CAHIERS MAGELLANES-NS
Volume 06 Issue 2
2024

ISSN:1624-1940
DOI 10.6084/m9.figshare.2632597
http://magellanes.com/

MOLECULAR DOCKING STUDIES ON CYTOCHROME C IMMOBILIZED MESOPOROUS FE(III)-PHOSPHATE

Papigari Padma[1], Prasanna Sheela Menga[2], Chelimela Narsaiah[1], Boga Ramya Sree[1], Talari Gopiraj[1], Satyanarayana Muvarapu[1], Chandra Sekhar Vasam[1*]

[1]Department of Pharmaceutical Chemistry, Telangana University, Nizamabad 503322 India.
[2]Department of Biotechnology, Telangana University, Nizamabad 503322 India

***Corresponding Author**: Chandra Sekhar Vasam
*E-mail: csvasamsa@gmail.com

Abstract: The results of molecular docking study on Cytochrome immobilized mesoporous Fe(III)-phosphate (FePO) using Autodock4.2software is described. The non-covalent i.e., supramolecular interactions of a high-resolution three-dimensional structure of horse heart cytochrome C, (PDB_ID 1HRC) with the monomeric (M1) and polymeric structure (M2) of mesoporous FePO units are deduced along with the binding energies. The results visualized the preferential binding orientation between the amino acid side chains of Cytochrome C and the phosphate units of M1/ M2 structures assisted by various supramolecular interactions, specifically hydrogen bonding, hydrophobic interactions at low pH conditions (Figure 1 and 2). Molecular docking of monomeric FePO 'M1' on Cytochrome C without Heme molecules in the active site was also constructed to understand the interactions (Figure 3).

Keywords: Molecular Docking, Cytochrome C, Iron(III) Phosphates, Biomolecule immobilization, Non-covalent interactions.

1. Introduction: Iron(III) phosphate ($FePO_4$) is a common chemical with a variety of uses in materials, agriculture, and catalysis [1-3]. The compound can be found in the environment, foods, and water. Further, Strengite, Cacoxenite and Beraunite are the few examples of naturally occurring minerals of $FePO_4$ [4-6]. Nevertheless, a variety of synthetic FePO materials have been reported so far for various applications in Science & Technology [7-9]. The FePO materials exhibits different physical structures that are usually connected by FeO_4 and PO_4 tetrahedral sites. Porous crystalline FePO materials are a type of advanced materials that have found exciting applications in materials and heterogeneous catalysis [10-12].

Indeed, the discovery of mesoporous materials by Mobil Oil Corporation for petrochemical industry application [13] revolutionized the application various inorganic structured materials in heterogeneous catalysis [14] and biocatalysis [15]. As a consequence, an interest was generated to promote the synthesis and catalytic application of mesoporous metal phosphates not only petrochemical industry applications, but also in industrial organic synthesis [16-18] and biomolecule immobilization [19, 20]. Immobilization of biomolecules such as proteins, enzymes, sugars on porous (micro-/ meso-/ macro-porous) inorganic materials is an emerging area of research useful in biocatalysis and biotechnology [21, 22]. Both physical and chemical methods are prescribed for the immobilization of biomolecules on

ISSN:1624-1940
DOI 10.6084/m9.figshare.2632597
http://magellanes.com/

the solid inorganic materials [23, 24]. Physical adsorption method is recommended as a simple way of immobilization of certain biomolecules on solid inorganic materials [23]. Depending on the nature and structure of solid inorganic materials, a variety of non-covalent interaction evolves between the biomolecule and solid inorganic materials. The non-covalent interactions such as hydrogen bonding, Van der Waals forces, electrostatic/ columbic attraction forces, hydrophilic and hydrophobic interactions play important role during the immobilization of certain biomolecules on solid inorganic materials via physical adsorption [24, 25]. Various spectroscopic and microscopic data analysis provides good evidence for the immobilization of biomolecules on solid inorganic materials. Nevertheless, the type of non-covalent interactions that are operating between the biomolecules and solid inorganic materials is not fully understood. Recently the molecular docking study, a powerful computational tool, has been emerged a useful technique to provide clear evidence for the evaluation of type of non-covalent interactions evolved during the above immobilization phenomenon [26-28].
This manuscript describes the results of molecular docking study performed on Cytochrome C immobilized mesoporous FePO. A special focus is given to deduce the type of non-covalent i.e. secondary interactions that are stabilizing the Cytochrome C immobilization on mesoporous FePO. The synthesis of mesoporous FePO was previously reported by one of us [9]. The essential role of normal Cytochrome C in various biological functions is well known. The results of such docking studies certainly helpful in designing useful supported biocatalysts and biosensors.

2. Experimental:
Molecular modelling studies of FePO monomer (M1) and FePO polymeric form (M2) were carried out using Autodock4.2software [29]. Docking of all compounds was carried out on structure of High-resolution three-dimensional structure of horse heart cytochrome C, (PDB_ID 1HRC) [30]. This protein PDB was retrieved from protein data bank. During the docking we have used 0.375Å grid box parameters as centre: x = 46.699, y = 23.289, z = 5.656 and grid box size: x=84, y=82, z=94. While docking 25 conformations were generated for each ligand by using default genetic algorithm. In this study, input preparation carried out using MGLtools-1.5.6 [29].

3. Results & Discussion:
Molecular docking studies
High-resolution three-dimensional structure of horse heart Cytochrome C has been selected for molecular docking. Docking studies have been carried for M1 and M2on Cytochrome C protein A chain. Thus, we have selected horse Cytochrome C(PDB_ID: 1HRC) protein for protein ligand binding analysis in this study. The surface of the Cytochrome C has four binding sites known as L-site, A-site, N-site and C-site [31, 32]. The L- site and A- site are having lysine residues to interact with the mitochondrial mimetic membranes [32]. Molecular docking studies with the grid covering total protein results the preference of the ligand molecule to interact with cytochrome C protein. The L-site with Lys25, Lys27 and His33 residues and the opposite to this site the A-site with Lys73 and Lys79 residues are actively involving in the lipid binding at low pH conditions [32].

CAHIERS MAGELLANES-NS
Volume 06 Issue 2
2024

ISSN:1624-1940
DOI 10.6084/m9.figshare.2632597
http://magellanes.com/

Molecule name	Binding affinity (in kcal/mol)
M1	-4.19
M2	-6.92

The Molecule M1 and M2 are docked on the protein with blind docking by taking the docking search space grid on total protein coverage results the molecules M1 and M2 are bound to L-site with good binding energies, and few conformations are at A-site with low binding energies compared to L-site binding. The L- site binding is considered for the analysis due a greater number of conformations with good binding energies.

The M1 molecule bound with the binding energy -4.19 kcal/mol and the Fe-phosphate monomeric unit forming hydrogen bond with the active site residues (figure 1). The oxygen atoms on Fe, are forming hydrogen bonds with side chain of Thr19 and main chain of His18, the other oxygens on Fe forming hydrogen bonds with side chains of Lys27 and main chain of Ala15. Similarly, the M2 molecules also bound at same location with the binding energy of -6.92 kcal/mol. The molecules also stabilized by forming hydrogen bonds and hydrophobic interactions (Figure 2). One of the phosphate oxygen is forming a bifurcated hydrogen bond with Lys27 and His18 residues. The side chain Glu21 is forming a bifurcated hydrogen bond with phosphorus atom and oxygen of the M2 molecules. The Thr19 and Tyr97 are also forming hydrogen bond with the M2 molecules. The other active site residues like Phe10, Val11, Ala15, Lys25 and Val20 residues are present.

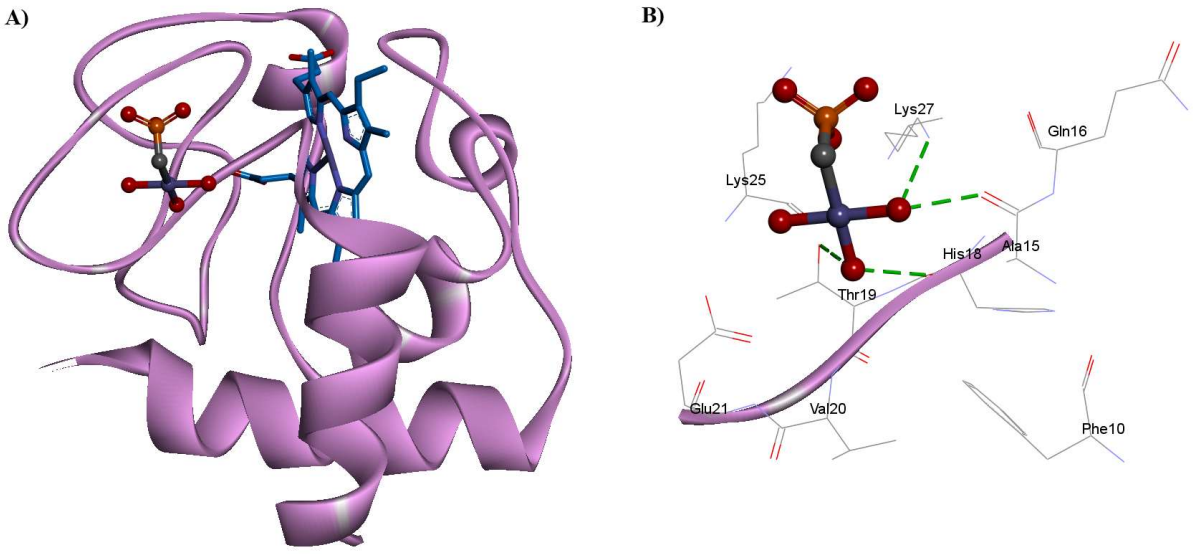

Figure 1: A) Molecular docking of Molecule M1 in to the active site (L-site) of Cytochrome C. B) active site protein amino acids are shown in lines and the molecule is shown in ball and sticks style. Hydrogen bonds are shown in green broken lines.

CAHIERS MAGELLANES-NS
Volume 06 Issue 2
2024

ISSN:1624-1940
DOI 10.6084/m9.figshare.2632597
http://magellanes.com/

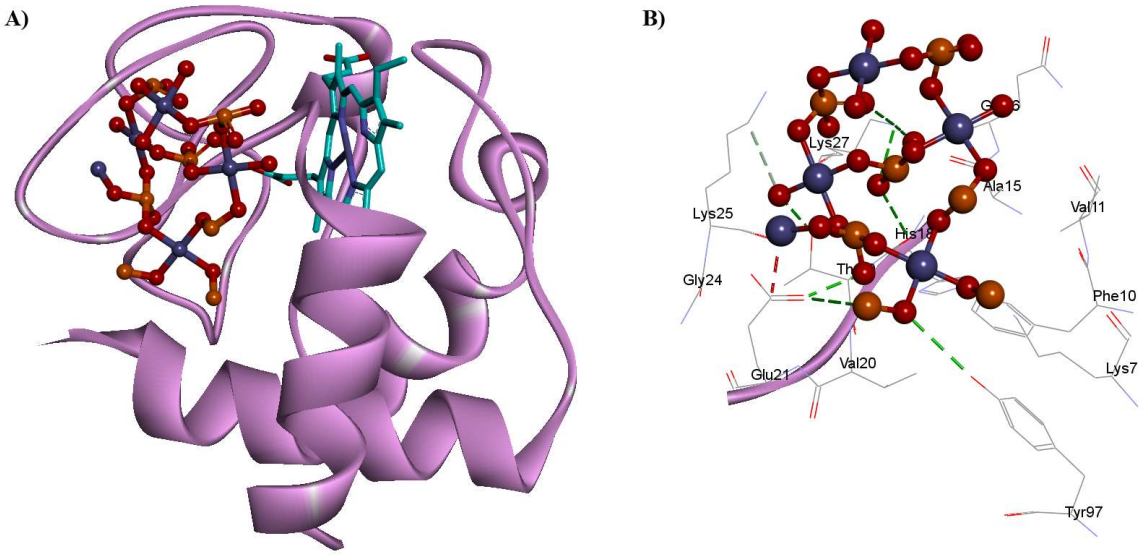

Figure 2: A) Molecular docking of Molecule M2 in to the active site (L-site) of Cytochrome C. B) active site protein amino acids are shown in lines and the molecule is shown in ball and sticks style. Hydrogen bonds are shown in green broken lines.

Molecular docking of Molecule M1 (FePO monomer) on Cytochrome C without Heme molecules in the active site carried out to understand the interactions. The Molecules M1 is binding into the Heme binding pocket with binding free energy of -4.6 kcal/mol. The molecule is making strong interactions with the residues of active site (Figure3). The Thr49 main chain and side chains are forming two hydrogen bonds with the M1 molecules oxygen atoms. One of the oxygens on the Fe atom is forming hydrogen bond with Tyr67 residue side chain. The Thr75 is likely to form a covalent bond with the iron metal ion. The hydrophobic interactions are observed from Ile75, Tyr48, Phe46, Thr28, and Asn52 residues. The molecule M2 also docked on the protein without Heme molecule replicate the binding of the molecule M2 on the Cytochrome C with Heme molecule and the binding free energy observed as -7.09 kcal/mol. The binding orientation and interactions are identical as shown in Figure 2.

ISSN:1624-1940
DOI 10.6084/m9.figshare.2632597
http://magellanes.com/

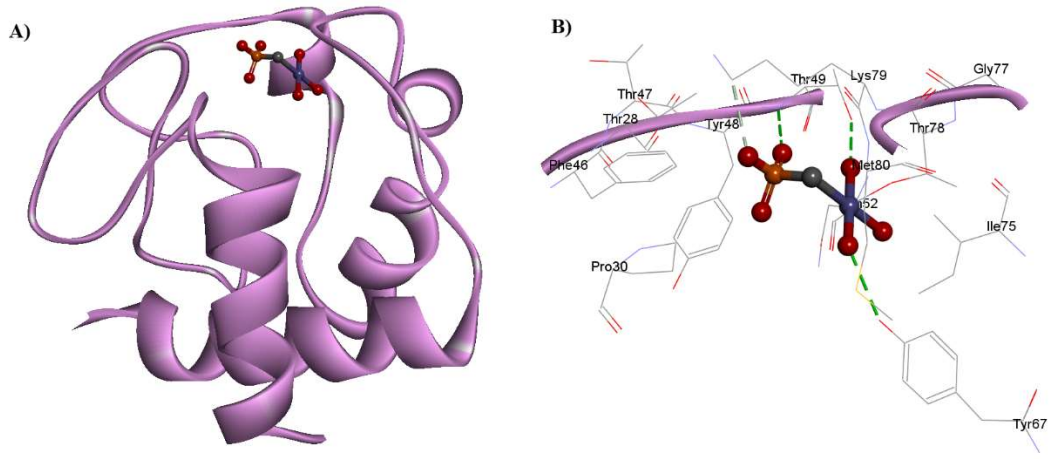

Figure 3: A) Molecular docking of Molecule M1 in to Heme binding site of Cytochrome C. B) active site protein amino acids are shown in lines and the molecule is shown in ball and sticks style. Hydrogen bonds are shown in green broken lines.

Conclusion: As per molecular docking studies, the Cytochrome C molecule can preferentially bind the monomeric and polymeric FePO units through non-covalent interactions. Therefore, physical adsorption method could be a good choice to immobilize the Cytochrome C type proteins on to the mesoporous materials to aid the various non-covalent interactions. The present study provided initiative about the firm immobilization of protein molecules on to the mesoporous supports in designing the biocatalysts and biosensors. Physical adsorption experiments are in progress in designing the real samples of Cytochrome C immobilized on our previously reported mesoporous FePO materials [9].

Conflict of Interest: There is no conflict of Interest

Acknowledgement: The authors acknowledge the support from TSCOST (Lr.No.03/TSCOST/DST-PRG/2021-22)

References:

1. B.Ozdogru, Y. Cha, B. Gwalani, V. Murugesan, M. Song, Omer Ozgur Capraz, *In Situ* Probing Potassium-Ion Intercalation-Induced Amorphization in Crystalline Iron Phosphate Cathode Materials, *Nano Lett.,* **2021**, *21*, 7579-7586. DOI: 10.1021 /acs.nanolett.1c02095
2. R. J. Saracanlao, T. Saelens, A. Voegelin, E. Smolders, M. Everaert, Recycled Iron Phosphates: A New Phosphorus Fertilizer for Paddy Rice, *Environ. Sci. Technol*., **2024**, *58*, 9250–9260. DOI:*10.1021/acs.est.4c02111.*
3. M. E.A. Drici, B. Amina, B. Redouane, B. Mohammed, B. Sumeya, M. Debdab, Iron phosphate nanoparticles as an effective catalyst for propargylamine synthesis, *React. Kinet. Mech. Catal.*, **2023**, *136*, 333-343.DOI:10.1007/s11144-023-02345-8

ISSN:1624-1940
DOI 10.6084/m9.figshare.2632597
http://magellanes.com/

4. J. T. Kloprogge, B. J. Wood, X-ray Photoelectron Spectroscopic and Raman microscopic investigation of the variscite group minerals: Variscite, strengite, scorodite and mansfieldite, *Spectrochim. Acta A; Mol. Biomol. Spectrosc*., **2017**, *185*, 163-172.DOI:10.1016/j.saa.2017.05.042.
5. Moore, P., Shen, J. An X-ray structural study of cacoxenite, a mineral phosphate. *Nature*, **1983**, *306*, 356–358.DOI:10.1038/306356a0
6. R. L. Frost, A. Lopez, R. Scholz, Y. Xi, C. Lana, The molecular structure of the phosphate mineral beraunite $Fe^{2+}Fe_5^{3+}(PO_4)_4(OH)_5{\cdot}4H_2O$ – A vibrational spectroscopic study, *Spectrochim. Acta A; Mol. Biomol. Spectrosc*., **2014**, *128*, 408-412.DOI:10.1016/j.saa.2014.02.198.
7. Z. Shi, S. Wei, T. Xie, Q. Liu, C. Au, S. Yin, High-throughput synthesis of high-purity and ultra-small iron phosphate nanoparticles by controlled mixing in a chaotic microreactor, *Chem. Eng. Sci.*, **2023**, *280*, 119084.DOI:10.1016/j.ces.2023.119084.
8. R. Lin, A. P. Amrute, F.Krumeich, K. Lazar, R. Hauert, M. Yulikove and J. Perez-Ramirez, Phase-controlled synthesis of iron phosphates via phosphation of β-FeOOH nanorods, *CrystEngComm*.,**2016**,*18*, 3174-3185. DOI:10.1039/C6CE00501B
9. S Kenane, CS Vasam, PP Knops-Gerrits, Vibrational Spectroscopy to Monitor Synthesis, Adsorption and Diffusion in Micro- and Mesoporous Metal Phosphates, *Springer Publications*, **2006**, 279-298.
10. M. A. Kumar, P. Selvam, Ionic Liquid Templated Ordered Hexagonal Mesoporous Iron Phosphate Molecular Sieves: A Highly Effective Heterogeneous Catalysts with Remarkable Selectivity for Phenol Hydroxylation, Reaction, *Chem. Asian J*., **2023**, *18*, e202300389.DOI:10.1002/asia.202300389
11. B. P.Bastakoti, Y. Li, S.Guragain, M. Pramanik, S. M. Alshehri, T. Ahamad, Z. Liu, Y. Yamauchi, Synthesis of Mesoporous Transition-Metal Phosphates by Polymeric Micelle Assembly, *Chem. Asian J.*, **2016**, *22*, 7463-7467.DOI:10.1002/chem.201600435
12. M. Pramanik, M. Imura, J. Lin, J. Kim, J. H. Kim and Y. Yamauchi, Shape-controlled synthesis of mesoporous iron phosphate materials with crystallized frameworks, *Chem. Commun.*,**2015**,*51*,13806-13809.DOI:10.1039/C5CC04356E
13. C.T. Kresge, J. C. Vartuli, W. J. Roth, M. E. Leonowicz,The discovery of ExxonMobil's M41S family of mesoporous molecular sieves,Editor(s): Osamu Terasaki, *Stud. Surf. Sci. Catal.*, **2004**, *148*, 53-72.DOI:10.1016/S0167-2991(04)80193-9.
14. R. Luque, A. Ahmad, S. Tariq, M. Mubashir, M. S. Javed, S. Rajendran, R.S. Varma, A. Ali, C. Xia, Functionalized interconnected porous materials for heterogeneous catalysis, energy conversion and storage applications: Recent advances and future perspectives, *Mater. Today*, **2024**, *73*, 105-129.DOI: 10.1016/j.mattod.2023.05.001.
15. Zhang Z, Zheng Y, Dou Z, Gu M, Sun M, Song J, Gao N, Cui F, Tian Y, Zhu G. Multivariate Porous Aromatic Frameworks with High Porosity and Hierarchical Structures for Enzyme Immobilization. *ACS Cent. Sci*.,**2023**, *9*, 488-493.DOI: 10.1021/acscentsci.3c00078.

ISSN:1624-1940
DOI 10.6084/m9.figshare.2632597
http://magellanes.com/

16. S. Lopez-Pedrajas, R. Estevez, R. Navarro, D. Luna, F.M. Bautista, Catalytic behaviour of mesoporous metal phosphates in the gas-phase glycerol transformation, *J.Mol. Catal. A: Chem*., **2016**, *421*, 92-101.DOI:10.1016/j.molcata.2016.05.015.
17. W. Guo, E. J. M. Hensen, W. Qi, H. J. Heeres, J. Yue, Titanium Phosphate Grafted on Mesoporous SBA-15 Silica as a Solid Acid Catalyst for the Synthesis of 5-Hydroxymethylfurfural from Glucose, *ACS Sust. Chem. Eng.*, **2022**, *10*, 31, 10157–10168.DOI:10.1021/acssuschemeng.2c01394.
18. M. P. Kapoor, S. Inagaki, and H. Yoshida, Novel Zirconium−Titanium Phosphates Mesoporous Materials for Hydrogen Production by Photoinduced Water Splitting, *J. Phys. Chem. B*., **2005**, *109*, 19, 9231-9238.DOI: 10.1021/jp045012b.
19. X. Zhang, L. Zhang, H. Liu, B. Cao, L. Liu, and W. Gong, Structure, morphology, size and application of iron phosphate, *Rev. Adv. Mater. Sci*.,**2020**, *59*,538–552.DOI:10.1515/rams-2020-0039.
20. O. G. da Silva, M. Alves, I. M. G. dos Santos, M. G. Fonseca, M. Jaber, Mesoporous calcium phosphate using casein as a template: Application to bovine serum albumin sorption, *Colloids Surf. B Biointerfaces*, **2017**, *158*, 480-487.DOI: 10.1016/j.colsurfb.2017.07.011
21. Z. Ashkan, R. Hemmati, A. Homaei, A.Dinari, M. Jamlidoost, A. Tashakor, Immobilization of enzymes on nanoinorganic support materials: An update, *Int. J. Biol. Macromol*., **2021**, *168*, 708-721.DOI:10.1016/j.ijbiomac.2020.11.127.
22. Mohamad NR, Marzuki NH, Buang NA, Huyop F, Wahab RA. An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes. *BiotechnolBiotechnol Equip*.,**2015** Mar 4;*29*(2):205-220. DOI: 10.1080 /13102818. 2015.1008 192.
23. Homaei A.A, Sariri R, Vianello F, Stevanato R. Enzyme immobilization: an update,*J. Chem Biol.***2013**, *6*, 185-205. DOI: 10.1007/s12154-013-0102-9.
24. Yasmin R. Maghraby, Rehan M. El-Shabasy, Ahmed H. Ibrahim, Hassan Mohamed El-Said Azzazy, Enzyme Immobilization Technologies and Industrial Applications, *ACS Omega,* **2023**, *8*, 5184–5196.DOI:10.1021/acsomega.2c07560.
25. Bilal, M., Asgher, M., Cheng, H., Yan, Y., & Iqbal, H. M. N., Multi-point enzyme immobilization, surface chemistry, and novel platforms: a paradigm shift in biocatalyst design, *Crit. Rev. Biotechnol*., **2019**, *39*, 202–219. DOI:10.1080/07388551.2018.1531822
26. U. Baig, M. A. Gondal, Md F. Alam, A. A. Laskar, M. Alam and H.Younus, Enzyme immobilization and molecular modeling studies on an organic–inorganic polypyrrole–titanium(IV)phosphate nanocomposite, *New J. Chem.*, **2015**,*39*, 6976-6986.DOI: 10.1039/c5nj01463h.
27. Bhattacharjee N, Alonso-Cotchico L, Lucas MF., Enzyme immobilization studied through molecular dynamic simulations. *Front. Bioeng. Biotechnol*.,**2023**, *11*,1200293. DOI:10.3389 /fbioe.2023.1200293.
28. Salha D, Andaç M, Denizli A., Molecular docking of metal ion immobilized ligands to proteins in affinity chromatography. *J. Mol. Recognit*.,**2021**, *34*, e2875. DOI:10.1002/jmr.2875.

ISSN:1624-1940
DOI 10.6084/m9.figshare.2632597
http://magellanes.com/

29. Morris, G.M.; Huey, R.; Lindstrom, W.; Sanner, M.F.; Belew, R.K.; Goodsell, D.S.; Olson A.J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility, *J. Comput. Chem.,* **2009**, *30*, 2785-2791.DOI: 10.1002/jcc.21256
30. Bushnell GW, Louie GV, Brayer GD. High-resolution three-dimensional structure of horse heart cytochrome c,*J Mol Biol*., **1990**, *214*, 585-95.DOI: 10.1016/0022-2836(90)90200-6.
31. Mohammadyani D, Yanamala N, Samhan-Arias AK, Kapralov AA, Stepanov G, Nuar N, Planas-Iglesias J, Sanghera N, Kagan VE, Klein-Seetharaman J. Structural characterization of cardiolipin-driven activation of cytochrome c into a peroxidase and membrane perturbation. *Biochim. Biophys. Acta Biomembr*., **2018,***1860*, 1057-1068.DOI: 10.1016 /j.bbamem .2018.01.009
32. Kawai C, Prado FM, Nunes GL, Di Mascio P, Carmona-Ribeiro AM, Nantes IL. pH-Dependent interaction of cytochrome c with mitochondrial mimetic membranes: the role of an array of positively charged amino acids. *J. Biol. Chem*., **2005**, *280*, 34709-17.DOI: 10.1074 /jbc.M41 2532200.