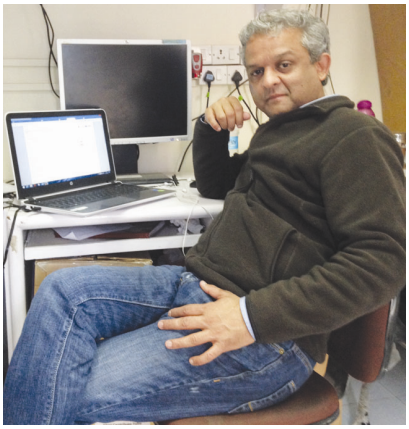
Amit Sharma



Malaria has been engaging the attention of scientists for many decades now. But some scientists have crossed these realms of uncertainty by identifying novel drug targets to curl increasing incidences of drug-resistance. Amit Sharma is a structural biologist whose passion is to understand the molecular function of malarial parasite proteins and highlight their structural features, thus leading to the discovery of structure-based inhibitors and for identifying specific motifs involved in protein–protein interaction in parasites, especially in *Plasmodium falciparum*.

Sharma completed his B S at Purdue University, USA and Ph D in protein crystallography (1995) from Northwestern University, USA. He then worked as a postdoctoral fellow in the area of structural biology (1995–2000), at The Institute of Molecular Medicine, Oxford University, UK. Later, he joined the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, as a Wellcome Trust International Senior Research Fellow, going on to become the Head of the Structural and Functional Biology Group at ICGEB in 2004. He is a recipient of many prestigious awards, including the Shanti Swarup Bhatnagar Award in Biological Sciences, B.M. Birla Science Prize and the Ranbaxy Research Award in the field of Medical Sciences.

In this interface with *Current Science*, he talked about his research interests, traversing the implications of malarial invasion in the body, malarial proteins and their characteristics, his take on Youyou Tu's achievements, malarial eradication and his vision for the near future.

Congratulations Dr Amit Sharma on being awarded the Infosys Prize 2015. Can you briefly describe your award-winning research? What motivated you to pursue it?

My laboratory focuses on unravelling the structural underpinnings of malaria parasite protein motors. Over the past 15 years, we have focused on dissecting key structural attributes of parasite proteins, with the overall aim of understanding and exploring them for target and drug discovery. Our work is necessarily multidisciplinary in nature, and we rely heavily on the latest tools in bioinformatics, biochemistry, cell biology, drug dockings and structural biology. We also have strong and enriching internal as well as external collaborations with high-quality researchers. Hence, the laboratory has been able to contribute towards the atomic mapping of several key parasitic proteins, which we hope will provide platforms for anti-malarial drug development.

My motivation has always been to discover and highlight the molecular bases of life, and it is inspired by the Darwinian understanding of modern biology. My intellectual position of anti-vitalism is fully satisfied by my work on understanding the atomic structures of proteins that drive cellular processes and life associated with them.

Can you explain the mechanism most malarial parasites use to invade the liver and red blood cells?

Malarial parasites have evolved specific protein families to enable them to recognize and enter host cells like hepatocytes and erythrocytes. Once the parasites are injected into host blood stream via bite of an infected mosquito, they rapidly zero into liver cells, where using several novel parasite proteins, they are able to attach and then enter into liver cells using receptors expressed on host liver cells. While many details of the liver-stage parasite proteins that enable this colonization remain unclear, it is evident that parasites like many other pathogenic organisms, hijack host receptor molecules for their own entry and survival. In case of *P. falciparum*, once the parasites exit the liver they re-enter the blood stream and then proceed to attach and invade erythrocytes. This process is critical for malaria parasites and for malaria control, as malaria-associated symptoms are linked to blood stage infection. Once again, these parasites have evolved specific protein families that recognize host red blood cell receptors and exploit them to allow entry of the parasites. In both contexts of liver and red blood cell infection, the parasite proteins involved are critical and hence the subject of intense investigation for anti-malarial therapeutic development.

What are the specific characteristics of key malarial proteins which can aid in their distinctive recognition?

A common feature of many *P. falciparum* proteins is their richness of specific amino acids like asparagine, and extensive sequence insertions in their protein domains. Although the structural, biochemical or pathogenic signficance of this richness in asparagines is still under investigation, insertions of specific sequence elements have been studied and shown to confer unique enzymatic attributes in some cases. The down side of these sequence pecularities is that *P. falciparum* proteins tend to be difficult to produce recombinantly for molecular biology studies. Another special feature of several malaria proteins is that they tend to utilize the same structural fold repeatedly for recognizing different receptors. This occurs simply by tinkering with the amino acid sequences, enough to alter biomolecular specificity but not the 3D architecture. We know that three-dimensional fold space is more conserved in evolution than primary sequence, and the parasite utilizes this fundamental facet to the fullest. It is akin to changing the paint on the walls in a house every so often, without compromising on the overall structure of the house.

Can you explain in detail the structure–function relationships for crucial parasite proteins? What is the mechanism with which some of these proteins cause DNA compactions and how do these interactions cause nucleosome assembly or disassembly?

Structure–function relationships refer to correlates in which biochemical attributes of a biomolecular machine can

be assigned to its three dimensional structure. For example, we had earlier shown how one of the *P. falciparum* nucleosome assembly proteins can specifically interact with linear sequence motifs within histones during the process of chromatin assembly. We had also drawn conclusions about the exclusive residency of either histones or DNA on these nucleosome assembly proteins, implying that either histones or DNA can be bound to them at a given time. These functional insights arose from structure–function correlates drawn from both biochemical and crystallographic data.

DNA replicates more than once at several stages in malaria parasites like *P. falciparum*, including when the parasite invades human liver cells, during blood stage infection, following fertilization and in oocysts during sporozoite formation. The parasite microgametocyte undergoes successive rounds of replication within minutes, raising its DNA content to octoploid levels before exflagellation. This rate of genome duplication is among the highest observed in eukaryotes. *P. falciparum*, like any other eukaryote, packages its DNA as a condensed DNA–protein complex called chromatin, where the repeating unit is a nucleosome. Both histone and DNA come together in an assembly to form nucleosome, and this process requires nucleosome assembly proteins. We have extensively characterized the two versions of nucleosome assembly proteins in *P. falciparum* over the years. *P. falciparum* chromatin (built of nucleosomes) is architecturally highly organized, and allows for clever regulation of its pathogenic genes. As an example, the 60-odd members of the *var* gene family drive antigenic variation and are central to pathogenic mechanisms that contribute to severe malaria. Our work on nucleosome assembly proteins highlighted the key roles played by two versions of these histone chaperones for the parasite. Importance of these proteins is underscored by attempted gene knockouts that indicated their essentiality and hence value for designing anti-malarial small-molecule inhibitors that focus on nucleosome assembly.

Why did you specifically choose Plasmodium falciparum for study, with Plasmodium vivax being just as detrimental?

Between *P. falciparum* and *P. vivax*, the former causes almost all malaria-associated deaths. Hence, the biomedical significance of *P. falciparum* is overwhelming. However, *P. vivax* is also a cause of morbidity in malaria endemic countries, and we now know that it can cause severe malaria-like symptoms. We have recognized over the past ten years that focusing on enzyme families which are essentially identical between these two human malaria parasites may allow easy co-opting of drug development efforts targeted at any one of them. Hence, our present theme of inhibiting protein synthesis as an avenue to kill parasites is equally valid for both (indeed possibly all five) human malaria parasites.

Can you elaborate on the relevance of tRNA synthetases in complex with inhibitors with respect to P. falciparum?

In 2009, we published the first report on the opportunity presented by pathogen-derived tRNA synthetases as new drug targets. Over the past six years, we and other laboratories worldwide have now validated this idea, and we currently have several promising anti-parasitic compounds that work via inhibition of pathogen-encoded tRNA synthetases. Our group has additionally solved several key structures of complexes between tRNA synthetases bound to these inhibitors. These studies have fuelled work on further derivatization of these inhibitors as future leads for potential drugs. It is noteworthy that given the extensive structure–function detailing of active sites of *P. falciparum* tRNA synthetases, their inhibitors are as likely to work against *P. vivax* as well.

How can blocking the charging of amino acids by tRNA synthetases inhibit cell growth in human pathogens?

Protein synthesis is a vital cellular process in all biological life and it relies crucially on several distinct biomolecular motors, including on tRNA synthetases. Each organism must have its full complement of (usually at least 20) tRNA synthetases for each of the twenty amino acids which constitute most proteins. Absence of even one tRNA synthetase, or blockage of its enzymatic action can hence abrogate growth via blocking protein translation on the whole. We and other researchers have identified several sets of compounds which specifically inhibit pathogen tRNA synthetase (as opposed to the host enzyme), and these provide a base for development of a new generation of anti-pathogenics which target this ubiquitous protein family. There is currently tremendous interest in utilizing tRNA synthetase inhibitors against tropical infectious diseases like malaria and back in a 2009 publication, we were the first group to highlight this family as a new focus for drug development. The field has moved rapidly over the past six years, and I expect several new tRNA synthetases to be targeted in the coming years.

What challenges did you face while purifying and crystallizing proteins from P. falciparum and how did you overcome them? Are the aforementioned challenges common for other species of Plasmodium?

Because of peculiar amino-acid distributions as well as a tendency to have long sequence insertions, many *P. falciparum* proteins have been notoriously difficult to produce recombinantly. We have managed with very poor protein production efficiencies, in some cases developed new protocols for protein re-folding, and tweaked around with several sets of recombinant systems to address these problems. Some of these problems are common within the *Plasmodium* genus, and only perseverance has allowed us to overcome the challenges.

Please share your opinion on malarial therapeutics with respect to Nobel laureate Youyou Tu's work. What is the status of India in developing significant drugs which can counter malaria? According to you, how long a time span would be required to eradicate malaria?

Dr Youyou's contributions are stupendous, groundbreaking and invaluable. My opinion on Youyou's work and achievements is that her work has paved the way for others to follow – on distilling traditional medicine clues into modern medicine realities. Youyou and her team's achievements have led to saving of million of lives, and she is a shining example of translational biology work. Indeed India, in its journey to exploit traditional medicine for modern therapeutic usage, will do well to follow her path.

Drug development is a strategic commitment from multiple stakeholders,

including academic researchers, pharmaceutical/biotech sectors and the government. In particular, it is an expensive business to initiate and complete, and requires resources and commitment beyond the usual 3–5 year research grant windows. In this context, the Indian community of malaria parasite researchers has contributed tremendous body of data that can be exploited by pharmaceutical partners/biotech companies for lead development. We hope that sustained funding from both government and possibly private sector within India will enable eventual development of an Indian antimalarial drug that is based on a new scaffold, or one that targets a novel pathway.

Eradication of an infectious disease is not the same as its elimination. In most cases, the correct reference frame is therefore of elimination and not eradication (which refers to freedom from disease globally, and for which the only successful example in the context of human health is smallpox). Elimination is a far more realistic goal that aims to reduce disease burden to near zero within geographical boundaries. For malaria, the feasible goals are determined on a country-to-country basis, and rely on each country to fullfil malaria control guidelines, such as implementation of insecticide-lined bednets, indoor residual insecticide spraying, rapid diagnotics and artemisinin-combination-based therapies. Indeed, it is evident that especially since 2000, malaria elimination has been a highly successful international programme, and it has saved close to six million lives already. Malaria elimination targets require multiple stakeholders, including the community, health professionals, city planners, government resources, international teams and most importantly, an adherence to the proposed guidelines. The world is indeed seeing fewer malaria-related deaths since 2000, and the number infected is also expected to further reduce compared to 2000. These efforts need to be sustained going forward, keeping in view the insidious threat of drug and insecticide resistance. In addition, it may be that malaria elimination is not feasible in some areas due to geographical or climactic factors. In these scenarios, effective malaria control measures become even more vital. Finally, malaria cases negatively correlate with wherewithal and so a genuinely developed egalitarian nation should either have very little or no indigenous malaria.

What is your research vision for the future and your message to young researchers?

A natural extension of our work is to apply the lessons learnt from drug target identification, elaboration and exploitation in the context of malarial parasites to other eukaryotic pathogens like *Toxoplasma gondii*, *Leishmania*, *Trypanosoma* and others. We have already initiated work along these lines and we hope to contribute to drug discovery against neglected tropical diseases too. The latest 'black box' in modern biology is the host microbiome that brings with it 10× more cells than human cells, and 100× more genetic material than the human genome. So, another highly exciting theme we have initiated in the laboratory is to catalogue the host microbiome in the context of various disease states. This new focus is both challenging and strategic, and it reflects my continued interest in unravelling the molecular basis of life. The microbiome is the new goldmine for biomolecules that drive life and disease.

I think young researchers worldwide are much smarter and wiser. They may do best without messages from our generation.

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