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COMPUTATIONAL ANALYSIS OF EVOLUTIONARY AND STRUCTURAL ASPECTS OF CRYPTOCHROMES AND FRATAXINS

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ABSTRACT

This thesis reports the work performed using Bioinformatics tools to understand evolutionary and structural aspects of Cryptochromes and Frataxins. Sequence alignments and sequence-based similarity networks demonstrate that CRY1 and CRY2 are the most homogeneous group members in the CRY/Photolyase family, showing clearly higher sequence identity when comparing with CRY-DASHs and DNA photolyases members. The FAD binding domain is conserved in vertebrates and insect CRYs, while DNA photolyase domain is only conserved in vertebrates with low residue conservation in insect CRYs. The low Photolyase Homology Region domain conservation among DNA Photolyases, suggests variable amino acid conservation for DNA repair activity function. Docking predictions show *Drosophila* Cryptochrome (dCRY) can accommodate the flavin type 8-HDF as second chromophore. Likewise Photolyases, dCRY conserved a hydrophobic side close to the flavin type cofactor. Supporting this prediction, residues W314, W413,W420 and W422 are properly located to play energy transfer between 8-HDF and FAD cofactors. Molecular Dynamics simulations demonstrate that human frataxin can play metal-binding through an acidic ridge on the alpha1 region. The simulations indicate the acidic residues Asp112, Glu111 and Glu108, and Glu100, Glu101 and Asp104 as potential metal binding sites, although low affinity seems to drive the binding nature. This finding is consistent with both downstream interactions in the Fe-S cluster formation and the iron “sensor” functions that frataxins may play.

Keywords: Cryptochromes; Second chromophore; Frataxins; Acidic ridge

Analisi computazionale degli aspetti evolutivi e strutturale di Criptocromi e Fratassina

RIASSUNTO

Questa tesi riporta il lavoro eseguito utilizzando strumenti bioinformatici per comprendere gli aspetti evolutivi e strutturali di Criptocromi e Fratassina. Allineamenti di sequenza e le reti di similarità basate su sequenza dimostrano che CRY1 e CRY2 sono i membri del gruppo più omogenei, che mostrano chiaramente maggiore identità di sequenza quando si confrontano con CRY-DASHs e DNA fotoliasi. Il dominio di legame FAD è conservato nei vertebrati e insetti, mentre il dominio DNA fotoliasi è conservato solo nei vertebrati, con bassa conservazione di residuo in insetti. La bassa conservazione del dominio della Regione Omologa di Fotoliasi tra DNA fotoliasi suggerisce una conservazione aminoacidica variabile per la funzione di attività di riparazione del DNA. Predizioni di Docking mostrano che *Drosophila* Cryptochrome (dCRY) è in grado di ospitare la Flavina 8-HDF come secondo cromoforo. Allo stesso modo che le fotoliasi, dCRY conserva un lato idrofobo vicino al cofattore di tipo Flavina. A supporto di questa previsione, i residui W314, W413, W420 e W422 sono opportunamente posizionati per eseguire la funzione di trasferimento di energia tra le cofattori 8-HDF e FAD. Simulazioni di dinamica molecolare hanno dimostrato che la fratassina umana può svolgere un ruolo nella funzione metallo-legame attraverso la regione acida alfa1. Le simulazioni hanno dimostrato che i residui acidi Asp112, Glu111 e Glu108; e Glu100, Glu101 e Asp104 possono legare un metallo, anche se la bassa affinità sembra guidare il carattere vincolante. Questo risultato è coerente con le interazioni nella formazione del cluster Fe - S e le funzioni di "sensore" di ferro che le fratassine possono svolgere.

Parole chiave: Criptocromi; Secondo cromoforo; Fratassina, Regione Acida

1. Introduction

Bioinformatics is the application of IT and computational methods used to tackle biological challenges. The vast amount of biological sequence/structural based data produced requires fast ways to interpret them. Computer-based tools have been developed to integrate and analyze information in the various databases. In this way, computer (dry lab) experiments can help researchers by simplifying and speeding up the analysis of biological data which would take much more time by traditional approaches. This characteristics made Bioinformatics spreading not only across molecular biology, but also into many general aspects of life science, including drug discovery, medicine and environmental science.

The reliability of many Bioinformatics tools predictions rely basically on availability and quality of data. A method developed today can have a better performance in the future when more data will be available, thought some data might not be publicly accessible. In order to avoid this, when developing prediction methods, simplifying assumptions need to be considered. Also, molecular dynamics related predictions need to simplify thermodynamics concepts due to computational time consuming. Regardless of simplifying assumptions, computer-based tools are a powerful tool to quickly understand biological relevant problems.

This thesis reports the work performed using Bioinformatics tools to understand evolutionary and structural aspects of specific proteins. In the first part, sequence alignment and sequence-based similarity networks have been used to understand the Cryptochrome/Photolyase family. These tools helped to infer differences among members of this family, serving to understand evolutionary aspects in this family. Also regarding Cryptochromes, docking predictions were performed to verify if *Drosophila* Cryptochrome (dCRY) can accommodate a second cofactor, contributing to a mechanistic understanding of the complex functional regulation underlying dCRY light activation. In the last part, structural aspects of human frataxin have been addressed. Through Molecular Dynamics simulations, it was verified if an acidic region in human frataxin has metal-binding capabilities. In this work it was possible to understand the iron-binding nature of frataxins.

2. Overview - Cryptochromes

2.1 Circadian Rhythms

Circadian rhythms are direct responses of cycle changes needs (for example, darkness and light) of organisms. Three main characteristics are required to characterize a circadian rhythm as a "clock": maintenance of the rhythm even in absence of external factors; light or dark reseting and temperature compensation of the circadian period. Given its importance, they can be found in all kingdoms of life. Circadian clock outputs, which are genetic timekeepers enabling organisms to maintain cycle rhythms, are believed to control many biological and physiological processes, like metabolism (Eckel-Mahan and Sassone-Corsi, 2013), physiology, behavior, learning, memory, aging and longevity, and even cell cycle.

2.2 Where Are Cryptochromes Expressed in Different Organisms?

Cryptochromes (CRY) are one of the molecular clocks found in many organisms. CRYs have been shown playing a direct role in the circadian rhythm as components of the circadian pacemakers. Cryptochromes were first discovered as blue-light and ultraviolet photoreceptors in plants. It is widely accepted Cryptochromes (CRY) are likely evolutionary descendants of DNA photolyases (subclass of CRY/Photolyase family proteins related with DNA repair function). However, since it is was believed there were no Cryptochromes in bacteria, but only in eukarya kingdom, this assumption has been cast doubt as Cryptochrome is also reported to be expressed in *Synechocystis* sp (Hitomi et al., 2000).

Members of CRY/Photolyase family can be found in bacteria, archaea and eukarya kingdoms, although Cryptochromes are mostly characterized in eukaryotes. *Drosophila* CRY is predominantly found in the nucleus (Stanewsky et al., 1998) (Emery et al., 1998), although it has also been found in the cytosol (Ceriani et al., 1999). Like *Drosophila*, also mammalians CRYs are predominantly in the nucleus (Kume et al., 1999) and can also be found in the cytosol (Thompson et al., 2003). Differently, plant

CRYs have shown only nuclear function (Guo et al., 1999).

CRYs occur in multiple tissues of *Drosophila.* In *Drosophila,* CRY is expressed in specific circadian oscillator neurons of the brain. Since CRYs are not expressed in all oscillator neurons, it is believed that these neurons are entrained by peripheral tissues (Helfrich-Förster et al., 2001).

Although CRYs are not expressed in every clock cell, they have circadian functions in relevant pacemaker neurons and in the compound eyes (Yoshii et al., 2008). CRYs in *Drosophila* show specificity for light dependent clock neurons (Yoshii et al., 2010), rather than temperature sensitives. However, signaling between peripheral and brain clocks seem to play an important role in *Drosophila* circadian rhythm. It has been demonstrated that brain clock neurons depend on signals from peripheral tissues (Sehadova et al., 2009). *Drosophila* CRY has been implicated in oscillations role in different peripheral tissues (Krishnan et al., 2001), although the importance of its function still remain unclear (Ivanchenko et al., 2001). Although CRYs presence in many peripheral clocks seems to play rhymicity, it does not show relevance in the core of rhythm generation in epidermal oscillators (Ito et al., 2008). Overall, as *Drosophila* CRY is expressed in multiple tissues, but seems to play different roles in different tissues, it indicates that dCRY may have tissue-specific role in circadian systems (Krishnan et al., 2001). Furthermore, CRY expression in pacemakers neurons and peripheral oscillators is likely controlled by different regulatory systems (Hao Zheng et al., 2008).

However, in vertebrates, CRYs have shown tissue expression differences. Since these organisms have two type of CRYs (CRY1 and CRY2), different expression in different organs have been identified (del Pozo et al., 2012). Moreover, mouse CRY (mCRY), along with rhodopsins, may play a circadian-photoreceptive role in the retina (Hall, 2000). Overall, while mammals and flies show differences between their central and peripheral clocks mechanisms, they do share similarities in the mechanism of their peripheral oscillators (Glossop and Hardin, 2002).

CRY1 and CRY2 of plants show primary function in the nucleus, while CRY3 is likely to function in chloroplasts and mitochondria (Kleine et al., 2003) (Yu et al., 2007) (Wu and Spalding, 2007). However, distinct functions during growth and development have been attributed to *Arabidopsis* CRY1 whether in the nucleus or in the cytoplasm

(Wu and Spalding, 2007). Rice CRY1 (OsCRY1), similarly than *Arabidopsis* CRY1, and CRY2 (OsCRY2) has been shown to have both cytoplasmic and nuclear localization (Matsumoto, 2003). Wheat CRY1 (TaCRY1), similarly than *Arabidopsis* and rice CRY1 is also expressed in both nucleus and cytoplasm, whereas TaCRY2 is found in the nucleus (Xu et al., 2008).

2.3 Binding Partners and Functional Differences

Cryptochromes are well-studied circadian clock in *Drosophila (Collins et al., 2005)*, mammals and plants. Since organisms have developed complex circadian clock mechanisms, differences have also been reported among CRY driven circadian rhythms.

In *Drosophila,* CRYs have been shown playing resetting and photoreception entrainment functions of the circadian rhythm (Emery et al., 1998). *Drosophila* CRY (dCRY) plays an important role regulating the circadian clock through synchronized interaction to downstream partners. One of dCRY partners is TIMELESS (TIM) (Busza et al., 2004), which has been shown playing important role mediating regulation of circadian clocks (Meissner et al., 2008). dCRY binding correlation to TIM and another interactor PERIOD (PER) has been reported even in ovarian tissues (Rush et al., 2006). The regulation of *Drosophila*'s circadian rhythm reveals complexity with different molecular components. dCRY, TIM and the F-box protein Jetlag can be found in the same pathway (Peschel et al., 2006). It is believed dCRY's interaction to TIM commits it to degradation. Although widely reported, interaction TIM-CRY is not always necessary to mediate neuron clock regulation (Fogle et al., 2011), which indicates dCRY plays different roles in different organs/tissues, whether they are peripheral or central clocks.

Another player in the circadian rhythm, PER is also a target for dCRY interaction (Rosato et al., 2001). Since PER is a CRY binding partner, and an interaction between PER-TIM has also been indicated (Kaushik et al., 2007), speculations that CRY could form a complex with TIM-PER have been suggested (Ceriani et al., 1999).

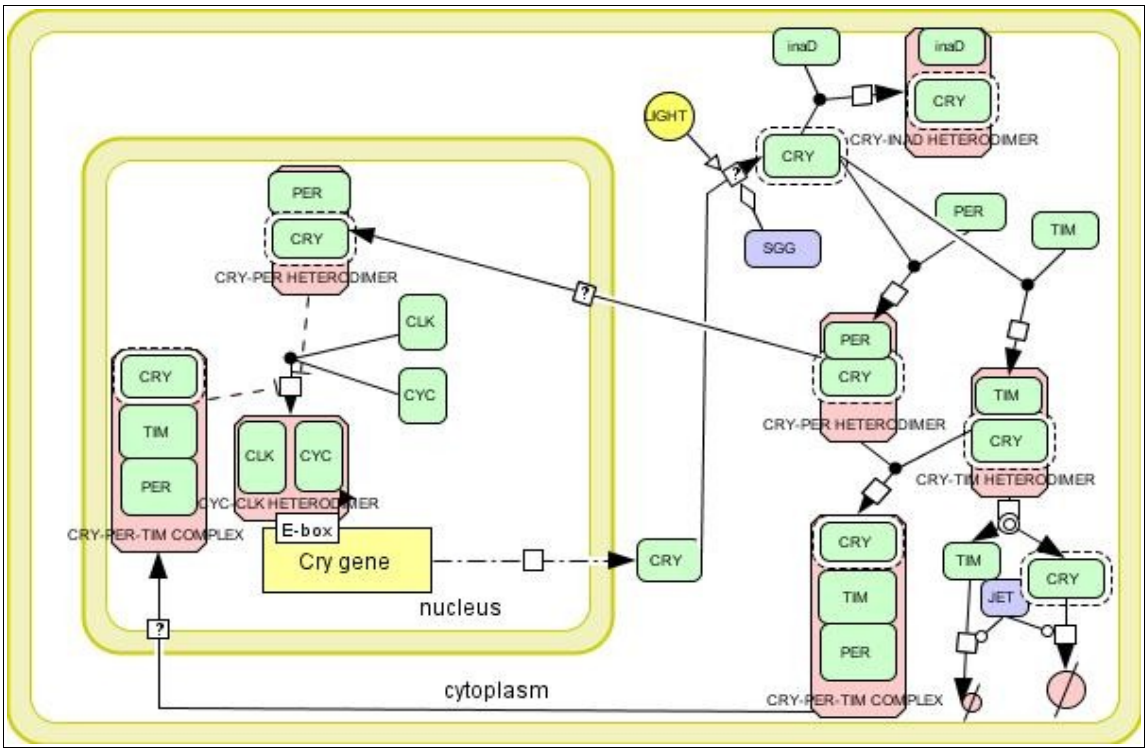

Figure 1. *Drosophila* Cryptochrome binding partners in the circadian rhythm.

dCRY's key function in the circadian rhythm is further confirmed when looking at its involvement in transcriptional repression of genes involved in the circadian clock (Collins et al., 2006). It has been demonstrated that dCRY together with PER can repress transcriptions of circadian oscillators key players by repressing CLK/CYC activity. The authors suggest that PER and CRY seem to control distinct steps on repression, with CRY initiating and PER maintaining repression.

Circadian clocks show some similarities and also differences between *Drosophila* and mammals. Mammals have two CRYs genes. CRYs role in mammals is well-studied in transcriptional repression. Similarly than *Drosophila* CRY, mammalian CRYs (mCRY1 and mCRY2) have been shown to inhibit the transcription activity of the CLOCK/BMAL1 heterodimer (Tamanini et al., 2007). Likewise dCRY, mCRYs impair CLOCK/BMAL1 (CLK/CYC in *Drosophila*) activity through interaction to PERs (Chaves et al., 2006) and also by impairing phosphorylation (Dardente et al., 2007). Although both PERs and CRYs seem to inhibit transcriptional activity of clock proteins, CRYs (especially CRY1) are stronger repressors than PER proteins. While they both together repress transcriptional activity, they also bind differently. PER2 binds to both BMAL1 and CLOCK whereas, CRY1 and CRY2 are only able to bind to BMAL1. Mammals also have TIMELESS, although is not considered a true ortholog of

Drosophila TIMELESS (dTIM). Despite its sequencial differences to dTIM, it has shown to preserve oscillation clock functions in mammals, although it has acquired dual function in mammals (Engelen et al., 2013).

Likewise mammals and insects, plants also have CRYs acting in photoreception in the circadian clock, but plants CRY1 and CRY2 function is related to growth and development (Kang et al., 2009) (Mao et al., 2005). CRY3 (member of CRY-DASH clade) of plants, shows intermediate functions between CRYs and Photolyases. Besides photosensor activity, it has been shown having DNA-repairing enzyme activity, which has been atributed only to Photolyases (Brudler et al., 2003) (Pokorny et al., 2008). Differently than mammals and insects, since plants do not have PER and TIM, plant CRYs photosensor activity shows different players. Some reported partners are: CIBs (CRY-interacting basic-helix-loop-helix), SPAs (SUPPRESSOR OF PHYA) and COP1 (CONSTITUTIVELY PHOTOMORPHOGENIC1), review (Liu et al., 2011). Also, differently than mammals and insects, no transcriptional repression has been atributed to plant CRY1 and CRY2, but instead this function seems to be transferred to the CRY-DASH members in plants (Coesel et al., 2009).

2.4 Structural Characteristics and Domain Organization of CRYs/Photolyases

Cryptochromes are a class of proteins having N-terminal domains with marked sequence similarity to DNA photolyases, an enzyme that removes UV light-induced DNA damage using visible light as an energy source (Sancar, 2003). Within the amino-terminal photolyase homology region (PHR), cryptochromes and photolyases have similar three-dimensional structures, characterized by an N-terminal α/β domain and a C-terminal α-helical domain. Along with sequence similarity, cryptochormes and photolyases are highly structurally similar. It has been found a total rmsd (root mean square deviation) of only 1.9 Å among cryptochromes and photolyases (Müller and Carell, 2009). Cryptochromes are divided in three subclasses: CRY1, CRY2 and CRY3 (also annotated as CRY-DASH), whereas DNA Photolyases are divided in two subclasses: DNA Photolyases and 6-4 Photolyases. Although a fourth CRY type (CRY4) member has been identified, little is known about this member. Members of this family

are not equally shared among organisms. For example, as plants have all members of this family, humans show only CRY1 and CRY2 members. Differences among CRYs and Photolyases can also be verified in their functions. As Photolyases have DNA repairing function, no DNA repairing activity has been atributed to CRYs. Only CRY-DASH, considered an intermediate class of the CRY/Phtolyase family, has been shown to have both DNA repairing and transcriptional repression activities (Brudler et al., 2003) (Pokorny et al., 2008).

Considering sequence-based correlations, when looking at PFAM database (Finn et al., 2006), CRYs of humans, insects and even plants show the same domain organization (Figure 2 and 3). CRYs show a common sequence-based prediction indicating a DNA Photolyase followed by a FAD binding domain.

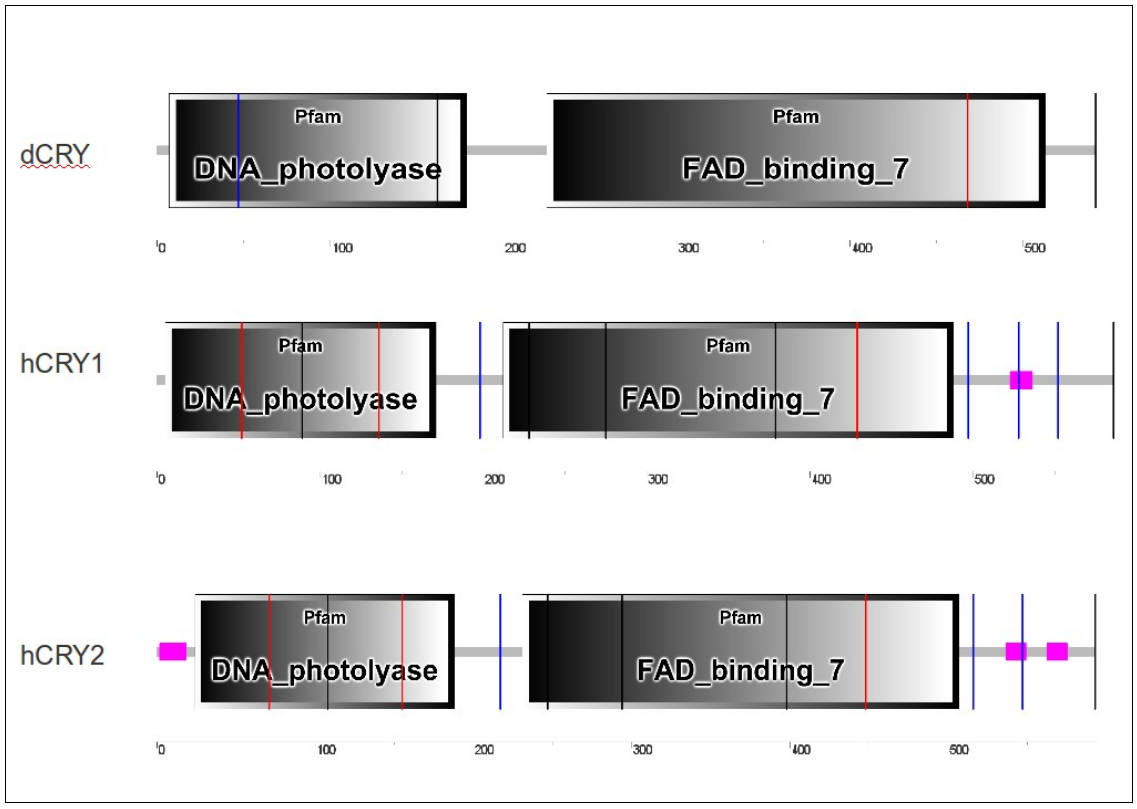

Figure 2. PFAM database prediction for *Drosophila* and human CRYs.

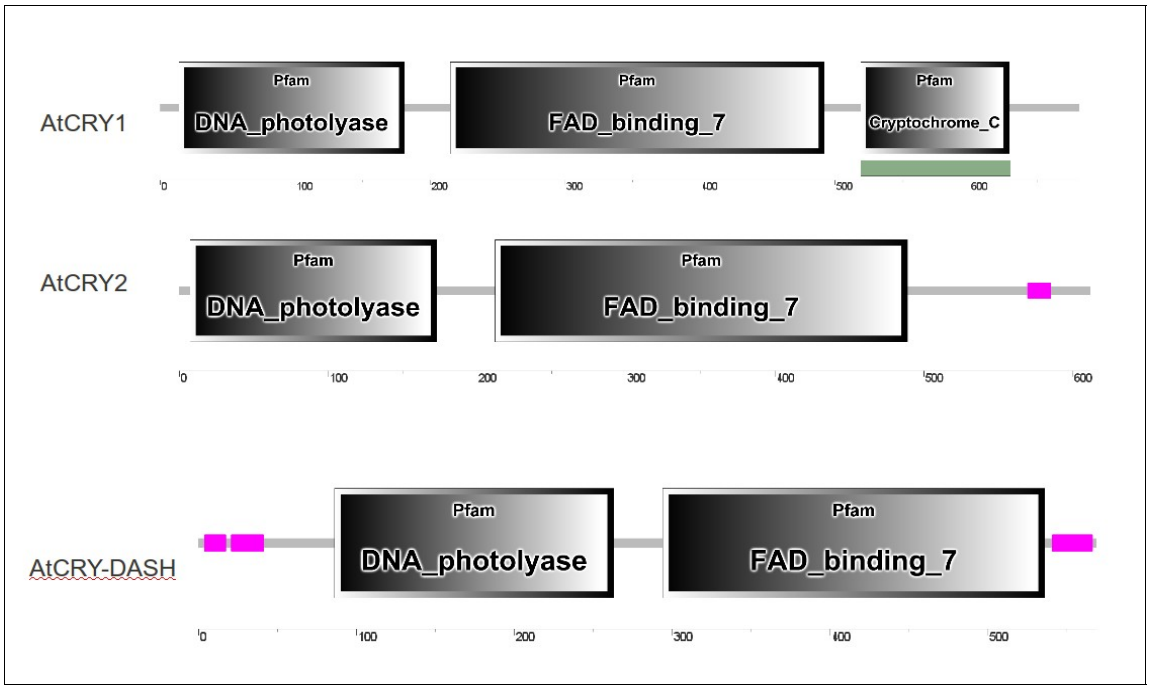

Figure 3. Domain organization of *Arabidopsis thaliana* CRYs.

However, while these two domains are highly conserved among CRY type members, Photolyase type members show some divergence. For example, since *E coli* DNA Photolyase and *Arabidopsis* 6-4 Photolyase share the two domains with CRYs, *Arabidopsis* DNA Photolyase only conserved the DNA Photolyase domain (Figure 4).

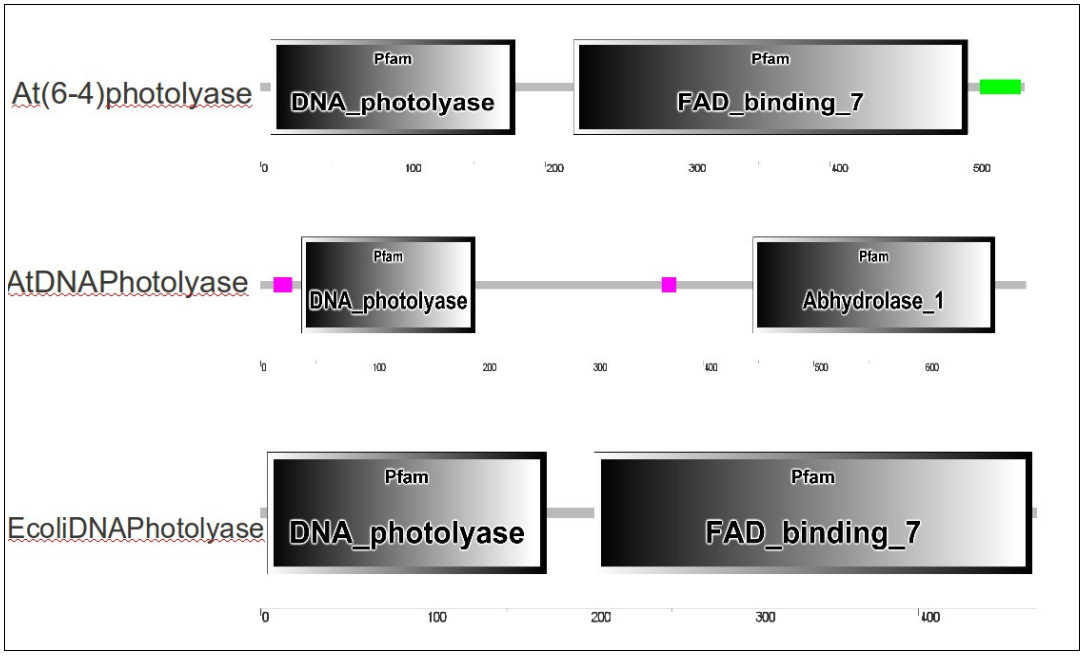

Figure 4. Domain organization of *Arabidopsis thaliana* DNA Photolyases and (6-4) Photolyases and *E coli* DNA Photolyases.

Although cryptochromes share a high degree of homology with photolyases, a defining characteristic of both plant and animal CRYs are C-terminal extensions of varying size, not present in photolyases. For example, in *Drosophila* CRY this C-terminal extension has about 40 residues, while *Arabidopsis* CRY1 has about 180 amino acids. It is widely accepted that the diversity and the acquirement of different C-termini is responsible for CRYs functional differences with photolyases and even among CRYs in different organisms.

In mammals, mCRY1 C-terminus is related with its function as circadian core oscillator while interacting to PER (Chaves et al., 2006). The authors further suggest that the complex interaction system to which mCRY1 is involved in order to maintain the circadian rhythm, makes necessary both the C-terminus and the PHR domain to fully exert its function. In a recent study, however, it has been suggested that mammals CRYs C-terminus is not absolutely required for repression activity, but can modulate period length (Khan et al., 2012). Differences among mCRY1 and mCRY2 have also been found, with mCRY1 having stronger repression activity than mCRY2.

Similarly, also in plant CRYs the C-terminus seems to have an important role in signaling. It has been suggested that their C-terminal extensions work as a "tail" triggering a conformational change, separating from the PHR domain and altering the interaction between CRYs and its signaling partners (Yang et al., 2000). Electrostatic repelling between the C-terminal region and the PHR domain seems to drive the signaling process.

In another well-studied model organism for CRYs, also in *Drosophila melanogaster* the C-terminus seems to play an important role when interacting to its circadian system partners. It has been shown that its C-terminus is important to play regulatory role with PER and TIM partners in pacemakers neurons (Dissel et al., 2004). In another C-terminal related study, the authors suggest that in addition to TIM-CRY interaction, this region also regulates CRYs stability and circadian photosensitivity (Busza et al., 2004). The authors further suggest that the PHR domain is sufficient for light detection and phototransduction, whereas the C-terminal region is not required for this activity. dCRY C-terminal region has also been shown to be important for the individual interaction to PER (Rosato et al., 2001).

2.5 Light and Temperature Dependencies and Magnetoreceptor Function

Several studies have shown light dependency for CRYs activity. dCRY has reported activity in the blue/near-UV region of the visible spectrum, with cut-off in about 500 nm and peak at 450 nm (VanVickle-Chavez and Gelder, 2007). In *Drosophila*, degradation of TIM via the F-box protein Jetlag (JET) is reported to be light-dependent. Light seems to trigger a conformational change in CRY allowing it to bind TIM, bringing it along with JET ubiquitination to the proteasome pathway for degradation (Naidoo et al., 1999). Also interaction of dCRY's to its circadian clock partners TIM and PER is light-driven (Ceriani et al., 1999) (Rosato et al., 2001). Although dCRY is well known for its light-dependent activity, light-independent function has also been found. It has been shown that dCRY can play photoreceptor-independent role in the *Drosophila* antennae, enhancing peripheral and central different oscillator mechanisms in *Drosophila* (Krishnan et al., 2001). Although not the core mechanism in the circadian clock activity, light-independent physical interaction between CRY and PER has also been found (Rosato et al., 2001). In addition, in *Drosophila,* besides light input, also temperature is reported to reset the clock system of the complex TIM-PER-dCRY. It has been suggested that light and temperature can synergistically drive the oscillation clock (Kaushik et al., 2007).

Contrasting with *Drosophila* CRY, mammals CRY1 and CRY2 act light-independently on repression by inhibiting activity of CLOCK-BMAL1 transcriptional activators (Griffin et al., 1999). However, a light dependent function has been atributed to both *Drosophila* and human CRYs. dCRY and human CRY2 (widely expressed in the retina), can function as magnetosensors (Gegear et al., 2008) (Foley et al., 2011), provinding information in a likely role of CRYs in the visual system. Moreover, fluorescence and EPR spectroscopies studies have demonstrated that human CRY1 can have light sensitivities functions (Hoang et al., 2008).

In plants, CRYs light sensitivity functions are similar than *Drosophila*. CRY2 interacts in a blue-light dependent manner with SPA1 and, regulating COP1 activity in *Arabidopsis* (Zuo et al., 2011). However, as plant CRYs are well known to have light-dependent activity, they also can have light independent function. Gene expression regulation has been reported to have blue light-independent activity (Yang et al., 2008).

Furthermore, CRY1 was found bound to COP1 in dark conditions (Yang et al., 2001). Differently than mammals and *Drosophila* CRYs, *Arabidopsis* CRYs have no response to magnetic fields in Cryptochrome-related plant growth (Harris et al., 2009), suggesting no magnetosensor activity to plant CRYs.

2.6 Phosphorylation and Degradation

Posttranslational modifications play an important role in regulating the circadian rhythm. Phosphorylation has been reported to be the most important posttranslational modification in circadian rhythms. Such phosphorylations usually function as activators/inactivators of protein states either by kinases or changing subcellular localization. Phosphorylation can also initiate protein degradation by the proteasome. Specific phosphorylation sites in the target proteins, after phosphorylated, are targets for the ubiquitin ligase complex.

In mammals, phosphorylation of mCRY1 C-terminal tail makes the circadian period to lengthen. Also, along with period length, phosphorylation has been atributed to CRYs stability, by preventing it to be degraded (Gao et al., 2013). Adenosine monophosphate-activated protein kinase (AMPK) has been shown to directly phosphorylate and destabilize mouse CRY1 in peripheral clocks (Lamia et al., 2009). Besides regulating the circadian rhythm by its own phosphorylation, CRYs can also set the pace of the clock by impairing transcriptional activators phosphorylation. It has been demonstrated that CRYs can regulate the clock by impairing the phosphorylation of the heterodimer CLOCK–BMAL1 (Dardente et al., 2007). Instead, mouse CRY2 has been reported to be phosphorylated in the liver by glycogen synthase kinase-3β (GSK-3β), destabilizing and contributing to its degradation (Kondratov et al., 2006) (Harada et al., 2005). Moreover, human CRYs have been reported to have autokinase activity (Özgür and Sancar, 2006), giving them further phosphorylation capabilities. Degradation is an important step in determining the clock rhythmicity. In mammals, this regulation has been shown to be performed by the SCF(Fbxl3) ubiquitin ligase complex, which ubiquitinates and brings CRY1 and CRY2 for degradation (Busino et al., 2007).

In a recent study, a complex of BRWD3 (as a substrate receptor) and a CRL4 E3 ligase, has been proposed to ubiquitylate dCRY in a light-dependent manner, before its

proteolysis by the proteosome, resetting the clock. dCRY, through the interaction to the F-box protein Jetlag (JET), has also been shown to be responsible to TIM degradation (Ozturk et al., 2013). Shaggy (SGG), which is the *Drosophila* ortholog of mammalian GSK3, can interact and stabilize dCRY, suggesting a possible phosphorylation step in its circadian clock function (Stoleru et al., 2007). SGG interaction to dCRY is also believed to be responsible to TIM phosphorylation, affecting its stability. Differently than human CRYs and *Arabidopsis* CRY1, dCRY lacks autokinase activity (Ozturk et al., 2009).

Similarly than human CRYs, *Arabidopsis* CRY1 catalyzes autophosphorylation, whereas CRY2 does not (Özgür and Sancar, 2006), suggesting different post-translational modifications pathways among plant CRYs. Likewise other organisms, *Arabidopsis* CRY2 also undergo ubiquitination-dependent degradation. E3 ubiquitin ligase CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), has been shown to be associated with CRY2 degradation, although additional E3 ubiquitin ligases can be involved (Shalitin et al., 2002) (Wang et al., 2001). This is further supported by the fact that SPA proteins, which are important for COP1 activity, show no direct involvement to CRY2 degradation.

2.7 Nuclear Localization Signals

Nuclear entry timing of circadian clocks plays an important step on regulating circadian oscillation. Circadian oscillation is driven by a transcription- and translation-based negative feedback loop, by impairing/allowing transcription of negative and positive regulators (Dunlap, 1999). Since the main molecular clocks can be found either in the nucleus or in the cytoplasm, timing transition between subcellular compartments drive the pace of the clock.

In mammals, PER has been shown to regulate transcriptional repression associated to CRY1. In fact, nuclear entry seems to be related with the presence of a nuclear localization domain (NLD) in rPER2, which has been demonstrated to establish nuclear accumulation of both rPER2 and CRY1 (Miyazaki et al., 2001). In another study, however, the authors suggest that in fact Nuclear Localization Signals (NLS) of both mPER2 and mCRY1 are required for nuclear localization. Besides from enter the nucleus associated to PER, the authors further suggest mCRY1 can have an alternative

route to shuttle to the nucleus (Chaves et al., 2006). The importance of the NLS motif for nuclear translocation is confirmed in another study, using *Xenopus laevis* CRYs as a model (Zhu et al., 2003). Moreover, also mCRY2 has shown its own capability to shuttle to the nucleus together with mPER2 mediated via importin alpha/beta nuclear imports (Sakakida et al., 2005).

Arabidopsis CRY2 nuclear localization is reported to be related with its C-terminal region, which contains a nuclear localization signal (Zuo et al., 2012) Two nuclear localization domains have been identified in rice CRY1 (OsCRY1) (Matsumoto, 2003). Moreover, wheat CRY1 (TaCRY1) has been reported to have nuclear localization domains (Xu et al., 2008).

To date, *Drosophila* CRY has not shown to have nuclear localization domains, enabling it to translocate to the nucleus. TIM has been demonstrated to have a nuclear localization signal and, its association to PER is believed to modulate circadian behavior and nuclear accumulation of both PER and TIM (Saez et al., 2011). It is possible that dCRY can enter the nucleus associated either to PER or TIM.

2.8 FAD

Cryptochromes and Photolyases are flavoenzymes showing flavin adenine dinculeotide (FAD) as main catalytic cofactor. This flavin cofactor is employed to regulate protein function, mediated by its redox state. FAD adopts a bent U-shape configuration in the CRY/Photolyase family. The cofactor can exist in four different redox states: oxidized (FAD), anionic semiquinone (FAD^{-}), neutral semiquinone (FADH•), and anionic hydroquinone ($FADH^{-}$). In Photolyases, FAD purify with a neutral flavin semiquinone (FADH•) state, which light will reduce to its active state $FADH^{-}$. In Cryptochromes, however, FAD active state is still under debate.

Insects type 1 CRYs (circadian photoreceptors) have been shown to interplay three different redox states: first reduced to $FAD^{\bullet -}$, then $FADH^{\bullet}$, and finally to $FADH^{-}$. Similarly, plant and *Escherichia coli* photolyases show the same redox functional states (Kao et al., 2008). Anionic hydroquinone flavin is also suggested to be the active state for CRYs to lead to a local conformation change and initiating signaling (Liu et al., 2013b). *In vitro*, tryptophans have been associated of electron transfer to the FAD

cofactor, although alternative pathways have been reported (Biskup et al., 2013) (Liu et al., 2013a). Moreover, although widely accepted that the oxidized form of FAD is the starting state, insects CRY1 have been demonstrated to have the deprotonated flavin anion radical as a ground state and not as the excited form. In this study the authors suggest that an artifact generated by exposure to air during purification could have formed the oxidized form o FAD (Öztürk et al., 2008). However, another study has demonstrated that *Drosophila* CRY and human CRY1 actually show an oxidized redox state *in vivo* as ground state, with light playing photoreduction to an intermediate state as signaling state (Hoang et al., 2008).

Similarly than insect and mammals CRYs, also in plant CRYs the oxidized form is believed to be the ground state. Purified *Arabidopsis* CRY1 contained oxidized FAD, which shows absorption peaks at the UV-A and blue-light spectrum (Lin et al., 1995). Light activation triggers a formation of a flavosemiquinone intermediate signaling state, which can be inactivated by green light (Bouly et al., 2007). Similarly, purified *Arabidopsis* CRY2 also contains oxidized FAD, which accummulate as semireduced flavin ($FADH^{\cdot}$) after light exposure (Banerjee et al., 2007). Oxidized flavin is also found in purified green alga *Chlamydomonas reinhardtii.* It has been shown that oxidized flavin is photoreduced to the neutral radical state by blue light and then to the fully reduced state by red light (Beel et al., 2012), suggesting a broader spectral activity to CRYs. Furthermore, accepted models deduce that the oxidized flavin state is actually the resting state, which accumulates in the dark. The back reoxidation reaction from the activated states to the oxidized form, reseting the system during darkness, has been suggested to occur depending on oxygen concentration. This restoration process back to oxidized flavin, is suggested to have magnetoreception potential via radical pairs (Müller and Ahmad, 2011). In another study the authors suggest that radical pairs formation is likely related to magnetosensor activity of CRY1 type. It is believed that re-oxidation of FAD to its inactive conformation plays radical pairs formation (Nießner et al., 2013).

2.9 Second Chromophore

The majority of the CRY/Photolyase family members in addition to the main

catalytic cofactor, also contain a second chromophore. Likewise the catalytic flavin cofactor, the second chromophore is non-covalently bound to the protein moiety. The second cofactor is believed to play energy transfer to the FAD catalytic cofactor working as antenna cofactor. Although most members of CRY/Photolyase family have a second cofactor, for some a second chromophore has not been identified yet (Kleiner et al., 1999). To date, members of the CRY/Photolyase family reportedly having a second cofactor, show either the folate type 5,10-methenyltetrahydrofolate (MTHF), or the flavins 7,8-didemethyl-8-hydroxy-5-deazariboflavin (8-HDF), FMN or even FAD as second chromophore. Additionally, synthetic flavins (8-demethyl-8-cyanoriboflavin, 8-demethyl-8-aminoriboflavin, and roseoflavin) have been demonstrated to incorporate into the antenna chromophore-binding site in *Thermus* photolyase (Klar et al., 2006).

It is thought that by harvesting light the second cofactors can increase the efficiency of repair and most likely photoactivation, through higher extinction coefficients than the catalytic cofactor (FAD) in the near UV and blue-light region (Hamm-Alvarez et al., 1989). It has been shown that in *Escherichia coli* photolyase energy transfer efficiencies are about 70% with the surface exposed folate type MTHF as antenna cofactor (Payne and Sancar, 1990). Similarly, MTHF in Cryptochrome 3 of *Arabidopsis thaliana* has been demonstrated having energy efficiencies between 78 and 87% (Song et al., 2006). Unexpectedly, energy transfer efficiency between 8-HDF, which is more internally located, and FAD in the *Anacystis nidulans* photolyase is reported to be close to 100% (Kim et al., 1992).

The promiscuity of the chromophore binding site has been demonstrated in *Thermus thermophilus* photolyase. *Thermus* photolyase has shown binding not only to flavin-mononucleotide (FMN), but also with 8-HDF and a novel synthetic flavin, 8-iodo-8-demethyl-riboflavin (8-IRF) (Klar et al., 2006). Similarly, 8-HDF is located at the same position in *Thermus* photolayse at the carboxyl edge of a beta-sheet in the alpha-beta domain, separated 17.5 Å (on average) to the FAD cofactor (Tamada et al., 1997). The average separation between FAD and 8-HDF, 8-IRF and FMN in *Thermus* photolyase was observed to be 15.4, 16.7 and 17.2 Å, respectively. A wide spectrum of possible antenna cofactors is also confirmed in a (6-4) Photolyase-like protein identified in *Agrobacterium tumefaciens,* which has been shown to have an organic 6,7-dimethyl-8-ribityllumazine (DMRL) as second chromophore (Zhang et al., 2013).

Furthermore, initially believed to be strictly limited to archael kingdom of life, flavin type antenna chromophores have also been found in eukaryotes. 7,8-Dimethyl-8-hydroxy-5deazaflavin (F_0) has been identified as light-harvesting cofactor in 6-4 Photolyase of *Drosophila melanogoster* (Glas et al., 2009). In plant 6-4 Photolyase however, although preserving potential flavin type antenna-binding sites, no second chromophore has been identified (Hitomi et al., 2009).

In the archeal photolyase of *Sulfolobus tokodaii* however, two FAD molecules have been identified. An unexpected FAD has been found placed at the second chromophore binding site of *Anacystis* and *Thermus* photolyases and proposed to work light-harvesting to the catalytic FAD cofactor. Similarly than *Anacystis* and *Thermus* photolyases, FAD light-harvesting cofactor has been found separated 17.2 Å to the catalytic cofactor. However, differently than the conformation found as catalytic cofactor in all members of the CRY/Photolyase family, where it shows a bent conformation, the proposed light-harvesting cofactor adopts a relatively straight configuration (Fujihashi et al., 2007).

The first folate antenna cofactor in the CRY/Photolyase family has been identified in *E. coli* photolyase. The light-harvesting cofactor 5,10 methenyltetrahydrofolylpolyglutamate (MTHF) is reported to be approximately 16.8 Å distant to the catalytic cofactor, FAD (Park et al., 1995). Placed at the same position, binding mode of MTHF of *Arabidopsis* CRY3 is not conserved with *E.coli.* Although having different residue conservation with MTHF in *E.coli,* they show similar conformation and a relative shorter distance to the catalytic cofactor (15.2 Å). In *Arabidopsis* CRY1 however, the authors were unable to identify any second chromophore associated to the structure (Brautigam et al., 2004). However, the authors suggest that purification processes can have impaired such finding, since in a previous work *Arabidopsis* CRY1 showed MTHF binding abilities (Malhotra et al., 1995). In *Drosophila* CRY controversial reports regarding the presence of a second chromophore have been found. Using biophysical methods it has been proposed that *Drosophila* CRY has only residual amounts of MTHF (Berndt et al., 2007), while in another study the authors have not found any second chromophore in dCRY (Selby and Sancar, 2012).

2.10 Methods

The main purpose in computational chemistry is the implementation of theoretical calculation models codes, in order to obtain mathematical models capable of simulating *in silico* the most likely chemical systems and chemical-physical mechanisms of matter. The molecules are placed ina particular environment, which is studied and described by a mathematical function based on the concept of an empirical force field. In molecular mechanics, electrons and nuclei are treated as a single entity having spherical symmetry, obtaining in this way, pseudo-atoms. In order to attribute different chemical characteristics such as hybridization states, absence/presence of charge and the valence, standard atom types must be created. It is a simplistic approach, representing the biggest limitation of molecular mechanics, where they are considered molecules. In addition, another weak point of this approximation is that we can only consider molecules in their fundamental, and not in their excited states. These obstacles, however, can be partially overcome through the adoption of a set of atom types that does not is limited to characterize the system according only to the atomic number. In fact, force fields aim to overcome this limitations through the adoption of a set of different atom types.

In molecular mechanics force field is the sum of several energy contributions necessary to describe the behavior of various types of atoms, atomic groups and bonds. The mathematical equation representing this sum varies, depending on the force field that you use. In general, the terms considered are:

$$E_{tot} = E_{stretching} + E_{bending} + E_{torsion} + E_{non\text{-}bonding\text{-}interaction} \ldots$$

1) *Stretching energy*: The most basic approach to describe a typical bond is Hooke's law:

$$E = \sum_{bonds} k_b (l - l_O)^2$$

For each type of binding specific values of k_b (Hooke's constant) and l_0,

parameter required to represent the equilibrium bond length, that is where the other terms of the equation of the force-field are set to zero, are assigned. The equation calculates the energy associated with changes in bond length in reference to the equilibrium situation. The model is not reliable when the bond length tends to values of dissociation.

2) *Bending energy:* Similarly in this case the deviation of the angles from their reference value is described by Hooke's harmonic potential law, referred to the deformation angle of a spring:

$$E = \sum_{angles} k_{\theta}(\theta - \theta_0)^2$$

The values of k_θ and θ_0 again assume constant values, and are referred as the constant angular deformation of the spring and the equilibrium value of the bond angle, respectively.

3) *Torsional energy:*

$$E = \sum_{dihedrals} A\,[1 + \cos(n\tau - \varphi)]$$

Most of structural variability and its energy is due to the complex synergy between torsional and non-binding contributions. The torsional energy has a cosine periodic behavior, where A defines the amplitude of the function n and its periodicity.

4) *Non-binding interactions:* In molecular mechanics, non-bonded atoms are defined as those separated by at least two atoms covalently bonded. The non-binding energy depends on the interactions between all possible non-bond pairs of atoms, i and j. The non-bonded forces include the Van Der Waals forces, described by the Lennard-Jones potential, and the forces of attraction-repulsion described by Coulomb's law. Altogether, this is considered in the equation:

$$E = \left(\sum_j - \frac{Aij}{r_{ij}}\right) + k_O\left(\sum_j \frac{q_i q_j}{r_{ij}}\right)$$

with

$$k_O = \frac{1}{4\pi\varepsilon_O}$$

AMBER99 is a simplified force field, suitable for calculations on macromolecules. The non-polar hydrogen atoms are treated implicitly, and therefore included in the description of the heavy atoms to which they are bound. The interactions between atoms separated by distances greater than a given value, said cut-off, are not considered. This type of simplifications result in a lower accuracy, but have the advantage of reducing drastically the timing calculation.

Energy minimization allows the system to be placed to a minimum relative energy closer to the initial situation. Such algorithms are based on the calculation of the first or second derivatives, allowing to reach the closest minimum, while to reach distant minimums, which are separated by energetical barriers compared to the initial situation, are more difficult toachieve as the necessary energetical gradient increases. There are several algorithms for energy minimization, based on the first or second derivatives:

1) *Steepest descent*: Steepest descent method is based on the calculation of the derivative before the potential energy function. The exploratory method used is called the line searching: every direction change is perpendicular to the previous one. This method is not characterized by optimal results, but can lead close to it, in the case where the initial situation is very far from the local minimum.

2) *Conjugate gradients*: The conjugate gradient method produces a set of directions that do not show the oscillatory behavior, which is characteristic of the steepest descent method, in proximity of the potential energy surface. Each step is stored in terms of location, eliminating the possibility of retracing a path exploratory already undertaken. In computational terms, this algorithm is much more expensive, as it must discriminate between courses already taken and possible paths to be taken.

3) *Truncated Newton* methods are based on the Newton-Raphson, which combines the calculation of the first derivative, provinding slope information, and second derivative, that instead provides direct information on the curvature of the potential energy function. At each change of coordinates, the algorithm analyzes the curvature of the adjacent potential, so as to find the optimal path to the biggest slope. Similarly than the conjugate gradients method, this method is computationally very

expensive.

Methods such as the steepest descent may be convenient to reach the minimum, as it is also much less expensive in terms of computational resources. If you want to get to a minimum with a fair degree of accuracy, methods such as conjugate gradients can be more advantageous, as the steepest descent, however, is much less precise (may prefer paths that lead to move away from the minimum), and in this particular case there is the risk that to reach the minimum will take a larger number of calculations and iterations.

2.10.1 Alignments

Alignment of more than two sequences has the advantage to find evolutionary differences, as the comparison of primary sequences allows a more direct cross-check of mutated amino acids. This procedure, calledmultiple alignment, however, is more complicated, since the number of possible combinations increases exponentially with the number of compared sequences.

In general, multiple alignments are carried out in four stages:

1) *Alignment of pairs of sequences*:

a) Progressive: the second sequence is aligned with the first, and the result is then aligned with the third and so on, until exhaustion of sequences.

b) Tree: the calculation is made considering a preliminary score assigned to the alignment of each pair of sequences. The sequences with the highest scores are re-aligned to each other. The last step divides the processed sequences in two groups, and the alignment score between them is defined as the maximum score obtained within each group.

2) *Cyclic realignment*: The score obtained from previous step is recalculated by a single cycle of realignment, in which each sequence is extracted in succession of the global alignment and realigned with all remaining sequences.

3) *Random iterative refinement*: Since the results of the two previous steps may be affected by the order in which the sequences are processed, the groups are randomly divided into two subgroups, which are realigned between them. If the alignment score after this operation improves, the new alignment is accepted, otherwise it is discarded.

4) *Structural realignment*: This step can be carried out only for the sequences that also contain information on the three-dimensional structure. For these sequences, a matrix of similarity between the coordinates aligns the tertiary structures, until the value of RMSD (Root Mean Square Deviation) does not increase more than the score of the alignment.

Mathematical matrices have been developed for the assignment of scores to the alignment of a particular residue amino acid with another. The identity matrix - non-identity score assigns 1 to identical pairs of amino acids, 0 to different pairs of amino acids.

The matrices based on mutation between amino acids derived from the observation of a set of homologous proteins, and the frequency with which a particular amino acid is replaced with another. The highest score is assigned to pairs of amino acids that are identical or different, but statistically favored by mutations. The lower scores on the other hand are assigned in the case of mutations infrequent or never verified before. There are various matrices, but the most used are PAM (Point Accept Mutation) and BLOSUM (Blocks Substitution Matrices). The PAM matrix , developed by Dayhoff (Dayhoff and Schwartz, 1978) was derived from the observation of point mutations found in natural selection, using a set of 71 groups of proteins homologous, and taking into consideration only those with a degree of similarity greater than 85%. The replacement is evolutionarily favored when the ratio between the number of substitutions observed and the expected number of substitutions is statistically high. The PAM matrix is however poor in the alignment of sequences with degree similarity less than 85%. In these cases, BLOSUM matrix assigns a score based on the observation of a large number of alignments made between structurally conserved regions within a family protein. BLOSUM matrix allows comparison between homologous regions of proteins also phylogenetically distant, with lower similarity percentages.

2.10.2 Docking.

A docking procedure gives some possible conformations of the ligand within the binding pocket, and assigns a score to each of these using different mathematical functions, called scoring functions. The ideal scoring function should return an accurate

value of the Gibbs free energy, and therefore should take into account the contribution of the enthalpic and entropic contribution that lead to the formation of the ligand-protein complex.

There are basically three types of scoring functions:

1) *Empirical scoring functions*: Are based on experimental physico-chemical properties. The scoring, or in the calculation of the free energy, take into account the contributions of the hydrophobic interactions, the bond hydrogen, the entropic effects and interactions with metal ions. This type of scoring function is calibrated by means of a multilinear regression that provides the coefficients of the equation, based on a training set of protein-ligand complexes of which are known binding affinities and three-dimensional structures.

2) *Knowledge-based scoring function*: It considers the functional groups present more frequently in the structures three-dimensional as favorable towards binding affinity. The free energy of the complex is then calculated as the sum of the free energy of all interatomic contacts, derived from the frequency of interatomic distances found in the database of three-dimensional structures obtained experimentally.

3) *Scoring functions force-field based*: The affinity is estimated considering the interactions of Van Der Waals forces and electrostatic interactions between all the atoms of the system, also between atoms of the same molecule.

For the generation of a complex ligand-protein, there are several algorithms. Deterministic algorithms, potentially able to guarantee the optimal solution are preferred rather than heuristic algorithms, which do not ensure that the solution truly represents the best, but instead can determine in a reasonable time.

The main algorithms for the generation of a complex are:

1) *Simulated annealing*: In computational chemistry, the simulated temperature increase allows the displacement of atoms from their initial position, reaching higher levels of energy. The slow cooling increases the chances of getting configurations with lower internal energy than the initial conformation. Various states of the conformational space are explored, generating random changes of the starting coordinates of the ligand.

2) *Tabu Search* is a stochastic algorithm, which through random changes of the value of the dihedral angles, allows the exploration of a large part of the conformational space accessible to the ligand. The chosen conformation is based on the calculation of

the value of rmsd between the cartesian coordinates of the two structures. If this value is lower than a cut-off, the two conformations are considered equal, they are not considered, forming a list. Series of new solutions are added to the list of results only if the value of the score is higher than the previous attempts. Otherwise, a new region of conformational space is explored.

3) *Genetic algorithm*: This stochastic algorithms is derived from evolutionary biology. Each possible conformation of the ligand-target is encoded by a corresponding gene. The state of the ligand corresponds to the genotype, and the atomic coordinates reflect the phenotype.

4) *ACO / Ant Colony Optimization*: This type of algorithm is based on the behavior of an ant colony. Whenever a new conformational space is explored, a "pheromone" is issued. The more frequently a conformation is explored, more "pheromone" is released, and more frequently it will be explored in subsequent iterations. If a conformation is not explored for a certain period of time, the pheromone disappears and new conformations are explored

5) *Divide and Conquer*: In this approach, the ligand is divided into fragments, which are individually subjected to a docking within the binding site. It seeks ultimately to build the most likely conformation by joining the poses of the various fragments..

6) *Incremental search*: The ligand is progressively assembled in the binding site. A main fragment is generated, which is placed into the binding site. The other fragments are added in an iterative way, in different orientations until obtaining the complete structure of the ligand.

2.11 References

Adamec, J., Rusnak, F., Owen, W.G., Naylor, S., Benson, L.M., Gacy, A.M., Isaya, G., 2000. Iron-dependent self-assembly of recombinant yeast frataxin: implications for Friedreich ataxia. Am. J. Hum. Genet. 67, 549–562.

Adinolfi, S., Iannuzzi, C., Prischi, F., Pastore, C., Iametti, S., Martin, S.R., Bonomi, F., Pastore, A., 2009. Bacterial frataxin CyaY is the gatekeeper of iron-sulfur cluster formation catalyzed by IscS. Nat. Struct. Mol. Biol. 16, 390–396.

Adinolfi, S., Trifuoggi, M., Politou, A.S., Martin, S., Pastore, A., 2002. A structural approach to understanding the iron-binding properties of phylogenetically different frataxins. Hum. Mol. Genet. 11, 1865–1877.

Allen, M.P., Tildesley, D.J., 1989. Computer simulation of liquids. Clarendon Press ; Oxford University Press, Oxford [England]; New York.

Aloria, K., Schilke, B., Andrew, A., Craig, E.A., 2004. Iron-induced oligomerization of yeast frataxin homologue Yfh1 is dispensable in vivo. EMBO Rep. 5, 1096–1101.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Baker, N.A., 2005. Improving implicit solvent simulations: a Poisson-centric view. Curr. Opin. Struct. Biol. 15, 137–143.

Banerjee, R., Schleicher, E., Meier, S., Viana, R.M., Pokorny, R., Ahmad, M., Bittl, R., Batschauer, A., 2007. The Signaling State of Arabidopsis Cryptochrome 2 Contains Flavin Semiquinone. J. Biol. Chem. 282, 14916–14922.

Bashford, D., Case, D.A., 2000. Generalized born models of macromolecular solvation effects. Annu. Rev. Phys. Chem. 51, 129–152.

Beel, B., Prager, K., Spexard, M., Sasso, S., Weiss, D., Müller, N., Heinnickel, M., Dewez, D., Ikoma, D., Grossman, A.R., Kottke, T., Mittag, M., 2012. A Flavin Binding Cryptochrome Photoreceptor Responds to Both Blue and Red Light in Chlamydomonas reinhardtii. Plant Cell Online 24, 2992–3008.

Berendsen, H.J.C., Grigera, J.R., Straatsma, T.P., 1987. The missing term in effective pair potentials. J. Phys. Chem. 91, 6269–6271.

Berndt, A., Kottke, T., Breitkreuz, H., Dvorsky, R., Hennig, S., Alexander, M., Wolf, E., 2007. A novel photoreaction mechanism for the circadian blue light photoreceptor Drosophila cryptochrome. J. Biol. Chem. 282, 13011–13021.

Biskup, T., Hitomi, K., Getzoff, E.D., Krapf, S., Koslowski, T., Schleicher, E., Weber, S., 2011. Unexpected Electron Transfer in Cryptochrome Identified by Time-Resolved EPR Spectroscopy. Angew. Chem. Int. Ed. 50, 12647–12651.

Biskup, T., Paulus, B., Okafuji, A., Hitomi, K., Getzoff, E.D., Weber, S., Schleicher, E., 2013. Variable Electron-Transfer Pathways in an Amphibian Cryptochrome:

Tryptophan versus Tyrosine-Based Radical Pairs. J. Biol. Chem.

Bou-Abdallah, F., Adinolfi, S., Pastore, A., Laue, T.M., Dennis Chasteen, N., 2004. Iron binding and oxidation kinetics in frataxin CyaY of Escherichia coli. J. Mol. Biol. 341, 605–615.

Bouly, J.-P., Schleicher, E., Dionisio-Sese, M., Vandenbussche, F., Straeten, D.V.D., Bakrim, N., Meier, S., Batschauer, A., Galland, P., Bittl, R., Ahmad, M., 2007. Cryptochrome Blue Light Photoreceptors Are Activated through Interconversion of Flavin Redox States. J. Biol. Chem. 282, 9383–9391.

Branda, S.S., Yang, Z.Y., Chew, A., Isaya, G., 1999. Mitochondrial intermediate peptidase and the yeast frataxin homolog together maintain mitochondrial iron homeostasis in Saccharomyces cerevisiae. Hum. Mol. Genet. 8, 1099–1110.

Brautigam, C.A., Smith, B.S., Ma, Z., Palnitkar, M., Tomchick, D.R., Machius, M., Deisenhofer, J., 2004. Structure of the photolyase-like domain of cryptochrome 1 from Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A. 101, 12142–12147.

Brooks, B.R., Brooks, C.L., 3rd, Mackerell, A.D., Jr, Nilsson, L., Petrella, R.J., Roux, B., Won, Y., Archontis, G., Bartels, C., Boresch, S., Caflisch, A., Caves, L., Cui, Q., Dinner, A.R., Feig, M., Fischer, S., Gao, J., Hodoscek, M., Im, W., Kuczera, K., Lazaridis, T., Ma, J., Ovchinnikov, V., Paci, E., Pastor, R.W., Post, C.B., Pu, J.Z., Schaefer, M., Tidor, B., Venable, R.M., Woodcock, H.L., Wu, X., Yang, W., York, D.M., Karplus, M., 2009. CHARMM: the biomolecular simulation program. J. Comput. Chem. 30, 1545–1614.

Brooks, B.R., Bruccoleri, R.E., Olafson, B.D., States, D.J., Swaminathan, S., Karplus, M., 1983. CHARMM: A program for macromolecular energy, minimization, and dynamics calculations. J. Comput. Chem. 4, 187–217.

Brudler, R., Hitomi, K., Daiyasu, H., Toh, H., Kucho, K., Ishiura, M., Kanehisa, M., Roberts, V.A., Todo, T., Tainer, J.A., Getzoff, E.D., 2003. Identification of a New Cryptochrome Class: Structure, Function, and Evolution. Mol. Cell 11, 59–67.

Bulteau, A.-L., O'Neill, H.A., Kennedy, M.C., Ikeda-Saito, M., Isaya, G., Szweda, L.I., 2004. Frataxin Acts as an Iron Chaperone Protein to Modulate Mitochondrial Aconitase Activity. Science 305, 242–245.

Busi, M.V., Zabaleta, E.J., Araya, A., Gomez-Casati, D.F., 2004. Functional and

molecular characterization of the frataxin homolog from Arabidopsis thaliana. FEBS Lett. 576, 141–144.

Busino, L., Bassermann, F., Maiolica, A., Lee, C., Nolan, P.M., Godinho, S.I.H., Draetta, G.F., Pagano, M., 2007. SCFFbxl3 controls the oscillation of the circadian clock by directing the degradation of cryptochrome proteins. Science 316, 900–904.

Busza, A., Emery-Le, M., Rosbash, M., Emery, P., 2004. Roles of the Two Drosophila CRYPTOCHROME Structural Domains in Circadian Photoreception. Science 304, 1503–1506.

Campanella, A., Isaya, G., O'Neill, H.A., Santambrogio, P., Cozzi, A., Arosio, P., Levi, S., 2004. The expression of human mitochondrial ferritin rescues respiratory function in frataxin-deficient yeast. Hum. Mol. Genet. 13, 2279–2288.

Case, D.A., Cheatham, T.E., 3rd, Darden, T., Gohlke, H., Luo, R., Merz, K.M., Jr, Onufriev, A., Simmerling, C., Wang, B., Woods, R.J., 2005. The Amber biomolecular simulation programs. J. Comput. Chem. 26, 1668–1688.

Case, D.A., Cheatham, T.E., Darden, T., Gohlke, H., Luo, R., Merz, K.M., Onufriev, A., Simmerling, C., Wang, B., Woods, R.J., 2005. The Amber biomolecular simulation programs. J. Comput. Chem. 26, 1668–1688.

Cavadini, P., O'Neill, H.A., Benada, O., Isaya, G., 2002. Assembly and iron-binding properties of human frataxin, the protein deficient in Friedreich ataxia. Hum. Mol. Genet. 11, 217–227.

Ceriani, M.F., Darlington, T.K., Staknis, D., Más, P., Petti, A.A., Weitz, C.J., Kay, S.A., 1999. Light-dependent sequestration of TIMELESS by CRYPTOCHROME. Science 285, 553–556.

Chaves, I., Pokorny, R., Byrdin, M., Hoang, N., Ritz, T., Brettel, K., Essen, L.-O., van der Horst, G.T.J., Batschauer, A., Ahmad, M., 2011. The cryptochromes: blue light photoreceptors in plants and animals. Annu. Rev. Plant Biol. 62, 335–364.

Chaves, I., Yagita, K., Barnhoorn, S., Okamura, H., van der Horst, G.T.J., Tamanini, F., 2006. Functional evolution of the photolyase/cryptochrome protein family: importance of the C terminus of mammalian CRY1 for circadian core oscillator performance. Mol. Cell. Biol. 26, 1743–1753.

Chothia, C., Lesk, A.M., 1986. The relation between the divergence of sequence and

structure in proteins. EMBO J. 5, 823–826.

Coesel, S., Mangogna, M., Ishikawa, T., Heijde, M., Rogato, A., Finazzi, G., Todo, T., Bowler, C., Falciatore, A., 2009. Diatom PtCPF1 is a new cryptochrome/photolyase family member with DNA repair and transcription regulation activity. EMBO Rep. 10, 655–661.

Collins, B.H., Dissel, S., Gaten, E., Rosato, E., Kyriacou, C.P., 2005. Disruption of Cryptochrome partially restores circadian rhythmicity to the arrhythmic period mutant of Drosophila. Proc. Natl. Acad. Sci. U. S. A. 102, 19021–19026.

Collins, B., Mazzoni, E.O., Stanewsky, R., Blau, J., 2006. Drosophila CRYPTOCHROME is a circadian transcriptional repressor. Curr. Biol. CB 16, 441–449.

Cook, J.D., Bencze, K.Z., Jankovic, A.D., Crater, A.K., Busch, C.N., Bradley, P.B., Stemmler, A.J., Spaller, M.R., Stemmler, T.L., 2006. Monomeric Yeast Frataxin is an Iron Binding Protein. Biochemistry (Mosc.) 45, 7767–7777.

Correia, A.R., Wang, T., Craig, E.A., Gomes, C.M., 2010a. Iron binding activity in yeast frataxin entails a trade off with stability in the ?1/?1 acidic ridge region. Biochem. J. 426, 197–203.

Correia, A.R., Wang, T., Craig, E.A., Gomes, C.M., 2010b. Iron binding activity in yeast frataxin entails a trade off with stability in the ?1/?1 acidic ridge region. Biochem. J. 426, 197–203.

Dardente, H., Fortier, E.E., Martineau, V., Cermakian, N., 2007. Cryptochromes impair phosphorylation of transcriptional activators in the clock: a general mechanism for circadian repression. Biochem. J. 402, 525.

Dayhoff, M.O., Schwartz, R.M., 1978. Chapter 22: A model of evolutionary change in proteins, in: In Atlas of Protein Sequence and Structure.

Delatycki, M.B., Williamson, R., Forrest, S.M., 2000. Friedreich ataxia: an overview. J. Med. Genet. 37, 1–8.

Del Pozo, A., Vera, L.M., Sánchez, J.A., Sánchez-Vázquez, F.J., 2012. Molecular cloning, tissue distribution and daily expression of cry1 and cry2 clock genes in European seabass (Dicentrarchus labrax). Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 163, 364–371.

Dissel, S., Codd, V., Fedic, R., Garner, K.J., Costa, R., Kyriacou, C.P., Rosato, E.,

2004a. A constitutively active cryptochrome in Drosophila melanogaster. Nat. Neurosci. 7, 834–840.

Dissel, S., Codd, V., Fedic, R., Garner, K.J., Costa, R., Kyriacou, C.P., Rosato, E., 2004b. A constitutively active cryptochrome in Drosophila melanogaster. Nat. Neurosci. 7, 834–840.

Dunlap, J.C., 1999. Molecular Bases for Circadian Clocks. Cell 96, 271–290.

Eckel-Mahan, K., Sassone-Corsi, P., 2013. Metabolism and the Circadian Clock Converge. Physiol. Rev. 93, 107–135.

Emery, P., So, W.V., Kaneko, M., Hall, J.C., Rosbash, M., 1998. CRY, a Drosophila clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. Cell 95, 669–679.

Engelen, E., Janssens, R.C., Yagita, K., Smits, V.A.J., van der Horst, G.T.J., Tamanini, F., 2013. Mammalian TIMELESS Is Involved in Period Determination and DNA Damage-Dependent Phase Advancing of the Circadian Clock. PLoS ONE 8, e56623.

Faraj, S.E., Venturutti, L., Roman, E.A., Marino-Buslje, C.B., Mignone, A., Tosatto, S.C.E., Delfino, J.M., Santos, J., 2013. The role of the N-terminal tail for the oligomerization, folding and stability of human frataxin. FEBS Open Bio 3, 310–320.

Feig, M., Brooks, C.L., 3rd, 2004. Recent advances in the development and application of implicit solvent models in biomolecule simulations. Curr. Opin. Struct. Biol. 14, 217–224.

Ferrara, P., Apostolakis, J., Caflisch, A., 2002. Evaluation of a fast implicit solvent model for molecular dynamics simulations. Proteins 46, 24–33.

Finn, R.D., Mistry, J., Schuster-Böckler, B., Griffiths-Jones, S., Hollich, V., Lassmann, T., Moxon, S., Marshall, M., Khanna, A., Durbin, R., Eddy, S.R., Sonnhammer, E.L.L., Bateman, A., 2006. Pfam: clans, web tools and services. Nucleic Acids Res. 34, D247–D251.

Fogle, K.J., Parson, K.G., Dahm, N.A., Holmes, T.C., 2011. CRYPTOCHROME Is a Blue-Light Sensor That Regulates Neuronal Firing Rate. Science 331, 1409–1413.

Foley, L.E., Gegear, R.J., Reppert, S.M., 2011. Human cryptochrome exhibits light-

dependent magnetosensitivity. Nat. Commun. 2, 356.

Foury, F., Pastore, A., Trincal, M., 2007. Acidic residues of yeast frataxin have an essential role in Fe-S cluster assembly. EMBO Rep. 8, 194–199.

Frenkel, D., Smit, B., 2002. Understanding molecular simulation from algorithms to applications. Academic Press, San Diego.

Fujihashi, M., Numoto, N., Kobayashi, Y., Mizushima, A., Tsujimura, M., Nakamura, A., Kawarabayasi, Y., Miki, K., 2007. Crystal Structure of Archaeal Photolyase from Sulfolobus tokodaii with Two FAD Molecules: Implication of a Novel Light-harvesting Cofactor. J. Mol. Biol. 365, 903–910.

Gakh, O., Adamec, J., Gacy, A.M., Twesten, R.D., Owen, W.G., Isaya, G., 2002. Physical Evidence that Yeast Frataxin Is an Iron Storage Protein†. Biochemistry (Mosc.) 41, 6798–6804.

Gakh, O., Park, S., Liu, G., Macomber, L., Imlay, J.A., Ferreira, G.C., Isaya, G., 2006. Mitochondrial iron detoxification is a primary function of frataxin that limits oxidative damage and preserves cell longevity. Hum. Mol. Genet. 15, 467–479.

Gao, P., Yoo, S.-H., Lee, K.-J., Rosensweig, C., Takahashi, J.S., Chen, B.P., Green, C.B., 2013. Phosphorylation of the Cryptochrome 1 C-terminal tail regulates circadian period length. J. Biol. Chem. jbc.M113.509604.

Gegear, R.J., Casselman, A., Waddell, S., Reppert, S.M., 2008. Cryptochrome mediates light-dependent magnetosensitivity in Drosophila. Nature 454, 1014–1018.

Gentry, L.E., Thacker, M.A., Doughty, R., Timkovich, R., Busenlehner, L.S., 2013. His86 from the N-terminus of frataxin coordinates iron and is required for Fe-S cluster synthesis. Biochemistry (Mosc.) 52, 6085–6096.

Gilson, M.K., Davis, M.E., Luty, B.A., McCammon, J.A., 1993. Computation of electrostatic forces on solvated molecules using the Poisson-Boltzmann equation. J. Phys. Chem. 97, 3591–3600.

Glas, A.F., Maul, M.J., Cryle, M., Barends, T.R.M., Schneider, S., Kaya, E., Schlichting, I., Carell, T., 2009. The archaeal cofactor F0 is a light-harvesting antenna chromophore in eukaryotes. Proc. Natl. Acad. Sci.

Glossop, N.R.J., Hardin, P.E., 2002. Central and peripheral circadian oscillator mechanisms in flies and mammals. J. Cell Sci. 115, 3369–3377.

González-Cabo, P., Vázquez-Manrique, R.P., García-Gimeno, M.A., Sanz, P., Palau, F.,

2005. Frataxin interacts functionally with mitochondrial electron transport chain proteins. Hum. Mol. Genet. 14, 2091–2098.

Griffin, E.A., Staknis, D., Weitz, C.J., 1999. Light-Independent Role of CRY1 and CRY2 in the Mammalian Circadian Clock. Science 286, 768–771.

Guex, N., Peitsch, M.C., 1997. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis 18, 2714–2723.

Guo, H., Duong, H., Ma, N., Lin, C., 1999. The Arabidopsis blue light receptor cryptochrome 2 is a nuclear protein regulated by a blue light-dependent post-transcriptional mechanism. Plant J. Cell Mol. Biol. 19, 279–287.

Hall, J.C., 2000. Cryptochromes: sensory reception, transduction, and clock functions subserving circadian systems. Curr. Opin. Neurobiol. 10, 456–466.

Hamm-Alvarez, S., Sancar, A., Rajagopalan, K.V., 1989. Role of enzyme-bound 5,10-methenyltetrahydropteroylpolyglutamate in catalysis by Escherichia coli DNA photolyase. J. Biol. Chem. 264, 9649–9656.

Hao Zheng, Ng, F., Yixiao Liu, Hardin, P.E., 2008. Spatial and circadian regulation of cry in Drosophila. J. Biol. Rhythms 23, 283–295.

Harada, Y., Sakai, M., Kurabayashi, N., Hirota, T., Fukada, Y., 2005. Ser-557-phosphorylated mCRY2 Is Degraded upon Synergistic Phosphorylation by Glycogen Synthase Kinase-3β. J. Biol. Chem. 280, 31714–31721.

Harris, S.-R., Henbest, K.B., Maeda, K., Pannell, J.R., Timmel, C.R., Hore, P.J., Okamoto, H., 2009. Effect of magnetic fields on cryptochrome-dependent responses in Arabidopsis thaliana. J. R. Soc. Interface 6, 1193–1205.

Helfrich-Förster, C., Winter, C., Hofbauer, A., Hall, J.C., Stanewsky, R., 2001. The circadian clock of fruit flies is blind after elimination of all known photoreceptors. Neuron 30, 249–261.

Hemsley, M.J., Mazzotta, G.M., Mason, M., Dissel, S., Toppo, S., Pagano, M.A., Sandrelli, F., Meggio, F., Rosato, E., Costa, R., Tosatto, S.C.E., 2007. Linear motifs in the C-terminus of D. melanogaster cryptochrome. Biochem. Biophys. Res. Commun. 355, 531–537.

Hess, B., Bekker, H., Berendsen, H.J.C., Fraaije, J.G.E.M., 1997. LINCS: A linear constraint solver for molecular simulations. J. Comput. Chem. 18, 1463–1472.

Hess, B., Kutzner, C., van der Spoel, D., Lindahl, E., 2008. GROMACS 4: Algorithms

for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation. J. Chem. Theory Comput. 4, 435–447.

He, Y., Alam, S.L., Proteasa, S.V., Zhang, Y., Lesuisse, E., Dancis, A., Stemmler, T.L., 2004. Yeast frataxin solution structure, iron binding, and ferrochelatase interaction. Biochemistry (Mosc.) 43, 16254–16262.

Hitomi, K., DiTacchio, L., Arvai, A.S., Yamamoto, J., Kim, S.-T., Todo, T., Tainer, J.A., Iwai, S., Panda, S., Getzoff, E.D., 2009. Functional motifs in the (6-4) photolyase crystal structure make a comparative framework for DNA repair photolyases and clock cryptochromes. Proc. Natl. Acad. Sci. 106, 6962–6967.

Hitomi, K., Okamoto, K., Daiyasu, H., Miyashita, H., Iwai, S., Toh, H., Ishiura, M., Todo, T., 2000. Bacterial cryptochrome and photolyase: characterization of two photolyase-like genes of Synechocystis sp. PCC6803. Nucleic Acids Res. 28, 2353–2362.

Hoang, N., Schleicher, E., Kacprzak, S., Bouly, J.-P., Picot, M., Wu, W., Berndt, A., Wolf, E., Bittl, R., Ahmad, M., 2008a. Human and Drosophila Cryptochromes Are Light Activated by Flavin Photoreduction in Living Cells. PLoS Biol 6, e160.

Hoang, N., Schleicher, E., Kacprzak, S., Bouly, J.-P., Picot, M., Wu, W., Berndt, A., Wolf, E., Bittl, R., Ahmad, M., 2008b. Human and Drosophila Cryptochromes Are Light Activated by Flavin Photoreduction in Living Cells. PLoS Biol 6, e160.

Höltje, H.-D., 2008. Molecular modeling: basic principles and applications. Wiley-VCH, Weinheim.

Huang, J., Dizin, E., Cowan, J.A., 2008. Mapping iron binding sites on human frataxin: implications for cluster assembly on the ISU Fe-S cluster scaffold protein. J. Biol. Inorg. Chem. JBIC Publ. Soc. Biol. Inorg. Chem. 13, 825–836.

Huang, M.L.-H., Becker, E.M., Whitnall, M., Suryo Rahmanto, Y., Ponka, P., Richardson, D.R., 2009. Elucidation of the mechanism of mitochondrial iron loading in Friedreich's ataxia by analysis of a mouse mutant. Proc. Natl. Acad. Sci. U. S. A. 106, 16381–16386.

Huynen, M.A., Snel, B., Bork, P., Gibson, T.J., 2001. The phylogenetic distribution of frataxin indicates a role in iron-sulfur cluster protein assembly. Hum. Mol.

Genet. 10, 2463–2468.

III, C.L.B., Karplus, rtin, Pettitt, B.M. (Eds.), 2007. Dynamical Simulation Methods, in: Advances in Chemical Physics. John Wiley & Sons, Inc., pp. 33–58.

Ito, C., Goto, S.G., Shiga, S., Tomioka, K., Numata, H., 2008. Peripheral circadian clock for the cuticle deposition rhythm in Drosophila melanogaster. Proc. Natl. Acad. Sci. 105, 8446–8451.

Ivanchenko, M., Stanewsky, R., Giebultowicz, J.M., 2001. Circadian photoreception in Drosophila: functions of cryptochrome in peripheral and central clocks. J. Biol. Rhythms 16, 205–215.

Jorgensen, W.L., Chandrasekhar, J., Madura, J.D., Impey, R.W., Klein, M.L., 1983. Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. 79, 926–935.

Kang, C.-Y., Lian, H.-L., Wang, F.-F., Huang, J.-R., Yang, H.-Q., 2009. Cryptochromes, Phytochromes, and COP1 Regulate Light-Controlled Stomatal Development in Arabidopsis. Plant Cell Online 21, 2624–2641.

Kao, Y.-T., Tan, C., Song, S.-H., Öztürk, N., Li, J., Wang, L., Sancar, A., Zhong, D., 2008. Ultrafast Dynamics and Anionic Active States of the Flavin Cofactor in Cryptochrome and Photolyase. J. Am. Chem. Soc. 130, 7695–7701.

Kaushik, R., Nawathean, P., Busza, A., Murad, A., Emery, P., Rosbash, M., 2007. PER-TIM Interactions with the Photoreceptor Cryptochrome Mediate Circadian Temperature Responses in Drosophila. PLoS Biol 5, e146.

Khan, S.K., Xu, H., Ukai-Tadenuma, M., Burton, B., Wang, Y., Ueda, H.R., Liu, A.C., 2012. Identification of a Novel Cryptochrome Differentiating Domain Required for Feedback Repression in Circadian Clock Function. J. Biol. Chem. 287, 25917–25926.

Kim, S.T., Heelis, P.F., Sancar, A., 1992. Energy transfer (deazaflavin .fwdarw. FADH2) and electron transfer (FADH2 .fwdarw. T.ltbbrac..rtbbrac.T) kinetics in Anacystis nidulans photolyase. Biochemistry (Mosc.) 31, 11244–11248.

Klar, T., Kaiser, G., Hennecke, U., Carell, T., Batschauer, A., Essen, L.-O., 2006. Natural and Non-natural Antenna Chromophores in the DNA Photolyase from Thermus Thermophilus. ChemBioChem 7, 1798–1806.

Klar, T., Pokorny, R., Moldt, J., Batschauer, A., Essen, L.-O., 2007. Cryptochrome 3

from Arabidopsis thaliana: Structural and Functional Analysis of its Complex with a Folate Light Antenna. J. Mol. Biol. 366, 954–964.

Kleiner, O., Butenandt, J., Carell, T., Batschauer, A., 1999. Class II DNA photolyase from Arabidopsis thaliana contains FAD as a cofactor. Eur. J. Biochem. 264, 161–167.

Kleine, T., Lockhart, P., Batschauer, A., 2003. An Arabidopsis protein closely related to Synechocystis cryptochrome is targeted to organelles. Plant J. 35, 93–103.

Kondapalli, K.C., Kok, N.M., Dancis, A., Stemmler, T.L., 2008. Drosophila Frataxin: An Iron Chaperone during Cellular Fe-S Cluster Bioassembly. Biochemistry (Mosc.) 47, 6917–6927.

Kondratov, R.V., Kondratova, A.A., Lee, C., Gorbacheva, V.Y., Chernov, M.V., Antoch, M.P., 2006. Post-translational regulation of circadian transcriptional CLOCK(NPAS2)/BMAL1 complex by CRYPTOCHROMES. Cell Cycle Georget. Tex 5, 890–895.

Korb, O., Stützle, T., Exner, T.E., 2006. PLANTS: Application of Ant Colony Optimization to Structure-Based Drug Design, in: Dorigo, M., Gambardella, L.M., Birattari, M., Martinoli, A., Poli, R., Stützle, T. (Eds.), Ant Colony Optimization and Swarm Intelligence, Lecture Notes in Computer Science. Springer Berlin Heidelberg, pp. 247–258.

Krishnan, B., Levine, J.D., Lynch, M.K., Dowse, H.B., Funes, P., Hall, J.C., Hardin, P.E., Dryer, S.E., 2001. A new role for cryptochrome in a Drosophila circadian oscillator. Nature 411, 313–317.

Kume, K., Zylka, M.J., Sriram, S., Shearman, L.P., Weaver, D.R., Jin, X., Maywood, E.S., Hastings, M.H., Reppert, S.M., 1999. mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell 98, 193–205.

Lamia, K.A., Sachdeva, U.M., DiTacchio, L., Williams, E.C., Alvarez, J.G., Egan, D.F., Vasquez, D.S., Juguilon, H., Panda, S., Shaw, R.J., Thompson, C.B., Evans, R.M., 2009. AMPK Regulates the Circadian Clock by Cryptochrome Phosphorylation and Degradation. Science 326, 437–440.

Lane, D.J.R., Richardson, D.R., 2010. Frataxin, a molecule of mystery: trading stability for function in its iron-binding site. Biochem. J. 426, e1–e3.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.

Lesuisse, E., Santos, R., Matzanke, B.F., Knight, S.A.B., Camadro, J.-M., Dancis, A., 2003. Iron use for haeme synthesis is under control of the yeast frataxin homologue (Yfh1). Hum. Mol. Genet. 12, 879–889.

Lin, C., Robertson, D.E., Ahmad, M., Raibekas, A.A., Jorns, M.S., Dutton, P.L., Cashmore, A.R., 1995. Association of flavin adenine dinucleotide with the Arabidopsis blue light receptor CRY1. Science 269, 968–970.

Liu, H., Liu, B., Zhao, C., Pepper, M., Lin, C., 2011. The action mechanisms of plant cryptochromes. Trends Plant Sci. 16, 684–691.

Liu, H.-Y., Kuntz, I.D., Zou, X., 2004. Pairwise GB/SA Scoring Function for Structure-based Drug Design. J. Phys. Chem. B 108, 5453–5462.

Liu, Z., Tan, C., Guo, X., Li, J., Wang, L., Sancar, A., Zhong, D., 2013a. Determining complete electron flow in the cofactor photoreduction of oxidized photolyase. Proc. Natl. Acad. Sci. U. S. A. 110, 12966–12971.

Liu, Z., Zhang, M., Guo, X., Tan, C., Li, J., Wang, L., Sancar, A., Zhong, D., 2013b. Dynamic determination of the functional state in photolyase and the implication for cryptochrome. Proc. Natl. Acad. Sci. U. S. A. 110, 12972–12977.

Li, X., Wang, Q., Yu, X., Liu, H., Yang, H., Zhao, C., Liu, X., Tan, C., Klejnot, J., Zhong, D., Lin, C., 2011. Arabidopsis cryptochrome 2 (CRY2) functions by the photoactivation mechanism distinct from the tryptophan (trp) triad-dependent photoreduction. Proc. Natl. Acad. Sci. U. S. A. 108, 20844–20849.

MacKerell, A. D., Bashford, D., Bellott, Dunbrack, R. L., Evanseck, J.D., Field, M.J., Fischer, S., Gao, J., Guo, H., Ha, S., Joseph-McCarthy, D., Kuchnir, L., Kuczera, K., Lau, F.T.K., Mattos, C., Michnick, S., Ngo, T., Nguyen, D.T., Prodhom, B., Reiher, W.E., Roux, B., Schlenkrich, M., Smith, J.C., Stote, R., Straub, J., Watanabe, M., Wiórkiewicz-Kuczera, J., Yin, D., Karplus, M., 1998. All-Atom Empirical Potential for Molecular Modeling and Dynamics Studies of Proteins†. J. Phys. Chem. B 102, 3586–3616.

Majeux, N., Scarsi, M., Apostolakis, J., Ehrhardt, C., Caflisch, A., 1999. Exhaustive

docking of molecular fragments with electrostatic solvation. Proteins 37, 88–105.

Malhotra, K., Kim, S.-T., Batschauer, A., Dawut, L., Sancar, A., 1995. Putative Blue-Light Photoreceptors from Arabidopsis thaliana and Sinapis alba with a High Degree of Sequence Homology to DNA Photolyase Contain the Two Photolyase Cofactors but Lack DNA Repair Activity. Biochemistry (Mosc.) 34, 6892–6899.

Mao, J., Zhang, Y.-C., Sang, Y., Li, Q.-H., Yang, H.-Q., 2005. A role for Arabidopsis cryptochromes and COP1 in the regulation of stomatal opening. Proc. Natl. Acad. Sci. U. S. A. 102, 12270–12275.

Matsumoto, N., 2003. Functional Analysis and Intracellular Localization of Rice Cryptochromes. PLANT Physiol. 133, 1494–1503.

Meissner, R.-A., Kilman, V.L., Lin, J.-M., Allada, R., 2008. TIMELESS Is an Important Mediator of CK2 Effects on Circadian Clock Function In Vivo. J. Neurosci. 28, 9732–9740.

Miyazaki, K., Mesaki, M., Ishida, N., 2001. Nuclear Entry Mechanism of Rat PER2 (rPER2): Role of rPER2 in Nuclear Localization of CRY Protein. Mol. Cell. Biol. 21, 6651–6659.

Müller, M., Carell, T., 2009. Structural biology of DNA photolyases and cryptochromes. Curr. Opin. Struct. Biol. 19, 277–285.

Müller, P., Ahmad, M., 2011. Light-activated Cryptochrome Reacts with Molecular Oxygen to Form a Flavin–Superoxide Radical Pair Consistent with Magnetoreception. J. Biol. Chem. 286, 21033–21040.

Musco, G., Stier, G., Kolmerer, B., Adinolfi, S., Martin, S., Frenkiel, T., Gibson, T., Pastore, A., 2000. Towards a structural understanding of Friedreich's ataxia: the solution structure of frataxin. Struct. Lond. Engl. 1993 8, 695–707.

Naidoo, N., Song, W., Hunter-Ensor, M., Sehgal, A., 1999. A role for the proteasome in the light response of the timeless clock protein. Science 285, 1737–1741.

Nair, M., Adinolfi, S., Pastore, C., Kelly, G., Temussi, P., Pastore, A., 2004. Solution Structure of the Bacterial Frataxin Ortholog, CyaY: Mapping the Iron Binding Sites. Structure 12, 2037–2048.

Nießner, C., Denzau, S., Stapput, K., Ahmad, M., Peichl, L., Wiltschko, W., Wiltschko, R., 2013. Magnetoreception: activated cryptochrome 1a concurs with magnetic

orientation in birds. J. R. Soc. Interface 10, 20130638.

O'Neill, H.A., Gakh, O., Park, S., Cui, J., Mooney, S.M., Sampson, M., Ferreira, G.C., Isaya, G., 2005. Assembly of Human Frataxin Is a Mechanism for Detoxifying Redox-Active Iron†. Biochemistry (Mosc.) 44, 537–545.

Özgür, S., Sancar, A., 2006. Analysis of Autophosphorylating Kinase Activities of Arabidopsis and Human Cryptochromes†. Biochemistry (Mosc.) 45, 13369–13374.

Ozturk, N., Selby, C.P., Song, S.-H., Ye, R., Tan, C., Kao, Y.-T., Zhong, D., Sancar, A., 2009. Comparative Photochemistry of Animal Type 1 and Type 4 Cryptochromes. Biochemistry (Mosc.) 48, 8585–8593.

Öztürk, N., Song, S.-H., Özgür, S., Selby, C.P., Morrison, L., Partch, C., Zhong, D., Sancar, A., 2007. Structure and Function of Animal Cryptochromes. Cold Spring Harb. Symp. Quant. Biol. 72, 119–131.

Öztürk, N., Song, S.-H., Selby, C.P., Sancar, A., 2008. Animal Type 1 Cryptochromes ANALYSIS OF THE REDOX STATE OF THE FLAVIN COFACTOR BY SITE-DIRECTED MUTAGENESIS. J. Biol. Chem. 283, 3256–3263.

Ozturk, N., VanVickle-Chavez, S.J., Akileswaran, L., Gelder, R.N.V., Sancar, A., 2013. Ramshackle (Brwd3) promotes light-induced ubiquitylation of Drosophila Cryptochrome by DDB1-CUL4-ROC1 E3 ligase complex. Proc. Natl. Acad. Sci. 110, 4980–4985.

Panchenko, A.R., Kondrashov, F., Bryant, S., 2004. Prediction of functional sites by analysis of sequence and structure conservation. Protein Sci. 13, 884–892.

Pandolfo, M., Pastore, A., 2009. The pathogenesis of Friedreich ataxia and the structure and function of frataxin. J. Neurol. 256 Suppl 1, 9–17.

Park, H.W., Kim, S.T., Sancar, A., Deisenhofer, J., 1995. Crystal structure of DNA photolyase from Escherichia coli. Science 268, 1866–1872.

Pastore, C., Franzese, M., Sica, F., Temussi, P., Pastore, A., 2007. Understanding the binding properties of an unusual metal-binding protein--a study of bacterial frataxin. FEBS J. 274, 4199–4210.

Payne, G., Sancar, A., 1990. Absolute action spectrum of E-FADH2 and E-FADH2-MTHF forms of Escherichia coli DNA photolyase. Biochemistry (Mosc.) 29, 7715–7727.

Peschel, N., Veleri, S., Stanewsky, R., 2006. Veela defines a molecular link between Cryptochrome and Timeless in the light-input pathway to Drosophila's circadian clock. Proc. Natl. Acad. Sci. 103, 17313–17318.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., Ferrin, T.E., 2004. UCSF Chimera--a visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1612.

Phillips, J.C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., Chipot, C., Skeel, R.D., Kalé, L., Schulten, K., 2005. Scalable molecular dynamics with NAMD. J. Comput. Chem. 26, 1781–1802.

Pils, B., Copley, R.R., Schultz, J., 2005. Variation in structural location and amino acid conservation of functional sites in protein domain families. BMC Bioinformatics 6, 210.

Pokorny, R., Klar, T., Hennecke, U., Carell, T., Batschauer, A., Essen, L.-O., 2008. Recognition and repair of UV lesions in loop structures of duplex DNA by DASH-type cryptochrome. Proc. Natl. Acad. Sci. 105, 21023–21027.

Prischi, F., Giannini, C., Adinolfi, S., Pastore, A., 2009. The N-terminus of mature human frataxin is intrinsically unfolded. FEBS J. 276, 6669–6676.

Prischi, F., Konarev, P.V., Iannuzzi, C., Pastore, C., Adinolfi, S., Martin, S.R., Svergun, D.I., Pastore, A., 2010. Structural bases for the interaction of frataxin with the central components of iron-sulphur cluster assembly. Nat. Commun. 1, 95.

Rosato, E., Codd, V., Mazzotta, G., Piccin, A., Zordan, M., Costa, R., Kyriacou, C.P., 2001. Light-dependent interaction between Drosophila CRY and the clock protein PER mediated by the carboxy terminus of CRY. Curr. Biol. 11, 909–917.

Rush, B.L., Murad, A., Emery, P., Giebultowicz, J.M., 2006. Ectopic CRYPTOCHROME Renders TIM Light Sensitive in the Drosophila Ovary. J. Biol. Rhythms 21, 272–278.

Ryckaert, J.-P., Ciccotti, G., Berendsen, H.J.C., 1977. Numerical integration of the cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. J. Comput. Phys. 23, 327–341.

Saez, L., Derasmo, M., Meyer, P., Stieglitz, J., Young, M.W., 2011. A key temporal delay in the circadian cycle of Drosophila is mediated by a nuclear localization signal in the timeless protein. Genetics 188, 591–600.

Sakakida, Y., Miyamoto, Y., Nagoshi, E., Akashi, M., Nakamura, T.J., Mamine, T., Kasahara, M., Minami, Y., Yoneda, Y., Takumi, T., 2005. Importin alpha/beta mediates nuclear transport of a mammalian circadian clock component, mCRY2, together with mPER2, through a bipartite nuclear localization signal. J. Biol. Chem. 280, 13272–13278.

Sancar, A., 2003. Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. Chem. Rev. 103, 2203–2237.

Schmucker, S., Martelli, A., Colin, F., Page, A., Wattenhofer-Donzé, M., Reutenauer, L., Puccio, H., 2011. Mammalian Frataxin: An Essential Function for Cellular Viability through an Interaction with a Preformed ISCU/NFS1/ISD11 Iron-Sulfur Assembly Complex. PLoS ONE 6, e16199.

Sehadova, H., Glaser, F.T., Gentile, C., Simoni, A., Giesecke, A., Albert, J.T., Stanewsky, R., 2009. Temperature entrainment of Drosophila's circadian clock involves the gene nocte and signaling from peripheral sensory tissues to the brain. Neuron 64, 251–266.

Selby, C.P., Sancar, A., 2012. The second chromophore in Drosophila photolyase/cryptochrome family photoreceptors. Biochemistry (Mosc.) 51, 167–171.

Shalitin, D., Yang, H., Mockler, T.C., Maymon, M., Guo, H., Whitelam, G.C., Lin, C., 2002. Regulation of Arabidopsis cryptochrome 2 by blue-light-dependent phosphorylation. Nature 417, 763–767.

Shan, Y., Cortopassi, G., 2012. HSC20 interacts with frataxin and is involved in iron-sulfur cluster biogenesis and iron homeostasis. Hum. Mol. Genet. 21, 1457–1469.

Shan, Y., Napoli, E., Cortopassi, G., 2007. Mitochondrial frataxin interacts with ISD11 of the NFS1/ISCU complex and multiple mitochondrial chaperones. Hum. Mol. Genet. 16, 929–941.

Söderberg, C.A.G., Shkumatov, A.V., Rajan, S., Gakh, O., Svergun, D.I., Isaya, G., Al-Karadaghi, S., 2011. Oligomerization Propensity and Flexibility of Yeast Frataxin Studied by X-ray Crystallography and Small-Angle X-ray Scattering. J. Mol. Biol. 414, 783–797.

Song, S.-H., 2006. Photodynamics of phototropin and cryptochrome blue-light

photoreceptors. Universität Regensburg.

Song, S.-H., Dick, B., Penzkofer, A., Pokorny, R., Batschauer, A., Essen, L.-O., 2006. Absorption and fluorescence spectroscopic characterization of cryptochrome 3 from Arabidopsis thaliana. J. Photochem. Photobiol. B 85, 1–16.

Stanewsky, R., Kaneko, M., Emery, P., Beretta, B., Wager-Smith, K., Kay, S.A., Rosbash, M., Hall, J.C., 1998. The cryb mutation identifies cryptochrome as a circadian photoreceptor in Drosophila. Cell 95, 681–692.

Stemmler, T.L., Lesuisse, E., Pain, D., Dancis, A., 2010. Frataxin and Mitochondrial FeS Cluster Biogenesis. J. Biol. Chem. 285, 26737–26743.

Stillinger, F.H., Rahman, A., 2003. Improved simulation of liquid water by molecular dynamics. J. Chem. Phys. 60, 1545–1557.

Still, W.C., Tempczyk, A., Hawley, R.C., Hendrickson, T., 1990. Semianalytical treatment of solvation for molecular mechanics and dynamics. J. Am. Chem. Soc. 112, 6127–6129.

Stoleru, D., Nawathean, P., Fernández, M. de la P., Menet, J.S., Ceriani, M.F., Rosbash, M., 2007. The Drosophila Circadian Network Is a Seasonal Timer. Cell 129, 207–219.

Suzek, B.E., Huang, H., McGarvey, P., Mazumder, R., Wu, C.H., 2007. UniRef: comprehensive and non-redundant UniProt reference clusters. Bioinformatics 23, 1282–1288.

Tamada, T., Kitadokoro, K., Higuchi, Y., Inaka, K., Yasui, A., de Ruiter, P.E., Eker, A.P.M., Miki, K., 1997. Crystal structure of DMA photolyase from Anacystis nidulans. Nat. Struct. Mol. Biol. 4, 887–891.

Tamanini, F., Chaves, I., Bajek, M.I., van der Horst, G.T.J., 2007. Structure function analysis of mammalian cryptochromes. Cold Spring Harb. Symp. Quant. Biol. 72, 133–139.

Taylor, R.D., Jewsbury, P.J., Essex, J.W., 2003. FDS: flexible ligand and receptor docking with a continuum solvent model and soft-core energy function. J. Comput. Chem. 24, 1637–1656.

Thompson, C.L., Bowes Rickman, C., Shaw, S.J., Ebright, J.N., Kelly, U., Sancar, A., Rickman, D.W., 2003. Expression of the blue-light receptor cryptochrome in the human retina. Invest. Ophthalmol. Vis. Sci. 44, 4515–4521.

VanVickle-Chavez, S.J., Gelder, R.N.V., 2007. Action Spectrum of Drosophila Cryptochrome. J. Biol. Chem. 282, 10561–10566.

Verlet, L., 1967. Computer "Experiments" on Classical Fluids. I. Thermodynamical Properties of Lennard-Jones Molecules. Phys. Rev. 159, 98–103.

Wang, H., Ma, L.-G., Li, J.-M., Zhao, H.-Y., Deng, X.W., 2001. Direct Interaction of Arabidopsis Cryptochromes with COP1 in Light Control Development. Science 294, 154–158.

Wang, J., Wang, W., Kollman, P.A., Case, D.A., 2006. Automatic atom type and bond type perception in molecular mechanical calculations. J. Mol. Graph. Model. 25, 247–260.

Wang, T., Craig, E.A., 2008. Binding of Yeast Frataxin to the Scaffold for Fe-S Cluster Biogenesis, Isu. J. Biol. Chem. 283, 12674–12679.

Waterhouse, A.M., Procter, J.B., Martin, D.M.A., Clamp, M., Barton, G.J., 2009. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics 25, 1189–1191.

Wu, G., Spalding, E.P., 2007. Separate functions for nuclear and cytoplasmic cryptochrome 1 during photomorphogenesis of Arabidopsis seedlings. Proc. Natl. Acad. Sci. 104, 18813–18818.

Xu, P., Xiang, Y., Zhu, H., Xu, H., Zhang, Z., Zhang, C., Zhang, L., Ma, Z., 2008. Wheat Cryptochromes: Subcellular Localization and Involvement in Photomorphogenesis and Osmotic Stress Responses. PLANT Physiol. 149, 760–774.

Yang, H.Q., Tang, R.H., Cashmore, A.R., 2001. The signaling mechanism of Arabidopsis CRY1 involves direct interaction with COP1. Plant Cell 13, 2573–2587.

Yang, H.-Q., Wu, Y.-J., Tang, R.-H., Liu, D., Liu, Y., Cashmore, A.R., 2000. The C termini of Arabidopsis cryptochromes mediate a constitutive light response. Cell 103, 815–827.

Yang, Y.-J., Zuo, Z.-C., Zhao, X.-Y., Li, X., Klejnot, J., Li, Y., Chen, P., Liang, S.-P., Yu, X.-H., Liu, X.-M., Lin, C.-T., 2008. Blue-Light-Independent Activity of Arabidopsis Cryptochromes in the Regulation of Steady-State Levels of Protein and mRNA Expression. Mol. Plant 1, 167–177.

Yoon, T., Cowan, J.A., 2003. Iron-sulfur cluster biosynthesis. Characterization of frataxin as an iron donor for assembly of [2Fe-2S] clusters in ISU-type proteins. J. Am. Chem. Soc. 125, 6078–6084.

Yoon, T., Dizin, E., Cowan, J.A., 2007. N-terminal iron-mediated self-cleavage of human frataxin: regulation of iron binding and complex formation with target proteins. J. Biol. Inorg. Chem. JBIC Publ. Soc. Biol. Inorg. Chem. 12, 535–542.

Yoshii, T., Hermann, C., Helfrich-Förster, C., 2010. Cryptochrome-positive and -negative clock neurons in Drosophila entrain differentially to light and temperature. J. Biol. Rhythms 25, 387–398.

Yoshii, T., Todo, T., Wülbeck, C., Stanewsky, R., Helfrich-Förster, C., 2008. Cryptochrome is present in the compound eyes and a subset of Drosophila's clock neurons. J. Comp. Neurol. 508, 952–966.

Yu, X., Klejnot, J., Zhao, X., Shalitin, D., Maymon, M., Yang, H., Lee, J., Liu, X., Lopez, J., Lin, C., 2007. Arabidopsis Cryptochrome 2 Completes Its Posttranslational Life Cycle in the Nucleus. Plant Cell Online 19, 3146–3156.

Zhang, F., Scheerer, P., Oberpichler, I., Lamparter, T., Krauß, N., 2013. Crystal structure of a prokaryotic (6-4) photolyase with an Fe-S cluster and a 6,7-dimethyl-8-ribityllumazine antenna chromophore. Proc. Natl. Acad. Sci. 110, 7217–7222.

Zhu, H., Conte, F., Green, C.B., 2003. Nuclear Localization and Transcriptional Repression Are Confined to Separable Domains in the Circadian Protein CRYPTOCHROME. Curr. Biol. 13, 1653–1658.

Zuo, Z.-C., Meng, Y.-Y., Yu, X.-H., Zhang, Z.-L., Feng, D.-S., Sun, S.-F., Liu, B., Lin, C.-T., 2012. A study of the blue-light-dependent phosphorylation, degradation, and photobody formation of Arabidopsis CRY2. Mol. Plant 5, 726–733.

Zuo, Z., Liu, H., Liu, B., Liu, X., Lin, C., 2011. Blue Light-Dependent Interaction of CRY2 with SPA1 Regulates COP1 activity and Floral Initiation in Arabidopsis. Curr. Biol. 21, 841–847.

3. CRY/Photolyase Family Sequence-based Analysis

3.1 Introduction

Cryptochrome/Photolyase family members are flavoproteins which show DNA repairing and/or photoreception functionality. While Photolyases are believed to play DNA repairing, Cryptochromes are involved in photoreception of entrainment circadian rhythms in animals, and light-related development in plants (Chaves et al., 2011). Despite their close structural similarity (Müller and Carell, 2009) and possible DNA binding, no photolyase activity has been atributed to cryptochromes (Öztürk et al., 2007). Differences are also reported on their FAD cofactor functionality (Hoang et al., 2008). CRY-DASHs, the most recent CRY class members, are an intermediate group showing multifunctionality in DNA repairing activity and transcriptional repression (Brudler et al., 2003) (Pokorny et al., 2008). Functionally, members of this family are divided in five groups: CRY1, CRY2, CRY3 (also annotated as CRY-DASH), DNA Photolyases and (6-4) Photolyases. Family members show basic N-terminal DNA photolyase and FAD binding domains (Figure 5), also known as Photolyase Homology Region (PHR). DNA photolyase domain binds a light harvesting cofactor, while FAD binding domain is correlated with the binding to the family main cofactor. The second cofactor is found at the interface between these two domains, with flavin cofactors more internally located, and the pterin type being surface exposed. As all family members contain FAD as cofactor, they diverge on the presence of a second cofactor. While some photolyases use 5,10-methenyltetrahydrofolate (MTHF) and others show flavin type like 7,8-didemethyl-8-hydroxy-5-deazariboflavin (8-HDF), FMN and FAD as second cofactor, there are some of them showing no second chromophore. Moreover, since all members of this family share a PHR domain, they diverge in the C-terminal region, with the length varying from 40 to about 240 residues. In Cryptochromes, functional differences have also been atributed to their C-terminus. While *Drosophila* Cryptochrome show some residues in its C-terminal "tail" being relevant when interacting to clock proteins, mammals CRYs C-terminal region is not relevant for clock function (Dissel et al., 2004) (Khan et al., 2012) (Hemsley et al., 2007) . Further functional differences have been verified with mammals CRYs showing light-

independent clock function, whereas *Drosophila* CRY play light dependent circadian rhythm entrainment (Griffin et al., 1999). It is widely accepted that tertiary structures are more conserved than sequences (Chothia and Lesk, 1986). Moreover, functional sites can have variable amino acid conservation in the protein domain families(Panchenko et al., 2004) (Pils et al., 2005). In this work we performed a qualitative study using sequence similarity and conservation profiles to understand the differences and similarities among CRY/photolyase family members, relating them with functional aspects.

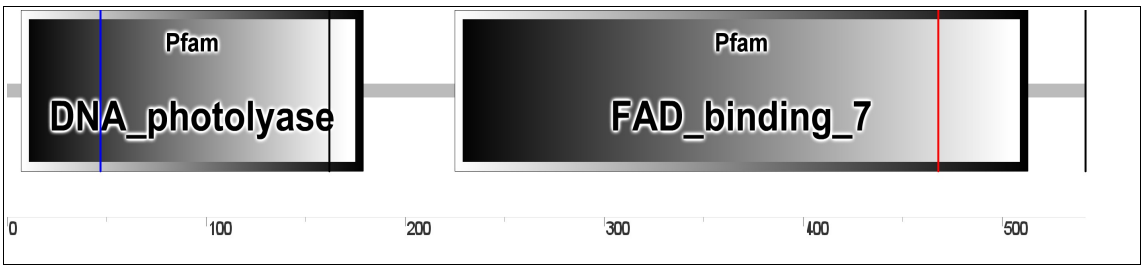

Figure 5. dCRY showing PFAM predicted domains.

3.2 Methods

In order to find the closest PHR (Photolyase Homology Region) domain sequences, PSI-BLAST (Altschul et al., 1997) has been run using dCRY as query against the highly manually annotated and non-redundant SwissProt protein sequence database (Suzek et al., 2007). After 3 iterations, 56 sequences have been taken, considering only the sequences annotated as CRY1 (15 sequences), CRY2 (6 sequences), CRY-DASH (15 sequences), (6-4) Photolyase (1 sequence) and DNA Photolyase (19 sequences), ranging from bacteria, archaea and eukarya kingdoms (Table 1).

TABLE 1
SwissProt Sequences Utilized in This Study

CRY/Photolyase family member	Organism and number of residues	Kingdom
CRY1	Drosophila melanogaster/1-542	Animal
CRY1	Drosophila_pseudoobscura/1-540	Animal
CRY1	Anopheles_gambiae/1-545	Animal

CRY1	Aedes_aegypti/1-545	Animal
CRY1	Mus_musculus/1-606	Animal
CRY1	Sylvia_borin/1-620	Animal
CRY1	Gallus_gallus/1-621	Animal
CRY1	Erithacus_rubecula/1-620	Animal
CRY1	Homo_sapiens/1-586	Animal
CRY1	Rattus_norvegicus/1-588	Animal
CRY1	Macaca_fascicularis/1-586	Animal
CRY2	Gallus_gallus/1-582	Animal
CRY2	Mus_musculus/1-592	Animal
CRY1	Spalax_judaei/1-587	Animal
CRY2	Rattus_norvegicus/1-594	Animal
CRY2	Homo_sapiens/1-593	Animal
CRYD	Synechocystis_sp._(strain_PCC_6803_/_Kazusa)/1-489	Bacteria
CRYD	Gloeobacter_ violaceus/1-500	Bacteria
CRYD	Danio_rerio/1-520	Animal
(6-4)_photolyase	Arabidopsis_thaliana/1-556	Plant
CRYD	Rhodopirellula_baltica/1-488	Bacteria
CRYD	Xenopus_laevis/1-523	Animal
CRYD	Natronomonas_pharaonis/1-474	Archaea
CRYD	Arabidopsis_thaliana/1-569	Plant
CRYD	Solanum_lycopersicum/1-577	Plant
DNA_photolyase	Synechocystis_sp._(strain_PCC_6803_/_Kazusa)/1-488	Bacteria
DNA_photolyase	Synechococcus_sp._(strain_ATCC_27144_/_PCC_6301_/_SAUG_1402/1)/1-484	Bacteria
CRYD	Oryza_sativa/1-582	Plant
CRY2	Arabidopsis_thaliana/1-612	Plant
DNA_photolyase	ECOLI/1-472	Bacteria
DNA_photolyase	Neurospora_crassa/1-642	Fungi
DNA_photolyase	Vibrio_cholerae/1-469	Bacteria
DNA_photolyase	Salmonella_typhimurium/1-473	Bacteria
CRY1	Sinapis_alba/1-501	Plant

DNA_photolyase	Halobacterium_salinarum/1-481	Bacteria
DNA_photolyase	Buchnera_aphidicola/1-483	Bacteria
CRYD	Ostreococcus_tauri/1-546	Plant
DNA_photolyase	Buchnera_aphidicola/1-478	Bacteria
CRY1	Culex_quinquefasciatus/1-499	Animal
CRY1	Arabidopsis_thaliana/1-681	Plant
DNA_photolyase	Streptomyces_griseus/1-455	Bacteria
DNA_photolyase	YEAST/1-565	Fungi
CRYD	Idiomarina_loihiensis/1-449	Bacteria
CRYD	Vibrio_cholerae/1-461	Bacteria
CRYD	Neurospora_crassa/1-745	Fungi
CRYD	Vibrio_parahaemolyticus/1-445	Bacteria
CRYD	Gibberella_zeae/1-678	Fungi
CRY2	Vibrio_cholerae/1-504	Bacteria
DNA_photolyase	Thermus thermophilus(strain_HB27_/_ATCC_BAA-163_/_DSM_7039)/1-420	Bacteria
DNA_photolyase	Thermus_thermophilus_(strain_HB8_/_ATCC_27634_/_DSM_579)/1-420	Bacteria
DNA_photolyase	Carassius_auratus/1-556	Animal
DNA_photolyase	Potorous_tridactylus/1-532	Animal
DNA_photolyase	Arabidopsis_thaliana/1-496	Plant
DNA_photolyase	Oryza_sativa/1-506	Plant
DNA_photolyase	Methanothermobacter_marburgensis/1-444	Archaea
DNA_photolyase	Methanothermobacter_thermautotrophicus/1-445	Archaea

Similarity network was obtained through PANADA webserver. To the analysis of the closest groups, different sequence identity thresholds have been considered.

Multiple sequence alignment was performed through Jalview (Waterhouse et al., 2009)web interface, using ClustalW (Larkin et al., 2007) default parameters.

Residue conservation was taken by Jalview assessment, which considers amino acids physico-chemical properties to calculate conservation (Figure 6).

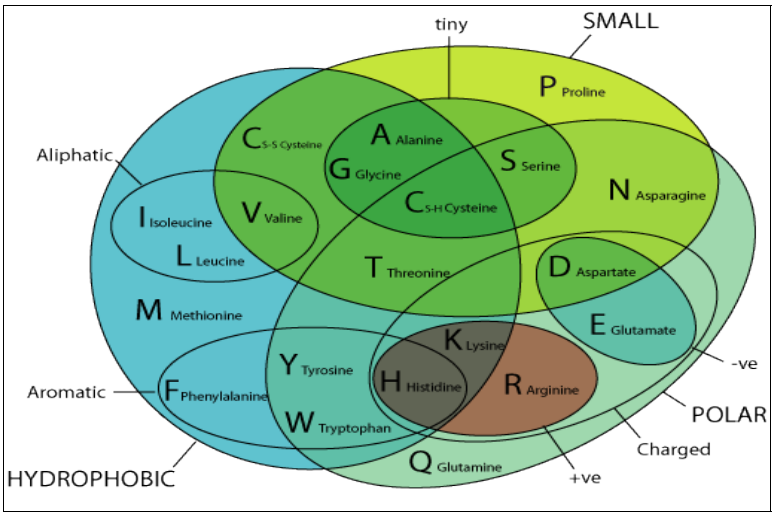

Figure 6. Chemical and physical properties of amino acids.

3.3 Results

PANADA similarity network has been used to verify sequence correlation among CRY/Photolyase family. When considering a threshold of 60 % identity, a group of CRY1 and CRY2 of Animal vertebrates is identified (Figure 7). This group forms a very densed sequence similarity network. An unexpected *Oryza sativa* DNA photolyase sequence is found close to the CRY1 and CRY2 vertebrates group.

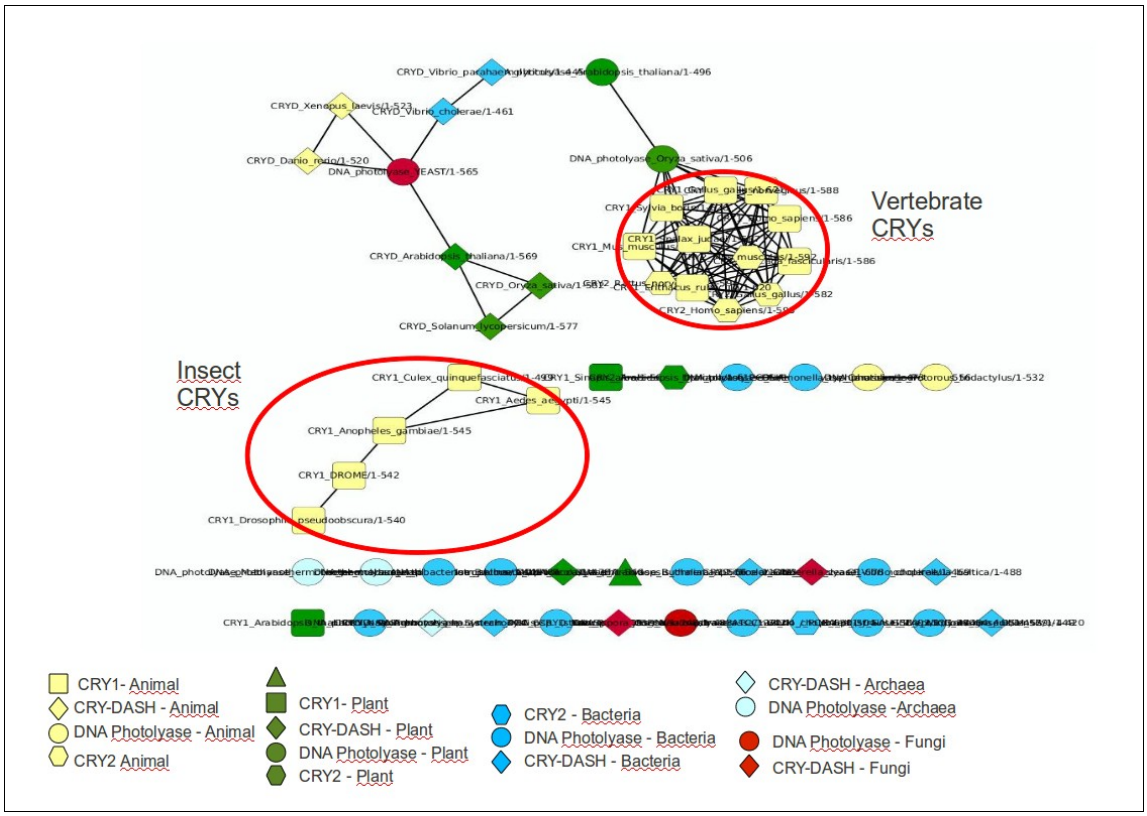

Figure 7. PANADA similarity network considering sequence identity within 100-60 %. Groups of Insect and vertebrate CRYs are identified.

The strong relationship among them can be further verified when looking at a multiple sequence alignment, where highly PHR domain sequence conservation is present. Differently, the C-terminal region is not conserved (Figure 8).

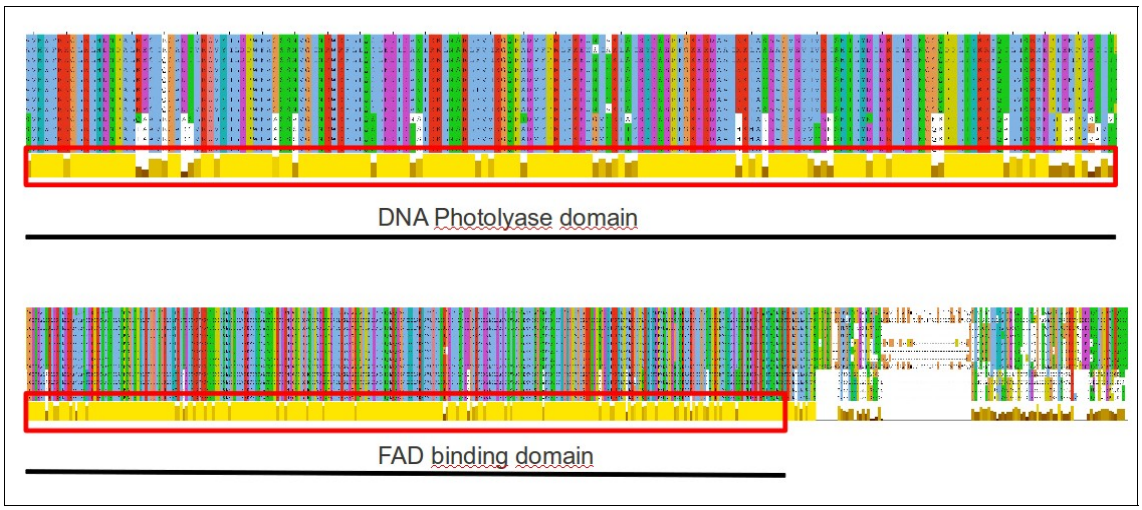

Figure 8. Multiple Sequence Alignment of only CRY1 and CRY2 vertebrates. PHR domain conservation is identified in red retangle.

Within 100-60% sequence identity, a second group of insect CRYs can be identified. The multiple sequence alignment of these five insect sequences shows the FAD binding region of the PHR domain mostly conserved, with no conservation of the DNA photolyase domain (Figure 9). Furthermore, differently than vertebrate CRYs, insects C-terminus show some conservation.

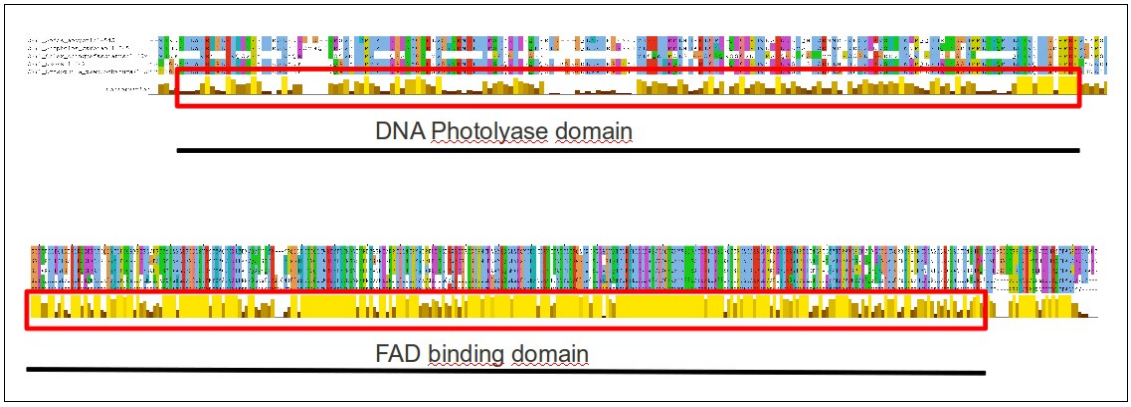

Figure 9. Insect CRYs multiple sequence alignment. *Drosophila* Cryptochrome DNA photolyase and FAD binding domains conservation are identified in red retangle.

Lowering the low bound of sequence identity to 45%, a group of CRY-DASH sequences can be found (Figure 10). While at this threshold CRY-DASHs are more closely related, DNA photolyases show low similarity.

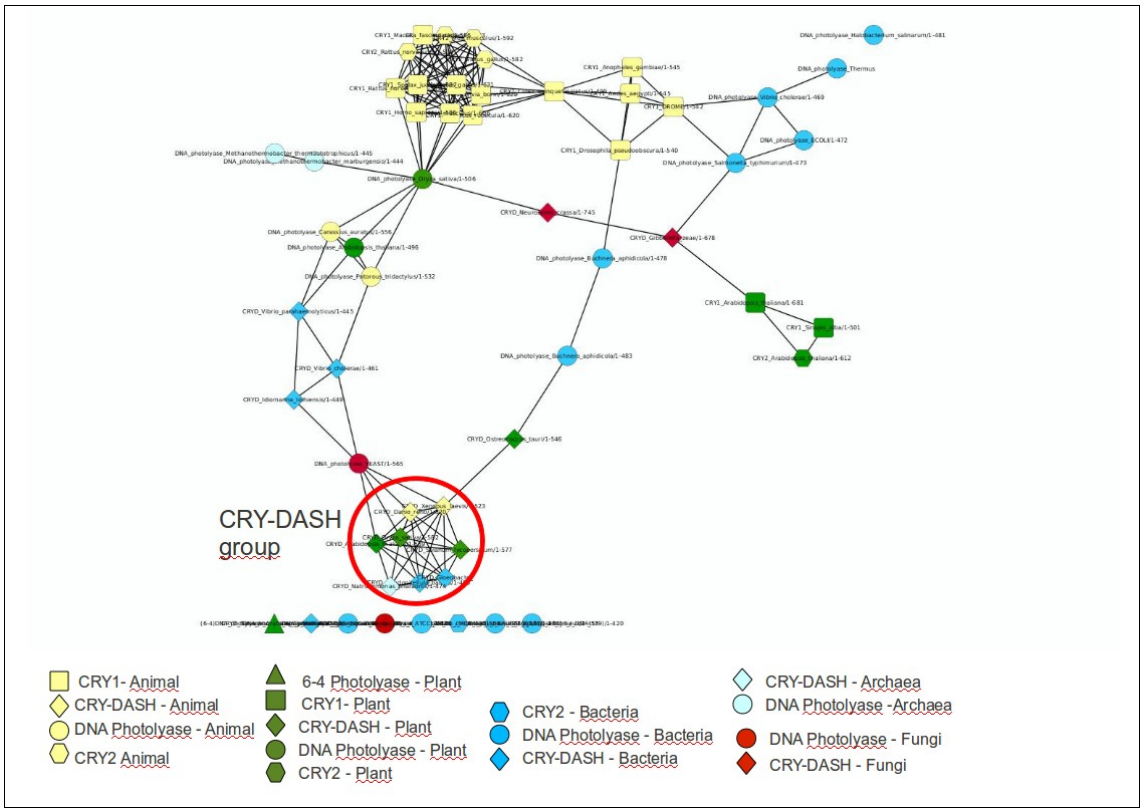

Figure 10. Similarity network considering sequence identity within 100-45%. A group of CRY-DASH is identified.

CRY-DASHs show low conservation of residues in the DNA Photolyase domain, with a slightly higher conservation in the FAD binding domain and no conservation in the C-terminal region (Figure 11).

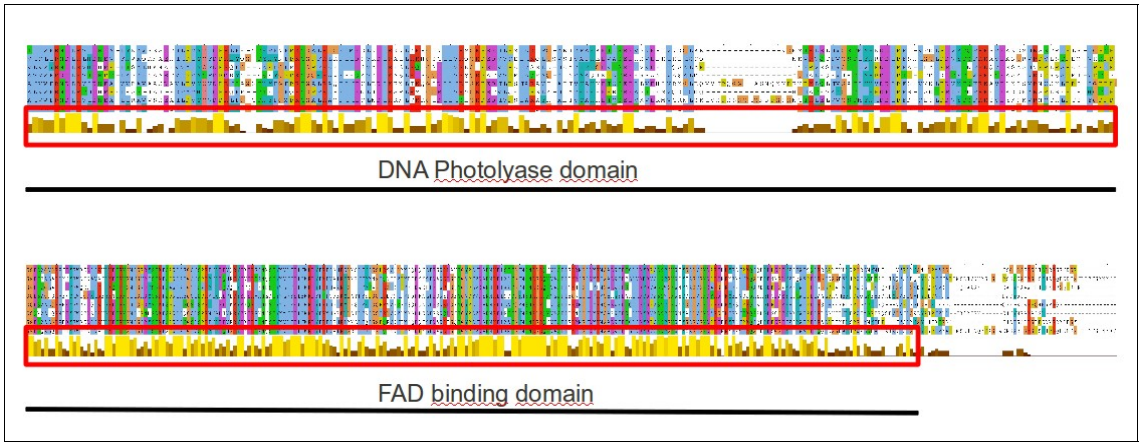

Figure 11. Multiple sequence alignment of CRY-DASHs, showing DNA Photolyase and FAD binding domains conservation in red retangle.

Residues R16, G273, R315, G356, T358, G365, G374, R381 and D412 (dCRY numerated) are highly conserved among all members of CRY/Photolyase family (56

sequences of this work) (Figure 12). D412, R381 and R315 are located close to the FAD cofactor, while G273 and R16 are part of the DNA photolyase domain.

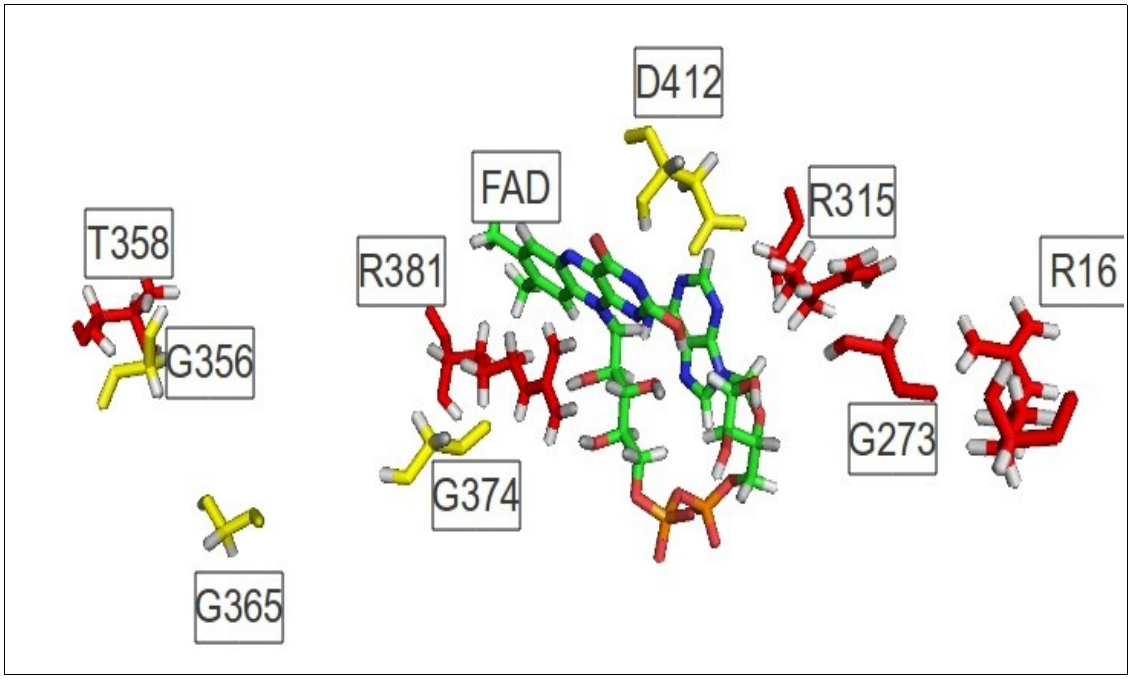

Figure 12. dCRYstructure showing highest conserved residues among 56 sequences of CRY/Photolyase family, with FAD cofactor (in green) at the center.

When considering only insect and vertebrate CRYs, fewer residues are conserved in the DNA photolyase domain than the highly conserved FAD binding domain (Figure 13). The majority of conserved residues in the DNA photolyase domain are located at the interface with the FAD binding domain. The FAD binding domain shows conserved residues spread across the domain with many residues also outside the FAD cofactor binding region.

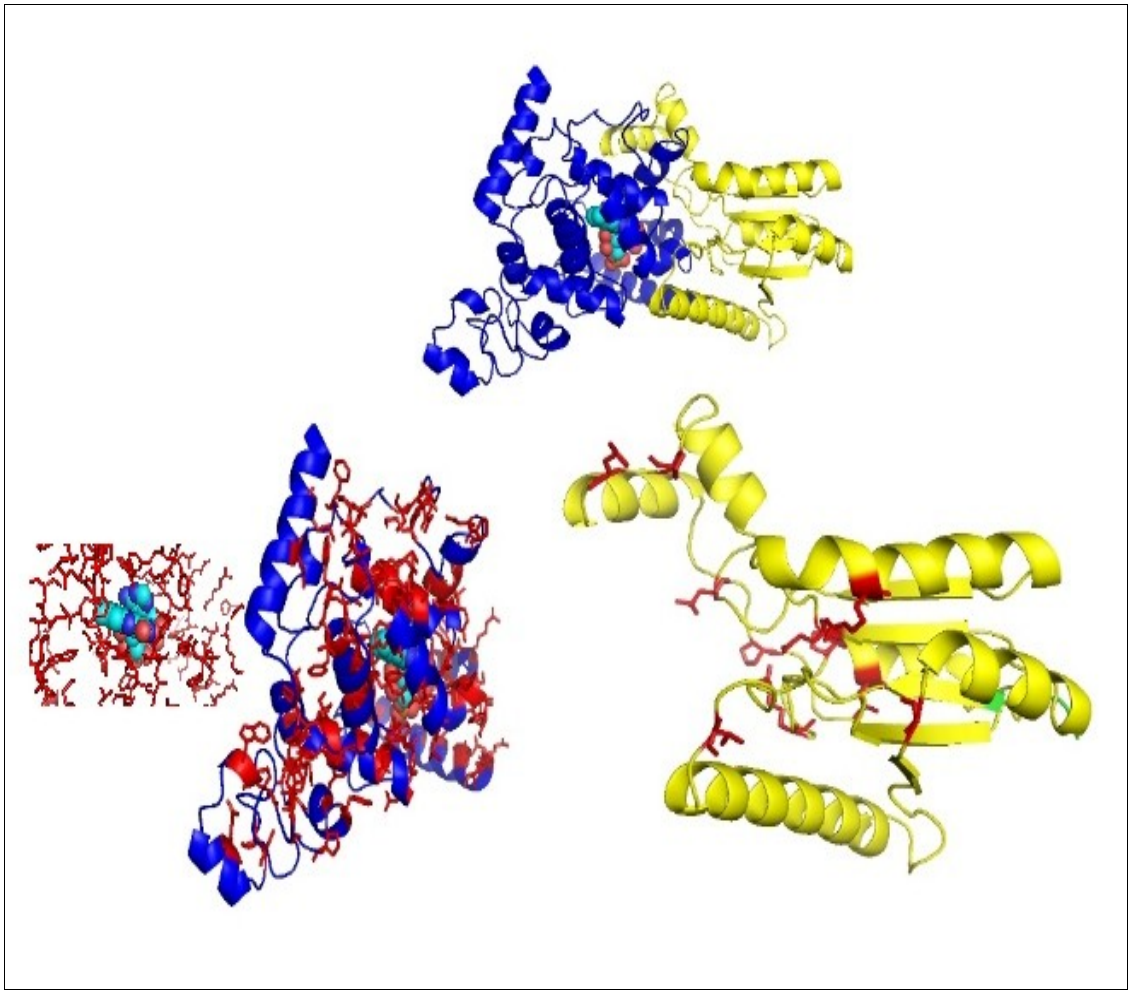

Figure 13. dCRY showing DNA photolyase (in yellow) and FAD binding (in blue) domains with FAD cofactor located at the center (in spheres). Insect and vertebrates highest conserved residues (in red) are showed on the DNA photolyase (on the right at the botom) and FAD binding (on the left at the botom) domains. A close-up view of FAD binding region is also shown.

3.4 Conclusions

Cryptochromes/photolyases are a large family of flavoproteins with sequences present in Bacteria, Archaea and Eukaryotes. They show a very similar structure with two domains: an N-terminal α/β domain (DNA photolyase) and a C-terminal α-helical domain (FAD binding). Despite their high structural conservation, they show divergent sequence conservation profiles. CRY1 and CRY2 are by far the most homogeneous group members, showing clearly higher sequence identity when comparing with CRY-DASHs and DNA photolyases. Vertebrates CRY1 and CRY2 show the highest sequence

identity correlation, with insect CRYs also forming a correlated group. However, while CRY-DASHs show some sequence identity among them, DNA photolyases are weakly correlated.

While FAD binding domain is conserved in vertebrates and insect CRYs, DNA photolyase domain is only conserved in vertebrates with low residue conservation in insects CRYs. The C-terminal "tail" downstream to the FAD binding domain, which is present in CRYs, shows conservation only in insects. The DNA photolyase domain shows low residue conservation even among DNA photolyase members. CRY-DASHs show homogeneity group with an intermediate conservation profile between CRY1/CRY2 and DNA photolyases.

Our analysis reveals different amino acid conservation profiles, enhancing amino acid conservation heterogeneity among members of CRY/Photolyase family. Our findings suggest that DNA repairing activity shows highly variable amino acid conservation on both DNA photolyase and FAD binding domains. On the other hand, CRYs photoreception activity has highly PHR domain conservation in vertebrates and high FAD binding domain conservation in insects, suggesting FAD binding region surroundings as an important aspect for its photoactivity. We further suggest insects C-terminus apparent conservation can explain their role in circadian rhythm entrainment interacting to clock proteins, consistent with previous findings. In addition, mammal CRYs low conservation may be correlated to their dispensable C-terminal extension, which has also been reported to be disordered.

3.5 References

Adamec, J., Rusnak, F., Owen, W.G., Naylor, S., Benson, L.M., Gacy, A.M., Isaya, G., 2000. Iron-dependent self-assembly of recombinant yeast frataxin: implications for Friedreich ataxia. Am. J. Hum. Genet. 67, 549–562.

Adinolfi, S., Iannuzzi, C., Prischi, F., Pastore, C., Iametti, S., Martin, S.R., Bonomi, F., Pastore, A., 2009. Bacterial frataxin CyaY is the gatekeeper of iron-sulfur cluster formation catalyzed by IscS. Nat. Struct. Mol. Biol. 16, 390–396.

Adinolfi, S., Trifuoggi, M., Politou, A.S., Martin, S., Pastore, A., 2002. A structural approach to understanding the iron-binding properties of phylogenetically

different frataxins. Hum. Mol. Genet. 11, 1865–1877.

Allen, M.P., Tildesley, D.J., 1989. Computer simulation of liquids. Clarendon Press ; Oxford University Press, Oxford [England]; New York.

Aloria, K., Schilke, B., Andrew, A., Craig, E.A., 2004. Iron-induced oligomerization of yeast frataxin homologue Yfh1 is dispensable in vivo. EMBO Rep. 5, 1096–1101.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Baker, N.A., 2005. Improving implicit solvent simulations: a Poisson-centric view. Curr. Opin. Struct. Biol. 15, 137–143.

Banerjee, R., Schleicher, E., Meier, S., Viana, R.M., Pokorny, R., Ahmad, M., Bittl, R., Batschauer, A., 2007. The Signaling State of Arabidopsis Cryptochrome 2 Contains Flavin Semiquinone. J. Biol. Chem. 282, 14916–14922.

Bashford, D., Case, D.A., 2000. Generalized born models of macromolecular solvation effects. Annu. Rev. Phys. Chem. 51, 129–152.

Beel, B., Prager, K., Spexard, M., Sasso, S., Weiss, D., Müller, N., Heinnickel, M., Dewez, D., Ikoma, D., Grossman, A.R., Kottke, T., Mittag, M., 2012. A Flavin Binding Cryptochrome Photoreceptor Responds to Both Blue and Red Light in Chlamydomonas reinhardtii. Plant Cell Online 24, 2992–3008.

Berendsen, H.J.C., Grigera, J.R., Straatsma, T.P., 1987. The missing term in effective pair potentials. J. Phys. Chem. 91, 6269–6271.

Berndt, A., Kottke, T., Breitkreuz, H., Dvorsky, R., Hennig, S., Alexander, M., Wolf, E., 2007. A novel photoreaction mechanism for the circadian blue light photoreceptor Drosophila cryptochrome. J. Biol. Chem. 282, 13011–13021.

Biskup, T., Hitomi, K., Getzoff, E.D., Krapf, S., Koslowski, T., Schleicher, E., Weber, S., 2011. Unexpected Electron Transfer in Cryptochrome Identified by Time-Resolved EPR Spectroscopy. Angew. Chem. Int. Ed. 50, 12647–12651.

Biskup, T., Paulus, B., Okafuji, A., Hitomi, K., Getzoff, E.D., Weber, S., Schleicher, E., 2013. Variable Electron-Transfer Pathways in an Amphibian Cryptochrome: Tryptophan versus Tyrosine-Based Radical Pairs. J. Biol. Chem.

Bou-Abdallah, F., Adinolfi, S., Pastore, A., Laue, T.M., Dennis Chasteen, N., 2004. Iron

binding and oxidation kinetics in frataxin CyaY of Escherichia coli. J. Mol. Biol. 341, 605–615.

Bouly, J.-P., Schleicher, E., Dionisio-Sese, M., Vandenbussche, F., Straeten, D.V.D., Bakrim, N., Meier, S., Batschauer, A., Galland, P., Bittl, R., Ahmad, M., 2007. Cryptochrome Blue Light Photoreceptors Are Activated through Interconversion of Flavin Redox States. J. Biol. Chem. 282, 9383–9391.

Branda, S.S., Yang, Z.Y., Chew, A., Isaya, G., 1999. Mitochondrial intermediate peptidase and the yeast frataxin homolog together maintain mitochondrial iron homeostasis in Saccharomyces cerevisiae. Hum. Mol. Genet. 8, 1099–1110.

Brautigam, C.A., Smith, B.S., Ma, Z., Palnitkar, M., Tomchick, D.R., Machius, M., Deisenhofer, J., 2004. Structure of the photolyase-like domain of cryptochrome 1 from Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A. 101, 12142–12147.

Brooks, B.R., Brooks, C.L., 3rd, Mackerell, A.D., Jr, Nilsson, L., Petrella, R.J., Roux, B., Won, Y., Archontis, G., Bartels, C., Boresch, S., Caflisch, A., Caves, L., Cui, Q., Dinner, A.R., Feig, M., Fischer, S., Gao, J., Hodoscek, M., Im, W., Kuczera, K., Lazaridis, T., Ma, J., Ovchinnikov, V., Paci, E., Pastor, R.W., Post, C.B., Pu, J.Z., Schaefer, M., Tidor, B., Venable, R.M., Woodcock, H.L., Wu, X., Yang, W., York, D.M., Karplus, M., 2009. CHARMM: the biomolecular simulation program. J. Comput. Chem. 30, 1545–1614.

Brooks, B.R., Bruccoleri, R.E., Olafson, B.D., States, D.J., Swaminathan, S., Karplus, M., 1983. CHARMM: A program for macromolecular energy, minimization, and dynamics calculations. J. Comput. Chem. 4, 187–217.

Brudler, R., Hitomi, K., Daiyasu, H., Toh, H., Kucho, K., Ishiura, M., Kanehisa, M., Roberts, V.A., Todo, T., Tainer, J.A., Getzoff, E.D., 2003. Identification of a New Cryptochrome Class: Structure, Function, and Evolution. Mol. Cell 11, 59–67.

Bulteau, A.-L., O'Neill, H.A., Kennedy, M.C., Ikeda-Saito, M., Isaya, G., Szweda, L.I., 2004. Frataxin Acts as an Iron Chaperone Protein to Modulate Mitochondrial Aconitase Activity. Science 305, 242–245.

Busi, M.V., Zabaleta, E.J., Araya, A., Gomez-Casati, D.F., 2004. Functional and molecular characterization of the frataxin homolog from Arabidopsis thaliana. FEBS Lett. 576, 141–144.

Busino, L., Bassermann, F., Maiolica, A., Lee, C., Nolan, P.M., Godinho, S.I.H., Draetta, G.F., Pagano, M., 2007. SCFFbxl3 controls the oscillation of the circadian clock by directing the degradation of cryptochrome proteins. Science 316, 900–904.

Busza, A., Emery-Le, M., Rosbash, M., Emery, P., 2004. Roles of the Two Drosophila CRYPTOCHROME Structural Domains in Circadian Photoreception. Science 304, 1503–1506.

Campanella, A., Isaya, G., O'Neill, H.A., Santambrogio, P., Cozzi, A., Arosio, P., Levi, S., 2004. The expression of human mitochondrial ferritin rescues respiratory function in frataxin-deficient yeast. Hum. Mol. Genet. 13, 2279–2288.

Case, D.A., Cheatham, T.E., 3rd, Darden, T., Gohlke, H., Luo, R., Merz, K.M., Jr, Onufriev, A., Simmerling, C., Wang, B., Woods, R.J., 2005. The Amber biomolecular simulation programs. J. Comput. Chem. 26, 1668–1688.

Case, D.A., Cheatham, T.E., Darden, T., Gohlke, H., Luo, R., Merz, K.M., Onufriev, A., Simmerling, C., Wang, B., Woods, R.J., 2005. The Amber biomolecular simulation programs. J. Comput. Chem. 26, 1668–1688.

Cavadini, P., O'Neill, H.A., Benada, O., Isaya, G., 2002. Assembly and iron-binding properties of human frataxin, the protein deficient in Friedreich ataxia. Hum. Mol. Genet. 11, 217–227.

Ceriani, M.F., Darlington, T.K., Staknis, D., Más, P., Petti, A.A., Weitz, C.J., Kay, S.A., 1999. Light-dependent sequestration of TIMELESS by CRYPTOCHROME. Science 285, 553–556.

Chaves, I., Pokorny, R., Byrdin, M., Hoang, N., Ritz, T., Brettel, K., Essen, L.-O., van der Horst, G.T.J., Batschauer, A., Ahmad, M., 2011. The cryptochromes: blue light photoreceptors in plants and animals. Annu. Rev. Plant Biol. 62, 335–364.

Chaves, I., Yagita, K., Barnhoorn, S., Okamura, H., van der Horst, G.T.J., Tamanini, F., 2006. Functional evolution of the photolyase/cryptochrome protein family: importance of the C terminus of mammalian CRY1 for circadian core oscillator performance. Mol. Cell. Biol. 26, 1743–1753.

Chothia, C., Lesk, A.M., 1986. The relation between the divergence of sequence and structure in proteins. EMBO J. 5, 823–826.

Coesel, S., Mangogna, M., Ishikawa, T., Heijde, M., Rogato, A., Finazzi, G., Todo, T.,

Bowler, C., Falciatore, A., 2009. Diatom PtCPF1 is a new cryptochrome/photolyase family member with DNA repair and transcription regulation activity. EMBO Rep. 10, 655–661.

Collins, B.H., Dissel, S., Gaten, E., Rosato, E., Kyriacou, C.P., 2005. Disruption of Cryptochrome partially restores circadian rhythmicity to the arrhythmic period mutant of Drosophila. Proc. Natl. Acad. Sci. U. S. A. 102, 19021–19026.

Collins, B., Mazzoni, E.O., Stanewsky, R., Blau, J., 2006. Drosophila CRYPTOCHROME is a circadian transcriptional repressor. Curr. Biol. CB 16, 441–449.

Cook, J.D., Bencze, K.Z., Jankovic, A.D., Crater, A.K., Busch, C.N., Bradley, P.B., Stemmler, A.J., Spaller, M.R., Stemmler, T.L., 2006. Monomeric Yeast Frataxin is an Iron Binding Protein. Biochemistry (Mosc.) 45, 7767–7777.

Correia, A.R., Wang, T., Craig, E.A., Gomes, C.M., 2010a. Iron binding activity in yeast frataxin entails a trade off with stability in the ?1/?1 acidic ridge region. Biochem. J. 426, 197–203.

Correia, A.R., Wang, T., Craig, E.A., Gomes, C.M., 2010b. Iron binding activity in yeast frataxin entails a trade off with stability in the ?1/?1 acidic ridge region. Biochem. J. 426, 197–203.

Dardente, H., Fortier, E.E., Martineau, V., Cermakian, N., 2007. Cryptochromes impair phosphorylation of transcriptional activators in the clock: a general mechanism for circadian repression. Biochem. J. 402, 525.

Dayhoff, M.O., Schwartz, R.M., 1978. Chapter 22: A model of evolutionary change in proteins, in: In Atlas of Protein Sequence and Structure.

Delatycki, M.B., Williamson, R., Forrest, S.M., 2000. Friedreich ataxia: an overview. J. Med. Genet. 37, 1–8.

Del Pozo, A., Vera, L.M., Sánchez, J.A., Sánchez-Vázquez, F.J., 2012. Molecular cloning, tissue distribution and daily expression of cry1 and cry2 clock genes in European seabass (Dicentrarchus labrax). Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 163, 364–371.

Dissel, S., Codd, V., Fedic, R., Garner, K.J., Costa, R., Kyriacou, C.P., Rosato, E., 2004a. A constitutively active cryptochrome in Drosophila melanogaster. Nat. Neurosci. 7, 834–840.

Dissel, S., Codd, V., Fedic, R., Garner, K.J., Costa, R., Kyriacou, C.P., Rosato, E., 2004b. A constitutively active cryptochrome in Drosophila melanogaster. Nat. Neurosci. 7, 834–840.

Dunlap, J.C., 1999. Molecular Bases for Circadian Clocks. Cell 96, 271–290.

Eckel-Mahan, K., Sassone-Corsi, P., 2013. Metabolism and the Circadian Clock Converge. Physiol. Rev. 93, 107–135.

Emery, P., So, W.V., Kaneko, M., Hall, J.C., Rosbash, M., 1998. CRY, a Drosophila clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. Cell 95, 669–679.

Engelen, E., Janssens, R.C., Yagita, K., Smits, V.A.J., van der Horst, G.T.J., Tamanini, F., 2013. Mammalian TIMELESS Is Involved in Period Determination and DNA Damage-Dependent Phase Advancing of the Circadian Clock. PLoS ONE 8, e56623.

Faraj, S.E., Venturutti, L., Roman, E.A., Marino-Buslje, C.B., Mignone, A., Tosatto, S.C.E., Delfino, J.M., Santos, J., 2013. The role of the N-terminal tail for the oligomerization, folding and stability of human frataxin. FEBS Open Bio 3, 310–320.

Feig, M., Brooks, C.L., 3rd, 2004. Recent advances in the development and application of implicit solvent models in biomolecule simulations. Curr. Opin. Struct. Biol. 14, 217–224.

Ferrara, P., Apostolakis, J., Caflisch, A., 2002. Evaluation of a fast implicit solvent model for molecular dynamics simulations. Proteins 46, 24–33.

Finn, R.D., Mistry, J., Schuster-Böckler, B., Griffiths-Jones, S., Hollich, V., Lassmann, T., Moxon, S., Marshall, M., Khanna, A., Durbin, R., Eddy, S.R., Sonnhammer, E.L.L., Bateman, A., 2006. Pfam: clans, web tools and services. Nucleic Acids Res. 34, D247–D251.

Fogle, K.J., Parson, K.G., Dahm, N.A., Holmes, T.C., 2011. CRYPTOCHROME Is a Blue-Light Sensor That Regulates Neuronal Firing Rate. Science 331, 1409–1413.

Foley, L.E., Gegear, R.J., Reppert, S.M., 2011. Human cryptochrome exhibits light-dependent magnetosensitivity. Nat. Commun. 2, 356.

Foury, F., Pastore, A., Trincal, M., 2007. Acidic residues of yeast frataxin have an

essential role in Fe-S cluster assembly. EMBO Rep. 8, 194–199.

Frenkel, D., Smit, B., 2002. Understanding molecular simulation from algorithms to applications. Academic Press, San Diego.

Fujihashi, M., Numoto, N., Kobayashi, Y., Mizushima, A., Tsujimura, M., Nakamura, A., Kawarabayasi, Y., Miki, K., 2007. Crystal Structure of Archaeal Photolyase from Sulfolobus tokodaii with Two FAD Molecules: Implication of a Novel Light-harvesting Cofactor. J. Mol. Biol. 365, 903–910.

Gakh, O., Adamec, J., Gacy, A.M., Twesten, R.D., Owen, W.G., Isaya, G., 2002. Physical Evidence that Yeast Frataxin Is an Iron Storage Protein†. Biochemistry (Mosc.) 41, 6798–6804.

Gakh, O., Park, S., Liu, G., Macomber, L., Imlay, J.A., Ferreira, G.C., Isaya, G., 2006. Mitochondrial iron detoxification is a primary function of frataxin that limits oxidative damage and preserves cell longevity. Hum. Mol. Genet. 15, 467–479.

Gao, P., Yoo, S.-H., Lee, K.-J., Rosensweig, C., Takahashi, J.S., Chen, B.P., Green, C.B., 2013. Phosphorylation of the Cryptochrome 1 C-terminal tail regulates circadian period length. J. Biol. Chem. jbc.M113.509604.

Gegear, R.J., Casselman, A., Waddell, S., Reppert, S.M., 2008. Cryptochrome mediates light-dependent magnetosensitivity in Drosophila. Nature 454, 1014–1018.

Gentry, L.E., Thacker, M.A., Doughty, R., Timkovich, R., Busenlehner, L.S., 2013. His86 from the N-terminus of frataxin coordinates iron and is required for Fe-S cluster synthesis. Biochemistry (Mosc.) 52, 6085–6096.

Gilson, M.K., Davis, M.E., Luty, B.A., McCammon, J.A., 1993. Computation of electrostatic forces on solvated molecules using the Poisson-Boltzmann equation. J. Phys. Chem. 97, 3591–3600.

Glas, A.F., Maul, M.J., Cryle, M., Barends, T.R.M., Schneider, S., Kaya, E., Schlichting, I., Carell, T., 2009. The archaeal cofactor F0 is a light-harvesting antenna chromophore in eukaryotes. Proc. Natl. Acad. Sci.

Glossop, N.R.J., Hardin, P.E., 2002. Central and peripheral circadian oscillator mechanisms in flies and mammals. J. Cell Sci. 115, 3369–3377.

González-Cabo, P., Vázquez-Manrique, R.P., García-Gimeno, M.A., Sanz, P., Palau, F., 2005. Frataxin interacts functionally with mitochondrial electron transport chain proteins. Hum. Mol. Genet. 14, 2091–2098.

Griffin, E.A., Staknis, D., Weitz, C.J., 1999. Light-Independent Role of CRY1 and CRY2 in the Mammalian Circadian Clock. Science 286, 768–771.

Guex, N., Peitsch, M.C., 1997. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis 18, 2714–2723.

Guo, H., Duong, H., Ma, N., Lin, C., 1999. The Arabidopsis blue light receptor cryptochrome 2 is a nuclear protein regulated by a blue light-dependent post-transcriptional mechanism. Plant J. Cell Mol. Biol. 19, 279–287.

Hall, J.C., 2000. Cryptochromes: sensory reception, transduction, and clock functions subserving circadian systems. Curr. Opin. Neurobiol. 10, 456–466.

Hamm-Alvarez, S., Sancar, A., Rajagopalan, K.V., 1989. Role of enzyme-bound 5,10-methenyltetrahydropteroylpolyglutamate in catalysis by Escherichia coli DNA photolyase. J. Biol. Chem. 264, 9649–9656.

Hao Zheng, Ng, F., Yixiao Liu, Hardin, P.E., 2008. Spatial and circadian regulation of cry in Drosophila. J. Biol. Rhythms 23, 283–295.

Harada, Y., Sakai, M., Kurabayashi, N., Hirota, T., Fukada, Y., 2005. Ser-557-phosphorylated mCRY2 Is Degraded upon Synergistic Phosphorylation by Glycogen Synthase Kinase-3β. J. Biol. Chem. 280, 31714–31721.

Harris, S.-R., Henbest, K.B., Maeda, K., Pannell, J.R., Timmel, C.R., Hore, P.J., Okamoto, H., 2009. Effect of magnetic fields on cryptochrome-dependent responses in Arabidopsis thaliana. J. R. Soc. Interface 6, 1193–1205.

Helfrich-Förster, C., Winter, C., Hofbauer, A., Hall, J.C., Stanewsky, R., 2001. The circadian clock of fruit flies is blind after elimination of all known photoreceptors. Neuron 30, 249–261.

Hemsley, M.J., Mazzotta, G.M., Mason, M., Dissel, S., Toppo, S., Pagano, M.A., Sandrelli, F., Meggio, F., Rosato, E., Costa, R., Tosatto, S.C.E., 2007. Linear motifs in the C-terminus of D. melanogaster cryptochrome. Biochem. Biophys. Res. Commun. 355, 531–537.

Hess, B., Bekker, H., Berendsen, H.J.C., Fraaije, J.G.E.M., 1997. LINCS: A linear constraint solver for molecular simulations. J. Comput. Chem. 18, 1463–1472.

Hess, B., Kutzner, C., van der Spoel, D., Lindahl, E., 2008. GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation. J. Chem. Theory Comput. 4, 435–447.

He, Y., Alam, S.L., Proteasa, S.V., Zhang, Y., Lesuisse, E., Dancis, A., Stemmler, T.L., 2004. Yeast frataxin solution structure, iron binding, and ferrochelatase interaction. Biochemistry (Mosc.) 43, 16254–16262.

Hitomi, K., DiTacchio, L., Arvai, A.S., Yamamoto, J., Kim, S.-T., Todo, T., Tainer, J.A., Iwai, S., Panda, S., Getzoff, E.D., 2009. Functional motifs in the (6-4) photolyase crystal structure make a comparative framework for DNA repair photolyases and clock cryptochromes. Proc. Natl. Acad. Sci. 106, 6962–6967.

Hitomi, K., Okamoto, K., Daiyasu, H., Miyashita, H., Iwai, S., Toh, H., Ishiura, M., Todo, T., 2000. Bacterial cryptochrome and photolyase: characterization of two photolyase-like genes of Synechocystis sp. PCC6803. Nucleic Acids Res. 28, 2353–2362.

Hoang, N., Schleicher, E., Kacprzak, S., Bouly, J.-P., Picot, M., Wu, W., Berndt, A., Wolf, E., Bittl, R., Ahmad, M., 2008a. Human and Drosophila Cryptochromes Are Light Activated by Flavin Photoreduction in Living Cells. PLoS Biol 6, e160.

Hoang, N., Schleicher, E., Kacprzak, S., Bouly, J.-P., Picot, M., Wu, W., Berndt, A., Wolf, E., Bittl, R., Ahmad, M., 2008b. Human and Drosophila Cryptochromes Are Light Activated by Flavin Photoreduction in Living Cells. PLoS Biol 6, e160.

Höltje, H.-D., 2008. Molecular modeling: basic principles and applications. Wiley-VCH, Weinheim.

Huang, J., Dizin, E., Cowan, J.A., 2008. Mapping iron binding sites on human frataxin: implications for cluster assembly on the ISU Fe-S cluster scaffold protein. J. Biol. Inorg. Chem. JBIC Publ. Soc. Biol. Inorg. Chem. 13, 825–836.

Huang, M.L.-H., Becker, E.M., Whitnall, M., Suryo Rahmanto, Y., Ponka, P., Richardson, D.R., 2009. Elucidation of the mechanism of mitochondrial iron loading in Friedreich's ataxia by analysis of a mouse mutant. Proc. Natl. Acad. Sci. U. S. A. 106, 16381–16386.

Huynen, M.A., Snel, B., Bork, P., Gibson, T.J., 2001. The phylogenetic distribution of frataxin indicates a role in iron-sulfur cluster protein assembly. Hum. Mol. Genet. 10, 2463–2468.

III, C.L.B., Karplus, rtin, Pettitt, B.M. (Eds.), 2007. Dynamical Simulation Methods,

in: Advances in Chemical Physics. John Wiley & Sons, Inc., pp. 33–58.

Ito, C., Goto, S.G., Shiga, S., Tomioka, K., Numata, H., 2008. Peripheral circadian clock for the cuticle deposition rhythm in Drosophila melanogaster. Proc. Natl. Acad. Sci. 105, 8446–8451.

Ivanchenko, M., Stanewsky, R., Giebultowicz, J.M., 2001. Circadian photoreception in Drosophila: functions of cryptochrome in peripheral and central clocks. J. Biol. Rhythms 16, 205–215.

Jorgensen, W.L., Chandrasekhar, J., Madura, J.D., Impey, R.W., Klein, M.L., 1983. Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. 79, 926–935.

Kang, C.-Y., Lian, H.-L., Wang, F.-F., Huang, J.-R., Yang, H.-Q., 2009. Cryptochromes, Phytochromes, and COP1 Regulate Light-Controlled Stomatal Development in Arabidopsis. Plant Cell Online 21, 2624–2641.

Kao, Y.-T., Tan, C., Song, S.-H., Öztürk, N., Li, J., Wang, L., Sancar, A., Zhong, D., 2008. Ultrafast Dynamics and Anionic Active States of the Flavin Cofactor in Cryptochrome and Photolyase. J. Am. Chem. Soc. 130, 7695–7701.

Kaushik, R., Nawathean, P., Busza, A., Murad, A., Emery, P., Rosbash, M., 2007. PER-TIM Interactions with the Photoreceptor Cryptochrome Mediate Circadian Temperature Responses in Drosophila. PLoS Biol 5, e146.

Khan, S.K., Xu, H., Ukai-Tadenuma, M., Burton, B., Wang, Y., Ueda, H.R., Liu, A.C., 2012. Identification of a Novel Cryptochrome Differentiating Domain Required for Feedback Repression in Circadian Clock Function. J. Biol. Chem. 287, 25917–25926.

Kim, S.T., Heelis, P.F., Sancar, A., 1992. Energy transfer (deazaflavin .fwdarw. FADH2) and electron transfer (FADH2 .fwdarw. T.ltbbrac..rtbbrac.T) kinetics in Anacystis nidulans photolyase. Biochemistry (Mosc.) 31, 11244–11248.

Klar, T., Kaiser, G., Hennecke, U., Carell, T., Batschauer, A., Essen, L.-O., 2006. Natural and Non-natural Antenna Chromophores in the DNA Photolyase from Thermus Thermophilus. ChemBioChem 7, 1798–1806.

Klar, T., Pokorny, R., Moldt, J., Batschauer, A., Essen, L.-O., 2007. Cryptochrome 3 from Arabidopsis thaliana: Structural and Functional Analysis of its Complex with a Folate Light Antenna. J. Mol. Biol. 366, 954–964.

Kleiner, O., Butenandt, J., Carell, T., Batschauer, A., 1999. Class II DNA photolyase from Arabidopsis thaliana contains FAD as a cofactor. Eur. J. Biochem. 264, 161–167.

Kleine, T., Lockhart, P., Batschauer, A., 2003. An Arabidopsis protein closely related to Synechocystis cryptochrome is targeted to organelles. Plant J. 35, 93–103.

Kondapalli, K.C., Kok, N.M., Dancis, A., Stemmler, T.L., 2008. Drosophila Frataxin: An Iron Chaperone during Cellular Fe-S Cluster Bioassembly. Biochemistry (Mosc.) 47, 6917–6927.

Kondratov, R.V., Kondratova, A.A., Lee, C., Gorbacheva, V.Y., Chernov, M.V., Antoch, M.P., 2006. Post-translational regulation of circadian transcriptional CLOCK(NPAS2)/BMAL1 complex by CRYPTOCHROMES. Cell Cycle Georget. Tex 5, 890–895.

Korb, O., Stützle, T., Exner, T.E., 2006. PLANTS: Application of Ant Colony Optimization to Structure-Based Drug Design, in: Dorigo, M., Gambardella, L.M., Birattari, M., Martinoli, A., Poli, R., Stützle, T. (Eds.), Ant Colony Optimization and Swarm Intelligence, Lecture Notes in Computer Science. Springer Berlin Heidelberg, pp. 247–258.

Krishnan, B., Levine, J.D., Lynch, M.K., Dowse, H.B., Funes, P., Hall, J.C., Hardin, P.E., Dryer, S.E., 2001. A new role for cryptochrome in a Drosophila circadian oscillator. Nature 411, 313–317.

Kume, K., Zylka, M.J., Sriram, S., Shearman, L.P., Weaver, D.R., Jin, X., Maywood, E.S., Hastings, M.H., Reppert, S.M., 1999. mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell 98, 193–205.

Lamia, K.A., Sachdeva, U.M., DiTacchio, L., Williams, E.C., Alvarez, J.G., Egan, D.F., Vasquez, D.S., Juguilon, H., Panda, S., Shaw, R.J., Thompson, C.B., Evans, R.M., 2009. AMPK Regulates the Circadian Clock by Cryptochrome Phosphorylation and Degradation. Science 326, 437–440.

Lane, D.J.R., Richardson, D.R., 2010. Frataxin, a molecule of mystery: trading stability for function in its iron-binding site. Biochem. J. 426, e1–e3.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson,

J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.

Lesuisse, E., Santos, R., Matzanke, B.F., Knight, S.A.B., Camadro, J.-M., Dancis, A., 2003. Iron use for haeme synthesis is under control of the yeast frataxin homologue (Yfh1). Hum. Mol. Genet. 12, 879–889.

Lin, C., Robertson, D.E., Ahmad, M., Raibekas, A.A., Jorns, M.S., Dutton, P.L., Cashmore, A.R., 1995. Association of flavin adenine dinucleotide with the Arabidopsis blue light receptor CRY1. Science 269, 968–970.

Liu, H., Liu, B., Zhao, C., Pepper, M., Lin, C., 2011. The action mechanisms of plant cryptochromes. Trends Plant Sci. 16, 684–691.

Liu, H.-Y., Kuntz, I.D., Zou, X., 2004. Pairwise GB/SA Scoring Function for Structure-based Drug Design. J. Phys. Chem. B 108, 5453–5462.

Liu, Z., Tan, C., Guo, X., Li, J., Wang, L., Sancar, A., Zhong, D., 2013a. Determining complete electron flow in the cofactor photoreduction of oxidized photolyase. Proc. Natl. Acad. Sci. U. S. A. 110, 12966–12971.

Liu, Z., Zhang, M., Guo, X., Tan, C., Li, J., Wang, L., Sancar, A., Zhong, D., 2013b. Dynamic determination of the functional state in photolyase and the implication for cryptochrome. Proc. Natl. Acad. Sci. U. S. A. 110, 12972–12977.

Li, X., Wang, Q., Yu, X., Liu, H., Yang, H., Zhao, C., Liu, X., Tan, C., Klejnot, J., Zhong, D., Lin, C., 2011. Arabidopsis cryptochrome 2 (CRY2) functions by the photoactivation mechanism distinct from the tryptophan (trp) triad-dependent photoreduction. Proc. Natl. Acad. Sci. U. S. A. 108, 20844–20849.

MacKerell, A. D., Bashford, D., Bellott, Dunbrack, R. L., Evanseck, J.D., Field, M.J., Fischer, S., Gao, J., Guo, H., Ha, S., Joseph-McCarthy, D., Kuchnir, L., Kuczera, K., Lau, F.T.K., Mattos, C., Michnick, S., Ngo, T., Nguyen, D.T., Prodhom, B., Reiher, W.E., Roux, B., Schlenkrich, M., Smith, J.C., Stote, R., Straub, J., Watanabe, M., Wiórkiewicz-Kuczera, J., Yin, D., Karplus, M., 1998. All-Atom Empirical Potential for Molecular Modeling and Dynamics Studies of Proteins†. J. Phys. Chem. B 102, 3586–3616.

Majeux, N., Scarsi, M., Apostolakis, J., Ehrhardt, C., Caflisch, A., 1999. Exhaustive docking of molecular fragments with electrostatic solvation. Proteins 37, 88–105.

Malhotra, K., Kim, S.-T., Batschauer, A., Dawut, L., Sancar, A., 1995. Putative Blue-Light Photoreceptors from Arabidopsis thaliana and Sinapis alba with a High Degree of Sequence Homology to DNA Photolyase Contain the Two Photolyase Cofactors but Lack DNA Repair Activity. Biochemistry (Mosc.) 34, 6892–6899.

Mao, J., Zhang, Y.-C., Sang, Y., Li, Q.-H., Yang, H.-Q., 2005. A role for Arabidopsis cryptochromes and COP1 in the regulation of stomatal opening. Proc. Natl. Acad. Sci. U. S. A. 102, 12270–12275.

Matsumoto, N., 2003. Functional Analysis and Intracellular Localization of Rice Cryptochromes. PLANT Physiol. 133, 1494–1503.

Meissner, R.-A., Kilman, V.L., Lin, J.-M., Allada, R., 2008. TIMELESS Is an Important Mediator of CK2 Effects on Circadian Clock Function In Vivo. J. Neurosci. 28, 9732–9740.

Miyazaki, K., Mesaki, M., Ishida, N., 2001. Nuclear Entry Mechanism of Rat PER2 (rPER2): Role of rPER2 in Nuclear Localization of CRY Protein. Mol. Cell. Biol. 21, 6651–6659.

Müller, M., Carell, T., 2009. Structural biology of DNA photolyases and cryptochromes. Curr. Opin. Struct. Biol. 19, 277–285.

Müller, P., Ahmad, M., 2011. Light-activated Cryptochrome Reacts with Molecular Oxygen to Form a Flavin–Superoxide Radical Pair Consistent with Magnetoreception. J. Biol. Chem. 286, 21033–21040.

Musco, G., Stier, G., Kolmerer, B., Adinolfi, S., Martin, S., Frenkiel, T., Gibson, T., Pastore, A., 2000. Towards a structural understanding of Friedreich's ataxia: the solution structure of frataxin. Struct. Lond. Engl. 1993 8, 695–707.

Naidoo, N., Song, W., Hunter-Ensor, M., Sehgal, A., 1999. A role for the proteasome in the light response of the timeless clock protein. Science 285, 1737–1741.

Nair, M., Adinolfi, S., Pastore, C., Kelly, G., Temussi, P., Pastore, A., 2004. Solution Structure of the Bacterial Frataxin Ortholog, CyaY: Mapping the Iron Binding Sites. Structure 12, 2037–2048.

Nießner, C., Denzau, S., Stapput, K., Ahmad, M., Peichl, L., Wiltschko, W., Wiltschko, R., 2013. Magnetoreception: activated cryptochrome 1a concurs with magnetic orientation in birds. J. R. Soc. Interface 10, 20130638.

O'Neill, H.A., Gakh, O., Park, S., Cui, J., Mooney, S.M., Sampson, M., Ferreira, G.C.,

Isaya, G., 2005. Assembly of Human Frataxin Is a Mechanism for Detoxifying Redox-Active Iron†. Biochemistry (Mosc.) 44, 537–545.

Özgür, S., Sancar, A., 2006. Analysis of Autophosphorylating Kinase Activities of Arabidopsis and Human Cryptochromes†. Biochemistry (Mosc.) 45, 13369–13374.

Ozturk, N., Selby, C.P., Song, S.-H., Ye, R., Tan, C., Kao, Y.-T., Zhong, D., Sancar, A., 2009. Comparative Photochemistry of Animal Type 1 and Type 4 Cryptochromes. Biochemistry (Mosc.) 48, 8585–8593.

Öztürk, N., Song, S.-H., Özgür, S., Selby, C.P., Morrison, L., Partch, C., Zhong, D., Sancar, A., 2007. Structure and Function of Animal Cryptochromes. Cold Spring Harb. Symp. Quant. Biol. 72, 119–131.

Öztürk, N., Song, S.-H., Selby, C.P., Sancar, A., 2008. Animal Type 1 Cryptochromes ANALYSIS OF THE REDOX STATE OF THE FLAVIN COFACTOR BY SITE-DIRECTED MUTAGENESIS. J. Biol. Chem. 283, 3256–3263.

Ozturk, N., VanVickle-Chavez, S.J., Akileswaran, L., Gelder, R.N.V., Sancar, A., 2013. Ramshackle (Brwd3) promotes light-induced ubiquitylation of Drosophila Cryptochrome by DDB1-CUL4-ROC1 E3 ligase complex. Proc. Natl. Acad. Sci. 110, 4980–4985.

Panchenko, A.R., Kondrashov, F., Bryant, S., 2004. Prediction of functional sites by analysis of sequence and structure conservation. Protein Sci. 13, 884–892.

Pandolfo, M., Pastore, A., 2009. The pathogenesis of Friedreich ataxia and the structure and function of frataxin. J. Neurol. 256 Suppl 1, 9–17.

Park, H.W., Kim, S.T., Sancar, A., Deisenhofer, J., 1995. Crystal structure of DNA photolyase from Escherichia coli. Science 268, 1866–1872.

Pastore, C., Franzese, M., Sica, F., Temussi, P., Pastore, A., 2007. Understanding the binding properties of an unusual metal-binding protein--a study of bacterial frataxin. FEBS J. 274, 4199–4210.

Payne, G., Sancar, A., 1990. Absolute action spectrum of E-FADH2 and E-FADH2-MTHF forms of Escherichia coli DNA photolyase. Biochemistry (Mosc.) 29, 7715–7727.

Peschel, N., Veleri, S., Stanewsky, R., 2006. Veela defines a molecular link between Cryptochrome and Timeless in the light-input pathway to Drosophila's circadian

clock. Proc. Natl. Acad. Sci. 103, 17313–17318.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., Ferrin, T.E., 2004. UCSF Chimera--a visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1612.

Phillips, J.C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., Chipot, C., Skeel, R.D., Kalé, L., Schulten, K., 2005. Scalable molecular dynamics with NAMD. J. Comput. Chem. 26, 1781–1802.

Pils, B., Copley, R.R., Schultz, J., 2005. Variation in structural location and amino acid conservation of functional sites in protein domain families. BMC Bioinformatics 6, 210.

Pokorny, R., Klar, T., Hennecke, U., Carell, T., Batschauer, A., Essen, L.-O., 2008. Recognition and repair of UV lesions in loop structures of duplex DNA by DASH-type cryptochrome. Proc. Natl. Acad. Sci. 105, 21023–21027.

Prischi, F., Giannini, C., Adinolfi, S., Pastore, A., 2009. The N-terminus of mature human frataxin is intrinsically unfolded. FEBS J. 276, 6669–6676.

Prischi, F., Konarev, P.V., Iannuzzi, C., Pastore, C., Adinolfi, S., Martin, S.R., Svergun, D.I., Pastore, A., 2010. Structural bases for the interaction of frataxin with the central components of iron-sulphur cluster assembly. Nat. Commun. 1, 95.

Rosato, E., Codd, V., Mazzotta, G., Piccin, A., Zordan, M., Costa, R., Kyriacou, C.P., 2001. Light-dependent interaction between Drosophila CRY and the clock protein PER mediated by the carboxy terminus of CRY. Curr. Biol. 11, 909–917.

Rush, B.L., Murad, A., Emery, P., Giebultowicz, J.M., 2006. Ectopic CRYPTOCHROME Renders TIM Light Sensitive in the Drosophila Ovary. J. Biol. Rhythms 21, 272–278.

Ryckaert, J.-P., Ciccotti, G., Berendsen, H.J.C., 1977. Numerical integration of the cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. J. Comput. Phys. 23, 327–341.

Saez, L., Derasmo, M., Meyer, P., Stieglitz, J., Young, M.W., 2011. A key temporal delay in the circadian cycle of Drosophila is mediated by a nuclear localization signal in the timeless protein. Genetics 188, 591–600.

Sakakida, Y., Miyamoto, Y., Nagoshi, E., Akashi, M., Nakamura, T.J., Mamine, T., Kasahara, M., Minami, Y., Yoneda, Y., Takumi, T., 2005. Importin alpha/beta

mediates nuclear transport of a mammalian circadian clock component, mCRY2, together with mPER2, through a bipartite nuclear localization signal. J. Biol. Chem. 280, 13272–13278.

Sancar, A., 2003. Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. Chem. Rev. 103, 2203–2237.

Schmucker, S., Martelli, A., Colin, F., Page, A., Wattenhofer-Donzé, M., Reutenauer, L., Puccio, H., 2011. Mammalian Frataxin: An Essential Function for Cellular Viability through an Interaction with a Preformed ISCU/NFS1/ISD11 Iron-Sulfur Assembly Complex. PLoS ONE 6, e16199.

Sehadova, H., Glaser, F.T., Gentile, C., Simoni, A., Giesecke, A., Albert, J.T., Stanewsky, R., 2009. Temperature entrainment of Drosophila's circadian clock involves the gene nocte and signaling from peripheral sensory tissues to the brain. Neuron 64, 251–266.

Selby, C.P., Sancar, A., 2012. The second chromophore in Drosophila photolyase/cryptochrome family photoreceptors. Biochemistry (Mosc.) 51, 167–171.

Shalitin, D., Yang, H., Mockler, T.C., Maymon, M., Guo, H., Whitelam, G.C., Lin, C., 2002. Regulation of Arabidopsis cryptochrome 2 by blue-light-dependent phosphorylation. Nature 417, 763–767.

Shan, Y., Cortopassi, G., 2012. HSC20 interacts with frataxin and is involved in iron-sulfur cluster biogenesis and iron homeostasis. Hum. Mol. Genet. 21, 1457–1469.

Shan, Y., Napoli, E., Cortopassi, G., 2007. Mitochondrial frataxin interacts with ISD11 of the NFS1/ISCU complex and multiple mitochondrial chaperones. Hum. Mol. Genet. 16, 929–941.

Söderberg, C.A.G., Shkumatov, A.V., Rajan, S., Gakh, O., Svergun, D.I., Isaya, G., Al-Karadaghi, S., 2011. Oligomerization Propensity and Flexibility of Yeast Frataxin Studied by X-ray Crystallography and Small-Angle X-ray Scattering. J. Mol. Biol. 414, 783–797.

Song, S.-H., 2006. Photodynamics of phototropin and cryptochrome blue-light photoreceptors. Universität Regensburg.

Song, S.-H., Dick, B., Penzkofer, A., Pokorny, R., Batschauer, A., Essen, L.-O., 2006.

Absorption and fluorescence spectroscopic characterization of cryptochrome 3 from Arabidopsis thaliana. J. Photochem. Photobiol. B 85, 1–16.

Stanewsky, R., Kaneko, M., Emery, P., Beretta, B., Wager-Smith, K., Kay, S.A., Rosbash, M., Hall, J.C., 1998. The cryb mutation identifies cryptochrome as a circadian photoreceptor in Drosophila. Cell 95, 681–692.

Stemmler, T.L., Lesuisse, E., Pain, D., Dancis, A., 2010. Frataxin and Mitochondrial FeS Cluster Biogenesis. J. Biol. Chem. 285, 26737–26743.

Stillinger, F.H., Rahman, A., 2003. Improved simulation of liquid water by molecular dynamics. J. Chem. Phys. 60, 1545–1557.

Still, W.C., Tempczyk, A., Hawley, R.C., Hendrickson, T., 1990. Semianalytical treatment of solvation for molecular mechanics and dynamics. J. Am. Chem. Soc. 112, 6127–6129.

Stoleru, D., Nawathean, P., Fernández, M. de la P., Menet, J.S., Ceriani, M.F., Rosbash, M., 2007. The Drosophila Circadian Network Is a Seasonal Timer. Cell 129, 207–219.

Suzek, B.E., Huang, H., McGarvey, P., Mazumder, R., Wu, C.H., 2007. UniRef: comprehensive and non-redundant UniProt reference clusters. Bioinformatics 23, 1282–1288.

Tamada, T., Kitadokoro, K., Higuchi, Y., Inaka, K., Yasui, A., de Ruiter, P.E., Eker, A.P.M., Miki, K., 1997. Crystal structure of DMA photolyase from Anacystis nidulans. Nat. Struct. Mol. Biol. 4, 887–891.

Tamanini, F., Chaves, I., Bajek, M.I., van der Horst, G.T.J., 2007. Structure function analysis of mammalian cryptochromes. Cold Spring Harb. Symp. Quant. Biol. 72, 133–139.

Taylor, R.D., Jewsbury, P.J., Essex, J.W., 2003. FDS: flexible ligand and receptor docking with a continuum solvent model and soft-core energy function. J. Comput. Chem. 24, 1637–1656.

Thompson, C.L., Bowes Rickman, C., Shaw, S.J., Ebright, J.N., Kelly, U., Sancar, A., Rickman, D.W., 2003. Expression of the blue-light receptor cryptochrome in the human retina. Invest. Ophthalmol. Vis. Sci. 44, 4515–4521.

VanVickle-Chavez, S.J., Gelder, R.N.V., 2007. Action Spectrum of Drosophila Cryptochrome. J. Biol. Chem. 282, 10561–10566.

Verlet, L., 1967. Computer “Experiments” on Classical Fluids. I. Thermodynamical Properties of Lennard-Jones Molecules. Phys. Rev. 159, 98–103.

Wang, H., Ma, L.-G., Li, J.-M., Zhao, H.-Y., Deng, X.W., 2001. Direct Interaction of Arabidopsis Cryptochromes with COP1 in Light Control Development. Science 294, 154–158.

Wang, J., Wang, W., Kollman, P.A., Case, D.A., 2006. Automatic atom type and bond type perception in molecular mechanical calculations. J. Mol. Graph. Model. 25, 247–260.

Wang, T., Craig, E.A., 2008. Binding of Yeast Frataxin to the Scaffold for Fe-S Cluster Biogenesis, Isu. J. Biol. Chem. 283, 12674–12679.

Waterhouse, A.M., Procter, J.B., Martin, D.M.A., Clamp, M., Barton, G.J., 2009. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics 25, 1189–1191.

Wu, G., Spalding, E.P., 2007. Separate functions for nuclear and cytoplasmic cryptochrome 1 during photomorphogenesis of Arabidopsis seedlings. Proc. Natl. Acad. Sci. 104, 18813–18818.

Xu, P., Xiang, Y., Zhu, H., Xu, H., Zhang, Z., Zhang, C., Zhang, L., Ma, Z., 2008. Wheat Cryptochromes: Subcellular Localization and Involvement in Photomorphogenesis and Osmotic Stress Responses. PLANT Physiol. 149, 760–774.

Yang, H.Q., Tang, R.H., Cashmore, A.R., 2001. The signaling mechanism of Arabidopsis CRY1 involves direct interaction with COP1. Plant Cell 13, 2573–2587.

Yang, H.-Q., Wu, Y.-J., Tang, R.-H., Liu, D., Liu, Y., Cashmore, A.R., 2000. The C termini of Arabidopsis cryptochromes mediate a constitutive light response. Cell 103, 815–827.

Yang, Y.-J., Zuo, Z.-C., Zhao, X.-Y., Li, X., Klejnot, J., Li, Y., Chen, P., Liang, S.-P., Yu, X.-H., Liu, X.-M., Lin, C.-T., 2008. Blue-Light-Independent Activity of Arabidopsis Cryptochromes in the Regulation of Steady-State Levels of Protein and mRNA Expression. Mol. Plant 1, 167–177.

Yoon, T., Cowan, J.A., 2003. Iron-sulfur cluster biosynthesis. Characterization of frataxin as an iron donor for assembly of [2Fe-2S] clusters in ISU-type proteins.

J. Am. Chem. Soc. 125, 6078–6084.

Yoon, T., Dizin, E., Cowan, J.A., 2007. N-terminal iron-mediated self-cleavage of human frataxin: regulation of iron binding and complex formation with target proteins. J. Biol. Inorg. Chem. JBIC Publ. Soc. Biol. Inorg. Chem. 12, 535–542.

Yoshii, T., Hermann, C., Helfrich-Förster, C., 2010. Cryptochrome-positive and -negative clock neurons in Drosophila entrain differentially to light and temperature. J. Biol. Rhythms 25, 387–398.

Yoshii, T., Todo, T., Wülbeck, C., Stanewsky, R., Helfrich-Förster, C., 2008. Cryptochrome is present in the compound eyes and a subset of Drosophila's clock neurons. J. Comp. Neurol. 508, 952–966.

Yu, X., Klejnot, J., Zhao, X., Shalitin, D., Maymon, M., Yang, H., Lee, J., Liu, X., Lopez, J., Lin, C., 2007. Arabidopsis Cryptochrome 2 Completes Its Posttranslational Life Cycle in the Nucleus. Plant Cell Online 19, 3146–3156.

Zhang, F., Scheerer, P., Oberpichler, I., Lamparter, T., Krauß, N., 2013. Crystal structure of a prokaryotic (6-4) photolyase with an Fe-S cluster and a 6,7-dimethyl-8-ribityllumazine antenna chromophore. Proc. Natl. Acad. Sci. 110, 7217–7222.

Zhu, H., Conte, F., Green, C.B., 2003. Nuclear Localization and Transcriptional Repression Are Confined to Separable Domains in the Circadian Protein CRYPTOCHROME. Curr. Biol. 13, 1653–1658.

Zuo, Z.-C., Meng, Y.-Y., Yu, X.-H., Zhang, Z.-L., Feng, D.-S., Sun, S.-F., Liu, B., Lin, C.-T., 2012. A study of the blue-light-dependent phosphorylation, degradation, and photobody formation of Arabidopsis CRY2. Mol. Plant 5, 726–733.

Zuo, Z., Liu, H., Liu, B., Liu, X., Lin, C., 2011. Blue Light-Dependent Interaction of CRY2 with SPA1 Regulates COP1 activity and Floral Initiation in Arabidopsis. Curr. Biol. 21, 841–847.

4. *Drosophila* Cryptochrome Second Chromophore

4.1 Background

Cryptochrome/Photolyase family proteins are UV/blue light activated thought to have evolved from DNA photolyase family. While some organisms such *Arabidopsis thaliana* express all members of photolyase/cryptochromes family, some such humans show only cryptochromes subclass members. The proteins of this family share in common FAD as a cofactor either for DNA repairing or photoreception in the circadian clock. Aditionally, proteins of this family can make use of a second chromophore, working as an antenna cofactor. However, the presence and the type of antenna chromophore diverge among this wide family. While some photolyases use 5,10-methenyltetrahydrofolate (MTHF) and others show 7,8-didemethyl-8-hydroxy-5-deazariboflavin (8-HDF), FMN and FAD as second chromophore, there are some of them showing no second chromophore (Table 2). Moreover, to date, CRY-DASH of *Arabidopsis thaliana* is the only Cryptochrome subclass crystal structure showing an antenna cofactor. We have carried out a computational analysis considering structural aspects of this family regarding the presence of a second chromophore and related them to *Drosophila* Cryptochrome (dCRY). Our results contribute to a mechanistic understanding of the complex functional regulation underlying dCRY light activation.

TABLE 2
Presence of Second Chromophores on the CRY/Photolyase Family Members So Far Identified

CRY/Photolyase family member	Organism	Second Chromophore	Method used
CRY1	*Drosophila melanogaster*	MTHF	Biophysical method
CRY2		none	
CRY-DASH (CRY3)	*Arabidopsis thaliana*	MTHF	X-ray diffraction
	Ostreococcus tauri	MTHF	Biophysical method
Photolyase	*E. coli*	MTHF	X-ray diffraction
	Thermus thermophilus	HDF	X-ray diffraction
	Sulfolobus tokodaii	FAD	X-ray diffraction
	Anacystis nidulans	HDF	X-ray diffraction

4.2 Methodology

In order to verify dCRY flexibility in the binding site, NAMD (Phillips et al., 2005) has been used for molecular dynamics simulation of dCRY, taking the crystal structure (PDB code 3TVS) as starting structure. The structure after 10 ns of Molecular Dynamics (MD) has been taken for docking predictions. To a wider analysis, docking has been performed taking mouse CRY2 (PDB code 4I6E) in addition to *Drosophila* Cryptochrome full lenght structure. Docking predictions have been carried out using PLANTS (Korb et al., 2006) programme and water molecules present on the binding site were considered. UCSF Chimera (Pettersen et al., 2004) was used for protein and cofactor preparation and energy minimization of the predicted docking conformations. AMBER (Case et al., 2005)force field was considered for protein charging while Gasteiger (Wang et al., 2006) has been used for cofactor charging. The cofactor binding site of photolyases was set the binding center for the docking predictions. The default parameter chemplp has been set as interaction scoring function and the docking prediction with the highest score was considered for the evaluation. Since H13 and S46 on dCRY structure (after 10 ns of MD) are oriented towards the likely binding site, their

side-chains were set flexible for the docking predictions. In order to verify cofactor binding residue conservation, sequences have been taken through a PSI-BLAST (Altschul et al., 1997) sequence identity search against SwissProt database (Suzek et al., 2007), considering *Drosophila* cryptochrome photolyase homology region (PHR) as query sequence. Jalview (Waterhouse et al., 2009) was used for multiple sequence alignment through its web service interface using Clustal default parameters.

4.3 dCRY-8-HDF Docking Prediction Results

dCRY crystal structure shows no space to accommodate a cofactor where photolyases show 8-HDF second chromophore (Figure 14). MD has shown dCRY triggers a conformational change in the loop where residues S46 and A47 are located, giving enough space to accommodate a cofactor like 8-HDF (Figure 14) although H13 points toward the binding site. This structure was used for docking predictions of dCRY and 8-HDF.

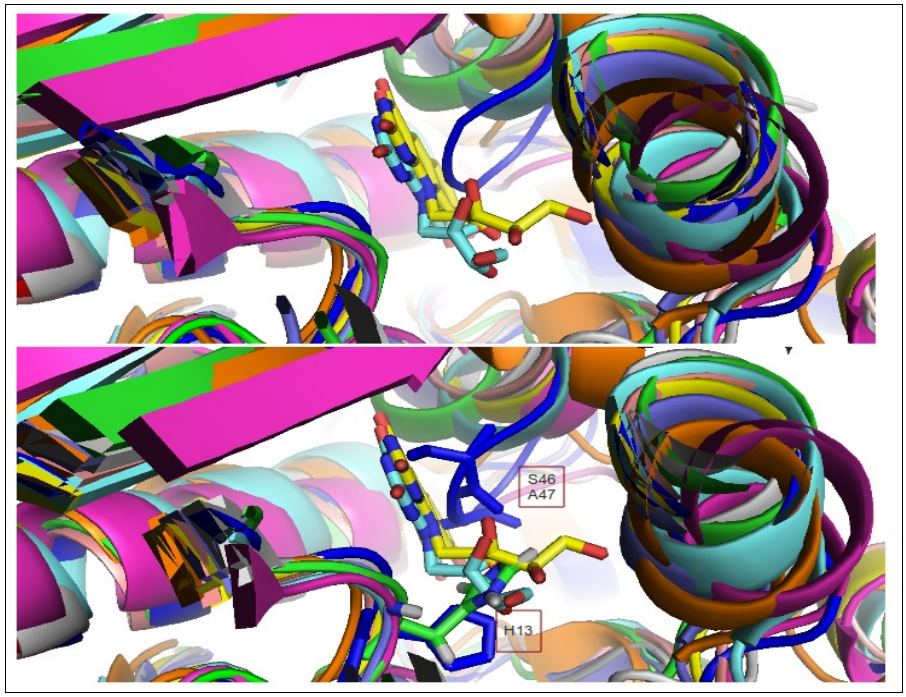

Figure 14. Superposition of dCRY (after 10ns Molecular Dynamics simulation, in green), dCRY crystal structure (PDB code 3TVS, in blue), *Anacystis* photolyase (PDB code 1TEZ, in yellow), *Thermus* photolyase (in cyan) with others crystal structures of this study showing the antenna binding site. The residues on dCRY overlaping 8-HDF antenna cofactor are identified.

Docking prediction for dCRY-8-HDF binding shows 8-HDF cofactor oriented perpendicularly to the orientation of 8-HDF on Photolyases (Figure 15). dCRY shows a tighter binding accommodation size than Photolyases with H13 pointing towards the binding site and impairing a similar orientation. On the same position *Thermus* Photolyase shows G10, while *Anacystis* photolyase shows R11, both oriented away from the ligand-binding site.

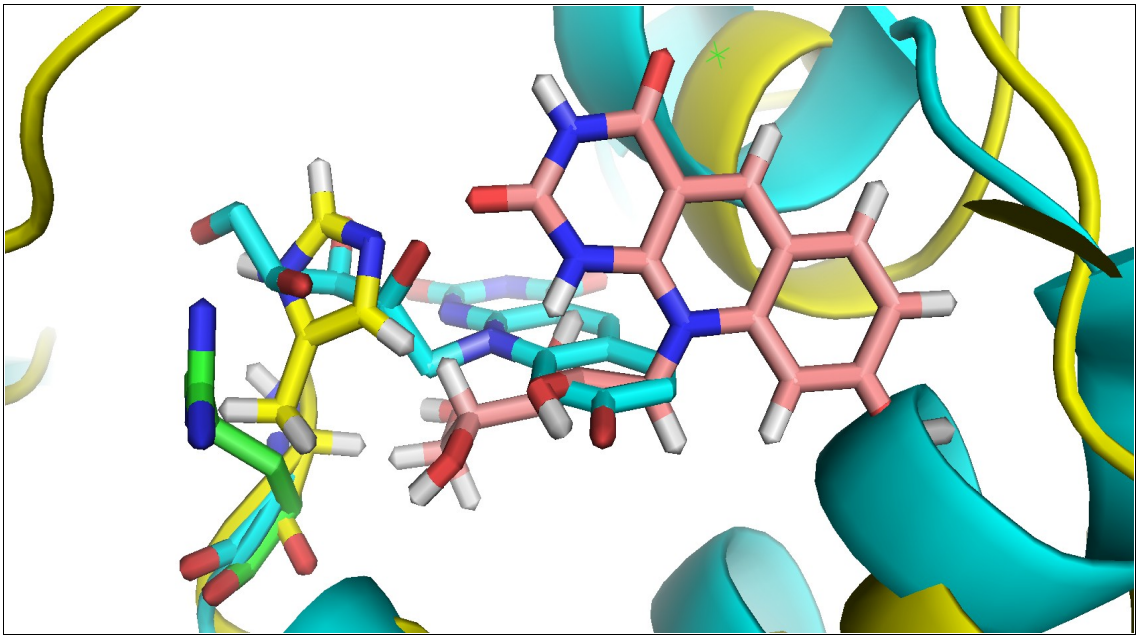

Figure 15. Superposition of dCRY (in yellow) and the predicted docking to 8-HDF(in salmon) with *Thermus* photolyase (in cyan). H13 on dCRY overlaping 8-HDF of photolyase is also shown (in yellow), with *Thermus* photolyase G10 (in cyan) and *Anacystis* Photolyase R11 (in green).

It is thought that the light-harvesting cofactor transfers the excitation energy to the catalytic cofactor. It is widely accepted that a tryptophan triad plays energy transfer (ET) between cofactors, although a diversity of pathways and different conservation have been reported (Li et al., 2011) (Biskup et al., 2013). In addition to a diversity of ET pathways among CRY/Photolyase family, orientation and solvent accessibility of involved residues, rather than distance to the core of the pathway has also been reported to be critical for its function (Biskup et al., 2011). dCRY shows residues W314, W413,W420 and W422 properly located to play energy transfer between 8-HDF and FAD cofactors (Figure 16). Additionaly, these residues are highly conserved among insect and vertebrates CRYs (Figure 17). Energy transfer efficiency between cofactors is

reported to be slightly higher on photolyase with 8-HDF chromophore (Kim et al., 1992) than CRY-DASH having surface exposed MTHF as antenna cofactor (Song et al., 2006). The overlaping between the absorption spectra of the two cofactors is believed to play energy transfer function between them (Park et al., 1995). While MTHF shows peak absorption at 380 nm, 8-HDF reportedly shows absorption at 445 nm. Oxidized FAD has shown absorption at 470 nm while absorption around 600 nm has been atributed to neutral semiquinone form of FAD (Song, 2006).

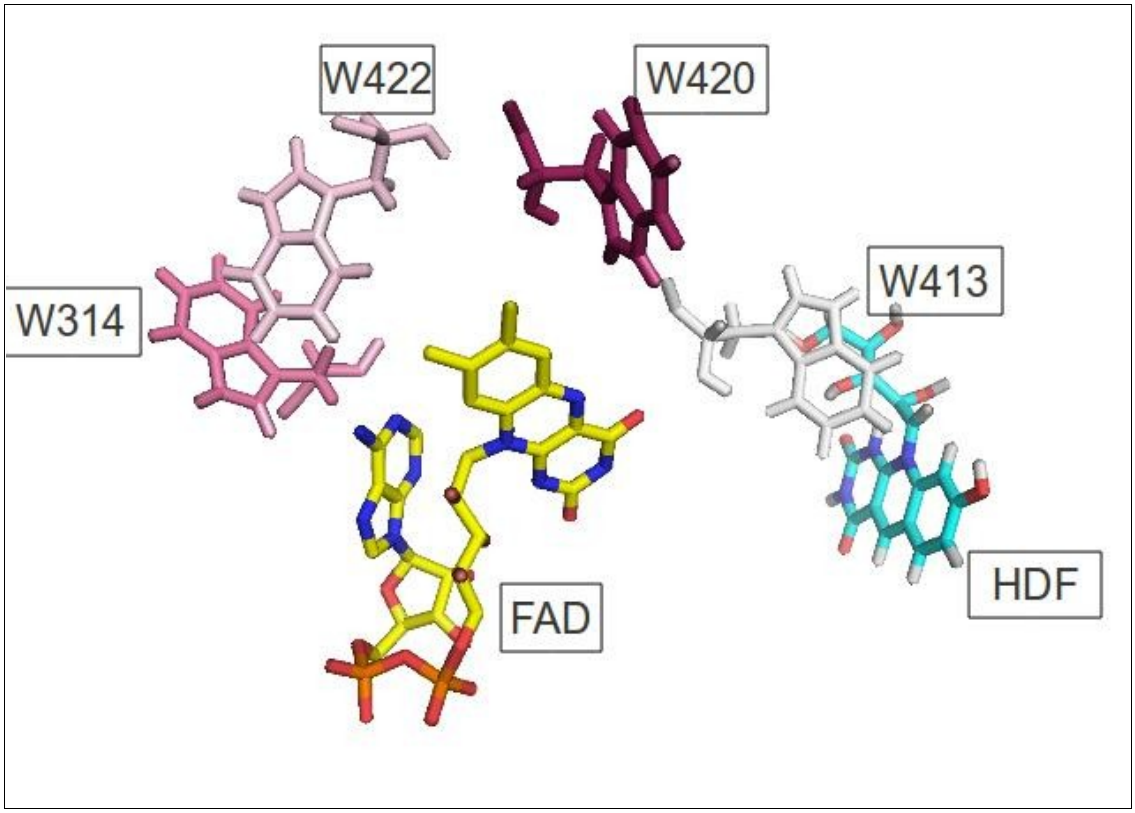

Figure 16. dCRY tryptophans located close and between the predicted 8-HDF conformation (in cyan) and FAD (in yellow) cofactors.

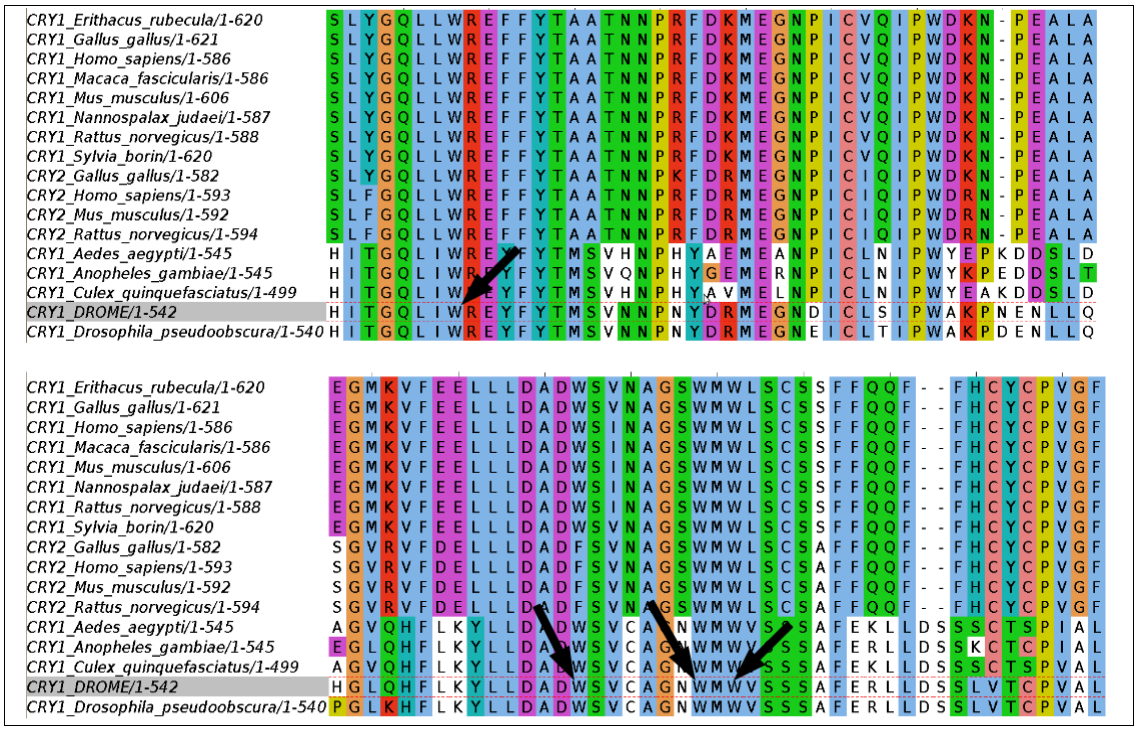

Figure 17. Multiple sequence alignment of Insect/vertebrates CRYs pointing out W314, W413, W420 and W422 on dCRY.

R56 in dCRY is oriented closely to the isoalloxazine moiety of 8-HDF. 8-HDF docking prediction for mCRY2 shows a similar orientation to dCRY (Figure 18) with R69 located where dCRY shows R56. Similarly, *Anacystis* and *Thermus* photolyases show R46 and R51, respectively, on the same position, with the former having crucial binding role to a flavin type cofactor. This Arginine is conserved among CRYs members of insect and vertebrates CRYs (Figure 19).

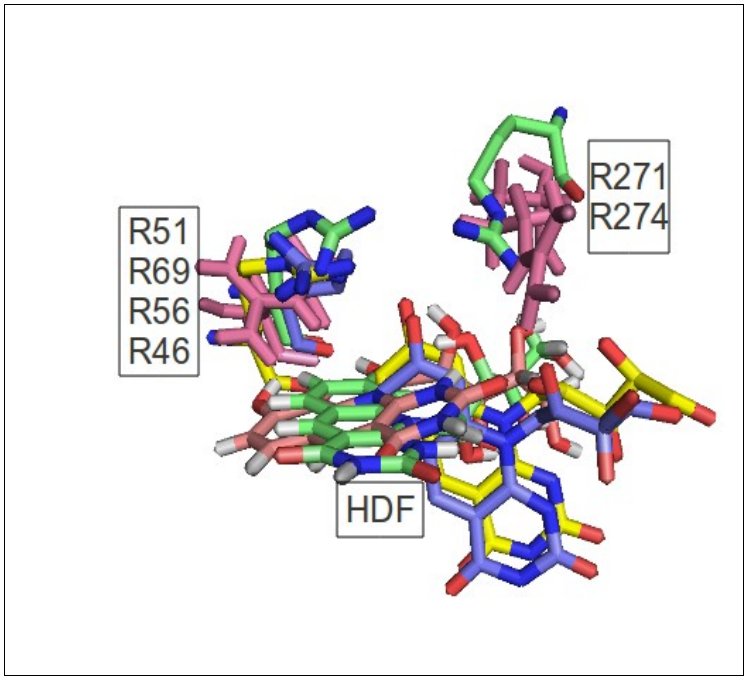

Figure 18. Superposition of dCRY (in salmon) and mCRY2 (in green) docking to 8-HDF. For comparison, 8-HDF of crystal structures of *Anacystis* (yellow) and *Thermus* (blue) Photolyases are also shown.

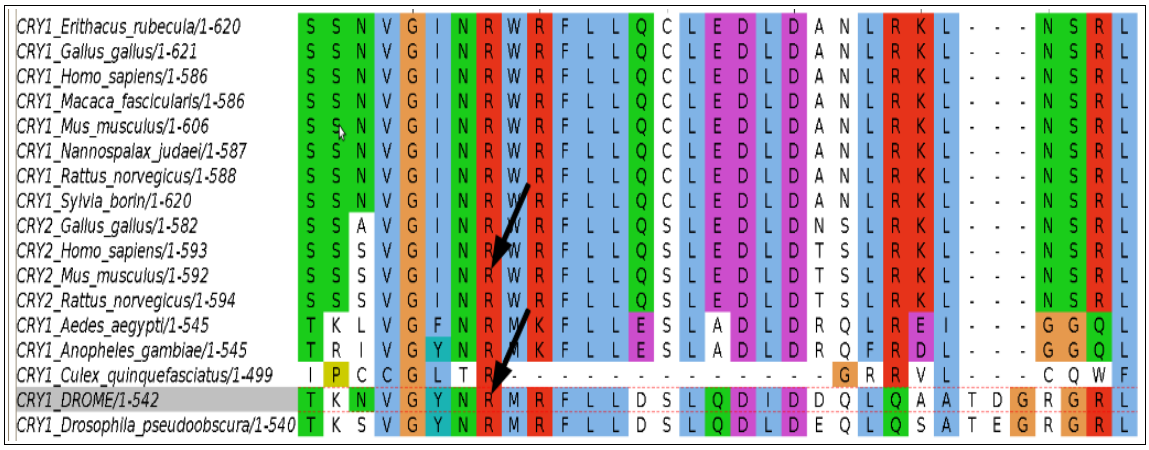

Figure 19. Multiple sequence alignment of insect and vertebrate CRYs showing conservation of dCRY R56 and mCRY2 R69.

At the oposite side of the isoalloxazine moiety, R271 together with H13 are also closely positioned for cofactor binding function (Figure 20). Mouse mCRY2 shows R274 on the same position (Figure 18). Also this Arginine is conserved among insect and vertebrates CRYs (Figure 21).

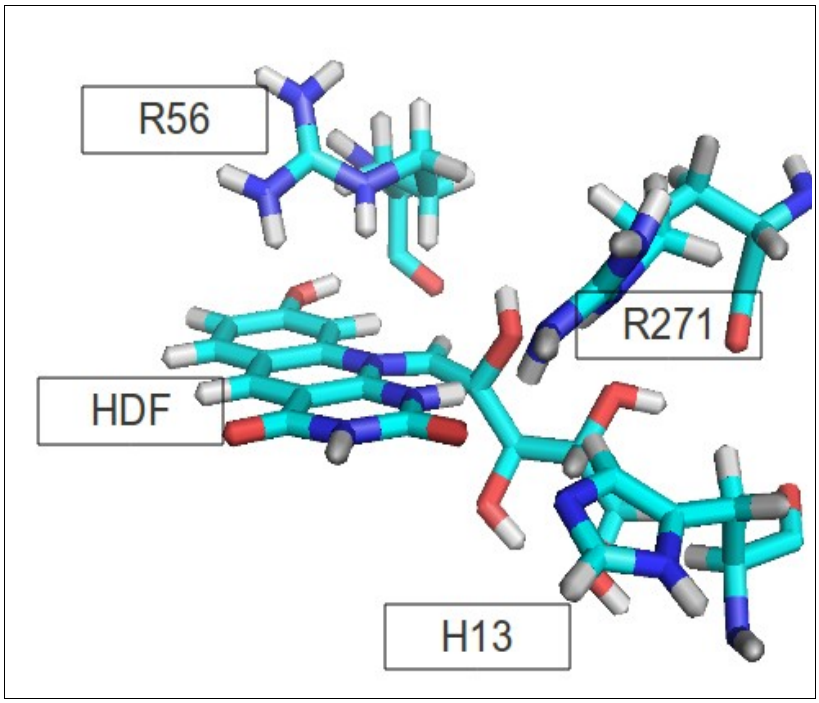

Figure 20. dCRY showing R56, R271 and H13 along with 8-HDF.

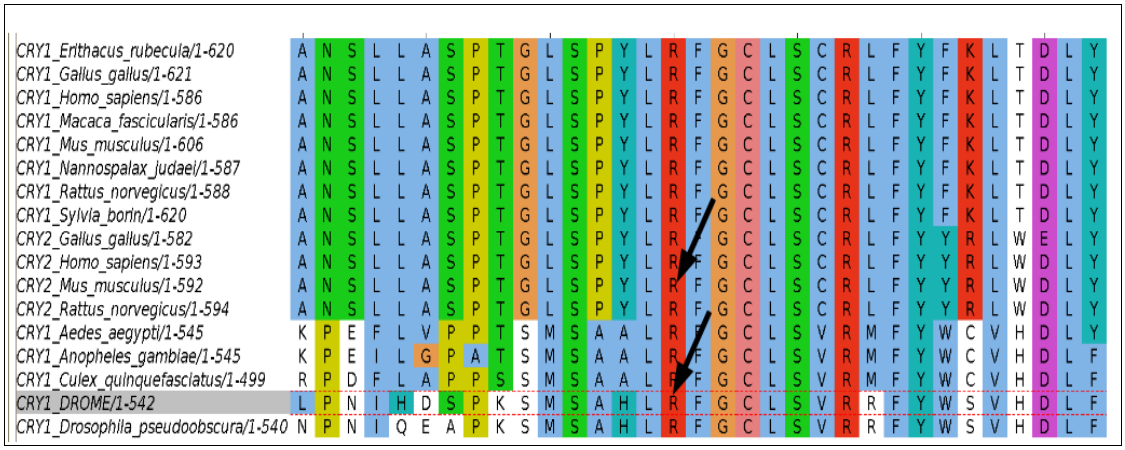

Figure 21. Insect and Vertebrates multiple sequence alignment showing conservation of dCRY R271. R271 dCRY and R274 mCRY2 are indicated.

Our docking prediction similarly than *Anacystis* and *Thermus* photolyases also predicts a hydrophobic side for interaction to 8-HDF. Likewise L55, F35 and F249 in the *Anacystis* photolyase, and F50 together with V33 and L218 in the *Thermus* photolyase, the hydrophobic part of W413 on dCRY is forming a hydrophobic side along with F272 and L55 (Figure 22).

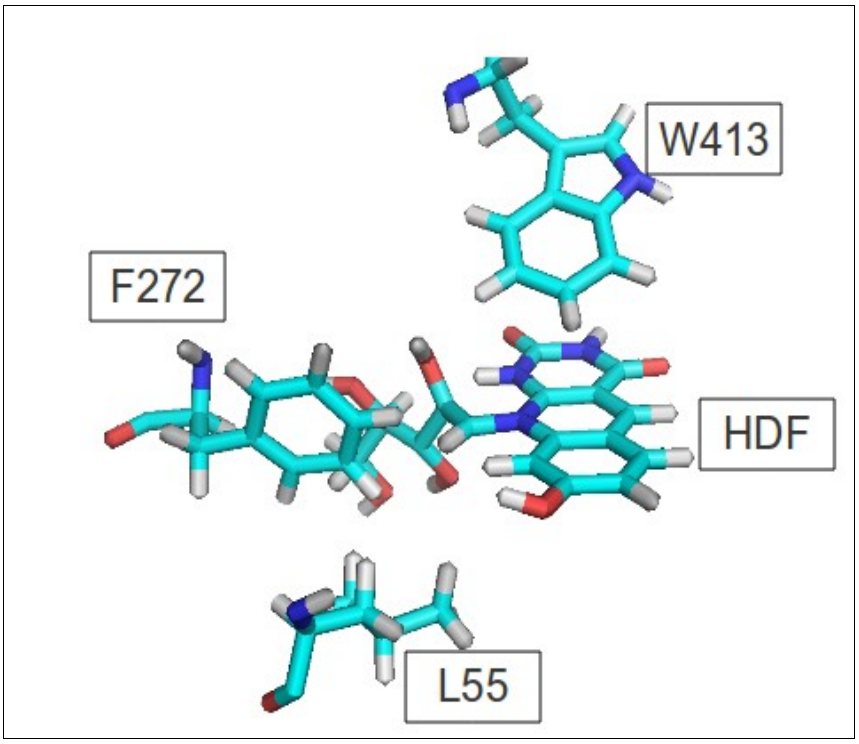

Figure 22. dCRY hydrophobic side formed by W413, L55 and F272.

4.4 Concluding Remarks

Our work suggests dCRY can have 8-HDF as antenna cofactor. Conservation profile shows that CRYs have maintained among insect/vertebrates the residues surrounding 8-HDF as possible cofactor. The presence of an antenna cofactor is further supported by a well conserved tryptophan path suitably located to play energy transfer between cofactors. Furthermore, Arginine key residues interacting to the cofactor on photolyases are conserved and located closely to the isoalloxazine moiety also on dCRY and in the predicted docking conformation for mCRY2. As this work suggest a flavin antenna cofactor for dCRY, we suggest it is worth further investigation to confirm 8-HDF as second chromophore.

4.5 MTHF as a Second Chromophore

In order to confirm MTHF as second cofactor to dCRY, docking prediction has been performed. After energy minimization of the docking predicted conformation, K50 points towards MTHF indicating a possible binding function (Figure 23). Differently

than *At*CRY-DASH (Klar et al., 2007) and *E. coli* photolyase, dCRY has no glutamate coordinating the pterin ring of MTHF. E116 is not properly oriented to play a similar role in dCRY. Moreover, differently than residues surrounding 8-HDF cofactor, residues on the MTHF binding site are poorly conserved. Overall, the poor conservation for MTHF binding sites, the lack of considerable number of residues properly oriented to bind a surface exposed cofactor, make it difficult to confirm MTHF as a second chromophore for dCRY as previously suggested (Berndt et al., 2007). However, although our work suggests unlikely, we cannot rule out MTHF as dCRY antenna cofactor.

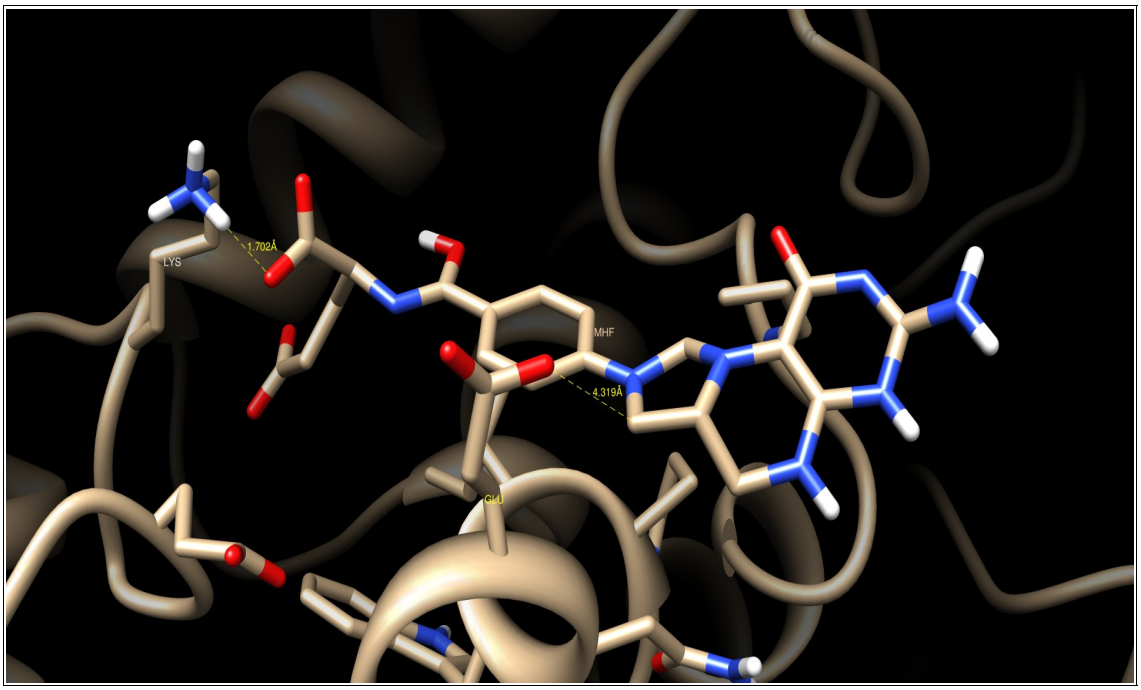

Figure 23 . dCRY-MTHF docking close-up view pointing out residues K50 and E116.

4.6 References

Adamec, J., Rusnak, F., Owen, W.G., Naylor, S., Benson, L.M., Gacy, A.M., Isaya, G., 2000. Iron-dependent self-assembly of recombinant yeast frataxin: implications for Friedreich ataxia. Am. J. Hum. Genet. 67, 549–562.

Adinolfi, S., Iannuzzi, C., Prischi, F., Pastore, C., Iametti, S., Martin, S.R., Bonomi, F., Pastore, A., 2009. Bacterial frataxin CyaY is the gatekeeper of iron-sulfur cluster formation catalyzed by IscS. Nat. Struct. Mol. Biol. 16, 390–396.

Adinolfi, S., Trifuoggi, M., Politou, A.S., Martin, S., Pastore, A., 2002. A structural approach to understanding the iron-binding properties of phylogenetically different frataxins. Hum. Mol. Genet. 11, 1865–1877.

Allen, M.P., Tildesley, D.J., 1989. Computer simulation of liquids. Clarendon Press ; Oxford University Press, Oxford [England]; New York.

Aloria, K., Schilke, B., Andrew, A., Craig, E.A., 2004. Iron-induced oligomerization of yeast frataxin homologue Yfh1 is dispensable in vivo. EMBO Rep. 5, 1096–1101.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Baker, N.A., 2005. Improving implicit solvent simulations: a Poisson-centric view. Curr. Opin. Struct. Biol. 15, 137–143.

Banerjee, R., Schleicher, E., Meier, S., Viana, R.M., Pokorny, R., Ahmad, M., Bittl, R., Batschauer, A., 2007. The Signaling State of Arabidopsis Cryptochrome 2 Contains Flavin Semiquinone. J. Biol. Chem. 282, 14916–14922.

Bashford, D., Case, D.A., 2000. Generalized born models of macromolecular solvation effects. Annu. Rev. Phys. Chem. 51, 129–152.

Beel, B., Prager, K., Spexard, M., Sasso, S., Weiss, D., Müller, N., Heinnickel, M., Dewez, D., Ikoma, D., Grossman, A.R., Kottke, T., Mittag, M., 2012. A Flavin Binding Cryptochrome Photoreceptor Responds to Both Blue and Red Light in Chlamydomonas reinhardtii. Plant Cell Online 24, 2992–3008.

Berendsen, H.J.C., Grigera, J.R., Straatsma, T.P., 1987. The missing term in effective pair potentials. J. Phys. Chem. 91, 6269–6271.

Berndt, A., Kottke, T., Breitkreuz, H., Dvorsky, R., Hennig, S., Alexander, M., Wolf, E., 2007. A novel photoreaction mechanism for the circadian blue light photoreceptor Drosophila cryptochrome. J. Biol. Chem. 282, 13011–13021.

Biskup, T., Hitomi, K., Getzoff, E.D., Krapf, S., Koslowski, T., Schleicher, E., Weber, S., 2011. Unexpected Electron Transfer in Cryptochrome Identified by Time-Resolved EPR Spectroscopy. Angew. Chem. Int. Ed. 50, 12647–12651.

Biskup, T., Paulus, B., Okafuji, A., Hitomi, K., Getzoff, E.D., Weber, S., Schleicher, E., 2013. Variable Electron-Transfer Pathways in an Amphibian Cryptochrome:

Tryptophan versus Tyrosine-Based Radical Pairs. J. Biol. Chem.

Bou-Abdallah, F., Adinolfi, S., Pastore, A., Laue, T.M., Dennis Chasteen, N., 2004. Iron binding and oxidation kinetics in frataxin CyaY of Escherichia coli. J. Mol. Biol. 341, 605–615.

Bouly, J.-P., Schleicher, E., Dionisio-Sese, M., Vandenbussche, F., Straeten, D.V.D., Bakrim, N., Meier, S., Batschauer, A., Galland, P., Bittl, R., Ahmad, M., 2007. Cryptochrome Blue Light Photoreceptors Are Activated through Interconversion of Flavin Redox States. J. Biol. Chem. 282, 9383–9391.

Branda, S.S., Yang, Z.Y., Chew, A., Isaya, G., 1999. Mitochondrial intermediate peptidase and the yeast frataxin homolog together maintain mitochondrial iron homeostasis in Saccharomyces cerevisiae. Hum. Mol. Genet. 8, 1099–1110.

Brautigam, C.A., Smith, B.S., Ma, Z., Palnitkar, M., Tomchick, D.R., Machius, M., Deisenhofer, J., 2004. Structure of the photolyase-like domain of cryptochrome 1 from Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A. 101, 12142–12147.

Brooks, B.R., Brooks, C.L., 3rd, Mackerell, A.D., Jr, Nilsson, L., Petrella, R.J., Roux, B., Won, Y., Archontis, G., Bartels, C., Boresch, S., Caflisch, A., Caves, L., Cui, Q., Dinner, A.R., Feig, M., Fischer, S., Gao, J., Hodoscek, M., Im, W., Kuczera, K., Lazaridis, T., Ma, J., Ovchinnikov, V., Paci, E., Pastor, R.W., Post, C.B., Pu, J.Z., Schaefer, M., Tidor, B., Venable, R.M., Woodcock, H.L., Wu, X., Yang, W., York, D.M., Karplus, M., 2009. CHARMM: the biomolecular simulation program. J. Comput. Chem. 30, 1545–1614.

Brooks, B.R., Bruccoleri, R.E., Olafson, B.D., States, D.J., Swaminathan, S., Karplus, M., 1983. CHARMM: A program for macromolecular energy, minimization, and dynamics calculations. J. Comput. Chem. 4, 187–217.

Brudler, R., Hitomi, K., Daiyasu, H., Toh, H., Kucho, K., Ishiura, M., Kanehisa, M., Roberts, V.A., Todo, T., Tainer, J.A., Getzoff, E.D., 2003. Identification of a New Cryptochrome Class: Structure, Function, and Evolution. Mol. Cell 11, 59–67.

Bulteau, A.-L., O'Neill, H.A., Kennedy, M.C., Ikeda-Saito, M., Isaya, G., Szweda, L.I., 2004. Frataxin Acts as an Iron Chaperone Protein to Modulate Mitochondrial Aconitase Activity. Science 305, 242–245.

Busi, M.V., Zabaleta, E.J., Araya, A., Gomez-Casati, D.F., 2004. Functional and

molecular characterization of the frataxin homolog from Arabidopsis thaliana. FEBS Lett. 576, 141–144.

Busino, L., Bassermann, F., Maiolica, A., Lee, C., Nolan, P.M., Godinho, S.I.H., Draetta, G.F., Pagano, M., 2007. SCFFbxl3 controls the oscillation of the circadian clock by directing the degradation of cryptochrome proteins. Science 316, 900–904.

Busza, A., Emery-Le, M., Rosbash, M., Emery, P., 2004. Roles of the Two Drosophila CRYPTOCHROME Structural Domains in Circadian Photoreception. Science 304, 1503–1506.

Campanella, A., Isaya, G., O'Neill, H.A., Santambrogio, P., Cozzi, A., Arosio, P., Levi, S., 2004. The expression of human mitochondrial ferritin rescues respiratory function in frataxin-deficient yeast. Hum. Mol. Genet. 13, 2279–2288.

Case, D.A., Cheatham, T.E., 3rd, Darden, T., Gohlke, H., Luo, R., Merz, K.M., Jr, Onufriev, A., Simmerling, C., Wang, B., Woods, R.J., 2005. The Amber biomolecular simulation programs. J. Comput. Chem. 26, 1668–1688.

Case, D.A., Cheatham, T.E., Darden, T., Gohlke, H., Luo, R., Merz, K.M., Onufriev, A., Simmerling, C., Wang, B., Woods, R.J., 2005. The Amber biomolecular simulation programs. J. Comput. Chem. 26, 1668–1688.

Cavadini, P., O'Neill, H.A., Benada, O., Isaya, G., 2002. Assembly and iron-binding properties of human frataxin, the protein deficient in Friedreich ataxia. Hum. Mol. Genet. 11, 217–227.

Ceriani, M.F., Darlington, T.K., Staknis, D., Más, P., Petti, A.A., Weitz, C.J., Kay, S.A., 1999. Light-dependent sequestration of TIMELESS by CRYPTOCHROME. Science 285, 553–556.

Chaves, I., Pokorny, R., Byrdin, M., Hoang, N., Ritz, T., Brettel, K., Essen, L.-O., van der Horst, G.T.J., Batschauer, A., Ahmad, M., 2011. The cryptochromes: blue light photoreceptors in plants and animals. Annu. Rev. Plant Biol. 62, 335–364.

Chaves, I., Yagita, K., Barnhoorn, S., Okamura, H., van der Horst, G.T.J., Tamanini, F., 2006. Functional evolution of the photolyase/cryptochrome protein family: importance of the C terminus of mammalian CRY1 for circadian core oscillator performance. Mol. Cell. Biol. 26, 1743–1753.

Chothia, C., Lesk, A.M., 1986. The relation between the divergence of sequence and

structure in proteins. EMBO J. 5, 823–826.

Coesel, S., Mangogna, M., Ishikawa, T., Heijde, M., Rogato, A., Finazzi, G., Todo, T., Bowler, C., Falciatore, A., 2009. Diatom PtCPF1 is a new cryptochrome/photolyase family member with DNA repair and transcription regulation activity. EMBO Rep. 10, 655–661.

Collins, B.H., Dissel, S., Gaten, E., Rosato, E., Kyriacou, C.P., 2005. Disruption of Cryptochrome partially restores circadian rhythmicity to the arrhythmic period mutant of Drosophila. Proc. Natl. Acad. Sci. U. S. A. 102, 19021–19026.

Collins, B., Mazzoni, E.O., Stanewsky, R., Blau, J., 2006. Drosophila CRYPTOCHROME is a circadian transcriptional repressor. Curr. Biol. CB 16, 441–449.

Cook, J.D., Bencze, K.Z., Jankovic, A.D., Crater, A.K., Busch, C.N., Bradley, P.B., Stemmler, A.J., Spaller, M.R., Stemmler, T.L., 2006. Monomeric Yeast Frataxin is an Iron Binding Protein. Biochemistry (Mosc.) 45, 7767–7777.

Correia, A.R., Wang, T., Craig, E.A., Gomes, C.M., 2010a. Iron binding activity in yeast frataxin entails a trade off with stability in the ?1/?1 acidic ridge region. Biochem. J. 426, 197–203.

Correia, A.R., Wang, T., Craig, E.A., Gomes, C.M., 2010b. Iron binding activity in yeast frataxin entails a trade off with stability in the ?1/?1 acidic ridge region. Biochem. J. 426, 197–203.

Dardente, H., Fortier, E.E., Martineau, V., Cermakian, N., 2007. Cryptochromes impair phosphorylation of transcriptional activators in the clock: a general mechanism for circadian repression. Biochem. J. 402, 525.

Dayhoff, M.O., Schwartz, R.M., 1978. Chapter 22: A model of evolutionary change in proteins, in: In Atlas of Protein Sequence and Structure.

Delatycki, M.B., Williamson, R., Forrest, S.M., 2000. Friedreich ataxia: an overview. J. Med. Genet. 37, 1–8.

Del Pozo, A., Vera, L.M., Sánchez, J.A., Sánchez-Vázquez, F.J., 2012. Molecular cloning, tissue distribution and daily expression of cry1 and cry2 clock genes in European seabass (Dicentrarchus labrax). Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 163, 364–371.

Dissel, S., Codd, V., Fedic, R., Garner, K.J., Costa, R., Kyriacou, C.P., Rosato, E.,

2004a. A constitutively active cryptochrome in Drosophila melanogaster. Nat. Neurosci. 7, 834–840.

Dissel, S., Codd, V., Fedic, R., Garner, K.J., Costa, R., Kyriacou, C.P., Rosato, E., 2004b. A constitutively active cryptochrome in Drosophila melanogaster. Nat. Neurosci. 7, 834–840.

Dunlap, J.C., 1999. Molecular Bases for Circadian Clocks. Cell 96, 271–290.

Eckel-Mahan, K., Sassone-Corsi, P., 2013. Metabolism and the Circadian Clock Converge. Physiol. Rev. 93, 107–135.

Emery, P., So, W.V., Kaneko, M., Hall, J.C., Rosbash, M., 1998. CRY, a Drosophila clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. Cell 95, 669–679.

Engelen, E., Janssens, R.C., Yagita, K., Smits, V.A.J., van der Horst, G.T.J., Tamanini, F., 2013. Mammalian TIMELESS Is Involved in Period Determination and DNA Damage-Dependent Phase Advancing of the Circadian Clock. PLoS ONE 8, e56623.

Faraj, S.E., Venturutti, L., Roman, E.A., Marino-Buslje, C.B., Mignone, A., Tosatto, S.C.E., Delfino, J.M., Santos, J., 2013. The role of the N-terminal tail for the oligomerization, folding and stability of human frataxin. FEBS Open Bio 3, 310–320.

Feig, M., Brooks, C.L., 3rd, 2004. Recent advances in the development and application of implicit solvent models in biomolecule simulations. Curr. Opin. Struct. Biol. 14, 217–224.

Ferrara, P., Apostolakis, J., Caflisch, A., 2002. Evaluation of a fast implicit solvent model for molecular dynamics simulations. Proteins 46, 24–33.

Finn, R.D., Mistry, J., Schuster-Böckler, B., Griffiths-Jones, S., Hollich, V., Lassmann, T., Moxon, S., Marshall, M., Khanna, A., Durbin, R., Eddy, S.R., Sonnhammer, E.L.L., Bateman, A., 2006. Pfam: clans, web tools and services. Nucleic Acids Res. 34, D247–D251.

Fogle, K.J., Parson, K.G., Dahm, N.A., Holmes, T.C., 2011. CRYPTOCHROME Is a Blue-Light Sensor That Regulates Neuronal Firing Rate. Science 331, 1409–1413.

Foley, L.E., Gegear, R.J., Reppert, S.M., 2011. Human cryptochrome exhibits light-

dependent magnetosensitivity. Nat. Commun. 2, 356.

Foury, F., Pastore, A., Trincal, M., 2007. Acidic residues of yeast frataxin have an essential role in Fe-S cluster assembly. EMBO Rep. 8, 194–199.

Frenkel, D., Smit, B., 2002. Understanding molecular simulation from algorithms to applications. Academic Press, San Diego.

Fujihashi, M., Numoto, N., Kobayashi, Y., Mizushima, A., Tsujimura, M., Nakamura, A., Kawarabayasi, Y., Miki, K., 2007. Crystal Structure of Archaeal Photolyase from Sulfolobus tokodaii with Two FAD Molecules: Implication of a Novel Light-harvesting Cofactor. J. Mol. Biol. 365, 903–910.

Gakh, O., Adamec, J., Gacy, A.M., Twesten, R.D., Owen, W.G., Isaya, G., 2002. Physical Evidence that Yeast Frataxin Is an Iron Storage Protein†. Biochemistry (Mosc.) 41, 6798–6804.

Gakh, O., Park, S., Liu, G., Macomber, L., Imlay, J.A., Ferreira, G.C., Isaya, G., 2006. Mitochondrial iron detoxification is a primary function of frataxin that limits oxidative damage and preserves cell longevity. Hum. Mol. Genet. 15, 467–479.

Gao, P., Yoo, S.-H., Lee, K.-J., Rosensweig, C., Takahashi, J.S., Chen, B.P., Green, C.B., 2013. Phosphorylation of the Cryptochrome 1 C-terminal tail regulates circadian period length. J. Biol. Chem. jbc.M113.509604.

Gegear, R.J., Casselman, A., Waddell, S., Reppert, S.M., 2008. Cryptochrome mediates light-dependent magnetosensitivity in Drosophila. Nature 454, 1014–1018.

Gentry, L.E., Thacker, M.A., Doughty, R., Timkovich, R., Busenlehner, L.S., 2013. His86 from the N-terminus of frataxin coordinates iron and is required for Fe-S cluster synthesis. Biochemistry (Mosc.) 52, 6085–6096.

Gilson, M.K., Davis, M.E., Luty, B.A., McCammon, J.A., 1993. Computation of electrostatic forces on solvated molecules using the Poisson-Boltzmann equation. J. Phys. Chem. 97, 3591–3600.

Glas, A.F., Maul, M.J., Cryle, M., Barends, T.R.M., Schneider, S., Kaya, E., Schlichting, I., Carell, T., 2009. The archaeal cofactor F0 is a light-harvesting antenna chromophore in eukaryotes. Proc. Natl. Acad. Sci.

Glossop, N.R.J., Hardin, P.E., 2002. Central and peripheral circadian oscillator mechanisms in flies and mammals. J. Cell Sci. 115, 3369–3377.

González-Cabo, P., Vázquez-Manrique, R.P., García-Gimeno, M.A., Sanz, P., Palau, F.,

2005. Frataxin interacts functionally with mitochondrial electron transport chain proteins. Hum. Mol. Genet. 14, 2091–2098.

Griffin, E.A., Staknis, D., Weitz, C.J., 1999. Light-Independent Role of CRY1 and CRY2 in the Mammalian Circadian Clock. Science 286, 768–771.

Guex, N., Peitsch, M.C., 1997. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis 18, 2714–2723.

Guo, H., Duong, H., Ma, N., Lin, C., 1999. The Arabidopsis blue light receptor cryptochrome 2 is a nuclear protein regulated by a blue light-dependent post-transcriptional mechanism. Plant J. Cell Mol. Biol. 19, 279–287.

Hall, J.C., 2000. Cryptochromes: sensory reception, transduction, and clock functions subserving circadian systems. Curr. Opin. Neurobiol. 10, 456–466.

Hamm-Alvarez, S., Sancar, A., Rajagopalan, K.V., 1989. Role of enzyme-bound 5,10-methenyltetrahydropteroylpolyglutamate in catalysis by Escherichia coli DNA photolyase. J. Biol. Chem. 264, 9649–9656.

Hao Zheng, Ng, F., Yixiao Liu, Hardin, P.E., 2008. Spatial and circadian regulation of cry in Drosophila. J. Biol. Rhythms 23, 283–295.

Harada, Y., Sakai, M., Kurabayashi, N., Hirota, T., Fukada, Y., 2005. Ser-557-phosphorylated mCRY2 Is Degraded upon Synergistic Phosphorylation by Glycogen Synthase Kinase-3β. J. Biol. Chem. 280, 31714–31721.

Harris, S.-R., Henbest, K.B., Maeda, K., Pannell, J.R., Timmel, C.R., Hore, P.J., Okamoto, H., 2009. Effect of magnetic fields on cryptochrome-dependent responses in Arabidopsis thaliana. J. R. Soc. Interface 6, 1193–1205.

Helfrich-Förster, C., Winter, C., Hofbauer, A., Hall, J.C., Stanewsky, R., 2001. The circadian clock of fruit flies is blind after elimination of all known photoreceptors. Neuron 30, 249–261.

Hemsley, M.J., Mazzotta, G.M., Mason, M., Dissel, S., Toppo, S., Pagano, M.A., Sandrelli, F., Meggio, F., Rosato, E., Costa, R., Tosatto, S.C.E., 2007. Linear motifs in the C-terminus of D. melanogaster cryptochrome. Biochem. Biophys. Res. Commun. 355, 531–537.

Hess, B., Bekker, H., Berendsen, H.J.C., Fraaije, J.G.E.M., 1997. LINCS: A linear constraint solver for molecular simulations. J. Comput. Chem. 18, 1463–1472.

Hess, B., Kutzner, C., van der Spoel, D., Lindahl, E., 2008. GROMACS 4: Algorithms

for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation. J. Chem. Theory Comput. 4, 435–447.

He, Y., Alam, S.L., Proteasa, S.V., Zhang, Y., Lesuisse, E., Dancis, A., Stemmler, T.L., 2004. Yeast frataxin solution structure, iron binding, and ferrochelatase interaction. Biochemistry (Mosc.) 43, 16254–16262.

Hitomi, K., DiTacchio, L., Arvai, A.S., Yamamoto, J., Kim, S.-T., Todo, T., Tainer, J.A., Iwai, S., Panda, S., Getzoff, E.D., 2009. Functional motifs in the (6-4) photolyase crystal structure make a comparative framework for DNA repair photolyases and clock cryptochromes. Proc. Natl. Acad. Sci. 106, 6962–6967.

Hitomi, K., Okamoto, K., Daiyasu, H., Miyashita, H., Iwai, S., Toh, H., Ishiura, M., Todo, T., 2000. Bacterial cryptochrome and photolyase: characterization of two photolyase-like genes of Synechocystis sp. PCC6803. Nucleic Acids Res. 28, 2353–2362.

Hoang, N., Schleicher, E., Kacprzak, S., Bouly, J.-P., Picot, M., Wu, W., Berndt, A., Wolf, E., Bittl, R., Ahmad, M., 2008a. Human and Drosophila Cryptochromes Are Light Activated by Flavin Photoreduction in Living Cells. PLoS Biol 6, e160.

Hoang, N., Schleicher, E., Kacprzak, S., Bouly, J.-P., Picot, M., Wu, W., Berndt, A., Wolf, E., Bittl, R., Ahmad, M., 2008b. Human and Drosophila Cryptochromes Are Light Activated by Flavin Photoreduction in Living Cells. PLoS Biol 6, e160.

Höltje, H.-D., 2008. Molecular modeling: basic principles and applications. Wiley-VCH, Weinheim.

Huang, J., Dizin, E., Cowan, J.A., 2008. Mapping iron binding sites on human frataxin: implications for cluster assembly on the ISU Fe-S cluster scaffold protein. J. Biol. Inorg. Chem. JBIC Publ. Soc. Biol. Inorg. Chem. 13, 825–836.

Huang, M.L.-H., Becker, E.M., Whitnall, M., Suryo Rahmanto, Y., Ponka, P., Richardson, D.R., 2009. Elucidation of the mechanism of mitochondrial iron loading in Friedreich's ataxia by analysis of a mouse mutant. Proc. Natl. Acad. Sci. U. S. A. 106, 16381–16386.

Huynen, M.A., Snel, B., Bork, P., Gibson, T.J., 2001. The phylogenetic distribution of frataxin indicates a role in iron-sulfur cluster protein assembly. Hum. Mol.

Genet. 10, 2463–2468.

III, C.L.B., Karplus, rtin, Pettitt, B.M. (Eds.), 2007. Dynamical Simulation Methods, in: Advances in Chemical Physics. John Wiley & Sons, Inc., pp. 33–58.

Ito, C., Goto, S.G., Shiga, S., Tomioka, K., Numata, H., 2008. Peripheral circadian clock for the cuticle deposition rhythm in Drosophila melanogaster. Proc. Natl. Acad. Sci. 105, 8446–8451.

Ivanchenko, M., Stanewsky, R., Giebultowicz, J.M., 2001. Circadian photoreception in Drosophila: functions of cryptochrome in peripheral and central clocks. J. Biol. Rhythms 16, 205–215.

Jorgensen, W.L., Chandrasekhar, J., Madura, J.D., Impey, R.W., Klein, M.L., 1983. Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. 79, 926–935.

Kang, C.-Y., Lian, H.-L., Wang, F.-F., Huang, J.-R., Yang, H.-Q., 2009. Cryptochromes, Phytochromes, and COP1 Regulate Light-Controlled Stomatal Development in Arabidopsis. Plant Cell Online 21, 2624–2641.

Kao, Y.-T., Tan, C., Song, S.-H., Öztürk, N., Li, J., Wang, L., Sancar, A., Zhong, D., 2008. Ultrafast Dynamics and Anionic Active States of the Flavin Cofactor in Cryptochrome and Photolyase. J. Am. Chem. Soc. 130, 7695–7701.

Kaushik, R., Nawathean, P., Busza, A., Murad, A., Emery, P., Rosbash, M., 2007. PER-TIM Interactions with the Photoreceptor Cryptochrome Mediate Circadian Temperature Responses in Drosophila. PLoS Biol 5, e146.

Khan, S.K., Xu, H., Ukai-Tadenuma, M., Burton, B., Wang, Y., Ueda, H.R., Liu, A.C., 2012. Identification of a Novel Cryptochrome Differentiating Domain Required for Feedback Repression in Circadian Clock Function. J. Biol. Chem. 287, 25917–25926.

Kim, S.T., Heelis, P.F., Sancar, A., 1992. Energy transfer (deazaflavin .fwdarw. FADH2) and electron transfer (FADH2 .fwdarw. T.ltbbrac..rtbbrac.T) kinetics in Anacystis nidulans photolyase. Biochemistry (Mosc.) 31, 11244–11248.

Klar, T., Kaiser, G., Hennecke, U., Carell, T., Batschauer, A., Essen, L.-O., 2006. Natural and Non-natural Antenna Chromophores in the DNA Photolyase from Thermus Thermophilus. ChemBioChem 7, 1798–1806.

Klar, T., Pokorny, R., Moldt, J., Batschauer, A., Essen, L.-O., 2007. Cryptochrome 3

from Arabidopsis thaliana: Structural and Functional Analysis of its Complex with a Folate Light Antenna. J. Mol. Biol. 366, 954–964.

Kleiner, O., Butenandt, J., Carell, T., Batschauer, A., 1999. Class II DNA photolyase from Arabidopsis thaliana contains FAD as a cofactor. Eur. J. Biochem. 264, 161–167.

Kleine, T., Lockhart, P., Batschauer, A., 2003. An Arabidopsis protein closely related to Synechocystis cryptochrome is targeted to organelles. Plant J. 35, 93–103.

Kondapalli, K.C., Kok, N.M., Dancis, A., Stemmler, T.L., 2008. Drosophila Frataxin: An Iron Chaperone during Cellular Fe-S Cluster Bioassembly. Biochemistry (Mosc.) 47, 6917–6927.

Kondratov, R.V., Kondratova, A.A., Lee, C., Gorbacheva, V.Y., Chernov, M.V., Antoch, M.P., 2006. Post-translational regulation of circadian transcriptional CLOCK(NPAS2)/BMAL1 complex by CRYPTOCHROMES. Cell Cycle Georget. Tex 5, 890–895.

Korb, O., Stützle, T., Exner, T.E., 2006. PLANTS: Application of Ant Colony Optimization to Structure-Based Drug Design, in: Dorigo, M., Gambardella, L.M., Birattari, M., Martinoli, A., Poli, R., Stützle, T. (Eds.), Ant Colony Optimization and Swarm Intelligence, Lecture Notes in Computer Science. Springer Berlin Heidelberg, pp. 247–258.

Krishnan, B., Levine, J.D., Lynch, M.K., Dowse, H.B., Funes, P., Hall, J.C., Hardin, P.E., Dryer, S.E., 2001. A new role for cryptochrome in a Drosophila circadian oscillator. Nature 411, 313–317.

Kume, K., Zylka, M.J., Sriram, S., Shearman, L.P., Weaver, D.R., Jin, X., Maywood, E.S., Hastings, M.H., Reppert, S.M., 1999. mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell 98, 193–205.

Lamia, K.A., Sachdeva, U.M., DiTacchio, L., Williams, E.C., Alvarez, J.G., Egan, D.F., Vasquez, D.S., Juguilon, H., Panda, S., Shaw, R.J., Thompson, C.B., Evans, R.M., 2009. AMPK Regulates the Circadian Clock by Cryptochrome Phosphorylation and Degradation. Science 326, 437–440.

Lane, D.J.R., Richardson, D.R., 2010. Frataxin, a molecule of mystery: trading stability for function in its iron-binding site. Biochem. J. 426, e1–e3.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.

Lesuisse, E., Santos, R., Matzanke, B.F., Knight, S.A.B., Camadro, J.-M., Dancis, A., 2003. Iron use for haeme synthesis is under control of the yeast frataxin homologue (Yfh1). Hum. Mol. Genet. 12, 879–889.

Lin, C., Robertson, D.E., Ahmad, M., Raibekas, A.A., Jorns, M.S., Dutton, P.L., Cashmore, A.R., 1995. Association of flavin adenine dinucleotide with the Arabidopsis blue light receptor CRY1. Science 269, 968–970.

Liu, H., Liu, B., Zhao, C., Pepper, M., Lin, C., 2011. The action mechanisms of plant cryptochromes. Trends Plant Sci. 16, 684–691.

Liu, H.-Y., Kuntz, I.D., Zou, X., 2004. Pairwise GB/SA Scoring Function for Structure-based Drug Design. J. Phys. Chem. B 108, 5453–5462.

Liu, Z., Tan, C., Guo, X., Li, J., Wang, L., Sancar, A., Zhong, D., 2013a. Determining complete electron flow in the cofactor photoreduction of oxidized photolyase. Proc. Natl. Acad. Sci. U. S. A. 110, 12966–12971.

Liu, Z., Zhang, M., Guo, X., Tan, C., Li, J., Wang, L., Sancar, A., Zhong, D., 2013b. Dynamic determination of the functional state in photolyase and the implication for cryptochrome. Proc. Natl. Acad. Sci. U. S. A. 110, 12972–12977.

Li, X., Wang, Q., Yu, X., Liu, H., Yang, H., Zhao, C., Liu, X., Tan, C., Klejnot, J., Zhong, D., Lin, C., 2011. Arabidopsis cryptochrome 2 (CRY2) functions by the photoactivation mechanism distinct from the tryptophan (trp) triad-dependent photoreduction. Proc. Natl. Acad. Sci. U. S. A. 108, 20844–20849.

MacKerell, A. D., Bashford, D., Bellott, Dunbrack, R. L., Evanseck, J.D., Field, M.J., Fischer, S., Gao, J., Guo, H., Ha, S., Joseph-McCarthy, D., Kuchnir, L., Kuczera, K., Lau, F.T.K., Mattos, C., Michnick, S., Ngo, T., Nguyen, D.T., Prodhom, B., Reiher, W.E., Roux, B., Schlenkrich, M., Smith, J.C., Stote, R., Straub, J., Watanabe, M., Wiórkiewicz-Kuczera, J., Yin, D., Karplus, M., 1998. All-Atom Empirical Potential for Molecular Modeling and Dynamics Studies of Proteins†. J. Phys. Chem. B 102, 3586–3616.

Majeux, N., Scarsi, M., Apostolakis, J., Ehrhardt, C., Caflisch, A., 1999. Exhaustive

docking of molecular fragments with electrostatic solvation. Proteins 37, 88–105.

Malhotra, K., Kim, S.-T., Batschauer, A., Dawut, L., Sancar, A., 1995. Putative Blue-Light Photoreceptors from Arabidopsis thaliana and Sinapis alba with a High Degree of Sequence Homology to DNA Photolyase Contain the Two Photolyase Cofactors but Lack DNA Repair Activity. Biochemistry (Mosc.) 34, 6892–6899.

Mao, J., Zhang, Y.-C., Sang, Y., Li, Q.-H., Yang, H.-Q., 2005. A role for Arabidopsis cryptochromes and COP1 in the regulation of stomatal opening. Proc. Natl. Acad. Sci. U. S. A. 102, 12270–12275.

Matsumoto, N., 2003. Functional Analysis and Intracellular Localization of Rice Cryptochromes. PLANT Physiol. 133, 1494–1503.

Meissner, R.-A., Kilman, V.L., Lin, J.-M., Allada, R., 2008. TIMELESS Is an Important Mediator of CK2 Effects on Circadian Clock Function In Vivo. J. Neurosci. 28, 9732–9740.

Miyazaki, K., Mesaki, M., Ishida, N., 2001. Nuclear Entry Mechanism of Rat PER2 (rPER2): Role of rPER2 in Nuclear Localization of CRY Protein. Mol. Cell. Biol. 21, 6651–6659.

Müller, M., Carell, T., 2009. Structural biology of DNA photolyases and cryptochromes. Curr. Opin. Struct. Biol. 19, 277–285.

Müller, P., Ahmad, M., 2011. Light-activated Cryptochrome Reacts with Molecular Oxygen to Form a Flavin–Superoxide Radical Pair Consistent with Magnetoreception. J. Biol. Chem. 286, 21033–21040.

Musco, G., Stier, G., Kolmerer, B., Adinolfi, S., Martin, S., Frenkiel, T., Gibson, T., Pastore, A., 2000. Towards a structural understanding of Friedreich's ataxia: the solution structure of frataxin. Struct. Lond. Engl. 1993 8, 695–707.

Naidoo, N., Song, W., Hunter-Ensor, M., Sehgal, A., 1999. A role for the proteasome in the light response of the timeless clock protein. Science 285, 1737–1741.

Nair, M., Adinolfi, S., Pastore, C., Kelly, G., Temussi, P., Pastore, A., 2004. Solution Structure of the Bacterial Frataxin Ortholog, CyaY: Mapping the Iron Binding Sites. Structure 12, 2037–2048.

Nießner, C., Denzau, S., Stapput, K., Ahmad, M., Peichl, L., Wiltschko, W., Wiltschko, R., 2013. Magnetoreception: activated cryptochrome 1a concurs with magnetic

orientation in birds. J. R. Soc. Interface 10, 20130638.

O'Neill, H.A., Gakh, O., Park, S., Cui, J., Mooney, S.M., Sampson, M., Ferreira, G.C., Isaya, G., 2005. Assembly of Human Frataxin Is a Mechanism for Detoxifying Redox-Active Iron†. Biochemistry (Mosc.) 44, 537–545.

Özgür, S., Sancar, A., 2006. Analysis of Autophosphorylating Kinase Activities of Arabidopsis and Human Cryptochromes†. Biochemistry (Mosc.) 45, 13369–13374.

Ozturk, N., Selby, C.P., Song, S.-H., Ye, R., Tan, C., Kao, Y.-T., Zhong, D., Sancar, A., 2009. Comparative Photochemistry of Animal Type 1 and Type 4 Cryptochromes. Biochemistry (Mosc.) 48, 8585–8593.

Öztürk, N., Song, S.-H., Özgür, S., Selby, C.P., Morrison, L., Partch, C., Zhong, D., Sancar, A., 2007. Structure and Function of Animal Cryptochromes. Cold Spring Harb. Symp. Quant. Biol. 72, 119–131.

Öztürk, N., Song, S.-H., Selby, C.P., Sancar, A., 2008. Animal Type 1 Cryptochromes ANALYSIS OF THE REDOX STATE OF THE FLAVIN COFACTOR BY SITE-DIRECTED MUTAGENESIS. J. Biol. Chem. 283, 3256–3263.

Ozturk, N., VanVickle-Chavez, S.J., Akileswaran, L., Gelder, R.N.V., Sancar, A., 2013. Ramshackle (Brwd3) promotes light-induced ubiquitylation of Drosophila Cryptochrome by DDB1-CUL4-ROC1 E3 ligase complex. Proc. Natl. Acad. Sci. 110, 4980–4985.

Panchenko, A.R., Kondrashov, F., Bryant, S., 2004. Prediction of functional sites by analysis of sequence and structure conservation. Protein Sci. 13, 884–892.

Pandolfo, M., Pastore, A., 2009. The pathogenesis of Friedreich ataxia and the structure and function of frataxin. J. Neurol. 256 Suppl 1, 9–17.

Park, H.W., Kim, S.T., Sancar, A., Deisenhofer, J., 1995. Crystal structure of DNA photolyase from Escherichia coli. Science 268, 1866–1872.

Pastore, C., Franzese, M., Sica, F., Temussi, P., Pastore, A., 2007. Understanding the binding properties of an unusual metal-binding protein--a study of bacterial frataxin. FEBS J. 274, 4199–4210.

Payne, G., Sancar, A., 1990. Absolute action spectrum of E-FADH2 and E-FADH2-MTHF forms of Escherichia coli DNA photolyase. Biochemistry (Mosc.) 29, 7715–7727.

Peschel, N., Veleri, S., Stanewsky, R., 2006. Veela defines a molecular link between Cryptochrome and Timeless in the light-input pathway to Drosophila's circadian clock. Proc. Natl. Acad. Sci. 103, 17313–17318.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., Ferrin, T.E., 2004. UCSF Chimera--a visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1612.

Phillips, J.C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., Chipot, C., Skeel, R.D., Kalé, L., Schulten, K., 2005. Scalable molecular dynamics with NAMD. J. Comput. Chem. 26, 1781–1802.

Pils, B., Copley, R.R., Schultz, J., 2005. Variation in structural location and amino acid conservation of functional sites in protein domain families. BMC Bioinformatics 6, 210.

Pokorny, R., Klar, T., Hennecke, U., Carell, T., Batschauer, A., Essen, L.-O., 2008. Recognition and repair of UV lesions in loop structures of duplex DNA by DASH-type cryptochrome. Proc. Natl. Acad. Sci. 105, 21023–21027.

Prischi, F., Giannini, C., Adinolfi, S., Pastore, A., 2009. The N-terminus of mature human frataxin is intrinsically unfolded. FEBS J. 276, 6669–6676.

Prischi, F., Konarev, P.V., Iannuzzi, C., Pastore, C., Adinolfi, S., Martin, S.R., Svergun, D.I., Pastore, A., 2010. Structural bases for the interaction of frataxin with the central components of iron-sulphur cluster assembly. Nat. Commun. 1, 95.

Rosato, E., Codd, V., Mazzotta, G., Piccin, A., Zordan, M., Costa, R., Kyriacou, C.P., 2001. Light-dependent interaction between Drosophila CRY and the clock protein PER mediated by the carboxy terminus of CRY. Curr. Biol. 11, 909–917.

Rush, B.L., Murad, A., Emery, P., Giebultowicz, J.M., 2006. Ectopic CRYPTOCHROME Renders TIM Light Sensitive in the Drosophila Ovary. J. Biol. Rhythms 21, 272–278.

Ryckaert, J.-P., Ciccotti, G., Berendsen, H.J.C., 1977. Numerical integration of the cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. J. Comput. Phys. 23, 327–341.

Saez, L., Derasmo, M., Meyer, P., Stieglitz, J., Young, M.W., 2011. A key temporal delay in the circadian cycle of Drosophila is mediated by a nuclear localization signal in the timeless protein. Genetics 188, 591–600.

Sakakida, Y., Miyamoto, Y., Nagoshi, E., Akashi, M., Nakamura, T.J., Mamine, T., Kasahara, M., Minami, Y., Yoneda, Y., Takumi, T., 2005. Importin alpha/beta mediates nuclear transport of a mammalian circadian clock component, mCRY2, together with mPER2, through a bipartite nuclear localization signal. J. Biol. Chem. 280, 13272–13278.

Sancar, A., 2003. Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. Chem. Rev. 103, 2203–2237.

Schmucker, S., Martelli, A., Colin, F., Page, A., Wattenhofer-Donzé, M., Reutenauer, L., Puccio, H., 2011. Mammalian Frataxin: An Essential Function for Cellular Viability through an Interaction with a Preformed ISCU/NFS1/ISD11 Iron-Sulfur Assembly Complex. PLoS ONE 6, e16199.

Sehadova, H., Glaser, F.T., Gentile, C., Simoni, A., Giesecke, A., Albert, J.T., Stanewsky, R., 2009. Temperature entrainment of Drosophila's circadian clock involves the gene nocte and signaling from peripheral sensory tissues to the brain. Neuron 64, 251–266.

Selby, C.P., Sancar, A., 2012. The second chromophore in Drosophila photolyase/cryptochrome family photoreceptors. Biochemistry (Mosc.) 51, 167–171.

Shalitin, D., Yang, H., Mockler, T.C., Maymon, M., Guo, H., Whitelam, G.C., Lin, C., 2002. Regulation of Arabidopsis cryptochrome 2 by blue-light-dependent phosphorylation. Nature 417, 763–767.

Shan, Y., Cortopassi, G., 2012. HSC20 interacts with frataxin and is involved in iron-sulfur cluster biogenesis and iron homeostasis. Hum. Mol. Genet. 21, 1457–1469.

Shan, Y., Napoli, E., Cortopassi, G., 2007. Mitochondrial frataxin interacts with ISD11 of the NFS1/ISCU complex and multiple mitochondrial chaperones. Hum. Mol. Genet. 16, 929–941.

Söderberg, C.A.G., Shkumatov, A.V., Rajan, S., Gakh, O., Svergun, D.I., Isaya, G., Al-Karadaghi, S., 2011. Oligomerization Propensity and Flexibility of Yeast Frataxin Studied by X-ray Crystallography and Small-Angle X-ray Scattering. J. Mol. Biol. 414, 783–797.

Song, S.-H., 2006. Photodynamics of phototropin and cryptochrome blue-light

photoreceptors. Universität Regensburg.

Song, S.-H., Dick, B., Penzkofer, A., Pokorny, R., Batschauer, A., Essen, L.-O., 2006. Absorption and fluorescence spectroscopic characterization of cryptochrome 3 from Arabidopsis thaliana. J. Photochem. Photobiol. B 85, 1–16.

Stanewsky, R., Kaneko, M., Emery, P., Beretta, B., Wager-Smith, K., Kay, S.A., Rosbash, M., Hall, J.C., 1998. The cryb mutation identifies cryptochrome as a circadian photoreceptor in Drosophila. Cell 95, 681–692.

Stemmler, T.L., Lesuisse, E., Pain, D., Dancis, A., 2010. Frataxin and Mitochondrial FeS Cluster Biogenesis. J. Biol. Chem. 285, 26737–26743.

Stillinger, F.H., Rahman, A., 2003. Improved simulation of liquid water by molecular dynamics. J. Chem. Phys. 60, 1545–1557.

Still, W.C., Tempczyk, A., Hawley, R.C., Hendrickson, T., 1990. Semianalytical treatment of solvation for molecular mechanics and dynamics. J. Am. Chem. Soc. 112, 6127–6129.

Stoleru, D., Nawathean, P., Fernández, M. de la P., Menet, J.S., Ceriani, M.F., Rosbash, M., 2007. The Drosophila Circadian Network Is a Seasonal Timer. Cell 129, 207–219.

Suzek, B.E., Huang, H., McGarvey, P., Mazumder, R., Wu, C.H., 2007. UniRef: comprehensive and non-redundant UniProt reference clusters. Bioinformatics 23, 1282–1288.

Tamada, T., Kitadokoro, K., Higuchi, Y., Inaka, K., Yasui, A., de Ruiter, P.E., Eker, A.P.M., Miki, K., 1997. Crystal structure of DMA photolyase from Anacystis nidulans. Nat. Struct. Mol. Biol. 4, 887–891.

Tamanini, F., Chaves, I., Bajek, M.I., van der Horst, G.T.J., 2007. Structure function analysis of mammalian cryptochromes. Cold Spring Harb. Symp. Quant. Biol. 72, 133–139.

Taylor, R.D., Jewsbury, P.J., Essex, J.W., 2003. FDS: flexible ligand and receptor docking with a continuum solvent model and soft-core energy function. J. Comput. Chem. 24, 1637–1656.

Thompson, C.L., Bowes Rickman, C., Shaw, S.J., Ebright, J.N., Kelly, U., Sancar, A., Rickman, D.W., 2003. Expression of the blue-light receptor cryptochrome in the human retina. Invest. Ophthalmol. Vis. Sci. 44, 4515–4521.

VanVickle-Chavez, S.J., Gelder, R.N.V., 2007. Action Spectrum of Drosophila Cryptochrome. J. Biol. Chem. 282, 10561–10566.

Verlet, L., 1967. Computer "Experiments" on Classical Fluids. I. Thermodynamical Properties of Lennard-Jones Molecules. Phys. Rev. 159, 98–103.

Wang, H., Ma, L.-G., Li, J.-M., Zhao, H.-Y., Deng, X.W., 2001. Direct Interaction of Arabidopsis Cryptochromes with COP1 in Light Control Development. Science 294, 154–158.

Wang, J., Wang, W., Kollman, P.A., Case, D.A., 2006. Automatic atom type and bond type perception in molecular mechanical calculations. J. Mol. Graph. Model. 25, 247–260.

Wang, T., Craig, E.A., 2008. Binding of Yeast Frataxin to the Scaffold for Fe-S Cluster Biogenesis, Isu. J. Biol. Chem. 283, 12674–12679.

Waterhouse, A.M., Procter, J.B., Martin, D.M.A., Clamp, M., Barton, G.J., 2009. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics 25, 1189–1191.

Wu, G., Spalding, E.P., 2007. Separate functions for nuclear and cytoplasmic cryptochrome 1 during photomorphogenesis of Arabidopsis seedlings. Proc. Natl. Acad. Sci. 104, 18813–18818.

Xu, P., Xiang, Y., Zhu, H., Xu, H., Zhang, Z., Zhang, C., Zhang, L., Ma, Z., 2008. Wheat Cryptochromes: Subcellular Localization and Involvement in Photomorphogenesis and Osmotic Stress Responses. PLANT Physiol. 149, 760–774.

Yang, H.Q., Tang, R.H., Cashmore, A.R., 2001. The signaling mechanism of Arabidopsis CRY1 involves direct interaction with COP1. Plant Cell 13, 2573–2587.

Yang, H.-Q., Wu, Y.-J., Tang, R.-H., Liu, D., Liu, Y., Cashmore, A.R., 2000. The C termini of Arabidopsis cryptochromes mediate a constitutive light response. Cell 103, 815–827.

Yang, Y.-J., Zuo, Z.-C., Zhao, X.-Y., Li, X., Klejnot, J., Li, Y., Chen, P., Liang, S.-P., Yu, X.-H., Liu, X.-M., Lin, C.-T., 2008. Blue-Light-Independent Activity of Arabidopsis Cryptochromes in the Regulation of Steady-State Levels of Protein and mRNA Expression. Mol. Plant 1, 167–177.

Yoon, T., Cowan, J.A., 2003. Iron-sulfur cluster biosynthesis. Characterization of frataxin as an iron donor for assembly of [2Fe-2S] clusters in ISU-type proteins. J. Am. Chem. Soc. 125, 6078–6084.

Yoon, T., Dizin, E., Cowan, J.A., 2007. N-terminal iron-mediated self-cleavage of human frataxin: regulation of iron binding and complex formation with target proteins. J. Biol. Inorg. Chem. JBIC Publ. Soc. Biol. Inorg. Chem. 12, 535–542.

Yoshii, T., Hermann, C., Helfrich-Förster, C., 2010. Cryptochrome-positive and -negative clock neurons in Drosophila entrain differentially to light and temperature. J. Biol. Rhythms 25, 387–398.

Yoshii, T., Todo, T., Wülbeck, C., Stanewsky, R., Helfrich-Förster, C., 2008. Cryptochrome is present in the compound eyes and a subset of Drosophila's clock neurons. J. Comp. Neurol. 508, 952–966.

Yu, X., Klejnot, J., Zhao, X., Shalitin, D., Maymon, M., Yang, H., Lee, J., Liu, X., Lopez, J., Lin, C., 2007. Arabidopsis Cryptochrome 2 Completes Its Posttranslational Life Cycle in the Nucleus. Plant Cell Online 19, 3146–3156.

Zhang, F., Scheerer, P., Oberpichler, I., Lamparter, T., Krauß, N., 2013. Crystal structure of a prokaryotic (6-4) photolyase with an Fe-S cluster and a 6,7-dimethyl-8-ribityllumazine antenna chromophore. Proc. Natl. Acad. Sci. 110, 7217–7222.

Zhu, H., Conte, F., Green, C.B., 2003. Nuclear Localization and Transcriptional Repression Are Confined to Separable Domains in the Circadian Protein CRYPTOCHROME. Curr. Biol. 13, 1653–1658.

Zuo, Z.-C., Meng, Y.-Y., Yu, X.-H., Zhang, Z.-L., Feng, D.-S., Sun, S.-F., Liu, B., Lin, C.-T., 2012. A study of the blue-light-dependent phosphorylation, degradation, and photobody formation of Arabidopsis CRY2. Mol. Plant 5, 726–733.

Zuo, Z., Liu, H., Liu, B., Liu, X., Lin, C., 2011. Blue Light-Dependent Interaction of CRY2 with SPA1 Regulates COP1 activity and Floral Initiation in Arabidopsis. Curr. Biol. 21, 841–847.

5. Overview - Frataxins

5.1 Iron Binding Related Functions

Frataxin is a mitochondrial protein present from bacteria to humans (Adinolfi et al., 2009) (Huang et al., 2008) and even plants (Busi et al., 2004). It is widely accepted that frataxin is involved in iron metabolism. The inability to produce frataxin, leading to disruption of cellular iron homeostasis, has been atributed to Friedreich's ataxia (FRDA), an autosomal recessive cardio- and neurodegenerative disorder (Delatycki et al., 2000). In fact, the metabolic defect in FRDA causes iron loading resulting from mitochondrial iron storage pathway deregulation (Huang et al., 2009).

The exact function of frataxins in iron metabolism remains unclear. One emerging consensus is that frataxin may function as iron chaperone or "donor" interacting with various partners in an iron-dependent manner. Frataxins interact with the scaffolding protein Isu, iron, and the cysteine desulfurase Nfs1, centralizing them in the mitochondrial Fe-S cluster (Stemmler et al., 2010). It has been demonstrated that frataxin facilitates iron use for Fe-S cluster formation on Isu (Yoon and Cowan, 2003) (Kondapalli et al., 2008). Human, bacterial and yeast monomeric frataxins have been demonstrated to bind 7, 2 and 2 Fe(II) atoms, respectively (Bou-Abdallah et al., 2004) (Cook et al., 2006) (Yoon and Cowan, 2003). Ferrous iron concentration is key in the interaction of yeast frataxin (Yfh1) to the scaffolding proteins (Isu) and the cysteine desulfurase (Nfs1) (Pandolfo and Pastore, 2009). Yeast frataxin homolog (Yfh1) has been shown to indirectly regulate iron uptake by interacting and controlling the activity of a metalloprotease involved in iron utilizing proteins (Branda et al., 1999). Also bacterial frataxin CyaY has iron donor function during [Fe-S] cluster assembly on the scaffold protein IscU in the presence of IscS and cysteine (Yoon and Cowan, 2003). Another study, however, has found that CyaY binds IscS (of the IscS/IscU complex) and the recognition does not require iron, occuring through electrostatic interactions with complementary charged residues (Prischi et al., 2010). Similarly, also human frataxin interacts with either Isu or ferrochelatase in an iron-dependent manner (Pandolfo and Pastore, 2009). Mammalian frataxin has also been shown to interact to the mitochondrial chaperone HSC20 in an iron-dependent manner, increasing the players in

the iron-sulfur complex pathway (Shan and Cortopassi, 2012). The N-terminus of human frataxin has been shown to have a key role in the Fe-S cluster biosynthesis pathway in mitochondria, targeted to the mitochondrial matrix by a N-terminal mitochondrial import signal (Yoon et al., 2007). The N-terminal region is required for high affinity iron coordination and iron assembly of Fe-S clusters by ISCU as part of the Fe-S cluster biosynthetic complex (Gentry et al., 2013). Surprisingly, iron inhibited the interaction between mammal frataxin and ISD11, which has been demonstrated to be a component of the eukaryotic Nfs1/ISCU iron–sulfur biogenesis complex. Nickel, instead, increased frataxin/ISD11 binding (Shan et al., 2007). In addition, another study has found a non iron-dependent interaction of mammalian frataxin to the preformed ISCU/NFS1/ISD11 iron-sulfur assembly complex (Schmucker et al., 2011), rather than interacting with them individually. The iron chaperone function is further identified in *Drosophila* model organism. *Drosophila* frataxin homologue (Dfh) can delivery Fe(II) to Isu, which is required for Fe–S cluster biosynthesis (Kondapalli et al., 2008).

Furthermore, in line with iron chaperone idea, frataxins can interact to other proteins in the Fe-S cluster assembly. Yeast frataxin has been shown to interact to aconitase, preventing the aconitase $[4Fe\text{-}4S]^{2+}$ cluster from disassembly, by transfering Fe(II) directly to aconitase (Bulteau et al., 2004). Also, yeast frataxin mediates iron use by ferrochelatase in haeme synthesis (He et al., 2004) (Lesuisse et al., 2003). Moreover, a key role of frataxin in the mitochondrial electron transport chain in humans has been suggested. Physical interaction between yeast and human frataxin with succinate dehydrogenase complex subunits confirmed a functional relationship between them (González-Cabo et al., 2005). Iron-specific and Fe-S cluster phenotypes have also been atributed to frataxins as a result of forced expression of mitochondrial ferritin (Campanella et al., 2004).

The bacterial frataxin ortholog CyaY, however, has been suggested to function as iron sensor regulating Fe-S cluster formation. It is proposed to fine-tune the quantity of Fe-S formed to the availability of the apo acceptors (Adinolfi et al., 2009). This sensor role for frataxins is consistent with their low iron binding affinities. It has been observed that bacterial frataxin ortholog CyaY has relatively modest iron affinity (Nair et al., 2004), with human frataxin showing even weaker binding affinity (Yoon and Cowan, 2003). In addition, a solution structure of human frataxin has been demonstrated having

no iron-binding and aggregation tendencies (Musco et al., 2000).

5.2 Oligomers/Monomers Formation

In additon to iron chaperone, another hypothesis suggests that frataxins may function as iron scavengers. It has been proposed that recombinant yeast frataxin (mYfh1p) can self-assembly in an iron-dependent manner, indicating a physiological role for frataxin in mitochondrial iron sequestration and bioavailability. Although yeast frataxin has iron-dependent assemblying characteristics, the authors have not detected any iron bound to yeast monomers, indicating that two or more subnunits are necessary to initial Fe^{2+} binding. In fact, mYfh1p trimer has been the smallest iron-containing species detected (Adamec et al., 2000). However, another study has found that the presence of magnesium or calcium salts, in mitochondrial physiological concentrations, can stabilize Yfh1 in the iron bound monomeric state against oligomerization. It is believed that the environmental conditions to bind the metal together with low iron binding affinity, is in agreement with frataxin's role as iron chaperone (Cook et al., 2006). Similarly, the bacterial frataxin ortholog CyaY also is predominantly monomeric up to to 1 : 20 protein-to-iron concentration, showing a small amount of agregate at a 1 : 10 ratio (Adinolfi et al., 2002).

In contrast, human frataxin (HsFtx) monomer only minimally assembled in presence of increasing iron concentrations *in vitro,* but showed normal assemblying in physiological concentrations. It has been suggested that human frataxin has structural and functional features of an authentic iron storage protein. Although monomeric HsFtx does not self-assemble even at high iron concentrations, a ~59-mer HsFtx homooligomer can bind approximately 10 iron atoms under aerobic and salt-free conditions (Cavadini et al., 2002). The N-terminal tail of human frataxin, which is not conserved throughout frataxins evolution, has been suggested to participate in the stabilization of a competent state for its oligomerization process (Faraj et al., 2013). In fact, HsFtx N-terminal region seems play an important role in iron binding agregation. The full-length mature HsFtx (residues 56 to 210) will self-assemble in the absence of metal, while the truncated form (lacking the initial 22 residues) does not self-assemble in absence of iron (O'Neill et al., 2005) (Cavadini et al., 2002).

Frataxin's iron storage hypothesis is further consistent when considering its ability to homooligomerize. Yfh1 forms a 48-multimeric homooligomer that can bind up to 50 iron atoms per protein monomer, when under high metal-to-protein stoichiometries, in the presence of oxygen and the absence of salt (Gakh et al., 2002). In a similar size and iron loading capacity, bacterial frataxin CyaY can also agregate under low-salt, aerobic, and iron-overloaded solution conditions (Adinolfi et al., 2002). Furthermore, another study has shown that CyaY tetramers can be formed when Fe(II) is added anaerobically and multiple protein aggregates are formed upon oxidation of the bound Fe(II), indicating a protective role in mitochondria against iron-induced oxidative damage (Bou-Abdallah et al., 2004).

5.3 Structural Aspects

Frataxins from bacteria, yeast and human show high structural similarity. The basic structure is composed of two α-helices at the N- and C-terminal ends of the protein that are flanked by an antiparallel β-sheet of five to seven strands in a 'sandwich' motif (Pandolfo and Pastore, 2009).

Besides oligomerization, the N-terminal tail of human frataxin can also have stability and folding functions. ANS fluorescence emission studies have demonstrated that a non-covalently interaction between the N-terminus tail and the rest of the protein is possible, indicating an N-terminal conformational plasticity (Faraj et al., 2013). In fact, a previous report have indicated that mature human frataxin has a flexible and an intrinsically unfolded N-terminal tail (Prischi et al., 2009). It is also thought that the N-terminus prevents frataxin from binding and chaperoning Fe to its target proteins through the interaction of the positive charges of this region, that interact electrostatically with unique carboxyl rich iron-binding sites. The N-terminal region is believed to play an iron-mediated cleavage reaction, exposing anionic Fe binding sites in the core protein and converting frataxin into its functional state (Yoon et al., 2007). The N-terminus of yeast frataxin has also shown to have a key role on oligomerization. It has been demonstrated that the N-terminal region is highly flexible and the main building block (trimer) is the result of the stabilization of the N-terminus with its neighboring monomers (Söderberg et al., 2011). Differently than eukaryotes frataxins,

bacterial frataxin does not have the N-terminal extension attributed to a mitochondrial import signal (Nair et al., 2004).

Regarding iron binding sites, solution studies in bacterial frataxin CyaY have demonstrated the involvement of the negatively charged residues in α1 and β1 (a semi-conserved acidic ridge) in cation binding. Geometrical observations indicated that the residues involved are surface exposed, with the involvement of the side chains of Asp and Glu residues. The authors further suggest that the binding mechanism is exclusively electrostatically driven and the overall charge density is more important, rather than precise positions of negatively charged residues (Pastore et al., 2007). In addition, the residues Glu18, Glu19 and Asp22, which are spatially adjacent and part of the conserved negatively charged patch, have been shown to be relevant in iron promoted protein agregation. Considering that CyaY's agregation is iron-specific, mutation studies demonstrated that these residues are key to bacterial frataxin agregation and Fe^{2+} ion binding to the monomers (Nair et al., 2004) (Adinolfi et al., 2002). Also in yeast frataxin, acidic residues D86, E90 and E93 have showed oligomerization role *in vitro,* but these residues are not related with binding to the scaffold protein Isu (Aloria et al., 2004). In another study residues D86, E89, E90, D101 and E103 in the acidic ridge affected iron detoxification but have not affected the iron chaperone function of the protein (Gakh et al., 2006). However, in another study the authors found that D86, E89, D101 and E103 act cooperatively in the interaction between Yfh1 and Isu1 *in vivo*, suggesting that in fact assembly of Fe–S clusters is the primary event of the conserved acidic ridge, with iron detoxification being the second event (Foury et al., 2007). Structural and conformational aspects have also been atributed to the acidic ridge between α-helix 1 and β-sheet 1 in yeast frataxin. Alteration in five residues in the acidic ridge, has been reported to increase Yfh1's resistance to thermal denaturation (i.e. stability) and resistance to proteolytic degradation (i.e. conformational plasticity), suggesting this iron-binding region is susceptible to an activity-stability trade off (Correia et al., 2010). The authors suggest that this structure–function compromise might facilitate binding to the ISC complex partners (Correia et al., 2010). In human frataxin, the acidic ridge has been associated with the preformed ISCU/NFS1/ISD11 complex. More specifically, the authors suggest that part of the acidic ridge (residues E108, E111 and D124) could be involved with interaction with NFS1 (Schmucker et al.,

2011).

5.4 Methods

Molecular Dynamics (MD) simulations are based on classical mechanics and provides information on the microscopic dynamic behavior, depending on time and the individual atoms that make up the system (Höltje, 2008).

Based on molecular mechanics (MM), molecules are treated as a collection of atoms that can be described by newtonian forces, or are treated as a collection of particles held together by harmonic or elastic forces. A complete set of interaction potentials between the particles is referred as a "force field" (III et al., 2007). The fundamentals of force field have been writen in a previous section of this thesis.

1) *Classical Molecular Dynamics*

Molecular Dynamics simulations are based on numerical solutions, step by step, by the equation of motion that can be described for a simple atomic system by:

$$F_i = m_i a_i$$

Where Fi is the force acting on each particle of the system in an instant of time t, and ai is the acceleration of atom i of mass m_i. Once defined the forde field, it is possible to calculate the forces on each atom, by calculating the first derivative of the potential energy, obtained from the chosen force field in relation to the positions of those atoms. Particles acceleration can be obtained by:

$$-\frac{dV}{dr_i} = m_i \frac{d^2 r_i}{dt^2}$$

From this, by integrating the equation of motions, we can obtain the velocities, which provides the position change of the atom. With the new positions and velocities of each particle, you have the potential and kinetic energies of the system. Applying this procedure successively, we obtain what is called the "trajectory" that is the set of positions and velocities of each particle over time.

2) *Integration of the equation of motion*

The integration of the equations of motion is performed using algorithms based

on the finite difference methods, where integration is divided into small time intervals (steps Integration), δt, allowing to simulate the movements with higher frequency of the system, which are mainly the vibrations of the bonds. One of the most used methods in molecular dynamics to integrate the equation of motion is the Verlet algorithm (Verlet, 1967), which uses the positions and accelerations in specific time t of the atoms in the positions from the previous step, r (t -δt) to determine new positions at the time t+δt, through the equation:

$$r(t + \delta t) = 2r(t) - r(t - \delta t) + a(t)\delta t^2$$

3) *Statistical ensemble*

An ensemble is the set of configurations and properties maintained constant during the integration of Newton's equations representing the state system (Allen and Tildesley, 1989). One of the first ensemble options is the micro-canonical or NVE (number of particles, volume and total energy constant during the simulation). Other alternatives to this ensemble have been developed, where one can separately control the temperature, T, and pressure, P, instead of the total energy (E).

4) *Initial conditions, energy minimization and equilibrium*

It is appropriate initially to locate the particles in a cristaline network position, which avoids overlaping between them, by generating a simulation box that can adopt different geometries. Simulation results are practically independent of the type of geometry used for the simulation boxes. The most commonly used are cubic, the parallelepiped and truncated octahedral.

Before starting the MD simulations, the system must be minimized to eliminate bad contacts between atoms. Energy minimization, also known as the geometry optimization is a technique which aims to find a set of coordinates that minimize the potential energy of the system of interest. By adjusting the atomic positions, the process relaxes the distortions in the chemical bonds, in the angles between bonds and van der Waals contacts . The minimized system has small forces acting on each atom and therefore is suitable as a starting point to start the MD simulations.

After energy minimization of the system, it is gradually heated to the temperature (T0) of interest, assigning initial velocities to all particles by a Maxwell-Boltzmann

distribution.

To adjust the initial conditions to the termodynamics equilibrium, the first steps of a MD constitute the "equilibrium period ". In this phase the sytem properties are not constant. From that point it is possible to generate MD trajectories and calculate the different properties for the system of interest.

5) *Constraints and Restraints*

Molecular systems have movements that occur at different time scales, considering both intramolecular and intermolecular forces. Usually the latter are in higher order of magnitude than the first, so that the vibrational movements are much faster than the translational and rotational ones. Thus the integration steps should be increased, resulting in a substantial increase in computational time. However, if the fastest movements are frozen (subtracted from calculation of interactions), constraining the degrees of freedom corresponding to the more frequent movements, one can achieve greater efficiency through the use of a larger integration time step in the equation of motion substantially reducing the computational cost (Frenkel and Smit, 2002). The constraints algorithms most commonly used are SHAKE (Ryckaert et al., 1977) and LINCS (Hess et al., 1997).

6) *Periodic boundary conditions*

In MD simulations, the systems typically have 10^2-10^6 particles, which is insignificant compared to the number of atoms contained in macroscopic systems (fractions of Avogadro's number). Thus, in MD simulations the number particles on the surface of the system is much higher than macroscopic system, causing undesirable surface effects. To minimize the surface effects and obtain the macroscopic properties from trajectories of MD simulations, periodic boundary conditions are used (Frenkel and Smit, 2002). Thus, surface effects are eliminated and the considered particles feel forces effect as if they were inside the system. In this technique, atoms of the system are placed in a box (usually cubic), and the original box is replicated in all directions of space. During the simulations, when an atom moves in the original box, its periodic image in one of the image boxes moves in the same way. If an atom get out of the original box, its image enters the opposite face of the image box at the same speed. Thus, the total number of atoms in the center box and the system as a whole is maintained.

7) *Molecular Dynamics of solvated systems*

There are two solvation models that can be used to perform MD simulations of solvated systems: the explicit and implicit solvation models. In the former, hundreds or thousands of solvent molecules are incorporated explicitly into the system as an additional component. Various potentials were developed and parameterized to explicitily describe water molecules. The most commonly used are the TIP family (Transferable Intermolecular Potentials), developed by Jorgensen and colleagues (Jorgensen et al., 1983), the simple point charge (Berendsen et al., 1987), and the ST2 model (Stillinger and Rahman, 2003).

In some cases, such as systems with large proteins, it is necessary to use implicit solvation models, reducing the computational cost of simulations. In general, an implicit solvation treatment considers the average influence of the solvent through direct solvation free energy estimates, defined as the reversible work necessary to transfer the solute in a fixed configuration from the vacuum to the solution. The method does not consider the degrees of freedom of the solvent explicitly, but they are treated as continuous medium. Empirical methods such as solvent accessible surface area (SASA) (Ferrara et al., 2002), are quick and easy ways for energy solvation evaluation with accuracy comparable to theoretical models. In the SASA approach, the free energy of solvation of the solute is expressed as the sum of the atomic contributions, weighted by their areas exposed to the solvent. The contribution of each atom is quantified by a surface coefficient, which reflects the hydrophobicity or hydrophilicity of the type of atom considered.

The continuous model of solvation has been used to describe the electrostatic solvation. In this approach the solute is considered an embedded cavity in a dielectric medium. The corresponding electrostatic solvation free energy can be calculated accurately by the Poisson-Boltzmann (PB) equation (Gilson et al., 1993) (Baker, 2005), or by the generalized Born model (GB) (Still et al., 1990)(Bashford and Case, 2000). In studies with macromolecules associated to ligands, the generalized Born model is the most commonly used, due of its lower computational cost (Majeux et al., 1999) (Liu et al., 2004) (Taylor et al., 2003).

The GB / SA combination has been recognized as an excellent choice for the implicit treatment of solvation in biomolecular simulations (Feig and Brooks, 2004).

Optimization of implicit solvation methos have been implemented in programs of molecular modelling like CHARMM (MacKerell et al., 1998) (Brooks et al., 1983) and AMBER (Case et al., 2005).

5.5 References

Adamec, J., Rusnak, F., Owen, W.G., Naylor, S., Benson, L.M., Gacy, A.M., Isaya, G., 2000. Iron-dependent self-assembly of recombinant yeast frataxin: implications for Friedreich ataxia. Am. J. Hum. Genet. 67, 549–562.

Adinolfi, S., Iannuzzi, C., Prischi, F., Pastore, C., Iametti, S., Martin, S.R., Bonomi, F., Pastore, A., 2009. Bacterial frataxin CyaY is the gatekeeper of iron-sulfur cluster formation catalyzed by IscS. Nat. Struct. Mol. Biol. 16, 390–396.

Adinolfi, S., Trifuoggi, M., Politou, A.S., Martin, S., Pastore, A., 2002. A structural approach to understanding the iron-binding properties of phylogenetically different frataxins. Hum. Mol. Genet. 11, 1865–1877.

Allen, M.P., Tildesley, D.J., 1989. Computer simulation of liquids. Clarendon Press ; Oxford University Press, Oxford [England]; New York.

Aloria, K., Schilke, B., Andrew, A., Craig, E.A., 2004. Iron-induced oligomerization of yeast frataxin homologue Yfh1 is dispensable in vivo. EMBO Rep. 5, 1096–1101.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Baker, N.A., 2005. Improving implicit solvent simulations: a Poisson-centric view. Curr. Opin. Struct. Biol. 15, 137–143.

Banerjee, R., Schleicher, E., Meier, S., Viana, R.M., Pokorny, R., Ahmad, M., Bittl, R., Batschauer, A., 2007. The Signaling State of Arabidopsis Cryptochrome 2 Contains Flavin Semiquinone. J. Biol. Chem. 282, 14916–14922.

Bashford, D., Case, D.A., 2000. Generalized born models of macromolecular solvation effects. Annu. Rev. Phys. Chem. 51, 129–152.

Beel, B., Prager, K., Spexard, M., Sasso, S., Weiss, D., Müller, N., Heinnickel, M., Dewez, D., Ikoma, D., Grossman, A.R., Kottke, T., Mittag, M., 2012. A Flavin

Binding Cryptochrome Photoreceptor Responds to Both Blue and Red Light in Chlamydomonas reinhardtii. Plant Cell Online 24, 2992–3008.

Berendsen, H.J.C., Grigera, J.R., Straatsma, T.P., 1987. The missing term in effective pair potentials. J. Phys. Chem. 91, 6269–6271.

Berndt, A., Kottke, T., Breitkreuz, H., Dvorsky, R., Hennig, S., Alexander, M., Wolf, E., 2007. A novel photoreaction mechanism for the circadian blue light photoreceptor Drosophila cryptochrome. J. Biol. Chem. 282, 13011–13021.

Biskup, T., Hitomi, K., Getzoff, E.D., Krapf, S., Koslowski, T., Schleicher, E., Weber, S., 2011. Unexpected Electron Transfer in Cryptochrome Identified by Time-Resolved EPR Spectroscopy. Angew. Chem. Int. Ed. 50, 12647–12651.

Biskup, T., Paulus, B., Okafuji, A., Hitomi, K., Getzoff, E.D., Weber, S., Schleicher, E., 2013. Variable Electron-Transfer Pathways in an Amphibian Cryptochrome: Tryptophan versus Tyrosine-Based Radical Pairs. J. Biol. Chem.

Bou-Abdallah, F., Adinolfi, S., Pastore, A., Laue, T.M., Dennis Chasteen, N., 2004. Iron binding and oxidation kinetics in frataxin CyaY of Escherichia coli. J. Mol. Biol. 341, 605–615.

Bouly, J.-P., Schleicher, E., Dionisio-Sese, M., Vandenbussche, F., Straeten, D.V.D., Bakrim, N., Meier, S., Batschauer, A., Galland, P., Bittl, R., Ahmad, M., 2007. Cryptochrome Blue Light Photoreceptors Are Activated through Interconversion of Flavin Redox States. J. Biol. Chem. 282, 9383–9391.

Branda, S.S., Yang, Z.Y., Chew, A., Isaya, G., 1999. Mitochondrial intermediate peptidase and the yeast frataxin homolog together maintain mitochondrial iron homeostasis in Saccharomyces cerevisiae. Hum. Mol. Genet. 8, 1099–1110.

Brautigam, C.A., Smith, B.S., Ma, Z., Palnitkar, M., Tomchick, D.R., Machius, M., Deisenhofer, J., 2004. Structure of the photolyase-like domain of cryptochrome 1 from Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A. 101, 12142–12147.

Brooks, B.R., Brooks, C.L., 3rd, Mackerell, A.D., Jr, Nilsson, L., Petrella, R.J., Roux, B., Won, Y., Archontis, G., Bartels, C., Boresch, S., Caflisch, A., Caves, L., Cui, Q., Dinner, A.R., Feig, M., Fischer, S., Gao, J., Hodoscek, M., Im, W., Kuczera, K., Lazaridis, T., Ma, J., Ovchinnikov, V., Paci, E., Pastor, R.W., Post, C.B., Pu, J.Z., Schaefer, M., Tidor, B., Venable, R.M., Woodcock, H.L., Wu, X., Yang, W., York, D.M., Karplus, M., 2009. CHARMM: the biomolecular simulation

program. J. Comput. Chem. 30, 1545–1614.

Brooks, B.R., Bruccoleri, R.E., Olafson, B.D., States, D.J., Swaminathan, S., Karplus, M., 1983. CHARMM: A program for macromolecular energy, minimization, and dynamics calculations. J. Comput. Chem. 4, 187–217.

Brudler, R., Hitomi, K., Daiyasu, H., Toh, H., Kucho, K., Ishiura, M., Kanehisa, M., Roberts, V.A., Todo, T., Tainer, J.A., Getzoff, E.D., 2003. Identification of a New Cryptochrome Class: Structure, Function, and Evolution. Mol. Cell 11, 59–67.

Bulteau, A.-L., O'Neill, H.A., Kennedy, M.C., Ikeda-Saito, M., Isaya, G., Szweda, L.I., 2004. Frataxin Acts as an Iron Chaperone Protein to Modulate Mitochondrial Aconitase Activity. Science 305, 242–245.

Busi, M.V., Zabaleta, E.J., Araya, A., Gomez-Casati, D.F., 2004. Functional and molecular characterization of the frataxin homolog from Arabidopsis thaliana. FEBS Lett. 576, 141–144.

Busino, L., Bassermann, F., Maiolica, A., Lee, C., Nolan, P.M., Godinho, S.I.H., Draetta, G.F., Pagano, M., 2007. SCFFbxl3 controls the oscillation of the circadian clock by directing the degradation of cryptochrome proteins. Science 316, 900–904.

Busza, A., Emery-Le, M., Rosbash, M., Emery, P., 2004. Roles of the Two Drosophila CRYPTOCHROME Structural Domains in Circadian Photoreception. Science 304, 1503–1506.

Campanella, A., Isaya, G., O'Neill, H.A., Santambrogio, P., Cozzi, A., Arosio, P., Levi, S., 2004. The expression of human mitochondrial ferritin rescues respiratory function in frataxin-deficient yeast. Hum. Mol. Genet. 13, 2279–2288.

Case, D.A., Cheatham, T.E., 3rd, Darden, T., Gohlke, H., Luo, R., Merz, K.M., Jr, Onufriev, A., Simmerling, C., Wang, B., Woods, R.J., 2005. The Amber biomolecular simulation programs. J. Comput. Chem. 26, 1668–1688.

Case, D.A., Cheatham, T.E., Darden, T., Gohlke, H., Luo, R., Merz, K.M., Onufriev, A., Simmerling, C., Wang, B., Woods, R.J., 2005. The Amber biomolecular simulation programs. J. Comput. Chem. 26, 1668–1688.

Cavadini, P., O'Neill, H.A., Benada, O., Isaya, G., 2002. Assembly and iron-binding properties of human frataxin, the protein deficient in Friedreich ataxia. Hum.

Mol. Genet. 11, 217–227.

Ceriani, M.F., Darlington, T.K., Staknis, D., Más, P., Petti, A.A., Weitz, C.J., Kay, S.A., 1999. Light-dependent sequestration of TIMELESS by CRYPTOCHROME. Science 285, 553–556.

Chaves, I., Pokorny, R., Byrdin, M., Hoang, N., Ritz, T., Brettel, K., Essen, L.-O., van der Horst, G.T.J., Batschauer, A., Ahmad, M., 2011. The cryptochromes: blue light photoreceptors in plants and animals. Annu. Rev. Plant Biol. 62, 335–364.

Chaves, I., Yagita, K., Barnhoorn, S., Okamura, H., van der Horst, G.T.J., Tamanini, F., 2006. Functional evolution of the photolyase/cryptochrome protein family: importance of the C terminus of mammalian CRY1 for circadian core oscillator performance. Mol. Cell. Biol. 26, 1743–1753.

Chothia, C., Lesk, A.M., 1986. The relation between the divergence of sequence and structure in proteins. EMBO J. 5, 823–826.

Coesel, S., Mangogna, M., Ishikawa, T., Heijde, M., Rogato, A., Finazzi, G., Todo, T., Bowler, C., Falciatore, A., 2009. Diatom PtCPF1 is a new cryptochrome/photolyase family member with DNA repair and transcription regulation activity. EMBO Rep. 10, 655–661.

Collins, B.H., Dissel, S., Gaten, E., Rosato, E., Kyriacou, C.P., 2005. Disruption of Cryptochrome partially restores circadian rhythmicity to the arrhythmic period mutant of Drosophila. Proc. Natl. Acad. Sci. U. S. A. 102, 19021–19026.

Collins, B., Mazzoni, E.O., Stanewsky, R., Blau, J., 2006. Drosophila CRYPTOCHROME is a circadian transcriptional repressor. Curr. Biol. CB 16, 441–449.

Cook, J.D., Bencze, K.Z., Jankovic, A.D., Crater, A.K., Busch, C.N., Bradley, P.B., Stemmler, A.J., Spaller, M.R., Stemmler, T.L., 2006. Monomeric Yeast Frataxin is an Iron Binding Protein. Biochemistry (Mosc.) 45, 7767–7777.

Correia, A.R., Wang, T., Craig, E.A., Gomes, C.M., 2010a. Iron binding activity in yeast frataxin entails a trade off with stability in the ?1/?1 acidic ridge region. Biochem. J. 426, 197–203.

Correia, A.R., Wang, T., Craig, E.A., Gomes, C.M., 2010b. Iron binding activity in yeast frataxin entails a trade off with stability in the ?1/?1 acidic ridge region. Biochem. J. 426, 197–203.

Dardente, H., Fortier, E.E., Martineau, V., Cermakian, N., 2007. Cryptochromes impair phosphorylation of transcriptional activators in the clock: a general mechanism for circadian repression. Biochem. J. 402, 525.

Dayhoff, M.O., Schwartz, R.M., 1978. Chapter 22: A model of evolutionary change in proteins, in: In Atlas of Protein Sequence and Structure.

Delatycki, M.B., Williamson, R., Forrest, S.M., 2000. Friedreich ataxia: an overview. J. Med. Genet. 37, 1–8.

Del Pozo, A., Vera, L.M., Sánchez, J.A., Sánchez-Vázquez, F.J., 2012. Molecular cloning, tissue distribution and daily expression of cry1 and cry2 clock genes in European seabass (Dicentrarchus labrax). Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 163, 364–371.

Dissel, S., Codd, V., Fedic, R., Garner, K.J., Costa, R., Kyriacou, C.P., Rosato, E., 2004a. A constitutively active cryptochrome in Drosophila melanogaster. Nat. Neurosci. 7, 834–840.

Dissel, S., Codd, V., Fedic, R., Garner, K.J., Costa, R., Kyriacou, C.P., Rosato, E., 2004b. A constitutively active cryptochrome in Drosophila melanogaster. Nat. Neurosci. 7, 834–840.

Dunlap, J.C., 1999. Molecular Bases for Circadian Clocks. Cell 96, 271–290.

Eckel-Mahan, K., Sassone-Corsi, P., 2013. Metabolism and the Circadian Clock Converge. Physiol. Rev. 93, 107–135.

Emery, P., So, W.V., Kaneko, M., Hall, J.C., Rosbash, M., 1998. CRY, a Drosophila clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. Cell 95, 669–679.

Engelen, E., Janssens, R.C., Yagita, K., Smits, V.A.J., van der Horst, G.T.J., Tamanini, F., 2013. Mammalian TIMELESS Is Involved in Period Determination and DNA Damage-Dependent Phase Advancing of the Circadian Clock. PLoS ONE 8, e56623.

Faraj, S.E., Venturutti, L., Roman, E.A., Marino-Buslje, C.B., Mignone, A., Tosatto, S.C.E., Delfino, J.M., Santos, J., 2013. The role of the N-terminal tail for the oligomerization, folding and stability of human frataxin. FEBS Open Bio 3, 310–320.

Feig, M., Brooks, C.L., 3rd, 2004. Recent advances in the development and application

of implicit solvent models in biomolecule simulations. Curr. Opin. Struct. Biol. 14, 217–224.

Ferrara, P., Apostolakis, J., Caflisch, A., 2002. Evaluation of a fast implicit solvent model for molecular dynamics simulations. Proteins 46, 24–33.

Finn, R.D., Mistry, J., Schuster-Böckler, B., Griffiths-Jones, S., Hollich, V., Lassmann, T., Moxon, S., Marshall, M., Khanna, A., Durbin, R., Eddy, S.R., Sonnhammer, E.L.L., Bateman, A., 2006. Pfam: clans, web tools and services. Nucleic Acids Res. 34, D247–D251.

Fogle, K.J., Parson, K.G., Dahm, N.A., Holmes, T.C., 2011. CRYPTOCHROME Is a Blue-Light Sensor That Regulates Neuronal Firing Rate. Science 331, 1409–1413.

Foley, L.E., Gegear, R.J., Reppert, S.M., 2011. Human cryptochrome exhibits light-dependent magnetosensitivity. Nat. Commun. 2, 356.

Foury, F., Pastore, A., Trincal, M., 2007. Acidic residues of yeast frataxin have an essential role in Fe-S cluster assembly. EMBO Rep. 8, 194–199.

Frenkel, D., Smit, B., 2002. Understanding molecular simulation from algorithms to applications. Academic Press, San Diego.

Fujihashi, M., Numoto, N., Kobayashi, Y., Mizushima, A., Tsujimura, M., Nakamura, A., Kawarabayasi, Y., Miki, K., 2007. Crystal Structure of Archaeal Photolyase from Sulfolobus tokodaii with Two FAD Molecules: Implication of a Novel Light-harvesting Cofactor. J. Mol. Biol. 365, 903–910.

Gakh, O., Adamec, J., Gacy, A.M., Twesten, R.D., Owen, W.G., Isaya, G., 2002. Physical Evidence that Yeast Frataxin Is an Iron Storage Protein†. Biochemistry (Mosc.) 41, 6798–6804.

Gakh, O., Park, S., Liu, G., Macomber, L., Imlay, J.A., Ferreira, G.C., Isaya, G., 2006. Mitochondrial iron detoxification is a primary function of frataxin that limits oxidative damage and preserves cell longevity. Hum. Mol. Genet. 15, 467–479.

Gao, P., Yoo, S.-H., Lee, K.-J., Rosensweig, C., Takahashi, J.S., Chen, B.P., Green, C.B., 2013. Phosphorylation of the Cryptochrome 1 C-terminal tail regulates circadian period length. J. Biol. Chem. jbc.M113.509604.

Gegear, R.J., Casselman, A., Waddell, S., Reppert, S.M., 2008. Cryptochrome mediates light-dependent magnetosensitivity in Drosophila. Nature 454, 1014–1018.

Gentry, L.E., Thacker, M.A., Doughty, R., Timkovich, R., Busenlehner, L.S., 2013. His86 from the N-terminus of frataxin coordinates iron and is required for Fe-S cluster synthesis. Biochemistry (Mosc.) 52, 6085–6096.

Gilson, M.K., Davis, M.E., Luty, B.A., McCammon, J.A., 1993. Computation of electrostatic forces on solvated molecules using the Poisson-Boltzmann equation. J. Phys. Chem. 97, 3591–3600.

Glas, A.F., Maul, M.J., Cryle, M., Barends, T.R.M., Schneider, S., Kaya, E., Schlichting, I., Carell, T., 2009. The archaeal cofactor F0 is a light-harvesting antenna chromophore in eukaryotes. Proc. Natl. Acad. Sci.

Glossop, N.R.J., Hardin, P.E., 2002. Central and peripheral circadian oscillator mechanisms in flies and mammals. J. Cell Sci. 115, 3369–3377.

González-Cabo, P., Vázquez-Manrique, R.P., García-Gimeno, M.A., Sanz, P., Palau, F., 2005. Frataxin interacts functionally with mitochondrial electron transport chain proteins. Hum. Mol. Genet. 14, 2091–2098.

Griffin, E.A., Staknis, D., Weitz, C.J., 1999. Light-Independent Role of CRY1 and CRY2 in the Mammalian Circadian Clock. Science 286, 768–771.

Guex, N., Peitsch, M.C., 1997. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis 18, 2714–2723.

Guo, H., Duong, H., Ma, N., Lin, C., 1999. The Arabidopsis blue light receptor cryptochrome 2 is a nuclear protein regulated by a blue light-dependent post-transcriptional mechanism. Plant J. Cell Mol. Biol. 19, 279–287.

Hall, J.C., 2000. Cryptochromes: sensory reception, transduction, and clock functions subserving circadian systems. Curr. Opin. Neurobiol. 10, 456–466.

Hamm-Alvarez, S., Sancar, A., Rajagopalan, K.V., 1989. Role of enzyme-bound 5,10-methenyltetrahydropteroylpolyglutamate in catalysis by Escherichia coli DNA photolyase. J. Biol. Chem. 264, 9649–9656.

Hao Zheng, Ng, F., Yixiao Liu, Hardin, P.E., 2008. Spatial and circadian regulation of cry in Drosophila. J. Biol. Rhythms 23, 283–295.

Harada, Y., Sakai, M., Kurabayashi, N., Hirota, T., Fukada, Y., 2005. Ser-557-phosphorylated mCRY2 Is Degraded upon Synergistic Phosphorylation by Glycogen Synthase Kinase-3β. J. Biol. Chem. 280, 31714–31721.

Harris, S.-R., Henbest, K.B., Maeda, K., Pannell, J.R., Timmel, C.R., Hore, P.J.,

Okamoto, H., 2009. Effect of magnetic fields on cryptochrome-dependent responses in Arabidopsis thaliana. J. R. Soc. Interface 6, 1193–1205.

Helfrich-Förster, C., Winter, C., Hofbauer, A., Hall, J.C., Stanewsky, R., 2001. The circadian clock of fruit flies is blind after elimination of all known photoreceptors. Neuron 30, 249–261.

Hemsley, M.J., Mazzotta, G.M., Mason, M., Dissel, S., Toppo, S., Pagano, M.A., Sandrelli, F., Meggio, F., Rosato, E., Costa, R., Tosatto, S.C.E., 2007. Linear motifs in the C-terminus of D. melanogaster cryptochrome. Biochem. Biophys. Res. Commun. 355, 531–537.

Hess, B., Bekker, H., Berendsen, H.J.C., Fraaije, J.G.E.M., 1997. LINCS: A linear constraint solver for molecular simulations. J. Comput. Chem. 18, 1463–1472.

Hess, B., Kutzner, C., van der Spoel, D., Lindahl, E., 2008. GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation. J. Chem. Theory Comput. 4, 435–447.

He, Y., Alam, S.L., Proteasa, S.V., Zhang, Y., Lesuisse, E., Dancis, A., Stemmler, T.L., 2004. Yeast frataxin solution structure, iron binding, and ferrochelatase interaction. Biochemistry (Mosc.) 43, 16254–16262.

Hitomi, K., DiTacchio, L., Arvai, A.S., Yamamoto, J., Kim, S.-T., Todo, T., Tainer, J.A., Iwai, S., Panda, S., Getzoff, E.D., 2009. Functional motifs in the (6-4) photolyase crystal structure make a comparative framework for DNA repair photolyases and clock cryptochromes. Proc. Natl. Acad. Sci. 106, 6962–6967.

Hitomi, K., Okamoto, K., Daiyasu, H., Miyashita, H., Iwai, S., Toh, H., Ishiura, M., Todo, T., 2000. Bacterial cryptochrome and photolyase: characterization of two photolyase-like genes of Synechocystis sp. PCC6803. Nucleic Acids Res. 28, 2353–2362.

Hoang, N., Schleicher, E., Kacprzak, S., Bouly, J.-P., Picot, M., Wu, W., Berndt, A., Wolf, E., Bittl, R., Ahmad, M., 2008a. Human and Drosophila Cryptochromes Are Light Activated by Flavin Photoreduction in Living Cells. PLoS Biol 6, e160.

Hoang, N., Schleicher, E., Kacprzak, S., Bouly, J.-P., Picot, M., Wu, W., Berndt, A., Wolf, E., Bittl, R., Ahmad, M., 2008b. Human and Drosophila Cryptochromes Are Light Activated by Flavin Photoreduction in Living Cells. PLoS Biol 6,

e160.

Höltje, H.-D., 2008. Molecular modeling: basic principles and applications. Wiley-VCH, Weinheim.

Huang, J., Dizin, E., Cowan, J.A., 2008. Mapping iron binding sites on human frataxin: implications for cluster assembly on the ISU Fe-S cluster scaffold protein. J. Biol. Inorg. Chem. JBIC Publ. Soc. Biol. Inorg. Chem. 13, 825–836.

Huang, M.L.-H., Becker, E.M., Whitnall, M., Suryo Rahmanto, Y., Ponka, P., Richardson, D.R., 2009. Elucidation of the mechanism of mitochondrial iron loading in Friedreich's ataxia by analysis of a mouse mutant. Proc. Natl. Acad. Sci. U. S. A. 106, 16381–16386.

Huynen, M.A., Snel, B., Bork, P., Gibson, T.J., 2001. The phylogenetic distribution of frataxin indicates a role in iron-sulfur cluster protein assembly. Hum. Mol. Genet. 10, 2463–2468.

III, C.L.B., Karplus, rtin, Pettitt, B.M. (Eds.), 2007. Dynamical Simulation Methods, in: Advances in Chemical Physics. John Wiley & Sons, Inc., pp. 33–58.

Ito, C., Goto, S.G., Shiga, S., Tomioka, K., Numata, H., 2008. Peripheral circadian clock for the cuticle deposition rhythm in Drosophila melanogaster. Proc. Natl. Acad. Sci. 105, 8446–8451.

Ivanchenko, M., Stanewsky, R., Giebultowicz, J.M., 2001. Circadian photoreception in Drosophila: functions of cryptochrome in peripheral and central clocks. J. Biol. Rhythms 16, 205–215.

Jorgensen, W.L., Chandrasekhar, J., Madura, J.D., Impey, R.W., Klein, M.L., 1983. Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. 79, 926–935.

Kang, C.-Y., Lian, H.-L., Wang, F.-F., Huang, J.-R., Yang, H.-Q., 2009. Cryptochromes, Phytochromes, and COP1 Regulate Light-Controlled Stomatal Development in Arabidopsis. Plant Cell Online 21, 2624–2641.

Kao, Y.-T., Tan, C., Song, S.-H., Öztürk, N., Li, J., Wang, L., Sancar, A., Zhong, D., 2008. Ultrafast Dynamics and Anionic Active States of the Flavin Cofactor in Cryptochrome and Photolyase. J. Am. Chem. Soc. 130, 7695–7701.

Kaushik, R., Nawathean, P., Busza, A., Murad, A., Emery, P., Rosbash, M., 2007. PER-TIM Interactions with the Photoreceptor Cryptochrome Mediate Circadian

Temperature Responses in Drosophila. PLoS Biol 5, e146.

Khan, S.K., Xu, H., Ukai-Tadenuma, M., Burton, B., Wang, Y., Ueda, H.R., Liu, A.C., 2012. Identification of a Novel Cryptochrome Differentiating Domain Required for Feedback Repression in Circadian Clock Function. J. Biol. Chem. 287, 25917–25926.

Kim, S.T., Heelis, P.F., Sancar, A., 1992. Energy transfer (deazaflavin .fwdarw. FADH2) and electron transfer (FADH2 .fwdarw. T.ltbbrac..rtbbrac.T) kinetics in Anacystis nidulans photolyase. Biochemistry (Mosc.) 31, 11244–11248.

Klar, T., Kaiser, G., Hennecke, U., Carell, T., Batschauer, A., Essen, L.-O., 2006. Natural and Non-natural Antenna Chromophores in the DNA Photolyase from Thermus Thermophilus. ChemBioChem 7, 1798–1806.

Klar, T., Pokorny, R., Moldt, J., Batschauer, A., Essen, L.-O., 2007. Cryptochrome 3 from Arabidopsis thaliana: Structural and Functional Analysis of its Complex with a Folate Light Antenna. J. Mol. Biol. 366, 954–964.

Kleiner, O., Butenandt, J., Carell, T., Batschauer, A., 1999. Class II DNA photolyase from Arabidopsis thaliana contains FAD as a cofactor. Eur. J. Biochem. 264, 161–167.

Kleine, T., Lockhart, P., Batschauer, A., 2003. An Arabidopsis protein closely related to Synechocystis cryptochrome is targeted to organelles. Plant J. 35, 93–103.

Kondapalli, K.C., Kok, N.M., Dancis, A., Stemmler, T.L., 2008. Drosophila Frataxin: An Iron Chaperone during Cellular Fe-S Cluster Bioassembly. Biochemistry (Mosc.) 47, 6917–6927.

Kondratov, R.V., Kondratova, A.A., Lee, C., Gorbacheva, V.Y., Chernov, M.V., Antoch, M.P., 2006. Post-translational regulation of circadian transcriptional CLOCK(NPAS2)/BMAL1 complex by CRYPTOCHROMES. Cell Cycle Georget. Tex 5, 890–895.

Korb, O., Stützle, T., Exner, T.E., 2006. PLANTS: Application of Ant Colony Optimization to Structure-Based Drug Design, in: Dorigo, M., Gambardella, L.M., Birattari, M., Martinoli, A., Poli, R., Stützle, T. (Eds.), Ant Colony Optimization and Swarm Intelligence, Lecture Notes in Computer Science. Springer Berlin Heidelberg, pp. 247–258.

Krishnan, B., Levine, J.D., Lynch, M.K., Dowse, H.B., Funes, P., Hall, J.C., Hardin,

P.E., Dryer, S.E., 2001. A new role for cryptochrome in a Drosophila circadian oscillator. Nature 411, 313–317.

Kume, K., Zylka, M.J., Sriram, S., Shearman, L.P., Weaver, D.R., Jin, X., Maywood, E.S., Hastings, M.H., Reppert, S.M., 1999. mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell 98, 193–205.

Lamia, K.A., Sachdeva, U.M., DiTacchio, L., Williams, E.C., Alvarez, J.G., Egan, D.F., Vasquez, D.S., Juguilon, H., Panda, S., Shaw, R.J., Thompson, C.B., Evans, R.M., 2009. AMPK Regulates the Circadian Clock by Cryptochrome Phosphorylation and Degradation. Science 326, 437–440.

Lane, D.J.R., Richardson, D.R., 2010. Frataxin, a molecule of mystery: trading stability for function in its iron-binding site. Biochem. J. 426, e1–e3.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948.

Lesuisse, E., Santos, R., Matzanke, B.F., Knight, S.A.B., Camadro, J.-M., Dancis, A., 2003. Iron use for haeme synthesis is under control of the yeast frataxin homologue (Yfh1). Hum. Mol. Genet. 12, 879–889.

Lin, C., Robertson, D.E., Ahmad, M., Raibekas, A.A., Jorns, M.S., Dutton, P.L., Cashmore, A.R., 1995. Association of flavin adenine dinucleotide with the Arabidopsis blue light receptor CRY1. Science 269, 968–970.

Liu, H., Liu, B., Zhao, C., Pepper, M., Lin, C., 2011. The action mechanisms of plant cryptochromes. Trends Plant Sci. 16, 684–691.

Liu, H.-Y., Kuntz, I.D., Zou, X., 2004. Pairwise GB/SA Scoring Function for Structure-based Drug Design. J. Phys. Chem. B 108, 5453–5462.

Liu, Z., Tan, C., Guo, X., Li, J., Wang, L., Sancar, A., Zhong, D., 2013a. Determining complete electron flow in the cofactor photoreduction of oxidized photolyase. Proc. Natl. Acad. Sci. U. S. A. 110, 12966–12971.

Liu, Z., Zhang, M., Guo, X., Tan, C., Li, J., Wang, L., Sancar, A., Zhong, D., 2013b. Dynamic determination of the functional state in photolyase and the implication for cryptochrome. Proc. Natl. Acad. Sci. U. S. A. 110, 12972–12977.

Li, X., Wang, Q., Yu, X., Liu, H., Yang, H., Zhao, C., Liu, X., Tan, C., Klejnot, J., Zhong, D., Lin, C., 2011. Arabidopsis cryptochrome 2 (CRY2) functions by the photoactivation mechanism distinct from the tryptophan (trp) triad-dependent photoreduction. Proc. Natl. Acad. Sci. U. S. A. 108, 20844–20849.

MacKerell, A. D., Bashford, D., Bellott, Dunbrack, R. L., Evanseck, J.D., Field, M.J., Fischer, S., Gao, J., Guo, H., Ha, S., Joseph-McCarthy, D., Kuchnir, L., Kuczera, K., Lau, F.T.K., Mattos, C., Michnick, S., Ngo, T., Nguyen, D.T., Prodhom, B., Reiher, W.E., Roux, B., Schlenkrich, M., Smith, J.C., Stote, R., Straub, J., Watanabe, M., Wiórkiewicz-Kuczera, J., Yin, D., Karplus, M., 1998. All-Atom Empirical Potential for Molecular Modeling and Dynamics Studies of Proteins†. J. Phys. Chem. B 102, 3586–3616.

Majeux, N., Scarsi, M., Apostolakis, J., Ehrhardt, C., Caflisch, A., 1999. Exhaustive docking of molecular fragments with electrostatic solvation. Proteins 37, 88–105.

Malhotra, K., Kim, S.-T., Batschauer, A., Dawut, L., Sancar, A., 1995. Putative Blue-Light Photoreceptors from Arabidopsis thaliana and Sinapis alba with a High Degree of Sequence Homology to DNA Photolyase Contain the Two Photolyase Cofactors but Lack DNA Repair Activity. Biochemistry (Mosc.) 34, 6892–6899.

Mao, J., Zhang, Y.-C., Sang, Y., Li, Q.-H., Yang, H.-Q., 2005. A role for Arabidopsis cryptochromes and COP1 in the regulation of stomatal opening. Proc. Natl. Acad. Sci. U. S. A. 102, 12270–12275.

Matsumoto, N., 2003. Functional Analysis and Intracellular Localization of Rice Cryptochromes. PLANT Physiol. 133, 1494–1503.

Meissner, R.-A., Kilman, V.L., Lin, J.-M., Allada, R., 2008. TIMELESS Is an Important Mediator of CK2 Effects on Circadian Clock Function In Vivo. J. Neurosci. 28, 9732–9740.

Miyazaki, K., Mesaki, M., Ishida, N., 2001. Nuclear Entry Mechanism of Rat PER2 (rPER2): Role of rPER2 in Nuclear Localization of CRY Protein. Mol. Cell. Biol. 21, 6651–6659.

Müller, M., Carell, T., 2009. Structural biology of DNA photolyases and cryptochromes. Curr. Opin. Struct. Biol. 19, 277–285.

Müller, P., Ahmad, M., 2011. Light-activated Cryptochrome Reacts with Molecular

Oxygen to Form a Flavin–Superoxide Radical Pair Consistent with Magnetoreception. J. Biol. Chem. 286, 21033–21040.

Musco, G., Stier, G., Kolmerer, B., Adinolfi, S., Martin, S., Frenkiel, T., Gibson, T., Pastore, A., 2000. Towards a structural understanding of Friedreich's ataxia: the solution structure of frataxin. Struct. Lond. Engl. 1993 8, 695–707.

Naidoo, N., Song, W., Hunter-Ensor, M., Sehgal, A., 1999. A role for the proteasome in the light response of the timeless clock protein. Science 285, 1737–1741.

Nair, M., Adinolfi, S., Pastore, C., Kelly, G., Temussi, P., Pastore, A., 2004. Solution Structure of the Bacterial Frataxin Ortholog, CyaY: Mapping the Iron Binding Sites. Structure 12, 2037–2048.

Nießner, C., Denzau, S., Stapput, K., Ahmad, M., Peichl, L., Wiltschko, W., Wiltschko, R., 2013. Magnetoreception: activated cryptochrome 1a concurs with magnetic orientation in birds. J. R. Soc. Interface 10, 20130638.

O'Neill, H.A., Gakh, O., Park, S., Cui, J., Mooney, S.M., Sampson, M., Ferreira, G.C., Isaya, G., 2005. Assembly of Human Frataxin Is a Mechanism for Detoxifying Redox-Active Iron†. Biochemistry (Mosc.) 44, 537–545.

Özgür, S., Sancar, A., 2006. Analysis of Autophosphorylating Kinase Activities of Arabidopsis and Human Cryptochromes†. Biochemistry (Mosc.) 45, 13369–13374.

Ozturk, N., Selby, C.P., Song, S.-H., Ye, R., Tan, C., Kao, Y.-T., Zhong, D., Sancar, A., 2009. Comparative Photochemistry of Animal Type 1 and Type 4 Cryptochromes. Biochemistry (Mosc.) 48, 8585–8593.

Öztürk, N., Song, S.-H., Özgür, S., Selby, C.P., Morrison, L., Partch, C., Zhong, D., Sancar, A., 2007. Structure and Function of Animal Cryptochromes. Cold Spring Harb. Symp. Quant. Biol. 72, 119–131.

Öztürk, N., Song, S.-H., Selby, C.P., Sancar, A., 2008. Animal Type 1 Cryptochromes ANALYSIS OF THE REDOX STATE OF THE FLAVIN COFACTOR BY SITE-DIRECTED MUTAGENESIS. J. Biol. Chem. 283, 3256–3263.

Ozturk, N., VanVickle-Chavez, S.J., Akileswaran, L., Gelder, R.N.V., Sancar, A., 2013. Ramshackle (Brwd3) promotes light-induced ubiquitylation of Drosophila Cryptochrome by DDB1-CUL4-ROC1 E3 ligase complex. Proc. Natl. Acad. Sci. 110, 4980–4985.

Panchenko, A.R., Kondrashov, F., Bryant, S., 2004. Prediction of functional sites by analysis of sequence and structure conservation. Protein Sci. 13, 884–892.

Pandolfo, M., Pastore, A., 2009. The pathogenesis of Friedreich ataxia and the structure and function of frataxin. J. Neurol. 256 Suppl 1, 9–17.

Park, H.W., Kim, S.T., Sancar, A., Deisenhofer, J., 1995. Crystal structure of DNA photolyase from Escherichia coli. Science 268, 1866–1872.

Pastore, C., Franzese, M., Sica, F., Temussi, P., Pastore, A., 2007. Understanding the binding properties of an unusual metal-binding protein--a study of bacterial frataxin. FEBS J. 274, 4199–4210.

Payne, G., Sancar, A., 1990. Absolute action spectrum of E-FADH2 and E-FADH2-MTHF forms of Escherichia coli DNA photolyase. Biochemistry (Mosc.) 29, 7715–7727.

Peschel, N., Veleri, S., Stanewsky, R., 2006. Veela defines a molecular link between Cryptochrome and Timeless in the light-input pathway to Drosophila's circadian clock. Proc. Natl. Acad. Sci. 103, 17313–17318.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., Ferrin, T.E., 2004. UCSF Chimera--a visualization system for exploratory research and analysis. J. Comput. Chem. 25, 1605–1612.

Phillips, J.C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., Chipot, C., Skeel, R.D., Kalé, L., Schulten, K., 2005. Scalable molecular dynamics with NAMD. J. Comput. Chem. 26, 1781–1802.

Pils, B., Copley, R.R., Schultz, J., 2005. Variation in structural location and amino acid conservation of functional sites in protein domain families. BMC Bioinformatics 6, 210.

Pokorny, R., Klar, T., Hennecke, U., Carell, T., Batschauer, A., Essen, L.-O., 2008. Recognition and repair of UV lesions in loop structures of duplex DNA by DASH-type cryptochrome. Proc. Natl. Acad. Sci. 105, 21023–21027.

Prischi, F., Giannini, C., Adinolfi, S., Pastore, A., 2009. The N-terminus of mature human frataxin is intrinsically unfolded. FEBS J. 276, 6669–6676.

Prischi, F., Konarev, P.V., Iannuzzi, C., Pastore, C., Adinolfi, S., Martin, S.R., Svergun, D.I., Pastore, A., 2010. Structural bases for the interaction of frataxin with the central components of iron-sulphur cluster assembly. Nat. Commun. 1, 95.

Rosato, E., Codd, V., Mazzotta, G., Piccin, A., Zordan, M., Costa, R., Kyriacou, C.P., 2001. Light-dependent interaction between Drosophila CRY and the clock protein PER mediated by the carboxy terminus of CRY. Curr. Biol. 11, 909–917.

Rush, B.L., Murad, A., Emery, P., Giebultowicz, J.M., 2006. Ectopic CRYPTOCHROME Renders TIM Light Sensitive in the Drosophila Ovary. J. Biol. Rhythms 21, 272–278.

Ryckaert, J.-P., Ciccotti, G., Berendsen, H.J.C., 1977. Numerical integration of the cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. J. Comput. Phys. 23, 327–341.

Saez, L., Derasmo, M., Meyer, P., Stieglitz, J., Young, M.W., 2011. A key temporal delay in the circadian cycle of Drosophila is mediated by a nuclear localization signal in the timeless protein. Genetics 188, 591–600.

Sakakida, Y., Miyamoto, Y., Nagoshi, E., Akashi, M., Nakamura, T.J., Mamine, T., Kasahara, M., Minami, Y., Yoneda, Y., Takumi, T., 2005. Importin alpha/beta mediates nuclear transport of a mammalian circadian clock component, mCRY2, together with mPER2, through a bipartite nuclear localization signal. J. Biol. Chem. 280, 13272–13278.

Sancar, A., 2003. Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. Chem. Rev. 103, 2203–2237.

Schmucker, S., Martelli, A., Colin, F., Page, A., Wattenhofer-Donzé, M., Reutenauer, L., Puccio, H., 2011. Mammalian Frataxin: An Essential Function for Cellular Viability through an Interaction with a Preformed ISCU/NFS1/ISD11 Iron-Sulfur Assembly Complex. PLoS ONE 6, e16199.

Sehadova, H., Glaser, F.T., Gentile, C., Simoni, A., Giesecke, A., Albert, J.T., Stanewsky, R., 2009. Temperature entrainment of Drosophila's circadian clock involves the gene nocte and signaling from peripheral sensory tissues to the brain. Neuron 64, 251–266.

Selby, C.P., Sancar, A., 2012. The second chromophore in Drosophila photolyase/cryptochrome family photoreceptors. Biochemistry (Mosc.) 51, 167–171.

Shalitin, D., Yang, H., Mockler, T.C., Maymon, M., Guo, H., Whitelam, G.C., Lin, C., 2002. Regulation of Arabidopsis cryptochrome 2 by blue-light-dependent

phosphorylation. Nature 417, 763–767.

Shan, Y., Cortopassi, G., 2012. HSC20 interacts with frataxin and is involved in iron-sulfur cluster biogenesis and iron homeostasis. Hum. Mol. Genet. 21, 1457–1469.

Shan, Y., Napoli, E., Cortopassi, G., 2007. Mitochondrial frataxin interacts with ISD11 of the NFS1/ISCU complex and multiple mitochondrial chaperones. Hum. Mol. Genet. 16, 929–941.

Söderberg, C.A.G., Shkumatov, A.V., Rajan, S., Gakh, O., Svergun, D.I., Isaya, G., Al-Karadaghi, S., 2011. Oligomerization Propensity and Flexibility of Yeast Frataxin Studied by X-ray Crystallography and Small-Angle X-ray Scattering. J. Mol. Biol. 414, 783–797.

Song, S.-H., 2006. Photodynamics of phototropin and cryptochrome blue-light photoreceptors. Universität Regensburg.

Song, S.-H., Dick, B., Penzkofer, A., Pokorny, R., Batschauer, A., Essen, L.-O., 2006. Absorption and fluorescence spectroscopic characterization of cryptochrome 3 from Arabidopsis thaliana. J. Photochem. Photobiol. B 85, 1–16.

Stanewsky, R., Kaneko, M., Emery, P., Beretta, B., Wager-Smith, K., Kay, S.A., Rosbash, M., Hall, J.C., 1998. The cryb mutation identifies cryptochrome as a circadian photoreceptor in Drosophila. Cell 95, 681–692.

Stemmler, T.L., Lesuisse, E., Pain, D., Dancis, A., 2010. Frataxin and Mitochondrial FeS Cluster Biogenesis. J. Biol. Chem. 285, 26737–26743.

Stillinger, F.H., Rahman, A., 2003. Improved simulation of liquid water by molecular dynamics. J. Chem. Phys. 60, 1545–1557.

Still, W.C., Tempczyk, A., Hawley, R.C., Hendrickson, T., 1990. Semianalytical treatment of solvation for molecular mechanics and dynamics. J. Am. Chem. Soc. 112, 6127–6129.

Stoleru, D., Nawathean, P., Fernández, M. de la P., Menet, J.S., Ceriani, M.F., Rosbash, M., 2007. The Drosophila Circadian Network Is a Seasonal Timer. Cell 129, 207–219.

Suzek, B.E., Huang, H., McGarvey, P., Mazumder, R., Wu, C.H., 2007. UniRef: comprehensive and non-redundant UniProt reference clusters. Bioinformatics 23, 1282–1288.

Tamada, T., Kitadokoro, K., Higuchi, Y., Inaka, K., Yasui, A., de Ruiter, P.E., Eker, A.P.M., Miki, K., 1997. Crystal structure of DMA photolyase from Anacystis nidulans. Nat. Struct. Mol. Biol. 4, 887–891.

Tamanini, F., Chaves, I., Bajek, M.I., van der Horst, G.T.J., 2007. Structure function analysis of mammalian cryptochromes. Cold Spring Harb. Symp. Quant. Biol. 72, 133–139.

Taylor, R.D., Jewsbury, P.J., Essex, J.W., 2003. FDS: flexible ligand and receptor docking with a continuum solvent model and soft-core energy function. J. Comput. Chem. 24, 1637–1656.

Thompson, C.L., Bowes Rickman, C., Shaw, S.J., Ebright, J.N., Kelly, U., Sancar, A., Rickman, D.W., 2003. Expression of the blue-light receptor cryptochrome in the human retina. Invest. Ophthalmol. Vis. Sci. 44, 4515–4521.

VanVickle-Chavez, S.J., Gelder, R.N.V., 2007. Action Spectrum of Drosophila Cryptochrome. J. Biol. Chem. 282, 10561–10566.

Verlet, L., 1967. Computer "Experiments" on Classical Fluids. I. Thermodynamical Properties of Lennard-Jones Molecules. Phys. Rev. 159, 98–103.

Wang, H., Ma, L.-G., Li, J.-M., Zhao, H.-Y., Deng, X.W., 2001. Direct Interaction of Arabidopsis Cryptochromes with COP1 in Light Control Development. Science 294, 154–158.

Wang, J., Wang, W., Kollman, P.A., Case, D.A., 2006. Automatic atom type and bond type perception in molecular mechanical calculations. J. Mol. Graph. Model. 25, 247–260.

Wang, T., Craig, E.A., 2008. Binding of Yeast Frataxin to the Scaffold for Fe-S Cluster Biogenesis, Isu. J. Biol. Chem. 283, 12674–12679.

Waterhouse, A.M., Procter, J.B., Martin, D.M.A., Clamp, M., Barton, G.J., 2009. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics 25, 1189–1191.

Wu, G., Spalding, E.P., 2007. Separate functions for nuclear and cytoplasmic cryptochrome 1 during photomorphogenesis of Arabidopsis seedlings. Proc. Natl. Acad. Sci. 104, 18813–18818.

Xu, P., Xiang, Y., Zhu, H., Xu, H., Zhang, Z., Zhang, C., Zhang, L., Ma, Z., 2008. Wheat Cryptochromes: Subcellular Localization and Involvement in

Photomorphogenesis and Osmotic Stress Responses. PLANT Physiol. 149, 760–774.

Yang, H.Q., Tang, R.H., Cashmore, A.R., 2001. The signaling mechanism of Arabidopsis CRY1 involves direct interaction with COP1. Plant Cell 13, 2573–2587.

Yang, H.-Q., Wu, Y.-J., Tang, R.-H., Liu, D., Liu, Y., Cashmore, A.R., 2000. The C termini of Arabidopsis cryptochromes mediate a constitutive light response. Cell 103, 815–827.

Yang, Y.-J., Zuo, Z.-C., Zhao, X.-Y., Li, X., Klejnot, J., Li, Y., Chen, P., Liang, S.-P., Yu, X.-H., Liu, X.-M., Lin, C.-T., 2008. Blue-Light-Independent Activity of Arabidopsis Cryptochromes in the Regulation of Steady-State Levels of Protein and mRNA Expression. Mol. Plant 1, 167–177.

Yoon, T., Cowan, J.A., 2003. Iron-sulfur cluster biosynthesis. Characterization of frataxin as an iron donor for assembly of [2Fe-2S] clusters in ISU-type proteins. J. Am. Chem. Soc. 125, 6078–6084.

Yoon, T., Dizin, E., Cowan, J.A., 2007. N-terminal iron-mediated self-cleavage of human frataxin: regulation of iron binding and complex formation with target proteins. J. Biol. Inorg. Chem. JBIC Publ. Soc. Biol. Inorg. Chem. 12, 535–542.

Yoshii, T., Hermann, C., Helfrich-Förster, C., 2010. Cryptochrome-positive and -negative clock neurons in Drosophila entrain differentially to light and temperature. J. Biol. Rhythms 25, 387–398.

Yoshii, T., Todo, T., Wülbeck, C., Stanewsky, R., Helfrich-Förster, C., 2008. Cryptochrome is present in the compound eyes and a subset of Drosophila's clock neurons. J. Comp. Neurol. 508, 952–966.

Yu, X., Klejnot, J., Zhao, X., Shalitin, D., Maymon, M., Yang, H., Lee, J., Liu, X., Lopez, J., Lin, C., 2007. Arabidopsis Cryptochrome 2 Completes Its Posttranslational Life Cycle in the Nucleus. Plant Cell Online 19, 3146–3156.

Zhang, F., Scheerer, P., Oberpichler, I., Lamparter, T., Krauß, N., 2013. Crystal structure of a prokaryotic (6-4) photolyase with an Fe-S cluster and a 6,7-dimethyl-8-ribityllumazine antenna chromophore. Proc. Natl. Acad. Sci. 110, 7217–7222.

Zhu, H., Conte, F., Green, C.B., 2003. Nuclear Localization and Transcriptional Repression Are Confined to Separable Domains in the Circadian Protein

CRYPTOCHROME. Curr. Biol. 13, 1653–1658.

Zuo, Z.-C., Meng, Y.-Y., Yu, X.-H., Zhang, Z.-L., Feng, D.-S., Sun, S.-F., Liu, B., Lin, C.-T., 2012. A study of the blue-light-dependent phosphorylation, degradation, and photobody formation of Arabidopsis CRY2. Mol. Plant 5, 726–733.

Zuo, Z., Liu, H., Liu, B., Liu, X., Lin, C., 2011. Blue Light-Dependent Interaction of CRY2 with SPA1 Regulates COP1 activity and Floral Initiation in Arabidopsis. Curr. Biol. 21, 841–847.

6. Human Frataxin Metal Binding Simulations

6.1 Introduction

Frataxin is a mitochondrial protein conserved from bacteria to humans. Mutations in Frataxin gene have been associated with Friedreich's ataxia (FRDA) neurodegenerative disease. Patients with FRDA show Fe-S cluster assembly deficiency and mitochondrial iron ion accumulation. Several studies have shown frataxin can play Fe-S cluster assemblying (Prischi et al., 2010) activity through delivery of iron (Cook et al., 2006)(Pandolfo and Pastore, 2009) (Huynen et al., 2001). It is believed that frataxin has increased iron binding affinity when playing complex formation (Huang et al., 2008).

While yeast and bacterial frataxin crystal structures show metal binding (Pastore et al., 2007), it is still unclear if human frataxin has iron binding capabilities. There are reports indicating human frataxin has no tendency to bind iron (Musco et al., 2000), whereas it has been also reported that monomeric human frataxin can bind up to seven iron ions (Yoon and Cowan, 2003). However, to date, there is no human frataxin crystal structure demonstrating metal binding capabilities. Furthermore, frataxins have been suggested to have low iron-binding specificity with the possibility of accommodating any divalent/trivalent cation in the same pocket (Pastore et al., 2007).

Main studies carrie on about whether frataxin is an iron scavenger (Adamec et al., 2000) and/or an iron-mediated Fe-S cluster assemblier or even to have ferritin-like iron storage properties. Furthermore, different roles have been atributed with a study suggesting frataxins in fact work as an iron “sensor” instead of iron chaperone due to its low binding affinity nature (Adinolfi et al., 2009).

Biophysical experiments have shown that several acidic residues located in the alpha1helix region can play a role in iron binding (Correia et al., 2010)and Fe-S cluster assembly. However, this role is unclear among frataxin homologs with this acidic region in yeast frataxin showing conflicting functions in Fe-S cluster formation (Wang and Craig, 2008) (Foury et al., 2007). Another study however, suggest that besides iron binding function, the acidic ridge region can further have structural flexibility function (Lane and Richardson, 2010).

Although iron binding seems to be relevant to frataxin's function, and the acidic ridge is certainly involved in this, the nature and specificity of the iron-binding site(s) remain unclear. In order to investigate human frataxin iron binding sites, we carried out Molecular Dynamics (MD) simulations exploring metal binding in specific sites in the acidic alpha1 helix region (Figure 24).

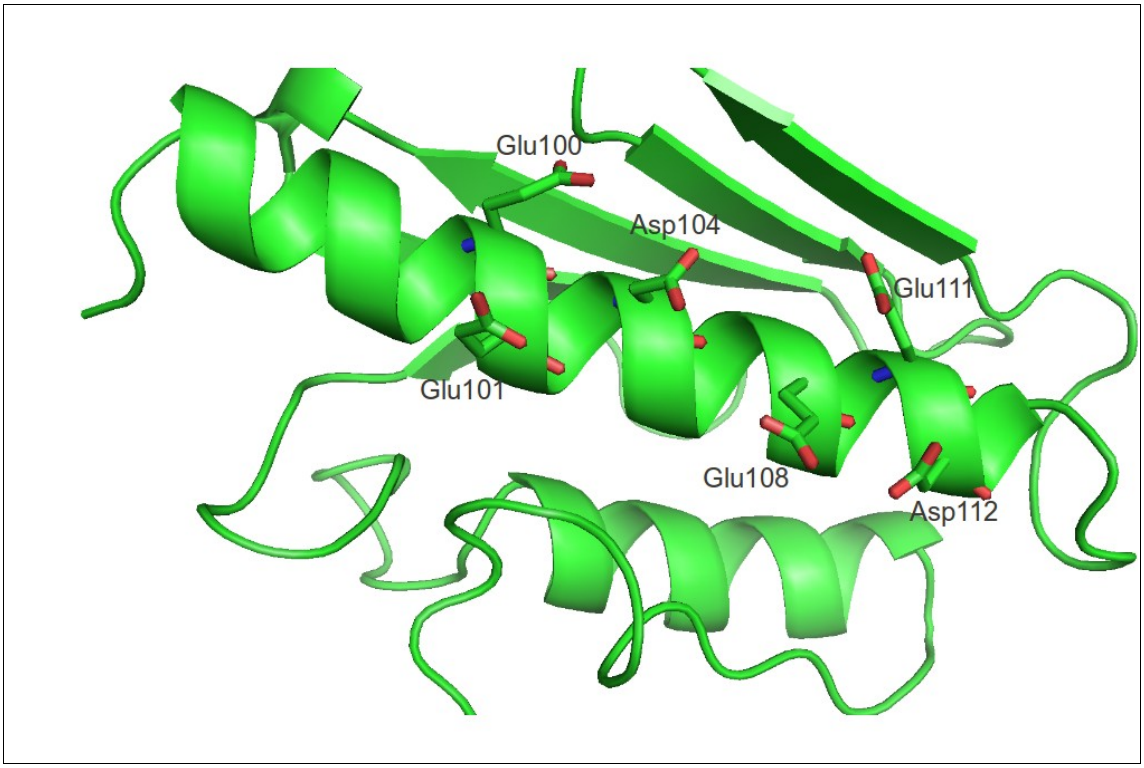

Figure 24. Human Frataxin crystal structure showing acidic ridge region. Acidic amino acids focus of this study are shown as sticks.

6.2 Methods

Gromacs (Hess et al., 2008) programme was used for Molecular Dynamics (MD) simulations. CHARMM (Brooks et al., 2009) force field was considered. All MD simulations in this work have been shown stability after 5 ns, when RMSD levels off to ~0.1 nm, and these conformations were considered for analysis. Human frataxin (PDB code 3S4M) was used as starting structure. To find low energy torsional states, Swiss-PDB Viewer (Guex and Peitsch, 1997) was used.

6.3 Results

Two potential metal binding sites can be identified in the acidic ridge region. Residues Glu112, Glu111 and Glu 108 are closely located to play metal binding. The Zinc atom placed at the center of these three residues and their low energy torsional states pointing to the metal have been chosen for the MD analysis. 5 ns of MD simulation showed these three residues pointing towards the metal, indicating metal binding function (Figure 25).

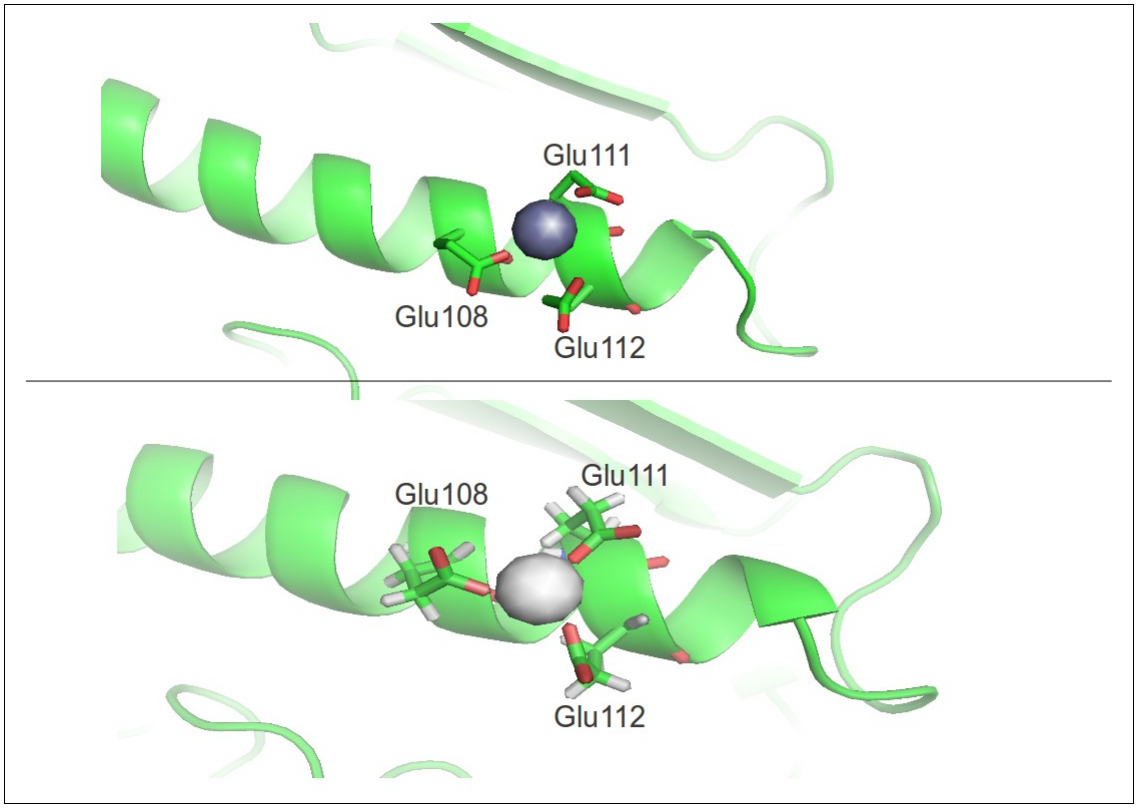

Figure 25. Glu112, Glu111 and Glu 108 residues considering low energy torsional states (at the top) and after 5 ns of MD (at the bottom).

Residues Glu100, Glu101 and Asp104 form another potential iron binding site. Similarly, after 5 ns MD simulation also these three residues showed metal binding capabilities pointing towards the metal (Figure 26).

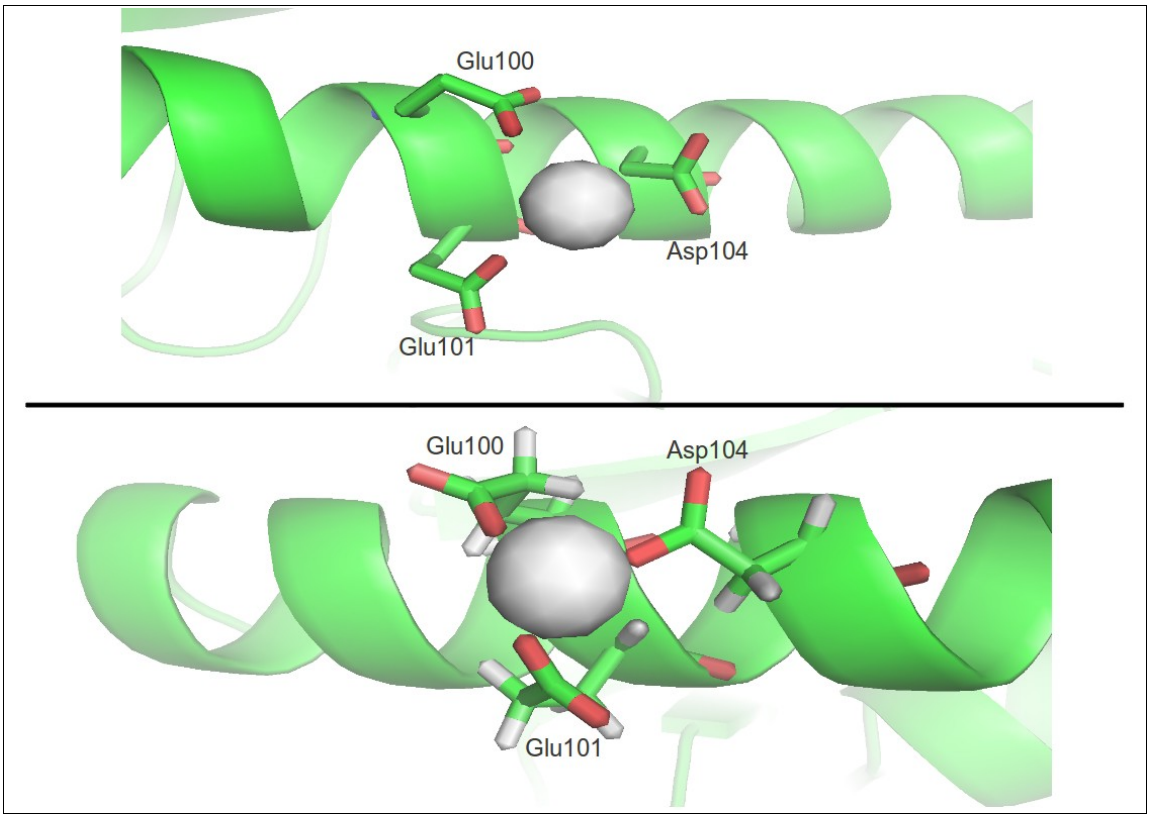

Figure 26. Glu100, Glu101 and Asp104 residues considering low energy torsional states (at the top) and after 5 ns of MD (at the bottom).

Since frataxin can have multiple metal bindings, we performed MD simulations considering the two above metal binding sites at the same time. We noticed that when running MD simulation without preparing the involved residues with their low energy torsional states, only two residues in each site point towards the metal. However, when running MD simulation considering residues low energy torsional states pointing to the metal, all three residues on each site point towards the metal after 5 ns (Figure 27).

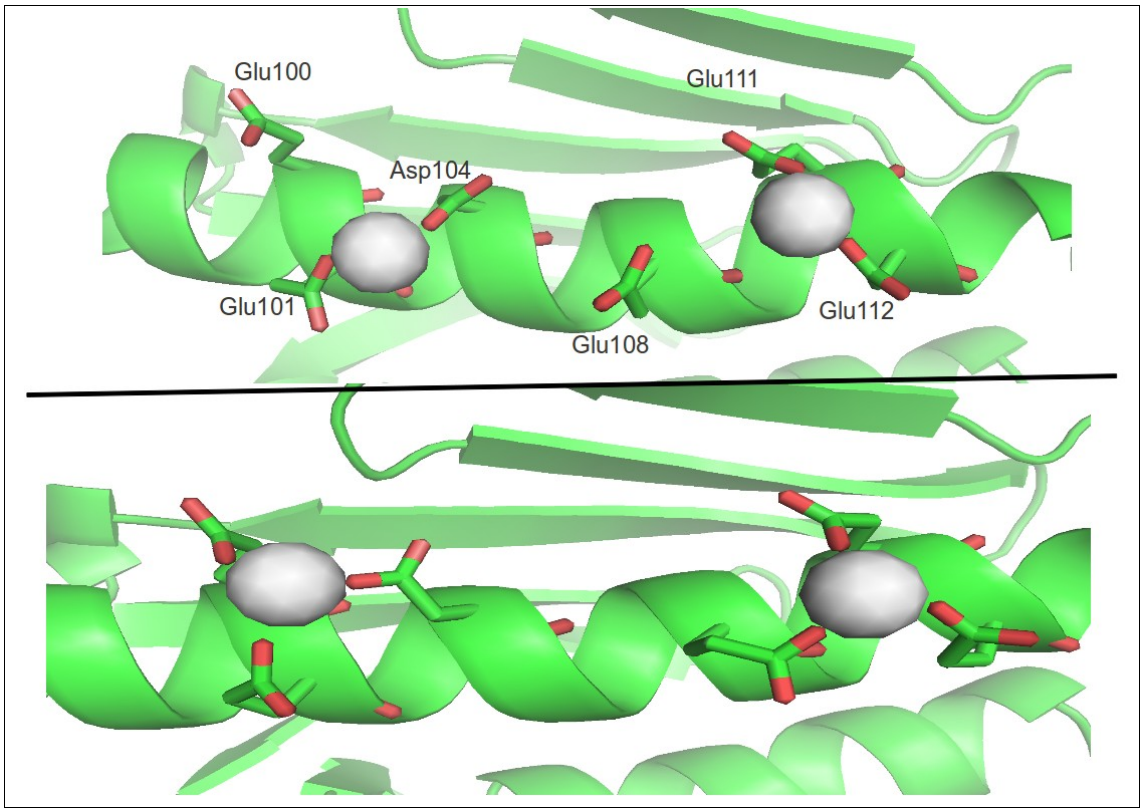

Figure 27. Two putative metal binding sites Glu112, Glu111 and Glu108, and Glu100, Glu101 and Asp104. At the top: Zinc atom binding after 1 ns without low energy torsional states consideration; At the bottom: Zinc atom binding after 5 ns when considering low energy torsional states.

6.4 Conclusions

We suggest human frataxin can play metal binding interactions through the Glu112, Glu111 and Glu108, and Glu100, Glu101 and Asp104 sites in its acidic ridge region. Our work further suggests that while they can play metal binding, low affinity seems to drive the binding nature. Moreover, as previously suggested it is possible that these interactions become stronger when frataxin plays downstream interactions in the Fe-S cluster formation. The nature of binding also supports the iron "sensor" function frataxins may play. Overall, since this work focus in the alpha1 acidic region, we cannot rule out that iron can be bound elsewhere. It is also possible that differently than transferrins, which show invariant binding sites, iron-binding to frataxins occur at various locations with moderate-affinity binding characteristics in an anionic surface. Finally, similarly than yeast frataxin, iron-binding behaviour can be linked to a structural flexibility of the protein that may be important for the downstream

interactions. This work brings further information in the nature of the controversial iron binding function role of frataxins.

6.5 References

Adamec, J., Rusnak, F., Owen, W.G., Naylor, S., Benson, L.M., Gacy, A.M., Isaya, G., 2000. Iron-dependent self-assembly of recombinant yeast frataxin: implications for Friedreich ataxia. Am. J. Hum. Genet. 67, 549–562.

Adinolfi, S., Iannuzzi, C., Prischi, F., Pastore, C., Iametti, S., Martin, S.R., Bonomi, F., Pastore, A., 2009. Bacterial frataxin CyaY is the gatekeeper of iron-sulfur cluster formation catalyzed by IscS. Nat. Struct. Mol. Biol. 16, 390–396.

Brooks, B.R., Brooks, C.L., 3rd, Mackerell, A.D., Jr, Nilsson, L., Petrella, R.J., Roux, B., Won, Y., Archontis, G., Bartels, C., Boresch, S., Caflisch, A., Caves, L., Cui, Q., Dinner, A.R., Feig, M., Fischer, S., Gao, J., Hodoscek, M., Im, W., Kuczera, K., Lazaridis, T., Ma, J., Ovchinnikov, V., Paci, E., Pastor, R.W., Post, C.B., Pu, J.Z., Schaefer, M., Tidor, B., Venable, R.M., Woodcock, H.L., Wu, X., Yang, W., York, D.M., Karplus, M., 2009. CHARMM: the biomolecular simulation program. J. Comput. Chem. 30, 1545–1614.

Cook, J.D., Bencze, K.Z., Jankovic, A.D., Crater, A.K., Busch, C.N., Bradley, P.B., Stemmler, A.J., Spaller, M.R., Stemmler, T.L., 2006. Monomeric Yeast Frataxin is an Iron Binding Protein. Biochemistry (Mosc.) 45, 7767–7777.

Correia, A.R., Wang, T., Craig, E.A., Gomes, C.M., 2010. Iron binding activity in yeast frataxin entails a trade off with stability in the ?1/?1 acidic ridge region. Biochem. J. 426, 197–203.

Foury, F., Pastore, A., Trincal, M., 2007. Acidic residues of yeast frataxin have an essential role in Fe-S cluster assembly. EMBO Rep. 8, 194–199.

Guex, N., Peitsch, M.C., 1997. SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis 18, 2714–2723.

Hess, B., Kutzner, C., van der Spoel, D., Lindahl, E., 2008. GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation. J. Chem. Theory Comput. 4, 435–447.

Huang, J., Dizin, E., Cowan, J.A., 2008. Mapping iron binding sites on human frataxin:

implications for cluster assembly on the ISU Fe-S cluster scaffold protein. J. Biol. Inorg. Chem. JBIC Publ. Soc. Biol. Inorg. Chem. 13, 825–836.

Huynen, M.A., Snel, B., Bork, P., Gibson, T.J., 2001. The phylogenetic distribution of frataxin indicates a role in iron-sulfur cluster protein assembly. Hum. Mol. Genet. 10, 2463–2468.

Lane, D.J.R., Richardson, D.R., 2010. Frataxin, a molecule of mystery: trading stability for function in its iron-binding site. Biochem. J. 426, e1–e3.

Musco, G., Stier, G., Kolmerer, B., Adinolfi, S., Martin, S., Frenkiel, T., Gibson, T., Pastore, A., 2000. Towards a structural understanding of Friedreich's ataxia: the solution structure of frataxin. Struct. Lond. Engl. 1993 8, 695–707.

Pandolfo, M., Pastore, A., 2009. The pathogenesis of Friedreich ataxia and the structure and function of frataxin. J. Neurol. 256 Suppl 1, 9–17.

Pastore, C., Franzese, M., Sica, F., Temussi, P., Pastore, A., 2007. Understanding the binding properties of an unusual metal-binding protein--a study of bacterial frataxin. FEBS J. 274, 4199–4210.

Prischi, F., Konarev, P.V., Iannuzzi, C., Pastore, C., Adinolfi, S., Martin, S.R., Svergun, D.I., Pastore, A., 2010. Structural bases for the interaction of frataxin with the central components of iron-sulphur cluster assembly. Nat. Commun. 1, 95.

Wang, T., Craig, E.A., 2008. Binding of Yeast Frataxin to the Scaffold for Fe-S Cluster Biogenesis, Isu. J. Biol. Chem. 283, 12674–12679.

Yoon, T., Cowan, J.A., 2003. Iron-sulfur cluster biosynthesis. Characterization of frataxin as an iron donor for assembly of [2Fe-2S] clusters in ISU-type proteins. J. Am. Chem. Soc. 125, 6078–6084.negatively charged residues in α1 and β1 (a semi-conserved acidic ridge).

7. Conclusions

This study aimed to better understand evolutionary and structural aspects of Cryptochrome/Photolyase family and Frataxins. Although CRY/photolyase family members share a high degree of similarity, they show different functions. CRYs (CRY1 and CRY2) show a higher degree of similarity than CRY-DASHs and DNA Photolyases. Also, while CRY1 and CRY2 are a homogeneous group member, unexpectedly, DNA Photolyases show no sequence-based correlation. The Photolyase Homology Region (PHR) domain displays different conservation profiles, with the FAD binding domain being conserved among insect and vertebrate CRYs and the DNA Photolyase domain showing conservation only among vertebrate CRYs. Considering DNA Photolyases low conservation in the PHR domain, we suggest that DNA repairing activity has variable amino acid conservation. On the other hand, consistent with previous reports, the C-terminal region conservation among insects seems to be related with binding to downstream partners in the circadian rhythm.

CRY/Photolyase family members diverge about the presence and the type of a second chromophore. CRY-DASH of *Arabidopsis thaliana* is the only Cryptochrome subclass crystal structure showing an antenna cofactor. Our analysis revealed that *Drosophila* CRY (dCRY) can accommodate the flavin type 8-HDF as an antenna cofactor. Residues surrounding 8-HDF are conserved among insects/vertebrates CRYs, also by a hydrophobic side, which is present also in Photolyases. Furthermore, tryptophan residues are properly located to play energy transfer between FAD and the antenna cofactor. Alltogether, our work suggest dCRY can have 8-HDF as second chromophore and we further suggest that it is worth further experiments to confirm this finding.

Negatively charged residues in α1 and β1 (a semi-conserved acidic ridge) of frataxins have been widely associated with iron-binding function. This work demonstrated that human frataxin can play metal-binding through its acidic ridge. The acidic residues Asp112, Glu111 and Glu108, and Glu100, Glu101 and Asp104 in human frataxin are surface exposed and properly located to play metal-binding. Since our analysis support the weak nature of binding, we suggest that human frataxin has versatile iron-binding functions. The binding nature is consistent with both downstream

partners interactions in the Fe-S cluster formation, oligomerization and even “sensor” roles attributed to frataxins. Overall, this work besides confirming the alpha1 acidic region as potential iron-binding in human frataxin, brings further information on the binding nature of frataxins.