classical legume lectin fold described above. Both these proteins are homologous to another glycosylated lectin EcorI (whose structure was available in the Protein Data Bank), though differing in their glycosylation sites. The difference in the quaternary arrangement of the EcorL dimer from the one observed in the ConA tetramer (which assembles to from a twelve stranded contiguous beta sheet), was ascribed to the glycosylation of EcorL, on the

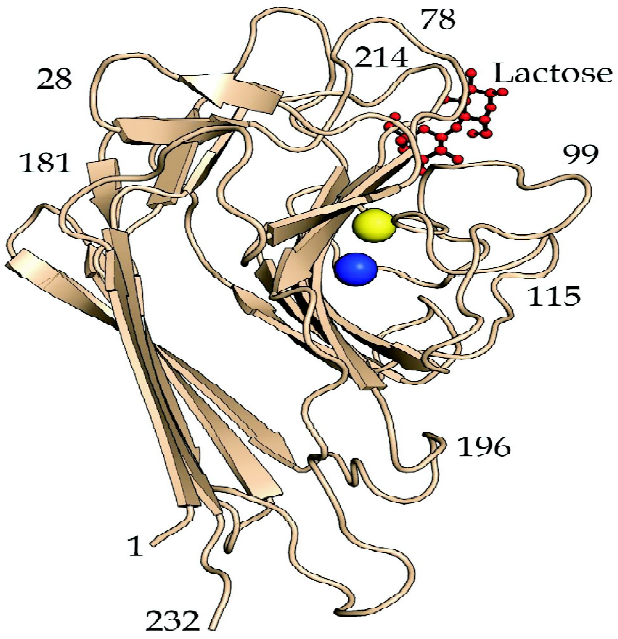

Figure 5: **The legume lectin fold as observed in a PNA – lactose complex (PDB Code: 2PEL). The blue and yellow spheres represent manganese and calcium ions respectively.**

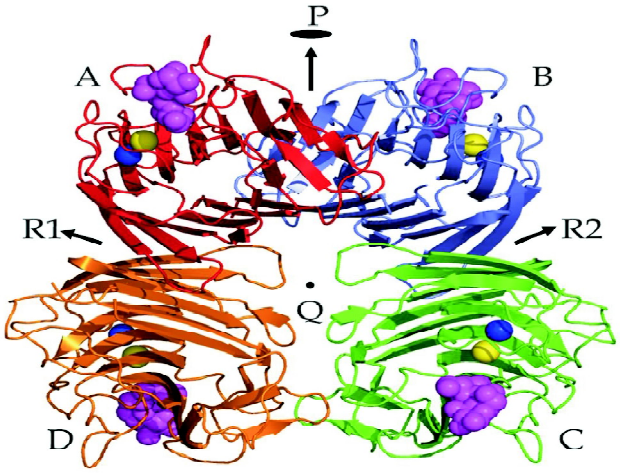

Figure 6: **Quaternary structure of PNA. Arrows represent local two fold axes. The four subunits have been shown in different colours. The blue and yellow spheres represent manganese and calcium ions respectively and the magenta space filling spheres represent bound lactose molecules (PDB Code : 2PEL).**

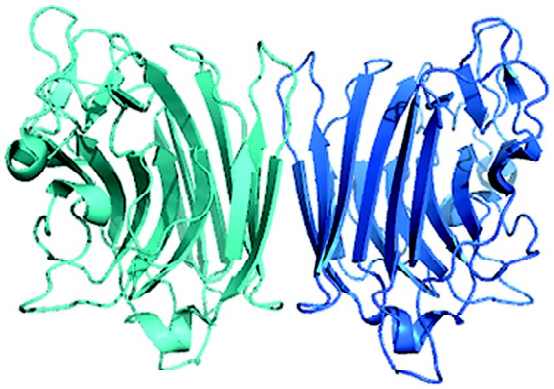

Figure 7: **Structure of WBAI (PDB Code: 1WBL)**

assumption that the covalently linked complex sugar probably hindered ConA type dimeric association in case of EcorL. The structures of WBAI and WBAII, however, were found to have a quaternary association similar to EcorL, despite differences in their respective glycosylation sites, thereby indicating that differences in the geometry of subunit association arose primarily from factors intrinsic to the concerned proteins. The issue was clinched when it was found that the dimeric association of recombinant non-glycosylated EcorL was identical to that of the native protein. Thus, Vijayan sums up the general principle as (Prabu *et al.*, 1999), *' ..it turns out that legume lectins are a family of proteins in which minor changes in essentially the same tertiary structure, lead to large changes in quaternary association.'*

Next, Vijayan and his group turned their attention to a completely novel lectin fold in the x-ray analysis of jacalin, one of the two lectins found in jackfruit seeds. Upon the *de novo* crystal structure solution of the heavily glycosylated jacalin (Sankaranarayanan *et al.*, 1996), each monomer was found to consist of three Greek key motifs arranged in the form of a prism with an internal three fold symmetry, the β prism I fold (Figure 8), and the assembly of four such monomers with 222 symmetry constituted the entire jacalin tetramer. Interestingly, the carbohydrate binding site was located on only one of the Greek key motifs. Lectin crystallography in Vijayan's group had by now entered its fully mature stage and a steady succession of structures followed. Crystal structures of artocarpin (Figure 8), the second lectin from jackfruit (Pratap *et al.*, 2002) and banana lectin (Singh *et al.*, 2005) both belonging to the β prism I fold, were solved, in addition to garlic lectin (Chandra *et al.*, 1999; Ramachandraiah *et al.*, 2002) which belonged to the β prism II fold. The β prism II fold is also internally three fold symmetric, each module consisting of a 4 stranded β-sheet, the β strands running nearly perpendicular to the internal triad axis, in contrast to β prism I, wherein it is parallel. Similar to legume lectins, these proteins also display a wide variety of quaternary arrangements determined by minor modifications in their tertiary structures as jacalin, artocarpin are tetrameric, heltuba (another protein of the same class; Bourne *et al.*, 1999) octameric and banana, calsepa (Bourne *et al.*, 2004) lectins are dimers (Figure 9). Variability in β prism II fold type proteins are also in evidence as garlic protein is dimeric in contrast to the homologous snow drop lectin which is a tetramer. In terms of molecular evolution, comparison of β prism I and β prism II lectins appears to indicate that initially an unique single domain Greek key carbohydrate binding motif underwent successive gene duplication and fusion events to subsequently diverge into the two distinct though related folds (Sharma *et al.*, 2007).

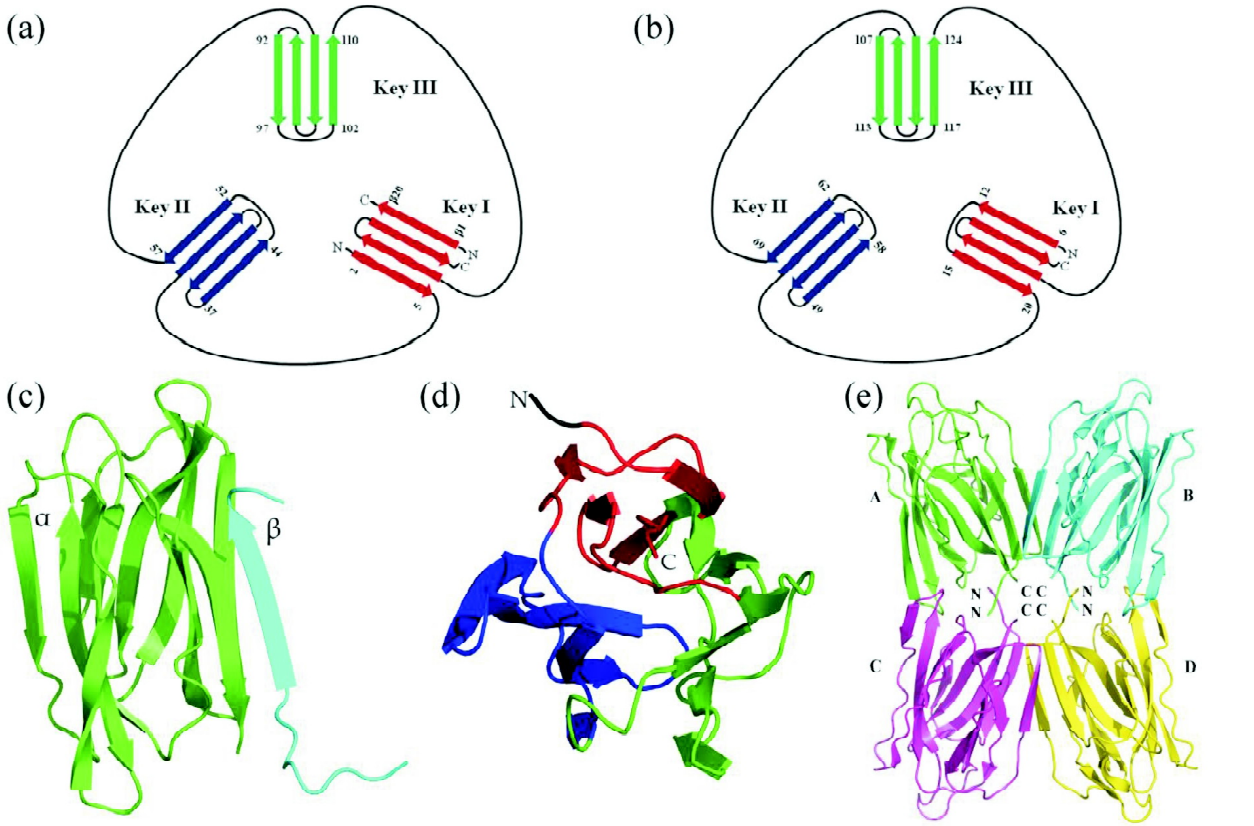

Figure 8: **Topology of the polypeptide chain(s) in (a) jacalin and (b) artocarpin. (c) Side view of the jacalin subunit (PDB Code : 1KUJ) (d) View of the artocarpin subunit down the molecular threefold axis (PDB Code : 1J4S) (e) Quaternary association in artocarpin (PDB Code : 1J4S).**

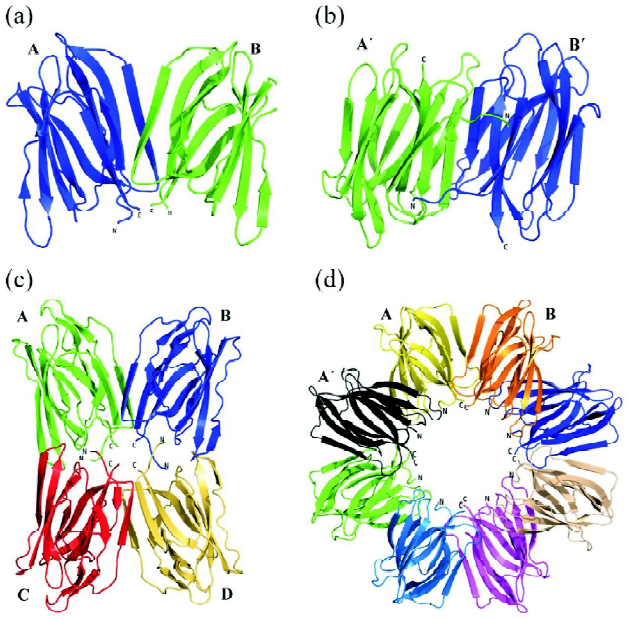

Figure 9: **Quaternary structure in (a) banana lectin (PDB Code : 1X1V) (b) calsepa (PDB Code : 1OUW) (c) artocarpin (PDB Code : 1J4S) and (d) heltuba (PDB Code : 1C3K).**

By now with so many lectin structures available, an exhaustive study was conducted on the structural basis of carbohydrate recognition in these molecules. The sugar binding site in PNA, as in other legume lectins is composed of four distinct loops (A, B, C and D) and crystal structures of PNA complexed with nearly a dozen sugars, coupled with molecular dynamics simulations clarified the role of each of these loops, their conservation or variability across other legumes and delineated the exquisite network of protein-sugar interactions which enables these proteins not only to distinguish between different sugars but also between the isomeric forms of the same carbohydrate (Banerjee *et al.*, 1996; Ravishankar *et al.*, 1997; Ravishankar *et al.*, 1998a; Ravishankar *et al.*, 1999; Pratap *et al.*, 2001; Natchiar *et al.*,2006). One notable result to emerge from these studies is the role of water molecules in these interaction networks, modulating sugar affinity. For example, the interactions between PNA and lactose on one hand and T-antigen on the other are nearly identical but for two additional water bridges between the T-antigen and PNA which increases their mutual affinity 20-fold (with respect to lactose). So comprehensive was the understanding of lectin-sugar interactions that it was possible to modify the sugar specificity of PNA by site directed mutagenesis (Sharma *et al.*, 1996; Sharma *et al.*, 1998; Adhikari *et al.*, 2001). The other lectins were also found to display their own idiosyncrasies in generating sugar specificities. Post-translational modification of jacalin bifurcates the single polypeptide chain of its precursor into two (long chain A and the shorter chain B), which in effect creates a new N-terminus capable of effecting additional contacts, enabling jacalin to distinguish between mannose and galactose. In the absence of any post- translational modification, artocarpin generates a different pattern of specificities for oligosaccharides, in comparison to homologous heltuba lectin (though they have similar affinities for mannose), by altering the length of a strategically placed loop (Jeyaprakash *et al.*, 2004). Similarly, dimeric garlic and tetrameric snowdrop lectin (Hester *et al.*, 1995) are both mannose-binding, yet snowdrop lectin is anti-retroviral (in contrast to garlic lectin) as a consequence of its affinity for N-linked carbohydrates such as GP120, due to a binding site which lies at the interface of two garlic lectin type dimers in the snowdrop tetramer. It would have become amply clear that to date, the most systematic analysis of the structural determinants of lectin – sugar specificity, has come from Vijayan's group. The entire corpus of work with regard to lectin crystallography is not only important for its scientific content but also that it served as a training field for a whole generation of crystallographers. The exemplary contribution of Suguna in the lectin project also needs to be mentioned.

The structural studies on lectins have been extended to two largely unexplored domains, lectins consisting of β-trefoil domains as in type II RIPs typified by ricins (Montfort *et al.*, 1987) and mycobacterial lectins. Two lectins from mycobacteri*a* have already been crystallized (Patra *et al.*, 2010; Patra *et al.*, 2011) and the structure of one of them (the lectin domain of MSMEG_3662 from *M.*19 *smegmatis,* in complex with mannose and methyl-α-mannose) has now been determined (Patra *et al.*, 2014). A succession of biologically interesting and significant results is expected to come from this virgin area in the coming years.

Crystallography of proteins from *Mycobacterium tuberculosis*

By now Vijayan was firmly entrenched into what can be termed 'mega science', largely driven by sophisticated technology and highly intensive in resources and trained manpower. However, despite being surrounded by the glamour of big science, the obligation of scientists to the society which funds their research (and livelihood) was never really far from his mind. It was time to take up a project, in which progress could perhaps alleviate the hardships of ordinary people. The ability of most, if not all pathogenic organisms to develop resistance to commonly used drugs is a universal phenomenon and the shelf life of a therapeutic agent could be anywhere between 10-15 years, after which its potency against the intended parasite begins to diminish. One such instance is tuberculosis (TB: caused by *Mycobacterium tuberculosis*), which accounted for 8.8 million new cases and 1.5 million associated deaths in 2010, mostly occurring in developing countries (WHO Report, 2011). Some of the reasons associated with the return of TB are the emergence of resistant strains, co-morbidity with AIDS and the generally compromised immune system of socially handicapped sections of the populace in third world countries. The first crystallographic analysis of TB protein *M tuberculosis RecA (*MtRecA) from India was from Vijayan's lab (Datta *et al.,* 2000).

MtRecA (Chandran *et al.,* 2012) is a multifunctional eubacterial DNA binding protein involved in recombination and DNA repair. The molecule exhibits a tendency to aggregate into filaments and exists in two states, ATP bound 'active' filaments when bound to DNA and 'inactive' filaments when the molecule exists in isolation. The active filaments are considerably longer in length than their inactive counterparts. MtRecA is composed of three domains (Figure 10), the single helical N-terminal domain (residues 1-30), followed by the M domain (31-269) which forms the main body of the protein and terminating in the 59 residue C-domain, consisting of three helices and three β strands. The M domain belongs to the 'P-loop containing NTPase fold' and consists of a twisted β sheet flanked by helices. The N and C terminal domains

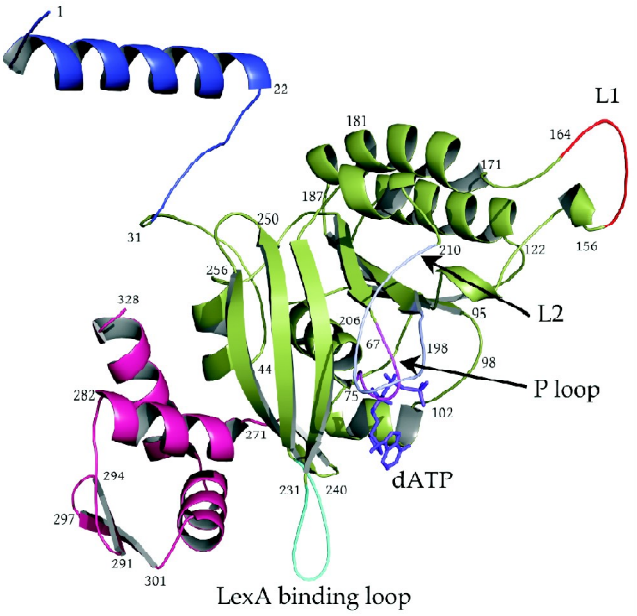

Figure 10: **The structure of MtRecA. The N-,M- and C-domains are denoted in blue, green and pink, respectively. Different functionally important regions in the M-domain are indicated (PDB Code: 1MO6).**

are instrumental in the higher order aggregation of the molecule whereas the M domain is responsible for nucleotide binding, utilizing the embedded P-loop. The native structure in conjunction with a series of complexes (with ADP-AlF4, ATPγS and dATP, all of which are non hydrolysable analogues of ATP and ADP) delineated the allosteric transitions of the molecule from the inactive to the active state and identified a 'switch' region which signals ATP binding and triggers the cascade of events transforming the molecule to its DNA binding active state. Thus, the work involving MtRecA is also of general scientific interest with regard to protein function in addition to drug targeting in the mycobacterial genome.

The next mycobacterial protein identified for structural studies was the single-stranded DNA-binding protein (SSB) essential for DNA replication, repair and recombination. The DNA binding domain of SSB from *Mycobacterium tuberculosis* (MtuSSB) was solved employing a combination of isomorphous replacement and anomalous scattering techniques (Saikrishnan *et al*., 2003). MtuSSB is a tetramer with 222 symmetry with each individual subunit consisting of two domains. The N-terminal domain which has the

OB fold consisting of three long β-hairpin loops extends from globular core composed of a five stranded β-barrel capped by an α-helix on one end. Somewhat similar to the scenario in legume lectins, the quaternary association in MtuSSB exhibits considerable variability when compared with the homologous proteins in human and *E. coli* (Figure 11)and the state of affairs was succinctly summarized by Vijayan thus (Saikrishnan *et al*., 2003), '*The crystal structures of MtuSSB, for the first time, reveal significant structural variations among tetrameric SSBs. It is possible, in theory, to take advantage of these variations to generate peptidomimics of the DNA that bind selectively and irreversibly to regions unique to MtuSSB and disable this essential protein*.' These structural variations have since been thoroughly explored (Arif and Vijayan, 2012). What is really remarkable is that once Vijayan targeted any scientific problem, the sheer volume of high impact scientific results which emanated from his laboratory is truly exemplary. Within the spatial constraints of this essay only some representative results (and publications) have been briefly discussed (or cited). Needless to add, those results which have been mentioned only *en passant* (or have been omitted) in this article, are not of any less importance. The full spectrum of his work on mycobacterial proteins include ribosome recycling factor (Saikrishnan *et al.,* 2005), peptidyl-tRNA hydrolase (Selvaraj *et al.,* 2007; Selvaraj *et al.*, 2011), DNA binding protein in stationary phase (Roy *et al.,* 2008), pantothenate kinase (Chetnani *et al.,* 2010), uracil DNA glycosylase (Kaushal *et al.,* 2008a) and RuvA (Prabu *et al.,* 2009). K. Muniyappa, U. Varshney, D. Chatterji and A. Surolia have been Vijayan's collaborators in the research on mycobacterial proteins.

By this time the work on mycobacterial proteins had expanded considerably and the 'Bangalore group' had joined an international consortium on TB structural genomics. Following Bangalore, the structural work on proteins from the mycobacterial genome were initiated in several institutions throughout India including IMTECH (Chandigarh), CDFD (Hyderabad), NII (New Delhi), CDRI (Lucknow), CCMB (Hyderabad) and a host of other institutions. It is a matter of great pride for Vijayan that the number of structures coming from Indian laboratories constituted about 10% of the structures determined worldwide (Arora *et al.,* 2011).

Recognition and role model

As the corpus of work began to grow and its merit recognized, Vijayan became the recipient of several prestigious awards beginning with the

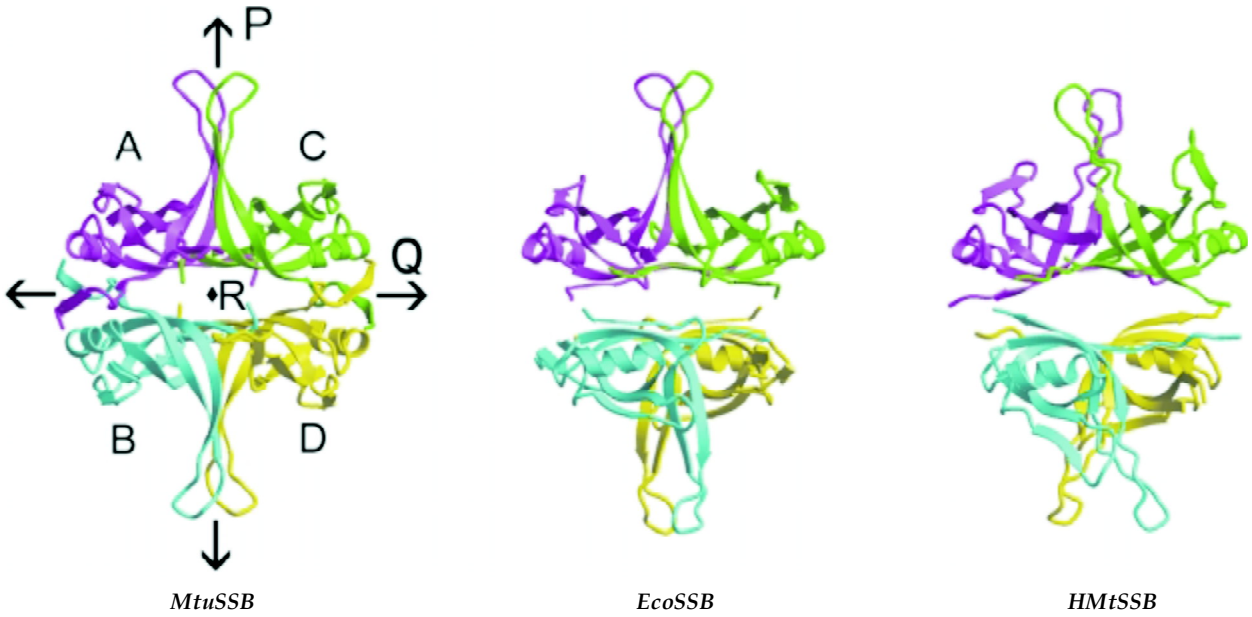

Figure 11: **Comparison of SSB's from *Mycobacterium tuberculosis* (MtuSSB : PDB Code 1UE1), *E. coli* (EcoSSB: PDB Code 1SRU) and human (HMtSSB : 3ULL).**

Bhatnagar Prize (1985), GN Ramachandran Medal (1994), FICCI award in Life Sciences (1995 – 1996), Om Prakash Bhasin Award (2000), Padma Shri (2004), Goyal Prize in Life Sciences (2005), Distinguished Alumni Award Indian Institute of Science (2005), Sir Devaprasad Sarvadhikari Medal University of Calcutta (2009) and the Champion of CSIR Award (2012) , to name a few. Internationally, he was instrumental in organizing several sessions, delivering invited talks in the annual conferences organized by the International Union of Crystallography (IUCr) and was the Chairman, Commission on Biological Macromolecules (IUCr) from 1993 – 1996. He was appointed the Associate Director of Indian Institute of Science in 2000, a position which he held till 2004 and was also elected the President of the Indian National Science Academy from 2008 – 2010 (Figure 12). However, this long list of awards and recognitions do not really manifest the enormous influence which Vijayan exercised as a mentor (guiding about seventy students and post docs) in the development of structural biology in India. He was in large measure responsible for the flowering of macromolecular crystallography in the country, when beginning with only about 2 centers (at the start of his career) there are today around twenty to thirty centers of structural biology in the India, whose leaders relate to him in the *guru – shishya parampara* in some form or the other (student, student's student, post doc, post doc's student etc.). Several of his students and post docs have gone onto become distinguished scientists in their own right such as Tej Pal Singh (AIIMS, New Delhi), Dinakar M Salunke (Regional Centre for Biotechnology, Gurgaon), C. G. Suresh (NCL, Pune), Shekhar Mande (NCCS, Pune), H. G. Nagendra (SMVIT, Bangalore), Rajan Sankaranarayanan (CCMB, Hyderabad), R. Ravishankar, J.V. Pratap (CDRI, Lucknow) and Nagasuma Chandra (IISc, Bangalore), to name only a few.

Figure 12: **Vijayan as President, Indian National Science Academy welcoming Prime Minister Manmohan Singh at the inaugural function of the platinum jubilee celebrations of the Academy.**

His human qualities

In the midst of many responsibilities, his first love however remains the nitty gritty of every day research and nothing would please him more than a sudden solution to a complex crystallographic problem which had absorbed his concentrated attention over an extended period of time. His enormous intellectual discipline enable him to give his undivided attention to a scientific problem placed before him, despite the many pulls and pressures of diverse administrative engagements. Truly, Vijayan belongs to the old school of scientists who believe that excellence in science should be pursued with a passion, be given unreserved value, and that the scientific discipline (in its most comprehensive form) should form one of the bulwarks of national regeneration. The world will no doubt remember Vijayan as a great scientist, who in addition to his many scientific achievements served as the nucleus for the flowering of macromolecular crystallography in this country. However, for those of us who had the good fortune to be close to him, if only for a brief period of time, will remember him primarily for his human and ethical qualities. For him science is essentially a human activity where the primary focus should be on men and women engaged in the pursuit of scientific understanding. Machines, computers and the rapidly expanding paraphernalia of scientific activity are of secondary importance. In this era of increasing competition he would repeatedly impress on us that good science must be done 'pleasantly', or in other words in spite of all the pressures one should preserve those qualities of heart which make a good human being. In many instances, we saw him to give up a problem and stand aside only on the request of a less endowed colleague, who would like to focus on the same

area of work. He repeatedly urged us to take pleasure in the day to day practice of science and would recount how Dorothy would rejoice even in an apparently minor success, which for someone of her stature could be considered to be a trifle. '*Trifles make perfection, and perfection is no trifle*' (a quote attributed to Michelangelo) exemplify his attitude to all works great and small. He combines in himself single-minded *shraddha* for the pursuit of science coupled with a compassionate concern for his students and all those who come within the orbit of his influence.

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