Name	TONY HOLDER
Position	Former Senior Group Leader, now Visiting Scientist (Group closed Dec 2020)
Year joined *(Crick or founder institute)*	1998

Career History

1969-1972: University of East Anglia – BSc (Hons) Biological Sciences
1972-1975: University of Leeds – PhD in Genetics
1975-1978: Department of Plant Physiology, The Carlsberg Laboratory, Copenhagen, Denmark
1978-1988: Senior Scientist, Dept of Immunochemistry/Molecular Biology, The Wellcome Research Laboratories, Beckenham, Kent
1988-2015: Head, Division of Parasitology, MRC National Institute for Medical Research, Mill Hill, London

Major Awards, Honours and Prizes

Membership of external committees, editorial boards, review panels, SABs etc

2011-2016: Wellcome Trust Expert Review Group – Pathogen biology and disease transmission (Chair from 2014)
1983- present: Editorial board Molecular and Biochemical Parasitology (Managing Editor 1994-2000)
2019: Joint organiser of Biochemical Society Focused Meeting on BMP signalling in Cancer II, Oxford UK

Lab Name	***Malaria Parasitology Laboratory***

Research programme and achievements

The principle focus of my research is to illuminate some of the cellular and molecular processes that underpin the intra-erythrocytic development of the human malaria parasite *Plasmodium falciparum,* and how it recognises and invades red blood cells. These fundamental studies underpin translational studies to develop new therapeutic approaches for malaria.

Studies on parasite ligand interaction with erythrocyte surface receptors have shown the key importance of certain protein complexes that are exported to the parasite surface during erythrocyte recognition and invasion, where they participate in binding and establishing the junction between parasite and host cell, which is essential for invasion. Host antibodies can block these interactions, implicating these parasite proteins as candidates for malaria vaccine development.

Invasion is driven by an actomyosin motor (called the glideosome) located below the parasite plasma membrane and anchored in an unusual membranous structure of flattened vesicles called the inner membrane complex (IMC). We have recently

characterized the components of the glideosome and the IMC and provided some unique insights into their structure and function.

Our recent studies, employing chemical biology approaches, on the intracellular development have focused on the importance of protein post-translational modifications and their role in the transition between the different parasite stages.
We have shown N-myristoyl transferase (NMT) to be a validated target for small molecule inhibitors in work together with Ed Tate and others, which has led to considerable interest from pharma and Medicines for Malaria Venture. Structure-function studies of the enzyme and inhibitors, coupled with genetic studies in the parasite have revealed the on-target specificity and mechanism of action of small molecule inhibitors. These molecular tools, together with recent advances in genetic manipulation of the parasite, have enable us to dissect the importance of individual NMT substrates. For example, myristoylation of GAP45, a key component of the glideosome that powers parasite motility and cell invasion, is not required for glideosome assembly but is essential for its function.

We have shown that the transition between intra-erythrocytic and extracellular phases of parasite development is accompanied by massive changes in ubiquitylation of many cellular proteins, such as components of the IMC. In the first step to understand the importance of this protein modification for the parasite we have shown that ubiquitin activating enzyme is essential for parasite development, using specific small molecule probes and genetic tools.

Together with Rita Tewari in Nottingham, we have begun to dissect aspects of mitosis and meiosis throughout the parasite life cycle, which display a number of unusual features. For example, during intra-erythrocytic development the haploid parasite undergoes asexual replication via closed mitosis and nuclear division to form a syncitium, followed by cytokinesis at the end of the cycle; during male gametogenesis, DNA replication, endoreduplication, karyokinesis and gamete budding from haploid gametocyte to eight flagellate male gametes occurs within 15 minutes. With physicians at University College Hospital, Ibadan, Nigeria, we have established the ‘Childhood Malaria Research Group’ to bring our scientific approaches to study severe disease, particularly severe childhood malaria anaemia. The first studies have now been published.

Research outputs

Green, J. L., Wu, Y., Encheva, V., Lasonder, E., Prommaban, A., Kunzelmann, S., Christodoulou, E., Grainger, M., Truongvan, N., Bothe, S., Sharma, V., Song, W., Pinzuti, I., Uthaipibull, C., Srichairatanakool, S., Birault, V., Langsley, G., Schindelin, H., Stieglitz, B., Snijders, A. P., and Holder, A. A. (2020) ***Ubiquitin activation is essential for schizont maturation in Plasmodium falciparum blood-stage development.*** **PLoS Pathog 16, e1008640. DOI: 10.1371/journal.ppat.1008640**
This study describes the ubiquitome of several stages of the intra-erythrocytic development and extracellular stage of the malaria parasite in the blood stream. It highlights the remarkable changes in ubiquitylation that occur and a number of very interesting substrates. Using a chemical biology approach we show the importance of the first step in the pathway and the consequences of its inhibition during intra-erythrocytic development.

Schlott, A. C., Mayclin, S., Reers, A. R., Coburn-Flynn, O., Bell, A. S., Green, J., Knuepfer, E., Charter, D., Bonnert, R., Campo, B., Burrows, J., Lyons-Abbott, S., Staker, B. L., Chung, C. W., Myler, P. J., Fidock, D. A., Tate, E. W., and Holder, A. A. (2019) ***Structure-Guided Identification of Resistance Breaking Antimalarial N-Myristoyltransferase Inhibitors.*** **Cell Chem Biol 26, 991-1000 e1007. DOI: 10.1016/j.chembiol.2019.03.015**

This study defines the interaction of NMT-inhibitors with the enzyme and the importance of an amino acid substitution in the active site for resistance to inhibition. These structure-function studies are complemented with genetic modification of the parasite to establish on-target specificity and mode of action of the inhibitors.

Knuepfer, E., Wright, K. E., Kumar Prajapati, S., Rawlinson, T. A., Mohring, F., Koch, M., Lyth, O. R., Howell, S. A., Villasis, E., Snijders, A. P., Moon, R. W., Draper, S. J., Rosanas-Urgell, A., Higgins, M. K., Baum, J., and Holder, A. A. (2019) *Divergent roles for the RH5 complex components, CyRPA and RIPR in human-infective malaria parasites.* PLoS Pathog 15, e1007809. DOI: **10.1371/journal.ppat.1007809**
A novel protein complex, essential for parasite recognition and binding of host erythrocytes is described. We recently developed *Plasmodium knowlesi*, a zoonotic infection, as a convenient *in vitro* system in which genetic manipulation can be performed easily; this study uses this system to understand parasite invasion, and is particularly relevant to the most prevalent human malaria parasite, *Plasmodium vivax,* which is refractory to study *in vitro*.

Green, J. L., Wall, R. J., Vahokoski, J., Yusuf, N. A., Ridzuan, M. A. M., Stanway, R. R., Stock, J., Knuepfer, E., Brady, D., Martin, S. R., Howell, S. A., Pires, I. P., Moon, R. W., Molloy, J. E., Kursula, I., Tewari, R., and Holder, A. A. (2017) *Compositional and expression analyses of the glideosome during the Plasmodium life cycle reveal an additional myosin light chain required for maximum motility.* J Biol Chem 292, 17857-17875. DOI: 10.1074/jbc.M117.802769
Parasite motility and cell invasion is driven by an actomyosin motor called the glideosome. This study characterizes the glideosome, identifies some new components and describes functional studies on the unusual type XIV myosin that is at its heart.

Moon, R. W., Sharaf, H., Hastings, C. H., Ho, Y. S., Nair, M. B., Rchiad, Z., Knuepfer, E., Ramaprasad, A., Mohring, F., Amir, A., Yusuf, N. A., Hall, J., Almond, N., Lau, Y. L., Pain, A., Blackman, M. J., and Holder, A. A. (2016) *Normocyte-binding protein required for human erythrocyte invasion by the zoonotic malaria parasite Plasmodium knowlesi.* Proc Nat Acad Sci, USA 113, 7231-7236. DOI: **10.1073/pnas.1522469113**
We identify a single protein that is essential for the recognition and binding of human erythrocytes during invasion by this zoonotic parasite. As malaria control measures in South East Asia largely continue to be successful against *Plasmodium falciparum,* targeting *P.knowlesi* becomes increasingly important. The study points to a protein that is a candidate antigen for vaccine development.