J Mol Model (2005) 11: 265–270
DOI 10.1007/s00894-005-0244-y

ORIGINAL PAPER

V. Renugopalakrishnan · Miguel Ortiz-Lombardía
Chandra Verma

Electrostatics of Cytochrome-c assemblies

Received: 19 September 2004 / Accepted: 4 January 2005 / Published online: 3 May 2005

Abstract Electrostatic potentials along with computational mutagenesis are used to obtain atomic level insights into Cytochrome-c in order to design efficient bionanosensors. The electrostatic properties of wild type and mutant Cytochrome-c are examined in the context of their assembly, i.e. are examined in the absence and presence of neighboring molecules from the assembly. An intense increase in the positive potential ensues when the neighboring molecules are taken into account. This suggests that in the extrapolation of electric field effects upon the design of assemblies, considering the properties of only the central molecule may not be sufficient. Additionally, the influence of the uncharged residues becomes quite diminished when the molecule is considered in an assembly. This could pave the way for making mutants that might be more soluble in different media used in the construction of devices.

Keywords Cytochrome-c · Bionanosensors · Poisson–Boltzmann electrostatic surface · Assemblies

V. Renugopalakrishnan
Bionanotechnology Group,
Department of Biomedical Engineering,
College of Engineering,
Florida International University,
Miami, FL 33174, USA

V. Renugopalakrishnan
Harvard Med School,
Children's Hospital,
Boston, MA 02115, USA

M. Ortiz-Lombardía · C. Verma
Structural Biology Laboratory,
Department of Chemistry,
University of York, York,
YO10 5YW, UK

C. Verma (✉)
Bioinformatics Institute,
30 Biopolis Street,
#07-01 Matrix,
Singapore, 138671
E-mail: chandra@bii.a-star.edu.sg

Introduction

Biological molecules play numerous roles in vivo in sensing the environment and in information processing. There is increasing interest in the integration of some of these molecules with solid-state materials, such as optical wave guides, electrodes and micro-fabricated structures to produce bioelectronic devices. Some of the most developed of these are biosensors where the biological materials (often proteins or nucleic acids) can be deposited as thin films (as sensors) to extend the sensitivity and selectivity of the latter towards chemical or biological substances. We can pack a large number of independent sensors in a small area, thus substantially increasing the signal-to-noise ratio (simply, by statistical averaging). The large signal-to-noise ratio is the critical factor that makes this protein template structure the most robust of all the sensor types currently available. Additionally, the template is in the nanoscale range, thus making it fairly portable. The redox potential of the Cytochrome family of proteins varies over a range of 800 mV from −400 (Cytochrome-c_3) to +400 mV (Cytochrome b_{559}). These properties depend upon the ability of biomolecules to carry and transport charge; this implies that the assemblies will be governed in their behavior by electrostatics. A large body of work has been carried out on the determinants of electron transfer processes in biomolecules [1–3]. Towards building models of this, computer simulations have provided a substantial body of information [4–6]. Current state-of-the-art computer simulation techniques have become quite powerful at revealing information on the structure–function relationships of proteins by providing unique and rich details of atomic fluctuations [7]. They are regularly contributing to the elucidation of molecular mechanisms underpinning fundamental biology [8]. In this study, we construct computer models to understand some of the underlying issues raised above.

We have adopted a twofold strategy to address the issue of obtaining insights from in silico modeling of the

Cytochrome super family of proteins towards the construction of bionanoscale sensors. These are based on two approaches: (a) we gain insights from analyzing the assemblies of the proteins from their crystal structures, in particular we focus on the electrostatic interactions between the protein molecules; (b) we examine the equilibrium properties including conformational changes of these proteins through molecular dynamics simulations. We then hope to build a comprehensive picture of how mutations influence the conformations of the molecules and the associated electrostatics of interactions between the molecules. Such rational site-directed mutagenesis will aid in enhancing the redox potential and increase the efficiency of such biomolecules in biosensor devices.

We imagine a device used for bionanosensor as a compilation of replicated units (these could be identical units or some suitable combinations of interacting units) stacked/arranged in ordered arrays in two or three dimensions. In such a scenario, parameters such as the inter-subunit spacing of the units, assuming that there are no agents that will mediate the information, will determine the efficiency of processes such as electron transfer. One method to optimize the inter-subunit spacing is to examine the distributions that are available from experimental data such as X-ray crystallography. This information is readily available from crystal structures of proteins. This information can, on the one hand, be used in the design of mutants of the native protein structures in order to make the systems more stable and efficient. Additionally, on the other hand, the assemblies of molecules that make a crystal can give us an idea of the intermolecular interactions that stabilize and govern the experimental associations and hence provide invaluable clues towards the design of assemblies. Of course the construction of such devices will also have to take into account variables such as temperature and pressure. The influence of these parameters on the structure and function of a native protein and its mutant variants can be examined using atomic-scale molecular dynamics simulations [9].

Since these processes involve the transportation of charges, it is obvious that the electrostatics characterizing the systems and the concomitant conformational equilibria will play a predominant role [10–12]. Towards providing a useful description of the thermodynamics and functional properties of biomolecules in terms of their electrostatic properties, developments of the Poisson–Boltzmann representations have been tremendously useful [13–15]. Traditionally, their use in understanding interactions within a protein or between proteins has been applied successfully to monomers or dimers or oligomers. Here we apply these methods for the first time to our knowledge to examining the changes and electrostatic properties that ensue between monomers and assemblies in a crystal. As we do not have any relevant experimental methods to compare our data against yet, the following is only an exploratory and hence qualitative study. Again, for this reason, we have deliberately not examined possible mutation-induced conformational changes in this study (a more detailed study is currently being carried out), particularly since it is known that several mutations lead to small local conformational changes [16]. However, given the rapidly increasing interest in the design and assembly of bionanodevices, we hope that the results are interesting enough to stimulate further work by others in this area.

Methods

The crystal structure of Cytochrome-c (PDB entry 1hrc.pdb) [17, 18] resolved at 1.9 Å was used for our models. This structure belongs to the P43 space group. It consists of one heme group with an Fe atom at its center linked to the protein through coordinating His and Met residues. The heme group is associated with the protein through Cys14 and Cys17. The protein consists of 12 acidic residues (Asp/Glu) and 21 cationic residues (Arg/Lys). With Fe in the +2 state. This makes the system highly positively charged (+11 eu), thereby making it ideally suited to accepting a negative charge. We examined the electrostatic properties of the molecule in isolation (in investigations of electrostatic properties of biomolecules, this is the current trend) and when it is surrounded by molecules within 3 Å of the central molecule in the crystal lattice. The protein was represented by a dielectric of 4 while the solvent water was represented as a continuum with a dielectric of 80. The protein was assumed to be at neutral pH and hence all the ionizable residues were assumed to be in their standard ionization states. Formal charges were applied and no computational "titration" to determine the p *K*as of residues [15] carried out. The ionic strength was set to 0.0 as we are investigating properties related to the construction of devices and it is not clear whether salt will play any role in this. The program Delphi [19, 20] was used to compute the electrostatic potentials and the figures were generated using the UCSF Chimera package from the Computer Graphics Laboratory, University of California, San Francisco (supported by NIH P41 RR-01081) [21, 22]. Generation of the crystallographic copies was carried out using the program Coot [23]. There are three regions we have identified as test cases to examine the effects of mutations. We examined the effects based upon the hypothesis that electron-transfer rates will be enhanced if we make Cytochrome-c more positively charged. Since the electron probably tunnels from the donor to the heme region of Cytochrome-c, we decided to examine regions somewhat removed from the entrance to the heme. These were centered on residues Gln12, Asp50, Asn70 and Glu90. In order to examine the influence of a positively charged residue at these regions, we placed a formal unit charge at the terminal regions of these side-chains. Additionally a hydrophobic lid region extending from 81 to 85 was chosen; this region is probably involved in modulating short-range interactions that enable the two systems to dock for

efficient electron transfer. Figure 1 shows the arrangement of the Cytochrome-c molecules in the crystal out of which we extracted a central molecule (in yellow) surrounded by three copies (in green).

Results and discussion

We first examined the available structural data to develop ideas about the determinants of complex formation by Cytochrome-c. Then we looked at the computed electrostatics in the context of the available data.

Similar to a hard-wired electronic circuit, the protein Cytochrome-c transfers electrons between two nodes that are membrane-proteins. It collects electrons from one membrane protein, Cytochrome bc1 (pdb entry 1kyo; on the left in Fig. 2) [24], and delivers them to another membrane protein, Cytochrome oxidase (PDB entry 1oco; on the right in Fig. 2); [25] in a process that underpins the cellular energy-production cycle. The electrons flow across several charged groups within each node with Cytochrome-c, providing the shuttle.

We look at the crystal structure of the complex between Cytochrome-c and Cytochrome-bc1 (on the left in Fig. 2 and shown in secondary structural detail on the left in Fig. 3. The location of Cytochrome-c is highlighted by the circle. We examined this structure in detail by zooming into the area including and near Cytochrome-c (on the right in Fig. 3). Cytochrome-c is shown in pink while the bc1 complex is in yellow. At these small distances (nanometre scales), electrons tunnel directly from one carrier to the next. The heme groups, shown with spheres at each atom (the iron atoms in yellow) are the carrier centers for the electrons. At this close approach between the two proteins, the hemes are brought into proximity to enable the electrons to tunnel from one heme to the next in nanoseconds.

The significance of the large number of charged groups, dominated by cationic residues, is examined by computing the electrostatic potentials, which are then mapped onto the surface of Cytochrome-c. Figure 4 shows the central molecule of the crystal in the center with the three regions outlined for mutations shown in red (Asp50 and Glu90), yellow (Gln12 and Asn70) and the ''hydrophobic lid'' in brown; the heme group is shown in green. The left column shows the electrostatic potential mapped onto the surface when the Cytochrome-c is examined by itself, while the right column shows the electrostatic potential mapped onto the surface of the central Cytochrome-c in the presence of the electric field generated by the surrounding four molecules in the crystal lattice. The first feature that is apparent in all four cases is the intense increase in the positive potential that ensues when the neighboring molecules are taken into account. This suggests that in the extrapolation of electric field effects upon the design of assemblies, care has to be taken when only the central molecule's properties are considered. In addition it seems that the influence of the non-charged residues becomes quite diminished when the molecule is considered in an assembly.

We of course have to include additional data regarding the detailed mechanism underlying interactions between the proteins in complexes such as the one described above. In solution (and in the cell) there are three events that occur in succession: formation of the protein/protein complex (and the incumbent conformational changes); transfer of electrons between the redox centers of the donor and acceptor proteins and dissociation of the protein/protein complex. Parameters that control the associations of proteins are quite complex and poorly understood, although some insights relating to the specific electrostatic interactions (and the residues implicated) and the non-specific non-polar interactions have been elucidated from computer simulation studies [26–28].

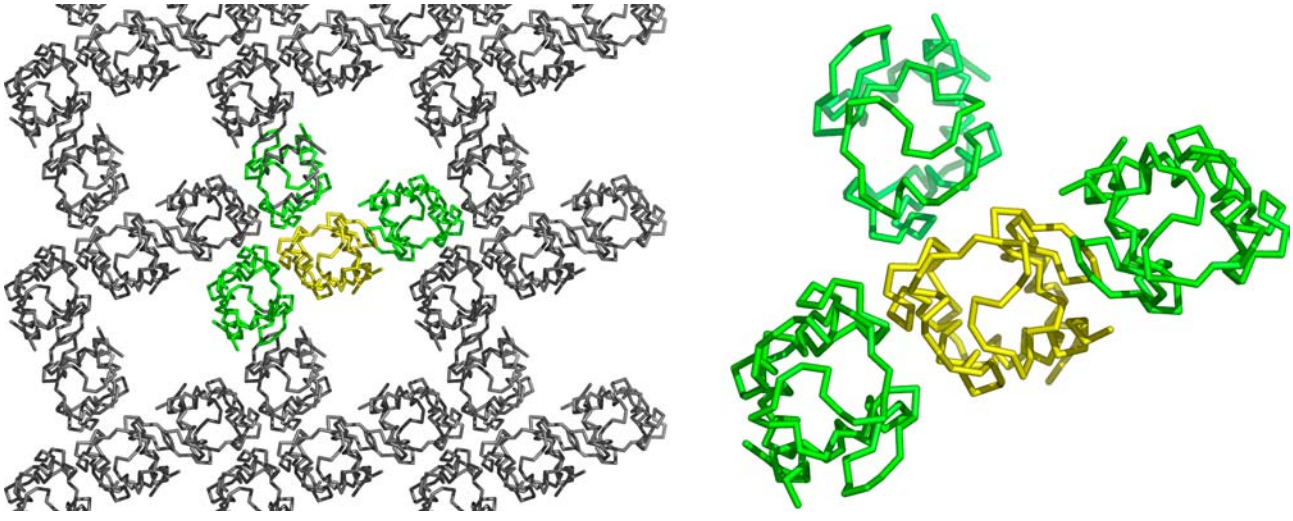


Fig. 1 The crystal lattice generated from the symmetry group P43 that characterizes the structure 1hrc of Cytochrome-c (on the *left*); the central four molecules shown in *yellow* and *green* and *enlarged* on the *right* have been used for the present calculations

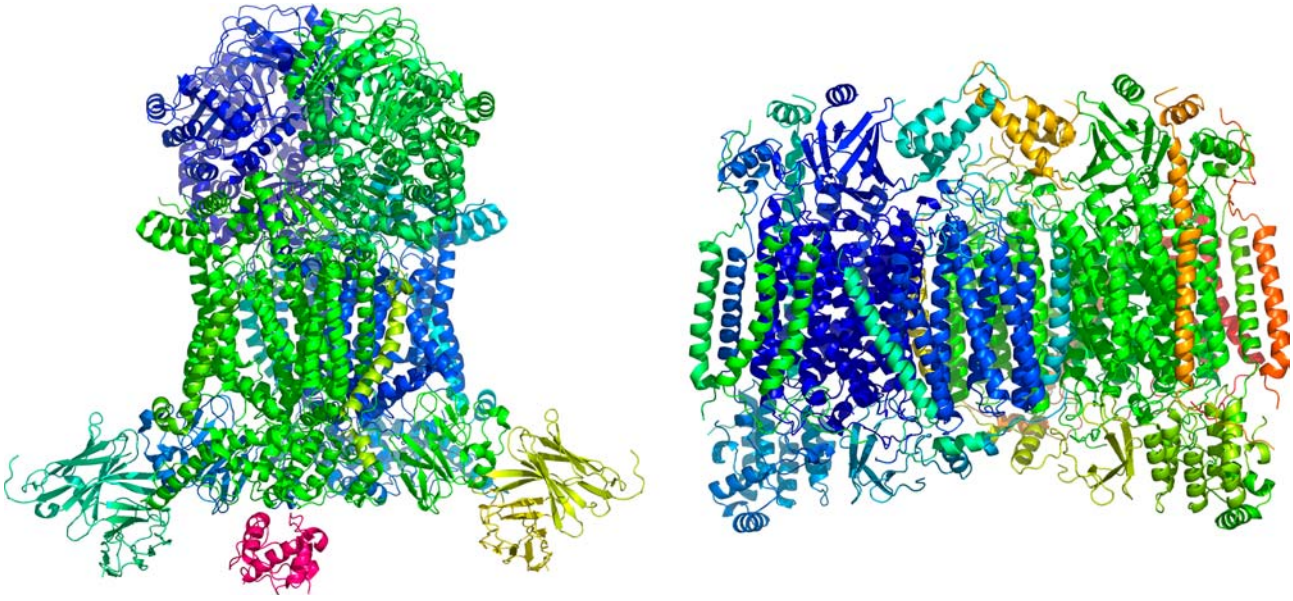
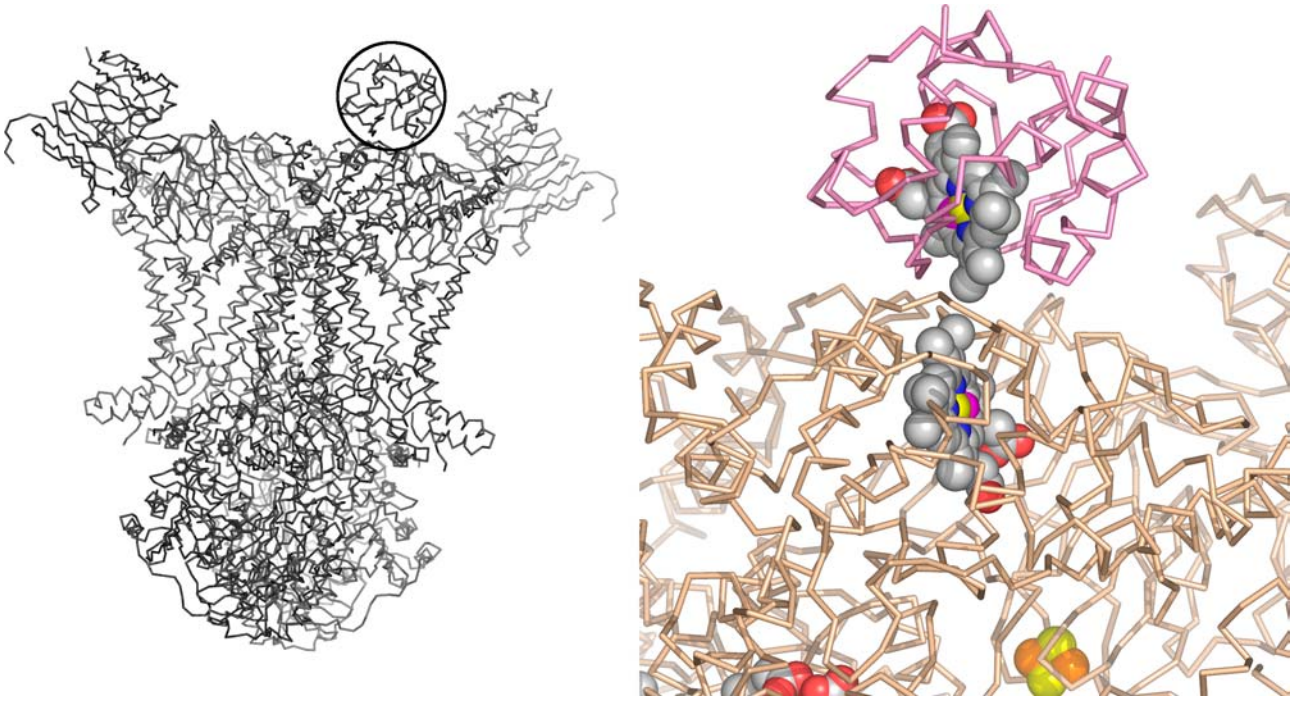


Fig. 2 The Cytochrome bc1 complex is shown on the left from PDB entry 1kyo and the Cytochrome oxidase complex is shown on the right from PDB entry 1oco (Illustration taken from Molecule of the month 2000 by David S. Goodsell of The Scripps Research Institute; http://www.rcsb.org/pdb/molecules/pdb36_2.html; reprinted with permission)

In Cytochrome-c features that may help modulate the capture and release of electrons could be either the carrier of the electron (the heme group), the amino acids surrounding the heme, the collective motions of the protein as a whole and some appropriate combination of the three [29, 30]. Additionally, one could design small molecules that could act as electron carriers between the designed proteins. This could lead to greater flexibility in the designs as it obviates the need for orientational dependence between the protein subunits. On the other hand, the residues surrounding the heme group may be mutated in order to modulate the electron carrying/

Fig. 3 On the *left* is the secondary structure representation of the crystal structure of the complex between Cytochrome b1 complex-Cytochrome-c (PDB entry1kyo.pdb). Cytochrome-c is shown *circled*. On the *right* is the close up of the interface of the complex of Cytochrome-c (at the *top*) and the large Cytochrome bc1 complex at the *bottom*. The protein chain in Cytochrome-c is shown in *pink tubes* and the protein chains in the bc1 complex are shown in *beige*. The hemes are shown with *spheres* at each atom, with the iron atoms in *yellow*, S atoms (in the Fe–S cluster in *orange*, C atoms in *grey*, N atoms in *blue* and O atoms in *red* (figure adapted from David Goodsell of the Scripps Research Institute; http://www.rcsb.org/pdb/molecules/pdb36_3.html; reprinted with permission)



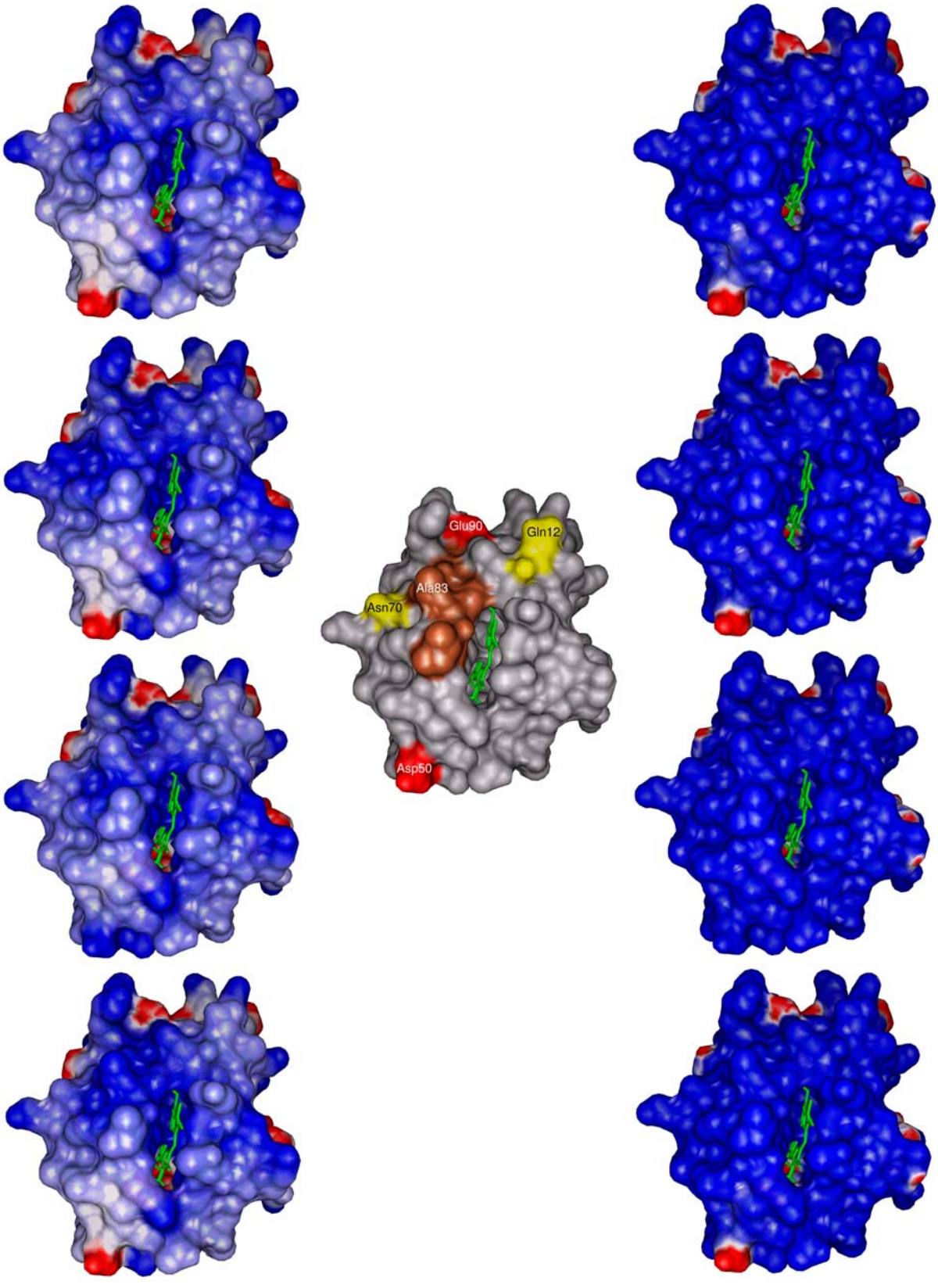


Fig. 4 The electrostatic potential, calculated using the program DELPHI [20] mapped on to the surface of Cytochrome-c when it is considered by itself (in the *left column*) and in the presence of the electrostatic field generated by the presence of the surrounding four molecules on the *right* (see Fig. 3). The potentials range from –10 kT in *red* to +10 kT in *blue*. The *central* figure shows the regions that have been mutated to positively charged residues by placing a unit positive charge at the terminal atom of the respective side chain. The figures range from the wild type in the *first row*, followed by the Gln12, Asn70, Asp50, Glu90 and Ala83 mutants

transfer potentials of Cytochrome-c [3, 31]. This could be complemented with mutations at regions that modulate protein–protein interactions at sites that are remote from the actual seat of the electron; additionally they could modulate the properties through collective motions [9]. Moreover, we are currently investigating the differences in potentials between neighboring molecules and how it might influence the electron-transfer properties. The present study looked at one instance of the influence of a certain assembly only as it was purely exploratory. This is currently being extended to a more systematic analysis, including the influence of dimers, trimers, etc. Unfortunately (to our knowledge) the experimental data that do exist [27, 28] on these systems are not suitable to test or benchmark our hypotheses. However, we hope that the results are sufficiently interesting in the current interests in bionanotechnology that it will lead to further and detailed experimental and theoretical investigations.

Summary

Structural examinations of the electron-transfer protein Cytochrome in isolation and in association with its partners suggest that in the design of devices that are constructed from assemblies of the protein, the electrostatic potentials are enhanced in the presence of other molecules in an assembly. This needs to be taken into account while modeling and constructing nanodevices for data storage/transmission using molecules such as Cytochrome-c as the replicating unit.

Acknowledgements The majority of the work was carried out at the Structural Biology Laboratory, University of York, UK. The bionanotechnology group expresses its thanks to Florida International University for support of the research described in this paper. BII is an A-STAR institute. We thank the referees for valuable comments.

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