

polarizable Cs<sup>+</sup> ions, you have the recipe for a good ionic conductor.

For a couple of decades, CsHSO<sub>4</sub> has been the model high-temperature proton conductor. But no one tested it in a real fuel cell. They may have been discouraged by its softness in the superionic phase, its solubility in water, the difficulty of synthesizing the compound with electrodes attached and the narrow operating window available (between 140 °C and its melting/decomposition temperature above 200 °C). Haile *et al.*<sup>1</sup> have now responded to the increasing clamour for intermediate-temperature solid electrolytes, and proton conductors in particular, and tried out CsHSO<sub>4</sub> in a laboratory set-up.

They made a fuel cell by sandwiching the solid acid between two electrode layers made from CsHSO<sub>4</sub> powder mixed with platinum and carbon and a volatile organic. The entire fuel cell was compressed to provide good contact between the electrolyte and electrode layers. On heating, the organic component evaporated, leaving behind a porous electrode structure with the possibility of multiple contacts between the fuel (or oxygen, at the other end of the cell), the electrolyte and the electrodes.

Next came the question of whether the solid acid electrolyte was stable when exposed to the operating conditions of the fuel cell: a temperature of 160 °C, hydrogen gas at one electrode and oxygen at the other. The laboratory fuel cell performed stably over several days, burning the world's cleanest fuel — hydrogen — and emitting the most environmentally friendly effluent, pure water.

Haile and her colleagues<sup>1</sup> have been studying for some time another class of proton conductors, in which the protons are not part of the structure, but merely defects dissolved in water-containing atmospheres. The breakthrough for these materials came in the early 1980s when Iwahara and co-workers showed that chemically doped SrCeO<sub>3</sub> and other perovskite-related oxides became proton-conducting in humid atmospheres and at high temperatures up to 800 °C. Iwahara demonstrated fuel cells and other devices based on a number of perovskite-related oxides, and conceived the term protonics for the rapidly expanding field of solid-state proton conductors<sup>4</sup>.

These high-temperature perovskite conductors lose protons at high temperatures, so the optimum temperature of proton conduction is a compromise between proton concentration and mobility. The proton conductivity typically peaks at around 400–600 °C. From these temperatures and down to those of solid acids such as CsHSO<sub>4</sub> (160 °C) there is a gap in fuel-cell temperatures for which, as yet, there is no real candidate for a solid electrolyte. This is an important temperature range because it should allow good materials stability, fast

reaction kinetics, and manageable heat recovery for a wide variety of applications.

Haile *et al.*<sup>1</sup> are applying for a patent for fuel cells based on solid acid salts, but are otherwise realistically modest about their results. The CsHSO<sub>4</sub> electrolyte they used was a millimetre or more thick, whereas real applications will require micrometre-thin films to reduce the resistance of the electrolyte layer. The performance of the electrodes may also be of concern; slow charge transfer appears to cause further drops in voltage. Overall, the laboratory fuel cell delivers a modest 44 milliwatts per square centimetre, which compares unfavourably with the output of state-of-the-art PEFCs and SOFCs. More importantly, one can imagine that operation times of tens of thousands of hours will reveal degradation of the electrolyte due to creep, evaporation and reduction of sulphate in the solid acid by the fuel. From a practical

point of view, accidental overheating would melt or decompose the electrolyte and water flooding would easily dissolve it.

Nonetheless, Haile *et al.* have proved the concept; true solid-state proton conductors can indeed be put to work in fuel cells, and the relevance of protonics has been extended into the intermediate temperature range. If CsHSO<sub>4</sub> is not the most practical electrolyte, then there is a rich chemistry of solid acids and hydroxides with much yet to discover. ■

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#### Molecular motors

## Doing a rotary two-step

Mark J. Schnitzer

By spinning around, the ATP synthase converts energy from electrochemical to chemical form for storage. A cunning assay reveals intricate details of the rotation.

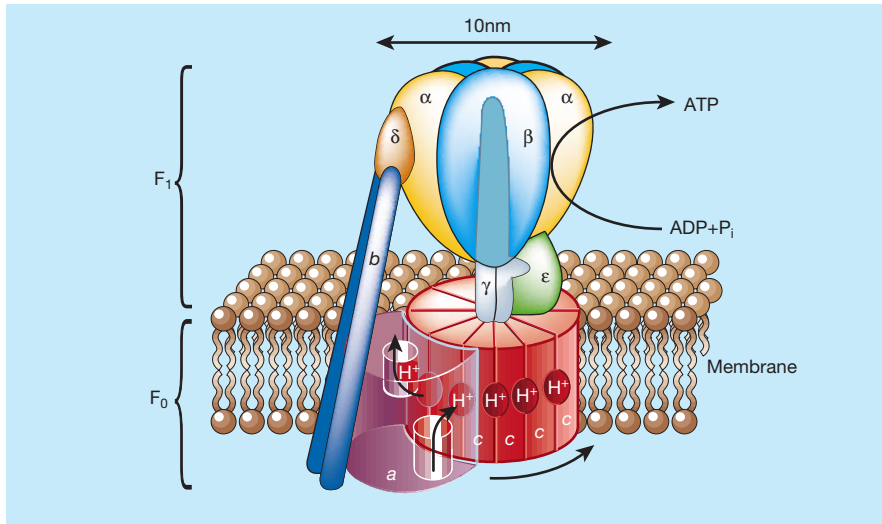
How do organisms power themselves? This simple question marked the start of a decades-long quest that led eventually to a rotary enzyme called ATP synthase. Using energy derived from proton gradients created during photosynthesis or respiration, this enzyme produces ATP (adenosine triphosphate) molecules — the main cellular energy store. A challenge now is to understand how ATP synthase works. It is thought to contain two rotary motors, called F<sub>0</sub> and F<sub>1</sub>, which are rotationally coupled through their drive shafts. This device uses motion, in the form of 120° rotary steps by F<sub>1</sub>, as an intermediate, converting energy from electrochemical through mechanical to chemical form<sup>1</sup>. On page 898 of this issue<sup>2</sup>, Yasuda and colleagues describe their elegant studies of the F<sub>1</sub> motor, and show that each rotary step actually consists of two smaller substeps. More generally, their work highlights some central issues concerning energy flow in proteins.

The F<sub>0</sub> and F<sub>1</sub> motors have specialized functions (Fig. 1). The F<sub>0</sub> motor is bound to membranes of energy-generating cellular structures such as mitochondria, and channels protons through its rotor and non-rotating stator to drive rotation. Meanwhile, the stator of F<sub>1</sub> catalyses the production of ATP from ADP (adenosine diphosphate) and inorganic phosphate, provided that F<sub>0</sub> drives the F<sub>1</sub> rotor with sufficient torque

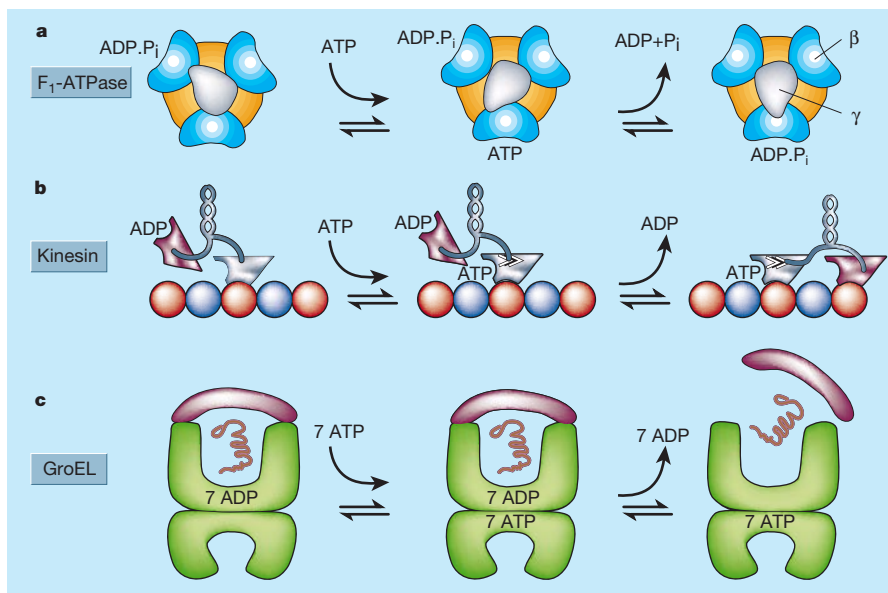
(rotary force). The catalytic sites of F<sub>1</sub> are within the three so-called β-subunits, which are arranged with tripartite symmetry in the stator. Both F<sub>0</sub> and F<sub>1</sub> can operate in reverse, and isolated F<sub>1</sub> is called the F<sub>1</sub>-ATPase because, in the absence of torque from F<sub>0</sub>, it hydrolyses ATP to power reverse rotation.

There is only indirect evidence that F<sub>0</sub> can drive rotation<sup>3–5</sup>. But rotation of F<sub>1</sub> sub-complexes has been visualized directly *in vitro*, by fixing the stator to a glass surface and using a micrometre-sized filament attached to the rotor's γ-subunit as a marker<sup>6,7</sup>. With this assay, 120° rotary steps can be detected by monitoring the movement of the filament at low ATP concentrations, such that the binding of ATP to F<sub>1</sub> is the rate-limiting event between steps<sup>7</sup>. The distribution of times between steps is consistent with one ATP molecule being hydrolysed per step.

Once a step begins, the time taken for the 120° turn depends on the frictional drag imposed by the filament and so on the filament's length. Drag also smoothes out any rapid fine structure within the protein's 120° movement when monitored via the filament. This obscures how rotor movements are linked to hydrolysis events, which probably occur in reverse during ATP synthesis. So, to break the speed barrier imposed by the filament, Yasuda *et al.*<sup>2</sup> have revved up the assay by using a bead with a diameter of a mere 40 nanometres as the marker. Using



**Figure 1** ATP synthase — energy converter. The enzyme consists of two motors,  $F_0$  and  $F_1$ . The transmembrane  $F_0$  motor has one  $a$ , two  $b$  and nine to twelve  $c$  subunits. The soluble  $F_1$  motor has three  $\alpha$  and three  $\beta$  subunits, and one each of the  $\epsilon$ ,  $\delta$  and  $\gamma$  subunits. The  $F_0$  rotor (consisting of the  $c$  subunits) and the  $F_1$  rotor ( $\epsilon$  and  $\gamma$  subunits) are connected, as are the respective stators (the  $F_0$  stator comprises the  $a$  and  $b$  subunits; the  $F_1$  stator consists of the  $\delta$  subunit and the three  $\alpha$  and three  $\beta$  subunits). During ATP synthesis,  $F_0$  channels protons across the membrane to drive rotation, which in  $F_1$  occurs as  $120^\circ$  rotational steps. Yasuda *et al.*<sup>2</sup> show that each  $120^\circ$  step is composed of two smaller substeps of  $90^\circ$  and  $30^\circ$ . Modified from refs 14, 15.



**Figure 2** Molecular machines that use ATP binding at one catalytic site to trigger a large conformational change and the release of ADP from another catalytic site.  $P_i$  is inorganic phosphate. a,  $F_1$ -ATPase, with the catalytic  $\beta$ -subunits in dark blue, the  $\alpha$ -subunits in yellow, and the  $\gamma$ -subunit in light blue. When ATP binds to one  $\beta$ -subunit, the  $\gamma$ -subunit rotates by  $90^\circ$ . ADP and  $P_i$  are released from another  $\beta$ -subunit, and the  $\gamma$ -subunit rotates a further  $30^\circ$ . b, Kinesin, a linear motor with two catalytic heads (purple and grey) that move along a microtubule filament (red and blue). ATP binding to the front head triggers a conformational change (shown by double arrowheads) in its adjoining neck linker domain<sup>9</sup> and induces the rear head to step forward and to release ADP. c, The chaperone GroEL (green), its co-chaperone GroES (purple) and a substrate protein (squiggly line). GroES acts as a cap, containing the substrate protein within GroEL while the substrate folds. GroEL has two rings, each of which bind seven ATP or ADP molecules. Cooperative binding of seven ATP to one ring triggers the other ring to release GroES, the substrate protein and seven ADP molecules.

a high-speed camera to capture footage of bead rotation, the authors find that, when attached to the  $\gamma$ -subunit off the rotational axis, the bead spins in an eccentric orbit during ATP hydrolysis. Rotational frictional

drag is so slight that, at saturating ATP concentrations,  $120^\circ$  steps begin roughly every 2 milliseconds, and an entire step is complete within about 0.25 milliseconds.

A potential problem with these rapid

speeds is that they might make it hard to detect substeps within a step, given that the camera acquires data only twice during a  $120^\circ$  step. However, it was known through the Nobel-Prize-winning work of Paul Boyer<sup>8</sup> that a large fraction of the energy liberated during the ATP-hydrolysis cycle is released when ATP binds tightly to one catalytic site. Most of the remaining energy is freed about 2 milliseconds later, when the products of hydrolysis (ADP and inorganic phosphate) are released from another catalytic site (Fig. 2a). It seemed plausible that these large energy changes might be accompanied by large conformational changes in  $F_1$ . If those changes involved rotational substeps, then it might prove possible to increase the average time between successive substeps to at least 2 milliseconds. The trick would be to pause rotation after the product-release substep, by lowering the ATP concentration enough to delay the next ATP-binding substep by 2 milliseconds or more. So, each substep would now follow a pause.

Yasuda *et al.*<sup>2</sup> adopt this tactic, and records of motion at  $20 \mu\text{M}$  ATP or below reveal  $90 \pm 10^\circ$  and  $30 \pm 10^\circ$  substeps, separated by about 2 milliseconds. The  $90^\circ$  movement must be the binding substep — its mean initiation time following a  $30^\circ$  movement depends on ATP concentration. Histograms of initiation times reveal the delay between  $90^\circ$  and  $30^\circ$  rotations, and show a time distribution consistent with two kinetic transitions, each requiring about 1 millisecond. A remaining challenge is to correlate these transitions and the substeps with structural forms of the catalytic  $\beta$ -subunits.

ATP synthase was one of the first enzymes known to show cooperative interactions between its catalytic sites. We now have explicit proof<sup>2</sup> that ATP binding to  $F_1$  triggers rotation.  $F_1$  thereby joins a growing list of molecular machines that use ATP binding at one catalytic site to induce product release from another catalytic site through large mechanical movements (Fig. 2b, c). Two other examples are the motor protein kinesin and the chaperone molecule GroEL, which helps newly formed protein chains to fold into the correct shape. Kinesin has two catalytic head regions that somehow allow the molecule to advance in discrete 8-nanometre steps. ATP binding at one head triggers a conformational change in the protein, allowing ADP to be released from the other head<sup>9</sup> and stepping to occur. GroEL has two ring domains; binding of seven ATP molecules to one ring triggers the other ring to release the substrate protein<sup>10</sup> and seven ADP molecules.

In all of these proteins, how does energy flow outwards from an ATP-binding site to initiate movement and product release? Energy must travel between catalytic sites — about 5 nanometres in  $F_1$ -ATPase<sup>11</sup>, some 9 nanometres in GroEL<sup>10</sup>, and potentially

up to 16 nanometres in kinesin<sup>9</sup>. Detailed modelling<sup>12</sup> of  $F_1$  indicates that ATP binding might trigger elastic bending in the catalytic  $\beta$ -subunit, which then applies torque to the  $\gamma$ -subunit in the rotor. In kinesin, a rigid helical structure may act as a mechanical relay between a phosphate-sensing loop at the ATP/ADP-binding site and a linker region that is usually mobile but which becomes immobilized upon ATP binding<sup>9</sup>. Energy flow in GroEL is poorly understood. Indeed, questions about all three enzymes remain; for example, do mechanical signals generally occur as elastic strain, as in  $F_1$ , or can some protein domains become thermally activated upon ATP binding? Temperature-dependent kinetic studies and efficiency considerations may help to separate entropic and enthalpic contributions, but energy flow will probably remain murky until investigated further. The hope is that new experimental approaches, such as those relying

on protein databases to enable statistical comparisons of closely related proteins<sup>13</sup>, will speed our understanding. ■

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### Fundamental physics

## Newton rules (for now)

Frank Wilczek

Three hundred years after Newton explained the falling of an apple and the motion of the planets, physicists are beginning to test his universal law of gravity down to micrometre distances — with interesting results.

Newton's formula for the force of gravitational attraction is the oldest, and perhaps the most revered, universal law of physics. Yet it is not sacred. The experimental support for Newton's force law at sub-centimetre distances is surprisingly weak. There are theoretical suggestions about why and how deviations from Newton's law might arise at these distances. A group of experimentalists at the University of Washington, Seattle, has tackled the question, using ingenious but surprisingly low-tech, small-science techniques to test newtonian gravity down to 200  $\mu\text{m}$ . As they report in *Physical Review Letters*<sup>1</sup>, Newton's law still rules, which puts some theoretical speculation about large 'extra dimensions' on the ropes.

In modern physics, the precise form of Newton's formula for the gravitational force — proportional to mass, inversely proportional to distance squared — is rooted in profound principles. Force proportional to mass means acceleration independent of mass. Thus Newton's law implies that the motion induced by gravity is independent of the material properties of the body it acts upon. This idea is deeply engrained in Einstein's theory of general relativity, in which gravitational fields (produced by matter) change the geometry of space-time, causing it to become curved. It is the curvature of space-time that controls the natural acceler-

ation of bodies. The inverse square form of Newton's law is a consequence of the field equations for gravity. The form of these equations, in turn, is dictated by the general principles of quantum field theory (specifically, the requirement that they derive from a local lagrangian function).

How, then, might deviations arise? One way is through the existence of extra, non-gravitational forces. Indeed, we know that there are other forces in physics, even at macroscopic distances. For example, there are  $1/r^2$  electric forces between charged bodies, and  $1/r^4$  magnetic forces (with complicated angular dependence) between magnetic bodies, where  $r$  is the separation. Even between unscreened neutral, non-magnetic bodies there are attractive short-range  $1/r^7$  van der Waals forces. If our goal is to test the foundations of physics, however, our focus must be on non-electric, non-magnetic bodies, and forces beyond van der Waals.

Within the framework of quantum field theory, forces are associated with the exchange of virtual particles. Thus new forces are associated with new particles. The range of the force depends inversely on the mass of the particle. If the particle mass is  $m$ , then the range of the corresponding force is  $\hbar/mc$ , where  $\hbar$  is Planck's constant and  $c$  is the speed of light. Numerically, a particle of mass  $2 \times 10^{-5}$  electron volts (25 billion times lighter than the electron) yields a force

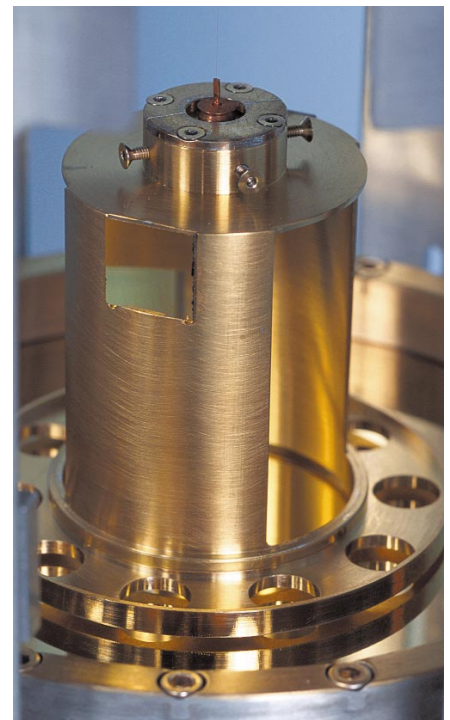


Figure 1 Apparatus used at the University of Washington<sup>1</sup> to test Newton's inverse square law. They measured the motion of a metal ring suspended above a rotating disk to show that Newton is correct down to ring-disk separations of at least 200  $\mu\text{m}$ . The photograph shows the metal ring, which has ten holes bored in it, suspended from a torsion pendulum. The rotating disk below the metal ring is hidden from view by a sheet of metal foil that minimizes electrical forces on the pendulum. As the disk rotates, the gravitational force causes the ring to twist back and forth ten times for each revolution of the disk. The twisting of the pendulum is measured by reflecting a laser beam from a small mirror attached to the upper part of the pendulum. The entire apparatus is coated in gold to prevent electrical forces interfering with the weak gravitational signal.

with a range of 1 centimetre. Thus in looking for deviations from Newton's force law — specifically new forces operating at super-molecular but sub-centimetre distances — we are probing for the existence of extremely light, extremely weakly interacting particles<sup>2,3</sup>.

Many examples of such particles have been suggested, with various motivations. So-called axions<sup>4</sup> arise in attempts to explain the accurate time-reversal symmetry of the 'strong force' — the short-range interaction that holds protons and neutrons together in nuclei. Dilatons arise in superstring theory (a fundamental theory of elementary particles that requires extra dimensions). Familons and modulons arise in attempts to understand the origin of the subtle differences between particles in different families — for example the difference between the electron and its heavier cousins, the muon and tau leptons<sup>5</sup>.

None of these exotic particles has yet been