3D Electron Microscopy Methods and Applications

Structures from Atomic Scale to Mesoscale

Taimin Yang
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Abstract
The crystal structure determines the physical properties of a material. The structure can be analysed at different levels, from atomic level, mesoscale level, all the way up to the macroscale level. Transmission Electron Microscope (TEM) is a powerful tool for studying the structure of materials at atomic scale level and mesoscale level because of the short wavelength of the electrons. At atomic scale level, structure determination using TEM can be performed in diffraction mode. The recent developments in 3D electron diffraction methods make structure determination from nano- and micron-sized crystals much easier than before. However, due to the strong interactions, electrons can be scattered multiple times through the crystal, causing the measured intensities to be less accurate than that in the X-ray case.

In this thesis, we use the continuous rotation electron diffraction (cRED) developed in our group to investigate the structure of materials and the accuracy of this method. In the third chapter, we use cRED method to determine the structure of two aluminophosphate zeolites, PST-13 and PST-14. We presented that these structures can be built from two pairs of enantiomeric structural building units. In the fourth chapter, we show that despite the inaccuracy in measured intensities originated from dynamical effect, it is still possible to determine the structure accurately. We show that the atomic coordinates of ZSM-5 and sucrose crystal structure determined by multiple electron diffraction datasets is identical to that determined from X-ray data or neutron data. We also assessed the linearity between calculated structure factor and observed structure factor and use this as a coarse assessment indicator for diffraction data quality for protein crystals.

Apart from atomic structure, mesoscale structures, such as mesopores, can also determine the property of materials. For the 3D structures of these nanoscale structures, we can also use TEM electron tomography techniques to investigate. In chapter five, we performed electron tomography for two different materials with mesoporous structure and illustrated the formation mechanism of mesoporous magnesium carbonate and the internal tunnel structure of hierarchical TS-1 zeolite.

Keywords: 3D Electron microscopy, continuous rotation electron diffraction, structure determination, data quality, electron tomography, mesoporous materials, zeolites.

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3D ELECTRON MICROSCOPY METHODS AND APPLICATIONS
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Methods and Applications
Structures from Atomic Scale to Mesoscale

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To my family.
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Abstract

The crystal structure determines the chemical and physical properties of a material. The structure can be analysed at different levels, from atomic level, mesoscale level, all the way up to the macroscale level. Transmission Electron Microscopy (TEM) is a powerful tool for studying the structure of materials from atomic to macroscale levels. At atomic scale level, structure determination using TEM can be performed in diffraction mode. The recent developments in 3D electron diffraction methods make structure determination from nano- and micron-sized crystals much easier than before. However, due to the strong interactions, electrons can be scattered multiple times through the crystal, causing the measured intensities to be less accurate than those in the X-ray case.

In this thesis, we use the continuous rotation electron diffraction (cRED) developed in our group to investigate the structure of materials and the accuracy of this method. In the third chapter, we use cRED method to determine the structure of two aluminophosphate zeolites, PST-13 and PST-14. These structures can be built from two pairs of enantiomeric structural building units. In the fourth chapter, we show that despite the inaccuracy in measured intensities originated from dynamical effects, it is still possible to determine the structures accurately. We show that the atomic coordinates of ZSM-5 and sucrose crystal structures determined by multiple cRED datasets are very similar to those determined from X-ray or neutron diffraction data. We also assessed the linearity between calculated structure factors and observed structure factors and use this as a coarse assessment indicator for diffraction data quality for protein crystals.

Apart from atomic structures, structures at meso- and macroscales, such as meso- and macropores, also affect the property of materials. Electron tomography can be used to determine the 3D pore structures. In chapter five, we applied electron tomography to study the 3D pore structures of two types of materials, mesoporous magnesium carbonate (MMC) and hierarchical zeolite ZSM-5. We illustrated the formation mechanism of MMC and the internal pore structure of hierarchical zeolite ZSM-5.

**Keywords:** 3D Electron microscopy, continuous rotation electron diffraction, structure determination, data quality, electron tomography, mesoporous materials, zeolites
List of papers

This thesis is based on the following papers:

Paper I:

Two Aluminophosphate Molecular Sieves Built from Pairs of Enantiomeric Structural Building Units


My contribution: I conducted the experimental TEM work, obtained the initial structure models of PST-13 and PST-14 using cRED data and refined the structure models using cRED data. I made major contributions in writing of the structure determination and refinement part.

Paper II:

On the quality of the continuous rotation electron diffraction data for accurate atomic structure determination of inorganic compounds


My contribution: I conducted the experimental TEM work and collected cRED datasets on different crystals of ZSM-5. I compared the data quality of these cRED datasets as well as the refined structure models. I made major contributions in writing of the structure refinement and structure comparison part.

Paper III:

Electron Crystallography Method for Rapid and Accurate Structure Determination of Small Organic Molecules
Hongyi Xu, Taimin Yang, Steve Waitschat, Andrew Kentaro Inge, Norbert Stock and Xiaodong Zou

In manuscript

**My contribution:** I refined the structure of sucrose and ABTC, and made major contributions in writing of the manuscript.

Paper IV:

**A Rare Lysozyme Crystal Form Solved Using Highly Redundant Multiple Electron Diffraction Datasets from Micron-Sized Crystals**

H. Xu, H. Lebrette, T. Yang, V. Srinivas, S. Hovmöller, M. Hogböm, and X. Zou


**My contribution:** I wrote a python script to compare the experimental structure factors (Fobs) and calculated structure factors (Fcalc) for multiple datasets. I was involved in the writing and discussions of the manuscript.

Paper V:

**Nanostructure and pore size control of template free synthesized mesoporous magnesium carbonate**


**My contribution:** I was responsible for the electron tomography part of the manuscript. I collected electron tomography tilt series on mesoporous magnesium carbonate material and reconstruct the 3D pore structure of this material. I was involved in the writing and discussions of the manuscript.

Paper VI:

**Hierarchical MFI zeolite synthesized via regulating the kinetic of dissolution-recrystallization and their catalytic properties**

J. Lin, T. Yang, C. Lin and J. Sun


**My contribution:** I was responsible for the electron tomography part of the manuscript. I collected electron tomography tilt series data and reconstructed
the 3D pore structure. I was involved in the writing and discussions of the manuscript.

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Papers not included in the thesis:

Paper VII:

**A porous cobalt tetraphosphonate metal-organic framework: accurate structure and guest molecule location determined by continuous rotation electron diffraction**

B. Wang, T. Rhauderwiek, A. K. Inge, H. Xu, T. Yang, Z. Huang, N. Stock and X. Zou


**My contribution:** I refined one of the datasets using DIALS and improved the data refinement result.

Paper VIII:

**Hierarchical Shell-Like ZSM-5 with Tunable Porosity Synthesized by using a Dissolution–Recrystallization Approach**

J. Lin, M. O. Cichocka, F. Peng, T. Yang, and J. Sun


**My contribution:** I helped with the electron tomography data collection and data processing.

Paper IX:

**PST-29: A Missing Member of the RHO family of Embedded Isoreticular Zeolites**


Chem. Mater., 2018, 30 (19), 6619–6623 DOI: 10.1021/acs.chemmater.8b03311

**My contribution:** I collected electron diffraction data for PST-29 and solved and refined the structure.
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# Abbreviation

<table>
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<th>Description</th>
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<tbody>
<tr>
<td>3D</td>
<td>Three dimensional</td>
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<tr>
<td>ED</td>
<td>Electron diffraction</td>
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<td>RED</td>
<td>Rotation Electron Diffraction</td>
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<tr>
<td>cRED</td>
<td>Continuous Rotation Electron Diffraction</td>
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<tr>
<td>TEM</td>
<td>Transmission Electron Microscope</td>
</tr>
<tr>
<td>HRTEM</td>
<td>High resolution transmission electron microscopy</td>
</tr>
<tr>
<td>BF</td>
<td>Bright field</td>
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<tr>
<td>STEM</td>
<td>Scanning Transmission Electron Microscope</td>
</tr>
<tr>
<td>EFTEM</td>
<td>Energy-filtered transmission electron microscopy</td>
</tr>
<tr>
<td>EDS</td>
<td>Energy dispersive spectroscopy</td>
</tr>
<tr>
<td>EELS</td>
<td>Electron energy loss spectroscopy</td>
</tr>
<tr>
<td>SXRD</td>
<td>Single crystal X-ray diffraction</td>
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<td>PXRD</td>
<td>Powder X-ray diffraction</td>
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<tr>
<td>ET</td>
<td>Electron Tomography</td>
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<tr>
<td>MCC</td>
<td>Mesoporous magnesium carbonate</td>
</tr>
<tr>
<td>WBEF</td>
<td>Weak beam dark field</td>
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<tr>
<td>HAADF</td>
<td>High angle annular dark field</td>
</tr>
<tr>
<td>ADF</td>
<td>Annular dark field</td>
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1. Introduction

The properties of a material are determined by its structure. The structure can be studied at different length scales, from atomic to macroscale. At atomic scale, crystalline materials are composed of periodically-arranged atoms, molecules or ions in three dimensions and the properties of the material are determined by how the atoms are arranged. In the 20th century, one of the most important advancements in understanding the periodic arrangement of atoms was brought by William Henry Bragg and William Lawrence Bragg in 1913[1], [2]. They used X-ray as illumination source and obtained single crystal X-ray diffraction patterns from crystals. This is now the most commonly used technique for structure determination of crystalline materials. However, this method typically requires crystals to be larger than $5\mu m \times 5\mu m \times 5\mu m$ in size, because of the weak interaction between X-ray photons and materials. In many cases, the size of synthesized crystals can only reach a few hundred nanometers or even less.

For crystals that are a few hundred nanometers or less in size, electrons are preferred as the source for diffraction experiments since they have much stronger interaction ($10^4$ times higher than X-ray) with atoms in the crystal. The first attempt of using electron diffraction to do structure analysis of crystals was in 1937-1938, by a group of Soviet scientists[3]. They built an electron diffractometer with relatively low accelerating voltages to collect data from different types of crystals, such as metal salts, metal nitrides, metal carbides and clay minerals[3]. The structure analyses of these materials using electron diffraction data were based on the kinematical approximation. Electron diffraction was also used by Cowley for solving a few structures[4], [5]. Cowley and Moddie developed a theoretical approach based on n-beam dynamical diffraction theory in 1957[6]. Because of the strong interaction between incident electrons and crystal, an already diffracted electron has high probability (depending on sample thickness) to be diffracted again. Multiple scattering will alter the measured intensities of reflections. As a consequence, the structure determined from electron diffraction data may be influenced[7]. The theory showed that if there are strong dynamical effects, the intensities of electron diffraction are not related to the square of structure factors in a linear relationship[7].

In diffraction experiments, the structure factor phases are not measured. The missing information is usually obtained by Patterson method, direct methods, charge flipping, molecular replacement or isomorphous replacement. With TEM, it is possible to obtain high resolution transmission electron
microscopy (HRTEM) images. The structure factor phases can be extracted by analysing these HRTEM images. The first attempt to obtain amplitude and phase information from TEM images was done by DeRosier and Klug at the Medical Research Council (MRC) laboratory of Molecular Biology in Cambridge, UK[8]. Later Henderson and Unwin applied this image reconstruction method to obtain the 3D structure of purple membrane from 7Å TEM images and electron diffraction data[9]. This method was also used for interpreting images of inorganic crystals by Klug. Hovmöller’s group developed the method further for solving crystal structures of inorganic materials, such as K₇Nb₁₅W₁₃O₈₀[10], Cs₈Nb₅₄(O,F)₁₄₆[11], Na₃Nb₂O₈₁F[12]. In HRTEM images of crystals, crystallographic structure factor phases are preserved. However, they are affected by contrast transfer function (CTF), beam tilt, crystal tilt and different kinds of aberrations from imperfect electromagnetic lenses. In addition, the signal to noise ratio of HRTEM images is relatively low compared with electron diffraction patterns. Thus, electron diffraction often contains information of the structure to higher resolution. HRTEM imaging requires higher electron dose to obtain sufficient signal-to-noise ratio[13] which makes it challenging to work with beam sensitive materials, such as zeolites, metal-organic frameworks (MOFs) and proteins.

In the 70s, Douglas Dorset, one of the pioneers in electron crystallography showed that it was possible to use electron diffraction alone to determine crystal structures. He applied direct phasing methods for the ab initio structure determination of n-hexatriacontane and 1,2-dipalmitoyl-rac-glycerophosphoethanolamine from 2D electron diffraction patterns [14]. He also demonstrated ab initio structure determination of a wide range of organic compounds, such as alkanes, alkane derivatives and lipids [15]. In the 80s, many inorganic materials, such as β-Ti₂Se, Zr₂Se, Ti₅Se₂ and v-AlCrFe[16], [17], were determined by Hovmöller’s group in Stockholm. In all these cases, electron diffraction data were obtained from a few 2D low-indexed oriented zone-axis patterns. Later, electron diffraction patterns were combined with HRTEM images using maximum entropy and likelihood ranking method to extend HRTEM images to higher resolution[18]. In 2007, Kolb’s group developed 3D electron diffraction method called automated diffraction tomography (ADT)[19], [20]. This method can produce high complete and quasi-kinematical data by coupling precession electron diffraction and goniometer tilt[21]. In 2008, Hovmöller and Zou’s group developed 3D electron diffraction method call rotation electron diffraction (RED)[22]. In this method, diffraction data is collected by combining electron beam tilt at many very small steps, with rotation of the crystal in a few but large steps[23]. The development of these two methods allowed a large number of inorganic structures (more than 100 structures in our lab alone) to be determined from nano-sized and submicron-sized crystals[24].
RED was also shown to be capable of phase identification and structure solution of multi-phasic crystalline powder samples[25]. With the development of direct-electron detection and CMOS technologies, the sensitivity, background and the read-out speed of the camera have been greatly improved[26], [27]. The hybrid detectors made it possible for researchers to collect continuous rotation electron diffraction data from submicro-sized 3D protein crystals[28] at Abraham’s group. Later, they determined structures of two organic pharmaceutical compounds by continuous rotation electron diffraction[26]. Around the same time, 3D electron diffraction methods were applied for micron- and nano-sized protein crystals. This was independently implemented by several groups: Gonen’s group[29]–[32], Yonekura’s group[33]–[35] and Abrahams’ group[36]. In 2016, Gemmi applied continuous rotation electron diffraction (fast electron diffraction tomography) to collect high quality electron diffraction data from inorganic materials [37], [38]. In 2018, Gruene et.al. used single crystal electron diffraction to study nano-sized zeolite silicate-1 and ZSM-5 and found these nano-sized crystals were single crystals and not intergrown[39]. Later they applied this method to small organic compounds and successfully determined the structure of a monoclinic polymorph of paracetamol and a methylene blue derivative[40]. They also applied a method for reducing dynamical electron scattering and revealed the position of hydrogen atoms in two pharmaceutical compounds, IRELOH and EPICZA[41]. All these developments were based on simple kinematical approximation approach without considering the dynamical effects.

In 2015, Palatinus demonstrated the feasibility of a robust structure refinement algorithm based on Bloch-wave theory and were able to account for dynamical effects[42]–[44]. The most significant advancement was the dynamical diffraction theory in precession electron diffraction. The structures refined against electron diffraction data by dynamical refinement achieved R values that are comparable to those refined against X-ray diffraction data. In addition, the differences in interatomic distances between structural models determined from electron and X-ray diffraction data were below 0.06 Å on average. In 2017, Palatinus et al. showed that it was possible to determine hydrogen positions in both organic (paracetamol) and inorganic crystals using dynamical refinement[44]. One of the disadvantages of the method is that the calculation demands increase exponentially with the number of independent atoms in the unit cell. As a consequence, it is not yet possible to use dynamical refinement to refine structures that have more than 50 individual atoms in the asymmetric unit such as complex zeolites, MOFs or covalent organic frameworks (COFs). Currently the dynamical refinement method has been successful on precession electron diffraction tomography data. It is not yet optimized for data collected using the continuously rotation method, which is widely used in both X-ray and electron diffraction.
Another method used for reducing dynamical electron scattering is a general likelihood-based computational approach proposed by Abrahams’ group[41]. This method reduces the adverse effects of dynamical scattering and in turn improves model accuracy. The hydrogen positions in the structure could be revealed after applying this method.

The arrangement of the atoms is not the only factor that determines the properties of a material. For example, zeolites are used as catalysts for fluid catalytic cracking (FCC) and hydro-cracking (HC) in oil refining industry. When zeolites are used in reactions involving bulky molecules, the catalytic activity is limited by the diffusion distance of the molecules in the zeolite crystal. An effective way of improving the diffusion is to produce zeolites with additional mesopores. The mesopores facilitate the diffusion of bulky molecules in both reactants and products[45]–[50]. It was shown that the catalytic performance of TS-1 zeolite crystals with hierarchical mesopores is much better than solid TS-1 zeolite crystals[51]. Both of them have the same framework structure, but for TS-1 zeolite with mesopores in the crystal, the reactant can easily diffuse into the mesopores and participate in the catalytic reactions within the zeolite crystal. Moreover, the shape and size of the crystals are other important factors which affect the catalytic property[45]. To obtain 3D structural information in nanoscale, electron tomography is one of the feasible methods. Tomography is used for revealing hidden structures that are not directly accessible by projected 2D images. The theory of tomography was first established by a mathematician called Johan Radon in 1917[52]. His paper illustrated how to project a high dimensional object into a lower-dimension space, known as Radon transform. Its inverse function lay the mathematical foundation of tomographic techniques. The first use of tomography in electron microscopy was introduced by David DeRosier and Aaron Klug[8], [53], [54]. Later, Hart illustrated that it was possible to improve the signal-to-noise ratio in a projection by averaging re-projected images calculated from a tilt series of micrographs[55]. Based on decades of developments, electron tomography can now be performed in a large variety of imaging and spectroscopy modes, such as bright field (BF), weak beam dark field imaging (WBDF), high angle annular dark field (HAADF), annular dark field (ADF), energy filtered TEM imaging (EFTEM), energy dispersive spectroscopy (EDS) and electron energy loss spectroscopy (EELS)[56]–[67].

The main purpose of this thesis is to apply various 3D electron microscopy methods, from diffraction to imaging, to study different types of crystals, from inorganic and organic compounds to protein. The thesis is organized as follows. The second chapter provides a brief introduction about the key concepts in 3D electron diffraction techniques and 3D electron tomography. In chapter 3, two 3D electron diffraction techniques were used for solving two molecular sieve structures. The two techniques are compared. Their
advantages and disadvantages are discussed. In chapter 4, the precision and accuracy of continuous rotation electron diffraction are analysed in the case of inorganic materials and proteins. In chapter 5, the mesoporous structures of two materials are studied using electron tomography. Their catalytic performance of the materials is improved by introducing the meso-pores in the crystals. In the last chapter, we summarize the results discussed in the thesis and provide future perspectives on the applications and developments of 3D electron microscopy techniques. In conclusion, transmission electron microscopy techniques provide comprehensive and straightforward views of the structure of materials from atomic scale to meso-scale. We hope this thesis can promote 3D TEM methods to researchers and students.
2. Methods

In this chapter, the instruments, theories, methods and software used in electron diffraction and electron tomography are reviewed. Firstly, basic theories of crystallography and TEM are introduced, such as Bragg’s law, atomic scattering factors and structure factors. Secondly, the data collection methods developed in our group, namely RED and cRED, and its data processing procedures are introduced. Thirdly, the basic concepts and theories of electron tomography, as well as the experimental procedures and software used in electron tomography are discussed in detail.

2.1 Electron Crystallography

2.1.1 Basics of Crystallography

A crystal structure in real space can be built by packing unit cells by pure translation. The unit cell parameters describe the dimensions of this repeating unit, which can be represented by six parameters: a, b, c, α, β, γ. Except for translation symmetry, there are other symmetries, such as rotation axis, mirror plane and inversion centre. Based on unit cell parameters and symmetries, crystal can be classified into seven crystal systems. They are triclinic, monoclinic, orthorhombic, tetragonal, trigonal, hexagonal and cubic. When considering lattice centring (primitive, C-centred, body-centred and face-centred), there are 14 Bravais lattices. 230 space groups will be generated by combining all the possible symmetry elements.

2.1.1.1 Bragg’s law

Bragg’s law was proposed by W.H Bragg and W.L. Bragg in 1913, which holds true for X-rays, neutrons and electrons. The law describes that when incident waves are scattered in a perfect crystal, the scattered waves will remain in phase only if the path difference is an integer number of the incident wavelength. In formula, it is:

\[ 2d \sin \theta = n\lambda, \]

Here \( \theta \) means the angle between the incident beam and lattice planes and \( n \) is an integer number. The variable \( d \) is the space between the lattice planes. A series of parallel lattice planes in real space represent a point in reciprocal lattice. As the equation shows, the maximum value that \( \sin \theta \) can take is 1. So the minimum theoretical \( d \) value that a diffraction experiment can resolve is half of the incident wavelength. Since the wavelength of electrons in TEMs is very small, it is an ideal radiation source for diffraction experiment.
Bragg’s law is probably the most important formula in crystallography. However, only through this formula it is not enough to solve the structure from diffraction data. The formula can only identify the possible position where you can find a diffraction spot in the reciprocal space. The law also assumes that the crystal is perfect (no mosaicity, defects and disorder).

2.1.1.2 Atomic scattering factor

Atomic scattering factor is a measure of the scattering power of an isolated atom. It is also called atomic form factor. In crystallography, we normally only consider the elastic interaction between the incident wave and the atom. Inelastically scattered wave is treated as background noise.

Electron scattering factor is the Fourier transform of the electrostatic potential of an isolated atom. For X-ray scattering, atomic form factor is the Fourier transform of the electron density.

The atomic scattering factors for X-rays can be described theoretically using wave functions. They are usually fitted by a sum of multiple Gaussian functions, as shown in the following formula:

\[
f^{(X)} \left( \frac{\sin \theta}{\lambda} \right) = \sum_{i=1}^{N} a_i e^{-b_i \left( \frac{\sin \theta}{\lambda} \right)^2} + c \quad \text{eq. 2-2}
\]

Where \( a_i, b_i, c \) is the coefficients for fitting. \( N \) can be either 4 or 5, depending on how many Gaussian functions are used to fit the scattering factor curve calculated by discrete Fourier transform. In SHELXL, the scattering factor is fitted by four Gaussian functions[68].

Atomic scattering factors for electrons can be calculated from those for X-rays using the Mott-Bethe formula:

\[
f^{(e)} \left( \frac{\sin \theta}{\lambda} \right) = \frac{m_0 e^2}{8 \pi^2 h^2} \left[ Z_0 - f^{(X)} \left( \frac{\sin \theta}{\lambda} \right) \right] \left( \sin \frac{\theta}{\lambda} \right)^2 \quad \text{eq. 2-3}
\]

Where \( Z_0 \) is the atomic number of this atom, \( m_0 \) is the mass for an electron. The formula is valid for an isolated atom, where both the electron density and the atomic potential are spherical symmetric. It assumes elastic interaction between the incident electron and the isolated atom. In reality, electrons can have inelastic interaction with atoms. For a small fraction of the electrons, they can even get back-scattered by the atom. In low resolution range, the Mott formula becomes less accurate because \( Z_0 - f^{(X)} (\sin \theta / \lambda) \) and \( \sin \theta / \lambda \) are both close to zero for neutral atoms. Therefore, this formula is not suitable for calculating \( f^{(e)}(0) \) for electrons. In order to solve this
problem, the following formula was proposed by Ibers[69] to calculate $f(e)(0)$:

$$f(e)(0) = \frac{4\pi^2 me^2}{3\hbar^2} Z\langle r^2 \rangle$$  \hspace{1cm} \text{eq. 2-4}

In this equation, $\langle r^2 \rangle$ is the mean-square radius of the atom.

The electron scattering factor will be greatly affected by the ionicity of the atoms, especially in low resolution range (<0.3Å⁻¹). It means that low resolution electron diffraction data may be used to reveal the charge state of an atom. Doyle and Turner showed that the electron scattering factor for ions can be written as[70]:

$$f(e)\left(\frac{\sin \theta}{\lambda}\right) = \frac{m_0 e^2}{8\pi^2\hbar^2} \left[Z_0 - f(x) \left(\frac{\sin \theta}{\lambda}\right)\right] + \frac{m_0 e^2}{8\pi^2\hbar^2} \frac{\Delta Z}{\left(\frac{\sin \theta}{\lambda}\right)^2}$$ \hspace{1cm} \text{eq. 2-5}

where $\Delta Z = Z - Z_0$ represents the ionic charge. The second term of this formula is the contribution from the unscreened Coulomb potential of the ionic charge.

2.1.1.3 Structure factor

Structure factor, similar to atomic scattering factor, can also be described mathematically with Fourier transform. For X-ray diffraction, it is the Fourier transform of electron density for the whole crystal. For electron diffraction, it is Fourier transform of the electro-static potential for the whole crystal. The unit of the structure factor amplitude depends on the incident radiation sources.

The formula to calculate structure factor is:

$$F(hkl) = \sum_{j=1}^{N} f_j(hkl) \times \exp[2\pi i (hx_j + ky_j + lz_j)]$$ \hspace{1cm} \text{eq. 2-6}

where hkl is the index of a reflection and $x_j$, $y_j$, $z_j$ are the fractional coordinates of atom j in the unit cell and N is the number of atoms in the unit cell. The intensity of the diffraction spot is directly related to the corresponding structure factor. From the formula, we know that $F(000)$ is equal to the sum of all the scattering factors in one unit cell when theta is equal to 0.
2.1.1.4 Darwin’s formula

The intensity of a reflection is not simply the square of the structure factor. There are several other factors influencing the intensity of a diffraction spot. For rotation data, the accurate relationship is written in Darwin’s formula[71]–[73]:

\[
I = I_{\text{beam}} \times r_e^2 \times \frac{V_{\text{xtal}}}{V_{\text{cell}}} \times \frac{\lambda^3 L}{\omega V_{\text{cell}}} \times P \times A \times |F|^2, \quad \text{eq. 2-7}
\]

for X-ray diffraction, \( I \) is the integrated spot intensity (photons/spot), \( I_{\text{beam}} \) is the intensity of the incident beam (photons/s/m²), \( r_e \) is radius of electron (2.818*10⁻¹⁵ m), \( V_{\text{xtal}} \) is the volume of crystal which is illuminated by the incident beam (m³), \( V_{\text{cell}} \) is the volume of the crystal unit cell (m³), \( \lambda \) is the wavelength (m), \( \omega \) is the rotation speed of the crystal (in radius/s), \( L \) is the Lorentz factor (speed/speed), \( P \) is the polarization factor (photons/photons), \( A \) is the X-ray transmittance of the path through the crystal. When data processing software is processing a sweep, one of the most important steps is to define the scaling for each diffraction spot.

For continuous rotation electron diffraction, the same formula can still be applied because the geometry of the data collection is identical to that in X-ray. However several values need to be changed.

### 2.1.2 Rotation Electron Diffraction (RED) and Continuous Rotation Electron Diffraction (cRED)

The RED method combines coarse goniometer tilts (usually around 2° to 3°) with finer electron beam tilt steps (usually 0.1° to 0.4°)[22], [25], as shown in Figure 2-1(a). When sampling the reciprocal lattice, between each fine-step beam tilt, there will still be a small missing wedge. Because of the design of the sample holder and the limited rotation range of the goniometer on TEM, individual dataset is often not complete. RED data can be collected using either selected area electron diffraction (SAED) or nano beam electron diffraction (NED). For a typical RED dataset, more than 1000 diffraction patterns are collected in just over one hour. The data collection software for RED is REDc and data processing software for RED is REDp[22]. In principle, the RED software package can be installed on any types of TEMs, provided it has the appropriate interface to control the deflectors of the TEM, goniometer rotation and the camera. In our lab, RED data is usually collected on a side-entry Gatan Orius SC200D camera installed on a JEOL JEM-2100 LaB₆ TEM operated at 200kV, coupled with a single-tilt tomography holder. In general, RED datasets are collected from crystals with less than 1μm in thickness. Typically, the goniometer tilt step is kept at
2° and beam tilt step is maintained at 0.1°. The overall tilt range is often kept above 100° to achieve high data completeness.

Compared with the RED method, cRED minimizes the small missing wedge between the beam tilt steps, as shown in Figure 2-1(b). During cRED data acquisition, the goniometer is continuously rotated at a constant speed. At the same time, a very fast detector is used for collecting diffraction frames continuously. Thus, a highly sensitive and low dead time camera is essential for this method. The typical collection time of cRED data is less than 5 mins which makes it possible to study electron beam sensitive materials, such as zeolites, MOFs, small organic molecules and proteins [74]–[78]. These samples rapidly transform from crystalline to amorphous under the radiation of the electron beam. Therefore, data collection time must be minimized. Our cRED data collection is performed in a 200kV JEOL JEM-2100 LaB₆ TEM equipped with a hybrid detector (Amsterdam Scientific Instrument, Timepix) dedicated to electron diffraction. The detector has a readout time between as low as 8 ms [28]. The detector is made of 512*512 pixels (55 μm pixel size). The typical exposure time per frame used in cRED data collection is 0.3 – 0.5 s. For room-temperature experiments, a JEOL high-tilt
tomography holder is used, while for cooling experiment, a Gatan high-tilt cryo-transfer holder (Model 914) is used. In order to analyse vacuum sensitive samples, they are cooled down to liquid nitrogen temperature before being inserted into the TEM. From RED to cRED, the data quality has been greatly improved, thanks to the short data collection time and full integration of diffraction intensities. However, a missing cone still exists due to the design of goniometer, sample holder and TEM grid. Since the geometry of cRED data collection is identical to single crystal X-ray diffraction, cRED data is processed using both REDp and sophisticated software developed for X-ray crystallography, such as DIALS[79], MOSFLM[80]–[82] and XDS[83]. Due to dynamical scattering, the space group cannot always be unambiguously determined based on statistics from electron diffraction data. Instead, systematic absences are manually analysed using REDp. X-ray data processing software is then used for unit cell determination, scaling, indexing and reflection intensities integration to produce an HKL file.

2.1.3 Using DIALS for processing cRED Data

As mentioned above, there are mainly three free software packages for data integration and processing in X-ray crystallography. There are also some commercial packages, such as Crysalispro, HKL3000 and d*TREK. Among all these software, DIALS is the most customizable data reduction software. DIALS, short for Diffraction Integration for Advanced Light Sources, is developed by researchers at Diamond Light Source, CCP4 and Lawrence Berkeley National Laboratory. It is a collaborative framework for diffraction data processing, which has been widely used in X-FEL, X-ray and electron crystallography [84]–[87], [79]. DIALS is written in Python and C++, using cctbx package for core crystallographic calculations. Python is one of the most popular programming languages for rapid prototyping and scientific research, because it has many libraries and can be easily understood. In DIALS, the computationally intensive tasks are handled by C++ libraries. With Boost.Python library as the interface between C++ and Python, users can easily call the functions written in C++ using Python. The main purpose of DIALS is to allow the development of a wide range of algorithms within a single framework. The workflow of DIALS can be decomposed into a number of independent processes. The communication between these processes is through data files. This architecture allows the implementation of standalone algorithms based on other data processing software, and the testing of individual units in the DIALS software. The data processing flow chart is presented in Figure 2-2.
There are several parameters in DIALS that are worth adjusting during data processing. The first one is “sigma_m” (m = mosaicity). This parameter represents the mosaicity of the measured crystal. Larger mosaic spread will lead to larger volume of diffraction spot, so that the diffraction spot will appear in more frames and the size of the spot on each frame will become larger. Since the estimate of mosaicity is often convoluted with the intrinsic beam divergence, the parameter sigma_b (b = beam divergence) should also be considered carefully when inputting the value of sigma_m. For electron diffraction data, sometimes the software cannot properly estimate the value of sigma_m and sigma_b. Therefore, it is important to input appropriate values for sigma_m and sigma_b, which will enhance I/sigma significantly. The second parameter that requires user attention is “scan_varying=True”. Dials.refine implements a scan_varying model which refines the diffraction geometry of input experiment against the input indexed reflections, including unit cell parameters, beam intensity, position of central spot and deviation of tilt angles. This model assumes that these parameters change gradually and continuously during data collection. For electron diffraction, the application of this scan varying model can improve the spot prediction and improve the I/sigma and Rmerge for the final integration.

2.1.4 Structure Solution and Refinement

After data integration and obtaining the reflection file in HKL or MTZ format, the next step is to solve the structure factor phase problem to obtain the structure solution. Typically for electron diffraction data, direct method (SHELXS, SHELXT[68], [88], SIR2014[89]) or simulated annealing (SIR2014[89]) is used for solving crystal structures of inorganic or small
organic molecules. The structure model is then refined using SHELXL. During structure refinement against electron diffraction data, one should pay attention to the parameters EXT1 and SWAT, which are used for correcting/weighting the calculated structure factor amplitudes by multiplying $k*[1+0.001*F_c^2*\lambda^3*x/sin(2\theta)]^{1/4}$ and $F_c^2/(1-g*\exp[-8\pi^2U(sin\theta/\lambda)^2])[90]$, respectively. By adding EXT1 or SWAT, the refinement result can generally be improved. On the other hand, for phasing crystal structures of macromolecules, molecular replacement (Phaser) [91] is the dominant method. The structures are refined using CCP4 package or Phenix package. Coot is used for visualizing the electrostatic potential map and difference map[92].

The typical $R_1$ values for refinement against electron diffraction data is ranging from 11% to 30%, which is quite high comparing to those obtained with X-ray data. The most common and widely accepted reason for explaining the high $R_1$ values is dynamical effects or multiple scattering. Until recently, many researchers believed that the structures determined from electron diffraction are not accurate because of the high $R_1$ values.

The second explanation for the high $R_1$ is due to the fact that TEMs are not optimized for continuous rotation experiments. During goniometer tilt, the movement of the crystal out of the selected area/aperture may cause inaccuracy in measured intensities, the sample height change may lead to deviations in unit cell parameters and the misalignment of the crystal with respect to the rotation axis may cause error in the oscillation angles. Furthermore, at high tilt angle, the sample holder may change the magnetic field between the polar pieces, introducing further error.

The third reason that the data processing software is not optimized for electron diffraction. For electron diffraction, the inelastically scattered electrons are mainly distributed at low angle and result in high background in diffraction patterns. In addition, the small crystal size itself is a source of error. It is challenging for the data processing software to perform background and mosaicity estimation. For those crystals with very large unit cells, the total number of unit cells in the whole crystal is limited. On the other hand, when the crystal size is small, the mosaicity of the crystal is expected to be reduced. However experimental results show that the mosaicity of the crystals studied with electron diffraction is often quite high, which indicates that the traditional mosaicity model in X-ray crystallography cannot be applied to electron diffraction directly. In summary, the relatively high $R_1$ value is not only because of dynamical effects. The real situation is more complex for electron diffraction. Electron diffraction methods require further improvements in TEM instrumentation, data collection strategies, theoretical foundations, data processing software and structure determination software.
2.2 Electron Tomography

Computed Tomography is a powerful tool for non-destructive imaging of the interior of an object. This technique can be used in many situations, ranging from X-ray CT scanner to TEM. Electron tomography is an imaging technique to visualize the 3D structure of an object in nano- and meso-scale using electrons as illumination source. Unlike diffraction techniques described above, the information provided by electron tomography is directly in real space. Electron tomography can be performed in different modes for different applications, such as BF-TEM, WBDF, HAADF-STEM, ADF-STEM, BF-STEM, EFTEM, EDS and EELS.

2.2.1 Basics of Tomography

Mathematically, projection is a dimension reduction process. For a two dimensional object, \( f(x, y) \), this process is defined as:

\[
P_\theta(t) = \int_{t_{\theta,t}} f(x, y) \, ds = \iint_{\mathbb{R}^2} f(x, y) \delta(x \cos \theta + y \sin \theta - t) \, dx \, dy, \quad \text{eq. 2-8}
\]

The rays are defined as \( x \cos \theta + y \sin \theta = t \). \( P_\theta(t) \) is the 2D projection of the 3D object. The Radon transform operation converts the data into ‘Radon space’ \((l, \theta)\). A single projection, \( P_\theta(t) \), is a discrete sampling of the Radon transform. Tomography is a two-stage process involving acquisition of images and combining those acquired images to reconstruct 3D information. Here Figure 2-3 is a schematic diagram to show the process.

For tomography data collection in our lab, we use a JEOL JEM-2100F FEG microscope equipped with a Gatan ultrascan. We typically collect tilt series in bright field mode since the material used in our experiment has limited ability to diffraction, which minimizes the diffraction contrast. The typical total tilt angle is 120° to 130° with a tilt step of 1°, while the typical exposure per image is between 1 to 2 s. A small objective aperture is used to filter the diffracted reflections at back focal plane to further reduce diffraction contrast. The processing software we use to align and reconstruct the tilt series is the ETomo module in IMOD software[93]–[95].
During the acquisition of a tilt series, projected TEM images are acquired in a step-wise fashion with an interval of a few degrees. As long as the angle interval is small enough (around 1°), the missing wedge between the frames can be neglected. In electron tomography, the biggest problem is the existence of so-called “missing cone”, which can be as large as 60 degrees. This means around one third of the information was lost for the reconstruction. The most severe artefact produced by the missing wedge is the elongation in the direction parallel to the electron beam. There are some iterative algorithms that can reduce the artefacts by applying boundary conditions or optimizing constrains during the reconstruction. Furthermore, the missing cone also greatly reduces the resolution of the reconstruction.

After the acquisition of tilt series, the individual image of the whole tilt series should be aligned with respect to each other. This step can severely affect the resolution of the reconstruction. Each image must be shifted onto a common tilt axis, requiring both translation operation and rotation operation of the images. The most common method is cross-correlation. This method is very simple and does not need the pre-treatment of the sample grid. Although cross-correlation method is effective in correcting for translational movement, manual intervention is still required to compensate for distortions, such as rotational misalignment, magnification change or other distortions. Better alignment results can be achieved by applying gold nanoparticles (<5nm) on the TEM grid as fiducial markers, which makes it
possible to correct for rotation axis variation, magnification variation and other distortion of the image during rotation of the goniometer.

After alignment of the images, 3D reconstruction can be performed. The most common method is Filtered Back Projection (FBP) or simultaneous iterative reconstruction technique (SIRT). The process of FBP is shown in Figure 2-3. With the increasing number of back projected rays, the shape of the sample will be shown in the area where it has the most overlapped back projected rays. FBP is an analytic solution and is based on the following formula:

$$f(x, y) = \int_0^\pi \left\{ \int_{-\infty}^{\infty} P_\theta(q) |q| e^{2\pi i q(x \cos \theta + y \sin \theta)} dq \right\} d\theta, \quad \text{eq. 2-9}$$

Here, a coordinate transformation from Cartesian coordinate system to polar coordinate system is required. As a consequence, when calculating numerically, errors will be produced and it is detrimental to the reconstruction quality. Miao developed equally sloped tomography method to overcome this problem[96]. By combining pseudo-polar fast Fourier transform and acquiring the tilt series at equal slope increments, the reconstruction can be improved.

SIRT is an algebraic reconstruction method. In SIRT, the object is modelled by a discrete representation of the object function. The projection operation is modelled as a projection matrix $W$, where $W = (w_{ij}) \in \mathbb{R}^{M \times N}$ is a sparse matrix that collects all coefficients related to the projection operation. The problem of tomography becomes solving the system of linear equation $W \bar{x} = \bar{p}$. The problem looks quite simple but the projection matrix is usually ill-conditioned and incomplete. Noise and discretization error will impose great error in the linear system, which makes the linear system inconsistent. As a consequence, finding the analytical solution for this equation becomes infeasible. Therefore, algebraic methods usually find the solution $\bar{x}$ which can minimize the projection distance $\|W \bar{x} - \bar{p}\|$ using different kinds of norm. The SIRT minimize the L_2-norm of this projection distance.

$$x^* = \arg \min \left( \|W \bar{x} - \bar{p}\|_2^2 \right), \quad \text{eq. 2-10}$$

This minimization problem can be calculated using an iterative approach:

$$x^{(k+1)} = x^k + CW^T R (p - W x^{(k)}), \quad \text{eq. 2-11}$$

where $C$ is defined as the diagonal matrix with the inverse column sums of $W$ and $R$ is defined as the diagonal matrix with the inverse row sums of $W$. 

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2.2.3 Problems in Electron Tomography

The first problem is the existence of the missing cone, which has a long history. With the current TEM design, the sample cannot be tilted by ±90°. Various techniques have been proposed and implemented to alleviate the elongation effect brought by the missing cone, such as dual-axis tilting [97], unique design of the holder geometry [98] and application of prior knowledge during reconstruction [58], [59], [64], [99]. For example, the iterative algorithm, such as SIRT, provides access to combine the iterative process with additional operations during the iterative process, such as discretization and image segmentation. These additional operations can alleviate the elongation effect of missing wedge and reduce the number of required images as prior knowledge is applied in the reconstruction process.

The second limitation is radiation damage. The useful signal is produced by the electron-specimen interaction. However, this interaction will damage the material. There are several modes of radiation damage. The first one is radiolysis from inelastic scattering of electrons. In this case, electrons interact with atoms inelastically by transferring kinetic energies to the atoms. The next one is knock-on damage and sputtering. High energy electrons can introduce displacement of atoms or point defects by knocking out atoms in the crystal lattice. The third mode of radiation damage is heating effect from phonons. When electrons pass through the material, atoms can be perturbed by the electrons so that phonons are created. The last possible cause is contamination from the decomposition of adsorbed hydrocarbon molecules.
3. Structure determination of PST-13 and PST-14 (Paper I)

PST-13 and PST-14 are aluminophosphate molecular sieves synthesized in the Centre for Ordered Nanoporous Materials Synthesis at Pohang University of Science and Technology. PST-13 has quite narrow synthesis window, while PST-14 is the calcined product of PST-13[75]. Both PST-13 and PST-14 crystals are thin rectangular plates with non-uniform width and length, and thicknesses of less than 100 nm.

In this chapter, I will first present the determination of PST-13 and PST-14 structures by step-wise RED method[22]. Then I will discuss about cRED data collection on these crystals[74] using a cryo-holder to cool down the sample in the TEM at 100K. The differences between the structure determination results by these two different protocols are presented and discussed.

3.1 Structure solution of PST-13 and PST-14 using RED data

Initially, RED data was collected under ambient temperature for PST-13 and PST-14. The crystals were crushed and dispersed in ethanol. After ultrasonication, a droplet of the suspension was taken and put on a TEM copper grid. After the liquid had evaporated, the grid was loaded onto the tomography holder, which allows a maximum tilt of $\pm 75^\circ$. For RED data collection, the average tilt range was above $120^\circ$ with goniometer rotation steps of $2.0^\circ$ and a fine beam tilt step of $0.40^\circ$. The exposure time was 2 seconds per frame. Data collection time for individual datasets was around an hour.

Figure 3-1 shows the ($h0l$) slice from the reconstructed reciprocal lattice of PST-14. There are severe streaks along the c-axis. However, the ($hk0$) plane shows sharp diffraction spots. It means there is no disorder along the a*- and b*- directions, the structure is severely disordered only along the c*-direction. It is also possible that electron radiation or vacuum has created disorders in the structure. First, the average structure of PST-14 was solved by ignoring the streaks between diffraction spots. The unit cell parameters of the averaged structure according to REDp are $a = 14.3$ Å, $b = 14.4$ Å, $c = 18.1$ Å, $\alpha = 90.7^\circ$, $\beta = 90.5^\circ$, $\gamma = 90.9^\circ$. PST-14 is tetragonal with the space group $P 4 2_{1} c$, determined from the systematic absences of reflections observed in the reciprocal lattice reconstructed from the RED data. After structure solution and refinement using SHELXT and SHELXL, an average
structure is obtained. The final $R_1$ value is 0.199 for reflections up to at 1.5 Å resolution. The connections between the T atoms are reasonable and AlO$_6$ and PO$_4$ can be arranged alternatively. This indicates that the framework and space group determination is correct. In the averaged structure, no six-coordinated Al atoms can be found. From TG-DTA analysis, PST-14 can easily lose water. When the sample was heated to 100°C, the sample lost as much water as 15% of its weight. It indicates the PST-14 has superior water absorption capability.

![Figure 3-1](image)

Figure 3-1. a) $^{27}$Al NMR spectrum of PST-13 and PST-14 in hydrated and dehydrated states. b,d) Morphology of a PST-14 crystal (b) and selected area electron diffraction pattern taken along the c-axis (d). c) $(h0l)$ slice cut from the reconstructed 3D reciprocal lattice of PST-14.

Figure 3-2 shows the reconstructed 3D reciprocal lattice of PST-13. We note that the resolution of the reconstructed RED data of PST-13 decays over time, indicating there is severe radiation damage during data collection. Unlike PST-14, there are fewer streaks in the reciprocal lattice of PST-13, suggesting the structure has less disorder.

The unit cell parameters of PST-13 determined from REDp are $a = 14.4$ Å, $b = 14.5$ Å, $c = 18.7$ Å, $\alpha = 90.348^\circ$, $\beta = 89.831^\circ$, $\gamma = 90.734^\circ$. PST-13 is tetragonal with the space group $P42_1c$. After structure solution and refinement using SHELXT and SHELXL, the obtained structure is similar to the framework of PST-14. Once more, there are only four-connected Al atoms in the structure.
3.2 Structure determination of PST-13 and PST-14 using cRED data under cryogenic temperature

Based on the RED experiments, we suspect that disorders in PST-13 and PST-14 crystals are created when they are dehydrated in the vacuum of the TEM. Therefore, it may be necessary to use a cryo-transfer holder to cool the sample to liquid nitrogen temperature and stabilize the water molecules in the pores before loading the sample into the TEM. After this, cRED was used to collect data for PST-13 and PST-14 crystals. The average tilting range of individual datasets was between 105° and 120°. The rotation speed was kept at 0.45°/s. The data collection time for each dataset was only a few minutes, minimizing the radiation damage.

The datasets were processed by DIALS software. DIALS uses a generalized linear model to estimate the background, a profile fitting method for full reflection integration, and a summation method for treating partial reflections. After data integration and scaling, a list of \( hkl \) intensities will be generated, which are used for structure solution and refinement.

Figure 3-3 shows the structure of PST-13. In the structure solved by cRED under liquid nitrogen temperature, we can find Al-O-Al bridges in the structure and some five-coordinated Al atoms which are colored in green in Figure 3-3. These atoms are located in the diagonal of the double four-ring \((d4r)\) building units and connected to neighboring Al atoms. For the structure of PST-14, we can also find octa-coordinated Al atoms in the structure, as shown in Figure 3-4. These atoms are coordinated with two water molecules that are located in the channel. It explains the exceptional water absorption property of this material. When the sample is dehydrated, the six-coordinated Al atoms become four-coordinated. Meanwhile, disorders are created in the structure. The structure of PST-13 contains a 3D...
pore system, which consists of straight (4.5 × 3.9Å) and wavy (4.5 × 3.1Å) 8 ring channels that intersect with each other, which forms layer A (Figure 3-5). Layer B is transformed from layer A by rotating it 90° around the c-axis. Perpendicular to the c-axis, there are 8-ring apertures (3.3 × 3.3Å) in body-centered tetragonal arrangement.

Upon calcination, the Al-O-Al bridges break down and the edge-sharing 3- and 5-ring are transformed to 6-ring, producing PST-14. The transformation of the structure is similar to that from AlPO₄-53(A) to AlPO₄-53(B). PST-14 also has straight (4.5 × 4.2Å) and wavy (4.5 × 3.5Å) 8-ring channels. The stacking arrangement of the intersecting straight and wavy channel is the same with PST-13. Furthermore, PST-14 has eight distinct T atoms and a framework density of 16.9/1000Å³.

Figure 3-3. The Structure of PST-13 (The location of penta-coordinated Al atom in the framework of PST-13 highlighted in green)

Figure 3-4. The structure of PST-14 (The location of 6-coordinated Al atom in the framework of PST-14 highlighted in blue)
In the structure of PST-14, there are two types of secondary building units, which are 1,5-open d4r units and 1,6-open d4r units, as shown in Figure 3-6. These new secondary building units, denoted as por-1 and por-2, are mirror images of each other and they are non-superimposable. Two enantiomeric por-1 and por-2 are alternatively connected to one another, along $a$-axis and $b$-axis. Then one 1D chain is connected to its mirror 1D chain, forming four and six rings and d4r between two neighboring chains. The 3D structure is formed by stacking 2D layers in the sequence of AA $\uparrow+90^\circ$ AA $\uparrow+90^\circ$ ..., here $t$ means translation operation by 0.5($a+b$) and 90° means rotation operation around $c$-axis for 90°.

![Figure 3-5](image1.png)

Figure 3-5. The tunnel structure of PST-13 and PST-14 viewed from $b$-direction and $c$-direction. Reproduced by permission of Wiley-VCH.

![Figure 3-6](image2.png)

Figure 3-6. a) The tetrahedral [4$^2$6$^2$] por-1 (left; 1,5-open d4r) and por-2 (right; 1,6-open d4r) units coloured in navy and orange, respectively. b) The building chain (left) and layer (right) comprised of por-1 and por-2 units with a strict alternation. Two adjacent building chains are related to each other by mirror symmetry. The bonds interconnecting all por-1 and por-2 units are indicated by black dashed lines. Reproduced by permission of Wiley-VCH.
3.3 Conclusions

The structures of two new aluminophosphate molecular sieves were solved by RED at ambient condition and cRED with sample cooling technique, respectively. By combining cRED and cooling, we were able to determine the correct structures of PST-13 and PST-14, which are consistent with NMR experiments. The structure determination results clearly show the great advantages of cRED and sample cooling for beam sensitive materials. The electron dose applied to the material can be very low, leading to improved accuracy and precision. For porous material that can easily endure structure transformation in vacuum condition, it is advisable to cool down the sample to below -70°C before inserting the sample into the microscope. Cooling can stabilize the water or organic molecules in the pore, thus keeping the original structure intact.
4. Assessment of precision and accuracy of continuous rotation electron diffraction data (Paper II, III and IV)

For the structure determination software in small molecule X-ray crystallography, such as SHELX and SIR2014, kinematical approximation is widely applied. For X-ray diffraction, it is a proper assumption. However, for electron diffraction, because of dynamical effects, the refinement quality indicator used in X-ray crystallography will usually reach an unacceptable high level (usually >20%). As mentioned in the introduction, dynamical effects is an important factor in determining the quality of electron diffraction data. As a result, many researchers have been arguing that electron diffraction is an unreliable technique for structure determination. In order to validate the structure models determined from 3D ED data, the refinement result from powder X-ray diffraction (PXRD) is usually required as supporting evidence. However, structure refinement against PXRD data is often challenging for complex structures, multiphase samples or disordered structures.

Recently, a dynamical refinement method has been developed for taking dynamical scattering into consideration when performing structure refinement against 3D ED data. The method is implemented in software called “Jana”. By using this method, the accuracy of the structure model can be improved, and the R value can be comparable to single crystal X-ray diffraction data (SCXRD). In a recent work, researchers show that hydrogen positions can be located by using dynamical refinement on 3D precession ED data. Although the result of this method sheds new light on electron crystallography, the amount of calculation increases exponentially with the complexity of the structures. Furthermore, precession data collection speed is not as fast as cRED meaning it may not be suitable for beam sensitive materials.

For protein X-ray crystallography, the tolerance for R value is much larger than small molecule X-ray crystallography. Typically, R-values larger than 20% are acceptable. Since the crystal structures of protein are rather flexible, the resolution of most protein data cannot easily reach below 0.9 Å, even using synchrotron X-ray as radiation source.

In this chapter, we evaluated the deviation in atomic coordinates between structures refined against cRED data and those refined against X-ray diffraction data. Firstly, we used a zeolite, ZSM-5, to conduct the comparisons. The structure of ZSM-5 was solved 30 years ago by single crystal X-ray crystallography [100]. The X-ray model of ZSM-5 is used as the reference model[101]. We then calculate the differences in atomic coordinates between the crystal structures determined from cRED datasets.
and the reference model. Secondly, we evaluate the accuracy of the crystal structure of sucrose determined by cRED against that determined from single crystal X-ray diffraction data, as well as that from neutron diffraction data[102]. Thirdly, we analyse the correlation between the calculated structure factor amplitude (F_{calc}) and the observed structure factor amplitude (F_{obs}) of the lysozyme crystal structure solved using electron diffraction data.

### 4.1 Assessment of cRED data quality of inorganic materials

The inorganic material that we choose as an example is as-made ZSM-5 (Pnma, a = 20.022 Å, b = 19.899 Å, c = 13.383 Å determined by SCXRD, see the International Zeolite Association (IZA) Database). There are 38 symmetry-independent atoms in the unit cell, of which 12 are Si atoms and 26 are O atoms. There are four O atoms located at special positions. A total of four datasets were collected using cRED from crystals of size ranging from 600 to 1400 nm, as shown in Figure 4-1. The total tilt range of the crystals was between 90° and 114°.

The cRED datasets were processed using DIALS. After using dials.find_spots to search for diffraction spots on individual diffraction

![Figure 4-1. TEM images of the four as-made ZSM-5 crystals used for cRED data collection. Crystals in (a)–(d) correspond to data sets 1–4, respectively. Reproduced by permission of IUCR](image-url)
frames, dials.index was used for the determination of the unit cell. Next, the unit-cell parameters were refined against each frame to optimize the geometry model, which minimizes the deviation between predicted spots and spots in the frames. The integration of the diffraction intensities was performed by dials.integrate. The results were further processed by aimless for data scaling and correction. The data reduction statistics for these four datasets are shown in Table 4-1.

Table 4-1. Data reduction statistics for the four cRED datasets for as-made ZSM-5 using DIALS. $R_{\text{merge}}$, $R_{\text{p.i.m}}$ and $CC_{1/2}$ are calculated as defined by Diederichs & Karplus (1997), Weiss (2001) and Karplus & Diederichs (2012).

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<td>19816/3879</td>
<td>20710/3597</td>
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</tbody>
</table>

The ZSM-5 crystals diffracted to between 0.831Å and 0.856Å for all four datasets. During the integration of reflections, the resolution was cut to 0.850 Å based on the data statistics. The average unit-cell parameters obtained from the four datasets are $a=20.23(5)$Å, $b=19.95(3)$Å, $c=13.43(3)$ Å, which differs by approximately 0.5% from the unit cell parameters obtained from SCXRD ($a = 20.022(4)$ Å, $b = 19.899(4)$ Å, $c = 13.383(3)$ Å). As shown in Table 4-2, the total number of reflections range from 17633 (dataset 1) to 22901 (dataset 4) and the number of unique reflections range from 3542 (data set 1) to 4565 (data set 4). The $R_{\text{merge}}$ values range from 0.191 (dataset 2) to 0.264 (dataset 4).
The structure of ZSM-5 was solved using SHELXT. All of the framework atoms Si/Al and O were located directly and assigned correctly using any of the four datasets. Since in ZSM-5, the Si/Al ratio is high (greater than 3), and the form factors of Si and Al are quite similar and these atoms share the

Table 4-2. Continuous RED experimental parameters and crystallographic details of the refinement from four datasets of as-made ZSM-5. The unit-cell parameters were obtained from dials.refine. For comparison, the same unit cell ($a = 20.022\,\text{Å}$, $b = 19.899\,\text{Å}$, $c = 13.383\,\text{Å}$ from SCXRD) and the same data resolution ($d = 0.870\,\text{Å}$) were used in all structure refinements.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of frames</td>
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<td>430</td>
<td>445</td>
<td>496</td>
</tr>
<tr>
<td>Tilt range (°)</td>
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<td>98.9</td>
<td>102.4</td>
<td>114.1</td>
</tr>
<tr>
<td>Frame width (°/frame)</td>
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<td>0.23</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>Tilt speed (°/s)</td>
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<td>0.45</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>$a$ (Å)</td>
<td>20.398(9)</td>
<td>20.284(6)</td>
<td>19.929(15)</td>
<td>20.305(19)</td>
</tr>
<tr>
<td>$b$ (Å)</td>
<td>19.922(9)</td>
<td>19.903(6)</td>
<td>19.846(15)</td>
<td>20.120(20)</td>
</tr>
<tr>
<td>$c$ (Å)</td>
<td>13.383(6)</td>
<td>13.384(4)</td>
<td>13.459(10)</td>
<td>13.490(14)</td>
</tr>
<tr>
<td>$\alpha$ (°)</td>
<td>89.708(8)</td>
<td>89.913(3)</td>
<td>89.637(8)</td>
<td>90.141(8)</td>
</tr>
<tr>
<td>$\beta$ (°)</td>
<td>89.461(7)</td>
<td>89.331(3)</td>
<td>88.558(5)</td>
<td>89.975(17)</td>
</tr>
<tr>
<td>$\gamma$ (°)</td>
<td>89.027(9)</td>
<td>89.023(3)</td>
<td>89.906(5)</td>
<td>89.563(14)</td>
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<tr>
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<td>0.87</td>
<td>0.87</td>
<td>0.87</td>
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<tr>
<td>Total/unique reflections</td>
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<td>17564/3390</td>
<td>17621/2925</td>
<td>19842/3795</td>
</tr>
<tr>
<td>Observed reflections ($F_o&gt;4\sigma(F_o)$)</td>
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<td>2082</td>
<td>2172</td>
<td>2402</td>
</tr>
<tr>
<td>Completeness</td>
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<td>0.773</td>
<td>0.667</td>
<td>0.865</td>
</tr>
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<td>$R_{int}$</td>
<td>0.2321</td>
<td>0.1909</td>
<td>0.2055</td>
<td>0.2649</td>
</tr>
<tr>
<td>$R_1$ ($F_o&gt;4\sigma(F_o)$)</td>
<td>0.1953</td>
<td>0.1965</td>
<td>0.2327</td>
<td>0.2648</td>
</tr>
<tr>
<td>$R_1$ (all)</td>
<td>0.2400</td>
<td>0.2321</td>
<td>0.2515</td>
<td>0.2867</td>
</tr>
<tr>
<td>Goodness-of-fit on $F^2$</td>
<td>1.132</td>
<td>1.131</td>
<td>1.177</td>
<td>1.121</td>
</tr>
<tr>
<td>ADRA (Å)</td>
<td>0.07(3)</td>
<td>0.05(3)</td>
<td>0.08(4)</td>
<td>0.08(4)</td>
</tr>
</tbody>
</table>
same position, so it is very hard to distinguish between Si and Al atoms. In order to make the comparisons with model obtained by SCXRD, the space group and unit cell parameters obtained from the SCXRD data were used for the refinement of all cRED datasets. The structure solution results from cRED data are used as the starting model for refinement. We note that the final atomic coordinates refined against the same hkl file, but from different starting models were identical. In other words, the refinement converges to the same result for the same dataset, despite slight differences in the starting model. The structure refinement details and the average deviations between the atomic coordinates and the reference X-ray structure are summarized in Table 4-2.

In the refinement of ZSM-5, the resolution is limited to 0.870Å for all four datasets. The number of refined parameters is 150. The numbers of reflections included in the refinement process range from 2925 to 3795 for these four datasets. We note that the refinement of ZSM-5 does not give negative atomic displacement parameters (ADPs). However, some of the Si-O distances in the refined structure model are not reasonable. Therefore, 48 DFIX restraints are applied to make Si-O bond within the reasonable distance range (1.61Å). In addition, for electron diffraction data, SHELXL sometimes has problems in estimating the overall scale factor. By using the extinction correction parameter (EXTI parameter in SHELXL) and an appropriate weighting factor (WGHT parameter in SHELXL), we were able to greatly improve the ADPs. The $R_1$ values also improved significantly, and ranged from 0.2321 to 0.2867 for the four datasets.

For structure model evaluation, we followed Palatinus’s evaluation method and calculated the average deviation from reference atoms (ADRA), which is the arithmetic mean of atomic coordinate deviation. In Table 4-3, the deviations of atomic positions for all symmetry-independent atoms and ADRA are listed. The average deviations from the reference model (ADRAs) are 0.07(3) Å, 0.05(3) Å, 0.08(4) Å and 0.08(4) Å for data sets 1, 2, 3 and 4, respectively (Table 4-3). The average Si-O bond distance is 1.59(1) Å (minimum 1.573 Å and maximum 1.636 Å), the average O-Si-O angle is 109(3)° (minimum 101.6° and maximum 118.7°) and the Si-O-Si angles range between 142° and 175° for all four structural models. These values are consistent with those published in the literature. The ADRA values suggest that despite high $R_1$ values, the final refined structures are accurate and reliable. In Figure 4-2, we superimpose the structural models refined against cRED datasets with the reference SCXRD model in order to visually compare their differences in atomic positions. From the figure, we can observe that most of the silicon atoms have almost perfect overlap. For those oxygen atoms with large deviations from the reference model, their positions refined against the different cRED datasets are very consistent and the deviations are quite small. However, the average atomic positions obtained
from the four models deviate from those of the reference model. This could be due to the different synthesis conditions as well as different data collection conditions of cRED and SCXRD, which are in vacuum and in air, respectively. Performing the measurements under vacuum with an electron beam may cause partial removal or disorder of the guest molecules in the pores, which could result in slight structural changes.

Figure 4-2. Comparison between the four refined structure models of as-made ZSM-5 from the four different datasets and the reference structure model. The structures are stacked together, viewed along (a) [001] and (b) [010]. The Si and O atoms in the reference ZSM-5 structure are shown in green and purple while those in the refined structure models from the experimental data in blue and red, respectively. A 10-ring window in the structure is shown in (c) and the magnified part. Reproduced by the permission of IUCr
Table 4-3. The atomic position deviations between the refined as-made ZSM-5 structure models (1-4) and the reference model from IZA database.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si1 (Å)</td>
<td>0.0312</td>
<td>0.0240</td>
<td>0.0449</td>
<td>0.0726</td>
<td>0.043(21)</td>
</tr>
<tr>
<td>Si2 (Å)</td>
<td>0.0683</td>
<td>0.0477</td>
<td>0.0604</td>
<td>0.0763</td>
<td>0.063(12)</td>
</tr>
<tr>
<td>Si3 (Å)</td>
<td>0.0663</td>
<td>0.0564</td>
<td>0.0619</td>
<td>0.0539</td>
<td>0.060(6)</td>
</tr>
<tr>
<td>Si4 (Å)</td>
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<td>0.0331</td>
<td>0.0428</td>
<td>0.0364</td>
<td>0.043(11)</td>
</tr>
<tr>
<td>Si5 (Å)</td>
<td>0.0540</td>
<td>0.0435</td>
<td>0.0701</td>
<td>0.0601</td>
<td>0.057(11)</td>
</tr>
<tr>
<td>Si6 (Å)</td>
<td>0.0710</td>
<td>0.0363</td>
<td>0.0852</td>
<td>0.0441</td>
<td>0.059(23)</td>
</tr>
<tr>
<td>Si7 (Å)</td>
<td>0.0556</td>
<td>0.0385</td>
<td>0.0837</td>
<td>0.0436</td>
<td>0.055(20)</td>
</tr>
<tr>
<td>Si8 (Å)</td>
<td>0.0746</td>
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<td>0.039(12)</td>
</tr>
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<td>Si10 (Å)</td>
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<td>0.0222</td>
<td>0.0450</td>
<td>0.0190</td>
<td>0.029(12)</td>
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<td>Si11 (Å)</td>
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<td>0.0257</td>
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<td>0.0244</td>
<td>0.019(10)</td>
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<tr>
<td>Si12 (Å)</td>
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<td>O1 (Å)</td>
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<td>O3 (Å)</td>
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<td>0.0508</td>
<td>0.1116</td>
<td>0.0957</td>
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<tr>
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</tr>
<tr>
<td>O8 (Å)</td>
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<td>0.0249</td>
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<td>0.0884</td>
<td>0.064(31)</td>
</tr>
<tr>
<td>O9 (Å)</td>
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<tr>
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<td>0.095(13)</td>
</tr>
<tr>
<td>O11 (Å)</td>
<td>0.0740</td>
<td>0.0428</td>
<td>0.0398</td>
<td>0.0820</td>
<td>0.060(21)</td>
</tr>
<tr>
<td>O12 (Å)</td>
<td>0.1313</td>
<td>0.1401</td>
<td>0.1389</td>
<td>0.2076</td>
<td>0.154(36)</td>
</tr>
<tr>
<td>O13 (Å)</td>
<td>0.1625</td>
<td>0.1047</td>
<td>0.1447</td>
<td>0.2067</td>
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</tr>
<tr>
<td>O14 (Å)</td>
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<td>0.0691</td>
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</tr>
<tr>
<td>O15 (Å)</td>
<td>0.0230</td>
<td>0.0110</td>
<td>0.0533</td>
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<td>0.052(49)</td>
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<td>0.0292</td>
<td>0.0500</td>
<td>0.1073</td>
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<tr>
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<td>0.0946</td>
<td>0.0335</td>
<td>0.055(31)</td>
</tr>
<tr>
<td>O18 (Å)</td>
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<td>0.0500</td>
<td>0.0771</td>
<td>0.1137</td>
<td>0.081(26)</td>
</tr>
<tr>
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<td>0.0963</td>
<td>0.082(19)</td>
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<td>0.1080</td>
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<td>0.1023</td>
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<td>0.0665</td>
<td>0.0398</td>
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<tr>
<td>ARDA (Å)</td>
<td>0.07(3)</td>
<td>0.05(3)</td>
<td>0.08(4)</td>
<td>0.08(4)</td>
<td>0.07(3)</td>
</tr>
<tr>
<td>$R_1(F_o&gt;4\sigma(F_o))$</td>
<td>0.1953</td>
<td>0.1965</td>
<td>0.2327</td>
<td>0.2648</td>
<td>0.2223</td>
</tr>
<tr>
<td>$R_1$(all)</td>
<td>0.2400</td>
<td>0.2321</td>
<td>0.2515</td>
<td>0.2867</td>
<td>0.2526</td>
</tr>
</tbody>
</table>
By comparing ADRA values with the $R_1$ values from the final refinement results, we found that there is no strong correlation between $R_1$ values and ADRA values. Lower $R_1$ value does not necessarily lead to lower ADRA value (better model). Higher $R_1$ value does not necessarily lead to higher ADRA value (worse model). Therefore, when evaluating the accuracy of the structure model obtained from electron diffraction data, ADRA should be considered. The $R_1$ value should not be sorely used to evaluating the quality of the structure model.

4.2 Assessment of cRED data quality of small organic molecule crystals

Sucrose is chosen as example for assessing the quality of cRED data of organic crystals and the structure model refined against cRED data. Sucrose crystals were obtained from sugar cubes used for coffee and tea. Several small grains of the sugar cubes were crushed in mortar and dispersed on a conventional TEM copper grid. The TEM grid was then loaded on a TEM tomography holder and inserted into the microscope for cRED data collection. The crystals were continuously rotated at a constant speed of 1.13°/s. By using an exposure time of 0.3s/frame, approximately 190 frames were collected in each dataset covering a rotation range of around 60° on average. The data collection time for each crystal was less than 1min, while the accumulated electron dose was estimated to be less than 5 e⁻/Å². In order to improve the completeness and redundancy, a total of 17 datasets were processed and merged using XDS and XSCALE[103]. The highest resolution of these cRED data reached beyond 0.80 Å, as shown in Figure 4-3. After the structure was solved using SHELXT, the structure was then refined with SHELXL. A total of 244 parameters were refined using only five restraints. The final $R_1$ value is 0.1438 for $F_o > 4\sigma(F_o)$ and 0.1666 for all 3203 reflections, as shown in Table 4-4. The atomic coordinates of the refined sucrose structure are compared with those of a structure determined by neutron diffraction and deposited in the CCDC organic structure database[102]. One reason for selecting this structure as the reference is that neutron diffraction can determine the position of hydrogen atoms reliably. It allows us to investigate the feasibility of refining hydrogen atom positions using electron diffraction data.

In Figure 4-3, we show the existence of intramolecular hydrogen bonding. Most of the hydrogen atoms are located at reasonable positions. However, a number of hydrogen atoms have exceptionally large ADPs. These hydrogen atoms are located on functional groups without forming hydrogen bonds with other atoms. These hydrogen atoms therefore can rotate freely. The positions of hydrogen bonded hydrogen atoms are more stable. Their ADPs
are also quite reasonable. We are also able to observe the hydrogen bonding network in the sucrose crystal, as shown in Figure 4-4. These hydrogen bond lengths are quite reasonable, ranging from 2.69 Å to 2.94 Å. Moreover, the average deviations from the reference model (ADRA$s$) are 0.05(2) Å for C and O atoms (Table 4-5). The average bond lengths after refinement are 1.51(3) Å for C-C bonds and 1.40(2) Å for C-O bonds (Table 4-6). All these averaged bond lengths are quite accurate.

Figure 4-3. (a) A typical electron diffraction pattern of sucrose crystal with a typical sucrose microcrystal (as an inset). (b) Asymmetric unit of the sucrose structure model refined against the merged cRED data.

Figure 4-4. Details of the sucrose structure. The hydrogen bonding network is highlighted in blue.
Table 4-4. Data statistics and refinement statistics for sucrose.

<table>
<thead>
<tr>
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<th>Sucrose</th>
</tr>
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<tbody>
<tr>
<td>Number of crystals</td>
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</tr>
<tr>
<td>a</td>
<td>11.19(7)</td>
</tr>
<tr>
<td>b</td>
<td>9.06(7)</td>
</tr>
<tr>
<td>c</td>
<td>8.04(12)</td>
</tr>
<tr>
<td>α [°]</td>
<td>90</td>
</tr>
<tr>
<td>β [°]</td>
<td>103.6</td>
</tr>
<tr>
<td>γ [°]</td>
<td>90</td>
</tr>
<tr>
<td>Resolution [Å]</td>
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<tr>
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<tr>
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<td>90.7* (32.0*)</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>Unique reflections</td>
<td>3203</td>
</tr>
<tr>
<td>Space group</td>
<td>P2_1</td>
</tr>
<tr>
<td>Completeness</td>
<td>0.995 (0.964)</td>
</tr>
</tbody>
</table>

Table 4-5. Deviations of atomic positions between the structures of sucrose determined from cRED data and the reference structure.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
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<td>C1</td>
<td>0.027</td>
<td>C9</td>
<td>0.047</td>
<td>O5</td>
<td>0.092</td>
</tr>
<tr>
<td>C2</td>
<td>0.076</td>
<td>C10</td>
<td>0.060</td>
<td>O6</td>
<td>0.033</td>
</tr>
<tr>
<td>C3</td>
<td>0.022</td>
<td>C11</td>
<td>0.065</td>
<td>O7</td>
<td>0.090</td>
</tr>
<tr>
<td>C4</td>
<td>0.029</td>
<td>C12</td>
<td>0.063</td>
<td>O8</td>
<td>0.059</td>
</tr>
<tr>
<td>C5</td>
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<td>0.035</td>
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</tr>
<tr>
<td>C6</td>
<td>0.046</td>
<td>O2</td>
<td>0.041</td>
<td>O10</td>
<td>0.031</td>
</tr>
<tr>
<td>C7</td>
<td>0.064</td>
<td>O3</td>
<td>0.079</td>
<td>O11</td>
<td>0.036</td>
</tr>
<tr>
<td>C8</td>
<td>0.071</td>
<td>O4</td>
<td>0.057</td>
<td>Average</td>
<td>0.05(2)</td>
</tr>
</tbody>
</table>
4.3 Assessment of cRED data quality of protein crystals

As-grown thin needle-shaped lysozyme crystals are shown in Figure 4-5. Under an optical microscope, the crystals are clustered like a sea urchin or a hedgehog. A drop of the buffer containing the lysozyme crystals is transferred to a TEM grid, blotted and vitrified in liquid ethane using a Vitrobot. The size of these needle-like crystals is 0.5 - 2 μm in width, 0.1 - 0.5 μm in thickness and > 10 μm in length. These crystals are too small for single crystal X-ray diffraction study but they are perfect for electron diffraction. The needle-like crystals can be easily located on the TEM grid in imaging mode. Furthermore, the thickness of these crystals is suitable for cRED data collection.

Since the lysozyme crystals were sub-micrometer sized, it is difficult to tell which hanging drop contains good crystals using a conventional optical microscope. A feasible way to solve the screening problem is using the negative staining technique to check the quality of the crystals. The typical imaging resolution of a negatively stained protein crystal can reach 25 Å, which is sufficient for viewing the lattice fringes of the crystals. Figure 4-5 shows an HRTEM image of a negatively stained needle-shaped lysozyme crystal as an example of this sample screening method.

Table 4-6. Bond lengths found in the crystal structure of sucrose refined against the cRED data.

<table>
<thead>
<tr>
<th>Atom1</th>
<th>Atom2</th>
<th>Length [Å]</th>
<th>Atom1</th>
<th>Atom2</th>
<th>Length [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>O1</td>
<td>1.427</td>
<td>C1</td>
<td>C3</td>
<td>1.533</td>
</tr>
<tr>
<td>C1</td>
<td>O9</td>
<td>1.374</td>
<td>C2</td>
<td>C4</td>
<td>1.477</td>
</tr>
<tr>
<td>C2</td>
<td>O2</td>
<td>1.432</td>
<td>C3</td>
<td>C5</td>
<td>1.515</td>
</tr>
<tr>
<td>C3</td>
<td>O3</td>
<td>1.367</td>
<td>C4</td>
<td>C6</td>
<td>1.510</td>
</tr>
<tr>
<td>C4</td>
<td>O1</td>
<td>1.410</td>
<td>C5</td>
<td>C7</td>
<td>1.552</td>
</tr>
<tr>
<td>C4</td>
<td>O4</td>
<td>1.391</td>
<td>C6</td>
<td>C8</td>
<td>1.556</td>
</tr>
<tr>
<td>C5</td>
<td>O5</td>
<td>1.375</td>
<td>C7</td>
<td>C9</td>
<td>1.488</td>
</tr>
<tr>
<td>C6</td>
<td>O6</td>
<td>1.360</td>
<td>C8</td>
<td>C10</td>
<td>1.482</td>
</tr>
<tr>
<td>C7</td>
<td>O7</td>
<td>1.416</td>
<td>C9</td>
<td>C11</td>
<td>1.517</td>
</tr>
<tr>
<td>C8</td>
<td>O8</td>
<td>1.423</td>
<td>C10</td>
<td>C12</td>
<td>1.489</td>
</tr>
<tr>
<td>C9</td>
<td>O9</td>
<td>1.399</td>
<td>C-C Average</td>
<td>1.51(3)</td>
<td></td>
</tr>
<tr>
<td>C10</td>
<td>O4</td>
<td>1.437</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C11</td>
<td>O10</td>
<td>1.411</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C12</td>
<td>O11</td>
<td>1.420</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-O Average</td>
<td>1.40(2)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
After sample screening, the optimized crystallization condition can be determined. The cryo-EM sample preparation was carried out using a FEI Vitrobot Mark IV. A drop of 4μL of buffer containing the lysozyme crystals was pipetted onto a TEM grid. The excess liquid is blotted away. After the blotting, the grid was plunged into liquid ethane, allowing the remaining sample to vitrify. The whole procedure was performed at a humidity of approximately 75%. The procedure aims to leave a very thin layer of ice around the protein crystal to protect it from dehydration in the TEM. The frozen sample was then transferred to a Gatan high-tilt cryo-transfer holder (Model 914) and inserted into TEM.

During data collection, we kept the rotation speed for the data collection to 0.45°/s. The exposure time of each frame was 2s, so that each frame covers 0.90° of the reciprocal lattice. Data with a total rotation range around 54° could be collected over 60 frames within 2 mins on each lysozyme crystal. The starting angle for each rotation is varied from -60° to +30° because there is preferred orientation of the lysozyme crystals on TEM grid.

The hen egg-white lysozyme crystal structure was solved using PHASER [91] by molecular replacement using the atomic coordinates of the X-ray model (PDB: 1AKI) as a starting model. A well-contrasted solution was obtained with 2 molecules per asymmetric unit in the space group $P2_12_12$. Crystallographic refinement is performed by PHENIX using the atomic
scattering factors for electrons, applying flexible torsion-angle non-
crystallographic symmetry restraints and translation liberation screw-motion (TLS) parameterisation. In order to analyse the effects of data merging on structure determination, six different datasets were produced. They are the best single dataset (denoted 1-crystal dataset), merged ED data from 4 crystals, 10 crystals with the highest I/\sigma, 10 randomly selected crystals, 20 crystals with the highest I/\sigma and 33 crystals, respectively (denoted 4-crystal dataset, 10-best crystal dataset, 10-random crystal dataset, 20-best crystal dataset and 33-crystal dataset, respectively).

Figure 4-6 shows the relationships between $F_{\text{calc}}$ and $F_{\text{obs}}$ of the final model refined against different datasets. From the plots, we can observe that the linearity of $F_{\text{calc}}$ and $F_{\text{obs}}$ is improved with the increasing number of merged datasets. Because of the multiple scattering of electron diffraction, some of the weak reflections will be enhanced and some of the strong reflections will be suppressed. This effect will change the relation between $F_{\text{calc}}$ and $F_{\text{obs}}$ change from linear to parabola. By merging a large number of datasets, the parabola relation can be partially compensated. In turn, the electrostatic potential map will be improved. As shown in Figure 4-7, when the number of merged datasets increases, the Na$^+$ ion and neighbouring water molecules coordinated with the Na$^+$ ion can be resolved.

Figure 4-6. Scatter plot of $F_{\text{model}}$ and $F_{\text{obs}}$ for increasing number of merged datasets
Figure 4-7. Improvement of detail definition in electrostatic potential map with increasing number of merged datasets, showing more details about water molecules and Na⁺ ion in the structure

4.4 Conclusions

By using cRED datasets collected from the aluminosilicate zeolite ZSM-5, its structure solution is rather easy and straightforward. Different datasets collected from crystals of different sizes and orientations produce similar structural models. Accurate structures can be determined from each of the datasets. Although multiple scattering of electrons have a great influence on the crystallographic statistics (mainly $R_{\text{merge}}$, $R_1$), the refined structures have low ADRA values when compared with the reference X-ray models. The refined atomic positions deviate from the reference model by an average of only 0.07(4) Å. We conclude that accurate structures can be obtained from cRED data using kinematical refinements.

In the sucrose example, we were able to solve and refine the structure by using the data merged from 17 crystals. We were able to observe the hydrogen-bond network in the refined crystal structure. Furthermore, we compare the structure determined from cRED data to the reference structure solved from neutron diffraction. It is found that the average ADRA is 0.05(2) Å for all the non-H atoms. In the structure, the C-O bond length is 1.40(2) Å and C-C bond length is 1.51(3) Å, which are quite reasonable.

For evaluating the quality of the lysozyme structure determined by using cRED data, we cannot use the same method as used in small molecule
crystallography. Instead, we use the linearity between the $F_{\text{calc}}$ and $F_{\text{obs}}$ calculated by PHENIX. We also evaluate the quality of the structure model from the electrostatic potential map after structure refinement. We show that by merging multiple datasets, the data quality and refinement result can be greatly improved.
5. Using electron tomography to reveal the internal structure of materials in nanoscale (Paper V, Paper VI)

Electron tomography (ET) is a widely used technique to reveal 3D structures of materials of size ranging from 5 nm to 5 μm. Atomic resolution ET can reach very high resolution (<1.5Å) to resolve for example individual atoms in a platinum nanoparticle. On the other hand, the resolution of conventional ET is relatively low (around 1-5 nm) and can resolve the shape or internal structure of cell and mesoporous materials. The conventional electron tomography has wider applications than atomic resolution ET because the later requires very small nanosized samples.

In this chapter, electron tomography is applied to two materials. The first sample is a mesoporous magnesium carbonate (MMC) material. The other sample is a hierarchical zeolite ZSM-5. We use electron tomography to reveal the inter-connectivities of the mesopores and formation mechanisms of both materials.

5.1 Reveal the formation mechanism of mesoporous magnesium carbonate (MMC) material (Paper IV)

In this study, transparent and crystal-like MMC material is synthesized in surfactant-free routine. The typical synthesis procedure of transparent MMC includes three steps: (i) sol–gel synthesis, (ii) powder formation and (iii) degassing. In a typical synthesis, 15 g of magnesium oxide is mixed with 225 cm³ of methanol in a 350 cm³ pressure reaction vessel. The vessel is sealed and a CO₂ pressure of around 4 bar is applied. After the formation of an optically clear, yellow liquid, the solution is stirred at 75 rpm in a ventilated area at controlled temperatures. During the evaporation of methanol, the reaction mixture first thickened into a gel. With further evaporation, the gel then broke up into small, wet powder-like compounds. The wet powder is then dried under nitrogen flow at 85 °C. At last, the dried powder is kept at 150 °C for 3 h and 250 °C overnight.

From Figure 5-1, HRTEM images show that on a nanometer scale, the transparent MCC particles are aggregates of randomly oriented crystalline nanoparticles of approximately 2-5 nm in size, denoted as Type I nanoparticle. By Fourier transform, we can see that the d-spacing of these crystalline nanoparticles corresponds to MgO. These Type I nanoparticles will aggregate and form Type II nanoparticles with size of around 30 nm. Figure 5-2 shows six reconstructed tomogram slices of a typical Type II nanoparticle. From the tomograms reconstructed from the tilt series, mesopores can be identified throughout the entire MMC particle. Both
irregularly-shaped and regularly-shaped pores are observed. Some of these pores are interconnected.

Figure 5-1. (a) 3D-Orthoslice representation of the reconstructed tomogram of MMC. (b) HRTEM image of an MMC particle showing randomly oriented MgO nanocrystals surrounded by amorphous regions. The crystalline parts are highlighted in red, (c) an enlarged view of a MgO nanocrystal marked in panel (b), (d) Fourier transform (FT) of the HRTEM image in panel (b) with the rings (marked) corresponding to MgO. Reproduced by permission of The Royal Society of Chemistry.

Figure 5-2. Six consecutive slices covering a thickness of 2.5 nm obtained from the tomogram reconstructed from the tilt series of TEM images of an MMC particle. Reproduced by permission of The Royal Society of Chemistry.
The results from the various characterization methods suggest that the material is formed by aggregation of different types of nanoparticles (Type I and Type II) in the reaction mixture containing also methanol with dissolved Mg$^{2+}$ and CO$_3^{2-}$. When methanol is evaporated from the reaction mixture, these aggregated nanoparticles are fixed in place, possibly by an additional layer of amorphous MgCO$_3$ that precipitated from the reaction mixture upon drying (as illustrated in Figure 5-3). Mesopores in the materials are created by the trapped CO$_2$ bubbles, released by the decomposition of MgCO$_3$.

Figure 5-3. Schematic representation of the mechanism and energy input (e.g. temperature or stirring) dependence related to the pore formation of the mesoporous material. Reproduced by permission of The Royal Society of Chemistry.

5.2 Reveal the internal pore structure of hierarchical zeolite TS-1 (Paper V)

Zeolites are microporous materials and have many applications, such as catalysis, separation and adsorption. In industrial applications, the diffusion process becomes the bottleneck for reactions containing bulky molecules because the pores of the zeolites are not large enough. Therefore, introducing mesopores into zeolite crystals provides a possible solution for the diffusion problem. In our study, we synthesized hierarchic MFI framework zeolite crystals, TS-1, using a temperature programmed dissolution-recrystallization post-treatment method.

TS-1 is synthesized using precursor with composition 1SiO$_2$: 0.02TiO$_2$: 0.3TPAOH: 50H$_2$O at 175°C. After 48h, the reactor is cooled down. The white powders are separated by centrifugation and washed using ethanol. The product is then dried at 80°C overnight and calcined at 550°C for 12h. For the post-treatment step, 2g of calcined TS-1 is added to 20ml of TPAOH. The obtained mixture is transferred to the autoclave. The autoclaves are first put into an oven which is preheated to the temperature of
120°C for 2 h, then the temperature is increased to 175°C for 24h. The obtained samples are denoted as TS-1-120(24)-175(24h). In comparison, TS-1-H is prepared by applying post-treatment at 175°C for 24h.

Figure 5-4. SEM and TEM images of TS-1-H and TS-1-120(2 h)-175(24 h). Reproduced by permission of Elsevier.

Figure 5-5. Six consecutive slices covering a thickness of 2.5 nm obtained from the tomogram reconstructed from the tilt series of TEM images of TS-1-120(2 h)-175(24 h). Reproduced by permission of Elsevier.
As shown in Figure 5-4, the TEM images show that there are mesopores in both samples. SEM images show that TS-1-120(2h)-175(24h) has more mesopores exposed to the outer surface of the particles. The distribution of the mesopores inside TS-1-120(2h)-175(24h) is studied by electron tomography. Three different slices of the tomogram are shown in Figure 5-5. These slices from the tomogram reveal homogenous distribution of mesopores inside the crystal after a temperature programmed post-treatment. In addition, we are able to confirm that some of these mesopores are exposed to the surface of particles.

The ammoximation of cyclohexanone is used to test the catalytic properties of the hierarchical TS-1. Figure 5-6 shows that the hierarchical TS-1 has superior catalytic activity compared to the conventional TS-1, denoted as TS-1-C. This is due to the presence of mesopores. The conversion of TS-1-120(2 h)-175(24 h) catalysed reaction reaches up to 85% after 90 mins, thanks to the enhanced diffusion in the presence of open mesopores.

5.3 Conclusions

In this chapter, we use electron tomography technique to investigate the formation mechanism of a mesoporous magnesium carbonate material and the mesopore connectivity of hierarchical TS-1. Mesoporous magnesium carbonate is formed from the hierarchical aggregation of MgO nanocrystals and MgCO₃ nanoparticles that are approximately 2-5 nm in size. These 2-5 nm nanoparticles first form ~30 nm nanoparticles, then aggregate to form
MMC particles. The resulting aggregates are transparent powders with high porosity. The mesopores in MMC are formed in the degassing process.

For hierarchical TS-1 zeolites, the mesopores are formed by preferential etching of the crystals. The temperature of the etching process will affect whether the mesopores are exposed to the outer surface of the crystals. Temperature programmed dissolution/recrystallization has been proven to be an effective and simple way to synthesize hierarchical MFI zeolite with open pores.
6. Conclusions

In summary, this thesis described the recent developments and applications of 3D electron diffraction and 3D electron tomography.

3D electron diffraction is a powerful tool for atomic structure determination. In the third chapter, we compared the structure determination results of two novel aluminophosphate zeolites from both RED and cRED methods. The structure determination of PST-13 and PST-14 using cRED shows that cRED can obtain more accurate structures than RED. PST-13 has hydroxyl groups in the framework. Each of these hydroxyl groups bridges with two Al atoms. PST-13 will irreversibly transform to PST-14 with loss of bridging Al-O-Al groups and OSDA molecules upon calcination. Both PST-13 and PST-14 structures contain a 3D pore system made up of intersecting straight and wavy 8-ring channels. Both structures can be built from pairs of enatiomeric building units, which are 1,5-open \(d_4r\) and 1,6-open \(d_4r\).

In addition, we investigated how accurate the structure determination result is using ZSM-5 as an example. We acquired four different cRED datasets for ZSM-5. Although both \(R_{int}\) and \(R_1\) values are quite high, the atomic positions of structural models refined against the cRED data are very accurate. The refined atomic positions deviate from the SCXRD model by only 0.07(4)Å on average. Moreover, we demonstrated the accuracy for the atomic positions of a small organic molecule sucrose obtained from cRED data. In total we collected 17 datasets and merged these datasets into a new dataset. The result shows the atomic position deviation of sucrose structure from cRED data from a reference structure model in the CCDC database is around 0.05(2)Å. For protein structures, we investigated the correlation between calculated structure factor and observed structure factor to check if there is linearity relationship. If the relationship is closer to parabolic, then the data quality will be lower. Another way is evaluating the electrostatic potential map after structure refinement, to see if it is possible to find out more details about the structure.

Apart from investigating 3D atomic structures, 3D nanoscale structures can be investigated by TEM using electron tomography. In this thesis, two samples were given to show the 3D nanoscale structures from electron tomography. Mesoporous magnesium carbonate was formed from the hierarchical aggregation of MgO nanocrystals and MgCO\(_3\) nanoparticles that are approximately 2-5 nm in size. These 2-5 nm nanoparticles first form ~30 nm nanoparticles, then these ~30 nm nanoparticles aggregate to form MMC particles. The resulting aggregates are transparent powders with high porosity. The mesopores in MMC were formed in the degassing process. For hierarchical TS-1 zeolites, the mesopores were formed by preferential...
etching of the crystals. Different temperature during the etching process will affect whether mesopores will connect to the outer surface of the crystals or not. Temperature programmed dissolution/recrystallization has been proven to be an effective and simple way to synthesize hierarchical MFI zeolite with open pores.
7. Future Perspectives

There are still many projects that I wish to carry out but I have not done yet because of the limited time of my PhD study.

The first one is investigating the influence of dynamical effects on the structure determination. An electron diffraction simulator is needed. With an electron diffraction simulator, we can obtain the \textit{hkl} file with dynamical scattering and use ordinary X-ray diffraction refinement software to study the effect of multiple scattering on the atomic coordinates, atomic occupancy and atomic displacement parameters.

The second one is investigating how to use electron diffraction data to determine the chirality of crystals. The chirality of crystal is very important for drug design and research. Even though no anomalous scattering can be observed in electron diffraction, the dynamical effect of electron diffraction will be very useful in distinguishing chirality between different crystals.

Another thing is investigating the effect of ions on the result of refinement. In many crystals, the atoms are charged. For X-ray diffraction, charges will not have significant impact on electron density. However, for electron diffraction, charges will have severe influence on the ED intensities of reflections, especially at low resolution range. Currently, electron diffraction at low resolution suffers from inelastic scattering background. The background will affect the signal from ionization. Therefore a TEM with an in-column Omega-filter is necessary if one wants to refine structures with ions. Another difficulty is the dose of incident electron should be limited because electron beam can change the ionization state of the ions. For instance, it may neutralize a positively charged ion. Therefore, the dose of electron should be controlled very carefully.
8. Sammanfattning

Kristallstrukturen bestämmer materialets kemiska och fysikaliska egenskaper. Strukturen kan analyseras på olika nivåer, från atomnivå, mesoskala nivå, hela vägen upp till makroskala nivå. Transmissionselektronmikroskopii (TEM) är ett kraftfullt verktyg för att studera materialets struktur från atomär till makroskal nivå. På atomnivå kan strukturbestämning med användning av TEM utföras i diffaktionsläge. Den senaste utvecklingen i 3D-elektrondiffaktionsmetoder gör strukturbestämning av kristaller i nano- och mikronstorlek mycket enklare än tidigare. På grund av de starka interaktionerna kan emellertid elektroner spridas flera gånger på sin väg genom kristallen, vilket medför att de uppmätta intensiteterna är mindre noggranna än i röntgendiffraktion.

I denna avhandling använder vi den kontinuerliga rotations elektrondiffaktionen (cRED) som utvecklats i vår grupp för att undersöka materialstrukturer och noggrannheten av strukturbestämning med denna metod. I det tredje kapitlet använder vi cRED-metoden för att bestämma strukturen för två aluminofosfat zeoliter, PST-13 och PST-14. Vi visar att dessa strukturer kan byggas från två par enantiomera strukturbyggnader. I det fjärde kapitlet visar vi att trots att felaktigheten i uppmätta intensiteter härstör från dynamiska effekter är det fortfarande möjligt att bestämma strukturen noggrant. Vi visar att atomkoordinaterna för ZSM-5 och sackaroskristallstrukturen bestämd av multipla elektrondiffaktionsdataset är mycket nära med de som bestäms från röntgen eller neutrondiffaktionsdata. Vi bedömde också linjärityeten mellan beräknade strukturfaktorer och observerade strukturfaktorer och använde detta som en indikator för att bedöma diffaktionskvaliteten för proteinkristaller.

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References


[26] E. van Genderen et al., “Ab initio structure determination of nanocrystals of organic pharmaceutical compounds by electron diffraction at room temperature using a Timepix quantum area direct


