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REVIEW

The F_OF_1 ATP synthase: from atomistic three-dimensional structure to the rotary-chemical function

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Abstract Molecular motors are multi-subunit complexes that are indispensable for accomplishing various tasks of the living cells. One such molecular motor is the F_OF_1 ATP synthase that synthesizes ATP at the expense of the membrane proton gradient. Elucidating the molecular origin of the motor function is challenging despite significant advances in various experimental fields. Currently atomic simulations of whole motor complexes cannot reach to functionally relevant time scales that extend beyond the millisecond regime. Moreover, to reveal the underlying molecular origin of the function, one must model the coupled chemical and conformational events using physically and chemically meaningful multiscaling techniques. In this review, we discuss our approach to model the action of the F_1 and F_O molecular motors, where emphasis is laid on elucidating the molecular origin of the driving force that leads to directional rotation at the expense of ATP hydrolysis or proton gradients. We have used atomic structures of the motors and used hierarchical multiscaling techniques to generate low dimensional functional free energy surfaces of the complete mechano-chemical process. These free energy surfaces were studied further to calculate important characteristics of the motors, such as, rotational torque, temporal dynamics, occurrence of intermittent dwell states, etc. We also studied the result of mutating various parts of the motor domains and our observations correspond very well with the experimental findings. Overall, our studies have generated a cumulative understanding of the motor action, and especially highlight the crucial role of electrostatics in establishing the mechano-chemical coupling.

Keywords Bioenergetics · Multiscale computer simulation · ATP synthase · Rotational torque · Coarse grained model

Editor's note: Govindjee (e-mail: gov@illinois.edu)

Since 1960, I have been fascinated by the process by which plants, algae and bacteria use light energy in uphill electron transport producing reducing power and ATP, both needed to produce food and biomass, via carbon reactions. By then, the path of carbon was elucidated by Andrew A. Benson, James Al Bassham and Melvin Calvin, for which Calvin received the 1961 Nobel Prize in Chemistry. Since then I have been enamored by several other discoveries, also recognized by Nobel Prizes in Chemistry. These include (i) understanding that ATP synthesis might be powered by proton motive force (Peter Mitchell: 1978); (ii) determination of three-dimensional structure of a photosynthetic reaction center from anoxygenic bacteria (Johannes Deisenhofer, Hartmut Mitchell and Robert Huber: 1988); (iii) adiabatic theory of electron transfer process in chemistry and biology (Rudolph A. Marcus: 1992); (iv) presentation of a firm basis for a rotary mechanism of ATP synthesis (Paul D. Boyer and John E. Walker: 1997). In view of the complexity of all

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We dedicate this review to **Klaus Schulten** (1947–2016), an innovator in the use of computational methods to study the chemical and biological processes driving living cells; he provided many biological insights into how cells function.

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biological reactions, it seemed necessary to have reliable structure based models to understand life as it really is. Michal Levitt, Martin Karplus, and Arieh Warshel (one of the coauthors of the current review) were indeed recognized by the 2013 Nobel Prize in Chemistry for the development of multi-scale models for complex chemical systems. And now, the 2016 Nobel Prize for chemistry was given to Jean Pierre Sauvage, Fraser Stoddart, and Bernard (Ben) L. Feringa, for progress toward synthetic molecular motors. Efforts toward design of these synthetic machines have brought us closer to a deep understanding of energy transduction in general. In view of this background, I invited **Shayantani Mukherjee and Arieh Warshel** to discuss their approach to model how ATP synthase works, and focus on the molecular origin of the driving force that leads to directional anti-clockwise rotation of F_1 at the expense of ATP hydrolysis or clockwise rotation of F_O at the expense of the proton motive force. The authors clearly point out the crucial difference between light driven energy transduction, constrained by the Einstein relation for absoption and stimulated and spontaneous emission, and chemically or osmotically driven energy transduction which is constrained by microscopic reversibility. In my opinion, it is a unique and wonderful Educational Review on how living organisms make ATP, the "energy currency of life". I recommend it to all graduate students and beginning researchers in biology, chemistry, biochemistry and biophysics.

We were fortunate that **Wolfgang Junge**, one of the topmost authorities on the mechanism of ATP synthesis in chloroplasts, agreed to review this paper (he is well known for establishing that indeed membrane potential can drive ATP synthesis & had provided key experiments concerning the rotary mechanism of ATP synthase in chloroplasts). All of Junge's suggestions have been incorporated in the manuscript that is published here. Junge stated in his review "…Authors have included limitations of their own work as well as of related theoretical work; these are very clearly stated in this neat review. ....it strongly argues in favor of "simply" electrostatic steering of the directionality and the mode of the rotary stepping in this enzyme." For the revised version, Junge wrote "It is excellent as it stands." We were equally fortunate to have **R. Dean Astumian**, an authority on chemically driven molecular motors and pumps, as a second reviewer, who wrote: "The paper is excellent, especially as a teaching tool for beginning researchers interested in working toward deep understanding of biological free-energy transducing systems. The quantitative energy landscape approach pioneered by A. Warshel and S. Mukherjee is the correct way to explore effects of structural features on thermodynamics and kinetics of energy transduction, and the authors have described this approach at a conceptually deep but easy to understand level."

Introduction

Molecular machines are ubiquitous for the functioning of living cells. Some of the well-known examples are DNA/RNA polymerases and Ribosomes working as the replication, transcription and translation machineries, ATP synthases supplying the cell with its energy needs, and cytoskeletal-motor proteins conducting intracellular transport and cellular motility. The hallmark of these cellular machines is their capability to drive thermodynamically uphill processes for prolonged time, despite being situated within the stochastic cellular environment where every atom of the molecular machine undergoes incessant random collision with its environment. Although, under the influence of randomizing forces (Brownian motion), the organization principles (see below) working on the molecular machine can result in directionality, high efficiency and capability to generate power (Astumian 1997; Junge et al. 2009; Keller and Bustamante 2000; Wang and Oster 1998). One of the key organization principles stems from the non-equilibrium distribution of the chemical species within the cellular compartments and is predominantly entropic in nature. For example, different cellular concentrations of ATP and ADP molecules within the cell or different concentrations of protons on both sides of the cellular membrane give rise to concentration dependent thermodynamic force, which is transduced internally within the molecular machine through preferred binding, catalysis and release of chemical species or through facilitated transport of charged species. (See Jagendorf (1975, 2002) and Ort and Melandri (1982) for historical perspective on ATP synthesis in chloroplasts.) The catalytic or charge transport free energy is further transduced to directed conformational changes within the molecular machine. This emphasizes another important organization principle, which is the ability of the molecular machine to couple the chemical or charge transport free energy to directed mechanical motions. Under the influence of these two key organization principles, systems like F_OF_1 ATP synthase produce directional mechanical motion at the expense of chemical or charge transport free energies or vice versa.

A typical biological machine is a macromolecule assembled from multiple subunits of proteins and/or nucleic acids and can undergo largescale mechanical motions or conformational changes during the complete course of its functional cycle. In parallel, chemical events such as ATP or GTP hydrolysis occurs within designated sites of the macromolecule, known as the catalytic centers, which provide the much-needed thermodynamic drive to propel the system continually along a specific direction. The efficacies of these molecular machines depend on extracting meaningful work out of the chemical free energy expended per cycle. Hence, to achieve high efficiency and minimize futile expenditure of chemical energy, it is imperative that the chemical events should be tightly coupled to the mechanical or the conformational changes occurring within the macromolecule. Typically, the chemical events are much faster and occur locally within the catalytic centers, while the mechanical or conformational events encompass much larger space and can be much slower than the former. To work efficiently, molecular machines must be able to accommodate events occurring within disparate spatiotemporal ranges, spanning timescales of picoseconds to seconds and spatial scales of nanometers to micrometers.

Although, it looks like a daunting task to be able to decipher the language of the cell's molecular machines, combined efforts from several experimental techniques, such as, biochemical and biophysical studies of the catalytic processes, three-dimensional structure determination of the whole macromolecule and single molecule experiments have shed considerable light on how these systems achieve their goals of directionality, efficiency and power generation within the stochastic cellular environment. Apart from experiments, substantial understanding has also been gained through theoretical and computational modeling efforts, although there is still a huge gap in achieving a quantitative understanding of molecular motors from the viewpoint of its three-dimensional atomic structure. The structure–function connection of molecular motors should result in a clear understanding of the energetics and its relationships to the mechano-chemical events occurring during the functional cycle. Such quantitative understanding would elucidate nature's design principles working through the complex three-dimensional structure of the molecular machine. In this review, we would discuss our recent work that attempts to unravel the workings of one such cellular machine, the F_OF_1 ATP synthase starting from its atomistic structure. The approach discussed has been based on elucidating the free energy surfaces of the complete functional cycle of a molecular machine including the mechanical/conformational and chemical events, as opposed to approaches that focus on modeling few parts of the whole complex or specific events occurring within the system, albeit describing them in much more details.

The F_OF_1 ATP synthase

Function of the complete molecular motor

The F_OF_1 ATP synthase is nature's smallest rotary motor that synthesizes ATP molecules or the chemical "fuel" required to sustain various processes of the cell (Boyer 1997; Dimroth et al. 2006; Junge and Nelson 2015; Walker 2013). Recent efforts like cryo-EM techniques have generated low-resolution density maps of the complete molecule and have yielded near-complete model of the bovine mitochondrial F_OF_1 (shown in Fig. 1) in combination with computational approaches (Zhou et al. 2015). The complete molecular machine is a combination of two separate motors, namely, the membrane bound F_O and the cytoplasmic F_1. The dual action of F_O and F_1 working in tandem leads to ATP synthesis within the cells under favorable thermodynamic conditions (dictated by intracellular concentrations of ATP, ADP, Pi and proton concentrations on both sides of the membrane), even though synthesizing ATP from ADP and P_i is a thermodynamically uphill process. During the synthesis cycle, the membrane bound F_O gains free energy by conducting proton transport (PT) from the electropositive or more acidic side of the cell (extracellular or the P side) to the electronegative side (cytoplasmic side or the N side). The gain in PT free energy is used to rotate the membrane embedded rotor portion of F_O (known as the c-ring) in clockwise direction as viewed from the P side. This mechanical rotation generates torque, which leads to rotation of the cytoplasmic rotor (also known as the central stalk) of the F_1 in a clockwise direction when viewed from the membrane side. The mechanical rotation of the central stalk in turn induces the stator part of F_1 (also known as the catalytic crown) to undergo cyclic conformational changes favoring the uphill process of ATP synthesis within its catalytic centers.

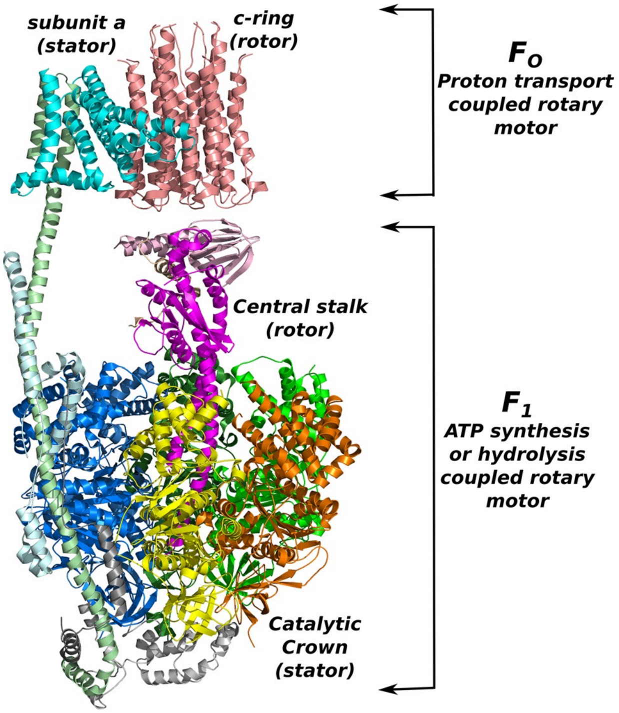

Fig. 1 A near complete structural model of the bovine mitochondrial F_OF_1 ATP synthase (Zhou et al. 2015)

The F_OF_1 is a reversible motor where reversing the conditions in the appropriate chambers such as ligand concentrations can lead to ATP hydrolysis in F_1 connected to an F_O working as a proton pump (Junge and Nelson 2015; Walker 2013). In the reverse direction, the free energy gained from hydrolyzing ATP rotate the central stalk of F_1 in the opposite direction (anti-clockwise when viewed from the membrane side), which can induce the c-ring of F_O to rotate in the opposite direction. The F_O under the action of the torque generated by F_1 can now act as a proton pump transporting protons from the N to the P side. Outside the cells, the two conjoined motors can also operate on their own, where the F_1 (without the torque generated by F_O) only shows anti-clockwise rotation at the expense of ATP hydrolysis, while the F_O shows clockwise rotation at the expense of PT from the P side to the N side. To understand the action of the combined motor, one must first elucidate the structure–function relationship behind the ATP hydrolysis powered rotation observed in F_1. Similarly, one must also understand the principles working in F_O to generate the PT-coupled-rotation. Understanding the coupling between mechanical (rotation) and chemical/proton motive forces in these two separate modules will provide a better picture of the complete F_OF_1 working under different thermodynamic constraints or cellular conditions.

ATP hydrolysis driven rotation of the F_1

The first crystal structure of F_1 was produced by John Walker and his coworkers (Abrahams et al. 1994), which revealed the architecture of the cytoplasmic motor for the first time. Alongside, pioneering work was done to reveal the kinetics and thermodynamics of the enzyme as it visits different chemical states during the hydrolysis cycle (Boyer 1993; Weber and Senior 1997). These authors suggested that the catalytic centers in the crown undergo cooperative conformational changes to perform ATP binding, ATP hydrolysis and release of ADP and P_i. These cooperative and cyclic conformational and chemical changes occurring in the crown induces mechanical rotation of the F_1 central stalk. Decades later, the first single molecule experiment showed the rotary motion of the stalk as F_1 performed its ATP hydrolysis process (Noji et al. 1997). Since then, various rotary-chemical aspects of the F_1 motor have been revealed through other important studies, where the rotational path has been traced out in further details and the average torque generated by the system has been calculated (Adachi et al. 2007; Masaike et al. 2008; Panke et al. 2001; Watanabe et al. 2014; Yasuda et al. 2001).

The structure of the F_1 motor (only the catalytic crown and the central stalk) is shown in Fig. 2a, while a schematic representation of the cooperative and cyclic chemical/conformational changes occurring in the crown are

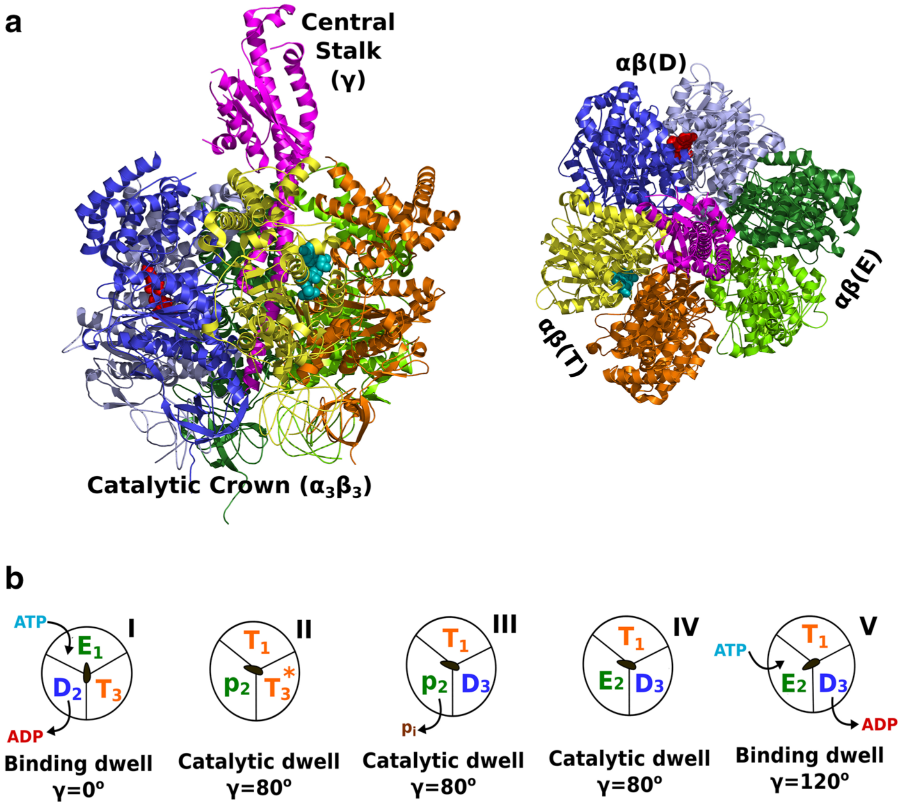

Fig. 2 **a** The structure of the F_1 motor (only the catalytic crown and the central stalk) is shown, where three catalytic dimers in distinct conformations are marked as E (empty), T (ATP bound) and D (ADP bound). The β subunits containing the catalytic centers are depicted in *deep green, orange* and *deep blue*, for dimers E, T and D, respectively. The α subunits of the same dimers E, T and D are shown in *light green, yellow* and *light blue*, respectively. The bound nucleotide in the T catalytic center is shown in *red* and the nucleotide in the D catalytic center is shown in *cyan*. **b** A schematic representation of the cooperative and cyclic chemical/conformational changes occurring in the three catalytic centers of the crown are depicted. Each schematic represents the states adopted by the F_1 motor during its 120° rotation and hydrolysis of a single ATP molecule

depicted in Fig. 2b. The crown of the F_1 is composed of three pairs of catalytic subunits, each composed of a single α and β subunit. The three catalytic centers can assume distinct conformational states, namely, the empty or open state where the subunit is devoid of any nucleotides (designated as E), the loose state where the hydrolysis products ADP and P_i are bound at the catalytic center (designated as D), and the tight state where ATP is bound at the catalytic center (designated as T) (Junge and Nelson 2015; Walker 2013). As the hydrolysis cycle proceeds, the catalytic subunits change their conformation from E → T → D in a cyclic and cooperative manner. Conformational changes in the crown rotate the central stalk in anti-clockwise direction that is partly embedded within the cavity created by the crown. The stalk has been observed to rotate in steps of 120° per ATP hydrolyzed by the crown and it takes a full rotation of 360° after hydrolyzing three ATP molecules into ADP and P_i molecules. Detailed single molecule studies further revealed that the 120° rotation is not continuous, but rather it occurs in sub-steps of 90°/30° (Yasuda et al. 2001) or 80°/40° (Masaike et al. 2008). Apparently, the stalk stops for a considerable time (in the range of milliseconds) after it has rotated for 90° or 80° and this dwell is known as the "catalytic dwell" as it is coupled to the catalytic and P_i release events in the crown. After the "catalytic dwell", the stalk undergoes the remaining portion of the 120° rotation and then waits for an ATP molecule to bind to one of its empty catalytic subunits. Accordingly, the second dwell occurring after the 30° or 40° sub-step is known as the "ATP waiting dwell". The continuous stepped rotation as a result of sequential hydrolysis of multiple ATP molecules leads to an observable torque within the system that has been measured in many experiments to be in the range of 40–55 pN.nm (picoNewton.nanometer) (Noji et al. 1997; Panke et al. 2001). This fact further reflects that F_1 is a highly efficient enzyme where the free energy released due to ATP hydrolysis is transduced efficiently into mechanical torque (Junge et al. 2009).

Proton transport driven rotation of the F_O

The F_O motor is the membrane embedded counterpart that is predominantly composed of a rotor unit (c-ring) and a stator unit (subunit a) (Dimroth et al. 2006; Junge and Nelson 2015). The c-ring spans the membrane from the P to the N sides and is composed of variable number of alpha-helices depending on the species, while the subunit a faces part of the c-ring forming the c-ring/subunit a interface that spans the membrane. The helices of the c-ring contain highly conserved Asp or Glu residues that form a ring of ionizable residues situated in the middle of the membrane. As the c-ring rotates, any one of its multiple Asp/Glu residues always faces a single highly conserved positively charged Arg(+) residue in the subunit a, that is also situated almost half way within the membrane. We know that the interface formed by the c-ring and the subunit a defines the major elements of the PT channels, while the conserved Asp/Glu and the Arg(+) residues are involved in capturing and releasing protons from the channels. Due to difficulties associated with solving membrane protein structures, a complete high resolution structure of the F_O is still missing, although comparatively good quality structures are available for the c-ring (Meier et al. 2005). Recent cryo-EM generated structures show the subunit a to be almost in a horizontal configuration to the membrane surface (Allegretti et al. 2015; Zhou et al. 2015) (shown in Fig. 1 and in Fig. 3a). An early attempt to solve the complete c-ring/subunit a structure led to an NMR model (Rastogi and Girvin 1999) where the helices of the subunit a show predominantly vertical configuration to that of the membrane surface (see Fig. 3b). This structure was superseded recently by the cryo-EM generated structures, mentioned above. In order to generate a quantitative structure–function relationship of the F_O, it is important to have high quality structural information of the c-ring/subunit a interface, as the protons are transported from the P to the N sides along this interface.

Even in the absence of a complete F_O structure, the crucial elements of the PT coupled rotation was predicted in early pioneering work (Junge et al. 1997; Vik and Antonio 1994), where the proton paths from the P to N sides were considered to traverse through two broken half channels (shown schematically in Fig. 3c and the PT through the half channels is shown in Fig. 3d). Junge and others postulated that when the c-ring undergoes clockwise rotation under the influence of stochastic Brownian motion, one of its multiple Asp/Glu residues comes closest to the Arg(+) of the stator subunit a. This leads to deprotonation of the Asp/Glu residue and the proton is released via the half-channel facing the N side of the membrane (illustrated in step II of Fig. 3d). Further clockwise rotation of the c-ring, promoted through stochastic Brownian motion, removes the deprotonated Asp/Glu(−) residue away from the Arg(+) while positioning another oncoming Asp/Glu near the Arg(+) (step III of Fig. 3d). Meanwhile, the previously deprotonated Asp/Glu(−) leaves the interface and starts to enter the membrane environment whose strong hydrophobic nature puts a high thermodynamic penalty on the penetration of any charged residues. This leads the Asp/Glu to quickly reprotonate by taking up a proton from the P side half channel as it enters the membrane environment (depicted in step IV of Fig. 3d). Hence, a clockwise rotation of the F_O continues to proceed in small steps, while proton is being taken up from the P side (low pH and high

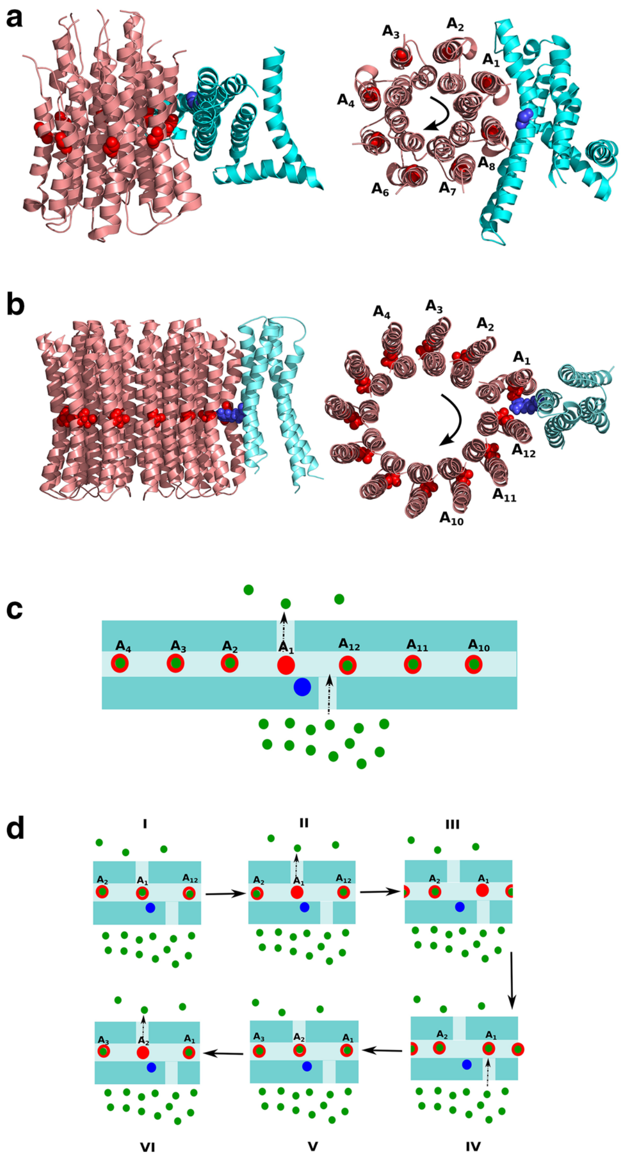

Fig. 3 **a** A recent structural model of the bovine mitochondrial F_O obtained by fitting atomic structures to cryo-EM maps (Zhou et al. 2015). The c-ring is depicted in *salmon*, the subunit a is in *cyan*, the conserved chain of ionizable residues are denoted by *red*, while the conserved Arg(+) on subunit a is shown in *blue*. **b** An old structural model of the F_O obtained using NMR techniques (Rastogi and Girvin 1999). The *color* scheme is the same as in **a.** **c** A schematic representation of the proton transport half channels on the N and P sides of the membrane. The membrane is shown as a *cyan rectangle*, while the ionizable residues are shown as *red circles*. The *blue circle* represents the stator Arg(+), while the *smaller green circles* represents the protons. The concentration of protons is higher on the P side compared to the N side and this allows proton uptake from the P half channel and proton release through the N half channel. **d** A schematic representation of the PT coupled c-ring rotation pathway. The *color* scheme is the same as in **c**

proton density) and released to the N side (high pH and low proton density) by the same Asp/Glu after almost a full turn of c-ring rotation.

Deciphering the molecular structure–function relationship in F_OF_1: challenges and achievements

Atomistic simulation methodologies have made immense progress in the last few decades in understanding the structure–function relationships of biomolecules (Warshel 2014). With significant progress made in hardware design, distributed computing strategies and efficient sampling methodologies, microseconds long simulations of small or medium sized biomolecules have become a reality. However, substantial bottleneck is faced while exploring cellular processes that involve large macromolecules functioning through intricately coupled chemical and mechanical events. One of the major challenges arise due to the huge size of the system, which typically needs enormous computational resources to simulate the macromolecule at the atomistic level (that is, by modeling every atom of the molecule along with the immediate environmental details of water, ions and/or membrane). This difficulty is further compounded by the fact that small time scale simulations for larger macromolecules that are typically feasible with today's computational resources still fail to delineate the mechano-chemical (or rotary-chemical) action of molecular motors. Small time scale simulations produce atomistic trajectories in the range of few hundreds of nanoseconds (or few microseconds) and thus only capture the local dynamical nature of the system. This is not sufficient to elucidate the kinetic and thermodynamic principles of the complete molecular motor whose mechanical events span across larger spatial and temporal regimes. Moreover, to gain structure-based knowledge of molecular motors, one must not rely on the mechanical or dynamical aspects of the system alone, but should also exploit ways to couple the chemical or PT events to the mechanical events.

Despite several challenges, there have been many efforts to study the action of F_1 (Bockmann and Grubmuller 2002; Czub and Grubmuller 2011; Nam et al. 2014; Okazaki and Hummer 2013, Pu and Karplus 2008), F_O (Pogoryelov et al. 2010) as well as in other related systems (Singharoy et al. 2017) using atomistic or coarse-grained (CG) description. These studies have revealed interesting structural details about the key chemical and mechanical states, which could be used to infer about some of the experimentally known facts of the system. To circumvent the difficulty of simulating long time scale processes, some studies have resorted to the use of large external forces (or torques) to accommodate the structural changes required for the motor action (Bockmann and Grubmuller 2002, Pu and Karplus 2008). This has allowed for an understanding of how the 3D structures dynamically adapt to unrealistic large forces that are not present in the actual cellular systems, but nonetheless failed to produce any complete knowledge of the mechano-chemical coupling in terms of its structure based energetics. Furthermore, such approaches, based on brute-force simulation of macromolecules, do not provide any knowledge of the basic physical principles that operate behind the molecular motor action. Without a way to properly quantify the physical principles, brute-force simulation approaches will not be able to discriminate several probable hypotheses about the molecular driving force; for example, whether a molecular motor is predominantly driven by steric or electrostatic considerations, or whether a Brownian Ratchet or Powerstroke mechanism is at play, and how does the three-dimensional structural arrangement encode for these different mechanisms at work? Finally, a quantitative structure–function approach should also be able to elucidate experimentally observed facts about the motor, such as, dwell states and dynamical nature, torque, efficiency, and how these observations change with alterations and/or mutations of key structural elements.

Understanding F_1 and F_O through quantitative functional free energy surfaces

To achieve quantitative understanding of biological motors, we can attempt to understand the free energetics of the mechanical and chemical events, using experimentally solved three-dimensional structures of the system. Furthermore, a consistent mathematical language of the mechano-chemical action can be developed to generate detailed structure based understanding of the motor function and to compare the feasibility of possible hypotheses based on molecular mechanisms. We have adopted such an approach in our efforts to study the structure–function relationship behind the mechano-chemical coupling in molecular motors. We used a hierarchical multiscaling technique where crucial information from each level is used to model the next higher level in progressively lower resolution. The following describes our multiscale approach to study the F_1 (Mukherjee et al. 2015; Mukherjee and Warshel 2011, 2015), while similar approaches were also adopted to study the F_O (Mukherjee and Warshel 2012) and other molecular systems, such as the DNA helicase (Liu et al. 2009), ribosome-translocon (Rychkova et al. 2013) and the myosin V motor (Mukherjee and Warshel 2013; Mukherjee et al. 2017). In case of F_1 we use known crystal structures (Menz et al. 2001) to build atomic models of the key states of the rotary-chemical cycle, such as catalytic subunits in E, T and D states and the central stalk in 0°, 120° and 240° rotated states. Using these atomic structures of key states, we model the large-scale conformational changes (for example, subunit E changing to T, subunit T changing to D, subunit D changing to E and the stalk rotating from 0° to 120°) by linearly interpolating the atomic coordinates of the whole system from the initial to the target states. Short time-scale molecular dynamics (MD) simulations were performed to relax the key and the intermediate structures, mainly to relieve the side chain short contacts. We note that the linear interpolation approach provides an approximate conformational path. Improved methodologies with more sampling can be adopted to map a path that lies closer to the least free energy path along the chosen conformational coordinate(s). Although, considering the cost of simulating large macromolecules and all its intermediate conformations, we have used the less costly linear interpolation scheme for the study of F_1 and F_O. These conformational changes are long time scale processes extending to the milliseconds regime and it is still highly resource intensive to perform free energy calculations using all-atom simulations with current computational resources. We also emphasize that the key element in our method is not only to simulate the dynamical path of the conformational changes, but rather to generate the conformational free energy profile of the most probable path. On a similar note, we further suggest that a linear interpolation scheme will provide a conformational path close to that selected by a targeted MD simulation (TMD) frequently used by the community to study biomolecular conformational changes. TMDs apply large force on the initial structure that depends on the deviation of the initial from the final structure. If such a forced simulation is done with finite sampling (which is often the case for large macromolecules), the trajectory will most likely result in a path close to a linearly interpolated path.

In the next stage of the multiscaling approach, we use a coarse grained (CG) description of the atomic structures to calculate the conformational/rotational free energy profile of the F_1. The CG model in our studies (Messer et al. 2010; Rychkova et al. 2013) uses a coarser description of the amino acid side chains where each of them is represented with a single particle. The CG force field also incorporates a special treatment of the electrostatics, which not only models the coulomb-type interactions between charged species, but also includes the effect of environmental screening coming from other polar and hydrophobic residues surrounding the charged species. In this way, the CG model allows one to calculate both the conservative part of the electrostatic forces arising due to charge–charge interactions (coulombic) and the part arising due to entropic contributions stemming from the solvent reorganization around the charged species (Warshel et al. 2006). This enhanced treatment of electrostatics (coulombic + entropic) allows the CG model to provide effective electrostatic free energy differences of large scale conformational changes, which is otherwise challenging to obtain, using all-atom simulation techniques. In addition, the treatment of the ionizable, polar and nonpolar residues allows the CG model to handle proteins embedded in different molecular environments, such as parts of the protein solvated by water while the rest embedded in the membrane (for example, the c-ring/subunit a interface of F_O). The free energies obtained using the CG model also allow us to discriminate between the electrostatic and hydrophobic contributions in a residue specific manner, thus providing a clear structure based implication of the driving principle.

The final step in our multiscaling approach is to build a quantitative description of the rotary-chemical cycle operating in F_1. To achieve this, thermodynamics and kinetics (relative free energies and activation barriers) of the ligand binding (ATP binding), bond-breaking chemistry (ATP hydrolysis) and product release (ADP and P_i release) steps are calculated from known experimental rates, while the conformational/rotational free energies are obtained from the CG free energetics described previously. These free energies and activation barriers of the chemical and conformational/rotational steps are used to generate a low-resolution surface description of the complete rotary-chemical cycle in F_1. Performing Langevin Dynamics (LD) on this low resolution effective free energy surface with realistic diffusion coefficients can yield quantitative knowledge about the system in real space and time and the results could be compared with known experimental data. The following sections review the main results gained from our studies of F_1 and F_O adopting the multiscale approach described above.

The stepped rotation, directionality and catalytic dwell of F_1

One of the most intriguing questions one can ask about F_1 ATPase is: what is the structural and energetics basis of the stepped rotation (Masaike et al. 2008; Yasuda et al. 2001) of the central stalk during ATP hydrolysis (that is, each rotation of 120° broken into 80°/90° and 40°/30° sub-steps). Another important question is: why does the F_1 central stalk rotate in the anti-clockwise direction while hydrolyzing ATP, but does the opposite while synthesizing ATP. Furthermore, why does the motor reside in intermittent dual states that are strongly connected to ATP hydrolysis (catalytic dwell after 80°/90° rotation) or ligand binding (ATP binding dwell after 40°/30° rotation) events (Adachi et al. 2007). Our initial inspiration towards modeling F_1 was to understand its directionality and stepped rotary-chemical function from the viewpoint of its three-dimensional structure, where one can delineate the molecular contributions that underscore the thermodynamically feasible free energetics of the conformational, rotation and catalytic processes (Fig. 4a shows the authors at work). We approached these questions by modeling the F_1 ATPase functional cycle using the hierarchical multiscaling method described above. The CG model allowed us to produce a good estimate of the conformation/rotation free energy surface of the central stalk rotating a complete 360° turn and the crown changing its conformation through three ATP hydrolysis cycles. The part of the conformation/rotation free energy arising due to electrostatics is shown in Fig. 4b (Mukherjee and Warshel 2011). To our surprise, we observe that the surface clearly shows a stepped path starting from the lower left corner of the map and traversing diagonally to the upper right corner. This implies that the electrostatics of the rotor–stator interactions in F_1 allows a least free energy path with sub-steps close to 80° and 40°. It also reveals that the 80° central stalk rotation has lower energetic barrier, while the 40° rotation is strongly coupled to the conformational changes of the crown subunits and the free energy barrier of this sub-step is much higher than the former.

Apart from elucidating the stepped rotational pattern, the free energy map also revealed the energetic basis of directional rotation during ATP hydrolysis. This fact is illustrated in Fig. 4c, where two rotational cycles of F_1 is shown. The horizontal axis (left to right) in this plot shows the central stalk rotation from $-360° \rightarrow 0° \rightarrow 360°$ (spanning a rotational period of $2\Delta\theta$ where $\Delta\theta = 360°$), while the vertical axis (top to bottom) shows the conformational changes undergone by the three catalytic centers during hydrolysis of three ATP molecules (spanning a chemical cycle of $\Delta\xi$ where $3\ \text{ATP} \rightarrow 3\ \text{ADP} + 3\ \text{P}_i$). To facilitate our understanding of how Fig. 4c reveals the directional nature of F_1 rotation, we consider the area enclosed within the rectangle drawn with green dashed line. A single molecule of F_1 starting at 0° and state $D_1E_2T_3$ (top central part of the map) can move in two different directions that either represent the anti-clockwise (towards 360°) or the clockwise (towards −360°) rotational direction in real 3D space. The motor encounters much lower free energy barrier (ε) when it moves over the path indicated by the white dashed line (i.e., from $0° \rightarrow 360°$), while much higher barrier (ε^*) is encountered when it tries to move in the opposite direction ($0° \rightarrow -360°$). The difference in ε and ε^* dictates the energetically most feasible direction of rotation for the hydrolytic motor (Astumian et al. 2016) and ensures that F_1 kinetically "prefers" an anti-clockwise rotational path over the clockwise path while hydrolyzing ATP. Furthermore, the release of the hydrolysis free energy coupled to the rotation/conformation changes ensures that the motor continually rotates in an anti-clockwise direction if it can bind ATP (that is, under conditions when the molar concentration of [ATP] is higher than the products [ADP] and [Pi] within the cellular compartment). We note here that the CG free energy map for F_1 has been calculated using a specific crystallographic structure (Menz et al. 2001) out of many available structures. Further, this structure has a slightly rotated central stalk position compared to the ensemble of other structures (Walker 2013). It is most likely that the CG

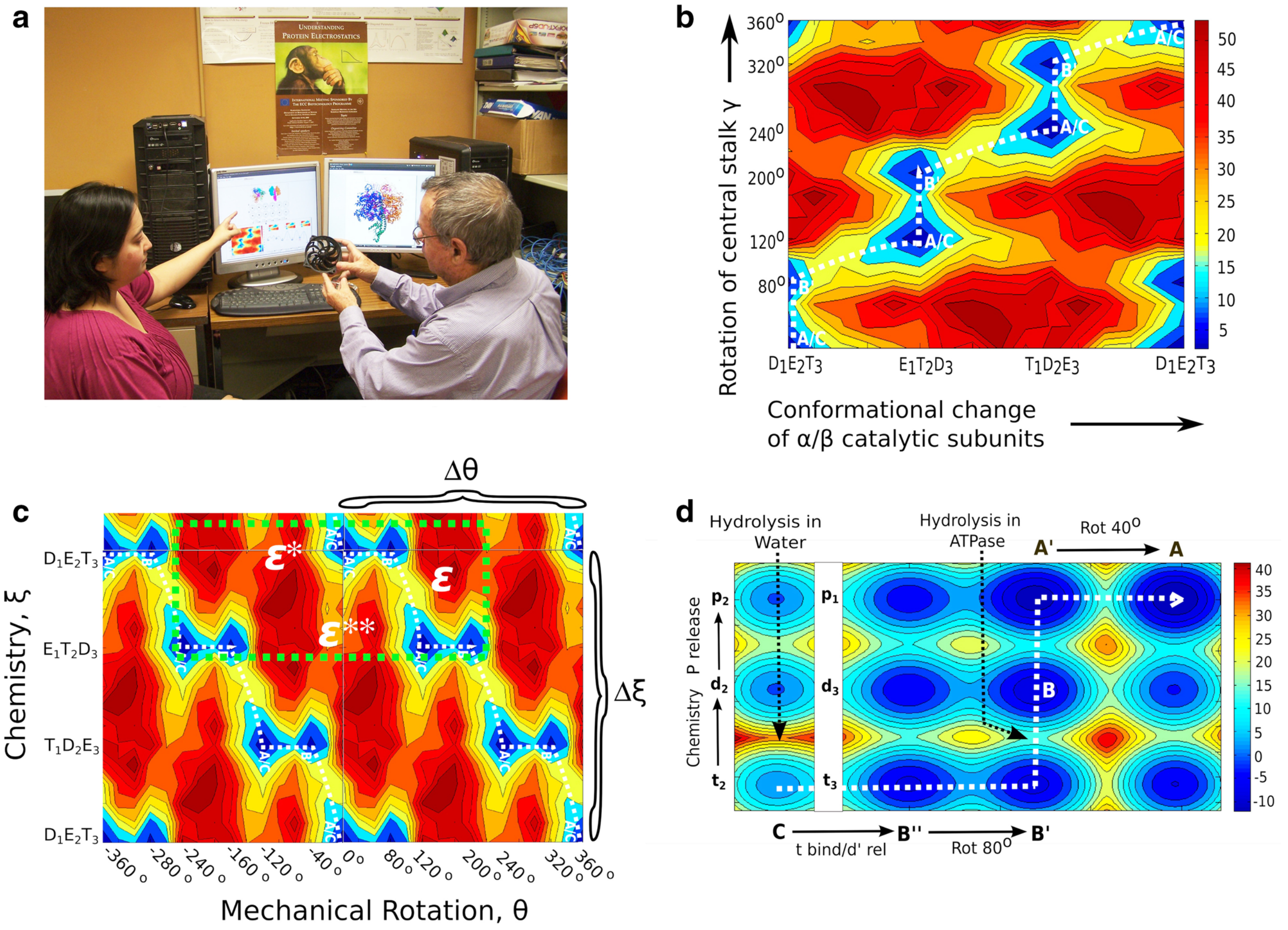

Fig. 4 **a** A photo of the authors trying to understand the rotary-chemical coupling of F_1 ATPase. **b** The CG electrostatic free energy map of the conformation/rotation cycle of F_1. **c** The free energy map for two complete rotational turn of the central stalk is shown. The area enclosed within a *rectangle* marked by *green dashed line* shows two different directions that F_1 can adopt. It encounters much lower free energy barrier (ε) when moving over the path indicated by *white dashed line* (i.e., from 0° → 360°), while much higher barrier (ε*) is encountered when it tries to move in the opposite direction (0° → −360°). **d** The low dimension effective functional free energy surface for the coupled mechano-chemical process in F_1. Figures **b** and **d** are taken from Mukherjee and Warshel (2011) and Figure **c** is taken from Astumian et al. (2016)

map, if calculated using other available structures, would still exhibit the pattern of stepped rotation, albeit starting from a different state. That is, the map calculated with a different F_1 X-ray structure may start at the catalytic dwell represented by state B instead of the ATP waiting dwell represented by state A/C in Fig. 4b. Here we stress the fact that the stepped rotational feature observed in our calculations is not dependent on the choice of any specific X-ray structure over others. The most interesting fact in our study is that a stepped free energy pattern with clear indication of a preference towards the anti-clockwise rotation is observed using a single X-ray structure (Mukherjee and Warshel 2011), and not by comparing two different structures with different central stalk orientations or by using CG models without capturing the chemical environment of the system (Pu and Karplus 2008). *In our view, the CG free energy map of* Fig. 4 *reveals a novel aspect of the molecular mechanism, namely, the role of electrostatics in guiding the rotary-chemical functionality of F_1molecular motor, which has been overlooked before*.

The question we asked next is: why does F_1 show a catalytic dwell after 80° and how the catalytic step is connected to the conformation/rotation behavior. To answer this question, we must formulate the coupled conformation/rotation-chemical free energy surface in a quantitatively meaningful way and infer about the most probable pathways that can occur on such a rotary-chemical coupled surface. There has been an insightful study of ATP hydrolysis occurring in one of the catalytic sites of F_1 using hybrid Quantum Mechanics-Molecular Mechanics (QM/MM) approach that highlighted detailed atomic level interactions occurring during the catalytic step (Dittrich et al. 2003). Another study used the Empirical Valence Bond (EVB) approach to understand the ATP synthesis process in different conformational states of the catalytic subunit and found that the conformational changes are converted to electrostatic interactions between the protein and the ligands facilitating ATP synthesis (Strajbl et al. 2003). Although these studies with atomistic details meticulously examined the catalytic step or even looked at the catalytic subunit conformational changes and its connection to catalysis, they could not provide the complete rotary-chemical process of the F_1 motor. To decipher the rotary-chemical surface, we used experimentally known rates of the ATP hydrolysis process to infer the chemical free energies and activation barriers instead of doing time-consuming QM/MM or EVB calculations of the reaction pathways. The chemical free energies and activation barriers inferred from experiments were further coupled to the relevant conformational free energies obtained from the CG surface to generate a low-dimensional effective surface of the rotary-chemical process (shown in Fig. 4d). The mathematical framework of the EVB method designed initially to study chemical transformation between reactants and products (Warshel and Weiss 1980) was extended to model the low-dimensional surface where multiple chemical and conformational states of the F_1 motor were represented on a single 2D surface. The conformation/rotation states of F_1 during 120° central stalk rotation is shown along the horizontal axis, while the chemical transformation from ATP (designated as t) to ADP (designated as d) and Pi (designated as p) is shown in the vertical axis of Fig. 4d. To understand the dynamics of the motor from the energy landscape of Fig. 4d, we can start at the state marked C where F_1 resides with its central stalk at 0° and an ATP molecule resides in water. The system cannot move in the vertical direction from t → d as the chemical barrier of hydrolyzing ATP in water is huge. When ATP binds F_1 (at state B″) the hydrolysis barrier is drastically reduced, but is still higher than the energetic barrier for the 80° stalk rotation. Thus, despite having an ATP molecule bound at one of the catalytic centers, the central stalk of F_1 undergoes 80° rotation before hydrolysis can occur and the system moves to state B′. At this stage, F_1 faces a very high conformation/rotation barrier associated with the next 40° rotation of the central stalk. As the kinetic barrier for transitioning from B″ → B′ along the horizontal direction is higher than the barrier for hydrolysis, the system now undergoes ATP hydrolysis by moving from t → d in the vertical direction. This is followed by the release of the P_i (d → p) leading to a large downhill change in the free energy. This allows the system to override the high energetic barrier of the 40° rotation and F_1 continues from A′ → A at the upper part of the map after ATP hydrolysis and Pi release. It is evident from Fig. 4d that the catalytic dwell must occur after 80° rotation, and the coupling of the downhill free energy change due to hydrolysis and Pi release leads to further 40° rotation, which was energetically costly before the F_1 crown hydrolyzed ATP. It is worthwhile to note that the released Pi is not the same that has been produced due to ATP hydrolysis in the same catalytic center. Rather, F_1 crown consists of three catalytic centers and during a single 120° rotation, one center binds ATP, while another cleaves an already bound ATP from a previous cycle, and yet another center releases the P_i from previously hydrolyzed products. Atomistic simulations could shed light on some of the structural details of the different chemical steps and its coordination in F_1 (Czub and Grubmuller 2014; Nam et al. 2014; Okazaki and Hummer 2013), although a quantitative picture of the structure-based energetics of the whole rotary-chemical cycle was missing before our work (Mukherjee and Warshel 2011). We note that in our work, the conformation/rotation free energies are calculated using a CG model of the F_1 motor with all essential parts, but the ATP binding, hydrolysis and ADP or Pi release free energies have been calculated from experimental rates. Despite adopting an empirical treatment of the ligand binding, hydrolysis and product release steps, our multiscaling approach deciphered the conformational landscape and provided a mathematical basis to couple the chemical and mechanical aspects of the complete molecular motor. The methodology described here focuses on elucidating the functional free energy landscape of the whole cycle and can thus provide novel molecular level insights on the organization principles of molecular motors.

The central stalk and torque generation in F_1

The continuous stepped rotation of the central stalk at the expense of ATP hydrolysis in F_1 can be observed experimentally in single molecule studies (Noji et al. 1997) and the torque can be calculated for such systems (Noji et al. 1997; Panke et al. 2001). The amount of free energy expended through ATP hydrolysis and the calculated torque because of the stepped rotation implies that the motor is highly efficient, as most of the chemical free energy is transformed into rotational work with minimal waste due to internal friction. In several experiments, the role of the central stalk was studied by deleting parts of it and measuring the torque of the resultant system (Chiwata et al. 2014; Furuike et al. 2008). We have attempted to understand the role of the central stalk in the rotary-chemical coupling by theoretically modeling such truncated systems and then calculating the CG conformation/rotation free energy surfaces (Mukherjee and Warshel 2015). Figure 5 shows the CG electrostatics maps of the truncated-F_1 systems, where different parts of the central stalk have been altered to model the deletion effects. In general, our results show that the top alpha-helical part and the bottom globular part

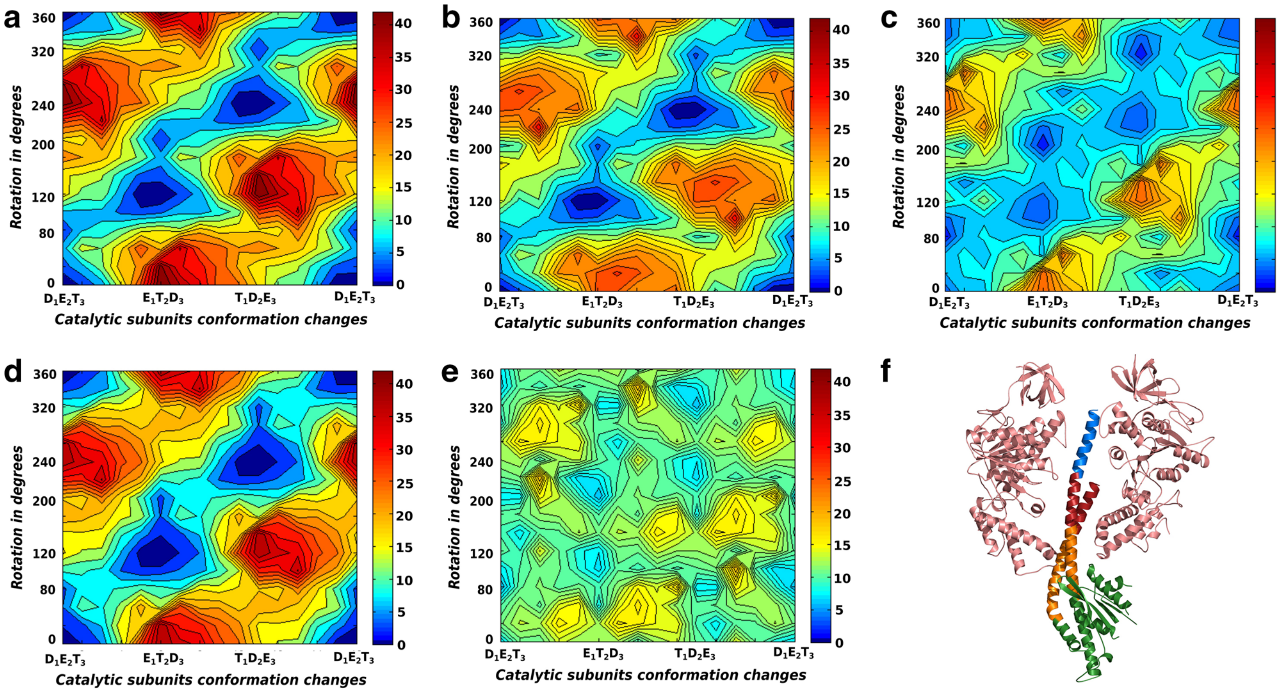

Fig. 5 The CG electrostatic free energy surfaces for the central stalk-truncated systems where; **a** *blue*, **b** *red*, **c** *orange*, **d** *green* and **e** *red* and *orange* parts of the central stalk have been mutated to remove side chain contributions from those regions. **f** The *color* scheme used to denote different deletion regions of the F_1 central stalk are shown. Only two crown subunits are retained in this figure for clarity. This figure has been taken from Mukherjee and Warshel (2015)

of the central stalk are not important in coupling the conformational changes of the crown catalytic centers to the rotational motion of the stalk. We still observe stepped rotational pattern when the free energy contributions from the top and the bottom parts of the stalk (blue and green parts of the stalk in Fig. 5f) are excluded. Rather, the most important region controlling the rotary-chemical coupling and torque production is the middle part that involves the bulge in the alpha helix of the central stalk (orange part of the stalk in Fig. 5f). This region of the stalk lies close to the loop region of the catalytic centers (known as the DELSEED loop) and highlights the importance of the electrostatics- mediated interactions stemming from the area. The region of the stalk just above the DELSEED loop (red part in Fig. 5f) is also important and together these two regions (orange and red in Fig. 5f) contribute significantly in establishing the stepped rotational feature of F_1. Our observations conform to the experimental data very well (Chiwata et al. 2014) and further support our idea of electrostatics mediated rotary-chemical coupling in the F_1 ATPase system. Next, we have calculated the torque in the wild-type F_1 using the CG free energy surfaces (Mukherjee and Warshel 2015), which is in very close agreement to experimental observations (Noji et al. 1997; Panke et al. 2001). Overall, our study indicates that any specific residue or any specific side chain interaction does not play any key role in the rotary-chemical behavior. Rather, the overall change in the electrostatics mediated solvation free energy induced by the conformational change of the crown catalytic centers surrounding the middle part of the central stalk during ATP hydrolysis cycle provides the energetic basis for the rotational motion.

The proton transport driven rotation in F_O

Obtaining a structure-based energetics of the proton transport (PT) driven rotation in F_O motor is challenging due to several factors. As discussed earlier, an atomistic resolution structural complex of the F_O c-ring, along with the subunit a, is still missing, although we have high-resolution atomistic structures of the c-ring alone (Meier et al. 2005; Pogoryelov et al. 2009). Cryo-EM generated maps are now available where parts of the subunit a protein backbone could be deciphered (Allegretti et al. 2015; Zhou et al. 2015). Another challenge for studying F_O is the huge size and the heterogeneous chemical environment of the system, where most of the system is embedded in the membrane, while crucial parts (especially the c-ring/subunit a interface) are exposed to water and ions. Atomic simulations have been used to study parts of the system that highlighted the importance of the Asp/Glu(−)–Arg(+) salt bridge (Pogoryelov et al. 2010), although a complete understanding of the PT coupled rotation has not been achieved from the structural perspective. We have attempted to delineate the F_O PT coupled rotation using similar multiscaling technique applied to F_1 ATPase. Our study was done before the advent of the new cryo-EM maps and instead used an NMR structure (Rastogi and Girvin 1999), which had oriented the subunit a vertically against the c-ring (Fig. 3b). Although it is very important to test whether our results hold for the cryo-EM F_O structure, we focus here on the importance of approaching a complex multidimensional problem using our hierarchically simplifying modeling techniques. Instead of approaching the problem using brute-force molecular dynamics simulations, our methodology focuses on extracting the most relevant free energetics of the key steps and then studies the combined system using a low dimensional functional free energy surface. This approach, as already demonstrated for the F_1 ATPase, can provide a much complete picture of the large macromolecular complexes and a structure-based molecular level understanding of the coupled chemical-conformational processes. A similar approach was adopted to study F_O, where we studied the PT coupled rotation process using our CG model that has been developed to capture the free energy changes of protein side chains embedded in different chemical environments, such as, water and/or membrane (Vicatos et al. 2014). This feature of the CG model allowed us to study the PT process in F_O by generating several rotated frames of the c-ring structure and then calculating the free energy of transporting a proton from the P side to the N side of the membrane (Mukherjee and Warshel 2012). Figure 6 shows the calculated free energy map, where the horizontal axis represents the sequentially rotated frames of the c-ring with the stator Arg(+) fixed in space, while the vertical axis represents the transport of a single proton from the P side to the N side of the membrane. This map clearly shows that a Asp/Glu residue sitting close to the Arg(+) can give off a proton to the N side via the low free energy path on the top half of the map, but the same charged Asp/Glu(−) cannot immediately reprotonate via the P side due to a large electrostatic barrier. The reprotonation of Asp/Glu(−) via the P side can only occur after the c-ring has rotated to avoid the electrostatic barrier imposed from the subunit a Arg(+). The map also reveals that the proton paths on the N and P sides are disjointed into two half channels and the effort of the c-ring to coordinate the PT using these two disjointed half channels gives rise to the rotation. Overall, the above results support the idea presented in earlier research (Junge et al. 1997; Vik and Antonio 1994) and further provide an energetics basis of the F_O functionality from a three-dimensional structural perspective. However, it would be highly interesting to see whether the key elements of the dislocated proton half channels and the electrostatic barrier are maintained for the new cryo-EM structure of the complete c-ring/subunit a model. We anticipate that these key elements will also be present in the free energy surface of the new c-ring/subunit a structure, as they are crucial for the PT coupled mechanical rotation, while the specific free energies and energy barriers of the PT pathways and rotational motion might change considerably. We further note that the inclusion of water molecules in the c-ring/subunit a interface while modeling using the NMR structure contributes considerably towards the difference in the free energies of the proton in the N and P side channels (Mukherjee and Warshel 2012). The near horizontal arrangement of the subunit a in the cryo-EM structures might preclude the need for such an ambiguous water inclusion strategy to solvate the protons in the half channels. Moreover, it is possible that the near horizontal arrangement of the subunit a helices allows for a different spatial separation of the N and P side half channels than seen for the NMR structure, which would directly dictate the sub-step features of the c-ring rotation. The development of a multiscale model for F_O based on the correct atomic level structure would certainly lead to a better understanding of the motor dynamics. Moreover, it would facilitate our understanding about the compliance between the two motors that leads individual F_1 and F_O to function efficiently as a single energy transducing

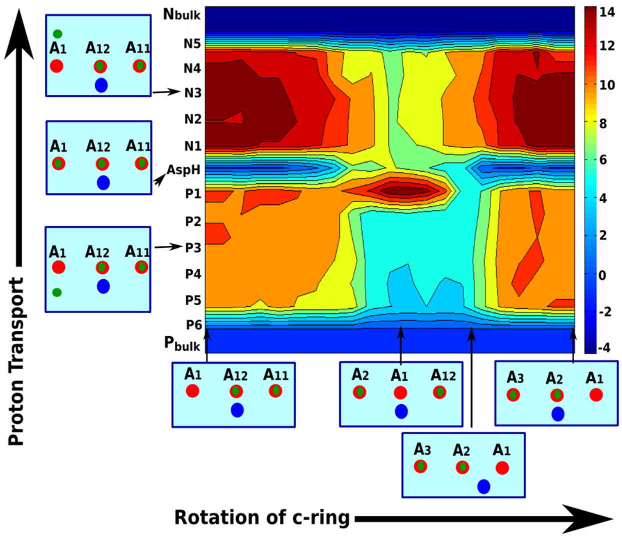

Fig. 6 The electrostatic map for the proton transport (PT) coupled c-ring rotation in F_O. The rotation of the c-ring helices is shown on the *X-axis*, while the schematics on the *X-axis* denotes the state of the system, using a similar *color* scheme as in Fig. 3. The position of the proton along the P and N sides of the membrane are shown on the *Y axis*, while the schematics on the *Y-axis* denotes the position of the proton in the system, using a similar *color* scheme as in Fig. 3. Here, P_{bulk} and N_{bulk} represents the outer solvent exposed area on the P and N sides of the membrane, while $P_{1,\ldots,6}$ and $N_{1,\ldots,5}$ represent internal positions of the proton on the P and N sides of the Asp–Arg pair, respectively. This figure is taken from Mukherjee and Warshel (2012)

rotary system (Junge and Nelson 2015; Junge et al. 2009; Walker 2013).

Design principles of light-activated and chemical catalysis-activated molecular machines

It is interesting to compare the operating principles of molecular systems that harness light energy to drive its functional cycle with those that use thermally activated chemical or charge transport free energies as the thermodynamic fuel. The molecular machineries of life have evolved to use both types of energy sources, although the underlying organizational principles for light-activated and catalysis-activated systems should be considered very carefully (Astumian 2016; Astumian et al. 2016). One of the most well-known examples of light-activated molecular system is the photosynthetic apparatus of plants, algae and cyanobacteria, which harnesses solar energy in the form of photons and transduces it into a form of chemical energy that is a more viable energy source for most other types of cellular processes (Mirkovic et al. 2017). The most important distinction of the photosynthetic apparatus is the presence of light sensitive ligands or chromophores. These molecules (electron donors, D) can reach excited states upon photon absorption ($D^0 \rightarrow D^*$) and can then funnel the energy to other near-by centers (electron acceptors, A) to generate light-induced charge separation ($D^+ \ldots A^-$). The process of light absorption and excitation is very fast (in the range of femtoseconds), while the dissipation of excited state energy ($D^* \ldots A^0$) to other centers ($D^+ \ldots A^-$) is a comparatively longer process. A free energy diagram of such a light-induced charge separation process is shown in Fig. 2 of Warshel and Schlosser (1981) or Fig. 11 of Astumian et al. (2016). The light-induced charge transfer will be efficient if the forward pathway forming the final charge-separated state ($D^+ \ldots A^-$) is a significantly downhill process and is much faster than the back reaction towards the initial ground state of the system ($D^0 \ldots A^0$). Since excitation from the ground to the excited state and vice versa are faster processes, the step following the initial charge-separation must be competitive for efficient light-induced charge separation system. This can only be controlled by significantly lowering the energy of the charge-separated state ($D^+ \ldots A^-$) in comparison to the previous state. It is important to note that an efficient light-induced charge separation cannot be obtained without lowering the energy of the final state, as the kinetic barrier would not allow faster propagation of the forward pathway in comparison to the back reaction.

In contrast to light-induced mechanism that relies on the dynamics between ground and excited states, catalysis driven motors (such as F_OF_1 and most of the other cellular machineries) transduce a predominantly entropic energy source stored in the form of non-equilibrium distribution of chemical or charged species. The molecular motor using this type of thermally activated energy source works by gating the chemical free energy through cycling between ligand-specific conformational states. Such a case is illustrated in Fig. 4c for the rotary-chemical process of F_1, where the minima or the stable intermediate states (0°, 80°, 120°, 200°, 240°, …) are energetically almost similar. It is the relative barriers along the forward (designated as ε) and backward (designated as ε^* and ε^{**}) paths that decide the directionality and efficiency of the motor. In other words, the design principles of a molecular motor would depend on the type of the energy source utilized by the system to operate its functional cycle. Keeping the above discussed principles in mind, light-activated systems can reach high efficiency when using an energy ratchet principle, while thermally activated systems rely on kinetic control or a Brownian ratchet mechanism (Astumian et al. 2016).

Concluding remarks

Understanding the molecular origin of the rotary-chemical function and energy transduction in F_OF_1 ATP synthase is a difficult challenge despite significant advances in three-dimensional structure elucidation, single molecule spectroscopy and kinetic studies. A major part of the difficulty is due to huge size of the system that forbids atomistic simulation of the complete motor at functionally relevant time scales, while another bottleneck is to model the coupled chemical and conformational events that span diverse ranges of the spatiotemporal regime. In this review, we have discussed our approach to model the action of the F_1 ATPase and the F_O protomotive motors, where emphasis is laid on elucidating the molecular origin of the driving force that leads to unidirectional rotation at the expense of ATP hydrolysis or proton gradient. We have used high-resolution crystallographic structure of the F_1 motor and used a hierarchical multiscale technique to generate low dimensional functional free energy surface of the complete rotary-chemical process. The same hierarchical multiscale approach has also been used to learn about the proton-transport-coupled-rotation in F_O motor, although the NMR structure used for the study has been recently disproved. The central feature of our multiscale approach is to draw meaningful characteristics of the motors from the mechano-chemical free energy surfaces. For example, our methodology has deciphered the physical reason behind directionality, established the reason behind stepped rotation with intermittent dwell states observed during motor dynamics, calculated rotational torque from the free energy surfaces and further studied the effect of mutating different parts of the motor domains.

These studies have generated a cumulative understanding of the organization principles behind the motor action. *Our research, reviewed here, has highlighted the role of electrostatics in establishing the directionality and the stepped rotational feature of the mechano-chemical coupling*. The methodology discussed here provides a quantitative way to model the action of complex macromolecules, and provides a structure-based understanding of the thermodynamics and kinetics principles working within these systems.

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