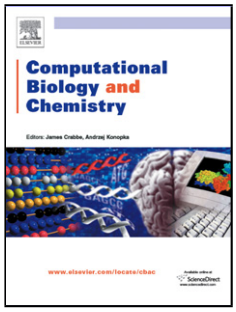

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PII: S1476-9271(13)00055-8
DOI: http://dx.doi.org/doi:10.1016/j.compbiolchem.2013.05.004
Reference: CBAC 6268

To appear in: *Computational Biology and Chemistry*

Received date: 23-4-2013
Revised date: 29-5-2013
Accepted date: 30-5-2013

Please cite this article as: Masone, D., Aloia, F.C., Del Pópolo, M.G., H-bond refinement for electron transfer membrane-bound protein-protein complexes: cytochrome c oxidase and cytochrome c552., *Computational Biology and Chemistry* (2013), http://dx.doi.org/10.1016/j.compbiolchem.2013.05.004

H-bond refinement for electron transfer membrane-bound protein-protein complexes: cytochrome c oxidase and cytochrome c552.

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Abstract: *In this study we propose a protocol to evaluate membrane-bound cytochrome c oxidase - cytochrome c552 docking candidates. An initial rigid docking algorithm generates docking poses of the cytochrome c oxidase – cytochrome c552, candidates are then aggregated into a 512-DPPC membrane model and solvated in explicit solvent. Molecular dynamic simulations are performed to induce conformational changes to membrane-bound protein complexes. Lastly each protein-protein complex is optimized in terms of its hydrogen bond network, evaluated energetically and ranked. The protocol is directly applicable to other membrane-protein complexes, such as protein-ligand systems.*

Keywords: *cytochrome c; docking; membrane; DPPC; protein-protein; molecular dynamics;*

1. Introduction

The aim of the present work is to find a protein-protein complex model for cytochrome c oxidase / cytochrome c552 applying a combination of three different methods. First, a rigid docking algorithm is used to efficiently explore the configurational protein-protein space. Second, large scale molecular dynamics is used to embed the protein-protein docking candidates into a membrane model. Finally, a hydrogen bond network refinement algorithm is used for proper energetic ranking of the docking candidates.

Electron transfer is a fundamental process in biology, chemistry and physics and an important stage in many enzymatic cycles (Beratan et al., 1992; Langen et al., 1995). The process involves the flow

of electron density from a donor to an acceptor molecule (Kendrew et al., 1958; Dawson et al., 1998). It plays a central role in many biochemical processes and for this reason, a detailed understanding of electron transfer reactions at the molecular level is of essential importance. Not surprisingly, computational techniques are an attractive tool in mapping electron transfer mechanisms (Balabin and Onuchic, 2000; Gehlen et al., 1996). Obtaining an atomic description of the transfer pathway is however a difficult task, at both the experimental and theoretical levels. The process can involve a short pathway, *e.g.* from a substrate or a cofactor directly bound in the vicinity of the acceptor group, or rather large pathways, *e.g.* across protein-protein complexes, where the donor and acceptor might be relatively distant from each other. Molecular-level structural details are thus extremely relevant to electron transfer processes and should be taken into account in docking algorithms in order to increase the accuracy of the protocols, and to generate more reliable and realistic docking candidates for electron transfer reactions in biological molecules.

Cytochrome c552 is a soluble heme protein playing a crucial role in the mitochondrial respiratory chain and is responsible for electron transfer with its redox partners, such as: cytochrome c reductase, cytochrome c oxidase, and cytochrome c peroxidase (Kim, 2000; Berini 2011). Cytochrome c552 is located in the inter membrane space and weakly binds to the inner mitochondrial membrane. As pointed out in previous studies cytochrome c552 is released from the mitochondria to the cytosol in response to different apopotosis-inducing agents, (Kluck et al., 1997). The role of cytochrome c552 in this important cellular process and the nature of cytochrome c oxidase interactions with model lipid membranes has motivated several studies (El Kirat and Morandat, 2009; Rytömaa et al., 1992).

Some studies have shown that cytochrome c oxidase dissociates from lipid membranes at high

ionic strengths, (Rytömaa, 1994), although it is not yet clear whether cytochrome c oxidase binds to the membrane due to sufficient intermembrane ionic strength that obstruct electrostatic protein-protein recognition, (Cortese et al., 1991). Other studies have proposed that cytochrome c oxidase remains partially bound to the membrane even at high ionic strengths (Cortese et al., 1995; Cortese et al., 1998), and that the protein interacts with phospholipids via hydrophobic forces (Rytömaa et al., 1992; Snel et al., 1994). Yet, other studies have demonstrated that a partially inserted cytochrome c oxidase can be found in the inner mitochondrial membrane with an important role in apoptosis (Ott et al., 2007). Kostrzewa et al. (2000) showed that when cytochrome c552 binds to negatively charged lipids it orients lysine residues towards the membrane surface, exposing the heme group to electron transfer.

To the present the molecular structure of the Paracoccus denitrificans protein-protein complex cytochrome c oxidase / cytochrome c552 has no crystallographic solution. However an NMR study by Wienk et al. (2003) showed small chemical shift changes during the interaction of cytochrome c552 and the soluble CuA domain in cytochrome c oxidase. In this work equivalent effects were found for fully reduced and oxidized systems, showing that the protein-protein interaction is practically independent of the redox states of the binding partners. Moreover, the relevance of several positive lysine residues in the long-range electrostatic protein-protein recognition processes was highlighted. The cited study has also agreed with previous works by Witt et al. (1998) and Drosou et al. (2002) and to previous models proposed for the Paracoccus denitrificans cytochrome c552 and cytochrome c oxidase system (Witt et al., 1998a, 1998b).

Flock and Helms (Flock and Helms, 2002), proposed a computational rigid-body docking model based on FTDock followed by energy minimization. The study found cytochrome c552 in two critically

different orientations (~95°) when docked against the two or the four subunit structures of the Paracoccus denitrificans oxidase. Additionally it was suggested that the bound complex exists as a dynamic ensemble of different orientations. However, this docking model predicts that some residues located at the binding interface do not show the chemical shifts reported in the work of Wienk et al. (2003).

2. Materials and Methods

In the soil bacterium Paracoccus denitrificans the unusually fast terminal electron transfer step (Tipmanee and Blumberger, 2012) from heme a in cytochrome bc1 (complex III of the respiratory chain) to heme a3 in cytochrome c oxidase (complex IV of the respiratory chain) is mediated by membrane bound cytochrome c552 (Berry and Trumpower, 1985; Turba et al., 1995). The reduced cytochrome c552 and the subunit II of cytochrome c oxidase hydrophilic domain interact electrostatically through positively charged lysine residues on cytochrome c552 surface and the binuclear Cu_A center in subunit II of cytochrome c oxidase. Lysine residues around the exposed heme edge in cytochrome c552 are thought to be responsible for long range electrostatic protein-protein recognition (Witt et al., 1998a). Previous studies have shown the importance of the interaction of this lysine cluster in cytochrome c552 with negatively charged lipids (Rytömaa et al., 1992). Further analysis (Witt et al., 1998b) has shown minor interfacial conformational changes that allow and extra electron transfer from the reduced heme in cytochrome c552 to the Cu_A center in subunit II of cytochrome c oxidase. Residue tryptophan 121 (the electron transfer entry site) on the interface of subunit II of cytochrome c oxidase mediates the heme-copper electronic transfer process (Witt et al., 1998b). These observations are consistent with previous studies (Briggs and Capaldi, 1978; Millet et al. 1982; Lalla et al., 2001).

In the present work we describe a computational protocol that allows the analysis of protein-protein complexes bound to a membrane patch. The method can be easily extrapolated to other membrane-bound systems, such as protein-ligand and cell penetrating peptides. Figure 1 shows a block diagram detailing the process:

Figure 1

2.1. Protein-protein docking of cytochrome c oxidase and cytochrome c552.

Using HEX (Ritchie and Kemp, 2000) we have generated 100 initial rigid-body docking poses, in the atomistic space, for the membrane unbound form of the complex (pdb codes 1ar1 and 1ql3). Rigid docking was performed in vacuum. These docking candidates were then geometrically clustered and filtered to fulfill a distance condition for electron transfer to be possible: only docking candidates with heme-copper distance < 20Å were kept for further analysis. From HEX data we kept 19 candidates for further refinement with molecular dynamics, (see figure 2).

Figure 2

2.2 Pore opening in the membrane patch.

We performed molecular dynamics in GROMACS (Van der Spoel et al., 2005) patched with Plumed (Bonomi et al., 2009) to implement a collective reaction coordinate in order to open a pore in a 512 DPPC membrane patch of 12nm length (see figure 3) solvated in explicit water. We followed Topekina et al. (2004) description for pore formation, where the local density of the lipids is lowered by a reaction coordinate defined as:

$$\xi = \frac{\Sigma - \Sigma_0}{\Sigma_M - \Sigma_0} \quad (1) \quad \text{with} \quad \Sigma = \sum_i \tanh(S r_i) \quad (2)$$

where r_i is the XY plane distance projection from every membrane atom to the pore center, S controls the size of the pore, $\sum_M$ is the total number of atoms in the membrane (25 600) and $\sum_0$ = 25 000 is the equilibrium value of sigma from an unrestrained simulation. As defined, the reaction coordinate allows for a maximum pore size when $\xi=1$, corresponding to a 3nm radius pore. This collective reaction coordinate is able to control the local density of the membrane lipids, without explicitly affecting lipids reorientation during the pore-opening process. In fact, lipid reorientation occurs spontaneously at long simulation times, as the resulting pore is hydrophilic.

Figure 3

2.3 Drag cytochrome c552 - cytochrome c oxidase candidates into the membrane patch.

During the pore opening process we dragged the protein complex candidates into the membrane sufficiently fast, in order not to allow too many water molecules to enter the pore. This was done using a second reaction coordinate by pulling the center of mass of the protein-protein candidate towards the membrane center (see figure 4). The water molecules that entered the pore were deleted and the system was re-equilibrated.

Figure 4

The need for pore opening becomes mandatory to facilitate the protein membrane aggregation process. Moreover, an incomplete reaction coordinate only dragging the protein into the membrane (such as the distance between membrane's and cytochrome's centers of mass) might not allow lipids to

accommodate sufficiently fast and might generate always-changing energy profiles. Hence, a collective reaction coordinate that predicts and takes into account as many conformational changes as possible, allows sampling the system more efficiently in computational terms. This is the case for the simultaneous pore-opening and protein-dragging coordinate we have used.

2.4 Pore closing and equilibration of each protein-protein membrane aggregated system.

The pore-opening / protein-dragging processes took less than 1ns of simulation time. However the protein-membrane system requires almost 5ns to equilibrate, in order to allow the interface between the protein and the membrane to relax while the pore gradually closes. In this way we take into account the effect of the membrane on the protein complex structure. As pointed out by Heimburg et al. (1999) membrane lipids' respond reorganizing themselves when exposed to binding macromolecules. Figure 5 shows membrane reorganization due to protein complex aggregation.

Figure 5

2.5 Hydrogen bond network optimization and scoring of docking candidates after membrane

induced conformational changes.

Each docking candidate was then removed from the membrane patch and evaluated energetically in vacuum. This last step was performed with a hydrogen bond network optimizer, 3DRefine (Bhattacharya and Cheng, 2013) since, as shown in a previous work (Masone et al., 2012), energetic evaluation of docking candidates improves dramatically when hydrogen bonds are optimized. From this last process the best candidates are identified.

2.6 Simulation details

The crystal structure of the fully-oxidized two-subunit cytochrome c oxidase from Paracoccus denitrificans at 2.7Å resolution is available from the Protein Data Bank (PDB) under the code 1AR1 (Ostermeier et al., 1997). This crystallographic model includes a Mg2+ ion, between subunits I and II linked to His403, Asp404 and Glu218 and a Ca2+ ion interacting with His59, Gly61, Gln63 and Glu56. Analogously, the 1.4Å resolution crystal structure of the soluble domain of cytochrome c552 from Paracoccus denitrificans in the reduced state can be found under the code 1QL3 (Harrenga et al., 2000).

In order to generate rigid docking candidates HEX was run online through its freely available web server [http://hexserver.loria.fr/index.php] using both electrostatics and shape complementarity. The search order parameter was set to 25 and no origin or interface residues were indicated in order not to bias

the search and to explore in an equal manner the configurational protein-protein space. Range angles for both receptor and ligand proteins were set to the maximum value of 180^{o} and the step size was set to 7.5. The filtering of the docking candidates was done by geometrically discarding any configuration not satisfying the distance condition R<20Å, where R is the distance between the centers of mass of cytochrome c552 heme group and Cu ions in cytochrome c oxidase. This step is necessary due to the docking approach we chose, meaning that the docking exploration was unbiased and only guided by shape complementarity and electrostatics interactions. It is a post-processing task to discard candidates not fulfilling the imposed geometric conditions that are mandatory for the electron transfer to take place.

The membrane patch used for protein aggregation consisted of 512 zwitterionic dipalmitoylphosphatidycholine (DPPC) phospholipids modeled with Berger's force-field (Berger et al., 1997). The whole system was solvated in a SPC water box of almost 70,000 water molecules. Large scale molecular dynamic simulations were then performed using GROMACS 4.5.5 patched with Plumed 1.3, for collective reaction coordinates implementation. The simulation temperature was set to 323K in all cases, using a Berendsen barostat and a V-rescale thermostat. Also, there were no area fluctuation constrains for the membrane in any direction. Simulations were extended to 5ns for equilibration purposes for each one of the 19 docking candidates generated by HEX and embedded in the membrane.

As hydrogen bonds have shown to be important in docking scoring (Masone et al., 2012), and since these interactions may stabilize secondary structure beta-sheets and alpha-helices in protein-ligand docking candidates (Williams and Ladbury, 2003), we used a dedicated algorithm for hydrogen bond network optimization as a refinement step after docking. The structural refinement was performed with the recently available free access web server 3DRefine [http://sysbio.rnet.missouri.edu/3Drefine/], which

inexpensively combines hydrogen bond optimization with energy minimization. Not surprisingly bonding between hydrogen atoms and electronegative elements may result in a smaller distance than the sum of the van der Waals radii, it is that hydrogen bonds are not so easy to model for classical molecular mechanics programs (Ponder and Case, 2003). Importantly, molecular dynamics simulations have shown greater stability when started from optimized structures instead of unoptimized ones (Hooft et al., 1996). All figures were generated with VMD, (Humphrey qt al., 1996).

3. Results and Discussion

Our cytochrome c552 and cytochrome c oxidase protein-protein conformation agrees well with experimental NMR results from Wienk et al. (2003) and Witt et al. (1998a). In this model, positive lysine residues located on cytochrome c552 surface in the surroundings of the heme group are responsible, upon binding, for the long-range electrostatics that allows protein-protein recognition. Additionally, another recent NMR study by Sakamoto et al. (2011) of the reduced and oxidized forms of cytochrome c552 has reinforced the importance of lysine residues (Lys13, Lys72, Lys86, Lys27, and Lys87) on the surface of cytochrome c552 for protein-protein recognition. Finally, and as extensively described by Witt et al. (1998b), the importance of residue TRP121 of subunit II of cytochrome c oxidase as the entry site in the electron transfer process is highlighted and shown in figure 6.

Figure 6

As described by Wienk et al. (2003), most cytrochrome c552 surface lysine residues certainly contribute to protein-protein recognition, as over 40% of our rigid-body docking candidates are concentrated in the vicinity of the heme-copper bridge, due to the dominant electrostatic interactions between positively charged lysine residues and patches of opposite charge above the Cu_A center of cytochrome c oxidase, that lead protein-protein recognition. However, most lysine residues remain out of the contact surface between protein partners and do not necessarily form part of the final protein-protein complex interface (see figure 7). Consequently, we have found differences between our predictions and the ones by Flock et al. (2002), where a docking model was also proposed but not in agreement with results by Wienk et al. (2003). In particular, this docking model relies on multiple electrostatic contacts between cytochrome c552 and cytochrome c oxidase, but some of the positively charged residues on the cytochrome c552 surface predicted to be part of the protein-protein interface did not show noticeable chemical shifts in the study by Wienk et al. (2003), ending up with a docking model with a highly rotated ligand protein.

Figure 7

Figure 8 shows the conformational changes of the protein complex when relaxed in its membrane-bound form. It is observed how cytochrome c552 rotates to form the heme-cooper bridge.

Figure 8

When using crystallographic complexes from the PDB for computer simulations, an important problem is the positioning of every hydrogen atom. Here ambiguities arise depending on the software used to this end. Examples of usual problems are the asparagine and glutamine side-chains, which contain a terminal amide group that can fully rotate 180^{o} with no relevant changes in the electron density. Also, histidines' imidazole rings can exhibit three possible protonation patterns, while aspartates and glutamates may be neutral or negatively charged, with the hydrogen on the two terminal side-chain oxygens. To efficiently reposition a protein's hydrogen bond network, the force-field used for the energetic calculations needs to properly describe every possible hydrogen configurations and their interaction energies. Running protein-docking simulations with badly oriented or mis-protonated side-chains, can lead to an increase of false positives, as well as to the impossibility to discriminate near-native poses (Webster, 2000). Hence, information from the chemical environment, specially the highly interdependent hydrogen bond network, needs to be included in simulation protocols. Figure 9 shows H-bond network optimization and energy minimization for each of 19 docking candidates after membrane-bound molecular dynamics. The main energy differences between the candidates are due to the amount of hydrogen bond formation during optimization.

Figure 9

Three similar candidates (with a ligand protein C_α RMSD< 10Å between them) are identified as having the lowest energies after optimization. For the three of them residue TRP121 mediates the electron transfer and have cytochrome c552 surface lysine residues mostly outside the protein-protein interface.

Our protocol has shown to be a potentially useful tool for testing experimental models. When some information about the binding process is known, a simple rigid-body docking algorithm based on electrostatics and shape complementarity is enough to efficiently explore the configurational protein-protein space. In a second step it is easy to reduce the amount of generated poses by limiting the distance between the binding sites belonging to binding cofactors where electron transfer takes place. Major conformational changes are induced to protein-protein complexes, in both side-chains and backbone by equilibration under their membrane-bound form. Finally, minor interface rearrangements are produced by hydrogen-bond optimizations and energy minimizations so that docking candidates can be properly ranked in vacuum. Importantly we have obtained lowest energies for the complexes corresponding to the geometrically most favorable electron transfer protein-protein candidates.

Acknowledgments

This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). The authors would like to thank the Instituto de Ciencias Básicas (ICB) - Universidad Nacional de Cuyo (UNCUYO) and Universidad Nacional de Córdoba (UNC) for supercomputing time.

References

Balabin, I., Onuchic, J., 2000. Dynamically controlled protein tunneling paths in photosynthetic reaction centers. Science 290, 114-117.

Beratan, D.N., Onuchic, J.N., Winkler, J.R., Gray, H.B., 1992. Electron-tunneling pathways in proteins. Science 258, 1740.

Berendsen, H.J.C., van der Spoel, D., van Drunen, R., 1995. GROMACS: A message-passing parallel molecular dynamics implementation. Computer Physics Communications 91(1–3), 43–56.

Berger, O., Edholm, O., Jähnig, F., 1997. Molecular dynamics simulations of a fluid bilayer of dipalmitoylphosphatidylcholine at full hydration, constant pressure, and constant temperature. Biophys J. 72(5), 2002-2013.

Berry, E.A., Trumpower, B.L., 1985. Isolation of ubiquinol oxidase from Paracoccus denitrificans and resolution into cytochrome bc1 and cytochrome c-aa3 complexes. J. Biol. Chem. 260(4), 2458-2467.

Bertini I., Chevance S., Del Conte R., Lalli D., Turano P., 2011. The Anti-Apoptotic Bcl-xL Protein, a New Piece in the Puzzle of Cytochrome C Interactome. PLoS ONE 6, 4.

Bhattacharya D., Cheng J., 2013. 3Drefine: Consistent protein structure refinement by optimizing hydrogen-bonding network and atomic-level energy minimization. Proteins: Structure, Function and Bioinformatics 81(1), 119–131.

Bonomi, M., Branduardi, D., Bussi, G., Camilloni, C., Provasi, D., Raiteri, P., Donadio, D., Marinelli, F., Pietrucci, F., Broglia, R.A., Parrinello, M., 2009. PLUMED: a portable plugin for free energy calculations with molecular dynamics, Comp. Phys. Comm. 180, 1961.

Briggs, M., Capaldi, R., 1978. Cross-linking studies on a cytochrome c –cytochrome c oxidase complex. Biochem. Biophys. Res. Com. 80, 553–559.

Cortese, J.D., Voglino, A.L., Hackenbrock, C.R., 1991. Ionic strength of the intermembrane space of intact mitochondria as estimated with fluorescein-BSA delivered by low pH fusion. J. Cell Biol. 113(6), 1331-1340.

Cortese, J.D., Voglino, A.L., Hackenbrock, C.R.,1995. Persistence of cytochrome c binding to membranes at physiological mitochondrial intermembrane space ionic strength. Biochim. Biophys. Acta 1228(2-3), 216-228.

Cortese, J.D., Voglino, A.L., Hackenbrock, C.R., 1998. Multiple conformations of physiological membrane-bound cytochrome c. Biochemistry 37, 6402-6409.

Dawson, J., 1988. Probing structure-function relations in heme-containing oxygenases and peroxidases.

Science 240, 433-439.

Drosou, V., Reincke, B., Schneider, M., Ludwig, B., 2002. Specificity of the interaction between the Paracoccus denitrificans oxidase and its substrate cytochrome c: comparing the mitochondrial to the homologous bacterial cytochrome c(552), and its truncated and site-directed mutants. Biochemistry 41, 10629-10634.

El Kirat, K., Morandat, S., 2009. Cytochrome c interaction with neutral lipid membranes: influence of lipid packing and protein charges. Chemistry and Physics of Lipids 162, 17–24.

Flöck, D., Helms, V., 2002. Protein-protein docking of electron transfer complexes: cytochrome c oxidase and cytochrome c. Proteins 47, 75–85.

Gabb, H.A., Jackson, R.M., Sternberg, M.J.E., 1997. Modelling Protein Docking using Shape Complementarity, Electrostatics and Biochemical Information. Journal of Molecular Biology 272(1), 106-120.

Gehlen, J., Daizadeh, I., Stuchebrukhov, A., Marcus, R., 1996. Tunneling matrix element in Ru-modified blue copper proteins: pruning the protein in search of electron transfer pathways. Inorg. Chim. Acta 243, 271.

Harrenga, A., Reincke, B., Ruterjans, H., Ludwig, B., Michel, H., 2000. Structure of the soluble domain c522 from Paracoccus denitrificans in the oxidized and reduced states. J. Mol. Biol. 295, 667–678.

Heimburg, T., Angerstein, B., Marsh, D., 1999. Binding of peripheral proteins to mixed lipid membranes:

The effect of local demixing upon binding. Biophys. J. 76, 2575–2586.

Hooft, R.W.W., Sander, C., Vriend, G., 1996. Positioning hydrogen atoms by optimizing hydrogen-bond networks in protein structures. PROTEINS 26, 363-376.

Humphrey, W., Dalke, A., Schulten, K., 1996. VMD - Visual Molecular Dynamics. J. Molec. Graphics 14.1, 33-38.

Kendrew, J.C., Bodo, G., Dintzis, H.M., Parrish, R.G., Wyckoff, H., Phillips, D.C., 1958. A Three-Dimensional Model of the Myoglobin Molecule Obtained by X-Ray Analysis. Nature 181, 662.

Kim, U., Kim, Y.S., Han, S., 2000. Modulation of Cytochrome c-Membrane Interaction by the Physical State of the Membrane and the Redox State of Cytochrome c. Bull. Korean Chem. Soc. 21, 4.

Kluck, R.M., Bossy-Wetzel, E., Green, D.R:, Newmeyer, D.D., 1997. The Release of Cytochrome c from Mitochondria: A Primary Site for Bcl-2 Regulation of Apoptosis. Science 275, 1132-1136.

Kostrzewa, A., Pali, T., Froncisz, W., Marsh, D., 2000. Membrane location of spin-labeled cytochrome c determined by paramagnetic relaxation agents. Biochemistry 39, 6066–6074.

Lalla, A., Alleyne, T., Andrews, Y., 2001. Understanding the mechanism of action of cytochrome c oxidase in normal and disease states: shark heart enzyme, potential model. West Indian Med. J. 50, 111–116.

Langen, R., Chang, I.J., Germanas, J.P., Richards, J.H., Winkler, J.R., Gray, H.B., 1995. Electron-

Tunneling in Proteins - Coupling through a b-Strand. Science 268, 1733.

Masone, D., Cabeza De Vaca, I., Pons, C., Fernandez Recio, J., Guallar, V., 2012. H-bond network optimization in protein–protein complexes: Are all-atom force field scores enough? Proteins: Structure, Function And Genetics 80, 818-824.

Millet, F., Darley-Usmar, V., Capaldi, R., 1982. Cytochrome c is cross-linked to subunit II of cytochrome c oxidase by a water- soluble carbodiimide. Biochemistry 21, 3857–3862.

Ostermeier, C., Harrenga, A., Ermler, U., Michel, H., 1997. Structure 2.7A resolution of the Paracoccus denitrificans two-subunit cytochrome c oxidase complexed with an antibody F-V fragment. Proc. Natl. Acad. Sci. USA. 94, 10547–10553.

Ott, M., Gogvadze, V., Orrenius, S., Zhivotovsky, B., 2007. Mitochondria, oxidative stress and cell death. Apoptosis 12(5), 913-22.

Ponder, J., Case, D., 2003. Force fields for protein simulations. Adv. Prot. Chem. 66, 27-85.

Rytömaa, M., Mustonen, P., Kinnunen, P.K., 1992. Reversible, nonionic, and pH-dependent association of cytochrome c with cardiolipin-phosphatidylcholine liposomes. J Biol Chem. 267(31), 22243-8.

Rytömaa, M., Kinnunen P.K., 1994. Evidence for two distinct acidic phospholipid-binding sites in cytochrome c. J Biol Chem. 269(3), 1770-4.

Ritchie D.W., Kemp, G.J.L., 2000. Protein Docking Using Spherical Polar Fourier Correlations. PROTEINS: Struct. Funct. Genet. 39, 178-194.

Sakamoto, K., Kamiya, M., Imai, M., Shinzawa-Itoh, K., Uchida, T., Kawano, K., Yoshikawa, S., Ishimori, K., 2011. NMR basis for interprotein electron transfer gating between cytochrome c and cytochrome c oxidase. Proc. Natl. Acad. Sci. USA 108(30), 12271-6.

Snel, M.M.E., de Kruijff, B., Marsh, D, 1994. Interaction of spin-labeled apocytochrome *c* and spin-labeled cytochrome *c* with negatively charged lipids studied by electron spin resonance. Biochemistry 33, 7146-7156.

Tipmanee, V., Blumberger, J., 2012. Kinetics of the terminal electron transfer step in cytochrome c oxidase. J Phys Chem B. 116(6), 1876-83.

Tolpekina, T.V., den Otter, W.K., Briels, W.J., 2004. Nucleation free energy of pore formation in an amphiphilic bilayer studied by molecular dynamics simulations. J. Chem. Phys. 121, 12060.

Turba, A., Jetzek, M., Ludwig, B., 1995. Purification of Paracoccus denitrificans cytochrome c552 and sequence analysis of the gene. Eur. J. Biochem. 231(1), 259-265.

Webster, D.M., 2000. Protein structure prediction: methods and protocols, first edition. Humana Press.

Williams, M.,Ladbury, J., 2003. Protein-Ligand Interactions, From Molecular Recognition to Drug Design. WILEY-VCH Verlag GmbH & Co.

Wienk, H., Maneg, O., Lücke, C., Pristovsek, P., Löhr, F., Ludwig, B., Rüterjans, H., 2003. Interaction of cytochrome c with cytochrome c oxidase: an NMR study on two soluble fragments derived from Paracoccus denitrificans. Biochemistry 42(20), 6005-12.

Witt, H., Malatesta, F., Nicoletti, F., Brunori, M., Ludwig, B., 1998a. Cytochrome-c-binding site on cytochrome oxidase in Paracoccus denitrificans. Eur. J. Biochem. 251, 367–373.

Witt, H., Malatesta, F., Nicoletti, F., Brunori, M., Ludwig, B., 1998b. Tryptophan 121 of subunit II is the electron entry site to cytochrome-c oxidase in Paracoccus denitrificans. J. Biol. Chem. 273, 5132–5136.

Van der Spoel, D., Lindahl, E., Hess, B., Groenhof, G., Mark, A.E., Berendsen, H.J., 2005. GROMACS: fast, flexible, and free. J Comput Chem 26, 1701–1718.

Figure legends:

Figure 1: Block diagram of the computational protocol.

Figure 2: Superposition of the 19 protein-protein docking candidates, HEME groups highlighted in yellow, Cu_A ions in red. All docking candidates fulfill a geometric condition for the electron-transfer to be possible.

Figure 3: Pore opening in the membrane patch (water molecules not shown). DPPC heads (P) are highlighted in yellow.

Figure 4: Protein complex dragging into the membrane (water molecules not shown). Heme groups and Cu_A ions are highlighted.

Figure 5: Membrane reorganization due to protein complex aggregation. Red spheres around the protein complex are the phosphate groups of DPPC lipids that have rotated towards the protein.

Figure 6: Residue TRP121 residue (yellow) as the entry site in the electron transfer process. Heme groups and Cu_A ions.

Figure 7: Membrane-bound docking candidate, (water molecules not shown). Most lysine residues

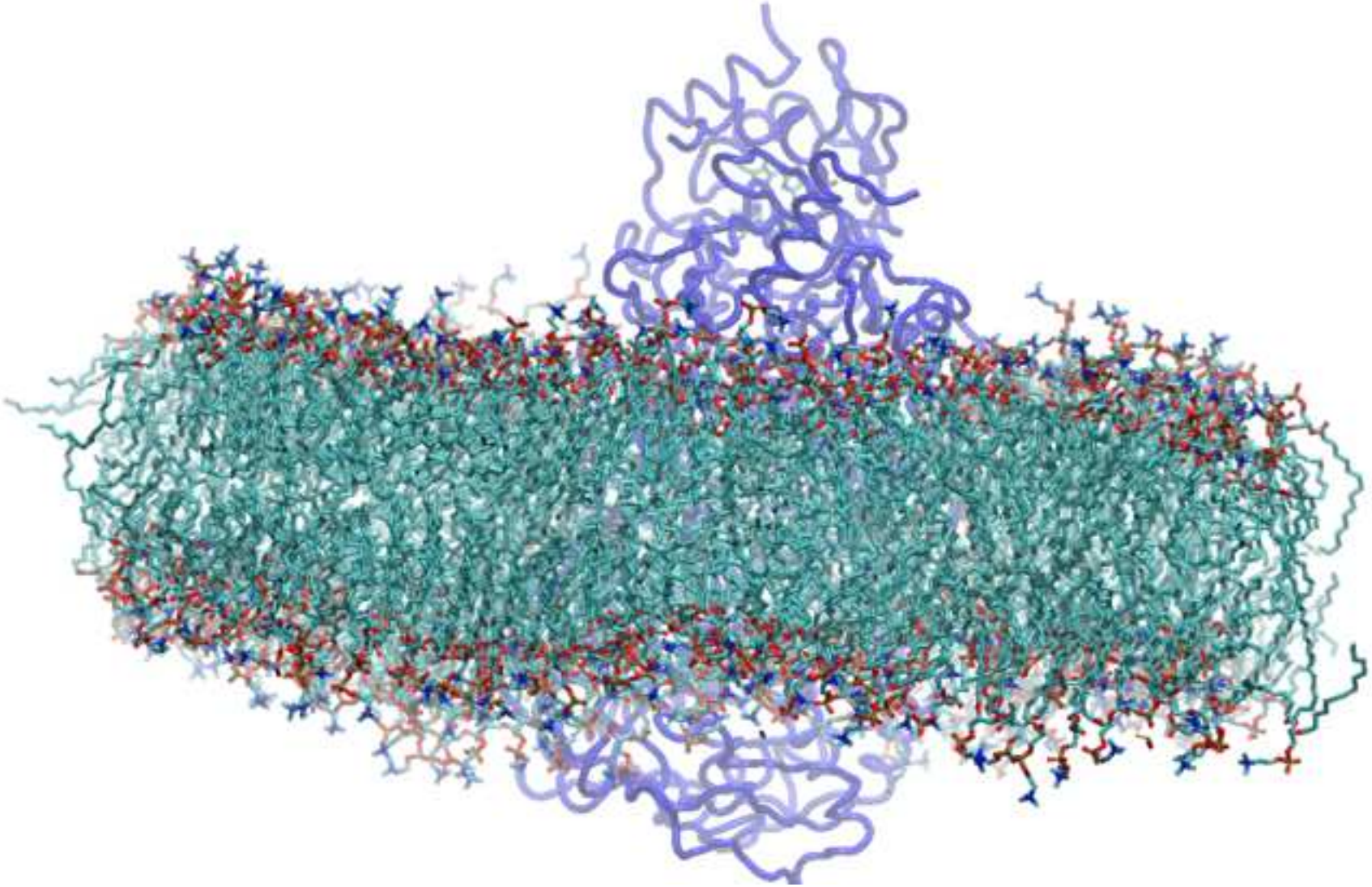
(yellow) remain out of the interface. HEME group and Cu_A ions are highlighted.

Figure 8: Membrane-bound protein complex conformational changes, (top) docking candidate before molecular dynamic relaxation, (bottom) the same docking candidate but after molecular dynamic relaxation.

Figure 9: H-bond optimization and energy minimization for 19 docking candidates.

Cytochrome c552 surface lysine residues are crucial for proteinprotein recognition.
Residue TRP121 behaves as the entry site in the electron transfer process.
Hydrogen bond network optimizations need to be included in simulation protocols.

***Graphical Abstract (for review)**



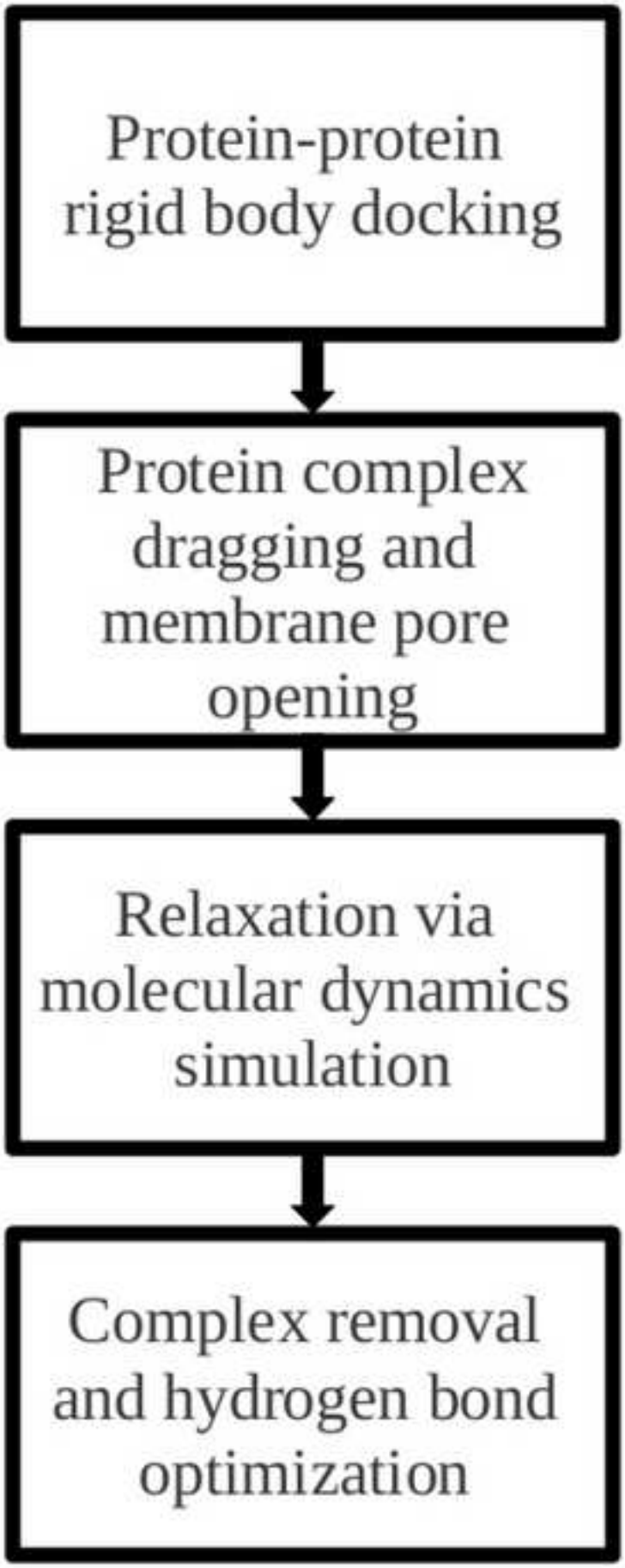
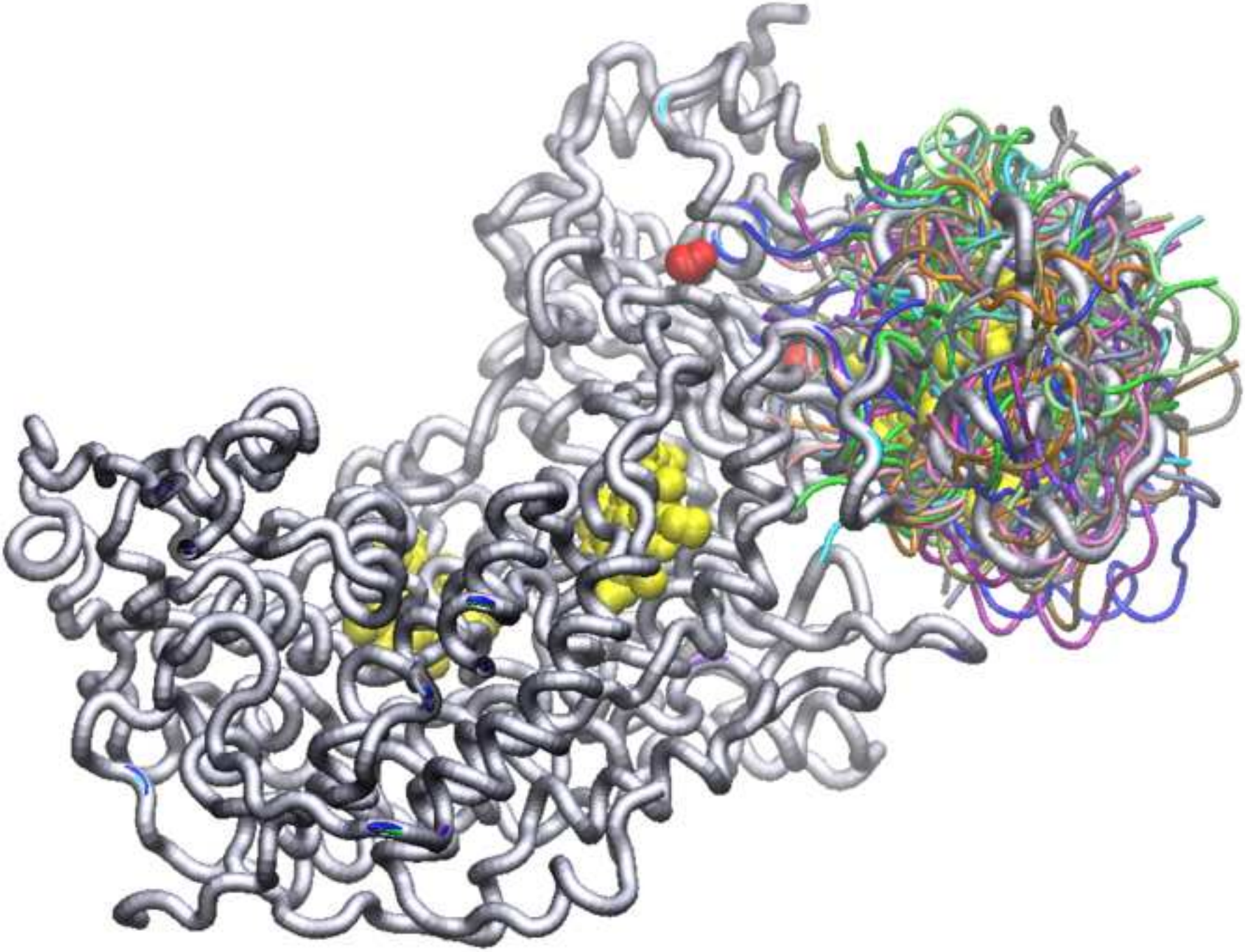
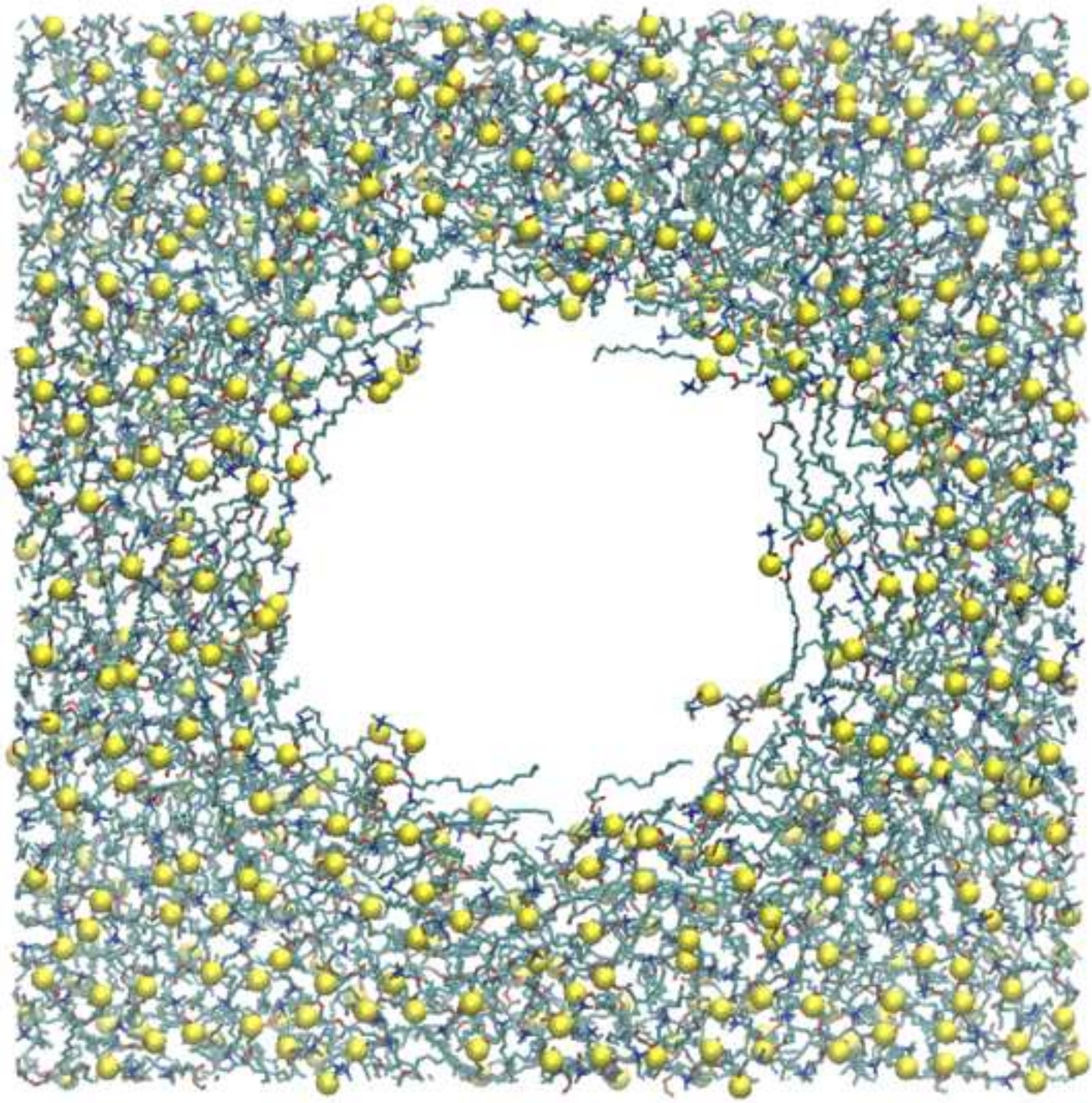


Figure2





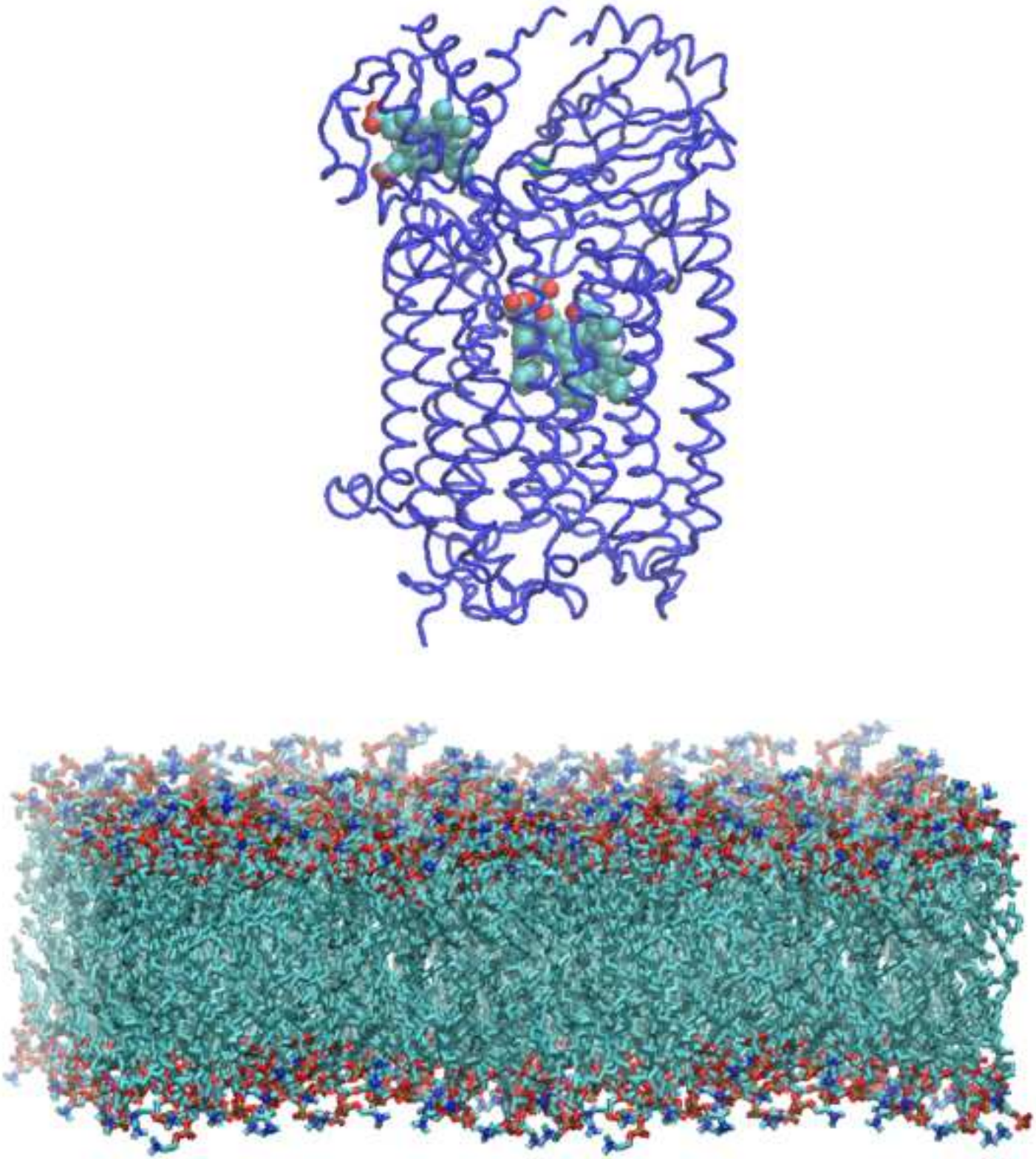


Figure5

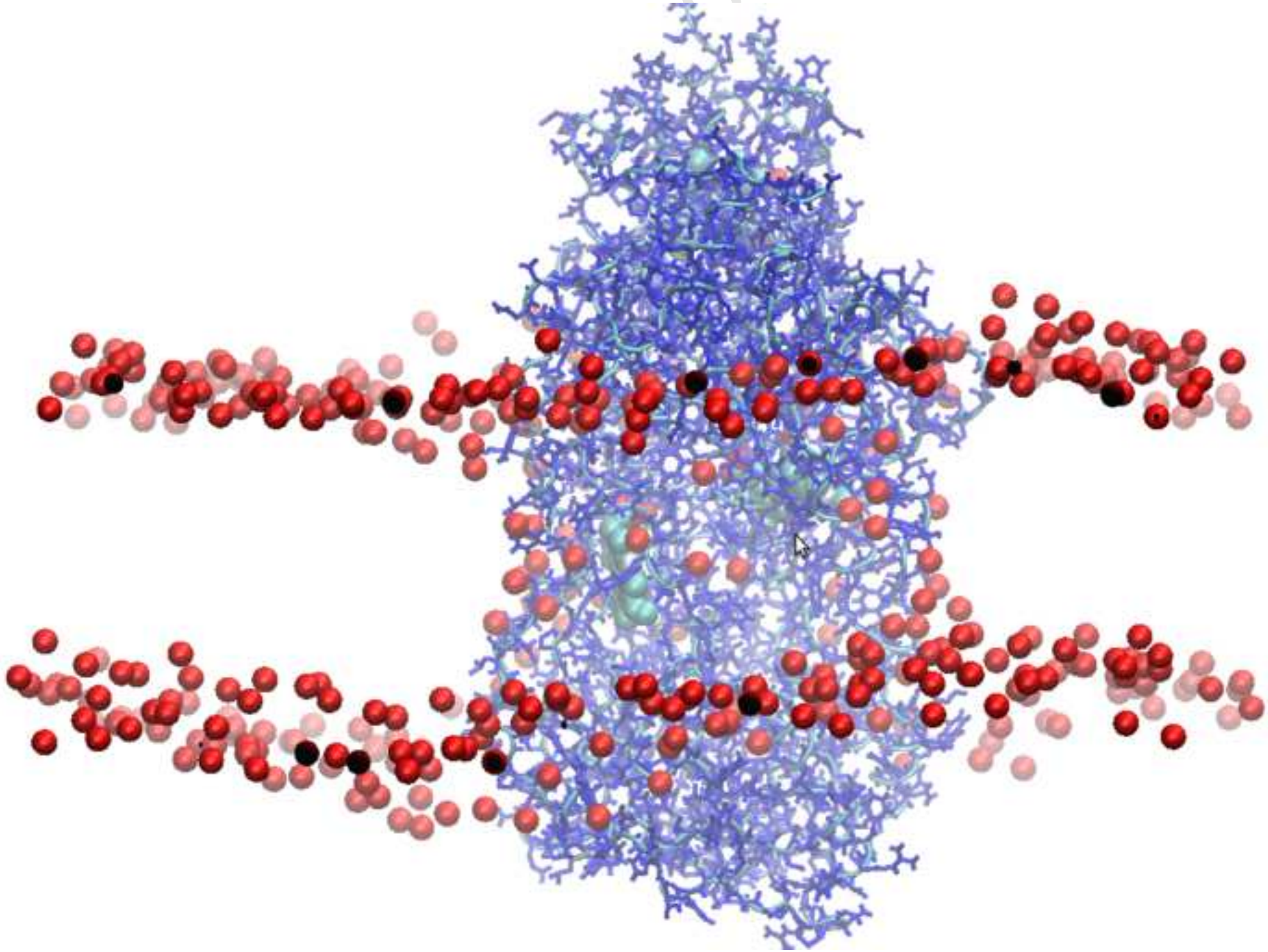


Figure6

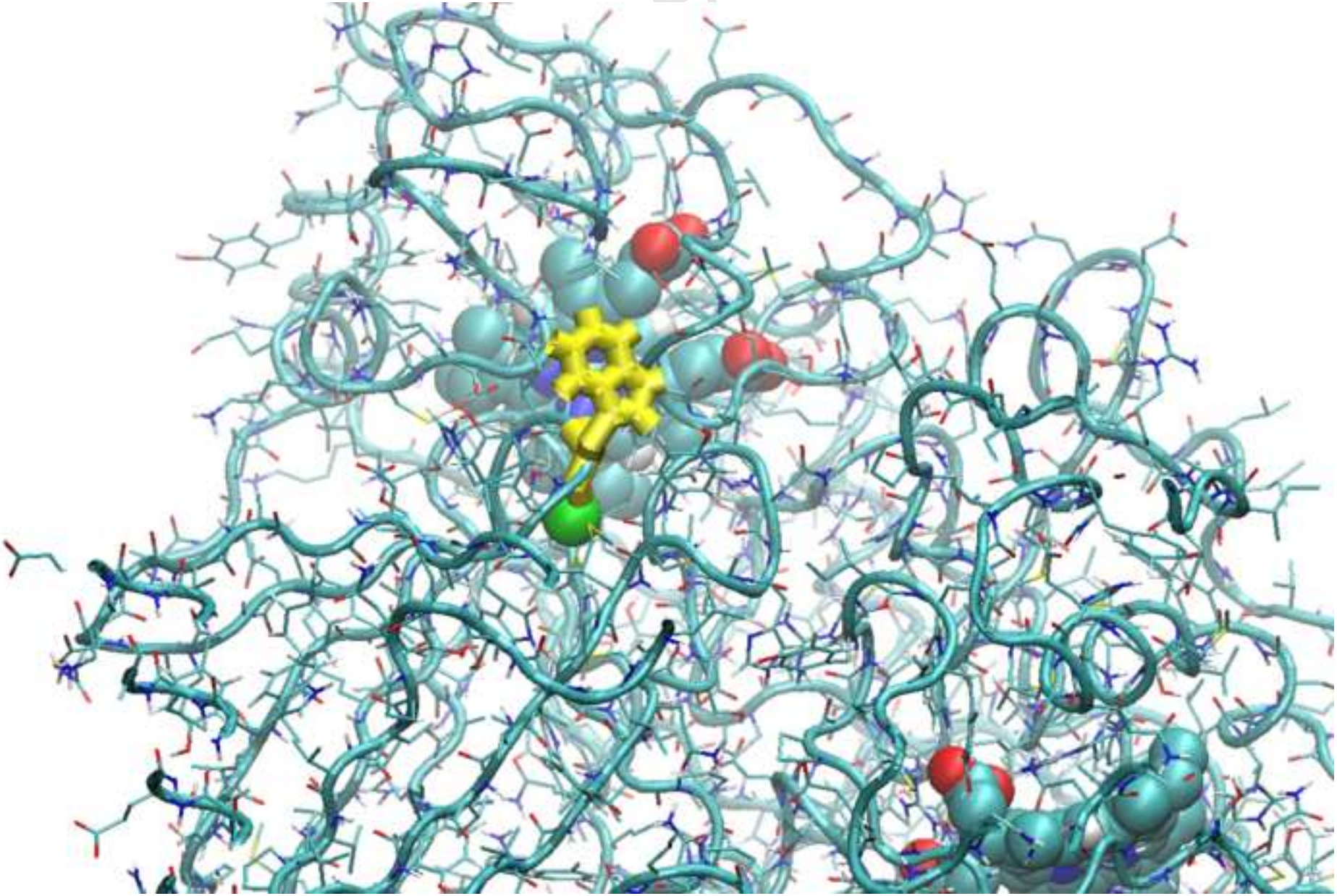
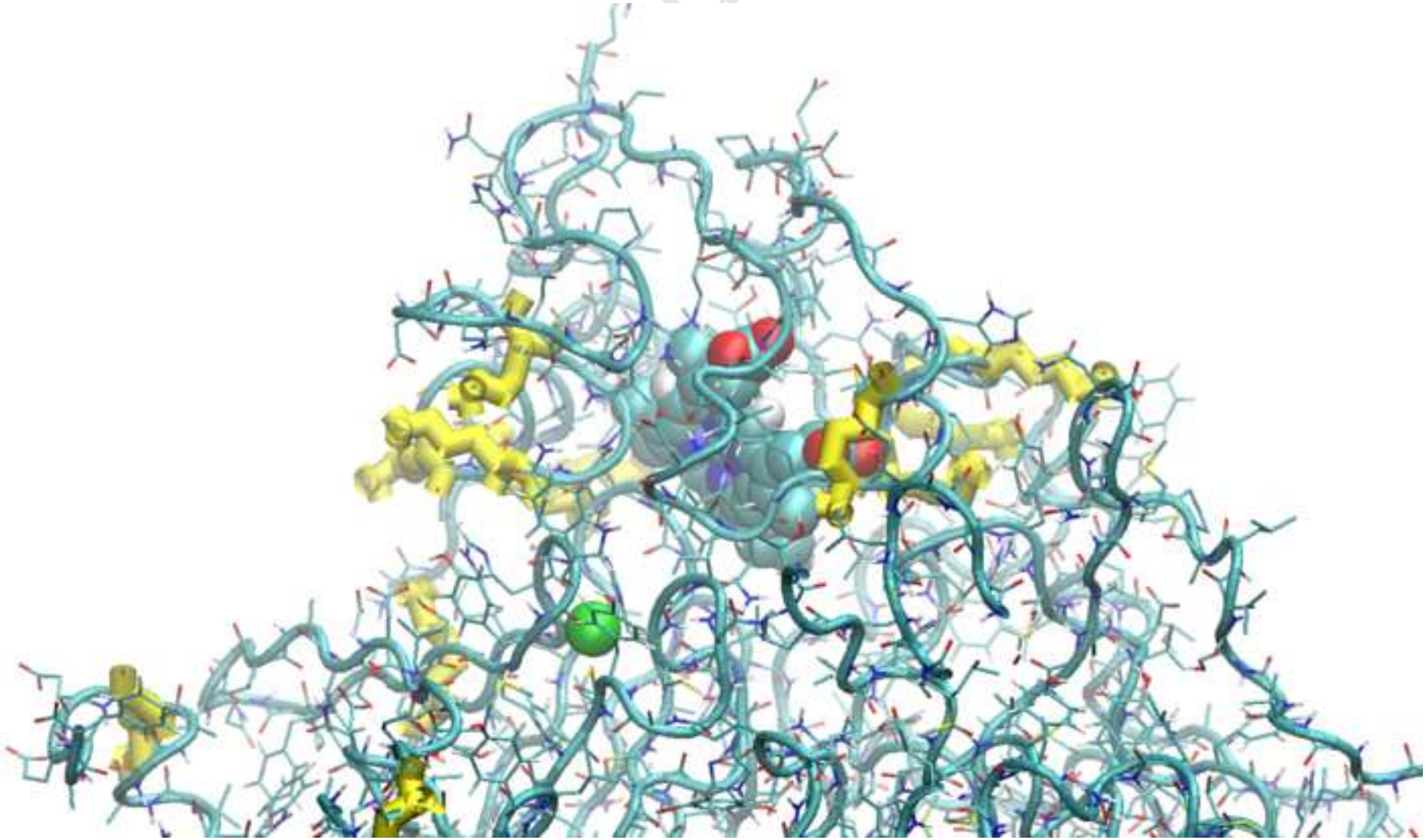


Figure7



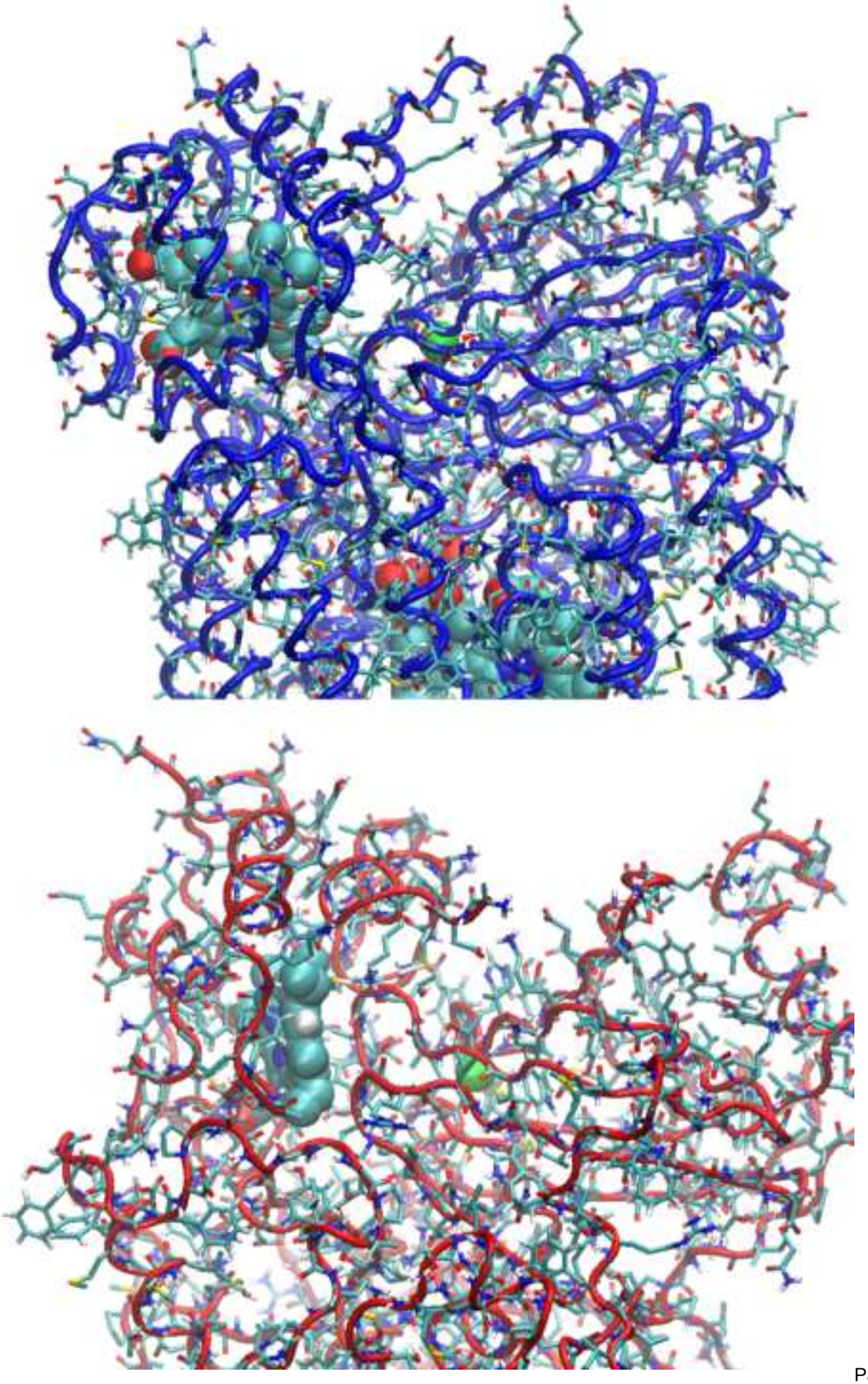
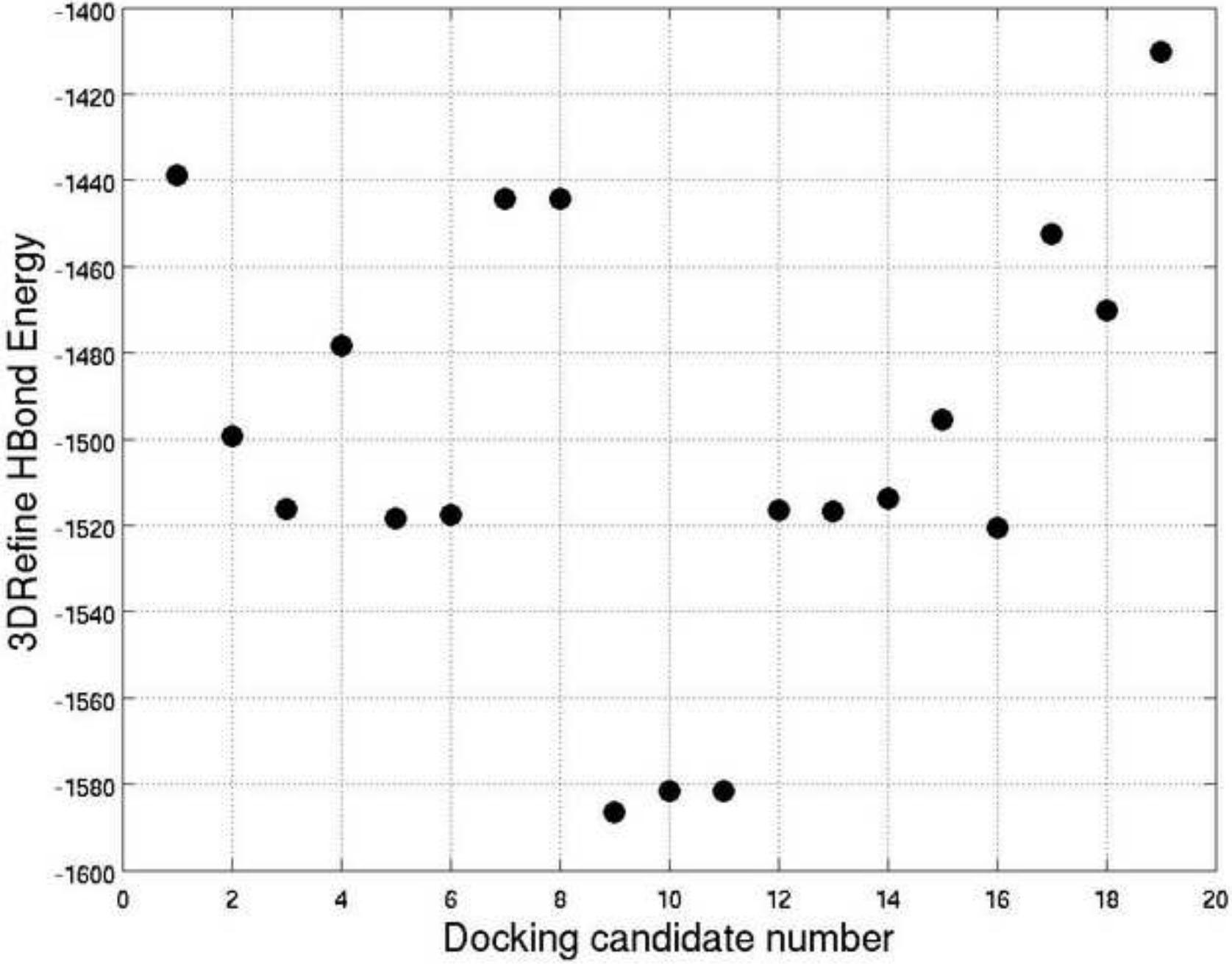


Figure9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