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Cryo-Crystallography: Diffraction at Low Temperature and More

Piero Macchi

Abstract This chapter comments on the motivations and the methods of crystallographic studies at low temperature. Cry-crystallography is a brunch of Crystallography, a science that is too often confused with a technique. On the other hand, the scientific background to study crystal phases at low temperature is here provided, together with a survey of many possible techniques that provide complementary or supplementary information. Several applications are discussed, in particular in relation with highly accurate studies like electron density determination or phase transition mechanisms.

Keywords Electron density · Low temperature · Phase transitions · X-ray diffraction

Contents

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1 What Is "Cryo-Crystallography"?

Low temperature crystallography is a discipline deeply entangled with the techniques of X-ray diffraction on cooled samples and very often *cryo-crystallography* and *low temperature diffraction* are used as synonymous. This reflects a more general problem of crystallography, a *science* often considered just at the level of a *technique*, a confusion that hopefully this book will contribute to remove.

Cryo-crystallography is much more than a technique, although it relies on sophisticated instrumentation and practices. Studying crystals at variable temperature opens new frontiers to the chemistry and physics of a substance in its solid state, crystal form. This extended knowledge can be used to obtain a more comprehensive picture of the phase diagram of a compound or more precise information on some properties of a material and an even more detailed image of a crystal structure down to subatomic level. Molecular chemists mainly appreciate the possibility to obtain more precise structures (which often just means easier to publish), whereas solid state chemists or physicists should be attracted by the additional opportunities offered by crystallographic investigations in a wider temperature range, as outlined above.

Cryo-crystallography clearly involves the study of materials at low temperature; however in general there is no obvious discontinuity between lowering and raising the temperature from ambient conditions, because much information becomes available after applying temperature gradients, no matter which sign. In some other cases, *low* temperature means making the image of a crystal structure more clear by reducing the thermal motion of atoms and molecules and therefore improving the Bragg diffraction from the crystal. However, *low* is ambiguous and it is definitely "material dependent," as we will see below. Another important point to clarify is that diffraction is not the only technique that could shed light on the behavior of a crystalline material as a function of the temperature. Other investigations could give complementary information, perhaps on a different size or time scale than diffraction.

In this chapter the author will focus on the theoretical aspects of crystals at low temperature, on the techniques to cool crystals, and on some illustrative examples of cryo-crystallographic studies.

2 Why Cryo-Crystallography? What Happens to Crystals at Low Temperature?

In a seminal review article, Larsen [1] described the state-of-the-art of the cryogenic techniques most commonly adopted for X-ray diffraction and lucidly showed the applications to all kind of crystallographic studies, stimulated by a variety of research interests. Already at that time, the availability of liquid nitrogen cryostats in many laboratories was highlighted, emphasizing the possibility to carry out many

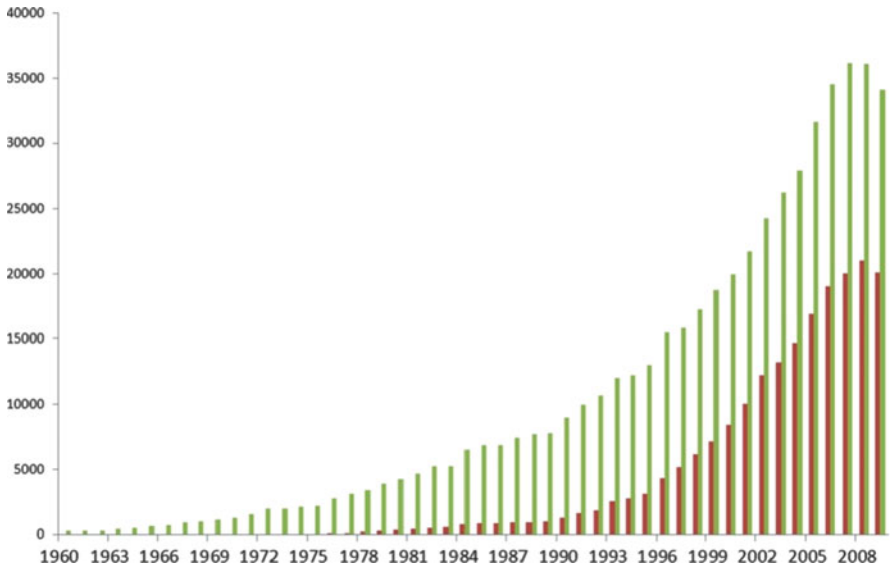

Fig. 1 The total number of crystal structures published since 1960 (*green bars*) and the number of those collected at temperature below 273 K (*red bars*). Data taken from the Cambridge Structural Database [2, 3] (the last bar is year 2009, but it is not complete)

experiments on single crystals or polycrystalline powders at low temperature, at variance from the past. This is of course even truer nowadays: a simple inspection of the database containing all the characterized organic/organometallic crystal structures [2, 3] shows how the percentage of experiments carried out below ambient temperature has increased over the years (Fig. 1) and it is now well above 50%, compared to 2% in 1960 and 15% in 1990. On the other hand, it is surprising that data collections below 80 K (that require more sophisticate and expensive techniques) are just 0.2% of the total number of experiments, with a decreasing trend. This tells us that many studies on crystals at low temperature are carried out just because easy and economic equipment is available in a laboratory, but without a genuine scientific interest in cryo-crystallographic studies. Were the scientific interests more pronounced, we would have witnessed a growth of experiments at very low temperature as well.

The change of the usual conditions for X-ray diffraction experiments may affect the interpretation of some results, especially by scientists outside crystallography. In fact, while one should appreciate the increased precision and accuracy of structures determined at low temperature, a drawback is that earlier benchmarks could be altered by sudden or progressive changes of experimental procedures. For example, theoretical chemists often use crystallographic tables reporting bonding distances or angles to test the quality of theoretical methods adopted for ab initio calculations. Most of those entries are given without any correction for thermal libration (see below), which means that they are significantly affected by atomic thermal motion (producing

inaccurate distances). As a consequence, the benchmark for theoreticians has changed somewhat, hopefully in the direction of higher accuracy, but earlier conclusions should be reconsidered, being based on different and less accurate observations. A side problem of low temperature "routine" crystal structure determination is that there is not a standard low temperature and experiments are carried out over a large range (90–173 K or even higher temperature) although using the same kind of liquid nitrogen devices. Structural correlation analysis [4] may also be significantly affected by the different temperatures of data collections because fragments retrieved from structures under largely different librational conditions certainly introduce systematic differences in the statistical analyses.

In his review, Larsen [1] focused on X-ray diffraction data at low temperature, mainly from a single crystal, and he outlined the following major advantages: (1) reducing the radiation damage; (2) improving the resolution; (3) decreasing systematic effects of the thermal motion. The fields of application were wide and basically covered all spectrums of crystallographic studies with X-ray diffraction: (1) structure determination of proteins; (2) structure determination of unstable small molecules; (3) phase transitions; (4) accurate electron density mapping.

Fifteen years later these fields are still central in crystallography and sample cooling is of equal importance for data quality. More applications can be envisaged and the great advantages offered by rapid data acquisitions may stimulate new and more sophisticated studies. However, data are often collected at low temperature without a clear scientific purpose, which is due to the insane attitude of contemporary scientists to use all possible guns in their hands to shoot the little mouse of a research target in order to impress reviewers and editors of a journal or panels of funding agencies. In this sense, the automatic checkup of crystal structure qualities provided by some software and recommended or even requested by many journals is one of the main causes, because high standards are suggested even when not requested. Data collections at room temperature are often adequate enough and efficiently provide the (little) information which is requested, like the structure of a molecule and its packing in the solid state.[1] A cryo-crystallographic study is necessary when the objective of a research study is more challenging and really requires exhaustive analyses of the physical-chemical behavior of a given crystal species.

In the following we will mainly analyze the thermodynamics of a perfect crystal at low temperature and the physics of diffraction at low temperature, in order to understand better the expectations from a cryo-crystallographic study, eventually discussed in Sect. 4.

[1] Sometimes an X-ray diffraction experiment is carried out only with the purpose of ascertaining the chemical composition of a given solid.

2.1 Crystals at Low Temperature

The definition of *crystal* is itself a developing concept, as demonstrated by the ongoing discussions [5, 6]. Most of the theoretical background proposed in this chapter is valid for a *perfect crystal*, i.e., an infinite mathematical object with an idealized crystal structure (*ideal crystal*) in thermodynamic equilibrium at a given pressure P and temperature T. In textbooks, only the gas phase thermodynamics is usually discussed in detail, whereas little attention is paid to the solid state. A full thermodynamic treatment of solids is beyond the scope of this chapter and the reader is referred to specific books on the subject, for example [7].

In chapter by Gavezzotti, the theoretical approaches to crystallography have been discussed and the reader has learnt methods to compute the potential energy of *ideal crystals* at various levels of approximation, with the purpose of estimating lattice energies [113]. It is important to stress that the crystal potential contains information on the nature of bonding in the molecular species and the type of intermolecular interactions, but calculations are typically carried out assuming no thermal energy, therefore in the hypothetical conditions of $T = 0$ K and neglecting the zero point motion. This means that lattice dynamics (hence entropic terms) are not explicitly considered. While this could be sufficient when searching the optimal structure of a molecular crystal or grossly estimating the relative stability of different polymorphs, from the experimental point of view one should not forget what are the consequences of dynamics and the effect of temperature. Einstein [8] first and Debye [9] later proposed approximated models to compute the free energy F of *perfect crystals* formed by N atoms, assumed to be harmonic oscillators:

$$F = \Phi_0 + E_{\text{vib}}(T) + TS(T), \tag{1}$$

where Φ_0 is the crystal potential (for example, computed as described in [113]), E_{vib} is the energy of the harmonic oscillators, and $S(T)$ is their entropic contribution. In Einstein approximation the oscillators are independent and no energy dispersion calculation is necessary. Debye's model, instead, assumes the crystal to be an isotropic elastic medium.

We will not discuss the theories of lattice dynamics [10] in detail, but we will just recall that atomic motion in crystals leads to characteristic equations that relate the frequencies ωs of waves traveling in the solid with vectors of correlated mass-weighted atomic displacements. Solutions to these Newton type equations give the three N dispersion relations for phonons, typically divided into 3 *acoustic* branches and 3N−3 *optic* branches (where N is the total number of atoms). Each dispersion relation describes an individual phonon along a given direction in wave vectors space. The three *acoustic* branches are associated with translations of the entire structure for a traveling wave of infinite wavelength; otherwise, they propagate in the crystal like acoustic waves. The *optic* modes are responsible for the atomic displacements about equilibrium positions, which are typically represented by a tensor **U** (with components $U_{ij} = \langle \Delta x_i \Delta x_j \rangle$) in relation to the unit cell

translational vectors. The **U** tensor affects the so-called Debye Waller factor $T(\mathbf{S})$, which is a kind of "damping factor" for the radiation scattered by an atom, as will be discussed in Sect. 2.2:

$$T(\mathbf{S}) = \exp\left(\frac{-8\pi^2\langle u_T^2\rangle \sin^2\vartheta}{\lambda^2}\right). \tag{2}$$

S is the scattering vector, u_T is the atomic displacement parameter in this simplified notation assumed to be isotropic, θ is the scattering angle, and λ the wavelength of the incident radiation. The atomic displacement depends on the temperature, and hence so does the Debye–Waller factor. If an atom is modeled by a classical oscillator, then the atomic displacement would change linearly with temperature:

$$\langle u_T\rangle = \frac{k_B T}{\bar{\omega}^2 m}. \tag{3}$$

m is the atomic reduced mass and k_B is the Boltzmann constant. However, in a quantum mechanical system, at low temperature the oscillator is temperature independent:

$$\langle u_T\rangle = \frac{\hbar}{2\bar{\omega}m}. \tag{4}$$

The typical behavior of an atomic displacement parameter is represented by the curve plotted in Fig. 2. This trend tells us that below the turn point ($\Theta_E/2$) atomic vibrations are not only smaller but also quite constant.

The important message from Einstein or Debye models is that vibrations of atoms in a crystal contribute to Entropy S and to Heat Capacity C; therefore they affect the thermodynamic equilibrium of a crystal by modifying both the Free energy F, which

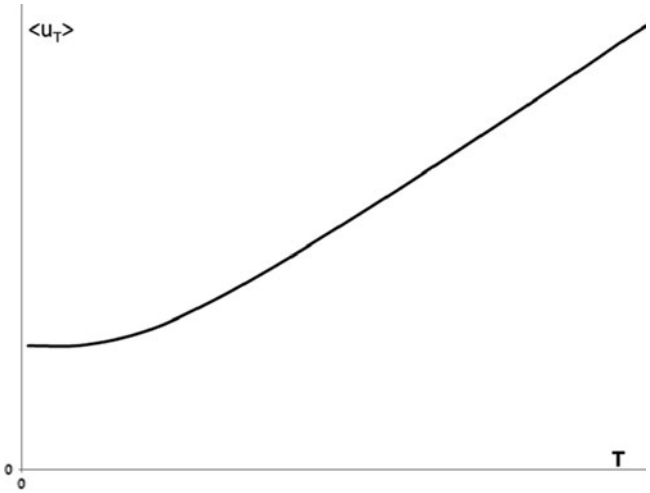

Fig. 2 The mean square displacement of a single harmonic oscillator as a function of temperature. Units are arbitrary because $\langle u_T\rangle$ and T values depend on the frequency of the oscillator and the mass of the particle. The plot shows that at low temperature the displacement is almost constant, whereas at high temperature it varies linearly with T. The change of regime occurs approximately at $\Theta_E/2$

determines the actual stability of a solid state phase at a given pressure, and temperature.

The *optic* modes can be further "decomposed" into *internal* and *external* when a molecular crystal is considered. Internal modes are associated with much higher energy (> 300 cm^{-1}), due to the intra-molecular chemical bonding beside some functional groups in a molecule which could be associated with large and independent librations (for example CX_3 groups, where X = H, Halogen, CH_3, etc.). External modes are instead associated with inter-molecular interactions and they are of much lower energy (< 300 cm^{-1}) and are therefore active even at low temperature. The atomic displacement of a molecular crystal, especially at low temperature, is mainly due to the external modes. This is well illustrated by comparing experimentally observed C_p and C_v of the crystal form of benzene against the calculated C_v from experimentally measured **u**s, after separating *internal* and *external* modes [11]. This calculation is possible assuming Debye and Einstein models for independent oscillators and gives the interesting result displayed in Fig. 3: up to $T = 100$ K the deviation is sufficiently small to conclude

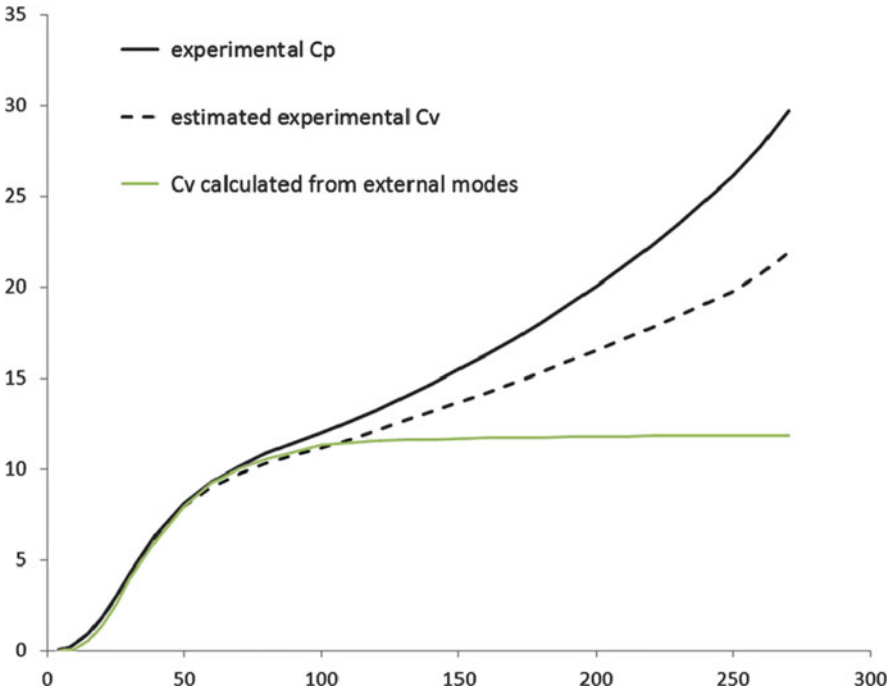

Fig. 3 Experimental heat capacities of benzene [11], C_v is obtained from observed C_p after subtracting the expansion work, computed using the experimentally determined bulk modulus. The C_v estimated from molecular translational and librational lattice modes (obtained from neutron diffraction ADP's) is also plotted. Note that these *external* modes well reproduce the observed C_v up to ca. 100 K. Above this temperature the *internal* modes are active and C_v exceeds the classical limit of 3 $k_B T$

that only *external* modes affect the heat capacity, but above that temperature this approximation does not hold any longer.

Because of the important distinction between internal and external modes, Bürgi proposed [12] for molecular crystals the so-called *molecular mean field model*, where each molecule is considered as a collection of independent oscillators in the average potential produced by other molecules in the crystal. The hypothesis that internal modes are less active than external ones leads to the *rigid body model*, where the atomic displacement parameters are assumed to be caused just by pure libration, pure translation, and coupled translation–libration of an isolated, rigid molecule [13]. In this respect, Hirshfeld [14] proposed a criterion to assess that atomic displacement tensors are meaningful when refined from X-ray or neutron diffraction data: two atoms covalently bonded to each other should have equal displacement along the bond direction within a given tolerance (that includes the effects of bending modes). This criterion is still applied when testing the quality of a structure determination and refinement for a molecular crystal, although weaker chemical bonds and unbalanced atomic masses in a bond produce breakdown of the Hirshfeld criterion, without implying incorrect structural refinement. This is, for example, the case in most of metal–ligand bonds of an organometallic molecule [15, 16].

So far, the models presented have assumed harmonic oscillators; however atoms in real crystals move in a substantially anharmonic potential. This has a number of implications for the properties of a material and a number of complications for the modeling, as the description of atomic displacement parameters becomes much more difficult [17, 18]. One of the first consequences of anharmonicity is the thermal expansion/contraction of the crystal lattice. In fact, a crystal composed of atoms moving in a purely harmonic potential would not expand or contract due to increased or decreased vibrations. In a harmonic potential, the force necessary to compress the crystal are equal in modulus to that necessary to expand it. However, the crystal potential is quite poorly harmonic, given that compression of atoms at distances smaller than their equilibrium position is energetically more expensive than expansion. The potential of a chemical bond has harmonic behavior only at the bottom of the energy potential well. Thus, anharmonic behavior is expected for all perfect crystals, but the nature of the species may play an important role. For example, covalent solids display harmonic behavior up to temperatures much higher than organic molecular crystals. In fact, weaker intermolecular interactions are associated with very flat and poorly harmonic potentials. Deviations from harmonicity are summarized by the Grüneisen parameter γ [19] through which one can explain the zero pressure equation of state of a material:

$$\gamma = \frac{V\beta}{k_T C_V} = \frac{V\beta}{k_S C_P} = \left(\frac{-d\ln\Theta_{E,D}}{d\ln V}\right). \tag{5}$$

Here V is the crystal volume, k_T and k_S are the isothermal and adiabatic *compressibility* (i.e., the contraction under pressure), β is the *expansivity* (expansion/contraction with temperature), C_p and C_v are heat capacities, and $\Theta_{E,D}$ are the Einstein or Debye Temperatures. Because β is only weakly temperature dependent,

from (5) one learns that the Grünesein parameter depends on the temperature, similar to heat capacity. Softer materials have larger γ and lower Debye Temperatures (Θ_D). For $T << \Theta_D$, heat capacity of dielectric materials increases with $(T/\Theta_D)^3$, whereas for $T >> \Theta_D$ the heat capacity becomes constant and larger (proportional to the number of oscillators; see also Fig. 3). As a consequence, in the "T^3 regime," the expansion of a crystal is much smaller. In addition, many lattice modes are not yet activated; thus atomic displacements are smaller and closer to their zero point limits. The reader will immediately appreciate that these two features play important roles for the study of crystals as they affect properties of the material (first of all the diffraction itself). Note that for metals, instead, the T^3 regime is only approximated because the electronic contribution to heat capacity ($\div T$) is not considered in the Debye (or Einstein) model.

The free energy of a phase is extremely important to explain phenomena like *phase transitions*, i.e., transformations of one solid state phase into another. Phase transitions are quite connected with *polymorphism*, i.e., the observation of two or more solid state phases of the same substance. On some occasions, in fact, kinetic effects allow observation of more than one polymorph at the same temperature and pressure conditions. Of course only one is thermodynamically stable, but transformation might be indefinitely slow, the most obvious example being transformation of diamond to graphite at ambient conditions. Along a given temperature range, it is possible that one solid state structure transforms into another at a given *critical* temperature (T_c). The same is true for pressure scans, of course (as will be discussed in [114]). Occurrence of a phase transition indicates that the free energy associated with a given structure has become lower than the other. In crystallography, there are a number of classifications of phase transitions that are sometimes dedicated to specific materials and are difficult to generalize. A unified description is not available, although a recent review article by Herbstein [20] has tried to rationalize different point of views and descriptors.

While cooling or warming crystals, phase transitions are not uncommon, but they are often overlooked. Many experiments can be useful to characterize a phase transition, as we will see in Sects 3 and 4. Diffraction, microscopy, spectroscopy, and calorimetry can all provide information on the structure, the energy, or the properties of a crystal. Their sudden change is the most obvious effect of a phase transformation and a matter of interesting study for scientists.

2.2 Low Temperature and X-Ray Diffraction from Ideal Crystals

The X-ray intensity diffracted from a single crystal and measured at the detector can be approximated as [21]

$$I_{\text{meas}}(\mathbf{S}_i) \approx I_0 v Q(\mathbf{S}_i) y \mathrm{A}(1+\alpha) + \sum_{m \neq i} p_m I_0 v Q(\mathbf{S}_m) + B, \tag{6}$$

where A is the transmission coefficient, y is the extinction coefficient [22], α is the correction for thermal diffuse scattering (TDS) [23], v is the sample volume, $\boldsymbol{B}$ is the *background*, and $p_m Q(\mathbf{S_m})$ is the contribution to the wave scattered along the direction $\mathbf{S_i}$ from all other vectors $\mathbf{S_m}$ through the so-called multiple scattering. The integrated reflectivity $Q(\mathbf{S})$ per volume of the crystal is

$$Q(\mathbf{S}_i) = \frac{a^2\lambda^3}{V^2}\frac{P}{\sin 2\theta}\left|F_{\text{Bragg}}(\mathbf{S}_i)\right|^2. \tag{7}$$

$a = e^2/mc^2$, λ is the wavelength, V is the cell volume, P is the polarization factor, and $\boldsymbol{F}$ is the structure factor. After applying several corrections, it is then possible to obtain the Bragg intensity.

The structure factor is the Fourier transform of the thermally averaged electron density:

$$F(\mathbf{S}_i) = \int_V \langle\rho(\mathbf{r})\rangle e^{2\pi i \mathbf{S}_i \mathbf{r}} \mathrm{d}\mathbf{r}. \tag{8}$$

Within an atomistic approximation, the structure factor can be expressed in terms of the atomic form factors, mean positions and mean-square displacements:

$$F(\mathbf{S}_i) = \sum_a f_a(\mathbf{S}) \exp(2\pi i \mathbf{S}_i \cdot \mathbf{r}_a) T_a(\mathbf{S}_i). \tag{9}$$

$T(\mathbf{S})$ is the Debye–Waller factor introduced in (2). The atomic form factors are typically calculated from the spherically averaged electron density of an atom in isolation [24], and therefore they do not contain any information on the polarization induced by the chemical bonding or by the interaction with electric field generated by other atoms or molecules in the crystal. This approximation is usually employed for routine crystal structure solutions and refinements, where the only variables of a least square refinement are the positions of the atoms and the parameters describing the atomic displacements. For more accurate studies, intended to determine with precision the electron density distribution, this procedure is not sufficient and the atomic form factors must be modeled more accurately, including angular and radial flexibility (Sect. 4.2).

As outlined above (6), there are many factors that affect the measured intensities, and therefore in a typical X-ray diffraction experiment there are many sources of systematic errors. The accuracy of the parameters obtained by X-ray crystal structure analysis depends on the measuring procedure, the strategy of the data-collection, the treatment of measured intensities to extract Bragg structure factors, the quality of the crystal sample, and its handling. It is important to recognize that cooling the sample cannot solve all inherent defects of a diffraction experiment, although it is true that it could enormously improve the quality of the data. First and most important, lower temperature reduces the atomic displacements, and consequently produces more Bragg scattering. Moreover, radiation damage of the crystals and dynamical disorder (if present) may be significantly reduced. Therefore, it is typical to read that *low temperature* improves the quality of the data, but the meaning of *low* depends on the material under study. One could use the Debye temperature as a kind of benchmark.

Table 1 Debye temperatures for some elemental solids and simple compounds. Data are obtained from thermal measurements at low temperature [25]

Solid	Θ_D (K)
Na	158
Si	640
Al	428
P	105
Ar	93
K	91
Fe	457
Cu	343
Cs	38
C (diamond)	2,230
KCl	235
NaCl	321
H_2O	218[a]
LiF	732

[a]From [26]

Crystals held together by strong forces between atoms and molecules will certainly have higher phonon frequencies (especially of external modes), and therefore higher Θ_D. In Table 1, we can see some known Debye temperatures obtained from experimental heat capacities measurement for some elements or simple compounds. We clearly see that Debye temperature can be quite low (such as for crystals of noble gases and alkali metals) or relatively higher (such as for transition metals and ionic solids). Very few data are available for most molecular crystals. The practical meaning of recommending $T < \Theta_D$ is that only translational and librational modes of an ideally rigid molecule in the crystal are actually activated. If the temperature is significantly lower than Θ_D ($T \sim 0.1\ \Theta_D$) then the Debye T^3 regime is valid. For most molecular crystals, in neutral electrostatic conditions and in the absence of strong intermolecular interactions like hydrogen bonding, the temperature should be well below 100 K. In reality it is enough to de-activate substantially the optic modes in order to maximize the benefits of low temperature as we will see in the following.

2.2.1 Resolution

As we saw in (9), the structure factors are affected by the temperature through the so-called Debye Waller factor $T(\mathbf{S})$. As temperature decreases, the diffracted intensity increases because $T(\mathbf{S})$ grows, especially for large diffraction angles. For a molecular crystal of an organic compound, the isotropic atomic displacement parameter can be reduced by a factor of 2–3 from room temperature down to 100 K (for example from 0.05 to 0.02 Å^2), corresponding to ca. 100 times larger intensity for a reflection at 0.5 Å resolution. The ratio could be even higher if the temperature is further reduced to 10 K, taking advantage of even smaller displacement parameters. A reflection at high diffraction angle is typically very weak because only core electrons contribute to the structure factor and, as discussed, Debye

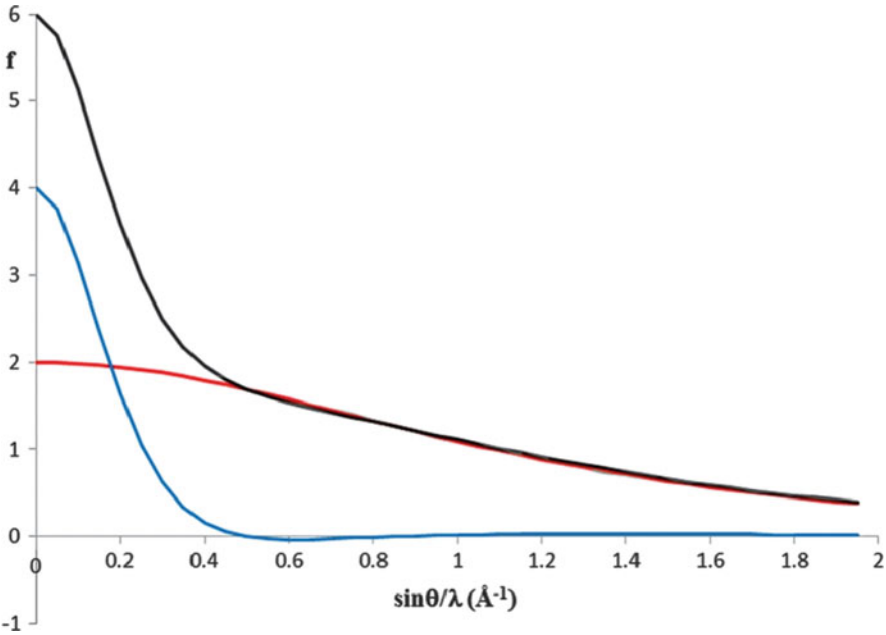

Fig. 4 The atomic form factor of a C atom (in $1s^2 2s^2 2p^2$ electronic configuration). Core electron scattering is in *blue*, Valence electron scattering is in *red* and total scattering in *black*

Waller factor implies a larger penalty to high scattering angles. Therefore, reducing the damping produced by $T(\mathbf{S})$ could bring the intensity of a high resolution reflection from hardly observable (at ambient temperature) to significant (at low temperature). For reflections at lower resolution the gain is smaller, but also less important because these reflections are inherently more intense (given the atomic form factors; see Fig. 4). It should be noted that for ionic crystals and minerals, the atomic displacements are already quite small at room temperature, and therefore the low temperature gain is not so large.

An additional advantage of the very low temperature is the smaller dependence of $\langle u^2 \rangle$ on the temperature. Thus, fluctuations of the temperature during an experiment are less troublesome. It should be taken into account that experiments without temperature control might easily experience ± 5 °C fluctuations (night/day variation, heating caused by other machines, etc.). This produces a fluctuation of 1–2% of the intensity for a reflection at 1.0 Å resolution and up to 10% for a reflection at 0.5 Å resolution (assuming it is measurable).

2.2.2 Thermal Diffuse Scattering

As seen in (6), the integrated intensities contain a contribution from Bragg diffraction as well as from diffuse scattering and background. TDS is caused by energy

exchange between the scattered radiation and the low-frequency lattice vibrational modes. The inelastic TDS has an opposite trend with respect to elastic scattering, which decreases as a function of θ. A proper estimation and correction of TDS is difficult and approximated algorithms are used in data correction procedures to extract pure Bragg intensities. The TDS depending on optic modes is typically included in the background, being substantially constant across the diffraction peak. The contribution from acoustic modes, instead, should be corrected separately with first- and second-order contributions [23] depending on the diffraction angle θ, the wavelength λ, the temperature, and the elastic constant of the crystal. Modes with frequency larger than 100 cm^{-1} are practically in the ground state at $T \approx 100$ K [27]; however, in molecular crystals librational modes may have smaller frequencies, suggesting that lower temperatures are necessary. TDS may significantly affect the correct estimation of atomic displacement parameters. At $T \approx 10$ K, instead, TDS is practically negligible up to the resolution normally obtained in an X-ray diffraction experiment.

2.2.3 Anharmonicity

The harmonic approximation assumes that forces between pairs of atoms are linearly proportional to their displacements. However, the potential is typically not harmonic and the **u** tensor should be replaced by a cumulant expression [17, 18], which significantly increases the number of parameters and may introduce additional correlation in a least squares refinement. At low temperature, anharmonic effects are typically negligible. Anyway, a good test to detect anharmonic motion is to check the thermal *expansivity* of the crystal by measuring accurate unit cell parameters at various temperatures. As outlined in Sect. 2.1, the equation of state is predicted from the Grüneisen parameter γ which basically accounts for the deviations of the atomic oscillators from the equilibrium positions expected from pure harmonic motion. Macchi and Sironi [28] for example showed the close relationship between anharmonic motion of some atoms and total strain of an organometallic molecular crystal. It is interesting that those atoms significantly modify their equilibrium position as a function of temperature (another indication of large anharmonicity).

A separate discussion of hydrogen atoms is merited, especially when involved in hydrogen bridges. The potential experienced by an H atom is highly anharmonic, especially when the hydrogen bonding is stronger. For this reason the atomic thermal parameters are usually very difficult to refine even from neutron diffraction experiments (the hydrogen scattering of X-rays being quite scarce). In general one can consider the thermal parameters of hydrogen as highly approximated anyway. Some procedures are known to calculate hydrogen displacement parameters from refined thermal motion of other atoms in the molecule, under rigid body assumption and including known high frequency stretching of X–H bonds (from infra-red spectroscopy or theoretical calculations [29]) or otherwise estimated from neutron diffraction works [30].

2.2.4 Radiation Damage and Crystal Instability

It may be that an important source of systematic errors can be corrected by monitoring standard intensities at regular intervals during data collection. Chemical damage of organic materials by X-rays and other forms of ionizing radiation is classified as *primary* or *secondary*. Primary damage is caused by interaction between the radiation beam and electrons of the compounds and it mainly depends on the radiation dose [31]. On the other hand, secondary damage, caused by the reactions of the resulting radiolytic products is typically reduced at low temperature [32, 33], though not completely removed. Another source of instability is the loss of solvated molecules that might occur even at temperatures well below the boiling point of this solvent (thus at temperatures at which the vapor tension of the solvent as pure liquid would be very small). De-solvation often damages the crystal, creating extended defect or fractures that severely affect the sample quality. Of course, by lowering the temperature all these phenomena can be reduced or even completely avoided for the entire duration of an experiment. Cooling the sample may not be the only way to solve the problem; in fact coating the sample properly, such as with a poly-fluorinated oil, or enclosing it in a closed capillary, may also be adopted. These alternatives allow temperature dependent investigation of the sample (even above room temperature).

Protective oil is a good alternative to glue for crystal mounting because of the large residual stress that epoxies can cause. Sensitive species must be handled with special care, because exposure to oxidative atmosphere or anyway to ambient temperature may produce irreversible damage. For this reason, mounting the sample in an inert atmosphere within glove boxes is sometimes necessary. If the sample must be kept at low temperature while under the microscope, special equipment is necessary as proposed by Stalke [34] or by Hope [35].

3 Cryo-Crystallographic Techniques

There are several techniques to cool crystal sample in order to carry out crystallographic studies and they depend on: (1) which investigation is carried out; (2) what is the target temperature; (3) how rapid shall be the experiment; (4) the available budget. In the following we will analyze these methods.

3.1 Single Crystal Diffraction

The diffraction from single crystals is certainly one of the most accurate techniques to obtain detailed information on the disposition of atoms in a solid. The crystals typically are very small ($\sim 10^{-3}$ mm^3), and they are selected and mounted in air on very small supports like glass fibers (or other amorphous and low absorbing

materials), centered on the goniometer of a diffractometer, and then exposed to the X-rays. Therefore, cooling a single crystal means removing a very small amount of heat from a very small sample (rotated in many positions during the experiment). The air medium around the crystal is often a problem. The humidity of the air, in fact, is enough to cause icing, whatever the method adopted to cool the sample.

The rapidity of currently available diffractometers equipped with area detectors allows employing the so-called open flow systems, where a cryogenic fluid (He or N_2) is flushed onto the sample without recycling. It is clear that these methods could be very expensive; however, liquid N_2 is typically available at low cost in many laboratories. The most commonly adopted systems use liquid nitrogen both to obtain an N_2 gas flow and to cool it to a temperature around the boiling point of nitrogen (77 K) [36]. The temperature is then adjusted by electric warming of the gas flow (in principle even above room temperature, guaranteeing uninterrupted data collection over a very wide temperature range). Earlier systems regulated the gas stream temperature by adjusting the evaporation of liquid nitrogen, a procedure that however required large amount of N_2 (especially to reach lower temperatures).

In the last decade, open flow systems working with helium have been introduced, despite the much higher costs of liquid or gaseous He. In experiments carried out at Synchrotron work stations, the measurements are very rapid. This makes less prohibitive the costs of experiments carried out with open flow He cryostats. Modern equipment for X-ray diffraction in laboratories (using rotating anode generators, multilayer optics, and area detectors) also allows rapid data collection. Therefore, open flow He systems are not so uncommon in university laboratories. Helium gas flow systems work using evaporation of the cryogenic medium [37, 38], consumption of which is proportional to the required temperature. These systems offer all the advantages of open flow systems, namely optical access to the sample, rapid or even flash cooling, low background of the gas stream. However, they also have some inherent defects: large consumption of the cryogen (that makes them inadequate for very long measurements), the necessity to have a warm outer stream to avoid turbulence and ice formation. Some problems could be created by a thermocouple in the gas stream, used to measure the flow temperature, because it could induce turbulence and break the laminar flow, which is particularly fragile for a He stream.

A different solution is a He gas stream refrigerated through conduction by a two-stage closed-cycle cooler, which works with compression/expansion cycles of He gas. The advantage of the two-stage cooler is the higher temperature stability, but the disadvantage is the base temperature ($>$10 K) and the temperature loss to reach the sample (ca. 15 K). The smaller amount of cryogen used with this system makes consumption very low, but the escaping attitude of low density He requires an open cup at the end of the nozzle to bring the flow onto the sample. The cup could be produced by a very light material (like beryllium) which is quite transparent to the X-rays. Proper windows for incoming and outgoing primary beams will decrease the scattering and therefore the background. An outer warm and dry stream is necessary to prevent icing formation.

The two-stage (or even three-stage) closed cycle systems were actually invented to cool the crystals directly through conduction, keeping the samples in closed

devices (like Be cups), evacuated to prevent icing. These systems are still quite common in laboratories and especially in large scale facilities. They offer excellent accuracy, the possibility to reach very low temperature without trouble, virtually no consumption of cryogenic medium, and long term stability, but they have some disadvantages when compared to open flow systems: (1) direct optic access is not possible (unless using the system proposed by Samson et al. [39]); (2) scattering from the rather thick cups must be considered and eliminated [40]; (3) rapid quenching of the sample is not possible. The two-stage devices are however extremely recommended for long measurements.

Most of the devices presented in this discussion could be used in both X-ray or neutron diffraction experiments, though taking into account that neutron diffraction measurements are typically longer and carried out on larger samples.

As has become clear, all devices may introduce some kind of error during data collection. Therefore proper corrections should be applied when analyzing the data. In particular, absorption of cups surrounding the crystal should be taken into account. Extra scattering is also present, but this is usually eliminated with proper masks (physical or digital) to avoid detection of spurious intensities. The scattering by the gas stream is usually a minor problem that increases the background in the same way as a glass fiber support. This is typically well accounted for from background determination of the image integrating software. Formation of ice, instead, would create more serious problems due to the increased and structure background produced and the absorption of incoming and diffracted beams.

Although this chapter is dealing with cryo-crystallography, as anticipated above, many studies of crystals over a large temperature range would be useful, and then one could also consider techniques to heat crystals, which are quite developed in large scale facilities and also available on the laboratory scale. Liquid nitrogen gas flow instruments are also typically able to reach temperatures above ambient (up to 500 K) and therefore are quite adequate for the study of organic crystals, taking into account that only a few species would remain solid (and crystalline) above those limits. For refractory materials, instead, more sophisticated heating is necessary, able to reach $T = 1{,}500$ K or more, using flame-heated inert gas [41, 42], electrically heated gas streams [43], or radiative heat transfer [44].

3.2 Powder Diffraction

Many kinds of high and low temperature chambers are available for diffraction of polycrystalline materials. In general, the equipment is based on similar technology as for single crystal diffraction techniques, although a great advantage is given by the reduced (or even null) movements of the sample holder. Therefore, low temperatures can be obtained by conduction in a much simpler way, using radiation transparent windows in the chamber to reduce absorption and background [45, 46]. High frequency induction and high vacuum are necessary to reach safely very high temperatures (for example above 2,000 K [47]).

3.3 Other Techniques

As mentioned above, cryo-crystallography is not just diffraction at low temperature and many other investigations could be carried out to obtain precious information on the chemical physics of a crystal. For example, differential scanning calorimetry (DSC) indicates the occurrence and the nature of a transformation, like a solid–solid phase transition. DSC instruments are quite common in many chemistry laboratories and low temperature attachments are easily installed. They work with cold nitrogen streams or otherwise removing the heat from the sample through conduction. DSC detects the heat of a transition, i.e., generated by the discontinuity in the enthalpy at the critical temperature; see Fig. 5. Some phase transformations are not associated with detectable heat of transition, but they show instead a discontinuity in the specific heat C_p. Modulated temperature DSC is a more recent technique [48, 49] that allows separation of reversible and irreversible heat exchange during the process. This could be useful to recognize processes controlled by thermodynamic equilibrium or by kinetic effects and the occurrence of hysteresis during phase transformations.

Another very useful technique for cryo-crystallographic studies is optical microscopy, in particular under polarized light. Cooling stages can be easily

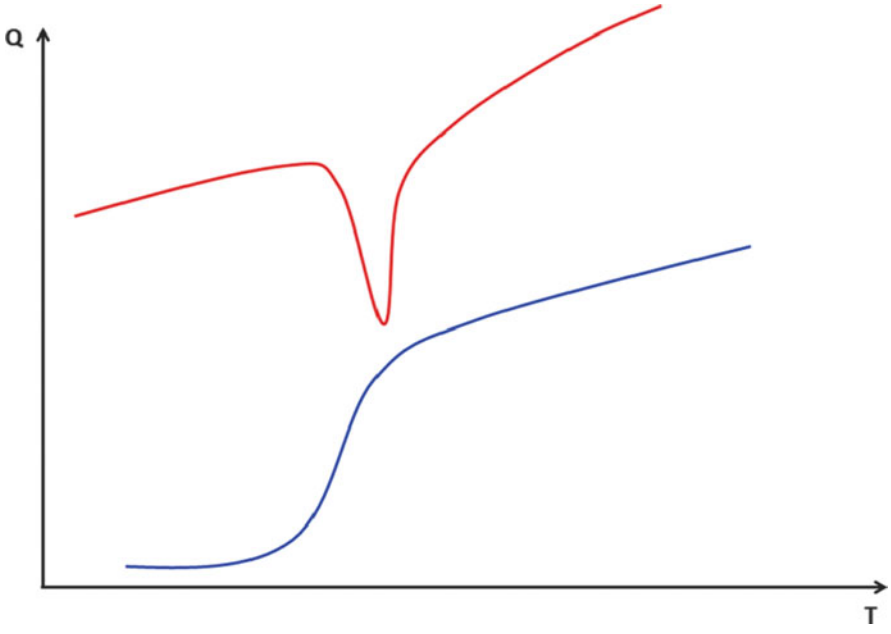

Fig. 5 The typical DSC diagram for solid state phase transition with latent heat (*red plot*) or without latent heat (*blue plot*). The scale is not the same; in general the curve for a second-order transition (*blue plot*) is associated with smaller changes of heat capacity (and therefore more difficult to detect)

attached on an optical microscope, allowing temperature ranges from 100 K up to very high temperatures. The hot/cold stage could be coupled with a calorimeter, allowing simultaneous heat capacity measurements. One very useful observation is the birefringence of the crystal that depends on the lattice symmetry and therefore can be sensitive to changes like structural phase transitions. The birefringence is easily observed using (partial) polarized light, cross polarizers, or otherwise a rotating polarizer (which gives the greatest amount of information) [50–53].

Computational chemistry is of course another technique to obtain theoretical information on *perfect crystals* at variable temperature. The background for this approach has been introduced in [113] and will not be further discussed here. It is important to stress that cryo-crystallography is not necessarily an experimental science, because predictions or explanations obtained from theoretical modeling are equally important in modern studies.

4 Cryo-Crystallographic Studies

Sometimes crystallographers consider that measuring a crystal at very low temperature is a kind of *panacea*, able to solve all defects of the sample, all kinds of experimental errors, and enhance the response indefinitely. Young students might be disappointed to learn that these miracles do not take place. A bad crystal sample remains as such even at 10 K, and sometimes it becomes even worse because the cooling process and the residual stress induced by a temperature gradient may produce further damage to the sample. Many other kinds of experimental problems and sources of error (for example absorption, extinctions, disorder, etc.) are not attenuated by the low temperature.

So, what can a scientist expect from a crystallographic study at low temperature? We give in the following a bunch of examples that cannot be fully comprehensive but should illustrate the potential of cryo-crystallography.

4.1 Crystal Structure Solution and Refinement

As anticipated, lower temperature increases the number of observations from an X-ray diffraction data collection (at constant radiation dose). This is however just one of the advantages that could improve a structure solution or a refinement. In fact, a reduced thermal motion usually implies a more reliable “standard” model, given that for smaller atomic displacements the harmonic approximation is more appropriate and less correlation is found between variables within a least squares refinement. This returns higher precision of the parameters calculated from those variables (for example bond distances, bond angles, etc.).

In X-ray diffraction experiments on small organic/organometallic molecules, it is less likely that low temperature will be really necessary to *solve* a crystal

structure, unless the species is unstable in air or under the X-rays. In this case, the low temperature could be one of the ways to reduce the damage (the easiest but not necessarily the best). Refinements of low temperature datasets are certainly easier because the increased resolution limit enables a larger observations/parameters ratio. However, if a crystal structure is severely affected by poor long range order (static disorder), a large part of the scattering which concentrates outside the Bragg position is often understood just as uninformative background noise. If diffuse scattering due to static disorder is large, any attempt to decrease the temperature with the purpose of obtaining more observable intensities would just be a desperate move and unlikely to succeed. On the contrary, one should provide thermal energy to increase long range order, for example through high temperature annealing.

The situation is different when the disorder is dynamical in nature, as it might occur when molecules have peripheral functional groups with enough flexibility to show large libration (in the gas phase and likely in the solid state as well). For example, substituted methyls are often associated with a relatively flat potential for rotation about the pseudo threefold axis. This implies very large displacements of the three carbon atoms. Sometime, the flat potential might display two or more minima and the dynamic disorder can somehow be "localized" with two or more competing conformations (see Fig. 6). By lowering the temperature the dynamic disorder can be significantly reduced or suppressed. In fact, one molecular conformation becomes favored over the other(s) either because the shape of the potential is itself modified or because the thermal energy is much reduced and less stable conformations become unavailable.

Other typical disorder conformations in the solid state are those of E-stilbenes or azobenzenes, where the two atoms of the central double bond are often involved in a complicated dynamic process (called *pedal motion*; see Fig. 6). Many crystallographic studies have been dedicated to analyze this kind of structural feature, including theoretical modeling of the dynamics; see, for example, Harada and Ogawa [54] and references therein. It is important to stress that this kind of dynamics severely affect the equilibrium positions of atoms refined from X-ray (or neutron) diffraction data, especially if the disorder cannot be satisfactorily modeled. As a consequence, geometrical parameters calculated from refined coordinates are typically quite incorrect (with severe underestimation of bond lengths). This is in general true when the thermal motion of a given molecule in a crystal is large. Therefore, a correction for libration, translation, and coupled translation/libration is necessary [13] to extract reliable bond distances from a set of refined coordinates. Unfortunately this correction is seldom applied and theoretical chemists often use uncorrected geometries as benchmark experimental results to test ab initio calculations.

For minerals and inorganic samples, low temperature is almost useless to improve structure solution and only marginally relevant to improve the refinement, unless dealing with host–guest materials like zeolites. In facts, for harder materials ambient temperature is already quite comparable and sometimes lower than the Debye temperature. Therefore, resolution is seldom a limitation for structure refinement of minerals at ambient temperature. On the contrary, for macromolecules and especially for proteins, the low temperature significantly increases the number of

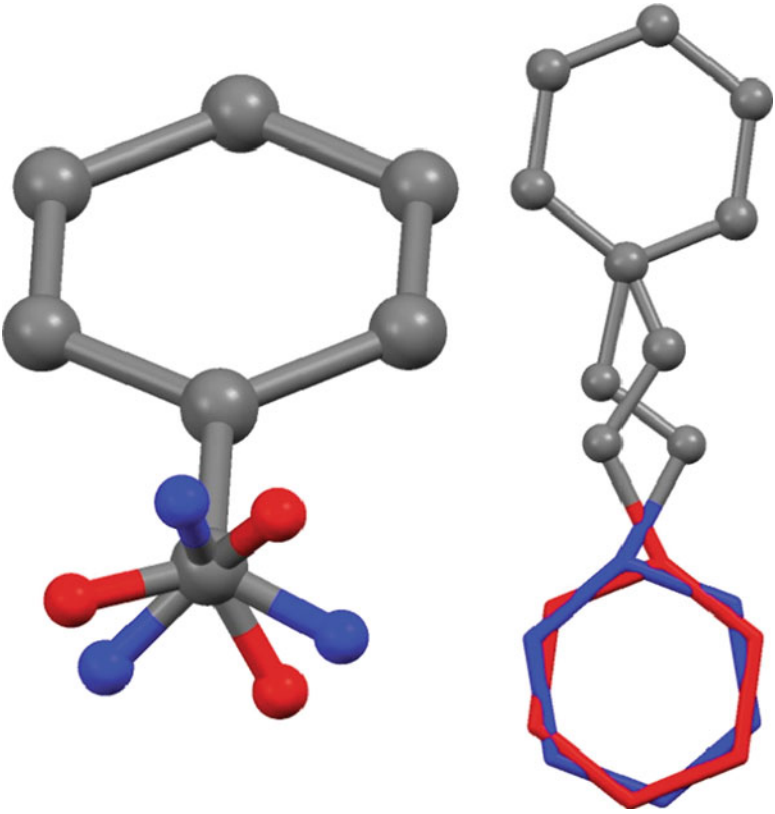

Fig. 6 The typical disorder of CX_3 peripheral groups about the pseudo threefold axis (*left*) and the typical pedal motion disorder about the central double bond in E-stilbene kind of molecules (*right*)

observations, especially because of the larger amplitudes of motions in these samples. In addition, proteins are often damaged by the incident radiation and cryo-protection is vital. As a consequence, the low temperature is a must for protein crystallographers [32], because it is relevant for the structure solution, not only for the refinement. However, cryo-crystallographic studies are not without disadvantages. For example, the crystal quality might be damaged by the cooling, in particular flash cooling. Moreover, some debate is open on the actual biological relevance of structures determined at 100 K, but intended to answer questions about phenomena occurring at 300 K [55].[2] In macromolecular crystallography, another debate is ongoing concerning the improvements when using helium as cryogenic agent [56] and therefore measuring the data at temperatures well below 80 K.

[2] More generally, one may ask whether a (macro)molecule frozen in the solid state can really be representative of the structure in solution, where the molecule is in fact active.

Interesting applications of low temperature single crystal diffraction have been presented in the field of metal organic frameworks (MOF) [57], porous materials based on metal *connectors* and organic *linkers*. Some large pore MOFs are able to host and exchange molecules like N_2, CO_2, noble gases, etc. The main problem is locating sites where the gas might be trapped, given the very weak interaction between the guest and the host framework. Therefore, diffraction on cooled species could reveal sites available to N_2 and Ar in channels of MOF-5, a structure with cubic array of $Zn_4O(CO_2)_6$ units connected by phenylene linkers [58].

Sometimes low temperature is claimed to be important to locate H atoms. This could be true in the case of organic species, where a reduced thermal motion of Hs may enhance electron density peaks in Fourier maps. These peaks compete against lone pair peaks of some atoms (especially O or N) and against peaks inside chemical bonds. At low temperature and using only low resolution data, the residuals due to lone pairs are smaller than residuals due to H atoms, yet missing in the structural model. In the case of organometallic molecules, expectations should instead be much lower, because the main source of residual peaks in a Fourier map are uncorrected absorption effects, which might overlap with potential sites for H atoms, especially those in the vicinity of a metal (like an agostic hydrogen, a hydride, etc.) [59].[3] Therefore, reducing the thermal motion of H may not be sufficient if careful absorption correction is not applied.

A problem affecting X-ray diffraction pattern of real crystals very close to ideality is *extinction* that is the manifest breakdown of the kinematic approximation. Within the mosaic crystals theory [60] we recognize a *primary extinction* (attenuation of the incident beam within a given crystal domain) and a *secondary extinction* (power loss due to the diffraction in the blocks traversed by the incident beam before it reaches the particular block under consideration). *Primary extinction* depends on the size of the domain and on the amplitude of the structure factors. The "critical thickness," beyond which extinction is negligible, inversely depends on λ. *Secondary extinction* depends on the degree of perfection of the crystal, hence on the misalignment of the domains (or mosaicity). The critical *mosaic spread*, above which the effect is negligible, depends on λ. Extinction may be severely anisotropic, especially if the crystal is under stress, which increases the mosaicity. As a matter of fact, an empirical way to decrease extinction is to stress the crystal by cooling and warming it, though the crystal may break during this procedure. For example, the mosaic spread of a crystal of KHC_2O_4 [61] studied at low temperature with three consecutive experiments was initially 3.3″ (first experiment with MoKα

[3] In [59] the authors reported the structure of a tri-osmium complex containing a hydride and clearly stated that a low temperature X-ray diffraction experiment would not be useful to locate the hydride if an accurate absorption correction is not carried out. Curiously, a few years before they had contacted Prof. A. Sironi and myself at the University of Milan proposing a low temperature data collection on that compound, with the purpose of locating the not so clearly visible hydride. As evident from [59], we were able to convince them on the real problems connected with the location of hydrogens close to heavy metals.

radiation at 125 K), 7.6″ (second experiment with AgKα radiation at 11 K), and 11.3″ (third experiment, AgKα at 11 K). The larger mosaicity implies that fewer reflections are significantly affected by extinction. As a matter of facts, an old technique used by macromolecular crystallographers was in fact shocking the crystal with rapid cooling in liquid nitrogen. The variation of extinction should be taken into account when repeating low temperature experiments on the same sample, sometimes producing scarce reproducibility of the data.

4.2 Accurate Electron Density

The possibility of probing the electronic structure of atoms by means of X-ray was recognized quite soon after the first diffraction experiments. In 1915, P. Debey wrote: "*It seems to me that experimental study of the scattered radiation, in particular from light atoms, should get more attention, since along this way it should be possible to determine the arrangement of the electrons in the atoms*" [62]. However, it was only in the mid-1960s that experimentally determined electron density maps could be obtained by combining X-ray and neutron diffraction techniques [63]. This breakthrough was certainly due to the improvements of the data measuring (using computer-controlled diffractometers and scintillator counters instead of photographic films), to the advent of neutron diffraction and the possibility to cool crystals, and to the significant improvement in data reduction and correction. Since then, methods of accurate determination of electron density have much improved together with models for mapping electron density from X-ray intensities [64]. We will now outline the advantages offered by low temperature measurements in this area, taking into account of course that for some materials cooling is absolutely mandatory, whereas for others it is not, although it could anyway be important to improve the quality of the data.

The mean thermal electron density in the unit cell can be computed by Fourier summation, over the reciprocal lattice vectors **S**, of the X-ray crystal structure factors:

$$\rho(\mathbf{r}) = 1/V \sum_{\mathbf{S}} \mathbf{F}(\mathbf{S}) \exp(-2\pi i \mathbf{S} \cdot \mathbf{r}_p). \tag{10}$$

Due to termination of the series, however, $\rho(\mathbf{r})$ is severely affected by ripples. In addition, especially in the case of non-centrosymmetric crystals, the phase of vector $\mathbf{F}(\mathbf{S})$ is not known with precision and this affects a correct reconstruction of the density. Therefore, Fourier summation cannot be used for precise and accurate mapping of electron density. On the other hand, a model is necessary to overcome these limitations and to produce a function that is sufficiently close to the real, quantum mechanical $\rho(\mathbf{r})$ in all regions of the crystal.

In the 1970s, among many other approaches, the method of multipolar expansion of atomic electron density was recognized as the most applicable and accurate.

Several formulations were proposed [65, 66], but the intuitive notation introduced by Hansen and Coppens [67] afterwards became the most popular. Within this method, the electron density of a crystal is expanded in atomic contributions. The expansion is better understood in terms of rigid *pseudoatoms*, i.e., atoms that behave structurally according to their electron charge distribution and rigidly follow the nuclear motion. A *pseudoatom* density is expanded according to its electronic structure, for simplicity reduced to the *core* and the *valence* electron densities (but in principle each atomic shell could be independently refined). Thus,

$$\rho(\mathbf{r}) = \sum_{\text{atoms}} \rho_i(\mathbf{r} - \mathbf{r}_i),$$

$$\rho_i(\mathbf{r}) = P_{i,\text{core}}\rho_{i,\text{core}}(\mathbf{r}) + P_{i,\text{valence}}\kappa_i^3\rho_{i,\text{valence}}(\kappa\mathbf{r}) + \sum_{l=0,l\,\text{max}}\left[\kappa_i'^3 R_l(\kappa'\mathbf{r})\sum_{m=0,l} P_{lm\pm}y_{lm\pm}(\mathbf{r}/r)\right] \tag{11}$$

The parameters $P_{lm\pm}$, P_{core}, and κ can be refined within a least square procedure, together with positional and thermal parameters of a normal refinement to obtain a crystal structure. In the Hansen and Coppens model, the valence shell is allowed to contract or expand and to assume an aspherical form [last term in (11)], as it is conceivable when the atomic density is deformed by the chemical bonding. This is possible by refining the κ and κ' radial scaling parameters and population coefficients $P_{lm\pm}$ of the multipolar expansion. Spherical harmonics functions $y_{lm\pm}$ are used to describe the deformation part. Several software packages [68–71] are available for multipolar refinement of the electron density and some of them [68, 70, 72] also compute properties from the refined multipolar coefficients.

As anticipated, the multipolar model is not the only technique available to refine electron density from a set of measured X-ray diffracted intensities. Alternative methods are possible, for example the direct refinement of reduced density matrix elements [73, 74] or even a wave function constrained to X-ray structure factor (XRCW) [75, 76]. Of course, in all these models an increasing amount of physical information is used from theoretical chemistry methods and of course one should carefully consider how "experimental" is the information obtained.

An X-ray atomic orbital (XAO) [77] method has also been adopted to refine electronic states directly. The method is applicable mainly to analyse the electron-density distribution in ionic solids of transition or rare earth metals, given that it is based on an atomic orbital assumption, neglecting molecular orbitals. The expansion coefficients of each atomic orbital are calculated with a perturbation theory and the coefficients of each orbital are refined to fit the observed structure factors keeping the orthonormal relationships among them. This model is somewhat similar to the valence orbital model (VOM), earlier introduced by Figgis et al. [78] to study transition metal complexes, within the Ligand field theory approach. The VOM could be applied in such complexes, within the assumption that the metal and the

ligands are linked by "low overlap" bonds between the atomic orbitals; therefore the electron density around metals or in the ligand shell can be treated as a perturbation of the atomic density. This assumption is also at heart for the determination of orbital coefficients from multipolar model as introduced by Coppens et al. [79].

All the above methods are somehow based on an orbital hypothesis. In fact, in the multipolar model, the core is typically frozen to the isolated atom orbital expansion, taken from Roothan Hartree Fock calculations (or similar [80]). Although the higher multipoles are not constrained to an orbital model, the radial functions are typically taken from best single ζ exponents used to describe the valence orbitals of a given atom [81]. Even tighter is the link to the orbital approach in XRCW, XAO, or VOM as described above. Obviously, an orbital assumption is not at all mandatory and other methods have been developed, for example those based on the Maximum Entropy Method (MEM) [82–86] where the constraints/restraints come from statistical considerations.

The role of low temperature in an experimental determination of electron density is multiple. The most important is certainly the reduction of thermal agitation of atoms, which makes the *pseudoatom* approach a more reliable approximation. As for normal structure refinements, smaller thermal motion means less correlation among parameters, hence higher reliability of the final model. Lower thermal motion also means that the harmonic approximation is more valid, as anticipated in Sect. 2.2. Although it is possible to go beyond the harmonic approximation, it should be considered that a model including, for example, a Grahm Charlier expansion [87] would be extremely costly because of the very large number of parameters. Mallinson et al. [88] could show that residuals due to anharmonic motion are somewhat similar to residuals of deformation density, especially when dealing with transition metals. This would of course create confusion between true electron density features and residuals due to atomic displacements exceeding the model, with obvious consequences for the interpretation of the results. It is important to warn that the physical significance of the refined Grahm Charlier parameters should be verified. In fact, it might easily occur that these coefficients are refined to nonsense values, implying, for example, negative nuclear probability at the equilibrium position (see the manual of the XD2006 package [68]).

Additional advantages of low temperature in electron density refinements are connected with the higher accuracy of the measured intensities, in particular at high resolution. It is important to stress that features of the bonding electron density are very likely not recorded at such resolution, which is typically dominated by core electron scattering (Fig. 4). However, the larger intensity at high angle can be very important to increase the precision of a refinement, including more reflections to refine atomic positions and thermal factors (apart for H atoms). As a matter of facts, a data/parameter ratio above 10 is often recommended; however in many cases the *effective* ratio is much smaller because variables introduced in (11) are mainly refined from low angle data [89]. Thus, if some parameters of a model (positions and thermal motion) could insist more on high angle observations, the correlation among variables would be significantly reduced.

Another reason why high angle reflections are better measured at low temperature is the decreased thermal diffuse scattering (see Sect. 2.2) which allows a more accurate integration of those intensities.

After listing all the benefits of low temperature on accurate electron density refinement, the reader might ask what temperature is really necessary. Of course, the lower the better; however some electron density studies at room temperature or even above have been reported. For example, Tanaka et al. have investigated electron density of lanthanide borides [90–92] at variable temperatures using XAO methods to determine the electronic configuration of the metal. Those studies addressed significant changes of the order and occupation of some electronic states (associated with 5d or 4f orbitals of Ce or Sm) as a function of temperature. Experiments of this kind are not so frequent and they would be quite impossible on molecular crystals. On the other hand, results of Tanaka et al. show that it would be interesting investigating temperature induced changes of electron density, even spanning the regime of high temperature.

We do not discuss here the very many applications of electron density studies which are summarized in many recent books on the subject [93–96].

4.3 Phase Transitions and Polymorphism

As introduced above, different forms of the same molecule can be observed in the solid state. The phenomenon is known as *polymorphism*, i.e., the concurrent presence of more crystal forms, only one of which is thermodynamically stable at a given pressure and temperature. However, more polymorphs can be observed simultaneously when kinetic conditions allow formation of metastable phases together with (or even in the absence of) the thermodynamically stable one. It might even occur that metastable phases are not recognized as such, simply because the most stable polymorph is (as yet) unknown. This might produce the extraordinary phenomenon of *disappearing polymorphs* [97].

At particular *critical* points (T_C, P_C) on the phase diagram of a substance, two phases can be found in thermodynamic equilibrium. Therefore, upon application of a pressure or a temperature gradient, a transformation occurs from one phase into the other. This is a *phase transition*, in many aspects similar to a transformation implying the change of aggregation state. However, the extent of the changes in a solid to solid transformation is much smaller. For example, latent heat or latent volumes associated with the transformations are quite small, sometimes even difficult to detect.

The number of cryo-crystallographic studies on phase transitions is quite large nowadays. It became common with the availability of single crystal diffractometers equipped with digital area detectors that allow very rapid data collections. They are optimal for repeated measurements at variable temperatures of the same crystal in a reasonable amount of time. Thus, the accurate and detailed structural information typically available from a single crystal X-ray diffraction experiment could be

associated with other useful information like, for example, heat capacity and heat exchange measurements (available from DSC experiments) or spectroscopic signals.

The nature and the mechanisms of phase transitions in solids are still matters of discussion among crystallographers, as nicely summarized in a recent review [20]. The reader is referred to textbooks for a comprehensive overview of this long term debate [98–100]. Here, we summarize the qualitative nature of the main changes that are possible in a solid to solid phase transition.

4.3.1 Symmetry

The symmetry of the crystal system could change during a phase transition, because of a transformation of the lattice into a super-lattice or sub-lattice, a change in the space group type, or both.[4] A change of symmetry (in $\mathbf{R}^3$ space) is a sufficient condition to assess occurrence of phase transformation, but it is not a necessary condition. It is not rare, however, to observe iso-symmetric phase transitions, i.e., transformations where the space group type and the lattice type remain unchanged, but two different structures are in equilibrium at the critical point. As a consequence the sole determination of the space group may not be sufficient to detect a phase transition, unless the thermal *expansivity* is calculated (which usually requires measures over a wide temperature range). A discontinuity of the cell volume [hence of the molar volume, $V(T)$] or a discontinuity of the *expansivity* [first derivative of $V(T)$] would reveal a phase transition. For the second-order type of transition (see below) it is necessary that a group–subgroup relationship is maintained between the space groups of the two phases [94, 95].

4.3.2 Molecular Structure

Changes of molecular structure as a function of temperature could reveal a phase transition. However, caution should be applied because molecular geometries can change together with crystal shrinking or expansion without implying formation of a new phase [101]. So what kind of structural changes are compatible with a phase transformation? If a crystallographic symmetry element appears/disappears through the phase transition, then the space group type would change and the phase transition is evident. Iso-symmetric phase transitions are instead caused by a

[4] It is important here to talk about *space group type*, not just *space group*. In fact, the *space group* is determined by the combination of lattice and symmetry operators. When crystallographers report a given *space group*, in reality they refer to a *space group type* (i.e., the coincidence of symmetry operations with those cataloged in the International Tables of crystallography, regardless the actual lattice dimensions). This distinction is particularly important when discussing phase diagrams and in particular it is fundamental to appreciate the exact meaning of *iso-symmetric* phase transition.

discontinuity of molecular or supramolecular geometries, for example a tautomerism, an intra-crystal oxidation/reduction, etc. It is important to note that sometimes it is not so easy to establish the discontinuous nature of the changes. For example, a proton shift within a strongly hydrogen bonded system might occur continuously [102] due to softer changes of the crystal potential, which come from the resonance between two (or more) electronic configurations. However, a discontinuity could also occur [103, 104] when the two electronic configurations of an HB system produce two separate minima on the potential energy surface. In this case, two distinct phases are recognized and a transition is established at (T_c, P_c).

4.3.3 Order of Transition

When the free energies F of the two crystal structures are identical, the system is at a critical point. The identity of F does not imply identical functions (otherwise the two phases would be indistinguishable). Therefore, at the critical point first derivatives of F might differ and therefore enthalpy, volume, and entropy of the two phases would be different. These transformations are first-order phase transitions, according to Ehrenfest [105]. A discontinuous enthalpy implies heat exchange at the transition temperature, which can easily be measured with DSC experiments. A discontinuous volume is evident under the microscope or, more precisely, with diffraction experiments on single crystals or powders. Some phase transitions are however characterized by continuous first derivatives of the free energy, whereas the second derivatives (specific heat, compressibility, or thermal expansivity, etc.) are discontinuous. These transformations are second-order transitions and are clearly softer.

To exemplify first- or second-order phase transitions, the behavior of $Co_2(CO)_6(As(C_6H_5)_3)_2$ [106] (**1**) (see Fig. 7) and $Ni(C_2H_8N_2)_3(NO_3)_2$ (**2**) [107] (see Fig. 8) are illustrated. In **1**, a transition occurs on cooling the crystals from ambient condition at ca. 210 K. The space group type (R-3) does not change, but the *c*

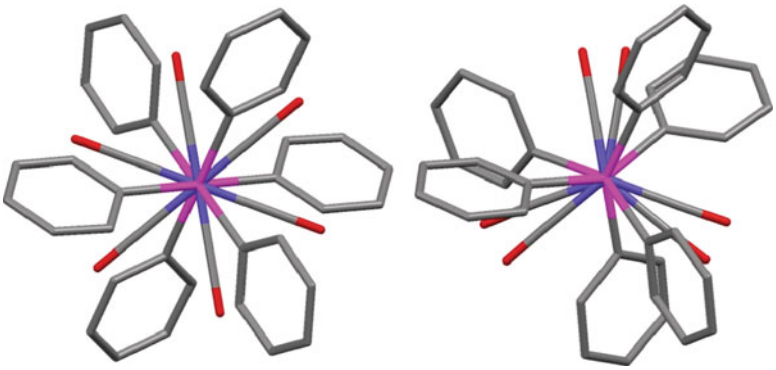

Fig. 7 The molecular transformations occurring in $Co_2(CO)_6(XPh_3)_2$ (X = P, As) from ambient conditions (*left*) to low temperature or high pressure (*right*). The conformational change occurs gradually and at (T_c, P_c) the two structures coincide

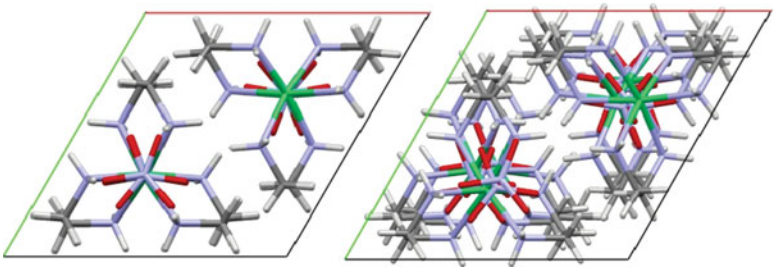

Fig. 8 The changes occurring to $[Ni(CH_2NH_2NH_2CH_2)_3][NO_3]_2$ from ambient conditions (*left*) to low temperature (*right*). The molecular geometry remains almost unchanged, but the molecule moves away from a crystallographic threefold axis producing a 3_1 helix. The phase change occurs abruptly and at (T_c, P_c) the two structures are different and in equilibrium

axis of the hexagonal lattice doubles below a critical temperature. At molecular level, the molecular symmetry is reduced from −3 to 3 and therefore the perfectly *staggered* conformation. Free rotation is now possible for the two $Co(CO)_3As(C_6H_5)_3$ moieties about the Co–Co axis. As temperature is lowered, the molecular conformation further changes, maintaining the threefold symmetry and without reaching the -6 m^2 of a perfectly *eclipsed* conformation. It is interesting that the group–subgroup relationship at the critical temperature is such that no low temperature phase is actually possible above T_c, because the two phases are simply identical. Therefore this is an example of second-order type phase transition, described in detail by Landau [99]. No discontinuity of molar volume, enthalpy, and entropy are expected, as in fact demonstrated by X-ray diffraction and DSC measurements. Sometime these transitions are called *continuous* to emphasize this soft nature, but one should remember that expansivity and specific heat are discontinuous. The high temperature phase could exist below T_c, but no hysteresis is observed. Notably the same kind of phase transition is observed for the isomorphic species $Co_2(CO)_6(P(C_6H_5)_3)_2$ (**3**) [108] and both **1** and **3** undergo similar transitions at high pressure.

$Ni(C_2H_8N_2)_3(NO_3)_2$ is quite different – the space group type and the lattice change at T_c (ca. 106 K). The transition show discontinuity of the cell volume and, as expected, there is a latent heat of transition. Notably at the critical temperature the two phases are structurally different and therefore they are in equilibrium at that temperature. A minor hysteresis is observed.

As mentioned above, sometimes two phases are recognized even though no space group type change is actually observed. Often these transformations are associated with competition between two different electronic configurations, or otherwise between two molecular conformations. The latter are, for example, order/disorder transitions caused by a group in a molecule subjected to weakly bounding potential surface, and therefore showing multiple minima. Below a critical temperature the higher energy conformer may be inaccessible. More interesting is instead the competition between different electronic configurations. This implies different bonding in a molecule (or supramolecular synthon). Notable examples are *spin crossover* materials, i.e., molecular compounds with the ability

to switch between a paramagnetic high spin state (HS) and a diamagnetic low-spin ground state (LS) [109]. This does not necessarily imply a change of crystal symmetry, but it clearly modifies the molecular geometry, hence the packing.

Phase transitions are not only characterized by atomic or molecular structural changes – they can also be characterized by significant modifications in the microstructure and domains and at a much larger size scale. One notable example has been recently reported by Glazer et al. [110] using linear birefringence measurements in $LiTaO_3$ and $LiTa_xNb_{1-x}O_3$ crystals at high temperature.

In this chapter combinations of low/high temperature and pressure have not been discussed. However it might be clear that this would offer even further possibilities to expand the knowledge on the phase diagram of a given species. At the same time, further accuracy could be obtained from high pressure experiments if atomic displacements are significantly reduced. Most of these applications can be exploited in the future with the continuous improvements of high pressure techniques and scientific research in this area (see [114] for more details).

4.4 Thermodynamics from Multi-Temperature Diffraction

As has become clear in previous sections, atomic thermal parameters refined from X-ray or neutron diffraction data contain information on the thermodynamics of a crystal, because they depend on the atom dynamics. However, as diffracted intensities (in kinematic approximation) provide magnitudes of structure factors, but not their phases, so atomic displacement parameters provide the mean amplitudes of atomic motion but not the "phase" of atomic displacement (i.e., the relative motion of atoms).[5] This means that vibrational frequencies are not directly available from a model where U_{ij} parameters are refined. However, Bürgi demonstrated [111] that such information is in fact available from sets of U_{ij}s refined on the same molecular crystals at different temperatures.

Using a rigid body approximation, i.e., assuming that molecules move in crystals without correlation between external (low frequency) and internal (high frequency) modes, Cruickshank demonstrated in 1956 how the atomic displacements (at just one temperature) could be used together with infra-red spectroscopy to obtain information on the entropy of crystalline naphthalene [112]. A partial rigid body approximation could also be applied to some functional groups in a molecule, having much lower rotational barriers (for example methyl, *tert*-butyl groups attached to some rigid aromatic skeleton).

Bürgi generalized the model, assuming a temperature independent high frequency term accounting for displacements due to the internal modes. By means of multi-

[5] This problem is sometimes referred to as the *second phase problem* in crystallography.

temperature experiments and refinement, the internal displacements can be separated from the external ones. In fact the atomic displacement parameters are expressed as

$$\Sigma^X(T) = \mathbf{AgV}\delta(T)\mathbf{V^T g^T A^T} + \varepsilon^X. \quad (12)$$

The matrices **g** and **A** transform the normal modes with frequencies v_i and eigenvectors **V** into atomic displacements Σ^X (T), ADPs are the 3 × 3 diagonal blocks of Σ^X, and ε^X is a temperature-independent term accounting for the high-frequency vibrations (internal modes).

The potentiality of multi-temperature studies has not been exploited much so far and only a few complete works have been reported in the literature; however it is likely that more applications could be proposed in the future.

5 Comments and Personal Perspectives

A typical paper on cryo-crystallographic applications is usually concluded by the author's encouragement to study more and more samples at lower and lower temperature. The message from this chapter is instead somewhat different and can be summarized as follows: (1) *use temperature critically and think carefully when it is necessary to measure structures or properties of crystals at lower temperature*; (2) *use all the additional information available when studying the sample at low temperature*; (3) *do not limit the temperature scans in the range below ambient conditions* (even when studying organic crystals).

Temperature offers an additional point of view on the structure of a material, and that's why it should be used with careful thinking and not randomly. In this respect, it is important to stress that what is the *ambient temperature* for the experimentalist may have no special meaning for the material under study. On the other hand, a given species may be of little interest at low temperature apart from the data quality improvement (this is especially true for compounds biological interest).

In this chapter we have emphasized that experiments on crystals at low temperature may provide information on the chemical bonding and on the thermodynamics of a system. A tight connection is evident between theoretical chemistry and cryo-crystallography.

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