News & views

Microbiology

Hit movie of tuberculosis drug halting ATP synthesis

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Structural data revealing how an anti-tuberculosis drug works could aid efforts to improve therapeutic options for the disease. The findings also uncover aspects of how the drug's target, the ATP synthase enzyme, operates. **See p.143**

The 2017 Nobel Prize in Chemistry was awarded for the development of an imaging method called cryo-electron microscopy. On bestowing the prize, the Royal Swedish Academy of Sciences stated that this technique has "moved biochemistry into a new era". On page 143, Guo *et al.*[1] provide a compelling glimpse into this new age.

The authors' work reveals how a drug called bedaquiline, which has revolutionized the treatment of drug-resistant tuberculosis[2,3], interacts with its target. The drug binds to the ATP synthase enzyme of the microorganism *Mycobacterium tuberculosis* that causes tuberculosis[4]. Adding to a rapidly growing body of work elucidating the structure of ATP synthases by cryo-electron microscopy[5,6], the details presented by Guo and colleagues – and particularly the videos (see Supplementary Videos 1 and 2 of ref. 1) generated from their structural data – are breathtaking in their ability to reveal how this macromolecular machine works. Moreover, the authors show how the drug binds to the enzyme and disrupts its synthesis of the molecule ATP, providing crucial information that had eluded detection through other, more conventional structural and biochemical investigative techniques.

ATP synthases are found in every domain of life, and their basic structure is evolutionarily conserved. These amazing protein complexes harness a gradient of protons (positively charged hydrogen ions) established across the cellular membrane, and couple the protons' movement across the membrane to the synthesis of ATP, the main currency of cellular energy[5,6]. These macromolecular assemblies have a common architecture comprising an F_1 head region (Fig. 1) – which can either produce or break down (hydrolyse) ATP – lodged above a membrane-embedded 'wheel', the rotating F_0 rotor. The F_1 head and F_0 rotor are connected by a central stalk (the enzyme's γ-subunit) in the centre of the wheel and by a peripheral stalk that is in contact with F_0 in the lipid membrane. Driven by proton movement, the peripheral stalk rotates around the wheel so that individual active sites of the enzyme in the F_1 head are energized and drive ATP synthesis; if the direction of rotation is reversed, ATP is hydrolysed instead.

The ATP synthase of *M. tuberculosis* consists of nine types of subunit (α, β, γ, δ, ε, a, b, b′ and c), from which the F_0 rotor (comprising one a-, one b-, one b′- and nine c-subunits) and F_1 head (three α, three β, one γ, one δ and one ε) are assembled. Unlike other bacterial ATP synthases, this version of the enzyme lacks ATP-hydrolysis activity[7,8]. However, the molecular basis of this distinctive feature, which is thought to enable these mycobacteria to conserve energy[9], has remained elusive[10].

Guo and colleagues now provide an answer to this puzzle with their discovery that the enzyme uses a 'hook and ratchet' mechanism to prevent ATP hydrolysis. The hook is formed from an extension of an α-subunit of the F_1 head, and this can catch a region of the γ-subunit at a central part of the F_0 rotor (Fig. 1). The binding of the hook to this ratchet prevents F_0 from rotating in the reverse direction that drives ATP hydrolysis. If, instead, F_0 rotates in the opposite direction, which leads to ATP synthesis, this releases the hook.

However, the more-crucial advance made by this study is the insight it provides into exactly how bedaquiline interferes with enzyme function. Genetic analysis[4,11] had already shown that the c-subunit, which forms the ring at the heart of the F_0 rotor, is bedaquiline's target. Biochemical analysis[11] indicated that the drug has only low (millimolar) affinity for

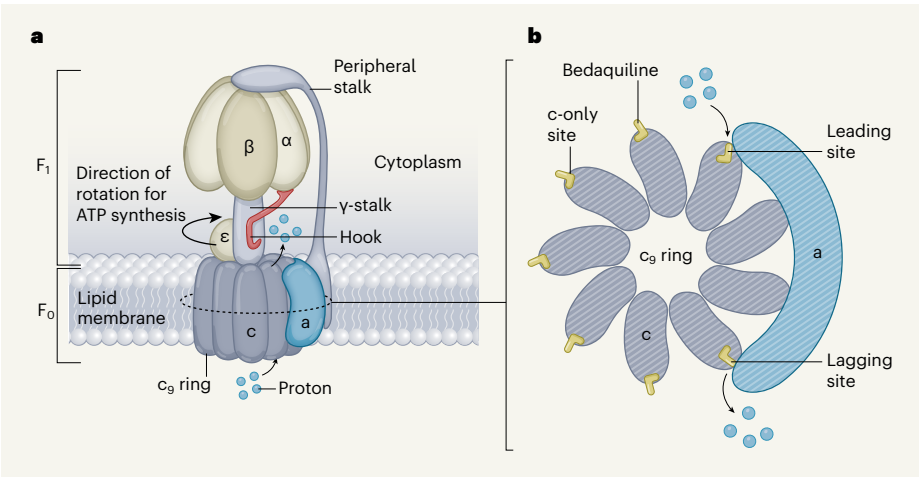

Figure 1 | The ATP synthase enzyme of the bacterium *Mycobacterium tuberculosis*. Guo *et al.*[1] report structural data obtained using cryo-electron microscopy. **a**, The molecule ATP (not shown) can be generated as the enzyme rotates when transporting protons (hydrogen ions) across the lipid membrane into the cytoplasm. The F_1 region of ATP synthase consists of a peripheral stalk, a γ-stalk and α-, β- and ε-subunits. The F_0 region includes a ring of nine c-subunits and an a-subunit. The authors discovered a 'hook' structure on the α-subunit that could bind to the γ-stalk and prevent rotation in the reverse direction that would cause the breakdown (hydrolysis), rather than the synthesis, of ATP. **b**, The authors determined how the anti-tuberculosis drug bedaquiline binds to the enzyme, as shown in this top-down view of the horizontal section of the F_0 region indicated by the dotted line in **a**. Guo and colleagues report that the drug binds better to the c-subunits in the complex where protons normally enter or exit (leading and lagging drug-binding sites, respectively) than to the other c-subunits (c-only drug-binding sites).

purified c-subunits studied *in vitro*, whereas bedaquiline's affinity is a million times higher (in the nanomolar range) when tested in *M. tuberculosis* cells[4]. A previous structural study[12] of the c-ring, using X-ray crystallography, suggested that one bedaquiline molecule binds to each of the nine c-subunits, and identified key features of the binding interaction that the drug makes with the c-ring's proton-binding sites.

By contrast, Guo and colleagues revealed (Fig. 1) seven bedaquiline molecules bound to seven of the nine c-subunits; the other two potential c-subunit binding sites were obstructed by the adjacent a-subunit. Five of the bedaquiline molecules were bound to c-subunits (at 'c-only' sites) in the same type of binding as previously revealed by X-ray analysis[12], whereas the other two sites in c-subunits that bound to bedaquiline had further interactions with subunit a. Designated as leading and lagging sites, respectively, these two sites are located in c-subunits that, in the absence of a drug to hinder enzyme function, would have either just picked up a proton in the proton-entry channel of the c-ring (the leading site) or just deposited a proton in the proton-exit channel of the c-ring (the lagging site).

When the authors washed the complex to remove the drug, bedaquiline disappeared from the images of the five c-only sites but remained clearly visible in the leading site, with some also remaining in the lagging site. The reason for this became clear when the authors looked at where the drug interacted with the protein: the leading site was created by large movements of specific amino-acid residues in the c- and a-subunits that formed a deeper and more extensive binding pocket for the drug compared with that of the c-only binding site. This difference provides a probable explanation for the striking difference between the drug's potency *in vitro* and *in vivo*. By binding particularly tightly to the leading and lagging sites that are created as the c-ring rotates, bedaquiline jams the rotation of the ring, thereby blocking proton transport and halting ATP synthesis.

It is unsurprising that a binding site's location with regard to the peripheral stalk should affect the possible conformations at that site, but it is nonetheless beautiful to actually observe the difference it makes to drug binding. The structural details of the leading-site pocket offer information for researchers wanting to develop an improved version of bedaquiline. But most of us will be riveted by the movies made possible by cryo-electron microscopy, showing ATP synthase at work. It highlights exactly how complex this machine — the basis of almost all life — actually is, while simultaneously revealing a vulnerability that bedaquiline exploits. The 2017 Nobel committee recognized how crucial it is to be able to visualize biochemical processes in three dimensions; we hope they are proudly watching the movies in this paper.

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Computational materials science

The complexity of dense amorphous silicon

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Transitions between amorphous forms of solids and liquids are difficult to study. Machine learning has now provided fresh insight into pressure-induced transformations of amorphous silicon, opening the way to studies of other systems. **See p.59**

Machine-learning approaches are being developed to produce accurate simulations of the structure and chemical bonding of disordered solids and liquids, modelling a sufficient number of atoms to enable direct comparison with experimental data. On page 59, Deringer *et al.*[1] report their use of this approach to probe the structure of amorphous silicon under compression, as the element transforms from semiconducting to metallic states. Their work demonstrates that the structural transformations of amorphous forms of materials can take place much more gradually than those between crystalline phases, and can involve the formation of nanostructured domains and localized atomic arrangements that are not found in any of the crystalline states.

Silicon is one of a small class of elements whose density increases on melting[2]. This unusual behaviour is shared with crystalline ice, which floats on top of liquid water. Such unexpected reversal of solid and liquid densities has been linked to a phenomenon called polyamorphism — the ability of a substance to exist as different amorphous phases that have distinct structures and properties.

Liquid silicon is a metallic electrical conductor, whereas solid silicon is a semiconductor in ambient conditions, a fact that underpins its use in technologies ranging from computer chips to solar panels. The solid can adopt either a crystalline or a structurally disordered amorphous form at room temperature and pressure; in both cases, each atom bonds to four others in a tetrahedral arrangement. However, both the crystalline and amorphous solids transform into denser structures under compression, a process that is accompanied by a transition to metallic conducting behaviour.

In the 1970s, calorimetric experiments were carried out to study the energy changes that accompany the transformations between amorphous and crystalline forms of silicon during heating and cooling[3]. Analysis of the results suggested that two amorphous forms of silicon exist, with a phase transition between them. Simulations have since suggested that silicon transforms from a low-density amorphous (LDA) phase, in which the coordination number — the number of neighbouring atoms around each silicon atom — is four, to a high-density amorphous (HDA) phase whose structure is similar to that of metallic liquid silicon[3,4]. The LDA–HDA transition has been observed both during rapid heating of the amorphous solid and on compression of amorphous silicon at ambient temperature[5–7].

Structural transformations between crystalline phases of silicon are readily observed using diffraction methods[8], but those involving the amorphous state are more difficult to study because they occur less abruptly as