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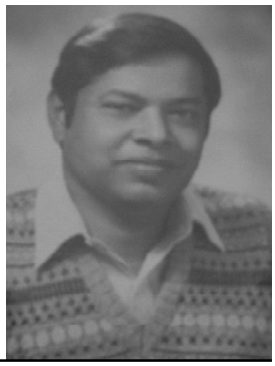

Soldiers of Science: A Profile

DR. BABLU BHATTACHARYYA: A JOURNEY OF FOUR DECADES WITH TUBULIN

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Abstract: Dr. Bhabatarak Bhattacharyya, a chemist turned biologist, is an eminent and distinguished scientist in the field of biophysical chemistry. He devoted his entire life in understanding a very interesting cytoskeletal protein, tubulin that polymerizes to form microtubules. He discovered a novel chaperone activity of tubulin and elucidated the folding/unfolding pathways of tubulin. He characterized the binding of different anticancer drugs to tubulin and most importantly contributed in unfolding the mystery of colchicine binding to tubulin. His contribution towards the scientific community will always be cherished and appreciated.

***Keywords*:** Dr. Bablu Bhattacharyya; Indian scientist; biophysical chemistry; tubulin

The journey begins

Dr. Bhabatarak Bhattacharyya (Photo) is a distinguished and eminent Indian scientist in the field of Biophysical Chemistry. Popularly known as "Bablu" in the Indian Scientific community, he started his scientific career with Prof. Umashankar Nandi, Department of Physical Chemistry, Indian Institute of Science (IISc), Bangalore as a graduate student. Prof. Nandi had a strong influence in shaping Bablu's scientific career in the chemistry of biomolecules. However, it was actually Dr. Bhupati Ranjan Bhattacharyya, his dearest elder brother, who was responsible for his changeover from a classical chemist to a researcher in Biology. Dr. Bhattacharyya earned his bachelor's degree in Chemistry in 1965 and subsequently his master's degree in 1967 in Physical Chemistry from the University of Calcutta. His basic interest lied in the chemistry of the physical principles, which was nurtured greatly in the classrooms of Physical Chemistry at the century old Rajabazar Science College, Kolkata (then Calcutta), that he later substantially applied in his research in the biological system.

Being a Chemistry graduate, it was not an easy decision for Bablu to choose a career in Biology. Basically, Chemistry was in his heart, but it was Biology, that acquired his mind. Probably, this tug of war between Chemistry and Biology brought him in a laboratory for his doctorate research where chemistry and biology walked together. His research career as a Ph.D. student started on the fundamental problems of biological systems that can be solved by his much-beloved principles of chemistry and chemistry-based tools. During his Ph.D. in Prof. Umashankar Nandi's lab, first at the Indian Association for the Cultivation of Science (IACS), Jadavpur and later at the IISc, Bangalore, Dr. Bhattacharyya worked on the basic biochemistry of 'DNA-small molecule interaction'. He was officially a Ph.D. student of IACS and received his Doctorate degree from the Calcutta University.

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The first mark of excellence

Though Dr. Bhattacharyya's scientific journey started as a Ph.D. scholar, his actual scientific voyage that was indeed full of excitement and immense enthusiasm started when he joined the research group of Dr. J. Wolff at the National Institute of Health (NIH), Bethesda, USA as a post-doctoral researcher. Dr. Wolff was then considered to be one of the stalwarts in the area of eukaryotic cytoskeletal protein, tubulin. Dr. Bhattacharyya was probably the first person in Dr. Wolff's lab who started investigating the biology of tubulin and microtubules using biophysical chemistry or rather physical chemistry as the basic tool. Being a scientist with medical (MD) degree, Dr. Wolff's interest was more on the clinical aspects of tubulin. He was a bit unsure if this Indian postdoctoral fellow with expertise in Physical Chemistry would be of any help in his research. Very soon, Dr. Wolff got convinced that he had hired an excellent researcher for his lab. In fact, Dr. Bhattacharyya proved himself to be the most talented and productive postdoctoral researcher that Dr. Wolff ever had.

Colchicine is one of the most widely studied anti-mitotic agents. While working in the Wolff lab, Dr. Bhattacharyya monitored for the first time, the binding of tubulin to colchicine using fluorescence spectroscopy. He still cherishes the eventful day when he made this discovery. He recalls that he was investigating the binding of tubulin with colchicine using a fluorescence spectrophotometer, his favorite instrument that brought hundreds of papers and many awards and laurels to him. He was unable to detect any significant fluorescence of colchicine immediately after it was mixed with tubulin. After a few hours of exhaustive titration experiments, he took a break for a coffee, leaving the titration mixture of tubulin and colchicine in the cuvette channel. After returning from the coffee break, he witnessed a beautiful fluorescence pattern of colchicine in the mixture. Then, he realized that the binding reaction between tubulin and colchicine is very slow and it needs about 15-30 minutes to get a detectable fluorescence.

He further proved that tubulin-colchicine binding is almost a completely irreversible reaction and it needs about 30 min and physiological temperature to attain the effective binding. This elegant finding was published in PNAS (USA) (Bhattacharyya and Wolff, 1974) and very shortly, it became a highly acknowledged reference for thousands of researchers in the field of tubulin-binding agents. Besides this novel finding, Dr. Bhattacharyya published numerous remarkable papers including one in the journal 'Nature' (Bhattacharyya and Wolff, 1976) during his five years research (1972-1976) as a post-doc in Dr. Wolff's laboratory and later as an invited research scientist for some time. With such an impressive scientific career, it was not difficult for him to obtain a faculty position in the USA, those days. In fact, many of his contemporaries were settling their independent laboratories in the USA. However, Dr. Bhattacharyya always had the dream to nurture science in India. He was so keen and confident that he did not even hesitate to start his career with a position of a CSIR pool officer in the Biochemistry Department, Bose Institute.

The Indian journey

Dr. Bhattacharyya is the first person who started research on eukaryotic cytoskeleton in India. In that sense, he cultivated tubulin-research in the country. It is really remarkable that he has published more than hundred papers on different aspects of a single protein, tubulin. His papers are published in international peer-reviewed and highly acclaimed journals like the Journal of Biological Chemistry, Biochemistry, and Journal of Medicinal Chemistry, etc (Bhattacharyya and Wolff, 1984; Gupta *et al*., 2005; Chakraborti *et al*., 2011). Dr. Bhattacharyya has produced 24 doctorates in his career of 30 years. Many of them are established as independent investigators, including the authors of this article, at national universities and research institutes like IIT, IISER, DBT institutes, Calcutta University as well as abroad. His scientific contributions and research excellence have been highly appreciated in the form of awards and recognitions. He received Shanti Swarup Bhatnagar award in 1989 from Council of Scientific and Industrial Research (CSIR), Government of India. He is a fellow of all the National Science Academies, National Academy of Sciences (NASI), Indian Academy of Sciences (IAS), Indian National Science Academy (INSA) and also received the prestigious Ramana

fellowship. He is indeed a shining star of Indian protein science.

Summary of research contribution in tubulin

Dr. Bhattacharyya's research has explored a number of critical areas in the field of tubulin-microtubules and has made an immense contribution that is highly acclaimed by the experts in the field worldwide. Throughout his career, he focused on the microtubule related studies and protein folding. Continuously, he dived deeper in his area of interest. He embraced many new techniques and collaborations to reveal the microtubule-structure, function, activity and its various inhibitors during his line of work. He has been working on tubulin, for more than 40 years and still publishes high quality research work in a consistent manner. Some of his major scientific contributions in recent years are summarized below.

Discovery of Molecular Chaperone-like activity of tubulin

Microtubules have been reported to function in various cellular processes such as cell division, maintaining cell polarity, providing a structural framework to the cells. The C-terminal tails of tubulin have been thought to regulate tubulin assembly by binding to other microtubule associated proteins. He has shown that the C-terminal tail of tubulin also regulates the association between alpha tubulin and beta tubulin (Panda *et al.*, 1992). While his major research interest has still been on the pharmacological aspects of tubulin, during the last 10-15 years, his work led to the identification of an unexplored biological property of tubulin, the molecular chaperone activity. The structural features of tubulin that makes it to function as chaperone are not known. Dr. Bhattacharya's group for the first time showed that tubulin exhibits structure and functions similar to many other known molecular chaperones. Tubulin suppresses both the thermal and non-thermal aggregation of various proteins. For example, it prevents the aggregation of equine liver alcohol dehydrogenase, insulin and crystallins, the soluble eye lens proteins. The result showed that tubulin could act as a chaperone for many unrelated proteins. Several approaches showed that the acidic C-terminal of tubulin was responsible for the chaperone activity of tubulin *in vitro* (Guha *et al.*, 1998). After showing the role of tubulin as a suppressor of protein aggregation, his group also studied whether like other chaperones, tubulin could protect the biological activity of proteins against thermal denaturation. A possible role of tubulin in the folding of other proteins was examined. Tubulin could increase the yield of a protein by assisting its folding. His group was successful in establishing the fact that the tubulin also prevents the loss of biological activity of proteins during thermal stress as studied in the case of alcohol dehydrogenase and malic dehydrogenase. They showed that tubulin prevents aggregation of proteins during the refolding of a protein from the denatured state by forming a stable complex with the denatured substrate. These results suggested that tubulin, in addition to its role in mitosis, cell motility, intracellular transport, or other cellular activities may have a significant role in protein folding and protection from the thermal stress (Manna *et al.*, 2001).

Effect of other chaperones on tubulin

Other than the chaperone activity of tubulin, Dr. Bhattacharyya's group has tried to reveal the effect of other chaperones on the assembly of tubulin. It was interesting to know through his work that chaperones like alpha-crystallin, HSP16.3, HSP70, and alpha (s)-casein from different sources inhibit tubulin self-assembly or polymerization in a dose-dependent manner, independent of the assembly inducers used. Interestingly, the chaperones could inhibit tubulin polymerization completely only when they were added before the initiation of polymerization, but not when added at the early stage of elongation phase of polymerization. This important study suggested that chaperones bind to the tubulin at the protein-protein interaction sites that are involved in the nucleation phase of self-assembly of tubulin (Mitra *et al.*, 2007).

Structure-function relationship of microtubule-binding drugs

While working on the structure and activity relationship of tubulin, Dr. Bhattacharyya has

shown the importance of B-ring analogs (with C-7 substituent) of colchicine in binding to tubulin. Due to the flexibility of the C-terminal tails of αβ-tubulin, this region plays an inhibitory role in the polymerization of soluble tubulin to the microtubules. He has proved that a B-ring substituent of colchicine binds to alpha-tubulin and induces significant conformational changes in it. The B-ring is responsible for the pH dependent binding of colchicine to tubulin. It was found that the C-terminal of alpha subunits interacts indirectly with the main body thereby making the interaction between tubulin and colchicine sensitive to pH. This result also suggested the tail-body interaction of tubulin (Chakraborty *et al.*, 2004). It has been known that the A and C rings of colchicine are responsible for its binding to tubulin. Dr. Bhattacharyya for the first time showed that a biologically active bifunctional colchicine analogue could be designed where the drug binds to tubulin through its A and B rings, while the C ring remains inactive. By substitution of a hydrophobic dansyl group on the B-ring side chain (C7 position) of isocolchicine, he showed that the virtually inactive isocolchicine can regain its lost biological activity (Das *et al.*, 2005). Podophyllotoxin possesses anti-tubulin activity. It exhibits partial structural similarities with colchicine and hence acts as a competitor for the colchicine binding site on tubulin. Dr. Bhattacharyya has established the involvement of the oxalone moiety as well as the lactone ring of podophyllotoxin in tubulin binding, by methoxy substitution reactions. From these results, an involvement of oxalone, as well as the lactone ring, of the drug in a specific orientation inclusive of ring A is indicated for podophyllotoxin-tubulin interaction. The result suggested that podophyllotoxin is a bifunctional ligand like colchicines and interacts with tubulin with more than one binding site. (Gupta *et al.*, 2006).

Diverse research contributions: drug associated

Expanding the work of tubulin-drug interaction, Dr. Bhattacharyya has also worked on the stability of upcoming and potential drug candidates. He has reported that curcumin loses its activity in a reducing environment. But, the replacement of diketone moiety of curcumin with isoxazole and pyrazole not only makes it extremely stable at physiological pH and reducing atmosphere, but also retains its activity to kill cancer cells under serum-depleted condition. Interestingly, these curcumin derived molecules also show better anti-oxidant activity than curcumin. This finding suggested that isoxazole and pyrazole curcumins could be a good candidate for the discovery of drug in future (Chakraborti *et al.*, 2013). He continued to further characterize different curcumin analogues for their functional aspects. Some of the major findings document that curcumin behaves as a bifunctional ligand. The curcumin analogs modified at the diketone position and at the phenolic position were found to be less effective whereas benzylidiene derivative was more effective than curcumin in inhibiting the assembly of tubulin. Structure-activity studies of benzylidiene derivative of curcumin suggested that the tridented nature of this compound is responsible for its higher affinity to tubulin compared to curcumin.

Several compounds of different structures are found to bind to tubulin either at the vinblastine or colchicine binding site on tubulin. Bablu was always fascinated by the mystery of the colchicine site. To solve the query of the unique properties of compounds that are structurally diverse but still possess a comparable affinity to tubulin at the colchicine binding site, he used two structurally different antitubulin compounds namely colchicine and TN16. An analysis of their binding modes indicated that in the complex states both colchicine and TN16 can move within the binding pocket; although in two different ways. This study provides an idea that the better fitting of the ligand into its binding pocket is significant when focusing on the ligand-receptor interactions for the discovery of new drug molecules (Chakraborti *et al.*, 2012) Vertebrate tubulin has several isotypes. Tubulin isotypes are suggested to have roles in maintaining microtubule dynamics and they also contribute in the development of drug resistance. These isotypes differ in their C-terminal amino acid sequences and their abilities to bind to colchicine. An analysis of the beta isotypes of tubulin suggested some differences in the main body sequence that are likely to be responsible for the

differential colchicine-binding kinetics of tubulin isotypes. Through homology modeling of all beta-tubulin isotypes, he proved that the isotypes differed from each other in the amino acids surrounding the A ring of the colchicine-binding site on beta-tubulin. He also showed that the C-terminal tail of alpha tubulin is responsible for higher activation energy of colchicine-tubulin interaction. It is also involved in the pH dependent binding of colchicine to tubulin. Removal of the negatively charged C-terminal tail of alpha-tubulin as well as its neutralization using a specific 14 amino acid peptide decreased the activation energy and the pH dependency of the binding of colchicine to tubulin.

The tubulin legend: teacher and humanitarian

Indeed, Dr. Bablu Bhattacharyya has led a career with passion for science, unraveling several important aspects of tubulin behavior. While we have put emphasis on Dr. Bhattacharyya's scientific journey, this endeavor will be incomplete if we do not acknowledge his humanitarian side. Whoever has known Bablu, vouches for his kindness and patience. His simplicity is the keynote of his elegance. He stands out by his sweet disposition, soft and pleasant personality, simplicity and friendliness. Even after being so busy with research and administrative work, he has always been concerned and involved with his student's progress and career. He has supervised 24 graduate students and has always been their philosopher and guide. No wonder, many of his students are now faculty and scientists at several institutes in India and abroad. He is full of life with never say die attitude. Because of his untiring and inspiring abilities, he has been able to produce excellent quality research papers using relatively simple infrastructure and facilities. The scientific community will fondly remember his contributions in protein folding, protein-ligand interactions and biochemical understanding of tubulin. For his pioneering contributions in microtubules/tubulin, he will be remembered as a scientist who initiated and successfully cultivated microtubule cytoskeleton research in India.

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