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Computational Simulation of the Docking of Prochlorothrix Hollandica Plastocyanin to Photosystem I: Modeling the Electron Transfer Complex

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Computational Simulation of the Docking of *Prochlorothrix hollandica* Plastocyanin to Photosystem I: Modeling the Electron Transfer Complex

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ABSTRACT We have used several docking algorithms (GRAMM, FTDOCK, DOT, AUTODOCK) to examine protein-protein interactions between plastocyanin (Pc)/photosystem I (PSI) in the electron transfer reaction. Because of the large size and complexity of this system, it is faster and easier to use computer simulations than conduct x-ray crystallography or nuclear magnetic resonance experiments. The main criterion for complex selection was the distance between the copper ion of Pc and the P700 chlorophyll special pair. Additionally, the unique tyrosine residue (Tyr^{12}) of the hydrophobic docking surface of *Prochlorothrix hollandica* Pc yields a specific interaction with the lumenal surface of PSI, thus providing the second constraint for the complex. The structure that corresponded best to our criteria was obtained by the GRAMM algorithm. In this structure, the solvent-exposed histidine that coordinates copper in Pc is at the van der Waals distance from the pair of stacked tryptophans that separate the chlorophylls from the solvent, yielding the shortest possible metal-to-metal distance. The unique tyrosine on the surface of the *Prochlorothrix* Pc hydrophobic patch also participates in a hydrogen bond with the conserved Asn^{633} of the PSI PsaB polypeptide (numbering from the *Synechococcus elongatus* crystal structure). Free energy calculations for complex formation with wild-type Pc, as well as the hydrophobic patch Tyr^{12}Gly and Pro^{14}Leu Pc mutants, were carried out using a molecular mechanics Poisson-Boltzman, surface area approach (MM/PBSA). The results are in reasonable agreement with our experimental studies, suggesting that the obtained structure can serve as an adequate model for *P. hollandica* Pc-PSI complex that can be extended for the study of other cyanobacterial Pc/PSI reaction pairs.

INTRODUCTION

Plastocyanin (Pc) is a small (10 kDa) protein, which functions as a shuttle of electrons from cytochrome b_6f to photosystem I (PSI) in the light reactions of photosynthesis. Pc is a β-sheet type 1 blue copper protein, and the copper ion is located in the "northern region" of the protein with the ligand His^{87} (poplar numbering) protruding into the solvent. This residue, implicated in the transfer of the electron from copper to PSI, is surrounded mainly by small hydrophobic amino acids, usually referred to as the Pc hydrophobic patch. The solution structure of *Prochlorothrix hollandica* Pc was solved recently (Babu et al., 1999). It was shown that it exhibits a unique hydrophobic patch; instead of conserved Gly and Leu residues at positions 12 and 14 (corresponding to positions 10 and 12 in the poplar protein), it has Tyr and Pro, respectively.

PSI is a multisubunit pigment-protein complex that provides the reduction potential necessary for conversion of oxidized form of nicotinamide adenine dinucleotide to reduced nicotinamide adenine dinucleotide. Crystallographic studies indicate that the lumenal site of PSI is essentially flat except for a 10-Å wide hydrophobic cleft between two major transmembrane subunits of PSI, PsaA and PsaB (Schubert et al., 1997). This cleft formed by two α helices, A/B-ij(2) of loops A/B-ij was suggested as a binding site for Pc (Fromme et al., 1994). Site-directed mutagenesis studies suggest that this region is involved in interaction with Pc (Sun et al., 1999; Sommer et al., 2002).

The docking complex is a reversible, specific assembly of the two proteins. During diffusion, proteins guided by long-range electrostatic forces tend to align their dipole moments in favorable orientation, steering two proteins toward the correct encounter complex (Gabdoulline and Wade, 2001). This steering effect, although not very specific, significantly enhances rates of diffusional collision (Zhou, 1993). Upon formation of a loose encounter complex, proteins undergo rotational and vibrational motions exploring conformational space (Northrup et al., 1988). During this process, at the optimal configuration of nuclear coordinates, electron transfer occurs. This electron transfer complex can be predicted based on available biological information. After formation of the encounter complex, short-range electrostatic forces act to enable formation of a more specific complex. These short-range forces include hydrophobic interactions, hydrogen bonds, dipole-dipole interactions, and salt bridges. Thus, the specificity of the association depends on structural properties of protein-protein interfaces, which should be geometrically and chemically complementary. To have favorable free energy of interaction, the enthalpic contribution attributable to desolvation of amino acids, formation of novel H-bonds, and van der Waals and electrostatic interactions should offset the decrease in entropy from the loss of translational and rotational degrees of freedom upon binding.

Depending on the type of the organism these forces have different contribution to the Pc and PSI docking.

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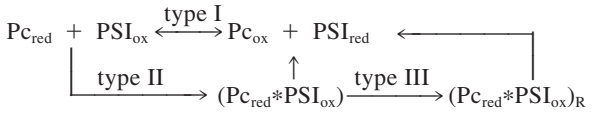
Based on the laser-flash kinetic analysis, Hervas et al. (1995) proposed three different kinetic mechanisms for the



SCHEME 1

Pc-PSI association (Scheme 1). The type I collisional mechanism exhibits a high rate constant such that the transient complex can not be measured kinetically. It is driven by long-range electrostatic forces and is ionic strength-dependent. This is the simplest mechanism of rigid body association and is observed for Pc/PSI interactions in many cyanobacteria, such as *Anabaena*. It was shown that a single arginine residue at position 88 in *Anabaena* Pc plays an important role in electrostatic steering of Pc to PSI (Molina-Heredia et al., 2001). By contrast, the more evolved type II mechanism, observed in *P. hollandica*, includes the formation of a detectable transient complex. The kinetics of complex formation are independent of ionic strength, suggesting that hydrophobic forces drive the protein association (Navarro et al., 2001). Finally, the reduction of PSI in chloroplast systems can be described by a type III mechanism that involves formation of a specific transient complex and its rearrangement before electron transfer. It was shown that salt bridges to the PsaF subunit of PSI play a role in this mechanism (Hippler et al., 1998).

To understand better the kinetics of the functional interactions between Pc and PSI, it is necessary to have the structure of their docking complex. The determination of the docking complex by x-ray crystallography and nuclear magnetic resonance (NMR) techniques is a formidable task because of the transient nature of the complex of the electron transfer proteins. Though the structure of the complex of Pc with the soluble part of cytochrome *f* was thoroughly studied by NMR (Ubbink et al., 1998) and molecular simulations (Ullmann et al., 1997), the high-resolution crystal structure of PSI was unavailable until recently (Fromme et al., 2001). The huge size of water-insoluble PSI makes the application of NMR and crystallography for structural determination of the docking complex far more difficult. In this work we use computational approaches for this purpose.

There are several methods available for the study of protein docking (Janin, 1995). Because molecular recognition consists of geometrical and chemical aspects, the computational algorithms for molecular recognition can be separated into geometry-based docking procedures that attempt to find the best steric fit between two molecules (GRAMM, Vakser and Aflalo, 1994; FTDOCK, Gabb et al., 1997), and approaches based on the minimization of the energy of interaction (AUTODOCK, Morris et al., 1998). The former perform exhaustive six-dimensional search of all possible conformations, the latter carry out statistical sampling of the conformational space. Some programs (DOT, Mandell et al., 2001) attempt to combine both approaches. All these algorithms have good predicting ability, as a root mean standard distance of the obtained complexes was within 2.5 Å from known crystal structures. The free energies of interaction were calculated using a novel molecular mechanics (MM)/Poisson-Boltzman, surface area (MM/PBSA) approach (Srinivasan et al., 1998a, b).

This paper provides an initial attempt to determine the computational structure of the Pc-PSI complex competent for electron transfer. In addition, we used several docking methods to obtain reliable docking structures. Overall, this enabled us to evaluate the efficacy of each method.

MATERIALS AND METHODS

Structure of PSI

Gene fragments encoding for PsaA and PsaB subunits of PSI were isolated by PCR with the following primers: for PsaA: 5′CTACACCGCTTGGCTATCGC, 3′GGACTCAATCAGCTCTTGCC; for PsaB: 5′CCCAAGGCCGTGCAGTGGGTG, 3′CCGTTGATCAACTGGGCC. The gene fragments were cloned into the pCRT7/CT TA cloning vector (Invitrogen, Carlsbad, CA) and then sequenced by automated methods (Cleveland Genomics, Cleveland, OH). The gene sequences were submitted to GenBank under accession number AY026898. The sequence alignment of the lumenal loops responsible for docking of Pc was carried out using the ClustalW program (Thompson et al., 1994). Based on the high degree of sequence identity (Fig. 1), it can be assumed that *P. hollandica* PSI has the same structure as the PSI in other related cyanobacteria. The 2.5-Å resolution crystal structure of *S. elongatus* was used as a basis for our homology modeling (Jordan et al., 2001). The A/B-ij helices were used as an input for docking programs. *P. hollandica* amino acid residues varying from the *S. elongatus* structure were manually replaced using Swiss-PDB Viewer. GRAMM, FTDOCK, DOT, and AUTODOCK were used to obtain reliable structures of the docking complex, and the parameters for each computation are provided below (see Results and Discussion). The best structure was used for free energy calculations.

Molecular dynamics

Only the lumenal loop region of PSI (residues 616 to 668 from the PsaA subunit and 603 to 648 from the PsaB subunit) was used in molecular dynamics studies to decrease the number of atoms. The ends were fixed using the BELLY option (Case et al., 2000) to preserve the conformation of the docking site. The structure and charges of Pc were described earlier (Babu et al., 1999). To understand the role of the unique Tyr^{12} and Pro^{14} residues, molecular dynamics were carried out for the computed complex of PSI with the wild-type (WT) Pc, as well as the Tyr^{12}Gly and Pro^{14}Leu mutants; the resulting coordinates were generated by Swiss-PDB Viewer. All energy minimizations and molecular dynamic simulations were carried out using the SANDER module of AMBER 6.0 package of programs (Case et al., 2000). The complex was solvated with a water box protruding for 10 Å in each direction from the molecule. The long-range electrostatics were treated with the particle mesh Ewald method (Darden et al., 1993). The SHAKE option (Ryckaert et al., 1977) was used to constrain all the bond length allowing for 2.0 fs time step. Nonbonded van der Waals interactions

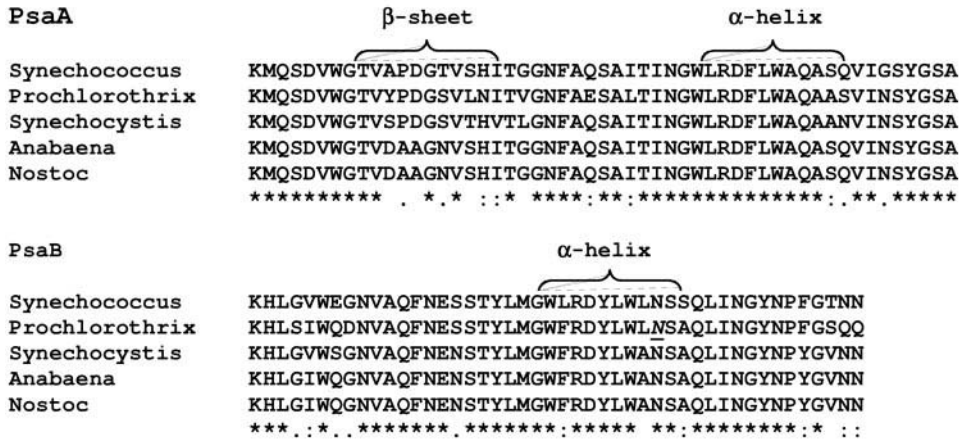


FIGURE 1 Sequence alignment of the A/B-ij loops of the PsaA and PsaB subunits for the following cyanobacteria: *S. elongates* (high-resolution crystal structure available), *P. hollandica*, *Synechocystis* sp. 6803, *Anabaena variabilis*, and *Nostoc*. The Asn[633] from PsaB involved in hydrogen bonding with Tyr[12] Pc is italicized and underlined.

were cutoff beyond 8.5 Å. The complex was minimized with steepest descent and the water box equilibrated for 30 ps at 300 K, keeping the complex fixed. Next, the complex was minimized and the production run for 100 ps at 300 K was for data collection. Snapshots were recorded every 100 fs. Every fifth snapshot of the last 80 ps was used for energy calculations. The free energies of interaction were calculated using the MM/PBSA approach (Srinivasan et al., 1998a,b). The MM energies were calculated with the ANAL module of AMBER 6.0. The electrostatic contribution to solvation free energy was calculated using DELPHI (Honig and Nicholls, 1995; Gilson and Honig, 1998). The interior dielectric constant of 1 was used for the protein because the AMBER force field was parameterized with dielectric constant 1 (Wang and Kollman, 2000). The exterior dielectric constant was set to 80 for the solvent. The solvent-accessible surface area was obtained with MSMS (Sanner et al., 1996) from which the nonpolar contribution to solvation energy was estimated from the following dependence $\Delta G_{\text{nonpolar}} = 0.00542$ * solvent-accessible surface area + 0.92 kcal/mol (Sitkoff et al., 1994).

RESULTS AND DISCUSSION

Criteria for the docking complex

As there is no evidence that there is any significant conformational change upon binding, Pc and PSI are treated as rigid bodies to allow for the application of docking algorithms. Furthermore, such algorithms currently can not treat large-scale structural changes. It has been shown that electron transfer to PSI occurs through the solvent-exposed nitrogen of *P. hollandica* Pc His[85] at the hydrophobic patch, involved in the docking interaction with PSI (Haehnel et al., 1994). The two α-helices of the two PsaA and PsaB subunits at the docking pocket of the PSI have two conserved tryptophan residues (PsaA Trp[655] and PsaB Trp[631]) that come together into van der Waals contact right below the edge of special pair of chlorophylls P700. It was shown that mutation of one of these tryptophans affects the interaction between PSI and the alternative, isofunctional electron donor cytochrome c_6 (Sun et al., 1999). The mutation of Trp[627] from PsaB of *Chlamydomonas reinhardtii* (analogous to Trp[631] in *S. elongatus*) completely abolished formation of complex between Pc and PSI and significantly reduced the rate of electron transfer between these proteins (Sommer et al., 2002). These data suggest that these tryptophans lie on the electron transfer path from the copper to P700 and that the Pc should be docked with His[85] in proximity to these tryptophans to minimize the donor/acceptor distance. This biological information was the main constraint for the complex selection. Additionally, the unique structure of the *P. hollandica* hydrophobic patch makes the interaction between Pc and PSI more specific. This implies that the docking site should also accommodate bulky Tyr[12] of *P. hollandica*. Transfer of Pc hydrophobic patch from solvent into the docking site of PSI displaces protein-bound water molecules into the bulk solvent. This induces local rearrangement in water hydrogen-bonding network, resulting in increase of entropy (Head et al., 1996). The amount of liberated water is proportional to the interface accessible surface area. The interface accessible surface area also reflects the steric fit between proteins. The study of protein-protein interfaces (Lo Conte et al., 1999) reveals that the average size of the recognition site is $\sim 1600 \pm 400$ Å^2, suggesting that the bigger the area, the more stable the complex. In the context of the current study, it has been shown previously that *P. hollandica* Pc forms hydrophobic complex with PSI stable enough to be detected by kinetically (Navarro et al., 2001). Thus, our docking complex should possess the following characteristics: the shortest metal-to-metal distance with His[85] located below P700, a cleft to accommodate the protruding tyrosine residue and the highest area of interface among the obtained complexes.

Comparison of the docking algorithms and resulting structures

All algorithms for the search of the best geometric complementarity used in this work (GRAMM, FTDOCK, DOT) are based on the molecular recognition algorithm developed by Katchalski-Katzir et al. (1992). It estimates surface complementarity between two proteins treated as rigid bodies. The atomic coordinates of the two proteins obtained

from PDB files are projected onto a three-dimensional grid, yielding a digital representation. Small positive numbers are assigned to the surface of the bigger molecule (receptor) and large negative numbers are assigned to its interior to penalize for penetration in the core of the protein. Next, the smaller molecule (ligand) is translated and rotated around the receptor searching through all the conformational space in six dimensions. At each rotational step the correlation function using Fourier transformation is calculated. The correlation function evaluates the degree of the geometric match between two molecules. Thus, the best geometric fit yields the highest score, and low scores represent the poor matches, as a result of penetration in the interior. In all the methods the PSI (receptor) was held fixed and the Pc (ligand) was manipulated to explore all possible orientations.

Global range molecular matching (GRAMM)

The GRAMM algorithm is the program for docking of the protein structures of varying accuracy. Although high-accuracy structures provide high-accuracy complexes, a large number of possible conformations between two proteins results in increased number of false-positive matches and increased computational time. Based on the fact that protein-protein interfaces are more hydrophobic (Jones and Thornton, 1996) than the rest of the protein surface, the simplified approach, called hydrophobic docking, was proposed by Vakser and Aflalo (1994). This approach exhibits a higher signal-to-noise ratio and decreases computational time. Also, to overcome the problem of conformational changes of amino acid side chains affecting the docking prediction (Vakser, 1996c), and the problem of inaccuracies in the protein structures, a low-resolution algorithm was developed (Vakser 1995, 1996a; Vakser and Nikiforovich, 1995). It was shown that docking of the molecules lacking high-resolution details (<7 Å) can overcome the multiplicity of the local minima and effectively find the global minimum (Vakser, 1996b, 1997; Vakser et al., 1999). The above mentioned geometry matching algorithm was interpreted in terms of the energy based on long-distance atom-atom potentials (Vakser, 1996a). In our studies we have used the high-resolution generic docking with grid step 2.5 Å, grid size 64 Å. The ligand was rotated with 10°-angle intervals. The energy score for repulsion was 30, the energy score for attraction was -1. The 100 structures that yielded the best score were selected and analyzed visually. Surprisingly, the structure of the docking complex that best incorporated all the above-mentioned criteria was the first one with highest best energy score (146).

FTDOCK

FTDOCK is another method that evaluates not only shape complementarity, but also electrostatic complementarity (Gabb et al., 1997; Aloy et al., 1998). First, it performs the global search using a slightly modified molecular recognition algorithm, which takes coulombic interactions into account. The obtained docking structures are ranked by empirical residue level pair-pair potentials, which reflect observed amino acid contacts between two proteins in nonhomologous complexes obtained from the solved crystal structures (Moont et al., 1999). Next, structures are filtered by the distance constraints using available biological information. To take into account the conformational changes of amino acid side chains, protein interfaces are refined by selecting the most probable position of a side chain from a rotamer library and performing energy minimization (Koehl and Delarue, 1994; Jackson et al., 1998). The PSI and Pc were projected onto $126 \times 126 \times 126$ grid with a 0.985 grid step and a global surface thickness of 1.3 Å. The rotation angle step was made 10° to make it consistent with GRAMM. 15,840 rotations were evaluated in total. The generated complex structures were ranked using pair potentials (RPscore), and the 6-Å distance constraint between His^{85} and the Trp analogous to PsaB Trp^{631} in *S. elongatus* was used to filter the correct complex. After such screening, only eight structures with the very low pair potentials score of -0.140 corresponded to our criteria.

DOT (Daughter of TURNIP)

DOT is a program that combines both geometry and energy minimization approaches. The van der Waals energy is obtained from the geometric matching algorithm mentioned above (Mandell et al., 2001). The solvent continuum electrostatic model is used to calculate the electrostatic energy by solving the Poisson-Boltzmann equation. The $128 \times 128 \times 128$ potential grid with a 1-Å step was generated for PSI using University of Houston Brownian Dynamics program (Davis et al., 1991; Madura et al., 1995). The solvent dielectric was set to 80, the protein dielectric -4, temperature 300 K, solvent and ionic radius -1.4 Å. From the composite energy term (the sum of electrostatic and van der Waals energies) the partition function was computed to derive the free energy of interaction. The free energy of interaction for the best complex structure was -11.7 kcal/mol, which is in the same order as the experimental values calculated from the binding constant (-5.7 kcal/mol) (Navarro et al., 2001).

AUTODOCK3.0

AUTODOCK is an example of a program that attempts to find the complex with minimal interaction energy. Earlier versions used Monte Carlo simulated annealing to sample the docking conformations (Goodsell and Olson, 1990;

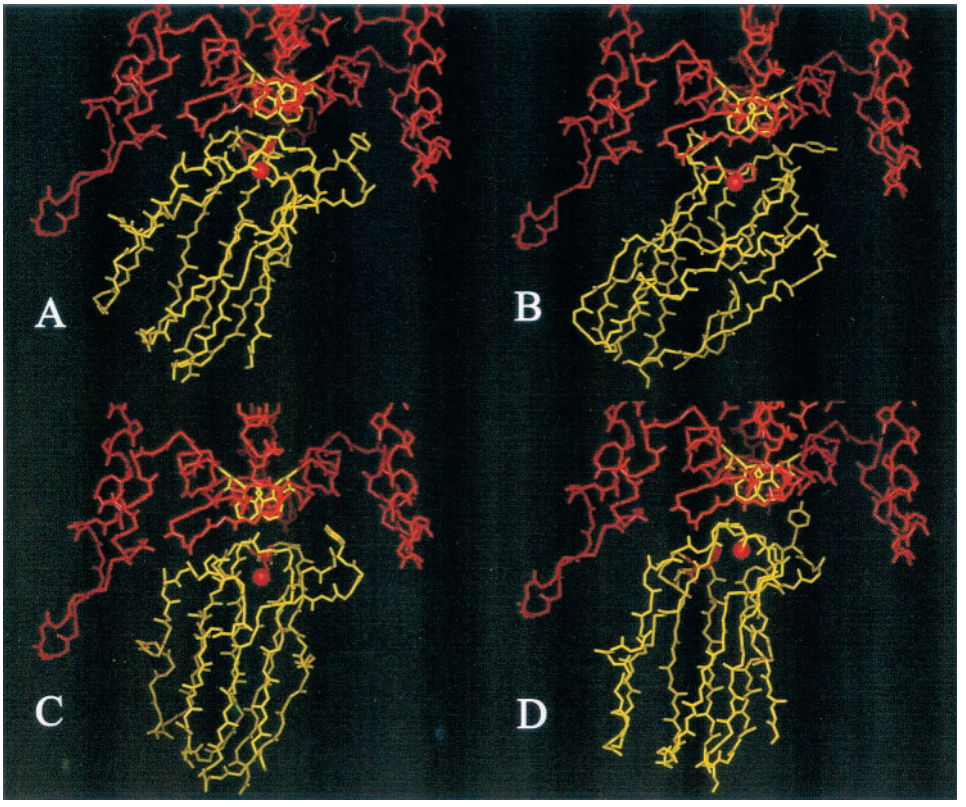


FIGURE 2 The best structures for the complex of *Prochlorothrix* Pc with PSI obtained by: (*A*) GRAMM; (*B*) FT-DOCK; (*C*) DOT; and (*D*) AUTODOCK. The side chains are not shown, except for tyrosine at position 12 of the Pc. Pc and residues PsaA Trp^{655} and PsaB Trp^{631} are shown in yellow. The copper center, Pc His^{85}, and the PSI core backbone are in red

Morris et al., 1996). Later, a Lamarckian genetic algorithm was introduced, which was shown to be more efficient than the simulated annealing (Morris et al., 1998). This algorithm was used in our simulation of the Pc-PSI association. The Pc was initially placed 5 Å away from the docking site of PSI. In this method, the ligand (Pc) is represented as a chromosome and its translation and orientation toward PSI is represented as genes in that chromosome. The atomic coordinates of the protein in the docking complex represent its phenotype. A random population of 75 individuals was generated and the interaction energy (fitness) of ligand with receptor (PSI) was calculated. The crossovers and mutations introduced randomly in the population and the individuals with the best fitness were selected to produce the next generation. The rate of cross-over was set to 0.8 and the rate of mutation to 0.04. The translational step was equal to 1 Å, and the rotational step equal to 5°. In the Lamarckian algorithm, the best individuals undergo a local search, which is analogous to energy minimization and is based on the Solis and Wets algorithm (1981). The local search finds the local minimum (best fitness) and then the position of the ligand (phenotype) is converted back to translational and orientation values (genotype). Because of the size of the system, the number of energy evaluations was reached faster and the next run was initiated. Total number of runs performed was 20. The results were clustered into several groups with a 1.0-Å cluster tolerance. Of 20 structures obtained, 9 structures clustered in one group, with a mean docked energy of −99.8 kcal/mol, matched our criteria for docking complex.

Analysis of docking complexes

The best structures obtained by above-mentioned algorithms were visualized and compared with one another (Fig. 2). The protein-protein interfaces of the docking complexes were further analyzed by the Protein-Protein Interaction Server (http://www.biochem.ucl.ac.uk/bsm/PP/server; Jones and Thornton, 1995, 1996). The results are presented in Table 1. All complexes have almost the same ratio of polar to nonpolar amino acids at the interface. The prevalence of nonpolar amino acids is the characteristic trait of the docking interface (Sheinerman et al., 2000). The highest interface-accessible surface area (Hubbard, 1992) and the shortest metal-to-metal distance necessary for effective electron transfer was yielded by the structure obtained by the GRAMM algorithm. The high area of the surface contact is probably attributable to interaction with a β-sheet region of the PsaA loop (Figs. 1 and 2). Also in this structure, a hydrogen bond of 2.9 Å is identified between Tyr^{12} of Pc and Asn corresponding to PsaB Asn^{633} in *S. elongatus*. The Pc His^{85} involved in electron transfer is at the van der Waals distance to the pair of stacked tryptophans homologous to Trp^{655} of PsaA and Trp^{631} of PsaB, suggesting their possible interaction (Fig. 3). These data

TABLE 1 Analysis of protein-protein interfaces for the structures obtained by GRAMM, FTDOCK, DOT, AUTODOCK

	GRAMM	FTDOCK	DOT	AUTODOCK
Interface–accessible surface area (Å^2)*	1005.63	570.38	806.25	618.91
% Polar atoms in interface	34.17	34.52	29.29	27.81
% Nonpolar atoms in interface	65.80	65.40	70.70	72.10
Metal–to–metal distance (Å)†	20.40	21.48	20.99	23.26
RMSD (Å)‡	0	8.81	6.52	6.59

*Accessible surface area is calculated by rolling a sphere probe of radius 1.4 Å over the van der Waals surface of the protein (Hubbard, 1992).
†The metal to metal distance was calculated with InsightII/Accelrys
‡The RMSD was calculated relatively to the GRAMM structure

render the GRAMM-derived structure the best of the four obtained complexes. Thus, this structure was used for the free energy calculations. Last, from root mean standard distance it can be inferred that other structures are 6–8 Å from the GRAMM, mostly because of rotation around C_2 axis of symmetry of the PSI.

It should be noted that the stability and specificity of complex should be balanced in such a way that will allow efficient electron transfer and rapid dissociation at the same time, because stable complexes can sometimes be trapped in unproductive local minima. From this point of view, the structure obtained by DOT program can be optimal, because it has less surface contact with PSI and is thus less stable. Nevertheless, in DOT, FTDOCK, and AUTODOCK complexes, Pc His^{85} is displaced from the suggested electron transfer pathway (Fromme et al., 2001). For AUTODOCK and FTDOCK complexes there was a gap between van der Waals surfaces of Pc His^{87} and the stacked tryptophans, which would affect electron transfer. These complexes could possibly represent an initial recognition complex.

Free energy of interaction

The evaluation of the free energy of interaction by rigorous approaches, involving free energy perturbation and thermodynamic integration, is computationally intensive. To decrease the computation time a new approach, MM/PBSA, was proposed by Kollman and colleagues (Srinivasan et al., 1998a,b). The free energy (ΔG_{bind}) is evaluated as a sum of the free energy of interaction in gas phase (ΔG_{gas}) and free energy of solvation (ΔG_{sol}). The gas phase free energy is a

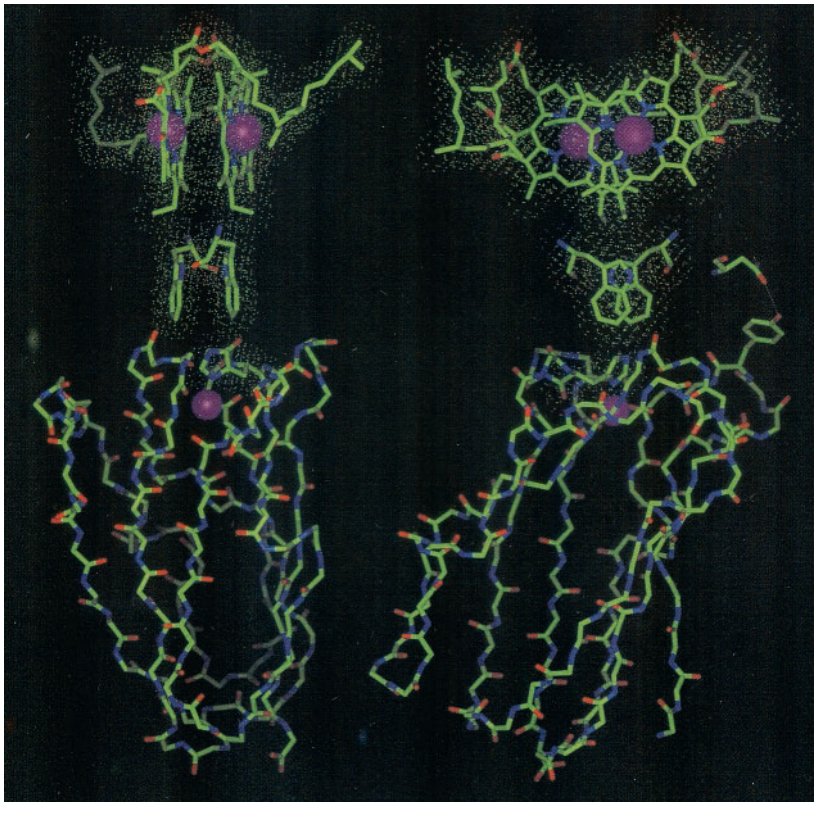


FIGURE 3 Structure of the Pc-PSI complex obtained by GRAMM: front and side view. The van der Waals surface is shown by dots. The His^{85} of the Pc is right below pair of stacked Trp (analogous to Trp^{655} for PsaA and Trp^{631} for PsaB) adjacent to special pair of chlorophylls. The metal centers are in purple. Also shown is the hydrogen bond between Asn^{633} and Tyr^{12}.

TABLE 2 Free energies of interaction between Pc and PSI calculated by MM/PBSA. WT, wild–type Pc, Y12G − Tyr12→Gly12 mutant, P14L − Pro14→Leu14 mutant

	ΔG_{inf}^{elec}	ΔG_{inf}^{vdw}	ΔG_{gas}	ΔG_{sol}^{nonpol}	ΔG_{sol}^{elec}	ΔG_{sol}	ΔG_{bind}	$\Delta\Delta G_{bind}$
WT	−363.6	−119.5	−483.1	−14.6	323.4	308.8	−174.3	0
Y12G	−379.3	−104.1	−483.4	−13.6	316.06	302.4	−181.0	−6.7
P14L	−379.7	−122.9	−502.6	−14.9	340.2	325.2	−177.3	−3.0

$\Delta G_{gas} = \Delta G_{int}^{elec} + \Delta G_{int}^{vdw}$
$\Delta G_{sol} = \Delta G_{sol}^{nonpol} + \Delta G_{sol}^{elec}$
$\Delta G_{bind} = \Delta G_{gas} + \Delta G_{sol}$

sum of electrostatic (ΔG_{int}^{elec}) and van der Waals (ΔG_{int}^{vdw}) energies calculated from MM. The solvation free energy is composed of nonpolar solvation energy (ΔG_{sol}^{nonpol}) estimated via accessible surface area and electrostatic solvation energy (ΔG_{sol}^{elec}) calculated by solving the Poisson-Boltzman equation with DelPhi program. This is represented in the following thermodynamic cycle (Scheme 2; Kollman et al., 2000).

$$\begin{array}{ccc} Pc_{gas} + PSI_{gas} & \xrightarrow{\Delta G_{gas}} & Pc{*}PSI_{gas} \\ \downarrow \Delta G_{sol}^{Pc} \quad \downarrow \Delta G_{sol}^{PSI} & & \downarrow \Delta G_{sol}^{PcPSI} \\ Pc_{aq} + PSI_{aq} & \xrightarrow[\Delta G_{bind}]{} & Pc{*}PSI_{aq} \end{array}$$

SCHEME 2

Thus, $\Delta G_{bind} = \Delta G_{gas} + \Delta G_{sol}^{PcPSI} - \Delta G_{sol}^{PSI} - \Delta G_{sol}^{Pc} - T\Delta S$, where $\Delta G_{gas} = \Delta G_{int}^{elec} + \Delta G_{int}^{vdw}$, and $\Delta G_{sol} = \Delta G_{sol}^{nonpol} + \Delta G_{sol}^{elec}$. Because we are interested only in relative binding free energies of WT and mutant proteins, the entropic contribution to free energy is not taken into consideration. Assuming that no conformational change occurs upon docking of Pc to PSI, the energies for complex, ligand, and receptor were evaluated from the trajectory of a complex, instead of using separate trajectories. This approach has proven to yield accurate predictions when compared with experimental data (Massova and Kollman, 1999). The inadequacies of the model are cancelled when the difference of the free energies is calculated (Wang et al., 2001). The results of the free energy calculations for the complex of PSI with WT, $Tyr^{12}Gly$, and $Pro^{14}Leu$ mutants are presented in Table 2. The free energy of −174.3 kcal/mol for the WT can be compared with those obtained for the complex of Pc with cytochrome *f* in related studies, −285.3 kcal/mol (De Rienzo et al., 2001) and −355.6 kcal/mol (Ullmann et al., 1997). The relative free energy of the $Tyr^{12}Gly$ mutant is −6.7 kcal/mol lower than the WT, which is the result of lower solvation energy (302.4 kcal/mol compared with 308.8 kcal/mol of the WT). The presence of Tyr^{12} also increases nonpolar solvation energy. In agreement with this calculation, it has been recently determined experimentally that this mutant has a modestly higher binding constant (Navarro et al., 2001).

It is worth noting that the internal electrostatic energy (−379.7 kcal/mol) and electrostatic solvation energy (340.2 kcal/mol) are significantly different for the $Pro^{14}Leu$ mutant than for the WT (−363.6 and 323.4 kcal/mol, respectively). This fact can account for the unique reactivity of this mutant in the electron transfer. It was shown by laser-flash photolysis experiments that it has a threefold higher electron transfer constant than the WT (Navarro et al., 2001). It was suggested earlier that the replacement of Pro^{14}, which has a rigid backbone, with Leu affects the flexibility and geometry of the copper site and the reorganization energy, making $Pro^{14}Leu$ mutant a better electron donor (Navarro et al., 2001). Thus, the results of the free energy calculations are in reasonable agreement with the experimental observations, further suggesting that the docking structure obtained is an adequate representation of the functional complex.

CONCLUSION

In this work, a combination of docking algorithms, molecular dynamics, and free energy calculations using MM/PBSA was presented. All algorithms have predicted complexes that are very similar, but the structure of a complex that best corresponded to the available biological information was obtained by the GRAMM algorithm, which was the fastest and easiest compared with the others. This work showed that geometric algorithms, which deal mostly with van der Waals interactions, were more effective in prediction than those concerned with energy considerations. This can probably be explained by the hydrophobic nature of the complex of interest. The obtained docking structures are slightly different from one another, but they still can be functionally effective because of the transient nature of the complexes formed by electron transfer proteins. The free energy calculations provided useful insights into experimental data obtained earlier, which in turn speak for the adequacy of the predicted complex. This work also suggests, in concert with others (Fromme et al., 2001), that the pathway of electron from Pc His^{85} to the special pair of chlorophylls in PSI could pass through the pair of tryptophans from PsaA and PsaB subunits stacked at the van der Waals distances. Finally, it should be noted that the predic-

tion of the docking complex was made possible by the unique surface conformation provided by Tyr^{12} of the *Prochlorothrix* Pc hydrophobic patch. This structure can thus be extended to understanding the docking mechanism seen in other Pc/PSI reaction pairs.

REFERENCES

Aloy, P., G. Moont, H. A. Gabb, E. Querol, F. X. Aviles, and M. J. Sternberg. 1998. Modelling repressor proteins docking to DNA. *Proteins.* 33:535–549.

Babu, C. R., B. F. Volkman, and G. S. Bullerjahn. 1999. NMR solution structure of plastocyanin from the photosynthetic prokaryote. *Prochlorothrix hollandica. Biochemistry.* 38:4988–4995.

Case, D. A., D. A. Pearlman, J. W. Caldwell, T. E. Cheatham, III, W. S. Ross, C. L. Simmerling, T. A. Darden, K. M. Merz, R. V. Stanton, A. L. Cheng, J. J. Vincent, M. Crowley, D. M. Ferguson, R. J. Radmer, G. L. Seibel, U. C. Singh, P. K. Weiner, and P. A. Kollman. 2000. *AMBER6*. University of California: San Francisco.

Darden, T. A., D. M. York and L. G. Pedersen. 1993. Particle mesh Ewald: an N log (N) method for Ewald sums in large systems. *J. Chem. Phys.* 98:10089–10092.

Davis, M. E., J. D. Madura, B. A. Luty, and J. A. McCammon. 1991. Electrostatics and diffusion of molecules in solution: simulations with the University of Houston Brownian Dynamics Program. *Comput. Phys. Commun.* 62:187–197.

De Rienzo, F., R. R. Gabdoulline, M. C. Menziani, P. G. De Benedetti, and R. C. Wade. 2001. Electrostatic analysis and Brownian dynamics simulation of the association of plastocyanin and cytochrome *f. Biophys. J.* 81:3090–3104.

Fromme, P., P. Jordan, N., and N. Krauss. 2001. Structure of photosystem I. *Biochim. Biophys. Acta.* 1507:5–31.

Fromme, P., W. D. Schubert, and N. Krauss. 1994. Structure of photosystem I: suggestions on the docking sites for plastocyanin, ferredoxin and the coordination of P700. *Biochim. Biophys. Acta.* 1187:99–105.

Gabb, H. A., R. M. Jackson, and M. J. Sternberg. 1997. Modelling protein docking using shape complementarity, electrostatics and biochemical information. *J. Mol. Biol.* 272:106–120.

Gabdoulline, R. R., and R. C. Wade. 2001. Protein-protein association: investigation of factors influencing association rates by brownian dynamics simulations. *J. Mol. Biol.* 306:1139–1155.

Gilson, M. K., and B. Honig. 1998. Calculation of the total electrostatic energy of a macromolecular system: solvation energies, binding energies, and conformational analysis. *Proteins.* 4:7–18.

Goodsell, D. S., and A. J. Olson. 1990. Automated docking of substrates to proteins by simulated annealing. *Proteins.* 8:195–202.

Haehnel, W., T. Jansen, K. Gause, R. B. Klosgen, B. Stahl, D. Michl, B. Huvermann, M. Karas, and R. G. Herrmann. 1994. Electron transfer from plastocyanin to photosystem I. *EMBO J.* 13:1028–1038.

Head, R. D., M. L. Smythe, T. I. Oprea, C. L. Waller, S. M. Green, and G. R. Marshall. 1996. VALIDATE: a new method for the receptor-based prediction of binding affinities of novel ligands. *J. Amer. Chem. Soc.* 118:3959–3969.

Hervas, M., J. A. Navarro, A. Diaz, H. Bottin, and M. A. De la Rosa. 1995. Laser-flash kinetic analysis of the fast electron transfer from plastocyanin and cytochrome c_6 to photosystem I. Experimental evidence on the evolution of the reaction mechanism. *Biochemistry.* 34:11321–11326.

Hippler, M., F. Drepper, W. Haehnel, and J. D. Rochaix. 1998. The N-terminal domain of PsaF: precise recognition site for binding and fast electron transfer from cytochrome c_6 and plastocyanin to photosystem I of *Chlamydomonas reinhardtii. Proc. Natl. Acad. Sci. U.S.A.* 95: 7339–7344.

Honig, B., and A. Nicholls. 1995. Classical electrostatics in biology and chemistry. *Science.* 268:1144–1149.

Hubbard, S. J. 1992. Analysis of protein-protein molecular recognition. Ph.D. thesis. University of London.

Jackson, R. M., H. A. Gabb, and M. J. Sternberg. 1998. Rapid refinement of protein interfaces incorporating solvation: application to the docking problem. *J. Mol. Biol.* 276:265–285.

Janin, J. 1995. Protein-protein recognition. *Prog. Biophys. Mol. Biol.* 64:145–166.

Jones, S., and J. M. Thornton. 1995. Protein-protein interactions: a review of protein dimer structures. *Prog. Biophys. Mol. Biol.* 63:31–65.

Jones, S., and J. M. Thornton. 1996. Principles of protein-protein interactions. *Proc. Natl. Acad. Sci. U.S.A.* 93:13–20.

Jordan, P., P. Fromme, H. T. Witt, O. Klukas, W. Saenger, and N. Krauss. 2001. Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution. *Nature.* 411:909–917.

Katchalski-Katzir, E., I. Shariv, M. Eisenstein, A. A. Friesem, C. Aflalo, and I. A. Vakser. 1992. Molecular surface recognition: determination of geometric fit between proteins and their ligands by correlation techniques. *Proc. Natl. Acad. Sci. U.S.A.* 89:2195–2199.

Koehl, P., and M. Delarue. 1994. Application of a self-consistent mean field theory to predict protein side-chains conformation and estimate their conformational entropy. *J. Mol. Biol.* 239:249–275.

Kollman, P. A., I. Massova, C. Reyes, B. Kuhn, S. Huo, L. Chong, M. Lee, T. Lee, Y. Duan, W. Wang, O. Donini, P. Cieplak, J. Srinivasan, D. A. Case, and T. E. Cheatham, III. 2000. Calculating structures and free energies of complex molecules: combining molecular mechanics and continuum models. *Accounts Chem. Res.* 33:889–897.

Lo Conte, L., C. Chothia, and J. Janin. 1999. The atomic structure of protein-protein recognition sites. *J. Mol. Biol.* 285:2177–2198.

Madura, J. D., J. M. Briggs, R. C. Wade, M. E. Davis, B. A. Luty, A. Ilin, J. Antosciewicz, M. K. Gilson, B. Bagheri, L. R. Scott, and J. A. McCammon. 1995. Electrostatics and diffusion of molecules in solution: simulations with the University of Houston Brownian Dynamics Program. *Comput. Phys. Commun.* 91:57–95.

Mandell, J. G., V. A. Roberts, M. E. Pique, V. Kotlovyi, J. C. Mitchell, E. Nelson, I. Tsigelny, and L. F. Ten Eyck. 2001. Protein docking using continuum electrostatics and geometric fit. *Protein Eng.* 14:105–113.

Massova, I., and P. A. Kollman. 1999. Computational alanine scanning to probe protein-protein interactions: a novel approach to evaluate binding free energies. *J. Amer. Chem. Soc.* 121:8133–8143.

Molina-Heredia, F. P., M. Hervas, J. A. Navarro, and M. A. De la Rosa. 2001. A single arginyl residue in plastocyanin and in cytochrome *c*(6) from the cyanobacterium *Anabaena* sp. PCC 7119 is required for efficient reduction of photosystem I. *J. Biol. Chem.* 276:601–605.

Moont, G., H. A. Gabb, and M. J. Sternberg. 1999. Use of pair potentials across protein interfaces in screening predicted docked complexes. *Proteins.* 35:364–373.

Morris, G. M., D. S. Goodsell, R. Huey, and A. J. Olson. 1996. Distributed automated docking of flexible ligands to proteins: parallel applications of AutoDock 2.4. *J. Comput. Aided Mol. Des.* 10:293–304.

Morris, G. M., D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, and A. J. Olson. 1998. Automated docking using a Lamarckian genetic algorithm and empirical binding free energy function. *J. Comput. Chem.* 19:1639–1662.

Navarro, J. A., E. Myshkin, M. A. De la Rosa, G. S. Bullerjahn, and M. Hervas. 2001. The unique proline of the *Prochlorothrix hollandica* plastocyanin hydrophobic patch impairs electron transfer to photosystem I. *J. Biol. Chem.* 276:37501–37505.

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

Ryckaert, J. P., G. Ciccotti, and H. J. Berendsen. 1977. Numerical integration of the Cartesian equations of motion of a system with constraints: molecular dynamics of *n*-alkanes. *J. Comput. Phys.* 23: 327–320.

Sanner, M. F., A. J. Olson, and J. Spehner. 1996. Reduced surfaces: an efficient way to compute molecular surfaces. *Biopolymers.* 38:305–320.

Schubert, W. D., O. Klukas, N. Krauss, W. Saenger, and P. Fromme. 1997. Photosystem I of *Synechococcus elongatus* at 4 Å resolution: comprehensive structure analysis. *J. Mol. Biol.* 272:741–769.

Sheinerman, F. B., R. Norel, and B. Honig. 2000. Electrostatic aspects of protein-protein interactions. *Curr. Opin. Struct. Biol.* 10:153–159.

Sitkoff, D., K. A. Sharp, and B. Honig. 1994. Accurate calculation of hydration free energies using macroscopic solvent models. *J. Phys. Chem.* 98:1978–1988.

Solis, F. J., and R. J. Wets. 1981. Minimization by random search techniques. *Math. Oper. Res.* 6:19–30.

Sommer, F., F. Drepper, and M. Hippler. 2002. The luminal helix l of PsaB is essential for recognition of plastocyanin or cytochrome c6 and fast electron transfer to photosystem I in *Chlamydomonas reinhardtii*. *J. Biol. Chem.* 277:6573–6581.

Srinivasan, J., J. Miller, P. A. Kollman, and D. A. Case. 1998a. Continuum solvent studies of stability of RNA hairpin loops and helices. *J. Biol. Struct. Dyn.* 16:671–682.

Srinivasan, J., T. E. Cheatham, III, P. Cieplak, P. A. Kollman, and D. A. Case. 1998b. Continuum solvent studies of the stability of DNA, RNA, and phosphoramidate-DNA helices. *J. Amer. Chem. Soc.* 120: 9401–9409.

Sun, J., W. Xu, M. Hervas, J. A. Navarro, M. A. Rosa, and P. R. Chitnis. 1999. Oxidizing side of the cyanobacterial photosystem I. Evidence for interaction between the electron donor proteins and a luminal surface helix of the PsaB subunit. *J. Biol. Chem.* 274:19048–19054.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22:4673–4680.

Ubbink, M., M. Ejdeback, 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. *Structures.* 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. Amer. 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.

Vakser, I. A. 1995. Protein docking for low-resolution structures. *Protein Eng.* 8:371–377.

Vakser, I. A., and G. V. Nikiforovich. 1995. Protein docking in the absence of detailed molecular structures. *In* Methods in Protein Structure Analysis. M. Z. Atassi and E. Appella, editors. Plenum Press, New York. 505–514.

Vakser, I. A. 1996a. Long-distance potentials: an approach to the multiple-minima problem in ligand-receptor interaction. *Protein Eng.* 9:37–41.

Vakser, I. A. 1996b. Low-resolution docking: prediction of complexes for underdetermined structures. *Biopolymers.* 39:455–464.

Vakser, I. A. 1996c. Main-chain complementarity in protein-protein recognition. *Protein Eng.* 9:741–744.

Vakser, I. A. 1997. Evaluation of GRAMM low-resolution docking methodology on the hemagglutinin-antibody complex. *Proteins.* 1(Suppl.): 226–230.

Vakser, I. A., O. G. Matar, and C. F. Lam. 1999. A systematic study of low-resolution recognition in protein-protein complexes. *Proc. Natl. Acad. Sci. U.S.A.* 96:8477–8482.

Wang, W., and P. A. Kollman. 2000. Free energy calculations on dimer stability of the HIV protease using molecular dynamics and a continuum solvent model. *J. Mol. Biol.* 303:567–582.

Wang, J., P. Morin, W. Wang, and P. A. Kollman. 2001. Use of MM-PBSA in reproducing the binding free energies to HIV-1 RT of TIBO derivatives and predicting the binding mode to HIV-1 RT of efavirenz by docking and MM-PBSA. *J. Amer. Chem. Soc.* 123:5221–5230.

Zhou, H. X. 1993. Brownian dynamics study of the influences of electrostatic interaction and diffusion on protein-protein association kinetics. *Biophys. J.* 64:1711–1726.