# In Silico Studies of Fullerene C60 with Zebrafish Proteins Prostacyclin Synthase and S100z Calcium Binding Protein

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## ABSTRACT

Nanotechnology offers many societal benefits and this have prompted the rapid growth of engineered nanomaterials. Fullerene ($C_{60}$) due to its unique properties has become an important nanomaterial in biomedical and biotechnological applications. Once in water, Fullerene forms stable suspended aggregates and thus become bioavalable to aquatic biota. The fate and transport of fullerene in the aquatic environment is poorly understood.Little data are available on the molecular interactions of fullerene with native proteins of zebra fish (Daniorerio) which is a universally accepted experimental model. In this study, we made an attempt to assess the binding mode of fullerene with two key zebrafish proteins viz. Prostacyclin synthase (cytochrome P450 8A1) and S-100Z Calcium binding protein using Autodock 4.0. The data indicates that Fullerene potentially binds to the active sites of both the proteins and this may induce conformational changes in the native protein structure there by altering the function,which might have a toxic effect to fish in survival. Further in vivo studies are required to evaluate the toxic impact on the expression and function of above proteins.

## Keywords:

Fullerene (C60), Prostacyclin synthase, S-100Z Calcium binding protein, Docking, Autodock 4.0.

## 1. Introduction

Fullerene ($C_{60}$) is made up of hollow carbon molecules arranged in a cage of interlocking pentagons and hexagons like the patches on the soccer ball with flexible chemical reactivity (1,2). Fullerene ($C_{60}$) with 7.2 $A^o$ in diameter is similar in size to steroid hormones or peptide alpha helices, and thus considered as ideal molecules to serve as Ligand for enzymes and receptors (3). Fullerene is shown to exhibit bioactivity in antibacterial, neuroprotection and DNA cleavage etc (4-8). Although data is still emerging on the toxicity of engineered nanomaterials, research done so far indicates that

Fullerene is capable of inhibiting the growth of prokaryotic cells, certain enzymes like glutathione reductase and neuronal nitric oxide synthase (9-11). The unique properties of fullerenes have also risen to another concern, their potential risks to biological systems. Research on fullerene is critical given the high production of these materials and their possible harmful ecological effects.

Little data are available on the molecular interactions of fullerene with native proteins of Zebrafish (*Danio rerio*) which is universally accepted experimental model. Zebrafish (*Danio rerio*) a small tropical fish which lives in rivers of India, Northern Pakisthan and Nepal and Bhutan in South Asia is widely used model in many areas of biological research. Furthermore, *Danio rerio* is an excellent system for chemical toxicity testing (12-13).

Prostacyclin synthase (CYP8A1) (5.3.99.4) is located on chromosome6 which catalyses the rearrangement of ProstaglandinH2(PGH2)toprostaglandinI2,thisendoplamic reticulum membrane protein catalyzes the conversion of prostaglandin $H_2$ to Prostacyclin (Prostaglandin $I_2$), a potent vasodilator and inhibitor of platelet aggregation. An imbalance of Prostacyclin contributes to the development of myocardial infarction, stroke, and atherosclerosis in humans (Fig. 1) (14). S-100 protein is a family of low molecular weight protein found in vertebrates characterized by two calcium binding sites of the helix-loop-helix ("EF-hand type") S100 is normally present in cells derived from the neural crest (Schwanncells,melanocytes,andglialcells),chondrocytes, adipocytes,myoepithelial cells, macrophages, Langerhans cells, dendritic cells and have been implicated in a variety of intracellular and extracellular functions. S100 proteins are involved in regulation of protein phosphorylation, transcription factors, $Ca^{2+}$ homeostasis, the dynamics of cytoskeleton constituents, enzyme activities, cell growth and differentiation, and the inflammatory response (15, 16).

This study is an attempt to evaluate the binding mode of Fullerene $C_{60}$,with two key zebrafish proteins namely Prostacyclin synthase Cytochrome ($P_{450}$8A1 5.3.99.4) and S100Z calcium binding protein using AutoDock 4.0.

## 2. MATERIALS AND METHODS

### Docking Studies

To study the nature of interactions and binding mode of zebra fish proteins and fullerene. Docking was performed using Auto dock 4.0

### 2.1 Preparation of protein

PDB (17) is a single worldwide archive of structural data of biological macromolecules, established in Brookhaven national laboratories (BNL) in 1971. It contains structural information of the macromolecules determined by x-ray crystallography's and NMR methods.The Zebra fish protein structures of Prostacyclin synthase (Cytochrome P450 8A1) with PDB ID 3B98 (Figure2) and, S100Z calcium binding protein with PDB ID 2Y5I (Fig. 2) were retrieved from PDB.

### 2.2 Preparation of Ligand

The 3D structure of Fullerene C60 (Ligand) (Fig. 3) with ID110185was retrieved from ChemSpider (18).Chemspider is a free chemical structure database providing fast access to over 25 million structures, properties and associated information. By integrating and linking compounds from more than 400 data sources, ChemSpider enables researchers to discover the most comprehensive view of freely available chemical data from a single online search. It is owned by the Royal Society of Chemistry.ChemSpider builds on the collected sources by adding additional properties, related information and links back to original data sources. ChemSpider offers text and structure searching to find compounds of interest and provides unique services to improve this data by curation and annotation and to integrate it with users' applications.

### 3. Active Site Analysis:

Q-site Finder (19), an online tool which uses hydrophobic probes, was used to predict possible binding sites.

### 4. Auto dock 4.0

Autodock (20) is the first docking package to model the ligand with full conformational stability. The package consists of two sequentially applied programs, Autogrid and Autodock. Autogrid is initially used to calculate noncovalent energy of interaction between the rigid part of the receptor and a probe atom that is located at various points of the lattice. Autodocks main strengths are receptor flexibilty, blind docking, Precalculated grid maps on abinding site, free energy scoring function based on linear regression analysis, the AMBER force field and a large set of Protein ligand complexes with known inhibition .

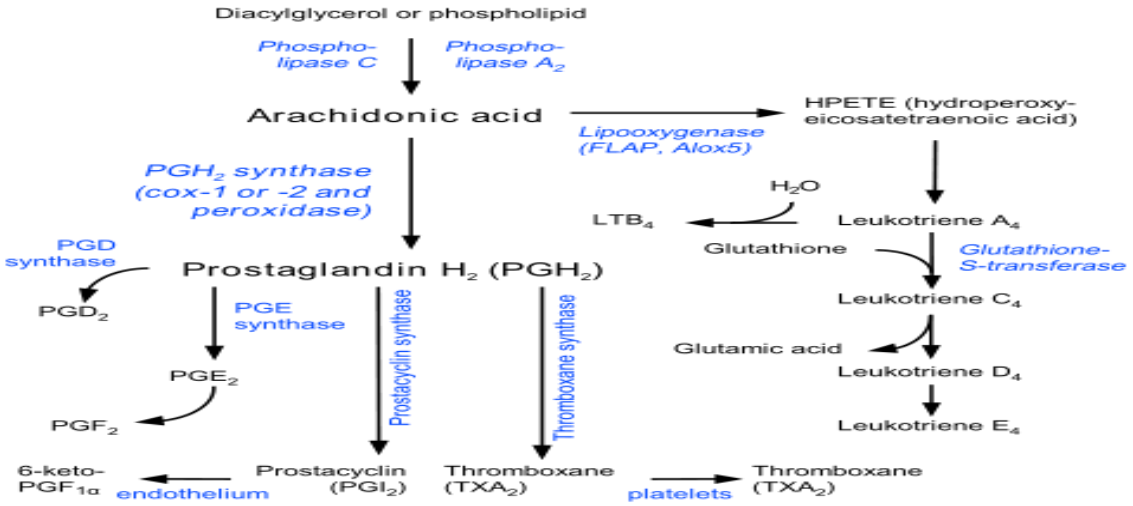


**Fig. 1: Cyclooxygenase , lipoxygenase and epoxygenase pathways leading to the formation of eicosanoids from arachidonic acid. Fullerene binds to prostacyclin synthase and may inhibits formation of prostacyclin ($PGI_2$)**


**Fig. 2: A. Prostacyclin Synthase (PDB ID 3B98); B. S- 100 Z Calcium Binding Protein (PDB ID 2Y5I)**

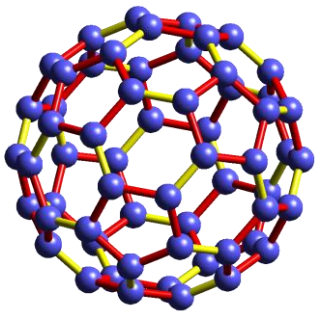

**Fig.3: Fullerene ($C_{60}$)**

## 3.0 RESULTS AND DISCUSSION

Prostacyclin synthase and S100z calcium binding protein are important enzymes related to metabolism in Zebrafish. Prostacyclin synthase is a endoplasmic reticulum membrane protein catalyzes the conversion of prostaglandin H2 to prostacyclin (Prostaglandin I2) a potent vasodilator through arachidonic acid pathway.Our Study indicates that Fullerene($C_{60}$) binds to the active site of Prostacyclinsynthase using Autodock 4.0.The binding energy was found to be -11.96 Kcal/mol as shown in Table I. The interactive residues of prostacyclin synthase with fullerene is depicted in Table 2.

Fullerene interacts with the residues,PHE126,VAL273,THR274,ASN277,CYS421,GLY423 Which are similar residues of Prostacyclin synthase, binding to selective substrates which is depicted in Table2.,where the residues are bolded.The interactive residues of prostacyclin synthase with fullerene are depicted in structure view is represented in Fig 4A . Hence ,we speculate that Fullerene could inhibit the activity of Prostacyclin synthase leading to inhibition of vasodilation and ultimately effecting the survival of fish on the long term .

S100 protein is alow molecular weight protein found in vertebrates characterized by two calcium binding sites of helix-loop-helix (“ EF-handtype “) involved in regulation ofproteinphosphorylation,transcriptionfactors,$ca^{2+}$homeostasis , the dynamics of cytoskeleton constituentsOur study also indicates that Fullerene ($C_{60}$) binds to active site of S100Z Calcium binding protein using Autodock 4.0 . The binding energy was found to be -3.78K cal/mol as shown in table I . The interactive residues ofS100 Z Calcium binding protein with Fullerene is depicted in Table 2. Fullerene interacts wth the residues ARG 97,GLN 93,LEU 82 Etc. The interactive residues of S100Z Calcium binding protein with fullerene are depicted Structure view is represented in Fig4 B .Hence we speculate that Fullerene could inhibit the activity of S100Z Calcium binding protein Which might have toxic effect on fish .

Our docking study also suggests that Fullerene binds to Prostacyclin synthase with more specificity compared to S100Z, based on the binding energies (Table 1), and the number of identical residues ( Prostacyclin synthase seven residues common between the substrate and Fullerene compared to S100Z which is three residues) binding between the selective substrate and Fullerene

(Table2)in the active site .Because of this the production of prostacyclin synthesis may decrease and lead to loss of vasoprotecting activity due to increase in vasoconstriction ,aggregation and platelet activation in fish.

S100 Z Calcium Binding ,the binding of Fullerene may effect in regulation of protein phosphorylation,transcription factors ,$ca^{++}$ homeostatsis ,which may have deleterious effect on the activity of fish in nature and environment.

**Table. 1: Energy value (Kcal/mol) of Proteins obtained by Auto dock 4.0 upon binding with Fullerene**

| SNo | Protein | Binding energy Kcal/mol |
|---|---|---|
| 1 | Prostacyclin synthase | -11.96 |
| 2 | S100z calcium binding protein | -3.78 |

**Table. 2: Residues found in the binding pocket of respective proteins using Q-site finder and residues in the active site region**

| S.No | Enzyme | Residues found in the binding pocket of proteins using Q-site finder | Fullerene bind to residues in the active site region |
|---|---|---|---|
| 1 | Prostacyclin synthase (3B98) | ALA122,**PHE126**,MET138,LEU171,PHE172,THR174,GLY175,VAL179,TRP245,TRP273,**VAL273**,**THR274**,GLN275,GLY276,**ASN277**,ALA278,GLY279,ALA281,LEU,321,LEU324,TRP414,**CYS421**,**PRO422**,**GLY423**,PHE426,ALA427,ALA431,ILE465 | **PHE126,VAL273,THR274, ASN277, CYS421, GLY423,** |
| 2 | S100Z Calcium binding protein (2Y5I) | PHE90,**GLN93**,GLN94,**ARG97**,SER98LEU38,LEU42,PHE45,LEU46,LEU54,ILE58,LEU78,**LEU82** | **LEU82, GLN 93, ARG97.** |

*** Bolded Residues are identical residues with interacting with selective substrate and Fullerene for prostacyclin synthase and S100Z calcium binding protein in the active site.**

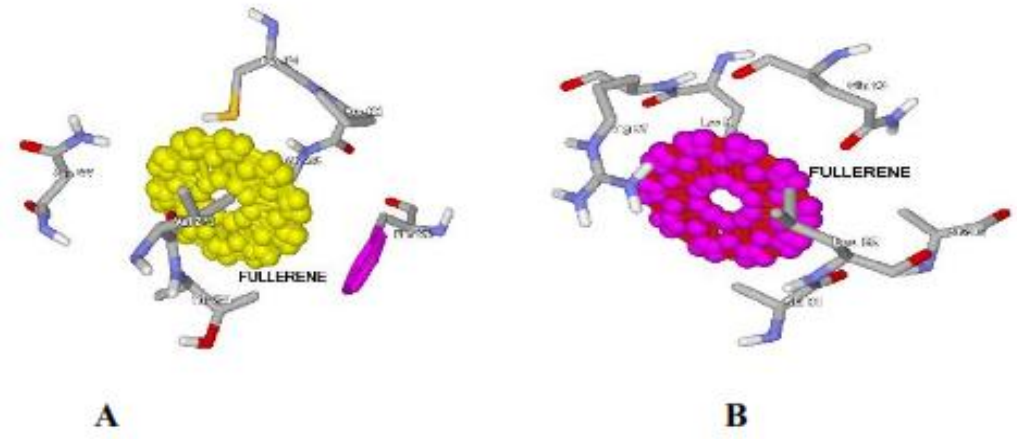


**Fig .4: A. Fullerene $C_{60}$ interaction with prostacyclin synthase 3B98, The ligand which is in ball and stick model shown in yellow color and the active site residues of protein are labelled and shown in stick model;**
**B. Fullerene interaction with S 100 Z calcium binding Protein, The ligand which is in ball and stick model shown in pink color and the active site residues of protein are labeled and shown in stick model**

## 5.0 CONCLUSION

Fullerene ($C_{60}$) binds to same residues as selective substrates for Prostacyclin synthase and S100Z Calcium binding protein in the active sites suggests that Fullerene could inhibit the activity of Prostacyclin synthase and S100Z activity. For further risk assessment of fullerene toxicity repeated studies following various animal models, methods of administration, and mechanisms of toxicity should be conducted.

## 6.0 ACKNOWLEDGMENTS

My sincere thanks to Dr. Rajashekar and Rajitha who helped me in doing work and also Sreenidhi institute of science and technology for providing Facilties to do my work .

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