The stalks of the motor A1A0 ATP synthase come into the focus : structural insight into subunits E, F and H

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**THE STALKS OF THE MOTOR A_1A_0 ATP SYNTHASE
COME INTO THE FOCUS: STRUCTURAL INSIGHT
INTO SUBUNITS E, F AND H**

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SCHOOL OF BIOLOGICAL SCIENCES
2010**

The stalks of the motor A_1A_0 ATP synthase come into the focus: structural insight into subunits E, F and H

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SCHOOL OF BIOLOGICAL SCIENCES

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Abbreviations

CPMG	Carr-Purcell-Meiboom-Gill
CSA	Chemical shift anisotropy
hetNOE	Heteronuclear Overhauser effect
HSQC	Heteronuclear single quantum correlation
BMRB	Biological magnetic resonance bank
CSI	Chemical shift index
NOE	Nuclear Overhauser effect
NOESY	Nuclear Overhauser effect spectroscopy
NMR	Nuclear magnetic resonance
PDB	Protein data bank
Ppm	Parts per million
RMSD	Root mean square deviation
R ₁	Longitudinal or spin-lattice relaxation rate constant
R ₂	Transverse or spin-spin relaxation rate constant
TOCSY	Total correlation spectroscopy
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
BSA	Bovine Serum Albumin
CD	Circular Dichroism
DTT	Dithiothreitol
<i>E. coli</i>	<i>Escherichia coli</i>
EDTA	Ethylenediaminetetraacetic acid
FPLC	Fast protein liquid chromatography
IPTG	Isopropyl- β -D-thiogalactoside
NTA	Nitrilotriacetic acid
PAGE	Polyacrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
PDB	Protein Data Bank
SAXS	Small Angle X-ray Scattering
SDS	Sodium dodecyl sulphate
Tris	Tris-(hydroxymethyl) aminomethane
A-ATP synthase	Archaeal ATP synthase

Abstract

A_1A_O adenosine triphosphate (ATP) synthases from archaea represent the second class of ATP synthases due to their unusual structural and mechanistic features, which may be due to archaea's unique phylogenetic position in the tree of life. The enzyme uses both H^+ and Na^+ ion gradients across the membrane to synthesize ATP. A_1A_O ATP synthases comprises subunits in the stoichiometry of $A_3:B_3:C:D:E_x:F:H_2:a:c_x$. The water soluble A_1 domain ($A_3:B_3$) has a pseudo-hexagonal arrangement of major subunits A and B and it is responsible for ATP synthesis or hydrolysis. The energy provided for or released during the process of ATP hydrolysis or synthesis is transmitted via the stalk parts to the membrane-bound A_O domain to facilitate ion conduction. The stalk parts have an important role for the regulation of this class of enzymes and are separated into a central part (C:D:F) and peripheral parts ($E_x:H_2:a_N$). In order to get a detailed picture, structural characterization has been done for the central stalk subunit F and the peripheral stalk subunits H and E. The solution structure of the central stalk subunit F was determined by NMR spectroscopy, revealing a distinct two-domain structure, with the N-terminal 78 residues and a flexible C-terminal part formed by the residues 79-101. The two domains are loosely associated, providing new insights into energy coupling between A_O and A_1 .

The subunit H is a component of one of the two peripheral stalks connecting the upper water soluble A_1 ATPase and the membrane bound subcomplex A_O . Small angle X-ray scattering (SAXS) was used to determine the first low-resolution structure of this molecule in solution. The protein is dimeric and has a boomerang-like shape, composed of two arms of 12.0 and 6.8 nm in length. NMR analysis together with cross-linking and mutagenesis experiments has shown that subunit H is divided into a C-terminal coiled-coil domain and an N-terminal domain formed by adjacent helices. The solution structure of the peptide comprising the last twenty amino acids of subunit H, H_{85-104} , and cross linking analysis at the C-terminus of entire H subunit indicate that the C-terminus is important for the structure formation of subunit H. To understand how the N-terminal domain of H is formed, the solution NMR structure of H_{1-47} was solved, revealing an α -helix between residues 15–42 and a flexible N-terminal stretch. The α -helix includes a kink that would bring the two helices of the C-terminus into the coiled-coil arrangement.

Subunit E is another essential peripheral stalk subunit in A_1A_O ATP synthase. To get into the details, the N-terminal structure of subunit E, the truncated construct E_{1-52} of *M. jannaschii* A_1A_O ATP synthase was solved by NMR spectroscopy. The structure indicates a possible helix-helix

interaction with subunit H. NMR titration experiments were performed between full length / truncated domains of subunits E, H and F. The data provide for the first time, structural insights of subunits E, H and F and their arrangement within the A₁A₀ ATP synthase.

1. Introduction

1.1 Overview on archaea

Archaea are considered to be among the oldest organisms on the planet. They form a separate evolutionary kingdom located relatively near to the root of the evolutionary tree (SCHÄFER *et al.*, 1999; DEPPENMEIER, 2002). Figure 1.1 shows that archaea are located in a distinct evolutionary branch from eukarya and bacteria. It was speculated that they may have a separate energy conserving mechanism from eukarya or bacteria and may represent an efficient form of energy conserving mechanism. In addition, they can live under extremely harsh conditions, at temperatures above 100 °C, tolerance of extreme pH values (pH 1-12) and salt concentrations (up to 5M) (MÜLLER *et al.*, 2005). These facts have been increasing the interest about archaea and their special kind of cellular adaptation mechanism that can stabilize their

Figure 1.1: The phylogenetic tree of life (LEWALTER *et al.*, 2006).

proteins in extremely hot, acidic, alkaline or saline environments. They have also unique metabolic pathways utilising low energy substrates. However, they have a wide variety of energy transducing mechanisms. Respiration, anaerobic respiration with nitrate, fermentation, anaerobic photorespiration, sodium motive methyl transfer reactions and proton reducing hydrogenase are all found in archaea (MÜLLER *et al.*, 2005). There are phylogenitically three distinct groups of archaea: the Crenarchaeota (extremely thermophilic and thermoacidophilic organisms), the Euryarchaeota (e.g. methanogenic and extremely halophilic bacteria) and the Korarchaeota

(unculturable microbes from terrestrial hot springs) (DEPPENMEIER, 2002). Archaea such as methanogens and halobacteria have chemiosmotic mechanisms of energy conservation. The process of chemiosmosis is characterized by the formation of electrochemical potential of hydrogen ions or sodium ions (SCHÄFER *et al.*, 1999). The process of chemiosmosis can be represented by the following equation for the proton motive force:

$$\Delta\mu_{H^+} = RT \cdot \ln ([H^+]_i / [H^+]_o) + F \cdot \Delta\Psi$$

where $\Delta\mu_{H^+}$ is the proton gradient of electrochemical potential, F is the Faraday constant, $\Delta\Psi$ is the membrane potential, R is the gas constant, T is the absolute temperature, and the subscripts "i" and "o" refer to the inside and outside respectively, of the cell or other membrane-enclosed compartment. In archaea the electrochemical potential of hydrogen or sodium ions can be translocated by redox systems, by methyl transfer reaction as in methanogenesis or by light as in photophosphorylating halobacteria (SCHÄFER *et al.*, 1999).

Methanogenesis is a unique pathway carried out by a group of strictly anaerobic archaea. They convert hydrogen and carbon dioxide to methane. Studies on methanogenesis have revealed that biological production of methane occurs via a series of reactions involving unique enzymes, cofactors and electron carriers found only in methanogens (Figure 1.2). The process is accompanied by a free energy change of -131 KJ/mol CH_4 allowing the synthesis of two mol of ATP under standard conditions (DEPPENMEIER, 2002). In nature, less than one third of an ATP can be produced per mol of CH_4 due to the marginal hydrogen concentration in the atmosphere. Thus, methanogens live close to the thermodynamic limit and have a unique energy conservation mechanism by using both sodium and proton gradients (DEPPENMEIER, 2002) across the membrane for the synthesis of ATP by a unique class of ATP synthase, the A-ATP synthases. These energy producers have unusual structural and functional features which make them a

Figure 1.2: The process of methanogenesis in archaea (RUDOLF *et al.*, 2008).

Figure 1.3: The arrangement of subunits in the F-ATP synthase (A) (MÜLLER *et al.*, 2003) and A-ATP synthase (B) (GRÜBER *et al.*, 2008). One α subunit has been removed from the F₁ section to reveal the subunit γ within the $\alpha_3\beta_3$ headpiece. The same has been done in case of A-ATP synthase where one B subunit was removed to reveal subunit D. The A subunit is attached to subunit N-terminal domain (E_N) by a peripheral stalk composed of subunit H, whereby the C-terminal part of E (E_C) is in close proximity to the coupling subunit D. *, show some of the cross-links that have been generated to probe the function and location of these subunits (SCHÄFER *et al.*, 2006b).

distinct class of enzymes from F₁F₀ ATP synthase (F-ATP synthase) of bacteria, chloroplasts and mitochondria (MÜLLER *et al.*, 2003). A-ATP synthases are only functionally related to F-ATP synthases as both of them can synthesize ATP (GRÜBER, 2003; GRÜBER *et al.*, 2008), but in terms of amino acid sequences of major subunits and overall subunit composition (Figure 1.3) they are different from F₁F₀ ATP synthases.

1.2 Overview on ATP synthases

1.2.1 F-ATP synthases

F₁F₀ ATP synthases are the best understood enzymes in the family of ATP synthases. They are found in the plasma membrane of bacteria, chloroplast thylakoid membrane in plants, mitochondrial inner membrane in animals, plants and also in the plasma membrane of human endothelial cells (CAPALDI *et al.*, 2002). The 550-650 kDa complex has eight subunits in

prokaryotes and in mammals it is composed of 16-18 subunits (CAPALDI *et al.*, 2002). The additional subunits are seen in generally stalk region of the enzyme. F_1F_0 ATP synthases are composed of a soluble F_1 domain and a membrane bound F_0 domain. The F_1 part has five subunits in the stoichiometry of $\alpha_3:\beta_3:\gamma:\delta:\epsilon$. The $\alpha_3:\beta_3$ head piece of the F_1 domain contains three catalytic nucleotide binding sites of β subunits at the interface of the adjacent α subunit. ATP synthesis / hydrolysis occurs in the $\alpha_3:\beta_3$ sector and energy provided for or released is transferred to the F_0 domain. Subunits a, b, c in the stoichiometry 1:2:10-14 form the F_0 part, which is responsible for mediating the ion flow through the cytoplasmic membrane (STOCK *et al.*, 2000).

The F_1 and F_0 part are connected by a central stalk, consisting of subunits γ and ϵ , which forms the key rotary element in the enzymes catalytic mechanism. Crystallographic models show that the coiled-coil structure of γ is located asymmetrically in the $\alpha_3:\beta_3$ headpiece (HAUSRATH *et al.*, 1999; GIBBONS *et al.*, 2000). The bottom of γ subunit is in contact with the external loop of subunit c . The second rotary subunit ϵ consists of a β sandwich and a helix-loop-helix domain. It is reported that the subunit ϵ of F-ATP synthase plays dual functions in the coupling of catalysis to proton transport as well as regulation of catalysis events (FENIOUK *et al.*, 2006; KATO *et al.*, 2007; YAGI *et al.*, 2007; IINO *et al.*, 2009; TSUMURAYA *et al.*, 2009). The N-terminal β sandwich domain connects the c -oligomer to the γ subunit, whereas α -helical C-terminus has the regulatory role in ATP synthesis by adopting either a closed or extended conformation (Figure 1.4) (FENIOUK *et al.*, 2006; YAGI *et al.*, 2007; TSUMURAYA *et al.*, 2009). In the extended conformation, the C-terminal domain of subunit ϵ interacts with the $\alpha_3\beta_3$ hexamer, thereby inhibits the catalysis. The interaction is caused by the positively charged residues in C-terminal domain of ϵ with the negatively charged acid residues of β DELSEED segment in the C-terminus of subunit β (FENIOUK *et al.*, 2006). Crystal structure refined at 2.1 Å resolution of a complex of subunit ϵ and the central domain of the subunit γ (residues 11-258) sheds a new light on how the catalytic site events in the F_1 part are coupled to proton translocation in the F_0 part (RODGERS *et al.*, 2000). In the complex structure, the β -sandwich domain of subunit ϵ is aligning parallel to the long pair of coiled-coil α -helices consisting of N-terminal residues 12-56 and C-terminal residues 212-247 and plays an important role in the binding of γ - ϵ complex to subunit c ring. Together with the eccentric rotation of the coiled-coil helices and the helix-sheet-helix domain of subunit γ , which is in the opposite side of the coiled-coil from β -sandwich domain of subunit ϵ , may influence the conformations and nucleotide binding affinities at the catalytic sites.

The peripheral stalk consists of subunits δ and b and functions as a stator unit, to experience the elastic strain imposed by the rotation (SENIOR *et al.*, 2006; WALKER *et al.*, 2006; WEBER, 2006). In *E. coli* two copies of b subunit and one copy of δ subunit form the

Figure 1.4: Crystal structures of the F₁ ATPase (subunit α , β and γ are in blue, green and gray color respectively) showing two different conformations of subunit ϵ (red): closed form (PDB code: 1E79) (GIBBONS *et al.*, 2000) (A) and extended form (PDB code: 1JNV) (HAUSRATH *et al.*, 2001) (B). The C-terminal α -helices of the ϵ subunit (enclosed by black lines).

peripheral stalk. The elongated shape and helical character of the b subunit in dimeric form have been explained experimentally in a number of studies (DEL RIZZO *et al.*, 2006; WEBER, 2006; WOOD *et al.*, 2007; PRIYA *et al.*, 2008). It extends from the top of the F₁ domain into and across the membrane to associate with subunit a (DUNN *et al.*, 2000; BHATT *et al.*, 2005; WEBER, 2006). The soluble part of the subunit b has three distinct domains: first the tether domain corresponding to the region between the membrane and beneath the F₁ sector; next, the dimerization domain, which extends up to the interface of subunits α and β ; third, the C-terminal F₁ binding domain required for the interaction of the peripheral stalk and F₁. The crystallographic structure of the major part of the dimerization domain, b_{62-122} , revealed an α -

helix with a length of 9.0 nm (DEL RIZZO *et al.*, 2006). Small angle X-ray scattering and analytical ultracentrifuge studies indicated that the soluble domain of subunit *b* is dimeric in solution (PRIYA *et al.*, 2008). Subunit *b* is described to be connected to the δ subunit via the C-termini (RODGERS *et al.*, 1997; MCLACHLIN *et al.*, 2000). Most recently, the NMR solution structure of the segments b_{30-82} and $b_{140-156}$, which interacts with the C-terminus of subunit δ (δ_{91-177}), has been determined by NMR spectroscopy (PRIYA *et al.*, 2008; PRIYA *et al.*, 2009). Structural features of the N-terminal domain of subunit δ (residues 1-134) available from NMR studies, show a six helix bundle (WILKENS *et al.*, 1997). Immuno-electron microscopy and NMR titration experiments showed that subunit δ sits at the top of the F_1 near the N-termini of subunits α and β , and holds the F_1 with respect to F_0 part in proper position for the efficient rotational energy coupling (WILKENS *et al.*, 1997; WILKENS *et al.*, 2005).

1.2.2 A-ATP synthase

The A_1A_0 ATP synthases are made of ten subunits in the stoichiometry of $A_3:B_3:C:D:E_x:F:G:H_2:a:c_x$ (GRÜBER *et al.*, 2001; MÜLLER *et al.*, 2003; GRÜBER *et al.*, 2008). Like F_1F_0 ATP synthase, they contain a water soluble (A_1) domain and a membrane anchored (A_0) domain (MÜLLER *et al.*, 2003; WEBER, 2006; GRÜBER *et al.*, 2008). ATP synthesis and ATP cleavage occurs in the A_3B_3 headpiece of the A_1 sector. The energy provided for or released during the process of ATP hydrolysis or synthesis is provided to the membrane-bound A_0 domain to facilitate ion conduction. The stalk parts are responsible for the energy coupling between the catalytic site events in the A_3B_3 hexamer with the ion conduction through A_0 domain (MÜLLER *et al.*, 2003; GRÜBER *et al.*, 2008; VONCK *et al.*, 2009).

1.2.2.1 Structure of A_1A_0 ATP synthase by electron microscopy from *M. jannaschii*

Despite several attempts in the past years, the structure of an intact A_1A_0 was determined only in 2004 by electron microscopy by Coskun *et al.* The first two dimensional projection of the enzyme (Figure 1.5) revealed several important structural basics toward a detailed understanding of the functions of the enzyme (COSKUN *et al.*, 2004). All of the projections from the microscopy clearly indicated the tripartite structure. There is a headpiece, a membrane embedded domain and a connecting stalk region. The connecting stalk region consists of a central stalk and two peripheral stalks. There is a hexagonal and alternate arrangement of subunit A and subunit B densities. The most important organizational feature for the hexagon (10.2 nm in diameter and 10.8 nm in height) is asymmetric from the top. One of the interesting features in the structure of

A_3B_3 head piece is the knob like structures formed by the N-terminal non homologous region (NHR; 80-90 amino acids) of the three catalytic A subunits. These knob like structures are

Figure 1.5: Structure of A_1A_0 ATP synthase by electron microscopy. 2-D Projection of the of the A_1A_0 complex from *M. jannaschii* (COSKUN *et al.*, 2004).

believed to connect the peripheral stalk to the A_3B_3 headpiece. They are absent in the amino acid sequence of homologous β subunits of F-ATP synthases (WILKENS *et al.*, 1998; KARRASCH *et al.*, 1999; BÖTTCHER *et al.*, 2000). The length of the central stalk is around 8 nm. More insight in the structure reveals a collar structure, which interacts with the two peripheral stalks. This structure together with some biochemical analysis resulted in a model in which the subunits C, D, and F form the central stalk domain (Figure 1.3B). The small angle X-ray scattering structure of the A_1 ATPase from *Methanosarcina mazei* Gö1 (GRÜBER *et al.*, 2001) pointed out that the shape and length of the central stalk in the A_1 ATPase is significantly different from F_1 ATPase and it is more elongated in the A_1 ATP synthase. This explains also the distinct class of A-ATP synthase enzyme from F-ATP synthase.

1.2.2.2 Structure and function of individual domains of the A_1A_0 ATP synthase

1.2.2.2.1 The A_3B_3 headpiece

The head domain consists of a nucleotide binding subunits A and B (corresponding β and α subunits from F-ATP synthase) arranged in a pseudo-hexagonal fashion (COSKUN *et al.*, 2002; COSKUN *et al.*, 2004; COSKUN *et al.*, 2004; GRÜBER *et al.*, 2008). Although the high

resolution structure for the head piece of A_1A_O ATP synthase has not been solved yet, the structures of subunit A and subunit B have been solved individually by X-ray crystallography at 2.55 Å and 1.5 Å resolution, respectively (MAEGAWA *et al.*, 2006; SCHÄFER *et al.*, 2006a). In general the structure of subunit A and B consists of three domains: N-terminal β barrel, an α - β domain and a C-terminal α -helical bundle. The important difference between the structures of the two subunits is the presence of a knob-like structure in catalytic subunit A which is made from an insertion of 90 amino acids. This insertion, called non homologous region (NHR) is absent in the homologous β subunit of F_1F_O ATP synthases. It was first seen in the electron micrographs of the intact A_1A_O ATP synthase and believed to connect the peripheral stalk to the A_3B_3 headpiece (COSKUN *et al.*, 2004). Comparison of subunit A and B sequences reveals that subunit A has a phosphate-binding loop (P-loop) sequence GXXGXGKTV, which is conserved at the nucleotide binding sites of the related α and β subunits of F-ATP synthases (WALKER *et al.*, 1982; NELSON *et al.*, 1989; SARASTE *et al.*, 1990; WILMS *et al.*, 1996). Subunit B can also bind to nucleotides, although it does not have an ideal P-loop sequence (SCHÄFER *et al.*, 2006a; KUMAR *et al.*, 2009; MANIMEKALAI *et al.*, 2009). Nucleotide binding capability of both subunits A and B have been tested by photo-affinity ATP labeling and fluorescence correlation spectroscopy (FCS) experiments. The results showed that both subunits can bind to nucleotides and there is a higher affinity for subunit A. It is still unclear about the function of nucleotide binding events to subunit B. Cross-linking study with EDC in presence of nucleotides indicates that coupling of nucleotide binding to subunit B might cause structural alterations in the neighboring subunit B and F (SCHÄFER *et al.*, 2006b; GAYEN *et al.*, 2007). This B-F interaction is proposed to play an important role linking the nucleotide binding events to the ion translocation in the A_O part (GAYEN *et al.*, 2007; RAGHUNATHAN *et al.*, 2009).

1.2.2.2.2 The stalk domain (C:D:F:H₂:E_X)

The central stalk of the A_1A_O ATP synthases descends from the bottom of the headpiece A_3B_3 to the inside of the *c*-ring. The central stalk composed of C, D and F subunits with molecular masses of 36, 25 and 12 kDa, respectively. Subunit C is characteristic only for A_1A_O ATP synthases and was described as a spacer unit that plays important roles in the coupling and rotational events for the synthesis of ATP and ion translocation (MÜLLER *et al.*, 2003; IWATA *et al.*, 2004; NUMOTO *et al.*, 2004; GRÜBER *et al.*, 2008). The overall structure of subunit C contains twenty helices and is separated into three domains (IWATA *et al.*, 2004; NUMOTO *et al.*, 2004). The domains have a similar fold and topology consisting of two long helices and four

shorter helices. All the helices run roughly parallel to form a tapered cylinder. The taper of the subunit C penetrates to the central cavity up to the mid point of the *c*-ring. This is further supported by the electrostatic charge distribution of subunit C. Topside is hydrophobic and the bottom side is charged, which is suitable to bind with the *c*-ring surface (NUMOTO *et al.*, 2004). Docking study showed that some structural changes are necessary for efficient interaction of subunit C with the *c*-ring (MURATA *et al.*, 2005). Subunit D has been described as a structural and functional homolog of subunit γ of F_1F_0 ATP synthases (ABRAHAMS *et al.*, 1994). Its central α -helix is surrounded by the three A and B subunits, arranged alternately like the segments of an orange. It passes through a large internal cavity connected to the crevices formed by the A and B subunits (Figure 1.3B). The function of this subunit is supposed to change the conformations of the nucleotide binding pockets in the A_3B_3 headpiece upon rotation, like the role of γ subunit in the F_1F_0 ATP synthases (GRÜBER *et al.*, 2001; COSKUN *et al.*, 2002). Subunit F is comprised of a globular domain with a length of 4.5 nm and hook like domain with a length of 3.0 nm as revealed by the small angle X ray scattering structure (SCHÄFER *et al.*, 2006a). Thus, subunit F with a length of 7.5 nm would partially span the width of the central stalk. The shape is almost identical to subunit ϵ of *Escherichia coli* F_1F_0 ATP synthase (UHLIN *et al.*, 1997). Cross linking studies with subunit F of A_1A_0 ATP synthase from *M. mazei* revealed that the hook like domain of F subunit is interacting with subunit B in a nucleotide binding dependant manner (SCHÄFER *et al.*, 2006b). Other than the central stalk, the first structure of the complete A_1A_0 ATP synthase obtained showed the presence of two peripheral stalks, running from the knobs on A_1 to a level of the A_0 domain (COSKUN *et al.*, 2004). The potential candidates of these two parts are subunits H and *a*, respectively. Subunit H (~12 KDa) is the smallest subunit in enzyme complex. Sequence based predictions suggest that the subunit H has an α -helical structure and can form a homo dimeric coiled-coil structure (BIUKOVIĆ *et al.*, 2007). The experimental data also revealed the presence of a collar-like structure that interacts with both peripheral stalks and is in direct neighborhood to the central stalk via the rotary subunit D. The remaining hydrophilic subunit E is the most likely candidate for constituting this collar domain (BERNAL *et al.*, 2004; COSKUN *et al.*, 2004). The size of the E subunit is around 23 kDa having two domains, a highly helical N-terminal domain and a C-terminal domain composed of short helices and β sheets. The C-terminal domain of subunit E (E₈₁₋₁₉₈) from *Pyrococcus horikoshii* OT3 has been solved by crystallography at 1.85 Å resolution (LOKANATH *et al.*, 2007). The structure consists of four antiparallel β strands and

six helices, revealing an elongated shape with a distance of 75 Å. The stoichiometry of subunits H and E in A-ATP synthase is still not established. Some studies have revealed that subunits H and E form the hetero dimer and constitute the peripheral stalks of the enzyme (KISH-TRIER *et al.*, 2008; KISH-TRIER *et al.*, 2009; VONCK *et al.*, 2009). Both the peripheral stalks and the collar-like structure are believed to be involved in constituting the stator region of the A₁A₀ ATP synthase, which is believed to have important roles in enzyme assembly as well as energy coupling between A₁ and A₀ (SCHÄFER *et al.*, 2006b). They may even have potential regulatory roles in interaction with other subunits within the enzyme complex (LOKANATH *et al.*, 2007).

1.2.2.2.3 The ion pumping domain A₀

The A₀ domain represents the membrane embedded rotor, containing only two subunits subunit *a* (stator) and subunit *c* (rotor). It has a molecular mass of 75 kDa with a hydrophilic N terminal and a hydrophobic C-terminal domain. The hydrophilic sector is believed to be similar in function to subunit *b* of F₁F₀ ATP synthases and are made of helices; the hydrophobic C-terminus has seven transmembrane helices similar to subunit *a* from F₁F₀ ATP synthase (MÜLLER *et al.*, 2003). The most important feature is a conserved arginine residue which is believed to be an essential positive charge for ion translocation (GRAHAM *et al.*, 2003; QI *et al.*, 2007). In most of the archaea, subunit *c* has two transmembrane helices (IHARA *et al.*, 1997; STEINERT *et al.*, 1997). Four and six transmembrane helices of subunit *c* were also seen for *M. thermoautotrophicus*, *M. jannaschii* respectively, because of the gene duplication and triplication events, followed by the fusion of the gene copies (RUPPERT *et al.*, 1999). It is still not completely understood how the *c* subunit plays a role in ion translocation and its coupling to ATP synthesis. In 2005, Murata *et al.* has solved the crystallographic structure of subunit *c* from *Enterococcus hirae* A-ATP synthase (MURATA *et al.*, 2005). The subunit *c* consists of five helices (H₀-H₄), out of which H₁-H₄ are transmembrane segments. Ten *c* subunits are arranged in two concentric rings where H₁ and H₃ are alternate in the inner ring and H₂ and H₄ are alternate in the outer ring (Figure 1.6). One of the interesting features in the structure of the *c*-ring, is that the lower and upper part of the helices H₂ and H₄ bend outward from the axis from the ring, whereas bends in the H₁ and H₃ helices are negligible. These structural features broaden the radius of the cytoplasmic and the periplasmic side with respect to the central region. H₀ helices are pointing inward on the opposite side of the membrane, narrowing the internal diameter of the ring. The ring diameter differs greatly from the *c* ring of bacterial F ATP synthases (STOCK *et*

al., 1999; VONCK *et al.*, 2002). This fact points out the differences of central stalks between these ATP synthases and its association with the upper part of the ring. In ATP synthases subunit C is supposed to be interposed between the central stalk (IWATA *et al.*, 2004) and the ring, whereas in F ATP synthases there is a direct contact between the central stalk (γ , δ , ϵ subunits) and the ring (STOCK *et al.*, 1999). The structure of subunit *c* also gives important aspects towards the ion specificity of A_1A_O ATP synthases. Residues from H₂, H₃, H₄ (T64, Q65, Q110 and E139) are important for the sodium binding pocket. The change of torsional angle of E139 might allow the exchange of sodium ion with the bulk phase (MURATA *et al.*, 2005). The clockwise rotation of the *c*-ring brings an occupied sodium binding site to the *c*-ring – subunit *a* interface. R573 of subunit *a*, which is very close to the sodium binding pocket, disrupts the hydrogen bonding network and releases the ion into the periplasm. Further rotation will allow the binding of another sodium ion by disrupting the arginine – glutamic acid interaction (MURATA *et al.*, 2005).

Figure 1.6: Structure of subunit *c* from *Enterococcus hirae*. (A) Ribbon representation of the structure of the *c* subunit. 10 protomers of the *c* subunit form the ring structure. (B) shows a view from the cytoplasmic side of the membrane and (C) a side view of the *c*-ring (MURATA *et al.*, 2005).

1.2.2.3 Conformational changes due to nucleotide binding in the A_1A_O ATP synthase

Small angle X-ray scattering studies showed that significant structural alterations occur to subunits A and B due to nucleotide binding in the A_1A_O ATP synthase from *M. mazei* Gö1 (COSKUN *et al.*, 2002). The value of the radius of gyration decreases slightly after binding to Mg-AMP-PNP and increases after binding Mg-ATP, Mg-ADP (COSKUN *et al.*, 2002). Such conformational changes of the quaternary structure are consistent with alterations in the closely related F_1 -ATPase, as demonstrated in a recent crystallographic structure of the bovine F_1 -

ATPase (MENZ *et al.*, 2001). In the F_1 -ATPase significant changes in the lower part of the nucleotide binding domain and the C-terminal domain of the catalytic β subunit have been observed due to the nucleotide binding. Cross linking of central stalk subunit D to catalytic subunit A depends also on type of nucleotide present in the solution. Mg-ADP and Mg-ATP favors the cross-link formation, whereas Mg-ADP + Pi, Mg-AMP-PNP and the absence of nucleotide disrupts the cross link (COSKUN *et al.*, 2002). These results point out the close proximity of subunit A and D and will allow the coupling between the catalytic site events in A via D to the other central stalk subunit C and F. Subunit F can be cross-linked to subunit B depending on the type of nucleotide. The similar nucleotide dependant movement is also seen in case of subunit H and subunit A (SCHÄFER *et al.*, 2006b). Therefore, the conformational changes in the A_1 headpiece depending on the type of the nucleotide might correlate with structural alterations in the stalk region. This can be expected when the stalk couples ATP hydrolysis/synthesis to ion conduction (MÜLLER *et al.*, 2003).

Thus, more structural and biochemical investigations are needed in order to understand this fascinating enzyme especially the coupling stalk regions. In the present study isolated stalk subunits were cloned, expressed and purified. Structural characterizations were done with nuclear magnetic resonance (NMR) spectroscopy. NMR spectroscopy has been used as a tool to determine the three dimensional structure, dynamics as well as protein-protein interactions.

1.3 Outline of the thesis

The A_1A_0 adenosine triphosphate (ATP) synthase from archaea uses the ion gradients generated across the membrane sector (A_0) to synthesize ATP in the A_3B_3 domain of the A_1 sector. The crystal structure of a large portion of A_1 has been solved. However, the structure of the central portion of the enzyme, through which conformational changes in A_0 are communicated to A_1 , has until now remained elusive. To get a detailed picture in this region the solution structure of the subunit F, N-and C-terminal domain of subunit H and N-terminal domain of subunit E (E_{1-52}) of the A_1A_0 ATP synthase were determined. Subunit F from *M. mazei* Gö1 exhibits a distinct two-domain structure, with the N-terminal having 78 residues and residues 79-101 forming the flexible C-terminal part. The two domains are loosely associated with more flexibility relative to each other. The flexibility of the C-terminal domain is confirmed by NMR dynamics studies. In addition, NMR structure of the isolated C-terminus of subunit F points out that the flexibility of the C-terminus enables subunit F to switch between an elongated and closed state and thereby provides new insights into energy coupling between A_0 and A_1 .

Subunit H of the A_1A_O ATP synthase is a component of one of the two peripheral stalks connecting the A_1 and A_O domain. The structural and functional role of this subunit is not known properly since its precise arrangement inside the A_1A_O complex is still an unresolved issue. The goal of this project is to over express, produce the recombinant protein, which could be used to derive the structural and biochemical information. Circular dichroism (CD) spectroscopy revealed that subunit H is comprised of 78% α -helix and a coiled-coil arrangement. To understand the importance of C-terminus for the stabilization of the quaternary structure of the protein, structure of a short length peptide (H_{85-104}) in the C-terminus of subunit H has been solved and three mutants of subunit H (H_{I93C} , H_{L96C} and H_{L98C}) were expressed, purified, and analyzed by cross-links and CD experiments. To understand how the N-terminal domain of H is formed the solution NMR structure of H_{1-47} was solved, revealing an α -helix between residues 15–42 and a flexible N-terminal stretch. The α -helix includes a kink that would bring the two helices of the C-terminus into the coiled-coil arrangement.

The structural and functional role of another stalk subunit, subunit E is also essential in understanding the function of A_1A_O enzyme. To get into details, NMR titration experiments were performed revealing that the N-terminal residues M1–6, K10, E11, A15, V120 and E24 of H_{1-47} interact specifically with the N-terminal domain E_{1-100} of subunit E. A more detailed picture regarding the residues of E_{1-100} involved in this association was obtained by titration studies using the N-terminal peptides E_{1-20} , E_{21-40} and E_{41-60} . These data indicated that the N-terminal tail E_{41-60} interacts with the N-terminal amino acids of H_{1-47} . In order to understand the E-H assembly and the N-terminal structure of E, the truncated construct E_{1-52} of *M. jannaschii* A_1A_O ATP synthase was determined by NMR spectroscopy. The protein is 60.5 Å in length and forms an α helix between the residues 8–48. The molecule is amphipathic with a strip of hydrophobic residues, which could lead to a possible helix-helix interaction with neighboring subunit H. Analysis of 1H – ^{15}N heteronuclear single quantum coherence (HSQC) spectra of the central stalk subunit F in the presence and absence of $E_{101-206}$ show no obvious interaction between the C-terminal domain of E and subunit F. The data presented provided, for the first time, structural insights into the interaction of subunits E and H, and their arrangement within A_1A_O ATP synthase.

2.1 Materials

2.1.1 Chemicals

All the chemicals used for the study were of at least analytical grade. Chemicals were purchased from the following companies:

Buffers and salts	Sigma (St. Louis, MO, USA) USB (Sampscott, MA) Calbiochem (Darmstadt, Germany) Fluka (Sigma, Buchs Germany) Roth (Karlsruhe, Germany) Serva (Heidelberg, Germany)
DTT	Hoefer (San Francisco, CA, USA)
Ni ²⁺ -NTA	QIAGEN (Hilden)
Pefabloc ^{SC}	BIOMOL (Hamburg, Germany)
PMSF	Sigma (St. Louis, MO, USA)
¹⁵ NH ⁴ Cl	Cambridge Isotopes Lab (USA)
¹³ C Glucose	Cambridge Isotopes Lab (USA)
LB Media	BD (Sparks, MD, USA)
<i>Electrophoresis Chemicals</i>	
(Agarose, SDS, Glycine, APS etc.)	Bio-Rad (Hercules, CA, USA)
Antibiotics	Calbiochem, Sigma and Gibco (Invitrogen)
IPTG	Fermentas
BSA	GERBU (Heidelberg, Germany)

2.1.2 Molecular biology materials

Genomic DNA	
<i>M. jannaschii</i> (43067D)	ATCC, Manassas, VA 20108, USA.
Primers	1 st Base and Research Biolabs (Singapore)
Peptides	NTU proteomics core facility, School of Biological Sciences, Nanyang Technological University, Singapore.
Pfu and T4 DNA Polymerase	Fermentas (Glen Burnie, MD, USA)
<i>NcoI</i> , <i>SacI</i>	Fermentas and New England Biolabs
T4 DNA Ligase	Fermentas and NEB
Miniprep Plasmid Kit	Qiagen (Hilden, Germany)

Nucleobond AX mediprep Kit	MN & Co (Düren, Germany)
<i>Escherichia coli</i> expression strains	DH5 α , BL21 (DE3)
pET-9d1	GRÜBER <i>et al.</i> , 2002.
pET-24 (+)	Novagene (Darmstadt, Germany)

2.1.3 Chromatography

2.1.3.1 Ion exchange

RESOURCE TM Q (6 ml)	Amersham Biosciences (New York city, USA)
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2.1.3.2 Gel filtration

Superdex 75 HR (10/30)	GE Healthcare (Uppsala, Sweden)
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2.1.3.3 Instruments and accessories

Akta FPLC, Akta Prime	GE Healthcare (Uppsala, Sweden)
Millex Filters (0.45 μ M)	Millipore (Bradford, USA)
Syringe, needles and accessories	BD Biosciences

2.1.3.4 Protein concentration, estimation

Centriprep YM10	Millipore (Co-cork, Ireland)
Amicon ulta (3-30kDa)	Millipore (Co-cork, Ireland)
BCA Assay Kit	Pierce (Rockford, IL, USA)

2.1.4 Other instrumentation

PCR Thermocycler:	
Biometra T personal	Biometra
Biometra T gradient	Biometra
Sonoplus Sonicator	Bendelin
Micropulser Electroporator	Bio-Rad
Ultraspec 2100 Pro Spectrophotometer	Amersham Biosciences

2.1.5 Computer software

Vector NTI 10.3.0	Invitrogen
SPARKY	University of California (San Francisco, USA)
Topspin 1.3	Bruker Biospin

CAYANA 2.1	Kimmo Paakkonen and Peter Günter (Japan)
TALOS	Gabriel Cornilescu <i>et al</i> (NIH, USA)
MOLMOL	Koradi <i>et al</i> (ETH, Zürich)
NMRpipe	Delaglio <i>et al</i> (NIH, USA)
nmrDraw	Howard Hughes Medical Institute, USA
PyMOL v0.99	DeLano Scientific LLC, USA

2.2 Methods

2.2.1 Cloning of subunit H of A₁A₀ ATP synthase from *M. jannaschii*

To amplify the Atp H coding region, oligonucleotide primers 5'-GGA ACC ATG GGC GTT AGT GTT ATG-3' (forward primer) and 5'-AAT CAC CTG AGC TCT TAA ATC TCA AGA ATC-3' (reverse primer), incorporating *Nco*I and *Sac*I restriction sites (underlined) were designed. Isolated pure genomic DNA of *Methanocaldococcus jannaschii* ATCC[®] 43067D was used as a template for the amplification of the subunit H gene. Polymerase chain reaction (PCR) was set up in a total volume of 50 µl on ice as mentioned below with the appropriate concentration of constituents as:

Reagents	Amount
Pfu buffer (10x)	5 µl
DNTP's (2 mM)	1.5 µl
Primers (100 µM)	2 x 0.5 µl
Template (genomic DNA)	1 µl
MilliQ water	40.5 µl
Pfu DNA polymerase	1 µl

All reagents were accurately pipetted into a PCR tube kept on ice all the time, mixing of the reagents was properly done by short centrifugation. PCR thermocycler (Biometra T personal) was preheated to 95 °C before reaction tubes were placed inside the machine. The following PCR thermocycler program was used for the amplification. The cycle denaturation, annealing and extension steps were repeated for another 29 cycles. Total time taken for the PCR program was 1h 43 min 49 sec.

Cycle steps	Temperature	Time
Lid	99 °C	
Initial denaturation	96 °C	3 min
Cycle denaturation	96 °C	30 sec
Annealing	58 °C	45 sec
Extension	72 °C	60 sec
Final extension	72 °C	10 min
End	4 °C	

The amplified PCR product was stored at -20 °C. In order to check the quality of the PCR amplified product, 5 µl of the samples were applied onto an analytical agarose gel (1%) and after that, the gel was kept in ethylene dibromide solution for 20 min and observed under UV light. The size of the PCR product was confirmed with the appropriate DNA marker. Remaining reaction mixture was applied onto a preparative agarose gel. The portions corresponding to the correct PCR product were cut out immediately and purified by gel extraction kit (QIAGEN) as per the manufacturers' protocol. The product was finally eluted in 30 – 50 µl volume of water or Tris-HCl

buffer of pH 8.0. 1 µl of gel extracted PCR product was applied onto 1% analytical agarose gel for estimating the purity of the sample. Purified DNA was double digested overnight at 37 °C by taking appropriate amount of *Nco*I and *Sac*I enzymes. After overnight digestion, the reaction mixture was again purified by enzyme reaction purification kit from QIAGEN. Subsequently, ligation reaction between vector (Figure 2.2.1) and amplified PCR product was setup at room temperature. The reagents used were as follows:

Figure 2.2.1: Vector Map modified pET-9d1 (+) (GRÜBER *et al.*, 2002) vector showing full map and unique multiple cloning sites (MCS) that has been used to clone various genes.

Ligase buffer (10x)	1.5 µl
Vector (V)	50-100 ng
Insert (I)	Variable (1-5 times of vector)
T4 Ligase	1 µl
MilliQ water	variable
Total	15 µl

The ligation mixture was kept at room temperature for 60 min. The reaction was terminated by precipitation of DNA from rest of the reaction mixture by adding 85 µl of Milli Q water to make a final volume of 100 µl. 1 ml of butanol was added into the mixture and mixed thoroughly. Then the mixture was centrifuged at 13,000 x *g* (using Eppendorf mini-centrifuge) for 10 min. Supernatant was discarded gently and the pellet was resuspended in 150 µl of 70%

ethanol and centrifuged again for 5 min. Supernatant was removed and the pellet was re-dissolved in 10 µl of elution buffer (10 mM Tris-HCl, pH 8.5) (EB buffer, QIAGEN) or Milli Q water. 5 – 10 µl of ligation mixture was used for the transformation into *Escherichia coli* DH5α cells. On the next day colonies were picked from the transformation plate and grown at 37 °C. The plasmid DNA was isolated using standard protocol (QIAGEN mini-prep kit) and subsequently double digested and applied onto 1% agarose gel, in order to confirm the ligated insert. Size of insert and vector were compared with appropriate controls and markers. Verified plasmid was finally transformed into *E. coli* BL21 (DE3) cells for protein production and they were grown on 30 µg/ml kanamycin-containing Luria-Bertoni (LB) agar plates. *E. coli* BL21 expression strains were purchased from Novagene (Darmstadt, Germany).

2.2.2 Cloning of other constructs

Similar strategy was used for the cloning of three mutants (H_{I93C}, H_{L96C} and H_{L98C}) of subunit H of A₁A_O ATP synthase from *M. jannaschii*. The primers used for current study are described below. Since the mutations sites are in the C-terminal of subunit H, I have introduced the mutations in the reverse primers (marked underline) in each case. PCR reactions and PCR program were set up according to the methods described in section 2.2.1, except for the annealing temperature of the PCR reaction, which in case of H_{L98C} mutant was set at 55 °C.

Construct	Primers
I93C	Forward 5'-CGC GAC ACC ATG GGC GTT AGT GTT ATG GAA GCA ATA-3' Reverse 5'-CCG AGC TCT TAA ATC TCA AGA ATC TCA GAC AAT TTC AAC GAA AGG <u>CAC</u> TTA ACC T-3'
L96C	Forward 5'-CGC GAC ACC ATG GGC GTT AGT GTT ATG GAA GCA ATA-3' Reverse 5'-CCG AGC TCT TAA ATC TCA AGA ATC TCA GAC AAT TT <u>G</u> <u>CAC</u> GAA AGT ATC-3'
L98C	Forward 5'-CGC GAC ACC ATG GGC GTT AGT GTT ATG GAA GCA ATA-3' Reverse 5'-CCG AGC TCT TAA ATC TCA AGA ATC TCA GAA <u>CAT</u> TTC AAC GA-3'

2.2.3 Protein production and purification of subunit H and its mutants of A₁A₀ ATP synthase from *M. jannaschii*

2.2.3.1 Induction test

From the selection plate, five colonies were taken and grown till the OD₆₀₀ reaches to 0.6 at 37 °C by constant shaking at 180 - 200 r.p.m. (Infors HT Minitron shaker). Induction test was carried out by using 1 mM IPTG for a period of 3 h at 37 °C. Respective controls under identical conditions were also taken, where IPTG was not added. Then the cells were centrifuged at 11,000 x g for 5 min at 4 °C and resuspended properly in 40 µl lysis buffer (98 µl 4 x Laemmli buffer, 4.1 µl DTT and 423.5 µl H₂O). Resuspended samples were heated at 95 °C for 5 min and were loaded in 17 % SDS polyacrylamide gel (Figure 2.2.2).

Figure 2.2.2: Induction test of subunit H: Uninduced (-) and induced samples for two colonies of subunit H from *M. jannaschii*. Induction test was done with 1 mM IPTG at 37 °C. Whole cell lysate was heated in 1 x lysis buffer at 95 °C for 5 mins and then applied onto 17 % SDS gel.

2.2.3.2 Solubility test of recombinant proteins

The solubility of the protein was tested in different buffers. 50 ml cultures were harvested and appropriate concentration of IPTG was added when the OD₆₀₀ of the culture reached to 0.6 to 0.7. Cells were separated into five equal fractions and pelleted down by centrifugation at 10,000 x g for 10 min. In order to prevent degradation, appropriate concentrations of Pefabloc^{SC} (2-8 mM) and/or PMSF (1-2 mM) were added. In the case of H mutant proteins, which have cysteine residues, reducing agent DTT (0.5 mM in the case of Ni²⁺-NTA resin column and 1 mM in the case of Resource Q and Superdex 75 columns) was used. The cell pellet was completely resuspended and sonicated at 20% power with KE76 tip of sonicator (Bandelin Sonoplus) three times for one minute each. A cooling interval of two minutes was kept between each cycle. After sonication, the pellet and the supernatant were separated by centrifugation and the pellet was

Figure 2.2.3: Solubility test results from H subunit of A₁A₀ ATP synthase from *M. jannaschii*. Pellet (p) and supernatant (s) in various buffers is shown in the gel.

again suspended in the same buffer. Samples from both the pellet and supernatant were loaded onto a 17 % SDS gel (Figure 2.2.3).

The *E. coli* cells having the gene *atp H* was grown on 30 µg/ml kanamycin-containing Luria-Bertoni (LB) agar plates. To express subunit H, liquid cultures were shaken in LB medium containing kanamycin (30 µg/ml) for about 20 h at 30 °C until an optical density OD₆₀₀ of 0.6-0.7 was reached. To induce expression, the cultures were supplemented with IPTG to a final concentration of 1 mM. Following incubation for another 4 h at 30 °C, the cells were harvested by centrifugation at 6,000 x g for 20 min at 4 °C. Afterwards the protein was purified by following the scheme described below.

Figure 2.2.4: Scheme for the purification of subunit H of A₁A₀ ATP synthase from *M. jannaschii*.

The mutants of subunit H (H_{I93C}, H_{L96C} and H_{L98C}), were isolated using a Ni²⁺-NTA resin column as described above using the buffer of 50 mM Tris-HCl (pH 7.5), 250 mM NaCl and 0.5 mM DTT. After that the sample was applied on an ion-exchange column (Resource Q, 6 ml; Amersham Biosciences). The protein was eluted by a linear NaCl-gradient (0 M - 1 M) with a flow rate of 1 ml/min. Finally the sample was applied to a size exclusion column (Superdex 75 HR 10/30; GE Healthcare).

2.2.4 Protein quantification by bicinchoninic acid method

The bicinchoninic acid (BCA) assay is available in kit form from Pierce (Rockford, IL, USA.). The protein concentration was estimated as per the manufacturer's instructions. BSA (0 – 250 µg/ml) was used as standard. In general, two dilutions of protein samples were taken and each of them was measured in triplicate to avoid any error in the measurement. Optical density was measured at 562 nm against blank.

2.2.5 Circular Dichroism spectroscopy

Circular dichroism (CD) is used to determine the secondary structure content of proteins and peptides. Steady-state CD spectra were measured in the far-UV light (185-260 nm) using a Chirascan spectropolarimeter (Applied Photophysics) under continuous purging of N₂ gas. Spectra were collected in a 60 µl quartz cell (Hellma) with a path length of 0.1 mm, at 20 °C and a step resolution of 1 nm. The readings were taken with the average of 1 sec at each wavelength, and the recorded ellipticity values were the average of three determinations for each sample at a concentration of 2 mg/ml. The spectrum for the buffer was subtracted from the spectrum of the protein. CD values were converted to mean residue ellipticity in units of deg x dmol⁻¹ x aa⁻¹ using the software Chirascan version 1.2 (Applied Photophysics) by giving appropriate cell path length, molecular weight, number of amino acid residues and protein concentration values. This baseline corrected spectrum was used as input for computer methods to obtain predictions of secondary structure.

2.2.6 Nuclear magnetic resonance (NMR) spectroscopy

2.2.6.1 Isotopic labeling (¹⁵N, ¹³C) of proteins

Reagents:

Minimal Media (M9): 42 mM Na₂NPO₄
22 mM KH₂PO₄

8.5 mM NaCl
 1 g/l $^{15}\text{NH}_4\text{Cl}$
 0.1 mM CaCl_2
 2 mM MgSO_4
 10 % D-Glucose
 30 μM FeCl_3
 5 ng/l Thiamin
 Antibiotic
 LB Media

For the production of uniformly labelled ^{15}N and ^{15}N and ^{13}C recombinant proteins in *E. coli* BL21 (DE3), M9 minimal media was used. All unlabeled reagents were standard laboratory reagents grade. All solutions were prepared using Milli-Q water (Millipore, Billerica, MA). 50 ml of overnight culture was grown. The cells were pelleted down by centrifugation at $5,000 \times g$ at room temperature. Then the pellet was washed two times with LB media following a final wash in 1 x M9 media and finally resuspended in 10 ml M9 media. 1 ml of final resuspension was inoculated per 500 ml. Cells were shaken at 37 °C till an OD_{600} of 0.6 - 0.7 was achieved. After that the culture was induced with 1 mM IPTG for 4 h or at 37 °C. At the end, cells were harvested and frozen in liquid nitrogen and stored at – 80 °C until purification.

2.2.6.2 NMR data collection and processing

The NMR samples were prepared in 25 mM $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ (pH 6.5) and 0.1% NaN_3 . 10% D_2O was added for the NMR-lock signal. All NMR experiments were performed at temperatures ranging from 10 °C to 35 °C on a Bruker Avance 600 MHz and 700 MHz spectrometers equipped with 5 mm triple-resonance ($^1\text{H}/^{15}\text{N}/^{13}\text{C}$) single-axis gradient probes, and a cryo-probe for both spectrometers. Different protein concentrations were used for the measurements. For HSQC experiment, 0.01 mM to 3 mM concentration of the protein was used. For the triple resonance backbone experiments, generally 0.5-1 mM protein sample was used. Appropriate pulse calibrations and other parameters including temperature, buffer and protein concentration were optimized before making final measurements. Homo-nuclear experiments included 2D ^1H , ^1H -NOESY, and TOCSY experiments for samples in H_2O and $^2\text{H}_2\text{O}$ (spectral widths of about 9615.85 Hz in both dimensions, using a WATERGATE scheme to suppress the water signal for the H_2O samples and pre-saturation of the residual water signal during recovery time for the $^2\text{H}_2\text{O}$ samples). For 2D TOCSY, a 80 ms mixing time and for 2D NOESY, a 300 ms mixing time were used for the data collection. Hetero nuclear experiments included 2D ^{15}N -HSQC, 2D ^{13}C -HSQC, 3D HNCA, HNCB, HN(CO)CA, HNCACB, CBCA(CO)NH, HNCACO, HCCH-TOCSY HCCCONH, 3D ^{15}N -NOESY-HSQC, 3D ^{13}C NOESY-HSQC. In all

of the above experiments, spectral widths were set to 9.6 kHz for protons, 2.8 kHz for ^{15}N and 6-12 kHz for ^{13}C . The 3D ^{15}N - ^1H NOESY-HSQC and 3D ^{13}C - ^1H NOESY-HSQC mixing time of 100 ms were used for NOESY assignment of the subunit F protein from *M. mazei* Gö1. 3D ^{15}N - ^1H NOESY-HSQC of 200 ms mixing time were collected for the proteins H₁₋₄₇ and E₁₋₅₂. All the three-dimensional experiments made use of pulsed-field gradients for coherence selection and artifact suppression, and utilized gradient sensitivity enhancement schemes. Quadrature detection in the indirectly detected dimensions was achieved using either the States/TPPI (time-proportional phase incrementation) or the echo/anti-echo method. Baseline corrections were applied wherever necessary. The proton chemical shift was referenced to the methyl signal of 2, 2-dimethyl-2-silapentane-5-sulphonate (Cambridge Isotope Laboratories) as an external reference to 0 ppm. The ^{13}C and ^{15}N chemical shifts were referenced indirectly to DSS. All the NMR spectra were processed either using nmrPipe/nmrDraw (DELAGLIO *et al.*, 1995) or Bruker Avance spectrometer in-built software Topspin. Peak-picking and data analysis of the Fourier transformed spectra were performed with the SPARKY program (GODDARD *et al.*, 1997). The translational diffusion rates were measured by monitoring 1D ^1H signal decay due to molecular diffusion in the z-direction of the sample using PFGs at variable concentrations of H₁₋₄₇, H₁₋₆₁, H₁₋₆₉ and H₁₋₈₀, respectively. In each experiment, the PFG strength was linearly incremented from 2 to 95 G/cm, with a translational diffusion delay of 200 ms and total encoding and decoding gradient durations of 5 ms. The diffusion rates were estimated using TopSpin (Bruker BioSpin).

2.2.6.3 Resonance assignment

In the initial stage of any investigation of nuclear magnetic resonance data of a protein, each resonance associated with a particular nucleus in the protein molecule must be assigned. The information useful for the sequence specific assignment for a protein is of three types based on scalar couplings, dipolar coupling and the chemical shift. Different strategies are employed for the resonance assignments depending on the type of the sample: unlabeled proteins or isotopically labeled proteins (CAVANAGH *et al.*, 1995).

Even for a small protein it is impossible to resolve all resonance lines in a one-dimensional (1D) spectrum. Assignments can be obtained using the sequential assignment approach based on homonuclear two-dimensional (2D) correlation spectroscopy (COSY) or total correlation spectroscopy (TOCSY) (Braunschweiler *et al.*, 1983) and 2D nuclear Overhauser effect spectroscopy (NOESY). TOCSY experiment is used to identify the resonance position within

each amino acid spin system and the NOESY experiment is used for the sequential assignment of the spin systems present in the amino acids. For a protein/peptide NMR spectrum, amide hydrogens are generally the best resolved set of resonances and resonate between 6-12 ppm. The side chain resonance assignment are generally done by examining the peaks in the backbone amide regions. This process is known as the sequential assignment strategy developed by Wüthrich and his co workers (WÜTHRICH, 1990).

For the sequential assignment strategy of larger molecules (>10 kDa.) this above assignment approach is not useful because overlaps of the NMR signals complicates the assignment. Such systems require the addition of a third dimension by the replacement of the naturally abundant isotope ^{14}N with ^{15}N and ^{12}C by ^{13}C . Here, assignment is based on triple resonance experiments allowing further decrease in the overlap of NMR signals. There are six triple resonance experiments commonly used in chemical shift assignments of the backbone atoms of the protein: HNCA, HN(CO)CA, HNCACB, CBCA(CO)HN, HNCO and HN(CA)CO. The HNCA experiment gives the chemical shifts of the $^{13}\text{C}^\alpha$ and the previous amino acid's $^{13}\text{C}^\alpha$,

Figure 2.2.5: Transfer of magnetization in the HNCA (A) and HN(CO)CA (B) experiments. HNCA experiment provides sequential connectivities by making use of ^{15}N - $^{13}\text{C}^\alpha$ J-coupling (7 - 11 Hz). HN(CO)CA experiment provides sequence correlations by transferring the coherence via the intervening ^{13}CO spin (CAVANAGH *et al.*, 1995).

while the HN(CO)CA gives only the previous amino acid's $^{13}\text{C}^\alpha$. Sequential assignment can then be undertaken by matching the shifts of each spin system's own and previous carbons. Figure 2.2.5 shows the transfer of magnetization in the HNCA and HNCOCA. Figure 2.2.6 shows N = 128.682 ppm plane of HNCA and HN(CO)CA experiments. In HNCA plane each amide ^1H chemical shift corresponds to two $^{13}\text{C}^\alpha$ chemical shifts. The peak with the stronger intensity is the amino acid's own $^{13}\text{C}^\alpha$, while the weaker intensity peak belongs to the previous amino acid's $^{13}\text{C}^\alpha$. In case of HN(CO)CA experiment only one peak is appearing for each amide ^1H shifts. An example of the assignment by these two experiments is also shown in Figure 2.2.6.

Figure 2.2.6: ^{15}N = 128.682 ppm plane of the HNCA experiment (A); ^{15}N = 128.682 ppm plane of the HN(CO)CA experiment (B); An example of assignment of 4 residues (K22, V23, Y24 and E25) by HNCA and HN(CO)CA experiments in case of subunit F of A_1A_O ATP synthase from *M. mazei* Gö1 (C).

The next four experiments are grouped in pairs in a similar way, providing a mean of double-checking the chemical shift data. The details of transfer of magnetization as well as an example of assignment of the same four residues of subunit F is shown in figure 2.2.7 in case of HNCACB and CBCA(CO)NH experiments. These two experiments take into account both the $^{13}\text{C}^\alpha$ as well as $^{13}\text{C}^\beta$ values for the sequential assignments of residues. They are extremely useful in case of degeneracy of $^{13}\text{C}^\alpha$ values. In addition, $^{13}\text{C}^\alpha$ and $^{13}\text{C}^\beta$ values can provide information about the type of amino acid. In the same way, the experiments HNCO, HN(CA)CO are also used in the backbone assignment of the proteins. HNCO experiments correlates the amide ^1H and ^{15}N chemical shifts of one amino acid to the ^{13}CO chemical shifts of the previous residue by using ^{15}N - ^{13}CO J-coupling ($\sim 15\text{Hz}$). HN(CA)CO experiment can give correlations of ^1H and ^{15}N spins to the ^{13}CO chemical shifts of the previous and same residue. Thus, HNCO experiment when used in conjunction with HN(CA)CO experiment, it provides a method to sequentially assign the amide ^1H , ^{15}N and ^{13}CO resonances.

Figure 2.2.7: Transfer of magnetization in the HNCACB (A) and CBCA(CO)NH (B). HNCACB experiment correlates the $^{13}\text{C}\alpha$ and $^{13}\text{C}\beta$ resonances with the amide ^1H and ^{15}N resonances of the same and the previous residue whereas the CBCA(CO)NH experiment correlate the $^{13}\text{C}\alpha$ and $^{13}\text{C}\beta$ resonances with the amide ^1H and ^{15}N resonances of the previous residue (CAVANAGH *et al.*, 1995). An example of assignment of 4 residues (K22, V23, Y24 and E25) by HNCACB and CBCA(CO)NH experiments in case of subunit F of A_1A_0 ATP synthase from *M. mazei* Gö1(C).

After successfully assigning the backbone of residues in ^1H - ^{15}N HSQC spectrum by using three sets of experiments (HNCA and HN(CO)CA; HNCACB and CBCA(CO)HN; HNCO and HN(CA)CO) side chain (^{13}C and ^1H) chemical shifts were assigned by using mainly three experiments: (H)CC(CO)NH-TOCSY, H(CC)(CO)NH-TOCSY and HCCH TOCSY (Figure

2.2.8). The first two experiments (H)CC(CO)NH-TOCSY, H(CC)(CO)NH-TOCSY provide the correlation of ^1H and ^{15}N amide resonances of one residue with the ^{13}C side chain and ^1H side chain resonances, respectively, of the previous residue. The 3D HCCH-TOCSY experiment is specifically designed to correlate side-chain aliphatic proton and ^{13}C resonances via ^{13}C - ^1H and ^{13}C - ^{13}C J-coupling constants (CAVANAGH *et al.*, 1995).

Figure 2.2.8: Transfer of magnetization in the (H)CC(CO)NH-TOCSY (A), H(CC)(CO)NH-TOCSY (B) and HCCH TOCSY (C) (CAVANAGH *et al.*, 1995).

2.2.6.4 NMR spectroscopy and structure calculation

The process of protein structure determination by NMR spectroscopy (Figure 2.2.9) involves three basic steps: first, the assignment of every resonance to a nuclear spin, second, the measurement of structural restraints and third, the calculation and subsequent refinement of structures that satisfy the experimental restraints. The most important restraint is NOE. NOE cross peak intensities depends on the cross relaxation rate, proportional to the inverse sixth power of the distance between the two interacting protons (CAVANAGH *et al.*, 1995). Thus, NOE intensities are used as distance restraints in the structure calculation. In NOESY experiment mixing time is very important. Intra residue and sequential NOE intensities were obtained from 100 ms NOESY spectra for subunit F and 200 ms NOESY spectra for H_{1-47} and E_{1-52} in order to minimize the effects of spin diffusion. The additional His-residues of the His-tag at the N-

terminus, which are essentially unstructured, were not used in the structure calculation. In case of subunit F, the ^1H sequential assignments were achieved mainly using homo-nuclear 2D NOESY ($t_m = 100$ ms) and TOCSY spectra in both H_2O and $^2\text{H}_2\text{O}$ solutions at 600 MHz at 15 °C; this approach was adopted because labeled protein samples became available only later in the work. Once labeled protein is available, assignments were extended to include backbone ^{15}N and ^{13}C signals using ^{15}N NOESY-HSQC, ^{13}C NOESY-HSQC, as well as triple resonance backbone experiments (HNCA, CBCA(CO)NH). The distance restraints for the structure calculation were collected from 3D ^{15}N - ^1H NOESY-HSQC and ^{13}C - ^1H NOESY-HSQC by manual and automatic assignments for which CYANA 2.1 was used. The experiments used for H_{1-47} and E_{1-52} were ^{15}N HSQC, HNCACB, CBCA(CO)NH, H(CC)(CO)NH-TOCSY and ^{15}N NOESY-HSQC. Dihedral angle restraints were calculated from chemical shifts using Torsion Angle Likelihood Obtained from Shift and sequence Similarity (TALOS) (CORNILESCU *et al.*, 1999) and the overall secondary structure were predicted from Chemical Shift Index (CSI) and NOE pattern. Upper bound for all NOE distance restraints were initially set to 5 Å and adjusted for non-stereospecifically assigned methylene and methyl protons using the method described originally for DYANA and detailed by Güntert (GÜNTERT *et al.*, 1997). Experimental evidence for hydrogen bonds was obtained from hydrogen/deuterium exchange in per-

deuterated buffer at 15 °C over a period of 24 h for subunit F. Amide protons that are associated in regulatory secondary structural elements are generally longer lived than those present in the flexible part of the protein. Thus, the slow amide exchange rate can be used as a measure for the presence of hydrogen bond. For the determination of the H/D exchange factors, subunit F sample was lyophilized and resuspended in 100% D_2O . A 2D ^1H - ^{15}N -HSQC spectra were obtained immediately after the lyophilized protein was dissolved in 0.5 ml of D_2O . The dead time of the experiment was about 30 min. The structure calculations were performed with the CYANA 2.1

Figure 2.2.9: A flow chart illustrating the general strategy for determining the three dimensional structure by high resolution NMR spectroscopy. Structure calculations are done in an iterative manner where calculated structures are used to identify more NOEs.

program package (GÜNTERT *et al.*, 1997), which uses simulated annealing in combination with molecular dynamics in torsion angle space. With the first round of structure calculations, only unambiguous long-range NOE constraints were used to generate a low-resolution fold for the structure. Assignments of ambiguous NOE cross-peaks were made by applying a structure-aided filtering strategy in repeated rounds of structure calculations. Starting *ab initio*, 100 conformers were calculated in 8,000 annealing steps each. The program MOLMOL (KORADI *et al.*, 1996) was used to visualize the result of ensemble of minimized conformers.

2.2.6.5 Correlation time (τ_c) and relaxation measurements

The correlation time was estimated using the equation:

$$\tau_c = ([6(T_1/T_2) - 7] / 4)^{1/2} / \Omega_N 2\pi$$

where T_1 and T_2 are the mean longitudinal and transverse relaxation times, and Ω_N is the Larmor frequency of ^{15}N at the field strength of 14.6 T and 16.4 T were used here. The ^{15}N longitudinal and transverse relaxation time constants, T_1 and T_2 , respectively, were determined by collecting a time series of ^{15}N -HSQC spectra with sensitivity enhancement. ^{15}N relaxation measurements were acquired on Bruker Avance 600 and 700 MHz spectrometer equipped with cryo probe at appropriate temperature using the triple-axis gradient enhanced and sensitivity-enhanced ^1H - ^{15}N HSQC experiment provided in the Bruker pulse sequence library.

For T_1 measurements, the spectra were collected with relaxation delays of 5, 40, 80, 130, 210, 330, 470, 630, 800 and 1000 ms, with repeat experiments at 40 ms and 130 ms for error estimations. For T_2 measurements, data were acquired with delays of 14.4, 28.8, 43.2, 57.6, 72.0, 86.4, 100.8, 115.2, 129.6, 144.0 and 158.4 ms with duplicate points at 43.2 and 72.0 ms. The delay between 180 pulses in the Carr–Purcell–Meiboom–Gill (CPMG) pulse train for T_2 measurements were fixed to 0.9 ms. A total of 2,048 complex data points with 128 complex increments were collected for the relaxation experiments. The relaxation rate constants were determined by fitting the cross-peak intensities to a mono exponential function.

T_1 and T_2 spectra were recorded with spectral widths of 9,615.85 Hz sampled over 2,048 complex points in the ω_2 (^1H) dimension, and 2,405.12 Hz over 128 complex points in the ω_1 (^{15}N) dimension with 16 scans for each increment in the indirect dimension. The relaxation delay for both T_1 and T_2 measurement was 1.5 sec.

The steady-state NOE spectra were acquired with a spectral width of 9,615.85 Hz over 2,048 Hz complex points in ω_2 (^1H) dimension and 2405.12 Hz over 128 complex points in the

ω_1 (^{15}N) dimension. ^{15}N decoupling during acquisition was achieved using a GARP-4 pulse sequence (SHAKA *et al.*, 1985). The field strength of the CPMG refocusing train about 3.3 kHz and 1.2 ms delay was used between the refocusing pulses (PERVUSHIN *et al.*, 1997). The effects of cross relaxation between ^{15}N - ^1H dipolar and ^{15}N chemical shift anisotropy were removed by applying 180° pulses during relaxation delay. ^1H - ^{15}N steady-state NOE values were measured with two different data sets, one collected with no initial proton saturation and a second with initial proton saturation. The proton saturation period was set to 3 sec.

2.2.6.5.1 Data processing and analysis

The HSQC spectra were processed using nmrPipe software (DELAGLIO *et al.*, 1995). Peak intensities were measured and analyzed using the peak picking routine built into SPARKY (GODDARD *et al.*, 1997). The T_1 and T_2 spectra were processed using a sine bell apodization function shifted by 90° for both dimension. The final sizes of the matrices were 2048×128 real points after zero filling in both the dimension and the Fourier transformation. An automated baseline correction was applied in both dimensions. The spectra were referenced to DSS signal. T_1 and T_2 values were obtained by fitting peak intensities using single exponential decay:

$$I(t) = I_0 \exp(-t/T_{1,2})$$

Where $I(t)$ is the peak intensity, t is the time, I_0 is the intensity at time 0.

The heteronuclear steady-state ^{15}N - ^1H NOE values were obtained from the ratios of the peak intensities in the saturated spectrum to those in the unsaturated spectrum. In order to suppress time- or temperature-dependent effects, the spectra were acquired in an interleaved mode with incremented relaxation delays. All experiments were recorded with 256 t_1 increments of 2,048 data points in t_2 . The spectral widths were set to 9,586.15 Hz (^1H), 2,406 Hz (^{15}N) at 600 MHz. Errors were estimated by evaluating the standard deviation of the NOE, σ_{NOE} .

$$\sigma_{\text{NOE}}/\text{NOE} = (\sigma I_{\text{sat}}/I_{\text{sat}})^2 + (\sigma I_{\text{unsat}}/I_{\text{unsat}})^2)^{1/2}$$

Where σI_{sat} and σI_{unsat} are the standard deviation of the noise in the spectra.

2.2.6.6 Binding studies with NMR

NMR spectroscopy is extensively used to identify the important residues for protein protein interaction (TAKEUCHI *et al.*, 2006; VAYNBERG *et al.*, 2006). With respect to other technique NMR have the advantage of monitoring interactions at amino acid level. ^1H - ^{15}N HSQC spectrum of labeled protein was used as starting point at optimized condition. Then the ligand protein/peptide was added in increasing amount at the same temperature and buffer conditions. A series of ^1H - ^{15}N HSQC spectra were recorded at each concentration of the binding partner. 100 - 500 μM of ^{15}N labeled protein was used for the reference spectrum. Unlabeled binding partner was added in increasing amounts to a molar ratio of 1:1 to 1:2 and chemical shift perturbations were monitored in ^1H ^{15}N HSQC spectrum (Figure 2.2.10). Experiments were performed on Bruker Avance 600/700 machine using Topspin for acquisition and processing of spectra. Respective spectra were overlapped to monitor chemical shift changes, further analysis were done in SPARKY (KNELLER *et al.*, 1997).

Figure 2.2.10: Illustration of peak position change (red color) in ^1H - ^{15}N HSQC spectrum upon addition of binding partner (CAVANAGH *et al.*, 1995).

1.2.7 Fluorescence measurements.

A *Varian Cary Eclipse* spectrofluorimeter was used, and all experiments were carried out at 20 °C. The samples were excited at 295 nm, and the emission was recorded from 310 to 380 nm with excitation and emission bandpasses set to 5 nm. For titration of the tryptophan fluorescence of peptide F₈₁₋₁₀₁ with ATP, the emission wavelength was 338 nm. Before use, peptide F₈₁₋₁₀₁ and increasing amounts of ATP were incubated in a buffer of 50 mM Tris/HCl (pH 8.0) for 5 mins.

3. Results

3.1 Solution structure of subunit F of A_1A_0 ATP synthase from *Methanosarcina mazei* Gö1

3.1.1 Introduction

Catalytic-site events (ATP synthesis and ATP hydrolysis) are connected to ion translocation through conformational changes of the subunits in the stalk region of the enzyme, A₁A₀ ATP synthase. Subunit F, the smallest subunit in A₁ ATPase, is a part of the central stalk domain of A₁A₀ ATP synthase (COSKUN *et al.*, 2002; SCHÄFER *et al.*, 2006b) and interacts with subunit B in the A₁ part, depending on the type of nucleotide bound to the enzyme. This important observation was noticed from the cross linking studies of the enzyme A₁A₀ ATP synthase from *Methanocaldococcus jannaschii* (SCHÄFER *et al.*, 2006b). When the enzyme was incubated with 5 mM MgAMP-PNP at 4 °C before EDC treatment, a 63 kDa B-F product was formed. By contrast there was lower cross-link formation in the presence of MgATP. The cross-link product completely disappeared when the enzyme was incubated with MgADP or MgADP + Pi. MALDI mass spectroscopy identified that the zero length cross-link product was formed between the C-terminal region ₃₈₈DLVAVVGEEALTDR₄₀₁ and ₈₂IDPVKELIR₉₀ of subunit B and subunit F, respectively. Interestingly, the peptide ₃₈₈DLVAVVGEEALTDR₄₀₁ is at a similar position to the so called DELSEED region of the nucleotide binding subunits α and β of F₁F₀ ATP synthases (AGGELER *et al.*, 1998). The cross-link study with EDC identified that subunit F is also in close contact to subunit C and subunit D. Thus, structure characterization of subunit F is very essential, in order to understand its functions in the A₁A₀ complex. Most recently, the shape of subunit F of the A₁A₀ ATP synthase from the archaeon *Methanosarcina mazei* Gö1 in solution was determined by small angle X-ray scattering, indicating that the protein is elongated and organized into two well-defined domains (SCHÄFER *et al.*, 2006b). Here, the three dimensional structure of F subunit of the A₁A₀ ATP synthase from *M. mazei* Gö1 as well as dynamical properties of this subunit in solution has been determined by NMR spectroscopy.

3.1.2 Resonance assignments of subunit F of the A₁A₀ ATP synthase

In order to determine the three dimensional structure it is important to do resonance assignment for ¹H, ¹⁵N and ¹³C atoms. The sequential resonance assignments of the backbone amide groups are presented in a 2D ¹H/¹⁵N-HSQC spectrum (Figure 3.1.1), indicating backbone HN and ¹⁵N cross-peaks of individual residues. A well-dispersed HSQC (Heteronuclear Single Quantum Coherence) spectrum suggests a folded structure for the protein. The folding of subunit F was also evident from the 1D-¹H NMR spectra of the protein, showing a good dispersion of amide peaks as well as up-filled shifted methyls (Figure 3.1.2). Assignments for the backbone HN, ¹⁵N, ¹³C $^{\alpha}$, ¹³C $^{\beta}$ and ¹³C' resonances were achieved by a combined analysis of the triple-

also clarifies that the amide exchange rate is faster in these regions (Figure 3.1.3).

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The $C^\alpha H$ and $C^\beta H$ proton resonances and side-chain carbon and proton resonances were assigned from the $H(CCO)NH$, $C(CO)NH$ and $HCCH$ -TOCSY spectra. In the 1H - ^{15}N HSQC spectrum, different line widths were observed for a number of residues. In order to investigate in detail, HSQC spectra at various concentrations ranging from 0.1 to 1 mM were taken at identical NMR conditions.

Figure 3.1.2: One dimensional 1H NMR spectrum of subunit F recorded at 288 K in 25 mM PO_4 buffer (pH 6.5) on Bruker Avance 600 MHz machine. Nice dispersion of resonance peaks in HN region, α -protons and uphill shifted methyl peaks are the characteristics of natively folded protein. Strong peaks at 4.7 ppm represents water signal.

Analysis of these spectra indicated that there is no measurable difference either in the line-widths or in the chemical shifts of 1H and ^{15}N resonances. Moreover, small-angle X-ray scattering (SAXS) data also indicated that the protein is monomeric in the solution (SCHÄFER *et al.*, 2006b) at the concentration used. These experiments clearly suggest that the broadness of the peak in 2D HSQC is due to the conformational changes of the protein, altering the environment of a nuclear spin and modulating chemical shift. When this process is on the microsecond to millisecond timescale and the chemical shift changes are large, the NMR signal broadens.

Figure 3.1.3: Exchange rate (h^{-1}) was plotted against residues for subunit F. The exchange rate has been calculated from the hydrogen deuterium exchange experiment where the protein was dissolved in 100% D_2O and the loss of intensity of the signal was monitored through 1H - ^{15}N HSQC experiment over time.

Figure 3.1.4: 2D ^1H - ^{15}N HSQC of subunit F with different peak shapes and intensities are indicating chemical exchange phenomena. The unlabelled peaks are single sharp resonances having line width of about 25-30 Hz. The peaks marked in single letter amino acid residue with asterisks indicate residues undergoing chemical exchange. The peaks marked in single letter amino acid residue with subscripts *a* and *b* indicate multiple peaks for the same residue. For example, A35, K101, and V45 have multiple peaks and are marked with subscripts *a* and *b* (GAYEN *et al.*, 2007).

Three different types of line shapes of the peaks were observed in the HSQC spectrum. There are single intense peaks and single peaks with exchange broadening and two or more peaks appear with skewed populations. Figure 3.1.4 represents the 2D ^1H - ^{15}N HSQC of subunit F, where the unlabelled peaks are single sharp resonances. 1D projection of the representative HSQC peaks are shown in Figure 3.1.5. Single sharp peaks have been shown for residues S9, V61, L62 and E68 peaks with exchange broadening as well as two or more peaks for the same residue have been shown for E10, A18, K22, G46 and A35, N52, G78, G96 respectively. Interestingly, all these single sharp peaks are found to be clustered in the middle of the protein sequence representing residues in β_2 , α_2 , β_3 , α_3 and β_4 secondary structural elements (Figure 3.1.5B). The intensities of the peaks are very low for the C-terminal residues, indicating that this region is flexible compared to the N-terminal domain. This difference in intensity of HSQC peaks is clear evidence that the protein undergoes conformational exchange on the microsecond to millisecond time scale. Structures of subunit F were calculated for one major conformer having intense peaks.

Figure 3.1.5: (A) 1D projection of representative ^1H - ^{15}N HSQC peaks of subunit F are plotted in the same Hz/pt scale. The column 1 shows single sharp peaks, column 2 single peak with exchange broadening and column 3 shows two or more peaks for the same residue. The residue along with the sequence number is marked in the left side of the peak. (B) Bar diagram showing intensities of the HSQC cross peaks as a function of residue position of the subunit F. Intensities were measured using SPARKY (GAYEN *et al.*, 2007).

3.1.3 Solution structure of subunit F of A_1A_0 ATP synthase from *M. Mazei* Gö1

The three dimensional solution structure of subunit F shows that the protein is an elongated molecule. The structure was calculated on the basis of a total of 1399 nontrivial NMR-derived distance restraints. Figure 3.1.6 shows an overlay of the 10 lowest energy structures of the protein and the statistics are given in table 3.1.1.

Figure 3.1.6: Backbone (N, $\text{C}\alpha$, C') (A) and ribbon (B) diagrams of 10 low-energy NMR-derived structures of subunit F of the A_1A_0 ATP synthase from *M. mazei* Gö1 (GAYEN *et al.*, 2007).

Total number of NMR restraints	1399
Unambiguous NOE peaks	
<i>Intraresidue (i=j)</i>	301
<i>Sequential (i-j =1)</i>	377
<i>Medium-range (2 ≤ i-j ≤ 4)</i>	261
<i>Long-range (i-j > 4)</i>	297
Dihedral angle constraints	163
Hydrogen-bond distance restraints	16
No. of restraint violations ¹	
<i>Total number of restraint violations > 0.5 Å</i>	0
<i>Total number of dihedral angle violations > 5 °</i>	0
Ramachandran plot² (%)	
<i>Residues in most favored regions</i>	70.6
<i>Residues in additionally allowed regions</i>	28.9
<i>Residues in generously allowed regions</i>	0.4
<i>Residues in disallowed regions</i>	0.1
Average r.m.s.d. to Mean (Å)	
<i>Backbone (residues 1–78)</i>	0.39 ± 0.09
<i>Heavy atoms (residues 1–78)</i>	0.78 ± 0.08

¹There are no distance violations greater than 0.5 Å or dihedral-angle violations greater than 5°. All residues are included in the final ensemble. ²Ramachandran plot was for all the residues.

Table 3.1.1: Structural statistics for the ten selected structures of subunit F of the A₁A₀ ATP synthase after energy minimization (GAYEN *et al.*, 2007).

These structures have an overall r.m.s.d. of 0.39 ± 0.09 Å for backbone atoms and 0.78 ± 0.08 Å for all heavy atoms in the well-ordered regions of the three-dimensional structures (residues 1-78) of the protein. High r.m.s deviations with loose C-terminal domain of subunit F reflect the difficulty in determination due to the lack of distance and dihedral restraints. The dimensions of the elongated subunit F (78.7 Å) are approximately 24.2 Å x 28.5 Å x 38.7 Å for the N-terminal part. The C-terminal tail has a length of about 40 Å. All these structures have energies lower than 3.7 kcal mol⁻¹, no NOE violations greater than 0.3 Å and no dihedral violations greater than 5°.

Ramachandran plot of residues ϕ and Ψ angles of subunit F has been shown in figure 3.1.7. The secondary structure prediction from chemical shift index (CSI) method in figure 3.1.8A indicates that there is an alternating arrangement of four α -helices and four β -strands and the residues 86-94 have a low probability to form a helix. However, no NOEs has been detected in this region. The location of relevant NOE restraints for α -helices and β strands are shown in figure 3.1.8B and 3.1.8C. From the figure 3.1.8C, it is clear that the structure of subunit F has been calculated mostly using the NOE restraints from the N-terminal domain (GAYEN *et al.*, 2007).

Figure 3.1.7: Ramachandran plot of residues ϕ and ψ angles of subunit F. 70.6% in most favored regions, 28.9% in additionally allowed regions, 0.4% in generously allowed regions and 0.1% in disallowed regions. This plot has been made from CYANA (GÜNTERT *et al.*, 1997).

Figure 3.1.8: (A) Secondary structure prediction from chemical shifts of subunit F from *M. mazei* using PECAN software (EGHBALNIA *et al.*, 2005). The positive vertical axis represents the probability of helix and the negative vertical axis represents the probability of sheet structure. Values near zero represents the random coil values. (B) and (C) Plot of relevant distance restraints used in the structure calculation by CYANA (GÜNTERT *et al.*, 1997).

Figure 3.1.9: Amino acid sequence of the subunit F with a survey of the short- and medium range NOE connectivities, which were used to establish the sequence-specific ^1H NMR assignment and to identify elements of regular secondary structure (GAYEN *et al.*, 2007)

The overall topology of the solution structure of subunit F is divided into a well-defined N-terminal domain, formed by an α/β fold and an extended C-terminal tail at its very end (Figure 3.1.7). The four-stranded β -sheet of the N-terminal part are oriented parallel to each other and are separated due to the alteration with the three α -helices. These α -helices are made up by the residues S9 - A18, A31 - V39 and V61 - E68, whereby the residues L3 - G7, K22 - T26, V45 - N52 and T73 - A76 form the β -strands ($\beta 1$ - $\beta 4$) (shown in figure 3.1.10).

Figure 3.1.10: Schematic representation of the secondary structure of subunit F is shown. Dotted lines indicate inter strand hydrogen bonds and solid lines indicate long-range ^1H - ^1H distances in 4 parallel β -strands of the subunit F (GAYEN *et al.*, 2007).

3.1.4 Intramolecular dynamics of the C-terminal domain

NMR relaxation experiments of proteins provide uniquely detailed and quantitative information about the conformational dynamics on the pico- and nanosecond time scale. There are NMR spin relaxation experiments of ^{15}N , ^{13}C or ^2H labeled protein, which are used to determine the longitudinal T_1 , transverse T_2 , heteronuclear NOE and other relaxation pathways. ^{15}N amide relaxation analysis of subunit F was done in order to get details about the flexibility of the C-terminal domain relative to the $(\alpha/\beta)_4$ part in more detail, which might be important for the coupling function of this subunit (GAYEN *et al.*, 2007). A first indication that the N- and C-terminal domains of subunit F are in loose contact in the solution structure is the observation that there are no long range NOEs between residues of the N- and C-terminal domains. Furthermore, a backbone ^{15}N - ^1H NOE spectrum was recorded and backbone relaxation parameters were measured. As shown in figure 3.1.5B, the peaks in the C-terminal regions are weakened, indicating that the two domains of the F subunit tumble as two different entity. Figure 3.1.11B-D, F-H illustrates the longitudinal R_1 and transverse R_2 relaxation rate constants and R_2/R_1 ratios. R_1 and R_2 relaxation rates as well as ^{15}N - ^1H NOE were determined at two different field strengths of 14.1 and 16.4 T respectively. Both R_1 and R_2 rates are fairly constant across

Figure 3.1.11: Comparison of NMR data collected on 600 MHz (A-D) and 700 MHz (E-H) spectrometers. (A, E) Steady-state ^1H - ^{15}N NOE enhancement data. The less intense bar graph indicates that this portion (80-101) of the protein is very dynamic. (B-D, F-H) Experimental longitudinal (R_1) and transverse (R_2) ^{15}N relaxation rates and R_2/R_1 ratios obtained for subunit FMm at 600.13 and 700.13 MHz, respectively. Almost all residues in the well-ordered region exhibit a rather uniform dynamic behavior, suggesting low backbone mobility for this region (1-78) of the protein structure. All C-terminal residues show a rather non uniform dynamic behavior, suggesting high backbone mobility for this C-terminal region of the protein structure. The error bars represent the SD (GAYEN *et al.*, 2007).

the primary sequence for the N-terminal $(\alpha/\beta)_4$ part. But in the C-terminal end of the protein (around 20 amino acids) there is a discernible increase in R_1 and a decrease in the R_2 values was observed. The average values of the relaxation parameters R_1 and R_2 and NOEs in the ordered regions (residues 1-78) were calculated at 14.6 and 16.4 T are 1.104, 23.469 and 0.774 sec^{-1} and 1.174, 16.334 and 0.809 sec^{-1} respectively. The non uniformity in the R_1 and R_2 relaxation rate constants signifies that the protein is tumbling anisotropically. Overall correlation times of 14.5 ns for 14.6 T and 9.94 ns at 16.4 T were obtained for this subunit F protein according to the equation described in the section 2.2.6.5. The backbone dynamics of subunit F was also

investigated by model-free analysis taking the relaxation parameters at two different field strengths of 14.1 and 16.4 T. The results for the model-free analysis are shown in figure 3.1.12A-C. The residues in the N-terminal globular part have $S^2 \sim 0.8$ indicating fairly rigid overall structure. Whereas, the flexible residues from the C-terminal loop has $S^2 < 0.6$. To examine further the presence of conformational exchanges on the micro- to millisecond time scale in subunit F, a rough estimation of the amount of conformational motions in the backbone of subunit F has been made from the difference in the R_2 values obtained for the two extreme values of $\nu_{\text{CPMG}}^{\text{min}} - \nu_{\text{CPMG}}^{\text{max}}$. As shown in figure 3.1.12D, a difference between the two experiments was observed in a number of residues in subunit F with significant positive values of $R_2(\nu_{\text{CPMG}}^{\text{min}}) - R_2(\nu_{\text{CPMG}}^{\text{max}})$, indicating that the conformational exchange occurs at multiple backbone sites in the protein. The corresponding residues probed by CPMG experiments were located primarily in the three different regions of the protein, in the N-terminal, loop1- α 1-loop2 (residues 8-23), in the middle part, β 3-loop 3 (residues 47-53) and in β 4 (residues 75, 76, and 79) and the C-terminal part of subunit F, suggesting a chemical exchange phenomenon in these regions.

Figure 3.1.12: (A-C) Plots from model free analysis. (D) Conformational exchange in subunit F of the methanogenic A_1A_0 ATP synthase. Measurement of backbone conformational motions from the difference values of $R_2(\text{CPMG})$ at minimal and maximal CPMG pulse repetition rates. $R_2(\nu_{\text{CPMG}}^{\text{min}}) - R_2(\nu_{\text{CPMG}}^{\text{max}})$ are displayed as a function of the subunit F sequence. Positive values are expected for residues that undergo conformational exchange.

3.1.5 Solution Structure of C-term of Subunit F (F_{81-101})

The NMR structure of subunit F revealed a globular N-terminal and an elongated C-terminal (GAYEN *et al.*, 2007). However, the secondary structure prediction from chemical shifts shows that there is a propensity to form a helix structure for the residues 86-94 in the entire subunit F (Figure 3.1.8A). Here, I investigated the structure of the 21 mer peptide from the C-terminal end of F subunit (F_{81-101}) using NMR spectroscopy.

Figure 3.1.13: NMR structure of the peptide F_{81-101} (A) superimposition of twenty lowest energy structures. (B) Cartoon representation of a single peptide structure showing orientations of R88, K92, D98 and W100 side chains.

Distance restraints

Total	255
Intraresidue ($i - j = 0$)	53
Sequential ($ i - j = 1$)	102
Medium-range ($2 \leq i - j \leq 4$)	66
Long-range ($ i - j \geq 5$)	34

Average number of violations

Distance violations $> 5 \text{ \AA}$	0
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Ramachandran plot (%)

Residues in most favoured regions	63.2
Residues in additionally allowed regions	36.8
Residues in generously allowed regions	0.0
Residues in disallowed regions	0.0

Average RMSD to Mean ($\text{\AA}$)

Residue 3-21, backbone (C^α , C' , and N)	0.339 ± 0.31
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Table 3.1.2: Structural statistics for F_{81-101} (RAGHUNATHAN *et al.*, 2009).

Using the standard procedure for sequential assignment based on homonuclear TOCSY and NOESY experiments, all the residues of the peptide were assigned. Figure 3.1.13 shows an overlay of the twenty lowest energy structures of the peptide (F₈₁₋₁₀₁), which have an overall r.m.s.d. of 0.491 ± 0.31 . All these structures have energies lower than $-100 \text{ kcal mol}^{-1}$, no NOE violations greater than $0.3 \text{ \AA}$ and no dihedral violations greater than 5° were observed. The statistics are given in Table 3.1.2. The secondary structure prediction using the ^1H chemical shifts is shown in figure 3.1.14A. The connectivity diagram of the peptide is indicative of a helical conformation with the sequential HN---HN, H α ---HN(*i*, *i*+3), H α ---HN(*i*, *i*+4), and H α ---H β (*i*, *i*+3) connectivities (Figure 3.1.14B & C). The peptide displays a short flexible N-terminal tail of the amino acids G81 – T85 and helix structural motif formed by the residues S86 to A94 (Figure 3.1.13).

Figure 3.1.14: (A) secondary structure prediction using H α chemical shifts. (B) NOESY connectivity diagram and (C) HN-HN region of the NOESY spectra for F₈₁₋₁₀₁ (RAGHUNATHAN *et al.*, 2009)

The solution structure of F₈₁₋₁₀₁ presented exhibits a defined loop formation at its very N-terminus, being part of the glycine rich sequence $_{78}\text{GCSGSGSTS}_{86}$, reflecting a phosphate loop (P-loop) motif GXXGXGKTV (WALKER *et al.*, 1982). Surface representation of the NMR structure showed that there is both positive and negatively charged surface area. Residues R88, K92 and K101 formed the positively charged area whereas residues E89 and D98 formed the negatively charged surface area. Zero-length cross-link data (SCHÄFER *et al.*, 2006b) and

Figure 3.1.15: Electrostatic potential surface representation of the NMR structure of F₈₁₋₁₀₁. Red and blue represent negatively and positively charged areas, respectively.

intrinsic tryptophan fluorescence spectroscopy (GAYEN *et al.*, 2007) showed that the association of subunit B and F occurs via the C-termini of both subunits. Thus, it might be possible that cationic regions on the extended C-terminal of subunit F may interact with the anionic patches on the ventral face of the subunit B. In order to examine whether ATP can bind to isolated F₈₁₋₁₀₁, ¹H-¹⁵N HSQC experiment was done in absence and presence of different concentration of ATP. A overlaid ¹H-¹⁵N HSQC spectra of F₈₁₋₁₀₁ in absence and presence of increasing concentration of ATP is shown in figure 3.1.16. HSQC peak positions of most of the residues are shifted with the addition of ATP. The maximum chemical shift change occurs for residues G83, S84, T85, V95, V97, W100 and K101, whereas residues L87, R88, E89, D98 and L99 have medium chemical shift change and residues S86, K90, I91 and A94 have no/minimum chemical shift change. The change of peak position can occur due to ATP binding as well as binding induced conformational change. Thus, it is difficult to conclude from the results which residues are directly involved the binding. However, the results suggest that the isolated peptide F₈₁₋₁₀₁ can bind to ATP. A model fitting of ATP into the F₈₁₋₁₀₁ structure reveals close contacts of the nucleotide to the P-loop as well as cation- π and π - π interaction of R88 and W100 with the adenine-ring respectively (Figure 3.1.16). α -, β - and γ -phosphate are in close neighborhood to the residues S84, T85 and S82 with distances of distance 3.04 Å, 2.30 Å and 4.02 Å, respectively.

Figure 3.1.16: (A) Overlay of 2D ¹H-¹⁵N-HSQC spectra of F₈₁₋₁₀₁ in the absence (red) and presence (blue, yellow and green) of 8.33 mM, 16.6 mM and 30 mM ATP. The spectrum was collected in a Bruker Avance 600 MHz spectrometer in 25 mM sodium phosphate buffer (pH 6.5), 40% TFE at 25°C. (B) ATP binding model for the peptide: pink, orange and light pink color residues represent the maximum, medium and no/minimum chemical shift change respectively in the HSQC spectrum upon ATP addition (RAGHUNATHAN *et al.*, 2009).

The NMR-titration experiment of E₈₅₋₁₀₄ with ATP has shown that the intrinsic Trp residue in F₈₁₋₁₀₁ undergoes a shift in the HSQC spectra after addition of ATP. In order to quantitatively evaluate the binding of the nucleotide the intrinsic tryptophan fluorescence quenching at 338 nm has been performed and the result is shown in Figure 3.1.17. The titration curve has a hyperbolic shape from which a binding constant (K_d) of 560 μ M could be determined.

Figure 3.1.17: Fluorescence titration of F81-101 with the ATP. Excitation was at 295 nm and the emission was measured at 338 nm.

3.2 Structural characterization of subunit H of A_1A_0 ATP synthase from *Methanocaldococcus jannaschii*

3.2.1 Introduction

The energy coupling between the A₁ headpiece, A₃:B₃ and membrane-embedded ion translocating part A₀ domains occurs via the stalk part(s) (GRÜBER *et al.*, 2008). The central stalk is made of subunits C, D and F whereas the subunit H is believed to be a part of the peripheral stalk of the enzyme (MÜLLER *et al.*, 2003; COSKUN *et al.*, 2004). Cross-linking studies of A₁A₀ ATP synthase subunits isolated from *M. jannaschii* using the cross-linker ehtyl-3-(dimethylaminopropyl)-carbodiimide (EDC), which can induce cross-linking between carboxyl and amino groups, demonstrated cross-linking of subunits B-F, D-E, A-H, and A-B-D. Among them, crossing-linking between subunits H and A, B and F were found to be nucleotide dependent (SCHÄFER *et al.*, 2006b). Cross-linking of D-E and A-B-D is independent of the nucleotide present. The nucleotide-dependency of A-H crossing-linking that occurs through the C-terminus of subunit H and the N-terminal domain of subunit A is opposite to that of B-F, as the presence of MgADP + Pi seems to induce A-H binding but inhibit B-F binding, while the presence of the non-hydrolyzable ATP analogue, MgAMP-PNP readily induce cross-linking of B-F but not A-H (SCHÄFER *et al.*, 2006b). Understanding the structural and functional role of H subunit is essential since the precise arrangement of this subunit inside the A₁A₀ complex is still an unresolved issue (GRÜBER *et al.*, 2008). The goal of the present work was to produce and purify the peripheral stalk subunit H of the A-ATP synthase and to examine the structural and biochemical features of this stalk subunit in solution.

3.2.2 Expression and purification of subunit H

Subunit H was cloned into a modified pET9d-His₃ (GRÜBER *et al.*, 2002) expression vector by using the genomic DNA from *M. jannaschii* ATCC® #43067DTM as the template for PCR amplification. The details of the PCR program and reaction mixture was described under section 2.2.1. Figure 3.2.1 shows a double restriction digestion with *Nco*I and *Sac*I enzymes. Recombinant H subunit was purified as described under section 2.2.3. The protein was

Figure 3.2.1: Cloning of subunit H. DNA agarose gel (1 %) picture showing double restriction digestion with *Nco*I and *Sac*I enzymes of atpH obtained after ligation of PCR fragment atpH to pET9d-1 (released insert and vectors are shown by arrow).

found entirely within the soluble fraction. A Ni²⁺-NTA resin column and an imidazole-gradient (25 – 200 mM) in buffer consisting of 50 mM Tris/HCl (pH 8.5) and 250 mM NaCl was used to separate subunit H from the main contaminating proteins (Figure 3.2.2). Subunit H eluting at

125 to 200 mM imidazole fractions were collected and subsequently applied to a Superdex 75 HR 10/30 column (GE Healthcare), in order to remove traces of minor contaminants and imidazole. Analysis of the isolated protein by SDS-PAGE revealed the high purity of the subunit. SDS-gels were stained with Coomassie Brilliant Blue G250. Protein concentrations were determined by the bicinchoninic acid assay (BCA; Pierce, Rockford, IL. USA). The SDS-PAGE of the produced recombinant subunit H revealed a prominent band of 12.5 kDa revealing the high purity of the subunit as shown in figure 3.2.3.

Figure 3.2.2: Ni^{2+} -NTA affinity enrichment of protein from whole cell lysate in buffer 50 mM Tris/HCl, 250 mM NaCl, pH 8.5 using an imidazole gradient (0 – 200 mM) for elution of Ni^{2+} -NTA bound protein. Fractions from 125 – 200 mM imidazole were pooled together for further purification step.

Figure 3.2.3: Purification of protein by gel filtration column Superdex 75 HR 10/30. 125 - 200 mM Ni^{2+} -NTA fractions were pooled and applied and the fractions under the half width of the peak (black) in the chromatogram were collected. Insert shows 1 μl of gel filtration purified protein on a 17 % SDS gel

3.2.3. Secondary structure determination of subunit H

The secondary structure content of this subunit was determined from circular dichroism spectra, measured in a far uv range between 185-260 nm. The CD spectra of subunit H measured at room temperature is shown in figure 3.2.4. Spectra were collected in a 60 μl quartz cell (Hellma) at 18 °C at a step resolution of 1 nm. CD spectroscopy of subunit H was performed in a buffer 50 mM Tris/HCl (pH 7.5) and 250 mM NaCl. The spectrum for the buffer was subtracted from the spectrum of the protein. This baseline corrected spectrum was used as input for computer methods to obtain predictions of secondary structure (BOHM *et al.*, 1992). The minima at 222 and 208 nm and the maximum at 192 nm indicate the presence of α -helical structures in the protein. The secondary structure content was calculated to be 78% α -helix and 10% random coil. This result is consistent with secondary structure predictions based on subunit H amino-acid sequence. The molar ellipticity values at 208 nm (corresponding to the α -helix

(B)

Since non-interacting helices typically give ratios of around 0.8, whereby interacting ones have ratios close to 1, the CD spectrum presented indicates, that many of the residues in subunit H are in coiled-coil interaction. Figure 3.2.4B shows the predicted secondary structure by online PSIPRED software, which uses a position specific scoring matrix generated by PSI-BLAST for secondary structure calculation by a two stage neural network algorithm (MCGUFFIN *et al.*, 2000).

Probability

indicate that this sequence has high propensity to form a two-stranded instead of three-stranded coiled-coil motif in between amino acid residues 25-98.

AtpH	1	MSVSVMEAIKEVKLAEEQAVKEIEBAKNRAEQIKABAIIEEAKKLIAEAE	60
Vma10p	1	MSQKNGIATLLQAEKEAHEIVSKARKYRQDKLKQAKTDAAKEIDSYKIQKDKELKEFEQ	69
sub. b	24	YVWPPLMAAIEKRQKEIADGLASAERAHKDLDLAKASATDOLKKAKAEAOVITIEQANKRRSQILDEAKA	103
AtpH	61	EAKKLVEEMIKKAEBAKKEAEKILEETEKEIKEIISI AKVKILSLKLSILEI.....	104
Vma10p	70	KNAGGVGELEKKAEAGVOGELAEIKKIAEKKDDVVKILITVVKPSAEVHINALM.....	116
sub. b	104	EAEQERTKIVAQAQAEIEAEERKRAREELRKQVAILAVAGAETIERSVDEAANSDIVDKLVLAEL	156

Figure 3.2.6: Sequence alignment of subunits H (A-ATP synthase) and G (V-ATPase) and the soluble domain of subunit *b* (F-ATP synthase) from *M. jannaschii*, *S. cerevisiae*, and *E. coli*, respectively. Alignment was generated using AlignX (Vector NTI v9 InforMax). Identical and homologous amino acids are highlighted in black and gray shading, respectively (BIUKOVIĆ *et al.*, 2007).

3.2.4 Low resolution shape and domain structure of subunit H

X-ray solution scattering patterns of subunit H and truncated form of subunit H, H₁₋₉₈ (BIUKOVIĆ *et al.*, 2007) were kindly measured and analyzed by Prof. Grüber. The radius of gyration (R_g) of subunit H and H₁₋₉₈ were found to be 5.7 ± 0.2 nm and 5.5 ± 0.2 nm respectively, and maximum dimensions (D_{max}) is 20.1 ± 0.3 nm and 15.1 ± 0.3 nm, respectively. Comparison of the forward scattering with the values obtained from a reference solution of bovine serum albumin

Figure 3.2.7: (A) Low-resolution structure of subunit H determined from SAXS data. (B) Superposition of the shapes of subunit H (green) and the truncated H₁₋₉₈ form (blue), determined from solution X-ray scattering data (BIUKOVIĆ *et al.*, 2007).

(BSA; 66 ± 2 kDa) yields a molecular mass of 25 ± 2 kDa, independently of the concentration used (2, 4, and 12 mg/ml) for subunit H. The determined mass is about twice the value calculated from the amino acid sequence and indicates that subunit H is dimeric in solution. The obtained shape for subunit H yields a good fit to the experimental data in the entire scattering range. Subunit H appears as a boomerang-shaped molecule of two distinct parts, with about 12.0 and 6.8 nm in length. The low-resolution shape of the H₁₋₉₈ (BIUKOVIĆ *et al.*, 2007) form is also determined and superimposed on subunit H in figure 3.2.7B. Like the entire H subunit, the H₁₋₉₈

form has an extended boomerang shape. However, comparison of the two models indicates that the truncation of six amino acids at the C-terminus of subunit H results in a shortening of the upper arm of the molecule, which seems to induce a twisting of the upper part. The shortening of the truncated protein H₁₋₉₈ is reflected by its decreased $D_{\max}$ value from X-ray scattering measurement. An intriguing observation is that the dimensions and shape of the lower domain of both molecules do not alter, implying that the C-terminus of subunit H is located in the upper domain and that this domain is important for the stabilization of the quaternary structure of the protein.

3.2.5 Solution structure of C-terminal of Subunit H (H₈₅₋₁₀₄)

To further characterize the C-terminus of subunit H, which is interacting with the N-terminal region of subunit A (K_d , 206 nM) (BIUKOVIĆ *et al.*, 2007), attention was focused to the short length peptide of the very C-terminus of subunit H (residues 85-104). Secondary structure prediction of this region of protein shows presence of both helix and strand in the structure (MCGUFFIN *et al.*, 2000). The peptide was dissolved in phosphate buffer pH 6.5 and NMR experiments were recorded on 600 MHz Bruker Avance spectrometer at a peptide concentration of 1 mM.

3.2.5.1 Assignment of the peptide H₈₅₋₁₀₄

Amino acids in the primary sequence of peptide H₈₅₋₁₀₄ were sequentially assigned as per the standard procedure. 2D TOCSY and NOESY experiments were recorded at a mixing time of 80 ms and 300 ms respectively. 2D TOCSY and 2D NOESY raw data was processed using in-built Topspin software 1.3 (Bruker biospin). All the

amino acids were sequentially assigned. Primary sequence amino acid marking was followed by the assignment of cross peaks by overlaying TOCSY and NOESY spectra using the software SPARKY (GODDARD *et al.*, 1997). Secondary structure prediction was done by using the HA chemical shifts which shows α -helical structure in the middle of the protein (Figure 3.2.8). Identified cross peaks in HN-HN region are shown in the figure 3.2.9A. HN-HN, H α -HN(i, i+3), H α -HN(i, i+4), and H α -H β (i, i+3) connectivities were plotted from the assigned NOESY spectrum (Figu3.2.9B) also supports α -helical formation in the C-terminus.

Figure 3.2.8: Secondary structure prediction using H α chemical shifts.

Figure 3.2.9: (A) Assignment of cross-peaks in the NOESY spectrum of H_{85-104} . Figure A shows NOESY cross-peaks in the HN-HN region of the spectrum. Peak picking was done in Sparky 3.110 software and cross peaks were identified based on TOCSY and NOESY spectrum. (B) The NOESY connectivity plot of peptide H_{85-104} indicative of the residues connected in space revealing the presence of helical structure in the middle of the peptide (residues 90-100).

3.2.5.2 Structure calculation of H_{85-104}

Structure calculation was carried out by Cyana 2.1 package (GÜNTERT *et al.*, 1997). Distance restraints were obtained from the assigned 2D NOESY spectra. Pseudo atom corrections were considered when stereo specific assignments could not be made. In the helix region, the values of ϕ dihedral angles were given as $60^\circ \pm 30$. In disordered regions the ϕ angles

Figure 3.2.10: Superimposition of 20 lowest energy NMR structures (A) and an average structure (B) of H_{85-104} calculated by the program CYANA (GÜNTERT *et al.*, 1997). The figures (C) and (D) show the molecular surface electrostatic potential of peptide H_{85-104} generated by Pymol, where the positive potentials are drawn in blue and negative in red.

were constrained between -180° to -20° to maintain good stereochemistry of the calculated structures. Out of the 100 structures generated, 20 lowest energy structures were taken for further analysis. In total, an ensemble of 20 calculated structures resulted in an overall r.m.s.d. of 0.45 Å for the backbone atoms and 1.67 Å for the heavy atoms. All these structures have energies lower than $-100 \text{ kcal mol}^{-1}$ and there are no NOE violations greater than 0.3 Å and no dihedral violations greater than 5° . The summary of the statistics for 20 structures are shown in table 3.2.1. The calculated structure displays a flexible N- and C-terminal region (residues 85-89, residues 101-104 respectively) and an α -helical region (residues 90-100) as shown in figure 3.2.10A - B. Molecular surface electrostatic potential of the peptide is shown in figure 3.2.10C - D. The charge distribution of the peptide is amphiphilic, with the positive charge (K90, K92 and K97) spreads on one side while the hydrophobic surface on the other side, formed by the residues I88, I93, L94 and I101. The only negatively charged amino acid E100 is at the hydrophobic side of the helix.

Total number of residues	20
Total number of NMR restraints	164
Intraresidual ($ i-j =0$)	33
Sequential ($ i-j =1$)	49
Short range ($ i-j \leq 1$)	82
Medium-range ($2\leq i-j \leq 5$)	31
Long-range ($ i-j > 5$)	0
Dihedral angle constraints	31
Ramachandran plot statistics (%)	
Residues in most favoured regions	85.0
Residues in additionally allowed regions	15.0
Residues in generously allowed regions	0
Residues in disallowed region	0
Structural precision for well ordered region	
r.m.s.d. backbone (residues 90–100)	0.45 Å
r.m.s.d. heavy atoms (residues 90–100)	1.67 Å

Table 3.2.1: Structural statistics for H₈₅₋₁₀₄

3.2.6 Examination of disulfide bond formation by cross linking and mutagenesis of subunit H from *M. jannaschii*

In this study, I have focused further on the C-terminus to determine the important role of specific residues to form the coiled-coil domain of subunit H. The solution structure of H₈₅₋₁₀₄ shows a helix formed by residues K90-E100. In the helix region of H₈₅₋₁₀₄, there is a triangular orientation of residues I93, L96 and L98 (Figure 3.2.11). In order to go into details, I have made three mutants of subunit H (H_{I93C}, H_{L96C} and H_{L98C}), where in each case a cysteine residue is introduced in place of I93, L96 and L98 respectively. This will nicely allow to analyze different inter subunit disulfide pattern as well as the proximity of those residues in the dimeric subunit H.

Figure 3.2.11: NMR solution structure of H₈₅₋₁₀₄ showing different orientations of three residues I93, L96 and L98 (A) side view, (B) top view. The positions of I93, L96 and L98 are shown in blue color.

3.2.6.1 Expression and purification of H_{I93C}, H_{L96C} and H_{L98C}

Genomic DNA of *Methanocaldococcus jannaschii* ATCC 43067D was used as template for the PCR. H_{I93C}, H_{L96C} and H_{L98C} was successfully cloned in modified pET9-d1 expression vector (GRÜBER *et al.*, 2002) as described in section 2.2.2. Expression was carried in routinely used host BL21 (DE3) *E. coli* strains. Screening tests pointed out that the protein is produced in high quantity and quality, when expressed in BL21 (DE3) *E. coli* strains at 37 °C for 4 h with 1 mM IPTG. Mutant proteins were purified as described under section 2.2.3.2. Ni²⁺-NTA affinity chromatography was used in the first step of purification. Distinct bands of proteins were identified in the fractions 150 to 400 mM on SDS gel (Figure 3.2.12). To remove residual minor contaminations and imidazole, protein was additionally applied onto an anion-exchange column RESOURCETM Q followed by a Superdex 75 HR 10/30 column. Prior to injecting on the RESOURCETM Q column, fractions were diluted appropriately in 50 mM Tris/HCl, pH 7.5 buffer to produce a final NaCl concentration less than 100 mM. The column the flow rate was kept low (1 ml/min) to allow binding of the protein to the matrix.

Figure 3.2.12: Expression and purification of subunit H mutants, H_{I93C}, H_{L96C} and H_{L98C}.

Linear gradient of buffer A (50 mM Tris/HCl, pH 7.5) and buffer B (50 mM Tris/HCl, 1 M NaCl, pH 7.5) were used for the elution of the protein. Appropriate fractions were collected and then again applied to a Superdex 75 HR 10/30 column. Fractions of constant volumes (200 μ l) were collected and applied onto an SDS gel. Then, the fractions were pooled, concentrated and subsequently applied onto an SDS gel (Figure 3.2.12) to check the purity and quantity of the recombinant protein.

3.2.6.2 Dimerization interactions of subunit H mutants

To analyze the proximity of I93, L96 and L98 residue of the first and second α -helices of the dimeric H subunit, a cysteine residue is introduced in place of these residues generating the mutants H_{I93C}, H_{L96C} and H_{L98C} of the entire subunit H. Cross-link formation in the absence and in presence of CuCl₂ (1 μ M) is shown in figure 3.2.13. In the case of mutant H_{I93C}, H_{L96C} ~15% of the protein formed a dimer. In contrast, when the residue L98, located at the opposite site of helix in H₈₅₋₁₀₄ structure (Figure 3.2.11) was exchanged by a cysteine residue, 100% of the protein formed dimer. Almost there is no difference when the mutant proteins were incubated in the absence and presence of oxidizing agent.

Figure 3.2.13: Cross-linking of the H_{I93C}, H_{L96C} and H_{L98C} and subunit H using CuCl₂. Protein were incubated with 1 μ M CuCl₂ for 30 min at 4 °C. The reaction was stopped by the addition of EDTA. The samples were applied to a 17% total acrylamide and 0.4% cross-linked acrylamide gel. Lanes: 1, protein marker; 2, 3, 4 and 5, untreated proteins H_{I93C}, H_{L96C} and H_{L98C} and subunit H respectively; 6, blank; 7, 8, 9 and 10, treated proteins H_{I93C}, H_{L96C} and H_{L98C} and subunit H respectively.

3.2.6.3 CD spectroscopy of subunit H mutants

CD spectra of the mutant proteins (Figure 3.2.14) shows minima at Θ 222 and Θ 208 and maxima at Θ 192, indicative of significant α -helical contents of the proteins. 2 mg/ml protein concentrations were used for the measurement of CD spectra from 180-260 nm. The details of the measurements have been described in the section 2.2.5. H_{I93C} and H_{L96C} mutants showed almost identical spectrum, suggesting the helical content and nature of helix interactions is not changed due to mutations. In contrast, there is a decrease in absorption at 208 and 222 nm for

H_{L98C} mutant in comparison with H_{I93C} and H_{L96C} mutants. The result indicate that a slight decrease in the fraction of residues in helical content for H_{L98C}.

Figure 3.2.14: CD Spectroscopy of subunit H mutants, H_{I93C} (blue), H_{L96C} (brown) and H_{L98C} (green). CD spectrum of mutant proteins were measured in a buffer (50 mM Tris, 250 mM NaCl, pH 7.5) at room temperature in a Hellma quartz cuvette (60 μ l volume) on Chirascan spectropolarimeter (Applied Photophysics) at a step resolution of 1 nm in the far UV range of the spectrum.

3.2.7 NMR study of the N-terminal domain of subunit H (H₁₋₄₇)

Subunit H of A₁A_O ATP synthase from *M. jannaschii* has a boomerang like shape in solution as determined from SAXS data (BIUKOVIĆ *et al.*, 2007). It is divided into two arms 120 and 60 Å in length. Now, I focused on determining the regions of the N-terminal arm of subunit H. In this context, H₁₋₄₇ construct was prepared by Dr. Goran Biuković, which was used for the structure determination by NMR spectroscopy. Since subunit H is proposed to be a part of the peripheral stalk of the A₁A_O ATP synthase due to its boomerang shape, this N-terminal construct H₁₋₄₇ will enable us to determine the possible neighboring subunit(s) in the complex.

3.2.7.1 Resonance assignments of H₁₋₄₇

The crucial step to analyze protein by NMR spectroscopy is the process of sequential assignment. Assignments of amino acids in the N-terminal part of H₁₋₄₇ were performed by backbone ¹⁵N and ¹³C signals using ¹⁵N NOESY-HSQC as well as triple resonance backbone experiments (HNCACB, CBCA(CO)NH). The C ^{α} H, C ^{β} H proton resonances and some of the side chain methyl resonances were assigned from the ¹⁵N-edited NOESY-HSQC. An example of the sequential assignment for the continuous residues (from Met₁ to Lys₁₀) is shown in figure 3.2.15. The backbone amide (¹⁵N and ¹H_N) resonances for 47 possible residues were completely assigned. The assignments of the resolved backbone residues of H₁₋₄₇ are presented on a 2D ¹H-

^{15}N heteronuclear single quantum correlation (HSQC) spectrum (Figure 3.2.15B). Overall the limited chemical shift dispersion of only 1.5 ppm for the amide proton resonances may indicate predominantly helical conformation of H_{1-47} .

Figure 3.2.15: (A) Strip plots from the HNCACB (top) and ^{15}N NOESY-HSQC (bottom) spectra of H_{1-47} . Sequential $\text{C}\alpha$, $\text{C}\beta$ and $\text{H}\alpha$ connectivity for the 1st 10 residues M1 - K10 are shown in lines. (B) $^1\text{H}/^{15}\text{N}$ -HSQC spectrum of H_{1-47} in 25 mM sodium phosphate buffer (pH 6.5) at 288 K. Backbone and side-chain (sc) amide assignments are shown for each residue. Signals from side-chain NH_2 groups are connected by horizontal lines (GAYEN *et al.*, 2008).

3.2.7.2 Solution structure of the N-terminal domain H_{1-47}

The three dimensional NMR structure of subunit H_{1-47} was calculated based on a total of 468 unequivocally assigned NOEs-derived distance constraints including 133 intraresidual, 180 sequential and 155 medium range intermonomeric NOEs. Figure 3.2.16 shows 10 lowest energy conformers of H_{1-47} superimposed using backbone atoms of residues 15-42 and statistics is shown in table 3.2.2. The NOE patterns together with the chemical shift index (CSI) reveal a helical region in the middle of the protein. CSI consist of a single helix in middle of protein with some flexible residues (M1 to M6 and L44 to C47) at both terminus as shown in figure 3.2.17. The analysis of the ramachandran plots shows 81.5 % residues in the most favored regions, 18.5 % are in the additionally allowed regions. The regular α -helical conformation was detected for residues A15 to K42 with a small kink in the centre of the molecule. In the solution structure, H_{1-47} has a total length of about 60 Å, including the α -helical structure of 43 Å in length. Figure

3.2.18 shows a bar diagram summarizing the backbone and the backbone/sidechain NOE connectivities of H₁₋₄₇.

Figure 3.2.16: Ribbon diagram of the NMR solution structure of H₁₋₄₇. (A) best fit superimposition of the 10 lowest-energy NMR structures (B) average structure of H₁₋₄₇ (BIUKOVIĆ *et al.*, 2009).

Total number of NMR restraints	526
Intraresidual ($ i-j =0$)	133
Sequential ($ i-j =1$)	180
Short range ($ i-j \leq 1$)	313
Medium-range ($2\leq i-j \leq 5$)	155
Long-range ($ i-j > 5$)	0
Dihedral angle constraints	58
Ramachandran plot statistics (%)	
Residues in most favoured regions	81.5
Residues in additionally allowed regions	18.5
Residues in generously allowed regions	0
Residues in disallowed regions	0
Structural precision for well ordered region	
r.m.s.d. backbone (residues 15–42):	0.95 Å
r.m.s.d. backbone (residues 15–33):	0.68 Å
r.m.s.d. backbone (residues 33–42):	0.61 Å

Table 3.2.2: Statistics for the 10 final structural models of H₁₋₄₇ (BIUKOVIĆ *et al.*, 2009)

Figure 3.2.17: The amino acid sequence of H₁₋₄₇ and the secondary structure elements, based on ¹³Cα chemical shifts with respect to its random coil values (GAYEN *et al.*, 2008).

Figure 3.2.18: Summary of structurally important intra monomer NOESY connectivities of H₁₋₄₇ (BIUKOVIĆ *et al.*, 2009).

3.2.7.3 Concentration dependence of ¹H, ¹⁵N chemical shifts and ¹⁵N relaxation parameters for H₁₋₄₇

The change of ¹H and ¹⁵N chemical shifts was monitored through ¹H ¹⁵N HSQC experiments. The spectra are taken as a function of concentration ranging from 0.125 to 3 mM.

An increase in concentration of H₁₋₄₇ results in an overall change in the HSQC spectrum (Figure 3.2.19). The variation of the cumulative ¹H and ¹⁵N shifts at highest and lowest studied concentrations is shown in figure 3.2.20. Since only one set of peaks is observed for all residues in the ¹H-¹⁵N HSQC spectrum, the resonances must be average values corresponding to monomeric H₁₋₄₇ and the oligomeric H₁₋₄₇ in fast exchange in the chemical shift time scale. The largest shifts observed for the continuous N-terminal stretch of residues comprising M6 to A19, where structural consolidation upon dimer formation is observed by ¹⁵N relaxation data. The results showed that there are several important residues affected by the formation of dimer, *e.g.* A19, I23, E24, A26, K27, Q32, I33, K34, E36, A37 and E39.

Figure 3.2.19: ¹H-¹⁵N HSQC spectra of H₁₋₄₇ at 10 °C in 25 mM phosphate buffer, pH 6.5 at highest (red) and lowest (blue) concentrations (BIUKOVIĆ *et al.*, 2009).

The relaxation rates in proteins are very much sensitive to overall rotational diffusion properties as well as shape and size of the protein molecule in solution. Under fast exchange conditions between monomeric and dimeric states, ¹⁵N relaxations rates become powerful reporters of the

Figure 3.2.20: Combined amide (^1H) and nitrogen (^{15}N) chemical shift changes ($[(\Delta^1\text{H}_\text{N})^2 + (\Delta^{15}\text{N}_\text{ppm}/6.51)^2]^{0.5}$) for H_{1-47} at concentrations between 3 and 0.125 mM. Orange colored bars represent the residues forming the dimeric interface of H_{1-47} . Charged residues exhibiting large shifts, which are not directly situated at the dimeric interface, are marked in cyan (BIUKOVIĆ *et al.*, 2009).

average rotational diffusion properties of the contributing oligomeric states. Thus, ^{15}N R_1 and R_2 relaxation rates and ^1H - ^{15}N NOE were measured in order to provide direct estimate of backbone mobility in monomeric as well as in the oligomeric forms of H_{1-47} . Generalized order parameters (S^2), internal correlation times (τ_e), and conformational exchange-induced line broadening (R_ex) were calculated from these data by the Lipari and Szabo model free approach. S^2 order parameters reflect spatial restriction of ^1H - ^{15}N bonds on the ps to ns time scale, and range from 0 for unconstrained motion to 1 for completely rigid proteins. Figure 3.2.21 shows the obtained ^{15}N R_1 and R_2 relaxation rates and ^1H - ^{15}N NOE as well as the generalized order parameters obtained in the Lipari-Szabo model free analysis. The corresponding model free statistics are listed in table 3.2.3. With the exception of the terminal residues, the S^2 values for H_{1-47} at high and low concentrations are rather uniform, ranging between 0.66 and 0.85. The resulted order parameters are shown in figure 3.2.21D. Their magnitude is characteristic of well-folded proteins, demonstrating that fast internal motions are limited for residues 15 to 42. The τ_e values are in the picosecond range, as it is typically found in well-folded proteins. Within the model of the isotropic rotational

Figure 3.2.21: (A) ^{15}N R_1 , (B) R_2 relaxation rates, (C) ^1H - ^{15}N heteronuclear NOEs for the H_{1-47} at 10 °C and (D) Lipari-Szabo order parameter S^2 versus residue number at 3 mM (brown) and 0.125 mM (blue) concentration of H_{1-47} (BIUKOVIĆ *et al.*, 2009).

diffusion, the rotational diffusion properties are contained in a single characteristic time constant, the rotational correlation time. Almost quantitative doubling of the rotational correlation time from 16 ns to 25 ns for the protein between high and low concentrations unequivocally establishes dimerization process. The latter is accompanied by structural consolidation at the region 4-21 as evidenced by the increase of the corresponding order parameters.

Res	S^2	S^2_{err}	τ_c	$\tau_{c\text{err}}$	R_{ex}	Res	S^2	S^2_{err}	τ_c	$\tau_{c\text{err}}$	R_{ex}
1	0.134	0.026	1303.000	33.406	4.177	1	0.169	0.024	1472.200	66.548	1.631
2	0.162	0.029	1223.900	28.266	0.000	2	0.155	0.021	1223.300	20.914	2.399
3	0.207	0.029	1112.100	22.296	0.000	3	0.216	0.027	1373.100	50.169	0.000
4	0.274	0.038	1260.300	45.214	0.000	4	0.343	0.034	1214.400	29.979	0.173
5	0.472	0.053	1141.100	57.016	0.003	5	0.397	0.039	1248.600	37.605	1.032
6	0.436	0.102	1001.900	88.053	0.000	6	0.496	0.047	1264.700	48.225	0.488
7	0.47	0.048	1060.200	45.824	0.337	7	0.504	0.045	1186.500	40.782	0.542
8	0.488	0.054	1001.500	50.128	0.004	8	0.526	0.048	1232.500	51.168	0.127
9	0.583	0.052	1007.000	70.816	0.227	9	0.532	0.042	1215.800	46.885	0.143
10	0.572	0.091	1011.500	141.310	0.000	10	0.601	0.053	1200.600	57.916	3.658
11	0.608	0.084	972.200	147.930	0.000	11	0.605	0.049	1443.200	102.780	1.097
12	0.528	0.092	1042.000	103.510	0.000	12	0.618	0.045	1478.300	118.440	0.212
13	0.504	0.101	1127.100	142.950	0.000	13	0.582	0.06	1425.500	96.525	0.000
14	0.536	0.092	1157.800	133.480	0.000	14	0.512	0.072	1448.200	90.142	0.000
15	0.751	0.056	926.890	223.230	0.317	15	0.639	0.044	1409.000	106.190	1.253
16	0.666	0.062	1184.400	167.780	1.067	16	0.648	0.046	1787.500	173.450	0.019
17	0.767	0.056	896.630	251.050	0.283	17	0.667	0.045	1540.400	150.020	0.001
18	0.772	0.053	929.980	224.850	0.236	18	0.683	0.043	1587.300	169.450	0.155
19	0.762	0.065	1062.500	206.240	0.000	19	0.688	0.046	1774.000	176.600	4.004
20	0.793	0.048	967.600	264.160	0.000	20	0.714	0.041	1488.300	158.610	0.586
21	0.751	0.055	1099.000	232.180	0.077	21	0.651	0.047	1708.100	177.380	0.635
22	0.816	0.056	1051.100	313.210	0.000	22	0.722	0.044	1527.900	158.620	0.844
23	0.766	0.062	1225.900	281.630	0.000	23	0.666	0.041	1425.800	100.070	0.000
24	0.796	0.06	1260.200	310.540	0.000	24	0.695	0.047	2000.000	159.520	0.119
25	0.752	0.061	1081.400	193.460	0.000	25	0.677	0.038	1791.600	173.290	0.063

Table 3.2.3: Model free parameters for H₁₋₄₇ at 0.125 and 3 mM concentration (BIUKOVIĆ *et al.*, 2009).

26	0.793	0.053	940.080	271.890	1.897	26	0.72	0.044	1718.300	195.590	0.843
27	0.882	0.041	1123.500	532.840	0.002	27	0.645	0.041	2000.000	140.460	1.682
28	0.798	0.054	1075.200	278.350	2.579	28	0.703	0.046	2000.000	156.440	1.131
29	0.817	0.052	1341.500	406.630	1.592	29	0.647	0.047	1430.000	108.780	0.388
30	0.786	0.052	1296.000	292.560	0.055	30	0.679	0.045	2000.000	146.660	0.186
31	0.891	0.042	1071.300	577.200	0.675	31	0.729	0.04	2000.000	167.060	0.089
32	0.82	0.052	1256.400	368.020	1.430	32	0.691	0.046	2000.000	161.530	1.481
33	0.735	0.056	1513.500	277.440	0.068	33	0.675	0.047	1927.000	160.250	0.015
34	0.843	0.046	1039.500	406.890	3.867	34	0.708	0.046	1444.100	135.590	2.871
35	0.735	0.067	1310.400	242.620	0.000	35	0.689	0.044	1745.100	178.170	0.391
36	0.734	0.054	1277.900	197.970	0.488	36	0.668	0.041	1920.200	151.870	0.156
37	0.696	0.051	1251.600	177.220	2.933	37	0.676	0.044	2000.000	140.410	0.215
38	0.753	0.055	1052.600	205.080	0.398	38	0.668	0.048	1773.000	184.110	0.087
39	0.805	0.052	1012.700	296.740	2.365	39	0.674	0.045	2000.000	147.930	0.284
40	0.729	0.054	1048.700	163.420	0.013	40	0.648	0.044	1787.500	172.180	0.019
41	0.72	0.058	1195.900	202.990	0.004	41	0.65	0.048	2000.000	151.380	0.481
42	0.609	0.056	1250.600	110.450	0.018	42	0.601	0.045	1951.400	140.950	0.486
43	0.56	0.055	1197.500	85.930	0.147	43	0.555	0.043	1739.100	152.460	0.288
44	0.407	0.047	1191.200	47.663	1.879	44	0.332	0.041	1488.300	88.449	0.000
45	0.299	0.04	1099.200	31.582	0.000	45	0.333	0.033	1114.000	17.067	2.376
46	0.134	0.023	829.590	22.395	0.000	46	0.148	0.019	827.740	17.820	0.000
47	-	-	-	-	-	47	0.052	0.008	556.380	29.833	0.000

Table 3.2.3 (continued): Model free parameters for H₁₋₄₇ at 0.125 and 3 mM concentration (BIUKOVIĆ *et al.*, 2009).

3.2.7.4 Measurement of diffusion coefficients

The ¹⁵N relaxation measurements as well as the corresponding model free analysis demonstrated two times increase of the rotational correlation time of H₁₋₄₇ with the increase of its concentration from 0.125 to 3 mM, directly demonstrating its dimeric state at concentrations higher than 3 mM. To estimate the dimerization equilibrium constant we have performed NMR pulsed field gradient (PFG) diffusion experiments in a dilution series, revealing the equilibrium between monomeric and dimeric states of H₁₋₄₇. The results clearly indicate that H₁₋₄₇ diffuses significantly faster at low concentration and the monomer-dimer equilibrium of H₁₋₄₇ exists at a

protein concentration < 3mM. We have also determined the diffusion coefficients for the constructs, H₁₋₆₁, H₁₋₆₉ and H₁₋₈₀ at different concentrations (shown in figure 3.2.22A-D). In all of the cases, the decrease in the experimentally determined diffusion coefficient with the increasing concentration suggests the dimer formation of the proteins. The results indicate that H₁₋₆₁, H₁₋₆₉ and H₁₋₈₀ have a higher tendency to form dimer than H₁₋₄₇. The diffusion coefficient of H₁₋₄₇, H₁₋₆₁, H₁₋₆₉ and H₁₋₈₀ were measured as function of protein concentration. Solution for NMR analysis was prepared by dissolving the proteins in 50 mM Tris, 250 mM NaCl. The dimer dissociation constant for H₁₋₄₇ was estimated by direct fit of translation diffusion velocities. H₁₋₆₁, H₁₋₆₉ and H₁₋₈₀ show a stronger tendency of dimer formation.

Figure 3.2.22: Change of diffusion coefficient against concentration for H₁₋₄₇ (A), H₁₋₆₁ (B), H₁₋₆₉ (C) and H₁₋₈₀ (D), respectively (BIUKOVIĆ *et al.*, 2009).

3.3 Interaction of subunit H with subunit E and the NMR solution structure of the N-terminus E₁₋₅₂ of A₁A₀ ATP synthase from *Methanocaldococcus jannaschii*

3.3.1. Introduction

Single particle analysis of the A₁A_O ATP synthase has revealed two peripheral stalks, and a collar-like structure (BERNAL *et al.*, 2004; COSKUN *et al.*, 2004; VONCK *et al.*, 2009). The most likely candidates are the subunits H, E and *a*, respectively (SCHÄFER *et al.*, 2006b; GRÜBER *et al.*, 2008). Both the peripheral stalks and the collar-like structure are involved in constituting the stator region of the enzyme, which is believed to have important roles in enzyme assembly as well as energy coupling between A₁ and A_O (GRÜBER, 2003). Subunit H has a boomerang-like shape, which is divided into two arms of 120 Å and 60 Å in length (BIUKOVIĆ *et al.*, 2007). An NMR solution structure of the 60 Å long N-terminal arm, composed of the N-terminal residues 1-47 (H₁₋₄₇), has been solved, revealing an α-helical feature (BIUKOVIĆ *et al.*, 2009). The crystallographic structure of the C-terminal part (residues 81 -198) of subunit E of the A-ATP synthase from *Pyrococcus horikoshii* OT3 has been reported, showing that the C-terminal domain of E consists of four antiparallel β-strands and six α-helices (LOKANATH *et al.*, 2007). The goal of this work was to analyze the interaction between H₁₋₄₇ and the N- as well as C-terminal domain of the proposed neighboring subunit E. In the crystal structure the N-terminal domain of subunit E was not observed (LOKANATH *et al.*, 2007). In order to go into details of the N-terminal domain of subunit E, the construct E₁₋₅₂, containing the N-terminal 52 residues, of the A₁A_O ATP synthase from *M. jannaschii* was designed for NMR structural studies. The solution structure of E₁₋₅₂ has been solved using nuclear magnetic resonance spectroscopy.

3.3.2. NMR titration of H₁₋₄₇ with the N- and C-terminus of E subunit

NMR titration experiments using ¹H-¹⁵N HSQC spectra were used to characterize the interactions between subunit H₁₋₄₇ and the two segments, E₁₋₁₀₀ and E₁₀₁₋₂₀₆ from *M. jannaschii*, respectively. The subunit E constructs (E₁₋₁₀₀ and E₁₀₁₋₂₀₆) have been cloned and purified by Dr. Asha Balakrishna of our laboratory. Two sets of titrations were performed: HSQC spectra of ¹⁵N labeled H₁₋₄₇ at a fixed concentration of 100 μM, were recorded in the absence or in the presence of increasing amounts of unlabeled E₁₋₁₀₀ and E₁₀₁₋₂₀₆ separately. Figure 3.3.1A shows the complete and figure 3.3.1B shows sections from the overlaid 2D ¹H-¹⁵N HSQC spectrum of the truncated form H₄₇ (shown in blue) and the H₄₇ - E₁₋₁₀₀ complex (shown in red), recorded in the presence or absence of E₁₋₁₀₀. A number of residues show significant chemical shift perturbations upon binding of ¹⁵N labeled H₁₋₄₇ to E₁₋₁₀₀. As can be seen, there is strong chemical shift change in the N-terminal region of the primary sequence of H₄₇ (residues 1-6, 10-11, 15, 20 and 24). By

comparison, when equimolar amounts of the C-terminal domain of subunit E, E₁₀₁₋₂₀₆, was added to H₁₋₄₇ no significant change in the spectrum could be detected (Figure 3.3.2A).

(A)

(B)

(C)

Figure 3.3.1: (A) Overlay of 2D ^1H - ^{15}N -HSQC spectra of H₁₋₄₇ in the absence and presence of 1.5 equivalents of unlabelled E₁₋₁₀₀. (B) Sections from the overlaid spectra of H₁₋₄₇ in the absence (red) and presence (black) of 1.5 equivalents of unlabelled E₁₋₁₀₀. (C) Gel picture showing H₁₋₄₇ (lane 1), E₁₋₁₀₀ (lane 2) and E₁₀₁₋₂₀₆ (lane 3) (GAYEN *et al.*, 2008).

Figure 3.3.2: (A) Overlay of 2D ^1H - ^{15}N -HSQC spectra of H₁₋₄₇ in the absence (*red*) and presence (*black*) of 1.5 equivalent of E₁₀₁₋₂₀₆. (B) Overlay of 2D ^1H - ^{15}N -HSQC spectra of H₁₋₄₇ in the absence (*red*) and presence (*black*) of 1.5 equivalent of E₄₁₋₆₀ peptide (GAYEN *et al.*, 2008).

In order to map the region of E₁₋₁₀₀ involved in the interaction with H₁₋₄₇, the latter has been titrated with the peptides $_1\text{MKLMGVDKIK-SKILDDAKAE}_{20}$ (E₁₋₂₀), $_{21}\text{ANKIISEAEA-EKAKILEKAK}_{40}$ (E₂₁₋₄₀) and $_{41}\text{EEAEKRKAEL-LKKGEKEAEM}_{60}$ (E₄₁₋₆₀), which are synthesized and purified at the Division of Chemical Biology and Biotechnology, School of Biological Sciences, Nanyang Technological University, Singapore. The HSQC spectrum of ^{15}N labeled H₁₋₄₇ in the presence of the peptide E₄₁₋₆₀ shows chemical shift changes of the N-terminal amino acids (Figure 3.3.2B), similar to the ones observed in the presence of the entire E₁₋₁₀₀ domain (Figure 3.3.1A). In contrast, no change of the spectra were observed in NMR-experiments in which ^{15}N labeled H₁₋₄₇ was supplemented with the peptides E₁₋₂₀, E₂₁₋₄₀, respectively.

3.3.3. NMR titration of subunit F and the C-terminus E₁₀₁₋₂₀₆

The solution structure of subunit F of the methanogenic A₁A₀ ATP synthase using NMR, exhibits a distinct two-domain structure, with a globular N-terminal having 78 residues and the residues 79-101 forming the C-terminal tail (GAYEN *et al.*, 2007). The N-terminal domain of F is proposed to be close to the bottom of the rotary D subunit (GRÜBER *et al.*, 2008), which is indicated to be in close proximity with the C-terminal part of subunit E (GRÜBER *et al.*, 2008). Based on this, we examined the ability of interaction of subunit F and E₁₀₁₋₂₀₆ by NMR titration

experiments. When E₁₀₁₋₂₀₆ was titrated against the labeled subunit F, no significant chemical shift changes could be observed, indicating that there is no interaction between both proteins.

Figure 3.3.3: Overlay of 2D ¹H-¹⁵N-HSQC spectra of F subunit in the absence (*red*) and presence (*black*) of 1.5 equivalent of unlabeled E₁₀₁₋₂₀₆. The spectrum was collected in a Bruker Avance 600 MHz spectrometer in 25 mM sodium phosphate buffer (pH 6.5) at 288 K (GAYEN *et al.*, 2008).

3.3.4. NMR solution structure of N-terminus of subunit E (E₁₋₅₂) of A₁A₀ ATP synthase from *M. jannaschii*

3.3.4.1 Resonance assignment of E₁₋₅₂

Assignment for the backbone HN, N, C^α, C^β and C' resonances of the E₁₋₅₂ was achieved by a combined analysis of the triple resonance HNCACB, CBCA(CO)NH, and HNCO spectra. Figure 3.3.1 shows the good quality of the assigned HSQC spectrum indicating backbone HN and ¹⁵N cross peaks of all residues of the E₁₋₅₂. Overall, the limited chemical shift dispersion of only ~2.0 ppm for the amide proton resonances (Figure 3.3.4) may indicate predominantly helical conformations of E₁₋₅₂ as shown for other helical proteins (GIRVIN *et al.*, 1998; TAYLOR *et al.*, 2000).

Figure 3.3.4: ^1H - ^{15}N HSQC spectrum of subunit E₁₋₅₂ of the A₁A₀ ATP synthase from *M. jannaschii* in 25 mM sodium phosphate buffer (pH 6.5) at 308 K. Backbone and side chain (sc) amide assignment is shown as line for residue N₂₄. (Insert) SDS gel showing the sample of purified E₁₋₅₂ (GAYEN *et al.*, 2009).

3.3.4.2 NMR derived 3-dimensional structures of E₁₋₅₂

A total of 545 inter-proton distance restraints (169 intraresidual, 152 sequential and 146 medium range) were used in the structure calculation of E₁₋₅₂, respectively, by molecular dynamics and simulated annealing. From 100 calculated structures, 20 with the lowest total energy were selected and used for structural analysis. Figure 3.3.5A represents an overlay of the 20 lowest-energy structures of E₁₋₅₂ and the statistics are given in table 3.3.1. A bar diagram summarizing the backbone and the backbone/sidechain NOE connectivities of E₁₋₅₂ is shown in figure 3.3.6. The 20 structures have an overall r.m.s.d. of 0.44 Å for backbone atoms and 1.10 Å for all heavy atoms in the helical regions (8-48) of the three dimensional structures of the protein. Figure 3.3.5B-D shows various representation of the solution structure of E₁₋₅₂. The protein adopts a regular α -helical conformation extending from residues V8 to R48. The helical conformation is not straight rather its N-terminal part (residues V8 – I26) and C-terminal part (I27 – R48) are slightly bend at opposite directions (Figure 3.3.5B-D). NH secondary chemical shifts of E₁₋₅₂, showing a 3-4 residue periodicity (Figure 3.3.7), support the existence of helix curvature consistent with many amphipathic and/or curved α -helices (WAGNER *et al.*, 1983; KUNTZ *et al.*, 1991; ZHOU *et al.*, 1992; NIELSEN *et al.*, 1996).

Figure 3.3.5: Ribbon diagram of the NMR structure of E₁₋₅₂. (A) Best fit superimposition of the 20 lowest-energy NMR structures. Side views (B), (C) and top view (D) of the average structure of E₁₋₅₂ (GAYEN *et al.*, 2009).

The curvature of helix is caused by the differences in the H-bonding on opposite faces of the helix. Short H-bonds are correlated with low-field shifts, observed for residues K10, K14,

Total number of NMR restraints	545
Intraresidual ($ i-j =0$)	169
Sequential ($ i-j =1$)	152
Short range ($ i-j \leq 1$)	321
Medium-range ($2\leq i-j \leq 5$)	146
Long-range ($ i-j > 5$)	0
Dihedral angle constraints	78
Total number of restraint violations $> 0.3\text{ \AA}$	0
Ramachandran plot statistics (%)	
Residues in most favoured regions	91.4
Residues in additionally allowed regions	8.6
Residues in generously allowed regions	0
Residues in disallowed regions	0
Average RMSD to mean ($\text{\AA}$)	
Backbone (residues 8–48)	$0.44 \pm 0.17\text{ \AA}$
Heavy atoms (residues 8–48)	$1.10 \pm 0.17\text{ \AA}$

Table 3.3.1: Statistics for the 20 final structural models of E₁₋₅₂ (GAYEN *et al.*, 2009).

Figure 3.3.6: Summary of structurally important NOE connectivities derived from CYANA (GÜNTERT *et al.*, 1997; GAYEN *et al.*, 2009).

Figure 3.3.7: (A) Secondary chemical shifts for NH protons for E₁₋₅₂ plotted against the residue sequence number for the E₁₋₅₂ (GAYEN *et al.*, 2009).

D17, E22, K25, E29, E33, K36, K40 and K43 and long H-bonds with shifts to higher field are observed for residues D9, K2, I15, A19, A23, I27, A30, A35, L38, A41 and A45. The highest bending angles calculated from local helix axes fitted to CA atoms of ((i-3) - i) and (i - (i+3)) is in the region of K10 - S13 using HELANAL algorithm (KUMAR *et*

al., 1996). This is further supported by the observation of $d_{\alpha N(i,i+2)}$ NOE connectivities (Figure 3.3.6) near the N-terminus of the helix D9 – K12 and N24 – K25, indicating fraying or a turn at this end of the α -helix.

All the structures have energies lower than - 4 kcal·mol⁻¹ calculated by GROMOS force field as implemented in SwissPDB viewer (GUEx *et al.*, 1997). There is no NOE violations

greater than 0.5 Å and no dihedral violations greater than 5°. Analysis of the ramachandran plots shows 91.4 % residues in the most favored regions, 8.6 % in the additionally allowed regions.

Figure 3.3.8: (A) The amino acid sequence of E₁₋₅₂ and the secondary structure elements based on $^{13}\text{C}^\alpha$ - $^{13}\text{C}^\beta$ (B) Ribbon representation of E₁₋₅₂ showing the hydrophobic amino acids colored in blue. (C, D) Surface representation of E₁₋₅₂. Red and blue represent negatively and positively charged areas, respectively, calculated with the program PyMOL (<http://www.pymol.org>) (GAYEN *et al.*, 2009).

In the solution structure, E₁₋₅₂ has a total length of 60.5 Å. $^{13}\text{C}^\alpha$ and $^{13}\text{C}^\beta$ chemical shifts depend on local backbone geometry. Their deviations from random coil values, termed secondary shifts, provide sensitive probes to detect the secondary structural propensities. The combined carbon ($\Delta^{13}\text{C}^\alpha - \Delta^{13}\text{C}^\beta$) secondary shifts of E₁₋₅₂ as a function of the protein sequence (Figure 3.3.8A) show a continuous stretch of positive combined carbon secondary shifts for almost all the residues. The middle of the protein (residues 8-48) appears to have higher positive values demonstrating the presence of a helical structure in the region. The helical domain of E₁₋₅₂

presents the structural characteristics of an amphipathic helix (Figure 3.3.8C - D) with the hydrophobic residues I11, I15, A19, A23, I26, A30, I37, A41 and A45 facing one side and the hydrophilic amino acids D9, K10, K14, D17, K20, K25, E29, E31, K36, E39, E43 and K47 making the second face of the helix.

4. Discussion

4.1 Solution structure of subunit F from *M. mazei* Gö1

High resolution crystal structures of catalytic and nucleotide binding subunits A (MAEGAWA *et al.*, 2006) and B (SCHÄFER *et al.*, 2006a) respectively, of the A-ATP synthase provides information on the nucleotide binding events in the A₁ part. However, the dual function of ATP synthesis or ATP hydrolysis in the A₁ domain and the ion transport in the A₀ domain is combined with a complicated coupling process. The central stalk subunits C, D and F play the leading roles in the coupling event. These subunits undergo conformational changes and/or rearrangements during enzyme function. Thus, in order to understand the molecular mechanism by which these subunits play their biological role, it is very much important to know their structure at atomic level. The first low resolution shape of subunit F from the archaea *Methanosarcina mazei* Gö1 in solution determined by small angle X-ray scattering has been published in 2006 (SCHÄFER *et al.*, 2006b). The shape of the protein is elongated (shown in figure 4.1.1) (7.5 nm) divided in two parts: a globular part with a length of about 4.5 nm and a hook like domain of about 3.0 nm in length (SCHÄFER *et al.*, 2006b). The structure of subunit F determined by NMR spectroscopy has similar dimensions (7.8 nm) and shape. It also reveals two well defined domains (GAYEN *et al.*, 2007). The N-terminal region forms the globular part and the C-terminus forms the hook like part. The N-terminal globular domain is made up by an alternating arrangement of four β -strands (β 1-4) and three α -helices (α 1-3). The four β -strands are oriented parallel to each other. The surface representation of subunit F indicates that the four-stranded β -sheet in the N-terminal part forms a hydrophobic surface (Figure 4.1.2). As subunit F of the A-ATP synthase is in close contact with rotating subunit D (COSKUN *et al.*, 2004), the hydrophobic surface might mediate the interaction of subunits F and D.

Consequently, the positively and negatively charged surface of the bottom of the N-terminal domain of subunit F would be oriented to the central stalk subunit C and thereby toward the membrane side. Such positioning of subunit F relative to subunit D and subunit C allows the

Figure 4.1.1: Structural model of the non-catalytic B subunit and the coupling subunit F. The atomic model of subunit B from the *M. mazei* Gö1 A₁A₀ ATP synthase derived from the X-ray coordinates determined recently (SCHÄFER *et al.*, 2006a) (PDB 2c61). The first low resolution shape of subunit F of A₁A₀ ATP synthase from *M. mazei* Gö1 was determined by solution X-ray scattering (SCHÄFER *et al.*, 2006b). The C-terminal region interacting with subunit F is labeled by sticks and balls (SCHÄFER *et al.*, 2006b).

positively charged area in the C-terminus of subunit F to interact with the negatively charged residues of the C-terminus of subunit B. Recently, the interaction of subunit F with the subunit B was proved by intrinsic tryptophan fluorescence experiment indicating a binding constant (K_d) of 332 nM, assuming that subunit B is interacting with subunit F in a 1:1 molecular ratio. Since, both subunits have one native tryptophan residue at the C-terminus, W430 and W100 for subunits B and F, respectively, the tryptophan of subunit F (W100) was substituted with tyrosine and isoleucine residue in order to measure the binding characteristic with subunit B. The fluorescence intensity dropped by 19% upon addition of mutant FW100Y with subunit B, indicating interaction between W430 of subunit B and the Y100 of mutant FW100Y. In comparison, a fluorescence quenching of only 5% was observed in case of mutant FW100I (GAYEN *et al.*, 2007). These results pointed out a π - π interaction of adenine rings of tryptophans at the C-terminus of both subunits.

Figure 4.1.2: Charge distributions on the surfaces of subunit F of the methanogenic A_1A_0 ATP synthase. Red and blue areas are negatively and positively charged areas, respectively, calculated with the program MOLMOL (KORADI *et al.*, 1996). At the left (A) the arrow shows the positively charged tail, which forms a cross-link with the C-terminus of subunit B (GAYEN *et al.*, 2007). The hydrophobic area, which is proposed to interact with the central subunit D, is emphasized by a white arrow (B). A ribbon representation of the F subunit in an orientation similar to that of the surface structure is shown in (C). (D) Surface representation of the N-terminal domain from the membrane side (GAYEN *et al.*, 2007).

The fact that the C-terminus of subunit F is flexible in comparison to the N-terminal domain was revealed by the direct measurements of backbone dynamics including R_1 , R_2 and two-dimensional ^1H - ^{15}N heteronuclear NOE experiments. The N-terminal domain (residues 1-78) showed uniform NOE, R_1 and R_2 values indicating that N-terminal domain form a rigid body. On the other hand C-terminus (residues 79-101) exhibited completely different and more

flexible dynamical properties as can be seen in smaller NOE, longer R_1 and shorter R_2 . This property makes subunit F to undergo rearrangements during energy coupling as they are described for the proposed homologue ϵ of the *E. coli* F_1F_0 ATP synthase (TSUMURAYA *et al.*, 2009). Subunits F and ϵ have the same overall appearance and domain structure (SCHÄFER *et al.*, 2006b). However, subunit F of *M. mazei* Gö1 shows a sequence identity of 9% to subunit ϵ of *E. coli* F_1F_0 ATP synthase and the secondary and tertiary structures of subunit ϵ are also different from subunit F. Figure 4.1.3 shows structures of subunit ϵ from *E. coli* F_1F_0 ATP synthase and subunit F. The N-terminal domain of subunit ϵ forms a β sandwich with six stranded sheets (β_1 -6) and the C-terminal domain formed by two helices (α_1 -2) arranged in a anti parallel coiled-coil structure (UHLIN *et al.*, 1997). Studies on isolated subunit ϵ and in complex with the truncated γ subunit revealed, that the C-terminal domain of subunit ϵ is also flexible and can undergo the rearrangement between two different conformations – the closed / hairpin-folded and extended states (WILKENS *et al.*, 1995; RODGERS *et al.*, 2000). The conformations of this subunit could reversibly change between two states depending on the ATP concentration (YAGI *et al.*, 2007). The C-terminus of subunit ϵ extends along the axial helices of subunit γ in the extended state and inhibits the conformational change in subunit β and thereby inhibits the clockwise rotation of subunit γ (YAGI *et al.*, 2007). As a result, both ATP synthesis and hydrolysis are suppressed. On the other hand, when the C-terminus fold back to the hairpin-folded state by an arm like motion, subunit ϵ does not have any direct interaction with the $\alpha_3\beta_3$ ring (YAGI *et al.*, 2007) and thereby has no effect on the coupling process.

Figure 4.1.3: Ribbon representation of subunit ϵ of the F_1F_0 ATP synthase from *E. coli* (A) (PDB code 1AQT; (UHLIN *et al.*, 1997)) and subunit F of the A_1A_0 ATP synthase from *M. mazei* Gö1 (B) (PDB code 2OV6; (GAYEN *et al.*, 2007)).

In a similar way, the structure of F_{81-101} displays a closed form compared to the extended form in the whole subunit F. The structure revealed that there is a short flexible N-terminal tail of the amino acids G81 – T85 and a helical structural motif formed by the residues S86 to A94. To investigate the stability of this closed form of the C-terminal end of subunit F and of the elongated form determined previously, 100 ns molecular dynamics (MD) simulation was performed on both structures using the continuum representation of bulk solvent water (RAGHUNATHAN *et al.*, 2009). The results reveal that there is an almost two-fold higher mobility among the residues 86-94 when elongated than when closed and helical. In addition, the solution structure of F_{81-101} reveals two cation- π interactions, one is between conserved R88 and W100 and the second is between K92 and W100. The distance between the pairs R88-W100 and K92-W100 was monitored during the simulations to check for the presence of cation- π interactions. The results demonstrate that the separation between R88-W100 and K92-W100 varies between 5-15 Å for the retracted and elongated states. Together, these observations highlight the intrinsic flexibility of the C-terminus, which in turn enables inter-conversions between the elongated and the closed states with a periodicity of ~30 ns (RAGHUNATHAN *et al.*, 2009). The flexible C-terminal tail enables the subunit F to undergo up and down movements relative to its neighboring nucleotide binding subunit B and functions as a coupling element by connecting the nucleotide-binding subunit with the ion-translocating part. The fact that the conformational change in the C-terminal domains of these proteins is not surprising, since similar kind of conformational change was also observed for Ras-related nuclear (Ran)-GTPase proteins (LEE *et al.*, 2005; HAO *et al.*, 2008) during nuclear protein import (Figure 4.1.4). Its C-terminal extension, consisting of an unstructured linker and a 16 residue α -helix, contacts the core of the protein in Ran-GDP state and is pointing away from the core in Ran-GTP state, thereby playing a crucial role of its binding to the transport factors. Taken together, these facts suggest that this kind of conformational change may be a general form of rearrangements in the family of hydrolases and or synthases.

Based on these observations, subunit F in its elongated form was tried to fit into the A_1 complex. Figure 4.1.5 shows a nice fitting of structure of subunit F inside the complex, and suggests a possible mechanism of the coupling function in the elongated state of subunit F. It is reported previously that subunit F formed a cross-linked product with subunit B inside the methanogenic A_3B_3DF subcomplex (COSKUN *et al.*, 2002), A_1 ATPase (COSKUN *et al.*, 2004), and A_1A_O ATP synthase (SCHÄFER *et al.*, 2006b), depending on whether MgATP or the nonhydrolyzable ATP analogue MgAMP-PNP is bound to the enzyme. The peptides involved in

Figure 4.1.4: (A) A model of extended form and closed form of the C-terminus of the NMR structure of subunit F from *M. mazei* Gö1 A-ATP synthase inside an envelope, representing the conformational mobility of the C-terminus of F, as derived from backbone dynamics and two-dimensional ^1H - ^{15}N heteronuclear NOE experiments. (B) The two structures of Ran proteins from two different organisms showing the conformational change of the C-terminal helix: the first one is the structure of a Q69L mutant of Ran from dog with a bound GDP molecule (PDB id 1byu, (STEWART *et al.*, 1998); the second structure corresponds to Ran from human in complex with human RanBP2 and a non-hydrolysable GTP analogue (PDB id 1rrp, (VETTER *et al.*, 1999).

this EDC (1-ethyl-3-[(dimethylamino) propyl]carbodiimide) induced zero-length cross-link of B and F are $_{390}\text{EALSERDTK}_{399}$ and $_{88}\text{REKIK}_{92}$ respectively (SCHÄFER *et al.*, 2006b). Peptide $_{390}\text{EALSERDTK}_{399}$ is a sequence homolog of the so-called C-terminal DELSEED region of nucleotide-binding subunit α of the F-ATP synthases (GIBBONS *et al.*, 2000) which undergoes a rotational movement of 6° after binding of nucleotides (AGGELER *et al.*, 1998; MENZ *et al.*, 2001; FENIOUK *et al.*, ; VENKATARAMAN *et al.*, 2006; MANIMEKALAI *et al.*, 2009). Therefore, the close proximity of the sequences $_{88}\text{REKIK}_{92}$ of the C-terminus of subunit F and the C-terminal residues of B (Figure 4.1.5) might induce a conformational change in the lower C-terminal region of B with the subsequent closing of the binding pocket for the nucleotide and thereby may inhibit ATP synthesis/hydrolysis activities. It has been shown that the B-F interaction is absent in the presence of $\text{MgADP} + \text{Pi}$ or MgADP , due to a relative movement of subunit F (SCHÄFER *et al.*, 2006b).

In summary, the first three-dimensional structure of the central stalk subunit F of the A-ATP synthase in solution provides the structural basis toward a fuller understanding of the mechanistical properties of this subunit inside this class of enzymes. Moreover, it gives more insight into the structural and thereby functional homologies and diversities of the coupling subunits ϵ and F of F- and A-ATP synthases respectively.

Figure 4.1.5: Proposed binding of subunit F inside the A_3B_3DF complex of the A_1 ATPase. *Pyrococcus horikoshii* A-ATP synthase subunit A (pink, PDB code 1vdz; (MAEGAWA *et al.*, 2006)) and *M. mazei* Gö1 A-ATP synthase subunit B (green, PDB code 2c61; (SCHÄFER *et al.*, 2006a)), as well as the bovine mitochondrial F-ATP synthase *c* subunit (violet, PDB code 1e1q, chain G; (GIBBONS *et al.*, 2000)), which is homologous to subunit D of the A-ATP synthase, were fitted into the electron density map of the A_1 ATPase (COSKUN *et al.*, 2004) and A_1A_0 ATP synthase (BERNAL *et al.*, 2004), obtained from single-particle analysis electron micrographs (SCHÄFER *et al.*, 2006b). The solution structure of subunit F (GAYEN *et al.*, 2007) of the *M. mazei* Gö1 A-ATP synthase is highlighted in red. The yellow and red arrows indicate the intrinsic Trp residue (Trp430) of subunit B and the contact region of subunits B and F, respectively. The figure was prepared using PyMOL (<http://www.pymol.org>) (GAYEN *et al.*, 2007).

4.2 Structural characterization of subunit H of A₁A₀ ATP synthase from *M. jannaschii*

The peripheral stalk subunit H of the A-ATP synthase predicted from its amino acid sequence is α -helical and has a high propensity to form a coiled-coil structure (BIUKOVIĆ *et al.*, 2007). The CD spectrum of subunit H is consistent with the predicted high helical content of subunit H (78%) and also with a high degree of coiling based on the $\Theta_{222}/\Theta_{208}$ ratio of 0.96 (BIUKOVIĆ *et al.*, 2007). Classically, the dimerization interface of a two stranded coiled-coil is made up by a periodicity of nonpolar core residues and solvent exposed polar residues (Cooper *et al.*, 1990), as reflected in the amino acid sequence of subunit H. Physical characterization of this subunit by small angle X-ray scattering supports unequivocally that subunit H is dimeric in solution (25 ± 2 kDa) and its N terminal part (hook like structure) (BIUKOVIĆ *et al.*, 2007) is rigid. This is believed to be involved in interaction with central stalk subunits. From recent studies using chemical cross linker it has been shown that subunit H is in close contact via the C-terminal peptide I74 to K80 with the N-terminal region T106 to R122 of the catalytic subunit A, depending on nucleotide-binding to the catalytic site (SCHÄFER *et al.*, 2006b). Therefore subunit H can be oriented inside the 2D projection with its C-terminus located close to the external surface of the A₁ headpiece (Figure 4.2.1). This peripheral stalk shows a clear connection to the collar and/or the central stalk. The collar consists of at least the subunit E, which is in direct neighborhood to the rotary subunit D via its peptides ₁₂₇LDEAAKK₁₃₄ and ₁₁₉AYSSKESEELVK₁₃₀ (SCHÄFER *et al.*, 2006b). The nucleotide dependent cross-link formation of the C-terminal region of subunit H and its close proximity via its N-terminus to the central stalk region leads to the speculation whether subunit H might act as a stator and/or as a coupling or regulatory domain in the A-ATP synthase.

Figure 4.2.1: Composite structure of the methanogenic A₁A₀ ATP synthase. The SAXS structure of subunit H (blue) was introduced by eye into the 2D projection of the enzyme complex (COSKUN *et al.*, 2004).

Domain features of subunit H were studied by using a series of deletions and disulfide formations between introduced cysteines (BIUKOVIĆ *et al.*, 2009). The results provide a clearer picture of the architecture of this subunit and helped in establishing that subunit H is separated into two domains. First, the C-terminal part of H, which consists of residues 54–104, is highly α -helical, and the two α -helices in this domain are arranged mostly in a coiled-coil fashion. This is reflected by the high $\Theta_{222}/\Theta_{208}$ ratio of the N-terminal deleted version of subunit H, H₄₇₋₁₀₅, which is higher compared to the C-terminal truncated constructs H₁₋₆₇, H₁₋₇₁, and H₁₋₇₈.

(BIUKOVIĆ *et al.*, 2009). It is also in line with the reduced α -helical content of H₁₋₉₁, H₁₋₇₈, H₁₋₇₁ and H₁₋₆₇, respectively, which corresponds with the deletion size of the C-terminal segment. The existence of subunit H as a pair of parallel helices with a coiled-coil arrangement in region 54–104 is also supported by data showing that cysteine mutants introduced at positions 54, 61, 69, and 80 of the truncated proteins had a strong tendency to form disulfides (BIUKOVIĆ *et al.*, 2009). This is further supported by the NMR solution structure of a short length peptide of the very C-terminus of subunit H (residues 84–103) and the cross-linking study with the mutants H_{I92C}, H_{L95C} and H_{L97C}. The results showed that a complete disulfide product between two cysteines at positions 97 and 97' of the first and second helices were generated, whereas a lower dimer product were seen for the cysteines at positions 92 and 95. These results clearly tell that residue 97 is a part of the helix-helix interface and the residues 92 and 95 are in the opposite side of the helix, which is in accordance to the solution structure of H₈₅₋₁₀₄. Thus, the C-terminus of subunit H is very important for the stabilization of the structure of entire subunit H, which is in line to our previous results, where truncation of six amino acids at the C-terminus of subunit H resulted twisting of the upper arm in the low resolution shape determined from SAXS analysis (BIUKOVIĆ *et al.*, 2007).

The second structural domain found is formed by the N-terminal segment (residues 1–47), which is confirmed by the NMR solution structure of H₁₋₄₇. The $\Theta_{222}/\Theta_{208}$ value of H₁₋₄₇ is 0.6 supporting the predicted absence of coiled-coil assembly in the N-terminal domain (BIUKOVIĆ *et al.*, 2009). This is further supported by the higher dissociation constant of H₁₋₄₇ compared to the constants of the longer constructs H₁₋₆₁, H₁₋₆₉ and H₁₋₈₀, respectively. In order to understand further how the N-terminal domain of subunit H is formed, I solved the NMR solution structure of H₁₋₄₇. The structure of H₁₋₄₇ exhibits an α -helix formed between amino acids 15–42. Fitting the structure over residues 15–33 (r.m.s.d. 0.68) and 33–42 (r.m.s.d. 0.61) shows, that each region forms an α -helix and the 10 structures agree well with each other over these stretches. By comparison, fitting over the length of the protein, including residue 33, gives less agreement (r.m.s.d. 0.95) because of a small kink in the centre of the molecule. In the solution structure, H₁₋₄₇ has a total length of 61.9 Å, including the α -helical structure of 42.8 Å in length. The NMR structure of H₁₋₄₇ reveals a strip of hydrophobic alanine residues made by A19, A26 and A37 with a slight twist to each other (Figure 4.2.2). The proximity of alanine residues of the first and second α -helix of the dimeric subunit H, A19, A26 and A37, respectively, have been analyzed recently in our laboratory by replacing alanine residue with a cysteine residue, generating the subunit H mutants H_{A19C}, H_{A26C} and H_{A37C} (BIUKOVIĆ *et al.*, 2009). When the mutants were

incubated in the absence of an oxidizing reagent a small amount of dimer formation were observed for mutant H_{A19C}, which was decreased in the case of the mutant H_{A26C} to H_{A37C}. In the case of mutant H_{A19C} about 50% of the protein formed a dimer, when the protein was supplemented with the oxidizing agent. By comparison, the amount of cross-link products was dropped when the mutant protein H_{A26C} or H_{A37C} have been used. In contrast, when the alanine residue 35, located at the opposite site of A37, was exchanged by a cysteine, no disulfide formation could be detected under the conditions used. These results indicated a closer proximity of residues 19 and 19' of both helices and supports the small twist the three alanine residues (19, 26, 37) have relative to each other in the α -helical structure (Figure 4.2.2). By contrast, the absence of any dimeric product of the H_{A35C} mutant is in line with the opposite location of this amino acid relative to the alanine-strip shown in the structure, and implies that this residue is located at the outside of the helix-helix assembly. Taken together, the low coiled-coil content seen in the CD spectra as well as the low dimeric formation indicate that the major part of the two N-terminal segments (H₁₋₄₇ and H_{1-47'}) might run in a more adjacent arrangement to each other and coming closer at the end of H₁₋₄₇ and H_{1-47'}.

Figure 4.2.2: Ribbon diagram of the NMR solution structure of H₁₋₄₇. Side (A) and top (B) view of the average structure of H₁₋₄₇ illustrating the relative positions of alanine residues 19, 26 35 and 37 inside the α -helix, which are marked in blue color (BIUKOVIĆ *et al.*, 2009).

This feature is consistent with the disulfide product seen in H₁₋₄₇ which became stronger in the seven residue longer construct H₁₋₅₄ (BIUKOVIĆ *et al.*, 2009). The structural switch from a more adjacent into a coiled-coil assembly of both helices inside the dimeric H subunit becomes facilitated by the small kink at residue 33. This kink is characterized by a break in the chemical shift index (CSI) pattern for the helix at this position and reflects also part of the bend in the low resolution solution structure of the boomerang-like shape of subunit H (Figure 4.2.3). The overall length (61.9 Å) and structure of H₁₋₄₇ is remarkably similar with the shorter N-terminal arm of the boomerang shape of H as shown in figure 4.2.3. The shape volume enables both helices, H₁₋₄₇ and H₁₋₄₇ to fit in (BIUKOVIĆ *et al.*, 2009).

The oligomerization state of H₁₋₄₇ has also been investigated based on overall correlation time from NMR relaxation analysis and on translational diffusion coefficients at different concentrations. The ¹⁵N relaxation measurements as well as the corresponding model free analysis demonstrated two times increase of the rotational correlation time of H₁₋₄₇ with the increase of its concentration from 0.125 mM to 3 mM, directly demonstrating its dimeric state at concentrations higher than 2 mM. NMR pulsed field gradient (PFG) diffusion experiments in a dilution series, reveal the equilibrium between monomeric and dimeric states of H₁₋₄₇. The results clearly indicate that H₁₋₄₇ diffuses significantly faster at low concentration and the monomer-dimer equilibrium of H₁₋₄₇ exists at a protein concentration

< 3mM. Stimulated by the data of H₁₋₄₇, the diffusion coefficients for the constructs, H₁₋₆₁, H₁₋₆₉ and H₁₋₈₀ at different concentrations were also measured. In all of the cases, the decrease in the experimentally determined diffusion coefficient with the increasing concentration suggests the dimer formation of the proteins. The results indicate that H₁₋₆₁, H₁₋₆₉ and H₁₋₈₀ have a higher tendency to form dimer than H₁₋₄₇.

Based on the cross-link data as well as the change in chemical shift data, I modeled the dimeric structure for H₁₋₄₇, shown in figure 4.2.4. NMR measurements with H₁₋₄₇ as function of concentration point out that H₁₋₄₇ forms a dimer at concentrations above ~2 mM. The ¹H and ¹⁵N chemical shift perturbations indicate that the residues A19, I23, E24, A26, K27, I33,

Figure 4.2.3: The H₁₋₄₇ structure is superimposed on the low resolution structure of the dimeric H subunit derived from solution SAXS data (BIUKOVIĆ *et al.*, 2007).

Figure 4.2.4: (A) Location of the interacting residues at the proposed dimeric interface of H_{1-47} in a helical wheel representation, which was prepared using the program Drawcoil (<http://www.gevorggrigoryan.com/drawcoil>). residues in 'a' and 'd' positions are marked in beige and green color, respectively. Destabilizing residues Glu16, Glu40 for the dimeric H_{1-47} are highlighted by larger fonts (BIUKOVIĆ *et al.*, 2009). (B) Dimeric model of H_{1-47} based on cross linking data and NMR data. Residues in orange color exhibiting significant shifts represent the residues forming the dimeric interface of H_{1-47} . Charged residues exhibiting large shifts which are not directly situated at the dimeric interface are marked in cyan color.

K34 and A37 might be involved in dimer formation. Based on our structural model the charged residues Q32, E36 and E39 exhibiting comparably large shifts, might not be directly situated at the putative dimeric interface but rather their side-chain pK_a might be affected via proxy charged residues from the other monomer. The maximum chemical shift change has been observed for the residues 4 - 21 and residues 43 - 47. NMR secondary chemical shifts as well as NMR relaxation data have been shown that the protein has a flexible N- (residues 1 - 15) and a C-terminal region (residues 43 - 47). These chemical shifts are explained by structural consolidation at these stretches of amino acids as the direct consequence of dimerization. This follows from increased order parameters for the backbone HN vectors derived from ^{15}N relaxation data. In the relaxation data, the decreased values of R_1 as well as increased values of

R_2 suggest the dimer formation of H_{1-47} at higher concentrations ~ 3 mM. Extracted order parameters S^2 reflect spatial restriction of 1H - ^{15}N bonds on the ps to ns time scale, and range from 0 for unconstrained motion to 1 for completely rigid proteins. With the exception of the terminal residues, the S^2 values for H_{1-47} at high and low concentrations are rather uniform, ranging between 0.66 and 0.85. Their magnitude is characteristic of well-folded proteins, demonstrating that fast internal motions are limited for residues 15 to 42. The τ_e values are also in the picosecond range, as is typical for a well-folded protein. Almost quantitative doubling of the rotational correlation time from 16 ns to 25 ns for the protein between high and low concentrations unequivocally establishes dimerization process. The latter is accompanying by structure accretion at the region 4 - 21 as evident from an increase of the corresponding order parameters.

4.3 Interaction of subunit H with subunit E and the NMR solution structure of the N-terminus E_{1-52} of A_1A_O ATP synthase from *M. jannaschii*

Comparison of the shape of H and the C-terminal truncated form H_{1-98} , derived from SAXS data, allowed the assignment of subunit H in the position of the peripheral stalk in the 2D projection of the A-ATP synthase (BIUKOVIĆ *et al.*, 2007). This assignment was further confirmed by intrinsic tryptophan fluorescence experiment with subunits H and A (BIUKOVIĆ *et al.*, 2007). The sequence of subunit H lacks any aromatic amino acid and subunit A contains nine native tryptophan; seven of them lie buried inside subunit A, whereby residues W118 and W192 are located on the outside of the molecule. W118 is inside the N-terminal peptide involved in the A-H cross-link formation (SCHÄFER *et al.*, 2006b). The fluorescence intensity dropped by 25% and 20% after addition of subunit H and the N-terminal truncated H_{8-104} protein, respectively. In contrast, in the presence of the C-terminal truncated H_{1-98} , no significant quenching of the fluorescent signal could be detected. These results showed that the C-terminus of subunit H is interacting with the subunit A with a binding constant of 206 nM (BIUKOVIĆ *et al.*, 2007). Connected via its C-terminal arm to the catalytic A subunit, H exceeds the total distance of the A_1 headpiece and the central stalk (GRÜBER *et al.*, 2001; COSKUN *et al.*, 2004) and becomes oriented with its N-terminal arm close to the collar-like structure of the enzyme complex, predicted to be formed by subunit E (Figure 4.3.2) (SCHÄFER *et al.*, 2006b; BIUKOVIĆ *et al.*, 2007). Recently, an E-H complex has been described, using electrophoresis and mass spectrometry (LOKANATH *et al.*, 2007; KISH-TRIER *et al.*, 2009). In the NMR titration experiment presented, it is shown that it is the N-terminal domain (E_{1-100}) of subunit E

which specifically binds to the very N-terminus of H₁₋₄₇. The high α -helical degree of E₁₋₁₀₀ determined (71%) might indicate that the amino acid region E₄₁₋₆₀ (41EEAEKRKAEILKKGEKEAEM₆₀) of the E₁₋₁₀₀ domain joins with the N-terminal residues 1-6, 10-11, 15, 20 and 24 of H₁₋₄₇ (Figure 4.3.1) via a helix-helix interaction. The H₁₋₄₇/E₄₁₋₆₀-binding was also proved by fluorescence correlation spectroscopy (FCS) (GAYEN *et al.*, 2008). A binding constant of 3.0 μ M of bound Atto488-E₄₁₋₆₀ to H₁₋₄₇ was calculated. In contrast, no binding could be observed with the C-terminal form, E₁₀₁₋₂₀₆. As observed by circular dichroism spectroscopy, Trier and Wilkens (KISH-TRIER *et al.*, 2009) discussed that truncated forms of subunit E and H, E₂₋₈₃ and H₂₋₉₁, from *Thermoplasma acidophilum* A₁A_O ATP synthase form a coiled-coil interaction, reflected by molar ellipticity values at 208 nm and at 222 nm in a ratio close to 0.99. The solved NMR structure of E₁₋₅₂ with its hydrophobic strip in the α helical segment between residues 8-48 forms a perfect surface for an hydrophobic and helix-helix interaction between subunit E and H and the periodicity of Ala and/or Ile residues may indicate a coiled-coil interaction of both proteins. There is biochemical evidence that subunit F of the A-ATP synthase is in close contact to subunit D (COSKUN *et al.*, 2002; GAYEN *et al.*, 2007). As demonstrated by the solution structure, the four-stranded β -sheet in the N-terminal part of subunit F forms a hydrophobic surface, which is suggested to mediate the interaction of both subunits (GAYEN *et al.*, 2007). Such positioning of subunit D relative to F might bring the latter in close proximity to the C-terminal domain of subunit E. However, the data presented show no obvious interaction of F and E₁₀₁₋₂₀₆, indicating that subunit E is mainly interacting with subunit H and D via its N- and C-terminal part, respectively.

In summary, the presented data support the view that the α -helical subunit H forms one of the two peripheral stalks of the enzyme, with its C-terminus connected to the N-terminal part of the catalytic A subunit and its N-terminus in close contact to the N-terminus of subunit E, with the latter being in close connection via its C-terminus to subunit D (Figure 4.3.2). The nucleotide

Figure 4.3.1: The structural model of H₁₋₄₇ enables to assign the residues involved in subunit E-H formation. Previously the residues M₁ to M₆, K₁₀ to E₁₁, A₁₅, V₂₀ and E₂₄ of H₁₋₄₇, marked in stick representation encircled with dotted spheres, have been shown to interact with the N-terminal tail E₄₁₋₆₀ of subunit E (BIUKOVIĆ *et al.*, 2009).

dependent cross-link formation of A-H, its close proximity via its N-terminus to subunit E, and the D-E neighborhood leads to the speculation whether both subunit H and E might be involved in coupling and/or regulatory events in the A-ATP synthase.

Figure 4.3.2: The arrangements of the subunits in the model A_1A_0 ATP synthase. One B subunit has been removed from the A_1 section to reveal the D subunit within the A_3B_3 hexamer. *P. horikoshii* A-ATP synthase subunit A (green, pdb 1vdz, *M. mazei* Gö1 A-ATP synthase subunit B (blue, pdb 2c61), and *M. mazei* Gö1 A-ATP synthase subunit F (olive red, pdb 20V6) were fitted into the model of the A_1 ATPase and A_1A_0 ATP synthase, obtained from single particle analysis electron micrographs. The figure was prepared using PyMOL (<http://www.pymol.org>). (B) The A subunit is attached to subunit N-terminal domain by a peripheral stalk composed of subunit H, whereby the C-terminal part of E is in close proximity to the coupling subunit D.

5. Conclusion

The A_1A_O adenosine triphosphate (ATP) synthase is a fascinating enzyme in cellular bioenergetics responsible for the production of life molecule ATP from ADP and phosphate in archaea. A_1A_O is a complex of two rotary motors A_1 and A_O and the ATP synthesis/hydrolysis reaction that is reversibly catalyzed by A_1 is coupled with proton transport across membrane-embedded A_O . Subunit F is an important subunit in the central stalk of the enzyme. The conformational change in the stalk region is important for both the catalytic events (ATP synthesis or hydrolysis) as well as ion translocation. The solution structure of subunit F is separated into a well structured N-terminal domain (78 residues) and an extended C-terminal tail (22 residue). The N-terminal domain is composed of a four-stranded parallel β -sheet structure and three α -helices and has a dimension of 24.2 Å x 28.5 Å x 38.7 Å. The length of the C-terminal tail is 40 Å. The entire subunit F tumbles in two different entities as the two domains are loose in contact. This is confirmed by NMR dynamics studies. To get a deeper insight, I have determined also the solution structure of the peptide from the C-terminus (residues 81-101). The solution structures together with the molecular dynamics study explain the possible role of the C-terminus of subunit F in the catalysis process.

The H subunit of the A_1A_O ATP synthase is a component of one of the stator domain connecting the A_1 and A_O sector and has an important role in the assembly and function of the enzyme. In order to get details about the stalk part of the enzyme subunit H was efficiently expressed and purified. CD spectroscopy of subunit H showed a 78% α -helix and a coiled-coil arrangement. Cross linking studies using a series of deletions and disulfide formation between introduced cysteines indicate that this peripheral stalk is divided into two structural parts. The solution structure of the peptide comprising the last twenty amino acids of subunit H, H_{85-104} , together with the mutagenesis, cross linking analysis at the C-terminus of entire H subunit indicate that the C-terminus is important for the structure formation of subunit H. In order to understand, how the N-terminal domain of subunit H is formed, the first solution NMR structure of H_{1-47} has been determined. The structure consists of an α -helix between residues 15 to 42 and a flexible N-terminal stretch (1-14). The α -helix includes a kink, which would bring the two helices of the C-terminus in the coiled-coil arrangement. The structure of H_{1-47} reveals a strip of hydrophobic alanine residues. NMR measurements with H_{1-47} as function of concentration clearly point out that H_{1-47} forms a dimer at concentrations above ~3 mM. The 1H and ^{15}N chemical shift perturbation experiment indicates that the residues A19, I23, E24, A26, K27, I33, K34 and A37 are involved in dimer formation. Based on our structural model the charged residues Q32, E36 and E39 exhibiting comparably large shifts, might not be directly situated at

the putative dimeric interface but rather their side-chain pK_a might be affected via proxy charged residues from the other monomer. However, the maximum chemical shift change has been observed for the residues 6-21 and residues 43-47. NMR secondary chemical shifts as well as ^{15}N relaxation data reveal that these regions are less structured as compared to the continuous central α -helix. Some structural consolidation at the N-terminus is seen as the direct consequence of dimerization evidenced by the increased order parameters.

Using the purified N- (E_{1-100}) and C-terminal segment ($E_{101-206}$) of subunit E, NMR titration experiments revealed that the N-terminal residues Met₁ to Met₆, Lys₁₀ to Glu₁₁, Ala₁₅, Val₂₀ and Glu₂₄ of H₁₋₄₇ interact specifically with the N-terminal peptide, E_{1-100} of subunit E. A more detailed picture regarding the residues of E_{1-100} involved in the interaction was obtained by titration experiments using the N-terminal peptides E_{1-20} , E_{21-40} and E_{41-60} . The data indicate that the N-terminal tail E_{41-60} interacts with the N-terminal amino acids of H₁₋₄₇ and has been confirmed by fluorescence correlation spectroscopy results. In order to understand further the E-H assembly the N-terminal structure of E_{1-52} of *M. jannaschii* was solved by NMR spectroscopy, showing an α -helix between the residues 8-48. The molecule is amphipathic with a strip of hydrophobic residues, which may associate with the neighboring subunit H via a possible helix-helix interaction.

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Appendix A

Chemical shift assignments of subunit F of A₁A₀ ATP synthase from *M. mazei* Gö1

The chemical shift assignment are listed in the NMR-STAR format (Columns are as follows: Atom shift assign ID, Residue author sequence code, Residue sequence code, Residue label, Atom name, Atom type, Chemical shift value, Chemical shift value error, Chemical shift ambiguity code)

1	1	9	PRO	HA	H	4.193	0.020	1	542	50	58	VAL	CB	C	30.110	0.400	1
2	1	9	PRO	HB2	H	2.092	0.020	2	543	50	58	VAL	CG1	C	18.840	0.400	1
3	1	9	PRO	HB3	H	1.842	0.020	2	544	50	58	VAL	CG2	C	18.730	0.400	1
4	1	9	PRO	HG2	H	1.762	0.020	1	545	50	58	VAL	N	N	128.100	0.400	1
5	1	9	PRO	HG3	H	1.762	0.020	1	546	51	59	MET	H	H	8.408	0.020	1
6	1	9	PRO	HD2	H	3.493	0.020	2	547	51	59	MET	HA	H	4.912	0.020	1
7	1	9	PRO	HD3	H	3.248	0.020	2	548	51	59	MET	HB2	H	2.133	0.020	1
8	1	9	PRO	C	C	174.100	0.400	1	549	51	59	MET	HB3	H	2.133	0.020	1
9	1	9	PRO	CA	C	60.950	0.400	1	550	51	59	MET	HG2	H	2.327	0.020	2
10	1	9	PRO	CB	C	29.320	0.400	1	551	51	59	MET	HG3	H	2.571	0.020	2
11	1	9	PRO	CG	C	24.540	0.400	1	552	51	59	MET	C	C	170.200	0.400	1
12	1	9	PRO	CD	C	47.880	0.400	1	553	51	59	MET	CA	C	51.840	0.400	1
13	2	10	MET	H	H	8.610	0.020	1	554	51	59	MET	CB	C	37.420	0.400	1
14	2	10	MET	HA	H	4.559	0.020	1	555	51	59	MET	CG	C	30.340	0.400	1
15	2	10	MET	HB2	H	1.895	0.020	2	556	51	59	MET	N	N	123.100	0.400	1
16	2	10	MET	HB3	H	2.049	0.020	2	557	52	60	HIS	H	H	10.310	0.020	1
17	2	10	MET	HG2	H	2.399	0.020	2	558	52	60	HIS	HA	H	4.370	0.020	1
18	2	10	MET	HG3	H	2.471	0.020	2	559	52	60	HIS	HB2	H	1.377	0.020	2
19	2	10	MET	C	C	173.000	0.400	1	560	52	60	HIS	HB3	H	2.484	0.020	2
20	2	10	MET	CA	C	52.240	0.400	1	561	52	60	HIS	HD2	H	7.273	0.020	1
21	2	10	MET	CB	C	29.840	0.400	1	562	52	60	HIS	HE1	H	7.191	0.020	1
22	2	10	MET	CG	C	29.420	0.400	1	563	52	60	HIS	C	C	175.100	0.400	1
23	2	10	MET	N	N	119.600	0.400	1	564	52	60	HIS	CA	C	53.730	0.400	1
24	3	11	GLU	H	H	8.242	0.020	1	565	52	60	HIS	CB	C	29.290	0.400	1
25	3	11	GLU	HA	H	4.525	0.020	1	566	52	60	HIS	N	N	123.800	0.400	1
26	3	11	GLU	HB2	H	1.867	0.020	2	567	53	61	ASN	H	H	8.728	0.020	1
27	3	11	GLU	HB3	H	1.765	0.020	2	568	53	61	ASN	HA	H	4.110	0.020	1
28	3	11	GLU	HG2	H	2.165	0.020	2	569	53	61	ASN	HB2	H	2.461	0.020	2
29	3	11	GLU	HG3	H	2.212	0.020	2	570	53	61	ASN	HB3	H	2.217	0.020	2
30	3	11	GLU	C	C	173.100	0.400	1	571	53	61	ASN	HD21	H	6.632	0.020	2
31	3	11	GLU	CA	C	53.600	0.400	1	572	53	61	ASN	HD22	H	7.400	0.020	2
32	3	11	GLU	CB	C	28.530	0.400	1	573	53	61	ASN	C	C	174.900	0.400	1
33	3	11	GLU	CG	C	33.620	0.400	1	574	53	61	ASN	CA	C	54.340	0.400	1
34	3	11	GLU	N	N	118.700	0.400	1	575	53	61	ASN	CB	C	36.970	0.400	1
35	4	12	LEU	H	H	8.406	0.020	1	576	53	61	ASN	N	N	126.600	0.400	1
36	4	12	LEU	HA	H	5.148	0.020	1	577	53	61	ASN	ND2	N	112.500	0.400	1
37	4	12	LEU	HB2	H	1.452	0.020	1	578	54	62	ASP	H	H	11.520	0.020	1
38	4	12	LEU	HB3	H	1.452	0.020	1	579	54	62	ASP	HA	H	4.172	0.020	1
39	4	12	LEU	HG	H	1.702	0.020	1	580	54	62	ASP	HB2	H	2.180	0.020	2
40	4	12	LEU	HD1	H	0.631	0.020	2	581	54	62	ASP	HB3	H	2.486	0.020	2
41	4	12	LEU	HD2	H	0.704	0.020	2	582	54	62	ASP	C	C	175.800	0.400	1
42	4	12	LEU	C	C	171.800	0.400	1	583	54	62	ASP	CA	C	54.590	0.400	1
43	4	12	LEU	CA	C	51.690	0.400	1	584	54	62	ASP	CB	C	37.050	0.400	1
44	4	12	LEU	CB	C	42.340	0.400	1	585	54	62	ASP	N	N	123.600	0.400	1
45	4	12	LEU	CG	C	29.650	0.400	1	586	55	63	ASP	H	H	7.239	0.020	1
46	4	12	LEU	CD1	C	23.490	0.400	1	587	55	63	ASP	HA	H	4.435	0.020	1
47	4	12	LEU	CD2	C	22.810	0.400	1	588	55	63	ASP	HB2	H	2.280	0.020	2
48	4	12	LEU	N	N	124.900	0.400	1	589	55	63	ASP	HB3	H	2.876	0.020	2
49	5	13	ALA	H	H	8.704	0.020	1	590	55	63	ASP	C	C	176.400	0.400	1
50	5	13	ALA	HA	H	5.439	0.020	1	591	55	63	ASP	CA	C	53.640	0.400	1
51	5	13	ALA	HB	H	1.299	0.020	1	592	55	63	ASP	CB	C	37.630	0.400	1
52	5	13	ALA	C	C	172.600	0.400	1	593	55	63	ASP	N	N	117.600	0.400	1
53	5	13	ALA	CA	C	47.370	0.400	1	594	56	64	ILE	H	H	7.530	0.020	1
54	5	13	ALA	CB	C	22.090	0.400	1	595	56	64	ILE	HA	H	3.306	0.020	1
55	5	13	ALA	N	N	123.000	0.400	1	596	56	64	ILE	HB	H	1.693	0.020	1
56	6	14	VAL	H	H	8.319	0.020	1	597	56	64	ILE	HG13	H	1.523	0.020	2
57	6	14	VAL	HA	H	5.021	0.020	1	598	56	64	ILE	HG2	H	0.714	0.020	1
58	6	14	VAL	HB	H	1.351	0.020	1	599	56	64	ILE	HD1	H	0.673	0.020	1
59	6	14	VAL	HG1	H	0.147	0.020	2	600	56	64	ILE	C	C	175.700	0.400	1
60	6	14	VAL	HG2	H	0.356	0.020	2	601	56	64	ILE	CA	C	62.780	0.400	1
61	6	14	VAL	C	C	170.600	0.400	1	602	56	64	ILE	CB	C	35.240	0.400	1
62	6	14	VAL	CA	C	57.040	0.400	1	603	56	64	ILE	CG1	C	26.290	0.400	1
63	6	14	VAL	CB	C	32.800	0.400	1	604	56	64	ILE	CG2	C	14.430	0.400	1
64	6	14	VAL	CG1	C	18.530	0.400	1	605	56	64	ILE	CD1	C	10.780	0.400	1
65	6	14	VAL	CG2	C	17.910	0.400	1	606	56	64	ILE	N	N	120.000	0.400	1
66	6	14	VAL	N	N	118.400	0.400	1	607	57	65	GLY	H	H	7.650	0.020	1

67	7	15	ILE	H	H	7.884	0.020	1	608	57	65	GLY	HA2	H	3.702	0.020	2
68	7	15	ILE	HA	H	5.190	0.020	1	609	57	65	GLY	HA3	H	3.763	0.020	2
69	7	15	ILE	HB	H	1.661	0.020	1	610	57	65	GLY	C	C	171.300	0.400	1
70	7	15	ILE	HG12	H	1.461	0.020	2	611	57	65	GLY	CA	C	43.800	0.400	1
71	7	15	ILE	HG13	H	1.473	0.020	2	612	57	65	GLY	N	N	102.700	0.400	1
72	7	15	ILE	HG2	H	0.908	0.020	1	613	58	66	ASN	H	H	6.995	0.020	1
73	7	15	ILE	HD1	H	0.669	0.020	1	614	58	66	ASN	HA	H	4.795	0.020	1
74	7	15	ILE	C	C	172.400	0.400	1	615	58	66	ASN	HB2	H	2.696	0.020	2
75	7	15	ILE	CA	C	56.630	0.400	1	616	58	66	ASN	HB3	H	2.881	0.020	2
76	7	15	ILE	CB	C	40.980	0.400	1	617	58	66	ASN	HD21	H	7.582	0.020	2
77	7	15	ILE	CG1	C	23.900	0.400	1	618	58	66	ASN	HD22	H	6.870	0.020	2
78	7	15	ILE	CG2	C	14.920	0.400	1	619	58	66	ASN	C	C	172.500	0.400	1
79	7	15	ILE	CD1	C	11.900	0.400	1	620	58	66	ASN	CA	C	50.180	0.400	1
80	7	15	ILE	N	N	125.700	0.400	1	621	58	66	ASN	CB	C	37.140	0.400	1
81	8	16	GLY	H	H	8.284	0.020	1	622	58	66	ASN	N	N	115.200	0.400	1
82	8	16	GLY	HA2	H	3.843	0.020	2	623	58	66	ASN	ND2	N	113.800	0.400	1
83	8	16	GLY	HA3	H	4.588	0.020	2	624	59	67	LEU	H	H	7.320	0.020	1
84	8	16	GLY	C	C	169.000	0.400	1	625	59	67	LEU	HA	H	4.356	0.020	1
85	8	16	GLY	CA	C	42.470	0.400	1	626	59	67	LEU	HB2	H	2.109	0.020	2
86	8	16	GLY	N	N	112.000	0.400	1	627	59	67	LEU	HB3	H	1.821	0.020	2
87	9	17	LYS	H	H	9.168	0.020	1	628	59	67	LEU	HG	H	1.496	0.020	1
88	9	17	LYS	HA	H	4.324	0.020	1	629	59	67	LEU	HD1	H	1.141	0.020	2
89	9	17	LYS	HB2	H	1.739	0.020	2	630	59	67	LEU	HD2	H	0.673	0.020	2
90	9	17	LYS	HB3	H	2.042	0.020	2	631	59	67	LEU	C	C	171.100	0.400	1
91	9	17	LYS	HG2	H	1.560	0.020	2	632	59	67	LEU	CA	C	50.600	0.400	1
92	9	17	LYS	HG3	H	1.546	0.020	2	633	59	67	LEU	CB	C	38.470	0.400	1
93	9	17	LYS	HD2	H	1.697	0.020	2	634	59	67	LEU	CG	C	24.920	0.400	1
94	9	17	LYS	HD3	H	1.658	0.020	2	635	59	67	LEU	CD1	C	22.950	0.400	1
95	9	17	LYS	HE2	H	2.895	0.020	2	636	59	67	LEU	N	N	120.200	0.400	1
96	9	17	LYS	HE3	H	3.275	0.020	2	637	60	68	PRO	HA	H	4.373	0.020	1
97	9	17	LYS	HZ	H	7.279	0.020	1	638	60	68	PRO	HB2	H	2.387	0.020	2
98	9	17	LYS	C	C	176.300	0.400	1	639	60	68	PRO	HB3	H	2.008	0.020	2
99	9	17	LYS	CA	C	53.530	0.400	1	640	60	68	PRO	HG2	H	1.747	0.020	1
100	9	17	LYS	CB	C	31.110	0.400	1	641	60	68	PRO	HG3	H	1.747	0.020	1
101	9	17	LYS	CG	C	22.520	0.400	1	642	60	68	PRO	HD2	H	3.273	0.020	2
102	9	17	LYS	CD	C	25.740	0.400	1	643	60	68	PRO	HD3	H	3.762	0.020	2
103	9	17	LYS	CE	C	39.160	0.400	1	644	60	68	PRO	C	C	174.500	0.400	1
104	9	17	LYS	N	N	119.300	0.400	1	645	60	68	PRO	CA	C	59.990	0.400	1
105	10	18	SER	H	H	9.347	0.020	1	646	60	68	PRO	CB	C	28.600	0.400	1
106	10	18	SER	HA	H	3.827	0.020	1	647	60	68	PRO	CG	C	24.850	0.400	1
107	10	18	SER	HB2	H	3.771	0.020	1	648	60	68	PRO	CD	C	47.440	0.400	1
108	10	18	SER	HB3	H	3.771	0.020	1	649	61	69	GLU	H	H	8.736	0.020	1
109	10	18	SER	C	C	174.500	0.400	1	650	61	69	GLU	HA	H	3.655	0.020	1
110	10	18	SER	CA	C	60.200	0.400	1	651	61	69	GLU	HB2	H	1.871	0.020	2
111	10	18	SER	CB	C	62.110	0.400	1	652	61	69	GLU	HB3	H	1.918	0.020	2
112	10	18	SER	N	N	116.700	0.400	1	653	61	69	GLU	HG2	H	2.160	0.020	1
113	11	19	GLU	H	H	9.453	0.020	1	654	61	69	GLU	HG3	H	2.160	0.020	1
114	11	19	GLU	HA	H	4.010	0.020	1	655	61	69	GLU	C	C	175.200	0.400	1
115	11	19	GLU	HB2	H	1.958	0.020	1	656	61	69	GLU	CA	C	58.120	0.400	1
116	11	19	GLU	HB3	H	1.958	0.020	1	657	61	69	GLU	CB	C	27.080	0.400	1
117	11	19	GLU	HG2	H	2.254	0.020	1	658	61	69	GLU	CG	C	33.260	0.400	1
118	11	19	GLU	HG3	H	2.254	0.020	1	659	61	69	GLU	N	N	124.400	0.400	1
119	11	19	GLU	C	C	174.200	0.400	1	660	62	70	VAL	H	H	8.448	0.020	1
120	11	19	GLU	CA	C	56.880	0.400	1	661	62	70	VAL	HA	H	3.687	0.020	1
121	11	19	GLU	N	N	118.900	0.400	1	662	62	70	VAL	HB	H	1.944	0.020	1
122	12	20	PHE	H	H	7.585	0.020	1	663	62	70	VAL	HG1	H	0.830	0.020	2
123	12	20	PHE	HA	H	4.651	0.020	1	664	62	70	VAL	HG2	H	0.880	0.020	2
124	12	20	PHE	HB2	H	3.169	0.020	2	665	62	70	VAL	C	C	174.200	0.400	1
125	12	20	PHE	HB3	H	3.288	0.020	2	666	62	70	VAL	CA	C	62.920	0.400	1
126	12	20	PHE	HD1	H	7.273	0.020	1	667	62	70	VAL	CB	C	28.290	0.400	1
127	12	20	PHE	HD2	H	7.273	0.020	1	668	62	70	VAL	CG1	C	19.050	0.400	1
128	12	20	PHE	HE1	H	7.583	0.020	1	669	62	70	VAL	CG2	C	17.730	0.400	1
129	12	20	PHE	HE2	H	7.583	0.020	1	670	62	70	VAL	N	N	116.300	0.400	1
130	12	20	PHE	C	C	174.300	0.400	1	671	63	71	LEU	H	H	6.729	0.020	1
131	12	20	PHE	CA	C	57.000	0.400	1	672	63	71	LEU	HA	H	4.175	0.020	1
132	12	20	PHE	N	N	119.700	0.400	1	673	63	71	LEU	HB2	H	1.510	0.020	2
133	13	21	VAL	H	H	8.154	0.020	1	674	63	71	LEU	HB3	H	1.760	0.020	2
134	13	21	VAL	HA	H	3.946	0.020	1	675	63	71	LEU	HG	H	1.400	0.020	1
135	13	21	VAL	HB	H	2.054	0.020	1	676	63	71	LEU	HD1	H	0.830	0.020	2
136	13	21	VAL	HG1	H	0.744	0.020	2	677	63	71	LEU	HD2	H	0.620	0.020	2
137	13	21	VAL	HG2	H	0.744	0.020	2	678	63	71	LEU	C	C	175.700	0.400	1
138	13	21	VAL	C	C	175.900	0.400	1	679	63	71	LEU	CA	C	53.690	0.400	1
139	13	21	VAL	CA	C	61.000	0.400	1	680	63	71	LEU	CB	C	39.770	0.400	1
140	13	21	VAL	CB	C	29.190	0.400	1	681	63	71	LEU	CG	C	27.120	0.400	1
141	13	21	VAL	CG1	C	16.500	0.400	1	682	63	71	LEU	CD1	C	22.680	0.400	1

142	13	21	VAL	CG2	C	16.500	0.400	1	683	63	71	LEU	CD2	C	21.280	0.400	1
143	13	21	VAL	N	N	108.300	0.400	1	684	63	71	LEU	N	N	120.100	0.400	1
144	14	22	THR	H	H	7.938	0.020	1	685	64	72	ARG	H	H	8.076	0.020	1
145	14	22	THR	HA	H	4.069	0.020	1	686	64	72	ARG	HA	H	3.624	0.020	1
146	14	22	THR	HB	H	3.702	0.020	1	687	64	72	ARG	HB2	H	1.729	0.020	2
147	14	22	THR	HG2	H	1.075	0.020	1	688	64	72	ARG	HB3	H	1.700	0.020	2
148	14	22	THR	C	C	173.200	0.400	1	689	64	72	ARG	HG2	H	1.516	0.020	2
149	14	22	THR	CA	C	65.210	0.400	1	690	64	72	ARG	HG3	H	1.386	0.020	2
150	14	22	THR	CB	C	66.110	0.400	1	691	64	72	ARG	HD2	H	3.102	0.020	2
151	14	22	THR	CG2	C	18.260	0.400	1	692	64	72	ARG	HD3	H	3.283	0.020	2
152	14	22	THR	N	N	118.600	0.400	1	693	64	72	ARG	HH11	H	6.524	0.020	1
153	15	23	GLY	H	H	8.301	0.020	1	694	64	72	ARG	HH12	H	6.524	0.020	1
154	15	23	GLY	HA2	H	3.708	0.020	1	695	64	72	ARG	HH21	H	6.970	0.020	1
155	15	23	GLY	HA3	H	3.708	0.020	1	696	64	72	ARG	HH22	H	6.970	0.020	1
156	15	23	GLY	C	C	175.500	0.400	1	697	64	72	ARG	C	C	175.500	0.400	1
157	15	23	GLY	CA	C	41.890	0.400	1	698	64	72	ARG	CA	C	57.770	0.400	1
158	15	23	GLY	N	N	107.300	0.400	1	699	64	72	ARG	CB	C	27.540	0.400	1
159	16	24	PHE	H	H	7.114	0.020	1	700	64	72	ARG	CG	C	25.440	0.400	1
160	16	24	PHE	HA	H	3.974	0.020	1	701	64	72	ARG	CD	C	41.260	0.400	1
161	16	24	PHE	HB2	H	2.632	0.020	2	702	64	72	ARG	N	N	119.100	0.400	1
162	16	24	PHE	HB3	H	2.831	0.020	2	703	64	72	ARG	NH2	N	83.04	0.400	1
163	16	24	PHE	HD1	H	6.488	0.020	1	704	65	73	LYS	H	H	8.100	0.020	1
164	16	24	PHE	HD2	H	6.488	0.020	1	705	65	73	LYS	HA	H	3.897	0.020	1
165	16	24	PHE	HE1	H	6.880	0.020	1	706	65	73	LYS	HB2	H	1.700	0.020	2
166	16	24	PHE	HE2	H	6.880	0.020	1	707	65	73	LYS	HB3	H	1.756	0.020	2
167	16	24	PHE	C	C	174.600	0.400	1	708	65	73	LYS	HG2	H	1.198	0.020	2
168	16	24	PHE	CA	C	58.320	0.400	1	709	65	73	LYS	HG3	H	1.350	0.020	2
169	16	24	PHE	CB	C	35.840	0.400	1	710	65	73	LYS	HD2	H	1.540	0.020	2
170	17	25	ARG	H	H	7.828	0.020	1	711	65	73	LYS	HE2	H	2.736	0.020	2
171	17	25	ARG	HA	H	4.157	0.020	1	712	65	73	LYS	HE3	H	2.814	0.020	2
172	17	25	ARG	HB2	H	1.832	0.020	2	713	65	73	LYS	HZ	H	7.443	0.020	1
173	17	25	ARG	HB3	H	1.847	0.020	2	714	65	73	LYS	C	C	175.500	0.400	1
174	17	25	ARG	HG2	H	1.627	0.020	2	715	65	73	LYS	CA	C	57.190	0.400	1
175	17	25	ARG	HG3	H	1.486	0.020	2	716	65	73	LYS	CB	C	29.310	0.400	1
176	17	25	ARG	HD2	H	3.127	0.020	1	717	65	73	LYS	CG	C	22.050	0.400	1
177	17	25	ARG	HD3	H	3.127	0.020	1	718	65	73	LYS	CD	C	26.700	0.400	1
178	17	25	ARG	HE	H	8.081	0.020	1	719	65	73	LYS	CE	C	39.510	0.400	1
179	17	25	ARG	HH21	H	7.242	0.020	1	720	65	73	LYS	N	N	119.100	0.400	1
180	17	25	ARG	HH22	H	7.242	0.020	1	721	66	74	ASN	H	H	7.614	0.020	1
181	17	25	ARG	C	C	177.800	0.400	1	722	66	74	ASN	HA	H	4.323	0.020	1
182	17	25	ARG	CA	C	56.650	0.400	1	723	66	74	ASN	HB2	H	2.706	0.020	2
183	17	25	ARG	CB	C	27.170	0.400	1	724	66	74	ASN	HB3	H	2.761	0.020	2
184	17	25	ARG	CG	C	25.62	0.400	1	725	66	74	ASN	HD21	H	6.752	0.020	2
185	17	25	ARG	N	N	120.200	0.400	1	726	66	74	ASN	HD22	H	7.085	0.020	2
186	17	25	ARG	NH2	N	83.28	0.400	1	727	66	74	ASN	C	C	175.800	0.400	1
187	18	26	LEU	H	H	7.999	0.020	1	728	66	74	ASN	CA	C	53.340	0.400	1
188	18	26	LEU	HA	H	3.987	0.020	1	729	66	74	ASN	CB	C	35.730	0.400	1
189	18	26	LEU	HB2	H	1.455	0.020	2	730	66	74	ASN	N	N	116.800	0.400	1
190	18	26	LEU	HB3	H	1.620	0.020	2	731	66	74	ASN	ND2	N	109.100	0.400	1
191	18	26	LEU	HG	H	1.474	0.020	1	732	67	75	LEU	H	H	8.317	0.020	1
192	18	26	LEU	HD1	H	0.718	0.020	2	733	67	75	LEU	HA	H	3.863	0.020	1
193	18	26	LEU	HD2	H	0.718	0.020	2	734	67	75	LEU	HB2	H	1.681	0.020	2
194	18	26	LEU	C	C	175.000	0.400	1	735	67	75	LEU	HB3	H	1.752	0.020	2
195	18	26	LEU	CA	C	54.230	0.400	1	736	67	75	LEU	HG	H	1.385	0.020	1
196	18	26	LEU	CB	C	38.770	0.400	1	737	67	75	LEU	HD1	H	0.657	0.020	2
197	18	26	LEU	CG	C	24.030	0.400	1	738	67	75	LEU	HD2	H	0.840	0.020	2
198	18	26	LEU	CD1	C	22.410	0.400	1	739	67	75	LEU	C	C	175.400	0.400	1
199	18	26	LEU	CD2	C	22.250	0.400	1	740	67	75	LEU	CA	C	55.230	0.400	1
200	18	26	LEU	N	N	119.700	0.400	1	741	67	75	LEU	CB	C	38.970	0.400	1
201	19	27	ALA	H	H	7.140	0.020	1	742	67	75	LEU	CG	C	23.820	0.400	1
202	19	27	ALA	HA	H	4.175	0.020	1	743	67	75	LEU	CD1	C	21.310	0.400	1
203	19	27	ALA	HB	H	1.228	0.020	1	744	67	75	LEU	CD2	C	20.980	0.400	1
204	19	27	ALA	C	C	174.400	0.400	1	745	67	75	LEU	N	N	119.200	0.400	1
205	19	27	ALA	CA	C	49.530	0.400	1	746	68	76	ASN	H	H	7.976	0.020	1
206	19	27	ALA	CB	C	15.960	0.400	1	747	68	76	ASN	HA	H	4.364	0.020	1
207	19	27	ALA	N	N	119.400	0.400	1	748	68	76	ASN	HB2	H	2.724	0.020	2
208	20	28	GLY	H	H	7.629	0.020	1	749	68	76	ASN	HB3	H	2.825	0.020	2
209	20	28	GLY	HA2	H	3.592	0.020	2	750	68	76	ASN	HD21	H	7.436	0.020	2
210	20	28	GLY	HA3	H	4.167	0.020	2	751	68	76	ASN	HD22	H	6.595	0.020	2
211	20	28	GLY	C	C	171.700	0.400	1	752	68	76	ASN	C	C	173.900	0.400	1
212	20	28	GLY	CA	C	42.700	0.400	1	753	68	76	ASN	CA	C	52.920	0.400	1
213	20	28	GLY	N	N	104.700	0.400	1	754	68	76	ASN	CB	C	36.180	0.400	1
214	21	29	ILE	H	H	7.449	0.020	1	755	68	76	ASN	N	N	116.700	0.400	1
215	21	29	ILE	HA	H	3.974	0.020	1	756	68	76	ASN	ND2	N	110.800	0.400	1
216	21	29	ILE	HB	H	1.558	0.020	1	757	69	77	GLU	H	H	7.590	0.020	1

217	21	29	ILE	HG12	H	1.363	0.020	2	758	69	77	GLU	HA	H	4.210	0.020	1
218	21	29	ILE	HG13	H	1.557	0.020	2	759	69	77	GLU	HB2	H	1.840	0.020	2
219	21	29	ILE	HG2	H	1.259	0.020	1	760	69	77	GLU	HB3	H	2.023	0.020	2
220	21	29	ILE	HD1	H	0.631	0.020	1	761	69	77	GLU	HG2	H	2.180	0.020	2
221	21	29	ILE	C	C	172.800	0.400	1	762	69	77	GLU	HG3	H	2.367	0.020	2
222	21	29	ILE	CA	C	57.760	0.400	1	763	69	77	GLU	C	C	173.700	0.400	1
223	21	29	ILE	CB	C	33.700	0.400	1	764	69	77	GLU	CA	C	53.160	0.400	1
224	21	29	ILE	CG1	C	30.690	0.400	1	765	69	77	GLU	CB	C	27.730	0.400	1
225	21	29	ILE	CG2	C	15.330	0.400	1	766	69	77	GLU	CG	C	33.710	0.400	1
226	21	29	ILE	CD1	C	9.573	0.400	1	767	69	77	GLU	N	N	115.100	0.400	1
227	21	29	ILE	N	N	121.200	0.400	1	768	70	78	SER	H	H	7.110	0.020	1
228	22	30	SER	H	H	8.182	0.020	1	769	70	78	SER	HB2	H	3.880	0.020	1
229	22	30	SER	HA	H	4.352	0.020	1	770	70	78	SER	HB3	H	3.880	0.020	1
230	22	30	SER	HB2	H	3.780	0.020	2	771	70	78	SER	C	C	172.700	0.400	1
231	22	30	SER	HB3	H	3.726	0.020	2	772	70	78	SER	CA	C	57.020	0.400	1
232	22	30	SER	C	C	173.400	0.400	1	773	70	78	SER	CB	C	60.660	0.400	1
233	22	30	SER	CA	C	57.410	0.400	1	774	70	78	SER	N	N	114.900	0.400	1
234	22	30	SER	CB	C	61.400	0.400	1	775	71	79	VAL	H	H	8.515	0.020	1
235	22	30	SER	N	N	121.500	0.400	1	776	71	79	VAL	HA	H	4.150	0.020	1
236	23	31	LYS	H	H	8.266	0.020	1	777	71	79	VAL	HB	H	1.970	0.020	1
237	23	31	LYS	HA	H	4.127	0.020	1	778	71	79	VAL	HG1	H	0.840	0.020	2
238	23	31	LYS	HB2	H	1.758	0.020	2	779	71	79	VAL	HG2	H	0.840	0.020	2
239	23	31	LYS	HB3	H	2.050	0.020	2	780	71	79	VAL	C	C	173.100	0.400	1
240	23	31	LYS	HG2	H	1.110	0.020	1	781	71	79	VAL	CA	C	59.950	0.400	1
241	23	31	LYS	HG3	H	1.110	0.020	1	782	71	79	VAL	CB	C	29.910	0.400	1
242	23	31	LYS	HD2	H	1.286	0.020	1	783	71	79	VAL	CG1	C	18.490	0.400	1
243	23	31	LYS	HD3	H	1.286	0.020	1	784	71	79	VAL	CG2	C	17.730	0.400	1
244	23	31	LYS	HE2	H	2.840	0.020	2	785	71	79	VAL	N	N	125.300	0.400	1
245	23	31	LYS	HE3	H	3.107	0.020	2	786	72	80	GLN	H	H	7.486	0.020	1
246	23	31	LYS	HZ	H	7.601	0.020	1	787	72	80	GLN	HA	H	4.505	0.020	1
247	23	31	LYS	C	C	170.200	0.400	1	788	72	80	GLN	HB2	H	1.860	0.020	2
248	23	31	LYS	CA	C	53.470	0.400	1	789	72	80	GLN	HB3	H	1.972	0.020	2
249	23	31	LYS	CB	C	27.810	0.400	1	790	72	80	GLN	HG2	H	2.162	0.020	2
250	23	31	LYS	CG	C	22.18	0.400	1	791	72	80	GLN	HG3	H	2.107	0.020	2
251	23	31	LYS	CD	C	28.33	0.400	1	792	72	80	GLN	HE21	H	7.582	0.020	2
252	23	31	LYS	CE	C	39.380	0.400	1	793	72	80	GLN	HE22	H	6.835	0.020	2
253	23	31	LYS	N	N	125.900	0.400	1	794	72	80	GLN	C	C	169.300	0.400	1
254	24	32	VAL	H	H	6.997	0.020	1	795	72	80	GLN	CA	C	50.750	0.400	1
255	24	32	VAL	HA	H	4.379	0.020	1	796	72	80	GLN	CB	C	27.080	0.400	1
256	24	32	VAL	HB	H	1.629	0.020	1	797	72	80	GLN	CG	C	31.180	0.400	1
257	24	32	VAL	HG1	H	0.504	0.020	2	798	72	80	GLN	N	N	121.200	0.400	1
258	24	32	VAL	HG2	H	0.550	0.020	2	799	72	80	GLN	NE2	N	113.200	0.400	1
259	24	32	VAL	C	C	172.200	0.400	1	800	73	81	PRO	HA	H	4.535	0.020	1
260	24	32	VAL	CA	C	57.340	0.400	1	801	73	81	PRO	HB2	H	1.395	0.020	2
261	24	32	VAL	CB	C	31.070	0.400	1	802	73	81	PRO	HB3	H	1.318	0.020	2
262	24	32	VAL	CG1	C	18.910	0.400	1	803	73	81	PRO	HG2	H	1.924	0.020	2
263	24	32	VAL	CG2	C	18.690	0.400	1	804	73	81	PRO	HG3	H	1.458	0.020	2
264	24	32	VAL	N	N	120.500	0.400	1	805	73	81	PRO	HD2	H	3.461	0.020	2
265	25	33	TYR	H	H	8.934	0.020	1	806	73	81	PRO	HD3	H	3.561	0.020	2
266	25	33	TYR	HA	H	4.582	0.020	1	807	73	81	PRO	C	C	173.300	0.400	1
267	25	33	TYR	HB2	H	2.930	0.020	2	808	73	81	PRO	CA	C	60.050	0.400	1
268	25	33	TYR	HB3	H	2.740	0.020	2	809	73	81	PRO	CB	C	31.530	0.400	1
269	25	33	TYR	HD1	H	6.530	0.020	1	810	73	81	PRO	CG	C	26.59	0.400	1
270	25	33	TYR	HD2	H	6.530	0.020	1	811	73	81	PRO	CD	C	47.280	0.400	1
271	25	33	TYR	HE1	H	6.930	0.020	1	812	74	82	THR	H	H	7.944	0.020	1
272	25	33	TYR	HE2	H	6.930	0.020	1	813	74	82	THR	HA	H	4.191	0.020	1
273	25	33	TYR	C	C	170.400	0.400	1	814	74	82	THR	HB	H	3.924	0.020	1
274	25	33	TYR	CA	C	53.730	0.400	1	815	74	82	THR	HG2	H	1.051	0.020	1
275	25	33	TYR	CB	C	35.880	0.400	1	816	74	82	THR	C	C	170.400	0.400	1
276	25	33	TYR	N	N	128.400	0.400	1	817	74	82	THR	CA	C	60.240	0.400	1
277	26	34	GLU	H	H	8.485	0.020	1	818	74	82	THR	CB	C	68.140	0.400	1
278	26	34	GLU	HA	H	5.111	0.020	1	819	74	82	THR	CG2	C	18.870	0.400	1
279	26	34	GLU	HB2	H	1.700	0.020	1	820	74	82	THR	N	N	116.900	0.400	1
280	26	34	GLU	HB3	H	1.700	0.020	1	821	75	83	VAL	H	H	8.812	0.020	1
281	26	34	GLU	HG2	H	1.942	0.020	2	822	75	83	VAL	HA	H	4.755	0.020	1
282	26	34	GLU	HG3	H	1.981	0.020	2	823	75	83	VAL	HB	H	1.845	0.020	1
283	26	34	GLU	C	C	174.500	0.400	1	824	75	83	VAL	HG1	H	0.661	0.020	2
284	26	34	GLU	CA	C	52.270	0.400	1	825	75	83	VAL	HG2	H	0.818	0.020	2
285	26	34	GLU	CB	C	27.810	0.400	1	826	75	83	VAL	C	C	172.600	0.400	1
286	26	34	GLU	CG	C	33.730	0.400	1	827	75	83	VAL	CA	C	58.970	0.400	1
287	26	34	GLU	N	N	125.500	0.400	1	828	75	83	VAL	CB	C	29.480	0.400	1
288	27	35	THR	H	H	8.791	0.020	1	829	75	83	VAL	CG1	C	20.890	0.400	1
289	27	35	THR	HA	H	4.603	0.020	1	830	75	83	VAL	CG2	C	17.460	0.400	1
290	27	35	THR	HB	H	3.955	0.020	1	831	75	83	VAL	N	N	129.100	0.400	1
291	27	35	THR	HG2	H	0.798	0.020	1	832	76	84	VAL	H	H	9.166	0.020	1

292	27	35	THR	C	C	171.900	0.400	1	833	76	84	VAL	HA	H	4.023	0.020	1
293	27	35	THR	CA	C	62.750	0.400	1	834	76	84	VAL	HB	H	1.802	0.020	1
294	27	35	THR	CB	C	67.010	0.400	1	835	76	84	VAL	HG1	H	0.700	0.020	2
295	27	35	THR	CG2	C	24.570	0.400	1	836	76	84	VAL	HG2	H	0.710	0.020	2
296	27	35	THR	N	N	119.100	0.400	1	837	76	84	VAL	C	C	169.600	0.400	1
297	28	36	PRO	HA	H	4.408	0.020	1	838	76	84	VAL	CA	C	58.750	0.400	1
298	28	36	PRO	HB2	H	1.975	0.020	2	839	76	84	VAL	CB	C	30.790	0.400	1
299	28	36	PRO	HB3	H	2.306	0.020	2	840	76	84	VAL	CG1	C	19.870	0.400	1
300	28	36	PRO	HG2	H	1.871	0.020	1	841	76	84	VAL	CG2	C	18.590	0.400	1
301	28	36	PRO	HG3	H	1.871	0.020	1	842	76	84	VAL	N	N	130.500	0.400	1
302	28	36	PRO	HD2	H	3.537	0.020	2	843	77	85	ALA	H	H	8.203	0.020	1
303	28	36	PRO	HD3	H	3.614	0.020	2	844	77	85	ALA	HA	H	4.978	0.020	1
304	28	36	PRO	C	C	172.400	0.400	1	845	77	85	ALA	HB	H	1.029	0.020	1
305	28	36	PRO	CA	C	61.070	0.400	1	846	77	85	ALA	C	C	174.500	0.400	1
306	28	36	PRO	CB	C	29.830	0.400	1	847	77	85	ALA	CA	C	46.810	0.400	1
307	28	36	PRO	CG	C	23.910	0.400	1	848	77	85	ALA	CB	C	17.720	0.400	1
308	28	36	PRO	CD	C	48.670	0.400	1	849	77	85	ALA	N	N	128.700	0.400	1
309	29	37	ASP	H	H	7.086	0.020	1	850	78	86	LEU	H	H	8.877	0.020	1
310	29	37	ASP	HA	H	4.679	0.020	1	851	78	86	LEU	HA	H	8.583	0.020	1
311	29	37	ASP	HB2	H	2.715	0.020	2	852	78	86	LEU	HB2	H	1.691	0.020	2
312	29	37	ASP	HB3	H	2.979	0.020	2	853	78	86	LEU	HB3	H	1.689	0.020	2
313	29	37	ASP	C	C	173.400	0.400	1	854	78	86	LEU	HD1	H	0.790	0.020	2
314	29	37	ASP	CA	C	49.210	0.400	1	855	78	86	LEU	HD2	H	0.958	0.020	2
315	29	37	ASP	CB	C	39.950	0.400	1	856	78	86	LEU	C	C	173.500	0.400	1
316	29	37	ASP	N	N	114.800	0.400	1	857	78	86	LEU	CA	C	50.780	0.400	1
317	30	38	ILE	H	H	8.643	0.020	1	858	78	86	LEU	CB	C	38.950	0.400	1
318	30	38	ILE	HA	H	3.799	0.020	1	859	78	86	LEU	CD1	C	23.650	0.400	1
319	30	38	ILE	HB	H	2.017	0.020	1	860	78	86	LEU	CD2	C	20.890	0.400	1
320	30	38	ILE	HG12	H	0.854	0.020	2	861	78	86	LEU	N	N	123.400	0.400	1
321	30	38	ILE	HG13	H	1.102	0.020	2	862	79	87	GLY	H	H	8.250	0.020	1
322	30	38	ILE	HG2	H	1.600	0.020	1	863	79	87	GLY	HA2	H	3.650	0.020	2
323	30	38	ILE	HD1	H	0.790	0.020	1	864	79	87	GLY	HA3	H	3.790	0.020	2
324	30	38	ILE	C	C	172.800	0.400	1	865	79	87	GLY	C	C	172.000	0.400	1
325	30	38	ILE	CA	C	63.710	0.400	1	866	79	87	GLY	CA	C	41.750	0.400	1
326	30	38	ILE	CB	C	32.630	0.400	1	867	79	87	GLY	N	N	106.700	0.400	1
327	30	38	ILE	CG1	C	26.620	0.400	1	868	80	88	GLY	H	H	8.227	0.020	1
328	30	38	ILE	CG2	C	14.800	0.400	1	869	80	88	GLY	HA2	H	3.994	0.020	2
329	30	38	ILE	CD1	C	9.635	0.400	1	870	80	88	GLY	HA3	H	3.990	0.020	2
330	30	38	ILE	N	N	120.600	0.400	1	871	80	88	GLY	C	C	171.400	0.400	1
331	31	39	PRO	HA	H	4.324	0.020	1	872	80	88	GLY	CA	C	42.220	0.400	1
332	31	39	PRO	HB2	H	2.040	0.020	2	873	80	88	GLY	N	N	107.600	0.400	1
333	31	39	PRO	HB3	H	2.219	0.020	2	874	81	89	SER	H	H	8.460	0.020	1
334	31	39	PRO	HG2	H	1.760	0.020	2	875	81	89	SER	HA	H	4.370	0.020	1
335	31	39	PRO	HG3	H	1.821	0.020	2	876	81	89	SER	HB2	H	3.995	0.020	2
336	31	39	PRO	HD2	H	3.652	0.020	2	877	81	89	SER	HB3	H	3.907	0.020	2
337	31	39	PRO	HD3	H	3.670	0.020	2	878	81	89	SER	C	C	172.600	0.400	1
338	31	39	PRO	C	C	177.100	0.400	1	879	81	89	SER	CA	C	55.880	0.400	1
339	31	39	PRO	CA	C	63.690	0.400	1	880	81	89	SER	CB	C	61.190	0.400	1
340	31	39	PRO	CB	C	27.770	0.400	1	881	81	89	SER	N	N	115.400	0.400	1
341	31	39	PRO	CG	C	25.170	0.400	1	882	82	90	GLY	H	H	8.515	0.020	1
342	31	39	PRO	CD	C	52.870	0.400	1	883	82	90	GLY	HA2	H	4.680	0.020	1
343	32	40	ALA	H	H	8.182	0.020	1	884	82	90	GLY	HA3	H	4.680	0.020	1
344	32	40	ALA	HA	H	4.160	0.020	1	885	82	90	GLY	C	C	171.600	0.400	1
345	32	40	ALA	HB	H	1.485	0.020	1	886	82	90	GLY	CA	C	42.800	0.400	1
346	32	40	ALA	C	C	178.000	0.400	1	887	82	90	GLY	N	N	110.900	0.400	1
347	32	40	ALA	CA	C	52.170	0.400	1	888	83	91	SER	H	H	8.161	0.020	1
348	32	40	ALA	CB	C	15.820	0.400	1	889	83	91	SER	HA	H	4.670	0.020	1
349	32	40	ALA	N	N	118.900	0.400	1	890	83	91	SER	HB2	H	3.850	0.020	1
350	33	41	THR	H	H	8.169	0.020	1	891	83	91	SER	HB3	H	3.850	0.020	1
351	33	41	THR	HA	H	4.342	0.020	1	892	83	91	SER	CA	C	55.930	0.400	1
352	33	41	THR	HB	H	3.747	0.020	1	893	83	91	SER	CB	C	60.920	0.400	1
353	33	41	THR	HG2	H	0.996	0.020	1	894	83	91	SER	N	N	115.500	0.400	1
354	33	41	THR	C	C	172.500	0.400	1	895	85	93	SER	HA	H	4.318	0.020	1
355	33	41	THR	CA	C	64.710	0.400	1	896	85	93	SER	HB2	H	3.937	0.020	1
356	33	41	THR	CB	C	65.240	0.400	1	897	85	93	SER	HB3	H	3.937	0.020	1
357	33	41	THR	CG2	C	18.770	0.400	1	898	85	93	SER	CA	C	55.880	0.400	1
358	33	41	THR	N	N	118.400	0.400	1	899	85	93	SER	CB	C	61.140	0.400	1
359	34	42	GLU	H	H	8.467	0.020	1	900	86	94	THR	H	H	8.172	0.020	1
360	34	42	GLU	HA	H	3.462	0.020	1	901	86	94	THR	HA	H	4.027	0.020	1
361	34	42	GLU	HB2	H	2.130	0.020	1	902	86	94	THR	HB	H	3.807	0.020	1
362	34	42	GLU	HB3	H	2.130	0.020	1	903	86	94	THR	CA	C	59.470	0.400	1
363	34	42	GLU	HG2	H	1.966	0.020	1	904	86	94	THR	CB	C	66.820	0.400	1
364	34	42	GLU	HG3	H	1.966	0.020	1	905	86	94	THR	N	N	115.500	0.400	1
365	34	42	GLU	C	C	175.200	0.400	1	906	87	95	SER	H	H	8.172	0.020	1
366	34	42	GLU	CA	C	57.750	0.400	1	907	87	95	SER	HA	H	4.320	0.020	1

367	34	42	GLU	CB	C	26.780	0.400	1	908	87	95	SER	HB2	H	3.930	0.020	1
368	34	42	GLU	CG	C	33.970	0.400	1	909	87	95	SER	HB3	H	3.930	0.020	1
369	34	42	GLU	N	N	121.400	0.400	1	910	87	95	SER	C	C	171.700	0.400	1
370	35	43	SER	H	H	7.806	0.020	1	911	87	95	SER	CA	C	55.890	0.400	1
371	35	43	SER	HA	H	3.991	0.020	1	912	87	95	SER	CB	C	61.000	0.400	1
372	35	43	SER	HB2	H	3.831	0.020	1	913	87	95	SER	N	N	123.600	0.400	1
373	35	43	SER	HB3	H	3.831	0.020	1	914	88	96	LEU	H	H	8.135	0.020	1
374	35	43	SER	C	C	174.200	0.400	1	915	88	96	LEU	HA	H	4.660	0.020	1
375	35	43	SER	CA	C	57.050	0.400	1	916	88	96	LEU	HB2	H	1.520	0.020	1
376	35	43	SER	CB	C	60.580	0.400	1	917	88	96	LEU	HB3	H	1.520	0.020	1
377	35	43	SER	N	N	112.100	0.400	1	918	88	96	LEU	C	C	174.100	0.400	1
378	36	44	ALA	H	H	7.814	0.020	1	919	88	96	LEU	CA	C	52.030	0.400	1
379	36	44	ALA	HA	H	3.807	0.020	1	920	88	96	LEU	CB	C	39.360	0.400	1
380	36	44	ALA	HB	H	1.182	0.020	1	921	88	96	LEU	CG	C	23.990	0.400	1
381	36	44	ALA	C	C	176.000	0.400	1	922	88	96	LEU	CD1	C	22.220	0.400	1
382	36	44	ALA	CA	C	52.390	0.400	1	923	88	96	LEU	CD2	C	20.580	0.400	1
383	36	44	ALA	CB	C	16.460	0.400	1	924	88	96	LEU	N	N	123.700	0.400	1
384	36	44	ALA	N	N	123.700	0.400	1	925	89	97	ARG	H	H	7.920	0.020	1
385	37	45	VAL	H	H	8.314	0.020	1	926	89	97	ARG	C	C	172.200	0.400	1
386	37	45	VAL	HA	H	3.078	0.020	1	927	89	97	ARG	CA	C	53.860	0.400	1
387	37	45	VAL	HB	H	2.065	0.020	1	928	89	97	ARG	CB	C	26.540	0.400	1
388	37	45	VAL	HG1	H	0.693	0.020	2	929	89	97	ARG	N	N	120.700	0.400	1
389	37	45	VAL	HG2	H	0.979	0.020	2	930	90	98	GLU	HA	H	4.089	0.020	1
390	37	45	VAL	C	C	174.400	0.400	1	931	90	98	GLU	HB2	H	1.915	0.020	2
391	37	45	VAL	CA	C	64.170	0.400	1	932	90	98	GLU	HB3	H	1.910	0.020	2
392	37	45	VAL	CB	C	28.950	0.400	1	933	90	98	GLU	HG2	H	2.128	0.020	2
393	37	45	VAL	CG1	C	21.780	0.400	1	934	90	98	GLU	HG3	H	1.968	0.020	2
394	37	45	VAL	CG2	C	20.040	0.400	1	935	90	98	GLU	CA	C	56.450	0.400	1
395	37	45	VAL	N	N	118.400	0.400	1	936	90	98	GLU	CB	C	27.280	0.400	1
396	38	46	ARG	H	H	7.843	0.020	1	937	90	98	GLU	CG	C	33.320	0.400	1
397	38	46	ARG	HA	H	3.683	0.020	1	938	91	99	LYS	H	H	7.848	0.020	1
398	38	46	ARG	HB2	H	1.764	0.020	2	939	91	99	LYS	HA	H	4.040	0.020	1
399	38	46	ARG	HB3	H	1.755	0.020	2	940	91	99	LYS	HB2	H	1.740	0.020	1
400	38	46	ARG	HG2	H	1.456	0.020	2	941	91	99	LYS	HB3	H	1.740	0.020	1
401	38	46	ARG	HG3	H	1.450	0.020	2	942	91	99	LYS	HE2	H	3.040	0.020	2
402	38	46	ARG	HD2	H	3.084	0.020	2	943	91	99	LYS	HE3	H	3.048	0.020	2
403	38	46	ARG	HD3	H	3.092	0.020	2	944	91	99	LYS	C	C	176.300	0.400	1
404	38	46	ARG	HH11	H	7.609	0.020	1	945	91	99	LYS	CA	C	54.240	0.400	1
405	38	46	ARG	HH12	H	7.609	0.020	1	946	91	99	LYS	CB	C	29.730	0.400	1
406	38	46	ARG	C	C	176.200	0.400	1	947	91	99	LYS	N	N	120.400	0.400	1
407	38	46	ARG	CA	C	57.790	0.400	1	948	92	100	ILE	H	H	7.660	0.020	1
408	38	46	ARG	CB	C	26.640	0.400	1	949	92	100	ILE	HA	H	3.724	0.020	1
409	38	46	ARG	CG	C	25.990	0.400	1	950	92	100	ILE	HB	H	1.829	0.020	1
410	38	46	ARG	CD	C	40.390	0.400	1	951	92	100	ILE	HG2	H	1.380	0.020	1
411	38	46	ARG	N	N	116.100	0.400	1	952	92	100	ILE	HD1	H	0.690	0.020	1
412	38	46	ARG	NH2	N	85.28	0.400	1	953	92	100	ILE	C	C	173.600	0.400	1
413	39	47	SER	H	H	7.607	0.020	1	954	92	100	ILE	CA	C	59.600	0.400	1
414	39	47	SER	HA	H	4.046	0.020	1	955	92	100	ILE	CB	C	39.520	0.400	1
415	39	47	SER	HB2	H	3.767	0.020	2	956	92	100	ILE	N	N	113.600	0.400	1
416	39	47	SER	HB3	H	3.760	0.020	2	957	93	101	LYS	H	H	8.048	0.020	1
417	39	47	SER	C	C	174.500	0.400	1	958	93	101	LYS	HA	H	3.713	0.020	1
418	39	47	SER	CA	C	59.020	0.400	1	959	93	101	LYS	HE2	H	2.980	0.020	1
419	39	47	SER	CB	C	60.310	0.400	1	960	93	101	LYS	HE3	H	2.980	0.020	1
420	39	47	SER	N	N	112.900	0.400	1	961	93	101	LYS	C	C	175.100	0.400	1
421	40	48	VAL	H	H	8.244	0.020	1	962	93	101	LYS	CA	C	58.43	0.400	1
422	40	48	VAL	HA	H	3.660	0.020	1	963	93	101	LYS	CB	C	27.740	0.400	1
423	40	48	VAL	HB	H	1.808	0.020	1	964	93	101	LYS	N	N	122.700	0.400	1
424	40	48	VAL	HG1	H	0.806	0.020	2	965	94	102	GLN	H	H	8.330	0.020	1
425	40	48	VAL	HG2	H	0.718	0.020	2	966	94	102	GLN	HA	H	3.890	0.020	1
426	40	48	VAL	C	C	175.300	0.400	1	967	94	102	GLN	HG2	H	2.136	0.020	2
427	40	48	VAL	CA	C	63.510	0.400	1	968	94	102	GLN	HG3	H	2.448	0.020	2
428	40	48	VAL	CB	C	28.150	0.400	1	969	94	102	GLN	HE21	H	7.500	0.020	2
429	40	48	VAL	CG1	C	21.720	0.400	1	970	94	102	GLN	HE22	H	6.720	0.020	2
430	40	48	VAL	CG2	C	20.840	0.400	1	971	94	102	GLN	CA	C	55.640	0.400	1
431	40	48	VAL	N	N	123.100	0.400	1	972	94	102	GLN	CB	C	25.950	0.400	1
432	41	49	LEU	H	H	8.071	0.020	1	973	94	102	GLN	N	N	119.300	0.400	1
433	41	49	LEU	HA	H	3.785	0.020	1	974	94	102	GLN	NE2	N	111.600	0.400	1
434	41	49	LEU	HB2	H	1.732	0.020	2	975	95	103	ALA	H	H	7.886	0.020	1
435	41	49	LEU	HB3	H	1.695	0.020	2	976	95	103	ALA	HA	H	4.172	0.020	1
436	41	49	LEU	HG	H	1.378	0.020	1	977	95	103	ALA	CA	C	52.040	0.400	1
437	41	49	LEU	HD1	H	0.614	0.020	2	978	95	103	ALA	CB	C	16.970	0.400	1
438	41	49	LEU	HD2	H	0.614	0.020	2	979	95	103	ALA	N	N	119.800	0.400	1
439	41	49	LEU	C	C	175.700	0.400	1	980	96	104	VAL	HA	H	4.271	0.020	1
440	41	49	LEU	CA	C	54.630	0.400	1	981	96	104	VAL	HB	H	2.215	0.020	1
441	41	49	LEU	CB	C	38.300	0.400	1	982	96	104	VAL	HG1	H	0.853	0.020	2

442	41	49	LEU	CG	C	24.110	0.400	1	983	96	104	VAL	HG2	H	0.796	0.020	2
443	41	49	LEU	CD1	C	22.560	0.400	1	984	96	104	VAL	C	C	174.000	0.400	1
444	41	49	LEU	CD2	C	19.100	0.400	1	985	96	104	VAL	CA	C	59.020	0.400	1
445	41	49	LEU	N	N	117.200	0.400	1	986	96	104	VAL	CB	C	30.650	0.400	1
446	42	50	GLU	H	H	6.986	0.020	1	987	96	104	VAL	CG1	C	18.820	0.400	1
447	42	50	GLU	HA	H	4.213	0.020	1	988	96	104	VAL	CG2	C	18.420	0.400	1
448	42	50	GLU	HB2	H	1.843	0.020	2	989	96	104	VAL	N	N	110.300	0.400	1
449	42	50	GLU	HB3	H	1.840	0.020	2	990	97	105	GLY	H	H	7.864	0.020	1
450	42	50	GLU	HG2	H	2.318	0.020	2	991	97	105	GLY	HA2	H	3.717	0.020	2
451	42	50	GLU	HG3	H	2.159	0.020	2	992	97	105	GLY	HA3	H	4.048	0.020	2
452	42	50	GLU	C	C	172.600	0.400	1	993	97	105	GLY	C	C	171.200	0.400	1
453	42	50	GLU	CA	C	53.240	0.400	1	994	97	105	GLY	CA	C	42.490	0.400	1
454	42	50	GLU	CB	C	27.590	0.400	1	995	97	105	GLY	N	N	108.900	0.400	1
455	42	50	GLU	CG	C	33.420	0.400	1	996	98	106	VAL	H	H	7.966	0.020	1
456	42	50	GLU	N	N	114.700	0.400	1	997	98	106	VAL	HA	H	4.170	0.020	1
490	46	54	VAL	H	H	7.214	0.020	1	998	98	106	VAL	CA	C	172.800	0.400	1
491	46	54	VAL	HA	H	3.755	0.020	1	999	98	106	VAL	CA	C	59.360	0.400	1
492	46	54	VAL	HB	H	1.989	0.020	1	1000	98	106	VAL	CB	C	29.830	0.400	1
493	46	54	VAL	HG1	H	0.655	0.020	2	1001	98	106	VAL	N	N	118.500	0.400	1
494	46	54	VAL	HG2	H	0.740	0.020	2	1002	99	107	ASP	H	H	7.714	0.020	1
495	46	54	VAL	C	C	172.500	0.400	1	1003	99	107	ASP	HA	H	4.649	0.020	1
496	46	54	VAL	CA	C	60.450	0.400	1	1004	99	107	ASP	HB2	H	2.304	0.020	2
497	46	54	VAL	CB	C	29.730	0.400	1	1005	99	107	ASP	HB3	H	2.614	0.020	2
498	46	54	VAL	CG1	C	19.900	0.400	1	1006	99	107	ASP	C	C	173.100	0.400	1
499	46	54	VAL	CG2	C	19.640	0.400	1	1007	99	107	ASP	CA	C	51.630	0.400	1
500	46	54	VAL	N	N	122.100	0.400	1	1008	99	107	ASP	CB	C	38.600	0.400	1
501	47	55	GLY	H	H	9.313	0.020	1	1009	99	107	ASP	N	N	122.600	0.400	1
502	47	55	GLY	HA2	H	3.767	0.020	2	1010	100	108	LEU	H	H	7.832	0.020	1
503	47	55	GLY	HA3	H	4.330	0.020	2	1011	100	108	LEU	HA	H	4.022	0.020	1
504	47	55	GLY	C	C	171.500	0.400	1	1012	100	108	LEU	HB2	H	0.994	0.020	2
505	47	55	GLY	CA	C	42.850	0.400	1	1013	100	108	LEU	HB3	H	1.282	0.020	2
506	47	55	GLY	N	N	114.800	0.400	1	1014	100	108	LEU	HG	H	0.874	0.020	1
507	48	56	ILE	H	H	7.564	0.020	1	1015	100	108	LEU	HD1	H	0.503	0.020	2
508	48	56	ILE	HA	H	4.871	0.020	1	1016	100	108	LEU	HD2	H	1.097	0.020	2
509	48	56	ILE	HB	H	1.496	0.020	1	1017	100	108	LEU	C	C	173.400	0.400	1
510	48	56	ILE	HG12	H	1.334	0.020	1	1018	100	108	LEU	CA	C	52.680	0.400	1
511	48	56	ILE	HG13	H	1.334	0.020	1	1019	100	108	LEU	CB	C	39.240	0.400	1
512	48	56	ILE	HG2	H	0.912	0.020	1	1020	100	108	LEU	CG	C	28.650	0.400	1
513	48	56	ILE	HD1	H	0.665	0.020	1	1021	100	108	LEU	CD1	C	23.080	0.400	1
514	48	56	ILE	C	C	175.7	0.400	1	1022	100	108	LEU	CD2	C	21.470	0.400	1
515	48	56	ILE	CA	C	56.590	0.400	1	1023	100	108	LEU	N	N	122.600	0.400	1
516	48	56	ILE	CB	C	38.570	0.400	1	1024	101	109	TRP	H	H	7.924	0.020	1
517	48	56	ILE	CG1	C	25.160	0.400	1	1025	101	109	TRP	HA	H	4.534	0.020	1
518	48	56	ILE	CG2	C	15.070	0.400	1	1026	101	109	TRP	HB2	H	2.991	0.020	2
519	48	56	ILE	CD1	C	10.830	0.400	1	1027	101	109	TRP	HB3	H	3.266	0.020	2
520	48	56	ILE	N	N	122.100	0.400	1	1028	101	109	TRP	HE1	H	9.985	0.020	1
521	49	57	LEU	H	H	8.706	0.020	1	1029	101	109	TRP	HE3	H	7.497	0.020	1
522	49	57	LEU	HA	H	5.030	0.020	1	1030	101	109	TRP	HZ2	H	7.540	0.020	1
523	49	57	LEU	HB2	H	1.462	0.020	2	1031	101	109	TRP	HZ3	H	7.612	0.020	1
524	49	57	LEU	HB3	H	1.265	0.020	2	1032	101	109	TRP	C	C	171.600	0.400	1
525	49	57	LEU	HG	H	0.827	0.020	1	1033	101	109	TRP	CA	C	55.370	0.400	1
526	49	57	LEU	HD1	H	0.679	0.020	2	1034	101	109	TRP	CB	C	26.860	0.400	1
527	49	57	LEU	HD2	H	0.645	0.020	2	1035	101	109	TRP	N	N	119.700	0.400	1
528	49	57	LEU	C	C	170.400	0.400	1	1036	101	109	TRP	NE1	N	129.100	0.400	1
529	49	57	LEU	CA	C	49.500	0.400	1	1037	102	110	LYS	H	H	7.295	0.020	1
530	49	57	LEU	CB	C	42.130	0.400	1	1038	102	110	LYS	HA	H	3.987	0.020	1
531	49	57	LEU	CG	C	25.410	0.400	1	1039	102	110	LYS	HB2	H	1.645	0.020	2
532	49	57	LEU	CD1	C	24.610	0.400	1	1040	102	110	LYS	HB3	H	1.569	0.020	2
533	49	57	LEU	CD2	C	21.030	0.400	1	1041	102	110	LYS	HG2	H	1.210	0.020	1
534	49	57	LEU	N	N	128.800	0.400	1	1042	102	110	LYS	HG3	H	1.210	0.020	1
535	50	58	VAL	H	H	9.018	0.020	1	1043	102	110	LYS	C	C	178.200	0.400	1
536	50	58	VAL	HA	H	4.906	0.020	1	1044	102	110	LYS	CA	C	55.250	0.400	1
537	50	58	VAL	HB	H	1.676	0.020	1	1045	102	110	LYS	CB	C	30.750	0.400	1
538	50	58	VAL	HG1	H	0.695	0.020	2	1046	102	110	LYS	CE	C	39.570	0.400	1
539	50	58	VAL	HG2	H	0.806	0.020	2	1047	102110	LYS	N	N	126.200	0.400	1	
540	50	58	VAL	C	C	171.500	0.400	1									
541	50	58	VAL	CA	C	58.380	0.400	1									

Appendix B

Chemical shift assignments of H₁₋₄₇ of A₁A₀ ATP synthase from *M. jannaschii*

The chemical shift assignment are listed in the NMR-STAR format (Columns are as follows: Atom shift assign ID, Residue author sequence code, Residue sequence code, Residue label, Atom name, Atom type, Chemical shift value, Chemical shift value error, Chemical shift ambiguity code

1	1	1	MET	H	H	8.769	0.020	1	248	25	25	GLU	H	H	8.537	0.020	1
2	1	1	MET	HA	H	4.510	0.400	1	249	25	25	GLU	HA	H	4.140	0.020	1
3	1	1	MET	HB2	H	2.075	0.020	2	250	25	25	GLU	HB2	H	2.094	0.020	2
4	1	1	MET	HB3	H	2.075	0.020	2	251	25	25	GLU	HB3	H	2.094	0.020	2
5	1	1	MET	HG2	H	2.612	0.020	2	252	25	25	GLU	HG2	H	2.320	0.020	2
6	1	1	MET	HG3	H	2.612	0.020	2	253	25	25	GLU	HG3	H	2.320	0.020	2
7	1	1	MET	CA	C	55.680	0.400	1	254	25	25	GLU	CA	C	58.780	0.400	1
8	1	1	MET	CB	C	33.310	0.400	1	255	25	25	GLU	CB	C	29.970	0.400	1
9	1	1	MET	CG	C	32.450	0.400	1	256	25	25	GLU	CG	C	36.620	0.400	1
10	1	1	MET	N	N	120.926	0.400	1	257	25	25	GLU	N	N	120.605	0.400	1
11	2	2	GLY	H	H	8.502	0.020	1	258	26	26	ALA	H	H	8.129	0.020	1
12	2	2	GLY	HA2	H	3.979	0.020	2	259	26	26	ALA	HA	H	4.166	0.020	1
13	2	2	GLY	HA3	H	3.979	0.020	2	260	26	26	ALA	HB	H	1.516	0.020	1
14	2	2	GLY	CA	C	45.600	0.020	1	261	26	26	ALA	CA	C	55.230	0.400	1
15	2	2	GLY	N	N	110.343	0.400	1	262	26	26	ALA	CB	C	18.470	0.400	1
16	3	3	VAL	H	H	8.040	0.020	1	263	26	26	ALA	N	N	122.943	0.400	1
17	3	3	VAL	HA	H	4.168	0.020	1	264	27	27	LYS	H	H	8.120	0.020	1
18	3	3	VAL	HB	H	2.076	0.020	1	265	27	27	LYS	HA	H	4.175	0.020	1
19	3	3	VAL	HG1	H	0.918	0.020	2	266	27	27	LYS	HB2	H	1.926	0.020	2
20	3	3	VAL	HG2	H	0.918	0.020	2	267	27	27	LYS	HB3	H	1.926	0.020	2
21	3	3	VAL	CA	C	62.380	0.400	1	268	27	27	LYS	HD2	H	1.540	0.020	2
22	3	3	VAL	CB	C	33.210	0.400	1	269	27	27	LYS	HD3	H	1.540	0.020	2
23	3	3	VAL	CG1	C	21.390	0.400	2	270	27	27	LYS	HE2	H	2.962	0.020	2
24	3	3	VAL	CG2	C	21.390	0.400	2	271	27	27	LYS	HE3	H	2.962	0.020	2
25	3	3	VAL	N	N	119.136	0.400	1	272	27	27	LYS	HG2	H	1.417	0.020	2
26	4	4	SER	H	H	8.549	0.020	1	273	27	27	LYS	HG3	H	1.417	0.020	2
27	4	4	SER	HA	H	4.482	0.020	1	274	27	27	LYS	CA	C	58.830	0.400	1
28	4	4	SER	HB2	H	3.878	0.020	2	275	27	27	LYS	CB	C	32.610	0.400	1
29	4	4	SER	HB3	H	3.878	0.020	2	276	27	27	LYS	CD	C	29.650	0.400	1
30	4	4	SER	CA	C	58.470	0.400	1	277	27	27	LYS	CE	C	42.310	0.400	1
31	4	4	SER	CB	C	64.880	0.400	1	278	27	27	LYS	CG	C	25.200	0.400	1
32	4	4	SER	N	N	120.034	0.400	1	279	27	27	LYS	N	N	120.716	0.400	1
33	5	5	VAL	H	H	8.304	0.020	1	280	28	28	ASN	H	H	8.310	0.020	1
34	5	5	VAL	HA	H	4.070	0.020	1	281	28	28	ASN	HA	H	4.575	0.020	1
35	5	5	VAL	HB	H	2.090	0.020	1	282	28	28	ASN	HB2	H	2.887	0.020	2
36	5	5	VAL	HG1	H	0.943	0.020	2	283	28	28	ASN	HB3	H	2.887	0.020	2
37	5	5	VAL	HG2	H	0.943	0.020	2	284	28	28	ASN	HD21	H	7.736	0.020	2
38	5	5	VAL	CA	C	63.230	0.400	1	285	28	28	ASN	HD22	H	6.953	0.020	2
39	5	5	VAL	CB	C	32.770	0.400	1	286	28	28	ASN	CA	C	55.350	0.400	1
40	5	5	VAL	CG1	C	21.370	0.400	2	287	28	28	ASN	CB	C	38.420	0.400	1
41	5	5	VAL	CG2	C	21.370	0.400	2	288	28	28	ASN	N	N	118.724	0.400	1
42	5	5	VAL	N	N	122.497	0.400	1	289	28	28	ASN	ND2	N	112.900	0.400	1
43	6	6	MET	H	H	8.407	0.020	1	290	29	29	ARG	H	H	8.301	0.020	1
44	6	6	MET	HA	H	4.428	0.020	1	291	29	29	ARG	HA	H	4.191	0.020	1
45	6	6	MET	HB2	H	2.052	0.020	2	292	29	29	ARG	HB2	H	1.927	0.020	2
46	6	6	MET	HB3	H	2.052	0.020	2	293	29	29	ARG	HB3	H	1.927	0.020	2
47	6	6	MET	HG2	H	2.555	0.020	2	294	29	29	ARG	HD2	H	3.243	0.020	2
48	6	6	MET	HG3	H	2.555	0.020	2	295	29	29	ARG	HD3	H	3.243	0.020	2
49	6	6	MET	CA	C	56.170	0.400	1	296	29	29	ARG	HG2	H	1.754	0.020	2
50	6	6	MET	CB	C	33.270	0.400	1	297	29	29	ARG	HG3	H	1.703	0.020	2
51	6	6	MET	CG	C	32.750	0.400	1	298	29	29	ARG	CA	C	58.390	0.400	1
52	6	6	MET	N	N	122.782	0.400	1	299	29	29	ARG	CB	C	30.510	0.400	1
53	7	7	GLU	H	H	8.350	0.020	1	300	29	29	ARG	CD	C	43.420	0.400	1
54	7	7	GLU	HA	H	4.204	0.020	1	301	29	29	ARG	CG	C	27.530	0.400	1
55	7	7	GLU	HB2	H	2.001	0.020	2	302	30	30	ALA	H	H	8.110	0.020	1
56	7	7	GLU	HB3	H	2.001	0.020	2	303	30	30	ALA	HA	H	4.175	0.020	1
57	7	7	GLU	HG2	H	2.288	0.020	2	304	30	30	ALA	HB	H	1.522	0.020	1
58	7	7	GLU	HG3	H	2.288	0.020	2	305	30	30	ALA	CA	C	55.230	0.400	1
59	7	7	GLU	CA	C	57.280	0.400	1	306	30	30	ALA	CB	C	18.560	0.400	1
60	7	7	GLU	CB	C	30.730	0.400	1	307	30	30	ALA	N	N	122.173	0.400	1
61	7	7	GLU	CG	C	36.570	0.400	1	308	31	31	GLU	H	H	8.230	0.020	1
62	7	7	GLU	N	N	122.264	0.400	1	309	31	31	GLU	HA	H	4.160	0.020	1
63	8	8	ALA	H	H	8.334	0.020	1	310	31	31	GLU	HB2	H	2.113	0.020	2
64	8	8	ALA	HA	H	4.267	0.020	1	311	31	31	GLU	HB3	H	2.113	0.020	2
65	8	8	ALA	HB	H	1.397	0.020	1	312	31	31	GLU	HG2	H	2.375	0.020	2
66	8	8	ALA	CA	C	53.180	0.400	1	313	31	31	GLU	HG3	H	2.375	0.020	2

67	8	8	ALA	CB	C	19.19	0.400	1	314	31	31	GLU	CA	C	58.380	0.400	1
68	8	8	ALA	N	N	125.161	0.400	1	315	31	31	GLU	CB	C	29.830	0.400	1
69	9	9	ILE	H	H	8.149	0.020	1	316	31	31	GLU	CG	C	36.610	0.400	1
70	9	9	ILE	HA	H	4.048	0.020	1	317	31	31	GLU	N	N	118.620	0.400	1
71	9	9	ILE	HB	H	1.873	0.020	1	318	32	32	GLN	H	H	8.160	0.020	1
72	9	9	ILE	HD1	H	0.902	0.020	1	319	32	32	GLN	HA	H	4.214	0.020	1
73	9	9	ILE	HG12	H	1.539	0.020	2	320	32	32	GLN	HB2	H	2.163	0.020	2
74	9	9	ILE	HG13	H	1.539	0.020	2	321	32	32	GLN	HB3	H	2.163	0.020	2
75	9	9	ILE	HG2	H	1.222	0.020	1	322	32	32	GLN	HE21	H	7.694	0.020	2
76	9	9	ILE	CA	C	62.030	0.400	1	323	32	32	GLN	HE22	H	6.895	0.020	2
77	9	9	ILE	CB	C	38.690	0.400	1	324	32	32	GLN	HG2	H	2.386	0.020	2
78	9	9	ILE	CD1	C	13.200	0.400	1	325	32	32	GLN	HG3	H	2.467	0.020	2
79	9	9	ILE	CG1	C	28.240	0.400	1	326	32	32	GLN	CA	C	58.100	0.400	1
80	9	9	ILE	CG2	C	17.830	0.400	1	327	32	32	GLN	CB	C	28.880	0.400	1
81	9	9	ILE	N	N	120.286	0.400	1	328	32	32	GLN	CG	C	33.820	0.400	1
82	10	10	LYS	H	H	8.282	0.020	1	329	32	32	GLN	N	N	121.642	0.400	1
83	10	10	LYS	HA	H	4.233	0.020	1	330	32	32	GLN	NE2	N	112.600	0.400	1
84	10	10	LYS	HB2	H	1.829	0.020	2	331	33	33	ILE	H	H	8.180	0.020	1
85	10	10	LYS	HB3	H	1.829	0.020	2	332	33	33	ILE	HA	H	3.927	0.020	1
86	10	10	LYS	HD2	H	1.506	0.020	2	333	33	33	ILE	HB	H	1.891	0.020	1
87	10	10	LYS	HD3	H	1.506	0.020	2	334	33	33	ILE	HD1	H	0.921	0.020	1
88	10	10	LYS	HE2	H	2.992	0.020	2	335	33	33	ILE	HG12	H	1.638	0.020	2
89	10	10	LYS	HE3	H	2.992	0.020	2	336	33	33	ILE	HG13	H	1.638	0.020	2
90	10	10	LYS	HG2	H	1.416	0.020	2	337	33	33	ILE	HG2	H	1.206	0.020	1
91	10	10	LYS	HG3	H	1.416	0.020	2	338	33	33	ILE	CA	C	63.620	0.400	1
92	10	10	LYS	CA	C	57.250	0.400	1	339	33	33	ILE	CB	C	38.530	0.400	1
93	10	10	LYS	CB	C	33.240	0.400	1	340	33	33	ILE	CD1	C	13.310	0.400	1
94	10	10	LYS	CD	C	29.470	0.400	1	341	33	33	ILE	CG1	C	28.370	0.400	1
95	10	10	LYS	CE	C	42.330	0.400	1	342	33	33	ILE	CG2	C	17.640	0.400	1
96	10	10	LYS	CG	C	25.070	0.400	1	343	33	33	ILE	N	N	121.132	0.400	1
97	10	10	LYS	N	N	125.057	0.400	1	344	34	34	LYS	H	H	7.964	0.020	1
98	11	11	GLU	H	H	8.394	0.020	1	345	34	34	LYS	HA	H	4.186	0.020	1
99	11	11	GLU	HA	H	4.217	0.020	1	346	34	34	LYS	HB2	H	1.892	0.020	2
100	11	11	GLU	HB2	H	2.002	0.020	2	347	34	34	LYS	HB3	H	1.892	0.020	2
101	11	11	GLU	HB3	H	2.002	0.020	2	348	34	34	LYS	HD2	H	1.537	0.020	2
102	11	11	GLU	HG2	H	2.282	0.020	2	349	34	34	LYS	HD3	H	1.537	0.020	2
103	11	11	GLU	HG3	H	2.282	0.020	2	350	34	34	LYS	HE2	H	2.978	0.020	2
104	11	11	GLU	CA	C	57.540	0.400	1	351	34	34	LYS	HE3	H	2.978	0.020	2
105	11	11	GLU	CB	C	30.700	0.400	1	352	34	34	LYS	HG2	H	1.450	0.020	2
106	11	11	GLU	CG	C	36.590	0.400	1	353	34	34	LYS	HG3	H	1.450	0.020	2
107	11	11	GLU	N	N	122.003	0.400	1	354	34	34	LYS	CA	C	58.380	0.400	1
108	12	12	VAL	H	H	8.220	0.020	1	355	34	34	LYS	CB	C	33.120	0.400	1
109	12	12	VAL	HA	H	3.988	0.020	1	356	34	34	LYS	CD	C	29.650	0.400	1
110	12	12	VAL	HB	H	2.081	0.020	1	357	34	34	LYS	CE	C	42.280	0.400	1
111	12	12	VAL	HG1	H	0.956	0.020	2	358	34	34	LYS	CG	C	25.250	0.400	1
112	12	12	VAL	HG2	H	0.956	0.020	2	359	34	34	LYS	N	N	122.920	0.400	1
113	12	12	VAL	CA	C	63.720	0.400	1	360	35	35	ALA	H	H	8.215	0.020	1
114	12	12	VAL	CB	C	32.680	0.400	1	361	35	35	ALA	HA	H	4.178	0.020	1
115	12	12	VAL	CG1	C	21.660	0.400	2	362	35	35	ALA	HB	H	1.509	0.020	1
116	12	12	VAL	CG2	C	21.660	0.400	2	363	35	35	ALA	CA	C	54.890	0.400	1
117	12	12	VAL	N	N	122.828	0.400	1	364	35	35	ALA	CB	C	18.710	0.400	1
118	13	13	LYS	H	H	8.385	0.020	1	365	35	35	ALA	N	N	123.352	0.400	1
119	13	13	LYS	HA	H	4.316	0.020	1	366	36	36	GLU	H	H	8.434	0.020	1
120	13	13	LYS	HB2	H	1.815	0.020	2	367	36	36	GLU	HA	H	4.145	0.020	1
121	13	13	LYS	HB3	H	1.815	0.020	2	368	36	36	GLU	HB2	H	2.075	0.020	2
122	13	13	LYS	HD2	H	1.670	0.020	2	369	36	36	GLU	HB3	H	2.075	0.020	2
123	13	13	LYS	HD3	H	1.670	0.020	2	370	36	36	GLU	HG2	H	2.303	0.020	2
124	13	13	LYS	HG2	H	1.455	0.020	2	371	36	36	GLU	HG3	H	2.466	0.020	2
125	13	13	LYS	HG3	H	1.455	0.020	2	372	36	36	GLU	CA	C	58.360	0.400	1
126	13	13	LYS	CA	C	56.990	0.400	1	373	36	36	GLU	CB	C	30.010	0.400	1
127	13	13	LYS	CB	C	33.030	0.400	1	374	36	36	GLU	CG	C	36.800	0.400	1
128	13	13	LYS	CD	C	29.300	0.400	1	375	36	36	GLU	N	N	119.725	0.400	1
129	13	13	LYS	CE	C	42.280	0.400	1	376	37	37	ALA	H	H	8.084	0.020	1
130	13	13	LYS	CG	C	25.060	0.400	1	377	37	37	ALA	HA	H	4.285	0.020	1
131	13	13	LYS	N	N	124.784	0.400	1	378	37	37	ALA	HB	H	1.507	0.020	1
132	14	14	LEU	H	H	8.360	0.020	1	379	37	37	ALA	CA	C	54.440	0.400	1
133	14	14	LEU	HA	H	4.272	0.020	1	380	37	37	ALA	CB	C	18.640	0.400	1
134	14	14	LEU	HB2	H	1.672	0.020	2	381	37	37	ALA	N	N	123.710	0.400	1
135	14	14	LEU	HB3	H	1.672	0.020	2	382	38	38	ILE	H	H	7.997	0.020	1
136	14	14	LEU	HD1	H	0.906	0.020	2	383	38	38	ILE	HA	H	3.953	0.020	1
137	14	14	LEU	HD2	H	0.906	0.020	2	384	38	38	ILE	HB	H	1.969	0.020	1
138	14	14	LEU	CA	C	56.450	0.400	1	385	38	38	ILE	HD1	H	0.914	0.020	1
139	14	14	LEU	CB	C	42.510	0.400	1	386	38	38	ILE	HG12	H	1.658	0.020	2
140	14	14	LEU	CD1	C	25.200	0.400	2	387	38	38	ILE	HG13	H	1.658	0.020	2
141	14	14	LEU	CD2	C	23.680	0.400	2	388	38	38	ILE	HG2	H	1.203	0.020	1

142	14	14	LEU	CG	C	27.470	0.400	1	389	38	38	ILE	CA	C	63.450	0.400	1
143	14	14	LEU	N	N	123.256	0.400	1	390	38	38	ILE	CB	C	38.540	0.400	1
144	15	15	ALA	H	H	8.301	0.020	1	391	38	38	ILE	CD1	C	13.370	0.400	1
145	15	15	ALA	HA	H	4.261	0.020	1	392	38	38	ILE	CG1	C	28.030	0.400	1
146	15	15	ALA	HB	H	1.450	0.020	1	393	38	38	ILE	CG2	C	17.780	0.400	1
147	15	15	ALA	CA	C	53.810	0.400	1	394	38	38	ILE	N	N	120.850	0.400	1
148	15	15	ALA	CB	C	18.960	0.400	1	395	39	39	GLU	H	H	8.100	0.020	1
149	15	15	ALA	N	N	124.067	0.400	1	396	39	39	GLU	HA	H	4.156	0.020	1
150	16	16	GLU	H	H	8.441	0.020	1	397	39	39	GLU	HB2	H	2.071	0.020	2
151	16	16	GLU	HA	H	4.195	0.020	1	398	39	39	GLU	HB3	H	2.071	0.020	2
152	16	16	GLU	HB2	H	2.077	0.020	2	399	39	39	GLU	HG2	H	2.317	0.020	2
153	16	16	GLU	HB3	H	2.077	0.020	2	400	39	39	GLU	HG3	H	2.317	0.020	2
154	16	16	GLU	HG2	H	2.261	0.020	2	401	39	39	GLU	CA	C	58.230	0.400	1
155	16	16	GLU	HG3	H	2.390	0.020	2	402	39	39	GLU	CB	C	29.990	0.400	1
156	16	16	GLU	CA	C	58.190	0.400	1	403	39	39	GLU	CG	C	36.270	0.400	1
157	16	16	GLU	CB	C	30.360	0.400	1	404	39	39	GLU	N	N	122.251	0.400	1
158	16	16	GLU	CG	C	36.730	0.400	1	405	40	40	GLU	H	H	8.435	0.020	1
159	16	16	GLU	N	N	120.401	0.400	1	406	40	40	GLU	HA	H	4.108	0.020	1
160	17	17	GLU	H	H	8.393	0.020	1	407	40	40	GLU	HB2	H	2.065	0.020	2
161	17	17	GLU	HA	H	4.168	0.020	1	408	40	40	GLU	HB3	H	2.065	0.020	2
162	17	17	GLU	HB2	H	2.080	0.020	2	409	40	40	GLU	HG2	H	2.329	0.020	2
163	17	17	GLU	HB3	H	2.080	0.020	2	410	40	40	GLU	HG3	H	2.329	0.020	2
164	17	17	GLU	HG2	H	2.275	0.020	2	411	40	40	GLU	CA	C	58.360	0.400	1
165	17	17	GLU	HG3	H	2.406	0.020	2	412	40	40	GLU	CB	C	30.090	0.400	1
166	17	17	GLU	CA	C	58.270	0.400	1	413	40	40	GLU	CG	C	36.330	0.400	1
167	17	17	GLU	CB	C	30.340	0.400	1	414	40	40	GLU	N	N	121.271	0.400	1
168	17	17	GLU	CG	C	36.820	0.400	1	415	41	41	ALA	H	H	8.054	0.020	1
169	17	17	GLU	N	N	120.884	0.400	1	416	41	41	ALA	HA	H	4.221	0.020	1
170	18	18	GLN	H	H	8.353	0.020	1	417	41	41	ALA	HB	H	1.483	0.020	1
171	18	18	GLN	HA	H	4.170	0.020	1	418	41	41	ALA	CA	C	54.180	0.400	1
172	18	18	GLN	HB2	H	2.100	0.020	2	419	41	41	ALA	CB	C	18.680	0.400	1
173	18	18	GLN	HB3	H	2.100	0.020	2	420	41	41	ALA	N	N	122.548	0.400	1
174	18	18	GLN	HE21	H	7.747	0.020	2	421	42	42	LYS	H	H	7.924	0.020	1
175	18	18	GLN	HE22	H	6.944	0.020	2	422	42	42	LYS	HA	H	4.168	0.020	1
176	18	18	GLN	HG2	H	2.414	0.020	2	423	42	42	LYS	HB2	H	1.878	0.020	2
177	18	18	GLN	HG3	H	2.414	0.020	2	424	42	42	LYS	HB3	H	1.878	0.020	2
178	18	18	GLN	CA	C	57.410	0.400	1	425	42	42	LYS	HD2	H	1.615	0.020	2
179	18	18	GLN	CB	C	29.080	0.400	1	426	42	42	LYS	HD3	H	1.615	0.020	2
180	18	18	GLN	CG	C	33.900	0.400	1	427	42	42	LYS	HE2	H	2.950	0.020	2
181	18	18	GLN	N	N	120.718	0.400	1	428	42	42	LYS	HE3	H	2.950	0.020	2
182	18	18	GLN	NE2	N	112.900	0.400	1	429	42	42	LYS	HG2	H	1.467	0.020	2
183	19	19	ALA	H	H	8.191	0.020	1	430	42	42	LYS	HG3	H	1.467	0.020	2
184	19	19	ALA	HA	H	4.272	0.020	1	431	42	42	LYS	CA	C	57.910	0.400	1
185	19	19	ALA	HB	H	1.481	0.020	1	432	42	42	LYS	CB	C	33.100	0.400	1
186	19	19	ALA	CA	C	54.310	0.400	1	433	42	42	LYS	CD	C	29.950	0.400	1
187	19	19	ALA	CB	C	18.820	0.400	1	434	42	42	LYS	CE	C	42.370	0.400	1
188	19	19	ALA	N	N	123.787	0.400	1	435	42	42	LYS	CG	C	25.710	0.400	1
189	20	20	VAL	H	H	8.000	0.020	1	436	42	42	LYS	N	N	118.809	0.400	1
190	20	20	VAL	HA	H	3.857	0.020	1	437	43	43	LYS	H	H	7.860	0.020	1
191	20	20	VAL	HB	H	2.128	0.020	1	438	43	43	LYS	HA	H	4.203	0.020	1
192	20	20	VAL	HG1	H	1.027	0.020	2	439	43	43	LYS	HB2	H	1.877	0.020	2
193	20	20	VAL	HG2	H	1.027	0.020	2	440	43	43	LYS	HB3	H	1.877	0.020	2
194	20	20	VAL	CA	C	64.950	0.400	1	441	43	43	LYS	HD2	H	1.731	0.020	2
195	20	20	VAL	CB	C	32.620	0.400	1	442	43	43	LYS	HD3	H	1.731	0.020	2
196	20	20	VAL	CG1	C	22.180	0.400	2	443	43	43	LYS	HE2	H	2.982	0.020	2
197	20	20	VAL	CG2	C	22.180	0.400	2	444	43	43	LYS	HE3	H	2.982	0.020	2
198	20	20	VAL	N	N	118.988	0.400	1	445	43	43	LYS	HG2	H	1.446	0.020	2
199	21	21	LYS	H	H	8.117	0.020	1	446	43	43	LYS	HG3	H	1.574	0.020	2
200	21	21	LYS	HA	H	4.197	0.020	1	447	43	43	LYS	CA	C	57.380	0.400	1
201	21	21	LYS	HB2	H	1.895	0.020	2	448	43	43	LYS	CB	C	33.140	0.400	1
202	21	21	LYS	HB3	H	1.895	0.020	2	449	43	43	LYS	CD	C	29.740	0.400	1
203	21	21	LYS	HD2	H	1.711	0.020	2	450	43	43	LYS	CE	C	42.350	0.400	1
204	21	21	LYS	HD3	H	1.711	0.020	2	451	43	43	LYS	CG	C	25.410	0.400	1
205	21	21	LYS	HE2	H	2.997	0.020	2	452	43	43	LYS	N	N	120.047	0.400	1
206	21	21	LYS	HE3	H	2.997	0.020	2	453	44	44	LEU	H	H	7.919	0.020	1
207	21	21	LYS	HG2	H	1.476	0.020	2	454	44	44	LEU	HA	H	4.336	0.020	1
208	21	21	LYS	HG3	H	1.476	0.020	2	455	44	44	LEU	HB2	H	1.685	0.020	2
209	21	21	LYS	CA	C	58.390	0.400	1	456	44	44	LEU	HB3	H	1.685	0.020	2
210	21	21	LYS	CB	C	32.910	0.400	1	457	44	44	LEU	HD1	H	0.915	0.020	2
211	21	21	LYS	CD	C	29.530	0.400	1	458	44	44	LEU	HD2	H	0.915	0.020	2
212	21	21	LYS	CE	C	42.420	0.400	1	459	44	44	LEU	CA	C	55.680	0.400	1
213	21	21	LYS	CG	C	25.070	0.400	1	460	44	44	LEU	CB	C	42.510	0.400	1
214	21	21	LYS	N	N	123.022	0.400	1	461	44	44	LEU	CD1	C	25.200	0.400	2
215	22	22	GLU	H	H	8.308	0.020	1	462	44	44	LEU	CD2	C	23.560	0.400	2
216	22	22	GLU	HA	H	4.173	0.020	1	463	44	44	LEU	CG	C	27.340	0.400	1

Appendix C

Chemical shift assignments of E₁₋₅₂ of A₁A₀ ATP synthase from *M. jannaschii*

The chemical shift assignment are listed in the NMR-STAR format (Columns are as follows: Atom shift assign ID, Residue author sequence code, Residue sequence code, Residue label, Atom name, Atom type, Chemical shift value, Chemical shift value error, Chemical shift ambiguity code

1	1	1	MET	H	H	9.714	0.020	1	260	27	27	ILE	H	H	8.357	0.020	1
2	1	1	MET	HA	H	4.455	0.020	1	261	27	27	ILE	HA	H	3.827	0.020	1
3	1	1	MET	HB2	H	2.135	0.020	2	262	27	27	ILE	HB	H	1.948	0.020	1
4	1	1	MET	HB3	H	2.135	0.020	2	263	27	27	ILE	HD1	H	0.937	0.020	1
5	1	1	MET	HG2	H	2.609	0.020	2	264	27	27	ILE	HG12	H	1.564	0.020	2
6	1	1	MET	HG3	H	2.609	0.020	2	265	27	27	ILE	HG13	H	1.779	0.020	2
7	1	1	MET	C	C	177.900	0.400	1	266	27	27	ILE	HG2	H	1.208	0.020	1
8	1	1	MET	CA	C	57.020	0.400	1	267	27	27	ILE	C	C	179.000	0.400	1
9	1	1	MET	CB	C	31.410	0.400	1	268	27	27	ILE	CA	C	64.620	0.400	1
10	1	1	MET	N	N	121.448	0.400	1	269	27	27	ILE	CB	C	37.770	0.400	1
11	2	2	ALA	H	H	7.905	0.020	1	270	27	27	ILE	N	N	119.945	0.400	1
12	2	2	ALA	HA	H	4.072	0.020	1	271	28	28	SER	H	H	8.155	0.020	1
13	2	2	ALA	HB	H	1.506	0.020	1	272	28	28	SER	HA	H	4.258	0.020	1
14	2	2	ALA	C	C	179.200	0.400	1	273	28	28	SER	HB2	H	4.080	0.020	2
15	2	2	ALA	CA	C	55.420	0.400	1	274	28	28	SER	HB3	H	4.080	0.020	2
16	2	2	ALA	CB	C	17.460	0.400	1	275	28	28	SER	C	C	175.800	0.400	1
17	2	2	ALA	N	N	121.462	0.400	1	276	28	28	SER	CA	C	61.530	0.400	1
18	3	3	VAL	H	H	7.612	0.020	1	277	28	28	SER	CB	C	62.570	0.400	1
19	3	3	VAL	HA	H	3.722	0.020	1	278	28	28	SER	N	N	115.157	0.400	1
20	3	3	VAL	HB	H	2.139	0.020	1	279	29	29	GLU	H	H	8.272	0.020	1
21	3	3	VAL	HG1	H	0.962	0.020	2	280	29	29	GLU	HA	H	4.164	0.020	1
22	3	3	VAL	HG2	H	0.962	0.020	2	281	29	29	GLU	HB2	H	2.326	0.020	2
23	3	3	VAL	C	C	179.200	0.400	1	282	29	29	GLU	HB3	H	2.168	0.020	2
24	3	3	VAL	CA	C	65.420	0.400	1	283	29	29	GLU	HG2	H	2.518	0.020	2
25	3	3	VAL	CB	C	31.260	0.400	1	284	29	29	GLU	HG3	H	2.518	0.020	2
26	3	3	VAL	N	N	115.271	0.400	1	285	29	29	GLU	C	C	178.200	0.400	1
27	4	4	LYS	H	H	7.656	0.020	1	286	29	29	GLU	CA	C	59.120	0.400	1
28	4	4	LYS	HA	H	4.135	0.020	1	287	29	29	GLU	CB	C	28.650	0.400	1
29	4	4	LYS	HB2	H	1.972	0.020	2	288	29	29	GLU	N	N	122.866	0.400	1
30	4	4	LYS	HB3	H	1.972	0.020	2	289	30	30	ALA	H	H	8.303	0.020	1
31	4	4	LYS	HD2	H	1.751	0.020	2	290	30	30	ALA	HA	H	4.206	0.020	1
32	4	4	LYS	HD3	H	1.751	0.020	2	291	30	30	ALA	HB	H	1.578	0.020	1
33	4	4	LYS	HE2	H	2.978	0.020	2	292	30	30	ALA	C	C	179.200	0.400	1
34	4	4	LYS	HE3	H	2.978	0.020	2	293	30	30	ALA	CA	C	55.120	0.400	1
35	4	4	LYS	HG2	H	1.539	0.020	2	294	30	30	ALA	CB	C	17.180	0.400	1
36	4	4	LYS	HG3	H	1.539	0.020	2	295	30	30	ALA	N	N	123.088	0.400	1
37	4	4	LYS	C	C	178.600	0.400	1	296	31	31	GLU	H	H	8.439	0.020	1
38	4	4	LYS	CA	C	58.660	0.400	1	297	31	31	GLU	HA	H	4.108	0.020	1
39	4	4	LYS	CB	C	31.630	0.400	1	298	31	31	GLU	HB2	H	2.176	0.020	2
40	4	4	LYS	N	N	119.781	0.400	1	299	31	31	GLU	HB3	H	2.176	0.020	2
41	5	5	LEU	H	H	8.051	0.020	1	300	31	31	GLU	HG2	H	2.401	0.020	2
42	5	5	LEU	HA	H	4.150	0.020	1	301	31	31	GLU	HG3	H	2.401	0.020	2
43	5	5	LEU	HB2	H	1.805	0.020	2	302	31	31	GLU	C	C	178.200	0.400	1
44	5	5	LEU	HB3	H	1.805	0.020	2	303	31	31	GLU	CA	C	58.950	0.400	1
45	5	5	LEU	HD1	H	1.008	0.020	2	304	31	31	GLU	CB	C	28.890	0.400	1
46	5	5	LEU	HD2	H	0.843	0.020	2	305	31	31	GLU	N	N	117.999	0.400	1
47	5	5	LEU	C	C	179.200	0.400	1	306	32	32	ALA	H	H	7.988	0.020	1
48	5	5	LEU	CA	C	57.160	0.400	1	307	32	32	ALA	HA	H	4.191	0.020	1
49	5	5	LEU	CB	C	41.450	0.400	1	308	32	32	ALA	HB	H	1.175	0.020	1
50	6	6	MET	H	H	8.081	0.020	1	309	32	32	ALA	C	C	179.200	0.400	1
51	6	6	MET	HA	H	4.315	0.020	1	310	32	32	ALA	CA	C	54.970	0.400	1
52	6	6	MET	HB2	H	2.174	0.020	2	311	32	32	ALA	CB	C	17.260	0.400	1
53	6	6	MET	HB3	H	2.174	0.020	2	312	32	32	ALA	N	N	122.398	0.400	1
54	6	6	MET	HG2	H	2.722	0.020	2	313	33	33	GLU	H	H	8.060	0.020	1
55	6	6	MET	HG3	H	2.572	0.020	2	314	33	33	GLU	HA	H	4.144	0.020	1
56	6	6	MET	C	C	177.900	0.400	1	315	33	33	GLU	HB2	H	2.188	0.020	2
57	6	6	MET	CA	C	57.400	0.400	1	316	33	33	GLU	HB3	H	2.188	0.020	2
58	6	6	MET	CB	C	32.380	0.400	1	317	33	33	GLU	HG2	H	2.381	0.020	2
59	6	6	MET	N	N	117.170	0.400	1	318	33	33	GLU	HG3	H	2.381	0.020	2
60	7	7	GLY	H	H	7.987	0.020	1	319	33	33	GLU	C	C	178.200	0.400	1
61	7	7	GLY	HA2	H	3.995	0.020	2	320	33	33	GLU	CA	C	58.950	0.400	1
62	7	7	GLY	HA3	H	3.995	0.020	2	321	33	33	GLU	CB	C	28.890	0.400	1

63	7	7	GLY	C	C	175.700	0.400	1	322	33	33	GLU	N	N	118.582	0.400	1
64	7	7	GLY	CA	C	46.050	0.400	1	323	34	34	LYS	H	H	7.986	0.020	1
65	7	7	GLY	N	N	107.082	0.400	1	324	34	34	LYS	HA	H	3.972	0.020	1
66	8	8	VAL	H	H	7.928	0.020	1	325	34	34	LYS	HB2	H	2.169	0.020	2
67	8	8	VAL	HA	H	3.807	0.020	1	326	34	34	LYS	HB3	H	1.992	0.020	2
68	8	8	VAL	HB	H	2.204	0.020	1	327	34	34	LYS	HD2	H	1.625	0.020	2
69	8	8	VAL	HG1	H	1.029	0.020	2	328	34	34	LYS	HD3	H	1.625	0.020	2
70	8	8	VAL	HG2	H	1.029	0.020	2	329	34	34	LYS	HE2	H	2.932	0.020	2
71	8	8	VAL	C	C	179.200	0.400	1	330	34	34	LYS	HE3	H	2.932	0.020	2
72	8	8	VAL	CA	C	65.270	0.400	1	331	34	34	LYS	HG2	H	1.593	0.020	2
73	8	8	VAL	CB	C	31.190	0.400	1	332	34	34	LYS	HG3	H	1.593	0.020	2
74	8	8	VAL	N	N	120.229	0.400	1	333	34	34	LYS	C	C	178.600	0.400	1
75	9	9	ASP	H	H	8.260	0.020	1	334	34	34	LYS	CA	C	58.110	0.400	1
76	9	9	ASP	HA	H	4.415	0.020	1	335	34	34	LYS	CB	C	30.980	0.400	1
77	9	9	ASP	HB2	H	2.726	0.020	2	336	34	34	LYS	N	N	119.109	0.400	1
78	9	9	ASP	HB3	H	2.726	0.020	2	337	35	35	ALA	H	H	7.977	0.020	1
79	9	9	ASP	C	C	179.300	0.400	1	338	35	35	ALA	HA	H	4.067	0.020	1
80	9	9	ASP	CA	C	56.950	0.400	1	339	35	35	ALA	HB	H	1.552	0.020	1
81	9	9	ASP	CB	C	40.240	0.400	1	340	35	35	ALA	C	C	179.200	0.400	1
82	9	9	ASP	N	N	119.778	0.400	1	341	35	35	ALA	CA	C	55.180	0.400	1
83	10	10	LYS	H	H	7.901	0.020	1	342	35	35	ALA	CB	C	16.830	0.400	1
84	10	10	LYS	HA	H	4.133	0.020	1	343	35	35	ALA	N	N	119.701	0.400	1
85	10	10	LYS	HB2	H	1.983	0.020	2	344	36	36	LYS	H	H	7.752	0.020	1
86	10	10	LYS	HB3	H	1.983	0.020	2	345	36	36	LYS	HA	H	4.098	0.020	1
87	10	10	LYS	HD2	H	1.692	0.020	2	346	36	36	LYS	HB2	H	1.996	0.020	2
88	10	10	LYS	HD3	H	1.692	0.020	2	347	36	36	LYS	HB3	H	2.093	0.020	2
89	10	10	LYS	HE2	H	2.977	0.020	2	348	36	36	LYS	HD2	H	1.733	0.020	2
90	10	10	LYS	HE3	H	2.977	0.020	2	349	36	36	LYS	HD3	H	1.733	0.020	2
91	10	10	LYS	HG2	H	1.542	0.020	2	350	36	36	LYS	HE2	H	2.949	0.020	2
92	10	10	LYS	HG3	H	1.542	0.020	2	351	36	36	LYS	HE3	H	2.949	0.020	2
93	10	10	LYS	C	C	178.600	0.400	1	352	36	36	LYS	HG2	H	1.592	0.020	2
94	10	10	LYS	CA	C	59.870	0.400	1	353	36	36	LYS	HG3	H	1.592	0.020	2
95	10	10	LYS	CB	C	31.780	0.400	1	354	36	36	LYS	C	C	178.600	0.400	1
96	10	10	LYS	N	N	119.866	0.400	1	355	36	36	LYS	CA	C	59.110	0.400	1
97	11	11	ILE	H	H	7.778	0.020	1	356	36	36	LYS	CB	C	31.780	0.400	1
98	11	11	ILE	HA	H	3.837	0.020	1	357	36	36	LYS	N	N	117.583	0.400	1
99	11	11	ILE	HB	H	2.005	0.020	1	358	37	37	ILE	H	H	7.960	0.020	1
100	11	11	ILE	HD1	H	0.917	0.020	1	359	37	37	ILE	HA	H	3.719	0.020	1
101	11	11	ILE	HG12	H	1.660	0.020	2	360	37	37	ILE	HB	H	2.044	0.020	1
102	11	11	ILE	HG13	H	1.660	0.020	2	361	37	37	ILE	HD1	H	0.911	0.020	1
103	11	11	ILE	HG2	H	1.188	0.020	1	362	37	37	ILE	HG12	H	1.576	0.020	2
104	11	11	ILE	C	C	179.000	0.400	1	363	37	37	ILE	HG13	H	1.798	0.020	2
105	11	11	ILE	CA	C	64.300	0.400	1	364	37	37	ILE	HG2	H	1.164	0.020	1
106	11	11	ILE	CB	C	37.140	0.400	1	365	37	37	ILE	C	C	179.000	0.400	1
107	11	11	ILE	N	N	120.208	0.400	1	366	37	37	ILE	CA	C	64.980	0.400	1
108	12	12	LYS	H	H	8.311	0.020	1	367	37	37	ILE	CB	C	37.460	0.400	1
109	12	12	LYS	HA	H	3.964	0.020	1	368	37	37	ILE	N	N	120.897	0.400	1
110	12	12	LYS	HB2	H	1.934	0.020	2	369	38	38	LEU	H	H	8.153	0.020	1
111	12	12	LYS	HB3	H	1.934	0.020	2	370	38	38	LEU	HA	H	4.106	0.020	1
112	12	12	LYS	HD2	H	1.642	0.020	2	371	38	38	LEU	HB2	H	1.846	0.020	2
113	12	12	LYS	HD3	H	1.642	0.020	2	372	38	38	LEU	HB3	H	1.587	0.020	2
114	12	12	LYS	HE2	H	2.933	0.020	2	373	38	38	LEU	HD1	H	0.906	0.020	2
115	12	12	LYS	HE3	H	2.933	0.020	2	374	38	38	LEU	HD2	H	0.906	0.020	2
116	12	12	LYS	HG2	H	1.433	0.020	2	375	38	38	LEU	C	C	179.200	0.400	1
117	12	12	LYS	HG3	H	1.433	0.020	2	376	38	38	LEU	CA	C	57.730	0.400	1
118	12	12	LYS	C	C	178.600	0.400	1	377	38	38	LEU	CB	C	40.980	0.400	1
119	12	12	LYS	CA	C	59.870	0.400	1	378	38	38	LEU	N	N	120.156	0.400	1
120	12	12	LYS	CB	C	31.780	0.400	1	379	39	39	GLU	H	H	8.115	0.020	1
121	12	12	LYS	N	N	119.425	0.400	1	380	39	39	GLU	HA	H	4.071	0.020	1
122	13	13	SER	H	H	7.964	0.020	1	381	39	39	GLU	HB2	H	2.206	0.020	2
123	13	13	SER	HA	H	4.154	0.020	1	382	39	39	GLU	HB3	H	2.206	0.020	2
124	13	13	SER	HB2	H	4.006	0.020	2	383	39	39	GLU	HG2	H	2.390	0.020	2
125	13	13	SER	HB3	H	4.006	0.020	2	384	39	39	GLU	HG3	H	2.390	0.020	2
126	13	13	SER	C	C	175.800	0.400	1	385	39	39	GLU	C	C	178.200	0.400	1
127	13	13	SER	CA	C	61.320	0.400	1	386	39	39	GLU	CA	C	59.260	0.400	1
128	13	13	SER	CB	C	62.540	0.400	1	387	39	39	GLU	CB	C	29.000	0.400	1
129	13	13	SER	N	N	112.107	0.400	1	388	39	39	GLU	N	N	119.628	0.400	1
130	14	14	LYS	H	H	7.681	0.020	1	389	40	40	LYS	H	H	7.951	0.020	1
131	14	14	LYS	HA	H	4.205	0.020	1	390	40	40	LYS	HA	H	4.095	0.020	1
132	14	14	LYS	HB2	H	1.973	0.020	2	391	40	40	LYS	HB2	H	1.989	0.020	2
133	14	14	LYS	HB3	H	1.973	0.020	2	392	40	40	LYS	HB3	H	1.989	0.020	2
134	14	14	LYS	HD2	H	1.755	0.020	2	393	40	40	LYS	HD2	H	1.734	0.020	2

135	14	14	LYS	HD3	H	1.755	0.020	2	394	40	40	LYS	HD3	H	1.734	0.020	2
136	14	14	LYS	HE2	H	2.956	0.020	2	395	40	40	LYS	HE2	H	2.993	0.020	2
137	14	14	LYS	HE3	H	2.738	0.020	2	396	40	40	LYS	HE3	H	2.993	0.020	2
138	14	14	LYS	HG2	H	1.547	0.020	2	397	40	40	LYS	HG2	H	1.595	0.020	2
139	14	14	LYS	HG3	H	1.547	0.020	2	398	40	40	LYS	HG3	H	1.595	0.020	2
140	14	14	LYS	C	C	178.600	0.400	1	399	40	40	LYS	C	C	178.600	0.400	1
141	14	14	LYS	CA	C	58.750	0.400	1	400	40	40	LYS	CA	C	59.380	0.400	1
142	14	14	LYS	CB	C	31.590	0.400	1	401	40	40	LYS	CB	C	31.820	0.400	1
143	14	14	LYS	N	N	123.289	0.400	1	402	40	40	LYS	N	N	120.185	0.400	1
144	15	15	ILE	H	H	8.163	0.020	1	403	41	41	ALA	H	H	8.550	0.020	1
145	15	15	ILE	HA	H	3.813	0.020	1	404	41	41	ALA	HA	H	4.120	0.020	1
146	15	15	ILE	HB	H	1.976	0.020	1	405	41	41	ALA	HB	H	1.540	0.020	1
147	15	15	ILE	HD1	H	0.907	0.020	1	406	41	41	ALA	C	C	179.200	0.400	1
148	15	15	ILE	HG2	H	1.174	0.020	1	407	41	41	ALA	CA	C	55.060	0.400	1
149	15	15	ILE	C	C	179.000	0.400	1	408	41	41	ALA	CB	C	17.490	0.400	1
150	15	15	ILE	CA	C	64.780	0.400	1	409	41	41	ALA	N	N	122.931	0.400	1
151	15	15	ILE	CB	C	37.300	0.400	1	410	42	42	LYS	H	H	8.257	0.020	1
152	15	15	ILE	N	N	119.789	0.400	1	411	42	42	LYS	HA	H	4.036	0.020	1
153	16	16	LEU	H	H	8.033	0.020	1	412	42	42	LYS	HB2	H	2.006	0.020	2
154	16	16	LEU	HA	H	4.183	0.020	1	413	42	42	LYS	HB3	H	2.006	0.020	2
155	16	16	LEU	HB2	H	1.838	0.020	2	414	42	42	LYS	HE2	H	2.948	0.020	2
156	16	16	LEU	HB3	H	1.589	0.020	2	415	42	42	LYS	HE3	H	2.948	0.020	2
157	16	16	LEU	HD1	H	0.904	0.020	2	416	42	42	LYS	HG2	H	1.542	0.020	2
158	16	16	LEU	HD2	H	0.904	0.020	2	417	42	42	LYS	HG3	H	1.542	0.020	2
159	16	16	LEU	C	C	179.200	0.400	1	418	42	42	LYS	C	C	178.600	0.400	1
160	16	16	LEU	CA	C	57.410	0.400	1	419	42	42	LYS	CA	C	57.040	0.400	1
161	16	16	LEU	CB	C	41.260	0.400	1	420	42	42	LYS	CB	C	31.700	0.400	1
162	16	16	LEU	N	N	119.388	0.400	1	421	42	42	LYS	N	N	119.296	0.400	1
163	17	17	ASP	H	H	8.210	0.020	1	422	43	43	GLU	H	H	7.979	0.020	1
164	17	17	ASP	HA	H	4.495	0.020	1	423	43	43	GLU	HA	H	4.070	0.020	1
165	17	17	ASP	HB2	H	2.939	0.020	2	424	43	43	GLU	HB2	H	2.010	0.020	2
166	17	17	ASP	HB3	H	2.760	0.020	2	425	43	43	GLU	HB3	H	2.010	0.020	2
167	17	17	ASP	C	C	179.300	0.400	1	426	43	43	GLU	HG2	H	2.370	0.020	2
168	17	17	ASP	CA	C	57.440	0.400	1	427	43	43	GLU	HG3	H	2.370	0.020	2
169	17	17	ASP	CB	C	40.470	0.400	1	428	43	43	GLU	C	C	178.200	0.400	1
170	17	17	ASP	N	N	120.521	0.400	1	429	43	43	GLU	CA	C	58.960	0.400	1
171	18	18	ASP	H	H	8.589	0.020	1	430	43	43	GLU	CB	C	28.940	0.400	1
172	18	18	ASP	HA	H	4.501	0.020	1	431	43	43	GLU	N	N	119.676	0.400	1
173	18	18	ASP	HB2	H	2.940	0.020	2	432	44	44	GLU	H	H	8.278	0.020	1
174	18	18	ASP	HB3	H	2.700	0.020	2	433	44	44	GLU	HA	H	4.069	0.020	1
175	18	18	ASP	C	C	179.300	0.400	1	434	44	44	GLU	HB2	H	2.159	0.020	2
176	18	18	ASP	CA	C	57.150	0.400	1	435	44	44	GLU	HB3	H	2.159	0.020	2
177	18	18	ASP	CB	C	39.550	0.400	1	436	44	44	GLU	HG2	H	2.366	0.020	2
178	18	18	ASP	N	N	121.775	0.400	1	437	44	44	GLU	HG3	H	2.366	0.020	2
179	19	19	ALA	H	H	8.393	0.020	1	438	44	44	GLU	C	C	178.200	0.400	1
180	19	19	ALA	HA	H	4.196	0.020	1	439	44	44	GLU	CA	C	58.960	0.400	1
181	19	19	ALA	HB	H	1.560	0.020	1	440	44	44	GLU	CB	C	28.940	0.400	1
182	19	19	ALA	C	C	179.200	0.400	1	441	44	44	GLU	N	N	118.681	0.400	1
183	19	19	ALA	CA	C	54.970	0.400	1	442	45	45	ALA	H	H	8.160	0.020	1
184	19	19	ALA	CB	C	17.040	0.400	1	443	45	45	ALA	HA	H	4.079	0.020	1
185	19	19	ALA	N	N	123.962	0.400	1	444	45	45	ALA	HB	H	1.547	0.020	1
186	20	20	LYS	H	H	8.277	0.020	1	445	45	45	ALA	C	C	179.200	0.400	1
187	20	20	LYS	HA	H	4.041	0.020	1	446	45	45	ALA	CA	C	54.910	0.400	1
188	20	20	LYS	HB2	H	1.983	0.020	2	447	45	45	ALA	CB	C	17.360	0.400	1
189	20	20	LYS	HB3	H	1.983	0.020	2	448	45	45	ALA	N	N	121.693	0.400	1
190	20	20	LYS	HD2	H	1.709	0.020	2	449	46	46	GLU	H	H	8.106	0.020	1
191	20	20	LYS	HD3	H	1.709	0.020	2	450	46	46	GLU	HA	H	4.083	0.020	1
192	20	20	LYS	HE2	H	2.940	0.020	2	451	46	46	GLU	HB2	H	2.164	0.020	2
193	20	20	LYS	HE3	H	2.940	0.020	2	452	46	46	GLU	HB3	H	2.164	0.020	2
194	20	20	LYS	HG2	H	1.543	0.020	2	453	46	46	GLU	HG2	H	2.415	0.020	2
195	20	20	LYS	HG3	H	1.543	0.020	2	454	46	46	GLU	HG3	H	2.415	0.020	2
196	20	20	LYS	C	C	178.600	0.400	1	455	46	46	GLU	C	C	178.200	0.400	1
197	20	20	LYS	CA	C	59.300	0.400	1	456	46	46	GLU	CA	C	58.170	0.400	1
198	20	20	LYS	CB	C	31.820	0.400	1	457	46	46	GLU	CB	C	28.920	0.400	1
199	20	20	LYS	N	N	118.639	0.400	1	458	46	46	GLU	N	N	117.210	0.400	1
200	21	21	ALA	H	H	7.989	0.020	1	459	47	47	LYS	H	H	7.905	0.020	1
201	21	21	ALA	HA	H	4.193	0.020	1	460	47	47	LYS	HA	H	4.106	0.020	1
202	21	21	ALA	HB	H	1.593	0.020	1	461	47	47	LYS	HB2	H	2.165	0.020	2
203	21	21	ALA	C	C	179.200	0.400	1	462	47	47	LYS	HB3	H	2.165	0.020	2
204	21	21	ALA	CA	C	54.910	0.400	1	463	47	47	LYS	HD2	H	1.710	0.020	2
205	21	21	ALA	CB	C	17.410	0.400	1	464	47	47	LYS	HD3	H	1.710	0.020	2
206	21	21	ALA	N	N	121.825	0.400	1	465	47	47	LYS	HE2	H	3.005	0.020	2

207	22	22	GLU	H	H	8.123	0.020	1	466	47	47	LYS	HE3	H	3.005	0.020	2
208	22	22	GLU	HA	H	4.166	0.020	1	467	47	47	LYS	C	C	178.600	0.400	1
209	22	22	GLU	HB2	H	2.170	0.020	2	468	47	47	LYS	CA	C	57.350	0.400	1
210	22	22	GLU	HB3	H	2.170	0.020	2	469	47	47	LYS	CB	C	32.200	0.400	1
211	22	22	GLU	HG2	H	2.363	0.020	2	470	47	47	LYS	N	N	119.153	0.400	1
212	22	22	GLU	HG3	H	2.363	0.020	2	471	48	48	ARG	H	H	7.846	0.020	1
213	22	22	GLU	C	C	178.200	0.400	1	472	48	48	ARG	HA	H	4.234	0.020	1
214	22	22	GLU	CA	C	58.840	0.400	1	473	48	48	ARG	HB2	H	1.950	0.020	2
215	22	22	GLU	CB	C	28.900	0.400	1	474	48	48	ARG	HB3	H	1.950	0.020	2
216	22	22	GLU	N	N	119.363	0.400	1	475	48	48	ARG	HD2	H	3.207	0.020	2
217	23	23	ALA	H	H	8.251	0.020	1	476	48	48	ARG	HD3	H	3.207	0.020	2
218	23	23	ALA	HA	H	4.109	0.020	1	477	48	48	ARG	HG2	H	1.728	0.020	2
219	23	23	ALA	HB	H	1.511	0.020	1	478	48	48	ARG	HG3	H	1.728	0.020	2
220	23	23	ALA	C	C	179.200	0.400	1	479	48	48	ARG	C	C	178.600	0.400	1
221	23	23	ALA	CA	C	55.100	0.400	1	480	48	48	ARG	CA	C	57.350	0.400	1
222	23	23	ALA	CB	C	16.900	0.400	1	481	48	48	ARG	CB	C	29.640	0.400	1
223	23	23	ALA	N	N	121.717	0.400	1	482	48	48	ARG	N	N	117.908	0.400	1
224	24	24	ASN	H	H	8.228	0.020	1	483	49	49	LYS	H	H	7.772	0.020	1
225	24	24	ASN	HA	H	4.460	0.020	1	484	49	49	LYS	HA	H	4.177	0.020	1
226	24	24	ASN	HB2	H	2.873	0.020	2	485	49	49	LYS	HB2	H	1.922	0.020	2
227	24	24	ASN	HB3	H	2.873	0.020	2	486	49	49	LYS	HB3	H	1.922	0.020	2
228	24	24	ASN	HD21	H	7.477	0.020	2	487	49	49	LYS	HG2	H	1.520	0.020	2
229	24	24	ASN	HD22	H	6.671	0.020	2	488	49	49	LYS	HG3	H	1.520	0.020	2
230	24	24	ASN	C	C	177.200	0.400	1	489	49	49	LYS	C	C	178.600	0.400	1
231	24	24	ASN	CA	C	55.760	0.400	1	490	49	49	LYS	CA	C	57.350	0.400	1
232	24	24	ASN	CB	C	38.330	0.400	1	491	49	49	LYS	CB	C	32.120	0.400	1
233	24	24	ASN	N	N	114.245	0.400	1	492	49	49	LYS	N	N	119.320	0.400	1
234	24	24	ASN	ND2	N	110.540	0.400	1	493	50	50	ALA	H	H	7.785	0.020	1
235	25	25	LYS	H	H	7.888	0.020	1	494	50	50	ALA	HA	H	4.365	0.020	1
236	25	25	LYS	HA	H	4.102	0.020	1	495	50	50	ALA	HB	H	1.480	0.020	1
237	25	25	LYS	HB2	H	2.111	0.020	2	496	50	50	ALA	C	C	179.200	0.400	1
238	25	25	LYS	HB3	H	2.009	0.020	2	497	50	50	ALA	CA	C	52.270	0.400	1
239	25	25	LYS	HD2	H	1.764	0.020	2	498	50	50	ALA	CB	C	18.650	0.400	1
240	25	25	LYS	HD3	H	1.764	0.020	2	499	50	50	ALA	N	N	121.668	0.400	1
241	25	25	LYS	HE2	H	2.935	0.020	2	500	51	51	GLU	H	H	7.924	0.020	1
242	25	25	LYS	HE3	H	2.935	0.020	2	501	51	51	GLU	HA	H	4.343	0.020	1
243	25	25	LYS	HG2	H	1.595	0.020	2	502	51	51	GLU	HB2	H	2.160	0.020	1
244	25	25	LYS	HG3	H	1.595	0.020	2	503	51	51	GLU	HB3	H	1.978	0.020	1
245	25	25	LYS	C	C	178.600	0.400	1	504	51	51	GLU	HG2	H	2.354	0.020	1
246	25	25	LYS	CA	C	59.390	0.400	1	505	51	51	GLU	HG3	H	2.354	0.020	1
247	25	25	LYS	CB	C	31.430	0.400	1	506	51	51	GLU	C	C	178.200	0.400	1
248	25	25	LYS	N	N	121.836	0.400	1	507	51	51	GLU	CA	C	56.370	0.400	1
249	26	26	ILE	H	H	7.949	0.020	1	508	51	51	GLU	CB	C	29.790	0.400	1
250	26	26	ILE	HA	H	3.828	0.020	1	509	51	51	GLU	N	N	118.979	0.400	1
251	26	26	ILE	HB	H	2.015	0.020	1	510	52	52	ILE	H	H	7.431	0.020	1
252	26	26	ILE	HD1	H	0.938	0.020	1	511	52	52	ILE	HA	H	4.108	0.020	1
253	26	26	ILE	HG12	H	1.572	0.020	2	512	52	52	ILE	HB	H	1.875	0.020	1
254	26	26	ILE	HG13	H	1.774	0.020	2	513	52	52	ILE	HD1	H	0.923	0.020	1
255	26	26	ILE	HG2	H	1.212	0.020	1	514	52	52	ILE	HG12	H	1.473	0.020	2
256	26	26	ILE	C	C	179.000	0.400	1	515	52	52	ILE	HG13	H	1.473	0.020	2
257	26	26	ILE	CA	C	64.310	0.400	1	516	52	52	ILE	HG2	H	1.168	0.020	1
258	26	26	ILE	CB	C	37.420	0.400	1	517	52	52	ILE	C	C	179.000	0.400	1
259	26	26	ILE	N	N	119.989	0.400	1	518	52	52	ILE	CA	C	62.830	0.400	1
									519	52	52	ILE	CB	C	39.260	0.400	1
									520	52	52	ILE	N	N	124.557	0.400	1

Author's publications during Ph.D period January 2006 to June 2010

1. Biuković, G., M. Rössle, **S. Gayen**, Y. Mu and G. Grüber (2007). *Small-Angle X-ray Scattering Reveals the Solution Structure of the Peripheral Stalk Subunit H of the A₁A_O ATP Synthase from Methanocaldococcus jannaschii and Its Binding to the Catalytic A Subunit*. *Biochemistry* **46**: 2070-2078.
2. **Gayen, S***, S. Vivekanandan*, G. Biuković, G. Grüber, and H. S. Yoon (2007). *Backbone ¹H, ¹³C, and ¹⁵N resonance assignments of subunit F of the A₁A_O ATP synthase from Methanosarcina mazei Gö1*. *Biomol. NMR Assign.* **1**: 23-25. (*Authors have equal contribution)
3. **Gayen, S***, S. Vivekanandan*, G. Biuković, G. Gruber and H. S. Yoon (2007). *NMR Solution Structure of Subunit F of the Methanogenic A₁A_O Adenosine Triphosphate Synthase and Its Interaction with the Nucleotide-Binding Subunit B*. *Biochemistry* **46**: 11684-11694. (*Authors have equal contribution)
4. **Gayen, S.**, A. M. Balakrishna, G. Biuković, W. Yulei, C. Hunke and G. Grüber (2008). *Identification of critical residues of subunit H in its interaction with subunit E of the A-ATP synthase from Methanocaldococcus jannaschii*. *FEBS J.* **275**: 1803-1812.
5. Rishikesan, S., R. Y. Thaker, R. Priya, **S. Gayen**, M. S. S. Manimekalai, C. Hunke and G. Grüber (2008). *Spectroscopical identification of residues of subunit G of the yeast V-ATPase in its connection with subunit E*. *Mol. Mem. Biol.* **25**: 400-410.
6. Priya, R., V. Tadwal, M. Rössle, **S. Gayen**, C. Hunke, W. Peng, J. Torres and G. Grüber (2008). *Low resolution structure of subunit b (b₂₂₋₁₅₆) of Escherichia coli F₁F_O ATP synthase in solution and the b-δ assembly*. *J. Bioenerg. Biomembr.* **40**: 245-255.
7. Rishikesan, S., **S. Gayen**, R. Y. Thaker, S. Vivekanandan, M. S. S. Manimekalai, Y. H. Yau, S. Greifman Shochat and G. Grüber (2009). *Assembly of subunit d (Vma6p) and G (Vma10p) and the NMR solution structure of subunit G (G₁₋₅₉) of the Saccharomyces cerevisiae V₁V_O ATPase*. *Biochim. Biophys. Acta-Bioenergetics* **1787**: 242-251.
8. Biuković, G*, **S. Gayen***, K. Pervushin and G. Grüber (2009). *The domain features of the peripheral stalk subunit H of the methanogenic A₁A_O ATP synthase and the NMR solution structure of H₁₋₄₇*. *Biophys. J.* **97**: 286-294. (*Authors have equal contribution)

9. **Gayen, S.**, A. M. Balakrishna and G. Grüber (2009). *NMR solution structure of the N-terminal domain of subunit E (E_{1-52}) of A_1A_O ATP synthase from *Methanocaldococcus jannaschii*. J. Bioenerg. Biomembr. **41**: 342-348.*
10. Priya, R., G. Biuković, **S. Gayen**, S. Vivekanandan and G. Grüber (2009). *NMR solution structure of the b30-82 domain of subunit b of Escherichia coli F_1F_O ATP synthase. J. Bacteriol. **191**: 7538-7544.*
11. **Gayen, S** and Grüber G (2009). *Disulfide linkage in the coiled coil domain of subunit H of A_1A_O ATP synthase from Methanocaldococcus jannaschii and the NMR structure of C-terminal segment H_{85-104} . FEBS Lett. **584**: 713-718.*
12. Devanathan, R, **S. Gayen**, G. Grüber and C. S. Verma (2009). *Cross-talk along the stalk: Dynamics of the interaction of Subunit B and Subunit F in A_1A_O ATP Synthase of Methanosarcina mazei Gö1. Biochemistry. **49**: 4181-4190.*
13. Balakrishna, A. M., M.S.S. Manimekalai, C. Hunke, **S. Gayen**, M. Rössle, J. Jeyakanthan, and G. Grüber (2010). *Crystal and solution structure of the C-terminal part of the Methanocaldococcus jannaschii A_1A_O ATP synthase subunit E revealed by X-ray diffraction and small-angle X-ray scattering. J. Bioenerg. Biomembr., in press.*

Conference presentation

1. Joint Third AOHUPO and Fourth Structural Biology and Functional Genomics Conference
NUS, Singapore from 4-7th Dec 2006.
Title: Solution structure of subunit F of A_1A_O ATP synthase from *Methanosarcina mazei*
Gö1.
2. 16th triennial conference for the international society of magnetic resonance (ISMAR 2007)
Kenting, Taiwan from 14th – 19th October 2007, pp218.
Title: Structural features of subunit H of A_1A_O ATP synthase in solution.