Brownian Dynamics Study of the Interaction between Plastocyanin and Cytochrome *f*

Douglas C. Pearson, Jr. and Elizabeth L. Gross
Department of Biochemistry and Biophysics Program, The Ohio State University, Columbus, Ohio 43210 USA

ABSTRACT The electrostatic interaction between plastocyanin (PC) and cytochrome *f* (cyt f), electron transfer partners in photosynthesis was studied using Brownian dynamics (BD) simulations. By using the software package MacroDox, which implements the BD algorithm of Northrup et al. (Northrup, S. H., J. O. Boles, and J. C. L. Reynolds. 1987. *J. Phys. Chem.* 91:5991–5998), we have modeled the interaction of the two proteins based on crystal structures of poplar PC and turnip cyt f at pH 7 and a variety of ionic strengths. We find that the electrostatic attraction between positively charged residues (K58, K65, K187, and R209, among others) on cyt f and negatively charged residues (E43, D44, E59, and E60, among others) on PC steers PC into a single dominant orientation with respect to cyt f, and furthermore, that the single dominant orientation that we observe is one that we had predicted in our previous work (Pearson, D. C., E. L. Gross, and E. S. David. 1996. *Biophys. J.* 71:64–76). This dominant orientation permits the formation of hydrophobic interactions, which are not implemented in the MacroDox algorithm. This proposed complex between PC and cyt f implicates H87, a copper ligand on PC, as the residue that accepts electrons from the heme on cyt f (and possibly through Y1 as we proposed previously). We argue for the existence of this single dominant complex on the basis of observations that the most favorable orientations of the interaction between PC and cyt f, as determined by grouping successful BD trajectories on the basis of closest contacts of charged residues, tend to overlap one another and have very close distances between the metal centers on the two proteins (copper on PC, iron on cyt f). We use this knowledge to develop a model for PC/cyt f interaction that places a reaction between the two proteins occurring when the copper-to-iron distance is between 16 and 17 Å. This reaction distance gives a good estimate of the experimentally observed rate constant for PC-cyt f interaction. Analysis of BD results as a function of ionic strength predicts an interaction that happens less frequently and becomes less specific as ionic strength increases.

INTRODUCTION

Plastocyanin (PC) and cytochrome *f* (cyt f) are reaction partners in photosynthetic electron transport. PC is a mobile electron carrier found in the lumen of the thylakoid in chloroplasts; it obtains an electron from cyt f, a member of the membrane-bound cytochrome b_6f complex, and transfers the electron to the P700 reaction center of the photosystem I complex (Gross, 1996).

PC (Fig. 1 *A*) is a "blue" copper protein of ~10 kDa, closely related to other copper proteins such as azurin and stellacyanin (Freeman, 1981). At this time, structure determinations exist for at least six different plastocyanins, including poplar PC in oxidized (Guss and Freeman, 1983) and reduced (Guss et al., 1986) forms. The copper center of PC is coordinated to four ligands—two histidine residues (H37, H87), a cysteine (C84), and a methionine (M92)—in a distorted tetrahedral geometry, and the overall structure of the protein is that of a Greek-key β-barrel (Gross, 1996). (For other reviews on PC, see Redinbo et al., 1994; Gross, 1993; Sykes, 1991.)

Electrostatic analysis of PC (Durell et al., 1990) reveals a large region of negative potential generated primarily by two patches of acidic residues (42–44 and 59–61). This patch surrounds one potential binding site for reaction partners, at tyrosine 83, as implicated by nuclear magnetic resonance studies (Cookson et al., 1980; Handford et al., 1980). Electron transfer at this site would involve a through-bond pathway to C84, which is a ligand to the copper. These NMR studies also implicate H87, which is surrounded by several hydrophobic residues and a region of positive potential (generated primarily by the positive charge on the copper atom), as a second possible reaction site. H87 is a ligand to the copper atom, so electron transfer at this site would be more direct. The H87 site has been dubbed "site 1" and the Y83 site "site 2" (Gross, 1996).

Cyt f (Fig. 1 *B*) is a membrane-bound protein of ~32 kD. A single α-helix spans the thylakoid membrane and keeps the protein anchored; however, the majority of the protein is exposed to the lumen. The crystal structure of the lumenal domain of turnip cyt f (Martinez et al., 1995) was the first to be determined; algal (Berry et al., 1997) and cyanobacterial (Carrell et al., 1997) structures have more recently been solved. Cyt f has two β-sheet folding domains; in turnip cyt f, there is a small domain of 63 residues and a large heme-binding domain of 189 residues. The unique ligation of the heme to the N-terminal tyrosine residue has been widely recognized (Martinez et al., 1995; Prince and George, 1995).

Electrostatic analysis of this cyt f structure (Pearson et al., 1996) reveals a large region of positive potential generated by the iron center and a large patch of positively charged residues, including lysines 58, 65, 66, and 187, and arginine

Received for publication 28 May 1998 and in final form 1 September 1998.
Address reprint requests to Dr. Elizabeth L. Gross, Department of Biochemistry, The Ohio State University, 484 West 12th Avenue, Columbus, OH 43210. Tel.: 614-292-948; Fax: 614-292-6773; E-mail: gross.3@osu.edu.

0006-3495/98/12/2698/14 $2.00

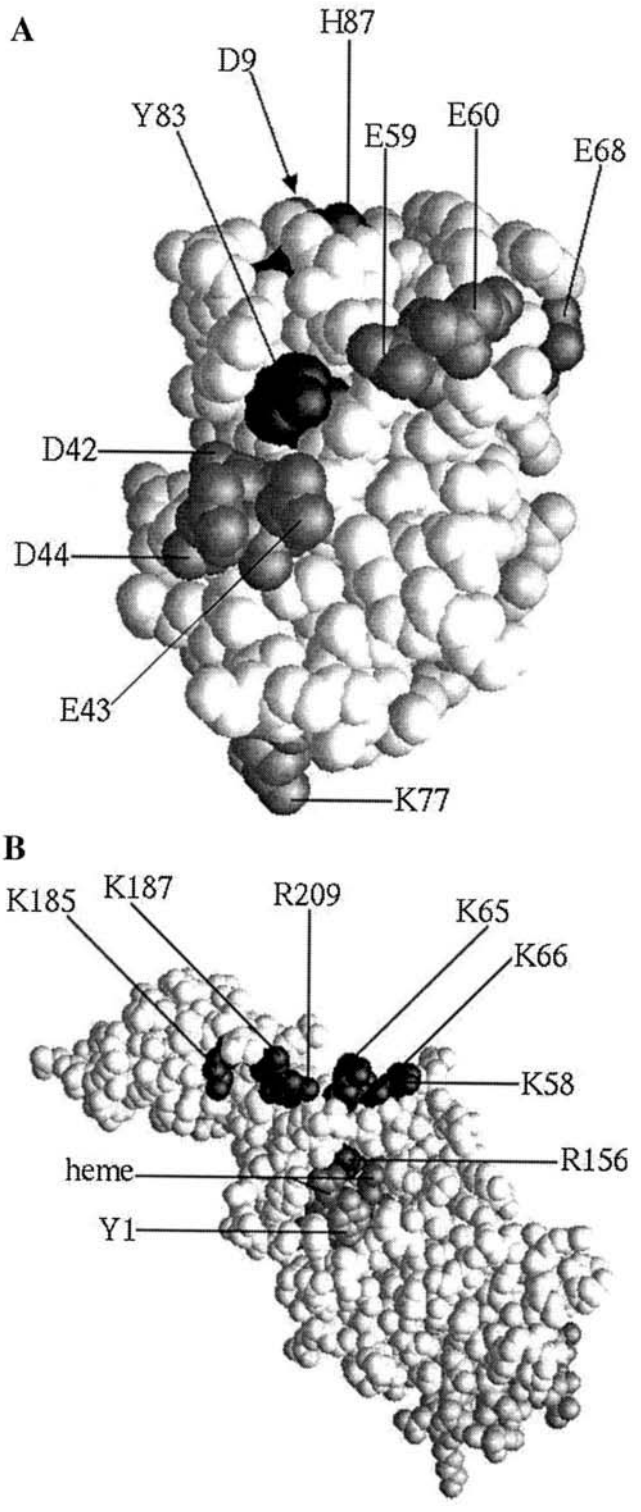

FIGURE 1 Molecular graphics representations of poplar plastocyanin (*A*; Guss et al., 1992) and turnip cytochrome *f* (*B*; Martinez et al., 1995) as prepared by GRASP (Nicholls et al., 1991) for Silicon Graphics IRIS workstations. Key residues of interest in this work are labeled.

209. The consensus on the interaction between PC and cyt f is that there is an electrostatic attraction between this positive patch and the aforementioned negative patch on PC (Frazao et al., 1995; Gross, 1996; Kannt et al., 1996; Ubbink et al., 1998). Experimentally, this idea has been confirmed in part by the work of Morand et al. (1989) in which covalent cross-links were observed between aspartate 44 on PC and lysine 187 on cyt f, and between residues 59 and 60 on PC and unknown positive charges on cyt f. In our previous work (Pearson and Gross, 1995; Pearson et al., 1996) we have proposed an electrostatic complex between PC and cyt f that reflects the results of Morand's cross-linking experiments. We proposed that electron transfer in this complex was unlikely because the metal centers were separated by >30 Å. The region around the heme itself is one overall of positive potential, especially at low ionic strengths, with a small area of negative potential at the exposed propionic acid side chains of the heme becoming apparent at higher ionic strengths. However, there are several hydrophobic residues in the area of the heme, especially on regions of the protein away from the positive patch. The apparent hydrophobic complementarity of the sites surrounding H87 on PC and Y1 and the heme on cyt f, in addition to the geometry of the molecules as observed in manual docking analysis, have led to a proposed second complex in which H87 on PC faces Y1 on cyt f in an electron-transfer-active orientation (Pearson et al., 1996). In this proposal, Y83 is all but eliminated as a possible electron transfer site for docking with cyt f. This proposal does appear to conflict with experiments conducted by He and co-workers (1991) that assert the importance of Y83 in electron transfer between PC and cyt f, as well as the work of Zhou et al. (1996) questioning the importance of Y1 on cyt f in electron transfer between the two proteins. Given that our previous work emphasizes manual methods of determining prospective complexes between the two proteins that can be influenced by human bias, as unbiased a means as possible to determine interaction sites and model the means of interaction between the two proteins was sought. The model of Northrup and co-workers (1988; also Northrup, 1996), who studied the interaction of cytochrome *c* (cyt c) and cytochrome *c* peroxidase (cyt c per), is especially relevant. Cyt c and cyt c per are electron transfer proteins that are attracted by electrostatic forces. By using an algorithm that placed cyt c and cyt c per a set distance apart and simulated the motion of cyt c toward cyt c per based on electrostatic interactions and a random factor designed to simulate Brownian motion, Northrup was able to model association trajectories between the two molecules and, with a sufficient sample size, reach some conclusions about the nature of docked complexes between the two molecules. This computational method is called Brownian dynamics (BD).

In the present study we apply Northrup's BD algorithm (Northrup et al., 1987) to the poplar PC/turnip cyt f interaction problem. This application has its strengths and weaknesses, the most obvious strength being the ability to predict complex formation between PC and cyt f in a less biased manner that considers the interaction of the molecules with their solvent. This algorithm uses electrostatic interactions

to determine the influences driving the attraction between the two proteins; this is quite reasonable for determining a trajectory from large separation differences where only monopoly or dipole attractions are important. However, the algorithm does not consider hydrophobic interactions, which are important in many protein-protein interactions (Tai et al., 1997; Vakser and Aflalo, 1994; Wells, 1996; Young et al., 1994) and have been proposed to play an important role in the final orientation of PC to an electron-transfer active position (Pearson et al., 1996). Observations from the present study, however, may demonstrate a need to revise this hypothesis as it applies specifically to PC and cyt f. In addition, this algorithm considers proteins only as rigid bodies. We know that proteins do not exist in single, rigid conformations, but in a collection of multiple conformational states, possibly with different functional roles (Frauenfelder et al., 1988), and that the reorganization of the nuclear coordinates within a protein is important in allowing electron transfer to go forward (Marcus and Sutin, 1985; Tollin et al., 1986; Moser et al., 1995). Therefore, these experiments only study the one conformation that is observed in the crystal structures of both PC and cyt f. If any other conformational states are relevant for these proteins' function, those states will not be considered. We plan to address both of these issues in future work.

Ullmann et al. (1997) have used a Monte Carlo sampling method to predict the formation of complexes between PC and cyt f, gaining results that agree with our previous work. Their approach has the advantage (as compared to this study) that molecular flexibility has been considered. However, the Monte Carlo method does not model macromolecular diffusion as the BD model does, and hence cannot be used to predict rate constants for the interaction between the two proteins. Moreover, the rigid-body model provides for calculations that are less computationally intensive, which leads to less use of computational resources. We argue that whatever benefits that consideration of molecular flexibility might provide, for purposes of this study, are not worth the computational time those benefits would require.

Most recently, as this work neared submission, NMR experiments from Ubbink et al. (1998) provided a partial structure determination for the PC/cyt f complex. We believe that this clearly important information does not diminish from the importance of this purely computational study; the fact that a less biased approach to finding potential complexes between these two proteins gives results similar to experimental work like Ubbink's provides evidence of the usefulness of the technique, which can be applied to other protein-protein interaction systems.

METHODS

Atomic coordinates

The atomic coordinates of oxidized poplar PC (Guss et al., 1992), *Anabaena* PC (Badsberg et al., 1996), *Alcaligenes denitrificans* azurin (Baker, 1988) and reduced cytochrome *f* (Martinez et al., 1996) were obtained from the Protein Data Bank at Brookhaven National Laboratory (Bernstein et al., 1977).

MacroDox and the Brownian dynamics algorithm

We used the software package MacroDox (Northrup, 1995) for our BD analysis. The details of the algorithms used in this package are given in Northrup et al. (1987).

For each protein studied in a MacroDox simulation, a modified Tanford/Kirkwood pK calculation (Matthew, 1985; Matthew and Gurd, 1986) is performed and the charges on each protein are set based on that calculation. At pH 7.0, the percent dissociation of the majority of the charges on both PC and cyt f will not be affected by the method used for the calculation since pH 7.0 is far away from the pK_a values and, thus, the modified Tanford-Kirkwood method is sufficient for these studies. Both of the histidines on PC and one of the two histidines on cyt f are uncharged because they are ligands to the metal center. The second histidine on cyt f is on the opposite side of the molecule from the putative binding site; thus, it will have a negligible effect on PC binding. Note that there is a charge of -1 on the sulfur atom of C84 (Durell et al., 1990).

A Poisson-Boltzmann calculation (Warwicker and Watson, 1982) is then performed to determine the electrostatic potential grid based on those assigned charges. The Poisson-Boltzmann electrostatic potential grid is the means by which the force in the BD algorithm is calculated. The Warwicker-Watson (1982) algorithm for solving the linearized Poisson-Boltzmann equation is used by MacroDox, which differs somewhat from the finite-difference algorithm of Nicholls and Honig (1991) utilized in GRASP and DelPhi that we have used in the past.

The general equation for the motion of a particle subject to Brownian motion in addition to an external (i.e., electrostatic) force that is used in BD algorithms is the Ermak-McCammon equation:

$$\mathbf{r} = \mathbf{r}_0 + \beta D\, \mathbf{F}(\mathbf{r}_0)\, \Delta t + \mathbf{R} \tag{1}$$

where $\mathbf{r}$ and $\mathbf{r}_0$ refer to the position of the particle (relative to some reference particle, in this case a receptor protein) after and before, respectively, the time step Δt, $\beta = (k_B T)^{-1}$, $\mathbf{F}(\mathbf{r}_0)$ is the external force on the molecule at r_0, D is the diffusion coefficient of the particle in question (in this case again, a relative quantity for the mutual diffusion of a ligand protein and a receptor protein), and $\mathbf{R}$ is a random (Brownian) vector with the following properties:

$$\begin{aligned} \langle \mathbf{R} \rangle &= 0 \\ \langle \mathbf{R}^2 \rangle &= 2\, D\, \Delta t \end{aligned} \tag{2}$$

It should be noted that the time difference Δt should be small enough so that there is minimal change in $\mathbf{F}$ in that time; that is, $\mathbf{F}(\mathbf{r}) \sim \mathbf{F}(\mathbf{r}_0)$. (McCammon and Harvey, 1987).

MacroDox implements this algorithm for each individual trajectory of a simulation, with $\mathbf{F}(\mathbf{r})$ being calculated based on the Poisson-Boltzmann potential map. The mobile ligand (PC) starts each trajectory a set distance b (89 Å in this study) away from the fixed receptor (cyt f); Fig. 2 gives a picture of this starting point. The value of 89 Å was chosen because this is farthest distance at which electrostatic interactions are important.

In our implementation of the BD algorithm for this work, MacroDox declares a trajectory successful if the two monitor atoms within the proteins (usually the copper center in PC and the heme iron in cyt f) come within x Å of one another (x, the value we refer to as the "reaction distance," was set to various values depending on the goals of the simulation) at any point before the mobile ligand passing outside a predefined outer escape radius c (200 Å from the fixed receptor). For each successful trajectory, the position of PC's closest approach to cyt f (as defined by the distance between monitor atoms) is recorded. Various values of b and c were also attempted (results not shown), and were found to return similar results to those given below.

After a preset number of trajectories (usually 1000 in this study) the run is concluded and the number of successful trajectories is used to determine

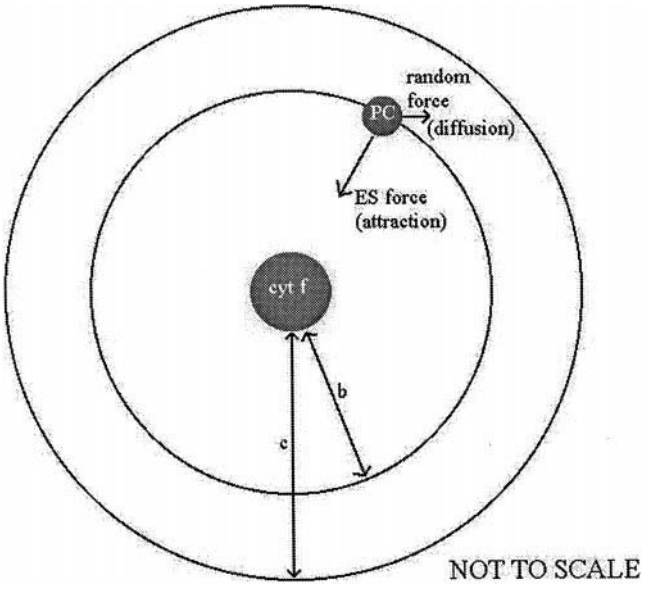

FIGURE 2 A representation of the initial conditions of the BD trajectory. The mobile ligand (PC, in this case) is centered to begin its trajectory on an imaginary sphere with radius b Å (89 Å in this work) with the fixed receptor (cyt f) at its center. The mobile ligand is subject to two forces: the electrostatic attraction between the two proteins and the random Brownian force. The closest approach of the mobile ligand to the fixed receptor is recorded, and the trajectory is concluded when the mobile ligand escapes a sphere of c Å (200 Å here).

a rate constant through Northrup's equations (1987), based on the theory of Smoluchowski (1916):

$$k = pk_{\mathrm{D}}(b)/[1 - (1 - p)\, k_{\mathrm{D}}(b)/k_{\mathrm{D}}(c)] \quad (3)$$

$$k_{\mathrm{D}}(s) = 4\pi sDN_{\mathrm{A}} \quad (4)$$

Here, p represents the number of successful trajectories as a fraction of total trajectories attempted and N_{A} represents Avogadro's number.

We have modified FORTRAN code from Northrup to sort successful trajectories by the three closest pairs of *charged* residues ("triplet contacts"). We use this program to complement the determination of p and k directly by MacroDox, as a means of demonstrating where PC is most likely to approach cyt f.

In this work we have taken multiple runs of MacroDox for each set of experimental criteria (here, varying ionic strengths; usually four runs, but nine runs in the case of the "standard" runs at 100 mM and the control runs at 10 M) using randomly generated random number seeds, and averaged results over those runs for "more reliable" predictions of the reaction fraction p and the rate constant k. As such, when standard errors are reported, they do not reflect the standard errors that MacroDox calculates for a single run, but rather errors we have calculated over a number of runs. Likewise, these errors do not necessarily reflect the real range of error in the prediction, but merely the theoretical deviation of the measurement.

A note must be made about the range of ionic strengths studied. As ionic strength was decreased, the number of successful complexes increased; the lifetime of the complexes in a single trajectory also increased, to the point where PC did not tend to leave the truncation radius. A run of 1000 trajectories at 50 mM ionic strength took ~6 h of CPU time, as opposed to 1 h for the standard 100 mM ionic strength run. Lower ionic strength runs, on the computational hardware we had access to, were impractical due to the extended use of CPU time.

Molecular representations

All representations of PC and cyt f in this work are prepared with the program GRASP (Nicholls et al., 1991), with the exception of the α-carbon traces presented in Figs. 3 and 6, which were generated with the program Quanta (1997).

RESULTS AND DISCUSSION

Standard runs

Two types of standard runs were established to differentiate between PC/cyt f complexes formed resulting from electrostatics and those from diffusion alone. The difference between these two runs was the pair of monitor atoms chosen: the copper atom on PC and the iron atom on cyt f, and the two atoms on each molecule closest to the center of mass (the γ2 carbon of I39 on PC, and the β carbon of N153 on cyt f). The former pair of monitor atoms was chosen for their biological importance; the distance between these two atoms must be minimized for electron transfer to occur, and therefore complexes that minimize the distance between these two atoms will be of the greatest biological importance. However, designing an experiment that will select complexes minimizing the distance at a proposed reaction site does not really tell whether the two molecules preferentially form complexes at that reaction site, which makes the experiment less powerful. Therefore, we select the most neutral monitor atoms possible on these two molecules, the two atoms closest to the center of mass on each molecule, purely for their position on the molecule and not for their biological importance. (Even this does not make the experiment totally unbiased; because cyt f is irregularly shaped, monitoring the distance between centers of mass will continue to favor complexes toward the center of the cyt f molecule. The main benefit comes from the change in

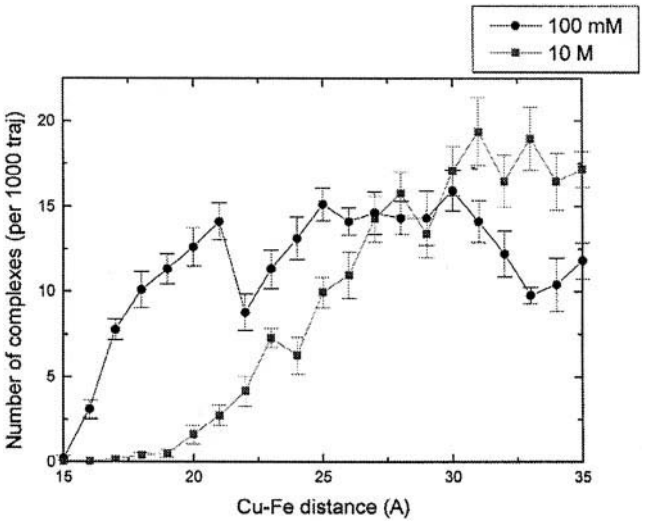

FIGURE 3 The distribution of distances between metal centers for all complexes with metal center distances of <35 Å for runs saving complexes based on minimized Cu-Fe distances at both 100 mM ionic strength and 10 M ionic strength.

monitor atom on PC, where complexes will no longer strongly favor the H87 face of PC by moving the monitor atom from the copper center to the center of mass.)

We realize that such general means of identifying complexes between these two molecules will generate many complexes that cannot be electron-transfer active, yielding an overestimate of the probability of interaction. At the same time, however, we assure that no potentially electron-transfer-active complexes are excluded, so that all possibilities can be analyzed.

Fig. 3 demonstrates graphically, through the distribution of complexes as a function of metal center distances, the results of nine MacroDox runs, of 1000 trajectories each, analyzing the interaction between poplar PC and turnip cyt f where complexes minimizing the distance between metal centers of the two molecules were obtained at 100 mM ionic strength (conditions under which electrostatic interactions are dominant) and 10 M ionic strength (representing a nonphysiological "random" interaction). (All distances were rounded up to the nearest whole number for this plot; for example, the number of complexes recorded at 20 Å represents all complexes with a metal center distance between 19 and 20 Å.) Through 21 Å, there is a clear tendency for a greater number of complexes because of electrostatic interactions through diffusion alone. The average probability that complexes will form with a metal center distance of <21 Å is 0.059 ∓ 0.005 when electrostatic interactions are present (at an ionic strength of 100 mM), when electrostatic interactions are not included (by increasing salt concentration to 10 M), that average probability is 0.005 ∓ 0.002. More strikingly, the average probability that complexes will form with a metal center distance of <17 Å is 0.011 ∓ 0.001 when electrostatic interactions are included, and 0.0001 ∓ 0.0001 when electrostatic interactions are eliminated.

This leads to the proposal that electrostatic interactions favor docks between PC and cyt f where the distance between metal centers is minimized. However, making this assertion based on a BD algorithm that searches for complexes with minimized metal centers is dubious. For this reason, a similar experiment was performed where the complex saved was not that with minimized metal center distances, but with minimized distance between atoms closest to the center of mass on the two proteins. The distribution of complexes from this run, *still as a function of metal center distance* despite the fact that complexes are no longer searched for based on that criteria, is given as Fig. 4. While the difference between electrostatics-aided complex formation and complex formation by diffusion alone is no longer as dramatic, the difference is still distinct. The probability of complexes with a Fe-Cu distance of <21 Å forming when analyzed by this method is 0.025 ∓ 0.004 when electrostatic interactions are included, as opposed to 0.005 ∓ 0.002 without electrostatic interactions; when this distance is decreased to 17 Å, the probabilities become 0.005 ∓ 0.001 with electrostatic interactions, 0.0003 ∓ 0.0003 without. Even when the bias of searching for complexes between PC and cyt f on the basis of distance between metal centers is eliminated, PC's preference to form closer complexes with cyt f through electrostatic interactions remains, especially for metal center distances of <20 Å.

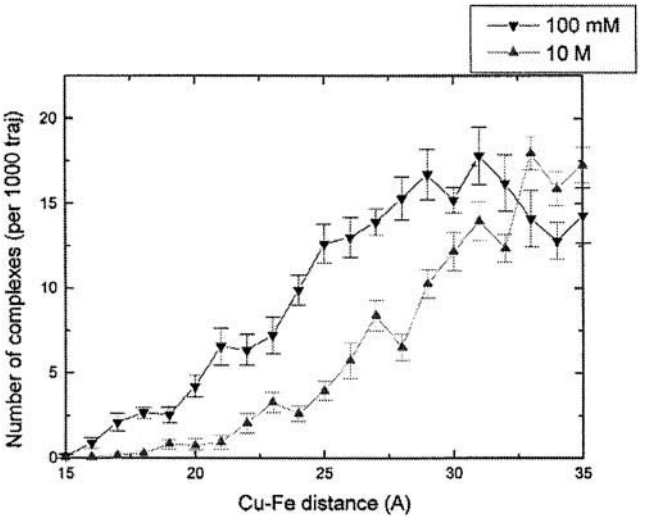

FIGURE 4 The distribution of distances between metal centers for all complexes with metal center distances of <35 Å for runs saving complexes based on minimized center of mass distances at both 100 mM ionic strength and 10 M ionic strength.

Many complexes are represented in these distributions, however, most of which are of orientations unrealistic for electron transfer. We now analyzed these complexes to determine whether there is a preferred binding site for electron transfer for PC on cyt f and whether there is also a preferred orientation for PC in docked complexes. To do this, we determined the three closest *charged* residue pairs for each successful complex and classified complexes on the basis of these "triplet contacts." Triplet contact analysis of the complexes presented in the distribution in Fig. 3 yielded the seven most frequently occurring triplets listed in Table 1. The idea that PC is steered by electrostatic interactions into complexes where the distance between metal centers is minimized is further supported by the average Fe-Cu distances for the most frequently occurring triplets: the top six each have average metal center distances of <20 Å. (As before, a similar table—not shown—was generated for the BD runs where the reaction distance between centers of mass determined when a complex was saved to ensure that the bias of searching for complexes based on minimized metal center distance did not significantly affect our evidence for the assertion that the formation of close complexes between PC and cyt f is electrostatically favored. As before, while the results are nowhere near as dramatic, there is a distinct tendency for the most popular triplets to be the ones with smallest Fe-Cu distances, usually <24 Å.) It is important to recognize that these complexes are formed solely by electrostatic interactions between the two molecules and do not reflect the effects that hydrophobic interactions, van der Waals interactions between the proteins,

TABLE 1 Best seven triplet contacts between cyt f and PC at 100 mM ionic strength

Triplets (cyt f res-PC res)			Number of Successes	*P* (Successes/ 9000)	Average Fe-Cu Distance (Å)
K65-E60	K187-E43	R209-E59	39	0.0043	18.0
K65-E60	K187-E43	heme-E59	24	0.0027	17.2
K65-E43	K187-D44	heme-E59	16	0.0018	18.2
K187-D44	R209-E43	heme-E59	12	0.0013	18.4
K65-E60	K185-D44	K187-E43	11	0.0012	19.1
R156-E59	K187-E43	heme-E60	11	0.0012	18.7
K58-D61	K65-E60	K122-K68	10	0.0011	24.5

etc., might have on a given complex. Nevertheless, assuming that hydrophobic interactions would further stabilize an electrostatically formed electron-transfer-active complex, we observe that electrostatic interactions alone tend to steer PC into a series of potentially electron-transfer-active complexes with cyt f.

It should be noted that these triplets provide confirmation of the cross-linking results of Morand et al. (1989); K187 on cyt f most frequently pairs with E43 or D44 on PC, while K58 and K60 most frequently pair with E60 or D61 (or, less frequently, E59). The Brownian dynamics results agree with our previous results based on electrostatic analyses (Pearson et al., 1996) as well as other work using chemical modification and mutagenesis techniques (Anderson et al., 1987; Lee et al., 1995; Kannt et al., 1996) asserting the importance of the basic patch on cyt f and the acidic patch on PC in the interaction between the two proteins.

To get a better picture of how these "triplet contacts" relate to differences in the orientation of PC with respect to cyt f in the docked complexes, we took 12 complexes with the four most frequently occurring triplets from a characteristic MacroDox run of 1000 trajectories and superimposed these complexes on one another graphically, aligning exactly the cyt f structures and comparing the orientation of the PC structures with respect to cyt f. This picture is given in Fig. 5. Immediately striking about this picture is the similarity of *all* of these complexes; in effect, they appear to represent a single consensus orientation of PC when complexed with cyt f. Observations from other runs and for other triplet contacts within this same run show that this single orientation is as dominant as it appears to be here. The implication of this observation is that the electrostatic interactions between PC and cyt f steer PC toward a *single* dominant orientation: one that is electron-transfer competent.

An observation that we did not expect is the similarity between this characteristic complex between PC and cyt f and our proposed manually docked "complex 2" (Pearson et al., 1996). We had originally proposed a distinctly electrostatic complex that was *not* electron-transfer competent as a pre-docking complex, followed by a reorganization into an electron-transfer-competent complex that was driven by hydrophobic interactions; we had also proposed that the electron-transfer-competent complex could not form by electrostatic interactions alone. We believe that the results presented here give significant evidence to refute that assertion (although clearly insufficient to state that electrostatic interactions are the *only* force drawing PC into an electron-transfer-competent complex with cyt f).

We observe, therefore, that the complexes that result most frequently from BD trajectories are among the best possible complexes for electron transfer and tend to have the lowest possible distance between metal centers; furthermore, electrostatic interactions tend to steer PC toward a *single dominant orientation* with respect to cyt f. Based on this, we can now argue that we can decrease the reaction distance and thereby tighten the conditions under which we

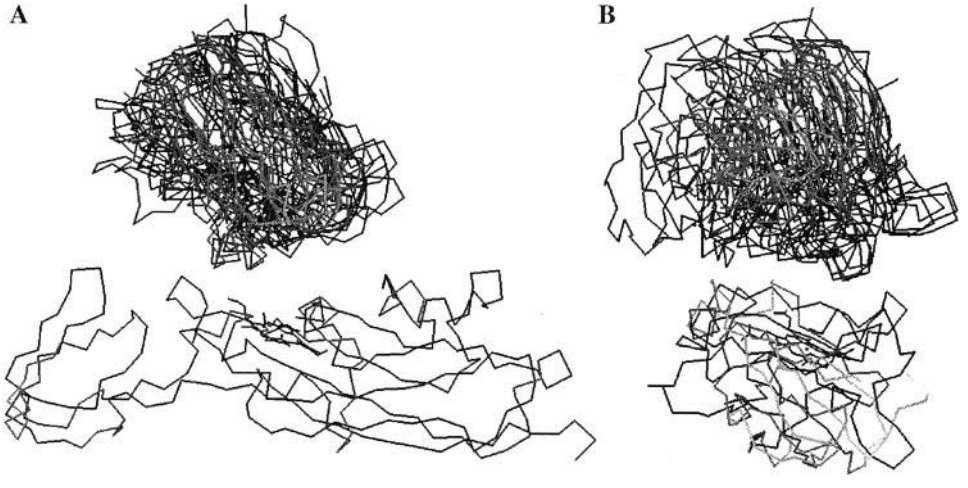

FIGURE 5 α-Carbon traces of 12 complexes between poplar PC and turnip cyt f generated by MacroDox for a single run of 1000 trajectories at pH 7 and 100 mM ionic strength, and selected randomly from the four best contact triplets given in Table 1. The complexes are superimposed on one another so that the cytochrome *f* coordinates overlay exactly to show the difference in positioning of PC in these 12 different approaches. *B* is a 90° rotation of *A*. The figure was prepared using the software package Quanta (MSI, 1996).

declare a trajectory "successful," and at the same time obtain the majority of these most frequently occurring complexes as classified by triplet contacts. This is discussed further below.

Further clarity with regard to the nature of the type of complex MacroDox generates most often comes from taking a sample complex between PC and cyt f (Fig. 6, Table 2) taken from the α-carbon trace and making observations similar to those we described when we created manually docked complexes in our previous work (Pearson et al., 1996). Residues within 6 Å of one another were determined to be in contact in that work, and those contacts were listed and compared. Table 2 lists these 6 Å closest contacts for this sample complex, and the complexes from Pearson et al. (1996) in which they appear. There are three important features to this sample complex: one, the close approach of H87 to the Y1/heme region on cyt f, further implicating H87 (and not Y83) as the more important residue for electron transfer; two, the electrostatic interaction between the first negative patch (E43, D44) on PC with its corresponding

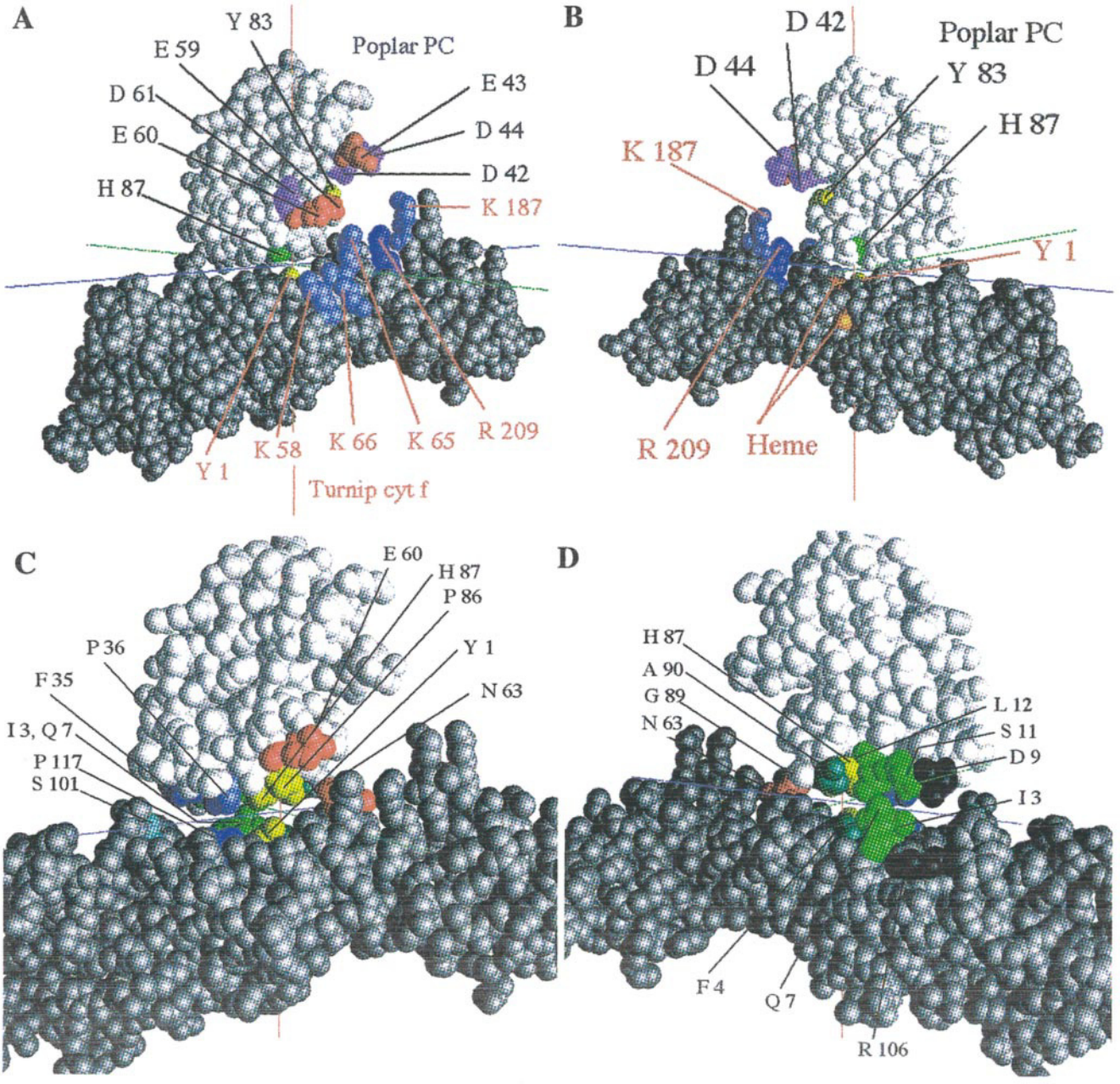

FIGURE 6 A characteristic complex taken from those in Fig. 5 shown in more detail to highlight some of the proposed intermolecular contacts between the two proteins. *A* and *B* give two rotations of a wide view of the complex, with electrostatic contacts highlighted (black for PC and red for cyt f). *C* and *D* give a closer view of the complex with nearest neighbors highlighted. Note the close proximity of H87 on PC to Y1 (and by connection, the heme) of cyt f.

TABLE 2 Dock table for example complex between PC and cyt f; prepared as in Pearson et al. (1996)

cyt f	PC	Presence of cyt f Residue in Complexes from Pearson et al. (1996)
Y1	P86, H87	Complex 2, Complex 3
I3	G10, L12	Complex 3
F4	L12, A90	Complex 2, Complex 3
Q7	G10, S11, L12	Complex 2, Complex 3
N63	E60	Complex 1, Complex 2
S101	F35	Complex 3
P117	G34, F35, P36	Complex 3

positive patch (K185, K187, R209) on cyt f, and between the second negative patch (E59, E60, D61) and its corresponding positive patch (K58, K65, R156) on cyt f; three, the alignment of hydrophobic residues (I3 and F4 on cyt f with L12 and A90 on PC; P117 on cyt f with F35 and P36 on PC) and possible hydrogen-bond partners (Q7 on cyt f with S11 on PC; N63 on cyt f with E60 on PC) surrounding the putative electron transfer sites on the respective molecules. In particular, the involvement of D44 on PC with K187 on cyt f and glutamines 59 and 60 on PC with positive residues on cyt f agree with experimental results of Morand et al. (1989), and the close proximity of L12 with a possible hydrogen-bonding partner in Q7 suggests agreement with the results of Modi et al. (1992). While not as close (as defined by distance between metal centers) as any of the manual docks in the previous paper (Pearson et al., 1996), this complex is strikingly similar to complex 2 as described in that paper, up to and including key matched residues between PC and cyt f that are supported by experimental evidence. Other closer complexes between PC and cyt f as generated by MacroDox are quite similar to this one, including similar pairings in their dock tables.

Please note that the complex shown in Fig. 6 represents the closest approach of PC to cyt f, as determined by distance between metal centers, for that particular trajectory. No attempt was made to "optimize" the dock—any possible hydrophobic or van der Waals interactions between the two proteins, outside of the simple criteria that the two proteins cannot enter each other's space, were not considered—so that in a "more real" docking simulation, some residues on the two molecules may approach each other even more closely.

In summary, we make the following arguments based on the modeling of the interaction between PC and cyt f at 100 mM ionic strength at pH 7: those complexes between PC and cyt f that are most likely to form are also most likely to have the lowest possible distance between metal centers, and are also therefore the best possible complexes for electron transfer. Electrostatic interactions will draw PC into a position where H87 on PC faces the Y1/heme region on cyt f, with hydrophobic interactions serving to "fine-tune" the interaction into the best possible position for electron transfer.

Defining the most reasonable reaction distance

As we argued above, the fact that BD simulations imply a preferred orientation for PC that minimizes the distance between metal centers allows us to develop a simple model for determining the type of interaction between PC and cyt f that is necessary for electron transfer. We do this, within the context of our desire for as simple a model as possible, by limiting the number of complexes MacroDox outputs by admitting only those complexes with a given maximum distance between metal centers. It is interesting, then, to see which reaction distances give the best agreement with the experimentally observed rate constant of $\sim 10^8$ M^{-1} s^{-1} (Gross et al., 1990; Qin and Kostic, 1992; Kannt et al., 1996). We assume, for this model, that the reaction between PC and cyt f is diffusion-limited and that a complex, when formed, does not dissociate before an electron transfer reaction occurs; in other words, for the reaction

$$PC_{ox} + cyt\,f_{red} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [PC_{ox}cyt\,f_{red}] \overset{k_{et}}{\longrightarrow} PC_{red} + cyt\,f_{ox} \qquad (5)$$

we make the assumptions that $k_{et} \gg k_1$ and $k_{et} \gg k_{-1}$. This assumption will provide meaningful results for this work, even if not strictly true, if we accept the more likely assertion that changes in ionic strength have far more effect on association and dissociation than they do on the intracomplex interactions (nuclear motions, etc.) that dictate k_{et} once the complex is formed.

Table 3 lists several possible reaction distances and the rate constants that those distances return. Reaction distances of 17 Å and 16 Å give rate constants on the order of 10^8 M^{-1} s^{-1}; it is also interesting to note that those are the smallest two (integer) reaction distances returning non-zero success probabilities P (and, therefore, non-zero rate constants). We are proposing in this model, therefore, that only the PC molecules that diffuse so that their copper center is as close as possible to the cyt f heme (<17 Å in the most permissive situation) will undergo the electron transfer reaction. For this implementation of the MacroDox algorithm, we propose that 3 to 10 electron transfer reactions happen for every 1000 "attempts" at an electron transfer reaction.

TABLE 3 Successes and rates for MacroDox runs of PC/cyt f interactions as a function of reaction distance

Reaction Distance [Fe-Cu distance (Å)]	Number of Successes	*P* (Successes/ Trajectories)	k (M^{-1} s^{-1})
35	2156	0.2396	6.58×10^9
20	406	0.0451	1.43×10^9
18	191	0.0212	6.84×10^8
17	100	0.0111	3.61×10^8
16	30	0.0033	1.09×10^8
15	0	0	0.00

TABLE 4 Successes and rates for MacroDox runs as a function of ionic strength and reaction distance (35 Å, 17 Å, or 16 Å)

	35 Å	17 Å			16 Å			
Ionic Strength (mM)	Number of Successes	Number of Successes	*P* (Successes/ Trajectories)	k (M^{-1} s^{-1})	Number of Successes	*P* (Successes/ Trajectories)	k (M^{-1} s^{-1})	Average Fe-Cu Distance (Å)
50	1061	101	0.02525	8.11×10^8	69	0.01725	5.57×10^8	23.34
60	1042	96	0.024	7.72×10^8	51	0.01275	4.14×10^8	23.74
70	1012	73	0.01825	5.89×10^8	31	0.00775	2.52×10^8	24.34
80	985	62	0.0155	5.02×10^8	24	0.006	1.96×10^8	24.64
90	1000	48	0.012	3.89×10^8	16	0.004	1.31×10^8	25.27
100	2156*	100*	0.01111	3.61×10^8	30*	0.00333	1.09×10^8	25.64
110	899	42	0.0105	3.41×10^8	12	0.003	9.81×10^7	25.45
125	906	22	0.0055	1.79×10^8	9	0.00225	7.36×10^7	26.14
150	884	20	0.005	1.63×10^8	6	0.0015	4.91×10^7	26.96
175	877	15	0.00375	1.23×10^8	3	0.00075	2.46×10^7	27.01
200	837	9	0.00225	7.36×10^7	1	0.00025	8.19×10^6	27.66
300	841	10	0.0025	8.17×10^7	5	0.00125	4.09×10^7	27.85
400	802	4	0.001	3.27×10^7	1	0.00025	8.19×10^6	28.33
500	776	3	0.0008	2.46×10^7	1	0.00025	8.19×10^6	28.33
10000	745	0	0	0	0	0	0	28.93

*Number of successes based on 9000 attempted trajectories, as opposed to 4000 for all other ionic strengths.

Complex formation as a function of ionic strength

For ionic strengths other than 100 mM, four runs of 1000 trajectories each were performed and the number of successful trajectories for reaction distances of 35, 17, and 16 Å, as well as the resulting complexes for each successful trajectory, were recorded. For these simulations, the ionic strength was varied between 50 mM and 500 mM. Values of ionic strength below 50 mM were not used because the trajectories never ended due to the fact that the very strong electrostatic forces prevented PC from leaving the 200 Å sphere. These results were compared to the 10 M control, where electrostatic interactions were eliminated. As above, the successes for the 17 Å and 16 Å reaction distances were converted to values for p and k. In addition, average values for the distance between metal centers for all successful trajectories (at $b = 35$ Å) were calculated. These data are tabulated in Table 4 and presented graphically in Fig. 7.

There is a clear decrease in rate constants k that BD predicts with increasing ionic strength at the 17 Å and 16 Å reaction distances. The dependence is much greater for these reaction distances than it is at the 35 Å distance. This indicates that Brownian forces predominate over electrostatic forces for larger reaction distances. This also suggests some interaction between the proteins will take place regardless of the magnitude or importance of electrostatic interactions; please note the number of "successes" at the 35 Å level remains more or less constant between ionic strengths of 500 mM and 10 M, when electrostatic interactions should have practically no importance.

To get a better picture of how the ionic strength impacts the nature of the complexes that form between PC and cyt f, a similar analysis to that done in the "standard case" of 100 mM ionic strength was performed for 50 mM, 300 mM, and 10 M ionic strength. Tables 5–7 give "triplet contact" analysis that parallels that done for 100 mM; this shows the marked increase in the number of more specific complexes that are formed at the lower ionic strengths, which lead to the vastly increased rate constant k, and by the same token the significant decrease in the total number of complexes, and the "more specific" complexes in particular, that are formed at the higher ionic strengths. Particularly, it should be noted that even the "more specific" complexes are no longer as tight as they are at lower ionic strengths: the most frequently occurring triplet at 50, 100, and 300 mM ionic strengths (K65 on cyt f paired with E60 on PC; K187 with E43; R209 with E59) returns an average Cu-Fe distance of 18.0 Å at 100 mM, as compared with 16.8 Å at 50 mM and 18.5 Å at 300 mM. The list of most popular complexes at 10 M bears no resemblance whatsoever to the lists for the lower ionic strengths, giving strong indication that the spec-

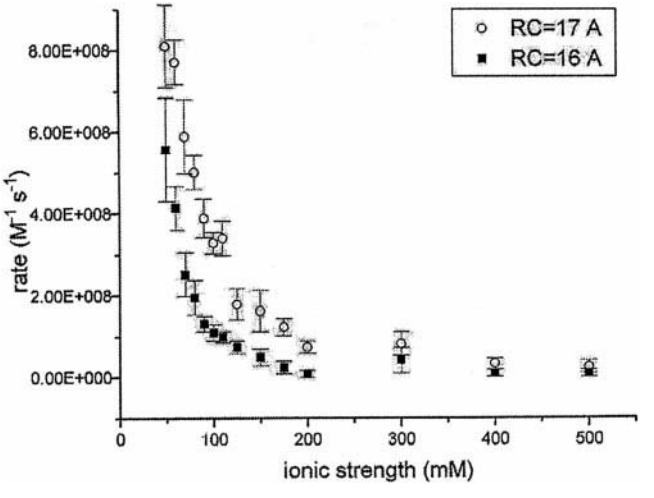

FIGURE 7 Calculated rates from MacroDox for the interaction based on reaction criteria of 16 and 17 Å as a function of ionic strength.

TABLE 5 Best seven triplet contacts between cyt f and PC at 50 mM ionic strength

Triplets (cyt f res-PC res)			Number of Successes	*P* (Successes/ 4000)	Average Fe-Cu Distance (Å)
K65-E60	K187-E43	R209-E59	50	0.0125	16.8
K65-E60	K187-E43	heme-E59	25	0.0063	17.2
K65-E60	R106-D9	K187-D43	19	0.0048	16.2
K58-E60	K65-E59	K187-D44	13	0.0033	17.2
R156-E59	K187-E43	heme-E60	13	0.0033	17.3
K187-D44	R209-E43	heme-E59	13	0.0033	16.9
K65-E43	K187-D44	heme-E59	12	0.0030	17.2

ificity of the interaction present at the lower ionic strengths has been lost, as one might predict for such a restriction on electrostatic interactions.

Again, α-carbon traces of the coordinates of the more specific complexes between PC and cyt f at 50 mM, 300 mM, and 10 M were created in the same way as they were for 100 mM ionic strength, and these traces are given in Fig. 8, *A–C*. In this figure, as before, 12 complexes between PC and cyt f from a single characteristic 1000-trajectory MacroDox run are taken. In the case of 50 mM ionic strength, an individual complex does not appear to be "tighter" than for 100 mM, it important to note that the number of "tight" complexes is greater at 50 mM. However, the distinct loss of specificity shown at higher ionic strengths, to some extent in the 300 mM case and radically so in the 10 M case, does give significant information about the degree that specificity is lost at higher ionic strengths and when electrostatic interactions are unimportant.

Previous work from this laboratory (Durell et al., 1990; Pearson et al., 1996) has emphasized the importance of ionic strength in the strength of the electrostatic attraction between the two proteins; in this light, these assertions are hardly surprising and only further serve to confirm experimental observations of the dependence of the interaction between PC and cyt f on salt concentration in vitro (Takabe et al., 1986; Anderson et al., 1987; Meyer et al., 1993).

Additional controls

Two additional sets of simulations were carried out to determine the importance of specific electrostatic interactions on the docking of PC with cyt f. In the first set of simulations, *Anabaena* PC (Badsberg et al., 1996) was substituted for poplar PC. *Anabaena* PC has a net charge of +1.13 as set up on MacroDox compared to −7.97 for poplar PC. It also has a large positive electrostatic field surrounding both site I and site II (E. L. Gross, unpublished observations). At 100 mM ionic strength and a reaction distance of 35 Å, 335 successes were observed of 4000 trajectories giving a probability *P* of 0.08375. These complexes have an average Cu-Fe distance of 29.7 Å, implying a greatly decreased specificity of interaction between the two molecules and, therefore, a decreased probability for electron transfer.

In the second set, azurin from *Alcaligenes denitrificans* (Baker, 1988) was substituted for poplar PC. Azurin lacks the negative charges found at site II in PC, and, therefore, should be a very good control for these simulations. Only 257 successes were observed of 4000 trajectories at 100 mM ionic strength and a 35 Å reaction distance. The average Cu-Fe distance was 30.8 Å, implying an even greater decrease in specificity.

In both cases, the success rate at reaction distances of 20 Å and less is zero, further strengthening the idea that electrostatic interactions do not provide the close dock necessary for electron transfer to occur. Fewer successful trajectories in both cases result than observed for poplar PC at 10 M ionic strength, indicating a total lack of electrostatic interactions leaving only the random Brownian component.

Further discussion

To maintain the simplicity of the model, we have not taken into rigorous account the intramolecular events that might lead up to an electron transfer reaction between PC and cyt f; we have instead chosen to model the reaction in a way such that we assume that an electron transfer reaction occurs every time the two metal centers on the proteins come within a predetermined distance; in other words, $k_{et} \gg k_1$ and $k_{et} \gg k_{-1}$ in the reaction scheme we outlined previ-

TABLE 6 Best four triplet contacts between cyt f and PC at 300 mM ionic strength

Triplets (cyt f res-PC res)			Number of Successes	*P* (Successes/ 4000)	Average Fe-Cu Distance (Å)
K65-E60	K187-E43	K209-E59	7	0.0018	18.5
R156-E59	K187-E43	heme-E60	5	0.0013	19.4
E10-D61	K187-E43	D162-K66	3	0.0008	30.3
K65-E60	K187-E43	heme-E59	3	0.0008	19.9

TABLE 7 Best four triplet contacts between cyt f and PC at 10 M ionic strength

Triplets (cyt f res-PC res)			Number of Successes	*P* (Successes/ 4000)	Average Fe-Cu Distance (Å)
K10-D9	R106-D8	D162-K66	4	0.0010	30.2
K58-E68	K65-D61	K122-K66	2	0.0005	34.1
R106-D9	D162-Cu	K187-E68	2	0.0005	29.8
R106-K66	D162-D8	Heme-D9	2	0.0005	24.5

ously. We believe that, while this is an oversimplification, it is a useful oversimplification for purposes of determining the orientation of the two proteins with respect to one another when an interaction occurs because the impact on ionic strength effects on the diffusion of PC and its association with cyt f (k_1 and k_{-1}) will be much greater than the ionic strength effects on reorganization of the complex for electron transfer (k_{et}). Furthermore, observations that the electron transfer rate depends greatly on the distance between electron transfer partners (Moser et al., 1992) provide further justification for choosing a distance-dependent distance for these models.

The recent structural determination of the PC/cyt f complex by Ubbink et al. (1998) gives strong support, in principle, to both the BD analysis presented here and to the Monte Carlo analysis of Ullmann et al. (1997) that predicted six possible energetically favorable families of PC/cyt f interactions, of which two were realistic electron-transfer configurations of the same type predicted in this work. The most significant result of Ubbink's work, the NMR evidence that residues 87–89 are nearest to the heme in the complex between the two proteins, agrees with our assertion that electrostatic interactions pull PC into a position where H87 faces the cytochrome heme. In addition, their computational determination of the ensemble of complexes between PC and cyt f shows reasonable similarity to the most frequently occurring complexes between the two proteins in BD analysis. We do take issue with building the assumption that the prominent acidic patch residues on PC (residues 42–44, 59, 60) interact with the large basic patch on cyt f (residues 58, 65, 66, 187) into the model that gives Ubbink's ensemble; we feel the rigid-body MD results would be even more convincing if electrostatic interactions over the whole protein were taken into account, as they are in this study.

Ubbink et al. (1998) propose a two-step model for the formation of the electron-transfer competent complex between PC and cyt f, similar to the model that we proposed previously (Pearson et al., 1996). This model is based in large part on chemical cross-linking studies between the two proteins based on that done by Morand et al. (1989). Results frequently cited (Qin and Kostic, 1993) indicate that the cross-linked complex is not electron-transfer competent, though other data (Takabe and Ishikawa, 1989; D. J. Davis, unpublished results) call this finding into question. Because of the capacity for electrostatic interactions alone to draw PC into a close contact with cyt f, the BD results we propose here might lead one to believe that a two-step model for attaining an electron-transfer-competent complex is not necessary. The predominant complex that we do see forming through electrostatic interactions is of the type that Ubbink et al. (1998) observe in their work. We continue to believe that the question of whether Morand's chemical cross-linked complex between PC and cyt f is electron-transfer competent is open, as is the question of whether pre-docking complexes are necessary to attain an electron-transfer-competent dock between the two proteins. We do not wish to imply that there is no role for other types of interactions, e.g., steric interactions and hydrophobic interactions, in the formation of complexes between PC and cyt f; we merely propose the possibility that electrostatic interactions alone would be sufficient for the maintenance of electron-transfer-competent docking between PC and cyt f.

The Monte Carlo analysis used by Ullmann et al. (1997) in their analysis of the PC/cyt f interaction, while giving similar results to ours, has a significantly different philosophical base from BD analysis; whereas BD is a simulation of a diffusion process, the Monte Carlo analysis is a energy-based search for most stable complexes. We find it reassuring that these two distinctly different methods of predicting complexes do yield similar results. Indeed, one of the results of the Ullmann work is the prediction of a most-efficient electron tunneling path through H87 and P86 on PC and a propionate side chain on cyt f, which very closely mirrors our own prediction here of the electron transfer pathway.

Ullmann et al. (1997) also propose a cation-π complex for electron transfer involving Y83 on PC and K65 on cyt f. We question this proposal for a number of reasons. As this idea relates to this work, we see little to no evidence of Y83 approaching close enough to K65 in complexes generated through BD simulations in sufficient frequency to make up a significant fraction of the electron-transfer events between the two proteins. In addition, the consistently observed reactivity between *Chlamydomonas* PC and lysine mutants of cyt f in vivo by Soriano et al. (1996, 1998), including mutants of K65, would imply that K65's importance is minimal in the interaction between the two proteins.

A question arises as to the role of electrostatic interactions for the docking of PC with cyt f in vivo. Soriano et al. (1996) showed that the removal of the five most important positive charges on *Chlamydomonas* cyt f had a negligible effect on cyt f oxidation in vivo under normal high ionic strength conditions. In contrast, Fernandez-Velasco et al. (1997) did observe inhibition of cyt f oxidation in permeabilized *Chlamydomonas* cells under low ionic strength

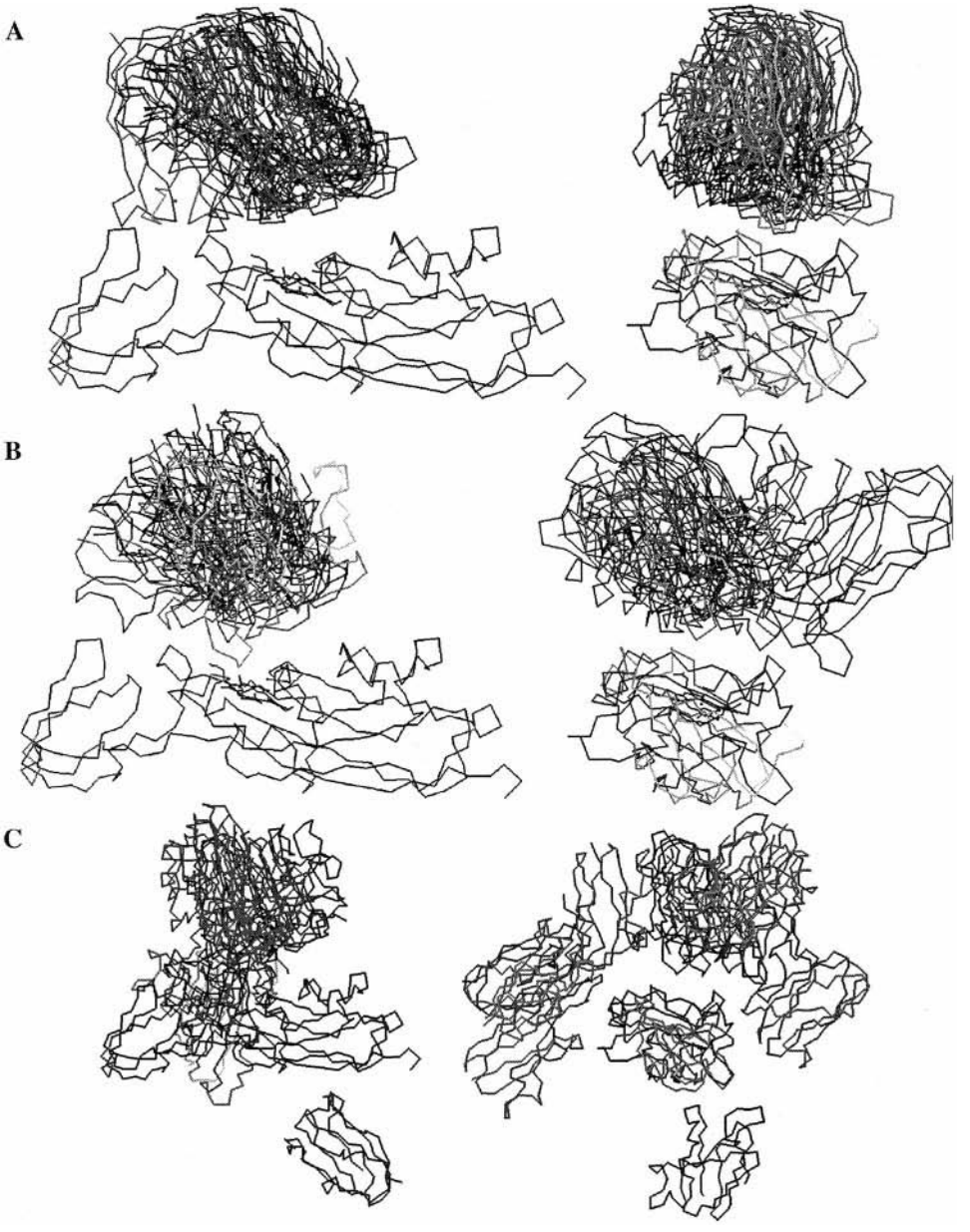

FIGURE 8 α-Carbon traces for 12 complexes between poplar PC and turnip cyt f at ionic strengths of (*A*) 50 mM, (*B*) 300 mM, and (*C*) 10 M. Traces were prepared as described for Fig. 5.

conditions. These observations agree with our results concerning the effect of ionic strength on the electrostatic interactions between PC and cyt f.

Soriano et al. (1998) have recently compared the effects of removing the positive charges on cyt f both in vivo and in vitro. The effects of removing the positive charges were much greater in vitro. On the basis of these results, they postulated that diffusional forces alone are capable of promoting docking of the two proteins in the small space of the lumen of the thylakoid. However, we have shown that diffusional forces alone (such as those observed at 10 M ionic strength) are insufficient to promote the correct distance and orientation of the two metal centers so as to promote efficient electron transfer. Moreover, in higher plant chloroplasts, 50% of the cyt f is localized within the grana stacks (Allred and Staehelin, 1986) requiring long-distance electron transport between the cyt f molecules in the grana stacks and photosystem I in the unstacked lamellae. Additionally, PC has been shown to migrate over long distances in spinach and pea chloroplasts (Haehnel et al., 1989). Therefore, electrostatic interactions have to be considered as a possible means of driving PC's migration.

CONCLUSIONS

Our observations of the electrostatic interactions between poplar plastocyanin and turnip cytochrome *f* through Brownian dynamics simulations lead us to propose a single dominant orientation of the complex between the two proteins. The nature of the interaction between positively charged residues on cytochrome *f* and negatively charged

residues on plastocyanin, somewhat surprisingly, steers PC into an orientation where the H87 ligand to the copper faces the Y1/heme region of cytochrome *f*, without need for hydrophobic interactions as we had previously proposed. These observations, along with the observation that this dominant orientation occurs at very close distances as measured between the metal centers of the two proteins, allow us to construct a model of the interaction between plastocyanin and cytochrome *f* based on this metal-center distance that agrees with experimentally observed bimolecular interaction rate constants.

REFERENCES

Allred, D. R., and L. A. Staehelin. 1986. Implications of cytochrome b_6/f location for thylakoid electron transport. *J. Bioenerg. Biomembr.* 18: 419–435.

Anderson, G. P., D. G. Sanderson, C. H. Lee, S. Durell, L. B. Anderson, and E. L. Gross. 1987. The effect of ethylenediamine chemical modification of plastocyanin on the rate of cytochrome *f* oxidation and P700 reduction. *Biochim. Biophys. Acta*. 894:386–396.

Badsberg, U., A. M. Jorgensen, H. Gesmar, J. J. Led, J. M. Hammerstad, L. L. Jespersen, and J. Ulstrop. 1996. Solution structure of reduced plastocyanin from the blue-green alga *Anabaena variabilis*. *Biochemistry.* 35:7021–7031.

Baker, E. N. 1988. Structure of azurin from *Alcaligenes denitrificans* refinement at 1.8 Å resolution and comparison of the two crystallographically independent molecules. *J. Mol. Biol.* 203:1071–1095.

Bernstein, F. C., T. F. Koetzle, G. J. B. Williams, E. F. Meyer, M. D. Brice, J. R. Rogers, O. Kennard, T. Shimanouchi, and M. Tatsumi. 1977. The Protein Data Bank: a computer-based archival file for macromolecular structures. *J. Mol. Biol.* 112:535–542.

Berry, E. A., L. S. Huang, Y. Chi, Z. Zhang, R. Malkin, and J. G. Fernandez-Velasco. 1997. The crystallization and structure of a soluble form of *Chlamydomonas reinhardtii* cytochrome *f*. *Biophys. J.* 72:125*a*. (Abstr.).

Carrell, C. J., B. G. Schlarb, C. J. Howe, D. S. Bendall, W. A. Cramer, and J. L. Smith. 1997. Structure of the soluble domain of cytochrome *f* from *Phormidium laminosum*. Midwest Photosynthesis Meeting abstracts, Marshall, IN.

Cookson, D. J., M. T. Hayes, and P. E. Wright. 1980. NMR studies of the interaction of plastocyanin with chromium(III) analogs of inorganic electron transfer reagents. *Biochim. Biophys. Acta*. 591:162–176.

Durell, S. R., J. K. Labanowski, and E. L. Gross. 1990. Modeling the electrostatic potential of plastocyanin. *Arch. Biochem. Biophys.* 277: 241–254.

Fernandez-Valasco, J. G., J. Zhou, and R. Malkin. 1997. The putative plastocyanin binding site in *Chlamydomonas reinhardii* cytochrome *f*. *Biophys. J.* 72:126*a*. (Abstr.).

Frauenfelder, H., F. Parak, and R. D. Young. 1988. Conformational substates in proteins. *Annu. Rev. Biophys. Biophys. Chem.* 17:451–479.

Frazao, C., C. M. Soares, M. A. Carrondo, E. Pohl, Z. Dauter, K. S. Wilson, M. Hervás, J. A. Navarro, M. A. De la Rosa, and G. M. Sheldrick. 1995. Ab initio determination of the crystal structure of cytochrome c_6 and comparison with plastocyanin. *Structure*. 3:1159–1169.

Freeman, H. C. 1981. Electron transfer in "blue" copper proteins. *Coord. Chem.* 21:29–52.

Gross, E. L. 1993. Plastocyanin: structure and function. *Photosynth. Res.* 34:359–374.

Gross, E. L. 1996. Plastocyanin: structure, location, diffusion, and electron transfer mechanisms. *In* Oxygenic Photosynthesis: the Light Reactions, D. Ort and C. Yokum, editors. Kluwer Academic Publishers, Dordrecht, the Netherlands. 413–429.

Gross, E. L., A. Curtiss, S. R. Durell, and D. White. 1990. Chemical modification of spinach plastocyanin using 4-chloro-3,5-dinitrobenzoic acid: characterization of four singly modified forms. *Biochim. Biophys. Acta*. 1016:107–114.

Guss, J. M., H. D. Bartnuik, and H. C. Freeman. 1992. Accuracy and precision in protein crystal structure analysis: restrained least-squares refinement of the crystal structure of poplar plastocyanin and 1.33 angstroms resolution. *Acta. Crystallogr., Sect. B.* 48:790–811.

Guss, J. M., and H. C. Freeman. 1983. Structure of oxidized poplar plastocyanin at 1.6 Å resolution. *J. Mol. Biol.* 169:521–563.

Guss, J. M., P. R. Harrowell, M. Murata, V. A. Norris, and H. C. Freeman. 1986. Crystal structure analyses of reduced (CuI) poplar plastocyanin at six pH values. *J. Mol. Biol.* 192:361–387.

Haehnel, W., R. Ratajczak, and H. Robenek. 1989. Lateral distribution and diffusion of plastocyanin in chloroplast thylakoids. *J. Cell. Biol.* 108: 1397–1405.

Handford, P. M., H. A. O. Hill, R. W.-K. Lee, R. A. Henderson, and A. G. Sykes. 1980. Investigation of the binding of inorganic complexes to blue copper proteins by proton NMR spectroscopy. I. The interaction between the $[Cr(phen)_3]^{3+}$ and $[Cr(CN)_5]^{3+}$ ions and the Cu(I) form of parsley PC. *J. Inorg. Biochem.* 13:83–88.

He, S., S. Modi, D. A. Bendall, and J. C. Gray. 1991. The surface-exposed residue Tyr 83 of pea plastocyanin is involved in both binding and electron transfer reactions with cytochrome f. *EMBO J.* 10:4011–4016.

Kannt, A., S. Young, and D. S. Bendall. 1996. The role of acidic residues of plastocyanin in its interaction with cytochrome *f*. *Biochim. Biophys. Acta*. 1277:115–126.

Lee, B. H., T. Hibino, T. Takabe, P. J. Weisbeek, and T. Takabe. 1995. Site-directed mutagenetic study on the role of negative patches on silene plastocyanin in the interactions with cytochrome *f* and photosystem I. *J. Biochem. (Tokyo)*. 117:1209–1217.

Marcus, R. A., and N. Sutin. 1985. Electron transfers in chemistry and biology. *Biochim. Biophys. Acta*. 811:265–322.

Martinez, S. E., D. Huang, M. Ponomarev, W. A. Cramer, and J. L. Smith. 1996. The heme center of chloroplast cytochrome *f* is linked to a buried five-water chain. *Protein Sci.* 5:1081–1092.

Martinez, S. E., D. Huang, A. Szczepaniak, W. A. Cramer, and J. L. Smith. 1995. Crystal structure of the chloroplast cytochrome *f* reveals a novel cytochrome fold and unexpected heme ligation. *Structure*. 2:95–105.

Matthew, J. B. 1985. Electrostatic effects in proteins. *Annu. Rev. Biophys. Biophys. Chem.* 14:387–417.

Matthew, J. B., and F. R. N. Gurd. 1986. Calculation of electrostatic interactions in proteins. *Methods Enzymol.* 130:413–436.

Meyer, T. E., Z. G. Zhao, M. A. Cusanovich, and G. Tollin. 1993. Transient kinetics of electron transfer from a variety of c-type cytochromes to plastocyanin. *Biochemistry*. 32:4552–4559.

Modi, S., M. Nordling, L. G. Lundberg, O. Hannson, and D. S. Bendall. 1992. Reactivity of cytochromes *c* and *f* with mutant forms of spinach plastocyanin. *Biochim. Biophys. Acta.* 1102:85–90.

Morand, L. Z., M. K. Frame, K. K. Colvert, D. A. Johnson, D. W. Krogmann, and D. J. Davis. 1989. Plastocyanin cytochrome *f* interaction. *Biochemistry*. 28:8039–8047.

Moser, C. C., J. M. Keske, K. Warncke, R. S. Farid, and P. L. Dutton. 1992. Nature of biological electron transfer. *Nature*. 355:796–802.

Moser, C. C., C. C. Page, R. Farid, and P. L. Dutton. 1995. Biological electron transfer. *J. Bioenerg. Biomembr.* 27:263–274.

Nicholls, A., and B. Honig. 1991. A rapid finite-difference algorithm, utilizing successive over-relaxation to solve the Poisson-Boltzmann equation. *J. Comp. Chem.* 12:435–445.

Nicholls, A., K. A. Sharp, and B. Honig. 1991. Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. *Proteins*. 11:281–296.

Northrup, S. H. 1995. MacroDox v.2.0.2: Software for the Prediction of Macromolecular Interaction. Tennessee Technological University, Cookeville, TN.

Northrup, S. H. 1996. Theoretical simulation of protein-protein interactions. *In* Cytochrome *c*: A Multidisciplinary Approach. R. A. Scott and A. G. Mauk, editors. University Science Publishers, Sausalito, CA. 543–570.

Northrup, S. H., J. O. Boles, and J. C. L. Reynolds. 1987. Electrostatic effects in the Brownian dynamics of association and orientation of heme proteins. *J. Phys. Chem.* 91:5991–5998.

Northrup, S. H., J. O. Boles, and J. C. L. Reynolds. 1988. Brownian dynamics of cytochrome *c* and cytochrome *c* peroxidase association. *Science*. 241:67–70.

Pearson, D. C., and E. L. Gross. 1995. The docking of cytochrome *f* with plastocyanin: three possible complexes. *In* Photosynthesis: From Light to Biosphere, Vol. II. P. Mathis, editor. Kluwer Academic Publishers, Dordrecht, the Netherlands. 729–732.

Pearson, D. C., E. L. Gross, and E. S. David. 1996. The electrostatic properties of cytochrome *f*: implications for docking with plastocyanin. *Biophys. J.* 71:64–76.

Prince, R. C., and G. N. George. 1995. Cytochrome *f* revealed. *TIBS*. 20:217–218.

Qin, L., and N. M. Kostic. 1992. Electron-transfer reactions of cytochrome f with flavin semiquinones and with plastocyanin. Importance of protein-protein electrostatic interactions and of donor-acceptor coupling. *Biochemistry*. 31:5145–5150.

Qin, L., and N. M. Kostic. 1993. Importance of protein rearrangement in the electron-transfer reaction between the physiological partners cytochrome *f* and plastocyanin. *Biochemistry*. 32:6073–6080.

Quanta Release 97. 1997. San Diego: Molecular Simulations, Inc. (MSI).

Redinbo, M. R., T. O. Yeates, and S. Merchant. 1994. Plastocyanin: structural and functional analysis. *J. Bioenerg. Biomembr.* 26:49–66.

Smoluchowski, M. V. 1916. Drei vortäge über diffusion, brownsche molekularbewegung und koagulation von kolloidteilchen. *Phys. Z.* 17: 557–571.

Soriano, G. M., M. V. Ponamarev, R. A. Piskorowski, L. I. Krishtallik, and W. A. Cramer. 1998. On the nature of cytochrome *f*/plastocyanin interactions. *Biophys. J.* 74:41*a*. (Abstr.).

Soriano, G. M., M. V. Ponomarev, G.-S. Tae, and W. A. Cramer. 1996. Effect of the interdomain basic region of cytochrome *f* on its redox reactions in vivo. *Biochemistry*. 35:14590–14598.

Sykes, A. G. 1991. Plastocyanin and the blue copper proteins. *Struct. Bond.* 75:175–224.

Tai, C. J., S. L. Lin, H. J. Wolfson, and R. Nussinov. 1997. Studies of protein-protein interfaces: a statistical analysis of the hydrophobic effect. *Protein Sci.* 6:53–64.

Takabe, T., and H. Ishikawa. 1989. Kinetic studies on a cross-linked complex between plastocyanin cytochrome f. *J. Biochem.* (*Tokyo*). 105: 98–102.

Takabe, T., K. Takenaka, H. Kawamura, and Y. Beppu. 1986. Charges on proteins and distances of electron transfer in metalloprotein redox reactions. *J. Biochem.* (*Tokyo*). 99:833–840.

Tollin, G., T. E. Meyer, and M. A. Cusanovich. 1986. Elucidation of the factors which determine reaction-rate constants and biological specificity for electron-transfer proteins. *Biochim. Biophys. Acta*. 853:29–41.

Ubbink, M., M. Ejdebäck, B. G. Karlsson, and D. S. Bendall. 1998. The structure of the complex of plastocyanin and cytochrome *f*, determined by paramagnetic NMR and restrained rigid-body molecular dynamics. *Structure*. 6:323–335.

Ullmann, G. M., E. W. Knapp, and N. M. Kostic. 1997. Computational simulation and analysis of dynamic association between plastocyanin and cytochrome f: consequences for the electron-transfer reaction. *J. Am. Chem. Soc.* 119:42–52.

Vakser, I. A., and C. Aflalo. 1994. Hydrophobic docking—a proposed enhancement to molecular recognition techniques. *Proteins*. 20: 320–329.

Warwicker, J., and H. C. Watson. 1982. Calculation of the electric potential in the active site cleft due to alpha-helix dipoles. *J. Mol. Biol.* 157: 671–679.

Wells, J. A. 1996. Binding in the growth hormone receptor complex. *PNAS*. 93:1–6.

Young, L., R. L. Jernigan, and D. G. Covell. 1994. A role for surface hydrophobicity in protein-protein recognition. *Protein Sci.* 3:717–729.

Zhou, J., J. G. Fernandez-Velasco, and R. Malkin. 1996. N-terminal mutants of chloroplast cytochrome *f*: effect on redox reactions and growth in *Chlamydomonas reinhardtii*. *J. Biol. Chem.* 271:6225–6232.