The Guy Foundation

WHAT BIOPHOTONICS CAN TELL US ABOUT THE BIOQUANTOME

Proceedings of the 1st Guy Foundation Symposium on Quantum Biology and Bioenergetics, Chedington Court, Dorset, UK, October 2-3, 2019

Available June 2020

Alistair Nunn &
Geoffrey Guy
(Eds.)

Driving innovation in medicine through quantum biology

CONTENTS

THE QUANTUM PREFACE: OF PHOTONS AND FIELDS AT THE ORIGINS OF LIFE – ALISTAIR NUNN

Quantum biology (QB) is as old as the discipline of quantum physics since its pioneers, such as Bohr, Schrödinger and Jordan, all put considerable thought into what it implied regarding the very nature of life itself. So what do we mean by QB?

Quantum physics is largely the study of the laws that govern the microscopic world at the level of atoms and smaller, hence at the sub-nanometre scale, which operate in very short time scales. It is defined by wave-particle duality, and other exotic effects such as superposition, entanglement, spin, tunnelling and coherence, which are not seen in the larger, classical Newtonian world. One of the reasons for this, which defines the boundary between the quantum and classical worlds, is that the more atoms that are involved in the defined quantum system, and the longer they are studied, the greater the chance of the "environment" (i.e., every other molecule, sub-atomic particle or photon surrounding it), have of disrupting their wave state – a process called decoherence. This is why experiments in the lab investigating the quantum world are often done at very low temperatures on very few atoms. However, under the right experimental conditions even very large molecules can show matter-wave interference and thus evidence of quantum superposition, such as oligoporphyrins (containing up to 2000 atoms, for example, see Fein et al., https://doi.org/10.1038/s41567-019-0663-9). So it is quite possible for "quantumness" at least in the laboratory, to exist at larger scales.

Thus it was thought for a long time that because biology is very warm and wet, the "quantumness" stayed firmly rooted at the atomistic scale as part of its chemistry of life, suggesting that evolution had not found ways to enhance it above normal scales of size and time – even if there were advantages to doing so. However, some questioned this, as theory indicated that at least some quantum effects, such as tunnelling or exciton transfer, could be enhanced by biological systems as they might offer an advantage. So it could be said that quantum biology is the exploration of the possibility that biology has evolved mechanisms to amplify and sustain quantum effects into the mesoscopic realm for extended time periods. This could be called the "bioquantome". These effects could enhance energy transfer and dissipation, chemical reactions, as well as signalling and environment sensing. To date, there is now strong evidence that photosynthesis uses a form of exciton transfer of energy, while modulation of proton tunnelling may be important in enzyme reactions. The mechanism, interestingly enough, may actually rely on the warm and wet biological environment inducing a form of resonance, which might be termed "quantum beating" that protects a small area from decoherence.

The idea for this first meeting was based on two key principles about life: it is both affected by, and generates, photons and electromagnetic fields. The average cell is full of molecules that can absorb photons, indeed, the ability of many to act as sunscreens has probably played a central role in evolution, in particular, the ability to absorb and dissipate ultraviolet light (UV) and take part in redox (molecules that can absorb light due to double bond structures are also good at transferring electrons and protons). Less known is that normal metabolism generates photons, for instance, during oxidative stress when electrons giving some of their energy up – a standard quantum principle. These are known as "biophotons".

Cells seem to produce a whole range of different energy photons (i.e., different wavelengths) and remarkably, this phenomena was first detected in the 1920s in onion roots (reviewed by Fritz-Albert Popp, https://www.ncbi.nlm.nih.gov/pubmed/15244259). However, as it is of very low intensity, it is not visible to the naked eye, which is probably partly due to the fact that cells also absorb most of these photons. The generation of electromagnetic fields is also a normal part of metabolism as life is constantly moving charged particles around, especially protons and electrons. In fact life depends on the generation of ionic gradients, which act as a source of energy to drive everyday processes. Evidence also continues to build that electromagnetic fields can also influence biological function, for instance, in determining morphology and cell movement, as well as function in the brain. The interaction between photons, matter and electromagnetic fields is of course what quantum physics is based on.

A powerful crossover point to biology is thus made via the mitochondrion, a central component of most eukaryotic cells, which is key in extracting the energy from electrons via the generation of a proton gradient, and is a prime source of electromagnetic fields and photons. As the mitochondrion, as well as the surrounding cell, probably evolved from some kind of endosymbiotic event between two prokaryotes, these systems are ancient and likely reflect the origins of life itself. In terms of medicine, mitochondrial dysfunction plays a role in multiple diseases, and is closely involved in the ageing process, as it is a hub of multiple processes. It is thus relevant that conditions that can enhance mitochondrial function, for instance, exercise and calorie restriction, as well as a healthy diet, seem to have very powerful anti-disease/ageing effects. Intriguingly, many plant secondary metabolites that are key in stress resistance seem to also modulate mitochondrial function and may have had their origins as sunscreens. Many are chromophores that have aromatic structures and conjugated double bond systems that can modulate redox and the proton gradient. This might suggest they could also modulate a proposed photonic/EM communication system, hinting at a very ancient mechanism of cellular control. Although some of this could be explained using classical physics, much of their properties are also describable using quantum mechanics, and a lot *cannot* be explained using conventional pharmacology.

This is the first proceedings of the first full meeting convened by the Foundation, and although not peer-reviewed, the research papers are written by leading scientists who publish regularly in their respective fields and who were invited to present at the meeting. The diverse topics highlight, we believe, the broad scope of the Foundation's intentions to bring together experts to broaden our understanding of the role of QB in medicine. For more details, visit our website (see below).

Professor Alistair VW Nunn, BSc, PhD

Director of Science, The Guy Foundation

www.theguyfoundation.org

INTRODUCTION TO THE GUY FOUNDATION – GEOFFREY GUY

The Guy Foundation has been set up to support and promote the investigation of quantum effects in biology, with the aim of improving our understanding of disease and thus medicine. Our belief is that significant quantum effects may well have not only been essential for life to get going, but also enabled it to grow in complexity by amplifying these effects both in space and time. For example, all life is based on iron-sulphur compounds that can display interesting tunnelling properties, which could be enhanced by the addition of proteins and chromophoric molecules. These molecules were all created by well understood geochemical/interstellar chemical processes long before life got going, which coupled with established thermodynamic mathematical principles involving self-organisation of dissipative structures in energy gradients, do provide the basis of a starting point for life. In short, if significant quantum effects are part of life, the failure to maintain this state probably plays a role in disease and thus, the ageing process.

Of course this raises a question, why hasn't anybody thought of using quantum mechanics to explain biology? Well, actually, as indicated in the preface, they had, right from the beginning from the days of the pioneers of quantum physics, and over the years, several leading scientists have discussed the possibilities that biology could be using significant quantum effects. Some, such as Roger Penrose, have even gone as far as suggesting it could explain consciousness itself, which, even today in the 21st century, is still far from being understood. In fact, with time, despite the 20th century optimism that by the 21st century mankind would have found cures for cancer and many other diseases, and possibly even for ageing itself, a deeper understanding of life seems to be still out of reach. It could be even further away as emerging global obesity appears to be *shortening* both a healthy and absolute life expectancy, which is resulting in spiralling health care costs across the planet. Despite mankind's emerging technical mastery of nature, we still have a very long way to go in terms of truly understanding it.

This therefore brings us neatly back to quantum mechanics and biology and the aims of the Foundation. Quantum mechanics is intuitively difficult to understand, and, as has been said, if you think you understand it, then you don't. Only now, after nearly 100 years, is technology reaching the point where one of the most difficult of concepts, quantum entanglement, can be tested. Einstein called it "spooky action at a distance", as he simply didn't believe it because it didn't fit with his general theory of relativity, and despite being one of the founders, he openly said that quantum mechanics had to be incomplete. He often argued with Niels Bohr over this. For many years the concept of "quantum realism" has stood quietly like a large elephant in the room, as many thought that only when a conscious observer observed something did its wave function collapse to give us the Newtonian universe we all understand. The latest experiments to test whether or not quantum entanglement exists continue to suggest that it clearly does, which indicate that two entangled particles, which share the same wave function, can still somehow communicate, instantaneously, even if they are on opposite sides of the galaxy. In fact, it is now finding uses, like other quantum effects, such as tunnelling, in everyday practical devices, such as eaves-dropper safe communication. Thus, it is likely that conventional biology, and quantum mechanics, despite the odd attempt to communicate, have largely passed as ships in the night for nearly a

century. The Foundation therefore aims to provide a platform and a forum for upstream pull through and downstream push through of the understanding of the role of quantum effects in biology in health and disease. We recognise these notions to be extremely avant-garde, oftentimes incomprehensible. However, we take a long view and are prepared to fund work which would be very difficult to be funded elsewhere and see ourselves as pioneers in a new wave of medicinal science.

Professor Geoffrey Guy MB BS, LRCP MRCS, LMSSA, DipPharmMed, BSc, DSc

Founder and Chairman of the Board of Trustees, The Guy Foundation

FULL PROCEEDINGS

ABOUT THESE PAPERS – ALISTAIR NUNN

Director of Science, The Guy Foundation

These papers have been written by the presenters at the meeting and the proceedings are free for anyone to download and read. They have not been officially peer-reviewed, but they have been cross-checked by each of the other authors so although they may contain some new ideas, there has been a level of peer oversight. We hope you enjoy these articles and that they stimulate some new thinking, and if you do cite them, please reference them to www.theguyfoundation.org, the Foundation's website.

PHOTONIC RESONANCE IN THE "MITOCYTOSOME" – PHILIP KURIAN

Principal Investigator and Founding Director Quantum Biology Laboratory, Howard University, Washington, DC 20059, USA

Light is fundamental to the origins and development of life. Out of need for protection from solar ultraviolet (UV) rays, organisms have employed a variety of mechanisms to absorb and transfer UV radiation, including with small plant-derived compounds. A hallmark of these compounds—shared by several basic constituents of proteins and nucleic acids—is their "aromatic" structure due to delocalized π electrons. Highly ordered networks of aromatic amino acids and nucleobases are integral to the formation of important cellular structures in mitosis and other biomolecular processes. My group and collaborators have predicted from quantum mechanical calculations that the native symmetries of such aromatic mega-arrays conspire to produce a superradiant effect [1], whereby a group of *N* quantum two-level systems interacts collectively with a common light field to emit high-intensity pulses with rate proportional to N^2. Our results have serious implications for mitochondrial function, as reactive oxygen species produced during aerobic respiration can emit ultraweak UV light. These UV photon emissions could be transmitted coherently by these aromatic lattices via supertransfer processes [1], thus potentially forming quantum optical signalling pathways in the cell. **Such a quantum optical superhighway in biology optimized for ultrafast processing would thus occur at the nexus of mitochondrial reticula and cytoskeletal networks, which I have dubbed the "mitocytosome."**

A number of biological systems exhibit collective, cooperative, and/or coherent behavior whose understanding is not only of fundamental interest but also of practical relevance. Allostery is most commonly known and is operative in respiration, where it regulates oxygen uptake by hemoglobin [2]. Coherent behavior, in which various chromophores exhibit collective or phase-synchronized behavior, is of more recent discovery when it comes to biological systems and is still the subject of debate [3-4]. In the photosynthetic system, the electrostatic type of coupling has been shown to give rise to energy transfer between the different chromophores involved in the capture and funnelling of energy towards the reaction center [5]. The extent and rates of such processes depend on the coupling between chromophores.

Superradiance (also called superfluorescence) is a phenomenon that occurs when a group of quantum two-level systems (TLS) acting as *N* emitters (e.g., excited atoms or molecules) interact with a common light field. If the wavelength of the light is much greater than the separation of the emitters, then the emitters interact with the light in a collective and coherent fashion. This causes the group to emit light as a high intensity pulse (with rate $\propto N^2$). This is drastically different from the expected exponential decay (with rate $\propto N$) of a group of independent atoms. Superradiant emission has been observed in distinctly different physical systems, such as gases [6], molecular aggregates and crystals [7], doped alkali halide crystals [8], nitrogen-vacancy centres in diamond [9], epitaxially grown quantum dots [10-12], and more recently in quantum dot superlattices [13]. In practice, observing superradiance is a challenge because of stringent requirements on the emissive material, such as high oscillator strength, small inhomogeneous line-broadening, and weak exciton dephasing. Ideally, to foster cooperative behaviour, structurally well-defined, long-range ordered, and densely packed arrays of TLS should be used.

Such lattices are possible with biological systems, which often have well-organized arrays of chromophores, yet superradiance has generally only been reported in the case of photosynthetic systems [14], whose shortened radiative rate of fluorescence has been used as a measure of the robustness of the exciton delocalization length. An interesting theoretical prediction of superradiance due to excitonic coupling between TLS has recently been made in the case of tubulin [1,15-16]. Microtubules share some similarities with photosynthetic antenna complexes, particularly in their ordered arrangement of photoactive molecules, namely the tryptophan (TRP) chomophores, whose aromatic (delocalized π electron) structure produces a strong transition dipole (6.0 debye), comparable with that of chlorophyll molecules, but absorbing in the UV rather than the visible range. Using native configurations of TRPs and a realistic model Hamiltonian describing light–matter interactions in the microtubule, we predicted that these TRP networks exhibit a superradiant lowest excitonic state, which represents an excitation fully extended on the chromophore lattice. Upon absorption of deep-UV light (~280 nm), energy transfer was predicted to occur along neuronal microtubules, and this exciton propagation can extend to dendritic length scales [16].

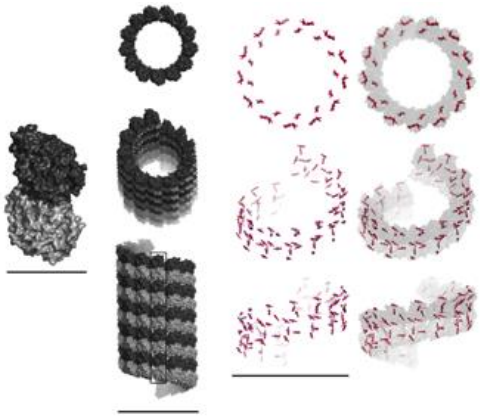

Figure 1 Tubulin proteins with scale bar ~5 nm (far left) polymerize into microtubules with scale bar ~25 nm (left) with highly ordered arrays of tryptophan amino acids (right and far right) that absorb radiation in the ultraviolet spectrum. Reproduced from [1].

A fascinating aspect of quantum optical behaviour is the observation of increasing superradiance with the scaling up of the size of chromophoric systems. For example, microtubules are hollow cylindrical structures whose walls are made up of polymerized tubulins. Microtubules have an external diameter of 25 nm and a wall thickness of 5 nm, which is composed of globular subunits of tubulin proteins. These subunits are organized in longitudinal, so-called protofilaments, parallel to the microtubule long axis (Figure 1, left). This shows that microtubules represent highly organized arrays of TRP two-level systems (Figure 1, right), owing to the eight TRPs in each tubulin. TRP represents an almost ideal TLS for superradiance studies because it has a large energy separation between its lowest L_a excited state and the ground state, its transition dipole and its excited state dipole are large, and it can be found in ordered or quasi-ordered arrangements in various biological systems.

An even larger biological architecture is the centriole, which is generally made of nine triplets of microtubules. Implicit to the phenomenon of superradiance is the concept of a coherence size for the emitting entity [7]. The coherence size is used to designate the number of molecules in the superradiant entity, and the radiative decay probability of the aggregate in the simplest case is related to the radiative decay probability of the incorporated monomeric species by a multiplicative factor corresponding to the coherence size. Therefore, while TRP is ubiquitous in most biological systems, tubulin, microtubules, and centrioles represent TRP networks that occur in the cell with the highest degree of organization into regular arrays, offering the ideal playground for identifying cooperative optical behaviour. TRP chromophores have been actively used in biological systems as a reporter of intra-protein dynamics. They have thus identified transient electric fields generated by bacteriorhodopsin photoexcitation [17-18], electron and energy transfer processes from photoexcited TRP to heme in hemoproteins [19-20], and hydration states on osmolytes [21-22].

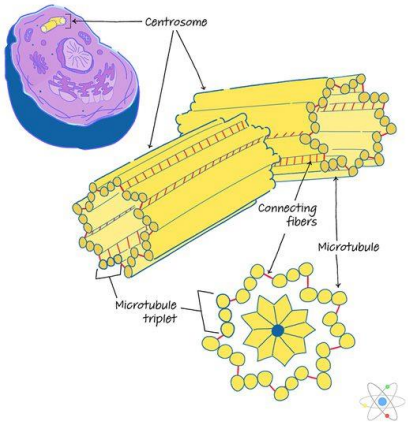

Figure 2: Centrosome complex of two centrioles with microtubule triplet structure. Reproduced from [27].

In a series of studies spanning a period of almost a quarter century, G. Albrecht-Buehler observed that living cells possess a spatial orientation mechanism located in the centrosome [23-26]. The centrosome is formed from an intricate arrangement of sets of microtubules, comprised of two perpendicular centrioles (Figure 2). This arrangement provides the cell with a primitive “eye” that allows it to locate the position of other cells within a two-to-three-degree accuracy in the azimuthal plane and with respect to the axis perpendicular to it [26]. Though it is still a mystery how the reception of electromagnetic radiation is accomplished by the centrosome, superradiant (and subradiant, below) behaviour in these microtubule aggregates may play a role.

Applying the techniques and methods of physics to biological systems has been very fruitful in recent years to shed new light on biological functions and to design new bio-inspired quantum devices for sensing, light harvesting, catalysis, quantum information processing, and quantum computation. There is now significant evidence that coherent effects play a role in different biological complexes, which has opened the possibility that quantum mechanics could play a functional role in biological systems. Most of the research on quantum biology has focused since 2007 on natural photosynthetic complexes. Such complexes are made of a network of chlorophyll molecules (photoactive in the visible range),

which are able to absorb sunlight and transport the excitation to a specific molecular aggregate (the reaction centre). Several studies have shown that coherent effects could play a fundamental role to enhance the light harvesting and energy transport efficiency of such systems.

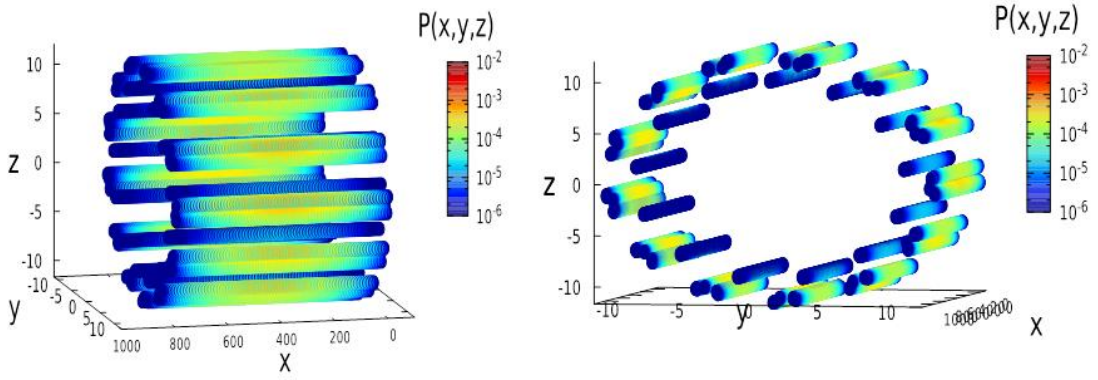

Figure 3. The quantum probability of finding the exciton on a tryptophan (TRP) of a microtubule segment of 100 spirals with 10,400 TRP molecules, is shown for the extended superradiant state in lateral view (left) and in cross section (right). Lengths are expressed in nanometers. Reproduced from [1].

Microtubules maintain the structural integrity of cells, they are involved in mitotic division, and they play an important role in neuronal signaling because very long (micron-scale) microtubules are generally stable in axons. As the role of photoexcitation in microtubules remains an open question, our recent studies of the optical properties of microtubules have been advanced by sophisticated methods typically used in quantum optics. In these studies [1], the existence of a superradiant ground state in the UV in microtubules has been predicted to emerge (Figure 3). This is evocative of what is usually found in photosynthetic complexes, which can show a superradiant state in the visible range in the low-energy portion of the spectrum. It is also intriguing that these highly symmetrical, light-absorbing structures have emerged to address two of the core processes at the origin of life: (1) photosynthesis, in order to capture sunlight for food storage, and (2) aerobic respiration, the biochemical inverse of (1), in which metabolic processes may be linked directly to UV, visible, and other ultraweak photon emissions via reactive oxygen species.

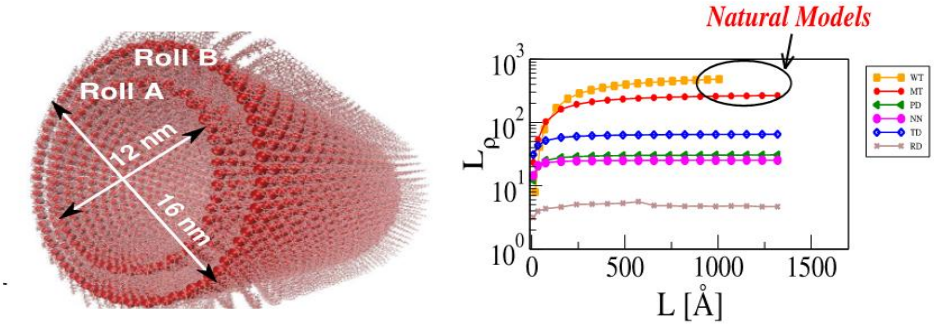

Figure 4. Photosynthetic nanotube (left) with natural models exhibiting the largest coherence lengths over many tube sizes (right). Reproduced from [28].

In photosynthetic molecular nanotubes from green sulphur bacteria (Figure 4), hundreds of thousands of chlorophyll molecules self-aggregate in a very symmetric and hierarchical way [28]. Such highly symmetric arrangements of molecules are able to enhance cooperative effects such as superradiance and supertransfer. It was shown that such naturally occurring nanotubes have the ability to support macroscopic coherent states, i.e., delocalized excitonic states coherently spread over many molecules, even at room temperature. The presence of macroscopic coherence in large molecular aggregates suggests the possibility of designing bio-inspired nanostructures able to exploit cooperative effects for efficient light harvesting, sensing, and energy transport. In a strikingly similar manner, the network of tryptophan molecules present in microtubules is able, due to their specific geometry, to create a superradiant ground state that can absorb efficiently in the UV. Even though the coupling between tryptophan molecules is an order of magnitude weaker than the coupling between chlorophyll molecules, large microtubule aggregates in neuronal bundles or in centrioles may sustain similar cooperative effects, such that quantum coherence might be preserved even at room temperature.

Delocalized excitonic states can have a much larger dipole strength than that of the constituent chromophores, and such giant transient dipoles can strongly couple to the electromagnetic field. Thus, these states are able to superabsorb light, i.e., they are able to absorb light at a rate which is much larger than the single-molecule absorbing rate. Indeed, the absorption rate of delocalized excitonic states can increase with the number of molecules over which the excitation is delocalized. Supertransfer is described in a similar way, with respect to movement of the excitation to an external molecular aggregate or between different parts of the same system. Specifically, an excitonic state delocalized on N molecules of one molecular aggregate can couple with an excitonic state delocalized on M molecules of a second aggregate with a coupling amplitude which is $\sqrt{NM}$ times larger than the coupling amplitude between single molecules belonging to different aggregates. Such supertransfer coupling is able to enhance the velocity of spreading of photoexcitations, and it has been shown to play an important role not only in photosynthetic complexes but also in other important biomolecular polymers, including cytoskeletal microtubules.

Interactions between the transition dipoles of photoactive molecules are in general very complicated, with the common coupling to the electromagnetic field more nuanced than simple dipole-dipole interactions, which are an effective description of a chromophoric

network only in the small-size limit (where the system size is much smaller than the excitation wavelength). However, for large aggregates one sometimes needs to go beyond the simple dipole-dipole interactions used in small aggregates. To do this, we have developed a model based on an effective non-Hermitian Hamiltonian interaction [1], commonly used in the quantum optics literature to study photoexcited molecular aggregates. This non-Hermitian description allows the possibility of donating the excitation back to the electromagnetic field through photon emission or of transferring excitation coherently between chromophores. Moreover, in the small-system-size limit it reduces to the familiar dipole-dipole interaction.

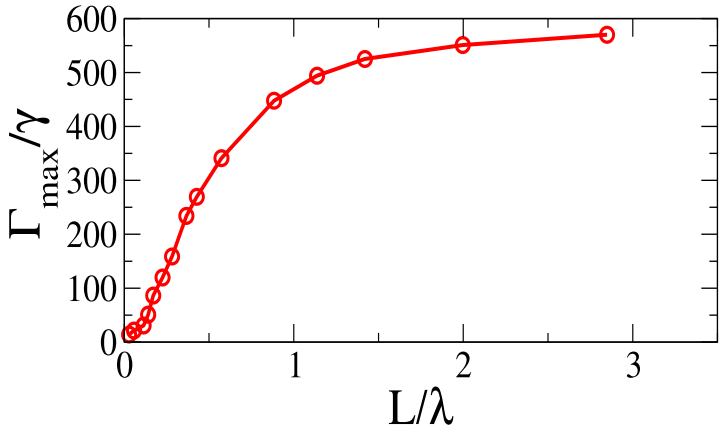

Figure 5. The maximum normalized decay width of a microtubule is shown as a function of its length *L* rescaled by the tryptophan excitation wavelength (280 nm). Reproduced from [1].

The imaginary part of the complex eigenvalues $\varepsilon = E - i\Gamma/2$ of such a non-Hermitian Hamiltonian determines the strength of the coupling of the excitonic states with the electromagnetic field and is connected with the dipole strength of the eigenstates of the system. While the coupling of a single aromatic molecule can be characterized by its decay rate γ, Γ determines the coupling of extended excitonic states with the electromagnetic field. Superradiant states are characterized by $\Gamma > \gamma$, while subradiant states are characterized by $\Gamma < \gamma$. Most importantly, for superradiant states, Γ should be proportional to the number of chromophores *N* for $L \leq \lambda$, where *L* is the system length and λ is the wavelength of the aromatic molecule optical transition, beginning to saturate for $L > \lambda$ (Figure 5). Because $\hbar/\Gamma$ is the lifetime of the excitonic eigenstate, larger values of Γ govern faster excitation decays. Since the process is symmetric under time-reversal, fast decaying states are also fast absorbing states. The advantage of this formalism, with respect to the simple dipole-dipole interaction, is that it allows us to consider system sizes that are larger than the wavelength of the absorbed light. This property becomes particularly important for UV excitation of biopolymeric structures like microtubules, which can generally be several orders of magnitude larger than the wavelength associated with the tryptophan molecular transitions (λ = 280 nm).

REFERENCES

1. Celardo, G. L. et al., On the existence of superradiant excitonic states in microtubules, New J. Phys. 21 023005 (2019).
2. Perutz, MF et al,. The stereochemical Mechanism of the cooperative effects in hemoglobin revisited, Annu. Rev. Biophys. Biomol. Struct. 27 1–34 (1998).
3. Scholes, G. D. et al., Using coherence to enhance function in chemical and biophysical systems, Nature 543, 647 (2017).
4. Chergui, M. Ultrafast photoinduced energy and charge transfer, Faraday Discuss., 216, 9 (2019).
5. Brixner, T. et al., Nature, 434, 625–628 (2005).
6. Skribanowitz, N., Herman, I. P., MacGillivray, J. C. & Feld, M. S. Observation of Dicke superradiance in optically pumped HF gas. Phys. Rev. Lett. 30, 309–312 (1973).
7. Spano, F. C. The spectral signatures of Frenkel polarons in H- and J-aggregates. Acc. Chem. Res. 43, 429–439 (2010).
8. Malcuit, M. S., Maki, J. J., Simkin, D. J. & Boyd, R. W. Transition from superfluorescence to amplified spontaneous emission. Phys. Rev. Lett. 59, 1189–1192 (1987).
9. Bradac, C. et al. Room-temperature spontaneous superradiance from single diamond nanocrystals. Nat. Commun. 8, 1205 (2017).
10. Scheibner, M. et al. Superradiance of quantum dots. Nat. Phys. 3, 106–110 (2007).
11. Miyajima, K., Kagotani, Y., Saito, S., Ashida, M. & Itoh, T. Superfluorescent pulsedemission from biexcitons in an ensemble of semiconductor quantum dots. J. Phys. Condens. Matter 21, 195802 (2009).
12. Dai, D. C. & Monkman, A. P. Observation of superfluorescence from a quantum ensemble of coherent excitons in a ZnTe crystal: evidence for spontaneous Bose-Einstein condensation of excitons. Phys. Rev. B 84, 115206 (2011).
13. Raino, G. et al, Superfluorescence from lead halide perovskite quantum dot superlattices, Nature 563, 671-675 (2018).
14. Monshouwer, R., Abrahamsson, M., van Mourik, F. & van Grondelle., R. Superradience and exciton delocalization in bacterial photosynthetic light-harvesting systems. J. Phys. Chem. B 101, 7241–7248 (1997).
15. Craddock T. J. A. et al, The feasibility of coherent energy transfer in microtubules, J. R. Soc. Interface 11 20140677 (2014).
16. Kurian, P. et al, Oxidative species-induced excitonic transport in tubulin aromatic networks: Potential implications for neurodegenerative disease, Journal of Photochemistry & Photobiology, B: Biology 175 109–124 (2017).

17. Schenkl, S.; van Mourik, F.; van der Zwan, G.; Haacke, S.; Chergui, M., Probing the ultrafast charge translocation of photoexcited retinal in bacteriorhodopsin. *Science 309* (5736), 917-920 (2005).

18. Leonard, J.; Portuondo-Campa, E.; Cannizzo, A.; van Mourik, F.; van der Zwan, G.; Tittor, J.; Haacke, S.; Chergui, M., Functional electric field changes in photoactivated proteins revealed by ultrafast Stark spectroscopy of the Trp residues. *P Natl Acad Sci USA 106* (19), 7718-7723 *(*2009).

19. Consani, C.; Aubock, G.; van Mourik, F.; Chergui, M., Ultrafast Tryptophan-to-Heme Electron Transfer in Myoglobins revealed by UV 2D spectroscopy. *Science 339*, 1586-1589 (2013).

20. Monni, R.; Al Haddad, A.; van Mourik, F.; Auböck, G.; Chergui, M., Tryptophan-to-heme electron transfer in ferrous myoglobins. *Proceedings of the National Academy of Sciences 112* (18), 5602-5606 (2015).

21. Shukla, N.; Pomarico, E.; Hecht, C. J. S.; Taylor, E. A.; Chergui, M.; Othon, C. M., Hydrophobic interactions of sucralose with protein structures. *Arch Biochem Biophys 639*, 38-43 (2018).

22. Shukla, N.; Pomarico, E.; Chen, L.; Chergui, M.; Othon, C. M., Retardation of Bulk Water Dynamics by Disaccharide Osmolytes. *The Journal of Physical Chemistry B 120* (35), 9477-9483 (2016).

23. Albrecht-Buehler, G. Phagokinetic tracks of 3T3 cells: Parallels between the orientation of track segments and of cellular structures which contain actin or tubulin. Cell 12, 333–339 (1977).

24. Albrecht-Buehler, G. Rudimentary form of cellular “vision.” PNAS 89, 8288–8292 (1992).

25. Albrecht-Buehler, G. Cellular infrared detector appears to be contained in the centrosome. Cell Motil. Cytoskeleton 27, 262–271 (1994).

26. Albrecht-Buehler, G.. Autofluorescence of Live Purple Bacteria in the Near Infrared. Experimental Cell Research 236, 43–50 (1997).

27. https://sciencing.com/centrosome-definition-structure-function-with-diagram-13717286.html

28. M. Gull; A. Valzelli; F. Mattiotti; M. Angeli; F. Borgonovi and G. L. Celardo. Macroscopic coherence as an emergent property in molecular nanotubes, New J. Phys. 21 013019 (2019).

OPTIMAL HEALTH: FROM QUANTUM BIOENERGETICS TO BODY FAT – JIMMY BELL

Mould R, Parkinson JRC, E Louise Thomas and Bell JD

Research Centre for Optimal Health, School of Life Sciences, College of Liberal Arts and Science, University of Westminster, 115 New Cavendish Street, London UK

The accepted definition of what an individual's "optimal health" represents has changed considerably over recent decades. Originally classified by a more disease-centric methodology, health was initially described as a relatively passive state defined solely by a lack of illness. However, such classification-by-absence can lead to the concept of optimal health straying into the more meta-physical realms of an individual's "well-being", thereby lacking the quantitative boundaries required for effective investigation (1). Over the years, the extraordinary successes of biomedical research have revealed the intricate details of individual disease pathogenesis. This has led to not only more effective treatments, but has also provided a much clearer understanding of the biological factors that can signal the anticipation and avoidance of morbidities, prior to manifestation. As a result, healthcare systems around the world have moved away from treatment per se, towards a more preventative approach (2). This paradigm shift in focus has led to profound clinical and financial benefits that will be required to sustain growing and ageing global populations.

At the core of this philosophy lies a re-evaluation of the term optimal health, whereby health is not simply defined as the absence of disease or illness but as an individual's ability to effectively respond to such insults. Nowhere is this approach more starkly exemplified than in the current COVID-19 pandemic, whereby responses to the virus range from completely asymptomatic to severe morbidity and death. What are the underlying health markers that will effectively indicate how patients will respond to the virus? How can targeted programs be developed to reduce the severity of symptoms?

OPTIMAL HEALTH, ACCELERATED AGEING AND BODY FAT

The search for sustained health and increased ***life-span*** has gone unabated from time immemorial. While the earliest known writing, as reflected by the *Epic of Gilgamesh*, gives prominence to the process of ageing and the limitations of a human life-span, in Greek mythology immortality is achieved by Tithonus, but cruelly marred by the unrelating ageing process of enfeeblement, ill health and dementia. Even then, they understood that a long life-span, without discontinuing the ageing process and maintaining optimal health, was futile. Today, the so called "health/anti-ageing industry" is worth trillions of dollars, with apparent little or no effect on the ageing process. If anything, while advances in technology, medicine, hygiene and nutrition have dramatically increased the average ***life-expectancy*** for most humans, accelerated ageing has become a common phenomenon for many.

It is now generally accepted that the life-span of humans appears to have a relatively fixed upper limit, one that does not seem to surpass 125 years. Indeed, leaving aside biblical references and unsubstantiated claims from some longed lived individuals, the French citizen Jeanne Louise Calment, with 122 years and 164 days, continues to be the longest-lived individual in human history. However, while the life-expectancy of individuals across

Europe has steadily increased in the last couple of centuries (now averaging of 81.5 years) most are not reaching their full life-span capacity, “wasting” over 40 years of potential existence. Coupled to this, is the fact that over 60% of the population lives today with one or more significant conditions or morbidities, in a process known as “morbidity expansion”, shortening their ***health-span***, in many cases, to less than half of their life-expectancy.

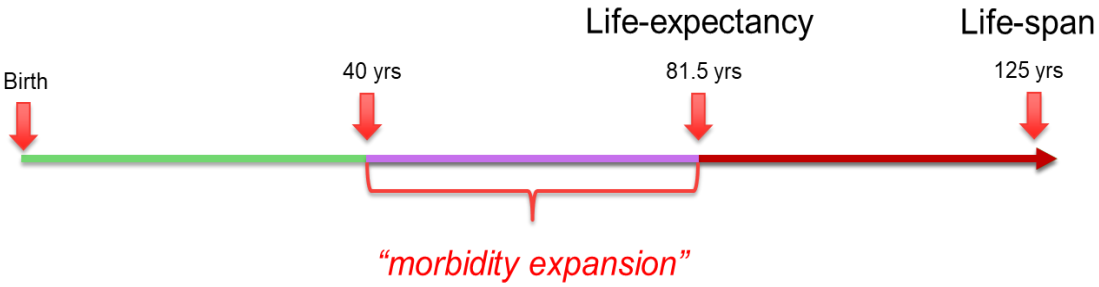

Figure 1 Life-span, Life-expectancy and Health-span. Despite a life-span capacity of c.a. 125 years, our average life-expectancy is still limited to 81.5 years, and even shorter health-span due to morbidity-expansion.

Lifestyle associated morbidities, including obesity, hypertension, some cancers, diabetes and multiform inflammatory dysfunctions have now been identified as key components of the processes driving accelerated ageing and the reduction of health-span. However, the underlying mechanism(s) that underpin these processes remain largely beyond our understanding, this despite the significant advances that have taken place in recent years. Obesity, especially central adiposity, was early on identified as a central component of accelerated ageing progression and health-span reduction. Yet how different adipose tissue depots contributed to these processes or even if they are their cause rather than simply the effect, was unclear.

Our initial foray into the field of optimal health arose from the development and application of magnetic resonance imaging (MRI) to the study of adiposity. MRI is the current gold standard technique for accurately measuring body fat distribution, allowing quantitative analysis of individual fat depots that are not represented by gross anthropometric measurements, such as BMI or waist circumference (**Figure 2**). This is important as increased accumulation of fat around the abdominal organs, termed visceral fat, and also ectopically within the liver and pancreas, are strongly associated with type 2 diabetes and cardiovascular disease (3). Conversely, increased abdominal subcutaneous adipose tissue appears to have a protective effect (4). In order to identify individuals at increased risk of succumbing to such conditions, we applied our imaging data to develop a phenotype based on increased visceral to subcutaneous abdominal fat measurements; the TOFI phenotype, thin on the outside, fat on the inside (5). Our research has continued to pursue the optimal balance of fat and muscle distribution that reflects a healthy, metabolically flexible individual.

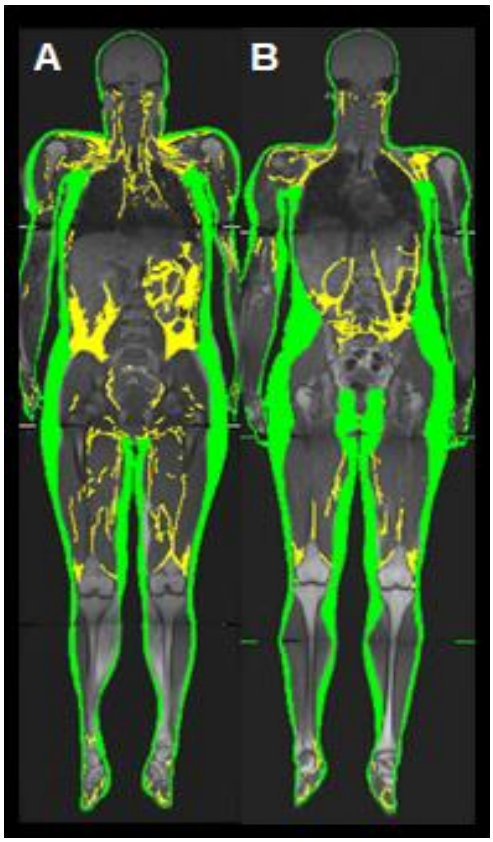

Figure 2. Whole body magnetic resonance imaging (MRI). MRI allows us to detect differences in fat deposition and provide a more accurate assessment of metabolic health. Subject A on the left has a lower volume of overall adipose tissue (in green), but more visceral fat (in yellow) in the abdomen compared to subject B on the right. These data indicate that despite being more obese by conventional measures, subject B is metabolically healthier than subject A.

One key factor linking specific fat depots and metabolic disease is inflammation; both the aforementioned visceral and ectopic fat stores are strongly linked to the production of key inflammatory markers and cytokines (6). Furthermore, numerous associated diseases are sensitive to and promote chronic inflammation; including cancers, features of the metabolic syndrome, cardiovascular disease and neurodegenerative conditions (7). As such, accurately measuring an individual's background inflammatory state is becoming a central focus of assessing health.

MITOCHONDRIA AND OPTIMAL HEALTH

Mitochondria are intrinsic to virtually all cellular processes and represent as close to something one could hope for as a master overseer and regulator of "optimal health". They generate numerous pro-inflammatory signals responsible for activating the immune system and generating inflammatory responses. Furthermore, mitochondria are key intermediates in the inflammatory response cycle and their dysfunction leads to many related disorders (8). Conversely healthy mitochondria reflect high levels of fitness and a healthy immune system.

The most prominent role of mitochondria lies in their regulation of bioenergetics via the production of ATP, but they also have critical roles in the production of metabolic and biosynthetic intermediates, the sequestration and release of intracellular calcium, the

production of reactive oxygen species (ROS), and apoptosis, the regulation of programmed cell death (9). Often overlooked, but highly relevant in the current climate is their role in the immune response to infections, including viruses, through the mitochondrial antiviral signalling proteins (MAVs) (10).

Once regarded as semi-autonomous, discrete organelles, we now know that the mitochondria are fully integrated in the cell; both physically, extending in dynamic networks stretching from the endoplasmic reticulum to the periphery, and functionally, by modulating nuclear gene expression. Importantly, we can quantitatively assess mitochondrial health by combining staining with microscopy techniques and novel imaging technology (**Figure 3**).

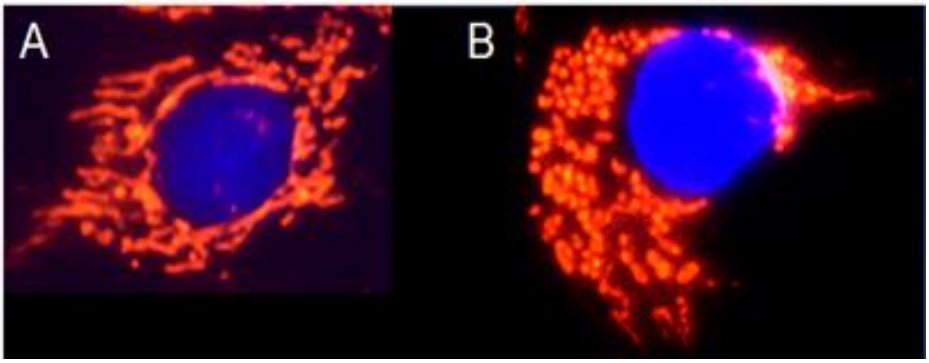

Figure 3 Assessing mitochondrial function. **(A)** Healthy mitochondria (red) form elongated networks around the nucleus (blue) that facilitates efficient cellular function. **(B)** The cell's mitochondria network has splintered into discrete, spherical units, a clear sign of stress

The level of mitochondrial stress is a key marker of cellular function; mild mitochondrial stress communicated to the nucleus can build a resistance to subsequent stresses via a process known as "mitohormesis" (11). Does the extent of mitohormesis define health? Perhaps the best examples to illustrate how are the mechanisms of mitochondrial ROS production. Mitochondrial ROS can act as both dangerous oxidants and essential signalling molecules; elevated ROS are implicit in the cellular response to a huge range of physiological insults, from obesity to infection. Yet at slightly higher levels, mitochondrial ROS activates various factors which in turn promote the expression of a number of genes that help a cell to protect itself against oxidative stress.

Clearly, there is a delicate equilibrium to mitochondrial ROS levels inside the cell, with the control of ROS production and disposal central to the fundamentally opposing biological pathways of protection or cell death. Indeed, the story of ROS does not end with oxidative stress and chemical signalling. The production of ROS itself yields a remarkable by-product; light. ROS, either directly or through oxidation of lipids, produce electronically-excited intermediates, which upon relaxation to their ground states release photons of light across the UV-visible spectrum (12). Emerging data has revealed that biophoton signalling may affect a number of cellular processes, including mitochondrial respiration and microtubule restructuring (13). Therefore, their intrinsic production by mitochondria indicates a potential role for biophotons in the function of this critical mediator of cellular metabolism.

CONCLUSION

Our research continues to explore the key role mitochondria play in defining not just cellular health, but the optimal health of an individual. Previous expertise in studying the effects of chronic conditions, such as obesity, diabetes and other features of the metabolic syndrome (8), has provided the basis for our novel research into the relationship and role of mitochondria in disease states. Furthermore, our current collaborations with experts in the fields of biophoton imaging and production may provide a means of identifying a completely unique cellular signalling process that could represent a way to effectively track and optimise an individual's optimal health.

REFERENCES

1. Ayres, J. S. The Biology of Physiological Health. Cell. 181, 250–269 (2020).
2. Fani Marvasti, F., and Stafford, R. From sick care to health care -reengineering prevention into the U.S. system. N. Engl. J. Med. 367, 889–991 (2012).
3. Despres, J.-P., Lemieux, I., Bergeron, J., Pibarot, P., Mathieu, P., Larose, E., Rodes-Cabau, J., Bertrand, O. F., and Poirier, P. Abdominal obesity and the metabolic syndrome: contribution to global cardiometabolic risk. Arterioscler. Thromb. Vasc. Biol. 28, 1039–1049 (2008).
4. Booth, A., Magnuson, A., and Foster, M. Detrimental and protective fat: body fat distribution and its relation to metabolic disease. Horm. Mol. Biol. Clin. Investig. 17, 13–27(2014).
5. Thomas, E. L., Parkinson, J. R., Frost, G. S., Goldstone, A. P., Dore, C. J., McCarthy, J. P., Collins, A. L., Fitzpatrick, J. A., Durighel, G., Taylor-Robinson, S. D., and Bell, J. D. The missing risk: MRI and MRS phenotyping of abdominal adiposity and ectopic fat. Obesity (Silver Spring). 20, 76–87 (2012).
6. Picard, F., and Deshaies, Y. Inflammation, ectopic fat and lipid metabolism: view from the chair. Int. J. Obes. Suppl. 2, S29–S30 (2012).
7. Liu, C. H., Abrams, N. D., Carrick, D. M., Chander, P., Dwyer, J., Hamlet, M. R. J., Macchiarini, F., PrabhuDas, M., Shen, G. L., Tandon, P., and Vedamony, M. M. Biomarkers of chronic inflammation in disease development and prevention: challenges and opportunities. Nat. Immunol. 18, 1175–1180 (2017).
8. Ponnalagu, D., and Singh, H. Insights Into the Role of Mitochondrial Ion Channels in Inflammatory Response. Front. Physiol. 11, 1–14 (2020).
9. Pfanner, N., Warscheid, B., and Weidemann, N. Mitochondrial proteins: from biogenesis to functional networks. Nat Rev Mol Cell Bio. 20, 267–284 (2019).
10. Mills, E., Kelly, B., and O'Neil, L. Mitochondria are the powerhouses of immunity. Nature, Immunol. 18, 488–498 (2017).
11. Nunn, A., Guy, G., and Bell, J. The quantum mitochondrion and optimal health. Biochem. Soc. Trans. 44, 1101–1110 (2016).
12. Le, M., McNeill, F. E., Seymour, C. B., Rusin, A., Diamond, K., Rainbow, A. J., Murphy, J., and Mothersill, C. E. Modulation of oxidative phosphorylation (OXPHOS) by radiation- induced biophotons. Environ. Res. 163, 80–87 (2018).
13. Rahnama, M., Tuszynski, J. A., Bokkon, I., Cifra, M., Sardar, P., and Salari, V. Emission of mitochondrial biophotons and their effect on electrical activity of membrane via microtubules. J. Integr. Neurosci. 10, 65–88 (2011).

F_1F_O ATP Synthase Rotary Mechanisms – Wayne Frasch

School of Life Sciences, Biomedicine and Biotechnology Faculty Group, Arizona State University, Tempe, USA

The F_1F_O ATP synthase (Fig 1A) is comprised of two rotary molecular motors that are attached by their rotors, and by their stators (1). The F_O motor, which is embedded in bioenergetic membranes, uses a non-equilibrium transmembrane gradient of protons (Na^+ in a few organisms) to power clockwise (CW) rotation of its ring of c-subunits relative to the stator proteins. The c-ring is attached to F_1 via subunit–ε, and the globular foot domain of subunit-γ. The γ-subunit coiled-coil domain serves as the drive shaft of the F_1 motor (Fig 1B, C), which penetrates into the core of the F_1 $(\alpha\beta)_3$-subunit ring where each αβ- heterodimer comprises a catalytic site (2).

When a proton gradient provides the energy to F_O to drive CW rotation, the F_1 drive shaft also rotates in this direction to induce conformational changes in the $(\alpha\beta)_3$-ring that result in the synthesis of ATP from ADP and inorganic phosphate (Pi). Each rotation of 120° induces the release of newly synthesized ATP from one catalytic site due to the staggered conformations of the three sites (3). In this manner, the F-type ATP synthase converts the energy from the non-equilibrium proton gradient into a non-equilibrium chemical gradient ($\Delta\mu_{ATP}$) in which the concentration ratio of ATP/ADP•Pi is far in excess of that found at equilibrium. A vast array of enzymes hydrolyze ATP as a means to use this non-equilibrium condition as an energy source, which sustains all life on earth.

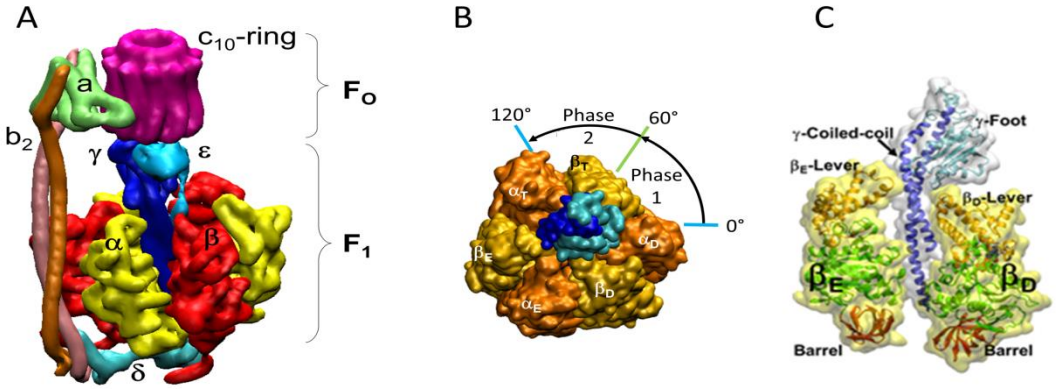

Figure 1. (**A**) Side view of cryo-EM structure of *E. coli* F_1F_O (PDB entry 5T4O). (**B**) Structure of bovine mitochondrial F_1 (PDB entry 1E79) viewed from the c-ring of F_O showing the subunit-γ coiled-coil (dark blue), and foot (light blue) domains. Subunit-ε is not shown. (**C**) Cross sectional side view of F_1 from **B** showing domain structures of subunits-β_E, β_D, and γ.

With rare exception, the F_1-ATPase motor can use the energy from a $\Delta\mu_{ATP}$ to drive anti-clockwise (ACW) rotation of subunit-γ in 120° power strokes that are separated by catalytic dwells during which ATP is hydrolyzed (4). This forces ACW rotation of F_O, which then

pumps protons across the membrane to create a proton gradient, which is used by some bacteria under specific environmental conditions. However, although F_O is much smaller than F_1, it is more powerful, and is able to minimize this wasteful expenditure of energy such that a significant ATP/ADP•Pi gradient is maintained under steady state conditions. Several organisms also employ regulatory mechanisms that minimize ATPase activity relative to the amount of ATP synthesized.

The F_1-ATPase can be purified from F_O, and studied as an ATPase-driven motor. Most of the catalytic residues for ATP synthesis/hydrolysis reside on subunit-β with the notable exception of the 'arginine finger' on subunit–α (2). In contrast to subunit-α, where ATP cannot be hydrolyzed and rarely dissociates, conformational changes in subunit-β result from ATP binding, hydrolysis, and release of ADP and Pi.

The $(\alpha\beta)_3$-ring is stabilized by a β-barrel domain on each subunit that assemble to form a crown (2). These subunits also contain an ATP-binding domain, and an α-helical 'lever' domain (Fig 1C). Most F_1 crystal structures (e.g. Fig 1B) represent a catalytic dwell conformation: a), with the γ-foot positioned above a β-subunit designated β_T that contains bound Mg-ATP; b), with the γ-coiled-coil interacting electrostatically with catalytic site β_E that is empty or contains bound Pi; and c), with the β_D catalytic site containing bound Mg-ADP (2,5). An obvious difference in the conformations of the three catalytic sites is that the position of the β_E-lever is extended, or open, relative to the closed lever conformation in β_T and β_D. As the result of each 120° power stroke, the γ-foot rotates ACW from its position above one β-subunit to the next, concurrent with conformational changes in the three β-subunits of $\beta_E \rightarrow \beta_T$, $\beta_T \rightarrow \beta_D$, and $\beta_D \rightarrow \beta_E$.

The first direct observation that the F_1-ATPase rotates was made by modifying the protein so that it could adhere to a microscope cover slip oriented with subunit-γ facing away from the surface (3). Subunit-γ was also modified to enable covalent attachment of a biotin molecule, which served as a means to attach a fluorescently decorated actin filament via streptavidin. Although a 12 nm diameter F_1 motor is too small to be observed by optical microscopy, the 2000 nm – 4000 nm long actin filaments attached to the F_1 rotor rotated upon addition of ATP, which was recorded using standard video at 30 frames sec^{-1}. This recording speed was sufficient to observe the slow rotation that resulted from the excessive drag created by the actin filament.

To capture detailed information concerning the much faster rotation that occurs when the F_1-ATPase is not rate-limited by drag, we used a 35x75 nm gold nanorod (AuNR) as a visible probe (6). We determined that the red and green photons scattered from the long and short axes, respectively, of an AuNR are polarized (7). Consequently, when viewed through a polarizing filter, the red and green scattered light intensities vary in a sinusoidal manner as a function of the rotary position of the AuNR relative to the polarizer orientation. Under these conditions, the AuNR attached to a rotating subunit-γ appears to blink red-green. To observe variations of subunit-γ angular velocity during a power stroke, rotations of single F_1-ATPases were recorded by aligning the microscope stage with a pin hole to collect light scattered from a single AuNR that blinks as the result of F_1-ATPase driven rotation (Fig 2A). The light was passed through a band pass filter to eliminate all but red light, and a polarizing filter that can be rotated. It was then focused on a single-photon avalanche detector to quantify intensity versus time. After the polarizer was rotated so that one of the three power strokes

began when the intensity of scattered light was at a minimum, rotation data was collected from each of ≥50 F_1 molecules for 5 sec. This duration was sufficient to capture rotation data from ~3,200 power strokes from each F_1 molecule that started from an intensity minimum (Fig 2B).

Intensity data were typically collected at 200 kHz (eq. to 200,000 frames sec^{-1}) or 5 μs per data point. Algorithms then determined the rotary position versus time by an $arcsine^{1/2}$ function, and the first derivative of these data determined the angular velocity versus rotary position for each power stroke. Average angular velocities were calculated for each 3° of power stroke rotation (8).

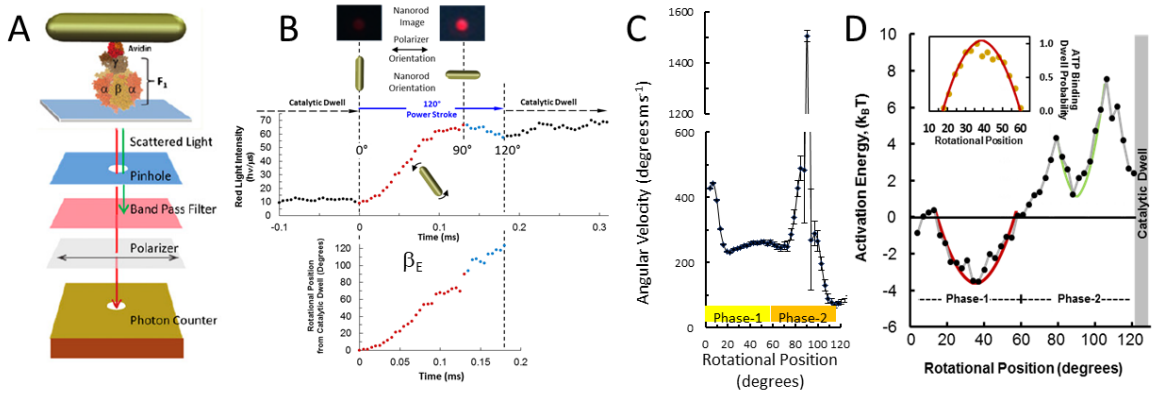

Figure 2. Single-molecule F_1-ATPase rotation measurements. (**A)** Experimental Schematic: subunit-γ biotinylated F_1 with his-tags attached to Ni-NTA cover slip, and to streptavidin-AuNR. Light scattered from the AuNR passed through a pinhole, band pass filter, and polarizer, then collected by an avalanche photodiode and sampled at 200 kHz. (**B)** Scattered light intensity vs. time during a power stroke, and catalytic dwells at saturating ATP concentrations. The polarizer was aligned perpendicular to the AuNR during the preceding catalytic dwell. Power Stroke rotational position vs. time calculated from using an $arcsine^{1/2}$ function. (**C**) Angular velocity vs. rotational position averaged from the first derivative of power stroke data like that of **B** averaged from thousands of power strokes obtained the same way. (**D**) Activation energy of the power stroke vs. rotation position determined by Årrhenius analysis of angular velocity data like that of **C** acquired between 16.3°C and 44.6°C. Inset: normalized probability of ATP-binding dwell formation at limiting ATP concentrations vs. rotational position.

During the power stroke, subunit-γ was found to exhibit several accelerations and decelerations at specific rotary positions (Fig 2C), which were grouped into 60° phases (9). The good fit of the Phase-2 (60° - 120°) angular velocity profile with molecular dynamics simulations of F_1 structures, which modelled the force applied to subunit-γ by ATP-binding dependent closure of the β_E lever (9,10), indicated that Phase-2 of the power stroke is primarily powered by ATP binding-dependent closure of the β_E lever that applies force to the γ-coiled-coil as to a crankshaft. At rate-limiting ATP concentrations, the average angular velocity during the first 60° (Phase-1) decreased. This resulted from the increased occurrence of an ATP-binding dwell where the duration was inversely proportional to ATP

concentration. Initial measurements suggested that the ATP-binding dwell occurred 40° after the start of the power stroke (4). However, with the increased resolution provided by the AuNR (Fig 2D, inset), we observed that ATP-binding dwells occur between 5° – 55° with a maximum probability at ~34° (11). Elevated ADP concentrations were found to induce a Phase-1 dwell like the ATP-binding dwell that results when ADP competes with ATP for binding to the empty catalytic site (9). High ADP also inhibited ADP release from the β_D catalytic site, which decreased Phase-2 velocities between rotary positions 80° – 110° with a maximum at ~95° (9).

The effects of temperature on the power stroke angular velocity profile were measured (Fig 2D), from which the energy profile of the power stroke was calculated by Årrhenius analysis (11). Although Phase-2 angular velocities increased with temperature, those of Phase-1 unexpectedly decreased. This latter dependence, which results in negative activation energies, clearly indicates that Phase-1 is powered by elastic energy. The negative activation energies occurred with a parabolic dependence between 5° – 55° with a maximum at ~34°. This parabolic dependence fit to the energy derived from a torsion spring with a spring constant of ~50 k_BT rad^{-2}, and matched the probability of ATP-binding dwell formation versus rotary position. Although activation energies were positive in Phase-2, a parabolic dip also occurred between 80° – 110° with a maximum at ~95° that fit to a spring constant of ~150 k_BT rad^{-2}. This parabolic dip was comparable to the dependence of angular velocity decreases observed at inhibitory ADP concentrations.

The plot of free energies of activation ($\Delta G^{\ddagger}$) versus rotational position was inversely proportional to the angular velocity profile of the power stroke (11). Simply, subunit-γ rotated more slowly over rotary positions with higher energy barriers, as expected. These measurements were carried out at saturating ATP and an ATP/ADP•Pi ratio with a $\mu\Delta_{ATP}$ of -31.25 k_BT. The efficiency of the reaction calculated from $\Delta G^{\ddagger}/\mu\Delta_{ATP}$ varied between 60% and 70% during the power stroke. These results were obtained under conditions in which the mechanism of the motor was rate-limiting. Higher efficiencies have been reported when the drag on the visible probe of rotation was rate-limiting.

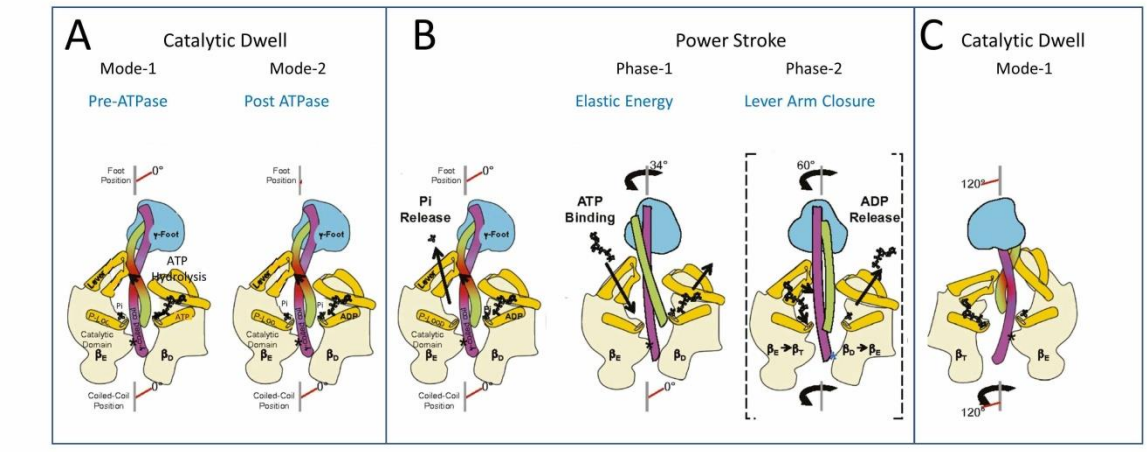

Figure 3. Elastic Coupling Mechanism of the F_1-ATPase Power Stroke. (A) Catalytic Dwell with ATP hydrolysis at β_D. **(B)** Power stroke Phase-1 starts with β_E-Pi release allowing γ-coiled-coil to unwind and rotate γ-foot. Power stroke Phase-2 initiated ATP binding to β_E, which closes the β_E– lever

upon subunit-γ. **(C)** Catalytic dwell begins, b-subunits change conformations, and ATP hydrolysis at new β_D rewinds torsion spring.

Our current understanding of the mechanism of *E. coli* F_1-ATPase driven rotation is summarized by the Elastic Coupling Model (11). During the catalytic dwell (Fig 3A), the β_D catalytic site hydrolyzes ATP to ADP and Pi during which elastic energy is stored when the γ-subunit coiled-coil is wound clockwise from its equilibrium position. Release of Pi from β_E ends the catalytic dwell (Fig 3B), which allows the coiled-coil to unwind and initiate Phase-1 of the power stroke. Binding of ATP to β_E, which occurs most often at 34°, provides the energy to complete Phase-2 of the power stroke. This binding induces a β_E conformational change that forces the closure of the β_E-lever domain to push subunit-γ to rotate by a cam-like action. Phase-2 rotation induces conformational changes $\beta_E \rightarrow \beta_T$, $\beta_T \rightarrow \beta_D$, and $\beta_D \rightarrow \beta_E$ of all three catalytic sites such that the motor is reset to the beginning of the next catalytic dwell (Fig 3C).

Mechanistic insight of the F_O motor was obtained by AuNR single-molecule studies (Fig 4A) using F_1F_O embedded in lipid bilayer nanodiscs (12-14). Upon addition of saturating ATP to induce F1-ATPase ACW rotation, some 120° power strokes rotated continuously comparable to those observed with purified F_1. However, other power strokes contained transient dwells that occurred every ~36° (Fig 4B). Since *E. coli* F_O contains a c_{10}-ring, this corresponds to the ability of subunit-a to stop F_1-ATPase driven rotation by binding to successive c-subunits in the ring (12). The 50 – 150 μsec duration of transient dwells is also consistent with the measured ensemble rates of proton translocation during ATP synthesis. In >70% of transient dwells, subunit-a not only stopped ACW rotation, but forced clockwise (CW) rotation in the ATP synthesis direction by as much as one c-subunit (13).

The pH dependence of transient dwell formation was determined between pH 5.0 and pH 9.0 (14). In ensemble experiments, optimal rates of ATP synthesis are achieved in membrane vesicles that have a ΔpH of 3 within the same pH range of 5 - 9 (15). The percent occurrence of transient dwells was determined from each single-molecule data set that comprised ~300 F_1-ATPase power strokes (Fig 4C), and the distribution of these percentages was plotted from data collected from ≥50 F_1F_O molecules (14). Between pH 7 – pH 9, the average occurrence of transient dwells was ~27%. This percentage increased with decreasing pH below pH 7 to an average maximum of ~55 % at pH 5. Based on these results, the pKa's of the subunit-a proton input and output channels to drive ATP synthesis were estimated to be 5.9 and 7.6, respectively, where both channels contribute to the force generated by the F_O motor to rotate CW and overcome the force of F_1-ATPase driven ACW rotation.

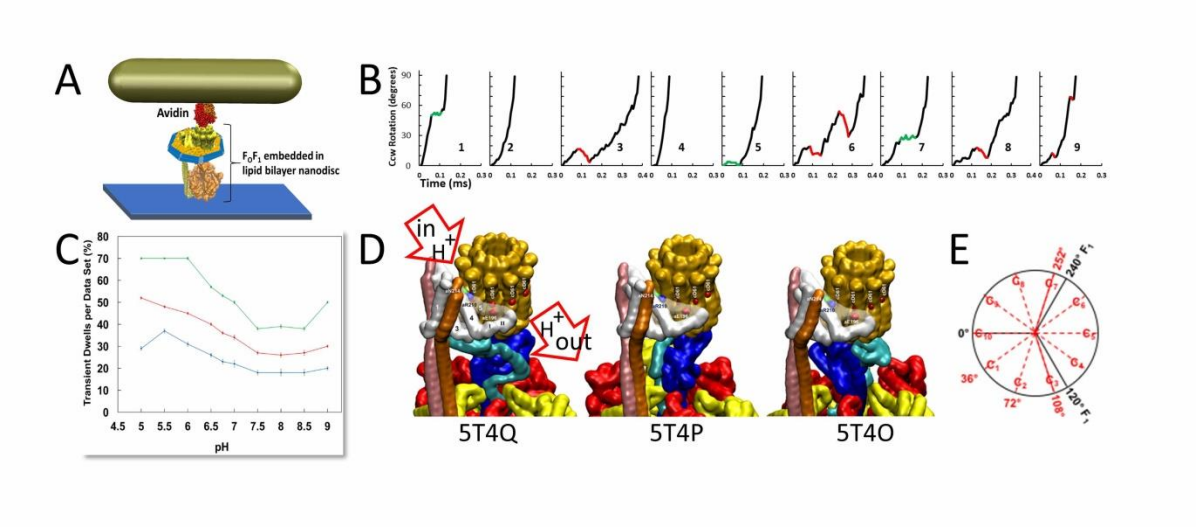

Figure 4. (**A**) Single-molecule rotation measurements of F_1F_O embedded in a lipid bilayer nanodisc. Anti-Clockwise rotation was initiated by addition of saturating ATP concentrations and rotation was detected as in Fig. 1. (**B**) Examples of rotational position vs. time of F_1F_O during F_1-ATPase-dependent power strokes at pH 5.0 where transient dwells: stop ACW rotation (power strokes 1, 5, 7); rotate CW in the ATP synthase direction (power strokes 3, 6, 8, 9); or are absent (power strokes 2, 4). (**C**) Dependence on pH of the average percent of transient dwells per data set with low (blue), medium (red), and high (green) probabilities of forming transient dwells. (**D**) Cryo-EM structures (PDB entries indicated) of the three states of autoinhibited *E. coli* F_1F_O showing the 120° rotary positions of subunit- γ relative to the peripheral stalk that each approximate an F_1 ADP-inhibited dwell. Proton input and output half-channels during ATP synthase-dependent CW rotation (red arrows). (**E**) Differences between the three 120° cryo-EM states vs. the 108° rotary alignment of c-subunits in the c_{10}-ring relative to subunit-a.

The distributions of transient dwell formation did not fit to a single Gaussian (Fig 4C), but fit best to the sum of three Gaussians with high, medium, and low average occurrences (14). Each Gaussian increased proportionally with decreasing pH, indicating that the low probability group was not converted to the medium, nor was the medium to the high. At pH 5.0, transient dwells occurred in an average of 70% of the power strokes, and 100% was observed in several of these data sets. These results are consistent with the fact that F_1-ATPase rotates in three 120° power strokes, while the c_{10}-ring rotates in 36° steps. Due to this disparity, 3 protons are consumed to synthesize one ATP during each of two catalytic sites, while 4 protons are required for ATP synthesis at the third catalytic site.

Three structural states of *E. coli* F_1F_O are evident by cryo-EM (Fig 4D) where subunit-γ is in one of three 120° rotary positions relative to the peripheral stalk that includes subunit-a (16). In the position between the two 3-proton steps, F_1 appears to be aligned with the c-ring. In the other two positions (Fig 4E), there is a positive and negative disparity of 12° (3 c-subunits x 36° = 108° versus 120° F_1 subunit-γ). Each single-molecule data set was derived from one F_1F_O molecule chosen at random from which only one of the three F_1 power strokes was examined (14). Since the distributions of transient dwells at any given pH was derived from ≥50 F_1F_O molecules, an average of 17 molecules from each of the three structural states were sampled. Consequently, the positive and negative strains imposed by

the 12° disparity in c_{10}-ring versus subunit-γ is evident as an increase and decrease, respectively, in the ability to form transient dwells (14,17).

The F-type ATP synthase is a member of a family of F-type rotary motors. Significant species-dependent structural differences include variations in the size of the c-ring that are currently known to range from 8 – 16 c-subunits, and differences in the subunits that comprise the peripheral stalk between F-types from mitochondria, chloroplast and eubacteria. In mitochondria, the folds in the inner membrane result from the formation of F-ATP synthase dimers, and tetramers that can form long chains. At least three different regulatory mechanisms exist that decrease ATP hydrolysis relative to synthesis. The F-type is also part of a superfamily of rotary motors that includes archaeal A_1A_O ATP synthases, or A/V-type ATP synthases, and eukaryotic vacuolar V_1V_O ATPases. Among other structural differences, the A-type and V-type contain two and three peripheral stalks, respectively. The V-type contains several additional subunits, and is only capable of ATP hydrolysis whereas only a few F-types are known thus far to be incapable of ATP hydrolysis. Other mechanistic differences include the absence of ATP-binding dwells in A-type and V-type ATPases, and a difference in the rotary position for Pi release in mitochondrial F-type relative to the motors from prokaryotes. At this time, the underlying molecular mechanisms that govern these differences are not understood.

REFERENCES

1. Spetzler, D., Ishmukhametov, R., Hornung, T., Martin, J., York, J., Jin-Day, L., and Frasch, W. D. (2012) Energy transduction by the two molecular motors of the F_1F_O ATP synthase. in *Photosynthesis: Plastid Biology, Energy Conversion and Carbon Assimilation* (Eaton-Rye, J. J., Tripathy, B. C., and Sharkey. T. D., eds.), Springer, Dordrecht, The Netherlands. pp 561-590
2. Abrahams, J. P., Leslie, A. G., Lutter, R., and Walker, J. E. (1994) Structure at 2.8 Å resolution of F_1-ATPase from bovine heart mitochondria. *Nature* **370**, 621-628
3. Noji, H., Hasler, K., Junge, W., Kinosita, K., Jr., Yoshida, M., and Engelbrecht, S. (1999) Rotation of Escherichia coli F_1-ATPase. *Biochem Biophys Res Commun* **260**, 597-599
4. Yasuda, R., Noji, H., Yoshida, M., Kinosita, K., and Itoh, H. (2001) Resolution of distinct rotational substeps by submillisecond kinetic analysis of F_1-ATPase. *Nature* **410**, 898-904
5. Bason, J. V., Montgomery, M. G., Leslie, A. G., and Walker, J. E. (2015) How release of phosphate from mammalian F_1-ATPase generates a rotary substep. *Proc Natl Acad Sci U S A* **112**, 6009-6014.
6. Spetzler, D., York, J., Daniel, D., Fromme, R., Lowry, D., and Frasch, W. (2006) Microsecond Time Scale Rotation Measurements of Single F1-ATPase Molecules. Biochemistry 45, 3117-3124
7. Hornung, T., Martin, J., Ishmukhametov, R., Spetzler, D., and Frasch, W. D. (2012) Microsecond resolution of Single Molecule Rotation Catalyzed by Molecular Motors, Methods in Molecular Biology 778, 273-289

8. Sielaff, H., Martin, J., Singh, D., Biukovic, G., Grüber, G., and Frasch, W. D. (2016) Power Stroke Angular Velocity Profiles of Archaeal A-ATP Synthase Versus Thermophilic and Mesophilic F-ATP Synthase Molecular Motors. J Biol Chem 291, 25351-25363

9. Martin, J. L., Ishmukhametov, R., Hornung, T., Ahmad, Z., and Frasch, W. D. (2014) Anatomy of F1-ATPase powered rotation. Proc Natl Acad Sci U S A 111, 3715-3720

10. Pu, J., and Karplus, M. (2008) How subunit coupling produces the gamma-subunit rotary motion in F1-ATPase. Proc Natl Acad Sci U S A 105, 1192-1197

11. Martin, J. L., Ishmukhametov, R., Spetzler, D., Hornung, T., and Frasch, W. D. (2018) Elastic coupling power stroke mechanism of the F1-ATPase molecular motor. Proc Natl Acad Sci U S A 115, 5750-5755

12. Ishmukhametov, R., Hornung, T., Spetzler, D., and Frasch, W. D. (2010) Direct observation of stepped proteolipid ring rotation in E. coli FOF1-ATP synthase. Embo Journal 29, 3911-3923

13. Martin, J., Hudson, J., Hornung, T., and Frasch, W. D. (2015) FO-driven Rotation in the ATP Synthase Direction against the Force of F1 ATPase in the FOF1 ATP Synthase. J Biol Chem 290, 10717-10728

14. Yanagisawa, S., and Frasch, W. D. (2017) Protonation-dependent stepped rotation of the F-type ATP synthase c-ring observed by single-molecule measurements. J Biol Chem 292, 17093-17100

15. Turina, P., Samoray, D., and Gräber, P. (2003) H+/ATP ratio of proton transport-coupled ATP synthesis and hydrolysis catalyzed by CFOF1-liposomes. EMBO Journal 22, 418-426

16. Sobti, M., Smits, C., Wong, A. S., Ishmukhametov, R., Stock, D., Sandin, S., and Stewart, A. G. (2016) Cryo-EM structures of the autoinhibited E. coli ATP synthase in three rotational states. eLife 5:e21598

17. Sielaff, H., Yanagisawa, S., Frasch, W. D., Junge, W., and Börsch, M. (2019) Structural Asymmetry and Kinetic Limping of Single Rotary F-ATP Synthases. Molecules 24, 504-529

THE QUANTUM BEAT OF LIFE & THE AMPLIFICATION OF A PRINCIPLE – ALISTAIR NUNN

Research Centre for Optimal Health, School of Life Sciences, College of Liberal Arts and Science, University of Westminster, 115 New Cavendish Street, London UK

INTRODUCTION

Are significant quantum effects important for life to exist and if so, were they necessary to get it started? It could be argued that this chicken and the egg paradox is dependent on scale, and of course, time. In biological terms, time, in the shape of evolution, would indicate that prokaryotes came before chickens or eggs, and in quantum terms, they are too big to be entangled and display wave-particle duality. But this doesn't stop some entertaining thought exercises in relation to Schrödinger's chicken* (Figure 1).

In size terms, quantum mechanical effects exist largely in the "nanoscopic" range, which is the world of atoms and small clusters of atoms at the nanometre scale. However, these can make their presence felt in the slightly bigger "mesoscopic" world, which is defined as somewhere ranging from tens of nanometres to a micrometre. At sizes above a micrometre, we enter the classical everyday "macroscopic" world. In biology, the mesoscopic domain is thus populated with large complexes, membranes, chromatin and enzymes, and sits between that described by quantum and classical mechanics [1]. This of course hints that some aspects of mesoscopic/macroscopic biology *could* be influenced by the nanoscopic quantum world if the conditions were right via some kind of quantum upscaling.

Manipulation of this mesoscopic boundary has generally only been achievable in physics laboratories as only here has it been possible to make fairly large clusters of atoms behave coherently at very low temperatures by preventing decoherence due to interaction with the outside environment. Because of this, it was thought by some that the "warm and wet" environment of biology meant that quantum effects were largely confined to the molecular/atomic world and no larger scale quantum effects could occur, apart from in physics laboratories. Others however, such as Schrödinger, did suggest that quantum effects could be "amplified" by biology for its own ends [2]. In fact, quantum superposition has been detected using matter-wave interferometers of much larger molecules of 2,000 atoms or more in physics laboratories, which is in the range of many proteins at around 27 kDa [3]. Thus it is certainly possible that nanoscopic quantum effects can upscale under the right conditions.

Thus the big question is: "does biology require quantum upscaling in order to function"? In effect, does biology require "supranormal" quantum effects to even work? Which then raises the question that if it does, did its origins require amplification of basic quantum principles, or did it do this later and thus its beginnings can be purely described by "classical" chemistry? In short, if quantum biology does exist, which came first, the chicken or the quantum?

In this short paper I build on the hypothesis that life has, indeed, embraced significant quantum effects, and has evolved mechanisms to modulate them at scales that might seem impossible at the temperatures it normally operates at. We suggest that it has achieved this by amplifying some fundamental quantum properties associated with particular molecules

that were generated before life began, in particular, chromophores and metallic compounds with semi-conductor properties. Today, biochemists tend to call them “cofactors”, which might be better termed “prefactors” – in effect, we suggest that some “core” seed molecules enabled life to begin as they had particular quantum properties around which amino acids and lipids accreted to enhance/upscale quantum effects. This not only has profound implications as to where life started, but how we understand processes such as ageing and disease.

Figure 1*. Quantum chickens. With regards chicken-related humour, in the quantum world the chicken-egg paradox could be explained via a wave function as it could be both a chicken and egg at the same time, and regarding the question about why the chicken crossed the road, it didn’t need to, as it was already on both sides at once. This of course answers a favourite children’s riddle: “what is the difference between a chicken” – answer, “one leg is both the same”. This of course is perfectly acceptable if it is described by a wave function.

THE EMERGENCE OF QUANTUM BIOLOGY

What is life? This is a question that Erwin Schrödinger, a key player in the development of quantum mechanics, asked in the 1940s about the role of this new field in explaining biology and whether or not things like “quantum coherence” were important [2], but the question had been asked even earlier by folk such as Pascual Jordan and Neils Bohr [4]. Hence, in a way, the birth of quantum physics was actually the birth of quantum biology. It has actually been said that quantum biology is the application of quantum theory to explain aspects of biology that classical physics fail to address accurately, in particular, charge transfer in electron transport, but also enzyme function, olfaction and bird navigation [5].

As any description of chemistry is ultimately based on quantum mechanics, it could therefore be said that biology is simply a classical macroscopic expression of the quantum world. But the real question is of course has biology, through evolution and natural selection, perhaps embraced some principles and amplified them as they are required to make biology “work”

by taking them further up the size scale in the mesocopic regimen that at first sight seem possible.

Physicists can generate quantum effects across larger scales, such as coherence, but only at very cold temperatures where the environment does not induce "decoherence"'. But has biology found a way around this problem in its "warm and wet" environment? Some say it has, for instance, Gábor Vattay has suggested that many biomolecules evolved at quantum criticality at a point between being a conductor and an insulator, suggesting a universal and unique system of charge transport, supporting the supposition that life exists as a complex system poised at the edge of chaos. This is, of course, very much a condensed-matter viewpoint [6]. This is exemplified by the ideas of Herbert Fröhlich that biology could be using coupled vibrational modes that could drive chemical reactions without creating heat stress [7].

All in all, there is both theoretical and now emerging evidence that there is a role for non-trivial quantum phenomena in biology, in particular to explain how light energy is captured in photosynthesis involving groups of chromophores. One possibility is that thermal vibrations can induce coherent exciton-vibration interactions, so called "quantum beating" [8,9]. This emerging concept, which can also be called environment-assisted quantum transport (ENAQT), is where the external environment enhances the transport efficiency of quantum particles and centres around the exciton transfer complex when referring to the connection between the antenna and reaction centre in photosynthesis [10].This quantum beating idea has also been extended to explaining consciousness and neural synchronisation involving microtubules [11]; a concept perhaps most (in)famously proposed by Roger Penrose [12]. In a way, this might imply that large scale effects, like consciousness, are built upon quantum resonance.

WHY LIFE NEEDS TUNNELLING AND THUS A BIOQUANTOME

Albert Szent-Györgyi once said: "life is nothing but an electron looking for a place to rest". He was of course referring to the emerging evidence about the role of electron transport in energy generation, which may, at least theoretically, involves electron tunnelling in the electron transport chain (ETC) [13-15]. As electron transfer is central to all biology, it could therefore be that electron tunnelling, certainly in the nanometre scale, could be critical via molecular tunnel junctions. One group does now appear to have observed quantum biological tunnelling (QBET) in cytochrome C using a biological tunnelling junction and spectroscopy [16]. However, it may not just be electrons that are tunnelling, but also protons – kinetic isotope experiments seem to suggest that proton tunnelling could also be a key part of enzymatic reactions, especially involving flavoprotein and quinoprotein systems [17,18].

Given that all life relies on using electron transfer to generate a proton gradient and that electron and proton transfer are intimately linked, it is possible that life has evolved to modulate quantum effects. Of course the "why" is a much deeper question, but may revolve around thermodynamics and the idea that life is a self-organising dissipative structure [19,20]. Thus, at the very highest level the bioquantome evolved to accelerate entropy in the Universe, which probably says something about a definition of life itself. It also says something about how far the amplification into the mescopic region, in terms of size, it might go; it may not need to result in coherence across a very large number of atoms, but it may

be more about tuning the coherence field in terms of size and duration in relation to controlling homeostasis to adapt to an every changing environment. In effect, turning amplified quantum effects on and off may be just as important.

SCHRÖDINGER'S 14 ÅNGSTROM LEGACY – A QUANTUM PILE UP IN HIS CAT?

Everybody is familiar with the idea of Schrödinger's cat, and maybe even of his chicken, but very few people will be familiar with Schrödinger's mitochondria. In his famous thought experiment he raised the rather seemingly non-intuitive concept that quantum physics might indicate that a cat in a box with a vile of cyanide linked to a radioactive isotope could be both dead and alive at once, and we wouldn't know until we opened the box, as its fate hinged on radioactive decay, a quantum-determined principle. The underlying premise, of course, is that observation of something precipitates the system into one particular state. However, what might actually be happening is dependent on what your favourite interpretation of quantum physics is, for instance, realism or non-realism and observer-induced quantum collapse [21].

Quite apart from this, it was rather fortuitous that he chose cyanide as a means of dispensing the cat (which one wonders would probably die from any other number of means, including lack of oxygen, lack of food, or radioactive poisoning and a visit from the animal welfare people – as well as having an aversion to the smell of almonds). Cyanide binds complex IV (cytochrome oxidase) of the ETC in the mitochondrion, so preventing electrons from making their final journey to oxygen. This of course has a number of immediate consequences, ranging from an electron pile up and the potential to generate free radicals to halting respiration, neither of which are particularly good for a cell. However, the fate of the mitochondrion would be dependent on the dose, and its state, and what it was doing at the time. Low doses of cyanide simply induce an adaptive response called hormesis, which might cause the demise of an individual mitochondrion, but upregulate the cells production of new ones and ways of dealing with the situation, including inducing glycolysis to generate energy without oxygen for a bit. Higher doses might activate apoptosis, so removing weaker cells, while even higher concentrations might damage the more oxygen sensitive organs, until at a high enough dose, the organism dies. Nothing in biology is really "on" or "off", but is a spectrum of possibilities, which of course does sound familiar to those versed in quantum theory.

Thus, in a way, Schrödinger's cat is actually a vastly more complex problem depending on the way you view it, and if the bioquantome idea is correct, it could even be that quite unknowingly, Schrödinger had designed an experiment within an experiment involving how we define life and quantum physics (figure 2). It would seem that the cat could also be in any number of intermediate states that could be determined by quantum tunnelling within its mitochondria. It has been suggested, at least for complex 1, that the rate of electron tunnelling can be tuned by the reduction status of the chain to maximise efficient energy conversion and minimise generation of free radicals, in effect redox tuning [14]. Electron tunnelling through ETCs seems to be dependent on cofactors being less that 14 Ångstroms (Å) apart, and within multi-electron catalytic transfer complexes such as cytochrome oxidase, less than 7Å. Interestingly, longer distances could enable fine tuning through impedance by

slowing tunnelling [22]. In effect, this might indicate that biology is tuning the mesoscopic quantum "status" to ensure homeostasis.

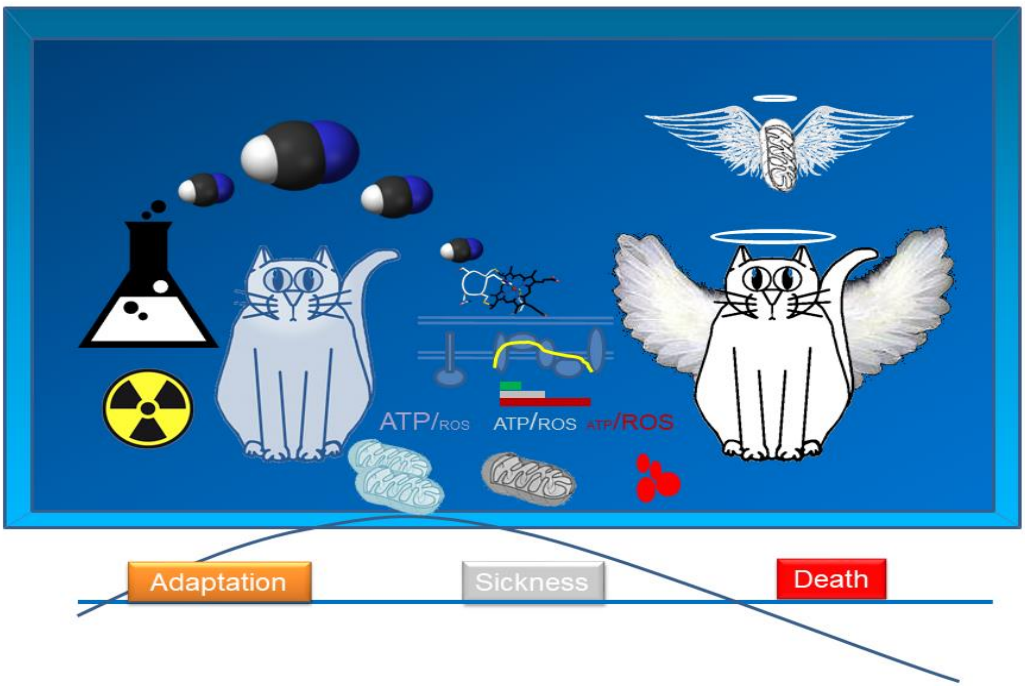

Figure 2. Schrodinger's mitochondria and the quantum pile up. Depending on dose, the cat could be in any number of states, ranging from enhanced through adaptation, to sick, to dead – each state would depend on how well its mitochondria could adapt, and thus, how many cells would survive or die. This effect is of course described by hormesis. In turn, the survival, or not, of each mitochondria may well depend on how well it can control tunnelling through its ETC. Slowing down electron transport may be just as important as speeding it up, as is redirecting the flow of electrons to somewhere safer and to use them as a signal. The yellow line is the path that electrons should take to oxygen, but depending on how much cytochrome C oxidase is inhibited, the degree of ETC reduction will vary. Total inhibition (red) will cause the ETC to stop, with loss of membrane potential and ATP production, a build-up of oxygen, and a huge generation of ROS. Inability to manage this will lead to death. Lesser inhibition could have a variety of outcomes, for instance, cells which are more used to hypoxia, or less dependent on respiration, and/or can be renewed, will either survive, or undergo apoptosis and the organ they are in will be rebuilt.

QUANTUM EFFECTS AT THE BEGINNING – CAN WE MAKE AN INFORMED GUESS ON ITS ROLE AT THE ORIGINS OF LIFE?

The proposed existence of a bioquantome is all well and good, but what of its beginnings? What clues are there to life embracing significant quantum effects to function? How life got going has probably fascinated our species ever since we first had the brainpower to start thinking about it. Today, there are a plethora of theories, but they generally fall into metabolism or replication first, with the former involving simple chemicals such as acetate, and the latter, molecules like RNA. There are also various discussions about where it started. For instance, did it start in deep sea alkaline vents, in geothermal ponds on land generated by meteorite impact, or in the first few meters of the sea? Furthermore, was

ultraviolet light essential and at what stage? Were the essential chemicals generated here on earth, and/or in circumstellar environments, and was the first life phototrophic or chemolithotrophic? However it started, it is probably best described as a thermodynamic dissipative self-organising structure [23-27]. The importance of self-organisation has been suggested by Tamulius and Grigalavicius in their concept of the “fatty acid world”, which is based on significant quantum mechanical effects such as quantum entanglement that promotes tunnelling and lead to self-reproducing photoactive fatty acid micelles driven by light energy; a key component of these are aromatic moieties such as squarine. Later, these micelles incorporated nucleic acids. This would hint at phototrophic beginnings, especially as there is evidence that some kind of photosynthetic life may have been around as long ago as 3.9 Gya [28,29].

Another strong theory is that it may have started in alkaline thermal vents, which at least for the purposes of this discussion, provides us with another starting point for the development of a bioquantome in relation to primordial catalysts. Alkaline thermal vents seem to supply most of the necessary conditions for prebiotic chemistry, including energy and a mineral boundary layer, and critically, iron (nickel) sulphur complexes that could have enabled electron transfer across a pH gradient and a way to tap into an energy supply that could generate basic metabolites. Evidence does seem to suggest that acetyl CoA pathway is one of the oldest. Thus the theory goes that the pores in submarine alkaline thermal vents enabled proton gradients across a Fe(Ni)S barrier that could drive the reduction of CO_2 by hydrogen [30]. Iron-sulphur clusters have some unique electronic properties due to anti-ferromagnetically coupled high-spin iron atoms that make them very good for electron transfer, and when combined with cysteine, water and other residues, seem to be key in enhancing electron tunnelling [31].

However, there is another interesting theory that the formation of these iron-sulphur structures may have also required UV light [32]. It may therefore be relevant that the ETC, which is essential for the generation of a proton gradient in all life, is very rich in “cofactors” that may have existed before life and were key in a prebiotic chemistry. For example, it is possible to make protocells that can transfer electrons from reduced nicotinamide adenine dinucleotide (NADH), via prebiotic iron-sulphur proteins, to ubiquinone – a process that is improved by insertion in a membrane. On exposure to hydrogen peroxide, this system can generate a proton gradient [33]. The relevance of UV is that many essential coenzymes display UV absorption spectra, such as the B vitamins [34,35]. In fact many of the central components of the ETC, such as the cytochromes and ubiquinone, as well as the flavins and NADH, all have associated absorption spectra, ranging from the UV to visible. Their spectra change according to their redox status, which certainly in the case of NAD(P)H and FAD+, as they fluoresce, has not only become a mechanism to image mitochondria, but also to monitor metabolism [36]. Critically, some phenolic antioxidants seem to utilise tunnelling to enhance their ability to prevent propagation of free radicals, such as ubiquinol and vitamin E, which does appear to rely on the ability of their double-bond structure to resonate [37]. In fact, it is well known that many plant secondary metabolites containing aromatic pie-electron systems (based on double-bond structures), such as those containing the phenolic moiety, are very good at both acting as sunscreens and acting as anti-oxidants [38,39].

In summary, it seems that a central component of all life, the ETC, contains elements that might depend on significant quantum effects to function, which may well have existed before life started, suggesting they were key in its inception. Moreover, many contain double bond structures that would support this, in particular, with an absorbance spectrum in the UV. Some researchers have suggested that the cristae of mitochondria were originally part of a light harvesting system [40], and that the components switched function as oxygen levels rose [41]. What seems to be clear is that life requires both iron-sulphur proteins, and compounds with conjugated double bond systems that seem to capitalise on quantum effects, and it is likely that both were available before life started. That many of these UV absorbing compounds could act as sunscreens, for instance, NAD [42], might suggest that life required both a proton-gradient and UV, the latter being key in generating both abiotic photochemistry and in providing the selective pressure to "kick start" life as a system to dissipate a large energy potential, which required quantum effects to enable this system operate at the edge of chaos. In effect, life is manipulating quantum effects at the mesoscopic scale to provide it with the necessary robustness to maintain structure that obeys the second law of thermodynamics. Clearly, one has to be careful as to the direction of causation, but Figure 3 summarises one possible idea on the origins of life and quantum mechanics. Whatever the origins of life actually turn out to have been (or be, if we consider life beyond Earth), it is possible that studying quantum effects might be key in helping us understand it.

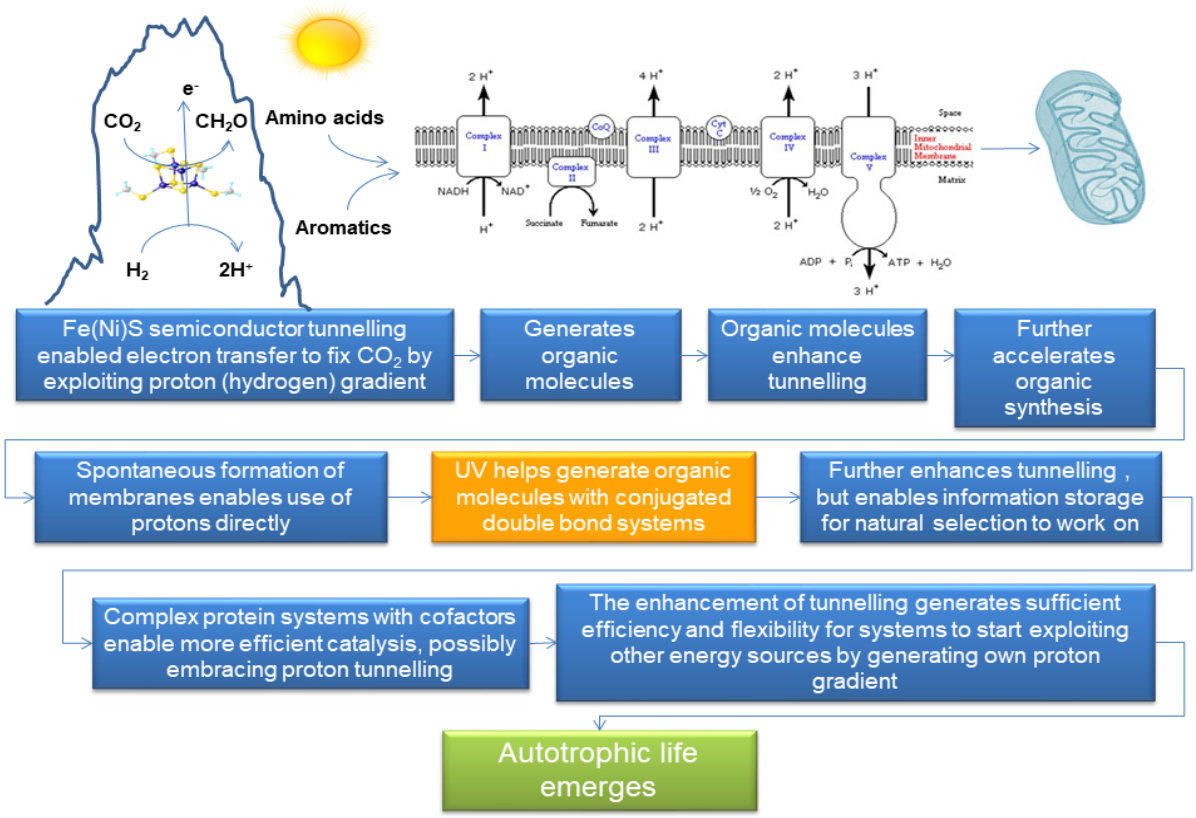

Figure 3. A quantum tunnel with UV kick start. If tunnelling enabled electrons to flow in iron-nickel-sulphur compounds and thus utilise a hydrogen energy gradient that could have existed in a thermal vent, then this could be one way life started. However, it is also possible that UV light was also essential to help drive the chemistry of dissipative molecules that could have accreted to enhance this process.

REFERENCES

1 Nakatsuji, N. Mesoscopic science, where materials become life and life inspires materials. A great opportunity to push the frontiers of life, materials, and biomaterials sciences. *Biomater. Sci.* **1**, 9-10, doi:10.1039/c2bm90001g (2013).

2 Schrodinger, E. *What is life? The physical aspect of the living cell.*, (Cambridge University Press, 1944).

3 Fein, Y. Y. *et al.* Quantum superposition of molecules beyond 25 kDa. *Nature Physics*, doi:10.1038/s41567-019-0663-9 (2019).

4 McFadden, J. & Al-Khalili, J. The origins of quantum biology. *Proc. R. Soc. A* **474**, doi:10.1098/rspa.2018.0674 (2018).

5 Marais, A. *et al.* The future of quantum biology. *J R Soc Interface* **15**, doi:10.1098/rsif.2018.0640 (2018).

6 Vattay, G. S., D.; Csabai,; Nassimi, A.; Kaufmann, S.A. Quantum Criticality at the Origin of Life. *J. Phys.: Conf. Ser 626 012023* (2015).

7 Srobar, F. Frohlich systems in cellular physiology. *Prague Med Rep* **113**, 95-104 (2012).

8 Lim, J. *et al.* Vibronic origin of long-lived coherence in an artificial molecular light harvester. *Nat Commun* **6**, 7755, doi:10.1038/ncomms8755 (2015).

9 O'Reilly, E. J. & Olaya-Castro, A. Non-classicality of the molecular vibrations assisting exciton energy transfer at room temperature. *Nat Commun* **5**, 3012, doi:10.1038/ncomms4012 (2014).

10 Zerah-Harush, E. & Dubi, Y. Universal Origin for Environment-Assisted Quantum Transport in Exciton Transfer Networks. *J Phys Chem Lett* **9**, 1689-1695, doi:10.1021/acs.jpclett.7b03306 (2018).

11 Craddock, T. J., Priel, A. & Tuszynski, J. A. Keeping time: could quantum beating in microtubules be the basis for the neural synchrony related to consciousness? *J Integr Neurosci* **13**, 293-311, doi:10.1142/S0219635214400019 (2014).

12 Hameroff, S. & Penrose, R. Consciousness in the universe: a review of the 'Orch OR' theory. *Phys Life Rev* **11**, 39-78, doi:10.1016/j.plrev.2013.08.002 (2014).

13 Hayashi, T. & Stuchebrukhov, A. A. Quantum electron tunneling in respiratory complex I. *J Phys Chem B* **115**, 5354-5364, doi:10.1021/jp109410j (2011).

14 de Vries, S., Dorner, K., Strampraad, M. J. & Friedrich, T. Electron tunneling rates in respiratory complex I are tuned for efficient energy conversion. *Angew Chem Int Ed Engl* **54**, 2844-2848, doi:10.1002/anie.201410967 (2015).

15 Moser, C. C., Farid, T. A., Chobot, S. E. & Dutton, P. L. Electron tunneling chains of mitochondria. *Biochim Biophys Acta* **1757**, 1096-1109, doi:10.1016/j.bbabio.2006.04.015 (2006).

16 Xin, H. *et al.* Quantum biological tunnel junction for electron transfer imaging in live cells. *Nat Commun* **10**, 3245, doi:10.1038/s41467-019-11212-x (2019).

17 Sutcliffe, M. J. *et al.* Hydrogen tunnelling in enzyme-catalysed H-transfer reactions: flavoprotein and quinoprotein systems. *Philos Trans R Soc Lond B Biol Sci* **361**, 1375-1386, doi:10.1098/rstb.2006.1878 (2006).

18 Layfield, J. P. & Hammes-Schiffer, S. Hydrogen tunneling in enzymes and biomimetic models. *Chem Rev* **114**, 3466-3494, doi:10.1021/cr400400p (2014).

19 Pulselli, R. M., Simoncini, E. & Tiezzi, E. Self-organization in dissipative structures: a thermodynamic theory for the emergence of prebiotic cells and their epigenetic evolution. *Biosystems* **96**, 237-241, doi:10.1016/j.biosystems.2009.02.004 (2009).

20 Prigogine, I. & George, C. The second law as a selection principle: the microscopic theory of dissipative processes in quantum systems. *Proc Natl Acad Sci U S A* **80**, 4590-4594 (1983).

21 Smolin, L. *Einstein's unfinished revolution; the search for what lies beyond the quantum*. (Allen Lane, Penguin Random House, 2019).

22 Moser, C. C., Page, C. C. & Dutton, P. L. Darwin at the molecular scale: selection and variance in electron tunnelling proteins including cytochrome c oxidase. *Philos Trans R Soc Lond B Biol Sci* **361**, 1295-1305, doi:10.1098/rstb.2006.1868 (2006).

23 Lane, N. *The Vital Question: Why is Life the way it is?* , (Profile Books Ltd, 2015).

24 Morowitz., E. S. a. H. J. *The origin and nature of life on earth.*, (Cambridge University Press, 2016).

25 Michaelian, K. Microscopic dissipative structuring and proliferation at the origin of life. *Heliyon* **3**, e00424, doi:10.1016/j.heliyon.2017.e00424 (2017).

26 26 Egel, R., Lankenau, D.-H. & Mulkidjanian, Y. Y. (Springer-Verlag, Berlin Heidelberg, 2011).

27 Chatterjee, S. A symbiotic view of the origin of life at hydrothermal impact crater-lakes. *Phys Chem Chem Phys* **18**, 20033-20046, doi:10.1039/c6cp00550k (2016).

28 Tamulis, A. & Grigalavicius, M. The emergence and evolution of life in a "fatty acid world" based on quantum mechanics. *Orig Life Evol Biosph* **41**, 51-71, doi:10.1007/s11084-010-9211-4 (2011).

29 Tamulis, A. & Grigalavicius, M. Quantum entanglement in photoactive prebiotic systems. *Syst Synth Biol* **8**, 117-140, doi:10.1007/s11693-014-9138-6 (2014).

30 Lane, N. Proton gradients at the origin of life. *Bioessays* **39**, doi:10.1002/bies.201600217 (2017).

31 Hayashi, T. & Stuchebrukhov, A. Electron tunneling pathways in respiratory complex I. The role of the internal water between the enzyme subunits. *J Electroanal Chem (Lausanne)* **660**, 356-359, doi:10.1016/j.jelechem.2011.03.014 (2011).

32 Bonfio, C. *et al.* UV-light-driven prebiotic synthesis of iron-sulfur clusters. *Nat Chem* **9**, 1229-1234, doi:10.1038/nchem.2817 (2017).

33 Bonfio, C. *et al.* Prebiotic iron–sulfur peptide catalysts generate a pH gradient across model membranes of late protocells. *Nat Cat* **1**, 616-623, doi:10.1038/s41929-018-0116-3 (2018).

34 Knak, A., Regensburger, J., Maisch, T. & Baumler, W. Exposure of vitamins to UVB and UVA radiation generates singlet oxygen. *Photochem Photobiol Sci* **13**, 820-829, doi:10.1039/c3pp50413a (2014).

35 Monteverde, D. R., Gomez-Consarnau, L., Suffridge, C. & Sanudo-Wilhelmy, S. A. Life's utilization of B vitamins on early Earth. *Geobiology* **15**, 3-18, doi:10.1111/gbi.12202 (2017).

36 Shuttleworth, C. W. Use of NAD(P)H and flavoprotein autofluorescence transients to probe neuron and astrocyte responses to synaptic activation. *Neurochem Int* **56**, 379-386, doi:10.1016/j.neuint.2009.12.015 (2010).

37 Inagaki, T. & Yamamoto, T. Critical role of deep hydrogen tunneling to accelerate the antioxidant reaction of ubiquinol and vitamin E. *J Phys Chem B* **118**, 937-950, doi:10.1021/jp410263f (2014).

38 Cockell, C. S. & Knowland, J. Ultraviolet radiation screening compounds. *Biol Rev Camb Philos Soc* **74**, 311-345 (1999).

39 Brunetti, C., Fini, A., Sebastiani, F., Gori, A. & Tattini, M. Modulation of Phytohormone Signaling: A Primary Function of Flavonoids in Plant-Environment Interactions. *Front Plant Sci* **9**, 1042, doi:10.3389/fpls.2018.01042 (2018).

40 Munoz-Gomez, S. A., Wideman, J. G., Roger, A. J. & Slamovits, C. H. The Origin of Mitochondrial Cristae from Alphaproteobacteria. *Mol Biol Evol* **34**, 943-956, doi:10.1093/molbev/msw298 (2017).

41 Dibrova, D. V., Shalaeva, D. N., Galperin, M. Y. & Mulkidjanian, A. Y. Emergence of cytochrome bc complexes in the context of photosynthesis. *Physiol Plant*, doi:10.1111/ppl.12586 (2017).

42 Nunn, A. V. W., Guy, G. W., Botchway, S. W. & Bell, J. D. From sunscreens to medicines: Can a dissipation hypothesis explain the beneficial aspects of many plant compounds? *Phytother Res*, doi:10.1002/ptr.6654 (2020).