



## Review

# The cytochrome *f*–plastocyanin complex as a model to study transient interactions between redox proteins

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This article is dedicated to the memory of Prof. António V. Xavier

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## ABSTRACT

**Transient complexes, with a lifetime ranging between microseconds and seconds, are essential for biochemical reactions requiring a fast turnover. That is the case of the interactions between proteins engaged in electron transfer reactions, which are involved in relevant physiological processes such as respiration and photosynthesis. In the latter, the copper protein plastocyanin acts as a soluble carrier transferring electrons between the two membrane-embedded complexes cytochrome *b<sub>6</sub>f* and photosystem I. Here we review the combination of experimental efforts in the literature to unveil the functional and structural features of the complex between cytochrome *f* and plastocyanin, which have widely been used as a suitable model for analyzing transient redox interactions. © 2011 Federation of European Biochemical Societies. Published by Elsevier B.V.**

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## 1. Introduction

Protein–protein interactions are key processes in the proper operation of living cells. Different kinds of interactions can be distinguished on the basis of protein binding affinities. Complexes characterized by low binding affinities occur in transient protein–protein interactions, which display high dissociation equilibrium constants ( $K_D$ ), within the range of  $\mu\text{M}$ – $\text{mM}$ , or lifetimes in the order of milliseconds [1,2].

Transient complexes are typical of physiological processes that require a compromise between binding specificity and turnover to run at an appropriate rate [3]. On the basis of a two-step model [4], the formation of a final complex first entails a primary unspecific

*Abbreviations:* BD, brownian dynamics; *Cb<sub>6f</sub>*, cytochrome *b<sub>6</sub>f*; *Cc<sub>6</sub>*, cytochrome *c<sub>6</sub>*; *Cf*, cytochrome *f*; CSP, chemical-shift perturbations; ET, electron transfer; EXAFS, extended X-ray absorption fine structure;  $k_2$ , bimolecular rate constant;  $K_D$ , dissociation equilibrium constant; MD, molecular dynamics; NIR, nitrite reductase; NMR, nuclear magnetic resonance; Pc, plastocyanin; PCS, pseudocontact shifts; *pI*, isoelectric point; PRE, paramagnetic relaxation enhancement; PSI, Photosystem I; RMSD, root mean square deviation; WT, wild type; XANES, X-ray absorption near edge structure; XAS, X-ray absorption spectrometry

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recognition as an encounter that can be transiently stabilized to yield a productive complex as an outcome.

Electron transfer (ET) reactions are excellent examples of transient complexes. In fact, soluble proteins mediate redox exchange between large membrane complexes in the photosynthetic and respiratory electron transport chains via short-lived interactions. For instance, in oxygenic photosynthesis there is a soluble metalloprotein that shuttles electrons from cytochrome *f* (*Cf*), which is a component of the membrane-embedded cytochrome *b<sub>6</sub>f* (*Cb<sub>6f</sub>*) complex, to P700, which is the special chlorophyll pair of Photosystem I (PSI) [5–7]. Either plastocyanin (Pc) or cytochrome *c<sub>6</sub>* (*Cc<sub>6</sub>*) can play such a role of electron carrier between *Cf* and PSI. Higher plants only contain Pc, whereas most cyanobacteria and green algae synthesize either Pc or *Cc<sub>6</sub>* depending on the relative availability of copper and iron, their respective cofactor metals [8,9].

*Cf* is anchored to the thylakoid membrane, within the *Cb<sub>6f</sub>* complex, by a C-terminal transmembrane helix leaving a 28-kDa N-terminal soluble portion exposed to the lumen with a clear two-domain structure. The large domain harbors the heme group, and the small domain possesses a patch of charged residues. *Cf* is considered an unusual *c*-type cytochrome because of its  $\beta$ -sheet-based structure, elongated form and particular heme axial

coordination with the N-terminus Tyr1 [10,11]. Pc, in its turn, is an 11-kDa cupredoxin with a  $\beta$ -barrel structure formed by eight  $\beta$ -strands and a small  $\alpha$ -helix, along with a copper centre coordinated by two histidines, one methionine and one cysteine [12].

The overall structures of both Cf and Pc are highly conserved from cyanobacteria to higher plants [13–15], but striking differences occur in some of their physical properties. The surface near the heme moiety in Cf is mainly hydrophobic for all organisms. However, a remarkable basic ridge is found in the small domain for higher plants and green algae turning to acidic in cyanobacteria. On the other hand, Pc relies on two functional sites: a hydrophobic patch surrounding the solvent-accessible histidine copper ligand, or the so-called “site 1”, and an electrostatically charged surface area, the so-called “site 2”, whose nature varies from one organism to another. Actually, site 2 is mainly acidic in plants and green algae, whereas it ranges from acidic to basic in cyanobacteria. This feature is a key for the dynamics of the complex and is thus herein reviewed.

The Cf–Pc complex has extensively been studied in the last years as a model to understand the nature of protein–protein interactions in ET chains. The main goal of this article is to briefly review not only our current understanding of the mechanism of ET in transient complexes [16], but also the different techniques used to analyze the structural features of such complexes [17] in an ample set of prokaryotic and eukaryotic organisms.

## 2. Kinetics within the Cf–Pc complex

Kinetic analyses provide information about the ET reaction mechanism, namely the limiting step and the nature of the partner interactions. Some years ago, our group proposed three different kinetic mechanisms to analyze the well-related interactions of PSI with Pc and  $Cc_6$  from a wide range of photosynthetic organisms [18–20]. These kinetic models can also be applied to the redox interactions between Cf and the two soluble carriers Pc and  $Cc_6$  [21–23]. Some of the currently available experimental data for the interaction between Cf and Pc are summarized in Table 1, where the values for the following constants are presented: bimolecular rate constant for complex formation ( $k_2$ ), equilibrium constant for association between partners ( $K_A$ ), and effective electron transfer rate constant ( $k'_{et}$ ). The interaction between Cf and  $Cc_6$  cannot be analyzed because of spectral overlapping of the two heme proteins.

Electrostatics determines the kinetics of the Cf–Pc reaction. In plants, such effect on the ET rate was mainly established by analyzing the interactions of lysine mutants at the basic ridge of turnip Cf with spinach and pea Pc [21]. Those charged residues at the basic

Cf patch are crucial for the *in vitro* interaction with Pc. Lysines 58, 65 and 187 of Cf directly interact with acidic residues at site 2 of Pc. The electrostatic attraction between the two partners results in a bell-shaped reaction rate dependence on ionic strength [16] (Fig. 1, upper panel). This behavior has been explained by assuming that the complex gets ‘locked’ in a non-productive electrostatic orientation at low ionic strength. As the ionic strength increases, the rearrangement of both proteins into the complex takes place in order to achieve a well-oriented and productive complex, which corresponds to the maximum of the bell. Further increase in ionic strength leads to complex dissociation [6]. It has recently been proposed that the expression ‘locked complex’ should be avoided as such a non-productive state at low ionic strength could rather correspond to a set of non-productive orientations slightly different in energy [4].

*In vitro* kinetic analyses of the interaction between Cf and Pc in cyanobacteria have mainly been addressed in the mesophilic *Nostoc* [22,24] and thermophilic *Phormidium* [23,25,26] species. The electron transfer rate constant between the two *Nostoc* proteins monotonically decreases with increasing ionic strength (Fig. 1, middle panel). The charge mutations at site 2 reduce the ability of Pc to oxidize Cf [22], whereas the mutations at the hydrophobic patch decrease its reactivity towards the heme protein. It is worth to mention that the charge mutation of Arg93 – a residue of Pc located at the interface between sites 1 and 2 – drastically diminishes the reaction rate, so revealing the crucial role of such amino acid in ET within the complex (Table 1). In contrast, the charge replacements at the small domain of Cf hardly affect the interaction with Pc [24] (Table 1), thereby suggesting that the specificity in the Cf–Pc interaction is mostly determined by the cupredoxin. This has been corroborated by NMR studies of the mixed Cf–Pc complexes between *Nostoc* and *Phormidium* cyanobacterial proteins [27].

The ET rate constant between the wild-type (WT) forms of the two *Phormidium* proteins slightly depends on ionic strength (Fig. 1, lower panel), but site-directed mutagenesis of certain charged residues of both proteins revealed a clear influence over the reaction rate (Table 1) [23,25]. Actually, a deeper analysis of the Cf–Pc complex by continuum electrostatics shows that net coulombic forces between protein charges are slightly repulsive [28]. Assuming a diffusion controlled reaction, the electrostatic forces do influence encounter complex formation. The process also involves specific hydrophobic interactions of aromatic residues in the N-terminal peptide of Cf [26].

Altogether, the major contribution to  $k_2$  in higher plants and *Nostoc* cyanobacterium is electrostatics, which leads to the encounter between proteins and to the formation of a well-defined

**Table 1**

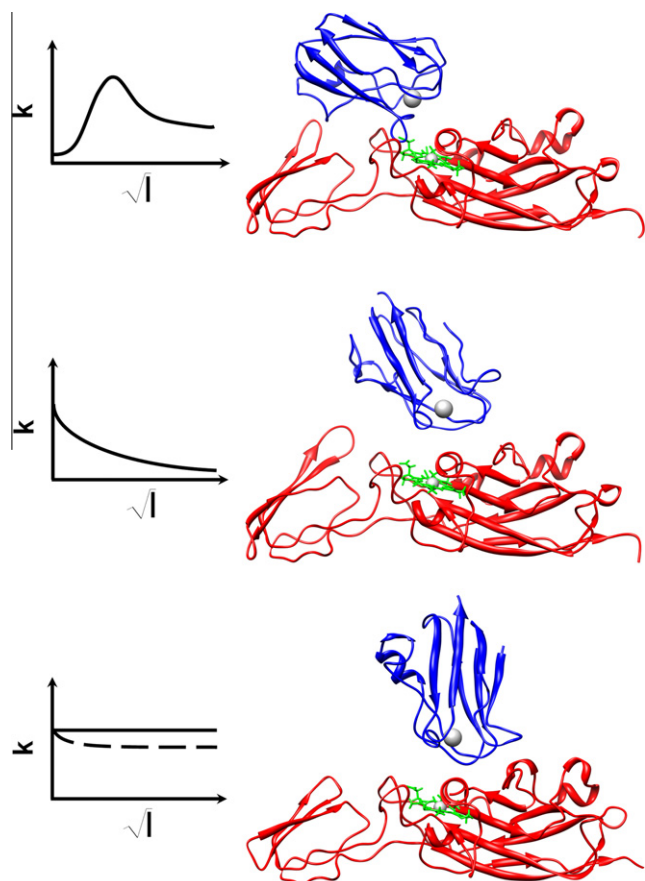
Kinetic data for the redox reaction between WT and mutant forms of Cf and Pc.

Cf	Pc	$k_2$ ( $10^7$ M <sup>-1</sup> s <sup>-1</sup> )	$k'_{et}$ ( $10^{-3}$ s <sup>-1</sup> )	$K_A$ ( $10^3$ M <sup>-1</sup> )	References
Turnip WT	Spinach WT	17.6 ± 2.2	~3 <sup>a</sup>	–	[21,22]
Turnip K187E	Spinach WT	2.5 ± 0.3	–	–	[21]
Turnip WT	Pea WT	17.5 ± 0.3	–	6.9 ± 0.18	[21]
Turnip K65Q	Pea WT	3.5 ± 0.1	–	0.55 ± 0.38	[21]
<i>Phormidium</i> WT	<i>Phormidium</i> WT	4.7 <sup>b</sup>	–	~0.3	[23,39]
<i>Phormidium</i> WT	<i>Phormidium</i> D44A	6.0 <sup>b</sup>	–	–	[23]
<i>Phormidium</i> WT	<i>Phormidium</i> R93E	1.0 <sup>b</sup>	–	–	[23]
<i>Phormidium</i> D63A	<i>Phormidium</i> WT	3.1 <sup>b</sup>	–	–	[25]
<i>Nostoc</i> WT	<i>Nostoc</i> WT	–	13.4	26 ± 1	[22,27]
<i>Nostoc</i> WT	<i>Nostoc</i> D54K	–	25.5	–	[22]
<i>Nostoc</i> WT	<i>Nostoc</i> R93E	–	1.8	–	[22]
<i>Nostoc</i> D64A	<i>Nostoc</i> WT	–	13.3	–	[24]
<i>Phormidium</i> WT	<i>Nostoc</i> WT	–	–	12 ± 1	[27]
<i>Prochlorothrix</i> WT	<i>Prochlorothrix</i> WT	~20	–	25 ± 2	[40]

$k_2$ , bimolecular rate constant for complex formation;  $K_A$ , equilibrium constant for association between partners;  $k'_{et}$ , effective electron transfer rate constant.

<sup>a</sup> French bean Pc was employed.

<sup>b</sup> The overall error of  $k_2$  with all *Phormidium* variants is estimated to be ≤5%.



**Fig. 1.** Comparison of Cf–Pc complexes from different organisms. *Right*, Superposition of the average structure, with the lowest energy value, calculated from the 10 best NMR structures of each complex. *Left*, profile of the ionic strength dependence of the observed rate constant in each case. *Upper*, poplar [36]; *middle*, *Nostoc* [22,38]; and *lower*, *Phormidium* [25,39]. Cf and Pc ribbons are in red and blue, respectively. The heme porphyrin ring is depicted in green sticks, whereas the copper and iron atoms are grey spheres.

functional complex. The opposite is valid for the thermophilic *Phormidium* cyanobacterium, in which non-polar interactions play the main role because of the small net contribution of electrostatics [23,26,28]. Nevertheless, the hydrophobic interactions are essential for ET in all complexes, since the reorganization of water molecules at the interface should impair the charge transfer process. In fact, the exclusion of water molecules yields a productive complex within which the ET takes place. Intriguingly, the reaction rates reach similar values under physiological salt concentration and temperature ( $k_2 \sim 10^8 \text{ M}^{-1} \text{ s}^{-1}$ ) despite the apparent differences among organisms [23].

*In vivo* approaches are quite scarce. In the alga *Chlamydomonas reinhardtii* [29,30], neutralization of Cf basic ridge has a negligible effect on the rate of protein re-oxidation, suggesting that electrostatic interactions within the Cf–Pc complex are not so relevant *in vivo* in this organism. Experimental conditions mimicking viscosity, macromolecular crowding and temperature have also been explored, but no relevant conclusions can be reached [31].

### 3. Defining the relative orientation between Cf and Pc

The structure of the Cf–Pc complex, as well as that of the Cf–Cc<sub>6</sub> one, has been analyzed by nuclear magnetic resonance (NMR) spectroscopy [17,32] by using the chemical and pseudo-contact shifts (PCS) experienced by the Pc signals as restraints for rigid-

body docking calculations. Diamagnetic, chemical-shift perturbations (CSP) are caused by changes in the chemical environment suffered by the atoms located at the binding interface. Structural restraints derived from CSP do not provide precise geometrical information about angles and distances between the two partners. However, measuring PCS renders geometrical restraints that may be sufficient to define the relative orientation of both proteins. PCS restraints are provided by the intrinsic paramagnetic probe of Cf, which supplies the oxidized iron atom ( $\text{Fe}^{3+}$ ) from the heme porphyrin ring. PCS depend on the axial and rhombic components of the magnetic susceptibility tensor and the angles that the vector joining the heme Fe atom of Cf to the target amide proton in Pc forms with them. Moreover, PCS are inversely proportional to the cubed distance between any particular nucleus of Pc and the paramagnetic center. Structure elucidation of the Cf–Pc complex for different organisms shown in this section fits well with the orientation of Cf within the Cc<sub>6</sub>f complex as determined by X-ray crystallography [11,33].

Some similar structural features, like interface areas of ca. 600–850 Å<sup>2</sup> per protein, are shared by the Cf–Pc complexes from prokaryotic and eukaryotic organisms. All the cases studied show that Cu–ligand His87 in Pc is close to iron-coordinating Tyr1 in Cf, thereby providing an efficient electron transfer pathway [34]. However, remarkable differences are found not only when comparing the structures of plant and cyanobacterial complexes, but also when the comparisons are made between complexes from cyanobacteria (Fig. 1).

Plant heterologous complexes between Cf from turnip and Pc from several other sources like spinach, parsley or poplar have been solved [35–37]. Their overall structures show a binding mode named *side-on* (Fig. 1, *upper panel*) in which the hydrophobic patches of the two proteins keep in close contact with each other and the complementary charged regions are juxtaposed. In other words, both Pc sites 1 and 2 are involved in the binding interface. The Fe–Cu distance varies from 10.9 Å in spinach to 13.9 Å in poplar and 13.0 Å in parsley. Actually, poplar and parsley Pc are slightly tilted with regard to the position of spinach Pc [36]. Within the poplar Cf–Pc complex, the approach of the side-chain of His87 from Pc to the heme ring at Cf is restricted by the loop containing this copper ligand. In contrast, the parsley Cf–Pc model shows a rotation of Pc, with respect to the spinach, in a direction opposite to that in poplar complex [37].

The cyanobacterial complexes have been analyzed using both Pc and Cf from the same source. The structure of the Cf–Pc complex from *Nostoc* [38] shows a relative orientation that resembles the plant *side-on* fashion, but with the charges reversed. Actually, the positive charged residues at site 2 of *Nostoc* Pc – which indeed are determinant in the isoelectric point (*pI*) of 8.4 for the whole protein – are keeping salt bridges with negative residues at the hinge and small domain of Cf. However, the *pI* of plant Pc is ca. 4.0 and its negatively charged site 2 interacts with the positive charged residues of Cf. The hydrophobic interactions involving the two metal centers of both proteins are also present (Fig. 1, *middle panel*). In contrast, Pc in the *Phormidium* complex is oriented in a *head-on* manner relative to Cf, with only site 1 (hydrophobic patch) making contact to Cf (Fig. 1, *lower panel*). In *Phormidium*, Cf lacks the typical basic ridge, whereas Pc has fewer charged residues at site 2 [39] than Pc from plants and shows a dipole moment aligned with the main molecule axis. This results in the attraction of the copper site towards Cf and repulsion of site 2. The resulting net coulombic term between the two proteins is repulsive, but is mostly compensated by solvent polarization phenomena to yield a small ionic strength effect [28]. The *Phormidium* complex shows a highly dynamic nature that could explain the lower precision of the solved structure – with an RMSD of 3.7 Å – compared with other Cf–Pc models. It could also be explained by differences in

the dynamic behavior of the complexes, a fact that is especially significant in those organisms that inhabit niches at high temperatures.

Crossed complexes between proteins from *Nostoc* and *Phormidium* revealed that it must be a low net electrostatic contribution in the latter complex [27], in agreement with results on ET kinetics. Although early NMR data [39] suggested the absence of electrostatic interactions, several transient conformations involving electrostatics could remain invisible by NMR, at least in the absence of paramagnetic relaxation enhancement measurements (PRE). Therefore, only the long-life conformation into the complex is detectable. In addition, studies of such mixed complexes show that the differences in interactions are mainly attributable to the surface properties of Pc [27]. The NMR analyses (PCS measurements) show that the Cf–Pc complex from *Prochlorothrix hollandica*, unlike the *Nostoc* Cf–Pc complex, exhibits a *side-on* orientation, in which Pc site 2 is not interacting with the small domain of Cf, thereby suggesting a dynamic nature that resembles the *Phormidium* complex [40].

An overall view of structural features for the well-defined oriented Cf–Pc complexes postulates a predominant *side-on* conformation highly dependent on electrostatics. As the electrostatic contribution becomes lower, the orientation of Pc relative to Cf shifts from *side-on* to *head-on*. All these observations are also in agreement with the ET proposed kinetic mechanisms.

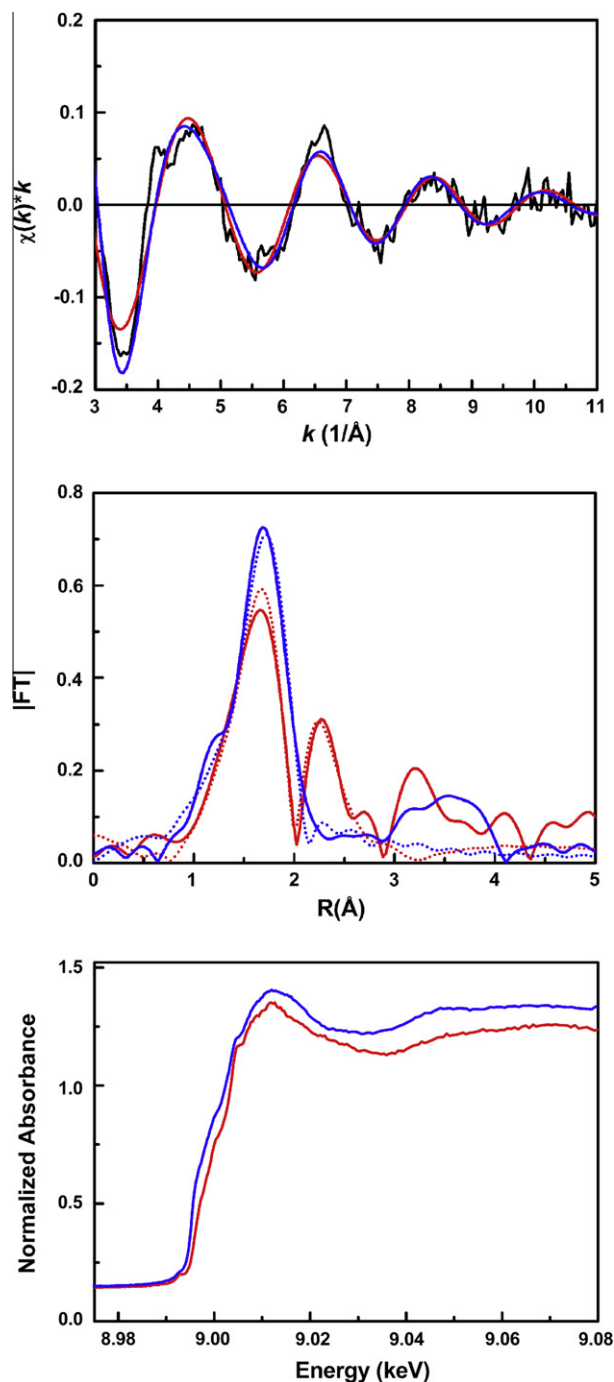
#### 4. Metal cofactors within the Cf–Pc complex

Although there are a lot of experimental data on the interaction between the two redox partners, the information on the effect of binding on the metal cofactors and their properties is rather scarce. In fact, the redox potential of Pc decreases in 30 mV upon binding to Cf [41]. Noteworthy, the way on how metal sites can adapt to changes in the protein matrix and modulate the ET reaction highly contributes to understand the transient complexes involved in this kind of processes.

The cyanobacterial *Nostoc* Cf–Pc complex has been analyzed by X-ray absorption spectroscopy (XAS). Both Fe and Cu K-edge XAS measurements of free and bound redox proteins have been studied in solution, using either the oxidized or reduced species [42,43].

In Cf, the Fe atom of the heme group is axially coordinated by two nitrogen atoms belonging to Tyr1 (N) and His26 (N<sub>ε2</sub>). Such atypical coordination geometry involves the N-terminal tyrosine, which also takes part in hydrophobic interactions with Pc, according to the structural elucidations of this transient complex [38]. XAS measurements reveal that the Fe coordination geometry remains unaltered upon binding to Pc. Getting a deeper insight into the data, a slight distortion of the metal (Fe<sup>2+</sup>) center geometry seems to happen when reduced Cf binds to reduced Pc. The resulting geometry is closer to that of oxidized Cf, either free or bound to oxidized Pc. Because of the smaller size of Fe<sup>3+</sup> compared to Fe<sup>2+</sup>, the first fits better than the second into the heme group as Fe<sup>2+</sup> is slightly out of the plane [44]. However, Fe<sup>2+</sup> could be driven back to the ring upon complex formation. Iron-N-terminus nitrogen bond in Cf is strong enough to prevent distortions over the coordination geometry of the metal center, so facilitating a stable binding site to Pc and enhancing ET.

The copper atom in Pc is coordinated by two nitrogen atoms from His39 and His92, as well as by two sulphur atoms from Cys89 and Met97. All of them are well-conserved residues placed at a protein loop that is at the interface with Cf [35,38,39]. Cu–K edge XAS shows a remarkable distortion in the trigonal pyramidal geometry of the copper coordination sphere upon binding to Cf, regardless of its redox state (Fig. 2, upper and middle panel). The main evidence for such a distortion is the contribution of the S<sub>δ</sub>



**Fig. 2.** Distortion of the copper geometry of Pc upon Cf binding in *Phormidium*. Upper, EXAFS spectra at the Cu K-edge of oxidized Pc upon binding to oxidized Cf (black) and fitting in the  $k$  space either considering that the contribution of S<sub>δ</sub>(Met97) is negligible (red,  $\chi^2$ : 16.97) or taking it into account (blue,  $\chi^2$ : 11.3). Middle, Fourier Transform modules of free Pc (blue) and Pc-bound to Cf (red), both in the reduced state. The best fits for the data are depicted in dotted lines following the same color-code. The contribution of the S<sub>δ</sub>(Met97) atom is considered for the fit in  $R$  space. Lower, XANES region of the Cu K-edge XAS spectra for free Pc (blue) and Pc-bound to Cf, both in the oxidized state (red).

atom from Met97 to the extended X-ray absorption fine structure (EXAFS) wave, indicating that the mobility of this side-chain is substantially restrained upon binding of Pc to Cf. Actually, the resulting tetrahedral structure of the copper center within the Cf–Pc complex exhibits a shorter Cu–S<sub>δ</sub>(Met97) distance with respect

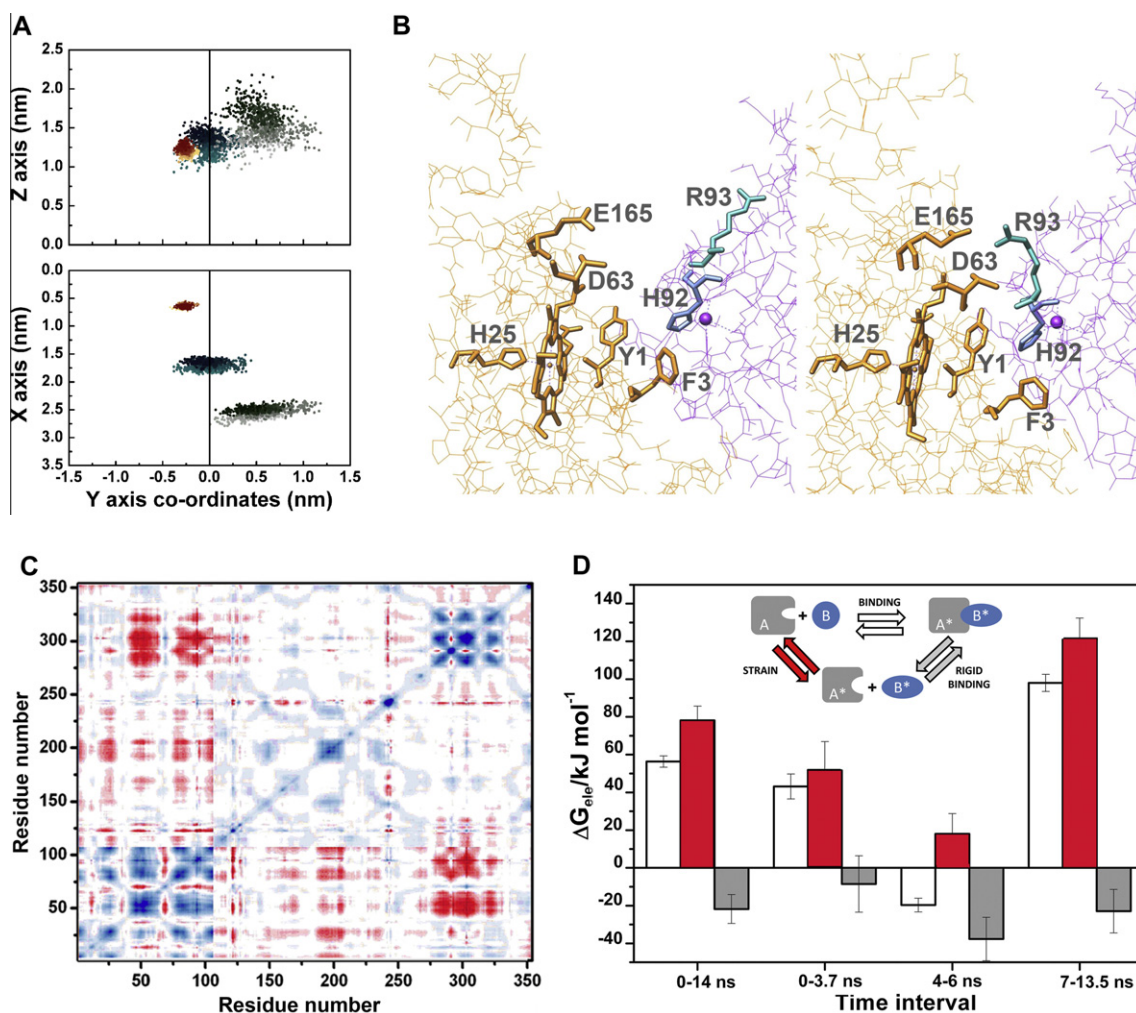


to the crystallographic structure of free Pc [45]. Noteworthy, the Cu–Sδ(Met97) distance of Cf-bound Pc by EXAFS resembles that of the crystal structure of oxidized nitrite reductase (NIR) [46], which belongs to the so-called “perturbed” copper centers. In addition, the electronic density around the copper atom increases when Pc binds to Cf in their oxidized states, according to the data from the X-ray absorption near edge structure (XANES) region (Fig. 2, lower panel). In fact, the redox potential of Pc becomes more negative upon binding to Cf [41]. As a result, the driving force for ET between both metalloproteins is significantly decreased by the Cf–Pc interaction. In addition, the observed geometrical changes in the first coordination sphere of copper within the Cf–Pc complex modulate the electron coupling along the different ET pathways and hence the redox reaction.

## 5. Theoretical approaches

The resulting data from analyzing the weak Cf–Pc complex by means of theoretical methods add useful information to our current knowledge of transient interactions.

The Cf–Pc complexes of a wide range of organisms, from cyanobacteria to plants, have been studied by Brownian Dynamics (BD) simulations using crystal or NMR structures. The role of electrostatic interactions in higher plant and algal complexes has been confirmed by BD simulations that are in good agreement with the  $k_2$  values for ET reactions previously reported [21,23,25]. The complex between turnip Cf and spinach Pc [47] and that between Cf and Pc from the green alga *Chlamydomonas reinhardtii* [48] have both been studied by performing systematic mutations of charged residues at the protein interfaces. These data support the model of an electrostatically driven encounter complex as a previous step of a final productive complex wherein efficient ET occurs. BD analyses with *Phormidium* Cf and Pc from different sources [49] show considerable variations in electrostatic forces, a finding that highlights the key role of a few charged residues close to His92 (His87 in plants and algae) for the complex interface. However, the computational studies show that Pc is highly mobile in all these complexes, opposite to its behavior in plant complexes. Still, hydrophobic interactions remain highly significant in all organisms.



**Fig. 3.** Molecular dynamics of the *Phormidium* Cf–Pc complex. (A) Drift of Pc around Cf along the MD trajectory in Ref. [28]. The dihedral first angle projections of the trajectories of iron from Cf (brown dots), copper (blue dots) and center of mass of Pc (grey dots) are represented with respect to the main axes and mass center of the large domain of Cf. (B) Interaction of the copper site and Arg93 in Pc with negative residues of Cf upon approaching of the two partners. Pc and copper center are in purple, and Cf in gold. The side chains of charged residues and the heme porphyrin ring are depicted in sticks following the same color-pattern. (C) Coordinate covariance matrix of a trajectory corresponding to the Cf–Pc complex obtained after aligning main chain atoms of the large domain of Cf. Residues 1–105 correspond to Pc, and 106–354 to Cf. Positive values are shown in blue, whereas negative are in red. (D) Analysis of continuum electrostatics averaged along different time intervals of a MD computation of the Cf–Pc complex. The insert summarizes the thermodynamic cycle used in the computation of the different terms in which  $\Delta G_{\text{ele-bind}}$  stands for the sum of free energies that account for distorting the free monomers to get the conformation they adopt in the complex,  $\Delta G_{\text{ele-strain}}$ , and their subsequent rigid-body association,  $\Delta G_{\text{ele-rigid}}$ .  $\Delta G_{\text{ele-bind}}$  is represented by white bars;  $\Delta G_{\text{ele-strain}}$  by red-filled bars;  $\Delta G_{\text{ele-rigid}}$  bars are colored in grey.

BD analyses are quite sensitive to small changes in conformation of the proteins that are being modeled. For instance, the Cf–Pc complex with spinach Pc adopted two different conformations when using distinct structural data sources [50]. On the basis of such finding, it was suggested that Pc may assume distinct conformations in solution so as to yield a productive ET complex.

BD and other docking approaches treat proteins as rigid bodies [40], whereas Molecular Dynamics (MD) calculations provide additional information about internal protein motions. Despite all the kinetic, structural and theoretical studies performed on the *Phormidium* Cf–Pc complex, there is certain controversy regarding NMR and functional data. Hence, MD simulations combined to continuum electrostatic calculations on this particular interaction [28] try to harmonize the two sets of data. The MD trajectories reveal that Pc tilts towards the small domain of Cf approaching site 2 to the heme protein (Fig. 3A). This involves the interaction of the positive charges of the copper site and Arg93 with the negatively charged residues at the loop of Cf surrounding the heme group (Fig. 3B). Thus, the relative orientation of Pc respect to Cf in the *Phormidium* complex can be redefined as a *tilted head-on* conformation (see Fig. 1, lower panel). However, the repulsive forces make both proteins swing in an opposite but concerted manner within the transient Cf–Pc complex, as inferred from the negative covariance between motions of Pc and those of the small domain of Cf (Fig. 3C). Interestingly, the conformation of Cf is strained upon binding of its partner and relaxes upon release. Although there are no direct contacts between Pc and the small domain of Cf, continuum electrostatic calculations indicate that the long-range electrostatic interactions between them are responsible for straining the conformation of the partners (Fig. 3D). The thermodynamic cycle shown in Fig. 3D reveals that  $\Delta G_{\text{ele-bind}}$  can be split in two terms if any one of the partners changes within the complex. The first term is the “strain energy” ( $\Delta G_{\text{ele-strain}}$ ), which accounts for the cost of structural changes needed for docking; the second one is the “rigid binding” ( $\Delta G_{\text{ele-rigid}}$ ) component, which represents the binding energy of the strained partners. Fig. 3D also shows that the time intervals corresponding to the two major ensembles of conformations (from 0 to 3.7 ns, and from 7 to 13 ns) are characterized by a positive  $\Delta G_{\text{ele-strain}}$  term and by large strain energy values ( $\Delta G_{\text{ele-bind}}$ ). However,  $\Delta G_{\text{ele-bind}}$  becomes negative, with a concomitant drop in  $\Delta G_{\text{ele-strain}}$ , in the time interval from 4 to 6 ns corresponding to the transition between these two major populations. An explanation for this binding energy decrease comes from the reaction field term that accounts for solvent polarization phenomena. In addition, the electrostatic strain may play a key role in breaking the complex off after the attraction between the copper surroundings and Cf is weakened upon charge transfer.

## 6. Conclusion and outlook

A multidisciplinary effort has been made in the last years to understand the behavior of transient ET complexes and, in particular, of the short-lived Cf–Pc complex from different organisms. In most cases, the functional and structural approaches coincide in postulating the relevance of long-range electrostatic interactions *in vitro*. The existence of an encounter complex may indeed play an important role in the Cf–Pc complexes, a finding that should be further explored in detail. Some controversy still exists over experimental data interpretation in the cyanobacterial model system from *Phormidium*, but theoretical simulations are providing valuable information to conciliate them. Actually, recent modeling developments could help to widen the scope of this subject. The bottleneck is to unveil the relevance of these data *in vivo*. Specifically, the role played by electrostatics on the ET process inside crowded cells needs further research.

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