Electrostatic strain and concerted motions in the transient complex between plastocyanin and *cytochrome f* from the cyanobacterium Phormidium laminosum

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Abstract:

Many fleeting macromolecular interactions, like those being involved in electron transport, are essential in biology. However, little is known about the behaviour of the partners and their dynamics within their shortlived complex. To tackle such issue, we have performed molecular dynamics simulations on an electron transfer complex formed by plastocyanin and *cytochrome f* from the cyanobacterium Phormidium laminosum.

Besides simulations of the isolated partners, two independent trajectories of the complex were calculated, starting from the two different conformations in the NMR ensemble. The first one leads to a more stable ensemble with a shorter distance between the metal sites of the two partners. The second experiences a significant drift of the complex conformation. Analyses of the distinct calculations show that the conformation of *cytochrome f* is strained upon binding of its partner, and relaxes upon its release. Interestingly, the principal component analysis of the trajectories indicates that plastocyanin displays a concerted motion with the small domain of *cytochrome f* that can be attributed to electrostatic interactions between the two proteins.

Keywords: *Cytochrome f*; Domain motions; Molecular dynamics; Plastocyanin; Transient protein complexes

1. Introduction

The complexes between redox proteins constitute a paradigm of short-lived complexes that are essential in a wide range of biological processes — namely, respiration, photosynthesis, nitrogen fixation, etc. Hence, it is of growing interest to unveil the factors that control their transient nature. In general, it is assumed [1–3] that the two reaction partners approach each other under the influence of long-range electrostatic attractive forces to form a dynamic ensemble of non-productive conformations known as the encounter complex, [4] wherein diffusion is partially restrained. Such state is in equilibrium with a single well-defined productive complex, in which the hydrophobic core at the interface provides the ideal medium for electron transfer. The whole process is not fully understood, but the predominant hypothesis [1,5] is that hydrophobic and van der Waals interactions are the major forces steering the encounter conformations towards the productive complex, as the electrostatic interactions are not specific enough to do it.

A well-known transient complex is that formed by *cytochrome f* (C*f*) and plastocyanin (Pc), two components of the photosynthetic electron transport chain. Pc is a small, soluble cupredoxin that shuttles electrons from Cf to photosystem I [2,3]. The structure of Pc is a beta-barrel with two functional surface areas [2,3,7–9]: one hydrophobic surrounding a copper-ligand histidine (site 1), and the other electrostatically charged (site 2). Cf, in its turn, is a subunit of *cytochrome* b_6f [6] that is anchored to this membrane complex by its hydrophobic, C-terminal alpha-helix. The soluble portion of C*f* shows a clear two-domain structure [10], wherein the large domain contains the heme group and the small domain includes a patch of charged residues known to interact with site 2 of Pc (Fig. 1).

The electron transfer from C*f* to Pc has been analysed by using stopped-flow [11–15] and fast kinetic techniques [7,16], often combined with site-directed mutagenesis of one or both partners. The resulting kinetic data have been very useful to analyse the interaction between Pc and Cf by means of theoretical methods, including Molecular Dynamics (MD) at sub-nanosecond time scale [17], rigid-body MD [18], Molecular Modelling [19] supported by NMR data and Brownian Dynamics (BD) simulations [20–28]. Last computations, in particular, are in good agreement with experimentally calculated reaction rates [21,24–26], thereby explaining many effects of ionic strength and mutations on the kinetics.

In addition to functional and theoretical data, the structure of the Pc–C*f* complex isolated from different organisms (ranging from cyanobacteria to plants) has been analysed [8,29,30]. The NMR chemical-shift perturbations reveal that site 1 of Pc is always required for the binding to C*f* but the involvement of the adjacent site 2 of Pc varies for each organism [9]. Actually, the tilt of Pc with respect to C*f* is closely related to the influence of electrostatic forces in binding, thus yielding the so-called "head-on" or "side-on" orientation. In the cyanobacterium Phormidium laminosum, the electron transfer between Pc and C*f* is slightly affected by salt addition [14,31] but ionic strength does not bear on the binding equilibrium, according to NMR data [29]. In the NMR structure of the Pc–C*f* complex from Phormidium [29] (Fig. 1), only Pc site 1 interacts with C*f* (head-on), whereas in higher plants, the electrostatic attractions play a key role and Pc site 2 is in close contact with the small domain of C*f* (side-on). Despite this variability in the binding mode, which in cyanobacteria relies mostly on the diversity of Pc rather than of C*f* [9,25], all the structures of the Pc–C*f* complexes show modest interface areas (530–850 $Å^2$ per protein), and have Pc site 1 lying near tyrosine 1 of C*f* [8].

As regards the dynamics of the two redox partners during the lifetime and further dissociation of the complex, little is known as yet. Recently, it has been shown that different conformations of Pc lead to different BDdata [22] and that the

geometry of the copper centre of Pc is perturbed upon binding to C*f* [32]. In addition, the role of the small domain of C*f* in Pc docking is not fully understood, and there still exist some discrepancies to reconcile structural and functional data, especially in Phormidium cyanobacterium.

Unlike BD and other experimental approaches [18,19] that treat proteins as rigid bodies, MD calculations can provide relevant information on the internal motions of the proteins that is complementary to experimental data. To the best of our knowledge, however, only one MD study of the Pc–C*f* complex has been reported [17] apart from calculations aimed at refining the structures of the partners [19]. Such MD study just reports short trajectories (200 ps) starting from partially solvated docking models, wherein Pc was simulated inside a water blob and most of C*f* was outside simulation limits.

Here, we report a series of MD and continuum electrostatic computations on the Pc–C*f* complex and the two isolated partners to unveil how the interaction between Pc and C*f* affects their relative motions within the transient complex and to clarify the role of the small domain of C*f* in the dynamics of the complex. The Phormidium Pc–C*f* complex shows different features that are interesting to analyse: First, the apparent lack of direct contact between the small domain of C*f* and Pc and, second, the above-mentioned contradiction between NMR and functional data as regards the electrostatics in the interaction between the two proteins. In addition, it displays a great dissociation constant and the NMR data show two sets of 3D conformations that differ in the tilt of Pc within the complex. Actually, the BD simulations with the Phormidium Pc–C*f* complex converge at a dynamic ensemble typical of encounter complexes [23], rather than at a single state, as do the complexes from other organisms.

Our results show that the dynamics of the small domain of C*f* is substantially affected upon binding of Pc to the large domain of C*f*. We show that this strain in the structure has an electrostatic nature. Moreover, the swinging motions of the C*f* small domain and Pc are concerted within the transient Pc–C*f* complex and due to repulsive electrostatic interactions between them.

2. Methods

2.1. Molecular dynamics

MD calculations were performed on the structures of Pc [33] and the soluble fragment of C*f* [34] from Phormidium, deposited at PDB [35] (access codes 1BAW and 1CI3, respectively) and two of the 10 best NMR structures of their complex [29] (gently provided by Dr. M. Ubbink). Simulations were carried out by using AMBER 8.0 [36] under the AMBER 95 force field [37] in a Dell Power Edge workstation. All calculations were run under periodic boundary conditions using an orthorhombic (minimum distance between protein and cell faces was initially set to 10 Å) cell geometry and PME electrostatics with a Ewald summation cut-off of 9 Å. Force field parameters corresponding to the oxidised copper site were those developed by Comba and Remenyi [38]. For the reduced heme moiety, Banci's parameters [39] were used, and a harmonic force of 200 kcal mol^{-1} $Å^{-2}$ – with an equilibrium distance of 2.44 Å – was applied to the bond between Fe and the N terminus of C*f* acting as the sixth ligand, to account for the stiffness of this bond observed by X-ray absorption values [40]. Counter-ions were added and all systems were solvated with TIP3P water molecules [41] (see Table SI-1 in Supplementary information), using the TLEAP module of AMBER. Protein side-chains were then energy minimised (250 steepest descent and 750 conjugate gradient steps) down to a RMS energy gradient of 0.0125 kJ mol^{-1} $Å^{-1}$, using the SANDER module of AMBER. Afterwards, solvent and counter-ions were subjected to 500 steps of steepest descent minimization followed by NPT-MD computations using isotropic molecule position scaling and a pressure relaxation time of 2 ps at 298 K. Temperature was regulated with Berendsen's heat bath algorithm [42], with a coupling time constant equal to 0.5 ps. After 100 ps simulation, the density of the system reached a plateau. Then, for each protein, the whole system was energy minimised and submitted to NVT-MD at 298 K, using 1.6 fs integration time steps. Snapshots were saved every 4 ps. SHAKE algorithm [43] was only used to constrain bonds involving hydrogen atoms.

2.2. Analysis of trajectories

Coordinate files were processed using both the PTRAJ module of AMBER and the VMD [44] package to perform alignments, root mean square deviation (RMSD) calculations and secondary structure determination by the Kabsch and Sander algorithm [45]. Data from the first 300 ps were considered as equilibration steps, and not included in any analysis. The geometries of the overall binding conformation and the pseudo-contact shifts (PS) of Pc bound to C*f* were analysed along the trajectories using the VMD tcl/tk console. These PS of Pc amides provide valuable geometric information relative to the orientation of the different atoms of the copper protein with respect to the heme moiety. In order to compare with experimental data [29] in which C*f* is in its oxidised paramagnetic state, PS were calculated from snapshots according to the following equation [30,46,47]:

wherein ....

and ....

are the axial and rhombic anisotropies of the magnetic susceptibility tensor, respectively; F is a scaling factor that accounts for the relative population of bound Pc in the NMR sample [29,30]; χ_{zz}, χ_{yy}, and χ_{xx}, are the diagonal elements

of the magnetic susceptibility tensor of the paramagnetic centre; Θ stands for the angle between χ_{zz} and the vector joining the heme Fe atom of C*f* to the target amide proton in Pc; and r is the distance between iron and the target nucleus of Pc. Ω corresponds to the angle between the metal-nucleus vector on the xy plane of the magnetic susceptibility reference system and its y axis [46,47]. Data concerning the magnetic anisotropy and the orientation of its components were taken from the literature [48]. It is worth noting that experimental $\Delta\delta_{PS}$ is obtained in samples wherein C*f* is oxidised, while in our calculations C*f* is reduced. This could lead to small differences between experimental values and those calculated from simulations.

To calculate the evolution of the relative orientations of Pc and the small domain of C*f*, relative to its large domain, the main (orthogonal) rotation axes of each set of atoms were calculated by singular value decompositions of their inertia moment matrixes (see Supplementary information for more details) in the VMD tk console. Given the three main, orthogonal axes of a reference set of atoms, wherein Z corresponds to its main axis, we can describe the relative orientation of a second set by three angles: pitch, yaw and roll. The pitch (see Fig. SI-1 in Supplementary information) of a group of atoms is defined as the angle between the main axis of this set (Z′) and the equatorial plane (XY) of the reference. Yawangle is the tilt of the main axis (Z′) of a given selection of atoms with respect to the reference ZX plane. Finally, a roll angle is defined as that between the secondary axis (Y′) of a set and the plane defined by the main axis of the reference and that of the atom set.

Principal component analysis (PCA) was performed using g_covar and g_anaeig modules of the GROMACS 3.2 package [49–52]. For this purpose, only the coordinates of Cα atoms were considered, and trajectories were formatted using VMD [44]. In the case of free Pc, backbone heavy atoms were used for aligning the structures, in order to remove translational and rotational diffusion. For the Pc–C*f* complex, several analyses were performed, using distinct alignments and time intervals of the trajectory, according to the subject of interest. The Dyndom program [53] was used to determine domain motions, the screw axis and the corresponding hinge residues. Standard parameters were used: The window size was 5 residues; the minimum domain size was 20 residues; and the cut-off for definition of effective hinge axis and mechanical hinge was 5.5 Å. Rotations of domains around a hinge axis perpendicular to the segment joining their mass centres are named closure motions [53]. Continuum electrostatic calculations were performed by finite difference discretisation of the Poisson–Boltzman equation in DelPhi [54] and represented in UCSF Chimera [55]. Binding electrostatics energies were calculated as described by Sheinerman and Honig [56].

3. Results

To characterise the dynamical features of the short-lived complex between Pc and C*f*, MD calculations of both the whole complex and the two partners in their free state have been performed. The initial backbone coordinates of Pc in the NMR model of the complex – once those of C*f* have been aligned – show a high RMSD (3.7 Å) [29], which is consistent with a highly dynamic complex [23] (see Fig. SI-2 in Supplementary information). Hence, two trajectories of the complex were calculated: The first started from the structure with the lowest deviation of Pc atoms (0.65 Å) from the model average (C-21.6) and the second one (C-14.6) began in one of the most perpendicular orientations of Pc with regard to C*f*, according to its pitch angle (14.6°). In both cases, copper to iron distances are fully consistent with fast electron transfer, though that in the C-21.6 conformation is shorter.

3.1. Stabilities of the simulations

The stabilities of the simulations were tested according to the geometrical properties shown in Table 1. The RMSD values in calculations corresponding to free Pc become stable along the first 300 ps, while ca. 1 ns is required in case of C*f*. A small RMSD drift (~10^{-2} Å ns^{-1}) can be observed, which is somewhat larger in some of the production steps corresponding to C*f*. However, no unfolding or swelling of the proteins is observed in any of the simulations, since their gyration radii do not change, as shown in Table 1. Furthermore, the larger RMSD values in C*f* simulations can be explained by small domain motions because its two domains show smaller RMSDs when analysed separately than the whole protein. On the other hand, protein loops from the small domain exhibit significant RMSD fluctuations (see Fig. SI-3 of the Supplementary information) unlike those regions with regular structure. Actually, the secondary structure of C*f* does not change along calculations (Fig. SI-4 in Supplementary information), remaining as reported in the DSSP [45] summary at RCSB [35].

The simulations of the complex show larger drifts from the starting structures than the trajectories of the free partners. Remarkably, C-14.6 shows a much larger deviation from the starting structure than C-21.6. According to data in Fig. 2A, this deflection is due to an evolution of the relative positions of the complex partners, rather than to any change in their own structures. In fact, the average RMSDs for Pc and C*f* backbone atoms are smaller in the C-21.6 trajectory and they remain around their respective averages (ca. 1.0 Å and 1.8 Å, respectively) during the calculations when analysed separately (Table 1). For C-14.6 trajectory, there is an overall increase in atomic fluctuations of the backbone atoms of C*f*. These fluctuations, however, do not explain the large RMSD of the C-14.6 complex with respect to its initial structure, which results from changes in the relative orientation of the two partners (Fig. 2A).

To illustrate the drift of Pc during the simulations, the large domain of C*f* was aligned along the trajectory corresponding to the Pc–C*f* complex, and the RMSDs between backbone atoms of Pc at different times were calculated (Fig. 2B). In the C-21.6 trajectory of the Pc–C*f* complex, we can distinguish two different states that are stable enough

to be analysed. However, the calculations starting on the C-14.6 conformation show a larger RMSD drift suggesting that each protein partner moves with respect to the other (see below).

Fig. 2C displays the evolution of Pc within the complex along the two MD calculations in Cartesian coordinates. For this purpose, the position of centres of mass of three groups of atoms (Pc, and the two metals with their corresponding first sphere ligands) has been plotted, using the main axes of the C*f* large domain as reference. This illustrates that the two trajectories differ in the relative position of Pc and its copper centre with respect to the heme moiety. Moreover, Fig. 2C reveals the fast evolution of the C-14.6 conformation towards a different state, opposed to the stability of the C-21.6 trajectory.

3.2. Accuracy of the molecular dynamics calculations of the Pc–C*f* complex

In order to evaluate the accuracy of the MD simulation, PS were calculated from the coordinates of snapshots along simulations, as explained in Methods, and compared to experimental data reported previously [29]. PS depend on the distance between a given atom and the paramagnetic centre, as well as on the orientation of iron-amide proton vectors relative to the main magnetic susceptibility components (see Methods), thereby allowing the assessment of structural data [46]. The equation includes a proportionality factor (F) that accounts for the ratio of Pc bound to C*f*. During our simulations,100% of Pc is bound, but only ca. 40% (F was set to 0.4) was interacting to C*f* for the conditions under which experimental PS were measured [29]. In our case, the optimum fit of PS requires a range of F values from 0.4 to 0.6, depending on the coordinate set analysed. Fig. 3 shows the overall agreement between experimental and theoretical data extracted from full MD trajectories.

In the starting NMR structures, the squared correlation coefficients between both sets of data were ca. 0.3 (Fig. 3A and E), whereas those for the full C-21.6 (Fig. 3B) and C-14.6 (Fig. 3F) trajectories were 0.5 and 0.1, respectively. Noteworthy, best fits (0.69 and 0.66) correspond to the two ensembles of the C-21.6 simulation (Fig. 3C and D, respectively). Indeed, data corresponding to the C-21.6 simulation (Fig. 3B) fit the experimental data better than the initial coordinate set (Fig. 3A) and very small differences are found when, instead of the full trajectory, only the time gap corresponding to any of the distinct states was used (Fig. 3C and D).

On the other hand, PS calculated from the C-14.6 simulation show significant divergences from experimental data that do not improve when sampling small intervals (Fig. 3G) instead of the full trajectory (0.1, Fig. 3F). The differences between experimental data and the C-14.6 trajectory are more evident along the sequence stretch between residues 60 and 70, with larger PS in the MD simulations.

3.3. Evolution of the complex conformation along simulations

The relative orientation of two atom sets depends on both, the positions of their mass centres (Fig. 2) and the angular orientation of their main axes. This is represented by three angles (explained in Methods and Fig. SI-1 in Supplementary information), which were calculated for both Pc (Fig. 4) and the small domain of C*f* (not shown), using the main axes of the large domain as reference. Noteworthy, pitch angles of Pc increase in both, C-21.6 and C-14.6,MD runs. Despite this, no direct contacts between site 2 of Pc and the small domain of C*f* were found along the trajectories — as might be expected for a side-on conformation [8,30,57]. Actually, although pitch angles were clearly higher (35 and 50° for C-21.6 and C-14.6, respectively) than the average calculated for the reported NMR structure (21.8±1.7°) at the end of the simulations, they were still lower than that (65.7±0.7°) measured for the Pc–C*f* complex from Nostoc cyanobacterium [8].

The distance between copper from Pc and iron from C*f* varies as long as the C-21.6 trajectory samples different conformations of the complex. After 4 ns without changes in this distance, a transition takes place wherein it first increases, reaching a maximum at 5.7 ns, and then it decreases below initial values. Then, it keeps steady along a 5 ns interval. Along with these changes, there is an increment of the yaw angle and a change in the roll angle (ca. 10°) that holds on the last part of the simulation. Such rearrangement of the complex can be summarised as a rigid-body motion of Pc. In fact, it does not affect atomic fluctuations (Fig. SI-3 in Supplementary information) of Pc backbone, except those of residues 91–96, which are influenced by the formation of a salt bridge between Arg93 from Pc and Glu165 from C*f*.

3.4. Motions of Pc and C*f*

As inferred from RMSD data, C*f* experiences domain motions. Actually, the small domain swings during MD simulations. In order to characterise these motions of C*f*, we have performed a principal component analysis of both free Cf and C-21.6 trajectories.

For free C*f*, the projection of the first eigenvector on the path resembles a cosine wave with a period two-fold the ratio between simulation time and the eigenvector index (see Fig. SI-5 in Supplementary information), as its high (0.60) cosine content [58] indicates. This value is much smaller in the following principal components, which show a Gaussian bell distribution (Fig. SI-6). According to the literature [58], high cosine contents indicate either a high degree of randomness in motion or that the trajectory is not long enough to sweep completely the corresponding principal component. However, the subspace overlaps between the first eight eigenvectors of the first half of the simulation,

Along with the whole eigenvector set of the second half is 0.62, indicates that the trajectory is long enough [59,60] to obtain an acceptable sampling of essential motions. Most principal components of the free C*f* trajectory show significant rigid-body rotations of the C*f* small domain — in the range of 10° (see Table SI-2 in Supplementary information). Fig. 5 represents the extreme coordinates of such motions for four eigenvectors and the corresponding hinge axis, as detected with Dyndom[53]. Remarkably, most of the loop between residues 154 and 171, which belongs to the C*f* large domain in most eigenvectors, behaves as part of the small domain in the third eigenvector, which shows the highest closure motion (94.6%). Hence, the electrostatic interaction between Arg93 from Pc and Glu165 in this loop of Cf could modulate the motions of the small domain.

Once Pc is bound to C*f*, domain motions within C*f* show substantial changes (see Tables SI-2 and SI-3 in Supplementary information). Hence, despite no direct contacts between site 2 of Pc and the small domain of C*f* are observed in Phormidium, we investigated whether the binding of Pc modulates the dynamics of this C*f* domain in our trajectories. Fig. 6 displays the positions of the mass centre of the C*f* small domain with respect to the large domain, in three MD calculations: free C*f*, C-21.6 trajectory and a third one corresponding to C*f* extracted from a snapshot of the C-21.6 computation. In the last case, C*f* was isolated from Pc, and again re-solvated and re-equilibrated before the production run. As inferred from Fig. 6, the presence of Pc modifies the position of the C*f* small domain, which tends to recover its initial coordinates when the copper protein is removed. This suggests that electrostatic interactions involving the small domain of C*f* and Pc are taking place within the complex, since there is no contact between them. Therefore, we performed covariance and principal component analysis on the full C-21.6 trajectory in order to characterise the most relevant motions in the complex. Fig. 7 shows the 3N×3N covariance matrix corresponding to Cα atoms of protein backbones. It is worth noting the negative covariance values between residues in Pc (residues 1 to 105) and the C*f* small domain (residues 276 to 338), as well as another region of C*f* placed near Pc binding site 1, from residues 85 to 112 (191 to 218), which is also rich in charged residues and displays a high mobility.

Principal component analysis on the C-21.6 full trajectory (Fig. 8) highlights two major states, in agreement with RMSD data. The first five eigenvectors account for 70% of the total amplitude. Similar to free C*f* trajectory, the highest cosine content (0.78)was found for the projection of the first eigenvector on the path (see Fig. SI-7 in Supplementary information). The following eigenvectors show much lower cosine contents. The subspace overlap of the first ten eigenvectors between consecutive 5 ns intervals (2–7, 7–12 ns) along the trajectory is 0.59. Moreover, if this analysis is performed on shorter consecutive intervals sampling one of the two major states instead of the full trajectory, the subspace overlaps are clearly better (ca. 0.65), indicating that calculations achieved a significant sampling [59,60] of motions (see Fig. SI-8 in Supplementary information).

The analysis of the first eigenvectors proves that concerted motions between Pc and the small domain are taking place within the complex. For instance, the first component shows, among others, a correlation between the swinging of the small domain with an increase in the pitch angle of Pc, a small rotation around its main axis, along with motions of the loop from Ala84 to Asn112 at the large domain of C*f* (Fig. 8). In addition, eigenvector 2 shows a correlation between movements of Pc and the C*f* sequence stretch from Lys185 to Ala191 located at a tip of the large domain of C*f* near the small domain.

3.5. Electrostatic calculations

In order to test whether electrostatics is responsible for the evolution of the complex conformation in the cyanobacterium Phormidium – for which controversial structural and functional data exist in the literature – we have performed continuous electrostatic calculations on the trajectories of the free partners as well as that of the C-21.6 complex. Although the data show some variability due to changes in conformation of the two partners along the trajectory, the average electrostatics plays a destabilising role on the binding process, as it has been observed with other protein complexes [56]. In fact, the global average electrostatic contribution to binding (ΔG_{ele}-bind) Pc to C*f* is 55.8±16.6 kJ mol^{-1}, as shown in Fig. 9. Remarkably, the Coulomb term (495.8±20.3 kJ mol^{-1}) is positive, favouring dissociation of the complex, whereas solvent polarisation effects (the corrected reaction field term; −428.7±18.9 kJ mol^{-1}) are stabilising (see Fig. SI-9 in Supplementary information). However, as shown in Fig. SI-9 in Supplementary information, Coulomb contribution substantially decreases during the second half of the simulation, indicating that charge–charge interactions are more favourable in the second ensemble of structures. As this effect is compensated for changes in reaction-field energies, a net increase in the binding energy is observed. Interestingly, our data concerning the contribution of ions in solution shows a small and negative value (−11.3±0.4 kJ mol^{-1}), in agreement with the mild ionic strength dependence of electron transfer rates between the two proteins at low salt concentrations [14].

A key point in the analysis of electrostatics [56] concerns whether or not binding involves a structural change within the two interacting partners. The thermodynamic cycle shown in Fig. 9 reveals that the $\Delta G_{ele\text{-}bind}$ can be split in two terms if the structure of any of the partners changes within the complex. The first term is the “strain energy” ($\Delta G_{ele\text{-}strain}$), which accounts for the cost of structural changes needed for docking; the second one is the “rigid binding” ($\Delta G_{ele\text{-}rigid}$) component that represents the binding energy of the strained partners. In the absence of any structural change, $\Delta G_{ele\text{-}strain}$ is negligible and, therefore, $\Delta G_{ele\text{-}rigid}$ becomes equal to the overall binding energy ($\Delta G_{ele\text{-}bind}$). We have previously shown that C*f* undergoes changes in its structure and dynamics upon binding to Pc, as well as both partners display a concerted motion. Hence, we investigated whether these modifications are correlated with changes in the average electrostatic contributions along the C-21.6 trajectory. Fig. 9 (lower panel) shows that the time intervals corresponding

to the two major ensembles of conformations (from0 to 3.7 ns and from 7 to 13 ns) are characterised by positive $\Delta G_{ele\text{-}bind}$ and large strain energy values ($\Delta G_{ele\text{-}strain}$). However, $\Delta G_{ele\text{-}bind}$ becomes negative, with a concomitant $\Delta G_{ele\text{-}strain}$ drop, in the time interval from 4 to 6 ns, corresponding to the transition between these two major populations. Most of this decrease in binding energy results from changes in the reaction-field term (see Fig. SI-9 in Supplementary information), that accounts for solvent polarisation phenomena.

4. Discussion

The aim of this work has been to study in depth the dynamic behaviour, at the nanosecond timescale, shown by two protein partners into a short-lived complex. As a model system, we have chosen a well-studied transient interaction, which takes place during the photosynthetic electron transfer reaction between C*f* and Pc, both from the cyanobacterium P. laminosum. One of the main reasons to select this organism is the ample controversial structural and functional data present in the literature, concerning the role of electrostatics in this interaction. Hence, MD simulations combined with continuum electrostatic calculations have been valuable tools to deal with this biological problem, highlighting the electrostatic nature of the concerted movements experienced by Pc and C*f*.

We have calculated two independent trajectories for the Pc–C*f* complex starting from two different set of coordinates: C-21.6 and C-14.6 conformations, with the lowest RSMD and the most perpendicular orientation, respectively. In the first (C-21.6), Pc displays a rigid-body swinging from the complex interface, thereby only experiencing small changes in its orientation relative to C*f*; in the second run (C-14.6), there is a substantial evolution of the complex. Actually, the initial C-21.6 structure clearly locates inside a well of the energy landscape, as inferred from both the tiny changes in the structure of the complex along the trajectory and the high overlap in the eigenvector space when comparing different time intervals. In fact, this also means that the calculated trajectory is long enough to obtain a representative sampling of the principal components [58,60,61]. Along its trajectory, C-21.6 conformer experiences a small transition to shift energy well. Therefore, the activation energy barrier between the sampled states may not be high, indicating that the energy basin contains several minima with common properties, instead of a single one. Principal components analysis shows correlations among swinging of the small domain of C*f*, changes of pitch and rotation of Pc around its main axis, and enhanced motions in flexible parts of the large domain of C*f*.

Our data, specifically those regarding the C-21.6 conformation, show a high agreement with the experimental ones previously reported. First, Fe–Cu distances are consistent with fast electron transfer. In addition, observed PS [29] match those derived from simulations (Fig. 3). The small differences can be attributed to the different redox state of C*f* in the simulations (Fe^{2+}) and the experimental assays (Fe^{3+}). As shown in Fig. 3, the fits between PS calculated from the two starting NMR structures along with the experimental ones are worse than those calculated from MD data. An explanation could be that the NMR structures were solved using only the axial components of the magnetic susceptibility tensors [29]. Finally, although the pitch angle increases along the C-21.6 trajectory, Pc mainly remains in "head-on" orientation with regard to the large domain of Cf. Actually, no direct contacts are observed between the small domain of C*f* and Pc, what distinguishes the NMR structure of this complex [29] from those [8,30,56] of other organisms.

Pc behaves as a rigid body, according to our analysis of its atomic fluctuations. Nevertheless, many residues placed at loops in the complex interface experience a substantial increase in their internal motions. This suggests that swinging motions of Pc within the complex force the interface to adapt continuously in order to keep favourable van der Waals interactions. The largest variation, however, corresponds to the rearrangement of the loop containing Arg93 to interact with Glu165 in the large domain of C*f*, that could explain the effects of Arg93Gln and Arg93Glu mutations in stopped-flow electron transfer kinetics [14] and on its ionic strength dependency. The shift of the guanidinium group of Arg93 from site 2 to the complex interface increases the electrostatic strain energy of Pc, but decreases the rigid binding contribution during the last half of the trajectory.

MD simulations reveal how both the domain motions of C*f* and the average position of its small domain are significantly affected by the presence of Pc. This represents the major structural change in C*f*, to which substantial electrostatic straining can be attributed. Therefore, although no direct contacts occur between Pc and the small domain of C*f*, continuum electrostatic calculations indicate that electrostatic interactions between them are responsible for straining the conformation of Cf. In addition, the negative covariance between motions of Pc and those of the small domain of C*f* is consistent with electrostatic computations showing that overall charge–charge interactions between the two partners are repulsive. Actually, Pc shows an asymmetric charge distribution with its positive pole docking amidst negative charges on C*f* surface. Hence, repulsion between the two partners may come from the negative part of Pc, which lies far from the interface. On one hand, attractive forces make Pc lean towards the small domain of C*f*, increasing its pitch angle and approaching its site 2 to the small domain of Cf. This involves the interaction of the positive charge of the oxidised copper site and Arg93 with the loop containing residues 154–173 from C*f*, whose motions are coupled, in turn, with those of the small domain. On the other hand, repulsive forces make both proteins swing in an opposite and disordered manner.

Despite the large amount of data concerning the mechanism of biological electron transfer [2,3,5] and the binding process, specifically, the phenomena triggering partners' dissociation once the electron transfer has taken place remain unveiled. Interestingly, the above scenario explains the role of electrostatics not only in determining binding

equilibrium but also in straining the structure of the partners and affecting their dynamics, as it is the case of the small domain of C*f* in the presence of Pc.

To conclude, MD reveals how the link between the two lumenal domains of free C*f* is highly flexible and how the small domain of C*f* is significantly affected upon binding of Pc to the large domain of C*f*. The swinging motions of the C*f* small domain and Pc are concerted within the transient Pc–C*f* complex in such a way that Pc tilts towards the small domain of C*f*, which is conversely displaced by the copper protein. Even though there is not a direct contact between the small domain of Cf and Pc within the transient complex, the long-range electrostatics between them may indeed play a key role in breaking the complex off by straining the structure.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version.

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