

Outstanding article (PDF category)

Coin tossing explains activity of opposite motors on phagosomes

We, The Mallik Lab, at Tata Institute of Fundamental Research are working towards understanding how tiny cellular material moves over large distances inside a living cell.

Cellular environment is extremely crowded and busy. Cells have several smaller compartments called organelles and various biomolecules, each of varying shapes and sizes. If we put a live cell under a microscope, we see that there is constant exchange and movement of material from one part of the cell to the other. This trafficking of biomolecules or “cargoes” as we call them is required for various life processes, such as - cell division, uptake of nutrients, migration of cells to the site of wound healing etc. Defects in transport of key molecules can often result in death or it can manifest in a number of diseases such as Alzheimer’s, Huntington’s etc.

We study the transport process with respect to infection. In our day-to-day life we encounter a variety of infectious agents. Our body has developed defense mechanisms to fight disease-causing agents without affecting our normal functions. When a foreign particle infects our body, our immune cells ingest these particles and trap them in a double-layered membrane structure, which is called a phagosome. This process is called phagocytosis. Phagosomes are eventually moved to the center of the cell for degradation. Motion of a phagosome from the site of engulfment to its site of killing is extremely important for effective clearance of the pathogen.

How does this motion of phagosome or other cargoes occur in the cell? This is brought about by the action of cellular “motors”. Motor proteins walk along pre-existing roads to deliver cargoes at their required locations. For simplicity, let us imagine the cargo as a cart, which requires a motor—say a horse, to drive its motion in one direction. There are two main types of motors that carry out long distance transport—like the horse and the bullock, which are Kinesin and Dynein. Both Kinesin and Dynein motors walk along same kind of tracks, however, they are quite different in their size, structure, as well as the direction in which they walk. Kinesin motors move cargoes towards cell periphery (a horse-cart moving in one direction) while Dynein generally moves them towards the center (a bullock-cart in the opposite direction).

To add to the cellular complexity, a large number of cargoes have both kinds of motors and actually move back and forth. So, we now imagine a cart with horses at one end and bullocks at

the other, both pulling back and forth to drive its motion in opposite directions. How these opposite motors work together to bring about molecules to the right place at the right time is hotly debated. We have recently addressed this question in our manuscript published in *Current Biology* in May 2018.

In our study, we examined the motion of phagosomes in their early stages of phagocytosis. These Early Phagosomes (EPs) display bidirectional back and forth motion due to presence of both Kinesin and Dynein motors. To study motor function on EPs, we extracted them out from immune cells using well-established protocols and made them walk on artificial constructed tracks. We analyzed their motion outside the cell using a special kind of microscopy called optical trapping. This technique provides tremendous amount of information as to how far motors can walk; the forces exerted by each motor type, their speed etc. Such experiments allow us to decipher properties of motors at a single phagosome level, a resolution that cannot be achieved when looking at the entire cell.

We investigated how could we explain back and forth motion of EPs? Do opposite motors depend on each other or do they pull against each other like in a tug of war? To make sense of how opposite motors behave on an EP, we specifically removed Dynein motors from the EP membrane. Surprisingly, upon Dynein removal, Kinesin neither performed better nor did it become worse. This suggested that both types of motors act independent of each other and do not require the opposite motor for their function. If motors function independently, what governs the choice of active motor - when do horses pull and when do bullocks pull the cart? Is there a pattern these motors follow to bring about motion?

To answer this, we analyzed a number of events where pulling force was generated by Kinesin (horses) and Dynein (bullocks) motors. We focused on event pairs for instance a KK pair where Kinesin was followed by another Kinesin event, or KD pair where a Kinesin was followed by a Dynein event, DK pair where Dynein was followed by Kinesin event and DD pair where Dynein was followed by another Dynein event. On performing statistical analysis of these pairs, we found that the number of each type of event pair is more or less similar. This suggests that all four types of event are equally likely to occur. This is similar to tossing of a coin where the probabilities of getting two heads (HH) or one head and then a tail (HT) or two tails (TT) or one tail and then a head (TH) are more or less equal. Thus, once a pulling event occurs, the choice

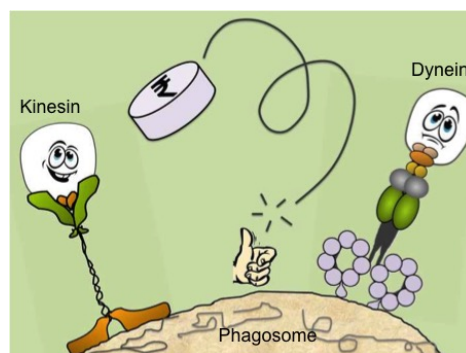
between Dynein and Kinesin for the next event is a random process. The system does not have any memory of the first event and thus, activation by either type of motor is equally likely.

We next asked whether this random choice of active motor explain bidirectional EP motion? If this is random, can we simulate this motion using modeling? Interestingly, we found that the back and forth EP motion is accurately explained by mathematical modeling when we take into account motor numbers on EP, their binding and unbinding rates and the geometry of phagosome where motors bind the track. These parameters somehow ensure that both Dynein and Kinesin events are equally possible resulting in back and forth EP motion. Such motion allows EPs to sample more intracellular space and interact with other organelles for exchange of components.

Thus, from our studies we have obtained basic parameters that make choice of motors a fair process. This may also be true for many other cellular cargoes. Further levels of regulation such as change in membrane composition; motor numbers or organization can bias this fair coin and accordingly change motion properties of phagosomes in the later stages. Our work, in general, addresses some fundamental questions by using a variety of approaches- we employ biological methods, biophysical force measurement techniques as well as mathematical modeling. Our work provides a holistic view in understanding of bidirectional cargo transport during early stages of phagosome motion as well as in situations when things go awry and result in infections.

The above work was performed by Myself, Ashwin D'Souza, Ashim Rai, Arpan Rai, Ranjith Padinhateeri and Roop Mallik.

Cartoon depicting a coin toss between Kinesin and Dynein motors on the phagosome



Picture credit- Roop Mallik

Adoption of Green Building for sustainable growth of rural India

Travelling to home town creates an excitement in a person of any age, education and status, it was summer vacation after completion of my first year of Master's in Civil Engineering. Since, the only way to reach my tiny village "Piapali" in Bastar district of Chhattisgarh State in central India was through railway in a break journey, first to Raipur and further from Raipur to Jagdalpur. Had a luxury to travel in AC-3 tier compartment due to tickets being sponsored by my grandfather to visit him at Piapali. Sitting in airconditioned train thinking about the beauty of nature and village-side life, it was a pleasant feeling which I realised after getting down the air-conditioned train to face the scorching heat of central India. Since, I had a connecting train from Raipur to Jagdalpur I had no other choice to wait in the waiting room which felt like sauna bath in the mid of May. After 16hrs of tiring journey from Raipur I reached Jagdalpur station and was happy to see my grandfather receive me, we reached our village and was excited and happy to meet my old aged grandmother, relatives and cousins. By the time it was 8pm, suddenly the electricity supply got shut down, after enquiring I came to know that our village is facing 16hrs of load shedding and electricity would resume only after that, had a sleepless night in a single fan which was running on inverter and whole family was compelled to sleep in one room which was creating suffocation due to very less ventilation in the room. Next morning onwards I realised that it was difficult to manage even menial thing in a village, potable water was sourced from Indravati river near to our house. Staying in an urban area with all luxuries of basic necessity like 24X7 water, electricity and other utility services I never thought about the wastage of specifically electricity and water until experienced it myself and concluded that, the balance of utilisation of natural resources defines the sustainability for long term of human existence. The balance between urban and rural area needs to be addressed for overall progress of any country. This thought gave a birth to a need to carry out research and development in sustainability, thus after returning, I zeroed down a research topic of my thesis. After going through various journal papers, research articles, technical magazines, books and further discussions with my guide, I started my journey on sustainability. Though, there were lot of research paper available but there were very few papers on Indian spectrum for green buildings. After researching I got to know that there were two major institutions in India for green building, out of which one was state sponsored and is known as Green Rating for Integrated Habitat Assessment (GRIHA) and Indian

Green Building Council (IGBC) which is a privately sponsored authority under Confederation of Indian Industry (CII) apart from others like, Leadership in Energy and Environment Design (LEED), Excellence in design for greater efficiencies (EDGE) and World Green Building Council (WGBC). Meanwhile in the first half of my thesis tenure I went to Vishakhapatnam to get training for IGBC and further appeared for IGBC accredited professional exam and cleared it first attempt in flying colours, my next goal was to take a training in GRIHA which I accomplished in Mumbai. After gaining a thorough knowledge of Green buildings I could not find a platform for calculating utility savings or reduced carbon footprints of green building as compared to the base case. Only EDGE had an online platform for the same and was giving results in an output format for submittals to concerned authorities, since EDGE is developed by International Finance Corporation (IFC) it lacked India specific requirements. Further to my discussion with guide about an idea of making a soft computing technique for IGBC and GRIHA code of practice, zeroed upon making a soft computing tool in Microsoft Excel for my master's thesis for appropriately calculating the credits with respect to the IGBC Abridge reference guide and preparing a case study using the same programme and comparing with an actual green certified building. The study included the green building concept like sustainable site planning, Building design optimization, Energy performance optimization, Renewable energy utilization, Water and Waste Management, Solid waste management, sustainable building material and construction technology, health, wellbeing and environmental quality. The benefits of green building have emerged which will prevent pollution, save energy and thereby saving on natural resources and expenditure during operation which results in approximately in 60% reduction in energy consumption, efficiency of human in offices and home increases with natural non-glare light and proper ventilation in the room, which results in reduction of respiratory diseases by 20% and increases the performance of the occupants upto 25%, finally results in low utility demands in green building. The environmental benefits include emission reductions, water conservation, strong water management, temperature moderation and waste reduction. The economic benefits include an energy and water savings, increase in property value due to lower operating cost and maintenance of building and decreased infrastructure strain i.e. less demand on local power grid and water supply. The indirect cost benefit includes improved attendance, increased productivity, sales improvement and development of local talent pool. The social benefit includes improved health due to better air circulation, proper lighting, lesser temperature variance etc., improved

attendance due to better environmental conditions, healthier life style and recreation by use of alternatives to personal driving such as bicycling and public transport which also adds to health and benefits of occupants. After completing my thesis of masters in civil engineering and ranking 3rd in the university I was elated and it resulted in building my self confidence to carry out further research by pursuing Doctor of philosophy (Ph.D.) in civil engineering. After taking due guidance from my able guide I decided to carry out my study on “Development of web-based decision tool for green building credit rating certification”, during the review of various literature in soft computing techniques for green building I carried out a critical appraisal of my literature and zeroed upon the gaps in the literature and thus defined the statement of problem for my study. Further to my above study I found that, in applying for green rating to the authorities, one has to engage various agencies in the field of energy modelling, water conservation, green consultants, project architects, project engineers, who thereby prepare hectic, large and complex documents to comply with the given intents so as to achieve the star rating of any green building, I intended to make a tool to meet the demands for quick, simple and free to use online web based decision tool to solve the complexities of the hidden methodology of resource efficiencies and cost savings in comparison to the base case without involvement of pocket burning expense by use of third party specialist to prepare and apply for green rating. My objectives of proposed study were to study all versions of GRIHA and IGBC rating system and to find out a methodology for appropriate calculations of credit points with respect to requirements as given in abridge reference guide. The above work required me to prepare programming concepts which included a complex conditional statement, looping of the same, implementation of logic flow diagram for problem solving and communications. After studying the above, I realised the need to master my self in programming tools like PHP: Hypertext Preprocessor, JQuery, Cascading style sheets (CSS), Hypertext Markup Language (HTML), Dot Net framework (.NET), C Sharp .NET, Dynamic-Link Library (DLL), Microsoft SQL Server and Java Script. On hardware front I had to study and find out the methods of storing data inputs through online and preparation of reports in the backend for further research and development, front end reports for the users. It was decided by me to make a close source online programme which could be used online so as to avoid copying and redistribution of my programme. The above study also required testing and deployment optimization by the way of algorithmic efficiencies, resource allocation, virtualizing, terminal server testing, power management, data centre power optimization technique, operating system support and means of

storage and cloud computing. The expected outcome of my study will be a web-based decision tool for all green rating system so as to meet the demands of easy, faster, reliable and affordable tool which shall be used to plan and estimate the design of resource efficiency in order to boost green building growth in emerging markets and backend data for in-house research and development. My dream to balance the gap of consumables like water, electricity and for sustainability in rural and urban areas shall come true by adaptation of green building certification in urban area which will reduced the demand of water and electricity and which can be directed to the villages of India.

Tales of fixing the tails

“I don’t like this face mask. It looks very ugly on me”, rebelled Vedant, a selfie-conscious teen, while having a family evening stroll in the smoggy lawns of IIT Delhi.

“Papa, why everyone walks-with-these-masks”, exclaimed Aarav in rhyming style, while jumping in the bandwagon of curiosity with his elder brother.

“Look, my boy”, I started to explain, but was cut short immediately by The lady of the house, “No technical jargon, please”, pleaded the student of literature.

“OK”, I promised in order to earn a green nod from my lady. “So, these masks save us from the pollutants present in the air. Vehicles around us exhale many dangerous gases from their tails”, I said.

“Oh, like dragons fire from their mouths”, wondered the movie buff Aarav.

“Yes, but that will be more fitting for chimneys of factories”, I responded.

“Can’t we fix these polluting tails and appear again like humans on our evening walks?”, was the most difficult question of the day, by visibly upset Vedant.

He awakened the researcher inside me and I responded enthusiastically, “Yes, people are trying and I am also playing a part in the solution. My research is focussed on developing materials for alternate power sources that are non-polluting and efficient.” I guess, the discussion made their Mom conscious and turning around she signalled for concluding the walk and talk. Obviously, we obliged with silent gestures of continuing later.

On the way back home, I remembered many intense discussions held in our research scholars’ room on the delicate situation of our environment. Overdependence of human race on the fossil resources for the survival and the unsustainable growth has nearly depleted the rich coffers of earth, which took millions of years to deposit, within a century. Moreover, our hunger for energy intensive essentials and comforts of life are severely polluting the air, water and land systems. Till date, the extraction of energy from these non-renewable fossil resources has been very inefficient, like in thermal power plants (roughly 30%) and auto-mobile engines (20-30%). Nuclear and hydro power sources have their own complications, viz. safety concerns and disastrous ecological footprints. Undoubtedly, we need cleaner and efficient sources of power to extend our survival on earth. Solar cells seem to be rising on global scale but they too struggle with their efficiency issues.

Fuel cells and specifically, Solid Oxide Fuel Cells (SOFCs) are among the most promising candidates, which produce power very efficiently (Approx. 80%) and more importantly, they release only pure water vapours from their tails (exhaust), when run on hydrogen and oxygen. They have been successfully tested on all conventional fuels including gasoline, LPG, coal and

biogas and have the added advantage of efficient extraction of power from these fuels as compared to conventional routes.

“So, what you develop in your lab?”, thus started the questionnaire of big boy, as we entered in our lobby.

“OK, just settle down and listen”, I started to explain the partial intricacy of my graduation journey to our school boys. “You know it well, how much we are dependent on electricity in our daily lives but do you know how much power is lost midway before it reaches our home? It is more than one third. Even the vehicles around us burn their fuel very inefficiently and worse, we also get life threatening pollutants like NO_x, SO_x and particulates from them.

Now, just imagine a power producing device that has no moving parts, no irritating noises and is thin as a paper of sheet. Interestingly, this compact device have a very clean tail (say exhaust) giving out pure water vapours. And it is not even like your gadgets’ battery that drain every now and then. As long as you supply it the fuels (say gases), you can generate clean electric power silently. A small suitcase size unit of it can power our entire house, non-stop. It is called solid oxide fuel cell or SOFC, in short”

“Oh, is it so?”, spoke the surprise in his eyes. Vedant continued, “So, is that you make in your lab?”.

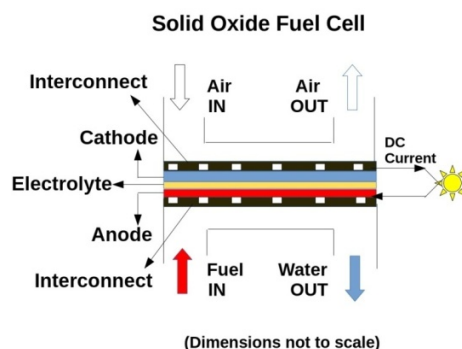
“Yes, we do that but partially. I mean, we develop and test the performance of some components of these solid oxide fuel cells”, I responded.

My response made him wonder, “Do you mean that this paper size fuel cell has many components, really?”.

“Of course, a single SOFC has a solid electrolyte sandwiched between two electrodes”, I told.

“Sandwich ! Oh yeah..hhh, my favourite”, shouted the younger one, making us laugh with his unwavering attention to catch possibly the only meaningful word that made sense to him.

“Look, this is a rough sketch of SOFC”, I drew on a paper, as shown below.



“To get some useful chunk of power, we need many cells because a single cell of one square centimetre area can generate only around 1 watt power. So, another component known as interconnect comes to our aid and depending on the requirement, we join multiple cells using these interconnects. Though, the electrolyte and electrodes of a SOFC are made up of ceramic materials (as they have to transport ions across them), the interconnects can be made using metals also, while operating at lower temperatures.”

“what do you mean by lower temperatures?”, interrupted Vedant.

“Oh, I forgot to tell you that latest generation SOFCs operate between 600 to 800 °C. Wait wait.., I got your ‘why’ signal. Because, the chemical reaction, rather the electro-chemical reaction (as it involves electrons) need that much temperature to take place. Actually, for this reaction, the ions need to be pushed across the reluctant solid electrolyte and thus, that much thermal push is necessary to overcome the ionic distaste of electrolyte.

Thus, single unit of SOFC is a layered structure consisted of an interconnect, anode, electrolyte and cathode, as you can see in the drawing. Combination of many such cells is known as the Cell Stack. Now, let me explain about my contribution in this context”, I set the pace.

“But papa, do we need some special fuel to run this device”, was the confusion of Vedant.

Also, I noticed that the younger one was already zooming in his dreams, so I tranquilized him with little heavier dose, “In ideal case, hydrogen gas on anode and oxygen gas on cathode will yield the maximum power output and pure water vapours from exhaust. But for practical purpose, air can be used at cathode side and many common fuels like biogas, syngas and gasoline can be efficiently utilized on anode side. Extra heat can also be put to use, if the situations demand”.

So, as I mentioned earlier, metals can also be used as interconnects. But, they face many restrictions in their selection. In addition to their good electrical conductivity, they must have matching thermal properties with other ceramic components and must be stable in both hydrogen and oxygen environments in such hot condition. My work is related to improve the properties of such metallic interconnects, so that the life and performance of SOFCs stack can be enhanced.”

“Hmmm..”, he nodded slowly.

“We fabricate specific grade stainless steel alloys and study their degradation behaviour under operating conditions of SOFCs. We have developed new and innovative routes to fabricate such alloys. Also, in our lab we test the interaction of these interconnects with cathode materials at high temperatures to look for their compatibility. And I can say that till now the results have been promising at lab scale”, I finished so and it was taken as my concluding gesture.

“Yes, I think that much is enough for today. Though, I can’t claim to understand everything you said but at least your ‘tales of fixing the tails’ seem interesting and are definitely part of the solution. I will read more about solid oxide fuel cells and get back to you with more doubts”, he hummed slowly and slipped into his bed swiftly.

“You people are still talking. Look at the clock! Switch off guys”, commanded the slumberous voice. Lights went off immediately.

Population and Ecology Symbiosis – Wetland, Macrophyte and Fish

The research story jotted below is a narration of a laboratory attempt to eradicate predicament of a fish population utilising the other entities in its own inhabiting environment. The work aims towards a transformation from harmful synthetic aqua-farming to sustained organic aqua-farming with an effort to reduce the discharge of toxic chemicals into the environment.

Aquaculture is one of the foremost and broadest economic sectors of India, contributing 1.1% to the total GDP and 10% to the total exports of the country which accounts for 6.3% of the global fish production. Wetlands are distinctive and awfully productive aquaculture ecosystems which are inundated by water permanently or seasonally. There are usually multiple numbers of flood plain wetlands in the lower delta of river Ganga, structured with hydric soil and harbouring unique flora and fauna. Parallely, this water bodies also bears ample responsibility for adverse environmental cause and impact linearity. Demands for high yield impels fisherman to use extensive synthetic chemicals which not only causes numerous pathogenic outbreak in wetland fisheries but also exerts detrimental health concern to producers as well consumers.

Holding this preview, the therapeutic traits of a well known wetland macrophyte *Ipomoea aquatica* Forssk. (Hindi: कलमी साग) was evaluated towards fin and tail rot disease of a cat fish, *Pangasius hypophthalmus*, commonly known as Pangus fish (Hindi: पंगास मछली).

The lyophilized and powdered leaf of *I.aquatica* was defatted and subjected to partition chromatography using organic solvents from low to high polarity. Finally the aqueous extract was obtained from the Methanol residue isolated from Chloroform : Water :: 4:1. The fractions were eluted through silica column and crystallized which was further flushed through Petroleum Ether : Chloroform 1:1.

The fish was obtained from Akaipur wetland, West Bengal. While still on stocking tank for acclimatization, the Pangus fish shoal was seen infected with fin and tail rot (पंख और पूंछ गलन). Though we had a preparation for fish challenge with different concentration of bacteria to optimise the LD50 but there could be no better experimental resource than the infected fish which

carried the infection right from its natural habitat. One of the most commonly seen disease symptoms rendering to high mortality in Indian fisheries is witnessed due to fin and tail rot which is largely caused by *Aeromonas* species viz; *A. hydrophila*, *A. veronii*, *A. popoffi* and sometimes by *Edwardsiella tarda* where again *A. hydrophila* mostly causes secondary infection by gas formation. These are gram-negative and mostly water-borne bacteria. In-order to confirm the bacterial strain, the infected parts were wiped with alcohol and tethered to streak on Muller Hilton Agar (MHA) plates followed by 16S rRNA sequencing which confirmed it to be *A.veronii*. Before going into In-Vivo assay, disc diffusion assay was performed on MHA plates where a



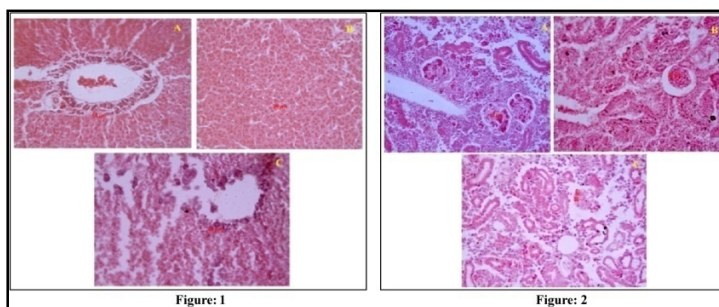
zone of inhibition of 14 mm was obtained (T₁).

The crystals obtained from Ipomoea leaves were hygroscopic in nature which aided to blending with commercial fish feed using egg white as binder and fed twice a day for seven days. A control group of infected fish was maintained which was fed with normal feed with egg white. Initially the results were very much promising but in latter days it did not show any curative

effects and the overall experiment to use *I.aquatica* leaves as a therapeutic source was not satisfactory or rather unsuccessful. Literature survey had plentiful studies on the prophylaxis trait of Ipomoea against cancer and some other diseases. Hence a remake of the previous experiment was deliberated in the simplest way possible to check its prophylaxis property instead of therapeutics, against fish disease. Fresh fish were segregated into two sets, one fed with the Plant sample + feed + egg white and the other group fed with normal feed + egg white each for 14 days then both the groups challenged intraperitoneal (IP) with the previously isolated and freshly sub-cultured bacteria (0.5 McFarland; 10µl/gm body weight) using insulin injection. The results were inferred with fresh uninfected fish.

Technically better results were observed than the previous trial and the clinical symptoms for the disease appeared after almost 17days for the first group fed with plant sample as compared to 54hrs 100% mortality for the second group. To understand the differences better, the liver and kidney histopathology of the normal, infected fish fed with feed + Plant sample and infected fish fed with normal feed was compared in Haematoxylin eosin stain after 54 hrs.

The liver histopathology (Figure: 1) reveals the normal liver section with intact portal triad, distinct bile duct and proportionate hepatocyte and pancerocyte (A); the infected



fish fed with plant material had almost the same histopathology like the normal with only exception of cytoplasmic vacuoles (B); the infected fish with normal feed showed disintegrated portal triad, disintegrated hepatocyte and (C) dissociation of the hepatocyte & pancerocyte.

The kidney histopathology (Figure: 2) reveals the normal kidney section with intact Bowman's capsule with Glomerulus with intact proximal tubules (A); the infected fish fed with plant material also was seen with prominent Bowman's capsule but some tubules were seen dilated (B); the infected fish with normal feed (C) showed disintegrated Bowman's capsule and the sloughing of the epithelial lining of the proximal and distal tubules.

The economical affairs of these researches are fairly cheap and within the periphery of the fish farmers which can serve as a convenient solution of problems faced by them or at least lend them some time to treat the diseased fishes by delaying natural mortality. We have also taken up three more floodplain wetland macrophytes in this aspect and expecting to find further worth mentioning results. Way forward to participatory rural appraisal towards efficacy of aquatic macrophytes in fish health.

The research is financed by Women Scientist Scheme (DST WOS-A), Department of Science & Technology, Govt. Of India and the research team consists of myself (Nabanita Chakraborty, DST WOS-A & PhD Scholar, ICAR-CIFRI, Barrackpore), Dr. Archan Kanti Das (Principal Scientist, ICAR – CIFRI, Barrackpore), Dr. Basudev Mandal (Assistant Professor, Vidyasagar University, WB), Dr. Ranjan Kumar Manna (Principal Scientist, ICAR – CIFRI, Barrackpore) and Dr. Basanta Kumar Das (Principal Scientist & Director, ICAR – CIFRI, Barrackpore).