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Review

Protein conformational changes involved in the cytochrome bc_1 complex catalytic cycle ☆

Jason W. Cooley *

Department of Chemistry, University of Missouri, Columbia, MO 65211-7600, USA

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ABSTRACT

Early structures of the cytochrome bc_1 complex revealed heterogeneity in the position of the soluble portion of the Rieske iron sulfur protein subunit, implicating a movement of this domain during function. Subsequent biochemical and biophysical works have firmly established that the motion of this subunit acts in the capacity of a conformationally assisted electron transfer step during the already complicated catalytic mechanism described within the modified version of Peter Mitchells Q cycle. How the movement of this subunit is initiated or how the frequency of its motion is controlled as a function of other steps during the catalysis remain topics of debate within the active research communities. This review addresses the historical aspects of the discovery and description of this movement, while attempting to provide a context for the involvement of conformational motion in the catalysis and efficiency of the enzyme. This article is part of a Special Issue entitled: Respiratory complex III and related bc complexes.

1. Background

The Rieske/cytochrome (cyt) *b* complex family of enzymes plays a central role in bioenergetic electron transport chains that carry out the task of oxidative and photophosphorylation. The two main branches of this family include the cyt bc_1 complex-type enzymes best known for their roles in respiratory (and purple bacterial photosynthetic) ET chains and the cyt b_6f complexes, which are typically found linking the respiratory and photosynthetic ET chains in plant chloroplasts and cyanobacteria. While the overall protein structural and cofactor architecture of these two families of complexes differ somewhat, the structures and their dynamics are loosely similar when it comes to the primary substrate oxidation reactions [1,2].

The cyt bc_1 complexes, on which this review will focus, all have the same catalytic core of three transmembrane subunits [3]. The central nucleus of the complex is a homodimer of the multi-transmembrane spanning proteinsubunit cytochrome *b*, named for its coordination of two *b*-type hemes. The two hemes, termed b_L and b_H for their low and high potentials, respectively, are found towards either the P or N side of the membrane within which they are embedded, explaining the alternate naming convention in the cytochrome b_6f complexes. A cyt *b* monomer harbors two binding sites for quinone moieties adjacent to either heme, where one site is associated with oxidation of a hydroquinone (Q_o site) and the other site (Q_i site) serves in the reduction of a quinone or semi-quinone molecule at the opposite side of the membrane. Each cyt *b* is associated with the lone transmembrane domain, or anchor, of the remaining two cofactor containing subunits, named the cyt c_1 and FeS for presence of a *c*-type cytochrome or a "Rieske" type [2Fe–2S] cluster in their soluble domains, respectively [3,4,2,5].

As the formal name implies, the family of enzymes serves to oxidize a hydroquinone (at the Q_o) site and reduce a soluble acceptor molecule (typically cytochrome *c*, plastocyanin, or a high potential iron protein). The mechanism of this oxidation and reduction is carried out by a modified form of Mitchell's proposed Q cycle. The modified Q cycle as we understand it now, involves the oxidation of a hydroquinone at the Q_o site where one of the two electrons is funneled to a high potential chain and the second to a low potential electron transport chain, who's final electron acceptors are located at opposing sides of the bioenergetic membrane [6–13]. The high potential chain is comprised of the [2Fe–2S] cluster of the FeS subunit and *c*-type heme of the cyt c_1 subunit, eventually being passed to a soluble mobile electron carrier cyt *c* molecule, whereas the low potential chain is comprised of the b_L, the b_H and the quinone bound at the Q_i site (Fig. 1). Several structures solved at the atomic level not only reiterated the nearly identical architecture of all of the catalytic homodimeric cores for this protein family. Further, these structures also hinted at the presence of a rotational and translational movement of the soluble portion of the FeS subunit, as it was either disordered, at the Q_o site or near the cyt c_1 surface depending upon the occupancy or the Q_i site [14–19,3,1,20] (Fig. 2). The presence of this movement and its import during multiple turnovers of the Q_o site was subsequently proven using complimentary biochemical approaches [21] and the elegant suggestion that the large scale domain movement acted as an intra-complex electron shuttle overcoming a

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* Tel.: +1 573 884 7525; fax: +1 215 852 7624.
E-mail address: cooleyjw@missouri.edu.

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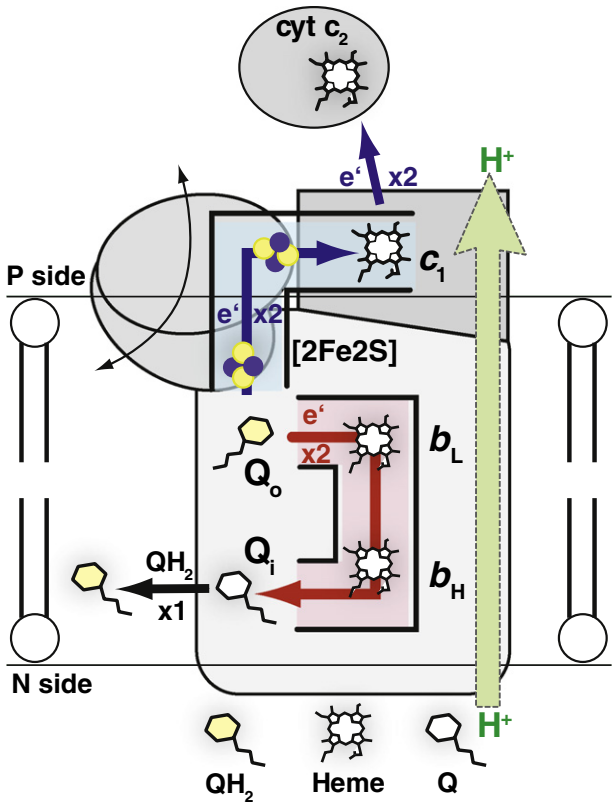

Fig. 1. Cartoon model of the turnover of the cyt bc_1 complex depicting the high (blue) and low (red) ET chains.

long inter-cofactor distance and unfavorable ET rate [22–26]. Since initial findings describing the domain movement and its function, many subsequent studies have dealt with whether this conformation change assisted ET reaction is controlled or even regulated in the context of the enzymes turnover. Specifically, questions of whether the domain movement is choreographed during the complicated sequence of events surrounding hydroquinone oxidation and subsequent Q_o site turnovers, or whether it is simply a tethered electron shuttle capable of diffusing at a rate slow enough to avoid short circuits and fast enough to not be rate limiting have remained.

2. FeS domain movement mechanics

How is the FeS head domain thought to move from the cyt *b* surface to the cyt c_1 surface? Shortly after the first series of structures were made available steered molecular dynamics was used to indicate that the rotational displacement was a feasible mechanism within the time constraints required for catalytic turnover [27]. This theory-based study revealed several potential interactions that could help stabilize intermediate positions observed in the coordinate files and help to guide the FeS subunit from the Q_o site at the cyt *b* surface to the surface of the cyt c_1 many of which were later verified experimentally [22–25,28,26]. Concurrent studies by the Yu lab, in which cysteine mutations in the cyt *b* and FeS head domain were able to abolish cyt *c* reduction in the presence of thiol reducing agents were able to prove conclusively that the motion of the FeS head domain was necessary for efficient electron transfer in the high potential chain of the enzyme and for multiple turnovers of the Q_o site to take place [21].

The atomic structures of the mammalian cyt bc_1 solved with various Q_o site occupants did envision subtle changes in the structure of the small α-helix formed in the amino acid sequence connecting the head domain and the transmembrane domain when the FeS metal center was near the cyt c_1 surface. This region became more coil-like when the head domain was near the Q_o site. Thus, this region became known as the “hinge” region of the FeS subunit implying that stabilizing this helical formation may be involved in promoting the movement of the head domain away from the surface of the cyt *b*. The structural changes in this region were subsequently confirmed indirectly by monitoring the protease accessibility of this site when differing Q_o site inhibitors known to change the FeS head domain position were introduced [29]. Several mutations in this hinge region have been made that had significant influence on the kinetics of cyt *c* re-reduction. The most well characterized are a series of amino acid insertions designed to extend the length of this loop with the aim of slowing the movement of the FeS head domain from the cyt *b* surface to thecyt c_1. This suite of mutations are often referred to as the +Ala insertion mutants (+1, 2 or 3 alanine residues). As it turns out, these mutants were integral in providing a biochemical mechanism for the domain movement and also in providing the first kinetic constraints associated with the movement during the catalytic cycle of the enzyme [23,24,26]. Thcs changes in the [2Fe−2S] cluster E_m were documented with altered equilibrium positions of the FeS position [24,28,30].

Interestingly, gain of function revertants to the + Ala hingeregion insertions were also isolated in which the resulting loci were found to be well away from the initial insertional mutations in the protein's sequence. These revertants implied that an intrinsic control over the equilibrium FeS head domain position was imparted by regions of the cyt *b* distant from the FeS head domain binding site at the cyt *b* surface. A number of the isolated mutations were found in the sequence of the cyt *b* subunit with specific legions found in the *ef* and *cd* extrinsic inter-transmembrane loops [23–25,28,31,32] as postulated by [27].

Due to the manifold technical constraints of this system, there has been very little work done to elucidate the biophysical forces involved with the head domain movement or how it is initiated. However, in general it is believed that the oxidation of the hydroquinone reduces the binding affinity of the head domain for the cyt *b* surface and the formation of the hinge helical region allows for the equilibrium position of

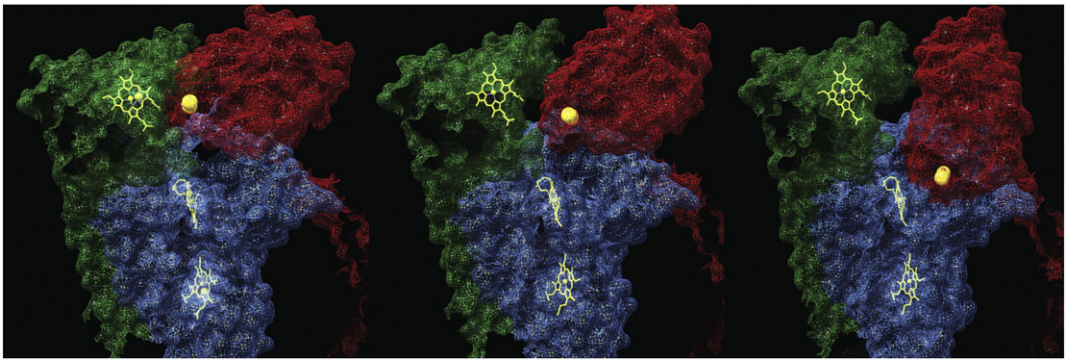

Fig. 2. Movement of the Rieske head domain as evidenced by solved structures with differing molecules in the Q_o site.

the soluble domain to favor those positions along the rotational displacement necessary for efficient reduction of the *c*-type heme of the cyt c_1 subunit. One attractive hypothesis that has been put forth is that the FeS head domain movement following hydroquinone oxidation is the result of a "spring loaded mechanism" [33]. In this theory, Crofts and coworkers have suggested that the strong hydrogen bond formed between the -nitrogen of the [2Fe–2S] cluster coordinating histidine and the hydroquinone at the Q_o site prior to the oxidation reaction is the bulk of the energy needed to facilitate binding of the FeS head domain to the cyt *b* surface. In essence, they have suggested that this bond represents the catch in a spring loaded mechanism, where, when the hydrogen bond is broken via the electron transfer from QH_2 to the [2Fe–2S] cluster, the "catch" is released and the spring, in the form of the hinge region helix formation, is sprung. This would unleash the potential energy of the spring, the inherent energy associated with forming the hinge region helix, thereby, sending the head domain vaulting off towards the cyt c_1 surface.

The spring loaded mechanism is an intuitively pleasing explanation for how the domain movement is controlled within the complicated catalytic cycle of the enzyme. However, the "strong" hydrogen bond hypothesis is based mostly upon observations of the head domain with the inhibitor stigmatellin, a molecule with very high affinity and thus very stable bond with the FeS head domain. The presence of a similar hydrogen bonding of the [2Fe–2S] cluster with the native substrate as with the inhibitor has beendocumented using pulsed EPR techniques, yet, differences in the influence of each hydrogen bond on the metal center E_m indicate that the bonds do not convey equivalent influences on the participating [2Fe–2S] cluster coordinating histidine residue [34–36]. These differences may be explained by differences in the relative number of FeS head domains found at the Q_o site with each Q_o site inhabiting molecule, with stigmatellin causing essentially 100% of the FeS head domain to at the proximal position versus somewhat less with native Q. This idea is reinforced by observations that the environment of the cyt *b* surface itself plays a role in altering the [2Fe–2S] cluster E_m. In fact, the affinity of the head domain for the cyt *b* surface remains high even when Q is oxidized and the [2Fe–2S] cluster is reduced and even in the absence of an interaction between the Q_o site occupant at all as evidenced by oriented EPR analyses [37,30]. Owing then to the propensity of the FeS head domain to be located at the cyt *b* surface [38], the energetics driving the release of the head domain from the surfaceof the cyt *b* may be somewhat more complex than the simple release of the head domain upon QH_2 oxidation and loss of Q_o site occupant [2Fe–2S] cluster interactions. The nature of the "spring", then, has yet to be fully realized. In order to understand what other forces may be at work in controlling this domain movement, other investigators have focused on the role of various interactions of the head domain at the Q_o site as well as along its trajectory form cyt xtitb to cyt c_1 surface as a means to understand how the movement is controlled or enacted.

3. Regions known to influence the FeS movement

As stated previously, the + $Ala_{(n)}$ insertion gain of function mutations exposed several interactions of the FeS head domain with the surface of the cyt *b* along the domain movement trajectory [23]. Several of these interactions were proposed from the aforementioned steered molecular dynamics simulations studies. However, the reversion of the +1Ala insertion mutant, +1Ala/L286F, was the first experimental evidence as to the role of the extrinsic *ef* loop which lies directly along the projected trajectory of the rotational displacement of the FeS head domain. These observations have been further borne out in cyt bc_1 complexes derived from various organismal sources, where the rate of photo initiated cyt *c* re-reduction were analyzed [39,31].

In effect, this loop can be thought of as the major barrier to this large scale conformational diffusion as the loop sits like a flap partially covering the more hydrophobic chasm that is the Q_o site. Darrouzet *et al.* came to the conclusion that bulky residues at the center of this extrinsic loop could effectively limit the rotational displacement away from the cyt *b* surface [23,25]. They also confirmed the proposal in [27] that residues in the *ef* loop are required to rotate out of the way of the FeS head domain in order for a complete translational rotation from cyt *b* to cyt c_1 surface to take place. The + $Ala_{(n)}$/L286F double mutants also revealed the role of the *ef* loop in limiting the FeS head domain equilibrium position to be near the Q_o site. When the gain of function L286F mutation was introduced into the +1Ala and +2Ala insertion mutants the raised midpoint potential of the [2Fe–2S] cluster observed in the parent strains was eliminated. This is correlated to an increased occupancy of the FeS head domain at the Q_o site pocket of the cyt *b* [23,25].In essence the *ef* loop appears to act as a valve for release of the FeS head domain from the cyt *b* surface to and fro from the cyt c_1 surface [23,25,39,31].

Gain of function revertants have also led to the discovery of additional sites away from the Q_o site forming cyt *b* surface [40–42,23–25,43]. Specifically, mutations generated in the FeS subunit that diminished formation of the Q_o site cyt *b*/QH_2/FeS complex yielded gain of function mutations located in the hinge region of the FeS subunit, again indicating the importance of this stretch of the *ef* loop in forming the proper active site [ES] complex [42,40].

Additional sites that are even more distant from the Q_o site can also influence the equilibrium FeS head domain position or Q_o site inhibitor binding [44–49,37,50,29,28,30,51]. Surprisingly, the nature and extent of the occupancy of the Q_i site, some 30 *Å* has been shown to influence the residency of FeS head domain at the the Q_o site or the cyt *b* surface proximal positions. This distant site influence over the equilibrium FeS head domain position was first hinted at using proteolytic cleavage assays of the FeS hinge region and the corresponding changes in the [2Fe–2S] E_m.

A more direct measurement of the Q_i mediated changes in the FeS head domain position used orientationally dependent EPR spectra of ordered chromatophore membrane from *Rhodobacter capsulatus*, which revealed that the ensemble environments of the [2Fe–2S] cluster are altered as a function of inhibitor binding at the Q_i site and even in the absence of Q_i site function [51,37,30]. In this case, addition of the Q_i site inhibitor antimycin A caused a heterogeneous orientation of the metal cluster versus the membrane plane, which in turn had a much more diverse set of spectral transition positions and shapes. Subsequent, application of oriented EPR techniques to antimycin A treated ordered membrane samples using the FeS head domain movement constrained + Ala_n mutants revealed two distinct populations of [2Fe–2S] cluster environments. This implied that one half of the FeS head domains remained tightly associated with the proximal cyt *b*, while the other half was free to explore alternative positions and environments [51,37]. The findings associated with changes in distance of the FeS versus the cyt *b* or cyt *c* were later confirmed via the use of distance dependent EPR measurements, where inter-paramagnetic distance changes implied that half of the [2Fe–2S] clusters changed their relative distance from the paramagnetic oxidized cyt c_1 heme iron [50].

These observations fueled hypotheses [52] that the dimeric turnover of the enzyme occurred via an alternating mechanism where each monomer turned over individually. Models were proposed in [51] which the alternation of sites was induced by the formation of a semiquinone species (represented by antimycin A binding) at the Q_i site of one monomer. The authors postulated that the presence of a semiquinone at the Q_i site of one monomer may facilitate that monomers FeS head domain to be found away from the cyt *b* surface, thus increasing the likelihood that oxidation of a hydroquinone would take place in the opposite monomer. This model implied a natural rationale for the postulated equilibration of electrons between the cyt *b* branches of the homodimer. A structural explanation for how this coupling may be carried out from Q_i to position of the FeS head domain was presented in [53] and is summarized in Fig. 3. Still other work using the atomic structures as a guiding principle, generated mutations in loops that make up the surface of the cyt *b* at the Q_o site, where the investigators

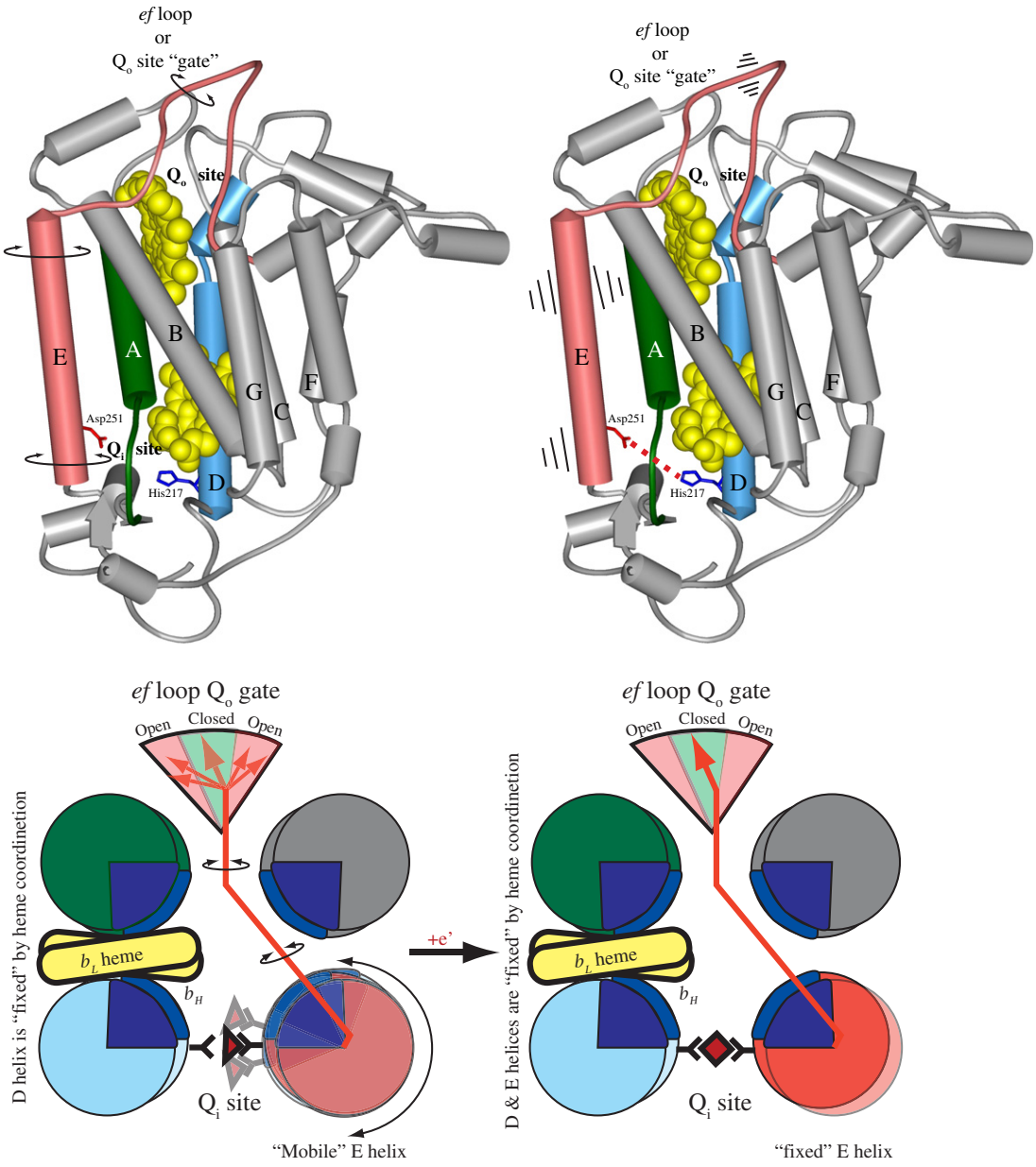

Fig. 3. Proposed scheme for Q_i site mediated changes in the equilibrium FeS head domain position.

found that the ability of these loops to slide or move at the surface as a function of the hydroquinone oxidation state at the Q_o influenced the position or efficiency of the FeS movement [32]. In all, it appears that the factors determining FeS head domain occupancy at the cyt *b* surface are more complicated than any one theory put forth thus far.

4. Implications for movement on Q_o site efficiency

Despite all of the evidence that movement of various portions of the cyt *b* influence the steady state position and frequency of the FeS domain movement, it remains unclear what if any the role of these lets call them fine tuning controls over the movement play in the catalytic mechanism of the enzyme or its control. For instance, while it is clear that the Q_i site occupancy influences roughly half of the FeS subunits positions, it is not clear why the enzyme would need to limit oxidation to either monomer of the dimer [51,50].

Several hypotheses have been raised regarding this issue with respect to whether electrons are free to equilibrate or not in the ET chains of the two cyt *b* monomers, but the general argument is that the kinetics of the ET reactions are predominantly controlled by the thermodynamic driving forces of the cofactors and their distances from one another [54]. This view would seem to preclude aneed for any change in the binding affinity of the FeS head domain for either oxidation–reduction relevant surface, i.e. the cyt *b* or cyt *c* surfaces, as the cyt *b* cofactor distance does not change. Additionally, FeS head domain trajectory oscillation should be well within the kinetic constraints placed on the system by the relatively slow ET rate from one b_L to another across the monomer–monomer interface. Regardless, looking at the data collected from several labs showing a clear influence of distinct regions of the enzyme on the FeS equilibrium position, a purely kinetic description of control is difficult to reconcile as it is curious to envision why such complicated mechanisms designed to control the changes in the steady state position of the head domain would be conserved in through organisms.

Thus, while we do not understand why the control of the head domain position is needed, it is present regardless of our perception of its necessity. Based upon this logic, it is easy to rationalize that we may not fully understand all of the conditions from which the enzyme must guard against deleterious side reactions during the course of the

enzyme's workday in a cellular environment. Conversely, one might argue that the control of this movement may be a case of biological over-engineering, for which there is very little selective pressure exerted or where the interwoven nature of the control mechanisms and the necessary structural requirements of the homodimeric enzyme precludes specific selective pressure of presumably "fine" tuning control mechanisms. It goes without saying that as we understand more and more about how and electrons are re-distributed within the dimeric complex and how fast, it is likely that we might in fact stumble upon the necessary reason for why the mobility of the FeS head domain needs to be controlled at all.

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