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Half a century of molecular bioenergetics

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Abstract

Molecular bioenergetics deals with the construction, function and regulation of the powerhouses of life. The present overview sketches scenes and actors, farsighted goals and daring hypotheses, meticulous tool-making, painstaking benchwork, lucky discovery, serious scepticism, emphatic believing and strong characters with weak and others with hard arguments, told from a personal, admittedly limited, perspective. Bioenergetics will blossom further with the search focused on both where there is bright light for ever-finer detail and the obvious dark spots for surprise and discovery.

Introduction

Early research into 'bioenergetics', the energy supply for life, started in the 18th Century. Jan Ingen-Housz [1] discovered that plants produce biomass at the expense of sunlight, the ultimate energy source, and water plus gases, the substrates. In his study on vegetables, Ingen-Housz noticed their "great power of purifying the common air in the sunshine and of injuring it in the shade and at night" [1]. It was a first appreciation of the production and re-consumption in the reaction cycle between photosynthesis and respiration of what was later coined 'oxygen' and 'carbon dioxide'. More than two centuries later, Karl Lohmann (in 1929) discovered ATP, Vladimir Engelhart (in 1935) found that it powers muscle activity, and Fritz Lipmann (between 1939 and 1941) emphasized "energy-rich phosphate bonds" as the main carriers of chemical energy in the cell. David Keilin [2] and Otto Warburg [3] were the first to discover proteins involved in respiration, namely cytochrome *c* and 'Atmungsferment', alias cytochrome *c* oxidase, respectively.

Max Perutz's programmatic article entitled 'Proteins, the machines of life' [4] set the path for today's molecular understanding of life. His work on the crystal structure of haemoglobin revealed, for the first time, structural determinants of protein function, here the mechanics of co-operative oxygen binding (for which he was awarded the Nobel Prize in Chemistry in 1962). At this time, only a few proteins involved in photosynthesis and respiration were known; none was structurally resolved or only crystallized. Those proteins were black boxes scattered over a wide open, but highly relevant, research field. It has attracted scientists from a broad range of disciplines.

Molecular bioenergetics started with the analysis of spectroscopic signatures and reaction rates. In 1955, elements of the respiratory electron transport from various substrates to oxygen were tracked by Britton Chance and Ron Williams [5,6] who monitored transients of pigment cofactors. Photosynthesis was more difficult to tackle owing to the higher speed of its partial reactions. This complication was then compensated by the benefit of non-invasive stimulation by short light pulses. In 1961, three biophysicists, Lou Duysens [7], Bessel Kok [8] and Horst Witt [9], independently concluded that green plant photosynthesis is powered by two photosystems which, acting in a serial electron transport chain, drive electrons from water to $NADP^+$. PSII (Photosystem II) oxidizes water to yield oxygen and protons. It reduces PSI (Photosystem I) which, in turn, reduces $NADP^+$ to NADPH. The comprehension between biophysicists, who studied spectroscopic transients, and biochemists, who were after the 'real' products, was almost nil. When confronted with Witt's reaction scheme at a conference in 1962, Warburg mused: "Could you tell us how the chemical mechanism of photosynthesis can be

Key words: cell respiration, electron transport, molecular bioenergetics, phosphorylation, photosynthesis, proton transport.

Abbreviations used: pmf, protonmotive force; PSI, Photosystem I; PSII, Photosystem II.

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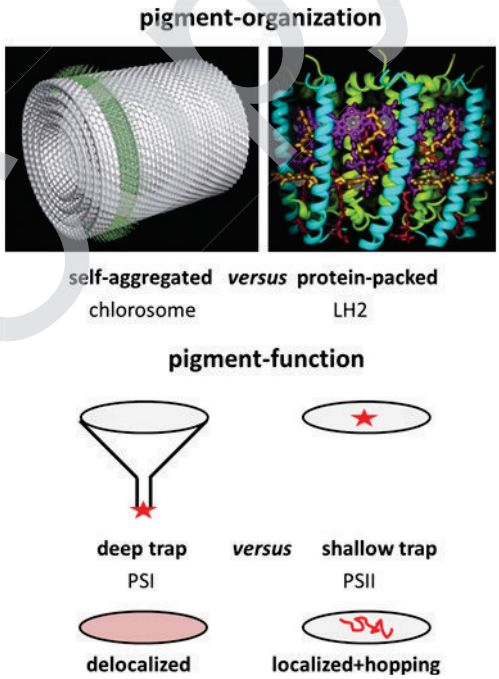
described on the basis of your spectroscopic observations?" Witt countered with a well-aimed jibe at his eminent critic, the pioneer of oxygen detection, by observing that "it would be difficult to deduce the mechanism of a combustion engine based only on sniffing the exhaust" (see [10]). The detailed analysis of the respective electron transport chains progressed rapidly owing to new tools in spectroscopy (e.g. pulsed lasers, EPR) and rapid kinetics, as pioneered by Manfred Eigen, Ronald Norrish, George Porter (joint winners of the Nobel Prize in Chemistry in 1967) and Britton Chance (see his fascinating account in [11]).

The atomic structure of the pertinent membrane proteins

Starting from Max Perutz's programme in 1945, it took more than two decades until the photosynthetic reaction centre of a purple bacterium was solubilized in functional form [12], and it took another two decades until Johann Deisenhofer, Robert Huber and Hartmut Michel (joint winners of the Nobel Prize in Chemistry in 1988) published a first structural model at 3 Å (1 Å = 0.1 nm) resolution [13]. It was the first structure of any membrane protein ever. A decade later, at a legendary Bioenergetics Gordon Conference in 1995, at which Hartmut Michel had already presented his structural model of bacterial cytochrome *c* oxidase [14], Shinya Yoshikawa described his yet to be published structure of a mammalian oxidase [15] (for its properties, see the article by Peter Rich and colleagues in this issue of *Biochemical Society Transactions* [15a]). Shortly before Yoshikawa's talk ended, the unexpected coincidence of two new structures was rightly underscored by fireworks for the Fourth of July celebrations outside the thin-walled audience. Today, structural models are available for all of the proteins of respiration and photosynthesis. The largest is PSI from green plants with a molecular mass of 660 kDa, hosting almost 200 chlorophyll molecules [16]. The similarly large ATP synthase is the most agile machine of all. By mechanic transmission, a rotary chemical generator [17] is mechanically coupled to a rotary electrochemical motor ([18,19] and see below). Whether complex I, a super-stoichiometric proton pump in mitochondria, operates by similarly pronounced mid-range mechanical interactions [20] has still to be established (see the articles by Leo Sazanov and Volker Zickermann in this issue of *Biochemical Society Transactions* [20a,20b]). PSII, the water–quinone oxidoreductase, has revealed its protein structure at 1.9 Å resolution (see [21] and references therein). When clocked by flashes of light, its catalytic Mn_4Ca cluster steps through four sequentially higher oxidation states until (in 1 ms) the reaction with bound water proceeds to yield dioxygen. The pooling of four oxidizing equivalents before initiating the four-electron reaction with water controls hazardous intermediates (e.g. hydroxyl radical and superoxide) on the way to dioxygen. The Mn_4Ca cluster proper has withstood unequivocal structural analysis because of its ready reduction during exposure of PSII crystals to X-rays [22]. For the time being, two other approaches, namely magnetic resonance spectroscopy {ENDOR (electron nuclear double resonance) [23]} and theoretical chemistry (density functional theory [24]), seem to converge towards one particular structural model of the metal centre and its ligands, including water (-derivatives). X-ray crystal structural analysis may soon take up and challenge or corroborate this concept by a novel 'probe before destroy' approach where a PSII crystal is exposed to the ultra-short and intense X-ray pulse (100 fs) of a free-electron laser [25]. Structural detail on the Mn_4Ca moiety with bound water derivatives is a requisite to disclose the detailed reaction mechanism of this 'holy grail' of photosynthesis.

Figure 1 | Light absorption, excitation energy transfer and trapping

High quantum yield despite large variations between antennae systems (see the text for details and references). Chlorosome structural model by Alfred Holzwarth (http://www.cec.mpg.de/forschung/heterogene-reaktionen/photochemistry.html) [148,149]; LH2 (light-harvesting complex 2) model by Richard Cogdell (http://www.gla.ac.uk/researchinstitutes/biology/staff/richardcogdell/researchinterests/lh2complex/lh2imagegallaries/lh2imagegallerywholecomplex/) [150].

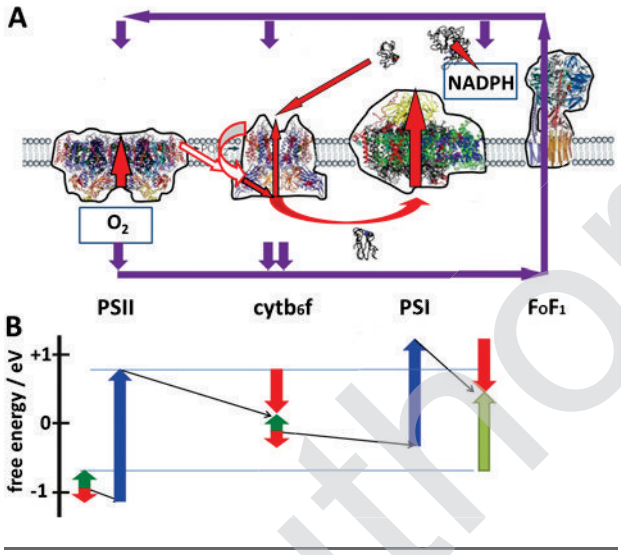


Common principles govern the transfer of excitation in photosynthesis and of electrons in photosynthesis and respiration

Molecular bioenergetics has blossomed into an unforeseen resolution of its machinery not only in space (2 Å), but also in time (<1 ps). The painstaking elucidation of complexity has been a prerequisite to fully appreciate the remarkable simplicity and robustness of Nature's engineering. Two examples of this follow.

(i) Antennae pigments capture light (Figure 1). The excitation energy migrates between some 100 pigment molecules until being trapped by the photochemically active

Figure 2 | Electron and proton transfer of oxygenic photosynthesis
(**A**) Architecture of the electron transport chain {Reproduced by permission from Macmillan Publishers Ltd: *Nature Reviews Molecular Cell Biology* [Nelson, N. and Ben-Sham, A. (2004) The complex architecture of oxygenic photosynthesis. Nat. Rev. Mol. Cell Biol. **5**(12): 971–982], © 2004. [151]}, and pathways for the transfer of electron (closed red arrows) and hydrogen (open red arrows). Sites of proton uptake and release plus the lateral proton transfer between pumps and the ATP synthase (purple arrows). (**B**) Energy profile in eV. The energy input by one quantum of red light into each of PSII and PSI is marked by blue arrows, energy dissipation by red arrows and the gain (i) in the form of the redox couple $^1/_4$ O_2 and $^1/_2$ NADPH by a light green arrow, and (i) additionally by electrogenic proton translocation by dark green arrows.



pigment cluster. Different types of pigments are involved, and the construction principles to bring them into close (although not too close) contact are diverse. In green bacteria, the chlorophyll molecules are self-aggregated [26]. In green plants and purple bacteria, they are embedded in a protein matrix [16,27]. The photochemically active pigment cluster, the trap, may be energetically lower relative to the antennae (deep trap as in PSI), at an equal level (shallow trap as in PSII) or even higher. The excitation energy may be delocalized (in some 10 fs) over many pigment molecules (coherent transfer) or hopping from one pigment to the other. "Lessons from Nature about solar light harvesting" have been presented [28]. Despite the large diversity of antennae construction, the quantum efficiency of energy trapping (at low light intensity) mostly exceeds 85 %.

(ii) The photochemical trap and the electron transport chain (Figure 2A). The trap drives electron transfer along a cascade of protein-embedded electron carriers. Starting from the first steps in the picosecond time domain (e.g. [29,30]) up to consecutive slower steps in milliseconds to seconds, the rate of the transfer between each pair of embedded electron carriers is exponentially related to their edge-to-edge distance. Chris Moser and Les Dutton [31] have analysed the rate of pairwise electron transfer in both respiration and photosynthesis. The exponential dependence of the rate on the distance holds over 13 orders of magnitude, for several donor–acceptor pairs, and in different protein environments. At a given edge-to-edge distance, the rate of electron transfer is only slightly affected by the electrostatic properties of the particular protein environment as described by Rudy Markus's theory of nuclear tunnelling [32] (winning him the Nobel Prize in Chemistry in 1992). When the free energy difference between the electron donor and the acceptor is properly tuned to the nuclear reorganization energy, the role of the protein scaffold is to tune the equilibrium rather than the forward rate. The very fast primary electron transfer steps in photosynthesis and the consecutive slower ones are each accompanied by a fall in free energy (Figure 2B) that favours the useful forward over wasteful back reactions. As has been pointed out by Bill Rutherford [33], energy efficiency is sacrificed for directionality (for the overall efficiency of photosynthesis, see below). Although the majority of electron transfer steps occurs between cofactors 'fixed' in their protein matrix, some steps are governed by random walk and electrostatic docking to the respective partner molecule (for plastocyanin, see [34]).

The enigmatic link between electron transport and ATP synthesis

At the time when the proteins involved in photosynthetic and respiratory electron transfer came into light, the construction principle of the embedding membrane was still obscure. It was assumed that proteins in biological membranes are rigidly layered on a lipid matrix. A particular role of the membrane for ATP synthesis was not in focus. In 1953, Bill Slater had seeded a general belief among biochemists that electron transfer generates a phosphorylated intermediate, (~P), which drives the synthesis of ATP [35]. It was based on a supposed similarity with glyceraldehyde-3-phosphate dehydrogenase, a soluble protein [36]. In 1961, two authors proposed very different concepts, both involving protons and the coupling membrane [36,37]. E.J.P. Williams, an inorganic chemist, proposed that the electron transport was coupled to proton injection into an "anhydrous" environment (e.g. the lipid core of the membrane), and that very low local pH shifted the equilibrium between phosphate, ADP and ATP towards the latter [37]. His article was the starter for the new *Journal of Theoretical Biology*. In the same year, Peter Mitchell, who had previously worked on the energy requiring translocation of metabolites across bacterial membranes [38], postulated the "coupling of photophosphorylation to electron and hydrogen transfer by a chemiosmotic type of mechanism" [39]. Without any empirical evidence, Peter Mitchell rightly foresaw in 1961 that vectorial electron transport crossed the membrane, and, coupled with proton uptake, hydrogen transfer and proton release, generated transmembrane pmf (protonmotive force) for the synthesis of ATP. Visionarily, he perceived "Proton-translocation phosphorylation in mitochondria, chloroplasts and bacteria (as) natural fuel cells and solar cells" [40].

The above three concepts for ATP synthesis, briefly stated as '(~P)', 'localized H^+' and 'delocalized H^+', became fiercely defended dogmata among disjunctive factions of bioenergeticists. To cope with overdoses of concentric attack against his view in conferences, Peter Mitchell used to ostentatiously remove his hearing aids. The discussion style of some leaders in the field, in loose terms strong characters with weak arguments, was indeed astounding for newcomers. On the other hand, youngsters found fertile grounds in this environment, or in Karl Popper's wording: "Critical thinking must have before it something to criticize, and this... ...must be the result of dogmatic thinking" [41]. Vigorous experimentation and fervent debates lined the path to 'the truth', until Peter Mitchell eventually received the Nobel Prize in Chemistry in 1978. "Opening Pandora's Box", the catchy title of a sociological analysis of this scientific debate [42], has been a thrilling venture extending into present days (see below).

Electrogenic proton pumps and a proton-translocating ATP synthase were only hypothetical until scientists in the photosynthesis field provided first evidence for Mitchell's hypothesis. In 1966, André Jagendorf published a straightforward test. He subjected broken chloroplasts to an acid–base jump and observed the formation of ATP [43]. The proponents of the (~P)-hypothesis were not convinced, of course, they argued that a pH jump might cause reverse electron-transport, formation of (~P) and then ATP. In 1968, Horst Witt and I characterized a spectroscopic signal as an intrinsic molecular voltmeter in the thylakoid membrane [44]. It was very rapidly formed with equal contributions from both photosystems and linked to proton transfer [45] (Figure 2A), and attributable to a functional unit of at least 100 [46] (later 10^5 [47]) electron transport chains. Baz Jackson and Tony Crofts found and calibrated a similar electrochromic signal in chromatophores of a purple bacterium [48]. Soon thereafter and in collaboration with Bernd Rumberg and Hartmut Schröder, I showed the following [49]: (i) the originally slow decay of the flash-light-induced voltage was accelerated under phosphorylating conditions; (ii) the extra charge flow was stoichiometrically correlated with the amount of ATP formed; (iii) an ionophore-induced electric conductance specific for alkali-cations competed with the conductance of the ATP synthase and diminished the ATP yield; and (iv) if the transmembrane voltage fell below a threshold, both the extra-conductance and ATP synthesis were inactivated. Later, it became clear that the deactivation of the oxidized chloroplast enzyme at subthreshold pmf prevents the hydrolysis of mitochondrial ATP by chloroplasts at night (see [50] for pmf regulation of the reduced and the oxidized ATP synthase). For photosynthesis in plants and bacteria, the above cited and further work had established the essentials of Mitchell's hypothesis, namely vectorial electron transport, electron–hydrogen loops (i.e. net proton pumping) and proton translocation linked to ATP synthesis. In oxygenic photosynthesis, the pmf accounts for approximately one-quarter of the useful work derived from sunlight, and the redox couple $^1/_2$ NADPH and $^1/_4$ O_2 for the rest (Figure 2B). In mitochondria, all useful work derived from reducing oxygen to water comes as pmf.

At this time, bioenergetics was dominated by students of mitochondria. For them, the evidence in favour of Mitchell's hypothesis resulting from photosynthesis research did not really count, and strong contrary winds blew against his view. In 1973, Dieter Oesterhelt discovered light-driven proton pumping by bacteriorhodopsin and ATP synthesis in halobacteria [51], and Ephraim Racker and Walter Stoeckenius reconstituted this proton pump with mitochondrial ATP synthase in liposomes [52]. Peter Mitchell added another facet to active proton translocation by electron–hydrogen loops, namely the protonmotive Q-cycle involving cytochrome $bc_1(f)$ [53]. Marten Wikström and Klaas Krab discovered extra proton pumping in cytochrome *c* oxidase in addition to the chemical proton consumption for water production [54].

In 1977, Peter Mitchell's pre-eminent critics eventually gave in. In a joint publication (truly a series of companion papers) Paul Boyer, Britton Chance, Lars Ernster, Ephraim Racker and Bill Slater, with Peter Mitchell alphabetically filed in, admitted that the chemiosmotic concept was probably right [55]. One year later, in 1978, Peter received the Nobel Prize in Chemistry. From then on, his concept has reflected back into and greatly fertilized the field of group translocation that had stimulated his original hypothesis. The lactose permease, Ron Kaback's lifelong devotion, is one example of this fertile branch of bioenergetics [56–59].

The mechanism of cyclic proton flow between pumps and the ATP synthase along both sides of the coupling membrane has remained a matter of debate until today. Several laboratories followed Williams's traits of proton injection into the hydrophobic core of the membrane. 'Localized coupling mechanisms' were proposed along either of two categories, intramembrane proton ducts and delayed escape of protons from the surface into the adjacent bulk phase. Whereas the evidence for the first has dwindled away, the latter merits a closer look. Studies on the propagation of a proton pulse along the surface of bacteriorhodopsin membranes have suggested a lateral diffusion coefficient by orders of magnitude less than in pure water (see, e.g., [60,61]). The observed slowing of pulse propagation is probably attributable to reaction diffusion, involving proton-buffering groups at the surface [62,63]. At the surface of a pure lipid membrane, the lateral diffusion coefficient is approximately half of its magnitude in bulk water [64]. Enhanced lateral mobility of protons at the surface over their mobility in bulk water has not been reported. However, there is good evidence for an energy barrier that slows the escape of protons from the membrane surface into the bulk, and this version of a localized coupling may be physiologically important. The barrier has been attributed to a layer of ordered water at the surface [65–68]. It would not matter in equilibrium (or a static head situation) as has been considered by Peter Mitchell. However, when stationary proton flow from proton pumps drives the ATP synthase, it provides greater pmf

between surface and surface than between bulk and bulk. This amendment to the original chemi-'osmotic' hypothesis may be particularly relevant for alkalophilic bacteria, as discussed elsewhere [66]. They perform ATP synthesis with a bulk-to-bulk pH difference that compensates for the electric potential difference, i.e. at virtually zero pmf [69]. It may also resolve a long-standing conflict over membrane-sequestered proton ducts. Dick Dilley's group has repeatedly reported the mismatch in thylakoids between bulk-to-bulk pmf and ATP synthesis (see, e.g., [70]). I, on the other side, observed full correspondence between proton flow away from the p-surface of the membrane and across the ATP synthase [71]. This was compatible when considering that a surface-attached pH indicator, Neutral Red, was used in the latter study. The emerging picture is the reasonably fast hopping of protons close to the surface, and between proton-binding groups (coined proton antenna in [72]). Take the extremely small aqueous volume of an isolated bacterial chromatophore of 30 nm internal radius [73]. pH 5 in the lumen implies the presence of 0.1 free proton in the average. The pH is nevertheless precisely defined by the rapid interchange of protons between many buffering groups. In chromatophores of purple bacteria, thylakoids of chloroplasts and cristae of mitochondria, Mitchell's concept of bulk-to-bulk has to be read as surface-to-surface pmf. It remains a delocalized coupling concept where many proton pumps serve many ATP synthase molecules.

Recently, the observed lateral segregation between proton pumps (e.g. cytochrome *c* oxidase, complex IV) and the F_oF_1-ATP synthase (complex V) in mitochondrial cristae has added a new flavour to the debate over localized versus delocalized (i.e. chemiosmotic) proton coupling, namely electrostatic focusing of protons into the ATP synthase [74,75]. In mitochondria, the proton pumps, complexes I, III and IV, are mainly found in the flat portions of crista membranes [76,77], whereas ribbons of F_oF_1 dimers line the rim [75,78,79]. A similar segregation holds true for thylakoids. Two groups have speculated that the placement of the ATP synthase in the highly curved rims serves to electrostatically focus protons into the ATP synthase, and to increase the pH portion of the pmf, both in mitochondria [75] and in thylakoids [74]. Both claims were based on electrostatic calculations for very low and non-physiological ionic strength. For physiological ionic strength, the electrostatic focusing of protons is negligible. Considering the realistic situation of steady proton flow from sources (e.g. cytochrome *c* oxidase) to sinks (the ATP synthase), one expects a more alkaline local pH at the sink than at the source, and not the opposite as has been claimed. In other words, the pH difference across the ATP synthase at the rim is less than the one across the flat area of the crista membrane hosting mainly proton pumps. This is another correction to Mitchell's original concept, albeit a minor one, because it only relates to the entropic component of the pmf, whereas the electrical component is rapidly delocalized because of high ionic strength (for thylakoids, see [47]).

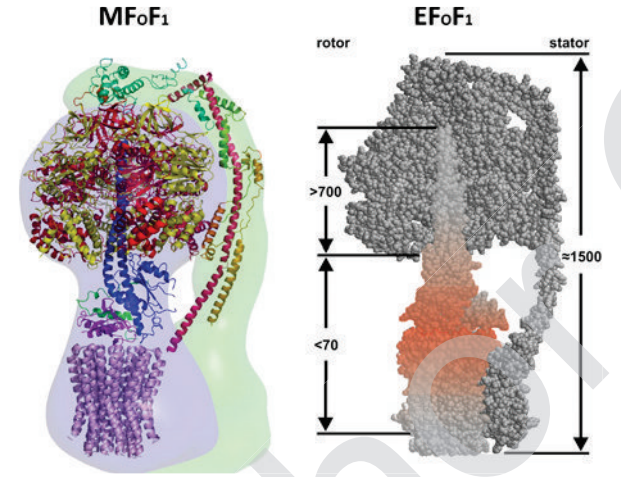
The rotary mechanism of the ion-translocating ATP synthase (F_oF_1)

When Peter Mitchell received the Nobel Prize 1978, little structural detail on the ATP synthase was available. It was known that the enzyme was bipartite with a membrane-bound proton-translocating portion, F_o, and a soluble portion, F_1, interacting with nucleotides and phosphate. It was obscure how proton flow might drive the formation of the anhydride bond between ADP and P_i. Both Mitchell [80] and Williams [81] had assumed that protons were channelled from F_o into F_1 where they interacted directly with bound phosphate to shift the equilibrium towards ATP. In contrast, Paul Boyer and his co-workers have found that the release of ATP (not its formation) requires energy input [82], that the exchange of ^{18}O between water and phosphate is independent of the pmf [83] and that ATP formation involves at least two equivalent reaction sites operating in alternation ([84] and see [85] for a similar proposal). A rotary mechanism with three reaction sites was considered as a possibility [86]. After the "conformational coupling in oxidative phosphorylation and photophosphorylation" by a binding change mechanism [87,88] was established, it became clear that F_1 contained three catalytic plus three non-catalytic binding sites for nucleotides [89]. For the F_1 portion, "a cyclical catalytic mechanism involving three catalytic sites" [90] was claimed by Alan Senior. Correspondingly, a cyclical element was also detected in the F_o-portion of the enzyme, namely a homo-oligomeric ring of the 'proteolipid', alias subunit *c* [91]. Graeme Cox suggested a proton-driven "conformational change by rotation of the *b*-subunit" relative to the *c*-ring in F_o [92], later extended to the *a*-subunit [93]. At the 7th European Bioenergetics Conference in Helsinki in 1991, Peter Pedersen [94] and John Walker presented their preliminary structural models of F_1, both showing a pseudo-hexagon of subunits α and β. It was compatible with a rotary mechanism of catalysis. At an EMBO conference in Freiburg in 1993, I presented a physical model to explain torque generation by proton flow through F_o [95]. It has been based on Brownian rotary fluctuations of the *c*-ring relative to subunit *a*, electrostatic constraints and two non-co-linear access channels for the proton to the ion-binding residue in the middle of one leg of the hairpin shaped *c*-subunit. An animation of its dynamics can be downloaded from my website (http://www.biologie.uni-osnabrueck.de/biophysik/junge/Media.html). The interplay of random Brownian motion and directed electrochemical driving force ('Langevin dynamics') is a common feature of all nanomotors as pioneered by Howard Berg's model for the proton drive of bacterial flagella [96].

In 1994, John Walker and his co-workers in Cambridge unveiled the first asymmetrical crystal structure of F_1 at 2.8 Å resolution [17]. It showed three, in principle, equivalent nucleotide-binding sites in the pseudo-hexagon of subunits $(\alpha\beta)_3$, and an asymmetrically placed central shaft (subunit γ). These sites were differently occupied {empty, with ADP and AMP-PNP (adenosine 5′-[β,γ-imido]triphosphate)}. The convex side of the central shaft faced the empty copy of

Figure 3 | Two structural models for the ATP synthase, F_oF_1

Left: the most complete model as of 2009 of the bovine ATP synthase. Reproduced with permission from Rees, D.M., Leslie, A.G. and Walker, J.E. (2009) The structure of the membrane extrinsic region of bovine ATP synthase. Proc. Natl. Acad. Sci. U.S.A. **106**(51), 21597–21601 [152]. Right: homology model of the *E. coli* ATP synthase (by Siegfried Engelbrecht). Adapted from Junge, W., Sielaff, H. and Engelbrecht, S. (2009) Torque generation and elastic power transmission in the rotary F_oF_1-ATPase. Nature **459**(7245), 364–370 [153]. The colour-coding relates to the torsional stiffness of domains, numbers given in units of pNnm as determined in [130,131], pink for compliant and grey for stiff domains.



subunit β, and, by pressing a lever on β, it held the respective site open. It made it obvious how the rotation of subunit γ would force the three catalytic sites to bind ATP, hydrolyse it into ADP and P_i, and eventually extrude the products in a cyclic mode. This first structure of the bovine mitochondrial F_1 has been followed with a long series of refined structures with different nucleotide (analogues) and inhibitors (see John Walker's Keilin Memorial Lecture article in the February 2013 issue of *Biochemical Society Transactions* [96a]). John Walker and Paul Boyer received the Nobel Prize in Chemistry in 1997. Although a complete structure of the holoenzyme is still lacking, plausible models are available. Figure 3 (left) shows the latest one from John Walker's laboratory.

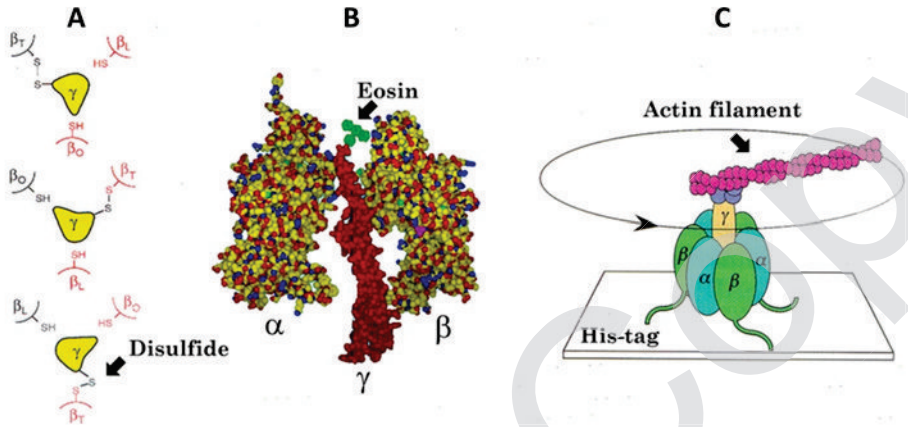
The first asymmetric F_1 structure opened the hunt for real-time detection of rotation. Richard Cross's laboratory was first [97] (Figure 4A). They reassembled F_1 from radioactively labelled subunits with one engineered cysteine residue on each copy of β and γ. When opening a pre-existing disulfide bridge on a given $\beta\gamma$ pair, and closing it again, with or without activity of the enzyme in the intermission, they found differently labelled pairs only when the enzyme was active (Figure 4A). This technique was not time-resolving, and could not discriminate between alternating and rotating motion. One year later, my group immobilized the $(\alpha\beta)_3$-hexagon, attached a photobleachable dye to the C-terminal end of subunit γ, and, using polarized photobleaching and recovery, detected the activity-linked rotation of subunit γ in some 10 ms [98] (Figure 4B). The data showed that the rotation was stepped with fewer than six steps [99]. Another year later, Masasuke Yoshida's and Kazuhiko Kinosita's laboratories joined forces and presented a video-micrographic rotation assay [100] (Figure 4C). By 'seeing is believing', it convinced most (but not all, see below) sceptics in the community, and became the gold standard in this field. They immobilized single molecules of F_1 head down on a solid support, attached a fluorescently labelled probe to the foot of subunit γ, and videographed its rotation relative to $(\alpha\beta)_3$, driven by ATP hydrolysis. They perfected the nanomechanical techniques to incredible precision. With a small probe (short actin filament or nanobead), and with a high-speed camera, the stepped rotation by 120° (substeps 40° and 80°) was resolved in real-time [101,102]. A masterpiece has been the detection of ATP production by driving single molecules with attached nanomagnet by a rotating magnetic field [103]. Extending this approach to F_oF_1, Masamitsu Futai's and my group have demonstrated that the *c*-ring of F_o co-rotates with subunit γ when the enzyme hydrolyses ATP [104,105]. Using FRET, Peter Gräber and Michael Börsch established a viable rotation assay for F_oF_1 embedded in liposomes ([106,107] and see the article by Michael Börsch in this issue of *Biochemical Society Transactions* [107a]). It has revealed the 36° stepping of the proton-driven *c*-ring of F_o [108]. Recently, Hiruyuki Noji's laboratory demonstrated rotation of $(\alpha\beta)_3$ driven by pmf in F_oF_1 with the *c*-ring immobilized on a solid supported membrane [109]. Wayne Frasch's group has used gold nanorods as probes and improved the time resolution to the range of microseconds [110,111].

The magnitude of the enzyme torque has been mostly calculated on the basis of the velocity of rotation and the supposed viscous drag on the probe in water [100,101]. Because the viscous flow coupling to the solid support was unknown, the torque was underestimated. This was overcome by using long actin filaments (typically 3 μm). It slowed the enzyme by orders of magnitude, and the torque was calculated from the curvature of the filament which served as a spring balance [112,113]. With 55 pNnm, the torque of the almost stalled enzyme matched the expectation for thermodynamic equilibrium between the chemical force of ATP hydrolysis by F_1 and the mechanical counterforce exerted by the spring which was attached to the *c*-ring of F_o [114]. Only under the almost stalled (near-equilibrium) conditions, is the efficiency of F_oF_1 almost 100 %; when running freely it is lower, of course.

The energy landscape of the enzyme has been recorded in real-time. The step size of 120° is differently phased depending on whether the enzyme is waiting for ATP or, under ATP saturation, waiting for the next catalytic step. Two groups have correlated the position of the central shaft during the dwells (40° and 80°) of the active enzyme with its position in the majority of crystal structures. Both arrived at the same conclusion. The position in the crystal of the bovine enzyme [17] resembles the position during the catalytic dwell of the active bacterial ATPase [115,116]. It was surprising because one of the three binding sites in the crystal was

Figure 4 | Techniques for monitoring the intra-enzyme rotation in the F_1-portion of the ATP synthase

See the text for details. Reproduced from *Trends in Biochemical Sciences* **22**(11) Wolfgang Junge, Holger Lill and Siegfried Engelbrecht, ATP synthase: an electrochemical transducer with rotary mechanics, 420–423, © 1997, with permission from Elsevier [95].



unoccupied as though waiting for ATP to bind. It has remained a challenge to solve this apparent inconsistency. Simulation of the Langevin dynamics based on a coarse-grained MD technique [117] is a promising approach.

Rotary ATP synthesis by F_1 calls for rotary proton transport by F_o. The earlier proposed physical mechanism for torque production by rotary proton transport [95,118] has remained plausible to this day. Like any other nanomotor, F_o functions by the interplay of stochastic thermal impact (Langevin force) and directed thermodynamic force, both coulombic and entropic. The structure and function of this rotary proton translocator is subject of very active research [119,119a].

The magnitude of rotary proton conduction of bacterial F_o has been determined by a single-molecule-per-vesicle approach [73]. If devoid of its F_1 counterpart, the proton conductance is 10 fS, ohmic up to 70 mV, and only a little pH-dependent over a wide range from pH 6.5 to 10. At 200 mV driving force, this conductance implies >12 000 protons or >1200 rounds/s in bacterial F_o. Compared with the less than 100 rounds/s of bacterial F_1 alone, F_o seems to be at quasi-equilibrium when coupled with its slower counterpart. It is noteworthy that Brownian rotation of the chloroplast enzyme in the thylakoid membrane (correlation time of ~100 μs [120]) is by one order of magnitude faster than the rotation of the load-free *c*-ring relative to subunit *a* in F_o. Friction of the spinning *c*-ring immersed in the lipid seems negligible.

How the proton stepping in F_o (with 8–15 steps per revolution depending on the organism [119,119a,121,122]) might be coupled to the different stepping by 120° (40° and 80°) in F_1 has been debated. George Oster's group had argued in favour of delicate fine-tuning of any step in F_1 to a corresponding step in F_o [123]. We have maintained that Nature's choice is simplicity and robustness, namely to kinetically decouple the detailed reaction steps in F_o and F_1 [112,124]. They work smoothly together via an elastic torque-transmission acting as an energy buffer. One stepper loads the elastic buffer and the other one draws energy whenever its next step is activated. It is the clue for this enzyme's ability to operate by the same principle in different organisms, namely on either protons or Na^+ ions [19], with different stator constructions [125–128], and with different gear ratios [119,121,122] (i.e. proton/ATP ratio). In mammalian mitochondria, the ring of *c*-subunits consists of eight copies [122], and 14 in chloroplasts [129]. In mitochondria, the enzyme operates at high and constant energy supply and runs at high speed, racer-like, and in chloroplasts it crawls slowly, tractor-like, under more variable and often low energy supply.

Which domains of the enzyme are responsible for the elastic buffer has been studied by fluctuation analysis [18,130,131]. Broadly speaking, there are two highly compliant domains: the rotor portion between the torque-generating domains on F_o and F_1 (torsion rigidity 70 pNnm [131]), and the hinge of the lever on subunit β (together they give rise to a stiffness of 35 pNnm in the active F_oF_1 [115]). The stator is much stiffer than the rotor (>1000 pNnm) even when the coiled coil of two *b*-subunits (*E. coli*) is prolonged by 11 amino acids or destabilized by inserting glycine residues [130]. A homology model of the *E. coli* enzyme colour-coded for compliant (red) and stiff (grey) domains is illustrated in Figure 3 (right). By solving the Fokker–Planck equation, Dimitry Cherepanov found that an elastic power transmission is a necessary prerequisite for a high turnover rate of a stepping nanomotor that drives a heavy load ([112,114] and see Figure 7 in [114]). The elastically compliant transmission allows this enzyme to operate with different gear ratios. If the elastic buffer is highly strained, say 200 mV electric driving force working against a blocked F_1, the elastic distortion of the compliant shaft varies accordingly, from 27° in animal mitochondria to 51° in chloroplasts [18].

In 2000, Dick McCarty listed some strange properties of the enzyme which he took as evidence against a rotary

mechanism [132]. It is now evident that they convey a stunning robustness of this rotary electro-mechano-chemical energy converter. All properties are compatible with a rotary mechanism, as has been shown in the cited articles, namely: truncation of γ does not inactivate ATPase [133–135], $(\alpha\beta)_3$ without γ can catalyse ATP hydrolysis [136], $(\alpha\beta)_3$-γ cross-links only slightly inhibit ATP hydrolysis [137,138], and the stator, b_2, can be extended or truncated in the middle without loss of function [130,139,140]. How F_o and F_1 and their respective cousins in the A- and the V-ATPase have evolved, and found each other to robustly co-operate is a matter of interesting speculation [141,142].

Is our knowledge on the ion-driven and rotary ATP synthase now ready and finished? Not at all, because a full structure of F_oF_1 at atomic resolution is not yet available, and the structural and dynamic knowledge has been assembled from different sources. Most important is the following, as a paradigm of Perutz's dream machines of life, the ATP synthase merits the most rigorous description in terms of basic physics and chemistry. A comprehensive characterization both by theory and experiment is more difficult to conduct with less extraverted enzymes. The experimental techniques are rapidly progressing, and theoretical tools as well, so it is hoped that molecular dynamics is going to overcome the nanosecond limit, and to address the micro- to milli-second time range of elementary reactions.

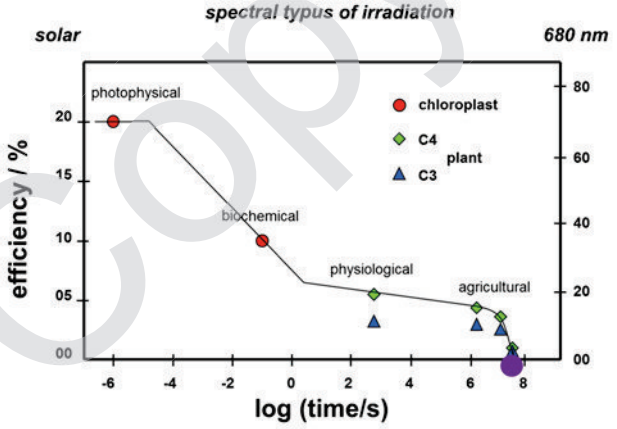
The efficiency of solar energy conversion by photosynthesis

Molecular bioenergetics addresses very basic and very ancient properties of life, mostly too basic for medical intervention, except for some hereditary deficiencies of the respiratory chain in mammals. One application sticks out in the light of the energy question, namely biosolar energy conversion into fuel and electricity. In the present article, the bio-inspired and biomimetic approaches are left out, but which is the energy conversion efficiency of photosynthesis proper?

Figure 5 illustrates the energy efficiency of photosynthesis on a logarithmic time scale. In their very first reactions (<1 μs), photosynthetic reaction centres of green plants (e.g. PSII) can chemically store approximately 20 % of the solar energy that impinges on the surface of the Earth. The low efficiency is a consequence of three features: (i) the Carnot efficiency of chlorophyll antennae in equilibrium with diffuse sunlight (~80 %), (ii) ~50 % loss by the extremely rapid dissipative internal conversion in chlorophyll a of 'blue' into 'red' excitation, and (iii) the availability for plant photosynthesis of only 50 % of the solar energy spectrum (see [143] and references therein). The primary energy conversion efficiency of 20 % compares well with the photophysical efficiency of single band-gap photovoltaic cells [144]. Higher efficiencies have been claimed (see, for example, the article by Matthias Rögner in this issue of *Biochemical Society Transactions* [144a]). Often they relate to excitation with monochromatic light (e.g. at 680 nm) as opposed to the full

Figure 5 | Energy conversion efficiency of oxygenic photosynthesis from reaction centre to crop as a function of time (logarithmic scale) following the absorption of a quantum of light

Left-hand scale: related to the full solar spectrum; right-hand scale: related to excitation with monochromatic light (680 nm). This graph resulted from discussions in 2009 between Jim Barber, Don Ort, Bill Parson and me at a U.S. Department of Energy meeting in Albuquerque (see [143,144,146] and the text for details).



solar spectrum (compare the right- and left-hand scales in Figure 5). From the reaction centre to the crop in the field, the efficiency falls further. From 20 % for the primary charge separation (<1 μs), it falls to ~10 % at the level of glucose formation (<1 s), to ~5 % for a plant in a growth chamber, and often to much less than 2 % as the yearly average both for energy crops in the field and aquatic micro-organisms (for productivity data, see, e.g., [145,146]). The energy efficiency for the conversion of biomass into liquid fuel, e.g. sugarcane or sugarbeet into bioethanol, is only 10 % or less. In overly optimistic estimates of the area required to fill our tanks with green fuel (see, e.g., Figure 1 in [145]), the energy costs for cultivation, harvest, storage and fuel fabrication have been neglected. If these costs are considered, current life-cycle analyses of biofuel production have revealed a solar energy efficiency of less than 0.2 % (see the purple dot in Figure 5). For most crops and fuel processes, the energy efficiency is even negative, i.e. more energy is to be invested than gained [147].

There are more energy-efficient ways than photosynthesis to directly or indirectly utilize sunlight for energy production, namely photovoltaic, photothermal and wind-energy converters. Take wind-generators as a benchmark. Their energy harvest factor ranges up to 40, it is the electric energy delivered over the energy spent for material, construction, operation and deconstruction during a lifetime of 20 years. Approximately 95 % of the area between wind-generators in a farm can be used for crop, cattle and timber. Related to the small, otherwise useless, footprint area, a modern generator yields an electric power density of 200–500 W/m^2 compared with a top energy-yielding plant, e.g. sugarcane in Brazil, with low caloric density of 4 W/m^2, and, if fuelled into an electric

power plant, even lower electric power density, <1.3 W/m^2. What humans consumed between 1900 and 2010 of fossil coal, oil and gas amounts to approximately 10 years of the present global productivity of photosynthesis on land, a negligible fraction of what has been turned over in half a billion years. How much exactly is still left in the ground is under debate; however, there is general agreement that the reserves of fossil fuels are limited.

The ever-rising power consumption of humankind, 16 TW in 2012, has reached almost 20% of the caloric equivalent of global photosynthesis (on land). For the time after peak-fossil, it implies that technical civilizations have to rely on technical energy sources. The products of present-day photosynthesis are insufficient in quantity and will soon become too valuable for being fuelled into combustion engines. They should be reserved for food, feed, fibre and industrial platform chemicals. Applied research in bioenergetics should be aimed at tuning, by breeding and molecular engineering, the product spectrum of photosynthetic and respiring organisms, rather than to focus on energy.

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