Chapter 4

STRUCTURAL INSIGHT INTO PROKARYOTIC HEAT SHOCK REGULATION
4.1 A STRUCTURAL INSIGHT INTO THE PROKARYOTIC HEAT SHOCK TRANSCRIPTION REGULATORY PROTEIN σ32 WITH THE HELP OF σ32-DnaK INTERACTION

The proper conformation of proteins and hence cellular survival is challenged by stress conditions like extreme heat which results in a massive aggregation of proteins inside both eukaryotic and prokaryotic cells. This response called the ‘heat shock response’ leads to the induction of almost all the universally conserved ‘heat shock genes’ which encode chaperones, proteases and other stress related proteins.

In *E. coli*, this regulation is mediated by *rpoH* gene product, the alternative sigma factor σ^32_. Its intracellular level is low and increases transiently after temperature up-shift [Straus et al. 1987]. The cellular concentration of σ^32_ is tightly controlled at four different levels: transcription and translation of the *rpoH* and activity and stability of σ^32_ protein. Heat induction of σ^32_ mainly occurs at the post transcriptional level. An extended secondary structure in the *rpoH* transcript blocks translation at low temperatures [Morita et al. 1999]. Thermal melting of that structure permits ribosome entry followed by translation initiation. Once produced the fate of σ^32_ is determined by its interaction with a number of other proteins including chaperones as DnaK, DnaJ, GrpE and GroEL/ES and proteases as ClpP family, HslUV, Lon and FtsH [Gamer et al. 1996; Tatsuta et al. 1998].

Under non-stress conditions, σ^32_ is neutralized by an interaction with DnaK and DnaJ proteins. This interaction serves two regulatory functions. It inactivates σ^32_ by preventing it from interaction with the RNA polymerase core enzyme and renders it susceptible to FtsH-mediated degradation [Tatsuta et al. 1998, Tomoyasu et al. 1998] (half-life of σ^32_ is < 1min) as over expression of σ^32_ is toxic [Guisbert et al. 2004]. Accumulation of unfolded proteins upon heat stress conditions titrates away the DnaK system, leaving behind free σ^32_, which associates with RNA polymerase and in turn initiates transcription of heat shock genes. Accumulation of σ^32_ only occurs in the initial phase (induction phase) of the heat shock response where the levels and half-life of σ^32_ increase transiently [Straus et al. 1987, Kanemori et al. 1999]. Elevated temperatures introduce a conformational change
in σ^{32} which specifically abolishes interaction with DnaK [Chattopadhyay et al, 2002]. It is assumed that the amino acid residues 190-205 in σ^{32} are responsible for σ^{32} specific function and become disordered at higher temperatures [Chattopadhyay et al, 2002]. The structurally altered sigma factor is rapidly turned over by cellular proteases. As a consequence, the cellular level of σ^{32} decreases and the heat shock response is shut off.

Several experiments for studying the dynamical properties as fluorescence resonance energy transfer, fluorescence anisotropy measurements and hydrogen / deuterium exchange have suggested significant structural flexibility in the core of σ^{32} [Rodriguez F et al. 2008]. This property is probably the main hindrance for the formation of good crystals and hence high resolution crystallographic 3D structure of the protein is still unavailable. As a result many structural and functional properties of σ^{32}, its interactions with the chaperones as well as with the proteases are still obscure.

In the present scenario an attempt has been made to analyze the structural biochemistry of σ^{32} protein along with its interactions with DnaK. We report a three dimensional model of σ^{32} built by homology modeling. We have docked the 3D structure of DnaK with the homology model of σ^{32}. This docked model is the first of its kind which have been used to elucidate a structural insight of the mechanism by which DnaK interacts with σ^{32} and regulate its activity and stability.

4.1.1 METHODS:

4.1.1.1 PROTEIN STRUCTURE DATABASES AND PDB

Structure databases archive, annotate and distribute sets of atomic coordinates in a systematic manner. The purpose of the structural databases is to organize and annotate the protein structures as summary of experimental results.

The Protein Data Bank (PDB) is a repository for three dimensional structural data obtained by X-ray crystallography, NMR spectroscopy or Cryo-electron microscope, of macromolecules, such as protein and nucleic acids. In 1971 at Brookhaven National
Laboratory PDB was established as public repository just with seven structures. Currently PDB is known by Worldwide Protein Data Bank, www PDB, a consortium that host deposition, annotation and distribution of coordinate structures.

4.1.1.2 PROTEIN STRUCTURE PREDICTION:

Protein structure determination by experimental techniques like X-ray crystallography, Cryo EM, NMR requires a significant amount of expertise, resources, time and sometimes with all these facility it becomes heard to determine structure experimentally due to some typical characters of the protein. Here structure prediction methods bridges between good determined structure and experimentally achieved information set. Two major approaches to-words three dimensional structure prediction are (a) comparative or homology modeling and (b) de novo or ab initio methods. Between these two approaches comparative modeling is the most reliable method and it shows good accuracy with experimentally determined facts.

4.1.1.3 HOMOLOGY MODELING

Two major foundation of homology modeling are:

(a) Three dimensional structure of protein can be predicted from their amino acid sequences.
(b) In the course of the evolution structures are more stable and rate of structural changes is much slower than sequence. This means structures are more conserved than sequences which reflect in the conserved fold patterns of protein.

Structure determination by homology modeling is done using four steps:
(a) Searching known structure (template) using to be modeled sequence (target),
(b) Alignment of the two aforementioned sequences,
(c) Building the model,
(d) Assessing the model.
4.1.1.4 HOMOLOGY MODELING BY MODELLER:

MODELLER is widely used computer program for homology modeling of protein structure. MODELLER generates comparative models by following spatial restraints:

(a) Homology-derived restraints on the distances and dihedral angles in the target sequence, extracted from its alignment with the template structure.

(b) Stereo chemical restraints such as bond length and bond angle preference, obtained from the CHARMM-22 molecular mechanics force field.

(c) Statistical preference for dihedral angles and non-bonded inter-atomic distances obtained from a representative set of known protein structures and some time optional manual restraints also implemented according to the necessity.

4.1.1.5 PROTEIN-PROTEIN DOCKING:

In biological system proteins rarely act in isolation but bind with other biomolecules to participate cellular process. Here docking methods comes into play and provide substantial structural knowledge about the complex, from which functional information can be drawn. Docking method can be described as a combination of two components:

(a) Developing a conformational search method to find possible docked conformation and
(b) Ranking such orientations in order to find the correct or nearly correct conformation using energy function.

4.1.1.6 SEQUENCE AND STRUCTURE FILES:
The amino acid sequence of $\sigma^{32}$ protein from *E. coli* K-12 was collected from Uniprot (accession numbers P0AGB3). The amino acid sequence of the $\sigma^{32}$ protein was used to search Brookhaven Protein Data Bank (PDB) [Rose et al. 2011] for suitable template(s) to build homology model using the BLAST software tool [Altschul et al. 1990]. The BLAST search picked up the crystal structure of the *T. thermophilus* RNA polymerase holoenzyme (PDB Code: 2A6H F chain) as the template with 40% sequence identity with $\sigma^{32}$ protein from *E. coli* K-12. The crystal template was used to model the three dimensional structure of the $\sigma^{32}$ protein from *E. coli* K-12 using the program Modeler in the Discovery Studio 2.5 Platform from the Accelrys. The model of $\sigma^{32}$ protein was then subjected to energy minimization using CHARMM force fields [Brooks et al. 1983] using steepest descent (SD) algorithm. Then the stereo-chemical qualities of the three dimensional models were checked using PROCHECK [Laskowski et al. 1993], and ERRAT [Colovos et al, 1993]. The software tools predicted a good model quality and no residues were found to be present in the disallowed regions of the Ramachandran Plot [Ramachandran et al. 1963]. In order to make $\sigma^{32}$–DnaK protein complex the model of the $\sigma^{32}$ protein was docked with the crystal structure of DnaK protein (PDB Code: 1DKX) using the program GRAMM [Tovchigrechko et al, 2006]. GRAMM produced seven different models of the $\sigma^{32}$–DnaK protein complex. Among them the best structure of the complex was chosen on the basis of the biological relevance as present in literature [Rodriguez et al. 2008]. The model of $\sigma^{32}$–DnaK protein complex was then protonated at pH 7.5 using Accelrys Discovery Studio 2.5 and then subjected to 2000 cycles of energy minimization using CHARMM force fields with steepest descent (SD) algorithm until the structure of the $\sigma^{32}$–DnaK protein complex reached the final energy derivative of 0.001 kcal / mole. The stereo-chemical qualities of the docked protein complex were again checked using PROCHECK and ERRAT. No residues were found to be present in the
disallowed regions of the Ramachandran Plot. Then molecular dynamics (MD) simulations were performed on the docked structure to predict the favorable binding interactions between $\sigma^{32}$ and DnaK proteins. The initial temperature of the dynamics run was kept constant at 303K. The system was solvated with water molecules. The $\sigma^{32}$–DnaK protein complex was then heated until the complex reached the temperature 316K. The same process had been followed for the modeled $\sigma^{32}$ protein (before docking to DnaK protein) at 303K and 316K temperatures. The final structures of $\sigma^{32}$ proteins as well as those of $\sigma^{32}$–DnaK protein complex at 303K and after heating at 316K were then analyzed to find out the possible modes of binding.

4.1.2 RESULTS & DISCUSSION

4.1.2.1 STRUCTURE OF SIGMA32

The $\sigma^{32}$ protein from *E. coli* is an all alpha protein with 284 amino acid residues. The protein had a total of thirteen helices (shown in figure 4.1 A). Structurally the protein might be considered to have four domains. All the domains were made up of helices connected together by loop regions. Overall the protein had similar structural arrangements of its secondary structural elements as in the protein RNA polymerase holoenzyme from *T. thermophilus* (PDB ID: 2A6H_F chain).

![Figure 4.1](image)

**Figure 4.1** (A) Three dimensional structure of prokaryotic transcription regulatory protein $\sigma^{32}$. (B) Six hydrogen bonds forms between DnaK and $\sigma^{32}$. DanK colored cyan and $\sigma^{32}$ colored red. (C) Superimposition of binding interface of DnaK docked $\sigma^{32}$ at 32°C (red) and 43°C (cyan).
4.1.2.2 STRUCTURAL CHANGES OF SIGMA32 WITH TEMPERATURE

It had been a well established fact that in *E. coli* upon temperature increase from normal to heat shock temperature, cellular level of σ^{32} protein increases [Kanemori et al. 1999]. Till date no detailed analysis of the conformational changes of σ^{32} at elevated temperature had been elucidated. In order to account for the loss of σ^{32} specific function from a structural perspective, the model of the σ^{32} protein was stepwise heated from 32^0C to beyond 42^0C. Initially no structural changes were observed but at around 43^0C the spatial arrangement of the protein were found to be significantly altered as measured by the r.m.s.d values (10.696 Å) of the backbone atoms of the proteins at these two different temperatures. This clearly indicated a huge change in the conformation of the σ^{32} protein at elevated temperature.

4.1.2.3 INTERACTIONS BETWEEN SIGMA32 AND DNAK

The three dimensional coordinates of the σ^{32} -DnaK protein complex had been generated using molecular docking technique with the help of the software tool GRAMM. When σ^{32} interacted with DnaK no significant changes occured in the σ^{32} structure (r.m.s.d. between σ^{32} in σ^{32} -DnaK complex and σ^{32} alone 0.2Å). Analysis of σ^{32} - DnaK protein complex showed that the interactions between the proteins had been stabilized mainly by H-bonds involving the side chain atoms of the proteins. The residues involved in interactions are shown in table 4.1 and fig 4.1 B. Interestingly, it had been observed that all the amino acid residues from σ^{32}, involved in binding DnaK fall in the region2 and region3 of σ^{32}. Though the aforementioned regions had previously been predicted to be involved in binding with DnaK and responsible for σ^{32} specific functions [Chattopadhyay et al, 2002; Rodriguez et al. 2008], the molecular details of the binding had not yet been established. From that point of view this was the first report regarding the structural details of σ^{32} as well as the interactions between σ^{32} and DnaK. Heating σ^{32} -DnaK protein complex beyond 42^0C, destroyed all six H-bond and resulted in total loss of interaction
between $\sigma^{32}$ and DnaK. This was because of the loss of structural arrangements in the binding interface of the $\sigma^{32}$ protein. This was exemplified by r.m.s.d value (8.276Å) of the backbone atoms of $\sigma^{32}$ protein above 42°C (Fig. 4.1 C). This might play a major role in decreasing the degradation rate of $\sigma^{32}$. Major structural change had been identified to occur in the binding interface of $\sigma^{32}$-DnaK protein complex. This result was consistent to earlier studies by Chattopadhyay et al. 2002, which reported that this abolition of $\sigma^{32}$-DnaK interaction is mainly due to the structural changes of $\sigma^{32}$ at heat shock temperature.

**Table 4.1: H-Bond of $\sigma^{32}$-DnaK complex: (A: $\sigma^{32}$, B: DnaK)**

<table>
<thead>
<tr>
<th>H-Bond</th>
<th>Donor Atom</th>
<th>Acceptor Atom</th>
<th>Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B:SER423:HN -</td>
<td>HN</td>
<td>OD2</td>
<td>1.93552</td>
</tr>
<tr>
<td>A:ASP215:OD2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B:HIS541:HD1 -</td>
<td>HD1</td>
<td>OD2</td>
<td>2.31651</td>
</tr>
<tr>
<td>A:ASP191:OD2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B:HIS541:HE2 -</td>
<td>HE2</td>
<td>O</td>
<td>1.95011</td>
</tr>
<tr>
<td>A:VAL198:O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B:GLN549:HE22 -</td>
<td>HE22</td>
<td>OD1</td>
<td>1.92394</td>
</tr>
<tr>
<td>A:ASP183:OD1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B:LYS556:HZ1 -</td>
<td>HZ1</td>
<td>O</td>
<td>1.95603</td>
</tr>
<tr>
<td>A:ALA69:O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B:GLN604:HE21 -</td>
<td>HE21</td>
<td>O</td>
<td>2.07719</td>
</tr>
<tr>
<td>A:GLY70:O</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
4.1.3 CONCLUSION:

The heat shock response mechanism is a very vital biochemical process and heat shock regulation is mainly controlled by $\sigma^{32}$ protein. The functionality of $\sigma^{32}$ is temperature dependent; at lower temperatures $\sigma^{32}$ is inactivated by its interactions with DnaK and this interaction is completely abolished above $42^\circ$C. Till date no molecular details of the interactions are available. In the present scenario, an attempt has been made to analyze first the structure of $\sigma^{32}$ obtained by comparative modeling techniques and then to study the interactions between $\sigma^{32}$ and DnaK. From this molecular modeling study we could specifically identify the binding interactions of $\sigma^{32}$ with DnaK and the mechanism of regulation of its activity and stability by DnaK. We also have predicted the three dimensional structure of $\sigma^{32}$ from *E.coli* which is the first of its kind. Our study may be useful for future mutational experiments in order to find out the possible roles of the amino acids in binding $\sigma^{32}$ with DnaK.

In this work an attempt had been made to analyze the probable molecular details of the interactions of $\sigma^{32}$ with DnaK. The three dimensional structure of $\sigma^{32}$ had been predicted using the homology modeling technique. The functions of $\sigma^{32}$ were dependent on temperature. Therefore, the modeled structure of $\sigma^{32}$ had been heated from $32^\circ$C to $43^\circ$C. It revealed a huge structural change beyond $42^\circ$C. The $\sigma^{32}$ was known to bind DnaK. In order to elucidate the mode of binding of $\sigma^{32}$ with DnaK, the three dimensional coordinates of the $\sigma^{32}$ and DnaK had been used to dock the two proteins together by molecular docking. Then interactions between $\sigma^{32}$ and DnaK had been calculated. The interaction scheme revealed that the presence of amino acid residues from the region 2 and region 3 of $\sigma^{32}$. So far this was the first report that deals with the detailed molecular biochemistry of temperature dependence of $\sigma^{32}$ and $\sigma^{32}$–DnaK interactions. Our study may therefore be useful to future genetic studies to elucidate the roles of the amino acid residues in the protein-protein interactions.
4.2 MUTUAL INTERACTION STUDY BETWEEN DNAK-GROEL-FTSH WITH HEAT SHOCK REGULATOR σ32 TO EXPLAIN PROKARYOTIC HEAT SHOCK REGULATION

The heat shock response (HSR) is mainly described as the response of the cell to combat with temperature up-shift [Morimoto et al, 1994]. The major consequence of this is the up-regulation of a group of proteins called heat shock proteins (hsp’s) and in Escherichia coli (E.coli) these proteins are generally regulated by the transcription factor σ32. The σ32 mediated HSR occurs after both up-shift and downshift in temperature [Straus et al, 1987]. There are three regulatory loops which control the output of HSR by altering the level and activity of σ32: (a) at elevated temperature translation of σ32 increases, (b) σ32 stability is transiently stabilized after shifting to high temperature (c) negative regulation followed by degradation of σ32 [Yura et al, 1999]. After production, the fate of σ32 is determined by the mutual interactions with a team of chaperones like DnaK/J, GroEL/S, GrpE and proteases as ClpP family, HslUV, Lon and FtsH [Guisbert et al, 2004; Tomoyasu et al, 1998; Gamer et al, 1996]. At physiological condition, σ32 is counterbalanced by the interaction with DnaK/J and GroEL/S which serves two functions: (a) to inactivate σ32 by preventing it from RNA polymerase core enzyme interaction, (b) to make it susceptible for the FtsH-mediated degradation [Gamer et al, 1996; Tatsuta et al, 1998]. Elevated temperatures introduce a conformational change in σ32 which specifically abolishes interaction with them [Chattopadhyay et al, 2002]. The degradation mechanism of σ32 however is a complex phenomenon. It had been an established fact that FtsH has a very poor unfoldase activity both in vivo and in vitro. Hence activity of FtsH depends upon the spontaneous unfolding of the protein [Herman et al, 2003; Dougan et al, 2002].

Even after thorough experimental investigation and review on the mechanism of heat shock chaperon network in E. coli by Guisbert et al [Guisbert et al, 2004, 2008], till date it is unclear whether the chaperones work together with FtsH to promote degradation of σ32 or the degradation occurs by other more indirect pathway. In this particular study we
tried to figure out the interactions of σ32 with some of the members of this chaperone and protease team using a structural bioinformatics approach in order to gain a deeper understanding of the heat shock regulation mechanism from a structural perspective. So far, this work is the first of its kind. Results from this study would therefore be beneficial to the understanding of the mechanism of heat shock response in *E. coli*.

### 4.2.1 METHODS

#### 4.2.1.1 SEQUENCE AND TEMPLATE SEARCH

There is no three dimensional structural report on sigma32 in PDB. So to build the structure of sigma32 we used the amino acid sequence of sigma32 protein from *E. coli* (Uniprot ID P0AGB3) and then we used this sequence to select the ideal template by PSI-BLAST using Protein Data Bank (PDB) and selected F chain of *T. thermophilus* RNA polymerase holoenzyme (PDB Code: 2A6H F chain) as template [Roy et al, 2012]. Like sigma32, the structure of *E. coli* FtsH was also needed to be modeled for our analysis. For this, first the sequence of *E. coli* FtsH protease domain from E. coli was extracted from UniProtKB (ID: 067077) [UniProt consortium]. This was then used to search for suitable template in PDB [Bernstein et al, 1977] using PSI-BLAST [Altschul et al, 1990] for homology modeling. The BLAST search result picked up the A chain of crystal structure of metalloprotease FtsH (PDB ID 2CE7) [Bieniossek et al, 2006] as closest match with the highest query coverage [58% of identity and 58% of query coverage].

#### 4.2.1.2 THREE DIMENSIONAL STRUCTURE FILE PREPARATION

2CE7-A chain for FtsH protease domain and 2A6H, F chain for sigma32 was then used as the templates to construct homology models of these proteins from *E. coli* using Discovery Studio package version 2.5. A total of 10 different models for FtsH protease domain were generated. The stereo-chemical qualities of the models were checked by
PROCHECK [Laskowski et al, 1993] and Verify3D [Lüthy et al, 1992]. The model which passed the Verify 3D criterion and had no residues in the Ramachandran plot [Ramachandran et al, 1963] was selected for further study. Reported crystal structure of DnaK (PDB ID 1DKX) [Zhu et al, 1996] and GroEL (PDB ID 1PCQ) [Chaudhry et al, 2003] have been used for the interaction study.

4.2.1.3 DOCKING OF DnaK-GroEL-FtsH WITH SIGMA32

All the models were then used separately to dock with σ32 using ClusPro 2.0 [Comeau et al, 2004] and Z-Dock [Chen et al, 2003] servers. The stereo-chemical qualities of the resultant docked complexes were again checked with PROCHECK and Verify3D and the best docked complex selected following the previous protocol was considered for further analysis. This complex was then energy minimized to ensure proper interactions. 4000 cycles of steepest descent [van der Spoel et al, 2011] energy minimization steps were applied with CHARMM force field [Brooks et al, 1983] until the structure reached a final derivative of 0.01 Kcal/mole. After minimization the structure was verified again with PROCHECK and Verify3D. This docked complex was subjected to molecular dynamics simulations with heating, equilibration and production run using simulation package of Discovery Studio version 2.5. σ32-GroEL and σ32-FtsH complexes at normal temperature and heat shock temperature are shown in figure 4.2 (a), 4.2 (b) and figure 4.3 (a), 4.3 (b) respectively.

4.2.2 RESULT & DISCUSSION

4.2.2.1 σ32 AND GroEL INTERACTION

The final pose of σ32-GroEL complex was selected by comparing the structures obtained from Z Dock as implemented in the ClusPro docking server. Final selected structure was then minimized using CHARMM force field and validated using PROCHECK and Verify3D.
tools to nullify structural errors. Analysis of σ32-GroEL protein complex shows that the interactions between these two proteins occur mainly by H-bonds involving the side chain atoms of the proteins at normal physiological temperature. When the complex is kept at heat shock temperature the number of H-bond tends to decrease [Fig. 4.4] which signifies that at elevated temperature σ32 and GroEL loses its complex assembly.

Figure 4.2 Depiction of interactions between (a) GroEL & σ32 (above) and (b) FtsH & σ32 at normal temperature (below). In both cases σ32 is colored yellow.
4.2.2.2 σ32 AND DnaK INTERACTION

Our group previously reported the mode of interactions between σ32 and DnaK elaborately [Roy et al, 2012]. There we clearly showed that σ32 and DnaK interaction is temperature dependent which is in agreement with the observations of Chattopadhyay et al [2002].

4.2.2.3 σ32 AND FtsH INTERACTIONS

The σ32-FtSH protein complex structure selected from all the possible poses (using the same protocol as in case of σ32-GroEL interactions) shows that σ32-FtSH protein complex has been stabilized mainly by H-bonds. Here we observe that number of H-bonds increases with temperature up-shift [Fig. 4.4]. It has already been studied that at heat shock temperature σ32 looses its compact conformation [Roy et al, 2012] and opens up at the temperature. This might favor its binding with FtsH. This increase in the binding affinity between FtsH and σ32 plays a major role in the FtsH mediated degradation of σ32.
4.2.2.4 STRUCTURAL INTERPLAY OF HEAT SHOCK REGULATION

Interactions between these proteins with heat shock regulatory protein σ32 suggest the fact that interactions of σ32 with both DnaK [Chattopadhyay et al, 2002, Roy et al, 2012] and GroEL are temperature dependent. In-vitro and in-vivo report suggests that GroEL controls the stability and activity of σ32 with similar efficiency as DnaK [Guisbert et al, 2004].

Figure 4.3 Depiction of interactions between (a) GroEL & σ32 (above) and (b) FtsH & σ32 at elevated temperature (below). In both cases σ32 is colored yellow.
In case of FtsH-σ32 complex, the number of H-bonds increases with temperature up-shift [Figure. 4.4]. This up-shift of temperature alters the structure of σ32 to an open conformation [Roy et al, 2012] favoring its binding to FtsH.

![Figure 4.4 Decrease and increase in hydrogen bonds during temperature upshift of (1) GroEL- σ32 complex and (2) FtsH- σ32 complex respectively.](image)

In-vitro experimental evidence suggests that FtsH cannot degrade σ32 in presence of chaperones [Tomoyasu et al, 2001]. Our results also suggest similar findings. In our model the binding surface of σ32 with FtsH has been found to overlap with the binding surface with DnaK and GroEL. This hardly leaves any chance of complex formation of the DnaK bound to σ32 with FtsH at normal temperature. Temperature up-shift tends to release σ32 from DnaK and GroEL and subsequently favors binding of FtsH. Increase in the number of H-bonding at higher temperature also indicates higher binding affinity between FtsH and σ32 which probably in-turn facilitates degradation of σ32 and controls the negative regulation.

4.2.3 CONCLUSION

Heat shock response in E. coli is mainly controlled by the alternative transcription factor σ32. This response leads to an up-regulation of heat shock proteins including chaperones and proteases. The activity and stability of σ32 is in turn altered through mutual
interactions with these heat shock proteins. The work reported here mainly deals with the docking of σ32 with the chaperone GroEL and protease FtsH. The findings of the above studies together with the σ32 – DnaK docking study reported earlier suggest that the binding of σ32 with GroEL and DnaK at normal temperature is stronger compared to those at high temperature. With rise in temperature σ32 adopts an open conformation and this probably favors binding with FtsH and renders it for degradation by FtsH.