the expectations of society," CEFIC stated (2003). "REACH has its good sides," van Ravenzwaay said, because it could become a starting point for more research in toxicology and an improved risk assessment. Moreover, REACH would finally shed light on the health and environmental risks of many chemicals that have been in use for decades but were never assessed by safety tests that are now mandatory. In doing so, it will finally end an almost century-long experiment on the human race, in which regulatory agencies have abstained from requiring sufficient safety data for a large number of chemicals. "One can consider the ethics of throwing 100,000 chemicals on humans without having them tested," Calabrese said. "We're using human society as a guinea pig."

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produced sufficiently accurate descriptions of crucial molecules for QM methods to unravel the details of key reactions, such as ATP hydrolysis.

QM has also made a significant impact on the study of photoreception and the detection of colour, on research into the sensing of magnetic location and directional information by migratory birds, and, most controversially, in understanding the processes underlying consciousness. The last example relies on certain unprovable assumptions about the scientific basis of perception, whereas research on catalytic reaction centres (such as analysing substrate binding) hinges on solving the Schrödinger wave equation. Described in 1926, and central to the theory of QM, this equation describes the probability that a given electron is in a particular location at a certain time (see sidebar). Such QM-based applications calculate the sequence of events at the atomic level by analysing the electronic properties during the formation and breakage of chemical bonds or the orientation of electron orbitals, as determined by their quantum wave function.

A quantum leap in biology

One inscrutable field helps another, as quantum physics unravels consciousness

The most esoteric research field in the natural sciences is probably quantum physics. Despite the fact that Werner Heisenberg first proposed its central concepts nearly 80 years ago, it continues to baffle physicists and to cause headaches among non-physicists. Even Albert Einstein was unwilling to accept the central tenet that everything is just a matter of possibilities; he famously dismissed Heisenberg's ideas by asserting that "God does not throw dice." As quantum physics seems too mystical to be relevant to anything as real as a living organism, it might come as a surprise that its first applications have arrived in biology, rather than physics.

The seeds of contemporary quantum biology were sown as early as 1930, a mere three years after Heisenberg postulated his uncertainty principle describing the inability to

measure related quantities exactly (see sidebar). At that time, Erich Hückel, a German chemist and physicist, developed simplified methods based on quantum mechanics (QM) for analysing the structure of unsaturated organic molecules, in particular to explain the state of electrons in aromatic compounds. But Hückel was too far ahead of his time, and his concepts went almost completely unrecognized until the 1950s, when the arrival of computers made it possible to perform more detailed calculations. It was not until the 1990s, however, that the field of quantum biology became established with the development of density functional theory (DFT), which allows accurate calculations of electronic structure (see sidebar). By that time, high-resolution structures of protein complexes obtained using X-ray crystallography and nuclear magnetic resonance

The validity of QM methods is not seriously disputed, but their high computational intensity precluded their use until the 1990s. Although computers had been used to simulate the function of proteins and their chemical reactions since the mid-1960s, these calculations were based on molecular mechanics (MM) techniques derived from Newton's laws of motion. These operate at the level of molecules rather than electrons, and describe the energy and forces associated with particular protein structures, by studying simpler model compounds that mimic the chemical groups in the constituent amino acids and other components.

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The weakness of MM methods is that they rely on making simple assumptions. For example, electrons are not considered directly, but are assumed to be in an optimum position determined by the location of their atomic nuclei. This process, based on the Born–Oppenheimer approximation of

A BACKGROUND IN PHYSICS

In the early nineteenth century, physics faced a crisis, as researchers made a number of observations on the behaviour of single photons and electrons that could not be explained by Newton's laws of mechanics. In 1926, the German physicist Werner Heisenberg finally solved these problems with his famous uncertainty principle, which has formed the cornerstone of quantum mechanics (QM) ever since. According to this principle, it is impossible to measure exactly all of a particle's quantities (such as mass, energy, position or momentum), simply because they do not have absolutely fixed values. Instead, they have a range of possible values within a probability distribution, but at normal space and timescales this range is relatively small. In everyday life, it is therefore possible to make exact measurements limited only by the sensitivity of the equipment. However, at small space and timescales, such as those that operate at the submolecular level, the impact of the uncertainty becomes much greater.

If, for example, researchers wanted to predict the location of a particle at a given time, they would measure its current position and its rate of change in position expressed as its momentum. The uncertainty principle states that the more accurately researchers determine one of these quantities, the less accurately they will know the other, which imposes a deterministic limitation on the accuracy of prediction. This uncertainty becomes significant not only within small spatial dimensions, but also at short time intervals. This is relevant for biology, given that many processes at the molecular level occur over short timescales. For example, the operation of molecular motors is coupled to a chemical reaction that occurs over a few femtoseconds.

Explaining enzymatic reactions requires the analysis of quantum effects because the core processes usually take place just one molecule at a time and are not bulk chemical reactions in a test tube. As such, they rely on the precise alignment of molecules, for example, when water molecules are split by the catalytic actions of four manganese ions and one calcium ion in photosynthesis. The electrons involved govern the resulting molecular interactions, and QM can be used to resolve their energies and, thus, the outcome. Similarly, photoreception involves the excitation of orbital electrons, and calculating the resulting energy change requires QM.

The uncertainty principle is applied to such problems by determining the energy levels of atoms or molecules using the wave equation, which was developed by the Austrian physicist Erwin Schrödinger. It encapsulates the uncertainty in any system as the probability of finding a given particle at a particular place. Schrödinger's equation is relevant for all chemical reactions or any interactions involving electrons, which can be described as an electromagnetic wave owing to the uncertainty of their position. When one electron interacts with another, such as in a chemical reaction, the waveform is said to collapse, as the electron assumes a definite position.

Density functional theory (DFT) replaces the individual electrons of a system, such as a molecule, with a single electronic density function to represent both the aggregate charge and the interactions between individual electrons. This means that the algorithm considers only three spatial dimensions when analysing quantum-level interactions between systems, irrespective of the number of electrons involved. Before DFT was first used in the 1990s, every electron had to be considered separately, restricting quantum-level analysis to the smallest interactions involving only a few atoms.

The Penrose–Hameroff model of consciousness uses the effect of quantum tunnelling to explain how several hundred neurons are able to simultaneously coordinate their firing rate. Quantum tunnelling exploits uncertainty about the position of an electron—with a high probability, it is near its atomic nucleus but it might also be at the far end of the galaxy, albeit with a much lower probability. By exploiting the uncertainty inherent in its wave nature, the electron appears to jump from one position on its probability wave to another, and seemingly hops over, or tunnels through, obstacles such as an atomic nucleus.

require the calculation of polarization effects—how molecules orientate themselves as they approach each other—which are determined by the behaviour of their orbitals.

Whenever electrons and their associated energies need to be considered explicitly, QM steps in. The same is true for studying reactions that involve the recognition of light (such as in the retina) or stimulation by light (as in photosynthesis), because these processes involve the excitation of electrons. QM methods, which are often described as *ab initio* because they work from first principles without using empirical techniques, also reveal the dynamics of reactions as they are taking place. They make it possible to determine and analyse intermediates, such as radicals and oxidation states of metal ions, that exist transiently before the finished products of the reaction are formed.

Rapidly increasing computational power combined with new methods, notably DFT to simplify calculations, makes it possible to apply QM methods to analyse enzymatic reactions. Before scientists began to use DFT widely in the 1990s, every electron in a system being analysed using QM had to be dealt with separately in each of the three spatial dimensions, whereas DFT combines all electrons into a single density function. For a system with N electrons this reduces the number of variables from $3N$ to 3, without, in principle, introducing any approximations.

Although DFT greatly reduces the degrees of freedom, QM calculations are still so computationally intensive that, even with contemporary supercomputers or computing grids, only a small number of atoms can be analysed at one time; the current maximum is around 100. Even more restrictive is the limit on the time-span of the simulation, according to Paolo Carloni, a professor at the International School for Advanced Studies in Trieste, Italy, who specializes in *ab initio* and MM simulations. "First-principle calculations of a system of, say, 100 atoms can cover up to a few tens of picoseconds," he said. However, most processes involving quantum effects occur over a much longer timescale, with many enzymatic reactions taking several milliseconds, for example. Researchers use statistical methods to extend the time range of QM methods, but this inevitably introduces errors.

There has been considerable success combining QM with traditional MM techniques to circumvent the limited scaling of

the Schrödinger equation, treats a complex molecule like an assembly of weights connected by springs. Therefore, when the MM algorithm calculates the energy required to stretch or compress a chemical bond, it applies a formula similar to Hooke's law of elastic springs under tension.

This works reasonably well for determining the geometry and total enthalpy of a molecule in isolation, but fails to describe reactions that involve binding or recognition in solution, as occurs in most crucial reactions in biology. Most reactions in nature

involve bond formation and breakage, with associated changes in electron organization that cannot be described accurately by classical mechanics because of the uncertainties involved. Similarly, reactions involving docking or molecular recognition in solution

Rapidly increasing computational power combined with new methods ... makes it possible to apply QM methods to analyse enzymatic reactions

the former. Such hybrid methods are now widely deployed in the study of enzyme reactions. The crucial part of the system under study (such as the active site of an enzyme complex or a molecule in solution) is analysed using QM methods, whereas the energy and forces for the remainder (such as the non-reacting part of a protein complex or the solvent molecules) are calculated using the traditional MM model. The idea is to use MM approximations for those parts that are sufficiently far removed from the active reaction area so as not to contribute significantly to the overall system.

Inevitably, hybrid QM/MM methods represent a compromise, and so require judicious application if they are to be sufficiently accurate. "I would say that the reliability of any QM/MM simulation strongly depends on the skills and thoroughness of the researcher doing the work, particularly during the planning/testing phase of the simulations," said Markus Dittrich from the University of Illinois at Urbana-Champaign, USA, who applies such methods to analyse ATP hydrolysis. "Once all the necessary steps have been taken, QM/MM simulations can give meaningful qualitative results, in some cases even quantitative ones, at least in my opinion." However, this requires significant testing and benchmarking for each system, as well as describing the QM/MM interface and the size of the part treated with the high-precision QM methods, according to Dittrich.

...QM calculations are still so computationally intensive that even with contemporary supercomputers or computing grids, only a small number of atoms can be analysed at one time...

Still, even hybrid methods require enormous computation power to analyse many biological structures and interactions, because of the large range of spatial dimensions and timescales involved. For example, molecular motor proteins coordinate chemical reactions over a few femtoseconds with mechanical motions taking place over microseconds or even milliseconds. Similarly, distances range from bond breaking in the catalytic binding site in a single angstrom to structural changes during molecular motion that span up to 10 nm.

No single computational approach can calculate over such ranges of time and distance; however, combining QM/MM with other classical techniques to focus on a small number of crucial variables has proved successful. Klaus Schulten and colleagues at the University of Illinois at Urbana-Champaign integrated a variety of methods, including QM/MM and molecular dynamics, to obtain new insights into the mechanism of the PcrA helicase molecular motor, which unwinds double-stranded DNA. PcrA uses the energy from ATP hydrolysis to skip along a single strand of DNA, one base pair at a time.

Schulten's breakthrough lay in determining the link between the mechanical motion and the binding and unbinding of PcrA with ATP as it skips along the DNA (Yu *et al.*, 2006). This link is mediated by a two-way conformational change in the protein. "When the protein binds, one part of the structure is a bit loose, and then the reverse happens when it unbinds," said Schulten. This sequence of flexing allows the protein to traverse the DNA.

As protein motors go, PcrA helicase is relatively simple, as it moves in a linear direction. Other motors involve more complex rotary motion. One of the best known and most sophisticated examples is the combination of F_0 and F_1 motors that work in tandem to synthesize or hydrolyse ATP. These motors convert energy between the

two forms in which it is stored in cells: as a transmembrane electrochemical gradient or in a chemical bond, such as the gamma phosphate bond in ATP. F_0 and F_1 act reversibly, with the former using the transmembrane electrochemical gradient to generate a rotary torque to drive ATP synthesis in the latter. The system can operate in reverse when F_1 hydrolyses ATP instead of producing it, generating torque that can then be harnessed by F_0 to pump ions 'uphill' against their transmembrane electrochemical gradient. As Schulten noted, this complex motion has yet to be fully explained, but the work on PcrA helicase will provide some clues. "When you look at the binding sites of ATPase and PcrA, you see that they are the carbon image of each other," said Schulten.

Another fundamental process that has benefited from the use of QM is photoreception. For years, researchers have been puzzled by how some animals, particularly migratory birds, use their retinal receptors not only for normal vision, but also to 'see' longer distances by measuring the direction and strength of the Earth's geomagnetic field. QM theories have been used to describe two mechanisms for this magnetoreception: the radical-pair mechanism and the magnetite-based mechanism. Originally believed to be competing, the

two explanations have since been found to be complementary.

In the radical-pair mechanism, a light-induced electron transfer in photopigments in a receptor in the eye creates a pair of excited electrons with a particular magnetic orientation or quantum spin state. The Earth's magnetic field affects the transition between these spin states, thus altering how the bird perceives colours. These radical-pair receptors amplify the Earth's weak magnetic signal using magnetic resonance, thus allowing the bird to detect it.

Under the magnetite-based mechanism, the Earth's field exerts a mechanical force on magnetite particles in the upper beaks of migrating birds. An increasing number of researchers now believe that the radical-pair mechanism provides directional information that is comparable to that from a magnetic compass, whereas the magnetite-based mechanism provides positional information as it measures the strength of the signal, which varies with location (Wiltschko & Wiltschko, 2006). However, further work will be needed to elucidate fully how the brain reconciles and processes information from the two magnetic sources alongside normal vision.

The debate over magnetoreception might not be settled, but there is broad agreement over the applicability of QM to this field. What is still disputed is its application to the study of consciousness. QM has always been inextricably linked to consciousness, given the vital role of the observer in making measurements and defining events, and consciousness itself can be explained with reference to QM according to a number of researchers. "It seems that consciousness operates very well in the classical realm," said Koichiro Matsuno, a professor in the Department of Bioengineering at Nagaoka University of Technology in Japan, and a leader in the QM field. "But one serious question would arise at this point. That is, how could one guarantee the robustness of such seemingly classical phenomena including our brain activities."

The most celebrated theory of quantum consciousness—linking events at the sub-atomic level with our perception of consciousness—was developed by the British mathematician and physicist Roger Penrose, and by Max Hameroff, a physician at the University of Arizona Medical Center in Tucson, USA (Hameroff & Penrose, 1996). In the quantum world, matter exists only as a

set of possibilities. 'Reality' emerges when the Schrödinger equation used to describe these possibilities 'collapses'—or moves from a probability wave form to a fixed state—which translates into a classical event, obeying rules such as Newton's laws of motion.

QM has always been inextricably linked to consciousness, given the vital role of the observer in making measurements and defining events, and consciousness itself can be explained with reference to QM...

According to the Penrose–Hameroff model, such quantum states of infinite possibilities exist in the tubulin subunits of microtubules in brain neurons and glia cells, in which they are isolated from their environment to prevent them from collapsing as a result of interacting with each other. Furthermore, the proteins and their associated quantum states are linked through quantum tunnelling, which allows particles to overcome energy and space–time barriers. Consciousness then occurs whenever a series of quantum states connected across neurons can no longer be preserved, and interact to yield a signal that the brain can recognize and respond to. This model explains the observation that consciousness seems to involve the simultaneous coordination of multiple neuronal signals.

The Penrose–Hameroff model has attracted criticism. Max Tegmark, an astrophysicist at the Massachusetts Institute of Technology in Boston, USA, suggested that the model could not work as the brain is simply too warm for quantum effects to occur (Tegmark, 2000). However, like Matsuno, Hameroff insists that no classical theory of consciousness has stood up to scrutiny. "Classical theories based on complexity, emergence, and so forth, have yet to make any testable predictions, and are not, as far as I can tell, falsifiable," he said. "Thus, although we are often criticized, we have a theory and our critics do not"—at least, that is, no theory that can be proved or disproved.

Hameroff has further attempted to define the relationship between complexity and consciousness, given that the phenomenon did not exist at the beginning of evolution and must have emerged at some point, either gradually or abruptly. His

explanation depends to some extent on the Penrose–Hameroff theory, and posits that consciousness arises at the boundary between classical states—events, such as neural signals, which can be recognized or processed as information—and the underlying quantum processes that generated them. On this basis, the threshold of complexity for consciousness was passed 540 million years ago in small worms, such as nematodes. Their neuronal network was sufficient to create quantum tunnel effects involving 100–1,000 neurons, which Hameroff considers enough to generate a single conscious event. This single event was defined by Libet *et al* (1991) to have a pre-conscious time of 500 ms—the time between the formation of a new waveform and its subsequent collapse. The basis for magnetoreception might have evolved even earlier, given that plants and animals have been shown to suffer when shielded from the Earth's magnetic field (Galland & Pazur, 2005).

Although the debate on the role of QM in consciousness persists, quantum physics has nevertheless made inroads into biology, and will further help biologists to understand other phenomena and mechanisms. If QM is the basis of reality, as some researchers believe, it should come as no surprise that it is intimately involved in all kinds of biological processes, even sensation and cognition.

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