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Binding interactions of porphyrin derivatives with Ca^{2+} ATPase of sarcoplasmic reticulum (SERCA1a)

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Abstract:
The use of Porphyrin derivatives as photosensitizers in Photodynamic Therapy (PDT) was investigated by means of a molecular docking study. These molecules can bind to intracellular targets such as P-type Ca^{2+} ATPase of sarcoplasmic reticulum (SERCA1a). CAChe software was successfully employed for conducting the docking of Tetraphenylporphinesulfonate(TPPS), 5,10,15,20-Tetrakis (4-sulfonatophenyl) porphyrinato Iron(III) Chloride (FeTPPS) and 5,10,15,20-Tetrakis (4-sulfonatophenyl) porphyrinato Iron(III) nitrosyl Chloride (FeNOTPPS) with Ca^{2+} ATPase from sarcoplasmic reticulum of rabbit. The results show that FeNOTPPS forms the most stable complex with Ca^{2+} ATPase.

Keywords: Porphyrin derivatives, Molecular docking, Photodynamic therapy, Photosensitizers.

Background:
A large number of photosensitizers have been used for Photodynamic therapy (PDT). They consist of Porphyrins, Chlorophylls and Dyes **[1, 2].** Other examples include Amino levulinic acid (ALA), Silicon Phthalocyanine, m-Tetra hydroxy phenylchlorin and mono-L-Aspartylchlorin. An important requirement for a photosensitizer is that it should have the ability to produce Reactive Oxygen Species (ROS). The predominantly formed ROS is Singlet Oxygen. In order to produce singlet oxygen, the energy of the triplet state of the photosensitizer must be higher than the energy needed to excite oxygen from its ground triplet state to the first singlet state (0.98 eV21). ROS induce controlled cell death via apoptosis or sudden cell death via necrosis **[3, 4].**

Small ligand docking is useful for the computational analysis of binding interactions between proteins and ligands **[5, 6].** Binding interactions are reported as Scoring Functions. Scoring Functions can describe the strength of intermolecular van der Waals and electrostatic interactions between all atoms of ligands such as Porphyrin derivatives in the complex with a receptor such as Ca^{2+} ATPase. Calcium ATPase transports Ca^{2+} ions across the cell membrane against a concentration gradient. The crystal structure of the Ca^{2+} ATPase of rabbit sarcoplasmic reticulum (SERCA1a) is known at 2.6 Å resolutions **(Figure 1a) [7, 8].** It is a 994 amino acid protein which contains a large cytoplasmic headpiece consisting of the A (actuator), N (nucleotide binding) and P (phosphorylation) domains. There are also ten transmembrane α-helices (M1–M10) and small luminal loops. The lengths of helices and the angle which they make with the membrane, is variable. Some of the helices (M2 and M5) are very long (60 Å), some are unwound (M4 and M6) and some have a kink in the middle (M10). In this study, molecular docking was employed to study the interactions with Ca^{2+} ATPase of Tetraphenyl porphine sulfonate (TPPS), 5,10,15,20-Tetrakis(4-sulfonatophenyl) porphyrinato Iron(III) Chloride (FeTPPS) and 5,10,15,20-Tetrakis(4-sulfonatophenyl) porphyrinato Iron(III) nitrosyl Chloride (FeNOTPPS) **(Figure 1b).**

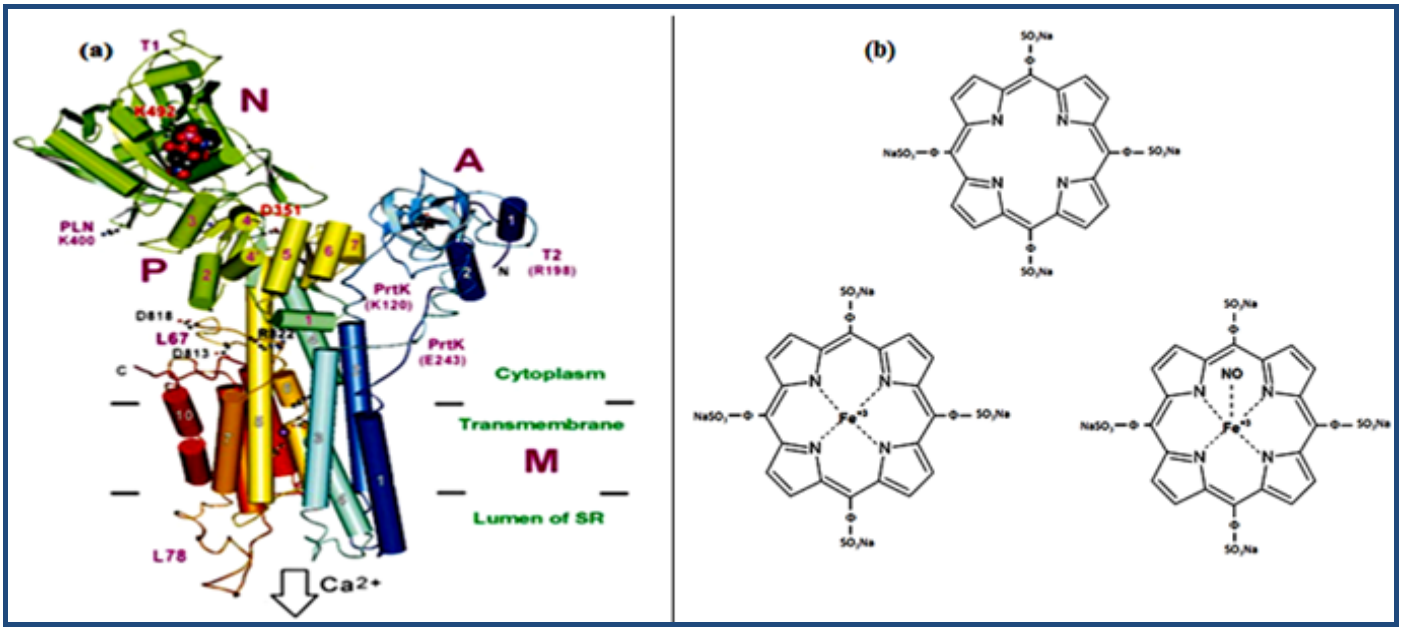

Figure 1: (a) Ribbon diagram based on the crystal structure of Ca^{2+} ATPase of sarcoplasmic reticulum (PDB Code: 1SU4) [7]. α-Helices are represented by cylinders and β-strands by arrows. Colors change gradually from the N-terminus (blue) to the C-terminus (red). The nucleotide-binding domain is labeled 'N', the activator domain is labeled 'A' and the phosphorylation domain is labeled 'P'; **(b)** Chemical structures of Tetraphenylporphinesulfonate (TPPS), 5,10,15,20-Tetrakis(4-sulfonatophenyl) porphyrinato Iron(III) Chloride (FeTPPS) and 5,10,15,20-Tetrakis(4 sulfonatophenyl)porphyrinato Iron(III)nitrosyl Chloride (FeNOTPPS)(counter clockwise from top).

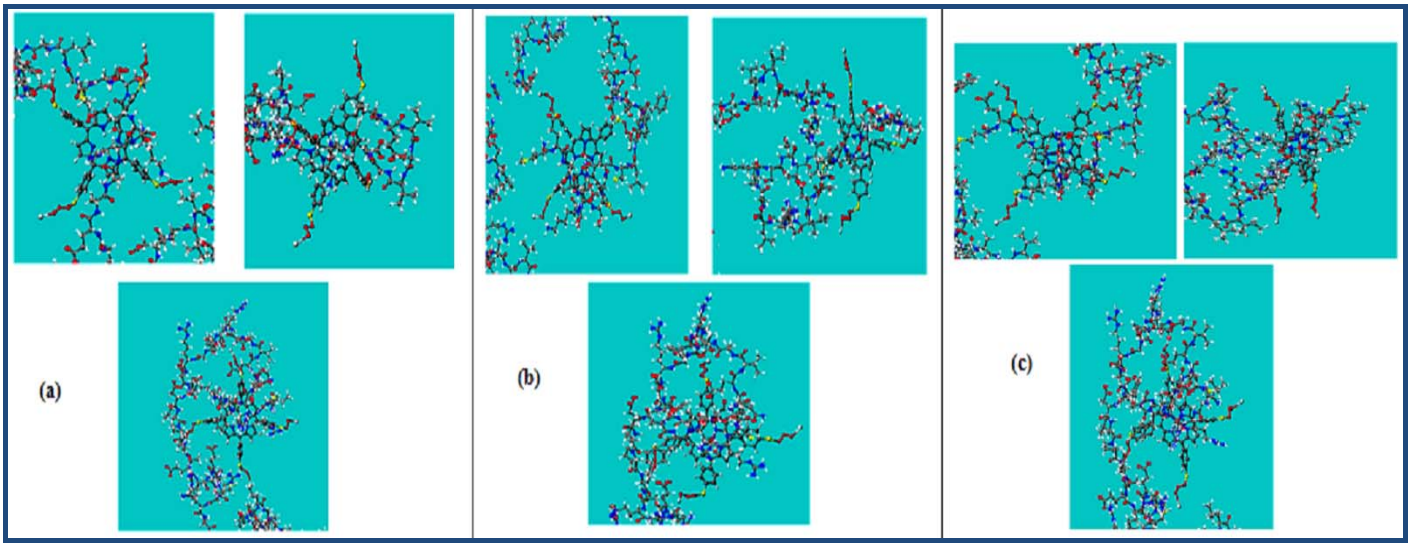

Figure 2: **(a)** Clockwise from the top, docking of TPPS with domain 1 (amino acid 1-150), domain 2 (amino acid 151-591) and domain 3 (amino acid 635-994) of Ca^{2+} ATPase of rabbit sarcoplasmic reticulum; **(b)** Clockwise from the top,docking of FeTPPS with domain 1 (amino acid 1-150), domain 2 (amino acid 151-591) and domain 3 (amino acid 635-994) of Ca^{2+} ATPase of rabbit sarcoplasmic reticulum; **(c)** Clockwise from top, docking of FeNOTPPS with domain 1 (amino acid 1-150), domain 2 (amino acid 151-591) and domain 3 (amino acid 635-994) of Ca^{2+} ATPase of rabbit sarcoplasmic reticulum.

Methodology:

Energy Minimization

All the three molecules, Tetraphenylporphinesulfonate (TPPS), 5,10,15,20-Tetrakis (4-sulfonatophenyl) porphyrinato Iron(III) Chloride (FeTPPS) and 5,10,15,20-Tetrakis (4-sulfonatophenyl) porphyrinato Iron(III)nitrosyl Chloride (FeNOTPPS), were constructed on a Silicon Graphics Octane2 workstation with IRIX 6.5 operating system. The energies of all the molecules were minimized using a MM3PRO force field **[9]** and Gasteiger-Hückel charges **[10]** with a convergence gradient of 0.05 kcal/mol/Å. For FeTPPS, the coordinate bonds of Fe^{+2} with pyrrole nitrogen were defined first before energy minimization. For FeNOTPPS, the coordinate bonds of Fe^{+2} with pyrrole nitrogen were defined first and then with nitric oxide. The total energies of TPPS, FeTPPS and FeNOTPPS after minimization

were 67.9, 90.9 and 125.8kcal/mol respectively. The breakup of energies is shown in **Table 1 (see supplementary material).**

Molecular Docking

CACHe program was used for docking TPPS, FeTPPS and FeNOTPPS in the crystal structure of Ca^{2+} ATPase (PDB code: 1SU4). These complexes were then subject to molecular dynamics simulation for duration of 10,000 fs then submitted for energy minimization using a MM3PRO force field **[9]** and Gasteiger-Hückel charges **[10]** with convergence gradient of 0.05 kcal/mol/Å for TPPS, FeTPPS and FeNOTPPS bound to Ca^{2+} ATPase. Individual docking studies of TPPS, FeTPPS and FeNOTPPS were then conducted using a trifurcated Ca^{2+} ATPase molecule divided into domain 1 (amino acid 1-150), domain 2 (amino acid 151-591) and domain 3 (amino acid 635-994) respectively **(Figures 2 a,b,c).**

Results & Discussion:

Photodynamic therapy is a medical treatment which uses the combination of light and a photosensitizer to kill cancer cells. When a Porphyrin derivative (photosensitizer) is introduced in the body, it reaches the interior of the cells where it is most likely present in a bound state. When these compounds reach a certain critical level in the diseased tissue, light is shone on the tissue to activate the Porphyrin-derivativesto produce the ROS (mostly singlet Oxygen) **[11].**

Singlet Oxygen can be produced by a chemical reaction **[12],** a gas phase discharge **[13]** or a photosensitization reaction **[14].** In PDT, the damage caused to cells by TPPS, FeTPPS and FeNOTPPS is due to singlet Oxygen production which results in cell death **[15]**. Singlet Oxygen can be produced either in the ER membrane from which it diffuses to the protein, or the photosensitizer itself can be bound to the Ca^{2+} ATPase. Single Oxygen has a short lifetime and a small radius of action. The binding site of Porphyrin-derivatives is likely within the Ca^{2+} ATPase. Initial diffusion of these compounds into the ER membrane followed by diffusion to binding sites within the transmembrane region of the Ca^{2+} ATPase is a possible mechanism.

In this study, molecular docking has been used to predict the binding orientation of Porphyrin-derivatives: TPPS, FeTPPS and FeNOTPPS, with respect to Ca^{2+} ATPase. The protocol used for docking comprised two parts: (i) initial introduction of a ligand in an active site; and (ii) assessment of the strength of binding by a Scoring Function. Ca^{2+} ATPase was not considered to undergo any significant conformational changes upon binding of the ligands **[16].** The strength of binding was determined by use of Scoring Functions that approximate the free energy of binding of a ligand to a receptor. Scoring Functions are expressed as a sum of separate terms that describe the various contributions to binding **[17, 18].** Generally, Scoring Functions are used to assess protein−ligand binding affinity in structure-based drug discovery. Empirical Scoring Functions (such as the one used by MM3PRO force field in CACHe) estimate the binding affinity by taking into account the various terms that can contribute to the binding free energy. These terms may include, for example, van der Waals interactions, hydrogen bonding, de-solvation effects, metal−ligand bonding, etc **[19-22].** A high value of the Scoring Function represents "tight" binding between the protein and the ligand and vice versa.

Table 1 gives the Scoring Functions **[11, 12]** obtained by Molecular Docking of TPPS, FeTPPS and FeNOTPPS with the three domains of Ca^{2+} ATPase. The values of Scoring Functions show that FeNOTPPS is energetically stable in domain 2 (amino acid 151-591) of Ca^{2+} ATPase **(Table 1).** According to the crystal structure of Ca^{2+} ATPase **(Figure 1a) [1, 2],** domain 2 contains 17 α-helices and 19 β-strands. Two of these α-helices are membrane spanning (M3 and M4). Domain 2 is also where the two Calcium-binding and a Nucleotide-binding (N) domains are located. Nucleotide-binding domain (N) is the largest of the three cytoplasmic domains. This domain stretches from Gln 360-Arg 604 and comprises a seven stranded antiparallel β-sheet with two helix bundles sandwiching it. Phe 487, which has been identified as an important residue for Nucleotide-binding, is present in this domain. Other residues such as Lys 515 and Lys 492 which are also important for Nucleotide-binding are located nearby. Given the comparable molecular sizes of ATP and Porphyrin derivatives, it is possible that FeNOTPPS is energetically stable in the Nucleotide-binding (N) domain **[23].**

Summation of Scoring Functions of TPPS/FeTPPS/FeNOTPPS in the various Ca^{2+} ATPase domains and division by three for the mean yielded Delta-values as shown in **Table 1.** The Delta-value for domain 2 is the highest which also proves that the molecules preferentially tend to dock into domain 2 (amino acid 151-591) of Ca^{2+} ATPase.

Conclusion:

Most of the photosensitizers currently being used in human clinical trials are porphyrin-based, so investigation of binding properties of TPPS, FeTPPS and FeNOTPPS and their potential application in photodynamic therapy, is important. A possible intracellular target for these molecules, after they have reached the interior of a cell, is Ca^{2+} ATPase of sarcoplasmic reticulum. Molecular docking revealed that interactions of FeNOTPPS with Ca^{2+} ATPase are relatively energetically favorable. It is possible that binding of FeNOTPPS to domain 2 (amino acid 151-591) stabilizes the Ca^{2+} ATPase in a confirmation that inhibits any further changes in conformation.

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Supplementary material:

Table 1: Total Energy, Scoring Functions and Delta-values after Docking of TPPS, FeTPPS and FeNOTPPS in different Domains of Ca^{2+} ATPase

Total Energy after Energy Minimizationof TPPS, FeTPPS and FeNOTPPS			
Parameter	**Total energy (kcal/mol)**		
	TPPS	**FeTPPS**	**FeNOTPPS**
Bond Stretching Energy	1.66	1.66	1.75
Angle Bending Energy	65.31	64.25	64.18
Torsional Energy	33.36	33.23	33.66
Out of Plane Bending Energy	0.19	0.19	1.12
1-4 van der Waals Energy	-1.45	-1.72	-1.81
Van der Waals Energy	-15.13	-14.27	-15.85
1-4 Electrostatic Energy	-6.88	-3.02	-2.90
Electrostatic Energy	-9.15	10.57	45.59
*__Scoring Function Valuesafter Docking in various Domains of Ca^{2+} ATPase__			
	Domain 1(amino acid 1-150)	**Domain 2** (amino acid 151-591)	**Domain 3** (amino acid 635-994)
TPPS	5660	9995	5404
FeTPPS	2209	10591	9281
FeNOTPPS	6438	14877	5707
Delta-values for various Domains of Ca^{2+} ATPase			
Delta-value (domain 1)	4769		
Delta-value (domain 2)	11821		
Delta-value (domain 3)	6797		

*Scoring Functions are used to predict the strength of the non-covalent interaction (also referred to as binding affinity) between two molecules after they have been docked together.Higher the value of the Scoring Function the higher the binding affinity.