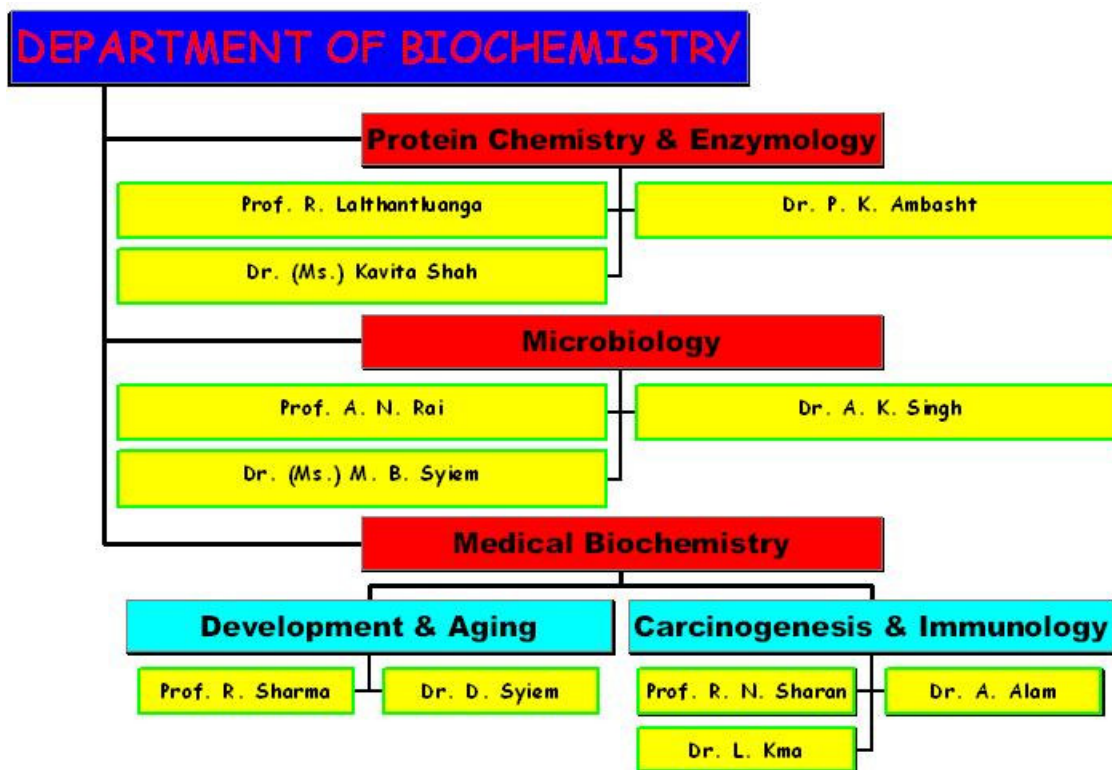


Major Research Groups and Activities

The following is the flow chart representation of the various research groups and the faculty involved in each group and sub-group in the broad areas of Biochemistry:



PROTEIN CHEMISTRY AND ENZYMOLOGY GROUP:

This group have been engaged in the study on different aspects of enzymes and proteins. Structural and functional proteins are integral parts of biological systems and serve vital roles in an organism. The functional capacities of several of them are correlated to their structural aspects. Initially, the group consisted of Prof R. Lalthantluanga and Dr. M. Y. Khan (left the department in 1993). In the year 2002, Dr. P. K. Ambasht and Dr. K Shah (both Lecturers) joined the group. The group looks at both the structural as well as functional/enzymatic aspects of protein molecules under different physiological conditions.

Prof. Lalthantluanga is interested in hemoglobin, an oxygen binding protein. The complete amino acid sequencing of the hemoglobins of *Chironomus* larva (*Chironomus thummi thummi*), Gayal (*Os frontalis*), Banteny (*Basjavanicus*) on yak (*Bus guinness*)

were determined. Their functional characterizations have been done in adult and fetal stages including their oxygen affinities. The investigation of different molecular bases that alter oxygen-binding, using ESR measurements is the prime objective for such studies.

Earlier, Dr. M. Y. Khan and his laboratory performed a systematic study on Cathepsin B and established that the enzyme could indeed play a role in intracellular protein degradation and can express its activity for a limited period even at physiological pH and ionic strength. A mechanism of inactivation by urea of cathepsin B was proposed with a probability of the presence of multidomain structure in the enzyme. Further, the presence of only one form of the enzyme in the buffalo kidney as against two forms found in the goat tissue indicated a probable species/tissue dependence of cathepsin B isoenzymes.

Dr. P. K. Ambasht has been working on enzymes like pyruvate kinase from mung beans, its physical characterization, kinetic properties, regulatory properties, active site functional groups and its interaction with enolase and lactate dehydrogenase. Previously pyruvate kinase and PEP-phosphatase activities were considered to reside on a single protein. Subsequently, with isolation of PEP-phosphatase free from pyruvate kinase and pyruvate kinase free from PEP-phosphatase have proved that these two are different proteins. Presently he is interested in enzyme immobilization studies with special emphasis on pyruvate kinase on different matrices and denaturation studies to elucidate the folding pattern.

Dr. K. Shah has been actively involved with research on proteins related to heavy metal stress in rice plants with special reference to cadmium metal. Earlier, she had purified and biochemically characterized the cadmium inducible protein binding complex from roots of rice plants. *In vitro* and *in vivo* enzymatic studies on the nucleolytic, proteolytic and phosphorolytic events as influenced by cadmium stress in rice. She studied the Nitrate reductase holoenzyme and antioxidant enzymes specially superoxide dismutases and peroxidases under Cd stress and the changes in the photosynthetic efficiency of growing rice seedlings. She also worked on phytochelatin induction in root culture tissues of *Rubia* and *Horseradish* with special emphasis to the biochemical changes brought about in the presence of Cd and glutathione on its biosynthetic enzyme γ -glutamyl cysteinyl transpeptidase and phytochelatin synthase using Radioisotopes and LC-MS. She showed for the first time that metal sulphur ratios influenced induced phytochelatin under metal stress. Presently she is interested in isolation and characterization of a pectin-binding cationic peroxidase from rice and its N-terminal protein sequencing. She had earlier characterized the similar type of peroxidase from Arabidopsis which has similarity with P32 of zucchini important for cell wall antioxidant property. This will also give an insight into the location of the peroxidase, its structural and functional roles in antioxidant activities.

MICROBIOLOGY GROUP:

This is an internationally recognized and leading group in India, working on **N₂-fixation** and related processes in free-living and symbiotic Cyanobacteria (Blue-Green

Algae). This is also the thrust area of research recognized by UGC under the DRS programme for this Department. This group has had research collaboration with UK, Sweden, Germany and Israel. A programme of collaborative research is under finalization with Moscow State University to work at White Sea Biological Research Station in Russia. A major project is currently underway on creation of new nitrogen-fixing symbioses. The group consists of Prof A. N. Rai, Dr. A. K. Singh (Reader) and Dr. M. B. Syiem (Senior Lecturer). Following are some of the contributions and areas of research undertaken and being undertaken by the group.

Cyanobacterial Symbioses: Using ^{15}N and ^{14}C kinetics, traced the routes of N and C metabolism; Immunogold localization of key enzymes of N and C metabolism; Showed that glutamine synthetase is repressed in heterocysts of the cyanobionts leading to the transfer of ammonia from cyanobionts to the host; Ability of cyanobionts to fix nitrogen even in darkness and requirement of PEP carboxylation to sustain it; Operation of malate-aspartate shuttle between cyanobiont and cycad host; Role of carboxysomes in CO_2 – fixation.

N_2 -fixation and Nitrogen Metabolism: Ammonia represses nitrogenase and heterocyst differentiation but nitrate represses only the heterocyst differentiation; N_2 ase and glutamine synthetase expression in heterocysts is linked and co-regulated; Uptake hydrogenase is membrane bound and present in heterocysts as well as vegetative cells; Characterized the transport system for ammonia, nitrate and amino acids; showed that glutamine synthetase is essential for use of amino acids as N source.

Cell Differentiation:

Akinetes- Factors leading to akinetes formation, metabolic activities of akinetes; Time sequence mapping of the reappearance of respiration, photosynthesis, nitrogenase and enzymes of C and N metabolism during akinete germination.

Heterocysts- Role of C nutrition in regulation of heterocyst differentiation; Absence of glutamate synthase in heterocysts; Absence of nitrate uptake and reductase in heterocysts; strategies for avoidance of competition for reductant and Mo, between nitrogenase and nitrate reductase, in heterocysts.

Non-heterocystous cyanobacteria: They have 10-20 times higher levels of nitrogenase protein than heterocystous forms; Insufficiency of oxygen protection mechanism in *Plectonema* for aerobic N_2 -fixation; Regulation of nitrogenase derepression and coordination with photosynthesis; Localization of various enzymes of C and N metabolism.

Biotechnological Applications: Photobiological production of ammonia by manipulation of GS and ammonium transport systems; Artificial symbioses between nitrogen-fixing cyanobacteria and crop-plants; Cell immobilization; Biofertilizer- special strains capable of profuse sporulation, N_2 -fixation in presence of combined nitrogen, and association with rice crops.

MEDICAL BIOCHEMISTRY GROUP

Development & Aging and Medicinal Plants sub-group:

This sub-group, comprising **Prof. R. Sharma** and **Dr. D. Syiem**, works on various aspects of development and aging and on the medicinal plants with anti-diabetic activities. The main area of research has been on the hormonal regulation and characterization of various enzymes of carbohydrate, amino acids and nucleotide metabolisms during development and aging of animals. A significant work has also been done on the glucocorticoid receptor (its level, activation and DNA-cellulose and nuclear DNA binding) changes during development and aging of rats and mice. It has been reported that glucocorticoid-mediated regulation of tyrosine aminotransferase and tryptophan oxygenase is also influenced by protein kinase C modulator (sphingosine and H-7) using rat hepatocytes as a model system. This was an addition to the emerging concept of cross-talk in signal transduction at that point wherein the steroid hormone action cascade got interdependence onto protein/peptide signaling cascade. Dietary restriction has been shown to increase longevity and postpones age-related pathologies in various groups of experimental animals. Recently, a systematic work on the dietary restriction regulation of adenosine deaminase, malate-aspartate shuttle enzymes and also on the glucocorticoid receptors at different phases of the mouse's life span. Dietary restriction induces glucocorticoid receptors and thereby helps better adaptation in aging animals.

Dr Syiem focuses his attention on the documentation of plants which have traditionally been used for diabetes, screening for their anti-diabetic properties using mouse model, and testing these extracts on specific enzymes of the polyol pathway known to be involve in cataract formation and other microvascular complications and also for their possible effects on enzymes which are responsible for the production of AGEs and Amadori product implicated with the pathogenesis of diabetic complications. In addition, scientific validation, isolation, characterization and identification of active chemical principle(s) using chromatographic techniques like TLC, HPLC and also other molecular tools like RIA and ELISA are being carried out by this group. Pharmacological and toxicity studies are also being carried out. As medicinal plants are mostly extracted from the wild, leading to species depletion, the group is also involve in interdisciplinary activity to encourage and promote conservation effort vis-à-vis medicinal plants which have been overexploited and threatened. The outcome of such research will hopefully result in bringing out new molecules which are more potent and with lesser adverse effects than those available in the market today. Further these products that can be made available locally and at prices that can be affordable to the rural underprivileged community.

Carcinogenesis and Immunology sub-group:

This sub-group has essentially been working on different but intricately interrelated aspects of carcinogenesis. While Prof. R. N. Sharan and Dr. L. Kma (Lecturer) are interested in chemical carcinogenesis, radiation induced DNA damage and its repair, Dr. A. Alam (Reader) is interested in cancer immunology.

Chemical carcinogenesis: Elucidation of molecular mechanisms of carcinogenesis in appropriate *in vivo* system has significant relevance to human welfare, as extrapolation of *in vitro* results to human situation is inappropriate. With this in mind, **hepatocarcinogenesis**, **ascites tumorigenesis** and **betel nut** induced general carcinogenesis mouse models have been established in the laboratory. The pioneering and

extensive work on betel nut induced cancer, a masticator of social relevance to this region, has especially been noticed and cited internationally. Our studies show that there is significant commonality in induced carcinogenesis at molecular level suggesting that a common strategy might control or reverse the process. In this, among others, cellular **poly-ADP-ribosylation** of chromosomal (especially histone) proteins has emerged as an important **biomarker** and **bioregulator** of cellular transformation *in vivo*. An ELISA based novel slot and Western blot immunoprobe assays of poly-ADP-ribosylated proteins has been developed in the laboratory for its qualitative and quantitative analyses. This is emerging as a potential tool for mass screening of cancer in large populations and has been recognized as one of the 'emerging technologies from Indian universities' by the **UGC-UNDP compilation** (2002). Currently, initial human trial is underway in collaboration with B. B. Cancer Institute, Guwahati for its possible deployment as a biochemical diagnostic tool for early **detection of cancer** (patent pending) especially from blood lymphocyte proteins. HMG proteins are other targets that are actively being pursued in this research.

Mutation in normal and cancer bearing human population: The blood samples and biopsies of normal and cancer bearing individuals of north-eastern region and experimental model (mice) are being screened for mutations in **p53 gene**, a tumor suppressor gene, as well as level of poly-ADP-ribosylations of total and individual proteins. Well-characterized regions of this gene, especially exon 3 and 4, have been shown to be most vulnerable to mutations. PCR amplification and NT analysis of these segments are underway. Other target genes for this study are BARC1 and 2 genes. **Transgenerational** effects are also being studied.

Radiation induced damage to DNA and its repair: Radiotherapy, the most widely used modality for cancer therapy, has inherent limitation - it also damages and kills neighboring normal cells/tissues. Thus, the clinical efficacy of radiotherapy is seriously undermined. To optimize the existing radiotherapy protocols, it is therefore important to understand the mechanisms of **radiation interaction** at molecular level. On the other hand, **genetic predisposition** and **genome instability** are increasingly becoming integral components of concern for human health and well being. Convincing evidences suggest that mutagenesis and carcinogenesis induced by radiation and chemicals are dependent on genomic constitution. Therefore, different individuals or groups of human population exhibit variable response to radiation and chemical interventions. The scientific evidences that some of these traits move transgenerationally further substantiate the concept that genomic configurations determine susceptibility of humans to diseases, particularly cancer. The nature and molecular basis of these, however, remain elusive. We have developed a plasmid DNA model to study strand break and other chemical damage to DNA by different quality of radiation and different radiomimetic chemical, free radical generating systems. Using *E. coli* (repair proficient, wild and deficient mutants) it has been shown the induction of DNA damage to plasmid DNA is influenced by the nucleotide sequence making the **GC-rich** segments relatively more vulnerable to radiation/chemical damage *in vitro*. *In vivo*, on the other hand, the plasmid sustained entirely different spectrum of damages in different *E. coli* mutants. Quantitative and quantitative differences among high and low LET, and UV-C radiation as well as

different radiomimetic chemical systems and their effects on gene expression is being actively studied.

Tumor immunotherapy: Tumor immunotherapy is a fast growing area of research and might be the answer to the growing menace that is cancer. One approach to cancer treatment is to augment the *natural defense system* of the body with extracted tumor-associated antigens (TAA), the objective being to stimulate the host's immune response against the tumor. The group working on cancer immunology has an aim to identify and characterize TAA in animals exposed to N-Nitrosodiethylamine (DEN) and N-Nitrosodibutylamine that are well established hepatocarcinogen. An over expressed high molecular weight glycoprotein (TAA) with a very high content of carbohydrates has been purified and partially characterized. The purified TAA was found highly immunogenic and could eventually be used to induce immune response in order to counter tumor regression. The interest in the study of these carcinogens stemmed from findings that N-Nitroso compounds present industrial occupational hazards. In recent years these compounds are of great concern, not only to industrial workers, but also to the population at large and have emerged as one of the most important classes of environmental carcinogens.

Liposome-mediated delivery of anti-tumor drugs/toxins: Liposomes are versatile vehicles in terms of structural characteristics and mode of drug and antigen incorporation. This creates a wide range of options for the design of effective liposomal drug formulations to induce tumoricidal effector mechanisms. This group have studied the effect on tumor of a free and liposome encapsulated AK-2123, a hypoxic cell radiosensitizer that has widely been used in combination with a number of cancer therapies such as thermotherapy, chemotherapy and radiotherapy. AK-2123 after incorporation into liposome afforded more efficient radiomodulatory effects than that of uncapsulated AK-2123. Thus, it is seen that treatment of cancer with a combination of radiation, a radiomodifier and a drug delivery system, opens a wide scope for exploitation for the improvement of existing cancer therapies. This group is also making an attempt to study the effect of liposome encapsulated ribosome inactivating proteins (RIPs) purified from seeds of *gelonium multiflorum* and *luffin cylindrical* on transformed hepatocytes *in vitro*. This may be a very effective approach for selective killing of cells by the toxin.



Research Scholars working in the department: (Back, L to R) Ashima Das, Kishore Chakma, Rajkumar Singh, Samrat Adhikari, Meriyani Oduyo, Rennie Lakadong, Sangeeta, James Wahlang, Pauza Khup, Daniel Nongbri, Danswarang Goyari, Thy Answer Challam, Suktilang Majaw; (Front, L to R) Thomas Iangjuh, Yashmin Choudhary, Chaitali Bhattacharjee, Amrita Bhattacharjee, Nonibala Devi, Nakhuru, Debipreeta Dutta