

derived from the complex susceptibility and the noise power spectral density, and so relates collective response to individual grain motion. This ratio turns out to be nearly constant for varying frequency, which enables D'Anna *et al.* to define an effective temperature for the grains. For a classical liquid, this ratio is equal to  $kT$  and is strictly independent of frequency. Using the average value of the ratio as their measure of thermal energy, the authors show that the temperature increases as the square of the driving-force amplitude — a result that would be naively expected if the bead velocities were linearly related to the container velocity, which in turn increases in direct proportion to the drive amplitude. These findings are intriguing, and they support results from other analyses of model systems that have indicated that the fluctuation–dissipation theorem, or a slightly modified version of it, applies to granular materials<sup>13–15</sup>. There are also signs that the temperatures obtained through application of the fluctuation–dissipation theorem<sup>13</sup> are compatible with the temperature obtained from a new form of statistical mechanics that is applicable to granular systems and possibly to other non-energy-conserving systems<sup>16</sup>.

D'Anna and colleagues' findings that granular ensembles with strong dissipation have a definable viscosity and approximately obey the fluctuation–dissipation theorem are exciting, but they do not yet definitively answer the question of how deep the similarities run between moving grains and ordinary

liquids. Some puzzles remain. Why does the effective temperature vary by approximately a factor of 10 for differently shaped probes? What influence does the probe have on the measurements? Why is the fluctuation–dissipation ratio an increasing function of frequency (albeit slowly)? The answers themselves may be mundane, but they might lead to deeper insights into the properties of granular materials and other related non-equilibrium subjects such as traffic flow, flocking, evolving networks and turbulence<sup>14</sup>. ■

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the two drugs most commonly used to treat malaria<sup>4</sup>. Hope for the containment of the disease now rests largely on a remarkable set of artemisinin drugs developed by Chinese scientists in the 1970s and early 1980s, which rapidly kill the malaria parasites.

The parasites are small 'protozoan' cells (the most prevalent species infecting humans are *Plasmodium falciparum* and *P. vivax*), which enter their human host through a mosquito bite. They first invade the liver and replicate there for two weeks, before beginning a cycle of red-blood-cell invasion, then growth, replication and red-cell destruction that leads to the disease symptoms. The artemisinin drugs are known to act specifically during this blood stage.

Artemisinin contains a structural feature called a peroxide bridge (Fig. 1), and this is believed to be the key to the drug's mode of action. Ferrous iron ( $\text{Fe}^{2+}$ ) catalyses the cleavage of this bridge, forming highly reactive free radicals<sup>5</sup>. The theory has been that these artemisinin-derived free radicals chemically modify and inhibit a variety of parasite molecules, resulting in parasite death<sup>5,6</sup>.

A rich source of intracellular  $\text{Fe}^{2+}$  is haem — an essential component of haemoglobin — and it has long been suspected that  $\text{Fe}^{2+}$ -haem is responsible for activating artemisinins inside the parasite. In support of this,  $\text{Fe}^{2+}$ -haem activates artemisinins in the test tube and haem–artemisinin complexes can be formed. This theory appealed to malariologists because it seemed to explain the specificity of the drug within the context of a unique aspect of parasite metabolism. During its growth and replication inside the red blood cell, the parasite ingests and degrades up to 80% of host-cell haemoglobin in a compartment called a food vacuole. This releases  $\text{Fe}^{2+}$ -haem, which is oxidized to  $\text{Fe}^{3+}$ -haematin and then aggregates within the food vacuole into an ordered crystalline pigment called haemozoin. A theory developed that the specific antimalarial effect of artemisinin was due to its entry into the parasite food vacuole and its interaction with  $\text{Fe}^{2+}$ -haem. Here, it would set off a 'cluster bomb' of free radicals, inhibiting several key parasite components and eventually resulting in parasite death.

This theory has been challenged<sup>7</sup>, however,

## Malaria

# To kill a parasite

Robert G. Ridley

Artemisinins have been used since ancient times to treat malaria. A new theory could explain how this age-old medicine is able to cause the death of the malaria parasite.

The Chinese herb qinghao (*Artemisia annua*) has long been used to treat malaria — Taoist manuscripts dating back to the third century describe the use of qinghao extracts to treat malaria-related fevers<sup>1</sup>. Over the past two decades, derivatives of the herb's active ingredient, artemisinin, have made an increasing contribution to malaria treatment. But the precise mechanism by which artemisinin derivatives kill the parasite has remained obscure. Writing on page 957 of this issue, Krishna and colleagues<sup>2</sup> propose a radical new theory to explain the molecular basis of the antimalarial activity of artemisinin.

Malaria remains a scourge of the developing world, killing over a million people each year and infecting around 500 million<sup>3</sup>. Most of the victims are children under the age of

five living in sub-Saharan Africa, but the disease also afflicts Southeast Asia, South America and the Indian subcontinent. The situation has worsened over recent years as resistance has developed against chloroquine and sulphadoxine–pyrimethamine,

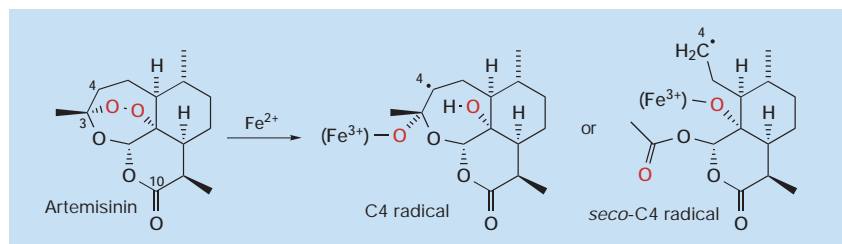


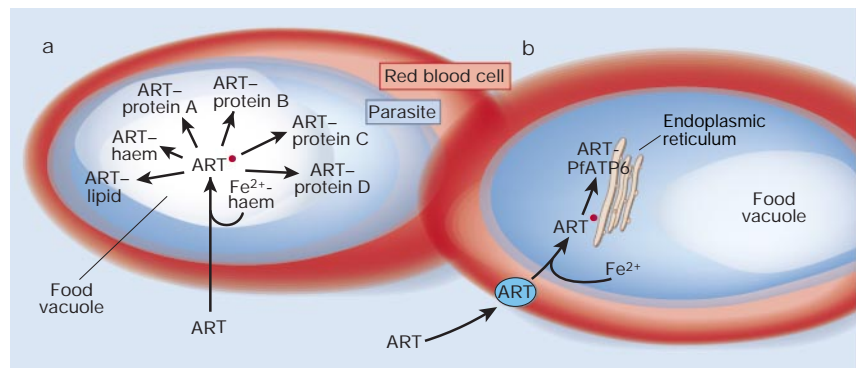
Figure 1 Structure of artemisinin. The molecule contains a peroxide bridge (red), which becomes cleaved when artemisinin interacts with ferrous iron ( $\text{Fe}^{2+}$ ). Cleavage creates 'C4' and 'seco-C4' free radicals, each capable of chemically modifying biological molecules.

based on chemical structure–activity considerations. The work of Krishna and colleagues<sup>2</sup> on *P. falciparum* now prompts a further rethink. Using a fluorescently labelled artemisinin derivative and powerful high-resolution confocal microscopy, the authors show that the artemisinins do not accumulate in the food vacuole, but are instead spread throughout the parasite. They further show that inhibitors that prevent haemoglobin degradation and the consequent release of haem do not interfere with the action of artemisinins. These inhibitors do antagonize the antimalarial properties of chloroquine, which is known to exert its effect through haem binding<sup>1</sup>. The authors' conclusion supports that of others<sup>7</sup>, that the activity of the artemisinins does not require haem.

If the artemisinins neither interact with haem nor localize in the food vacuole, how do they function? Krishna and colleagues present compelling evidence that the artemisinins work through irreversibly inhibiting a metabolic enzyme — the malarial calcium-dependent ATPase (PfATP6). PfATP6 is very similar to a mammalian ATPase (the sarco/endoplasmic reticulum calcium-dependent ATPase, SERCA), which, as its name suggests, is located in a membrane-enclosed intracellular compartment called the sarco/endoplasmic reticulum (ER). In the parasite, the ER is situated outside the food vacuole (throughout the parasite cytoplasm) — the same location as the proposed site of artemisinin action.

But another connection led the authors to propose that PfATP6 might be a molecular target for artemisinin. The mammalian enzyme SERCA is inhibited by a drug called thapsigargin that, although it lacks a peroxide bridge, has some structural similarities to the artemisinins. To test whether PfATP6 could be a molecular target for artemisinins, Krishna and colleagues introduced the malarial enzyme into frog eggs and assessed its activity in the presence of thapsigargin or artemisinin. Both drugs did indeed inhibit PfATP6. Importantly, thapsigargin also interfered with the action of artemisinin, indicating that both drugs were operating on malaria parasites through similar mechanisms. An iron chelator, desferrioxamine (which removes free iron from the cellular environment), also interfered with artemisinin action — it antagonized the inhibition of PfATP6, the inhibition of parasite growth and the accumulation of artemisinin inside the parasite. Taken together, these data indicate that an iron-dependent mechanism generates free radicals from artemisinin, which inhibit PfATP6, thereby slowing parasite growth (Fig. 2).

Krishna and colleagues make a strong case for their new hypothesis, but certain questions remain and some confirmatory experiments suggest themselves. We still do



**Figure 2** A new molecular target for an ancient antimalarial drug. These images represent **a** (trophozoite-stage) malarial parasite (blue) growing inside a red blood cell. **a**, It was originally supposed that artemisinin (ART) was transported to the food vacuole of the parasite (white), where it was converted into a free radical after an interaction with  $\text{Fe}^{2+}$ -haem. These free radicals were then thought to modify and inhibit haem, lipids and at least four proteins, resulting in parasite death. **b**, Krishna and colleagues<sup>2</sup> now propose that artemisinin is transported from the red blood cell into the parasite inside parasite-derived membrane vesicles. Once inside the parasite, artemisinin is activated by free iron, or another iron-dependent process, that occurs close to PfATP6 in the endoplasmic reticulum. The activated artemisinin specifically and irreversibly binds and inhibits PfATP6, and inhibits parasite growth.

not have definitive biochemical evidence that the activated artemisinins bind to PfATP6 inside the parasite at inhibitory concentrations. We also do not know whether irreversible binding occurs at one or more sites. The fact that resistance has not yet developed to the artemisinins suggests that their binding to PfATP6 is not affected by single point mutations in the target protein. So the free radicals generated from artemisinin might indeed modify multiple sites on a single target. Furthermore, other studies have shown that artemisinin can bind to several different parasite proteins<sup>5</sup>, so it is possible that the drug has other molecular targets, in addition to PfATP6. In short, further biochemical work with parasite material is needed.

Nevertheless, this study<sup>2</sup> will radically alter our thinking about the mode of action of artemisinins. PfATP6 is also now a prospective target for the development of

new antimalarial drugs. Identifying other antimalarials with the efficacy, tolerability and safety of the artemisinins will be a tall order, but the ever-growing threat of resistance to antimalarial drugs gives the work of Krishna and colleagues a practical significance beyond its undoubted academic merit.

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### Cell biology

## Tumour jailbreak

Kenneth M. Yamada

New work shows that a cage-like matrix of protein fibres around cells can inhibit the growth of tumours. But cancer cells producing the enzyme MT1-MMP can cleave this matrix and proliferate freely.

**T**umour cells resemble hardened criminals. They defy the body's social restraints, altering their behaviour and interactions to proliferate and spread as they please. The body also has potential physical restraints — the three-dimensional mesh-like matrices that provide support for normal cells — and Hotary *et al.*<sup>1</sup> now

report in *Cell* that these can prevent the proliferation of tumour cells in the laboratory. But if the cells are able to produce a specific matrix-cleaving enzyme, they can escape these physical restraints as well, becoming free to change shape and multiply.

One of the purposes of the 'extracellular matrix' is to provide structural support for