**Journal of Computational Methods in Molecular Design, 2014, 4 (2):45-50**

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**ISSN : 2231- 3176**
**CODEN (USA): JCMMDA**

# Modelling and characterization of *Chlamydia pneumonia* derived Heat Shock Protein 60

**Soundhara Rajan G[1 £], Ankita Sharma[1 £], Rupsi Kharb[1] and Sagarika Biswas[1]***

[1]*CSIR-Institute of Genomics & Integrative Biology, Mall Road, Delhi, India*
**£** *Authors contributed equally*

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**ABSTRACT**

*Coronary Artery Disease (CAD), a major cause of death worldwide, arises due to the interference in blood supply by the deposition of foam cell (fat laden macrophages) in the form of waxy plaque. Chlamydia pneumoniae is the microorganism associated with the respiratory infection and also its role in enhanced inflammation during atherosclerosis has been revealed significantly. Heat Shock Protein 60 (HSP 60) produced by this microorganism is contributing to the inflammation by increasing the expression of inflammatory mediators. Computational study may provide us the insight into the functional significance of this protein in CAD. So, homology based 3D model of chlamydial heat shock protein 60 (cHSP 60) was constructed via Modeller (version 9.12) followed by validation through PROCHECK, Verify 3D, WHAT-IF, ERRAT and PROVE for reliability. Interacting partners of cHSP 60 was identified using STRING server for elucidating possible role of this protein along with interacting partners in plaque formation. Molecular level analysis of the residues involved in the active enzymatic role was identified to study the non-canonical protein-protein interactions. Identification of the B and T cell epitopes provided the base to study the possible immune-modulatory effect of cHSP60. This structure can be utilized for the future drug designing in order to minimize the effect of inflammation.*

**Keyword:** Coronary Artery Disease, foam cells, plaques, *Chlamydia pneumoniae*, chlamydial Heat Shock Protein 60.

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## INTRODUCTION

Coronary Artery Disease (CAD) occurs by the deposition of fat as plaque under the endothelium layer of artery. The plaque hardens and narrows the arteries, reducing oxygenated blood supply to heart muscle leading to Atherosclerosis [1]. *Chlamydia pneumoniae* is the pathogen reported to be an active participant of CAD progression and is found to be present in the respiratory tract of humans that enters into the blood circulation during the chronic respiratory infection. Various epidemiological based studies indicated that the active role of *Chlamydia pneumoniae* in CAD progression is due to its viable presence detected in atherosclerotic plaque. The mannose binding lectins (MBL) in the blood binds the sugar residues on the surface of the invading micro-organisms and activates the complement system to achieve the innate immunity. The individuals with the variant allele of the *mbl2* gene tend to have the decreased MBL level leading it to the chronic infection by invading bacteria. *Chlamydia pneumoniae* infection has been strongly correlated with this allelic factors that tends to increase the host's inflammation response spontaneously [2]. Two factors such as chlamydial Heat Shock Protein 60 (cHSP 60) and chlamydial lipopolysachharide (LPS) are responsible for perpetuating the inflamed condition. cHSP60 is the main instigator among the specified chlamydial factors for inflammation during CAD [3]. Inflammation starts when the foam cells interact with the T cell that releases tissue factors and metalloproteinase. These tissue factors leads to the plaque rupture. After entering into the blood circulation, cHSP60 activates the platelets and clotting factors to form fibrin net like structures and ultimately leads to the thrombus formation [4]. cHSP 60 is capable of activating the inflammatory responses by activating the T cells, macrophages and mast cells that secrets cytokines such as IL-8,

ERK, TLR-4, TNF and Interferon-γ [5, 4]. These processes ultimately give rise to the endothelial dysfunction and proliferation of vascular smooth muscle cells [2].

Available treatment strategies involving the administration of antibiotics that targets *C.pneumoniae* have been failed to prevent coronary diseases indicating that there has been some other factors along with cHSP 60 protein that may be playing a role in the pathogenesis of Atherosclerosis [4]. To unlock the factors responsible for pathogenesis of Atherosclerosis, we oriented our studies on computational structure prediction and functional annotation of cHSP 60 along with its interacting partners. The study has been further coupled with the identification of ligand binding sites and antigenic determinants in order to predict its structure related to functional relevance in humans. It may lead us to use cHSP 60 as the centre of drug target studies. With the help of predicted model we can reveal the peptides that act as an antigen and can induce the activation of inflammatory processes. Active sites and ligand binding sites may help in understanding the functions, regulations and future drug designing against this protein. Establishment of interacting partners may help in better understanding of these partners that played their role in activating inflammation.

## MATERIALS AND METHODS

### Template identification and sequence analysis of cHSP 60 protein

cHSP 60 sequence (Uniprot ID: W3RRE0) was acquired from NCBI (www.ncbi.nlm.nih.gov). Sequence, templates having similarity with cHSP60 sequence, was obtained by utilizing BLAST-P [6]. Five reference templates have been used for the present study. 3D modelling from these templates was carried out by considering only un-gapped regions of identified templates.

### 3D model construction and quality analysis studies

High quality multiple templates are the primary ingredient for the protein model construction. 3D model of cHSP 60 was constructed through Modeller (Version 9.12) using reference templates to conduct homology modelling [7]. Loop modelling was carried out through Modloop server in order to remove bumps and steric clashes among non-bonded interactions. Predicted model was refined by minimizing the energy through Swiss PDB viewer (SPDBV). The PDB of this protein was visualized by PyMOL version 1.1 (http://pymol.sourceforge.net/) [8]. Stereo chemical quality of constructed model was analyzed via program PROCHECK [9]. 3D profiling of the residues of this protein was carried out by VERIFY3D [10]. ERRAT evaluated the overall quality factor [11]. Statistical significance of the predicted model was analysed through the SAVS [12].

### Active site prediction

Identification of active sites can provide the valuable information about the residues involved in physiochemical properties of protein that are needed for its function. Computed Atlas of Surface Topography of protein (CASTp) server (http://sts-fw.bioengr.uic.edu/castp/) was utilized for the prediction of active sites of the protein structure [13].

### Ligand binding site identification

An automated server 3DLigandSite (http://www.sbg.bio.ic.ac.uk/3dligandsite/) was utilized for the prediction of ligand binding sites. A structural library has been searched to identify the homologous structures with bound ligands by employing 3D structure of the cHSP 60. The annotated structures were superimposed onto the predicted 3D structure of cHSP 60 [14] for the prediction of ligand binding sites.

### Interaction based studies

STRING database (http://string-db.org/) has been utilized to find out the interacting partners of cHSP 60. The full description of a protein's function requires knowledge of all partner proteins with which it specifically interacts and STRING facilitates the analysis of biological processes at system level [15, 16]. It provides graphical representation of both physical and functional protein-protein interactions network that gives a high-level view of functional association with cHSP60 [17].

## RESULTS AND DISCUSSION

Five templates (1IOK, 4KI8, 2YEY, 2EU1, 1J4Z) were predicted through BLASTp program for homology based structure prediction. All non hydrogen main-chain and side-chain atoms are included to the generated three dimensional model of cHSP 60 (Figure 1).

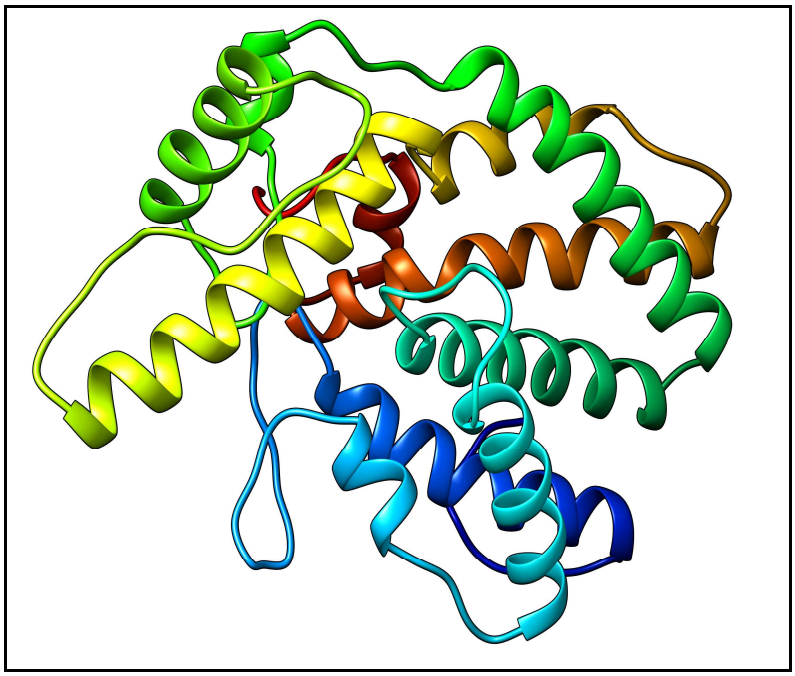

**Figure 1: 3D model of cHSP 60**

This model satisfies the stereochemistry and is validated through Ramachandran plot. This analysis indicates that 87.4% amino acid corresponds to the most favoured regions and 12.2% amino acid corresponds to the allowed regions, while the amino acid in the disallowed region is 0.0%. Analysis with VERIFY 3D shows that 44.87% residues has good environmental profile of protein due to the average 3D-1D score greater than 0.2. Overall quality factor predicted through ERRAT 2 was 94.444% thus validating the quality of predicted structure and its reliability for prediction of ligand binding sites and eventually in drug designing. This model directly correlates with the results of SOPMA that indicates 55.34% Alpha-Helix, 12.21% extended sheets, 9.16% Beta-turns and 23.28% random coil. Quality estimation of predicted model was carried out by comparing it with high resolution reference structures, obtained from X-Ray crystallographic analysis on the basis of Z-score. The Z-score "0" indicates good model and the Z-score of our predicted model have been calculated to be 6.789, which indicates that our model is statistically significant model. Total 21 active sites were elucidated in the structure with ideal parameters. Five pockets were selected on the basis of pocket volume to find out its residues around probe radius of 1.4Å (Table 1).

**Table 1: Colours represents the active sites of cHSP 60 and their physical parameters**

| Active site | Area (Å$^2$) | Volume (Å$^3$) |
|---|---|---|
| | 2493 | 4315 |
| | 149.7 | 152.9 |
| | 99 | 91.4 |
| | 148.7 | 129.3 |
| | 143.7 | 84.8 |

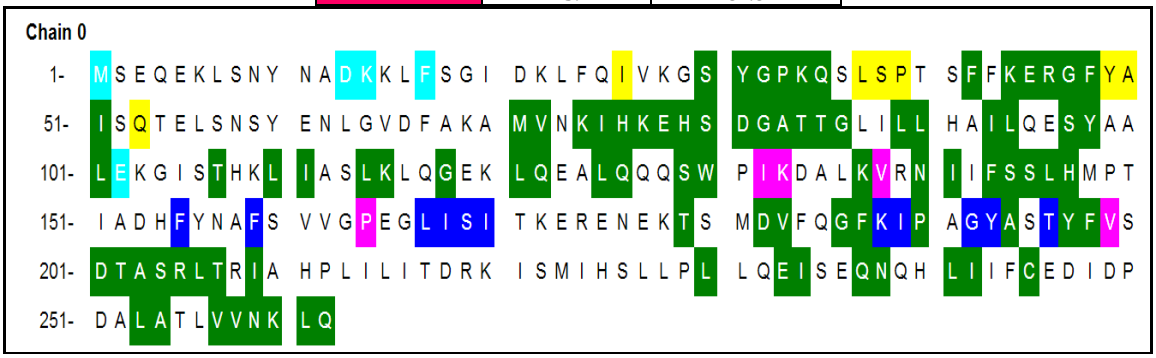


**Figure 2: Active sites of cHSP 60 are represented with different colours in predicted model**

The area of the largest active site has been found to be 2493Å$^2$ and volume has been estimated to be 4315Å$^3$. The amino acid residues comprising this surface pocket were represented by green colour (Figure 2). The surface antigenic peptide analysis of cHSP 60 indicates 13 antigenic determinants (Table 2). Besides the antigenic properties, the result also interprets the possibilities of these peptides to involve in protein-protein interactions.

**Table 2: Possible antigenic peptides observed in cHSP 60**

| S.No | Start position | End position | Peptide | Peptide length |
|---|---|---|---|---|
| **1.** | 17 | 30 | FSGIDKLFQIVKGS | 14 |
| **2.** | 33 | 41 | PKQSLSPTS | 9 |
| **3.** | 48 | 54 | FYAISQT | 7 |
| **4.** | 62 | 70 | NLGVDFAKA | 9 |
| **5.** | 85 | 103 | TGLILLHAILQESYAALEK | 19 |
| **6.** | 107 | 119 | THKLIASLKLQGE | 13 |
| **7.** | 122 | 130 | QEALQQQSW | 9 |
| **8.** | 133 | 149 | KDALKVRNIIFSSLHMP | 17 |
| **9.** | 152 | 170 | ADHFYNAFSVVGPEGLISI | 19 |
| **10.** | 181 | 202 | MDVFQGFKIPAGYASTYFVSDT | 22 |
| **11.** | 206 | 218 | LTRIAHPLILITD | 13 |
| **12.** | 222 | 234 | SMIHSLLPLLQEI | 13 |
| **13.** | 238 | 248 | NQHLIIFCEDI | 11 |

The cHSP 60 has 67.99% hydrophilic amino acids, 32% hydrophobic amino acids and .01% others. Total 5 ligand binding sites has been identified using 3DLigandSite (Table 3).

**Table 3: Description of the predicted ligand binding pocket in the cHSP60 model**

| Ligand cluster | Residues | Av dist | ASA |
|---|---|---|---|
| 1 | Tyr31 | 0.00 | 0.22 |
| | Gly32 | 0.00 | 0.19 |
| | Asp81 | 0.05 | 0.47 |
| | Gly82 | 0.04 | 0.21 |
| | Thr84 | 0.00 | 0.17 |
| | Thr85 | 0.00 | 0.25 |
| | Phe143 | 0.28 | 0.19 |
| | Gln237 | 0.17 | 0.22 |
| | Leu241 | 0.01 | 0.15 |
| | Asn259 | 0.04 | 0.76 |
| | Lys260 | 0.00 | 0.53 |
| | Leu261 | 0.00 | 0.74 |
| | Gln262 | 0.57 | 0.79 |

Isoelectric point of this protein has been found to be 6.51. The protein has good water solubility property with hydrophilic solvent accessible surface area being 14979.43 Å2.

Total 10 proteins have been identified as the possible partners of cHSP 60 by STRING database based on their similar pattern of expression with the confidence probability being 0.4. Co-expression of cHSP 60 with dnaK, thrS and pheT implicates the possible involvement in regulation of heat shock response; ATP regulated molecular process, drug binding, RNA binding and magnesium ion binding (Figure 3).

## CONCLUSION

*Chlamydia pneumoniae* is the suspect of inflammation in a lower respiratory tract infection and was first identified using electron microscopy in coronary atherosclerotic plaques [19]. *Chlamydia pneumoniae* is a Gram-negative obligate intracellular bacterium and has been found frequently in lesions of the aorta, iliac, carotid, and coronary arteries [20–22]. This pathogen generated interest in scientific community due to its active contribution in exacerbation of lesions reported in rabbit and mouse animal models [3]. Several studies have shown viable presence of *C.pneumoniae* in atherosclerotic plaques but not in adjacent normal tissues suggesting its main role in triggering the inflammatory response [20, 21]. Presence of *C.pneumoniae* in atherosclerotic lesions raises the doubt that whether the organism plays a causal role or is an innocent bystander [20, 23]. cHSP 60 is the protein secreted by this pathogen that belongs to 60kDa heat shock protein family [24]. This protein is implicated to be responsible for stimulating the immuno-pathogenic response in many diseases [25]. cHSP 60 provokes the proliferation of human vascular smooth muscle cells (SMC) via toll-like receptor 4, as a result; it contributes to the Atherosclerosis [26]. Evidence supporting a causal role of chlamydia in atherogenesis comes from recent reports that *C.pneumoniae* and chlamydial antigens can activate mononuclear phagocytes and vascular cells, including SMCs. A heat-stable

component of *C. pneumoniae* induces macrophage foam cell formation [27] and this effect is replicated by chlamydial lipopolysaccharide (LPS). cHSP 60 is a highly expressed chlamydial protein that can activate macrophages and vascular cells by mechanisms that are not well characterized [21]. Detailed structural annotation of cHSP 60 enables the in-silico studies of functional characterization of associated surface properties and molecular level interactions including drug designing. The identification and prediction of ligand binding sites implicated the residues that will provide prerequisite for docking studies for virtual drug design. It gives insight into the residues that serve biological purpose during the binding of compatible ligands [28]. The residues (30, 31, 32, 33, 46, 47, 48, 81, 82, 83, 84, 85, 258, 259, 260 and 261) form the ligand binding pockets. This surface pocket binds to the unfolded peptide representing the GroEL (molecular chaperon) activity that is required for the correct folding of many proteins in Chlamydia. This protein assembles into a homo-tetradecameric complex and exploits the co-chaperone protein GroES and ATP hydrolysis to aid in the proper folding of a various cytosolic proteins. Residues (216, 228) present in the active site exhibit the para-hydroxybenzoate hydroxylase enzyme activity that inserts oxygen into the substrates with the assistance of labile intermediate [29]. These intermediate performs essential role for the translocation of substrates into the solvent-shielded active site during catalysis.

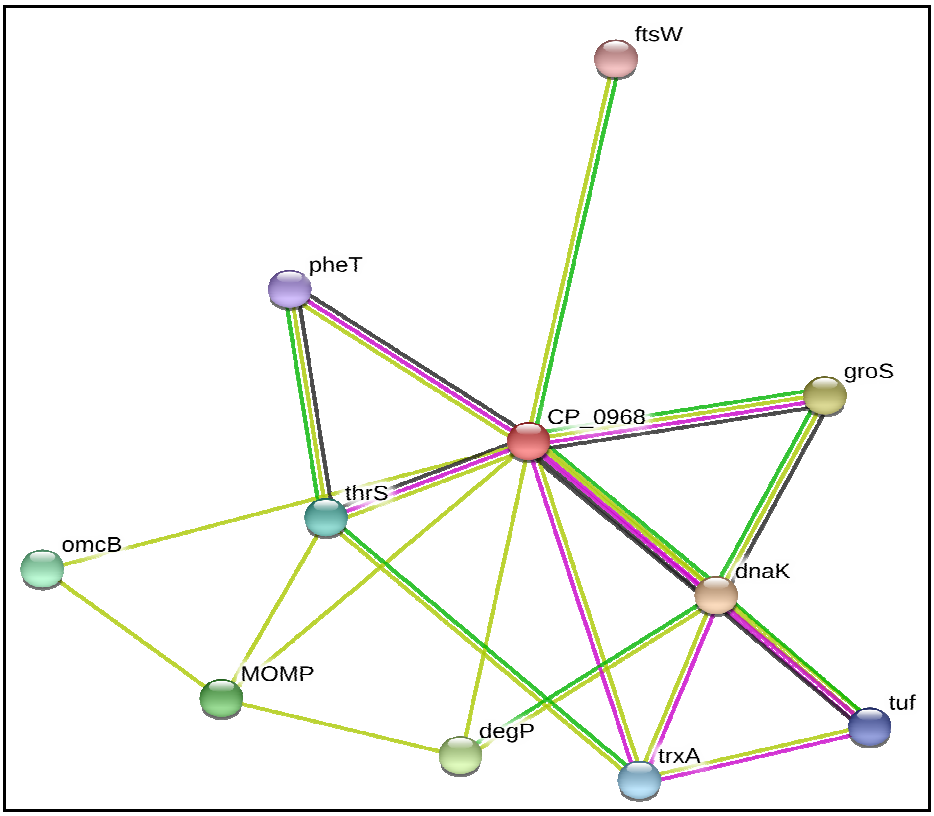


**Figure 3: Possible interacting partners of cHSP 60.**

Thus the molecular characterisation of the cHSP60 may provide a lead to the drug designing and virtual screening studies to identify the possible ligands that regulate the activity of cHSP 60. The result may lead us to obtain the clinical significance in treating inflamed conditions like CAD. This study can be further used in identification of the cHSP 60 regulated signalling pathways behind the activation of immuno-modulatory response during the plaque formation.

**Acknowledgments**
This work is funded by CARDIOMED project code: BSC0122 of Council of Scientific and Industrial Research (CSIR), Government of India, New Delhi, India.

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