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The temperature of flash-cooling has dramatic effects on the diffraction quality of nucleosome crystals

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Nucleosome core-particle crystals are routinely flash-cooled in liquid propane at temperatures of ~153 K, followed by transfer into a cold nitrogen-gas stream (~93 K). Analysis of diffraction data from crystals flash-cooled at different temperatures shows that the optimal temperature is ~153 K. The data quality worsens, with a concomitant reduction in the diffraction limit, at temperatures both higher and lower than 153 K. With some batches of crystals, significant shrinkage of the unit-cell volume is also observed at temperatures of 138 K and lower. The lattice shrinkage is always restricted to the *c* axis, concurrent with closer packing of two nucleosomes. Direct plunge-cooling of crystals in liquid nitrogen leads to loss of diffraction quality and resolution limit. Thus, in cases where flash-cooling into liquid nitrogen is detrimental to diffraction, optimizing cooling protocols at higher temperatures using liquid propane or other cryogens with similar properties may lead to dramatically improved results. In a related study, it is shown that a nucleosome crystal transported under 'cryocooled' conditions has higher mosaicity and yields inferior data quality in comparison to a crystal cryocooled at the synchrotron. For fragile crystals, transport in mother liquor and/or cryoprotectant followed by subsequent flash-cooling at the synchrotron may be the best procedure.

1. Introduction

In macromolecular crystallography, it is now standard practice to collect diffraction data at cryogenic temperatures (~100 K; Garman & Schneider, 1997; Pflugrath, 2004). Cryocrystallography dramatically reduces radiation damage to crystals, leading to diffraction data of better quality and higher resolution. It also facilitates the handling, storage and transport of crystals. The prolonged lifetime of cryocooled crystals has made the collection of multi-wavelength anomalous dispersion (MAD) data from single crystals a very common technique (Garman & Schneider, 1997; Hendrickson, 1991, 1999; Pflugrath, 2004). The crystals are usually harvested and stabilized in cryoprotectant solution prior to cryocooling. The various cryocooling procedures include cryogens such as liquid nitrogen, liquid propane, liquid ethane and tetrafluoromethane (CF_4). Flash-cooling directly in the cold nitrogen-gas stream is also common. While liquid nitrogen is commonly used to flash-cool crystals, there are a few examples for large macromolecular complexes where flash-cooling in cryogens such as liquid propane (at temperatures higher than that of liquid nitrogen) is the method of choice (for examples, see Hope *et al.*, 1989; Luger *et al.*, 1997; Sargent & Richmond, 2004). Some previous studies comparing the cooling rates for liquid nitrogen and liquid propane have shown that liquid

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propane has significantly higher cooling rates (Kriminski *et al.*, 2003; Teng & Moffat, 1998), while another study reported that the cooling rates for liquid nitrogen and liquid propane were similar (Walker *et al.*, 1998). These various studies involve intricate experimental setups and the results are not directly comparable. Thermocouples were also used as samples to obtain the measurements reported in these studies, which cannot be easily or directly extrapolated to macromolecular crystals. While the boiling point (231 K) and melting point (83 K) of liquid propane are well separated, the boiling point (77 K) and melting point (63 K) of liquid nitrogen are very close. Teng and Moffat reported that this narrow spread between the boiling and melting points for liquid nitrogen leads to 'film boiling' between 250 and 150 K whereby boiling of liquid nitrogen leads to the formation of an insulating film during flash-cooling. This 'film boiling' in turn reduces the efficiency of heat transfer in liquid nitrogen (Teng & Moffat, 1998). On the other hand, liquid-propane cooling is dominated by 'nucleate boiling' between 260 and 90 K, during which the coolant makes direct contact with the crystal. This leads to efficient heat-transfer and faster cooling rates over a much broader temperature range in liquid propane. Efficient heat exchange occurs with liquid nitrogen only when the temperature falls below 150 K (Teng & Moffat, 1998). Liquid ethane is also an effective coolant. Ethane has been historically used in cryo-electron microscopy and shares very similar properties to that of propane except that ethane is more difficult to liquefy than propane (the boiling point of ethane, 184.5 K, is lower than that of propane). Additionally, while the use of liquid ethane is allowed at most facilities, the use of liquid propane at synchrotron sources is restricted owing to safety issues and often necessitates the cryocooling of crystals in the home laboratory. Other cryogens include tetrafluoromethane (CF_4). CF_4 has been routinely used for several years as a cryogen in the Cryo-Xe-Siter device (Rigaku/MSC Inc.) used in the preparation of xenon derivatives (Jim W. Pflugrath, personal communications). The boiling and melting points for CF_4 are 145 and 86 K, respectively.

As seen above, liquid propane, ethane and CF_4 have a larger spread between boiling and melting points than liquid nitrogen, with liquid propane having the largest dynamic spread. This allows the optimization of cryocooling protocols at temperatures higher than liquid-nitrogen temperatures. Optimization of flash-cooling temperatures may be more critical than currently appreciated. Crystals of nucleosome core particles (nucleosomes) are one such example. The crystals in cryoprotectant are initially flash-cooled in liquid propane (at ~153 K) followed by subsequent exposure to the cold nitrogen-gas stream. This method is regularly used to obtain well diffracting nucleosome crystals (Luger *et al.*, 1997; Sargent & Richmond, 2004), although the mechanism underlying this observation remains largely unknown.

Here, we perform a systematic study to examine the effects of various propane temperatures on the diffraction quality of nucleosome crystals. We also compare the results obtained from crystals flash-cooled directly in liquid nitrogen with those obtained from our propane-cooling procedure at intermediate temperatures. Our results can be summarized as follows. Firstly, we do not observe ice formation for the entire temperature range studied here (113–193 K). The lack of ice nucleation during all our measurements suggests that the cryoprotectant is presumably in a 'vitrified' or 'glass' state in the temperature range tested, although the precise 'glass-transition' temperature (for a discussion of the phenomenon of glass formation in liquids, see Angell, 1995), which characterizes the vitrification properties of the cryoprotectant, has not been measured. This also suggests that the solvent, which is confined to narrow channels and cavities in the crystals (see §2.1 for a discussion of the solvent in the nucleosome crystals), remains in a glass state and does not crystallize within the studied temperature range. There have been similar reports on the absence of crystallization of solvent in narrow channels and cavities in protein crystals (Weik *et al.*, 2001). Secondly, the best diffraction data are obtained when the propane temperature is maintained at ~153 K during the flash-cooling step. Flash-cooling crystals at temperatures both above and below 153 K leads to significant deterioration in data quality, with a concomitant reduction in the diffraction limit. We also observe that around and below 138 K some crystals undergo a defined transition during which the *c* axis shrinks by 3.3%. Thirdly, we find that direct plunge-cooling of nucleosome crystals into liquid nitrogen yields irreproducible results in terms of the crystal unit-cell volume, diffraction quality and data resolution. Therefore, for fragile crystals, for crystals with high solvent content or for larger crystals with a tendency for lattice and/or mosaicity changes, optimizing cooling protocols at intermediate temperatures in cryogens such as liquid propane (or other cryogens with a dynamic temperature spread) may result in spectacularly improved results. Thus, cryogen, flash-cooling temperature and flash-cooling protocol need to be a variable in screening for optimal diffraction qualities, in addition to screening for crystallization conditions and cryoprotectant.

We also studied the effect of shipping a crystal in the 'frozen' state *versus* shipping it in mother liquor containing cryoprotectant. From this experiment, it appears that fragile crystals may be better preserved when transported under liquid conditions prior to cooling.

2. Materials and methods

2.1. Crystals

Preparation and crystallization of nucleosomes containing a palindromic 146 bp DNA fragment derived from human α-satellite DNA and four full-length *Xenopus laevis* histone proteins have been described previously (Dyer *et al.*, 2004, and references therein). The nucleosome crystals were grown by sitting-drop vapour diffusion. The crystals were grown in 1–2 weeks at 292 K in 40–45 m*M* $MnCl_2$, 35–38 m*M* KCl and 20 m*M* potassium cacodylate pH 6.0 containing ~20 μ*M* nucleosome core-particle complex. The sitting-drop technique yielded several crystals with dimensions of ~0.3 × 0.3 × 0.7 mm. The crystals belong to space group $P2_12_12_1$, with unit-

cell parameters as described in Tables 1 and 2. The crystals have a solvent content of 55%. The orthorhombic nucleosome crystals have narrow intermolecular solvent channels of ~15 Å in their longest dimension. The nucleosome core particle also has only a narrow central hole of ~10 Å along the superhelical axis, which is not completely hollow and is occupied by some of the histone-protein side chains (Luger *et al.*, 1997).

2.2. Flash-cooling procedure

The nucleosome crystals were harvested by stepwise soaking (~1–2 min each) of the crystals in mother liquor containing increasing concentrations of 2-methyl-2,4-pentanediol (MPD). The MPD concentration was changed gradually in 3%(*w*/*v*) increments *in situ*. The *in situ* harvesting procedure prevents repeated manipulation of these fragile crystals. The final cryoprotectant solution contains 24%(*w*/*v*) MPD and 5%(*w*/*v*) trehalose (often used in cryoprotectants as it reduces osmotic stress and prevents excessive crystal dehydration). The crystals were stable after soaking overnight in the final cryoprotectant solution.

Nucleosome crystals were routinely cryocooled in two steps. Firstly, liquid propane (propane tank with a diptube, instrument grade 99.5% purity, Scott Speciality Gases) was collected and pre-cooled in liquid nitrogen for a few minutes. An empty cryovial containing a stirbar and surrounded by a layer of insulating material was cooled in liquid nitrogen. The pre-cooled liquid propane was poured into the cryovial and allowed to gradually warm to 153 K or the desired final temperature, as shown in Figs. 1(*a*) and 1(*b*). Precooling the empty cryovial in liquid nitrogen ensures a slow and constant rate of increase in propane temperature (not shown). The slow kinetics of propane-temperature increase have been studied previously (Vernède & Fontecilla-Camps, 1999). The nucleosome crystal-cooling procedure takes advantage of the slow kinetics of propane for the precise control of propane temperature during flash-cooling and we exploit the same property in this study (as discussed below) for testing a wide range of propane temperatures during flash-cooling. The temperature of the propane in the vial is monitored using a thermocouple attached to a digital recorder. The length of the thermocouple inserted in the vial is identical to the length from the centre of the cryoloop to the base of the pin (Figs. 1*a* and 1*b*). This monitors the propane temperature at the height of the crystal in the vial. The crystal is flash-cooled by immersing in propane at ~153 K (or other desired final temperatures, see Table 1), as in Figs. 1(*a*) and 1(*c*). The crystal is then immediately transferred to the cold nitrogen-gas stream (93 K) for data collection, as in Fig. 1(*d*). The flash-cooled crystal can also be stored in liquid nitrogen for long-term storage, transport or subsequent data collection.

2.3. Data collection and refinement

Diffraction data from similar-sized crystals (~0.3 × 0.3 × 0.7 mm) that were flash-cooled at different propane temperatures (Table 1) were collected using our in-house rotating-anode RU-H3R generator and R-AXIS IV detector (Rigaku/MSC Inc.) at a wavelength of 1.5418 Å. Data comparing the effect of transport on cryocooled crystals (Table 2) were obtained at the Advanced Light Source, beamline 8.2.1, Berkeley, USA. Single measurements at each temperature are shown in Table 1, although data were collected from three crystals for each temperature and similar results were obtained from repeated measurements (not shown). Data were collected with identical data-collection strategies and parameters. Similarly, the two data sets reported in Table 2 were collected from two similar-sized fragments of one single long crystal. Diffraction data were integrated, scaled and merged with *HKL*2000 (Otwinowski & Minor, 1997). Mosaicity is defined in *HKL*2000 as the smallest angle through which the crystal can rotate about any axis or combination of axes while a reflection is still observed. Mosaicity estimates from the program also include contribu-

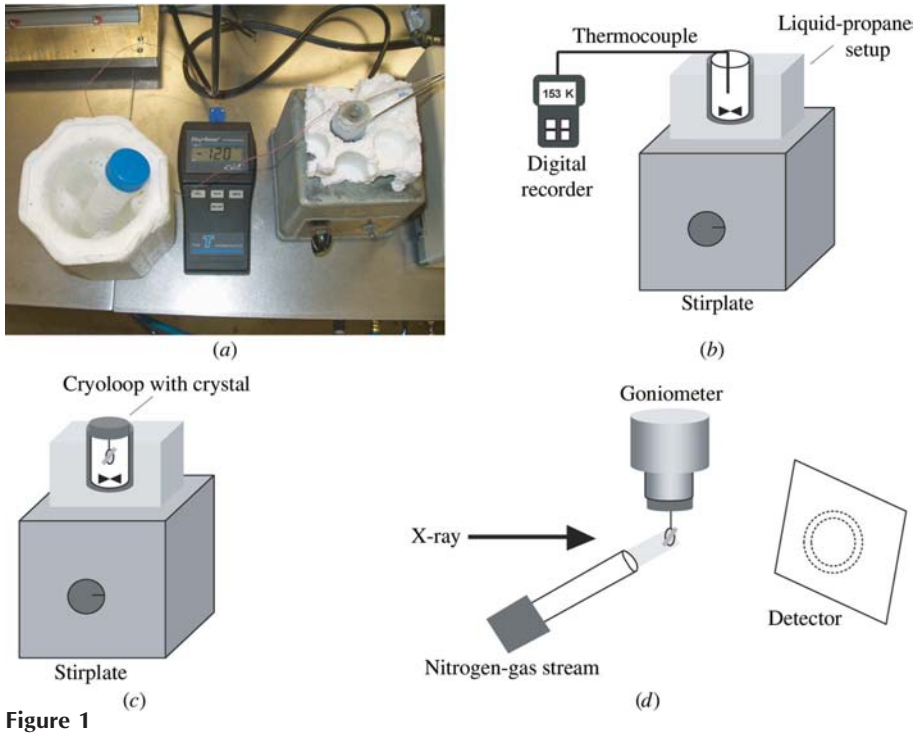

Figure 1
Experimental setup for flash-cooling nucleosome crystals in liquid propane. (*a*) Photograph of the propane flash-cooling setup. (*b*) Liquid propane (pre-cooled in liquid nitrogen) is allowed to slowly warm to around 153 K under continuous stirring. (*c*) The crystal is mounted in a cryoloop and flash-cooled by immersion in the cryovial when the propane temperature is ~153 K. (*d*) The flash-cooled crystal is quickly transferred to a cold nitrogen-gas stream (93 K).

Table 1
Effect of flash-cooling temperature on data quality.

Values in parentheses are for the highest resolution bin.

Data set†	193 K	173 K	153 K	143 K	138 K	133 K	113 K
Mosaicity (°)	0.7	0.6	0.8	0.6	0.7	0.9	0.8
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters (Å)							
a	106.0	106.0	106.3	106.1	105.3	105.2	104.9
b	110.1	109.6	109.8	109.6	109.3	109.4	109.2
c	181.9	181.9	181.9	182.0	175.6	175.4	175.1
Resolution range‡ (Å)	50–2.88 (2.98–2.88)	50–2.88 (2.98–2.88)	50–2.88 (2.98–2.88)	50–2.89 (2.99–2.89)	50–3.41 (3.62–3.41)	50–3.41 (3.62–3.41)	50–3.62 (3.90–3.62)
No. of unique reflections	43345	45523	44890	45425	27946	27927	20825
Completeness (%)	88.7 (77.9)	93.7 (88.3)	91.7 (78.6)	94.0 (83.7)	97.9 (93.5)	98.5 (96.6)	87.6 (90.4)
Multiplicity	3.0	3.0	3.1	3.1	3.2	3.1	4.0
Average $I/\sigma(I)$							
Overall	9.6	19.3	27.9	15.1	13.9	11.5	12.3
Highest bin	2.2	3.6	6.5	2.4	2.8	2.7	2.8
3.91–3.62 Å bin	6.5	15.4	26.2	12.3	4.6	4.0	2.8
2.98–2.88 Å bin	2.2	3.6	6.5	2.4	—	—	—
$R_{\rm merge}$§							
Overall	0.095	0.048	0.036	0.067	0.08	0.081	0.099
Highest bin	0.517	0.303	0.181	0.448	0.391	0.49	0.639
3.91–3.62 Å bin	0.063	0.147	0.041	0.088	0.244	0.265	0.639
2.98–2.88 Å bin	0.517	0.303	0.181	0.448	—	—	—

† Data sets were collected in-house at the indicated propane temperature during the flash-cooling step. ‡ The effective high-resolution limit reported is equal to the $d_{\rm edge}$ ($d_{\rm edge}$ = 2.89 Å) for data sets 193 K, 173 K, 153 K and 143 K. For data sets 138 K, 133 K and 113 K, the effective high-resolution limit was determined using the criterion $I/\sigma(I) \geq 2$. § $R_{\rm merge} = \sum|I_h - \langle I_h\rangle|/\sum I_h$, where $\langle I_h\rangle$ is the mean of the measurements for a single *hkl*.

Table 2
Effect of mode of crystal transport on data quality.

Values in parentheses are for the highest resolution bin.

Data set†	1 (flash-cooled in-house)	2 (flash-cooled at synchrotron)
Mosaicity (°)	0.7	0.3
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters (Å)		
a	106.2	106.2
b	109.5	109.6
c	181.8	181.8
Resolution range‡ (Å)	50–2.60 (2.70–2.60)	50–2.45 (2.54–2.45)
No. of unique reflections	64298	77169
Completeness (%)	97.3 (96.5)	99.0 (99.3)
Multiplicity	4.1	4.1
Average $I/\sigma(I)$	14.2 (3.1), 3.1 (2.70–2.60 Å bin)	21.2 (2.7), 5.1 (2.76–2.54 Å bin)
$R_{\rm merge}$§	0.083 (0.344), 0.344 (2.70–2.60 Å bin)	0.064 (0.546), 0.327 (2.76–2.54 Å bin)

† Data sets were collected at Advanced Light Source beamline 8.2.1. ‡ The effective high-resolution limit was determined using the criterion $I/\sigma(I) \geq 2$. § $R_{\rm merge} = \sum|I_h - \langle I_h\rangle|/\sum I_h$, where $\langle I_h\rangle$ is the mean of the measurements for a single *hkl*.

tions from the X-ray bandwidth and beam crossfire. An error model, as implemented in *HKL*2000, was used to allow χ_2 values (goodness-of-fit estimate) to converge to ∼1.0 for all data sets prior to calculation of the intensity statistics reported in Tables 1 and 2. Molecular replacement (using PDB code 1aoi as the original search model) and subsequent partial structure refinement for two of the structures (data sets 153 K and 138 K in Table 1) was carried out using *CNS* (Brünger *et al.*, 1998). Steps in refinement included rigid-body refinement, simulated annealing, positional minimization and grouped *B*-factor refinement. Crystal-packing analysis and structure comparisons were performed using the program *LSQMAN* from the Uppsala Software Factory (Kleywegt, 1996).

3. Results and discussion

3.1. Optimal propane flash-cooling temperature

The temperature for the propane flash-cooling step for nucleosome crystals has been previously reported by our group and others to be ∼153 K (Luger *et al.*, 1997; Muthurajan *et al.*, 2003; Sargent & Richmond, 2004). We performed a systematic and detailed analysis to investigate the effect of vitrification temperatures on the diffraction quality of nucleosome crystals. Three nucleosome crystals were flash-cooled at each of the various liquid-propane temperatures and diffraction data were obtained using an identical data-collection strategy for each crystal. All crystals were of uniform size

(~0.3 × 0.3 × 0.7 mm). Representative data from only one crystal for each temperature is shown in Table 1, although the observed data statistics were reproducible for the multiple measurements at each temperature.

A comparison of the various data sets reveals several temperature-dependent effects on the data quality, as shown in Table 1 and Fig. 2. The overall completeness and completeness in the highest resolution bin are both lower at extreme temperatures (193, 113 K) because of the rejection of a higher percentage of reflections following data reduction. A comparison of the average diffraction intensities and the $R_{\rm merge}$ values for all data sets reveals that flash-cooling crystals at a propane temperature of 153 K leads to the highest diffraction intensities with lowest $R_{\rm merge}$ values (Fig. 2). We observe a twofold to fivefold increase in the average diffraction intensities (Table 1; Fig. 2*a*) and an approximately threefold increase in the average $I/\sigma(I)$ (Table 1; Figs. 2*b* and 2*c*) at 153 K compared with both higher and lower temperatures. The $R_{\rm merge}$ values also reflect this trend, with data at 153 K yielding $R_{\rm merge}$ values which are around threefold lower than those at 193 and 113 K (Table 1; Figs. 2*d* and 2*e*). These differences are significant considering that the comparisons are drawn between crystals of similar sizes, with identical data-collection parameters, space groups and data redundancies. Further characterization of crystals at different temperatures with different detector distances showed a concomitant reduction in the diffraction limit at temperatures both above and below 153 K (not shown), even though the data shown in Table 1 were obtained only to $d_{\rm edge}$ = 2.89 Å in each case. Routinely, the observable diffraction limit deteriorates from ~2.2 Å at 153 K to ~3.2 Å at the extreme propane temperatures tested (not shown). Hence, our results reveal that ~153 K is the optimal cooling temperature for nucleosome crystals and that a temperature fluctuation of a few tens of

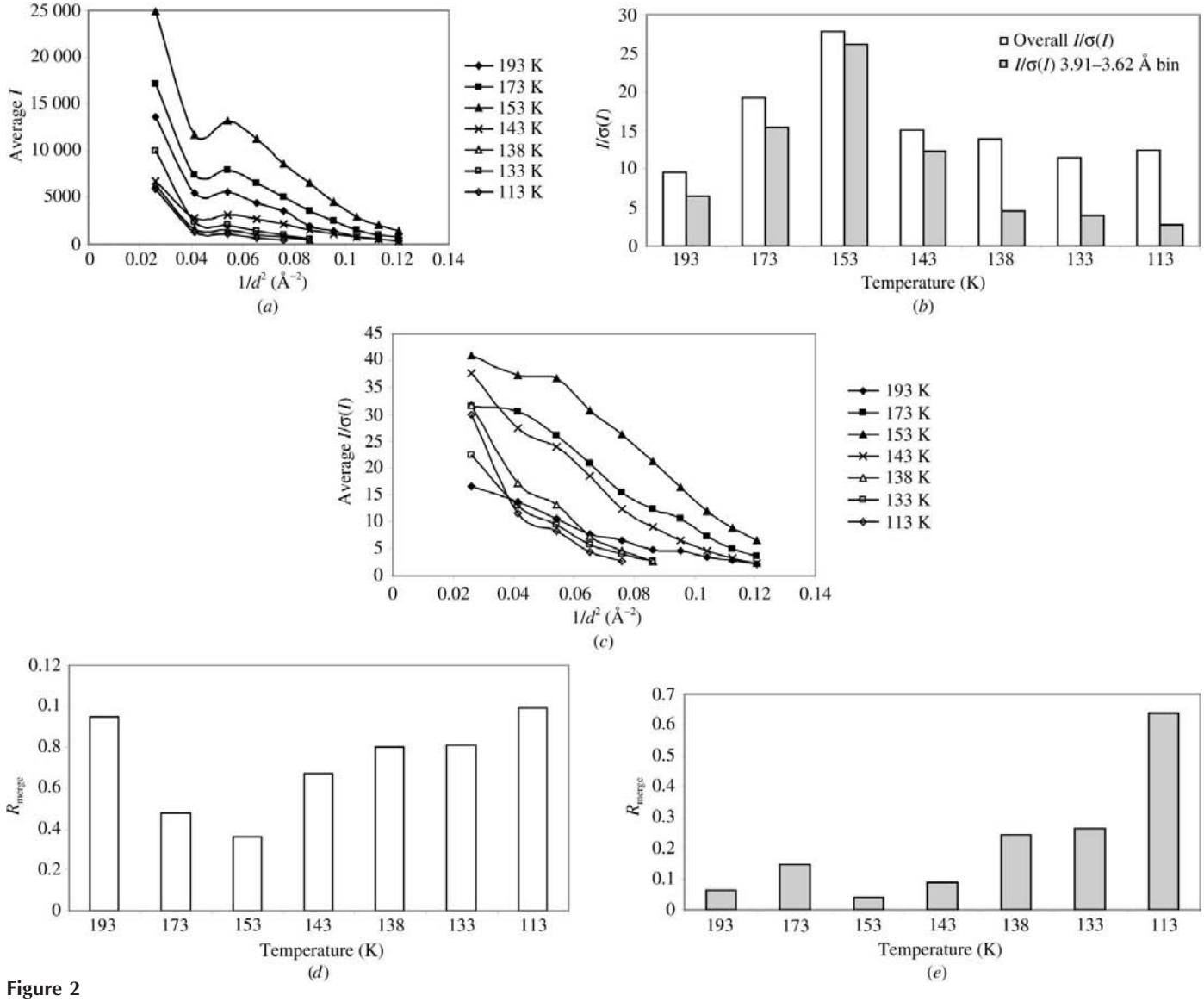

Figure 2
Plots showing the dependence of the data statistics (from Table 1) on propane flash-cooling temperature. A single representative data set is shown for each temperature. (*a*) Plot of average intensities from all reflections as a function of resolution. (*b*) $I/\sigma(I)$ values for the various data sets. (*c*) Average $I/\sigma(I)$ as a function of resolution. Overall $R_{\rm merge}$ (*d*) and $R_{\rm merge}$ in the 3.91–3.62 Å resolution bin (*e*) for the various data sets.

degrees above and below the optimal temperature has a detrimental effect on diffraction quality.

3.2. Crystal-packing analysis

In the course of these experiments, we also observed that the lattice spacing along the *c* axis changes in a temperature-dependent manner, while the *a* and *b* axes remain unchanged (Table 1; Fig. 3). The unit-cell length along the *c* axis is shortened by ∼3.3% (from ∼182 to ∼175 Å). It must be noted that lattice changes are only observed with some batches of nucleosome crystals tested. If they do occur, they are always observed at temperatures lower than 153 K. Although the basis for the lattice change is not clear, it may be related to the kinetics of nucleosome crystal flash-cooling and to the extent of thermomechanical stress induced during flash-cooling. It is a fairly common observation that mild dehydration of crystals prior to or during flash-cooling often improves diffraction. The effects of lattice changes on the diffraction quality, the crystal packing and the domain conformation of HIV-1 reverse transcriptase have been examined in detail and up to an 18% reduction in lattice volume following induced dehydration has been reported (Esnouf *et al.*, 1998). We show here that even a ∼3.3% reduction of the lattice volume (Table 1; Fig. 3) during flash-cooling in our case correlates with a worsening of diffraction data quality (Table 1; Fig. 2). This reduction in length from ∼182 to ∼175 Å stays constant, with no further decrease beyond this end-point at any of the lower temperatures tested.

In order to study how the shrinkage of the unit-cell *c* axis affects nucleosome structure and/or crystal packing, the structures obtained at 153 and 138 K (data sets in Table 1) were compared in detail. Both data sets were phased and the structures were partially refined as outlined in §2. Molecular replacement (using PDB code 1aoi as the original model) was used for initial phasing followed by partial refinement of the model as described in §2.3. The *R*-factor and $R_{\rm free}$ values following partial refinement are 22.0 and 26.9%, respectively, for data set 153 K and 24.7 and 32.2%, respectively, for data set 138 K. As shown in Table 1, while the unit cell *a* and *b* axes are similar for the two data sets, the *c* axis is shorter by ∼3.3% for data set 138 K, resulting in a decrease in the solvent content from ∼55 to ∼52% for data set 138 K.

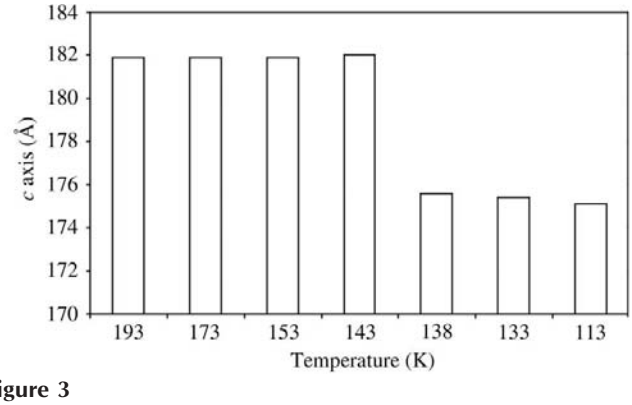

Figure 3
Lattice changes in nucleosome crystals. Reduction in *c*-axis length at lower propane temperatures.

We compared the two structures following a least-squares superposition of the nucleosome structure at 138 K on that obtained from the 153 K data set using the *LSQMAN* program from the Uppsala Software Factory (Kleywegt, 1996). The two structures superimposed with an overall root-mean-square deviation of 0.33 Å and inspection of the two structures revealed no global differences between them (not shown). Next, we compared the crystal packing between the two structures, which revealed some interesting changes. Fig. 4 summarizes our findings. The packing is altered along the *c* axis and neighbouring molecules pack much closer to each other along the *c* axis at ∼138 K (Fig. 4). More specifically, the outer strand phosphates on the nucleosomal DNA between neighbouring molecules are in close proximity as a consequence of the lattice change (for an example, see Fig. 4*d*). The negatively charged phosphates in close contact may cause repulsion and increased disorder within the crystal, which may partly explain the deterioration in diffraction in crystals with the reduced *c* axis (Table 1). Also, the molecules cannot pack any closer along the *c* axis owing to steric constraints. This explains why we do not observe further reduction beyond the *c*-axis length of ∼175 Å at any of those temperatures.

Nucleosome crystals are highly unstable, susceptible to radiation damage and are not amenable to data collection at room temperature. It is therefore not possible to collect complete data sets and obtain the mosaicity value and other data statistics for crystals at room temperature. However, we tested how nucleosome crystals diffracted when flash-cooled in liquid nitrogen. Five crystals were cooled by direct plunging into liquid nitrogen (data not shown). Again, as in the case of flash-cooling in liquid propane, flash-cooling in liquid nitrogen did not lead to ice nucleation in the crystals. All crystals were of similar size (∼0.3 × 0.3 × 0.7 mm). Flash-cooling the crystals by plunging into liquid nitrogen leads to irreproducible results. In some cases, a precise unit-cell shrinkage identical to that discussed above (shrinkage of the *c* axis from ∼182 to ∼175 Å) is observed. A severe deterioration of the data quality and a decrease in the diffraction limit is also observed in most cases in liquid nitrogen compared with that from crystals flash-cooled in liquid propane at 153 K. However, because of the lack of reproducibility, we made no further use of the data.

3.3. Effect of mode of crystal transport on data quality

We have previously observed that the mosaicity estimates obtained in-house for a freshly cooled nucleosome crystal are often lower than those obtained following transport of the same cryocooled crystal to the synchrotron. Since the estimate of mosaicity includes contributions from both the mosaic spread of the crystal and beam divergence, it is not possible to separate these factors when comparing data collected in-house and at the synchrotron for the same crystal. In order to study the effect of the mode of crystal transport on the diffraction quality, diffraction data from two similar-sized fragments from

one large crystal were compared. Crystal 1 was flash-cooled in-house and transported to the synchrotron in liquid nitrogen, while crystal 2 was transported in a harvesting tray in cryoprotectant solution and then mounted in a cryoloop and flash-cooled at the synchrotron (Table 2). The data sets were collected at the same beamline using identical data-collection strategies and processed with the same program (outlined in §2). As shown in Table 2, the two data sets are isomorphous. Most noticeably, the mosaicity of the crystal flash-cooled on-site is much lower (mosaicity 0.3) compared with that flash-cooled in-house (mosaicity 0.7). The diffraction limit, the diffraction intensities (∼1.5-fold higher) and the overall data quality (Table 2) were significantly better for the crystal cooled on-site, although the diffracting volumes for the two crystals were almost identical. Both crystals were flash-cooled in an identical manner in liquid propane at 153 K using our regular cryocooling protocol (as discussed previously). Our results suggest that better data quality and a higher diffraction limit are obtained when the crystal is transported in cryoprotectant and flash-cooled at the synchrotron. Dehydration

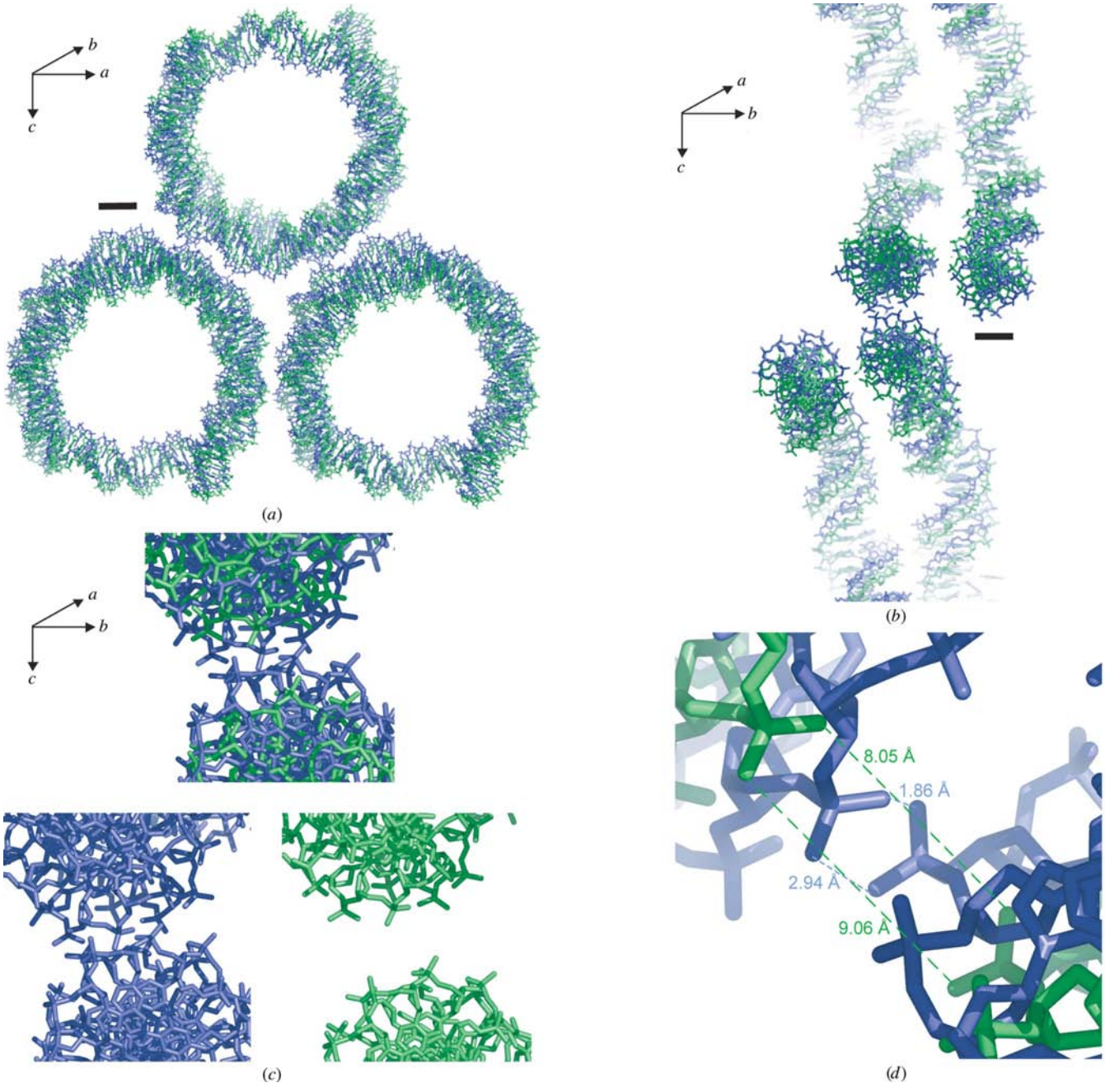

Figure 4
Comparison of crystal packing between crystals flash-cooled at propane temperatures of 153 K (green) and 138 K (blue). DNA alone is shown. Histone proteins have been omitted for clarity. (*a*) Crystal packing with view down the unit-cell *b* axis. Superposition of the two structures is shown. The scale bar is 15 Å. (*b*) Crystal packing with view down the *a* axis. The scale bar is 9 Å. (*c*) A magnified view of Fig. 3(*b*) is shown. The top panel shows a superposition of the two structures; the bottom panel shows the individual structures. (*d*) A magnified view of Fig. 3(*c*) (top panel) is shown.

or other lattice changes do not contribute to the differences, as such changes have not been previously observed for nucleosome crystals upon prolonged incubation in the cryoprotectant (also seen by the almost identical unit-cell parameters for the two data sets in Table 2). Only a single measurement in each case is reported in Table 2 and therefore several data sets will have to be obtained to analyse these effects in detail and to rule out contributions of any other variables to the observed differences. Therefore, for fragile crystals that are susceptible to mosaicity changes, it may be worthwhile to transport the crystal under liquid conditions (either in mother liquor and/or cryoprotectant solution) and subsequently flash-cool at the synchrotron.

4. Conclusions

In conclusion, our results show that plunge-cooling in liquid nitrogen is irreproducible, can cause significant damage to the nucleosome crystals and that limiting the magnitude of the temperature jump by plunge-cooling in liquid propane at intermediate temperatures (∼153 K) prevents or at least minimizes this damage. We routinely observe in our laboratory that plunge-cooling in liquid nitrogen works well for crystals of other macromolecules. In contrast, nucleosome crystals are fragile and have a tendency for high mosaicity and lattice changes during flash-cooling. Flash-cooling these crystals in liquid propane at an intermediate temperature of 153 K prior to data collection at 93 K is essential for optimal data quality. Dehydration during soaking and/or flash-cooling may be a common phenomenon with macromolecular crystals and thus careful observation of lattice changes may be helpful since it may either be advantageous or detrimental to diffraction. We conclude that the lack of 'film boiling' of liquid propane (which increases the cooling rates), the slow kinetics of propane temperature increase (which allows precise control of the temperature during cooling) and a delicate balance between the cooling rates and the thermomechanical stress at ∼153 K determine the optimal cryocooling temperature for nucleosome crystals harvested in the MPD-containing cryoprotectant.

In the second study, we find that the mode of crystal transport has an effect on the diffraction quality of nucleosome crystals. The crystal transported in cryoprotectant produced better diffraction data with a higher resolution limit compared with the crystal transported under flash-cooled conditions. Hence, it may be worthwhile to transport fragile crystals in cryoprotectant in order to minimize stress during transport.

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