Twisting up DNA may be the Achilles Heel of Malaria

If you have ever had an antibiotic from the doctor, the chances are you have taken a drug that targets a fascinating nanoscale machine found in bacteria that may also turn out to be the Achilles heel of other diseases such as malaria.

The antibiotics, called fluoroquinolones, target an enzyme that is found in all bacterial cells but not in humans. The enzyme is called "DNA gyrase" and it has a fascinating job in the cell – it coils up DNA. Because already a coil of one strand around the other (the famous double helix), this coiling of coils that gyrase carries out is called "supercoiling". Supercoiling is very important for the cell, being crucial for controlling the reading ("transcription") and copying ("replication") of genes.

In order to carry out its task of supercoiling DNA, gyrase has to break both strands of the DNA double helix and pass another double strand through the break before resealing it. This is a potentially suicidal step because broken DNA is lethal for the cell. Fluoroquinolones work by binding to gyrase just after it has broken the DNA and stops it from resealing the broken strands. This leads to cell death. Interestingly, as there are many gyrases in each cell but only a very few strand breaks are required to be lethal. As a result the fluoroquinolones are impressively deadly - they can kill cells at concentrations where there is no noticeable effect on the overall supercoiling activity of the enzyme – this is why they are such effective drugs.

DNA gyrase was once thought to be found only in bacteria and not in eukaryotic cells (such as human cells). A recent discovery changed this idea. Some single-celled eukaryotes called the Apicomplexa contain gyrase. Apicomplexa include important parasites causing human disease including *Toxoplasma gondii,* which causes toxoplasmosis and species of *Plasmodium, which* are responsible for malaria. How did bacterial DNA gyrase get into these cells? Close inspection reveals that these parasite cells contain small structures called apicoplasts. Apicoplasts were once free-living bacteria but found a permanent home in the ancestors of the Apicomplexa. However, they never lost their own bacterial DNA or their bacterial gyrase. Because apicoplast structures are vital for the survival of the cell, perhaps we could cure these parasitic diseases simply by administering fluoroquinolones. However, experiments have shown that this is not effective. This could be because the drugs cannot get access to the gyrase, which is buried deep in the apicoplast. Alternatively it could be because these special apicoplast gyrases are significantly different in structure compared to "normal" gyrases, meaning that the place where the drug binds has changed shape and the drug can no longer attach. If the first possibility is true it means that, in theory, gyrase-targeting drugs such as fluoroquinolone could cure diseases such as malaria – if a good delivery system can be devised. If the second point is true we need to design and test new drugs to target the apicoplast gyrases.

We aim to find out which of these possibilities is true. To answer the question the DNA gyrase proteins need to be made and purified. Once this is achieved their activities, their response to drugs and their structures can all be analysed.

Across the world, researchers have tried to achieve this but the apicoplast gyrases have proved difficult to produce for reasons that are not fully understood. It may be due to their special structures; they are much larger than "normal" bacterial enzymes and have large extra domains whose function is not fully understood. In our own work we became the first (and so far only) people in the world to successfully purify and test an apicoplast gyrase (from *Toxoplasma gondii*) but we have many more tests to carry out including understanding the structure of the enzyme. In addition we have not yet been able to make enough of the enzyme from *Plasmodium* to be able to test it. In our future work we will make more of these proteins and we will analyse their structure and the way in which they supercoil DNA to understand them in more detail. This will ultimately allow us to design and test new inhibitors and help us to defeat an important class of parasitic foe.