Macromolecular Crystallography in India. A Historical Overview

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Abstract | India has a distinguished tradition in crystallography and structural biology. However, biological macromolecular crystallography in the country has had a somewhat delayed start on account of paucity of adequate funds and insufficient interactions between crystallographers and biochemists. Preliminary results in the area began to appear in the early eighties. The support provided by the Department of Science & Technology in the mid eighties under its thrust area programme for macromolecular crystallographic studies at the Molecular Biophysics Unit of the Indian Institute of Science gave a major impetus to work in the area. The Bangalore centre also came to be recognised as a national nucleus for the development of the area in the country. Since then, over the years, biological macromolecular crystallography has grown into a major activity in India encompassing nearly 20 institutions and close to twice as many research groups. It is also now at the centre stage of modern biological research in India. The problems addressed by biological crystallographers in the country span a wide spectrum and their efforts have made considerable international impact. Collective initiatives such as those involving microbial pathogens and structure-based inhibitor design have also begun to emerge.

1. Introduction

Much of our understanding of the structure of matter at the atomic and molecular level has been derived from X-ray crystallography. After the discovery of the diffraction of X-rays by crystals in 1912 by von Laue, the emphasis in the early days of X-ray structure analysis, promoted primarily by William Bragg and Lawrence Bragg, was on inorganic materials. In the twenties and particularly in the thirties of the last century, the crystallography of organic compounds began to take shape. The most striking achievement of organic chemical crystallography was the structure solution of vitamin B_{12} almost exclusively using X-ray crystallography in the fifties, an achievement for which Dorothy Hodgkin was awarded the Nobel Prize in 1964. That was a time when crystallography of biological macromolecules was beginning to make its presence felt. Currently, the most spectacular applications of crystallography are in biology, although it continues to be a crucial and indispensable method in structural chemistry and materials science.

The recording of the X-ray diffraction pattern from pepsin crystals by J.D. Bernal and Dorothy Crowfoot (subsequently Hodgkin) in 1934 formally marked the beginning of biological macromolecular crystallography[1]. That was a time when even the chemical nature of proteins was not fully established and structural crystallography was at its infancy. A quarter of a century elapsed before the three dimensional structures of two related proteins, myoglobin[2] and haemoglobin[3], were established for the first time by John Kendrew and Max Perutz, a prolonged effort for which they are awarded the Nobel Prize in 1961. The structure solution of lysozyme[4], the first enzyme to be X-ray analysed, soon followed in 1964. The structures of a few more enzymes were

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reported in the sixties[5–9]. As a culmination of efforts spanning more than three decades, the structure of insulin, a protein hormone, was solved in 1969[10]. Thus by the dawn of the seventies, biological macromolecular crystallography had come of age. Subsequent developments in modern biology such as genetic engineering and technology for producing monoclonal antibodies, had a very positive impact on macromolecular crystallography, particularly in terms of the availability of materials. During the same period, the technology for the production and detection of X-rays also registered phenomenal growth. Still more remarkable has been the breath-taking progress in computation and visualization. All these scientific and technological developments ushered in a new revolution in macromolecular crystallography, which still continues. Macromolecular crystallography now is the most important component of molecular structural biology and is at the centre stage of modern biology.

2. Early efforts

The tradition of crystallographic research in India is long, thanks to the efforts of stalwarts like K. Banerjee, G.N. Ramachandran, S. Ramaseshan, A.R. Verma and many others. Many pioneering contributions in structural biology emanated from India primarily on account of the leadership provided by Ramachandran and his colleagues. A few Indians have also been involved in early macromolecular crystallography projects abroad. Yet, India had a comparatively late start in macromolecular crystallography primarily for two reasons. The level of normal research funding available in India till the mid-eighties was insufficient for setting up laboratories for such work. Secondly, the level of interactions between crystallographers and biochemists, a precondition for pursuing a healthy structural biology programme, was inadequate.

I was the first macromolecular crystallographer to return to India in 1971 after participating in the structure solution of insulin in the laboratory of Dorothy Hodgkin at Oxford. For reasons alluded to above, it was at that time impossible to initiate macromolecular work at the Indian Institute of Science (the Institute) where I returned to. My major effort for a long period after my return was in the crystallography of small biomolecules, with special emphasis on molecular interactions and supramolecular association. In the meantime, K. K. Kannan, a former student of the Institute along with me, returned to the Bhabha Atomic Research Centre, (BARC), Mumbai, in 1978 from Uppsala where he was involved in the structure analysis of carbonic anhydrase in the laboratory of Bruce Strandberg. A major break through in the efforts occurred when the Department of Science and Technology (DST), then under the secretaryship of S. Varadarajan, provided handsome support in 1983 under their Thrust Area Programme to the Bangalore group at the Molecular Biophysics Unit (MBU) of the Institute, then chaired by V. Sasisekharan. The Bangalore group had two mandates: one to build up a vibrant activity in the area at the institute and the second to serve as a national nucleus for the development of biological macromolecular crystallography in the country.

In the eighties, macromolecular crystallographic activities in India were confined to the Institute and BARC. The work at BARC was largely concerned with carbonic anhydrase including the enzyme from buffalo[11,12]. Much of the work at Bangalore was concerned with lectins[13–17] resulting from a collaboration with A. Surolia who moved from the Indian Institute of Chemical Biology, Kolkata, to MBU in 1981. The first paper resulting from this collaboration[13], that on the crystallization and preliminary studies on peanut lectin, was published in 1982. That marked the beginning of a major effort in the structural biology of lectins, with considerable impact on the development of the area in the country. Structural studies on protein hydration and its consequences, using a novel approach involving water-mediated transformations were also initiated during this period[18,19]. M. R. N. Murthy joined MBU in the early-eighties with his ambitious virus crystallography programme[20,21]. K. Suguna joined the lectin effort in 1988. Most of the investigations at Bangalore during the eighties, including those on lectins, were exploratory or preliminary in nature. The one definitive result to emerge during this period was concerned with additional binding sites in lysozyme[22], a result which was later confirmed using better data[23].

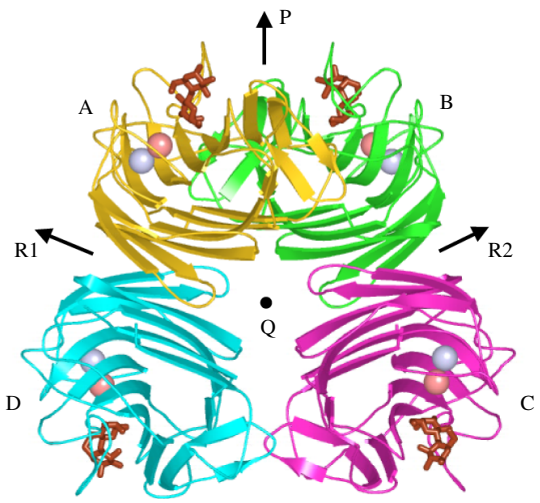
3. Consolidation

In the eighties work at Bangalore and BARC were carried out against many technological odds. During much of this period, the method of choice for recording X-ray diffraction data was oscillation photography. This method when used with an in-house rotating anode X-ray generator is in general more than an order of magnitude slower than when used with synchrotron sources, which India did not have (and still do not have!). However, position sensitive detectors began to be used as detectors in the eighties and the first area detector system for macromolecular crystallography was installed at MBU in the very early nineties. That led to the establishment of a national facility

for X-ray data collection from macromolecular crystals, with continuous support from the DST. This facility was of critical importance in the development of the area in the country in the initial stages. Computation was another bottle neck in the eighties, primarily because of the sanctions imposed by the United Sates and other countries. The situation began to ease by the early nineties. The Graphics Facility established by the Department of Biotechnology (DBT) at the Institute in 1990 when N. Seshagiri was the chairman of the DBT Task Force on Bioinformatics has been of great use in macromolecular crystallographic studies. Eventually, computation ceased to be a source of worry.

During the nineties, major results emerged from the X-ray work on lectins. The structure of peanut lectin was eventually solved and published in 1994[24]. This tetrameric legume lectin has an unusual open quaternary structure without the expected symmetry, which violates an accepted paradigm of protein architecture (Figure 1). This structure, along with those of winged bean lectins determined later[25,26], established that legume lectins are a family of proteins in which small alterations in essentially the same tertiary structure leads to large variations in quaternary association[27,28]. The structural basis of the carbohydrate specificity of peanut lectin has also been thoroughly examined[29–31]. In particular, it was demonstrated how water bridges can be used for generating ligand specificity[30]. A new lectin fold, the β-prism I fold, was discovered through X-ray analysis of jacalin[32] (Figure 2), one of the two lectins in jackfruit seeds. This structure also showed how a post translational modification can be used as a strategy for generating carbohydrate specificity. The work on jacalin paved the foundations for subsequent detailed investigations of β-prism fold lectins. Yet another major result to emerge during the decade was the structure of garlic lectin[33]. A comparison of this lectin and the related snowdrop lectin showed how oligomerisation can be used as a strategy for generating ligand specificity.

Figure 1: Quaternary structure of peanut lectin. Subunits A, B, C and D are indicated. P relates A and D to B and C by twofold symmetry while R1 relates A and D and R2 B and C. Q is an irrational screw axis which relates A and C, and B and D. P, R1 and R2 do not intersect among themselves, but they intersect with Q at different locations.



The ambitious programme of M.R.N. Murthy, in collaboration with H.S. Savithri, on viruses began to bear fruit in the nineties. In a major demonstration of crystallographic prowess, the three-dimensional structures of two icosahedral plant viruses, namely, Sesbania mosaic virus[34,35] and Physallis mottle virus[36] were determined (Figure 3). Viruses are among the largest objects dealt with by crystallographers and virus crystallography is practiced only in a few countries such as the United States, the United Kingdom, Sweden and Japan. Through the structure solution of the two viruses, Murthy and his colleagues elevated India to that exclusive group of countries. Furthermore, they used these two structures as a platform for subsequent detailed, thorough studies on virus assembly.

Definitive results on protein hydration and its consequences also began to emerge during this period. In particular, it was demonstrated in lysozyme and ribonuclease A that movements in the molecules that accompany partial dehydration are similar to the movements that occur during enzyme action[37–44]. The structure solution of xynalases by M. A. Viswamitra and S. Ramakumar of the physics department of the Institute during this period is also noteworthy[45,46].

The main thrust of the activities at BARC continued to be on carbonic anhydrase with emphasis on complexes with sulfonamide, metal ions etc., with a view to elucidating the mechanism of action of the enzyme[47–51]. Another interesting structure studied by Kannan and M. V. Hosur, who joined him later, was on the ribosome inactivating protein gelonin[52]. Hosur also had initiated work on a tethered mutant of HIV protease, which subsequently developed into a major project.

The early nineties also marked the beginning of X-ray crystallographic activities at the Saha Institute of Nuclear Physics (SINP), Kolkata under the leadership of J. K. Dattagupta. The first protein to be studied by them was a chymotrypsin inhibitor from winged bean seeds[53–55]. Towards the end of the nineties they started work on thermostable thiol proteases from a medicinal plant[56](Figure 4).

During this period, the Biophysics Department of the All India Institute of Medical Sciences

Figure 2: Tertiary (left) and quaternary (right) structure of Jacalin.

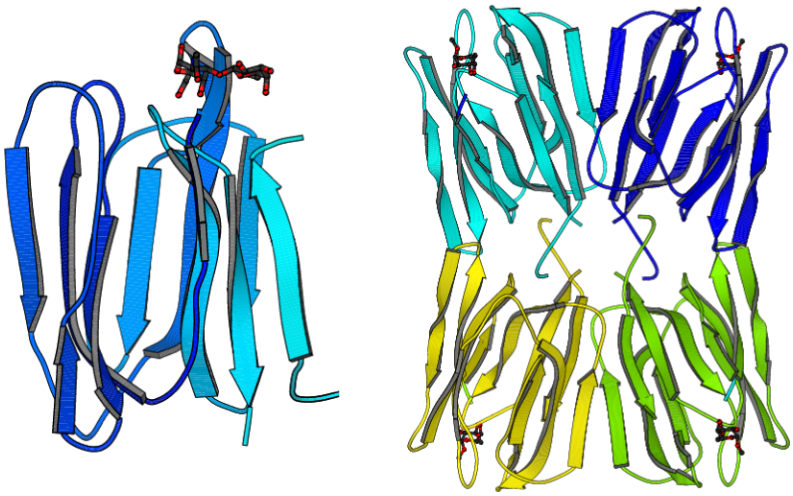
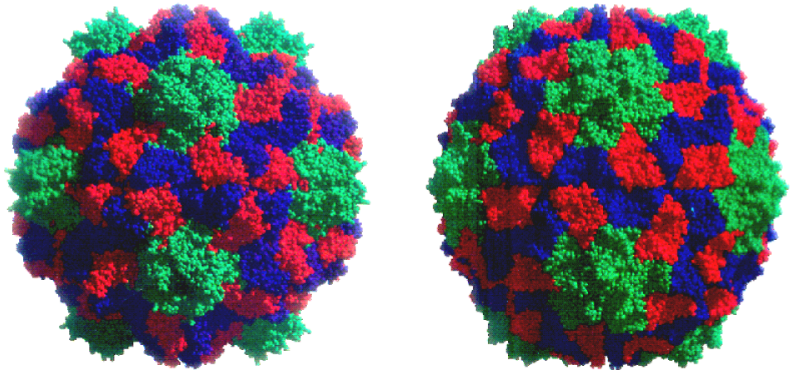


Figure 3: Structures of Physallis mottle virus and Sesbania mosaic virus. The figure was kindly made available by M. R. N. Murthy.



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(AIIMS), under the leadership of T. P. Singh, emerged as a major centre of macromolecular crystallography research. The work started with a bifunctional proteinaceous inhibitor from ragi[57,58]. A great deal of collaborative efforts were also carried out on the inhibitor complexes of proteinase K[59]. But the centrepiece of the AIIMS effort during this period was the detailed thorough studies on lactoferrin from a variety of mammals such as buffalo, camel and mare[60–63] (Figure 5). Also studied separately were the two lobes of the bilobal protein[64]. The inhibition of proteinase K by a peptide fragment of lactoferrin was characterized and related to the anti-microbial property of the milk protein[65]. Also studied was the structure of lactoperoxidase. Work was initiated on toxic phospholipase A2 (PLA2)[66,67], with and without bound molecules, which eventually developed into a major structure-based drug design programme.

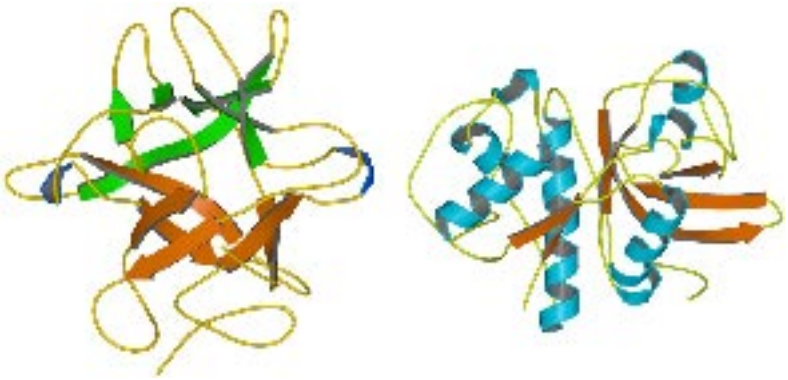
The initiation of macromolecular crystallography at the National Institute of Immunology (NII), New Delhi, by D. M. Salunke involved the structure solution of barstar[68] and rat ribonuclease[69]. The work on an anti-GnRH antibody by the NII group[70] was the harbinger of their remarkable subsequent investigations on molecular mimicry and antibody maturation. The first results in protein crystallography to emanate from the Madras University were from the work carried out by Vasantha Pattabi on different forms of trypsin[71,72]. Structural studies by N. Gautham at the University on the plasticity of Z-DNA also deserve special mention[73]. Work was initiated by C. G. Suresh at the National Chemical Laboratory, (NCL), Pune, through exploratory studies on a lectin from *Artocarpus hirsuta*[74]. As the beginning of a remarkable project on membrane proteins, crystallization and preliminary X-ray studies of OmpC were carried by S. Krishnaswamy of the Madurai Kamaraj University (MKU) during this period[75].

4. The current state

Towards the end of the nineties, there were less than 10 institutions in the country where macromolecular crystallography was being pursued or had been initiated. The expansion of the area in the current decade has been phenomenal. Work in the area is currently on in about 20 institutions involving nearly twice as many research groups. There was just one modern data collection facility (an area detector mounted on a rotating anode X-ray generator), the one at Bangalore, in the early nineties. There are now close to twenty such facilities which are functional or are being installed. Many macromolecular crystallography groups are located in multi-disciplinary institutions like the Institute, Indian Institutes of Technology and universities. In addition, most of the major modern biology laboratories in the country have biological macromolecular crystallography groups located in them. As in developed countries, macromolecular structural studies have become an integral part of modern molecular biological research in India.

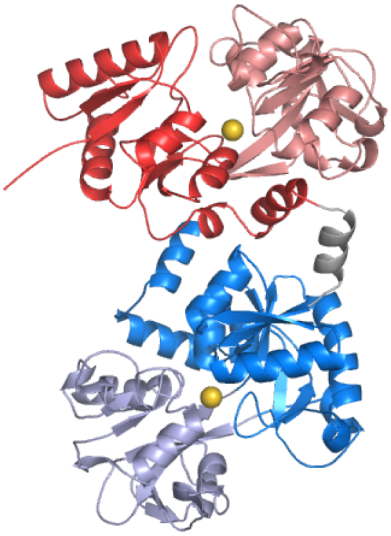
The current spread of macromolecular crystallographic research in the country is such that it is not possible to even refer to all the strands of these activities. The work on lectins continues at the Institute[76–92] and NCL[93]. So does that on plant viruses at the Institute[94–103]. Systems investigated using water-mediated transformations at the Institute now include multimeric proteins[104–109].

Figure 4: Structures of a chymotrypsin inhibitor (PDB code 4wbc) and a thiol protease from *Ervatamia coronaria* (1iwd).



Proteases and protease inhibitors continue to be investigated in different laboratories at SINP[110–114], the Institute[115,116] and the Madras University[117–119]. Extensive efforts on inhibitor design using PLA2[120–140] as targets (Figure 6) are in progress at AIIMS, in addition to work on lactoferrins[141–148] and other systems. Complex phenomena like molecular mimicry and antibody maturation are being addressed using X-ray crystallography at NII[149–159] (Figure 7). The work on membrane proteins started at MKU in the late nineteen nineties fructified into the structure solution of a complex of *E. coli* OmpC with the N-terminal region of camel lactoferrin. The work at BARC involving the tethered mutant of HIV protease scaled new heights in the current decade[160–165]. The work of Z-DNA continued at the Madras University[166–168]. The same is true about the work on Penicillin V Acylase at NCL[169,170]. Many of the recently initiated activities are concerned with proteins from microbial pathogens. They are later dealt with separately. In addition to them, there are other new programmes initiated by established groups. One of them is concerned with the structural genomics of human genetic disorders in blood being carried out at SINP[171]. Another is the work on proteins in human secretions, which has gathered momentum at AIIMS[172–176]. An important result to emerge from the newly established group of R. Sankaranarayanan at the Centre for Cellular and Molecular Biology (CCMB), Hyderabad is the structure solution of the editing domain of a threonyl-tRNA synthetase[177]. Yet another important structure solved recently is that of the coliphage lambda transcription activator protein CII by P. Chakrabarti at the Bose Institute[178]. A major new programme is concerned with bovine pancreatic PLA2 and is carried out by K. Sekar and his colleagues at the Institute[179–181]. That involves detailed studies on the mutants of the protein and its complexes. The structure analysis of the catalytic domain of the chick retinal neurite inhibitor-receptor protein tyrosine phosphatase, carried out by B. Gopal of the Institute is also noteworthy[182].

Figure 5: Typical structure of a mammalian lactoferrin (1bix).



5. Structural biology of microbial pathogens

By the end of the last decade, macromolecular crystallography in India had come of age with a critical mass of scientists working in the area. Several groups also began to address problems related to infectious diseases, an endeavour which

Figure 6: PLA2 from *Vipera russelli* complexed with vitamin E (1kpm).

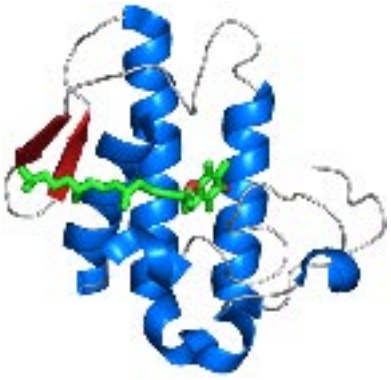
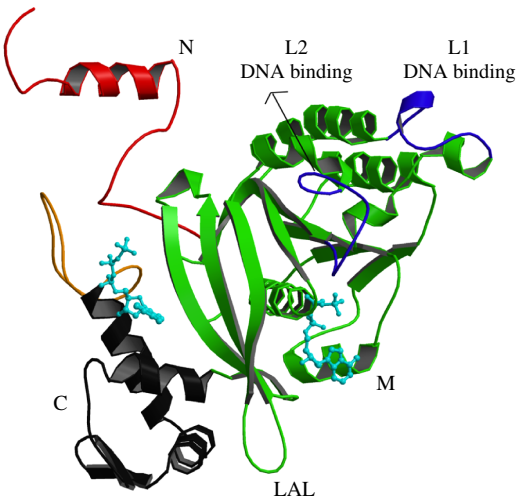


Figure 8: Structure of mycobacterial RecA. The bound nucleotides are indicated as ball-and-stick.



is very relevant to India. In particular, a concerted programme on TB proteins emerged in the wake of the publication of the sequence of the genome of *Mycobacterium tuberculosis*, the causative agent of TB, as part of a larger effort on the structural genomics of microbial pathogens. The overall strategy has been for each group to address a carefully chosen set of proteins, with a measure of loose academic networking among the groups. The first structure of a TB protein to be reported from India in 2000 was that of RecA determined at the Institute. By now the structures of about 20 TB proteins, and in one case those of many ligand complexes also, have been determined in different laboratories in the country and many more are on the anvil. Many of them are possible drug targets. It turns out that the X-ray analysis of nearly 10% of the TB proteins of known structure has been carried out in Indian laboratories.

The Institute has a long tradition in mycobacterial research. The structural studies on TB proteins undertaken at the Institute primarily seeks to build on the existing strengths. The main thrust

Figure 7: Complexes of Fab of germline antibody 36-55 with three independent dodecapeptides (2a6d, 2a6i, 2a6k).

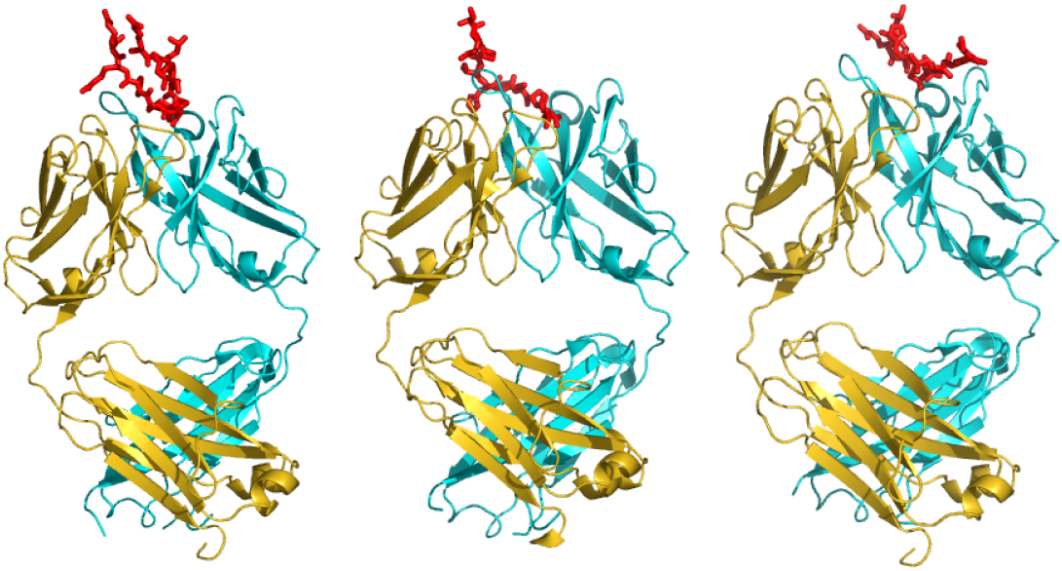


Figure 9: Structures of *M. tuberculosis* class III adenyl cyclase (1yk9) and two promoter recognition domains of the extra-cytoplasmic function σ factor σ^C (2o7g, 2o8x) (right).

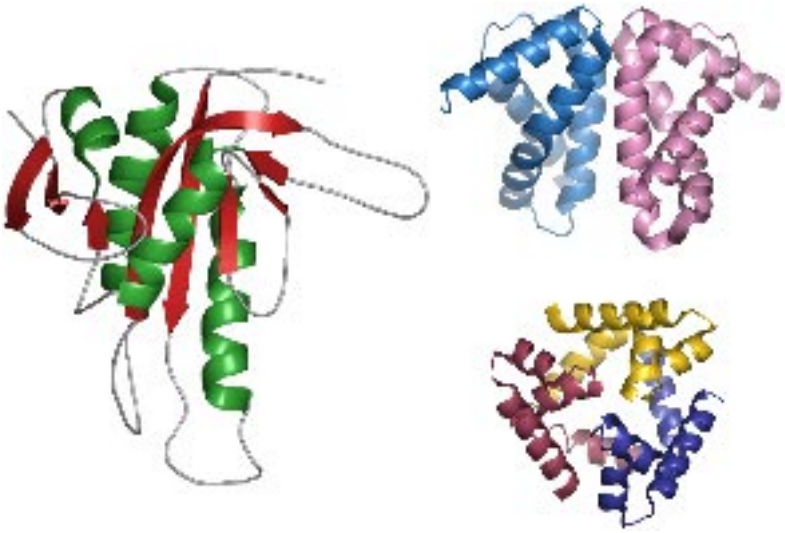
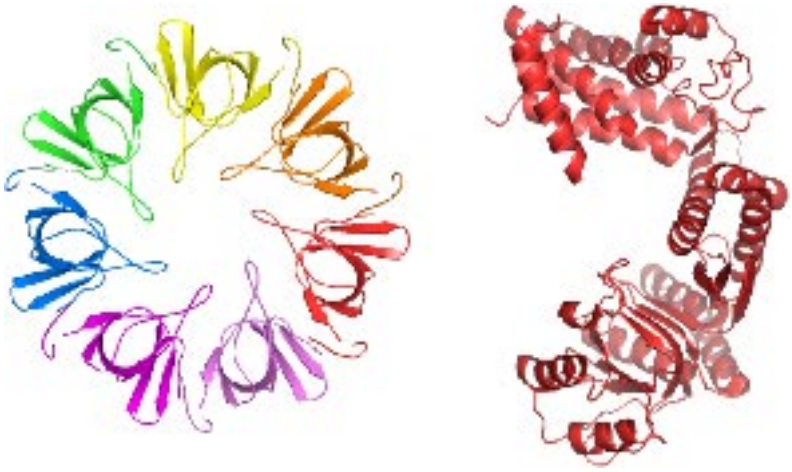


Figure 10: Structures of Chaperonin-10 (1hx5) (left) and GroEL (1sjp) (right) from *M. tuberculosis*.



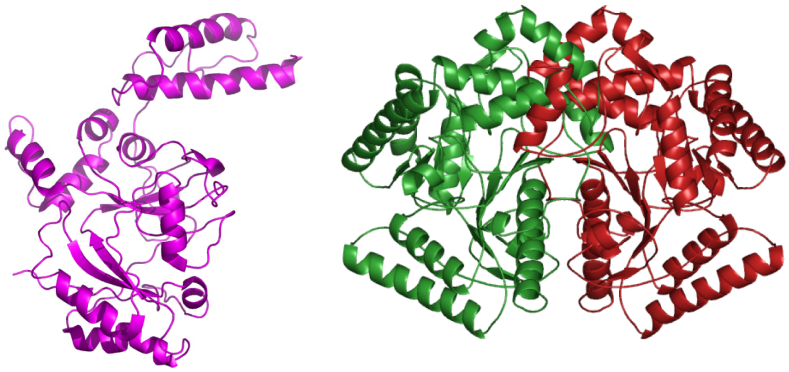
of the effort in the laboratory of this writer has been on proteins involved in DNA recombination and repair, and protein synthesis with recent forays into those involved in other metabolic pathways. In many instances, structural information on TB proteins has been supplemented by that on the corresponding proteins from *M. smegmatis*. The entire work has involved extensive collaboration with biochemists K. Muniyappa, U. Varshney, A. Surolia and D. Chatterjee. RecA, involved in homologus DNA recombination and repair and in SOS response, from *M. tuberculosis* and *M. smegmatis* has been studied with considerable thoroughness and these studies have yielded many novel results[183–187] (Figure 8). Single stranded DNA binding protein (SSB), again involved among other things in DNA repair, from both the bacteria have been analysed[188,189]. Yet another repair enzyme studied in this laboratory is uracil N-glycosylase. The proteins involved in protein synthesis X-ray analysed in this laboratory are ribosome recycling factor (RRF)[190] and peptidyl-tRNA hydrolase (Pth) from *M. tuberculosis*. Also determined is the structure of pantothenate kinase (PanK), the first enzyme in the coenzyme A synthesis pathway[191]. Although not directly relevant to TB, the studies on the DNA binding proteins from stationary phase cells (Dps) from *M. smegmatis* merit special mention[192,193]. Another notable contribution from the Institute is the structure determination of the class III adenylyl cyclase from *M. tuberculosis* by K. Suguna in collaboration with Sandhya Visweswariah[194]. In addition, the new group headed by B. Gopal has carried out structural studies on promotor recognition domains of the extra-cytoplasmic function σ factor σ^C [195] (Figure 9).

A major TB structural group is headed by Shekhar C. Mande who started his independent career at the Institute of Microbial Technology (IMTech), but subsequently moved to the Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad. The proteins studied by them have been chosen on the basis of a careful examination of the TB genome. Part of their work is concerned with chaperonins involved in protein folding, with particular reference to Chaperonin-10[196] and GroEL[197] (Figure 10). The other protein structures analysed by them, namely, alkylhydroperoxidase, thioredoxin reductase[198] and chorismate mutase[199], were chosen for their potential as drug targets.

The Central Drug Research Institute (CDRI), Lucknow, is another important centre for structural studies on TB proteins. An AdoMet-independent methyltransferase, determined by H. S. Subramanya and his colleagues at CDRI, was one of the early TB proteins to be analysed in this country[200]. The TB proteins analysed by the newly established group of R. Ravishankar include the adenylation domain of a NAD+ dependant DNA ligase[201,202] and lysine ε-aminotransferase[203] (Figure 11). The design of their inhibitors with a view to drug development, is also in progress.

An important TB protein to be structure analysed recently by R. Sankaranarayanan of CCMB in collaboration with Rajesh Gokhale of NII is a polyketide synthase[204] (Figure 12). The newly established group of Amit Das at the Indian Institute

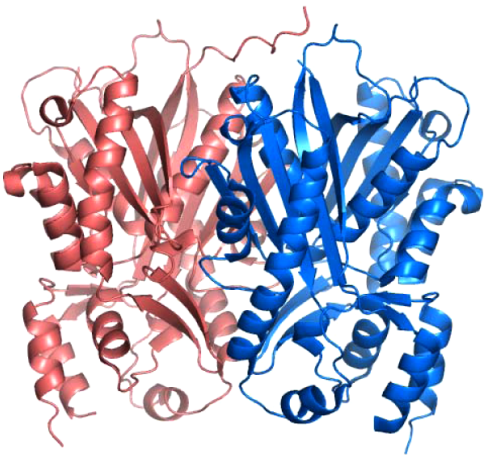
Figure 11: Structures of the adenylation domain of a NAD^+ DNA lygase (1zau) (left) and lysine ε-aminotransferase (2cin) (right) from *M. tuberculosis*.



of Technology, Kharagpur has also been active on TB proteins. They have already analysed the structure of tyrosine phosphastase from the pathogen[205].

There has been substantial work also on pathogenic bacteria such as *Salmonella typhimurium*, particularly by M. R. N. Murthy and H. S. Savithri[206–208]. Their work on plant viruses and that of M. V. Hosur on a HIV protease mutant have already been referred to. Yet another project concerned with viruses is that on rotavirus proteins, being pursued by K. Sugna in collaboration with C. Durga Rao[209]. Notable structural work has emerged from India on proteins from *Plasmodium falciparum*, a causative agent of malaria, much of it from Bangalore (Figure 13). Murthy and his colleagues in collaboration with H. Balaram and P. Balram have studied the triose phosphate isomerase from this parasite, its mutants and complexes[210–214]. Another protein from the parasite studied by the same group is adenylosuccinate synthetase[215]. Suguna and her colleagues, in collaboration with A. Surolia and N. Surolia, have been involved in structural work on *P. falciparum* proteins in the fatty acid synthesis pathway. The systems studied by them include enoyl-ACP reductase[216] and their complexes and β-hydroxyacyl ACP dehydratase[217,218]. Remarkable contributions pertaining to the malarial parasite have come from Amit Sharma of the International Centre for Genetic Engineering and Biotechnology (ICGEB). The structures solved by him and his colleagues include those of a gamatocyte protein essential for sexual development in the parasite[219] and a Duffy-binding-like domain involved in host receptor recognition[220] (Figure 14).

Figure 12: The structure of a polyketide synthase from *M. tuberculosis* (1ted).



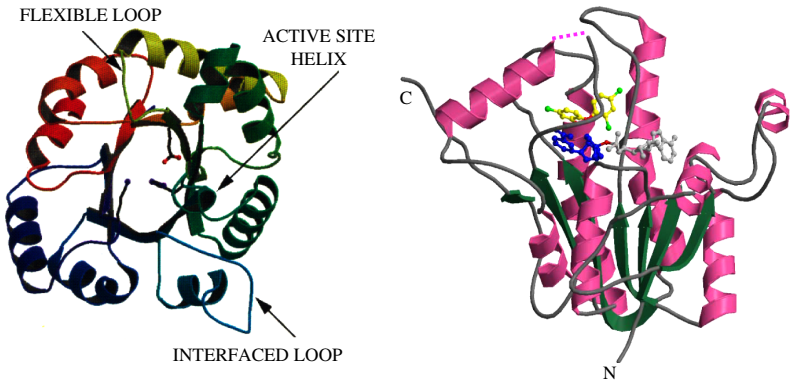
Leishmania donavani, the causative agent for "kala azar" is an almost unique Indian parasite. The first structure determination of a protein[221] from this parasite, that of cyclophilin, was recently carried out by R. Banerjee of SINP in collaboration with A. K. Datta of the Indian Institute of Chemical Biology, Kolkata. *Entamoeba histolica* is a pathogen which causes dysentery. Crystallographic studies on proteins from this organism have been recently initiated by S. Gaurinath at the Jawaharlal Nehru University (JNU), New Delhi, through the X-ray analysis of a calcium binding protein from it[222] in collaboration with A. Bhattacharya.

6. The way ahead

As mentioned earlier, macromolecular crystallography has now become an integral part of modern biology in India. The problems addressed by crystallographers in the country encompass almost the entire spectrum of biology at the molecular level. Much of the work has been pursued on individual initiative and it is difficult to predict the future course of the effort. However there have been a couple of common initiatives involving several workers.

The single most important factor that came in the way of realizing the full potential of the macromolecular crystallography activity in the country has been the absence of an indigenous synchrotron source. Almost 95% of the macromolecular structures solved in the world now are based on synchrotron data, but most of the structures solved in India are derived from lower quality in-house data. There has been a great deal of discussion about an Indian source from the late seventies, but we still do not have a usable facility although the construction of one was started at Indore some time ago. Encouragement from

Figure 13: Structures of triose phosphate isomerase (figure kindly supplied by M. R. N. Murthy) (left) and enoyl-ACP reductase (right) (1uh5) from *Plasmodium falciparum*.

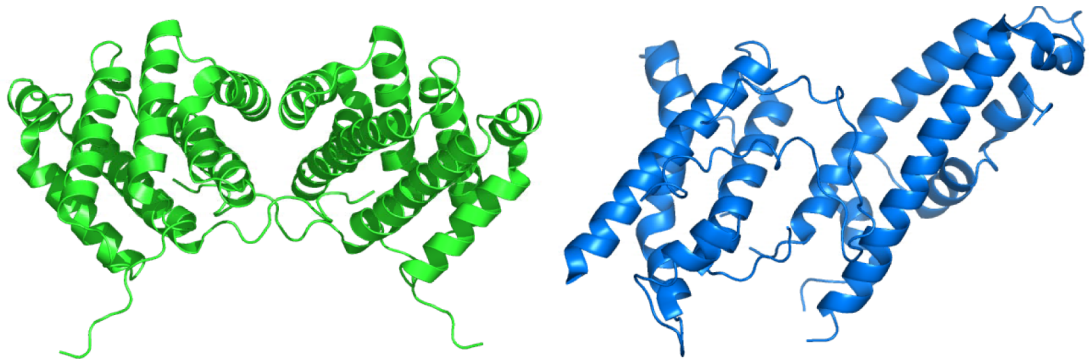


the user community, including macromolecular crystallographers, has helped in speeding up the effort at Indore. Hopefully, the facility will be commissioned soon. A consensus has also been reached on the need for a second facility, as it is unwise to depend on a single facility in view of the diverse synchrotron based activities which are developing in the country.

Developments in modern biology have also enabled attempts at rational design of drugs. One of the approaches in this direction is structure-based inhibitor design in which, for instance, inhibitors are developed for an essential enzyme on the basis of its three-dimensional structure. An inhibitor so designed may turn out to be a drug-lead for further effort. The successful examples of this approach are currently available drugs for AIDS and influenza. Most of the drugs in the market for AIDS are inhibitors of HIV protease or HIV reverse trasnscriptase. The role of crystallographers in the development of these drugs has been immense. The same is true in relation to the currently available drugs against influenza, which are inhibitors of the viral nuraminidase.

Structure based inhibitor design is at its infancy in India except in one laboratory, that of T.P. Singh at AIIMS, where considerable progress has been made in the design of potential drugs against pain and inflammation. With the concerted effort on proteins from microbial pathogens taking off the ground, efforts at designing inhibitors of these proteins, have begun in earnest. Viruses which cause AIDS and influenza are small organisms containing a handful of proteins. Bacteria and parasites typically contain thousands of proteins each. Each such organism is likely to have hundreds of potential drug targets. The problem is therefore extremely complex and difficult. At the same time it opens up a wide avenue for useful research. Eventually, we should aim at producing a basket of inhibitors for each important

Figure 14: Structures of a gametocyte protein (1n81) (left) and a Duffy-binding-like domain (2c6j) (right) from the malarial parasite.



protein studied in India. When a large number of inhibitors is produced, one or a few of them might turn out to be a drug or drugs. Eventually, it is desirable to develop inhibitor-design as a near-routine tool in the repertoire of medically oriented biological research.

Any research programme on pathogens rightly solicits questions regarding diagnostics, vaccines and drugs. However, while addressing these questions, it is also important to keep in mind the larger picture. Microbial pathogens are perhaps the only predators that humans have. With the advent of antibiotics, we thought we are on the way to conquering them. However, the microbes swiftly developed drug resistance and multiple drug resistance is a major problem in the treatment of infectious diseases. Thus the fight between pathogens and humans is a continuous one. In order to combat infectious agents on a long term basis, we need to understand well their basic biology. This long term goal should also be kept in mind.

The contributions of biological crystallographers in India is globally recognized. They are also at the vanguard of Indian biology. The growth of biological macromolecular crystallography in the country during the past quarter of a century has naturally given a great deal of satisfaction to the writer. All the same, we are conscious that we are yet to approach the kind of heights in structural biology that G. N. Ramachandran scaled a generation ago. However, the recent remarkable performance of Indian biological crystallographers, including the younger ones, leads to the confidence that they would begin to do so in the none too distant future.

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