buffer and salt conditions. These variations can be exploited in a systematic fashion for phasing by electron-density averaging, so long as (1) the shrinkage relationships among the different crystals are not merely isotropic and (2) the boundaries and NCS parameters among related segments can be determined. Perutz (Perutz, 1946; Bragg & Perutz, 1952) recognized the potential utility of such shrinkage stages for crystallographic phasing in studies of haemoglobin crystals with varying degrees of hydration.

Recent examples of structure solutions involving multidomain and multiple-crystal-form averaging include studies of HIV reverse transcriptase (RT) (Ren *et al.*, 1995; Ding *et al.*, 1995). Studies of HIV RT by Stuart and coworkers involved multidomain and multiple-crystal-form averaging using different soaking solutions (Esnouf *et al.*, 1995; Ren *et al.*, 1995), in some cases with dramatically improved diffraction resolution. Arnold and co-workers have applied multidomain and multiple-crystal-form averaging to studies of HIV RT, including a systematic application of averaging electron density between 'frozen' and 'unfrozen' crystal forms (Ding *et al.*, 1995; Das *et al.*, 1996). Tong *et al.* (1997) recently described electron-density averaging among multiple closely related crystal forms of the human cytomegalovirus protease that were obtained by treatment of the crystals with different soaking buffers containing differing levels of precipitants, such as salt and polyethylene glycol.

13.4.14. Programs

This review hopefully covers most aspects encountered when employing electron-density averaging, yet the authors have drawn liberally from their own experience. There are now a large number of averaging programs and procedures available, some more suitable for structure determinations of proteins with low NCS redundancy and improper relationships (Jones, 1992) and others particularly suitable for high NCS redundancy, such as is encountered in the study of icosahedral viruses. For large structures, phase determination can be a very time-consuming computer operation. Therefore, attempts have been made to parallelize some programs (Cornea-Hasegan *et al.*, 1995), although this may lead to difficulties in exporting the programs to new and different computers.

Recently described program packages for symmetry averaging have been successfully applied to a number of cases. General program systems for averaging that are well suited to cases with high NCS include *ENVelope* (Rossmann *et al.*, 1992) and *GAP* (Jonathan Grimes and David Stuart, unpublished results); these same packages have also been used for multiple-crystal-form averaging and problems with low symmetry. A number of the program packages have been conveniently integrated with interactive computer-graphics programs such as *O* (Jones *et al.*, 1991) and most permit molecular-envelope definition by a number of possible approaches. *RAVE* and *MAVE* (Kleywegt & Jones, 1994), programs for graphics-assisted averaging within and between crystal forms, also come with an array of tools for flexible map handling and envelope definition (Kleywegt & Jones, 1996). The program systems *DMMULTI* (Cowtan & Main, 1993) and *MAGICSQUASH* (Schuller, 1996), which both derive from the program *SQUASH* (Zhang, 1993), can simultaneously apply real-space (symmetry averaging and solvent levelling with or without histogram matching) and reciprocal-space (phase refinement by the Sayre equation) constraints for phase improvement and extension. The advantage of adding phasing by the Sayre equation is greater at higher resolution, but appears to be significant in some cases, even at relatively low resolution (Cowtan & Main, 1993). *MAGICSQUASH* has been used to determine a number of structures which required multiple-domain and multiple-crystal-form averaging (Schuller, 1996). The *DEMON/ANGEL* package allows noncrystallographic averaging among multiple crystal forms together with solvent flattening and histogram matching (Vellieux *et al.*, 1995). Other versatile programs for electron-density averaging include *AVGSYS* (Bolin *et al.*, 1993) and *PHASES* (Furey & Swaminathan, 1990, 1997), both of which have features for facilitating definition and refinement of NCS parameters.

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