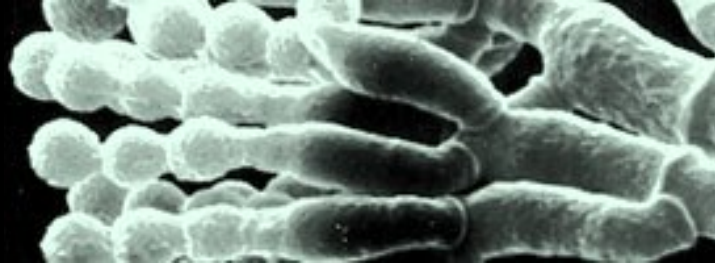
MiSAC matters Articles



An introduction to molecular biology approaches in parasitic protozoa:

3. Visualising proteins in protozoan parasites

Tansy Hammarton

Proteins are the workers of cells, carrying out a multitude of tasks to keep the cell alive and healthy. Previous articles in this series (MiSAC Articles *An introduction to molecular biology approaches in parasitic protozoa: 1. Investigating gene function in protozoan parasites – general approaches and challenges* and *2. Molecular genetic methods for analysing gene function in protozoan parasites*) have addressed how scientists can study protein function in protozoan parasites by using molecular biology to knockout, knockdown or overexpress the protein or its corresponding gene. Valuable insights into protein function can also be gained by viewing the protein within a parasite. Visualising proteins allows scientists to study where in the parasite the protein of interest (POI) is found, and whether its level of expression or its localisation changes over the life or cell division cycle of the parasite, or under different environmental conditions, which can provide clues as to its function (Fig. 1). For example, a protein found in the nucleus might play a role in gene transcription, or, if it is only expressed during cell division and localises to the mitotic spindle, might be expected to contribute to chromosome segregation at mitosis. Alternatively, a protein localising to the mitochondrion or Golgi apparatus might play a role in energy generation or protein trafficking, respectively. If a protein is only expressed when the parasite is exposed to stressful conditions (such as increased temperature or pH, or when nutrients are low), that protein might be involved in stress responses that help the parasite survive unfavourable conditions.

Visualising a POI also enables scientists to observe the effects of mutating it. By changing the sequence of the gene encoding it, it is possible to change specific amino acids within a POI or to delete a particular region of the POI. A mutation might interfere with the protein's activity, stability, localisation within the cell or ability to bind to other proteins, for example. Being able to detect the protein in the parasite can show whether its abundance or localisation has changed as a result of the mutation. This article will look at several methods for visualising proteins in parasites.

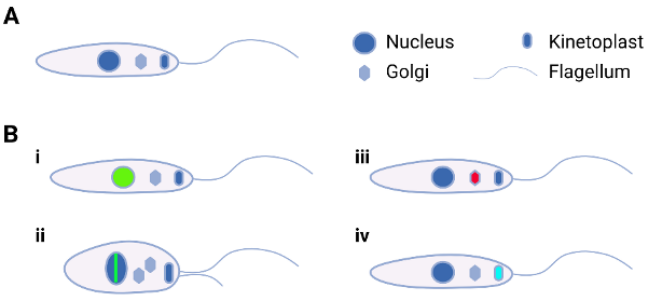


Figure 1. Visualising proteins.

A. Schematic of a protozoan parasite e.g., *Leishmania*. Some single copy organelles (nucleus, kinetoplast (containing the mitochondrial DNA), Golgi and flagellum) are shown.

B. Labelling proteins with e.g., a fluorescent protein or antibody, allows them to be visualised within the parasite cell. Fluorescent proteins come in different colours e.g., green (i and ii), red (iii) or cyan (iv). Sometimes the localisation of a protein gives clues as to its function e.g., a protein present in the nucleus (i) might be involved in gene transcription while a protein that only appears in the nucleus during cell division and appears to localise to the mitotic spindle (ii) might be required for dividing the DNA. Alternatively, a protein that localises to the Golgi (iii) could play a role in protein trafficking within the cell and a protein localising to the kinetoplast (iv) might be involved in packaging its DNA, while a protein localising to the mitochondrion more generally might be required for energy generation.

Created with BioRender.com.

This article is one of a number of MiSAC *matters Articles*, written by experts, which are up to date and relevant to microbiology in schools. MiSAC is grateful to all contributing authors'. E: microbe@misac.org.uk. W: *www.misac.org.uk.*

Immunofluorescence

Immunofluorescence (Fig. 2) is a technique that uses one or two antibodies to detect a POI. Chemicals are used to first fix (preserve) the parasite cell and make it permeable to allow antibodies to enter the cell. The fixed and permeabilised cells are then incubated with an antibody that specifically recognises and binds to the POI (the **primary antibody**), before any non-bound primary antibody is washed off. Sometimes, a fluorescent primary antibody is used, that can then be detected using fluorescence microscopy, but more commonly, the primary antibody is non-fluorescent and a fluorescent **secondary antibody** that recognises and binds to the primary antibody, is then added. Again, following a suitable incubation period, unbound antibody is washed off. Fluorescent antibodies are generated by linking a **fluorophore**, which fluoresces under UV light, to them. This fluorescence can be detected using a fluorescence microscope, which enables the location of the POI within the parasite to be visualised (Fig. 2A). Because more than one molecule of secondary antibody can bind to each molecule of primary antibody, using a secondary antibody amplifies the fluorescence signal, making it easier to visualise the POI.

It is also possible to visualise two or more proteins at the same time using immunofluorescence (Fig. 2B; known as **co-immunofluorescence**). Primary antibodies specific to each of the POIs, but which have been generated in a different animal species (e.g., rabbit versus sheep, mouse or chicken) are used. Then, secondary antibodies specific for each of the species and linked to different fluorophores are employed e.g., an anti-rabbit secondary with a green fluorophore plus an anti-sheep secondary with a red fluorophore. Thus, POI1 lights up in green, while POI2 lights up red when viewed under a fluorescence microscope. It is also possible to use primary antibodies that originate from the same animal species if the antibodies are of different subtypes and secondary antibodies specific for each antibody subtype are used, or if the primary antibodies are themselves each linked to a different fluorophore and used directly without secondary antibodies. Co-immunofluorescence can be used for colocalization studies – if POI1 and POI2 localisations overlap, it might indicate that the proteins are binding partners, although additional experiments (e.g., **immunoprecipitation** or **FRET**) need to be done to confirm direct interactions between two proteins. Co-immunofluorescence can also be used to determine whether a POI is present in a particular organelle e.g., the nucleus or the Golgi, by using antibodies against the POI and against a protein already known to be present in that organelle.

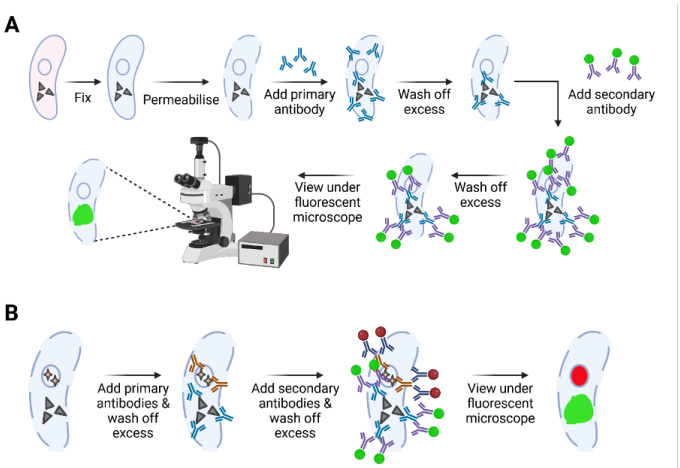


Figure 2. Immunofluorescence.

A. Schematic of the immunofluorescence method. The parasite is chemically fixed and permeabilised, which can either be performed in solution or on a microscope slide, before being incubated in a solution containing primary antibody that recognises the POI. Excess (unbound) primary antibody is then washed off before (usually) a secondary antibody is then added. The secondary antibody binds to the primary antibody and is conjugated (linked) to a fluorophore (green circles). After excess secondary antibody is washed off, the parasite is viewed under a fluorescent microscope and the location of the POI glows green.

B. Immunofluorescence can be performed for more than one POI at the same time (co-immunofluorescence). Following fixation and permeabilisation, the parasite is incubated in solutions of each primary antibody, which originate from different animal species, or are of different antibody subtypes. Excess primary antibody is washed off and the parasite cells are then incubated with secondary antibodies that are specific for antibodies from each animal species the primary antibodies originated from, or for the different subtypes of primary antibodies. Each secondary antibody is labelled with different fluorophores (red and green circles), allowing the location of each POI to be detected by fluorescence microscopy.

Created with BioRender.com

Although immunofluorescence is a valuable technique, it does have some drawbacks. Specific antibodies may not be available for the POI, and it can be a lengthy (3 months or more) and expensive process to generate such antibodies. A cheaper and, often, quicker alternative is to use molecular biology to attach a short peptide sequence (usually ~10 amino acids), called an **epitope tag,** to the POI (Fig. 3A). This allows the protein to be detected using commercially available antibodies that recognise the epitope tag following the steps outlined above. Many different proteins can be tagged with the same epitope in different cell lines and the same antibody can be used to detect each of the different proteins. However, sometimes adding an epitope tag can, despite its small size, interfere with the function, stability or localisation of a protein. It is good practice to compare the effects of epitope tagging at each end (N and C termini) of the POI, and/or to compare different types of epitope tags. Finally, immunofluorescence requires cells to be chemically fixed and permeabilised, which can sometimes introduce spurious results (or artefacts), so protein localisations should ideally be checked using more than one type of fixative.

Fluorescence tagging

An alternative approach that can be used in live (non-fixed) cells is **fluorescent tagging** (Fig. 3B). Here, molecular biology techniques are used to fuse the coding sequence for a fluorescent protein in frame to the gene of interest, resulting in the parasite producing a fluorescent version of the POI that can be visualised under a fluorescence microscope without the use of antibodies. This is a rapid and cheap technique for visualising proteins, which also allows the detection of the POI in real time, allowing its expression and localisation in the cell over time, or in response to changes in environmental conditions, or following incubation with a drug, to be monitored. The first fluorescent protein to be studied was green fluorescent protein (GFP), which was originally purified from the jellyfish, *Aequorea victoria.* The scientists Osamu Shimomura, Martin Chalfie and Roger Tsien shared the 2008 Nobel Prize in Chemistry for their work in discovering and developing GFP. Since its discovery, they and others have mutated GFP resulting in it fluorescing different colours; a red fluorescent protein (DsRed) from coral (*Discoma* spp.) was also discovered and subsequently mutated to generate new variants such as mRFP (monomeric red fluorescent protein) and mCherry and mStrawberry from the mFruit series (Figure 3C). The different fluorescent proteins not only differ in colour but also in brightness and stability under different conditions, allowing scientists to choose the most appropriate fluorescent protein for their application. However, the fluorescent proteins are quite big (typically ~28 kDa), and care has to be taken that they don't themselves alter the properties of the protein they are fused to. Again, the POI should ideally be tagged at each terminus and/or with different fluorescent tags, and results compared to improve the reliability of the data.

It is also worth noting that it is possible to detect fluorescent proteins in fixed cells by performing immunofluorescence using commercial antibodies that bind to the fluorescent tag.

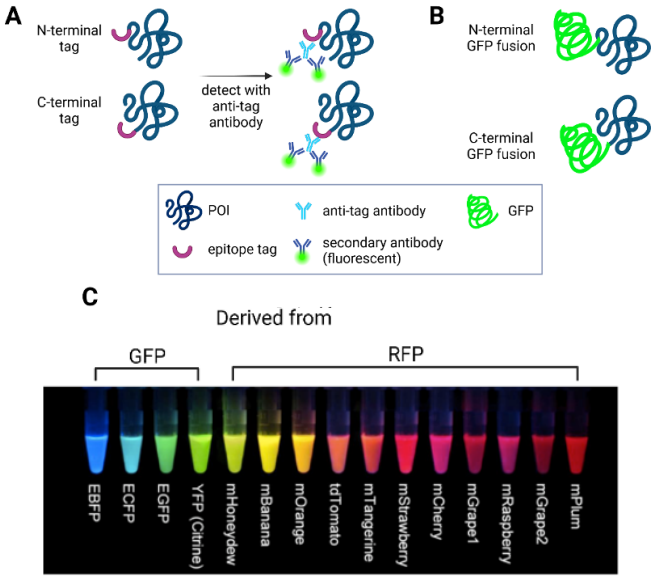


Figure 3. Protein tagging.

A. A short sequence of ~10 amino acids, known as an epitope tag, can be added to a POI using molecular genetic techniques. The tag may be added at either end of the protein, or even in the middle of the protein, and can be visualised by immunofluorescence of fixed and permeabilised cells using a commercially available antibody that recognises and binds to the epitope tag and a fluorescent secondary antibody that binds to the anti-tag antibody.

B. Alternatively, a fluorescent protein, such as green fluorescent protein (GFP), can be fused to the POI, again at either end, allowing direct visualisation of the POI in live cells. Note that GFP and other fluorescent proteins may be quite large in comparison with the POI being tagged.

C. A wide range of fluorescent proteins now exist. Some have been termed the mFruit series and have been developed by mutating either GFP or red fluorescent protein (RFP) to give them different spectral properties. Adapted from Roger Y. Tsien Nobel Lecture 2008.

Created with BioRender.com.

Case study: TrypTag project

TrypTag (http://tryptag.org) was an ambitious project that aimed to determine the localisation of >8700 protein-coding genes in the protozoan parasite *Trypanosoma brucei*, which causes sleeping sickness in humans and a related wasting disease, Nagana, in livestock in sub-Saharan Africa. As well as being an important human and animal pathogen, *T. brucei* is an early-branching eukaryote offering important insights into the evolution of eukaryotic cell biology. It also has multiple single-copy organelles (Fig. 4A) which replicate in a defined temporal and spatial order, and *T. brucei* is considered a model organism for the study of the biology of organelles such as the Golgi, basal body and flagellum in higher eukaryotes. Localising the majority of *T. brucei* proteins within the cell aimed to shed light on the protein composition and dynamics of individual organelles, as well as on any changes in protein localisation during the cell division cycle.

Attempts were made to tag 8721 proteins with a green fluorescent protein known as mNeonGreen. 89% (7,766) proteins were successfully tagged at at least one terminus, allowing their subcellular localisation to be determined and the protein composition of all organelles/structures to be determined (Billington *et al.*, 2023). Some proteins were found to localise differentially to either an old or a new organelle generated during the cell cycle, shedding light on the replication/synthesis of the organelles. Novel mitotic-spindle associated proteins were also identified, that were then shown to play important roles in mitosis, and >100 proteins were found to localise to the posterior cell tip, at least some of which may be important in abscission, the final stage of cytokinesis where the daughter cells finally separate. Such was the success of this project, that a sister project, LeishTag (http://www.leishtag.org/), is underway to study the localisation of proteins in the related parasite *Leishmania*, that either do not have an equivalent in *T. brucei* or are very divergent in sequence from their *T. brucei* counterpart.

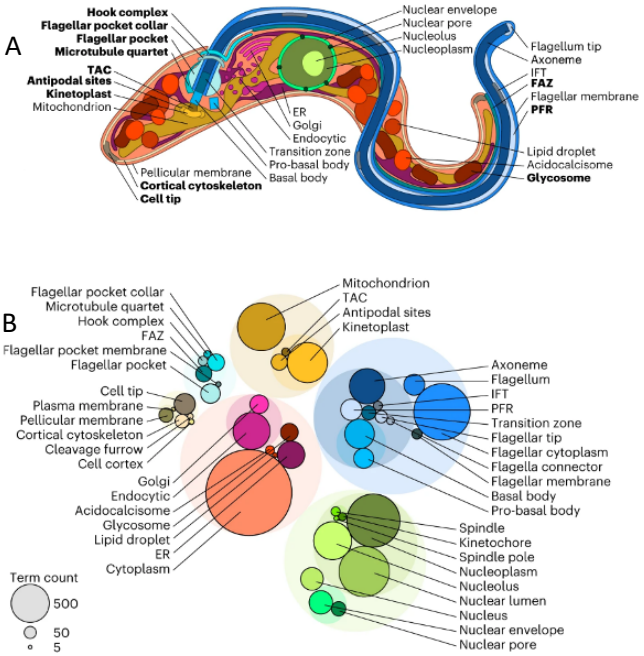


Figure 4. Data from the TrypTag project.

A. Cartoon of a procyclic (insect form) *Trypanosoma brucei* cell showing all of the organelles and structures. Structures in bold are those unique to, or particularly elaborate, in the *T. brucei* evolutionary lineage.

B. Depiction of the number of proteins found to localise to each compartment in the cell. The bigger the circle, the greater the number of proteins.

Abbreviations: TAC: tripartite attachment complex (set of filaments linking the mitochondrial genome to the basal bodies); ER: endoplasmic reticulum; IFT: intraflagellar transport; FAZ: flagellum attachment zone (attaches the flagellum to the cell body); PFR: paraflagellar rod (a structural component of the flagellum).

Figure adapted from Billington et al., 2023 and created with BioRender.com.

Chromobodies

A recent development allows antibodies to be used in live cells to visualise specific proteins. This technique uses a **nanobody**, a very small antibody originating from camels, alpacas or llamas. Unlike most species whose antibodies are composed of two heavy and two light chains, camelids also produce antibodies comprising of just two heavy chains. Nanobodies consist of just the antibody-binding domains of the heavy chain antibodies and are about a tenth of the size of a conventional IgG antibody. In chromobodies, the nanobody is fused to a fluorescent protein, such as GFP (Fig. 5A). Rather than add the **chromobody** directly to cells, like antibodies are added during immunofluorescence, molecular genetics are used to insert a DNA sequence encoding the chromobody into the parasite, directing the parasite to make the chromobody itself. The chromobody protein is then able to specifically bind its target without interfering with its function, allowing the target POI to be visualised by fluorescence microscopy.

Case study: visualising actin in Toxoplasma gondii

Actin is a protein present in all eukaryotic cells where it plays essential roles in maintaining and controlling cell shape and movement, as well as the transport of other proteins around the cell. Actin can either be present as globular monomers (single molecules; G-actin) or can form filaments composed of multiple actin molecules (F-actin). In the parasite *T. gondii*, which can cause toxoplasmosis in immunocompromised individuals or in foetuses *in utero*, actin is an essential protein. It has been shown to play a variety of roles, including in host cell invasion and egress, division of the apicoplast (a specialist organelle essential to parasites such as *Toxoplasma* and *Plasmodium*), transport of the secretory organelles known as dense granules and in cell replication. However, *Toxoplasma* actin is unusually unstable, and therefore only short filaments of actin can form, with the vast majority of cellular actin being in the globular G-actin form. Historically, it has been difficult to visualise the short F-actin filaments in the parasite, but using actin chromobodies, Periz *et al.*, 2017 were able to demonstrate that F-actin is present in the cytosol of *Toxoplasma* parasites. They also detected an extensive F-actin network linking individual parasites present and multiplying within the **parasitophorus vacuole** within the host cell, allowing the transport of vesicles between the parasite cells (Fig. 5B). This actin network is assembled and then disassembled in an ordered manner during the *Toxoplasma* replication cycle (Fig. 5C). This study highlights that even canonical (common) proteins can be divergent in parasites, and alternative methods are often needed to be able to study them and shed important light on their functions.

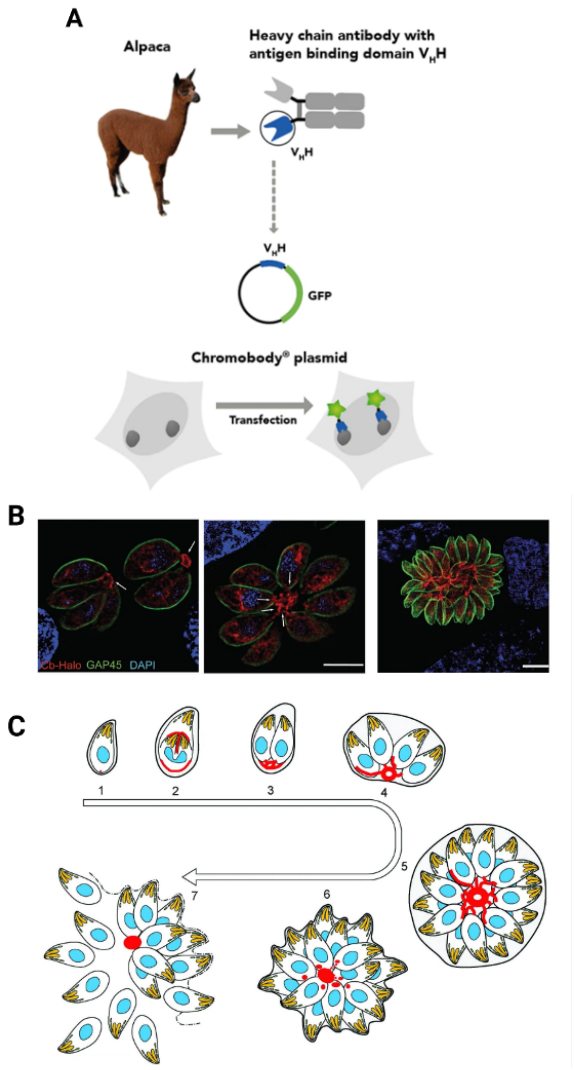


Figure 5. Detection of *Toxoplasma* actin with chromobodies.

A. Chromobody schematic. Chromobodies are derived from the antibodies of camelids, such as alpacas. These animals produce antibodies comprising of two heavy chains, each with a binding domain called V_HH. The coding sequence for the V_HH domain (the nanobody) recognising the target of interest e.g., actin, is inserted into a plasmid (circular DNA molecule) in frame with the coding sequence for a fluorescent protein such as GFP. This plasmid is then transfected into the cell type being studied, allowing the cell to synthesise the fluorescent chromobody. The nanobody recognises and binds to its protein target, and the fluorescent protein allows the location of the nanobody within the cell to be determined using fluorescent microscopy. Figure taken from https://www.ptglab.com/products/chromotek-nanobody-based-reagents/chromobodies/.

B. Fluorescence microscopy images of intracellular *Toxoplasma gondii* parasites stained for DNA (using the blue dye, DAPI), GAP45 (green, a protein found in the cytoskeleton of the parasite, just beneath the cell membrane, used to outline the cells) and actin (red – using an actin chromobody fused to either a Halo or Emerald fluorescent tag). Note that the host cell nuclei (large blue areas at edges of images) are also stained by DAPI, and that actin is detected both inside the parasites and extracellularly. White arrows indicate the extracellular actin filament networks that link parasites within the vacuole they reside in. These networks are maintained as increasing numbers of parasites are replicated (right panel). Scale bars (white bars): 5 µm (left) and 10 µm (right). Adapted from Periz *et al.*, 2017.

C. Schematic of actin filament formation during the replication cycle of *T. gondii*. (1) structure of the *Toxoplasma* cell. Blue: nucleus; yellow: secretory structures at the apical end of the parasite cell; dotted lines: cytoskeleton (also known as the Inner Membrane Complex or IMC); red: actin. Note that after the parasite invades the host cell, it replicates within a parasitophorus vacuole (2). New parasites are formed within the mother cell. Actin is initially found at the IMC and the basal (posterior) end of the parasite. Once the daughter cells are fully formed, the mother cell disintegrates and is recycled at the basal end of the daughter cells in a structure known as the residual body (3). Actin strongly localises to this region, and the filamentous network and ring structures start to form. Parasite replication continues (4-5) and the actin network links daughter cells together within the vacuole. Just before the parasites break out (egress) from the vacuole, the actin network breaks down, leaving a dot of actin at the posterior end of each daughter cell as well as an accumulation of actin at the residual body (6-7). Adapted from Periz *et al.*, 2017.

Created with BioRender.com.

Using fluorescent or luminescent parasites to study disease processes

In addition to labelling specific parasite proteins with fluorescent proteins, parasites can be genetically modified to express a fluorescent protein on its own that isn't tagged to another protein. In this case, the fluorescent protein is usually expressed throughout the cytoplasm of the parasite and provides an effective way of visualising parasites within their host, providing a tool to monitor parasite infections and the efficacy of anti-parasitic drugs. For example, tsetse flies were infected with a fluorescent *T. brucei* parasites in order to investigate parasite mating within the fly (Peacock *et al.*, 2009). Genetic exchange occurs during mating, although this is not obligatory for the life cycle of trypanosomes and it had long been suspected that mating occurred between parasites located within the tsetse salivary glands, but this had not been directly observed. By feeding tsetse flies mixtures of red and green fluorescent trypanosomes, it was demonstrated that some parasites in the salivary glands, but not elsewhere in the tsetse fly, became able to express both red and green fluorescence, with these parasites appearing yellow when red and green fluorescent images were overlayed (Fig. 6A). This indicated that the parasites had mated and exchanged genetic material within the salivary glands.

Special imaging techniques can also be used to detect fluorescent parasites within mammalian hosts to track how they infect and cause disease. However, because many animal cells and fur are themselves fluorescent, this makes it harder to detect the parasites, so it is much more common to use **luminescence** rather than fluorescence. Here, the parasites are genetically modified to express a luciferase enzyme, frequently one that emits light in the far-infra-red spectrum, away from the autofluorescence of the animal. Luciferases convert luciferin to oxyluciferin, emitting light in the process, and are found naturally in fireflies, various marine creatures as well as some bacteria. Experimental animals are first infected with the luciferase-expressing parasite, and then at a suitable timepoint after infection, are anesthetised, injected with luciferin and imaged using an In vivo Imaging System that can detect the light produced by the luciferase with high sensitivity. The same animal can be imaged repeatedly over several days, reducing the number of experimental animals needed, and allowing infections to be followed in real time as they develop (Fig. 6B). This approach can be used to determine the efficacy of experimental vaccines in preventing infection or experimental drugs in clearing infection.

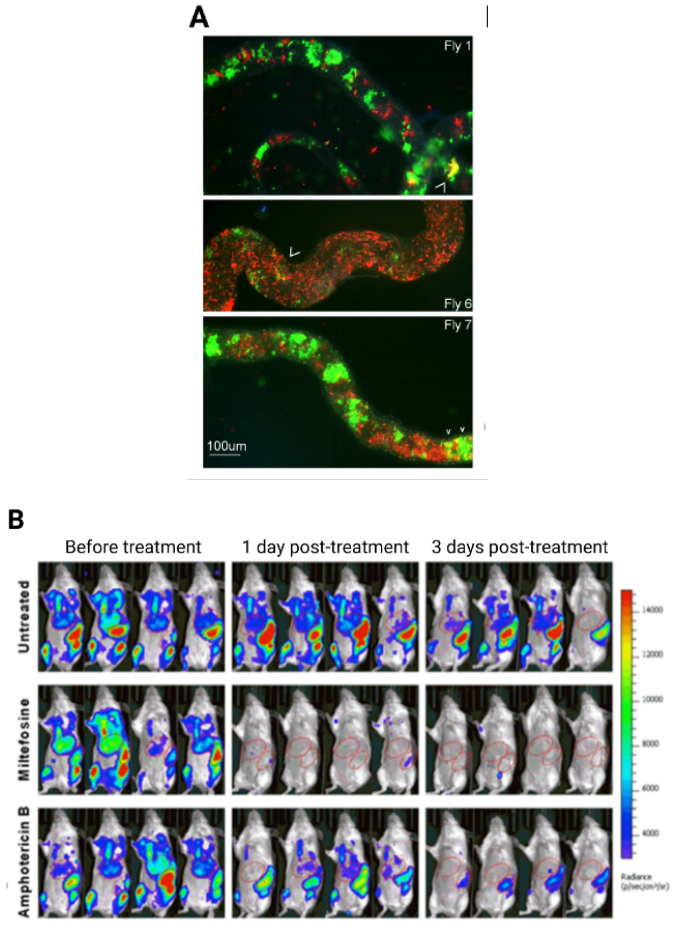


Figure 6. Visualising fluorescent or luminescent parasites within their host.

A. Tsetse flies were infected with a mixed population of green and red fluorescent *T. brucei* parasites. Following a suitable infection period, the fly salivary glands were dissected and imaged using a fluorescence microscope, with red and green images overlayed. Arrowheads indicate trypanosomes that mated and underwent genetic exchange within the salivary glands resulting in them expressing both red and green fluorescent proteins and appearing yellow in the image overlays. Image taken from Peacock *et al.*, 2009.

B. Mice were infected with luciferase-expressing *Leishmania infantum* parasites, and 15 days later were treated with drugs, either miltefosine or amphotericin B, for four days. A set of control mice were not treated with any drugs. Mice (four per treatment group) were injected with luciferin and imaged using an In Vivo Imaging System (IVIS) just before treatment started, and one and three days after treatment finished. A colour scale was applied according to the intensity of light emitted from the parasites going from purple (low radiance, few parasites) to red (high radiance, many parasites). Red ovals indicate the positions of the liver (larger oval) and spleen (smaller oval), which are key sites for parasite replication. Image adapted from Mendes Costa *et al.*, 2019.

Created with BioRender.com.

Glossary

Chromobody – a tiny antibody (or nanobody) tagged with a fluorescent protein. DNA encoding the chromobody protein is transfected into the cell, resulting in it synthesising the chromobody itself. The chromobody binds to the nanobody's target protein without interfering with its function, allowing it to be visualised by fluorescence microscopy.

Co-immunofluorescence – immunofluorescence performed for two or more proteins simultaneously. Often used to give an indication as to whether different POIs might interact with each other, or whether a POI localises to a particular cellular location e.g., nucleus or Golgi apparatus.

Epitope tag – a small peptide sequence of ~10 amino acids in length, which is recognised by specific antibodies that are commercially available. Fusing an epitope tag to a POI allows it to be detected by immunofluorescence without needing to generate a specific antibody to the POI itself.

Fluorescent tagging – the gene sequence encoding a POI is fused, in frame, at either its 5` or 3` end to the coding sequence for a fluorescent protein, resulting in a fluorescent fusion protein being expressed (made) by the parasite. This allows detection of the POI by fluorescence microscopy.

Fluorophore – a fluorescent molecule, often attached to an antibody to make it fluorescent.

FRET – fluorescence resonance energy transfer is a technique that can help investigate whether two POIs interact, as it has much greater resolution than fluorescent microscopy on its own. The two POIs are tagged in the same parasite cell line with different fluorescent proteins that have different spectral properties e.g., cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP). To visualise the proteins, a wavelength of light suitable to excite only one of them e.g., CFP, 414 nm, is applied. This excites the CFP, which emits light at 475 nm, that in turn, is able to excite YFP, if it is in close enough proximity, which then emits at 525 nm. FRET only works if the two fluorophores are brought to within 8-10 nm of each other (1 nanometer (nm) is one billionth of a metre), so if FRET is observed, it suggests that the proteins to which the fluorophores are fused interact.

Immunofluorescence – a cell biology technique whereby fluorescent antibodies that bind to a POI are

used to visualise the protein using a fluorescence microscope. Although it is possible to use a fluorescent primary antibody that recognises the protein of interest, it is more common to use a non-fluorescent primary antibody and then add a fluorescent secondary antibody that binds to the primary antibody. Because more than one secondary antibody molecule can bind to each primary antibody molecule, this amplifies the fluorescent signal.

Immunoprecipitation – a technique where an antibody recognising the POI is used to specifically extract the POI and any proteins it interacts with from a cell. Cells are lysed (broken open) and incubated with the antibody, which is typically attached to a resin or beads. After a suitable incubation period, any proteins that have not bound to the antibody are washed off the beads, resulting in just the POI plus any other cellular proteins that it binds to being retained on the beads. These cellular partners can then be identified using a variety of molecular techniques. Immunoprecipitation can be used to confirm that two particular proteins interact inside a parasite.

Luminescence – the emittance of light that does not involve heat. As described in this article, this is the result of an enzymatic reaction (bioluminescence) that involves the enzyme luciferase and its substrate luciferin and can be exploited to visualise parasites within their hosts with greater sensitivity than is possible using fluorescence (another form of luminescence).

Nanobody – a tiny antibody comprising just the antibody-binding portion of a camelid heavy chain antibody.

Parasitophorus vacuole – a vacuole or sac inside a host cell within which some parasites such as *Toxoplasma* and *Plasmodium* replicate.

Primary antibody – an antibody that directly recognises and binds to a POI.

Secondary antibody – an antibody that recognises the stalk (conserved portion) of a primary antibody. Secondary antibodies typically recognise primary antibodies from a particular animal species, or a subtype of primary antibody from a particular animal species. Often, antibodies are conjugated (linked) to a fluorophore which enables them to be used for immunofluorescence and detected using fluorescent microscopy.

References

Billington, K., Halliday, C., Madden, R. *et al.* Genome-wide subcellular protein map for the flagellate parasite *Trypanosoma brucei*. *Nat Microbiol* **8**, 533–547 (2023). https://doi.org/10.1038/s41564-022-01295-6

Mendes Costa, D., Cecílio, P., Santarém, N. *et al.* Murine infection with bioluminescent *Leishmania infantum* axenic amastigotes applied to drug discovery. *Sci Rep* **9**, 18989 (2019). https://doi.org/10.1038/s41598-019-55474-3

Peacock, L., Ferris, V., Bailey, M. *et al.* Intraclonal mating occurs during tsetse transmission of *Trypanosoma brucei*. *Parasites Vectors* **2**, 43 (2009). https://doi.org/10.1186/1756-3305-2-43

Periz, J., Whitelaw, J., Harding, C., *et al.* (2017) *Toxoplasma gondii* F-actin forms an extensive filamentous network required for material exchange and parasite maturation *eLife* 6:e24119. https://doi.org/10.7554/eLife.24119.001

AUTHOR PROFILE

Tansy Hammarton is a Senior Lecturer in the School of Infection & Immunity at the University of Glasgow. After obtaining a BA in Natural Sciences from the University of Cambridge and a PhD in Microbiology at the University of Manchester, she moved to Glasgow to study the cell cycle of kinetoplastid parasites. Her group's research currently focusses on the signalling molecules that regulate mitosis and cytokinesis in trypanosomes and *Leishmania*. Dr Hammarton also has a keen interest in public engagement, particularly in working with schools, and was awarded the 2018 Microbiology Society's Peter Wildy Prize Lecture for her work. She is currently the Microbiology Society's representative on MiSAC.