UC Irvine

UC Irvine Previously Published Works

Title

Atomic force microscopy applications in macromolecular crystallography.

Permalink

<https://escholarship.org/uc/item/36h6z67t>

Journal

Acta crystallographica. Section D, Biological crystallography, 57(Pt 8)

ISSN

0907-4449

Authors

McPherson, A
Malkin, Aj
Kuznetsov, YG
et al.

Publication Date

2001-08-01

DOI

10.1107/s0907444901008824

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

**A. McPherson,* A. J. Malkin,
Yu. G. Kuznetsov and M. Plomp**

Department of Molecular Biology and
Biochemistry, University of California, Irvine,
CA 92697-3900, USA

Correspondence e-mail: amcphers@uci.edu

Alexander McPherson is a Professor of Molecular Biology and Biochemistry at the University of California at Irvine. He received his PhD degree in the laboratory of Professor Michael Rossmann at Purdue University after completing his BS in Physics at Duke University. His postdoctoral experience was in the laboratory of Professor Alexander Rich at MIT where he worked on the structure of tRNA. He was an Assistant Professor at the Milton S. Hershey Medical Center, Pennsylvania State University and later Associate and Full Professor at the University of California Riverside until 1997. His principle interests are in the structures of intact monoclonal antibodies, virus structure and assembly, and macromolecular crystallization. Dr Alexander Malkin came to UC Riverside in 1989 from the Soviet Union, and Dr Yuri G. Kuznetsov from Russia in 1993. Both obtained their PhD degrees from Moscow State University and worked in the laboratory of Professor Alexander Chernov at the Institute for Crystallography in Moscow. There, they specialized in the physics of crystal growth. Both are accomplished atomic force microscopists with broad interests in the crystallization of macromolecules and the visualization of biological structure.

Atomic force microscopy applications in macromolecular crystallography

Atomic force microscopy (AFM) can be applied both *in situ* and *ex situ* to study the growth of crystals from solution. The method is particularly useful for investigating the crystallization of proteins, nucleic acids and viruses because it can be carried out in the mother liquor and in a non-perturbing fashion. Interactions and transformations between various growth mechanisms can be directly visualized as a function of supersaturation, as can the incorporation of diverse impurities and the formation and propagation of defects. Because the crystals can be observed over long periods, it is also possible to obtain precise quantitative measures of the kinetic parameters for nucleation and growth. Finally, AFM has allowed us to identify a number of previously unsuspected phenomena that influence nucleation, rate of growth and the ultimate perfection of macromolecular crystals. These are all features which are important in determining the ultimate resolution and quality of a crystal's diffraction pattern.

Received 18 February 2001

Accepted 29 May 2001

1. Introduction

Modern X-ray crystallography, particularly protein crystallography, is still beset with persistent practical problems that impede progress and annoy investigators. Most of these concern the properties and perversities of the samples themselves. It is now less how data is collected or its subsequent treatment and use that proves problematic, but the crystals themselves; how they are grown, how they are manipulated or handled in data collection and, most importantly, their diffraction qualities. The following questions are familiar to most of us.

(i) How can we grow larger more perfect crystals more reproducibly and more predictably? How can we eliminate twinning and disorder?

(ii) How can we grow and treat crystals to improve cryogenic mounting and data collection? What is the basis of cryo-annealing?

(iii) What can be performed to increase the diffraction resolution and reduce the mosaicity of crystals?

(iv) Can improvements be made in the properties of crystals, such as reduction of statistical disorder, to enhance the precision of our structure determinations?

One approach to these questions is trial-and-error screening of conditions; simply trying different procedures, reagents and parameters. This certainly has its place and often provides the most practical answers most rapidly. Another approach, of course, is to develop a more profound and comprehensive understanding of the fundamental nature of macromolecular crystals and their growth and, by discovering the sources of problems and origins of effects, gain insight into how they might be addressed.

A powerful technique has come into use in the study of macromolecular crystals and their growth that has considerably advanced our appreciation of their detailed physical properties: this is atomic force microscopy (AFM). First introduced to the field by Steve Durbin in 1992 (Durbin & Carlson, 1992; Durbin *et al.*, 1993), its subsequent application has substantially changed the way we view protein, nucleic acid and virus crystals. In addition to exposing the crystallization process to direct visualization, it has provided keen insights into many of the detailed phenomena on which crystallization relies and has suggested new bases on which the fundamental questions posed above may be addressed. It has, furthermore, added new emphasis to the importance of impurities and their consequences (Malkin, Kuznetsov & McPherson, 1996b; Giegé *et al.*, 1994), altered our ideas of the defect structures of crystals (Malkin, Kuznetsov, Glantz *et al.*, 1996) and revealed the mechanisms that regulate macromolecular crystal growth (Malkin *et al.*, 1995).

An attempt will be made here to describe briefly the fundamental ideas behind the application of AFM to protein, virus and nucleic acid crystals and to point out some areas where the method has been particularly productive. Some examples will also be provided along the way for purposes of illustration and inspiration.

2. Instrumentation

AFM instruments, as shown schematically in Fig. 1, are conceptually and technically simple in comparison with X-ray diffraction systems. Most modern instruments can be operated in both contact mode and tapping mode. In contact mode, a probe made of silicon nitride is impressed on the surface of

Figure 1

Schematic diagram of an atomic force microscope. The vertical deflection of the cantilever tip is amplified through a reflected laser beam and reported by a photoelectric detector. Scanning takes place in a fluid-filled cell of about 75 μl volume. The sample is translated in a raster manner by a piezoelectric positioner upon which the fluid cell is mounted. Shown in the insert is an STEM image of a cantilever tip.

interest and then scanned in a raster mode over the surface. The probe in AFM is a sharp stylus, similar to a minute phonograph needle, which has a tip radius of about 5–40 nm, although even sharper tips using carbon nanotubes are currently under development (Woolley *et al.*, 2000). The probe is mounted at the end of a short cantilever, typically 100–250 μm in length and having a low spring constant ($<1 \text{ N m}^{-1}$) to minimize the force between the tip and the sample during imaging. Scanning is realised by translating the sample beneath the probe, using piezoelectric positioning, along a continuous sequence of raster lines beneath the probe. As the probe tip passes over the surface, it interacts through aggregate atomic forces with structural features on the surface. The interactions cause the probe to be deflected. These exceedingly small displacements are amplified by deflections of a laser beam, which is reflected from the upper surface of the probe and detected by a split photodiode. Photoelectric circuitry converts the deflections into height information (Binnig *et al.*, 1986). The resulting data, recorded as a digital topological image, can then be presented in a number of visual formats.

Protein, nucleic acid and virus crystals can be investigated by AFM in their mother liquor as they grow, on a surface after air drying or in other fluids. The imaging which proves most valuable, of course, is in the growth medium. All imaging discussed here, unless otherwise noted, pertains to visualization in the mother liquor.

The AFM can be operated in both 'height' or 'deflection' modes. With the former, a feedback mechanism raises and lowers the sample and maintains the cantilever deflection nearly constant. The system monitors the piezo 'height' and produces the corresponding image information. In 'deflection' mode, the piezo remains stationary and actual cantilever deflection data are recorded. When the AFM is operated in 'height' mode, quantitatively accurate information on surface morphology is obtained. The 'deflection' mode of operation can be particularly useful for imaging surface features which vary widely over the field of observation.

Microfabricated cantilevers exert a significant pressure on the substrate surface. As one might expect, the quality of the image depends on the degree of force employed. The greater the force between tip and surface, the more sensitive the probe is to height variations. On the other hand, too great a force will perturb or damage the surface. While this may not be a severe limitation for hard surfaces such as conventional crystals, it is a major consideration when dealing with relatively soft biological materials.

Problems arising from unfavorable probe–surface interactions, particularly lateral force, have been obviated to some extent by the development of tapping-mode instruments (Hansma *et al.*, 1994). With tapping mode, the probe tip is not in continuous contact with the surface (contact mode) but oscillates up and down as it is scanned over the surface, essentially 'tapping' its way and gently sensing the heights of obstacles it encounters.

In tapping mode the vertical position of the sample is continually adjusted by the feedback mechanism to maintain

the amplitude of the freely oscillating probe a constant. Tapping mode minimizes contact between the probe tip and the sample surface and greatly reduces lateral forces. The tapping-mode technique has proven a significant advance as it has permitted the visualization of materials that would otherwise be too soft to tolerate contact-mode examination. A constraint that may present difficulties is that the specimen must be maintained immobile on the substrate of the fluid cell, which may be glass, cleaved mica or plastic. If the specimen moves owing to interaction with the probe, then no useful information is obtained.

3. Sample preparation and data acquisition

AFM can yield images of crystal surfaces having exceptional clarity and detail. Scan fields may range in size from less than 20 nm up to about 150 μm , with a spatial resolution on biological materials of a few nanometers and a height resolution better than 1. Thus, it provides precise visual detail over a size range that exceeds most other techniques. Its application extends over the range lying between individual macromolecules, which are accessible by X-ray crystallography, macromolecular assemblies amenable to electron microscopy, and living cells, which can just be seen using light microscopy (Allen *et al.*, 1997; Bustamante & Keller, 1995). Because visualization is carried out in a fluid environment, the specimens suffer no dehydration, as is generally the case with electron microscopy. Growing crystals can be observed over long periods as long as they remain immobilized. No fixing or staining is necessary. Contrast depends only on height variation.

The great power of AFM lies not in its imaging capability alone but from the non-perturbing nature of the probe interaction with the sample surface. Because the specimen is virtually ignorant of the presence of the probe, natural processes such as transport and growth appear to be minimally affected. Thus, the investigator can record not simply a single image but a sequence of images that may extend over hours or even days. This is ideal for the study of the growth of macromolecular crystals, which develop over just such periods. Imaging frequency depends on the scan rate of the probe. As scan speed increases, so does pressure on the sample surface that may produce damage, particularly for soft materials. For protein crystals, images are usually collected with a period of 1–5 min. For macromolecular crystal growth, a relatively slow process at low to moderate supersaturations, events on the surface impose no requirement for rapid scan speed. Thus, an extended series of good-quality images are generally accessible to the investigator.

When AFM is carried out in fluid cells, the fluid can be changed during the course of experiments without appreciably disturbing the specimen. This is of real value in the study of macromolecular crystallization because a frequent objective is to study growth processes under various conditions of supersaturation. This can be achieved with a single growing crystal because the mother liquor can be exchanged. Growth steps are visible on the surfaces of crystals and, because their

advancement is relatively slow, their rate of progression over the surface can be recorded in a temporal sequence of images. When rates are recorded as a function of temperature, salt concentration, supersaturation or some other variable, then growth-step velocities can be used to deduce thermodynamic and kinetic parameters such as the step free energy and the kinetic coefficient of steps (Kuznetsov *et al.*, 1999; Land *et al.*, 1997; Malkin, Kuznetsov & McPherson, 1996a; Malkin *et al.*, 1997; Yau, Petsev *et al.*, 2000; Yau, Thomas *et al.*, 2000). In the best of cases, individual virus particles and even single protein molecules can be observed as they are recruited into advancing step edges (Kuznetsov *et al.*, 1999; McPherson *et al.*, 2000).

In the AFM analysis of macromolecular crystallization some practical problems are common. Because biological crystals are fragile and often difficult to manipulate and because scanning occurs in an aqueous environment, it may be difficult to fix the crystals to a substrate. This can be overcome by nucleating and growing the crystals directly on the substrate, *i.e. in situ* analysis, or by ‘clamping’ larger crystals to the substrate beneath flexible carbon fibers. The greatest difficulty in obtaining images is the softness of the crystals and their susceptibility to scarring by the AFM tip. Tapping-mode operation can alleviate this problem in some instances, but even then softness may set the limit of resolution. Some crystals, such as lysozyme or thaumatin, are relatively hard and resistant to probe damage. Crystals of larger viruses on the other hand may be fragile and difficult to deal with.

Figure 2

Lattice-resolution AFM images of protein crystals. In (a) is a crystal of a murine IgG (Harris *et al.*, 1992) and in (b) a crystal of a complex of human–simian chimeric antibodies (Kuznetsov, Day *et al.*, 2000). In (c) is an AFM image of a thaumatin crystal and in (d) that same image after Fourier filtering. Scan sizes are (a) 200 $\times$ 200 (b) 100 $\times$ 100, (c) 300 $\times$ 300 and (d) 100 $\times$ 100 nm.

Cantilever tips are of crucial importance in imaging biological specimens. At the nanometer level, tips are irregular and often make multiple contacts with the surface under study. As a consequence, double images, multiple images, convoluted images and other types of artifacts can be obtained. Probe tips are also easily fouled by extraneous material such as protein precipitate, which significantly lessens their ability to sense surface detail. Mother liquors, therefore, must be scrupulously clean and well filtered. Vibration can affect high-resolution analyses and must be minimized in every way possible.

With the exception of their softness and fragility, macromolecular crystals are otherwise excellent systems for studying the general phenomenon of crystal growth. The particle size is relatively large, 3–10 nm diameters for most proteins, many times that for viruses. This is an order of magnitude or more larger than conventional molecules that crystallize. Thus, aggregates can be seen on the surfaces of crystals (Land *et al.*, 1995; Malkin *et al.*, 1999; Kuznetsov *et al.*, 1998) and even the mobility of individual molecules on the crystal surface can be recorded. The kinetics of growth of macromolecular crystals are several orders of magnitude slower than for conventional crystals; thus, the course of events during growth are compatible with the temporal resolution of the instrument. Unit cells are one to two orders of magnitude larger than for conventional crystals and this enhances the definition of growth steps, dislocations, the incorporation of impurities and the defect structure.

Macromolecular crystals are, of course, periodic. This is helpful because the eye averages when their images are

examined and otherwise minor features become evident. In addition, the underlying periodicity makes possible application of Fourier filtering and averaging processes that can yield improved images (Kuznetsov *et al.*, 1997; Brisson *et al.*, 1999). AFM may, in the best of cases, yield lattice-resolution images of crystals that even reveal some gross features of the macromolecules in the unit cells. Examples are shown in Fig. 2. From such images, it may be possible to deduce packing arrangements or even molecular orientations. Li and co-workers (Li, Nadarajah *et al.*, 1999; Li, Perozzo *et al.*, 1999) and Kuznetsov *et al.* (1999), for example, used AFM to analyze packing arrangements on the faces of lysozyme and thaumatin crystals, respectively, and from these deduced the probable mechanisms for molecule incorporation.

Because height information is preserved, enantiomorphic space groups can be resolved. For large asymmetric units, as occur for example in virus or ribosome crystals, it may be possible to derive initial phase information from the particles seen in their fully hydrated crystalline state. This may serve a valuable future role in X-ray crystallography as the field addresses ever larger structures.

4. Interpretation of AFM images

There appear to be a number of basic mechanisms by which protein, virus and nucleic acid crystals develop (that is, create and extend new growth layers) and all have been visualized *in situ* using AFM. In general, an individual crystal does not grow exclusively by a single mechanism, but may employ different

mechanisms on different crystal faces, on a single face at different supersaturations and, frequently, competing mechanisms simultaneously on one surface (Malkin *et al.*, 1995). For some crystals, a single mechanism may dominate over a very wide range of physical and chemical conditions, for example in most virus crystals (Kuznetsov, Day *et al.*, 2000), while others shift from one mechanism to another as a consequence of only small changes in crystallization conditions, *e.g.* tRNA (Ng *et al.*, 1997). In another interesting example (Yip *et al.*, 1998) it was shown by AFM that significant effects in the crystallization of insulin resulted from only minor changes in amino-acid sequence.

Of the mechanisms that have been verified for macromolecular crystals, most were known to pertain to various kinds of conventional crystals.

Figure 3

Screw dislocations on the surfaces of macromolecular crystals. (a) thaumatin, 15 × 15 μm; (b) canavalin, 10 × 10 μm; (c) Bence-Jones protein, 2 × 2 μm; (d) trypsin, 30 × 30 μm; (e) yeast phenylalanine tRNA, 23 × 23 μm; (f) the 50s subunit of bacterial ribosomes, 60 × 60 μm.

These were growth originating at screw dislocations (Burton *et al.*, 1951), as illustrated in Figs. 3 and 4, growth arising from two-dimensional islands shown in Fig. 5 (Chernov, 1984; Chernov *et al.*, 1988) and normal growth (Tiller, 1991). All of these sources provide growth steps in either a continuous (screw dislocations) or discontinuous (nucleation of two-dimensional islands) manner that then proceed, as recorded in Fig. 5, to extend tangentially over the face of the crystal by addition of molecules at their edges.

An unusual growth phenomenon and one that appears unique to macromolecular crystals because of the properties of concentrated protein solutions (Haas & Drenth, 1999; Piazza, 1999) is growth by three-dimensional nucleation (Malkin *et al.*, 1995). An example involving the protein trypsin is seen in Fig. 6. This process is manifested by the sudden appearance on crystal surfaces of multilayer stacks, each layer of which serves as a source for tangential expansion. The most plausible explanation for the appearance of these three-dimensional nuclei on surfaces is that they arise from liquid protein droplets (Ten Wolde & Frenkel, 1997; Kuznetsov *et al.*, 1998) that exist at the interfacial layer. These droplets, or massive aggregates, are presumably composed of thousands of molecules which exhibit short-range order principally mediated by non-specific hydrophobic interactions and random arrangements of hydrogen bonds, which then order into crystalline layers under the direction of the underlying lattice.

The tangential velocity of advancement of individual step edges can, with AFM, be recorded as a function of supersaturation. From such kinetic measurements, fundamental thermodynamic parameters of the crystallization process, *e.g.*

Figure 4
The development of complex screw dislocations on the surface of an orthorhombic trypsin crystal. Intervals between images are 67 s and the scan areas are $10 \times 10 \mu\text{m}$.

the surface free energy α , can be estimated (Chernov, 1984). Others can be determined from the rates of two-dimensional

Figure 5
Two-dimensional nuclei, or islands that ultimately merge with others to produce new growth layers, are seen on the surfaces of (a) thaumatin, $20 \times 20 \mu\text{m}$, (b) intact mouse antibody Mab 231, $11 \times 11 \mu\text{m}$, (c) glucose isomerase, $11 \times 11 \mu\text{m}$ and (d) turnip yellow mosaic virus, $7 \times 7 \mu\text{m}$.

Figure 6
A series of AFM images showing the development of a multilayer stack on a {101} face of an orthorhombic trypsin crystal. Two-dimensional nucleation is absent even on the very flat top of the stack. These plateaux of growth layers are common on most protein, nucleic acid and virus crystals.

Figure 7

Vacancies in the lattices, often several unit cells in extent, are seen in a variety of protein and virus crystals. (a) A crystal of an intact human–simian chimeric IgG complex (Kuznetsov, Day *et al.*, 2000), $1.0 \times 1.0 \mu\text{m}$; (b) bromegrass mosaic virus, $542 \times 542 \text{ nm}$; (c) satellite tobacco mosaic virus, $800 \times 800 \text{ nm}$; (d) a crystal of an intact mouse IgG immunoglobulin (Harris *et al.*, 1992), $6.5 \times 6.5 \mu\text{m}$.

Figure 8

A variety of grain boundaries and stacking faults typical in macromolecular crystals. (a) Thaumatin, $25 \times 25 \mu\text{m}$; (b) cucumber mosaic virus, $1.5 \times 1.5 \mu\text{m}$; (c) bovine trypsin, $40 \times 40 \mu\text{m}$; (d) satellite tobacco mosaic virus, $25 \times 25 \mu\text{m}$.

nucleation on surfaces and subsequent layer addition, both of which can be directly visualized using AFM. Critical two-dimensional nuclear sizes (Malkin *et al.*, 1999; Yau & Vekilov, 2000) can also be deduced as a function of supersaturation simply by measuring the sizes and even by counting the number of molecules comprising nascent growth islands that either persist and grow or dissolve and disappear. From AFM studies, it has become evident that while the mechanisms for growth of macromolecular crystals are essentially the same as for conventional crystals, the kinetic parameters, the rates that govern these processes, are two to four orders of magnitude less (McPherson *et al.*, 2000).

Among the most intriguing observations to emerge from AFM studies have been of the defects and dislocations that afflict macromolecular crystals, the consequences of those defects and their sources. Examples of two important classes of defects are presented in Figs. 7 and 8. From AFM analyses, it is again clear that the variety of defects and dislocations are much the same as for conventional crystals, but that the density of defects may be two to five orders of magnitude greater (Malkin, Kuznetsov & McPherson, 1996a). These patterns of defects may serve as a basis for a more refined understanding of mosaicity. Furthermore, the defects undoubtedly influence diffraction resolution and they provide the sources for mechanical stress introduced by cryogenic procedures. They may further supply a framework for understanding the phenomenon of crystal annealing.

The kinds of impurities incorporated by macromolecular crystals range from foreign protein molecules to aberrant

Figure 9

Incorporation of microcrystals from solution into larger actively growing crystals. (a) Thaumatin, $3 \times 3 \mu\text{m}$; (b) trypsin, $5 \times 5 \mu\text{m}$; (c) canavalin, $20 \times 20 \mu\text{m}$; (d) beef catalase, $42 \times 42 \mu\text{m}$. In (d) the catalase crystal has been etched to reveal the embedded microcrystals.

Figure 10

If the STMV crystal is still growing in its mother liquor, as it is here, then fibers are incorporated in their entirety into the crystal lattice as impurities and remain a permanent part of the virus crystal. At low resolution in (a) it is immediately apparent that the fibers always assume lattice directions on the surface of the crystal and are incorporated along crystallographic directions. (b) is a high-magnification image of the defect produced in the crystal in the neighborhood of an incorporated fiber. In (c) a particularly long fiber is seen interacting with the growth layers on the crystal surface. It lies on the original surface (1), blocks progress of a step edge (2), but is raised by one growth later (3) by progression of another growth step approaching from below. Scan areas are (a) 25×25 , (b) 1×1 and (c) $2 \times 2 \mu\text{m}$.

Figure 11

A crystal which has stopped growing frequently has a surface totally covered with impurities which then form a hard shell over the surface. If the shell is scraped away with the AFM tip as in (a) then, as seen by the growth islands forming on the freshly exposed surface, the crystal resumes growth. Scan areas are $7.5 \times 7.5 \mu\text{m}$.

virions in virus crystals, to dust and foreign particles (Land *et al.*, 1995; Yau, Petsev *et al.*, 2000) to fibers and microcrystals that sediment on growing crystal surfaces (Malkin, Kuznetsov & McPherson, 1996b; McPherson *et al.*, 1996; Malkin *et al.*, 1997). Examples, some rather surprising, are seen in Figs. 9 and 10. What is remarkable is the elastic properties of many macromolecular crystals. Unlike conventional crystals, they exhibit extreme tolerance for impurities of relatively large size, particularly microcrystals in random orientations (Malkin, Kuznetsov & McPherson, 1996a; Malkin *et al.*, 1997; McPherson *et al.*, 1996). In spite of the insults inflicted on the long-range order of growing surfaces, the crystals appear in most cases able to incorporate the contaminant and then continue growing around it without otherwise serious disruption.

In terms of X-ray diffraction effects, it seems entirely plausible that the extensive volume of the crystals affected by local stress and disorder instigated by impurities may be important. Mosaicity, for example, is a function of defect

structure and defect density. Disorder, both local and long range, is a determinant in diffraction resolution.

An additional effect of impurities may be the limitation of ultimate crystal size. As crystals grow, they deplete the solution of nutrient and supersaturation decreases. While tangential extension of layers, which is not kinetically limiting, may be relatively insensitive to a decrease in supersaturation, the initiation of new growth layers may be strongly affected. As a consequence, surfaces of crystals become progressively flatter and terraces between growth steps accumulate more impur-

ities in a given time. This then tends to retard growth even further. Eventually growth, relative to impurity accumulation, reaches a point where it can no longer be sustained. It becomes overwhelmed by impurity interference (Cabrera & Vermilyea, 1958). As seen in Fig. 11, these impurity layers eventually form a dense shell on the crystal surfaces which completely prevents further growth. If the impurity layer is scraped away with the AFM tip, however, the newly exposed crystal surface below immediately resumes growth.

Studies by AFM suggest that crystals have a broad range of properties and that they vary, as do conventional crystals, according to the molecules that comprise them and the interactions by which molecules interact with one another. At one end of the spectrum are crystals whose molecules form numerous strong geometrically well defined bonds in three dimensions. As a consequence, their lattices are highly selective in terms of particle size, integrity and conformation; that is, they depend upon stringent particle or molecular homogeneity. They have a high surface free energy α and their long-range order is very precise. At the other end of the spectrum are crystal lattices whose molecules form sparse, weak and geometrically imprecise bonds. Their lattices are promiscuously tolerant in terms of molecular size and conformation and they readily incorporate aberrant particles and contaminants. Those crystals have a low surface free energy and long-range order is poor. Most lattices, of course, lie somewhere in between. AFM, as evidenced now by many studies, is a useful approach for delineating and even quantitating many of these properties.

References

- Allen, S., Davies, M. C., Roberts, C. J., Tendler, S. J. B. & Williams, P. M. (1997). *Trends Biotechnol.* **15**, 101–105.
- Binnig, G., Quate, C. F. & Gerber, C. (1986). *Phys. Rev. Lett.* **56**, 930–933.
- Brisson, A., Bergsma-Schutter, W., Oling, F., Lambert, O. & Revskine, I. (1999). *J. Cryst. Growth*, **196**, 456–470.

- Burton, W. K., Cabrera, N. & Frank, F. C. (1951). *Philos. Trans. R. Soc. London Ser. A*, **243**, 299–310.
- Bustamante, C. & Keller, D. (1995). *Phys. Today*, **48**, 32–38.
- Cabrera, N. & Vermilyea, D. A. (1958). *Growth and Perfection of Crystals*. London: Chapman & Hall.
- Chernov, A. A. (1984). *Modern Crystallography*, Vol. III, *Crystal Growth*. Berlin: Springer-Verlag.
- Chernov, A. A., Rashkovich, L. N., Smoliskii, I. L., Kuznetsov, Yu. G., Mkrtchyan, A. A. & Malkin, A. I. (1988). *Growth of Crystals*, Vol. 15, edited by E. I. Givargizov & S. A. Grinberg, pp. 43–91. New York: Consultant Bureau.
- Durbin, S. D. & Carlson, W. E. (1992). *J. Cryst. Growth*, **122**, 71–79.
- Durbin, S. D., Carlson, W. E. & Saros, M. T. (1993). *J. Phys. D*, **26**, 128–135.
- Giegé, R., Lorber, B. & Theobald-Dietrich, A. (1994). *Acta Cryst. D*, **50**, 339–350.
- Haas, C. & Drenth, J. (1999). *J. Cryst. Growth*, **196**, 388–394.
- Hansma, P. K., Cleveland, J. P., Radmacher, M., Walters, D. A., Hillner, P. E., Bezanilla, M., Fritz, M., Vie, D., Hansma, H. G., Prater, C. B., Massie, J., Fukunage, L., Gurley, J. & Elings, V. (1994). *Appl. Phys. Lett.* **64**, 738–1740.
- Harris, L. J., Larson, S. B., Hasel, K. W., Day, J., Greenwood, A. & McPherson, A. (1992). *Nature (London)*, **360**, 369–372.
- Kuznetsov, Yu. G., Day, J., Newman, R. & McPherson, A. (2000). *J. Struct. Biol.* **131**, 108–115.
- Kuznetsov, Yu. G., Konnert, J., Malkin, A. J. & McPherson, A. (1999). *Surf. Sci.* **440**, 69–80.
- Kuznetsov, Yu. G., Malkin, A. J., Land, T. A., DeYoreo, J. J., Barba de la Rosa, A. P., Konnert, J. & McPherson, A. (1997). *Biophys. J.* **72**, 2357–2364.
- Kuznetsov, Yu. G., Malkin, A. J. & McPherson, A. (1998). *Phys. Rev. B*, **58**, 6097–6103.
- Land, T. A., DeYoreo, J. J. & Lee, J. D. (1997). *Surf. Sci.* **384**, 136–155.
- Land, T. A., Malkin, A. J., Kuznetsov, Yu. G., McPherson, A. & DeYoreo, J. J. (1995). *Phys. Rev. Lett.* **75**, 2774–2777.
- Li, H., Nadarajah, A. & Pusey, M. L. (1999). *Acta Cryst. D*, **55**, 1036–1045.
- Li, H., Perozzo, M. A., Konnert, J. H., Nadarajah, A. & Pusey, M. L. (1999). *Acta Cryst. D*, **55**, 1023–1035.
- McPherson, A., Malkin, A. J. & Kuznetsov, Yu. G. (2000). *Annu. Rev. Biophys. Biomol. Struct.* **29**, 361–410.
- McPherson, A., Malkin, A., Kuznetsov, Yu. G. & Koszelak, S. (1996). *J. Cryst. Growth*, **168**, 74–92.
- Malkin, A. J., Kuznetsov, Y. G., Glantz, W. & McPherson, A. (1996). *J. Phys. Chem.* **100**, 11736–11743.
- Malkin, A. J., Kuznetsov, Yu. G., Land, T. A., DeYoreo, J. J. & McPherson, A. (1995). *Nature Struct. Biol.* **2**, 956–959.
- Malkin, A. J., Kuznetsov, Yu. G., Lucas, R. W. & McPherson, A. (1999). *J. Struct. Biol.* **127**, 35–43.
- Malkin, A. J., Kuznetsov, Yu. G. & McPherson, A. (1996a). *Proteins Struct. Funct. Genet.* **24**, 247–252.
- Malkin, A. J., Kuznetsov, Y. G. & McPherson, A. (1996b). *J. Struct. Biol.* **117**, 124–137.
- Malkin, A. J., Kuznetsov, Y. G. & McPherson, A. (1997). *Surf. Sci.* **393**, 95.
- Ng, J. D., Kuznetsov, Yu. G., Malkin, A. J., Keith, G., Giegé, R. & McPherson, A. (1997). *Nucleic Acids Res.* **25**(13), 2582–2588.
- Piazza, R. (1999). *J. Cryst. Growth*, **196**, 415–423.
- Ten Wolde, P. R. & Frenkel, D. (1997). *Science*, **277**, 1975–1978.
- Tiller, W. A. (1991). *The Science of Crystallization: Macroscopic Phenomena and Defect Generation*. Cambridge University Press.
- Woolley, A. T., Cheung, C. L., Hafner, J. H. & Lieber, C. M. (2000). *Chem. Biol.* **7**, 193–204.
- Yau, S. T., Petsev, D. N., Thomas, B. R. & Vekilov, P. G. (2000). *J. Mol. Biol.* **303**, 667–678.
- Yau, S. T., Thomas, B. R. & Vekilov, P. G. (2000). *Phys. Rev. Lett.* **85**, 353–356.
- Yau, S. T. & Vekilov, P. G. (2000). *Nature (London)*, **406**, 494–497.
- Yip, C. M., Brader, M. L., DeFelippis, M. R. & Ward, M. D. (1998). *Biophys. J.* **74**, 2199–2209.