

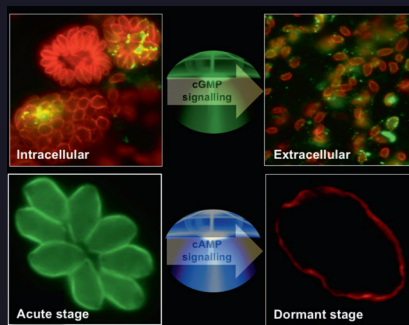
# Host-pathogen interplay in the limelight



Parasite vs Host

- ★ Optogenetics is established as a valuable tool in neurobiology, now researchers at Humboldt University in Berlin are striving to apply it in infection biology. This work will shed new light on intracellular pathogens, giving researchers new insights into pathogen-host interactions, as **Dr Nishith Gupta** explains

**The development of** optogenetics has opened up new possibilities in biological research, enabling scientists to use light to selectively turn specific cellular pathways on or off, and then assess the physiological response. However, while optogenetics has been widely applied in neurosciences, this is not yet the case in infection biology, something which Dr Nishith Gupta and his colleagues at Humboldt University in Berlin aim to change. "Having pioneered the applications of optogenetics, our goal now is to consolidate them for investigating the biology of intracellular pathogens and pathogen-host interactions," he says. This research involves the application of light to activate or deactivate chosen cellular pathways. "We do this by deploying light-regulated proteins in transgenic cells," explains Dr Gupta. "We engineer photo-responsive parasites or host cells, and switch the system by illuminating them with LEDs of specific colours, and then study the resulting phenomena." With a similar approach involving compatible gene-encoded biosensors, Dr Gupta's group can also monitor the dynamic oscillations of the subcellular signalling mediators and metabolites.



Images show activation of cyclic GMP and cyclic AMP signalling in *Toxoplasma gondii* by light exposure, which in turn cause the parasites to exit host cells (top panel), or differentiate into dormant stage (bottom panel).

## Illuminating parasites

Dr Gupta's team is applying this approach to investigate three parasites in particular, *Toxoplasma*, *Plasmodium* and *Eimeria*, all belonging to the protozoan phylum apicomplexa. These unicellular parasites are obligate intracellular pathogens, hence cannot survive without a host cell. Dr Gupta is looking at parasite-host interactions in a molecular context. "We have the host cell, and we have a parasite living inside that host cell. It is a complex intertwined system, where the challenge is to regulate

both entities independently of each other. We're investigating how the parasite co-opts, subverts, or modulates, the host cell, and also how it uses its own metabolic designs and signalling pathways to survive," he outlines. Some parasites, such as *Toxoplasma*, can cause either acute or chronic infection. "In acute infection, a parasite multiplies and kills the tissue," says Dr Gupta. "In chronic infection, the parasite hides within infected host cells, and the immune response cannot see it, so the parasite can live for a long time waiting for the right moment - that is, a decline in host immunity, when it can switch back to the acute stage."

The goal for the parasite is to replicate, or to undergo a dormant stage, so that it can survive for long periods before transmitting further. When a host organism is infected, the parasite has to adapt to a changing environment to ensure its continued existence. "It's constantly responding to environmental cues, and manipulating host-cell machinery," explains Dr Gupta. The strategic survival of the parasite is a topic of great interest to Dr Gupta, and lies at the heart of his group's research. "We would like to understand the molecular mechanisms for the reproduction,

adaptation, persistence and transmission of the parasite," he continues. "Once we know them better, we can then look forward to target underlying pathways to prevent or cure infections."

A central topic in research at this point is the acute cycle of a parasite, which typically involves invading a host cell, replicating inside it, and then exiting it by lysis. Calcium-, cyclic GMP-, cyclic AMP- and lipid-mediated-signalling play major roles in controlling the acute cycle. "Using optogenetic tools, we can activate or repress cyclic AMP or cyclic GMP signalling by light. Likewise, we can monitor calcium, cyclic AMP, cyclic GMP or lipids using gene-encoded biosensors," says Dr Gupta. Such light-sensitive proteins can be engineered in a parasite (or host cell), from which more can be learnt about the pathogen-host interactions. "We activate

being the natural victim of cancer."

A parasite's ultimate aim is to survive and reproduce, similarly to cancer cells. The point where the comparison seems to fall down is in the inter-host transmission, yet Dr Gupta says that recent research clearly shows that some forms of cancer have in fact evolved to transmit between hosts. "A series of very interesting high-profile original articles have been published over the last decade, and 3-4 cancer types that can transmit from 'infected' to healthy individuals have been found in nature," he describes. The scenario is intriguing as well as frightening at the same time. The parallels between cancer and parasite biology will be an important part of Dr Gupta's agenda in future, work which has important implications, from the laboratory bench to the hospital bedside.

The research group of **Dr Nishith Gupta** continues to play a prominent role in expanding our **understanding of intracellular parasitism and beyond**. He believes there is a pressing need to introduce innovative technologies into **parasitology** and bridge it with **other disciplines**.

signalling by light, and then look at which proteins downstream are (de)phosphorylated, and/or which genes are modulated. Once we've identified those mediators, we can then knock them out and see what happens to the parasite. The optogenetic approach becomes even more enlightening when combined with reverse genetics and pharmacological modulators," adds Dr Gupta.

### Bridging with cancer biology

The group is also involved in other strands of research, including exploring parallels between parasites and cancer cells. While parasitology and cancer biology are typically thought of as entirely separate areas of research, Dr Gupta says a convergence between them can be drawn. "When we look at which metabolic and immune pathways contribute to the growth of cancer cells, there are a lot of conceptual similarities between parasites and cancer cells. We would like to decipher these equivalences in molecular terms," he states. There are two ways to discern this, the first of which involves thinking of cancer cells as being a kind of new parasite species. "They also eat up your body, inside-out; they make use of certain metabolic pathways to fuel their growth; and they have developed ingenious strategies to dodge the immune system," explains Dr Gupta. "Alternatively, we can think of parasites as

### Going translational

While Dr Gupta continues to pursue further basic research in the aforementioned areas, his team is also seeking translational applications, which range from diagnostics to treatment and prevention. In the course of their research, Dr Gupta and his colleagues sometimes find that a particular enzyme or metabolite is used by a parasite but absent in humans, which could open up new possibilities in diagnosis. "We could potentially use those enzymes or metabolites as biomarkers to detect parasitic infections," he says. Another translational possibility arising from research is cases where a parasite cannot survive without a particular gene product essential for reproduction, which would provide an excellent drug target. "We can collaborate with chemists to synthesise certain chemicals that can selectively inhibit that essential protein and thereby kill the parasite," continues Dr Gupta. "Another aspect is prevention and prophylaxis. We can construct the parasite mutants that are heavily attenuated in their growth and virulence but potent enough in eliciting the immune response against challenging infections, so we can generate metabolically-attenuated vaccines." Future clinical developments will be based on this kind of fundamental research.

## OPTOBIOLOGY IN INFECTION

### Exposing pathogen-host intimacy by light

#### Project Objectives

Dr Gupta's group studies the survival strategies of a eukaryotic cell inhabiting another eukaryotic cell. Specifically, he has been investigating the metabolic interactions between intracellular parasites (namely *Toxoplasma*, *Eimeria*, *Plasmodium*) and mammalian host cells. The main objectives are to reveal the metabolic determinants and signalling mediators that underlie successful reproduction, adaptation and pathogenesis of these pathogens. His group has also pioneered the application of optogenetics in entwined models, particularly to study the cyclic nucleotides and calcium signalling in intracellular pathogens.

#### Project Funding

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Dr Nishith Gupta



**Dr Gupta** is currently working as a Heisenberg Research Group Leader in Berlin (Germany). He obtained his MSc in Biotechnology from Banaras Hindu University (India), and subsequently completed his PhD in Biochemistry from the University of Leipzig (Germany). He then undertook a postdoctoral training at the National Jewish Medical Research Centre in Denver (USA), followed by Habilitation in Biochemistry at the Humboldt University of Berlin.

