# Orchestration of algal metabolism by protein disorder

Helene Launay, Véronique Receveur-Bréchot, Frédéric Carriere, Brigitte Gontero

# Orchestration of algal metabolism by protein disorder

Hélène Launay, Véronique Receveur-Bréchot, Frédéric Carrière, Brigitte Gontero*

Aix Marseille Univ, CNRS, BIP UMR 7281, 31 Chemin Joseph Aiguier, Marseille Cedex 20, 13402, France

*Correspondence to Brigitte Gontero

Dr Brigitte Gontero, Aix Marseille Univ, CNRS, BIP UMR 7281, 31 Chemin Joseph Aiguier, Marseille Cedex 20, 13402, France

Email: bmeunier@imm.cnrs.fr

Tel: + 33 4 91 16 45 49

**Highlights (85 characters)**

- IDPs help algae to cope with extreme conditions
- IDPs from algae are under-represented in disordered protein databases
- Only IDPs involved in $CO_2$ metabolism have been studied so far (EPYC1, CP12, RCA…)
- Disorder is involved in the regulation of enzymes belonging to $CO_2$ metabolism
- Disorder is also important in algal chaperones

**Abstract**

Intrinsically disordered proteins (IDPs) represent a family of proteins that provide many functional advantages in a large number of metabolic and signaling pathways. Because of their high flexibility that endows them with pressure-, heat- and acid-resistance, IDPs are valuable metabolic regulators that help algae to cope with extreme conditions of pH, temperature, pressure and light. They have, however, been overlooked in these organisms. In this review, we present some well-known algal IDPs, including the conditionally disordered CP12, a protein involved in the regulation of $CO_2$ assimilation, as probably the best known example, whose disorder content is strongly dependent on the redox conditions, and the essential pyrenoid component 1 that serves as a scaffold for ribulose-1, 5-bisphosphate carboxylase/oxygenase. We also describe how some enzymes are regulated by protein fragments, called intrinsically disordered regions (IDRs), such as ribulose-1, 5-bisphosphate carboxylase/oxygenase activase, the $A_2B_2$ form of glyceraldehyde-3-phosphate dehydrogenase and the adenylate kinase. Several molecular chaperones, which are crucial for cell proteostasis, also display significant disorder propensities such as the algal heat shock proteins HSP33, HSP70 and HSP90. This review confirms the wide distribution of IDPs in algae but highlights that further studies are needed to uncover their full role in orchestrating algal metabolism.

**Keywords** : Calvin cycle, chaperone, CP12, EPYC, Intrinsically disordered protein, RuBisCO

**Abbreviations**

ADK: adenylate kinase; CBB: Calvin-Benson-Bassham; CCM: $CO_2$ concentrating mechanism; CD: circular dichroism; CP12: chloroplast protein of 12 kDa; EPYC1: Essential

Pyrenoid component 1; EST: expressed sequence tag; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; HCA: hydrophobic cluster analysis; HSP: heat shock protein; IDP: intrinsically disordered protein; IDR: intrinsically disordered region; LSU: large subunit; NMR: nuclear magnetic resonance; PGA: phosphoglyceric acid; PRK: phosphoribulokinase; ROS: reactive oxygen species; PTM: post translational modification; RuBisCO: Ribulose-1, 5-bisphosphate carboxylase/oxygenase; RuBP: Ribulose-1, 5-bisphosphate; RCA: RuBisCO activase; SAXS: small angle X-ray scattering; SSU: small subunit

# 1. Introduction

***Regulation of Algal Metabolism***

Algae comprise a hugely diverse, polyphyletic group of photosynthetic organisms including prokaryotic cyanobacteria and eukaryotic algae from the Archaeplastida, Chromalveolata, Cryptophyta, Haptophyta and Dinoflagellata [1]. At least 72,500 algal species are known [2] but this is probably a large underestimate. Algae contribute about 46% of global primary production, mainly in the ocean [3] and also play an important role in global biogeochemical cycles such as oceanic carbon sequestration [4]. Algae are ubiquitous in aquatic and moist environments and some of them are 'extremophiles' tolerating, *inter alia*, desiccation [5], high or low temperatures, high or low pH, high salinity, high pressure [6] and high concentrations of heavy metals [7, 8]. Some survival strategies involve the production of novel biochemical compounds and there is a recent impetus towards the valorization of algae metabolic products, such as polyunsaturated fatty acids, fatty acid esters, polysaccharides, and the production of enzymes for various applications [9].

In eukaryotic algae, as in plants, chloroplasts have a central role in metabolism. They all derive from a cyanobacterium, but the number of membranes around a chloroplast varies depending on the number of endosymbioses involved in their evolutionary history [10]. Most of the chloroplastic enzymes involved in the metabolic primary steps are nuclear-encoded [11, 12] and their activity can be regulated at the transcription, translation, or protein level. Notably, at the protein level, post-translational modifications, protein-ligand or protein-protein interactions can regulate enzyme activity [13, 14].

Algae comprise a large number of algal species with high biochemical and structural diversity, are ecologically important, and can survive and grow in a large range of different

environments. To understand the basic principles of how algae regulate their metabolism, and eventually support future biotechnological applications, we need to decipher the mechanisms of these processes at the molecular level. Among regulatory proteins, a peculiar class of proteins has emerged, the intrinsically disordered/ductile proteins (IDPs), or the proteins that contain intrinsically disordered regions (IDRs). In this review, we will discuss their role in the regulation of algal metabolisms.

***Reassessment of the protein structure function paradigm***

IDPs escape from the classic structure-function paradigm and are fully functional despite being fully or partially disordered. The principal feature of these IDPs or IDRs containing proteins is the absence of a unique stable conformation which results in a high structural flexibility [15, 16]. Nevertheless, IDPs can also present either small structural motifs, or have a significant propensity to fold into secondary structural elements [17, 18]. This propensity to fold is modulated by several factors such as post-translational modifications [19], protein or ligand binding [20-22], physico-chemical conditions such as redox-conditions [23-27], pH [28], temperature [29], binding to a membrane, adsorption at an interface [30], etc. Some IDPs are also named conditionally disordered proteins [31] when their disorder content and their function is directly modulated by different possible conditions, as redox-conditions. A large collection of names were found in the literature that describes these proteins, and the community eventually decided to keep the term IDP [32]. The term will be used in the rest of this review.

The signatures of IDPs are rather well-established, with a bias in their amino-acid composition and frequent sequences of low complexity [33, 34]. Based on the sequence peculiarities of IDPs, a plethora of disorder predictors has been developed during the last two decades, with increasing prediction accuracy, and are continually updated [35-37]. These

predictors have allowed large scale analyses of an ever growing number of fully sequenced and annotated genomes in all kingdoms of Life, which revealed the widespread and universal nature of IDPs. Several databases of IDPs have thus been implemented, which provide pre-computed disorder predictions of completely-sequenced genomes ($D^2P^2$; http://d2p2.pro/), of all UniProt entries (MobiDB; http://mobidb.bio.unipd.it/), or which reference only experimentally verified IDPs (DisProt, http://www.disprot.org/; IDEAL, http://idp1.force.cs.is.nagoya-u.ac.jp/IDEAL/).

A range of biophysical approaches are required to characterise experimentally the disordered properties of IDPs, often advantageously in association with state-of-the-art modelling tools to comprehend the large ensemble of probable conformational states that coexist in a given condition [16, 38-41]. IDPs have thus been characterised by small angle X-ray scattering (SAXS), nuclear magnetic resonance (NMR) spectroscopy, circular dichroism (CD) spectroscopy, protease sensitivity and many other techniques, to cite but a few (see Longhi, this issue, [16, 41-43], with each method having advantages and limitations.

The functions carried out by IDPs are diverse and complement those of ordered proteins. They play significant roles in many biological processes, such as control of the cell cycle, transcriptional activation, and signalling, and they frequently interact with or function as central hubs in protein interaction networks [44-48]. Moreover, correlation between disorder and post-translational modifications (PTMs) such as phosphorylation, methylation, acylation, glycosylation and ubiquitination has been reported [19, 49]. This is worth noting since PTMs can contribute to the diversification and functionality of proteomes by regulating different properties of proteins (stability, conformation, etc…). Hence IDPs via diverse PTMs participate in the regulation processes of the cell [19, 46, 50, 51].

***IDP phylogenic distribution***

Analysis of the proteomes using predictors of disorder revealed that the proportion of intrinsic disorder in proteomes is variable in the evolution tree. There is a low proportion of disorder in bacteria and archaea, a higher one in viruses, and a much larger one in eukaryotes, especially in higher vertebrates, including mammals [52-54]. Recently, it was shown that IDPs play a role in the evolution of multi-cellularity and cell type specification [48, 55].

By contrast, the distribution of IDPs among the different kingdoms of Life, that have been experimentally investigated, does not correlate with the distribution of predicted IDPs. Notably, in the experimentally-verified database DisProt [56], IDPs from photosynthetic organisms, and especially from algae, are under-represented while IDPs from bacteria are over-represented, with 19% of all IDPs. In the other databases, this number ranges from 0 to 8.1% [56]. This discrepancy in the IDP databases might reflect, in a predominant manner, a biased interest in scientific research for pathogenic organisms that overlooks the wide distribution of disorder in the tree of Life. Another bias might also result from the fact that large scale projects on structural genomics have used *E. coli* as the main expression system for high throughput production and screening of proteins, which naturally limits the efficient and correct production of some eukaryotic proteins.

***IDP in plants and algae***

A specific feature of IDPs is that they are able to maintain their functional states in a wide range of physiological conditions, unlike well-folded proteins which can be extremely sensitive to variation in their environment. Indeed, IDPs do not aggregate under high temperature or low pH, conditions in which structured proteins usually undergo denaturation and loss-of-function [29, 57, 58]. As mentioned above, algae encounter extreme conditions. Indeed, some algae can sink down to 4000 m in the deep sea and survive despite the high hydrostatic pressure [6]. Similarly to plant cells, algae can maintain their cellular volume by

imposing a turgor pressure inside their cell wall [5, 59]. Variation of pressure can be also detrimental for the conservation of functional cavities in folded enzymes and of macromolecular scaffolds [60]. For instance, it has been shown that the activity of Calvin-Benson-Bassham (CBB) cycle enzymes is strongly affected by high pressure [61]. IDPs, which are devoid of internal cavities, are expected to be more stable upon pressure increase, and indeed, α-synuclein [62] and the disordered region of titin [63] preserve their disordered characteristics at high pressure. It is likely that IDPs in general are more stable in these conditions but this needs further investigations.

Besides, the chloroplast proteome undergoes variations of physico-chemical conditions with redox switches [64]. The dark-light regulation of the photosynthetic pathway in the chloroplast is mainly modulated via redox transitions [65-67]. Thanks to their propensity to receive post-translation modifications that modify their conformational and functional states, IDPs are particularly suited to sense these changes of physico-chemical conditions, thereby regulating enzymes activity [68, 69]. For all these reasons, the properties of IDPs are valuable assets for the regulation of enzymes and metabolic pathways, in the broad range of environmental conditions that algae withstand.

Up to now, only few IDPs from photosynthetic organisms have been investigated, and they mainly come from the higher plant *Arabidopsis thaliana*, which has one of the most accurately annotated sequenced genome thanks to its small size. A recent analysis of 12 plant genomes revealed that the occurrence of disorder in plants is similar to that in many other eukaryotes [70, 71] though only few plant IDPs have been experimentally characterised (A. Covarrubias, this issue). These IDPs or IDR containing proteins are, in the vast majority, nuclear-encoded proteins, while chloroplast- and mitochondria-encoded proteins have a lower disorder trend as in Archae and Bacteria, which is in agreement with their phylogenetic origin.

In the model alga *Chlamydomonas reinhardtii*, a systematic search for disordered protein experimentally showed that this alga contains a large number of non-characterised proteins that have IDP-like properties [72]. Interestingly, the few IDPs that have been biophysically investigated so far are the ones involved in the regulation of their metabolic pathways. This bias arises from the keen interest in the metabolism of algae, which have a high potential in $CO_2$ assimilation and production of various added-value molecules.

In this review we shine a spotlight on the few known IDPs from algae that help these photosynthetic organisms to adjust their photosynthetic carbon metabolism to the prevailing conditions in their habitat. These IDPs are found in the chloroplast, where the primary step of photosynthesis occurs, and where $CO_2$ is assimilated and converted into building blocks to produce fatty acids, carbohydrates, proteins and various metabolites. Chaperones, which regulate the proteostasis, and therefore metabolism, through disordered regions, will also be discussed. Finally, we will extend the discussion to other algal predicted IDPs involved in metabolic regulation.

## 2. $CO_2$ acquisition and assimilation

During photosynthetic $CO_2$ assimilation, chemical energy, conveyed mainly by ATP and NAD(P)H, and supplied by electron transfer reactions through the photosystems I and II, is used by metabolic reactions of the CBB cycle. The efficiency of this assimilation relies on a fine tuning between energy demand and supply. Hence the CBB is highly regulated. This cycle functions in the light and is inactive in the dark. Some enzymes are active only under reducing conditions, corresponding to the light conditions, and are inactive under oxidising conditions corresponding to the dark [13, 65].

***RuBisCO activase (RCA)***

Ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) is the enzyme that initiates the CBB cycle by converting ribulose-1, 5-bisphosphate (RuBP) and carbon dioxide ($CO_2$) into two molecules of phosphoglyceric acid (PGA). In addition, RuBisCO catalyses a competitive reaction that converts RuBP in the presence of $O_2$, into one PGA and one toxic compound, phosphoglycolate [73]. RuBisCO is made up of eight large (LSU, RbcL) and eight small (SSU, RbcS) subunits, in algae and higher plants [74]. In order for RuBisCO to be active, it has long been suggested that a specific lysine residue on the LSU needs to be carbamylated [75]. Today, it is known that the mechanism for regulating RuBisCO is much more complex and involves not only changes in the carbamylation state of this lysine residue, but also conformational changes in the protein. These conformational changes are triggered by a protein called RuBisCO activase (RCA), which is a specific molecular chaperone and an $AAA^+$ ATPase [66]. RuBisCO active sites can be locked by sugar phosphate in the close inactive state and RCA prevents this inhibition by promoting ATP-dependent conformational changes that open closed sites, thus facilitating dissociation of inhibitory sugar phosphate

(Figure 1). Furthermore, the presence of RCA allows RuBisCO to be active even at sub-optimal $CO_2$ concentrations that would not normally permit carbamylation *in vivo* [76, 77].

Some algae in the charophyta phylum have multiple isoforms of RCA: two α-isoform of ~45-48 and 58 kDa, and a β-isoform of ~41-43 kDa [78] (Figure 2B). These isoforms are the product of alternative splicing and differ from each other by a larger and redox-regulated C-terminal extension on the α-isoforms. This C-terminal extension contains three or two regulatory cysteine residues that allow the protein to be activated by light through the action of a small redox protein, the thioredoxin f [78, 79]. Interestingly, RCAs from another phylum of microalgae, the chlorophyta (including *C. reinhardtii*), lack these two cysteine residues and therefore are unlikely to be redox-sensitive.

An *in silico* analysis [80] showed that the C-terminal extension of RCA isoforms is intrinsically disordered (Figures 1B and 2A). Several small but significant differences were observed between the intrinsic disorder propensities in RCAs from two chlorophyta algae, *Tetraselmis* sp. and *Ostreococcus tauri*, probably reflecting a different mode of interaction between their RCAs and RuBisCO [81]. The authors suggest that the difference in the disordered propensities can be ascribed to different evolution pressures on these two algae related to their motility. Indeed, *Tetraselmis* sp. is motile and can actively migrate to optimal habitat, in contrast to *O. tauri,* which is a non-motile microalga. For the latter, therefore, there is a higher evolution pressure on RCA to optimise photosynthesis under non-optimal conditions. However, one cannot exclude that other processes are involved. In other photosynthetic organisms such as in β cyanobacteria, the RCA lacks the N terminal domain necessary for RuBisCO activation found in plants and green algae [66, 78, 82]. In red lineage organisms, there is no RCA gene but it is substituted by another protein, called CbbX. In the purple bacterium *Rhodobacter sphaeroides*, CbbX possesses a short disordered C-terminal

extension (Figures 1C and 2A), the function of this part though, remains unelucidated [83]. The homologues of CbbX from Rhodophyta lack this C-terminal extension (Figure 2C).

***Essential Pyrenoid component 1 (EPYC1)***

In many algae, RuBisCO is present within a chloroplastic membraneless compartment that concentrates locally proteins involved in carbon fixation, and called a pyrenoid. Pyrenoids are involved in $CO_2$ concentrating mechanisms (CCMs) that are essential for aquatic organisms, which face low $CO_2$ concentrations much below the optimal concentration for RuBisCO activity [84, 85]. These CCMs increase $CO_2$ concentration around RuBisCO and thereby decrease $O_2$ competition. The pyrenoid is located in the chloroplast, and is surrounded by a starch sheath and traversed by membrane tubules that are continuous with the thylakoid membranes [86]. Recently, it was shown that in the model alga *C. reinhardtii*, RuBisCO accumulation and localization within the pyrenoid is mediated by a disordered repeat nuclear-encoded protein, called Essential Pyrenoid component 1 (EPYC1), previously identified as a low-$CO_2$ inducible protein (LCI5; Cre10.g436550) [87]. This protein is ubiquitous in pyrenoid-containing algae but is absent in pyrenoid-less algae. In addition to EPYC1, RuBisCO and its chaperone RCA, 135 to 190 putative CCMs proteins including some of their binding partner(s) were also found to be located in the membrane-less pyrenoid, by mass spectrometry analysis [88, 89]. Like many IDPs, EPYC1 is phosphorylated at low $CO_2$ concentration [90]. Furthermore EPYC1 was shown to interact with a predicted serine/threonine protein kinase (KIN4-2; Cre03.g202000) and two 14-3-3 kinases FTT1 and FTT2 [87]. These interactions with kinases probably regulate RuBisCO and EPYC1 interactions and activities.

EPYC1 protein displays characteristic features of IDPs. Its CD profile is typical of an IDP (Figure 3A), and it is resistant to denaturing conditions such as boiling for 15 min [91]. Its sequence contains four 60 amino-acids repeats, each domain being predicted to be

disordered (Figure 3C). The role for these tandem domain repetitions remains controversial. It was hypothesised that these low-complexity repeats allow EPYC1 to bind several RuBisCO (Figure 3B), which then drives formation of pyrenoid droplets with liquid-like properties [88]. However, it was later shown that a mutant of EPYC1 with a single repeat was still able to form membrane-less liquid-liquid phase separation droplets upon binding to RuBisCO *in-vitro*, suggesting that each repeat domain contains several binding sites to RuBisCO. *In-vitro* reconstitution of liquid-liquid phase separation droplets indicated that EPYC1 and active RuBisCO are necessary and sufficient for phase separation, and that the phase separation is governed by electrostatic interactions [91]. In line with this finding, it is known that proteins with IDRs and low complexity sequences tend to phase separate and to form membrane-less organelles [92]. The molecular nature of such membrane-less organelles is under intense scrutiny [93]. In the current model, the pyrenoid matrix is highly dynamic, in a liquid-phase that supports efficient molecular diffusion to increase $CO_2$ concentration and decrease the oxygenase activity of RuBisCO.

***CP12 and the regulation of the CBB cycle***

CP12 is a nuclear-encoded chloroplast protein of about 8.5 kDa that is present in many photosynthetic organisms, including cyanobacteria, algae, and plants [94, 95] as well as in cyanophages [96]. It has been especially well characterised in some model organisms such as the higher plant *A. thaliana*, the cyanobacterium *Synechococcus elongatus* and the green alga *C. reinhardtii* [97]. This small protein is a conditionally disordered protein that has been characterised by NMR, SAXS, CD, Förster resonance energy transfer, fluorescence correlation spectroscopy, mass spectrometry and molecular modelling [26, 27, 98-101]. CP12 is involved in the regulation of the CBB cycle but has multiple other faces, such as being a chaperone. CP12 from *C. reinhardtii* has two disulfide bridges, one at the N-terminus and one

at the C-terminus. While the C-terminal cysteine residues are conserved in all CP12 proteins, the N-terminal ones may be missing in some photosynthetic organisms, e.g. in rhodophytes, the glaucophyte *Cyanophora* and in *Synechococcus* [94, 102, 103]. The formation of the two disulfide bonds under dark or oxidizing conditions allows the algal CP12 to bind two enzymes from the CBB cycle, namely glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoribulokinase (PRK). The formation of this supramolecular ternary edifice results in the inactivation of these two enzymes and consequently leads to the arrest of the CBB cycle in the dark. In the light, CP12 is reduced and the breakage of its two disulfide bridges leads to the dissociation of the ternary complex, with the resulting dissociated forms of PRK and GAPDH being fully active. CP12 therefore participates in the regulation and in the "work on demand" of the CBB cycle that is active under light and inactive under dark.

While in the oxidised state, CP12 has some α-helices, in the reduced state it becomes fully disordered (Figure 4A and 4B) [27, 99, 104, 105]. However, in both reduced and oxidised states, CP12 is highly mobile. NMR, CD and SAXS showed that the algal oxidised CP12 is composed of three regions with distinct conformational sampling [27]: its C-terminus folds in a stable turn, ready for GAPDH binding, it is linked to the N-terminal domain through a randomly disordered linker, while the N-terminus oscillates on the millisecond timescale between helical and random states (Figure 4C). The existence of a conformational equilibrium between two states of the algal CP12 was also shown previously by ion mobility mass spectrometry [98]. The combined analysis of NMR and SAXS data enabled the quantification of the relative population of each state (40% random-coil sate and 60% helical). Upon binding to GAPDH, this equilibrium shifts towards more extended conformations (Figure 4D). In other words CP12 undergoes an induced unfolding. This phenomenon, called cryptic disorder, which contributes to decreasing the entropy cost of binding, might explain the very high affinity of CP12 for its partners, with a dissociation constant in the nM range [106]. This

GAPDH/CP12 sub-complex in turn enables the binding of PRK [107]. The idea that two proteins could form a high-affinity complex while remaining highly dynamic has long been "nearly heretical". However, a recent study has brought to light the case of two fully disordered proteins forming a dynamic complex of very high affinity (pM range), without any gain of structure in the two proteins [108]. This last example shows that disordered proteins might interact strongly without anchor sites, nor disorder-to-order transitions, opening a new concept and avenue in the field of IDPs.

CP12 can bind metal ions, such as $Cu^{2+}$, $Ni^{2+}$ [109] and $Ca^{2+}$ [110], as well as enzymes such as the FBP aldolase [111]. Of interest also, CP12 can play the role of a specific chaperone for GAPDH preventing its aggregation and inactivation by heat treatment [112]. Hence, CP12 is a versatile protein considered as a jack-of-all-trades but master of the CBB cycle [113], that indirectly regulates the $CO_2$ assimilation by interacting with PRK and GAPDH. This regulation by CP12 is conserved in different species, but the molecular mechanism of the interactions and the strength of binding between the PRK/GAPDH/CP12 complex differ among species. For example, in *A. thaliana* the dissociation constant for GAPDH/CP12 is in the µM range [114], and CP12 undergoes an induced folding upon binding to GAPDH, in contrast to *C. reinhardtii* [27]. The broad adaptation to multiple environmental conditions might explain these divergent molecular mechanisms.

***GAPDH-B and ADK3, two enzymes with C-terminal extensions homologous to CP12***

There are two main forms of chloroplast GAPDH. The first one, GapA, is present in the majority of photosynthetic organisms [102, 115] and forms $A_4$ homotetramers. The second isoform, GapB, is found in higher plants and in the alga *Ostreococcus*. It is extremely similar to GapA, but has a C-terminal extension (about 30 amino acids) homologous to CP12 [116, 117]. The GapB subunit forms functional $A_2B_2$ heterotetramers with the GapA subunit, as

well as inactive $A_8B_8$ oligomers, although the relevance and the role of these complexes remain disputed. However, it is well established that the disordered "tail" or the C-terminal extension on GapB endows GAPDH with autonomous redox regulation [115].

In *C. reinhardtii*, where only GapA exists, this autonomous regulation is impossible. However, as the first steps of glycolysis and the oxidative pathway of phosphate pentoses take place in the chloroplast [118], there is an obligatory need for a fine regulation of the activity of this $A_4$ isoform. It has been shown that the redox regulation of this chloroplast A4 GAPDH, depends on its interaction with CP12. Therefore, the regulation of GAPDH relies, as for RuBisCO, on IDRs either on the enzyme itself or on its partner, CP12. Since GAPDH is a moonlighting protein, with many cellular functions [119], fusion with this CP12-like tail may endows it with further other regulatory functions yet unknown and to be explored.

Other important enzymes are the adenylate kinases (ADKs). These enzymes maintain the energetic balance in cellular compartments including chloroplasts and mitochondria, by catalyzing the interconversion of AMP, ATP, and ADP (AMP+ATP↔ 2ADP). In *C. reinhardtii*, ten genes encoding ADKs are present. Within the chloroplast stroma of photosynthetic organisms, where the ATP:ADP ratio has to be kept at an optimal level to allow a sustainable rate of $CO_2$ fixation, ADK3 plays a major role in the regulation of concentrations of adenylates [120].

ADK3 from *C. reinhardtii* comprises two domains: a canonical domain, homologous to the *Escherichia coli* ADK (44% identity), and a "tail" of 25 residues, very close to the C-terminal portion of CP12 (68% identity) [121]. The kinetic parameters for the wild-type and truncated ADK3 (removing the C terminus, namely ADK3-ΔCP12) are very similar, indicating that the C-terminal extension of ADK3 does not affect the activity and kinetic properties of the enzyme, nor its global secondary structure. Since CP12-like extension

confers GAPDH redox regulation, it was hypothesised that it might be the same with ADK3. This redox regulation was not observed, but the CP12-like extension confers ADK3 with a high affinity for the chloroplast GAPDH [122]. Via this disordered region, ADK3 down-regulates the NADPH-dependent GAPDH activity, therefore stopping the consumption of NADPH by the CBB cycle. The C-terminal extension on ADK is also found in the sequence of ADK from other algae and might play the same role.

In addition, the C terminus of ADK3 has a strong effect on the thermal stability of the enzyme, and this effect is probably the result of its predicted disorder propensity, in agreement with IDPs heat-resistance [29, 57].

***Protection against oxidative damage***

The metabolism of microalgae is tightly redox regulated. On the other hand, the transient accumulation of reactive oxygen species (ROS) causes subsequent oxidative damage, protein unfolding and aggregation. Chloroplasts are organelles where molecular oxygen is produced during photosynthesis and where ROS production occurs, therefore protein-thiols protective mechanisms are active in these organelles. Under conditions causing oxidative stress, glutathionylation and nitrosylation play roles in enzyme regulation and signalling [123]. A proteomic study on *C. reinhardtii* has identified 225 proteins that are potential targets of glutathionylation, including 10 out of 11 enzymes of the CBB cycle [124], suggesting a central role of such modification in the global regulation of the carbon assimilation pathway. The role of CP12 in oxygen photoautotrophs beyond light/dark regulation, includes protection of PRK and GAPDH from oxidative stress [125] and a role in the glutathionylation of ADK3 that remains to be defined. Indeed, while ADK3 can be glutathionylated, the truncated ADK3-ΔCP12 cannot, even though the site of glutathionylation is not on the cysteine residues present in the C-terminal extension on ADK3 [121].

## 3. Molecular chaperones

Protein homeostasis, or proteostasis, is controlled by the so-called chaperones, in response to stress, and in normal conditions [126]. These proteins are thus essential for the "orchestration" of metabolism. Several molecular chaperones have now been identified and classified as conditionally disordered proteins [127]. The functions and underlying mechanisms of molecular chaperones are very well studied in bacteria and in the cytosol or endoplasmic reticulum of eukaryotic cells, but their role in the metabolic processes of the chloroplast have been overlooked.

An experimental search for IDPs in *C. reinhardtii* showed that chaperones and ribosomal proteins were the most represented IDPs [72]. Twenty proteins related to chaperone-function were identified, such as the heat-shock proteins HSP33, HSP40, HSP70s, FKBP, HSP90 co-chaperone, …. The disordered propensities of HSP70, HSP90 and HSP90 co-chaperone (Figure 5) have been shown experimentally in other organisms than algae [128], except for HSP33 that was characterised in *C. reinhardtii* [69].

HSP33 has been well studied in *E. coli* and is a redox chaperone that has a flexible C terminus that contains a conserved cysteine-rich center, Cys-X-Cys-$X_n$-Cys-X-X-Cys [23, 25]. These cysteine residues bind $Zn^{2+}$ under reducing conditions, thus maintaining the protein in its inactive form. In the oxidised form, the C-terminus unfolds after $Zn^{2+}$ release and the protein becomes active. To be active, this bacterial chaperone therefore needs to become disordered and this internal order-to-disorder transition is required to control substrate binding and release [129]. Interestingly, HSP33 is present in only two clades of eukaryotes: (i) Kinetoplastids and parasitic oomycetes and (ii) photosynthetic organisms, but only in algae and mosses, not in higher land plants [69]. In *C. reinhardtii*, and in general in the green algal lineage, the first cysteine residue of the conserved cysteine-rich center has been lost and

replaced by a glycine residue. As a result, the C-terminus of HSP33 has lost its ability to bind Zn in reducing conditions and is disordered in both oxidised and reduced forms, unlike the bacterial protein. Only few structural changes of HSP33 from *C. reinhardtii* have been observed experimentally between the oxidised and the reduced forms, as predicted (Figure 5A), and this was linked to a lower affinity for $Zn^{2+}$ [69]. In *C. reinhardtii*, HSP33 expression increases in response to light-induced oxidative stress and appears to participate in the rapid response to exposure of the algal cells to oxidizing conditions. This protein is therefore essential to algal proteostasis and metabolism.

In the cytosol of eukaryotic cells, the function of a plethora of proteins involved in signal transduction, like hormone receptors and kinases, depends on their folding by the HSP90 and HSP70 machineries [130, 131]. The HSP70s family comprises one subset of the best-studied chaperones in *C. reinhardtii*. These proteins play a role in all major subcellular compartments of the cell, and the HSP70B, HSP70C and HSP70A are targeted to the chloroplast, mitochondrion and cytosol, respectively [132]. The chloroplast HSP70B is induced either by light, or by heat or cold stress [132, 133]. Interestingly, the algal HSP70B that is highly homologous to other HSP70B from different species (see Table 1 in [132]) exhibits long disorder regions of more than 30 amino acid residues, and in particular a 71-amino acid disordered region at its C terminus (Figure 5B). Its flexibility allows binding and release of clients, in good agreement with its function. In *C. reinhardtii*, HSP70B and HSP90C can form a multimeric complex [134], although this complex has never been successfully reconstituted *in vitro* from the two recombinant proteins [135]. The algal HSP90C also bears a disordered C-terminus of about 50 aminoacid residues (Figure 5C). Both HSP70B and HSP90C were found in the *Chlamydomonas* phosphoproteome [136] indicating that they are phosphorylated as many IDPs. Despite their importance in the cell, these heat-

shock proteins, HSP70B, HSP90C and HSP33 remain understudied in *C. reinhardtii* and other algae compared to other organisms [137, 138].

## Algal IDPs in other cellular processes

The experimental search for putative IDPs in *C. reinhardtii* also revealed few enzymes with long intrinsically disordered segments, involved in the algal metabolism (Figure 6) that are nuclear-encoded and targeted to either the mitochondrion or the chloroplast [72]. These IDR-containing enzymes have not been studied so far to our knowledge. Yet, one can hypothesise that the IDRs of these enzymes may also play important roles in the metabolism. To cite but a few, the magnesium chelatase complex, involved in the light capture in the chloroplast, contain an IDR. This enzyme synthesises a precursor of chlorophyll. Downstream of the CBB, the synthetic pathway of the storage compound starch also involves an IDR-containing protein, the soluble starch synthase III (Figure 6B). Besides, four enzymes with long IDRs targeted to the mitochondrion were identified by Zhang *et al.:* the peptide methionine sulfoxide reductase, the dihydrolipoamide succinyltransferase oxoglutarate dehydrogenase E2 component (DLST-2OGDH) (Figure 6B), the F1F0 ATP-synthetase ε-subunit and the carbonic anhydrase CA-8, all involved in the respiration process.

## 5. Conclusions

The autonomous chloroplastic and mitochondrial genomes encode only a small subset of proteins and the majority of the proteins present in these organelles are nuclear-encoded and imported after their synthesis in the cytosol. This import process allows the optimization of metabolic pathways by IDPs, although the chloroplast and the mitochondrion originate from bacteria, which have a lower content in IDPs. Furthermore, IDPs, with their ductile properties, are very robust and well-suited to cope with the drastic environmental changes encountered

by algae (extreme changes in temperature, pressure, pH, light etc.) and tune the metabolic pathways according to these changes. The data obtained from the experimental study on *C. reinhardtii*, although not exhaustive [72], and the predictions from algal genomes [71] demonstrate the wide distribution of protein disorder in algae. Remarkably a study comparing the distribution of disorder in different cell types, from mammals (including *Homo sapiens*), plants, algae, and bacteria showed that the proteome of *C. reinhardtii* contains the highest number of residues with a disorder score [55].

Whereas very few studies have been undertaken hitherto, the growing interest in microalgae and their metabolic properties will undoubtedly trigger original studies on the role of IDPs in the regulation of cellular and metabolic processes in these microorganisms.

**Acknowledgements**

The authors are grateful to Pr Stephen Maberly who read our manuscript and made helpful comments.

**Funding**

Financial support is provided by CNRS, Aix Marseille Université, and the ANR SIGNAUX BioNRJ (ANR-15-CE05-0021-03).

**Figure captions**

**Figure 1**: **Mechanism of action of RCA**. **A:** Model of the interaction between RuBisCO and RCA and of the mechanism of action of RCA activation, figure extracted from [139]. The disordered C-terminal extension of RCA is indicated by the letter C. **B**: Model structure of RCA from the higher plant tobacco (*Nicotiana tabacum*), with C-terminal extensions not resolved in the electron microscopy images, represented by black lines (RCSB entry

3ZW6.pdb, [140]). **C**: Model structure of the red-type $AAA^+$ rubisco activase CbbX from the purple bacteria *R. sphaeroides*. The C-terminal extensions not resolved in the negative stain images are represented by a black line (RCSB entry 3ZUH.pdb [83]).

**Figure 2: Disorder propensity of the C-terminal extension of RuBisCO activase and CbbX. A.** Hydrophobic Cluster Analysis (HCA, [141]) plots of the C-terminal extension of rubisco activase and CbbX from the following organisms, from top to bottom: *C. reinhardtii* (accession number XP_001692244.1); *Chlorokybus atmophyticus,* isoform β (EST HO20293, [78]); *Klebsormidium flaccidum,* isoforms α2 and α1 (EST HO431775.1, [78]); the purple bacterium *Rhodobacter sphaeroides* (accession number AAC44827.1). **B**: Schematic alignment of RCA from cyanobacteria, chlorophyta and charophyta highlighting their C-terminal extension. Figure extracted from [78]. **C**: Schematic alignment of CbbX from purple bacteria, rhodophyta and heterokonta. Interestingly, and contrary to the green-type viridiplantae, the rhodophyta seem to have lost the C-terminal disordered extensions.

**Figure 3: Disorder in the essential pyrenoid component EPYC1 forming membraneless pyrenoid with RuBisCO. A**: The circular dichroism spectrum of EPYC1 indicates random-coil structural properties, in contrast to the spectra obtained for RuBisCO and lysozyme. Figure extracted from supplementary figure 1 of [91]. **B**: Schematic of the composition of the pyrenoid, with a ratio of EPYC1: RuBisCO from one to four. Among the 135-190 proteins that are present in the pyrenoid, we chose to represent RCA that is discussed in this review. C: Schematic of the EPYC1 protein sequence with its four repeating domains. The HCA plot of the first repeat is shown (*C. reinhardtii* sequence, accession number PNW77439.1).

**Figure 4: Redox conformational transitions of CP12 governing the light-dark CBB-cycle regulation**. **A**: Schematic of the redox transitions of CP12 and GAPDH-CP12 formation. The two pairs of cysteine residues of the green-lineage CP12 are redox regulated by the thioredoxins (Trx) that are reduced (under light) or oxidised (under dark). **B**: Under reducing conditions, CP12 is fully disordered and highly flexible. **C**: Under oxidizing conditions, the two pairs of cysteine residues form two disulfide bridges, and CP12 is partially disordered. The C-terminal bridge is in a stable structure, depicted in blue, while the N-terminal bridge is in a region that interconverts between two states on a millisecond timescale, depicted in red: a random-coil state and a helical hairpin. **D:** This latter region unfolds when the C-terminal region is bound to GAPDH.

**Figure 5: Three chloroplastic intrinsically disordered chaperons of *C. reinhardtii***. HSP33, HSP70b and HSP90C are predicted as containing disordered regions using Pondr-VLXT (A score higher than 0.5 indicates a propensity for structural disorder)[142]. **A**: Pondr-VLXT score of *C. reinhardtii* HSP33 (accession number, XP_001700446). The prediction of the reduced state of HSP33 is obtained using the mutant C274S,C309S,C312S. **B**: Pondr-VLXT score of *C. reinhardtii* HSP70b (accession number XP_001696432). **C**: Pondr-VLXT score of *C. reinhardtii* HSP90 (accession number XP_001702984.1).

**Figure 5: Examples of *C. reinhardtii* metabolic enzymes with long intrinsically disordered regions** . **A**: Schematic of the metabolic pathways which possess enzymes with long intrinsically disordered regions and that are heat-resistant. These enzymes have been identified by Zhang *et al.* [72]. Some enzymes are chloroplastic: the magnesium chelatase complex ($Mg^{2+}$ CC, accession number: XP_001700902.1), the soluble starch synthase III (SSS III, accession number: AAY42381.1); while others are targeted to the mitochondrion:

the peptide methionine sulfoxide reductase (PMSR, accession number: XP_001689879.1), the carbonic anhydrase 8 (CA-8, accession number: XP_001697606.1),the dihydrolipoamide succinyltransferase, the oxoglutarate dehydrogenase E2 component (DLST-2OGDH, accession number: XP_001692539.1) and the F1F0 ATP synthetase ε subunit (F1F0 ATP ε, accession number: XP_001702609.1). Other IDPs described in this review are also included, in particular those regulating the CBB cycle, and chaperones. **B**: The IUPred2A [143] disorder prediction for the mitochondrial DLST-2OGDH and the chloroplastic soluble starch synthase III are given as examples. A score higher than 0.5 indicates a propensity for structural disorder.

## References


[1] S. Markov, Algae, Magill's Encyclopedia of Science: Plant Life, Salem Press 2003, pp. 47-50.

[2] M.D. Guiry, How many species of alage are there?, Journal of Phycology 48(5) (2012) 1057-1063.

[3] C.B. Field, M.J. Behrenfeld, J.T. Randerson, P. Falkowski, Primary production of the biosphere: Integrating terrestrial and oceanic components, Science 281(5374) (1998) 237-240.

[4] J.L. Sarmiento, N. Gruber, Ocean Biogeochemical Dynamics, Princeton University Press 2006, pp. 1-526.

[5] A. Holzinger, U. Karsten, Desiccation stress and tolerance in green algae: consequences for ultrastructure, physiological and molecular mechanisms, Frontiers in Plant Science 4 (2013).

[6] S. Agusti, J.I. González-Gordillo, D. Vaqué, M. Estrada, M.I. Cerezo, G. Salazar, J.M. Gasol, C.M. Duarte, Ubiquitous healthy diatoms in the deep sea confirm deep carbon injection by the biological pump, Nature Communications 6 (2015) 7608.

[7] L.J. Rothschild, R.L. Mancinelli, Life in extreme environments, Nature 409(6823) (2001) 1092-1101.

[8] P. Varshney, P. Mikulic, A. Vonshak, J. Beardall, P.P. Wangikar, Extremophilic micro-algae and their potential contribution in biotechnology, Bioresource Technology 184 (2015) 363-372.

[9] S. Leu, S. Boussiba, Advances in the Production of High-Value Products by Microalgae, Industrial Biotechnology 10(3) (2014) 169-183.

[10] T. Cavalier-Smith, Membrane heredity and early chloroplast evolution, Trends Plant Sci 5(4) (2000) 174-182.

[11] B. Agne, F. Kessler, Protein transport in organelles: The Toc complex way of preprotein import, FEBS J 276(5) (2009) 1156-1165.

[12] Y.D. Paila, L.G.L. Richardson, D.J. Schnell, New insights into the mechanism of chloroplast protein import and its integration with protein quality control, organelle biogenesis and development, J Mol Biol 427(5) (2015) 1038-1060.

[13] B. Gontero, L. Avilan, S. Lebreton, Control of carbon fixation in chloroplasts, Annual Plant Reviews 22 (2006) 187-218.

[14] S.C. Maberly, C. Courcelle, R. Groben, B. Gontero, Phylogenetically-based variation in the regulation of the Calvin cycle enzymes, phosphoribulokinase and glyceraldehyde-3-phosphate dehydrogenase, in algae, J. Exp. Bot. 61 (2010) 735-745.

[15] R.B. Berlow, H.J. Dyson, P.E. Wright, Expanding the paradigm: Intrinsically Disordered Proteins and allosteric regulation, J Mol Biol 430(16) (2018) 2309-2320.

[16] A. Bhowmick, D.H. Brookes, S.R. Yost, H.J. Dyson, J.D. Forman-Kay, D. Gunter, M. Head-Gordon, G.L. Hura, V.S. Pande, D.E. Wemmer, P.E. Wright, T. Head-Gordon, Finding our way in the dark proteome, J Am Chem Soc 138(31) (2016) 9730-9742.

[17] R. Sharma, A. Sharma, G. Raicar, T. Tsunoda, A. Patil, OPAL+: Length-Specific MoRF Prediction in Intrinsically Disordered Protein Sequences, Proteomics 19(6) (2019) e1800058.

[18] J.A. Marsh, V.K. Singh, Z. Jia, J.D. Forman-Kay, Sensitivity of secondary structure propensities to sequence differences between alpha- and gamma-synuclein: implications for fibrillation, Protein Sci 15(12) (2006) 2795-2804.

[19] A. Bah, J.D. Forman-Kay, Modulation of intrinsically disordered protein function by post-translational modifications, J Biol Chem 291(13) (2016) 6696-6705.

[20] M. Arai, K. Sugase, H.J. Dyson, P.E. Wright, Conformational propensities of intrinsically disordered proteins influence the mechanism of binding and folding, Proc Natl Acad Sci U S A 112(31) (2015) 9614-9619.

[21] M. Fuxreiter, P. Tompa, Fuzzy complexes: a more stochastic view of protein function, Adv Exp Med Biol 725 (2012) 1-14.

[22] K. Sugase, H.J. Dyson, P.E. Wright, Mechanism of coupled folding and binding of an intrinsically disordered protein, Nature 447(7147) (2007) 1021-1025.

[23] C.M. Cremers, D. Reichmann, J. Hausmann, M. Ilbert, U. Jakob, Unfolding of metastable linker region is at the core of Hsp33 activation as a redox-regulated chaperone, J Biol Chem 285(15) (2010) 11243-11251.

[24] H. Fraga, J. Pujols, M. Gil-Garcia, A. Roque, G. Bernardo-Seisdedos, C. Santambrogio, J.J. Bech-Serra, F. Canals, P. Bernado, R. Grandori, O. Millet, S. Ventura, Disulfide driven folding for a conditionally disordered protein, Sci Rep 7(1) (2017) 16994.

[25] M. Ilbert, J. Horst, S. Ahrens, J. Winter, P.C. Graf, H. Lilie, U. Jakob, The redox-switch domain of Hsp33 functions as dual stress sensor, Nat Struct Mol Biol 14(6) (2007) 556-563.

[26] H. Launay, P. Barré, C. Puppo, S. Manneville, B. Gontero, V. Receveur-Bréchot, Absence of residual structure in the intrinsically disordered regulatory protein CP12 in its reduced state, Biochem Biophys Res Commun 477(1) (2016) 20-26.

[27] H. Launay, P. Barre, C. Puppo, Y. Zhang, S. Maneville, B. Gontero, V. Receveur-Brechot, Cryptic disorder out of disorder: Encounter between Conditionally Disordered CP12 and glyceraldehyde-3-phosphate dehydrogenase, J Mol Biol 430(8) (2018) 1218-1234.

[28] J. Yao, J. Chung, D. Eliezer, P.E. Wright, H.J. Dyson, NMR structural and dynamic characterization of the acid-unfolded state of apomyoglobin provides insights into the early events in protein folding, Biochemistry 40(12) (2001) 3561-3571.

[29] V. Csizmok, Z. Dosztanyi, I. Simon, P. Tompa, Towards proteomic approaches for the identification of structural disorder, Curr Protein Pept Sci 8(2) (2007) 173-179.

[30] A. Benarouche, J. Habchi, A. Cagna, O. Maniti, A. Girard-Egrot, J.F. Cavalier, S. Longhi, F. Carriere, Interfacial properties of NTAIL, an Intrinsically Disordered Protein, Biophys J 113(12) (2017) 2723-2735.

[31] U. Jakob, R. Kriwacki, V.N. Uversky, Conditionally and transiently disordered proteins: awakening cryptic disorder to regulate protein function, Chem Rev 114(13) (2014) 6779-6805.

[32] A.K. Dunker, M.M. Babu, E. Barbar, M. Blackledge, S.E. Bondos, Z. Dosztányi, H.J. Dyson, J. Forman-Kay, M. Fuxreiter, J. Gsponer, K.-H. Han, D.T. Jones, S. Longhi, S.J. Metallo, K. Nishikawa, R. Nussinov, Z. Obradovic, R.V. Pappu, B. Rost, P. Selenko, V. Subramaniam, J.L. Sussman, P. Tompa, V.N. Uversky, What's in a name? Why these proteins are intrinsically disordered, Intrinsically Disordered Proteins 1(1) (2013) e24157.

[33] H.J. Dyson, P.E. Wright, Intrinsically unstructured proteins and their functions, Nat Rev Mol Cell Biol 6(3) (2005) 197-208.

[34] J. Habchi, P. Tompa, S. Longhi, V.N. Uversky, Introducing protein intrinsic disorder, Chem Rev 114(13) (2014) 6561-6588.

[35] Y. Liu, X. Wang, B. Liu, A comprehensive review and comparison of existing computational methods for intrinsically disordered protein and region prediction, Brief Bioinform 20(1) (2019) 330-346.

[36] F. Meng, V.N. Uversky, L. Kurgan, Comprehensive review of methods for prediction of intrinsic disorder and its molecular functions, Cell Mol Life Sci 74(17) (2017) 3069-3090.

[37] J.T. Nielsen, F.A.A. Mulder, Quality and bias of protein disorder predictors, Sci Rep 9(1) (2019) 5137.

[38] M. Bonomi, G.T. Heller, C. Camilloni, M. Vendruscolo, Principles of protein structural ensemble determination, Curr Opin Struct Biol 42 (2017) 106-116.

[39] M. Krzeminski, J.A. Marsh, C. Neale, W.Y. Choy, J.D. Forman-Kay, Characterization of disordered proteins with ENSEMBLE, Bioinformatics 29(3) (2013) 398-399.

[40] V. Ozenne, F. Bauer, L. Salmon, J.R. Huang, M.R. Jensen, S. Segard, P. Bernado, C. Charavay, M. Blackledge, Flexible-meccano: a tool for the generation of explicit ensemble descriptions of intrinsically disordered proteins and their associated experimental observables, Bioinformatics 28(11) (2012) 1463-1470.

[41] P. Sormanni, D. Piovesan, G.T. Heller, M. Bonomi, P. Kukic, C. Camilloni, M. Fuxreiter, Z. Dosztanyi, R.V. Pappu, M.M. Babu, S. Longhi, P. Tompa, A.K. Dunker, V.N. Uversky, S.C. Tosatto, M. Vendruscolo, Simultaneous quantification of protein order and disorder, Nat Chem Biol 13(4) (2017) 339-342.

[42] V. Receveur-Brechot, J.M. Bourhis, V.N. Uversky, B. Canard, S. Longhi, Assessing protein disorder and induced folding, Proteins 62(1) (2006) 24-45.

[43] V. Receveur-Brechot, D. Durand, How random are intrinsically disordered proteins? A small angle scattering perspective, Curr Protein Pept Sci 13(1) (2012) 55-75.

[44] M.M. Babu, R. van der Lee, N.S. de Groot, J. Gsponer, Intrinsically disordered proteins: regulation and disease, Curr Opin Struct Biol 21(3) (2011) 432-440.

[45] A.K. Dunker, S.E. Bondos, F. Huang, C.J. Oldfield, Intrinsically disordered proteins and multicellular organisms, Semin Cell Dev Biol 37 (2015) 44-55.

[46] K.J. Niklas, S.E. Bondos, A.K. Dunker, S.A. Newman, Rethinking gene regulatory networks in light of alternative splicing, intrinsically disordered protein domains, and post-translational modifications, Front Cell Dev Biol 3 (2015) 8.

[47] A. Patil, H. Nakamura, Disordered domains and high surface charge confer hubs with the ability to interact with multiple proteins in interaction networks, FEBS Lett 580(8) (2006) 2041-2045.

[48] I. Yruela, C.J. Oldfield, K.J. Niklas, A.K. Dunker, Evidence for a strong correlation between transcription factor protein disorder and organismic complexity, Genome Biol Evol 9(5) (2017) 1248-1265.

[49] F.X. Theillet, C. Smet-Nocca, S. Liokatis, R. Thongwichian, J. Kosten, M.K. Yoon, R.W. Kriwacki, I. Landrieu, G. Lippens, P. Selenko, Cell signaling, post-translational protein modifications and NMR spectroscopy, J Biomol NMR 54(3) (2012) 217-236.

[50] A. Kurotani, T. Sakurai, In silico analysis of correlations between protein disorder and post-translational modifications in algae, Int J Mol Sci 16(8) (2015) 19812-19835.

[51] A. Kurotani, A.A. Tokmakov, Y. Kuroda, Y. Fukami, K. Shinozaki, T. Sakurai, Correlations between predicted protein disorder and post-translational modifications in plants, Bioinformatics 30(8) (2014) 1095-1103.

[52] Z. Peng, J. Yan, X. Fan, M.J. Mizianty, B. Xue, K. Wang, G. Hu, V.N. Uversky, L. Kurgan, Exceptionally abundant exceptions: comprehensive characterization of intrinsic disorder in all domains of life, Cell Mol Life Sci 72(1) (2015) 137-151.

[53] J.J. Ward, J.S. Sodhi, L.J. McGuffin, B.F. Buxton, D.T. Jones, Prediction and functional analysis of native disorder in proteins from the three kingdoms of life, J Mol Biol 337 (2004) 635-645.

[54] B. Xue, A.K. Dunker, V.N. Uversky, Orderly order in protein intrinsic disorder distribution: disorder in 3500 proteomes from viruses and the three domains of life, J Biomol Struct Dyn 30(2) (2012) 137-149.

[55] K.J. Niklas, A.K. Dunker, I. Yruela, The evolutionary origins of cell type diversification and the role of intrinsically disordered proteins, J Exp Bot 69(7) (2018) 1437-1446.

[56] M. Necci, D. Piovesan, Z. Dosztanyi, P. Tompa, S.C.E. Tosatto, A comprehensive assessment of long intrinsic protein disorder from the DisProt database, Bioinformatics 34(3) (2018) 445-452.

[57] M.S. Cortese, J.P. Baird, V.N. Uversky, A.K. Dunker, Uncovering the unfoldome: enriching cell extracts for unstructured proteins by acid treatment, J Proteome Res 4(5) (2005) 1610-8.

[58] A. Tantos, P. Tompa, Proteomic methods for the identification of intrinsically disordered proteins, Methods Mol Biol 896 (2012) 429-37.

[59] M.A. Bisson, G.O. Kirst, Osmotic acclimation and turgor pressure regulation in algae, Naturwissenschaften 82(10) (1995) 461-471.

[60] J. Roche, C.A. Royer, Lessons from pressure denaturation of proteins, J R Soc Interface 15(147) (2018).

[61] G. Prat-Gay, A. Paladini, M. Stein, R.A. Wolosiuk, The effect of high hydrostatic pressure on the modulation of regulatory enzymes from spinach chloroplasts, J Biol Chem 266(31) (1991) 20913-20921.

[62] J. Roche, J. Ying, A.S. Maltsev, A. Bax, Impact of hydrostatic pressure on an intrinsically disordered protein: a high-pressure NMR study of α-synuclein, Chembiochem: Chem Eur J 14(14) (2013) 1754-1761.

[63] J. Somkuti, Z. Martonfalvi, M.S. Kellermayer, L. Smeller, Different pressure-temperature behavior of the structured and unstructured regions of titin, Biochim Biophys Acta 1834(1) (2013) 112-118.

[64] L. Norén, P. Kindgren, P. Stachula, M. Rühl, M.E. Eriksson, V. Hurry, Å. Strand, Circadian and plastid signaling pathways are integrated to ensure correct expression of the CBF and COR genes during photoperiodic growth, Plant Physiol 171(2) (2016) 1392-1406.

[65] B.B. Buchanan, A. Holmgren, J.P. Jacquot, R. Scheibe, Fifty years in the thioredoxin field and a bountiful harvest, Biochim Biophys Acta 1820(11) (2012) 1822-1829.

[66] B. Gontero, M. Salvucci, Regulation of photosynthetic carbon metabolism in aquatic and terrestrial organisms by Rubisco activase, redox-modulation and CP12, Aquat Bot 118 (2014) 14-23.

[67] E. Jensen, R. Clement, S.C. Maberly, B. Gontero, Regulation of the Calvin-Benson-Bassham cycle in the enigmatic diatoms: biochemical and evolutionary variations on an original theme, Philos Trans R Soc Lond B Biol Sci 372(1728) (2017).

[68] S. Eriksson, N. Eremina, A. Barth, J. Danielsson, P. Harryson, Membrane-induced folding of the plant stress dehydrin Lti30, Plant Physiol 171(2) (2016) 932-943.

[69] N.a. Segal, M. Shapira, HSP33 in eukaryotes - an evolutionary tale of a chaperone adapted to photosynthetic organisms, Plant J 82(5) (2015) 850-860.

[70] I. Yruela, B. Contreras-Moreira, Protein disorder in plants: a view from the chloroplast, BMC Plant Biol 12 (2012) 165.

[71] I. Yruela, B. Contreras-Moreira, Genetic recombination is associated with intrinsic disorder in plant proteomes, BMC Genomics 14 (2013) 772.

[72] Y. Zhang, H. Launay, A. Schramm, R. Lebrun, B. Gontero, Exploring intrinsically disordered proteins in *Chlamydomonas reinhardtii*, Scientific Reports 8(1) (2018) 6805.

[73] C.R. Somerville, W.L. Ogren, A phosphoglycolate phosphatase-deficient mutant of Arabidopsis. , Nature 280 (1979) 833-836.

[74] T.C. Taylor, I. Andersson, Structure of a product complex of spinach ribulose-1,5-bisphosphate carboxylase/oxygenase, Biochemistry 36(13) (1997) 4041-4046.

[75] G.H. Lorimer, H.M. Miziorko, Carbamate formation on the epsilon-amino group of a lysyl residue as the basis for the activation of ribulose bisphosphate carboxylase by $CO_2$ and $Mg^{2+}$, Biochemistry 19(23) (1980) 5321-5328.

[76] A.R. Portis, M.E. Salvucci, W.L. Ogren, Activation of Ribulose bisphosphate Carboxylase/Oxygenase at physiological $CO_2$ and ribulose bisphosphate concentrations by Rubisco Activase, Plant Physiol 82(4) (1986) 967-971.

[77] T. Wunder, Z.G. Oh, O. Mueller-Cajar, $CO_2$ -fixing liquid droplets: towards a dissection of the microalgal pyrenoid, Traffic (2019) 380-389.

[78] R. Nagarajan, K.S. Gill, Evolution of Rubisco activase gene in plants, Plant Molecular Biology 96(1) (2018) 69-87.

[79] A.E. Carmo-Silva, M.E. Salvucci, The regulatory properties of Rubisco activase differ among species and affect photosynthetic induction during light transitions, Plant Physiol 161(4) (2013) 1645-1655.

[80] G. Thieulin-Pardo, L. Avilan, M. Kojadinovic, B. Gontero, Fairy "tails": flexibility and function of intrinsically disordered extensions in the photosynthetic world, Front Mol Biosci 2 (2015) 23.

[81] L. Sena, V.N. Uversky, Comparison of the intrinsic disorder propensities of the RuBisCO activase enzyme from the motile and non-motile oceanic green microalgae, Intrinsically Disord Proteins 4(1) (2016) e1253526.

[82] O. Mueller-Cajar, M. Stotz, A. Bracher, Maintaining photosynthetic CO2 fixation via protein remodelling: the Rubisco activases, Photosynth Res 119(1-2) (2014) 191-201.

[83] O. Mueller-Cajar, M. Stotz, P. Wendler, F.U. Hartl, A. Bracher, M. Hayer-Hartl, Structure and function of the AAA+ protein CbbX, a red-type Rubisco activase, Nature 479(7372) (2011) 194-9.

[84] P.D. Tortell, Evolutionary and ecological perspectives on carbon acquisition in phytoplankton, Limnol Oceanog 45(3) (2000) 744-750.

[85] J.N. Young, B.M. Hopkinson, The potential for co-evolution of CO2-concentrating mechanisms and Rubisco in diatoms, J Exp Bot 68(14) (2017) 3751-3762.

[86] B.D. Engel, M. Schaffer, L.K. Cuellar, E. Villa, J.M. Plitzko, W. Baumeister, Native architecture of the Chlamydomonas chloroplast revealed by in situ cryo-electron tomography, Elife 4 (2015).

[87] L.C.M. Mackinder, M.T. Meyer, T. Mettler-Altmann, V.K. Chen, M.C. Mitchell, O. Caspari, E.S.F. Rosenzweig, L. Pallesen, G. Reeves, A. Itakura, R. Roth, F. Sommer, S. Geimer, T. Muehlhaus, M. Schroda, U. Goodenough, M. Stitt, H. Griffiths, M.C. Jonikas, A repeat protein links Rubisco to form the eukaryotic carbon-concentrating organelle, Proc Natl Acad Sci USA 113(21) (2016) 5958-5963.

[88] E.S. Freeman Rosenzweig, B. Xu, L.K. Cuellar, A. Martinez-Sanchez, M. Schaffer, M. Strauss, H.N. Cartwright, P. Ronceray, J.M. Plitzko, F. Forster, N.S. Wingreen, B.D. Engel, L.C.M. Mackinder, M.C. Jonikas, The eukaryotic CO2-Concentrating organelle is liquid-like and exhibits dynamic reorganization, Cell 171(1) (2017) 148-162.

[89] Y. Zhan, C.H. Marchand, A. Maes, A. Mauries, Y. Sun, J.S. Dhaliwal, J. Uniacke, S. Arragain, H. Jiang, N.D. Gold, V.J.J. Martin, S.D. Lemaire, W. Zerges, Pyrenoid functions revealed by proteomics in *Chlamydomonas reinhardtii*, PloS One 13(2) (2018) e0185039.

[90] M.V. Turkina, A. Blanco-Rivero, J.P. Vainonen, A.V. Vener, A. Villarejo, $CO_2$ limitation induces specific redox-dependent protein phosphorylation in *Chlamydomonas reinhardtii*, Proteomics 6(9) (2006) 2693-2704.

[91] T. Wunder, S.L.H. Cheng, S.-K. Lai, H.-Y. Li, O. Mueller-Cajar, The phase separation underlying the pyrenoid-based microalgal Rubisco supercharger, Nature Commun 9 (2018).

[92] D.M. Mitrea, R.W. Kriwacki, Phase separation in biology; functional organization of a higher order, Cell Commun Signal 14 (2016) 1.

[93] S. Boeynaems, S. Alberti, N.L. Fawzi, T. Mittag, M. Polymenidou, F. Rousseau, J. Schymkowitz, J. Shorter, B. Wolozin, L. Van Den Bosch, P. Tompa, M. Fuxreiter, Protein phase separation: A new phase in cell biology, Trends Cell Biol 28(6) (2018) 420-435.

[94] R. Groben, D. Kaloudas, C.A. Raines, B. Offmann, S.C. Maberly, B. Gontero, Comparative sequence analysis of CP12, a small protein involved in the formation of a Calvin cycle complex in photosynthetic organisms, Photosynth Res 103(3) (2010) 183-194.

[95] D.N. Stanley, C.A. Raines, C.A. Kerfeld, Comparative analysis of 126 cyanobacterial genomes reveals evidence of functional diversity among homologs of the redox-regulated CP12 protein, Plant Physiol 161(2) (2013) 824-835.

[96] L.R. Thompson, Q. Zeng, L. Kelly, K.H. Huang, A.U. Singer, J. Stubbe, S.W. Chisholm, Phage auxiliary metabolic genes and the redirection of cyanobacterial host carbon metabolism, Proc Natl Acad Sci U S A 108(39) (2011) 757-764.

[97] P.E. Lopez-Calcagno, T.P. Howard, C.A. Raines, The CP12 protein family: a thioredoxin-mediated metabolic switch?, Front Plant Sci 5 (2014) 9.

[98] W. Kaaki, M. Woudstra, B. Gontero, F. Halgand, Exploration of CP12 conformational changes and of quaternary structural properties using electrospray ionization traveling wave ion mobility mass spectrometry, Rapid Commun Mass Spectrom 27(1) (2013) 179-186.

[99] E. Mileo, M. Lorenzi, J. Erales, S. Lignon, C. Puppo, N. Le Breton, E. Etienne, S.R. Marque, B. Guigliarelli, B. Gontero, V. Belle, Dynamics of the intrinsically disordered protein CP12 in its association with GAPDH in the green alga *Chlamydomonas reinhardtii*: a fuzzy complex, Mol Biosyst 9(11) (2013) 2869-2876.

[100] S.B. Moparthi, G. Thieulin-Pardo, J. de Torres, P. Ghenuche, B. Gontero, J. Wenger, FRET analysis of CP12 structural interplay by GAPDH and PRK, Biochem Biophys Res Commun 458(3) (2015) 488-493.

[101] S.B. Moparthi, G. Thieulin-Pardo, P. Mansuelle, H. Rigneault, B. Gontero, J. Wenger, Conformational modulation and hydrodynamic radii of CP12 protein and its complexes probed by fluorescence correlation spectroscopy, FEBS J 281(14) (2014) 3206-3217.

[102] J. Petersen, R. Teich, B. Becker, R. Cerff, H. Brinkmann, The GapA/B gene duplication marks the origin of Streptophyta (charophytes and land plants), Mol Biol Evol 23(6) (2006) 1109-1118.

[103] C. Oesterhelt, S. Klocke, S. Holtgrefe, V. Linke, A.P.M. Weber, R. Scheibe, Redox regulation of chloroplast enzymes in *Galdieria sulphuraria* in view of eukaryotic evolution, Plant Cell Physiol 48(9) (2007) 1359-1373.

[104] F. Gardebien, R.R. Thangudu, B. Gontero, B. Offmann, Construction of a 3D model of CP12, a protein linker, J Mol Graph Model 25(2) (2006) 186-195.

[105] E. Graciet, P. Gans, N. Wedel, S. Lebreton, J.M. Camadro, B. Gontero, The small protein CP12: a protein linker for supramolecular complex assembly, Biochemistry 42(27) (2003) 8163-8170.

[106] E. Graciet, S. Lebreton, J.M. Camadro, B. Gontero, Characterization of native and recombinant A4 glyceraldehyde 3-phosphate dehydrogenase. Kinetic evidence for confromation changes upon association with the small protein CP12, Eur J Biochem 270(1) (2003) 129-136.

[107] S. Lebreton, E. Graciet, B. Gontero, Modulation, via protein-protein interactions, of glyceraldehyde-3-phosphate dehydrogenase activity through redox phosphoribulokinase regulation, Journal of Biological Chemistry 278(14) (2003) 12078-12084.

[108] A. Borgia, M.B. Borgia, K. Bugge, V.M. Kissling, P.O. Heidarsson, C.B. Fernandes, A. Sottini, A. Soranno, K.J. Buholzer, D. Nettels, B.B. Kragelund, R.B. Best, B. Schuler, Extreme disorder in an ultrahigh-affinity protein complex, Nature 555(7694) (2018) 61-66.

[109] A. Delobel, E. Graciet, S. Andreescu, B. Gontero, F. Halgand, O. Laprevote, Mass spectrometric analysis of the interactions between CP12, a chloroplast protein., and metal ions: a possible regulatory role within a PRK/GAPDH/CP12 complex, Rapid Commun Mass Spectrom 19(22) (2005) 3379-3388.

[110] A.G. Rocha, U.C. Vothknecht, Identification of CP12 as a novel Calcium-binding protein in chloroplasts, Plants 2(3) (2013) 530-540.

[111] J. Erales, L. Avilan, S. Lebreton, B. Gontero, Exploring CP12 binding proteins revealed aldolase as a new partner for the phosphoribulokinase/glyceraldehyde 3-phosphate dehydrogenase/CP12 complex--purification and kinetic characterization of this enzyme from *Chlamydomonas reinhardtii*, FEBS J 275(6) (2008) 1248-1259.

[112] J. Erales, S. Lignon, B. Gontero, CP12 from *Chlamydomonas reinhardtii*, a permanent specific "chaperone-like" protein of glyceraldehyde-3-phosphate dehydrogenase, J Biol Chem 284(19) (2009) 12735-12744.

[113] B. Gontero, S.C. Maberly, An intrinsically disordered protein, CP12: jack of all trades and master of the Calvin cycle, Biochem Soc Trans 40(5) (2012) 995-999.

[114] L. Marri, P. Trost, X. Trivelli, L. Gonnelli, P. Pupillo, F. Sparla, Spontaneous assembly of photosynthetic supramolecular complexes as mediated by the intrinsically unstructured protein CP12, J Biol Chem 283(4) (2008) 1831-1838.

[115] P. Trost, S. Fermani, L. Marri, M. Zaffagnini, G. Falini, S. Scagliarini, P. Pupillo, F. Sparla, Thioredoxin-dependent regulation of photosynthetic glyceraldehyde-3-phosphate dehydrogenase: autonomous vs. CP12-dependent mechanisms, Photosynth Res 89(2-3) (2006) 263-275.

[116] E. Baalmann, R. Scheibe, R. Cerff, W. Martin, Functional studies of chloroplast glyceraldehyde-3-phosphate dehydrogenase subunits A and B expressed in Escherichia coli: formation of highly active A 4 and B 4 homotetramers and evidence that aggregation of the B 4 complex is mediated by the B subunit carboxy terminus, Plant molecular biology 32(3) (1996) 505-513.

[117] F. Sparla, P. Pupillo, P. Trost, The C-terminal extension of glyceraldehyde-3-phosphate dehydrogenase subunit B acts as an autoinhibitory domain regulated by thioredoxins and nicotinamide adenine dinucleotide, J Biol Chem 277(47) (2002) 44946-44952.

[118] W.C. Plaxton, The organization and regulation of plant glycolysis, Annu Rev Plant Physiol Plant Mol Biol 47 (1996) 185-214.

[119] M.A. Sirover, On the functional diversity of glyceraldehyde-3-phosphate dehydrogenase: biochemical mechanisms and regulatory control, Biochim Biophys Acta 1810(8) (2011) 741-751.

[120] A.U. Igamberdiev, L.A. Kleczkowski, Optimization of ATP synthase function in mitochondria and chloroplasts via the adenylate kinase equilibrium, Front Plant Sci 6 (2015) 10.

[121] G. Thieulin-Pardo, A. Schramm, S. Lignon, R. Lebrun, M. Kojadinovic, B. Gontero, The intriguing CP12-like tail of adenylate kinase 3 from *Chlamydomonas reinhardtii*, FEBS J 283(18) (2016) 3389-3407.

[122] Y. Zhang, H. Launay, F. Liu, R. Lebrun, B. Gontero, Interaction between adenylate kinase 3 and glyceraldehyde-3-phosphate dehydrogenase from *Chlamydomonas reinhardtii*, FEBS J 285(13) (2018) 2495-2503.

[123] M. Zaffagnini, M. Bedhomme, S.D. Lemaire, P. Trost, The emerging roles of protein glutathionylation in chloroplasts, Plant Sci 185-186 (2012) 86-96.

[124] M. Zaffagnini, M. Bedhomme, H. Groni, C.H. Marchand, C. Puppo, B. Gontero, C. Cassier-Chauvat, P. Decottignies, S.D. Lemaire, Glutathionylation in the photosynthetic model organism *Chlamydomonas reinhardtii*: a proteomic survey, Mol Cell Proteomics 11(2) (2012) M111 014142.

[125] L. Marri, G. Thieulin-Pardo, R. Lebrun, R. Puppo, M. Zaffagnini, P. Trost, B. Gontero, F. Sparla, CP12-mediated protection of Calvin-Benson cycle enzymes from oxidative stress, Biochimie 97 (2014) 228-237.

[126] R.A. Quinlan, R.J. Ellis, Chaperones: needed for both the good times and the bad times, Philos Trans R Soc Lond B Biol Sci 368(1617) (2013) 20130091.

[127] J.C. Bardwell, U. Jakob, Conditional disorder in chaperone action, Trends Biochem Sci 37(12) (2012) 517-525.

[128] J.C. Borges, T.V. Seraphim, P.R. Dores-Silva, L.R.S. Barbosa, A review of multi-domain and flexible molecular chaperones studies by small-angle X-ray scattering, Biophys Rev 8(2) (2016) 107-120.

[129] D. Reichmann, Y. Xu, C.M. Cremers, M. Ilbert, R. Mittelman, M.C. Fitzgerald, U. Jakob, Order out of disorder: working cycle of an intrinsically unfolded chaperone, Cell 148(5) (2012) 947-957.

[130] W.B. Pratt, D.O. Toft, Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery, Exp Biol Med 228(2) (2003) 111-133.

[131] S.K. Wandinger, K. Richter, J. Buchner, The Hsp90 chaperone machinery, J Biol Chem 283(27) (2008) 18473-18477.

[132] C. Drzymalla, M. Schroda, C.F. Beck, Light-inducible gene HSP70B encodes a chloroplast-localized heat shock protein in *Chlamydomonas reinhardtii*, Plant Mol Biol 31(6) (1996) 1185-1194.

[133] A. Maikova, Z. Zalutskaya, T. Lapina, E. Ermilova, The HSP70 chaperone machines of Chlamydomonas are induced by cold stress, J Plant Physiol 204 (2016) 85-91.

[134] M. Schroda, T. Muhlhaus, A 'foldosome' in the chloroplast?, Plant Signal Behav 4(4) (2009) 301-3.

[135] F. Willmund, M. Schroda, Heat shock protein 90C is a bona fide Hsp90 that interacts with plastidic HSP70B in *Chlamydomonas reinhardtii*, Plant Physiol 138(4) (2005) 2310-2322.

[136] H. Wang, B. Gau, W.O. Slade, M. Juergens, P. Li, L.M. Hicks, The global phosphoproteome of *Chlamydomonas reinhardtii* reveals complex organellar phosphorylation in the flagella and thylakoid membrane, Mol Cell Proteomics 13(9) (2014) 2337-2353.

[137] O. Genest, J.R. Hoskins, J.L. Camberg, S.M. Doyle, S. Wickner, Heat shock protein 90 from Escherichia coli collaborates with the DnaK chaperone system in client protein remodeling, Proc Natl Acad Sci U S A 108(20) (2011) 8206-8211.

[138] O. Genest, J.R. Hoskins, A.N. Kravats, S.M. Doyle, S. Wickner, Hsp70 and Hsp90 of E. coli Directly Interact for Collaboration in Protein Remodeling, J Mol Biol 427(24) (2015) 3877-3889.

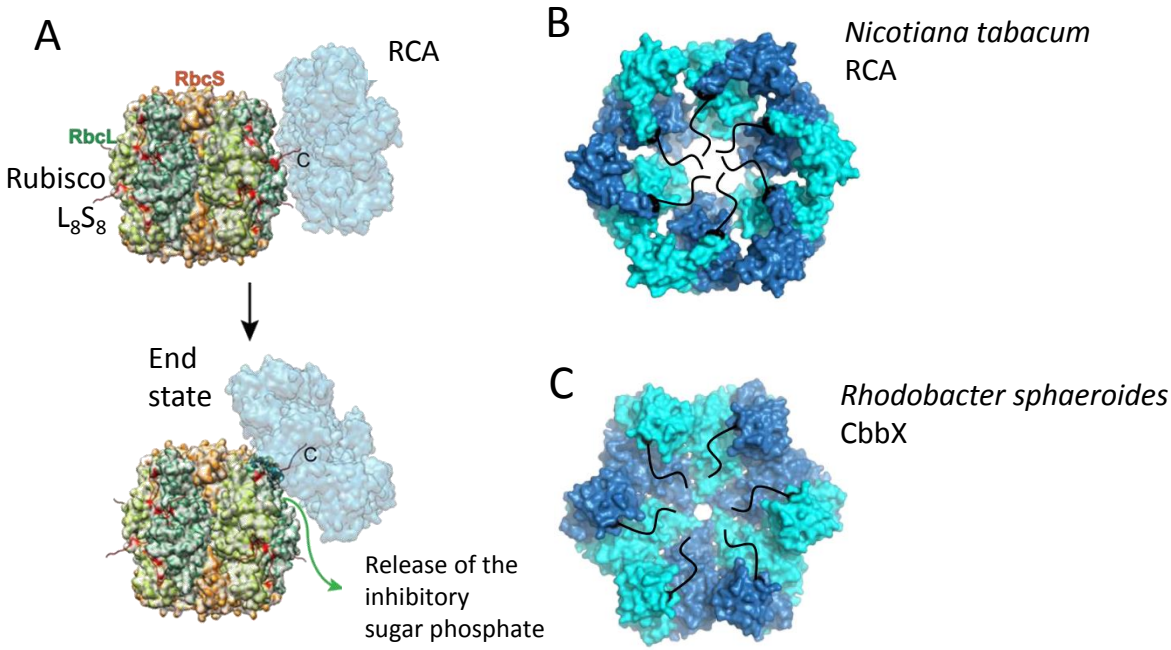
[139] J.Y. Bhat, G. Miličić, G. Thieulin-Pardo, A. Bracher, A. Maxwell, S. Ciniawsky, O. Mueller-Cajar, J.R. Engen, F.U. Hartl, P. Wendler, M. Hayer-Hartl, Mechanism of enzyme repair by the AAA+ chaperone Rubisco Activase, Molecular Cell 67(5) (2017) 744-756.e6.

[140] M. Stotz, O. Mueller-Cajar, S. Ciniawsky, P. Wendler, F.U. Hartl, A. Bracher, M. Hayer-Hartl, Structure of green-type Rubisco activase from tobacco, Nature Struct Mol Biol 18(12) (2011) 1366-1370.

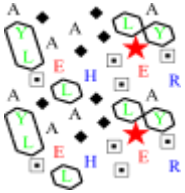
[141] T. Bitard-Feildel, A. Lamiable, J.P. Mornon, I. Callebaut, Order in Disorder as Observed by the "Hydrophobic Cluster Analysis" of Protein Sequences, Proteomics 18(21-22) (2018) e1800054.

[142] B. Xue, R.L. Dunbrack, R.W. Williams, A.K. Dunker, V.N. Uversky, PONDR-FIT: A meta-predictor of intrinsically disordered amino acids, Biochim Biophys Acta (Proteins and Proteomics) 1804(4) (2010) 996-1010.

[143] B. Mészáros, G. Erdős, Z. Dosztányi, IUPred2A: context-dependent prediction of protein disorder as a function of redox state and protein binding, Nucleic Acids Res 46(W1) (2018) 329-337.

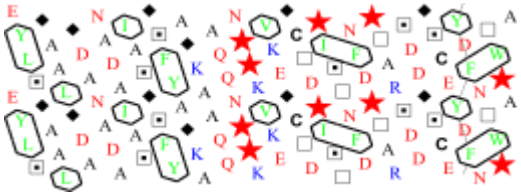

A
RCA
RbcS
RbcL
C
Rubisco
$L_8S_8$
End
state
C
Release of the
inhibitory
sugar phosphate
B
*Nicotiana tabacum*
RCA
C
*Rhodobacter sphaeroides*
CbbX

A Chlorophyta RCA C-ter
(*C. reinhardtii)*

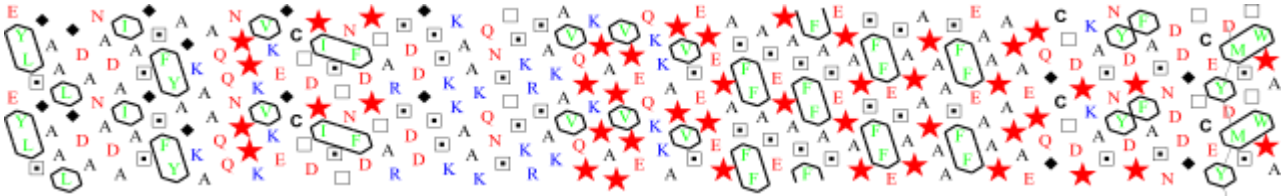


Charophyta β C-ter
(*Chlorokybus atmophyticus*)

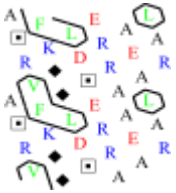
Charophyta α2 C-ter (*Klebsormidium flaccidum*)



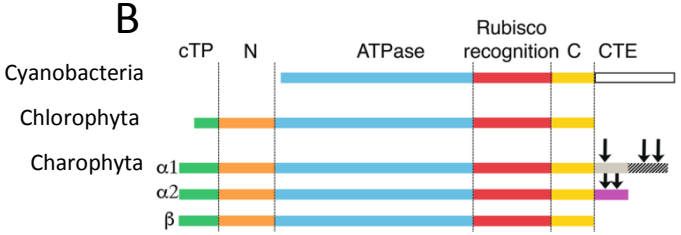
Charophyta RCA α1 C-ter (*K. flaccidum*)



Purple bacterium CbbX C-ter
(*Rhodobacter sphaeroides*)

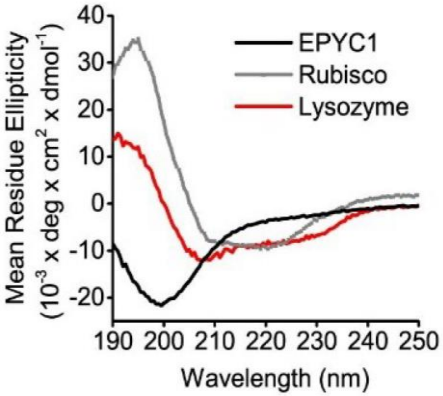


B

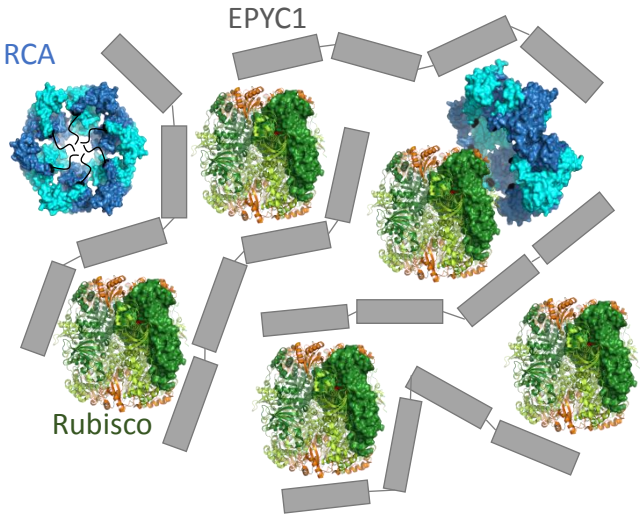



C

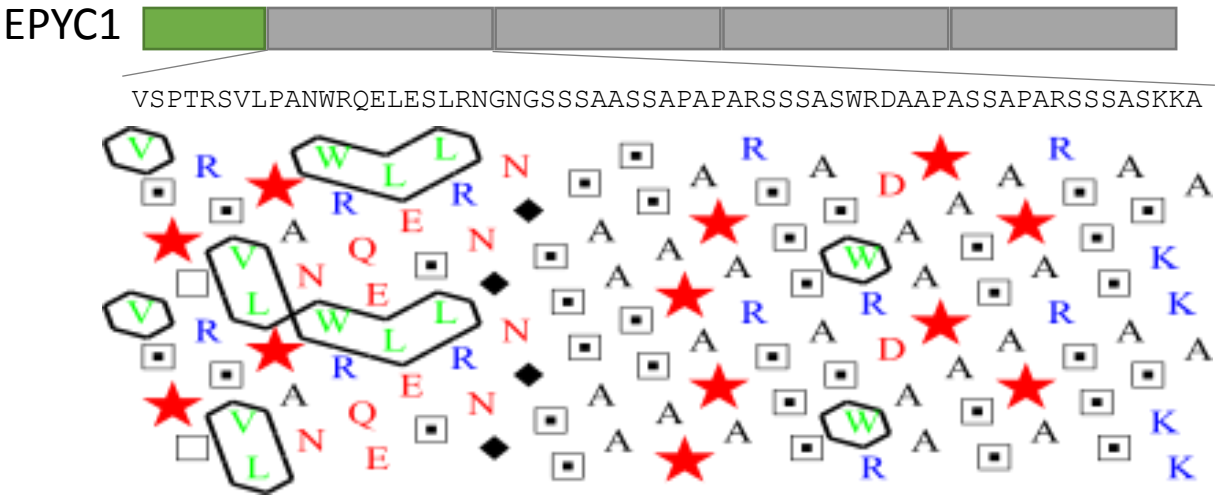
A

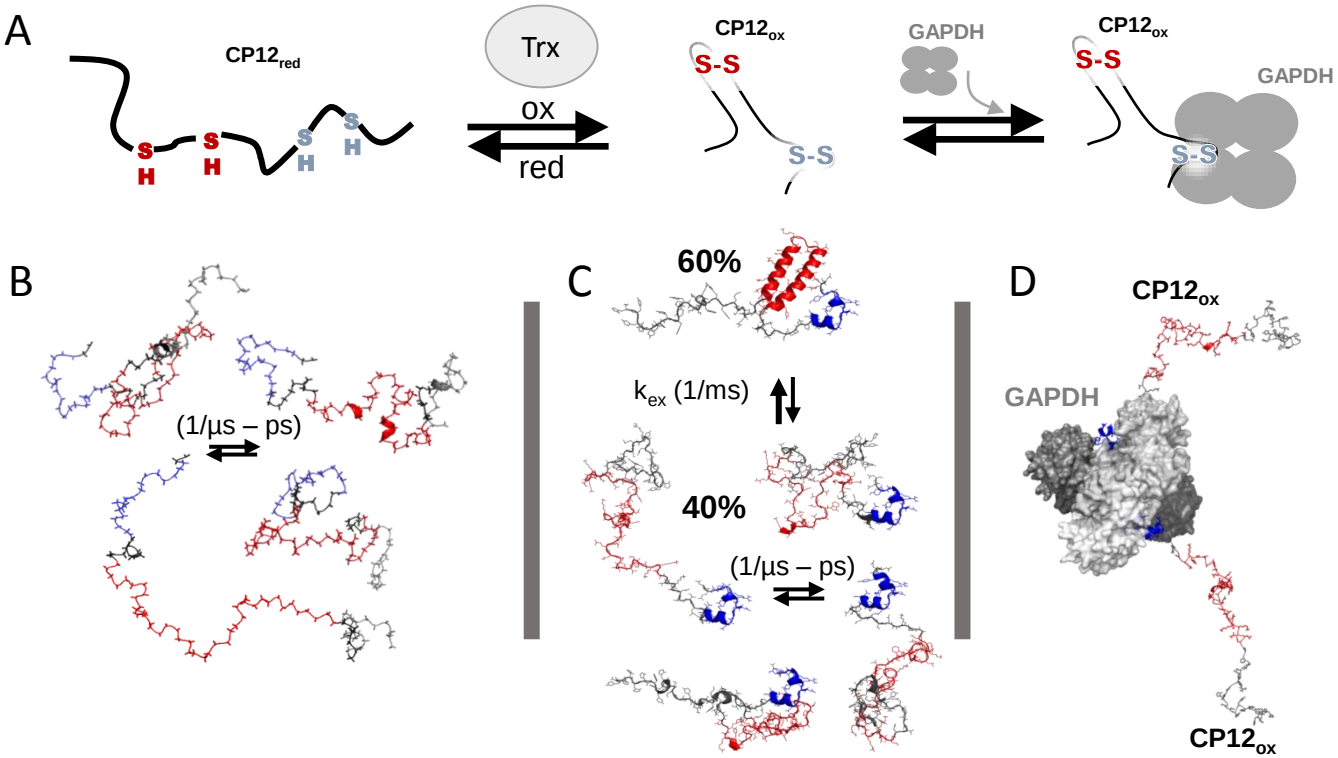

Mean Residue Ellipticity
($10^{-3}$ x deg x $cm^2$ x $dmol^{-1}$)
40
30
20
10
0
-10
-20
190 200 210 220 230 240 250
Wavelength (nm)
EPYC1
Rubisco
Lysozyme

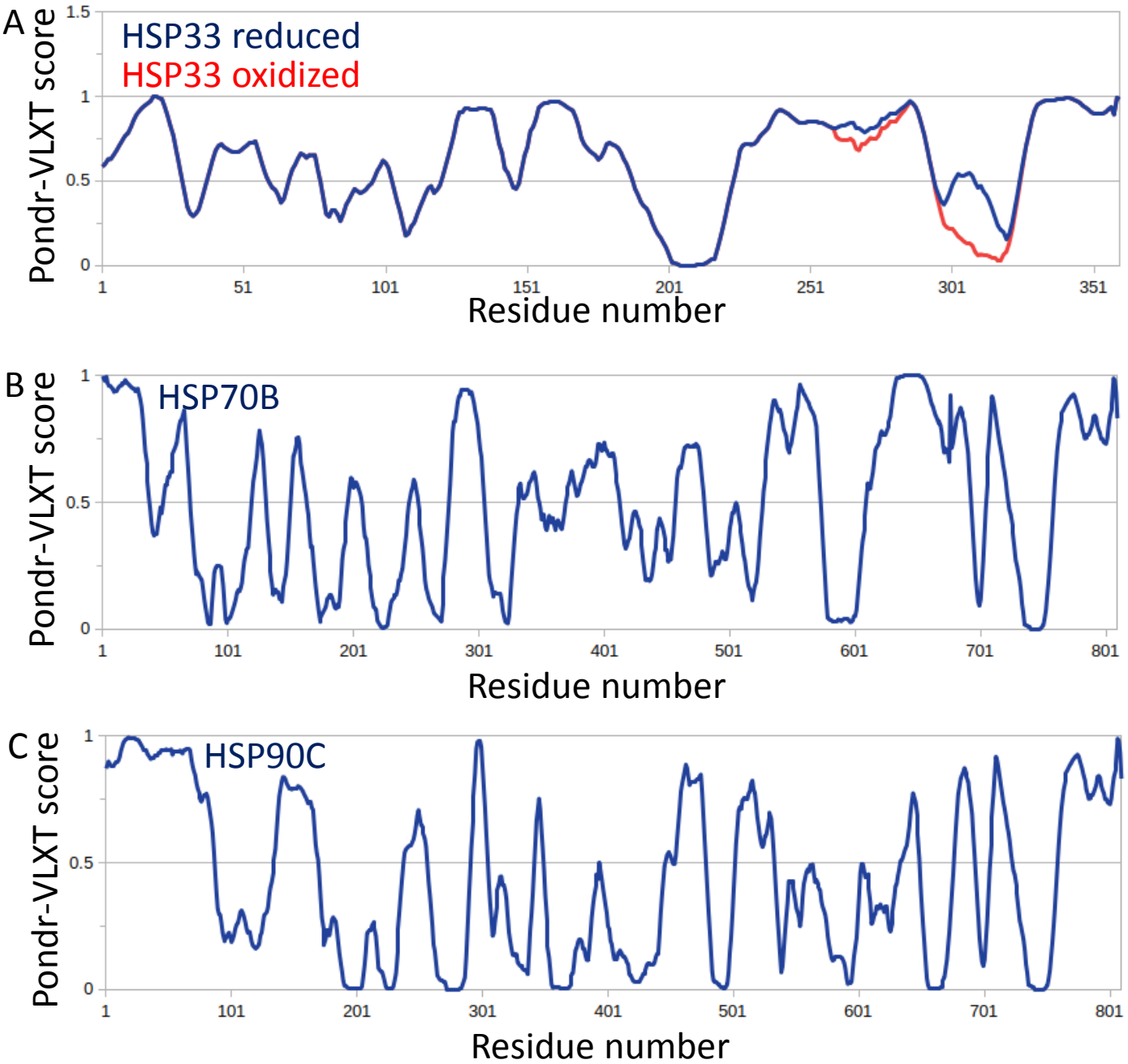
B

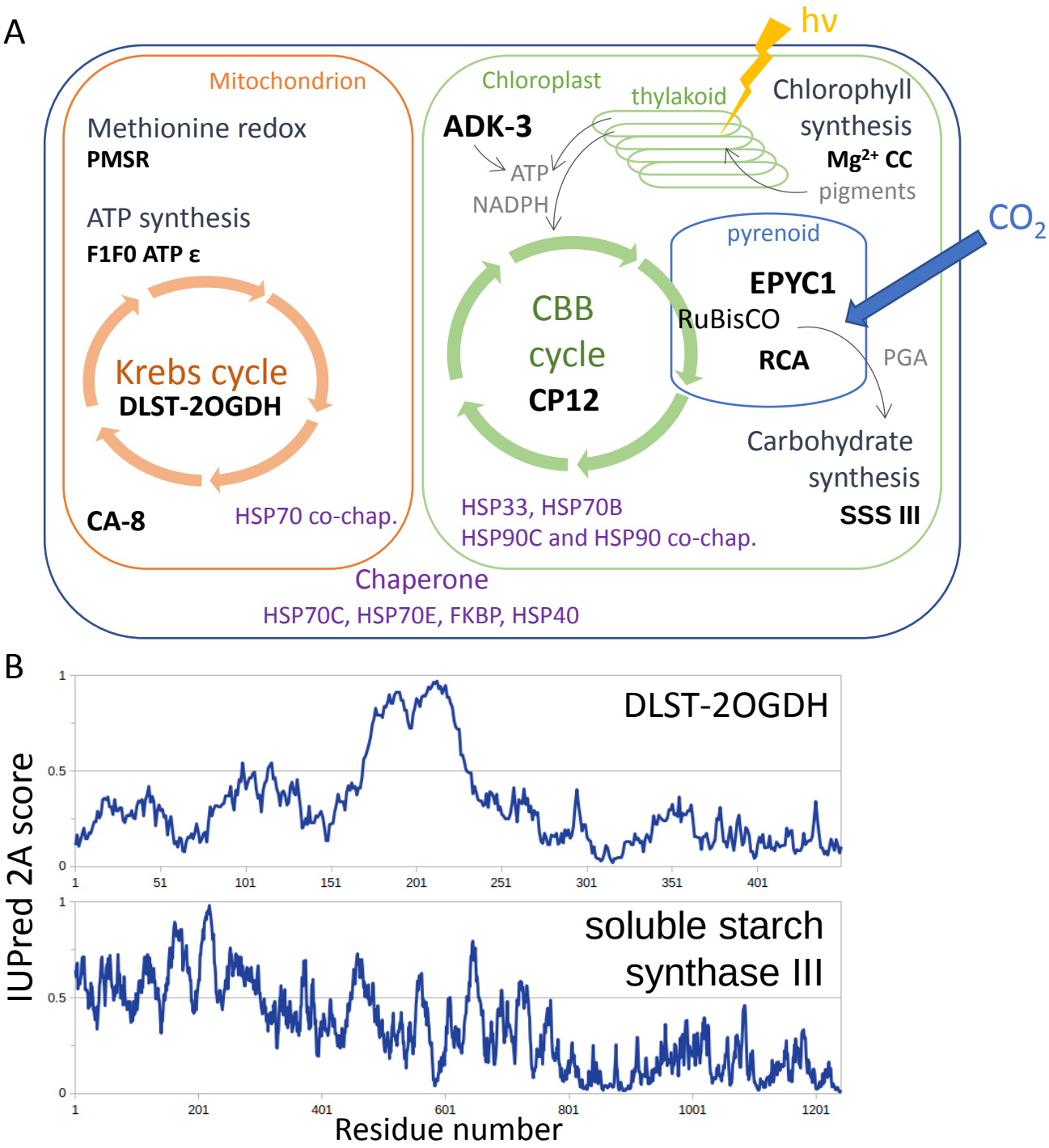

EPYC1
RCA
Rubisco

C


EPYC1
VSPTRSVLPANWRQELESLRNGNGSSSAASSAPAPARSSSASWRDAAPASSAPARSSSASKKA


A
CP12red
Trx
ox
red
CP12ox
S-S
GAPDH
CP12ox
S-S
GAPDH
S-S
B
(1/µs – ps)
C
60%
kex (1/ms)
40%
(1/µs – ps)
D
CP12ox
GAPDH
CP12ox


A
HSP33 reduced
HSP33 oxidized
Pondr-VLXT score
1.5
1
0.5
0
1
51
101
151
201
251
301
351
Residue number
B
HSP70B
Pondr-VLXT score
1
0.5
0
1
101
201
301
401
501
601
701
801
Residue number
C
HSP90C
Pondr-VLXT score
1
0.5
0
1
101
201
301
401
501
601
701
801
Residue number


A
hν
Mitochondrion
Methionine redox
PMSR
ATP synthesis
F1F0 ATP ε
Krebs cycle
DLST-2OGDH
CA-8
HSP70 co-chap.
Chloroplast
thylakoid
ADK-3
ATP
NADPH
Chlorophyll synthesis
Mg2+ CC
pigments
pyrenoid
EPYC1
RuBisCO
RCA
CO2
PGA
CBB cycle
CP12
Carbohydrate synthesis
SSS III
HSP33, HSP70B
HSP90C and HSP90 co-chap.
Chaperone
HSP70C, HSP70E, FKBP, HSP40
B
IUPred 2A score
DLST-2OGDH
soluble starch synthase III
Residue number