Appendix I

A.1 Protein crystallization

A.1.1 Principle of Protein Crystallization

A.1.1.1 Materials and Methods

Crystallization of macromolecules is the limiting step in protein crystallography. The principles of crystallization of macromolecules are analogous to those of small molecules. Protein molecules themselves are distinctive, composed of approximately 50% solvent though this may vary from 30 - 78% (Matthews, 1985). Proteins are labile, fragile, and sensitive to external environments owing to their high solvent content, and the weak binding energies between protein molecules in the crystal (Littlechild, 1991). The only optimal conditions suitable for their growth are those that cause little or no perturbation of their molecule properties. As a result, crystals must be grown from a medium where temperature is constant and within a broad range of pH 3 – 10 because complete hydration is an essential factor for maintaining the crystal structure integrity. There are many differences between small molecule crystals and protein crystals. In general, small molecule crystals are grown to large dimensions (1 – 100 cm), they are physically hard, brittle, easy to handle, have strong optical properties and can diffract X- rays intensively. This is because the small molecule crystals exhibit firm lattice interactions and highly ordered lattice arrangements within the crystal. In comparison, protein crystals are generally smaller in size (1 – 1000 μm), soft and crushed without difficulty, and with weak crystal forces. These kinds of crystals, which will redissolve if rehydration occurs, have weak optical properties and diffract X-rays weakly. They are also temperature sensitive, because protein solubility alters as a function of

temperature. In theory, as the temperature rises, the solubility of proteins increases, thus, no crystals are formed or crystals will be redissolved. When crystals are exposed to long-standing X-ray radiation, it can cause extensive damage of crystals. This is due to the weakening of lattice forces within the crystal structure (McPherson, 1982) and the X-rays can produce sufficient free radicals to cause specific chemical changes on the protein molecules such as the breaking of the disulfide bonds (Ravelli & McSweeney, 2000).

The crystallization of proteins from solution is a reversible equilibrium phenomenon. It contains three stages: nucleation, growth and cessation of growth. The formation of crystals is due to the decreasing free energy of the system while the formation of many new chemical bonds simultaneously outweighs the decreasing entropy of the system in order to grow a highly organized internal structure. In other words, the free energy of the system is reduced to its energy minimum and a thermodynamic driving force exists that provides for the ordering process of crystals (McPherson, 1982). The basic strategy of producing protein crystals is to generate a certain degree of super saturation in the solution. At the equilibrium point, the amount of protein molecules entering the solution is the same as the amount of protein molecules leaving the solution. This is referred to as the solubility limit of a protein. When the solubility of a protein is below this limit, the solution is under saturated. If the solubility is equal to the limit, the solution is saturated. Crystals can grow only when the solubility exceeds the limit. Every protein has a unique solubility. Decreasing the solubility of the protein is the most effective way to create supersaturation for crystal growth. Only in a non-equilibrium supersaturated solution, can a crystal grow. Supersaturation can be achieved by different approaches including altering the buffer pH,

temperature, protein concentration, dielectric constant of the medium and precipitant concentration in order to change the protein solubility to reach the condition that lies just above the supersaturation region (McPherson, 1998).

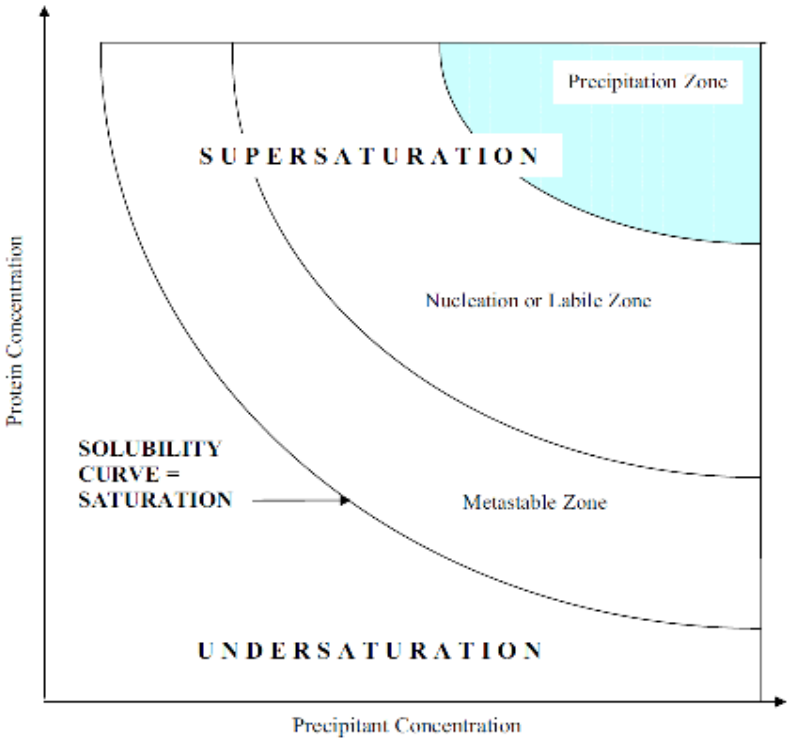

Figure A.1: The solubility phase diagram for crystallization from solution.

A classical explanation of crystal nuclei formation and growth can be visualized by the two-dimensional solubility phase diagram shown in Figure 1.8. The solubility curve divides the concentration space into under saturation and super saturation regions. In the under saturation zone, under the solubility curve, the protein will never crystallize. Above the solubility curve, this region can be subdivided into three zones according

to level of saturation and the kinetics required for reaching equilibrium. In the precipitation zone, excess protein does not remain in solution and exists as an amorphous precipitate the formation of precipitate implies that no crystals will form. Before a crystal can grow in solution, nucleation has to occur. Nucleation is the beginning of crystal formation. In this process, the nucleus of sufficient size must be formed to initiate aggregation in an ordered manner. In the nucleation zone or labile zone, there is a high probability that critical nuclei will form spontaneously in solution because this corresponds to an increased energy state of the system. In fact, the energy or probability barrier to the formation of the first nucleus allows the creation of a supersaturated solution. If the degree of aggregation is too high, the solution will be oversaturated and a precipitate will be formed. If the degree of aggregation is adequate, stable nuclei can continue to grow to larger size without forming precipitate, and then the crystal can be formed and grown. The detestable zone is ideal for the growth of crystals without nucleation of new crystals. In this zone nuclei will not form, but if nuclei are present or seed crystals are introduced then crystals may grow. When a crystal grows to a certain size, it will stop growing spontaneously. The crystallization of proteins is influenced by a numbers of factors, and each protein is unique. It is not possible to envisage the conditions that can cause the crystallization of a protein. The various parameters that affect crystallization are not independent of each other and their interrelation may be complicated and difficult to distinguish (McPherson, 1998). Finding a rational guideline to crystallize macromolecules successfully is not an easy task. The only way to do this is to identify the important components and refine each of them individually. In general, precipitant type and concentration, buffer type and pH, temperature, and sample concentration

are the most important factors for protein crystallization. They are considered first when performing crystallization experiments. Each parameter is manipulated independently to determine its effect on crystallization.

A.1.1.2 Crystallization Methods

There are at least seven practical methods used for macromolecule crystallization including vapor diffusion, bulk crystallization, batch, free-interface diffusion, dialysis, temperature-induced, and seeding. Among these methods, vapor diffusion and micro batch (a new developed method from an old technique) are the most popular means being utilized by crystallographers to obtain macromolecule crystals. The method of vapor diffusion is undoubtedly regarded as the most widely employed approach for crystallization. Nucleation occurs when the sample concentration increases as the droplet volume decreases by hydration-driven mechanisms. This is induced by the equilibration of water vapor between the sample droplet and the reservoir solution. The vapor diffusion technique is an ideal methodology for screening a broad spectrum of crystallization conditions. It can be used to optimize the size of crystals suitable for X-ray diffraction analysis. Vapor diffusion methods include hanging drop, sitting drop, sandwich, and capillary methods. The most common protocols are the hanging drop and sitting drop methods (McPherson, 1998). The hanging drop vapor diffusion method is an efficient means of screening crystallization parameters. The advantage of this method is that it requires only a small volume of droplet, which can be as low as 2 μL per experiment, so a minimum amount of sample is consumed for

screening and optimization of the crystallization conditions (McPherson, 1998). The reason for the popularity of the hanging drop method is the ease of performing the experiment, only a 24 well-plate (such as Linbro or VDX plate), grease and cover slides are needed. The principle of this approach is straightforward, a drop composed of a mixture of macromolecule sample and precipitating solution is placed in vapor equilibration with a reservoir solution of precipitant and buffer. To start the trial, the precipitating solution composed of precipitant, buffer, additive, etc, is dispensed into reservoir. Then equal volumes of the sample and reservoir solution are mixed onto the surface of siliconized glass cover slide (Figure 1.10). The drop has a lower concentration of precipitant than the reservoir solution, so water or volatile chemicals will escape from the drop into the reservoir solution to achieve system equilibrium inside the reservoir. Eventually this causes the sample inside the drop to become more concentrated until the precipitating concentration in the drop is almost equivalent to the reservoir concentration. The major benefits of using the hanging drop method are relative ease of mounting the crystal for X-ray diffraction experiments by inverting the cover slide with a pair of forceps. This method can be used to place multiple drops in each reservoir as well, thus saving time and material.

Sitting drop vapor diffusion method is performed using a 96 well depression plate. One ml of protein solution and 1 ML of reservoir solution are mixed together at the top of a ledge (Figure A.1.2). The plate is then sealed with sealing tape and placed inside the incubator for crystal growth. The basic principle of this method is quite similar to the hanging drop method but the differences are that 100 uL of reservoir solution is used

instead of 1000 uL and the droplet is not hung but sits on the depression platform. The advantage of sitting drop is that it requires a small amount of material and is ideal for screening a great number of different conditions by using the different screening kits, for example the grid screens and crystal screens supplied by different companies such as Sigma, Hampton Research, and Molecular Dimension.

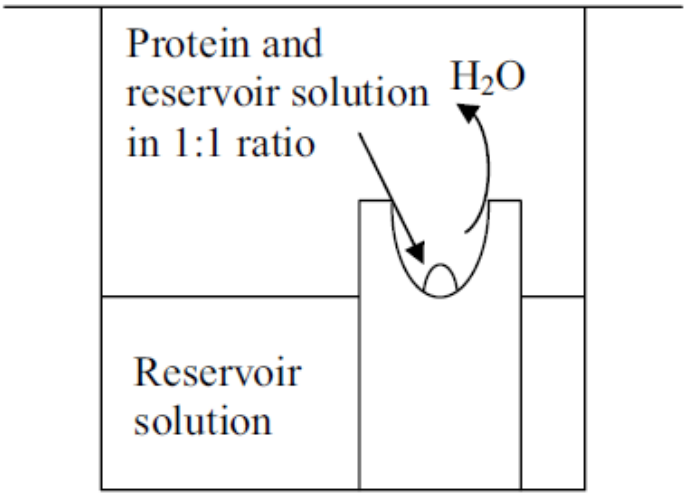

Figure A.1.2: The sitting-drop vapor diffusion method for protein crystallization (Hampton Research, 2001).

Micro batch method is another approach for rapid protein crystallization using micro-volumes of sample. The objective of this method is to reduce the consumption of sample by generating crystallization trials in tiny amounts. It is a new method developed from the oldest crystallization technique, "The Batch Method". The batch method has been the conventional crystallization strategy for over 150 years (McPherson, 1991). The most famous example of a protein

crystallized by this method is the crystallization of lysozyme (Forsythe *et al.*, 1997). The principle is simple and the procedure involves the direct mixing of the unsaturated protein solutions with precipitating solution. The batch method alters the protein solubility and changes the dielectric properties of the medium to create a supersaturated environment to generate the crystal. This method has been revived by preparing the crystallization samples under oil (Figure A.1.3). The typical final sample droplet volume is about 1 – 2 µL. The major advantages of this approach are automatic implementation in an efficient way, high accuracy; low sample consumption, time-saving screening, and the sample can be protected from evaporation, contamination and physical shock by oil (Chayen and Stewart, 1992).

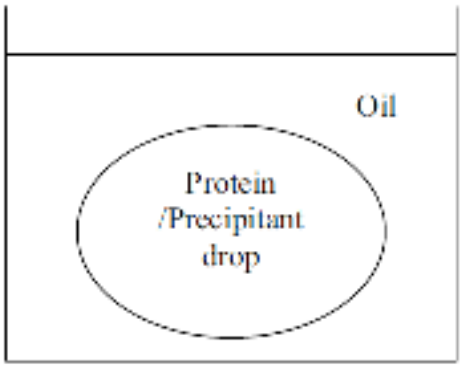

Figure A.1.3: The micro batch method for protein crystallization (HamptonResearch, 2001).

The fundamental distinction between the vapor diffusion and micro batch method is that the former is a dynamic system in which the conditions are changing throughout the whole crystallization process. Thus, there is little

control over the experiment once the trial has been set up. The latter process is non-dynamic, the final concentration of the sample has been determined precisely at the beginning of the experiment. The crystallization condition can be maintained with minimum fluctuation for the normal crystallization period of about 1–3 weeks (Chayen, 1998). Other crystallization methods that have been employed include container less crystallization (Chayen, 1996), crystallization in silica gels (Cudney *et al.*, 1994) and crystallization under microgravity (Littke and John, 1984). According to the BMCD database, vapor diffusion (62.7%), batch method (~12%) and seeding (5.47%)

A.1.1.3 Crystal Handling, Mounting, Cooling, Storage and Transportation

Crystal mounting for cryocrystallography can be carried out using capillary tubes, fine glass capillary tubes, thin glass spatulas, and thin loop films. However, the freestanding loop mounting technique has become the prevalent procedure for crystal mounting at cryogenic temperatures (Rodgers, 1997). Originally the loops were made from copper or tungsten wire with 1-2 mm diameters and 25– 75mm thickness, but now, they are made from fine nylon fibers with 0.05–1.0 mm diameters and 10 & 20mm thickness. These types of cryo-loops show minimal background diffraction due to the optically clear environment and the loops are thin enough to be convenient for fast freezing. The major advantages of using this approach to lift up, transfer, flash cool the crystal are the production of lower X-ray scatter and lower X-ray absorption. Uniform results can be obtained compared to the conventional mounting techniques such as capillary mounting (Teng,

1990). The loop itself serves as a platform to support the crystal in place and to keep it away from foreign material which has determental effects to the crystal. It is a good idea to select a loop size which is just wide enough to keep the crystal from dropping off. For instant data collection, the crystal is picked up and flash-cooled immediately. The cold stream is first deflected from the nitrogen gas nozzle, the crystal is placed on the monometer, and then the obstruction of the flow is removed immediately. If the crystal needs a faster rate of cooling, or will be sent away to synchrotron facilities for data analysis, the crystal can be rapidly plunged beneath the surface of liquid nitrogen for a few seconds to a few minutes. Next, the crystal is moved to a diffractometer for experiment or transferred to a storage tank for storage and transportation (Parkin and Hope, 1998). The other advantage of cryocrystallography is the potential for storing and transporting crystals as soon as they have been flash-cooled. Well-diffracted crystals can be kept and sent to a synchrotron after screening at an in-house facility. Another factor to be considered is that crystals can grow to a limiting size and subsequently degrade. So crystals can be flash cooled at their finest conditions until data collection is performed. Crystals must be kept at cryogenic temperatures, so a liquid nitrogen Dewar which is portable and provides protection from mechanical shock is highly recommended. In principle, once the crystals have been successfully cooled to cryogenic temperatures, they can be kept for an indefinite time (Parkin and Hope, 1998).

A.1.2 X-rays Diffraction

A.1.2.1 Proteins, Crystals and X-rays

X-rays are generated when electrons collide with the atoms of a metal target, e.g. copper. The electrons are liberated from a heated filament and accelerated by high voltage towards the metal target (Stout and Jensen, 1989). X-rays are a form of electromagnetic radiation, where wavelength is about 0.1–10 nm (1100 Å) on the electromagnetic spectrum (Petrucci *et al.*, 2002). The major reason that X-rays have been chosen to study the 3-D structures of proteins in crystallography because the range of wavelengths of X-rays we choose (0.5 Å–1.6 Å) is on the same order of magnitude as the bond length of the atoms within protein molecules. The bond length between atoms within a protein is about 0.15 nm or 1.5 Å, thus these wavelengths can be utilized to visualize the geometry and structure of protein molecules through X-ray diffraction (Blow, 2002). Individual atoms in a molecule can diffract X-rays; however, they are weak scatterers of X-rays. Therefore, X-rays may pass through a single molecule without any diffraction, so diffraction might be too weak to be detected by any instrument. However, one can solve the problem by analyzing a crystal diffraction pattern rather than a molecule. This is because a crystal is composed of a number of repeating patterns (unit cells) in a regular and ordered manner. Each molecule within the crystal therefore diffracts equally, and thus strong diffracted X-ray beams can be measured (Rhode, 2000).

A.1.2.2 Bragg's Law

W. L Braggs managed to visualize the scattering X-rays from a crystal by considering that the diffracted beams were reflected by planes passing through points of a crystal lattice. The diffracted X-rays are scattered by the crystal at a certain angle of reflection (θ) This reflection is analogous to that from a mirror, for which the angle of incident X-ray beam is equal to the angle of diffracted X-ray beam. The incident and the diffracted X-rays are in the same plane and the X-rays of wavelength (λ) are normal to a set of diffracting planes (FigureA.1.4). The constructive interference between X-rays scattered from successive planes in the crystal will only take place if the path difference (2d) between the X-rays is equivalent to an integral number of wavelength λ. That is the Bragg's law equation (Glusker and Trueblood, 1985):

$$n\lambda = 2d \sin\theta$$

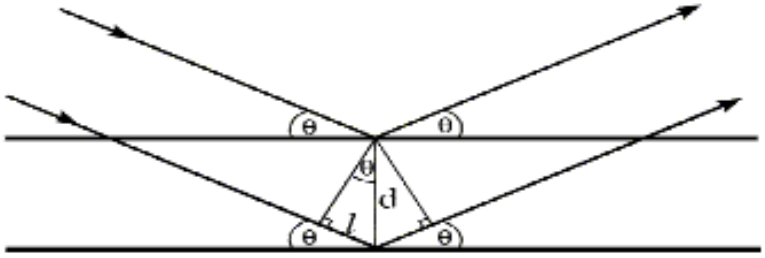

Figure 1.4: The geometry of diffraction and its relationship to Bragg's Law (Glusker and True blood, 1985).

In Bragg's law, if the wavelength and the diffraction angle of a reflection are known, the perpendicular distance between the lattice planes in the crystal (interplanar spacing, d) can be easily calculated. As the angle increases, *d* must become smaller for the path length to remain equal to one wavelength. The equation can be rearranged as

$$d = n\lambda / (2 \sin\theta).$$

The minimum interplanar spacing (dm), where dm= 1/2 (sinθmax), is also interpreted as the resolution of an electron density map. Since the maximum possible value of sinθ is 1, so the smaller the dm value, the higher the resolution will be of the X-ray diffraction pattern. For instance, if the radiation used for X-ray generation has a wavelength of 1.54 Å, then the maximum resolution that can be observed with this radiation would be 0.77 Å (Blundell and Johnson, 1976; Glusker *et al.*, 1994). Most proteins seldom diffract better than 1.5 Å (Glusker *et al.*, 1994). If a protein is diffracted to a high resolution level (above 2 Å), most fine macromolecular structures can be solved and refined by crystallography.

A.1.2.3 Asymmetric Unit, Space Group, Unit Cell and Bravais Lattices

Crystals can be characterized by three elements to precisely define the arrangement, coordination, and periodicity of the fundamental unit of which they are composed. These 3 elements are symmetry properties, repetitive features and distribution of the atoms in the repeating unit. Protein molecules are inherently asymmetric. The asymmetric unit is the smallest component in the crystal. The asymmetric unit may consist of one molecule, part of a molecule or several molecules

not related by symmetry. If only one molecule occupies a unit cell, then the cell itself is chiral and has no symmetry elements at all. This object is termed as the asymmetric unit because no part of it is systematically related to any other by crystallographic properties. That means it has no symmetry elements such as rotation axis or mirror plane (Figure A.1.5). In most cases, the unit cell contains more than one identical molecule or oligomeric complexes (dimmer, trimer, tetramer, etc.) in an arrangement that produces symmetry elements. So, the largest aggregate of molecule(s) in a cell that possesses no symmetry element but can be juxtaposed on other identical entities by symmetry operation is called the asymmetric unit (Rhode, 2000). A set of symmetry operations includes rotation, reflection, inversion, rotatory inversion, screw axes, glide plane, and translation. These operations can be applied to an asymmetric unit. Combination of all these elements in all possible ways generates a total of 230 unique, three-dimensional space groups of symmetry operation. These 230 space groups are described in *International Tables forX-ray CrystallographyVol.A*(Hahn,2002).

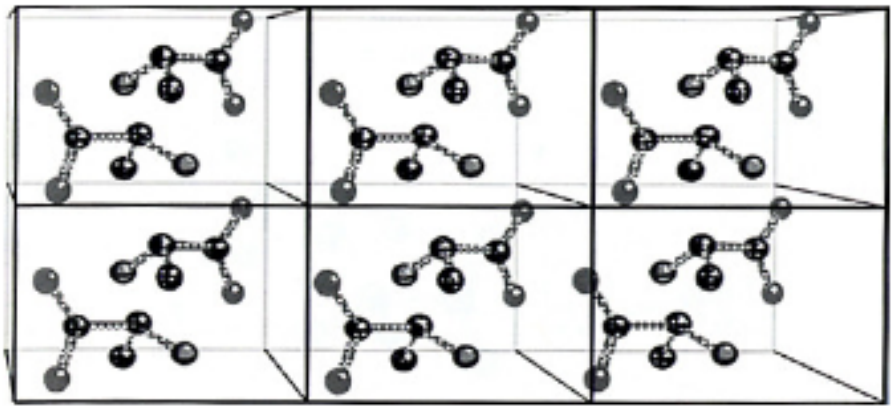

Figure A.1.5: There are six unit cells in this crystalline lattice. In each unit cell contains two molecules, the asymmetric unit is a dimer (Rhode, 2000;)

The unit cell is the basic building block of the crystal and is repeated infinitely in three dimensions. The directions of constructive interference depend only on the size and shape of the unit cell. The dimensions of a unit cell are designated by six parameters: the length of 3 unique edges (a, b, c) which run along x, y and z coordinates respectively, and 3 unique angles(α, β, γ) as indicated in Figure A.1.6.

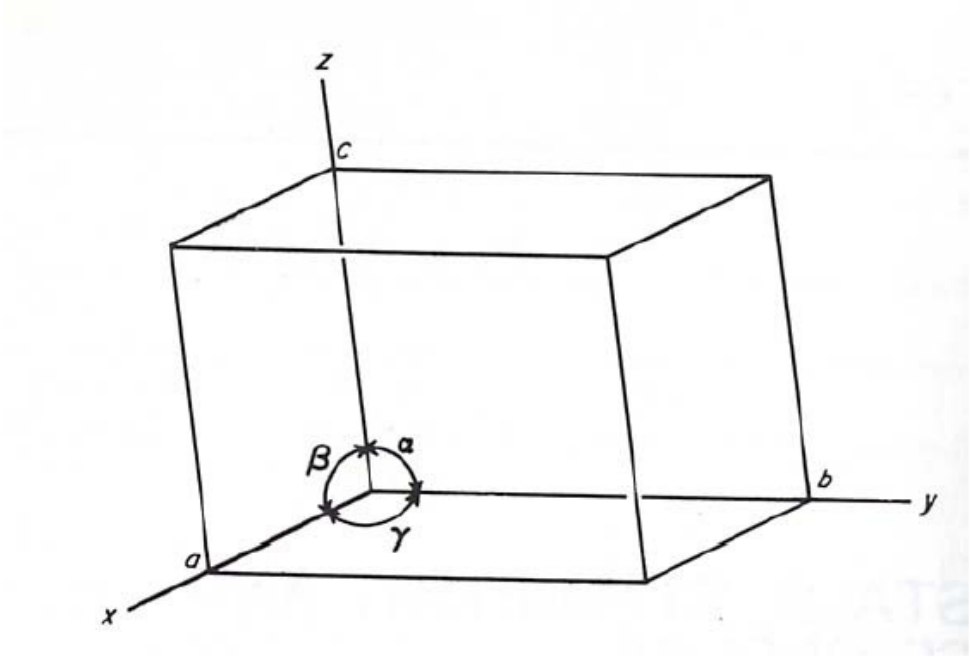

Figure A.1.6: The unit cell with edges, a, b, c and angles, α, β and γ (Stout and Jensen, 1989).

In virtually all cases, a crystallographer is concerned only with the content of the individual unit cell and the coordinate of the atoms within the unit cell. There are 14 allowable unit cell types classified as *Bravais lattice* to distinguish their characteristic The Bravais lattices themselves can be divided into five types of lattice, which are primitive (**P**), centered (**C**), body-centered (**I**) , face-centered (**F**) and rhombohedral (**R**) (McPherson, 2003; Stout and Jensen, 1989). Any crystal can be regarded as being established by consecutively translational repetition of the unit cell and its content along a, b, c by distance |a|, |b|, |c| respectively, until a continuous three–dimensional array of repeated unit cells in a regular

manner has been created (Glusker *et al.*, 1994; Glusker and True blood, 1985; Rhode, 2000). The simple symmetry operations and elements needed to describe unit cell symmetry are translation, rotation and reflection. The symmetry of a unit cell is described in 230 space groups (like P212121). The space group is a group of symmetry operations consistent with an infinitely extended, regularly repeating pattern. Protein is an asymmetric object since all amino acids except glycine have chirality. However, the D form of amino acids does not exist in proteins and only the L form does. Thus, there are less symmetry elements (mirror planes, inversion centers and glide planes) involved within the unit cell, and less space groups can be used to designate the protein. This limits the possible space groups to 65 out of the 230 mathematically possible ones (McRee, 1999). There are seven crystal systems used to classify the symmetry of the crystal, this corresponds to the seven fundamental shapes for unit cells, consistent with the 14 Bravais lattices as displayed in Table A.1 (Glusker *et al.*, 1994; Stout and Jensen, 1989).

Table A.1 The seven crystal systems (Glusker *et al.*, 1994; Stout and Jensen, 1989).

	Crystal System	Bravais Lattices	Lattice	Angle
1	Triclinic	P	a≠b≠c	α≠β≠γ
2	Monoclinic	P, C	a≠b≠c	α=γ= 90°≠β
3	Orthorhombic	P, C, I, F	a≠b≠c	α=β=γ=90°
4	Tetragonal	P, I	a=b≠c	α=β=γ=90°
5a	Trigonal	P	a=b≠c	α=β=90°,γ=120°
5b	Rhombohedral	R	a=b=c	α=β=γ<120°,≠90°
6	Hexagonal	P	a=b≠c	α=β=90°,γ=120°
7	Cubic	P, I, F	a=b=c	α=β=γ=90°

A.1.3 X-ray diffraction Data Collection

For data collection purposes, the most important factor required for evaluating data quality is the completeness of the X-ray data including all the indices and their associated intensities, with their standard uncertainties (Dauter, 1999). Two factors that influence the data completeness are the geometric and informative content. The geometric factor, arising from the symmetry of crystal lattice and the detector setup, is a quantitative factor related to a number of variables including the approach of angular rotating method, the selection of the total rotation range appropriate for the crystal symmetry, crystal-to-detector distance, crystal mosaiccity and beam divergence. The informative factor includes the quality of the data, the dynamic range of detector and the R-factor. The longer the exposure time, the greater the intensities and the signal-to-noise ratio, and the better the data quality obtained (Dauter, 1997; Dauter, 1999). The X-ray data quality for

macromolecular crystallography is assessed by a global indicator, the merging R-factor (Rmerge) or symmetry R-factor (Rsym). The merging Rfactor is defined by the following equation (Blundell and Johnson, 1976):

$$\text{Rmerge (I)} = \frac{\sum hkl \sum \text{i}/\ I\text{i}\ (hkl) - \{I(hkl)\}}{\sum hkl + \sum \text{i}\ I\text{i}\ (hkl)}$$

Where (*I*i(*hkl*)) is all observed intensities and - *I* (*hkl*). is the average value of all observed intensities The Rmerge value will be between 20 – 40% while the signal/noise (*I*/ /sigma(*I*)) falls around 1.0- 2.0 (Dauter, 1999). The quantity of merging R-factor is almost universally used for evaluating X-ray diffraction data.

References

Blow, D. M. (2002). Outline of Crystallography for Biologist, Oxford University Press, New York.

Blundell, T. L. and Jhonson, L. N (1976). Protein Crystallography. Academic Press,London and New York

Chayen, N. E. (1996). A novel technique for containerless protein crystallization, *Protein engineering* 9:927-929.

Chayen, N. E. (1998) Comparative Studies of Protein Crystallization by Vapour-Diffusion and Microbatch Techniques, *Acta Crystallographica Section D* 54:8-15.

Chayen, N. E., Shaw Stewart, P. D., Blow, D. M. (1992). Microbatch crystallization under oil-a new technique allowing many small-volume crystallization trials, J. Crystal Growth 122:176-180.

Cudney, R., Patel, S. and McPherson, A. (1994). Crystallization of macromolecules in silica gels, *Acta Crystallogr Biol D Crystallogr* 50:479-483.

Dauter, Z. (1997). Data Collection Strategy, *Methods Enzymol*. 276:326-344.

Dauter, Z. (1999). Data-collection strategies, *Acta Crystallogr D Biol Crystallogr* 55:1703-1717.

Forsythe, E. L., Snell, E. H. and Pusey, M. L. (1997). Crystallization of chicken egg-white lysozyme from ammonium sulfate, *Acta Crystallogr D Biol Crystallogr* 53:795-797.

Glusker, J. P. and True blood, F. N (1985). Crystal structure analysis. - A primer, Oxford University Press,New York.

Glusker, J. P., Lewis, M. and Rossi, M. (1994). Crystal Structure Analysis for Chemists and Biologists: Wiley-VCH. New York.

Hahn, T. (2002). International Tables for Crystallography.

Littke, W. and John, C. (1984). Materials: Protein Single Crystal Growth Under Microgravity, *Science* 225:203-204.

Littlechild, J. A. (1991). Protein crystallization: magical or logical: can we establish some general rules?, *Journal of Physics D: Applied Physics*, **24:** 111-118.

Matthews, B. W. (1985). Determination of protein molecular weight, hydration, and packing from crystal density, *Methods Enzymol* 114:176-187.

McPherson, A. (1982). Preparation and Analysis of Protein Crystals, John Wiley and Sons.

McPherson, A. (1991). A brief history of protein crystal growth, *J Cryst Growth* 110: 1-10.

McPherson, A. (1998). Crystallization of Biological Macromolecules, Cold Spring Harbor Laboratory Press.

McRee, D. E. (1999). Practical Protein Crystallography,Academic Press.

Parkin, S. and Hope, H. (1998). Macromolecular Cryocrystallography: Cooling, Mounting, Storage and Transportation of Crystals, *Journal of Applied Crystallography* 31:945-953.

Petrucci, R. H., Harwood, W. S. and Herring, F. G. (2002). *General Chemistry: Principles and Modern Applications.*: Pearson/ Prentice Hall, New Jersey.

Ravelli, R. B. G. and Mc Sweeney, S. M. (2000). The fingerprint that X-rays can leave on structures 8:315-328.

Rhode, G. (2000). Crystallography Made Crystal Clear: Academic Press.

Rodgers, D. W. (1997). Practical Cryocrystallography, *Methods Enzymol* 276:183-203.

Stout, G. H.and Jensen., L. H. (1989*).* X-Ray Structure Detemination: A Practical Guide: John Wiley and Sons.

Teng, T. Y. (1990). Mounting of crystals for macromolecular crystallography in a free-standing thin film, *Journal of Applied Crystallography* 23:387-391.

Appendix II

A.2.1 PDB Entry

The crystal structure of *Spatholobus parviflorus* seed lectin was deposited In PDB as

Title: Crystal structure of *Spatholobus parviflorus* seed lectin
PDB ID: 3IPV

A.2.2 Sequence Deposition

Sequences were deposited in UniProt Knowledgebase (UniProtKB) /Swiss-Prot, with accession numbers:

P86352 for Seed lectin alpha chain in *Spatholobus parviflorus*
P86353 for Seed lectin beta chain in *Spatholobus parviflorus*