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NMR Solution Structure of the Protein PsbQ from Photosystem II

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Linz, October 2013

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Abstract

The PsbQ protein (16.5 kDa) is an extrinsic protein found in the thylakoid membrane of higher plants and green algae. As a member of the Psb protein family, it is situated in the oxygen evolving center and takes part in the water splitting reaction. The stable oxygen production in photosystem II depends on the cooperation of PsbQ with other photosynthetic proteins, mainly PsbP. In order to identify the possible interaction sites, the tertiary structure in solution has to be determined. Although the X-ray crystallographic structure of PsbQ was determined previously, the conformation of residues 14-33 (so-called "missing link") was still unknown at the onset of this work. The initial backbone assignment as well as a secondary structure estimation were achieved recently. In this thesis the resonance assignment was extended and ^{15}N as well as ^{13}C NOESY-HSQC spectra were recorded to obtain structural constraints. The solution structure was determined using the program CYANA. The results obtained show that, while the four helix bundle domain is nearly identical compared to the available X-Ray crystallographic structure significant deviations occur in the N-terminal region. In particular, the residues 37-41, where a short β-strand had been proposed in the crystal structure, exhibit high α-helical propensity.

List of abbreviations and acronyms

AEBSF	4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride
APS	Ammonium peroxodisulfate
Bis-Tris	(Bis(2-hydroxyethyl)-amino-tris(hydroxymethyl)-methane)
CARA (software)	Computer Aided Resonance Assignment
CM	Carboxymethyl
CPU	Central Processing Unit
CUDA	Compute Unified Device Architecture
CYANA (software)	Combined assignment and dynamics algorithm for NMR applications
DEAE	Diethylaminoethyl
DPFGSE-NOE	Double Pulsed Field Gradient Spin Echo NOE
DSS	4,4-dimethyl-4-silapentane-1-sulfonic acid
DYANA (software)	Dynamics Algorithm for Nmr Applications
E. coli	Escherichia coli
EDTA	Ethylenediaminetetraacetic acid
HOESY	Heteronuclear Overhauser Effect Spectroscopy
HSQC	Heteronuclear single quantum coherence spectroscopy
IEC	Ion Exchange Chromatography
IPTG	Isopropyl β-D-1-thiogalactopyranoside
LB medium	Lysogenybroth medium
MD	Molecular Dynamics
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
NOESY	Heteronuclear Overhauser Effect Spectroscopy
PAGE	Polyacrylamide gel electrophoresis
PDB	Protein Data Bank
PSII	Photosystem II
RDC	Residual Dipolar Coupling
RF	Radiofrequency
RMSD	Root-mean-square deviation
ROESY	Rotational Frame Nuclear Overhauser Effect Spectroscopy
SDS	Sodium Dodecyl Sulphate
SE	Sulfoxyethyl
SEC	Size Exclusion Chromatography
SP	Sulfopropyl
TEAE	Triethyl amine
TEMED	Tetramethylethylenediamine
TMAE	Trimethylaminoethyl
TOCSY	Total Correlation Spectroscopy
Tris base	Tris(hydroxymethyl)aminomethane
TRNOE	Transferred Nuclear Overhauser Effect
VMD	Visual Molecular Dynamics
YASARA (software)	Yet Another Scientific Artificial Reality Application

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1. Introduction

1.1. Extrinsic proteins of Photosystem II

Photosystem II (PSII) is a multiprotein and pigment complex with a size of approximately 1100 kDa. PSII is located in thylakoid membrane of higher plants, green algae and cyanobacteria. Since the main function of PSII is the catalysis of the electron transfer from water molecule to the plastoquinone, it is classified as water-plastoquinone oxidoreductase. The whole PSII comprises 20 subunits which can be divided into two major groups.

The first group comprises intrinsic transmembrane proteins, which are tightly embedded within the phospholipid bilayer. These proteins are conserved across a wide range of photosynthetic species. Representatives, as for example PsbB and PsbC (also known as CP47 and CP43, respectively), are polypeptides capable of binding chlorophyll and beta-carotene in order to pass the excitation energy to the reaction center (1).

The second group consists of so-called extrinsic proteins. These proteins are, by contrast to the first group, loosely attached to the surface of phospholipid bilayer and can be found in varying forms among many different species. PsbQ (17 kDa), PsbP (23 kDa) and PsbR (10 kDa) belong to the group of extrinsic polypeptides exclusively found in green algae and higher plants. In cyanobacteria, these proteins are altered to PsbU (12 kDa), PsbV (15 kDa), CyanoQ and CyanoP (analogues of PsbP and PsbQ) (2). Their main function is the regulation of oxygen evolution during photosynthesis. The only common extrinsic protein in higher plants and cyanobacteria is PsbO (33 kDa), which is attached to the intrinsic PsbB (45 kDa). As of to date, high-resolution three-dimensional X-Ray crystallographic structures for PsbQ and PsbP from *Spinacia Oleracea* have been determined (3, 4). However both structures are incomplete and they were crystallized under non-native conditions. Additionally, to solve the phasing problem, zinc ions were incorporated into the crystals which could have affected the proper folding of the proteins.

An alternative method to obtain structural information at atomic resolution is nuclear magnetic resonance (NMR). It is the only way to determine solutions structure at atomic resolution and also yields information on global and local chain dynamics. The first NMR solution structure of a photosynthetic protein from PSII was derived in 2009 (5).

To the present day, the solution structures for Psb27, Psb28 and CyanoP proteins from PSII from *Synechocystis* species have been resolved (PDB entry 2KMF, 2 KVO and 2LNJ, respectively) (5-7).

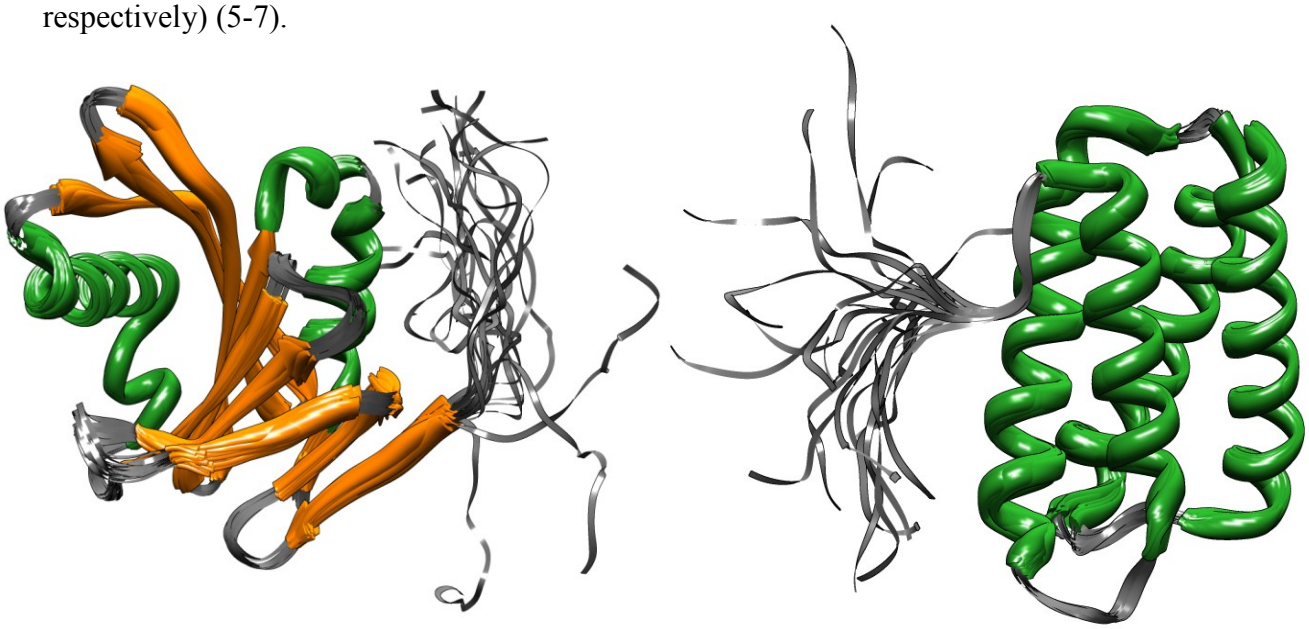

Figure 1: *NMR solution structure of CyanoP (left) and Psb27 (right) from Synechocystis sp. (5, 7).*

1.2. High resolution NMR spectroscopy

1.2.1. Theoretical background

The nuclear magnetic resonance spectroscopy is a technique, which allows observation of (bio)molecules at atomic resolution. It is based on the perturbation of nuclear magnetic spin within a constant magnetic field by employing electro-magnetic radio frequency (RF) pulses. The nuclear spin quantum number *I* is represented as a positive integer multiple of $^1/_2$. Nuclei with spin quantum number $I = 0$ have no spin and are called "NMR silent" (8). Unfortunately, the most abundant ^{12}C and ^{16}O isotopes (98.9% and 99.8%, respectively) are NMR silent and must be substituted by isotopes with $I = {}^1/_2$ (see Chapter 2.1.1) (9). Nuclei with $I > {}^1/_2$ (for example ^{14}N, ^{23}Na, ^{27}Al, ^{35}Cl, etc.) are known as "quadrupolar nuclei". The NMR properties of such nuclei are relatively complex, though very informative - especially in the context of solid-state NMR. But they are not generally used in 3D protein structure determination (10).

When a static magnetic field of strength B_0 is applied, the magnetic moment of a nucleus aligns only in $2I+1$ ways (for spin $^1/_2$ either parallel or antiparallel with respect to B_0). Since the nucleus is permanently spinning, the rotational axis must precess about this magnetic field (11). The rate of this precession is defined as Larmor velocity which is proportional to the field strength and the gyromagnetic ratio of a spin (8). As mentioned, the concept of nuclear magnetic resonance is based on the perturbation of the spin. In order to change the spin state, electromagnetic radiation pulse is applied to deliver a quantum of energy, which can be absorbed by the nucleus, changing its magnetic quantum numbers. However, when nuclei of the same nuclide resonate, their resonance frequencies are not necessarily the same. This phenomenon is called shielding effect and is caused by surrounding electron shells, whose charge and rotation produce an opposite magnetic field affecting the resonance frequency (9). Since the resonance frequency varies according to the electron density distribution and the signals of individual nuclei can be assigned according to the molecular structure (so called chemical shift).

Additionally, two types of magnetic interactions between two nuclear spins exist. Dipole-dipole coupling (also called dipolar coupling) results from direct interaction between two magnetic dipoles while J-coupling arises from hyperfine interactions between nuclei and local electrons. J-coupling is extremely important, since it contains information about the bond distance, angle and the molecular connectivity. Similarly, dipolar coupling contains also structural information but via cross relaxation, which finally results in the nuclear Overhauser effect, the ultimate source of inter-atom distance information (8).

1.2.2. The nuclear Overhauser effect

In 1953, Albert Overhauser proposed a theory, that perturbing electron magnetic resonance by saturation or inversion may change the intensity of nearby nuclear magnetic resonance transitions (12). This phenomenon was also found to exist between nuclei and later named "nuclear Overhauser effect" (NOE) (13). It was firstly experimentally proved in 1962 (14). The origin of the nuclear Overhauser effect relies on the transfer of nuclear spin polarization from one spin state to another spin state via cross relaxation.

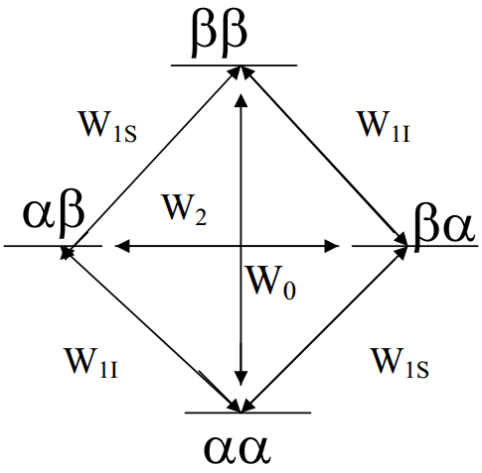

Figure 2: *The energy level diagram for a two spin system. For spin I and S three transition types can be distinguished. W_{1I} and W_{1S} transitions cause the flip of only one spin. The W_2 transition involves simultaneous flips of both spins, whereas W_0 consists of opposite spin transitions, ($\alpha \rightarrow \beta$) and ($\beta \rightarrow \alpha$)*

Since the dipole-dipole coupling acts through space, it depends on the distance between the nuclei. This kind of interaction is strongly distance dependent. The magnitude of the dipolar coupling constant is proportional to the inverse third power of the internuclear distance r. The NOE as a dissipative phenomenon depends on r^6. For this reason, the observable NOEs are usually restricted to a maximal distance of ~ 5 Å. Nowadays, a variety of different 2D and 3D experiments exploiting NOE have been developed in order to determine the 3D solution structure of biomolecules (15).

Abbreviation	Full name
NOESY	Nuclear Overhauser Effect Spectroscopy
HOESY	Heteronuclear Overhauser Effect Spectroscopy
TRNOE	Transferred Nuclear Overhauser Effect
ROESY	Rotational Frame Nuclear Overhauser Effect Spectroscopy
DPFGSE-NOE	Double Pulsed Field Gradient Spin Echo NOE experiment

Table 1: *Examples of two dimensional NMR spectra based on the NOE*

1.2.3. NMR solution structure determination

As mentioned previously, solution structure determination by NMR spectroscopy is an alternative to X-ray crystallography. The importance of this method is not only based on the structure determination but also lies in its ability to study intermolecular interactions and molecular dynamics pertaining to molecular function. Two major breakthroughs in NMR spectroscopy haven enabled the structure determination in solution.

The first one is the nuclear Overhauser effect. More recently exploitation of residual dipolar couplings (RDC) was introduced (16). This method is based on the partial alignment of a molecule in an anisotropic medium aligned in the magnetic field. This leads to an incomplete averaging of spatially anisotropic magnetic interactions (17). In contrast to traditional NOEs, which provide short range distance restraints, RDCs provide long-range orientational information. Since the analysis of NOEs is becoming complex with increasing protein size, the RDCs are commonly used for proteins larger than 25 kDa, in particular to resolve relative domain orientations.

The process of solution structure determination by NMR comprises many steps. Firstly, a (standard) set of assignment spectra (18) must be recorded and followed by the assignment of resonance frequencies (see chapters 2.4 and 2.5). The next step is the recording and assignment of observed NOE cross-peaks and/or RDC alignment tensors to atom pairs and subsequent conversion of these data into distance or orientation constraints. The key principle implemented in the software used here (19, 20) is starting from a random structure. Repetitive structure optimization in a field of constraints is performed with subsequent manual error corrections, which may arise from incorrect constraints or assignment ambiguities. The whole procedure is iterated until the number of deviated NOE constraints is minimal (lower the number, the more consistent structure ensemble is obtained). Since the constraints are defined as intervals (given as lower or upper limits) the calculations generate a number of conformers, that are "compatible" with the constraints. In contrast to X-ray crystallography, the result is always represented by a bundle of (usually 20) different conformers with independent atomic coordinates. In case of solution structure, the precision of the structure ensemble is determined by the root mean square deviation (RMSD) of the superimposed single structures. For two structures *A* and *B* with selected number of atoms *N*, the RMSD can be computed as follows:

$$RMSD = \sqrt{\frac{1}{N}\sum_{i=1}^{N}[(x_i^A - x_i^B)^2 + (y_i^A - y_i^B)^2 + (z_i^A - z_i^B)^2]}$$

2. Methods

2.1. Recombinant protein expression

In order to investigate a protein´s structure, function, modifications or it´s interactions, relatively large amount of the pure protein must be obtained. The isolation of a single protein from the a native organism is very time consuming and ineffective. Hypothetically, to obtain 3 mg of pure protein one would need to start with 10 kg of crude cellular extract. For this reasons only low soluble membrane and cytotoxic proteins, which can not be obtained by any other method, are isolated using this technique. In 1963 Robert B. Merrifield introduced a suitable technique for chemical peptide synthesis. The "solid phase peptide synthesis" involves the stepwise addition of protected amino acids to a growing peptide chain which is bound to the solid support (21). However, given the size and the complexity of the proteins, this method becomes realistically applicable only for polypeptides of up to 50 residues (22).

Nowadays, proteins are mostly prepared with help of genetically modified prokaryotic or eukaryotic organisms - so called expression systems. The most studied and used expression system are Gram negative bacteria, *Escherichia coli*. The protocol is based on the possibility to clone a foreign DNA fragment (template) into an open bacterial plasmid (vector). Commercially available vectors contain variety of auxiliary components as for example antibiotic resistance, reporter genes, protein purification tags and promoters. Antibiotic resistance is used to control the growth of unwanted bacterial strains during the cultivation process. Since certain antibiotics are present in the media, only cells that have a plasmid which codes for its resistance survive. Another necessary feature of bacterial vectors is an operon, which controls expression, usually the *lac* operon. When the IPTG (an allolactose analog) is added to the medium, it binds to the *lac* repressor and thus initiates the transcription of the cloned gene under the lac operon control. However, when the protein expression rate is too high, many proteins become insoluble as inclusion bodies that are tough to isolate and refold. To prevent this complication, the expression must often be curbed e.g. by lowering the incubation temperature.

Bacterial expression is simple and fast, because bacteria are easy to clone, culture and they can produce a high yield of protein. However prokaryotic systems lack the ability of post-translational modifications and the maximum protein size, which can conveniently be produced via bacterial expression, is limited to approx. 150 kDa.

2.1.1. Isotopic labeling of proteins

Since the two-dimensional NMR spectroscopy is only applicable for proteins up to ca. 100 residues, the development of three- and four-dimensional spectra was crucial for bigger target proteins. The naturally abundant isotopes ^{12}C and ^{14}N (nuclear spin I = 0 and I = 1, respectively) are not suitable for high resolution NMR, which occurs by spin-spin coupling. Therefore, they must be substituted by ^{15}N and ^{13}C isotopes (both spin I = 1/2), whose natural abundance is very low (0.37% and 1.11%, respectively). The isotopes must be added artificially to the cultivation media. For this purpose many different techniques has been developed so far.

The isotopic labels are introduced by using isotopically enriched specific nutrients in the growth medium. In most cases the basis will be a so-called "minimal medium" (most commonly M9), which contains only the essential salts and trace elements. The carbon or nitrogen sources must therefore be introduced using a variety of different compounds, like glucose and ammonium salts. Many commercially available *E. coli* strains have been consequently optimized to metabolize amino acid precursors which will serve as ^{13}C and ^{15}N donors. The most used carbon and nitrogen sources are nowadays ^{13}C *D*-glucose, ^{13}C glycerol, ^{13}C sodium acetate, ^{15}N ammonium chloride and ^{15}N ammonium sulfate.

The yield of the protein expression is highly dependent on the type of medium used. It is predictable that the labeled minimal media (M9) are going to have smaller yield than the classical rich non-labeled LB medium, which contains nutrient rich bacteriological peptone and yeast extract as a source of nitrogen and carbon. To increase the yield, rich labeled media, e.g. algal digest that grew on $^{13}CO_2$ and ^{15}N salts or mixture of synthetically labeled amino acids, can be prepared. This leads to an improvement of the expression, but on the other hand is much more expensive.

2.2. Recombinant protein extraction and isolation

When the protein is being expressed, it is stored either in the cytosol or in inclusion bodies (23). To get the protein into the solution, the cells or the tissue of interest must be broken. For this purpose several methods are available - either chemical or mechanical. The use of lysozyme (24) is widely used chemical method for the enzymatic bacterial cell wall disruption, but its labile stability and impossibility to use it at large scale makes this method

less convenient. Removing an excess of lysozyme can be another problem. Sonication, as a one of mechanical methods, is robust and cheap. It applies high frequency sound (20 - 50 kHz) to the sample and causes the disruption of cell walls and various organelles (25). The disadvantage of this method is the need to cool the sample, because the ultrasound generates a lot of heat.

2.2.1. Ammonium sulfate precipitation

Ammonium sulfate precipitation is an old, but very effective method for a crude separation (26). It is based on the ability of the protein to aggregate at high salt concentrations. Since each protein will aggregate at different salt concentration, the precipitate can be easily centrifuged and dissolved. However the re-dissolved sample has still high salt content, which needs to be removed before further purification.

Desired saturation [%]	4 °C		25°C	
	Amount [g]	**Final volume [ml]**	**Amount [g]**	**Final volume [ml]**
5	0.26	10.14	0.27	10.15
10	0.53	10.28	0.56	10.30
20	1.10	10.58	1.15	10.63
30	1.70	10.90	1.78	10.97
40	2.33	11.24	2.46	11.34
50	3.01	11.60	3.18	11.73
60	3.73	11.98	3.95	12.15
70	4.50	12.39	4.78	12.60
80	5.33	12.83	5.67	13.08
90	6.21	13.30	6.63	13.60
100	7.17	13.81	7.68	14.17

Table 2: *Overview of the ammonium sulfate amount needed to reach desired saturation for sample volume of 10 ml at different temperatures*

2.2.2. Dialysis

Dialysis is a suitable technique for removal of small undesired molecules from the protein concentrate through a semipermeable membrane (27). It is based on the diffusion principle, which results from the random movement of molecules in a solution. Small

molecules are able to freely pass through the membrane, whereas big molecules (in our case proteins) stay kept inside the dialysis tubing. This results in the concentration equilibrium among the solute volume. This technique is often used to refold and desalt proteins, because the proper salt concentration is a crucial parameter for further purification steps, as for example ion exchange chromatography (see chapter 2.3.1).

The ratio of buffer volume to the sample volume influences the repetitions needed to carry out the dialysis. When 10 mL of sample is dialyzed against 2 L of a buffer, the salt concentration decreases 200 folds. To effectively removed salt from 10 mL of a saturated solution, at least three repetitions are needed.

Another factor influencing the diffusion is the temperature. Since, for stability reasons, the recommended temperature for biological sample is about 4°C, the time required before switching to a fresh buffer varies in total between 12 - 24 hours.

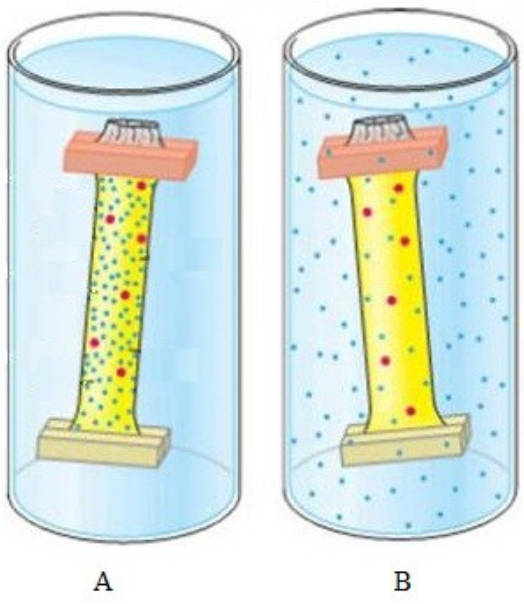

Figure 3: *Representation of dialysis for biological samples* (27)

2.3. Recombinant protein purification

A crucial prerequisite for protein NMR is to reach sufficient sample purity and quantity. The crude protein mixtures undergo many series of separations based on their properties as size, solubility, charge and specific binding capacity. For NMR purposes, two

optimized chromatographic steps are mostly sufficient to obtain relatively pure protein sample for spectra recording.

2.3.1. Ion exchange chromatography

Ion exchange chromatography (IEC) is commonly used technique for purification and separation of proteins (28). IEC columns have an outstanding protein binding capacity, resolution and flexibility. The stationary phase in an IEC system is a matrix with acidic or basic functional groups that interact with the analyte. Basic ion exchangers ("anion exchangers") have positively charged groups, while acidic ion exchangers ("cation exchangers") contain negatively charged groups. The functional sites of these exchangers, either positively or negatively charged, generate an electrostatic interaction between the protein and the stationary phase. Most of the separations are carried out in aqueous solutions. In some cases, the aqueous buffer is supplemented by and organic solvent. This prevents unwanted hydrophobic interactions with the matrix and an increase of electrostatic interactions.

Since the strength of the interaction depends (among other) on the net charge of the protein, the pH of the solvent is the most important parameter. If the pI value of the protein is far away from the pH of the solvent, the protein is strongly bound to the ion exchanger. But when the difference of the pH and the pI value is too big, other proteins may also bind. This makes the chromatography less affective and therefore the pH of the solvent is usually adjusted to be max. 2 units below/above the pI value of the protein.

	Name	Shortcut	pK_a	Functional group
Anion exchangers	Diethylaminoethyl	DEAE	5.8/9.1	$-OCH_2NH(C_2H_5)_2$
	Trimethylaminoethyl	TMAE	-	$-OCH_2CH_2N^+(CH_3)_3$
	Triethyl amine	TEAE	9.5	$-OCH_2N^+(C_2H_5)_3$
Cation exchangers	Carboxymethyl	CM	3.5-4.0	$-OCH_2COOH$
	Sulfoxyethyl	SE	2.0	$-OCH_2CH_2SO_3H$
	Sulfopropyl	SP	2.0 - 2.5	$-OCH_2CH_2CH_2SO_3H$

Table 3: *pK_a values and functional groups of ion exchangers*

The recuperation of the protein is done by addition of increasing the concentration of salt, usually NaCl, in the elution buffer. The protein is forced to compete for the ion exchanger due to the increasing salt concentration, which makes the binding weaker. There is no rule what salt concentration is suitable for releasing the sample out of the column. However, the majority of proteins is eluted before the ion concentration reaches 1M.

2.3.2. Size exclusion chromatography

Size exclusion chromatography (also called gel filtration) is an uncomplicated and straightforward method for protein separation according to the molecular size (and shape) (29). The separation is achieved by the exclusion of proteins of different size from the pores of the stationary phase, as they pass through a bed of porous particles. Therefore, proteins of bigger size can´t penetrate the pores and are eluted faster than proteins of smaller size, whose trajectories are longer.

The stationary phase of modern systems is made of particles from silica, hydrophilized vinyl polymers or highly crosslinked agarose with dextran (Figure 4). Highly efficient columns have a typical particle size in range of 5 - 50 μm. The smaller the particle size, the better the peak narrowing and the final resolution.

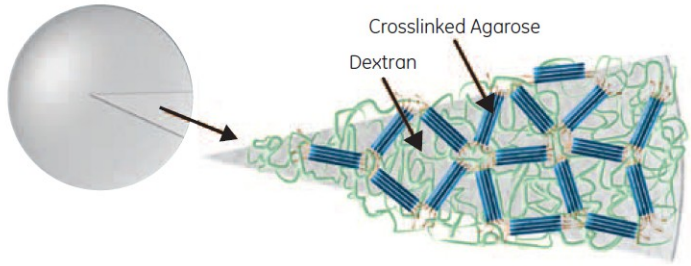

Figure 4: *Section of a Superdex particle. Dextran chains are linked to a highly cross linked agarose matrix* (30)

The important feature of the commercially available supports is their physical and chemical stability. The pH resistance of standard supports covers the range of pH 2 - 10. Some types of the high resolution supports (Superdex 30, 75 and 200) are nowadays able to have the pH stability even better. The only exception are the silica based materials which can be used in limited range of pH 2.5 - 7.0 (Gx000SW and Synchropak GPCx00).

2.3.3. SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (simply SDS-PAGE) is widely used method for simple separation of protein samples. The separation is based on the electrophoretic mobility in polyacrylamide gel in the presence of an electric field. Since the mobility is function of length, charge and conformation, the proteins must be denatured and have the same mass-charge ratio to be separated only according to their size. This is achieved by the addition of sodium dodecyl sulfate to the sample. SDS is a very strong denaturing agent which additionally provides a net negative charge. It is used in cooperation with 2-mercaptoethanol it can also reduce disulfide bonds (Figure 5). This method is often used as an analytical technique during purification to monitor the protein purity, size and approximate concentration.

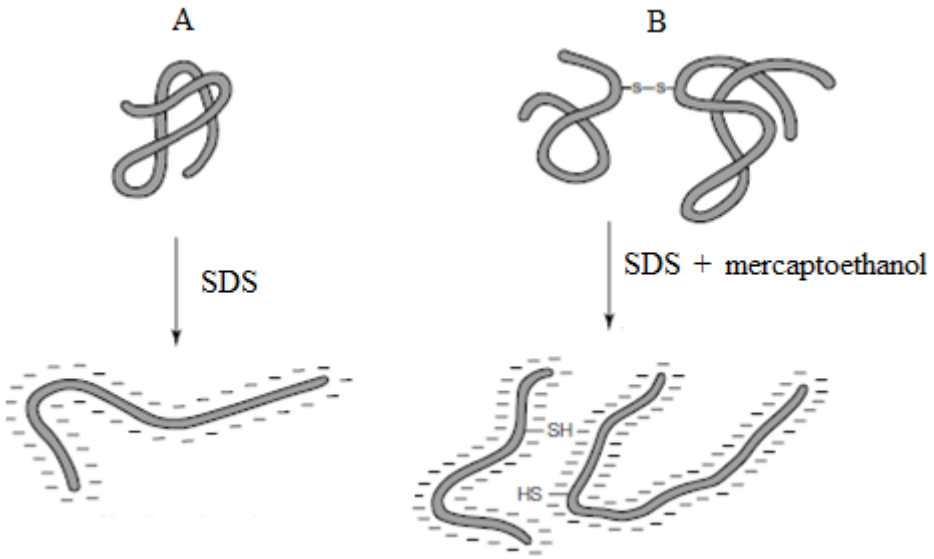

Figure 5: *The effect of sodium dodecyl sulfate and mercaptoethanol on single subunit protein A and protein B with two subunits joined by a disulfide bond* (31)

2.4. Multi-dimensional NMR experiments

Reliable sequence specific assignment of the NMR chemical shifts to particular spin systems in protein NMR spectra is an indispensable prerequisite for structure determination by NMR (32). In 1970´s, a Belgian scientist Jean Jeener developed a first two dimensional homonuclear NMR experiment. From this starting point, later experiments known as Correlation Spectroscopy (COSY) and Nuclear Overhauser Effect Spectroscopy (NOESY)

were developed (33, 34). This lead to the first NMR assignments of smaller proteins and the first solution structure in 1985 (35). However, due to the small ^{3}J (^{1}H-^{1}H) couplings and the increase of transverse relaxation with molecular weight, the magnetization transfer efficiency rapidly drops with increasing protein size. Also the probability of accidental signal overlap increases with number of atoms. Therefore simple two-dimensional homonuclear experiments couldn´t be used for proteins bigger than ca. 10kDa.

To avoid the spectral (near-) degeneracy, an additional dimension can be added. This can be achieved by the combination of two 2D experiments, which can be combined to create a new 3D experiment. 2D NOESY can be for example combined with 2D TOCSY to result in 3D NOESY-TOCSY. Such homonuclear spectra can be very instructive, but they are mostly nonselective and the number of individual signals is very large (for bigger proteins up to several thousands).

More suitable method is the use of heteronuclearly resolved homonuclear experiments. Similarly to the previous case, the heteronuclear 3D experiment is created from a combination of two 2D experiments. For example, 3D NOESY-HSQC is a fusion of heteronuclear 2D HSQC (^{1}H-^{15}N or ^{1}H-^{13}C) and homonuclear 2D NOESY (^{1}H-^{1}H). The consequence is the increased dimensionality with higher resolution, but without any increase of signals (peaks).

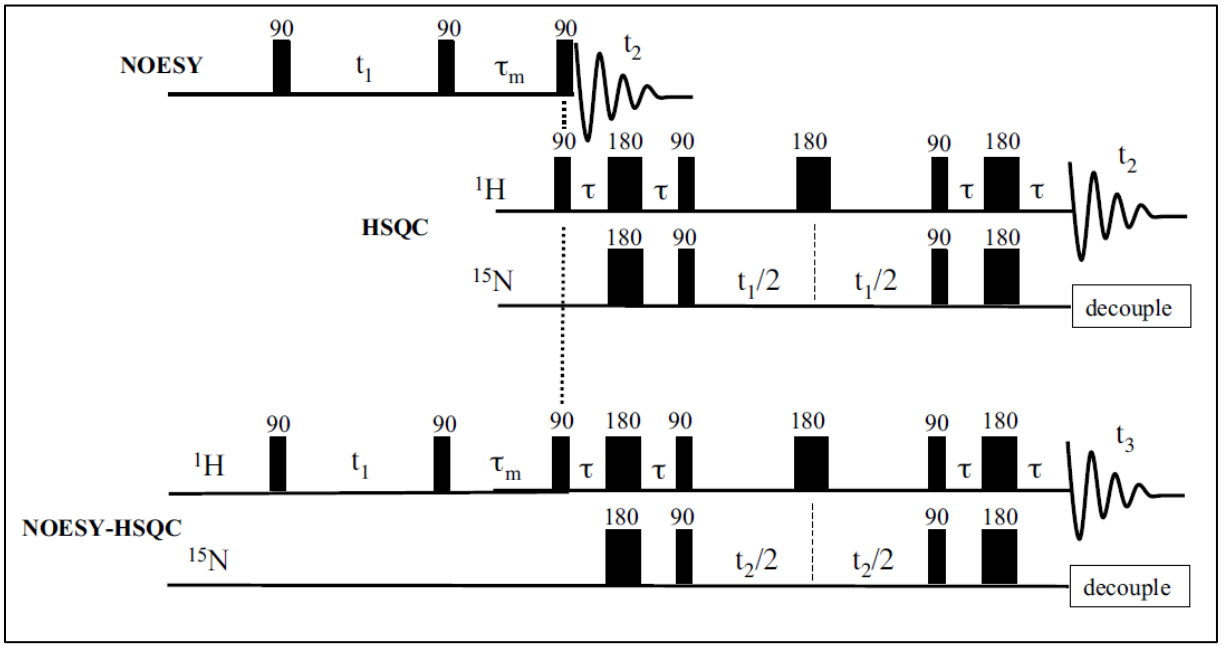

Figure 6: *Schematic derivation of 3D ^{15}N NOESY-HSQC* (36) from 2D experiments.

Nowadays, triple resonance experiments are routinely used for resonance assignment of proteins (≥ 10 kDa). In triple resonance experiments three types of atomic nuclei (typically ^{1}H,^{15}N and ^{13}C) are subjected to radio-frequency pulses. In contrast to homonuclear experiments, where small $^{3}J_{HH}$ couplings are involved, these experiments use rather $^{1}J_{NH}$, $^{1}J_{CH}$, $^{1}J_{CC}$, and $^{1,2}J_{CN}$ couplings. So the magnetization transfer mostly proceeds through multiple and efficient single bond magnetization steps. Such experiments are sensitive and give the possibility to separate signals according to their backbone amide frequencies (37). This first heteronuclear 3D technique was introduced in 1988 by Oschkinat (38). Fortunately, the price of ^{13}C and ^{15}N sources dropped in the last two decades, enabling the mass production of doubly labeled proteins necessary for this kind of experiments.

Protein/Size	Experiment	Information obtained
^{13}C, ^{15}N 80 - 150 amino acids	HNCO	sequential connectivity
	HN(CA)CO	sequential connectivity (combined with HNCO)
	HNCA	sequential connectivity $^{13}C_{\alpha}$ chemical shift constraints
	HN(CO)CA	(combine with HNCA)
	CBCA(CO)NH	sequential connectivity, $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ chemical shift constraints
	CBCANH	for smaller proteins (combine with CBCA(CO)NH)
	HNCACB	for bigger proteins (combine with CBCA(CO)NH)
	HBHA(CO)NH	$^{1}H_{\alpha}$ and $^{1}H_{\beta}$ assignment
	H(CCCO)NH	sidechain ^{1}H assignment
	(H)CC(CO)NH	sidechain ^{13}C assignment
^{2}H, ^{13}C, ^{15}N >160 amino acids ^{2}H decoupling	CT-HNCA	sequential connectivity
	HN(CO)CA	(combine with HNCA)
	CBCA(CO)NH	sequential connectivity, $^{13}C_{\alpha}$/$^{13}C_{\beta}$ chemical shifts
	CT-HNCACB	(combine with CBCA(CO)NH)
	C(CO)NH	sidechain ^{13}C assignment
	^{15}N-HSQC-NOESY-HSQC	sequential and long-range NH-NH NOE constraints

Table 4: *Overview of 3D triple resonance experiments (37)*

2.5. Computer Aided Resonance Assignment (CARA)

Once the cross-peaks in various multidimensional NMR spectra are observed and identified, they are assigned to amino acid residues sequence-specifically. This defines small fragments, which are further linked and mapped on the protein sequence. Nowadays, many programs are available to facilitate the spectral interpretation. One of these programs is

CARA, it stands for Computer Aided Resonance Assignment and was published by Rochus Keller in 2004 (20). It is a user friendly freeware application written in ANSI C++ (39) and supported on most available platforms. CARA enables fast peak picking together with simple representation of three-dimensional experiments. 3D spectra can be projected together with appropriate two-dimensional experiments (i.e. HNCA with ^{15}N HSQC) making the backbone assignment straightforward (Figure 7). Similar to an older predecessor - XEASY (40), CARA is equipped with possibility to quickly propose spin and find the best match when looking for NOESY constraints. The major advantage of CARA program is the possibility to define its own experiments and import extension scripts in "lua" language (41).

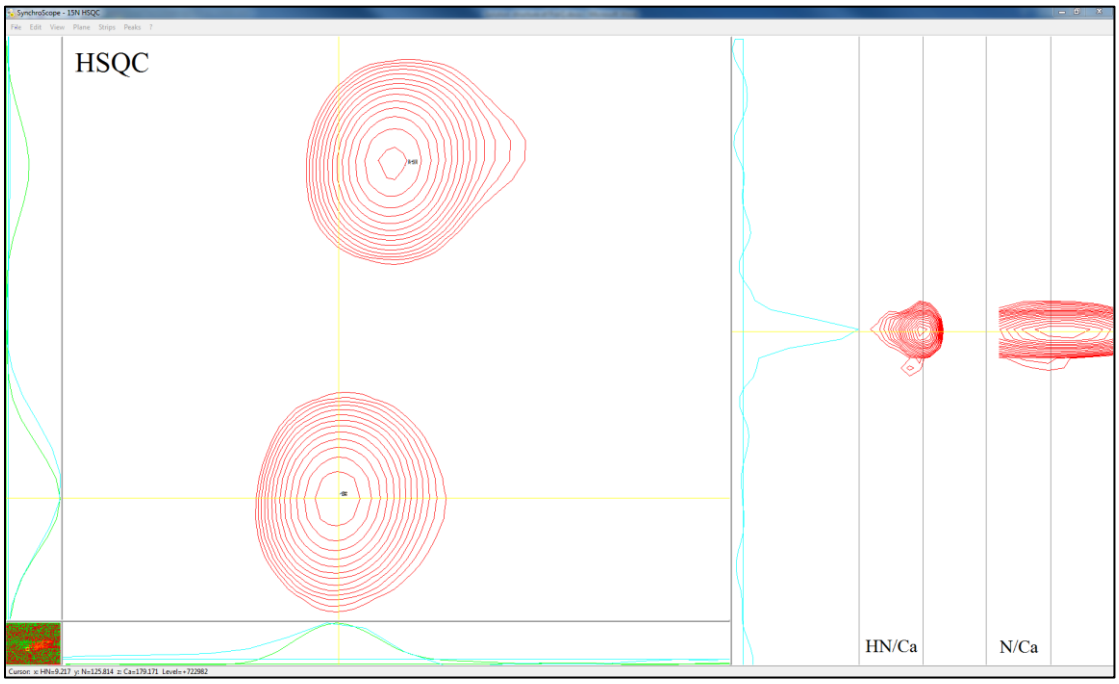

Figure 7: *Projection of ^{15}N HSQC with HNCA in Polyscope*

Recently, many programs have been developed to automate resonance assignment. Unfortunately, the accuracy of these programs is not satisfactory in order to get complete (backbone as well as sidechain) and error-free assignment. There are also semi-automated programs based on combination of the automatic protein backbone (H_α, C_α,C_β and HN shifts) and the manual sidechain assignment. Such programs as for example MARS or AutoAssign, provide for fast backbone assignments for proteins up to 400 residues with maximum accuracy of 96% (42, 43). However, the peak picking in each spectrum must be done manually and the resulting assignment still needs to be checked manually for possible mistakes.

2.6. Peak Integration

In order to convert the NOE cross-peaks into distance constraints, we need to determine their integrals. Since CARA supports peak integration only in 2D spectra, an additional software must be used to achieve the peak integration in 3D spectra. For this purpose NEASY-extension is build in the latest version of CARA (1.9.0, 2013-02-16). NEASY is an emulation of a functional subset of XEASY and provides backward compatibility with CARA (20, 40). Assigned peak lists and spectra can be therefore simply exported from CARA and treated in NEASY. Its advantage is the interactive integration which can be simply checked for mistakes as the structural calculation proceeds.

2.7. Secondary structure estimation

Information about the local structure are additional constraints required by CYANA. Many programs capable of NMR based secondary structure estimation are based on the relation between backbone chemical shifts and backbone torsion angles (ϕ and ψ) of a given residue according to a database of known polypeptides. TALOS+ and its newer version TALOS-N guarantee a high prediction rate of $\geq$ 88.5% with an errors smaller than 3.5% (44), (45). Their database consists of experimentally obtained secondary chemical shifts of 580 proteins and chemical shifts of 9523 proteins computed by SPARTA+ (46).The peak list (containing the HN, Hα, Cα, Cβ, CO and N chemical shifts of all assigned residues) is directly exported from CARA without any conversion and uploaded together with protein sequence. Both programs exist as software packages running exclusively on Linux or accessible as freely available web-based version. The prediction is in both cases done in a few minutes and the results can be displayed within a graphic interface.

2.8. Manually controlled structure calculation with CYANA

CYANA (Combined Assignment and Dynamics Algorithm for NMR Applications) is based on an algorithm developed for macromolecular structure determination, which also enables automated structure calculation together with automated NOESY cross-peak assignment (19). CYANA was introduced by members of Prof. Güntert´s group and arose from the pre-dating DYANA algorithm published in 1997 (47).

The NMR parameters, i.e. NOE integrals an coupling constants are translated to distance and torsion angle restraints. The driving force behind CYANA algorithm is the minimization of a target function V. The so called "target function" becomes zero when all restraints are fulfilled simultaneously, without any collision of non-bonding atom pairs [31]. To put it simply, a match between the given constraints and the absence of steric overlap is sought.

$$V = \sum_{c=u,l,v} W_c \sum_{(\alpha,\beta)\in I_c} (d_{\alpha\beta} - b_{\alpha\beta})^2 + W_a \sum_{i\in I_a} \left[1 - \frac{1}{2}\left(\frac{\Delta_i}{\Gamma_i}\right)^2\right] \Delta_i^2$$

Equation 1: The *CYANA target function [31]*. $\mathbf{b}_{\alpha\beta}$ = lower and upper bounds; $\mathbf{d}_{\alpha\beta}$ = distance between atoms α and β; $\boldsymbol{I_u}$, $\boldsymbol{I_l}$, $\boldsymbol{I_v}$ = sets of atom pairs with upper, lower or VdW distance bounds; $\boldsymbol{I_a}$ = set of restrained torsion angles; $\boldsymbol{w_a}$, $\boldsymbol{w_l}$, $\boldsymbol{w_v}$, $\boldsymbol{w_u}$ = weighting factors for different types of constraints; $\boldsymbol{\Gamma_i}$ = half width of forbidden range of torsion angle; $\boldsymbol{\Delta_i}$ = size of torsion angle constraint violation

Note that one, several or no structure may fulfill the V=0 condition. Due to the presence of experimental errors V=0 is rarely reached in practice. Therefore minimization of V is used to optimize structures. For this purpose a simulated annealing method (48) by molecular dynamics is applied. Since molecular dynamics uses kinetic energy as the driving force, barriers of the potential surface can be overcome and the probability to get accidentally trapped in an energetic local minimum is significantly reduced (19) as compared to pure gradient based descent methods. In order to save computation time, the number of degrees of freedom must be limited. In CYANA instead of Cartesian coordinates (frequently used in classical MD simulations) torsion angle space is used mainly for its reduced complexity. The number of degrees of freedom is thus reduced to the significantly smaller number of torsion angles. As a consequence the computation time is only linearly increasing with increasing atom number.

An essential step in the structure calculation is the conversion of NOESY peak intensities into distance constraints. CYANA provides for automatic conversion and calibration with help of an inbuilt CALIBA subroutine (49). CALIBA sorts the cross-peak intensities into three classes according to their origin. For each class, differently modified calibration functions are applied (Table 5) in order to achieve a more accurate distance calibration than a uniform $1/d^6$ dependence (which would only hold strictly for diatomic interactions).

Class	Peaks/Constraints	Calibration function
Backbone	HN/HA↔ HN/HA, HN/HA↔HB to max. dist of 5Å	$V = \frac{A}{d^6}$
Methyl	all methyl groups	$V = \frac{C}{d^4}$
Sidechain	all others	$V = \frac{B}{d^4}$

Table 5: *Overview of CALIBA calibration functions. A,B and C are constants derived from the assumed average distance between backbone atoms* (50).

As mentioned earlier, CYANA includes the CANDID algorithm (51) giving the possibility to automate NOESY assignment whose accuracy is enhanced by iterative cycles during the structure calculation. However the automatic way requires a list of NOESY cross-peak positions, their volumes and constraints from a third party software. So, the time requirements are comparable to the manual approach, where the NOESY spectra are manually (interactively) assigned and integrated. This manual method benefits from full control and simple identification of possible assignment mistakes during the calculation process.

2.9. Visualization and analysis of molecular structures

In addition to structure calculation, CYANA provides many other useful auxiliary functions as for example: format conversions, calculation of RMSD, checking of the peak consistency, etc. Unfortunately, CYANA doesn't have graphical user interface and can't display the standard Protein Data Bank (PDB) files (52). Hence, additional software must be used to display and analyze the result.

Visual Molecular Dynamics (VMD) is a program suitable for high quality visualization, modeling and analysis of macromolecules (53). VMD supports over 60 molecular file formats and has almost no limitation of molecular size and complexity. The latest version 1.9 (2013) works with structures of up to 100 million atoms and can be compiled to run in an immersive virtual reality environment. In order to handle such enormous load, VMD provides unlimited CPU multithreading and modern "Compute Unified Device Architecture" (CUDA).

An another widely used program for visualization of macromolecules is the "Yet Another Scientific Artificial Reality Application" (YASARA) (54). YASARA is known for its extreme simplicity while maintaining powerful features found in other professional programs. MD simulations, homology modeling and small-molecule docking experiments can be easily set up in a few seconds, hence it is mainly used for educational purposes. The weakness of this program is the less sophisticated definition of secondary structure. Since there is no deviation tolerance, only perfectly defined secondary structures are represented in "cartoon mode" that makes the program less suitable for initial structure estimation.

3. Experimental

3.1. List of Chemicals

Compound	Chemical formula	M [g·mol^{-1}]	Supplier	Purity
Acetic Acid	$C_2H_4O_2$	60.5	Merck	100%
Acrylamide/Bis-acrylamide 30%			Sigma-Aldrich	
AEBSF	$C_8H_{10}FNO_2S \cdot HCl$	239.69	neoLab	≥ 98%
Ammonium persulfate	$(NH_4)_2S_2O_8$	228.18	Sigma-Aldrich	≥ 98%
Ammonium sulfate	$(NH_4)_2SO_4$	132.14	Sigma-Aldrich	≥ 99%
Ammonium sulfate ^{15}N	$(^{15}NH_4)_2SO_4$	134.13	Sigma-Aldrich	≥ 98%
Ampicillin sodium salt	$C_{16}H_{18}N_3NaO_4S$	371.04	Fluka	≥ 99%
Agar - bacteriological			Merck	
Bacteriological Peptone			USB	
BIS-TRIS	$C_8H_{19}NO_5$	209.24	Sigma-Aldrich	≥ 98%
Bromophenol Blue sodium salt	$C_{19}H_9Br_4O_5SNa$	692	ICN Biomedicals Inc.	
Chloramphenicol	$C_{11}H_{12}Cl_2N_2O_5$	323.14	Fluka	≥ 99%
Coomassie Brilliant Blue R 250	$C_{45}H_{44}N_3O_7S_2 \cdot Na$	825.97	Serva	
Deuterium oxide	D_2O	20.027	Euriso-top	100%
di-Sodium hydrogen phosphate dihydrate	$Na_2HPO_4 \cdot 2H_2O$	177.99	Merck	≥ 98%
Dithiothreitol	$C_4H_{10}O_2S_2$	154.25	USB	
Ethylenediaminetetraacetic acid disodium salt dihydrate	$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$	372.24	Sigma-Aldrich	≥ 99%
Glucose D ^{13}C	$C_6H_{12}O_6$	186.16	Spectra Gases	≥ 99%
Glucose D(+)	$C_6H_{12}O_6$	180.16	Merck	
Glycerol for molecular biology	C_3H_8O	92.09	Sigma-Aldrich	≥ 99%
Glycine	C_2H_5NO	75.07	Sigma-Aldrich	≥ 99%
IsoproIsopropyl β-D-1-thiogalactopyranosidepyl	$C_9H_{18}O_5S$	238.3	Applichem	≥ 99%
Magnesium sulphate heptahydrate	$MgSO_4 \cdot 7H_2O$	246.47	Merck	≥ 99.5%
Methanol - distilled	CH_4O	32,04		
Potassium Chloride 3M	KCl aq.	74.55	VWR	

Compound	Chemical formula	M [g·mol^{-1}]	Supplier	Purity
Potassium dihydrogen phosphate	KH_2PO_4	136.09	Merck	≥ 99.5%
Sodium dodecyl sulfate	$C_{12}H_{25}NaO_4S$	288.38	Fluka	>99%
Sodium dihydrogen phosphate monohydrate	$NaH_2PO_4 \cdot H_2O$	137.99	Fluka	≥ 99%
Sodium Chloride	NaCl	58.44	Merck	≥99.97%
Tris base	$C_4H_{11}NO_3$	121.14	Sigma-Aldrich	≥99.9%
yeast extract			USB	

3.2. List of Solutions

3.2.1. Protein expression

Ampicillin stock solution, 100 mg·mL^{-1}

1 g of Ampicillin in 10 mL of 18 Ω H_2O/ -20°C

Cloramphenicol stock solution, 20 mg·ml^{-1}

200 mg of Chloramphenicol in 10 mL of Ethanol/ -20°C

IPTG stock solution, 1M

0.476 g of IPTG in 2 mL of 18 Ω H_2O/ -20°C

AEBSF stock solution, 0.1 M

36 mg of AEBSF in 1.5 mL of 18 Ω H_2O/ -20°C

LB-medium

10 g bacteriological peptone, 5 g yeast extract and 10 g NaCl in 1 L of 18 Ω H_2O, sterilized at 120 ºC, cooled down and 1 ml of Ampicillin and Chloramphenicol stock solutions added

LB-agar

same as LB-medium, but additionally 20 g of bacteriological agar added

M9 minimal medium - singly/doubly labeled

6 g $Na_2HPO_4 \cdot 2H_2O$, 3 g KH_2PO_4, 1.5 g $(^{15}NH_4)_2SO_4$, 0.5 g NaCl, 2 g of glucose, 1 mM $MgSO_4$, 1 mL of trace elements, 1 mL of Ampicillin and Chloramphenicol stock solutions, filtered though Millipore Express Plus 0.22 μm filter /for double labeled media 2 g of ^{13}C-D-glucose was used

3.2.2. Protein isolation

Resuspension buffer (20mM Tris, 1mM EDTA)

121 mg Trizma base, 18.6 mg EDTA and 500 μL of AEBSF stock solution in 50 mL of 18 Ω H_2O

Binding Buffer (20mM Tris, 1mM EDTA, pH = 6.5)

2.42 g Trizma base and 0.37 g EDTA in 1 L of 18 Ω H_2O, pH adjusted with conc. HCl to 6.5, filtered though Millipore Express Plus 0.22 μm filter

Elution Buffer (1M NaCl, 20mM Tris, 1mM EDTA, pH = 6.5)

2.42 g Trizma base, 58.44 g NaCl and 0.37 g EDTA in 1 L of 18 Ω H_2O, pH adjusted with conc. HCl to 6.5, filtered though Millipore Express Plus 0.22 μm filter

SEC buffer (20mM Tris, 200 mM NaCl, 1mM EDTA, pH = 8.0

2.42 Trizma base, 11.69 g NaCl and 0.37 g EDTA in 1 L of 18 Ω H_2O, pH adjusted with conc. HCl to 8.0, filtered though Millipore Express Plus 0.22 μm filter

2M Tris stock solution (pH = 8.8)

24.22 g of Trizma base in 0.1 L of 18 Ω H_2O, pH adjusted with conc. HCl/NaOH to 8.8

1M Tris stock solution (pH = 6.8)

12.11 g of Trizma base in 0.1 L of 18Ω H_2O, pH adjusted with conc. HCl/NaOH to 6.8

APS stock solution, 10%

1 g Ammonium persulfate in 10 mL of 18 Ω H_2O/ 4°C

SDS stock solution, 10%

10 g Sodium dodecyl sulfate in 10 mL of 18 Ω H_2O/ 4°C

Separation buffer for gel electrophoresis

75 mL of 2 M Tris stock solution, 4 mL of SDS stock solution, 21 mL of 18 Ω H_2O/ 4°C

Stacking buffer for gel electrophoresis

50 mL of 1 M Tris stock solution, 4 mL of SDS stock solution, 46 mL of 18 Ω H_2O/ 4°C

Running buffer for gel electrophoresis, 5x

15 g Trizma base, 75 g glycine, 5 g sodium dodecyl sulfate in 1 L of 18 Ω H_2O

Bromophenol blue, 1%

100 mg Bromophenol Blue sodium salt in 10 mL of 18 Ω H_2O/ 4°C

DTT stock solution, 5 M

0.77 g reduced Dithiothreitol in 1 mL of 18 Ω H_2O/ 4°C

Loading buffer for gel electrophoresis, 5x

0.6 mL of 1 M Tris stock solution, 5 mL of 50% glycerol, 2 mL of SDS stock solution, 1 mL of 1 % Bromophenol blue, 250 µL DTT stock solution and 1.15 mL of 18 Ω H_2O/ 4°C

Staining solution

1 g of Coomassie Brilliant Blue R 250 in 450 mL methanol, 100 ml acetic acid and 450 mL of 18Ω H_2O

Destaining solution

100 mL acetic acid, 100 mL methanol in 800 mL of 18Ω H_2O

3.2.3. NMR sample preparation

Buffer solution for NMR (20 mM NaH_2PO_4, 1 mM EDTA, pH = 7.0)

2.76 g sodium dihydrogen phosphate monohydrate, 0.37 g EDTA in 1 L of 18 Ω H_2O, pH adjusted with conc. HCl to 7.0, filtered though Millipore Express Plus 0.22 µm filter

DSS standard, 13.2 mM

2.6 mg labeled DSS in 1 mL of D_2O/ -20°C

3.3. List of instruments

Instrument	Type	Manufacturer
Autoclave	Certovclav EL	Certoclav
Centrifuge	Biofuge pico	Heraeus Instruments
	Biofuge Stratos	
	Megafuge 1.0 R	
Dyalysis membrane	Spectra/Por Dialysis Membrane MWCO 3.500	Spectrum Laboratories
Electrophoresis	EasyPhor PAGE mini	Biozym
Fast purification liquid chromatorgaphy	GradiFrac system	Pharmacia Biotech
Filters	Syringe filter 0.22 µm	GVS
	Express Plus 0.22µm	Millipore
	Amicon Ultra-15, 3000MWCO	
Chromatographic stationary phase	DEAE Sepharose Fast Flow	GE Healthcare
	HiLoad 16/60 Superdex 75 prep grade	Pharmacia Biotech
	SP Sepharose Fast Flow	GE Healthcare
Incubator	Shaking incubator 3033	GFL
NMR spectrometer	Avance III 700MHz, equipped with TCI cryo probe	Bruker
pH meter	290A with LIQ-glass electrode	Orion
Sonicator	Sonoplus HD 70	Bandelin electronic
UV-VIS spectrometer	HP 8453	Hewlett-Packard
	Uvikon 800	Kontron

3.4. PsbQ Expression in *E. coli*

E. coli BL21(DE3)pLysS cells transformed with JR2592 plasmid for PsbQ (provided from Institute of Nanobiology and Structural Biology, CAS, Nové Hrady) were diluted 1:10^6 and streaked on LB-agar plate (Ampicillin, Chloramphenicol both 20 $\mu g \cdot mL^{-1}$). The plates were incubated at 37 °C for 20 h. The accrued colonies were examined and one isolated colony was transferred into 20 mL of rich LB medium (Ampicillin, Chloramphenicol both 20 $\mu g \cdot mL^{-1}$) and grown overnight at 37 °C. This starting culture was used to inoculate 1 L of M9 (singly/doubly labeled) media. The initial optical density at 580 nm was measured and the measurement was carried out every hour to check the bacterial growth. When the OD reached 0.8, an IPTG solution (1 ml of stock solution per l L of medium) was added in order to induce the cells and promote the protein expression. The cultivation temperature was set to 28 °C to prevent the protein accumulation in inclusion bodies. The total cultivation time was 24 h.

3.5. Isolation and purification

The cell culture was harvested by centrifugation (4000 g, 45 min., 3 °C) and the remaining pellets were resuspended with 50 mL of binding buffer (enriched with 500 µL of AEBSF protease inhibitors stock solution). Resuspended cells were subjected to sonication (4x 15 min. at 70% power) and centrifuged at 4000 g for 1 h at 3 °C. The supernatant containing the released proteins was stored in refrigerator at 4 °C overnight.

3.5.1. Ammonium sulfate precipitation

The supernatant was kept on ice and 30.68 g of ammonium sulfate were added in portions while continuous stirring. The solution was centrifuged in order to retrieve the precipitate. The resulting pellet was dissolved in 10 mL of binding buffer.

3.5.2. Dialysis

The solution containing proteins was transferred into a dialysis membrane and tightly closed with clamps. The dialysis bag was soaked in 2 L of binding buffer and slowly stirred at 4 °C. The binding buffer was exchanged after 8 hours and the procedure was repeated three

times. The sample was extracted from dialysis bag, centrifuged at 4000 g for 1 h at 3 °C and supernatant stored at -20 °C.

3.5.3. SDS-PAGE

Two special glass plates were placed in the frame and together introduced into the casting stand. The mixture for separation gel was prepared (Table 6), immediately filled between the plates 1.5 cm below the top of the glass and overlaid with ethanol. After 20 minutes, ethanol was poured out and replaced by fresh stacking gel mixture (Table 6). The combs were inserted and the frame was placed into the electrophoretic chamber. When the stacking gel was polymerized, the electrophoretic chamber was filled with 1x running buffer and the comb was removed. The resulting wells were rinsed with running buffer.

Component	Separation gel (15%)	Stacking gel (4.5%)
Acrylamide/Bis-acrylamide 30%	2.50 mL	3.25 mL
18MΩ H_2O	1.25 mL	1.87 mL
Separation buffer	1.25 mL	-
Stacking buffer	-	1.87 ml
TEMED	5 µL	7.5 µL
10% APS	50 µL	75 µL

Table 6: *Composition of separation and stacking gels*

An appropriate sample amount (according to expected concentration, mostly 10-15 µL) was mixed with 5x loading buffer, incubated for 5 min. and loaded into the well. 4 µL of the protein standard (Precision Plus Protein All Blue Standards, Bio-Rad) were used to mark each gel. The electrophoretic chamber was closed, and constant voltage of 220V was applied for 50 min.

After the electrophoresis was finished, the cassettes were taken out and the gels were placed into the staining solution for 1 h. In order to get contrast between the sample and the surroundings, the gels were incubated in destaining solution at least for 2 h.

3.5.4. Ion exchange chromatography

The pI value of PsbQ is 9.25 and therefore it is a suitable candidate for cation exchanger. In order to achieve better separation two serially connected columns has been used. First column was packed with DEAE sepharose serving as an anion exchanger while the second with SP sepharose binds PsbQ at pH 6.5. The elution was done using 1M NaCl gradient only on SP sepharose column.

Prior to use, both columns were washed with deionized water and equilibrated with binding buffer. The protein sample was filtered through syringe filter (0.22 μm) and loaded onto the system under the lowered flow of 1 $mL \cdot min^{-1}$. When the sample was distributed on the columns the flow was increased to 2 $mL \cdot min^{-1}$. As soon as the flow through portion was finished, the columns were disassembled and SP sepharose column was subjected to gradient elution (0-60% elution buffer) and 2 mL fractions were collected. The course of chromatography was monitored via the UV-detection and selected fractions were inspected by SDS-PAGE.

Fractions containing PsbQ were pooled together, concentrated using Amicon ultra-15 centrifugal filter with 3 kDa cutoff to the final volume of 8 mL and the buffer was changed to SEC buffer.

3.5.5. Size exclusion chromatography

In order to improve the sample purity, size exclusion chromatography was performed. The column was packed with HiLoad Superdex 75 resin and thoroughly washed with SEC buffer. After equilibration the sample was loaded to the system under a continuous flow of 0.8 $mL \cdot min^{-1}$. When bigger proteins started to elute, the flow was lowered to 0.4 $mL \cdot min^{-1}$ and 2 mL fractions were collected. In the same way as for IEC, the size exclusion chromatography was monitored via UV-detection and selected fractions were examined via SDS-PAGE.

Fractions containing PsbQ were pooled together, washed with NMR buffer and concentrated to final volume of 1.5 mL via Amicon ultra centrifugal filter. The final concentration was determined by UV absorbance measurement at 280 nm. For NMR experiments, 220 μL of the sample was mixed with 24 μL of D_2O and 2 μL of DSS stock solution.

3.5.6. Concentration determination

The UV/VIS absorbance at 280 nm was measured in order to calculate the total protein concentration. 20 µl of sample were diluted in 600 µL of NMR buffer (degassed prior to use). The measurement was repeated three-times and the mean value was multiplied by dilution factor (31x) and divided by molar extinction coefficient (55).

3.6. Double and triple resonance experiments

Following NMR experiments were recorded at 700 MHz Avance III spectrometer with Ascend magnet and TCI cryoprobe at The Austro-Czech RERI-uasb NMR center in Linz and processed with Bruker software Topspin v.3.1.

Type	Experiment	Information obtained	min. labelling
2D spectra	^{15}N HSQC	H-N correlations	^{15}N
	^{13}C HSQC	H-C correlations	^{13}C
3D spectra	HC(C)H-COSY	neighboring CH groups within one residue	^{15}N, ^{13}C
	(H)CCH-TOCSY	all carbon atoms within one residue	^{15}N, ^{13}C
	TOCSY-HSQC	all hydrogen atoms within one residue	^{15}N
	^{15}N NOESY-HSQC	NOEs from one HN to all hydrogen atoms nearby	^{15}N
	^{13}C NOESY-HSQC	NOEs from one CH to all hydrogen atoms nearby	^{13}C
triple resonance spectra	(H)C(C)(CO)NH	all carbon atoms from previous residue	^{15}N, ^{13}C

Table 7: *Overview of recorded spectra, their informative contribution and stable isotope labeling required.*

^{15}N HSQC			**^{13}C HSQC**		
hsqcetf3gpsi2	F2	F1	hsqcetgpsi	F2	F1
Number of scans	8		Number of scans	8	
Size of FID	4096	192	Size of FID	4096	256
Spectral width [Hz]	9803.922	5322.899	Spectral width [Hz]	7002.801	11887.116
Acquisition time [sec]	0.208896	0.0180353	Acquisition time [sec]	0.2924544	0.010768

Table 8: *The acquisition parameters for ^{15}N HSQC and ^{13}C HSQC*

HC(C)H-COSY			
hcchcogp3d	F3	F2	F1
Number of scans	8		
Size of FID	4096	96	128
Spectral width [Hz]	7692.308	11887.102	4202
Acquisition time [sec]	0.266	0.0040	0.0152
Power level [W]	303	360	10.3
Power level for CPD/BB decoupling[W]	-	14.4	-
HC(C)H-TOCSY			
hcchdigp3d2	F3	F2	F1
Number of scans	16		
Size of FID	2048	64	128
Spectral width [Hz]	7002.801	11887.1	11887.1
Acquisition time [sec]	0.14623	0.00269	0.00538
Power level [W]	0.00323	360	10.3
Power level for CPD/BB decoupling[W]	-	14.4	-
Mixing time [ms]	22.6		
TOCSY-HSQC			
dipsihsqcf3gpsi3d	F3	F2	F1
Number of scans	16		
Size of FID	2048	68	160
Spectral width [Hz]	6996.269	2342.09	6996.27
Acquisition time [sec]	0.146361	0.01452	0.01143
Power level [W]	138	130	8.7
Power level for CPD/BB decoupling[W]	2.1563	-	-
Mixing time [ms]	80		
(H)C(C)(CO)NH			
hccconhgpwg3d3	F3	F2	F1
Number of scans	16		
Size of FID	2048	68	142
Spectral width [Hz]	7002.801	2342.09	10566.3
Acquisition time [sec]	0.14622	0.01452	0.00672
Power level [W]	138	-	8.7
Power level for CPD/BB decoupling[W]	2.15634	-	0.14754

Table 9: *The acquisition parameters for HC(C)H-COSY, HC(C)H-TOCSY, TOCSY-HSQC and (H)C(C)(CO)NH*

^{15}N NOESY-HSQC			
noesyhsqcf3gpwg3d	F3	F2	F1
Number of scans	8		
Size of FID	2048	48	512
Spectral width [Hz]	9803.92	1987.22	7423.53
Acquisition time [sec]	0.10445	0.01208	0.03448
Power level [W]	138	130	8.7096
Power level for CPD/BB decoupling[W]	2.1563	-	-
Mixing time [ms]	60		
^{15}C NOESY-HSQC			
noesyhsqcetgp3d	F3	F2	F1
Number of scans	8		
Size of FID	2048	100	160
Spectral width [Hz]	8196.7	10918.5	8196.7
Acquisition time [sec]	0.1249	0.0045	0.0097
Power level [W]	138	130	8.7096
Power level for CPD/BB decoupling[W]	-	6.8227	-
Mixing time [ms]	120		

Table 10: *The acquisition parameters for ^{15}N NOESY-HSQC and ^{13}C NOESY-HSQC*

3.7. Complementation of resonance assignment

Newly recorded NMR spectra (Table 7) were calibrated and imported into existing CARA project (BMRB entry 17357) (56). The assignment consistency and sample integrity was verified by the comparison of repeatedly recorded ^{15}N- and ^{13}C- HSQC spectra in between the new experiments. In order to increase the number of assigned backbone resonances, the ^{15}N HSQC spectrum was first projected together onto HNCO experiment. When an unassigned peak (^{15}N-^{1}H signal) in the HSQC spectrum correlated with a carbonyl resonance in the HNCO, a new spin system was proposed. The such defined spin systems were sequence specifically classified with help of HNCaCb and CbCa(CO)NH spectra. After the C_α and C_β chemical shifts were thus determined, HC(C)H-TOCSY and (H)C(C)(CO)NH experiments were used to complement the remaining sidechain carbon shifts and check their correctness in all residues. Similarly, the combination of HbHa(CbCaCo)NH, HC(C)H-COSY and TOCSY-HSQC spectra was employed to assign remaining proton backbone and sidechain resonances.

3.8. Secondary structure estimation

Once the assignment process was completed, the peak list was exported into BMRB NMR-Star format use a lua extension in the CARA terminal window and uploaded to the Talos-N prediction server (http://spin.niddk.nih.gov/bax/nmrserver/talosn/).The resulting .aco file was opened in a text editor and all dihedral angles, which were predicted with low accuracy (marked "BAD") together with the φ angles for prolines were deleted.

3.9. ^{15}N and ^{13}C NOESY assignment

The ^{15}N NOESY-HSQC spectrum was shift-calibrated with respect to ^{15}N HSQC and imported into CARA program. NOESY cross-peak resonances having unique chemical shifts were assigned first and used to calculate a preliminary structure. NOEs having two or more corresponding resonances were assigned with respect to the preliminary structure and prototypical NOESY patterns (57). Since each strip in ^{15}N NOESY-HSQC spectrum contains NOEs only from NH groups to other (non-NH) hydrogen atoms, additional NOEs were necessary to improve the quality of the structure. Hence, the ^{13}C NOESY-HSQC spectrum was calibrated with help of ^{13}C HSQC and imported into separate CARA project. The cross-peaks from sidechain C-H groups to nearby hydrogen atoms were thus assigned.

From each project, peak list (.peaks) and proton list (.prot) files were exported and loaded in NEASY extension. The integration was done manually by interactive integration function ("ii"). Sufficiently separated peaks were integrated easily, whereas the integrals of overlapping peaks were divided by the number of contributed signals. Such potentially problematic peaks were noted and taken into account during the structure calculation.

3.10. Structure calculation

CYANA version 3.0 was installed on an Ubuntu Linux 12.04 LTS distribution. All input files were saved in the same cyana directory as executing calc.cya macro file (Table 11).

	Description	Command
Input	read and calibrate first peaklist	read seq PsbQ
		read prot PsbQ-15N.prot unknown=warn
		read peaks PsbQ-15N-NOESY assigned integrated
		caliba avedis=3.4 vmin=10100.0
		write upl PsbQ-15N
	read and calibrate second peaklist	read seq PsbQ
		read prot PsbQ-13C.prot unknown=warn
		read peaks PsbQ-13C-NOESY assigned integrated
		caliba avedis=3.4 vmin=10085.0
		read upl PsbQ-15N append
	read angle constraints from Talos-N	read aco PsbQ-talos.aco
	perform gridsearch	habas angles="CHI1 CHI2*" tfcut=0.05
	remove redundant constraints and pseudoatom correction	distance modify
		distance unique
	number of used processors	nproc=8
	perform structure calculation	calc_all structures=400 anneal steps=10000
Output	number of generated structures	overview file=PsbQ structures=20 range=- pdb
	display upper distance and angle restraint cutoff in terminal window	cut_upl=0.2
		cut_aco=5
		stru viol
	calculate root mean square deviation	rmsd 1..149
	generate overall upper distance restraint file, plot of long range NOEs and distance statistics	write upl psbq-all
		longrangeplot
		distance stat

Table 11: *Overwiev of the calc.cya macro*

4. Results and discussion

4.1. Protein expression

After 5 hours at 37°C the cells reached optical density of 0.67 and could be induced with IPTG. Subsequently, the cell growth had an exponential progress for additional three hours. Similar behavior was also observed for doubly labeled minimal media. After 25 hours (at 28°C), the optical density reached 2 and the cultivation was stopped to prevent product degradation. Compared to the previous results (58), the cell growth in M9 minimal media reaches the optical density twice slower at 28°C than at 37 °C. On the other hand, this precaution prevents formation of inclusion bodies and facilitates purification and stability of the protein

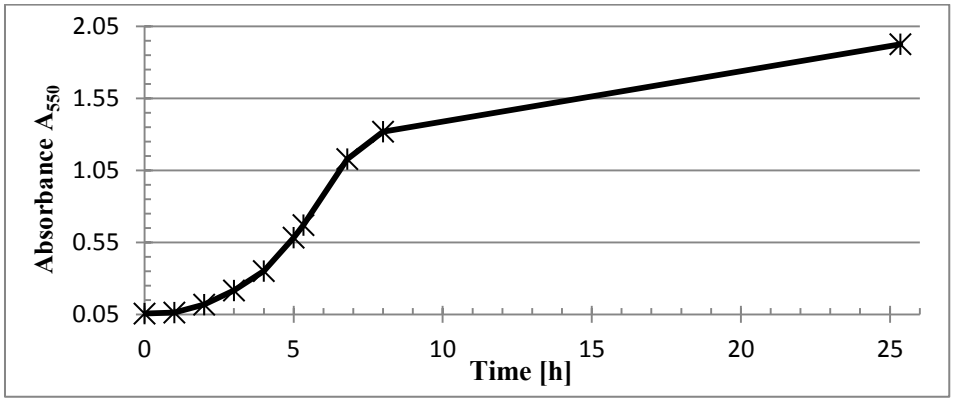

Figure 8: *Typical growth curve for BL21 E. coli cells in M9 minimal media at 28°C.*

4.2. Protein isolation and purification

After the cells were sonicated, the supernatant and the remaining pellet were analyzed by SDS-PAGE. Since the sonication was proved to be less efficient than expected (Figure 9), the procedure was repeated four times.

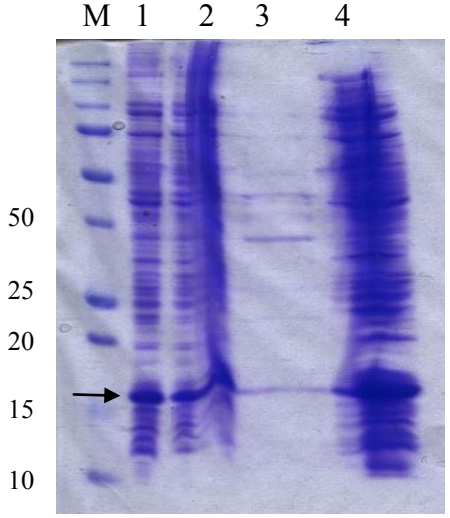

Figure 9: *SDS-PAGE of first two runs of cell lysis*
1 - supernatant after first sonication (10 µL)
2 - remaining pellet after first sonication(10 µL)
3 - supernatant after second sonication(10 µL)
4 - remaining pellet after second sonication (20 µL)
M - marker, arrow - PsbQ

4.2.1. Ion exchange chromatography

Since the protein concentration estimated by SDS-PAGE was relatively high, the sample was diluted to prevent possible aggregation on the column. In total 3 IEC purification runs (3 x 10 mL) were performed. In all cases, the PsbQ started to elute at 39% of elution buffer (Figure 10). It was verified by SDS-PAGE, that the majority of impurities had been separated (Figure 11). However, after the fractions were combined together and concentrated to 10 mL, some unwanted proteins appeared to be present on SDS-PAGE (Figure 12).

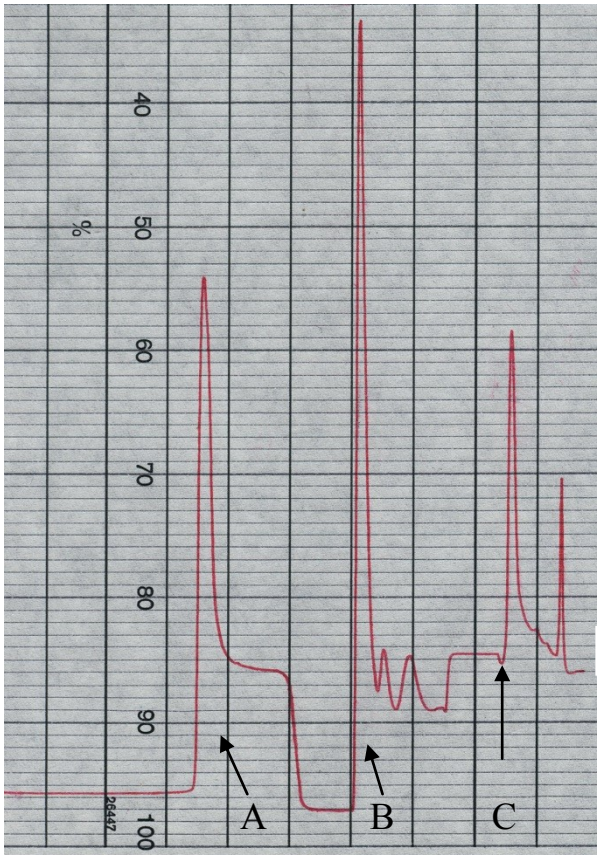

Figure 10: *Chromatogram of ion exchange chromatography*

A- flow-through
B- start of the gradient
C- elution of PsbQ

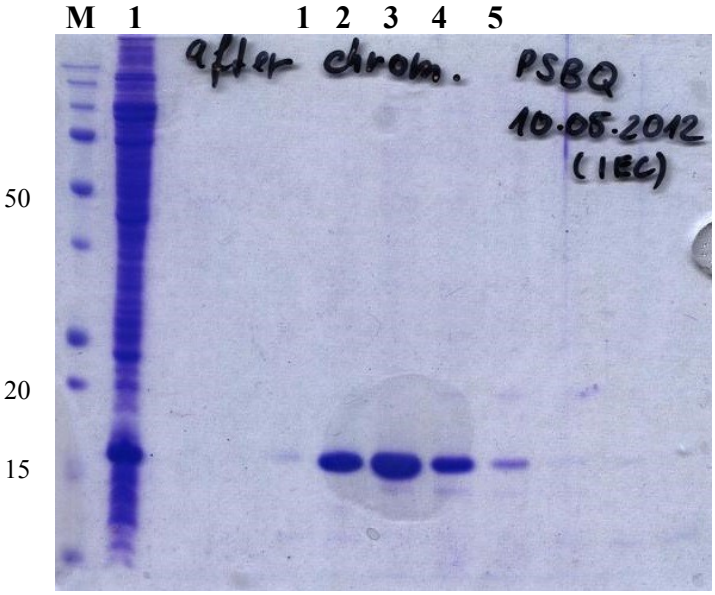

Figure 11: *SDS-PAGE of ion exchange chromatography, 1 - before IEC, 1 - 5 - elution of PsbQ, M - marker*

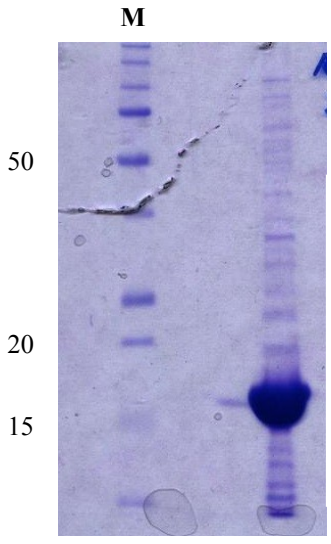

Figure 12*:* *SDS-PAGE of concentrated sample after IEC*

4.2.2. Size exclusion chromatography

In order to avoid column overloading, the size exclusion chromatography was done in two independent runs (each with 5 mL of concentrated sample). Because some of the impurities were of similar size as PsbQ, the flow rate was decreased to 0.4 $mL \cdot min^{-1}$ to ensure proper separation. 18 fractions of eluate were collected and inspected via SDS-PAGE. According to the gels, 11 fractions (in total 22 mL) containing PsbQ protein were selected and concentrated. The SDS-PAGE of the final product proved sufficient purity (Figure 14).

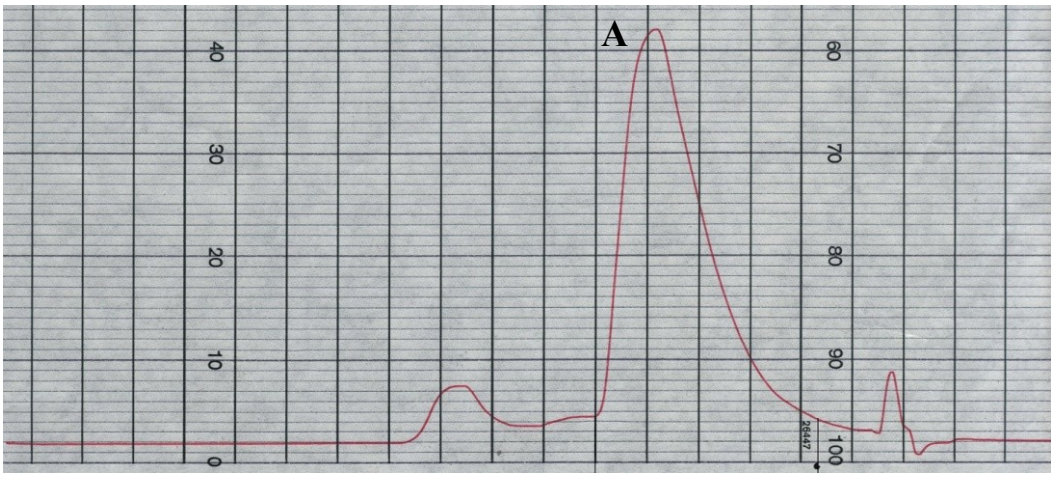

Figure 13: *Chromatogram of Size Exclusion Chromatography, A - elution of PsbQ*

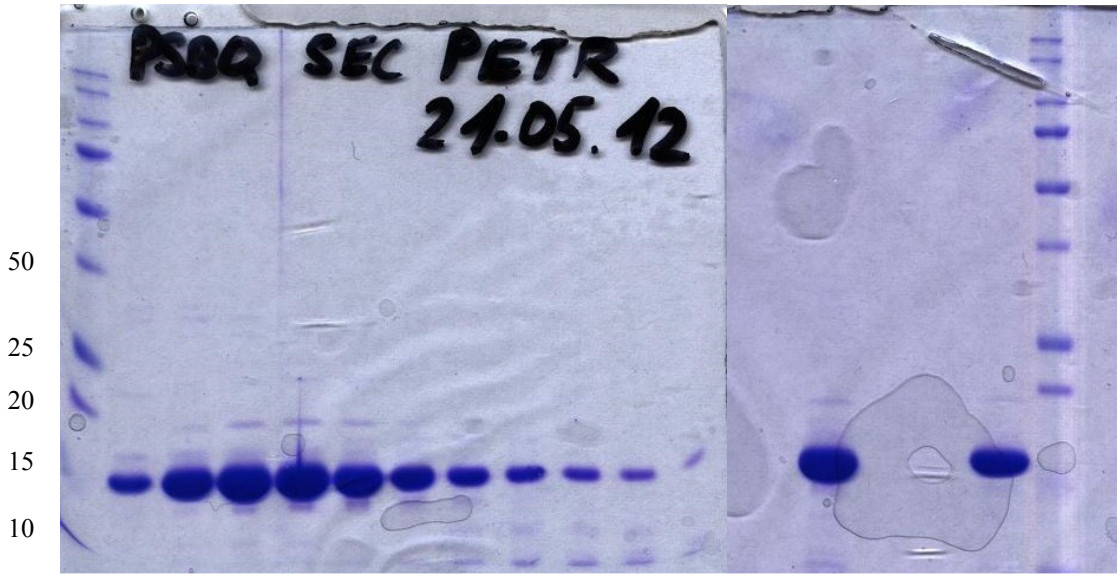

Figure 14: *A - SDS-PAGE of Size Exclusion Chromatography, B - concentrated fractions (left 20µL, right 15µL of sample)*

For both protein samples (singly/doubly labeled) the concentration was determined to be suitable in order to proceed with NMR experiments. However the yield of doubly labeled protein was significantly lower (Table 12). Since the optical density during the cultivation was comparable, the losses were mainly occurring during the sonication and purification steps.

Sample	Volume of M9 media	Concentration	Sample Volume	Total amount
^{15}N PsbQ	1 L	16.75 mg ·mL^{-1}	1.6 ml	26.80 mg
^{15}N ^{13}C PsbQ	1 L	7.62 mg ·mL^{-1}	1.5 ml	11.43 mg

Table 12: *Overview of singly and doubly labeled protein yields*

4.3. Complementation of resonance assignment

With the set of experiments described in chapter 3.6, the previous resonance assignment (56) could be enhanced from 55% to 75% (Table 13). Additional ^{15}N resonances were assigned to missing NH pairs in the ^{15}N HSQC spectrum. They were found with help of the HNCO spectrum exclusively in regions with high peak overlap. In most cases, such overlapping peaks in the HSQC spectrum showed additional carbonyl Cs belonging to previously unassigned amino acids. The ambiguity was resolved by crosschecking with (H)C(C)(CO)NH, HC(C)H-COSY and (H)CCH-TOCSY spectra. The sequence specific ordering of the newly found HN resonances was done with help of the CbCaNH and CbCa(CO)NH experiments. Since the dispersion of carbon chemical shifts is minimal, the majority of the newly found ^{13}C resonances was assigned with help of (H)CC(CO)NH experiment with respect to the BMRB chemical shift database.

Shifts	Completed		Missing		Completed [%]	
	A	B	A	B	A	B
^{1}H	471	730	527	268	47.2	73.1
^{13}C	481	581	257	157	65.1	78.7
^{15}N	125	143	78	60	61.6	70.4
Backbone	596	690	143	49	80.7	93.4
Sidechain	481	764	719	436	40.1	63.7

Table 13: *Comparison of enhanced res. assignment (B) with bmrb entry 173571 (A)*

The remaining hydrogen resonances were assigned by correlation of TOCSY-HSQC with HC(C)H-COSY. However, these spectra were less sensitive and contained lot of noise. In order to determine the exact ^{1}H chemical shift, the non-standard correlation with ^{15}N and ^{13}C NOESY-HSQC was employed (Figure 15). As the sidechain assignment proceeded, the CH resonances in ^{13}C HSQC spectra became assigned and could be correlated in ^{13}C NOESY-HSQC (Figure 16).

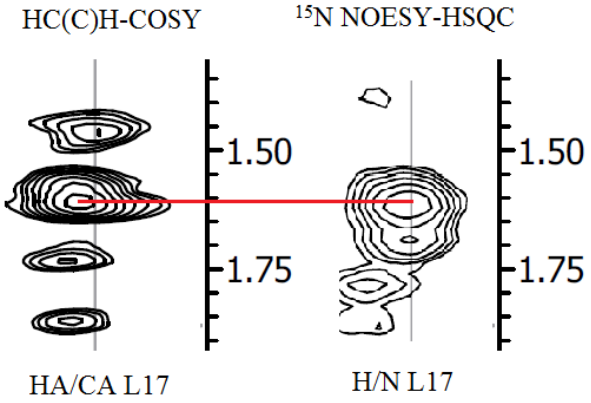

Figure 15: *Example of non standard correlation of ^{15}N NOESY-HSQC with HC(C)H-COSY to disambiguate the H_β chemical shift of leucine-17 residue.*

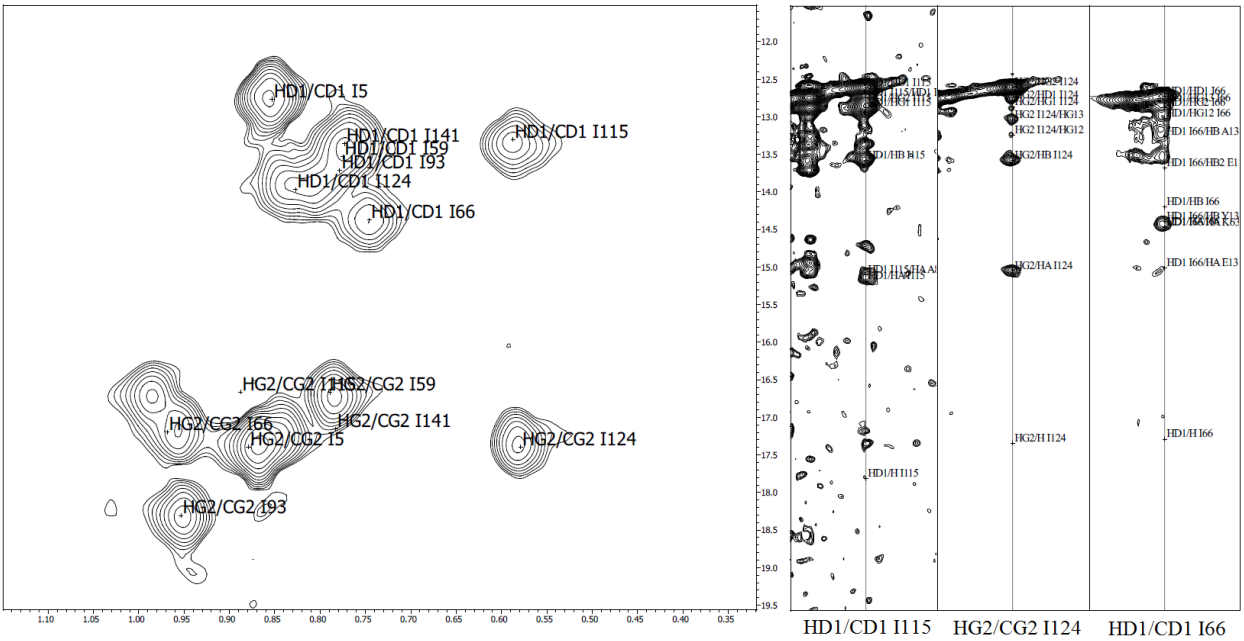

Figure 16: *^{13}C HSQC (left) correlation with ^{13}C NOESY-HSQC (right) of Ile 115, Ile 124 and Ile 66 in methyl region.*

4.4. Secondary structure estimation

Once the assignment was extended, the newly derived peak list was uploaded on the Talos-N prediction server. From the backbone chemical shifts, the program predicted five *α*-helices and one short *β*-strand (Figure 17). The *α*-helices (located within residues 36-39, 45-68, 71-94, 98 -123 and 127-147) were predicted with an average probability of more than 85%. Residues 5-7 were predicted to have *β*-strand propensity, however the prediction percentage was lower and closer to the values for random coil. In order to assess the prediction correctness, Ramachandran plot of all residues was inspected.

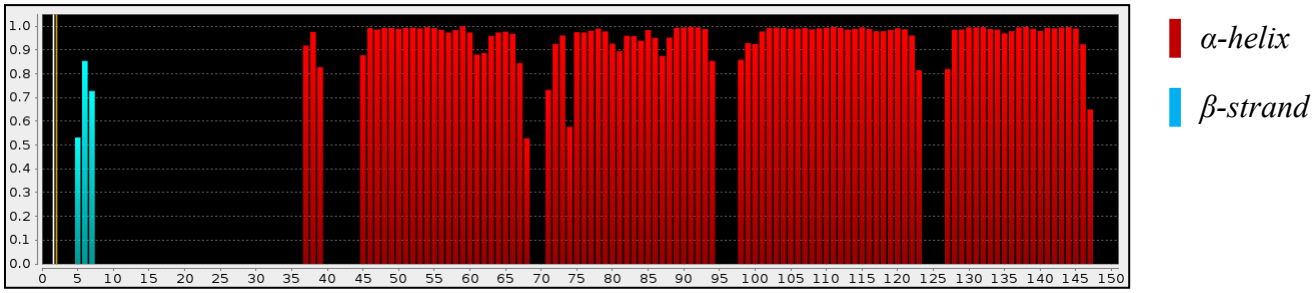

Figure 17: *Predicted secondary structure by Talos-N*

All dihedral angles with probability lower than 85% or close to the random coil value were discarded. Additionally, all remaining values (ϕ and ψ torsion angles) were increased by ±10° to provide more degrees of freedom during the structure calculation. This prevents from excessive dihedral constraint-dependence and puts more emphasis on the NOE derived distance constraints.

4.5. ^{15}N, ^{13}C NOESY assignment

In total 1035 NOESY cross peaks were assigned and integrated. The majority of these peaks was assigned in the ^{15}N NOESY-HSQC. This spectrum provided mostly intra-residual, short and medium distance NOEs. Remarkable was the cross-peak distribution along the protein sequence. In the predicted α-helical regions, the number of NOEs was significantly higher than in regions predicted as unstructured. Notably, NOEs indicative of α-helical structure [$d_{NN}(i,i+1)$, $d_{\alpha N}(i,i+1)$, $d_{\beta N}(i,i+1)$ and $d_{\alpha N}(i,i+3)$] were found in all five α helices proposed with Talos-N. However, not a single NOE corresponding to the Talos predicted *β*-strand was found (Appendix Figure 24).

The long range NOEs were found in ^{13}C NOESY-HSQC. Unfortunately, due to artifacts in this spectrum only a limited number of 138 cross-peaks could be identified. The experiment was repeated with a fresh protein sample, but the spectrum did not provide any additional information.

Total	Intra-residual	Short range	Medium range	Long range
1035	447	373	77	138

Table 14: *NOE upper distance constraints derived from ^{13}C and ^{15}N NOESY-HSQC*

4.6. Solution structure calculation

Based on 1035 upper distance and 211 torsion angle constraints derived from Talos-N, 400 structures were generated in 10000 annealing steps. An average target function of 6.54 ± 0.29 Å^2 was obtained for the 20 best CYANA conformers with maximal distance constraint violation of 0.44 Å. The mean backbone root mean square deviation (RMSD) for the overall 20 conformers was relatively high (3.31 ±0.54 Å) which is due to the presence of very flexible N-terminal part. Limiting the calculation to the stable 4-helix bundle of residues 49-148, the mean backbone RMSD was determined as 0.41 ± 0.07 Å.

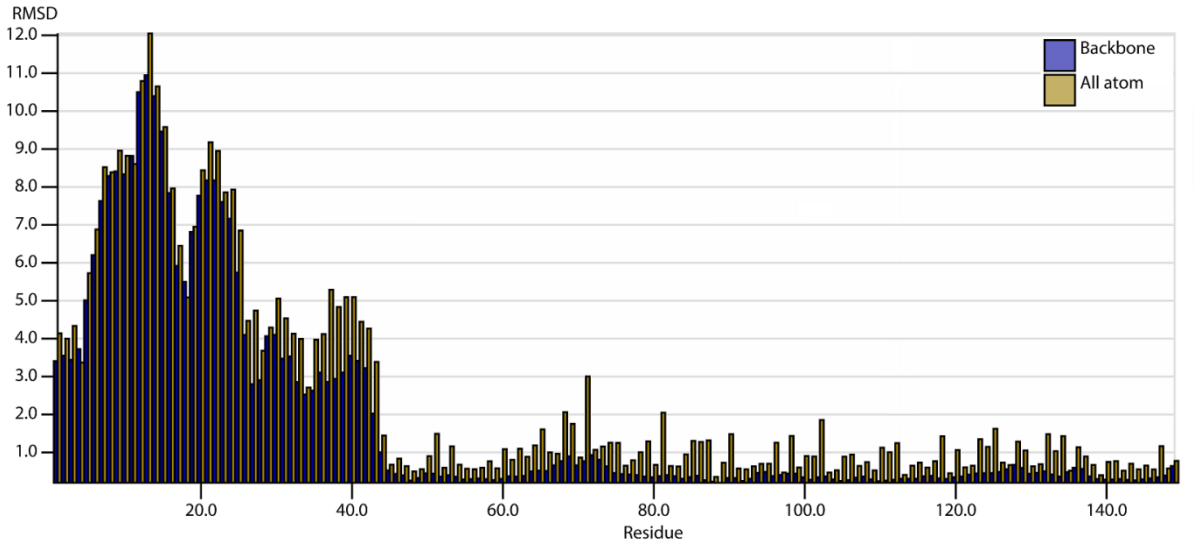

Figure 18*: RMSD distribution along the structure for 20 generated conformers*

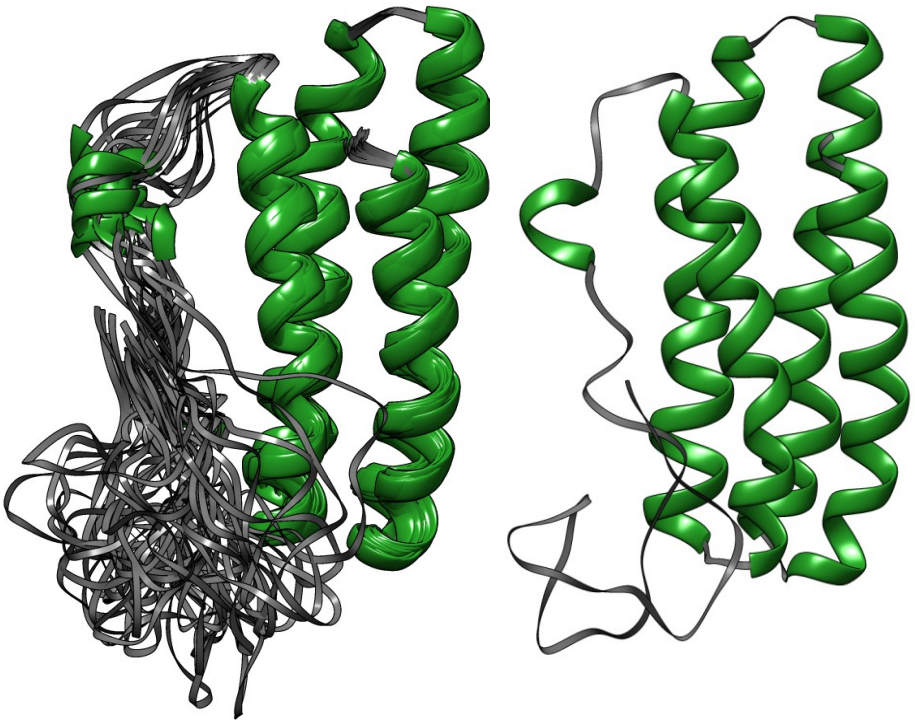

Figure 19: *Final solution structure of PsbQ. Ensemble of 20 structures with the lowest CYANA target function (left) and lowest energy single structure (right)*

In comparison with the most up-to-date available crystallographic structure (PDB entry 1VYK) the dynamic N-terminus of the molecule exhibits α-helical propensity at the location where a short β-strand has been proposed (res. 37 - 40) (4). The two β-strands present in the crystallographic structure had been included on the basis of MD simulation results (4), whereas the short α-helix was found from experimental NOE distance constraints in solution.

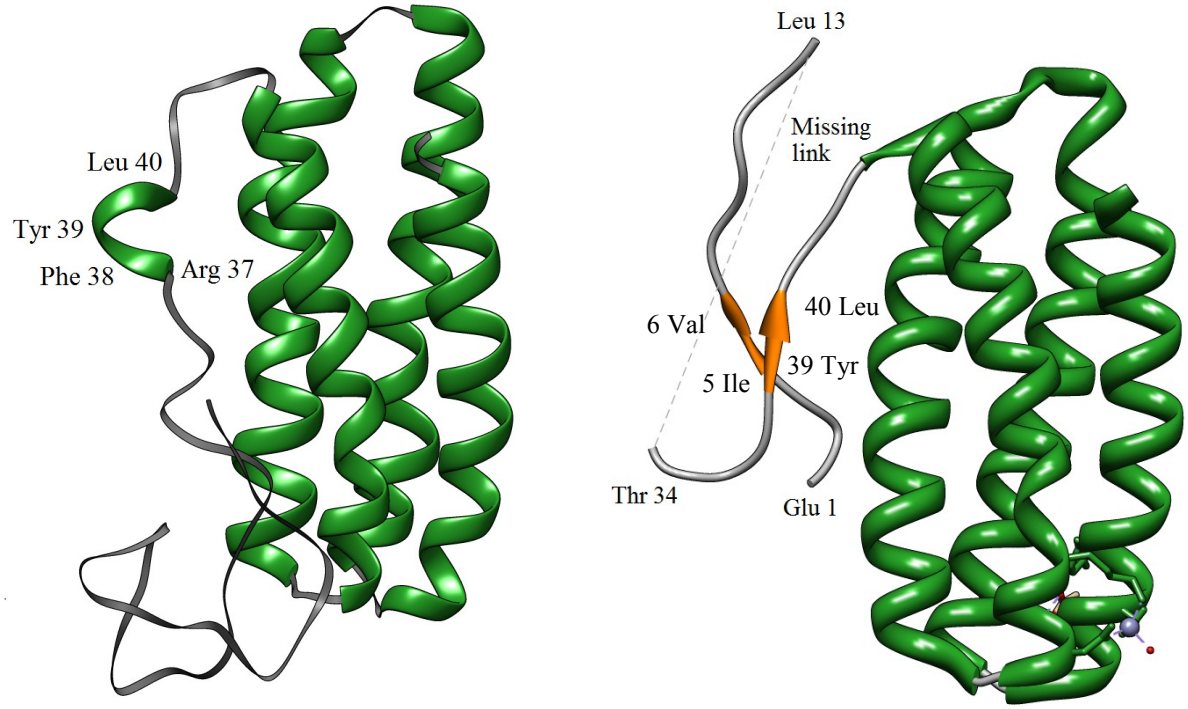

Figure 20: *Comparison between the NMR determined solution structure (left) and X-ray crystallographic structure (right)(4)*

So called "missing link", unresolvable in X-ray crystallography, has been fully assigned. However no NOEs present, which would give a clear structural information, have been found (Appendix Figure 23). This is in good accordance with dynamically disorder N-terminal region of the free PsbQ protein in solution.

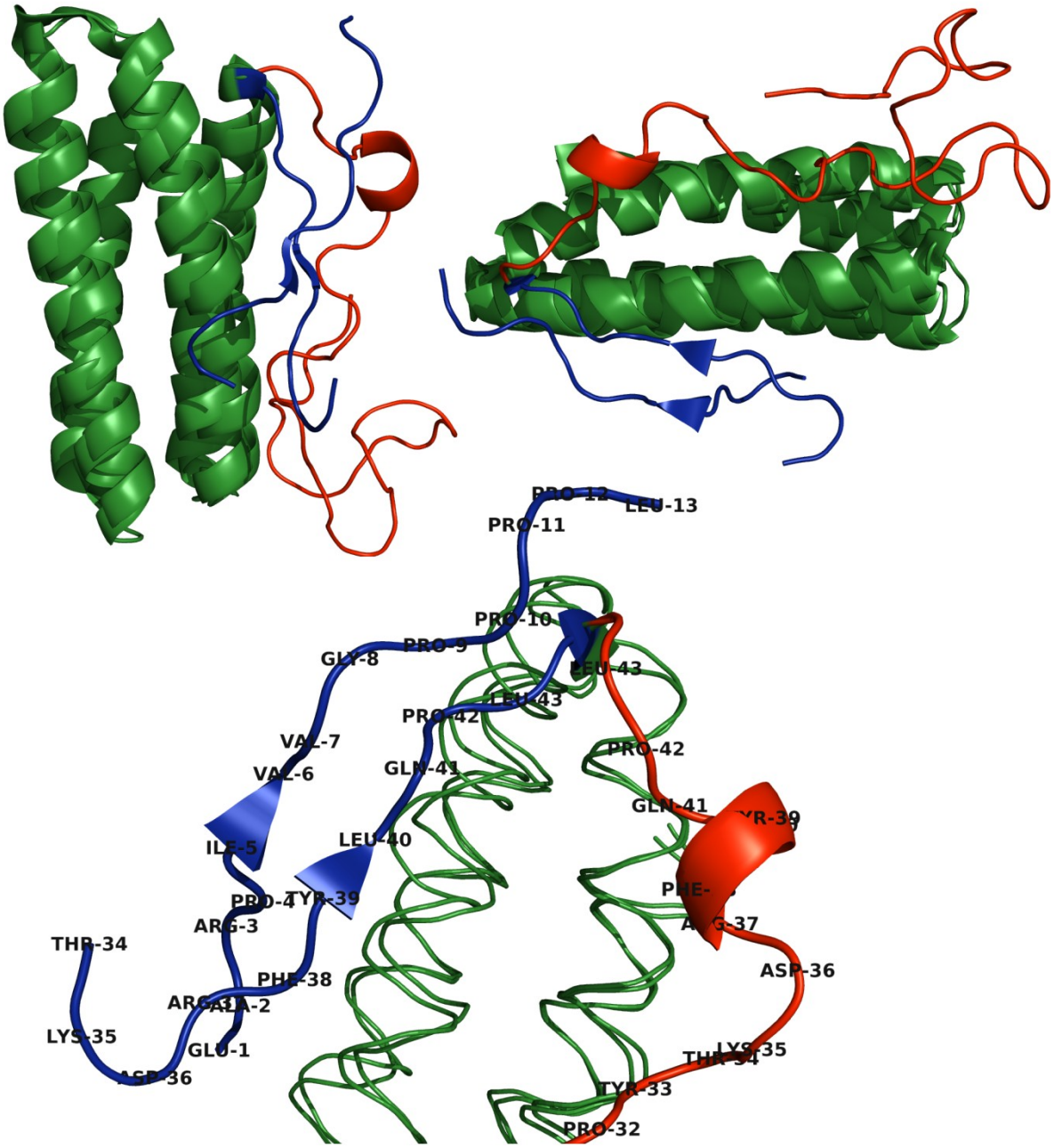

Figure 21: *Three different views of the alignment of the crystallographic structure (N-terminal part in blue) (4) and NMR solution structure determined in this Thesis (N-Terminal part in red) of PsbQ*

5. Conclusion

The protein PsbQ was successfully expressed in the *E. coli* expression system in both singly and doubly isotope labeled forms with an improved yield of 26.80 mg for ^{15}N-PsbQ and 11.43 mg ^{15}N^{13}C-PsbQ, respectively. Six different 3D NMR spectra were be recorded to extend the resonance assignment. Two heteronuclear filtered 3D NOESY spectra served to proceed to the structure calculation. The resonance assignment was extended from 80.7% to the final 93 % of the backbone and from 40.1% to 64 % of the sidechain resonances as compared to the previous results (56).

The secondary structure estimation by Talos-N (45) using the newly extended assignment confirmed the already known four α-helical bundle together with a small α-helix within residues 36-39. Only one from the two β-strands (forming a parallel β-sheet) present in the X-ray structure, only one was estimated to be present by Talos-N. However, the probability was low and the angle distribution in Ramachandran plot did not corroborate this prediction.

A ^{15}N NOESY-HSQC spectrum was recorded and assigned following the chemical shift matches along the protein sequence. In the ambiguous cases, the signals were assigned taking guidance from the predicted secondary structure. Most of the peaks assigned belong to the short-to-medium range NOEs. The N-terminal region of the protein exhibited a lack of NOE cross-peak signals compared to the regions with alpha helical propensity (Appendix Figure 22). An additional ^{13}C NOESY-HSQC spectrum was recorded to obtain long range NOEs. Unfortunately, due to the residual artifacts and noise, only a limited number of NOEs was found and assigned.

The peak lists from both NOESY spectra were integrated and imported to the torsion angle molecular program CYANA. In total 1035 NOESY cross peaks were used to calculate the final 20 structures from 400 conformers in 10000 annealing steps. After each run, the generated distance violations were inspected in order to improve the NOE peak integration.

Based on the experimentally obtained distance constraints, the resulting structure ensemble clearly indicated high flexibility of the N-terminal region of the PsbQ protein in solution, whereas the rest of the molecule remains rigid dominated. In total five α-helixes are present in all of the final 20 CYANA lowest target function structures. Four α-helixes forming a bundle correspond closely to the available crystallographic structure (4) the fifth (short)

α-helix is located within residues 37-40. This is a region, where a β-strand had been found in the crystal structure, where this strand forms a parallel β-sheet together with residues 4-6. The latter part is completely disordered in the solution structure. In the completely assigned "missing link" region (res. 14-33) no NOE signals were found, indicating high flexibility in accordance with preliminary relaxation data (58).

In future investigations it is proposed to perform further NOESY experiments at lower temperature and to take additional precautions to avoid the observed artifacts. Additional structure constraints could potentially be obtained after assigning the aromatic sidechains. Eventually one might, find NOEs between the dynamic N-terminus and the alpha helical bundle in the way allowing to restrict the conformational space of the N-terminus.

Future research on this protein will be mainly focused on the interactions with other proteins in PSII. The titration of isotopically labeled PsbQ with unlabeled PsbP and *vice versa* (and possibly PsbR) could be used to identify the interaction sites between these proteins in NOESY spectra. Since the NMR assignment for PsbP is ongoing and will be available soon, even some characteristic ^{1}H chemical shifts from unlabeled PsbP might be suitable to identify first contacts in NOESY experiments.

6. References

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7. Appendix

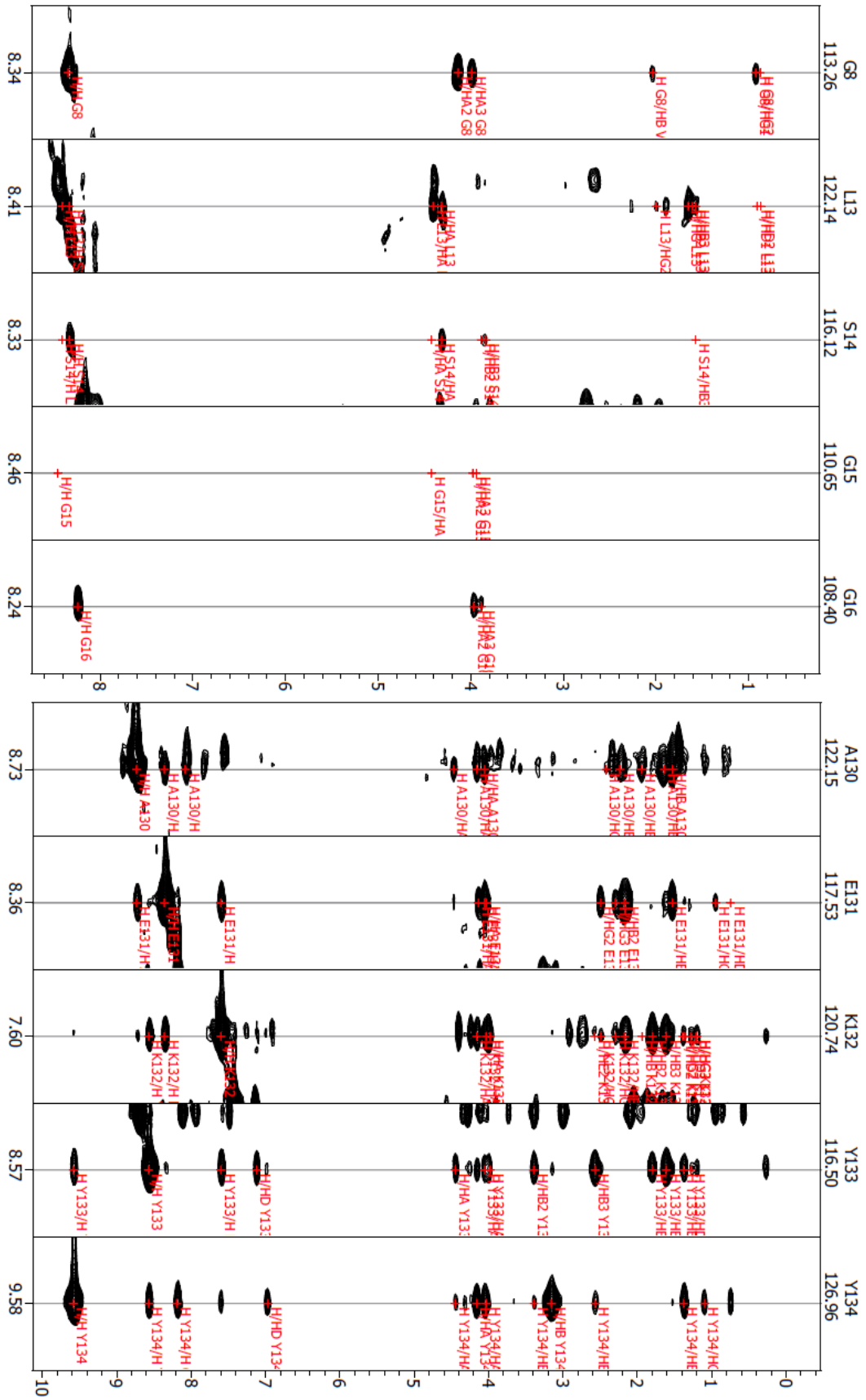

Figure 22: *The comparison of ^{15}N NOESY-HSQC strips for dynamic region (left) and for α-helical region (right)*

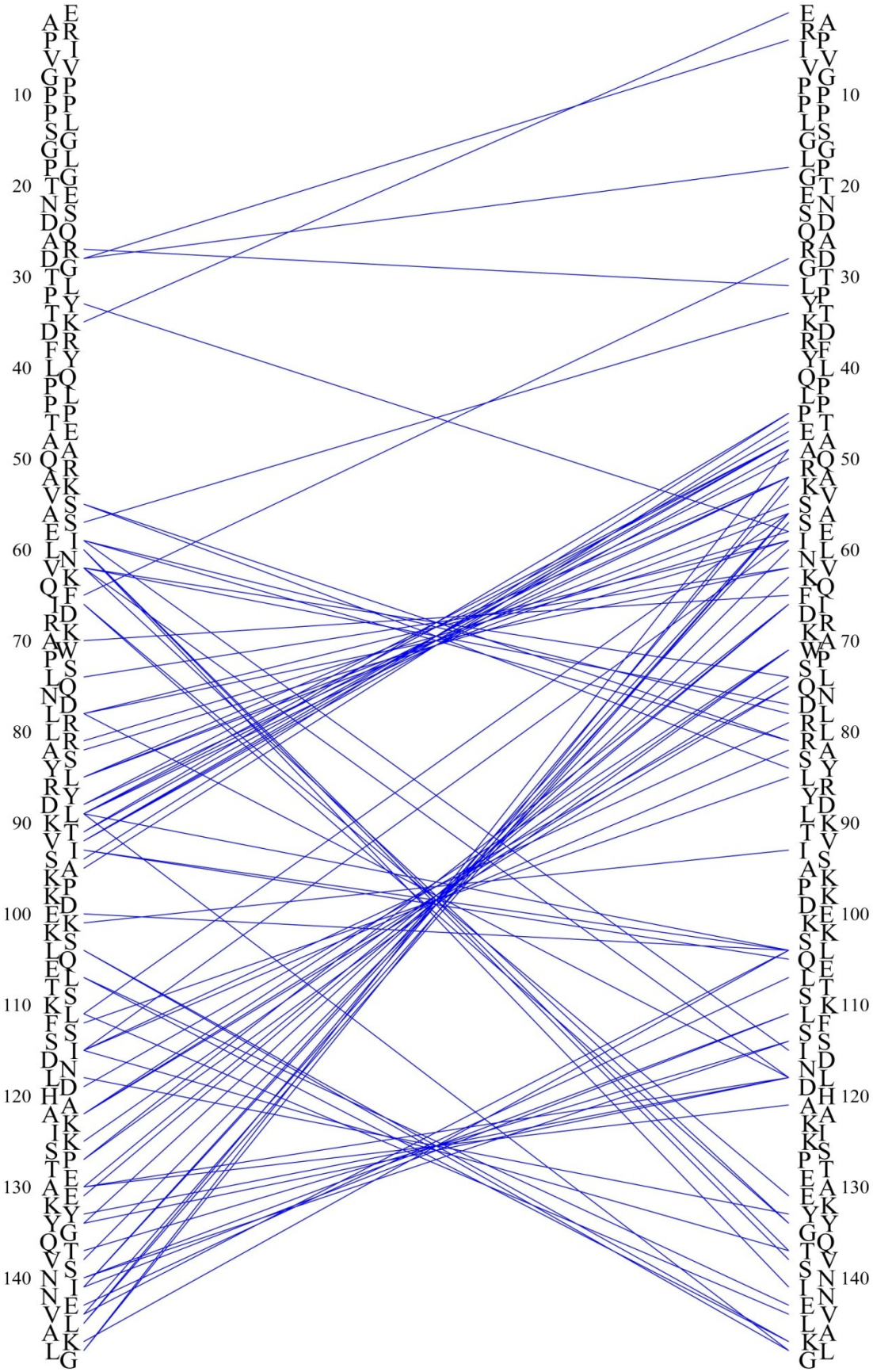

Figure 23: *The distribution of long range NOEs along the protein sequence*

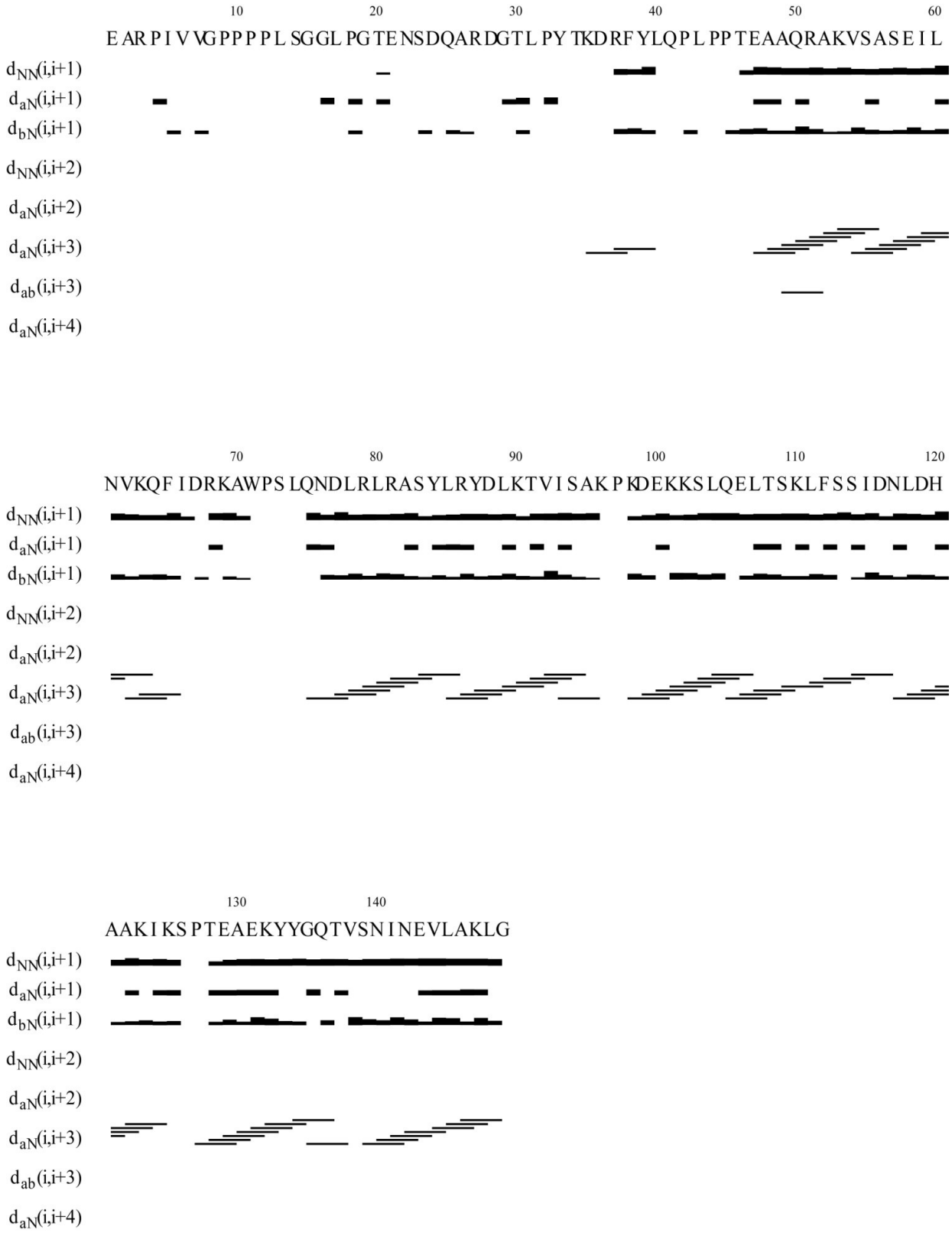

Figure 24: *The distribution of short and medium range NOEs along the protein sequence*

7.1. Resonance assignment of PsbQ (newly found assignments are **printed bold)**

_Atom_shift_assign_ID
_Residue_seq_code
_Residue_label
_Atom_name
_Atom_type
_Chem_shift_value
_Chem_shift_value_error
_Chem_shift_ambiguity_code

1	1	GLU	C	C	175.743	0.3	1
2	1	GLU	CA	C	56.271	0.3	1
3	1	GLU	CB	C	30.756	0.3	1
4	1	GLU	CG	C	36.119	0.3	1
5	1	GLU	HA	H	4.292	0.020	1
6	1	GLU	HB2	H	2.022	0.020	2
7	1	GLU	HB3	H	1.947	0.020	2
8	1	GLU	HG2	H	2.263	0.020	1
9	2	ALA	C	C	177.285	0.3	1
10	2	ALA	CA	C	56.524	0.3	1
11	2	ALA	CB	C	19.130	0.3	1
12	2	ALA	H	H	8.545	0.020	1
13	2	ALA	HA	H	4.303	0.020	1
14	2	ALA	HB	H	1.387	0.020	1
15	2	ALA	N	N	126.338	0.3	1
16	3	ARG	C	C	174.172	0.3	1
17	3	ARG	CA	C	53.152	0.3	1
18	3	ARG	CB	C	30.648	0.3	1
19	3	ARG	H	H	8.203	0.020	1
20	3	ARG	HA	H	3.882	0.020	1
21	**3**	**ARG**	**HB2**	**H**	**1.727**	**0.020**	**1**
22	3	ARG	N	N	122.153	0.3	1
23	4	PRO	C	C	176.611	0.3	1
24	4	PRO	CA	C	62.847	0.3	1
25	4	PRO	CB	C	31.976	0.3	1
26	4	PRO	CG	C	27.319	0.3	1
27	4	PRO	CD	C	50.528	0.3	1
28	4	PRO	HA	H	4.439	0.020	1
29	4	PRO	HB2	H	2.287	0.020	2
30	4	PRO	HB3	H	1.929	0.020	2
31	4	PRO	HG2	H	2.020	0.020	2
32	4	PRO	HG3	H	1.910	0.020	2
33	4	PRO	HD2	H	3.778	0.020	2
34	4	PRO	HD3	H	3.598	0.020	2
35	4	PRO	N	N	137.044	0.3	1
36	5	ILE	C	C	176.192	0.3	1
37	5	ILE	CA	C	61.048	0.3	1
38	5	ILE	CB	C	38.567	0.3	1
39	5	ILE	CG1	C	27.300	0.3	1
40	5	ILE	CG2	C	17.394	0.3	1
41	5	ILE	CD1	C	12.766	0.3	1
42	5	ILE	H	H	8.299	0.020	1
43	5	ILE	HA	H	4.138	0.020	1

44	5	ILE	HB	H	1.818	0.020	1
45	5	ILE	HG12	H	1.498	0.020	2
46	5	ILE	HG13	H	1.205	0.020	2
47	5	ILE	HG2	H	0.879	0.020	1
48	5	ILE	HD1	H	0.853	0.020	1
49	5	ILE	N	N	121.708	0.3	1
50	6	VAL	C	C	175.804	0.3	1
51	6	VAL	CA	C	62.102	0.3	1
52	6	VAL	CB	C	32.850	0.3	1
53	6	VAL	CG1	C	20.942	0.3	1
54	6	VAL	H	H	8.276	0.020	1
55	6	VAL	HA	H	4.143	0.020	1
56	6	VAL	HB	H	2.000	0.020	1
57	6	VAL	HG1	H	0.887	0.020	1
58	6	VAL	N	N	125.784	0.3	1
59	7	VAL	C	C	176.092	0.3	1
60	7	VAL	CA	C	62.148	0.3	1
61	7	VAL	CB	C	32.716	0.3	1
62	7	VAL	CG1	C	21.111	0.3	1
63	**7**	**VAL**	**CG2**	**C**	**20.476**	**0.3**	**1**
64	7	VAL	H	H	8.373	0.020	1
65	7	VAL	HA	H	4.139	0.020	1
66	7	VAL	HB	H	2.037	0.020	1
67	7	VAL	HG1	H	0.933	0.020	1
68	**7**	**VAL**	**HG2**	**H**	**0.921**	**0.020**	**1**
69	7	VAL	N	N	125.834	0.3	1
70	8	GLY	C	C	170.954	0.3	1
71	8	GLY	CA	C	44.224	0.3	1
72	8	GLY	H	H	8.329	0.020	1
73	8	GLY	HA2	H	4.165	0.020	2
74	8	GLY	HA3	H	3.994	0.020	2
75	8	GLY	N	N	113.433	0.3	1
76	9	PRO	C	C	174.457	0.3	1
77	9	PRO	CA	C	61.279	0.3	1
78	9	PRO	CB	C	30.772	0.3	1
79	9	PRO	N	N	135.780	0.3	1
80	10	PRO	C	C	174.298	0.3	1
81	10	PRO	CA	C	61.352	0.3	1
82	10	PRO	CB	C	30.301	0.3	1
83	10	PRO	N	N	136.956	0.3	1
84	11	PRO	C	C	174.799	0.3	1
85	11	PRO	CA	C	61.382	0.3	1
86	11	PRO	CB	C	30.856	0.3	1
87	11	PRO	N	N	136.418	0.3	1
88	12	PRO	C	C	177.050	0.3	1
89	12	PRO	CA	C	62.755	0.3	1
90	12	PRO	CB	C	31.867	0.3	1
91	12	PRO	CG	C	27.319	0.3	1
92	12	PRO	CD	C	50.243	0.3	1
93	12	PRO	HA	H	4.364	0.020	1
94	12	PRO	HB2	H	2.197	0.020	2
95	12	PRO	HB3	H	1.918	0.020	2
96	12	PRO	HG2	H	1.996	0.020	2
97	12	PRO	HG3	H	1.976	0.020	2
98	12	PRO	HD2	H	3.770	0.020	2
99	12	PRO	HD3	H	3.599	0.020	2

100	12	PRO	N	N	135.006	0.3	1
101	13	LEU	C	C	177.667	0.3	1
102	13	LEU	CA	C	55.386	0.3	1
103	13	LEU	CB	C	42.278	0.3	1
104	13	LEU	CG	C	27.002	0.3	1
105	13	LEU	CD1	C	24.788	0.3	1
106	13	LEU	CD2	C	23.607	0.3	1
107	13	LEU	H	H	8.390	0.020	1
108	13	LEU	HA	H	4.325	0.020	1
109	13	LEU	HB2	H	1.650	0.020	2
110	13	LEU	HB3	H	1.578	0.020	2
111	13	LEU	HG	H	1.619	0.020	1
112	13	LEU	HD1	H	0.905	0.020	1
113	13	LEU	HD2	H	0.883	0.020	1
114	13	LEU	N	N	122.277	0.3	1
115	14	SER	C	C	175.007	0.3	1
116	14	SER	CA	C	58.305	0.3	1
117	14	SER	CB	C	63.723	0.3	1
118	14	SER	H	H	8.314	0.020	1
119	14	SER	HA	H	4.435	0.020	1
120	14	SER	HB2	H	3.858	0.020	1
121	14	SER	HB3	H	3.858	0.020	1
122	14	SER	N	N	116.217	0.3	1
123	15	GLY	C	C	174.457	0.3	1
124	15	GLY	CA	C	45.319	0.3	1
125	15	GLY	H	H	8.436	0.020	1
126	15	GLY	HA2	H	3.993	0.020	2
127	15	GLY	HA3	H	3.938	0.020	2
128	15	GLY	N	N	110.768	0.3	1
129	16	GLY	C	C	173.717	0.3	1
130	16	GLY	CA	C	44.815	0.3	1
131	16	GLY	H	H	8.237	0.020	1
132	16	GLY	HA2	H	3.960	0.020	2
133	16	GLY	HA3	H	3.902	0.020	2
134	16	GLY	N	N	108.501	0.3	1
135	17	LEU	C	C	175.465	0.3	1
136	17	LEU	CA	C	53.028	0.3	1
137	17	LEU	CB	C	41.527	0.3	1
138	**17**	**LEU**	**CG**	**C**	**26.592**	**0.3**	**1**
139	**17**	**LEU**	**CD1**	**C**	**24.855**	**0.3**	**1**
140	**17**	**LEU**	**CD2**	**C**	**22.849**	**0.3**	**1**
141	17	LEU	H	H	8.180	0.020	1
142	**17**	**LEU**	**HA**	**H**	**4.602**	**0.020**	**1**
143	**17**	**LEU**	**HB2**	**H**	**1.595**	**0.020**	**1**
144	**17**	**LEU**	**HB3**	**H**	**1.595**	**0.020**	**1**
145	**17**	**LEU**	**HG**	**H**	**2.008**	**0.020**	**1**
146	**17**	**LEU**	**HD1**	**H**	**0.944**	**0.020**	**1**
147	**17**	**LEU**	**HD2**	**H**	**0.883**	**0.020**	**1**
148	17	LEU	N	N	122.717	0.3	1
149	18	PRO	C	C	177.505	0.3	1
150	18	PRO	CA	C	63.486	0.3	1
151	18	PRO	CB	C	31.975	0.3	1
152	18	PRO	CG	C	27.414	0.3	1
153	18	PRO	CD	C	50.571	0.3	1
154	18	PRO	HA	H	4.430	0.020	1
155	18	PRO	HB2	H	2.287	0.020	2

156	18	PRO	HB3	H	1.928	0.020	2
157	18	PRO	HG2	H	2.053	0.020	2
158	18	PRO	HG3	H	1.990	0.020	2
159	18	PRO	HD2	H	3.818	0.020	1
160	18	PRO	HD3	H	3.818	0.020	1
161	18	PRO	N	N	136.735	0.3	1
162	19	GLY	C	C	174.710	0.3	1
163	19	GLY	CA	C	45.286	0.3	1
164	19	GLY	H	H	8.614	0.020	1
165	19	GLY	HA2	H	4.004	0.020	2
166	19	GLY	HA3	H	3.979	0.020	2
167	19	GLY	N	N	109.845	0.3	1
168	20	THR	C	C	174.913	0.3	1
169	20	THR	CA	C	61.813	0.3	1
170	20	THR	CB	C	70.055	0.3	1
171	20	THR	CG2	C	21.539	0.3	1
172	20	THR	H	H	8.011	0.020	1
173	20	THR	HA	H	4.380	0.020	1
174	20	THR	HB	H	4.286	0.020	1
175	20	THR	HG2	H	1.198	0.020	1
176	20	THR	N	N	112.697	0.3	1
177	21	GLU	C	C	176.296	0.3	1
178	21	GLU	CA	C	56.887	0.3	1
179	21	GLU	CB	C	30.382	0.3	1
180	21	GLU	CG	C	36.077	0.3	1
181	21	GLU	H	H	8.735	0.020	1
182	21	GLU	HA	H	4.298	0.020	1
183	21	GLU	HB2	H	1.944	0.020	2
184	**21**	**GLU**	**HB3**	**H**	**2.038**	**0.020**	**2**
185	21	GLU	HG2	H	2.273	0.020	1
186	21	GLU	N	N	123.233	0.3	1
187	22	ASN	C	C	175.406	0.3	1
188	22	ASN	CA	C	53.126	0.3	1
189	22	ASN	CB	C	38.904	0.3	1
190	22	ASN	H	H	8.511	0.020	1
191	22	ASN	HA	H	4.727	0.020	1
192	22	ASN	HB2	H	2.843	0.020	2
193	22	ASN	HB3	H	2.752	0.020	2
194	22	ASN	N	N	119.605	0.3	1
195	23	SER	C	C	174.597	0.3	1
196	23	SER	CA	C	58.665	0.3	1
197	23	SER	CB	C	63.364	0.3	1
198	23	SER	H	H	8.325	0.020	1
199	23	SER	HA	H	4.438	0.020	1
200	23	SER	HB2	H	3.933	0.020	2
201	23	SER	HB3	H	3.837	0.020	2
202	23	SER	N	N	116.428	0.3	1
203	24	ASP	C	C	176.498	0.3	1
204	24	ASP	CA	C	54.861	0.3	1
205	24	ASP	CB	C	40.809	0.3	1
206	24	ASP	H	H	8.456	0.020	1
207	24	ASP	HA	H	4.574	0.020	1
208	24	ASP	HB2	H	2.706	0.020	2
209	24	ASP	HB3	H	2.647	0.020	2
210	24	ASP	N	N	122.299	0.3	1
211	25	GLN	C	C	176.011	0.3	1

212	25	GLN	CA	C	56.173	0.3	1
213	25	GLN	CB	C	28.924	0.3	1
214	25	GLN	CG	C	33.792	0.3	1
215	25	GLN	H	H	8.188	0.020	1
216	25	GLN	HA	H	4.265	0.020	1
217	25	GLN	HB2	H	2.117	0.020	2
218	25	GLN	HB3	H	1.970	0.020	2
219	25	GLN	HG2	H	2.356	0.020	1
220	25	GLN	N	N	120.032	0.3	1
221	26	ALA	C	C	178.001	0.3	1
222	26	ALA	CA	C	54.774	0.3	1
223	26	ALA	CB	C	18.904	0.3	1
224	26	ALA	H	H	8.242	0.020	1
225	26	ALA	HA	H	4.272	0.020	1
226	26	ALA	HB	H	1.390	0.020	1
227	26	ALA	N	N	124.260	0.3	1
228	27	ARG	C	C	176.263	0.3	1
229	27	ARG	CA	C	56.202	0.3	1
230	27	ARG	CB	C	30.782	0.3	1
231	27	ARG	CG	C	27.064	0.3	1
232	27	ARG	CD	C	43.216	0.3	1
233	27	ARG	H	H	8.206	0.020	1
234	27	ARG	HA	H	4.297	0.020	1
235	27	ARG	HB2	H	1.844	0.020	1
236	27	ARG	HG2	H	1.639	0.020	1
237	27	ARG	HD2	H	3.181	0.020	1
238	27	ARG	N	N	120.165	0.3	1
239	28	ASP	C	C	176.867	0.3	1
240	28	ASP	CA	C	54.396	0.3	1
241	28	ASP	CB	C	41.109	0.3	1
242	28	ASP	H	H	8.318	0.020	1
243	28	ASP	HA	H	4.578	0.020	1
244	28	ASP	HB2	H	2.702	0.020	2
245	28	ASP	HB3	H	2.657	0.020	2
246	28	ASP	N	N	120.786	0.3	1
247	29	GLY	C	C	174.550	0.3	1
248	29	GLY	CA	C	45.272	0.3	1
249	29	GLY	H	H	8.381	0.020	1
250	29	GLY	HA2	H	4.007	0.020	2
251	29	GLY	HA3	H	3.962	0.020	2
252	29	GLY	N	N	109.645	0.3	1
253	30	THR	C	C	174.550	0.3	1
254	30	THR	CA	C	62.164	0.3	1
255	30	THR	CB	C	69.933	0.3	1
256	30	THR	CG2	C	21.615	0.3	1
257	30	THR	H	H	8.118	0.020	1
258	30	THR	HA	H	4.291	0.020	1
259	30	THR	HB	H	4.183	0.020	1
260	30	THR	HG2	H	1.186	0.020	1
261	30	THR	N	N	113.478	0.3	1
262	31	LEU	C	C	175.354	0.3	1
263	31	LEU	CA	C	52.903	0.3	1
264	31	LEU	CB	C	41.753	0.3	1
265	**31**	**LEU**	**CG**	**C**	**26.938**	**0.3**	**1**
266	**31**	**LEU**	**CD1**	**C**	**24.979**	**0.3**	**1**
267	**31**	**LEU**	**CD2**	**C**	**23.226**	**0.3**	**1**

268	31	LEU	H	H	8.173	0.020	1
269	**31**	**LEU**	**HA**	**H**	**4.605**	**0.020**	**1**
270	**31**	**LEU**	**HB2**	**H**	**1.590**	**0.020**	**2**
271	**31**	**LEU**	**HB3**	**H**	**1.433**	**0.020**	**2**
272	**31**	**LEU**	**HG**	**H**	**1.650**	**0.020**	**1**
273	**31**	**LEU**	**HD1**	**H**	**0.910**	**0.020**	**1**
274	**31**	**LEU**	**HD2**	**H**	**0.870**	**0.020**	**1**
275	31	LEU	N	N	125.247	0.3	1
276	32	PRO	C	C	176.448	0.3	1
277	32	PRO	CA	C	63.134	0.3	1
278	32	PRO	CB	C	31.720	0.3	1
279	32	PRO	CG	C	27.233	0.3	1
280	32	PRO	CD	C	50.455	0.3	1
281	32	PRO	HA	H	4.363	0.020	1
282	32	PRO	HB2	H	1.772	0.020	2
283	**32**	**PRO**	**HB3**	**H**	**2.175**	**0.020**	**2**
284	32	PRO	HG2	H	1.946	0.020	1
285	32	PRO	HD2	H	3.793	0.020	2
286	32	PRO	HD3	H	3.554	0.020	2
287	32	PRO	N	N	135.635	0.3	1
288	33	TYR	C	C	175.914	0.3	1
289	33	TYR	CA	C	57.947	0.3	1
290	33	TYR	CB	C	38.379	0.3	1
291	33	TYR	H	H	8.099	0.020	1
292	33	TYR	HA	H	4.580	0.020	1
293	33	TYR	HB2	H	2.997	0.020	1
294	33	TYR	HB3	H	2.997	0.020	1
295	33	TYR	N	N	119.769	0.3	1
296	34	THR	C	C	174.362	0.3	1
297	34	THR	CA	C	61.287	0.3	1
298	34	THR	CB	C	67.500	0.3	1
299	34	THR	CG2	C	21.476	0.3	1
300	34	THR	H	H	7.839	0.020	1
301	34	THR	HA	H	4.263	0.020	1
302	34	THR	HB	H	3.762	0.020	1
303	34	THR	HG2	H	1.125	0.020	1
304	34	THR	N	N	115.311	0.3	1
305	35	LYS	CA	C	56.858	0.3	1
306	35	LYS	CB	C	32.619	0.3	1
307	**35**	**LYS**	**CG**	**C**	**24.653**	**0.3**	**1**
308	**35**	**LYS**	**CD**	**C**	**29.149**	**0.3**	**1**
309	**35**	**LYS**	**CE**	**C**	**41.973**	**0.3**	**1**
310	35	LYS	H	H	8.205	0.020	1
311	**35**	**LYS**	**HA**	**H**	**4.179**	**0.020**	**1**
312	**35**	**LYS**	**HB2**	**H**	**1.854**	**0.020**	**2**
313	**35**	**LYS**	**HB3**	**H**	**1.740**	**0.020**	**2**
314	**35**	**LYS**	**HG2**	**H**	**1.385**	**0.020**	**1**
315	**35**	**LYS**	**HD2**	**H**	**1.625**	**0.020**	**1**
316	**35**	**LYS**	**HD3**	**H**	**1.625**	**0.020**	**1**
317	**35**	**LYS**	**HE2**	**H**	**2.644**	**0.020**	**2**
318	**35**	**LYS**	**HE3**	**H**	**3.353**	**0.020**	**2**
319	35	LYS	N	N	122.839	0.3	1
320	37	ARG	C	C	178.008	0.3	1
321	37	ARG	CA	C	60.018	0.3	1
322	37	ARG	CB	C	29.870	0.3	1
323	**37**	**ARG**	**CG**	**C**	**27.321**	**0.3**	**1**

324	37	ARG	CD	C	43.100	0.3	1
325	37	ARG	H	H	8.917	0.020	1
326	37	ARG	HA	H	3.812	0.020	1
327	37	ARG	HB2	H	1.783	0.020	2
328	37	ARG	HB3	H	1.668	0.020	2
329	37	ARG	HG2	H	1.214	0.020	1
330	37	ARG	HD2	H	2.778	0.020	1
331	37	ARG	N	N	118.703	0.3	1
332	38	PHE	C	C	178.384	0.3	1
333	38	PHE	CA	C	61.929	0.3	1
334	38	PHE	CB	C	39.109	0.3	1
335	38	PHE	H	H	7.572	0.020	1
336	**38**	**PHE**	**HA**	**H**	**4.111**	**0.020**	**1**
337	**38**	**PHE**	**HB2**	**H**	**3.200**	**0.020**	**2**
338	**38**	**PHE**	**HB3**	**H**	**3.270**	**0.020**	**2**
339	**38**	**PHE**	**HD1**	**H**	**7.090**	**0.020**	**1**
340	**38**	**PHE**	**HD2**	**H**	**7.090**	**0.020**	**1**
341	**38**	**PHE**	**HE1**	**H**	**7.244**	**0.020**	**1**
342	**38**	**PHE**	**HE2**	**H**	**7.244**	**0.020**	**1**
343	38	PHE	N	N	117.884	0.3	1
344	39	TYR	CA	C	57.761	0.3	1
345	39	TYR	CB	C	40.854	0.3	1
346	39	TYR	H	H	7.795	0.020	1
347	**39**	**TYR**	**HA**	**H**	**4.514**	**0.020**	**1**
348	**39**	**TYR**	**HB2**	**H**	**2.636**	**0.020**	**2**
349	**39**	**TYR**	**HB3**	**H**	**2.703**	**0.020**	**2**
350	39	TYR	N	N	117.979	0.3	1
351	**40**	**LEU**	**CA**	**C**	**58.104**	**0.3**	**1**
352	**40**	**LEU**	**CB**	**C**	**40.334**	**0.3**	**1**
353	**40**	**LEU**	**CG**	**C**	**26.245**	**0.3**	**1**
354	**40**	**LEU**	**CD1**	**C**	**24.022**	**0.3**	**1**
355	**40**	**LEU**	**CD2**	**C**	**22.424**	**0.3**	**1**
356	**40**	**LEU**	**H**	**H**	**8.974**	**0.020**	**1**
357	**40**	**LEU**	**HA**	**H**	**4.078**	**0.020**	**1**
358	**40**	**LEU**	**HB2**	**H**	**2.152**	**0.020**	**1**
359	**40**	**LEU**	**HB3**	**H**	**2.152**	**0.020**	**1**
360	**40**	**LEU**	**HG**	**H**	**1.727**	**0.020**	**1**
361	**40**	**LEU**	**HD1**	**H**	**1.087**	**0.020**	**1**
362	**40**	**LEU**	**HD2**	**H**	**1.087**	**0.020**	**1**
363	**40**	**LEU**	**N**	**N**	**118.769**	**0.3**	**1**
364	41	GLN	C	C	173.826	0.3	1
365	41	GLN	CA	C	53.329	0.3	1
366	41	GLN	CB	C	29.121	0.3	1
367	42	PRO	C	C	176.576	0.3	1
368	42	PRO	CA	C	63.355	0.3	1
369	42	PRO	CB	C	32.179	0.3	1
370	42	PRO	CG	C	27.414	0.3	1
371	42	PRO	CD	C	50.532	0.3	1
372	42	PRO	HA	H	4.430	0.020	1
373	42	PRO	HB2	H	2.276	0.020	2
374	42	PRO	HB3	H	1.845	0.020	2
375	42	PRO	HG2	H	2.025	0.020	1
376	42	PRO	HD2	H	3.810	0.020	2
377	42	PRO	HD3	H	3.626	0.020	2
378	42	PRO	N	N	138.337	0.3	1
379	43	LEU	C	C	174.668	0.3	1

380	43	LEU	CA	C	52.202	0.3	1
381	43	LEU	CB	C	44.600	0.3	1
382	**43**	**LEU**	**CG**	**C**	**26.904**	**0.3**	**1**
383	**43**	**LEU**	**CD1**	**C**	**24.885**	**0.3**	**1**
384	**43**	**LEU**	**CD2**	**C**	**23.258**	**0.3**	**1**
385	43	LEU	H	H	8.436	0.020	1
386	**43**	**LEU**	**HA**	**H**	**4.308**	**0.020**	**1**
387	**43**	**LEU**	**HB2**	**H**	**2.025**	**0.020**	**2**
388	**43**	**LEU**	**HB3**	**H**	**1.909**	**0.020**	**2**
389	**43**	**LEU**	**HG**	**H**	**2.150**	**0.020**	**1**
390	**43**	**LEU**	**HD1**	**H**	**0.966**	**0.020**	**1**
391	**43**	**LEU**	**HD2**	**H**	**0.696**	**0.020**	**1**
392	43	LEU	N	N	123.331	0.3	1
393	44	PRO	C	C	175.261	0.3	1
394	44	PRO	CA	C	61.348	0.3	1
395	44	PRO	CB	C	30.811	0.3	1
396	44	PRO	N	N	133.601	0.3	1
397	45	PRO	C	C	178.342	0.3	1
398	45	PRO	CA	C	67.176	0.3	1
399	45	PRO	CB	C	32.897	0.3	1
400	45	PRO	HA	H	3.995	0.020	1
401	45	PRO	HB2	H	2.274	0.020	2
402	**45**	**PRO**	**HB3**	**H**	**2.116**	**0.020**	**2**
403	45	PRO	N	N	133.441	0.3	1
404	46	THR	C	C	176.671	0.3	1
405	46	THR	CA	C	66.476	0.3	1
406	46	THR	CB	C	67.930	0.3	1
407	46	THR	CG2	C	22.326	0.3	1
408	46	THR	H	H	8.726	0.020	1
409	46	THR	HA	H	4.000	0.020	1
410	46	THR	HB	H	4.160	0.020	1
411	46	THR	HG2	H	1.280	0.020	1
412	46	THR	N	N	113.415	0.3	1
413	47	GLU	C	C	179.288	0.3	1
414	47	GLU	CA	C	59.399	0.3	1
415	47	GLU	CB	C	30.360	0.3	1
416	47	GLU	CG	C	37.557	0.3	1
417	47	GLU	H	H	7.788	0.020	1
418	47	GLU	HA	H	4.151	0.020	1
419	47	GLU	HB2	H	2.186	0.020	2
420	47	GLU	HB3	H	2.056	0.020	2
421	47	GLU	HG2	H	2.431	0.020	2
422	47	GLU	HG3	H	2.274	0.020	2
423	47	GLU	N	N	122.656	0.3	1
424	48	ALA	C	C	179.169	0.3	1
425	48	ALA	CA	C	55.349	0.3	1
426	48	ALA	CB	C	18.440	0.3	1
427	48	ALA	H	H	9.216	0.020	1
428	48	ALA	HA	H	3.996	0.020	1
429	48	ALA	HB	H	1.466	0.020	1
430	48	ALA	N	N	125.745	0.3	1
431	49	ALA	C	C	178.795	0.3	1
432	49	ALA	CA	C	55.523	0.3	1
433	49	ALA	CB	C	18.137	0.3	1
434	49	ALA	H	H	7.876	0.020	1
435	49	ALA	HA	H	3.868	0.020	1

436	49	ALA	HB	H	1.560	0.020	1
437	49	ALA	N	N	118.860	0.3	1
438	50	GLN	C	C	178.779	0.3	1
439	50	GLN	CA	C	58.164	0.3	1
440	50	GLN	CB	C	28.550	0.3	1
441	50	GLN	CG	C	33.420	0.3	1
442	50	GLN	H	H	7.077	0.020	1
443	50	GLN	HA	H	4.094	0.020	1
444	50	GLN	HB2	H	2.202	0.020	2
445	50	GLN	HB3	H	2.166	0.020	2
446	50	GLN	HG2	H	2.571	0.020	1
447	**50**	**GLN**	**HE21**	**H**	**7.453**	**0.020**	**1**
448	**50**	**GLN**	**HE22**	**H**	**7.453**	**0.020**	**1**
449	50	GLN	N	N	114.588	0.3	1
450	51	ARG	C	C	178.701	0.3	1
451	51	ARG	CA	C	60.411	0.3	1
452	51	ARG	CB	C	30.500	0.3	1
453	**51**	**ARG**	**CG**	**C**	**26.621**	**0.3**	**1**
454	51	ARG	CD	C	43.846	0.3	1
455	51	ARG	H	H	8.050	0.020	1
456	51	ARG	HA	H	4.130	0.020	1
457	51	ARG	HB2	H	1.804	0.020	1
458	51	ARG	HG2	H	1.408	0.020	2
459	51	ARG	HG3	H	0.836	0.020	2
460	51	ARG	HD2	H	3.137	0.020	2
461	51	ARG	HD3	H	2.985	0.020	2
462	51	ARG	N	N	122.658	0.3	1
463	52	ALA	C	C	179.455	0.3	1
464	52	ALA	CA	C	54.248	0.3	1
465	52	ALA	CB	C	17.469	0.3	1
466	52	ALA	H	H	8.776	0.020	1
467	**52**	**ALA**	**HA**	**H**	**4.212**	**0.020**	**1**
468	**52**	**ALA**	**HB**	**H**	**1.462**	**0.020**	**1**
469	52	ALA	N	N	122.617	0.3	1
470	**53**	**LYS**	**CA**	**C**	**60.418**	**0.3**	**1**
471	**53**	**LYS**	**CB**	**C**	**32.119**	**0.3**	**1**
472	**53**	**LYS**	**CG**	**C**	**26.265**	**0.3**	**1**
473	**53**	**LYS**	**CD**	**C**	**29.745**	**0.3**	**1**
474	**53**	**LYS**	**CE**	**C**	**42.133**	**0.3**	**1**
475	**53**	**LYS**	**H**	**H**	**7.559**	**0.020**	**1**
476	**53**	**LYS**	**HA**	**H**	**3.809**	**0.020**	**1**
477	**53**	**LYS**	**HB2**	**H**	**1.976**	**0.020**	**1**
478	**53**	**LYS**	**HB3**	**H**	**1.976**	**0.020**	**1**
479	**53**	**LYS**	**HG2**	**H**	**1.528**	**0.020**	**1**
480	**53**	**LYS**	**HD2**	**H**	**1.900**	**0.020**	**1**
481	**53**	**LYS**	**HD3**	**H**	**1.900**	**0.020**	**1**
482	**53**	**LYS**	**HE2**	**H**	**3.127**	**0.020**	**2**
483	**53**	**LYS**	**HE3**	**H**	**3.082**	**0.020**	**2**
484	**53**	**LYS**	**N**	**N**	**115.660**	**0.3**	**1**
485	54	VAL	C	C	179.383	0.3	1
486	54	VAL	CA	C	66.657	0.3	1
487	54	VAL	CB	C	31.279	0.3	1
488	54	VAL	CG1	C	22.468	0.3	1
489	54	VAL	CG2	C	21.251	0.3	1
490	54	VAL	H	H	7.532	0.020	1
491	54	VAL	HA	H	3.740	0.020	1

492	54	VAL	HB	H	2.495	0.020	1
493	54	VAL	HG1	H	1.142	0.020	1
494	54	VAL	HG2	H	0.992	0.020	1
495	54	VAL	N	N	121.965	0.3	1
496	55	SER	C	C	176.311	0.3	1
497	55	SER	CA	C	62.028	0.3	1
498	55	SER	CB	C	62.351	0.3	1
499	55	SER	H	H	8.796	0.020	1
500	55	SER	HA	H	4.199	0.020	1
501	55	SER	HB2	H	3.945	0.020	1
502	55	SER	N	N	118.423	0.3	1
503	56	ALA	C	C	178.860	0.3	1
504	56	ALA	CA	C	55.349	0.3	1
505	56	ALA	CB	C	18.146	0.3	1
506	56	ALA	H	H	8.946	0.020	1
507	56	ALA	HA	H	3.994	0.020	1
508	56	ALA	HB	H	1.535	0.020	1
509	56	ALA	N	N	121.540	0.3	1
510	57	SER	CA	C	62.036	0.3	1
511	57	SER	CB	C	62.812	0.3	1
512	57	SER	H	H	7.914	0.020	1
513	**57**	**SER**	**HA**	**H**	**4.383**	**0.020**	**1**
514	**57**	**SER**	**HB2**	**H**	**4.070**	**0.020**	**2**
515	**57**	**SER**	**HB3**	**H**	**3.736**	**0.020**	**2**
516	57	SER	N	N	113.374	0.3	1
517	**58**	**GLU**	**CA**	**C**	**59.268**	**0.3**	**1**
518	**58**	**GLU**	**CB**	**C**	**33.192**	**0.3**	**1**
519	**58**	**GLU**	**CG**	**C**	**36.547**	**0.3**	**1**
520	**58**	**GLU**	**H**	**H**	**8.141**	**0.020**	**1**
521	**58**	**GLU**	**HA**	**H**	**4.140**	**0.020**	**1**
522	**58**	**GLU**	**HB2**	**H**	**2.087**	**0.020**	**1**
523	**58**	**GLU**	**HG2**	**H**	**2.518**	**0.020**	**2**
524	**58**	**GLU**	**HG3**	**H**	**2.275**	**0.020**	**2**
525	**58**	**GLU**	**N**	**N**	**121.798**	**0.3**	**1**
526	59	ILE	CA	C	65.564	0.3	1
527	59	ILE	CB	C	36.731	0.3	1
528	**59**	**ILE**	**CG1**	**C**	**30.660**	**0.3**	**1**
529	59	ILE	CG2	C	16.671	0.3	1
530	59	ILE	CD1	C	13.532	0.3	1
531	59	ILE	H	H	8.048	0.020	1
532	**59**	**ILE**	**HA**	**H**	**3.794**	**0.020**	**1**
533	59	ILE	HB	H	2.195	0.020	1
534	**59**	**ILE**	**HG12**	**H**	**1.011**	**0.020**	**1**
535	59	ILE	HG2	H	0.789	0.020	1
536	59	ILE	HD1	H	0.775	0.020	1
537	**59**	**ILE**	**N**	**N**	**122.257**	**0.3**	**1**
538	60	LEU	C	C	180.571	0.3	1
539	60	LEU	CA	C	57.838	0.3	1
540	60	LEU	CB	C	40.410	0.3	1
541	60	LEU	CG	C	27.384	0.3	1
542	60	LEU	CD1	C	25.040	0.3	1
543	60	LEU	CD2	C	24.217	0.3	1
544	60	LEU	H	H	7.680	0.020	1
545	60	LEU	HA	H	4.179	0.020	1
546	60	LEU	HB2	H	2.157	0.020	2
547	60	LEU	HB3	H	1.687	0.020	2

548	60	LEU	HG	H	2.007	0.020	1
549	**60**	**LEU**	**HD1**	**H**	**0.983**	**0.020**	**1**
550	**60**	**LEU**	**HD2**	**H**	**0.983**	**0.020**	**1**
551	60	LEU	N	N	117.387	0.3	1
552	61	ASN	C	C	176.499	0.3	1
553	61	ASN	CA	C	53.856	0.3	1
554	61	ASN	CB	C	38.786	0.3	1
555	61	ASN	H	H	7.649	0.020	1
556	61	ASN	HA	H	4.897	0.020	1
557	61	ASN	HB2	H	3.070	0.020	2
558	61	ASN	HB3	H	2.968	0.020	2
559	61	ASN	N	N	115.815	0.3	1
560	62	VAL	C	C	177.001	0.3	1
561	62	VAL	CA	C	63.404	0.3	1
562	62	VAL	CB	C	31.928	0.3	1
563	62	VAL	CG1	C	18.353	0.3	1
564	62	VAL	CG2	C	20.284	0.3	1
565	62	VAL	H	H	8.077	0.020	1
566	62	VAL	HA	H	4.487	0.020	1
567	62	VAL	HB	H	2.898	0.020	1
568	**62**	**VAL**	**HG1**	**H**	**1.406**	**0.020**	**1**
569	62	VAL	HG2	H	1.479	0.020	1
570	62	VAL	N	N	113.212	0.3	1
571	63	LYS	C	C	177.215	0.3	1
572	63	LYS	CA	C	59.342	0.3	1
573	63	LYS	CB	C	31.631	0.3	1
574	63	LYS	CG	C	24.398	0.3	1
575	63	LYS	CD	C	28.513	0.3	1
576	63	LYS	CE	C	42.073	0.3	1
577	63	LYS	H	H	7.065	0.020	1
578	63	LYS	HA	H	3.284	0.020	1
579	63	LYS	HB2	H	1.692	0.020	2
580	63	LYS	HB3	H	1.507	0.020	2
581	**63**	**LYS**	**HG2**	**H**	**1.385**	**0.020**	**2**
582	**63**	**LYS**	**HG3**	**H**	**1.155**	**0.020**	**2**
583	**63**	**LYS**	**HD2**	**H**	**1.191**	**0.020**	**2**
584	**63**	**LYS**	**HD3**	**H**	**1.050**	**0.020**	**2**
585	63	LYS	HE2	H	2.586	0.020	2
586	**63**	**LYS**	**HE3**	**H**	**2.519**	**0.020**	**2**
587	63	LYS	N	N	122.802	0.3	1
588	64	GLN	C	C	177.275	0.3	1
589	64	GLN	CA	C	58.142	0.3	1
590	64	GLN	CB	C	27.184	0.3	1
591	64	GLN	CG	C	31.888	0.3	1
592	64	GLN	H	H	7.633	0.020	1
593	64	GLN	HA	H	4.095	0.020	1
594	64	GLN	HB2	H	1.836	0.020	2
595	64	GLN	HB3	H	1.762	0.020	2
596	64	GLN	HG2	H	2.232	0.020	2
597	64	GLN	HG3	H	2.175	0.020	2
598	64	GLN	N	N	115.109	0.3	1
599	65	PHE	C	C	177.929	0.3	1
600	65	PHE	CA	C	59.000	0.3	1
601	65	PHE	CB	C	39.117	0.3	1
602	65	PHE	H	H	7.171	0.020	1
603	65	PHE	HA	H	4.339	0.020	1

604	65	PHE	HB2	H	3.415	0.020	2
605	65	PHE	HB3	H	2.892	0.020	2
606	65	PHE	N	N	117.172	0.3	1
607	66	ILE	C	C	174.811	0.3	1
608	66	ILE	CA	C	65.669	0.3	1
609	66	ILE	CB	C	37.624	0.3	1
610	**66**	**ILE**	**CG1**	**C**	**29.753**	**0.3**	**1**
611	**66**	**ILE**	**CG2**	**C**	**17.195**	**0.3**	**1**
612	**66**	**ILE**	**CD1**	**C**	**14.374**	**0.3**	**1**
613	66	ILE	H	H	7.381	0.020	1
614	**66**	**ILE**	**HA**	**H**	**3.275**	**0.020**	**1**
615	**66**	**ILE**	**HB**	**H**	**2.893**	**0.020**	**1**
616	**66**	**ILE**	**HG12**	**H**	**1.176**	**0.020**	**2**
617	**66**	**ILE**	**HG13**	**H**	**0.886**	**0.020**	**2**
618	**66**	**ILE**	**HG2**	**H**	**0.969**	**0.020**	**1**
619	**66**	**ILE**	**HD1**	**H**	**0.746**	**0.020**	**1**
620	66	ILE	N	N	120.163	0.3	1
621	**67**	**ASP**	**CA**	**C**	**57.242**	**0.3**	**1**
622	**67**	**ASP**	**CB**	**C**	**39.807**	**0.3**	**1**
623	**67**	**ASP**	**H**	**H**	**7.014**	**0.020**	**1**
624	**67**	**ASP**	**HA**	**H**	**4.223**	**0.020**	**1**
625	**67**	**ASP**	**HB2**	**H**	**2.544**	**0.020**	**1**
626	**67**	**ASP**	**HB3**	**H**	**2.544**	**0.020**	**1**
627	**67**	**ASP**	**N**	**N**	**112.431**	**0.3**	**1**
628	68	ARG	C	C	174.692	0.3	1
629	68	ARG	CA	C	55.497	0.3	1
630	68	ARG	CB	C	31.101	0.3	1
631	68	ARG	CG	C	27.355	0.3	1
632	68	ARG	CD	C	43.699	0.3	1
633	68	ARG	H	H	6.901	0.020	1
634	68	ARG	HA	H	4.337	0.020	1
635	68	ARG	HB2	H	1.975	0.020	2
636	68	ARG	HB3	H	1.544	0.020	2
637	68	ARG	HG2	H	1.651	0.020	1
638	68	ARG	HD2	H	3.120	0.020	2
639	**68**	**ARG**	**HD3**	**H**	**3.261**	**0.020**	**2**
640	68	ARG	N	N	113.824	0.3	1
641	69	LYS	C	C	174.137	0.3	1
642	69	LYS	CA	C	56.617	0.3	1
643	69	LYS	CB	C	29.435	0.3	1
644	69	LYS	CG	C	25.218	0.3	1
645	**69**	**LYS**	**CD**	**C**	**32.636**	**0.3**	**1**
646	69	LYS	CE	C	42.359	0.3	1
647	69	LYS	H	H	7.576	0.020	1
648	69	LYS	HA	H	4.247	0.020	1
649	69	LYS	HB2	H	1.955	0.020	2
650	69	LYS	HB3	H	1.713	0.020	2
651	**69**	**LYS**	**HG2**	**H**	**1.375**	**0.020**	**1**
652	**69**	**LYS**	**HG3**	**H**	**1.375**	**0.020**	**1**
653	**69**	**LYS**	**HD2**	**H**	**1.563**	**0.020**	**2**
654	**69**	**LYS**	**HD3**	**H**	**1.758**	**0.020**	**2**
655	**69**	**LYS**	**HE2**	**H**	**3.051**	**0.020**	**1**
656	**69**	**LYS**	**HE3**	**H**	**3.051**	**0.020**	**1**
657	69	LYS	N	N	118.878	0.3	1
658	70	ALA	C	C	177.429	0.3	1
659	70	ALA	CA	C	49.082	0.3	1

660	70	ALA	CB	C	17.344	0.3	1
661	70	ALA	H	H	8.021	0.020	1
662	70	ALA	HA	H	4.583	0.020	1
663	70	ALA	HB	H	1.272	0.020	1
664	70	ALA	N	N	122.931	0.3	1
665	71	TRP	C	C	175.088	0.3	1
666	71	TRP	CA	C	61.285	0.3	1
667	71	TRP	CB	C	30.581	0.3	1
668	71	TRP	H	H	7.032	0.020	1
669	**71**	**TRP**	**HA**	**H**	**4.686**	**0.020**	**1**
670	**71**	**TRP**	**HB2**	**H**	**2.300**	**0.020**	**2**
671	**71**	**TRP**	**HB3**	**H**	**1.901**	**0.020**	**2**
672	71	TRP	N	N	119.538	0.3	1
673	72	PRO	C	C	179.767	0.3	1
674	72	PRO	CA	C	65.943	0.3	1
675	72	PRO	CB	C	29.987	0.3	1
676	72	PRO	N	N	136.568	0.3	1
677	**74**	**LEU**	**CA**	**C**	**54.101**	**0.3**	**1**
678	**74**	**LEU**	**CB**	**C**	**42.003**	**0.3**	**1**
679	**74**	**LEU**	**CG**	**C**	**26.018**	**0.3**	**1**
680	**74**	**LEU**	**CD1**	**C**	**24.638**	**0.3**	**1**
681	**74**	**LEU**	**CD2**	**C**	**23.220**	**0.3**	**1**
682	**74**	**LEU**	**HA**	**H**	**3.882**	**0.020**	**1**
683	**74**	**LEU**	**HB2**	**H**	**1.679**	**0.020**	**1**
684	**74**	**LEU**	**HB3**	**H**	**1.679**	**0.020**	**1**
685	**74**	**LEU**	**HG**	**H**	**1.048**	**0.020**	**1**
686	**74**	**LEU**	**HD1**	**H**	**0.479**	**0.020**	**1**
687	**74**	**LEU**	**HD2**	**H**	**0.400**	**0.020**	**1**
688	**75**	**GLN**	**CA**	**C**	**62.216**	**0.3**	**1**
689	**75**	**GLN**	**H**	**H**	**7.778**	**0.020**	**1**
690	**75**	**GLN**	**HA**	**H**	**3.978**	**0.020**	**1**
691	**75**	**GLN**	**N**	**N**	**112.424**	**0.3**	**1**
692	**76**	**ASN**	**CA**	**C**	**58.712**	**0.3**	**1**
693	**76**	**ASN**	**CB**	**C**	**37.029**	**0.3**	**1**
694	**76**	**ASN**	**H**	**H**	**7.840**	**0.020**	**1**
695	**76**	**ASN**	**HA**	**H**	**4.490**	**0.020**	**1**
696	**76**	**ASN**	**HB2**	**H**	**3.339**	**0.020**	**2**
697	**76**	**ASN**	**HB3**	**H**	**3.176**	**0.020**	**2**
698	**76**	**ASN**	**HD21**	**H**	**6.932**	**0.020**	**1**
699	**76**	**ASN**	**HD22**	**H**	**6.932**	**0.020**	**1**
700	**76**	**ASN**	**N**	**N**	**122.906**	**0.3**	**1**
701	**77**	**ASP**	**CA**	**C**	**58.026**	**0.3**	**1**
702	**77**	**ASP**	**CB**	**C**	**42.061**	**0.3**	**1**
703	**77**	**ASP**	**H**	**H**	**8.751**	**0.020**	**1**
704	**77**	**ASP**	**HA**	**H**	**4.450**	**0.020**	**1**
705	**77**	**ASP**	**HB2**	**H**	**2.651**	**0.020**	**2**
706	**77**	**ASP**	**HB3**	**H**	**2.559**	**0.020**	**2**
707	**77**	**ASP**	**N**	**N**	**122.220**	**0.3**	**1**
708	**78**	**LEU**	**CA**	**C**	**58.847**	**0.3**	**1**
709	**78**	**LEU**	**CB**	**C**	**43.235**	**0.3**	**1**
710	**78**	**LEU**	**CG**	**C**	**25.852**	**0.3**	**1**
711	**78**	**LEU**	**CD1**	**C**	**23.735**	**0.3**	**1**
712	**78**	**LEU**	**H**	**H**	**8.679**	**0.020**	**1**
713	**78**	**LEU**	**HA**	**H**	**4.032**	**0.020**	**1**
714	**78**	**LEU**	**HB2**	**H**	**2.068**	**0.020**	**2**
715	**78**	**LEU**	**HB3**	**H**	**1.738**	**0.020**	**2**

716	**78**	**LEU**	**HD1**	**H**	**1.083**	**0.020**	**1**
717	**78**	**LEU**	**HD2**	**H**	**1.083**	**0.020**	**1**
718	**78**	**LEU**	**N**	**N**	**117.943**	**0.3**	**1**
719	**79**	**ARG**	**CA**	**C**	**59.390**	**0.3**	**1**
720	**79**	**ARG**	**CB**	**C**	**30.258**	**0.3**	**1**
721	**79**	**ARG**	**CG**	**C**	**27.593**	**0.3**	**1**
722	**79**	**ARG**	**CD**	**C**	**43.790**	**0.3**	**1**
723	**79**	**ARG**	**H**	**H**	**8.019**	**0.020**	**1**
724	**79**	**ARG**	**HA**	**H**	**4.015**	**0.020**	**1**
725	**79**	**ARG**	**HB2**	**H**	**1.903**	**0.020**	**1**
726	**79**	**ARG**	**HB3**	**H**	**1.903**	**0.020**	**1**
727	**79**	**ARG**	**HG2**	**H**	**1.625**	**0.020**	**1**
728	**79**	**ARG**	**HG3**	**H**	**1.625**	**0.020**	**1**
729	**79**	**ARG**	**HD2**	**H**	**3.153**	**0.020**	**1**
730	**79**	**ARG**	**N**	**N**	**115.864**	**0.3**	**1**
731	80	LEU	CA	C	57.952	0.3	1
732	80	LEU	CB	C	42.366	0.3	1
733	80	LEU	CG	C	26.524	0.3	1
734	80	LEU	CD1	C	24.919	0.3	1
735	80	LEU	CD2	C	23.666	0.3	1
736	**80**	**LEU**	**H**	**H**	**7.417**	**0.020**	**1**
737	80	LEU	HA	H	3.921	0.020	1
738	80	LEU	HB2	H	1.785	0.020	2
739	80	LEU	HB3	H	1.605	0.020	2
740	80	LEU	HG	H	1.107	0.020	1
741	80	LEU	HD1	H	0.784	0.020	1
742	80	LEU	HD2	H	0.710	0.020	1
743	**80**	**LEU**	**N**	**N**	**120.372**	**0.3**	**1**
744	81	ARG	C	C	179.411	0.3	1
745	81	ARG	CA	C	55.278	0.3	1
746	81	ARG	CB	C	32.458	0.3	1
747	81	ARG	CG	C	26.118	0.3	1
748	81	ARG	CD	C	41.978	0.3	1
749	81	ARG	H	H	8.397	0.020	1
750	81	ARG	HA	H	3.874	0.020	1
751	81	ARG	HB3	H	1.943	0.020	1
752	81	ARG	HG2	H	1.205	0.020	1
753	81	ARG	HG3	H	1.205	0.020	1
754	81	ARG	HD2	H	2.971	0.020	1
755	81	ARG	N	N	114.977	0.3	1
756	82	ALA	C	C	179.414	0.3	1
757	82	ALA	CA	C	55.958	0.3	1
758	82	ALA	CB	C	18.855	0.3	1
759	82	ALA	H	H	9.140	0.020	1
760	82	ALA	HA	H	4.207	0.020	1
761	82	ALA	HB	H	1.690	0.020	1
762	82	ALA	N	N	119.805	0.3	1
763	83	SER	CA	C	61.895	0.3	1
764	83	SER	CB	C	62.769	0.3	1
765	83	SER	H	H	7.751	0.020	1
766	**83**	**SER**	**HA**	**H**	**4.257**	**0.020**	**1**
767	**83**	**SER**	**HB2**	**H**	**4.036**	**0.020**	**1**
768	**83**	**SER**	**HB3**	**H**	**4.036**	**0.020**	**1**
769	83	SER	N	N	114.702	0.3	1
770	**84**	**TYR**	**CA**	**C**	**59.012**	**0.3**	**1**
771	**84**	**TYR**	**CB**	**C**	**42.023**	**0.3**	**1**

772	**84**	**TYR**	**H**	**H**	**7.045**	**0.020**	**1**
773	**84**	**TYR**	**HA**	**H**	**3.291**	**0.020**	**1**
774	**84**	**TYR**	**HB2**	**H**	**1.599**	**0.020**	**1**
775	**84**	**TYR**	**N**	**N**	**113.337**	**0.3**	**1**
776	85	LEU	C	C	177.337	0.3	1
777	85	LEU	CA	C	59.105	0.3	1
778	85	LEU	CB	C	41.663	0.3	1
779	85	LEU	CG	C	28.646	0.3	1
780	85	LEU	CD1	C	24.669	0.3	1
781	**85**	**LEU**	**CD2**	**C**	**25.915**	**0.3**	**1**
782	85	LEU	H	H	7.817	0.020	1
783	85	LEU	HA	H	3.790	0.020	1
784	85	LEU	HB2	H	2.384	0.020	2
785	**85**	**LEU**	**HB3**	**H**	**1.584**	**0.020**	**2**
786	**85**	**LEU**	**HG**	**H**	**1.739**	**0.020**	**1**
787	**85**	**LEU**	**HD1**	**H**	**1.084**	**0.020**	**1**
788	**85**	**LEU**	**HD2**	**H**	**1.189**	**0.020**	**1**
789	85	LEU	N	N	121.592	0.3	1
790	86	ARG	C	C	178.060	0.3	1
791	86	ARG	CA	C	60.341	0.3	1
792	86	ARG	CB	C	29.952	0.3	1
793	**86**	**ARG**	**CG**	**C**	**27.451**	**0.3**	**1**
794	**86**	**ARG**	**CD**	**C**	**43.050**	**0.3**	**1**
795	86	ARG	H	H	8.091	0.020	1
796	86	ARG	HA	H	3.812	0.020	1
797	86	ARG	HB2	H	1.964	0.020	2
798	86	ARG	HB3	H	2.102	0.020	2
799	**86**	**ARG**	**HG2**	**H**	**1.530**	**0.020**	**1**
800	86	ARG	HD2	H	2.980	0.020	1
801	86	ARG	N	N	115.275	0.3	1
802	87	TYR	C	C	177.599	0.3	1
803	87	TYR	CA	C	56.443	0.3	1
804	87	TYR	CB	C	38.620	0.3	1
805	87	TYR	H	H	8.164	0.020	1
806	87	TYR	HA	H	4.353	0.020	1
807	87	TYR	HB2	H	2.769	0.020	2
808	87	TYR	HB3	H	2.712	0.020	2
809	**87**	**TYR**	**HD1**	**H**	**7.036**	**0.020**	**3**
810	**87**	**TYR**	**HD2**	**H**	**6.900**	**0.020**	**3**
811	87	TYR	N	N	115.945	0.3	1
812	88	ASP	C	C	178.265	0.3	1
813	88	ASP	CA	C	57.924	0.3	1
814	88	ASP	CB	C	42.144	0.3	1
815	88	ASP	H	H	8.021	0.020	1
816	88	ASP	HA	H	4.440	0.020	1
817	88	ASP	HB2	H	2.647	0.020	2
818	88	ASP	HB3	H	2.554	0.020	2
819	88	ASP	N	N	120.649	0.3	1
820	89	LEU	C	C	178.075	0.3	1
821	89	LEU	CA	C	59.295	0.3	1
822	89	LEU	CB	C	43.159	0.3	1
823	89	LEU	CG	C	26.152	0.3	1
824	89	LEU	CD1	C	23.777	0.3	1
825	89	LEU	H	H	8.665	0.020	1
826	89	LEU	HA	H	4.002	0.020	1
827	89	LEU	HB2	H	1.751	0.020	1

828	89	LEU	HD1	H	1.083	0.020	1
829	89	LEU	N	N	118.115	0.3	1
830	90	LYS	C	C	179.669	0.3	1
831	90	LYS	CA	C	60.331	0.3	1
832	90	LYS	CB	C	31.573	0.3	1
833	**90**	**LYS**	**CG**	**C**	**29.370**	**0.3**	**1**
834	**90**	**LYS**	**CD**	**C**	**25.064**	**0.3**	**1**
835	**90**	**LYS**	**CE**	**C**	**41.957**	**0.3**	**1**
836	90	LYS	H	H	8.002	0.020	1
837	**90**	**LYS**	**HA**	**H**	**3.989**	**0.020**	**1**
838	**90**	**LYS**	**HB2**	**H**	**1.907**	**0.020**	**1**
839	**90**	**LYS**	**HB3**	**H**	**1.907**	**0.020**	**1**
840	**90**	**LYS**	**HG2**	**H**	**1.671**	**0.020**	**1**
841	**90**	**LYS**	**HG3**	**H**	**1.671**	**0.020**	**1**
842	**90**	**LYS**	**HD2**	**H**	**1.546**	**0.020**	**1**
843	90	LYS	N	N	115.802	0.3	1
844	**91**	**THR**	**C**	**C**	**179.536**	**0.3**	**1**
845	**91**	**THR**	**CA**	**C**	**66.642**	**0.3**	**1**
846	**91**	**THR**	**CB**	**C**	**68.477**	**0.3**	**1**
847	**91**	**THR**	**CG2**	**C**	**21.429**	**0.3**	**1**
848	**91**	**THR**	**H**	**H**	**7.365**	**0.020**	**1**
849	**91**	**THR**	**HA**	**H**	**3.844**	**0.020**	**1**
850	**91**	**THR**	**HB**	**H**	**4.321**	**0.020**	**1**
851	**91**	**THR**	**HG2**	**H**	**1.269**	**0.020**	**1**
852	**91**	**THR**	**N**	**N**	**117.273**	**0.3**	**1**
853	**92**	**VAL**	**C**	**C**	**178.807**	**0.3**	**1**
854	**92**	**VAL**	**CA**	**C**	**67.210**	**0.3**	**1**
855	**92**	**VAL**	**CB**	**C**	**32.844**	**0.3**	**1**
856	**92**	**VAL**	**CG1**	**C**	**22.385**	**0.3**	**1**
857	**92**	**VAL**	**CG2**	**C**	**21.294**	**0.3**	**1**
858	**92**	**VAL**	**H**	**H**	**7.900**	**0.020**	**1**
859	**92**	**VAL**	**HA**	**H**	**3.430**	**0.020**	**1**
860	**92**	**VAL**	**HB**	**H**	**2.147**	**0.020**	**1**
861	**92**	**VAL**	**HG1**	**H**	**1.070**	**0.020**	**1**
862	**92**	**VAL**	**HG2**	**H**	**0.903**	**0.020**	**1**
863	**92**	**VAL**	**N**	**N**	**118.866**	**0.3**	**1**
864	**93**	**ILE**	**CA**	**C**	**66.395**	**0.3**	**1**
865	**93**	**ILE**	**CB**	**C**	**38.361**	**0.3**	**1**
866	**93**	**ILE**	**CG2**	**C**	**18.306**	**0.3**	**1**
867	**93**	**ILE**	**CD1**	**C**	**13.715**	**0.3**	**1**
868	**93**	**ILE**	**H**	**H**	**8.976**	**0.020**	**1**
869	**93**	**ILE**	**HA**	**H**	**3.381**	**0.020**	**1**
870	**93**	**ILE**	**HB**	**H**	**1.903**	**0.020**	**1**
871	**93**	**ILE**	**HG13**	**H**	**0.931**	**0.020**	**1**
872	**93**	**ILE**	**HG2**	**H**	**0.954**	**0.020**	**1**
873	**93**	**ILE**	**HD1**	**H**	**0.778**	**0.020**	**1**
874	**93**	**ILE**	**N**	**N**	**118.869**	**0.3**	**1**
875	94	SER	C	C	174.264	0.3	1
876	94	SER	CA	C	61.437	0.3	1
877	94	SER	CB	C	62.666	0.3	1
878	94	SER	H	H	7.757	0.020	1
879	94	SER	HA	H	4.135	0.020	1
880	**94**	**SER**	**HB2**	**H**	**4.018**	**0.020**	**1**
881	**94**	**SER**	**HB3**	**H**	**4.018**	**0.020**	**1**
882	94	SER	N	N	112.225	0.3	1
883	95	ALA	C	C	177.789	0.3	1

884	95	ALA	CA	C	51.880	0.3	1
885	95	ALA	CB	C	19.915	0.3	1
886	95	ALA	H	H	7.159	0.020	1
887	95	ALA	HA	H	4.565	0.020	1
888	95	ALA	HB	H	1.536	0.020	1
889	95	ALA	N	N	121.015	0.3	1
890	96	LYS	C	C	174.077	0.3	1
891	96	LYS	CA	C	53.640	0.3	1
892	96	LYS	CB	C	32.234	0.3	1
893	**96**	**LYS**	**CG**	**C**	**24.022**	**0.3**	**1**
894	**96**	**LYS**	**CD**	**C**	**29.128**	**0.3**	**1**
895	**96**	**LYS**	**CE**	**C**	**41.876**	**0.3**	**1**
896	96	LYS	H	H	7.439	0.020	1
897	**96**	**LYS**	**HA**	**H**	**3.883**	**0.020**	**1**
898	**96**	**LYS**	**HB2**	**H**	**1.768**	**0.020**	**1**
899	**96**	**LYS**	**HB3**	**H**	**1.768**	**0.020**	**1**
900	**96**	**LYS**	**HG2**	**H**	**1.086**	**0.020**	**1**
901	**96**	**LYS**	**HD2**	**H**	**1.682**	**0.020**	**2**
902	**96**	**LYS**	**HD3**	**H**	**1.505**	**0.020**	**2**
903	**96**	**LYS**	**HE2**	**H**	**3.044**	**0.020**	**2**
904	**96**	**LYS**	**HE3**	**H**	**2.946**	**0.020**	**2**
905	96	LYS	N	N	120.570	0.3	1
906	97	PRO	C	C	177.275	0.3	1
907	97	PRO	CA	C	62.341	0.3	1
908	97	PRO	CB	C	32.393	0.3	1
909	97	PRO	CG	C	27.950	0.3	1
910	97	PRO	CD	C	48.435	0.3	1
911	97	PRO	HA	H	4.563	0.020	1
912	97	PRO	HB2	H	2.464	0.020	1
913	97	PRO	HG2	H	2.110	0.020	1
914	97	PRO	N	N	132.979	0.3	1
915	98	LYS	C	C	178.098	0.3	1
916	98	LYS	CA	C	60.833	0.3	1
917	98	LYS	CB	C	32.886	0.3	1
918	98	LYS	CG	C	24.464	0.3	1
919	98	LYS	CD	C	29.318	0.3	1
920	98	LYS	CE	C	41.859	0.3	1
921	98	LYS	H	H	8.627	0.020	1
922	98	LYS	HA	H	3.842	0.020	1
923	98	LYS	HB2	H	1.874	0.020	1
924	98	LYS	HG2	H	1.561	0.020	1
925	98	LYS	HG3	H	1.561	0.020	1
926	98	LYS	HD2	H	1.724	0.020	1
927	98	LYS	HE2	H	3.072	0.020	1
928	98	LYS	N	N	120.567	0.3	1
929	99	ASP	C	C	178.051	0.3	1
930	99	ASP	CA	C	56.997	0.3	1
931	99	ASP	CB	C	39.740	0.3	1
932	99	ASP	H	H	8.946	0.020	1
933	99	ASP	HA	H	4.515	0.020	1
934	99	ASP	HB2	H	2.770	0.020	2
935	99	ASP	HB3	H	2.647	0.020	2
936	99	ASP	N	N	117.836	0.3	1
937	100	GLU	C	C	178.194	0.3	1
938	100	GLU	CA	C	56.250	0.3	1
939	100	GLU	CB	C	31.097	0.3	1

940	100	GLU	CG	C	36.671	0.3	1
941	100	GLU	H	H	7.844	0.020	1
942	100	GLU	HA	H	4.298	0.020	1
943	100	GLU	HB2	H	2.031	0.020	1
944	100	GLU	HG2	H	2.282	0.020	1
945	**100**	**GLU**	**HG3**	**H**	**2.282**	**0.020**	**1**
946	100	GLU	N	N	120.622	0.3	1
947	101	LYS	C	C	178.250	0.3	1
948	101	LYS	CA	C	60.617	0.3	1
949	101	LYS	CB	C	32.857	0.3	1
950	101	LYS	CG	C	24.398	0.3	1
951	101	LYS	CD	C	29.896	0.3	1
952	101	LYS	CE	C	39.237	0.3	1
953	101	LYS	H	H	8.340	0.020	1
954	101	LYS	HA	H	3.834	0.020	1
955	101	LYS	HB2	H	1.929	0.020	1
956	**101**	**LYS**	**HB3**	**H**	**1.929**	**0.020**	**1**
957	101	LYS	HG2	H	1.422	0.020	1
958	**101**	**LYS**	**HD2**	**H**	**1.725**	**0.020**	**1**
959	**101**	**LYS**	**HD3**	**H**	**1.725**	**0.020**	**1**
960	101	LYS	HE2	H	2.980	0.020	1
961	101	LYS	N	N	120.722	0.3	1
962	102	LYS	C	C	179.027	0.3	1
963	102	LYS	CA	C	59.623	0.3	1
964	102	LYS	CB	C	32.441	0.3	1
965	102	LYS	CG	C	24.749	0.3	1
966	102	LYS	CD	C	29.316	0.3	1
967	102	LYS	CE	C	45.214	0.3	1
968	102	LYS	H	H	7.837	0.020	1
969	102	LYS	HA	H	4.147	0.020	1
970	102	LYS	HB2	H	1.954	0.020	1
971	102	LYS	HG2	H	1.550	0.020	2
972	**102**	**LYS**	**HG3**	**H**	**1.425**	**0.020**	**2**
973	102	LYS	HD2	H	1.727	0.020	1
974	102	LYS	N	N	117.984	0.3	1
975	103	SER	C	C	177.670	0.3	1
976	103	SER	CA	C	61.785	0.3	1
977	103	SER	CB	C	62.570	0.3	1
978	103	SER	H	H	7.995	0.020	1
979	103	SER	HA	H	4.269	0.020	1
980	103	SER	HB2	H	4.030	0.020	1
981	103	SER	HB3	H	4.030	0.020	1
982	103	SER	N	N	113.241	0.3	1
983	104	LEU	C	C	179.883	0.3	1
984	104	LEU	CA	C	57.785	0.3	1
985	104	LEU	CB	C	41.891	0.3	1
986	104	LEU	CG	C	27.414	0.3	1
987	104	LEU	CD1	C	24.988	0.3	1
988	104	LEU	CD2	C	24.742	0.3	1
989	104	LEU	H	H	8.066	0.020	1
990	104	LEU	HA	H	4.230	0.020	1
991	104	LEU	HB2	H	1.874	0.020	2
992	104	LEU	HB3	H	1.603	0.020	2
993	104	LEU	HG	H	1.730	0.020	1
994	104	LEU	HD1	H	0.949	0.020	1
995	104	LEU	HD2	H	0.900	0.020	1

996	104	LEU	N	N	121.281	0.3	1
997	105	GLN	C	C	178.833	0.3	1
998	105	GLN	CA	C	59.586	0.3	1
999	105	GLN	CB	C	28.333	0.3	1
1000	**105**	**GLN**	**CG**	**C**	**34.581**	**0.3**	**1**
1001	105	GLN	H	H	8.965	0.020	1
1002	**105**	**GLN**	**HA**	**H**	**4.097**	**0.020**	**1**
1003	**105**	**GLN**	**HB2**	**H**	**2.189**	**0.020**	**1**
1004	**105**	**GLN**	**HB3**	**H**	**2.189**	**0.020**	**1**
1005	**105**	**GLN**	**HG2**	**H**	**2.391**	**0.020**	**1**
1006	**105**	**GLN**	**HG3**	**H**	**2.391**	**0.020**	**1**
1007	105	GLN	N	N	122.396	0.3	1
1008	106	GLU	CA	C	59.582	0.3	1
1009	106	GLU	CB	C	29.476	0.3	1
1010	106	GLU	CG	C	36.731	0.3	1
1011	**106**	**GLU**	**H**	**H**	**8.199**	**0.020**	**1**
1012	106	GLU	HA	H	4.138	0.020	1
1013	106	GLU	HB2	H	2.104	0.020	1
1014	**106**	**GLU**	**HB3**	**H**	**2.104**	**0.020**	**1**
1015	106	GLU	HG2	H	2.260	0.020	1
1016	106	GLU	HG3	H	2.260	0.020	1
1017	106	GLU	N	N	120.590	0.3	1
1018	107	LEU	C	C	179.661	0.3	1
1019	107	LEU	CA	C	57.706	0.3	1
1020	107	LEU	CB	C	42.540	0.3	1
1021	107	LEU	CG	C	26.771	0.3	1
1022	107	LEU	CD1	C	24.940	0.3	1
1023	107	LEU	CD2	C	23.989	0.3	1
1024	107	LEU	H	H	7.953	0.020	1
1025	107	LEU	HA	H	4.150	0.020	1
1026	107	LEU	HB2	H	1.913	0.020	2
1027	107	LEU	HB3	H	1.636	0.020	2
1028	**107**	**LEU**	**HG**	**H**	**1.843**	**0.020**	**1**
1029	107	LEU	HD1	H	0.904	0.020	1
1030	**107**	**LEU**	**HD2**	**H**	**0.875**	**0.020**	**1**
1031	107	LEU	N	N	119.888	0.3	1
1032	108	THR	CA	C	67.775	0.3	1
1033	108	THR	CB	C	67.803	0.3	1
1034	**108**	**THR**	**CG2**	**C**	**22.796**	**0.3**	**1**
1035	108	THR	H	H	8.645	0.020	1
1036	**108**	**THR**	**HA**	**H**	**4.326**	**0.020**	**1**
1037	**108**	**THR**	**HB**	**H**	**3.729**	**0.020**	**1**
1038	**108**	**THR**	**HG2**	**H**	**0.916**	**0.020**	**1**
1039	108	THR	N	N	115.260	0.3	1
1040	**109**	**SER**	**CA**	**C**	**61.720**	**0.3**	**1**
1041	**109**	**SER**	**CB**	**C**	**62.277**	**0.3**	**1**
1042	**109**	**SER**	**H**	**H**	**8.285**	**0.020**	**1**
1043	**109**	**SER**	**HA**	**H**	**4.151**	**0.020**	**1**
1044	**109**	**SER**	**HB2**	**H**	**4.054**	**0.020**	**1**
1045	**109**	**SER**	**HB3**	**H**	**4.054**	**0.020**	**1**
1046	**109**	**SER**	**N**	**N**	**118.325**	**0.3**	**1**
1047	110	LYS	C	C	178.884	0.3	1
1048	110	LYS	CA	C	59.819	0.3	1
1049	110	LYS	CB	C	32.401	0.3	1
1050	110	LYS	CG	C	24.862	0.3	1
1051	110	LYS	CD	C	29.985	0.3	1

1052	110	LYS	CE	C	41.693	0.3	1
1053	110	LYS	H	H	7.582	0.020	1
1054	110	LYS	HA	H	4.128	0.020	1
1055	110	LYS	HB2	H	1.955	0.020	1
1056	110	LYS	HB3	H	1.955	0.020	1
1057	**110**	**LYS**	**HG2**	**H**	**1.425**	**0.020**	**1**
1058	**110**	**LYS**	**HG3**	**H**	**1.425**	**0.020**	**1**
1059	**110**	**LYS**	**HD2**	**H**	**1.713**	**0.020**	**1**
1060	110	LYS	HE2	H	2.973	0.020	1
1061	110	LYS	N	N	122.423	0.3	1
1062	111	LEU	C	C	177.917	0.3	1
1063	111	LEU	CA	C	57.832	0.3	1
1064	111	LEU	CB	C	40.927	0.3	1
1065	111	LEU	CG	C	26.428	0.3	1
1066	111	LEU	CD1	C	26.402	0.3	1
1067	**111**	**LEU**	**CD2**	**C**	**24.423**	**0.3**	**1**
1068	111	LEU	H	H	7.947	0.020	1
1069	111	LEU	HA	H	4.086	0.020	1
1070	111	LEU	HB2	H	2.133	0.020	2
1071	111	LEU	HB3	H	1.262	0.020	2
1072	111	LEU	HG	H	0.839	0.020	1
1073	111	LEU	HD1	H	0.592	0.020	1
1074	111	LEU	N	N	120.270	0.3	1
1075	112	PHE	C	C	177.965	0.3	1
1076	112	PHE	CA	C	62.640	0.3	1
1077	112	PHE	CB	C	37.138	0.3	1
1078	112	PHE	H	H	8.697	0.020	1
1079	112	PHE	HA	H	4.296	0.020	1
1080	112	PHE	HB2	H	3.413	0.020	2
1081	112	PHE	HB3	H	3.021	0.020	2
1082	112	PHE	N	N	116.713	0.3	1
1083	113	SER	CA	C	62.028	0.3	1
1084	113	SER	CB	C	62.214	0.3	1
1085	113	SER	H	H	8.110	0.020	1
1086	**113**	**SER**	**HA**	**H**	**4.490**	**0.020**	**1**
1087	**113**	**SER**	**HB2**	**H**	**4.092**	**0.020**	**2**
1088	**113**	**SER**	**HB3**	**H**	**4.045**	**0.020**	**2**
1089	113	SER	N	N	116.862	0.3	1
1090	**114**	**SER**	**CA**	**C**	**62.939**	**0.3**	**1**
1091	**114**	**SER**	**CB**	**C**	**63.291**	**0.3**	**1**
1092	**114**	**SER**	**H**	**H**	**8.165**	**0.020**	**1**
1093	**114**	**SER**	**HA**	**H**	**4.255**	**0.020**	**1**
1094	**114**	**SER**	**HB2**	**H**	**4.037**	**0.020**	**1**
1095	**114**	**SER**	**HB3**	**H**	**4.037**	**0.020**	**1**
1096	**114**	**SER**	**N**	**N**	**119.109**	**0.3**	**1**
1097	**115**	**ILE**	**CA**	**C**	**65.515**	**0.3**	**1**
1098	**115**	**ILE**	**CB**	**C**	**37.923**	**0.3**	**1**
1099	**115**	**ILE**	**CG1**	**C**	**24.641**	**0.3**	**1**
1100	**115**	**ILE**	**CG2**	**C**	**16.664**	**0.3**	**1**
1101	**115**	**ILE**	**CD1**	**C**	**13.301**	**0.3**	**1**
1102	**115**	**ILE**	**H**	**H**	**8.142**	**0.020**	**1**
1103	**115**	**ILE**	**HA**	**H**	**4.317**	**0.020**	**1**
1104	**115**	**ILE**	**HB**	**H**	**1.991**	**0.020**	**1**
1105	**115**	**ILE**	**HG12**	**H**	**0.975**	**0.020**	**1**
1106	**115**	**ILE**	**HG13**	**H**	**0.975**	**0.020**	**1**
1107	**115**	**ILE**	**HG2**	**H**	**0.888**	**0.020**	**1**

1108	**115**	**ILE**	**HD1**	**H**	**0.587**	**0.020**	**1**
1109	**115**	**ILE**	**N**	**N**	**123.650**	**0.3**	**1**
1110	116	ASP	C	C	179.669	0.3	1
1111	116	ASP	CA	C	57.845	0.3	1
1112	116	ASP	CB	C	39.389	0.3	1
1113	116	ASP	H	H	8.131	0.020	1
1114	116	ASP	HA	H	4.447	0.020	1
1115	116	ASP	HB2	H	2.937	0.020	1
1116	116	ASP	HB3	H	2.937	0.020	1
1117	116	ASP	N	N	120.384	0.3	1
1118	117	ASN	C	C	177.334	0.3	1
1119	117	ASN	CA	C	55.653	0.3	1
1120	117	ASN	CB	C	36.283	0.3	1
1121	117	ASN	H	H	7.953	0.020	1
1122	117	ASN	HA	H	4.320	0.020	1
1123	117	ASN	HB2	H	0.660	0.020	2
1124	**117**	**ASN**	**HB3**	**H**	**2.055**	**0.020**	**2**
1125	117	ASN	N	N	119.439	0.3	1
1126	118	LEU	C	C	176.811	0.3	1
1127	118	LEU	CA	C	57.806	0.3	1
1128	118	LEU	CB	C	40.608	0.3	1
1129	**118**	**LEU**	**CG**	**C**	**26.457**	**0.3**	**1**
1130	**118**	**LEU**	**CD1**	**C**	**26.106**	**0.3**	**1**
1131	**118**	**LEU**	**CD2**	**C**	**22.401**	**0.3**	**1**
1132	118	LEU	H	H	8.352	0.020	1
1133	118	LEU	HA	H	4.218	0.020	1
1134	118	LEU	HB2	H	2.155	0.020	2
1135	**118**	**LEU**	**HB3**	**H**	**1.677**	**0.020**	**2**
1136	**118**	**LEU**	**HG**	**H**	**0.995**	**0.020**	**1**
1137	**118**	**LEU**	**HD2**	**H**	**0.763**	**0.020**	**1**
1138	118	LEU	N	N	125.510	0.3	1
1139	119	ASP	C	C	178.310	0.3	1
1140	119	ASP	CA	C	57.993	0.3	1
1141	119	ASP	CB	C	41.848	0.3	1
1142	119	ASP	H	H	8.579	0.020	1
1143	119	ASP	HA	H	4.155	0.020	1
1144	119	ASP	HB2	H	3.098	0.020	2
1145	119	ASP	HB3	H	2.734	0.020	2
1146	119	ASP	N	N	120.222	0.3	1
1147	120	HIS	C	C	176.573	0.3	1
1148	120	HIS	CA	C	60.960	0.3	1
1149	120	HIS	CB	C	29.150	0.3	1
1150	120	HIS	H	H	8.170	0.020	1
1151	120	HIS	HA	H	4.135	0.020	1
1152	120	HIS	HB2	H	3.261	0.020	1
1153	**120**	**HIS**	**HB3**	**H**	**3.261**	**0.020**	**1**
1154	120	HIS	N	N	117.587	0.3	1
1155	121	ALA	C	C	178.239	0.3	1
1156	121	ALA	CA	C	54.684	0.3	1
1157	121	ALA	CB	C	17.844	0.3	1
1158	121	ALA	H	H	8.168	0.020	1
1159	121	ALA	HA	H	4.206	0.020	1
1160	121	ALA	HB	H	1.533	0.020	1
1161	121	ALA	N	N	121.398	0.3	1
1162	122	ALA	C	C	179.738	0.3	1
1163	122	ALA	CA	C	54.343	0.3	1

1164	122	ALA	CB	C	19.359	0.3	1
1165	122	ALA	H	H	7.822	0.020	1
1166	122	ALA	HA	H	3.877	0.020	1
1167	122	ALA	HB	H	1.751	0.020	1
1168	122	ALA	N	N	117.609	0.3	1
1169	123	LYS	C	C	178.828	0.3	1
1170	123	LYS	CA	C	59.565	0.3	1
1171	123	LYS	CB	C	32.068	0.3	1
1172	**123**	**LYS**	**CG**	**C**	**24.872**	**0.3**	**1**
1173	123	LYS	CD	C	29.413	0.3	1
1174	123	LYS	CE	C	42.048	0.3	1
1175	123	LYS	H	H	8.062	0.020	1
1176	123	LYS	HA	H	3.811	0.020	1
1177	123	LYS	HB2	H	1.975	0.020	2
1178	**123**	**LYS**	**HB3**	**H**	**1.829**	**0.020**	**2**
1179	**123**	**LYS**	**HG2**	**H**	**1.206**	**0.020**	**1**
1180	**123**	**LYS**	**HD2**	**H**	**1.701**	**0.020**	**1**
1181	**123**	**LYS**	**HD3**	**H**	**1.701**	**0.020**	**1**
1182	123	LYS	N	N	122.148	0.3	1
1183	124	ILE	C	C	174.838	0.3	1
1184	124	ILE	CA	C	61.063	0.3	1
1185	124	ILE	CB	C	37.448	0.3	1
1186	**124**	**ILE**	**CG1**	**C**	**26.254**	**0.3**	**1**
1187	124	ILE	CG2	C	17.394	0.3	1
1188	124	ILE	CD1	C	13.969	0.3	1
1189	124	ILE	H	H	7.457	0.020	1
1190	124	ILE	HA	H	4.128	0.020	1
1191	124	ILE	HB	H	1.994	0.020	1
1192	**124**	**ILE**	**HG12**	**H**	**0.954**	**0.020**	**1**
1193	**124**	**ILE**	**HG13**	**H**	**0.954**	**0.020**	**1**
1194	**124**	**ILE**	**HG2**	**H**	**0.579**	**0.020**	**1**
1195	**124**	**ILE**	**HD1**	**H**	**0.827**	**0.020**	**1**
1196	124	ILE	N	N	108.686	0.3	1
1197	125	LYS	C	C	174.814	0.3	1
1198	125	LYS	CA	C	55.940	0.3	1
1199	125	LYS	CB	C	28.903	0.3	1
1200	125	LYS	CG	C	24.749	0.3	1
1201	**125**	**LYS**	**CD**	**C**	**28.844**	**0.3**	**1**
1202	125	LYS	CE	C	40.390	0.3	1
1203	125	LYS	H	H	7.121	0.020	1
1204	125	LYS	HA	H	4.294	0.020	1
1205	125	LYS	HB2	H	1.733	0.020	1
1206	125	LYS	HB3	H	1.733	0.020	1
1207	**125**	**LYS**	**HG2**	**H**	**1.427**	**0.020**	**1**
1208	**125**	**LYS**	**HG3**	**H**	**1.427**	**0.020**	**1**
1209	**125**	**LYS**	**HD2**	**H**	**1.929**	**0.020**	**1**
1210	**125**	**LYS**	**HD3**	**H**	**1.929**	**0.020**	**1**
1211	125	LYS	HE2	H	2.992	0.020	1
1212	**125**	**LYS**	**HE3**	**H**	**2.992**	**0.020**	**1**
1213	125	LYS	N	N	120.011	0.3	1
1214	126	SER	C	C	173.482	0.3	1
1215	126	SER	CA	C	53.105	0.3	1
1216	126	SER	CB	C	63.922	0.3	1
1217	126	SER	H	H	7.274	0.020	1
1218	**126**	**SER**	**HA**	**H**	**5.117**	**0.020**	**1**
1219	**126**	**SER**	**HB2**	**H**	**3.597**	**0.020**	**1**

1220	**126**	**SER**	**HB3**	**H**	**3.597**	**0.020**	**1**
1221	126	SER	N	N	111.274	0.3	1
1222	127	PRO	C	C	179.083	0.3	1
1223	127	PRO	CA	C	65.934	0.3	1
1224	127	PRO	CB	C	32.327	0.3	1
1225	127	PRO	CG	C	28.176	0.3	1
1226	127	PRO	CD	C	50.724	0.3	1
1227	127	PRO	HA	H	4.491	0.020	1
1228	127	PRO	HB2	H	2.724	0.020	2
1229	127	PRO	HB3	H	2.281	0.020	2
1230	**127**	**PRO**	**HG2**	**H**	**2.044**	**0.020**	**1**
1231	**127**	**PRO**	**HG3**	**H**	**2.044**	**0.020**	**1**
1232	**127**	**PRO**	**HD2**	**H**	**3.805**	**0.020**	**2**
1233	**127**	**PRO**	**HD3**	**H**	**3.631**	**0.020**	**2**
1234	128	THR	C	C	178.503	0.3	1
1235	128	THR	CA	C	65.146	0.3	1
1236	128	THR	CB	C	67.844	0.3	1
1237	128	THR	CG2	C	22.921	0.3	1
1238	128	THR	H	H	7.833	0.020	1
1239	128	THR	HA	H	4.145	0.020	1
1240	128	THR	HB	H	4.143	0.020	1
1241	128	THR	HG2	H	1.304	0.020	1
1242	128	THR	N	N	109.654	0.3	1
1243	129	GLU	C	C	178.122	0.3	1
1244	129	GLU	CA	C	58.732	0.3	1
1245	129	GLU	CB	C	30.403	0.3	1
1246	129	GLU	CG	C	37.032	0.3	1
1247	129	GLU	H	H	8.077	0.020	1
1248	129	GLU	HA	H	4.096	0.020	1
1249	129	GLU	HB2	H	2.210	0.020	2
1250	129	GLU	HB3	H	2.169	0.020	2
1251	**129**	**GLU**	**HG2**	**H**	**2.539**	**0.020**	**1**
1252	129	GLU	N	N	122.672	0.3	1
1253	130	ALA	C	C	179.145	0.3	1
1254	130	ALA	CA	C	55.375	0.3	1
1255	130	ALA	CB	C	18.188	0.3	1
1256	130	ALA	H	H	8.733	0.020	1
1257	130	ALA	HA	H	4.082	0.020	1
1258	130	ALA	HB	H	1.528	0.020	1
1259	130	ALA	N	N	122.154	0.3	1
1260	131	GLU	C	C	179.714	0.3	1
1261	131	GLU	CA	C	60.181	0.3	1
1262	131	GLU	CB	C	29.454	0.3	1
1263	131	GLU	CG	C	36.457	0.3	1
1264	131	GLU	H	H	8.348	0.020	1
1265	131	GLU	HA	H	4.073	0.020	1
1266	131	GLU	HB2	H	2.138	0.020	1
1267	131	GLU	HG2	H	2.517	0.020	2
1268	131	GLU	HG3	H	2.283	0.020	2
1269	131	GLU	N	N	117.714	0.3	1
1270	132	LYS	C	C	180.047	0.3	1
1271	132	LYS	CA	C	59.266	0.3	1
1272	132	LYS	CB	C	32.176	0.3	1
1273	132	LYS	CG	C	23.686	0.3	1
1274	132	LYS	CD	C	29.490	0.3	1
1275	132	LYS	CE	C	41.658	0.3	1

1276	132	LYS	H	H	7.595	0.020	1
1277	132	LYS	HA	H	4.106	0.020	1
1278	132	LYS	HB2	H	1.937	0.020	1
1279	**132**	**LYS**	**HB3**	**H**	**1.937**	**0.020**	**1**
1280	**132**	**LYS**	**HG2**	**H**	**1.207**	**0.020**	**1**
1281	**132**	**LYS**	**HG3**	**H**	**1.207**	**0.020**	**1**
1282	**132**	**LYS**	**HD2**	**H**	**1.389**	**0.020**	**2**
1283	**132**	**LYS**	**HD3**	**H**	**1.278**	**0.020**	**2**
1284	132	LYS	HE2	H	2.585	0.020	1
1285	132	LYS	N	N	120.922	0.3	1
1286	133	TYR	C	C	179.167	0.3	1
1287	133	TYR	CA	C	62.647	0.3	1
1288	133	TYR	CB	C	36.957	0.3	1
1289	133	TYR	H	H	8.568	0.020	1
1290	133	TYR	HA	H	4.485	0.020	1
1291	133	TYR	HB2	H	3.406	0.020	2
1292	133	TYR	HB3	H	2.540	0.020	2
1293	**133**	**TYR**	**HD1**	**H**	**7.119**	**0.020**	**1**
1294	**133**	**TYR**	**HD2**	**H**	**7.119**	**0.020**	**1**
1295	133	TYR	N	N	116.687	0.3	1
1296	134	TYR	C	C	176.382	0.3	1
1297	134	TYR	CA	C	63.329	0.3	1
1298	134	TYR	CB	C	38.045	0.3	1
1299	134	TYR	H	H	9.591	0.020	1
1300	134	TYR	HA	H	4.180	0.020	1
1301	134	TYR	HB2	H	3.163	0.020	1
1302	**134**	**TYR**	**HB3**	**H**	**3.163**	**0.020**	**1**
1303	**134**	**TYR**	**HD1**	**H**	**6.971**	**0.020**	**1**
1304	**134**	**TYR**	**HD2**	**H**	**6.971**	**0.020**	**1**
1305	134	TYR	N	N	126.966	0.3	1
1306	135	GLY	C	C	177.239	0.3	1
1307	135	GLY	CA	C	47.426	0.3	1
1308	135	GLY	H	H	8.182	0.020	1
1309	135	GLY	HA2	H	4.046	0.020	2
1310	135	GLY	HA3	H	3.677	0.020	2
1311	135	GLY	N	N	106.437	0.3	1
1312	136	GLN	C	C	177.682	0.3	1
1313	136	GLN	CA	C	59.093	0.3	1
1314	136	GLN	CB	C	28.905	0.3	1
1315	136	GLN	CG	C	33.975	0.3	1
1316	136	GLN	H	H	7.615	0.020	1
1317	136	GLN	HA	H	4.150	0.020	1
1318	136	GLN	HB2	H	2.286	0.020	2
1319	136	GLN	HB3	H	2.115	0.020	2
1320	136	GLN	HG2	H	2.599	0.020	2
1321	136	GLN	HG3	H	2.501	0.020	2
1322	136	GLN	N	N	119.338	0.3	1
1323	137	THR	CA	C	68.317	0.3	1
1324	137	THR	CB	C	67.659	0.3	1
1325	**137**	**THR**	**CG2**	**C**	**21.626**	**0.3**	**1**
1326	137	THR	H	H	8.053	0.020	1
1327	**137**	**THR**	**HA**	**H**	**4.161**	**0.020**	**1**
1328	**137**	**THR**	**HB**	**H**	**4.007**	**0.020**	**1**
1329	**137**	**THR**	**HG2**	**H**	**1.282**	**0.020**	**1**
1330	137	THR	N	N	118.566	0.3	1
1331	**138**	**VAL**	**CA**	**C**	**67.231**	**0.3**	**1**

1332	**138**	**VAL**	**CB**	**C**	**31.430**	**0.3**	**1**
1333	**138**	**VAL**	**CG1**	**C**	**22.480**	**0.3**	**1**
1334	**138**	**VAL**	**CG2**	**C**	**20.687**	**0.3**	**1**
1335	**138**	**VAL**	**H**	**H**	**8.324**	**0.020**	**1**
1336	**138**	**VAL**	**HA**	**H**	**3.202**	**0.020**	**1**
1337	**138**	**VAL**	**HB**	**H**	**2.175**	**0.020**	**1**
1338	**138**	**VAL**	**HG1**	**H**	**0.484**	**0.020**	**1**
1339	**138**	**VAL**	**HG2**	**H**	**0.825**	**0.020**	**1**
1340	**138**	**VAL**	**N**	**N**	**121.457**	**0.3**	**1**
1341	139	SER	C	C	177.385	0.3	1
1342	139	SER	CA	C	61.748	0.3	1
1343	139	SER	CB	C	62.475	0.3	1
1344	139	SER	H	H	7.481	0.020	1
1345	139	SER	HA	H	4.189	0.020	1
1346	139	SER	HB2	H	4.007	0.020	2
1347	**139**	**SER**	**HB3**	**H**	**3.936**	**0.020**	**2**
1348	139	SER	N	N	113.316	0.3	1
1349	140	ASN	C	C	178.003	0.3	1
1350	140	ASN	CA	C	55.941	0.3	1
1351	140	ASN	CB	C	38.909	0.3	1
1352	140	ASN	H	H	8.467	0.020	1
1353	140	ASN	HA	H	4.554	0.020	1
1354	140	ASN	HB2	H	2.860	0.020	2
1355	140	ASN	HB3	H	2.551	0.020	2
1356	140	ASN	N	N	119.386	0.3	1
1357	141	ILE	C	C	177.242	0.3	1
1358	141	ILE	CA	C	65.195	0.3	1
1359	141	ILE	CB	C	36.563	0.3	1
1360	**141**	**ILE**	**CG1**	**C**	**30.598**	**0.3**	**1**
1361	141	ILE	CG2	C	17.155	0.3	1
1362	141	ILE	CD1	C	13.357	0.3	1
1363	141	ILE	H	H	9.189	0.020	1
1364	141	ILE	HA	H	3.549	0.020	1
1365	141	ILE	HB	H	2.174	0.020	1
1366	**141**	**ILE**	**HG12**	**H**	**1.703**	**0.020**	**2**
1367	**141**	**ILE**	**HG13**	**H**	**1.009**	**0.020**	**2**
1368	**141**	**ILE**	**HG2**	**H**	**0.783**	**0.020**	**1**
1369	141	ILE	HD1	H	0.773	0.020	1
1370	141	ILE	N	N	123.371	0.3	1
1371	142	ASN	C	C	178.762	0.3	1
1372	142	ASN	CA	C	56.407	0.3	1
1373	142	ASN	CB	C	37.344	0.3	1
1374	142	ASN	H	H	8.288	0.020	1
1375	142	ASN	HA	H	4.467	0.020	1
1376	142	ASN	HB2	H	2.987	0.020	2
1377	142	ASN	HB3	H	2.780	0.020	2
1378	**142**	**ASN**	**HD21**	**H**	**7.490**	**0.020**	**1**
1379	142	ASN	N	N	119.151	0.3	1
1380	143	GLU	C	C	179.037	0.3	1
1381	143	GLU	CA	C	59.643	0.3	1
1382	143	GLU	CB	C	29.635	0.3	1
1383	143	GLU	CG	C	36.844	0.3	1
1384	143	GLU	H	H	7.800	0.020	1
1385	143	GLU	HA	H	4.145	0.020	1
1386	143	GLU	HB2	H	2.183	0.020	1
1387	**143**	**GLU**	**HB3**	**H**	**2.183**	**0.020**	**1**

1388	**143**	**GLU**	**HG2**	**H**	**2.537**	**0.020**	**1**
1389	143	GLU	N	N	119.853	0.3	1
1390	144	VAL	C	C	177.908	0.3	1
1391	144	VAL	CA	C	66.941	0.3	1
1392	144	VAL	CB	C	31.571	0.3	1
1393	144	VAL	CG1	C	24.626	0.3	1
1394	144	VAL	CG2	C	20.971	0.3	1
1395	144	VAL	H	H	7.919	0.020	1
1396	144	VAL	HA	H	3.507	0.020	1
1397	144	VAL	HB	H	2.199	0.020	1
1398	144	VAL	HG1	H	0.880	0.020	1
1399	144	VAL	HG2	H	0.832	0.020	1
1400	144	VAL	N	N	119.901	0.3	1
1401	145	LEU	C	C	180.097	0.3	1
1402	145	LEU	CA	C	58.156	0.3	1
1403	145	LEU	CB	C	40.865	0.3	1
1404	145	LEU	CG	C	27.129	0.3	1
1405	145	LEU	CD1	C	25.727	0.3	1
1406	145	LEU	CD2	C	23.036	0.3	1
1407	145	LEU	H	H	8.982	0.020	1
1408	145	LEU	HA	H	3.916	0.020	1
1409	145	LEU	HB2	H	1.974	0.020	2
1410	145	LEU	HB3	H	1.481	0.020	2
1411	145	LEU	HG	H	1.193	0.020	1
1412	145	LEU	HD1	H	0.841	0.020	1
1413	145	LEU	N	N	118.426	0.3	1
1414	146	ALA	C	C	179.476	0.3	1
1415	146	ALA	CA	C	54.609	0.3	1
1416	146	ALA	CB	C	18.117	0.3	1
1417	146	ALA	H	H	7.513	0.020	1
1418	146	ALA	HA	H	4.216	0.020	1
1419	146	ALA	HB	H	1.528	0.020	1
1420	146	ALA	N	N	119.677	0.3	1
1421	147	LYS	C	C	177.596	0.3	1
1422	147	LYS	CA	C	55.474	0.3	1
1423	147	LYS	CB	C	32.716	0.3	1
1424	147	LYS	CG	C	24.844	0.3	1
1425	147	LYS	CD	C	28.141	0.3	1
1426	147	LYS	CE	C	42.269	0.3	1
1427	147	LYS	H	H	7.600	0.020	1
1428	147	LYS	HA	H	4.395	0.020	1
1429	147	LYS	HB2	H	1.917	0.020	2
1430	**147**	**LYS**	**HB3**	**H**	**2.031**	**0.020**	**2**
1431	**147**	**LYS**	**HG2**	**H**	**1.444**	**0.020**	**1**
1432	**147**	**LYS**	**HG3**	**H**	**1.444**	**0.020**	**1**
1433	147	LYS	HD2	H	1.695	0.020	2
1434	**147**	**LYS**	**HD3**	**H**	**1.640**	**0.020**	**2**
1435	147	LYS	HE2	H	2.968	0.020	1
1436	147	LYS	N	N	116.071	0.3	1
1437	148	LEU	C	C	177.001	0.3	1
1438	148	LEU	CA	C	55.952	0.3	1
1439	148	LEU	CB	C	42.332	0.3	1
1440	148	LEU	CG	C	25.717	0.3	1
1441	**148**	**LEU**	**CD1**	**C**	**23.343**	**0.3**	**1**
1442	148	LEU	CD2	C	22.823	0.3	1
1443	148	LEU	H	H	7.664	0.020	1

1444	148	LEU	HA	H	4.197	0.020	1
1445	148	LEU	HB2	H	1.601	0.020	1
1446	148	LEU	HG	H	1.852	0.020	1
1447	148	LEU	HD1	H	0.802	0.020	1
1448	148	LEU	N	N	119.200	0.3	1
1449	149	GLY	C	C	179.048	0.3	1
1450	149	GLY	CA	C	46.239	0.3	1
1451	149	GLY	H	H	7.560	0.020	1
1452	**149**	**GLY**	**HA2**	**H**	**3.775**	**0.020**	**2**
1453	**149**	**GLY**	**HA3**	**H**	**3.633**	**0.020**	**2**
1454	149	GLY	N	N	112.662	0.3	1